

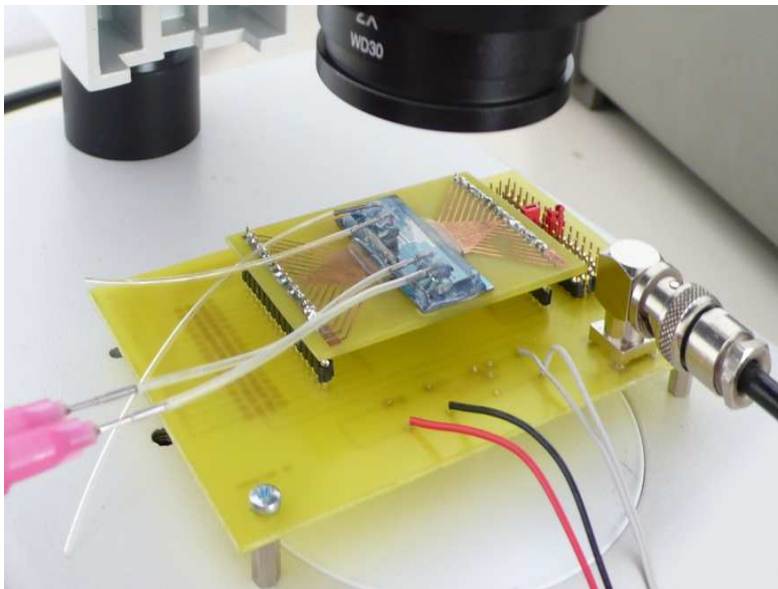
Challenges in Nanotechnologies for Health and Food Applications

P.P.Freitas^{1,5}, S.Cardoso¹, F.Cardoso¹, T.Dias¹, A.Chicharo¹, J.P.Amaral¹, J.Germano², T.Costa²,
M.Piedade², A.Sebastiao³, V.Pinto⁴

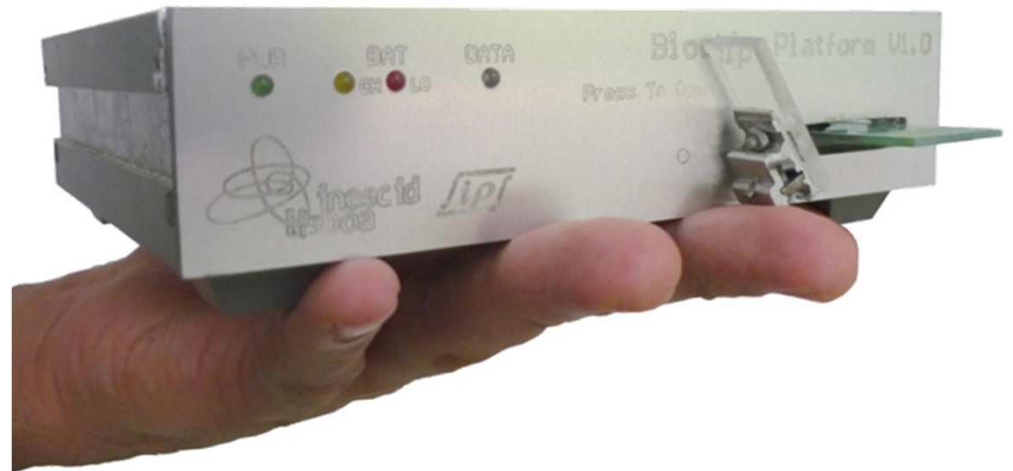
(1-INESC MN, 2-INESC ID, 3-IMM, 4-ICVS-UM)

V. Martins⁵, B.Espina⁵, R.Ferreira⁵, J.Gaspar⁵, Elvira Paz⁵, A.Vila⁵, J.Rivas⁵, C.Rodriguez⁵,
Y.Kolenko⁵, Jerome Borme⁵, Mariam Debs⁵, Margaret Costa⁵, Helder Rodrigues⁵ (5-INL)

Dynamic biosensor platform



Static multi-analyte biochip



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Challenges Nanomedicine Researchers Seek to Overcome

- **Diagnostics**
 - High sensitivity – comparable to or exceeding PCR, ELISA
 - Speed, multiplexing, portability, and decreased cost
 - Application of ultra high sensitivity systems in the clinic for improved disease diagnosis
- **Imaging**
 - Non-invasive monitoring of disease occurrence and progression
- **Therapeutics**
 - Improved specificity for drug delivery
 - Enabling nucleic acids and biologics for therapies
- **Regenerative Medicine**
 - Architectures that induce tissue regeneration as well as bone and organ repair



nanoZ

Nanotechnology Long-term Impacts
and Research Directions: 2000 – 2020

Chapter 7. Nanobiosystems, Medicine and Health

C.A. Mirkin, A. Nel
In collaboration with:

Barbara Baird, C. Carl Batt, David Grainger, Sanjiv Sam Gambhir, Demir Akin, Otto Zhou, J. Fraser Stoddart, Thomas J. Meade, Piotr Grodzinski, Dorothy Farrell, Harry F. Tibbals, Joseph De Simone, Shad Thaxton

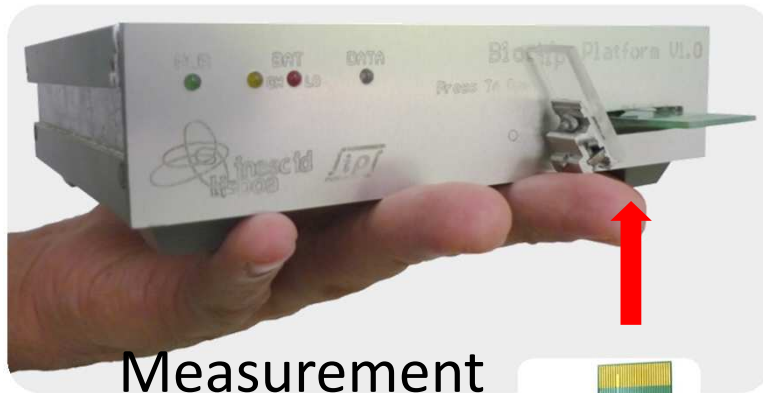
NSF, September 30, 2010

Point Of Care Diagnostic Platforms

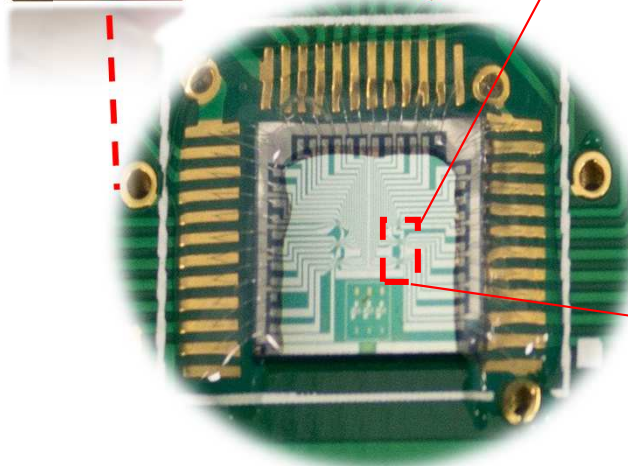
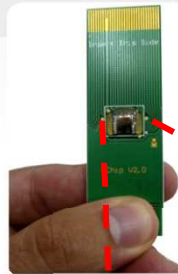
MR biochip static platform

Developed at INESC MN and INESC ID
2000-2012

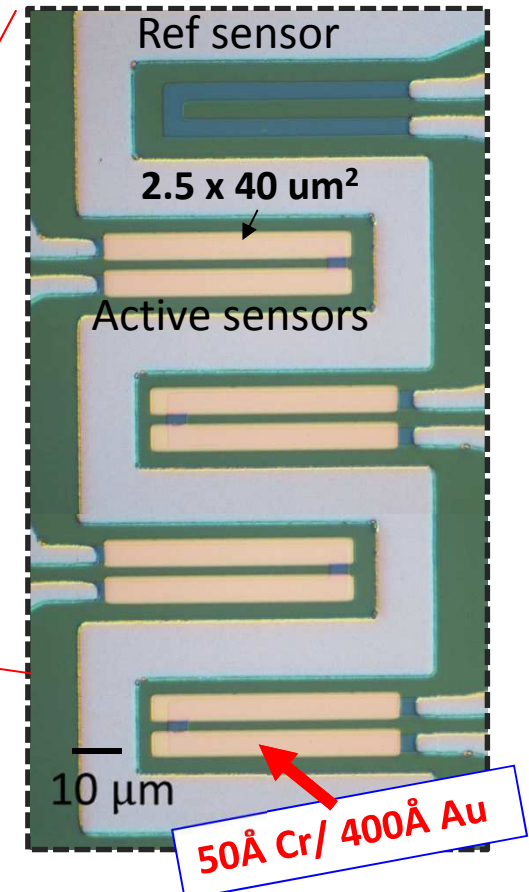
Being licensed to Magnonics, 2013
Patents pending (2)



Measurement platform



Chip area: 6 x 7.2 mm²



MR biochip

- 30 sensing units
 - 6 groups of
 - 5 sensors
- (4 bio-active + 1 reference)

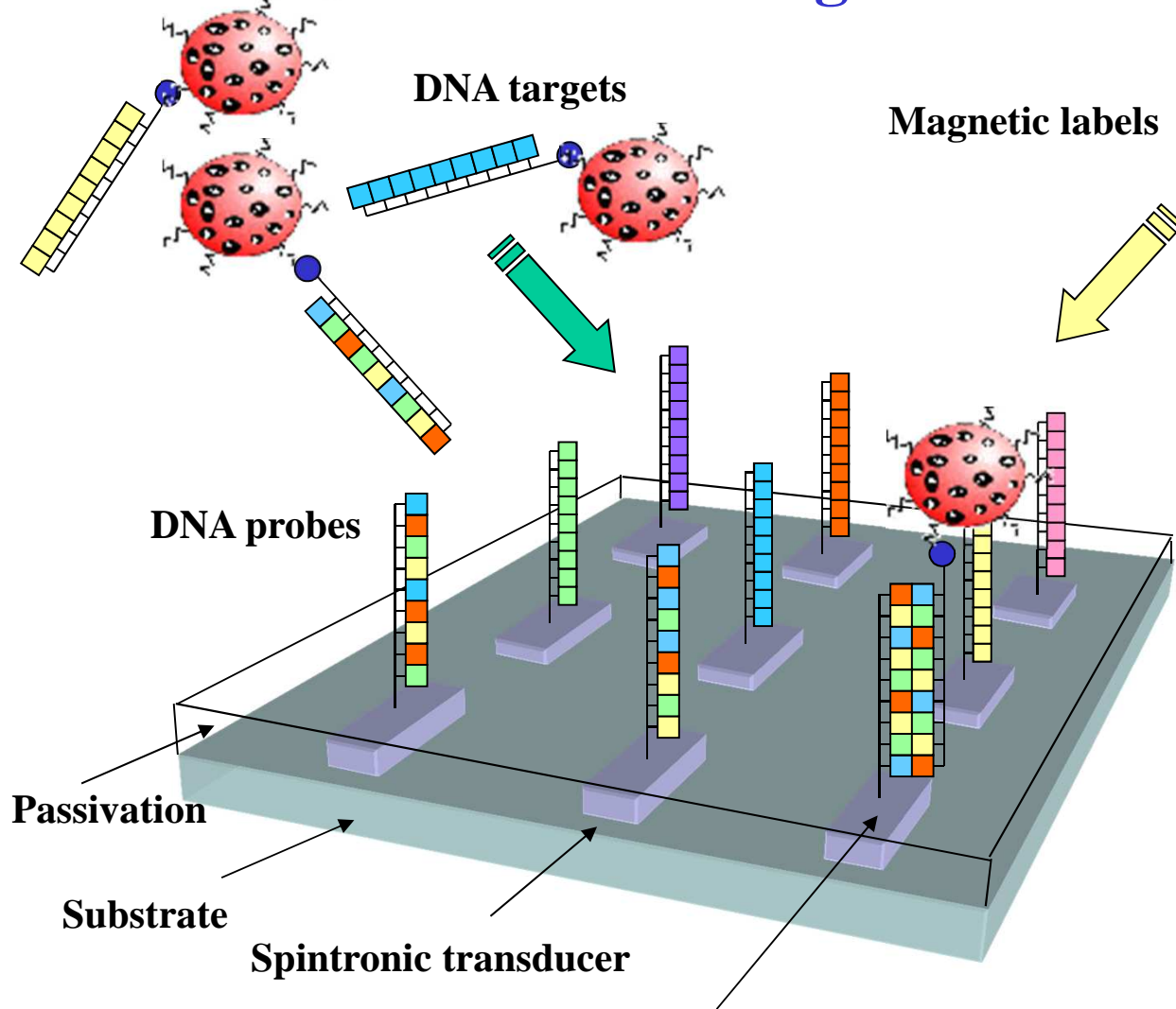
1-Scalable magnetoresistive biochips for point of care diagnostics

