NanoBiotechnology programme at the Joint Research Centre

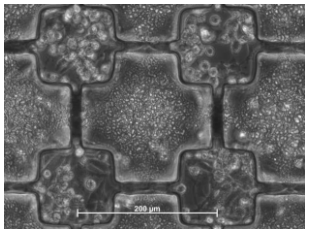
François Rossi

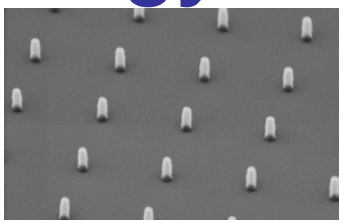
www.jrc.ec.europa.eu

Serving society
Stimulating innovation
Supporting legislation

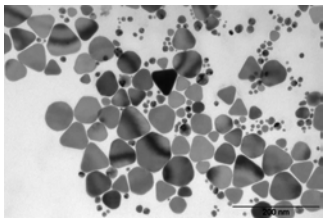
Joint Research Centre

NanoBiotechnology @ JRC





Chemistry

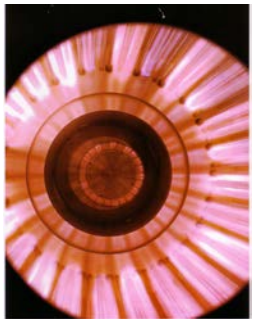


NP synthesis and characterisation

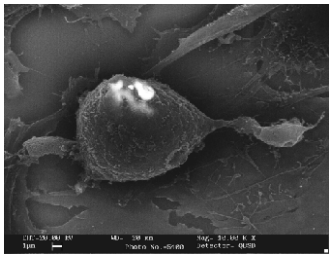
μFabrications
Nanostructures

Plasma polymers
Bio interfaces

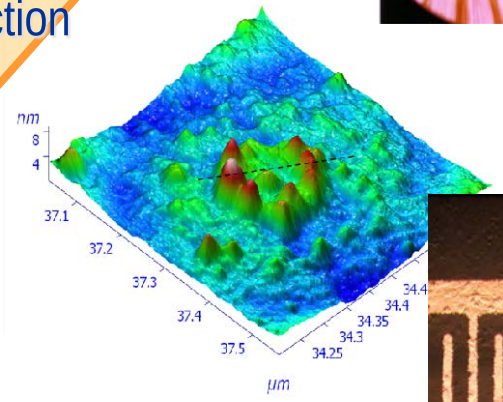
Physics/Materials

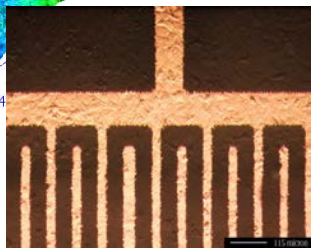


Nanotoxicology



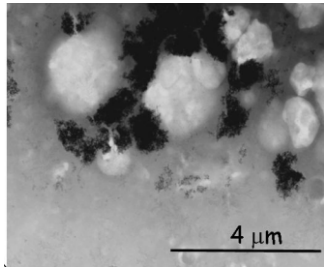
Molecular Detection
Sensors

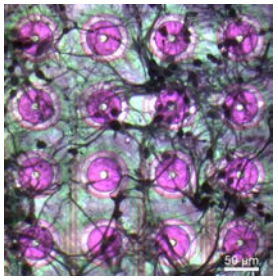


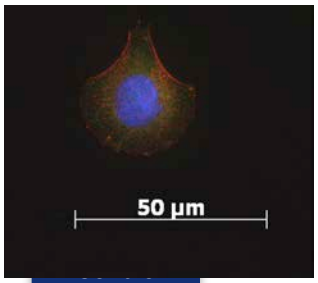


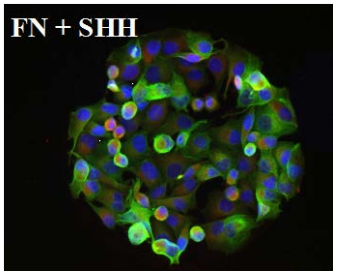
In vitro models
Stem cells culture

Biology



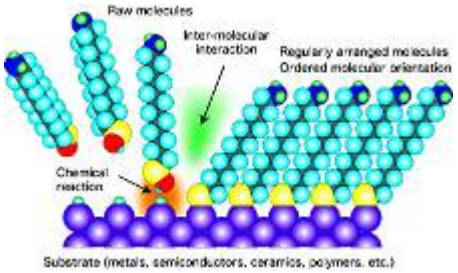




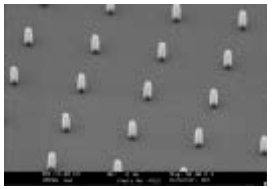


Surface chemistry

Micro-nano fabrication



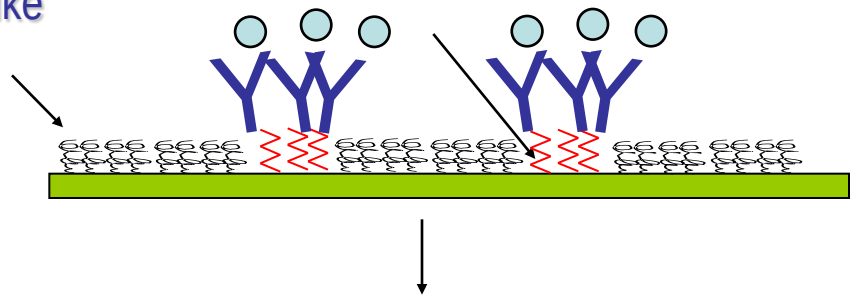
Surface engineering to Control surface Physical/chemical properties at micro and nanoscale



Nanostructures

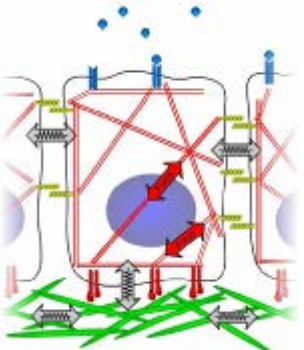
Anti-adhesive
PEO-like

Bio-adhesive = COOH/NH_2 …functionalities

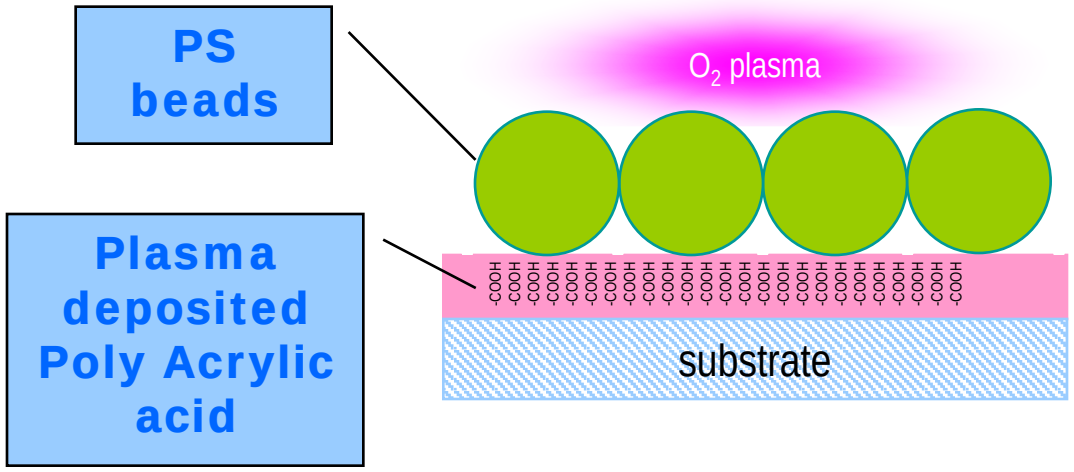


- Biosensing
- Protein chips

- To create cell microenvironment at cellular resolution favourable to preserve cell function on solid surfaces



Colloidal Lithography + Plasma Polymers



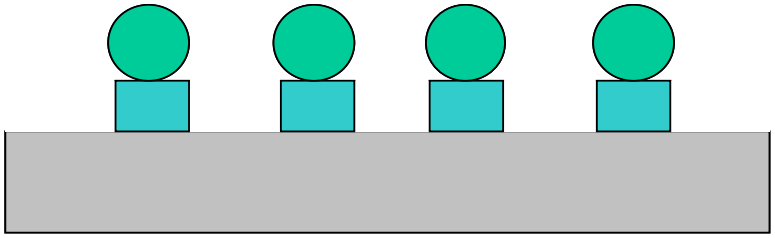
Ultrasound bath

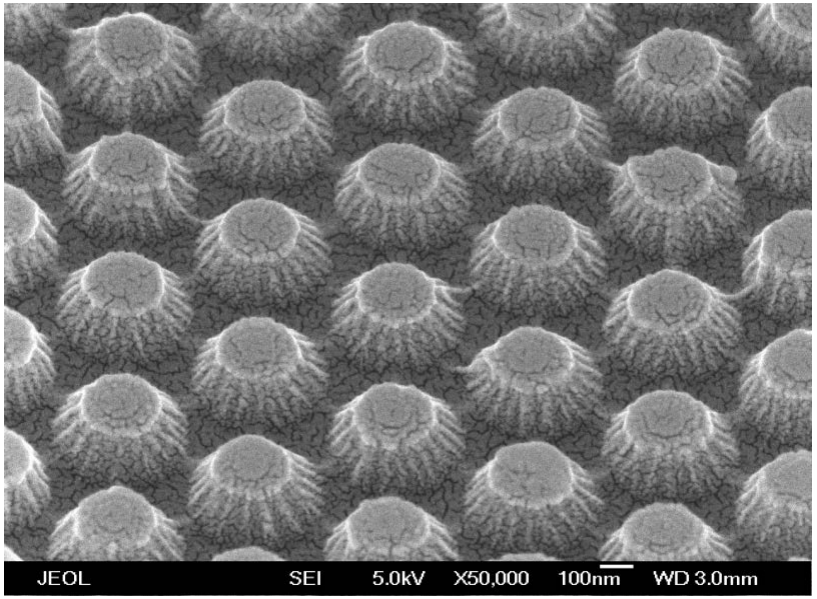
Valsesia, et al. *Nano Lett*., 4 (2004), 1047-1050
Valsesia,et al ., *Adv. Func. Mat. ,16* (2006),1242-1246

Process characterization

2)

