



**Trinity College Dublin**  
Coláiste na Tríonóide, Baile Átha Cliath  
The University of Dublin

# Nanomedicine for early diagnosis of cancer

**Yuri Volkov, PhD, MD, MA, FTCD**  
School of Medicine and AMBER Centre

NUOVE FRONTIERE NELLA TERAPIA DELLE MALATTIE ONCOLOGICHE ED ONCOEMATOLOGICHE

Treviso, November 20th-21st, 2015



# THE IRISH BIOMEDICAL LANDSCAPE

Biomedical and Nanotech organisation





# TCD: European Technology Platform in Nanomedicine Annual Event bringing the community together



Attracted 120 Nanomedicine key players for 3 days at the Trinity Translational Medicine Institute. First Translational Projects to go forward for Industrial support and funding.

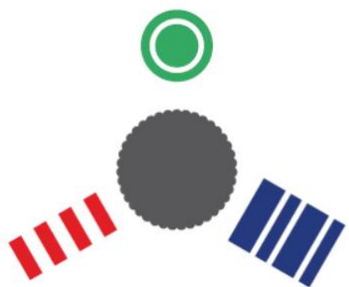
Featured in this month's *Nature Nanotechnology* 2015



# UNRESOLVED PROBLEMS IN CANCER DIAGNOSTICS

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- Sensitivity of detection (Yes/No)
- Test specificity (What is detected)
- Insufficient informative value (Single parameter tests)
- Significant volumes of biomaterial required
- Invasiveness of the methods
- Insufficient speed of achieving results
- Most accurate tests available only in hospital settings
- High costs of reliable diagnosis

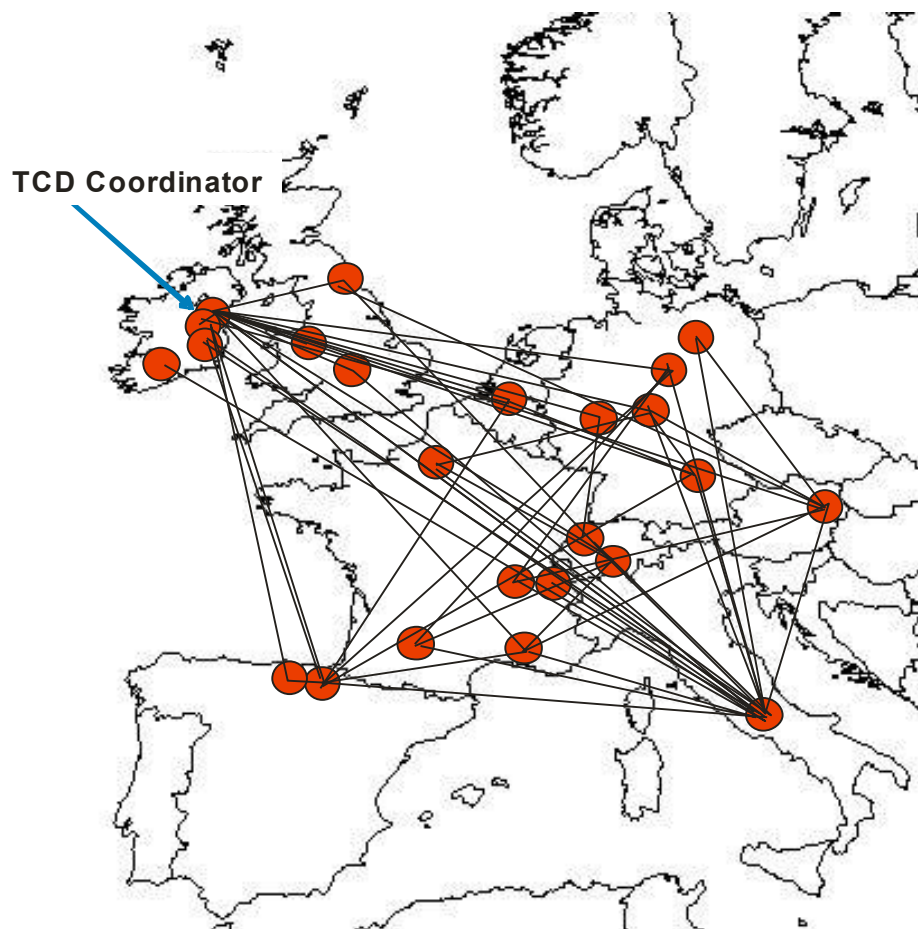


# NAMDIATREAM

NANOTECHNOLOGICAL TOOLKITS  
FOR MULTI-MODAL DISEASE DIAGNOSTICS  
AND TREATMENT MONITORING



## Pan-European Consortium



Trinity College Dublin, The University of Dublin

**22** Partners

**9** EU Countries:

Austria, Belgium, France, Germany,  
Ireland, Italy,  
Spain, Switzerland, UK

**12** Leading Academic and  
Research centres

**8** High-Tech SMEs

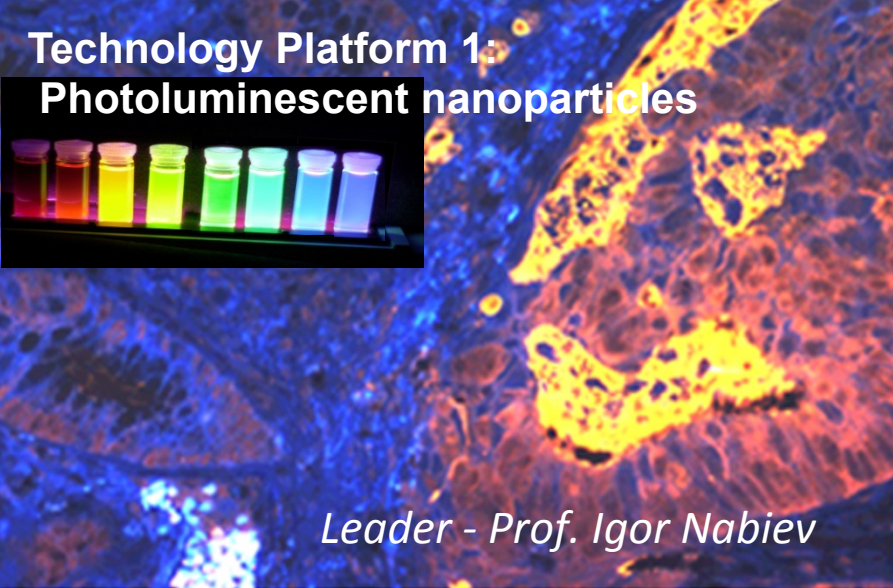
**2** Multi-National Companies

**13** Scientific disciplines



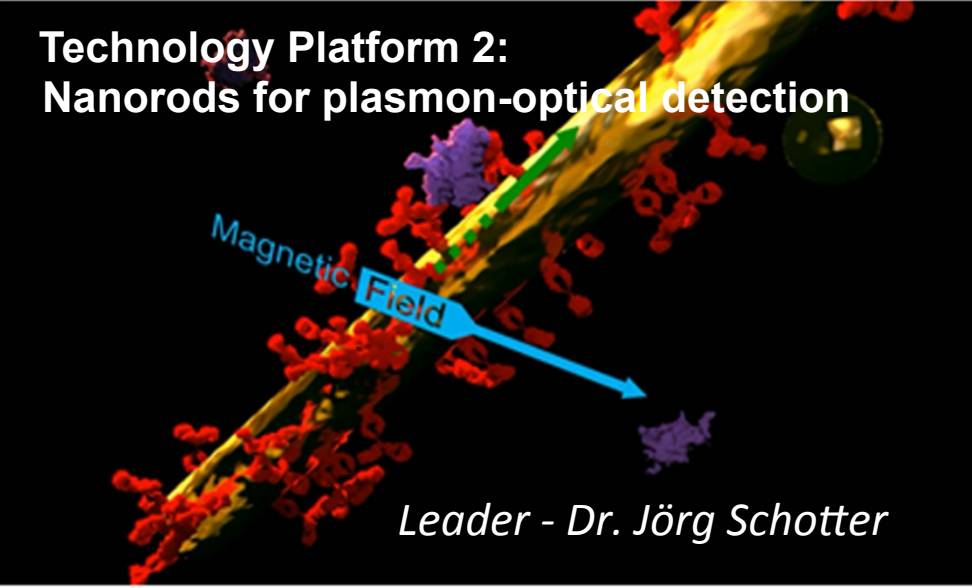
# ***NAMDIATREAM project: MULTI-MODAL DIAGNOSTIC APPROACHES IN ONCOLOGY***

