3RD YEAR (2019 – 2020) / 2ND TERM

NANOTECHNOLOGY/ LAB LECTURES

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NANOTECHNOLOGY

Definitions & terms

*** Nanoscience** is the study of phenomena and manipulation of materials at atomic, molecular and Micro-molecular scales, where properties differ significantly from those at a larger scale. **nanometer**, or **nm**, is 1 x 10-9 meters **or** one millionth of a millimeter

***Nanotechnology** is the understanding, manipulation, and control of matter at dimensions of roughly 1 to 100 nanometers, which is near-atomic scale, to produce new materials, devices, and structures which can be used in different areas : Biology, Physics, Chemistry, Material science, Electronics, Chemical Engineering, Information technology.

- **nanoparticles** typically refer to materials in which at least one dimension (e.g., **length, width, height, and/or diameter**) that are in the nanoscale

- may be dry particles, suspended in a gas (as a Nano-aerosol), suspended in a liquid (as a Nano-colloid or nanohydrosol), or embedded in a matrix (as a nanocomposite).

- can be composed of many different base materials (**carbon, silicon, and metals such as gold, silver cadmium, and selenium**).

WHAT'S SO SPECIAL ABOUT THE NANOSCALE?

- At the nanoscale, the **physical, chemical, mechanical, optical electrical and thermal & biological properties** of materials differ in **fundamental** & **valuable ways** from the properties of individual atoms and molecules or bulk matter

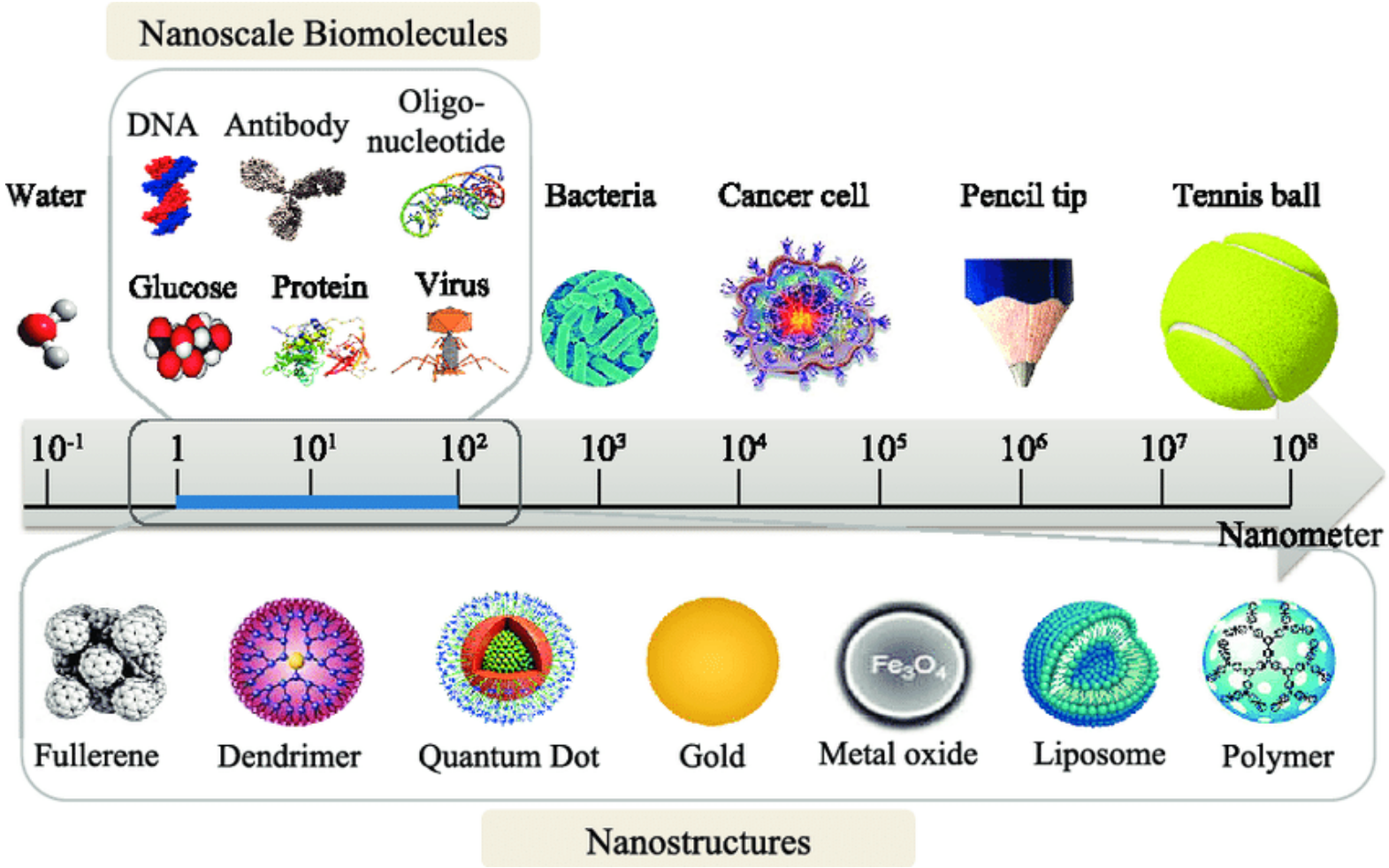
- Particles in the nanometer size may occur in **nature** (generated by natural events such as volcanic eruptions and forest fires) or as an **incidental byproduct** of existing industrial processes (generated during welding, metal smelting, automobile exhaust, and others).

