EXTENDED DYNAMIC RANGE CAPILLARY-DRIVEN MICROFLUIDICS

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ABSTRACT

We report a concept to increase the dynamic range of capillary driven microfluidics for use in point-of-care diagnostics [1]. The chip uses one-pipetting step of 20 µL of human serum spiked with C-reactive protein (CRP) at 100 ng/mL. A four-fold increase in signal strength is achieved by varying the flow rate in six different reaction chambers.

KEYWORDS: Point-of-care, diagnostics, dynamic range, capillary, microfluidics

INTRODUCTION

Binding of analytes to capture antibody (cAb) receptors is limited by their equilibrium constant. Assays are usually developed with the highest affinity antibodies to push detection limits by increasing the signal for low analyte concentrations. Conversely, the assay quickly saturates in signal for high analyte concentrations. Previous results on one-step immunoassays for detecting CRP suggest a dynamic range of 1 to 100 ng/mL [1]. We would like to detect less than 1 ng/mL or more than 100 ng/mL without using antibodies with different equilibrium constants [2]. By varying the flow rate, the time analytes can diffuse to the detection regions is changed, Figure 1. At the slowest flow rate, low concentration analytes can diffuse to the surface and increase the signal. At the fastest flow rate, analytes diffuse less to the surface decreasing the signal and the assay saturates at a higher concentration.

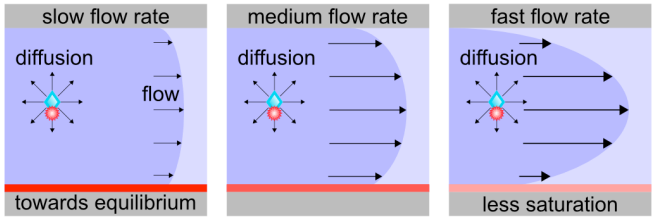

Figure 1: Concept of extending the dynamic range by varying the flow rate. With a slow flow rate, analytes have time to diffuse and bind to surface immobilized receptors, increasing the signal and lowering the lowest detectable concentration. With a fast flow rate, analytes have little time to diffuse to the surface and bind, decreasing the signal. Tests saturate at a higher analyte concentration, increasing the highest detectable concentration. The cross section of a microfluidic channel is here represented by the layer of cAb and captured analyte-dAb complex in red.

RESULTS AND DISCUSSION

This concept is implemented in the extended dynamic range chip, Figure 2. The chip contains a detection antibody (dAb) deposition zone. The Dean flow mixer provides a uniform quantity of dAb in the channel cross-section. Alternating mixing elements generate alternating chaotic vortices allowing proteins to mix by exploring the channel cross-section chaotically [3]. The channel is split and distributed into independent reaction chambers. During filling, the flow rate of the sample is the same across all channels. Each reaction chamber after junction point J has an increasing resistance (R_1 to R_6) determining the flow rate for the rest of the test. The experimental filling times are greater than the calculated filling times, table 1. This could be due to variability in the surface of the microfluidic channels, evaporation of the sample in the loading pad, or the approximate viscosity used for calculations.

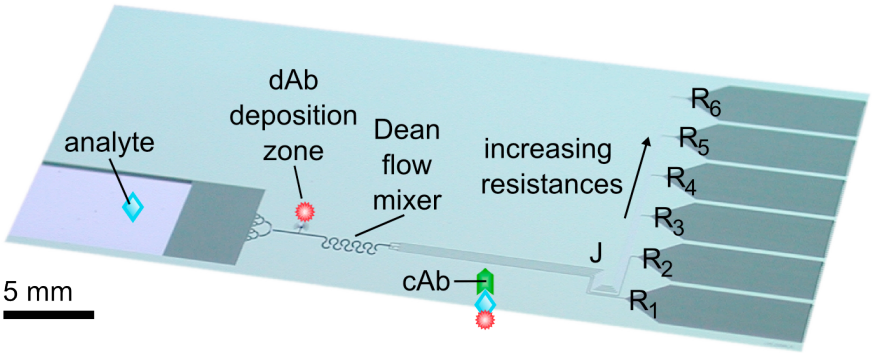

Figure 2: Extended dynamic range chip. Solution containing analytes is pipetted into the loading pad. dAb solution is previously spotted using and inkjet printer on the dAb deposition zone where dAbs are redissolved and CRP-dAb complex is formed. In the reaction chamber, cAbs are patterned on PDMS where the detection occurs. The channels before the capillary pumps have increasing resistances that vary the flow rates in the reaction chamber from fast (R_1) to slow (R_6) flow rates. The fastest path (R_1) increases the upper limit of detection and the slowest channel (R_6) lowers limit of detection.

Table 1. Calculated and experimental filling times

Channel	Resistance R_{FR} (m^{-3})	Flow rate D ($L\ s^{-1}$)	Calculated fill time	Experimental fill time
R_1	3.8×10^{17}	6.3×10^{-9}	8 min 3 s	10 min
R_2	7.5×10^{17}	3.2×10^{-9}	16 min 10 s	19 min
R_3	1.5×10^{18}	1.6×10^{-9}	32 min 23 s	36 min
R_4	1.8×10^{18}	1.4×10^{-9}	37 min 1 s	43 min
R_5	2.0×10^{18}	1.2×10^{-9}	42 min 11 s	60 min
R_6	2.2×10^{18}	1.1×10^{-9}	47 min 5 s	72 min

The operation of the chip begins when a serum sample is introduced in the loading pad, Figure 3. The sample gradually redissolves the dAb. In the 1st loop of the Dean flow mixer (a) the dAb concentration across the channel is a gradient between x_1 and x_2. At the 9th loop (b) the dAb between x_3 and x_4 has been mixed and spread across the channel cross-section. The channel is split by a flow distributor (c) with the same quantity of dAb entering each reaction chamber, between x_5 and x_6. The signal in the detection regions of the reaction chamber (d) imaged after 30 minutes is four times stronger in the slow flowing reaction chamber than in the fast flowing one, between x_7 and x_8. These results show that we can change the signal strength by changing the flow rate in microchannels while requiring only one sample handling step to perform the immunoassay. It will be interesting to test the chips with higher concentration samples and to see what is the highest detectable concentration that can be detected before the signal saturates. Alternatively, it will be interesting to test the chips with low concentration samples to determine the limit of detection in the slowest flowing microchannel.

