

# **Integration of Multiplexed Microfluidic Electrokinetic Concentrators with a Morpholino Microarray via Reversible Surface Bonding for Enhanced DNA Hybridization**

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## **Abstract**

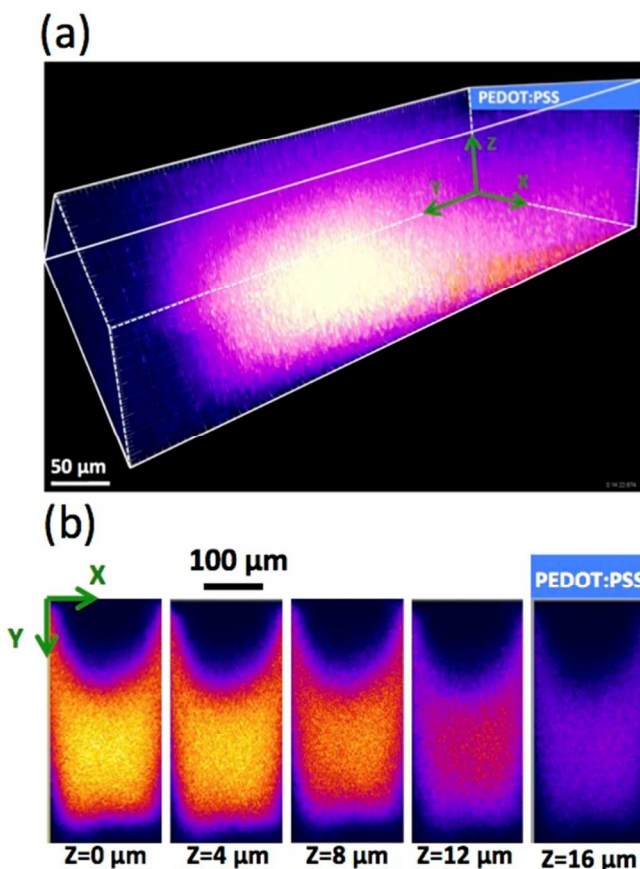
Supporting information provides: a video showing the automatic PEDOT:PSS printing process on PDMS channel, 3D confocal videos of the DNA plug during concentration experiments, confocal data of the vertical concentration profile of the plug in microfluidic concentrators with PEDOT:PSS at the top and bottom of the microchannel, estimation of the pH shift during ET concentration a 5-channel simultaneous DNA concentration video, and a measurement of the channel-to-channel and device-to-device variation.

## **S1. PEDOT:PSS printing on the PDMS**

Video S-1 shows the PEDOT:PSS printing step in 5 parallel PDMS microchannels. Prior to the printing step, the PDMS microchannels were rendered hydrophilic via confined corona plasma through the microchannels. The microchannels were then mounted onto the microplotter and the center position of each circular pattern in the microchannel is saved for subsequent access. The glass micropipette was then filled with PEDOT:PSS solution and sequentially driven to each one of the saved positions for in-channel membrane deposition (Video S-1). During this last step, the localized in-channel hydrophilicity allowed the PEDOT:PSS solution to spread inside the circular pattern and did not overflow into the connecting microchannel or over the channel edge which could compromise the reversible bonding between the PDMS chip and the substrate.

## S2. 3D confocal videos of the DNA plug

The microchannel volume was vertically scanned in 2  $\mu\text{m}$  step size. Video S-2 [last frame showed in Fig S-1 (a)], shows the formation of a DNA plug on a straight microchannel just next to a PEDOT:PSS membrane printed on top of the channel with no MOs. Video S-3 shows a DNA plug next to a PEDOT:PSS membrane printed at the bottom of a microchannel without MOs. Video S-4 shows a DNA plug next to a PEDOT:PSS membrane printed at the top of a microchannel with MO spots printed. The initial DNA concentration for all three experiments was 100 nM and the applied voltage was 75 V. These videos are all compressed time videos (15 min. total, real time). In these videos, the DNA plug starts forming near the membrane and the maximum concentration region of the plug slowly moves away from the membrane with time. In the videos, it is clear that the vertical concentration profile is not uniform and is highest at the bottom of the microchannel (colors representing different fluorescence intensities, from the highest to the lowest intensity: white, yellow, purple, blue).