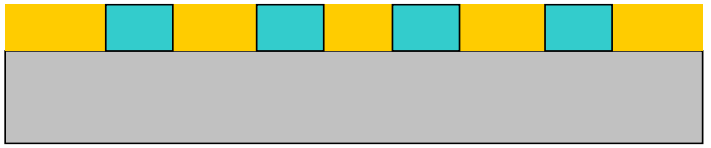
Plasma Etching (1:1)

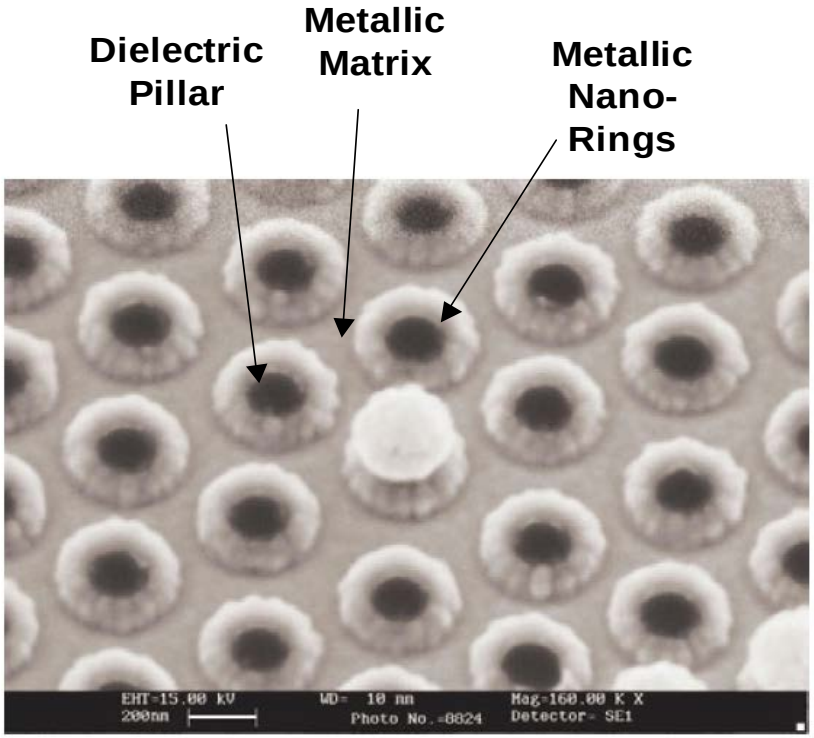




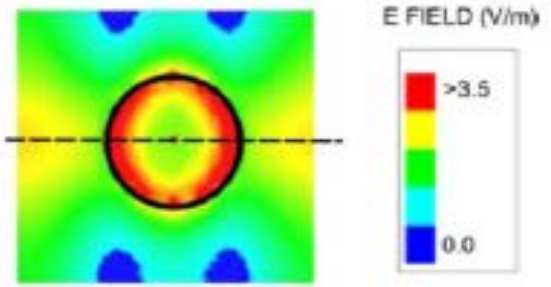
4)

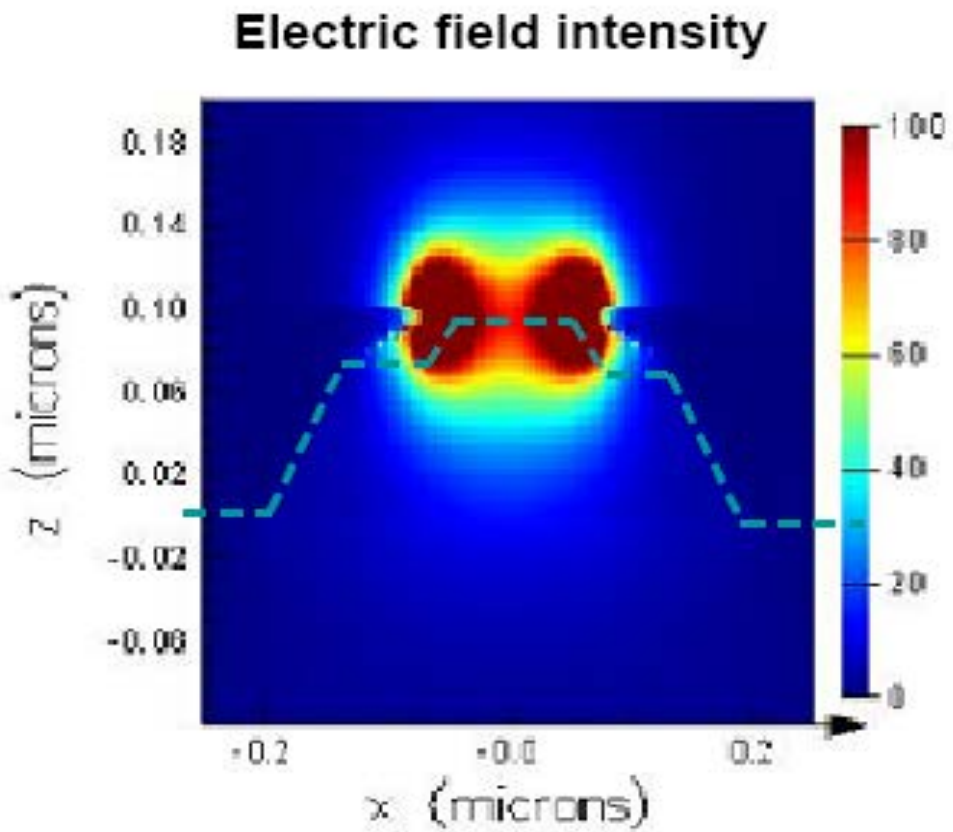
Lift-off

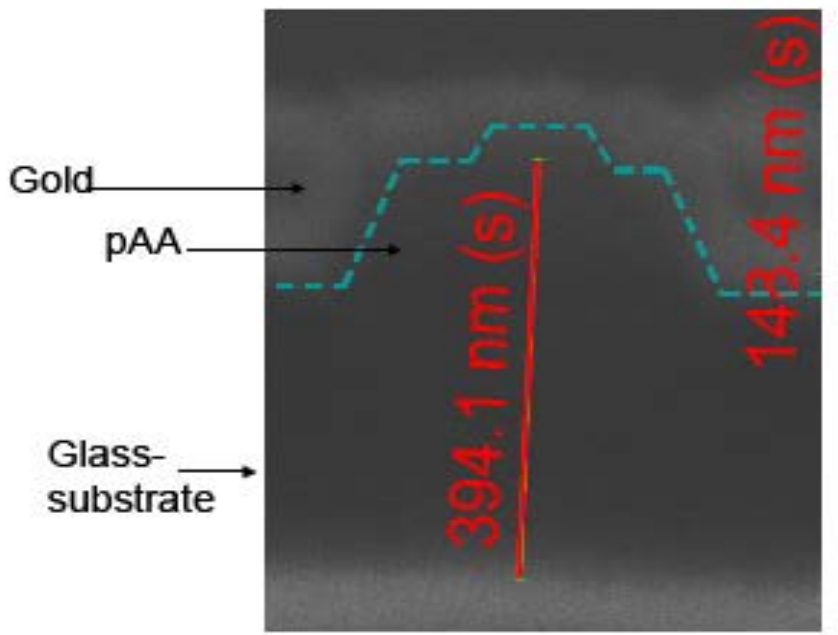




Plasmonic structures:
Improvement of the local sensitivity (x10)







Development of *in vitro* models

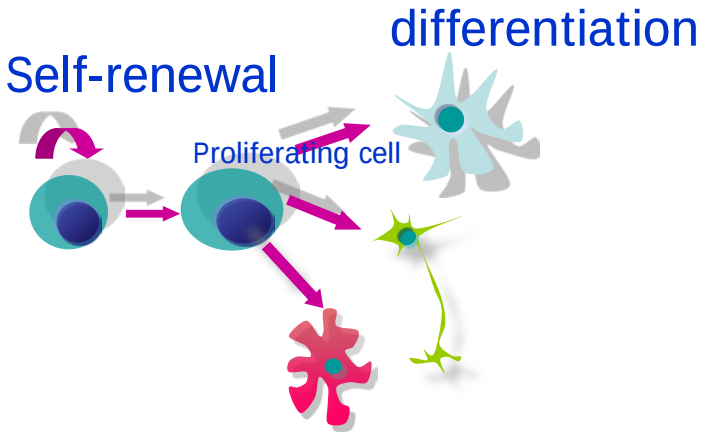
Based on
cell lines

?