**Technology Platform 1:  
Photoluminescent nanoparticles**



*Leader - Prof. Igor Nabiev*

**Technology Platform 2:  
Nanorods for plasmon-optical detection**



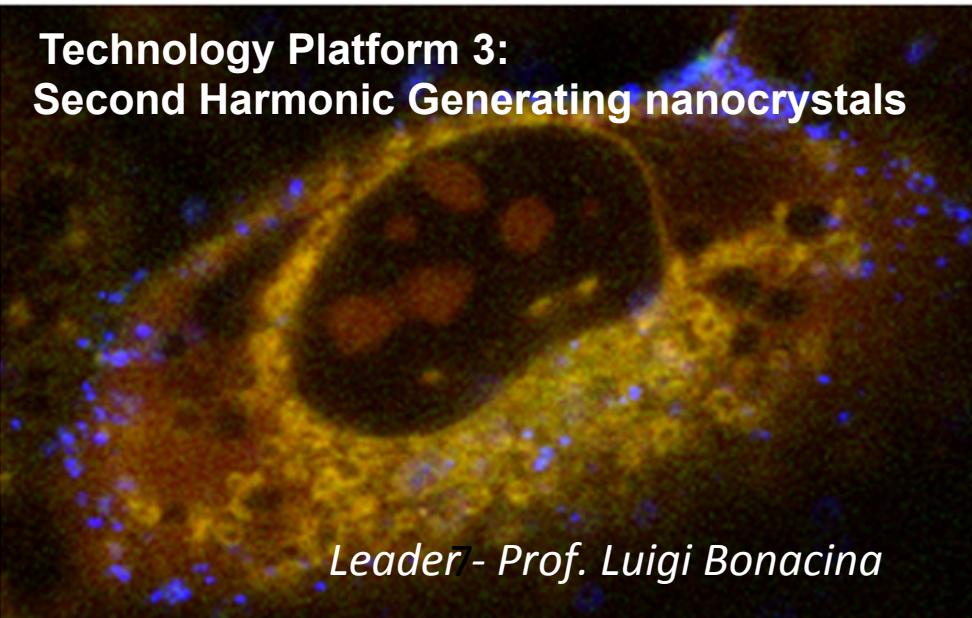
*Leader - Dr. Jörg Schotter*

**Technology Platform 4:  
Magnetically barcoded nanowires**



*Leader - Prof. Yuri Volkov*

**Technology Platform 3:  
Second Harmonic Generating nanocrystals**



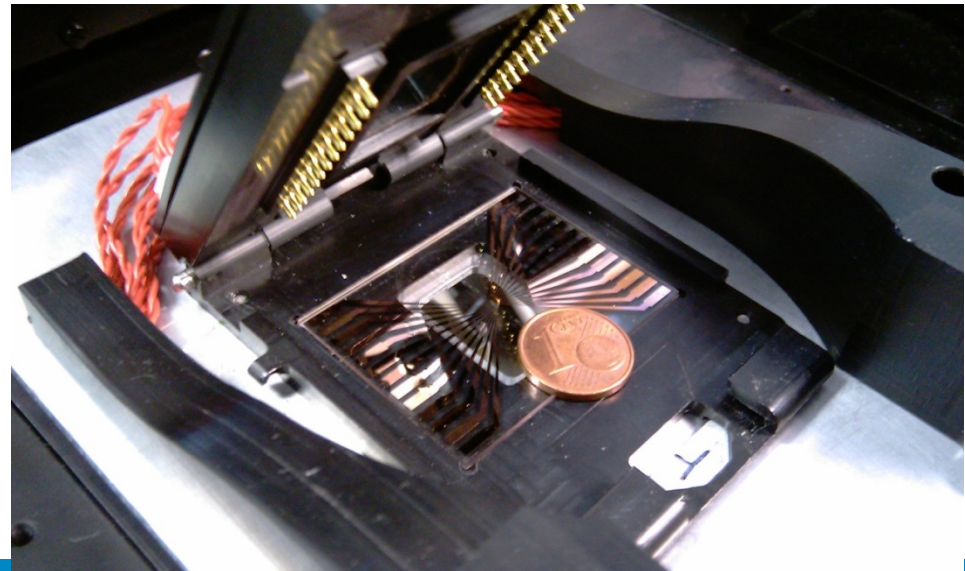
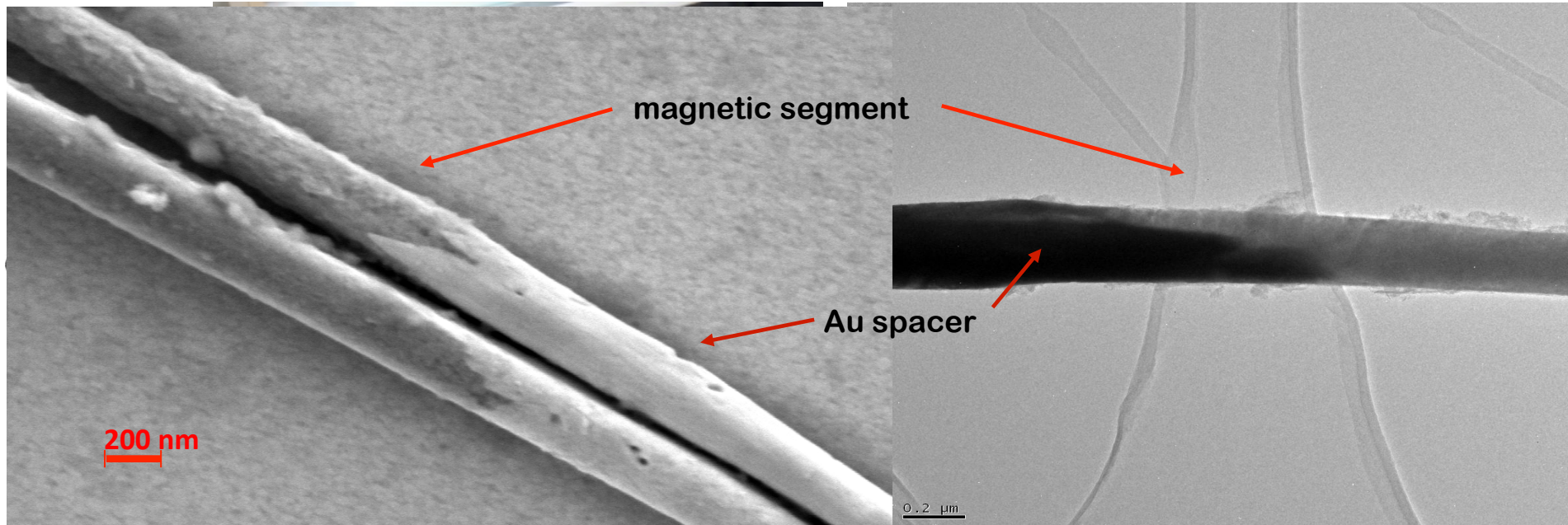
*Leader - Prof. Luigi Bonacina*

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# **NANOTECHNOLOGICAL SYSTEMS FOR MOLECULAR DIAGNOSTICS IN HOMOGENOUS SAMPLES**



# BARCODES ON MAGNETIC NANOWIRES: "THE MOLECULAR SUPERMARKETS"



# CANCER BIOMARKERS DETECTION USING MAGNETICALLY BARCODED NANOWIRES: THE TECHNOLOGICAL CONCEPT

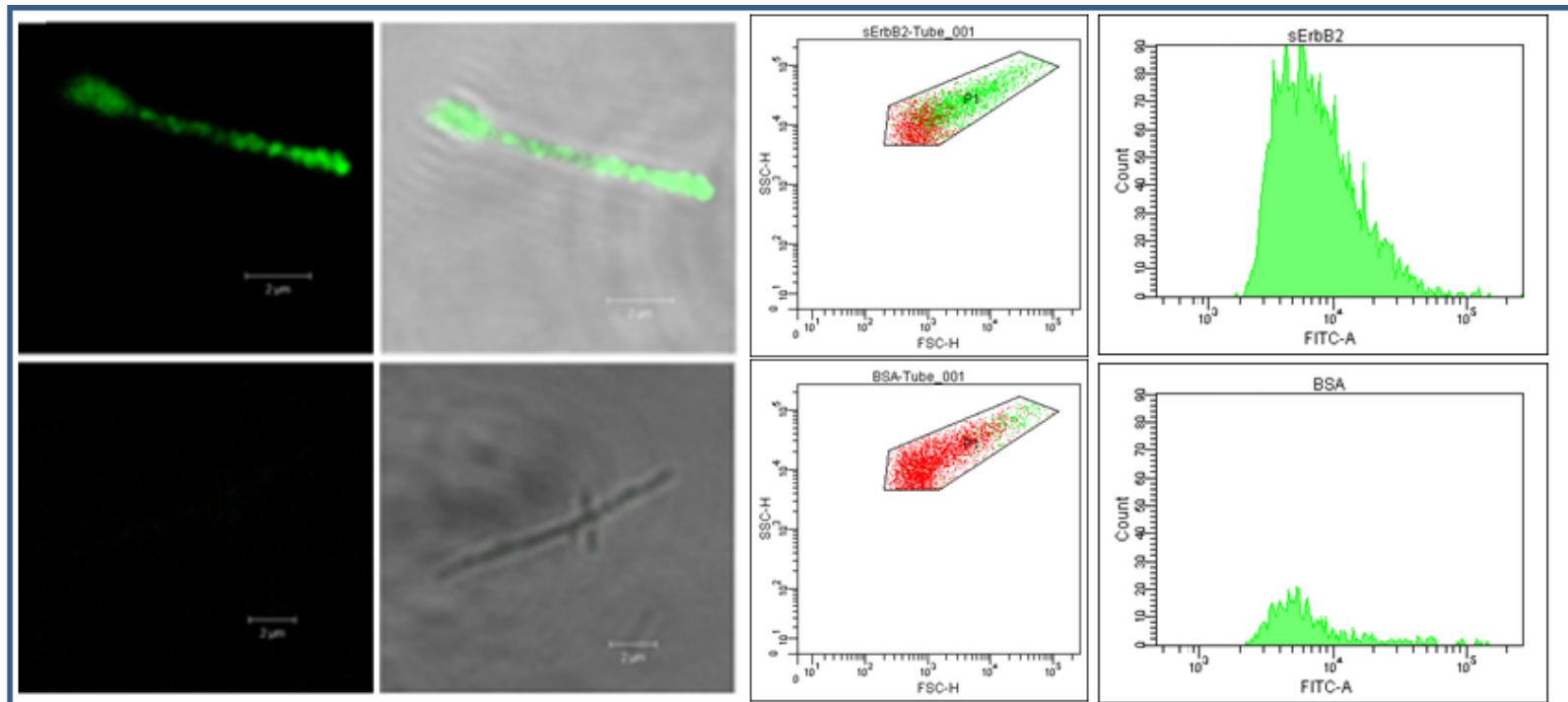
© Trinity College Dublin





I CAN NEVER FIND THE BARCODE ON THESE THINGS.

# DETECTION OF CANCER BIOMARKER sErbB2 USING MAGNETICALLY BARCODED NANOWIRES

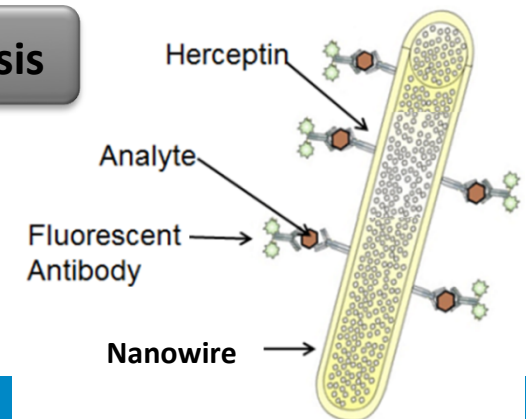


sErbB2  
Detected

BSA

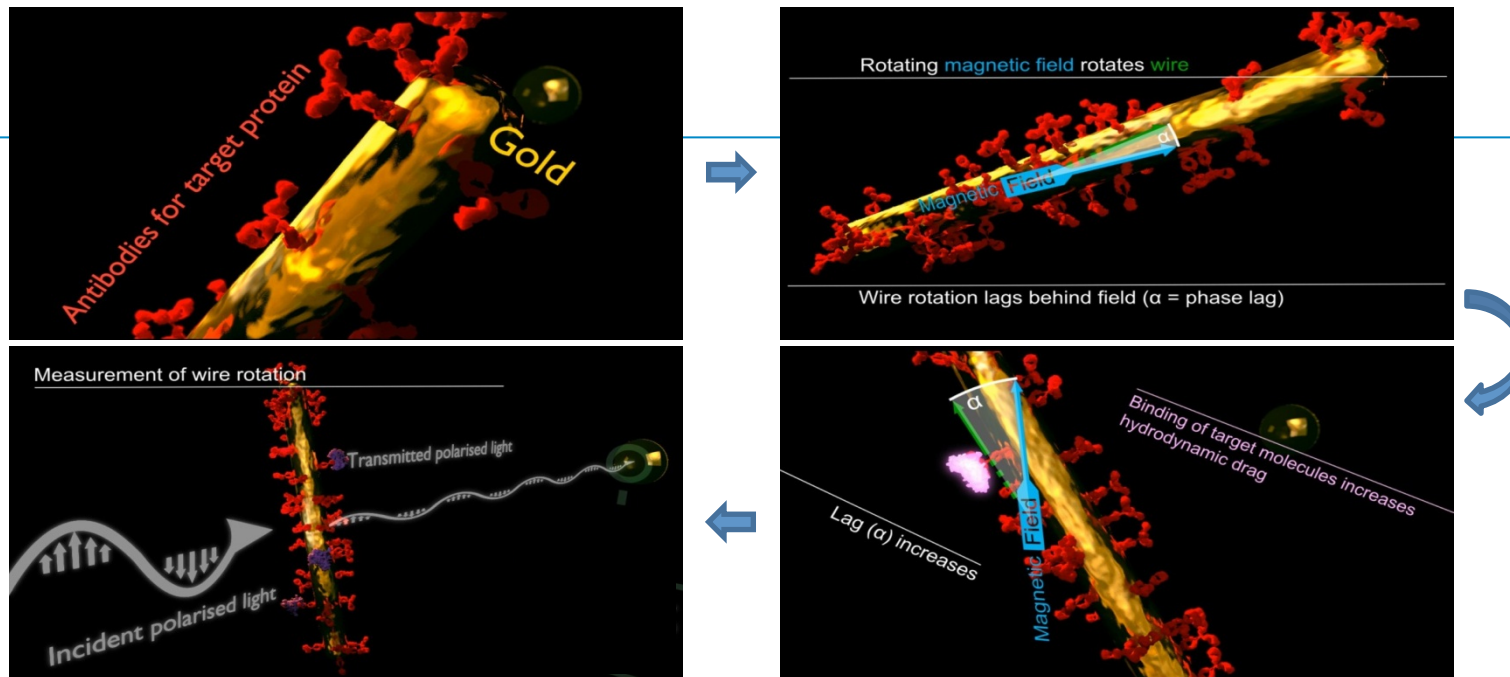
Qualitative Assessment

Quantitative Analysis





# MAGNETO-OPTICAL DETECTION OF SOLUBLE MOLECULAR MARKERS



- Homogeneous immunodiagnostics by optical detection of nanoparticle relaxation
- Based on antibody-functionalized magnetic nanorods orientated by external magnetic fields
- Binding of the biomarker to the magnetic carrier changes the nanowire orientation, enabling target detection

