[redacted] (NANOBIOTECHNOLOGY)

UNIT - I

Basic biology, principles and practice of microfabrication techniques:-

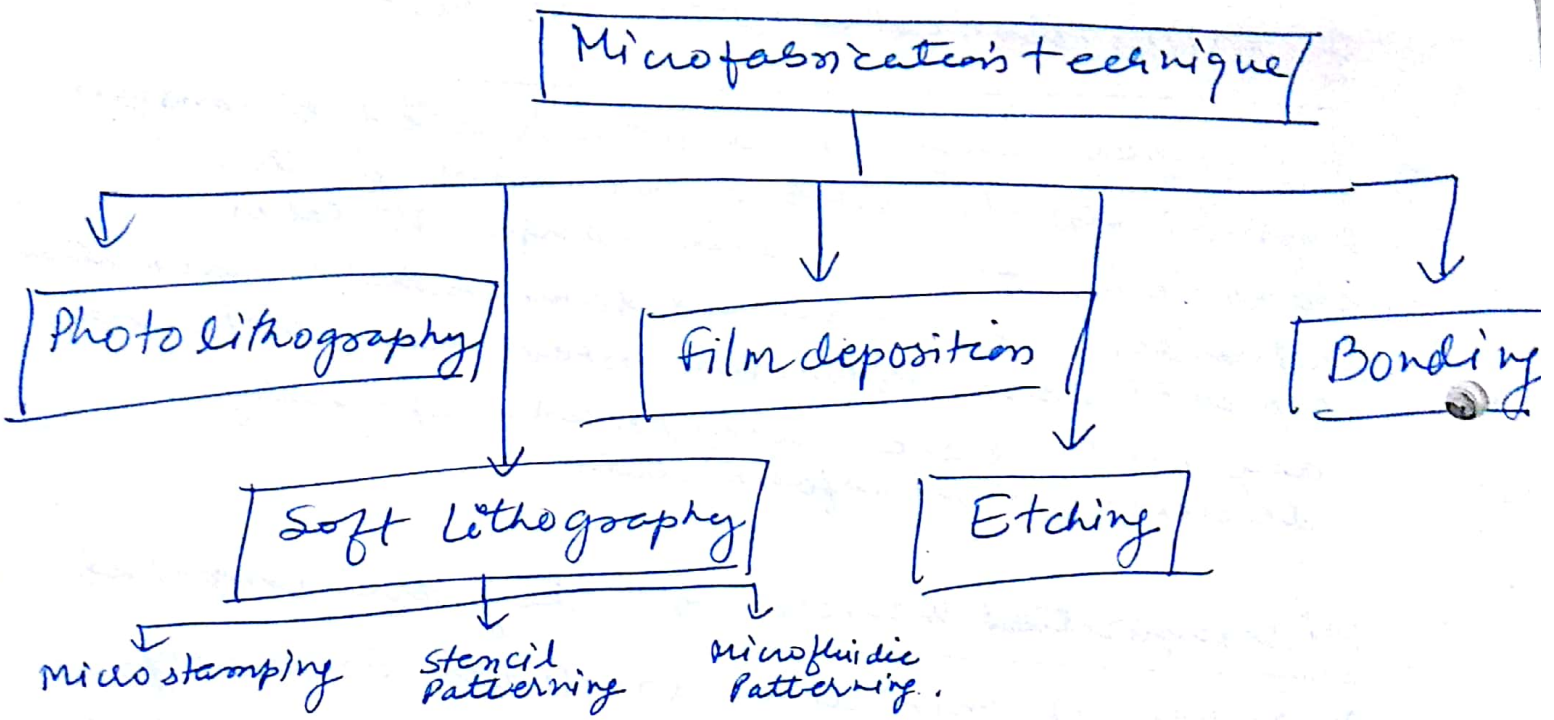
⇒ Microfabrication is a process used to construct physical objects with dimensions in the micrometer to millimeter range. It takes advantage of established semiconductor fabrication processes, used to make integrated circuits, and augments these with processes specially developed for microfabrication.

Microfabricated Devices ⇒ They are comprised of range of miniature structures, including moving parts such as cantilevers and diaphragms, static structures such as flow channels and wells, chemically sensitive surfaces such as proteins and cells, and electrical devices such as resistors and transistors.

