TAC meeting

Report on progress, ongoing work and outlook

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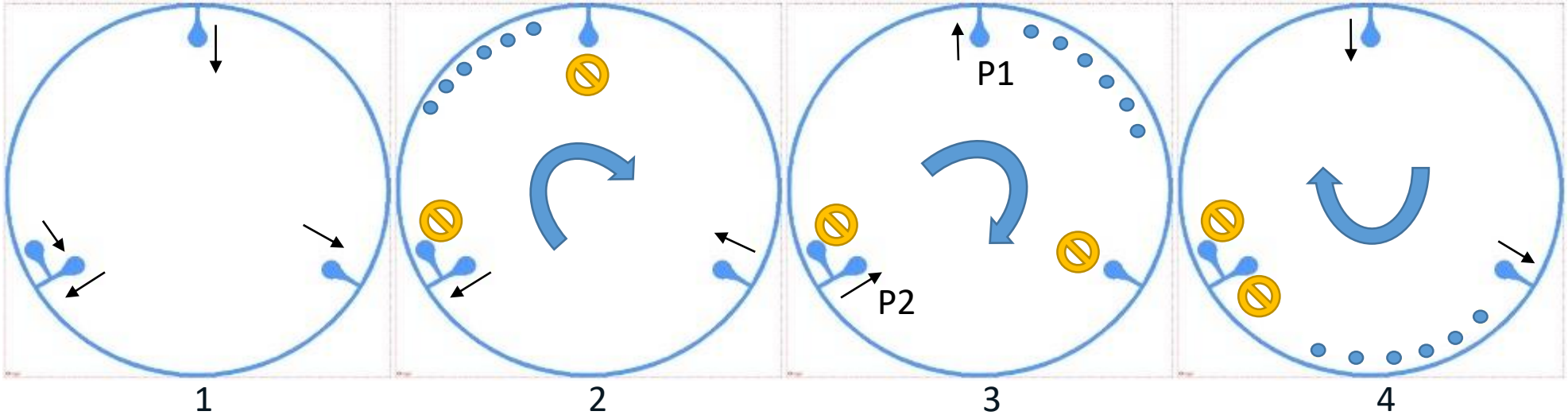
18.05.2021 Dresden

Development of circular microfluidics



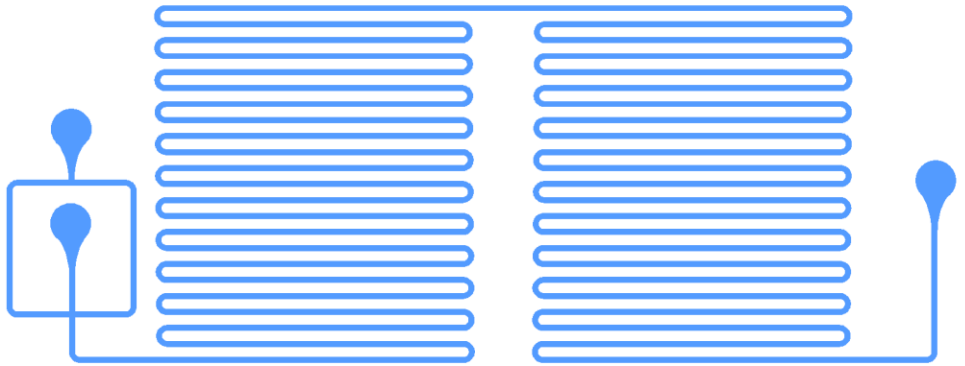
The aim was to design microfluidics to enable circulation:

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

Microfluidics

Switch from circular design to linear approaching “there and back” movement

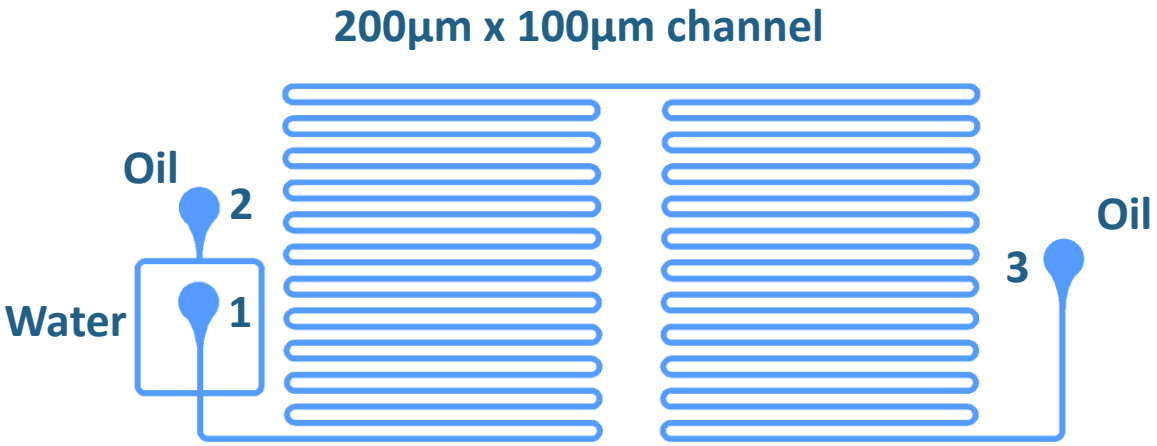
200µm x 100µm channel



- The device is faricated
- The manipulation test are done

An array of 85 droplets is generated and guided over the sensor area in “there and back” manner. The time duration of one cycle is about 6-8 minutes. Up for now is achieved 2 hour and 45 minute total incubation of ones generated droplets.