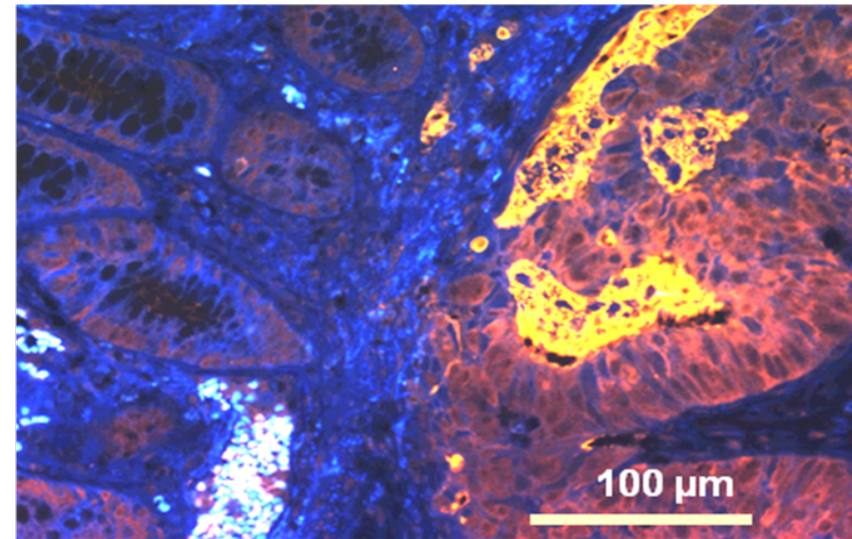
Dr. Joerg Schotter (AIT, Vienna)

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# **NANOTECHNOLOGICAL SYSTEMS FOR CELLULAR AND TISSUE DIAGNOSTICS**

# UNIQUE PROPERTIES OF QUANTUM DOTS APPLIED FOR THE DETECTION OF CANCER CELLS

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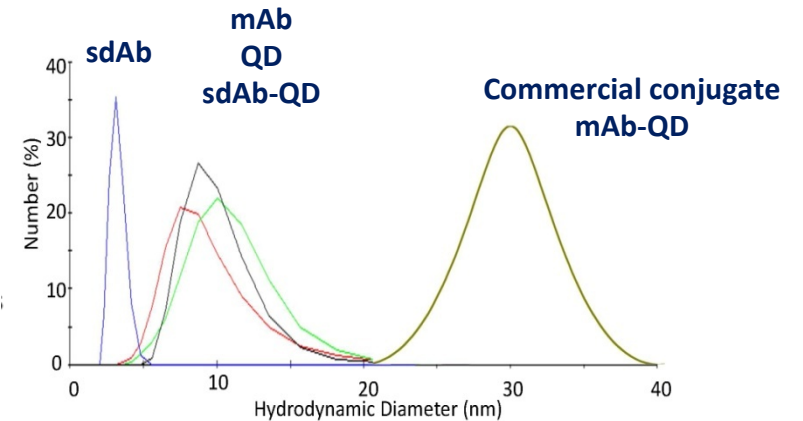
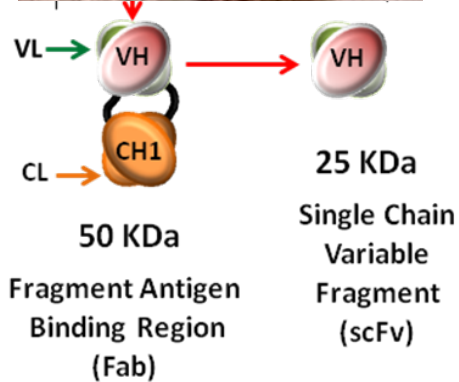
- Optical detection of rare disseminated and circulating malignant cells and cancer markers
- Based on ultras-small photoluminescent QDs tagged to highly specific single domain antibodies

**Prof. Igor Nabiev (TCD/URCA)**



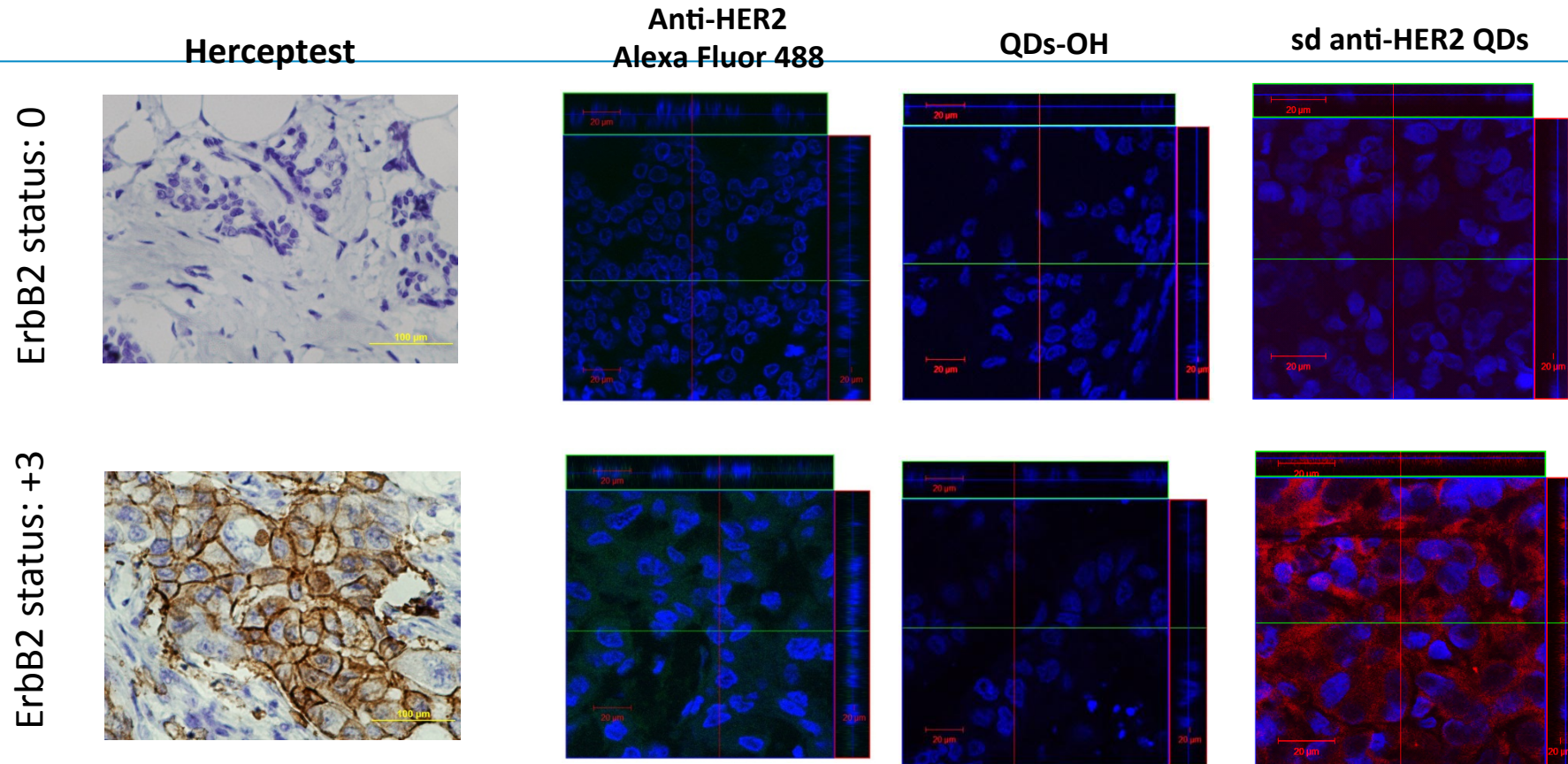
# SINGLE DOMAIN ANTIBODIES: MATCHING THE ADVANTAGES OF ULTRASMALL LUMINESCENT NANOPARTICLES

Immunoglobulin G (IgG)  
150 KDa



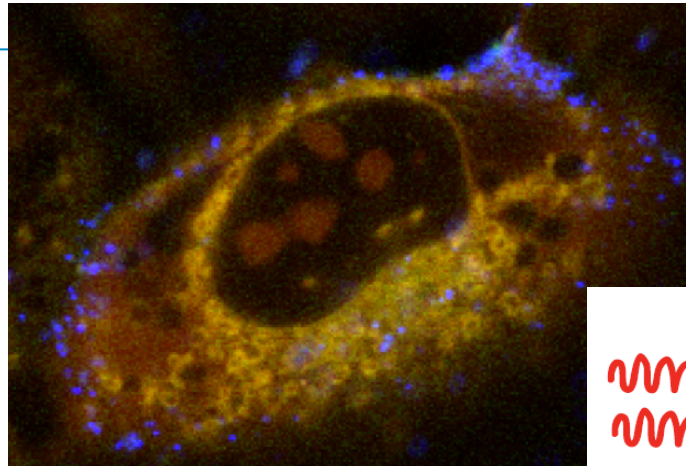
| Sample              | Hydrodynamic diameter (nm) |
|---------------------|----------------------------|
| QDs                 | 8.84 ± 2.9                 |
| sdAb                | 3.2 ± 0.52                 |
| Conjugates sdAbs-QD | 11.9 ± 2.9                 |
| IgG                 | 11.4 ± 2.5                 |
| Conjugates IgG-QD   | 30.3 ± 3.2                 |

# QUANTUM DOTS CONJUGATED WITH SINGLE DOMAIN ANTIBODIES FOR CANCER DIAGNOSTICS



ErbB2 detection with SD/anti-HER2 QDs in tissue biopsies from breast cancer patients

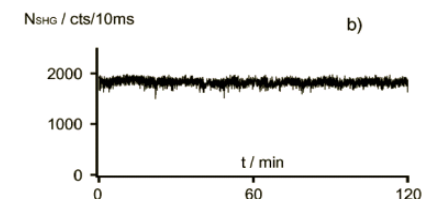
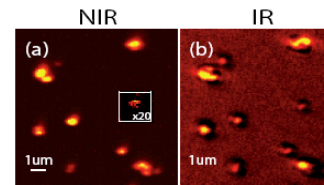
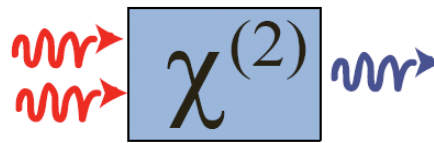
# SECOND HARMONIC GENERATION NANOCRYSTALS (SHG) FOR TISSUE DIAGNOSTICS



SOME EXAMPLES OF NON-CENTROSYMMETRIC CRYSTALS

FOR SHG APPLICATIONS:

$\text{BaTiO}_3$ ,  $\text{KNbO}_3$ ,  $\text{LiNbO}_3$ ,  $\text{KTiOPO}_4$  (KTP),  $\text{ZnO}$