**Figure S-1: (a) 3D reconstruction from the confocal fluorescence microscopy images in (b) of the DNA plug in a PDMS microchannel with a PEDOT:PSS membrane printed on top of the channel. The membrane boundary matches with the rightmost vertical facet of the rectangular prism domain in (a). Note: in (a) for clarity, the z-axis is magnified by 10× relative to the y- and x-axis.**

### S3. Vertical concentration profile for 100 nM DNA concentration on a deactivated superaldehyde glass slide without MO

Figure S-2 depicts the fluorescence intensity distribution along the vertical axis of the microchannel. The initial sample concentration was 100 nM. The imaging conditions used for 100 nM were 5% for laser power and HV=702 V for photomultiplier voltage.

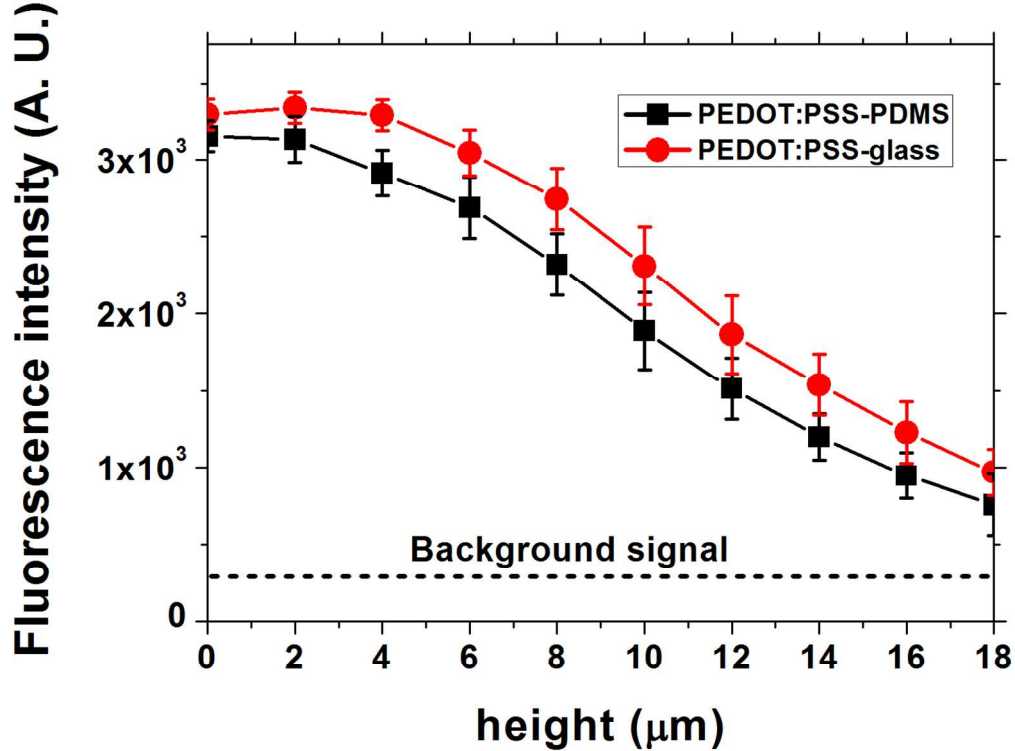


Figure S-2: Vertical intensity profile of the DNA plug as a function of the microchannel height, after applying 75 V across the microchannels for 15 min. These measurements were performed for devices with the PEDOT:PSS membrane at the top (squares, PEDOT:PSS-PDMS) of the microchannel and at the bottom (dots, PEDOT:PSS-glass). The results show that the membrane position does not affect significantly the plug profile when the initial DNA sample concentration was 100 nM. However, the fluorescence signal intensity was higher for PEDOT:PSS-glass devices than for PEDOT:PSS-PDMS devices.

#### S4. Vertical concentration profile for 100 nM DNA concentration on a deactivated superaldehyde glass slide with MO probes

Figure S-3 depicts the fluorescence intensity distribution along the vertical axis of microchannels with MO spots printed on the superaldehyde glass substrate. The initial DNA concentration was 100 nM. The imaging conditions used were 5% for laser power and HV=702 V for photomultiplier voltage.

