The Fate, Transport, Transformation and Toxicity of Manufactured Nanomaterials in Drinking Water

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Project Objectives

Goal: To understand the fate and significance of nanomaterials in drinking water

The objectives of this project are:

1) to characterize the fundamental properties of nanomaterials in aquatic environments
2) to examine the interactions between nanomaterials and pollutants and pathogens
3) to evaluate the removal efficiency of nanomaterials by drinking water unit processes
4) to test the toxicity of nanomaterials in drinking water using cell culture model system of the epithelium.

Project Timetable

Tasks	Scheduled Time
Literature preparation	January 2005
Nanomaterial Detection Methods	Oct 2004 – Mar 2005
Characterization of Nanomaterials in Water	Jan 2005 – Dec 2005
Adsorption of Dissolved Pollutants onto Nanoparticles	July 2006 – June 2007
Aggregation and Coagulation of Nanomaterials	Jan 2005 – Mar 2007
Nanoparticle Adsorption and Disinfectant Shielding of Virus	June 2006 – Mar 2007
Nanoparticle Toxicity Screening for Drinking Water	Jan 2005 – Dec 2006
Final Report	July 2007 - Oct 2007

I. Nanoparticle Characterization

Interesting note: Several papers investigate environmental applications of nanoparticles, but use 0.45 um filtration to remove the “nano”particles.

We have found for metal oxide nanoparticles:

- Nanoparticles placed in water are aggregated
- Aggregation due to electrostatics of dry powders, manufacturing process, and/or aggregation in solution
- Sonication temporarily dis-aggregates some nanoparticles
- Surfactants and/or solvents promote some dis-aggregation
- Nanoparticles purchased in solutions are less, but still, aggregated
- Solution = Producing nanoparticles in laboratory rather than commercial sources

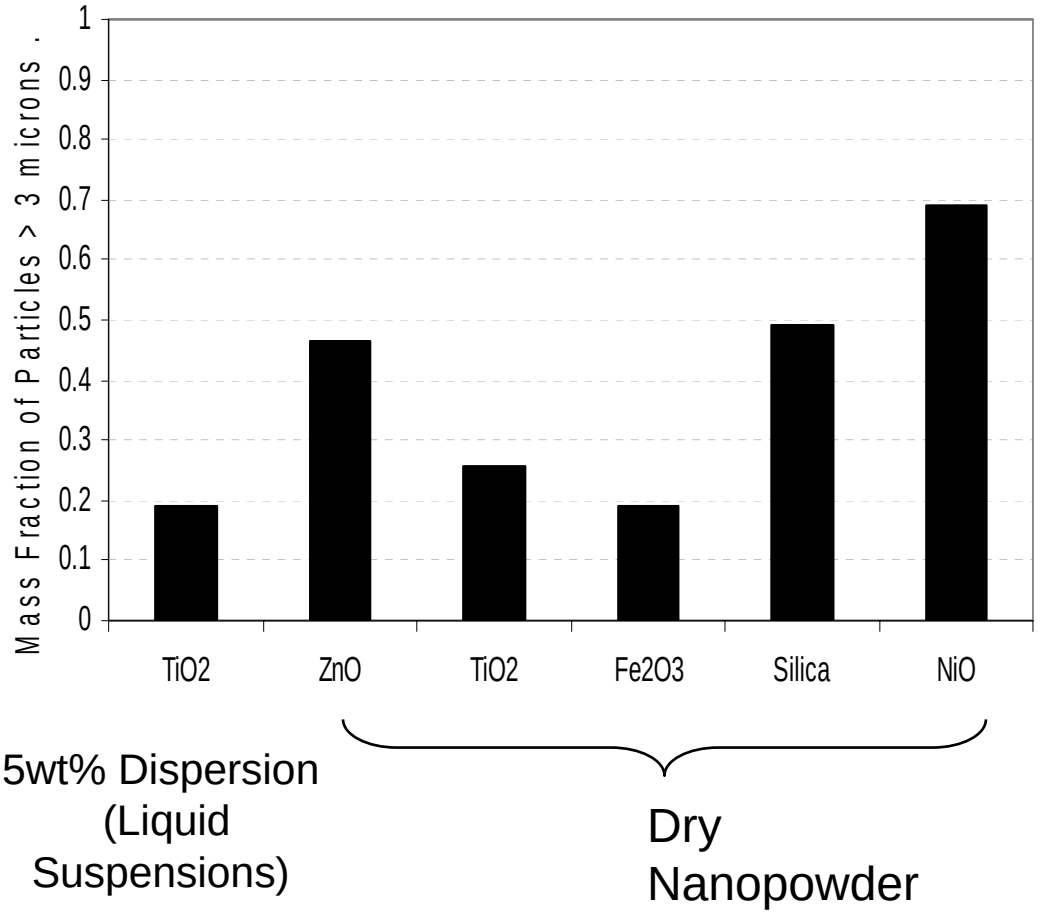
Challenge for all nanoparticle research: commercial nanoparticles in water are NOT nanoparticles (< 100 nm in at least one dimension)

Nanomaterials in Study

Nanoparticle	Vendor Reported Mean Particle Size	Density	Source
Titanium dioxide	< 40 nm	3.9g/mL	5wt% Dispersion in water
Aluminum oxide	< 20 nm	3.97g/mL	5wt% Dispersion in water
Zinc Oxide	50 ~ 70 nm	5.61g/mL	Nanopowder
Titanium dioxide	15 nm	3.9g/mL	99.7% Nanopowder
Iron (III) Oxide	5 ~ 25 nm	5.24g/mL	Nanopowder
Nickel Oxide	10 ~ 20 nm	6.67g/mL	99.8% Nanopowder
Silica	10 nm	2.6g/mL	99.5% Nanopowder

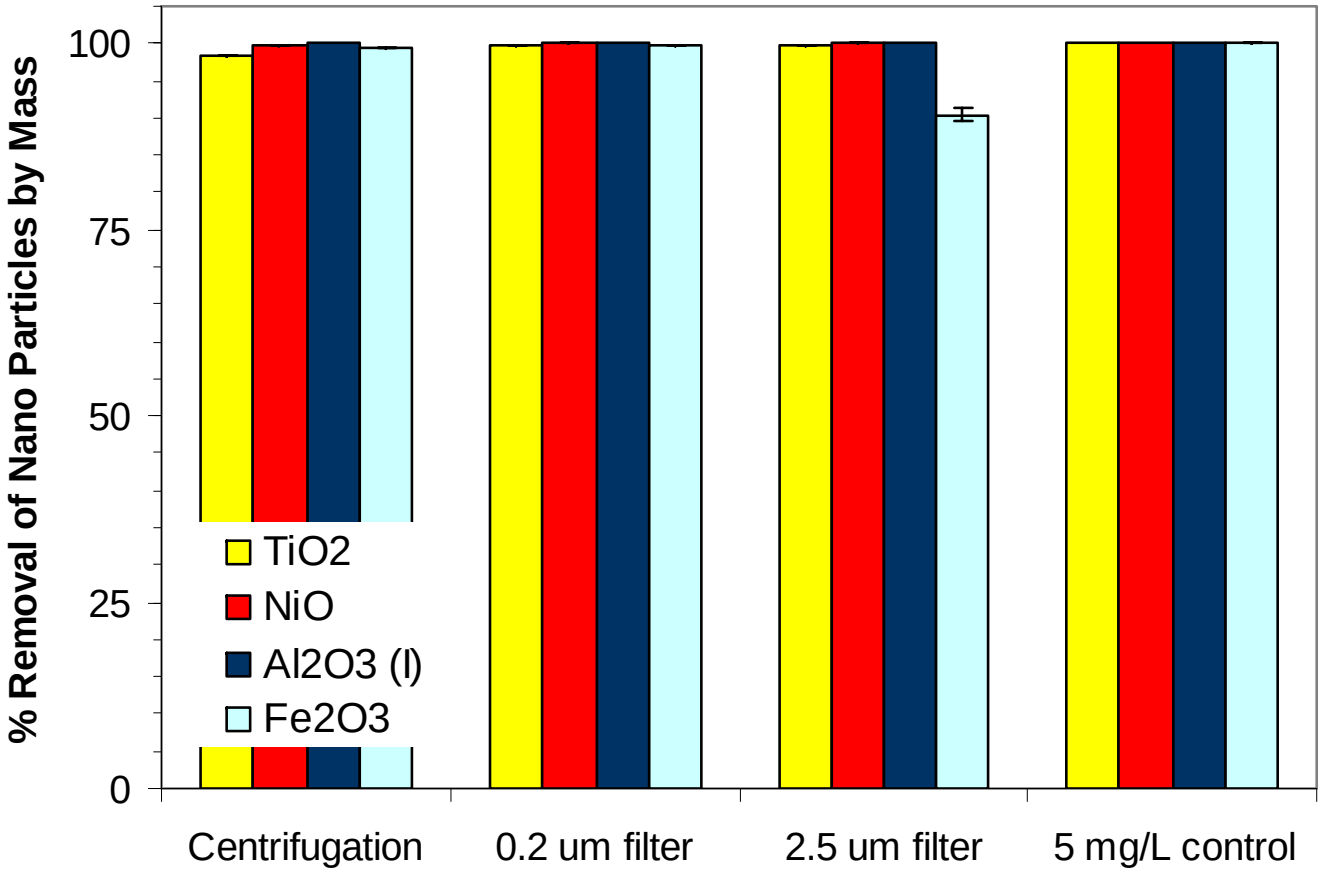
- All nanoparticles purchased as powders and liquid suspensions
- All values in above table are reported by Vendor.
- Other nanomaterials currently in use:
  - Several other commercial metal oxide nanoparticles
  - Carbon nanotubes and fullerenes
  - TiO2 nanotubes and nanoparticles – fabricated in ASU laboratory
  - Gold and/or cadmium quantum dots

