Supplementary Material (ESI) for Lab on a Chip

Eletronic Supplementary Information for B823541D:

Fluidic conduits for highly efficient purification of target species in EWOD-driven droplet microfluidics

1. Sample purification by serial dilution

In many biochemical assays, the purity of the target species ("TS") (e.g. specific cells, proteins, DNA, either by themselves or bound to beads) at certain steps is vital for their effectiveness. Concentration/separation of the TS from the non-target species ("nonTS") becomes critical in such assays. Unlike continuous microfluidics, where the TS is immobilized while wash-buffer is flowed through the channels to remove impurities (i.e. nonTS)[1], purification in droplet microfluidics (e.g. by EWOD) typically involves serial (i.e., repeated steps of) dilution of the nonTS[2,3], schematicaly described in Fig. S1. In each wash step, a buffer droplet is added to the sample (Fig. S1(1a)). The TS is actively collected in one region of this combined ("parent") droplet using one or more differentiating properties, such as magnetic, electric, optical or dimensional[4,5] (Fig. S1(1b)). The droplet is then split so that most of the TS are collected in one of the daughter droplets – "collected" droplet (left) in which TS is concentrated and "depleted" droplet (right) from which TS have been depleted (Fig. S1(1c-1d)).

To purify and concentrate TS, not only TS should be concentrated but also nonTS should be depleted from the collected droplet. The distribution of the nonTS between the two daughter droplets, on the other hand, is not actively controllable. It is governed by their initial distribution in the parent droplet and non-specific phenomena such as diffusion and fluidic movement that occur during the purification step. If the nonTS were uniformly distributed in the parent droplet, they would be distributed between the daughter droplets roughly in proportion to their volumes. Thus, even though many nonTS from the parent droplet will be removed in the form of the depleted droplet, the process would still leave a significant proportion of nonTS in the collected droplet. In order to improve the TS purity, therefore, collecting the TS across the incoming buffer droplet has been shown to be helpful[6], which is initially free from the nonTS (Fig. S1(1a-1b)). The droplet is then stretched (Fig. S1(1c)) and split (Fig. S1(1d)) into the collected and depleted droplets.

Even though the original distribution of nonTS in the parent droplet is made favorable for high purity by quickly pulling TS towards the buffer side of the droplet (Fig. S1(1b)), the non-specific transport and redistribution of the nonTS during the subsequent steps (Fig. S1(1b-1c)) leave a considerable amount of nonTS in the collected droplet (Fig. S1(1d)), requiring multiple wash cycles in series (Fig. S1(2a-3a)).

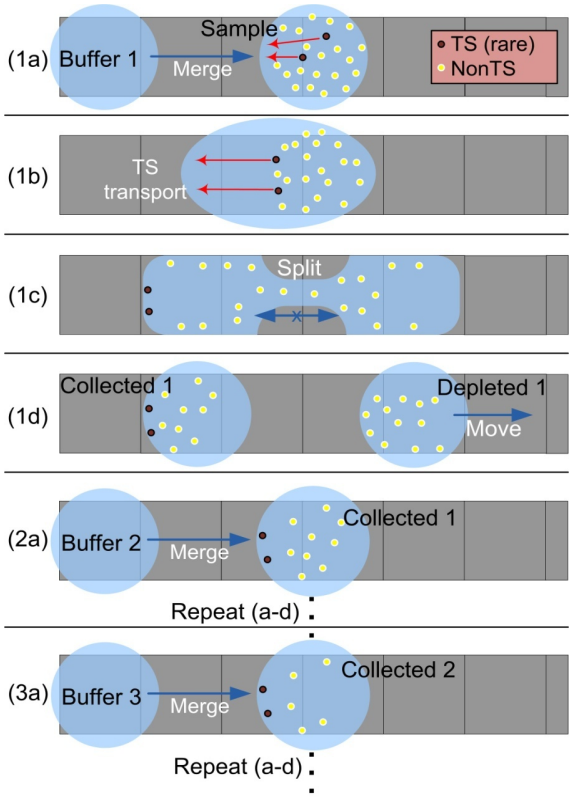

Fig. S1: Known technique of serial dilution to purify target species (TS) under a droplet microfluidic platform: (1a) Wash buffer is added to sample. (1b) TS (dark) is transported and collected to the buffer side (left) of the droplet. (1c) The droplet is then stretched and (1d) cut to form the collected (wherein TS are collected) and depleted (depleted of TS) droplets. Many nonTS (bright) are removed with the depleted droplet, but some nonTS enter collected droplet due to diffusion, and/or fluidics-driven transport. (2a, 3a) More wash-buffer is added and the above steps are repeated to improve TS purity by serial dilution of the nonTS.

