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

Supplementary Information

Efficient flowless separation of mixed microbead populations on periodic ferromagnetic surface structures

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S1. Example of movement of 4 µm beads over 8 µm beads (and 2 µm beads)

Microbead with a diameter approximately equal to the spacing between the patterns, i.e. 4 µm are moved in forward direction for a certain amplitude of applied magnetic field, as shown in Figure S1a. An applied field lower than a threshold field cannot move the beads, while a field higher than a certain limit changes the directional motion into a looping motion. On breaking the symmetry of the applied switching field, the opposite direction of motion of 4 µm beads can be enabled. Motion switches from a forward to a backward direction.

To investigate the separation possibility of two or more sizes of beads, experiments were performed on smaller 2 µm beads and larger 8 µm beads. The 2 µm beads do not move to the next pattern due to the too large spacing between the patterns. The motion of larger 8 µm beads, having a diameter twice the spacing between patterns, is also largely hindered, as shown in Figure S1b.

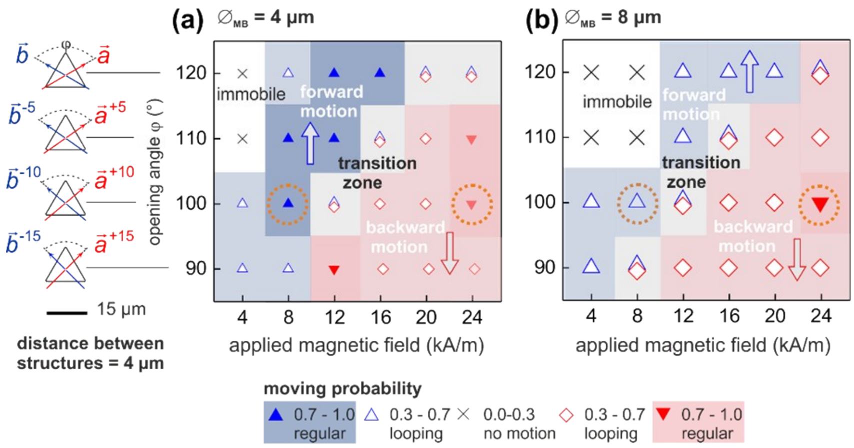
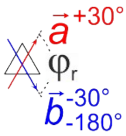
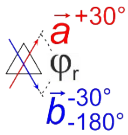


Figure S1. Phase diagrams depicting the regimes of forward and backward motion of 4 µm diameter (a) and 8 µm diameter beads (b) on magnetic patterns having an edge length of 15 µm with a distance of 4 µm between the patterns at magnetic field modulations of 1 Hz. 4 µm beads exhibit extended regimes of regular forward motion. In comparison, 8 µm beads demonstrate no pronounced forward motion. In both cases a small regime for regular backward motion exists.

S2. Applied magnetic field amplitude and direction for selective separation of microbeads populations.

The separation of microbeads from a mixture of microbead population is facilitated through inversion of one magnetic field direction. The amplitudes and directions of applied magnetic fields enabling the selective separation of microbeads are indicated in Table S1. 4 µm and 8 µm beads can be alternatively moved and locked by applying the matching magnetic field conditions.

Table S1. Selective motion of 4 µm and 8 µm beads under low and high amplitude of applied magnetic fields changing with the matching field states. Both type of beads can be moved and locked alternatively. *(A few smaller 2 µm beads were occasionally observed slipping along arrays at high field amplitudes. The motion is non-reproducible under these field conditions.)

Applied magnetic field	H_{ext} = 8 kA/m		H_{ext} = 24 kA/m	
field sequence				
Motion for ∅$_{MB}$ = 2 µm	×	×	× *	× *
Motion for ∅$_{MB}$ = 4 µm	×	×	⊥	↑
Motion for ∅$_{MB}$ = 8 µm	↑	×	↑	×

forward ↑
backward ⊥
immobile or locked ×

S3. Experimental results for quantitative separation of 4 µm and 8 µm beads populations

To compare with the state of the art, we define the separation efficiency of our system by employing the equation used by a very similar study.

