MOLOKO

Multiplex phOtonic sensor for pLasmonic-based Online detection of contaminants in milK

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CNR-ISMN

Food safety in the dairy sector

January 28th, 2021

PROJECT DETAILS

PROJECT REFERENCE: 780839

START/END: Jan 2018 – Jun 2021

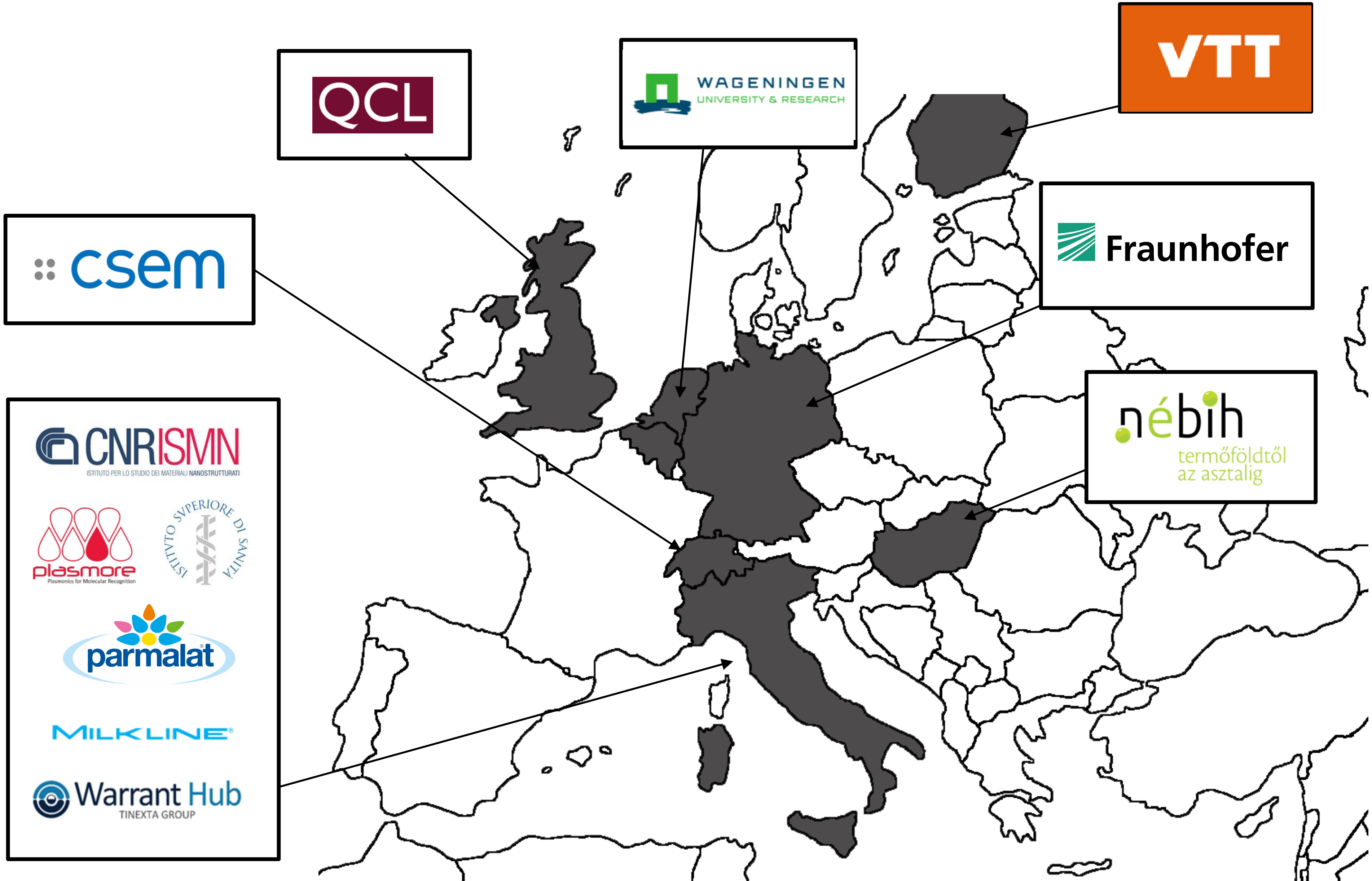
(*project extension to be determined*)

TOTAL COST: EUR 6,036,381.25

EU CONTRIBUTION: EUR 5,479,159

TOPIC: ICT-30-2017 Photonics KET 2017

PARTNERS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CNRISMN
ISTITUTO PER LO STUDIO DEI MATERIALI NANOSTRUTTURATI
plasmore
Plasmonics for Molecular Recognition
ISTITUTO SUPERIORE DI SANITÀ
parmalat
MILKLINE
Warrant Hub
TINEXTA GROUP

OBJECTIVES

Manufacturing, implementation and validation of a **self-managing and automatic miniaturized integrated photonic sensor**

Fast-response on-site monitoring of interest analytes for security and quality within **milk supply chain**

Multiplexing quantitative detection of up to 10 analytes: **food safety parametes** and **food quality parameters** (*antibiotics , toxins, antifraud analytes)*

User-friendly, **reusable** and highly-integrated **opto-microfluidic chip**

OBJECTIVES

Market-placement by direct comparison with respect to commercially-available standard analytical methods and optical biosensors

Self-monitoring the safety and quality standards in the value-chain of milk production and distribution directly by both the sector and non-technician operators

Possibility to **implement the device as in on-line analyser into milk process stream** by coupling with an (already-existing) automated technological platform for monitoring the whole milk chain

Cloud-based traceability given that all measurements can be accessed and tracked consistently along the complete production and delivery chain

AMBITIONS

1

To join the gap between **high sensitive/selective vs fast/handheld/low-cost sensors** for multiplexing on-process diagnostics in food safety.