Types of Microfabrication Techniques

A number of techniques are used for the fabrication of micron-scale devices. Some of these techniques have been adopted from the well established field of semiconductor but others have been specifically developed for microfabrication.

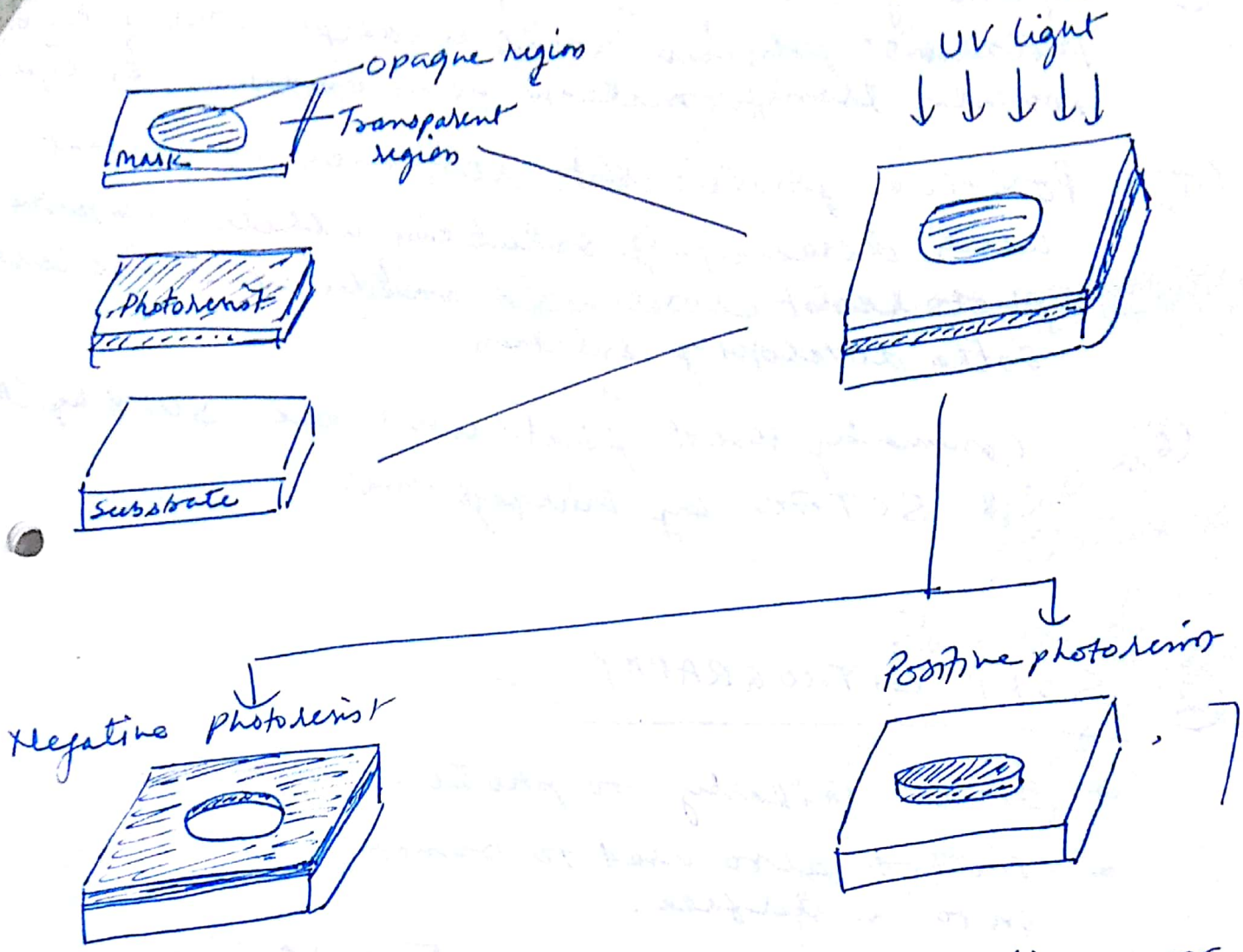
The microfabrication process utilizes these techniques in a sequencial manner to produce the desired structure.



1. PHOTOLITHOGRAPHY :-

- One of the most readily employed microfabrication techniques.
- It is used to create patterns in to a material.
- photolithographic process consists of a numbers of steps in which a desired pattern is generated on the surface of a substrate through exposure of regions of a light sensitive material to U.V. light.

