Status talk

Electrical and real-time label-free tracking of nano-bioreactors in multiphase microfluidics

Report on progress, ongoing work and outlook

Presenter:
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17.03.2022 Dresden

Agenda

Overview of previous work:

I. Monitoring of enzymatic reaction (β-galactosidase\ONPG) using FETs chip in droplets.

II. Circular microfluidics (CMF) development

- CMF
- Nano/micro electrode fabrication

Recent progress:

III. Monitoring of antibiotic effect on *E.coli* in droplets

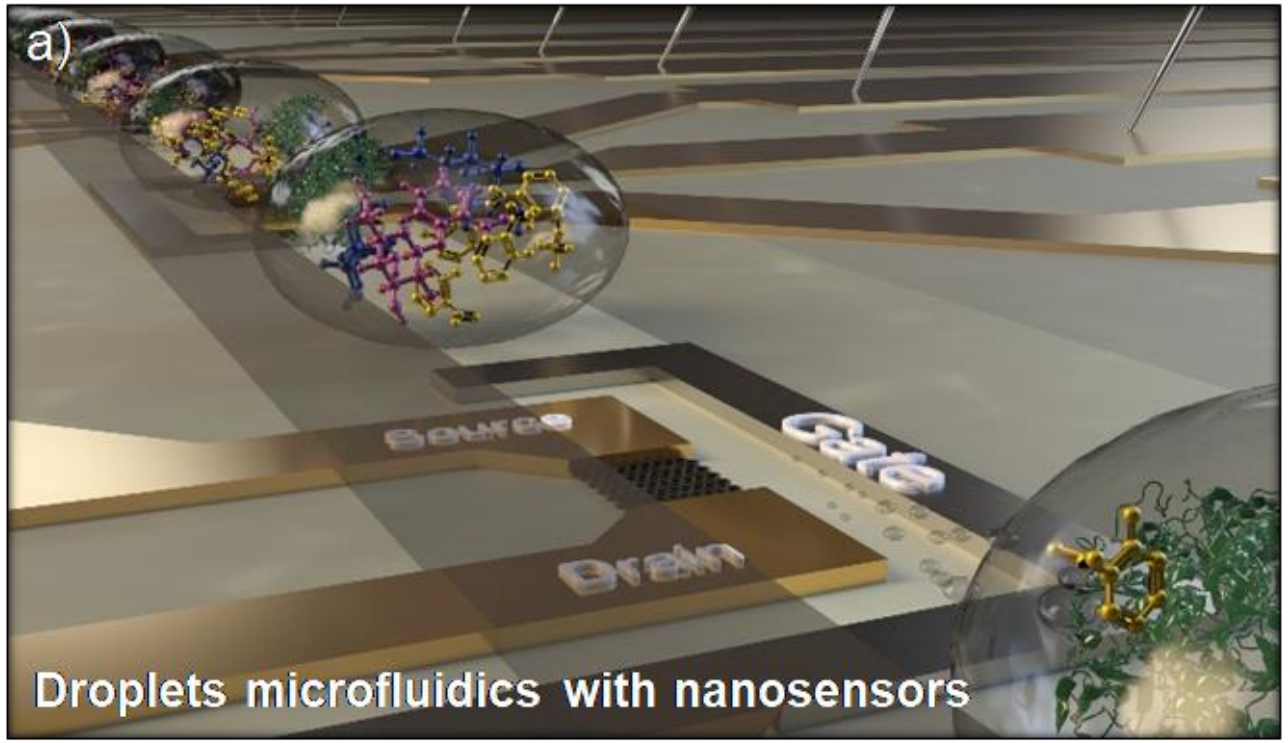
- E.coli in LB buffer and antibiotic effect on the culture growth
- E.coli in M9 buffer and antibiotic lysis effect monitoring

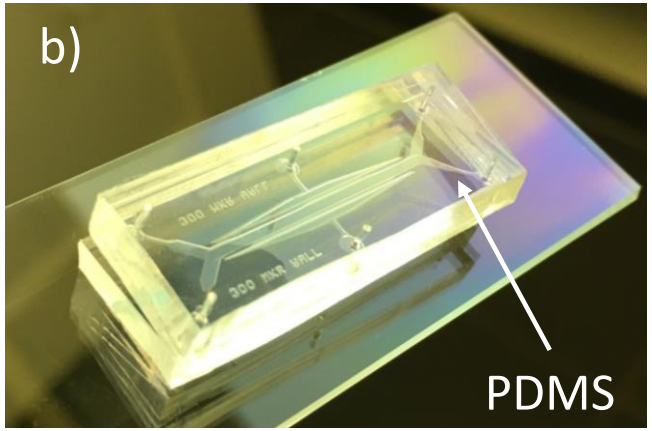
To do´s and outlook

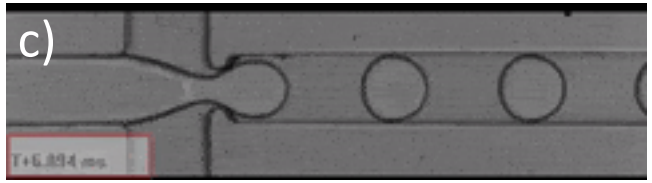
Microfluidics – what´s that?

Microfluidics - typically accociated with the *behaviour, manipulation* and precise *control* of *small* volumes *of fluids*.

Operating volumes: **µL, nL, pL, fL**, thus, the **channel** dimensions are in the range of **µm**.



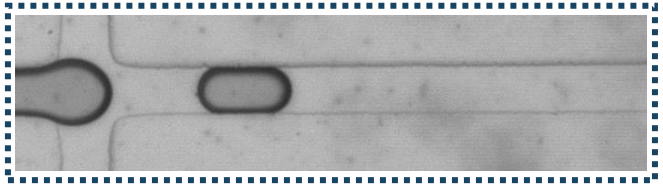




Motivation

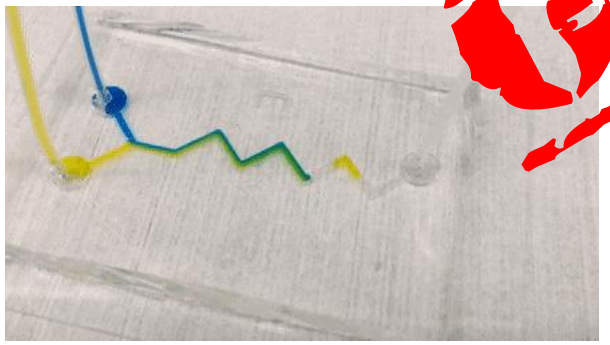
Conventional approach

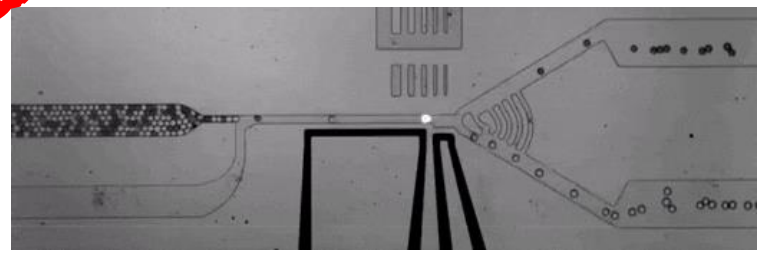
Droplet generation

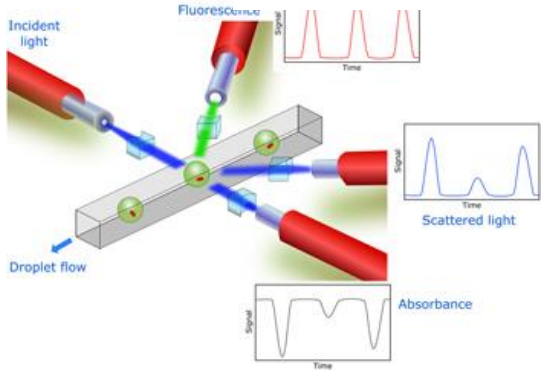


Functional part(s)

Mixing Sorting Detection

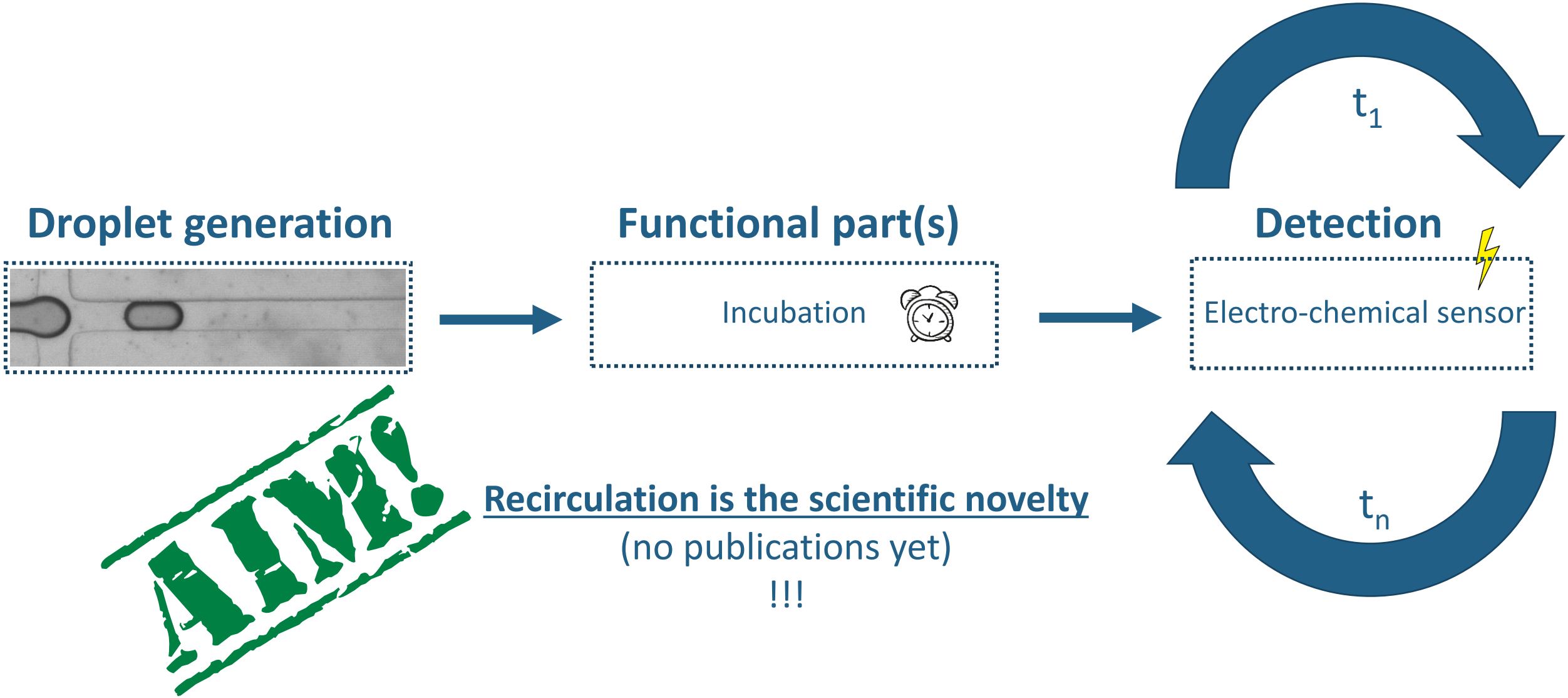






DRY WAY!