The properties of materials can be different at the Nanoscale for two main reasons:

1- Quantum effects can begin to dominate the behavior of matter at the Nanoscale

2- Nanomaterials have a relatively larger surface area when compared to the same mass of material produced in a larger form.

Properties of materials are **size-dependent** in this scale range. Thus properties such as **melting point, fluorescence, electrical conductivity, magnetic permeability, and chemical reactivity** change as a function of the size of the particle.


Nanoscale Biomolecules
DNA
Antibody
Oligo-nucleotide
Water
Glucose
Protein
Virus
Bacteria
Cancer cell
Pencil tip
Tennis ball
10^{-1}
1
10^{1}
10^{2}
10^{3}
10^{4}
10^{5}
10^{6}
10^{7}
10^{8}
Nanometer
Fullerene
Dendrimer
Quantum Dot
Gold
Fe_3O_4
Metal oxide
Liposome
Polymer
Nanostructures

Safe Handling of Nanomaterials

Due to the extremely small dimensions, large surface area and high reactivity of engineered Nano-materials they have the potential ability to penetrate living cells quite readily. As a result, their unique Nano-features may also make them potentially hazardous for human health and environmental safety.

TOXICITY OF NANOMATERIALS

1-Biological toxicity after entering of Nanomaterials to the body by one of the exposure primary routes(dermal , inhalation , ingestion , injection ,ocular)

2-Enviromental toxicity Because the transfer of some Nanomaterials to the environment leads to a kind of pollution known as nanomaterials related environmental pollution.

Nanomaterial Properties that effect Toxicity & their interactions with the biological systems

1- Solubility:

2- Surface area

3- Surface charge

4- Surface chemistry or **reactivity**

5- Particle Size:

6- Particle Shape:

7- Others like **Mass, Density & Number of Nanoparticles**

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Lab: 2 Outline of Best Practices for Safe Handling of Nanomaterials in research

The main safety concern arises from the lack of knowledge about the potential effects and impacts of Nano-sized materials on human health and the environment. Limited information on toxicology, & lack of optimal test methods make the risk assessment & management of nanotechnologies more difficult.

Due to the extremely **small** dimensions, **large** surface area and **high** reactivity of engineered Nano-materials (as nanotubes, nanowires & nanosheets ...etc.) they have the potential ability to penetrate living cells quite readily. As a result, their **unique Nano-features** may also make them potentially **hazardous** for human health and environmental safety. Therefore, intense research activity is being undertaken in various Research & Development Institutions, universities and industries across the world **to evaluate their toxicity and critical exposure levels.**

Studies using engineered nanoparticles (**ENPs**) in isolated non-human cell experiments showed DNA damage. Short-term ENP exposure in animals has also produced dose-dependent inflammatory responses and pulmonary fibrosis. Hence there is an urgent need to establish the long-term safety of ENPs in humans. Other Studies mentioned that the exposure to nanomaterials maybe cause a **range** of **acute & chronic** effects.

Toxicity of Nanomaterials

1- Biological toxicity: may occur in a cellular or system level after entering of Nanomaterials **to** the body by one of **the exposure primary routes (Table 1)**. Also the absorption of nanomaterials may happen via first interaction with biological components (cells and proteins). Consequently can cause toxic effects including allergies, fibrosis, asthma, metal fume fever, deposition in organs (causing defects and insufficiency in organs), inflammation, cytotoxicity, tissue damage, producing reactive oxygen species (ROS), DNA damage & cancer.

2- Environmental toxicity: Because the transfer of some Nanomaterials to the environment **leads to** a kind of pollution known as nanomaterials related environmental pollution.

Therefore before release of large amounts of nanomaterials into the environment, their solubility and degradability in soil and water **should be** investigated and basic information on their safety, toxicity, and compatibility with soil & aquatics be obtained.

Nanomaterial Properties that effect Toxicity & their interactions with the biological systems

The Cellular uptake mechanisms & dispersion of nanomaterials in biological environments depend on their physicochemical properties which are the main cause of cytotoxicity and side effects of these materials in the body.

1- Solubility:

Poorly soluble inhaled nanoparticles can cause oxidative stress, leading to inflammation, fibrosis, or cancer. Studies have shown significantly higher toxicity of Nano-metals when compared to Nano-ceramics, which has been attributed to higher dissolution rate in water.

2- Surface area:

Increasing nanomaterials reactivity with other molecules, Associated with lung inflammation in rats and mice and cancer in rats,

3- Surface charge: is the most interesting physicochemical feature of nanoparticle relating to cytotoxicity (neutral < anionic < cationic).

4- Surface chemistry or **reactivity** of nanoparticles may have a role in the generation of free radicals, which influences the overall surface reactivity and toxicity of ingested particles.

Ex; Hydrophobicity and the existence of lipophilic groups, the presence of metallic species or toxic components in nanomaterials.

