Cell-Based Toxicity Assay-on-Chip for the Next-Generation CMOS Technology

(Task Number: 425.037)

PIs:

- **Shyam Aravamudhan, Nanoengineering, NC A&T**
- **Shanthi Iyer, Nanoengineering, NC A&T**
- **Marinella Sandros, Nanoscience, UNC-Greensboro**
- **Ethan W. Taylor, Nanoscience, UNC-Greensboro**

Graduate Students:

- **Komal Garde, Nanoengineering, NC A&T**
- **Mubin Tarannum, Nanoengineering, NC A&T**
- **Karshak Kosaraju, Nanoscience, UNC-Greensboro**
- **Soodeh Ravari, Nanoscience, UNC-Greensboro**

Other Researchers:

- **Steven Crawford, Research Technician, Nanoengineering, NC A&T**

Cost Share (other than core ERC funding):

- **25% cost-share (in cash) from Joint School of Nanoscience and Nanoengineering (collaboration between NCA&T and UNC-Greensboro)**

Objectives

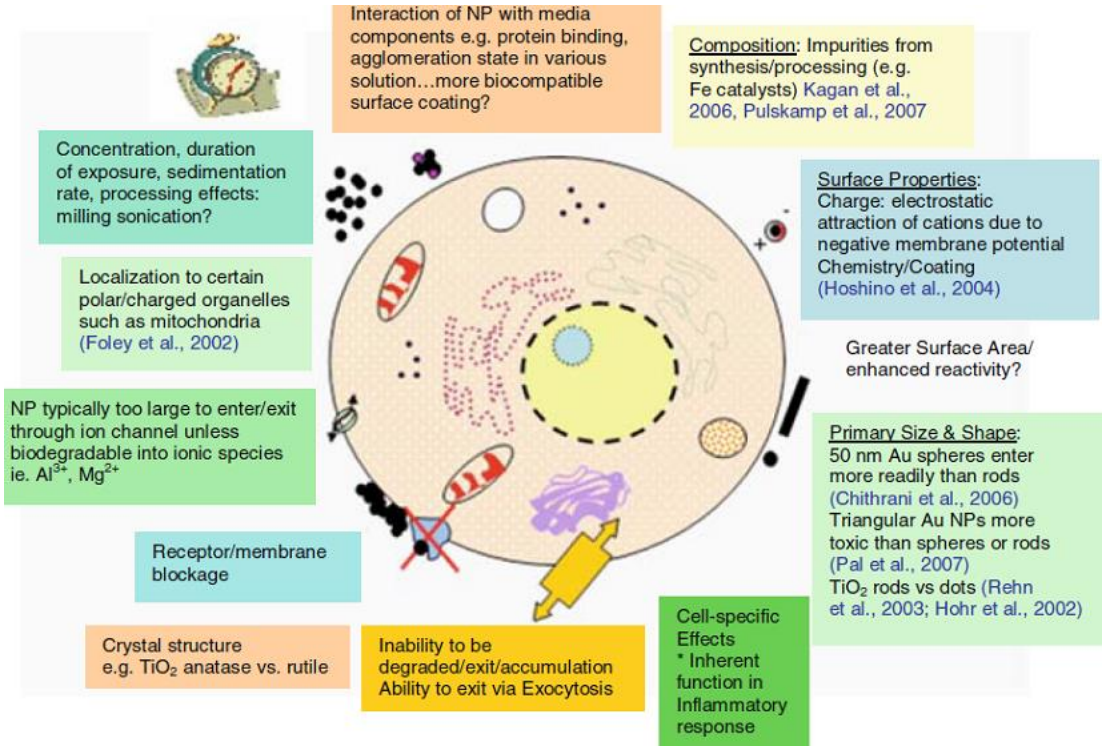
- **Long-term objectives**
  - Understand ESH of Engineered Nanomaterials (ENs) used/to be used in semiconductor industry, particularly CMP nanoparticles slurries and CNTs
  - Develop high-content assays-on-chip and analytical/microscopic methods to assess rapidly influence of physiochemical properties on ESH
- **Year 2 goals**
  - Optimize high-content assessment methods to monitor longitudinal CBCs (critical biological consequences) - both acute and delayed toxicity
  - Comprehensive physio-chemical characterization of 4 model slurries, namely colloidal silica, fumed silica, ceria and alumina NPs
  - Study the path/mechanism of cellular uptake and internalization of NPs (from slurries) on interaction with mammalian cells
- **Developed comprehensive longitudinal methods to study acute and delayed cellular uptake and internalization of NPs – ICP-OES, Confocal Raman and Comet Assays**
- **Validated novel assessments methods – ECIS, ultrastructural microscopy with conventional cell viability and toxicity assays**

ESH Metrics and Impact

1. **Reduction in the use or replacement of ESH-problematic materials (CMP NP slurries and CNTs)**
   - Conducted comprehensive physicochemical characterization of four model slurries and identified using toxicity and uptake assays relationship between slurry NP properties and toxic potential
2. **Reduction in emission of ESH-problematic material to environment**
   - Conducting CMP of oxide, III-V (GaAs) and Cu surfaces using the given modelslurries; analyze physicochemical and toxic properties of slurry waste after dilution/neutralization procedures
3. **Reduction in the use of natural resources (water and energy)**
   - Results from Metric 2 will inform us on efficient use of water for dilution/neutralization procedures
4. **Reduction in the use of chemicals**

Understanding Nanotoxicity

- Number of physicochemical properties and mechanisms occur at the surface of nanomaterial, on its interaction with and inside a cell, thus affecting toxicity (see figure)
- Variability is huge due to different properties, cell types, processing and assay conditions
- Studies with fully characterized model materials exposed to same cell types and conditions is ideal
- Establish dose-response relationships for predictive nanotoxicity and safe design of EN



Schematic representation of some possible interactions of nanomaterials with a cell in culture media (Schrand et al., 2009. Ch. in Safety of Nanoparticles, T.J. Webster (ed.))

Critical Questions to be Addressed

- **Focusing the research efforts, Schrurs et al. Nature Nanotechnology, 2012**
  - Are ENs more cytotoxic than their larger counterparts?
  - Does EN aggregation influence the cytotoxic activity?
  - Which properties of ENs drive their cytotoxic activity? Physiochemical properties such as size, distribution, aggregation, shape, composition, area, morphology, surface charge, chemistry, protein corona
  - Does cytotoxicity of ENs vary with cell type?
  - Do ENs penetrate into cells and how does intracellular trafficking of ENs occur?
  - Address technical interferences and positive control

Experimental Method and Approach

- **Nanoparticles (NPs) and NP slurries investigated**

Sample	Composition	pH	Size (nm)
Colloidal Silica (NS-0813-01)	3% precipitated silica; adjusted with acetic acid	2.5-4.5	50-60
Fumed Silica (NS-0813-02)	5% silica; adjusted with KOH	10	120-140
Ceria (NS-0813-03)	1% Ceria;	3-4	60-100
Alumina (NS-0813-04)	3% Alumina; adjusted with nitric acid	4.5-5	80-100

Pristine Particles	Size (nm)
Colloidal Silica (PS1)	80
Colloidal Silica (PS2)	200
Silica MPs (PS3)	1-3 µm
Ceria NPs (PC1)	50-105
Ceria MPs (PC2)	1-2 µm
Alumina NPs (PA1)	80
Alumina NPs (PA1)	<10 µm

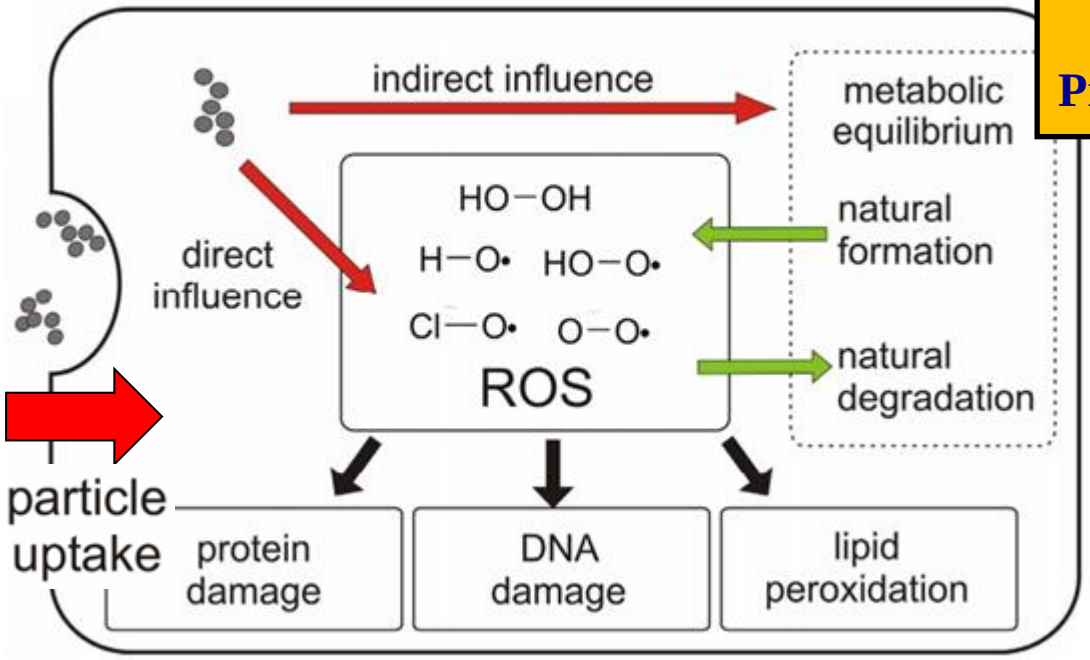
  - **Multi-Wall CNTs: Long- 3-5 nm x 5-20 µm; Short - 1-3 nm x 5-9 µm**
- **Cells studied**
  - **A549 adenocarcinomic human alveolar basal (lung) epithelial cells**
  - **NIH/3T3 fibroblasts and PC-12 neurons (previously)**

Experimental Pathway

I **Comprehensive Characterization of ENs**
(DLS, Zeta potential, BET, FTIR, XRD, Raman, ICP-OES, HRSEM, TEM)

High-Content Assays/Longitudinal Methods

II **Acute/delayed cytotoxicity (cell viability and membrane integrity) using MTT and LDH assays**



III **Acute/delayed cellular uptake and internalization**
(ICP-OES, Confocal Raman, ECIS, HRTEM, ROS)

IV **Delayed genotoxicity studies – Comet assays**

Safe EN design and use; Predictive toxicity

I. Characterization of Nanomaterials

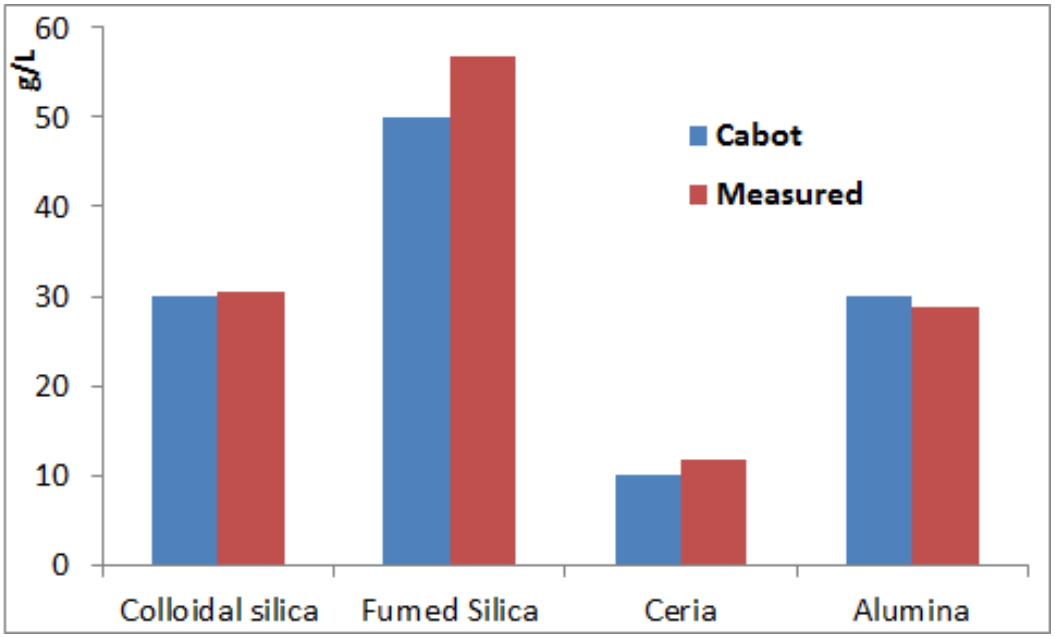
- **Particle Size Distribution (PSD) and Zeta Potential**

Slurry no.	Dilution	Diluent	PSD (d.nm)	Zeta (mV)
1 (50-60 nm)	10x	Water	36.63/0.1	-
	100x	Water	38.31/0.1	-
2 (120-140 nm)	100x	Water	124.2/0.1	-
	1000x	Water	122.4/0.1	-
	10x	Media	145.3/0.2	-
3 (60-100 nm)	100x	Water	145/0.2	42.7/8
	1000x	water	149.5/0.2	32.8/11.7
	100x	Media	193.9/0.2	-
4 (80-100 nm)	100x	Water	137.1/0.1	31.4/14
	1000x	Water	190.5/0.3	32.6/12
	100x	media	187.3/0.2	

- **Dynamic light scattering (DLS) in Malvern ZetaSizer Nano ZS instrument with HeNe laser (633 nm) and 175° scattering angle**
- **Refractive index - Alumina – 1.726, Ceria – 2.2, Silica – 1.47**
- **After dilution, samples sonicated for 30 minutes and pH measured**
- **Measured PSD/ZP for pristine NPs and MWCNTs with/without surfactants (10-30% of Pluronic, SDS and Dowfax)**
- **Both short/long MWCNTs gave stable dispersions in 10% SDS with Zeta Potential > 60 mV**

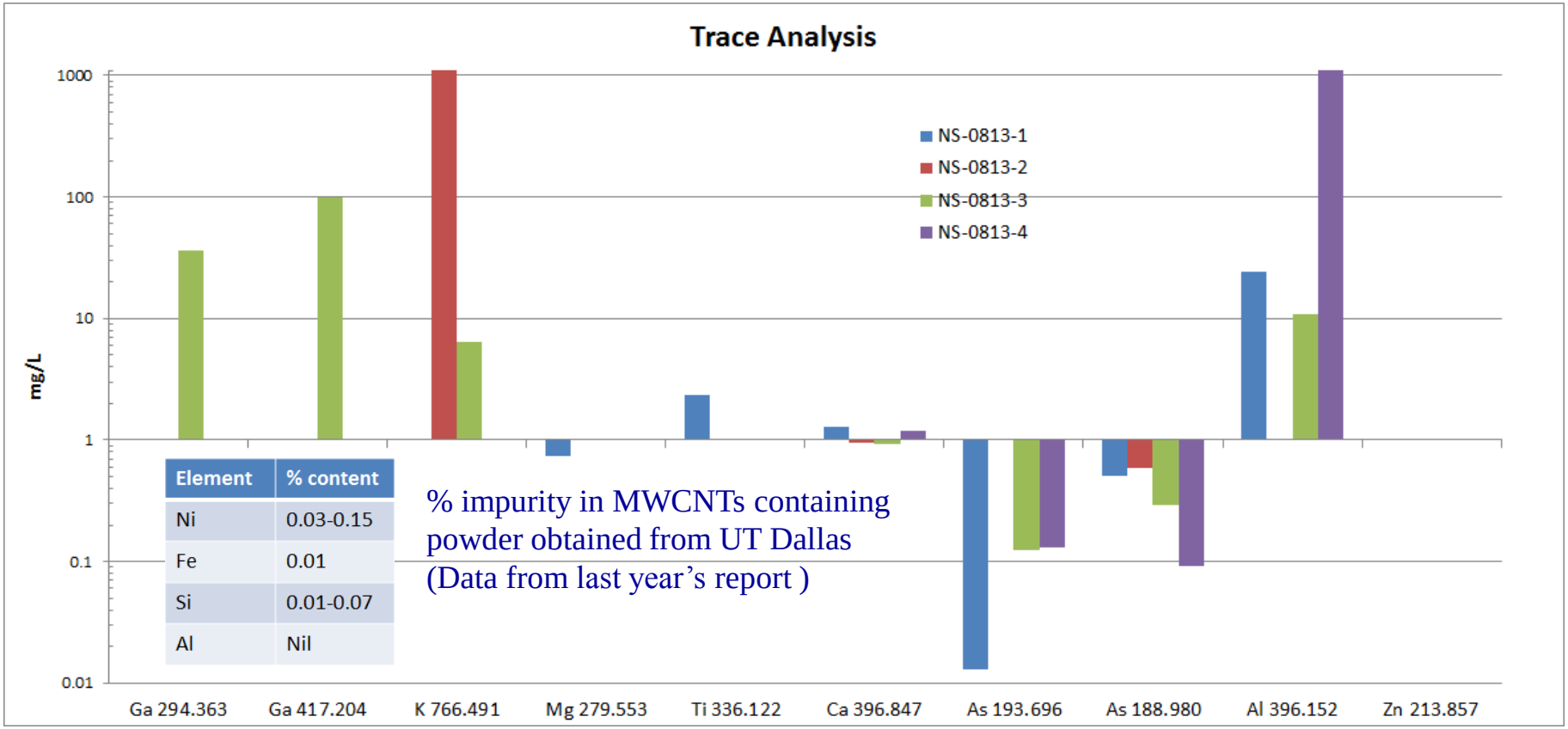
I. Characterization of Nanomaterials

- **Elemental analysis of slurries using ICP-OES (Varian 710 ES ICP Axial Spectrometer)**
- **Samples were digested as suggested by UA**
  - **Silica slurries in 1% HF**
  - **Ceria slurries in 70% HNO_3 and 30% H_2O_2**
  - **Alumina slurries in 3.65% HCl**



I. Characterization of Nanomaterials

- **Trace elemental analysis of slurries using ICP-OES**



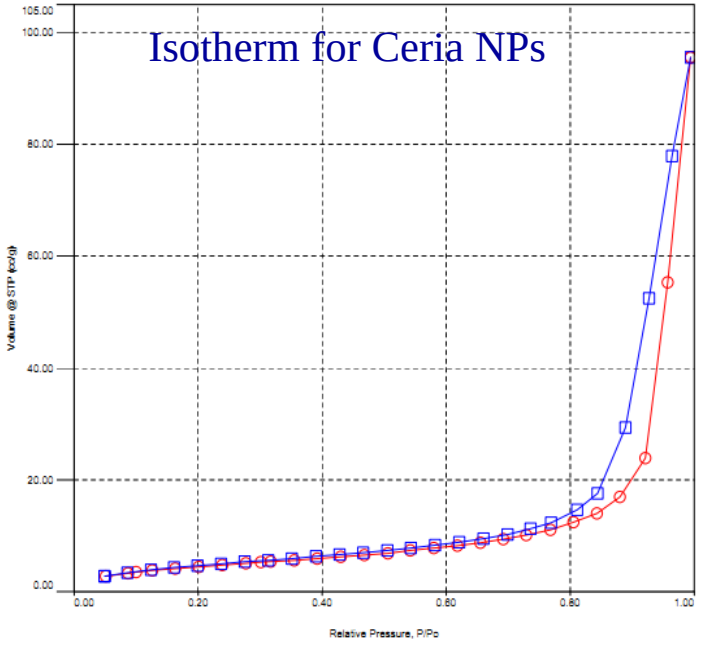
Element	% content
Ni	0.03-0.15
Fe	0.01
Si	0.01-0.07
Al	Nil

% impurity in MWCNTs containing powder obtained from UT Dallas (Data from last year's report)

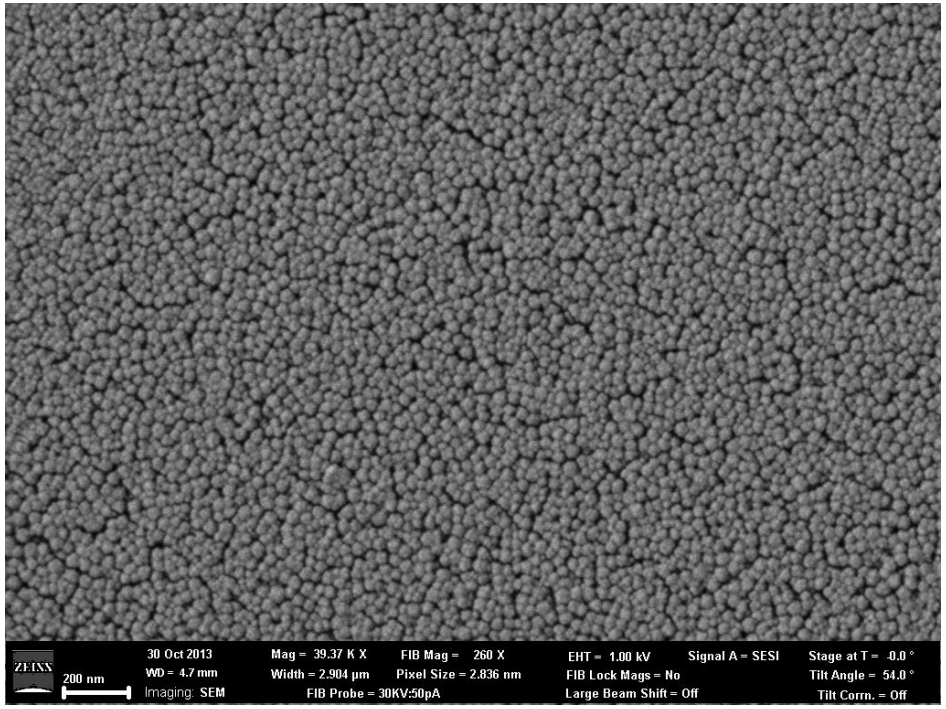
I. Characterization of Nanomaterials

- **NOVA quantachrome 2200e BET surface area analyzer**
- **Dried slurries were degassed for 4 hours after which nitrogen adsorption and desorption curves were obtained.**
- **Surface area was calculated using an extension of Langmuir Theory**
- **NPs in slurry 1, which are smaller in size as compared to NPs in slurry 2, have a larger surface area than that of slurry 2**

