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Development of a Lab-on-Chip Based Biosensor-array System for Life Detection on Mars and Icy Moons

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EPIC Meeting on Photonics for Space:
Opening New Horizons at Exail
Paris, 21-22 September 2023

Our Mission

LioniX International is a leading global provider of customized microsystem s ns, in particular integrated pho ed, in scalable production

Integrated Photonics is one of the key enablers for this

Why

Applyin echnologies to solve societal challenges

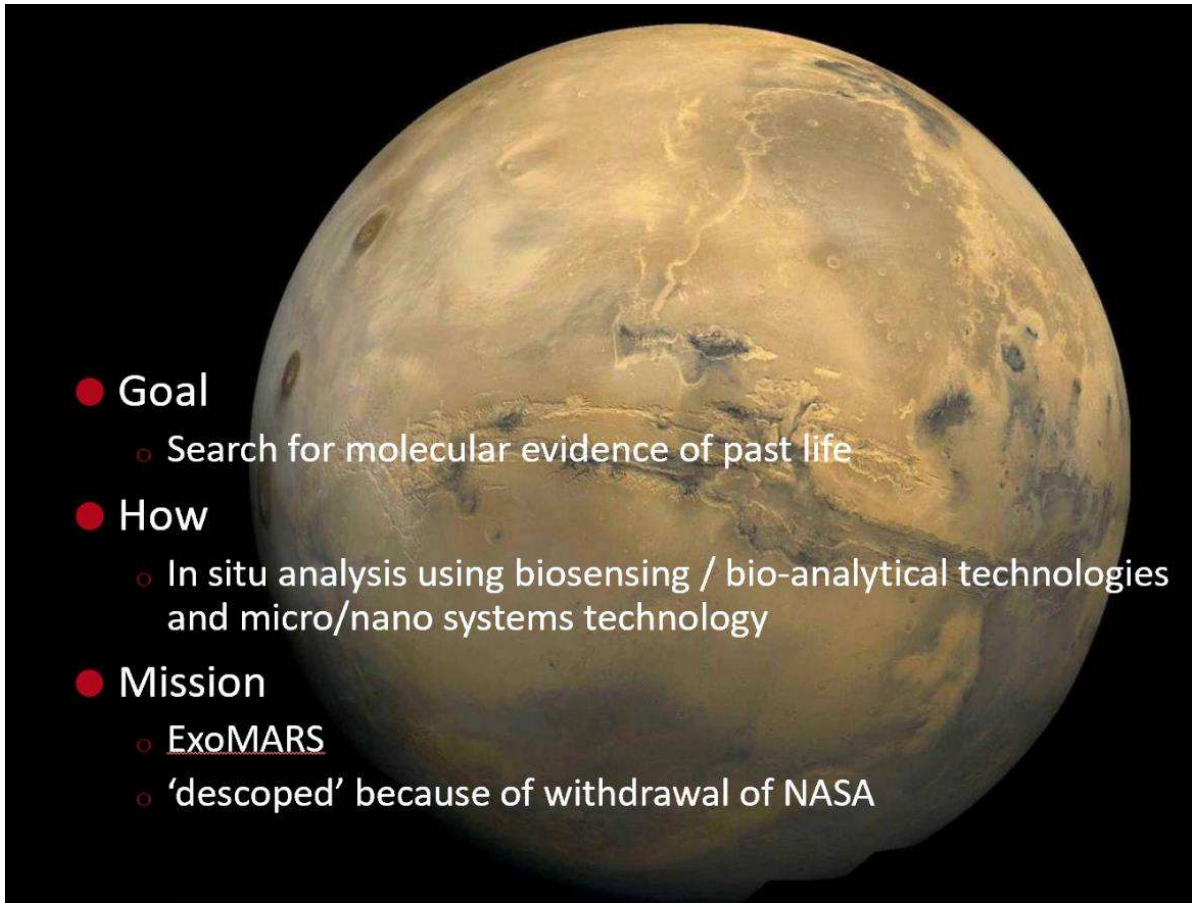
Who:

- Located in Enschede, The Netherlands
- 80 people and growing; 50% PhD
- Tele/datacom, Life Sciences, Metrology
- **Space: NSO - ESA (TRP-GSTP-ARTES) – EU Horizon**
- Proprietary Integrated Photonics platform
  - Special features for communications, sensing, lasers
- Development and production for applications in
  - Integrated photonics based light/signal/data processing: 5G/6G and **(RF) satellite** systems, visible light engines (AR/VR)
  - **Lab-on-a-Chip** and (bio)chemical sensor systems

Background: Life Marker Chip

- LMC Team Leaders
  - Mark Sims, Leicester Univ. (UK)
  - Dave Cullen, Cranfield Univ. (UK)
- LMC Instrument Team
  - LioniX, Dutch Space (NL), Leicester University, Cranfield University, Magna Parva (UK), Kayser Italia, SSTL (UK), and Imperial College (UK)

Netherlands Space Office

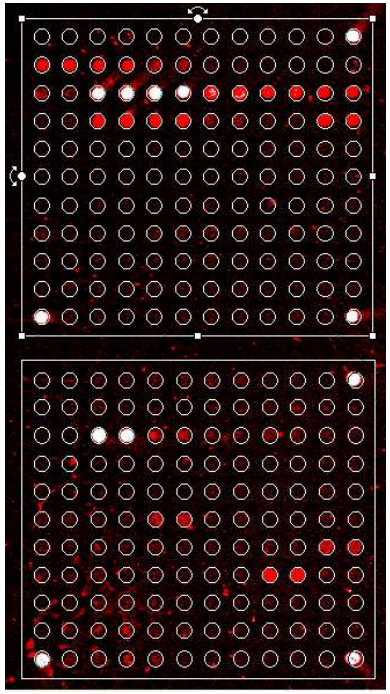


Life Biomarkers on Mars

Antibody microarray technology allows the attachment of thousands of probes in a few square centimetres on a solid support. Smaller reaction volumes and higher reaction kinetics, together to its great potential for miniaturization and robotization, make microarray technology a good system for *in situ* analysis of biomarkers in astrobiology.

Features

1. Capable to detect extant and extinct life
2. Multiple molecular detection in parallel
3. No special external calibration
4. Allows detection of broad molecular size range
5. Sensitivity: From ppb to ppt
6. Results are very easy to analyze
7. Biotechnology industry supports this technology.



Typical image with one hundred different antibodies as well as positive and negative control