5- Particle Size:

Nanoparticles have a greater chance of depositing in the lungs than micro-sized particles, and therefore have the potential to cause damage by acting directly at the site of deposition by translocating to other organs or by being absorbed through the blood.

Nanoparticles can penetrate the membrane barriers resulting in significant damages. For example, silver nanoparticles with size < 9 nm can penetrate the nuclear membrane of certain human cells nucleus and cause significant DNA damage or mutation.

6- Particle Shape:

Studies have clearly established that exposure to fibrous particles like asbestos increases the risk of fibrosis and cancer. Similarly, the tubular structure of carbon nanotubes is believed to cause inflammation and lesions in lungs.

7- Others like **Mass, Density & Number of Nanoparticles.**

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Lab3 :

Exposure pathways and safety measures

Type of Exposure	Pathway	Safety measures
Dermal	**Nanoparticles** can migrate through skin & circulate in the body while handling nanoparticle suspensions or Dry powders. Skin absorption is much less likely for solid bound or matrixed nanomaterials.	Wear gloves and lab coat while handling the nanoparticles.
Ingestion	**Ingestion** can occur if good hygiene practices are not followed. Nanoparticles might be absorbed & Transported within the body by the circulatory system.	Eating and drinking are not allowed in laboratories. Spills of nanoparticles should be quickly and properly cleaned-up.
Inhalation	**Respiratory** absorption through the mucosal lining of the trachea, bronchioles or the alveoli of the lung.	Nanoparticles are to be handled in a form that is not easily airborne, such as in solution or on a substrate. Use of respiratory air filters N100 or N95 is recommended.
Injection	**Exposure** by accidental injection (skin puncture), when working with animals or needles.	Wear gloves and lab coats, and apply the standard practices for working with sharp objects.
Ocular	**Exposure** to airborne nanoparticles placed near the eye, accidentally splashed onto the eye or Transferred from hands during rubbing of eyes.	Wear safety glasses, goggles, full facepiece respirator (Recommended when there is exposure to solvent or hot material). **Note: Do not wear contact lenses at work place**

A basic safety and health approach to reduce risk in the workplace should be adhered to during the interim period. The approach, in order, is as follows:

1. Elimination 2. Substitution 3. Engineering controls 4. Administrative, or Work Practices

5. Personal Protective Equipment

Engineering Controls

Are likely to be similar to those that are used in controlling aerosols (gases, dusts, chemical vapors, etc.) Such controls may include: Local exhaust ventilation (**LEV**) & Filtration (**it should be tested and have a current certification**).

Any process or procedure that creates the potential for nanoparticles to become airborne or aerosolized should be performed in one of the following LEVs:

1. Chemical fume hood (CFH)
2. Class II, Biosafety Cabinet (BSC) Type A2, B2 & B1
3. Glovebox

Note: Do not use laminar-flow clean benches for the control of nanoparticles due to these devices direct the air flow towards the worker

Administrative, or Work Practices

Good work practices involving nanoparticles should include, awareness of the following practices:

•Develop a site-specific standard operating procedure (SOP) for work involving nanoparticles
•Minimize the potential for inhalation exposure and skin contact
•Practice good personal hygiene (e.g. hand washing, etc.)
•Utilize appropriate procedures when utilizing laboratory equipment
•Follow the manufacturer instructions for the use or handling of nanoparticles. Review **MSDS**
•Handle, store, and transport nanoparticles (liquid/powder state) in a closed, sealed & labeled container
•Limit material quantity to what is needed

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Lab4 :

Selection & Use of Personal protective equipment (PPE)

Material, including clothing (e.g. gown, gloves, respirators, safety glasses), used to prevent exposure to or contamination of a person by chemical or biological matter. There are limited referenced guidelines for appropriate PPE for protection from nanoparticles. Because PPE is typically tested at certain particle size ranges. Ex; some protective clothing is tested at the 1 µm (1,000 nm) size range for particle penetration. In respirators, the 3 µm (3,000 nm) size range is used in respirator filter testing. Therefor the size of the nanoparticle may be a factor in determining appropriate PPE.

***Gloves;** Personnel should wear nitrile gloves (two layers of gloves) when handling nanomaterials.

***Respirators;** where engineering controls are not available or feasible, the Personnel should use appropriate Respirators. Like N-95 respirators, may provide some protection. The particle size of the nanoparticle should be evaluated in determining the appropriate respirator (penetrating particle size of the respirator).

Dust masks (and surgical masks) should not be used for protection from nanoparticles.

SPILLS & CLEANUP

The plan for handling spills and cleanup associated with nanoparticles should be in accordance with the work practices noted below, where applicable:

•Wear gloves, two layers, during cleanup

•Avoid creating any potential for generation (e.g. sweeping) of aerosols during the cleanup

•Utilize **wet** wiping methods during the cleanup (if the material is a powder/dry)

•Place absorbents down (if the material is a liquid)

•Ensure cleanup materials are not re-used (e.g. any towels used during the cleanup are disposed)

•Place cleanup materials in a Labelled & sealed plastic bag (double bag).