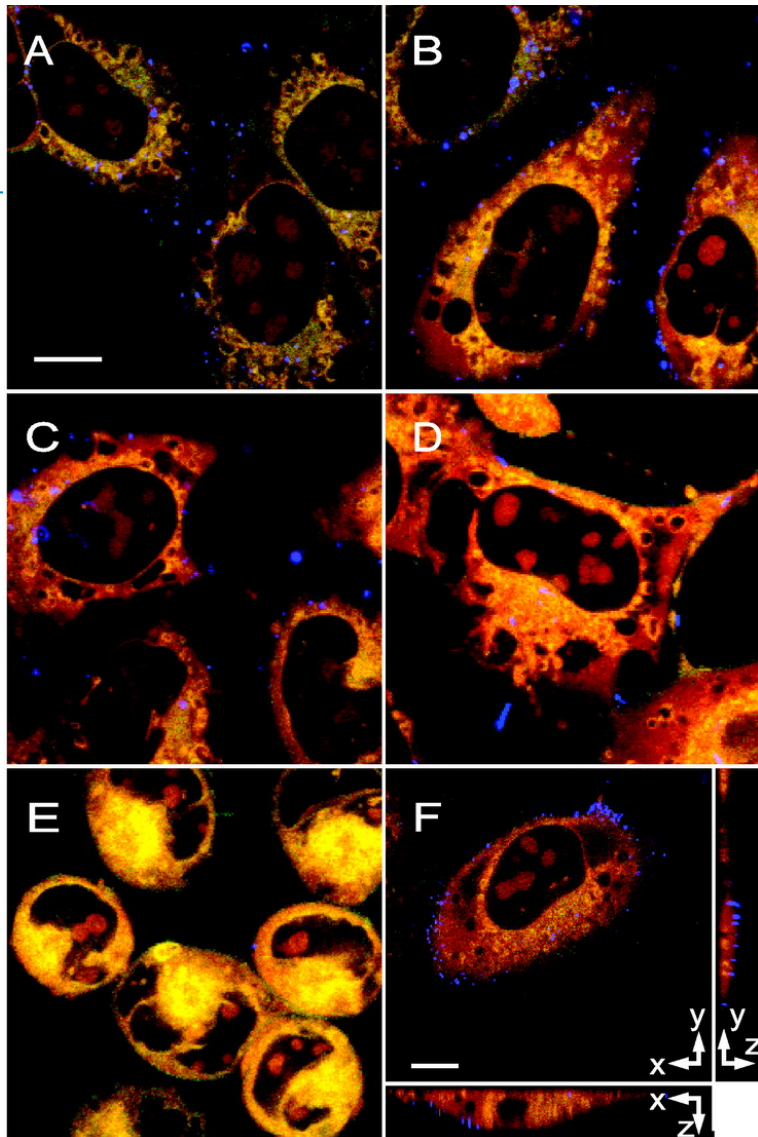


- Focused on diagnostic imaging based on nanoparticles with Second Harmonic Generation (SHG) properties
- SHG defines the capability of a material to **double the frequency** of incoming light
- Utilises nanocrystals which exhibit a strong nonlinear optical response
- Exploits non-resonant nature of SHG, making it resistant to bleaching and blinking

Prof. L. Bonacina and J.P. Wolf, University of Geneva



# APPLICATION OF *SHG* MATERIALS FOR TISSUE IMAGING AND DIAGNOSTICS



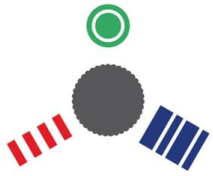
- **Inherently Nonlinear**
  - high spatial resolution
- **No Bleaching nor Blinking**
  - long-term tracking
- **Excitation Wavelength Tunability**
  - deeper penetration
  - reduced photo-damage
  - no background

BEAS-2B bronchial epithelial cells exposed for 5 h to (A) BaTiO<sub>3</sub>, (B) KNbO<sub>3</sub>, (C) LiNbO<sub>3</sub>, (D) KTP, (E) ZnO, and (F) z-KNbO<sub>3</sub> SHG-NPs

*Ronzoni et al., JOVE 2014*

# $\text{BiFeO}_3$ SYNTHESIS BY COMBUSTION

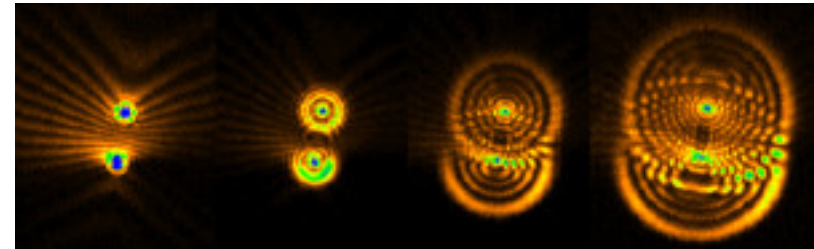
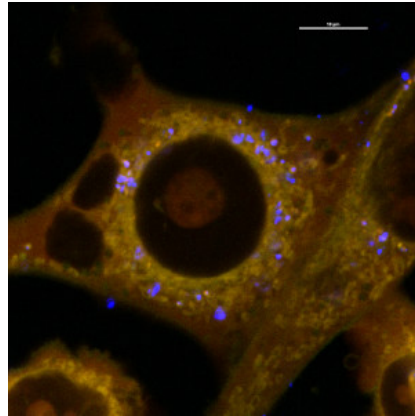
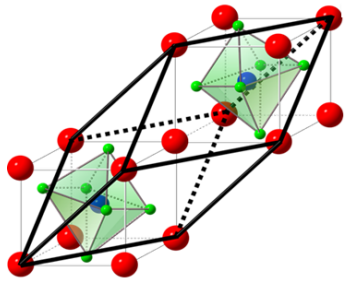




# NAMDIATREAM

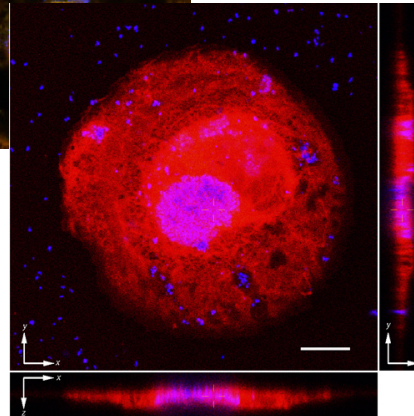
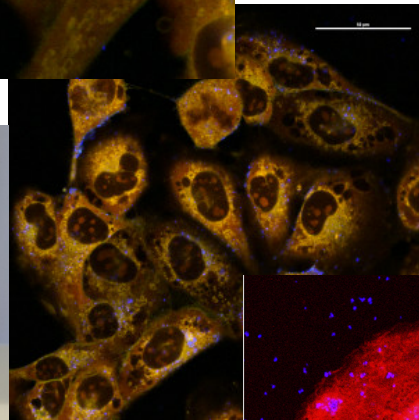
NANOTECHNOLOGICAL TOOLKITS  
FOR MULTI-MODAL DISEASE DIAGNOSTICS  
AND TREATMENT MONITORING

## Nanoprobes: **Bismuth ferrite (BFO)** nanocrystals and their fingerprint (interference pattern)



SHG NPs performance in diagnostics of cancer:  
sensitivity, specificity, imaging depth,  
non-toxic and safe to handle

### BFO as Commercial Product



Bismuth ferrite ( $\text{BiFeO}_3$ )



Barium titanate ( $\text{BaTiO}_3$ )



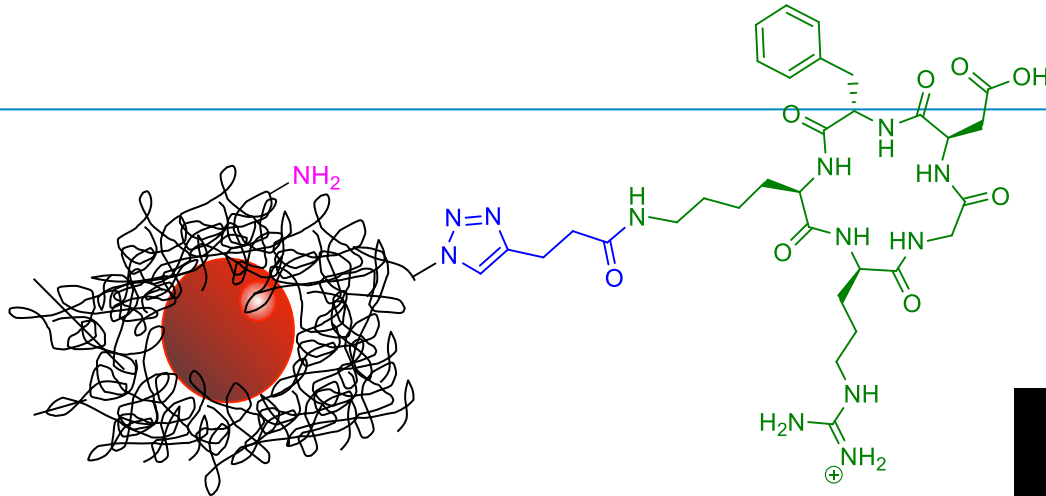
Potassium niobate  
( $\text{KNbO}_3$ )

Commercialised by



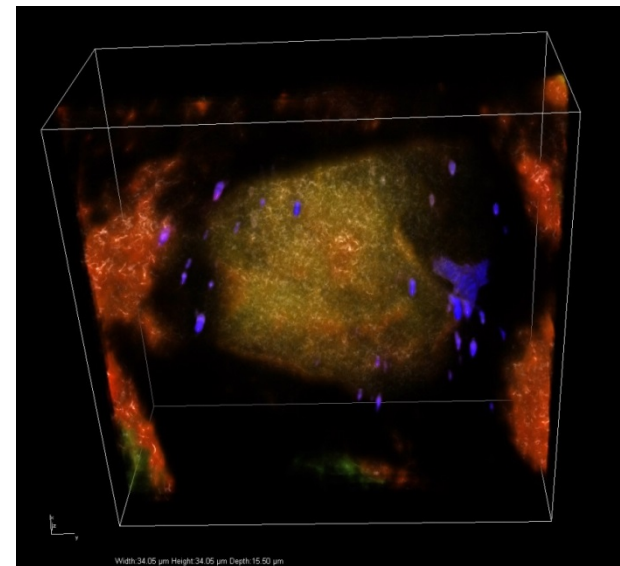


# FUNCTIONALISED SHG NANOPARTICLES FOR CANCER CELL LABELLING AND IMAGING

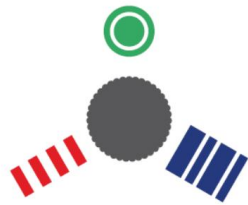


## BFO-cRGDfK

Human-derived prostate cancer cells (PC3) exposed to **BFO-cRGDfK** NPs and visualized using multiphoton imaging. Excitation at 790 nm. Blue : Second harmonic signal from NPs



# EURONANOFORUM 2015 – FUTURE FLASH! BEST EU-FUNDED PROJECT COMPETITION



**NAMDIATREAM**

NANOTECHNOLOGICAL TOOLKITS  
FOR MULTI-MODAL DISEASE DIAGNOSTICS  
AND TREATMENT MONITORING

NAMDIATREAM awarded the best NMP European project prize out of more than 1000 EU-funded projects at the EuroNanoForum (Riga, Latvia, June 2015)



