

تقنيات احيائية نانوية (عملي) Nanobiotechnology

التدريسين

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Lab: 1 Outline of Nanotechnology Elements, 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.

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.

Nanotechnology is Interdisciplinary area: Biology, Physics, Chemistry, Material science, Electronics, Chemical Engineering, Information technology.

A nanometer, or **nm**, is 1×10^{-9} meters or one millionth of a millimeter. Putting this size into perspective, a single human hair is about 80,000 nanometers in width, the length of Housefly ~ 1 **cm**, the size of Sand particle ~ 1 **mm**, the diameter of RBCs ~ 6-8 **µm**, the size of Proteins ~ 1-20 **nm** & the size of Hydrogen atom 0.04 **nm**.

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

Nanomaterials are generally in the 1-100 nm range and can be composed of many different base materials (**carbon, silicon, and metals such as gold, cadmium, and selenium**). Nanomaterials also have different shapes: referred to by terms such as **nanotubes, nanocapsules, nanofibers nanosprings, nanoplates, nanoparticle, nanobelts, nanofluids, nanowires, crystalline structures such as quantum dots, Dendrimers and fullerenes** (carbon 60).

Nanomaterials often exhibit very different properties from their respective bulk materials, So many benefits of nanotechnology **depend on the fact that it is possible to tailor** the structures of materials at extremely small scales to achieve specific properties, thus greatly extending the materials science toolkit. Using nanotechnology, materials can effectively be made **stronger, lighter, more durable, more reactive, more sieve-like, or better electrical conductors**, among many other traits.

Nanotechnology is helping to considerably improve, even revolutionize, many technology and industry sectors: **information technology, homeland security, medicine, transportation, energy, food safety, and environmental science**, among many others.

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

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.

So these phenomena are based on "quantum effects" and other simple physical effects such as expanded surface area. Also working at the nanoscale enables scientists to utilize the unique physical, chemical, mechanical, and optical properties of materials that naturally occur at that scale.

For example:

- Copper (opaque substance) becomes transparent
- Aluminum (stable material) becomes combustible
- Platinum (inert material) becomes a catalyst
- Silicon (insulator) becomes a conductor
- Gold (solid) turns into liquid at room temperature
- Gold absorbs certain wavelengths of light while reflecting others based on how photons interact with the surface of the material. In bulk, gold, like other metals, absorbs most light in the visible spectrum while reflecting infrared light giving it a shiny appearance.

As the size of the particles decreases, the surface area of gold is increased (Figure 1). This changes the spectrum of light that is absorbed and reflected.

Therefore a smaller gold nanoparticles exhibit a **red color** as they reflect red wavelengths and absorb shorter wavelengths of light. As gold nanoparticles increase in size, their color changes to a **blue** & then **purple**. The optical properties (and thus color) of certain nanostructures depend strongly on the nanostructures' sizes and shapes.

The size-dependent optical properties of gold nanostructures can be exploited for a variety of applications, including as sensors.

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

Ex, Nano particles can make materials more chemically reactive and affect their strength or electrical properties.

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

Ex, titanium dioxide and zinc oxide become transparent at the nanoscale and have found application in sunscreens.

When particles are created with dimensions of about 1–100 nanometers, the materials' properties change **significantly** from those at larger scales. 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.

Even the structure of materials changes with respect to Size, (e.g.; nanoparticles can also be arranged into layers on surfaces, providing a large surface area and hence enhanced activity, relevant to a range of potential applications such as catalysts).

