



# Cellular Entry of Gold Nanoparticles

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December 2017

# Outline

## □ Introduction

- Nanoparticles applications
- Gold Nanoparticles
- Surface Plasmon Resonance
- Nanoparticle-based drug delivery system
- Cellular entry of nanoparticles

## □ Comparative study on cellular entry of two different types of gold nanoparticles

- Preparation of nanoparticles
- Imaging techniques
- Results

## □ Possible effects of nanoparticle absorbance on biophysical properties

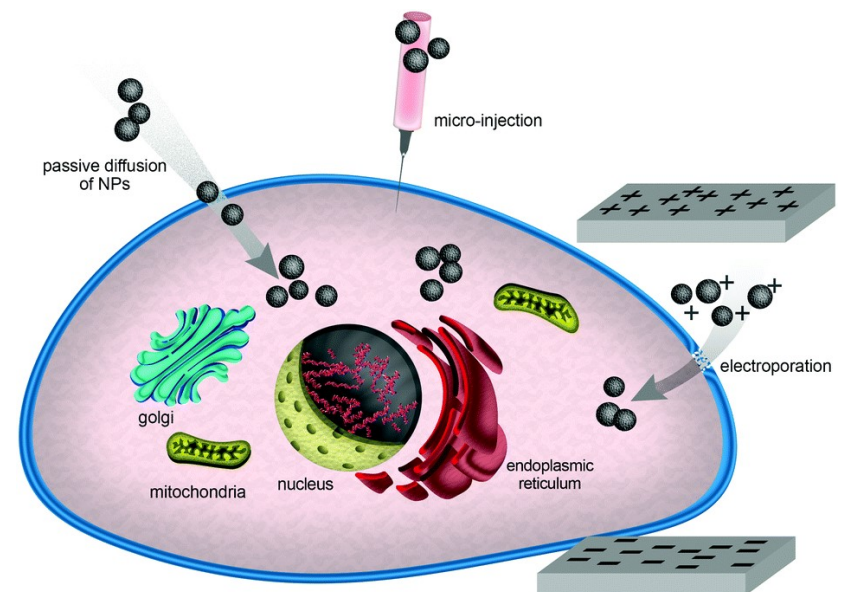
- Importance of biophysical properties
- Various methods for biophysical characterization
- Classical methods, MEMS-based methods, Microfluidic Methods
- Suspended- microfluidic for biophysical characterization

## □ Summary



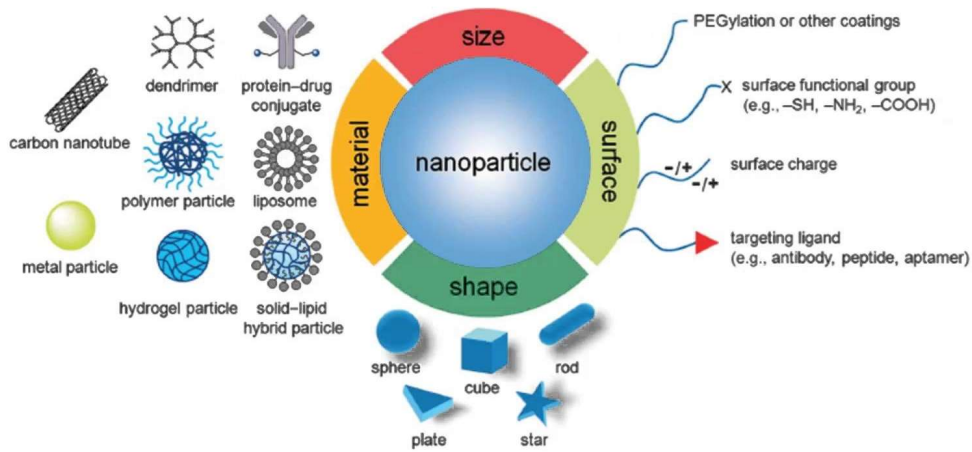
<http://bgr.com/2014/05/05/nanogold-paint-smartphones-biotech/>

# 1- Cellular Entry of Nanoparticles



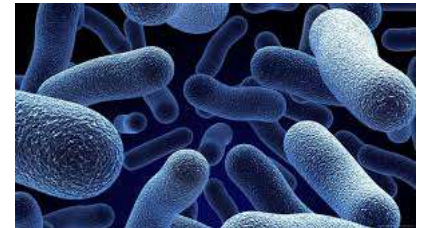
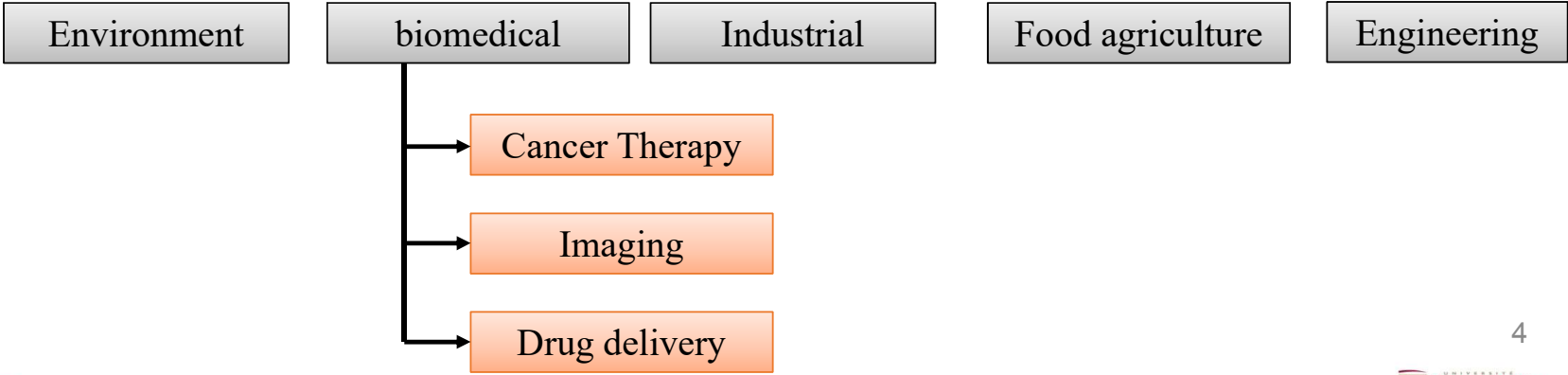
Behzadi et al, *Chem Sco Rev*, 2017

# Nanoparticles types/applications



Cheo et al, *Chem Sco Rev*, 2011

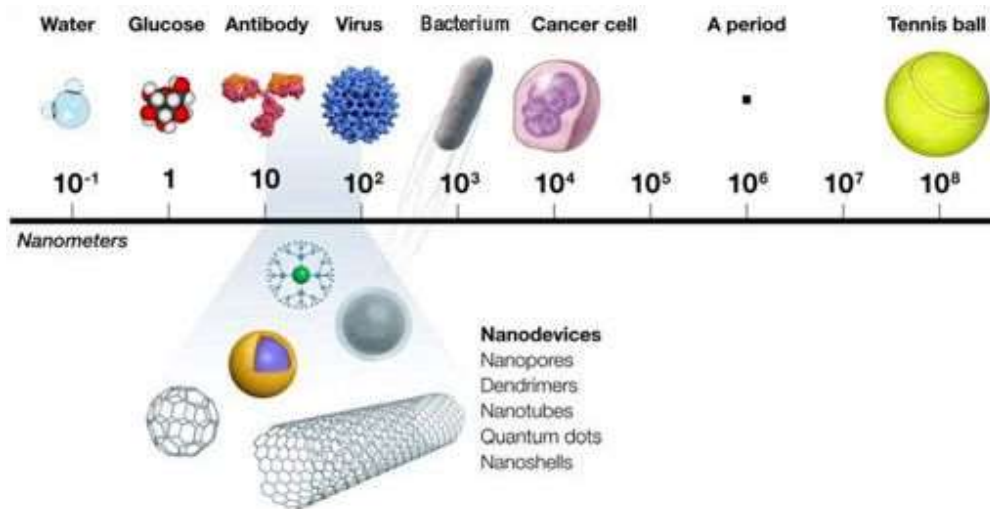
**Application**



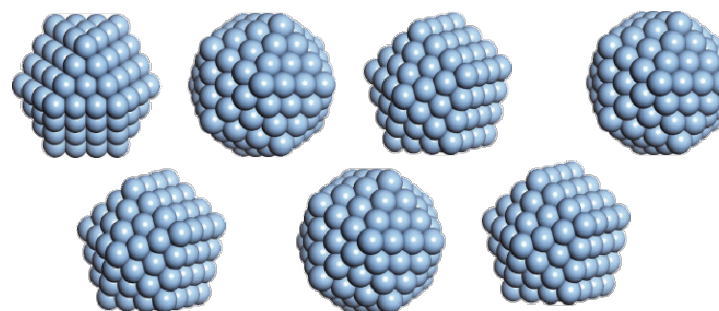
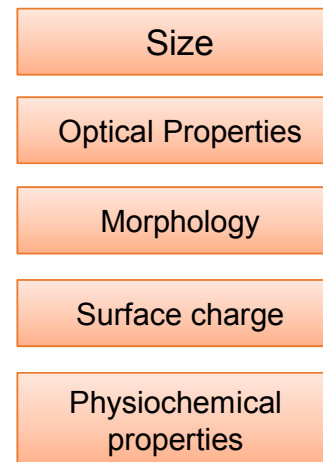
<http://nanogloss.com/nanoparticles>

# Nanoparticles

- Nanoparticles are small pieces of substances between 1 to 100 nanometers having various applications
- Nanoparticles are classified based on their properties



<http://tremblinguterus.blogspot.ca/>

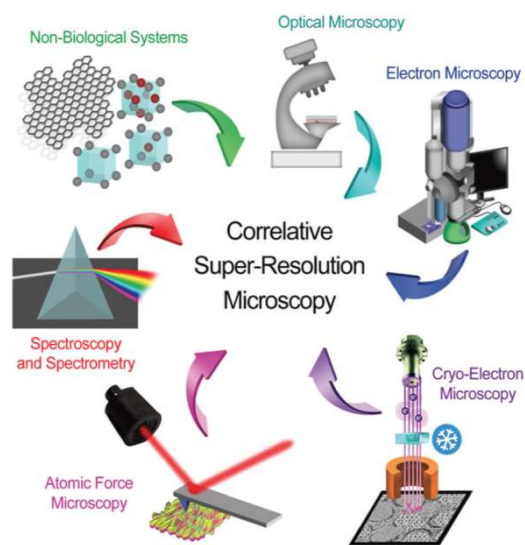


Joshua A et al. 2015, *The Journal of Physical of Chemistry C*

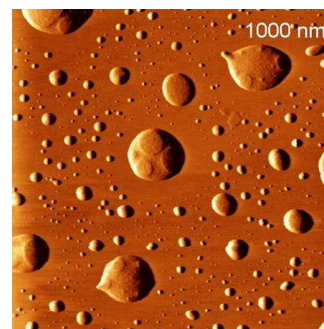
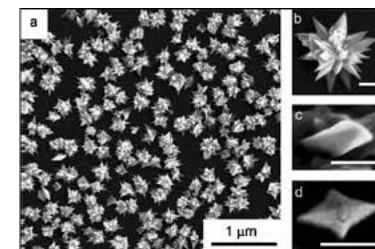
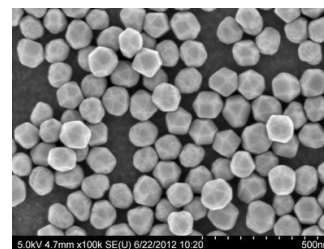


## How can you see Nanoparticles?

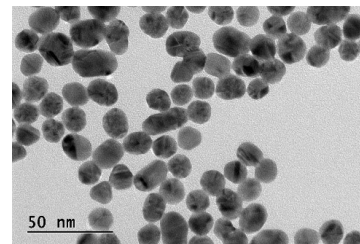
- We cannot see nanoparticles with regular microscopes
- ❑ Scanning Electron Microscopy (SEM)
- ❑ Atomic Force Microscopy (AFM)
- ❑ Transmission Electron Microscopy (TEM)



Behzadi et al, *Chem Sco Rev*, 2017



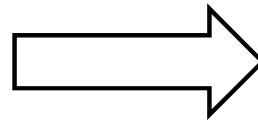
**silver nanoparticles**  
<http://www.nanoscopy.net/>



**Gold nanoparticles**  
 Imperial college  
 London

# Nanogold particles

- Gold Particles (AuNPs) have great potentials for biomedical applications



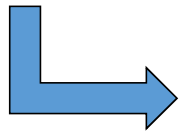
Bio-sensing

Therapeutic

Diagnosis

Drug Delivery

- AuNPs are tunable in term of :