Size of nanoparticles in water



- 10mg/L and 5mg/L nanoparticles in Nanopure water sonicated for 15 min
- Filter paper with 3 µm pore size and 110nm diameter
- 100ml and 50ml suspensions for filtration
- Concentrations of particles analyzed by digestion/AAS.
- Conclusion:
  - Significant mass of “nanoparticles” are aggregated and > 3um
  - DLS particle size instrument only measures particles < 3 um

At high nanoparticle concentration (1g/L) more aggregation occurs



C(NP) = 1 g/L

10 mM $NaHCO_3$

pH = 8.4 ± 0.2

Analysis by:

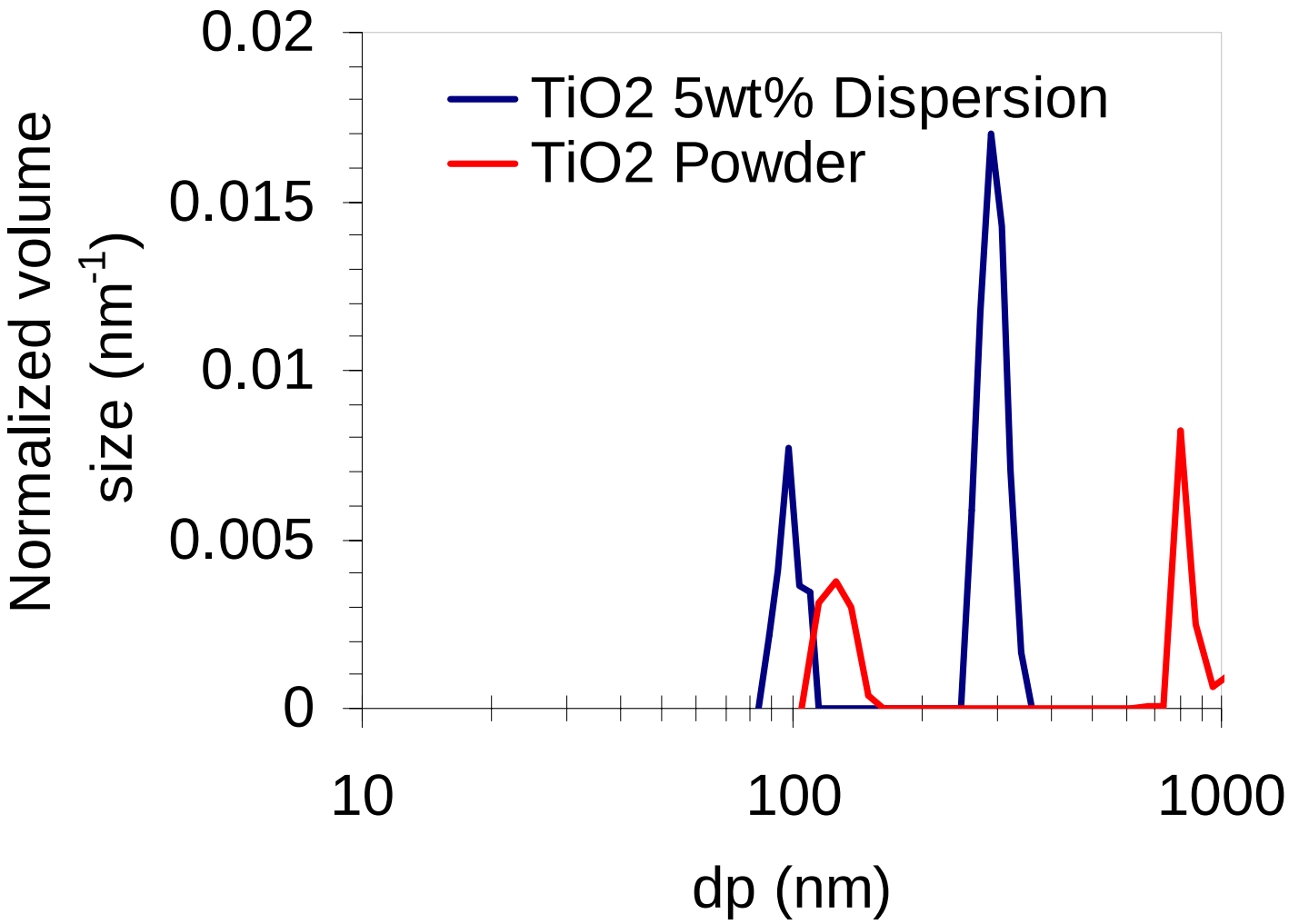
Digestion/GF-AAS

Centrifugation:

G > 1300

10 mg/L TiO_2 in Water

(ZetaPALS dynamic light scattering; < 3um only)