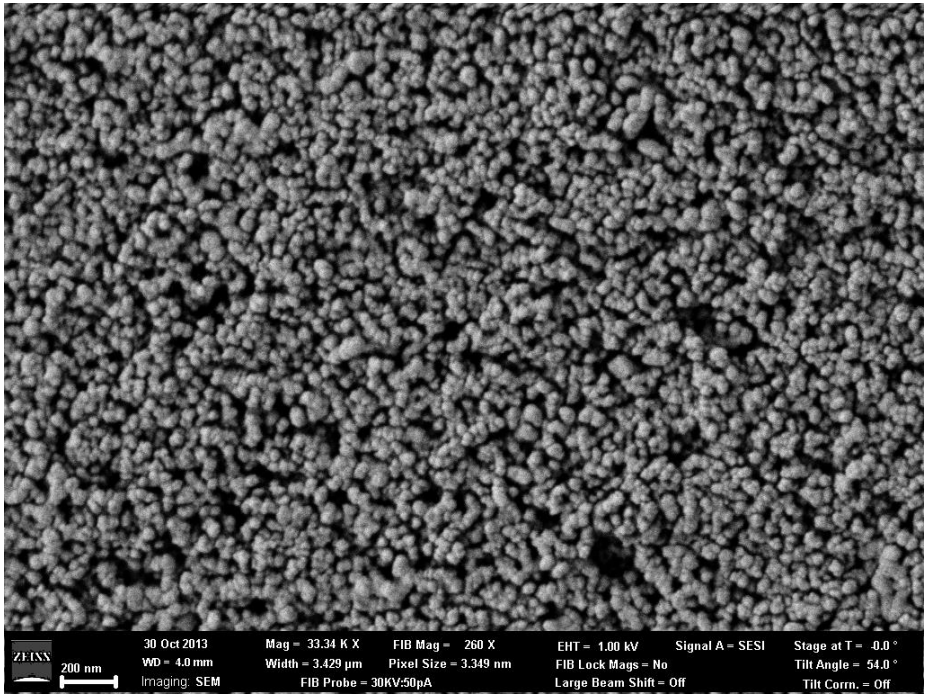
Slurry	Surface Area (m^2/g)
NS-0813-1 (S1)	99.509
NS-0813-2 (S2)	50.997
NS-0813-3 (S3)	16.979
NS-0813-4 (S4)	50.37



I. Characterization of Nanomaterials



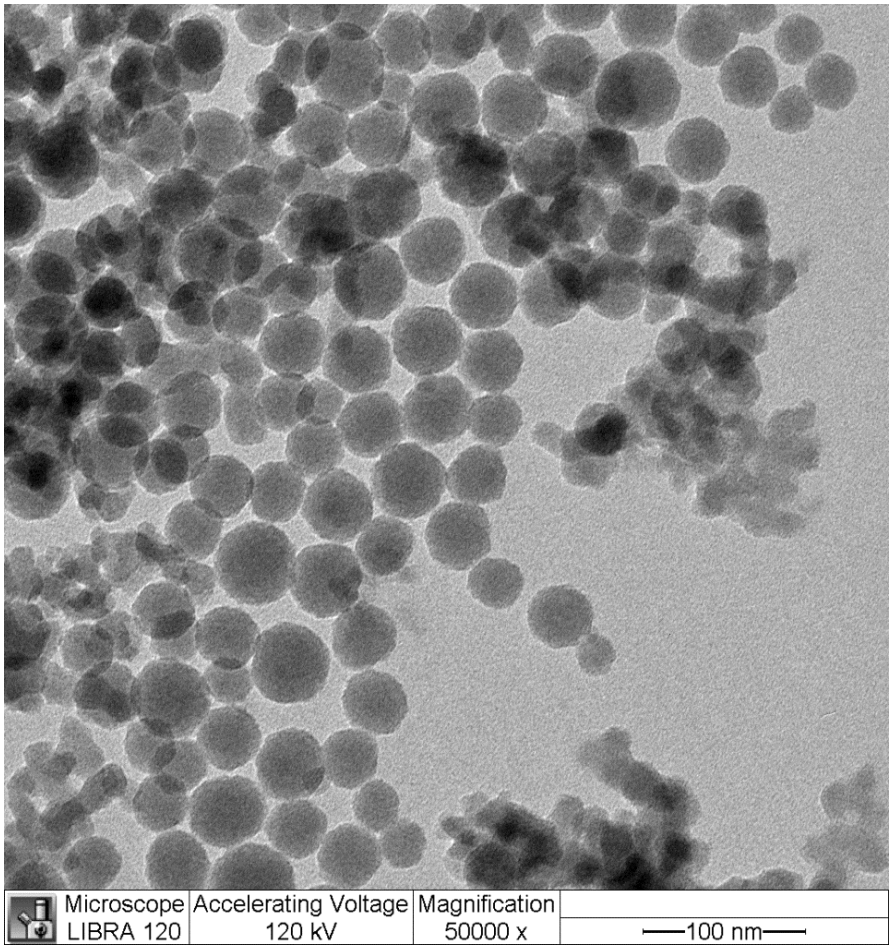
SEM of Silica NPs in Slurry 1



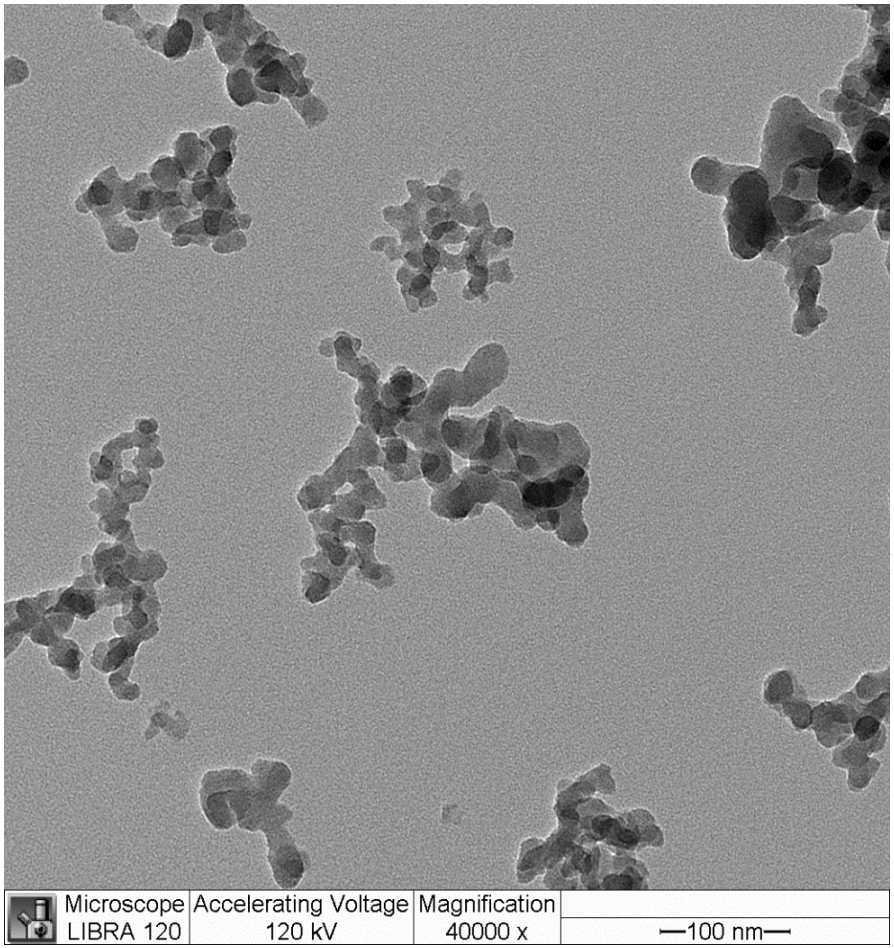
SEM of Silica NPs in Slurry 2

- **Drop of sample was directly placed on carbon tape (no dilution) and sputter coated with Au**

I. Characterization of Nanomaterials

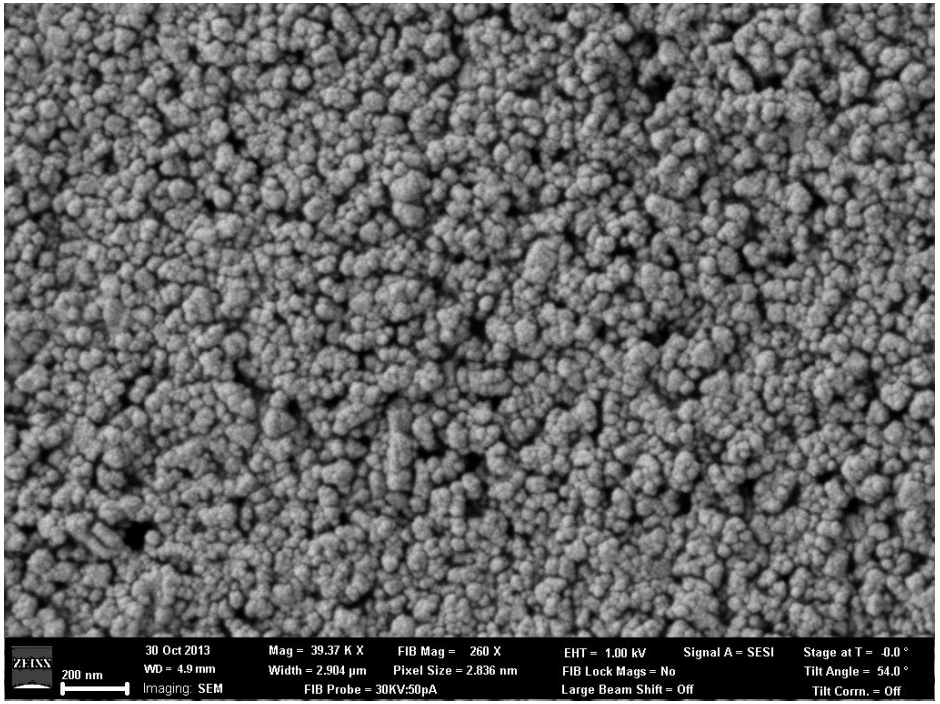


TEM of Silica NPs in Slurry 1

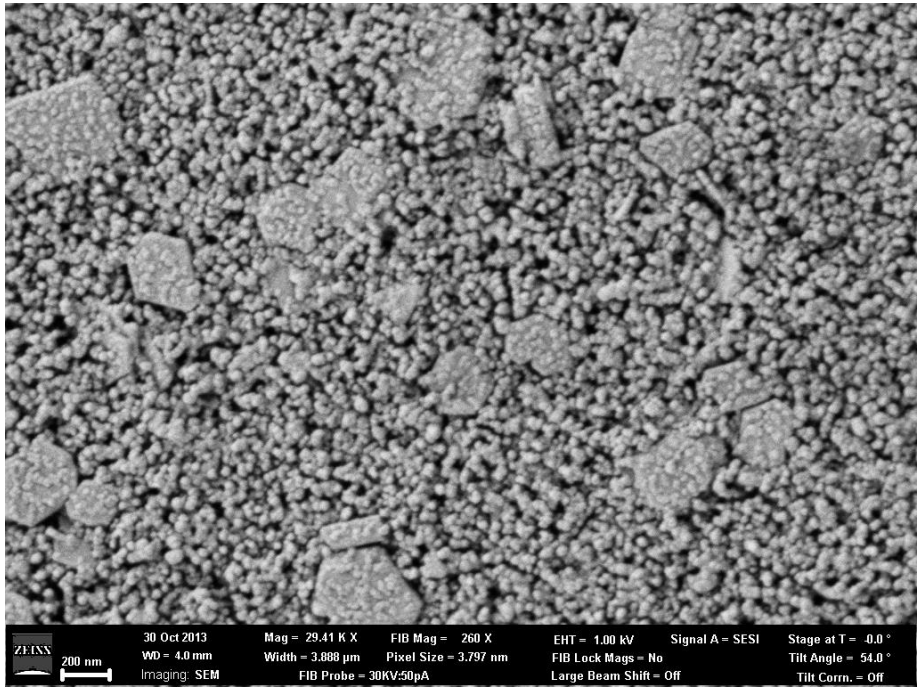


TEM of Silica NPs in Slurry 2

I. Characterization of Nanomaterials



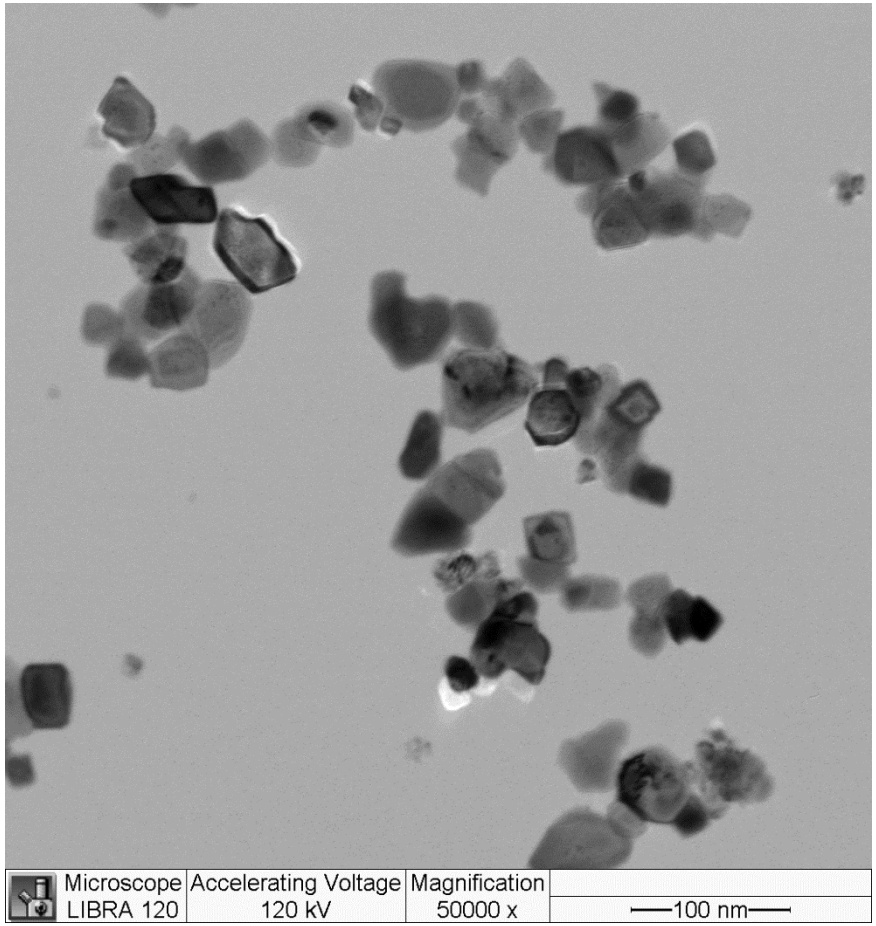
SEM of Ceria NPs in Slurry 3



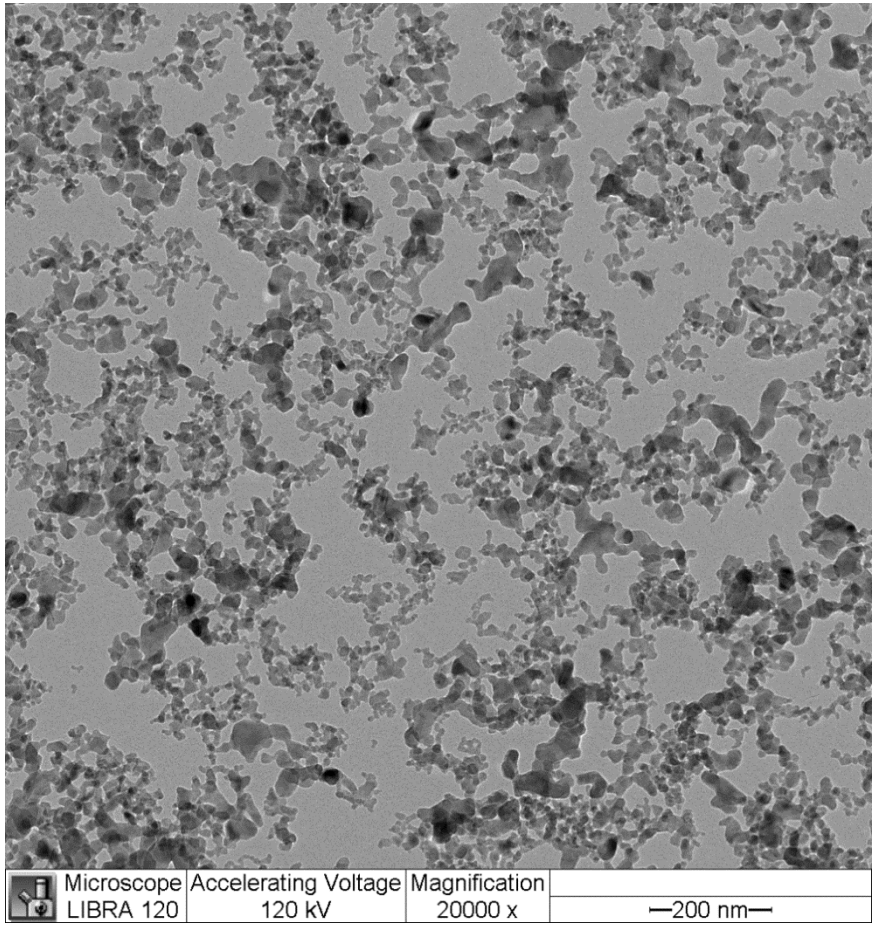
SEM of Alumina NPs in Slurry 4

- **Drop of sample was directly placed on carbon tape (no dilution) and sputter coated with Au**