Present life biomarkers

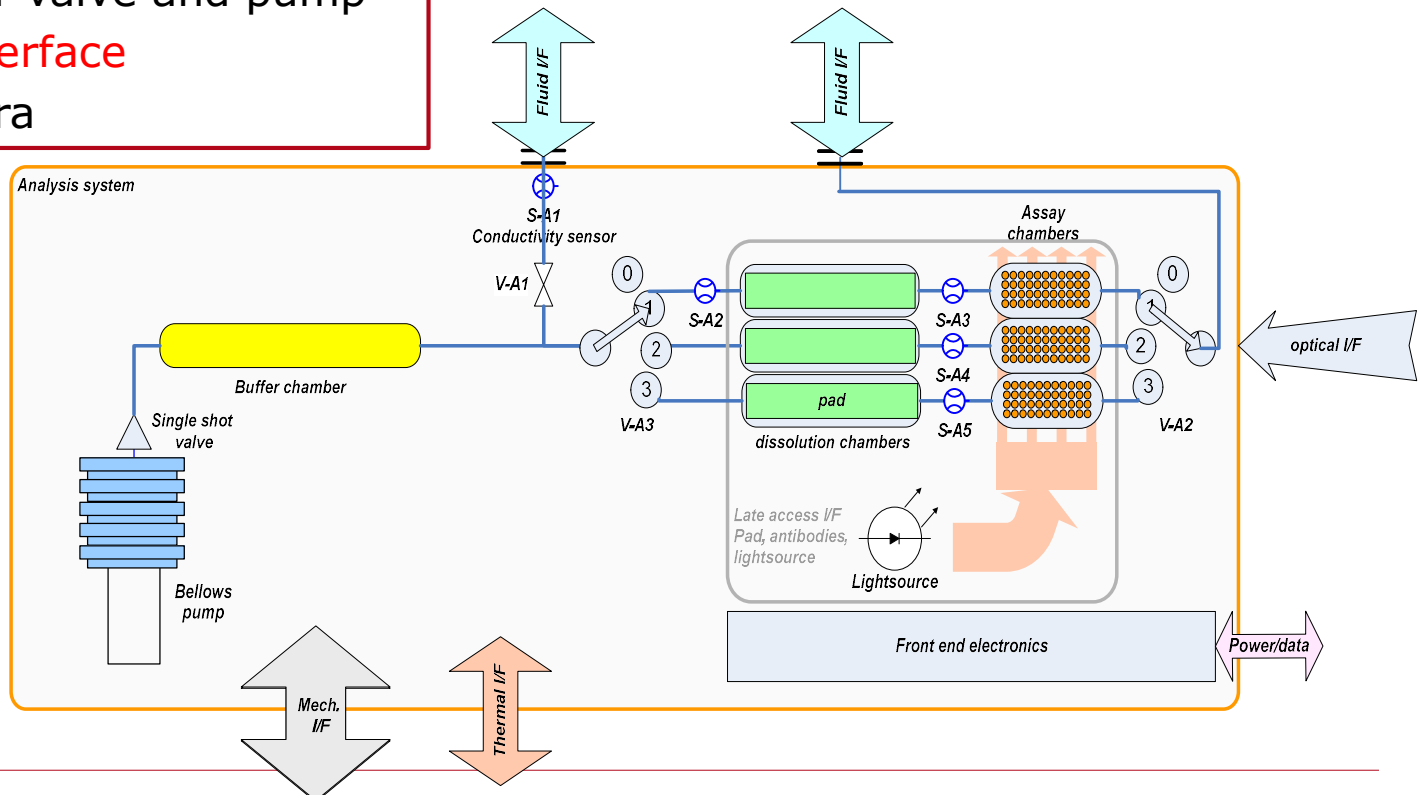
1. Whole cells,
2. Cellular debris, biofilms
3. Biopolymers

Past life biomarkers

1. Aliphatic Hydrocarbons.
2. Monocyclic hydrocarbons.
3. Tricyclic hydrocarbons.
4. Aromatic carotenoids.
5. Hopanoids and other pentacycic triterpanes.
6. PAHs.
7. Lipids – Steroids.
8. Porphyrins and maleimides.
9. Aminoacids (aa) and nucleotides.
10. Nucleotides and other metabolites
11. Polymers

LMC Analysis subsystem

- Based on Laser Induced Fluorescence antibody array
- Hybridly integrated microfluidic and photonic chip
- Miniaturized (chip-based) selector valve and pump
- External laser diode with fiber interface
- Bulky relay optics and CCD camera

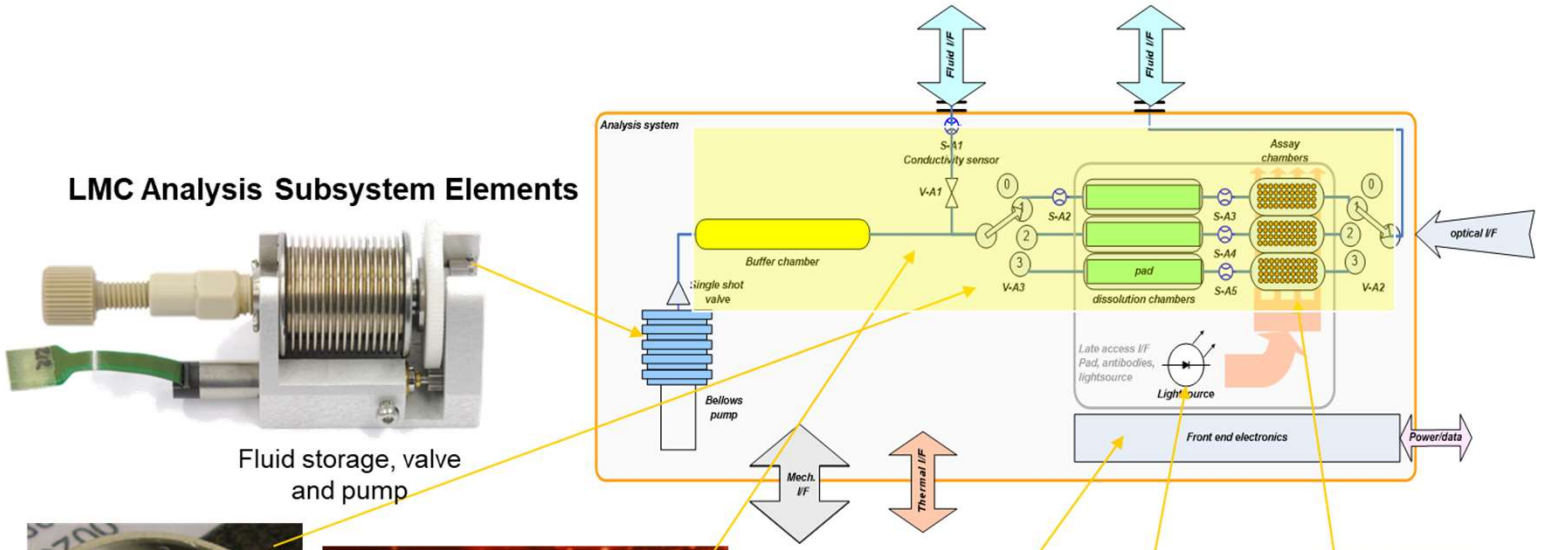


Chips system integration

Courtesy of Guus Borst
Dutch Space

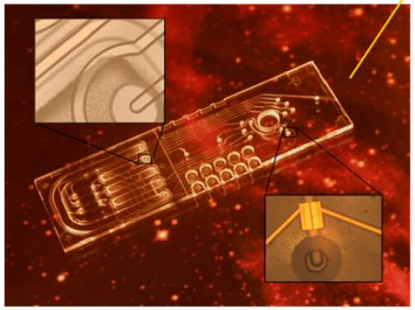
Status 2013:

Prototype TRL 5-6

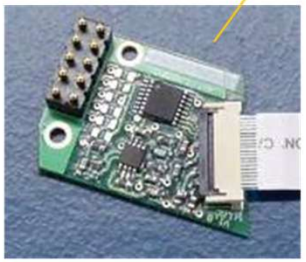


Fluid storage, valve and pump

Rotary valve to control the fluid over the chip

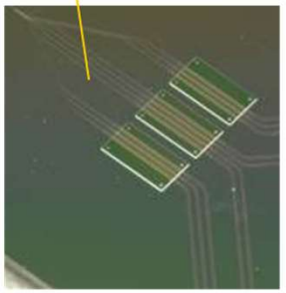


Glass chip with integrated fluid channels, chambers and sensors



Electronics to read out the sensors

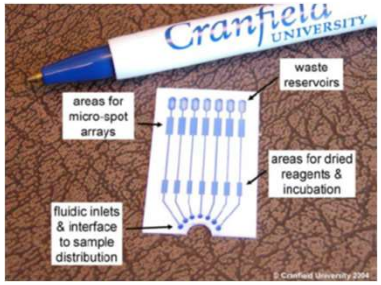
Laser



Waveguides to excite the attached antibodies

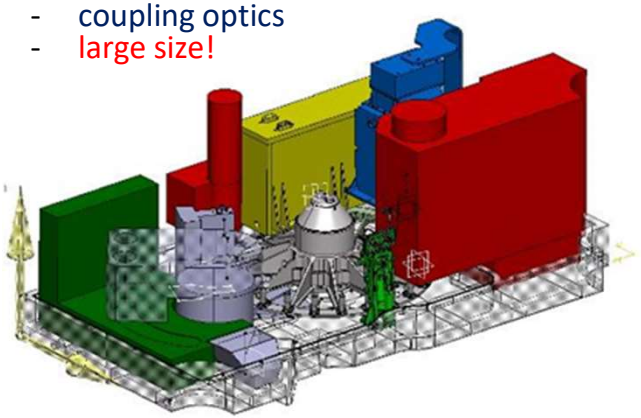
Micro/Nano/Bio Tech in LMC

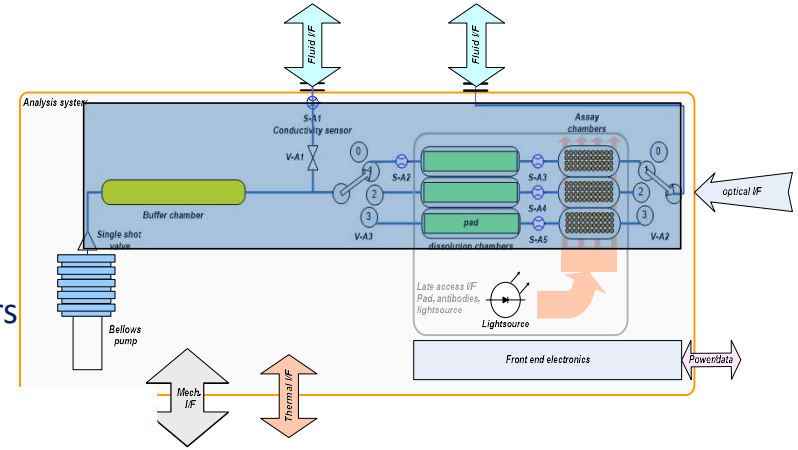
Antibody microarray
- competition assay format (3 chemistries)
- predosed dried chemicals
- single-use
- 4 modules of 10x10 spots



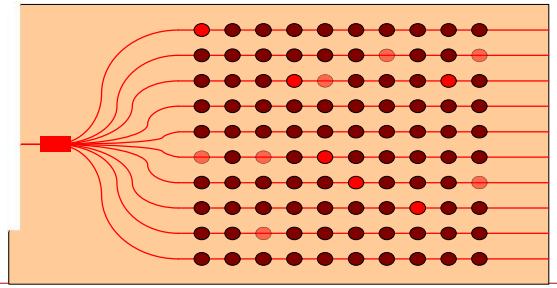
Microfluidics based core system
- based on micro/nano technologies
- micro channels: fluidic connections
- micro chambers: reagents, array, buffer
- micro sensors: electricial conductivity
- planar optical waveguides: excitation of dyes
- micro system integration: compact subsystem
- hybrid selector valve

System integration
- control subsystem
- sample pre-treatment
- lab-on-a-chip
- macrofluidics, pumps and reservoirs
- camera subsystem
  - camera
  - coupling optics
  - large size!





Photonic Integrated Circuit (PIC)
- Laser Induced Fluorescence (LIF)
- excitation by 'manifold' substrate
- external laser coupled with fiber



ESA-TRP IDOS (2020-23)- Objectives

INTEGRATED LABEL-FREE DETECTION IN OPTOFLUIDICS SYSTEMS FOR SAMPLE ANALYSIS (IDOS)

Partners: Kayser Italia (lead), LioniX International, Surfix Diagnostics

This activity aims at the development and feasibility demonstration of label-free optofluidic refractometric biosensor array with integrated sources and detectors to detect and quantify a selected set of biomarkers.

Other objectives consists of

- *Developing Preliminary concepts for future space application,*
- *Identifying critical issues including technical and safety challenges.*

The final output consists of a IDOS breadboard aiming to reach a target TRL 3/4, supporting the Analytical and experimental critical functions and/or the features of an experimental proof-of-concept.

- *Selected model biomarkers:*

Health: - *C-reactive protein (CRP) in blood serum;*

- *Human serum albumin (HSA) in urine;*

Life signature: - *DNA - synthetic probe/target hybridization couple, 16S related;*

- *Benzo(a)pyrene (B(a)P).*

- *Sample extraction and preparation by proven laboratory and LMC procedures.*

Label-free optofluidic biosensor platforms

Technology platform	Detection limit (RIU)	Integration capability (+/-)	Multiplexing capability (+/-)	Chip processing complexity
3.1 SPR - Surface Plasmon Resonance				
Prism coupled SPR	$10^{-5} - 10^{-8}$	-	-	n/a
Waveguide-based SPR	$10^{-5} - 10^{-6}$	+	+	+
Imaging SPR	$10^{-5} - 10^{-6}$	-	+	n/a
Localized SPR	10^{-5}	-	-	n/a
3.2 Optical fiber				
Optical fiber	$10^{-4} - 10^{-7}$	-	+/-	n/a
3.3 Auto-fluorescence				
Auto-fluorescence detection	Depends on analyte	+	-	+
3.4 Photonic crystal				
Photonic crystal	$10^{-3} - 10^{-5}$	-	+	n/a
3.5 MRR & Interferometers				
MRR MicroRing Resonator	$10^{-4} - 10^{-7}$	+	+	+
MZI Balanced Mach-Zehnder Interferometer	10^{-6}	+	+	-
aMZI Asymmetric Mach-Zehnder Interferometer	10^{-8}	+	+	+
Michelson interferometer	10^{-8}	-	-	n/a
3.5 Additional techniques				
Plasmonic SPR	$10^{-8} - 10^{-9}$	+	+	-
Bimodal interferometer	10^{-7}	-	+	-
Microbubble resonator	0.9 pg/ml	-	-	n/a
Non-optical resonator	100 pg/ml	-	-	n/a

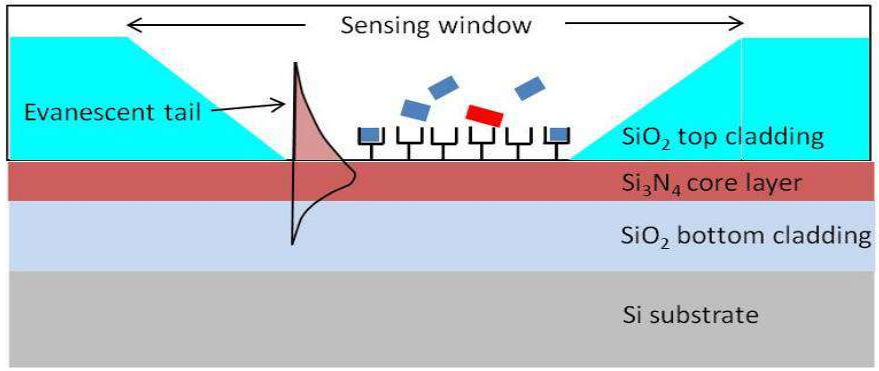
Optofluidic biosensors and property summary

- Study and evaluation of label-free photonic biosensor platforms
- New type interferometric sensor (proprietary)
- ***Asymmetric*** Mach-Zehnder Interferometer (aMZI)
  - Follow up of LMC development
  - Development funded in several EU projects
  - Promising results initiated ESA-TRP IDOS

"LMC v3.0"- How it works

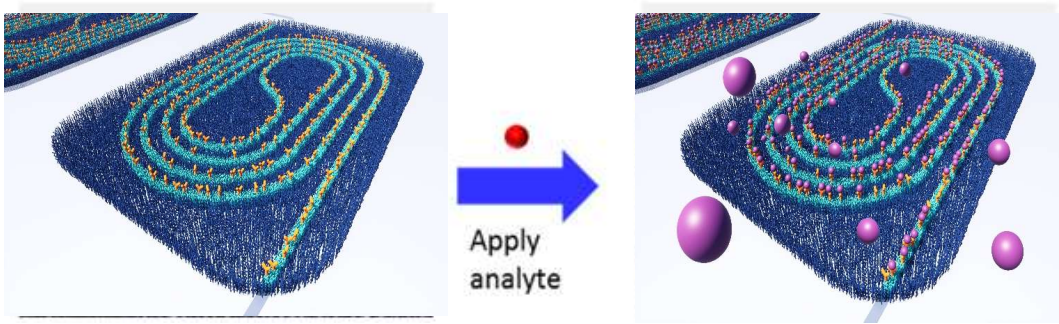
LMC boosted the development of an integrated photonics based biochip for non-space applications, i.e. medical!

