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# Optofluidics Developments for Life Detection on Mars and Icy Moons

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EPIC Meeting on Photonics at the Final  
Frontier at European Space Agency (ESA)  
ESTEC, Noordwijk, 13-14 September 2022

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# Our Mission

LioniX International is a leading global provider of customized microsystem solutions, in particular integrated photonic solutions, in scalable production.

**Integrated Photonics is one of the key enablers for this**

**Why**

Applying  
to solve

technologies

societal challenges



- Located in Enschede, The Netherlands
- >50 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 and **(RF) satellite** systems, visible light engines (AR/VR)
  - Lab-on-a-Chip and (bio)chemical sensor systems

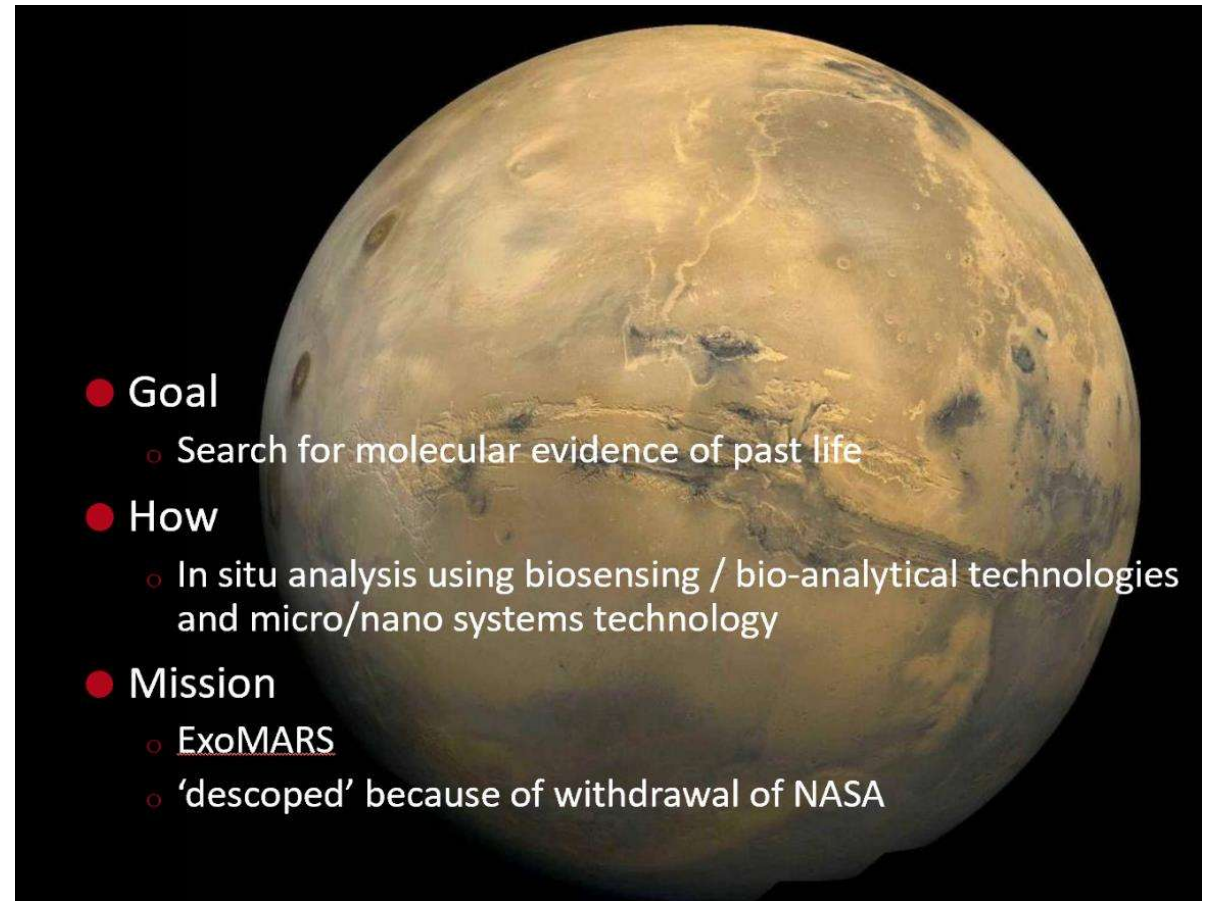


# Background: Life Marker Chip

- 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



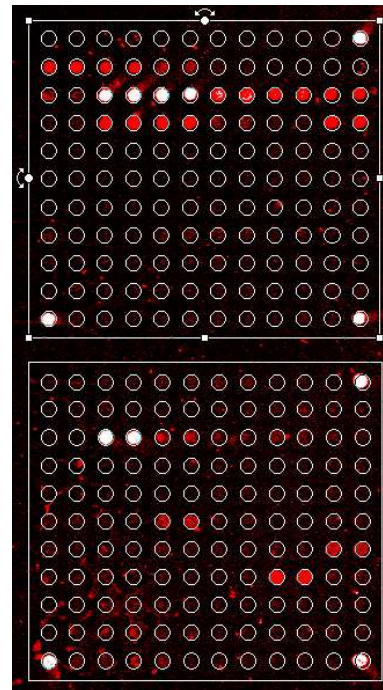
- Goal
  - Search for molecular evidence of past life
- How
  - In situ analysis using biosensing / bio-analytical technologies and micro/nano systems technology
- Mission
  - ExoMARS
  - 'descoped' because of withdrawal of NASA

