Supporting Information
Optofluidic Sensor for Inline Hemolysis Detection on Whole Blood

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Materials and Methods

System Description with Optical Transfer Functions

As shown in Figure S1, the waveguide with nano-filter section is illustrated and annotated. In general, I indicates the power of the light and G the optical transfer function. The incident intensity $I_{p,in}$ of the waveguide mode is given by

$$I_{p,in}(\lambda) = G_{in}(\lambda)\, I_0(\lambda). \tag{1}$$

I_0 is the initial intensity of the light before entering the grating couplers, G_{in} the efficiency of the coupling. Regarding the propagation loss within the region of the ridge-waveguide,

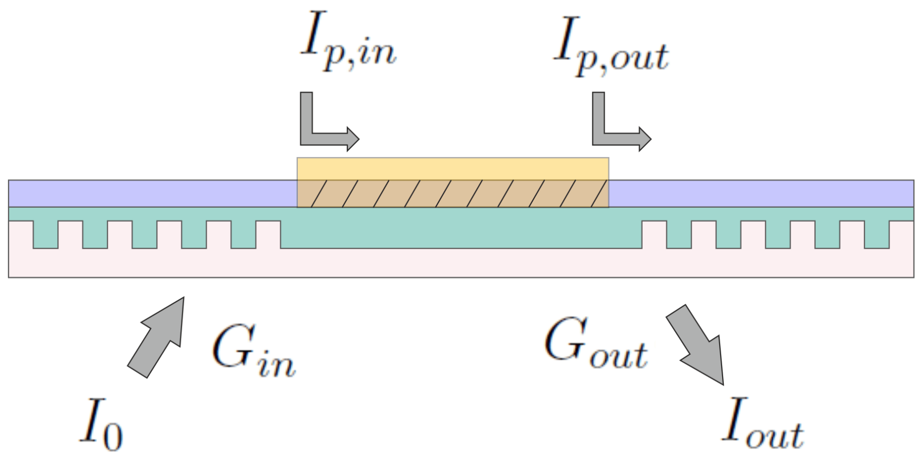

Figure S1: Illustration of the optical system

we can define

$$I_{p,out}(\lambda, n, l) = I_{p,in}(\lambda)\ \exp(-\eta(\lambda)\,\sigma nl - \alpha_0 l). \tag{2}$$

Here, the power transmitted through the waveguide is attenuated by the dielectric loss inside the waveguide and absorption by the specimen in the liquid according to Lambert-Beer's law:

$$I = I_0 \exp(\sigma nl), \tag{3}$$

where l is the length of the waveguide, σ and n the attenuation cross section and number density of the attenuating species, respectively. In the equation 2, $I_{p,out}(\lambda, n, l)$ denotes the output power of the waveguide, $I_{p,in}(\lambda)$ the input power, α_0 is the intrinsic loss of the waveguide, which should include scattering loss and material absorption loss - not from the detection species - along the whole waveguide length. A further parameter η is defined as the mode-overlap ratio, which has been put into the consideration as not 100% energy is interacting with the absorbing species as assumed in the Lambert-Beer's law. It is given as:

$$\eta = \frac{\iint_{evan} \mathbf{S}\cdot\mathbf{n}\ dx\,dy}{\iint_{tot} \mathbf{S}\cdot\mathbf{n}\ dx\,dy} \tag{4}$$

where $\mathbf{S} = \mathbf{E}\times\mathbf{H}$ is the Poynting vector of the mode field in the waveguide and $\mathbf{n}$ is the normal vector to the cross-section of the waveguide.

Here, since we used e as base instead of 10, we used notation of attenuation cross section and number density, while the more commonly used molar attenuation coefficient ε and species concentration c can be expressed with

$$\varepsilon(\lambda) = \frac{N_A}{ln10}\sigma(\lambda), \tag{5}$$

$$c = \frac{n}{N_A}. \tag{6}$$

For the output of the light, we obtain a similar relationship like the input side:

$$I_{out}(\lambda) = G_{out}(\lambda, \theta) I_{p,out}(\lambda). \tag{7}$$

Combing equations 1 to 7, we can obtain a description for the outcoming signal I_{out} like the following expression:

$$I_{out}(\lambda) = I_0(\lambda) G_{in}(\lambda, \alpha) exp(-\eta(\lambda)\sigma n l - \alpha_0 l) G_{out}(\lambda) \tag{8}$$