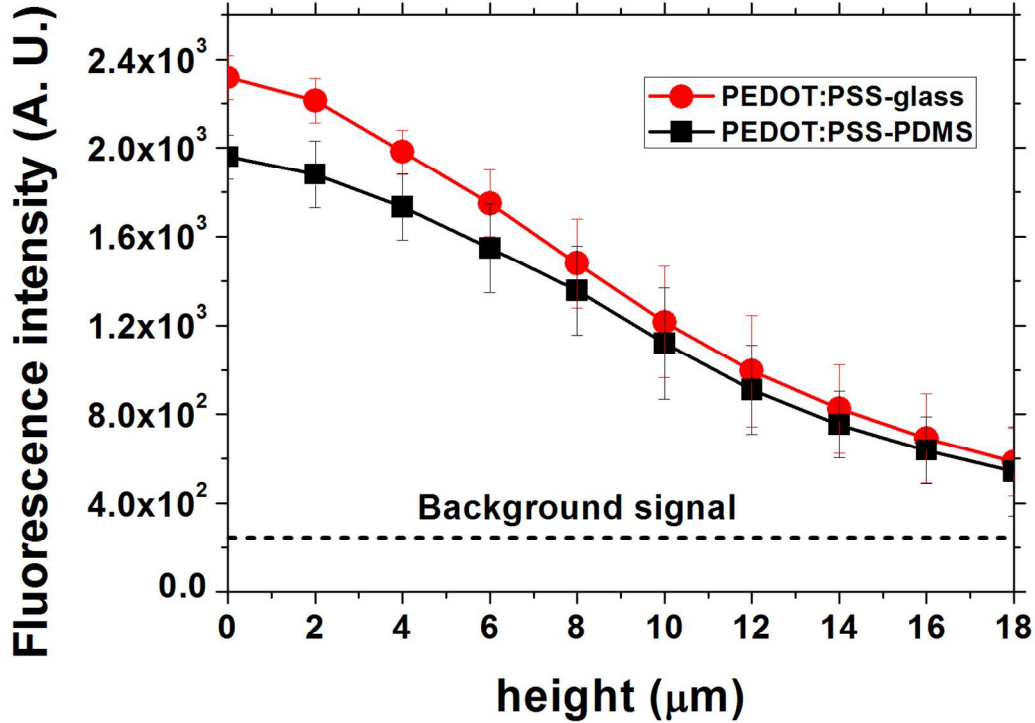


Figure S-3: Vertical intensity profile of the DNA plug as a function of the microchannel height, after applying 75 V across the microchannels for 15 min, during an MO-DNA hybridization experiment with an initial DNA concentration of 100 nM. These measurements were performed for devices with the PEDOT:PSS membrane at the top (squares, PEDOT:PSS-PDMS) of the microchannel and at the bottom (dots, PEDOT:PSS-glass). These results show that the MO spots did not affect significantly the plug signal intensity profiles. However, the data suggests that the presence of morpholino spots at the bottom of the microchannel slightly decreased the plug signal intensity in comparison with those devices with only deactivated superaldehyde glasses in agreement with the discussion on the electrophoretic mobilities of different substrates (compare with Fig. S-2).

### S5. Vertical concentration profile for 10 nM DNA concentration on a deactivated superaldehyde glass slide without MO

The imaging conditions used for 10 nM were 25% for laser power and HV=790 V for photomultiplier voltage.