- Evanescent-field, refractometric detection of capturing of analyte molecules on surface-bonded receptors (e.g. antibodies)
- Tiny mass changes detected by the evanescent tail of the light wave in the Si_3N_4 waveguide core (red)



- Nanochemistry based selective sensor surface functionalization
  - Improved bio-assay reproducibility
  - Prevention of (trace) analyte depletion by repelling surrounding, large surface

  → Enhancement of LoD: ~ 10-100x (in theory)

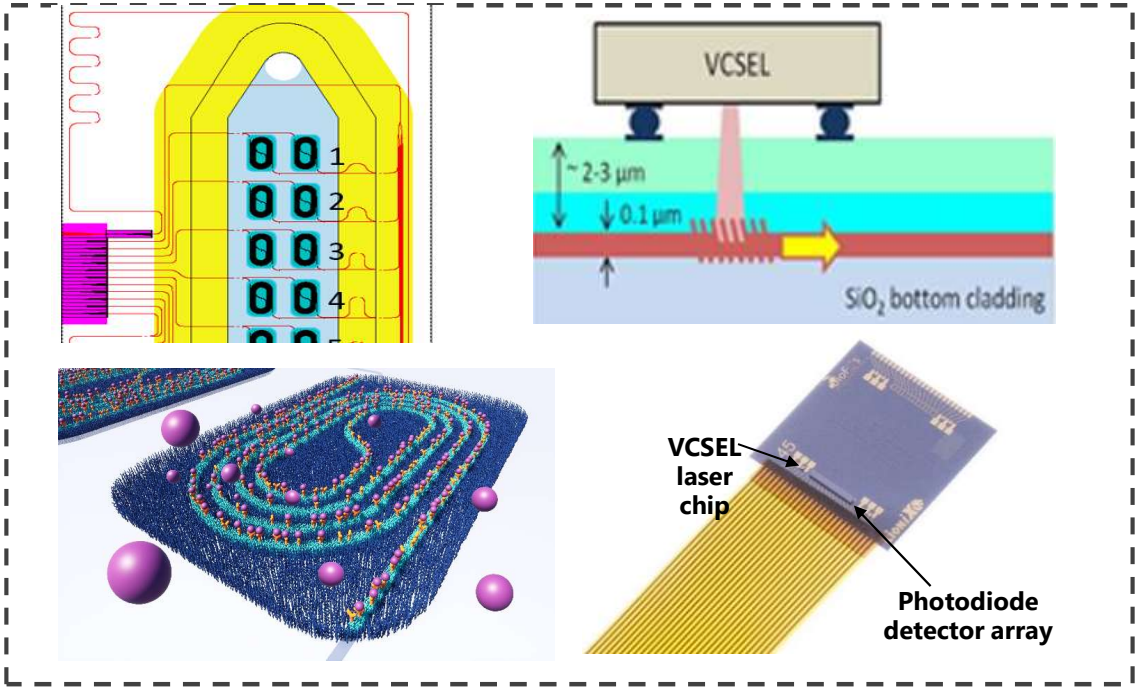


Innovation

Asymmetric Mach-Zehnder Interferometer (aMZI) platform

Ultrasensitive, hybridly integrated and wafer scale manufacturable photonic biosensor array chip module

- Direct analyte capture detection on biochip with hybrid ***integrated*** laser and detector:
  - ***label free***, no derivatization step of fluorescent dyes
  - ***no*** fiber interface required
  - ***no*** relay optics and CCD camera required
- Combination of innovative aMZI and nanochemistry based selective surface functionalisation
- Fast, ultrasensitive, accurate and multiplex (10 - 20) detection
- Applications:
  - (Point-of-care) diagnostics, detection and monitoring
  - Drug development
- Applicable in desktop, portable or hand-held system

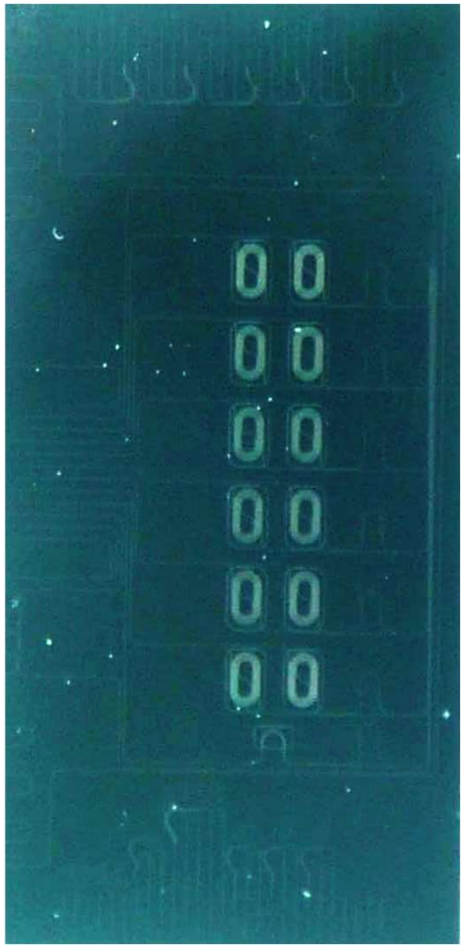


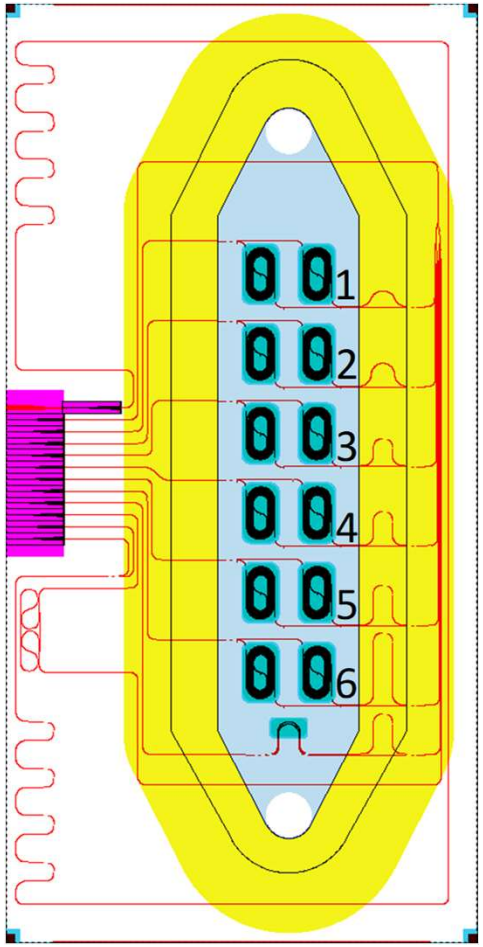
aMZIs with enhanced optical sensitivity

- Sensing path length: 12.5 mm
- Different asymmetry stretches
- Characterization of prototype sensors prove sensitivity enhancement (see table)