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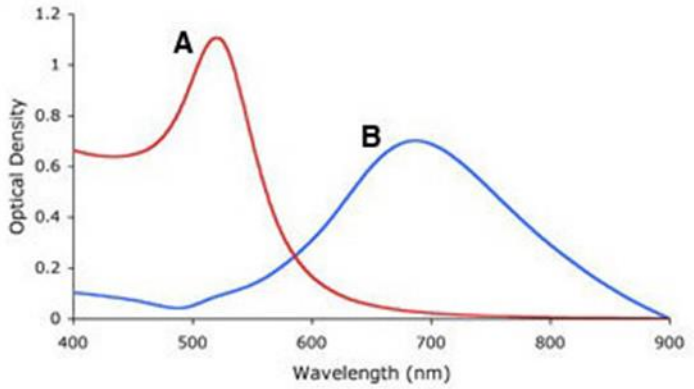
Nanotechnology

Lab 5: Equipment and tools for nanoparticles Characterization, Imaging, and Analysis

Different machines are used to study and characterize nanoparticles. In this lab, we will learn the basic concepts of these machines that used for this purpose as well as the advantages and disadvantages of each method.

1. Ultraviolet-Visible (UV-Vis) Spectroscopy:

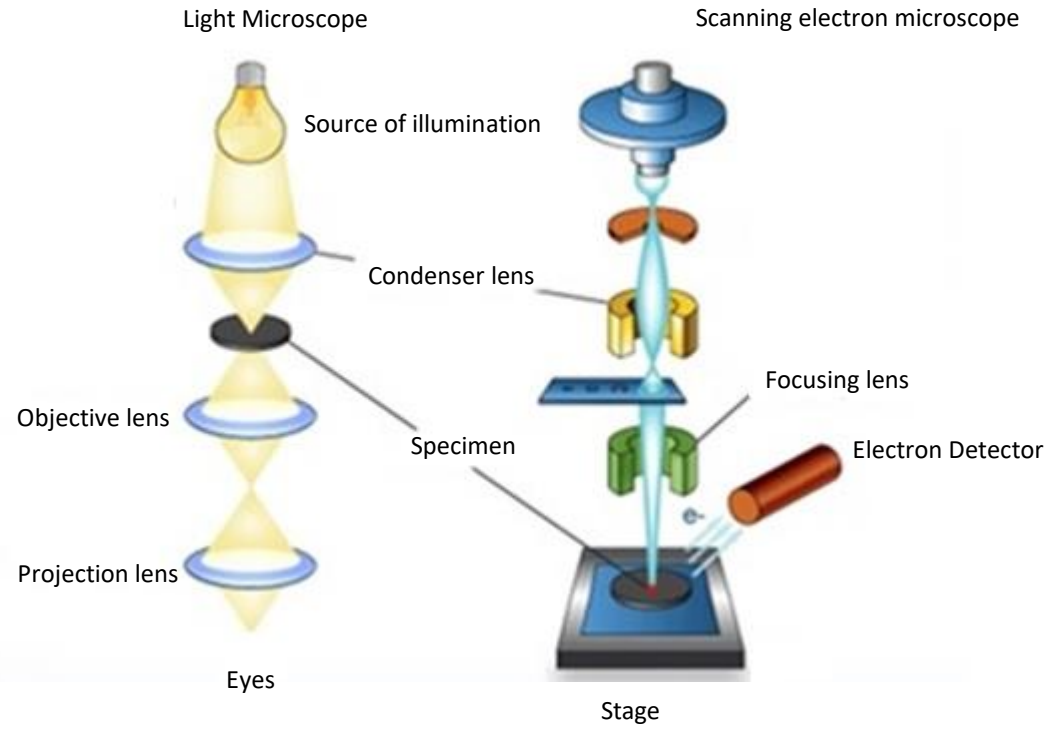
The optical density (OD), or absorbance of a given molecule depends on the size, shape and concentration. We can take advantage from this feature to recognize nanoparticles. For example, the peak optical density of gold (with 200 nm) is around 500 nm. While the peak optical density of gold nanoparticle (with 20 nm) is around 700 nm.



2. Scanning electron microscope (SEM):

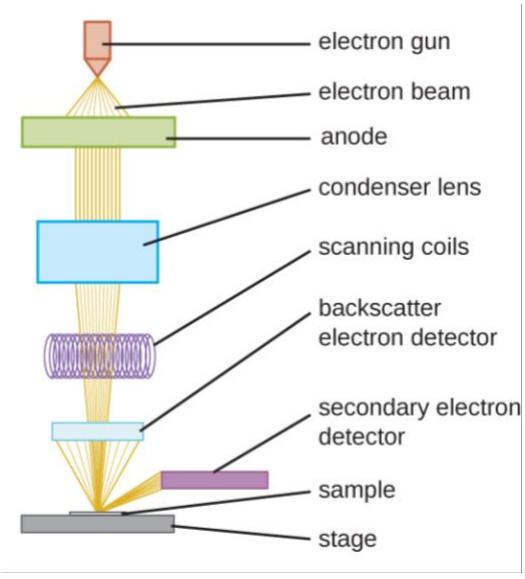
SEM involves shooting an electron beam at a specimen and observing the reactions on the specimen surface. When the electron hits a molecule on the surface, its energy is absorbed by