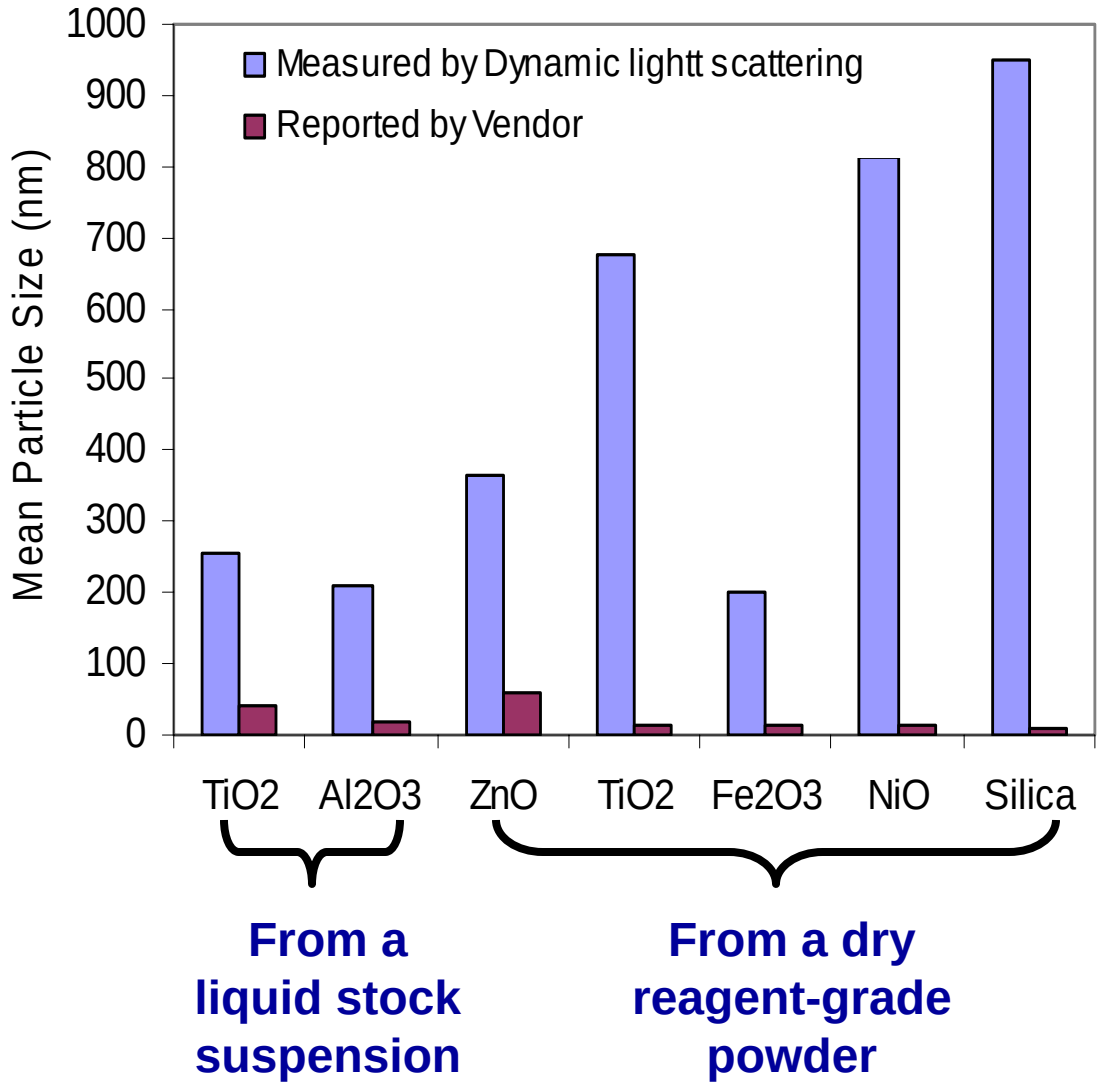


Conclusion: Nanoparticles do not appear to be discrete NP in water

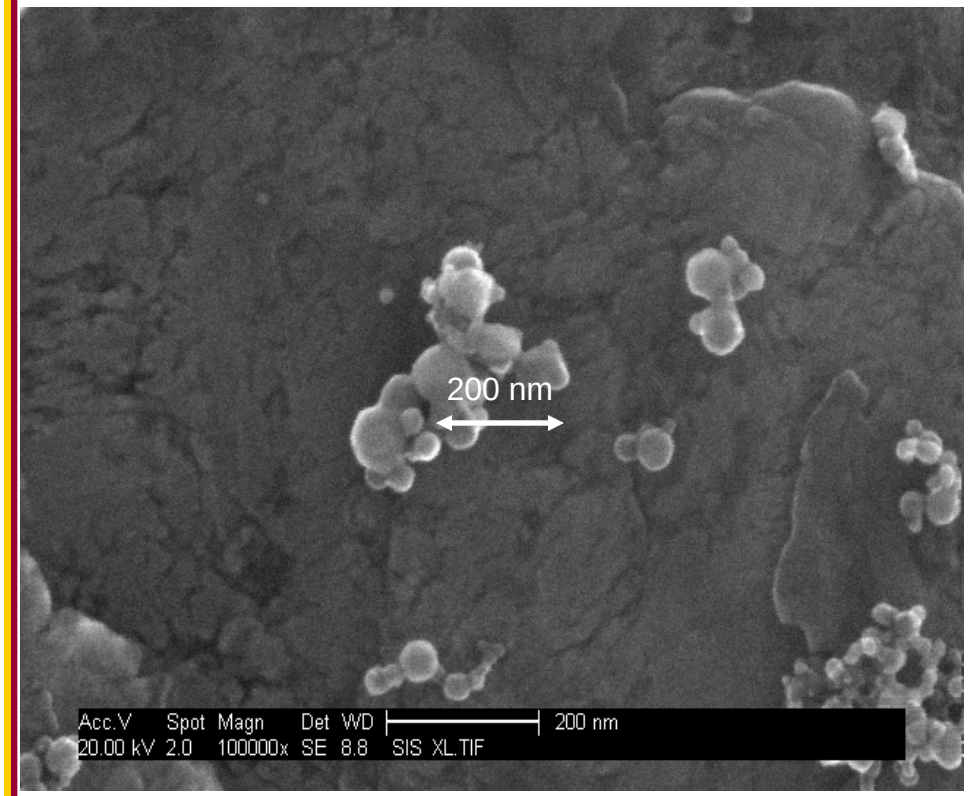
Size of nanoparticles in water

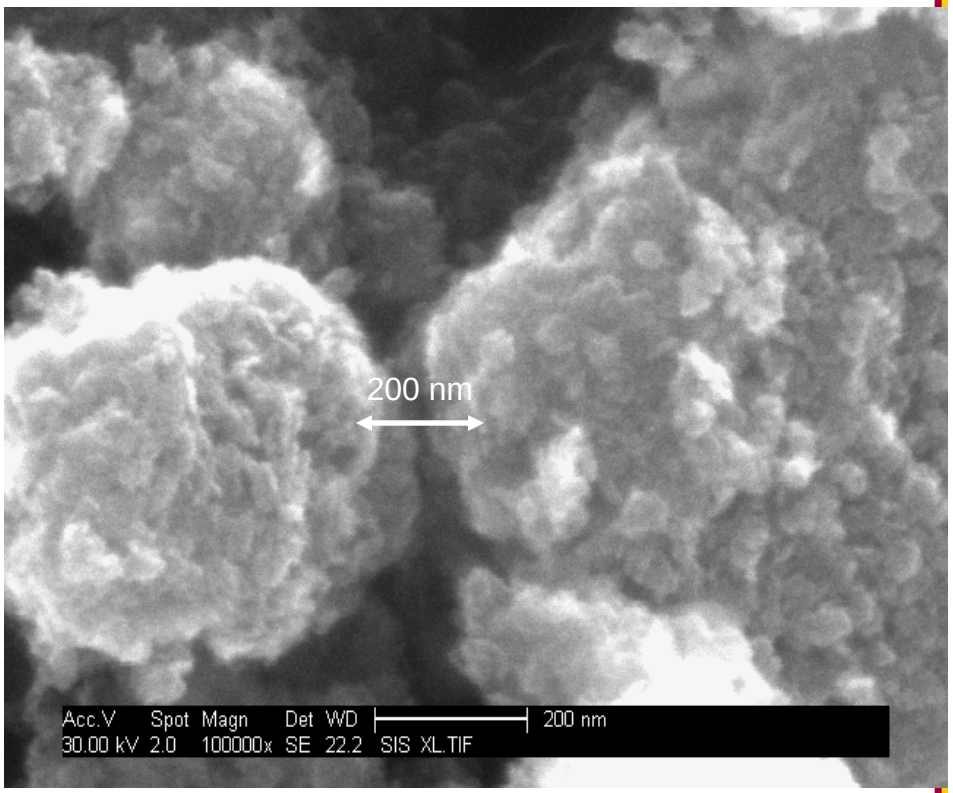
- Particle Size of nanoparticles were analyzed by Dynamic Light Scattering.
- 10mg/L nanoparticles in Nanopure water
- Sonicated for 15 min at 200 W/L and 20 kHz.
- Instrument range: 2nm ~ 3μm.

➢ Mean particle size is greater than reported by manufacturer.



SEM Analysis of Commercial TiO_2 NPs





TiO_2 NPs from **5wt% Dispersion in water**

TiO_2 NPs from a **dry powder**

Nanoparticles do not appear to be discrete NPs

Dispersion of aggregated NPs in water

- Sodium hexameta phosphate, Sodium dodecyl sulfate, Isopropanol, Acetone, Butonanone, Methanol, and Ethylene Glycol selected as a dispersant solvent.
- All suspensions sonicated for 15 mins

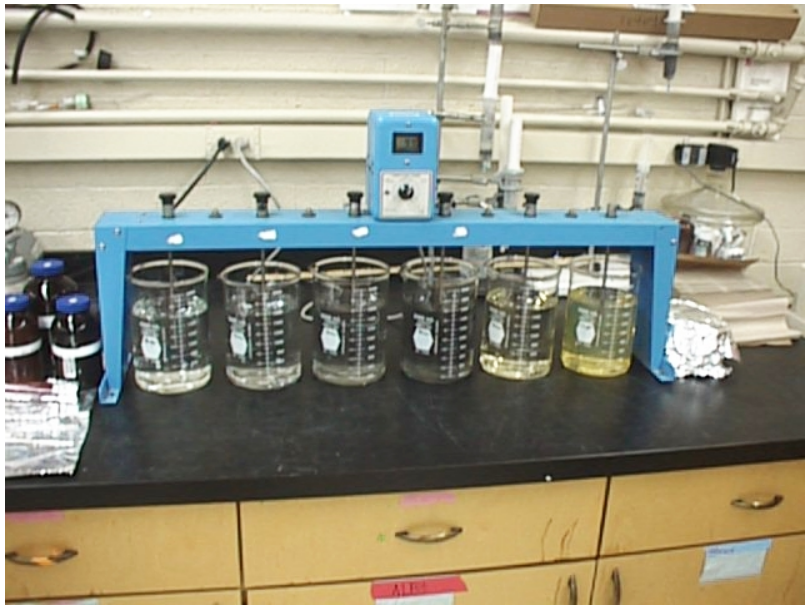
➢ With all above dispersants, mean particle sizes of TiO2 nanoparticles in Nanopure water still were much more than size of discrete nanoparticles.

