

Supplementary Information

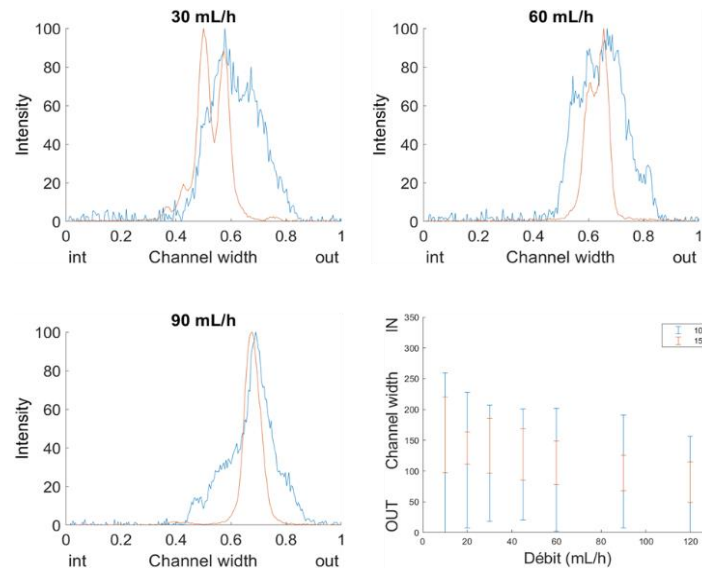
To evaluate the performance of the single focusing strategy, which consists of focusing a single population while allowing the smallest diameter population to circulate within the vortices, we designed a spiral channel (S0) in which the 15 μm beads have to focus into a single stream, while the 10 μm beads remain unfocused. To achieve this, the height and width of the channel were chosen to satisfy the confinement ratio $\frac{a}{D_h}$ (where a is the particle diameter, and D_h is the hydraulic diameter of the channel) and the lift-to-Dean force ratio R_f for the 15 μm population (Supplementary Table 1), while these conditions were not met for the 10 μm population ($\frac{a_p}{D_h} = 0.06 < 0.07$).

Spiral	10 μm beads		15 μm beads	
	a/D_h	R_f	a/D_h	R_f
S0	0.063	0.049	0.094	0.110

Supplementary Table 1: Confinement ratio and Lift-to-Dean ratio for the S0 spirals, calculated either below or above their respective focusing thresholds for 15 μm and 10 μm particles, respectively.

10 μm and 15 μm beads were injected in the channel and their fluorescence intensity have been measured in the channel outlet width. The distribution of fluorescent intensity for the two populations is shown in Supplementary Figure a) b) and c) at respectively 30, 60 and 90 mL/h. For all these flow rates, the fluorescence profile of the 10 μm beads in Device S0 appears larger compared to the sharp peaks of the 15 μm beads fluorescence. This indicates that the 10 μm beads are unfocused and can flow through the vortices, as predicted from the design criteria.

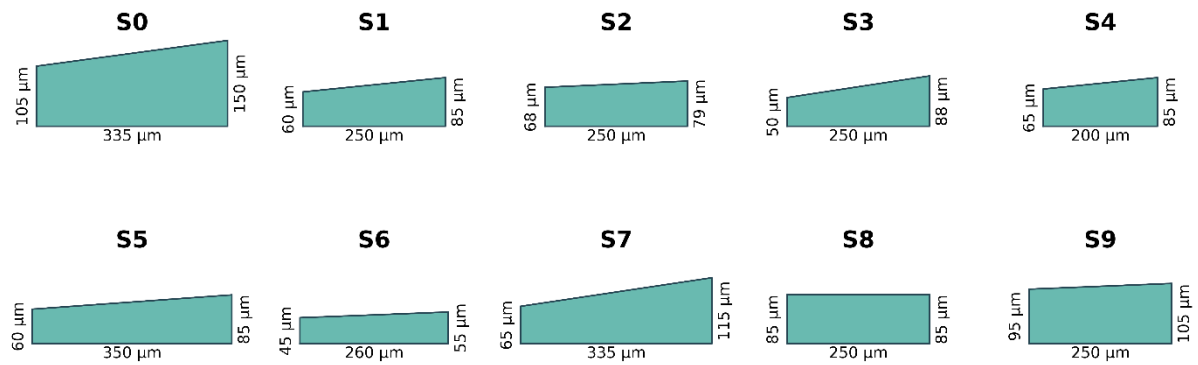
Moreover, the central positions of the two bead populations are very close to one another. On Supplementary Figure 1.d is represented the proportion of the channel width in which the fluorescence intensity of the two bead populations is greater than 20% of their maximum intensity, for different flow rates. At all tested flow rates, the peaks of the two populations significantly overlap, making separation impossible between 20 mL/h and 120mL/h.



