LIONIX

OUR CHIPS ENABLE YOUR PRODUCTS

Integration of label free optical detection in the Life Marker Chip instrument

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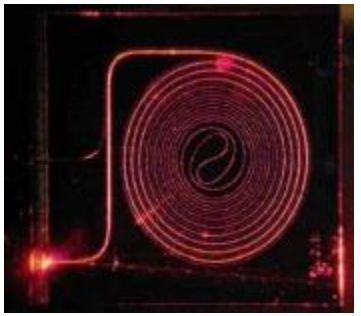
Who we are?

LioniX is a leading provider in co-development of products and manufacturing of components based on cutting-edge micro/nano technology for its (OEM) customers

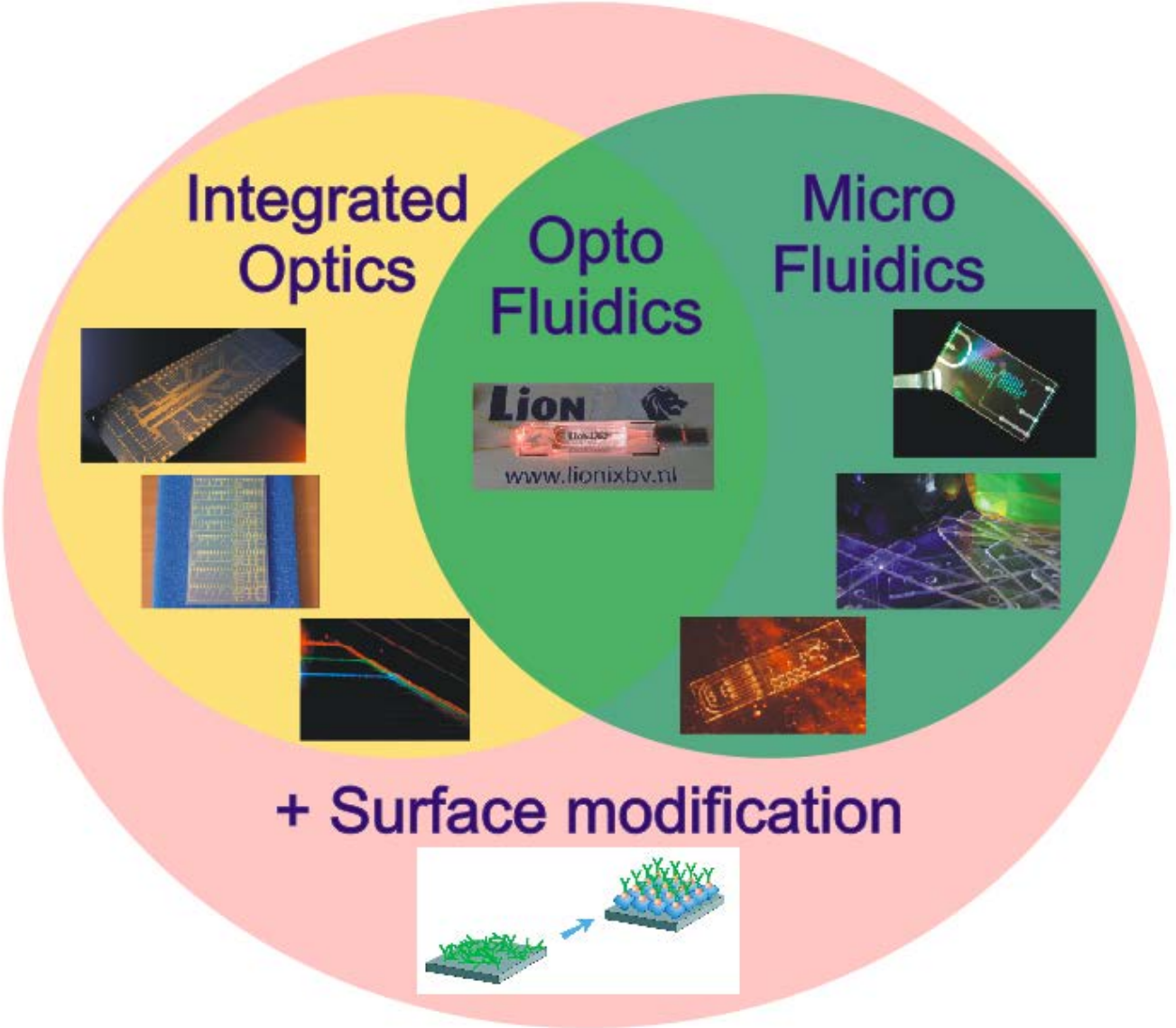
Overview LioniX

- Located at the Science Park University of Twente (NL)
  - Facility sharing with MESA+ Nanolab
- Sister company iX-factory at MST.factory Dortmund
- 30 people, mainly highly educated
- Private company (BV), profitable
- Shareholder Panthera Group BV (70 people)
  - Product spin-outs / sister companies
- Core technologies/activities
  - Custom development and production of micro/nano products
  - Lab-on-a-Chip / (bio)chemical sensor systems
  - Microphotonic data/signal processing (communications, spectrometry)



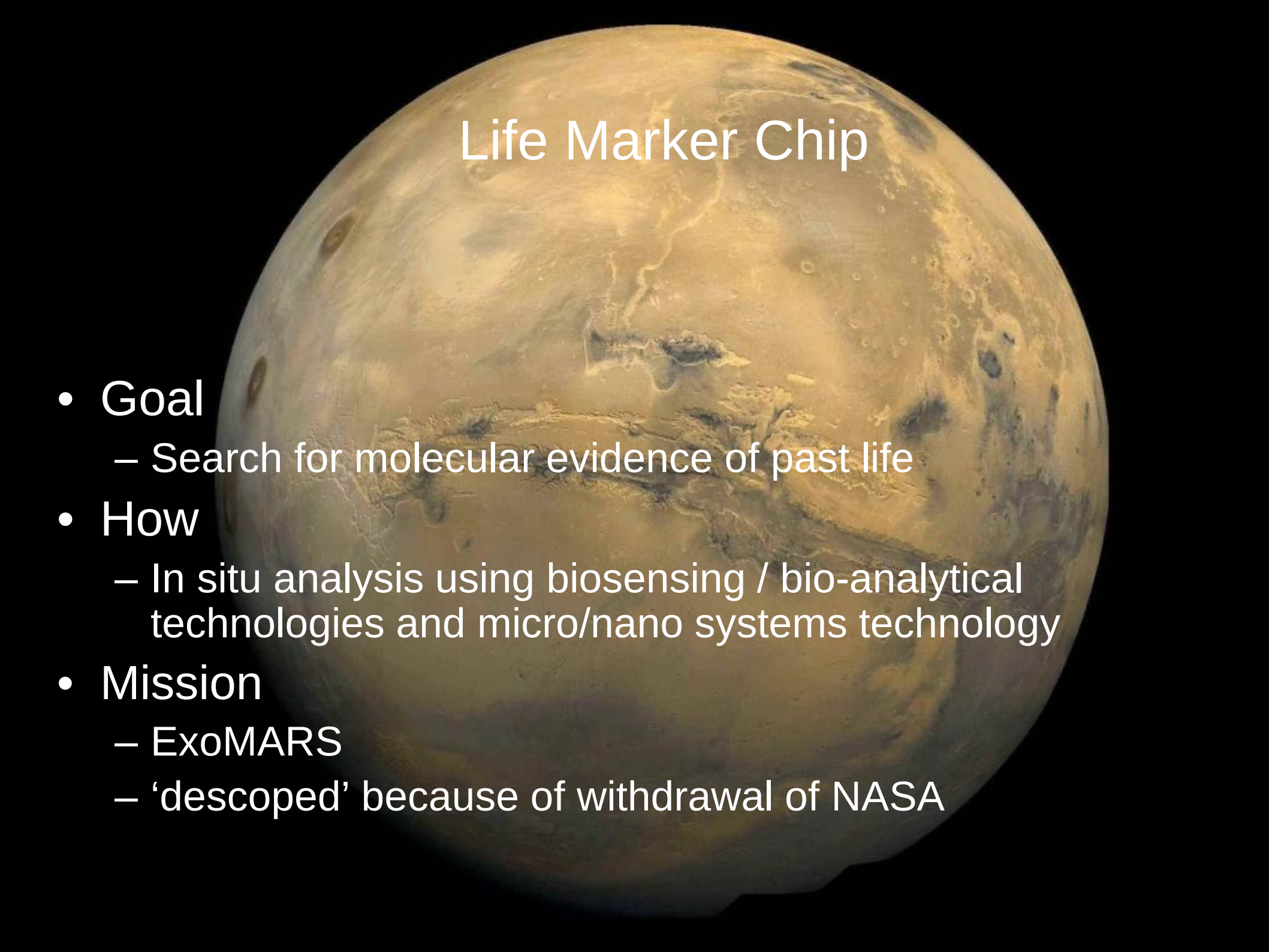



Core competences



Global presence

- Established worldwide customer base
- Representatives in UK, Scandinavia, Israel, USA, China, India, Korea



