Supplementary Information for “Actively Transporting Virus Like Analytes with Optofluidics for Rapid and Ultrasensitive Biodetection”

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5 Scaling Analysis

Figure S1. Schematics for the conventional and actively controlled flow models. (a) Analytical model for the conventional scheme is shown. Solution flows through a microfluidic channel of height $H_c$ and width $W_c$. The sensor of length $L_s$ and width $W_s$ is placed in the channel. (b) Analytical model for the actively controlled flow scheme is shown. A unit cell is considered in this case. The sensing area is taken as the gold side wall of the holes.

The conventional flow scheme is shown in Fig. S1a. The sensor is modeled as a flat square with width $W_s = 60 \mu m$ and length $L_s = 60 \mu m$. The micro-channel is of height $H_c = 100 \mu m$ and width $W_c = 100 \mu m$. A solution of analytes with a concentration of $c_0 = 100 nM$ and a diffusivity of $D = 7 \mu m^2/s$ (a typical diffusivity for 50 nm radius particles in water) is considered to flow with a rate of $Q = 5 \mu L/min$. Reaction rate is assumed to be $k_b = 7 \times 10^7 M^{-1}$ and the binding site density is $b_s = 2 \times 10^{15}$ sites/m$^2$.

Channel Peclet number, defined as the ratio of the particle convection rate by the flow to diffusion rate, can be calculated by$^1,2$

$$P_{e,con} = \frac{Q}{W_c D} = 1.2 \times 10^5 \quad \text{Equation 1}$$

The dimensionless mass transport flux delivered to the sensor surface is given by$^3$

$$F_{conv} = 0.81 \cdot \frac{6P_{e,con}(L_s/H_c)^2}{v} = 50 \quad \text{Equation 2}$$

In dimensional terms, the number of analytes transported to the sensor per second is

$$J_{conv} = Dc_0 W_s F_{conv} = 1.1 \text{ molecules/s} \quad \text{Equation 3}$$

Damkohler number, defined as the ratio of reactive to diffusive flux, can be calculated by

$$Da_{conv} = \frac{k_b h c L_s}{D F_{conv}} \approx 60 \quad \text{Equation 4}$$

Actively controlled flow model is shown in Fig. S1b. A single nanohole unit is considered in this model. The nanohole is patterned on a 100 nm thick SiN layer with a 100 nm gold layer. Since nanoholes are $D_{hole} = 200 nm$ in diameter and $P = 600 nm$ in period, there are $N_{hole} = 10^5$ nanoholes on a sensor. Therefore, the flow rate through each nanohole is $Q_{unit} = Q/N_{hole} = 5 \times 10^{-4}$ $\mu L/min$. For simplicity, the sensing area is taken as the gold inner sidewalls of the hole.

The channel Peclet number for the actively controlled flow scheme is

$$P_{e,act} = \frac{Q_{out}}{\pi RD} = 4 \times 10^3 \quad \text{Equation 5}$$

and mass transport flux $F_{act} \approx 23$. The number of analyte transported on the sensing area is $J_{act} \approx 6 \times 10^3$ molecules/s. Thus, in total there are $J_{active} = N_{hole} J_{act} = 60$ molecules being delivered to the sensor per second. The Damkohler number can be calculated from equation 4 as: $Da_{active} \approx 0.2$.

FEM Simulation

Figure S2. Numerical calculation domain for the conventional and the actively controlled flow models. (a) Numerical model for the conventional flow scheme is shown. (b) Numerical model for the actively controlled scheme is shown. Equation 8 applies to the blue highlighted sensing area as boundary condition.

Steady state flow profile is obtained by solving Navier-Stokes equations in a two-dimensional model.

$$\rho \left( \frac{\partial v}{\partial t} + v \cdot \nabla v \right) = -\nabla p + \mu \nabla^2 v \quad \text{Equation 6}$$

Where $\rho$ is the fluid density, $v$ is the flow velocity, $p$ is the pressure and $\mu$ is the dynamic viscosity.

In the simulation, microfluidic channels are scaled down to 20 $\mu m$ in length and 5 $\mu m$ in height (Fig. S2). The sensor is represented by 20 nanoholes. The opening on the left side of the channel is used as inlet to pump the analyte solution into the chamber. Solution is assumed to be water with density $\rho = 1000 kg/m^3$ and the flow rate is $v_0 = 5 \times 10^{-4} m/s$.

