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Size-selective detection in integrated optical interferometric biosensors

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Abstract: We present a new size-selective detection method for integrated optical interferometric biosensors that can strongly enhance their performance. We demonstrate that by launching multiple wavelengths into a Young interferometer waveguide sensor it is feasible to derive refractive index changes from different regions above the waveguide surface, enabling one to distinguish between bound particles (e.g., proteins, viruses, bacteria) based on their differences in size and simultaneously eliminating interference from bulk refractive index changes. Therefore it is anticipated that this new method will be ideally suited for the detection of viruses in complex media. Numerical calculations are used to optimize sensor design and the detection method. Furthermore the specific case of virus detection is analyzed theoretically showing a minimum detectable virus mass coverage of $4 \times 10^2$ fg/mm$^2$ (typically corresponding to $5 \times 10^3$ particles/ml).

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References and links

1. Introduction

Integrated optical (IO) biosensors have been demonstrated as a powerful detection and analysis tool for biosensing. Main advantages of IO biosensors are its high sensitivity, real-time and label-free measurements. Interferometric sensors [1–6], surface plasmon resonance (SPR)-based sensors [7,8], grating couplers [9,10], resonant optical microcavity sensors [11–13], and photonic crystal waveguide sensors [14,15], are several IO sensors which have been developed. Integrated optical interferometric biosensors sense refractive index (RI) changes, induced by analyte binding, occurring in the evanescent field. These sensors, including the Mach Zehnder interferometer and the Young interferometer (YI), show extremely high (10⁻⁷–10⁻⁸ refractive index units (RIU)) RI sensitivity. The YI is a strong candidate for point-of-care viral diagnostics, because of this high sensitivity and its multiplexing capability [1]. Measurements show short time delays, because no extensive sample treatment is needed. However, the utilization of the high sensitivity is often hampered by background signals arising from non-specific RI changes within the evanescent field. Any RI change within the evanescent field will contribute to the measured signal. Consequently, in addition to specific binding of the analyte, also non-specific binding and RI changes (e.g. due to temperature changes) in the fluid covering the waveguide (bulk) will be detected. To distinguish between specific and non-specific binding, selective chemical binding techniques are used in combination with washing steps and/or differential measurements. Nevertheless, non-specificity and bulk background changes still hamper successful application of these type of biosensors. Measurements done in body fluids such as blood serum show a high variability in
background and large non-specific binding. For that reason, a method to reduce the contribution to RI changes attributable to non-specific binding was developed [16]. By tuning the evanescent field of two different polarization modes a thin layer (20-30nm) was desensitized and the response to non-specific binding was reduced by a factor of hundred or more. Furthermore, a dual-wavelength operation of an integrated-optical difference interferometer was used to discriminate between binding of molecules and bulk RI changes or between binding of molecules and temperature changes [4]. In general however, the various background contributions to the signal are present simultaneously and therefore the existing methods that allow distinguishing only one of them from the signal are in practice not always sufficient. Previously we expanded the existing dual-wavelength approach [4] to a three wavelength approach that allows to discriminate several different background (bulk and temperature induced RI changes) contributions simultaneously [17]. Here we explore a similar approach for size-selective detection of analytes. The use of multiple wavelengths (3 or more) enables to probe RI changes at different distances from the sensor surface allowing to discriminate larger particles (e.g. viruses) from both smaller particles (e.g. proteins) and bulk contributions. We provide a theoretical basis for this method, we optimize the method for application to a YI sensor and we calculate the achievable detection limit. We anticipate that using the size-selective multiple-wavelength approach as presented here should significantly improve the background suppression. It should be noted that the method presented here will most likely not replace existing methods like bio-receptor layers and anti-fouling strategies to reduce non-specific binding [18], but is rather to be used in combination with these methods to yield enhanced specificity.

We focus on the detection of virus particles. The detection of virus particles in complex matrices such as serum is hampered by both bulk RI changes and non-specific binding, from which we need to discriminate simultaneously. We show that this is possible using the approach developed here because of the differences in size between virus particles (50-200 nm), proteins (1-10 nm) responsible for non-specific binding and bulk RI changes. Although we specifically develop this method for a YI sensor, the method is also applicable to other types of IO interferometric sensors.

A detailed theoretical analysis is given on the performance of this new method for two cases. The first case is aimed to distinguish between specific binding and bulk changes by using two wavelengths. The second case uses three wavelengths to distinguish virus binding from bulk contributions and non-specific binding. Optimized waveguide structures are calculated for each case.

2. Theoretical aspects

This section starts with the theory required to calculate the precision with which the RI change can be determined from the phase changes measured for the different wavelengths. This approach is used to optimize waveguide properties. Subsequently, the specific case of virus detection is treated. It should be noted that the precision (defined as the standard deviation $\sigma_{\Delta n}$ of subsequent measurements of the RI change) is the relevant parameter for indicating the performance of the sensor. Induced RI change due to virus binding should exceed $2 \times \sigma_{\Delta n}$ (95% confidence interval).
2.1 General theory

YI sensors are based on the evanescent field sensitivity of guided modes propagating through the waveguide structure of the sensor [19]. Figure 1 illustrates the working of the YI sensor. Monochromatic light is coupled into an optical channel waveguide and split into two channels, including a measurement and a reference channel. Binding events near the surface of the measurement channel result in an RI change $\Delta n$ at this surface. Consequently, the phase of the beam in the measuring channel changes, resulting in an alteration of the interference pattern that exist in the region of overlapping beams from the two channels. Assuming small RI changes such that the electric field distribution of the guided mode (mode profile) is not affected, the phase change $\Delta \varphi$ between two beams, propagating through any two channels, can be described by [20]:

$$\Delta \varphi = \frac{2\pi}{\lambda} \cdot l \cdot \Delta N_{\text{eff}} = \frac{2\pi}{\lambda} \cdot l \cdot \left(\frac{\partial N_{\text{eff}}}{\partial n}\right)_{\lambda} \cdot \Delta n,$$