Labelled targets

DNA targets

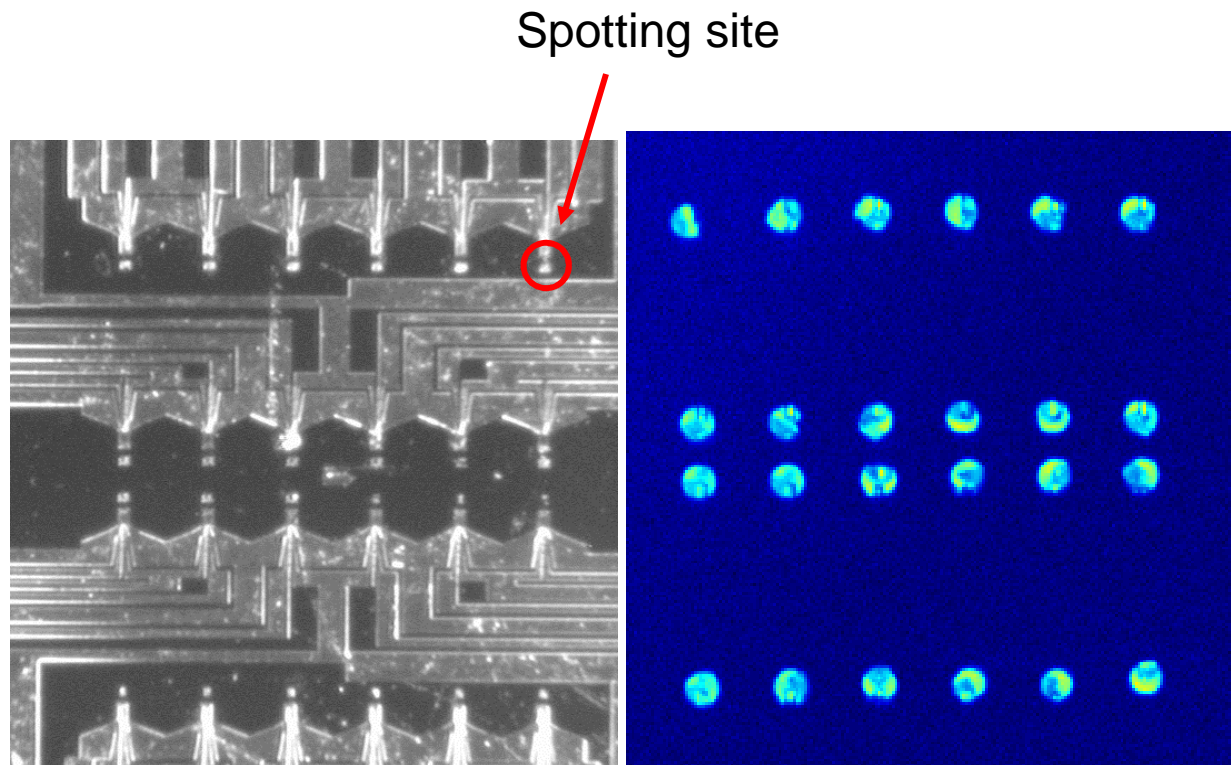
Magnetic labels

Hybridize first
Label after



Trends in Biotechnology, August 2004

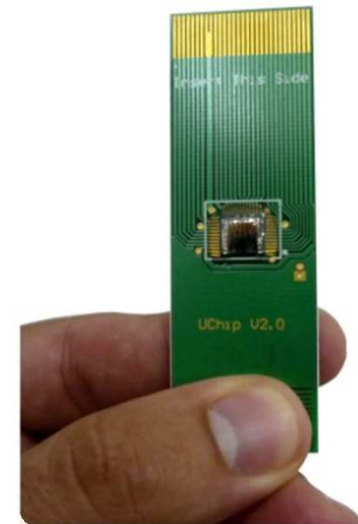
1-d) Spotting biological targets on the biosensing platform



1 μM Oligo solution, Cy5 labeled
200 pL droplets



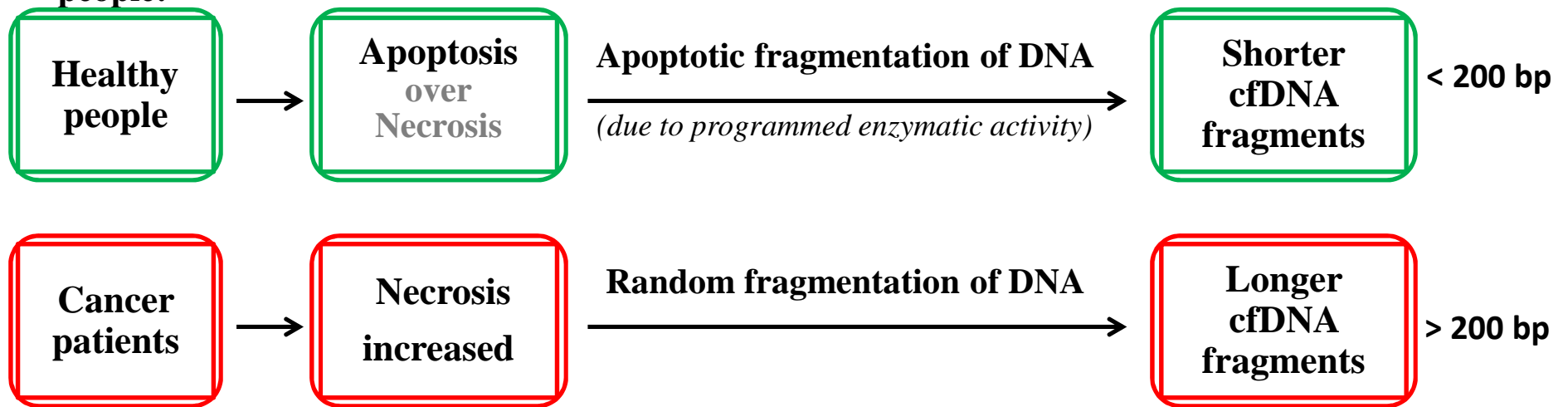
Gesim spotter



Disposable biochip

Application: Cell-free DNA as cancer biomarker?

- **Cell-free DNA:** DNA that can be found outside of cells in blood circulation. Results mainly from dying cells (apoptosis or necrosis)
- The cfDNA found in cancer patients is qualitatively different from what is found in healthy people:



$$\text{Integrity index:ratio} = \frac{\text{ALU247 (longer cfDNA fragment)}}{\text{ALU115 (shorter cfDNA fragment)}}$$



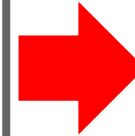
Universal cancer biomarker in therapy follow-up ?

Blood finger-prick

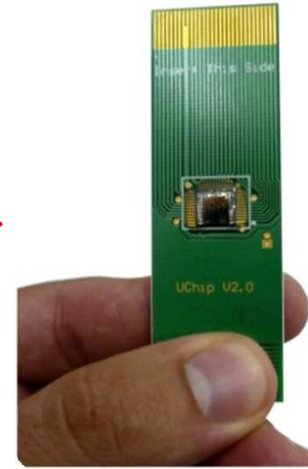


Sample preparation step

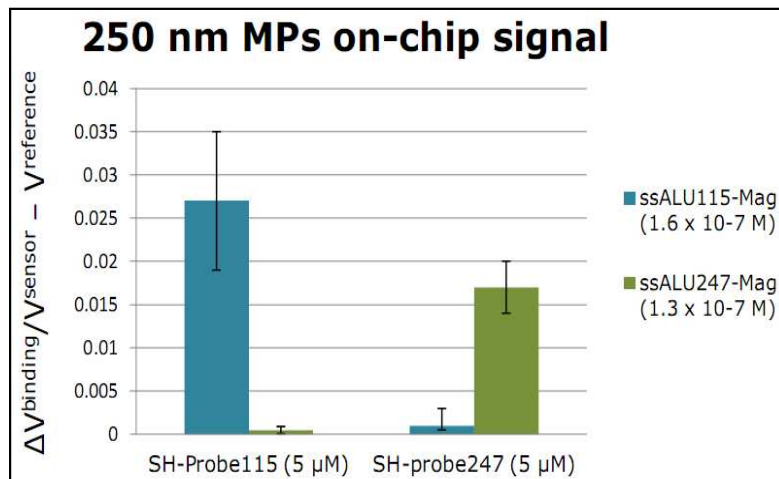
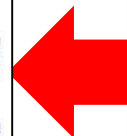
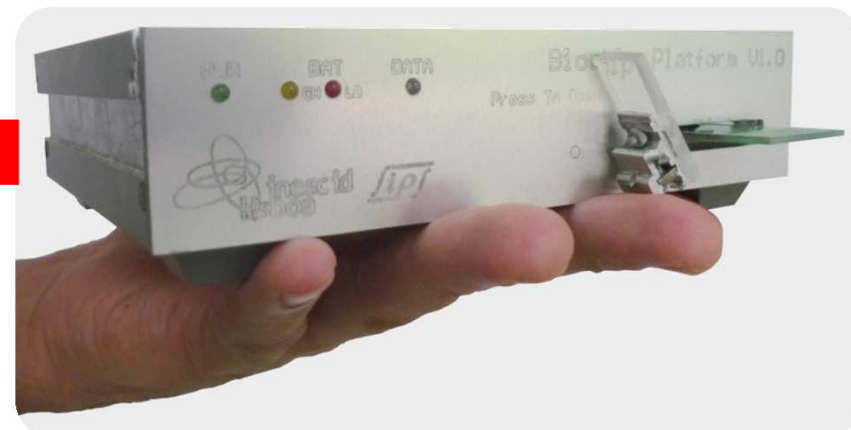
separation of plasma
from blood cells



