# Real Time Cell Electronic Sensing (RT-CES) for Nanotoxicity Evaluation

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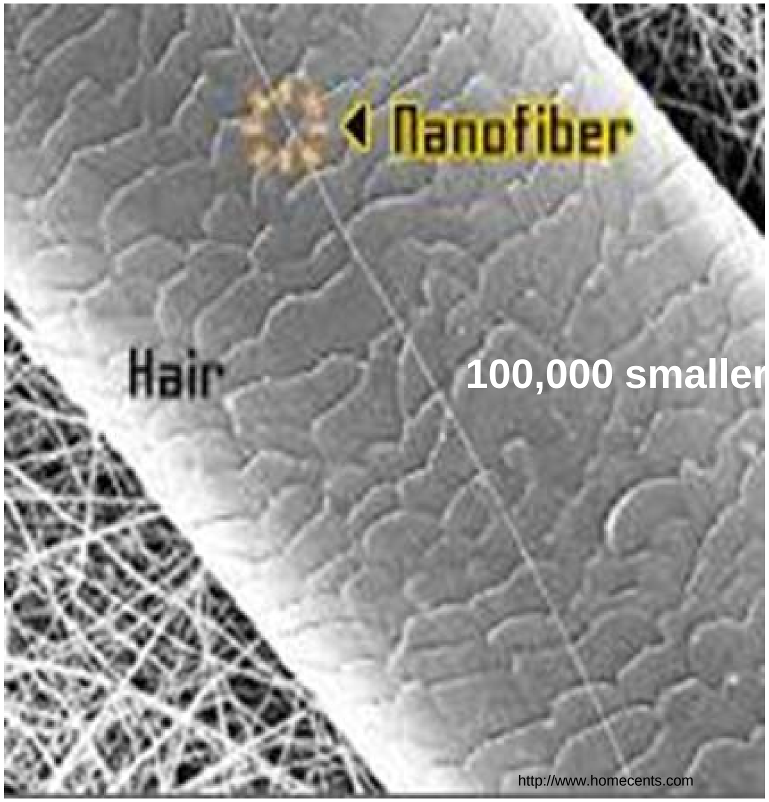
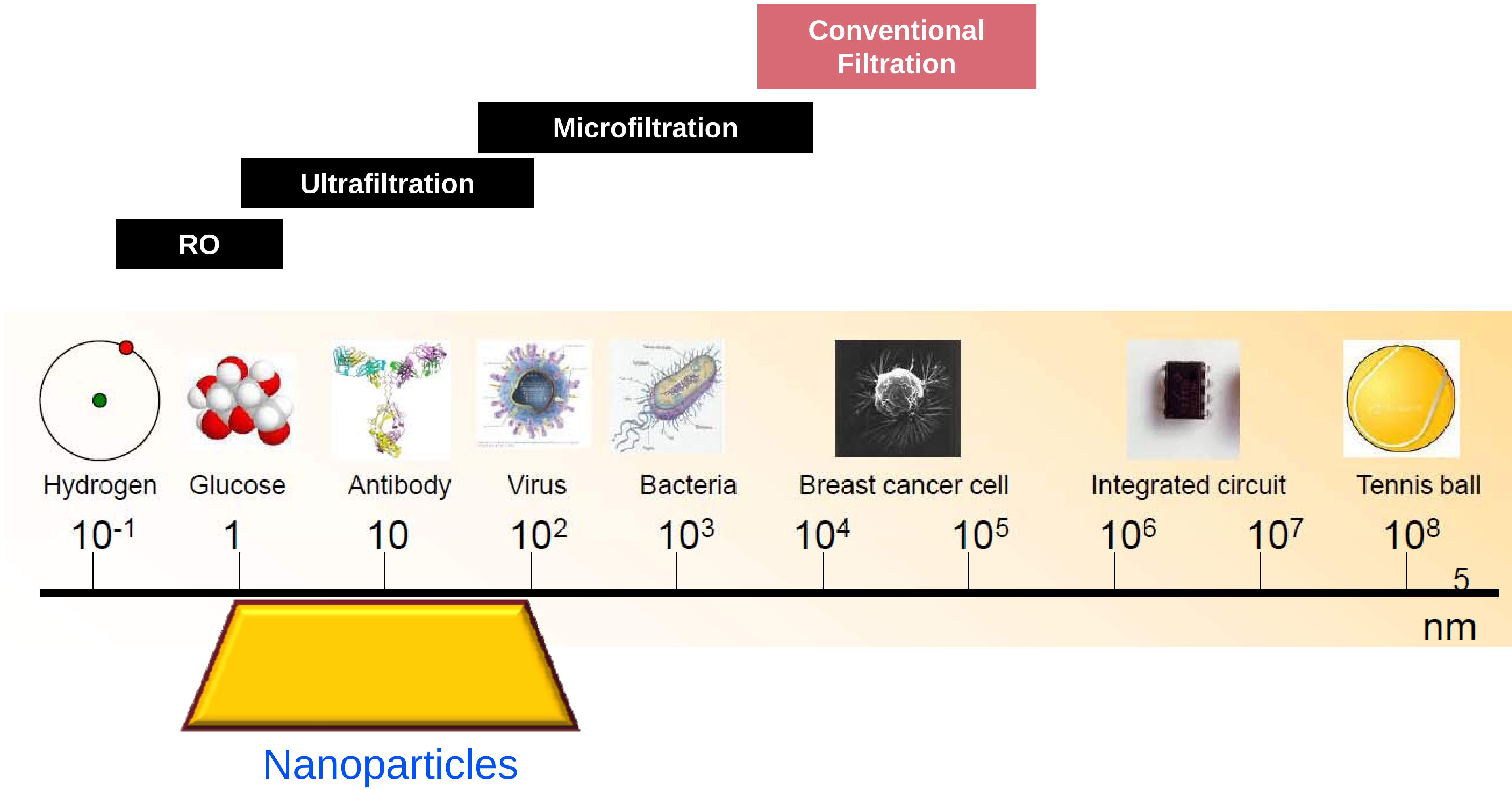
# NANOPARTICLES

- Nanoparticles (NPs) are particles sized in less than 100 nm.




**Nano = Dwarf (Greek) = $10^{-9}$**

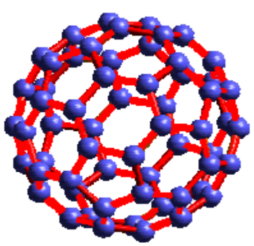
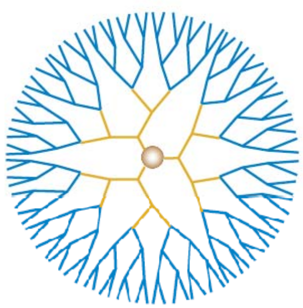
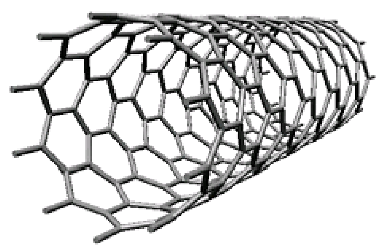
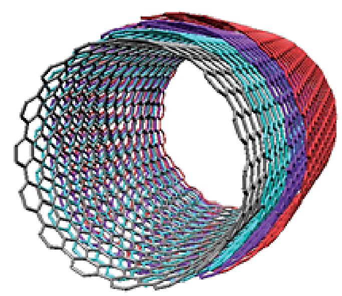
# INTRODUCTION



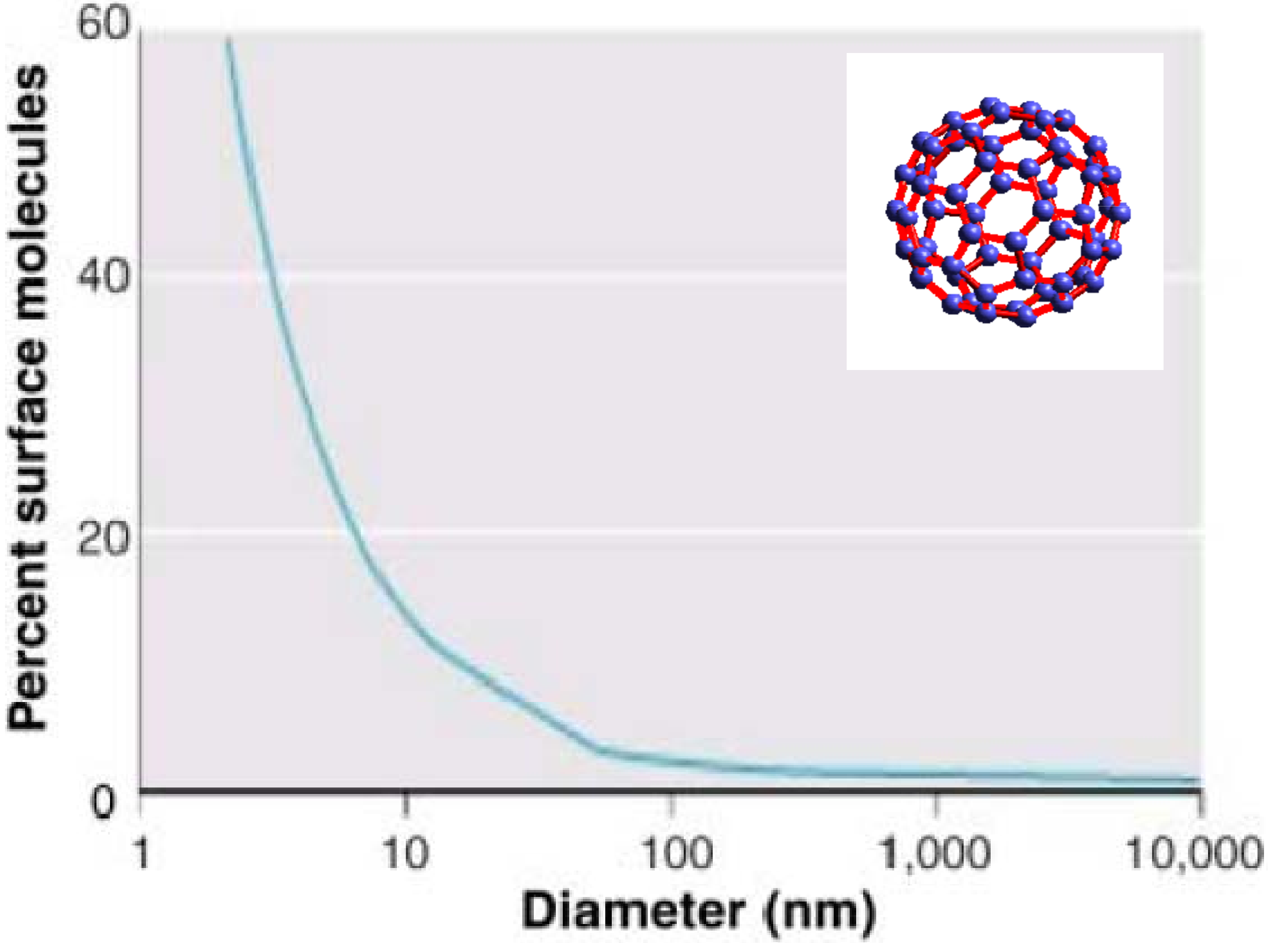
# Unique Properties of Nanoscale Materials

- **Small size**
- **High specific surface area** (> 100 $m^2/g$)
- **Quantum effects**