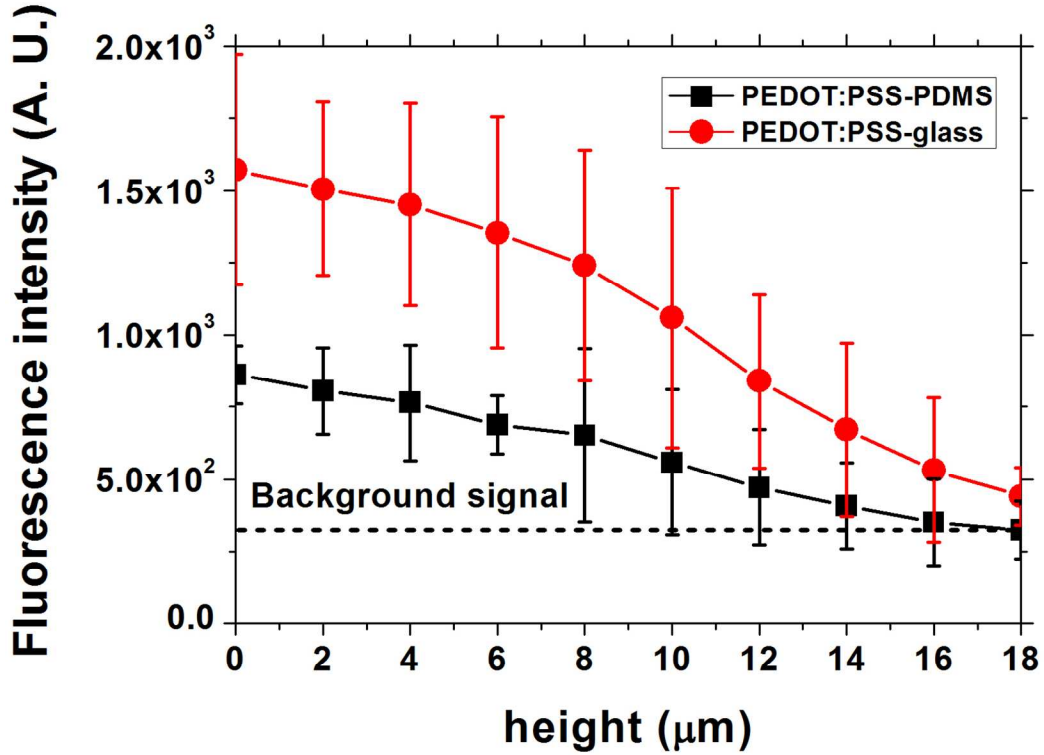


Figure S-4: Vertical intensity profile of the DNA plug as a function of the microchannel height, after applying 75 V across the microchannels for 15 min, for an initial DNA concentration of 10 nM. These measurements were performed for devices with the PEDOT:PSS membrane at the top (dots, PEDOT:PSS-glass) of the microchannel and at the bottom (squares, PEDOT:PSS-PDMS). The results show that, for an initial DNA sample of 10 nM, the membrane position in the channel impacts significantly the DNA plug signal intensity. In fact, PEDOT:PSS-glass devices show the double of the background deduced fluorescence intensity at the surface when compared with the PEDOT:PSS-PDMS devices.

