Supplementary Information (SI) for Lab on a Chip.
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Supplementary Information for

Parallel DLD Microfluidics for Chloroplast Isolation and Sorting

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1. Critical Diameter Estimation

The system operates based on a critical particle diameter (CD). Particles smaller than this diameter flow through the system without being displaced, while particles larger than this diameter are laterally displaced by cylindrical posts arranged in a specific geometry (Fig. 2d in the main manuscript). The critical diameter (CD) is calculated as [1]:

$$CD = 1.4G\varepsilon^{0.48}$$

Where G is the separation between the posts or the gap, and ε can be calculated as 1/N, where N is the number of posts within a period and can be calculated as:

$$N = \frac{\lambda}{|\Delta\lambda|}$$

Where λ is the array pitch, which can be derived from the sum of the pillar diameter plus the gap, and Δλ is the row pitch.

Table S1. Summary of calculated diameters for polystyrene microbeads used in characterization experiments. The table reports the nominal diameter, equivalent circular diameter obtained from micrograph measurements, as well as the corresponding standard deviation.

Nominal size (µm)	**Mean equivalent diameter (µm)**	**Standard deviation (µm)**
2	2.016	0.309
3	2.998	0.381
4	4.157	0.319
5	5.021	0.282

Table S2. Percentage of chloroplast recovery at the device outlets, expressed in terms of concentration (chloroplasts/µL). The initial sample was diluted to determine the chloroplast concentration prior to separation.

Sample	**Input (Chloroplasts /µL)**	**Total recovered (Chloroplasts /µL)**	**Recovery (mean ± SD)**
Chloroplasts (DLD)	83000	13650- 15600	17.9 % ± 1.3

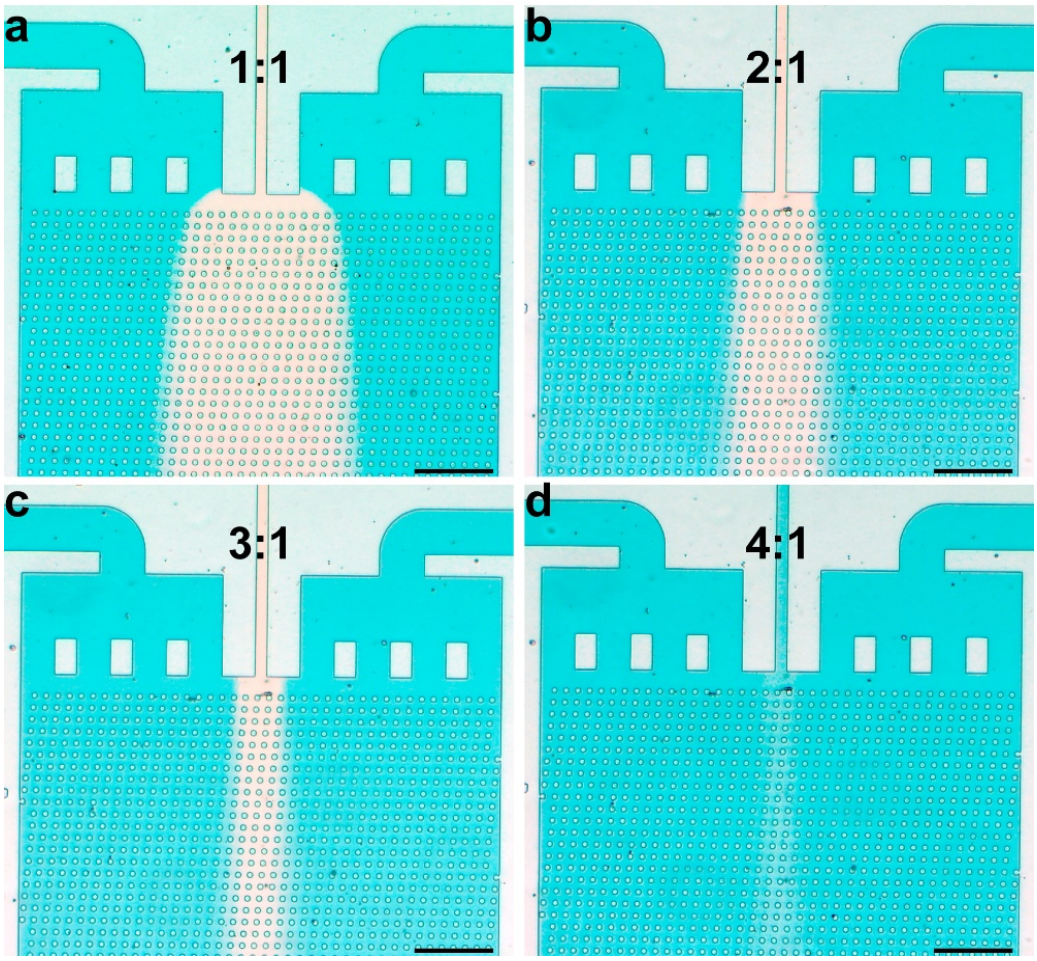

Figure S1. Micrographs of the DLD device inlet showing sample focusing with dyes at different sheath-to-sample flow rate ratios: (a) 1:1, (b) 2:1, (c) 3:1 and (d) 4:1. Scale bar: 300 μm.

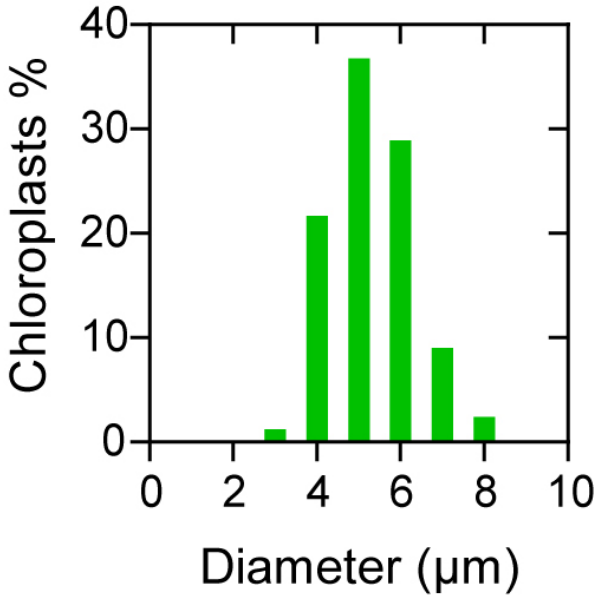

Figure S2. Size distribution of chloroplasts in the initial sample prior to injection into the microfluidic device.

Movie S1. Real-time video of chloroplast size-based isolation. The video begins by showing the entry of autofluorescent chloroplasts at the inlet of the microfluidic device. Subsequently, the final section of the device is shown, where the chloroplasts are separated based on their size.

References

1 J. McGrath, M. Jimenez and H. Bridle, *Lab Chip*, 2014, 14, 4139–4158.