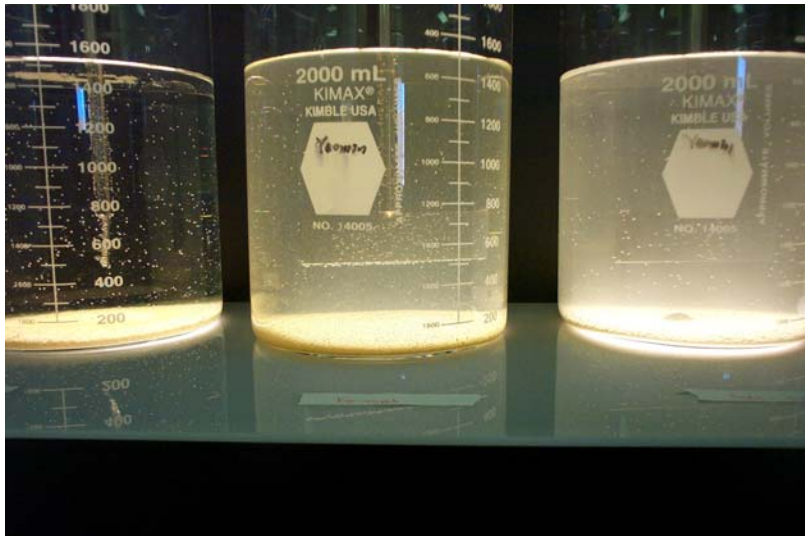
➢It is very difficult to disaggregate these NPs and obtain homogenous discrete nanoparticles.

Dispersant	Mean Particle Size
SDS (Sodium dodecyl sulfate)	530~570nm
(NaPO3)6 (Sodium hexameta phosphate)	560~580nm
Ethylene Glycol (10~100%)	300~550nm
Isopropanol (10%)	550~1050nm
Acetone (10%)	560~580nm
Butonanone (10%)	580~640nm
Methanol (10%)	550~570nm
Nanopure water (no dispersant)	540~600nm

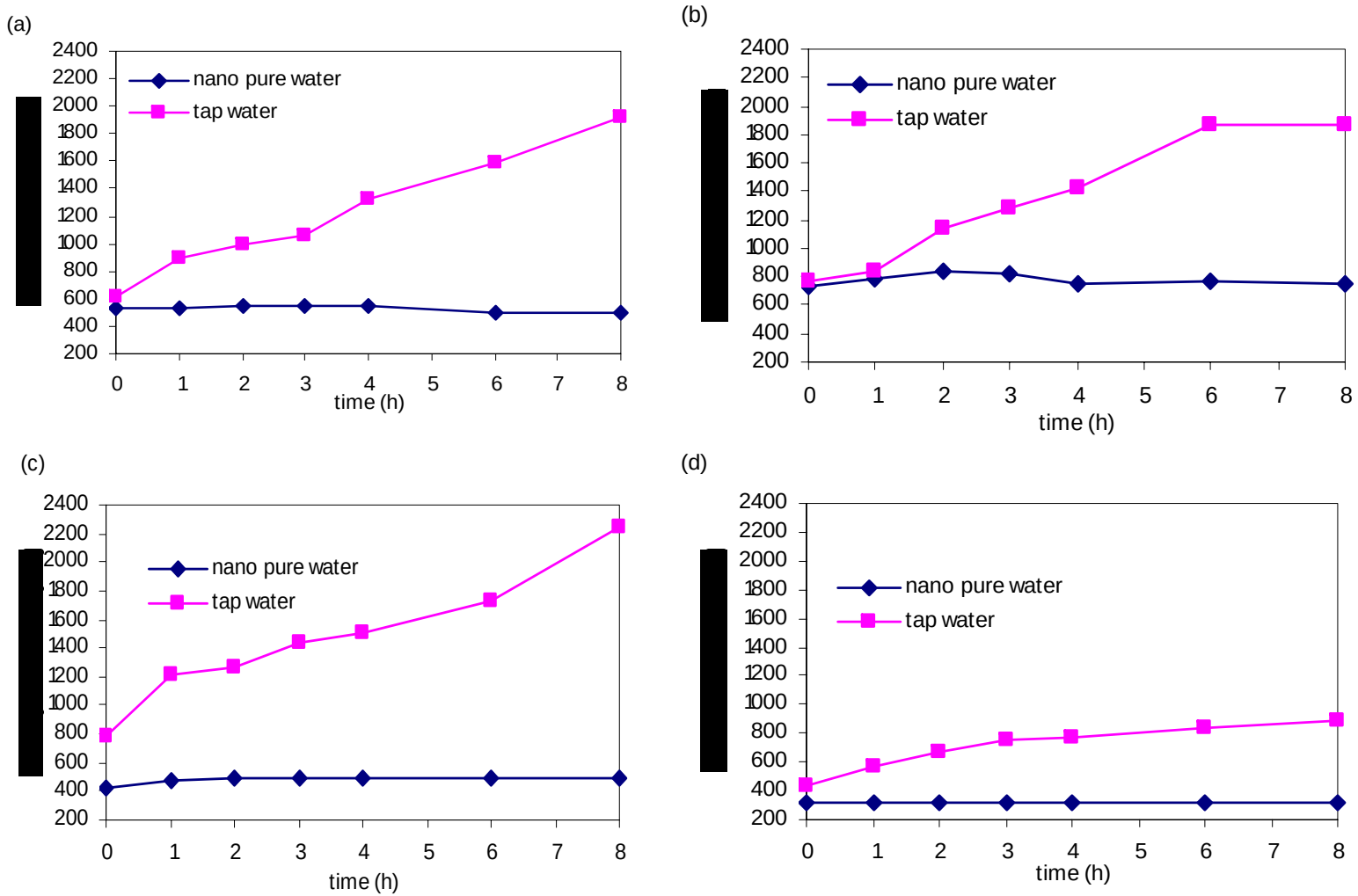
Mean particle size of TiO2 by Dynamic Light Scattering in Nanopure water w/o dispersants

