

NP Characterization

- Critical element of exposure/bioavailability/toxicity studies
 - <http://characterizationmatters.org/>
- Initial characterization of materials (easiest ??)
- During exposure testing
 - Before, during, and after exposures
- Stability Studies
 - Aggregation and Dissolution
- Environmental fate (most difficult ??)
 - Low concentrations of engineered NPs
 - Presence of natural NPs, colloids, and particles
 - Matrix interferences on analytical measurements

NP Characterization

- Sensitivity
 - How low can we go (ppb, ppt)
- Discrimination (selectivity)
 - Can we determine if a NP is natural or engineered
- Quantitation versus characterization

Characteristics

- Size
 - Average
 - Distributions
- Morphology
 - Shape
 - Heterogeneity
- Number
- Bulk Composition
 - Elemental
 - Mineralogical
- Surface composition
- Surface Charge
- Surface Area

Techniques Reviewed by:

Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles

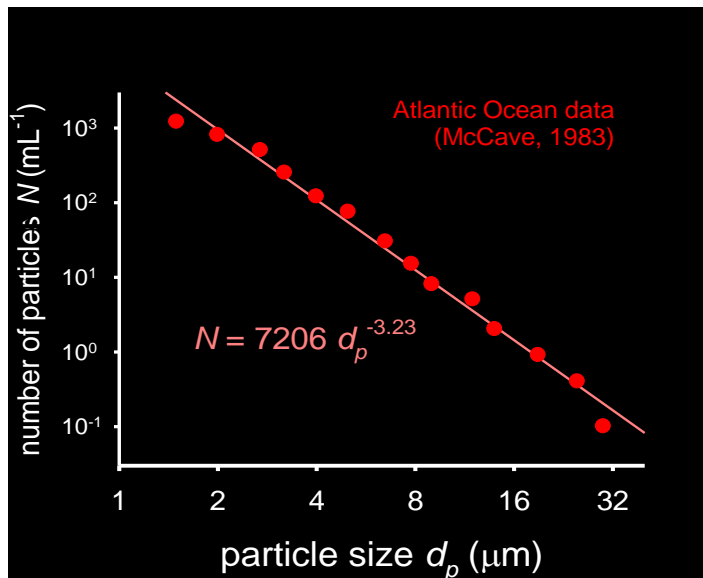
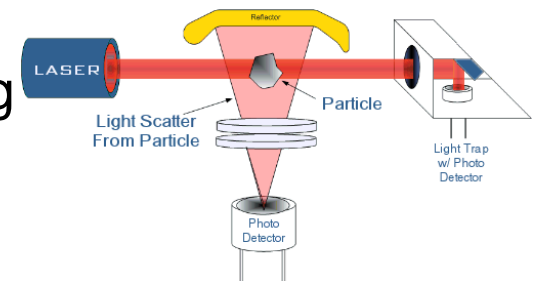
M.Hasselov, J. Readman, J. Ranville and K. Tiede
Ecotoxicology (2008) 17:344–361

Sample Preparation Considerations

- Dispersion is a critical consideration.
- Agglomerates:
 - may be present in sample or artifact of characterization procedure
 - Transient phenomena, so difficult to quantitate
 - Agglomeration more of a “yes or no” characteristic
 - In these systems what information does characterization give?????
- Dispersed NPs
 - “Fundamental” characteristic of NP
 - May not represent what organism is exposed to
 - Produced by physical and chemical means
 - Physical:
 - Ultrasonication (probes, baths)
 - Grinding
 - Stirring (long time periods may lead to surface modification)
 - Chemical
 - Stabilizers, surfactants (to be discussed later)
 - At time of NP synthesis or after NPs formed

Size Analysis: 1

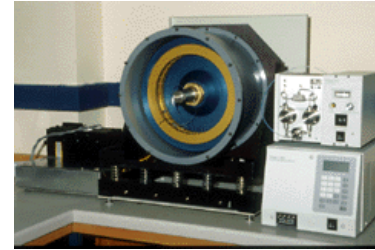
- Light scattering
 - Turbidity, static and dynamic light scattering
 - Usually an average value
- Light Absorption or fluorescence
- Single particle counting
 - Electrical impedance, light-blocking & scattering
 - Number based distribution
 - Usually limited to colloids $> 100\text{nm}$



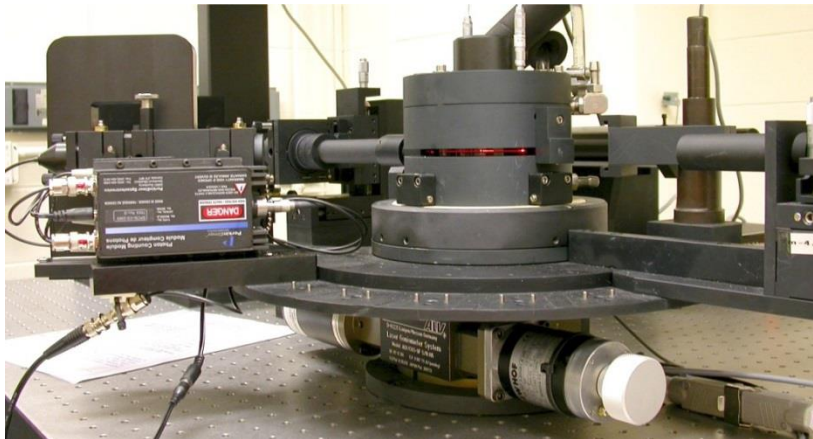
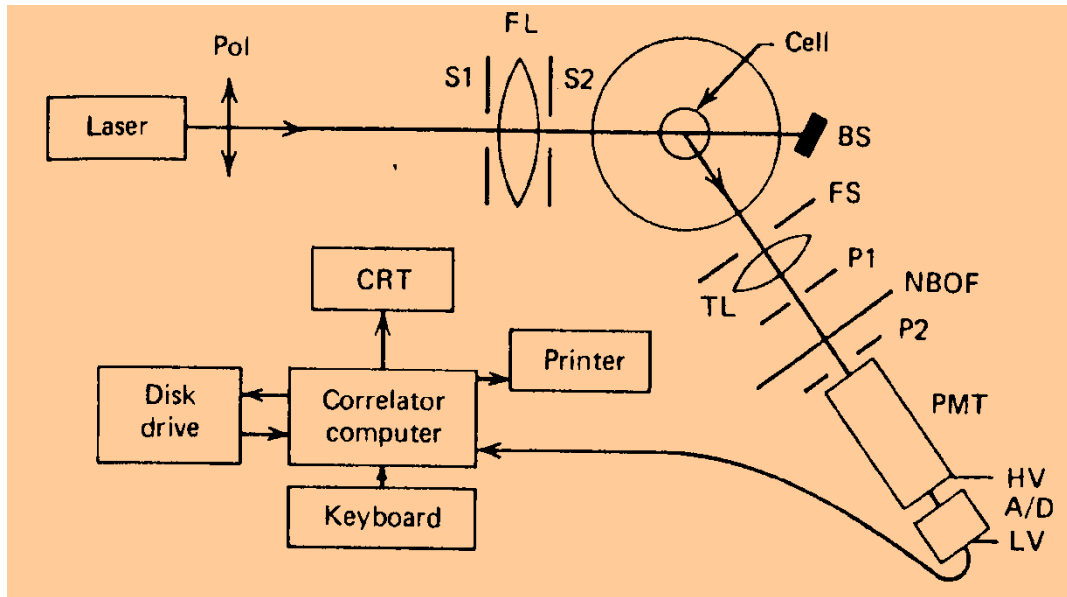
- Natural Colloids and particles
 - Larger natural abundance of smaller particles
 - Results from processes such as weathering, aggregation, settling
- NPs size distribution ?
 - Initial NP size may be monodisperse
 - Does it change?

Size Analysis: 2

- Size with composition
 - How does chemistry change with size?
 - Mixtures, weathering, etc.
 - fractionation
 - “chromatography”
 - Field flow fractionation (FFF)
 - Size exclusion chromatography (SEC)
 - hydrodynamic chromatography (HC)
 - Serial filtration, centrifugation
- Microscopy
 - Also provides shape information
 - Electron microscopy
 - Scanning (SEM)
 - Transmission (TEM)
 - Atomic Force microscopy

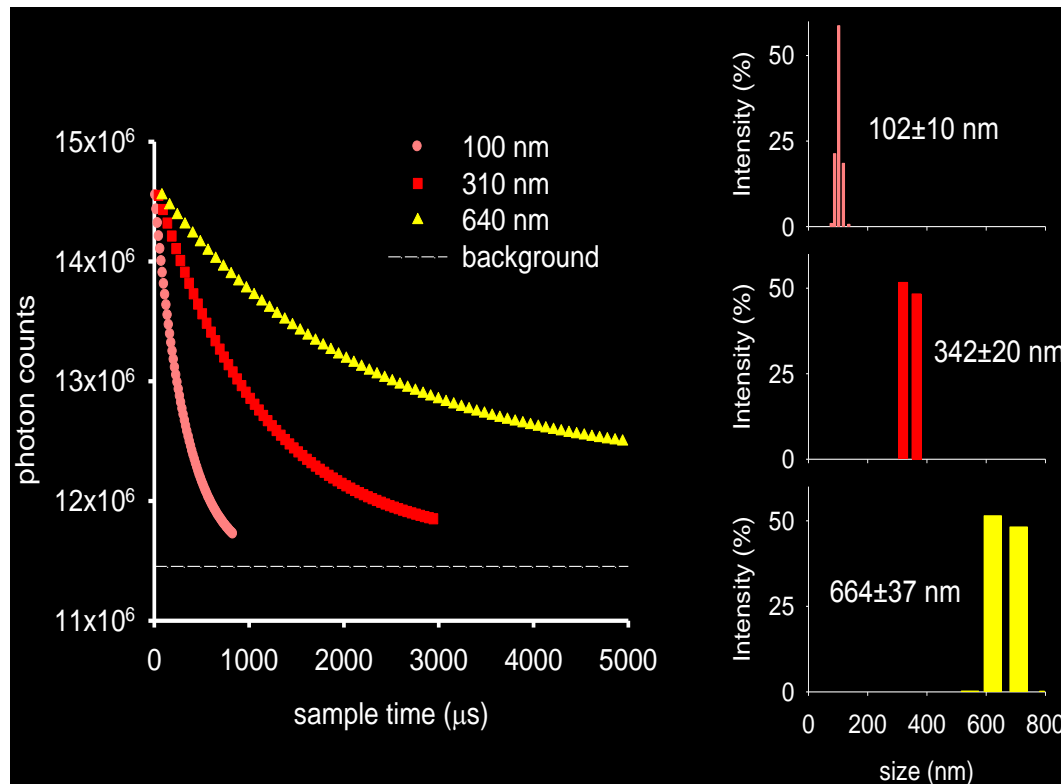


Size: Dynamic Light Scattering



- Very commonly applied
- Non-intrusive, minimal sample preparation
- Fast
- Often misinterpreted, especially for polydisperse samples
- Components
 - *laser* -- polarized monochromatic light
 - *cell* -- holds sample
 - *photomultiplier* -- multiangle
 - *correlator* -- computer for data analysis