  **(dual behavior, wave- and particle-like)** → unique mechanical, electronic, photonic and magnetic properties

# Increase of Surface Area with Decreasing Particle Size




Nel et al. Science, 2006, 311:622-627

# NPs - Applications

## Increasing industrial / commercial applications

- Catalysis
- Medicine
- Environmental technology
- Cosmetics
- Semiconductors
- Microelectronics

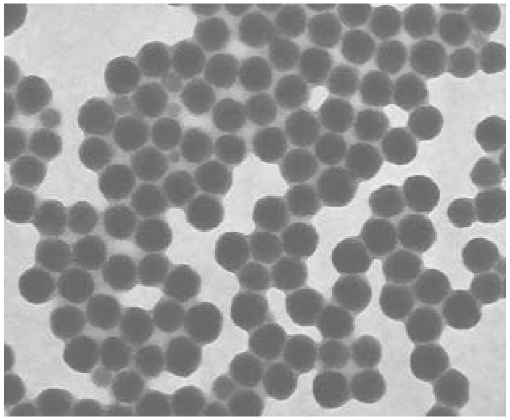

**Nanotechnology → 1 trillion US $ market by 2015.**

# NPs in Semiconductor Manufacturing

## CMP slurries

- $SiO_2$
- $Al_2O_3$
- $CeO_2$

## NPs for immersion lithography

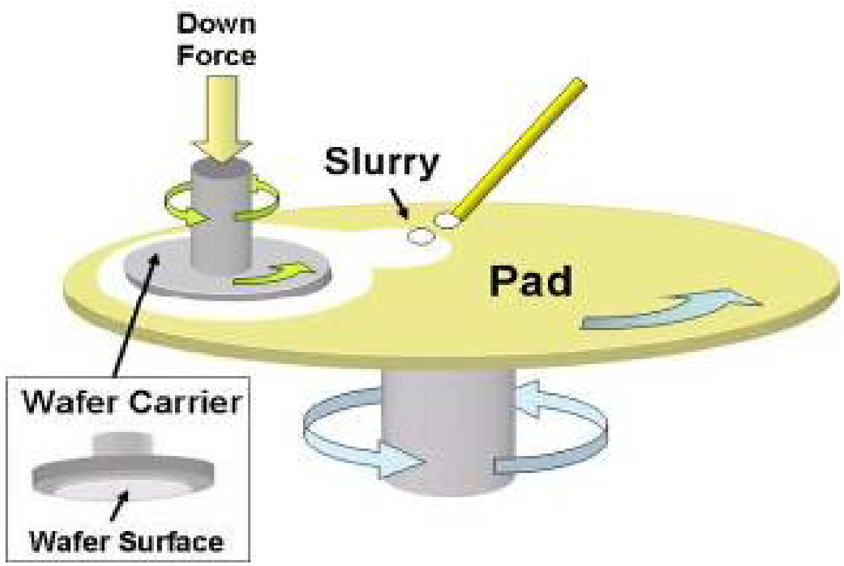


## Carbon nanotubes

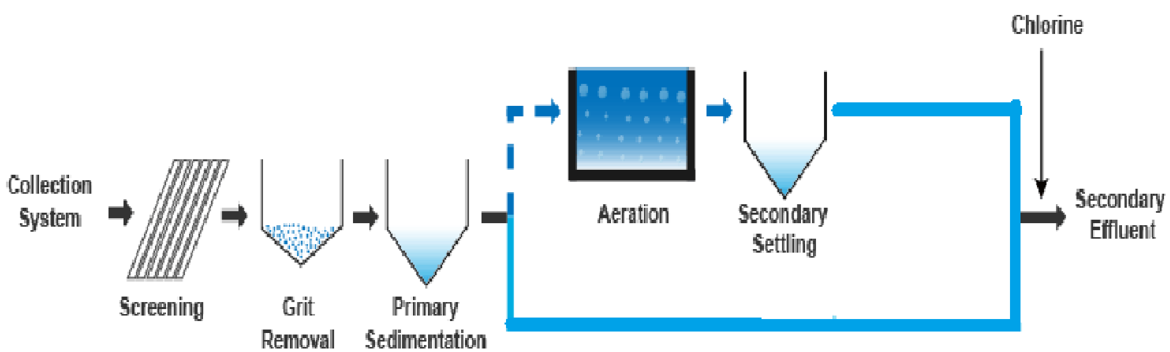
**Colloidal silica (10-130 nm)**

(Source: www.bjgrish.com)

# NPs in Chemo-Mechanical Planarization




**Untreated CMP effluent (50-500 mg/L)**



# Nanomaterials – ESH Concerns

Concern about the adverse effects of NPs on biological systems

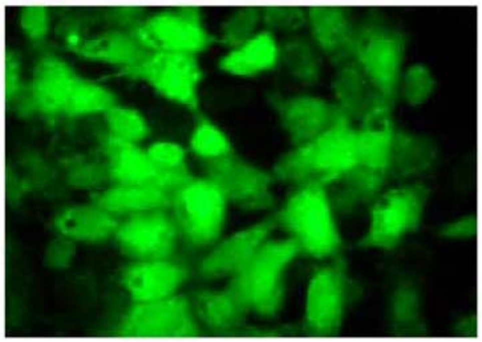
- ENM: unusual properties due to their small size
- Increasing evidence that some NP cause toxicity

Poor understanding of “nanotoxicity”

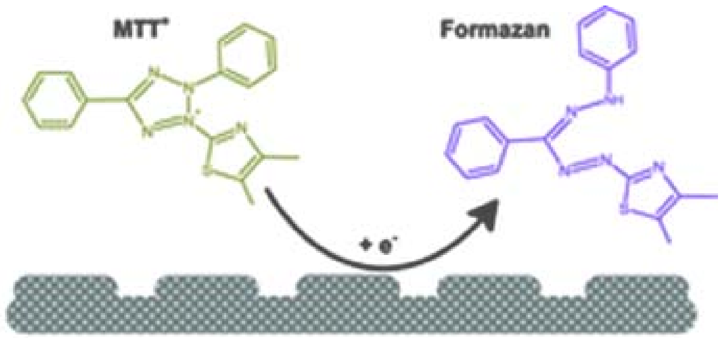
- Uncertainty about the real-life hazards of engineered NPs

Need for improved bioassays to evaluate “nanotoxicity”

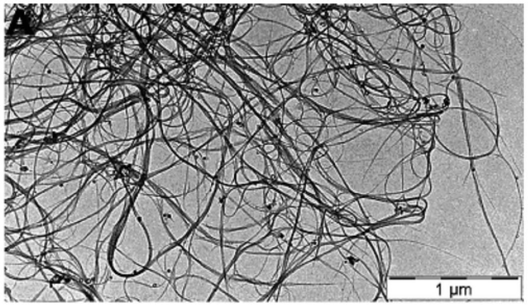
# Problems Assessing Nanotoxicity



Quenching of fluorescence



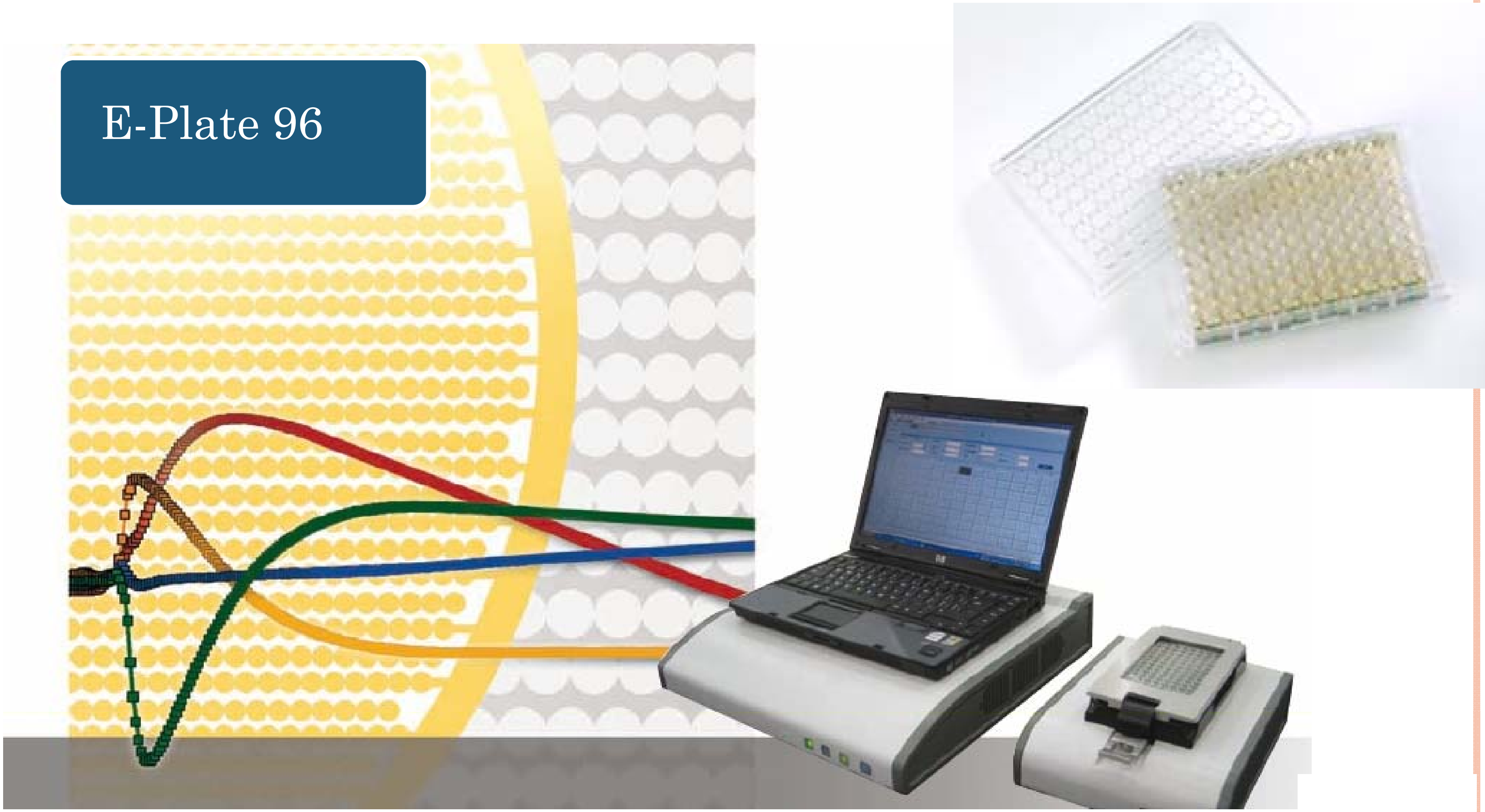

Reduction of MTT dye by NP surface



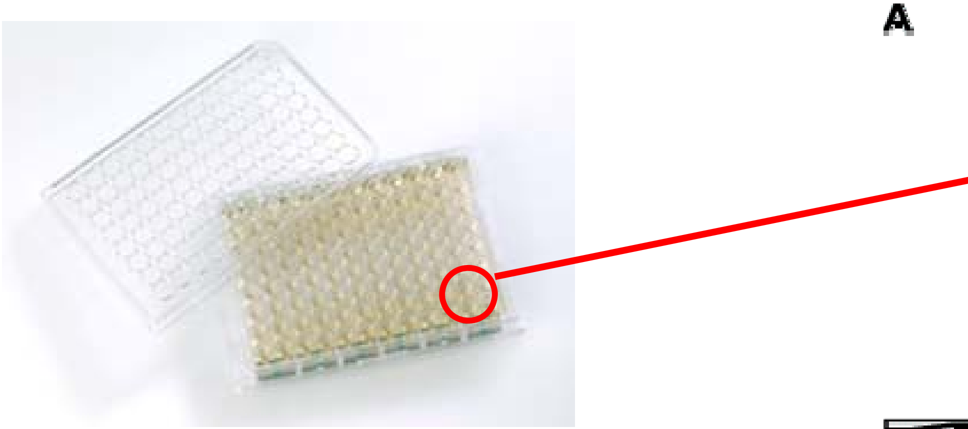

Sequestration of insoluble MTT dye by CNT

- **Interference in classical methods** dependent on colorimetric or fluorimetric measurements.
- **NP characterization** → most studies do not include characterization of NPs in biological medium.