Shape

Size

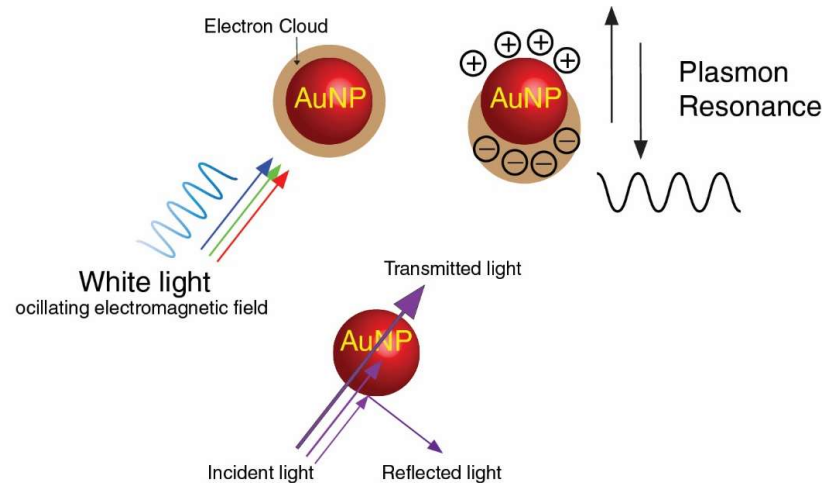
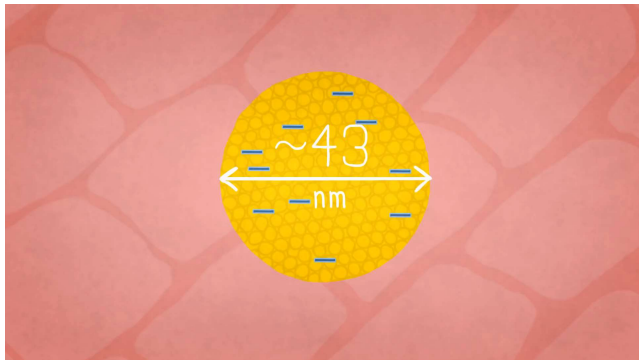
Surface  
chemistry

Aggregation  
State

Optical

## Gold Nanoparticles Properties: Surface Plasmon Resonance

- Optical Properties of metallic nanoparticles: Gold and Silver
- (The wavelength of the light are larger than the size of particles)  $R < \lambda$
- LSPR: Localized Surface Plasmon Resonance





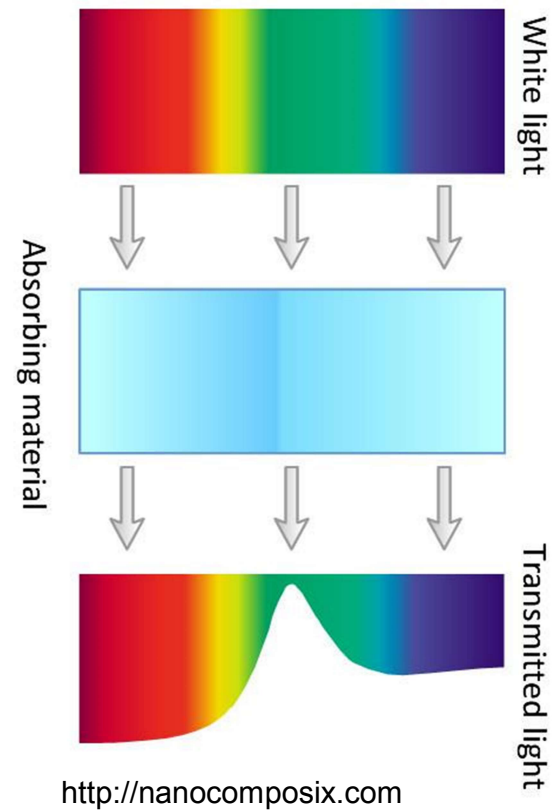
# Gold Nanoparticles Properties

- Colloidal Gold (Suspension of submicron particles of golds in fluid)



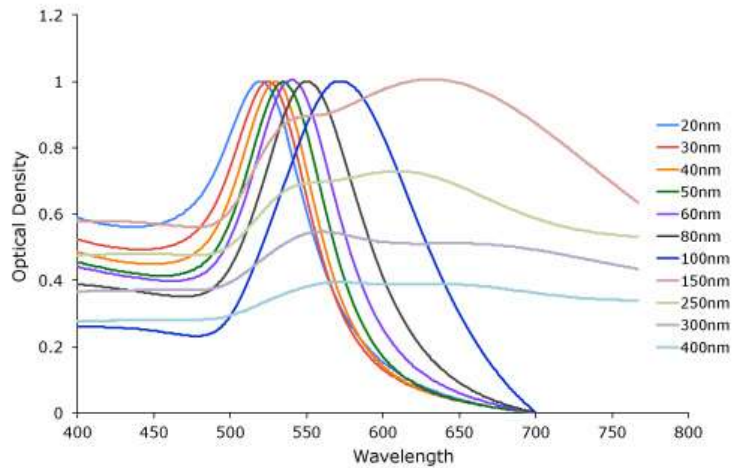
Increasing Size

Red ----- Blue



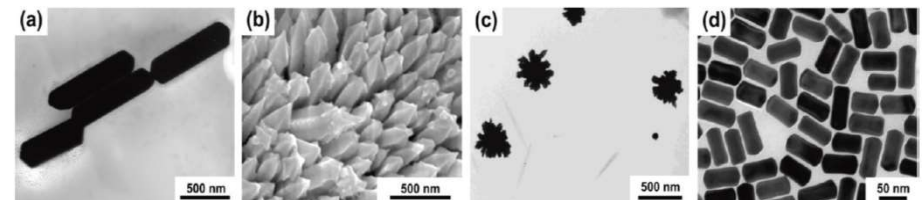
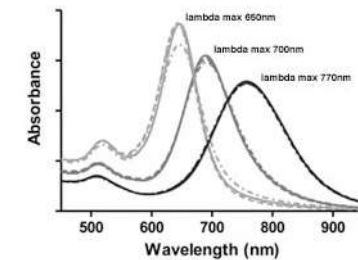
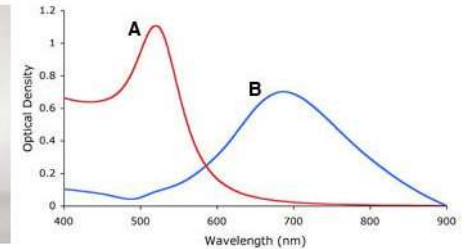
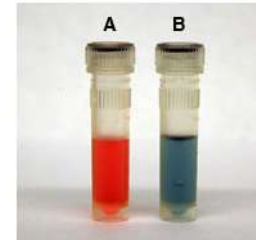
# Influence of gold nanoparticles properties on LSPR

Size  
Sharper to broader



<http://www.cytodiagnosics.com>

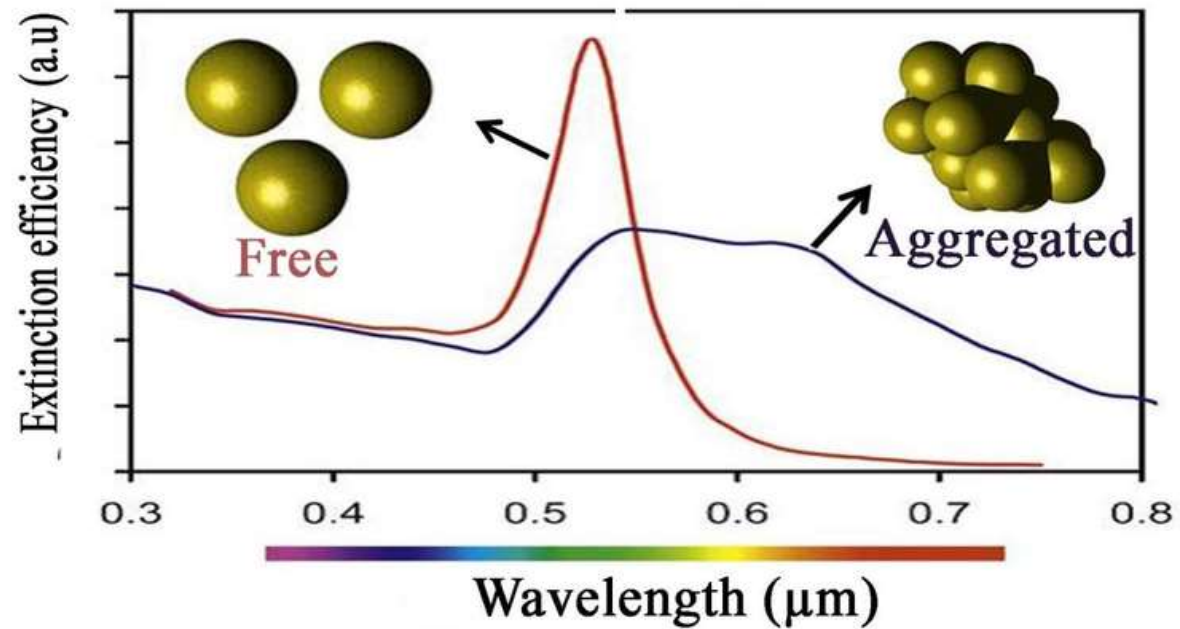
Shape



Senyuk et al, *Nano Letter*, (2012)

# Influence of gold nanoparticles properties on LSPR

- Aggregation



Kumar et al, *Pharmacy and Pharmaceutical Science*, 2014

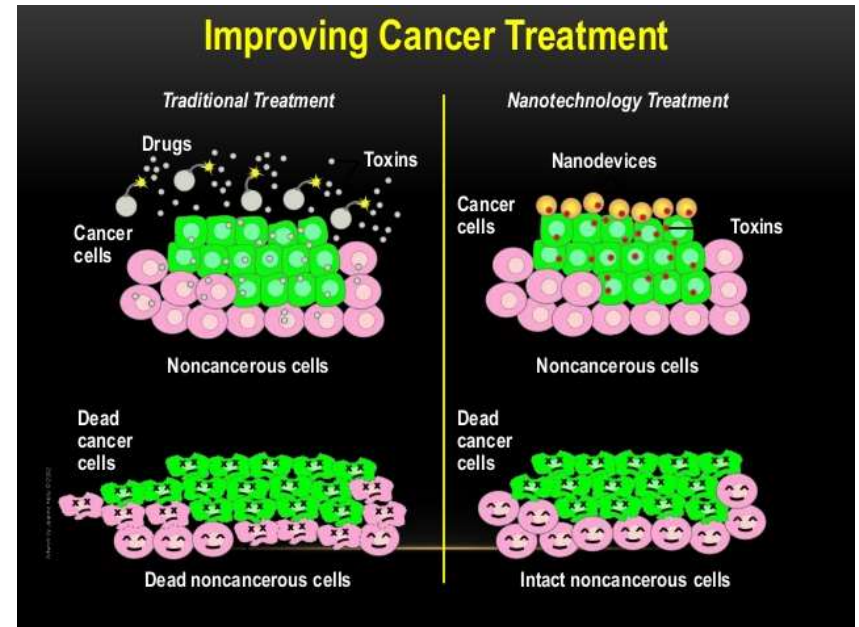
# Nanoparticles for drug-delivery

Nanoparticles can be absorbed, convolutely attached, or encapsulated into particles

## Limitation of conventional methods

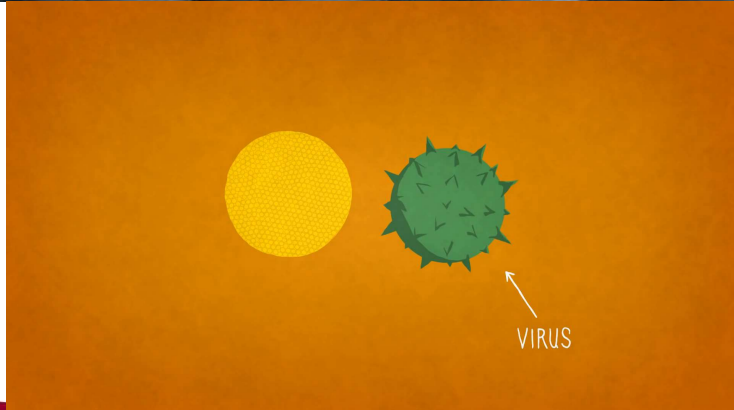
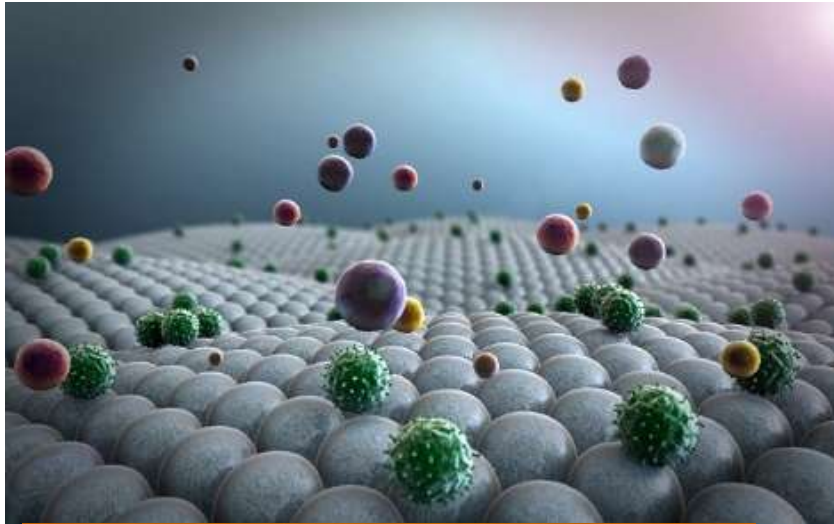


- Lack of selectivity toward cancerous cells
- Systemic toxicity
- Low therapeutic index
- Low circulation half-life
- Tendency to aggregate