2. Fabrication and experimental methods

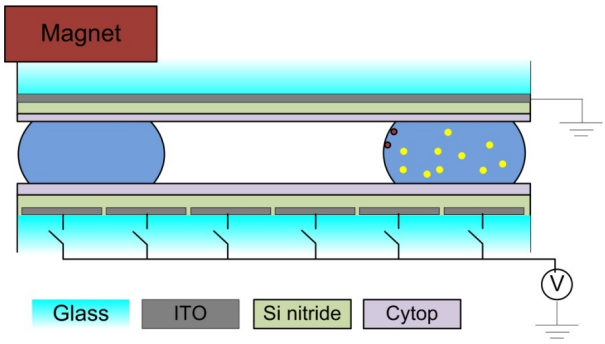

Fig. S2: Schematic cross-section of EWOD device used.

The proposed idea of the fluidic conduit was demonstrated for high purity collection of magnetic beads (MBs) from a mixed population of MBs and non-magnetic beads (nonMBs) on an EWOD device. MBs of 4.5 µm diameter (Invitrogen) were used as the TS, and fluorescent nonMBs of 5.2 µm diameter (Molecular Probes) were used as the nonTS. Pluronic F68 surfactant of 0.15% w/v (Sigma-Aldrich) in PBS was used for both the sample and the buffer droplets in all the experiments.

A standard two-plate EWOD device[7] was used for the experiments, schematically suggested in Fig. S2. For the bottom plate, EWOD electrodes were patterned into the 140 nm-thick indium-tin-oxide (ITO) layer on glass substrate. A 60 µm wide line going through the middle of the 1 mm x 1 mm EWOD electrodes defines the SE. A gap (1-1.5 mm) was left at the left end of the SE to ensure the breakage of the conduit during droplet splitting. Silicon nitride (1.1 µm thick) was deposited by plasma-enhanced chemical vapor deposition (PECVD) to form the dielectric layer, and Cytop® (~1 µm thick) was spun-coated to form the top hydrophobic layer. For the top plate, an unpatterned layer of ITO was coated with 100 nm silicon nitride and 50 nm Cytop®. Double-sided tape (~100 µm thick) was used as the spacer between the plates.

Droplet actuation was achieved by sequential application of voltage (70-80 V_{ac} @1 kHz) to the EWOD electrodes. Electronic control for the actuation sequence was controlled using LabVIEW (National Instruments) via a digital I/O device (DAQPad 6507, National Instruments). For magnetic collection, an NdFeB permanent magnet (½" dia x ½" thick) was placed on top of the device to the left. Droplet actuation movies were captured by a video camera (Panasonic KR-222) mounted onto an inverted fluorescence microscope (Nikon TE 2000U). To increase the field of view, a 0.45x demagnification optics was attached to the video camera. Better quality fluorescence images were taken using a cooled-CCD camera (Photometrics Coolsnap EZ) also mounted on the microscope, but without any demagnification optics.

