

## **Supporting Information**

### **Electrokinetic Lab-on-a-BioChip for Multi-Ligand/Multi-Analyte**

#### **Biosensing**

Ganeshram Krishnamoorthy, Edwin T. Carlen, Hans L. de Boer, Albert van den Berg and

Richard B. M. Schasfoort

BIOS Lab-on-a-Chip Group, MESA+ Institute for Nanotechnology, University of Twente,

P.O. Box 217, 7500 AE Enschede, the Netherlands. Tel.: 0031 53 4892724; Fax: 0031 53

4893595

Email: [g.krishnamoorthy@tnw.utwente.nl](mailto:g.krishnamoorthy@tnw.utwente.nl)

## Electrokinetics Simulation

The electrokinetic flow behavior, including flow rate and voltage distribution across the gold islands, was calculated using a numerical solution of the 2D Navier-Stokes equation with a commercial finite element software (Conductive media model, COMSOL, USA).<sup>1,2</sup> A lumped electrical circuit representation of the system is shown in Fig. 2d (main text). The electrical resistances  $R_1$  through  $R_4$  represent the equivalent electrical resistance of the buffer in each channel and  $I_1$  through  $I_4$  are electrical currents flowing from the inlet to the outlet in each channel, respectively. In this case, all channel inlets are connected to a single voltage source, and therefore, the total electrical current is the sum of all currents, (i.e.  $I_t = I_1 + I_2 + I_3 + I_4$ ). The resistances are estimated with  $R = L_c / \sigma A$ , where  $\sigma$  is the buffer conductivity and  $A$  is the cross sectional area of the microchannel. The channels are designed so that the equivalent electrical resistance of the buffer is the same in each channel ( $R_1 = R_2 = R_3 = R_4$ ), in order to maintain the same flow rate in each channel. Controlling and minimizing the current in each channel is important in order to avoid any electrochemical reaction with the gold islands, which can adversely affect the thin gold layer (and adhesion layer) and SPR measurements.<sup>3-5</sup> Channel dimensions are chosen such that the current in each microchannel  $I_i < 20$  mA and the electric field of across the length of the microchannels is  $E_{max} < 10$  V/mm to ensure that the thin gold sensing regions are not affected during operation.<sup>6</sup> A low conductivity (10 mM HEPES, pH-7.2,  $\sigma \approx 238$   $\mu$ S/cm) buffer is used to minimize current in the channels.

The initial boundary conditions for the numerical simulation of the EK flow rate were set according to the conditions mentioned above. The electroosmotic mobility was assumed to be  $5 \times 10^{-8}$  m<sup>2</sup>/Vs.<sup>7</sup> Figure SI-1a shows the typical electric potential profiles in the channels and Fig SI-1b shows the flow speed ( $v_f$ ) profile. The estimated flow speed is  $v_f \sim 50$   $\mu$ m/s in all

cases. The calculated volumetric flow rate according to the dimensions of the channel is  $Q_f \sim 26$  nL/min. The electric field across each channel is  $E_{in} \sim 9$  V/mm. We have found that a dextran coating over the gold islands can help to protect the gold layer. Experiments were performed in the new chip and are discussed in the following section.

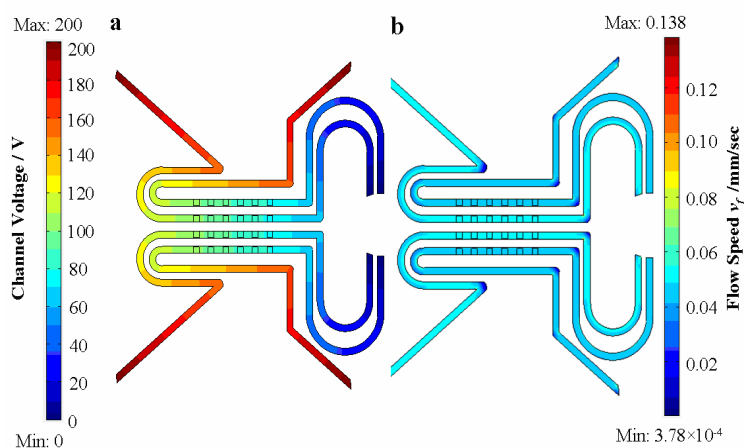


Figure-SI-1. Simulation of 2D Navier-Stokes equation with Conductive media model (COMSOL, USA). (a) Voltage profile across each microchannels. (b) Flow speed profile with  $V_{in}=200V$  resulting in  $v_f \sim 50 \mu\text{m/s}$

