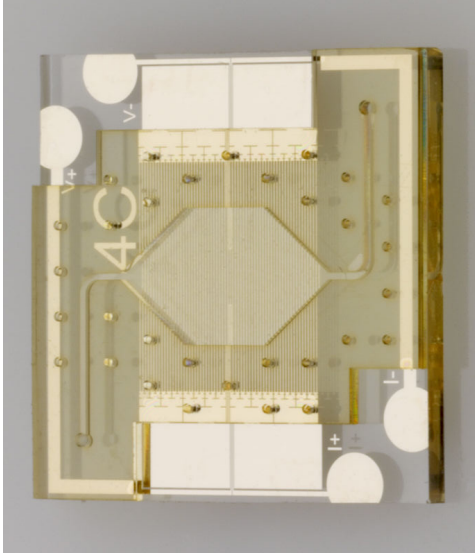


CONCENTRATION, LYSIS AND REAL-TIME PCR ON A SU-8 LAB ON A CHIP FOR RAPID DETECTION OF *Salmonella* spp. IN FAECES



M. Agirregabiria¹, D. Verdoy², G. Olabarria², J. Berganzo¹, J. Berganza², L. J. Fernandez¹, M. Pascual de Zulueta², K. Mayora¹, P. Aldamiz-Echevarria², and J. M. Ruano-López¹

Outline

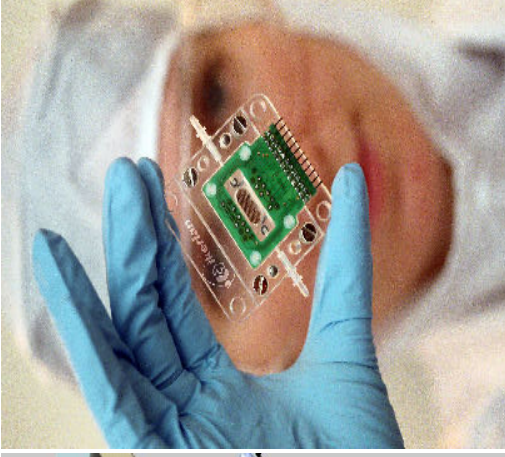
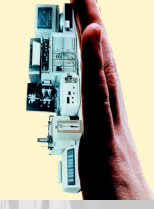
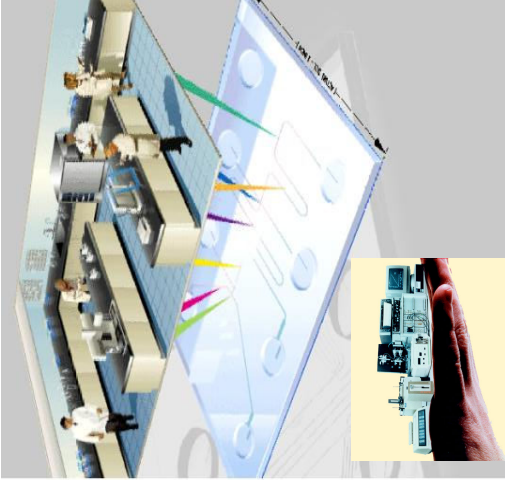
- ❖ **Introduction**
- ❖ **Goal**
- ❖ **Design**
- ❖ **Fabrication**
- ❖ **Experimental methodology**
- ❖ **Results**
- ❖ **Conclusions and future work**

Introduction

Real food, human,
environmental
sample preparation



Construction of rapid and quick laboratories



Sample preparation at low cost = Successful LOC

Outline

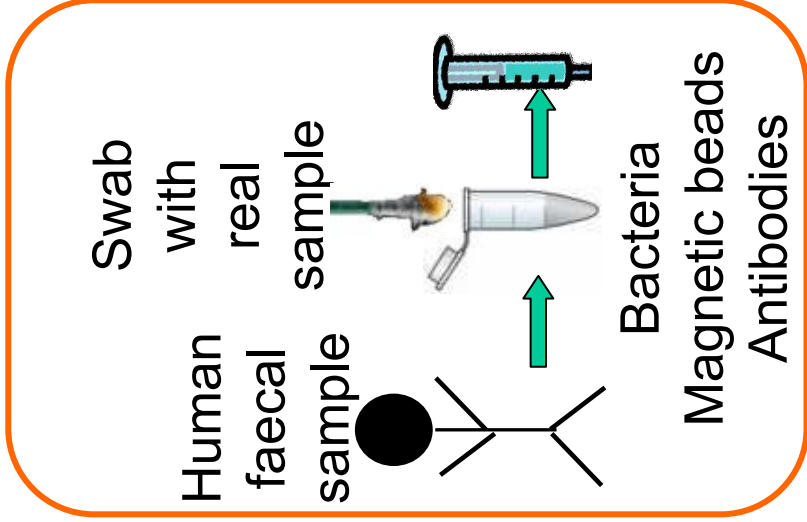
- ❖ Introduction

- ❖ **Goal**

- ❖ Design
- ❖ Fabrication
- ❖ Experimental methodology
- ❖ Results
- ❖ Conclusions and future work