Life Marker Chip

- 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

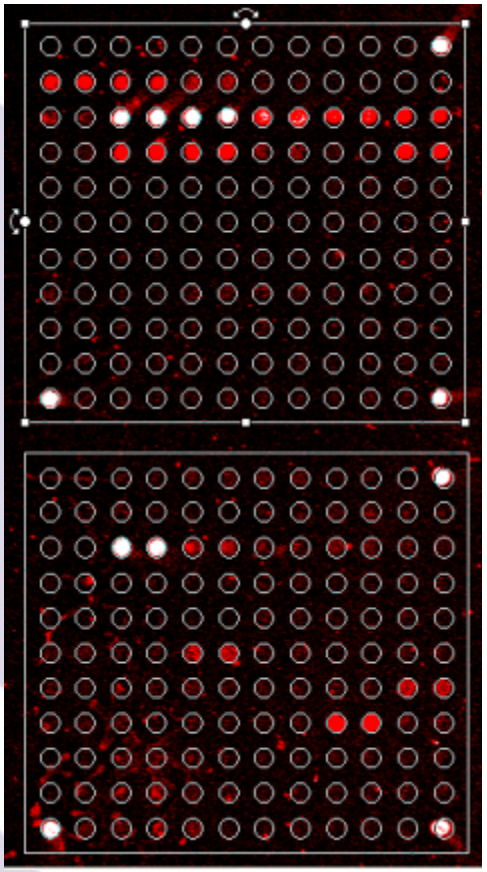
Courtesy of Mark Sims (LU) and Dave Cullen (CU)

Life Marker Chip

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

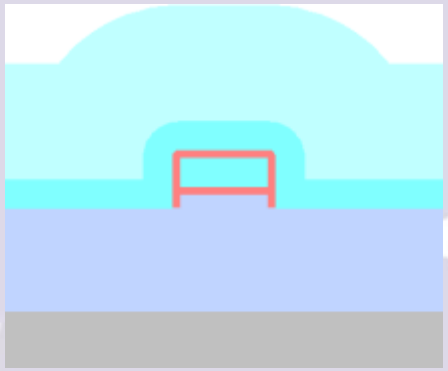
Present life biomarkers

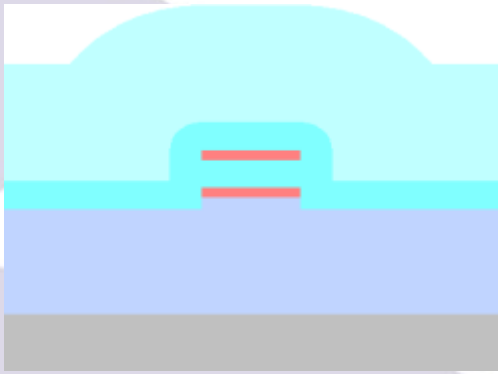
1. **Whole cells,**
2. **Cellular debris, biofilms**
3. **Biopolymers**

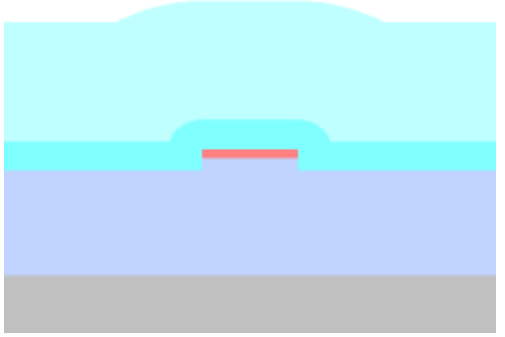
Past life biomarkers

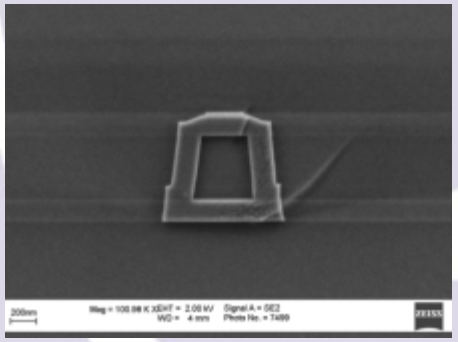
1. **Aliphatic Hydrocarbons.**
2. **Monocyclic hydrocarbons.**
3. **Tricyclic hydrocarbons.**
4. **Aromatic carotenoids.**
5. **Hopanoids and other pentacycic triterpanes.**
6. **PAHs.**
7. **Lipids – Steroids.**
8. **Porphyrins and maleimides.**
9. **Aminoacids (aa) and nucleotides.**
10. **Nucleotides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

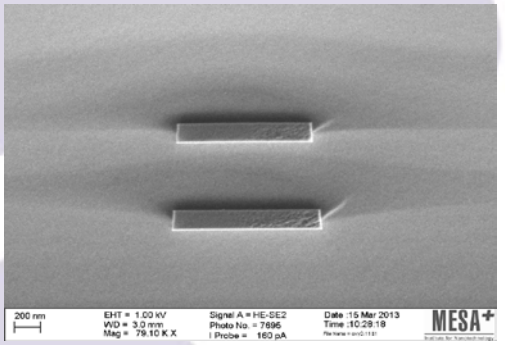
Planar waveguide technology
TriPleX™

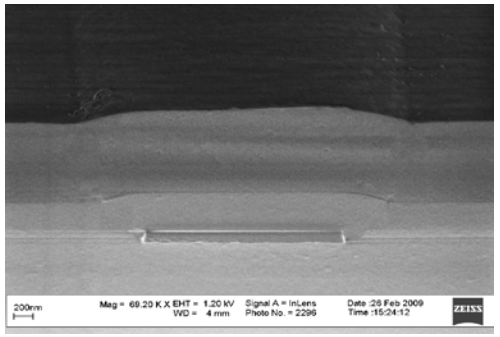




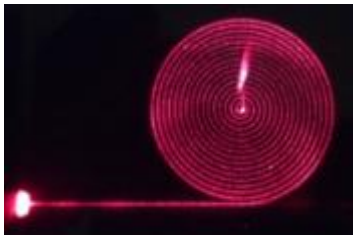






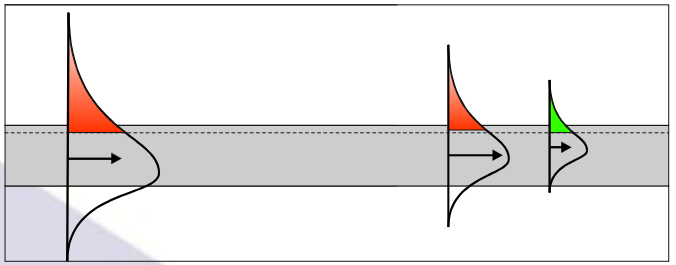


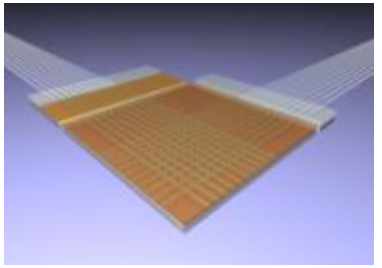
Adjustable polarization properties (sensors ⇔ data/telecom)
Low optical attenuation
Small bend radii (small footprint!)
Design by geometry



