Supplementary Information

Supplementary Note 1. Theoretical estimation of the sort purity and yield.

Supplementary Figure 1 shows our model for theoretically estimating the sort purity and yield. In this model, we assume that five parameters shown in the figure are important to estimate the result of sorting a particle of interest: the particle of interest, two neighboring particles (the particle right before the particle of interest and the particle right after the particle of interest), and two intervals between the successive particles. Each particle is classified as a target or non-target particle, while each interval is classified as an interval that is either shorter or longer than half of the sort window. In total, the size of the sample space is $2^5 = 32$. The probability of each parameter is given as follows: the probability that the particle of interest is a target particle is given by the target-particle concentration, $r = N_{\text{target}} / (N_{\text{target}} + N_{\text{non-target}})$, where N_{target} and $N_{\text{non-target}}$ are the number of target particles and the number of non-target particles in a sample, respectively; the probability that the particle of interest is a non-target particle is given by the non-target-particle concentration, $1 - r$; the probability that the interval of the successive particles is shorter than half of the sort window is given by Poisson-statistics-based equation $P_p(T < \tau) = 1 - e^{-\lambda\tau}$, where λ and τ are the throughput and half of the sort window, respectively; the probability that the interval of the successive particles is longer than half of the sort window is given by $1 - P_p(T < \tau)$. The product of the probabilities of all the five parameters gives the probability of each scenario.

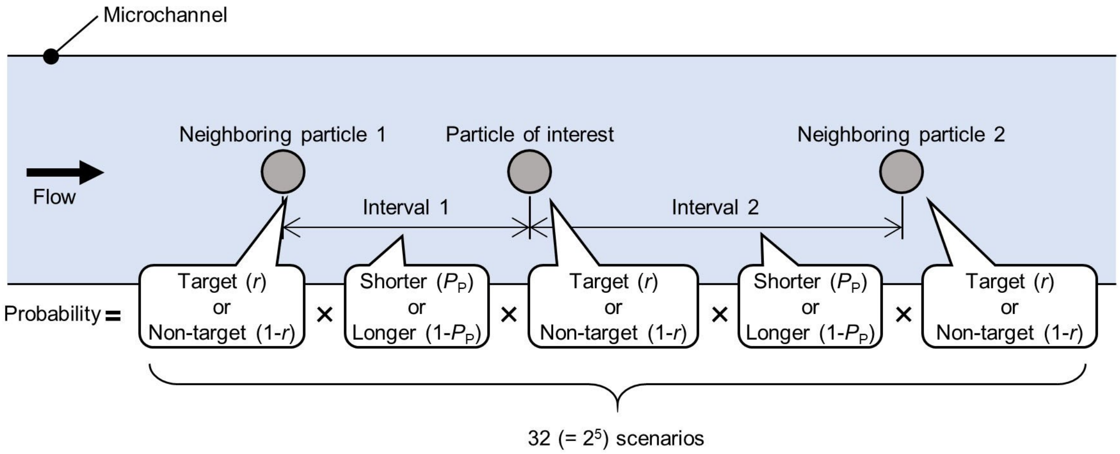
Supplementary Table 1 summarizes the sample space composed of the above probabilities and the result of sorting the particle of interest in each scenario. The sort results are classified into four categories: true positive (TP), false positive (FP), true negative (TN), and false negative (FN). The sort results are rationalized based on the working principles of the dual-membrane push-pull cell sorter. For example, scenario no. 9 in the table is described as follows. When the particle preceding the particle of interest arrives at the sort point, the push-pull cell sorter is activated because it is recognized as a target particle. This sort action does not affect the particle of interest because the interval between these two particles is longer than half of the sort window. When the particle of interest arrives at the sort point, the push-pull cell sorter is activated again to push the particle to the upper/lower side because it is recognized as a target particle. Simultaneously, the particle following the particle of interest is also pushed in the same direction because the interval between these particles is shorter than half of the sort window. After a time delay, which is shorter than half of the sort window in this scenario, the particle following the particle of interest arrives at the sort point. At this timing, the push-pull cell sorter is activated to push the particle to the lower/upper side, which is in the opposite direction to the previous sort action, resulting in these two particles back to the initial position (center of the channel) followed by flowing into the central waste channel, resulting in a FN event.

Based on Supplementary Table 1, we calculated the sort purity and yield using the definitions

$$Purity(\lambda, \tau, r) = \frac{\Sigma P_{TP}}{\Sigma P_{TP} + \Sigma P_{FP}},$$

$$Yield(\lambda, \tau, r) = \frac{\Sigma P_{TP}}{\Sigma P_{TP} + \Sigma P_{FN}},$$

where ΣP_{TP}, ΣP_{FP}, ΣP_{TN}, and ΣP_{FN} are given by the sum of the probabilities of the scenarios that result in the TP, FP, TN, and FN events, respectively. As shown in these equations, the sort purity and yield vary, depending on the throughput, sort window, and target-particle concentration. Note that we assumed that the sort timing was predictable.



Supplementary Figure 1. Model for theoretically estimating the sort purity and yield.

Supplementary Table 1. Sample space and expected sort results. TP, true positive; FP, false positive; TN, true negative; FN, false negative; P_p, P_p ($T < \tau$).

