

A Microfluidic Device for Immunoassay-Based Protein Analysis of Single *E. coli* Bacteria

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Abstract

We present a method suitable for quantitative analysis of intracellular proteins, metabolites and secondary messengers of single bacterial cells. The method integrates the concept of immunoassays on a microfluidic device that facilitates single cell trapping and isolating in a small volume of a few tens of picoliters. Combination of the benefits of microfluidic systems for single cell analysis with the high analytical selectivity and sensitivity of immunoassays enables the detection of even low abundant intracellular analytes which occur only at a few hundred copies per bacterium.

Key words Single-cell analysis, Bacteria analysis, *E. coli*, Immunoassay, Microfluidics, Enzyme activity

1 Introduction

Individual genetically identical cells subjected to the same microenvironment can differ considerably in their phenotypic properties [1–3]. This diversity originates mainly from the stochastic nature of cellular gene transcription and translation processes [4, 5]. Investigation of the underlying control mechanisms regulating the noise level in a cell population requires analysis of single cells [6, 7]. In facing the challenges of single cell analysis, microfluidic platforms offer particular advantages over conventional macro scale systems [8, 9]. Precise handling and control of small fluid volumes in the picoliter and nanoliter range allows transport and positioning of cells and cell lysates without sample dilution or loss [10]. Due to these benefits, several microfluidic devices highly suitable for single cell DNA and RNA analysis were presented in recent years [11–13]. In case of DNA and RNA analysis, the sensitivity can be greatly enhanced by amplification of the nucleic acids via polymerase chain reaction (PCR). For the detection of proteins in the low concentration range, the situation is more difficult, as there are no convenient signal amplification methods

available [14, 15]. Moreover, selectivity is a critical point in the choice of detection method, as there is a great variety of different proteins present in a cell. In previous work, we successfully performed enzyme-linked immunosorbent assays (ELISA) on-chip which enabled the protein analysis of single mammalian cells [16]. Due to the high sensitivity and selectivity provided by immunoassays, it was possible to quantify the amount of the housekeeping protein GAPDH in U937 cells as well as HEK293 cells and the amount of the secondary messenger cAMP in MLT cells. The above referenced projects illustrate the successful practical use of microfluidic devices for single cell analysis, but are limited with respect to the cell types. Most often, single cell analysis systems are optimized for mammalian cells that are relatively large and contain large analyte quantities compared to bacteria [17, 18]. Microfluidic platforms capable of handling small bacteria with on average a few hundred analyte copy numbers per cell are rarely found [19–21].

A better understanding of the origins of microbial phenotypic diversity and the underlying control mechanisms are of great importance for developing strategies against antibiotic or biocide resistant microorganisms. Therefore, we developed a system optimized for the analysis of single *E. coli* bacteria. For this purpose, the design of a previously used microfluidic device [16] was significantly downscaled in size. As a result, single *E. coli* bacteria could mechanically be trapped and isolated in microchambers with a volume as small as 155 pL [22]. As the analysis microchambers could be opened and closed repeatedly, different surface- and cell treatment steps could be performed successively. As a proof-of-concept study, an immunoassay for the quantification of the enzyme β -galactosidase in individual *E. coli* bacteria was carried out. However, the approach could be extended to any other proteins for which antibodies are available. In the following protocol the major procedures of the method including bacteria cultivation, device fabrication, immunoassay performance, and data acquisition are described in greater detail.

2 Materials

2.1 Bacteria Cultivation

1. LB Agar plates: Add 250 mL deionized water to a 500 mL media storage bottle, weigh out 14 g of premixed LB-Agar powder and stir the mixture vigorously for 30 min. Add deionized water to total volume of 400 mL, label with autoclave tap and autoclave for 20 min at 120 °C and 1 bar above atmospheric pressure. Let the mixture cool to 55 °C. Meanwhile, place a Bunsen burner and 16 sterile petri dishes (petri dish square with vents) on a bench top that has been cleaned carefully with ethanol. Light the Bunsen burner, flame the lid of

the LB agar bottle with one hand (wear gloves), remove the lid of the first petri dish with the other hand and pour about 25 mL LB agar into the petri dish until it is almost half filled. After filling, immediately replace the lid and do not move the petri dish until the LB Agar is solid. Repeat the process for the remaining petri dishes. Write name, date and content on the upper edge of the lids and store the hardened LB Agar plates in plastic bags at 4 °C in the fridge (*see Note 1*).