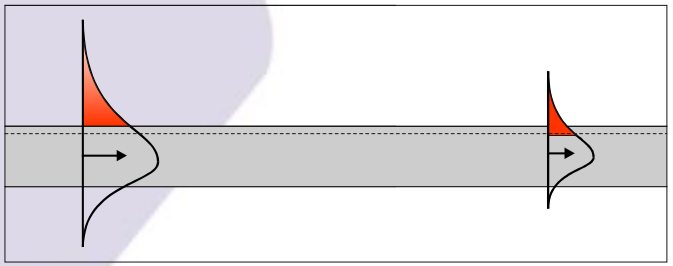
Opto-fluidics:
evanescent field based sensing types

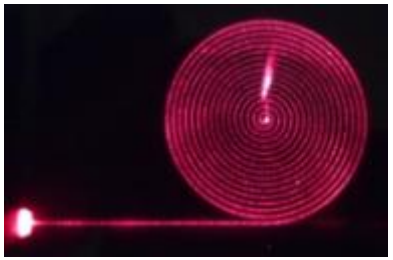
Fluorescence
- ◊ fluorescent light captured by waveguide through use of dye excitation
- ◊ detection with camera or evanescent field of detection waveguide



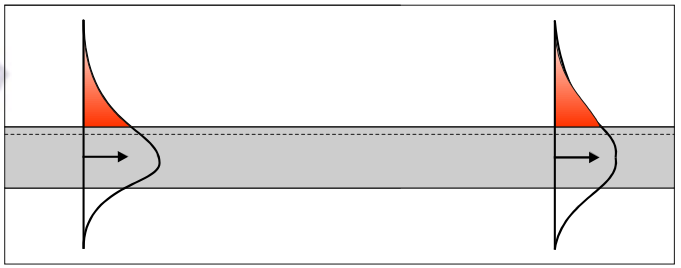


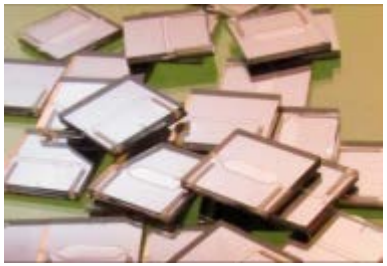
Absorbance (scattering)
- ◊ fluid/gas absorbs (scatters) light
- ◊ long interaction length
- ◊ compact 'spectro-sensor'





Refractive index (e.g. MRR*)
- ◊ modal field, Neff changes
- ◊ extremely compact and sensitive





*Published in IEEE JSTQE, p.1583-96, vol.18, no.5, Sept. 2012

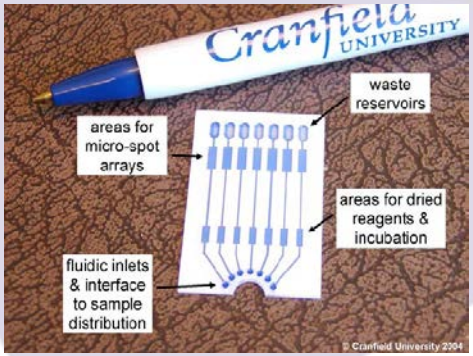
Considerations

- Fluorescence/LIF micro-array
  - (very) high sensitivity by labelling
  - very high number of spots but requires bulky camera (& optics),
  - ‘standard’ micro-array technology
- Integrated optics → photonic integrated circuits (PICs)
  - very compact by hybrid/heterogenouos integration of standard, robust technologies
  - very efficient fiber or hybrid VCSEL (butt) coupling
  - controlled fluorescence label excitation by evenescent field → reproducibility
  - ExoMARS-LMC baseline
- Evanescent field (EF) Refractive Index based sensing → LMC v2.0
  - Micro Ring Resonators in TriPleX™ platform (Silicon Nitride core)
  - label-free & no undesired affection of capturing process
  - excitation in VIS, no field losses in water (as in SOI based sensors)
  - very compact compared to LIF based LMC
  - limited in number of spots in practice