(1)

where $l$ is the length of the sensing window, $\lambda$ the vacuum wavelength of the guided light, $\Delta N_{\text{eff}}$ the effective RI change of the guided mode, $\Delta n$ the RI change in the region probed by the evanescent field and $\left(\frac{\partial N_{\text{eff}}}{\partial n}\right)_{\lambda}$ the sensitivity coefficient of $N_{\text{eff}}$ with respect to $n$, for a wavelength $\lambda$. Although not explicitly written, chromatic dispersion is taken into account (see Appendix A). Next, we define multiple layers above the core of the waveguide of which the RI change has to be determined (Fig. 2). Thicknesses of the defined layers can be chosen arbitrarily depending on the experiment, e.g. three layers to discriminate between non-specific protein binding, specific virus binding and bulk RI changes (see Fig. 3).
Fig. 2. Structure definition of waveguide with on top $N$ introduced imaginary layers and a guided mode profile (dashed line), where $d$ is the thickness and $n$ the refractive index.

Fig. 3. Guided mode profiles of three different wavelengths propagating through a waveguide structure with three layers introduced on top of the sensing window to distinguish between the non-specific protein binding, the specific virus binding, and the bulk solution changes.

The electric field distribution of the guided mode depends on the wavelength of the light (shorter wavelengths are more confined to the core than longer wavelengths, see Fig. 3). Consequently, the RI changes in the different layers can be determined by measuring the phase changes at a number of different wavelengths, provided the number of layers does not exceed the number of used wavelengths.

Consider $N_{layer}$ layers (see Fig. 2), and $N_{\lambda}$ number of different wavelengths. The measured phase changes can be written as (in analogy with Eq. (1)):

$$\Delta \varphi = M_{i} \cdot \Delta n$$

with

$$\Delta \varphi = \begin{bmatrix} \Delta \varphi_{1} \\ \vdots \\ \Delta \varphi_{N_{\lambda}} \end{bmatrix}, \quad \Delta n = \begin{bmatrix} \Delta n_{1} \\ \vdots \\ \Delta n_{N_{\lambda}} \end{bmatrix},$$

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Consider $N_{layer}$ layers (see Fig. 2), and $N_{\lambda}$ number of different wavelengths. The measured phase changes can be written as (in analogy with Eq. (1)):
with \( \Delta \phi_j \) the phase change measured at \( \lambda_j \), \( \Delta n_i \) the RI change in layer \( i \), and \( M_i \) (sensitivity matrix) defined as:

\[
M_i = 2\pi \cdot \begin{bmatrix}
\frac{1}{\lambda_1} S_{1,1} & \frac{1}{\lambda_2} S_{1,2} & \frac{1}{\lambda_3} S_{1,3} & \cdots & \frac{1}{\lambda_{N_{\text{layer}}}} S_{1,N_{\text{layer}}}
\frac{1}{\lambda_1} S_{2,1} & \frac{1}{\lambda_2} S_{2,2} & \cdots & \frac{1}{\lambda_{N_{\text{layer}}}^2} S_{2,N_{\text{layer}}}
\frac{1}{\lambda_1} S_{3,1} & \cdots & \frac{1}{\lambda_{N_{\text{layer}}}^3} S_{3,N_{\text{layer}}}
\vdots & \vdots & \ddots & \vdots
\frac{1}{\lambda_1} S_{N_{\text{layer}},1} & \frac{1}{\lambda_1} S_{N_{\text{layer}},2} & \cdots & \frac{1}{\lambda_{N_{\text{layer}}}^{N_{\text{layer}}}} S_{N_{\text{layer}},N_{\text{layer}}}
\end{bmatrix}
\]

where \( S_{i,j} \) is the sensitivity coefficient of the \( i \)th layer and \( j \)th wavelength (see Appendix B for an explicit expression of \( S_{i,j} \)). Equation (2) is rewritten to find the RI change in each layer:

\[
\Delta n_s = M_s^{-1} \Delta \phi_s
\]

where \( M_s^{-1} \) is the inverse of \( M_s \) for a square matrix and the right inverse of \( M_s \) for a non-square matrix (for \( N_j > N_{\text{layer}} \)). Equation (4) has a unique solution if \( \det(M_s) \neq 0 \). In that case, the RI change in layer \( i \) can be determined with a precision \( \sigma_{\Delta n_i} \) (defined as the standard deviation in \( \Delta n_i \)) depending on the precision \( \sigma_{\Delta \phi_j} \) of the measurement of \( \Delta \phi_j \), which is determined by experimental factors such as laser noise, camera noise, and temperature fluctuations. If the matrix \( M_s \) gets more singular the precision \( \sigma_{\Delta n_i} \) will worsen. Therefore it is essential to optimize the experimental configuration such that \( M_s \) does not get singular. \( M_s \) is determined by the sensitivity coefficients which in turn depend on the guided mode profiles. If the mode profiles are similar, the sensitivity coefficients will be almost equal and as a consequence, the matrix \( M_s \) gets more singular. Therefore the wavelengths should differ as much as possible in the workable wavelength range.