2. LB Broth (Lennox) medium: Add 16 g LB Broth (Lennox) powder microbial growth medium to a 1000 mL media storage bottle filled with 800 mL deionized water and stir rigorously for 20 min. Then, autoclave the solution for 20 min at 120 °C and 1 bar above atmospheric pressure. Store the autoclaved LB Broth (Lennox) medium at room temperature.
3. 40 wt% glucose stock solution: Add 320 g glucose to a 1000 mL media storage bottle filled with 480 mL deionized water. Add glucose slowly while stirring the solution vigorously (*see Note 2*). Then, autoclave the solution for 20 min at 120 °C and 1 bar above atmospheric pressure. Store the autoclaved solution at room temperature.
4. M9 minimal medium: Buy or prepare the following stock solutions in deionized water: M9 minimal salts (5×), 1 M MgSO₄ solution, and 1 M CaCl₂ solution.
5. Preparation of M9 Minimal medium: To a 1000 mL media storage bottle filled with 640 mL deionized water, add 160 mL M9 salts (5×), 0.8 mL MgSO₄ (1 M), and 0.08 mL CaCl₂ (1 M). Stir the solution for 10 min. Then, autoclave the solution for 20 min at 120 °C and 1 bar above atmospheric pressure. Store the completed M9 minimal medium at room temperature.
6. 10 wt% lactose stock solution: Add 80 g lactose to a 1000 mL media storage bottle filled with 720 mL deionized water. Stir the solution for 15 min. Then autoclave the solution for 20 min at 120 °C and 1 bar above atmospheric pressure. Store the autoclaved solution at room temperature.
7. *E. coli* bacteria of the strain K-12 MG1655.
8. 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) stock solution (100 μM in MeOH). Store at -20 °C.

2.2 Fabrication of the Microfluidic Device

1. Photoresist SU-8 2010.
2. Mr-Dev600 developer.
3. Silanization: 1*H*,1*H*,2*H*,2*H*-perfluorodecyl-trichlorosilane.
4. Poly(dimethylsiloxane) (PDMS) mixture: Poly(dimethylsiloxane) PDMS monomer and curing agent.

2.3 Surface Modification of the Microfluidic Device

1. Phosphate buffered saline (PBS): Buy or prepare a phosphate buffered saline solution with the following formulation of inorganic salts: KCl (200 mg/L), KH_2PO_4 (200 mg/L), NaCl (8000 mg/L), $\text{Na}_2\text{HPO}_4 \times 7\text{H}_2\text{O}$ (2160 mg/L). Dissolve all salts in deionized water. Filter the PBS sterile before use to avoid contamination of the microfluidic device with dust particles. Store at room temperature until usage.
2. Biotin-derivatized poly(L-lysine)-grafted poly(ethylene glycol) (PLL-g-PEG-biotin) solution: Mix 2.5 μL of the biotin-derivatized poly(L-lysine)-grafted poly(ethylene glycol) (PLL-g-PEG-biotin) stock solution (0.5 wt% in PBS) with 2.5 μL of the poly(L-lysine)-grafted poly(ethylene glycol) (PLL-g-PEG) stock solution (0.5 wt% in PBS) and 44 μL PBS in a 1.5 mL Eppendorf tube. Store the prepared solution at -20°C until usage.
3. Avidin solution: Mix 5 μL of the avidin stock solution (0.5 wt% in PBS) and 45 μL PBS in a 1.5 mL Eppendorf tube. Store the prepared solution at -20°C until usage.
4. Biotin-conjugated Protein G solution: Mix 5 μL of the biotin-conjugated Protein G stock solution (0.025 wt% in PBS) and 45 μL PBS in a 1.5 mL Eppendorf tube. Store the prepared solution at -20°C until usage.
5. Anti- β -galactosidase antibody solution: Mix 5 μL of the anti- β -galactosidase antibody stock solution (β -galactosidase mouse monoclonal IgG₁, Santa Cruz Biotechnology, USA, 50 $\mu\text{g}/\text{mL}$ in PBS) and 45 μL PBS in a 1.5 mL Eppendorf tube. Store the prepared solution at -20°C until usage.

2.4 Immunoassay

1. Lysis buffer: Buy or prepare the following stock solutions in deionized water: 10 mM Tris-HCl (pH 8) solution, 50 mM EDTA solution, and 1 mM NaCl solution.
Mix 5 mL of the 1 M NaCl solution with 1 mL of the EDTA stock solution and 44 mL of the 10 mM Tris-HCl (pH 8) stock solution in a 100 mL media storage bottle and store at 4°C until use for a maximum of 4 weeks. Directly before performing the immunoassay, weigh 5 mg lysozyme powder (lysozyme from chicken egg white) to a 2 mL Eppendorf tube and dissolve in 500 μL of the lysis buffer stock solution. It is important to prepare a fresh lysozyme lysis buffer solution for every experiment (*see Note 3*).
2. Substrate solution: Mix 1 μL of the fluorescein di- β -D-galactopyranoside (FDG) stock solution, with 399 μL PBS.

3 Methods

3.1 Bacteria Cultivation

All steps of the following protocol should be performed under sterile conditions.

1. For short-term maintenance and use, streak *E. coli* bacteria of the strain K-12 MG1655 with the lac operon on dry LB Agar plates. Incubate the plates, agar side up, at 37 °C for 24 h. Seal the plates with Parafilm to keep the moisture in the culture and store them at 4 °C for a maximum of 4 weeks (*see Note 4*).
2. Preparation of the preculture: Inoculate a 15 mL Falcon tube containing 3 mL of LB Broth (Lennox with additional 20 mM glucose) with a single *E. coli* colony of the previously prepared LB Agar plates. Carry out the incubation at 37 °C with constant shaking at 220 rpm for 8 h. Inoculate the preculture into a 15 mL Falcon tube containing 3 mL M9 minimal medium (with additional 20 mM lactose) at a dilution of 1–200 (*see Note 5*). Carry out the incubation at 37 °C with constant shaking at 220 rpm until the OD₆₀₀ is approximately 1.

