Supporting Information

An open space diffusive filter for simultaneous species retrieval and separation

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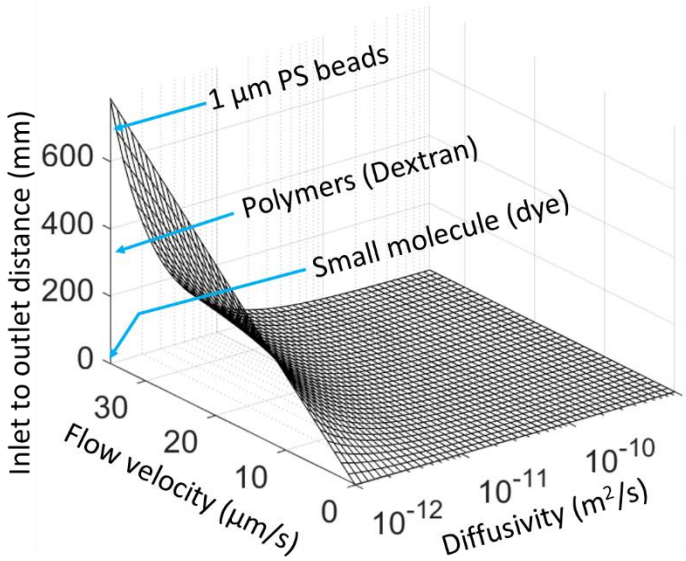

Figure S1*: Dependence of the inlet-to-outlet distance on species diffusivity and flow velocity for a fixed separation width of 150 µm. The separation distances required to separate certain representative species are also highlighted.*

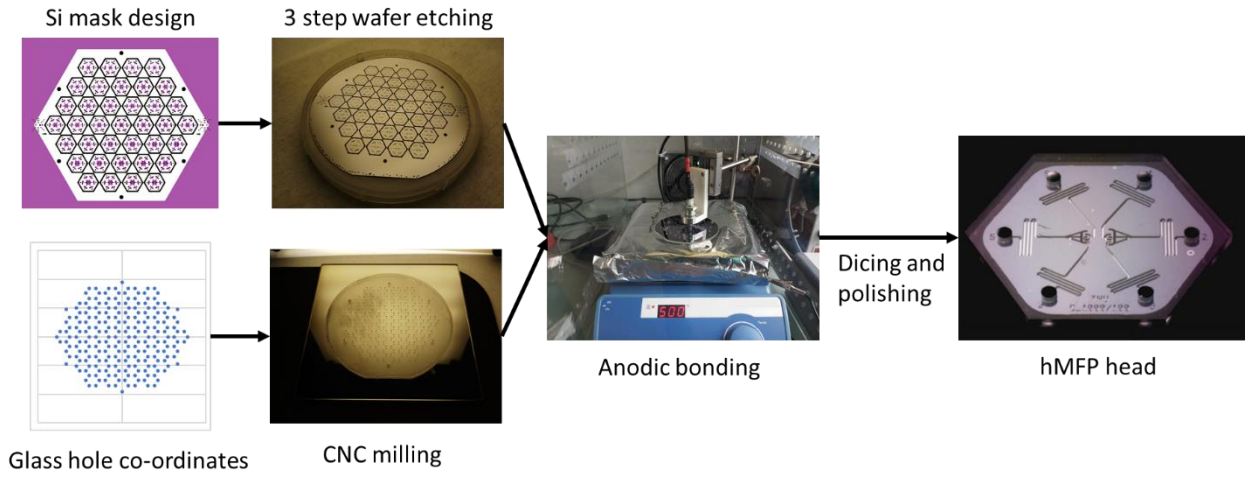

Figure S2: *Manufacturing process of the horizontally oriented microfluidic probe (hMFP) device.*

