

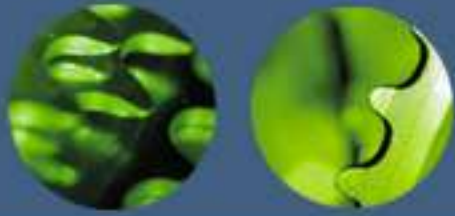


# RESPONSIBLE DEVELOPMENT OF NANOTECHNOLOGY IN SA

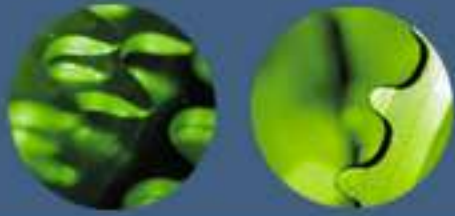
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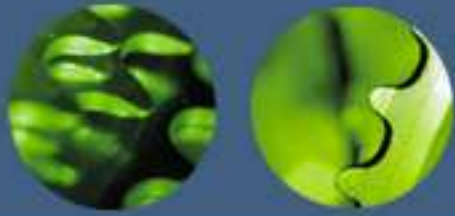
science  
& technology  
Department  
Science and Technology  
REPUBLIC OF SOUTH AFRICA



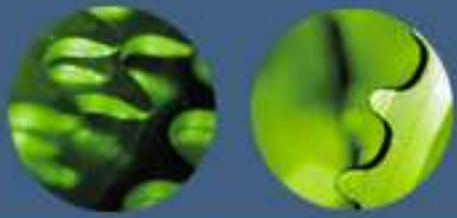
- DST's role in responsible development of nanotechnology in SA:
  - Standardization
  - Nanotechnology Ethics Committee
    - Involvement in the NanoCode project
  - Public Engagement Programmes
  - Nanotechnology HSE Research Platform



- DST's role in responsible development of nanotechnology in SA:
  - Standardization
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    - Involvement in the NanoCode project
  - Public Engagement Programmes
  - **Nanotechnology HSE Research Platform**

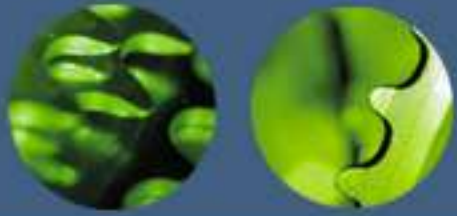


- Awareness of the increasing concerns on the effects of nanomaterials to humans and other biological life forms.
- A need to address the potential health, safety, and environment (HSE) aspects related to nanotechnology by DST.



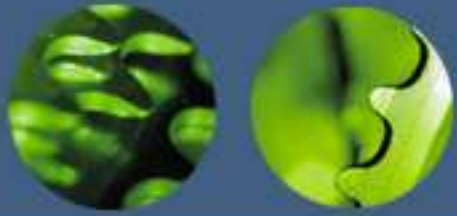
# Nanotechnology HSE Research Platform

- This is to ensure nanotechnology-driven benefits
  - Sociological (e.g. in water, health and energy).
  - Economical (e.g. advanced industrial manufacturing)
- Are exploited safely, responsibly, and sustainably.



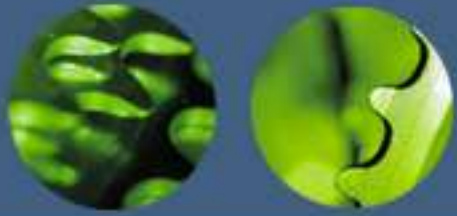
## **Nanotechnology National Research Plan**

- The National Research Plan (NRP) to achieve the objectives identified within the national Nano-HSE research platform.



## Nanotechnology HSE Research Plan

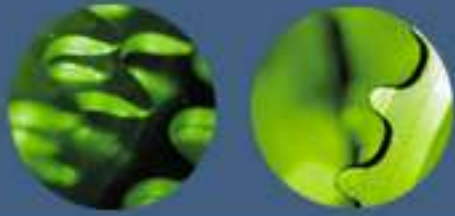
- Risk assessment of nanomaterials proposed in this strategic plan seeks to provide:
  - Information based on the analysis of scientific data to be generated from:
    - Hazard identification
    - Exposure assessment
    - Risk characterization



## Example: Gold nanoparticles

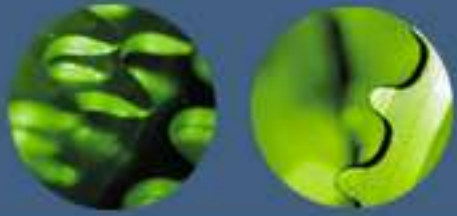
- Major Commercial use:
  - Currently used for Lateral flow diagnostics and Catalysis.
  - Expected to be also applied in therapeutics (e.g., cancer) and imaging.