I. Characterization of Nanomaterials



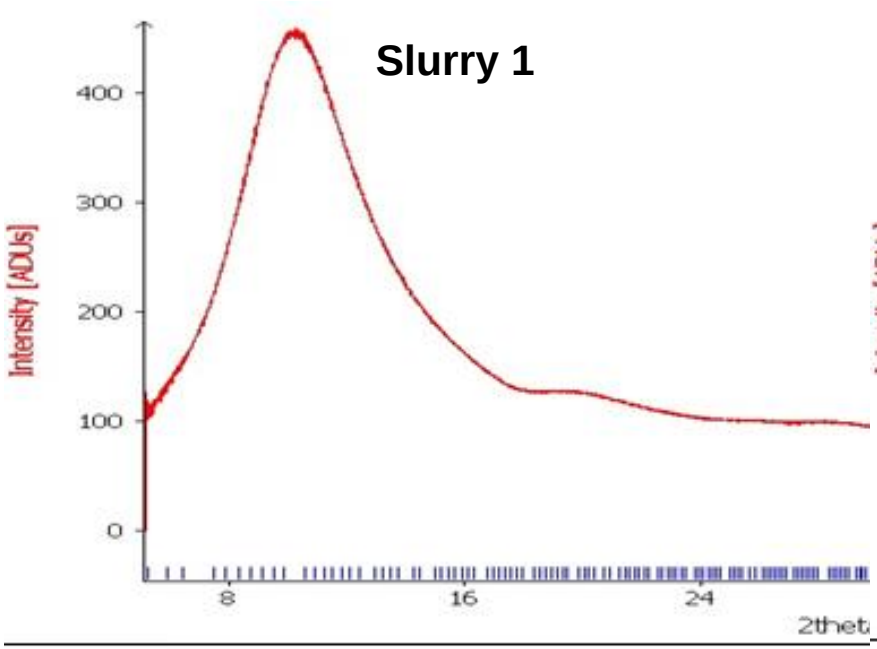
TEM of Ceria NPs in Slurry 3

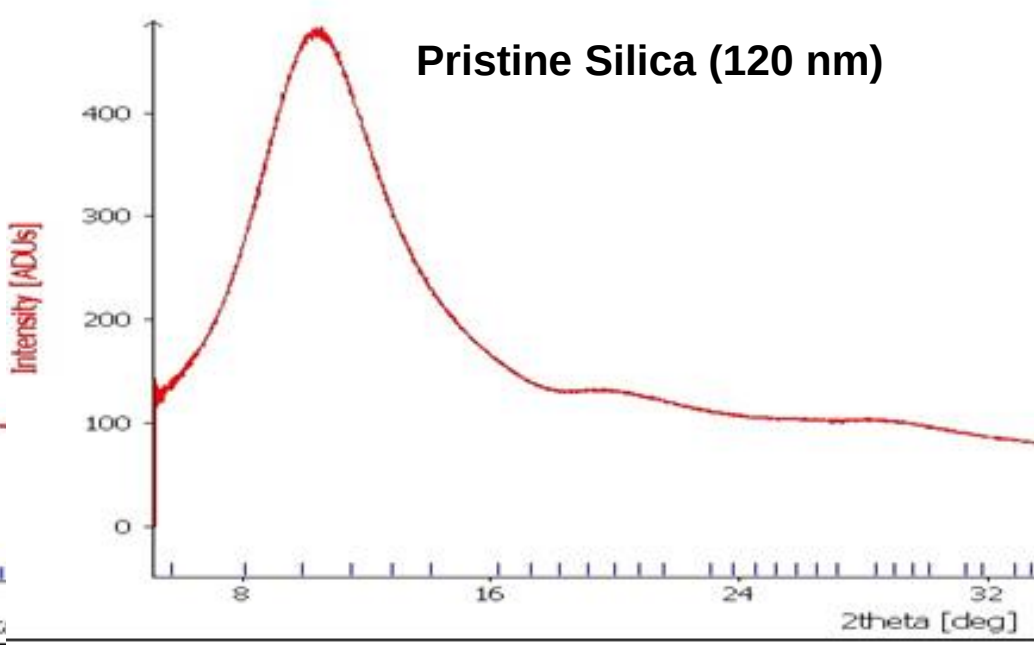


TEM of Alumina NPs in Slurry 4

I. Characterization of Nanomaterials

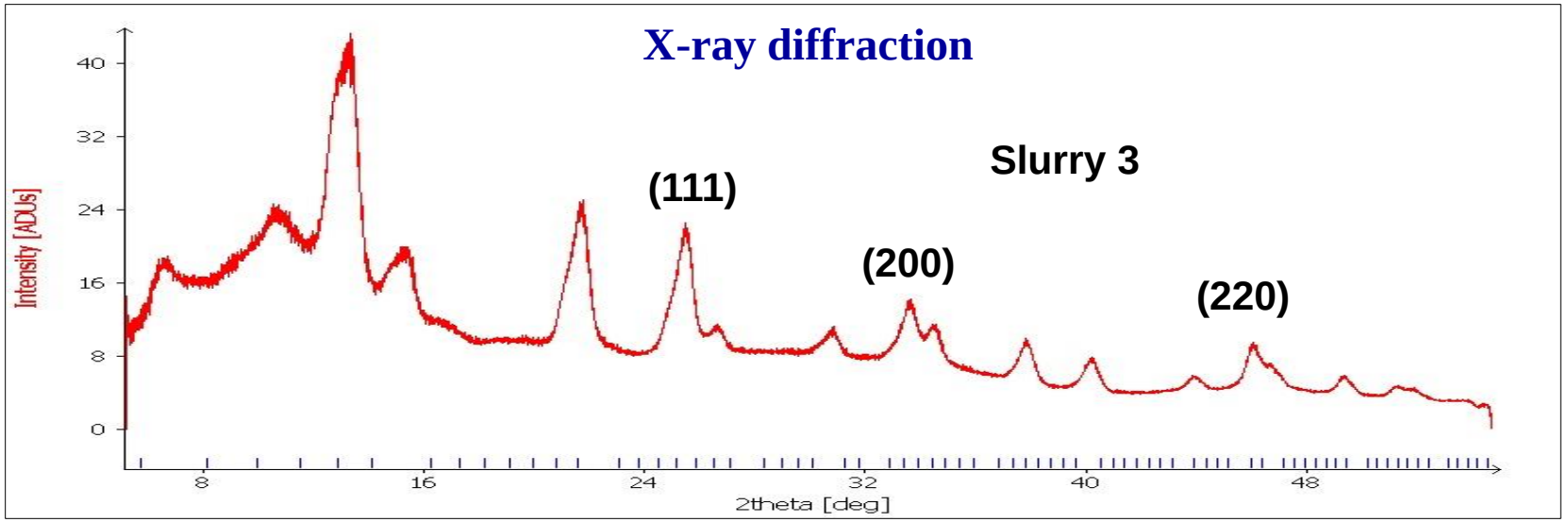
- **X-ray diffraction - Agilent-Gemini X-Ray Diffractometer; Molybdenum source (50mV and 30 mA)**
- **Slurries dried at 125 ºC till constant mass is obtained.**

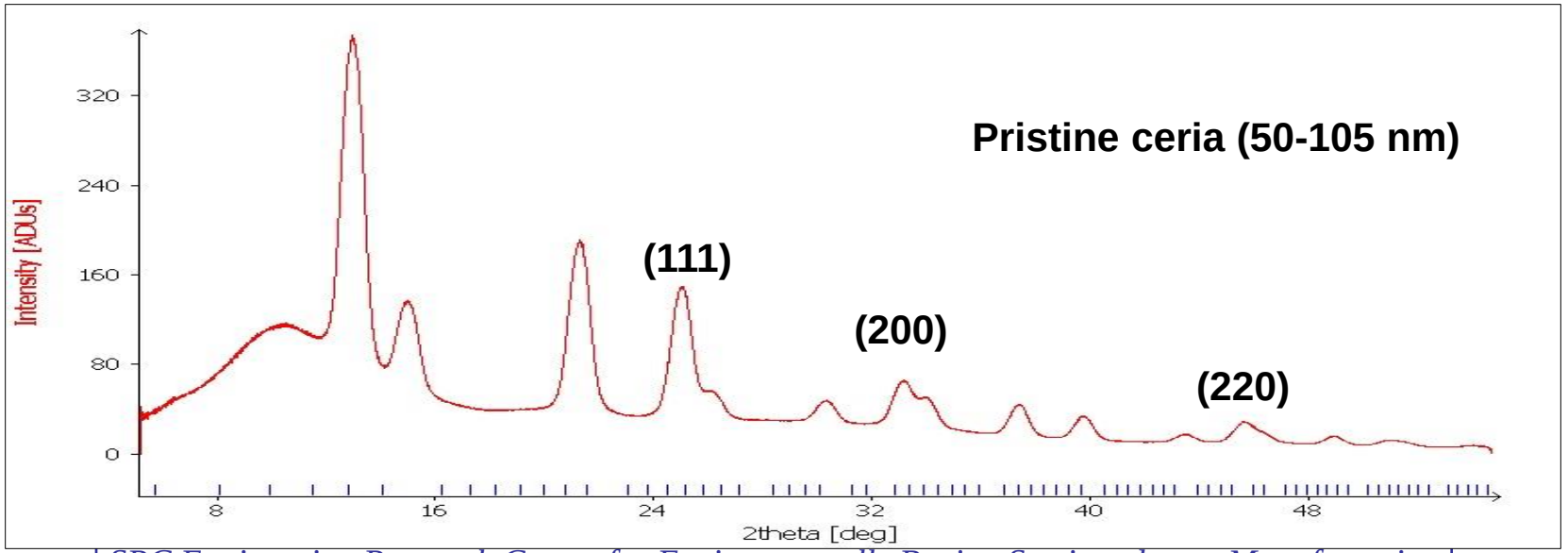




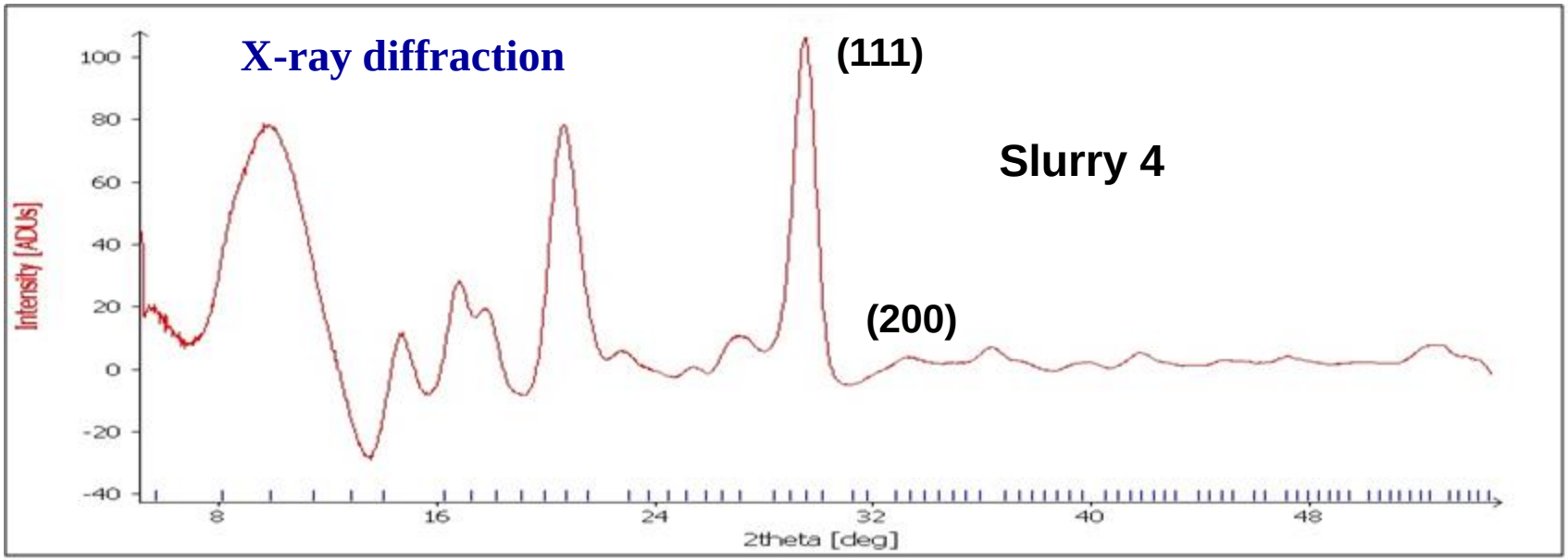
- **Broad peak in XRD pattern indicates amorphous silica structure.**

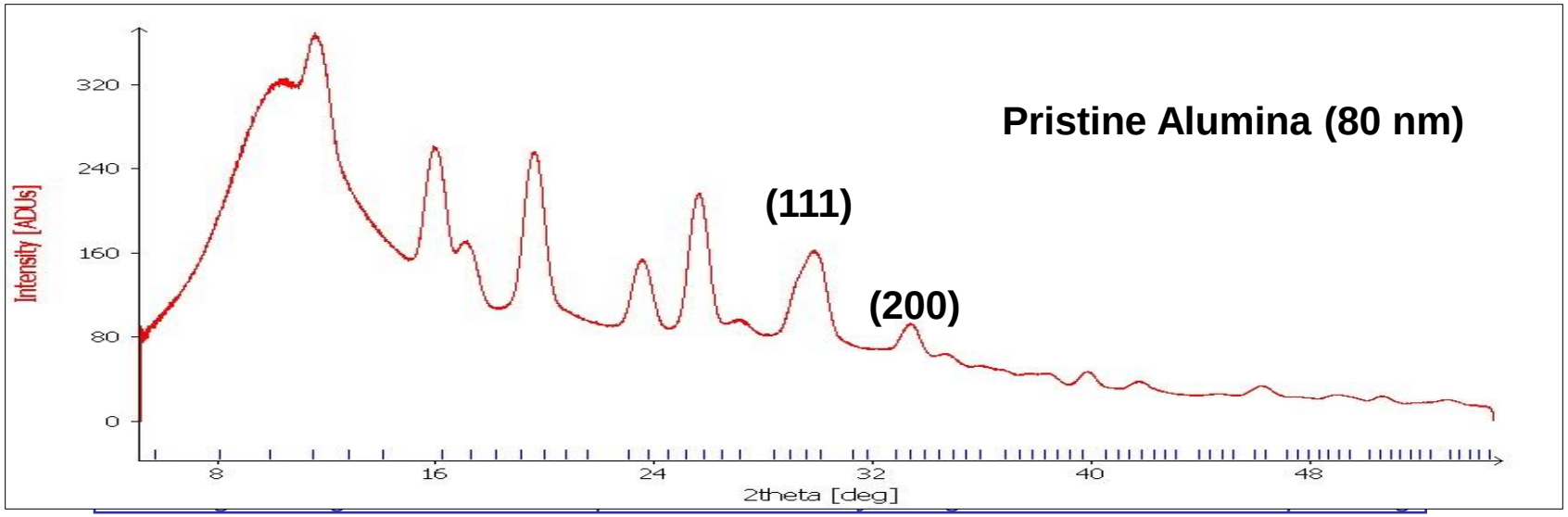
I. Characterization of Nanomaterials





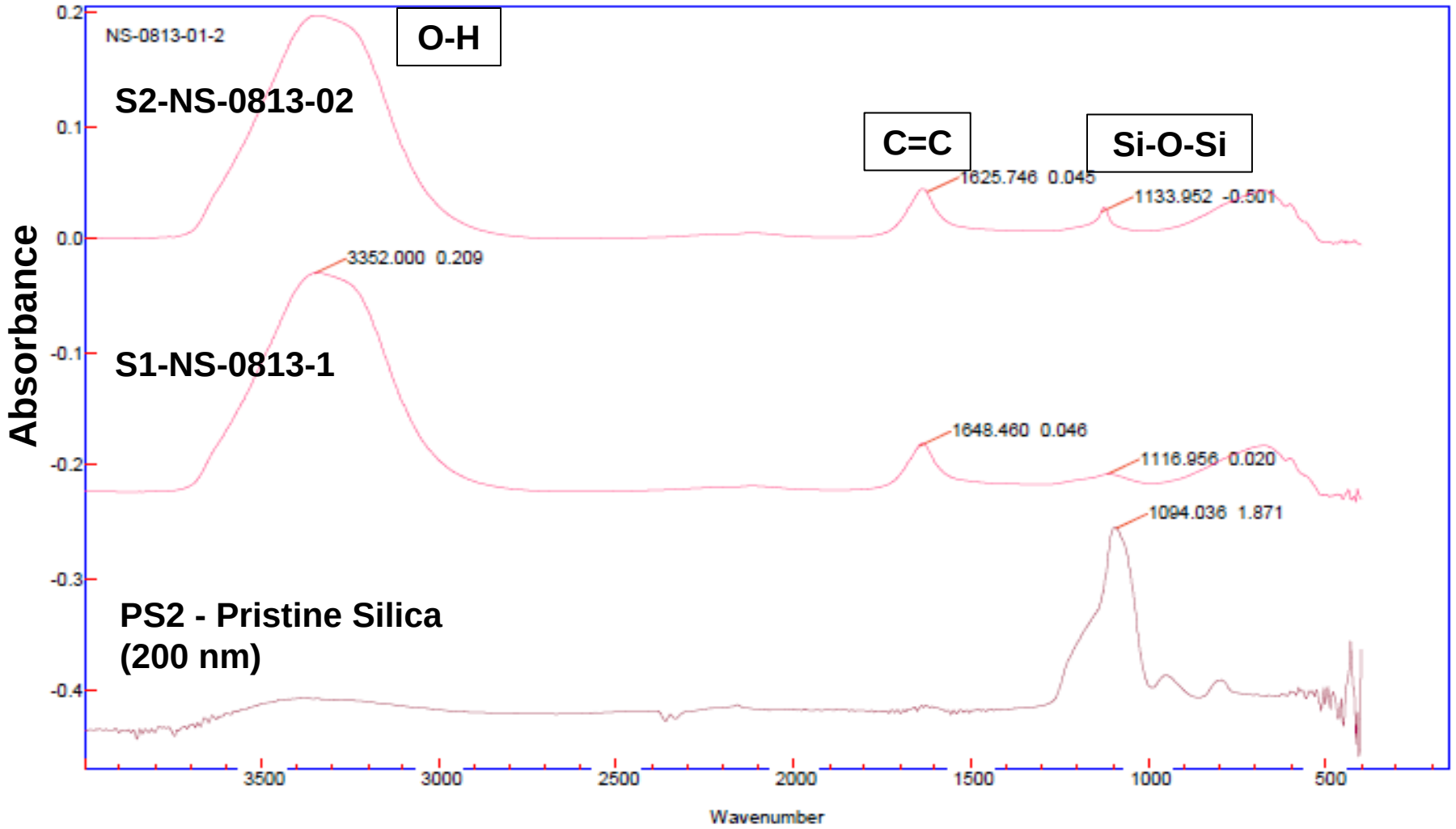
I. Characterization of Nanomaterials





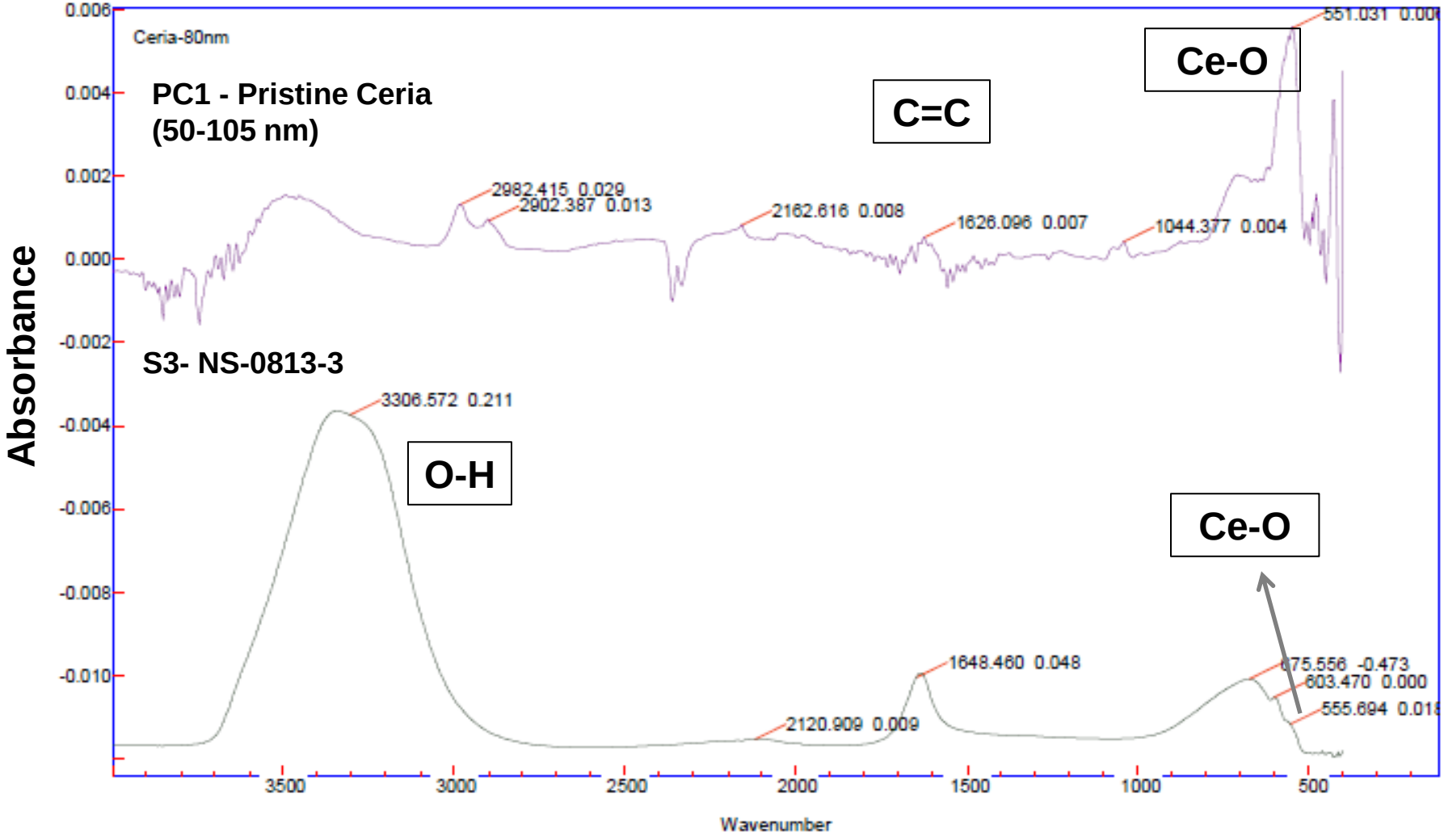
I. Characterization of Nanomaterials

- FT-IR spectroscopy – ATR on Varian 600 FT-IR spectrophotometer
- 16 scans; Si-O, Ce-O and Al-O at around 1100, 550 and 590 cm^{-1}