<https://www.cancer.gov/sites/ocnr/cancer-nanotechnology/treatment>

# Advantages of NPs for drug delivery



## Nanoparticle-based Drug Delivery System

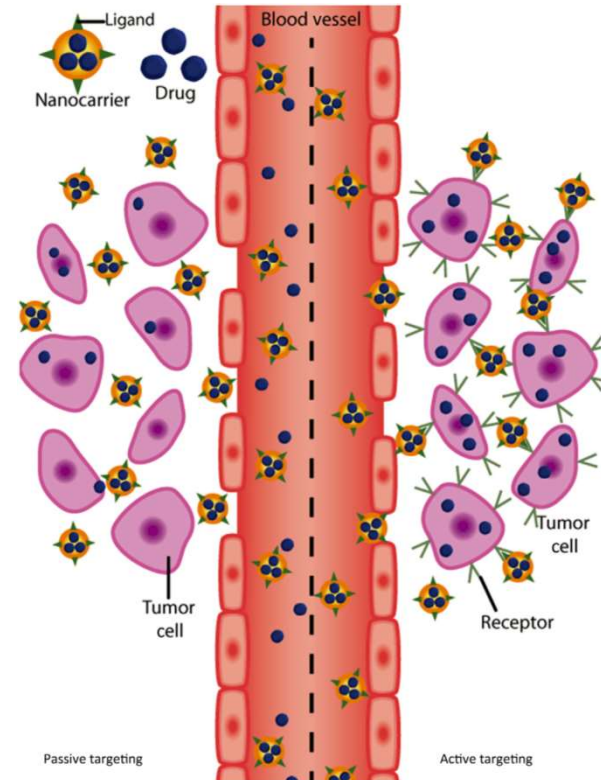
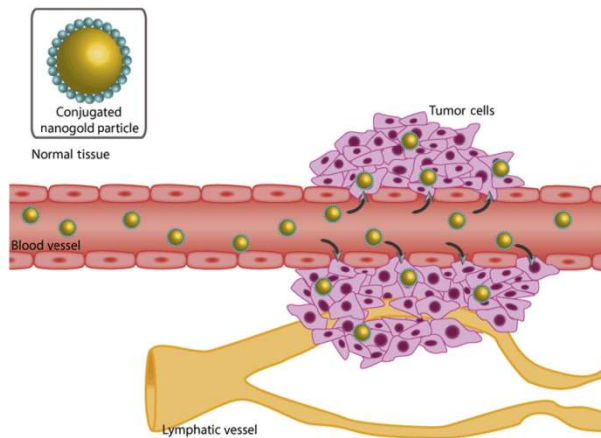
- Decreasing toxic side effects
- Increasing specific location
- Controlled contribution
- Reducing dose
- Increasing treatment efficiency
- Improving patient compliance



# Targeting Approaches

1- Active Targeting (Pre conjugated with antibodies, small molecules and peptides)

2- Passive Targeting (EPR)



Ajnai et al, *Journal of Experimental and Clinical Medicine* (2014)

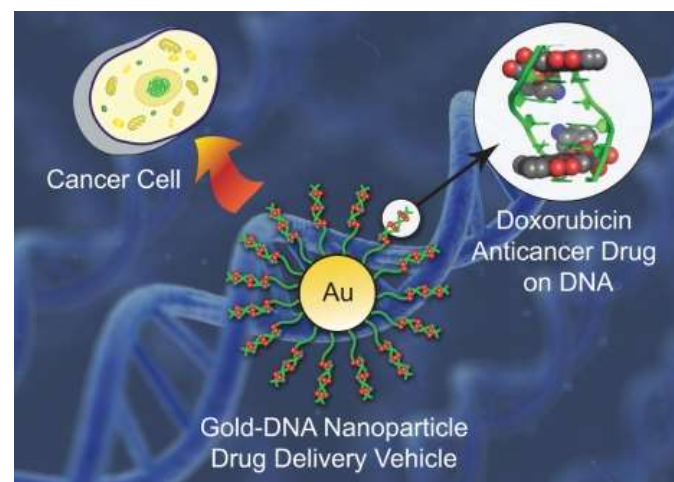
# Limitation of nanoparticle drug delivery system

Two determining factors should be taken into account before using nanoparticles as Drug Delivery system

## 1- Biocompatibility

- Not toxic (Exposure time, dose)  
Methods: viability of cell Function changes
- Accepted by body without rejection
- Inert or stable

## 2- Internalization ability (subcellular location)



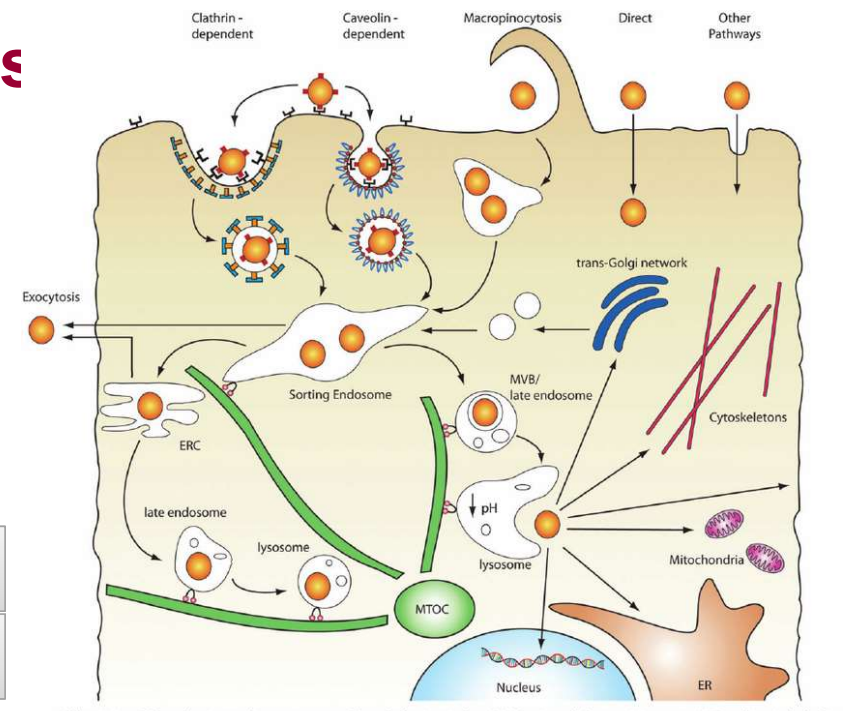
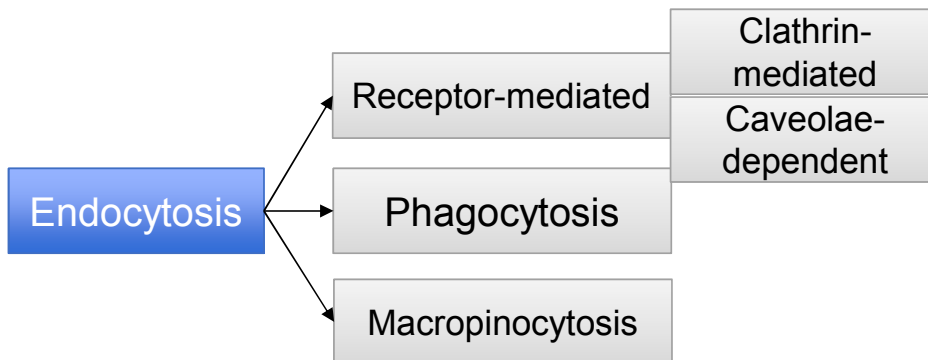
# Cellular uptake of Nanoparticles

Cell-specific targeting:

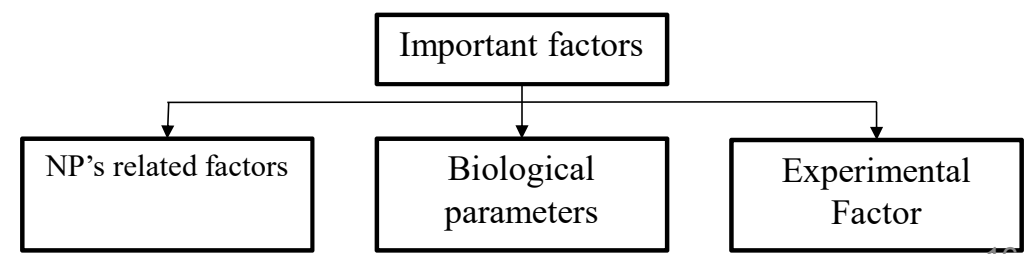
Attaching drugs to specially designed carries

(Drug can be absorbed, covalently attached or encapsulated into nanoparticles)

**Entry Mechanisms:**



Abbreviations: ERC, endocytic recycling compartment; ER, endoplasmic reticulum; MTOC, microtubule-organizing centre; MVB, multivesicular bodies; L. Chou et al, *Chemical Society Reviews* (2012)

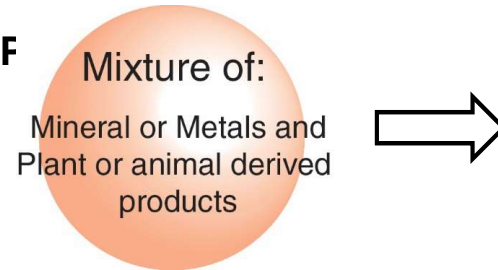


## 2- Comparative Study on cellular entry of Synthesized and Ayuverdic gold particles

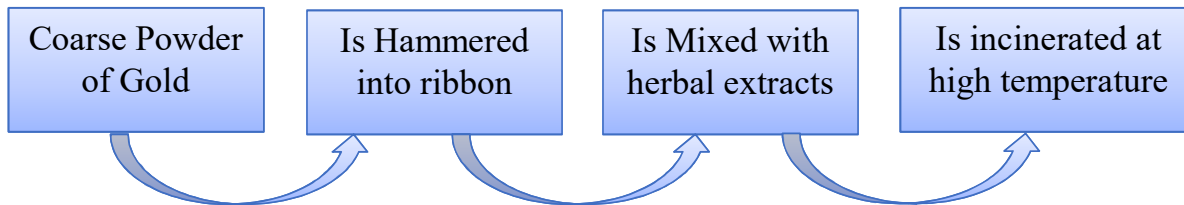
- Scientific Report, Nature (2017)
- Plasmonic, Springer (2017)
- Nanoscience and Nanotechnology, ASP, (2017)

# Synthesized gold nanoparticles and Ayuverdic particles

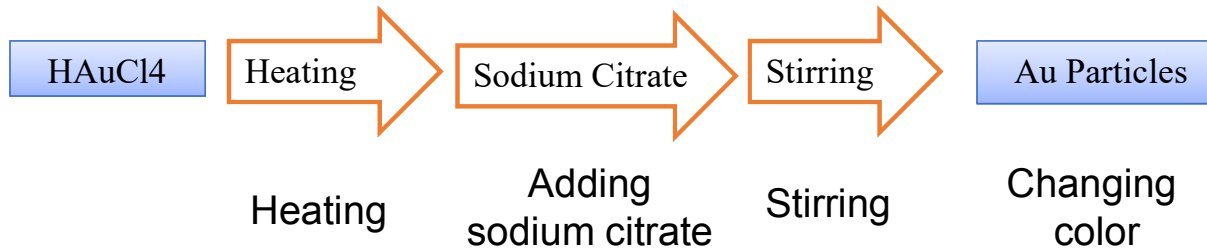
❑ **Incinerated Gold nanoparticles (IAuF)**  
 Traditional Indian approach:



**Swarna Bhasma Powder**  
 (<http://www.planetayurveda.com>)



❑ **Citrate-capped spherical nanoparticles (AuNPs)**  
 By the reduction of chlorauric acid with sodium citrate

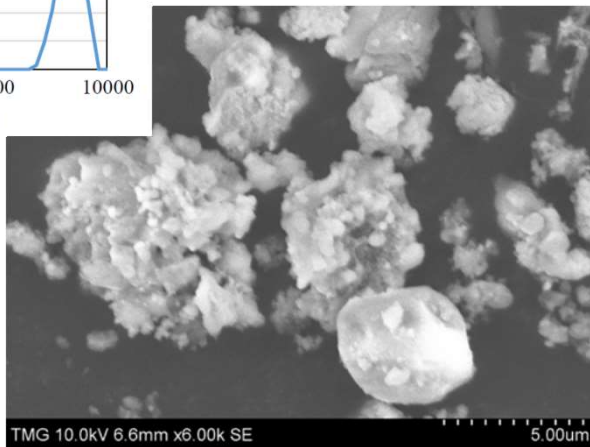
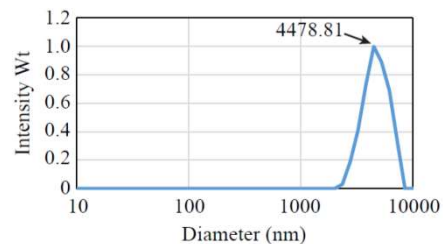


**Colloidal Gold**  
 (<https://dir.indiamart.com>)



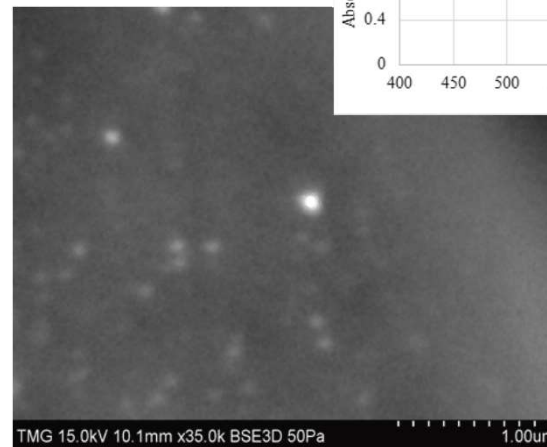
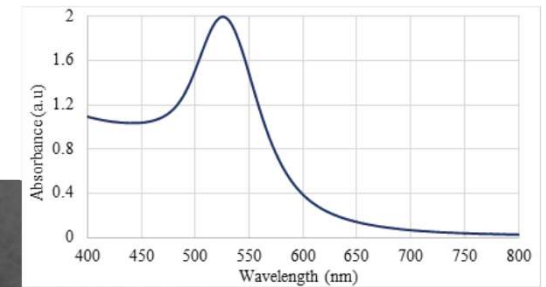
# Characterization of particles