Motivation

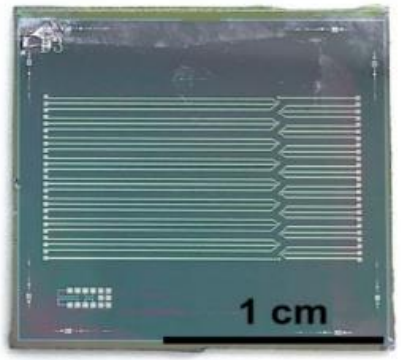


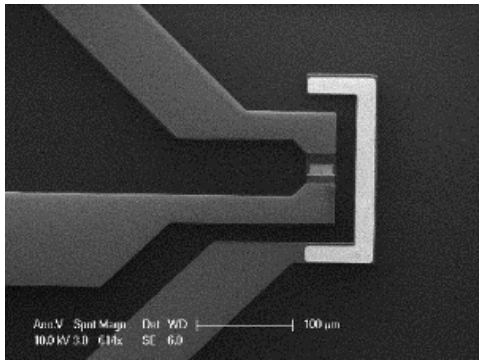
<u>Recirculation is the scientific novelty</u>

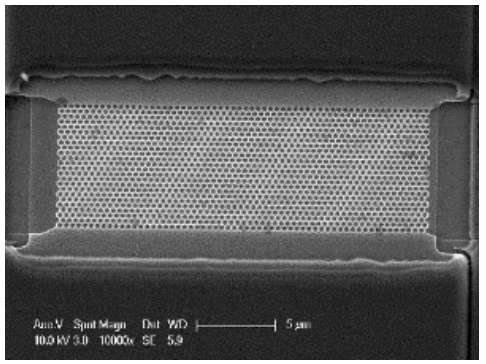
(no publications yet)

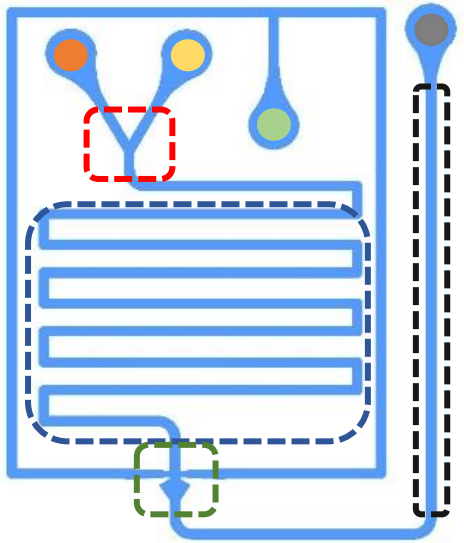
!!!

I. Monitoring of enzymatic reaction (β-galactosidase & ONPG) using FETs chip.





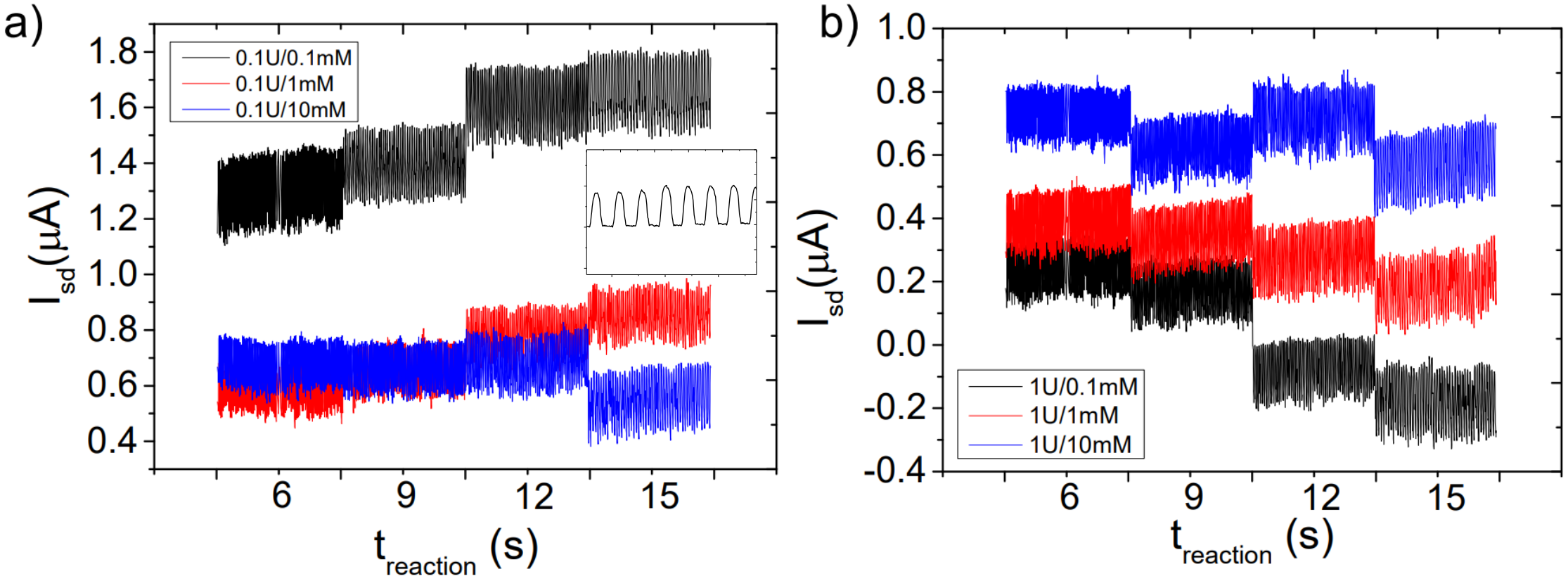




- **Mixing** of the reaction components directly on chip (red area)
- **Incubation** prior to **droplet generation** (blue and green areas)
- Changes of the flowrate changed the **detection** time (reaction time point) (black area)

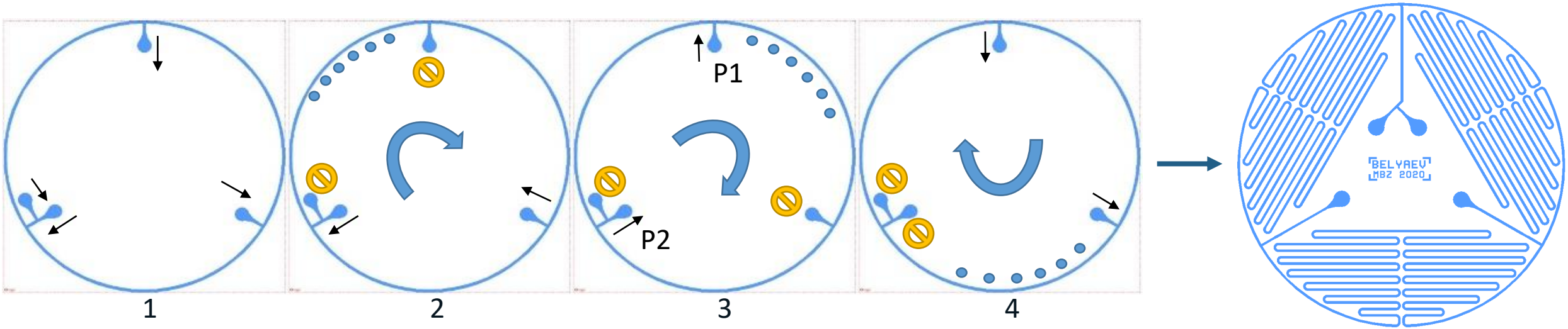
The result of this chapter was published in Micromachines 2020, 11(2), 138; https://doi.org/10.3390/mi11020138

Monitoring using FETs chip



Data obtained from the assay with the subtracted effect of streaming potential. (a) Reaction with increasing substrate concentration and constant enzyme concentration at (a) 0.1 U and (b) 1 U.

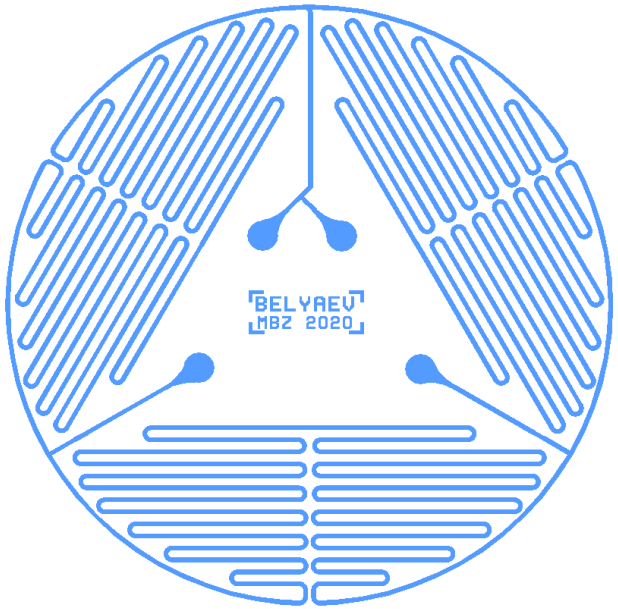
II. Circular microfluidics development



The aim was to design microfluidics **to enable recirculation**:

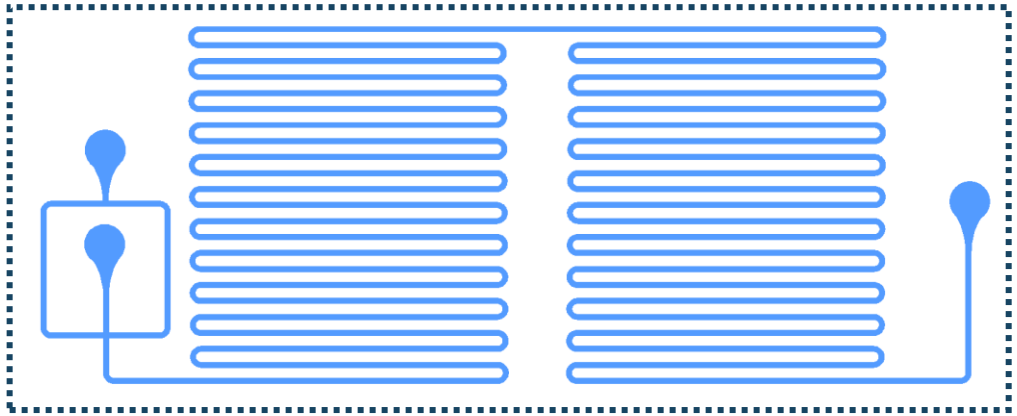
- no inlets/outlets → closed «patrol engine» approach
- precision expected to be the key parameter

II. Circular microfluidics development



Circular design
to
“there and back” movement

200µm x 100µm channel



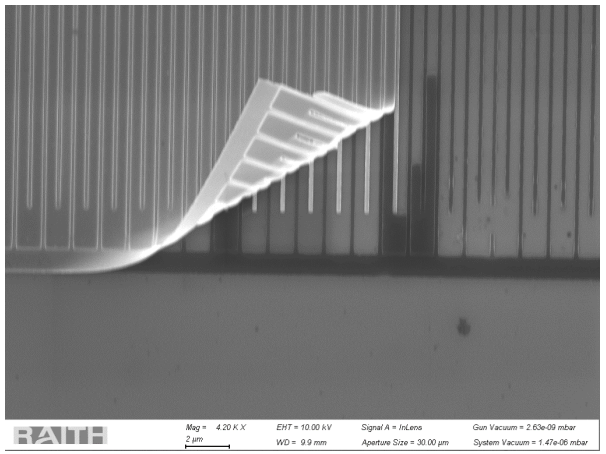
- The device was faricated
- The manipulation tests were done

An array of ~100 droplets is generated and guided over the sensor area in “there and back” manner. The time duration of **one cycle is about 5 minutes**. **Maximum** achieved **3 hours and 30 minutes total incubation** of ones generated droplets.

Nano-electrode chip fabrication & switch to µ-electrodes

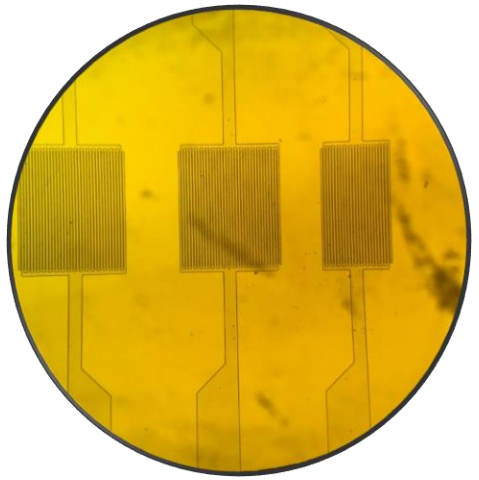
The nano-electrode chip was intended to be fabricated in the following manner:

1) EBL patterning on PMMA ✓
2) Development of PMMA ✓
3) Cr/Au deposition ✓
4) Lift-off in acetone ✘



Alternative protocol:

1) Cr/Au deposition ✓
2) PMMA spin-coating ✓
3) EBL patterning on PMMA ✓
4) Reactive ion etching (HZDR) ✘



Main (and unsolved) problem:
Lift-off !

Adjusting parameters:
- PMMA thickness
- Gold thickness
- E-beam dose and etc.

Switched to micro electrode fabrication via UV-lithography

III. Bacteria growth monitoring

Aim:

Allow monitoring of once generated array of droplets containing *E.coli* bacteria and investigate the effect of the antibiotic on the culture growth.

Experimental procedure:

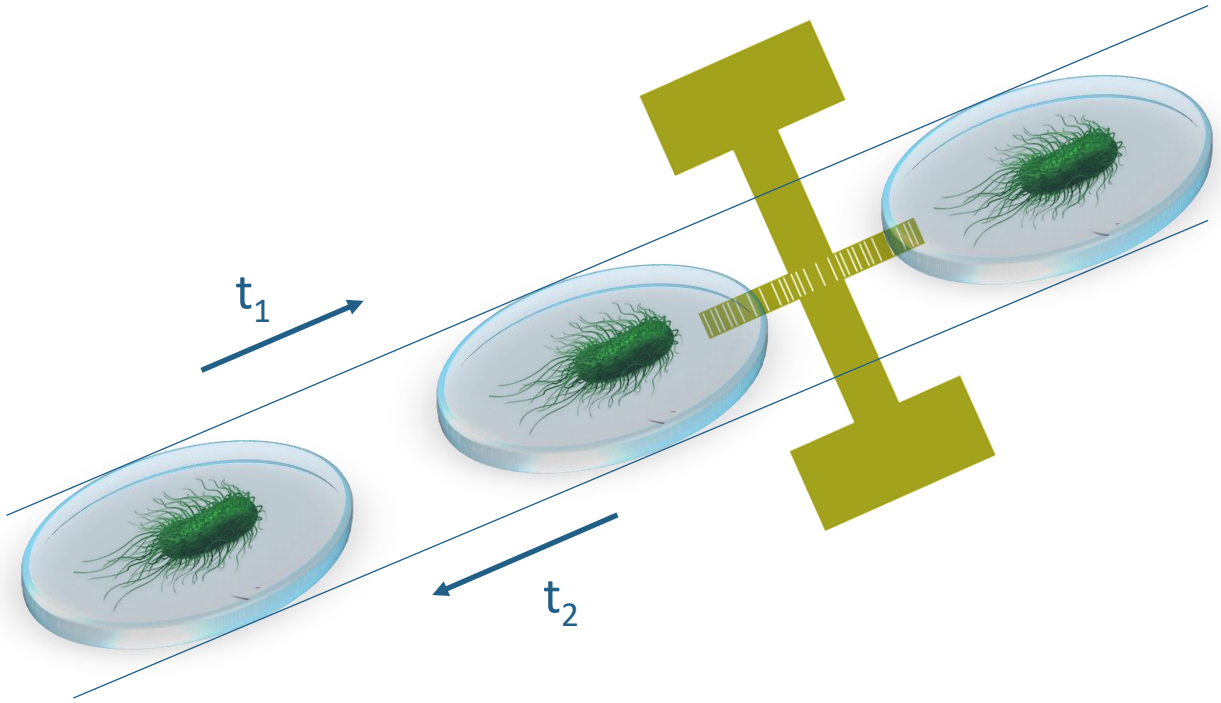
- *E.coli* in LB medium
- **[C] = OD_{600} x 5.1 x 10^8 cells/ml**
- Diluted to exponential growth phase concentration

Problems:

- The stability of the droplets containing LB were lower in comparison to the PBS or M9 buffer
- The difference in electrical signal between pure buffer and bacteria containing buffer was not observed

Solutions:

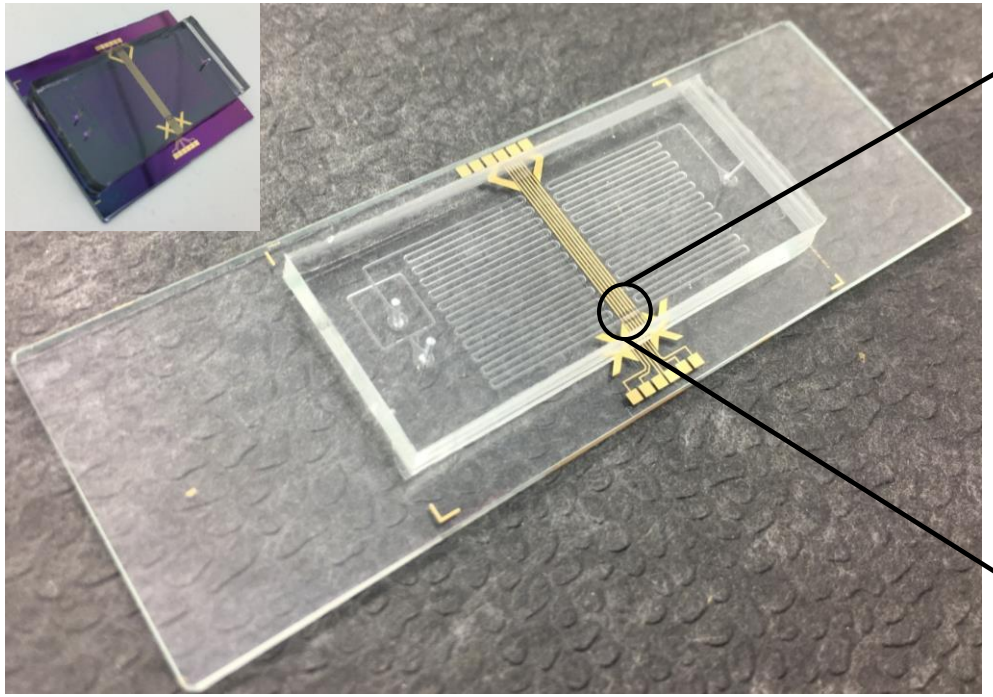
Switch from LB to M9 buffer for *E.coli* cultivation