## 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

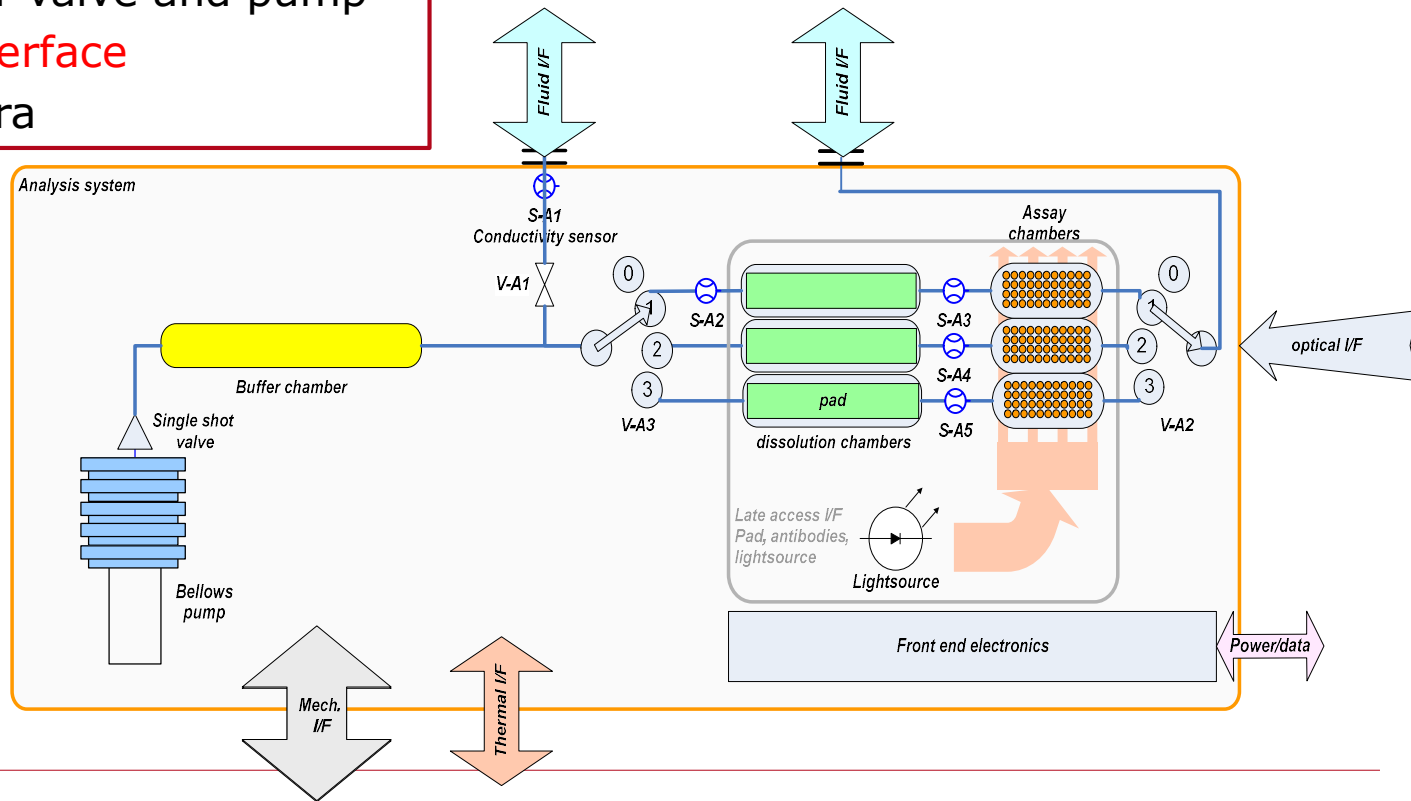
1. Whole cells,
2. Cellular debris, biofilms
3. Biopolymers