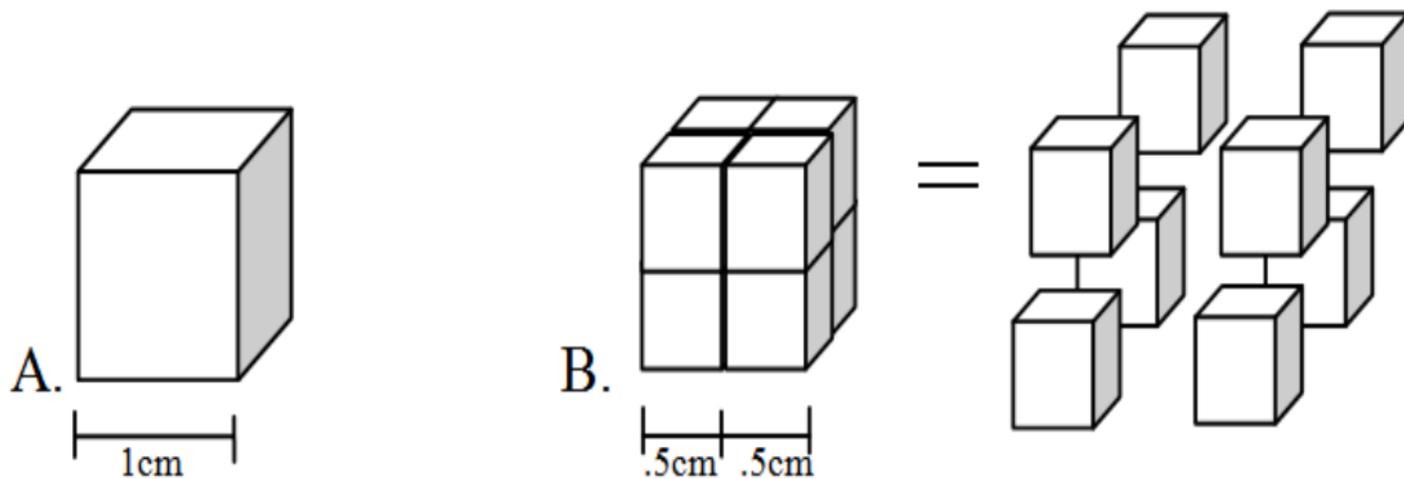
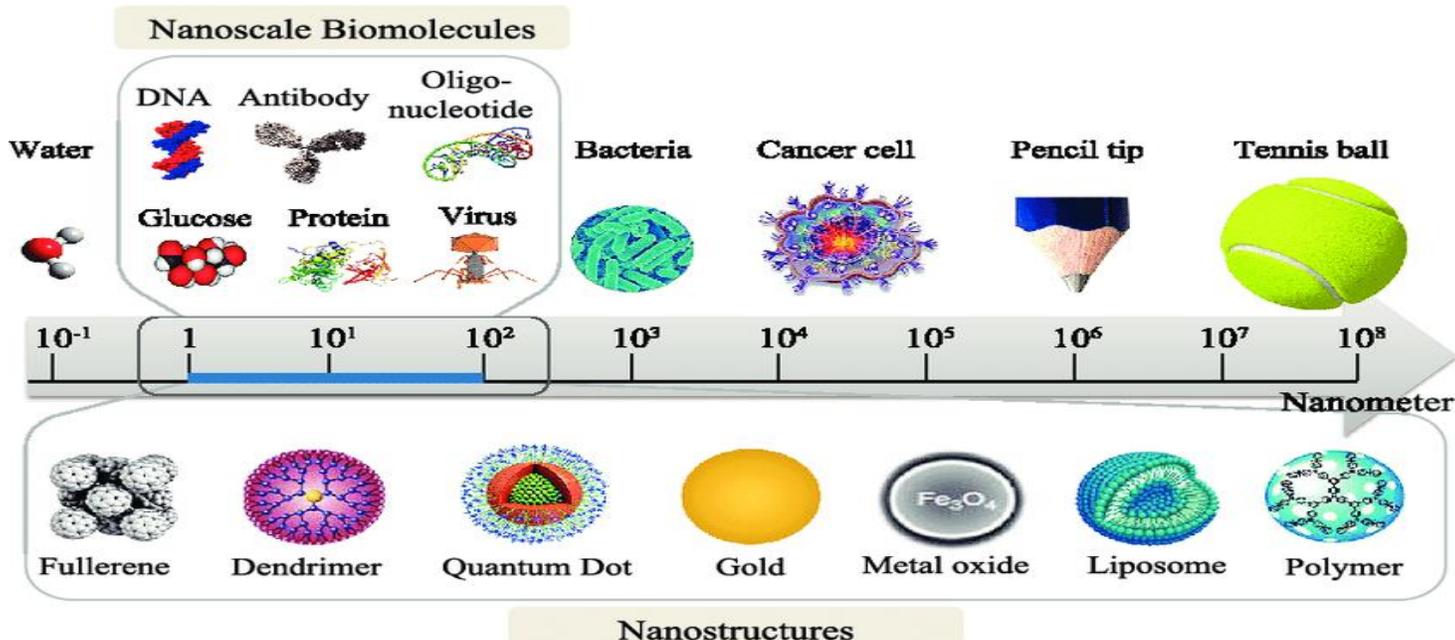


Figure 1. Sample A and Sample B contain the same amount of gold. The surface area of Sample A is 6 cm². Although the volume is the same, the total surface area of the 8 particles that make up Sample B is 12 cm².