Optimized sensitivity of 'State of the Art' MicroRing Resonator: ≈100 nm/RIU

#	Sensitivity (nm/RIU)
1	9900
2	4945
3	3295
4	2471
5	1977
6	988

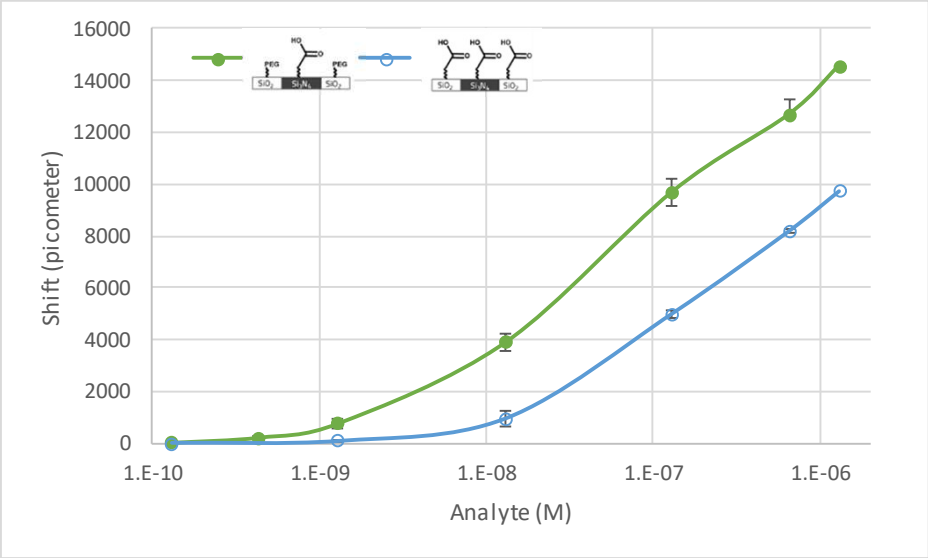




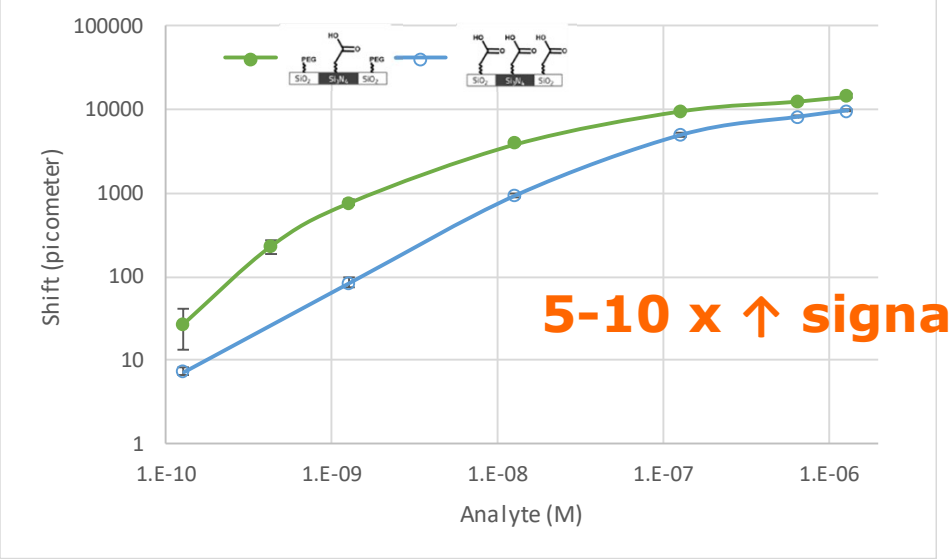
Selective functionalization

Comparison of selective versus not-selective

Shift as a function of analyte concentration (Anti-Mouse IgG) II



Single logarithmic



Double logarithmic

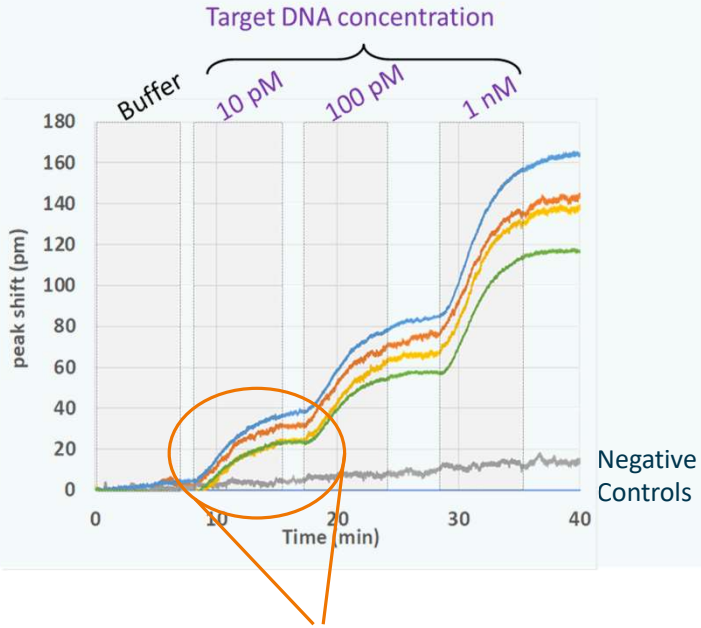
Examples applications

SURFIX

QURIN DIAGNOSTICS

DNA based early cancer detection

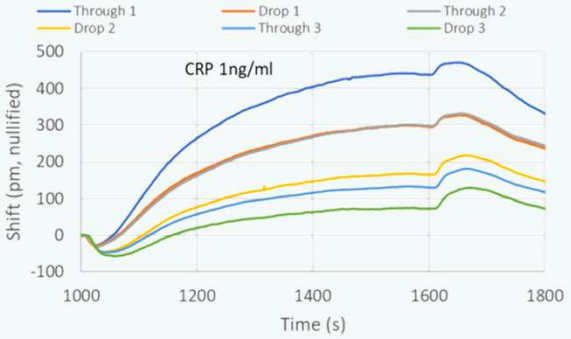
- Small fragments of hypermethylated DNA in urine
- Detection of low pM concentrations of small target DNA fragments (5-10 kDa) demonstrated



30-40 pm shift easily measured!!

Blood analysis
C-reactive protein (CRP)

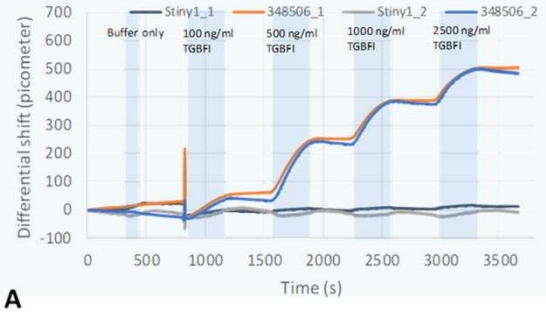
- Inflammatory marker in blood
- Clinical range ± 1ng/ml

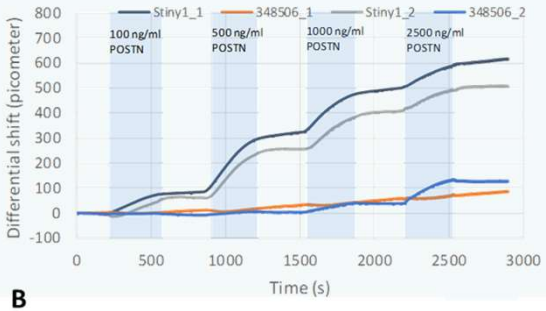


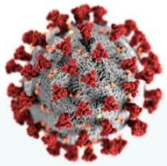
BIOCDx

Protein based cancer detection

- Multiplexed detection of recombinant TGFBI (A) and POSTN (B) demonstrated







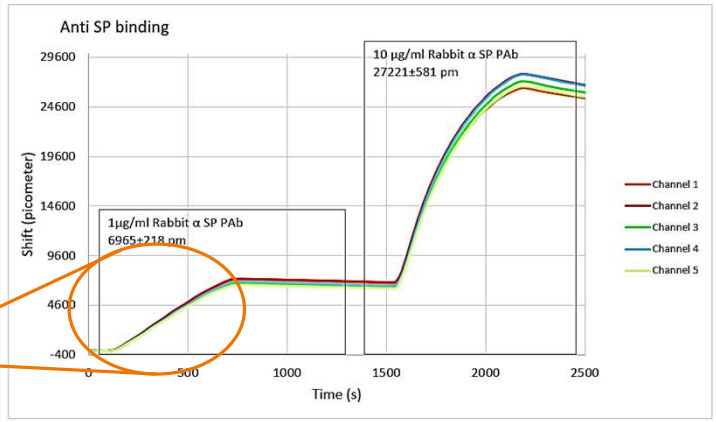
SARS-COV-2 serology and antigen testing

SURFIX

Serology test development

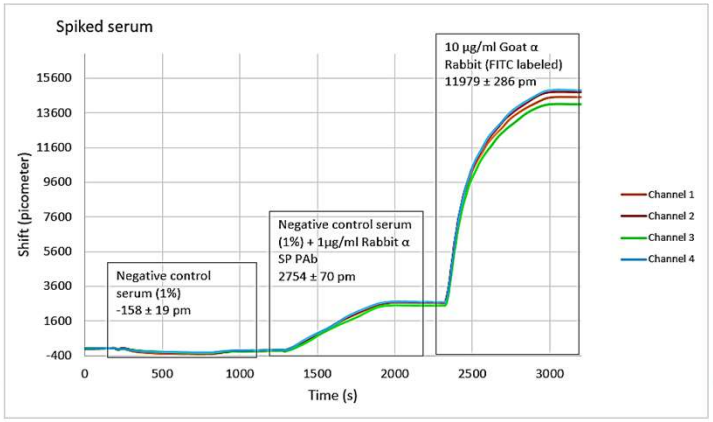
- Detection of AB against SP protein of SARS-COV-2 virus
- Clinical range ± 1µg/ml
- Y2022: Validated in blood serum samples!