3. Supplementary experimental results:

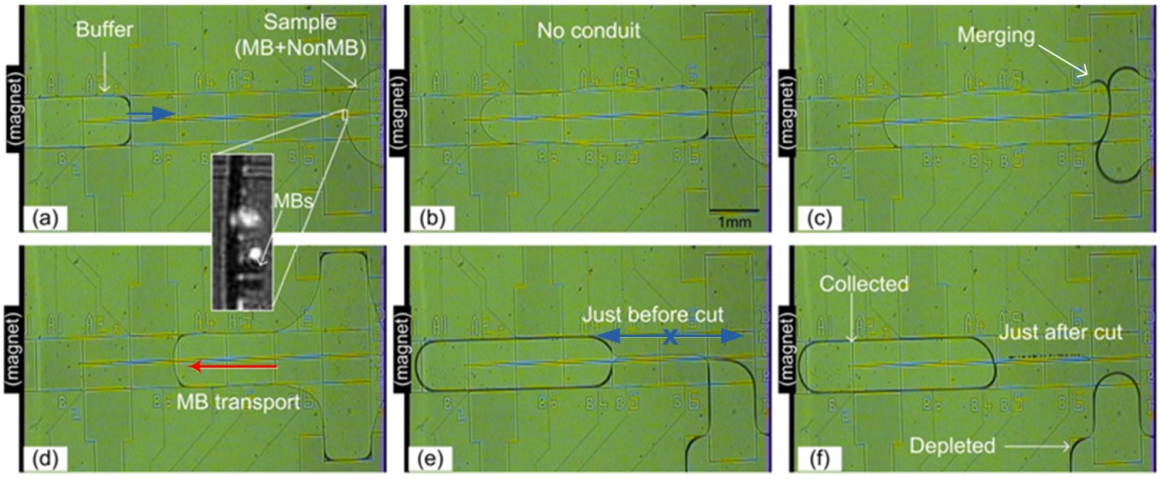

Fig. S3: Image sequence for magnetic separation without the use of droplet-conduit structures. All droplets contain 0.15% pluronic F68 in PBS. Magnet is placed at the left. (a-b) Buffer droplet is introduced from the left, towards the sample (right) of MBs and fluorescent nonMBs. The MBs (dark) are collected at the left edge (a-inset). (c) The droplets are merged, (d) allowing the MBs to be actively transported to the left edge of the combined ("parent") droplet. After collecting the MBs,(e) there is significant fluidic movement as the neck is formed and the droplet is stretched so as to (f) split it into the collected (left) containing the collected MBs, and depleted (right) droplets, depleted of the MBs.

More supplementary figures are provided, in addition to those in the main paper, for a clearer comparison between the magnetic separation without and with the fluidic conduits. Experiments were performed without and with the conduit to evaluate high purity separation of TS (i.e., MBs) from fluorescent nonTS (i.e., nonMB) using the slender fluidic conduit. Fluorescent beads were chosen instead of fluorescent or colored dyes due to the ease of quantification without the need for high-sensitivty detection systems, particularly since the nonTS concentration in the collected droplet can be quite low. In order to demonstrate the utility of fluidic conduit for purification of rare species, a low (~1:20) MB:nonMB ratio was chosen.

Fig. S3 shows the image sequence for the case without using the fluidic conduit. The buffer droplet is introduced from the left towards the sample droplet containing MBs and nonMBs, with the magnet placed at the left (Fig. S3(a-b)). On merging (Fig. S3(c)), the MBs are transported actively towards the magnet (wait time after merging was 10-20 s), to the leftmost edge of the combined parent droplet (Fig. S3(d)), while nonMBs may be passively redistributed by diffusion and fluidic movement. For the present device geometry and particle size, it turns out that the fluidically driven transport is particularly dominant, so that during the subsequent fluidic actuation for neck creation and pinch-off (Fig. S3(e)), many nonMBs enter the collected region (left). Upon splitting (Fig. S3(f)), therefore, while the collected droplet (left) has all the MBs, it also contains a significant number of nonMBs, undermining the purity (see Fig. S4).

Fig. 2 (in the main text), on the other hand, shows the sequence of images for magnetic separation using a fluidic conduit. As described in the main text, the slender conduit was forrmed using the stabilizing electrode (SE), following which the conduit-forming droplet was merged with the sample. After the MB transport, the conduit was split by stretching the droplet with the SE off.

Figs S4 (no conduit case) and S5 (with conduit case) show fluorescence images (along with some bright-field illumination) of the full collected (Figs. S4 (a), S5 (a)) and depleted (Figs. S4(c), S5(c)) droplets for both cases. Corresponding better quality images of representative portions of droplets for each case (Figs. S4(b,d), S5(b,d)) as used in the main text are also included in the figures.