PREFIX	SYMBOL	MULTIPLIER	EXPONENT FORM
exa	E	1, 000, 000, 000, 000, 000, 000	10 ¹⁸
peta	P	1, 000, 000, 000, 000, 000	10 ¹⁵
tera	T	1, 000, 000, 000, 000	10 ¹²
giga	G	1, 000, 000, 000	10 ⁹
mega	M	1, 000, 000	10 ⁶
kilo	k	1, 000	10 ³
hecto	h	100	10 ²
deca	da	10	10 ¹
Basic Unit	Basic Unit	1	10 ⁰
deci	d	0.1	10 ⁻¹
centi	c	0.01	10 ⁻²
milli	m	0.001	10 ⁻³
micro	μ	0.000,001	10 ⁻⁶
nano	n	0.000,000,001	10 ⁻⁹
pico	p	0.000,000,000,001	10 ⁻¹²
femto	f	0.000,000,000,000,001	10 ⁻¹⁵
atto	a	0.000,000,000,000,000,001	10 ⁻¹⁸



Nano-scale Effects on Properties

Properties	Examples
Catalytic	Better catalytic efficiency through higher surface-to-volume ratio
Electrical	Increased electrical conductivity in ceramics and magnetic nanocomposites, increased electric resistance in metals
Magnetic	Increased magnetic coercivity up to a critical grain size, superparamagnetic behaviour
Mechanical	Improved hardness and toughness of metals and alloys, ductility and superplasticity of ceramic
Optical	Spectral shift of optical absorption and fluorescence properties, increased quantum efficiency of semiconductor crystals
Sterical	Increased selectivity, hollow spheres for specific drug transportation and controlled release
Biological	Increased permeability through biological barriers (membranes, blood-brain barrier, etc.), improved biocompatibility

Carbon nanotubes (CNT) can have either single or multiple layers of carbon atoms arranged in a cylinder. The dimensions of typical single wall carbon nanotubes (SWCNT) are about 1-2 nm in diameter by 0.1 μm in length. Multiple wall carbon nanotubes (MWCNT) are 20 nm in diameter and 1 μm long. They have properties very different from bulk carbon or graphite. They have great tensile strength and are potentially the strongest, smallest fibers known. **CNT is 100,000 times thinner than a strand of hair & 20 times tougher than steel**

Quantum dots (QD) are nanocrystals containing 1000 to 100,000 atoms and exhibiting unusual "quantum effects" such as prolonged fluorescence. They are being investigated for use in immunostaining as alternatives to fluorescent dyes. The most commonly used material for the core crystal is **cadmium-selenium**, which exhibits bright fluorescence and high photo-stability.

Fullerenes are another category of carbon based nanoparticles. The most common type has a molecular structure of C_{60} which take the shape of a ball shaped cage of carbon particles arranged in pentagons and hexagons.

C_{60} molecules & buckyball. Molecules made up of 60 carbon atoms arranged in a series of interlocking hexagons and pentagons, forming a structure that looks similar to a soccer ball

Dendrimers are man-made molecules about the size of an average protein, and have a branching shape made of layers of polymers surrounding a central core.)

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.

Table 1: 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

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. Therefore 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:

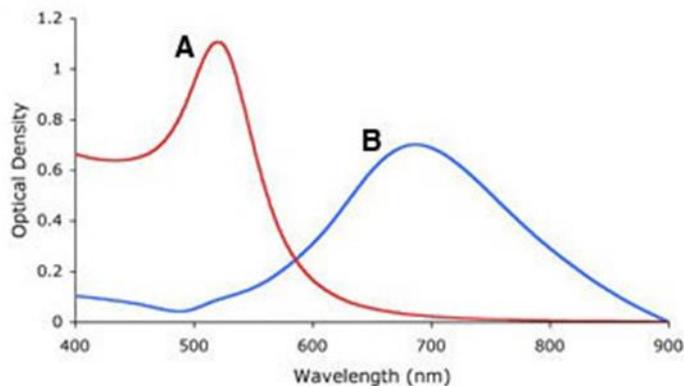
- Wear gloves, two layers, during cleanup
- Avoid creating any potential for generation (e.g. sweeping) of aerosols during the cleanup
- Utilize **wet** wiping methods during the cleanup (if the material is a powder/dry)
- Place absorbents down (if the material is a liquid)
- Ensure cleanup materials 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).