Plasma injected in the detection chip

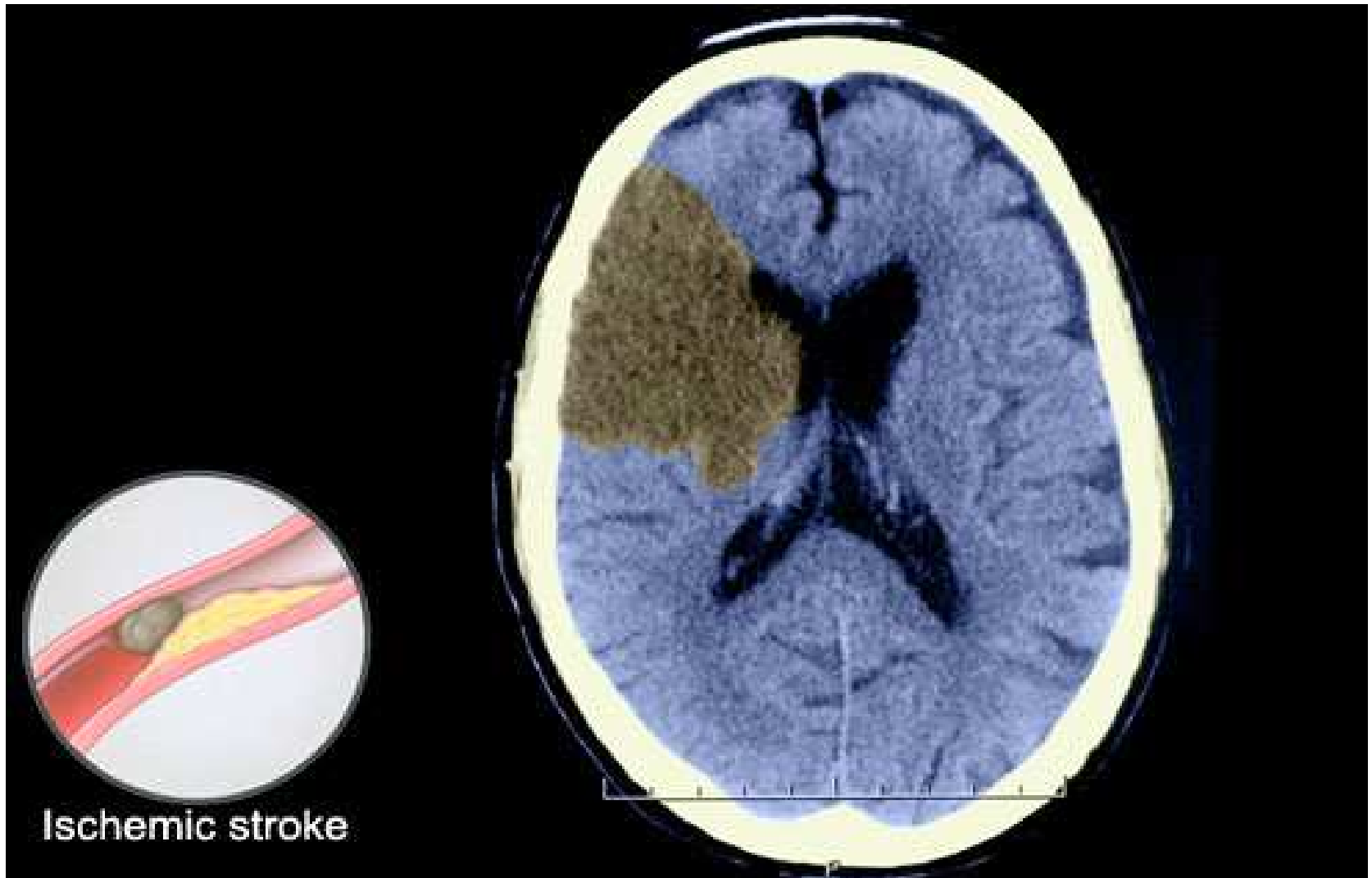


Measurement of the chip



- **2-Multiplexed detection of ischemic stroke (ICTUS) biomarkers**

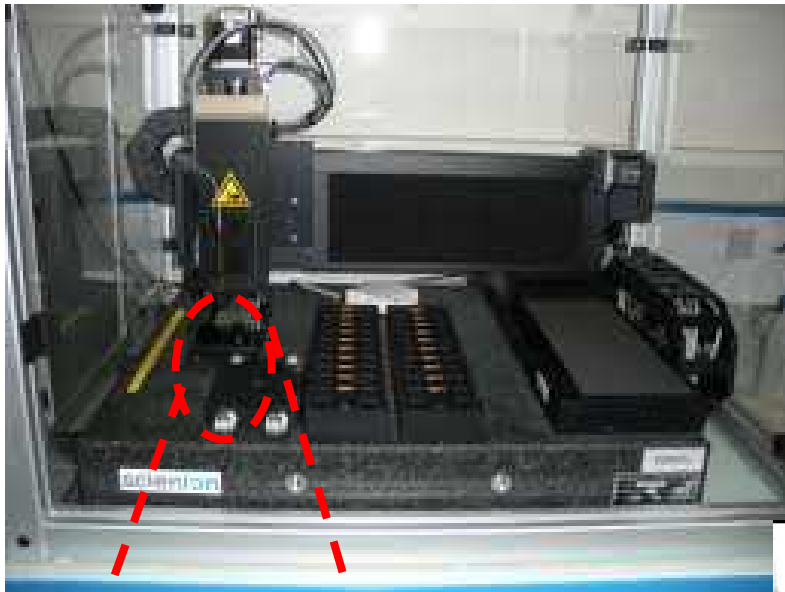
V.Romao, A.Vila, J.Rivas, P.P.Freitas, INL; T.Sobrinho, J.Castillo Sanchez, IDIS



Selected biomarkers (detection in blood)

- ➔ **Cellular Fibronectin** *Stroke. 2004; 35: 1671-1676*
Elevated plasma levels of cellular fibronectin (c-Fn) reflect vascular damage, so c-Fn might be a marker of secondary bleeding risk in cerebral ischemia.
- ➔ **IL-10** *Neuroimmunomodulation. 2010;17(4):223-8.*
Serum IL-10 was significantly higher in patients with severe neurological impairment; predictive of combined major adverse clinical outcomes.
- ➔ **Endotelina 1**
Endothelins are proteins of 21-amino acid that constrict blood vessels and raise blood pressure.
- ➔ **Metaloprotease 9 –MMP-9** *J Stroke Cerebrovasc Dis. 2011 Jan-Feb;20(1):47-54.*
Related to blood-brain barrier disruption, vasogenic edema formation, and hemorrhagic transformation
- ➔ **PDGF-CC** *Atherosclerosis. 2013 Jan;226(1):165-71*
Increased PDGF-CC levels after tPA treatment is associated with hemorrhagic transformation
- ➔ **Neuroserpin**
Primarily secreted by axons in the brain, and preferentially reacts with and inhibits tissue-type plasminogen activator