STEPS of PHOTOLITHOGRAPHY



(1) A substrate material, such as silicone or glass is coated with a layer of photoresist, or light sensitive polymer.

(2). A photomask, made by patterning with an opaque material the desired shape on a glass dish or other transparent material, is placed on top of the substrate and photoresist.

(3). This assembly is then irradiated with U.V. light

(4) Depending on the type of photo resist utilized photoresist polymer will undergo one of two possible transformations. upon exposure to light.

(5) Positive photo resist removed is contact with developing solution. while negative photo resist crosslinked and become insoluble in the developing solution.

(6) Commonly used photo resist are SU-8 by IBM & SOTEC by Microsystems.

(2) SOFT LITHOGRAPHY :-

⇒ It is similarly to photolithography.

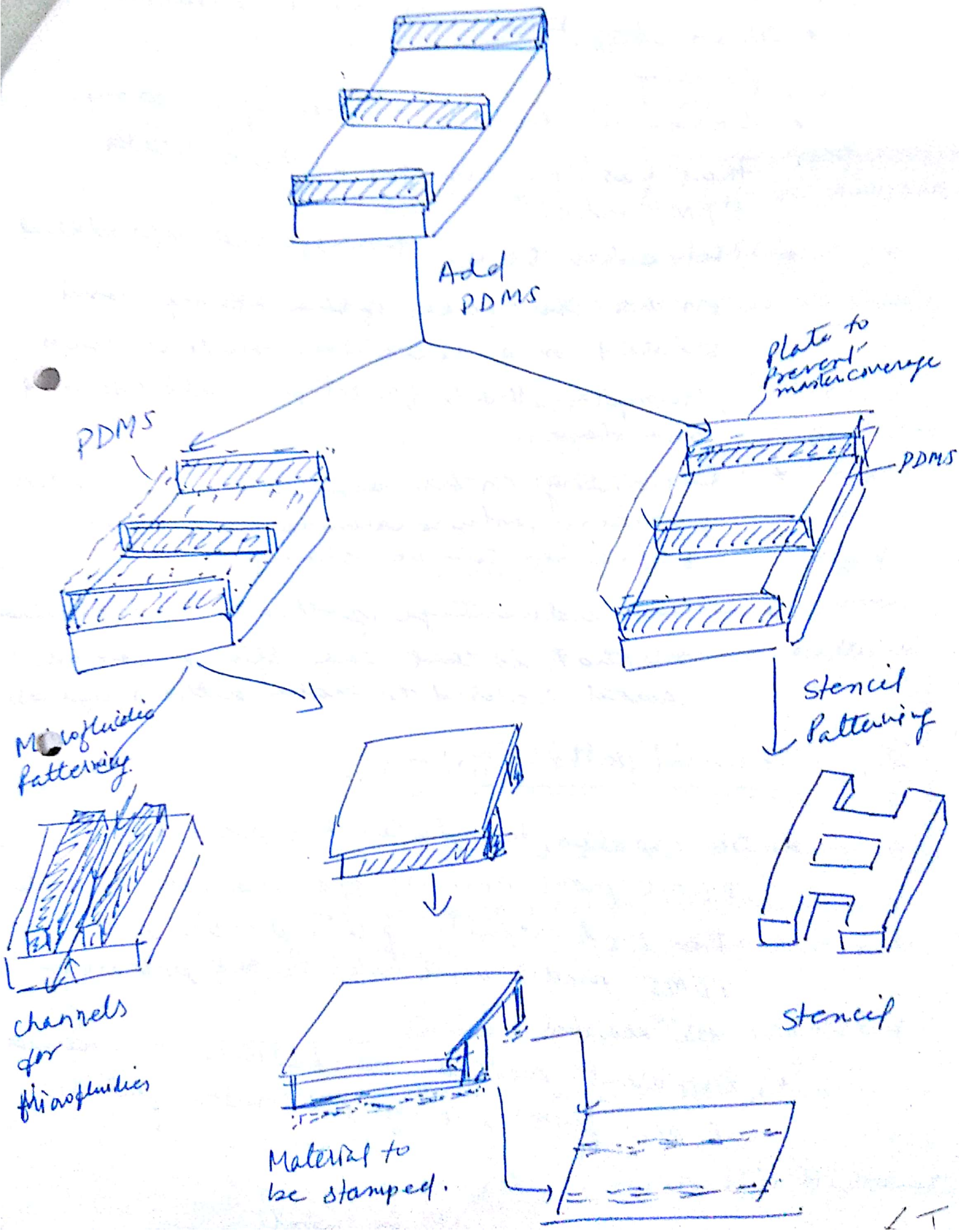
⇒ Method also used to transfer a pattern on to a surface.