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:

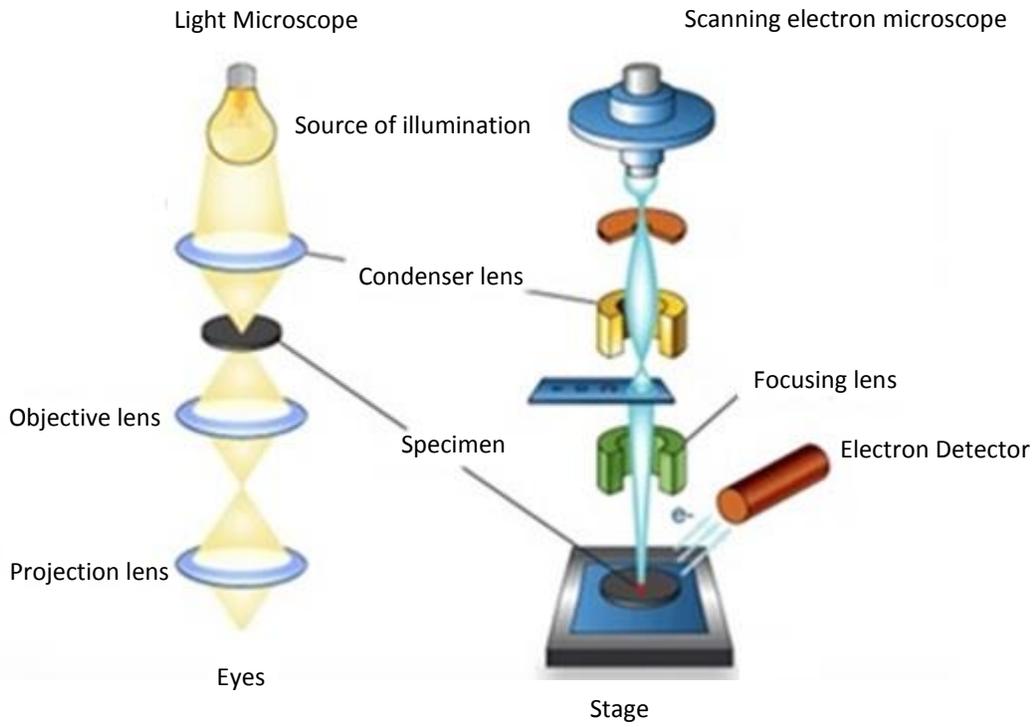
The optical density (OD), or absorbance of a given molecule depends on the size, shape and concentration. We can take advantage from this feature to recognize nanoparticles. For example, the peak optical density of gold (with 200 nm) is around 500 nm. While the peak optical density of gold nanoparticle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



A: Gold bulk (200 nm), B: Gold nanoparticle (20 nm)