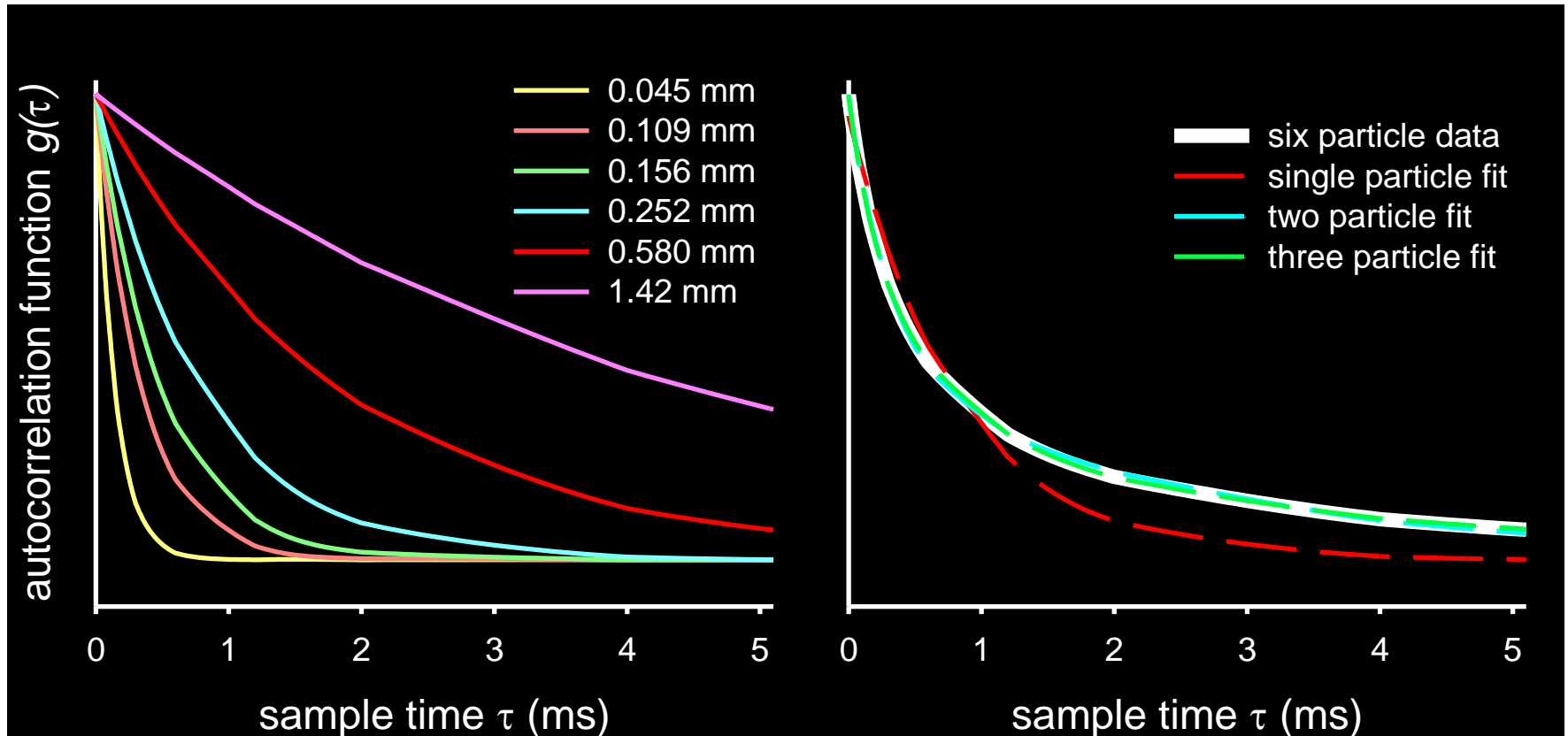
- Correlation function used to compute D
- Stokes-Einstein used to compute hydrodynamic diameter assuming spherical geometry



- For non-spherical particles, “equivalent spherical” diameter obtained.

Polydisperse Samples

- Mixtures of NPs
- Individual NPs and aggregates
- “Dust”
- Very problematic for DLS



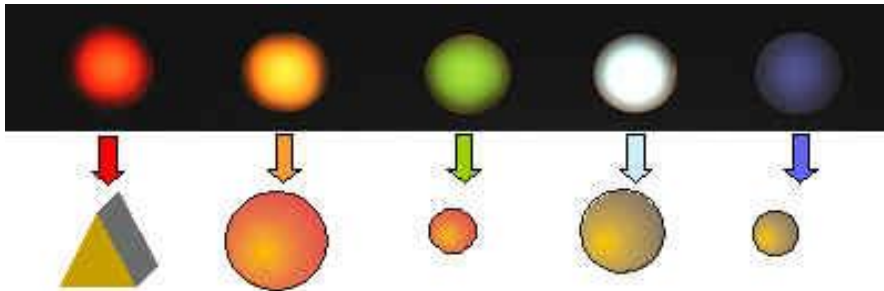
This figure illustrates the inability to analyze polydisperse samples

DLS Summary

- Dynamic light scattering
 - use DLS (PCS) with caution!!
 - confirm findings with SEM, AFM
 - useful for
 - Initial characterization of simple systems
 - coagulation studies with well-defined particle sizes
 - *Generally not useful* for complex systems

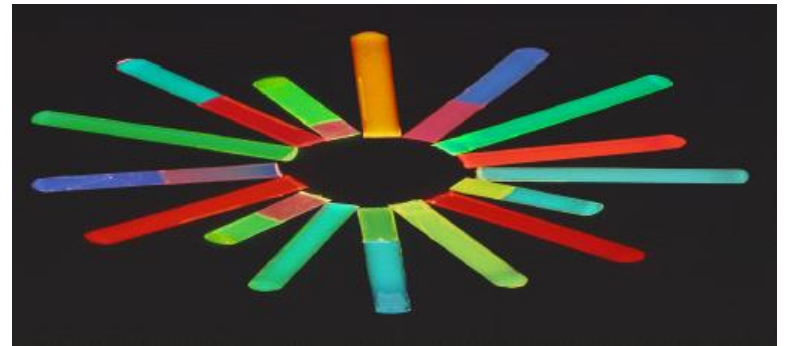
Size: Spectroscopy

- For some materials, size can be obtained from optical measurements
 - Absorption
 - Fluorescence
- Applicable for “pure” systems but difficult (impossible?) for environmental samples or NP mixtures



**Ag(12nm) Au(100nm) Au(50nm) Ag(90nm)
Ag(40nm)**

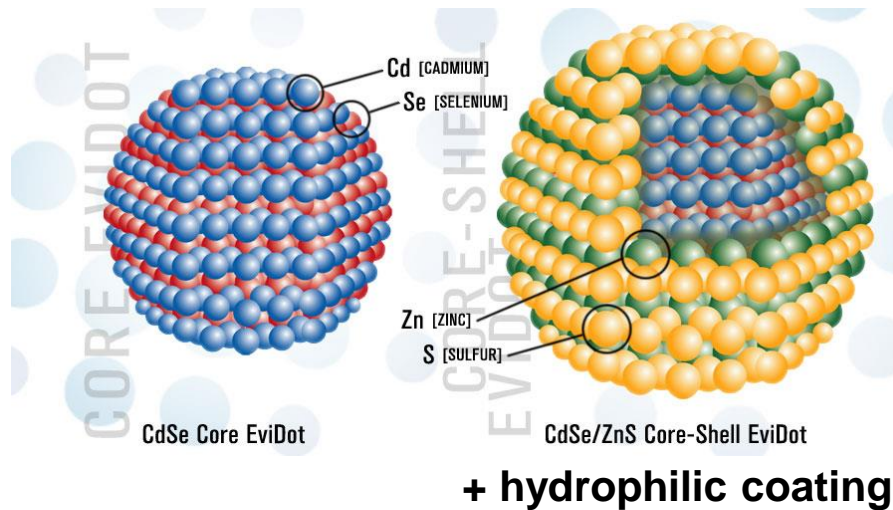
Colors of light scattered by solutions of nanoparticles of certain sizes *Science*, **2001**, 294, 1903



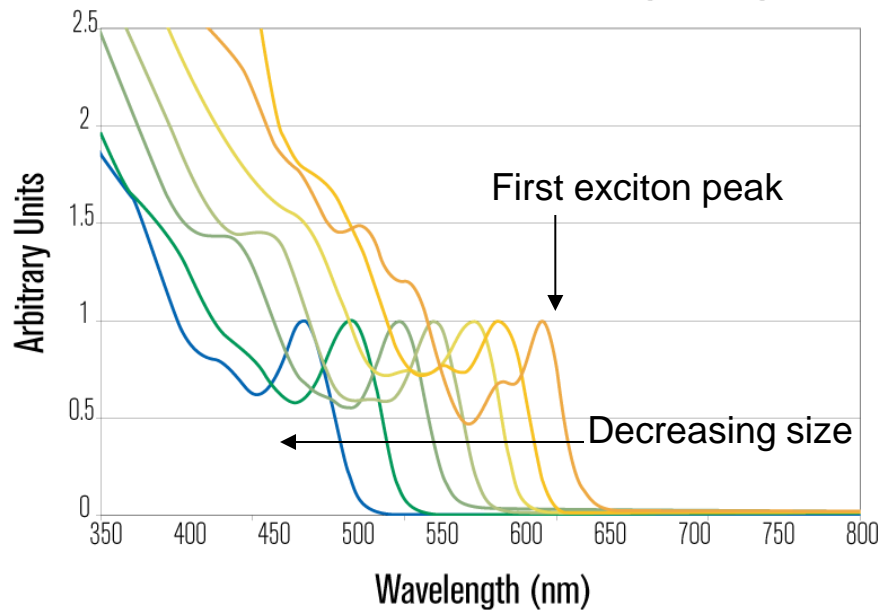
- Quantum Dots
- Semi-conductors
- Bright, photostable fluorophores
- Basic Structure
 - Metalloid core with protective shell
 - Added coating to make hydrophilic
- Sulfides, tellurides, selenides
- Used in biological imaging and optics



Optical properties depend on core size



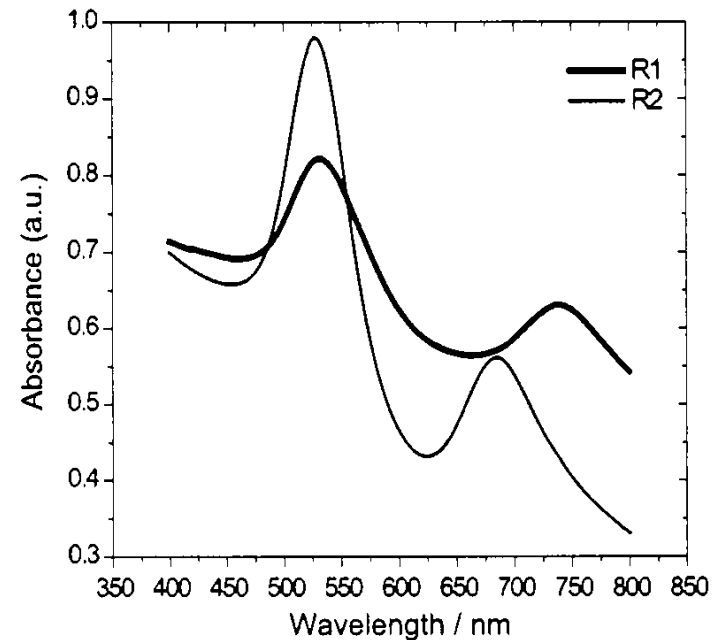
CdSe/ZnS Core-Shell EviDot Absorption Spectra