Goal

Complete pathogen detection on a chip: from real sample to detection



Sample collection



Bacteria concentration



Lysis and real-time PCR

Outline

❖ Introduction

❖ Goal

❖ **Design**

✓ Device design
✓ Biological reagents design

❖ Fabrication

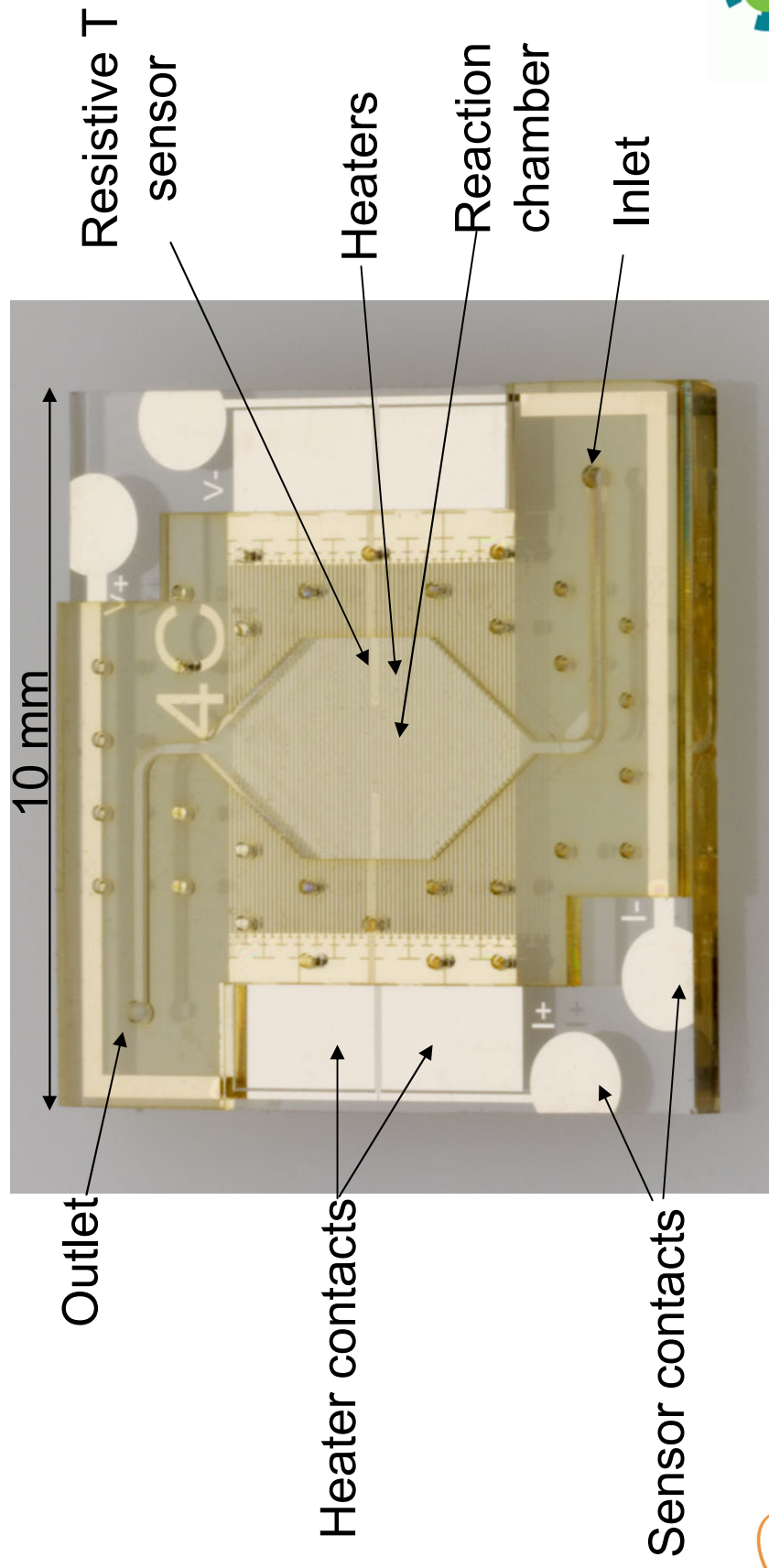
❖ Experimental methodology

❖ Results

❖ Conclusions and future work

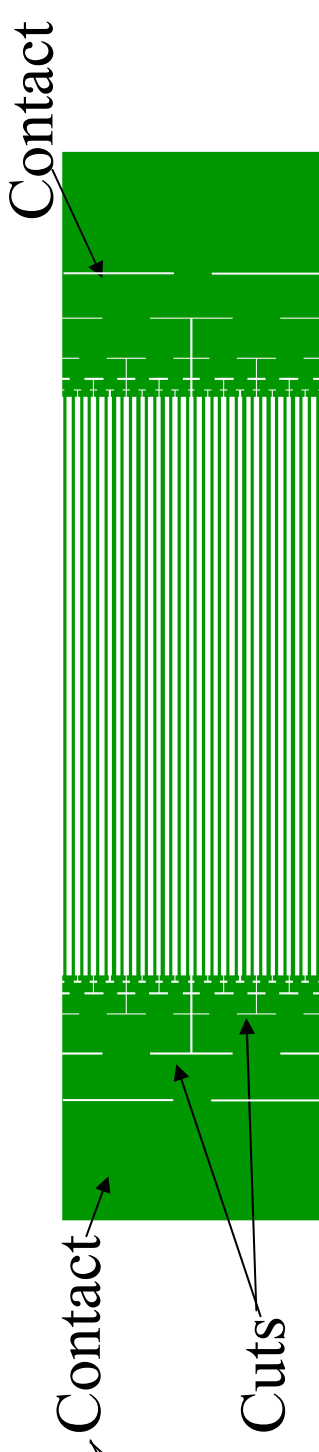
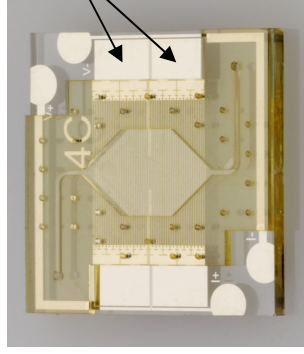
Device design I

- ❖ 200 μm high, 3 mm wide and 5.4 mm long chamber (2.5 μl)
- ❖ 2 parallel Ti/Pt heaters
- ❖ A resistive sensor



Device design II: Heater

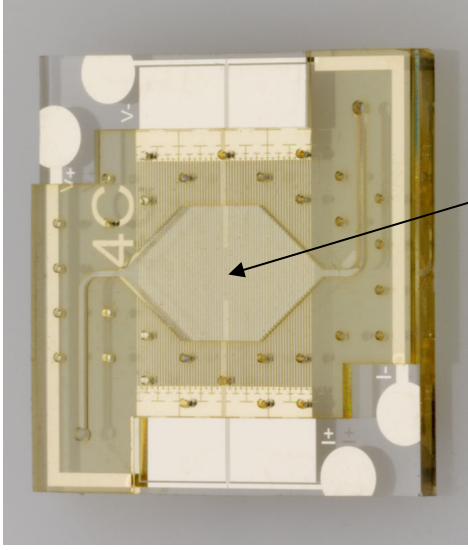
2 parallel heaters consisting of 32 heating elements
20 μ m wide, 5mm long and 50 μ m spaced