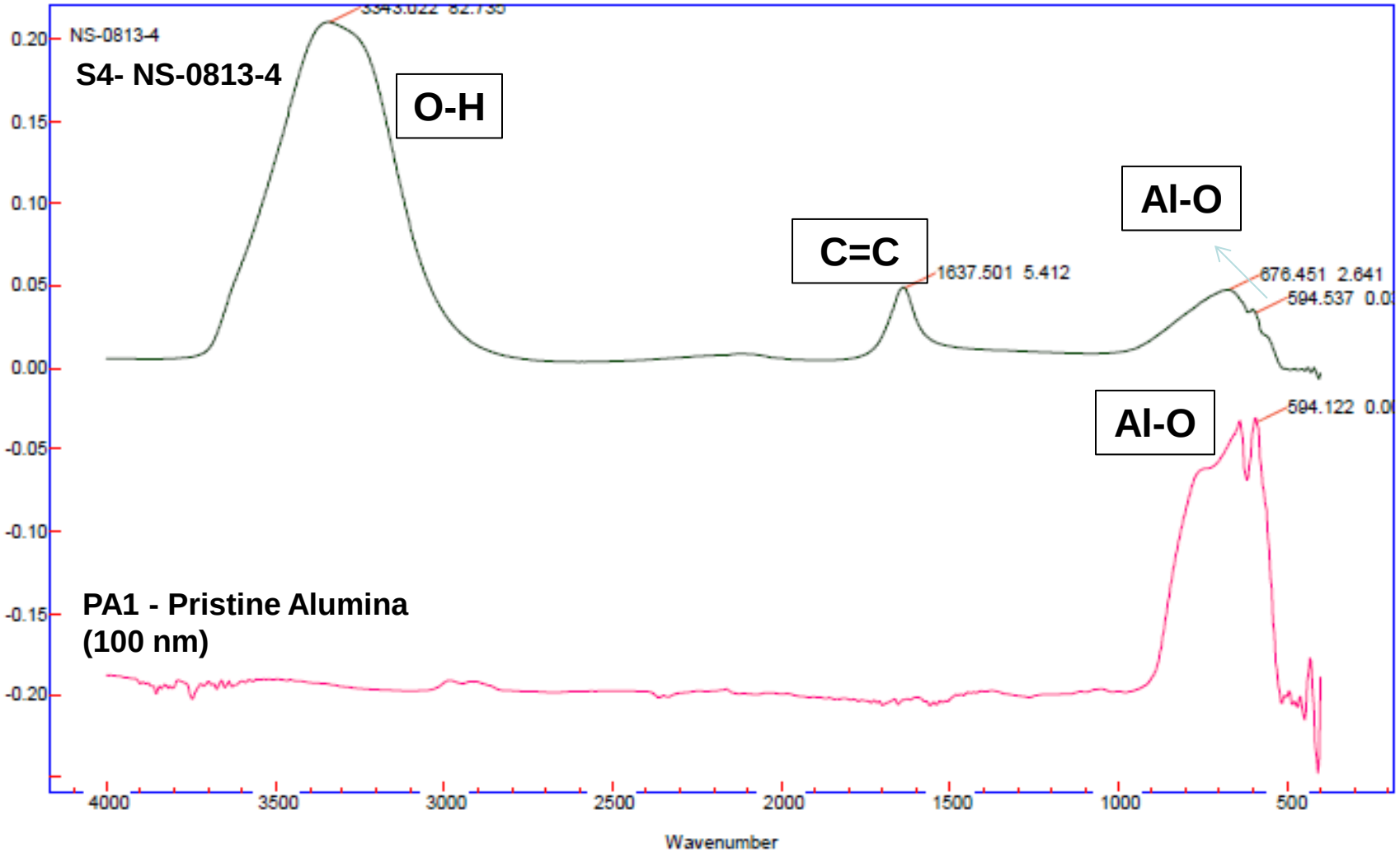
I. Characterization of Nanomaterials

- **FT-IR spectroscopy – ATR on Varian 600 FT-IR spectrophotometer**



I. Characterization of Nanomaterials

- **FT-IR spectroscopy – ATR on Varian 600 FT-IR spectrophotometer**



II. Cytotoxicity – Cell Viability and Integrity

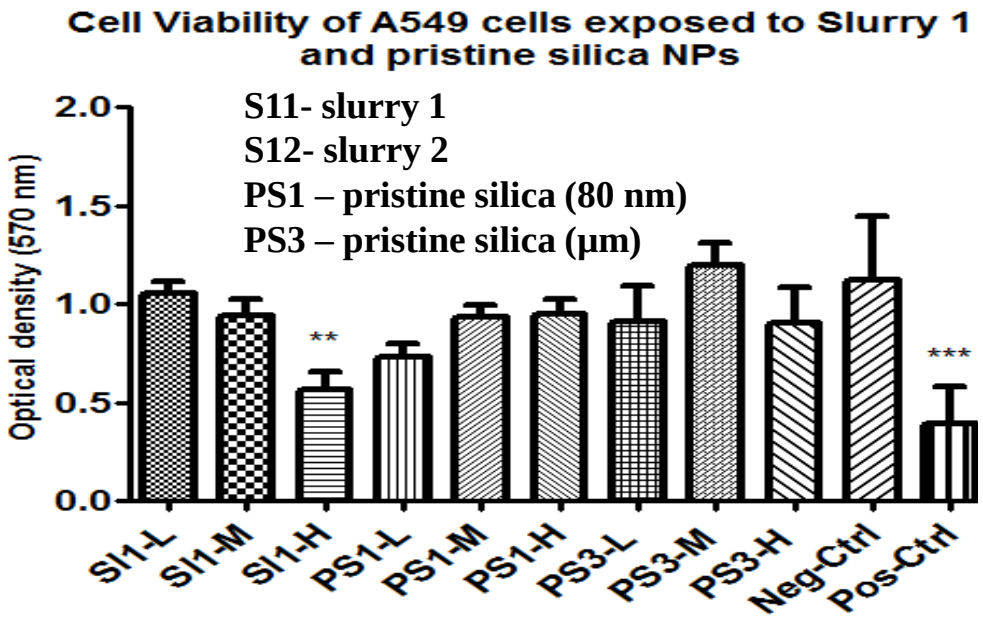
- **End-point cytotoxicity assays to assess the plasma membrane integrity and cell viability**
- **To address one key question**
  - Is cytotoxicity driven by composition, dose and time – acute or delayed response?
- **MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay**
  - Based on the conversion of MTT into formazan crystals (purple color) by living cells, which determines its mitochondrial activity. For most cells the total mitochondrial activity is a indicator of number of viable cells, a measure of in-vitro cytotoxicity
- **Lactate Dehydrogenase (LDH) assay**
  - Assessment of membrane integrity - by monitoring the passage of substances that are normally sequestered inside cells to outside.
- **Positive (H_2O_2 or lysis buffer) and negative (media) controls were incorporated to ensure the assays can detect cytotoxic activity**

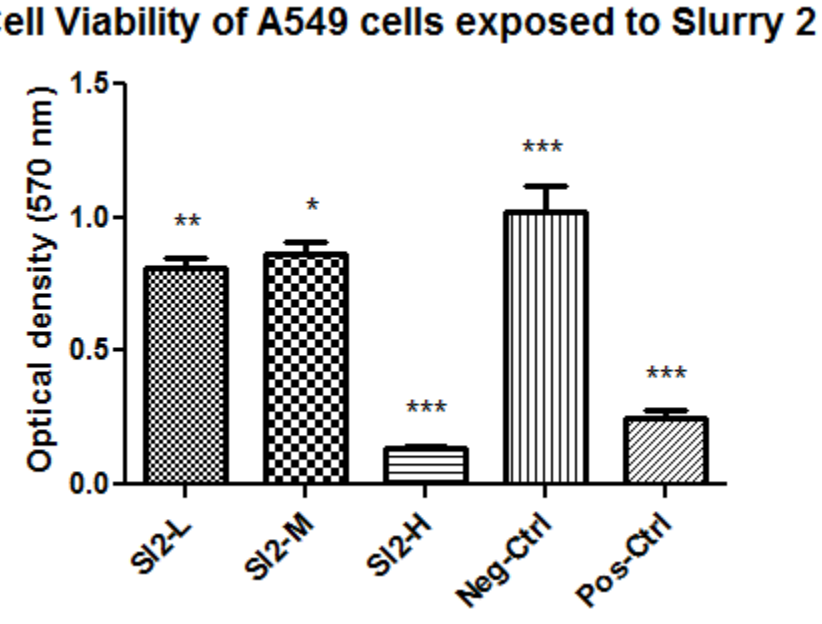
II. Cytotoxicity – Cell Viability and Integrity

Sample		Volume of slurry (in µL)	Concentration (mg/mL)	pH
Slurry 1	High (H)	10	2.03	7.55
	Medium (M)	1	0.203	7.74
	Low (L)	0.1	0.0203	7.78
Slurry 2	High (H)	10	3.34	8.2
	Medium (M)	1	0.334	7.83
	Low (L)	0.1	0.0334	7.76
Slurry 3	High (H)	10	0.52	7.7
	Medium (M)	1	0.052	7.74
	Low (L)	0.1	0.0052	7.73
Slurry 4	High (M)	10	2.01	7.72
	Medium (M)	1	0.201	7.81
	Low (L)	0.1	0.0201	7.85

II. Cytotoxicity – Cell Viability and Integrity

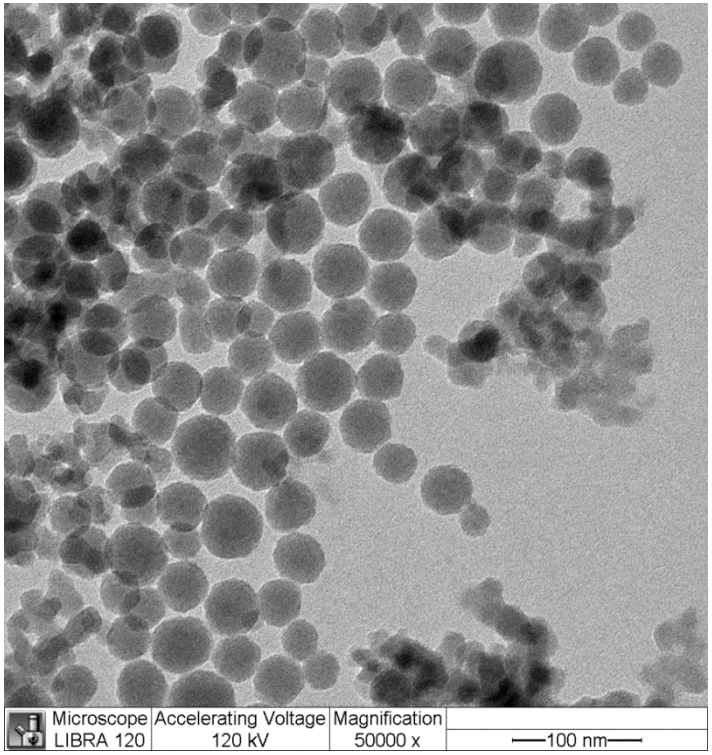
- A549 cells (density of 10,000/cm^2) exposed to specified amount of slurries and same concentration of pristine NPs for 48 hours; the assay is performed after another 24 hours
- Absorbance measured at 570 nm
- ANOVA (Analysis of Variance) - "one-way ANOVA" using a significance level of 0.05 (95% confidence intervals); *** - 99.9% confidence interval; ** - 99% confidence and * - 95% confidence interval



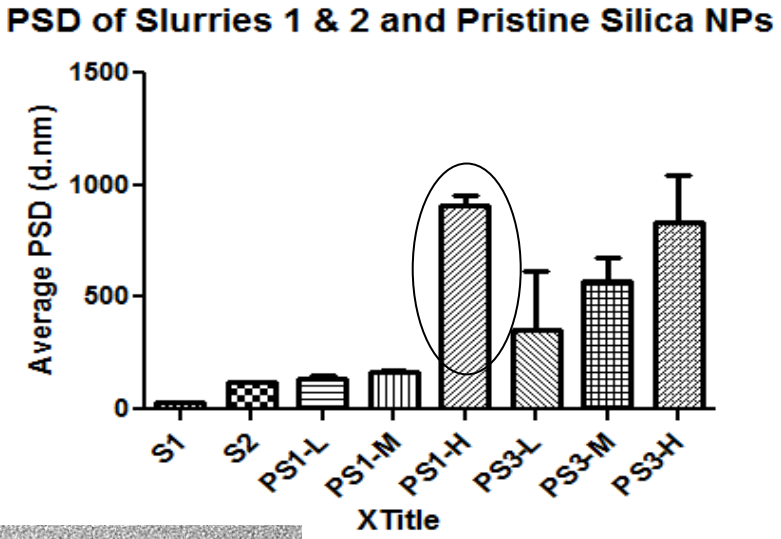


II. Cytotoxicity – Cell Viability and Integrity

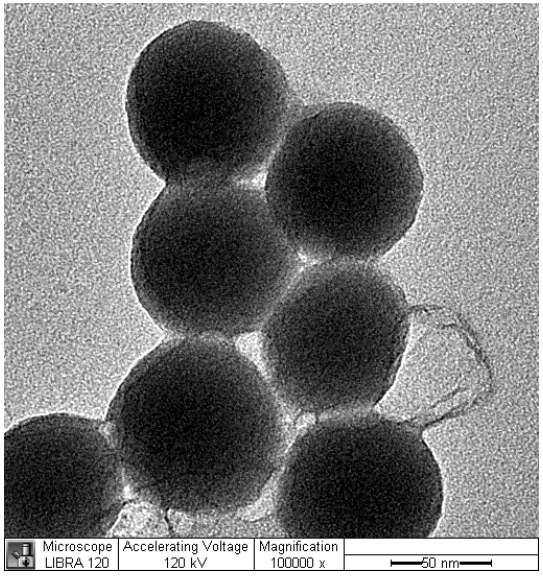
- **Aggregation of colloidal silica NPs in slurries and pristine colloidal silica NPs**



TEM of Colloidal Silica NPs in Slurry 1



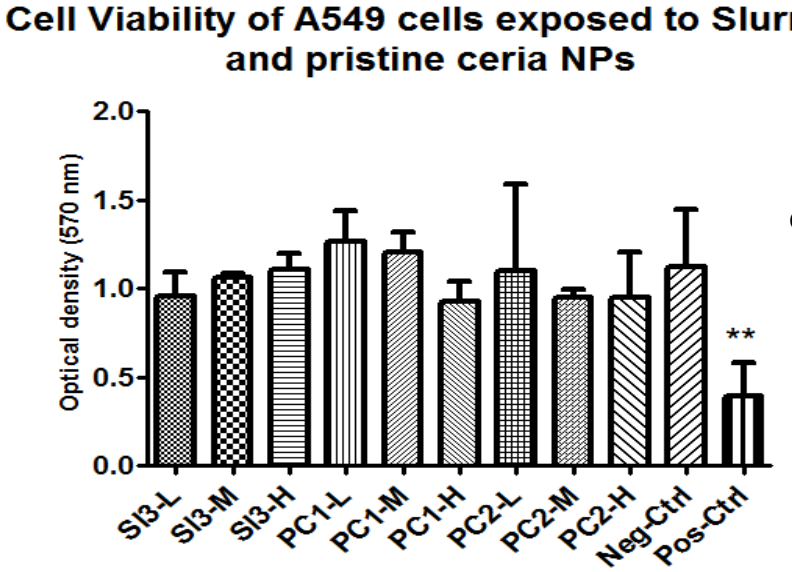
PS1 – pristine colloidal silica (80 nm)
PS3 – pristine colloidal silica (200 nm)



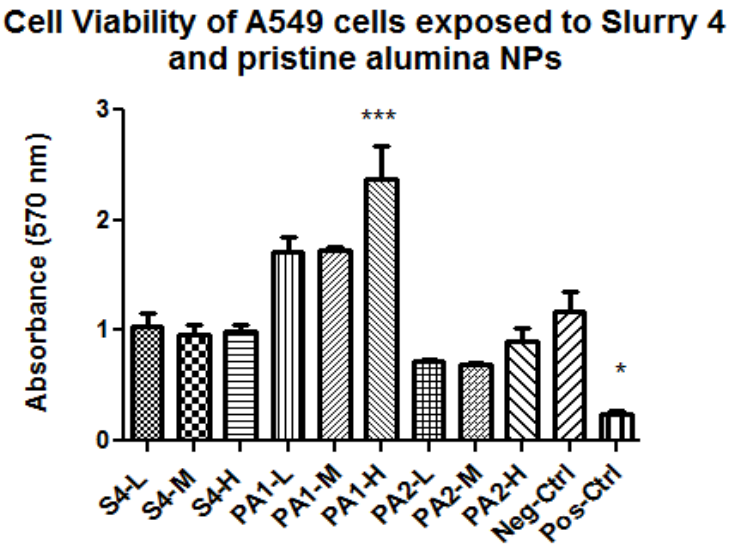
TEM of Pristine Colloidal Silica NPs of same size as silica in slurry 1

II. Cytotoxicity – Cell Viability and Integrity

- **Cells exposed to specified amount of slurries and same concentration of pristine NPs for 48 hours; the assay is performed after another 24 hours**
- **Absorbance measured at 570 nm**



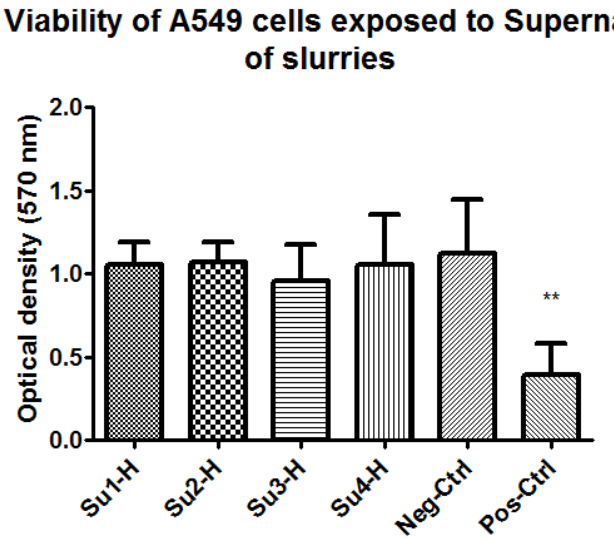
S13- slurry 3
S14- slurry 4
PC1 – pristine ceria (50-105nm)
PC2 – pristine ceria (µm)
PA1 – pristine alumina (80nm)
PA2 – pristine alumina (µm)

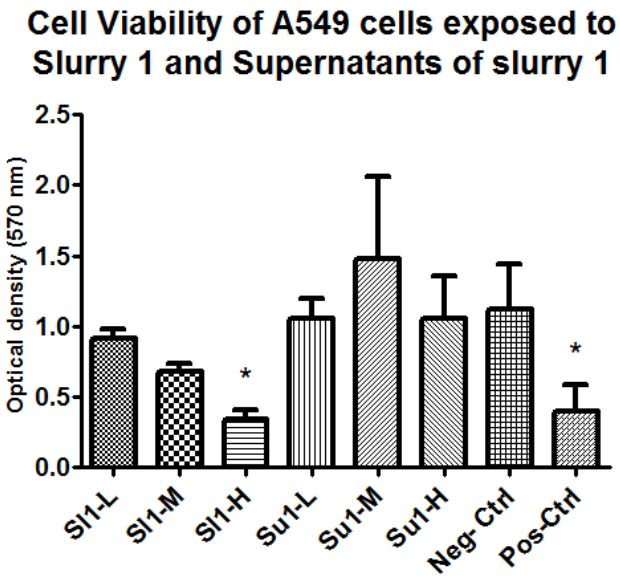


- Silica NP slurries showed a dose-dependent response, particularly high concentration of fumed silica showed high cytotoxicity, but pristine silica NPs of similar size/concentration showed no cytotoxicity (in fact exhibited the opposite)
- Ceria and Alumina NPs exhibit no cytotoxicity, compared to control cells

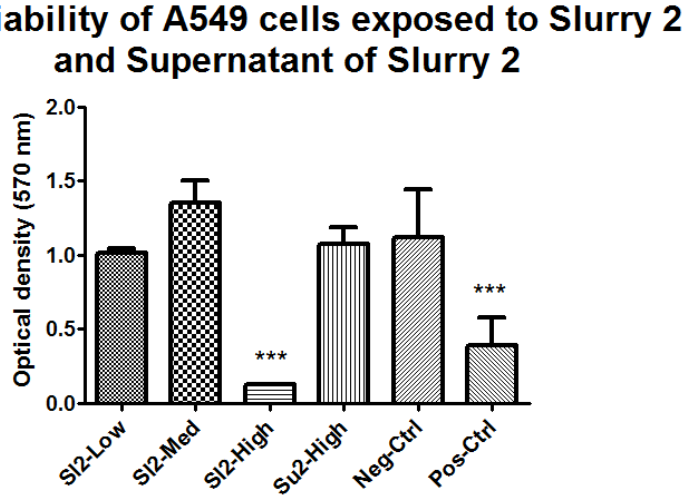
II. Cytotoxicity – Cell Viability and Integrity

- **Effect of slurry supernatants on A549 cell viability**
- **Supernatants were prepared by UTD by removing nanoparticles by centrifugation at 200,000g**
- **As stated earlier, the same MTT cell viability procedures were followed**