2

To define **effective and univocal standardization protocol** from farm to fork in the diary supply chain

3

To **avoid time-dependent interferences** with antibodies and milk fouling on the sensing interface

4

To **increase the overall method sensitivity** and instrument miniaturization

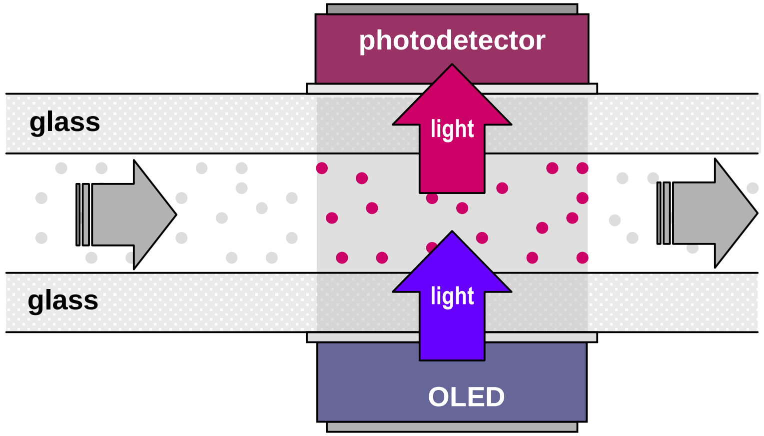
5

To respond to **user-driven specific needs** and new applications

6

To **increase reliability and specificity**

Optomicrofluidic sensor for healthcare diagnosis and food security



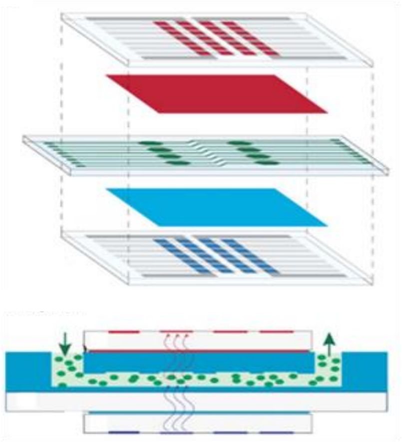
Schematic of a Lab-On-a-Chip device itegrating a microfluidic chip, organic LEDs and photodetectors

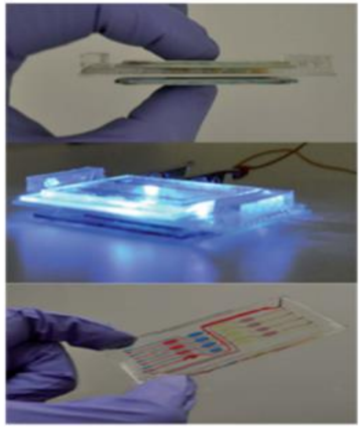
Major Pros:

- ❑ Avoidance of bulky optical light-sources/detectors and photonic components
- ❑ Higher level of components integration
- ❑ Real-time in-situ quantitative diagnostic tests

MOLOKO

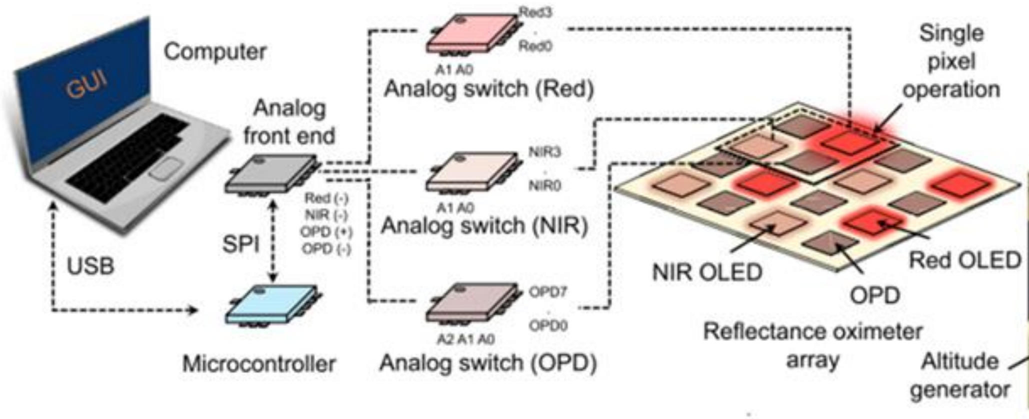
Organic optoelectronics into biosensors





Algal fluorescence sensor based on a microfluidic chip comprising:

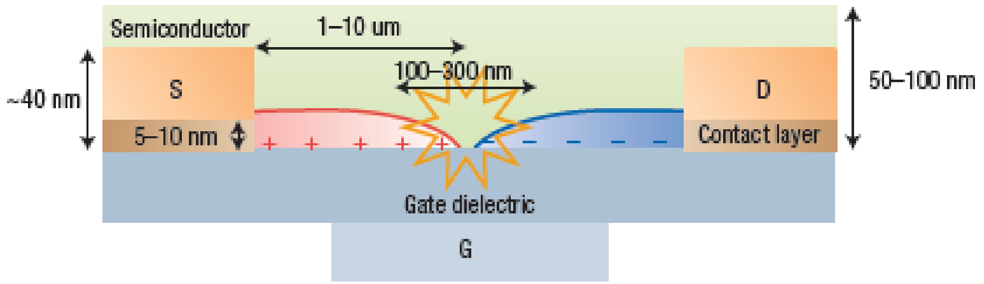
- ❑ *OLED*
- ❑ *OPD*
- ❑ *emission filter*
- ❑ *excitation filter*