We define the relative precision \( \Phi_i \) (describing the relative precision in determining the RI change in the \( i \)th layer) as the ratio between \( \sigma_{\Delta n_i} \) and \( \sigma_{\Delta \phi_j} \). Assuming \( \sigma_{\Delta n_i} = \sigma_{\Delta \phi j} \quad \forall \quad 1 \leq k \leq N_j \), the relative precision is a vector \( \Phi \) with the \( i \)th element \( \Phi_i \) given by (see Appendix C):

\[
\Phi_i = \frac{\sigma_{\Delta n_i}}{\sigma_{\Delta \phi j}} = \sqrt{\sum_{j=1}^{N_j} \left( M_s^{-1} \right)_{i,j}^2}
\]

\( \Phi_i \) is evaluated for different wavelengths, waveguide refractive indices and layer thicknesses. Given an experimentally determined precision in the phase measurements \( \sigma_{\Delta \phi j} \approx 10^{-4} \) fringes @ 1 Hz for the reported YI sensor [19]), the precision \( \sigma_{\Delta n_i} \) with which a RI change of a given layer \( i \) can be measured, can be calculated by multiplying the relative precision \( \Phi_i \) of the corresponding layer with the experimentally determined value of \( \sigma_{\Delta n_i} \).
2.2 Specific case of virus detection

Next, we treat the specific case of virus detection, to convert the RI changes obtained from a measurement into the more relevant virus mass coverage $C_v$. To discriminate between non-specific binding of proteins, specific virus binding and bulk solution changes we introduce three layers on top of our waveguide (see Fig. 3). We assume that layer 2 changes due to specific virus binding and bulk RI changes, whereas changes in layer 3 are only caused by bulk RI changes. It therefore follows that the RI changes due to specific virus binding are given by the difference in RI changes between layer 2 and 3. Multiplying this RI change with a constant $\beta$ converts an RI change into a virus mass coverage (see Appendix D):

$$C_v = \frac{1}{\beta}(\Delta n_2 - \Delta n_3),$$  

(6)

and the minimal detectable virus mass coverage given by:

$$\Delta C_v = \frac{\sqrt{2}}{\beta} \sigma_{\Delta n_{\text{virus}}},$$  

(7)

where $\beta$ is given by:

$$\beta = (n_{\text{virus}} - n_{\text{solution}}) \cdot d_{\text{virus}}^{-2} \cdot m_{\text{virus}}^{-1}.$$  

(8)

Assuming an RI of a virus $n_{\text{virus}} = 1.41$ [21] and the RI of a buffer solution $n_{\text{solution}} = 1.33$, a molecular weight of a single Adenovirus particle, $m_v = 1.75 \times 10^8$ Da [22] ($= 2.91 \times 10^{-1}$ fg), and the diameter of the Adenovirus $d_{\text{virus}} = 80$ nm, $\beta$ is equal to $1.76 \times 10^{-9}$ mm$^2$/fg.
3. Results and discussion

Fig. 4. Relative precision as a function of the core thickness and core refractive index (given at a wavelength of 550 nm) for a) the virus layer using two layers, b) the bulk using two layers, c) the virus layer using three layers, d) the bulk using three layers and e) the non-specific binding layer using three layers.

3.1. Optimization of the waveguide structure

First, the two layers case is treated, which is used to discriminate between analyte binding (layer 1) and RI changes of the bulk (layer 2). Layer 1, the “virus layer” has a defined thickness of 80 nm. The second layer is called “bulk” and is all the space above layer 1. The dependence of the relative precision on the core thickness $d_{\text{core}}$ is given (Fig. 4a,b) for three different core refractive indices. The refractive indices are chosen such that they correspond to those of real materials that are often used for waveguide fabrication; $n \approx 1.77$: aluminum oxide ($\text{Al}_2\text{O}_3$), $n \approx 2.02$: silicon nitride ($\text{Si}_3\text{N}_4$) and $n \approx 2.65$ titanium oxide ($\text{TiO}_2$). The used
wavelengths are \( \lambda_1 = 400 \text{ nm} \) and \( \lambda_2 = 700 \text{ nm} \). More details of the waveguide can be found in Table 1. For all simulations only zeroth order Transverse Electric (TE0) modes are taken into account. From Fig. 4(a,b) it is clear that the best performance (lowest value of \( \Phi \)) is obtained for high values of \( n_{\text{core}} \) combined with low core thicknesses. This holds for both the precision achieved for the virus layer and the bulk layer (note that at optimal thickness, the dependence on the core RI is marginal). This conclusion is important in choosing the waveguide materials, i.e. choosing Si\(_3\)N\(_4\) \((n = 2.034)\) as a core material is justified as it combines a high RI with excellent cleanroom processing possibilities. The interpretation of the calculated \( \Phi \) and its consequences for virus detection are discussed later.

### Table 1. Waveguide structure details for all the simulations, where the RI of the layers is determined by the Sellmeier equations (see Appendix A).