Probe antibody automated spotting

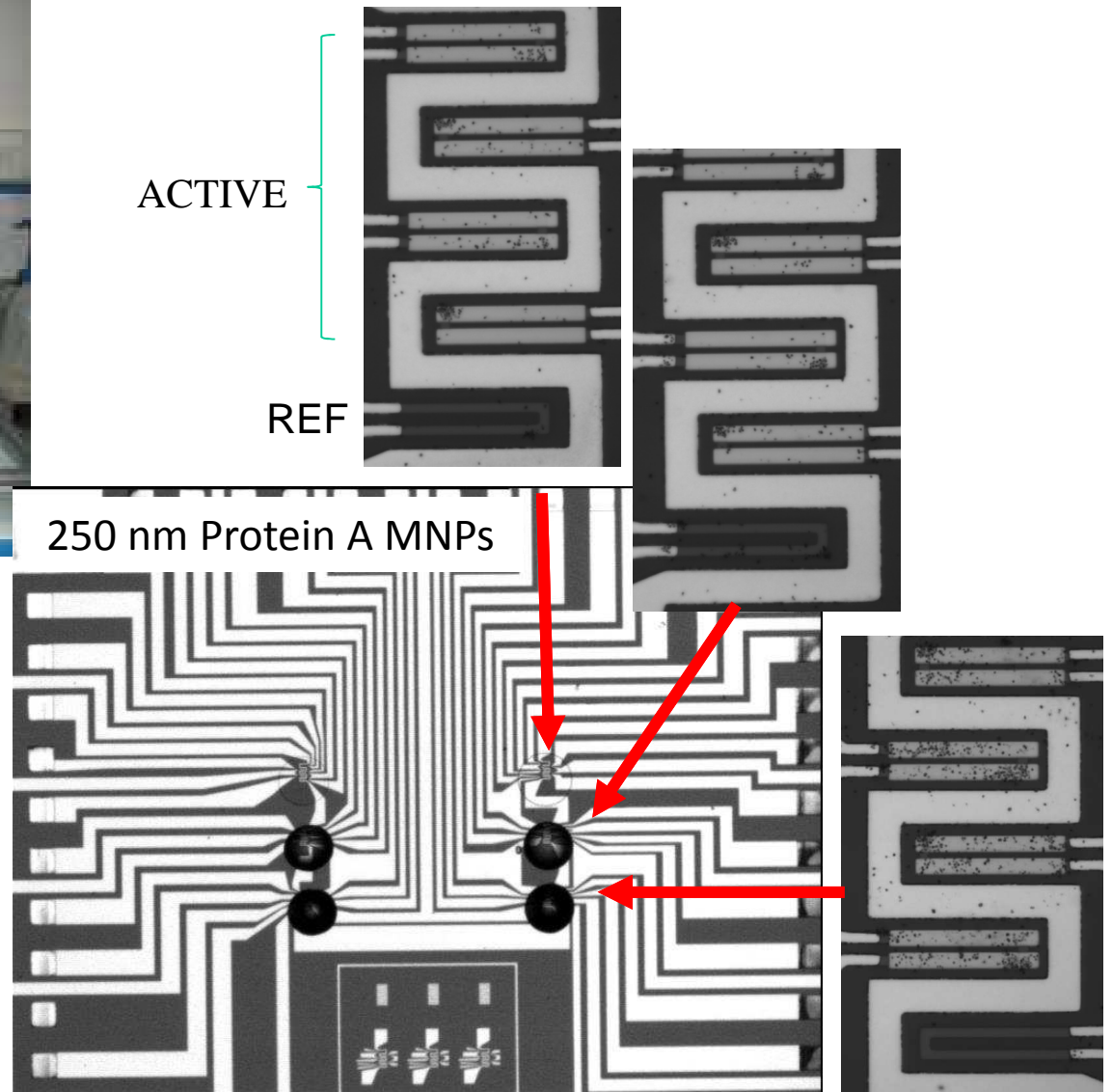


SciFlex-Arrayer S3

ACTIVE

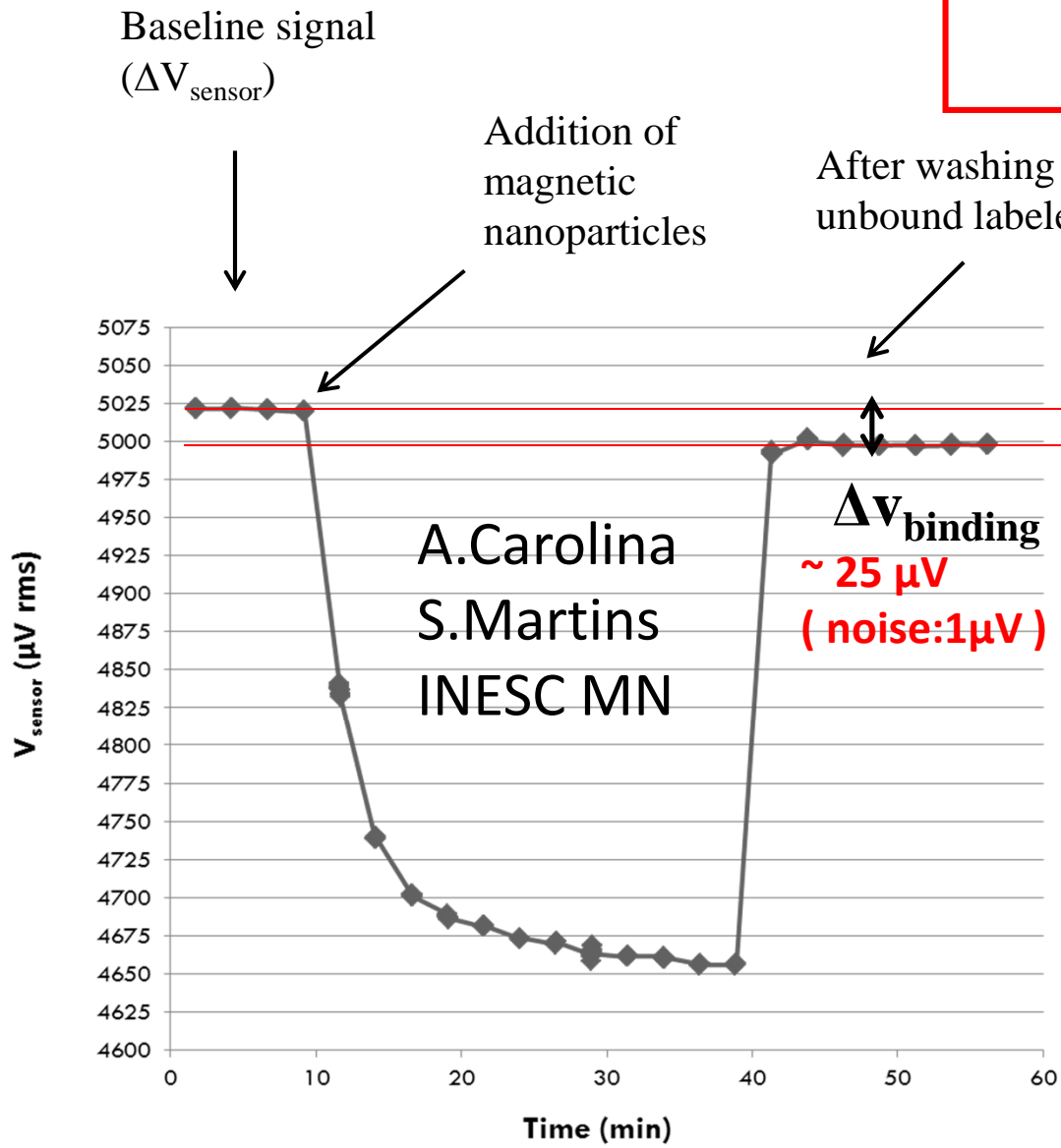
REF

250 nm Protein A MNPs

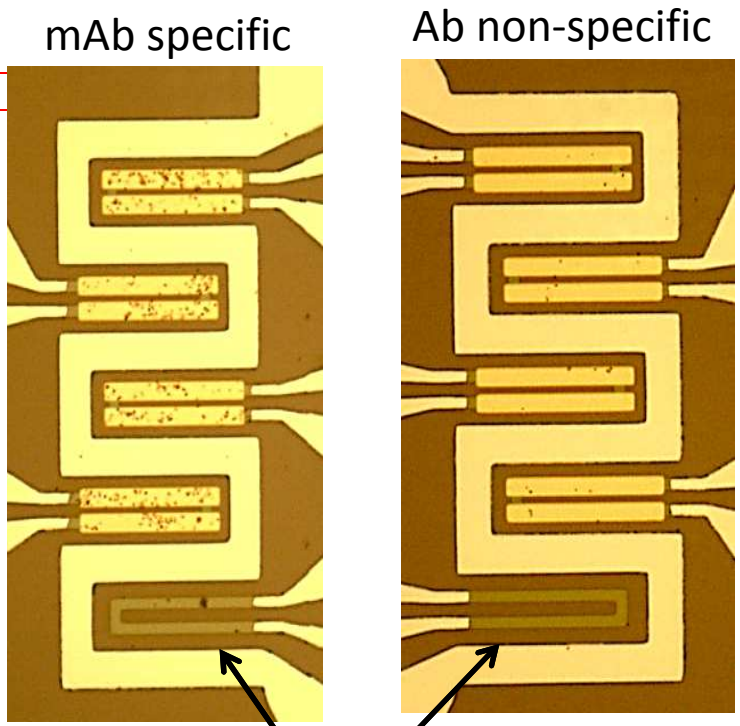


Proof-of-concept detection of IL-6

IL-6 concentration of 2.5 $\mu\text{g/mL}$
(in PB)



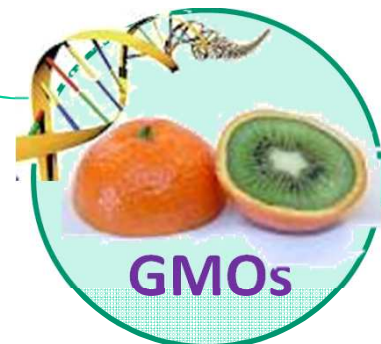
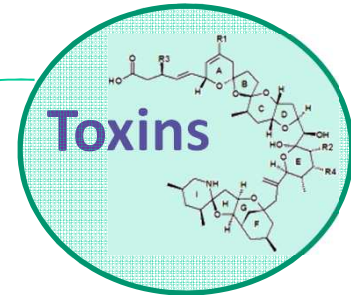
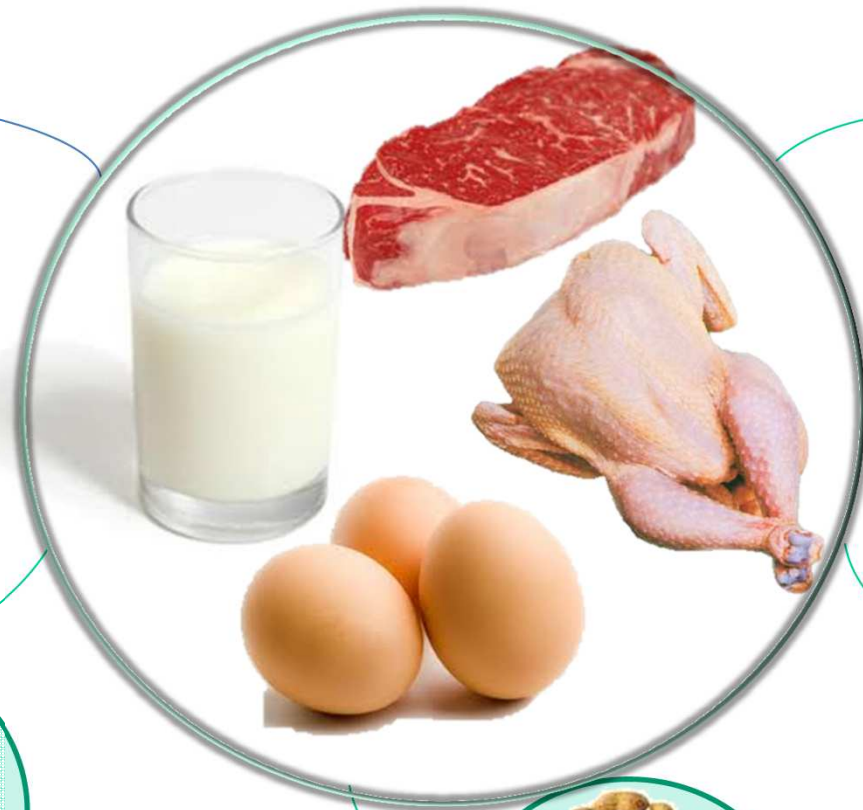
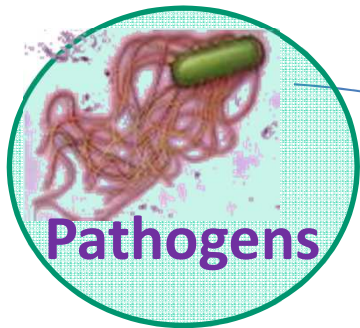
QUESTION:
Required Limits of Detection?
ng/ml



Reference sensor

FOOD QUALITY CONTROL

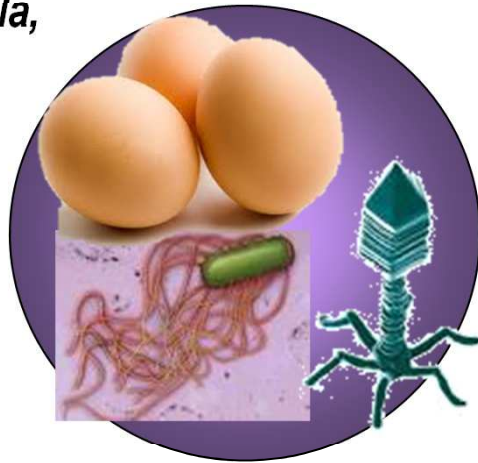
ANALYTES TO DETECT AND CONTROL IN FOOD



A SINGLE PLATFORM MULTIPLE APPLICATIONS: FOOD

Detection of food-borne pathogens (*Salmonella*, *Campylobacter*) with novel bioligands

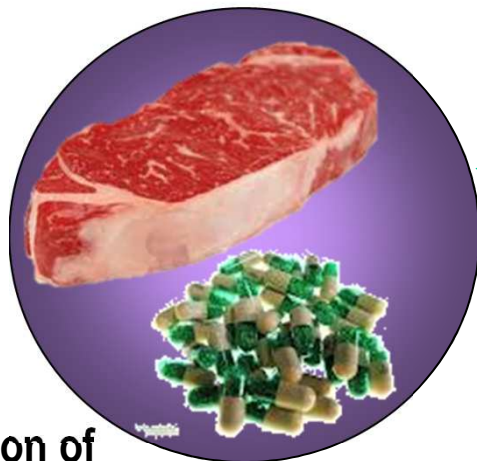
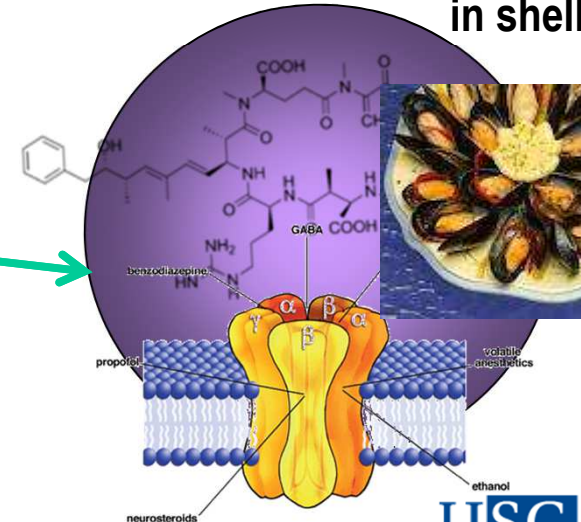
UM-DEB