## Scanning Electron Microscopy (SEM)



IAuPs

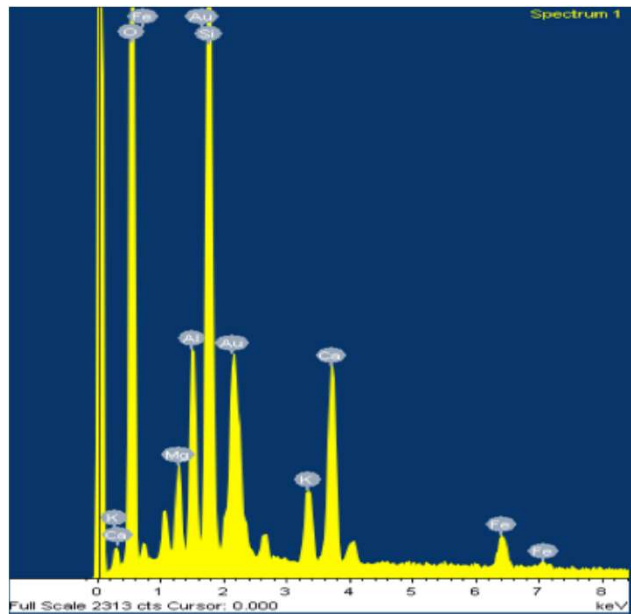
Average Size: 4500 nm (Dynamic Light Scattering)  
 Crystal size: 60 nm  
 Non-uniform



AuNPs

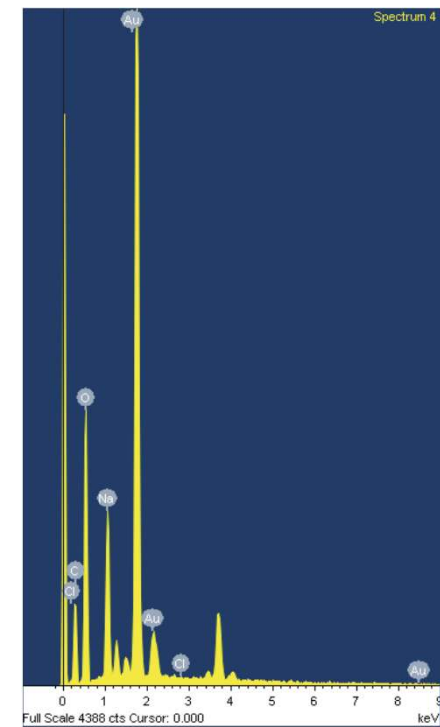
Average Size: 32 nm  
 uniform

# Elemental composition of AuNPs and IAuPs



EDS-SEM for IAuPS

	<b>AuNPs</b>	<b>IAuPs</b>
Au	56.88 %	89.6 ppm
Mg	1.8 %	0.273 ppm
Na	-	20.9 ppm



EDS-SEM for IAuPS

# Characterization of gold nanoparticles to cells

To test the toxicity and subcellular location

Two experiment test were performed

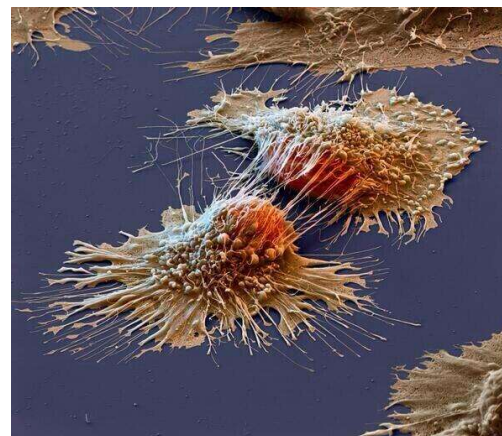
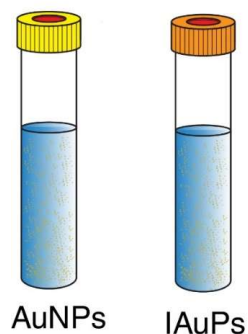
- 1- under different exposure time
- 2- under different doses

Two types of cell lines were chosen

- 1- HeLa (Cancerous cells)
- 2- HFF1- (Healthy cells)

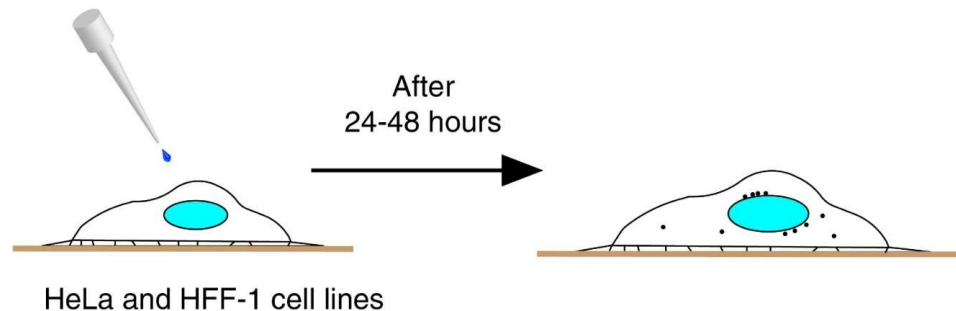
Test:

Localization, entry and impacts on human cells



<http://smashinglife.co.uk/cancer-cells-look/>

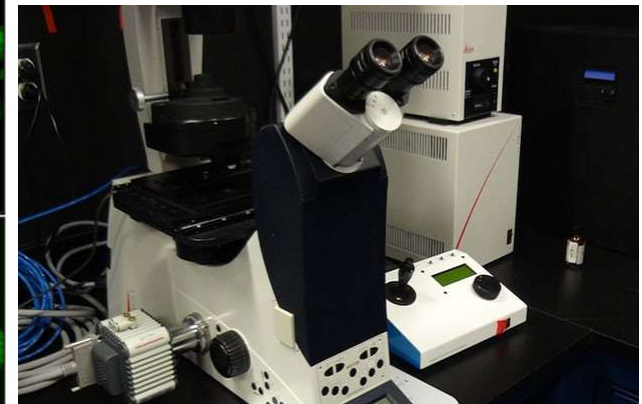
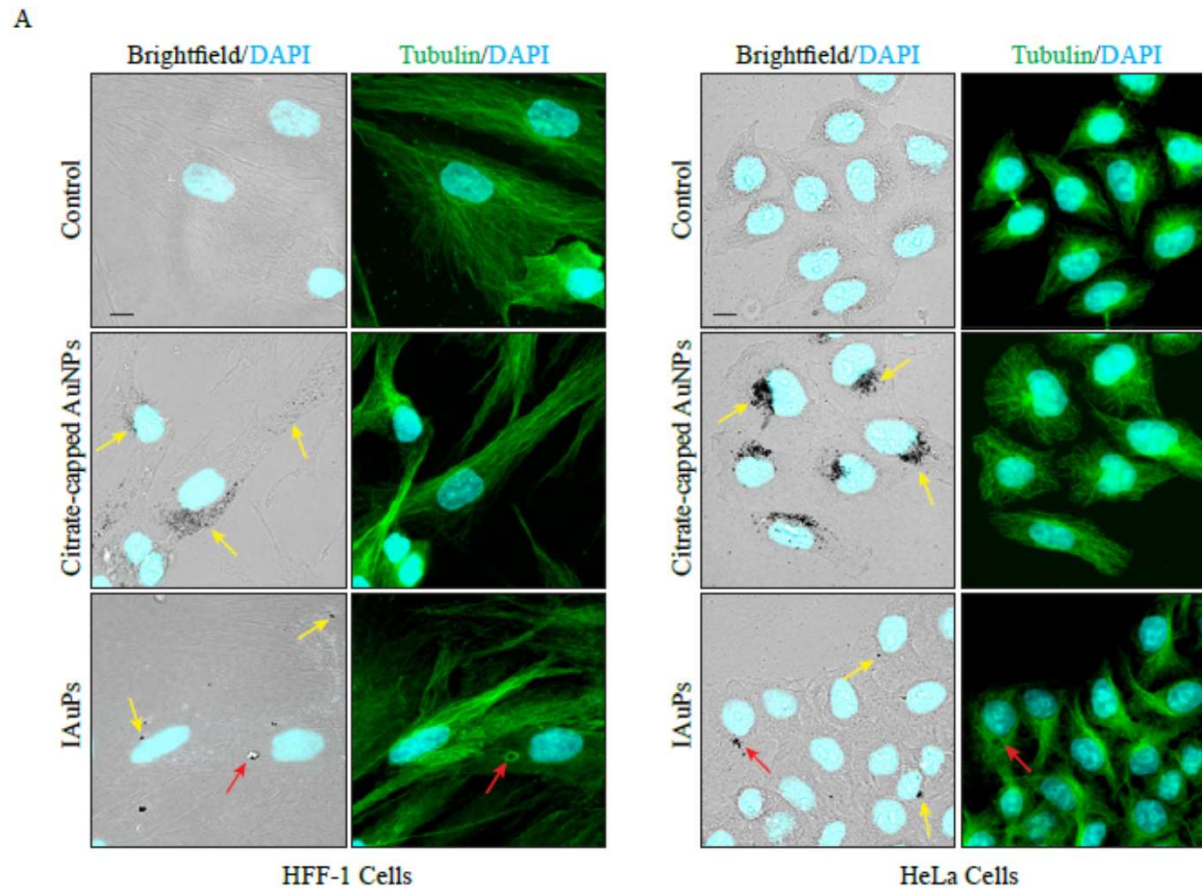
Nanoparticles



Imaging Techniques:

- Light Microscopy
- SEM
- Hyperspectral Imaging

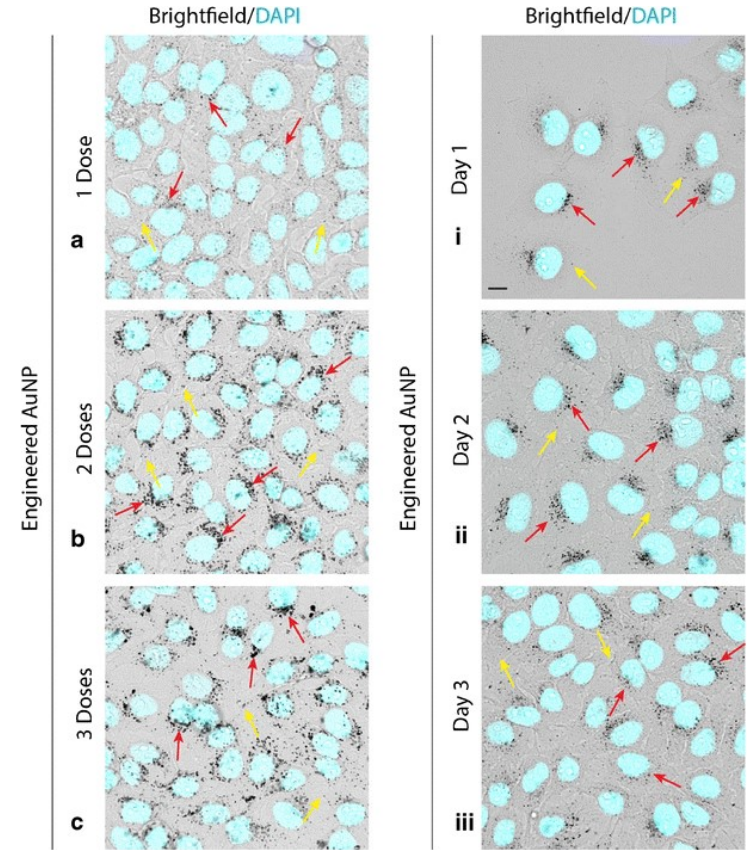
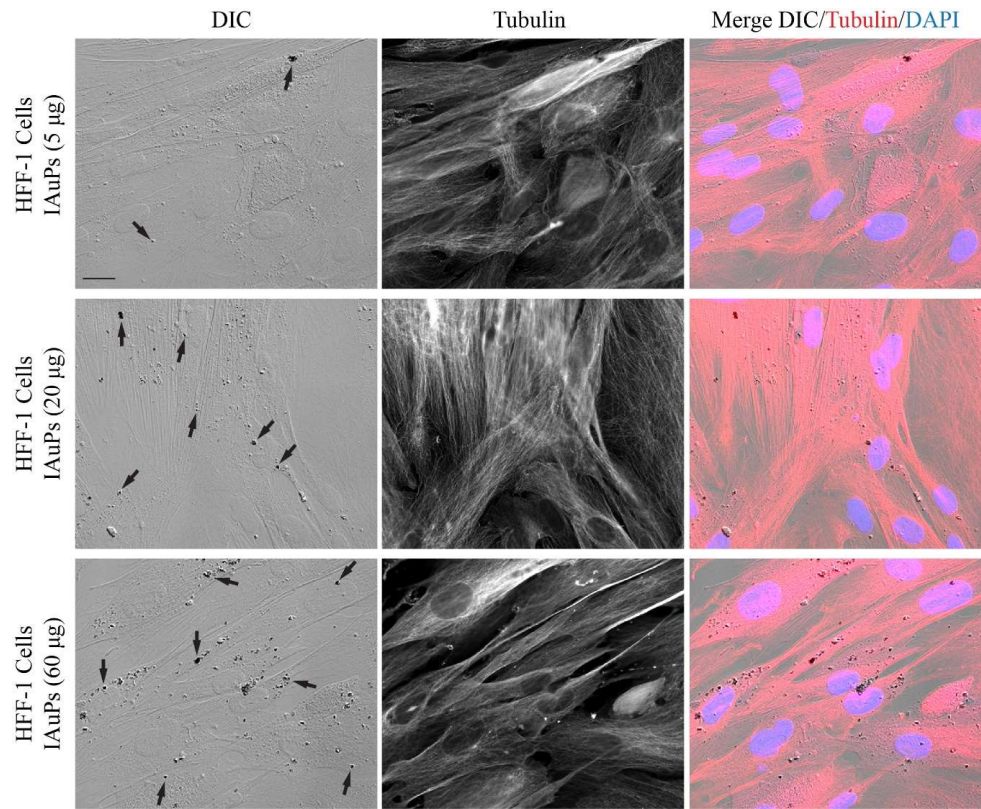
# Nanoparticle in cells (Light Microscopy)