Reflectance oximeter system, where each pixel comprising one red OLED, one near-infrared (NIR) OLED and two OPDs, is connected to an instrument for driving the devices

Lefèvre, et al. Lab Chip 2012, 12, 787–793.
Khan, Y. Et al. Proc. Natl. Acad. Sci. USA 2018, 115, E11015–E11024.
Toffanin S. et al. Nanomaterials 2020, 10(3), 480; https://doi.org/10.3390/nano10030480

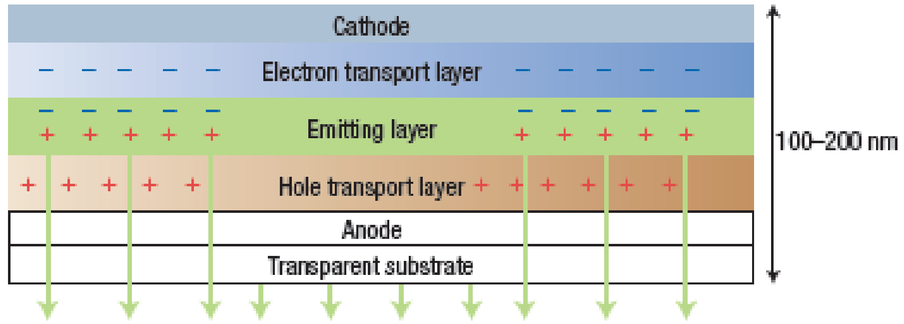
OLET vs OLED



E_{field} ~ 10^4 V/cm

HORIZONTAL TRANSPORT GEOMETRY

FIELD-EFFECT CHARGE TRANSPORT



E_{field} ~ 10^6 V/cm

VERTICAL TRANSPORT GEOMETRY

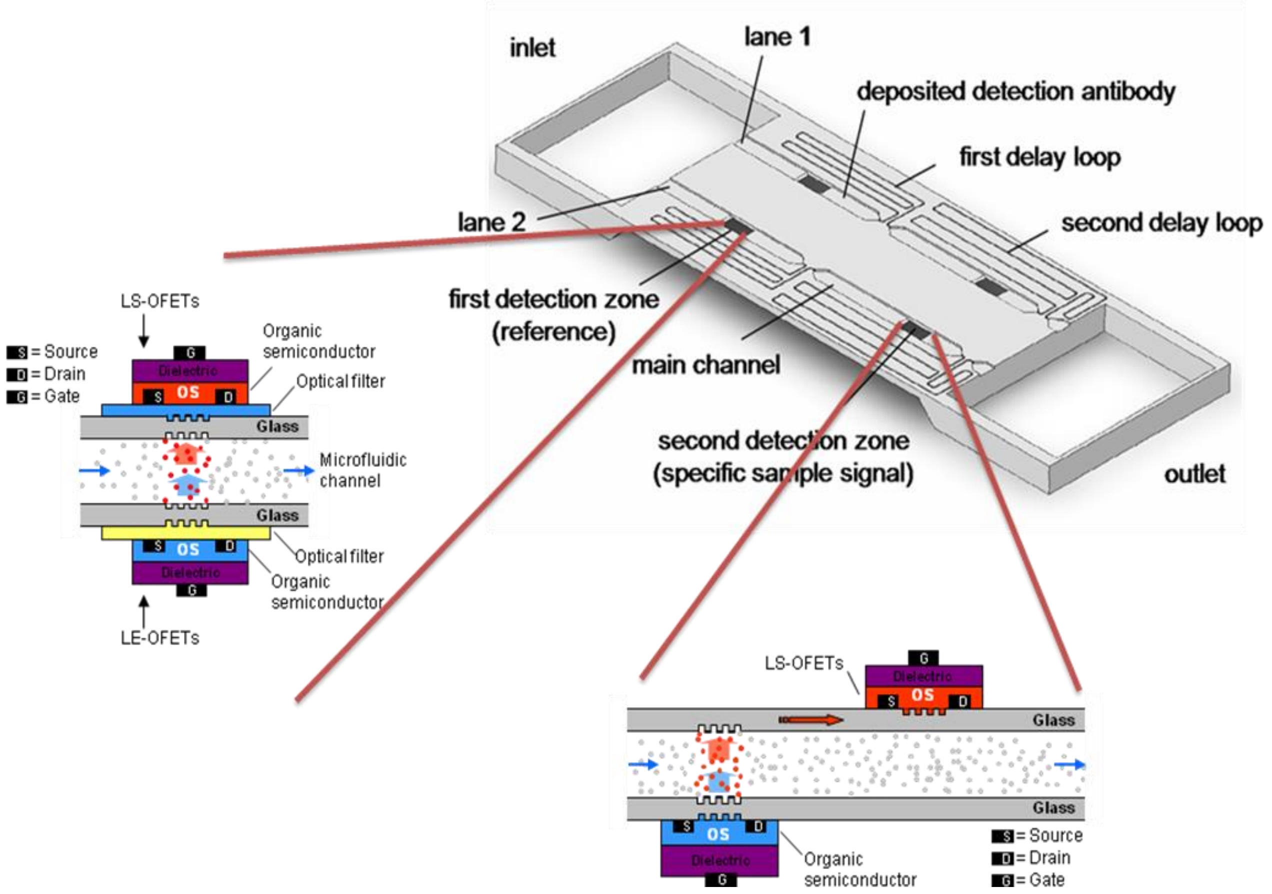
BULK CHARGE TRANSPORT

- ❑ Higher brightness

due to lower exciton quenching by interaction with charges, electric field and metal electrodes