3.2 Fabrication of the Microfluidic Device

The microfluidic device is composed of two poly(dimethylsiloxane) (PDMS) layers which are bonded on a glass slide to close the fluid channel (*see Fig. 1*). The top layer (pressure layer) consists of a set

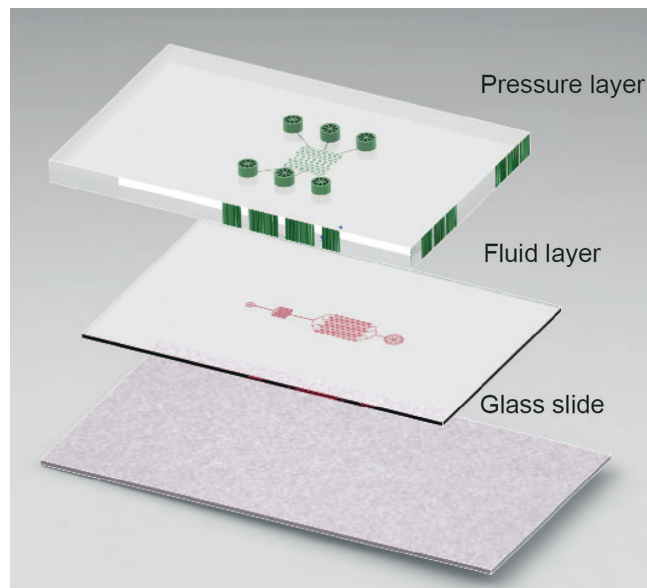


Fig. 1 Schematic drawing of the three different layers required to build the microfluidic device. Adapted from [22]

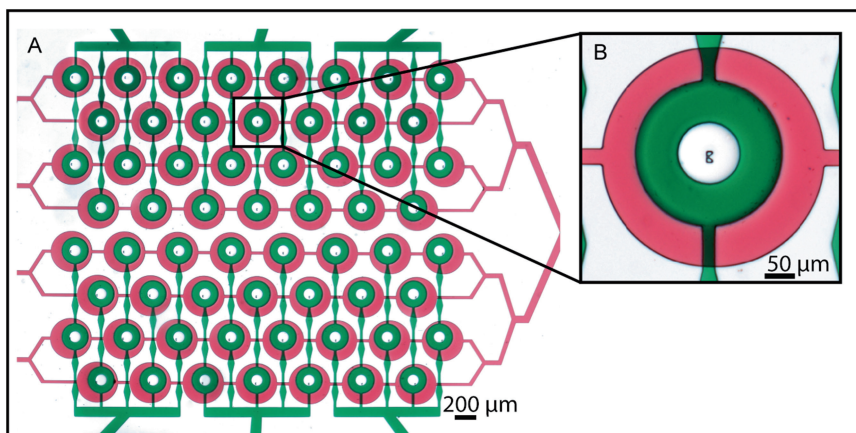


Fig. 2 (a) Bright-field image of the entire array. The fluid channels (height 10 μm) are filled with red food dye, the closed microchambers (volume 80 μL) are filled with colorless buffer solution and the pressure layer is filled with green food dye. (b) Magnified bright-field image of one microchamber. The centrally placed cell trap with a small gap size of 1 μm enables to trap individual *E. coli* bacteria

of 60 microchambers with integrated ring feature (*see* Fig. 2). The ring feature act as a valve, which when attenuated, isolates the content of the microchambers completely from the surrounding medium. The opening and closing of the valves is regulated by a homemade pressure control system (*see* **Note 6**) with nitrogen gas supply which is connected to the device by custom made metal connectors (*see* **Note 7**) and silicon tubing. To ensure precise opening times in the range of milliseconds, the pressure valves are operated by a LabVIEW program (*see* **Note 8**). The key feature of the device is a cell trap for mechanical trapping of single *E. coli* bacteria located in the center of each microchamber (*see* Fig. 2). The **steps 1–7** of the following protocol describe the fabrication of the two master forms needed for the production of the PDMS part of the microfluidic device and should be performed preferentially in a dust-free environment (clean room) (except **step 7**). The **steps 8–17** specify the actual device production in a standard laboratory environment.

1. For preparation of the master forms, dehydrate two silicon wafers (100 mm diameter) at 200 $^{\circ}\text{C}$ for 10 min. Let the wafers cool down, spin-coat with photoresist at 2500 rpm for 30 s so that a final height of 10 μm is achieved.
2. Soft bake at 95 $^{\circ}\text{C}$ for 240 s.
3. Exposure with UV light (130 mJ/cm^2 , measured at 365 nm) in a mask aligner and a transparency photomask.
4. Post exposure bake the photoresist at 95 $^{\circ}\text{C}$ for 240 s and develop for 3 min exposing the 10 μm high features.