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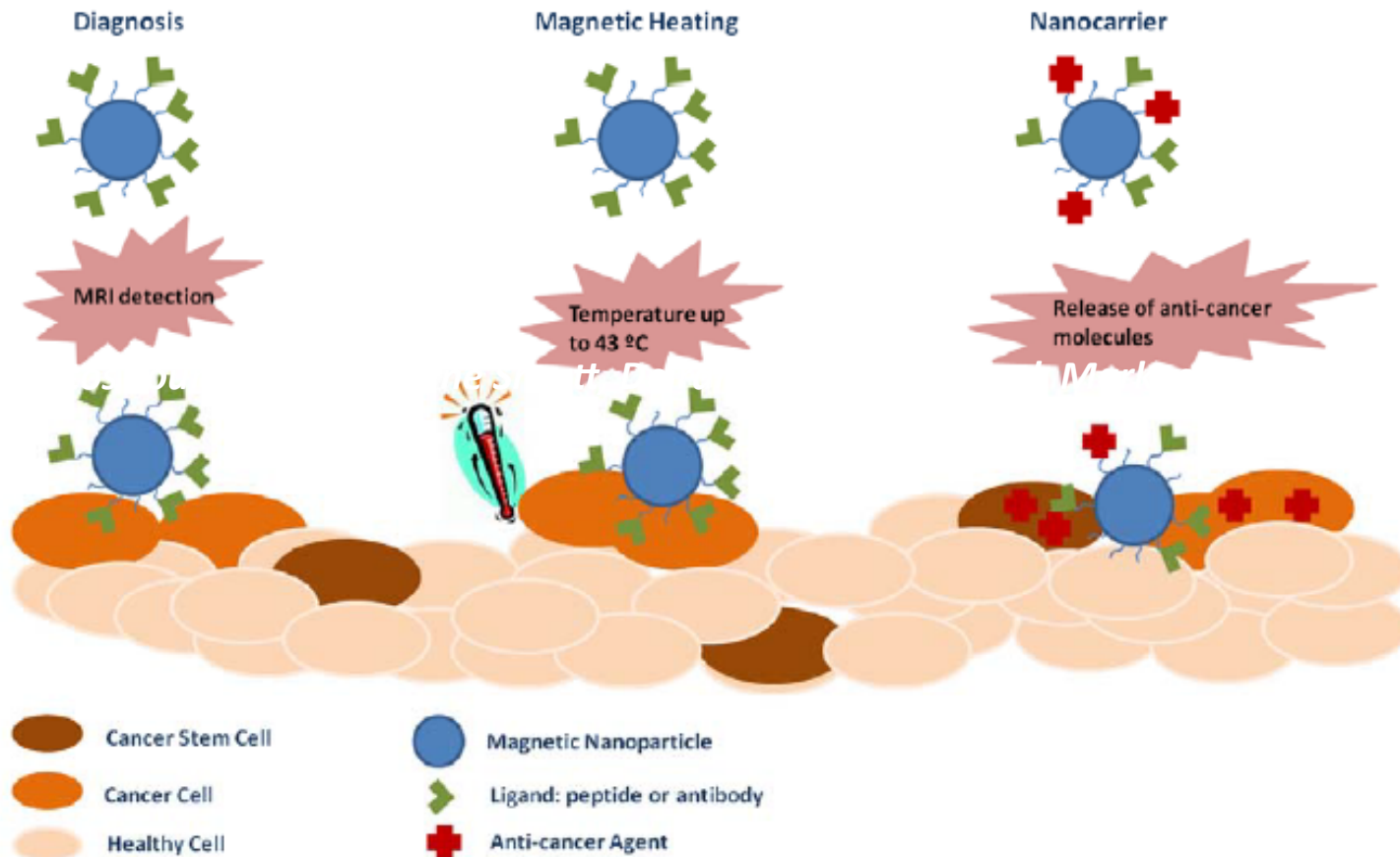
# **MULTIFUNCTIONAL SYSTEMS FOR CANCER DIAGNOSTICS AND TREATMENT (THERANOSTICS)**



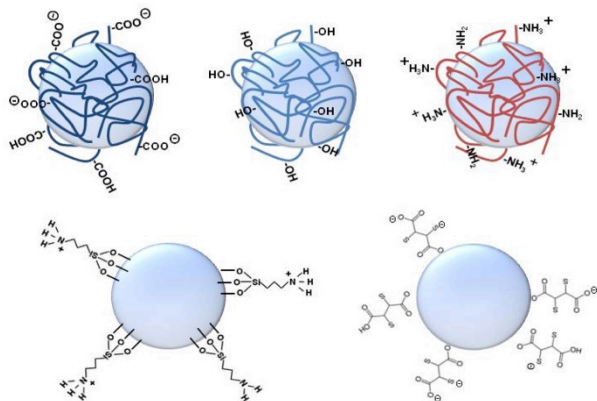
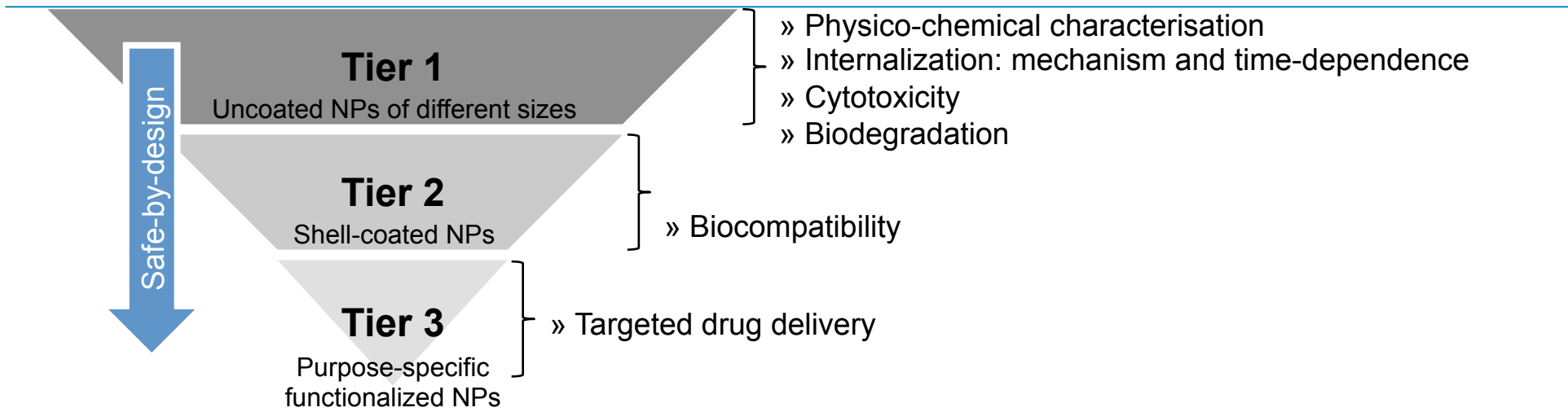
# MULTIFUN: MULTIFUNCTIONAL NANOTECHNOLOGY FOR SELECTIVE DETECTION AND TREATMENT OF CANCER

## THERAGNOSIS: MRI detection + Multimodal Therapeutic Approach

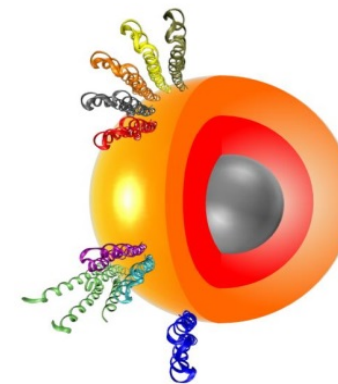
### Multimodal Therapeutic Approach



# THERANOSTIC TOOLS FOR CANCER TREATMENT: SAFE BY DESIGN APPROACH TO THE ASSESSMENT OF ENGINEERED NANOMATERIALS



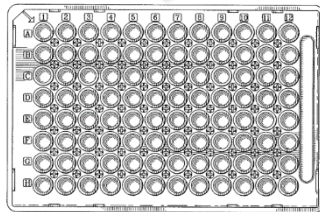
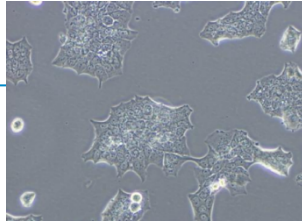
What is the best functional SPION for theranostic application?



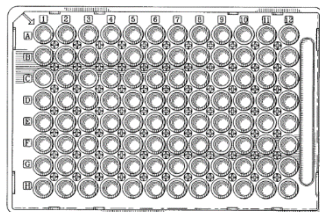
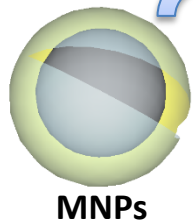
# Multiparametric in vitro characterisation of MNPs by High Content Screening assays: Experimental Workflow

GE Healthcare InCell 1000 Analyser

Cancer cell lines



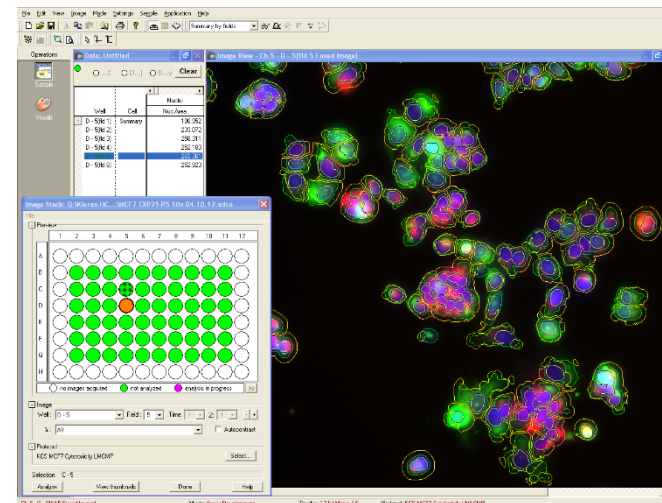
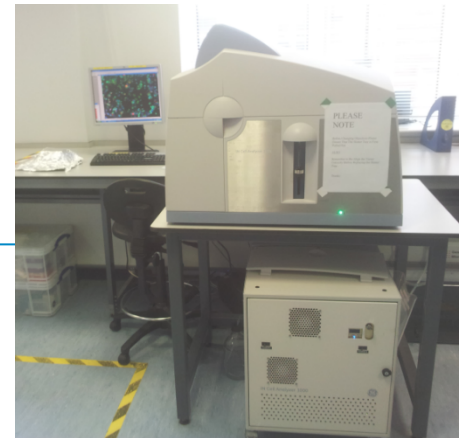
24 h



24 h – 72 h

Cells Fixed and Stained

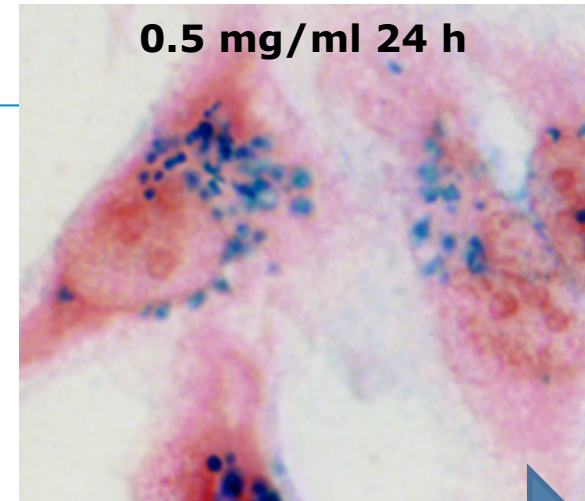
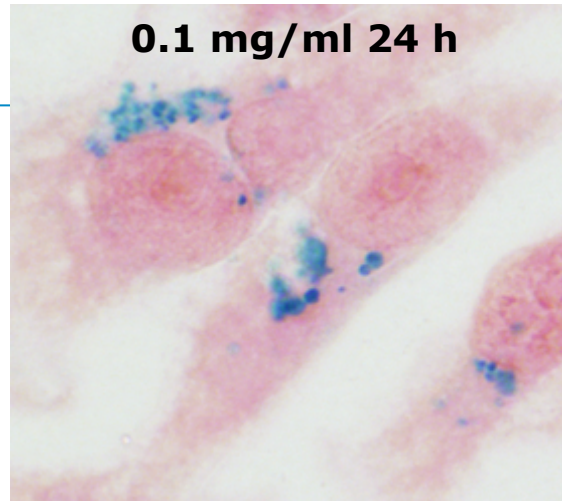
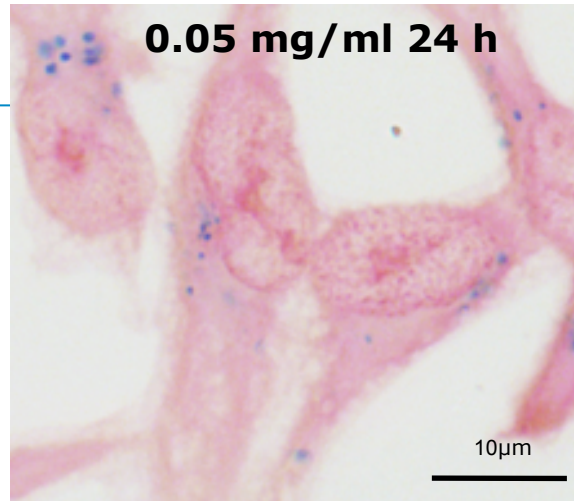
HCSA