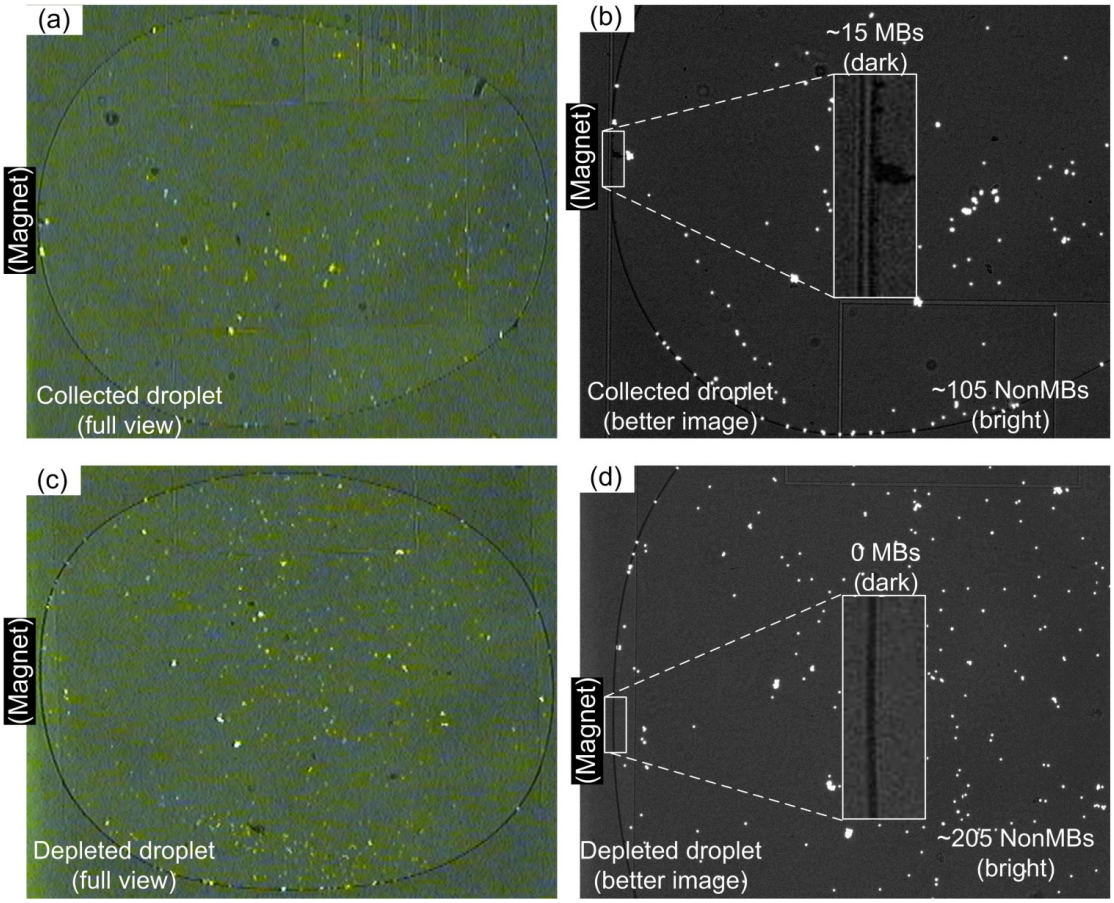

Fig. S4: High purity collection of MBs is not achieved in the case without conduit structure. Images were taken under fluorescence excitation but with some bright-field illumination to identify non-fluorescent features as well. The fluorescence nonMBs (bright) are easily counted. To distinguish the MBs (dark) from other dark features, a magnet was introduced and the magnetically responsive particles (i.e. MBs) were counted. (a,b) The collected droplet contains all MBs and some fluorescent nonMBs (~105). (c,d) The depleted droplet contains no MBs (0), and a majority of nonMBs (~205). Therefore, even though high collection efficiency is obtained, the purity is poor due to nonMB contamination into the collected droplet. Images (a and c) show the full droplets, while (b and d) are corresponding better quality images.