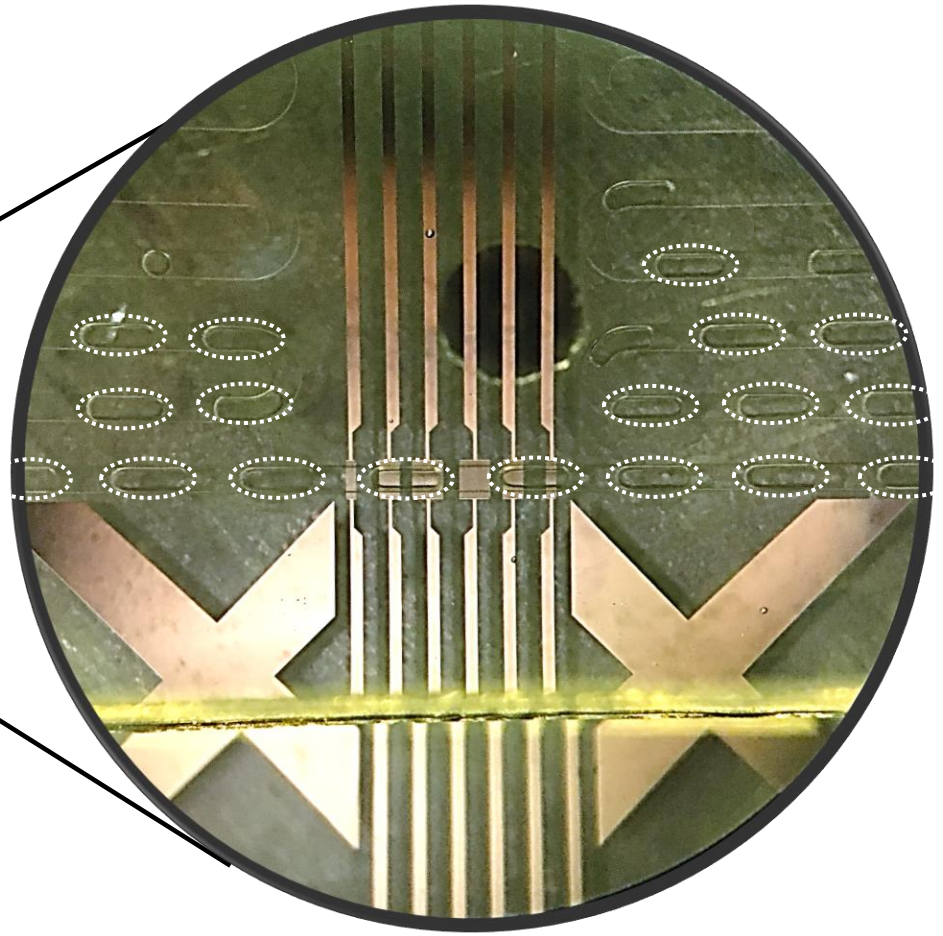


III. Bacteria growth monitoring

Droplet manupulation was automated using Nemesys syringe control software !

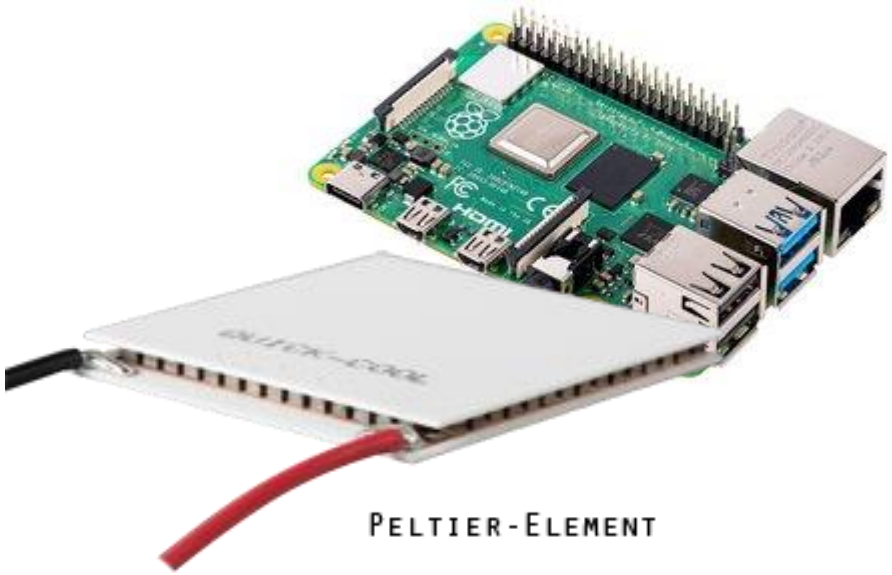
Fully automated droplet manipulation





Permanently sealed device: 200 µm X 100 µm channel, 6 electrode sensors on board with 6, 12 and 18 interdigitated micro-wire pairs (each duplicated).

Temperature control implementation



- In order to maintain temperature regime around 36 °C to allow rapid bacteria growth Peltier element was used.
- The temperature of the flowcell device was successfully controlled.

Occurred problems:

- Due to temperature change, the viscosity of the oil changed
- Jetting appeared
- Droplet stability significantly decreased (from 1 hour down to ~10 min)

The decision to change the use case assay was make.

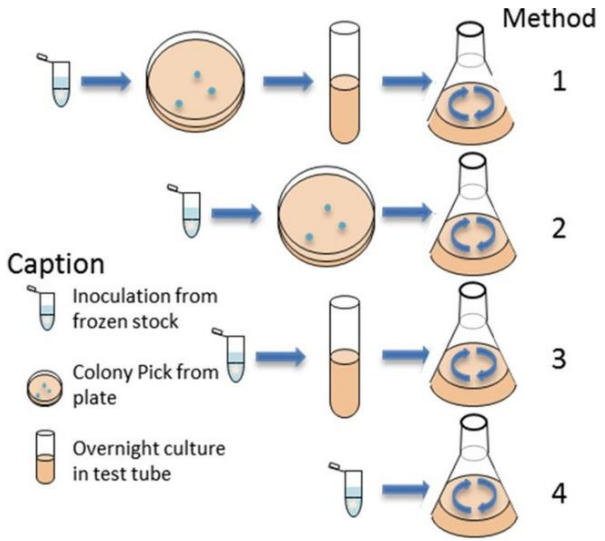
Bacteria growth monitoring → **Bacteria lysis monitoring (with antibiotic)**

Thanks to Dr. Riemenschneider the T-module was set up in MBZ.

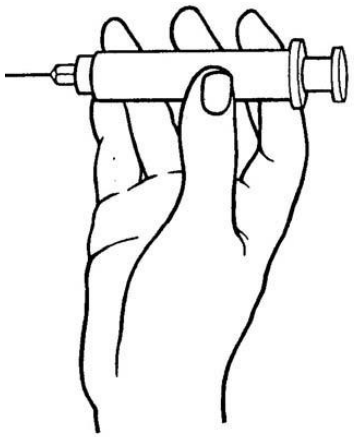
Final bacteria monitoring assay

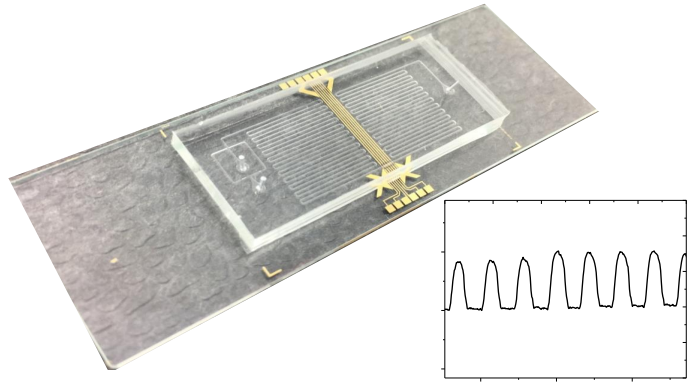
Aim:

Allow monitoring of once generated array of droplets containing *E.coli* bacteria and investigate the effect of Cefotaxime on the culture (membrane destruction).