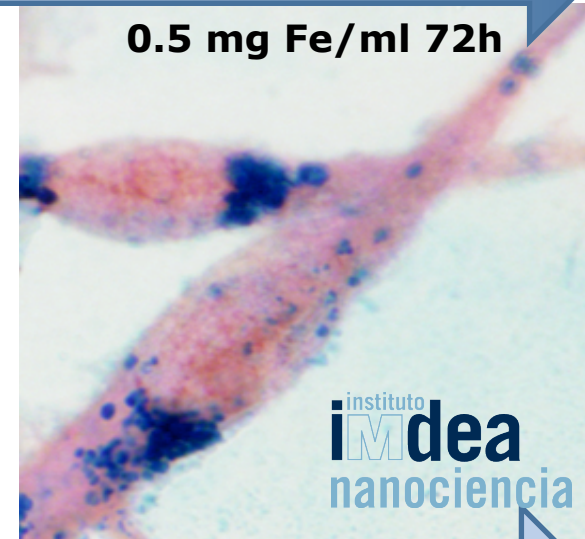
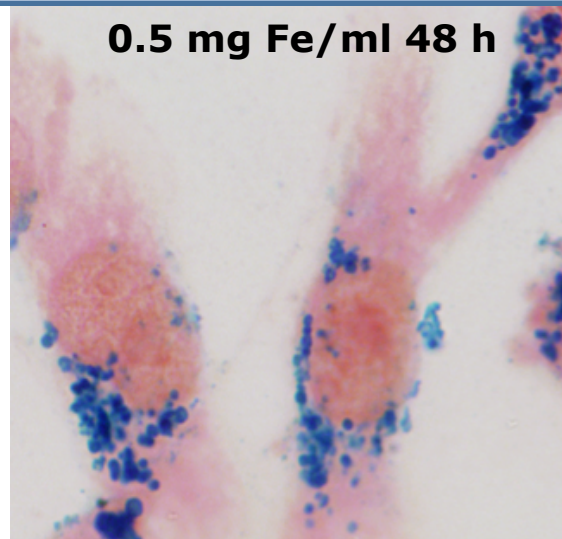
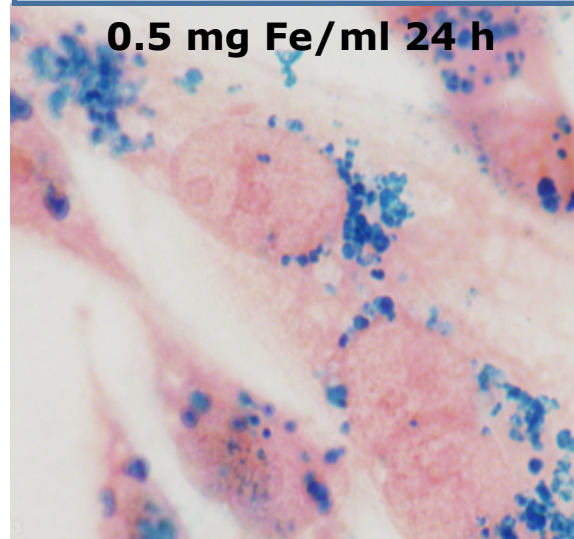
Data Mining and Heatmap Generation



# UPTAKE AND ACCUMULATION DYNAMICS OF MAGNETIC NANOPARTICLES IN MDA-MB-231 CANCER CELL LINE



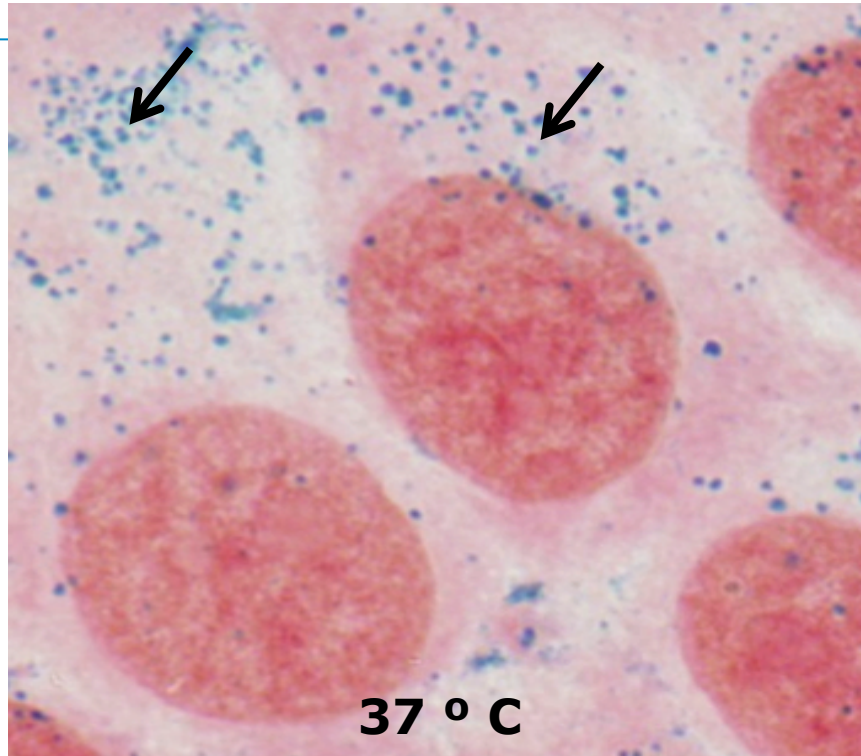
**MNPs (OD15) accumulation is concentration-dependent**



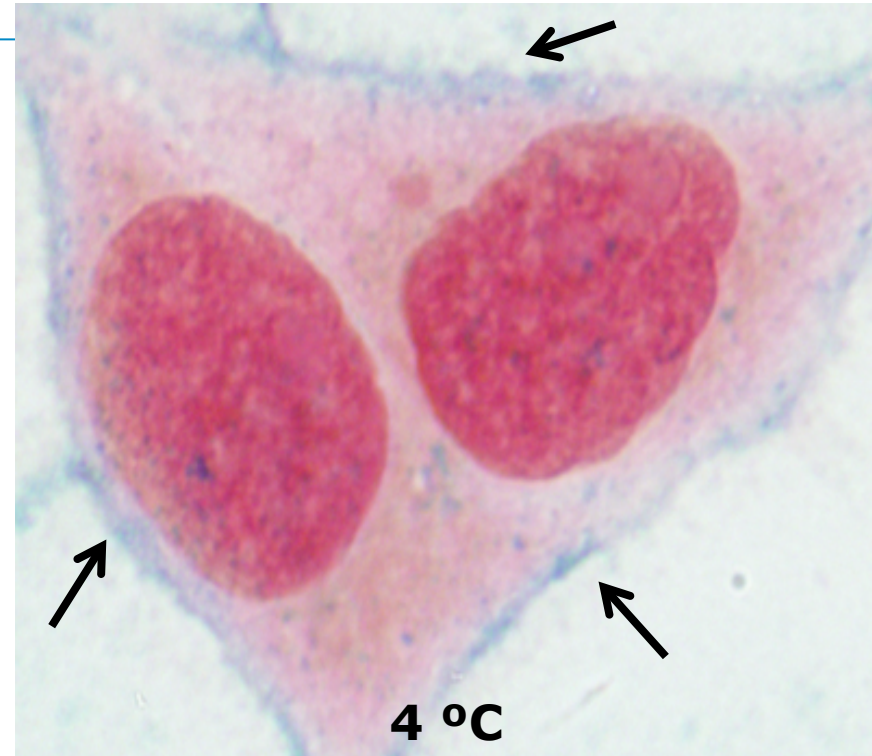
**The kinetics of accumulation indicated a saturation from 24 h**