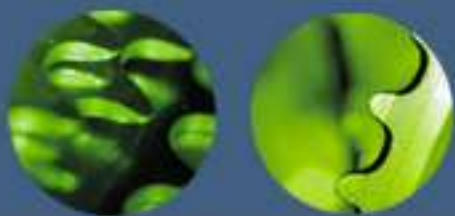
# OECD Nanomaterials

- Fullerenes (C60)
- Single-walled carbon nanotubes (SWCNTs)
- Multi-walled carbon nanotubes (MWCNTs)
- Silver nanoparticles
- Iron nanoparticles
- Titanium dioxide
- Aluminium oxide
- Cerium oxide
- Zinc oxide
- Silicon dioxide
- Dendrimers
- Nanoclays
- **Gold nanoparticles (Lead Sponsor: South Africa)**

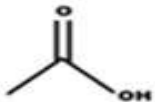

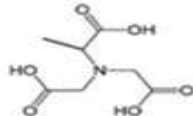
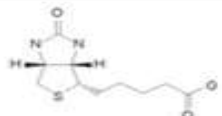



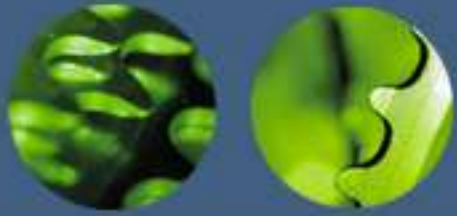
# Gold nanoparticles

- Gold nanoparticles developed at Mintek:
  - Colloids,
  - Monolayer protected clusters (MPCs)
  - Mixed Monolayer Protected Clusters (MMPCs)



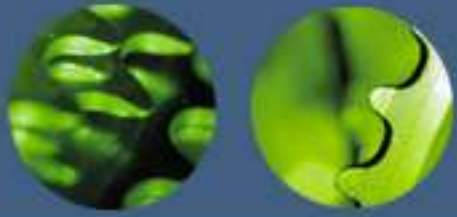
## Gold nanoparticles studied

<u>Ligand</u>	<u>Abbreviation</u>	<u>Structure of functional group</u>	<u>Charge (zeta potential)</u>
Uncoated, citrate stabilised	Au14nm		-34.8 mV
Carboxy-PEG	PCCOH		-32.4 mV
Azide-PEG	PAZ		-35 mV
Nitrilotriacetic acid-PEG	PNTA		-39.9 mV
Biotin-PEG	PBtn		-43.4 mV
Hydroxy-PEG	POH		-22.6 mV



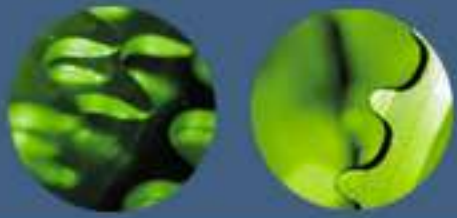
# OECD Sponsorship Programme

- **National Collaborators**
  - **NIOH/University of the Witwatersrand**
  - **Mintek**
  - **North West University**
  - **University of Johannesburg**



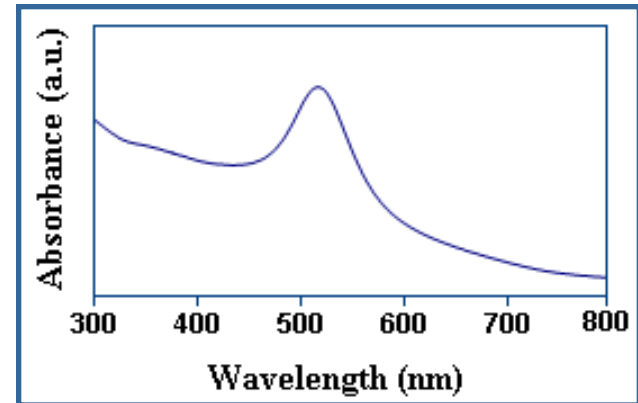
# Risk Assessment of Gold Nanoparticles

- Physicochemical properties
- Hazard identification
  - *In vitro*
  - Dose response relationship
  - *In vivo*
- Exposure assessment
- Risk characterization