Microfluidics: recirculation



Occurred problems:

- ✓ Jetting
- ✓ Not immediate droplet generation stopping
- ✓ Water dripping after stop
- ✓ Droplet leaking to the inlet 3
- ✓ Non stability of recirculation steps
- ❑ Droplet merging (after 60 min of incubation)

1) Droplet generation (0.5; 0.5; -1)
2) Stopping the generation (-0.187; 1; -1)
3) Guiding the array to the sensor area (-0.187; 0.687; -0.5)
4) Activation of recirculation algorithm (values slightly differ from device to device):
   (-0.187; -0.313; 0.5) switch in 6 min 45 sec to (-0.187; 0.687; -0.5)

Microfluidics: why we lost 4 weeks of work? Syringes!

What was the problem?
Random, unstable behaviour of liquids inside the devices (water, oil, LB buffer, bacteria).

During that period we:
Made about 70 hours of attempts to set recirculation parameters
Spent about 50 hours on fabrication of new devices, masks and chips
Specifically: changed the dimensions of the channel (from 100 to 50 µm), changed the substrate, changed to HFE oil), changed the algorithm MF manipulation (over and over again…)

Until one day we saw the leaking syringe…

With new syringes we manage to finish the system within 3 days.

Nano-electrode chip fabrication

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:

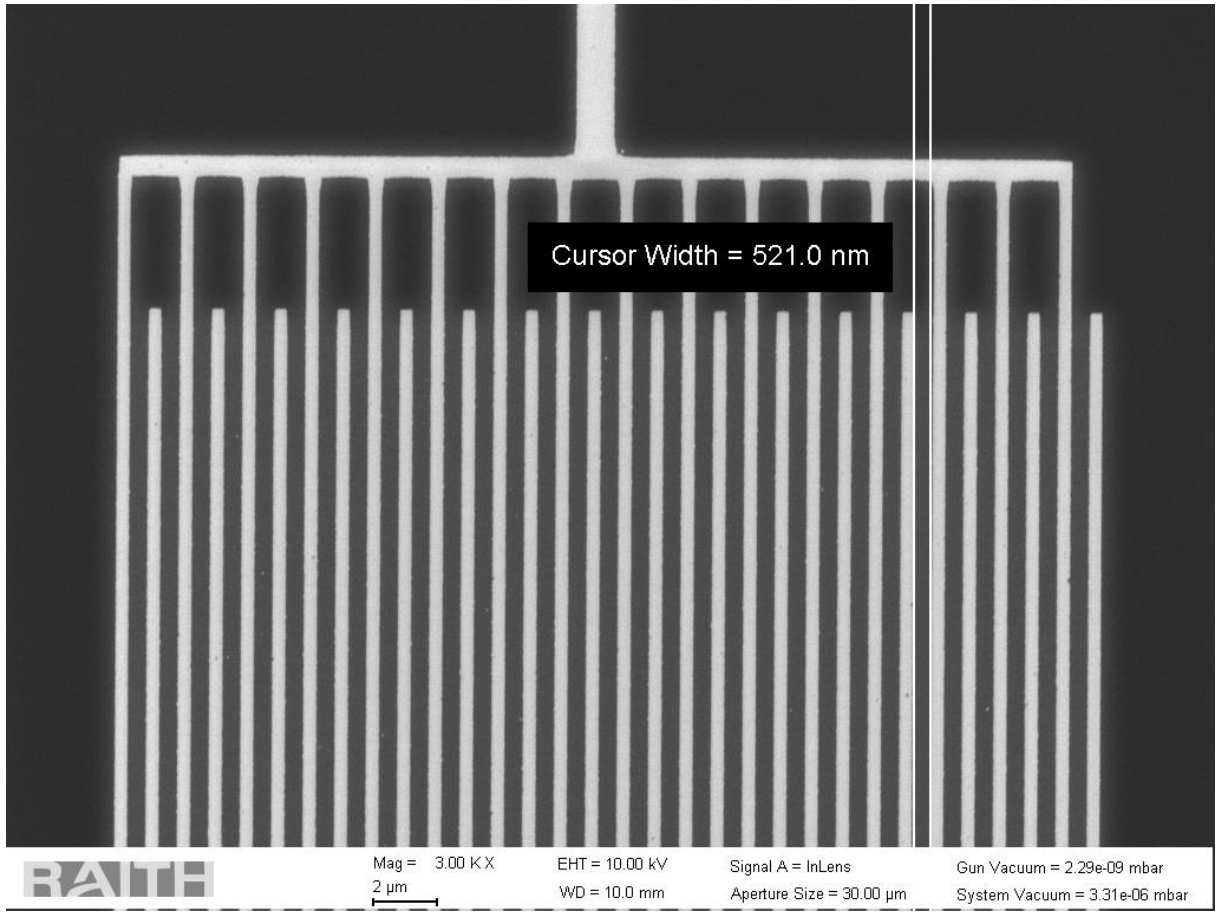
Lift-off !