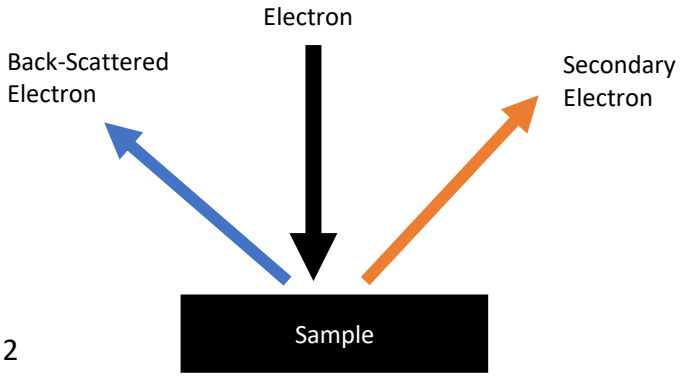
the molecule, which in turn emits a lower amount of energy. This energy can be in the form of a secondary, less energetic electron, a photon of light, or x-rays. Differentiation between these emissions is used to produce image.



Comparison between light and scanning electron microscope

Principle of SEM

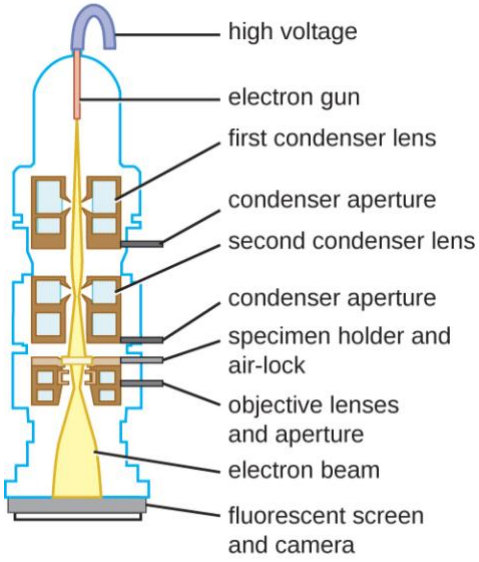


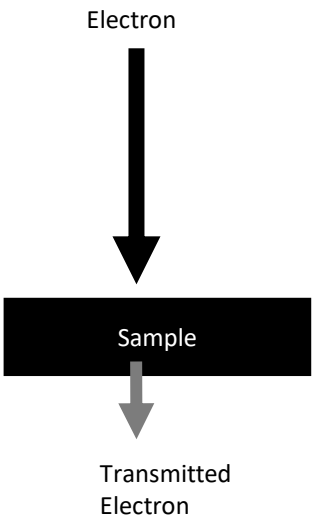


3. Transmission Electron Microscope (TEM)

TEM acts much like a typical brightfield microscope in the sense that it sends electrons through a specimen. The transmitted electrons are passed through an objective lens and then projected onto a material which can then be recorded photographically. This requires samples to be prepared in very thin slices in order to allow transmission of the electrons through transparent sections.

Principle of TEM





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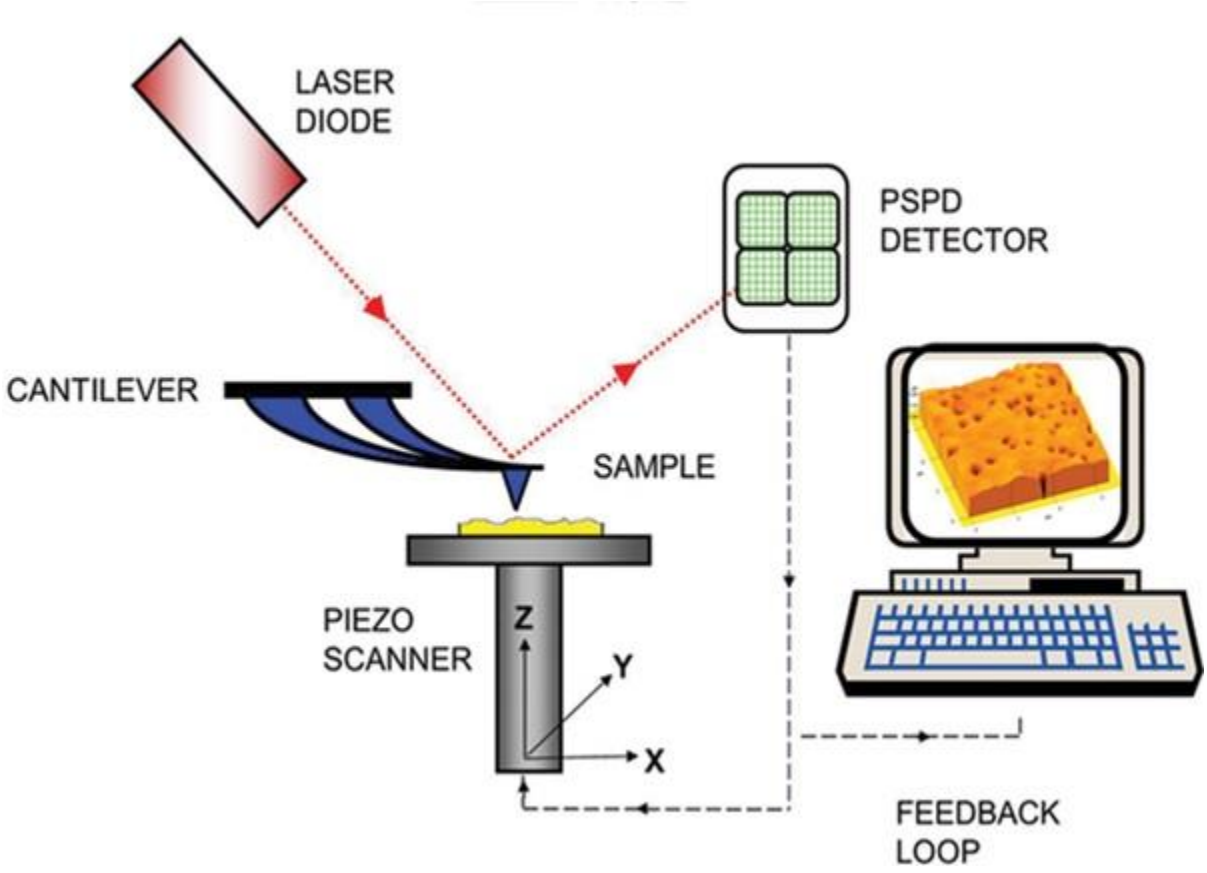
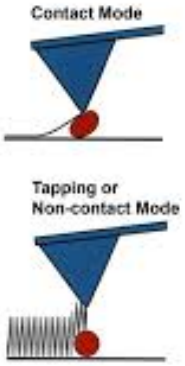
Lab 6:

1. **Atomic force microscopy (AFM):**

Atomic force microscopy (AFM) is a type of microscopy technique where the interactions between a sharp probe and a sample are used for imaging. The basic components of any AFM are electronic circuits, detection systems, and cantilever with probe. The free end of the probing cantilever is attached with an atomically sharp tip, (made of silicon) which is then brought into contact with the surface. The sharper your tip, the better is your resolution. The extent of interactions between the surface and the tip is measured in terms of cantilever displacements. The same is monitored using a laser attached to the back of the cantilever, whose beam is detected on a segmented photodetector.



AFM is primarily operated in 2 ways:



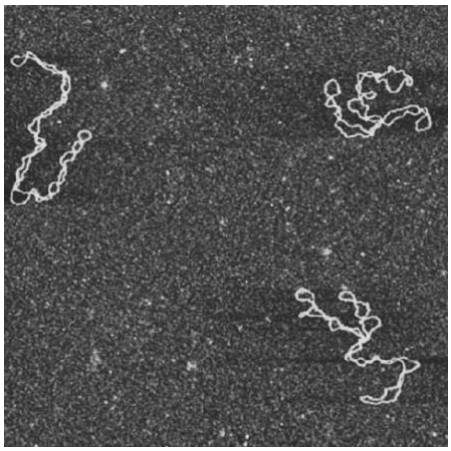
Contact mode: The probing tip comes in contact with the surface.

Non-contact mode: The tip does not contact the surface.

Limitations

The width of several molecules seen using the AFM may not be of the actual width. This is primarily due to 2 reasons.

The molecule undergoes relaxation on the substrate on which it is held.
Tip induced deformation in the sample.



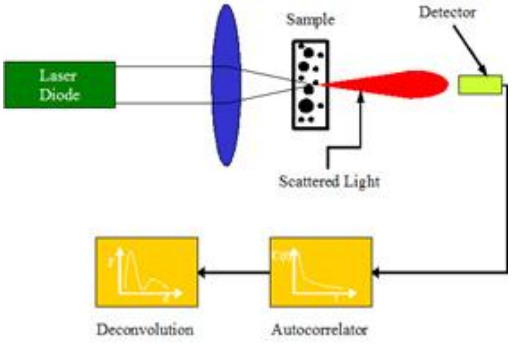
AFM images of supercoiled 5.6 Kb plasmid DNA.

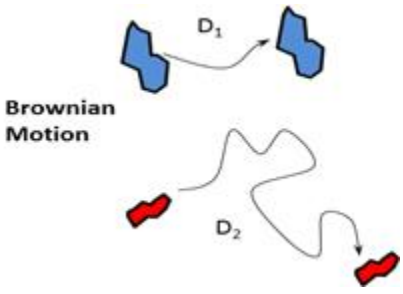
Uses

The dimensions of a DNA can be found out using AFM. Since the DNA is a 3D object, only its width isn't sufficient a parameter to describe it.

2. **Dynamic Light Scattering (DLS)**

DLS is also known as Photon Correlation Spectroscopy (PCS). DLS is used to size particles from below 5 nm to several microns. This technique operates on the principle that particles move randomly in gas or liquid i.e. undergo Brownian motion.





- **Synthesis nanoparticles**
  A number of approaches are available for the synthesis of nanoparticles for example, reduction in solution, photochemical and chemical reaction, physical methods (irradiation assisted), electrochemical, microwave assisted process and recently via green method (biological methods).