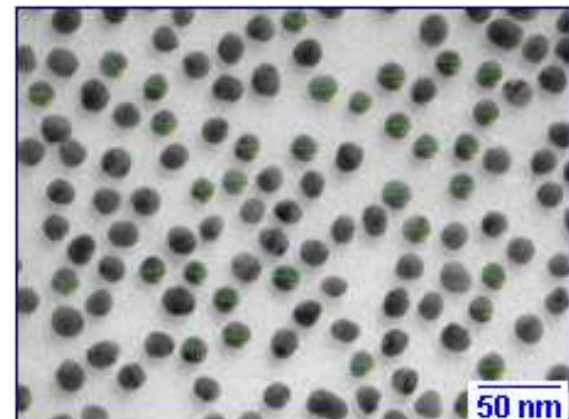


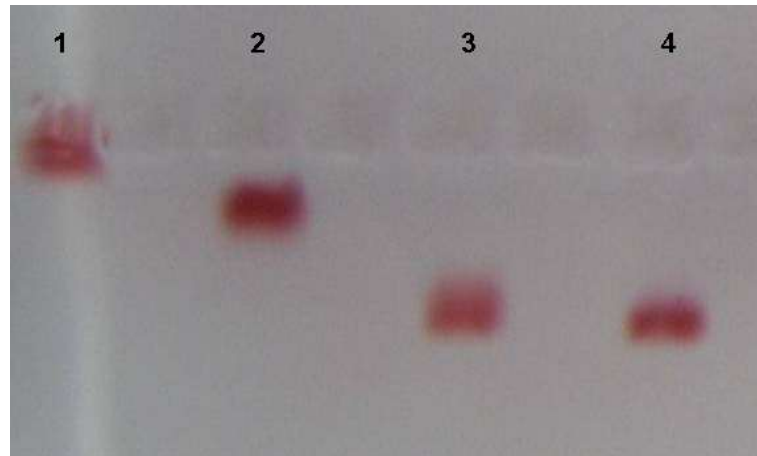
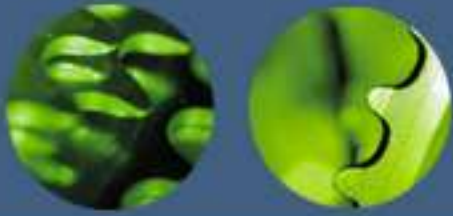
# Physicochemical Properties

- UV/Vis

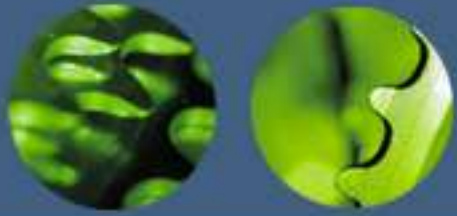


- TEM





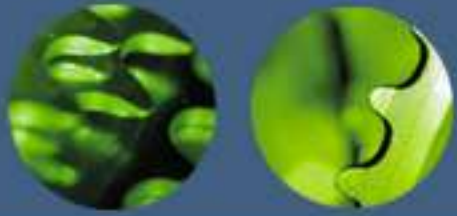
- Various ligands and their percentage loading is evaluated using electrophoresis.



## *In vitro* cell cultures

- BEAS-2B
- Hep-G2 cells
- Primary astrocytes
- U937 cells

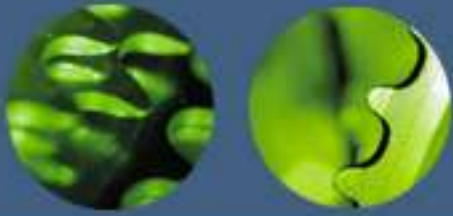




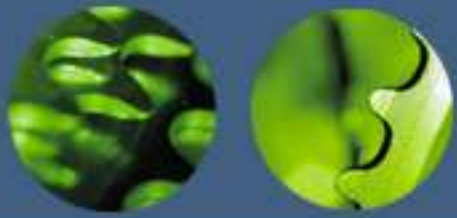
## *In vitro* tests

- Bacterial reverse mutation test
- Comet assay
- Intracellular localization
- Cytotoxicity tests



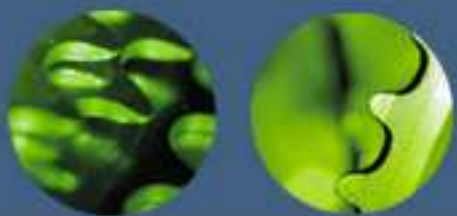


- Commonly accepted cytotoxicity tests:
  - Increased viability. (XTT)
  - Increased toxicity (LDH)
  - Increased toxicity (ATP)
- Oxidative stress:
  - DCFH-DA (Decrease ROS production and therefore decreased toxicity)



## Challenges faced with traditional *in vitro* toxicity assays

- Optical properties of gold nanoparticles could interfere:
  - Absorbance
  - Fluorescence
- Contradictory results due to interference of gold nanoparticles in the above test systems using the BEAS-2B cells.

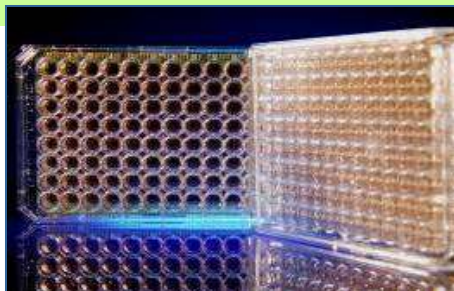


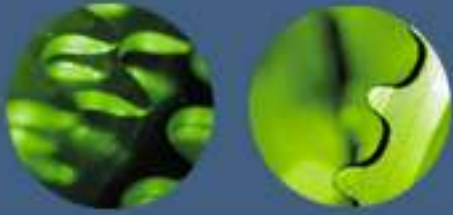
## Cytotoxicity using xCELLigence Real-Time Cell Analyzer (RTCA) system