Detection of pathogens in milk causing mastitis



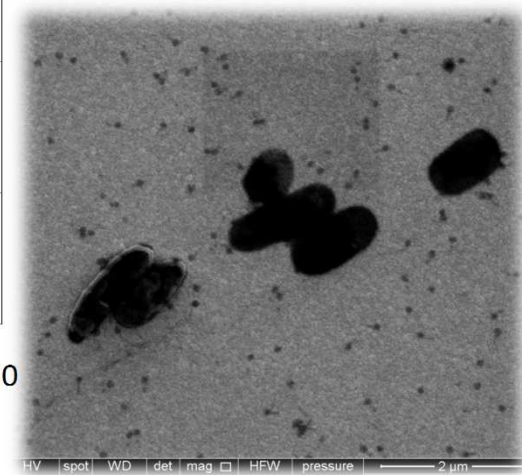
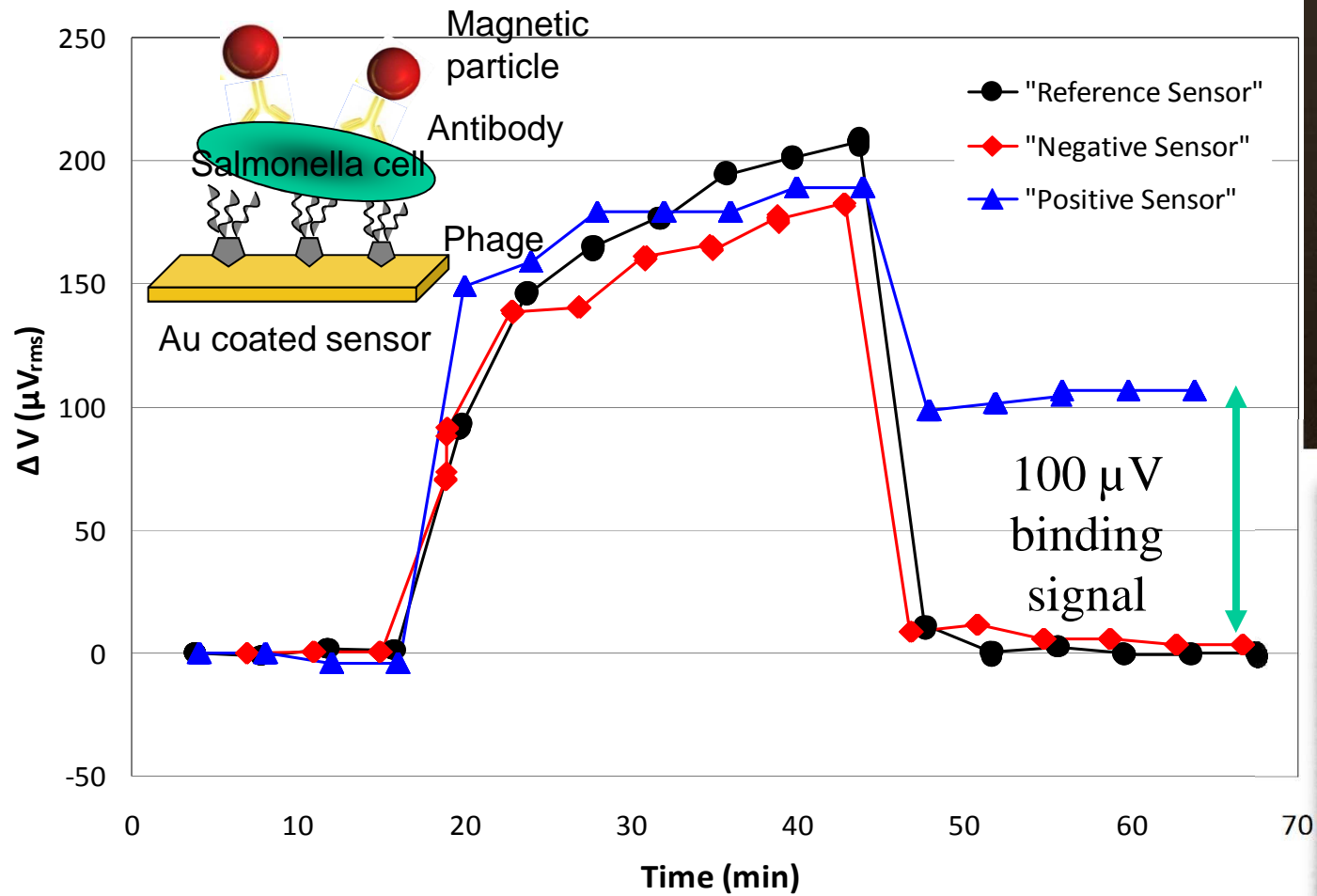
Detection of toxins in shellfish



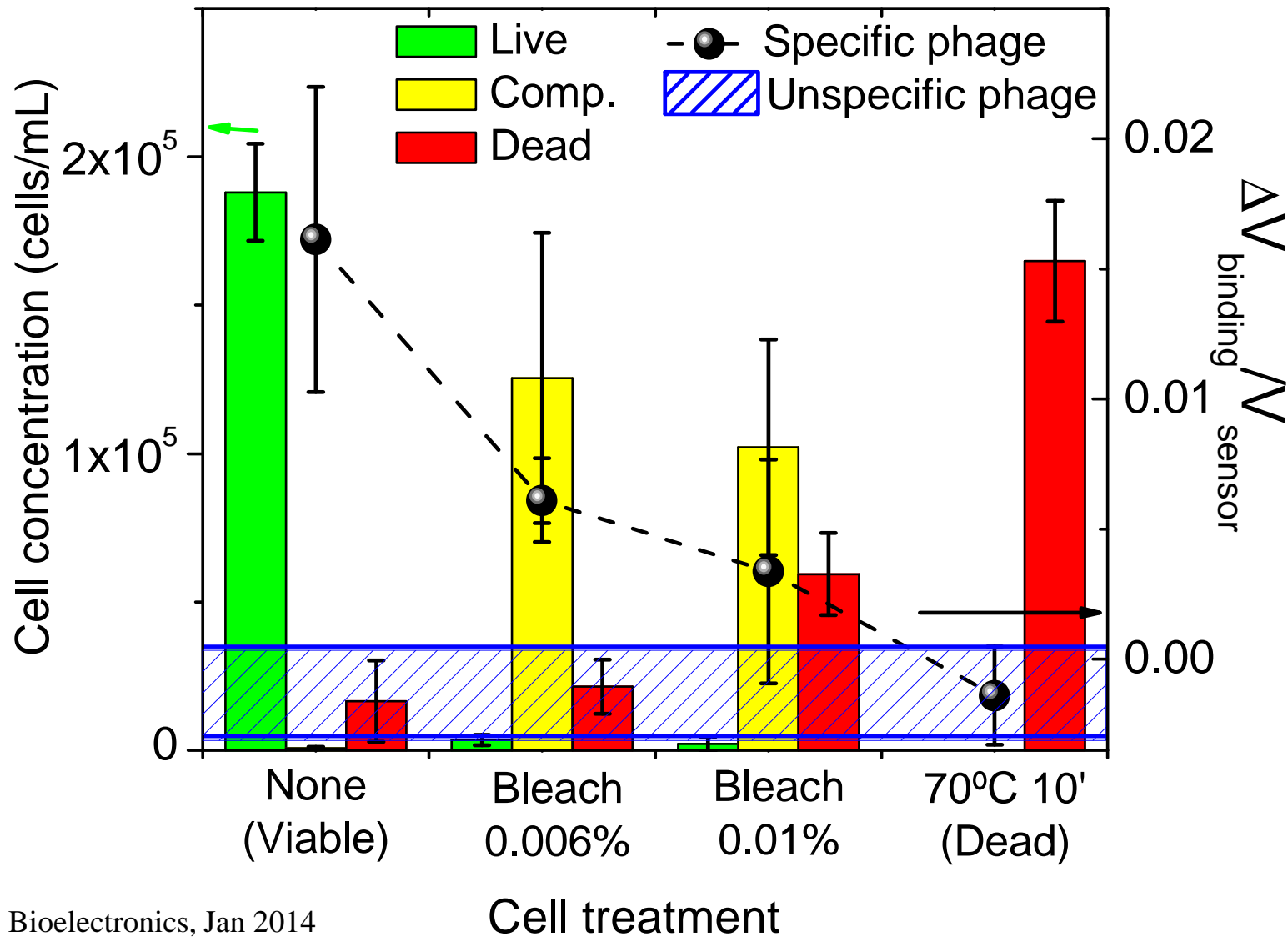
Detection of antibiotics in meat (U.Zaragossa)



A spintronic-based platform was used in combination with bacteriophages to detect Salmonella cells



The phage-based MR chip was able to distinguish Viable from VBNC and Dead Salmonella cells



Micro-immunoassays applied to detection of OTA for food safety

Need:

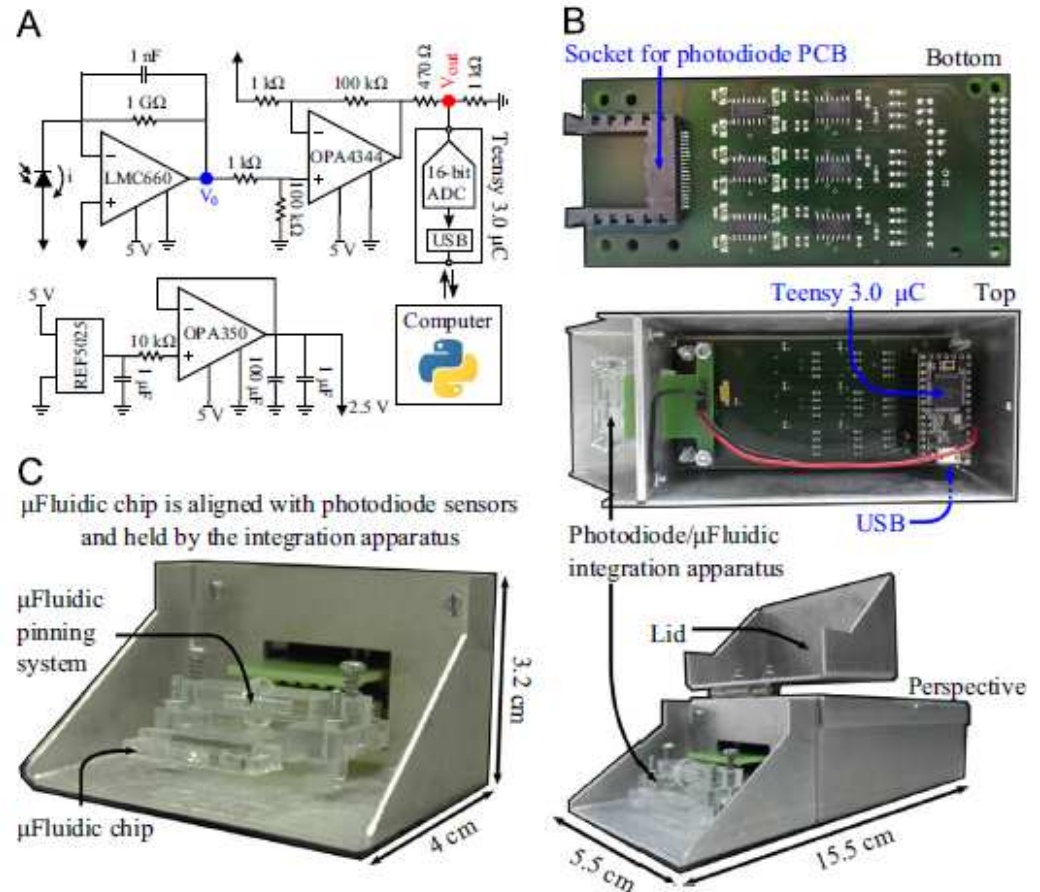
- a simple and inexpensive way of testing on site the presence of toxins in foodstuffs
- can be used as a screening tool to decide on further testing

PDMS disposable capillary biochip integrated with thin-film silicon photosensors for multi-toxin detection in the food production chain

Research for SMEs FP 7 projects OTASENS and DEMOTOX

P. Novo, G. Moulas, D.M.F. Prazeres, V. Chu, J. P. Conde, "Detection of ochratoxin A in wine and beer by chemiluminescence-based ELISA in microfluidics with integrated photodiodes", *Sensors and Actuators B: Chemical* **176**, 232-240 (2013).