- **The biological synthesis** are most acceptable than other methods for nanoparticles synthesis and provides advancement over physical and chemical method as it is cost effective environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, temperature, energy and toxic chemical. The use of environmentally benign material like plant extract (leave, flower, bark, seed, peels etc.), fungi and bacteria (enzyme and DNA) for the synthesis of nanoparticles.

Some metal nanoparticles synthesized by biological methods (plant and microorganisms)

No.	Material	Natural Resource	Part used	Size	Shape
1	AgNps	*Pseudomonas aeruginosa*	glycolipid	50nm	Spherical
2	AuNps	*Fusarium oxysporum*	*F.oxysporum* Biomass	40nm	Small rod shaped
3	AgNps	Onion (*Allium cepa*)	Onion (bulb)	33nm	Spherical
5	ZnoNPs	*Aloe vera*	Pulps	25nm	Spherical

- **Physical methods**:
  Most important physical approaches include evaporation-condensation and laser ablation and much other method show in **Table**. Various metal nanoparticles like silver and gold have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of silver nanoparticles distribution are the **advantages** of physical methods in comparison with chemical processes.

Approach	Method	Advantage
Physical	Tube furnace	Occurs at atmospheric pressure
	Ceramic heater	Use local heat source, prepared in high concentrations, can be used for calibration for nanoparticle measuring device
	Laser ablation	Absence of chemical reagents in solutions Gives pure and uncontaminated nanoparticle
	Arc discharge	Silver wires are used up as electrodes by providing DC voltage between them

- **Chemical methods**

The most common approach using to synthesis of nanoparticles is chemical reduction by other organic or inorganic reducing agents. In general, different reducing agents such as sodium citrate, ascorbate, sodium borohydride. This method have **advantage** as high-speed in formation nanoparticles, put highly toxic method.

- **Synthesis Silver nanoparticles by biological method (bacteria product)**

  Media and Fermentation Conditions for biosurfactant production
  Fermentation media Preparation based on Niladevi-Prema Design Medium prepared by mixing components such as:

  1. NaCl: 0.1 g/l, FeSO4: 0.01g/l, ZnSO4: 0.009 g/l, MgSO4: 0.002 g/l, CaCO3: 0.02 g/l, CuSO4: 0.001 g/l, KH2PO4: 0.5 g/l and K2HPO4 1g/l
  2. KNO3 as nitrogen source 1%.
  3. Olive Oil as carbon sources 1%.
  4. The PH was adjusted to 7.0 and then sterilized by autoclaving at 121℃ for 15min.
  5. After sterilization, the medium left to cool and inoculated 1% of the selected bacteria isolate (1×10^8 CFU, OD=0.5).
  6. Incubated medium in a shaker incubator at 30℃ at 120 rpm for 96h.

- **Extraction of biosurfactant**

Acid precipitation method was used to extraction of biosurfactant (BS). After end of incubation time the culture were centrifugation at 10000 rpm for 15 min, the pellets were discard and the supernatant was used for biosurfactant extraction. 2N HCl solution was used to acidify the supernatant containing BS, until pH 2. The mixture was then incubated for 24 hrs at 4℃. The precipitate formed was collected by separation funnel by adding chloroform and methanol (2:1) for partially purified, precipitate was then dried, in oven at 60℃ and after 24 hrs of drying process, a brown color precipitate was obtained.

- **Synthesis of silver nanoparticles**

Silver nitrate ($AgNO_3$, 99%) (Aldrich/Germany) was used in the preparation of the silver nanoparticles. Silver nanoparticles were synthesized according to a method described by Martinez-Gutierrez with modification. Method of synthesis are done by two solutions:

Solution (A) is prepared as follows: 0.02 gm (0.1 mmol) of $AgNO_3$ were dispersed by ultrasonication in 20 ml deionized water (DI) for 2 minutes. The interaction and production of nanoparticles to be done, need for reducing agent and stabilizer to

prevent aggregation. In addition, solution (B) which consists of (rhaminolipid) that extracted from local isolate, acts as capping stabilizer and reducing agent.

Solution (B) is prepared by dissolving 0.002 gm (w/v) in 20 ml DI water and dispersed by ultrasonication for 2 minutes.

The two solutions (A and B) are mixed by magnetic stirrer and exposed to the direct sunlight for about 5 min at pH 5. The solution contains silver nanoparticles, was separated and concentrated by centrifugation at 10,000 rpm for 15 min.

- **Synthesis Zno nanoparticles by plant (green tea)**

Zinc acetate dihydrate (Zn $(CH3COO)_2$ .2H2O) with 0.02 M was dissolved in 100 mL of De-ionized water and stirred until dissolved completely. 20 gm of green tea leaf, in dried form, was added to 100 mL of De-ionized water and boiled for 10 minutes. After cooling to room temperature, green tea was filtered through fennel with gauze and Whatman No. 1 paper. Green tea extract (1 ml) was mixed homogeneously with the prepared zinc acetate solution (100 ml) with adjusting of pH at 12 by NaOH and stirring for 2 hours.

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Nanotechnology

Lab 8: Nanoparticles Applications

Nanoparticles (NPs) have several applications in different fields such as medicine, industry, agricultural, and environment. In this lab, we will focus primarily on the nanoparticle's applications in health and medicine.