Leica DMI 6000 B inverted epifluorescence microscope



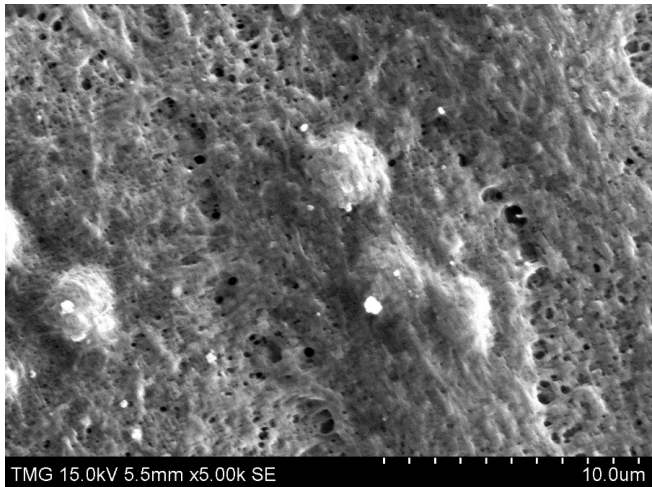
# Concentration and incubation time effects



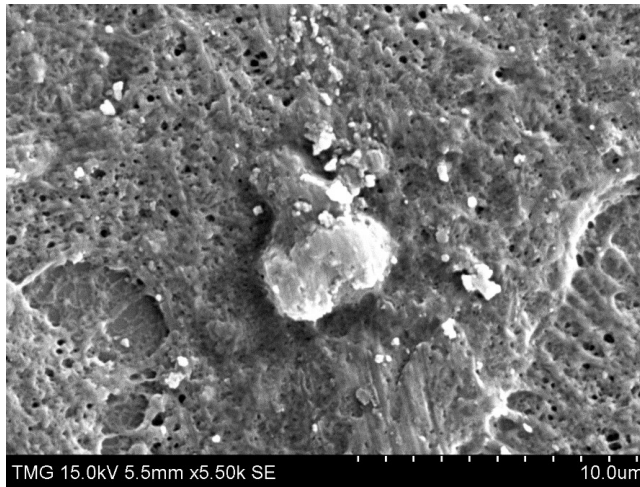


# AuNPs and I AuPs in Cells (SEM)

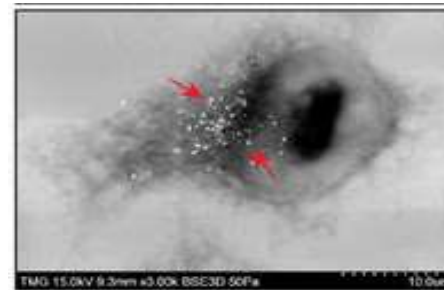
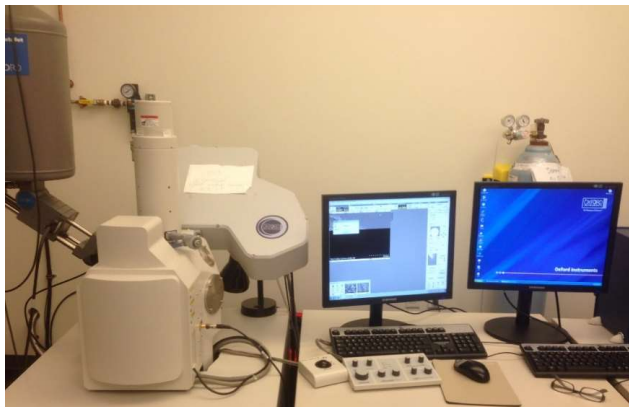
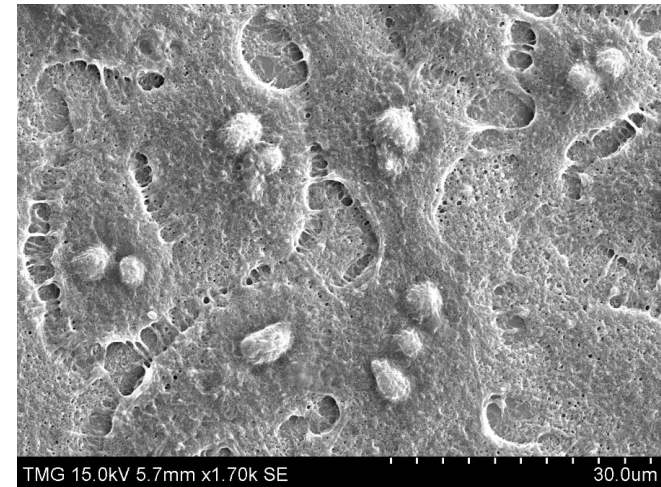
Low con. AuNPs in Hela



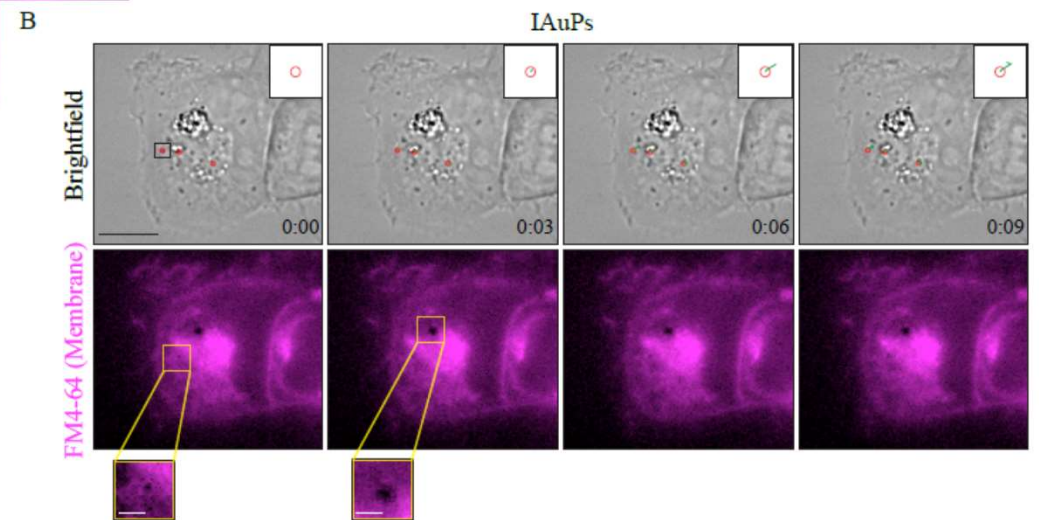
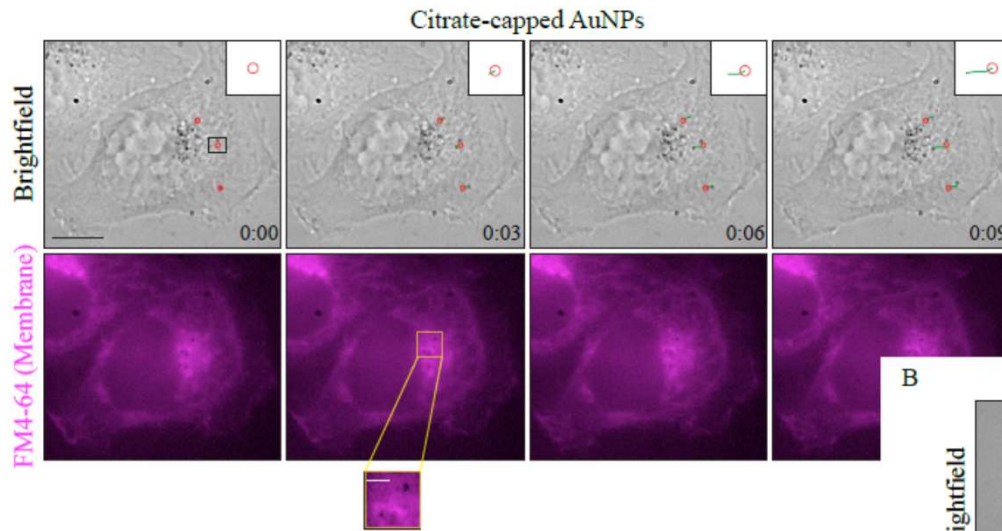
High con. AuNPs in HeLa



Control w/o AuNPs



# Nanoparticle in cells (Live Imaging)

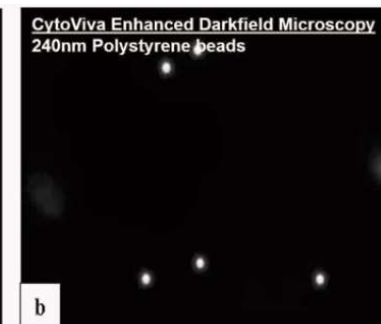
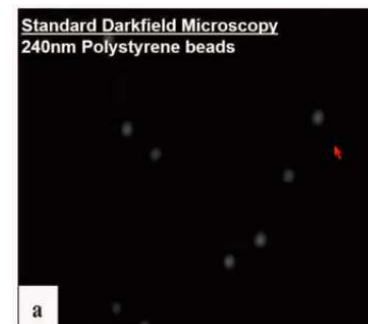
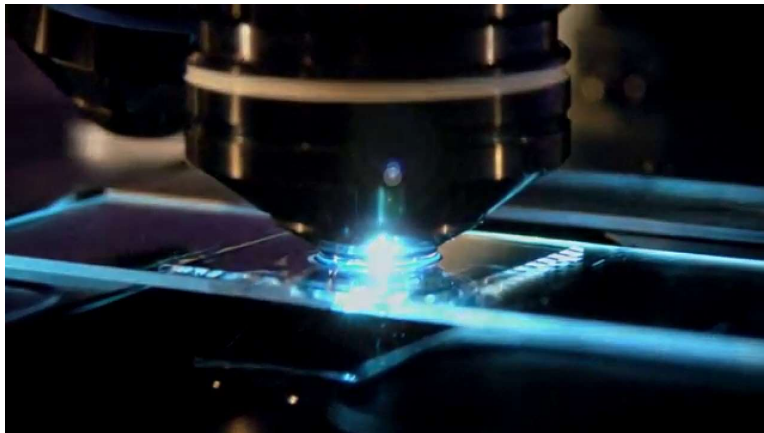


# Hyperspectral Microscopy CytoViva

Technology for characterization of nanomaterial in cells

Combination of:

- Hyperspectral imaging system
- Optical Microscope

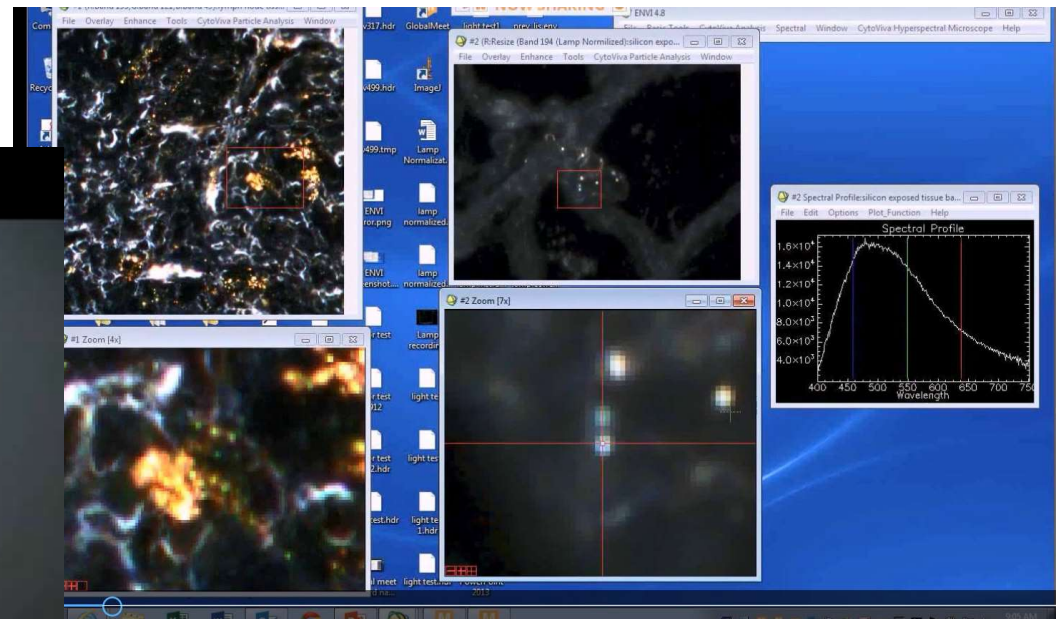


<https://cytoviva.com>



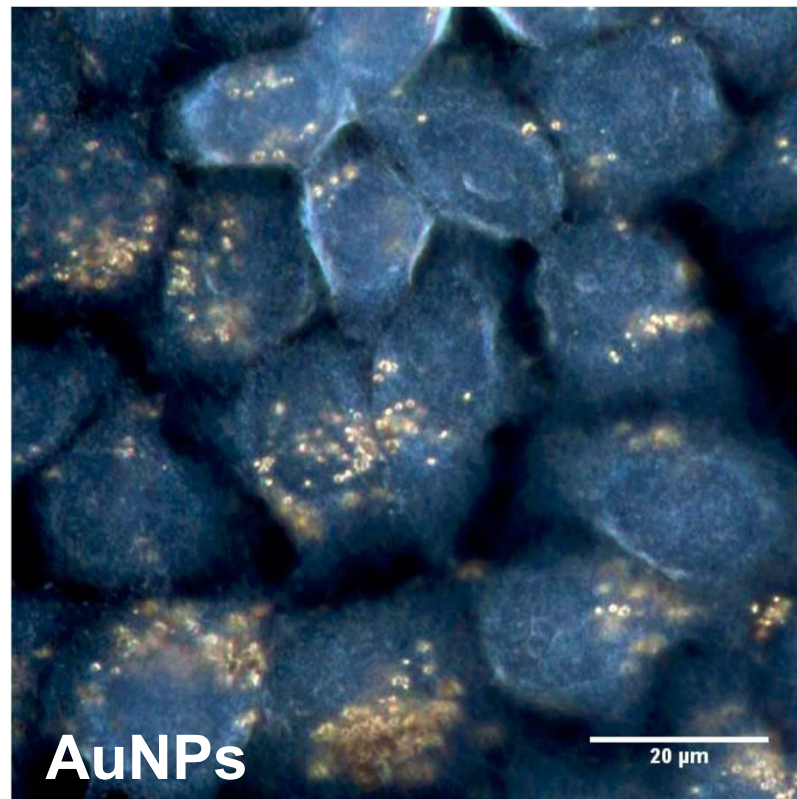
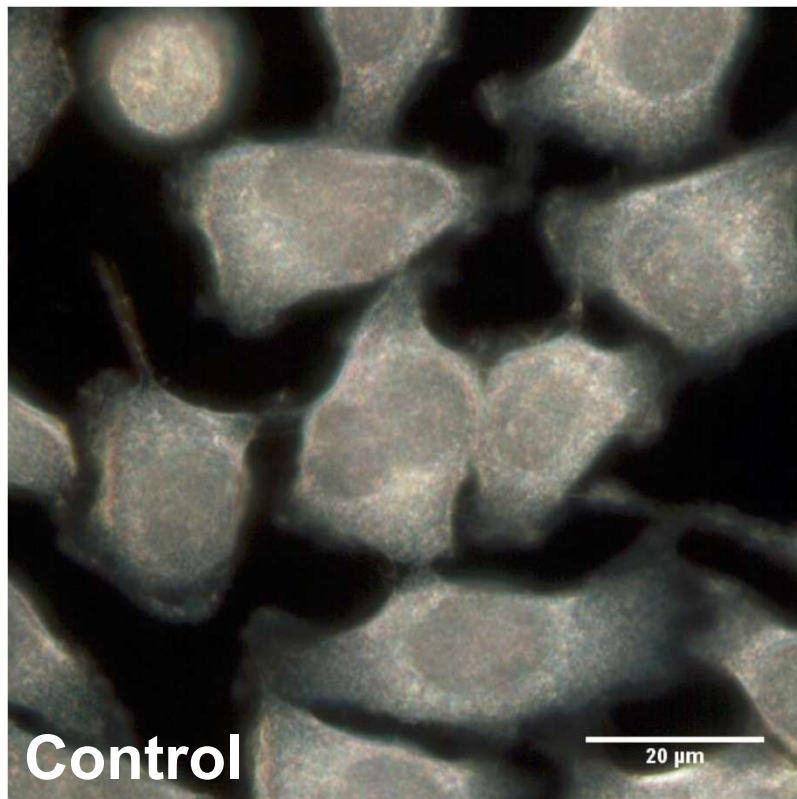
# Using Hyperspectral Microscopy for AuNPs in cells

- Presence of AuNPs
- Location of AuNPs



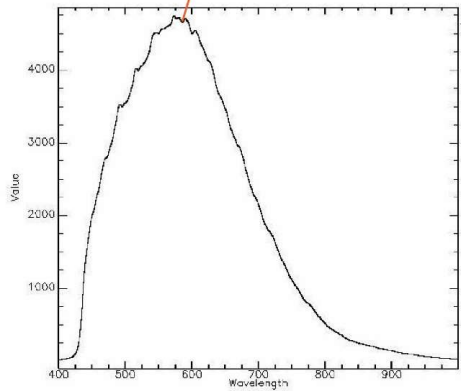
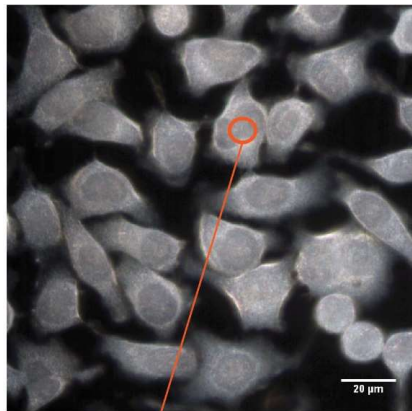
<https://cytoviva.com>

## Enhanced dark field imaging

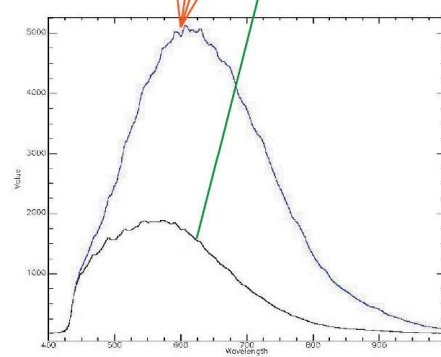
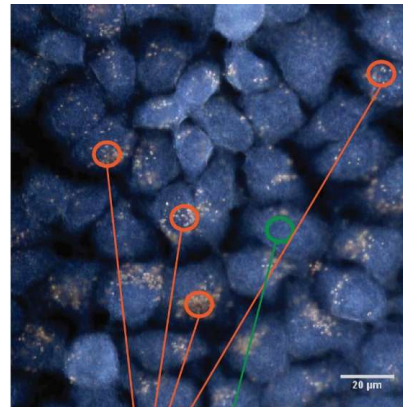




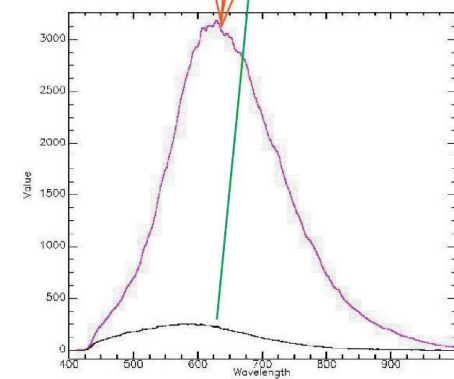
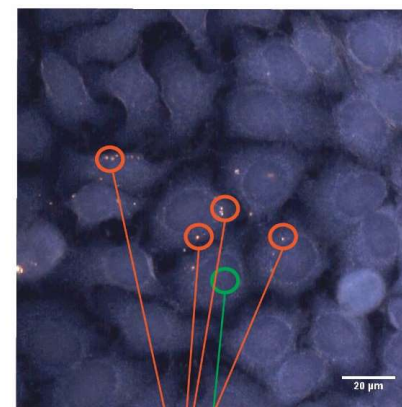
# Hyperspectral imaging of AuNPs and I AuPs in cells



Cell



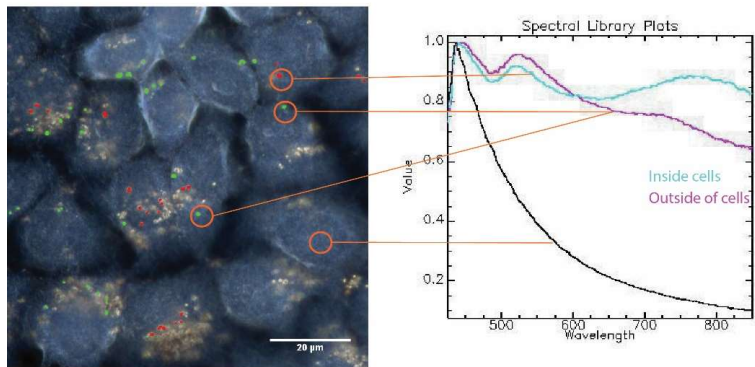
AuNPs  
600~700 nm



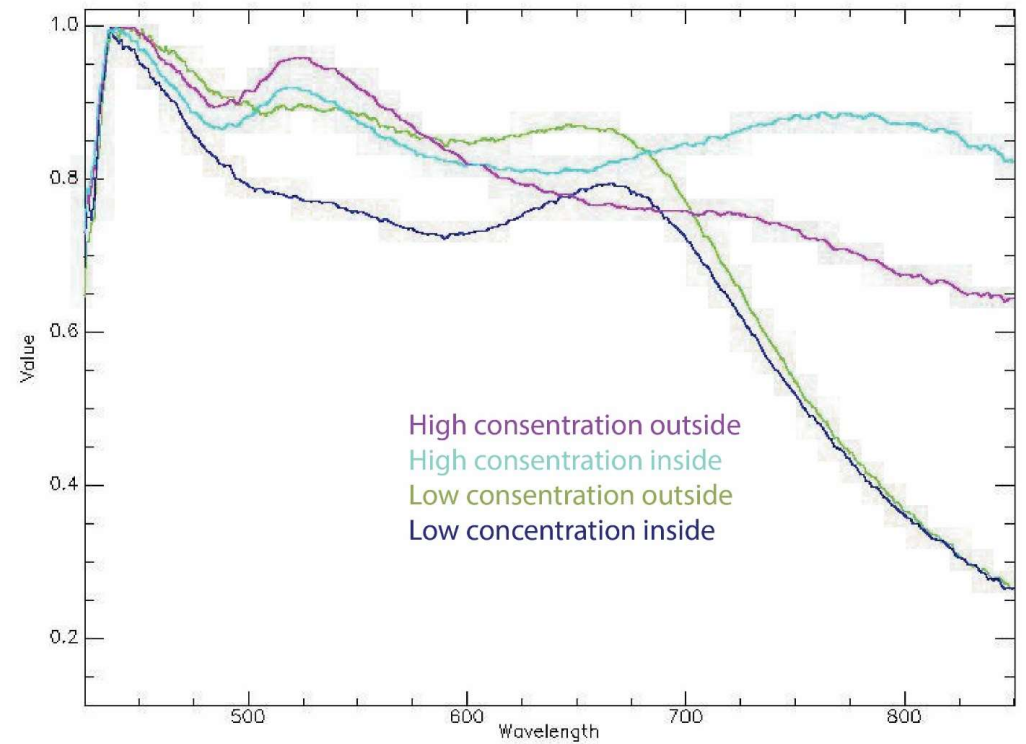
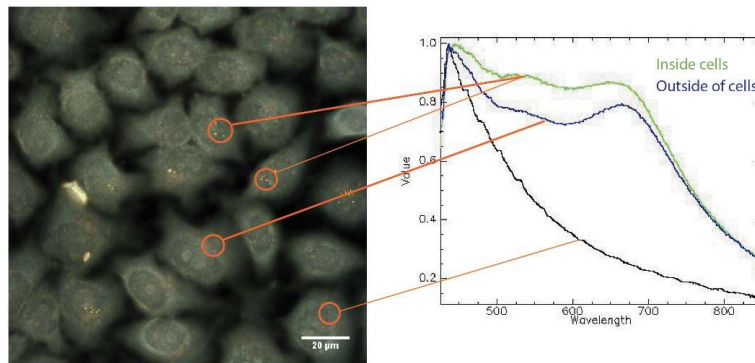
IAuPs  
600~625 nm

# Intracellular Localized Surface Plasmonic Sensing for Subcellular Diagnosis

A)



B)