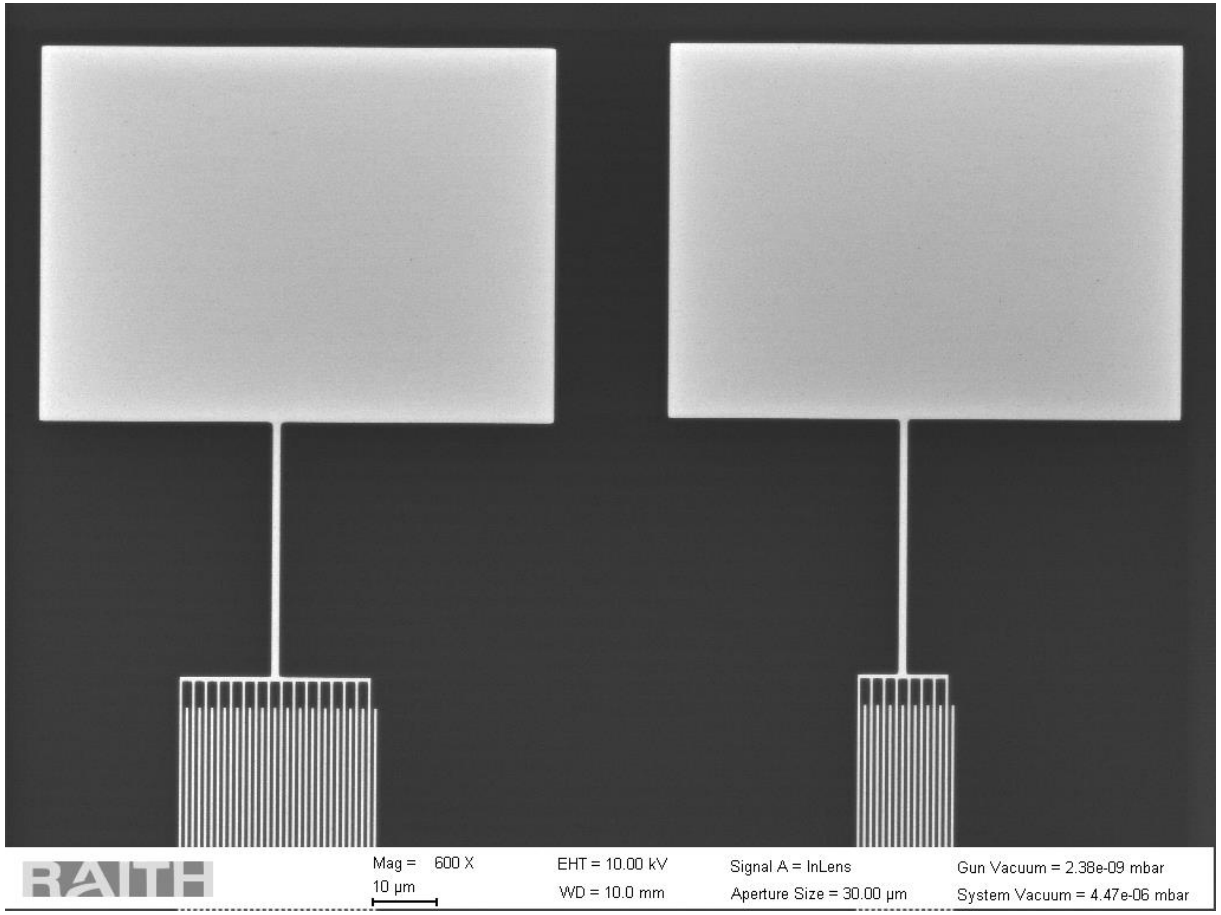
Adjusting parameters:

- PMMA thickness
- Gold thickness
- E-beam dose and etc.