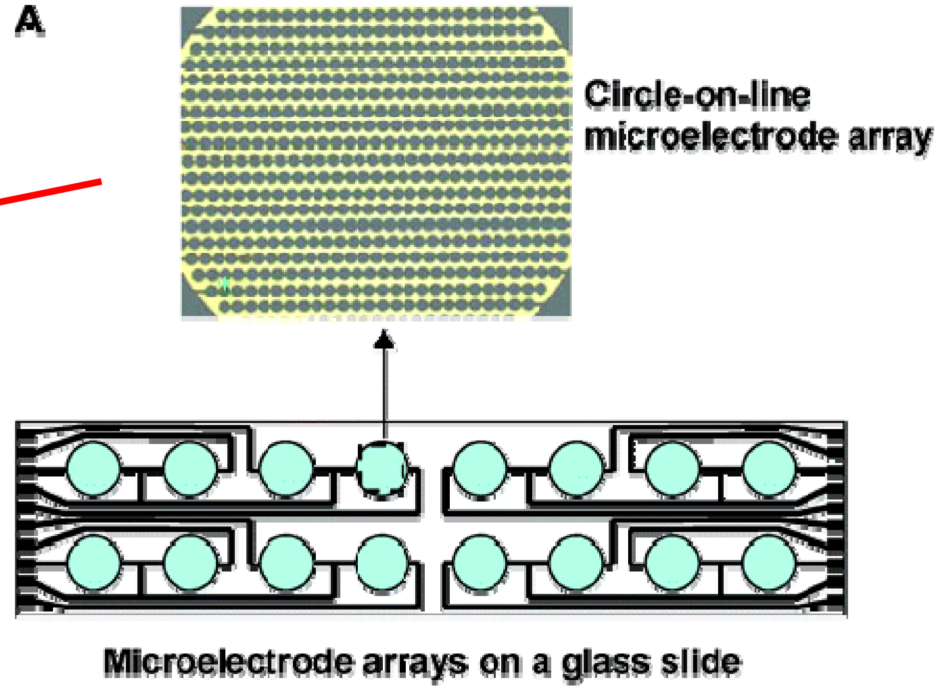
# Real Time Cell Electronic Sensing (RT-CES)

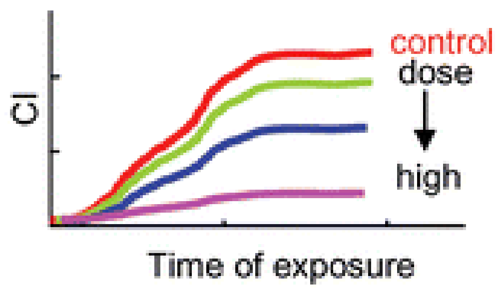


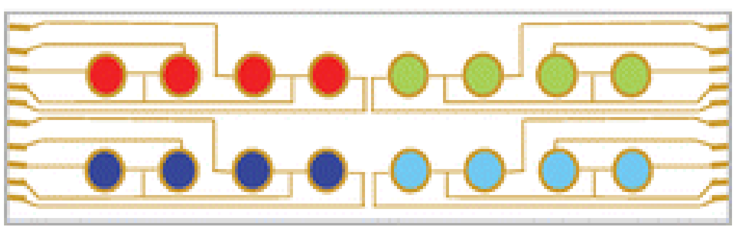

- The RT-CES system measures **electrical impedance** across interdigitated micro-electrodes integrated on the bottom of culture plates.

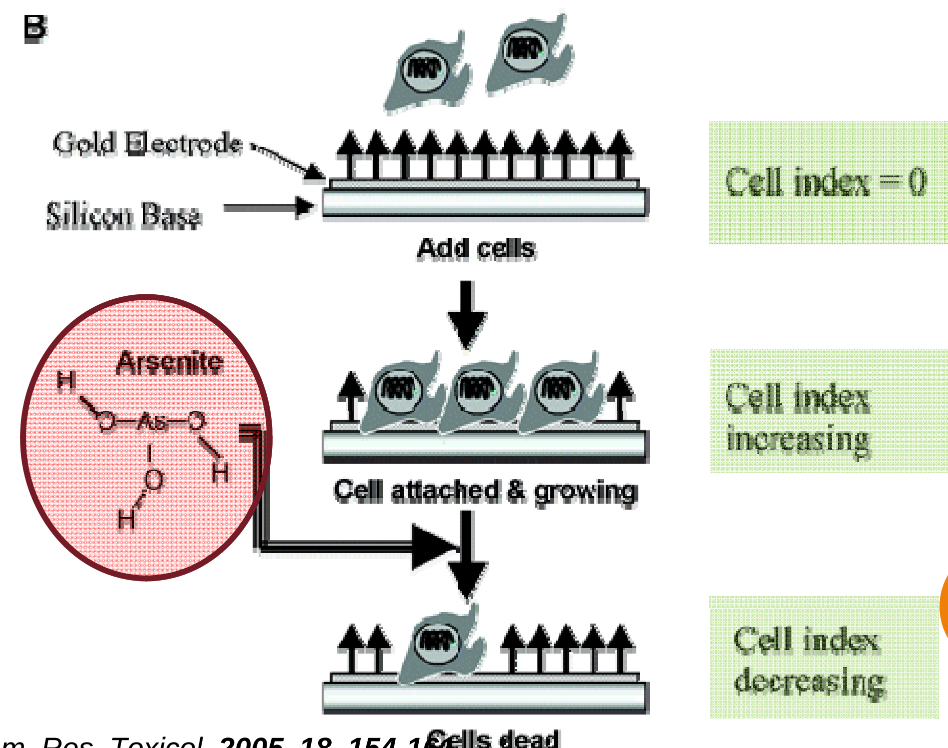


E-plate



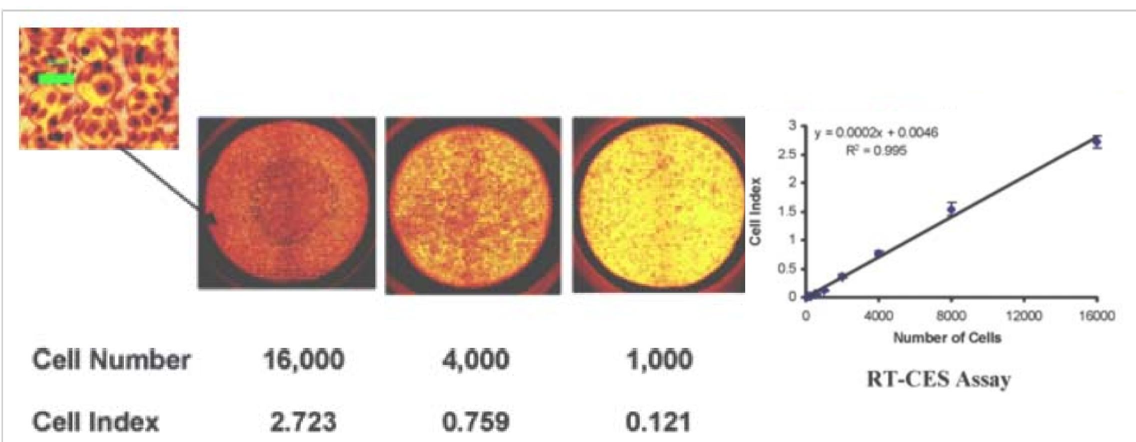



*Chem. Res. Toxicol.* ***2005, 18, 154-161***

# Cell Index

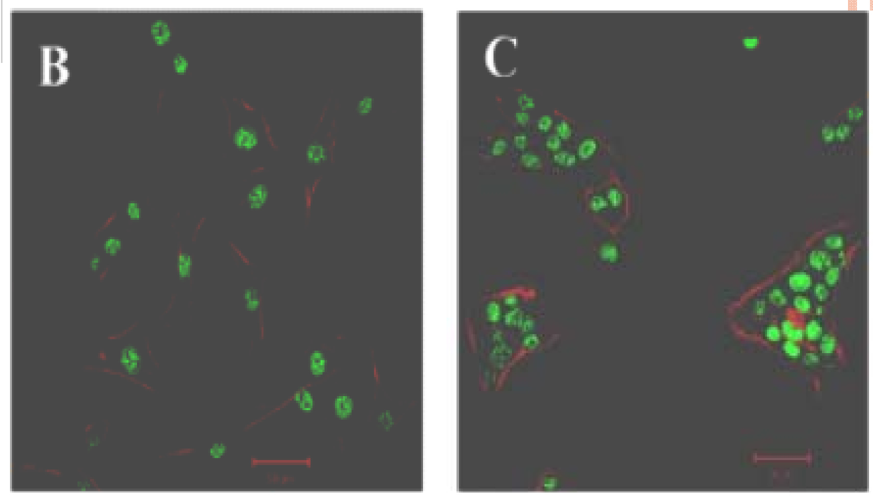
**CI**- Quantitative measure of the overall status of the cells:

- Cell number
- Cell adhesion and spreading
- Cell morphology




Increase in Cell Index with cell number

*Chem. Res. Toxicol.* ***2005, 18, 154-161***



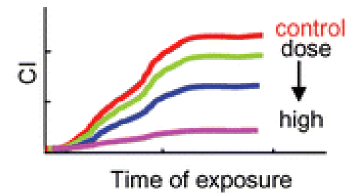

Cell morphology before (**B**) and after 3h treatment with As(III) (**C**)

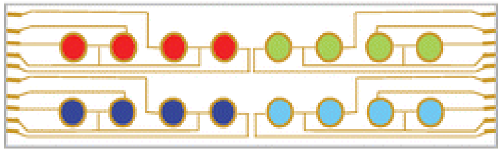
*Chem. Res. Toxicol.* ***2005, 18, 154-161***

# Impedance-based Real Time Cell Analysis

## Advantages

- (Fluorescent) **label free**
- Dynamic data of the **biological status** of the cells
- Noninvasive
- High throughput technique



## Limitations

- Requires **adherent** cells
- **Correlation** analysis between **RT-CES** and **classical toxicity** endpoints performed on a limited number of compounds
- Limited information of its applicability to NPs.

# OBJECTIVES

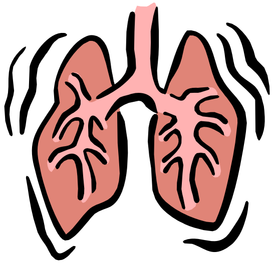
- Assess the applicability of a real time cell electronic sensing (RT-CES) technique based on impedance measurements to evaluate the cytotoxicity of **nanosized inorganic oxides** used in semiconductor manufacturing

- **Validation** of the RT-CES assay results: RT-CES *vs*. MTT

- Characterizing the aggregation of nanomaterials in the biological medium.