Spiked buffer

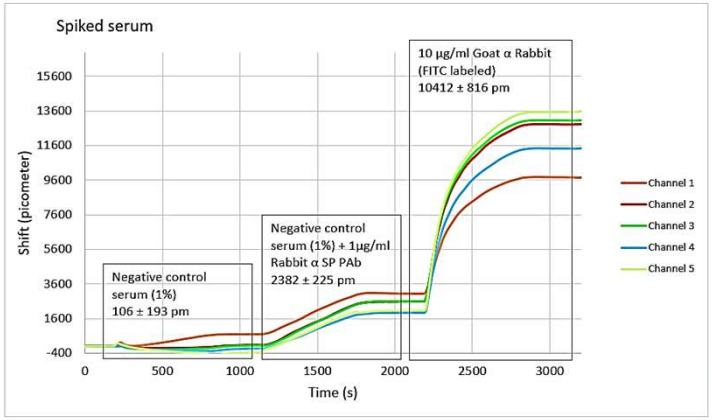


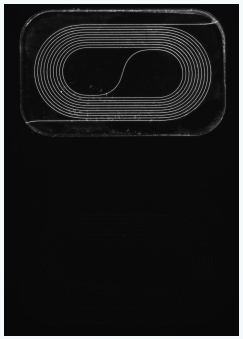
At clinical range almost 7000 pm shift is obtained
'there is plenty of room at the bottom'

Spiked serum (n = 1)



Spiked serum (n = 2)

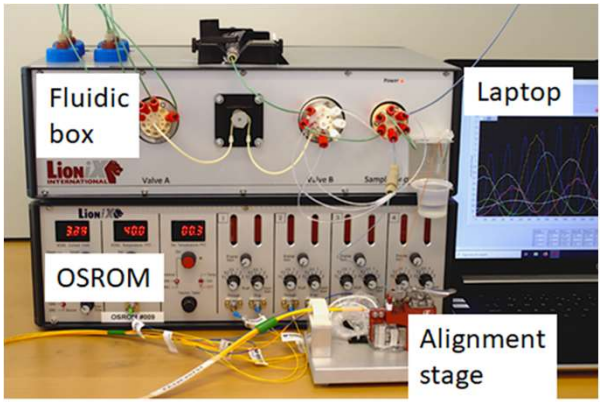




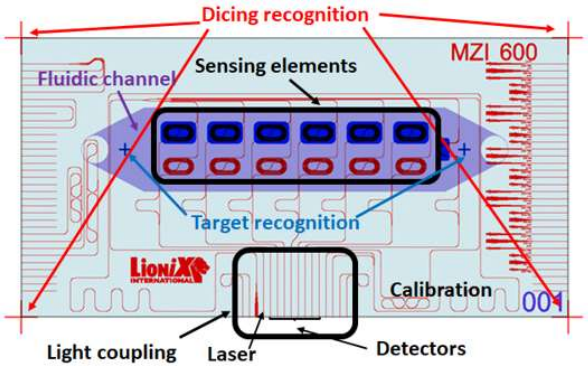
Serum matrix effects are filter out by reference (balanced) sensors

TRP-IDOS BB-1: Assay development

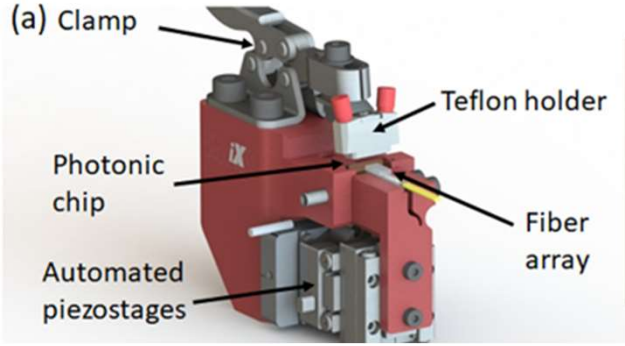
- Flexible experimental system
- Free space fiber light edge coupling of laser and detectors
- 'Naked' biosensor-array chip
- Automated positioning of fiber array
- Demo assays for CRP, HSA, DNA and B(a)P



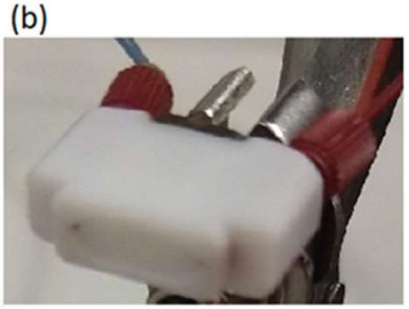
Experimental system



Schematic of biochip



Biochip mounting/holder

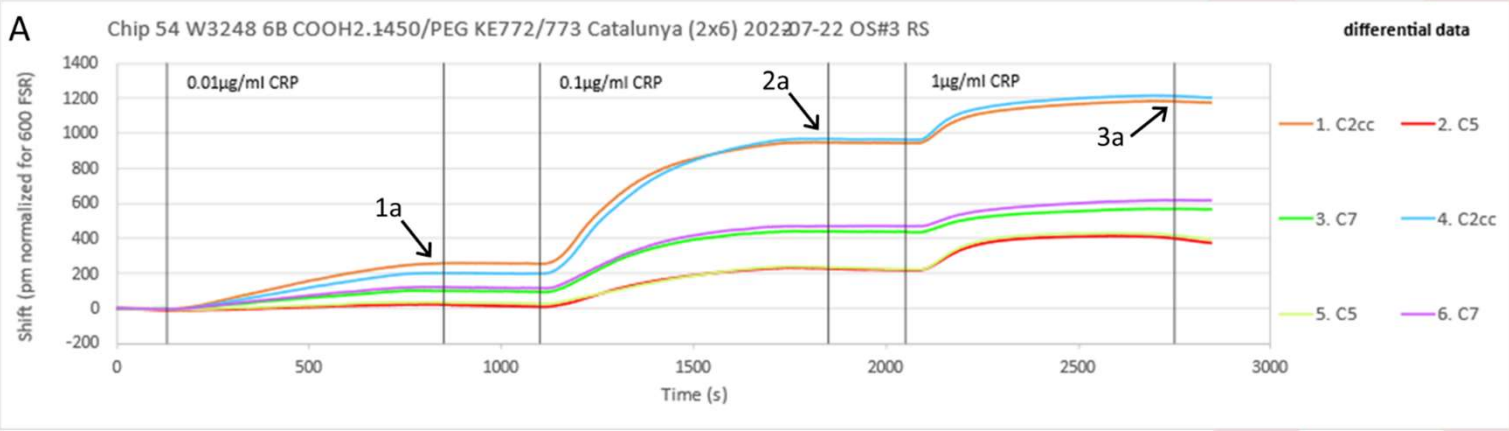


Fiber array holder

Biomarker assay results

C-Reactive Protein (CRP)

- Comparison of different capture antibodies
- Minor effect of matrix (synthetic serum analogue)
- Optimization of buffer composition