Switching to micro electrode via UV-lithography…

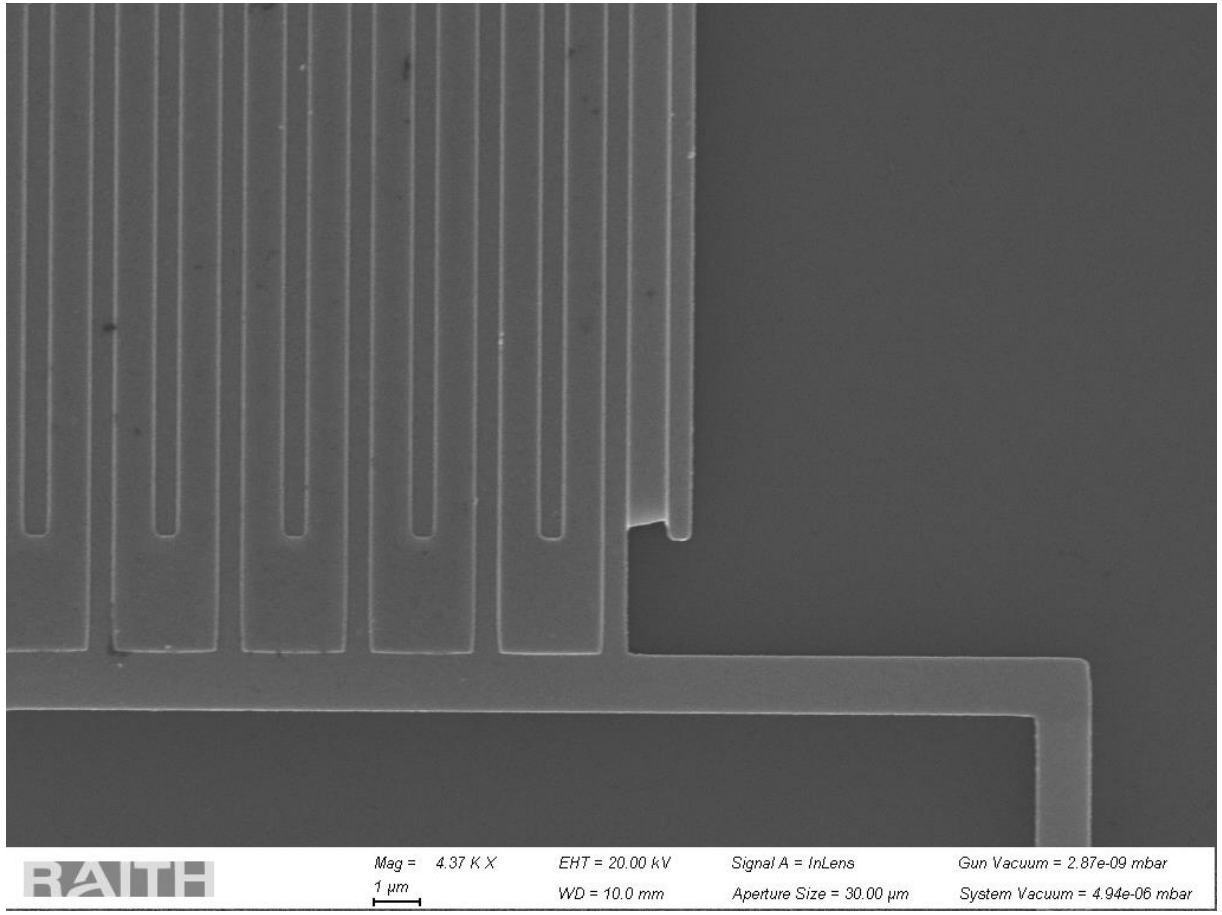
Nano-electrode chip fabrication

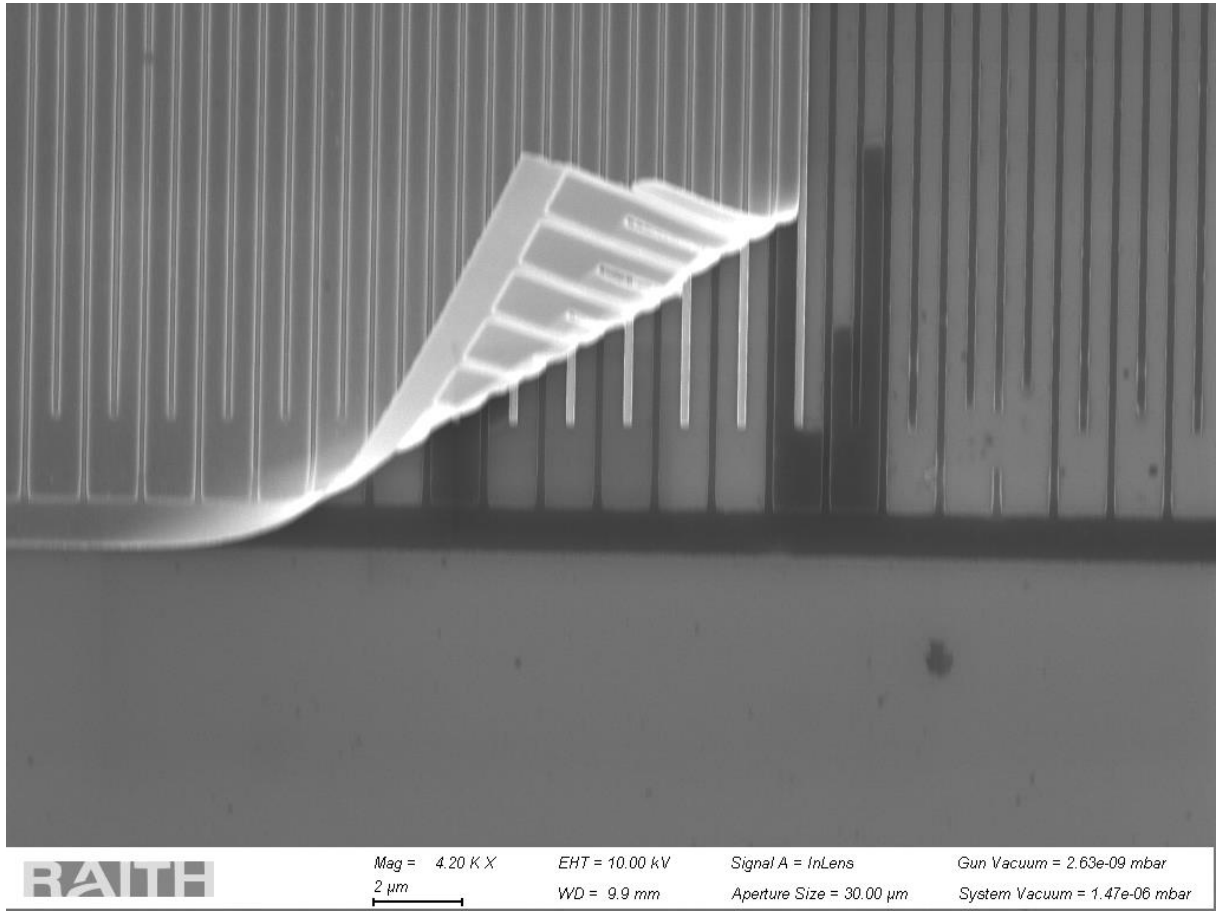




Patterned and developed PMMA

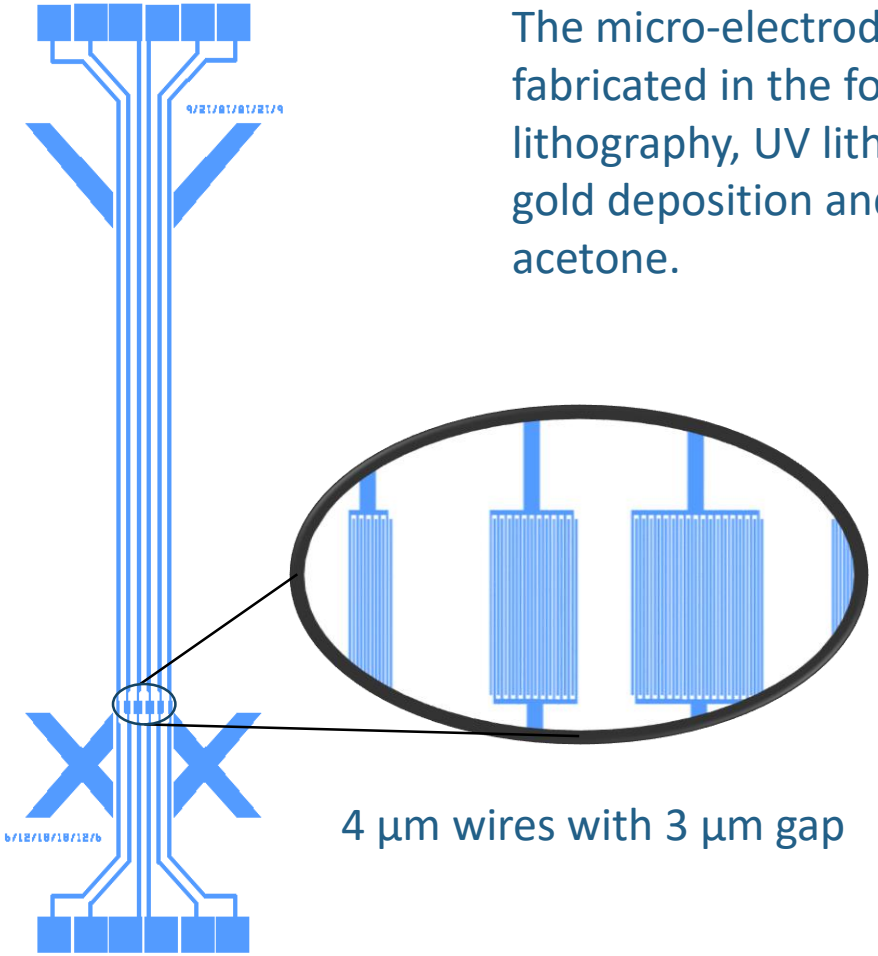
Nano-electrode chip fabrication