### Optical Microscopy Flow Profiling

The electrokinetic flow profile was observed with fluorescence microscopy in which four different concentrations (0, 0.5, 1.0 and 2.0  $\mu\text{g/ml}$ ) of alexafluor488 (Molecular probes, USA) were injected into the different microchannels simultaneously. The flow profile is shown in the Fig. SI-2a. The measured fluorescence intensity profiles at location A-A' is shown in Fig. SI-2b. The dark regions are caused by the gold islands blocking light from the inverted microscope. The intensities observed in  $C_2$  and  $C_3$  are not uniform and show a slight decrease of intensity near the channel walls. This is very important when choosing region of interests (ROIs) for the iSPR measurements, where it is best to have a uniform flow speed in the region where the iSPR measurement occurs. The inset of Fig. SI-2b is the relationship

observed for fluorescence intensity versus concentration of alexafluor488. The chip alignment of the microchannels to the gold-island imaging array is very important and misalignment can result in wasted sensing area and possible channel leakage. The difference between the channel dimensions and sensing array dimensions are very small in this case, the chip alignment problem was minor. This can be avoided by considering larger flow channel dimensions.

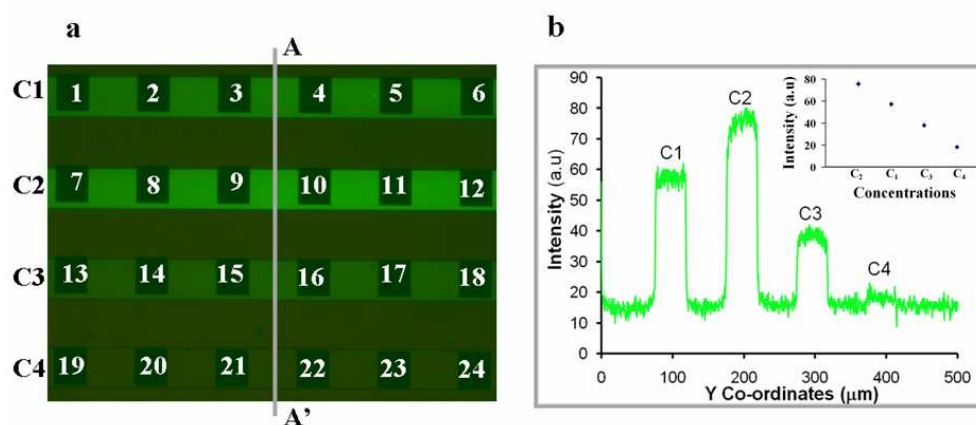


Figure-SI-2. (a) Fluorescence microscopy images of various concentrations of alexafluor488 in 10mM HEPES in all channels.  $C_1$  – 1:10 dilution;  $C_2$  – 1:5 dilution;  $C_3$  – 1:20 dilution (b) Fluorescence intensity measurements at A-A' (gray line) showing the highest intensity for the highest concentration ( $C_2$ ) and lowest intensity for the lowest concentration ( $C_4$ ). The inset plot shows the fluorescence intensity – concentration relationships.

## References

1. Krishnamoorthy, S.; Feng, J.; Henry, A.; Locascio, L.; Hickman, J. J.; Sundaram, S. *Microfluid. Nanofluid.*, **2006**, 2, 345-355.
2. Gao, Y.; Wong, T. N.; Chai, J. C.; Yang, C.; Ooi, K. T. *Int. J. Heat Mass Transfer*, **2005**, 48, 5103-5111.
3. Abelès, F.; Lopez-Rios, T.; Tadjeddine, A. *Solid State Commun.*, **1975**, 16, 843– 47.

4. Gordon II, J.G.; Ernst, S. *Surf. Sci.*, **1980**, 101, 499–506.
5. Kolb, D. M. *Prog. Surf. Sci.*, **1996**, 51, 109–173.
6. Krishnamoorthy, G.; Carlen, E. T.; Kohlheyer, D.; Schasfoort, R. B. M.; van den Berg, A. *Anal. Chem.*, **2009**, 81, 1957-1963.
7. Kohlheyer, D.; Unnikrishnan, S.; Besselink, G. A. J.; Schlautmann, S.; Schasfoort, R. B. M. *Microfluid. Nanofluid.*, **2005**, 1, 242–248.