Electronic Supplementary Material (ESI) for Lab on a Chip
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Supplementary Information

Measurement of magnetic beads redispersion in droplets

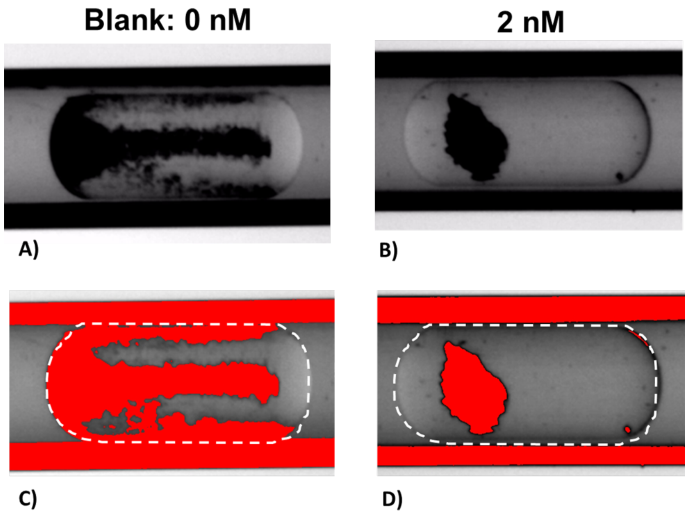

Figure S1. Droplet micrographs before (A, B) and after (C, D) image treatment using ImageJ software.

On the optical images, the surface occupied by the magnetic beads (MBs) in the droplet was used to quantify MBs redispersion. When observed in the transmission mode, MBs in the droplet create a contrast (dark pixels) that was analyzed using ImageJ software. The image analysis workflow is the following: i) grey level pictures from blank (A) and assay (B) are acquired, ii) droplets edges are manually selected (white dashes on figure S1 C and D) iii) an automatic thresholding procedure is used to measure the areas occupied by the MBs within the droplet (the contrast induced by the particles are used to define a grey level threshold that is visualized by red pixels on figure S1 C and D). iv) Finally, *the* MBs redispersion efficiency (surface of the red pixels/ surface of the droplet) was calculated by comparing the surface occupied by the MBs relative to the droplet surface.

S_{assay} and S_{blank} represent MBs redispersion with and without analyte respectively. The specific signal S was defined as: $S=1-(S_{assay}/S_{blank})$.

Influence of the surfactant content (percent) on the agglutination efficiency

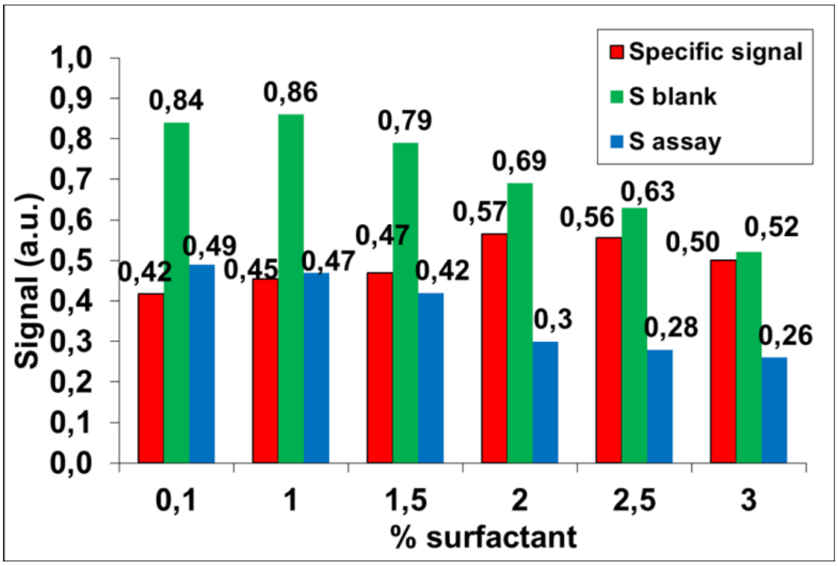

Figure S2. Influence of the surfactant percentage on S_{blank}, S_{assay} and the specific signal S, MBs and analyte concentrations were fixed at 2 mg/mL and 1000 pM, respectively. ***B****: 20 mT; flow rate: 0.4 µL/min.*