P. Novo, V. Chu, J.P. Conde, "Integrated optical detection of autonomous capillary microfluidic immunoassays: a hand-held point-of-care prototype", *Biosensors and Bioelectronics* **57**, 284-291 (2014).

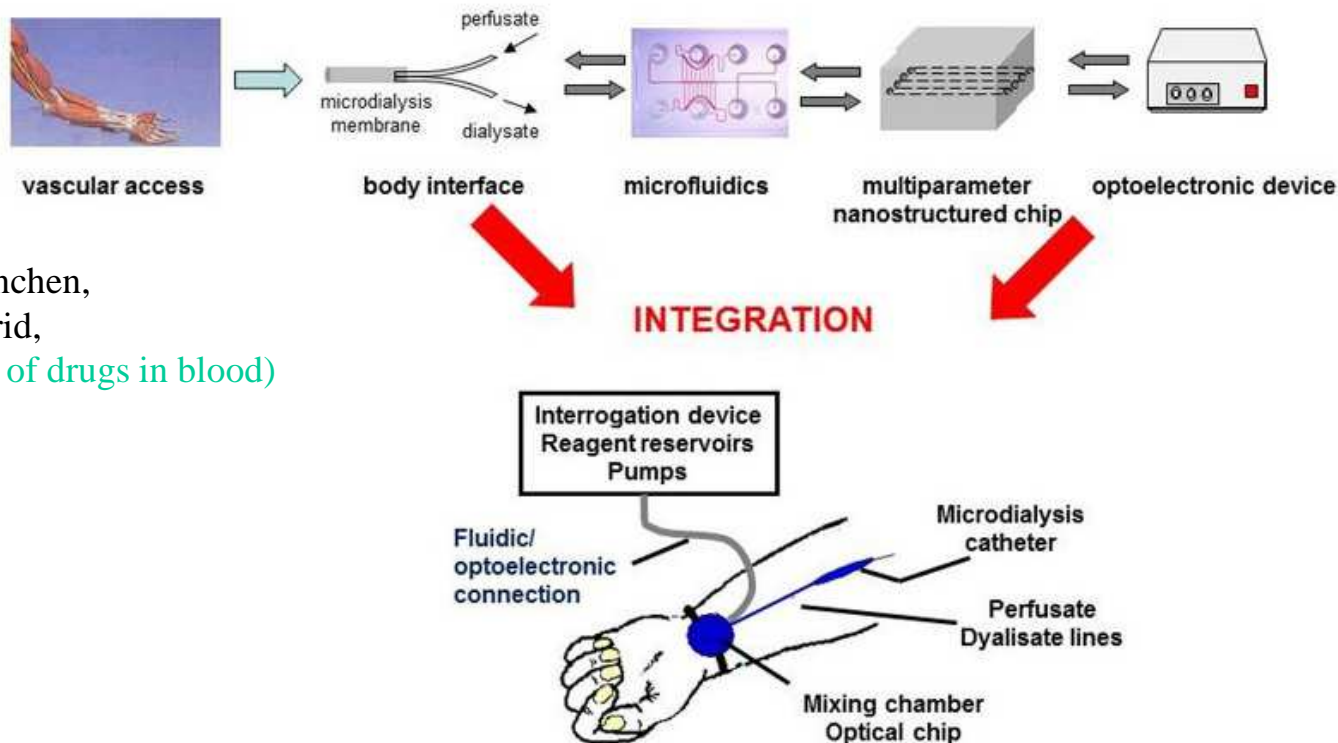


NANODEM Project FP7 (2012-2015)

NANODEM



NANOphtonic Device for Multiple therapeutic drug monitoring: immunosuppressants in blood



Partners:

CNR-Milan,
 University of Tübingen,
 Klinikum rechts Techn. Univ. München,
 Universidad Computense de Madrid,
 INESC MN, (magnetic separation of drugs in blood)
 University of Stuttgart ,
 Microfluidic ChipShop-D,
 Probe Sc.Ldt.
 DataMed S.r.L.

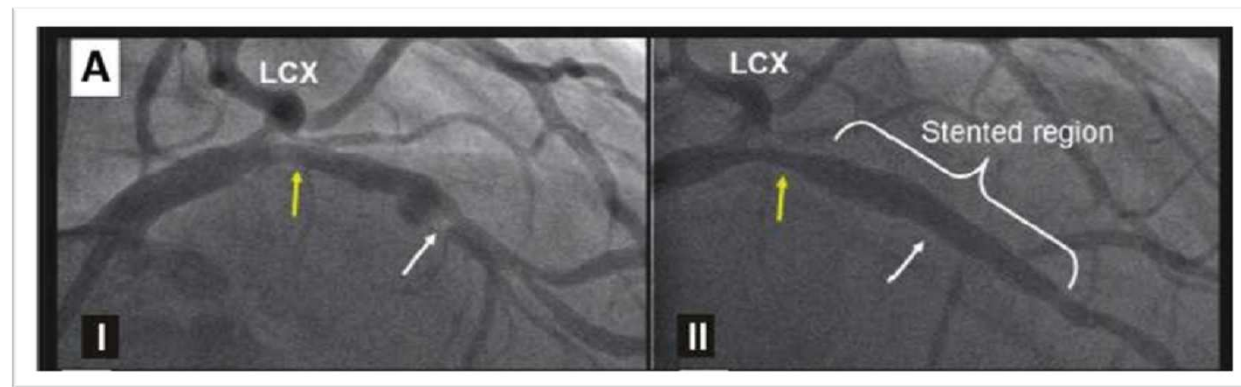
Drugs to be monitored

Project Ranking	Drug	Plasma protein binding [%]*	Therapeutic range in whole blood	Therapeutic range without bound protein	Comments - mainly found
1	Tacrolimus	99	5-25 ng/ml	0,05-0,25 ng/ml	intracellular
2	Cyclosporin A	90	100-250 ng/ml	10-25 ng/ml	intracellular
3	Sirolimus	92	12-20 ng/ml	0,96-1,6 ng/ml	intracellular
4	Everolimus	74	3-8 ng/ml	0,78-2,08 ng/ml	intracellular
5	Mycophenolic acid	83-97	2-5 µg/ml	60-150 ng/ml	Plasma

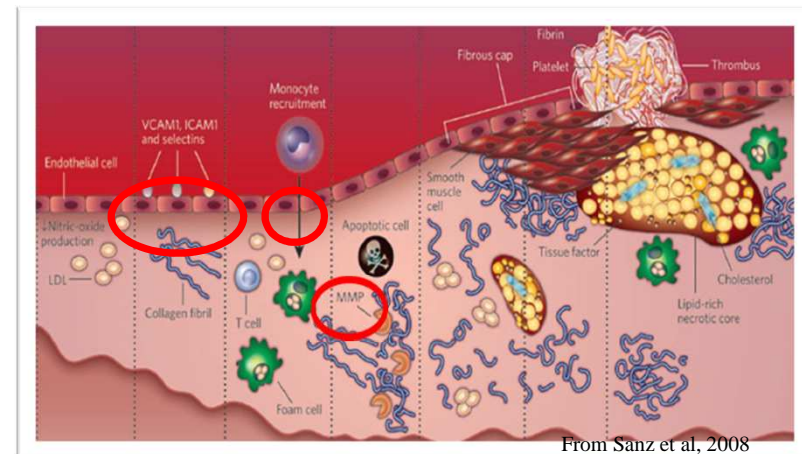
*bound to albumin but also to lipoproteins

Cardiovascular project

- With Coronary angiography
 - Detect the coronary artery responsible for the flow blockage
 - Other atherosclerotic plaques: **vulnerable or stable?**

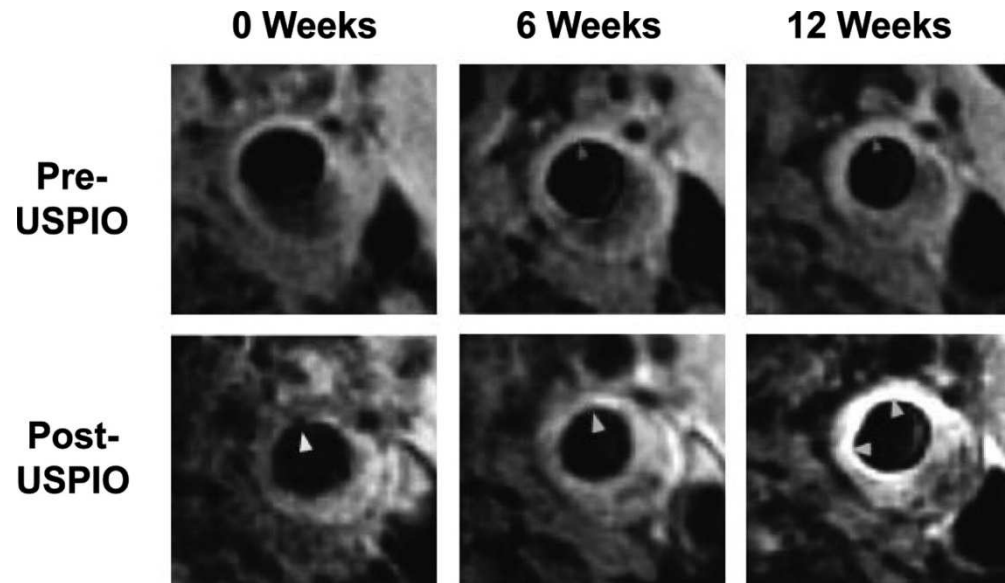
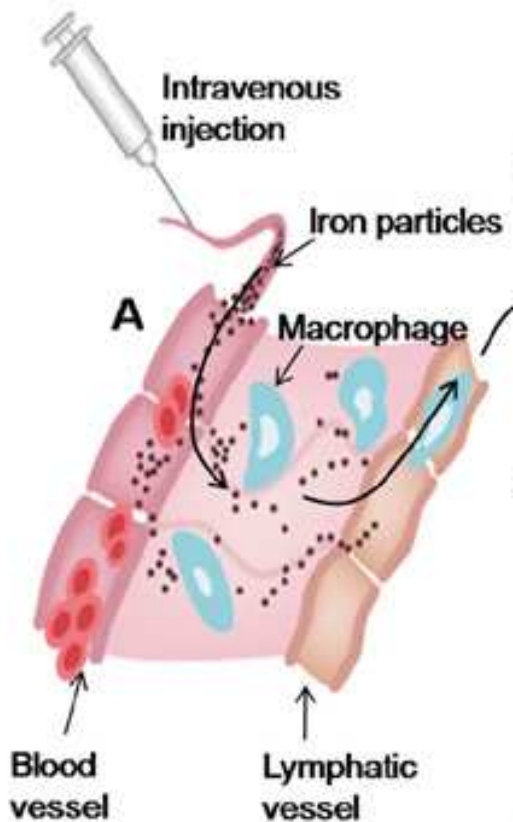


Goal:
In vivo analysis
of plaque vulnerability
through biomarker
detection:



**Molecular targets candidates:
ICAMP-1, MCP-1, MMP-9, MPO**

Imaging of human plaque inflammation with Ultrasmall Superparamagnetic Iron Oxide (USPIOs) as a magnetic resonance contrast agent.



Tang *et al*, 2009

For detection during surgery we need
A catheter based detection system.