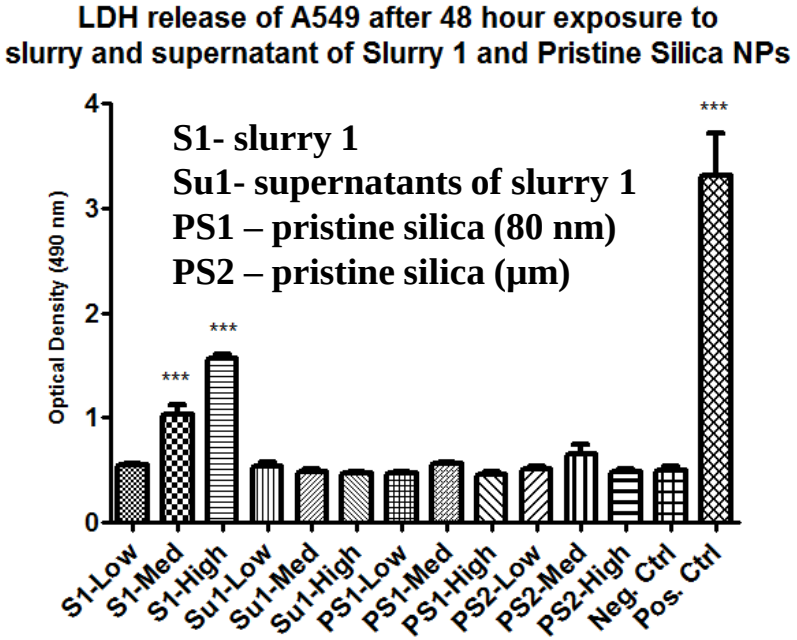
Su1- supernatant of slurry 1
Su2- supernatant of slurry 2
Su3- supernatant of slurry 3
Su4- supernatant of slurry 4

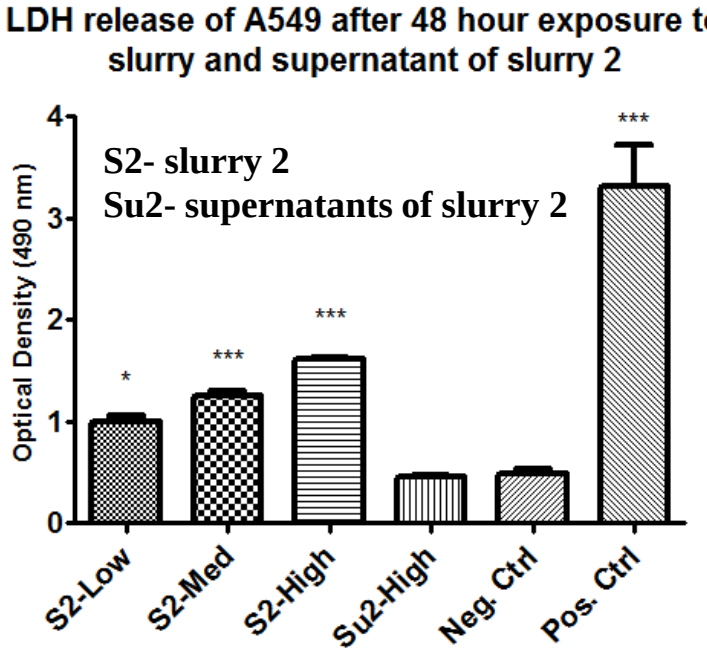


- Slurry supernatants showed no to minimal cytotoxicity, compared to control cells

II. Cytotoxicity – Cell Viability and Integrity

- **Lactate Dehydrogenase (LDH) assay**
  - **Assessment of membrane integrity - by monitoring the passage of substances that are normally sequestered inside cells to outside**
- **LDH assay was performed after 48 hours of NP exposure using the supernatants from the well plate**

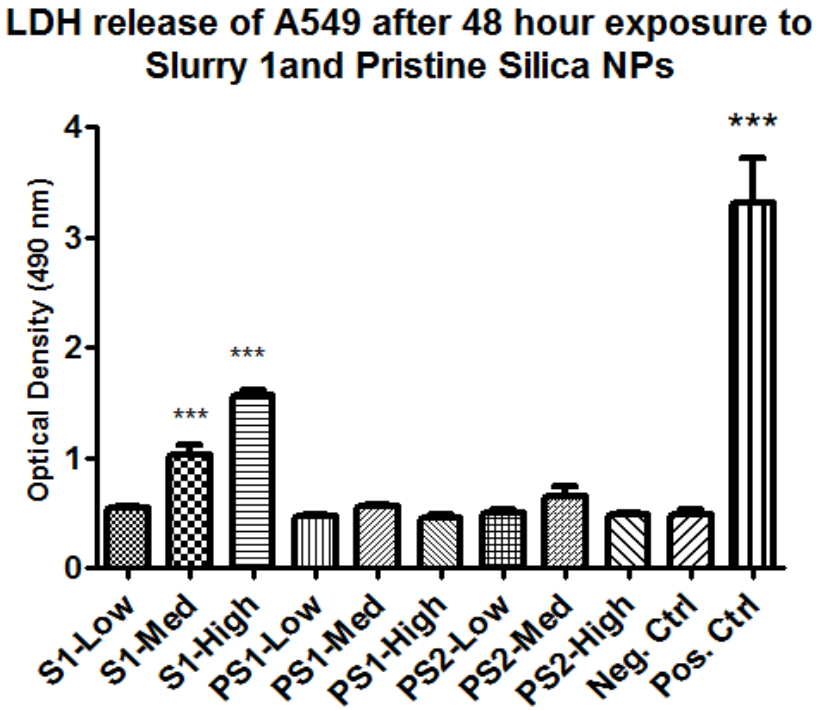


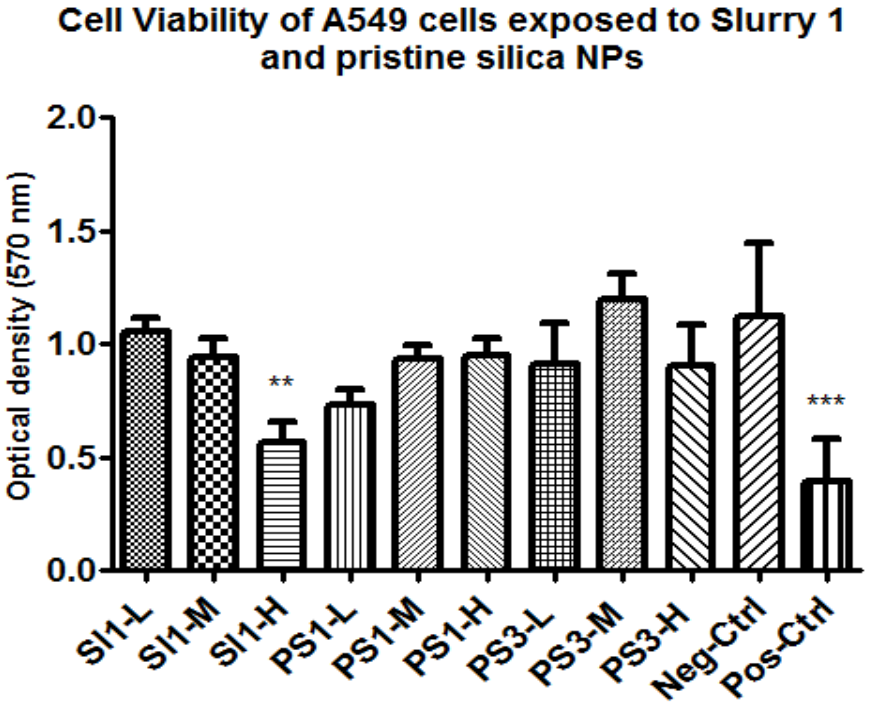


- **Similar to MTT assays, silica NP slurries showed a dose-dependent LDH response, compared to control cells and pristine micro and nanoparticles**

II. Cytotoxicity – Cell Viability and Integrity

- **Comparison of MTT and LDH assay for slurry 1 and colloidal pristine silica micro and nanoparticles**
- **Excellent correlation among the two cell viability and membrane integrity assays**

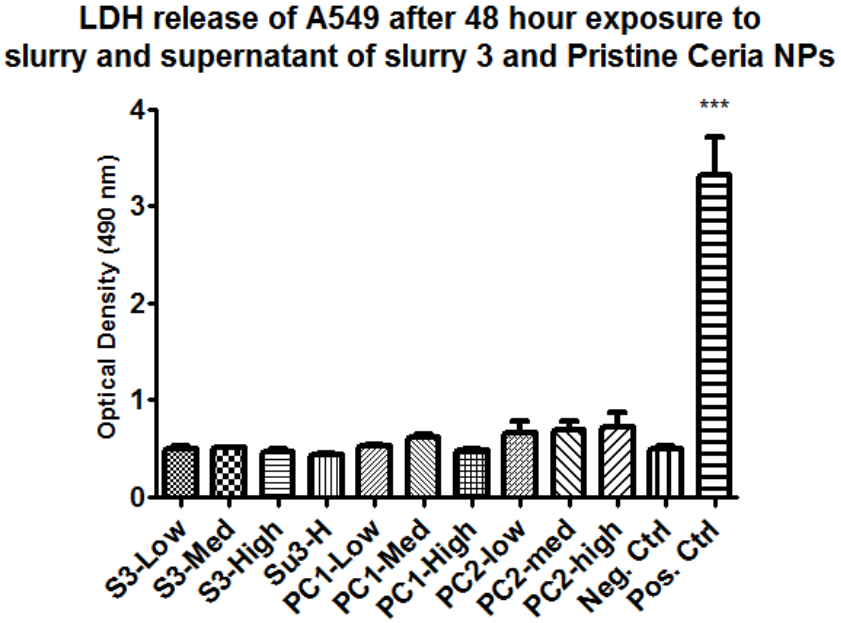


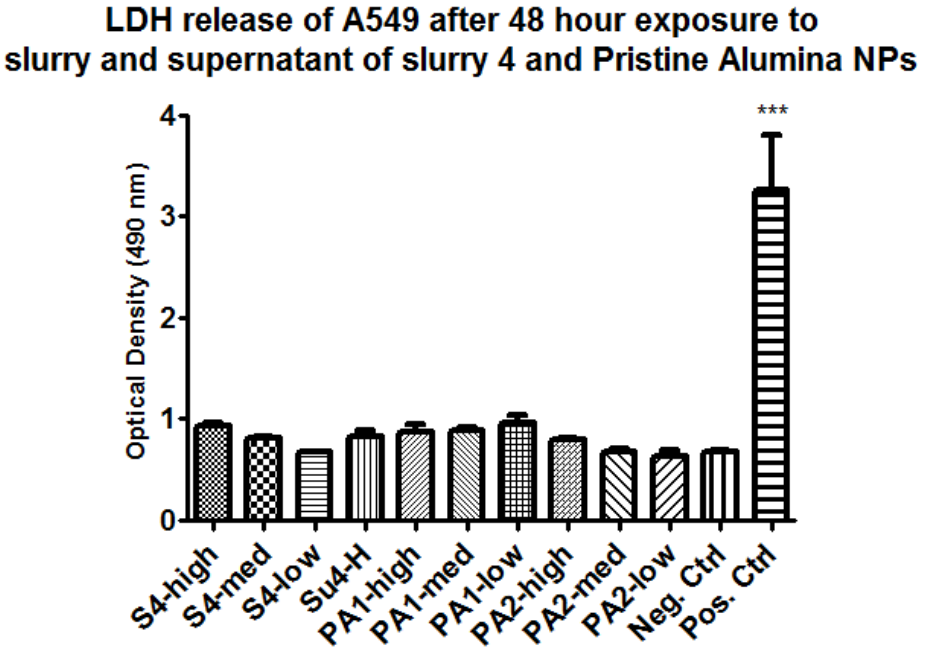


S1/S11 – slurry 1, PS1 – pristine silica (80 nm), PS2/PS3 – pristine silica (µm)

II. Cytotoxicity – Cell Viability and Integrity

- **LDH assay was performed after 48 hours of NP exposure using the supernatants from the well plate**



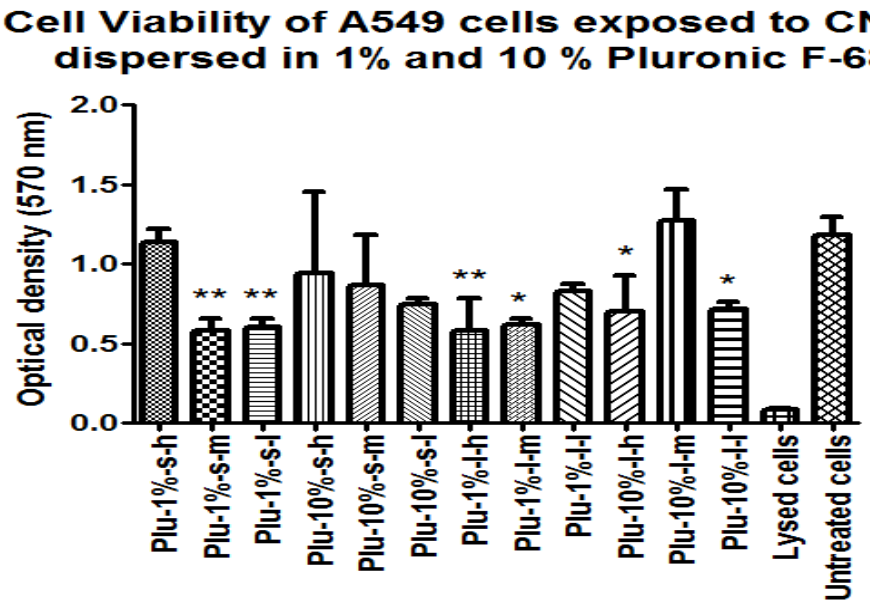


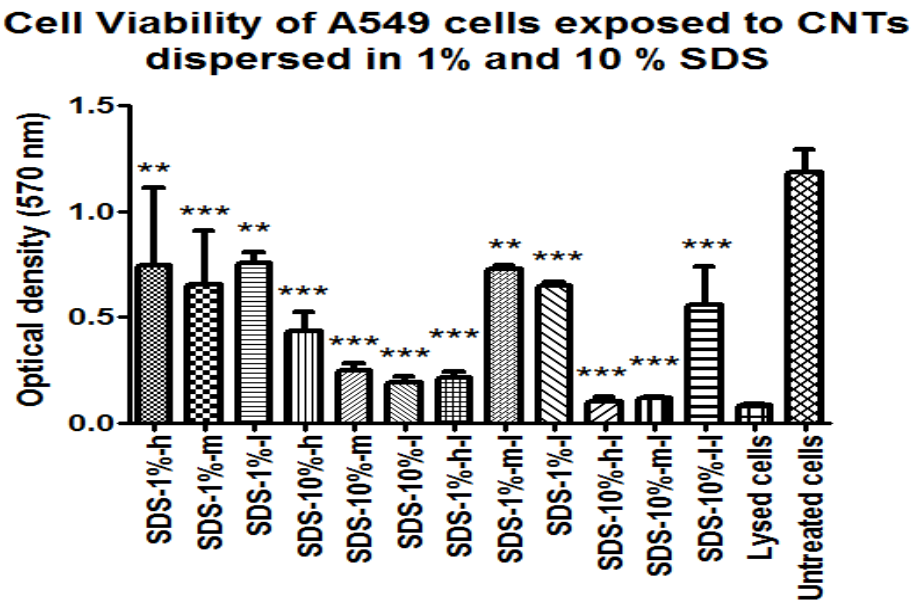
S3- slurry 3; S4- slurry 4; Su3 and Su4 – supernatants of slurry 3 and 4, PC1 – pristine ceria (50-105nm); PC2 – pristine ceria (μm); PA1 – pristine alumina (80nm); PA2 – pristine alumina (μm)

- **No significant differences are observed in LDH release for ceria and alumina particles, when compared to control cells**

II. Cytotoxicity – Cell Viability and Integrity

- **MTT assays with MWCNT (short and long), with or without surfactants to control the amount of aggregation**



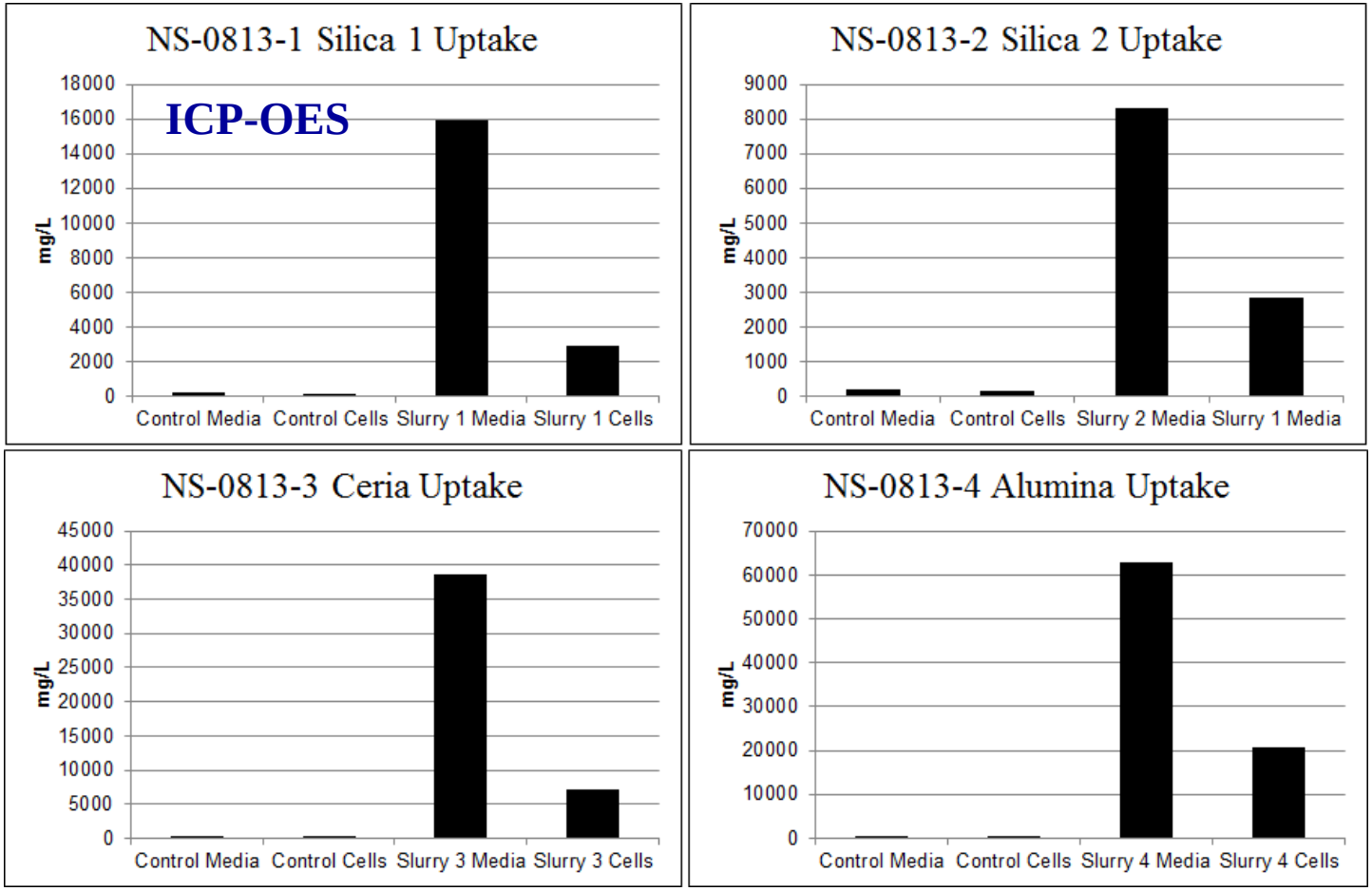


- **Dispersants used: SDS (288.4 g/mol), Pluronic (8400 g/mol) and Dowfax (569 g/mol)**
- **SDS showed better dispersion, particle size, zeta potential (stability)**
- **MTT assay performed after 48 hours of incubation. H-4 ug/mL, m-0.4 ug/mL and l-0.04 ug/mL**

III. Cellular Uptake and Internalization

- **Do ENs penetrate into cells? If so, where do they accumulate or internalize? What about Reactive Oxygen Species (ROS)?**
- **Studied cell uptake and internalization**
  - Inductively coupled plasma optical emission spectrometry (ICP-OES)
  - Confocal Raman Microscopy
  - Ultrastructural characterization using HRTEM and Helium Ion Microscopy (HeIM)
  - Electrochemical Cell Impedance Spectroscopy (ECIS)
- **ICP-OES is an analytical technique used for the detection of trace metals**
  - Intensity of the emission is indicative of the concentration of the element within the sample
  - Same exposure conditions were followed as in MTT/LDH, highest concentration of slurries were chosen
  - Before analysis, cells were washed and cells exposed to the slurries were then digested as per standard protocols