### Past life biomarkers

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

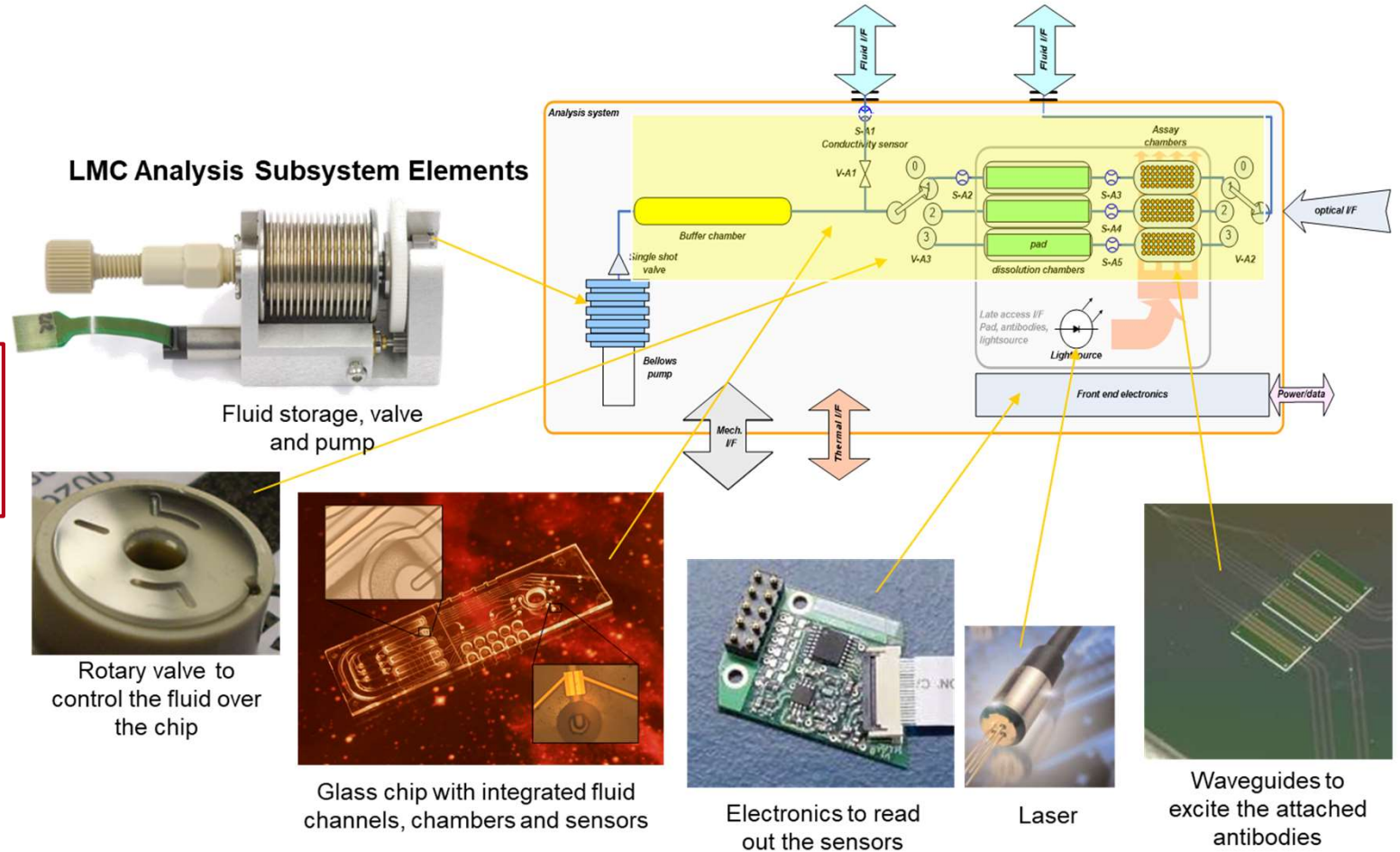
# LMC Analysis subsystem

- 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



Courtesy of Guus Borst  
Dutch Space

# Chips system integration



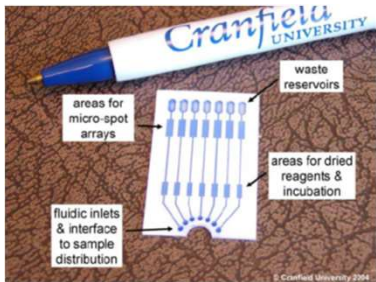
**Status 2013:**  
**Prototype TRL 5-6**



# Micro/Nano/Bio Tech in LMC

## Antibody microarray

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

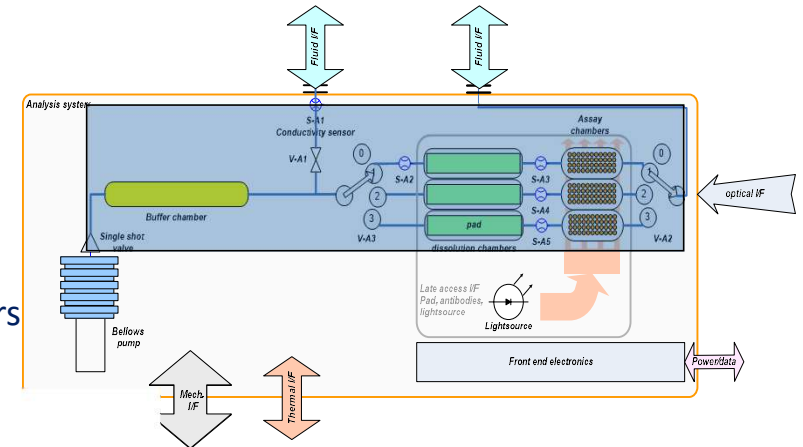
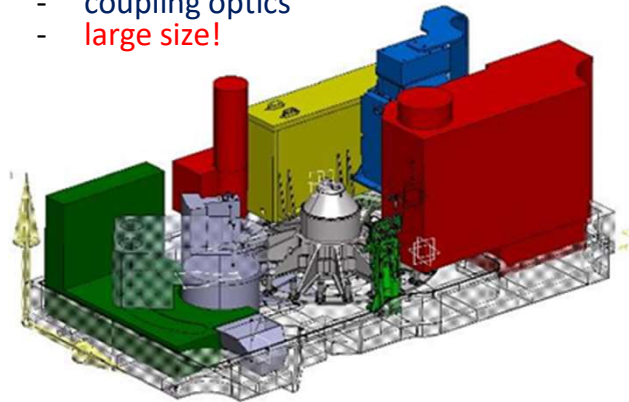


## Microfluidics based core system

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

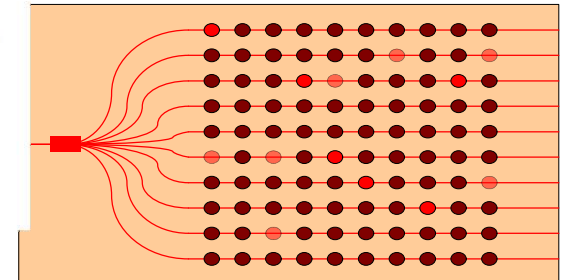
## System integration

- 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 (2020-22)- 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
<b>3.1 SPR - Surface Plasmon Resonance</b>				
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
<b>3.2 Optical fiber</b>				
Optical fiber	$10^{-4} - 10^{-7}$	-	+/-	n/a
<b>3.3 Auto-fluorescence</b>				
Auto-fluorescence detection	Depends on analyte	+	-	+
<b>3.4 Photonic crystal</b>				
Photonic crystal	$10^{-3} - 10^{-5}$	-	+	n/a
<b>3.5 MRR &amp; Interferometers</b>				
MRR MicroRing Resonator	$10^{-4} - 10^{-7}$	+	+	+
MZI Balanced Mach-Zehnder Interferometer	$10^{-6}$	+	+	-
<b>aMZI</b> <b>Asymmetric Mach-Zehnder Interferometer</b>	<b><math>10^{-8}</math></b>	<b>+</b>	<b>+</b>	<b>+</b>
Michelson interferometer	$10^{-8}$	-	-	n/a
<b>3.5 Additional techniques</b>				
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