Micro/Nano/Bio Tech in LMC

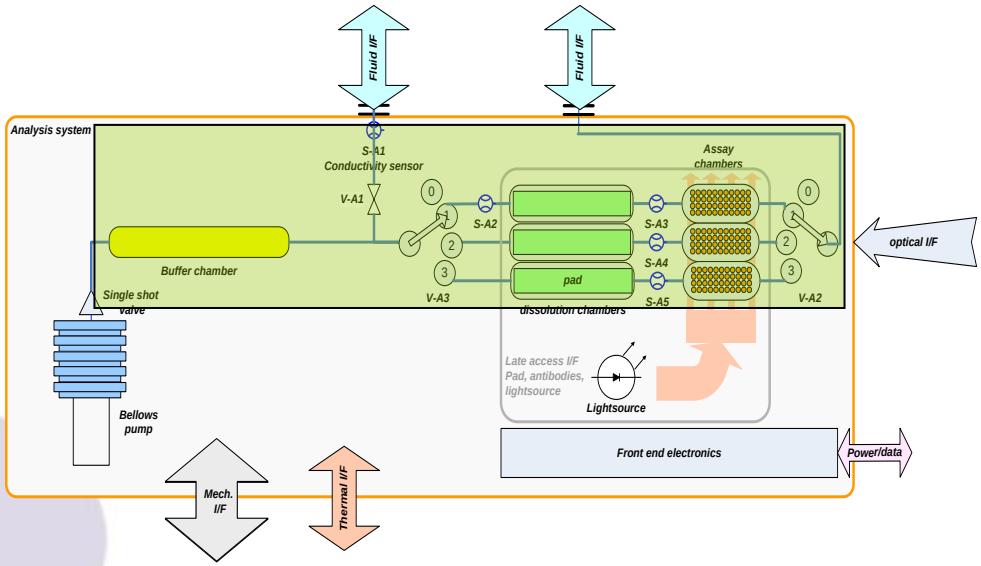
- **Antibody microarray**

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



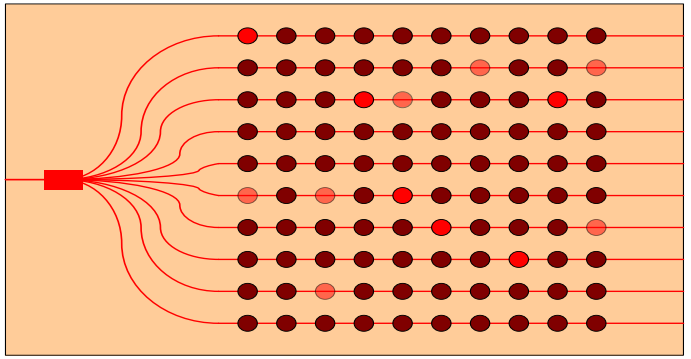
- **Microfluidics based core system**

- 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



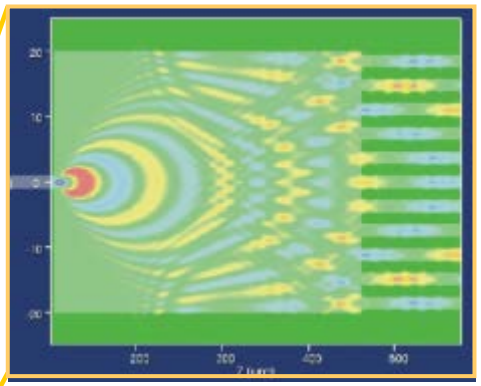
- **Integrated planar waveguides**

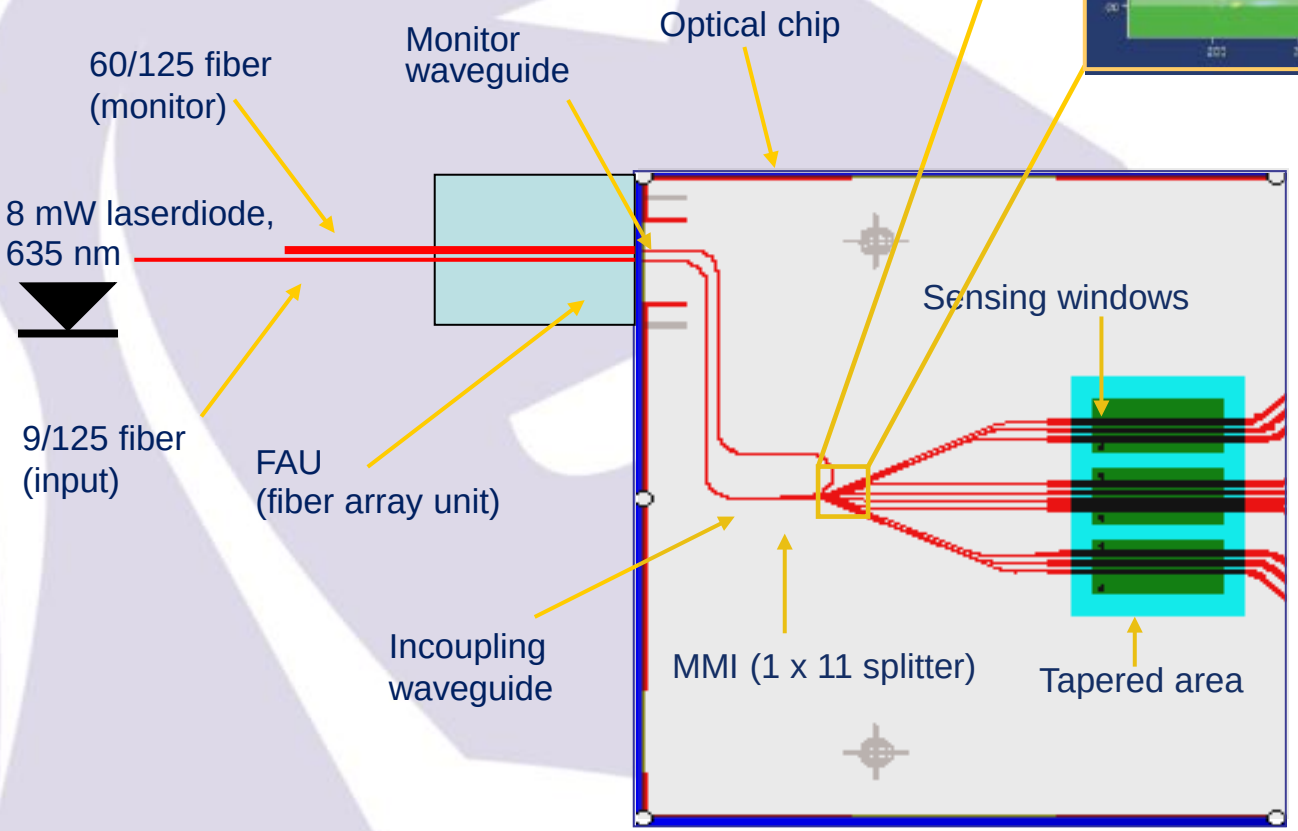
- Laser Induced Fluorescence (LIF)
- excitation by ‘manifold’ substrate

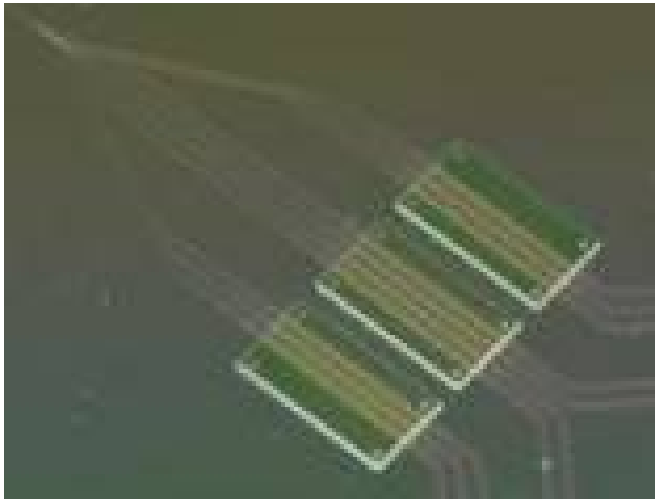


LMC optical chip and fiber connections

- MultiMmode Interferometer
- Core thickness tapering
  - 3 areas for optimization:
  - coupling, bending, sensing