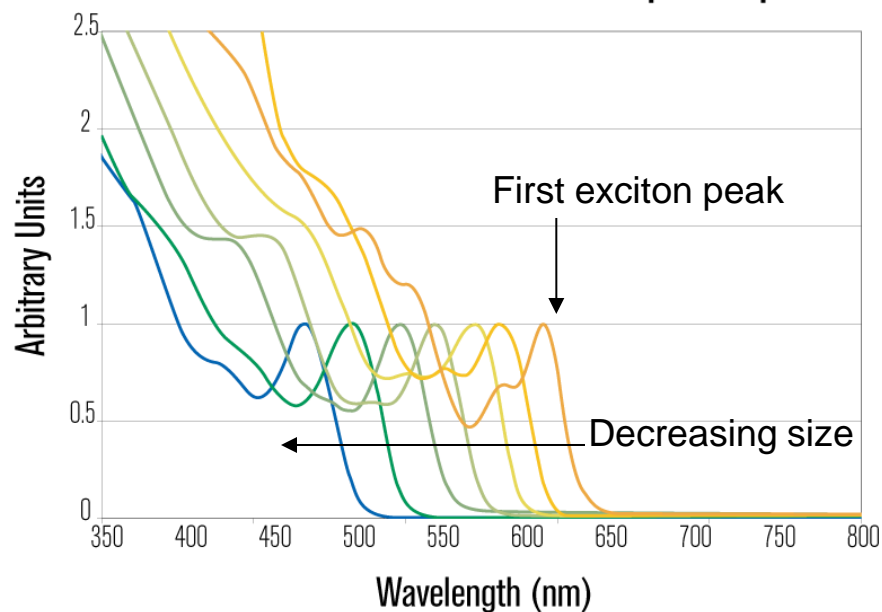
Evident Technologies Inc

Aspect Ratios
R1=4.6
R2=18

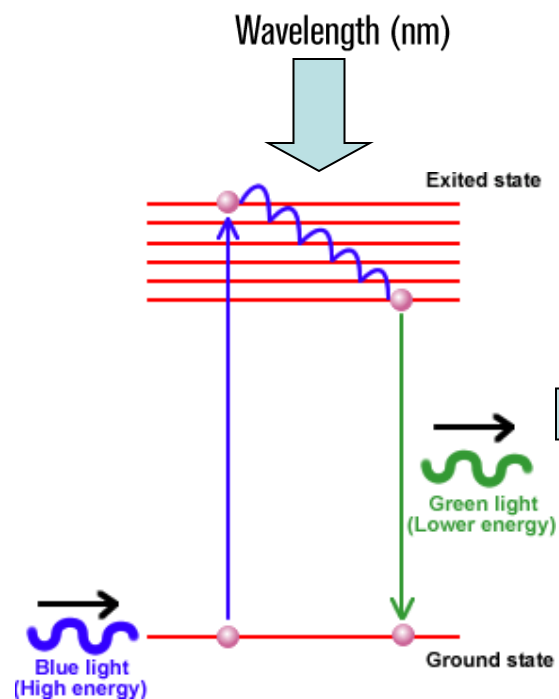
- Absorption arises from interaction of light with the electron cloud of atoms on surface
- Surface plasmon band (first exciton peak)
- Size of particle influences quantum confinement which results in different absorption wavelengths
- Rods (some aggregates) give two peaks



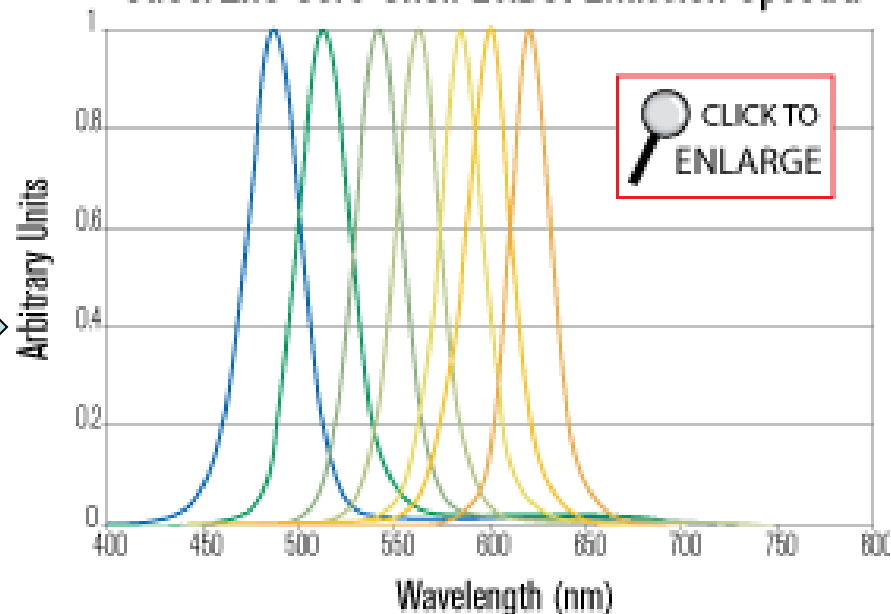
CdSe/ZnS Core-Shell EviDot Absorption Spectra



- Excitation with light of wavelength lower than expected emission

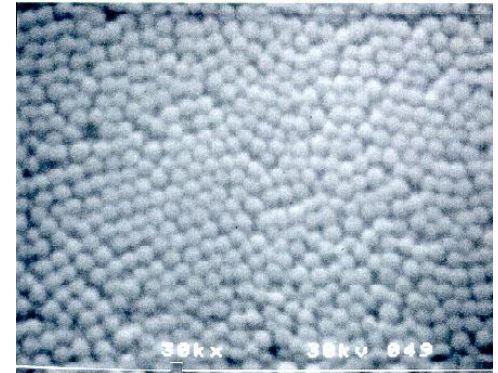


CdSe/ZnS Core-Shell EviDot Emission Spectra



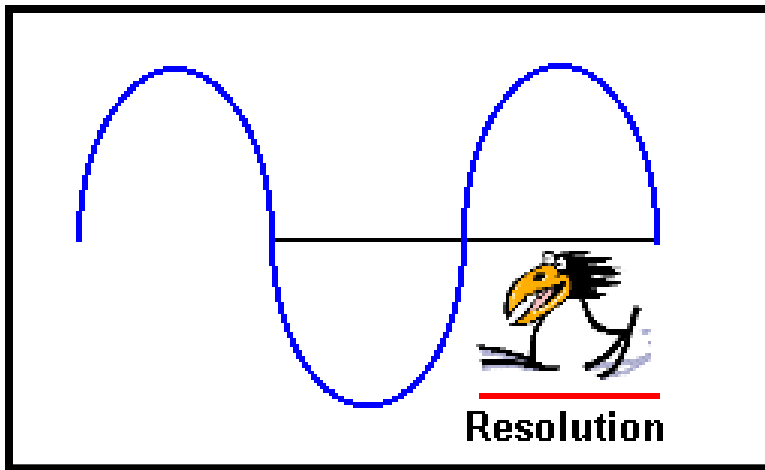
Size: Microscopy

- Microscopy
 - *Optical*, SEM, TEM, AFM
 - Direct observation of NPs
 - “seeing is believing”
 - Morphology (shape, heterogeneity)
 - Particle counting to get number-based size distributions
 - Number concentration (#/ml) difficult to quantify
 - SEM/TEM: Sample highly perturbed
 - Drying, coating, exposure to vacuum
 - SEM/TEM: Elemental composition for constituents at concentrations > than a few percent



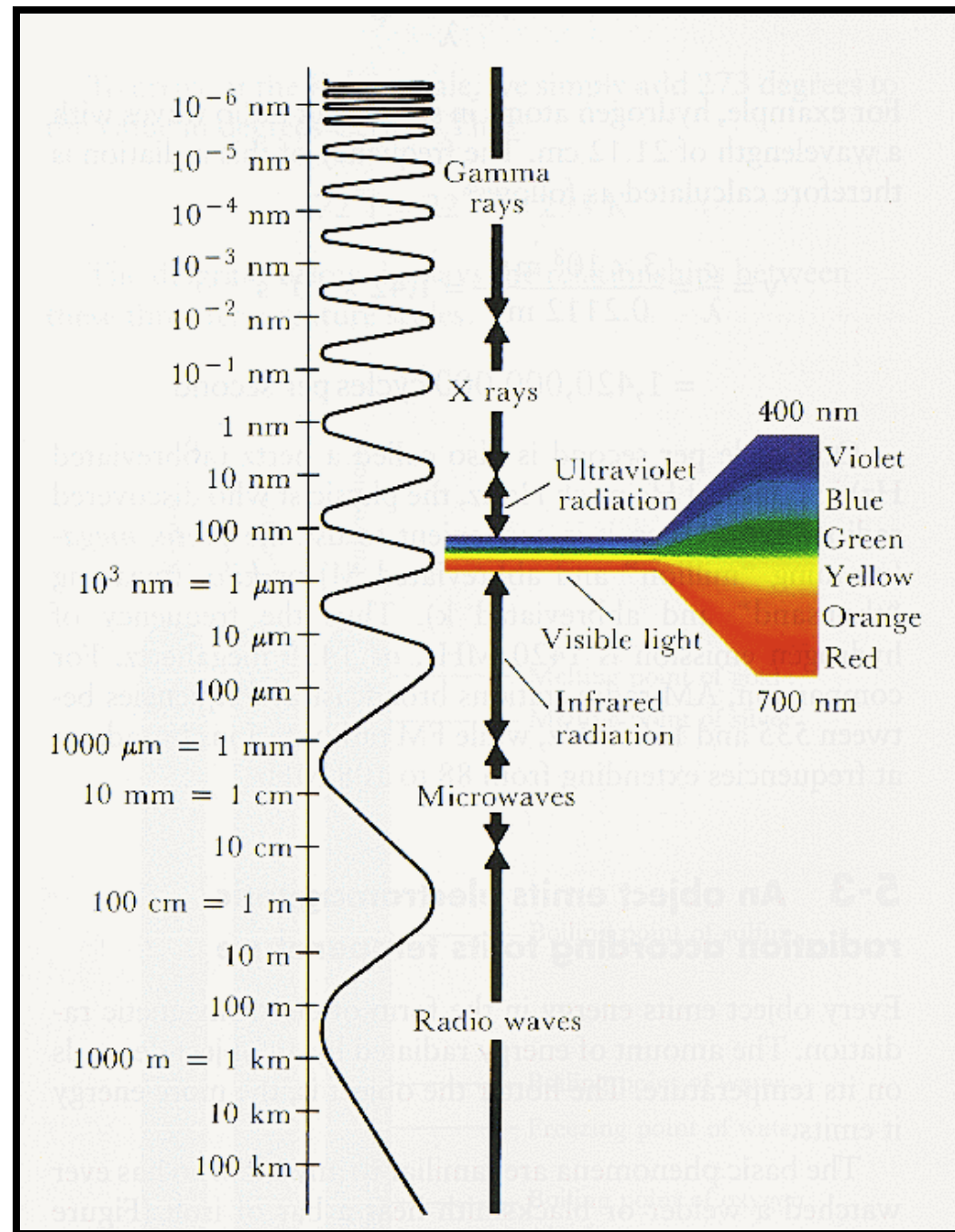
Microscopy

- **light microscopy:** photons interact with the specimen
- **electron microscopy:** electrons interact with the specimen: TEM and SEM
- **Atomic force microscopy** utilizes a variety of different interactions of a fine tip with the specimen. Atomic forces