Uniform current distribution = uniform temperature

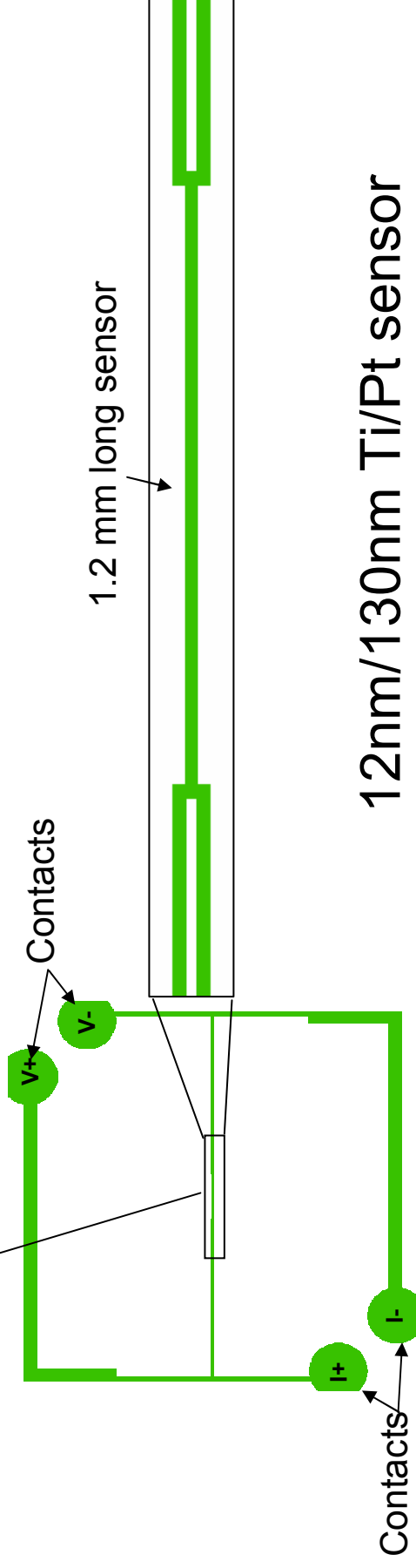


Device design III: sensor



Pt temperature sensor integrated
on chip

4 wire resistance measurement



12nm/130nm Ti/Pt sensor

Linear resistance/temperature behaviour

Outline

❖ Introduction

❖ Goal

✓ Device design

❖ Design

✓ Biological reagents design

❖ Fabrication

❖ Experimental methodology

❖ Results

❖ Conclusions and future work

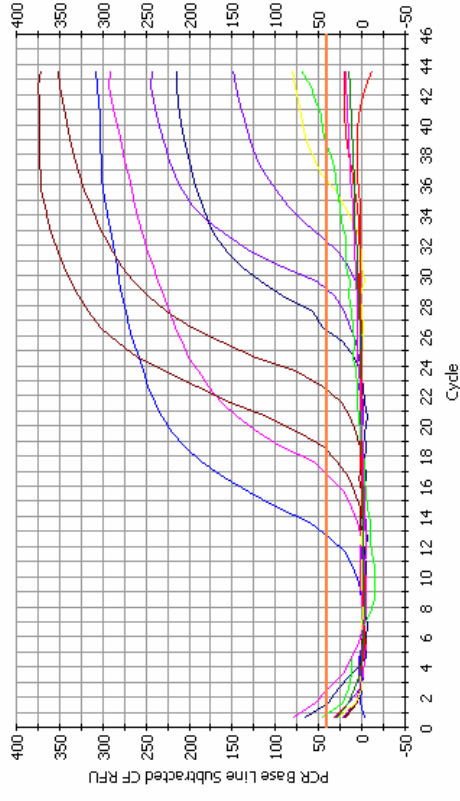
Biological reagents design

Optimization of PCR conditions and PCR mixture components using conventional methods

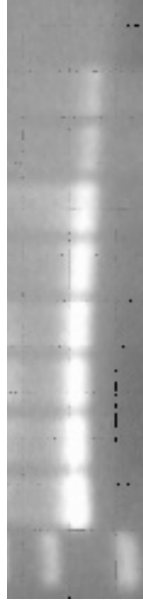


Salmonella genome copies/PCR C_T

| | |
|----------|------|
| Negative | N/A |
| 10E1 | 36.3 |
| 10E2 | 32.3 |
| 10E3 | 29 |
| 10E4 | 26.4 |
| 10E5 | 22.3 |
| 10E6 | 18.4 |
| 10E7 | 16.8 |
| 10E8 | 12 |