⇒ It utilizes a microstructure replica produced by molding a polymer, such as poly(dimethyl siloxene)(PDMS) to a master.

⇒ There are three main soft-lithography processes:

(1) Microstamping (2) stencil Patterning (3) Microfluidic Patterning.

Soft Lithography techniques



① Microstamping :-

* It is also known as micro contact printing
* Based on the construction of a stamp that has the desired pattern with PDMS mold.
* Molecules to be transferred are placed on the surface of the stamp and printed on a receiving surface upon stamping, thus forming a self assembled monolayer.
* Depending on the application, peptides proteins, polysaccharides and other molecules can be stamped.
* The advantage of this microfabrication method is that the stamp can be reused to make pattern replicas.

②. Stencil patterning :-

* It creates templates by preventing PDMS from covering the master template
* The end result of this process is a PDMS model with holes in the pattern of the master.
* Different methods can be used to prevent PDMS from covering the master features.

(2) Microfluidic Patterning :-

* It utilizes a PDMS mold to create microchannels against a substrate.
* These microchannels can then be used to pattern fluid materials onto a substrate.
* This techniques has been utilized for the patterning of cells in tissue engineering applications.
* One important feature of these microchannels is their ability to maintain seperate fluid streams through a single channel because of laminer flow.
* This characteristic can be utilized for the study of cellular response to the stimuli of different materials.

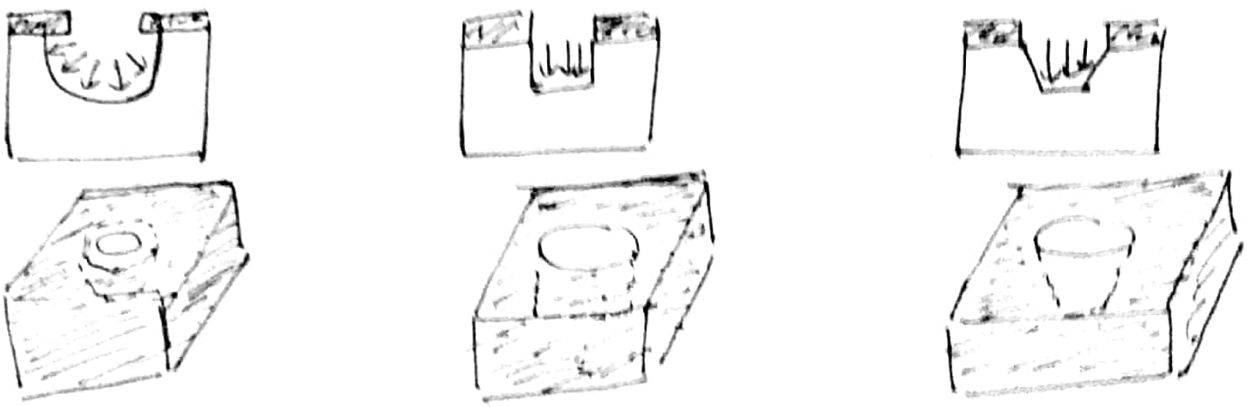
(3) FILM DEPOSITION :-

* Thin films are used for a variety of different purposes in microstructures — masking materials, structural materials etc.
* They are formed by either chemical reaction driven processes or physical processes.
* These films can play a structural or functional role in the design.

* Numerous types of materials are used for the generation of films.
* Most commonly used are plastics, silicon-containing compounds, metals and biomolecules.

(4) ETCHING :-

* It is the process that aims to create topographical features on a surface by selective removal of material, through physical or chemical means.
* The mechanisms used for etching utilize liquid chemicals or gaseous physico-chemical processes.
* Etching process is categorised into three groups:
  (1) Isotropic etching (2) Dry anisotropic etching
  (3) Wet anisotropic etching.