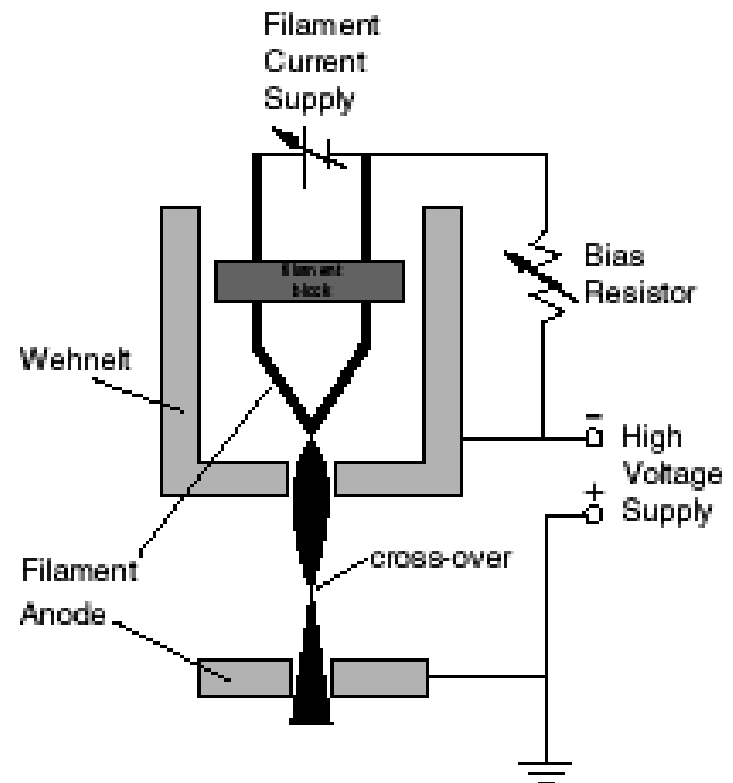
$$\text{Resolution} \approx \frac{1}{2} \lambda$$

- Visible Light
 - 100's of nm
- Electrons/x-rays
 - nm's



The way forward is to use electrons

- Short wavelength given by the [de Broglie](#) wavelength equation
 $E = hc/\lambda$
- e- beams can readily be [generated](#), using an [electron gun](#).
- Electrons can be generated. The kinetic energy they acquire is given by $E = Vq$
- because of their charge they can be focused by electromagnetic lenses.
- Energies in the keV, wavelengths in nm range



- Dry NPs
 - Add small amount of power in solvent
 - Ultrasonicate to disperse NPs
 - Place a small drop of suspension on a flat surface
 - Directly on SEM stub and evaporate

- Salts can crystallize

- NP Suspensions

- Filter suspension

- Depth (Polycarbonate) filters better than depth (cellulose) filter

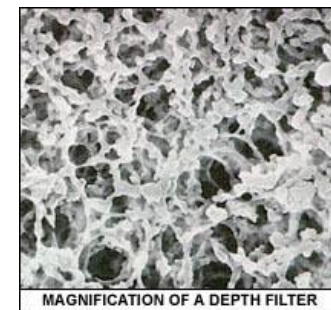
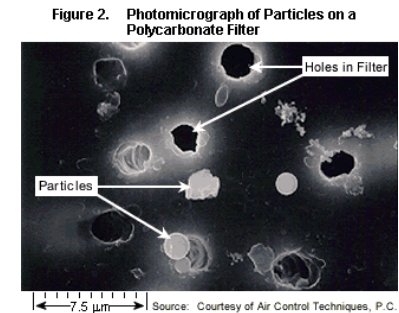
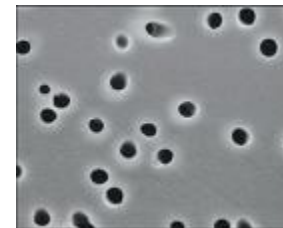
- Attach filter to stub

- Carbon tape, carbon glue

- Coat to prevent charging

- Dispersion is critical!!!

- Aggregates may form



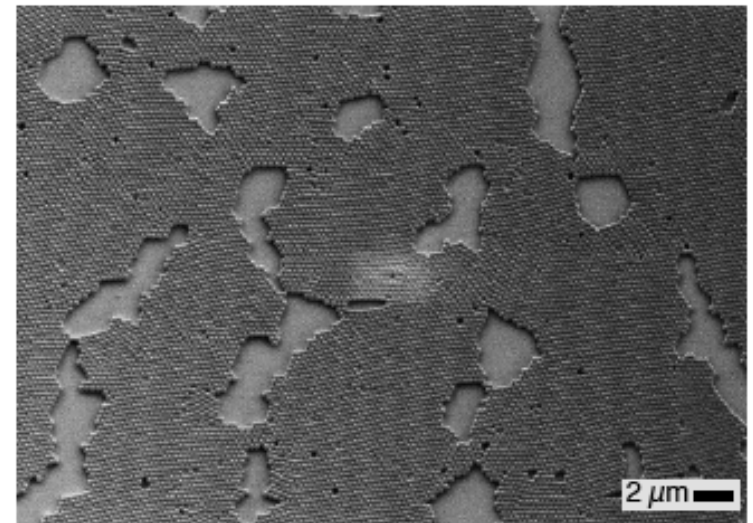
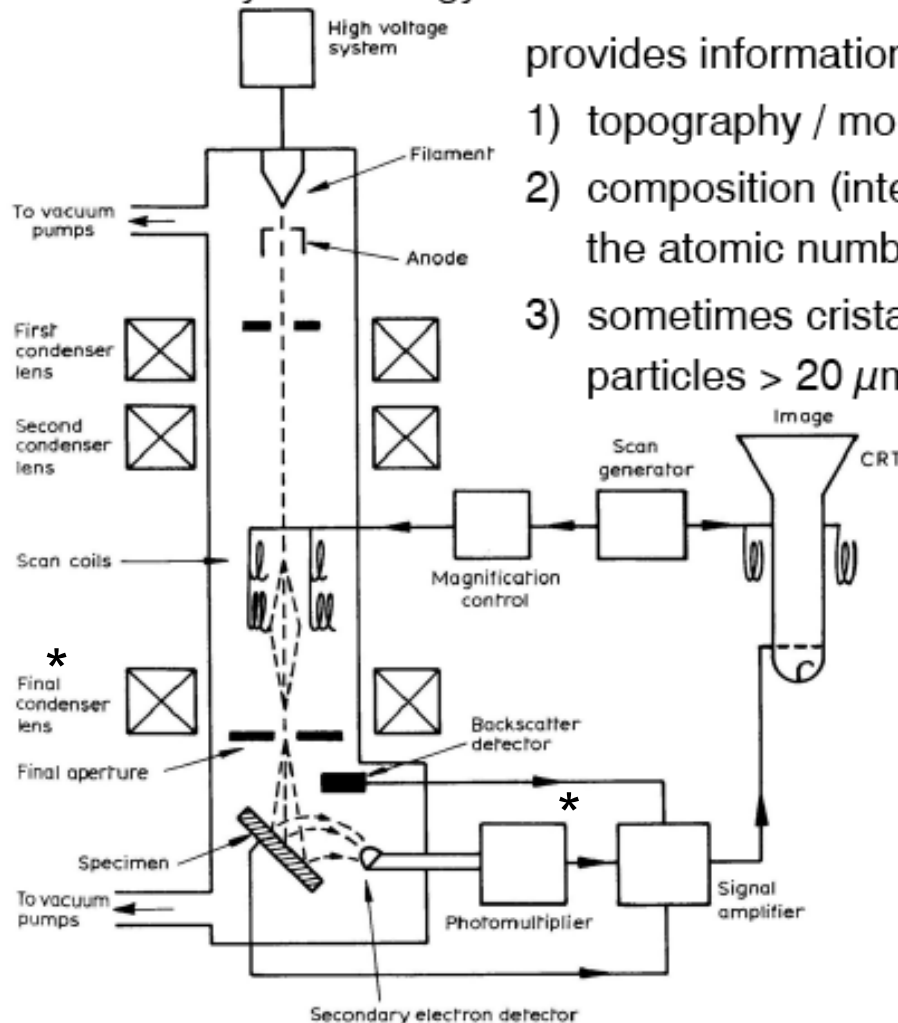
MAGNIFICATION OF A DEPTH FILTER

Imaging Methods: Scanning Electron Microscopy (SEM)

scanning of electron beam (0.2 – 30 keV) over a (usually conducting) specimen and detection of secondary low energy or backscattered electrons, resolution from mm down to about 5 nm

provides information on:

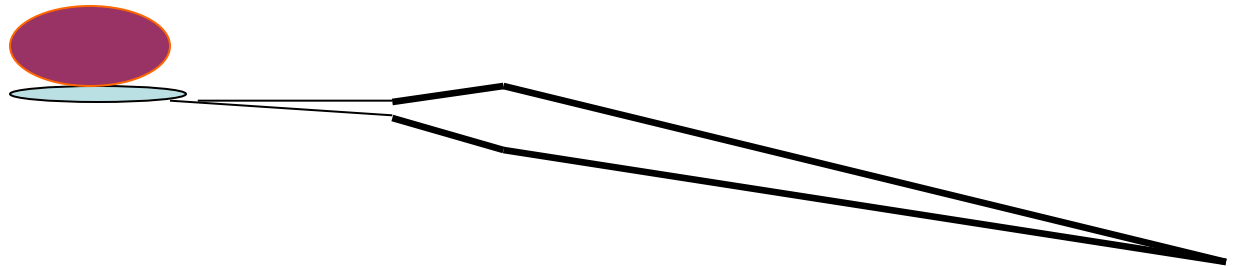
- 1) topography / morphology (surface profile, structural features)
- 2) composition (intensity of backscattered electrons correlates to the atomic number of elements within the sampling volume)
- 3) sometimes cristallographic information (single-crystal particles $> 20 \mu\text{m}$)



monolayer of colloidal polymer particles (280 nm)

TEM GRID PREPARATION

- Take a micropipette and put a droplet on top the grid. Let the solution sit on the grid.
- After 30 sec, wick away the rest of the solution with filter paper. Do not touch the grid!
- Finally let it sit until the grid dries off.