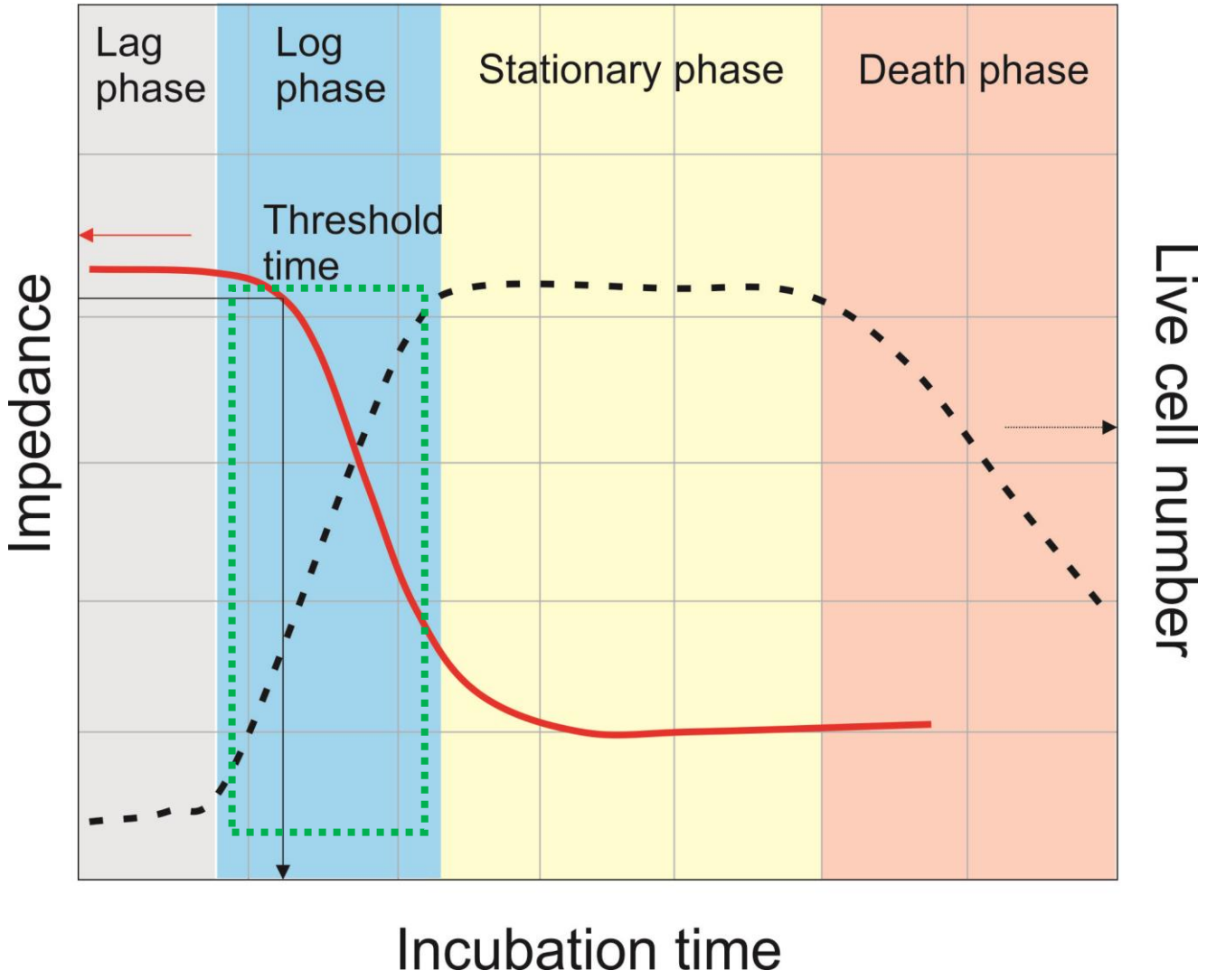
[C] dilution

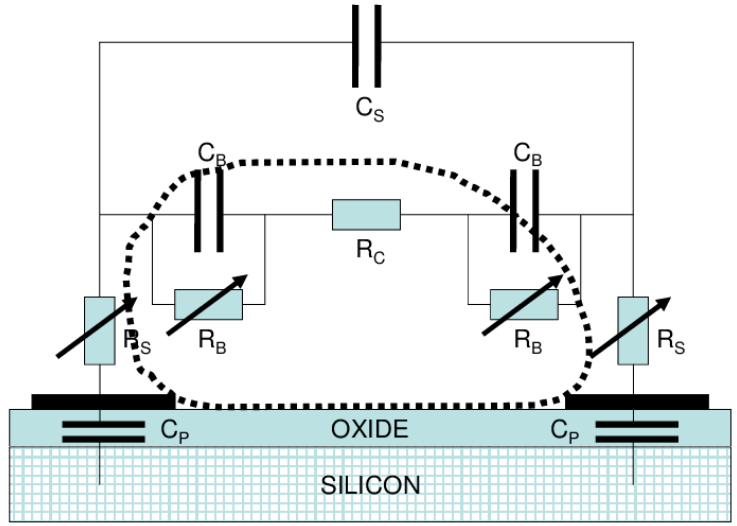




✓ Sensor characterisation → ✓ M9 incubation reference → ✓ *E.coli* incubation reference → ✓ Assay *E.coli* + Cefotaxime → 981 mb of .txt data

What is expected with respect to the signal?

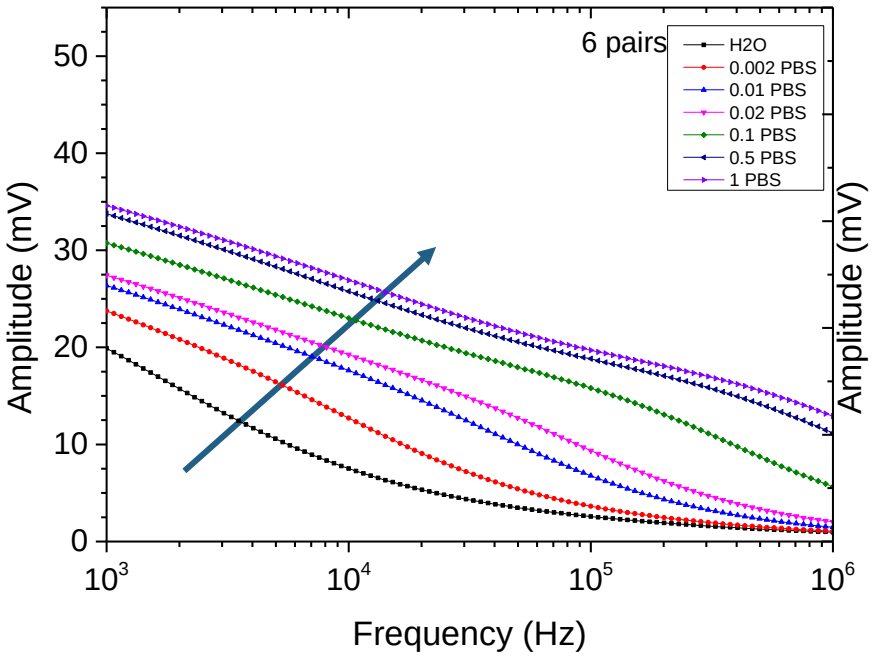




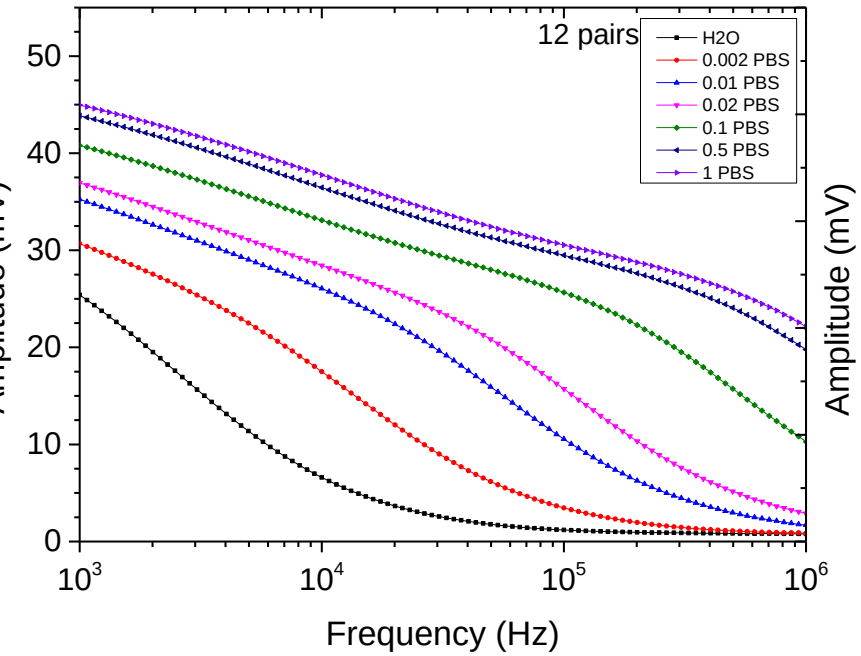
$$Z' = \frac{V_{in}}{V_{out}\sqrt{(\omega * C_{ref})^2 + \left(\frac{1}{R_{ref}}\right)^2}}$$

Microsensor characterisation: PBS sweep

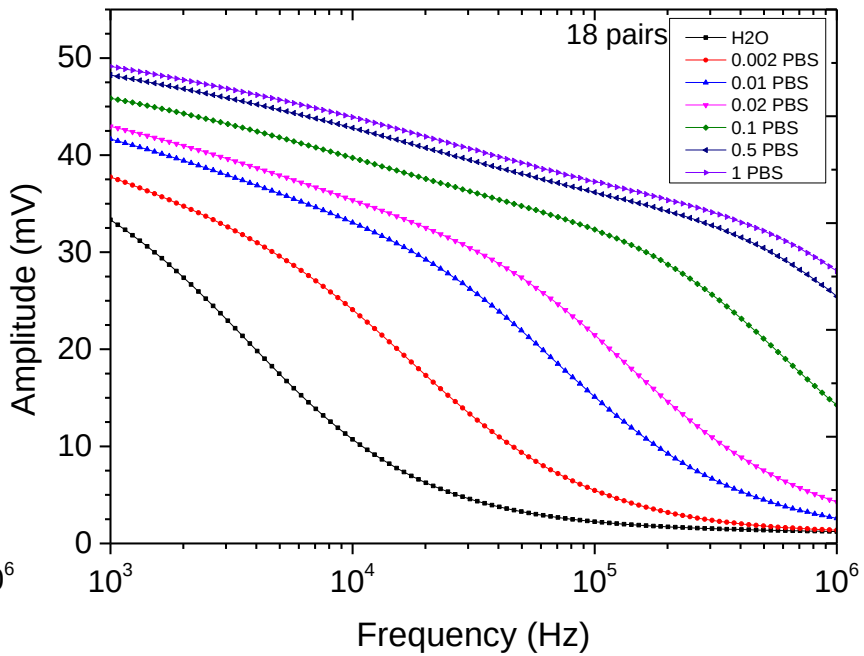
Sweeping range: 10^3Hz - 10^6Hz Dilutions from 10mM PBS