$$SE_4 = \frac{n_4}{N_4} P_4 + \left(1 - \frac{n_8}{N_8}\right) P_8$$

$$SE_8 = \frac{n_8}{N_8} P_8 + \left(1 - \frac{n_4}{N_4}\right) P_4$$

These equations give the separation efficiency of microbeads with 4 µm (SE_4) and 8 µm diameter (SE_8) by taking also the undesired moving beads and agglomerates into account. N_4 and N_8 are the number of total beads present in the initial mixture on the area of observation and n_4 and n_8 are the number of separated beads counted at the border of the area of observation. P_4 and P_8 are the percentage of both bead sizes in the initial mixture. The first terms in both equations define the separation of desired beads while second terms give the undesired beads. The separation efficiencies of 4 µm and 8 µm diameter beads calculated from six experiments each are shown in Table S2 and Table S3.

Table S2. The separation efficiencies of 4 µm beads with the number of beads before and after the separation of the 4 µm beads. The number of undesired 8 µm beads is also given.

Exp. no	Number of beads in initial mixture		Number of beads after Separation		$\frac{n_{4\mu m}}{N_{4\mu m}}$	$\frac{n_{8\mu m}}{N_{8\mu m}}$	Separation effciiency, $SE_{4\mu m}$	H_{ext} = OFF & ON
	$N_{4\,\mu m}$	$N_{8\,\mu m}$	$n_{4\,\mu m}$ = desired	$n_{8\,\mu m}$ = undesired				
1	220	25	separated = 193 non separated = 6 agglomerated = 21	locked = 13 slipped = 3 agglomerated = 9	193/220 (87%)	3/25 (12%)	**88%**	yes
2	81	28	= 49 = 8 = 24	= 10 = 1 = 17	49/81 (60%)	1/28 (3%)	**70%**	no
3	874	19	= 734 = 75 = 36	= 7 = 0 = 12	734/874 (83%)	0/19 (0%)	**84%**	no
4	186	17	= 172 = 8 = 6	= 7 = 2 = 8	172/186 (92%)	2/17 (11%)	**92%**	no
5	204	14	= 185 = 4 = 15	= 8 = 2 = 6	185/204 (90%)	2/14 (14%)	**90%**	yes
6*	196	27	= 177 = 3 = 16	= 19 = 1 = 7	177/196 (90%)	1/27 (3%)	**91%**	yes

Table S3. The separation efficiencies of 8 µm beads with the number of beads before and after the separation. The separation efficiencies of 8 µm beads shown along the number of beads before and after the separation for 8 µm. The number of undesired 4 µm beads are given.

Exp. no	**Number of beads in initial mixture**		**Number of beads after Separation**		$\frac{n_{4\mu m}}{N_{4\mu m}}$	$\frac{n_{8\mu m}}{N_{8\mu m}}$	Separation effciiency, **$SE_{8\mu m}$**	H_{ext} = OFF & ON
	4 µm	**8 µm**	**4 µm = immobile**	**8 µm = separated**				
1	27	22	single+immobile = 6 agglomerated = 21 slipped = 2	separated = 9 non separated = 4 agglomerated = 9	2/27 (7%)	9/22 (40%)	**69%**	yes
2	32	27	= 13 = 19 = 5	= 7 = 2 = 6	5/32 (15%)	7/27 (25%)	**58%**	no
3	17	28	= 5 = 12 = 0	= 20 = 2 = 6	0/17 (0%)	20/28 (71%)	**82%**	no
4	10	16	= 4 = 6 = 0	= 9 = 7 = 0	0/10 (0%)	9/16 (56%)	**73%**	no
5	7	16	= 3 = 4 = 0	= 11 = 3 = 2	0/7 (0%)	11/16 (68%)	**78%**	no
6*	19	27	= 3 = 13 = 3	= 16 = 4 = 7	3/19 (15%)	16/27 (59%)	**70%**	yes

The experiment #6 are shown as exemplary data in the paper, where the separated bead count for both bead sizes comes from one starting mixture.

By combining the bead count of all 6 experiments, the separation efficiency of 4 µm and 8 µm beads is SE_4 = ~86% and SE_8 = ~70%.