II. Removal of Nanoparticles in Simulated Water Treatment Systems



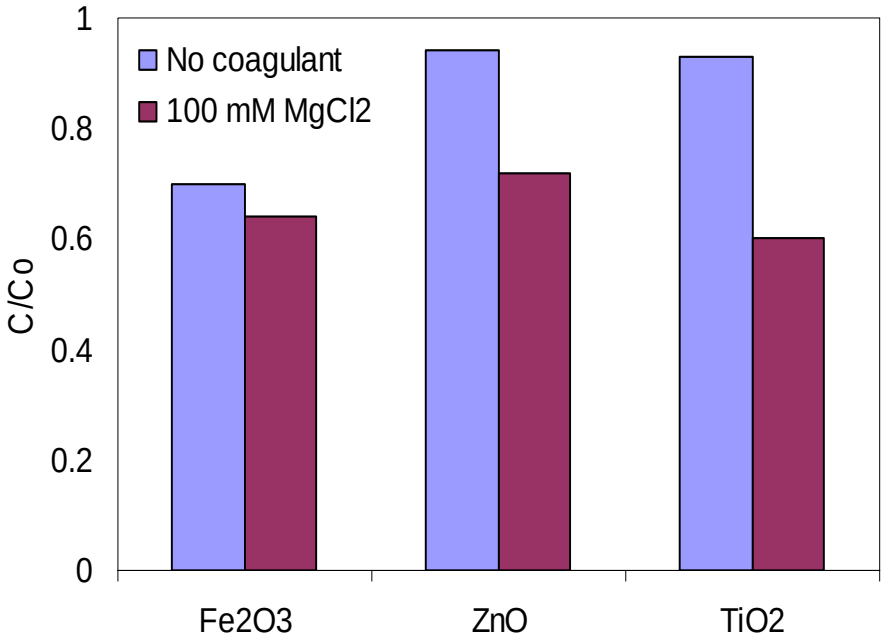


Further aggregation of nanoparticles in water

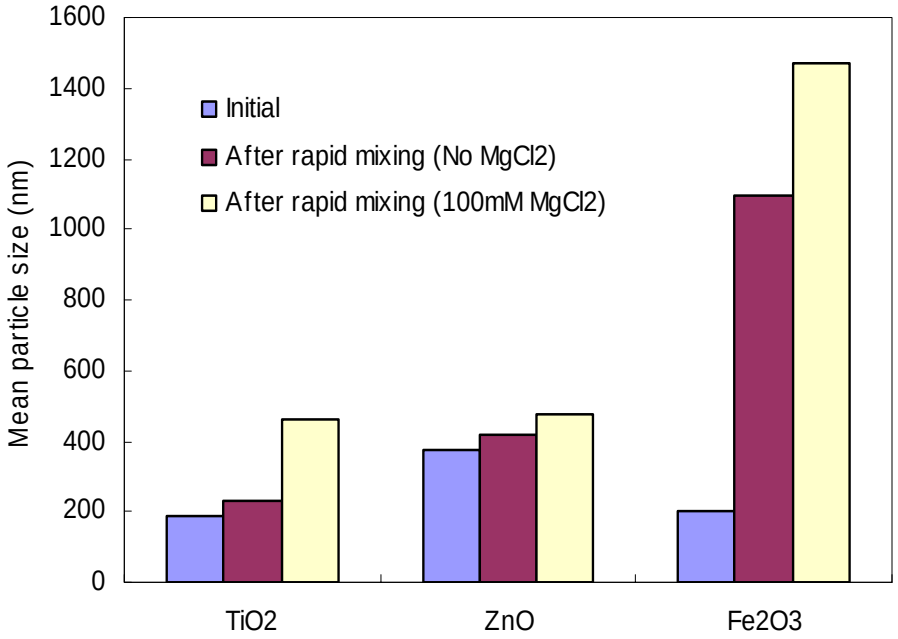


Mean particle size of 10mg/L nanoparticle suspensions with time. a.TiO_2 b. NiO c. Fe_2O_3 d.ZnO

Electric Double Layer Compresssion



Removal of nanoparticles in nano pure water without coagulant and with 100mM MgCl2



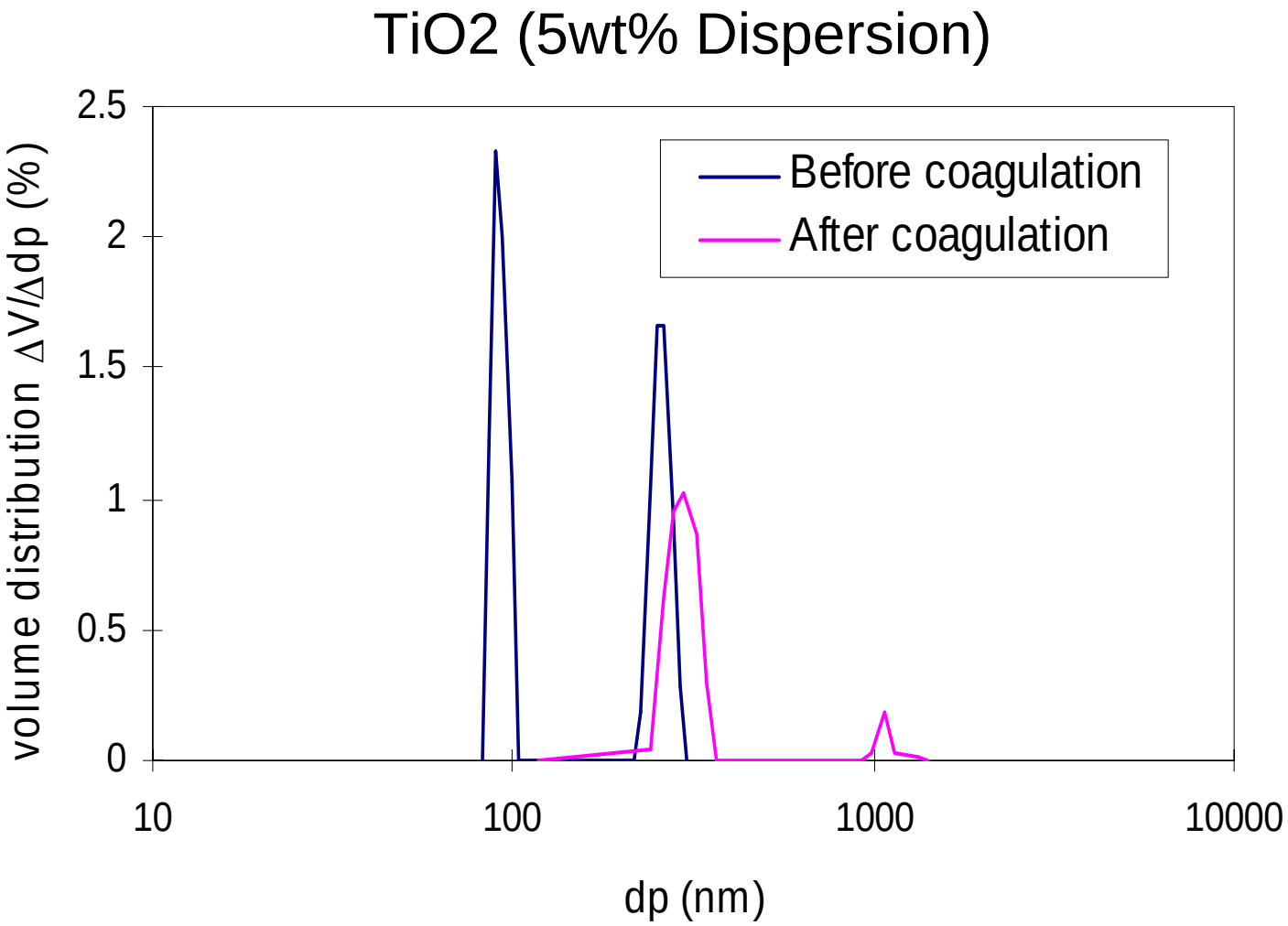
Mean nanoparticle size in nano pure water without coagulant and with 100mM MgCl2

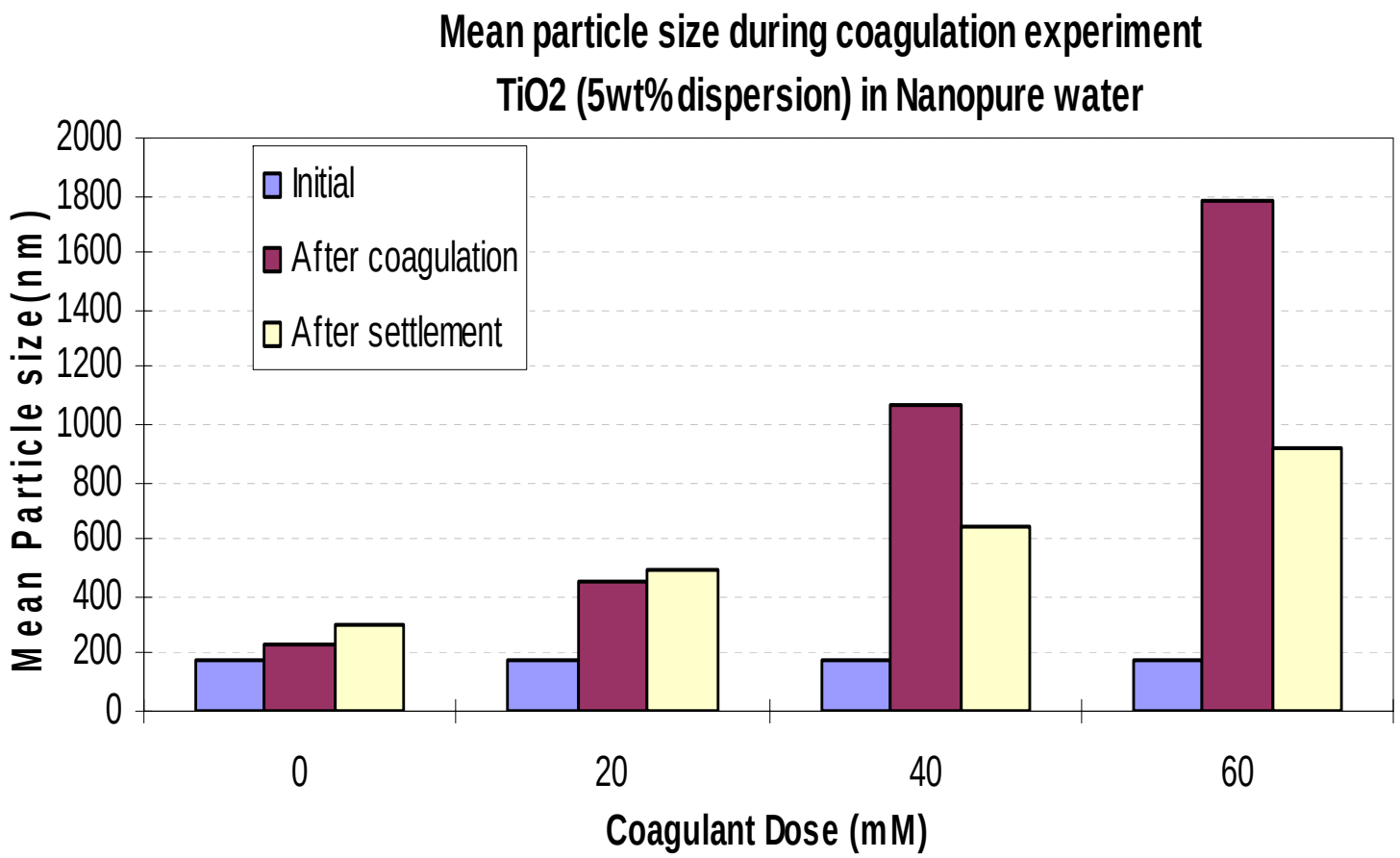
Test Conditions:

- 10 mg/L NPs in Nanopure water buffered by 10mM NaHCO3 (pH=8.1± 0.2)
- 100 mM $MgCl_2$ for EDL compression and NP destabilization
- NPs in supernatant measured by digestion/AAS after sedimentation period

Conclusions:

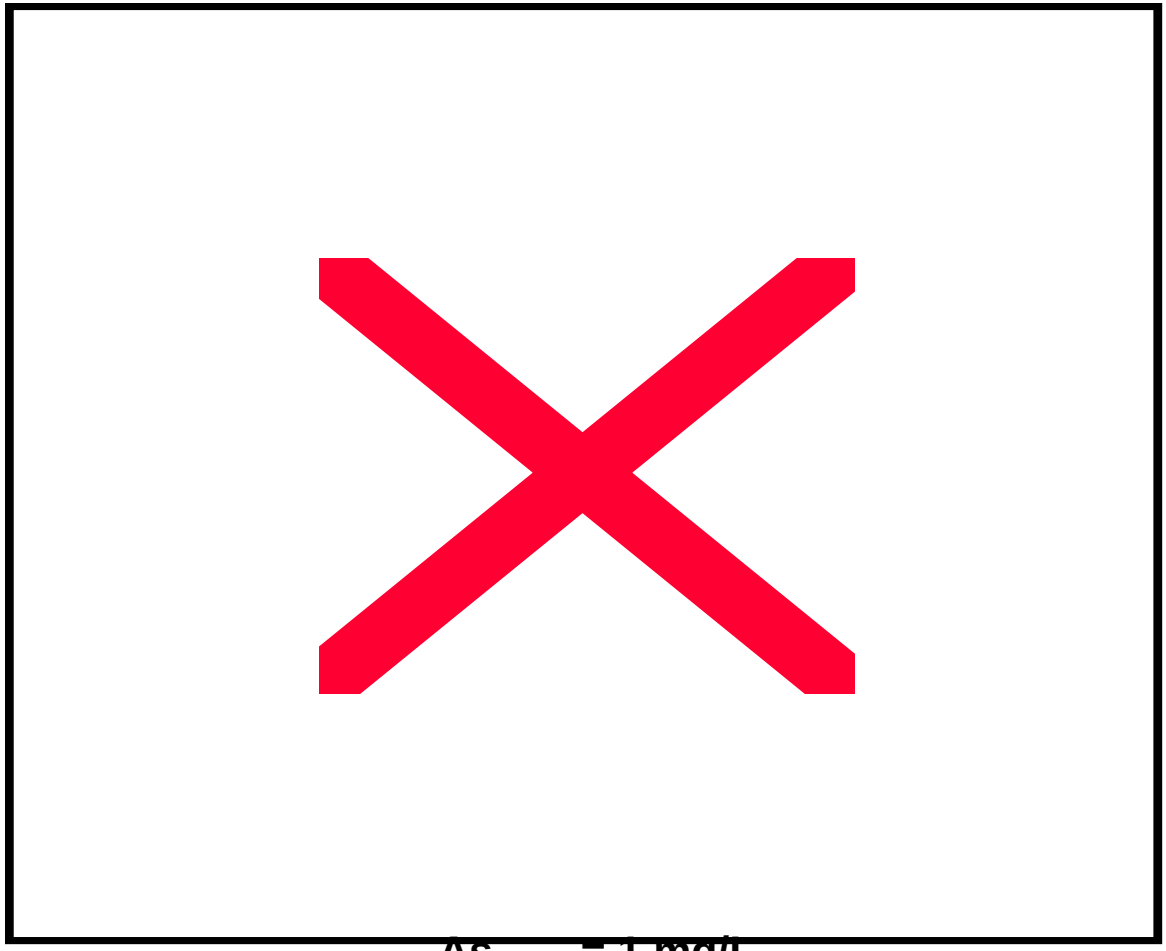
- Sedimentation removes aggregated NPs
- EDL compression leads to more aggregation for some NPs
- > 40% of NPs remain after sedimentation





III. Adsorption of Pollutants onto Nanoparticles

- Arsenic adsorption evaluated (see graphic)
- Adsorption of bacteriaphage (MS2 & PRD1):
  - Adsorption occurs onto positively charged NPs
  - Charge based upon zeta potential measurements



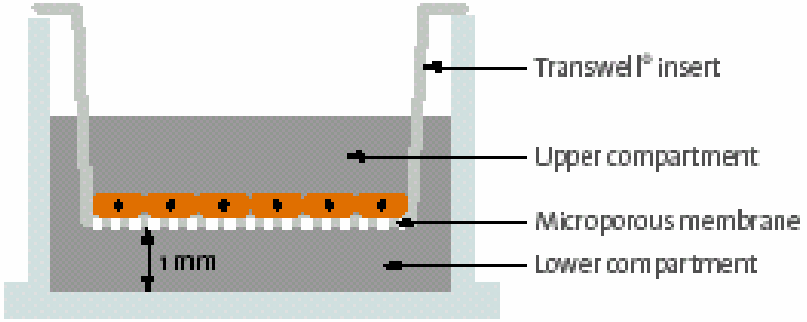
$As_{Initial}$ = 1 mg/L

C(NP) = 1 g/L pH = 8.0 ±0.4

IV. Toxicity of Nanoparticles

Objectives:

- Evaluate transport and necrotic effects of nanoparticles across epithelial layers (esophageal & intestinal)
- Uniform cells are established on semi-permeable support. Continuous cell structure leads to conductivity gradient across biofilm. Disruption of conductivity gradient inferred as detrimental impact to cells or biofilm structure



Filter Inserts

Human intestinal tissue model (Caco-2BBe) – from ATCC:

- Cells were transferred to semi-porous membranes and allowed to anchor and form tight junctions (three days).
- Transepithelial electrical resistance (TEER) was utilized to monitor the density and junctional complex of the cell monolayer.
- Cells were maintained in DMEM containing 10% fetal calf serum, penicillin/streptomycin/fungizone, and transferrin at 37°C, humidified air containing 10% CO2. Subsequently, the medium was changed every day after seeding onto the membrane.