8 7 6 5 4 3 2 1



158 pb →

Biological reagents design

- **Negative strains tested**

Campylobacter jejuni

Citrobacter freundii

Enterococcus faecalis

Enterobacter aerogenes

Escherichia coli

Listeria monocytogenes

Proteus vulgaris

Pseudomonas aeruginosa

Serratia marcescens

Staphylococcus aureus

- **Positive strains tested**

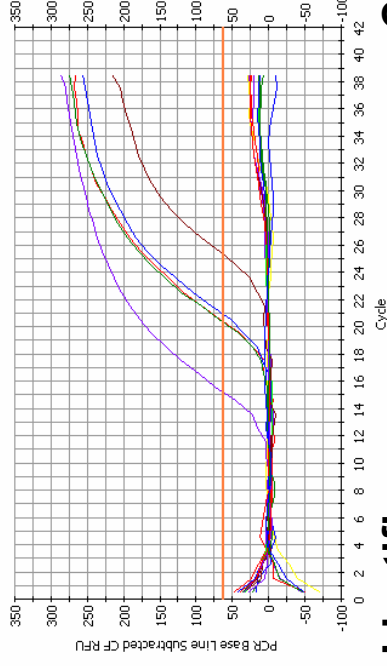
S. enteritidis 1209-251641

S. enteritidis 1216-251083

S. typhimurium 1212-251713

S. typhimurium 1210-251659

S. typhimurium CECT 443



Identifier **Ct**

C.jejuni N/A

S.Marcenses N/A

S.enteritidis 41 20.4

C.freundii N/A

S.aureus N/A

S.enteritidis 20.3

E.faecalis N/A

S.typhimurium 20.9

E.Aerogenes N/A

S.typhimurium 25.3

E.coli N/A

S.typhimurium 443 15.1

L.monocytogenes N/A

P.vulgaris N/A

Negative N/A

Outline

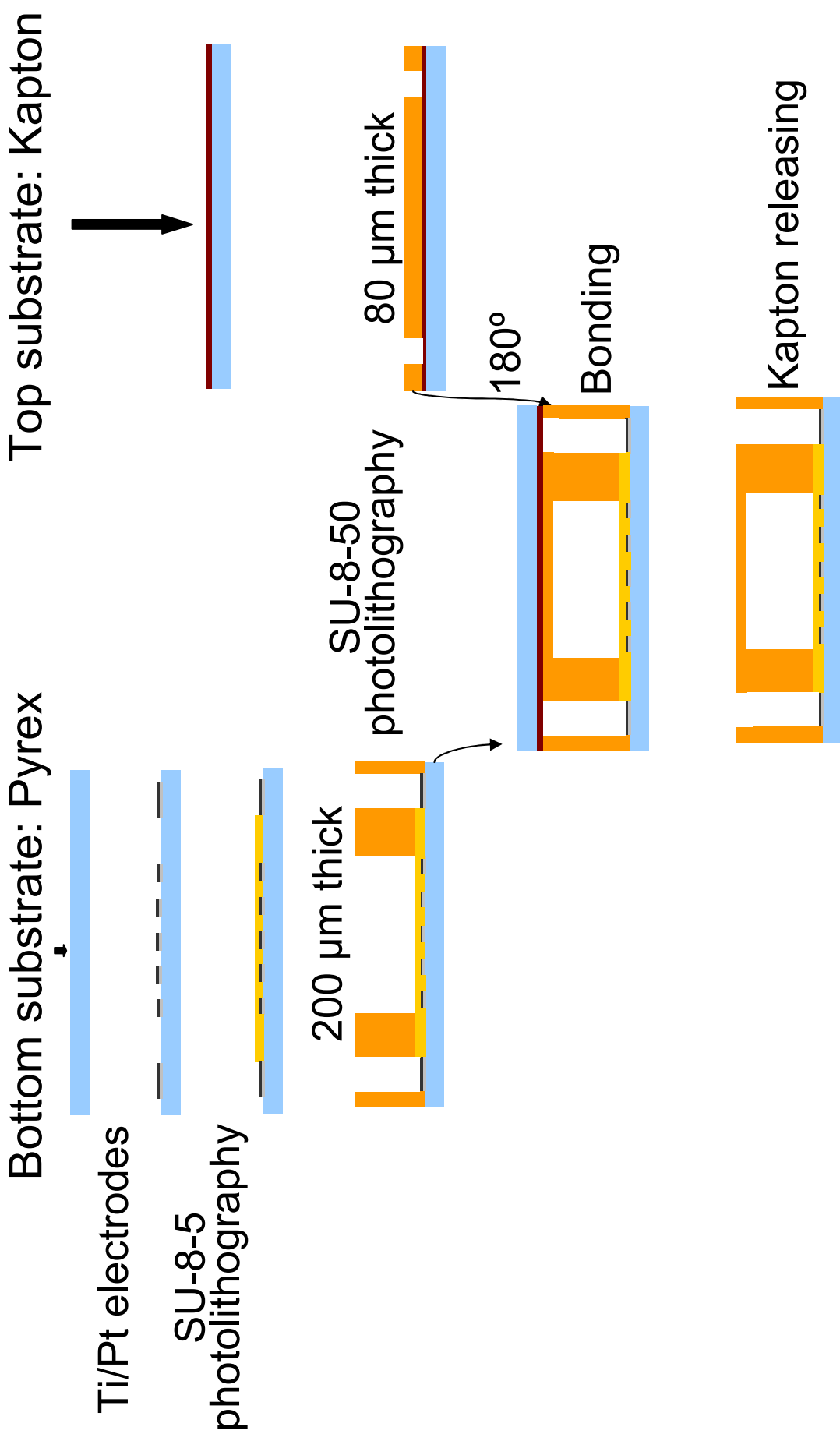
- ❖ Introduction
- ❖ Goal
- ❖ Design

❖ **Fabrication**

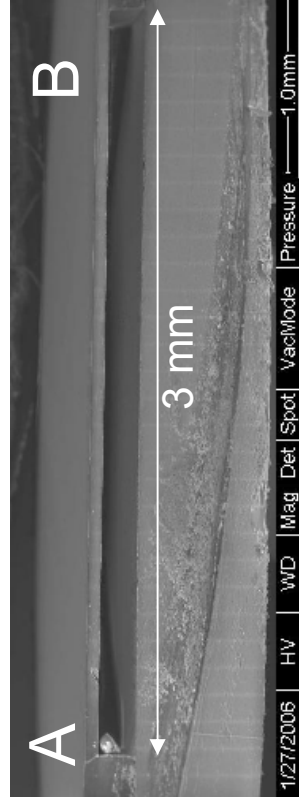
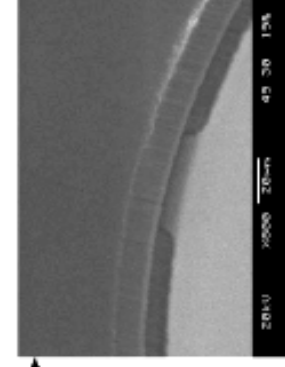
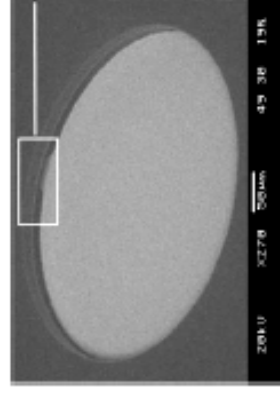
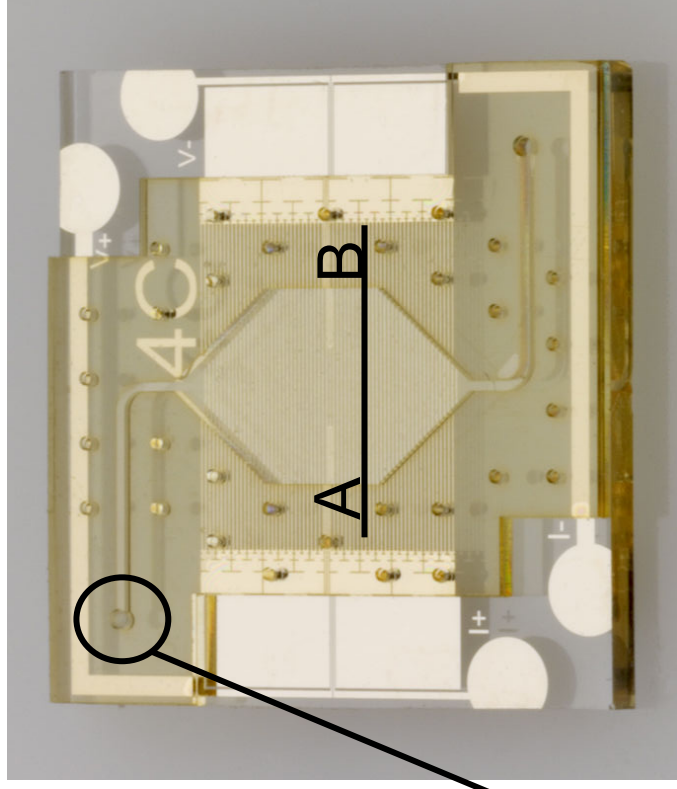
- ✓ **Fabrication process**
- ✓ **Calibration**
- ✓ **Washing**
- ✓ **Packaging**

- ❖ Experimental methodology
- ❖ Results
- ❖ Conclusions and future work

Fabrication process



Fabrication process



Calibration

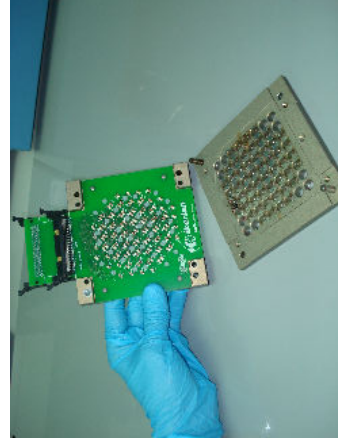
Average R at 30°C = 84.36Ω

Average TCR = 1979

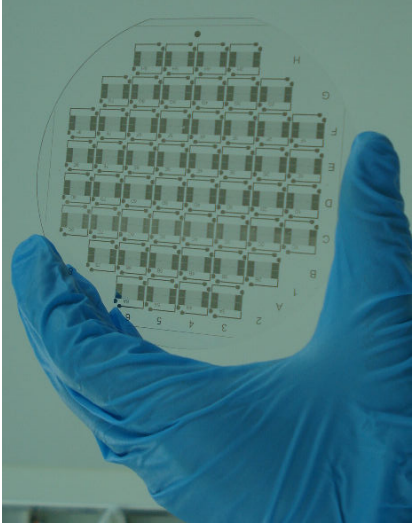
| | StD in the wafer (%) | StD between wafers (%) |
|-------|----------------------|------------------------|
| TCR | 0.86 | 3.08 |
| R (Ω) | 4.1 | 23.35 |

ΔT = 2.53°C

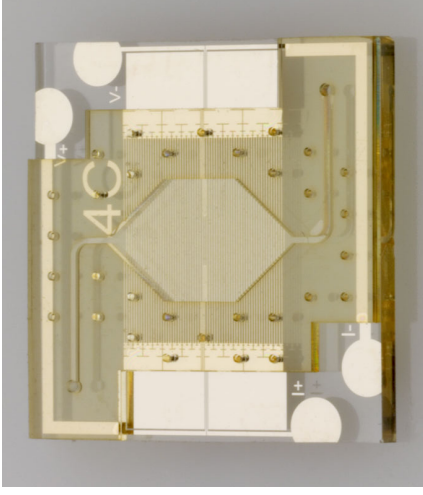
ΔT = 0.7°C



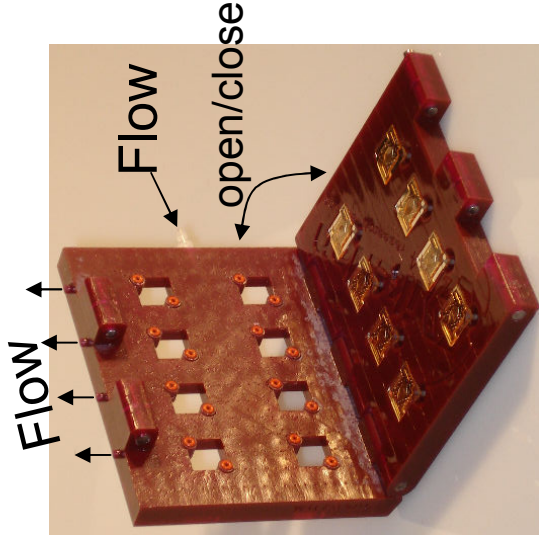
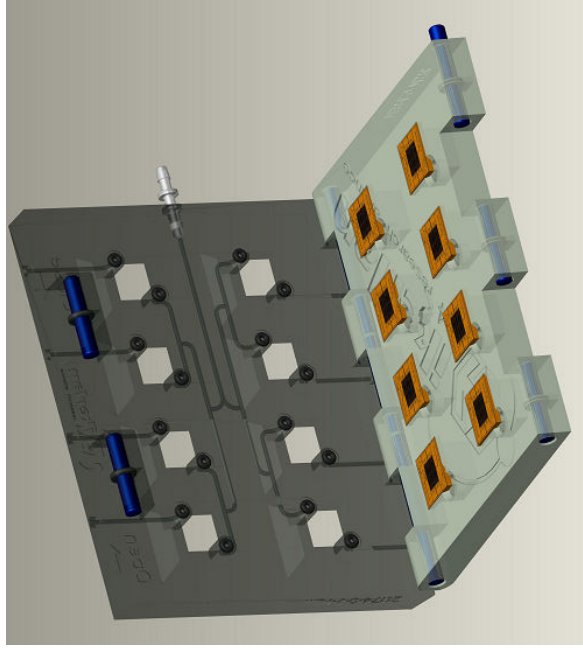
Washing