- ❑ Higher and balanced charge mobility (10^{-1} vs 10^{-4})
- ❑ Higher current densities (10 vs 10^{-3})
- ❑ Less manufacturing

Integrated FET photonic systems for biodiagnostics and sensing



Integration of field-effect transistor devices into microfluidic chip for in-situ detection:

Multifunctional OFETs
Nanophotonics

- *Both fluorescence and label-free detection is allowed*
- *Low-cost and portable device*
- *High throughput*
- *High sensitivity and high specificity*

PhotoFET Project (ICT-2009.3.8)

Disposable device for diagnosis of myocardial infarction + determination of severity

Photonic FET Approach

Major benefits:

- **Pre-fabrication** on the substrate:
  optimal registration between photonic devices and fluidic chip

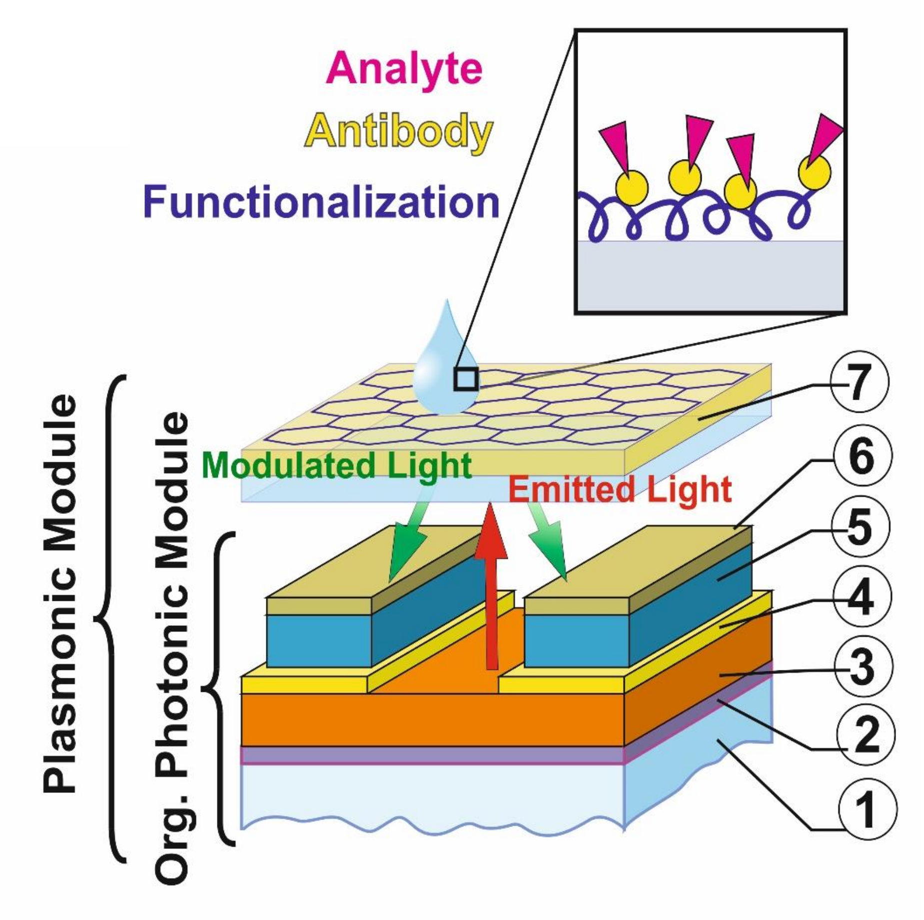
- Simplified photonic device **fabrication processing**:
  potentially single layer devices can be used

- Improved **optical coupling** and guiding:
  significant performance gains can be expected

- Straightforward **integration of auxiliary transistors**:
  improved electrical driving and signal amplification at the point-of-generation