A) High concentration B) Low concentration

## Breaking IAuPs to smaller particles

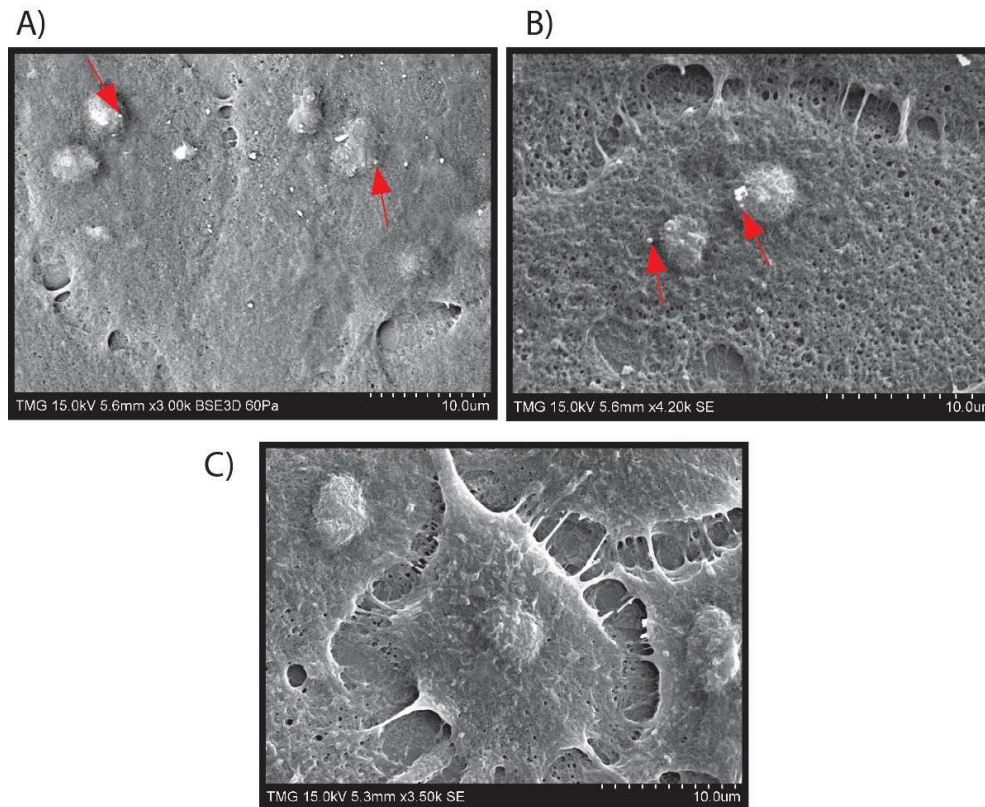
Sonication



Mixer



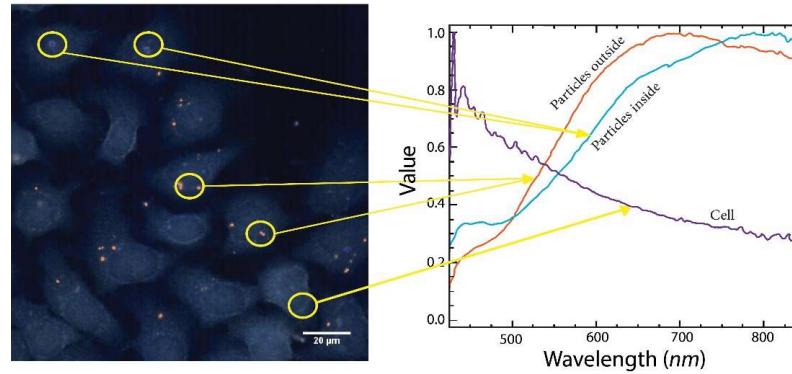
# Broken and unbroken particles in cells



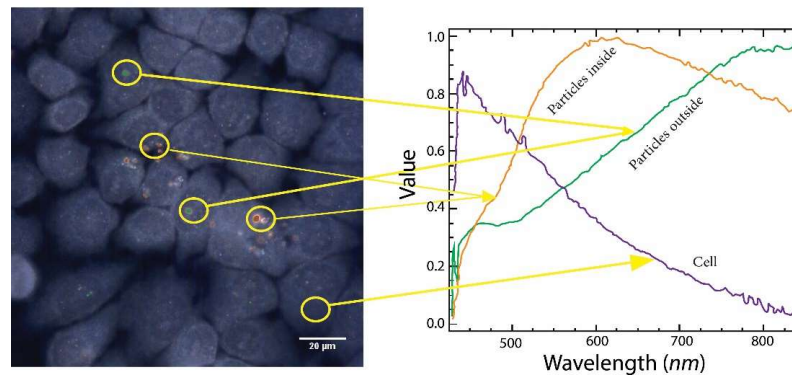


# Hyperspectral imaging of broken and unbroken I AuPs in cells

A) Unbroken I AuPs



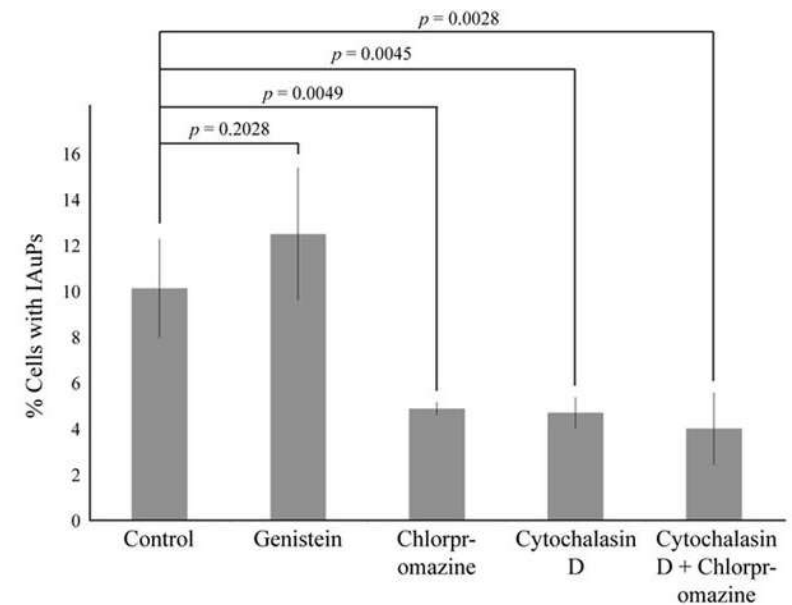
B) Broken I AuPs





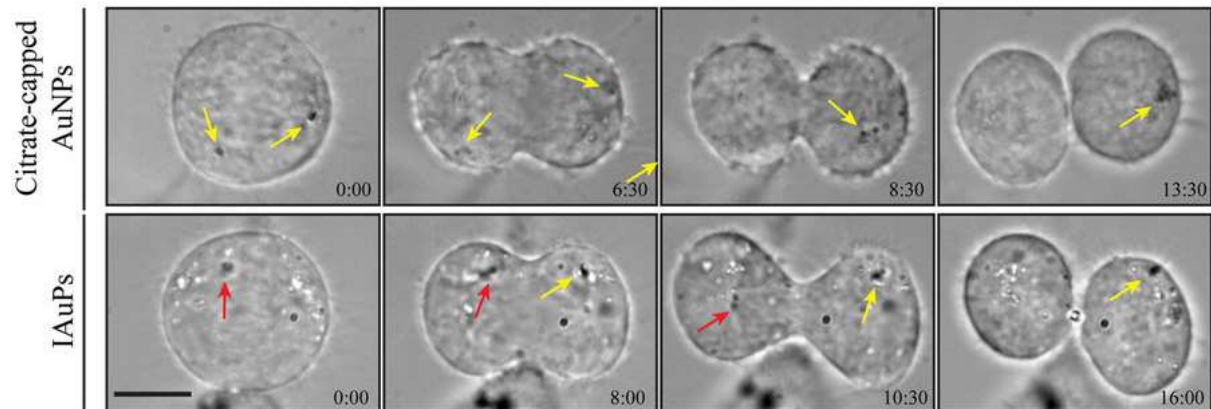
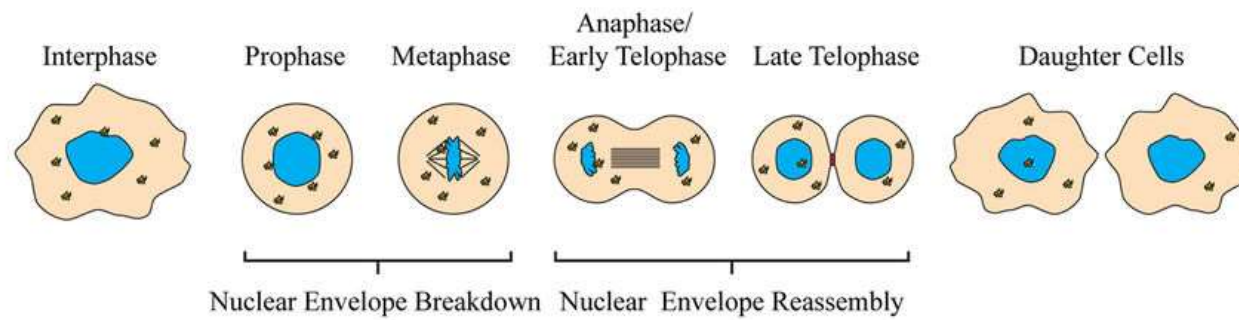
## Entry mechanism of IAuPs

Mechanisms	Before blocking	After blocking (changes)
Macropinocytosis	9.2 %	4.7% (-4.5%)
Clarotin-mediated	11.1 %	4.9% (-5.2%)
Both Macropinocytosis and Clarotin-mediated	9.2 %	4% (-5.2%)
Calveolin-mediated	~12 %	~ 12%

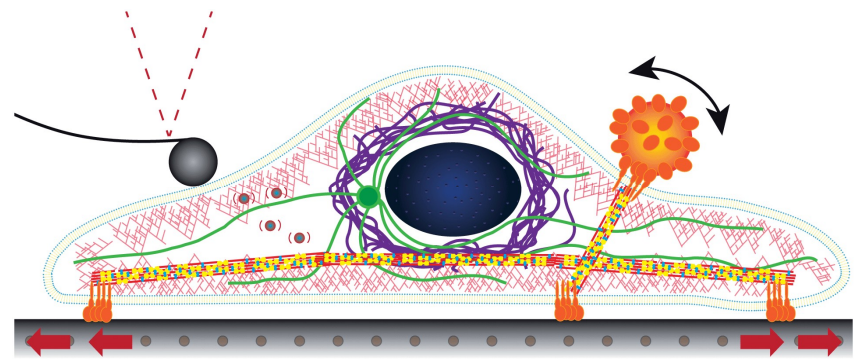


# Nanoparticles in Nucleus

A



### 3- Impacts of nanoparticles



Robyn et al, (2014)

## Biophysical properties of cells

Biophysical properties of cells?

**BIOMECHANICAL**, bioelectrical, biochemical

Important biophysical biomechanical properties

Size,

Viscoelastic properties

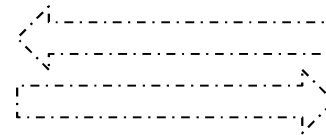
Mass

Friction

Density

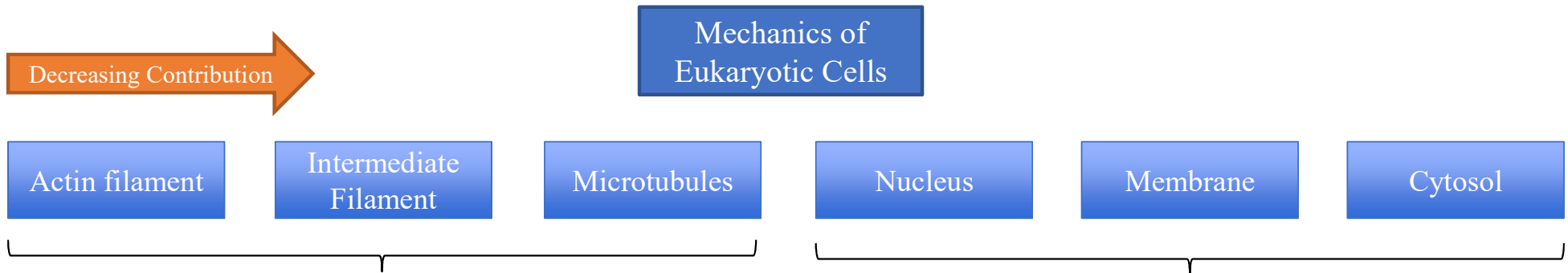
Biological  
Function

Biophysical of  
cells

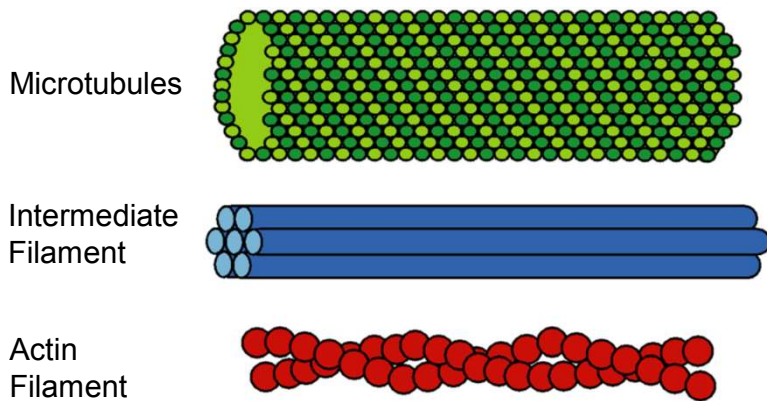


Mechanics

# Components and Mechanics of Cells (Eukaryotic cell)

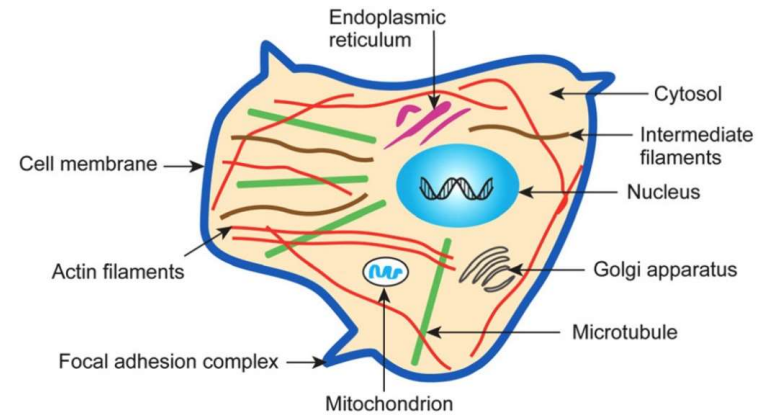


Cytoskeleton and Cellular Structure



Rodriguez et al, *Applied Mechanics Review* (2013)