OCT detection: SiO₂/Au NPs



Contents lists available at [ScienceDirect](#)

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



Interaction of polyacrylic acid coated and non-coated iron oxide nanoparticles with human neutrophils



Diana Couto^a, Marisa Freitas^a, Vânia Vilas-Boas^b, Irene Dias^a, Graça Porto^c,
M. Arturo Lopez-Quintela^d, José Rivas^e, Paulo Freitas^e, Félix Carvalho^b,
Eduarda Fernandes^{a,*}

^a REQUIMTE, Laboratory of Applied Chemistry, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

^b REQUIMTE, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal

^c Service of Clinical Hematology, Santo António Hospital, Porto, Portugal

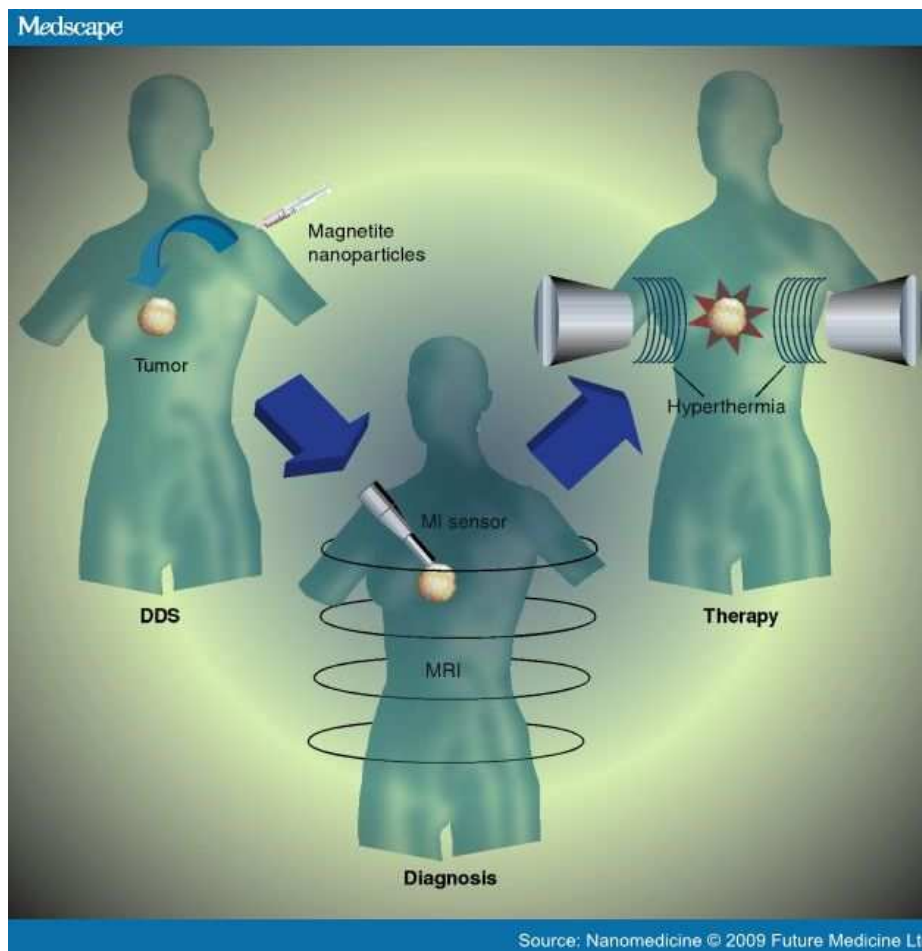
^d Laboratory of Nanotechnology and Magnetism, Institute of Technological Research, IIT, University of Santiago de Compostela (USC), Santiago de Compostela, Spain

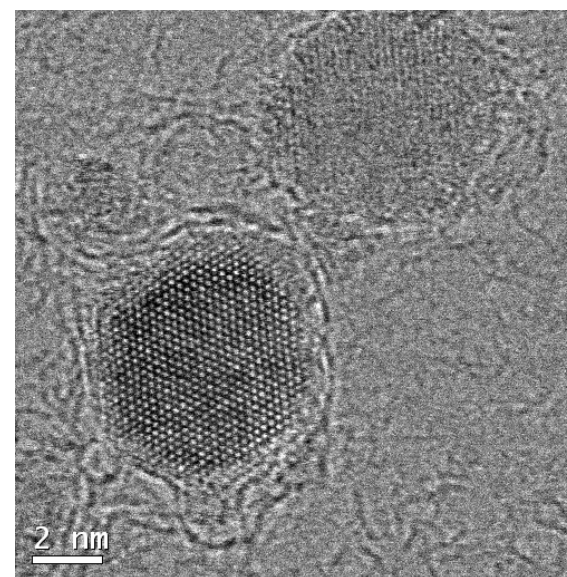
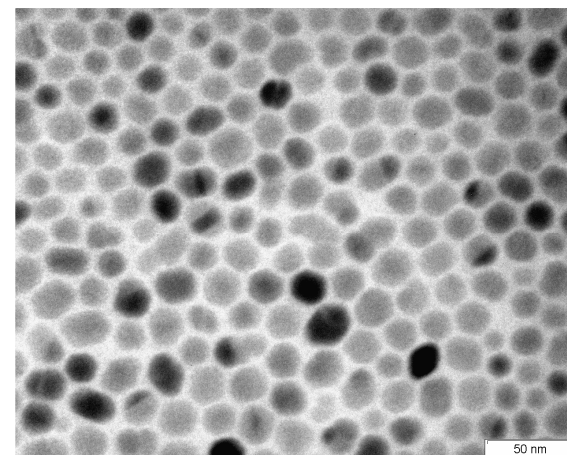
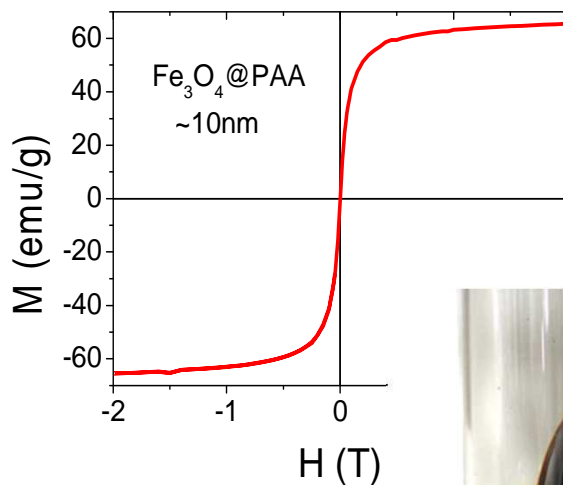
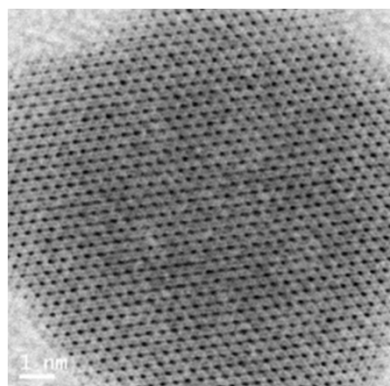
^e International Iberian Nanotechnology Laboratory, Braga, Portugal

H I G H L I G H T S

- Polyacrylic acid-coated iron oxide nanoparticles increase neutrophils' apoptosis.
- Non-coated iron oxide nanoparticles prevent neutrophils' apoptosis.
- Both nanoparticles trigger neutrophils' oxidative burst by NADPH oxidase activation.

Magnetic Hyperthermia

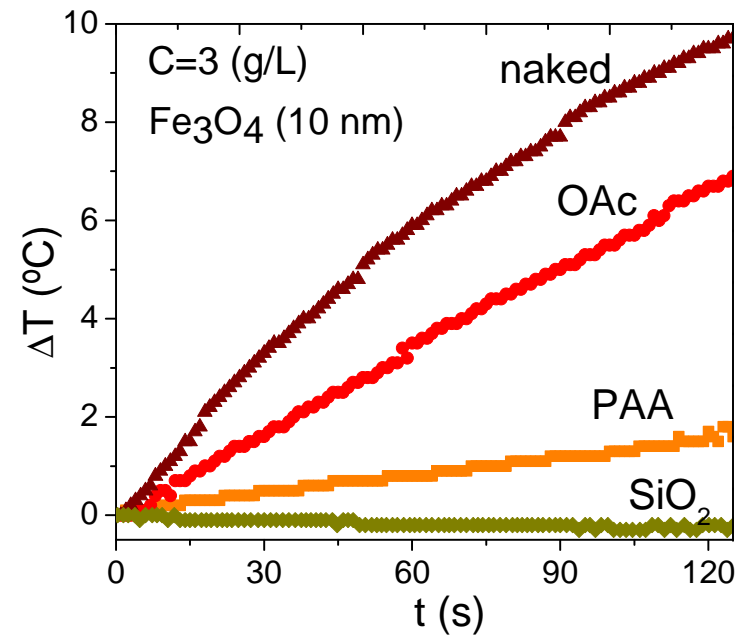
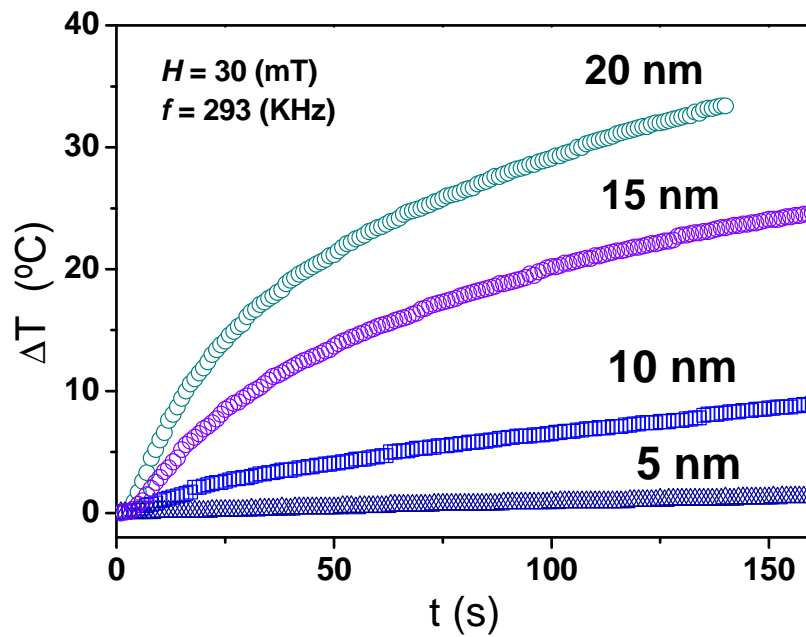




SPM iron oxide nanoparticles:

- Biocompatible and non-toxic
- Relatively high Ms
- No coercive forces or remanence
- Versatility

Hyperthermia response



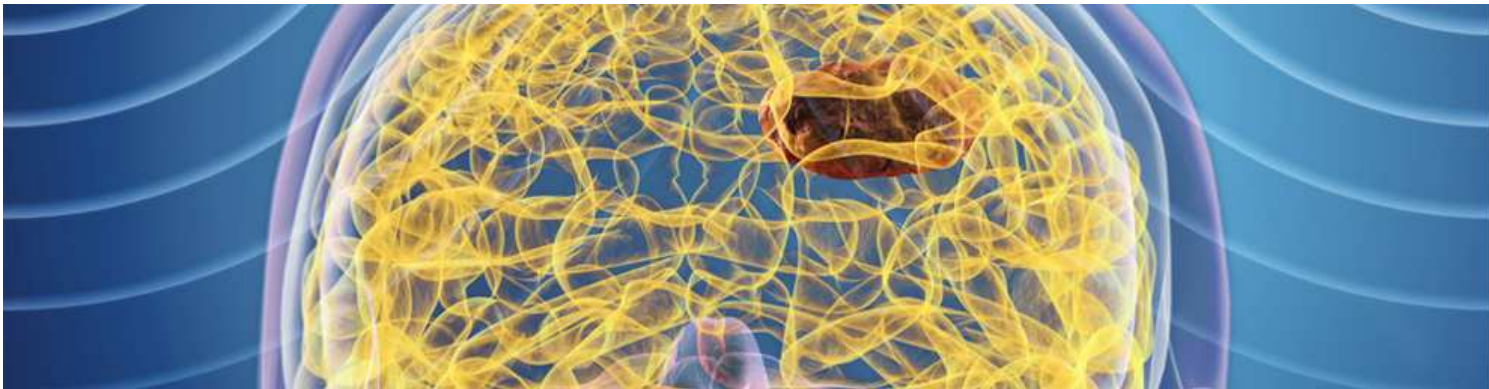
magforce®
THE NANOMEDICINE COMPANY



<http://www.magforce.de/en/home.html>

1st company worldwide to receive European approval for a medical product using nanoparticles