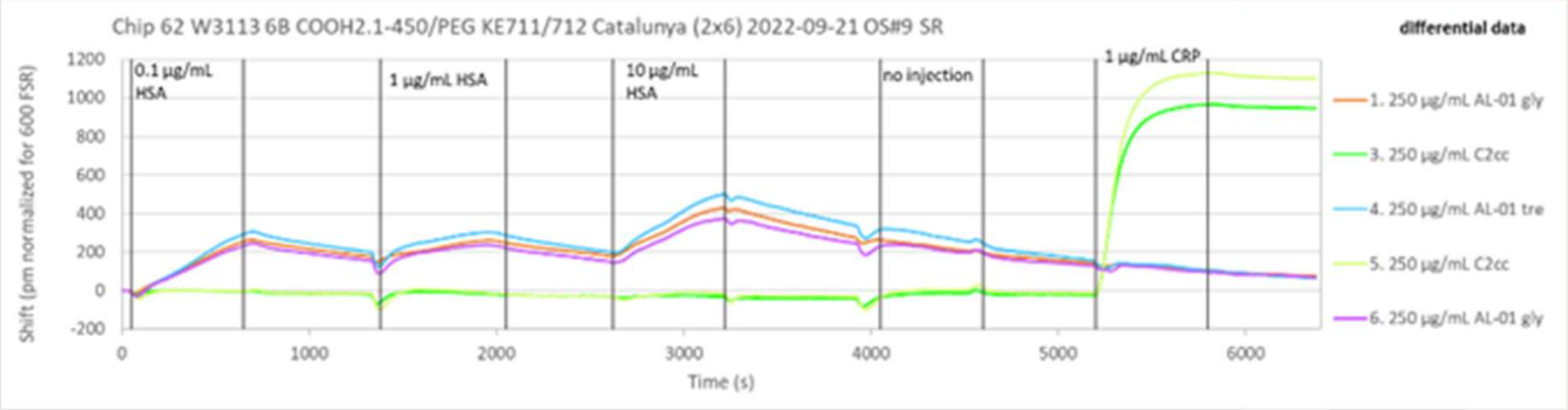
Conclusion

- Successful detection of CRP, LOD <0.01 µg/ml
- Highest sensitivity with antibody C2cc
- Quantitative detection of high concentrations requires further dilution

Biomarker assay results

Human Serum Albumin (HSA)

- Comparison of different immobilization (spotting) conditions
- Minor effect of matrix (synthetic urine)
- Optimization of buffer composition



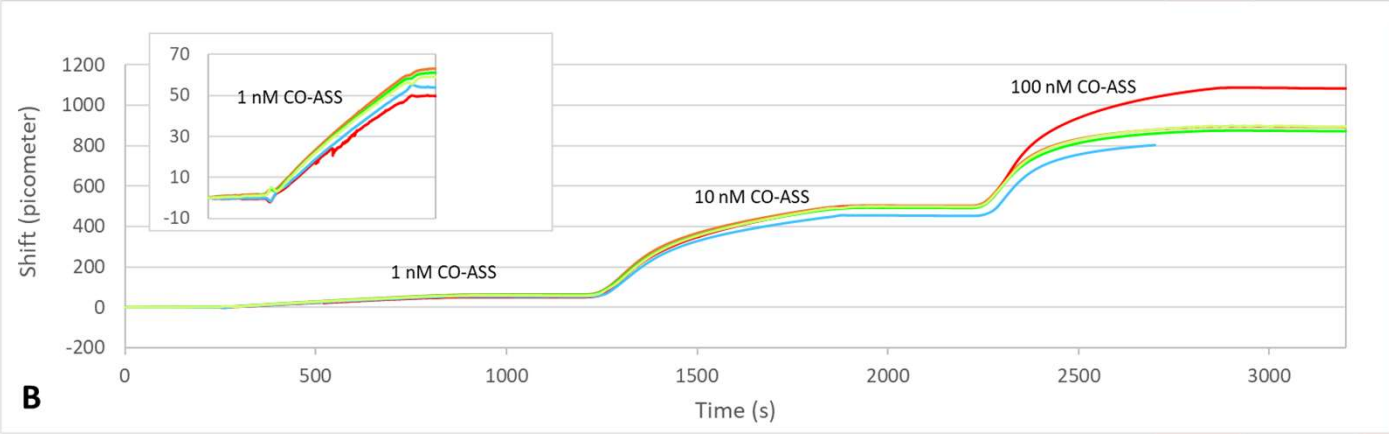
Conclusion

- Successful detection of HSA, LOD <0.01 µg/ml
- No major differences between tested conditions
- Slow signal equilibration, quantitation by analysing slope

Biomarker assay results

Bacterial DNA (Aeromonas)

- Direct detection of ssDNA
- Find optimum temperature for highest sensitivity



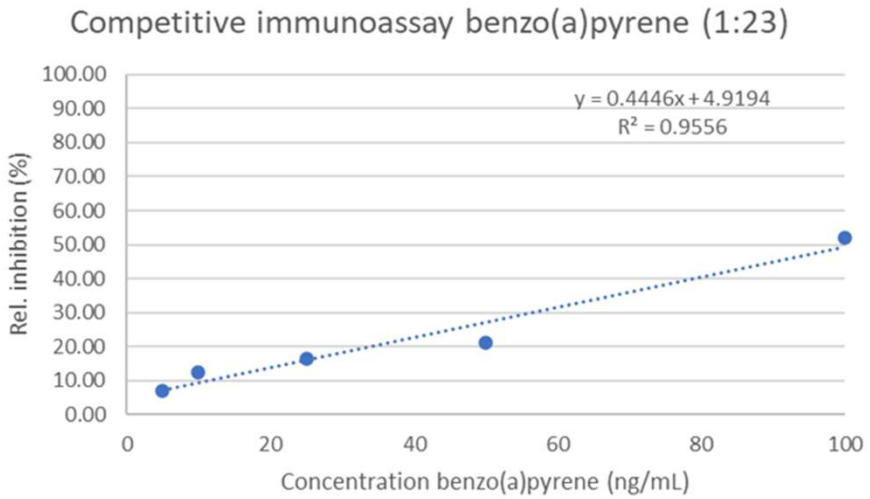
Conclusion

- Successful detection of DNA, LOD ~0.1 nM
- Future work
  - Improve sensitivity (solve issues with high T or alternative approach)
  - Detection of genomic DNA

Biomarker assay results

Benzo(a)Pyrene – B(a)P

- Synthesis and comparison of different B(a)P-protein conjugates

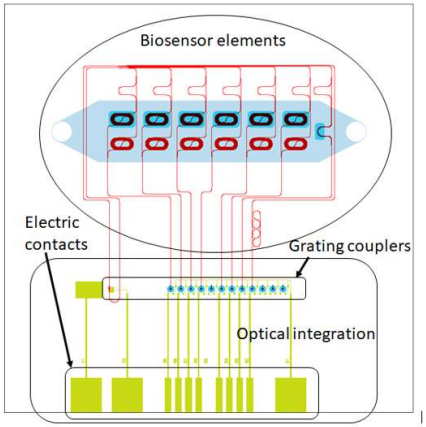


Conclusion

- Successful detection of B(a)P, LOD <5 ng/ml
- Low solubility of B(a)P in water is challenging
  - Organic solvents are not compatible with proteins used in assay
  - Investigate alternative receptors, e.g. aptamers or MIPs?