# ACTIVE INTERNALISATION OF MULTIFUNCTIONAL MAGNETIC NANOPARTICLES IN BT-474 CELL LINE



**MF66R MNPs inside cells**



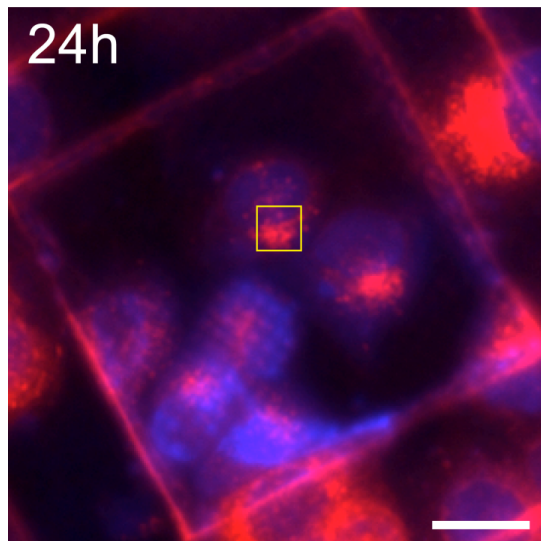
**MF66R MNPs at the cell membrane**

**MNPs enter into BT-474 cells by energy-dependent endocytosis**

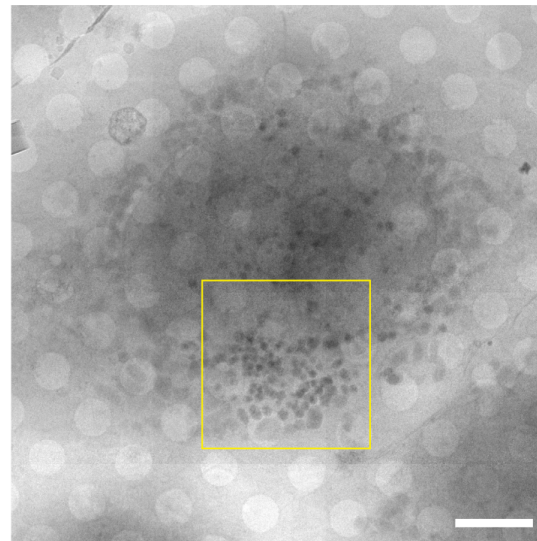
# ACCUMULATION OF MAGNETIC NANOPARTICLES IN MCF-7 CANCER CELL LINE

## Cryo-Soft X-ray Tomography (SXT)

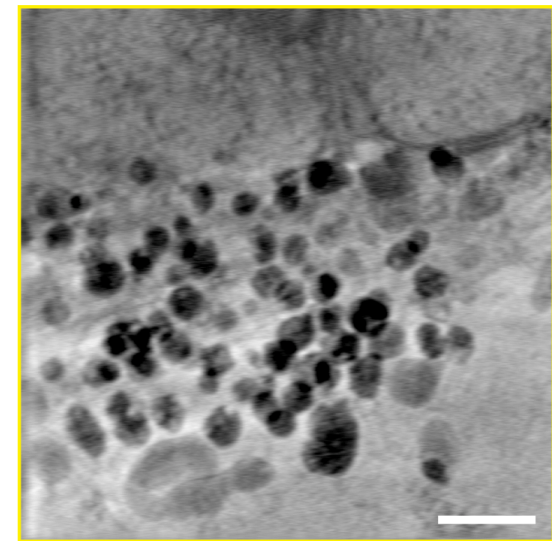
Cryo-optical fluorescence



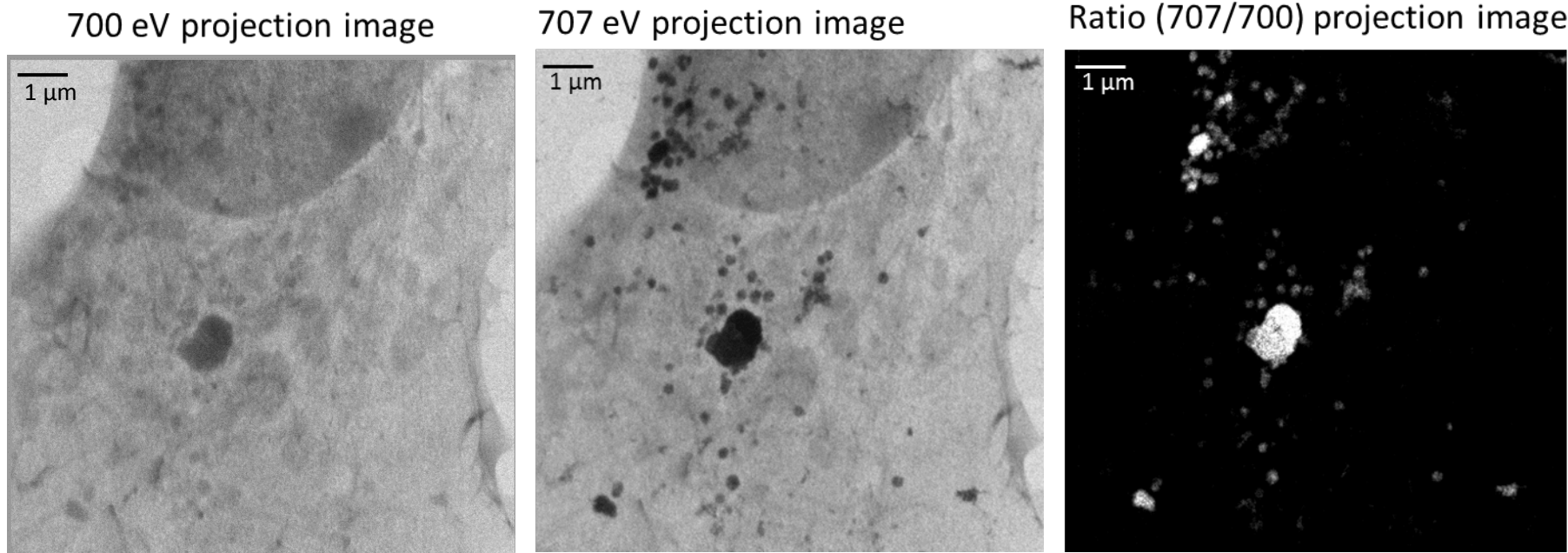
SXT-mosaic



SXT-slice



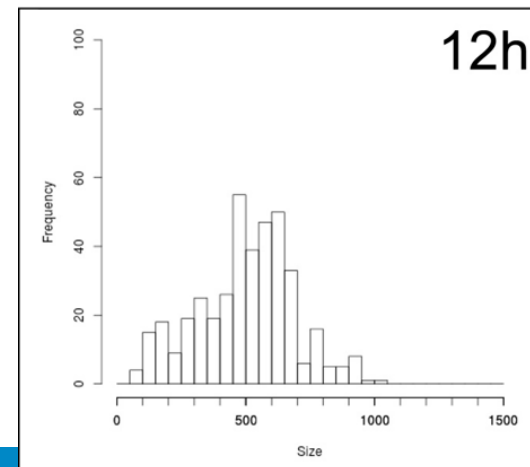
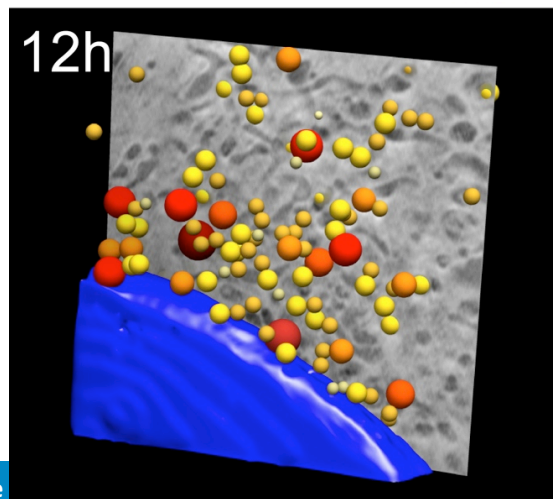
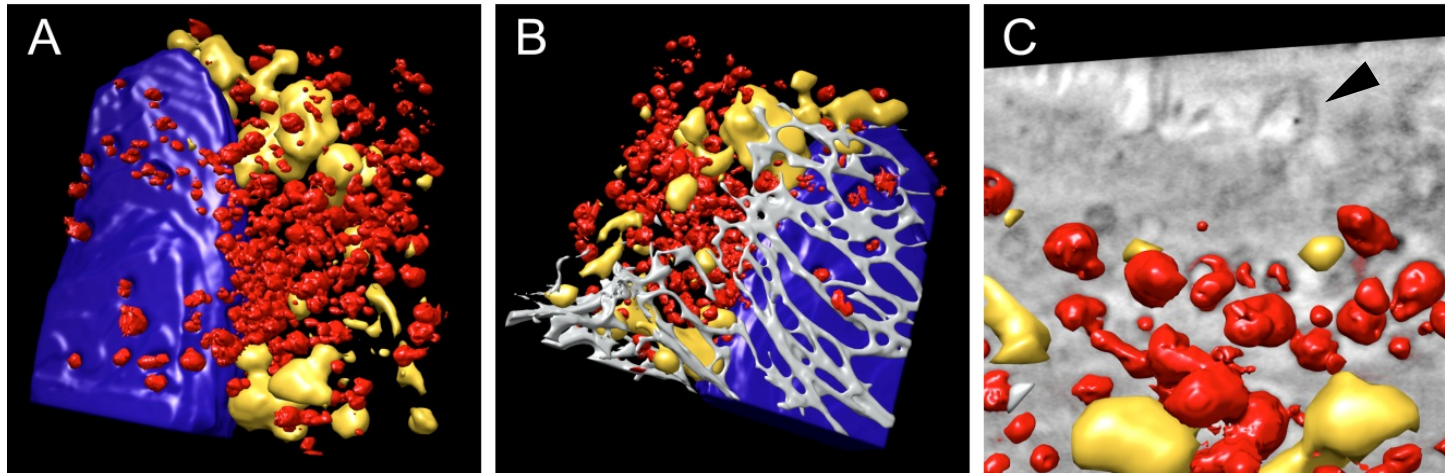
## IRON DETECTION AND 3-D CHARACTERIZATION BY X-RAY MICROSCOPY AND TOMOGRAPHIC RECONSTRUCTION (SXT)



### Spectroscopic imaging of Iron inside cells incubated with MNPs OD15

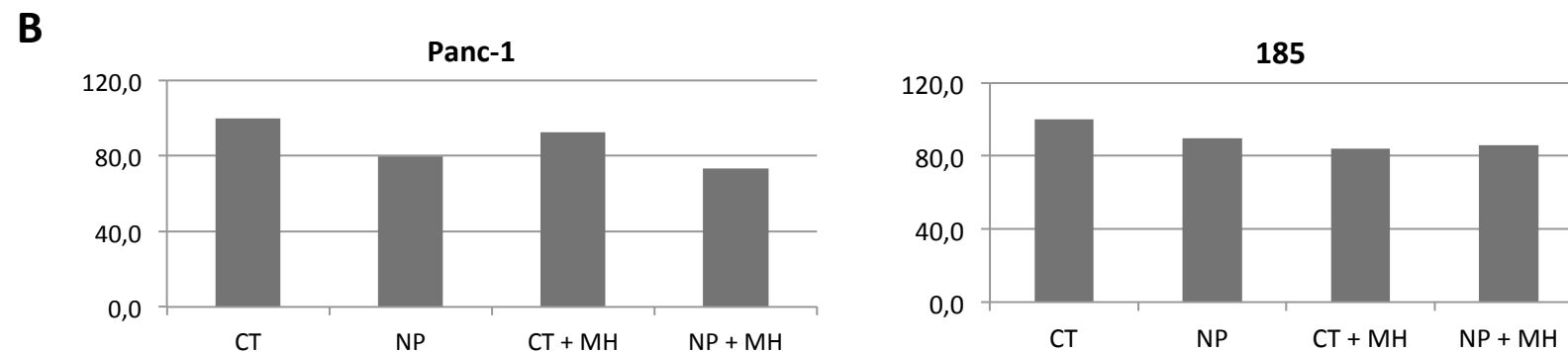
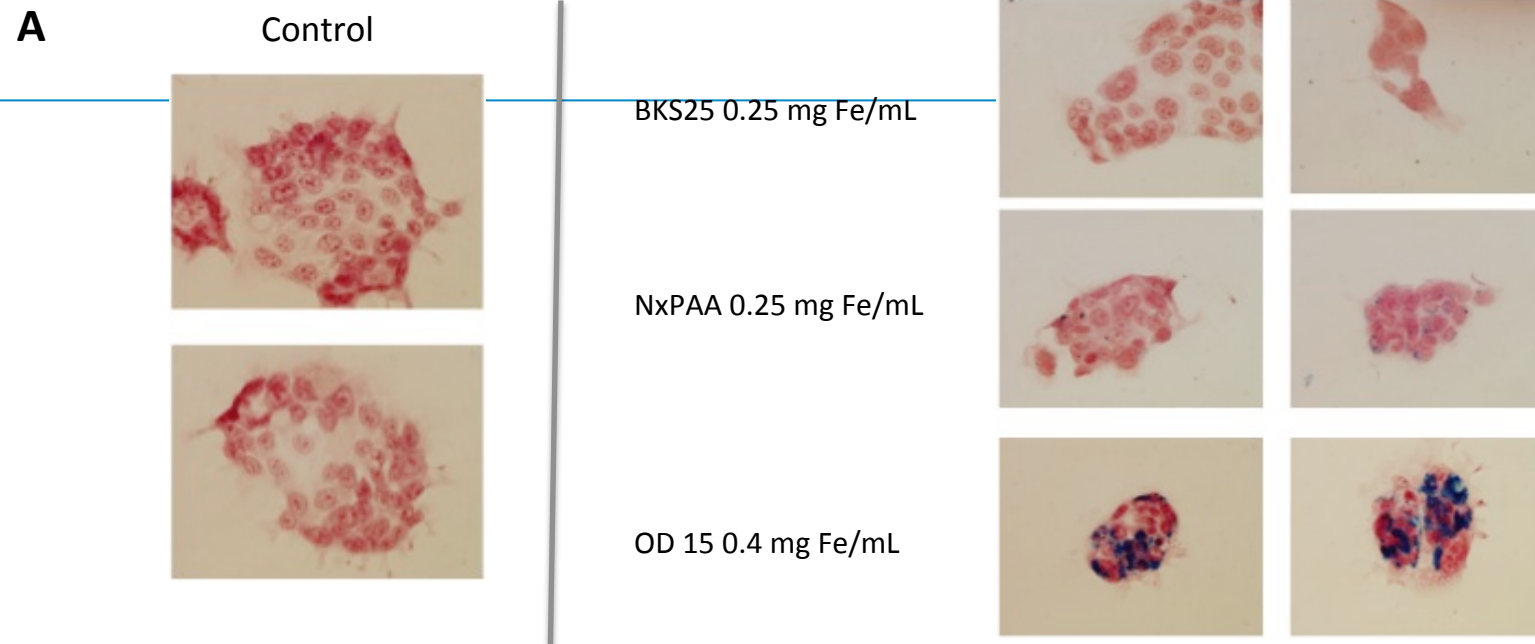