CONCEPT

Optoplasmonic module

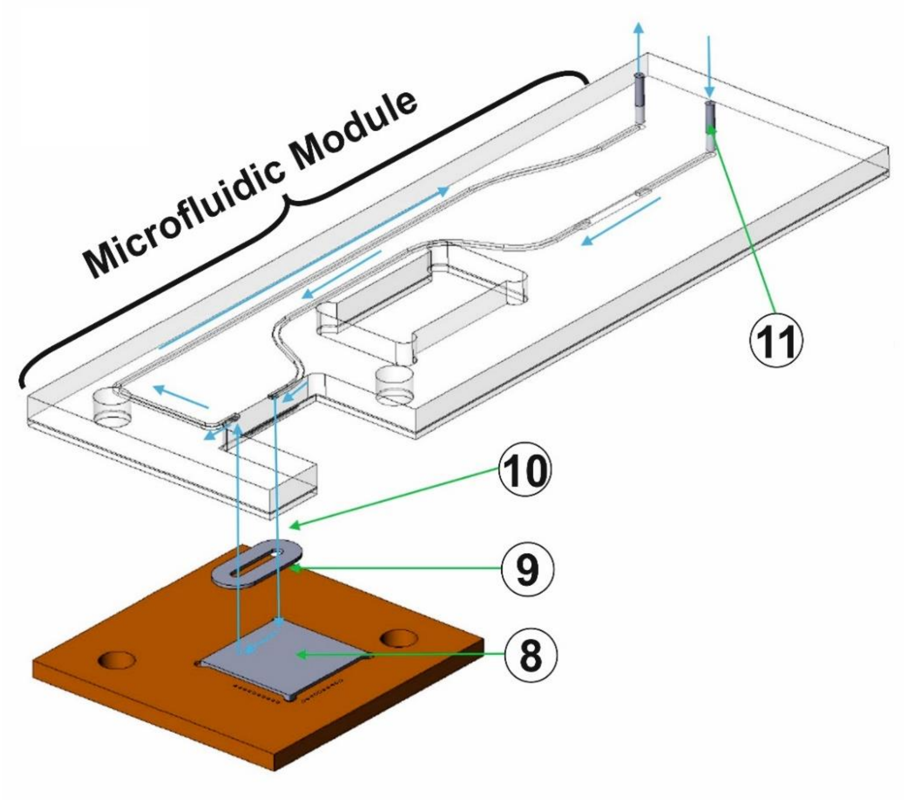


1. Chip substrate
2. Gate electrode and dielectric
3. OLET active layer
4. OLET source/drain and OPD anode
5. OPD organic stack
6. OPD cathode
7. Nanoplasmonic grating

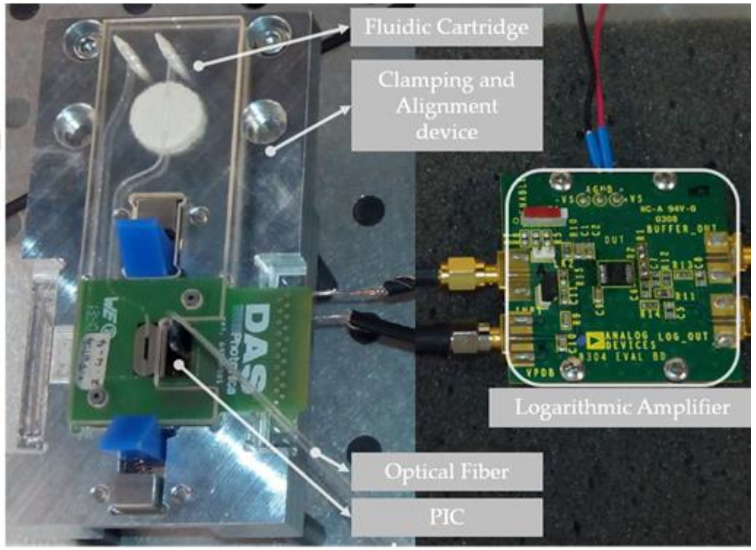
- ❑ Inherent Multiplexing
- ❑ Monolithic Integration
- ❑ Optic-less approach

CONCEPT

Manual/automated sensor

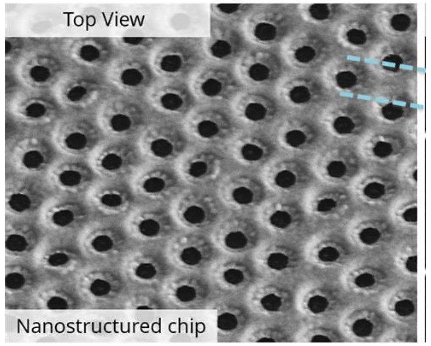


8. Optoplasmonic module
9. Sealing
10. Flow direction of the sample
11. Connection to the milk line



- ❑ Automatization of assay procedure
- ❑ Evaluation of flow velocities and reagent volumes
- ❑ Specification for cartridge development

SENSOR BUILDING BLOCKS

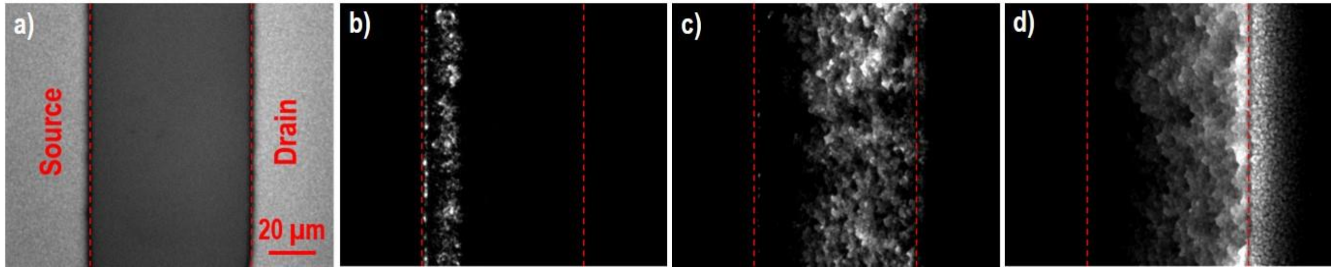


- Non-conventional nanostructured plasmonic surfaces: to detect refractive index changes onto grating surface opposite to where the excitation light is impinging