5. Hard bake the wafers for 3 h at 200 °C.
6. Confirm the heights of the SU-8 features with a step profiler.
7. Silanize the master forms by storing the wafers overnight in a desiccator with 50 µL 1*H*,1*H*,2*H*,2*H*-perfluorodecyl-trichlorosilane under reduced pressure of 100 mbar. After silanization, the production process of the master forms is finished.
8. For production of the poly(dimethylsiloxane) (PDMS) part of the device, mix carefully PDMS monomer and curing agent at a ratio of 10:1 (total of 60 g) in a plastic weighing dish by using a plastic spatula. Place the weighing dish in a vacuum degassing chamber and degas under reduced pressure of 50 mbar for 20 min.
9. Place the master form of the top part in the middle of the bottom part of a square petri dish and fix at the edges with tape.
10. Pour a 5 mm thick layer of PDMS mixture on the master form of the top part, place in a vacuum degassing chamber and degas under reduced pressure of 50 mbar for 20 min. Cure in the oven at 80 °C for 3 h.
11. For the bottom part containing the fluid channels, spin-coat the PDMS mixture on the master form at 2600 rpm (Spin Coater) for 60 s to build a 30 µm high PDMS coating. Afterwards cure in the oven at 80 °C for 1 h.
12. Cut the master form carefully out of the petri dish by using a scalpel. Remove the hardened PDMS from the master form (the PDMS should peel off automatically). Cut the top part to shape by a razor blade and punch holes for the pressure valves with a Biopsy puncher (1 mm diameter).
13. Place the tailored top part and the PDMS coated master form of the fluid layer in a plasma cleaner. Activate both chip parts by oxygen plasma (0.75 mbar, 18 W) for 45 s and align under a microscope. Pour PDMS around the composed device to facilitate later the peel off process.
14. Harden the composed device in the oven at 80 °C for 1 h.
15. Remove the composed device carefully from the master form, cut to shape by a scissor and punch two holes for the fluid inlet and outlet with a Biopsy puncher (1.5 mm diameter).
16. For assembling the PDMS part of the device and the glass slide (24×40×0.15 mm), place both in a plasma cleaner and activate by oxygen plasma (0.75 mbar, 18 W) for 45 s.
17. Place the PDMS part of the device on the glass slide and place the completed device on a hot plate at 50 °C for 5 min to allow bonding (*see Note 9*).

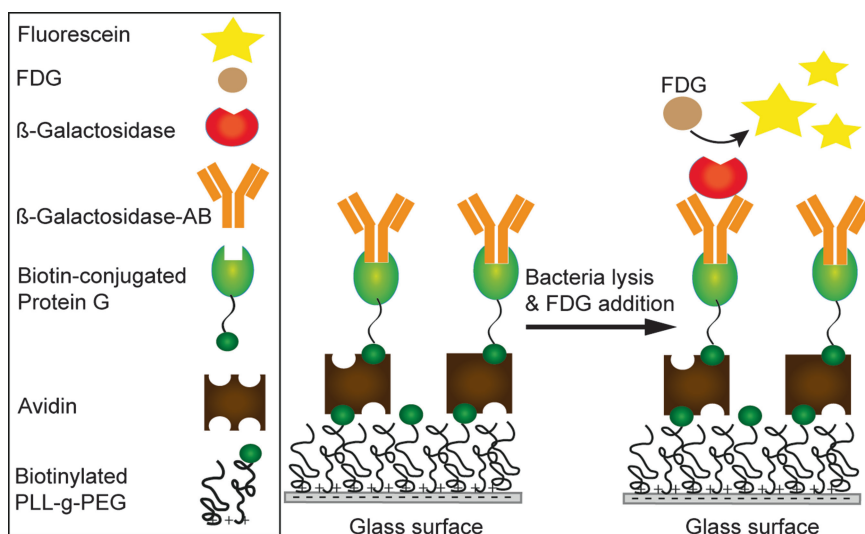


Fig. 3 Schematic drawing of the surface modification. β -galactosidase binding antibodies are immobilized on the surface. After bacteria lysis, the released β -Galactosidase is captured by the antibodies and converts the added substrate FDG into highly fluorescent fluorescein

3.3 Surface Modification of the Microfluidic Device (Fig. 3)

1. Centrifuge the prepared PLL-g-PEG-biotin solution into the device using a centrifuge ($800\times g$, 5 min) directly after finishing the bonding process (*see Note 10*). After 1 h of incubation time, flush the device with PBS using a syringe pump (*see Note 11*).
2. Flush the prepared avidin solution into device at a flow rate of $5\ \mu\text{L}/\text{min}$ for 10 min. After a 5 min flow-free incubation time (*see Note 12*), continue with the washing-step and flush the device with PBS at a flow rate of $10\ \mu\text{L}/\text{min}$ for 5 min.
3. Flush the prepared biotin conjugated Protein G solution into the device at a flow rate of $5\ \mu\text{L}/\text{min}$ for 10 min. After a 5 min flow-free incubation time, continue with the washing step and flush the device with PBS at a flow rate of $10\ \mu\text{L}/\text{min}$ for 5 min.
4. Flush the prepared anti- β -galactosidase antibody solution into the device at a flow rate of $5\ \mu\text{L}/\text{min}$ for 10 min. After a 5 min flow-free incubation time, continue with the washing step and flush the device with PBS at a flow rate of $10\ \mu\text{L}/\text{min}$ for 5 min. Store the surface modified device at $4\ ^\circ\text{C}$ until usage (*see Note 13*).