Isotropic Dry anisotropic Wet anisotropic

(2) Atomic Force Microscopy (AFM)

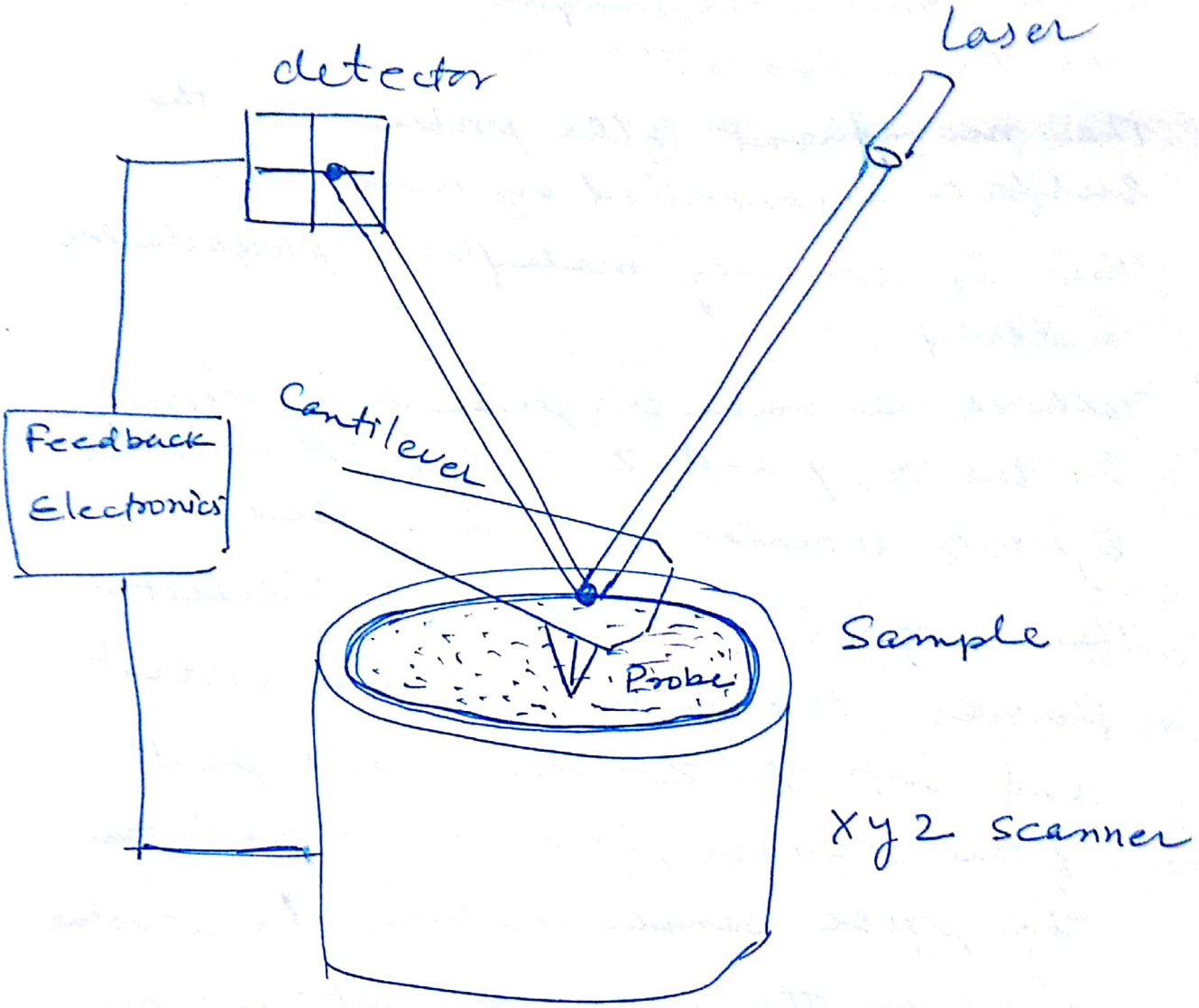
* AFM provides a 3D profile of the surfaces on a nanoscale, by measuring forces between a sharp probe (<10nm) and surface at very short distance (0.2–10nm).
* The probe is supported on a flexible cantilever.
* The AFM tip gently touches the surfaces and records the small force between the probe and the surface.
* The atomic force microscope is one kind of scanning probe microscopes are designed to measure local properties, such as light, ferction, magnetism with a probe.
* To acquire an image, the SPM raster-scans the probe over a small area of the sample, measuring the local property simultaneously.