Effects of chemicals on **human Health**

Primary cells

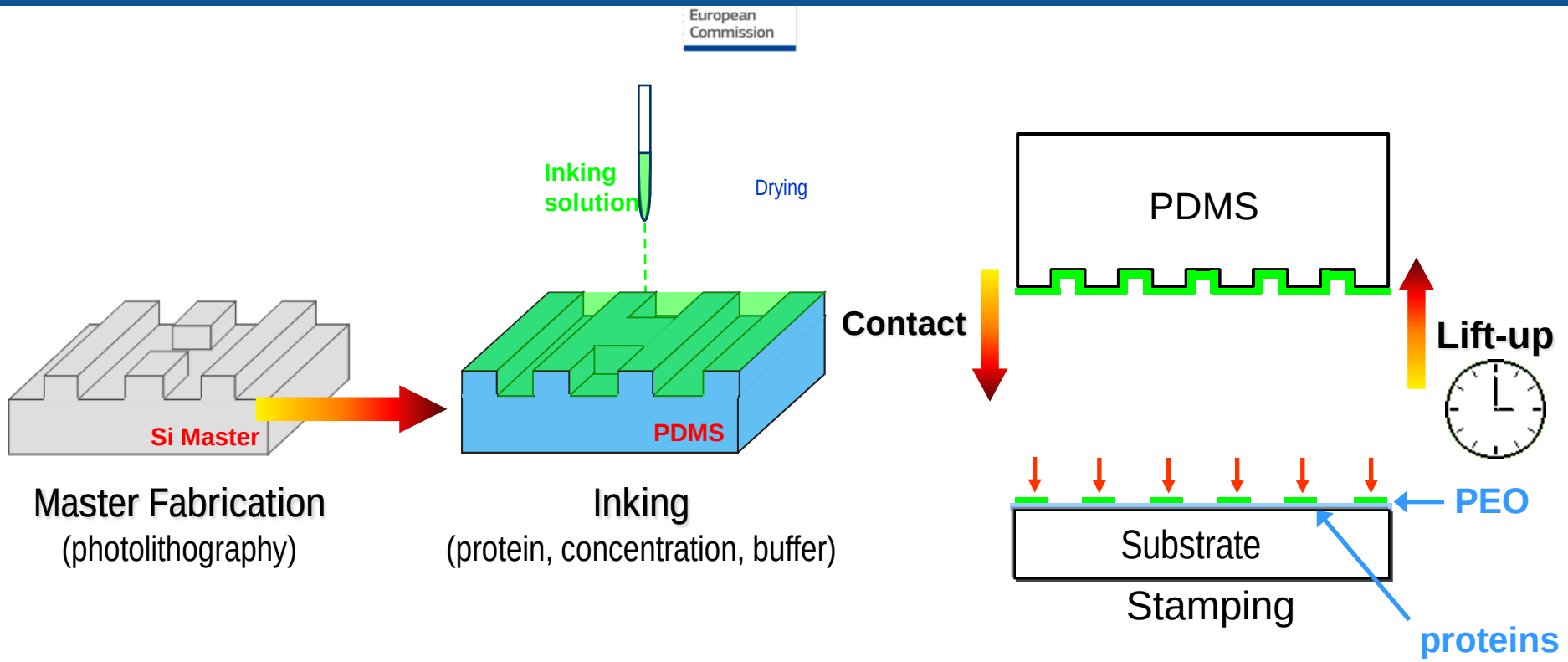
Stem Cell lines and their derivatives



- Embryonic (ethical issues)
- Adult (in tissues, blood…)

Toxicity Testing in the Twenty-first Century: A Vision and a Strategy : Committee on Toxicity and Assessment of Environmental Agents, National Research Council

Micro contact printing



Plasma-PEO immobilises proteins in dry conditions

- **Advantages:**
  - Direct patterning of relevant biomolecules on plasma PEO.
  - Direct fabrication of biological contrast: bio-adhesive/ bio-repellent

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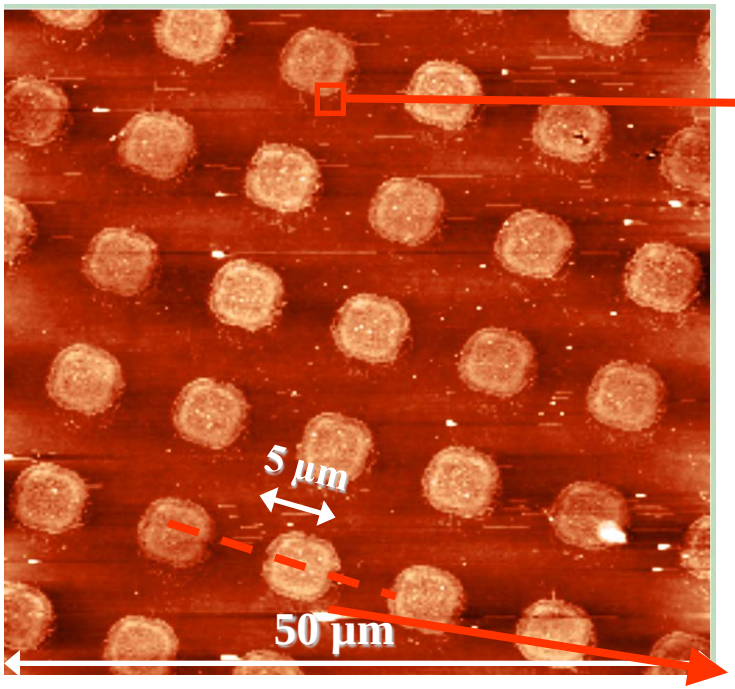
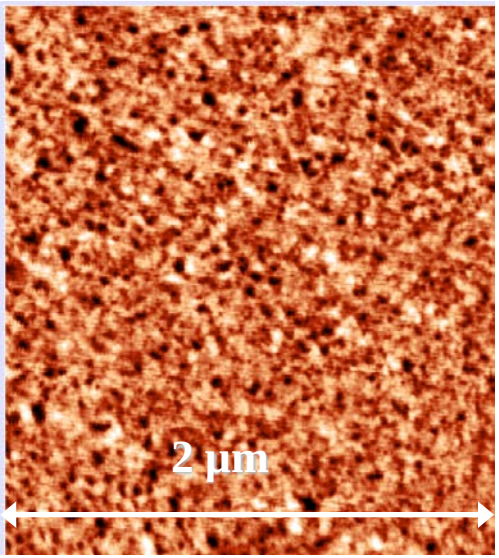
Ruiz et al. Microelectronic engineering 2007

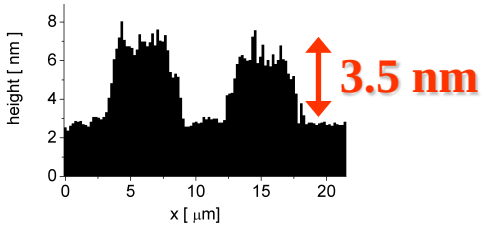
Microcontact printing of Fn patterns

AFM

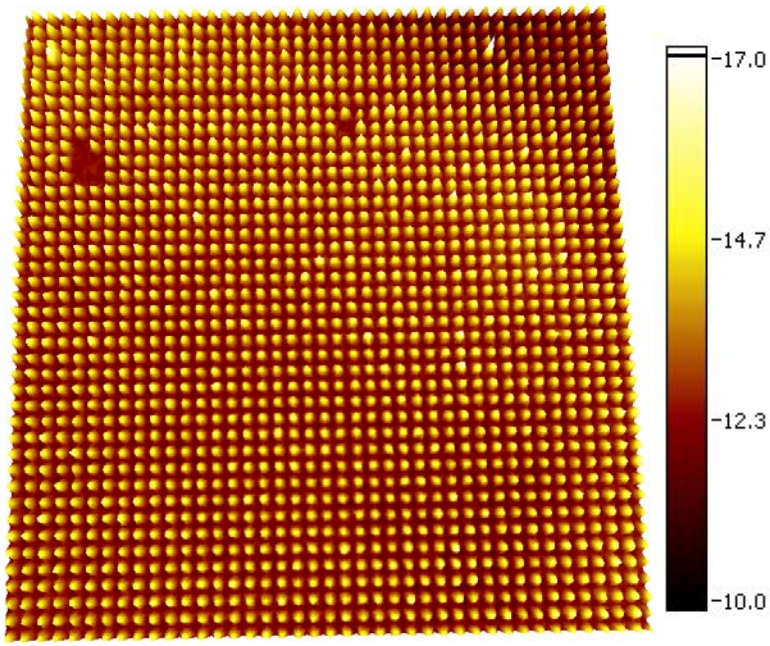


Morphology of the protein





Ellipsometry



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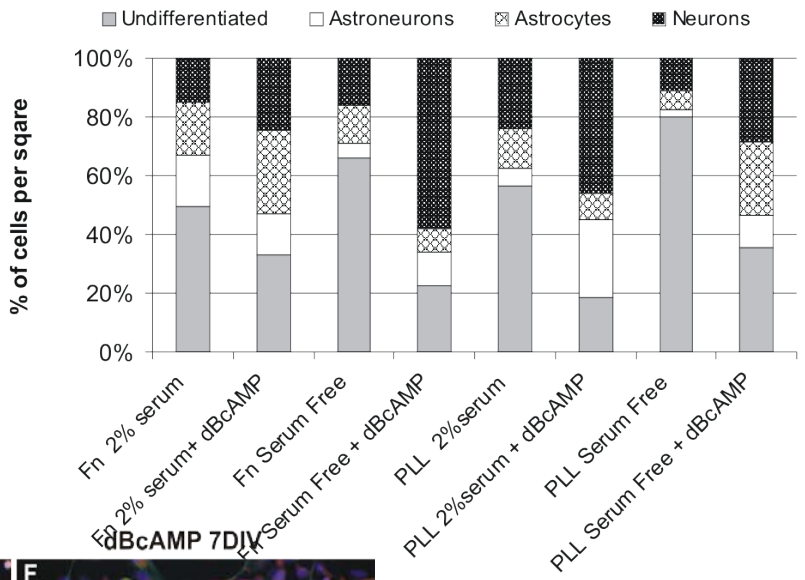
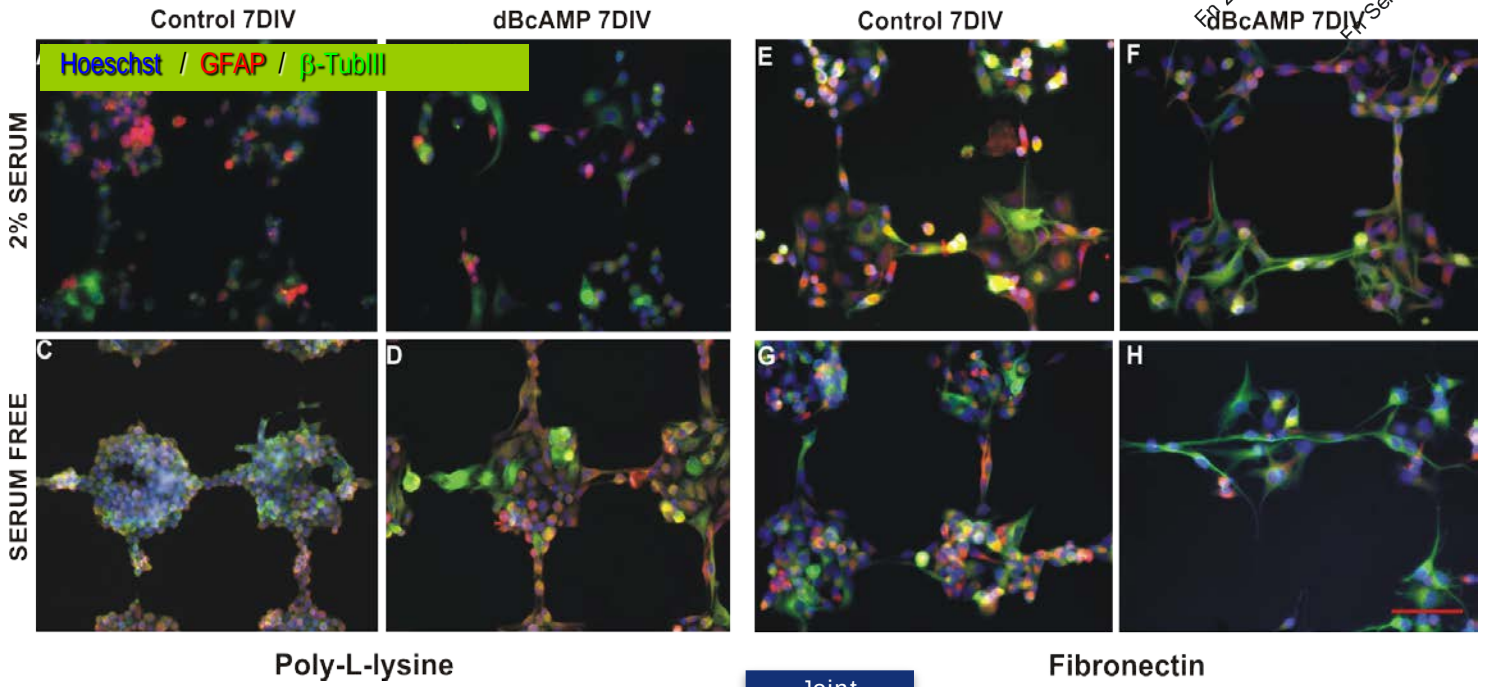
Ruiz et al: J Biomater Sci Polym Ed 2008