3.4 Dil-Staining and On-Chip Loading of *E. coli* Bacteria

1. Pipette $1\ \mu\text{L}$ DiI stock solution into a 2 mL Eppendorf tube. Add $150\ \mu\text{L}$ of the *E. coli* culture grown to an OD_{600} of 1 (*see Subheading 3.1*) and incubate at $37\ ^\circ\text{C}$ with constant shaking at 220 rpm for 5 min in a thermomixer.
2. Add $1849\ \mu\text{L}$ PBS and centrifuge for 3 min at 14.1 rcf. Discard the supernatant, resuspend the bacteria pellet with 2 mL of

fresh PBS, and repeat the centrifugation process (*see Note 14*). Discard again the supernatant and resuspend the bacteria cell pellet in 1.5 mL fresh PBS. Pipette 150 μL of the bacteria sample in a 2 mL Eppendorf tube, add 1350 μL PBS and mix by repeated aspirating and dispensing of the pipette tip.

3. For monitoring the trapping process of the *E. coli* bacteria and for collecting the data of the subsequently performed immunoassay, position the microfluidic device on a microscope equipped with a high-sensitivity camera (EMCCD camera).
4. Filter the *E. coli* bacteria, dilute the sample again 1 to 10 with PBS (Filter pore size of 10 μm) (*see Note 15*) and draw it into a 1 mm syringe. Load the *E. coli* bacteria into the device at a flow rate of 5 $\mu\text{L}/\text{min}$ for 5 min (*see Note 16*). Afterwards, flush the device with PBS at a flow rate of 5 $\mu\text{L}/\text{min}$ for 10 min (*see Note 17*).
5. Finally, control the number of single bacteria occupied microchambers by taking fluorescent images of each cell trap (60 \times water immersion objective with 1.5 zoom) and each microchamber (60 \times water immersion objective, exposure time 200 ms, excitation at 546 ± 12 nm, emission at 607 ± 80 nm). Close the microchambers to isolate the single trapped *E. coli* bacteria from the surrounding.

3.5 Performance of the Immunoassay

See Fig. 3 for schematic of assay. A detailed description of the preparation of the lysis buffer and the FDG solution can be found in the Subheading 2.4. Both solutions should be prepared and mixed together on the day the experiment is performed.

1. Flush the lysis buffer into the device at a flow rate of 5 $\mu\text{L}/\text{min}$ for 10 min (*see Note 18*).
2. Open the microchambers for 0.7 s to introduce the lysis buffer (*see Note 19*). For precise opening of the microchambers use the previously described LabVIEW program.
3. Flush the device with PBS at a flow rate of 5 $\mu\text{L}/\text{min}$ for 10 min (*see Note 20*).
4. Flush the FDG solution into the device at a flow rate of 5 $\mu\text{L}/\text{min}$ for 5 min.
5. Open the microchambers for 0.7 s to introduce the substrate FDG (*see Note 21*). For precise opening of the microchambers use the LabVIEW program.

3.6 Data Acquisition and Evaluation

1. Start to monitor the reaction process directly after the substrate FDG was added. Therefore, take pictures every 30 min within a period of 3 h of all microchambers occupied by a single *E. coli* bacterium (*see Fig. 4*) (EMCCD camera, 4 \times objective, exposure time 300 ms, gain $\times 100$ excitation: 470 ± 40 nm, emission: 525 ± 50 nm).

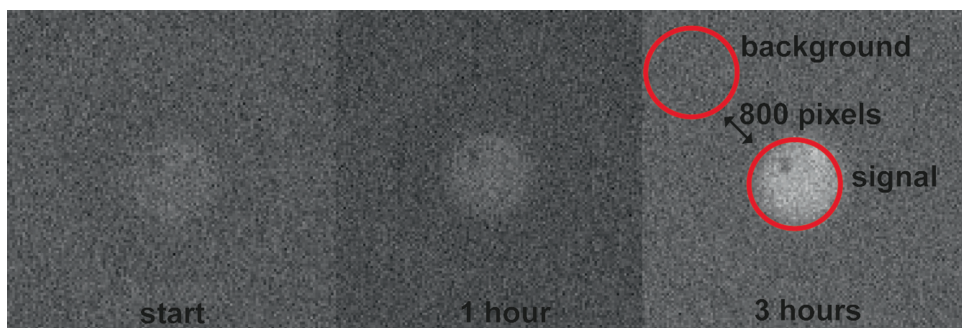


Fig. 4 Fluorescent images of the same microchamber enclosing the lysate of a single *E. coli*. The starting time is defined as the point in time the substrate FDG is added. The fluorescent signal inside the microchamber increases over time as the amount of fluorescent product of the enzymatic reaction increases. For background correction an image section directly beside the closed microchamber of identical pixel size is also analyzed and subtracted from the detected fluorescent data inside the microchamber