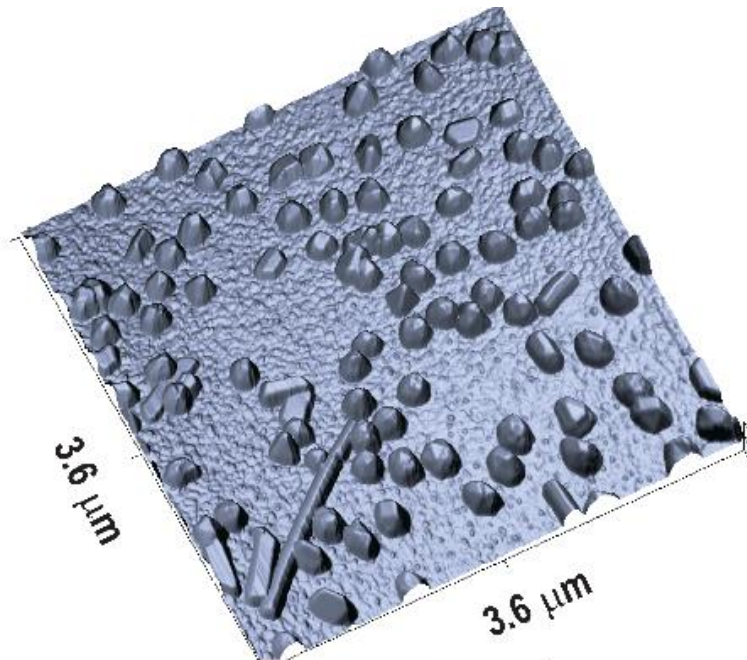


Atomic Force Microscopy

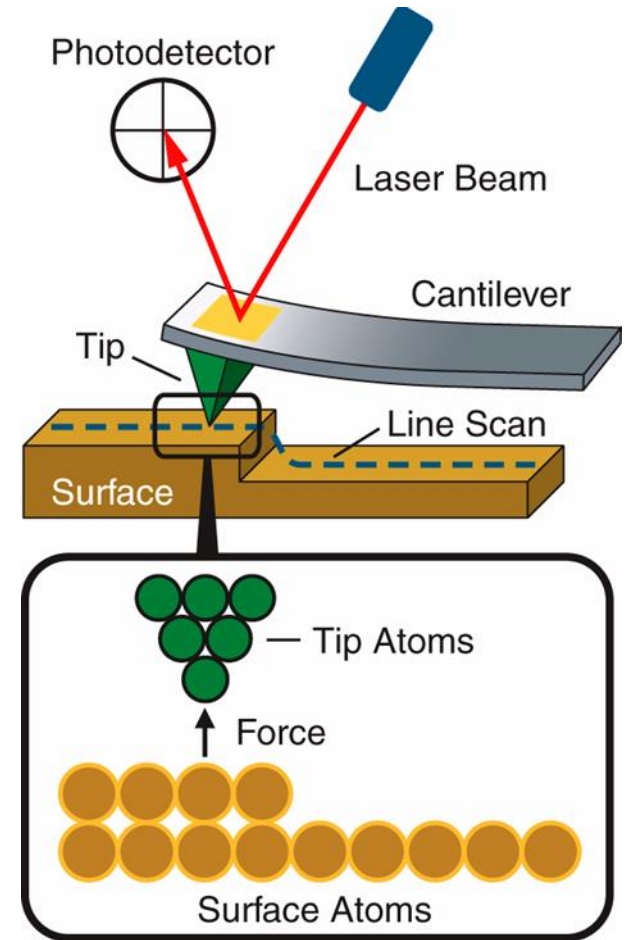
Can be performed in liquid

- Slower scanning rate compared to SEM/TEM
- Good web page on artifacts

• http://www.pacificnanotech.com/files/pdf_15_1076152090_0.pdf



Ag NPs



Size (+ composition): Fractionation

- Size-based separation methods
 - Size determined from elution time
 - Field flow fractionation
 - Size exclusion chromatography
- NP/colloid elemental composition vs size
 - Filtration/centrifugation
 - atomic absorption/emission/mass spectrometry
 - ICP-AES, ICP-MS
 - Hyphenated methods
 - FFF-ICP-MS
 - SEC-ICP-MS

Particles are rejected by mechanisms other than screening

- Rejection can occur due to:
 - Adsorption
 - Pore blockage
 - Surface aggregation
 - Cake formation
- Size distributions produced by serial filtration can be (usually) skewed
 - Amounts in smaller size fractions can be underestimated
 - Sample components look larger than they are
 - Dissolved vs NP

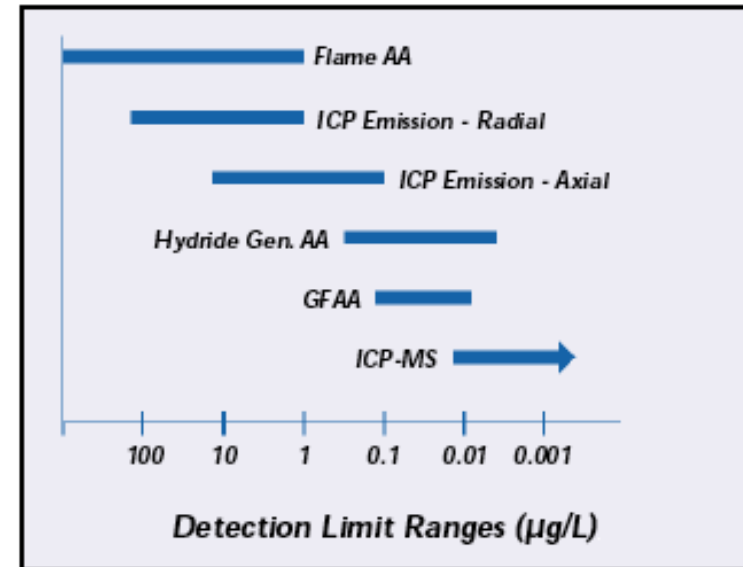
Chemical Analysis of Filtrates

ICP, ICP-OES, ICP-AES, ICP-MS—what's the difference?

- ICP
 - common to both techniques
 - Plasma is a very high temperature gas
 - relies on the fact that the plasma is a good atomization and ionization source
- MS or OES (AES)
 - Mass spectrometry, inherently low background, high sensitivity, we're counting ions here
 - Optical emission spectroscopy, detects elements based on characteristic emissions from atoms or ions, higher background, lower sensitivity

Detection Limits

ICP-AES Detection Limits (µg/L)																						
Li 0.3	Be 0.1																B 1	C 40	N na			
Na 3	Mg 0.1																Al 3	Si 4	P 30	S 30	Cl na	
K 20	Ca 0.02	Sc 0.3	V 0.5	Ti 0.5	Cr 2	Mn 0.4	Fe 2	Co 1	Ni 5	Cu 0.4	Zn 1	Ga 4	Ge 20	As 20	Se 50	Br na						
Rb 30	Sr 0.06	Y 0.3	Nb 5	Zr 0.8	Mo 3		Ru 6	Th 5	Pd 3	Ag 1	Cd 1	In 9	Sn 30	Sb 10	Te 10	I na						
Cs 10	Ba 0.1	La 1	Hf 4	Ta 15	W 8	Re 5	Os 0.4	Ir 5	Pt 10	Au 4	Hg 1	Tl 30	Pb 10	Bi 20								
			Ce 5	Pr 1	Nd 1		Sm 2	Eu 0.1	Gd 1	Tb 2	Dy 2	Ho 0.4	Er 1	Tm 0.6	Yb 0.3	Lu 0.2						
			Th 70		U 15																	



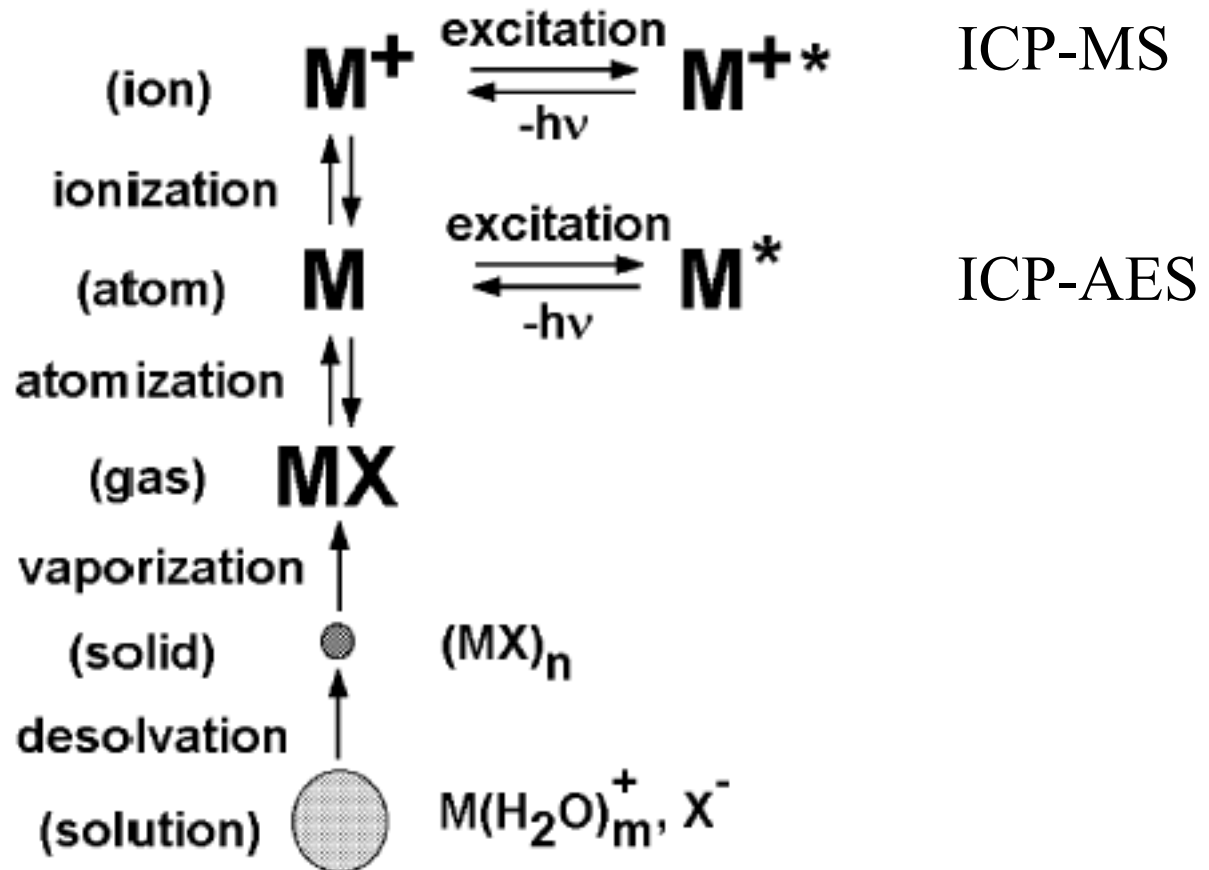
Linear Range

Figure 2-1. Periodic table with ICP-OES detection limits (side-on viewing). All detection limits are reported as 3σ and were obtained on a Perkin-Elmer Optima 3000 under simultaneous multi-element conditions with a side-viewed plasma. Detection limits using an axially-viewed plasma are typically improved by 5–10 times.

QuickTime™ and a
decompressor
are needed to see this picture.