# RT-CES *VS.* MTT BIOASSAYS

Human bronchial epithelial cells
(16HBE14o-)

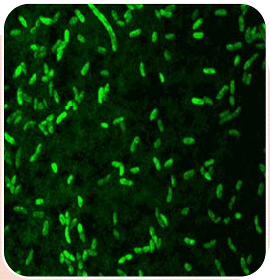


**RT-CES**

- Impedance based RT-CES assay:
- (xCELLigence, Roche)

**MTT**

- $O_2$ uptake
- Cell membrane integrity

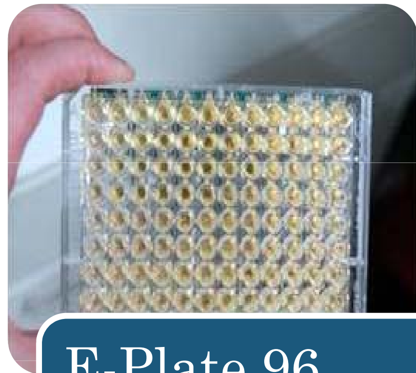


# IMPEDANCE-BASED RT-CES

**(RT-CES**, xCELLigence, Roche) - Lung epithelial cells: 16HBE14o-

**16 HBE culture**

- MEM[1] (10% FBS[2])
- 37ºC



**E-Plate 96**

- MEM (3.75% FBS)
- 100,000 cells/well
- 37ºC

**RT-CES system**

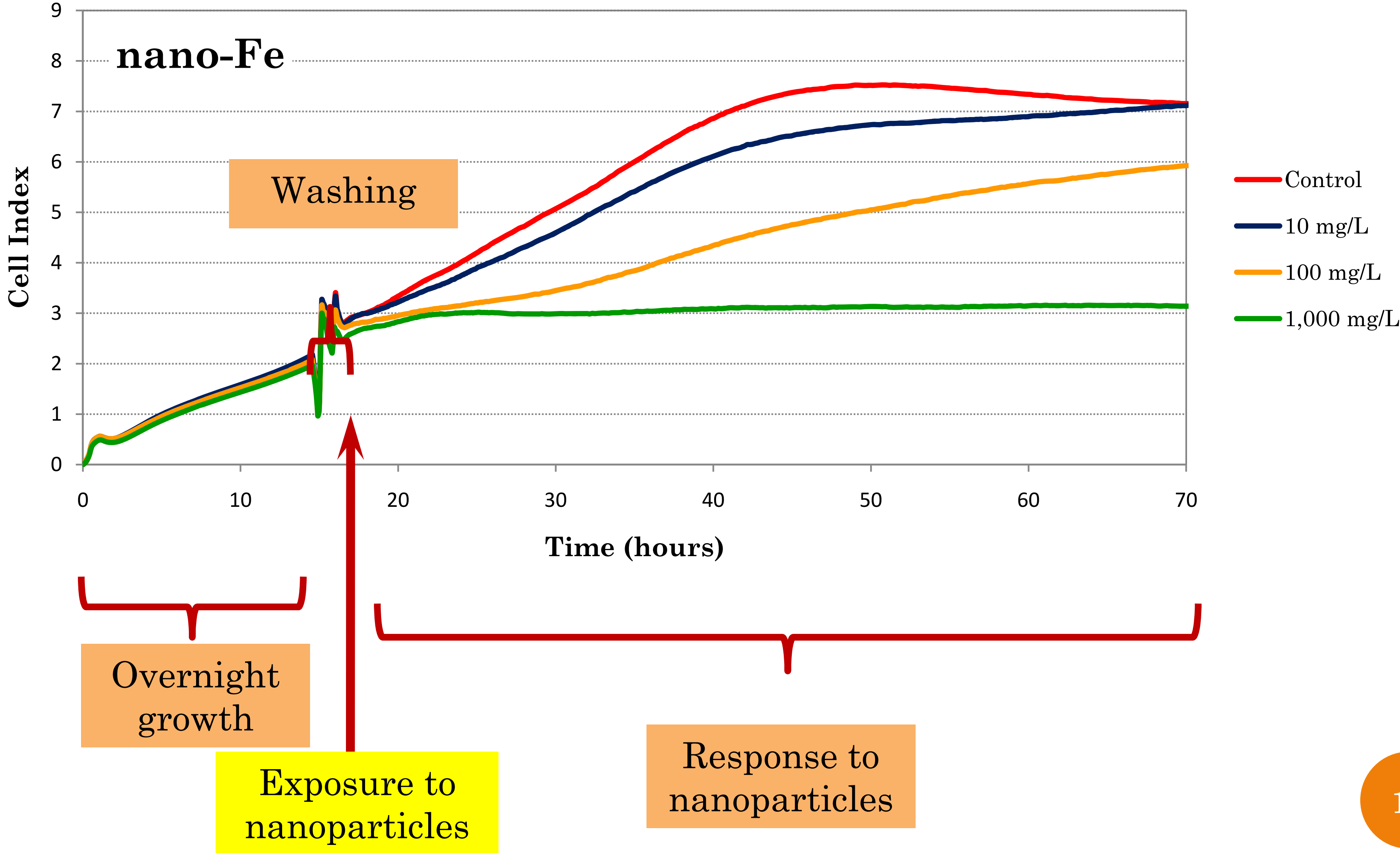
- 37ºC

[1] Minimum Essential Medium
[2] Fetal Bovine Serum

- Cells are transferred to the E-Plate at 100,000 cells/well.
- NPs are dosed after **16 h** of incubation.
- Cells are monitored for at least **48 h**.