After Au lift-off in acetone

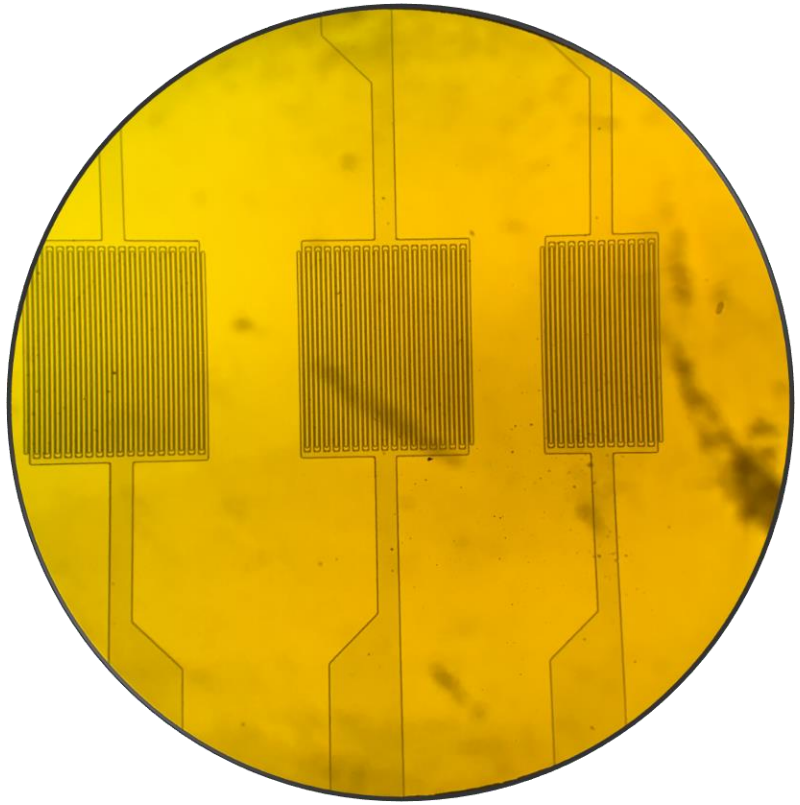
Micro-electrode chip: fabrication



The micro-electrode chips were fabricated in the following laser lithography, UV lithography, gold deposition and lift-off in acetone.