This system allows for label-free, real-time measurements of cell status

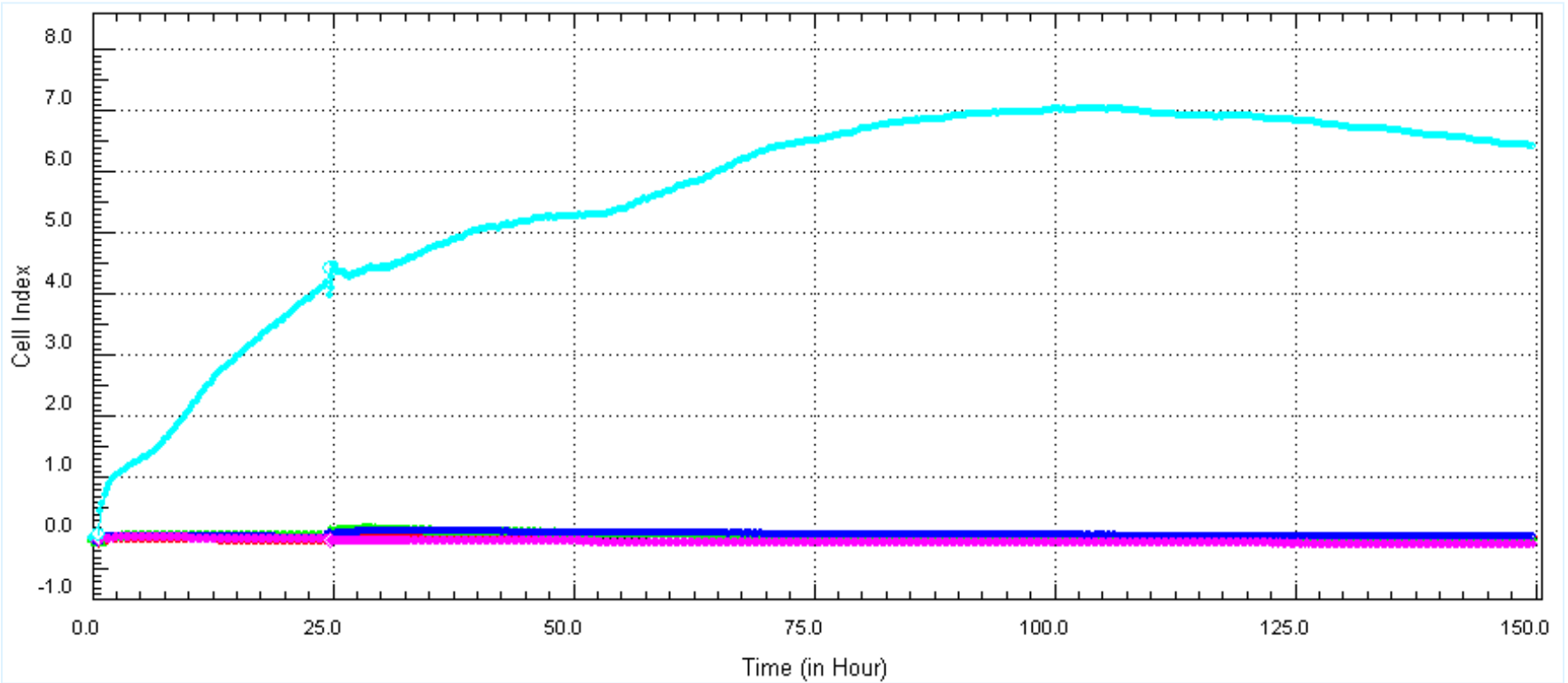
Comparable to traditional assays (MTT, LDH, NRU) but without potential problems of NPs interfering with the assay

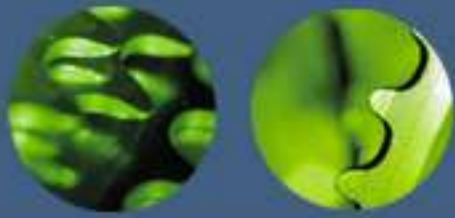
Compounds with similar activity produce similar cell response profiles which could be used as an indicator of a possible mechanism