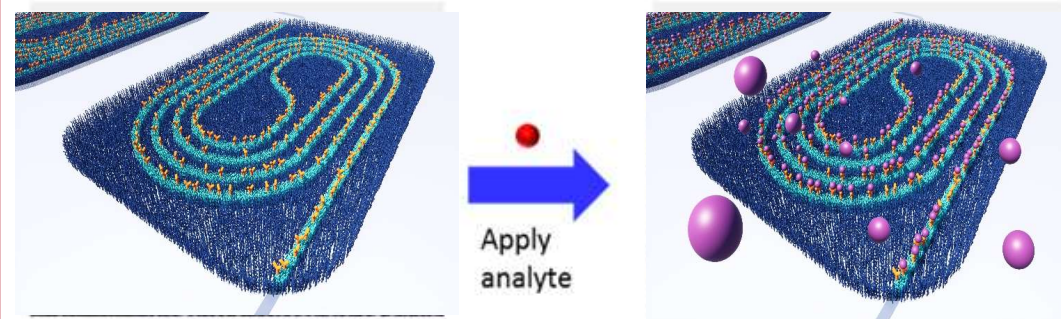
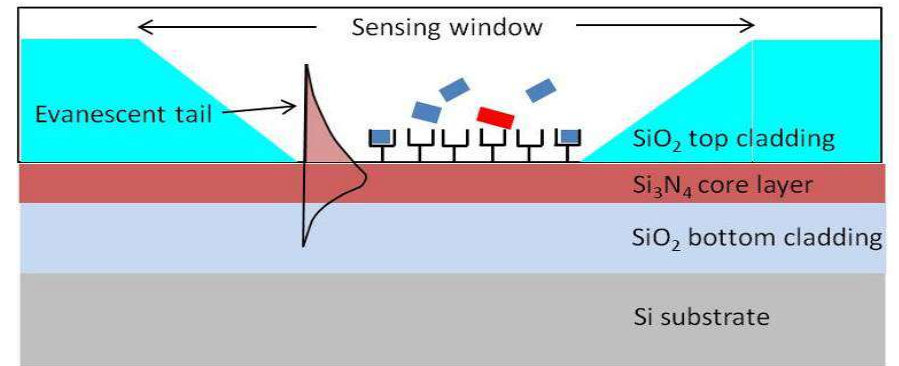
- Study and evaluation of label-free photonic biosensor platforms
- New type interferometric sensor (proprietary)
- **Asymmetric** Mach-Zehnder Interferometer (aMZI)
  - ❑ Follow up of LMC development
  - ❑ Development funded in several EU projects
  - ❑ Promising results initiated ESA-TRP IDOS

# “LMC v3.0” - How it works

## LMC boosted the development of an integrated photonics based biochip!

- 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  $\text{Si}_3\text{N}_4$  waveguide core (red)

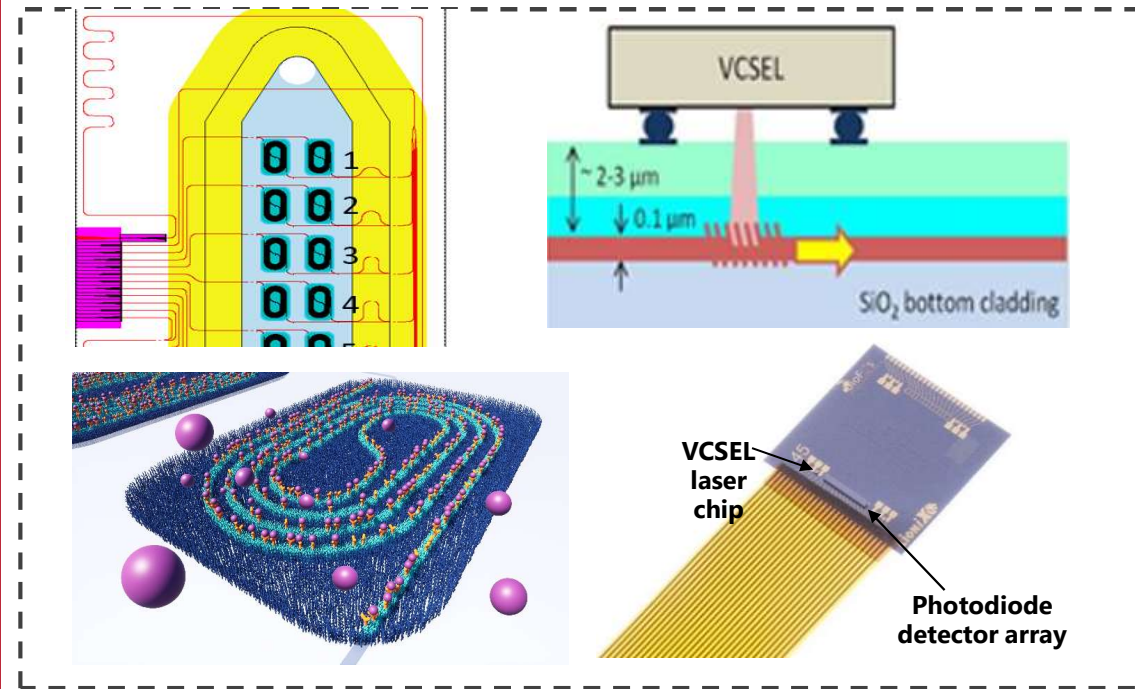
- Nanochemistry based selective sensor surface functionalization
  - Improved bio-assay reproducibility
  - Prevention of (trace) analyte depletion by repelling surrounding, large surface
- ➔ Enhancement of LoD: ~ 10-100x (in theory)