Stem cell differentiation

- PLL micropatterns allow maintaining the neural stem cells attached to the surface in non-differentiated state
- Attachment to Fn without serum promotes neuronal differentiation

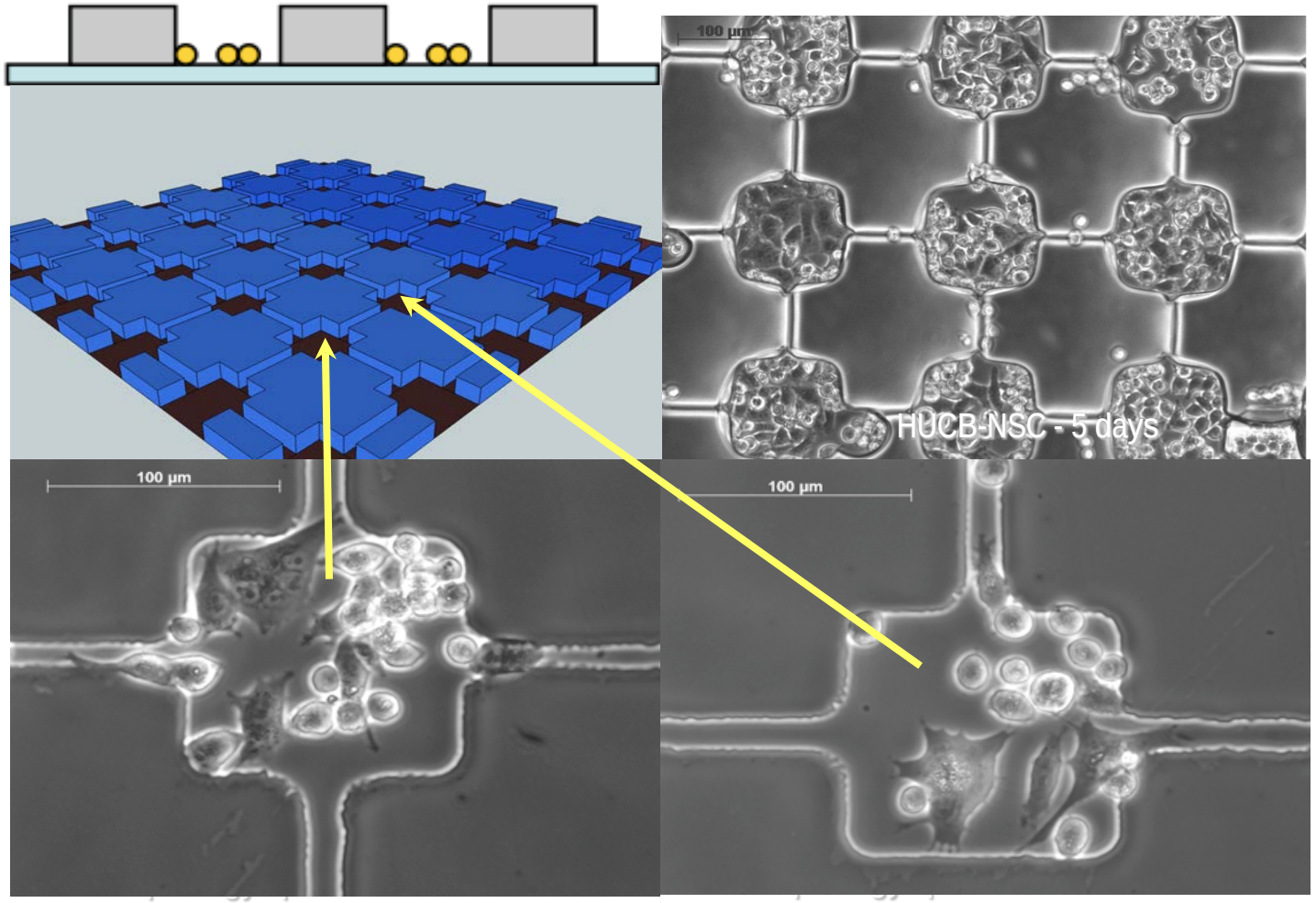




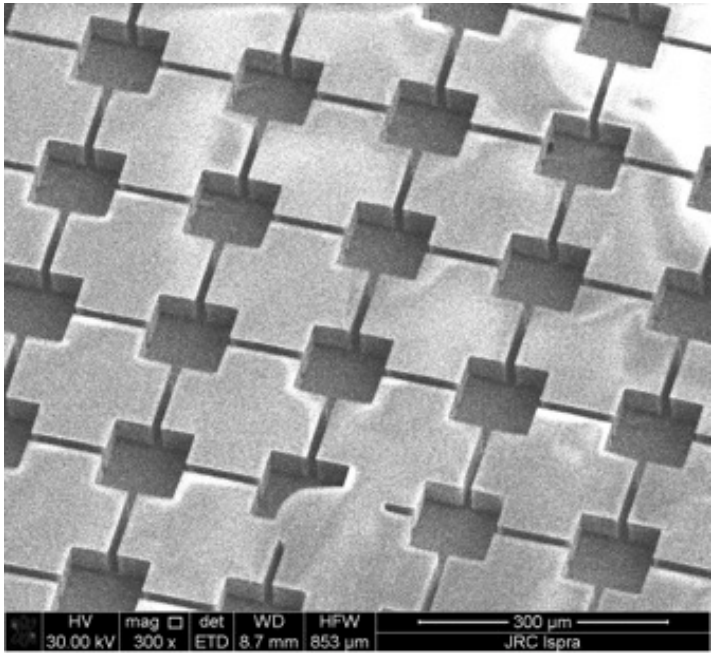
β-TubIII → neuronal , GFAP → astrocytes / stem cell, Hoechst → nuclei.