6 pairs



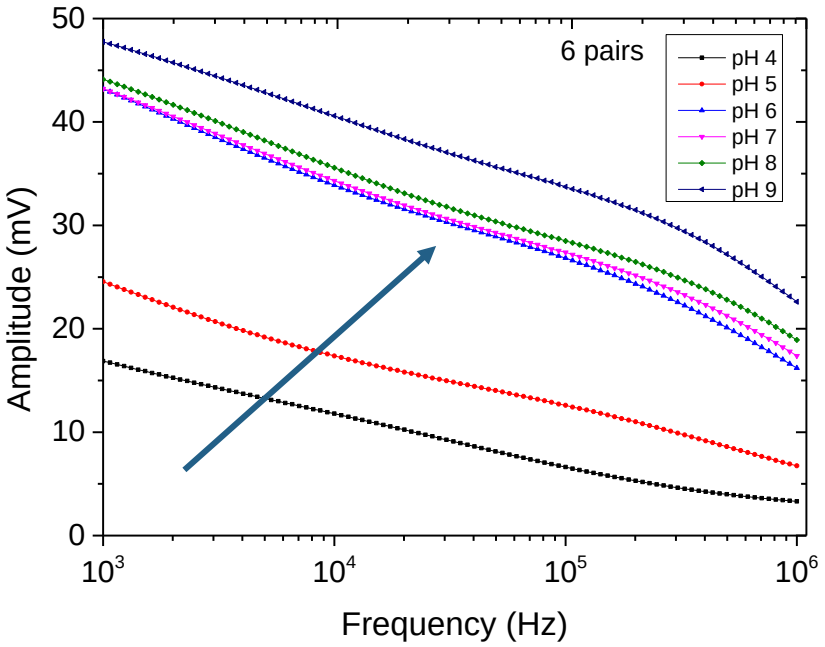
12 pairs



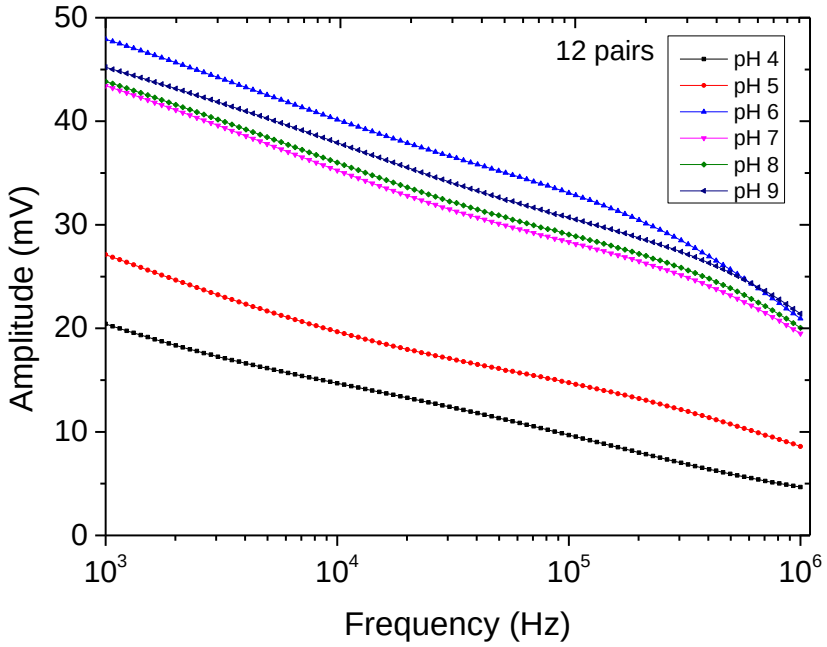
18 pairs

Microsensor characterisation: pH sweep

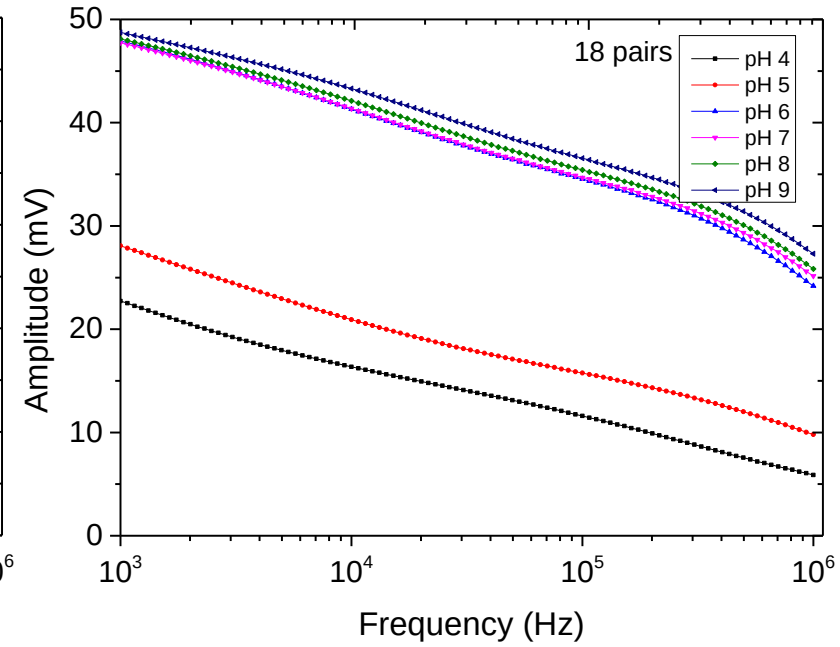
Sweeping range: 10^3Hz - 10^6Hz pH range: pH4 - pH9



6 pairs



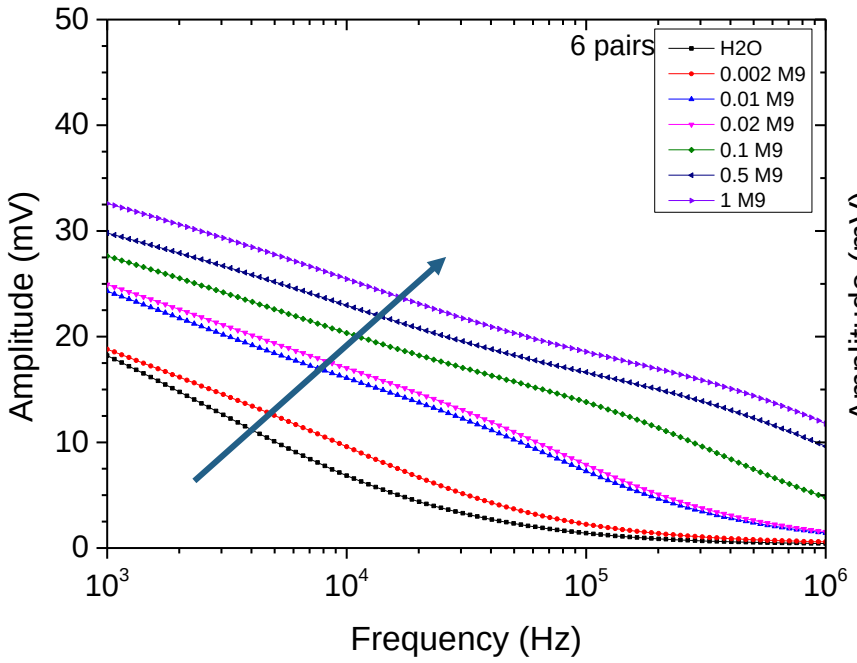
12 pairs



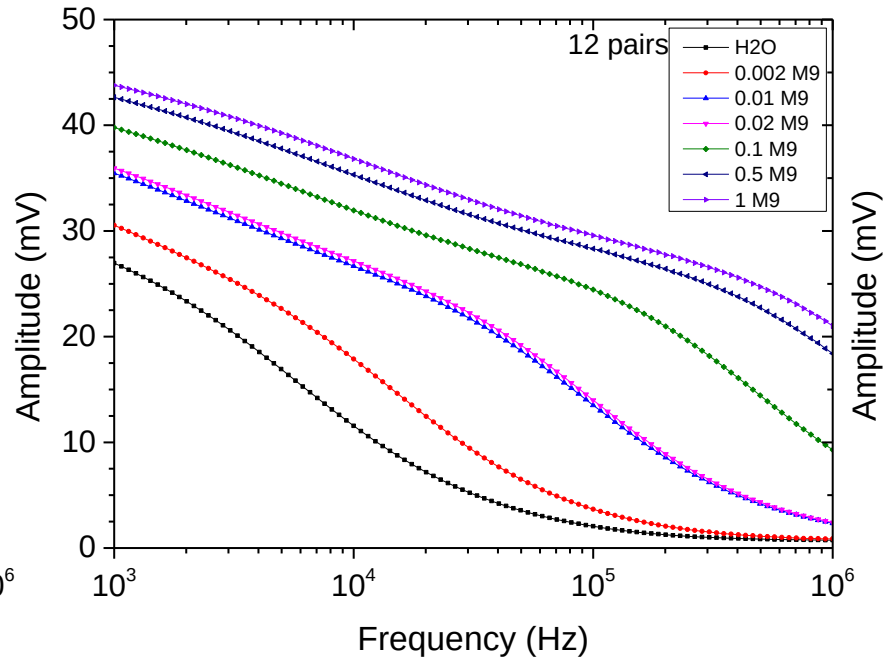
18 pairs

Microsensor characterisation: M9 sweep

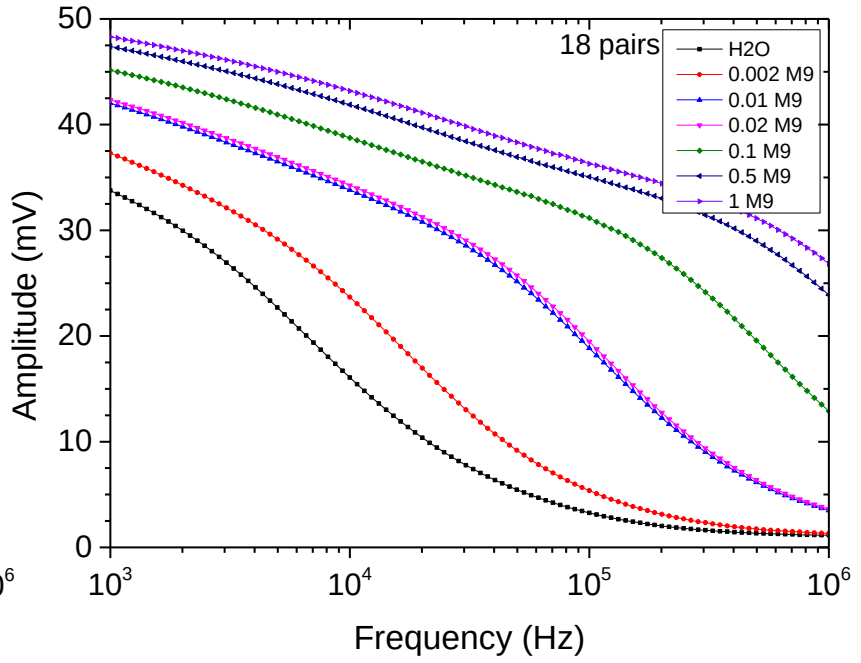
Sweeping range: 10^3Hz - 10^6Hz Dilutions from 10mM M9