Dicing 

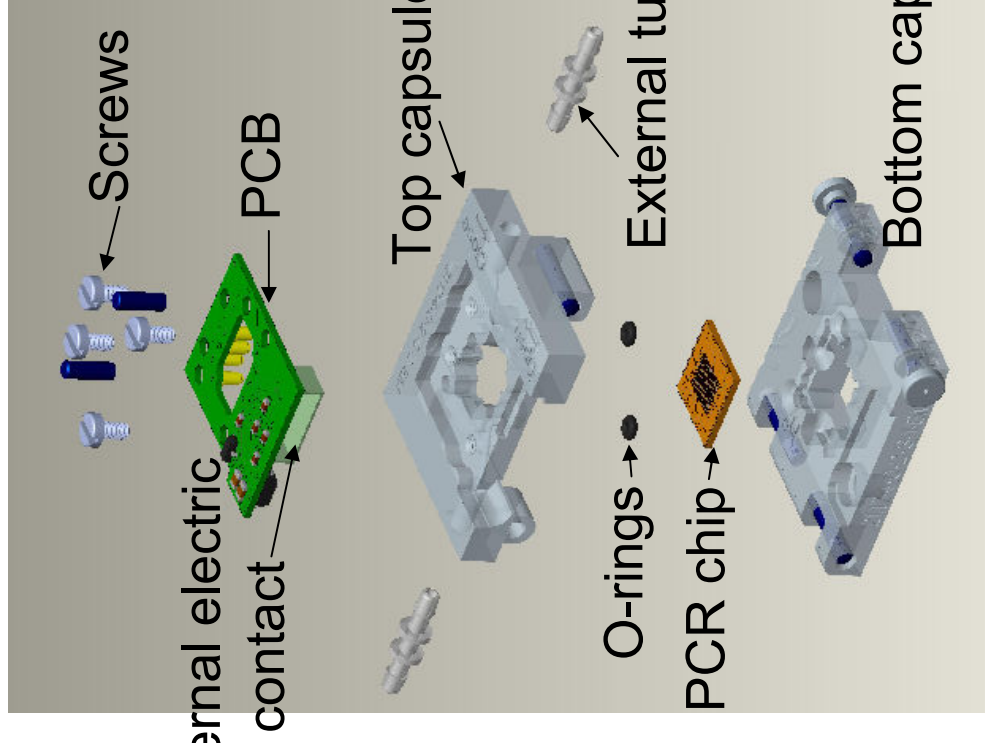
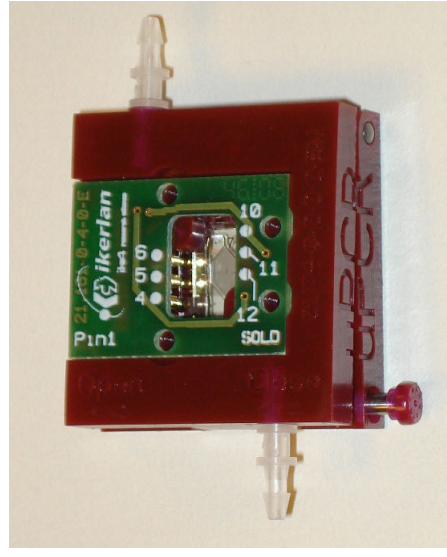
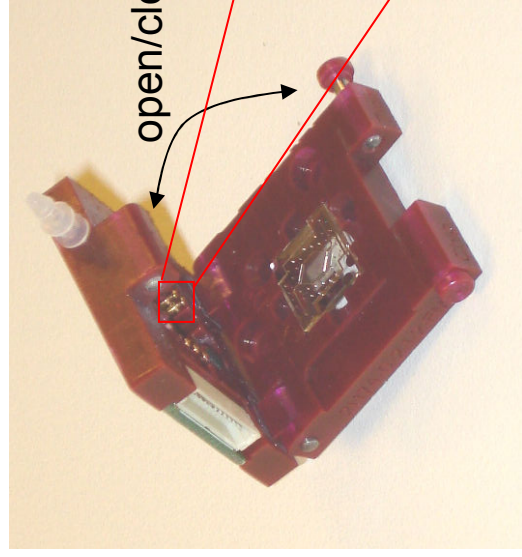


Washing: IPA + DI water



Packaging

- ❖ After Kapton releasing, easy electric/fluidic connection
- ❖ Without glue/wires, easy device replacement



Outline

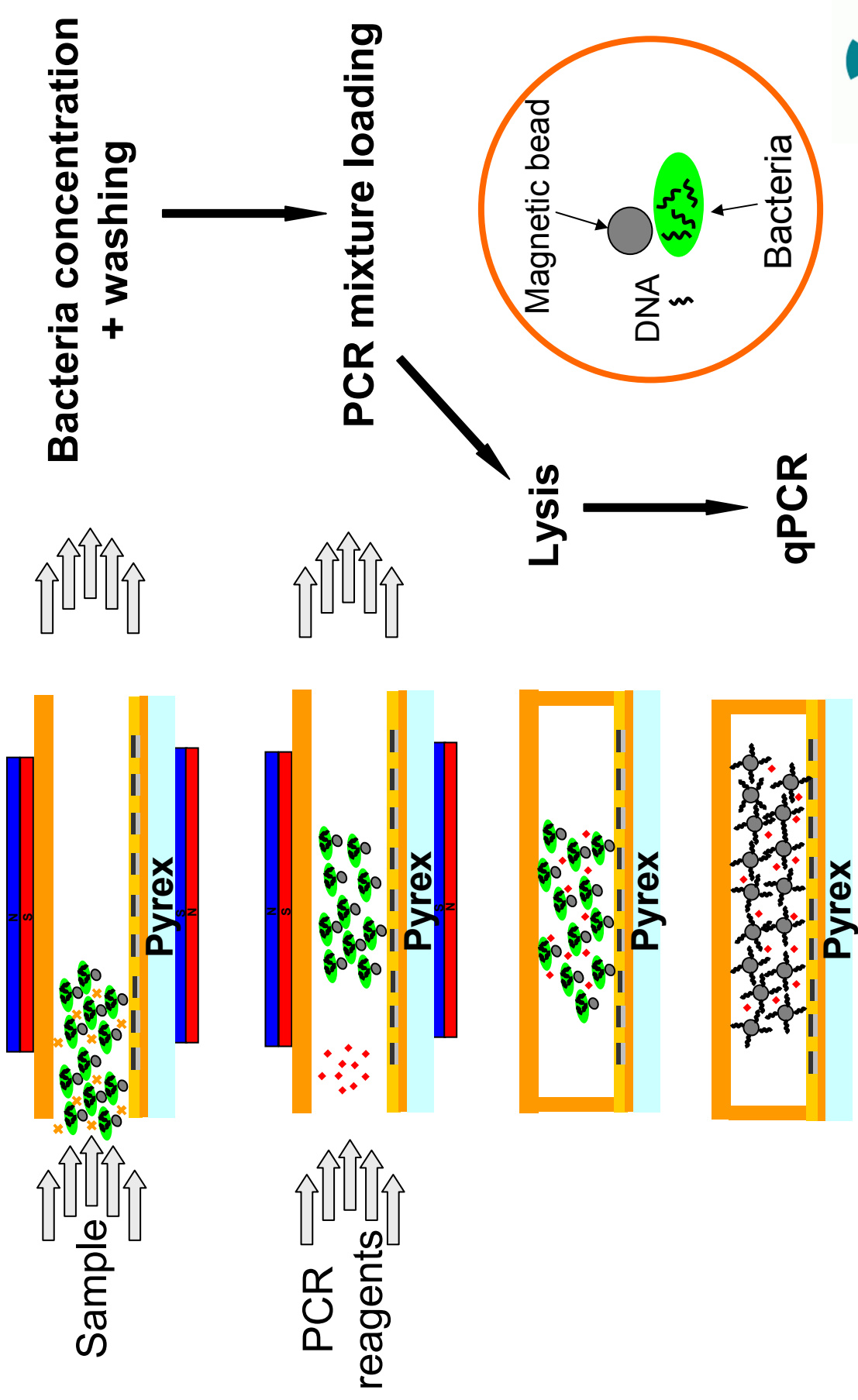
- ❖ Introduction
- ❖ Goal
- ❖ Design
- ❖ Fabrication