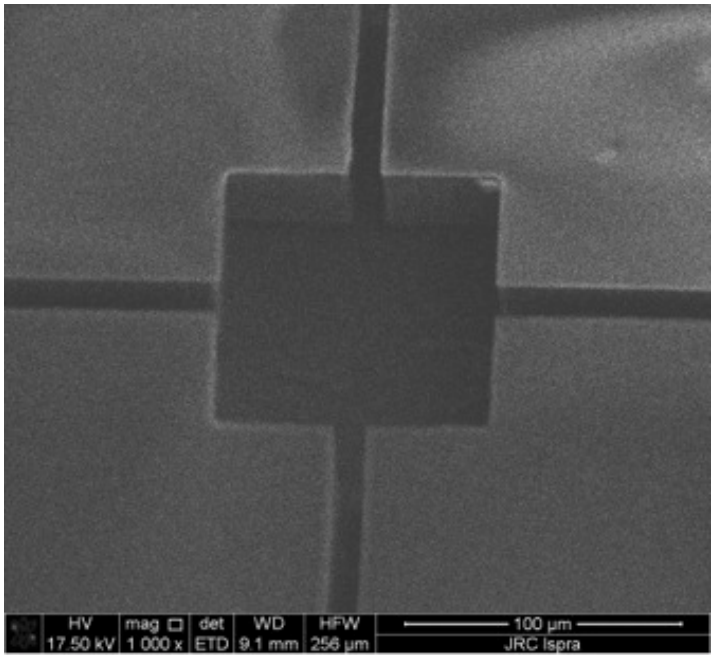
Ana Ruiz et al. Biomaterials 2008

HUCB-NSC cultured in 3D domains

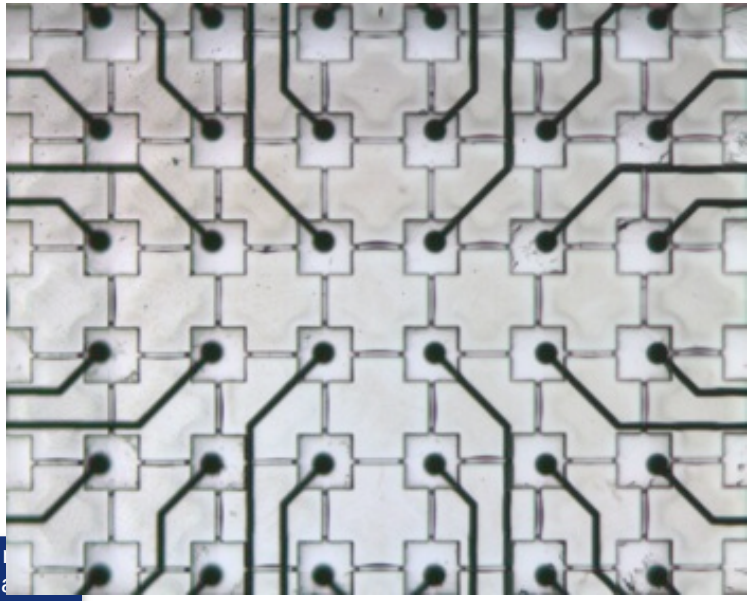


HUCB-NSC display neuronal like phenotype when cultured in PEG-3D scaffolds





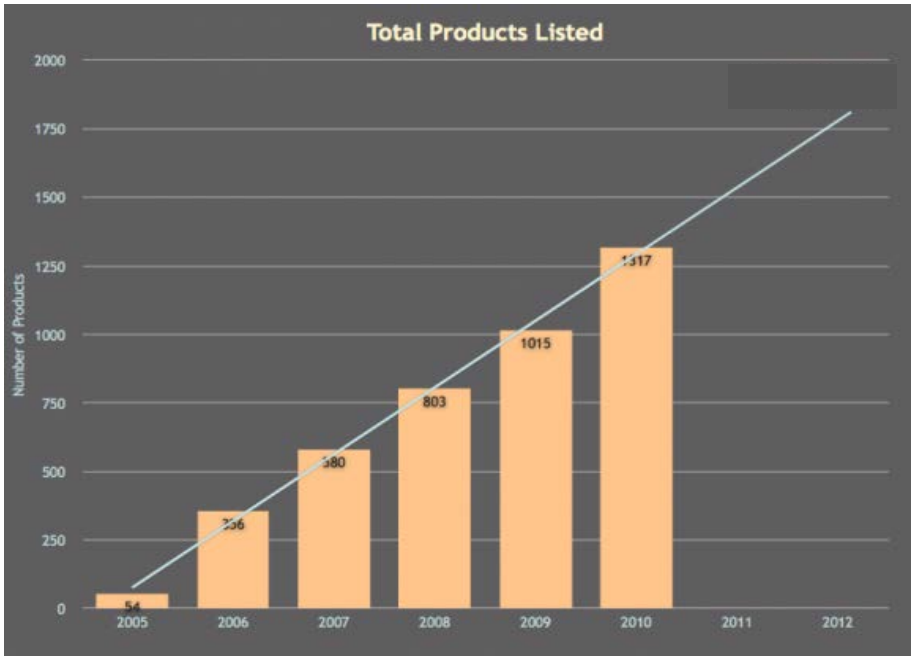
The cells can be easily aligned prior UV cross-linking with the MEA electrodes: Electrical monitoring of neurons groups

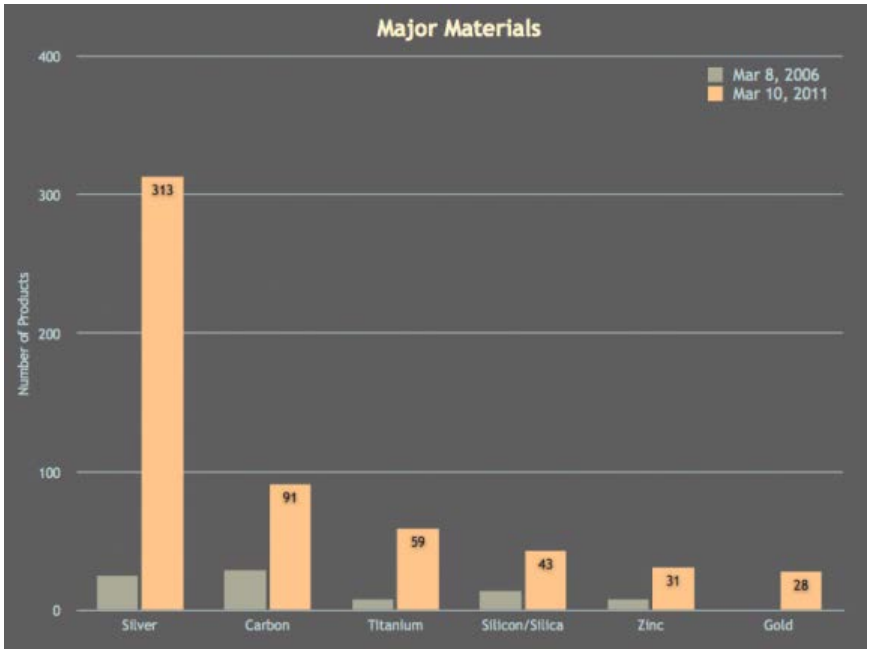


Joi
Resea
Centre

Nanomaterials and nanotoxicology

Nanotechnology consumer products on the market





Examples of product areas:

- Cosmetics / personal care
- Paints & coatings
- Household products
- Textiles
- Food (and ingredients)
- Food packaging and more…

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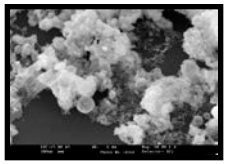
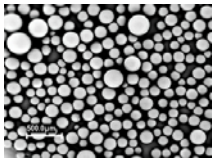
Ag NP:

Antimicrobial
Antifungal
Antiviral
Food Packaging
Wall paints
Biocide sprays
Textiles
Laundry detergents

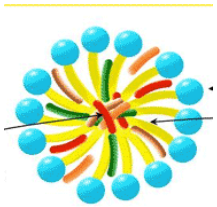
Nanomaterials : what makes them different?



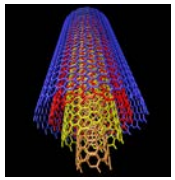
Size (1 - >100 nm)
Large specific surface



Special properties (electronic, mechanical, optical ...)

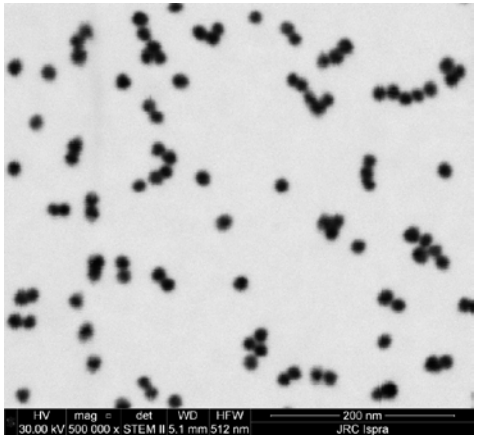


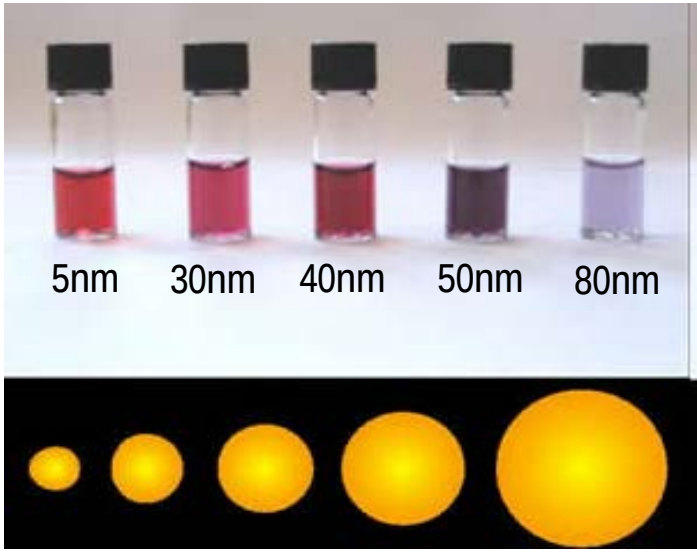
Chemical reactivity very different compared to bulk material



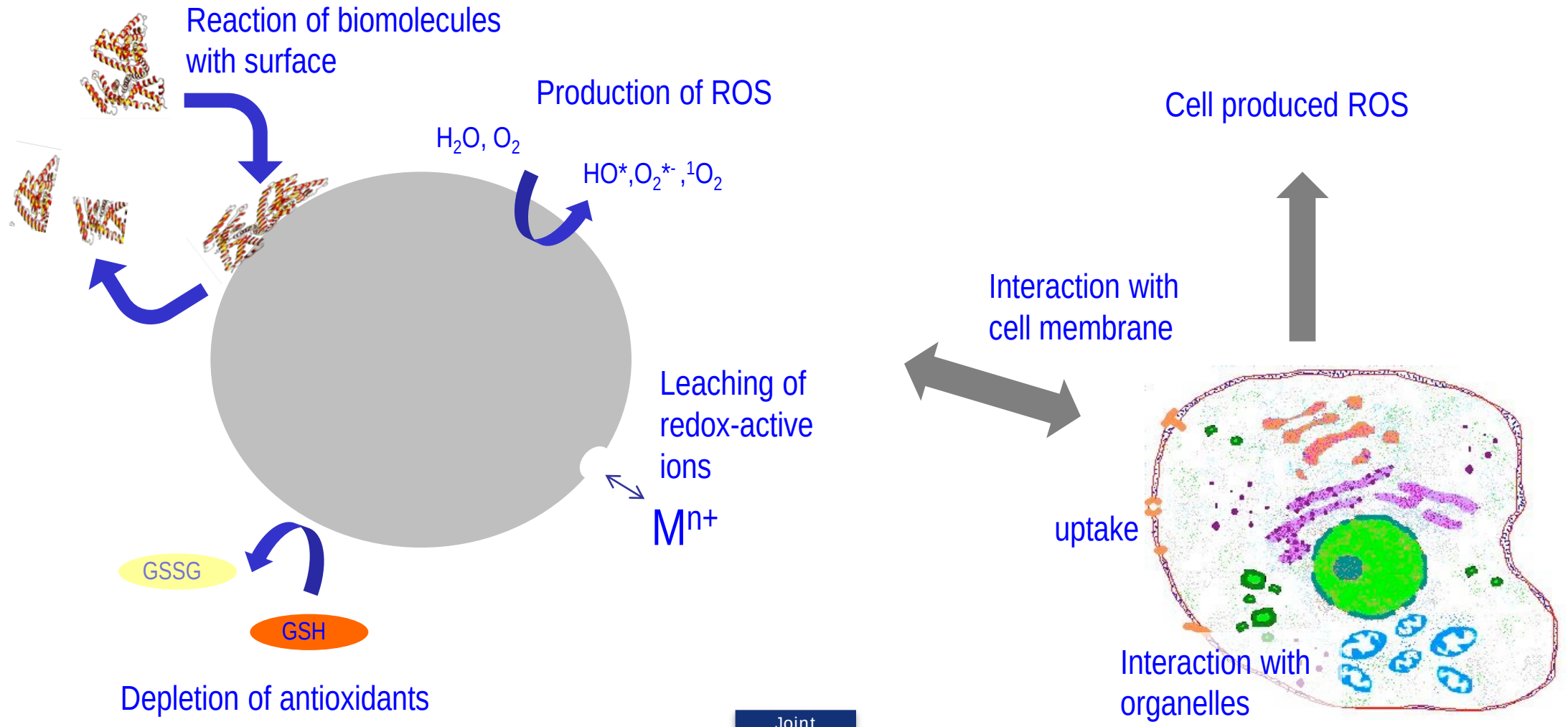
Matrix dependent properties

Many forms: fullerenes, graphene, nanotubes, nanocarriers, nanoemulsions...