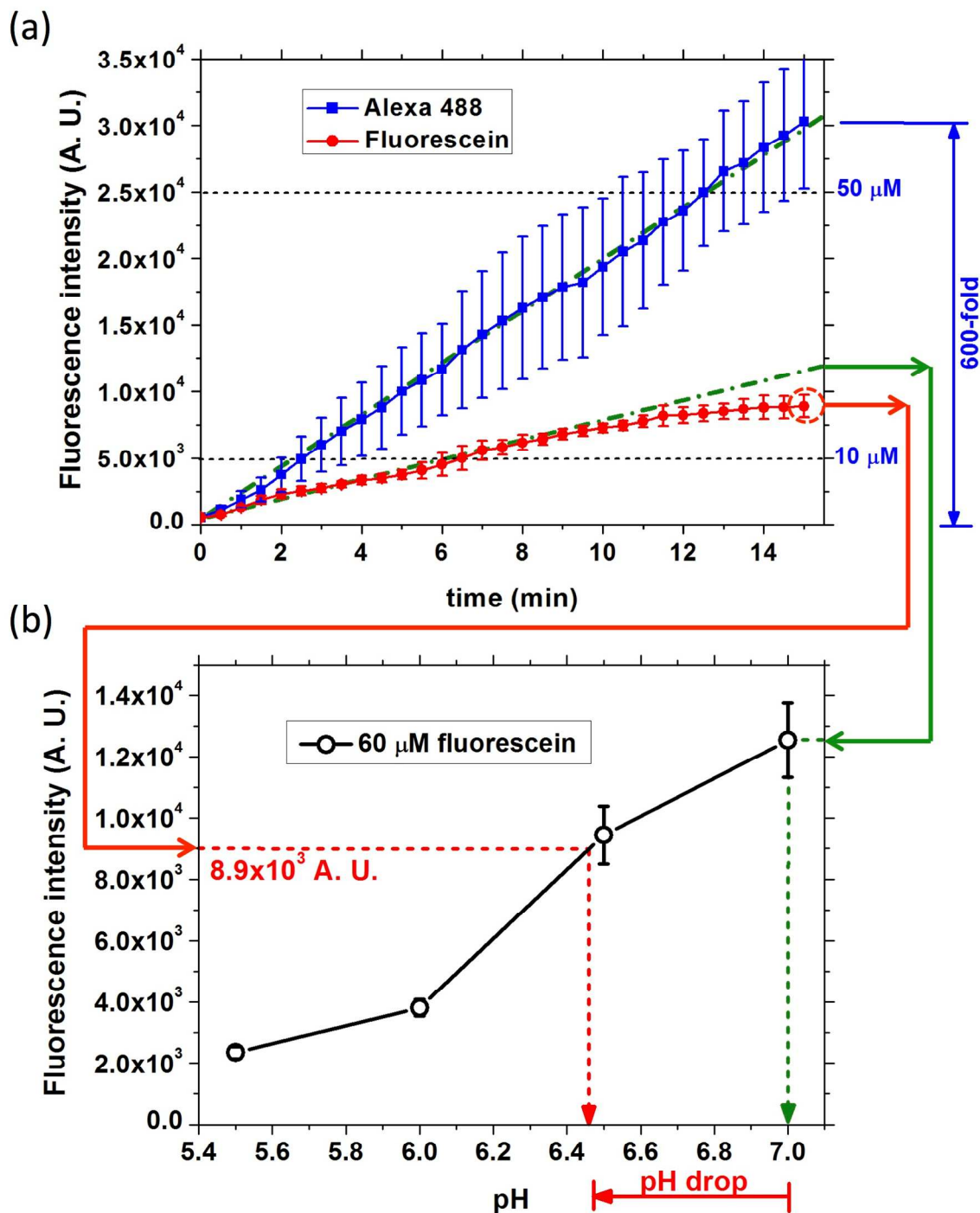
## S6. pH shift in the plug region during preconcentration

To estimate the pH variation during our electrokinetic concentration method, we separately concentrated two fluorescent dyes, Alexa Fluor 488 NHS Ester (ThermoFisher Scientific Cat. # A-20000, MW 643.4, pH insensitive from pH 4 to pH 10) and fluorescein (ThermoFisher Scientific, Cat. # F1300, MW 332.31, pH sensitive from pH 5 to pH 9<sup>1,2</sup>), and then compared their fluorescence signal intensities as a measure of concentration factor [see Figure S-5 (a) below]. With Alexa Fluor 488, the fluorescence intensity of the plug increased linearly with time, whereas the fluorescence intensity of fluorescein increased linearly (the lower fluorescence intensities are due to the lower quantum yield of fluorescein in comparison to Alexa Fluor 488) for 8 min. and reached a plateau after 12 min.. The linear regression line is represented as a dash-dot green line. We attributed this deviation from the initial linearity to a pH drop in the concentration region and not to a decline in the rate of molecular accumulation, as it was only observed with pH sensitive fluorescein. In fact, for Alexa Fluor 488, the accumulation of molecules occurs at a constant rate due to the constant applied EOF, and because its fluorescence is not affected by a pH change. Therefore, the fluorescence intensity of the plug increased linearly with time, as observed. Since EOF dominates the transport of the charged species in the microchannel, fluorescein should accumulate at the same rate as Alexa Fluor 488 during concentration; consequently the observed fluorescence intensity plateau can be explained as a result of a pH drop during electrokinetic concentration. As mentioned earlier, fluorescein is pH-sensitive and its fluorescence increases with higher pH, as shown in Figure S-5 (b) below.