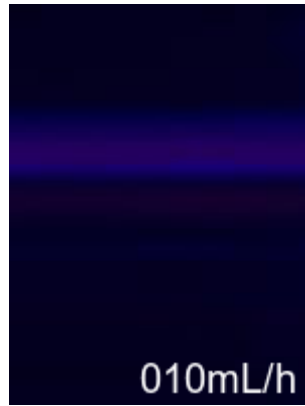
Supplementary Figure 1: Intensity repartition of 10 μm (blue) and 15 μm (orange) diameter beads throughout the channel width at the outlet of S0 device at a) 30, b) 60 and c) 90 mL/h. d) Proportion of the channel width in which the fluorescence intensity of the two bead populations is greater than 20% of their maximum intensity, for different flow rates.

a (μm)	Ratio	S0	S1	S2	S3	S4	S5	S6	S7	S8	S9
10	a/D_h	0.063	0.103	0.094	0.120	0.102	0.098	0.130	0.092	0.079	0.073
	R_f	0.049	0.221	0.164	0.346	0.212	0.186	0.443	0.155	0.098	0.077
15	a/D_h	0.094	0.155	0.140	0.180	0.153	0.146	0.196	0.138	0.118	0.109
	R_f	0.110	0.497	0.368	0.778	0.477	0.419	0.997	0.349	0.220	0.172

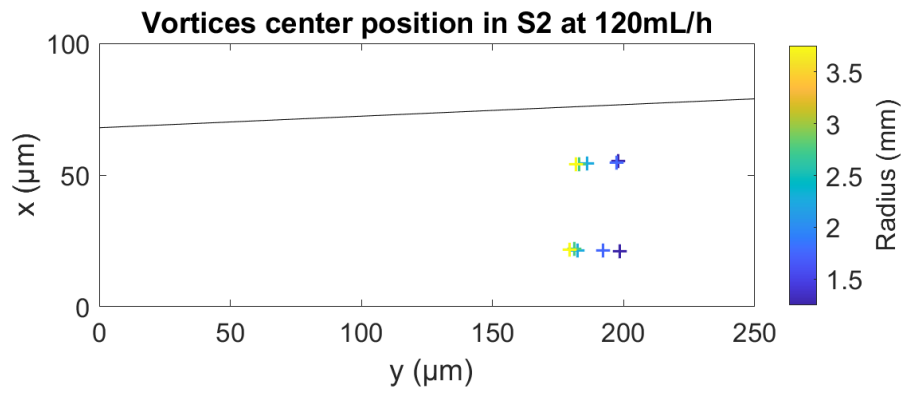
Supplementary Table 2: Confinement ratio a/D_h and ratio of the lift to Dean forces (R_f), calculated for tested spirals S0 to S1, for particles of 10 and 15 μm. a is the particle diameter, and D_h is the hydraulic diameter of the channel)



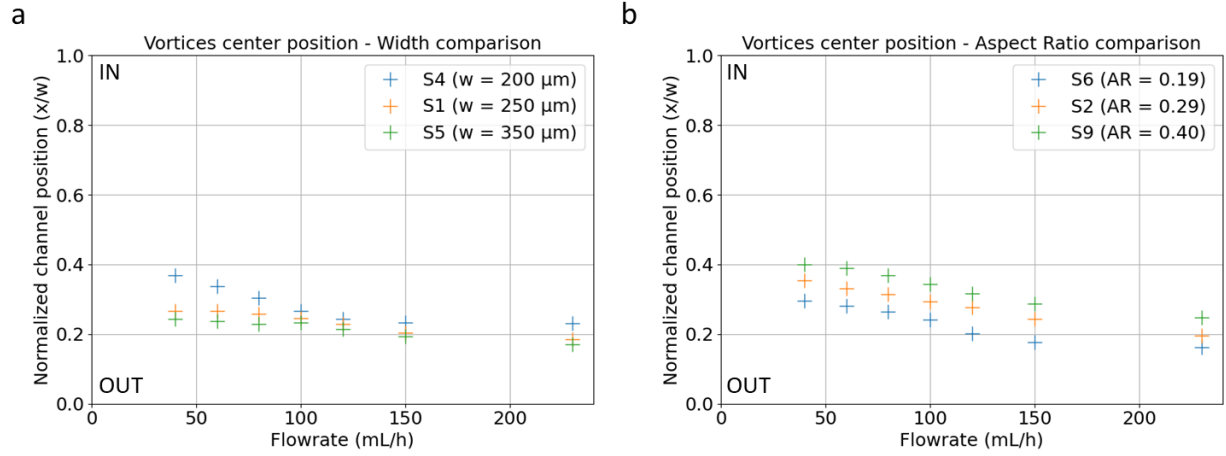
Supplementary Figure 2: Schematic representation of the spiral sections, presented at a uniform scale.