Voltohmmeter

Testing Procedures

Rejected Methodology

- 5% CO2
- Media absent of transferrin
- Membranes composed of polycarbonate
- Membranes composed of polyester
- 12mm membrane diameter
- 24mm membrane diameter
- 3.0 µm pore size in membrane
- Change media every three days
- Change media every two days

Adopted Methodology

- 10% CO2
- Media containing 10µg/mL transferrin
- Pre-coated collagen membranes
- 6.5mm membrane diameter
- 0.4µm pore size in membrane
- Change media daily

Optimization of Culture Conditions

Condition	Relative Growth					
	0.05	1	2	3	4	5
CO2 Level	5%					
	10%					
Transferrin (μg/mL)	0		10			
Filter diameter (mm)	24			12		6.5
Filter pore size (μm)	3	0.4				
Filter material	Polycarb	Polyester				
Filter casing	Untreated					Collagated-PTFE
Collogen coated	By hand		Precoated			
Frequency for media change-out (days)	3	2	1			

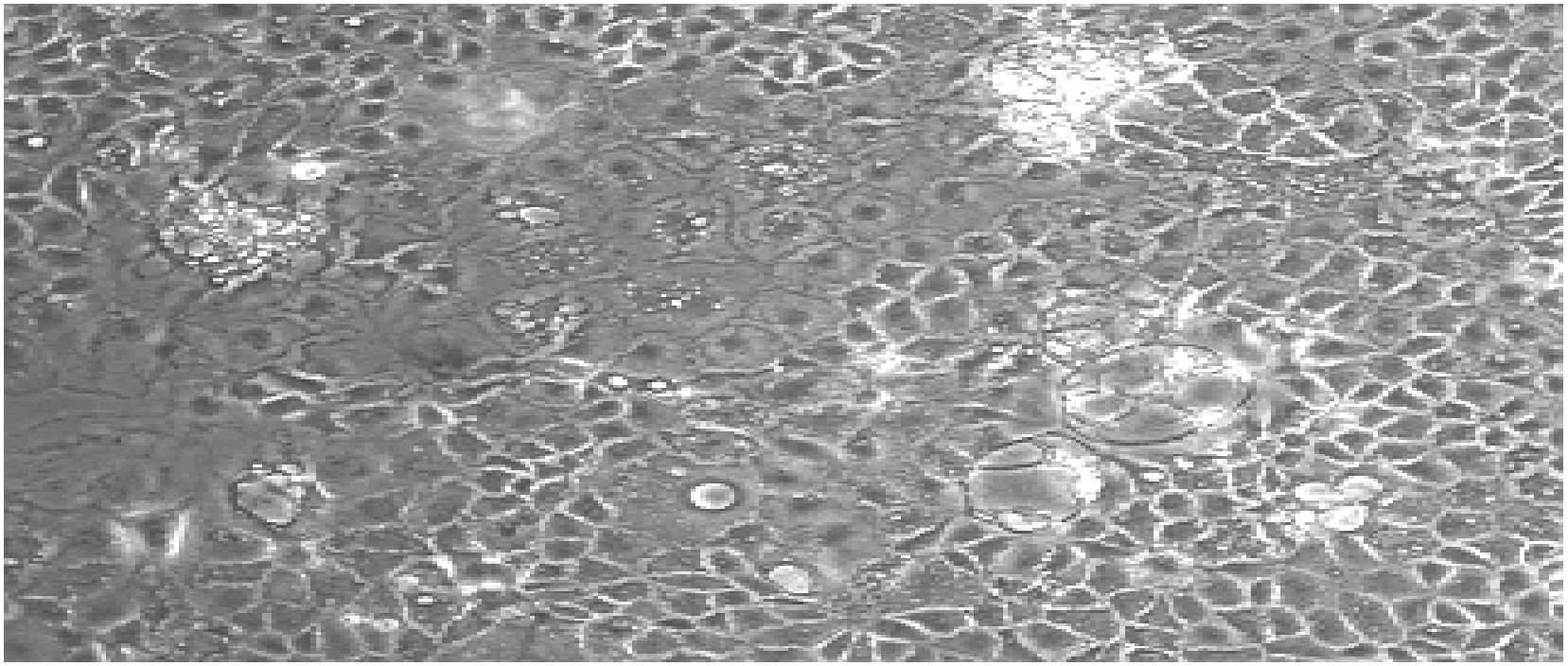
Optimization of Culture Conditions

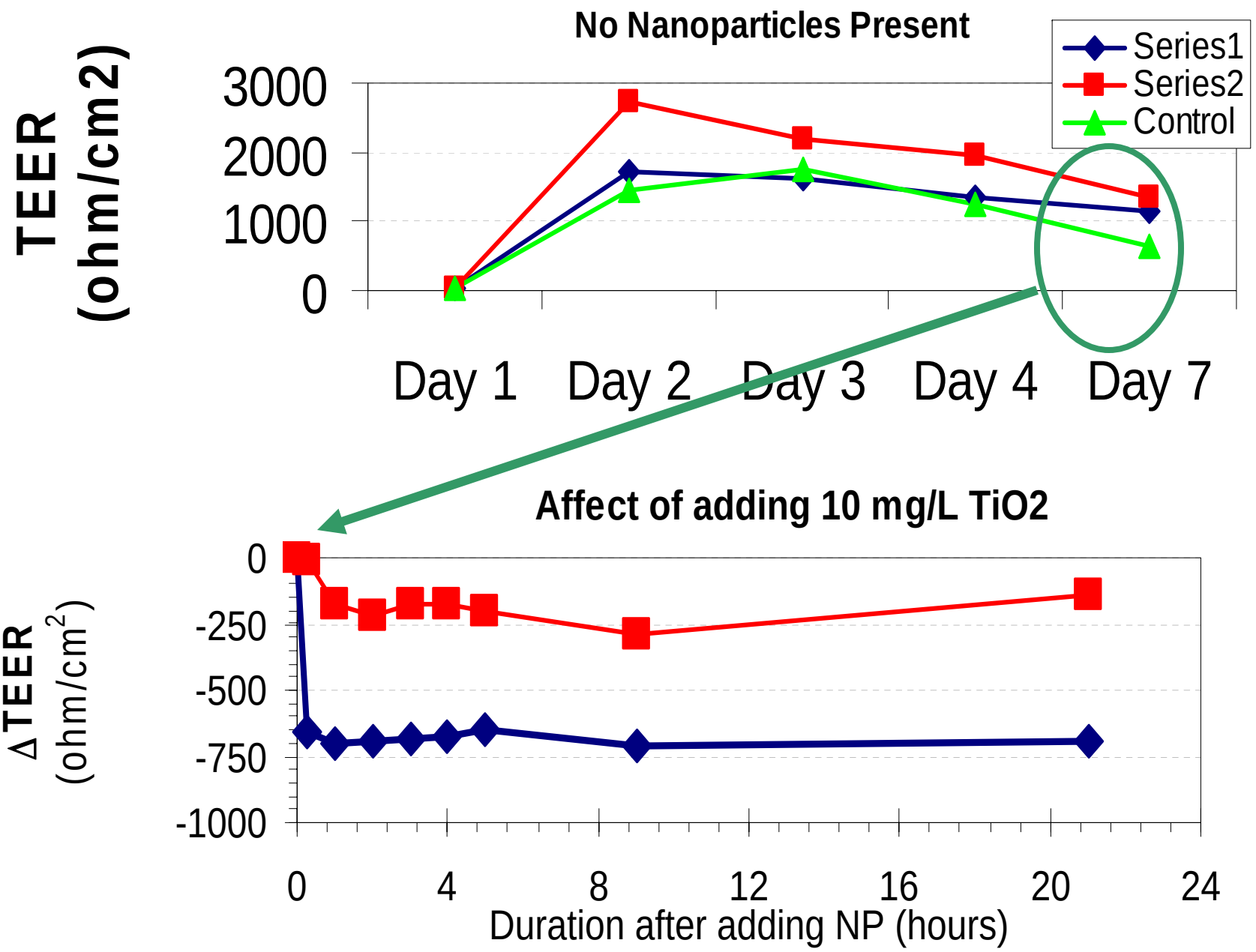
- CO_2 Level: no difference with 5% or 10%
- Transferrin addition: 10 μg/L significantly better than no addition (costly but required)
- Filter diameter: 6.5 mm best (6.5 > 12 > 24 mm)
- Filter material: Polyester better than polycarbonate
- Filter coating:
  - Collagen coated better than untreated
  - Commercially precoated better than hand precoated
- Frequency for media changeout: 1 day better than 2 or 3 days

Caco-2 cells

9 days of growth in culture (10x)