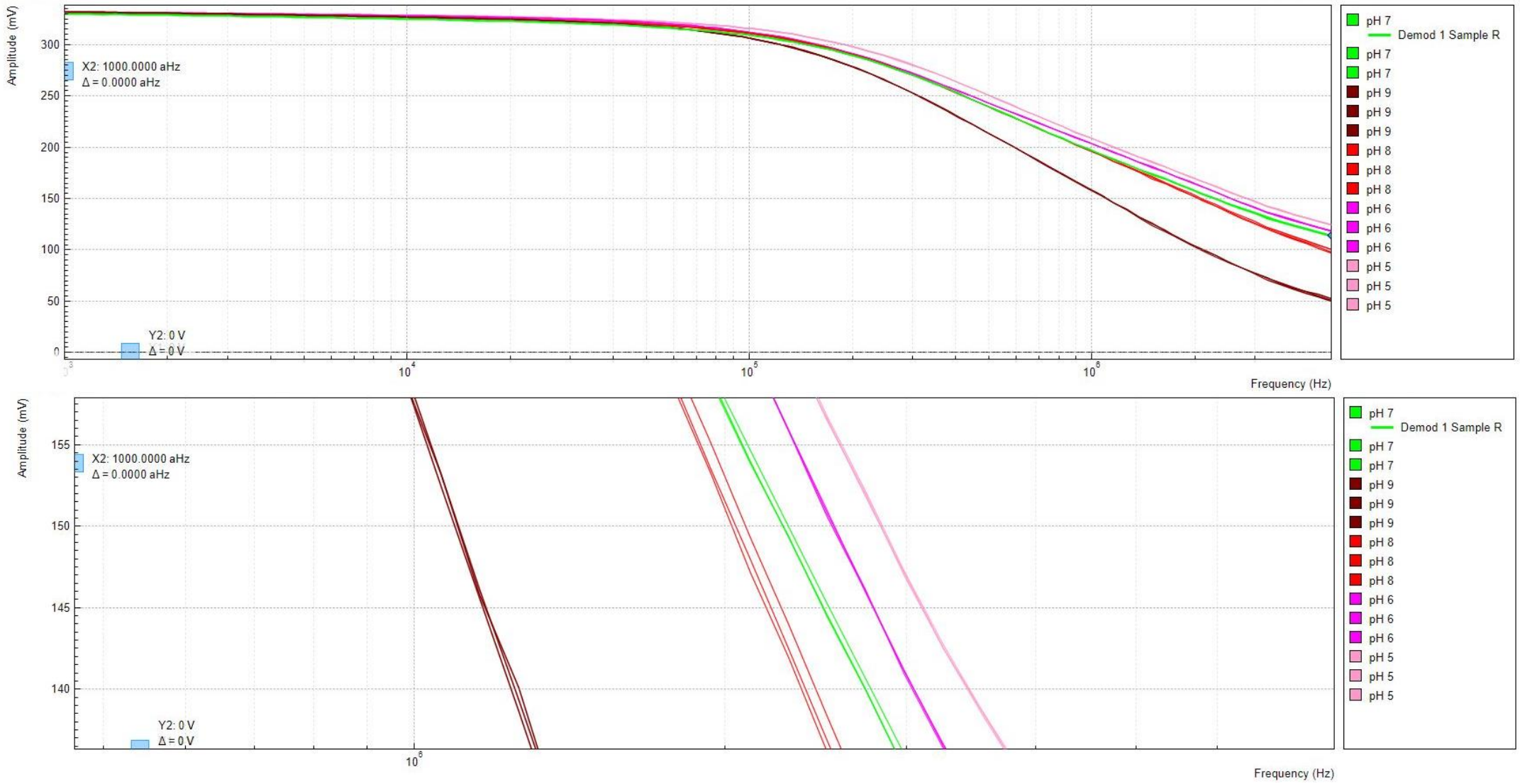
4 µm wires with 3 µm gap

The device was fabricated on Si and switched to glass due to adhesion problems

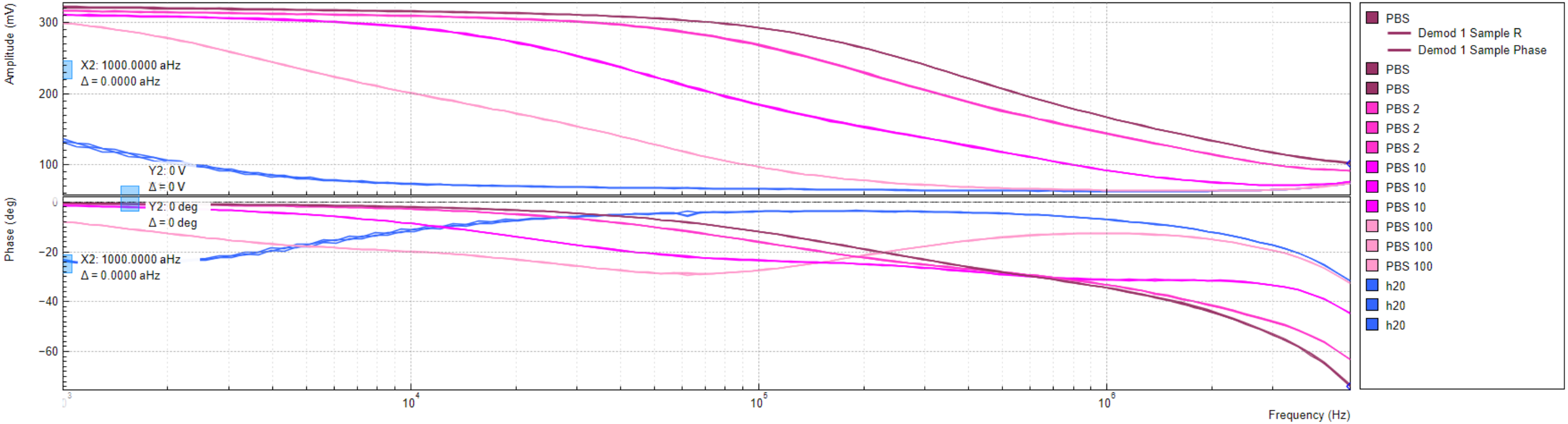


Minor problems occurred, all solved.