✓ Sample preparation
✓ Lysis and qPCR

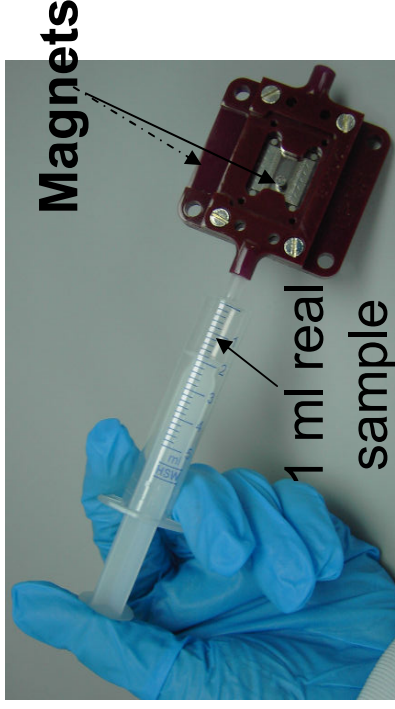
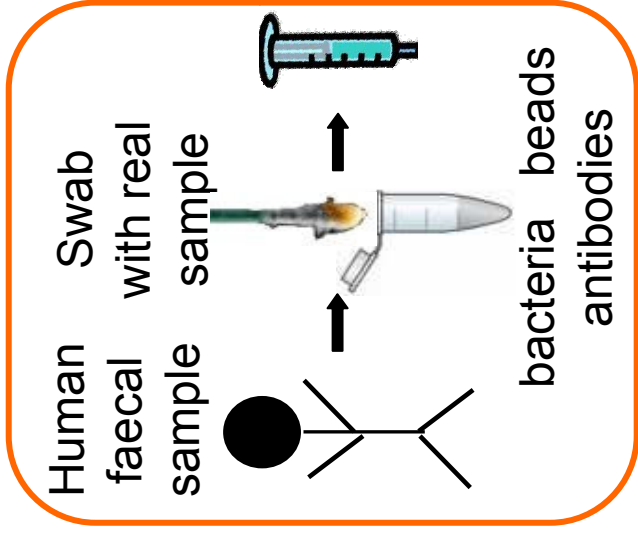
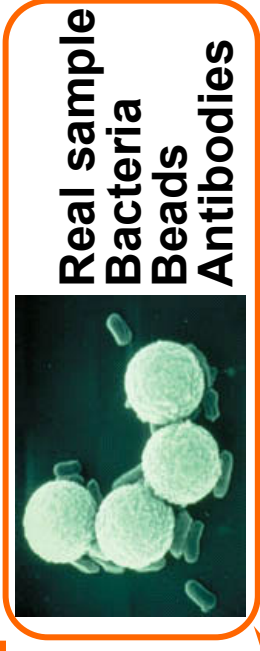
❖ **Experimental methodology**

- ❖ Results
- ❖ Conclusions and future work

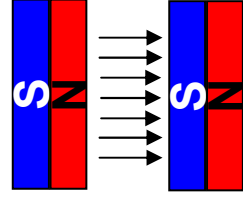
Experimental methodology



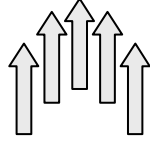
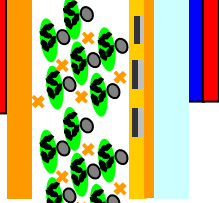
Sample preparation



Magnetic Field



Sample



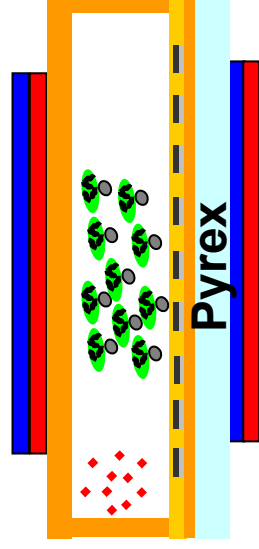
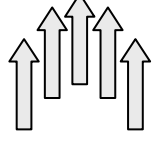
Lysis and qPCR



Remove magnets
Close inlet/outlet

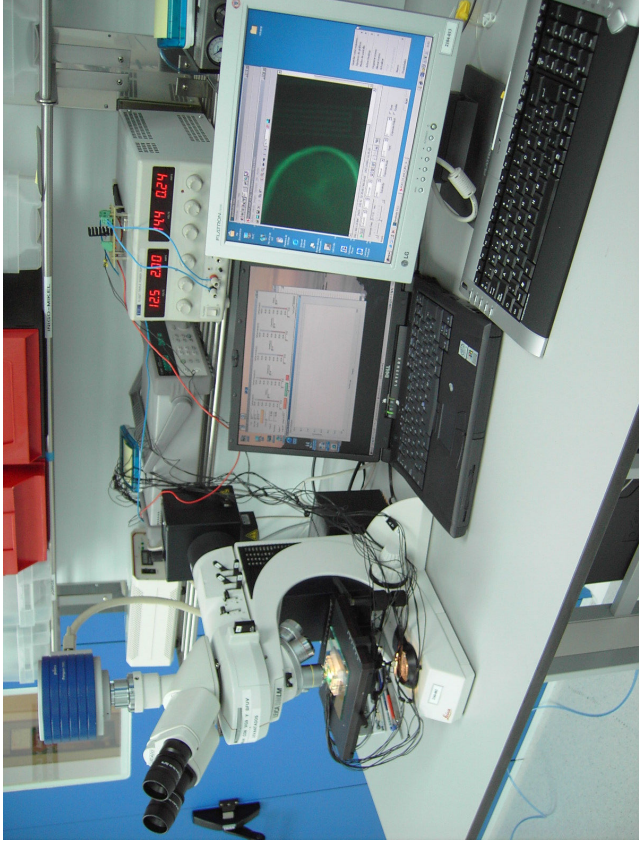


PCR
reagents

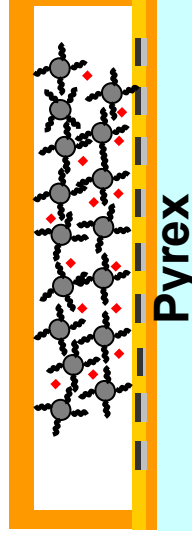


Lysis and qPCR

| PCR MIX | CONCENTRATION |
|---------------------------|---------------|
| 10X buffer | 1X |
| MgCl ₂ 25 mM | 3 mM |
| dNTPs 2mM | 200 µM |
| LHNS 25 µM | 0.5 µM |
| RHNS 25 µM | 0.5 µM |
| Template | 1.5 µM |
| Taq DNA polymerase 5U/ µl | 2.5 U |
| Probe (Cy5/BH Q2) 2 µM | 200 nM |
| H ₂ O | Up to 15 µl |