III. Cellular Uptake and Internalization

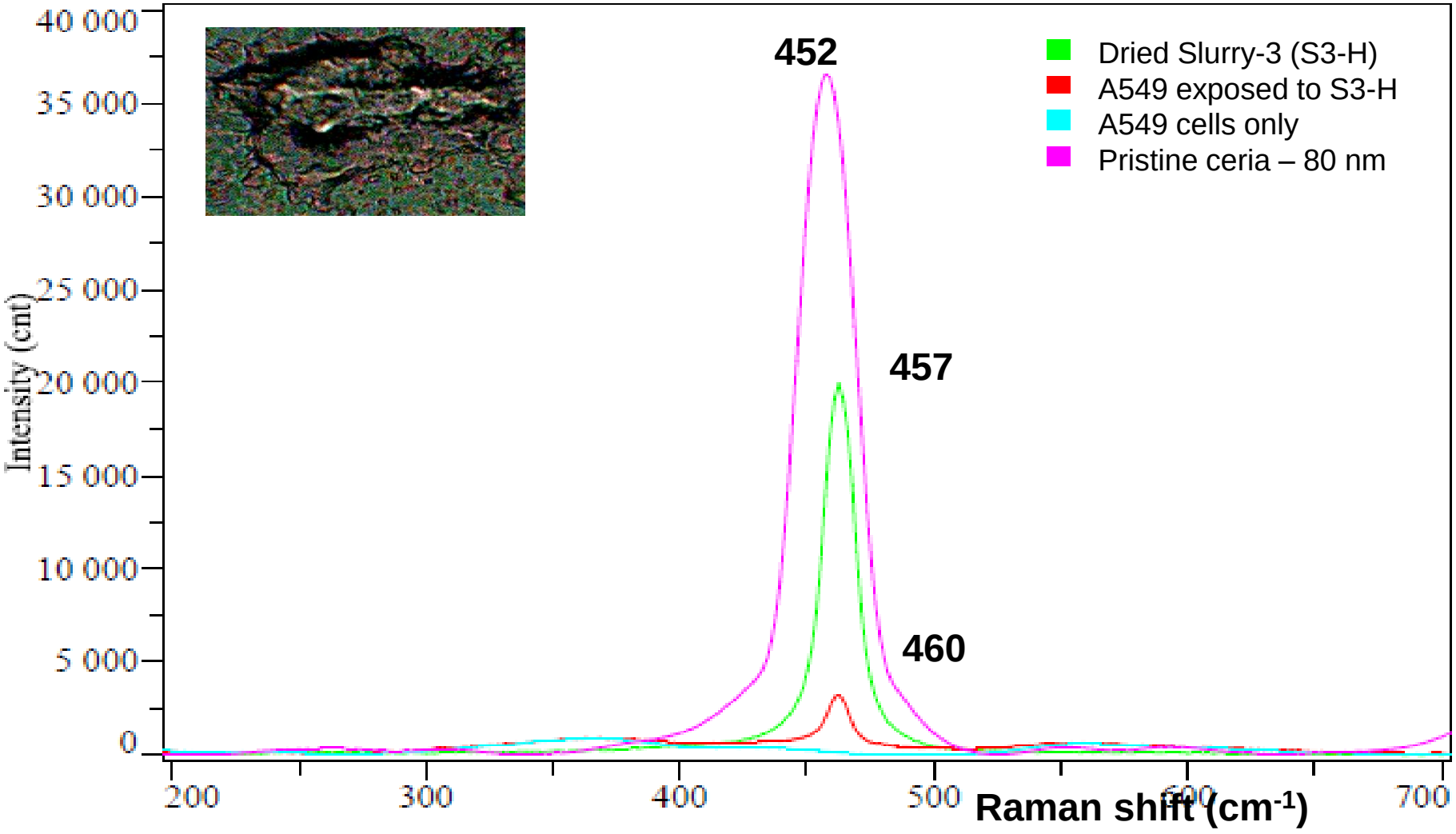


- **All NP slurries (high concentration) showed some amount of cellular uptake compared to controls, but just uptake does not necessarily lead to toxicity.**

III. Cellular Uptake and Internalization

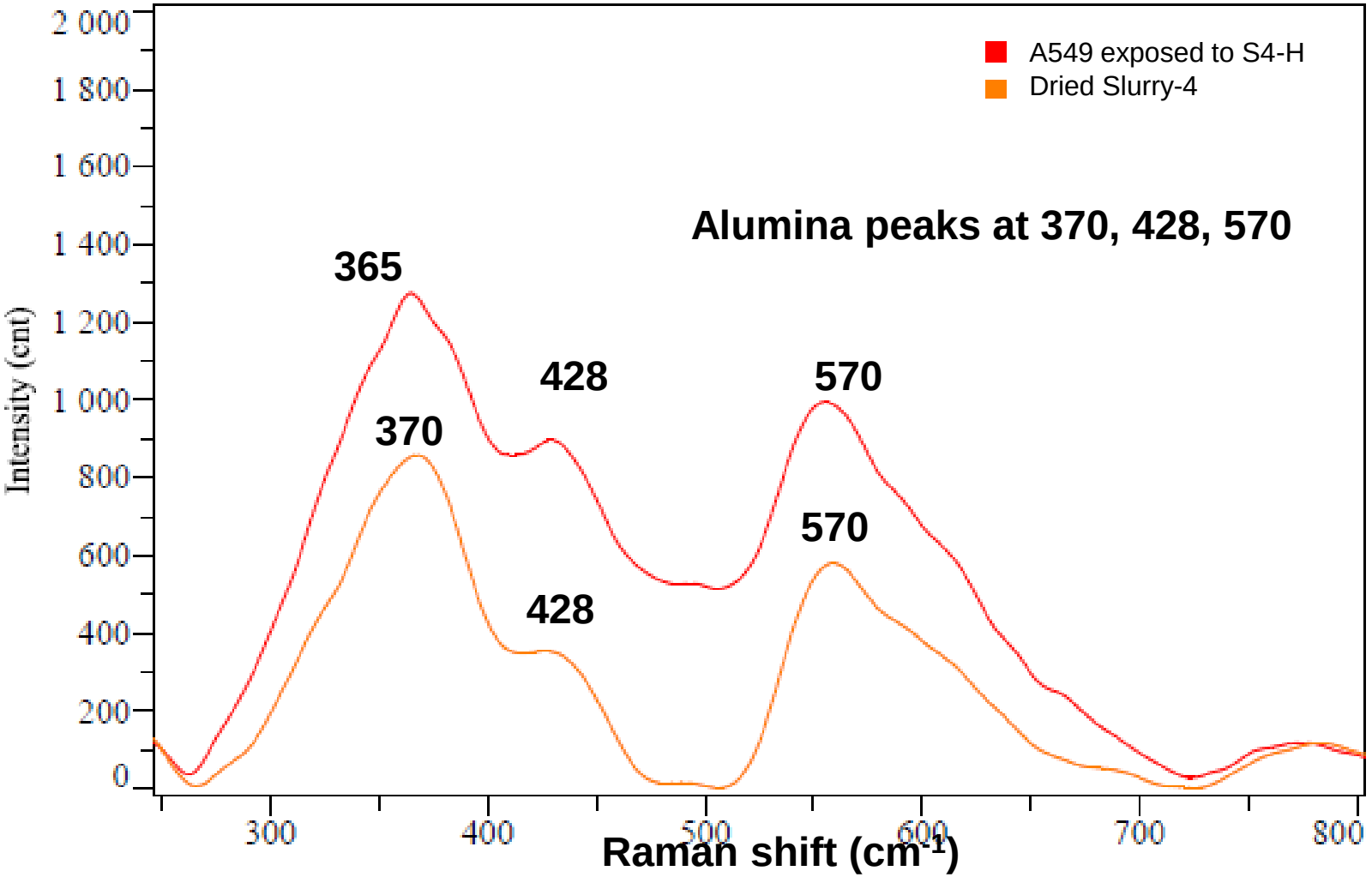
- **Confocal Raman, a non-invasive, non-destructive and label-free technique, was employed to study uptake and localization of NPs**
- **Horiba XploRA Raman Confocal Microscope System; Spot size – 1.12 µm**
- **NPs show Raman active vibration modes**
- **Ceria NPs - a single strong band located around 450-465 cm^{-1}**
- **For alumina NPs, from the 27 possible optical modes in the crystal structure, 7 modes are Raman active and 6 modes are infrared (IR) active**
- **Same A549 cell exposure conditions were followed as in MTT/LDH, highest concentration of slurries were chosen**
- **Before analysis, cells were washed to remove surface bound NPs**

III. Cellular Uptake and Internalization



- **Clear evidence of Ceria NP localization within A549 cells**

III. Cellular Uptake and Internalization

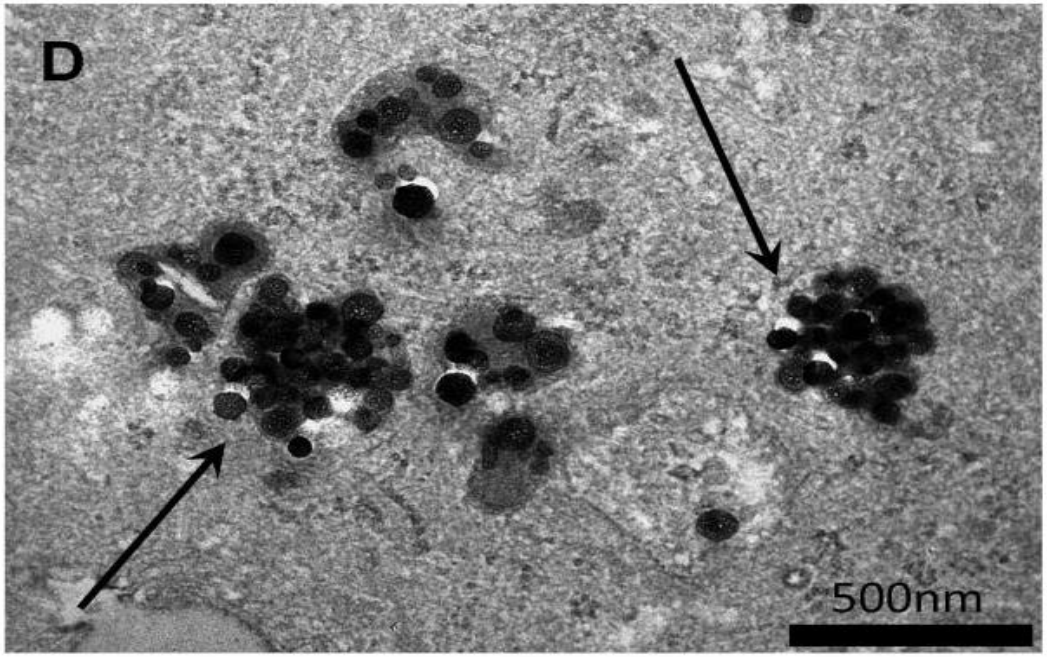


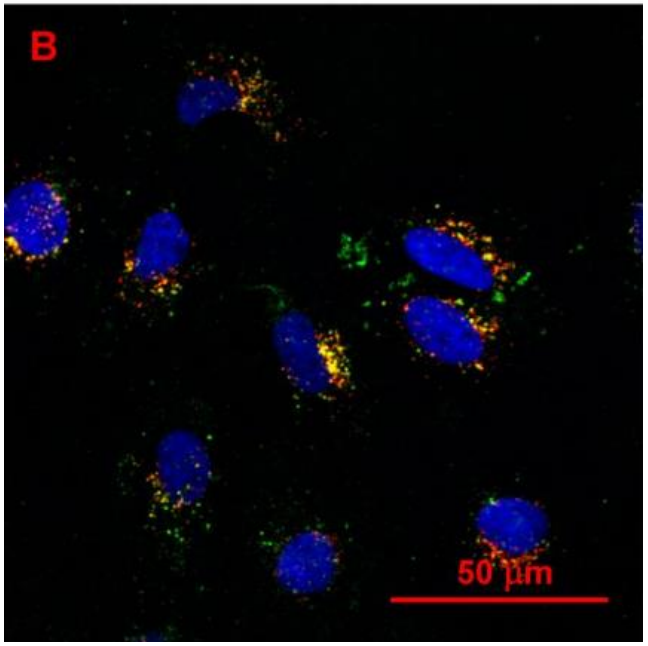
- **Evidence of Alumina NP localization within A549 cells**

III. Cellular Uptake and Internalization

- **Track/locate NPs internalized in cells via phagocytosis/endocytosis**
- **Currently, we are optimizing the critical protocol for ultrastructural characterization using HRTEM**
  - After following the same exposure procedure, cells are incubated for 48 h. Then they are prefixed with fixative (25% of 0.4M cacodylate buffer, 12.5% of 16% formaldehyde, 10% of 25% glutaraldehyde in water) at 4C for 4h and then washed.
  - Post fixation is done with 1% osmium tetroxide in 0.1 M PBS solution at 4C for 2h. Cells are then stained with 2% uranyl acetate at 4C for 1h and then dehydrated by gradient acetonitrile and absolute ethanol.
  - Infiltration with Spurr's resin is done for at least 6 hours. Then, individual samples are embedded in fresh 100% Spurr's in BEEM capsules at 70C overnight for polymerization
  - Next, sectioning (80-100 nm) is performed by using ultramicrotome. The samples are then ready for TEM ultrastructural characterization

III. Cellular Uptake and Internalization

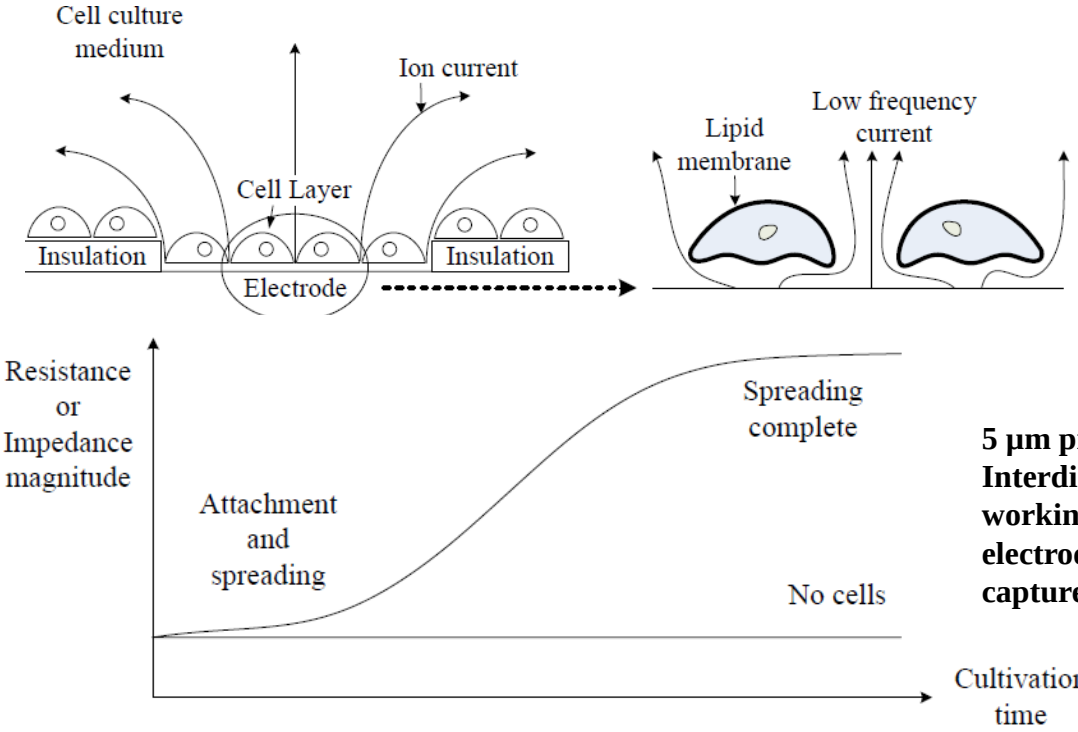




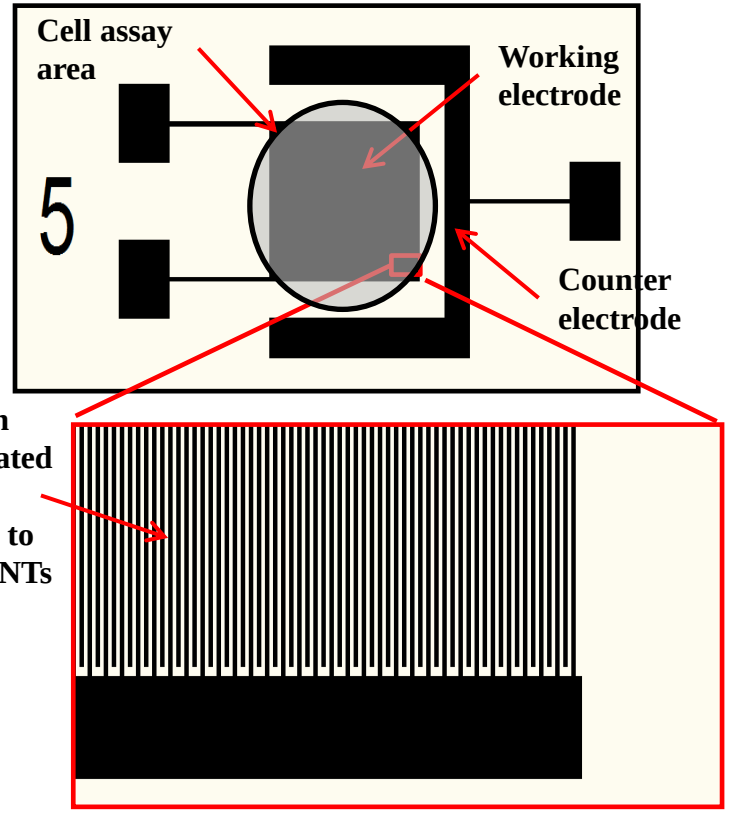
Typical TEM and confocal image of A549 cells after 24 h of exposure to 100 µg/ml silica NPs with green labels (100 nm). NPs are seen to localize in lysosomes but no evidence of reaching the nucleus. Red - immunostaining of lysosomes with LAMP1 antibody (secondary Alexa-647 antibody). Blue-DAPI staining of nuclei. Arrows indicate the localization of nanoparticles in the cells (From ref - Shapero, K., et al. (2011). Time and space resolved uptake study of silica nanoparticles by human cells. Molecular Biosystems, 7(2), 371-378.

III. Cellular Uptake and Internalization

- **Development and optimization of a high-content screening/monit method – Electrochemical Cell Impedance Spectroscopy (ECIS)**



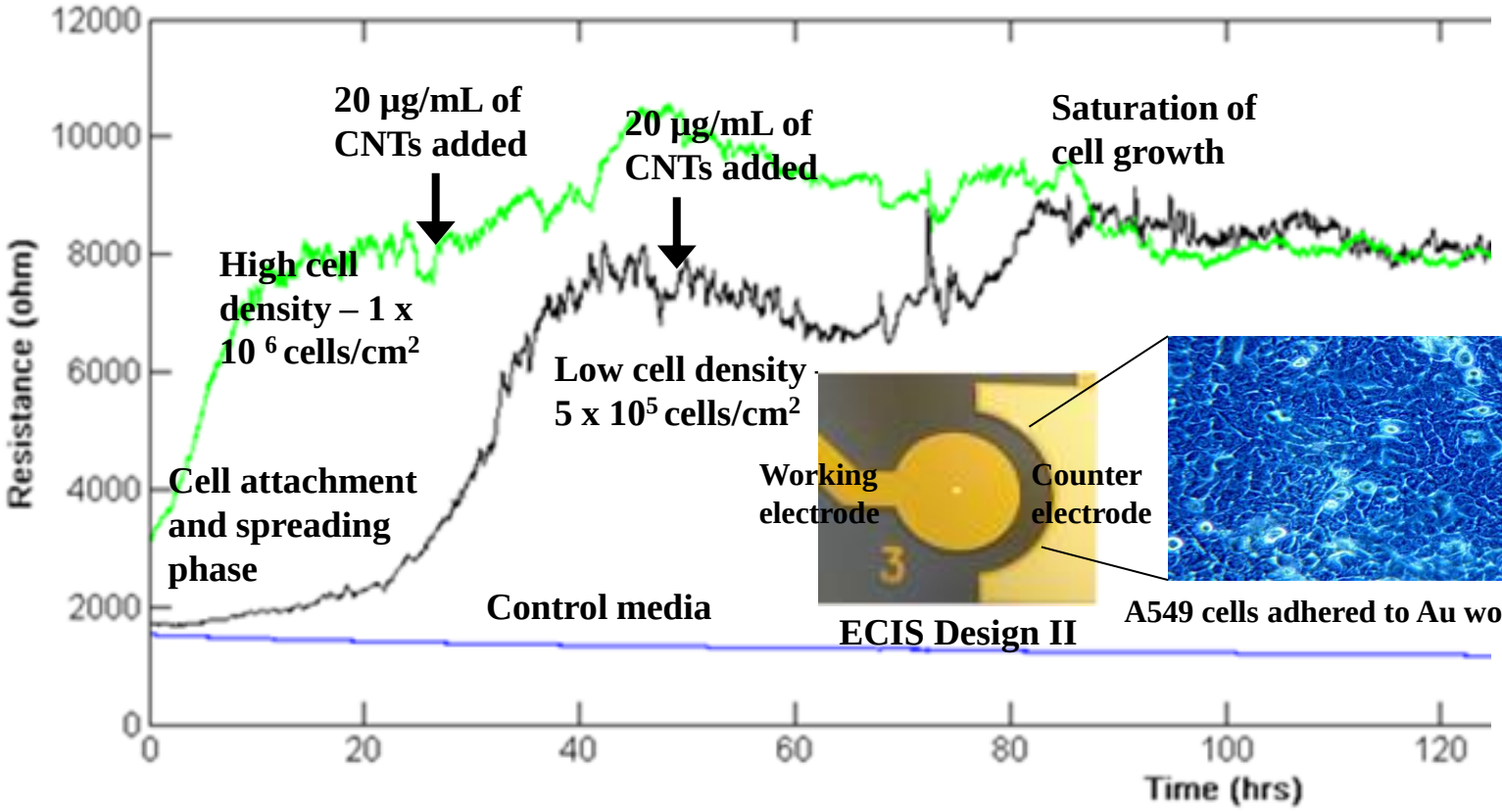
Schematic of microelectrode-based cell chip, below: impedance recorded at the frequency of 4 kHz during the cell growth on the electrode (Giaever and Keese 1993)