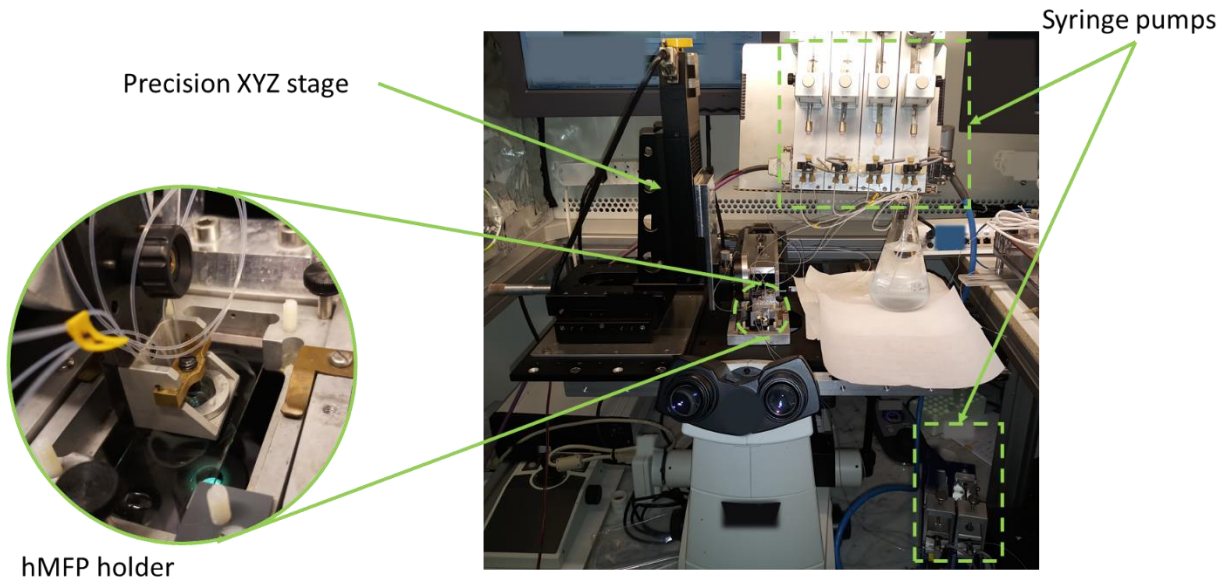

Figure S3*: Experimental setup showing the six syringe pumps to control the flow and the hMFP holder connected to a precision XYZ stage*

Supplementary Note 1: Flow profile in the absence of stabilisation apertures

In the absence of stabilisation apertures, a parallel flow profile could not be maintained in the MFP design, with the flow either not confined at all (Fig. S3a) or high aspiration to injection rate ratios (50:1) causing the flows to merge with a disrupted profile.

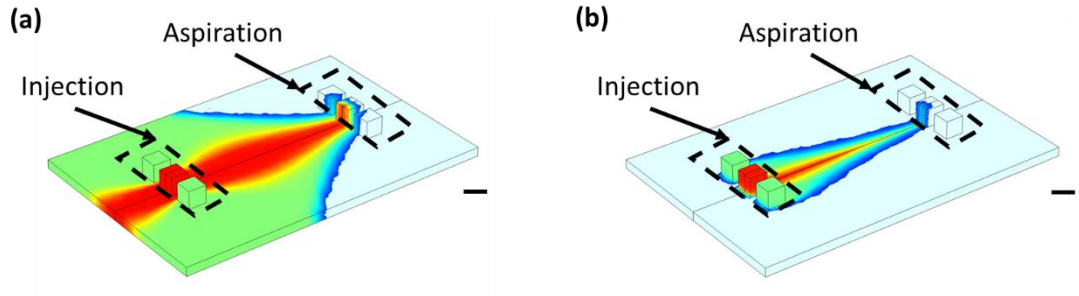

Figure S4*: Flow in the absence of stabilisation apertures (a) Non-confined flow at a 2:3 injection-to-aspiration ratio. (b) High aspiration-to-injection flow rate ratio (50:1) to confine flow with disrupted parallel profiles. Distinct colours represent different fluids in the central and side stream and the immersion liquid. All scale bars are 100 µm.*

Supplementary Note 2: Governing equations of the T-sensor

The governing equation (in a T-sensor) which enables assessment of the diffusion process is the advection - diffusion equation. In microfluidic channels, the 1D transient diffusion equation can be used, i.e.

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial t^2} \tag{S1}$$

for which the analytical solution can be expressed using the error function (erf) as[1]:

$$C(y,t) = \frac{C_0}{2}\left[1 - erf\left(\frac{y}{2\sqrt{Dt}}\right)\right] \tag{S2}$$

For a T-sensor, assuming a steady state condition and $\frac{\partial^2 C}{\partial y^2} \gg \frac{\partial^2 C}{\partial x^2}$ as diffusion dominates in the channel width rather than along the length, the 2D advection diffusion equation can be simplified as:

$$\frac{\partial C}{\partial t} + U\frac{\partial C}{\partial x} = D\frac{\partial^2 C}{\partial y^2} + D\frac{\partial^2 C}{\partial x^2}$$

$$\Rightarrow U\frac{\partial C}{\partial x} = D\frac{\partial^2 C}{\partial y^2} \tag{S3}$$