# IRON DETECTION AND 3-D CHARACTERIZATION BY X-RAY MICROSCOPY AND TOMOGRAPHIC RECONSTRUCTION (SXT)





# MNPS UPTAKE BY PRIMARY PANCREATIC CANCER CELLS



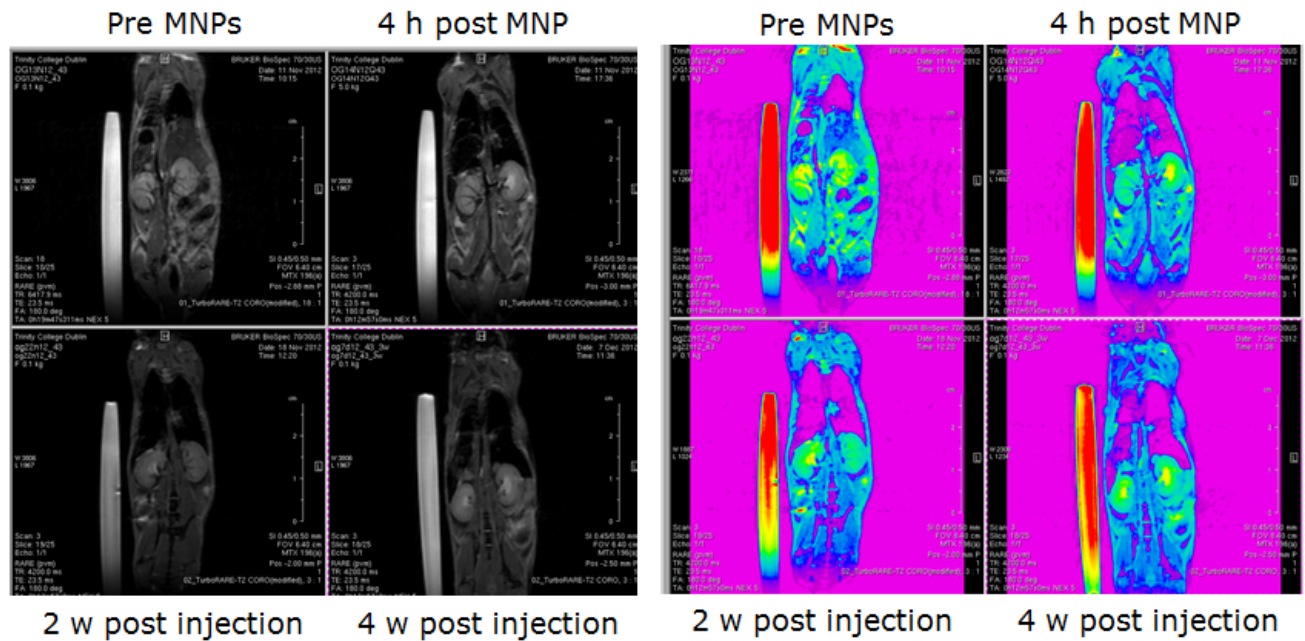
(A) Internalization of bare MNP in primary pancreatic cancer cells (Panc185), after a 24h incubation period. (B) Cell viability measured 24h after cells exposure to a magnetic field of 120 kHz y 40 mT.



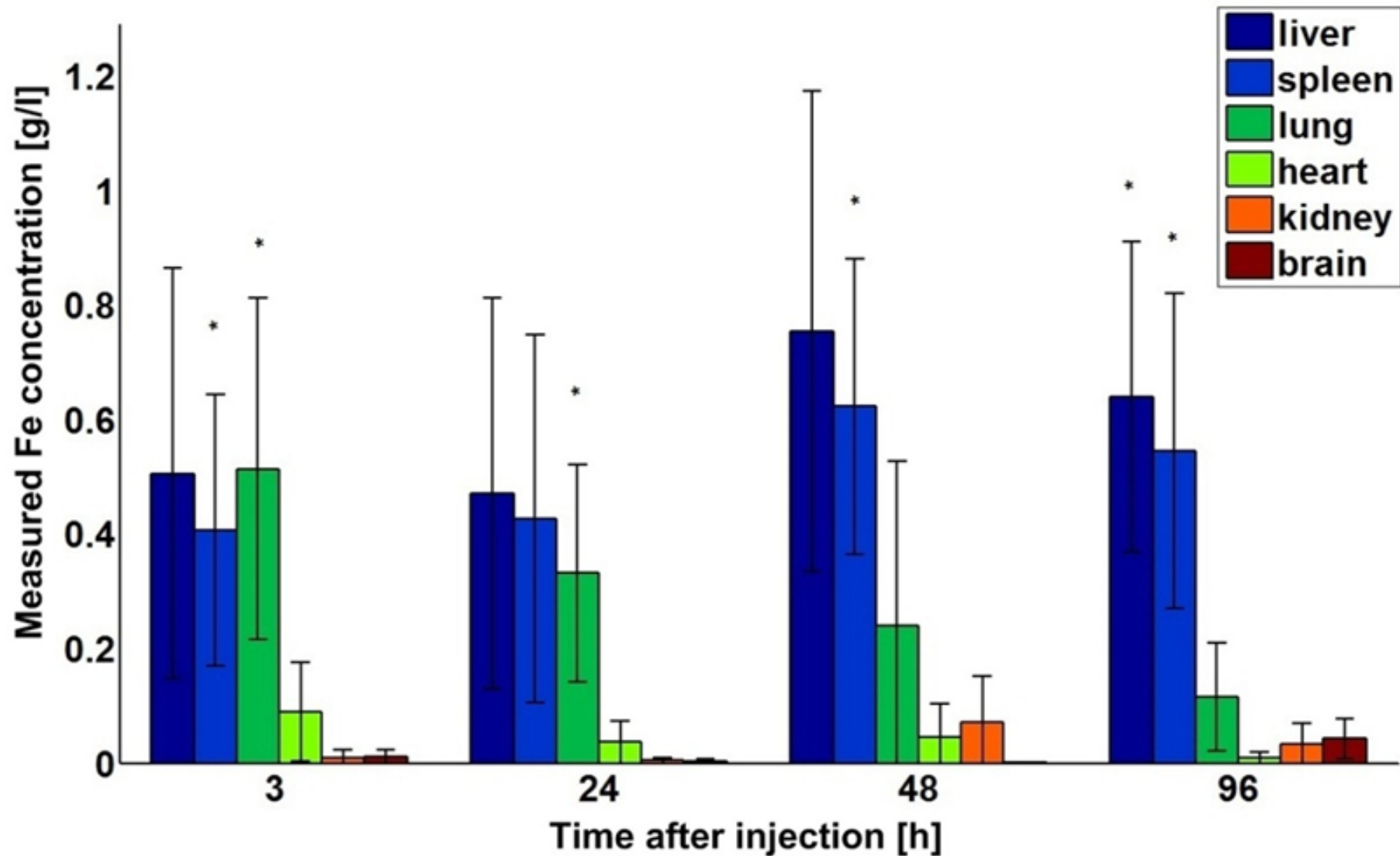
Bruker BioSpec 7T imaging system

SPIONS

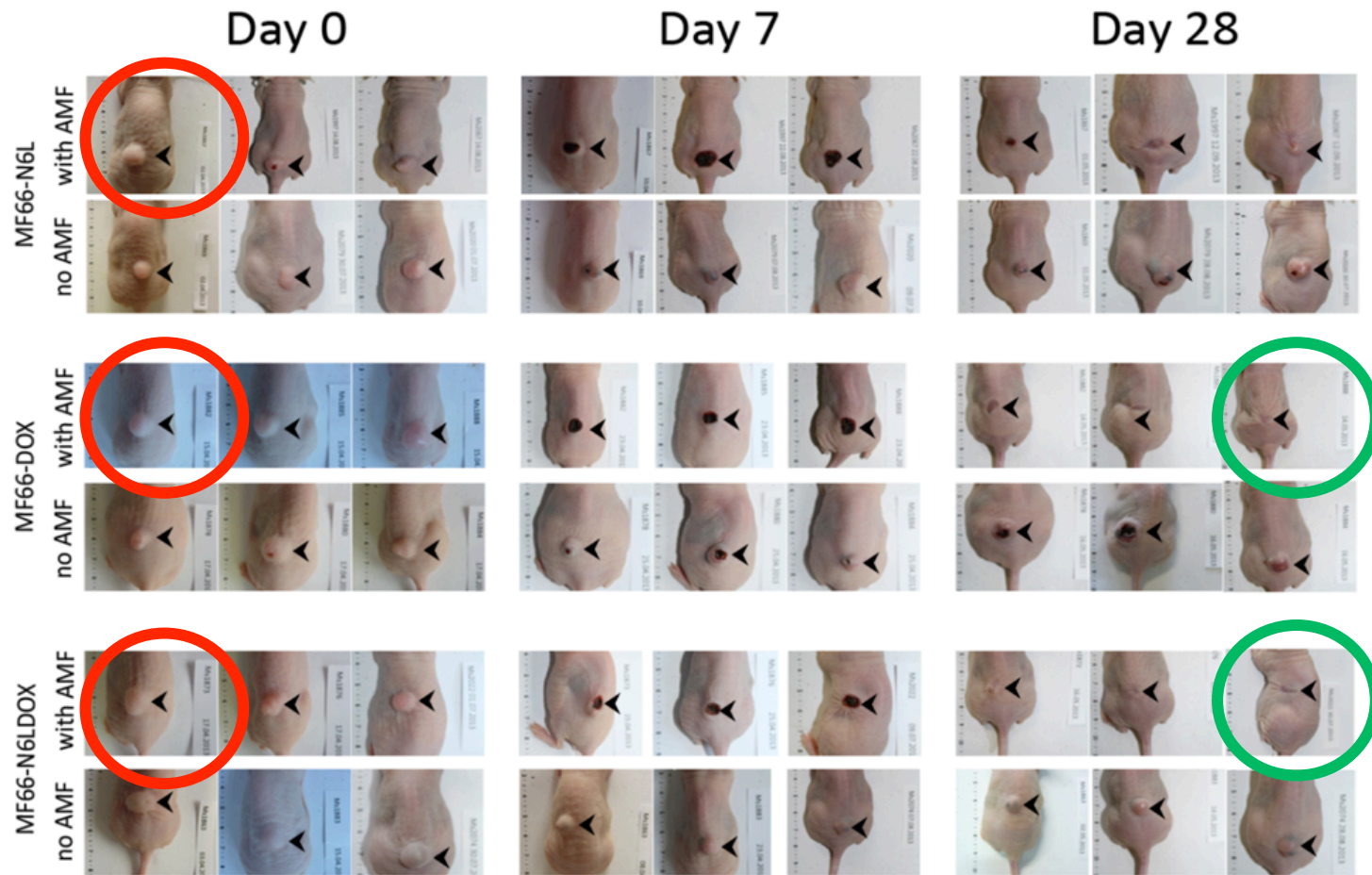
MRI imaging: Pharmacodynamics (0.33 mg/mouse)



# BIODISTRIBUTION OF MAGNETIC NANOCARRIERS *IN VIVO*



# APPLICATION OF MULTIFUNCTIONAL MAGNETIC NANOPARTICLES FOR *IN VIVO* CANCER TREATMENT IN COMBINATION WITH HYPRTHERMIA



Breast adenocarcinoma cell line MDA-MB-231. Therapeutic efficacy analyzed in subcutaneous MDA-MB-231 tumor-bearing female athymic nude mice.

Kossatz et al., 2015, Breast Cancer Research





# THE MULTI-NATIONAL TCD NANOMEDICINE TEAM TACKLING THE MULTI-FACTORIAL CHALLENGES



Trinity College Dublin, School of Medicine:

*Dania Movia, Anthony Davies, Tatsiana Rakovich, Ayokunmi Ajetunmobi, Namrata Jain, Kieran Crosbie-Staunton, Adriele Prina Mello, Navin Kumar Verma, Bashir Mohammed, Malgozhata Nowostavska, Omar Kazem Mahfoud, Erato Altoka, Gareth Clarke.*

# PARTNERS AND FUNDING

**MULTIFUN project: FP7 NMP-2010-4.0-1 (ref 262943)**  
[www.multifun-project.eu](http://www.multifun-project.eu)



**NAMDIATREAM project: FP7 NMP-2009-4.0-3**  
**(ref 24647)**  
[www.namdiatream.eu](http://www.namdiatream.eu)



**Trinity College Dublin**  
Coláiste na Tríonóide, Baile Átha Cliath  
The University of Dublin

**Thank You**