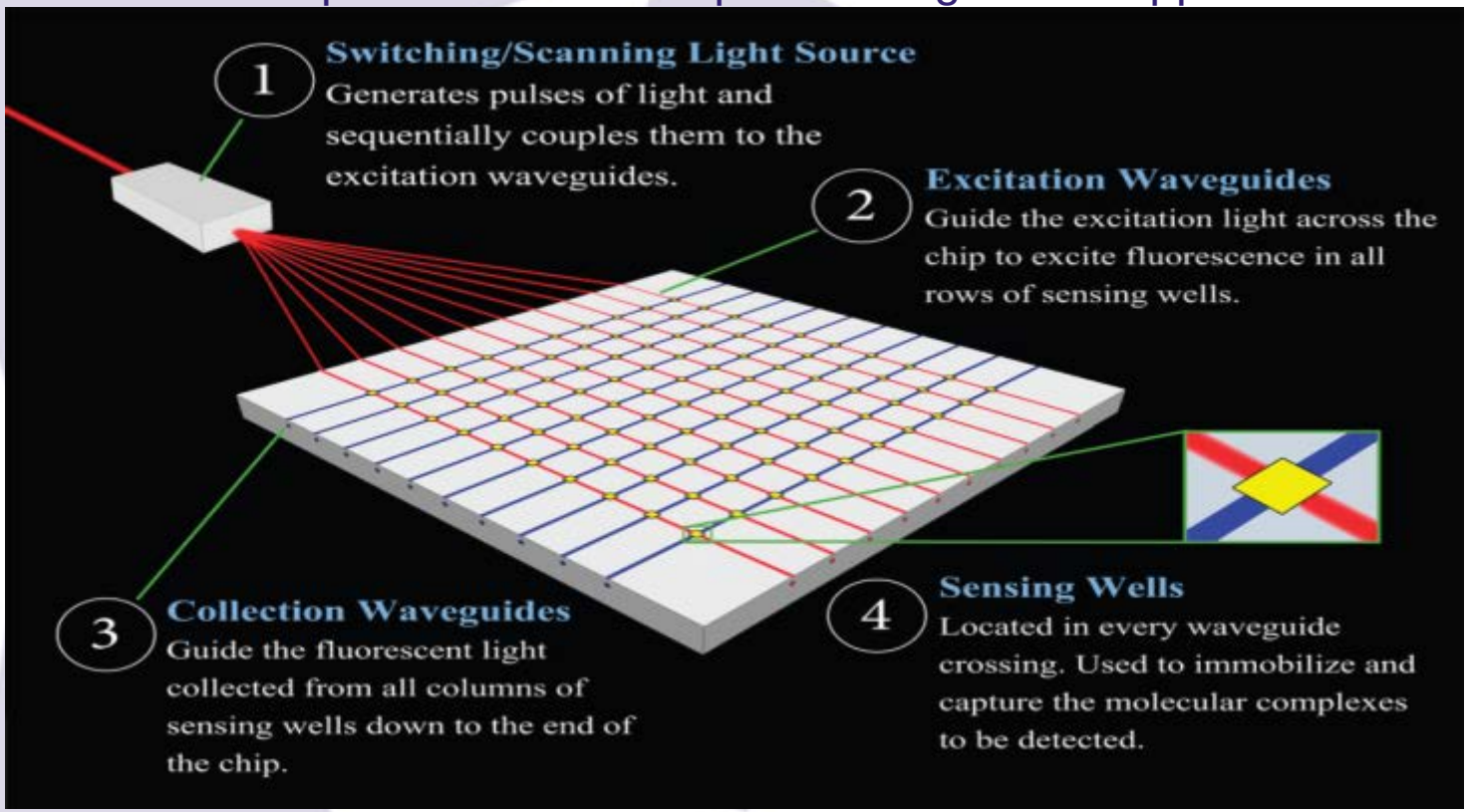


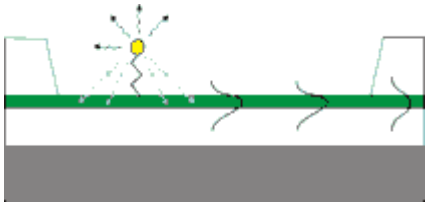


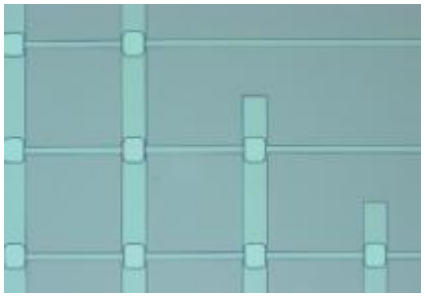


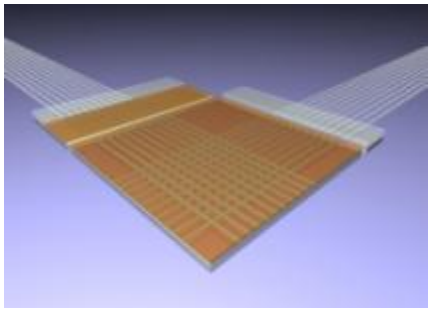
Fluorescence: excitation & detection

- Spin-off of LMC project
- Sensitive and cheap detection of molecular biomarkers
- 5 minutes Immuno and nucluic acid assays
- Very sensitive (1 fM or smaller)
- Detection in complex matrices as blood, whole serum and urine
- Suitable for point of care / companion diagnostics applications





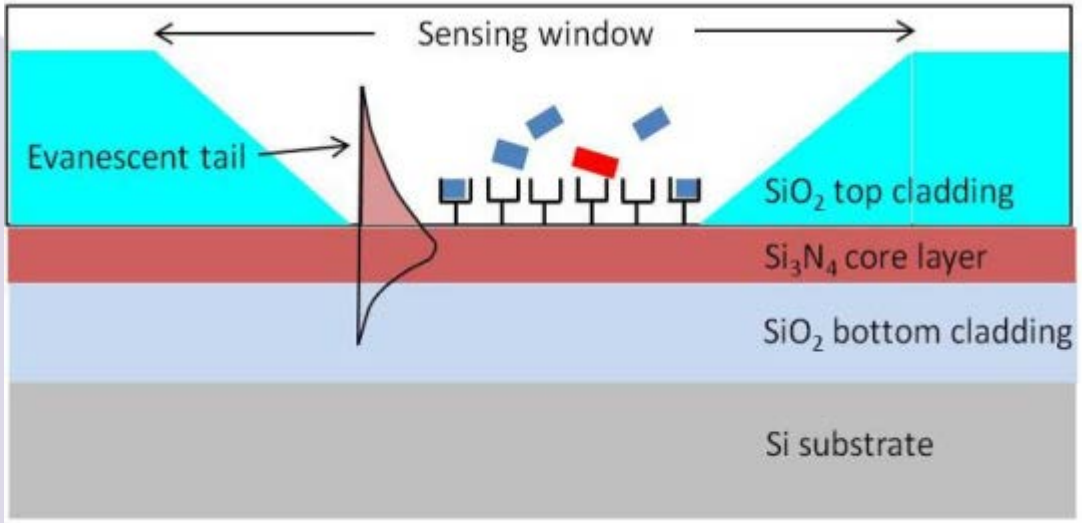


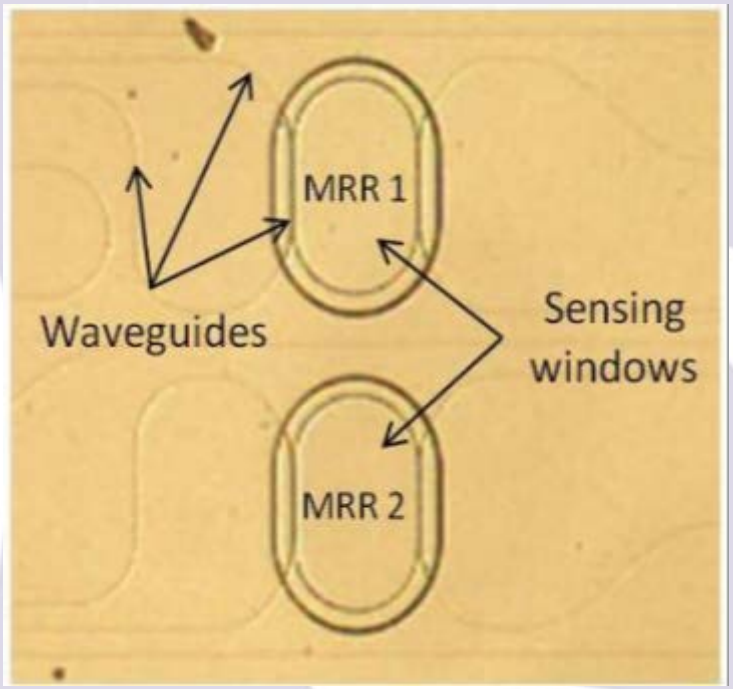


Courtesy of PLC Diagnostics

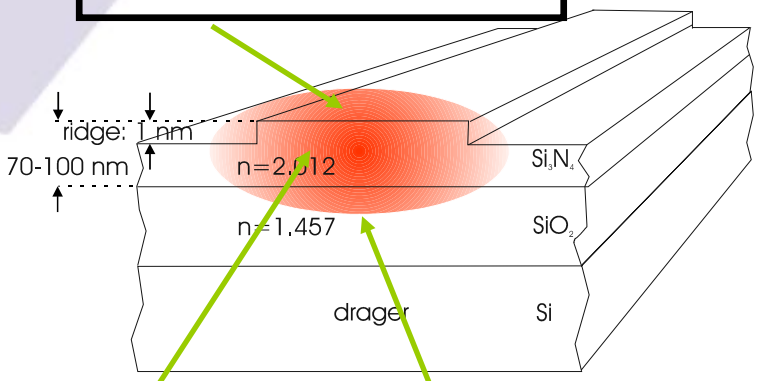
Refractive Index based sensing platform

Micro Ring Resonator (MRR)