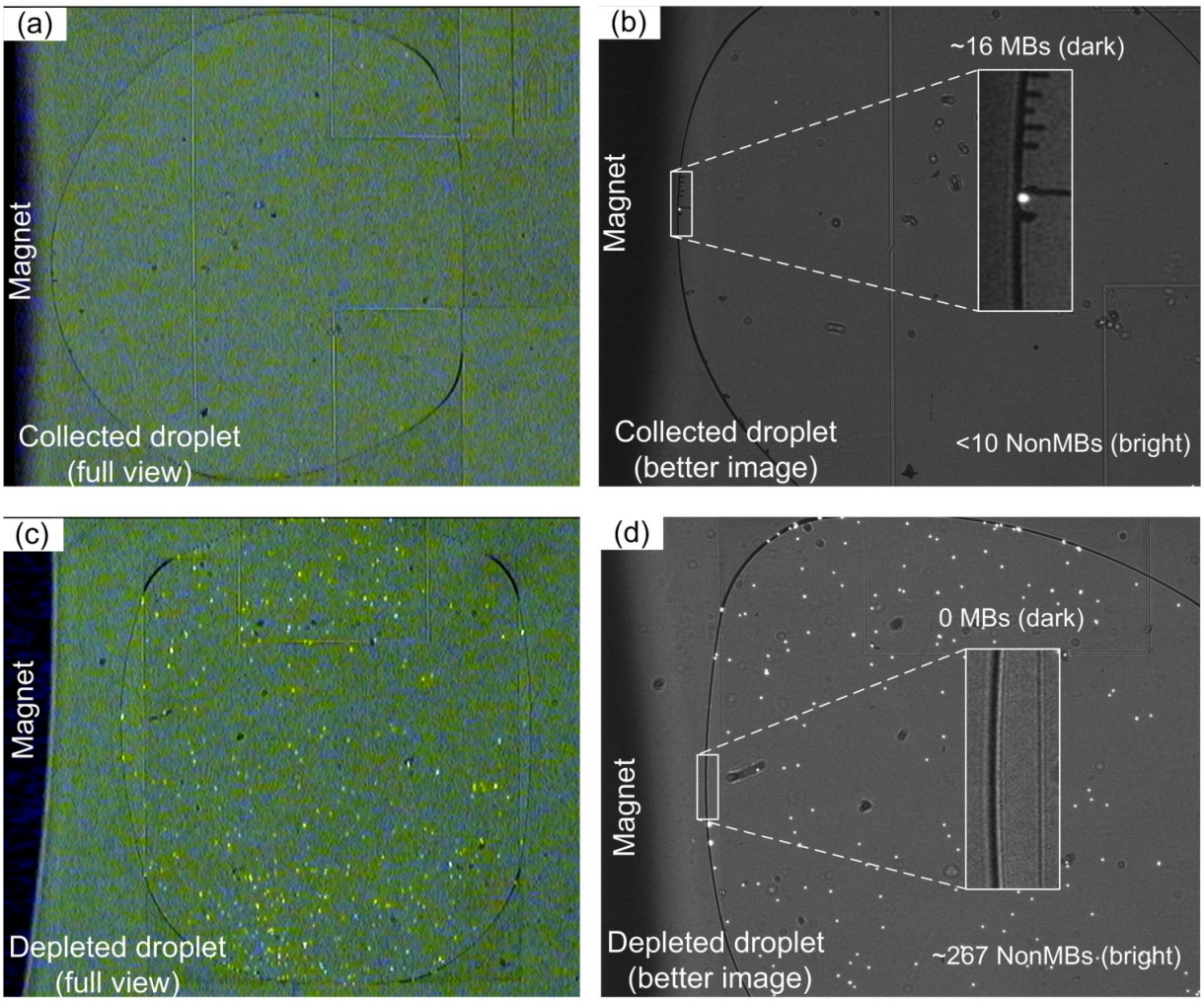

Fig. S5: High-purity separation is accomplished using droplet-conduit structures, as quantified by counting the MBs (dark) and nonMBs (bright) in the collected and depleted droplets. Images were taken under fluorescent excitation but with some bright-field illumination so as to visualize non-fluorescent features as well. To count MBs (and not dirt etc.), a magnet was introduced right next to a droplet edge, and the magnetically responsive MBs attracted to it were counted. (a,b) All the MBs (~16) and very few nonMBs (<10) were counted in the "collected droplet". (c,d) No MBs (0) and a majority of nonMBs (~267) were seen in the "depleted droplet". The results correspond to target (MB) collection efficiency of over 99% in the collected droplet, with nonMB concentration dropping to ~3% (or by more than 28 times) in just one step. Images (a and c) show the full droplets, while (b and d) are corresponding better quality images.

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References for ESI

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