Patent WO2010146160, 23-12-2010

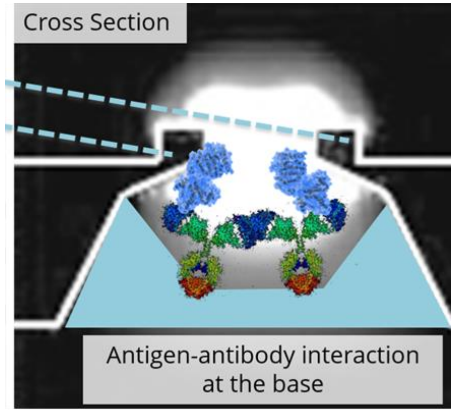
Patent WO 2013007448 A1, 18-01-2013

- Light-coupling in the detection due to electronically-controlled spatial modulation of emission zone in OLET



gate-voltage increase

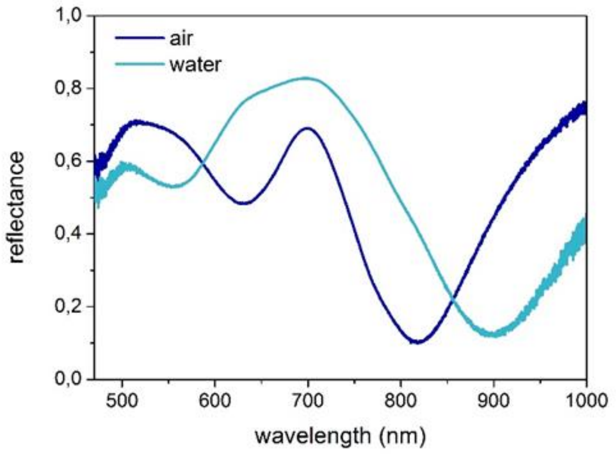
Laser Photonics Rev. 7, No. 6, 1011–1019 (2013)



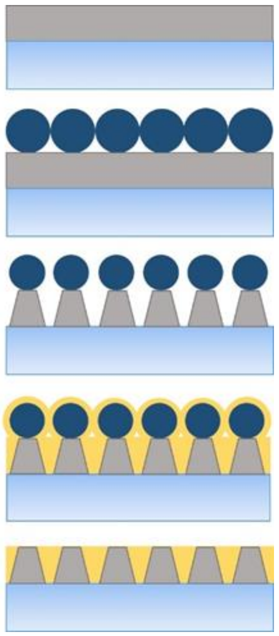
- Recombinant antibody technology for increasing the specificity of the assay and reducing the inhibiting matrix effect

Biosensors and Bioelectronics, 2006, 21: 1141

NANOPLASMONIC GRATING COMPONENT



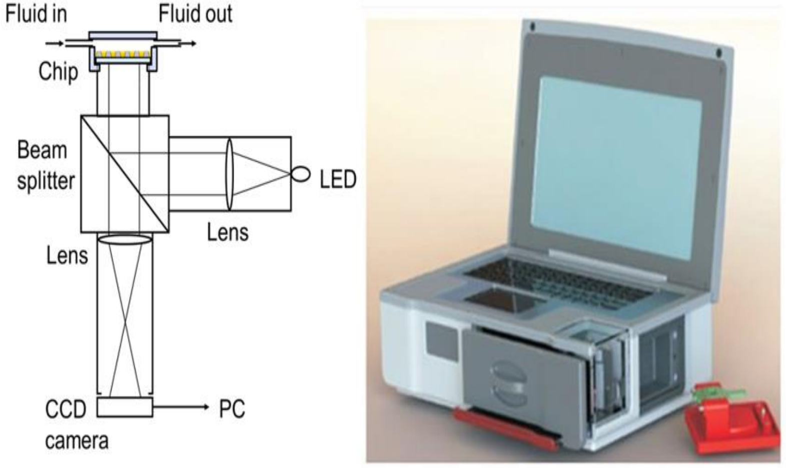
- ❑ **Wet Lithography method**
  - grid of polymeric pillars embedded in a gold matrix
  - subwavelength holes arrays generate an enhancement of the transmitted signal



- ❑ **Reflectance signal change during media change**
- ❑ **Conventional read-out**

  A fluidic flow cell with a plasmonic grating is put into the system

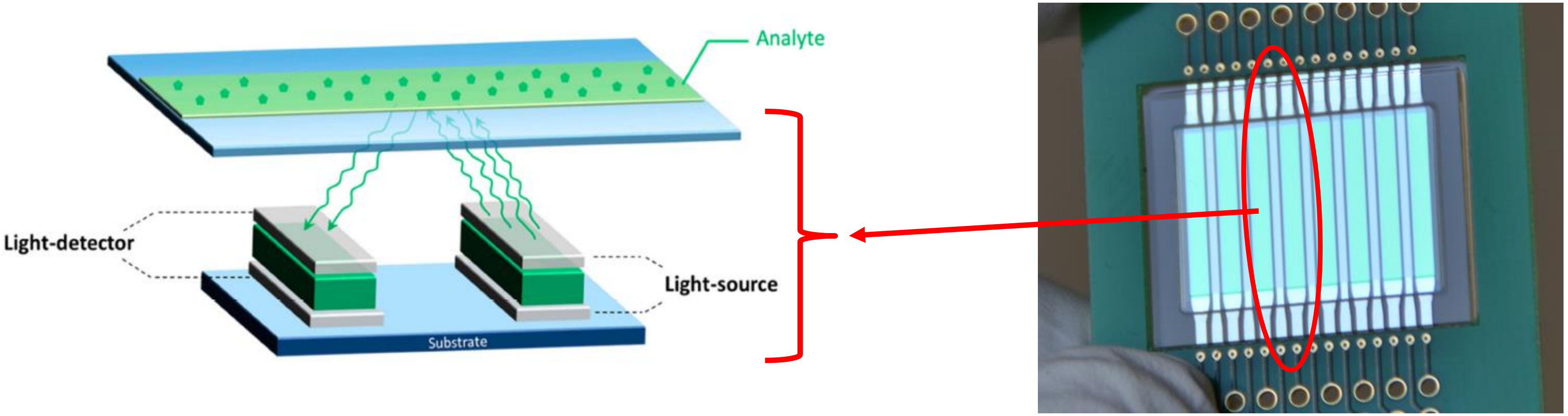
- ❑ **Standard Parameters**
  - Excitation wavelength 900nm
  - PMMA layer ~ 100 nm
  - Colloids diameter = 500 nm
  - Gold layer ~ 100 nm