Less contribution to mechanics of cells

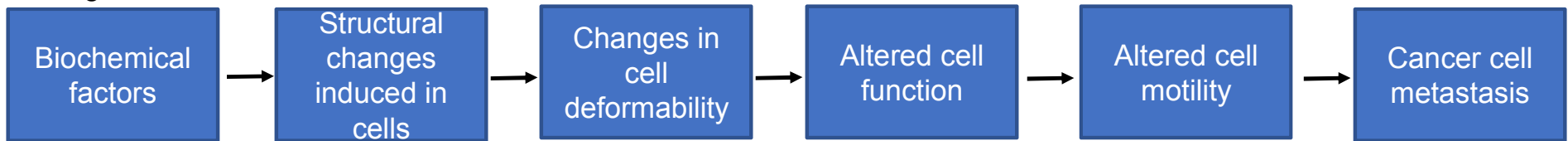


Suresh, *Acta Biomaterialia* (2007)



# Why studying Bio-Mechanical Properties of cells is important?

Bio-mechanical properties of cells during disease undergo changes

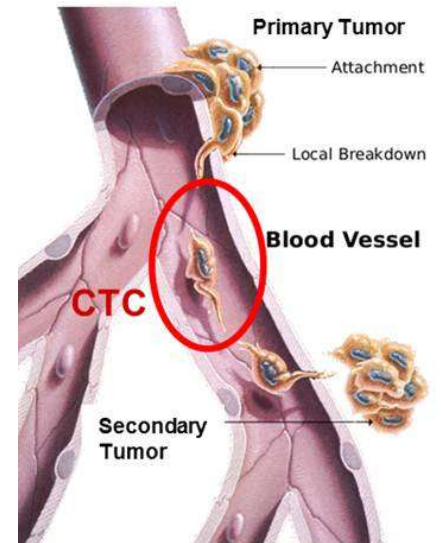
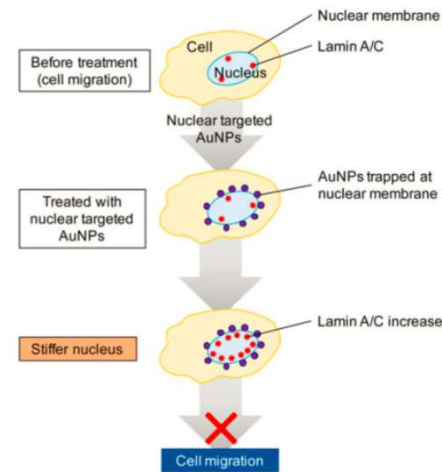


Suresh, *Acta Biomaterialia* (2007)

## Importance of Mechanical Properties

A powerful and label-free approach for diagnosis cancer at early stage

Evaluate the efficiency and effectiveness of medicine or nanoparticle-based drug delivery systems



Adapted from National Cancer Institute

# Different Methods for Deformability Characterization of Single Cells

Methods:

- Classical Methods,
- MEMS-based methods,
- Microfluidic-based methods

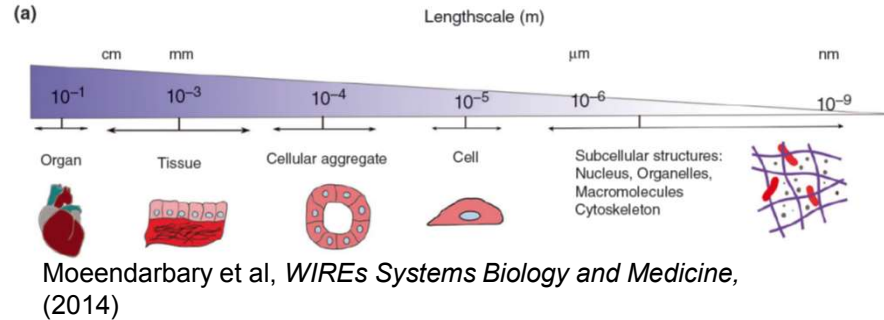
## Classical methods:

Main Advantage:

High –precision

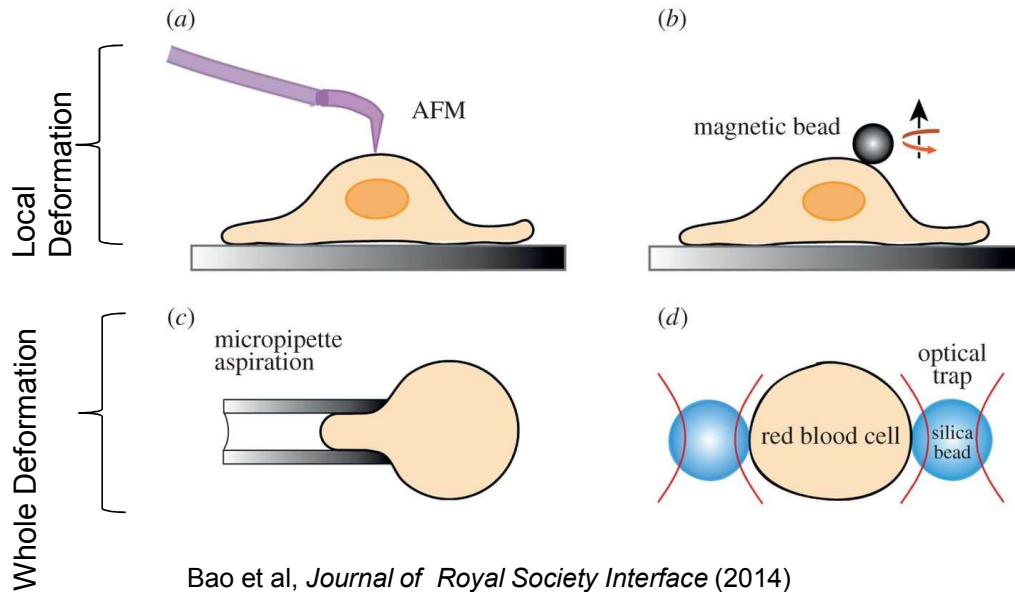
Main Limitation:

Low-throughput



Choice Criteria for cell mechanics:

- ✓ Size,
- ✓ Elasticity
- ✓ Precision
- ✓ Speed

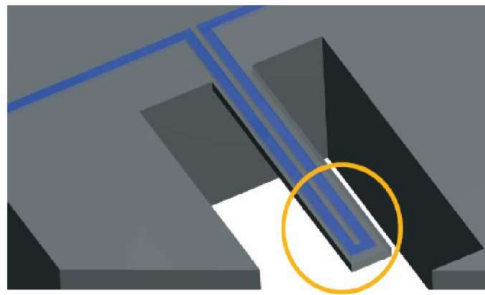


# Deformability characterization: MEMS-based systems

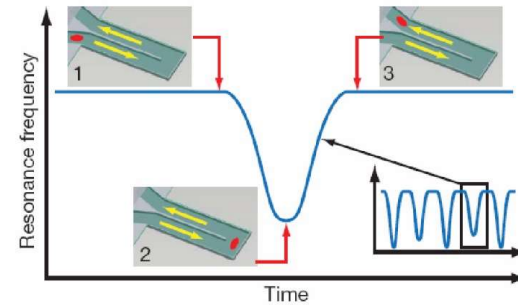
## Limitations:

Expensive  
External devices  
Non-Transparent

A)

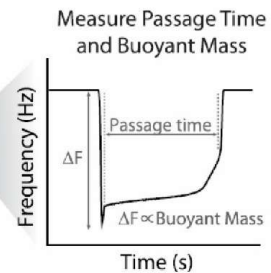
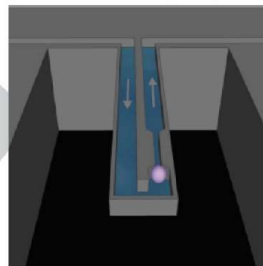


B)

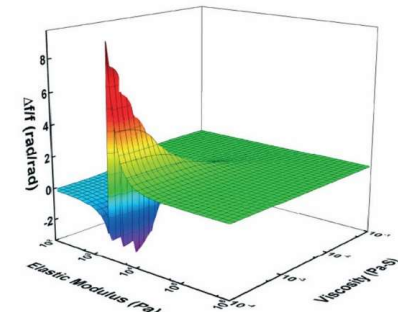
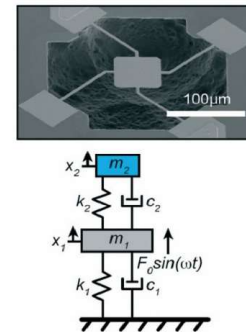


C)

Suspended Microchannel Resonator (SMR)

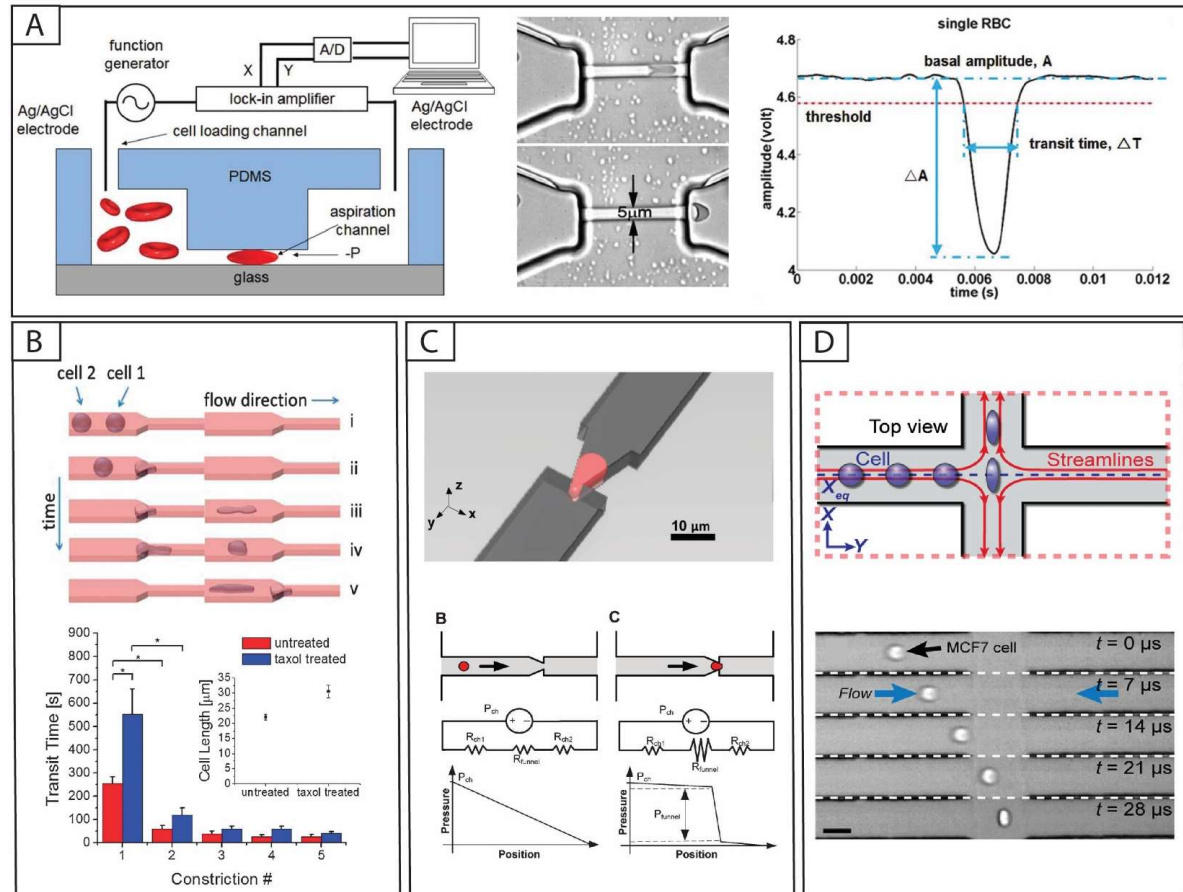


D)



# Microfluidic-based systems

- A) Constriction-induced deformation
- B) Multiple Constriction channel
- C) Micro- aspiration
- D) Hydrodynamic-induced deformation



## Advantages

High-throughput  
Easy fabrication

## Limitation

Low precision

## Nanoparticle uptake effects on biomechanical of cells

- NPs can provide desirable effects on cells

But! The intercellular effects of NPs in cells is unknown.

cell	Particles	Effects on stiffness
mesenchymal stem cells	Silica	Increased
Escherichia coli	Hematite NPs	Increased
iron oxide NPs	endothelial	Increased
Selenium NPs	MCF-7	Decreased



## Summary

- ❑ Advantages of nanoparticles have made them a good candidate for medical application
- ❑ Gold nanoparticles can be used for cancer diagnosis as well as cancer therapy
- ❑ Nanoparticle-based drug delivery system can provide advantages comparing to conventional methods
- ❑ Cellular entry and toxicity are two determining factors in choosing particles for drug-delivery systems
- ❑ Nanoparticles can enter cells through different mechanisms
- ❑ Nanoparticles absorption can alter biophysical properties of cells
- ❑ Resolution and throughput are two important factor for bio-mechanical characterization of cells