# RT-CES Bioassay

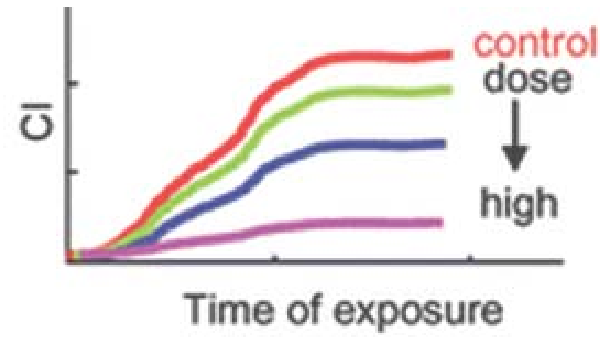
- Experiment stages



# RT-CES Bioassay

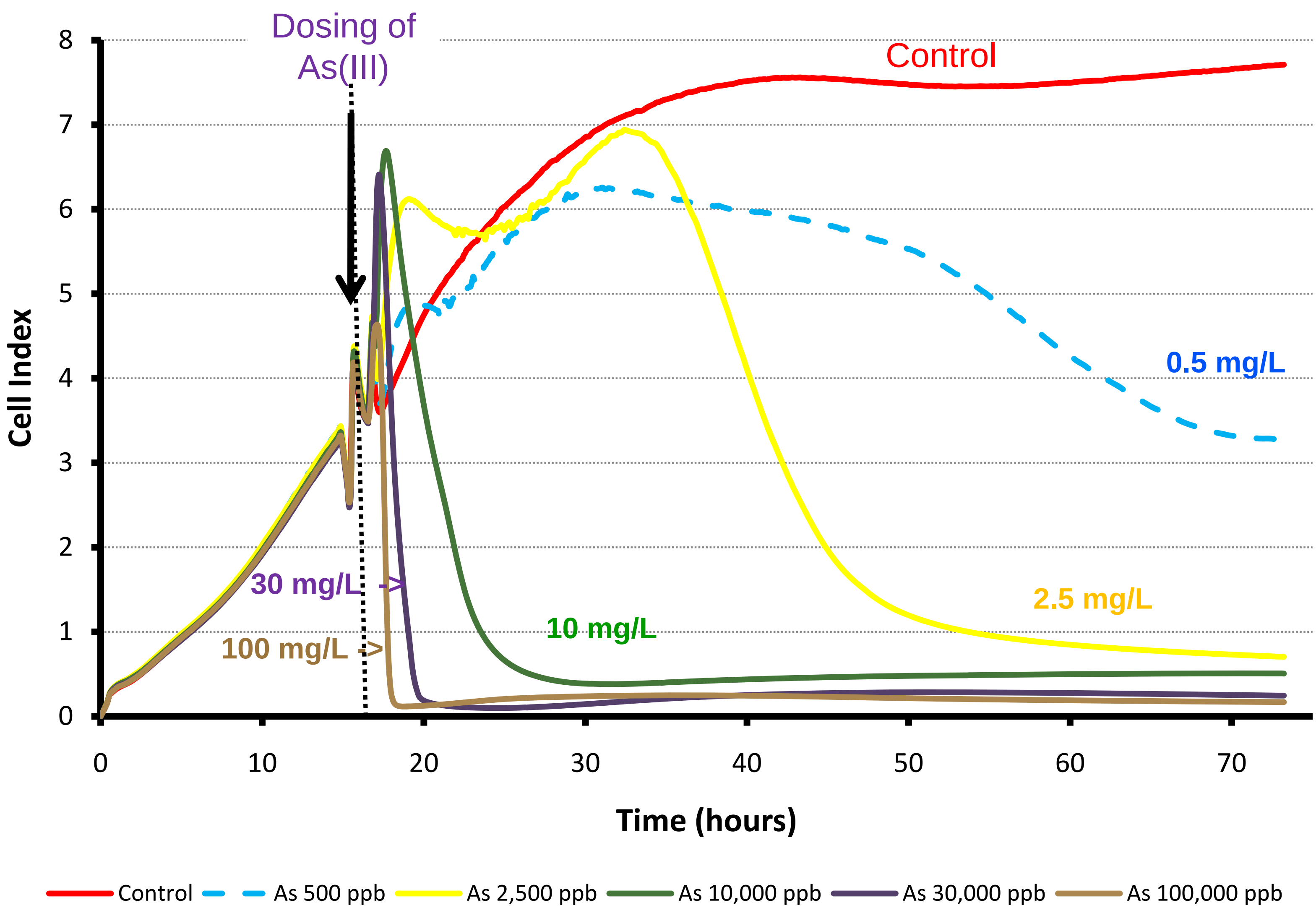
## CELL INDEX

$$CI = \max_{i=1...N}\left[\frac{R_{cell}}{R_b} - 1\right]$$

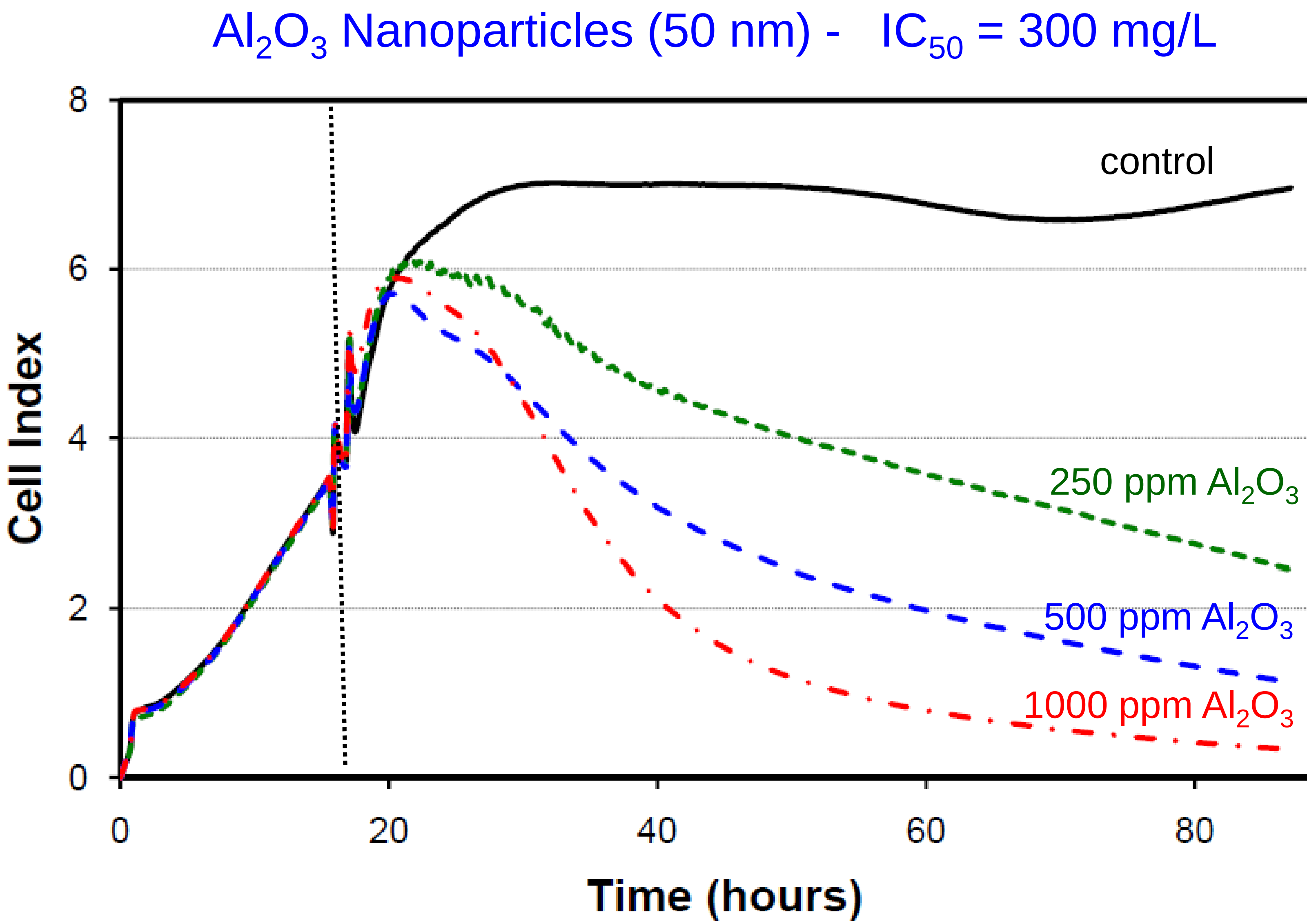



$R_{cell}$ = Electrode impedance with cells present
$R_b$ = Electrode impedance without cells
$N$= Number of points measured = 3

# Example Output RT-CES with As(III)

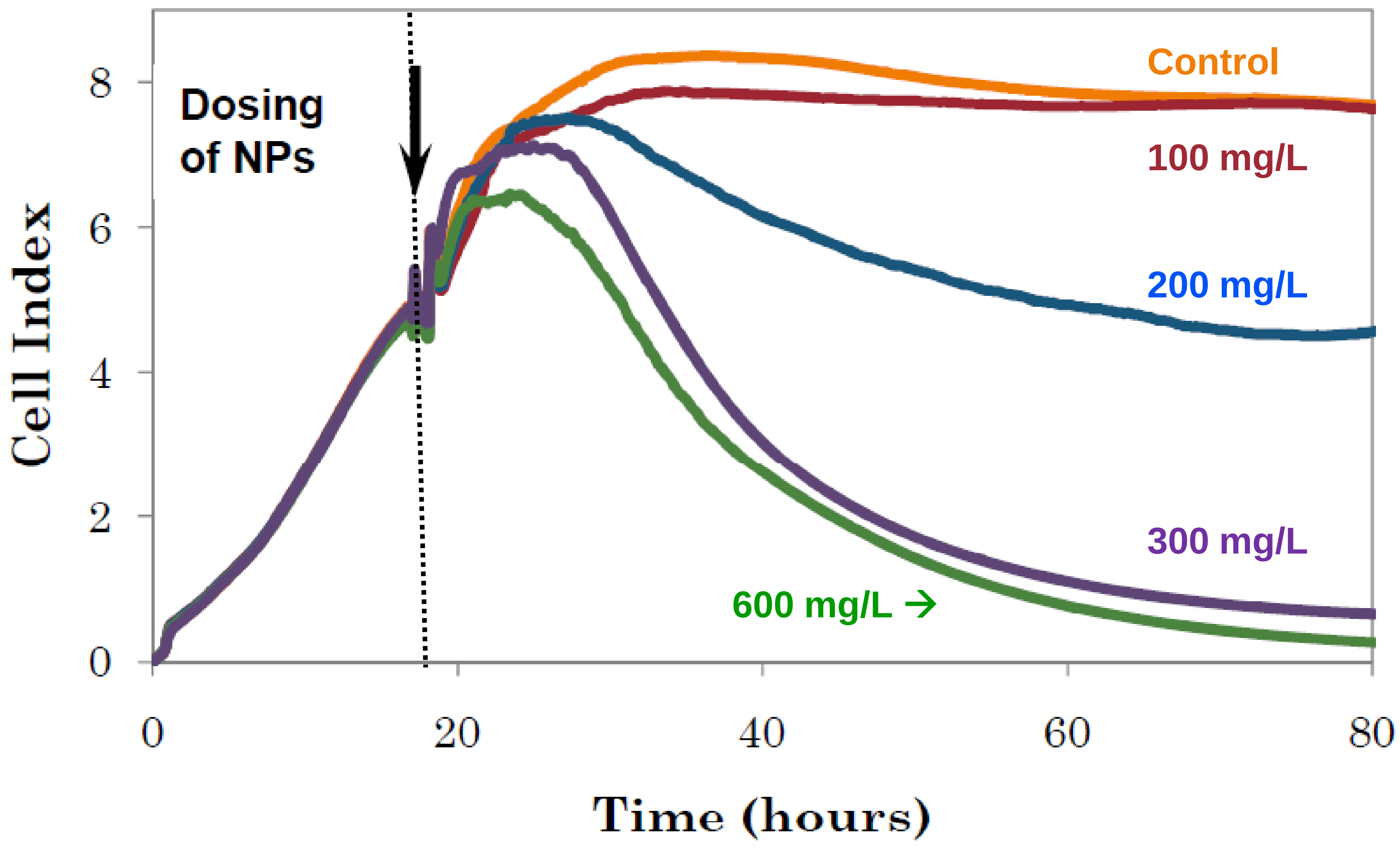


# RT-CES: $Al_2O_3$ Nanoparticles



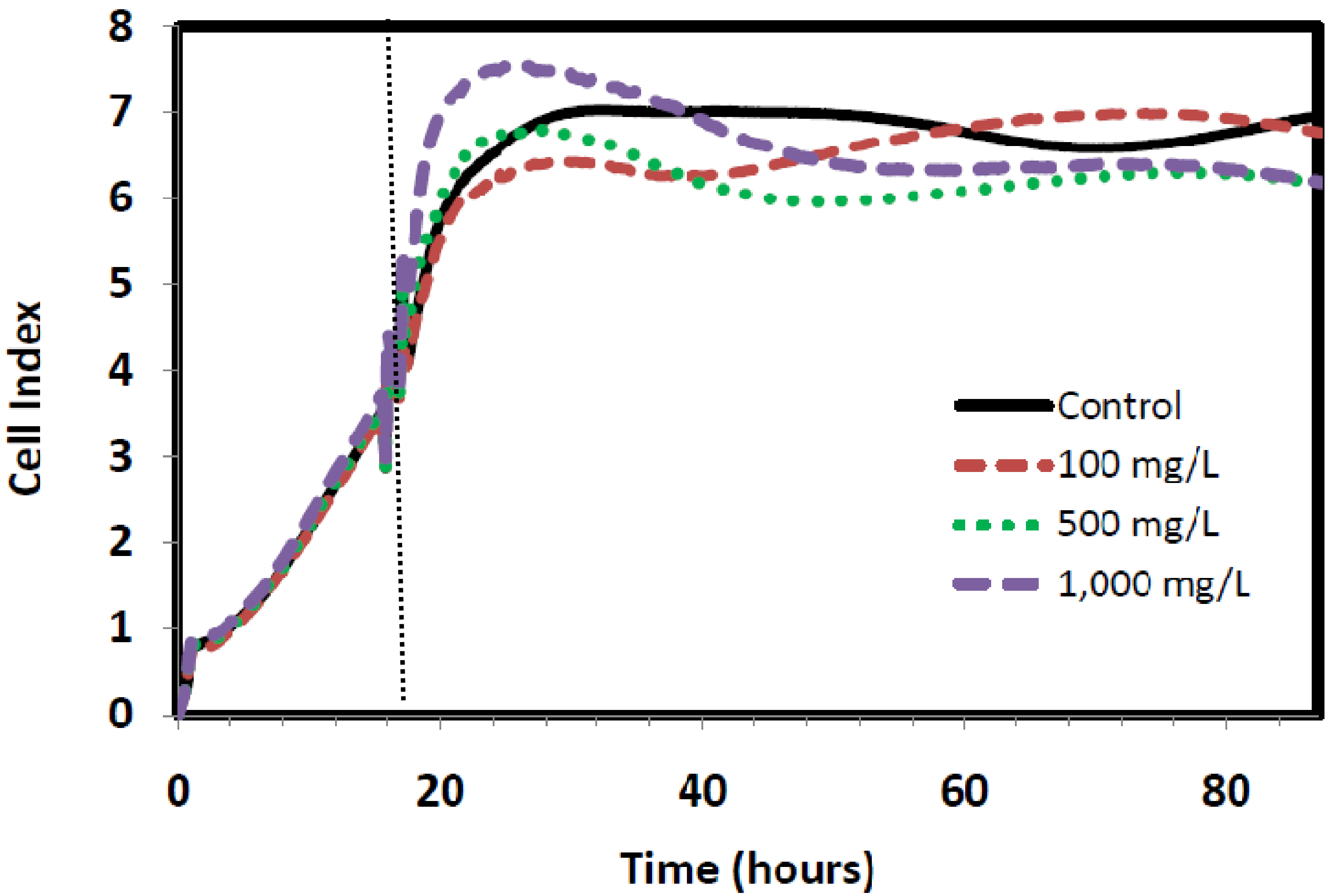
# RT-CES: $SiO_2$ nanoparticles

$SiO_2$ Nanoparticles (10-20 nm) - $IC_{50}$ = 225 mg/L

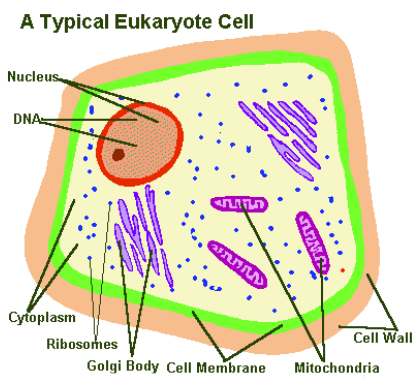


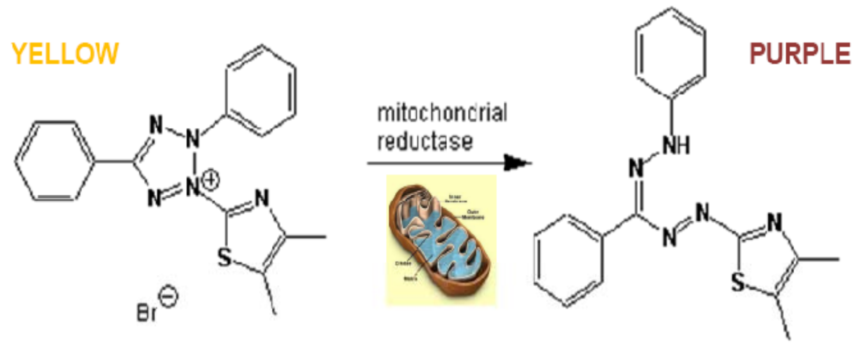
# RT-CES: $CeO_2$ nanoparticles

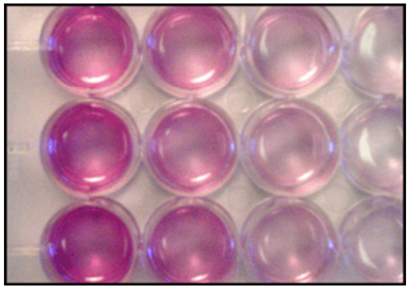
$CeO_2$ Nanoparticles (50 nm) - $IC_{50}$ > 1,000 mg/L