Scenario	Neighboring particle 1	Interval 1	Particle of interest	Interval 2	Neighboring particle 2	Sort result
1	r	$1-P_\mathrm{p}$	r	$1-P_\mathrm{p}$	r	TP
2	r	$1-P_\mathrm{p}$	r	$1-P_\mathrm{p}$	$1-r$	TP
3	r	$1-P_\mathrm{p}$	r	P_p	r	FN
4	r	$1-P_\mathrm{p}$	r	P_p	$1-r$	TP
5	r	$1-P_\mathrm{p}$	$1-r$	$1-P_\mathrm{p}$	r	TN
6	r	$1-P_\mathrm{p}$	$1-r$	$1-P_\mathrm{p}$	$1-r$	TN
7	r	$1-P_\mathrm{p}$	$1-r$	P_p	r	FP
8	r	$1-P_\mathrm{p}$	$1-r$	P_p	$1-r$	TN
9	r	P_p	r	$1-P_\mathrm{p}$	r	FN
10	r	P_p	r	$1-P_\mathrm{p}$	$1-r$	FN
11	r	P_p	r	P_p	r	TP
12	r	P_p	r	P_p	$1-r$	FN
13	r	P_p	$1-r$	$1-P_\mathrm{p}$	r	FP
14	r	P_p	$1-r$	$1-P_\mathrm{p}$	$1-r$	FP
15	r	P_p	$1-r$	P_p	r	TN
16	r	P_p	$1-r$	P_p	$1-r$	FP
17	$1-r$	$1-P_\mathrm{p}$	r	$1-P_\mathrm{p}$	r	TP
18	$1-r$	$1-P_\mathrm{p}$	r	$1-P_\mathrm{p}$	$1-r$	TP
19	$1-r$	$1-P_\mathrm{p}$	r	P_p	r	FN
20	$1-r$	$1-P_\mathrm{p}$	r	P_p	$1-r$	TP
21	$1-r$	$1-P_\mathrm{p}$	$1-r$	$1-P_\mathrm{p}$	r	TN
22	$1-r$	$1-P_\mathrm{p}$	$1-r$	$1-P_\mathrm{p}$	$1-r$	TN
23	$1-r$	$1-P_\mathrm{p}$	$1-r$	P_p	r	FP
24	$1-r$	$1-P_\mathrm{p}$	$1-r$	P_p	$1-r$	TN
25	$1-r$	P_p	r	$1-P_\mathrm{p}$	r	TP
26	$1-r$	P_p	r	$1-P_\mathrm{p}$	$1-r$	TP
27	$1-r$	P_p	r	P_p	r	FN
28	$1-r$	P_p	r	P_p	$1-r$	TP
29	$1-r$	P_p	$1-r$	$1-P_\mathrm{p}$	r	TN
30	$1-r$	P_p	$1-r$	$1-P_\mathrm{p}$	$1-r$	TN
31	$1-r$	P_p	$1-r$	P_p	r	FP
32	$1-r$	P_p	$1-r$	P_p	$1-r$	TN

Supplementary Table 2. Gene and primer sequences.

1POK synthetic gene	atgatcgattatacagctgccggcttcacgttgctgcaaggcgcacacttgtacgctcccgaagaccgggaatttgcgacgtgttggtcgcaaacggaaagataatagcagttgcttctaatataccatctgatatagtccccaactgcactgttgtagatctttcagggcagattctttgcccgggattcatcgatcaacacgtccatctcatcgggggcggaggggaagcgggaccgaccacccgaacaccagaggtggcattgagcaggctcaccgaagctggtgtcacatccgtggtgggtcttctcggtactgatagtatcagcagacatccggagagtcttctggccaagacccgggcgttgaacgaggaaggtatctcagcatggatgttgacaggtgcctaccacgttcccagccggacaattacgggatcagttgaaaaggatgtcgccatcatagacagggttataggtgttaaatgcgcgataagcgaccaccggtcagcagcacctgacgtgtaccatctggctaacatggccgctgagagtcgagtaggagggcttcttggtgggaaacctggcgtgactgtttttcacatgggcgactcaaagaaagcccttcaaccaatctacgatttgttggaaaattgtgatgtgccaatatcaaaacttctgccgactcatgtaaatcgcaacgttccccttttctaccaagcccttgagtttgcccgcaaagggggtacgattgacattactagttcaatcgatgagccggtagcccctgccgaagggattgcgagggcagttcaggctggcatacccctggcccgggtgacactcagtagtgacggaaacggttctcaaccgttcttcgatgatgaggggaaccttactcacataggagtcgccggatttgaaactcttttggagaccgtacaagtcctggtcaaagattacgacttctccatttccgacgcgctccgacctttgacaagtagtgttgccgggtttcttaatttgacagggaagggtgagatcctgcctggtaacgatgctgacttgctcgtgatgactcctgaactgcgcatcgaacaagtgtacgcgagggggaaacttatggttaaggatggtaaagcgtgtgtaaaaggcaccttcgagacggcc
1POK forward	5'- GACTGGCTAGCGCCACCATGATCGATTATACAGCTGCCGG-3'
1POK reverse	5'-CAGAACCGCCTCCGCCGGCCGTCTCGAAGGTGC-3'
EGFP forward	5'-GGCGGAGGCGGTTCTGGAGGCGGTGGGAGCATGGTGAGCAAGGGCGAG-3'
EGFP reverse	5'-CTAGACTCGAGTTACTTGTACAGCTCGTCCAT-3'