Micro-electrode chip: sweeping vs pH



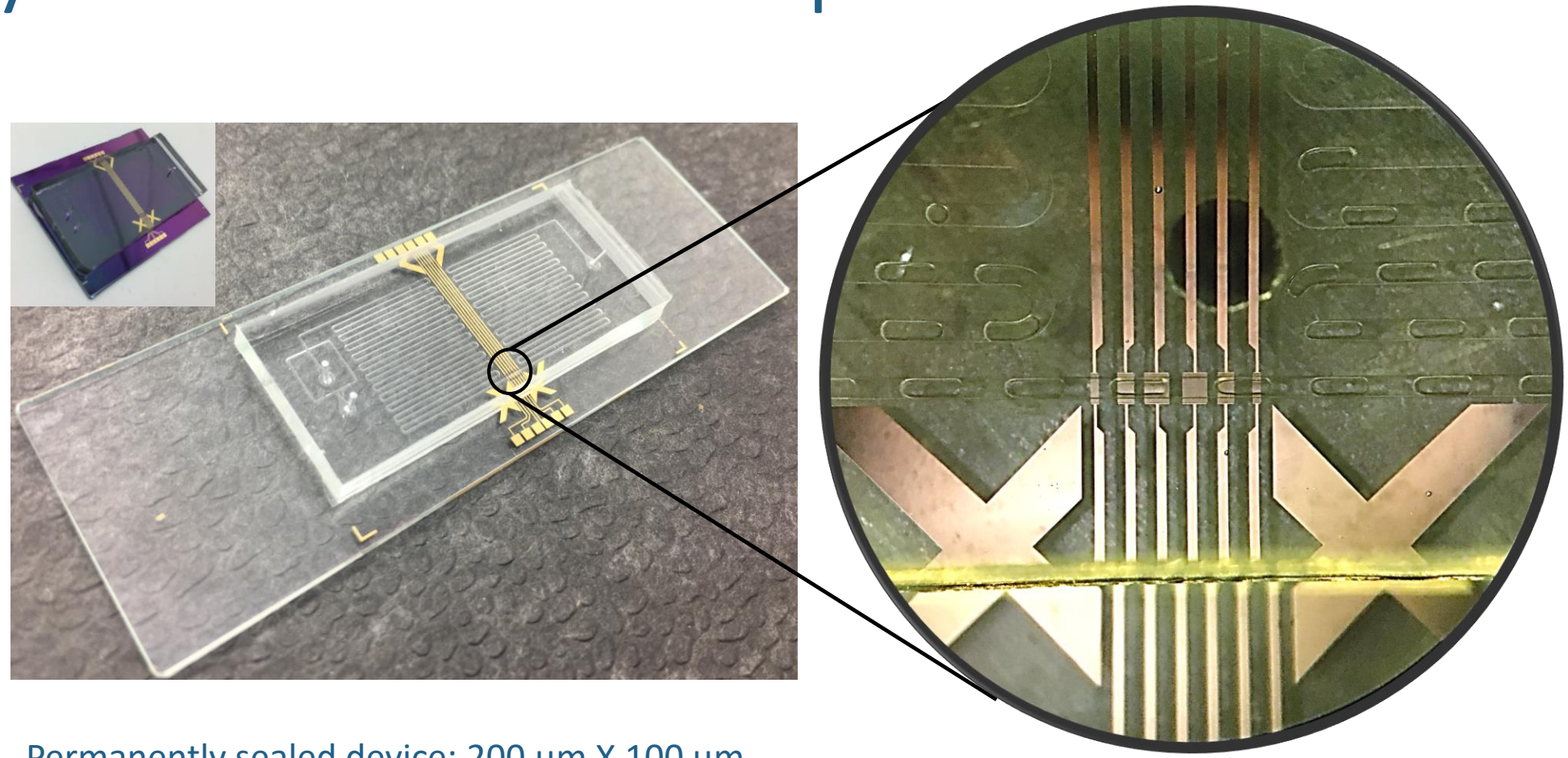
Micro-electrode chip: sweeping vs [PBS]



Final assembly of MF and electrode chip

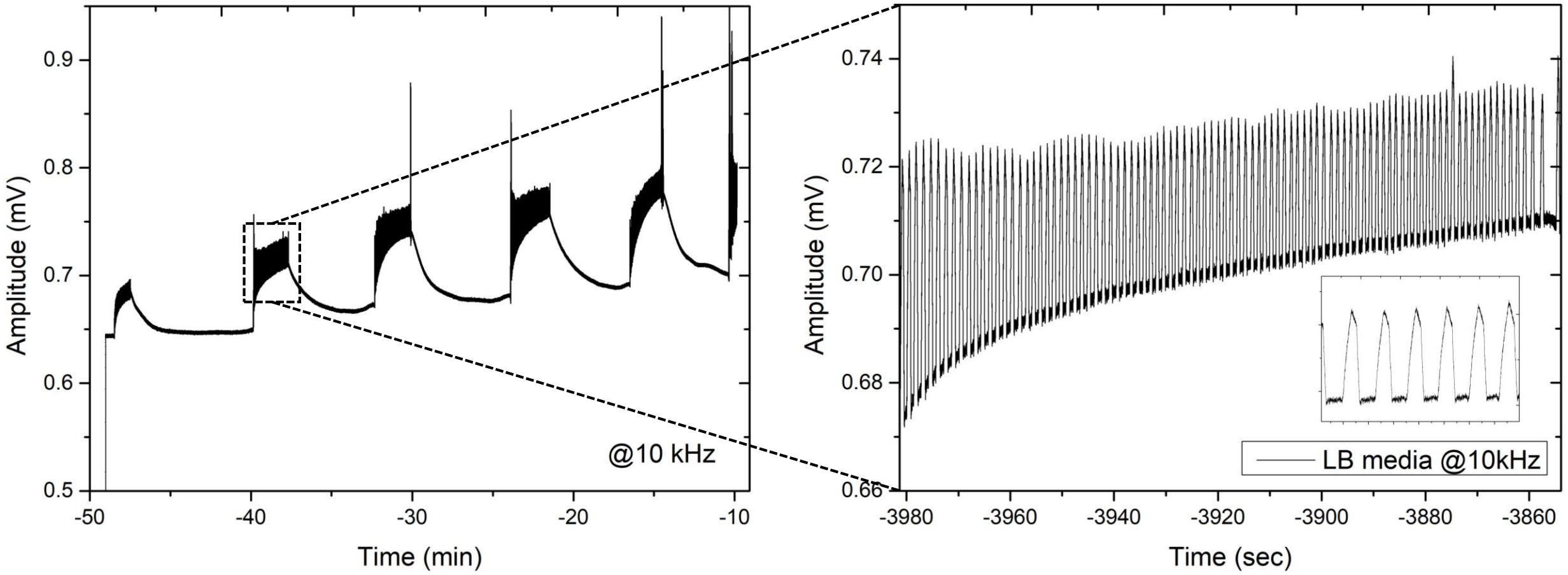
The PDMS flow-cell and the micro electrode chip were plasma treated @ 1 mbar, 100W for 8 sec.