## **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

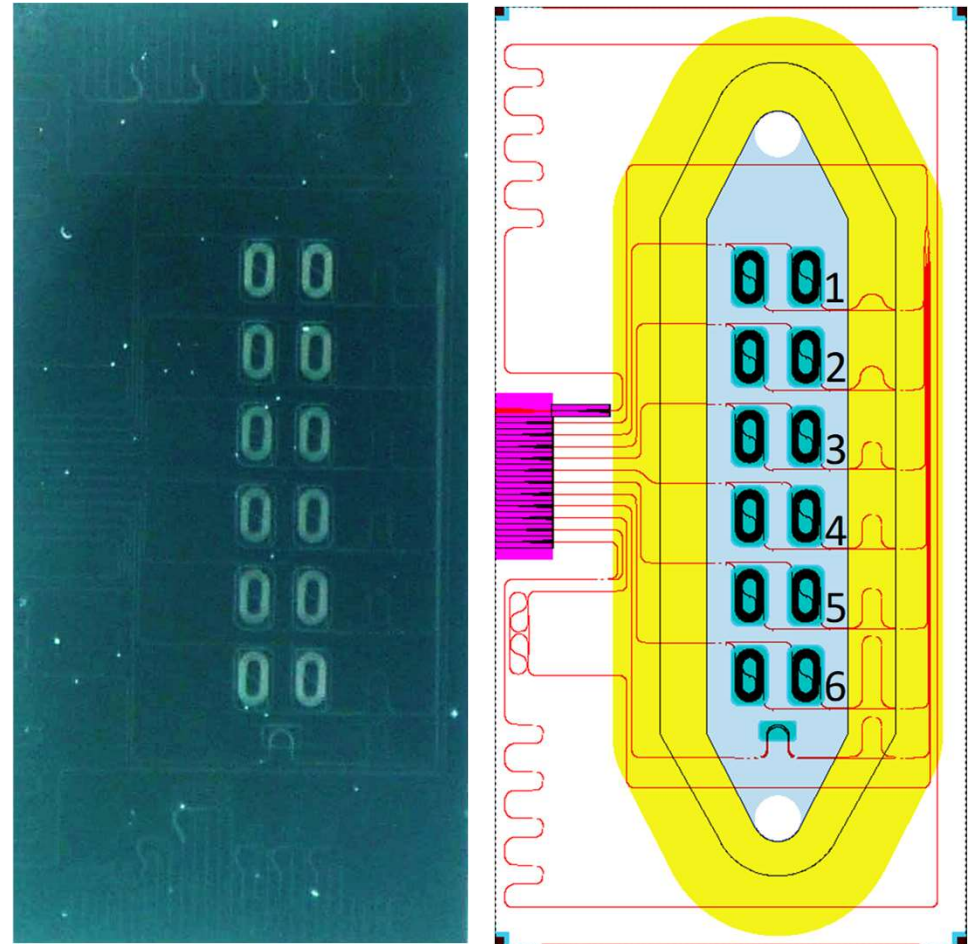


# aMZIs with enhanced optical sensitivity

- 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:  $\approx 100$  nm/RIU**

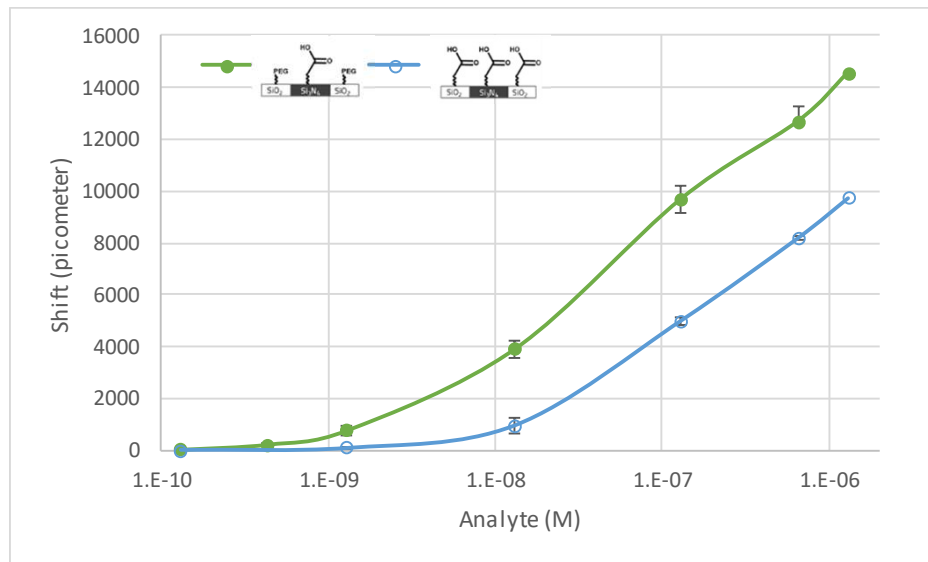
#	Sensitivity (nm/RIU)
1	9900
2	4945
3	3295
4	2471
5	1977
6	988