6 pairs



12 pairs

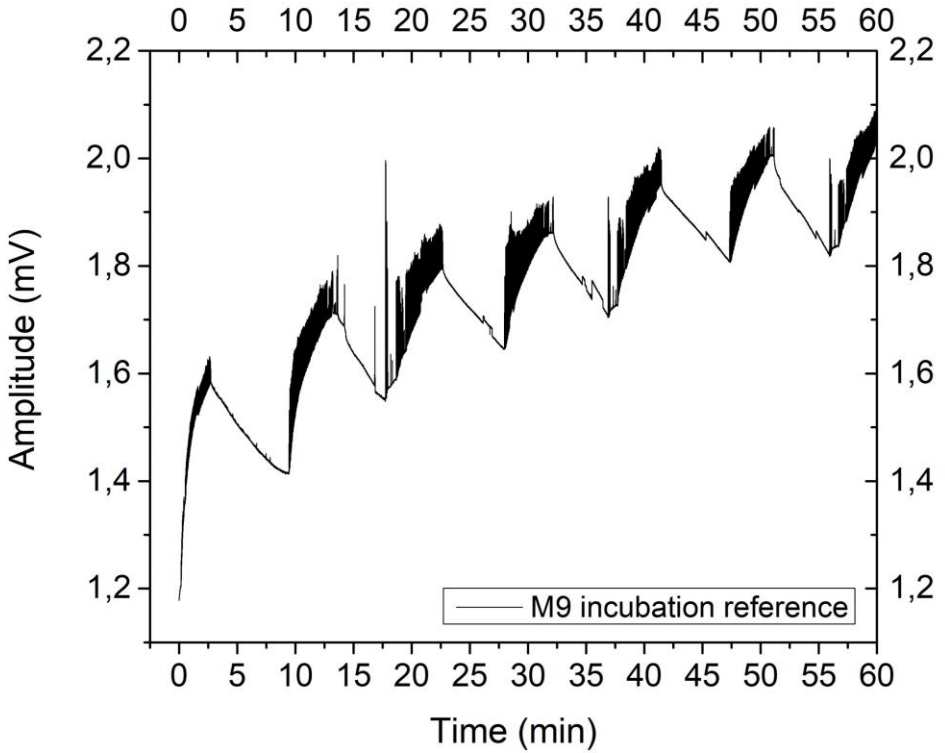


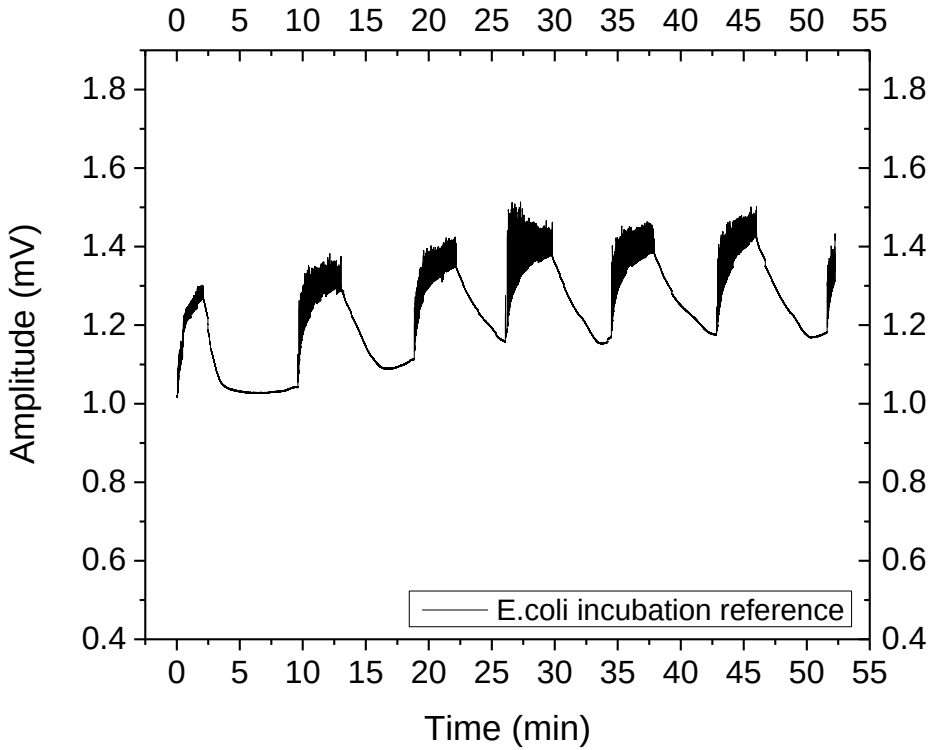
18 pairs

Incubation reference measurements

Measurement parameters:

- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ

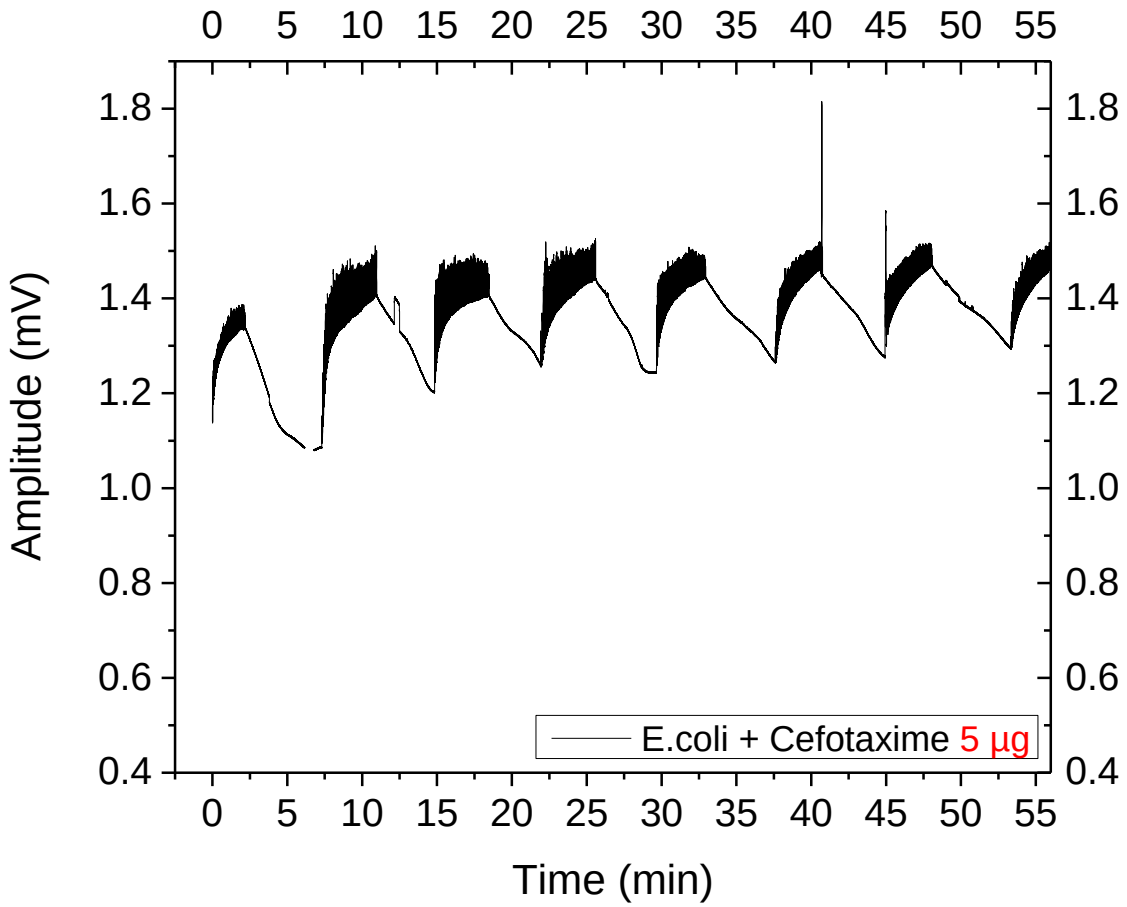




E.coli + *Cefotaxime* incubation 5 µg/ml

Measurement parameters:

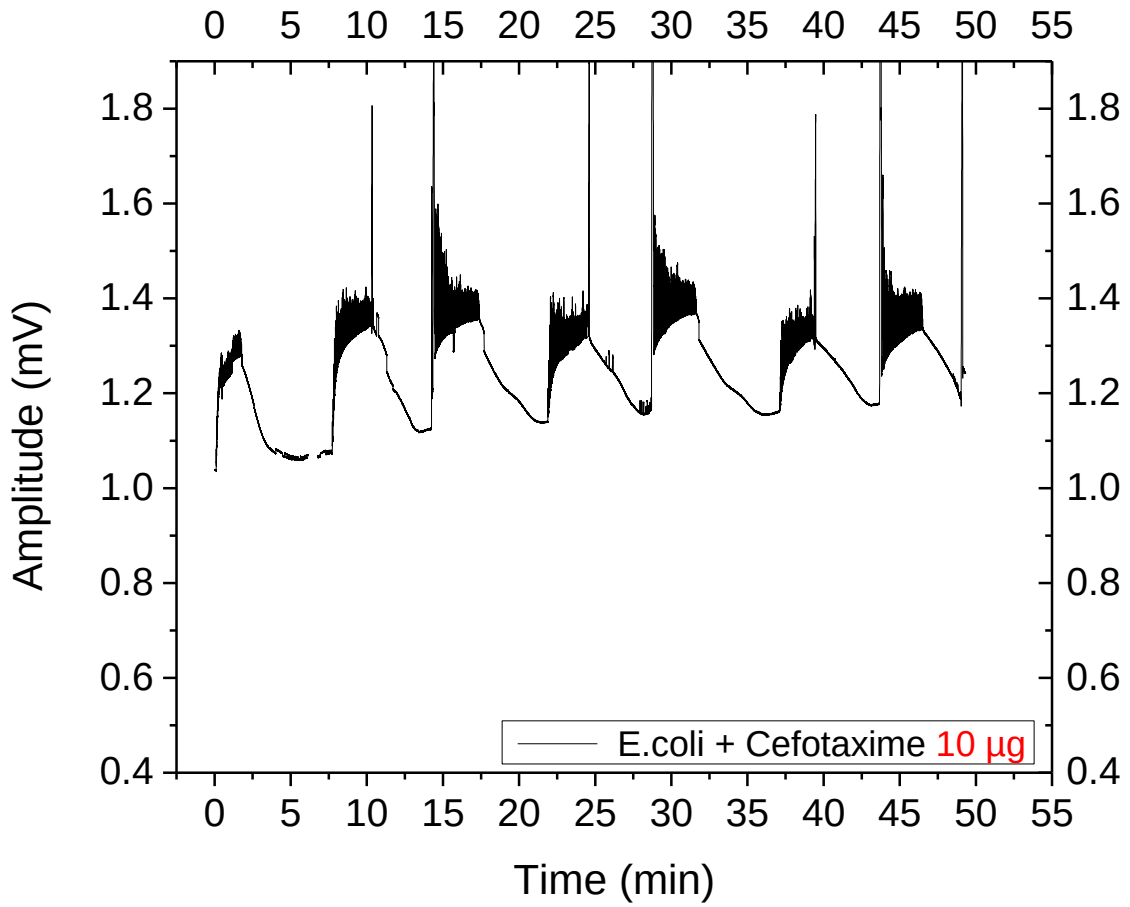
- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ



E.coli + *Cefotaxime* incubation 10 µg/ml

Measurement parameters:

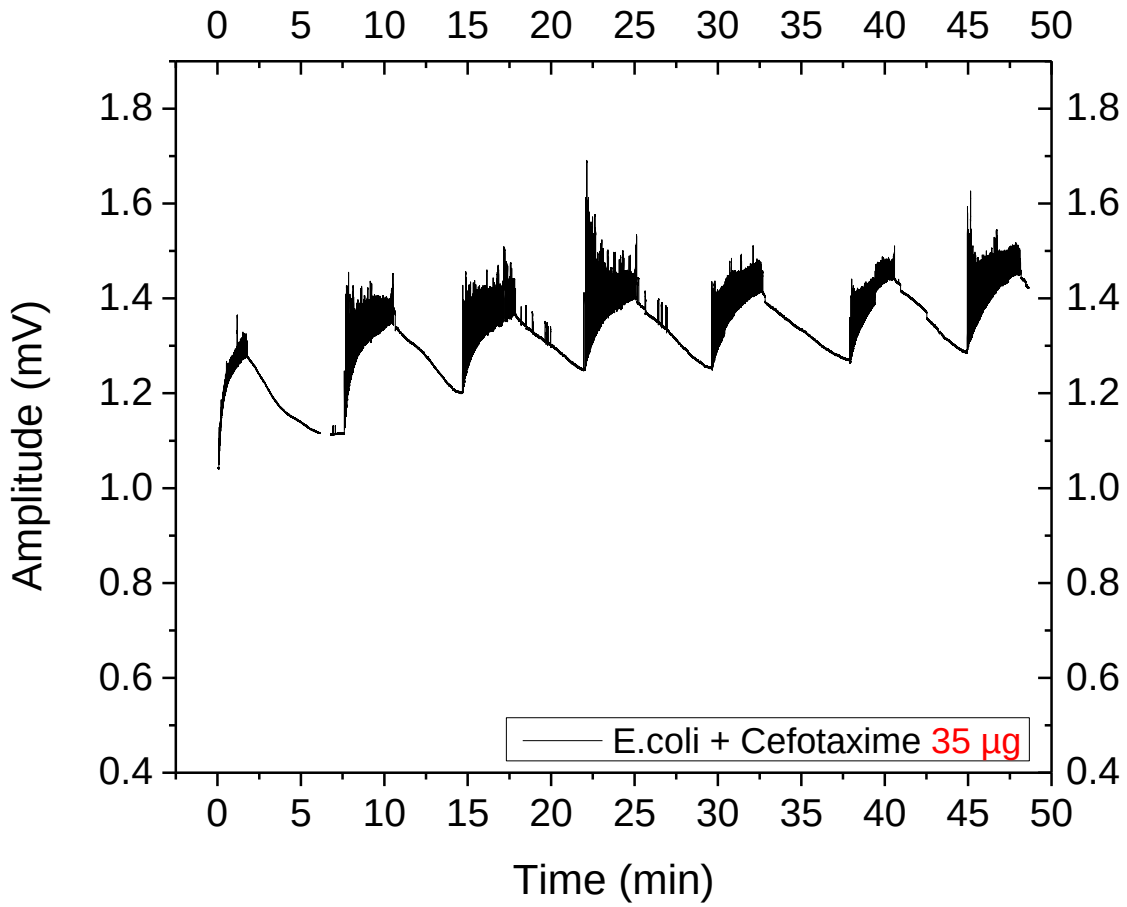
- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ



E.coli + *Cefotaxime* incubation 35 µg/ml

Measurement parameters:

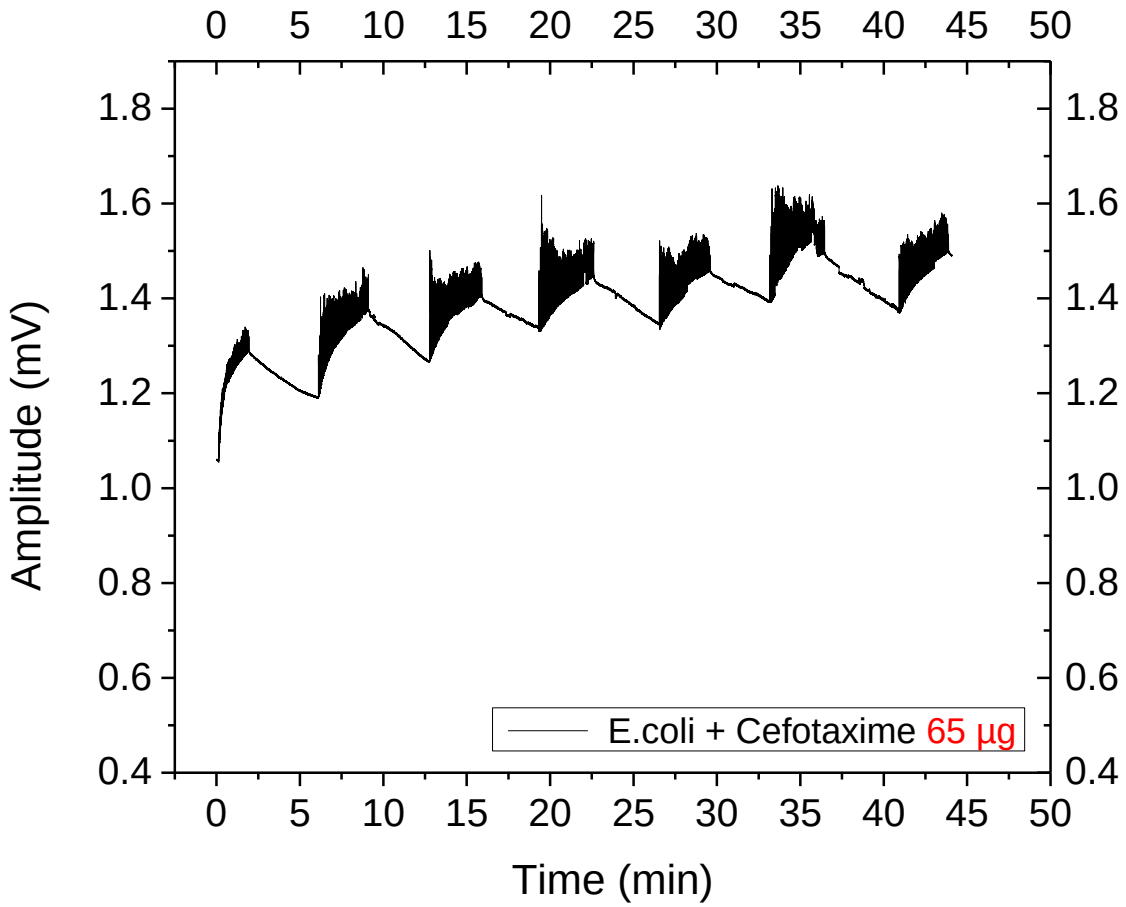
- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ



E.coli + *Cefotaxime* incubation 65 mg/ml

Measurement parameters:

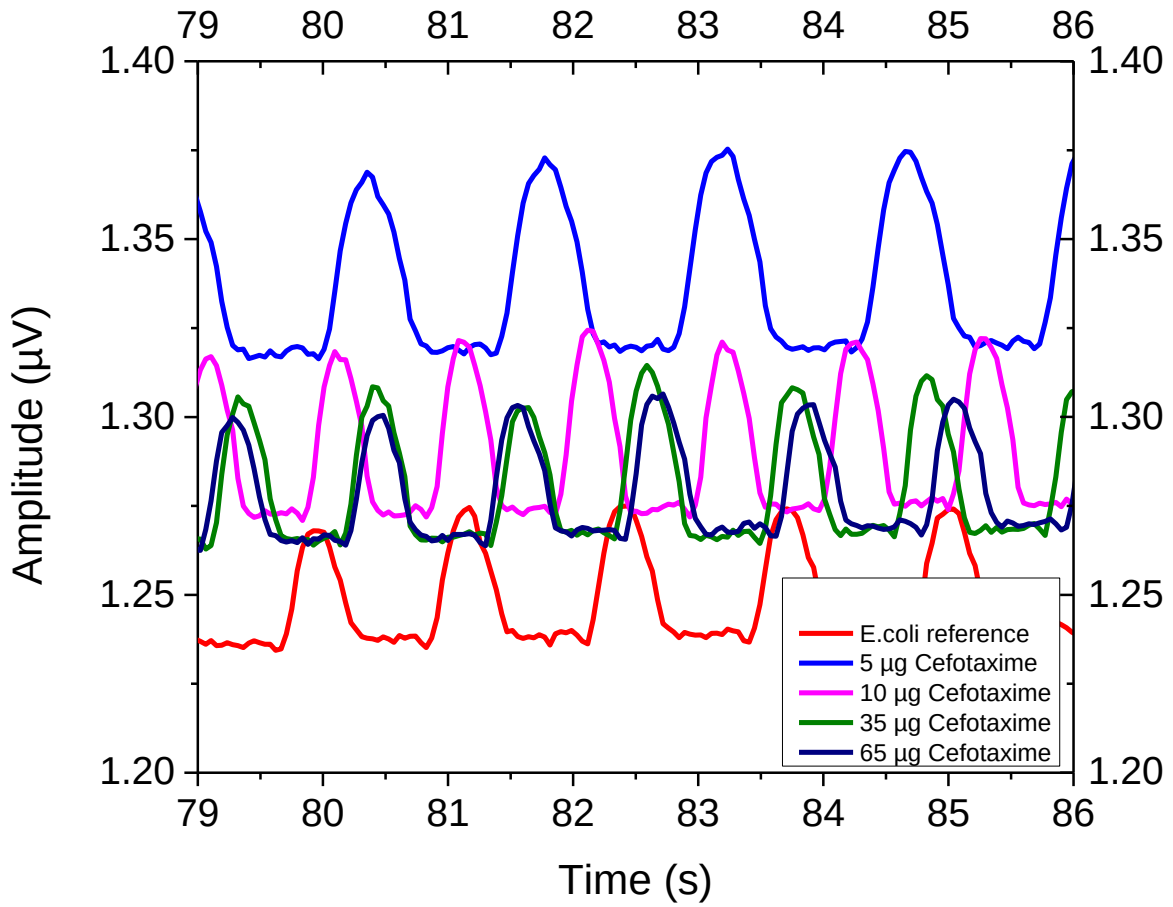
- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ



E.coli + *Cefotaxime* incubation

Measurement parameters:

- f=10 kHz
- C_{ref}=10pF
- V_{in}=0.5 V
- R_{ref}=1MΩ



Ongoing To Do`s:

Full data processing (highest priority):

- Analysis of the phase shift
- Peak analysis
- Impedance calculation

Work on the manuscript

Outlook:

- Switch from syringe to pressure pumps
- Direct measurement of impedance
- Adjustment of oil composition (i.e. surfactant) to higher temperatures
- Other possible biological assays to test with the developed system
- Machine learning implementation for signal pattern recognition

Thank you! Any questions?