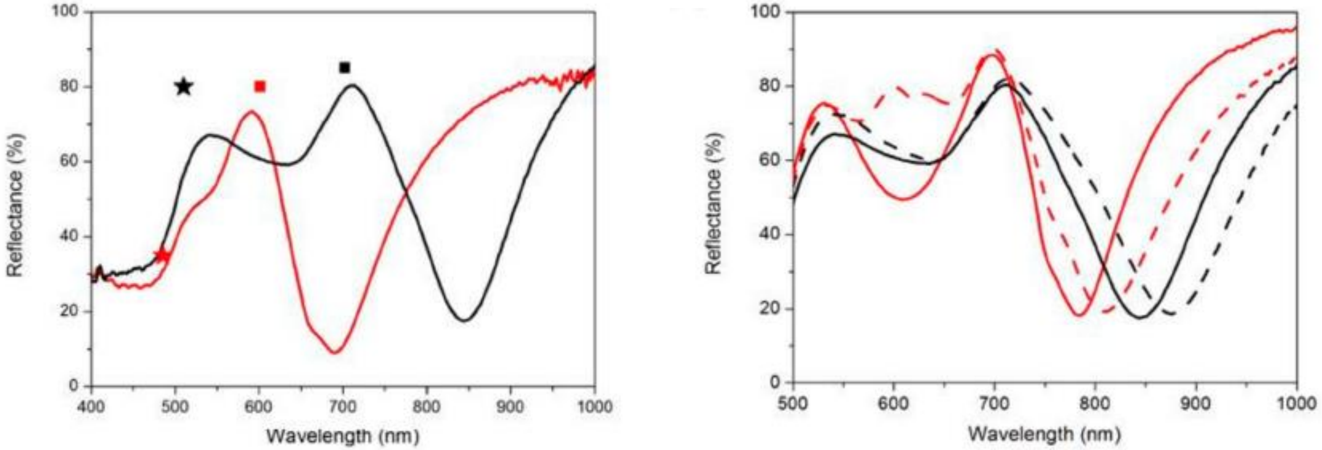


Conventional read out

Journal of Biomedical Optics 19(1), 017006 (January 2014)

THE SENSOR: ASSEMBLY AND EXPECTED OUTPUT



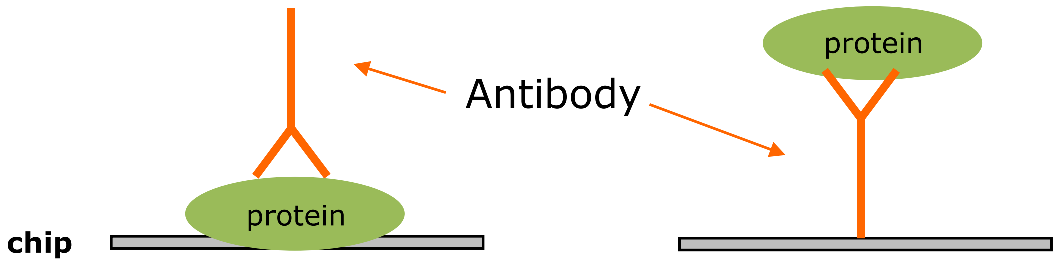


Modifications of the grating geometry - lattice pitch (*left*) and hole size (*right*):
different response to the **probing light** and the **medium** in contact with the grating surface

ASSAY CONSTRUCTION and FORMATS

Indirect assay

Direct assay



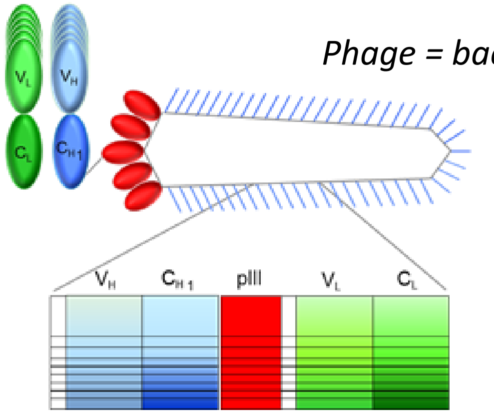
HMW targets
LMW targets

HMW targets

Biacore 3000

Antibody (Fab)

Phage = bacteria virus



Corresponding antibody gene inside the phage

Biotechnologies implemented:

- ❑ Monoclonal antibodies
- ❑ Novel recombinant antibodies discovered from antibody phage display libraries

MOLOKO TARGET ANALYSIS

- ☐ inherence to milk safety and milk quality
- ☐ typology of the detected compounds
- ☐ availability or feasibility for the realization of the immunoassays for the detection
- ☐ positioning with respect to the state-of-the-art detection ranges and MRLs evaluation (where possible) of the detection range by a similar detection system based on SPR detection principle

QUALITY

Lactoferrin (Lf)

κ-Casein (kC)