In real measurements, it's imperative to always have a reference signal obtained and compared with the signal taken with the species of interest. Usually, it's taken immediately before or after the measurement with rinse. Here, we define an output signal $I_{out,ref}(\lambda)$ to refer to the reference measurement taken by signal acquisition with rinse fluid. The measurement and reference signal can be expressed as:

$$I_{out,mea}(\lambda) = I_0(\lambda) G_{in,mea}(\lambda) exp(-\eta_{mea}(\lambda)\sigma_{mea}(\lambda) n_{mea} l - \alpha_{0,mea}(\lambda) l) G_{out,mea}(\lambda), \tag{9}$$

$$I_{out,ref}(\lambda) = I_0(\lambda) G_{in,ref}(\lambda) exp(-\eta_{ref}(\lambda)\sigma_{ref}(\lambda) n_{ref} l - \alpha_{0,ref}(\lambda) l) G_{out,ref}(\lambda) \tag{10}$$

By taking division of $I_{out,ref}$ and $I_{out,mea}$, following expression can be made:

$$\frac{I_{out,ref}(\lambda)}{I_{out,mea}(\lambda)} = \frac{G_{in,ref}(\lambda)G_{out,ref}(\lambda)}{G_{in,mea}(\lambda)G_{out,mea}(\lambda)} \cdot exp((\eta_{mea}(\lambda)\sigma_{mea}(\lambda)n_{mea} - \eta_{ref}(\lambda)\sigma_{ref}(\lambda)n_{ref})l + (\alpha_{0,mea}(\lambda) - \alpha_{0,ref}(\lambda))l) \quad (11)$$

If we assume $G_{in,mea}(\lambda) = G_{in,ref}(\lambda)$ and $G_{out,mea}(\lambda) = G_{out,ref}(\lambda)$ as the grating couplers are excluded from the context of liquid interaction and $\sigma_{ref}(\lambda)n_{ref} \to 0$ for non-absorbing reference measurement, then we have:

$$\frac{I_{out,ref}(\lambda)}{I_{out,mea}(\lambda)} = exp(\eta_{mea}(\lambda)\sigma_{mea}(\lambda)n_{mea}l + (\alpha_{0,mea}(\lambda) - \alpha_{0,ref}(\lambda))l) \quad (12)$$

Furthermore, if we assume the intrinsic loss of the waveguide stays the same, $\alpha_{0,mea}(\lambda) = \alpha_{0,ref}(\lambda)$, then:

$$\frac{I_{out,ref}(\lambda)}{I_{out,mea}(\lambda)} = exp(\eta_{mea}(\lambda)\sigma_{mea}(\lambda)n_{mea}l) \quad (13)$$

or

$$ln(\frac{I_{out,ref}(\lambda)}{I_{out,mea}(\lambda)}) = \eta_{mea}(\lambda)\sigma_{mea}(\lambda)n_{mea}l \quad (14)$$

We can also use molar attenuation coefficient η and species concentration c replacing the attenuation cross section and number density:

$$\chi = \log_{10}(\frac{I_{out,ref}(\lambda)}{I_{out,mea}(\lambda)}) = \eta_{mea}(\lambda)\varepsilon_{mea}(\lambda)c_{mea}l. \quad (15)$$

Taking the derivative of the concentration, we obtain an expression of sensitivity:

$$S = \frac{\partial\chi}{\partial c} = \eta_{mea}(\lambda)\varepsilon_{mea}(\lambda)l. \quad (16)$$

Here we can conclude some important facts regarding the sensitivity:

- For a given analyte with known extinction coefficient, the sensitivity of the waveguide sensor is proportional to the mode-overlap ratio η and the length of the waveguide.

- The change in refractive index of the media is not substantially influencing the sensitivity since it alters the mode-overlap ratio η only slightly.

- To obtain a better signal to noise ratio, we should maximize the signal $I_{out,mea}(\lambda)$, which can include strategies like 1) suppression of the intrinsic waveguide loss, 2) increase of the initial input optical, 3) improve the coupling efficiency.

- To be able to compare with the reference spectra e.g. taken by a cuvette measurement, the dependence of mode-overlap ratio η has to be taken into account since η is going to change with altering wavelength.