TRP-IDOS BB-2: Hybrid integration demo

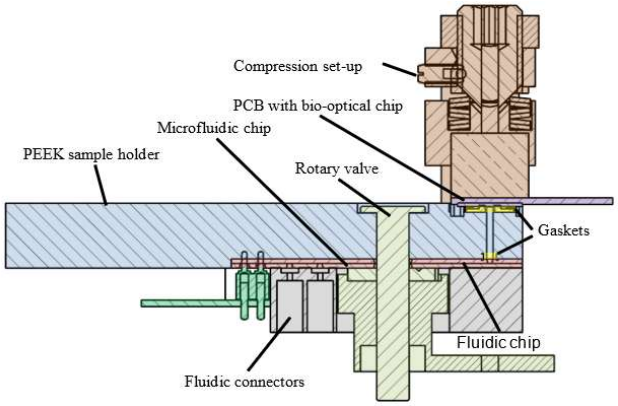
- Hybrid integrated laser and detector on biochip
- Bragg grating couplers on biochip
- Electrical interfacing on PCB by wirebonding
- Microfluidic (uF) sampling and selector valve in bonded sandwich chip module (LMC heritage)
- Demo assay: spiked samples with NaCl

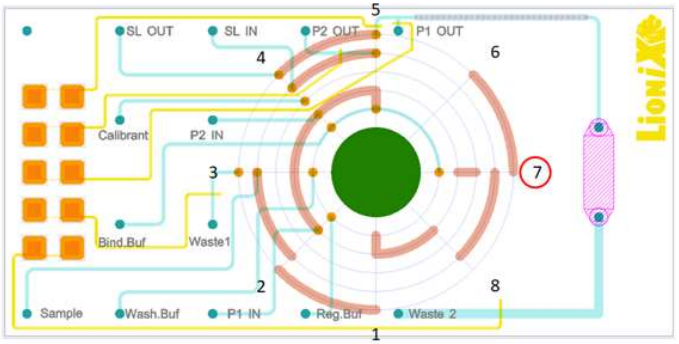


Hybrid biochip

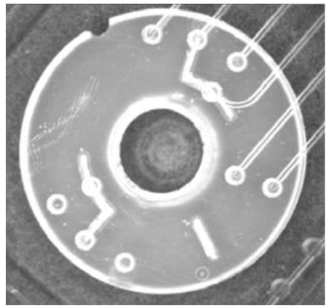

Biochip on PCB


uF sandwich chip





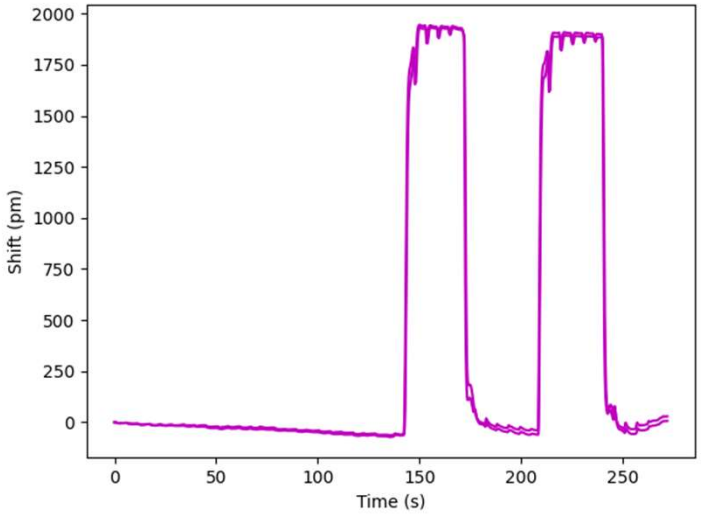
uF selector valve

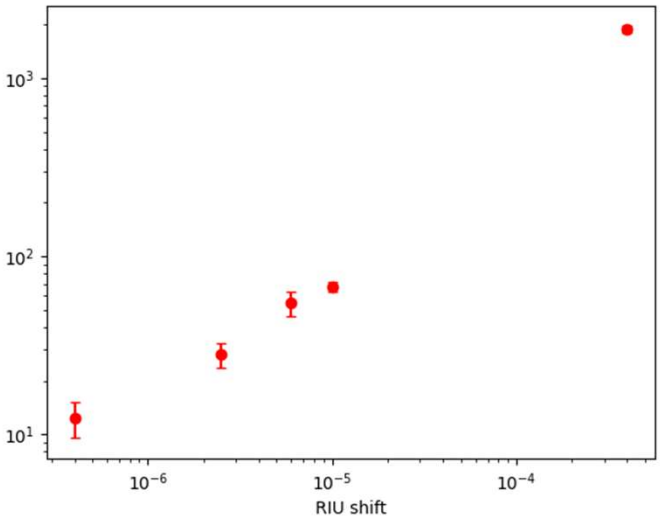

Teflon rotary slider

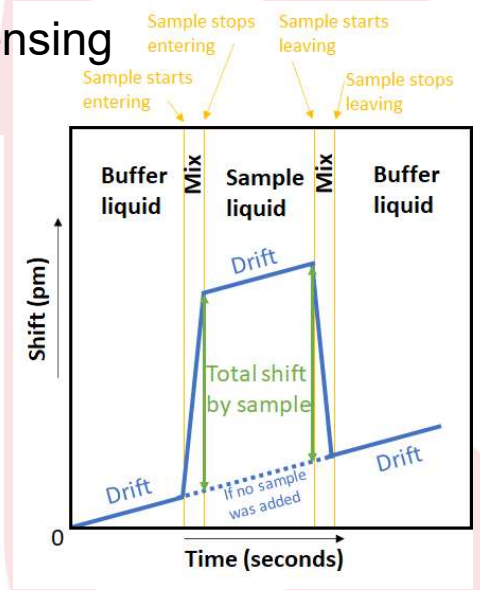
BB2 development

Validation using **salt concentrations**
Technology demonstrator: refractive index sensor

< 10^{-6} RIU can be easily measured
Improvements: application of (proprietary) tunable laser and *balanced* sensing







Development activities and challenges

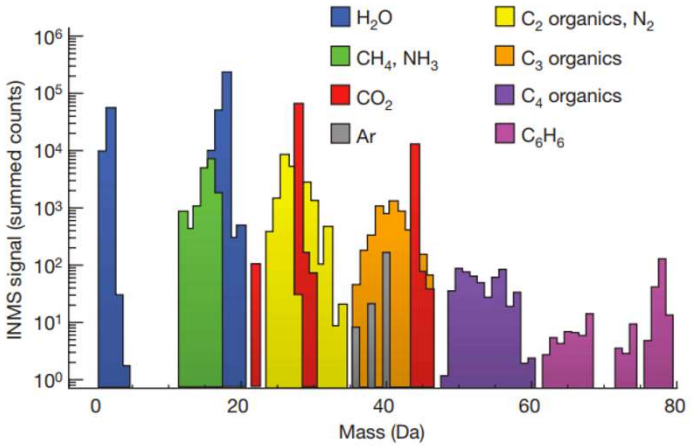
- **LMC science and technology as starting point**
- **System concept development and science case for space application**
  - Science cases – TU Delft - Vermeersen/Cazaux (present participation in JUICE – launched April 2023)
  - Sample extraction and preparation – depending on mission objective
- **Receptor technologies for life biomarkers**
  - Antibodies – state-of-the art, not ideal (lack of robustness, less appropriate for small biosignature molecule detection
  - Alternatives:
    - Aptamers (DNA constructs)
    - MIPs (Molecular Imprint Polymer)
- **Next step: From partly to fully integrated analysis breadboard**
  - Complete breadboard system for single experiment (GSTP?)

Science Case Icy Moons

- *Enceladus*, icy moon orbiting around Saturn, has a liquid ocean under its icy crust
- Water from the sub-surface ocean escapes through Geysers which have been visited by the Cassini mission *Porco 2014, Thomas 2016*
- The Cassini mission detected complex molecules in the plumes, reflecting the composition of the ocean *Postberg 2018, Khawaja et al. 2019*

Science Case Icy Moons

- Simple molecules have been observed – H2O, CO2, NH3, etc. with a mass spectrometer observing low masses (INMS).
- However, the mass spectrometer's resolution did not allow many identifications of molecules *Waite 2009*
- Fragments of large molecules have been observed and could be originating from large organic molecules
- Possible candidates such as $C_{100}H_{70}O_{22}N_3S_7$ *Postberg 2018*



Future

- **Planetary Exploration plan**
  - Objective: participation in future ESA Icy Moon mission (tbd)
  - Activities in NL
    - Running research on *Enceladus* NL (a.o. TU Delft)
    - User community: Dutch Platform Planetary Sciences (NPP), Origins Center
  - Collaboration in ESA framework
    - Science, technologies
- **Other mission/application options**
  - ESA Extraterrestrial Sample Infrastructure (EETSI)
  - Moon missions
- **Funding sources**
  - NL: NWO – GO, NSO Science Program
  - ESA – GSTP, follow up of TRP-IDOS
  - ESA Science Program