To calculate the pH drop during preconcentration, we first estimated the final fluorescein concentration (after 15 min.) by using the electrokinetic concentration curve of Alexa Fluor 488 (Figure S-5 (a), blue squares) together with an Alexa Fluor 488 fluorescence intensity vs. concentration calibration curve [in Figure S-5 (a), Alexa Fluor 488 reference concentration lines are displayed as dashed lines]. As shown in Figure S-5 (a), we obtained a concentration factor of ~600 times (concentration increased from 0.1  $\mu$ M to 60  $\mu$ M) for Alexa 488 dye after 15 min. of electrokinetic concentration. By assuming the same concentration factor of ~600 times for fluorescein, we calculated the final concentration of fluorescein to be 60  $\mu$ M starting from an initial concentration of 0.1  $\mu$ M. By measuring the fluorescence signal intensity of 60  $\mu$ M fluorescein in 0.1 x PBS titrated from pH 7.0 to pH 5.5 in 0.5 pH units step [see Figure S-5 (b)] and comparing the fluorescence intensity of the electrokinetically concentrated fluorescein dye after 15 min. from Figure S-5 (a) to that of 60



$\mu\text{M}$  fluorescein between pH 5.5 and 7.0 in Figure S-5 (b), we determined a pH drop of  $\sim 0.5$  pH units from the initial value of pH 7.0 through a linear interpolation. The fact that the linear regression line of the fluorescence signal intensity for fluorescein without pH change [green line in Figure S-5 (a)] results in similar fluorescence signal intensity after 15 min. of electrokinetic concentration compared to the fluorescence signal intensity of  $60 \mu\text{M}$  fluorescein at pH 7 in Figure S-5 (b) validates our accurate estimation of 600-fold increase of concentration for fluorescein.



**Figure S-5. Estimation of pH drop during electrokinetic concentration.** (a) 50 V were applied to concentrate 0.1  $\mu\text{M}$  Alexa Fluor 488 and fluorescein (both fluorescent dyes were diluted in 0.1X PBS, pH 7.0) for 15 min. in separate channels. The fluorescence signal intensity was acquired throughout the entire concentration period (exposure time was 100 ms and interval between each measurement was 30 s).

The linear approximations for the first 8 min. of concentration are depicted as the dash-dot green lines. A concentration enrichment factor of ~600 times (concentration increased from 0.1  $\mu\text{M}$  to 60  $\mu\text{M}$ ) was measured for pH-insensitive Alexa Fluor 488. The deviation from the linear regression line observed in the case of fluorescein results from its pH sensitivity. (b) To estimate the pH shift during electrokinetic concentration, 60  $\mu\text{M}$  fluorescein in 0.1 x PBS was titrated from pH 7.0 to 5.5 in 0.5 pH unit steps (fluorescence signal intensity between the experimental data points was linearly interpolated). From the comparison of the fluorescence signal intensity after 15 min. of electrokinetic concentration [Fig. S-5 (a), red dot at 15 min.] with the titration curve suggests a ~0.5 pH drop from pH 7.0.

### **S7. Simultaneous DNA concentration in 5 channels**

Video S-5 shows a 15 min. simultaneous preconcentration of the DNA sample next to PEDOT:PSS membranes printed on top in five different microchannels. After dispensing 100 nM DNA sample into the right reservoir, and the buffer solution into the left reservoir, the DNA sample was concentrated by applying 75 V across the channels. As can be seen in the movie, the DNA plugs in different channels formed at the same rate and remained close to the membranes during the whole 15 min period. Once the voltage was switched off, all the DNA plugs moved away from the membranes.

## S8. Channel-to-channel and device-to-device variation

Channel-to-channel deviation is low because the average fluorescence intensity and standard deviation values of the DNA plugs and hybridization results are similar among the different channels. The chip-to-chip reproducibility (4 multi-channel devices tested in total) is high since in general the error bars are small in comparison with the average fluorescence intensity values for both the DNA plugs and hybridization results. In fact, for 100 nM: the average intensity concentration was 173 040 and the standard variation was 41 025; the average hybridization intensity was 4206 and standard variation was 819. For 10 nM: the average intensity concentration was 780 and the standard variation was 232; the average hybridization intensity was 97 and standard variation was 23.

**Table S-1 Measurement of the chip-to-chip reproducibility for PEDOT:PSS-PDMS devices.**

<b>Concentration (nM)</b>	<b>Plug intensity (A. U.)</b>	<b>Hybridization signal (A. U.)</b>
100	173 040 ± 41 025	4 206 ± 819
10	780 ± 232	97 ± 23

## References

- (1) Chen, A. K.; Cheng, Z.; Behlke, M. A.; Tsourkas, A. *Anal. Chem.* **2008**, 80 (19), 7437–7444.
- (2) Emmart, E. W. *Arch. Biochem. Biophys.* **1958**, 73 (1), 1–8.