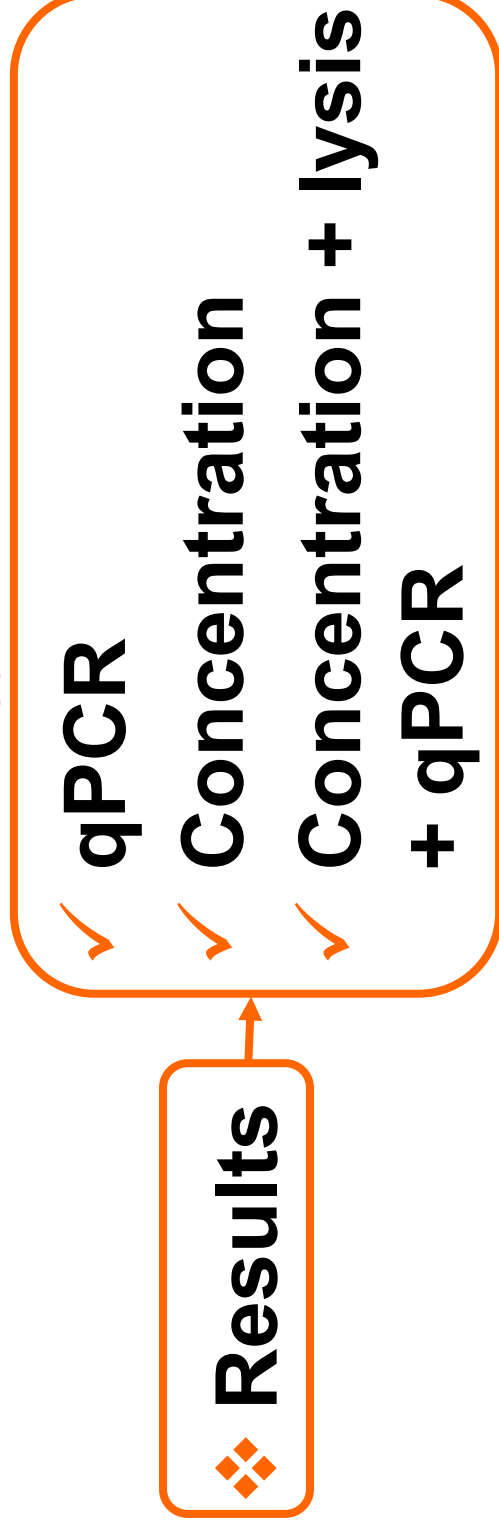


Thermocycling:
10 min at 94 °C
35 PCR cycles of 94 °C, 8 s; 50 °C,
8 s; and 72 °C, 8 s.



Outline

- ❖ Introduction
- ❖ Goal
- ❖ Design
- ❖ Fabrication
- ❖ Experimental methodology

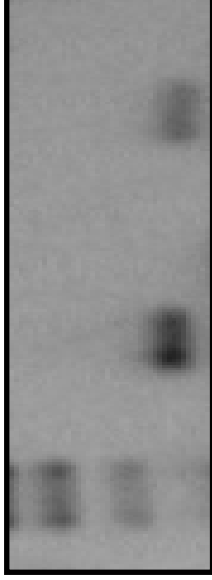


- ❖ Conclusions and future work

qPCR results I: inhibition effects

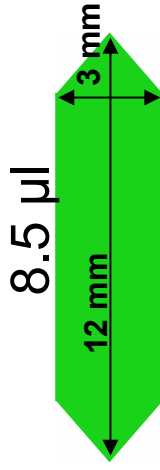
❖ Higher levels of polymerase enzyme are required to perform successfully qPCR assay.

- 1 Molecular weight marker
- 2/4 Polymerase units, optimized OFF chip
- 3/6 Increased Polymerase units, optimized **ON chip**

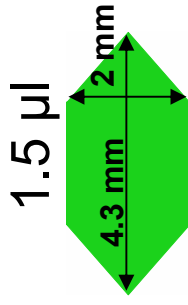
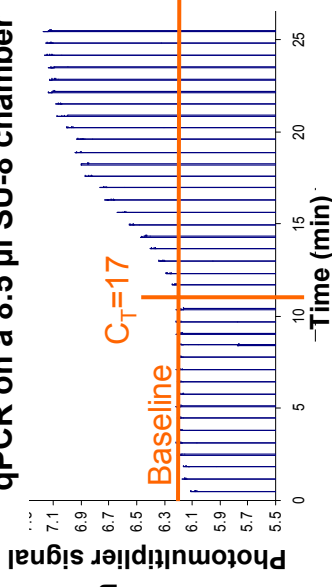


❖ To avoid PCR inhibition in a micro-SU8 chamber, BSA is added as a reagent to the PCR mixture.

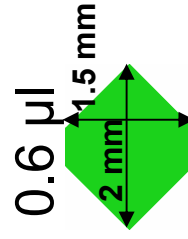
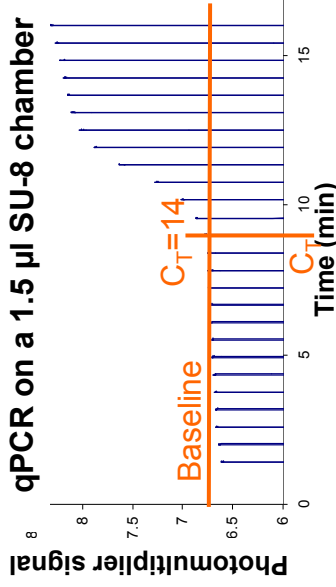
qPCR results II: volume reduction