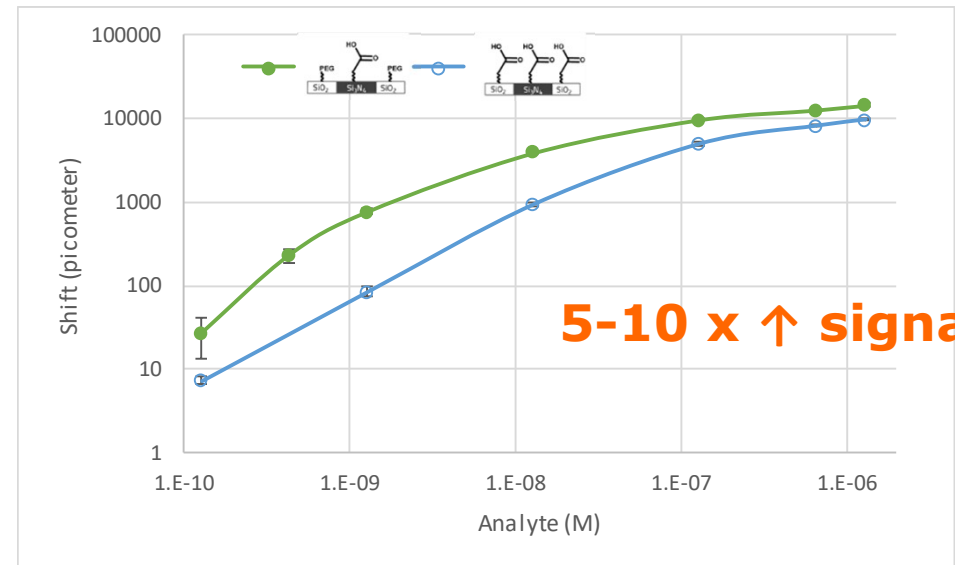
# Selective functionalization

## Comparison of selective versus not-selective

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



**Single logarithmic**



**Double logarithmic**

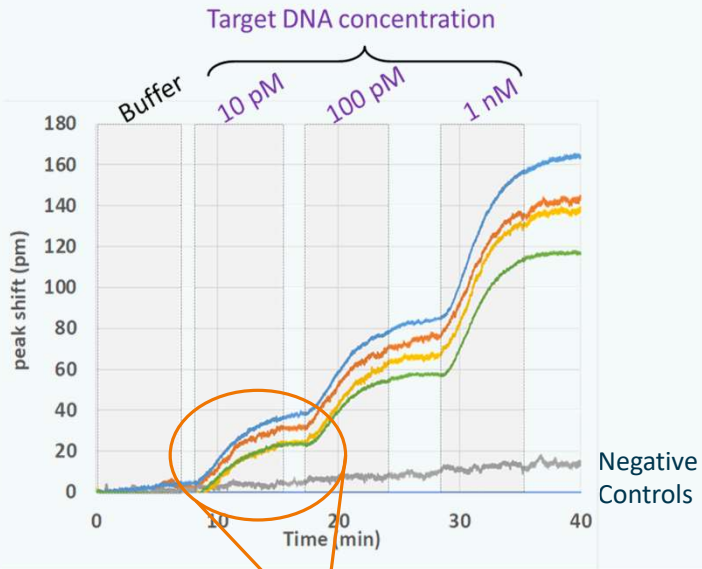
**5-10 x ↑ signal**

# Examples applications



## 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

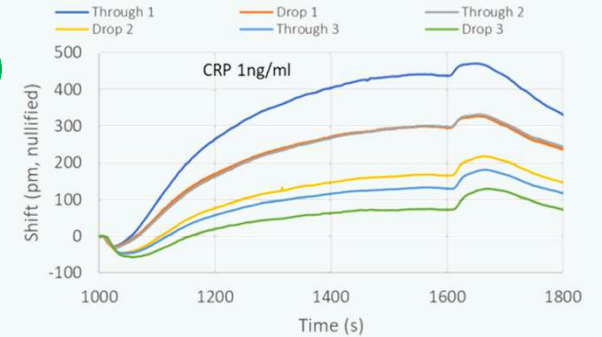


confidential

30-40 pm shift easily measured!!

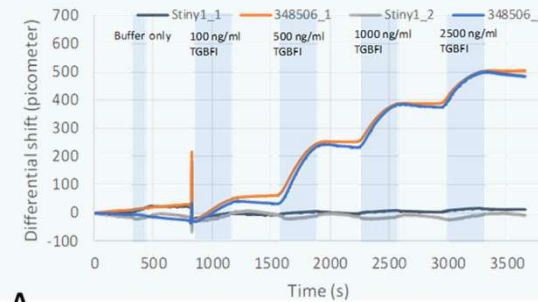
## Blood analysis C-reactive protein (CRP)