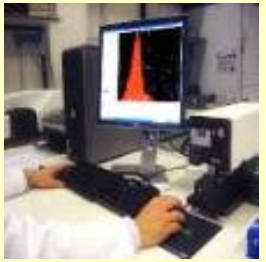
Chemistry/biochemistry related toxicity

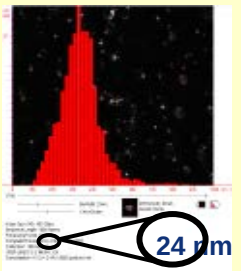


In vitro Nano-Toxicology at JRC

Synthesis of NM

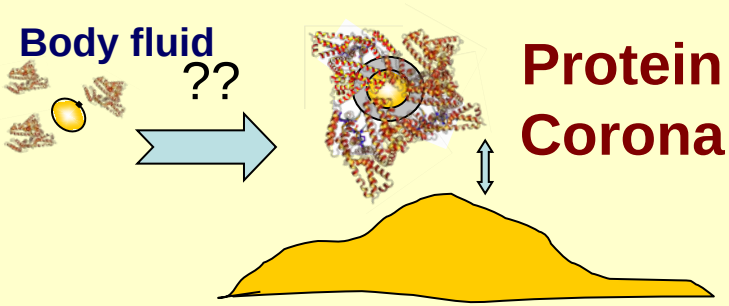
Characterisation



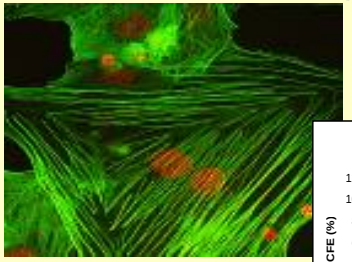


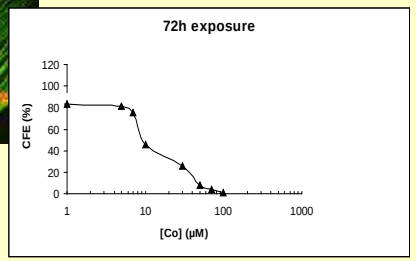
Dynamic light scattering, SEM,..

Uptake and cell response



Toxicity effects





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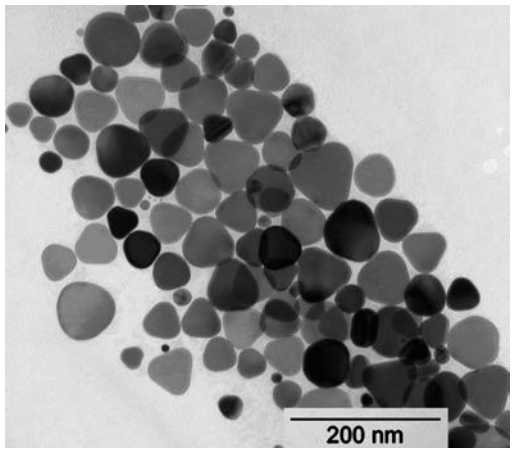
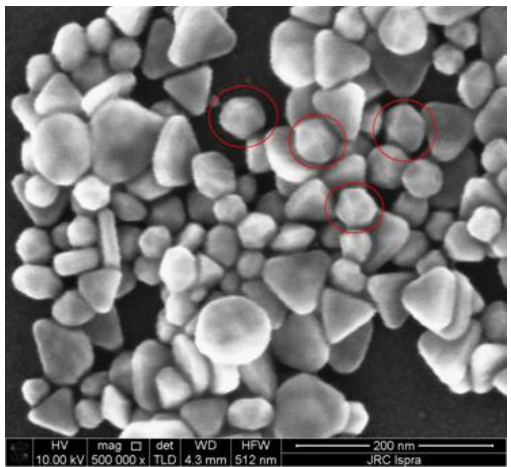
☞ Libraries of NM with controlled properties

Silver

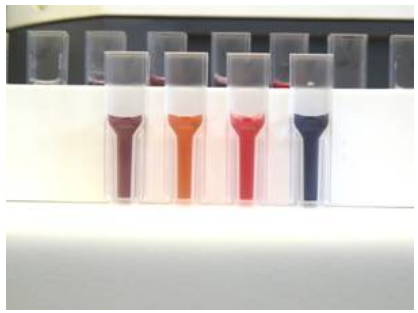
Prisms of 20-100 nm diameter range

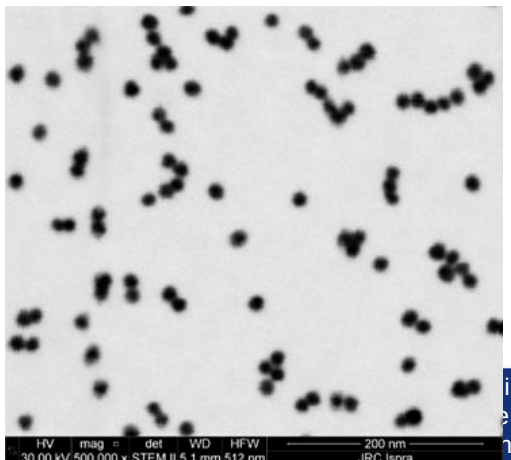




Gold

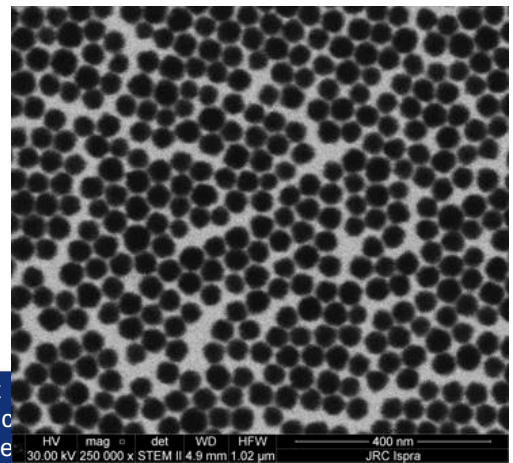
Spheres of 5-100 nm diameter range





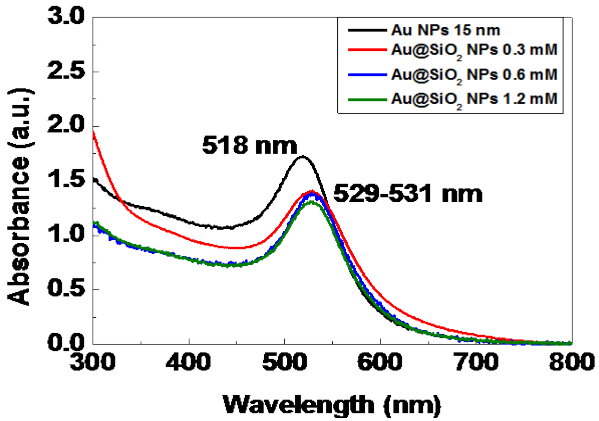
Silica

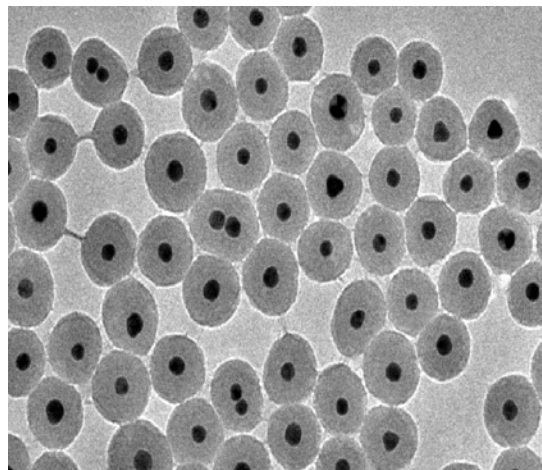
Doped amorphous NPs of 15-300 nm diameter range



Core shell

Au-SiO2 20-60 nm



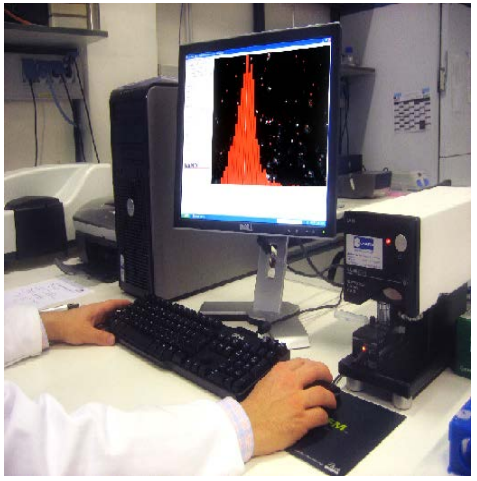


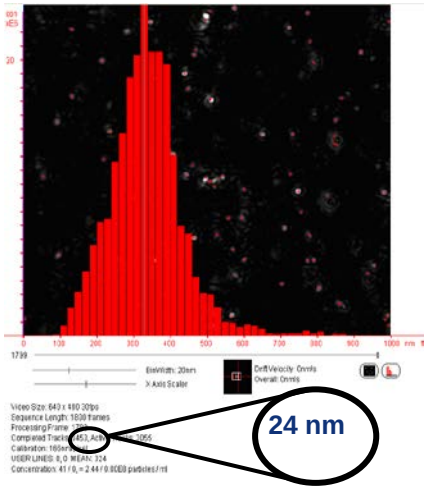
NP Characterisation

Size	**DLS, SEM, TEM, DSC,PTA,CPS**
Morphology	**SEM , STEM, TEM**
Phase	**XRD**
Surface Charge	**Zpotential**
Concentration, Purity, solubility	**ICP-MS**
Surface composition	**XPS, ToF SIMS**
Specific area	**BET,**
Chemistry	**FTIR, Raman**
…	

PLUS specific characterisation:

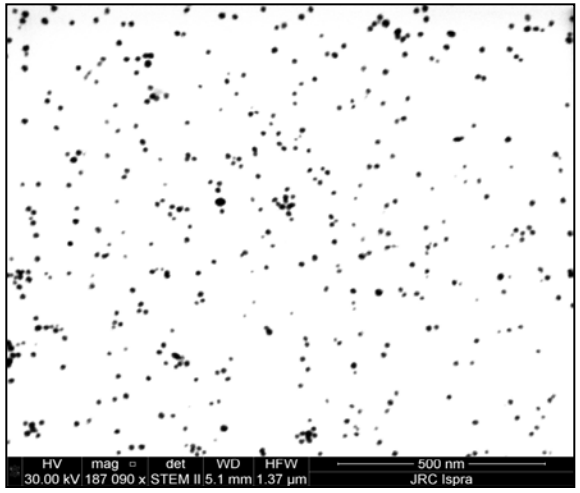
- **Behaviour in Culture Medium**
- **Binding with plasma proteins**
- **Dissolution, aggregation**
- **Sedimentation…**



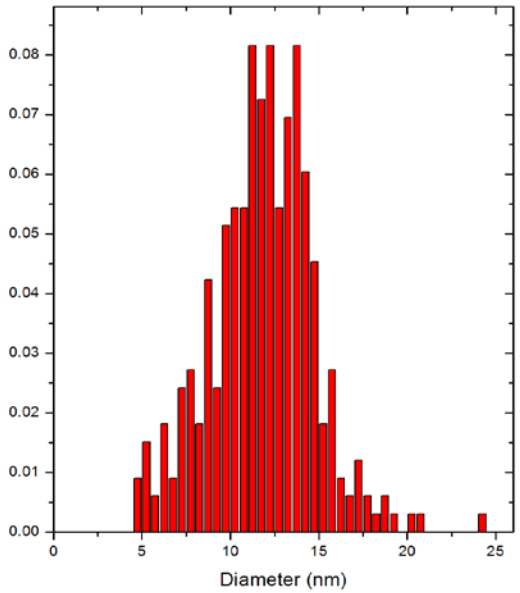


Direct Imaging-FIB-SEM

Image processing
and particle counting

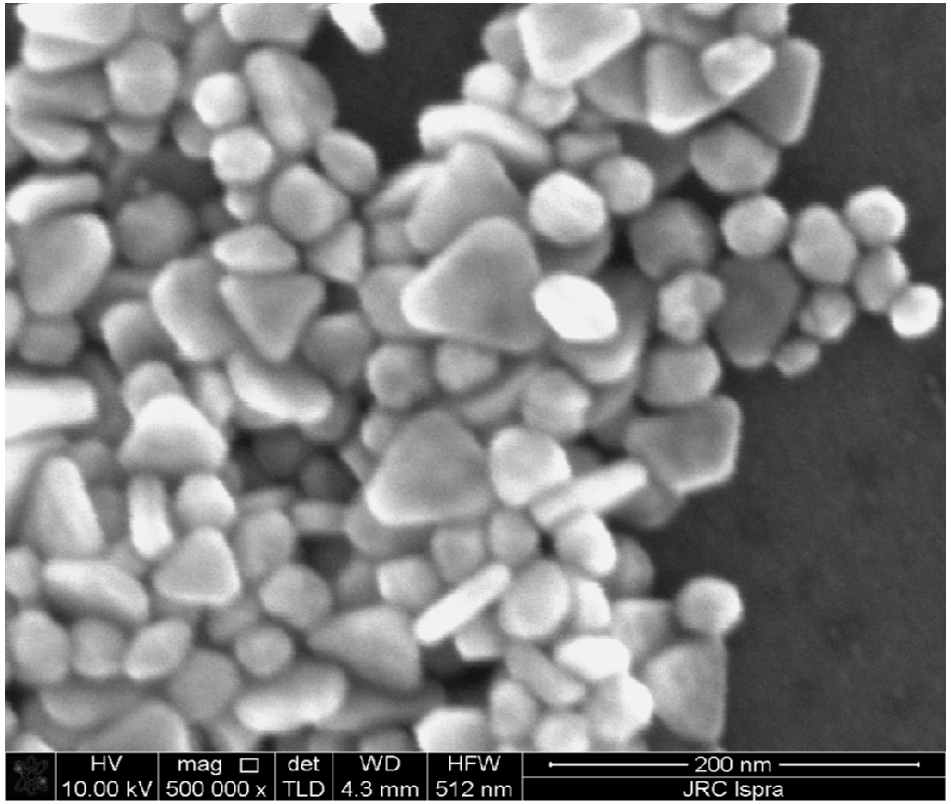


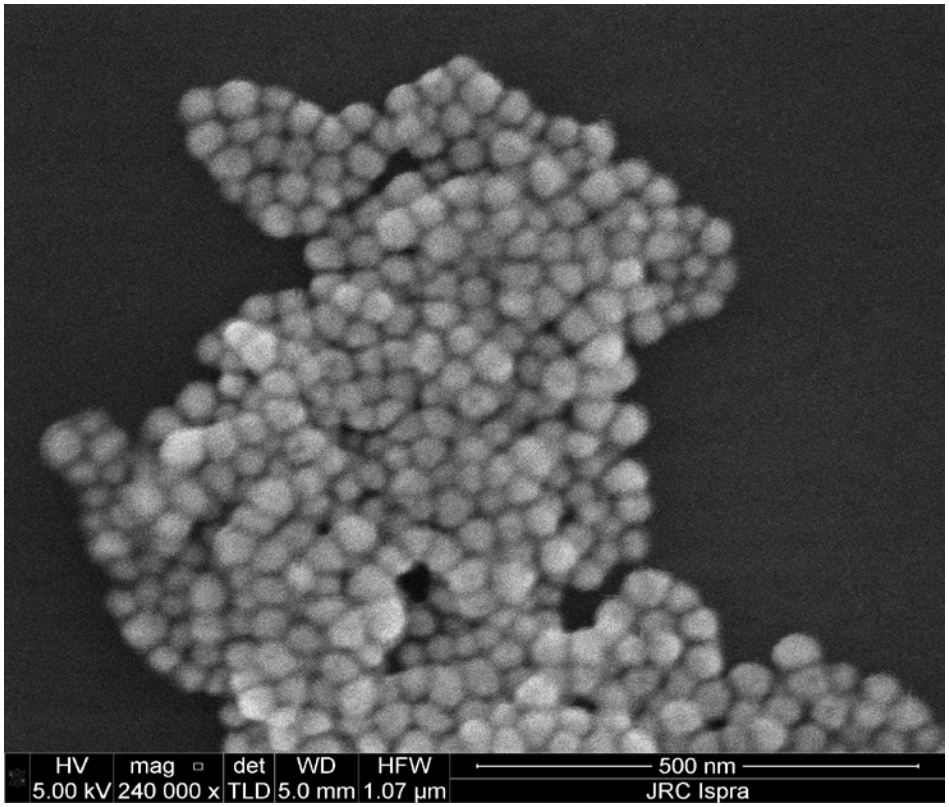
STEM Images contrast enhanced
for image processing