<table>
<thead>
<tr>
<th>Simulation description</th>
<th>Substrate material</th>
<th>Core material</th>
<th>( n_{\text{core}} )</th>
<th>( d_{\text{core}} ) (nm)</th>
<th>( d_{\text{virus layer}} ) (nm)</th>
<th>( d_{\text{bulk layer}} ) (nm)</th>
<th>( d_{\text{core}} ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Phi ) vs. ( d_{\text{core}} ) (two layers)</td>
<td>SiO(_2) (n = 1.461 ) @ ( \lambda = 550 \text{ nm} ) ([28])</td>
<td>Al(_2)O(_3), (n = 1.770 ) @ ( \lambda = 550 \text{ nm} ) ([27]), Si(_3)N(_4), (n = 2.024 ) @ ( \lambda = 550 \text{ nm} ), TiO(_2), (n = 2.648 ) @ ( \lambda = 550 \text{ nm} ), ([28])</td>
<td>Water @ 20(^\circ)C, (n = 1.335 ) @ ( \lambda = 550 \text{ nm} ) ([29])</td>
<td>30-200</td>
<td>-</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>( \Phi ) vs. ( d_{\text{core}} ) (three layers)</td>
<td>SiO(_2)</td>
<td>Al(_2)O(_3), Si(_3)N(_4), TiO(_2)</td>
<td>Water @ 20(^\circ)C</td>
<td>30-200</td>
<td>-</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>( \Phi ) vs. ( N_{\text{layer}} )</td>
<td>SiO(_2)</td>
<td>Si(_3)N(_4)</td>
<td>Water @ 20(^\circ)C</td>
<td>70</td>
<td>random values</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \Phi ) vs. ( N_{\text{layer}} )</td>
<td>SiO(_2)</td>
<td>Si(_3)N(_4)</td>
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<td>70</td>
<td>random values</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In order to distinguish between specific analyte bind, non-specific binding and bulk RI changes, three layers are required, a protein layer (non-specific binding), a virus layer, and a bulk layer. For details see Table 1. Figure 4(c-e) shows \( \Phi \) for the virus layer, the bulk and the non-specific binding layer respectively as a function of \( d_{\text{core}} \) for the different core materials. The three wavelengths are: \( \lambda_1 = 400 \text{ nm} \), \( \lambda_2 = 550 \text{ nm} \) and \( \lambda_3 = 700 \text{ nm} \). For optimal detection of specific binding, the relative precision of the “virus layer” and the “bulk” should be minimal. Figure 4(c) shows a minimum value of \( \Phi_{\text{viral layer}} = 6.12 \times 10^{-4} \text{ rad}^{-1} \) for a core material of TiO\(_2\) and \( d_{\text{core}} = 35 \text{ nm} \), and a two-fold higher value of \( \Phi_{\text{viral layer}} = 1.63 \times 10^{-3} \text{ rad}^{-1} \) for Si\(_3\)N\(_4\) as core material and \( d_{\text{core}} = 70 \text{ nm} \). Figure 4(d) shows a similar relative precision of the bulk for both conditions. For each core material, the core thickness should be chosen carefully.

### 3.2. Expanding the number of layers

With the use of an increasing number of wavelengths it is, in theory, possible to determine a complete RI change profile, i.e. the RI change as a function of distance to the waveguide surface. However the use of an increasing number of wavelengths results in mode profiles which are increasingly similar and therefore result in a worsening precision \( \sigma_{30} \). For example, by comparing the relative precisions for the two and three layers cases, we see an increase in \( \Phi \) of approximately one order of magnitude. In order to explore the limits, we calculated the \( \Phi \) as a function of the number of layers \( N_{\text{layer}} \). The RI change cannot be determined at distances from the waveguide surface where the evanescent field becomes very small. Therefore, we define our layers in the region starting at the surface and ending at 200 nm from the surface. The space above the 200 nm limit is considered as bulk. The thicknesses of the layers are randomly chosen with a minimum of 10 nm. \( \Phi \) is calculated for all layers and this calculation is repeated 1000 times (each time with different randomly chosen layer...
thicknesses). Next, the mean of all these calculated relative precisions $<\Phi>$ is calculated. The number of wavelengths is chosen equal to $N_{\text{layer}}$. For the core we chose Si$_3$N$_4$ with a thickness of 70 nm. For optimal settings, the wavelengths are spread maximally. This means that $\lambda_{\min} = 400$ nm and $\lambda_{\max} = 700$ nm. The wavelengths in between are equally divided within this range. Figure 5 shows $\Phi$ as a function of $N_{\text{layer}}$, where bulk is included in $N_{\text{layer}}$, but not in $\Phi$. The error bars are based on a 95% confidence interval of the 1000 calculated relative precisions and give an indication about the spread in the calculated relative precisions. A one-layer situation is included as a solid line in Fig. 5 (wavelength is set at 550 nm). Figure 5 shows that $\Phi$ increases exponentially with $N_{\text{layer}}$, meaning that $\Phi$ can be determined less precisely.

![Fig. 5. Mean relative precision as a function of the number of layers, which is equal to the number of wavelengths, for a core thickness of 70 nm and Si$_3$N$_4$ as core material.](image)

**3.3. Expanding the number of wavelengths**

The number of wavelengths should be equal to or larger than the number of layers that needs to be resolved. In the previous sections we explored several configurations of various number of layers and equal number of wavelengths. Here $\Phi$ is evaluated for the case $N_{\text{layer}} = 2$ and $N_{\lambda} \geq N_{\text{layer}}$. The two layers are defined as a “virus layer” of 80 nm and a “bulk” layer covering all the space above 80 nm. The wavelengths are in each case equally divided between 400 nm and 700 nm (for waveguide details see Table 1). Figure 6 shows $\Phi$ as a function of the number of wavelengths for $N_{\text{layer}} = 2$. The $\Phi$ for the “virus layer” only decreases a factor of $\approx2$ by adding eight extra wavelengths, where $\Phi$ of bulk layer decreases even less. It can be concluded that $\Phi$ decreases with an increasing number of wavelengths. In conclusion, adding more wavelengths than layers results in a marginal gain in $\Phi$. 