Processes Occurring in the Plasma:

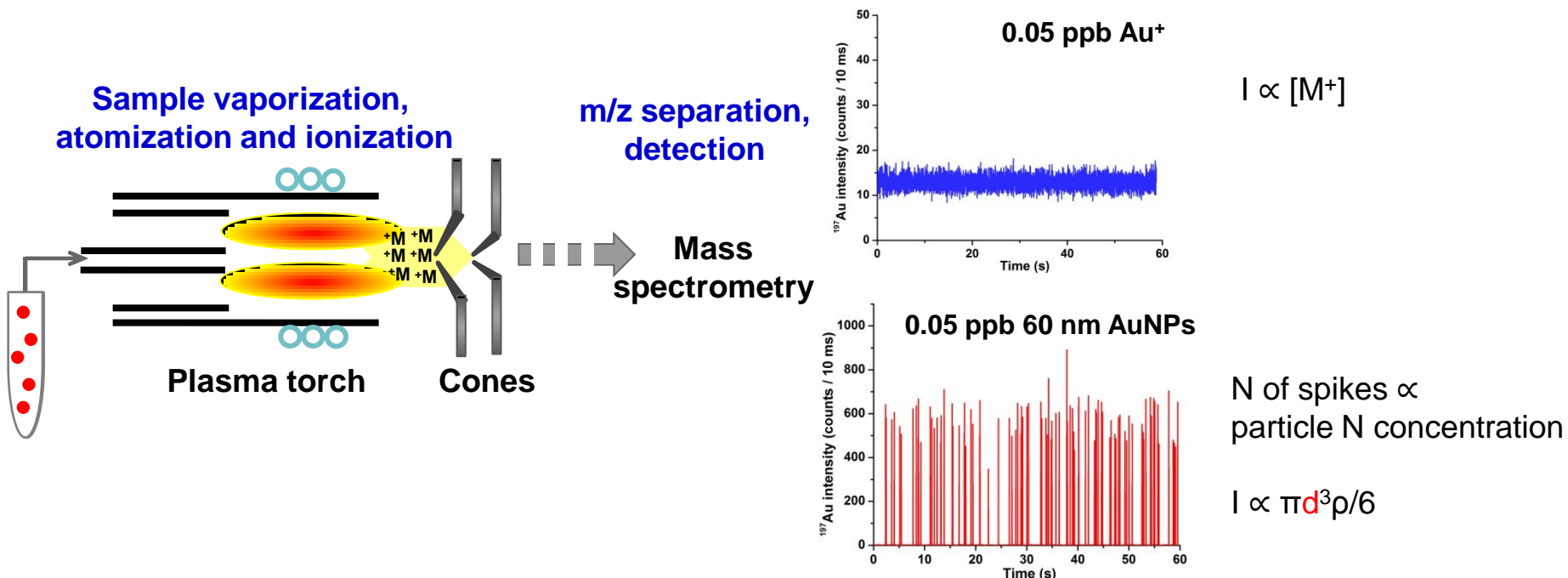
Excitation



Quantitative Analysis by ICP-MS

- Multielement calibration standards used for calibration curve
- Internal Standards needed to correct for drift
 - ‘Rare’ elements (in a solution) that are added to standards and samples at exactly the same concentration.
 - Li^6 , Sc, Ge, Y, Rh, In, Tb, Ho, Lu, Bi are often used
 - An effective internal standard cannot initially be present in the sample
 - often need to check this by scanning the sample first
- Quality control standards run often (5-10% of samples)

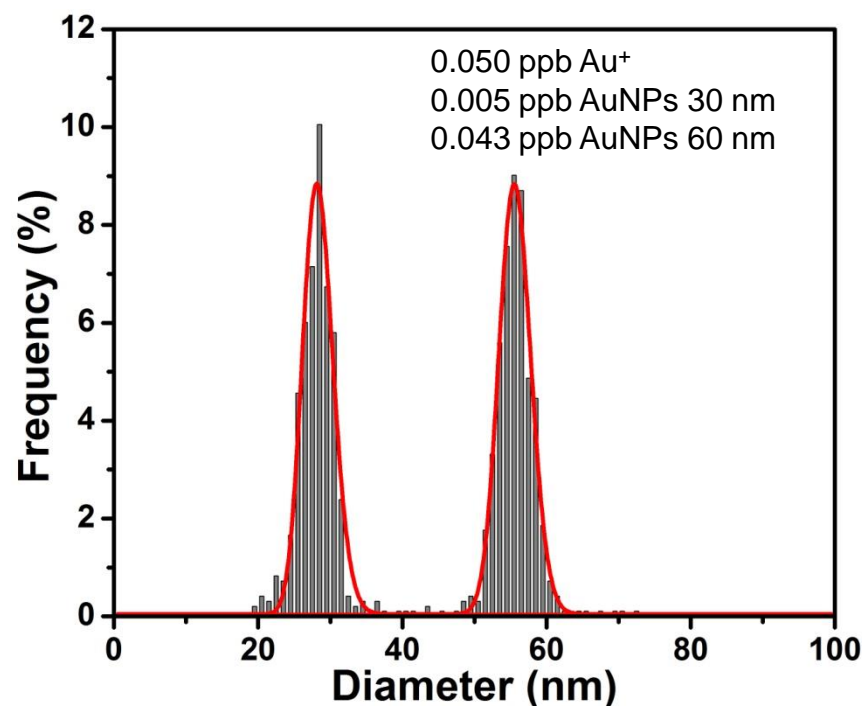
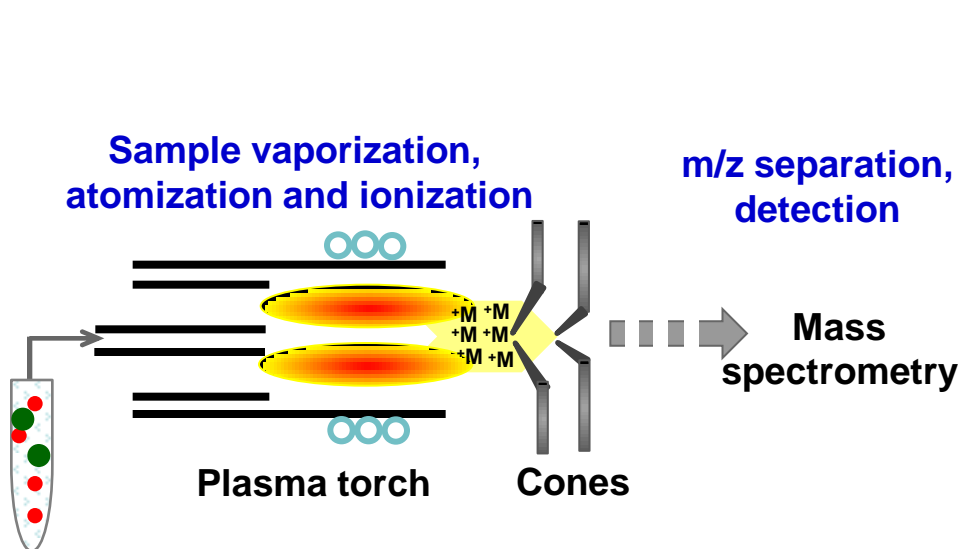
Single Particle ICP-MS (spICP-MS)



- Operates ICP-MS in time resolved analysis mode: a particle event lasts 0.4 ms, typical dwell time 3 - 10 ms
- Particle size distribution and number concentration

* *J. Anal. At. Spectrom.*, 2012, 27, 1143

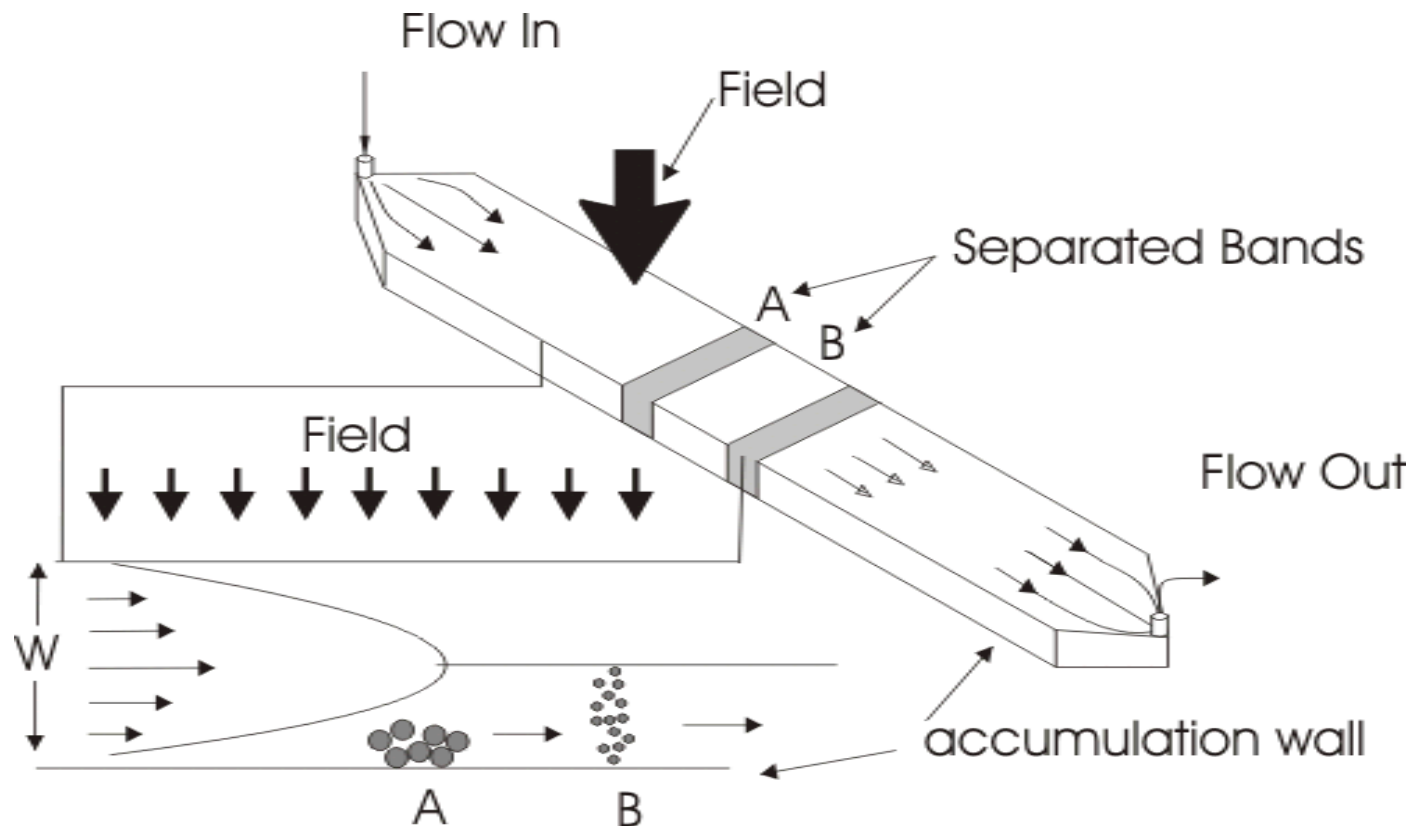
Single Particle ICP-MS (spICP-MS)