Histogram of particle size
distribution

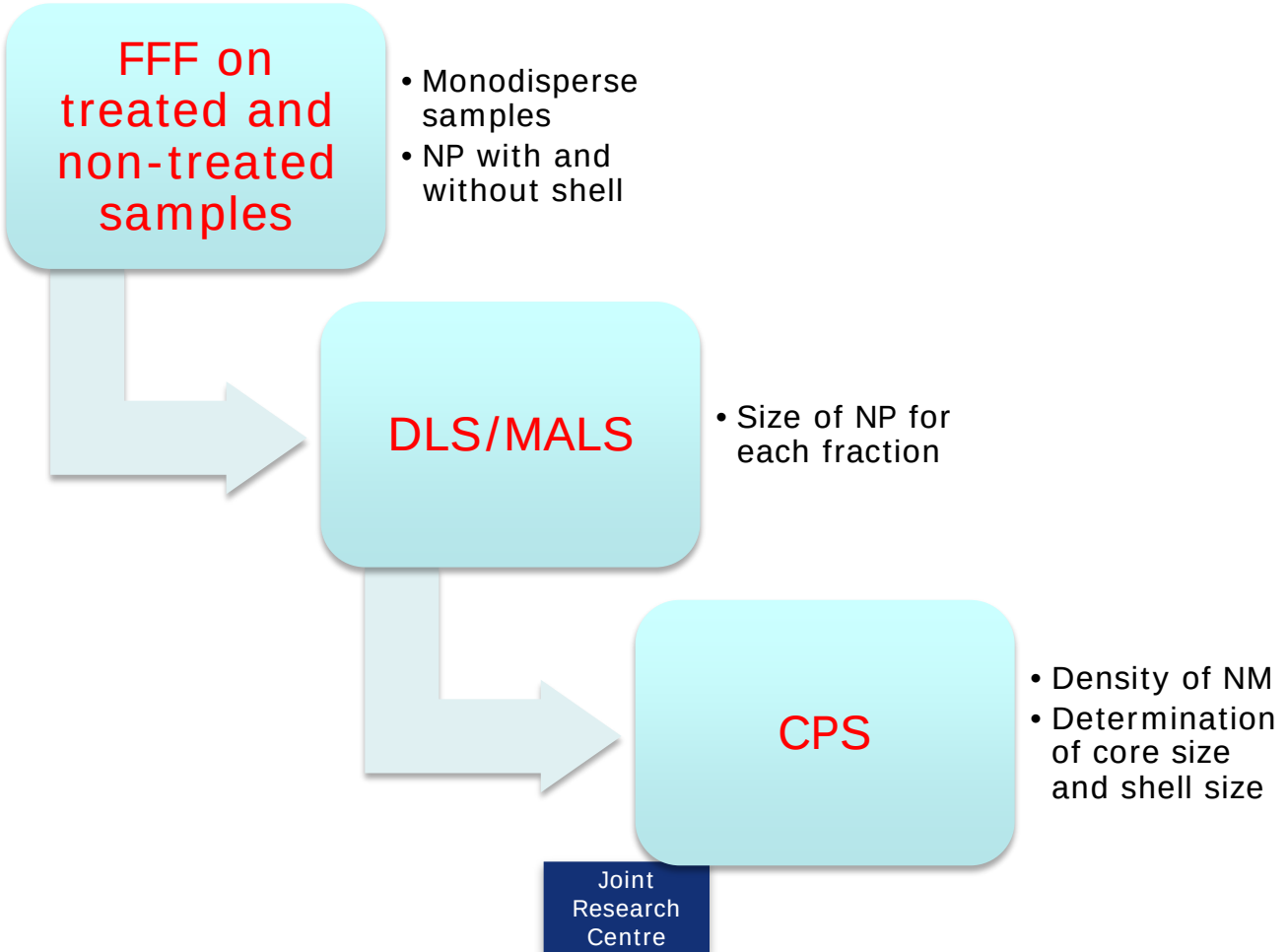
SEM images of badly behaved samples



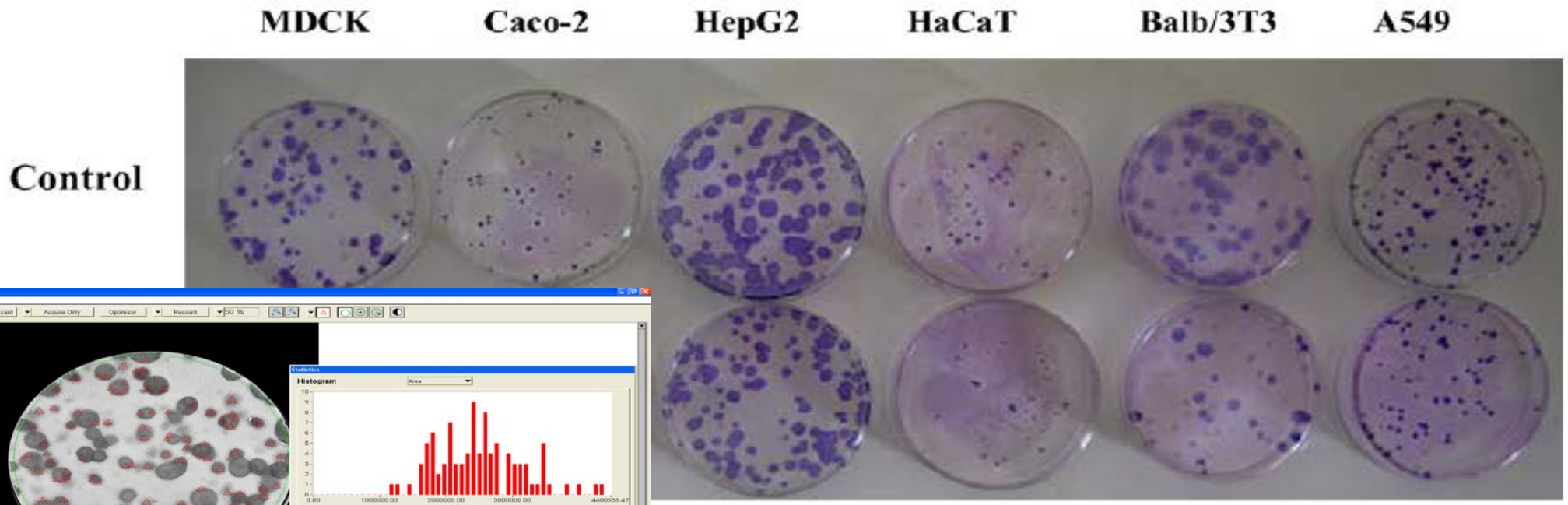


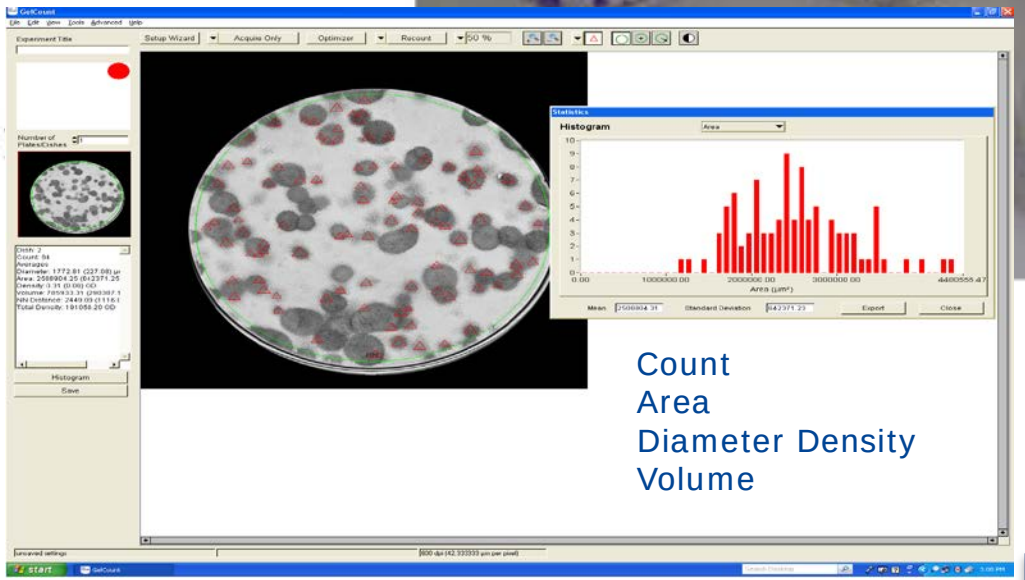
Ag nanoprisms-polymer coated:
PVP polymer must be (almost) completely removed or samples change morphology
Remove too much PVP and irreversible aggregation occurs: c.f. protein coated “soft” particles
Uniform layers difficult to produce (uncharged polymer): Statistical image analysis difficult

Characterisation of core-shell NM

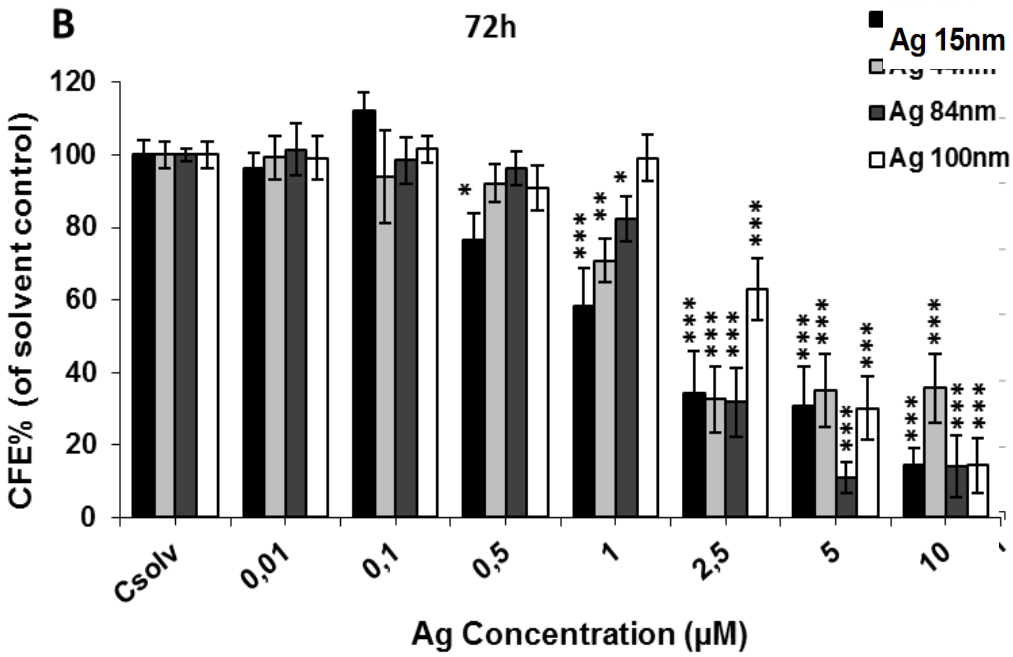


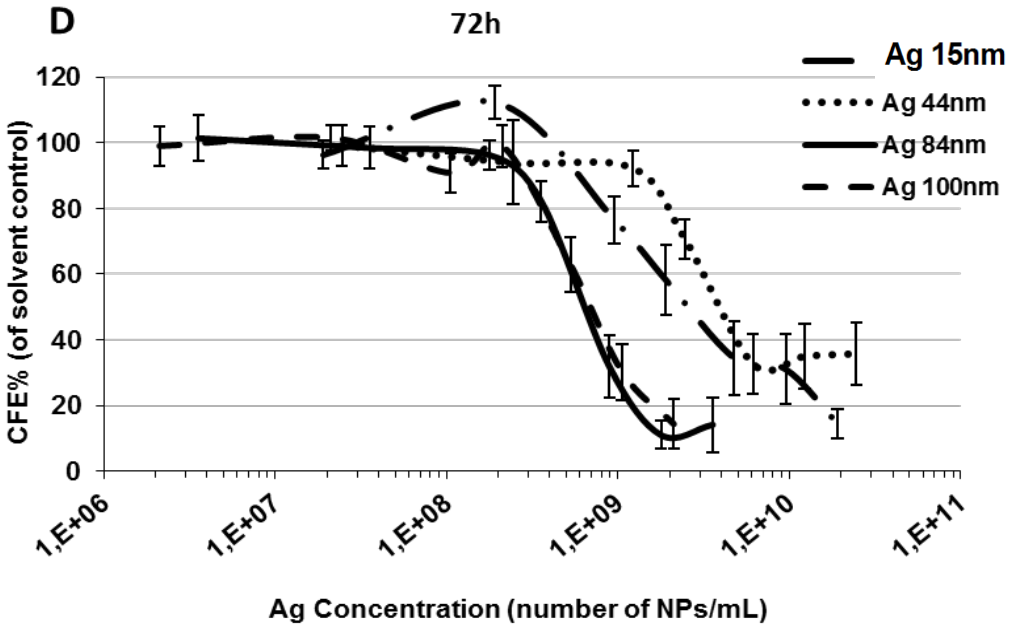
Colony Forming Efficiency Assay on six different cell lines





Count
Area
Diameter Density
Volume





Nanoparticles	IC50 values (µM)		IC50 values (Number of NPs/mL)	
	24 h	72 h	24 h	72 h
Ag 15nm	8.0	1.5	1.52E+10	2.85E+09
Ag 44nm	>10	1.7	>2.45E+10	4.16E+09
Ag 84nm	>10	1.9	>3.56E+09	6.76E+08
Ag 100nm	>10	3.2	>2.11E+09	6.75E+08

Conclusions

- NanoBiotechnologies provide an essential toolbox for design of innovative health applications and understanding E&H impact of nanomaterials
- Bio interfaces are at the core of our studies
- Interlaboratory studies are necessary to understand and optimise to nanotools and test methods
- Huge field of applications in detection/sensors, nanomedicine, nanotoxicology

European Commission

NanoBiotechnology group @ Ispra

Joint Research Centre