- Inflammatory marker in blood
- Clinical range  $\pm 1$  ng/ml

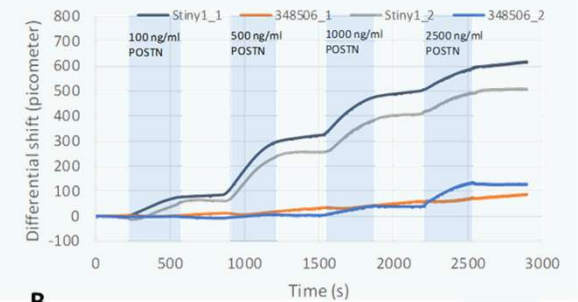


## Protein based cancer detection

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

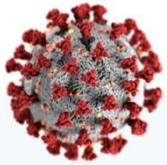


A



B





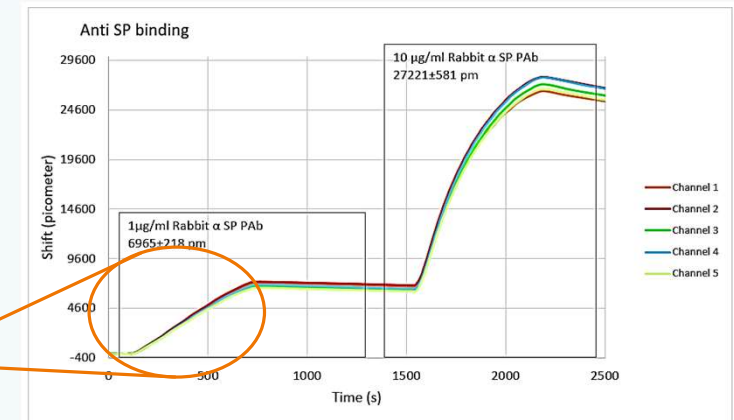
# SARS-COV-2 serology and antigen testing



## Serology test development

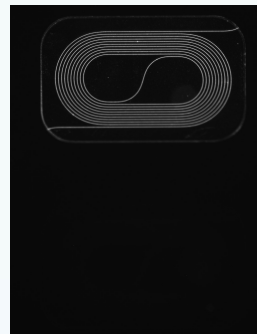
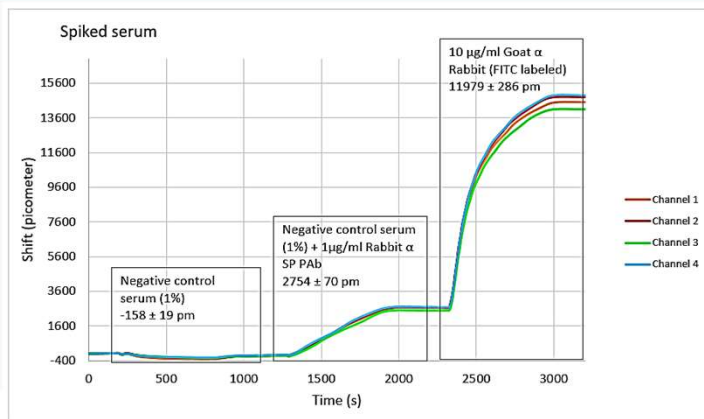
- Detection of AB against SP protein of SARS-COV-2 virus
- Clinical range  $\pm 1\mu\text{g/ml}$
- Y2022: Validated in blood serum samples!

## Spiked buffer

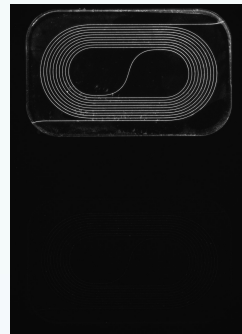
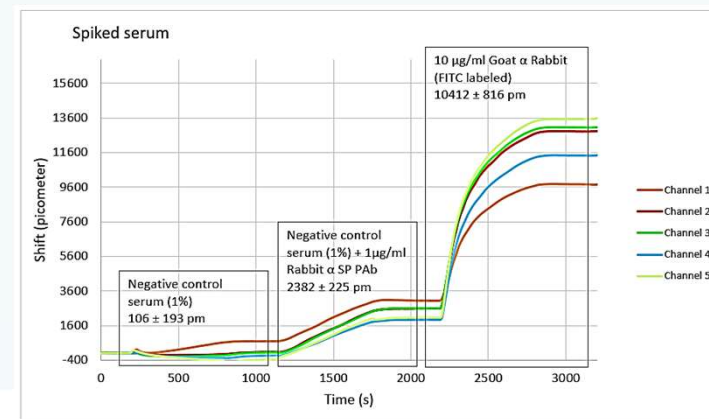


At clinical range almost 7000 pm shift is obtained  
'there is plenty of room at the bottom'