κ-Casein (B)

β-Casein A2A2 (CA2)

SAFETY

Streptomycin (Strep)

SEA/SEB

Aflatoxin M_1 (AFM_1)

Penicillins (Pen)

Cephalosporins (Ceph)

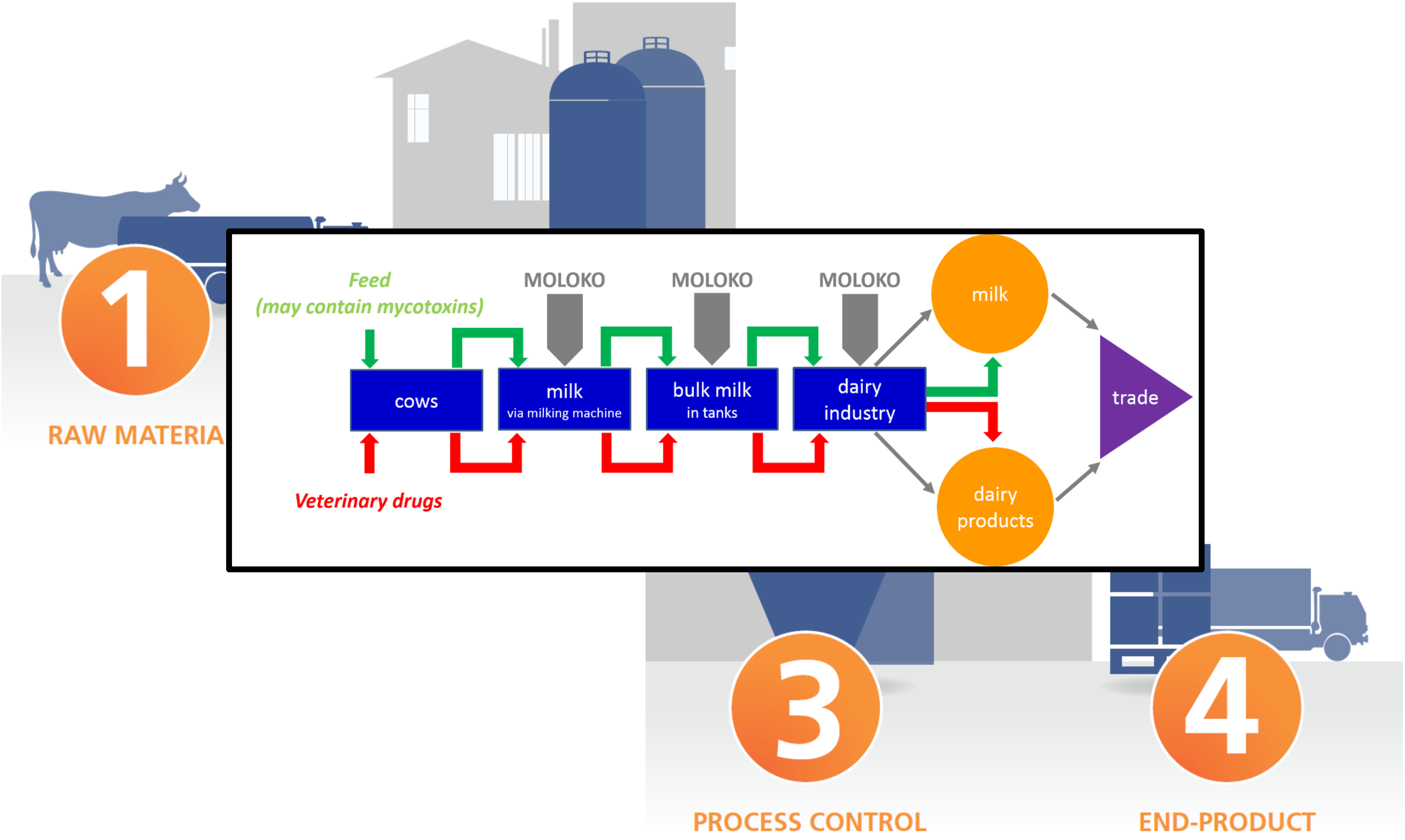
Tetracyclines (TET)

Quinolones (Quin)

Sulphonamides (Sulph)

Alkaline Phosphatase (AP)

ONLINE CONTROL IN MILK SUPPLY CHAIN



CONCEPT

The Sensor Device

- *Based on Surface Plasmonic Resonance (SPR) technology, detects variation of the refractive index on a gold nanostructured surface (nanoplasmonic grating)*

The Optics

- *Optic-less approach: planar light-source and light-detector are mounted on the underside of the nanostructured surface which detects changes on the surface*

Detecting Analytes

- On top surface of the gold nanostructured surface there are antibodies (immunoassay diagnostics)

Sampling

- *Microfluidics deliver the milk sample to the antibodies*

Detection

- *Any target molecules in the milk bind to the antibodies*

Analysis

- *The sensor detects how many of the antibodies are now bound to the target molecule (less than 10 minutes)*

Reuse

- *The surface is regenerated ready for the next test*

OUTLOOK

- ❑ Self-managing and automatic miniaturized integrated photonic sensor
- ❑ Monolithic integration of Organic PhOtonics and Nanoplasmonic technologies
- ❑ Multiplexing detection: up to 10 anlytes among QUALITY and SAFETY parameters
- ❑ Effectiveness and market-placement of the engineered functional prototype
- ❑ Cost-reduction along the severe logistically-spread value chain of milk

for more info

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THANKS FOR YOUR ATTENTION !

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