# MTT Bioassay



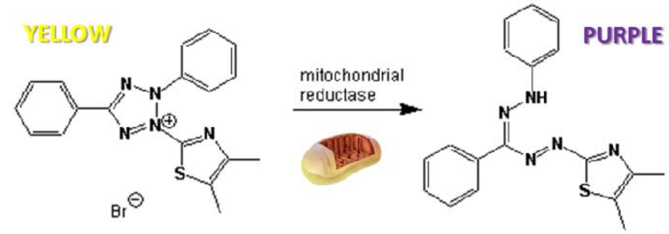



- Assay relies on the reduction by live cells of the water-soluble tetrazolium MTT salt to a colored formazan dye.
- Indicator of cell redox activity and viability

# MTT BIOASSAY

**Cells**
- MEM (10% FBS)
- 37°C, 5% $CO_2$

**24 wells plate**
- MEM (3.75% FBS)
- 500,000 cells/well



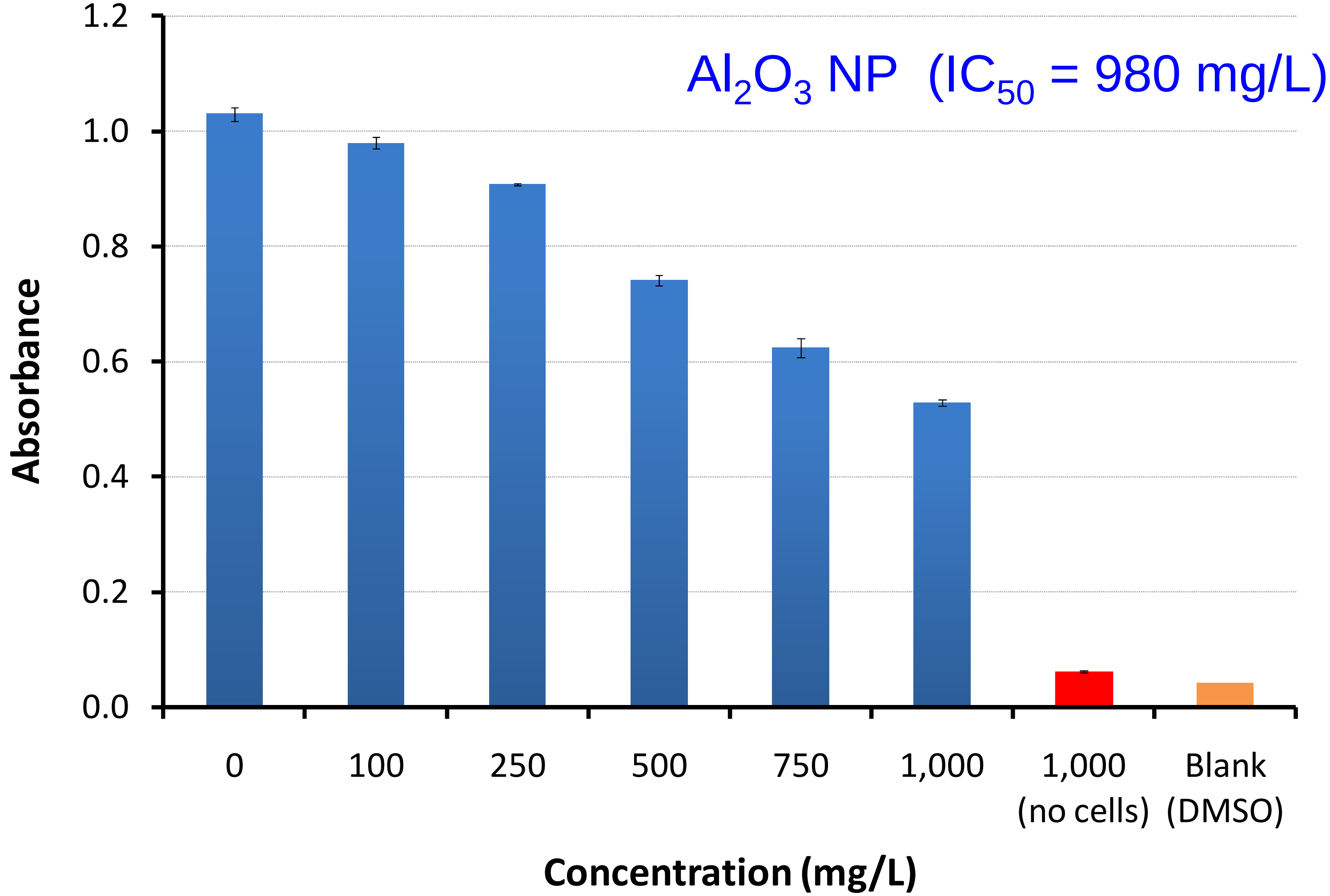

**Incubation**
- 37°C, 5% $CO_2$

**Staining**
- MTT reagent
- 0.4 mg/mL

**Measurement**
- Plate reader
- 550 nm

- Cells are transferred to a 24-well plate (5 $10^5$ cells/well)
- NPs dosed after **24** h of incubation
- After **48 h**, cells are washed and stained with MTT reagent