2. For the data analysis, analyze the micrographs with an imaging program like ImageJ (*see* **Note 22**). Measure the fluorescence signal in the microchamber by marking a circular area of around 800 pixels (*see* Fig. 4). For reliable evaluation of the fluorescence signal, it is essential to make a background correction for each data point. Therefore, measure the background signal separately for each analyzed microchamber. In this case, the background signal is the signal outside of the microchamber where no analyte is present. Generate a graphic (Excel) for each microchamber in which the background corrected fluorescence signal is plotted against time. The curve progression in case of an *E. coli* free chamber can be clearly distinguished from a chamber occupied by one or two *E. coli* (*see* Fig. 5).
3. It would be also possible to quantify the amount of enzyme present in a single *E. coli*. Therefore, perform the assay with different known enzyme concentrations (*see* as example Fig. 6) instead of with bacteria. Acquire and evaluate the data as described in **steps 1** and **2**. Perform a linear fitting (using Origin or Excel) of the plotted curve. The slope of the linear part at the beginning of data acquisition is a measure for the number of enzyme molecules present in a microchamber, as the enzymatic reaction is based on Michaelis–Menten kinetics. The Michaelis–Menten enzyme kinetics model states how the product formation speed correlates with the substrate starting concentration as well as the enzyme concentration [23]. As the substrate starting concentration is kept constant during the immunoassay, the product formation speed can be correlated directly with the β -galactosidase concentration.

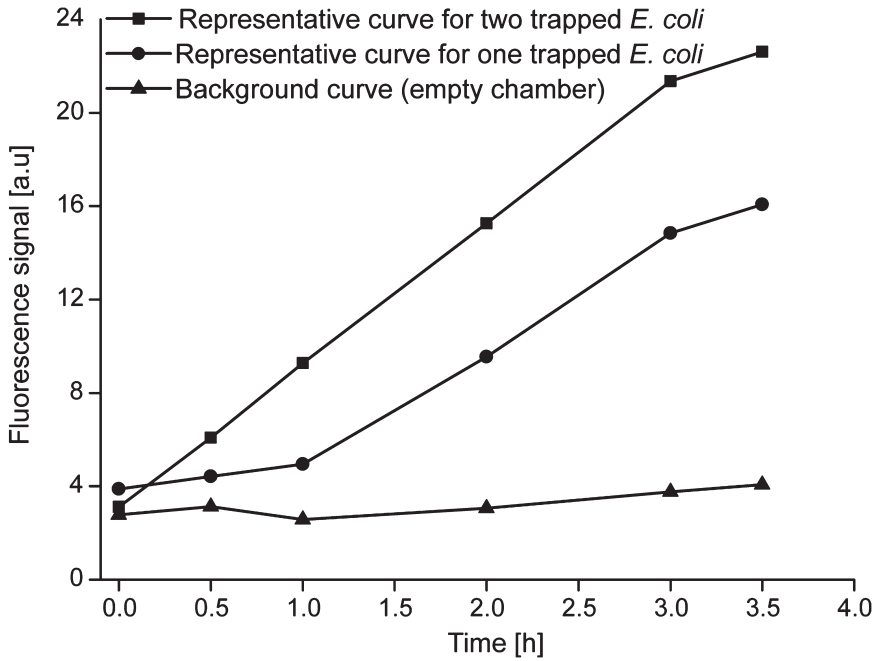


Fig. 5 Representative curves of the fluorescent signal over time progression in case of two, one, or zero lysed *E. coli* in the microchamber. The shown data originate from three different microchambers of the same microfluidic device

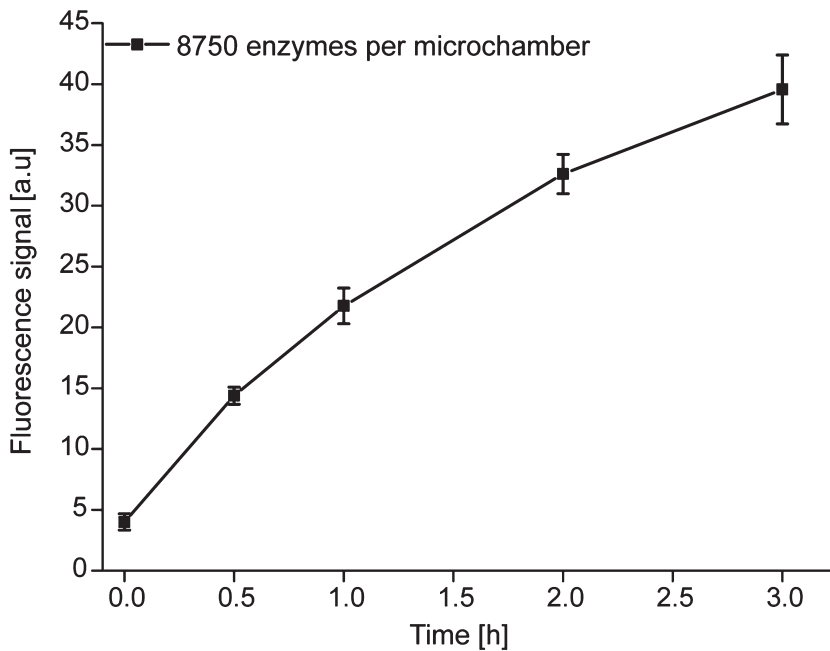


Fig. 6 Curve progression in the case where the immunoassay is performed with a known enzyme concentration of 8750 enzymes per microchamber. The slope of the curve is a measure for the number of enzymes present, and therefore, a set of curves of different known enzyme concentrations could be used to generate a calibration curve

4. To quantify the amount of enzymes present in a single *E. coli* bacterium, generate a calibration curve as described in **step 3** and insert the slope value of an actual *E. coli* experiment into the equation of the calibration curve.