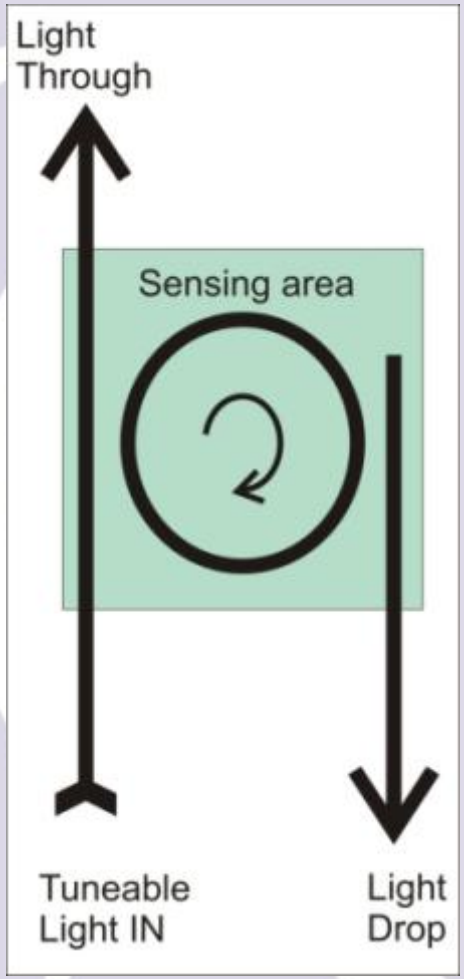
Evanescent field

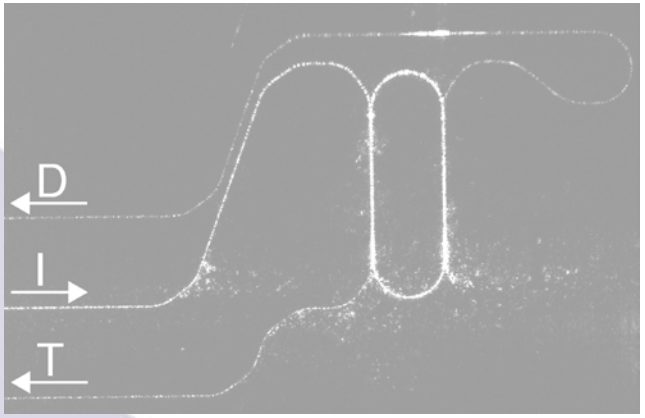


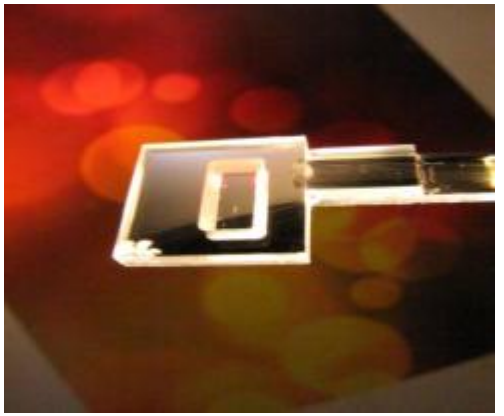
Core Si_3N_4

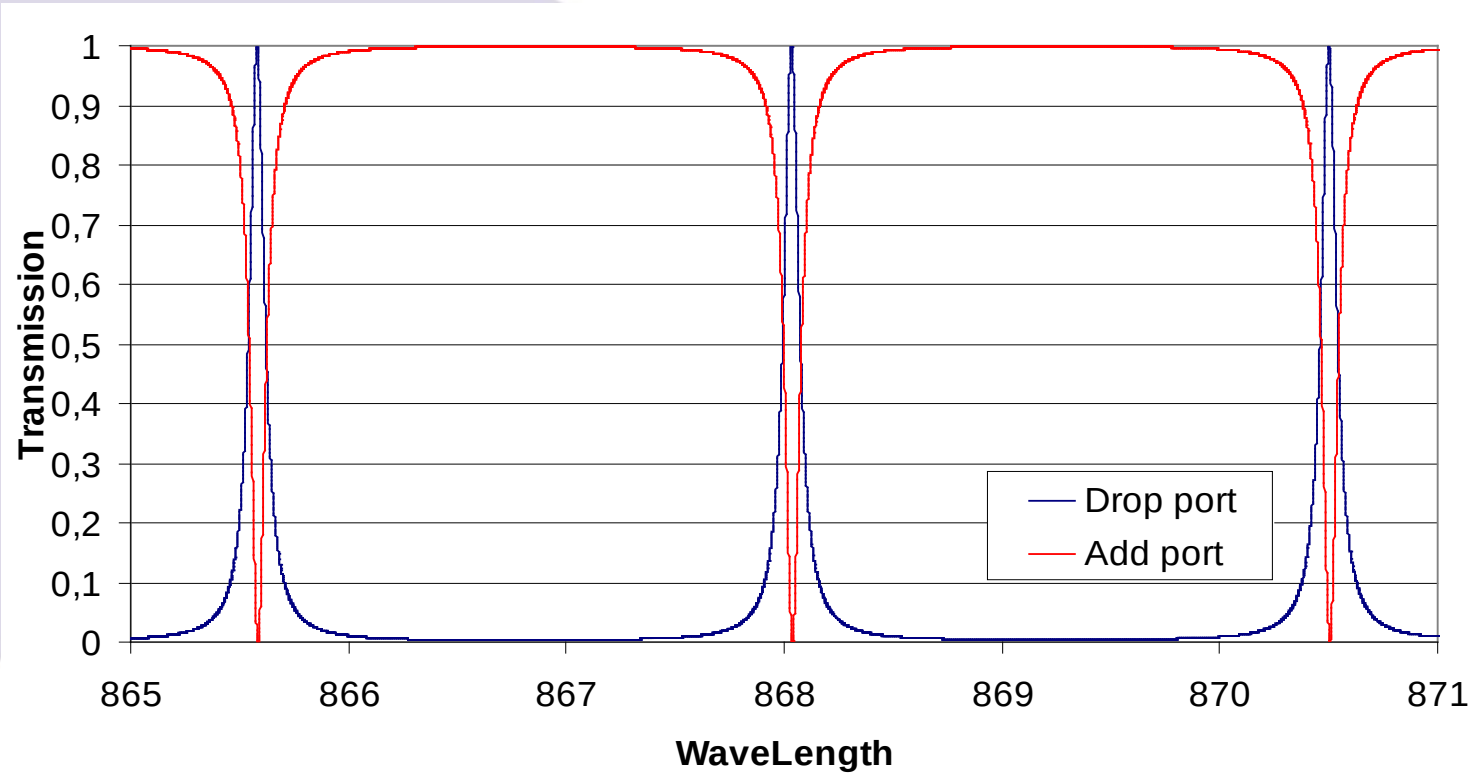
Cladding SiO2

MRR based sensing platform

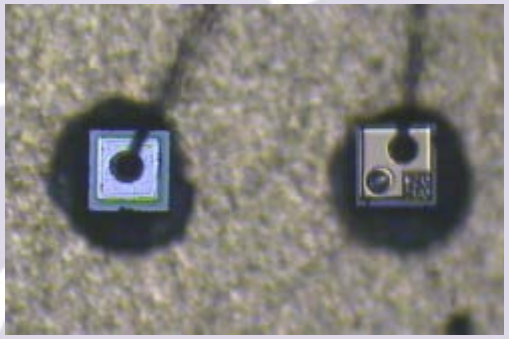


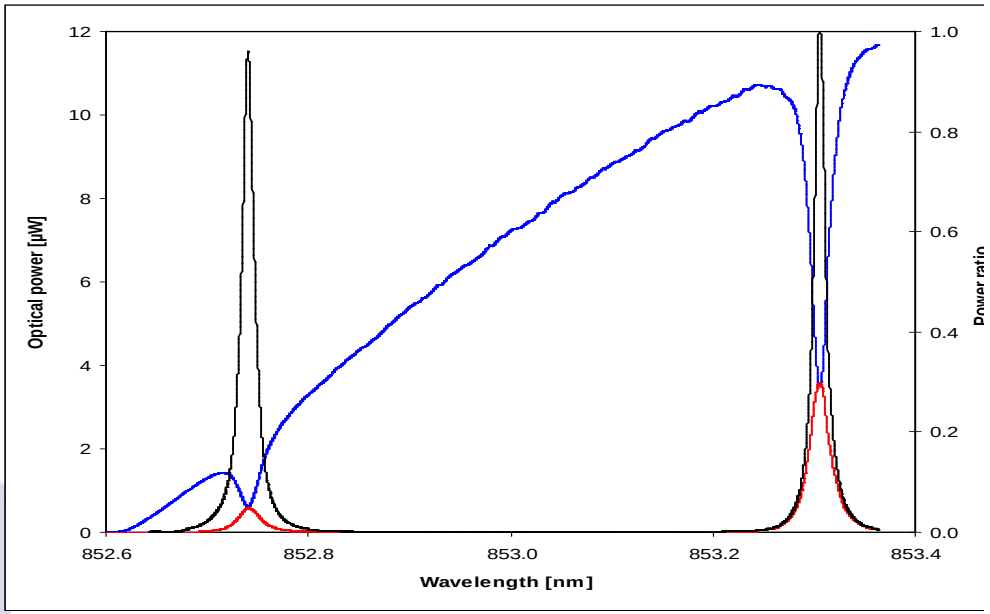




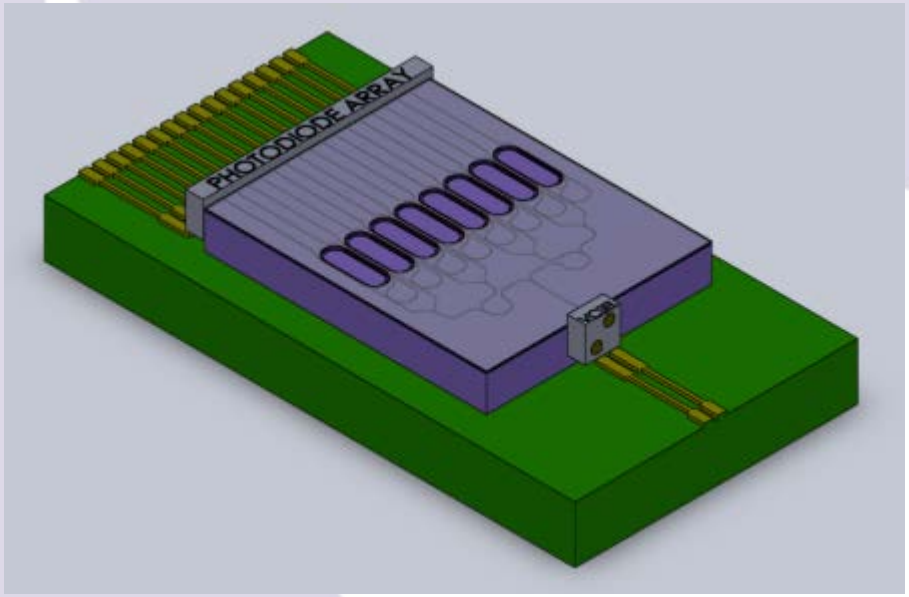


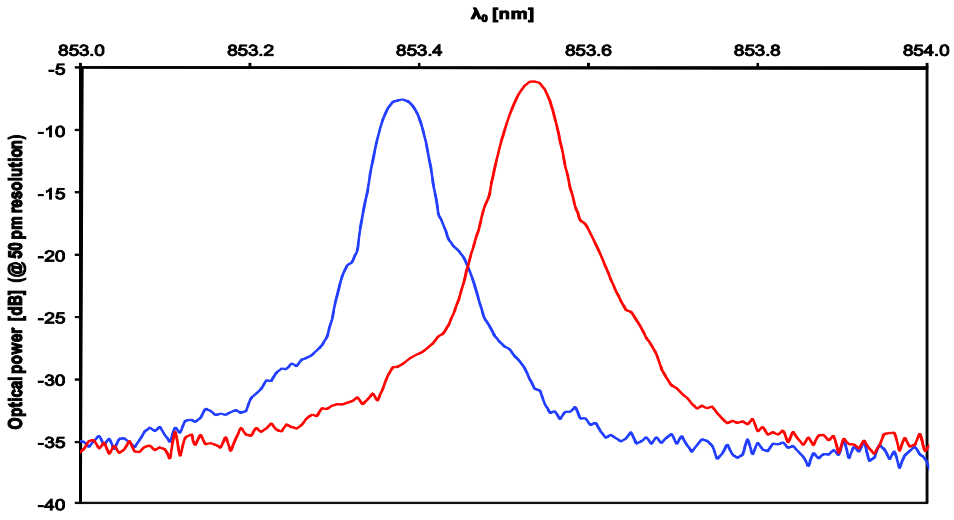