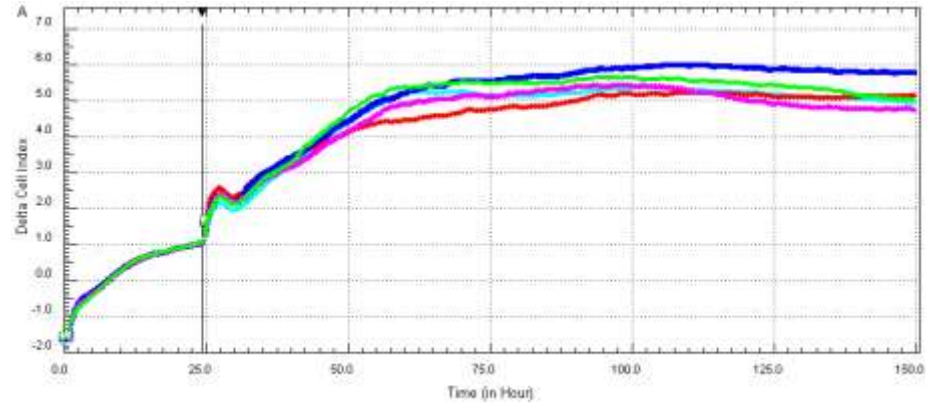


# Cell index and control wells

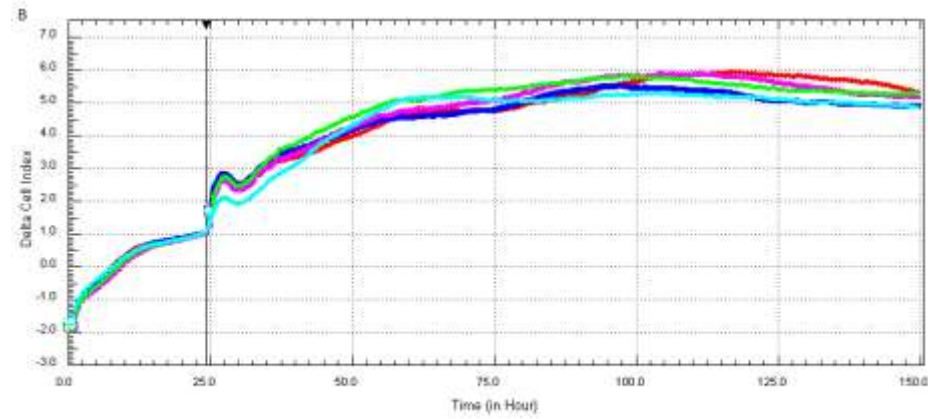




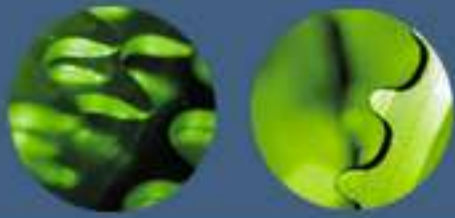
20 nm



40 nm



5nM; 2nM; 1nM; 0.5nM; untreated cells



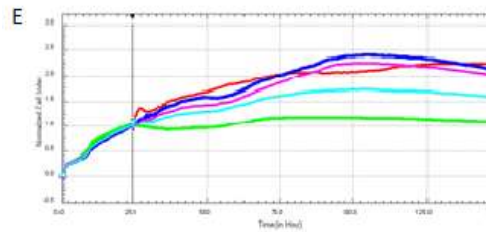
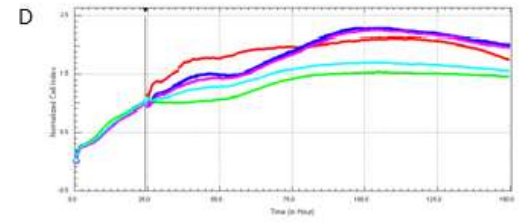
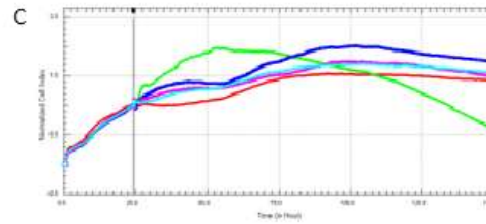
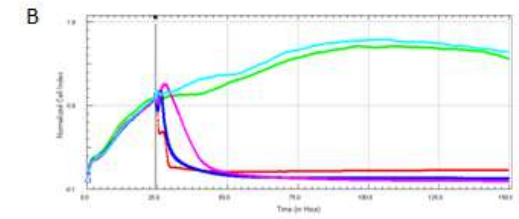
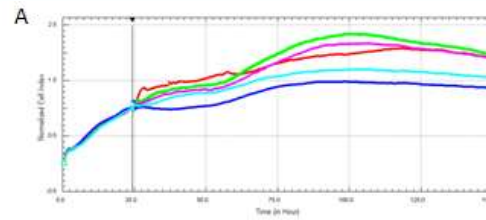
(A) PEG-COOH MPCs

(B) PEG-Azide MMPCs

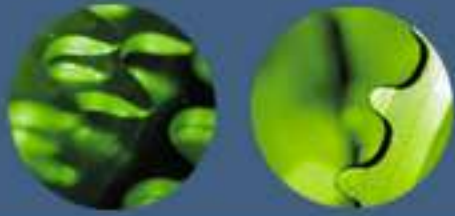
(C) PEG-NTA MMPCs

(D) PEG-Biotin MMPCs

(E) PEG-OH MPCs

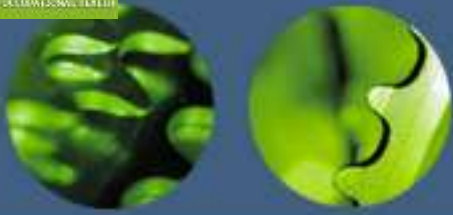


5nM; 2nM; 1nM; 0.5nM; untreated cells



- xCELLigence instrument allows for real-time cytotoxicity measurements with no problems with interference