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3.4. Virus detection

To illustrate the possibilities of the size-selective detection scheme, we discuss the example of virus detection. Using Eq. (7) in combination with the results from the three layer case, we calculate the minimal detectable virus mass coverage $\Delta C_v$. Multiplying the relative precisions from Fig. 4(c) for the virus layer with the known phase precision of approximately $10^{-4}$ fringes for the YI sensor [19] gives $\sigma_{\Delta C_v}$. For a core thickness of 70 nm and a core material of $\text{Si}_3\text{N}_4$ we find $\Delta C_v = 8 \times 10^2 \text{fg/mm}^2$.

For virus detection, the sensor should be capable of detecting viral concentrations in the clinically relevant range (e.g. $10^3$ to $10^6$ particles/ml for the HIV-1 virus) [23]. The phase change as measured with an YI sensor for a low HSV-1 virus concentration of $10^3$ particles/ml is $5 \times 10^{-2}$ fringes [1]. This phase change corresponds to an RI change of approximately $5 \times 10^{-5}$. The RI change should be determined with at least this precision. The minimum permitted relative precision $\phi_{\text{min}} = 5 \times 10^{-3} / 2\pi \times 10^{-4} \text{rad} \approx 8 \times 10^{-2} \text{rad}^{-1}$. The relative precisions of the virus layers for the two layer and three layer configurations (see Fig. 4) are below $\phi_{\text{min}}$, and so sufficient to detect a very low concentration ($10^3$ viral particles/ml). Figure 5 shows that we can go up to roughly four layers for a $\text{Si}_3\text{N}_4$ core of 70 nm before $\phi_{\text{min}}$ is exceeded. It should be noted that different viruses have different sizes. In Fig. 4 we analysed a specific situation of an 80 nm sized viral particle. However, calculations show that the values of the relative precision for viral particles of different sizes do not differ significantly, e.g. for viral particles of 160 nm the relative precision differs a factor of $\approx 2$.

3.5. Detection of analytes in complex matrices

Here we would like to discuss several issues related to the use of this method for the detection of e.g. virus particles in complex matrices such as blood, serum, urine or sputum. In our analysis we assumed a thin and uniform layer of proteins that are non-specifically bound to the sensor surface. However, the proteins responsible for non-specific binding can have various sizes, with some of them being larger than 10 nm, exceeding the thickness of the non-specific binding layer assumed in the calculations. In general, the majority of proteins in e.g. blood or serum are however much smaller than a virus particle. It is this majority, the excess of proteins compared to the number of virus particles that leads to measurable background due to non-specific binding. Small numbers of very large proteins can be discriminated by the specificity of the bio-receptor coating of the sensor surface.
Another concern is that several types of viruses can be present in a real sample all having the same size. The method presented here obviously cannot distinguish between particles with the same size and therefore it is important to always combine this method with a traditional affinity bio-receptor coating of the sensor surface in order to strongly enhance the binding of the specific virus of interest. Only in extreme cases where the concentration of the virus of interest is much lower than the concentration of other viruses could this result in the non-specific binding of other viruses exceeding the specific binding of the viruses of interest. In this case our method obviously cannot distinguish between the two different viruses.

Finally in a real sample the molecules are moving rather than being static. Brownian motion of molecules in the sampling volume gives rise to fluctuations in the refractive index. The expected fluctuations in the refractive index in a 10 nm thin layer above the sensor surface is calculated to be $10^{-9}$ RIU (corresponding to a phase noise of $10^{-6}$ fringes) for an average protein content of 10 g/ml (typical for blood). The calculated fluctuations are well below the detection limit of the sensor (two orders of magnitude smaller than the value used in the calculations) and as such we can conclude that fluctuations in the signal due to Brownian motion of proteins in the sample fluid can be neglected.

3.6. Implementation

For the implementation of this new method two aspects need special attention. First, multiple lasers emitting at different wavelengths should be coupled into a single channel waveguide of the YI sensor. This can be accomplished by combining the output of multiple monochromatic lasers using either free space optics (e.g. using appropriate dichroic mirrors) or by using fiber-optical combiners. Secondly, for each wavelength the phase change should be measured independently from the recorded interference pattern. This can be done in two ways. First, the interference pattern of each wavelength is measured separately. This can be done using either a multicolour video camera, or by introducing a dispersive element (e.g. a grating) in the detection path to separate the different interference patterns (one for each wavelength) spatially on a monochrome video camera. Alternatively, the same detection setup as used for single wavelength sensors can be used [2]. In this case a single interference pattern is recorded. To obtain the phase changes for each wavelength separately one can make use of the fact that the spatial frequency of the interference pattern depends on the wavelength of the used light. Therefore, the amplitude spectrum of the Fourier-transformed interference pattern now consists of well-separated spatial frequency peaks (one for each wavelength). Consequently, the phase change for a given wavelength can be monitored independently from the other wavelengths by selecting the corresponding spatial frequency in the phase spectrum of the Fourier-transformed interference pattern. We have successfully used a similar approach to simultaneously measure multiple phase changes from a multi-channel integrated optical YI sensor [24].

3.7. General discussion

Comparing the calculated detection limit (which is defined here as the minimum reliably detectable induced RI change which equals two times the precision) with existing methods we find that our method is comparable to the detection limit of optical biosensors based on SPR ($\approx 10^{-6}$ RIU) [7]. The detection limit also compares to reported results obtained with grating couplers ($\approx 10^{-6}$ RIU, 0.3 pg/mm²) [9], photonic crystals ($\approx 10^{-2}$ RIU) [15] and resonant optical microcavities sensors ($\approx 10^{-5} - 10^{-6}$ RIU) [12,13]. Although in this method the detection limit gets worse compared to single wavelength YI sensors, the performance is comparable to existing methods with the advantage that the sensor is capable of size selective detection which we believe yields a strong improvement in the specificity of the sensor.