ECIS Design I to monitor cell impedance

III. Cellular Uptake and Internalization

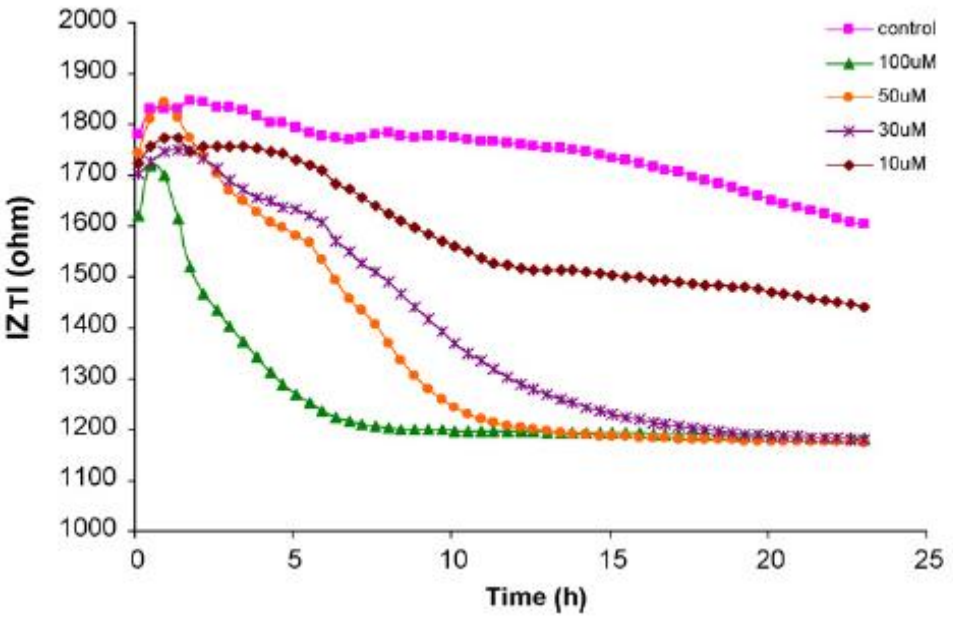
- Electrochemical Cell Impedance Spectroscopy (ECIS) of A549 cells
- 20 µg/mL of CNTs were added to the culture after cell spreading was complete



ECIS data for A549 cells exposed to CNTs measured at 4 KHz

III. Cellular Uptake and Internalization

- **When cells are treated to a cytotoxic agent such as sodium arsenite, impedance decreases, which can be directly correlated to toxicity**
- **As the concentration of sodium arsenite increases, the rate of decrease in impedance modulus also increased**
- **Similarly, ECIS measurements with cells exposed to toxic NPs can be longitudinally monitored using impedance data, as cells start to detach from electrode surface**



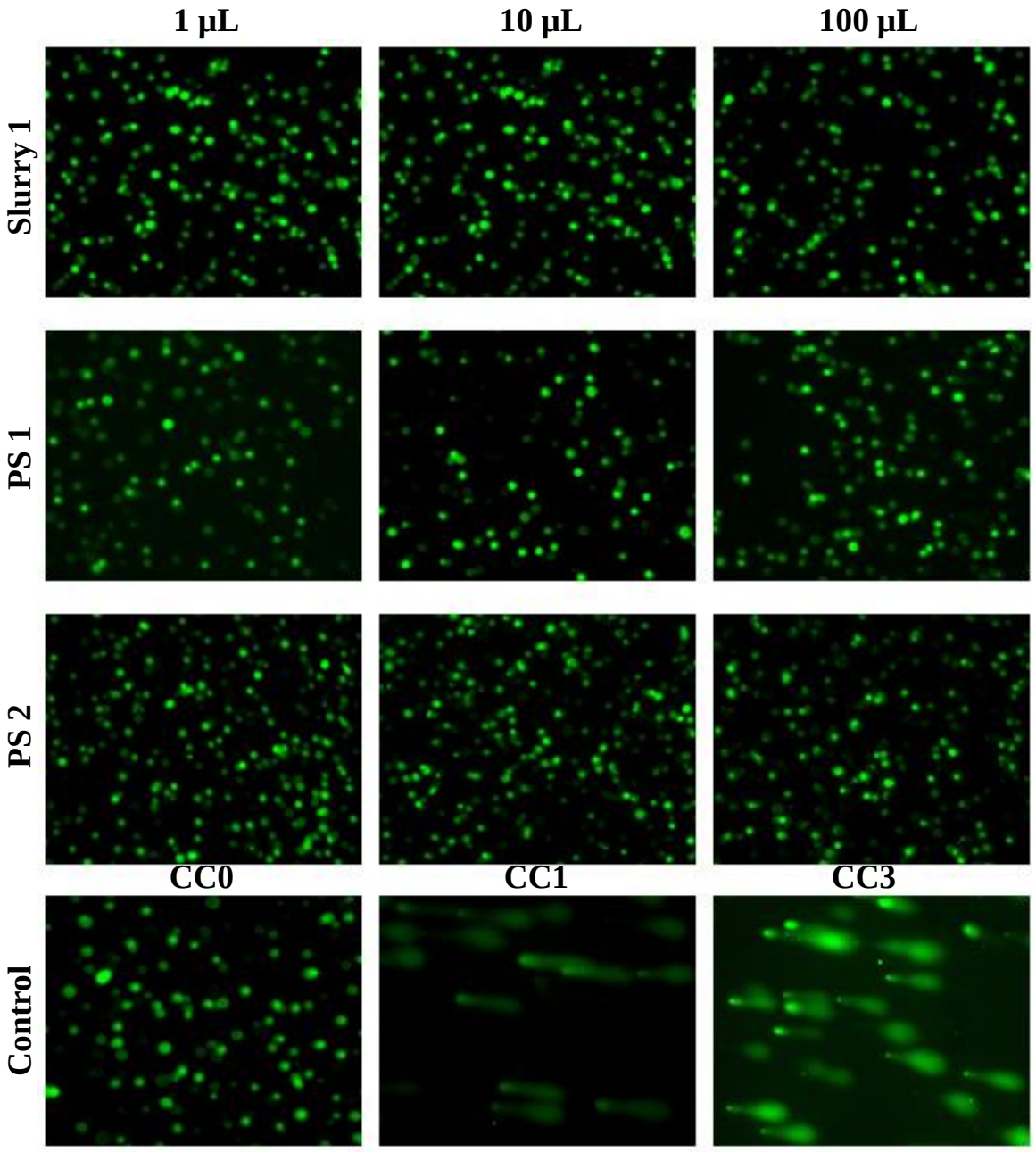
Monitoring of impedance modulus after addition of fresh media alone (control) and media containing 10, 30, 50 and 100µM sodium arsenite (Ceriotti, L., et al. (2007). Assessment of cytotoxicity by impedance spectroscopy. Biosensors and Bioelectronics, 22(12), 3057-3063.

IV. Genotoxicity Studies

- **Alkaline Comet Assays - To evaluate the DNA damage caused in cells**
- **Same assay and exposure conditions as previously stated for MTT/LDH**
- **Comet assay can detect nanoparticle-induced damage by measuring variations in DNA damage and repair capacity within a population of mammalian cells**
  - Nanoparticles may induce genotoxicity by interacting directly with DNA or through indirect means, by virtue of a number of factors including surface stress through direct particle influences on DNA, release of toxic ions from soluble NPs, or generation of oxidative stress
  - Assays is based upon the migration of DNA fragments out of nucleoids under the influence of electric field, the undamaged DNA is supercoiled, thus remains within the confines of nucleoid and does not migrate far out
  - Assay can also measure DNA single-strand breaks, DNA double-strand breaks, cross-links, base damage and apoptotic nuclei
- **Negative control, CC0, positive control with different level of damage - CC1, CC3**
- **Statistical Analysis**
  - SD from at least 90 cells with comet tail counts for each sample; averaging three independent experiments. negative control (student's t- test; $P < 0.00001$).
  - ANOVA (Analysis of Variance) - "one-way ANOVA" using a significance level of 99% confidence interval

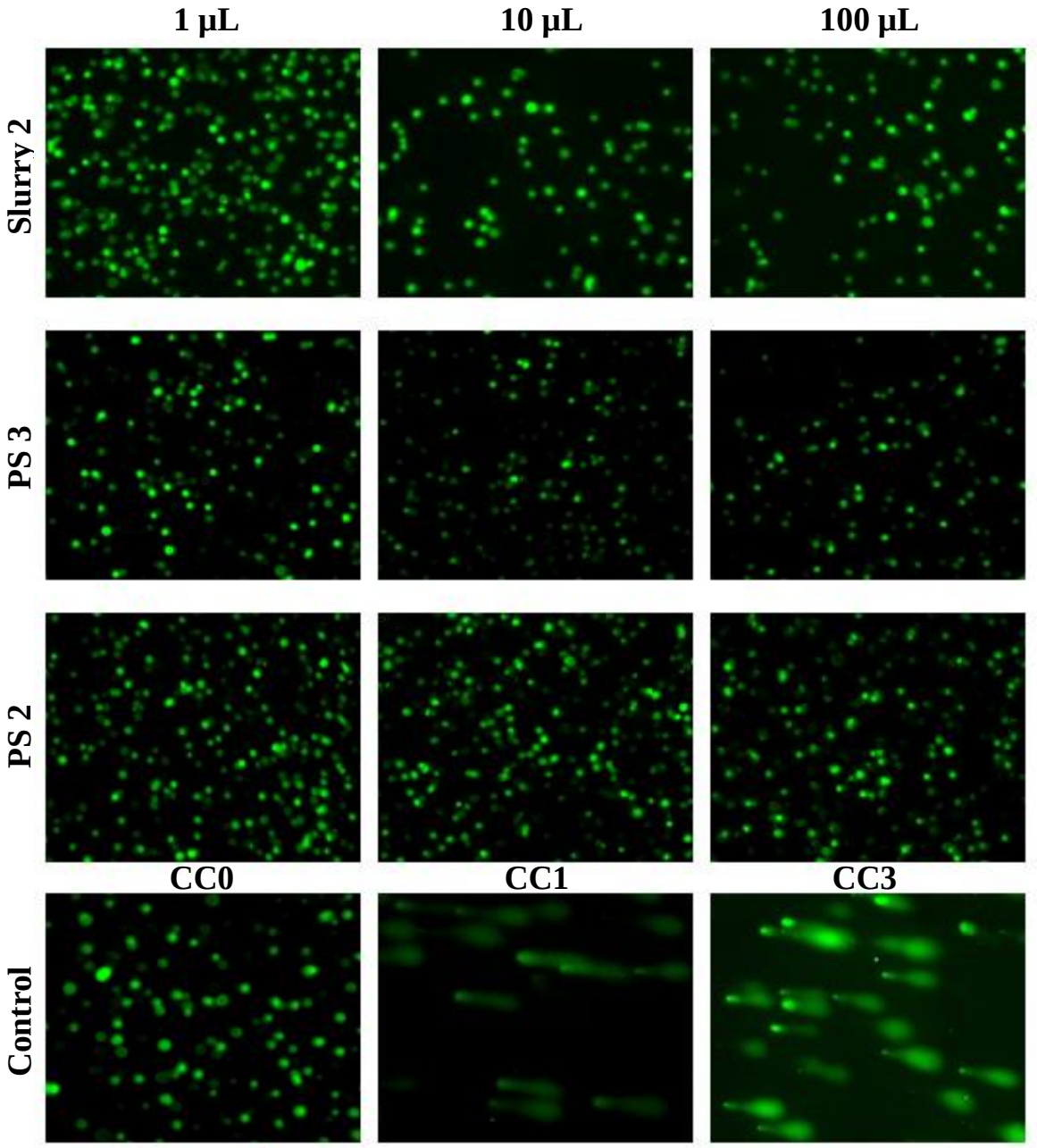
IV. Genotoxicity Studies

- **No detectable DNA damage from Comet assay on A549 cells on exposure to Slurry 1 and Pristine colloidal silica NPs and MPs, compared to positive control cells.**



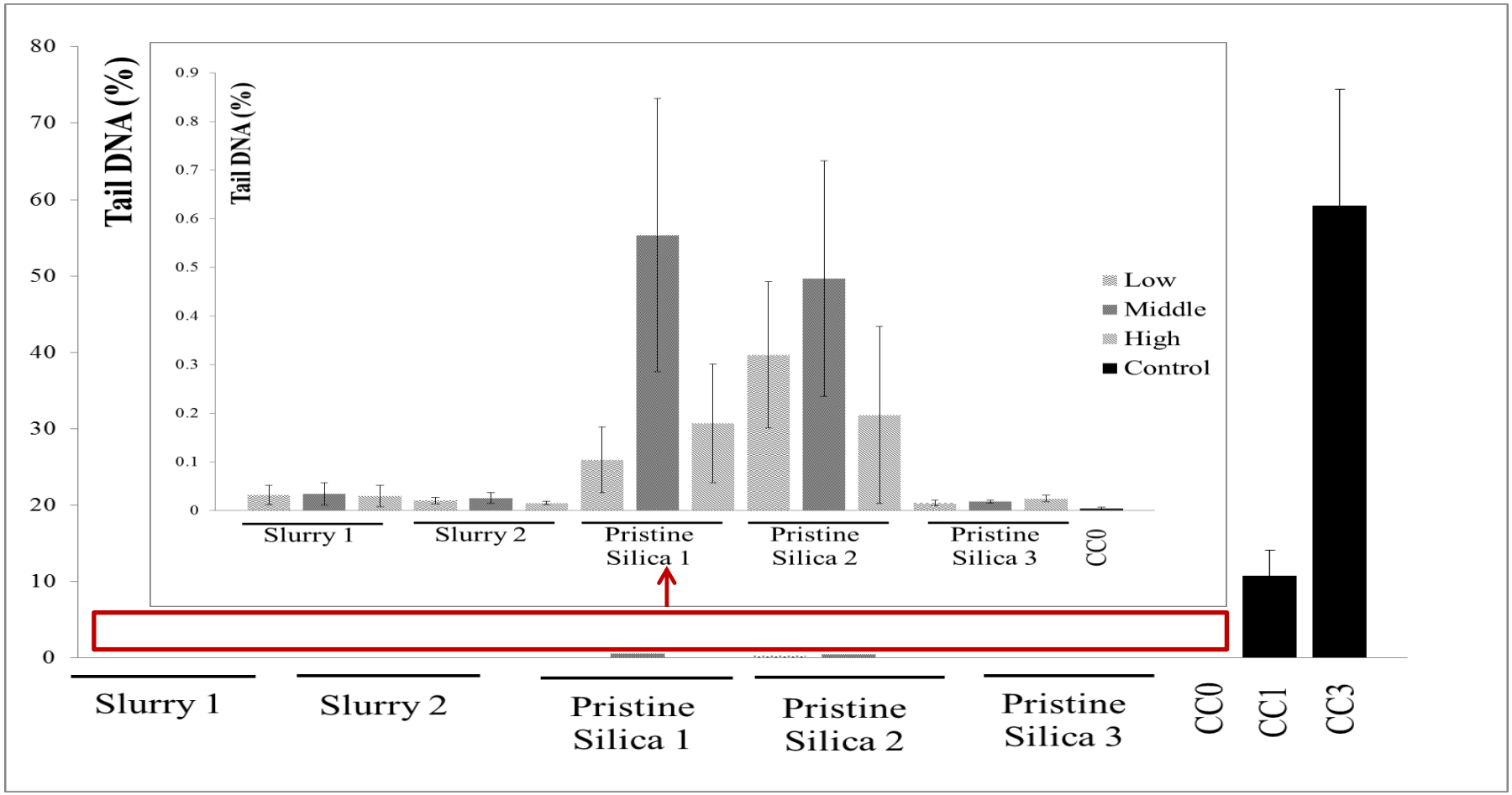
IV. Genotoxicity Studies

- No significant DNA strand breakage from Comet assay on A549 cells on exposure to Slurry 2, compared to positive control cells
- However, Slurry 2 showed significantly decreased cell population at high concentrations



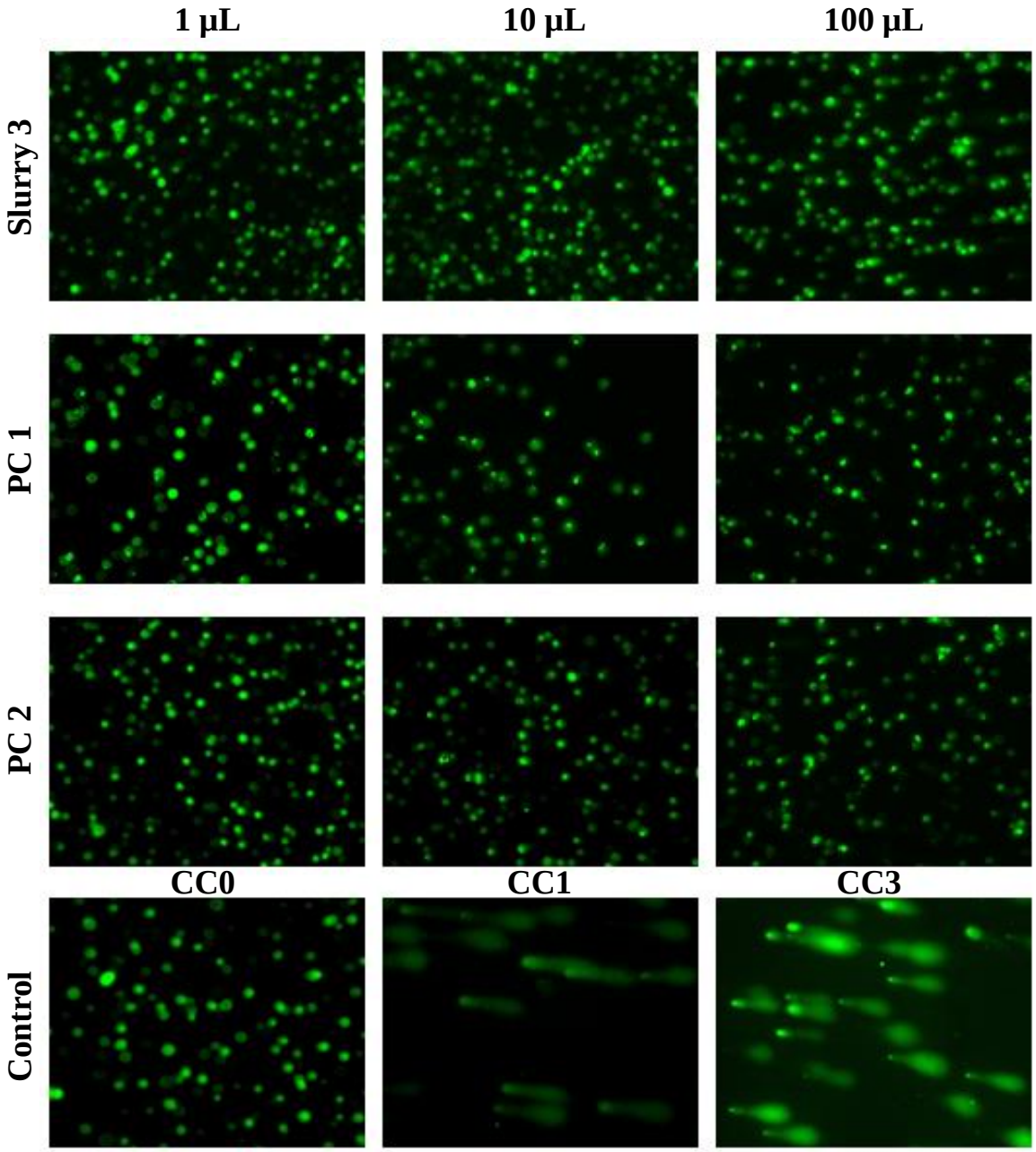
IV. Genotoxicity Studies

- **Statistical analysis for Slurry 1, Slurry 2, Pristine NPs and MPs**



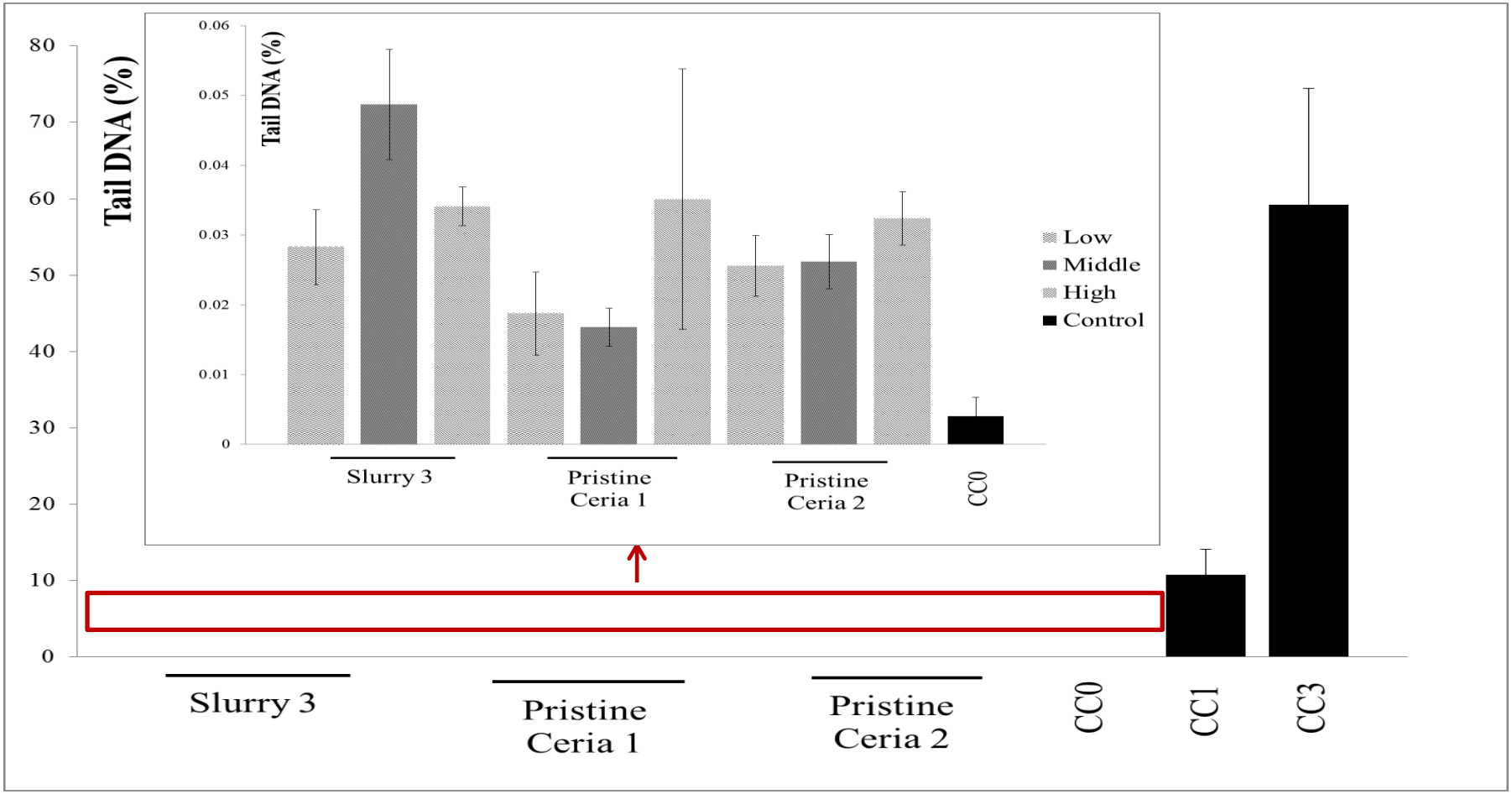
IV. Genotoxicity Studies

- No significant DNA strand breakage from Comet assay on A549 cells on exposure to Slurry 3, Pristine Ceria NPs and MPs, compared to positive control cells