Difficulties of drug delivery by classical ways:

- Delivering the appropriate dose of a particular active agent.
- Site-specific targeting of active agents.
- Reduce toxic systemic side effects.
- Drug bioavailability: specific places in the body and over period of time.
- Poor solubility drugs.
- Drug degradation.

Nanoparticles give many advantages when they used for drug delivery such as:

- NPs can alter the pharmacokinetics and bio-distribution of the drug.
- NPs carry drugs to specific sites.
- NPs are designed to avoid the body's defense mechanisms, thus help to prevent drug degradation.

- Drugs that are placed in the body can activate only on receiving a particular signal.
- A drug with poor solubility will be replaced by a drug delivery system.

Nano technology based drug delivery bases upon three facts:

1) Efficient encapsulation of the drugs.
2) Successful delivery of drugs to the targeted region of the body.
3) Successful release of that drug there.

The clinical application of nanotechnology in bacterial infection

- Polyethylene glycol (PEG) nanoparticles carrying antibiotics were used to target bacterial infection more precisely inside the body.

➢ Negative charge NP is not binding to nontarget cells or blood components at physiologic pH 7.4.

➢ Inflammation at a site of infection is acidic.

➢ The charge of NPs become positive (due to changing in pH) and binding to negatively charged bacteria.

The applications of nanotechnology in visualization and imaging

- Tracking molecules; luminescent tags were used to dye various numbers of molecules or cells. These tags are quantum dots attached to proteins which penetrate cell membranes.
- NPs, such as quantum dots can be used in conjunction with magnetic resonance imaging, to produce exceptional images of tumor sites. As compared to organic dyes, NPs are much brighter and need one light source for excitation. But quantum dots are usually made of quite toxic elements.

Applications of nanoparticles in immunity

- The buckyballs have been used to alter the allergy/ immune response. They prevent mast cells from releasing histamine into the blood and tissues.
- NPs can bind to free radicals better than any anti-oxidant available.
- Nanoparticles may also be used in inhalable vaccines in the future as the surface change of protein-filled nanoparticles impacts the ability of the nanoparticle to stimulate immune responses.

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Nanotechnology

Lab 9:

The Applications of nanotechnology in neurodegenerative disorders

It is difficult to deliver a drug by traditional ways to the infected regions in the brain because of the Blood Brain Barrier (BBB). NPs may represent promising solution in curing some important neurodegenerative disorders such as Alzheimer's disease and Parkinson’s disease.

The clinical applications of nanotechnology in operative dentistry

- Nano filled composite resin materials are believed to offer excellent wear resistance, strength, and ultimate aesthetics.
- In operative dentistry, nano fillers constitute spherical silicon dioxide (SiO2) particles with an average size of 5-40 nm.

The clinical applications of nanotechnology tissue engineering

- Nanotechnology can be applied to reproduce or repair damaged tissues. By using suitable nanomaterial-based scaffolds and growth factors, artificially stimulated cell proliferation, in organ transplants or artificial implants.

Application of nanotechnology in modified medicated textiles

- Using nanotechnology newer antibacterial cotton has been developed and used for antibacterial textiles.

- This technique has been advanced by a focus on inorganic nano structured materials that acquire good antibacterial activity and application of these materials to the textiles.

The clinical application of nanotechnology in stroke and heart attack

- Drug coated NP was used to dissolve blood clots by selectively binding to the narrowed regions in the blood vessels.
- Biodegradable NP aggregates were coated with tissue plasminogen activator (tPA) were injected intravenously which bind and degrade the blood clots.
- plasminogen activator catalyzes the activation of plasmin, which is important factor for breaking down of fibrin polymers formed during blood clotting.

The clinical application of nanotechnology in cancer

- NPs are used in cancer photodynamic therapy.
- NPs are inserted within the tumor in the body specifically by conjugating antibodies or peptides to the nano shell surface.
- NPs are illuminated with photo light from the outside.
- The particle absorbs light and if it is of metal, it will get heated due to energy from the light. High energy oxygen molecules are produced due to light which chemically react with and destroy tumors cell, without reacting with other body cells.

Applied Nanoparticles

The antibacterial test for nanoparticles (AgNPs)

- The antibacterial activity of AgNPs were investigated using gram-negative bacteria (*Escherichia coli*) and gram-positive bacteria (*Staphylococcus aureus*).
- The antimicrobial activity of AgNPs for each microorganism was determined by applying agar well diffusion technique.
- Using Rhaminolipid and synthetic AgNPs from (Hongwu, China) as negative control by concentration is same the concentration of green AgNPs that used in all experiment.
- Approximately, 25 mL of sterilized and cooled Müller Hinton agar medium was poured into sterilized Petri dishes and allowed to solidify at room temperature. The overnight growth test organisms were transferred and spread over the agar medium using a sterile cotton swab for each test microorganism and then wells were made. Concentration of green AgNPs, and negative control (Rhaminolipid and synthetic AgNPs) were added is same the concentration of green AgNPs to the wells.
- The AgNPs inoculated plates were incubated at 37 °C for 24 h.
- After incubation, the zone of inhibition around the well was measured.