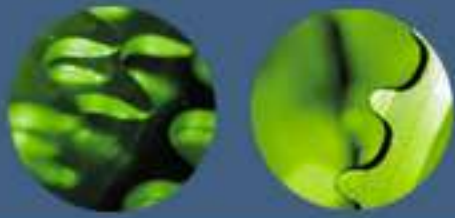


## Uptake of nanoparticles

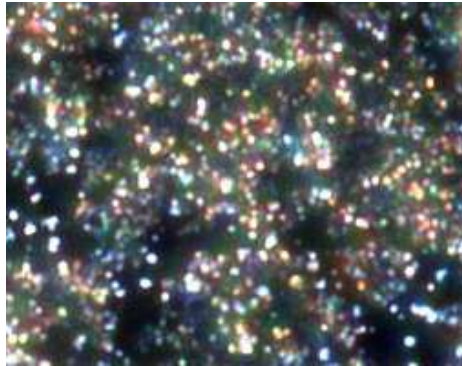


Techniques such as TEM  
and Raman microscopy  
We opted for the CytoViva  
Hyperspectral Imaging  
System:

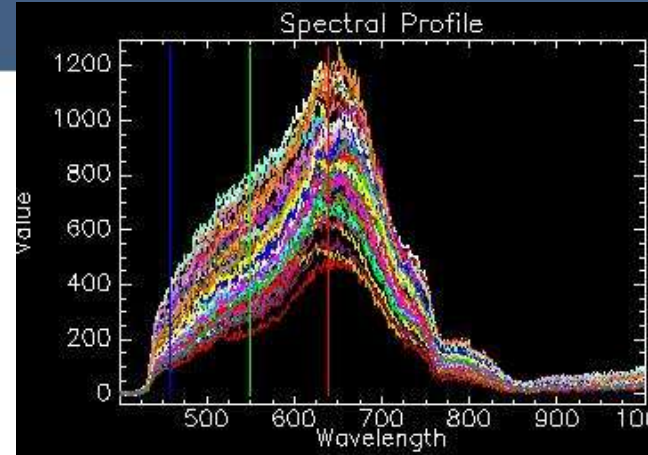
- High signal-to-noise  
darkfield images
- Spectral analysis (VNIR)



# Spectral libraries Spectral libraries



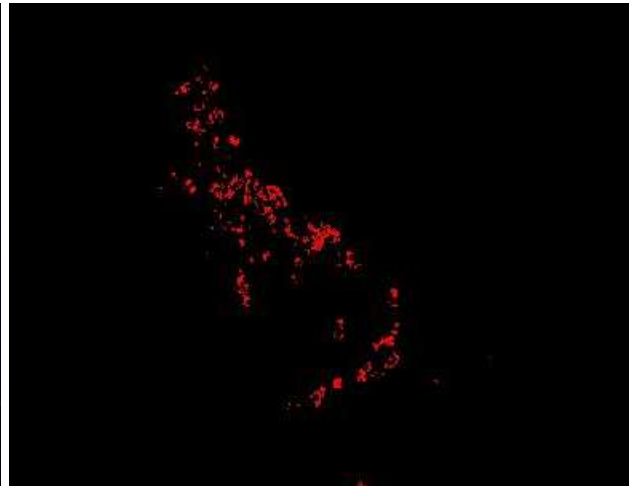
Spectral scan of Au14nm



Spectral profile of Au14nm



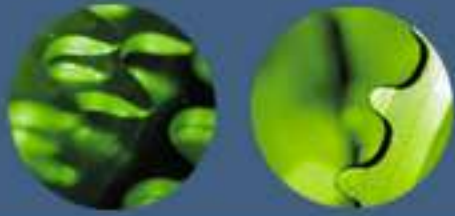
Spectral scan of cell incubated with Au14nm