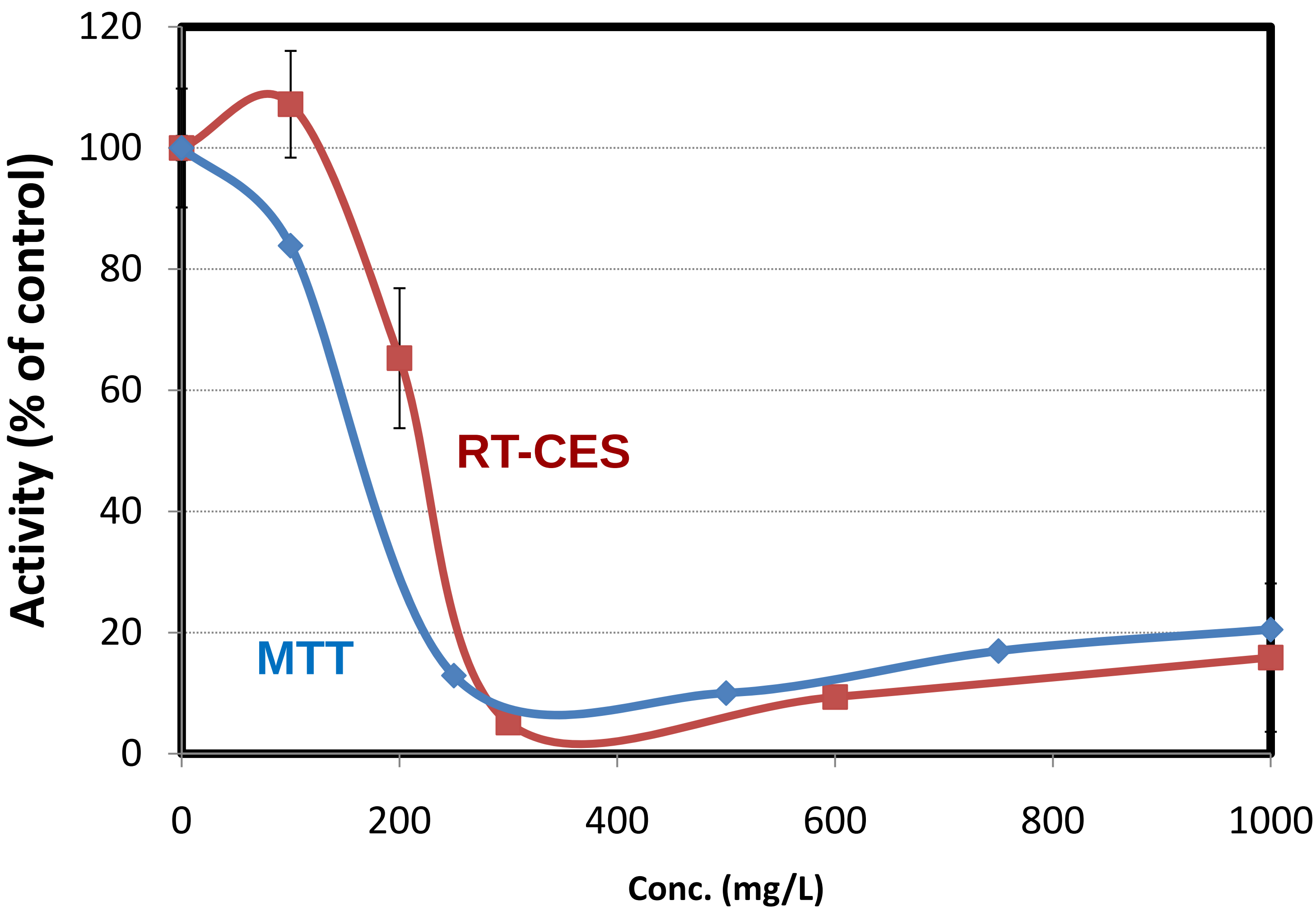
# MTT Bioassay: Nano- $Al_2O_3$



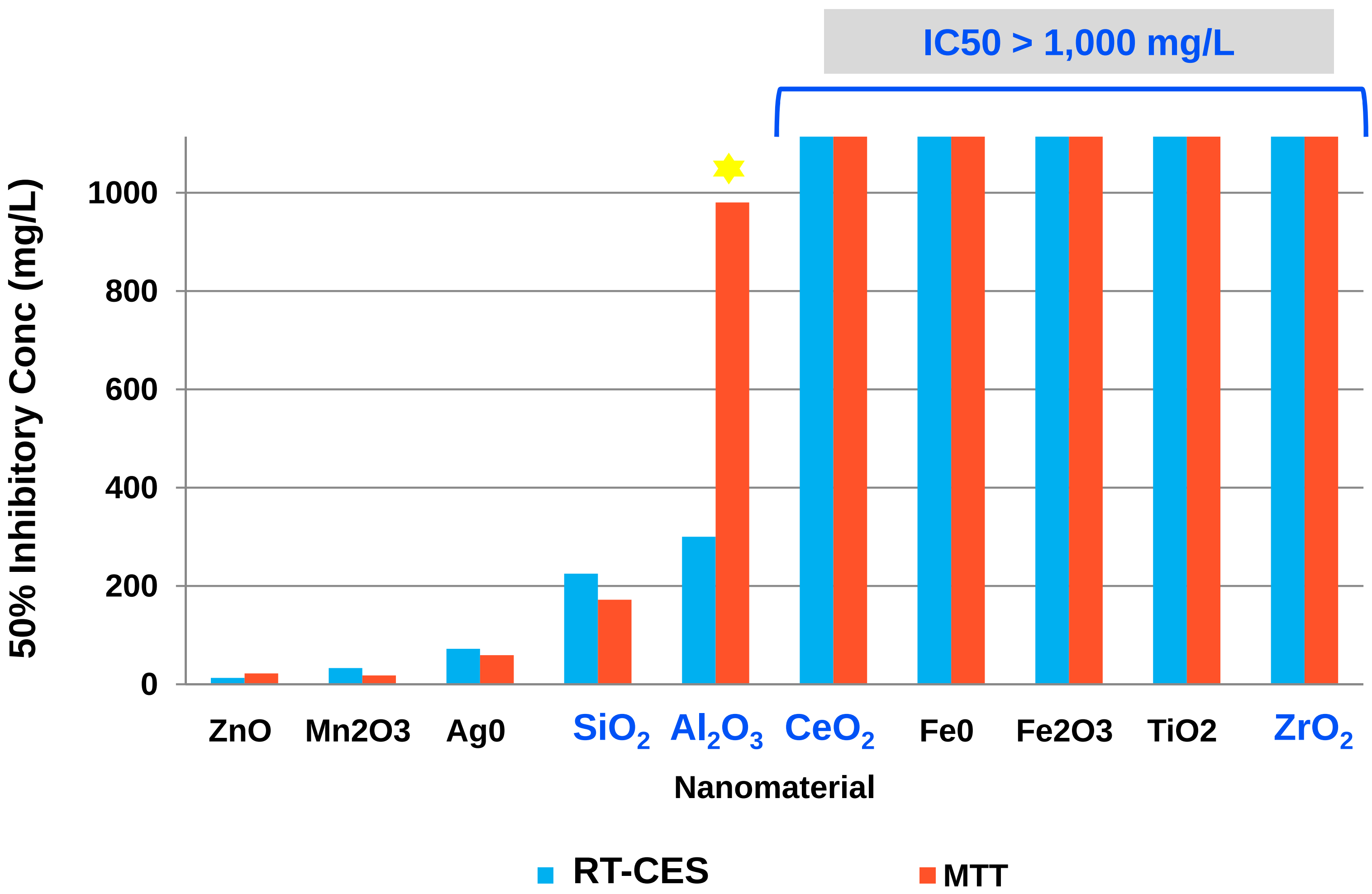

- NP did not interfere with the MTT analysis. Cell-free controls with the highest NP level caused a marginal increase of the absorbance relative to the DMSO control (2-3% of max. absorbance, depending on the NP used)

# RT-CES & MTT BIOASSAY: NANO- $SiO_2$

$SiO_2$ NPs- **$IC_{50}$** = 225 mg/L (RT-CES), 172 mg/L (MTT)



# Comparison RT-CES *vs* MTT Results




Conclusions: Good correlation between RT-CES and MTT results

$Al_2O_3$ and $SiO_2$ moderate toxicity, $CeO_2$ not toxic

# Aggregation of NPs in Biological Medium

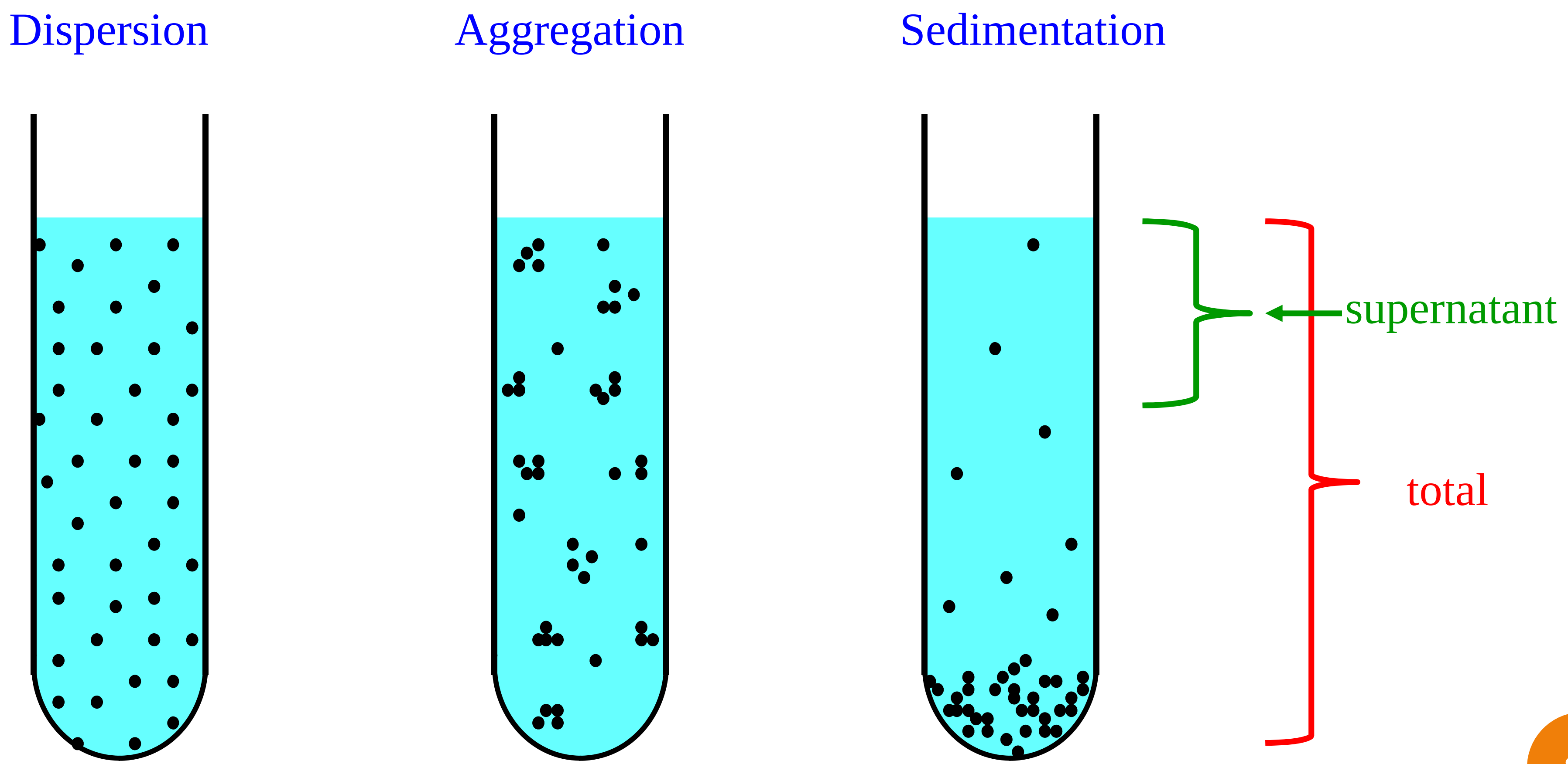
- Particle size distribution (PSD) & chemical analysis
  - Same conditions as in toxicity assays

Sampling

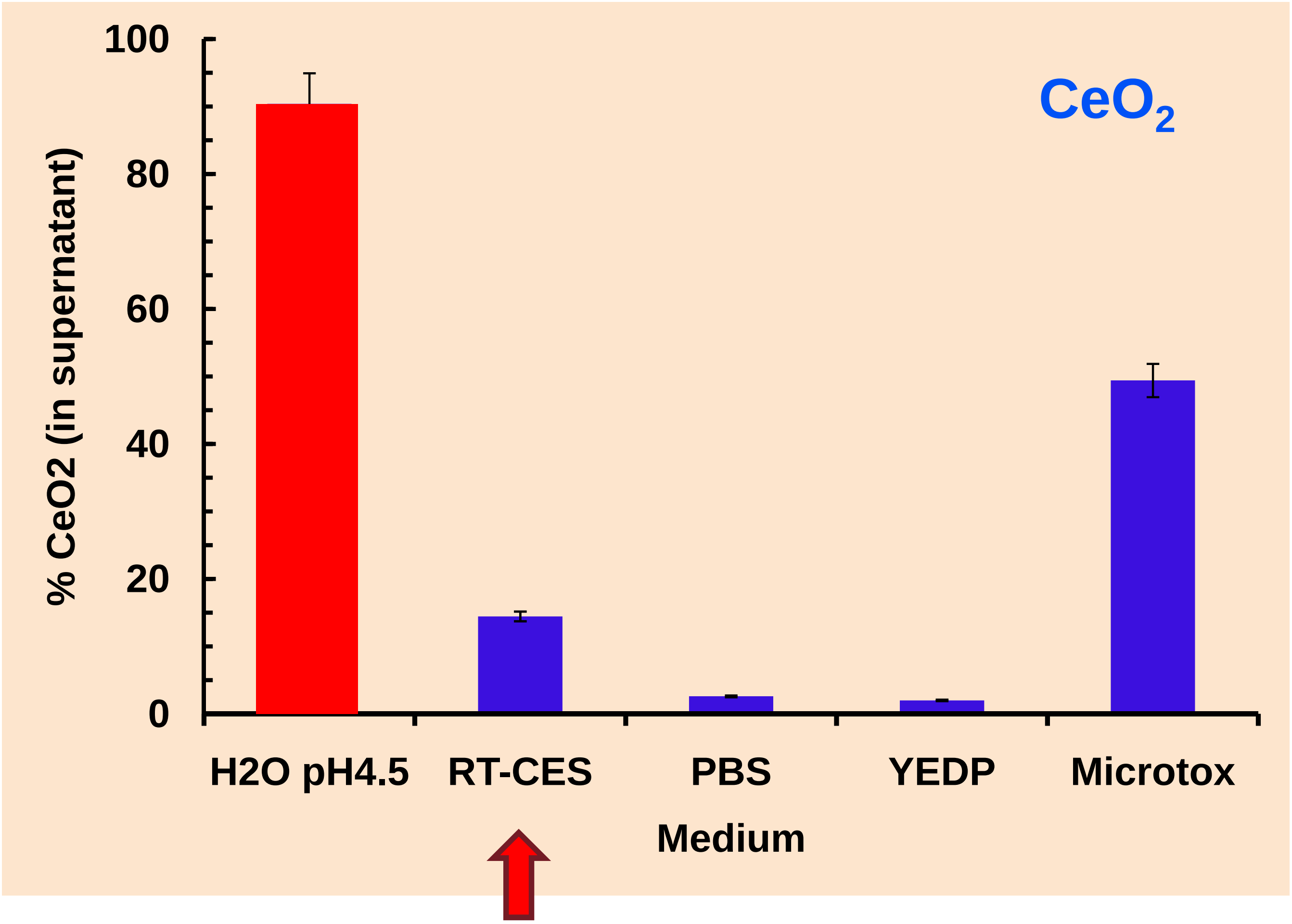
PSD & Zeta Potential (DLS)

Concentration (ICP-OES)