We further envision the potential of implementing improvements or alternative configurations to improve sensor performance. One possible improvement is to make use of other than TE0 modes. As discussed, the precision with which different layers can be resolved.
requires the use of mode profiles that have different field strengths in the different layers. Here we used different wavelengths to achieve this. Alternatively one can use dual polarizations [25], however it will not be possible to distinguish between specific binding, non-specific binding, bulk changes simultaneously using only two polarizations. On the other hand, higher order modes can be used. The difference between mode profiles of e.g. TE0 and TE1 at the same wavelength is larger than the difference between mode profiles of e.g. a TE0 mode at different wavelengths. Measuring the phase changes for different order modes at the same wavelength is possible [26] but is less trivial than detecting the same mode at different wavelengths. Alternatively it is possible to use a TE0 mode at one wavelength and a TE1 mode at another wavelength. The combination of modes and/or wavelengths that give the best results requires a systematic experimental investigation and is beyond the scope of the current paper.

Currently methods to increase specificity of the sensor are aimed to reduce non-specific binding by chemical treatment of the sensor surface (e.g. blocking of vacant positions by bovine serum albumin). The method described here can be used in addition to this blocking approach to further improve the specificity. Approaches to eliminate bulk RI changes also exist. Usually after interaction of the analyte with the sensor surface, the sample fluid is replaced by a clean buffer and the bulk contribution is obtained by comparing the RI before and after fluid replacement. However this approach has some disadvantages. First, no significant changes in binding of the analyte to the sensor surface should occur during the time of fluid replacement, which usually means long measuring times. Secondly this method is prone to errors as a change from sample to buffer solution sets a new equilibrium of the free analyte vs. surface-bound analyte. Also quite common in interferometric sensors is to use the reference channel to eliminate common RI changes. In this case a reference channel is modified identically to the sensing channel without the analyte specific antibody. Applying the sample to both channels simultaneously strongly reduces the bulk and non-specific contributions, however in practice this approach is not always sufficient especially in complex matrices like blood. Also here the size-selective approach can be used either alone or in addition to a reference channel to further increase the specificity.

4. Conclusions

In this paper we have described a new approach, based on the use of multiple wavelengths in combination with an integrated optical Young interferometer sensor, which allows detecting analytes based on size. The use of multiple wavelengths allows discriminating between RI changes from different locations. To simultaneously distinguish between specific binding, non-specific binding and bulk RI changes, a three layer system with three wavelengths is used. The required precision of the RI determines the spatial resolution of this new method. Assuming a phase precision of \(10^{-4}\) fringes, this new method has a minimum detectable virus mass coverage of \(4 \times 10^2\) fg/mm\(^2\). With a better phase precision it is even conceivable to reach a higher precision of \(\Delta n\) or to introduce an extra imaginary layer, resulting in a possibility to distinguish from another type of particle with a different size. Furthermore, applying this new method to the current sensor gains in specificity while the detection limit is still comparable to the detection limit of other existing methods. We believe that this method can strongly improve the performance of IO interferometric sensors by not appreciably affecting precision on the one hand (that is, retaining sufficient sensitivity to detect low virus concentrations), and by gaining selectivity of the sensor on the other hand.

Appendix A: Chromatic dispersion

Chromatic dispersion is included in the determination of the sensitivity coefficients. The sensitivity coefficients are determined by the mode profile of the light source, which depends on the wavelength of the light and the waveguide structure \((d_{\text{core}}, n_s, n_{\text{core}}, n_c)\). The refractive indices of the chosen materials as a function of the wavelength are given by the Sellmeier
equations, which are listed below in Table A.1. Chromatic dispersion in the formation of a layer of particles (e.g. viruses, proteins) on the surface is assumed to be negligible.

Table A.1. Sellmeier equations for the different materials used in the waveguide structure

<table>
<thead>
<tr>
<th>Material</th>
<th>Sellmeier equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al₂O₃</td>
<td>[ n(\lambda) = \left(1 + \frac{A\lambda^2}{\lambda^2 - B} + \frac{C\lambda^2}{\lambda^2 - D} + \frac{E\lambda^2}{\lambda^2 - F}\right)^{\frac{1}{2}}, \lambda \text{ in } [\mu m] \right.</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>A = 1.4313493, B = 0.0726631, C = 0.65054713, D = 0.1193242, E = 5.3414021, F = 18.028251</td>
<td></td>
</tr>
<tr>
<td>SiN₄</td>
<td>[ n(\lambda) = \left(1 + \frac{A\lambda^2}{\lambda^2 - B}\right)^{\frac{1}{2}}, \lambda \text{ in } [\mu m] \right.</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>A = 2.8960, B = 0.14010</td>
<td></td>
</tr>
<tr>
<td>TiO₂</td>
<td>[ n(\lambda) = \left(5.913 + \frac{A}{\lambda^2 - B}\right)^{\frac{1}{2}}, \lambda \text{ in } [\mu m] \right.</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>A = 0.2441, B = 0.0803</td>
<td></td>
</tr>
<tr>
<td>SiO₂</td>
<td>[ n(\lambda) = \left(1 + \frac{A\lambda^2}{\lambda^2 - B}\right)^{\frac{1}{2}}, \lambda \text{ in } [\mu m] \right.</td>
<td>[28]</td>
</tr>
<tr>
<td></td>
<td>A = 1.1008, B = 0.094025</td>
<td></td>
</tr>
<tr>
<td>Water (20°C)</td>
<td>[ n(\lambda) = \left(1 + \frac{A\lambda^2}{\lambda^2 - B} + \frac{C\lambda^2}{\lambda^2 - D} + \frac{E\lambda^2}{\lambda^2 - F} + \frac{G\lambda^2}{\lambda^2 - H}\right)^{\frac{1}{2}}, \lambda \text{ in } [\mu m] \right.</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>A = 5.684027565·10⁻¹, B = 5.101829712·10⁻³, C = 1.726177391·10⁻¹, D = 1.82115396·10⁻², E = 2.086189578·10⁻², F = 2.620722293·10⁻², G = 1.13074868·10⁻¹, A = 1.069792721·10⁻²</td>
<td></td>
</tr>
</tbody>
</table>