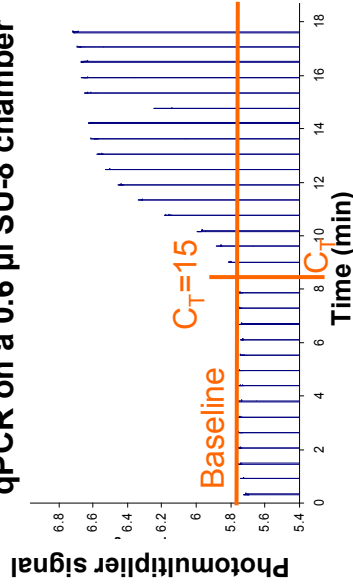
qPCR on a 8.5 µl SU-8 chamber



qPCR on a 1.5 µl SU-8 chamber



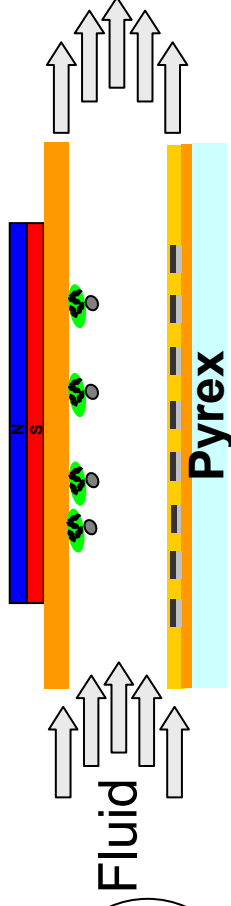
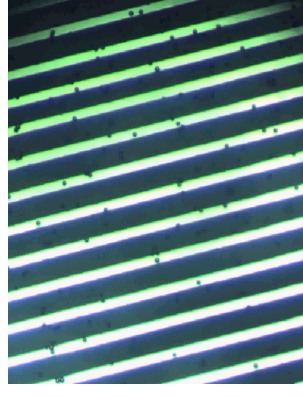
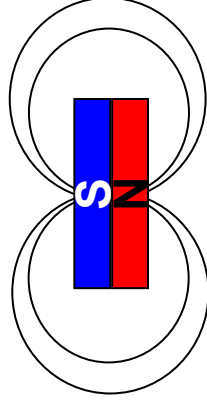
qPCR on a 0.6 µl SU-8 chamber



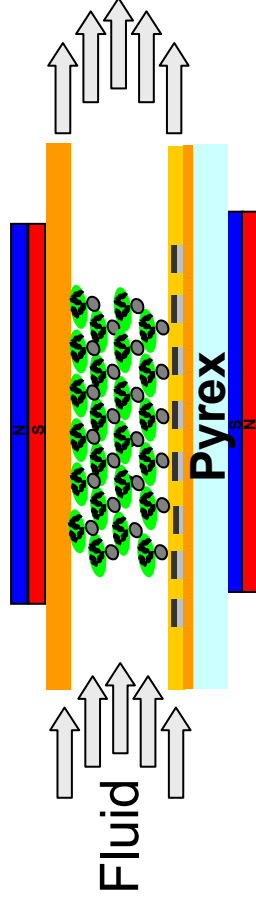
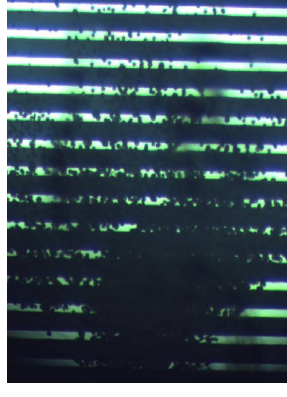
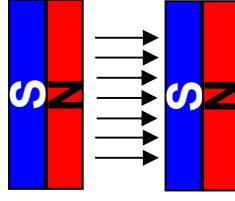
2.5 µl SU-8 chambers have been chosen for PCR optimisation to be able to extract the PCR product after thermocycling

Concentration results

Magnetic Field

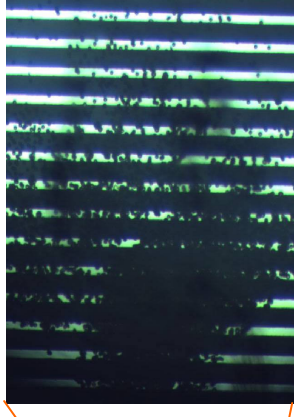


Magnetic Field

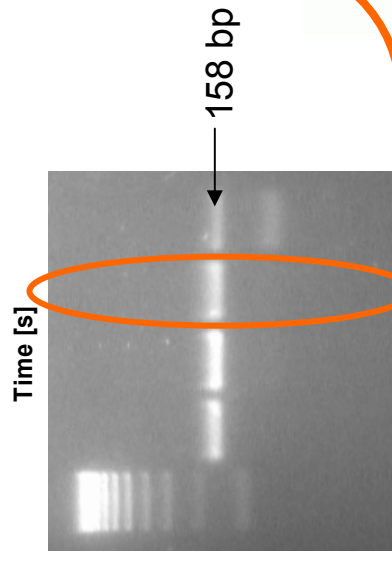
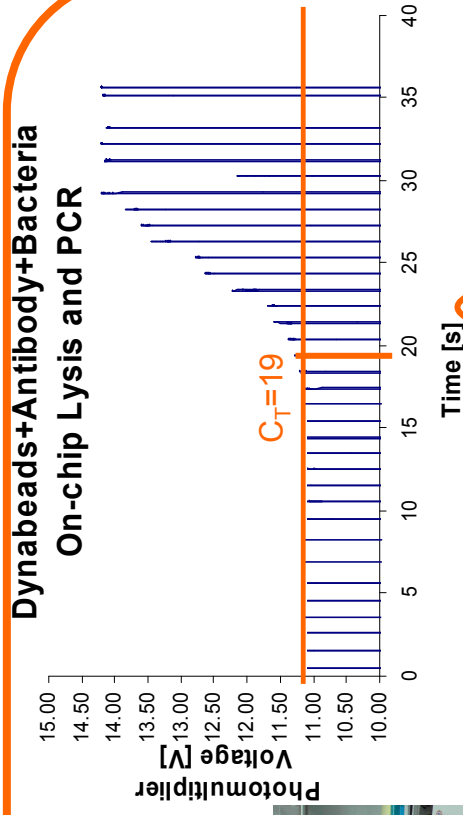
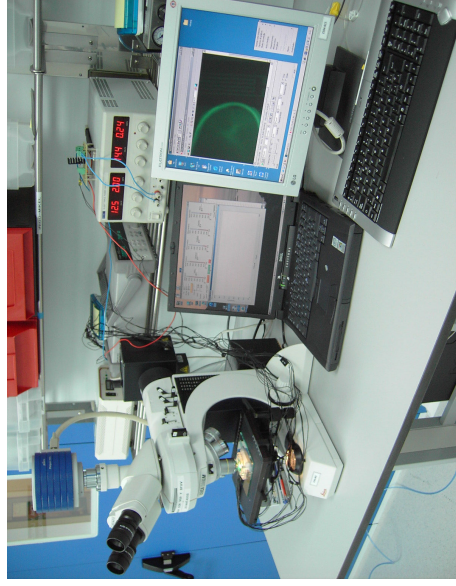


Concentration + lysis + qPCR

Real sample
concentration



Lysis + qPCR



Outline

- ❖ Introduction
- ❖ Goal
- ❖ Design
- ❖ Fabrication
- ❖ Experimental methodology
- ❖ Results

❖ **Conclusions and future work**

Conclusions

- ❖ The developed fabrication technology proves to be an excellent tool for rapid prototyping, as well as for mass production of low cost disposable PCR LOCs.
- ❖ Bacteria concentration from human faecal sample, lysis and DNA extraction from this concentrated sample, amplification of 158 bp molecular marker from *Salmonella* spp. genomic DNA and detection were successfully carried out on a single chip.
- ❖ The whole protocol, including *Salmonella* spp. concentration, lysis and qPCR was achieved in 25 min.
- ❖ 2 simple syringes, an SU-8 microdevice and optical detection system are enough for rapid pathogen detection.

Future work

- ❖ Further characterisation of on-chip concentration.
- ❖ Sensitivity measurements.
- ❖ Integration of microfluidic control.
- ❖ Integration of an internal positive control.
- ❖ Optical detection integrated on package.
- ❖ Exploration of immunoPCR, RT-PCR and NASBA.

Acknowledgements

- ❖ Basque Government for its financial support under the ETORTEK program.
- ❖ BIOEF for sample supply.
- ❖ Optolabcard european project.



OPTOLABCARD

www.optolabcard.com

Questions



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