# Aggregation of NPs in Biological medium

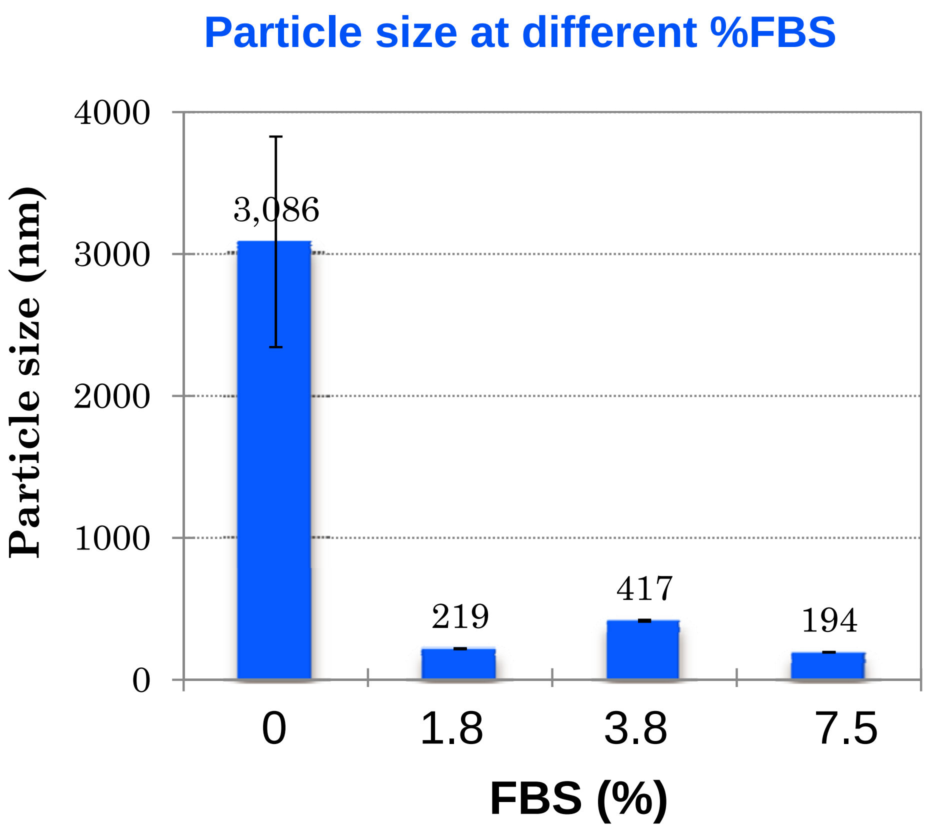


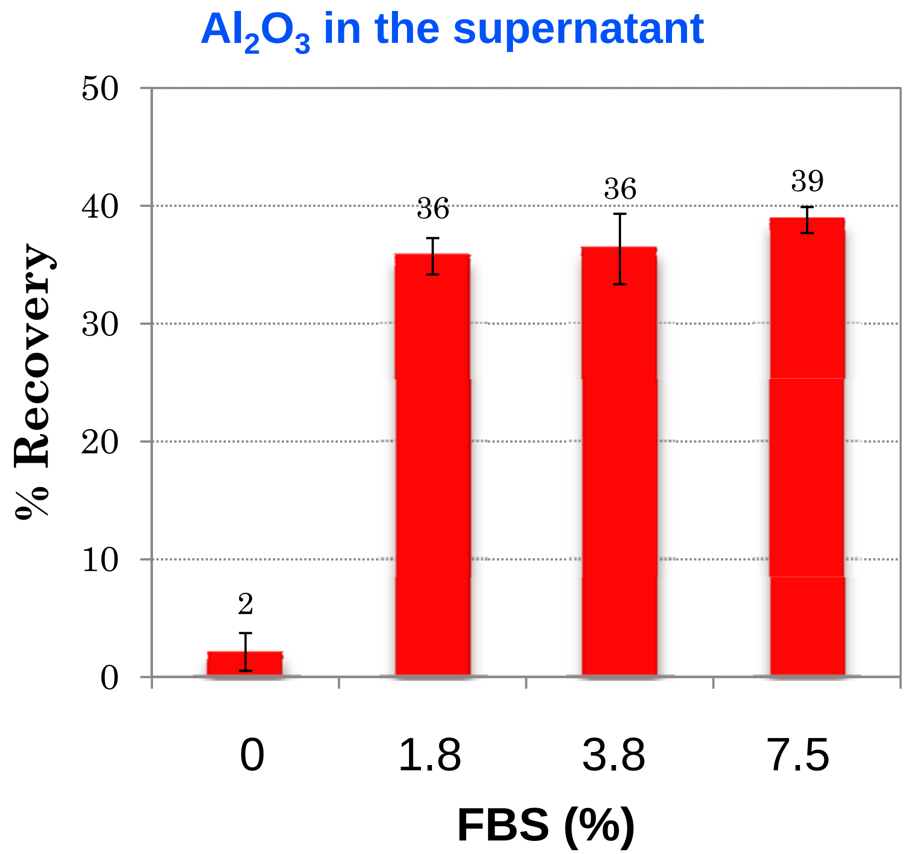
# Aggregation of NPs in Biological medium




In bioassay media, $CeO_2$ NP agglomerated and the concentration that is effectively dispersed decreased many-fold

# PROTEIN ADDITION (FBS)- Effect on Stability of $Al_2O_3$ NPs



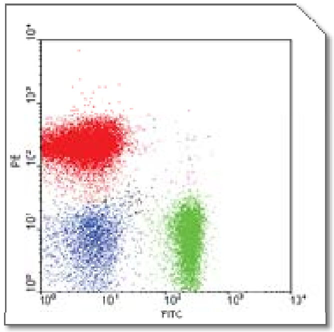

FBS in the growth medium (MEM) **stabilizes the $Al_2O_3$** NP dispersions

# CONCLUSIONS

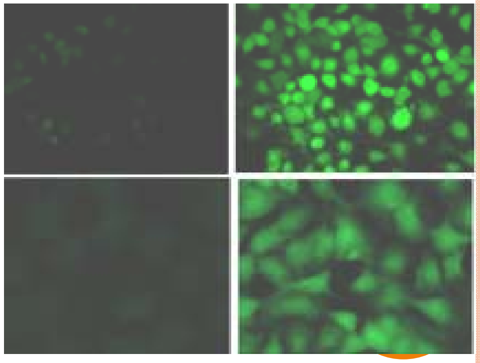
- RT-CES is a useful, high throughput technique for dynamic monitoring of NP cytotoxicity. The test relies on impedance measurements, avoiding interference problems often associated with colorimetric/fluorimetric tests.
- The inhibitory concentrations determined for NPs using the RT-CES technique correlated well with those generated by a commonly used cytotoxicity assay (MTT).
- $Al_2O_3$ and $SiO_2$ NPs showed **moderate toxicity** in the MTT and RT-CES assays, $CeO_2$ was not toxic at very high concentrations (1,000 mg/L)
- Most of the nanoscale inorganic oxides tested showed a high **tendency to aggregate in the RT-CES & MTT medium** resulting in micron-size aggregates that settled out of the dispersion.

# ONGOING RESEARCH:

## CYTOXICITY MECHANISMS OF INORGANIC OXIDE NPS

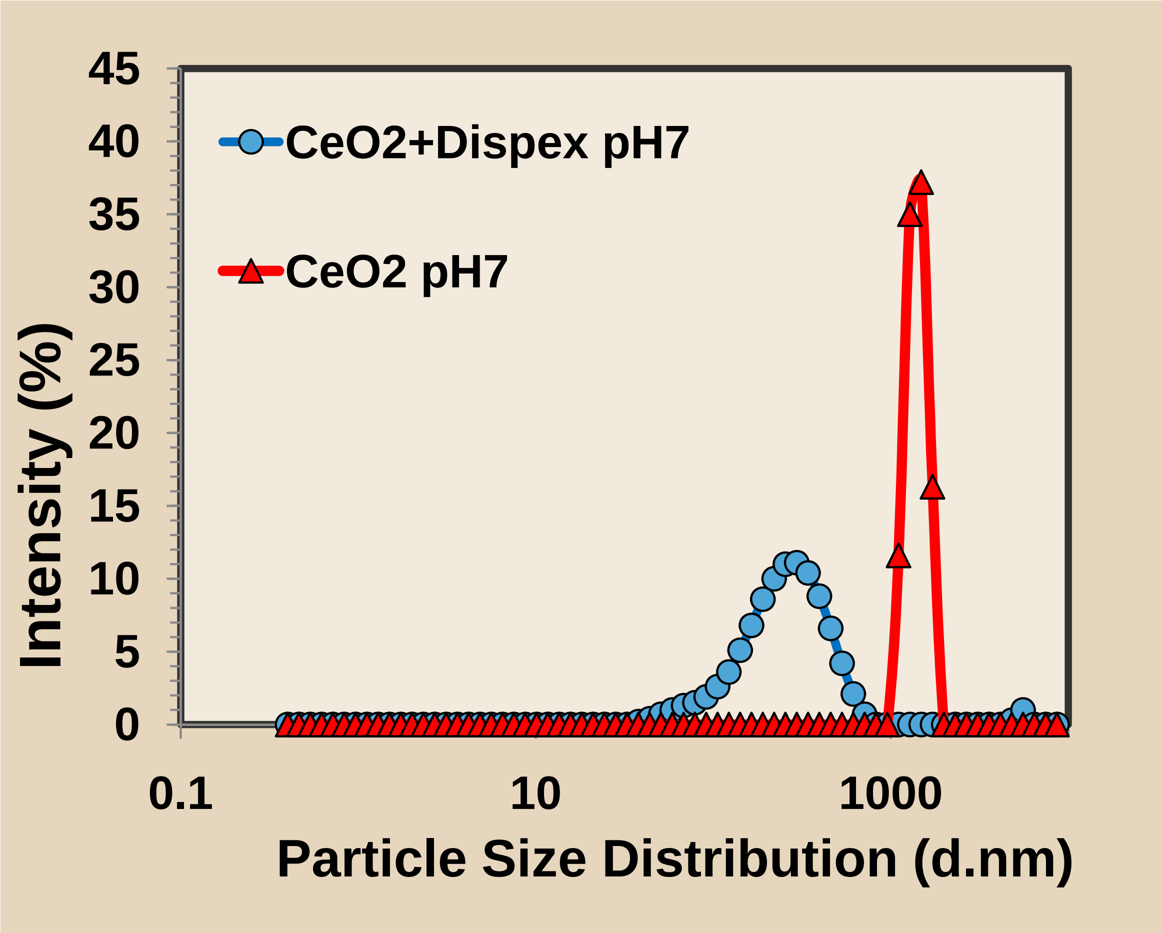


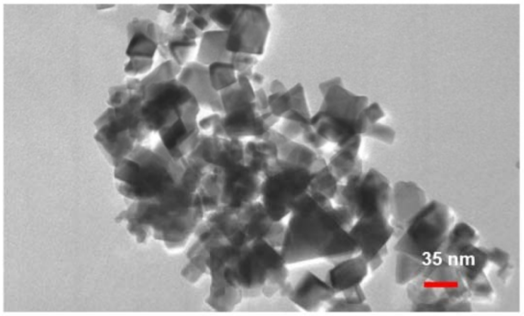
- Release of toxic products
- Disruption of cell membrane (flow cytometry)
- ROS formation (intra- and extracellular generation)
- Protein damage (ELISA bioassay)



# ONGOING RESEARCH:

## REDUCING AGGREGATION OF NPS IN BIOLOGICAL MEDIUM



# ACKNOWLEDGEMENTS


- SEMATECH / SRC
- UA Nanotoxicity group