Appendix B: Derivation sensitivity coefficient

In this section we derive the sensitivity coefficient \( S_{i,j} \). Based on a three layer waveguide with a substrate (\( n_s \)), a core (\( n_{core} \)), and a cladding (\( n_c \)), the sensitivity coefficient is given by the \( N_{eff} \) dependence on a RI change in the region probed by the evanescent field (cladding) and can be determined for TE polarization by [30]:

\[
\left( \frac{\partial N_{eff}}{\partial n_i} \right)_{\lambda_j} = \left( \frac{n_i}{N_{eff}} \right) \left( \frac{n_{core}^2 - N_{eff}^2}{n_{core}^2 - n_i^2} \right) \left( \frac{Y_{0c}(\lambda_j)}{d_{core} + Y_{0c}(\lambda_j) + Y_{0s}(\lambda_j)} \right),
\]

where \( d_{core} \) is the core thickness, \( Y_{0c} \) is the penetration depth of the electric field into the covering region on top of the waveguide, and \( Y_{0s} \) is the penetration depth of the electric field into the substrate, which are given by:

\[
Y_{0c,0s}(\lambda_j) = \left( \frac{\lambda}{2\pi} \right) \left( \frac{n_{eff}^2 - n_i^2}{N_{eff}^2 - n_i^2} \right)^{\frac{1}{2}},
\]

where \( \lambda \) the vacuum wavelength of the guided light. The sensitivity of the \( i \)th layer in the evanescent region can now be calculated as a fraction of the complete evanescent mode power present in this layer [31]:

\[
S_{i,j} = \frac{\int_{0}^{\lambda_j} \exp \left( -\frac{2z}{Y_{0c}(\lambda_j)} \right) dz}{\int_{0}^{\lambda_j} \exp \left( -\frac{2z}{Y_{0c}(\lambda_j)} \right) dz} \left( \frac{\partial N_{eff}}{\partial n_i} \right)_{\lambda_j},
\]
\[ z_0 = 0, \quad z_i = \sum_{n=1}^{i} d_n, \] where \( d_n \) is the thickness of layer \( n \). This finally results in this expression for the sensitivity coefficient for the \( i^{th} \) layer and the \( j^{th} \) wavelength:

\[
S_{i,j} = \left[ \exp\left( -\frac{2z_{i-1}}{Y_0(\lambda_j)} \right) \right] \cdot \left[ \exp\left( -\frac{2z_i}{Y_0(\lambda_j)} \right) \right] \cdot \left[ \frac{n_{\text{core}}^2 - N_{\text{eff}}^2}{n_{\text{core}}^2 - n_i^2} \right] \cdot \left( \frac{Y_{0i}(\lambda_j)}{d_{\text{core}} + Y_{0i}(\lambda_j) + Y_{0i}(\lambda_j)} \right).
\] (12)

Appendix C: Derivation relative precision

Here we derive an expression for the relative precision \( \Phi \), where \( \Phi \) is defined as the standard deviation in the RI change \( \sigma_{\Delta n} \) normalized by the standard deviation of the measured phase change. From Eq. (4) of the main text we have:

\[
\Delta n = M_{s}^{-1} \Delta \varphi.
\] (13)

The variance of \( \Delta n \) is given by:

\[
\sigma^2(\Delta n) = \left( M_{s}^{-1} \circ M_{s}^{-1} \right) \sigma^2(\Delta \varphi),
\] (14)

where \( \circ \) is the Hadamard product, which is an element-wise multiplication:

\[
\left( M_{s}^{-1} \circ M_{s}^{-1} \right)_{ij} = \left( M_{s}^{-1} \right)_{ij}^2.
\] (15)

So, the \( i^{th} \) element of vector \( \sigma(\Delta n) \) can be described by:

\[
\begin{bmatrix}
\sigma(\Delta n)_i
\end{bmatrix} = \sigma_{\Delta n_i} = \left[ \left( M_{s}^{-1} \circ M_{s}^{-1} \right) \sigma^2(\Delta \varphi) \right]_{ii}^{\frac{1}{2}} = \left[ \sum_{j=1}^{N} (M_{s}^{-1})_{ij}^2 \sigma^2_{\Delta \varphi_j} \right]^{\frac{1}{2}}.
\] (16)

By assuming an equal phase noise for every laser (\( \sigma_{\Delta \varphi_j} = \sigma_{\Delta \varphi} \)), it is possible to define the \( i^{th} \) element of the relative precision as:

\[
\Phi_i = \frac{\sigma_{\Delta n_i}}{\sigma_{\Delta \varphi}} = \frac{1}{\sigma_{\Delta \varphi}} \left[ \sum_{j=1}^{N} (M_{s}^{-1})_{ij}^2 \sigma^2_{\Delta \varphi_j} \right]^{\frac{1}{2}} = \left[ \sum_{j=1}^{N} (M_{s}^{-1})_{ij}^2 \right]^{\frac{1}{2}}.
\] (17)