VCSEL integration & read-out





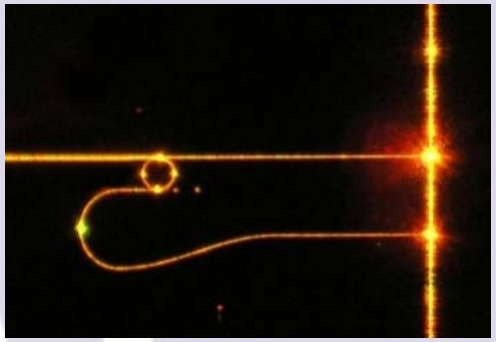
Butt-end coupling of VCSEL and photodiode array (in development)

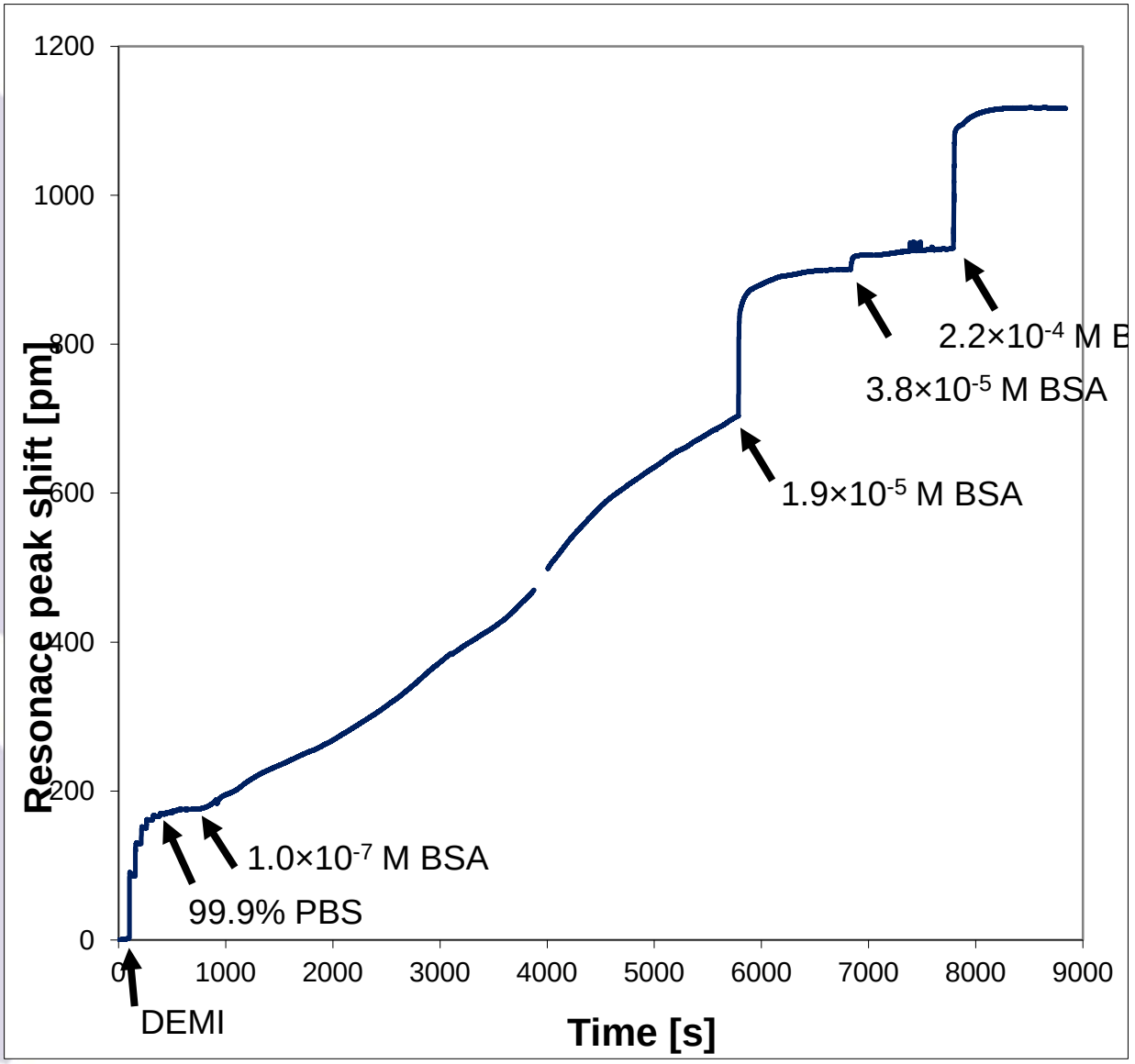




Wavelength modulation with VCSEL driving current

Example of immuno-assay with MRR





Experimental resolution:

0.1 pm

⇒ $\Delta n = 1 \cdot 10^{-6}$ RIU

⇒ surface sensitivity:

⇒ ~1 pm layer protein!