Sensitivity Regarding Pitch Size

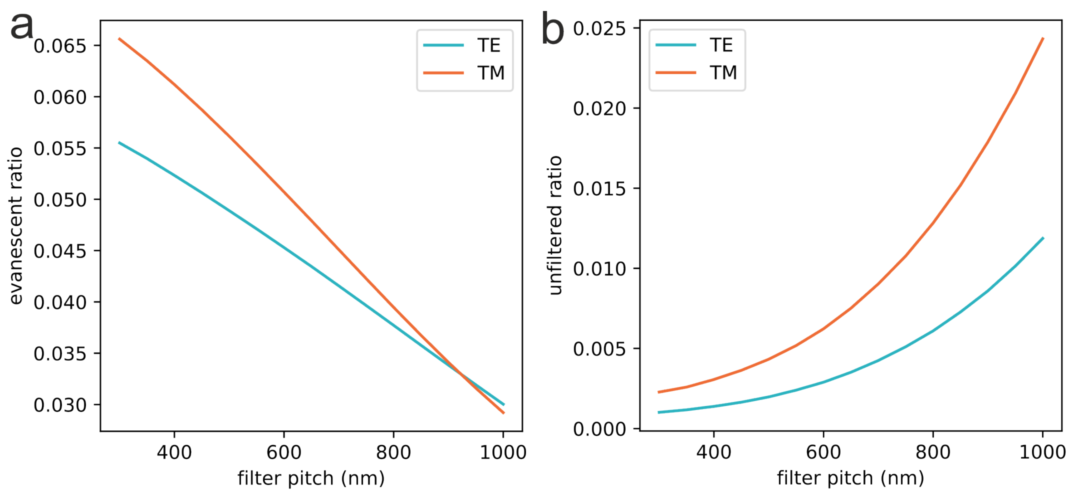

Figure S2: (a) Simulation result of the evanescent overlap ratio for TE and TM mode. 50% protrusion ratio were used. (b) Simulation result of the unfiltered ratio for TE and TM mode.

Pitch of the nano-filters were swept to investigate the sensitivity. The protrusion ratio of the filters is fixed as 50%. As shown in Figure S5a, in the range from 300 nm to 900 nm, the sensitivity of TM polarization is in higher than TE polarization. Both decrease monotonously with higher pitch size. On the other hand, the unfiltered ratio is increasing with larger pitch size as shown in Figure S5b. As a result the design was chosen to have a pitch size of 400 nm to maximize the sensitivity and filtering performance.

Cost Estimation of the Sensor

The sensors are fabricated with 4 inch wafers. For 50 sensors on a wafer, the cost for each single sensor is less than 1 EUR. Since the process is based on nanoimprint lithography, the other major cost of fabrication is given by the e-beam lithography for defining the master stamp. However, one stamp can be used thousands of times for imprint, thus the e-beam cost for making the stamp (3000 EUR) will be marginal when the the sensor is produced in large quantities (assuming 1000 times imprint). We estimate the cost per sensor in Table 1, where 50 sensors are made on single wafer.

Table 1: Cost Estimation

cost items	per wafer (EUR)	per sensor (EUR)
glass wafer	30	0.6
polymer and solvent	6	0.12
e-beam cost	3	0.06
total cost	39	0.78

Coupling and Calibration of Spectral Projection on Linear Camera

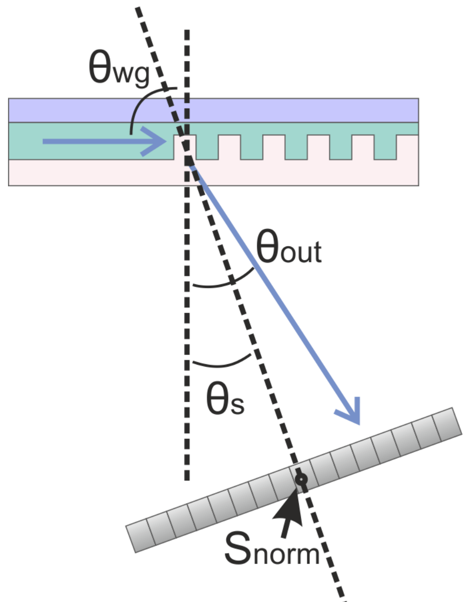

Figure S3: Illustration of the out-coupling from waveguide

We use the out-coupling from the waveguide to discuss the coupling condition and the the calibration method used to determine the spectrum. The coupling condition according to Bragg's condition is given as:

$$n_{out}\frac{2\pi}{\lambda_0}sin\theta_{out} + m\frac{2\pi}{d} = n_{wg}\frac{2\pi}{\lambda_0}sin\theta_{wg}, \tag{17}$$

where n_{out},$sin\theta_{out}$ are the refractive index of the outcoupling medium and the angle. λ_0 the wavelength of the light, m the diffraction order, d the periodicity of the grating, n_{wg} the effective refractive index of the waveguide, $sin\theta_{wg}$ the angle of the waveguide mode which should be 90 degree in this case. In our case, only the first diffraction order $m = 1$ is utilized and outcoupling medium is air so $n_{out} = 1$. Now we have:

$$sin\theta_{out} = n_{wg} - \frac{\lambda_0}{d}, \tag{18}$$

or

$$\theta_{out} = arcsin(n_{wg} - \frac{\lambda_0}{d}) \tag{19}$$

with $\lambda_0 = 420$ nm, $d = 368$ nm, and $n_{wg} = 1.555$ for TE polarization, which we obtained by numerical simulation with material refractive indices acquired by ellipsometry, we can obtain e.g. the outcoupling angle of 24.4° for the given condition. In this way we calculated all the angles from 400 nm to 600 nm, as shown in Figure S4a. As the effective refractive indices of the waveguide depends on material properties that are non-linear experimental data, the results are fitted with third order polynomial function to obtain an analytical expression for arbitrary angle and wavelength, which is defined as $f_\theta(\lambda_0) = \theta_{out}$ and $f_\theta^{-1}(\theta_{out}) = \lambda_0$.

For the calibration of the line sensor spectral projection, we have:

$$D_{norm} = \frac{(P_{\lambda 2} - P_{\lambda 1})w_{pixel}}{tan(\theta_s - \theta_{\lambda 2}) - tan(\theta_s - \theta_{\lambda 1})}, \tag{20}$$

where D_{norm} is the normal distance between the outcoulping point on sensor and to the normal intersection on sensor S_{norm}. $P_{\lambda 1}$, $P_{\lambda 2}$ are the pixel number of the two lasers with wavelengths of $\lambda 1$ and $\lambda 2$. w_{pixel} is the width of a pixel on the linear sensor,θ_s the angle between sensor element and the waveguide, $\theta_{\lambda 1}$, $\theta_{\lambda 2}$ the outcouple angles of the lasers. The distance of the laser spots relative to the S_{norm} position on the sensor can be expressed as:

$$S_{\lambda 1} = D_{norm} tan(\theta_s - \theta_{\lambda 1}), \tag{21}$$

$$S_{\lambda 2} = D_{norm} tan(\theta_s - \theta_{\lambda 2}). \tag{22}$$

To determine the wavelength of the light projected on pixel x, P_x, we have

$$\lambda_x = f_\theta^{-1}(\theta_s - arctan(\frac{(P_x - P_{\lambda 1})w_{pixel} + S_{\lambda 1}}{D_{norm}})) \tag{23}$$

Thus, we obtained an expression of the mapping individual pixel to the wavelength of the light by using two lasers as calibration. The results of the 405 nm and 450 nm lasers' projection are shown in Figure S4b. Furthermore, we compared the line camera projection with a the reference spectrum obtained by a commercial spectrometer (HR2000, Ocean Optics), as shown in Figure S4c and d.