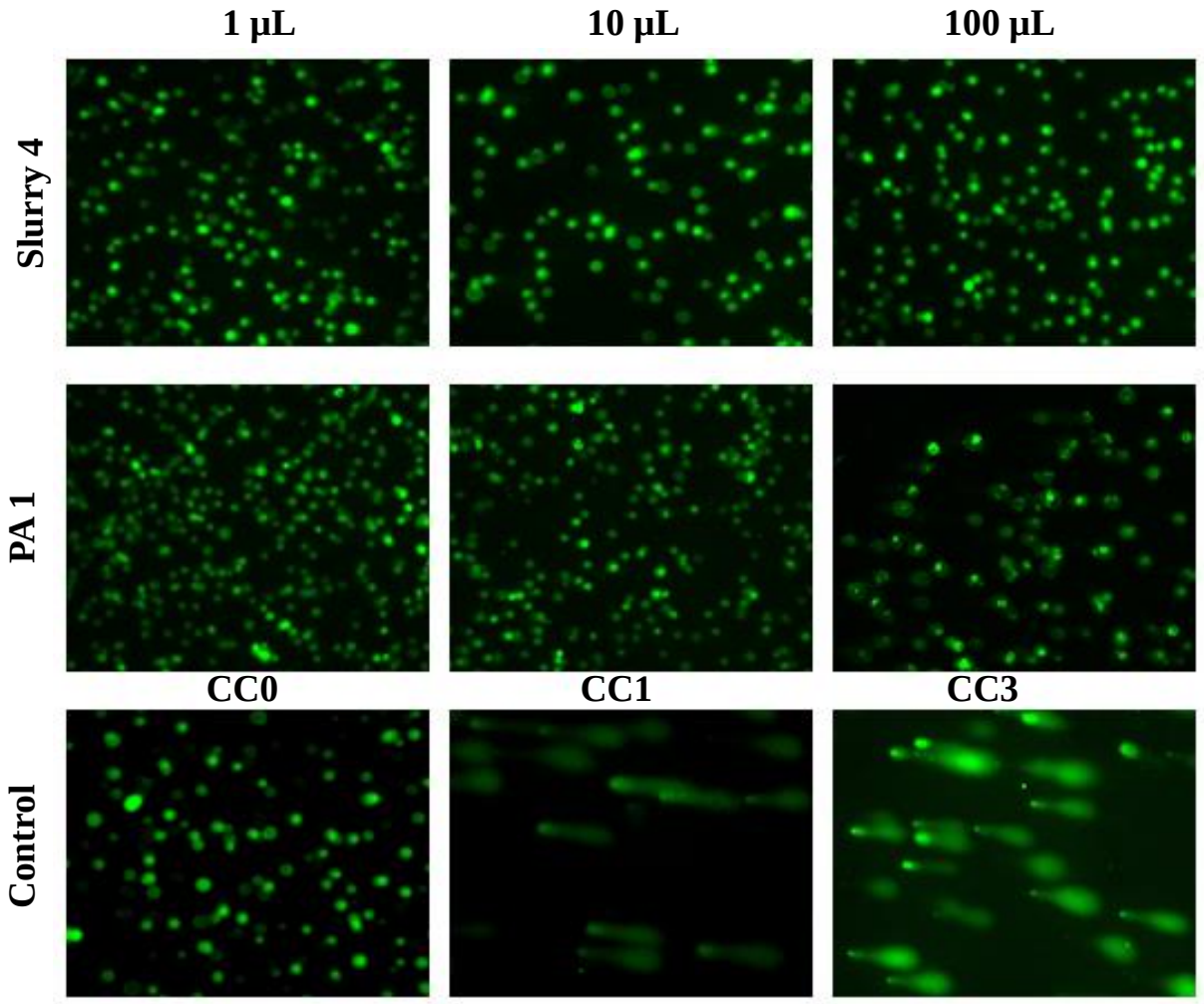
IV. Genotoxicity Studies

- **Statistical analysis for Slurry 3, Pristine Ceria NPs and MPs**



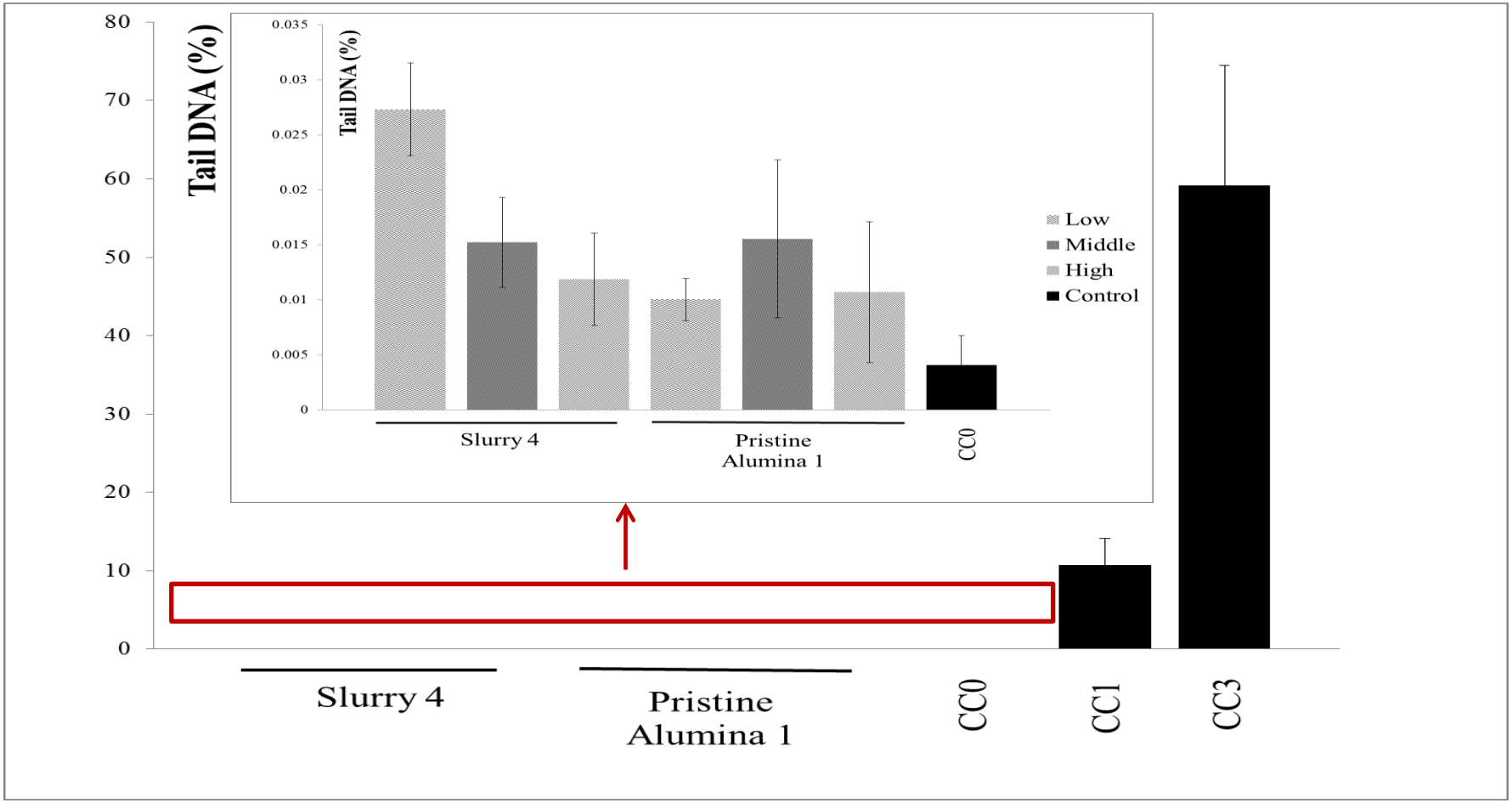
IV. Genotoxicity Studies

- **No significant DNA strand breakage from Comet assay on A549 cells on exposure to Slurry 4, Pristine Alumina NPs and MPs, compared to positive control cells**



IV. Genotoxicity Studies

- **Statistical analysis for Slurry 4, Pristine Alumina NPs and MPs**

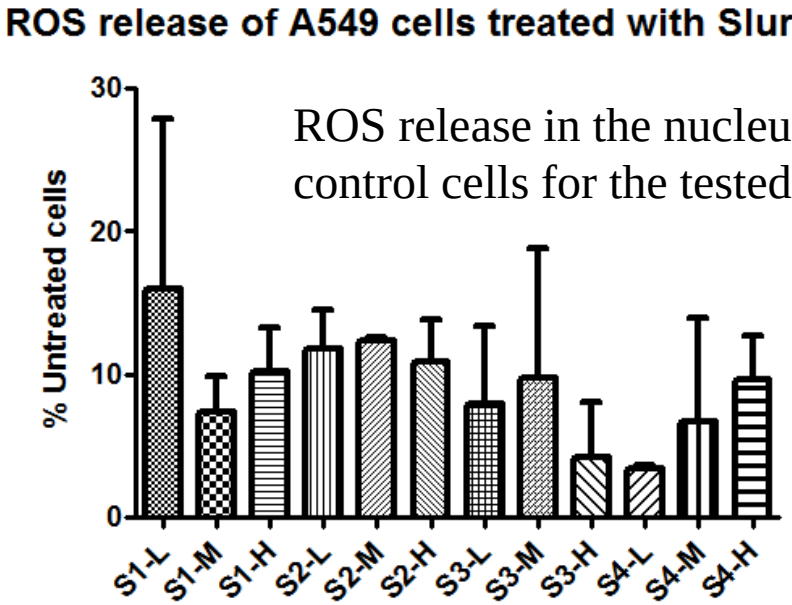


IV. Genotoxicity Studies

- **All slurries and pristine particles did not cause detectable DNA damage in the A549 cells**
- **However, high concentrations of slurry 2 (fumed silica) showed decrease in total cell population (live and dead). This may suggest the possibility of growth inhibition, possibly due to increased level of oxidative stress**
  - Interaction between NPs and cells (by contact or internalization) can increase production of ROS as a fast response, leading to apoptosis (cell death)

Preliminary Reactive Oxygen Species (ROS)

- ROS (and free radicals) are one of the primary NP toxicity mechanisms; may result in oxidative stress, inflammation, consequent damage to proteins, membranes and DNA
- CellROX® Green Reagent is a fluorogenic probe for measuring ROS in live cells. This cell-permeant dye is non-fluorescent while in reduced state and exhibits bright green photostable fluorescence upon oxidation by ROS
- ROS assay conditions were same as stated earlier; CellROX reagent was added at a concentration of 5 μM, incubated for 30 min at 37°C, then washed with PBS - 3 times
- Fluorescent intensity was measured at 485/520 nm (emission/excitation)



ROS release in the nucleus less than 20% of control cells for the tested concentrations

Preliminary CMP Results

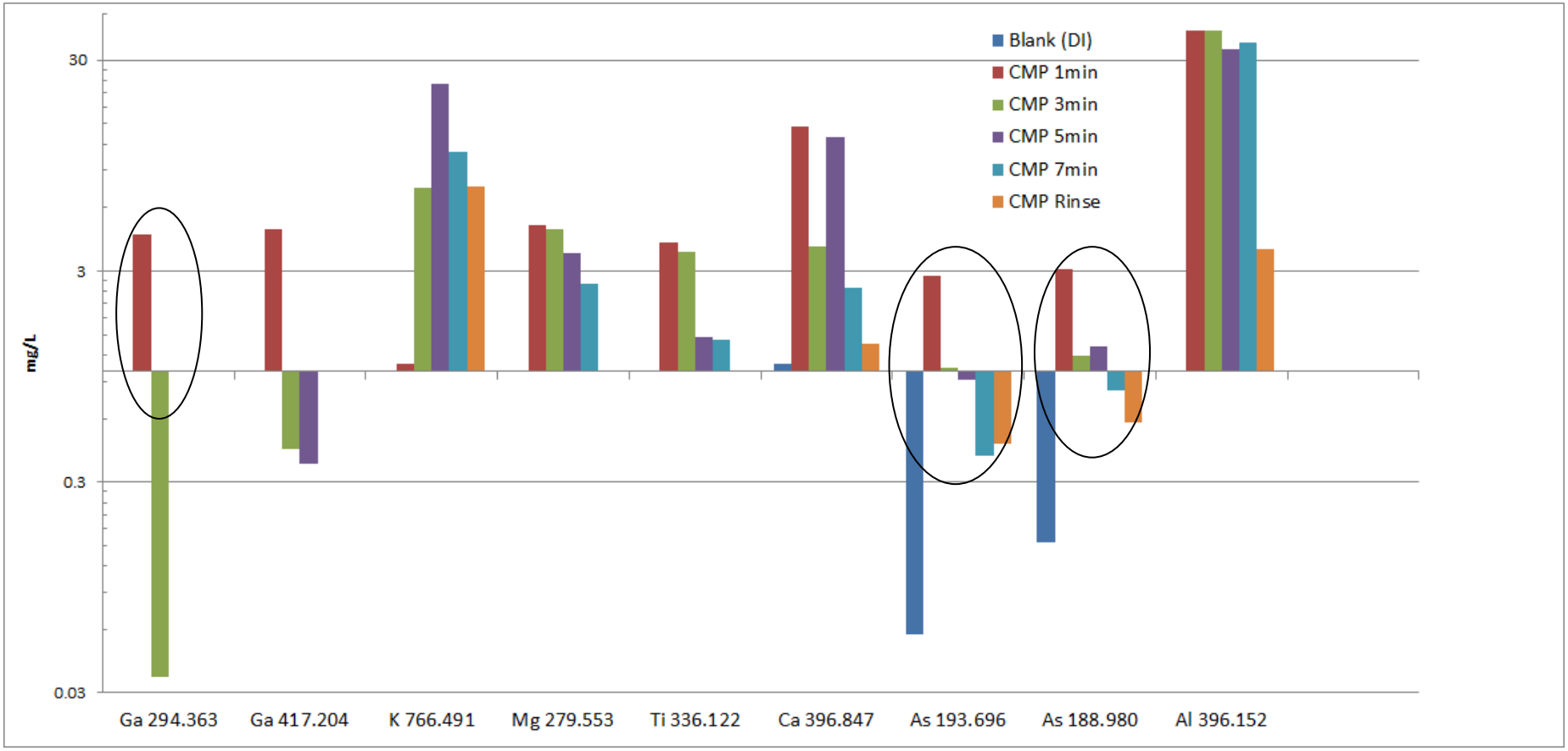
- Polished 6” GaAs substrates on IPEC Avanti 472 CMP
- Polishing conditions (ref. Babu group) – Colloidal silica slurry (S1), carrier/platen speed- 80/72 rpm, polishing time – 1 to 7 minutes, operating pressure - 2.5 psi; Dow® IC1000™ K-groove polishing pad, pad conditioning done in-situ
- Post-CMP clean on Lam Research Corp DSS-200 Series II Brush Cleaner
- ICP-OES analysis of pre- and post-CMP slurry/waste
- Ongoing efforts - study slurry/waste from CMP of GaAs and InGaAs buffer layers deposited by Molecular Beam Epitaxy (MBE)

IPEC Avanti 472 CMP. Inset shows polishing pad/surface

Lam DSS-200 Series II Brush Cleaner

Preliminary CMP Results

- **CMP colloidal silica slurry waste collected after polishing GaAs substrates**



ICP-OES trace elemental analysis after digestion of sl;urry waste in 1% HF

Summary of Results

- Comprehensive physical and chemical characterization of four model slurries, along with pristine micro and nanoparticles of comparable size and method of synthesis
- Conducted NP pathway or lifecycle on interaction with a mammalian cell
  - **Surface Interaction** - Cell viability and membrane integrity (MTT and LDH assays)
  - **Cellular uptake and i**nternalization (using ICP-OES, microscopy, Confocal Raman, ECIS and ROS)
  - **Interaction with nucleus** (NP-induced DNA damage, Comet assay)
- Results indicate cellular uptake for all NPs and toxic potential (at least for silica slurry) at higher NP concentration, however minimal to no toxicity due to NP alone (pristine NPs)
- Trace element studies did not explain silica slurry toxicity; more analysis of slurry constituents is required to understand source of silica toxicity
- Preliminary CMP on interaction of colloidal silica slurries with GaAs

Industrial Interactions and Technology Transfer

- **Monthly SRC/ERC Nanotoxicity consortium meetings with academic researchers and industry liaisons**
  - **Obtained 4 model slurries of silica, ceria and alumina NPs**
  - **Conducted round-robin studies with other consortium members on these slurries**
- **Hosted two teleseminars for SRC/ERC for Environmentally Benign Semiconductor Manufacturing during 2013**

Future Plans

Next Year Plans

- **Finish cellular uptake, internalization and fate studies to completely track and understand the pathway of NP inside a cell**
- **Use the validated longitudinal methods (ECIS, TEM microscopy, ICP-OES, Confocal Raman) to conduct delayed and chronic cellular (> 48 hours) response studies**
- **Conduct CMP of InGaAs, GaAs buffer layers, oxide and Cu surfaces on our CMP tool (IPEC Westech 472 along with post-CMP clean) using the model slurries. Analyze polished surfaces, post-CMP waste slurry for physiochemical factors and toxicity**

Long-Term Plans

- **Study ESH aspects (properties, interaction and toxicity) with real CMP samples and waste slurries.**
- **Generate dose-response relationship for slurries of different physiochemical properties including pH, oxidizers and charge**

Publications, Presentations, and Recognitions/Awards

- S. Aravamudhan, Talk at VCU, Richmond, VA, Feb 2014
- K. Garde, S. Aravamudhan, "Do Engineered Nanoparticles Penetrate into Cells?," J Nanomed Nanotechnol 2013, 4:5
- K. Garde, K. Kosaraju, S. B. Ravari, S. Aravamudhan, "Towards Understanding Toxicity of Engineered Nanomaterials," Ch. in Nanoscience and Nanoengineering: Advances and Applications, CRC Press, ISBN 9781482231199, 2014
- K. Kosaraju, K. Garde, S. Crawford, S. Aravamudhan, "Examining the Cellular Uptake of Engineered Nanomaterials," 225th ECS Meeting, Orlando, FL, May 11-16, 2014
- K. Kosaraju, K. Garde, S. Crawford, S. Aravamudhan, "Examining Cellular Uptake of CMP Nanoparticles," TECHCON 2014 (submitted)
- Funding from NSF/CBET - "Analysis of cardiac repolarization as a tool for the noninvasive assessment of cardiovascular system exposure to carbon and metallic Nanotubes," 2013-2015