Now, choosing x/U as a time-like coordinate, the T-sensor has the same governing equation as the transient 1D diffusion equation (**Equation S1**). Thus, the steady state concentration[2] in a T-sensor is,

$$C(x,y) = \frac{C_0}{2}\left[1 - erf\left(\frac{y}{2\sqrt{D\frac{x}{U}}}\right)\right] \tag{S4}$$

Supplementary Note 3: Geometry calculation for hMFP – separation of bead and dye

The hMFP geometry constrained the width $w \approx 150\ \mu m$ µm due to a channel size of 100 µm and an inter-channel spacing of 50 µm. Further the diffusivity is constrained as the diffusive species being used was fluorescein with $D = 4 \times 10^{-10}\ m^2/s$. Also due to available hardware, the minimum stable flow rate which could be generated was ≈ 30 nL/min. The surface distance (standoff) would affect flow velocity (due to a change in flow area) and would further affect reliability of operation over an uneven sample (too low standoff would cause collisions between hMFP and the sample). Here, a standoff of 100 µm was chosen to have a low flow rate and allow samples with uneven surfaces to be possibly used. So, for a channel size of 100 µm and a surface standoff of 100 µm, this constrained the flow velocity to $v_0 \approx 30\ \mu m/s$. Considering these constraints, the required confinement length was ≈*1 mm* as determined by rearranging Eq. 3.

Supplementary Note 4: Separation efficiency in different experimental runs

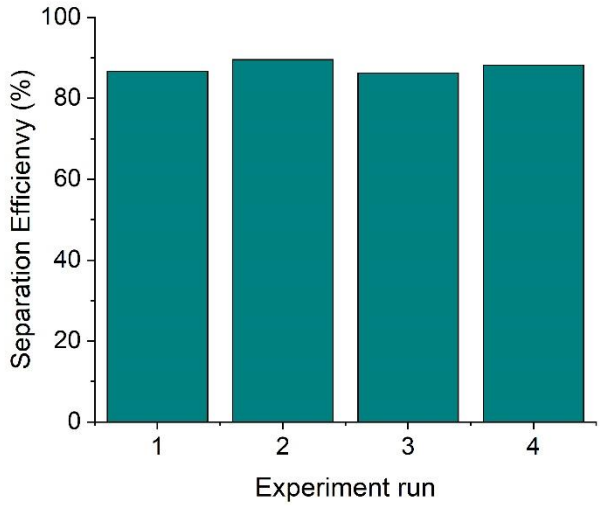

Figure S5*: Separation efficiency of the open space diffusive filter in different experimental runs.*

The same hMFP was used multiple times with a mixture of fluorescein and PS beads to assess reliability of the device over time. Separation efficiency was defined as the ratio of actual dye separated (from side aspiration streams) to the theoretical limit (66%). From these four cases, the separation efficiency was calculated to be $87.7 \pm 1.3\%$.

Supplementary Note 5: Diffusivity measurement in different experimental runs

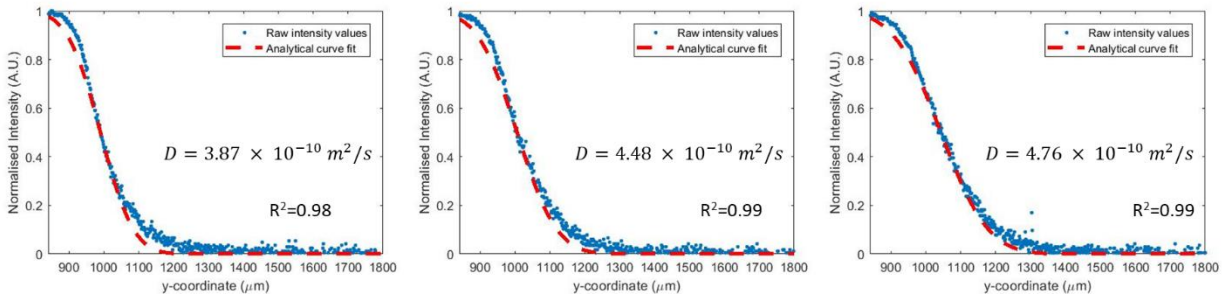

Figure S6*: Diffusivity measurement of fluorescein using the hMFP in different experimental runs*

The intensity of fluorescein was measured across the hMFP flow streams and fit to the theoretical model (**Equation S4**) to calculate diffusivities for different experimental runs.

1 H. Bruus, *Theoretical Microfluidics*, Oxford University Press, Cambridge, 2007.

2 P. Tabeling, *Introduction to microfluidics*, Oxford University Press, Cambridge, 2005.