4 Notes

1. Agar plates which do not contain antibiotics can generally be used as long as they are not contaminated or dried out. In order to prevent drying, wrap the lid of the petri dish with Parafilm.
2. Glucose at high concentrations can be solubilized more easily if the deionized water is gently heated.
3. Lysozymes are a type of glycoside hydrolases which disintegrate the cell wall of bacteria. They are an essential component of the *E. coli* lysis buffer. The ideal storage temperature for the enzyme is $-20\text{ }^{\circ}\text{C}$, and fresh, defrosted enzyme powder should be added to the buffer directly before use.
4. To ensure that the *E. coli* colonies stored on LB agar plates receive an optimal supply of nutrients, streak them out on fresh LB agar plates every 4 weeks.
5. It is advisable to inoculate the *E. coli* bacteria first from LB agar plates to a complex medium and afterwards to a defined medium. By preculturing the *E. coli* bacteria in a nutrient rich complex medium, it is ensured that the bacteria are in an optimal fitness state before the change of nutrient medium and carbon source occurs.
6. Key feature of the custom-made pressure control system is a magnetic valve block (nine valves in total) which is connected to a programmable logic controller which enables to operate the valves via the LabVIEW program. For detailed information see the schematic (Fig. 7).
7. The custom-made metal connectors are made of capillary tubes (stainless steel, inner diameter of 1.35 mm) with a 90° bend.
8. The LabVIEW program controls the sequentially opening and closing of the ring-shaped valves. For opening, the pressure is released of the affected valve within a defined period of time. For closing, the valve is pressurized again. The LabVIEW program enables to operate precisely opening times in the range of milliseconds.
9. Bonding of the PDMS-part of the device to the glass slide is a highly time-critical step, as the surface activation only lasts for a few minutes.

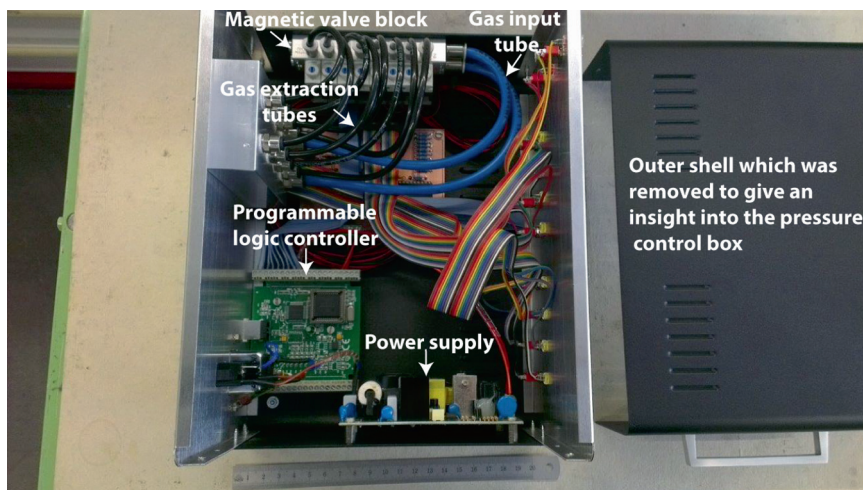


Fig. 7 Schematic of custom-made pressure control system

10. The first step of the surface modification is highly time-critical, as the glass slide of the device has to remain activated otherwise PLL-g-PEG-biotin will not attach to the glass surface. Only the first step of the surface modification protocol is time-critical, the **steps 2–4** can be performed without maintaining a strict time-frame, as long as drying-up of the surface is prevented.
11. All reagent solutions were pumped through the device by using a syringe pump guaranteeing a flow-rate accuracy of 0.5 %.
12. The flow-free incubation time is performed to ensure that the reagents flushed through the device have enough time to diffuse to the surface area of the device.
13. To prevent drying up of the modified surface, place PBS filled pipette tips in the inlet and outlet of the device. Store the device in a closed box containing a moisturized tissue at 4 °C in the fridge.
14. The DiI-labeled *E. coli* bacteria sample is washed twice to remove excess dye from the sample solution. Otherwise, the excess dye stains the PDMS when the bacteria sample is flushed into the device. As a result, the PDMS based cell trap is stained itself and it would be impossible to distinguish an empty cell trap from an occupied cell trap.
15. It is important to filter the *E. coli* bacteria sample before introducing it to the microfluidic device to prevent dust particles from the medium solution from blocking the cell traps. It is advisable to use filters with a pore size of 20 μm or 10 μm . Smaller pore sizes should be avoided as they also filter out the bacteria.