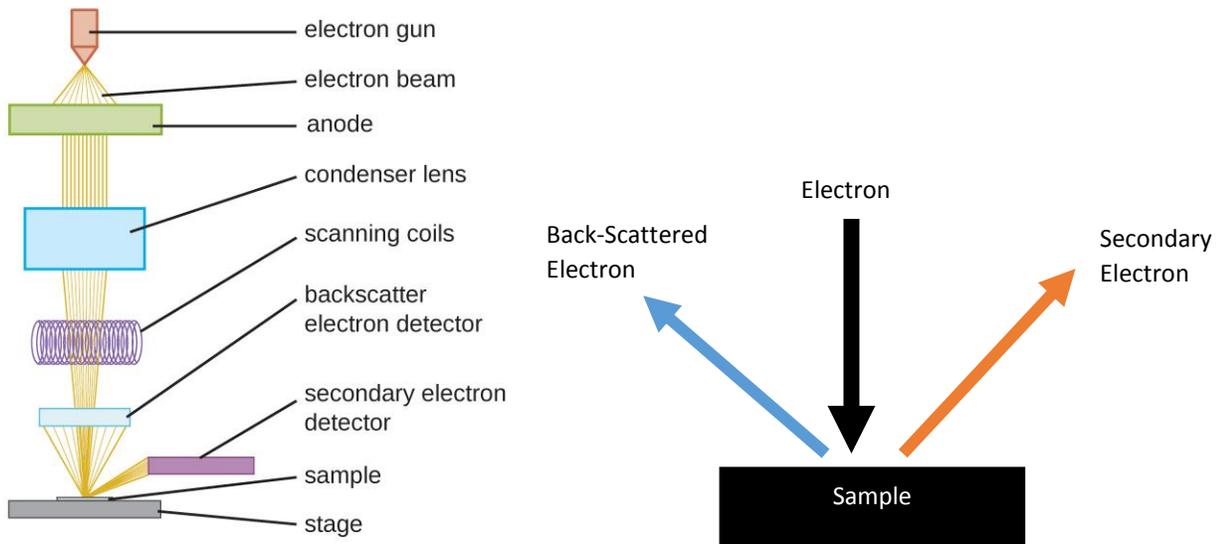
2. Scanning electron microscope (SEM):

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

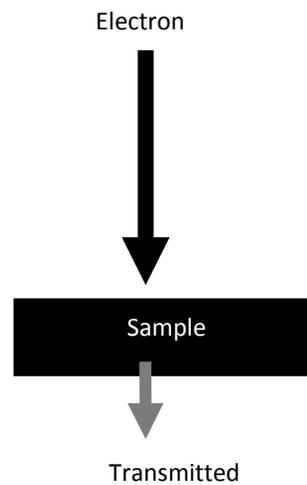
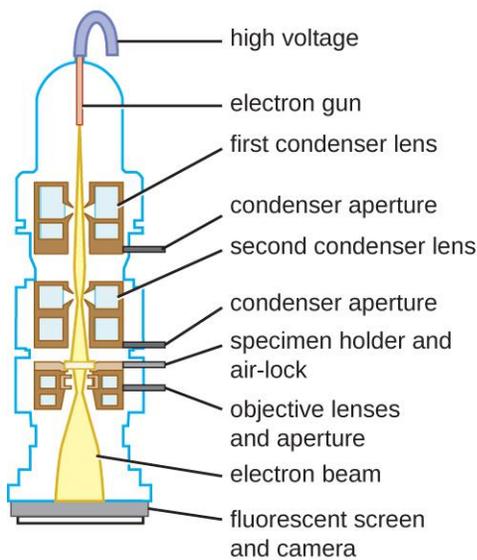
Principle of SEM