Spectral mapping



Overlay of images



# PEG-COOH

B



• 2 Hours

B

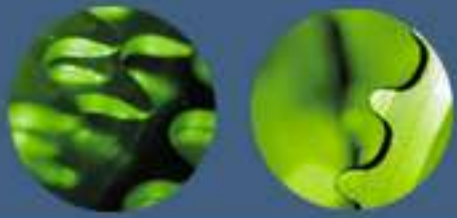


• 24 Hours

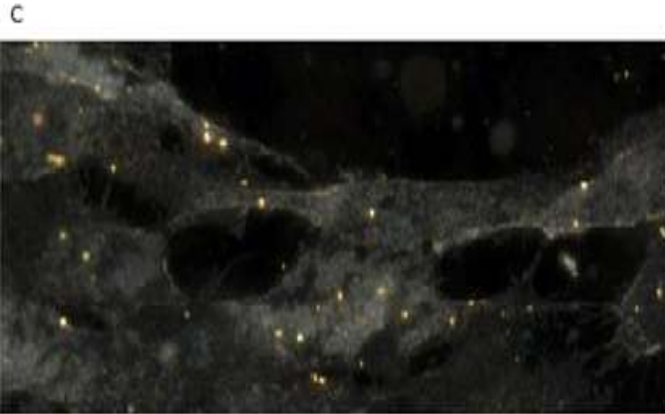
B



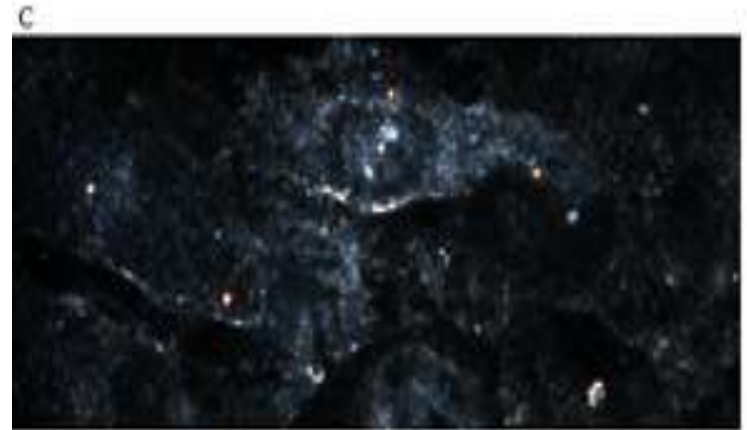
• 48 Hours



# PEG-nitrilotriacetic acid



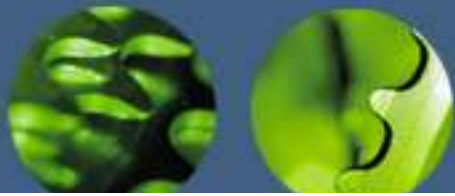
• 2 Hours



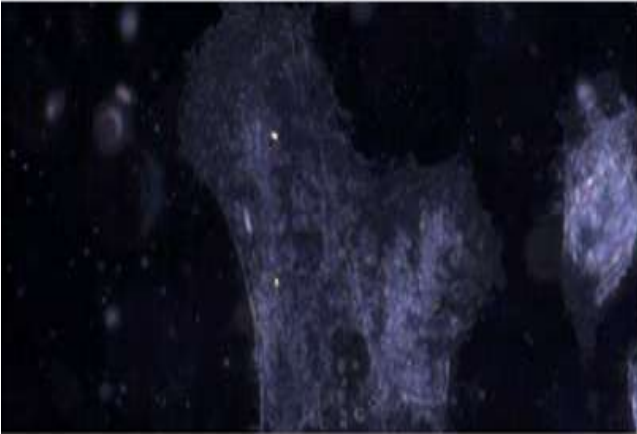
• 24 Hours



• 48 Hours



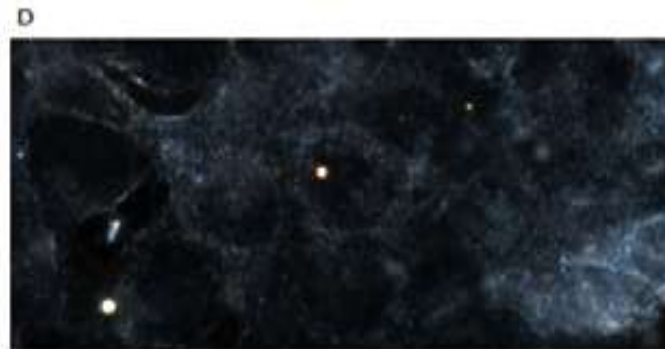
# PEG-Biotin



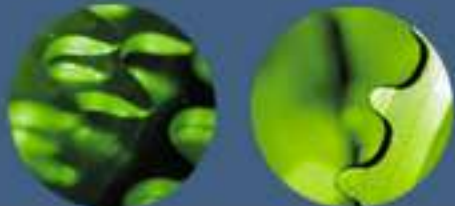
• 2 Hours



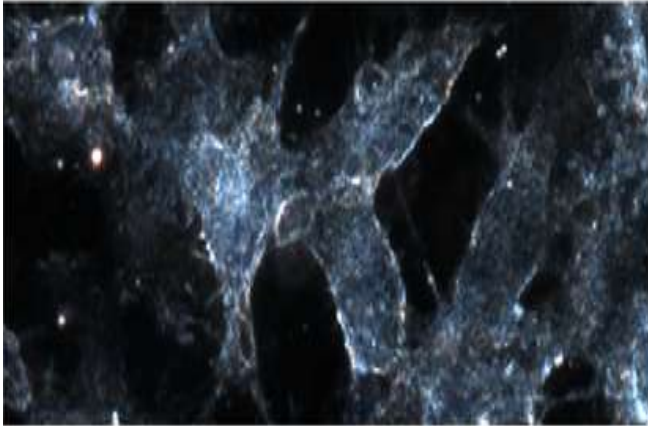
• 24 Hours



• 48 Hours



# PEG-OH



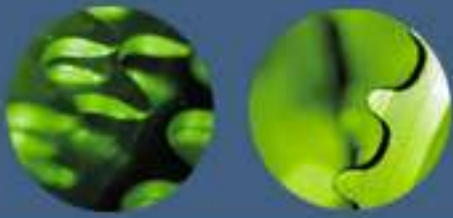
• 2 Hours



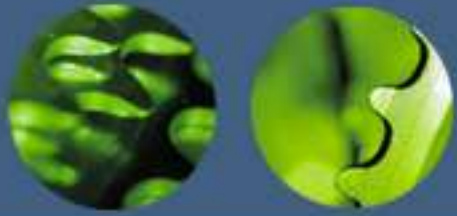
• 24 Hours



• 48 Hours



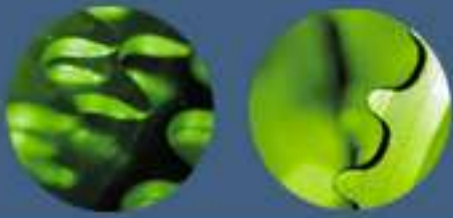
- We could achieve in single cell imaging of intracellular gold nanoparticle.
  - Cells need to be treated for microscopy.
- These were therefore not real-time imaging (living cells).



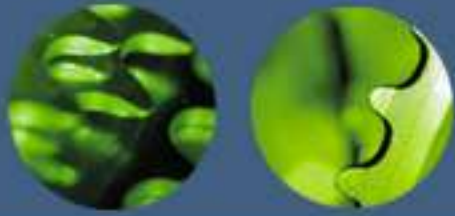
## **Biopersistence of gold nanoparticles**

- Biodegradability of gold nanoparticles is of great relevance.
- It is highly desirable to use real-time imaging that will help assess the intracellular location as well as fate of the particles over time.

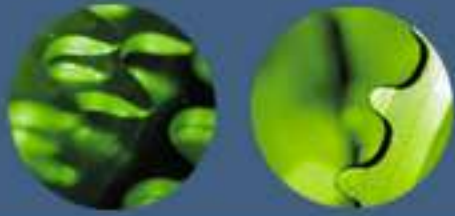




- In addition, the application of different methodologies need to be investigated to assess the stability and half-lives of the coating material and the functional groups in biological as well as in environmental media.

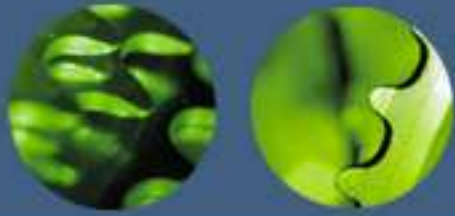


- **Biological *in vivo* Mammalian Toxicology**