17 billion iron oxide nanoparticles in just one milliliter of magnetic liquid

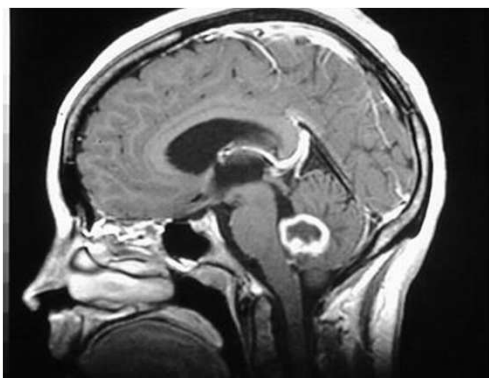


- NanoTherm® therapy in Charité-University hospital in Berlin

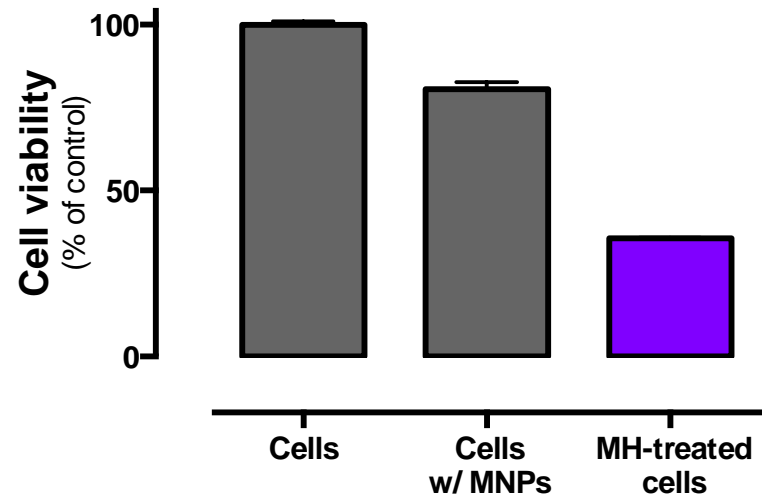
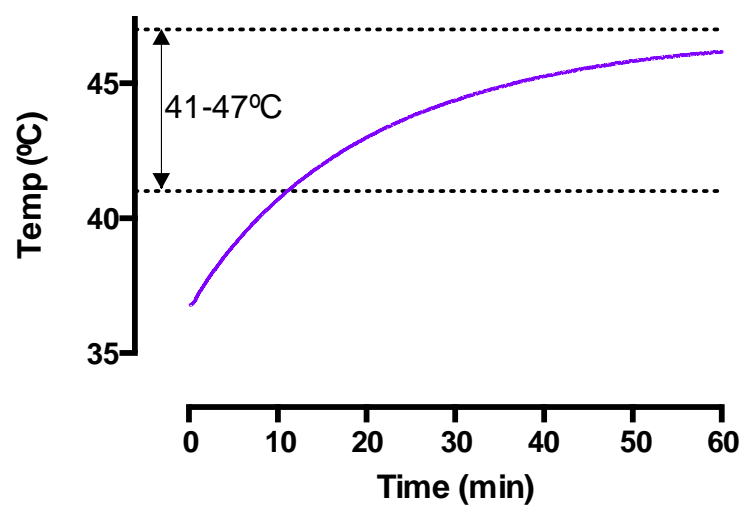
Functionalization of iron-oxide nanoparticles for the treatment of glioblastoma with magnetic hyperthermia

Vânia Vilas-Boas, Manuel Bañobre-Lopez, Yury Kolen'ko, Begoña Espiña, Verónica Martins and Félix Carvalho

- Glioblastoma multiforme (GBM) is the most malignant and frequent primary brain tumor;
- Current standard therapy for GBM includes surgery, radio- and chemotherapy;
- Despite aggressive treatments, survival rate is around 12-14 months, less than 10% of patients are still alive 5 years after diagnosis;
- Magnetic hyperthermia has been approved in Europe for the treatment of brain tumors.



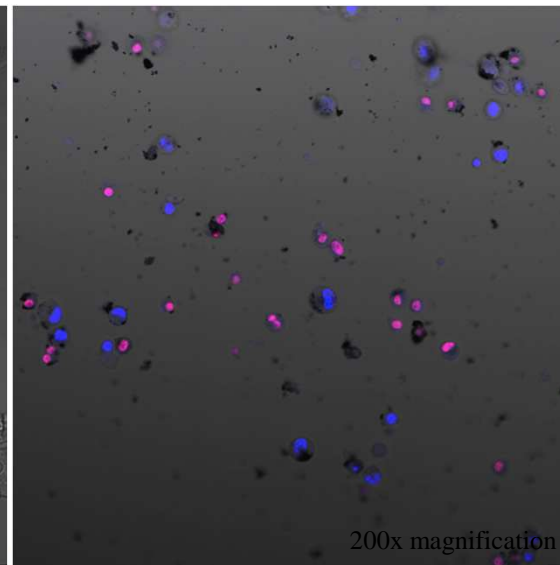
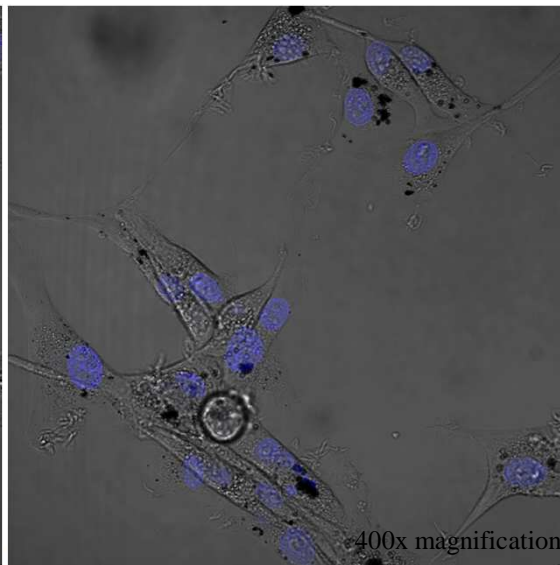
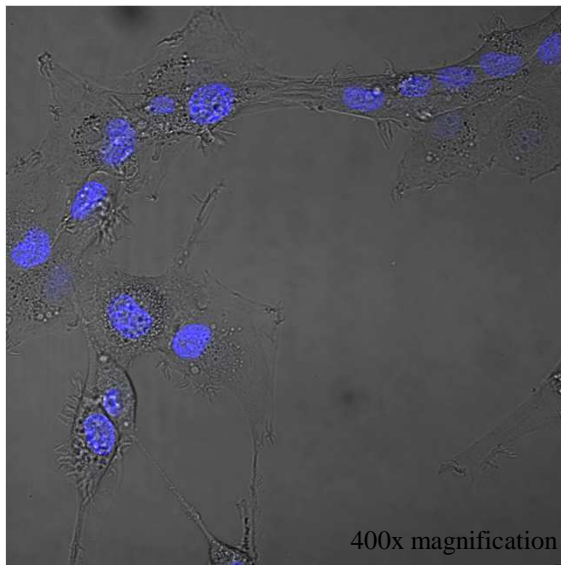
First magnetic hyperthermia studies in cells



Cells

Cells w/ MNPs

MH-treated cells



INL and the NANOREG project

D2.10 Protocol(s) for size-distribution analysis of primary NM particles in air, powder and liquids

Aim(s) or Objective(s) of deliverable

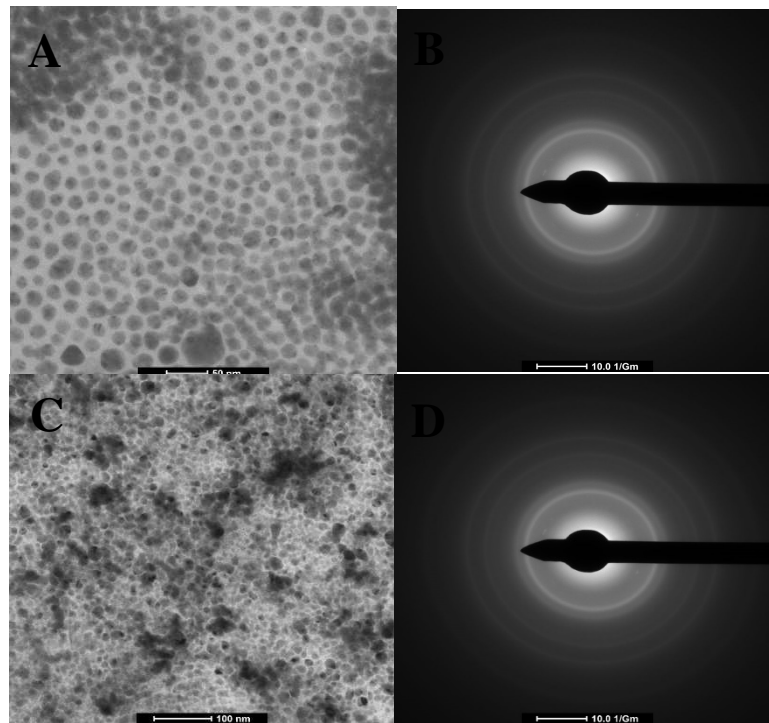
- Establish SOP to apply EC definition
 - Quantitative size (and shape) analysis
 - Number-based: per particle
 - Primary particles of manufactured NM.
 - Validation on reference and representative nanomaterials for application for regulatory use
 - Methods include TEM, SEM, NTA, FFF, SP-ICP-MS, ...

Pieter-Jan De Temmerman, Jeroen Lammertyn, Bart de Ketelaere, Vikram Kestens, Gert Roebben, Eveline Verleysen, Jan Mast (2013)
Measurement uncertainties of size, shape and surface measurements using transmission electron microscopy of near-monodisperse, near-spherical nanoparticles. J. Nanoparticle Res. Submitted September 2013



Goal: Examine the stability of NM-300K Ag nanoparticles on the grid
by Analytical TEM

To Identify Ag oxides or Ag sulphides if present



TEM images and corresponding diffraction patterns of NM-300K particles, recorded immediately after preparation of the TEM specimen on 15/10/2013 (A and B) and recorded on 06/03/2014 (C and D)

Data – CODA-CERVA, Jan Mast

Other ongoing work

- POC NMR spectroscopy chip for food quality analysis (olive oil, ...)
- DNA and protein extraction from complex samples:
LOC for olive oil, and later wine
 - Sample purification: filter based LOC for milk,oil, others
 - Sample purification: blood; immuno separation LOC (white cell removal)
 - Sample purification: wine, beer: phase separation LOC

Nuclear and aviation: NDT (defects on weldings, light molecule detection)

INESC MN



INL