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

Assessment of pathogen bacteria using periodic actuation

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Assessment of cell concentration and capture using fluorescence assays

Material and Methods

To assess the concentration of cells in suspension, cells were stained with the Acridine Orange fluorophore (AO) and measured with the fluorescence module of the Multimodal GloMax Reader (Promega). The fluorescence module has the excitation range: 465-485 nm and emission detector spanning: 515-575 nm.

AO is a fluorescent dye that can penetrate the cytoplasm and interact with nucleic acids (for DNA the excitation is at 502 nm and the emission at 525 nm, for RNA the excitation is at 460 nm and the emission at 650 nm)[1].

Cell labelling was performed by adding 1 µl of AO to a 50 µl volume of stock suspension and vortexing. The suspension was centrifuged at 6000 rpm for 1h and the AO containing supernatant was removed and replaced with PBS.

From a stock suspension of labelled cells (7.9×10^9 cells/ml), successive dilutions were made to obtain concentrations ranging from 10^2 to 10^7 cells/ml. Each 10 fold dilution was made by adding 50 µl of concentrated cell suspension to a volume of 450 µl of buffer solution and analysed in the multimode reader. The LOD for this method is evaluated at 10^4 cells/ml (see Table 1).

For microscopy experiments the cells and MB-clusters containing cells were observed using a Zeiss Axio Observer Z1 inverted microscope in transmission and epifluorescence mode. The fluorescence of the AO stained cells was observed using the halogen epifuorescence lamp and the 09 filter set with excitation at 450-490 nm and long pass emission filter above 515 nm. Pictures were taken using the 40x objective and an Andor EMCCD DU-885K camera.

Assessment of cell concentration and capture

The capture of the cells by the functionalized MB and the formation of MB-clusters were tested using AO labelled *E. coli*. The cell concentration and capture was assessed using luminometric measurements while the formation of clusters was investigated with microscopy observations.

The cell fluorescence was measured and plotted in respect to known cell concentration enabling calibration curves for assessment of target pathogen cells. Data is presented in the table S1.

Conc.(cell/ml)	Fluorescence (FSU)	SD(FSU)
10^7	160397	±624
10^6	19569	±28
10^5	2054	±5
10^4	298	±4

Table S1

To measure the capture efficiency, labelled cells were incubated with specific MB according to the sample preparation protocol. Afterwards the suspension was placed on a magnet for 2 minutes to collect all the MB. The supernatant containing unbound cells was removed and analysed with the multimode reader. The measured signal reflected the number of unbound cells and the capture efficiency was calculated with the formula:

$$C_{eff}\% = \left(1 - \frac{L_{sup} - L_{buff}}{L_{cell}}\right) \times 100, \quad \text{S(1)}$$

where L_{sup} is the fluorescence of the supernatant containing unbound cells, L_{buff} is the fluorescence of the buffer solution without cells, and L_{cell} is the fluorescence of the suspension of the cells at the corresponding concentration.

The measurements revealed a capture efficiency >80%. For example, for a sample containing 10^6 cell/ml incubated with specific MB, the fluorescence of the supernatant was 3191±126.7 FSU. That corresponds to a capture efficiency of 83 ±0.6%. It was not possible to measure the capture efficiency for cell concentrations smaller than 10^6 cell/ml since the fluorescence signal of the supernatant renders below detector sensitivity.

Assessment of cluster formation using microscopy

The formation of the clusters was validated using fluorescence microscopy (figure S1, B, C). Using AO stained cells we highlight the presence of the cells in specific MB- clusters (figure S1B inset) – cells are visible within the MB clusters.

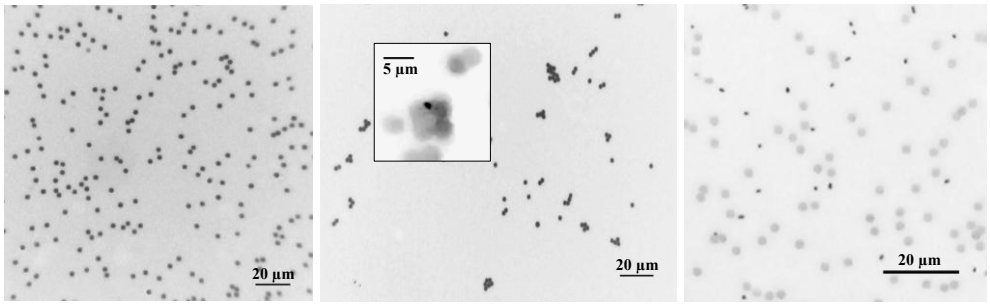

Figure S1 Microscopy observation of formation of MB clusters
A – MB 2.7 µm. **B.** 2.7 µm MB clusters in the presence of specific *E. coli O157:H7*; Inset Fluorescence image of 2.7 µm MB cluster in the presence of specific *E. coli O157:H7* cells; colors inverted (cells –black, MB 2.7 µm – grey) **C** – Fluorescence image in the presence of nonspecific *S. typhimurium* cells; colors inverted (cells –black, MB 2.7 µm – grey).

It is been shown that in the absence of target cells (figure S1A) and in the presence of nonspecific cells (figure S1C) the formation of MB-clusters is not induced.

Supplementary movie
An animation of the magnetic field gradient synchronized with a movie showing the oscillating magnetic beads in real time.

References

1. W. Martens-Habbena and H. Sass, *Applied and Environmental Microbiology*, 2006, **72**, 87–95.