3. Transmission Electron Microscope (TEM)

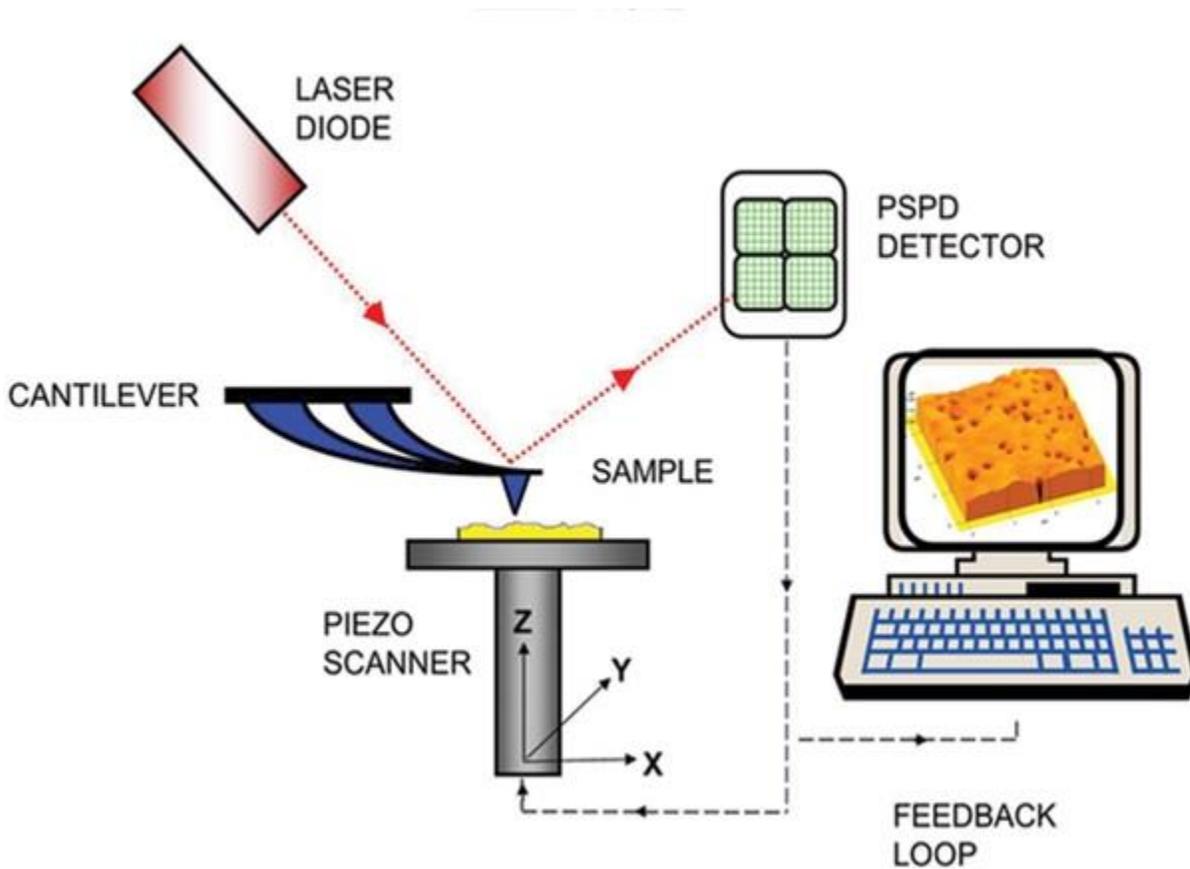
TEM acts much like a typical brightfield microscope in the sense that it sends electrons through a specimen. The transmitted electrons are passed through an objective lens and then projected onto a material which can then be recorded photographically. This requires samples to be prepared in very thin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

Principle of TEM



4. 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. 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.

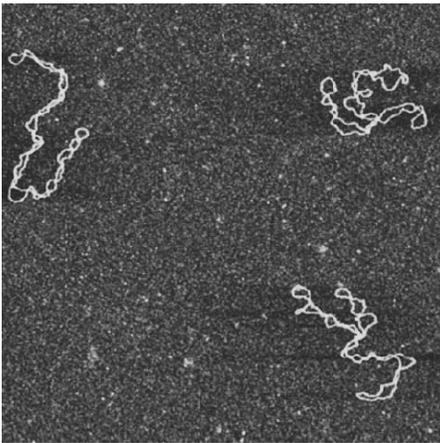


Limitations

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.



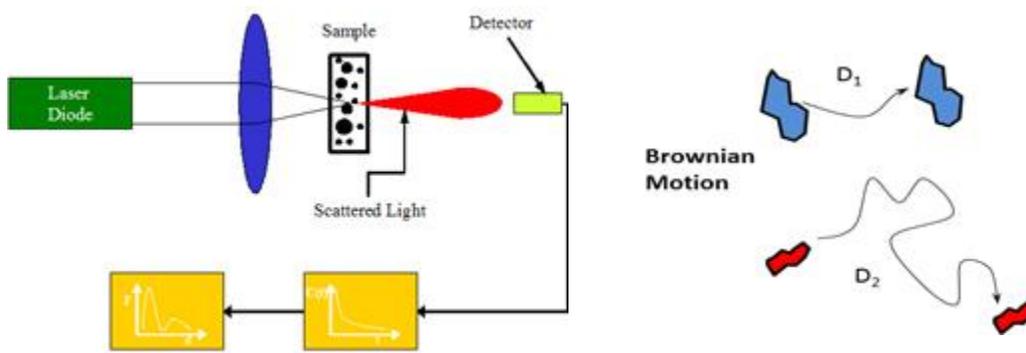
Uses

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.

AFM images of supercoiled 5.6 Kb plasmid DNA.

5. 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.



Nanotechnology

Lab 6: 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.

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.