- Operates ICP-MS in time resolved analysis mode: a particle event lasts 0.4 ms, typical dwell time 3 - 10 ms
- Particle size distribution and number concentration
- Simultaneous detection of both dissolved and particulate species
- Characterization of metal containing ENMs in natural matrix

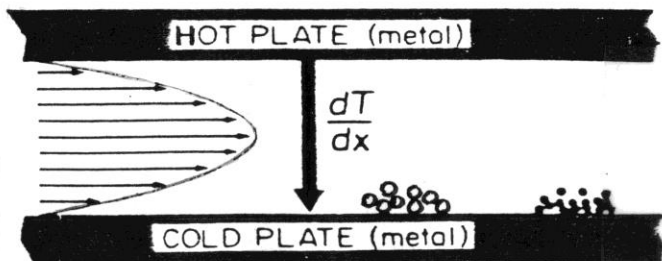
Flow field flow fractionation

- Separation technique like chromatography but works by a different mechanism
- Separates molecules and particles based on their size

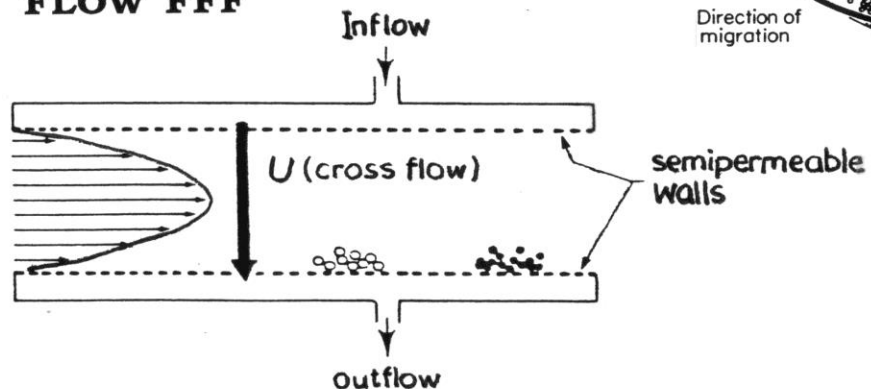


FFF Subtechniques: Field Type

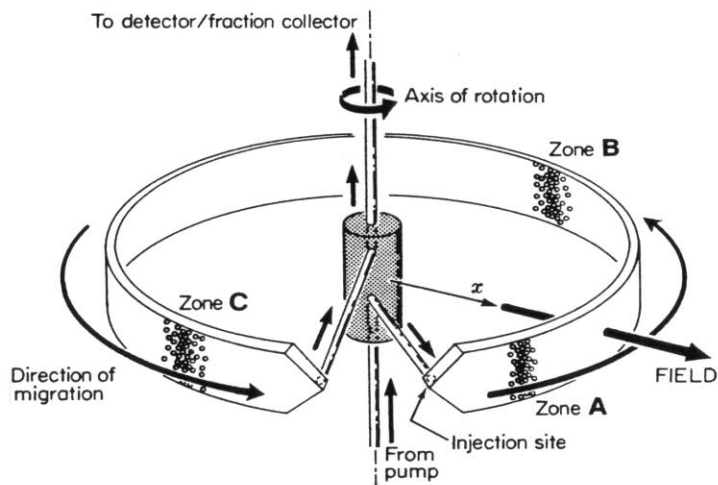
THERMAL FFF



FLOW FFF

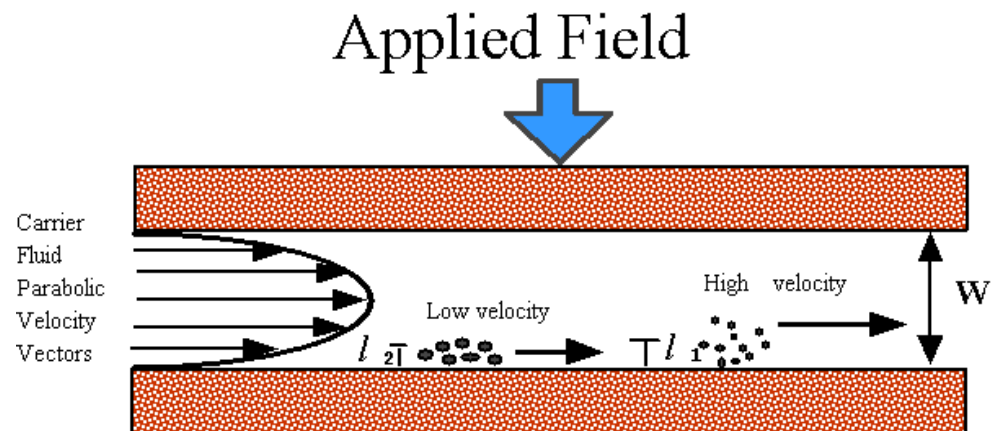


SEDIMENTATION FFF

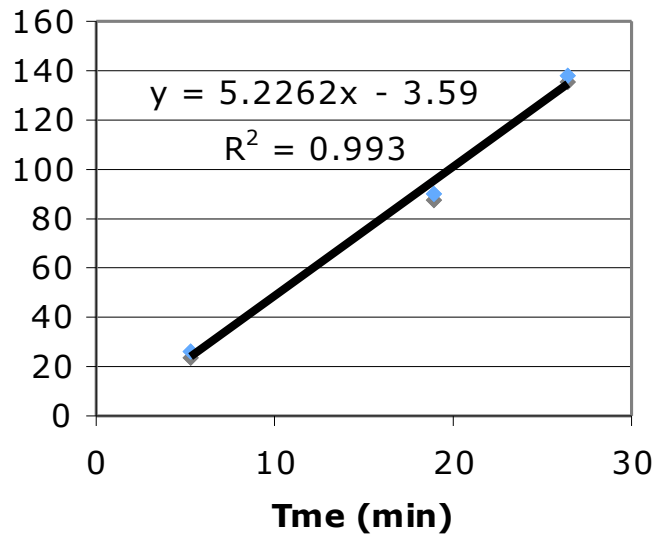
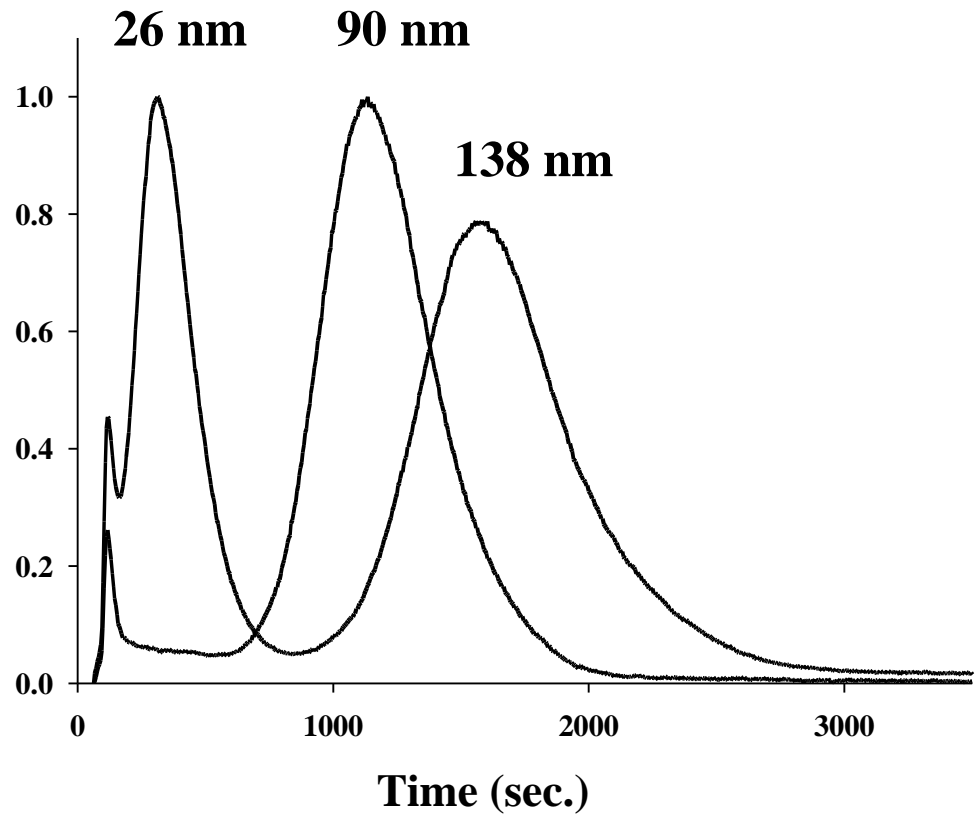


Principles of normal mode FFF

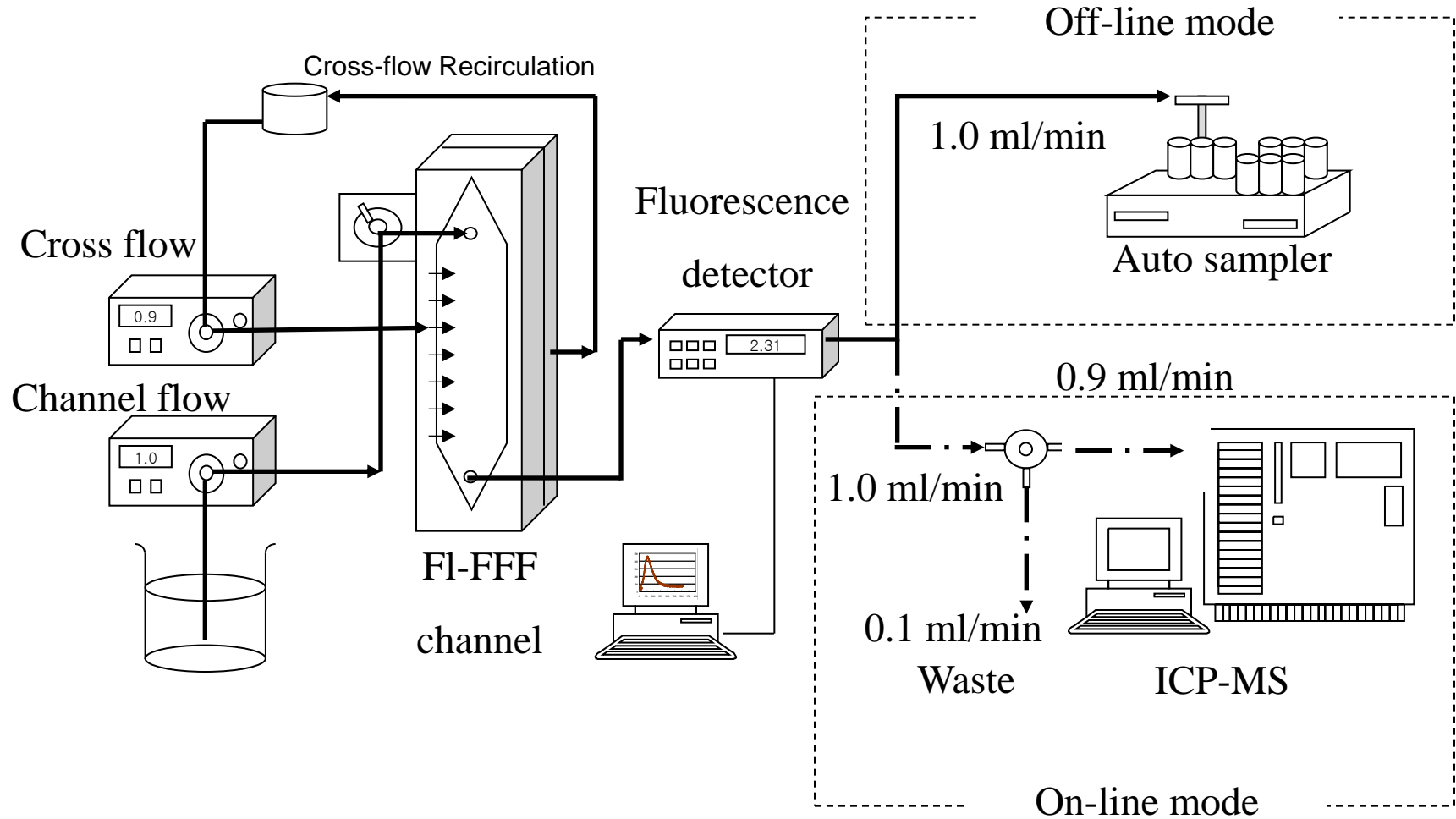
- Separates colloids (inorganic or organic solids from nm – 1 μ m size range)
- Inject sample into thin channel
- Apply perpendicular force
- Molecules/colloids are forced to accumulation wall.
- Diffusion (Brownian motion) acts against applied force
- Small molecules diffuse higher in the channel against the applied force and encounter faster channel flow
- Small molecules elute first