Then manually aligned and attached. Heated on the hotplate @75C under weight for 2 hours.



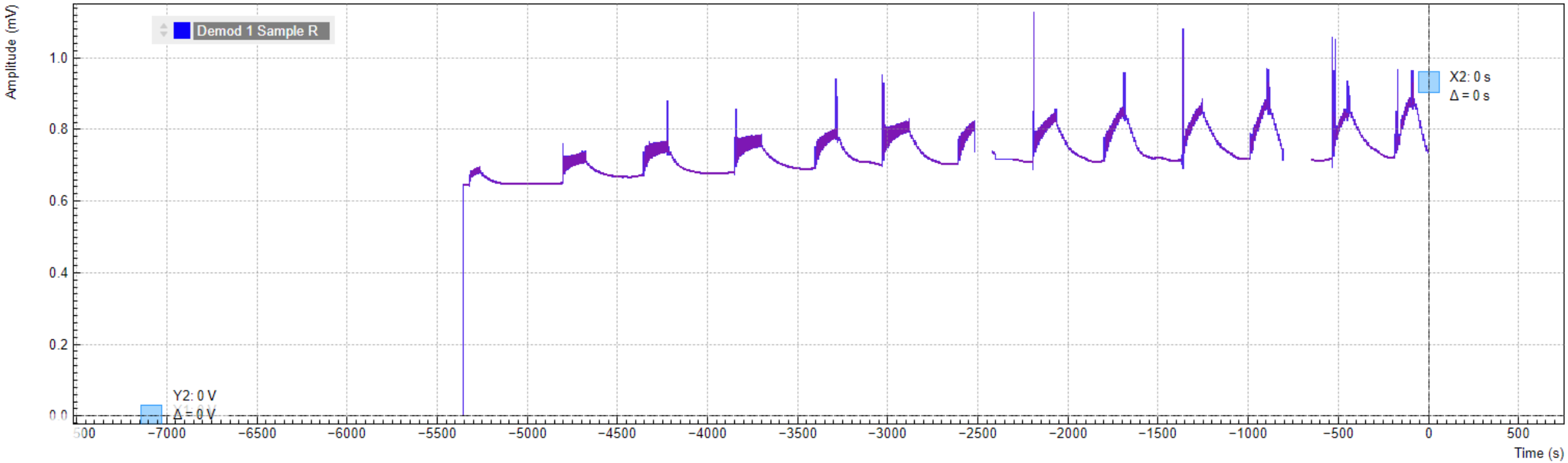
Permanently sealed device: 200 µm X 100 µm channel, 6 electrode sensors on board with 6, 12 and 18 interdigitated micro-wire pairs (each duplicated).

Impedance measurement at 10kHz (LB media)



Impedance measurement at 10kHz (LB media)

The monitoring of the LB media droplets during 1.5 hours



$Amplitude_{init}$ = 0.7 mV

$Amplitude_{90min}$ = 0.88 mV

Some data is lost…

Impedance measurement at 10kHz (*E.Coli*)

- Quick test was made without recirculation @10kHz.
- *E.Coli* (YFP) was diluted to the value of 5 cells per nl.
- The bacteria culture inside the syringe was warmed by IR lamp.
- Droplets were constantly generated and guided over the electrode for 1 hour.
- After ~ 1 hour the level of Amplitude changed **from 0.7-0.8 mV to 3 mV**

$Amplitude_{init}$ ~ 0.7 mV
$Amplitude_{60min}$ ~ 3mV

$OD600_{init}$ = 0.147A
$OD600_{60min}$ = 0.288A

Unfortunately the data is lost…

To do: final(ly) measurements!

- Resolve data problems (possibly exchange the measuring device)
- Measurements:
  - LB buffer monitoring as reference
  - *E.coli* YFP growth monitoring without the antibiotic (+OD600 value reference)
  - *E.coli* YFP growth monitoring with the antibiotic (+OD600 value reference)
  - *B9* growth monitoring without the antibiotic (+OD600 value reference)
  - *B9* growth monitoring with the antibiotic (+OD600 value reference)

- Work on the manuscript
- Work on the paper

The plan is to submit the version of the thesis until end of June (paper in parallel)

Thank you for attention!