The boundaries for the fluidic profile have the following conditions

\begin{align*}
\text{Inlet} & : v = v_0 \\
\text{Outlet} & : v = 0 \\
\text{The other boundaries} & : \nabla p = 0
\end{align*}
Analyte concentrations are calculated by solving a convection-diffusion equation:

$$\frac{\partial c}{\partial t} + \nabla \cdot (-D \nabla c + cv) = 0$$  \hspace{1cm} \text{Equation 7}$$

Where $D$ denotes the diffusivity of the analyte ($7 \mu m^2/s$), $c$ is the analyte concentration, and $v$ is the flow velocity we calculated using equation 6. The initial conditions for equation 7 is $c = c_0 = 100$ nM.

Boundary condition at the sensing surface (highlighted area in Fig. S2) is given by first-order Langmuir equation:

$$\frac{\partial c}{\partial t} = k_o c (b_o - c) - k_{off} c$$  \hspace{1cm} \text{Equation 8}$$

Where $c_s$ is the analyte concentration on the sensor, $b_o$ is the receptor density on the sensing surface (2×10^{12} sites/m^2), $k_o$ is the reaction association rate (7×10^7 M^{-1}), and $k_{off}$ is the disassociation rate (3×10^{-5} s^{-1}).

The other boundary conditions considered are as follows:

$$c = c_0 \hspace{1cm} \text{Inlet}$$
$$\nabla \cdot (-D \nabla c + cv) = \nabla \cdot cv \hspace{1cm} \text{Outlet}$$
$$\nabla \cdot (-D \nabla c + cv) = 0 \hspace{1cm} \text{The other boundaries}$$

Models are meshed using triangular elements (Max/Min element size 0.4/0.002 μm) and the vicinity of the sensing area is of refined sizes (Max/Min element size 0.1/0.0005 μm).

**DUV lithography**

![Figure S3. Wafer scale fabrication of the nanohole array sensors using DUV lithography.](image)

We implemented DUV lithography based fabrication (Fig. S3a) through the following four consecutive steps: (i) nanohole arrays are patterned in wafer scale using DUV stepper, (ii) pattern transfers to the SiN layer using reactive ion etching (RIE), (iii) free standing SiN membranes are formed using KOH wet etching, and (iv) direct metal deposition.

**DUV lithography**

ASML S500/300 DUV Stepper is used for the lithography. 52 sensor chips can be fabricated on a 4-inch wafer at a time. Each designed chip is 1 cm by 1 cm in size and contains 3 by 3 nanohole arrays.

**RIE dry etching**

The nanohole pattern is etched all the way through the SiN layer by SF₆ and Argon gas using photore sist as the hard mask. Resist residue is later removed with an oxygen plasma cleaning process leaving a clean patterned SiN layer.

**KOH wet etching**

We create standing SiN membranes using photolithographic and a wet etching processes. Initially, 2 μm thick MICROPOSIT™ positive photoresist is spin coated, and 750 μm x 750 μm apertures on the SiN layer are defined by photolithography with SUSS MicroTec MA/BA6 Mask Aligner. Photolithographic pattern is transferred to the backside of the wafer using a RIE process with Plasma Therm 790 RIE/PECVD System. Later, the chips are immersed in KOH solution to create free standing membranes. Approximately 150 μm x 150 μm and free standing SiN membranes are obtained once the etching stops at the patterned layer (Fig. S3b).

**Metal deposition**

An e-beam evaporator is used to deposit Ti (5 nm) and Au (125 nm) metal layers defining the plasmonic nanohole sensors as described above (Fig. S3c).

The optical responses of the nanohole arrays are the ultimate metrics for determination of the fabricated nanostructure qualities for our sensing applications. The transmission spectrum is obtained from the DUV fabricated arrays in DI water and the result is compared with the spectrum of the structure fabricated using EBL (Fig. S4). Both structures show strong resonances at the designed wavelength of 850 nm. The spectral line-width of the resonance is comparable to that of the arrays fabricated using EBL. These observations indicate the feasibility of DUV technique for large area patterning of nanohole arrays with optical qualities achievable by EBL.

**Reference**
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5. E. Fu, et al., Analytical chemistry, 2009, 81, 3407-3413