Calibration is alternative approach to FFF theory

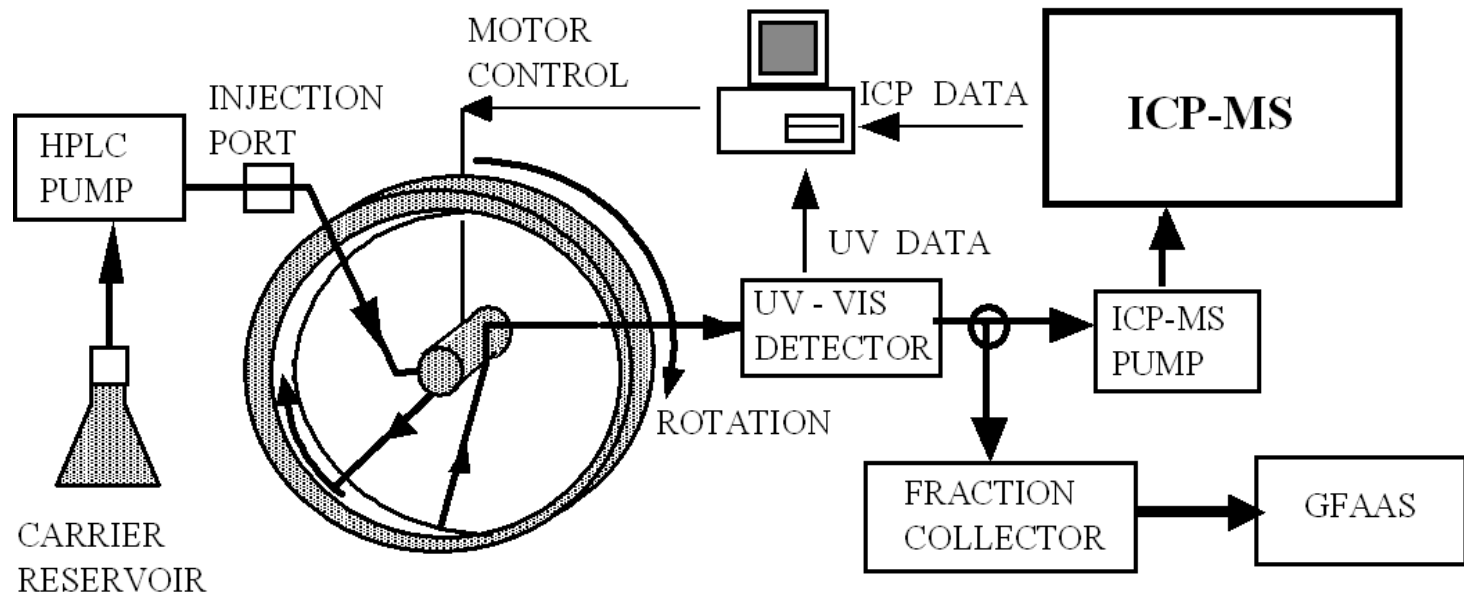


FI FFF - ICP - MS

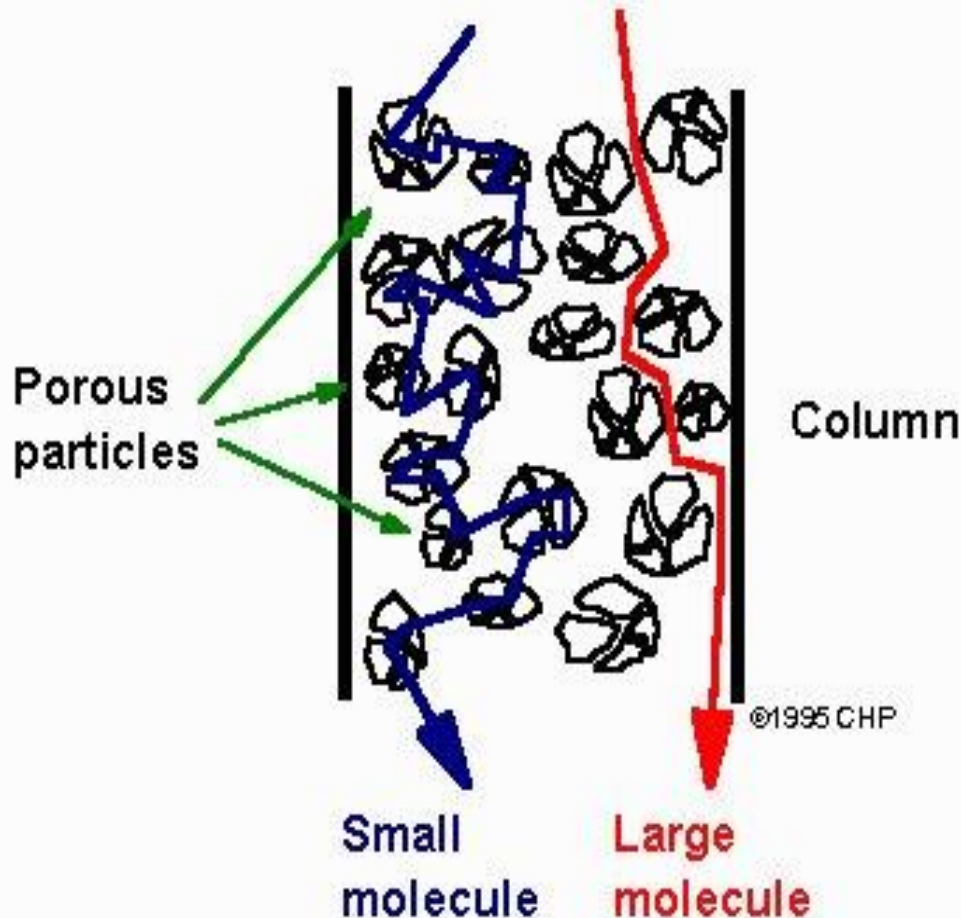


Sedimentation FFF (SdFFF-ICP-MS)

- **Gravitational field produced by centrifugation**
- **Effective mass separation**
- **Online addition of element-specific detector**



Size exclusion chromatography



Molecules greater than a certain size are excluded from all pores = **exclusion volume**.

Molecules smaller than a certain size access all pores = **permeation (total) volume**.

In between these limits larger molecules can't access some of the pore space so elute earlier than smaller molecules.

Surface Charge

– Important parameter for NP stability

- **Surface potentiometric titrations**

- Particle suspension in water is titrated with a strong acid and/or a strong base (i.e. NaOH, HCl)
- pH buffering capacity of the mineral surface is measured vs. pH
- Difference between added H^+/OH^- and measured pH provides information on exchange capacity of surface
- Must avoid mineral dissolution/precipitation, alteration reactions



- **Zeta potential measurements (microelectrophoresis)**

- Suspension is introduced to an electric potential gradient
- Particles will drift toward or away from the electrode, depending on sign of surface charge
- Velocity depends on applied field strength and magnitude of surface charge

Summary & applicability to environmental samples

Table 1. Comparison of Different Techniques for Detecting and Characterizing Nanoparticles		
Characterization Techniques	Features	Applicability to Environmental Systems
Scanning and/or Transmission Electron Microscopy	Visualization and semi-quantitative chemical analysis	Very useful for number distributions, can discriminate among particle shapes and types, laborious without automation, possible artifacts from high vacuum
Dynamic Light Scattering	Non intrusive, rapid and accurate measurement of particle size for monodispersed NPs	Generally unsuitable as it cannot discriminate among particle types, gives average size that is heavily biased to large particles
(Ultra)Filtration and/or Centrifugation	Provides elemental size distribution when coupled to chemical analysis	Filtration is very widely used but data is often over-interpreted, incomplete passage of particles through filters bias results to larger size, centrifugation is procedurally difficult and has low resolution for size separation
UV-VIS and Fluorescence Spectroscopy	UV absorbance and fluorescence are widely used for size analysis of quantum dots and other semiconductor NPs	Generally unsuitable as other species present in most natural waters interfere.
Field Flow Fractionation	High resolution size separation with wide dynamic range, when coupled with specific detectors can provide size distributions of chemical properties	Very applicable for polydisperse, heterogeneous systems when coupled to ICP or TEM, generally requires sample pre-concentration and size pre-fractionation
Real-Time Single Particle ICP (RTSP-ICP)	Element specific particle sizing and counting	Very useful due to low detection limit, potential of ICP-AES to discriminate among particles

Summary “of sorts” of common methods

Techniques	Applicability to Environmental Systems
Scanning and/or Transmission Electron Microscopy/ Atomic Force Microscopy	Very useful for number distributions, can discriminate among particle shapes and types, laborious without automation, possible artifacts from high vacuum, difficult to translate # observed to #/L, Needle in the haystack problem when TSS is high.
Dynamic Light Scattering	Generally unsuitable as it cannot discriminate among particle types, gives average size that is biased to large particles.
(Ultra)Filtration and/or Centrifugation	Gives mass-based distribution. Filtration is very widely used but data is often over-interpreted, incomplete passage of particles through filters bias results to larger size. Centrifugation is procedurally difficult and has low resolution for size separation. Methods most appropriate for Dissolved vs NP distinction.
UV-VIS and Fluorescence Spectroscopy	Generally unsuitable as other species present in most natural waters interfere.
Field Flow Fractionation	Very applicable for polydisperse, heterogeneous systems when coupled to ICP or TEM, generally requires sample pre-concentration and size pre-fractionation.
Real-Time Single Particle ICP (RTSP-ICP)	Very useful due to low detection limit, still early in the development stage.

All methods will be sensitive to sample pre-treatments