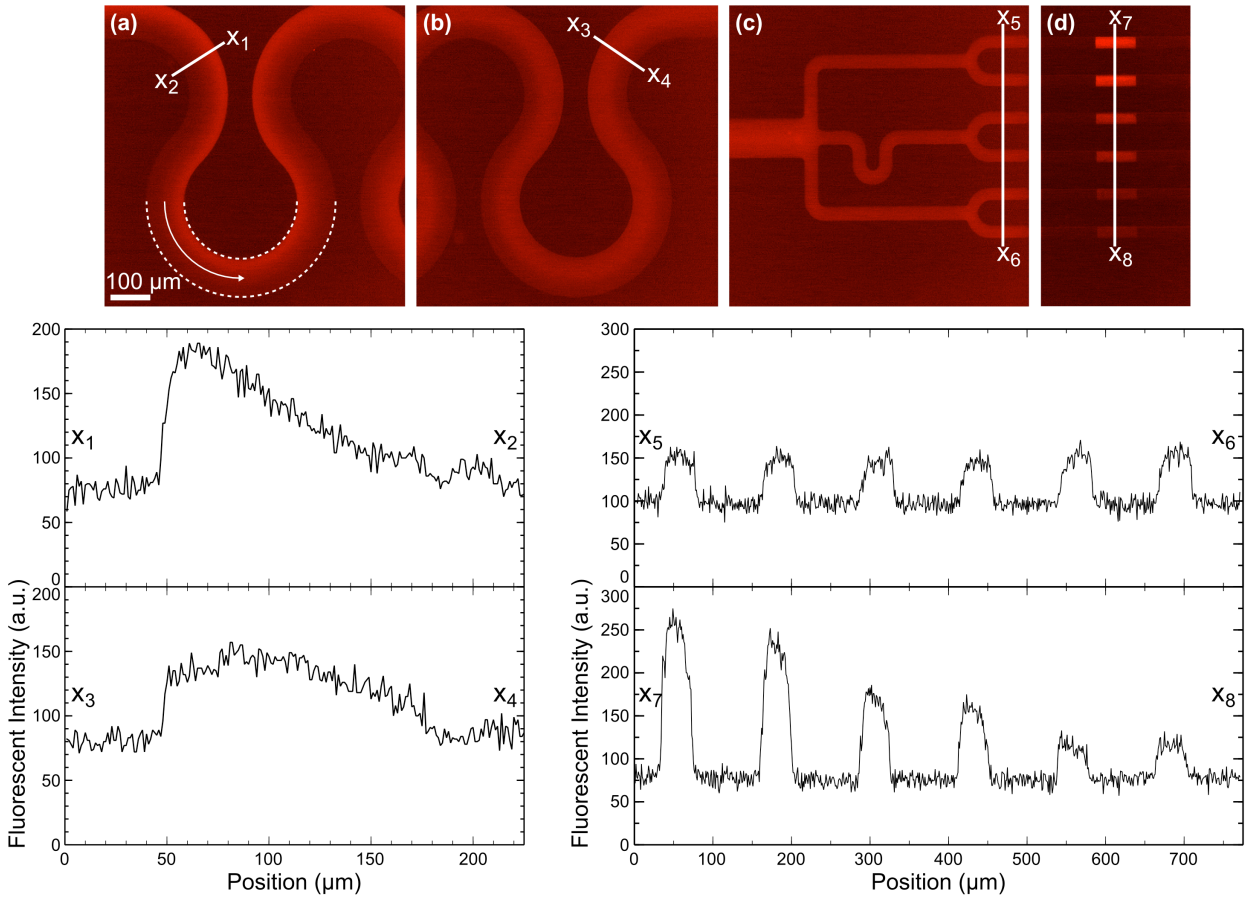

Figure 3: Mixing, distribution and detection. On one chip, 60 drops of dAb solution (10.8 nL) were deposited and 20 µL of human serum spiked with CRP at 100 ng/mL was pipetted. In the1st loop of the Dean flow (a) there is a gradient of concentration of dAb between x_1 and x_2, after the 9th loop (b) the dAbs are spread between x_3 and x_4. The channel is split into six by a flow distributor (c) with the same quantity of dAbs entering each reaction chamber between x_5 and x_6. The signal in the detection regions of the reaction chamber (d) imaged after 30 minutes is 4 times stronger in the reaction chamber with the slowest flow rate than in the reaction chamber with the fastest flow rate, between x_7 and x_8

CONCLUSION

We demonstrated the concept of extending the dynamic range by four time using capillary driven one-step immunodiagnostics chips. Microfluidic chips with a wider dynamic range are easier to use because they may minimize diluting and preconcentrating the sample and testing on multiple chips for samples that lie outside of the chips detectable concen-

tration range. We believe this improvement might increase even more the potential of using capillary-driven microfluidics in point-of-care diagnostics.

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