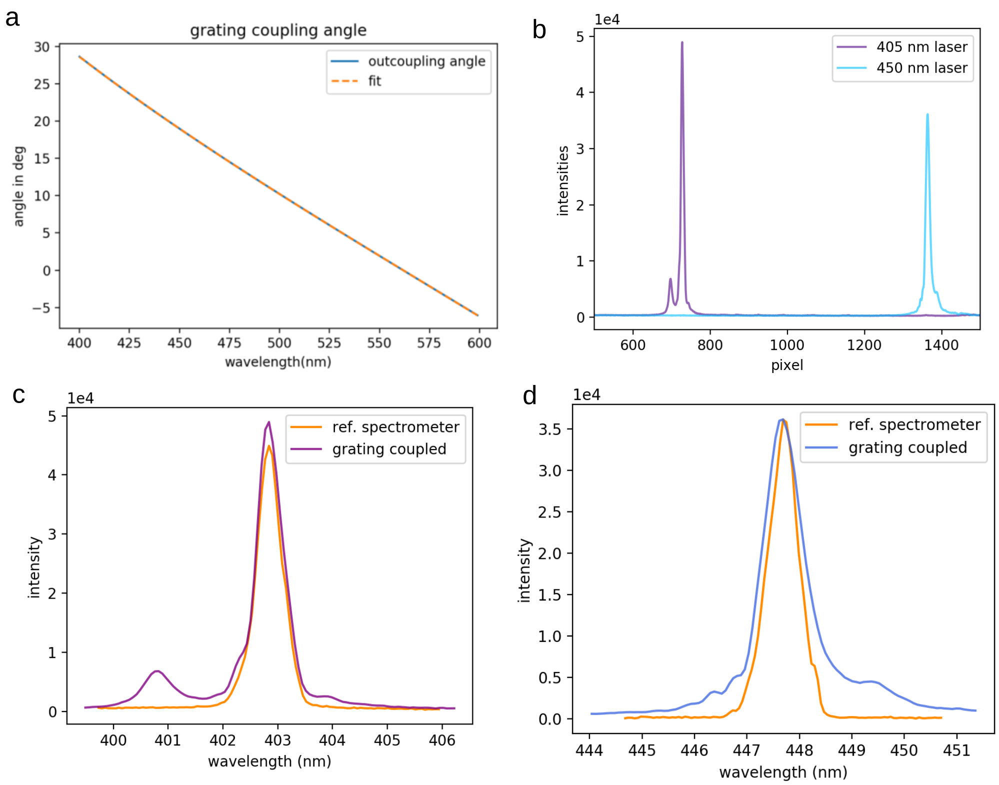

Figure S4: (a) Calculated outcoupling angles of the waveguide and polynomial fit. (b) Measurements with linear camera with two lasers at 405 nm and 450 nm. (c)(d) Comparison of spectral shape taken with the linear camera to the measurements with a commercial spectrometer.

The calibration was used to measure a dye solution (tartrazine) to assess the spectral shape. As shown in Figure S5a and b, the dashed lines are scaled reference measurements and solid line are the waveguide measurements. The overlap is very good of both in range of 415 - 430 nm.

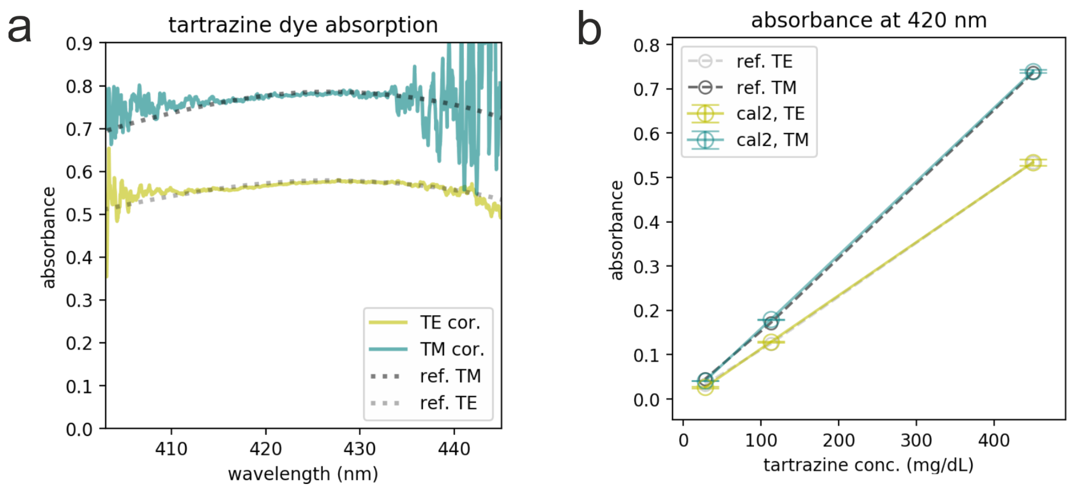

Figure S5: (a) Spectra of tartrzine dye solution measured with the calibrated line camera readout. (b) Concentration against the absorbance measured at 420 nm. Reference data were measured with photo-spectrometer and scaled to compare with the waveguide sensor.