Figure S2 shows that the surfactant percentage has a significant influence on the specific signal. Increasing surfactant percentage leads to a decrease of S_{blank} and S_{assay}. Indeed, when using a lower quantity of surfactant, the internal recirculation flows velocities were increased thus favoring S_{blank} but the resulting shear forces also favored the redispersion of beads aggregated in the presence of analyte, so S_{assay} increased too. Overall, the specific signal decreased. For higher surfactant percentages, specific aggregates were maintained while S_{blank} was slightly modified, thus increasing the specific signal. The optimum value was obtained for 2 %. Physically, the effect of surfactant concentration on flow recirculations can be interpreted as follows: an increase of surfactant percentage lead to a decrease of surface tension, and thus an increase in the thickness of the oil film between the droplet and the wall. The shear flow in the film increases, decreasing the difference of velocity between the droplet center of mass and its wall. As a consequence, recirculation flows inside the droplet are reduced.[1] The decrease of the specific signal for high surfactant concentrations may also be explained through Marangoni effects.[2] Interfacial shear associated with the recirculation flows inside and outside of the droplet may induce a non-uniform distribution of the surfactant that rigidifies the droplet interface and modifies internal recirculation flows.

Magnetic beads occupancy in a function of time after magnetic confinement

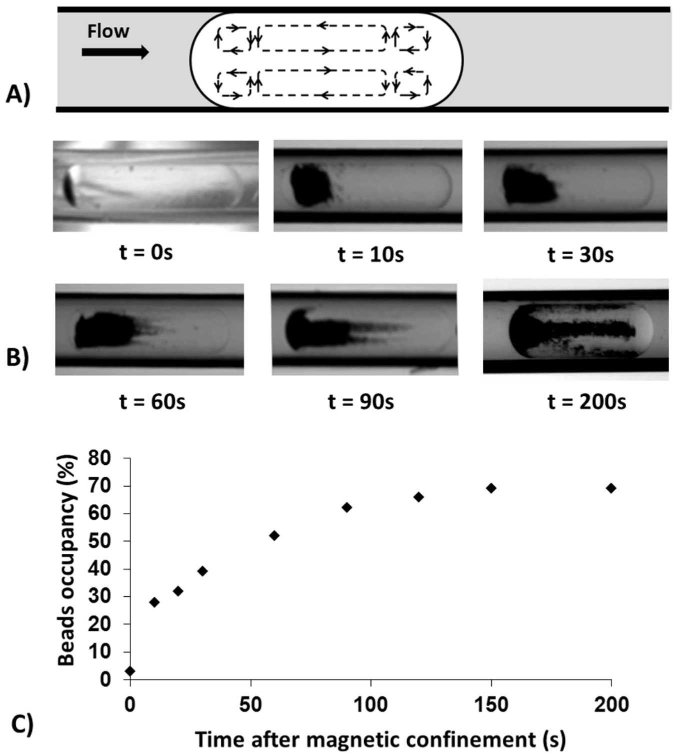

Figure S3. Schematic representation of the recirculation flows in a moving droplet. Two internal flows in the center of the droplet are surrounded by two co-rotating flows at the front and rear cap of the droplet (A). Micrographs of magnetic beads occupancy in the droplet as a *function of time after magnetic confinement (B). Curve representing variations of magnetic beads occupancy as a function of time after magnetic confinement (C). MBs concentration was fixed at 2 mg/mL.* ***B****: 20 mT; flow rate: 0.4 µL/min.*

As expected, the images of magnetic beads recirculation in the droplet underline that the beads are mainly redispersed by the main internal flows in the center of the droplet causing a particle spreading in the center part of the droplet and recirculation on the edges, close to the tubing. After 200s, the surface occupied by the particles reaches 70% of the surface of the droplet.