16. The bacteria trapping efficiency is higher at high flow rates. Therefore, do not reduce the flow-rate while flushing the bacteria sample into the device. Moreover, do not flush more than 25 μL of the bacteria sample into the device, as a high number of excess bacteria increase the risk of unintentional bacteria sticking to the PDMS outside the cell traps.
17. This long washing step is used to ensure that the bacteria sample is completely flushed out of the device. Thereby only bacteria physically trapped within the cell traps remain within the device.
18. As the lysis of *E. coli* bacteria can take up to 30 min, wait at least for 30 min before you insert the substrate FDG.
19. 0.7 s is the minimal opening time required to ensure a full fluid exchange within the microchamber at a flow rate of 5 $\mu\text{L}/\text{min}$. As complete lysis of *E. coli* bacteria takes up to 30 min, analyte loss by opening the chamber for 0.7 s is unlikely.
20. The lysis buffer could lead to a degradation of the substrate FDG. Therefore, it is important to ensure that the lysis buffer is flushed out completely before the substrate is introduced.
21. Opening the microchamber for introducing the substrate FDG also allows for cell lysate residues to be washed out. That means the substrate introducing step is also an integrated washing step.
22. <http://imagej.nih.gov/ij/>

References

1. Kaern M, Elston TC, Blake WJ et al (2005) Stochasticity in gene expression: from theories to phenotypes. *Nat Rev Genet* 6:451–464
2. Ito Y, Toyota H, Kaneko K et al (2009) How selection affects phenotypic fluctuation. *Mol Syst Biol* 5:1–7
3. Munsky B, Neuert G, Van Oudenaarden A (2012) Using gene expression noise to understand gene regulation. *Science* 336:183–187
4. Viney M, Reece SE (2013) Adaptive noise. *Proc R Soc B* 280:1–9
5. Hunt BG, Ometto L, Keller L et al (2013) Evolution at two levels in fire ants: the relationship between patterns of gene expression and protein sequence evolution. *Mol Biol Evol* 30:263–271
6. Taniguchi Y, Choi PJ, Li GW, Chen H et al (2010) Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* 329:533–538
7. Mazumder A, Tummler K, Bathe M et al (2013) Single-cell analysis of ribonucleotide reductase transcriptional and translational response to DNA damage. *Mol Cell Biol* 33:635–642
8. Dittrich PS, Manz A (2006) Lab-on-a-chip: microfluidics in drug discovery. *Nat Rev Drug Discov* 5:210–218
9. Klepárník K, Foret F (2013) Recent advances in the development of single cell analysis—a review. *Anal Chim Acta* 800:12–21
10. Kovarik ML, Gach PC, Orno DM et al (2012) Micro total analysis systems for cell biology and biochemical assays. *Anal Chem* 84:516–540
11. Zhang Y, Ozdemir P (2009) Microfluidic DNA amplification—a review. *Anal Chim Acta* 638:115–125
12. Lounsbury JA, Karlsson A, Miranian DC et al (2013) From sample to PCR product in under 45 minutes: a polymeric integrated microdevice for clinical and forensic DNA analysis. *Lab Chip* 13:1384–1393
13. Chang CM, Chang WH, Wang CH et al (2013) Nucleic acid amplification using microfluidic systems. *Lab Chip* 13:1225–1242

14. Wu M, Singh AK (2012) Single-cell protein analysis. *Curr Opin Biotechnol* 23:83–88
15. Bendall SC, Simonds EF, Qiu P et al (2011) Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. *Science* 332:687–696
16. Eyer K, Stratz S, Dittrich PS et al (2013) Implementing enzyme-linked immunosorbent assays on a microfluidic chip to quantify intracellular molecules in single cells. *Anal Chem* 85:3280–3287
17. Eyer K, Kuhn P, Dittrich PS et al (2012) A microchamber array for single cell isolation and analysis of intracellular biomolecules. *Lab Chip* 12:765–772
18. Chen Y, Zhang B, Feng H et al (2012) An automated microfluidic device for assessment of mammalian cell genetic stability. *Lab Chip* 12:3930–3935
19. Leung K, Zahn H, Leaver T et al (2012) A programmable droplet-based microfluidic device applied to multiparameter analysis of single microbes and microbial communities. *Proc Natl Acad Sci U S A* 109:7665–7670
20. Kim M, Isenberg BC, Sutin J et al (2011) Programmed trapping of individual bacteria using micrometer-size sieves. *Lab Chip* 11: 1089–1095
21. He M, Edgar JS, Jeffries GD et al (2005) Selective encapsulation of single cells and sub-cellular organelles into picoliter- and femtoliter-volume droplets. *Anal Chem* 77: 1539–1544
22. Stratz S, Eyer K, Kurth F, Dittrich PS (2014) On-chip enzyme quantification of single *Escherichia coli* bacteria by immunoassay-based analysis. *Anal Chem* 86: 12375–12381
23. Berg JM, Tymoczko JL, Stryer L (2002) The Michaelis-Menten model accounts for the kinetic properties of many enzymes. In: Freeman WH (ed) *Biochemistry*, 5th edn. Freeman W. H. and Company, New York



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