Confluent cells are present

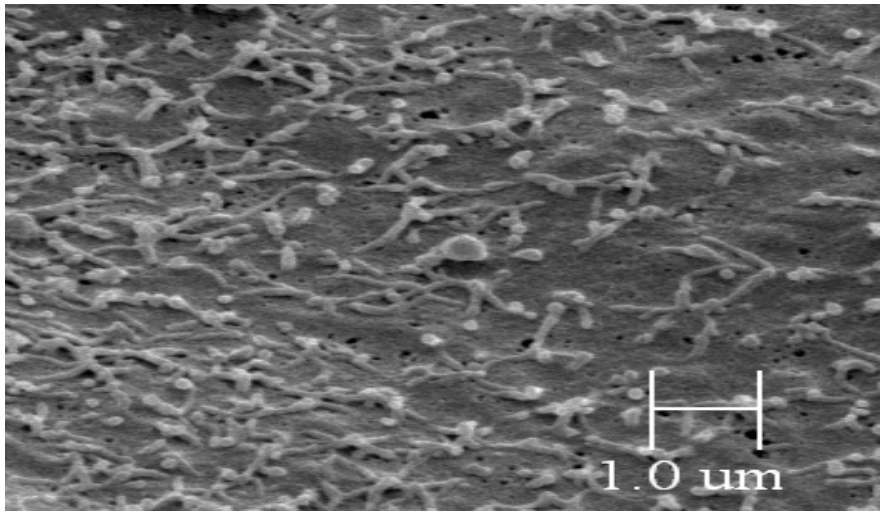




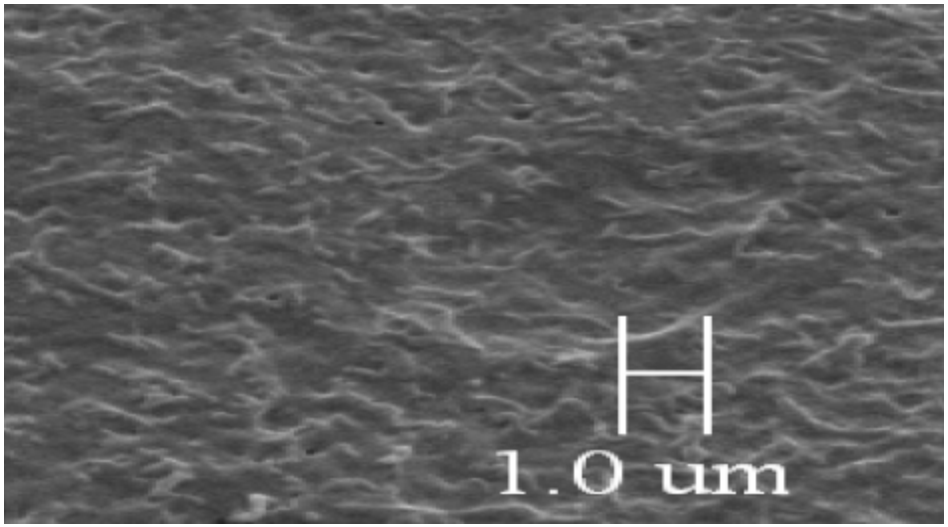
Scanning Electron Microscopy

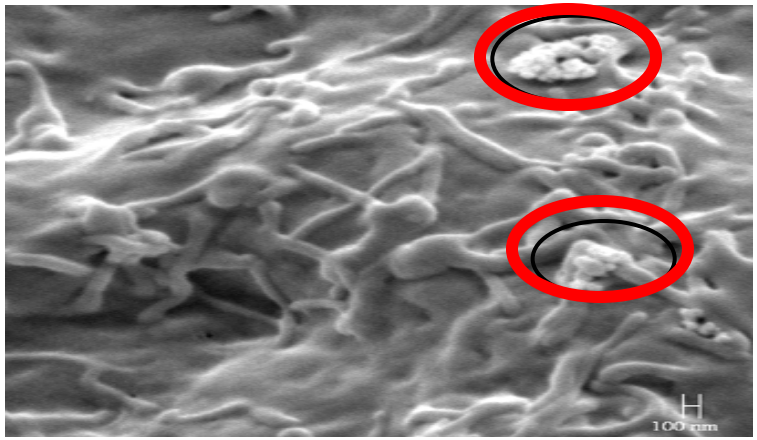
Nanoparticles may flatten down microvilla

Control



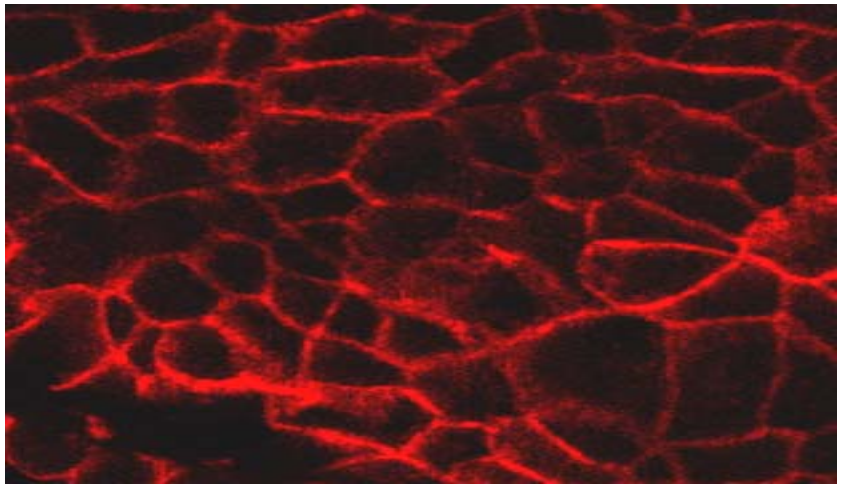
10ppm TiO_2



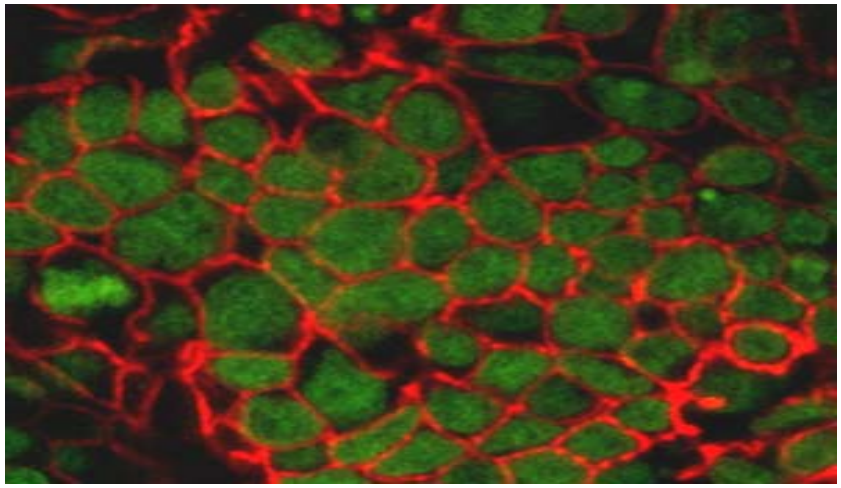


Confocal Scanning Laser Microscope

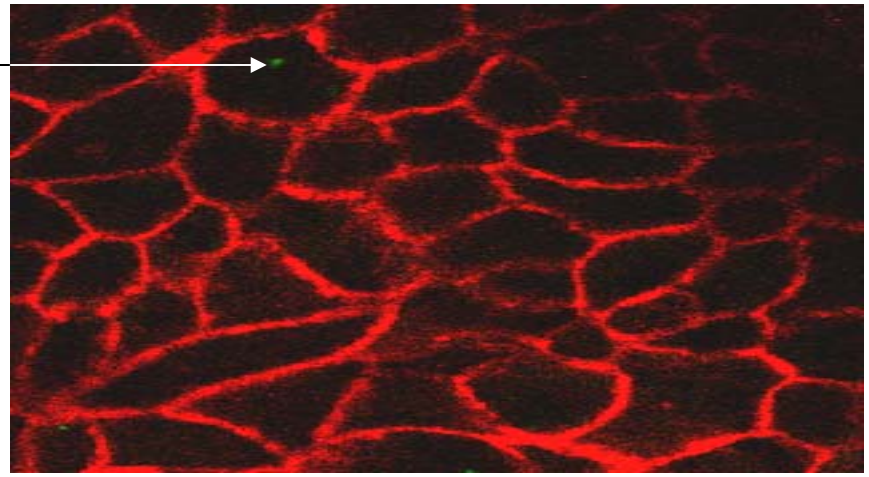
Red = gamma catenin



Green = Nuclei



Green =
Possible TiO_2
Particle
(no stain for nuclei)



Plan for Year 2

I. Nanoparticle Characterization
- Measure surface areas of commercial NPs
- Synthesize in-lab true NP and nanotubes
- Develop procedure to disaggregate commercial NPs
- Measure DLS on sample from WTP effluent

II. Nanoparticle removal during simulated water treatment
- Evaluate role of size, surface charge density, hydrophobicity, shape
- Removal after coag/sed and paper filter

III. Adsorption of pollutants onto nanoparticles
- Continue MS2/PRD1 work
- Evaluate adsorption of 1hydrophobic and 1 hydrophilic SOC

IV. Nanoparticle Toxicity
- Test the toxicity of various nanoparticles
- Determine effects of chronic and acute exposure to nanoparticles
- Examine viability (live vs. dead) of the cells after treatment
- Design esophageal and stomach models