Influence of magnetic flux density on agglutination

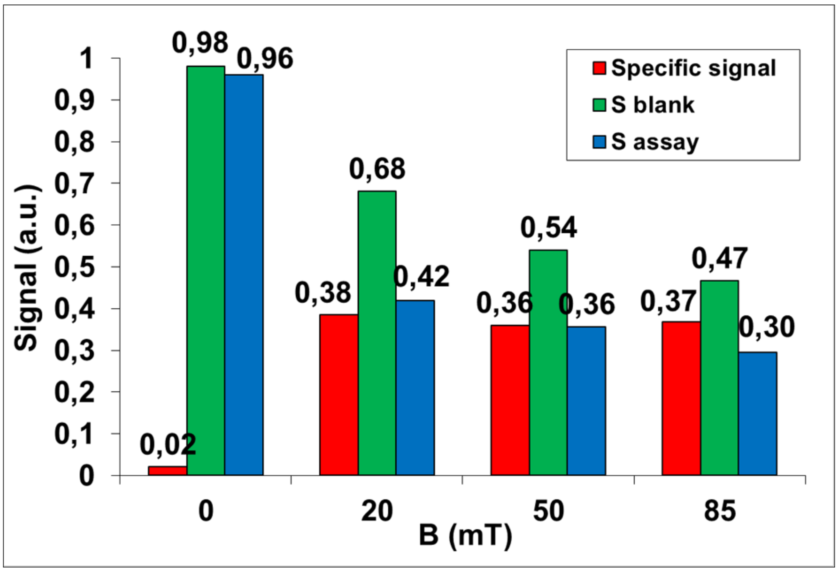

Figure S4. Influence of magnetic flux density on the specific signal, S_{blank} and S_{assay}. MB and analyte concentrations were set to 2 mg/mL and 650 pM, respectively.

Influence of flow rate on agglutination

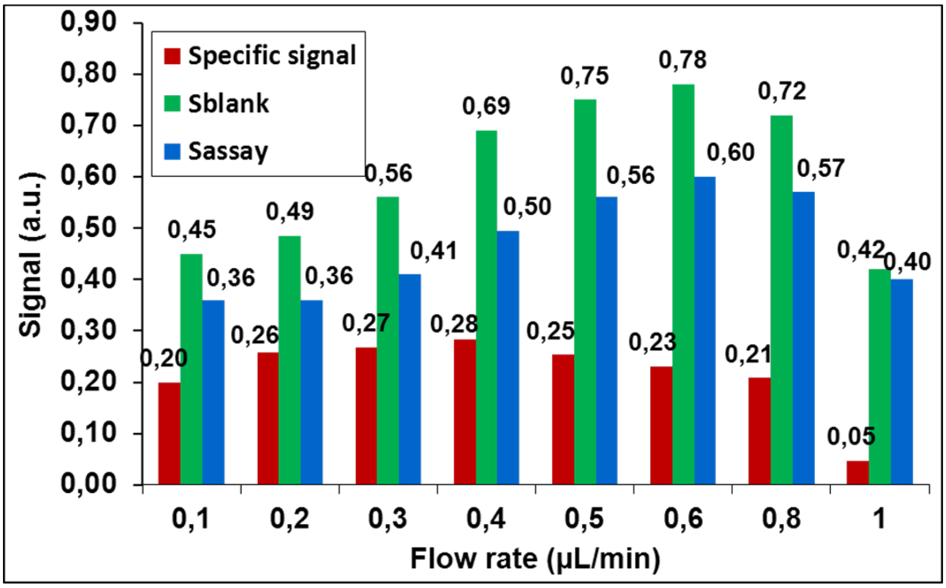

*Figure S5. Influence of the flow rate on the the specific signal S_{blank} and S_{assay} for a magnetic flux density **B** = 20mT. MBs and analyte concentrations were set to 2 mg/mL and 650 pM, respectively.*

References

1 F. Sarrazin, T. Bonometti, L. Prat, C. Gourdon and J. Magnaudet, *Microfluid. Nanofluid.*, 2008, **5**, 131–137.

2 J.-C. Baret, *Lab Chip*, 2012, **12**, 422–433.