Supplementary Video 1: Compiled fluorescent images of 10 and 15 μm diameter beads (in blue and red respectively) at different flowrate showing the inner to outer shift of position of the particles as the flowrate increases. (See attached file)

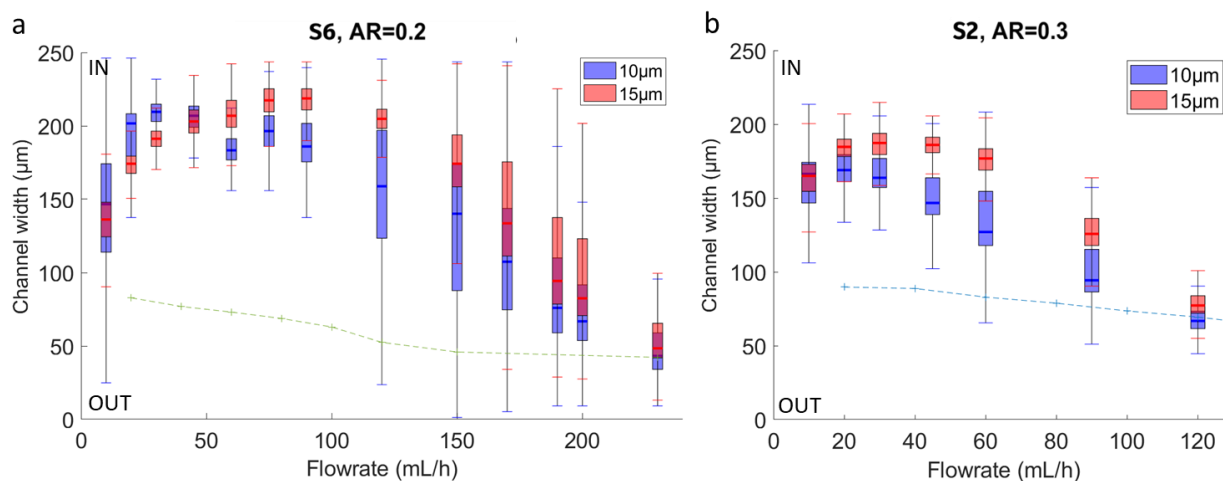


Supplementary Figure 3: Vortices center positions in S2 cross section at 120 mL/h at the outlet, depending on the radius of curvature. The lowest radius (1.25 mm, blue) corresponds to the most internal spire; and the bigger one (3.75 mm, yellow) corresponds to the last one, at the outlet. x and y axis represent respectively the width and height of the channel section.

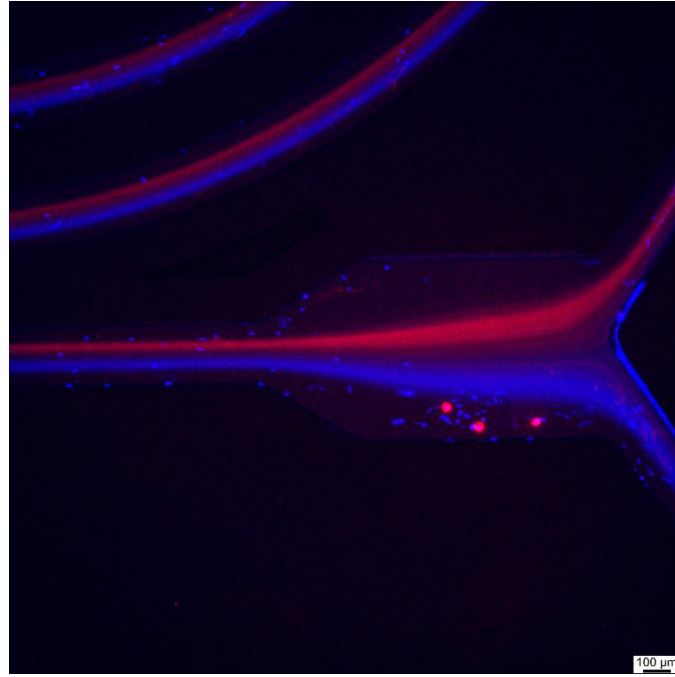


Supplementary Figure 4: Lateral position of vortex centers across the normalized channel width for varying flow rates. Effect of parameters of interest on vortex center location: a) channel width (w) in S4, S1 and S5. b) aspect ratio (AR) S6, S2 and S9.

The aspect ratio plays a crucial role in particle size-based positioning. The device S6 has the same width and slope as S2 but differs in height, resulting in a lower aspect ratio. This difference significantly affects the bead positions as shown in Supplementary Figure 5. In S6, the shift toward the outer position occurs at much higher flow rates than in lower-aspect-ratio channels. Moreover, in spiral S6, although both particle populations migrate closer to the inner wall, their streams remain too close to each other for effective size-based separation. At the flow rates of interest (around 50 mL/h), the larger aspect ratio of S6 appears to promote better separation.



Supplementary Figure 5: Fluorescence intensity position of 10 μm and 15 μm beads in the channel width at the spiral outlet in a) S6 and b) S2 (with respective aspect ratio (AR) of 0.2 and 0.3).



Supplementary Figure 6: Superimposed fluorescence image of WBC in blue (10^6 cell/mL, stained with Hoechst) and MCF7 in red (10^3 cell/mL, stained with red CellTracker, Invitrogen) flowing through the outlet of the channel.