Appendix D: Derivation virus mass coverage

For the specific case of virus detection, we derive here a set of RI changes into a virus mass coverage \( C_v \). On top of the core of the waveguide three imaginary layers are defined. The RI change in layer one \( \Delta n_1 \), is caused by non-specific binding of proteins, specific virus binding and bulk changes. Specific binding and bulk changes result in an RI change in layer two and the RI change in layer three is only caused by concentration changes of the bulk. In addition, we convert the RI change of the different layers into a protein mass coverage, \( C_p \), a virus mass coverage, \( C_v \), and a concentration change of the bulk \( C_b \). Therefore, the relation between \( C_p \), \( C_v \), \( C_b \) and \( \Delta n \) is given by:

\[
\begin{bmatrix}
\Delta n_1 \\
\Delta n_2 \\
\Delta n_3
\end{bmatrix} = M_c \cdot \begin{bmatrix}
C_p \\
C_v \\
C_b
\end{bmatrix}, \text{with } M_c = \begin{bmatrix}
\alpha & \beta & \gamma \\
0 & \beta & \gamma \\
0 & 0 & \gamma
\end{bmatrix}.
\] (18)
where \( \alpha, \beta, \) and \( \gamma, \) convert an RI change into respectively a protein mass coverage, a virus mass coverage and a bulk concentration change. We assume small concentration changes, so we can neglect changes in \( \alpha, \beta, \) and \( \gamma, \) because of the growth of viruses to the surface, which results in less space on the surface which can be covered by new virus particles. The protein mass coverage, virus mass coverage and bulk concentration change can be calculated by:

\[
\begin{bmatrix}
C_p \\
C_v \\
C_b
\end{bmatrix} = M_c^{-1} \begin{bmatrix}
\Delta n_1 \\
\Delta n_2 \\
\Delta n_3
\end{bmatrix}, \text{with } M_c^{-1} = \begin{bmatrix}
1/\alpha & -1/\alpha & 0 \\
0 & 1/\beta & -1/\beta \\
0 & 0 & 1/\gamma
\end{bmatrix}.
\]

From this the mass coverage \( C_v \) is given by:

\[
C_v = \frac{1}{\beta} (\Delta n_2 - \Delta n_3), \quad (20)
\]

where \( \Delta n_2 \) is composed of two contributions: \( \Delta n_2 = \Delta n_{\text{virus}} + \Delta n_{\text{bulk}} \), whereas \( \Delta n_3 = \Delta n_{\text{bulk}} \), which results in an equation for mass coverage:

\[
C_v = \frac{1}{\beta} \Delta n_{\text{virus}}. \quad (21)
\]

Next, we determine \( \beta \). We define an expression for \( C_v \) as:

\[
C_v = \frac{N_{\text{virus}} \cdot m_{\text{virus}}}{w \cdot l}, \quad (22)
\]

where \( N_{\text{virus}} \) is the number of virus particles bound the surface, \( m_{\text{virus}} \) the mass of a single virus particle, \( w \) the sensitivity window width (\( = 4 \mu m \) for current chip), and \( l \) the sensitivity window length (\( = 4 \text{ mm} \) for current chip). The maximum number of virus particles \( N_{\text{max}} \) that can be bound to the surface is determined as the ratio of the surface area of the sensor and the surface area of a single virus particle and can be approximated as \( N_{\text{max}} = \frac{w \cdot l}{d^2} \), where \( d \) is the diameter of the virus particle. If we define a layer of thickness equal to the diameter of the virus, than the corresponding maximum RI change \( \Delta n_{\text{virus}} \) in that viral layer equals \( \Delta n_{\text{virus}} = n_{\text{virus}} - n_{\text{solution}} \). If in an experiment an RI change is introduced by specific virus binding (denoted as \( \Delta n_{\text{virus}} \)), the number of viruses \( N_{\text{virus}} \) bound to the surface of the chip is calculated as:

\[
N_{\text{virus}} = \frac{\Delta n_{\text{virus}}}{\Delta n_{\text{max}}} N_{\text{max}} = \frac{\Delta n_{\text{virus}} \cdot w \cdot l}{(n_{\text{virus}} - n_{\text{solution}}) \cdot d^2}. \quad (23)
\]

Combining Eq. (21), Eq. (22), and Eq. (23), this results in an expression for \( \beta \):

\[
\beta = \left( n_{\text{virus}} - n_{\text{solution}} \right) \cdot d \cdot m_{\text{virus}}. \quad (24)
\]

Based on Eq. (20) (so \( C_v = f (\Delta n_2, \Delta n_3) \)) the minimal detectable virus mass coverage is given by:

\[
\Delta C_v = \left[ \left( \frac{\partial f}{\partial (\Delta n_1)} \right) \cdot \Delta (\Delta n_1) \right] \cdot \Delta (\Delta n_2) + \left[ \left( \frac{\partial f}{\partial (\Delta n_2)} \right) \cdot \Delta (\Delta n_2) \right] \cdot \Delta (\Delta n_3) = \frac{1}{\beta} \left[ |\Delta (\Delta n_1)| + |\Delta (\Delta n_2)| \right] \cdot \Delta (\Delta n_2). \quad (25)
\]

If we assume that the error in \( \Delta n_2, \Delta (\Delta n_2), \) is given by the precision in \( \Delta n_2 \) (relative precision multiplied by the precision in the phase change), \( \sigma_{\Delta n_2}, \) and that
\[ \sigma_{\text{det}} = \sigma_{\text{shut}} \approx \sigma_{\text{shun}} \], than the minimal detectable virus mass coverage can be approximated by:

\[ \Delta C_v = \frac{\sqrt{2}}{\beta} \sigma_{\text{shun}}. \] (26)

**Acknowledgments**

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