## Spiked serum (n = 1)

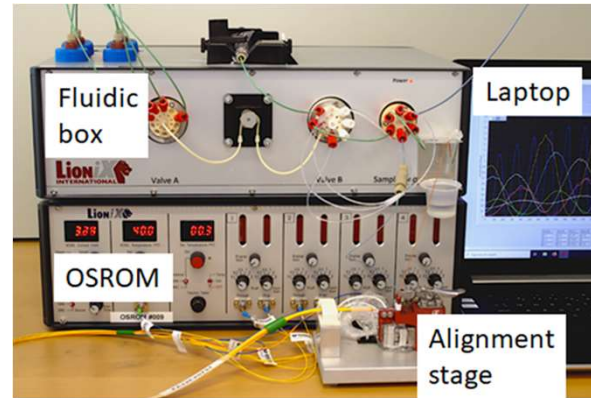


## Spiked serum (n = 2)

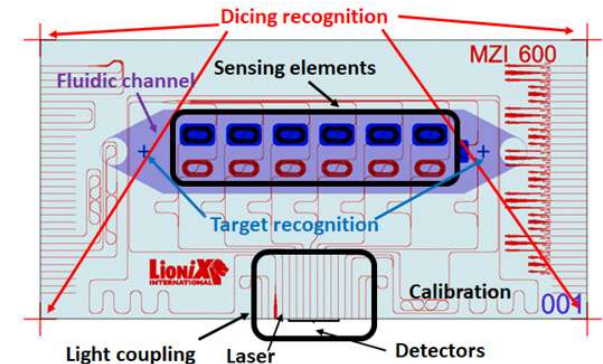


# TRP-IDOS BB-1: Assay development

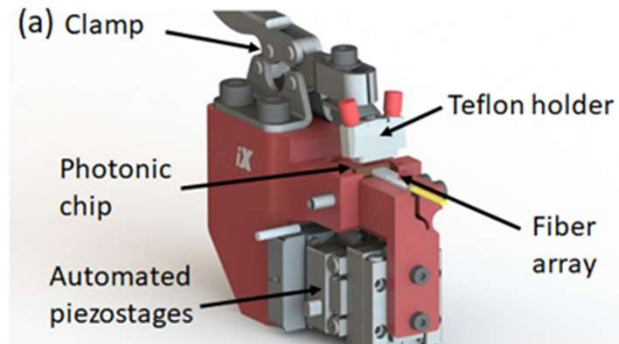
- Flexible experimental system
- Free space fiber light edge coupling of laser and detectors
- 'Naked' biosensor-array chip
- Automated positioning of fiber array
- Demo assays for CRP, HSA, DNA and B(a)P



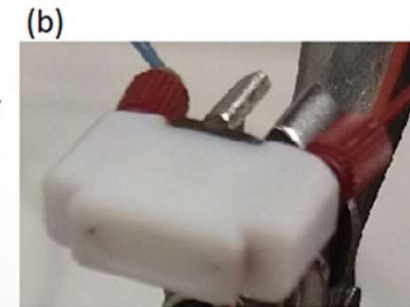
Experimental system



Schematic of biochip



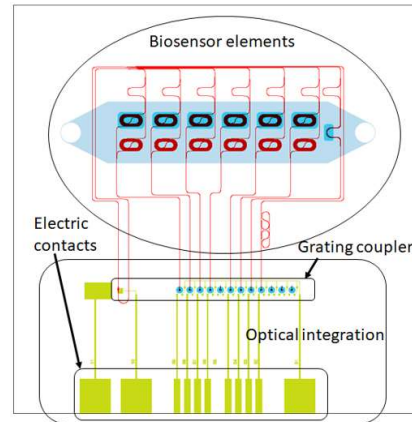
Biochip mounting/holder



Fiber array holder

# 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



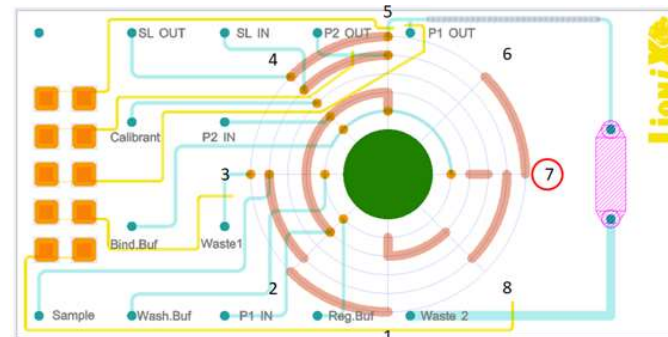
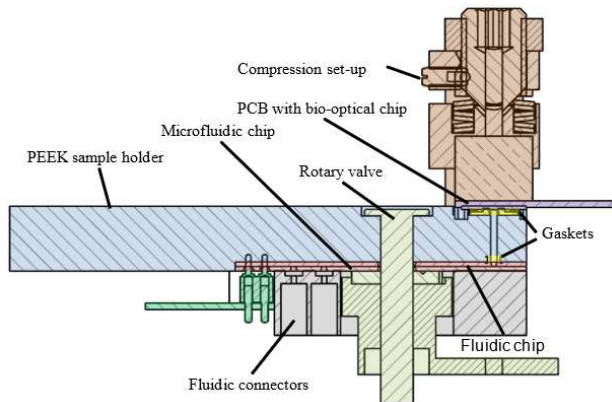
Hybrid biochip



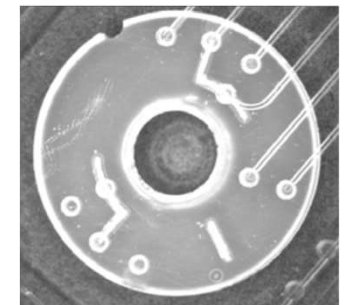
Biochip on PCB



uF sandwich chip



uF selector valve



Teflon rotary slider

# Development activities and challenges

- **LMC science and technology as starting point**
- **System concept development for space application**
  - Science cases – currently TUD-Vermeersen/Cazaux, UU-Ten Kate
  - Overall Life Marker system – Kayser Italia (It) (present lead IDOS)
  - Sample extraction and preparation – depending on mission objective
  - Planetary protection - ESA (NASA)
- **Receptor technologies for life biomarkers**
  - Antibodies – state-of-the art, not ideal (lack of robustness, less appropriate for small 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?)

- **Netherlands – Planetary Exploration plan**
  - Headline: Moons of Saturn, running research in NL
  - User community: Dutch Platform Planetary Sciences (NPP), Origins Center
  - Support of NSO
- **Other mission/application options**
  - ESA Extraterrestrial Sample Infrastructure (EETSI)
  - Moon missions
- **Funding sources**
  - National: NWO – GO, NSO Science Program
  - ESA – GSTP, follow up of TRP-IDOS (tbd in IDOS final report)
  - ESA Science Program