  - Acute toxicity, including dermal, inhalation and oral
  - Short term repeated dose
  - Sub-chronic toxicity
  - Long term or chronic
  - Reproductive toxicology
  - Carcinogenicity
  - Neurotoxicity
  - Local effects on
    - skin
    - eye
    - human skin
  - Allergic sensitisation
  - Toxicokinetics & metabolism

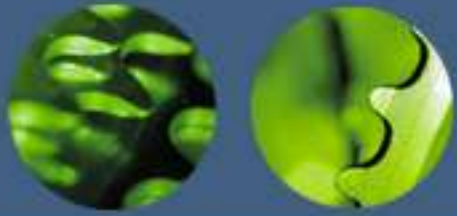


- Environmental Toxicology

- Determination of the short and long term effects on pelagic species (Algae, Crustaceans, Fish).
- Determination of the short and long term effects on sediment species.
- Determination of the short and long term effects on soil species.
- Determination of the effects on microorganisms.
- Determination of the effects on activated sludge.



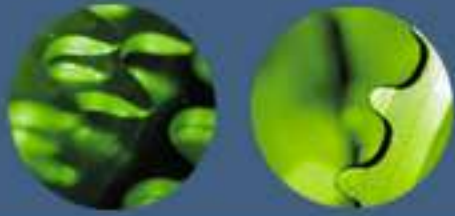
- Environmental Fate
  - Dispersion stability in water
  - Biotic degradation
  - Abiotic Degradability and Fate
  - Adsorption-Desorption
  - Bioaccumulation potential



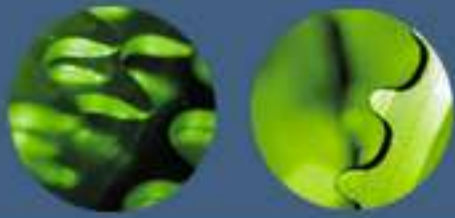
# Exposure assessment

- **Nano-ID**
- Fractions of 2 nm up to 20  $\mu\text{m}$
- Size-resolved samples are not altered, permitting compositional analysis using ICP-MS, AAS, and SEM/TEM





- Training within the gold nanoparticle risk assessment programme
  - Four Post Docs
  - 8 PhDs
  - 6 MScs
- Training within the National Research Plan (NRP)
  - Is determined as per new research programme.



- Collaborative work nationally and internationally in the risk assessment of:
  - Nanomaterials in general
  - Gold nanoparticles in particular