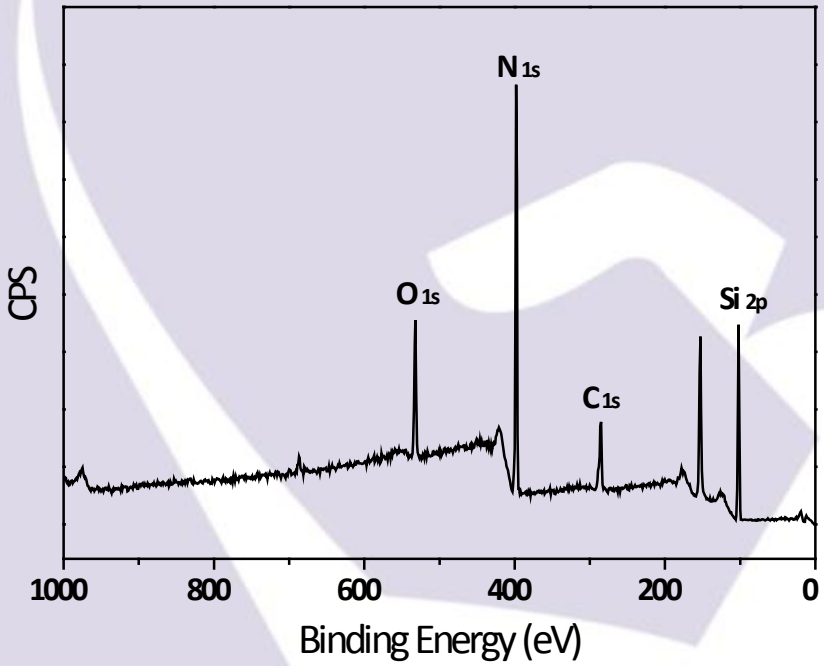
Reprinted from IEEE JSTQE, p. 1583-96, vol.18, no.5, Sept. 2012

Surface functionalisation

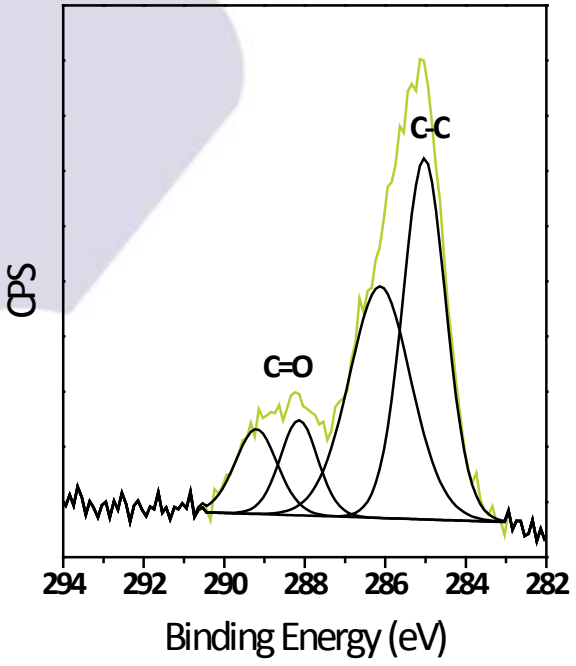
Modification with carboxylic acid functionality (COOH) (patented)

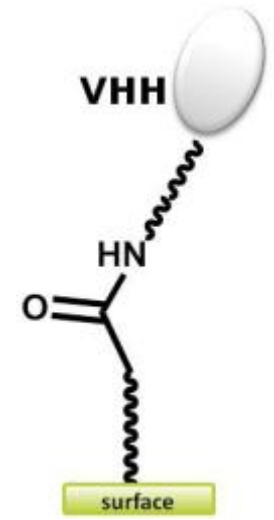
layer thickness ~1.5 nm

XPS wide scan (elemental analysis)



XPS C_{1s} narrow scan (chemical composition)





VHH-functionalized surface (part llama-antibody)

surfix
fixing surface properties

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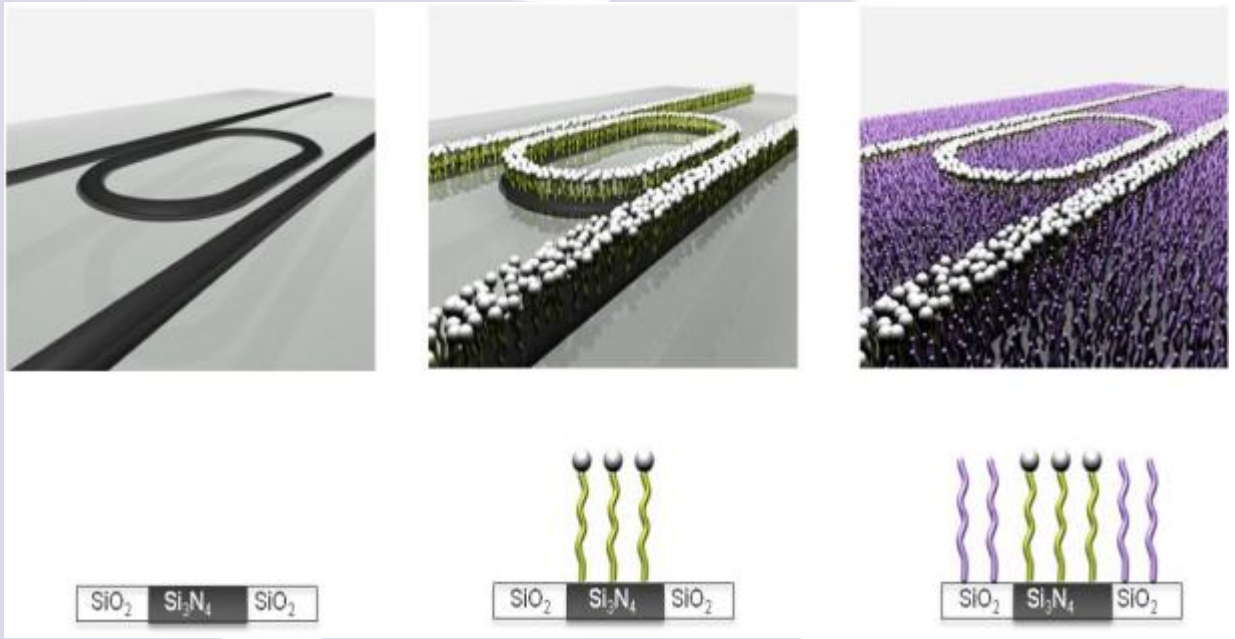
Selective surface functionalisation

Objectives:

- lower limit of detection (LoD)
  - specific binding only on relative small sensing surface
  - less depletion
- minimized non-specific binding
- higher reproducibility



Selective immobilization of fluorescently labeled streptavidin as indicator

surfix
fixing surface properties

OUR CHIPS ENABLE YOUR PRODUCTS

Spin-out applications

- LMC: *complete miniaturized system*
  - including sample pre-processing!
  - ‘flow through’, ‘closed’ systems
  - dedicated applications
  - compact and user-friendly
- LMC: *enabling technologies for ‘**field**’ analysis*
  - analysis in space (micro-gravity, life support systems)
  - medical (Companion Diagnostics - CDx))
  - food quality and safety
  - industrial process control
  - environmental and water
  - safety & security

Concluding remarks

- Innovative convergence of micro-nano-bio technologies in Life Marker Chip instrument
- Proprietary integrated optics technology TriPleX™
- Further integration of optical detection → LMC v2.0
- Surface functionalization key issue
- Developments in proprietary selective functionalization for substantial increase of sensitivity
- LMC as enabler for terrestrial applications

Acknowledgements

- Funding
  - Netherlands Space Office (NSO)
  - European Space Agency (ESA)

Netherlands Space Office

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