* AFM was a development of scanning Tunneling Microscopy (STM) intended to extend the possibilities of STM to insulating samples.

Principles of operation

* The basic principle of AFM is that a probe is maintained in closed contact with the sample surface by a feedback mechanism as it scans over the surface and the movement of the probe to stay at the same probe-sample distance is taken to be the sample topography.
* A variety of probes have been used but the most commonly used are microfabricated silicon (Si) or silicon nitride (Si_3N_4) cantilevers with integrated tips.
* The tips can be very sharp.
* Typically, probe radius varies from 5 to 20 nm.
* The bending of the cantilever normal to the sample surface is usually monitored by an optical lever.
* This system magnifies the normal bending of cantilever greatly and is sensitive to system-level movement.

* There are variety of modes of scanning, but in the simplest mode, the probes gently touches the sample as it moves over the surface.
* The movement of the probe over the surface is controlled by a scanner.
* This is normally made from a piezoelectric material.
* which can move the probe very precisely in the X, Y and Z axes, although other types of actuator are also used.
* The signal from the photo detector passes through a feedback circuit, and into the Z- movement part of the scanner, in order to maintain the probe-sample distance at a set value.
* Because the cantilever acts as spring, this fixed cantilever deflection means a fixed probe-sample force is maintained.
* The amount by which the scanner has to move in the Z axis to maintain the cantilever deflection is taken to be equivalent to the sample topography.



(Schematic operation of AFM)

Operating Modes

The most commonly used modes of operation of an AFM are:-

(A) Contact mode AFM
(B) Tapping Mode AFM
(C) Non contact Mode AFM.

(A) Contact Mode AFM

* < 0.5 nm probe surface seperation
* Spring constant of cantilever is less surface, the cantilever bends.
* The force on the tip is repulsive
* By maintaining a constant cantilever deflection, the force between the probe and the sample remains constant and an image of the surface is obtained

(B) Tapping mode AFM :-

* 0.5 - 2 nm probe surface seperation
* Image is similar to contact mode
* In this mode the cantilever is oscillated at its resonant frequency.
* The probe lightly taps on the sample surface during scanning, contacting the surface at the bottom of its swing.
* By maintaining a constant oscillation amplitude a constant tip sample interaction is maintained and an image of the surface is obtained.

(C) Non contact mode AFM :-

* The probe does not contact the sample surface.
* But oscillates above the adsorbed fluid layer on the surface during scanning.

* Using feedback loop to monitor changes in the amplitude due to attractive vander weale forces the surface topography can be measured.

LIMITATION :-

* The AFM can be used to study a wide variety of samples including biological samples such as walls of cells and bacteria.
* However there are limitations in achieving atomic resolution.
* The physical probe used in AFM imaging is not ideally sharp.
* As a result, An AFM image does not reflect the true sample topography, but rather represents the interaction of the probe with the sample surface.

(3) Biological production of metal nanoparticles :—

(1)

Nanoparticles :- They are the particles between 1 and 100 nanometer in size.

In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties.

Particles are also classified on the basis of diameter

(i) Ultrafine :- 1 and 100 nm

(ii) fine — 100 and 2500 nm

(iii) Coarse — 2500 and 10,000 nm.

Metal nanoparticles :- The term metal nanoparticles is used to described nano sized metals with dimensions (length, width or thickness) existing the size range 1-100 nm.

Synthesis of Nanoparticles / Metal nanoparticles

Nanoparticles are synthesized by using various methods there are

1. Chemical Synthesis
2. Physical Synthesis
3. Biological Synthesis

(1) Chemical Synthesis :-

It is valuable method as it takes tiny period of time for synthesis of large quantity of nanoparticles. Currently three chemical technique are used:-

- (A) Dispersion of preformed polymers
- (B) Polymerization of monomers
- (C) Ionic gelation or coacervation of hydrophillic polymers.

(2) Physical Synthesis :-

The metal nanoparticles are synthesized by evaporation-condensation process which might be carried out using a tube furnace

at atmospheric pressure. ~~Nanomaterial~~

Nanoparticles of different materials such as Ag, Au, PbS have been produced by using the evaporation-condensation technique.

(3) Biological Synthesis :- It is the method of production of nanoparticles/metal nanoparticles by using various biological agents These are:

(A) Nanoparticle synthesis by using Plant Extract.

(B) Nanoparticle synthesis by Bacteria

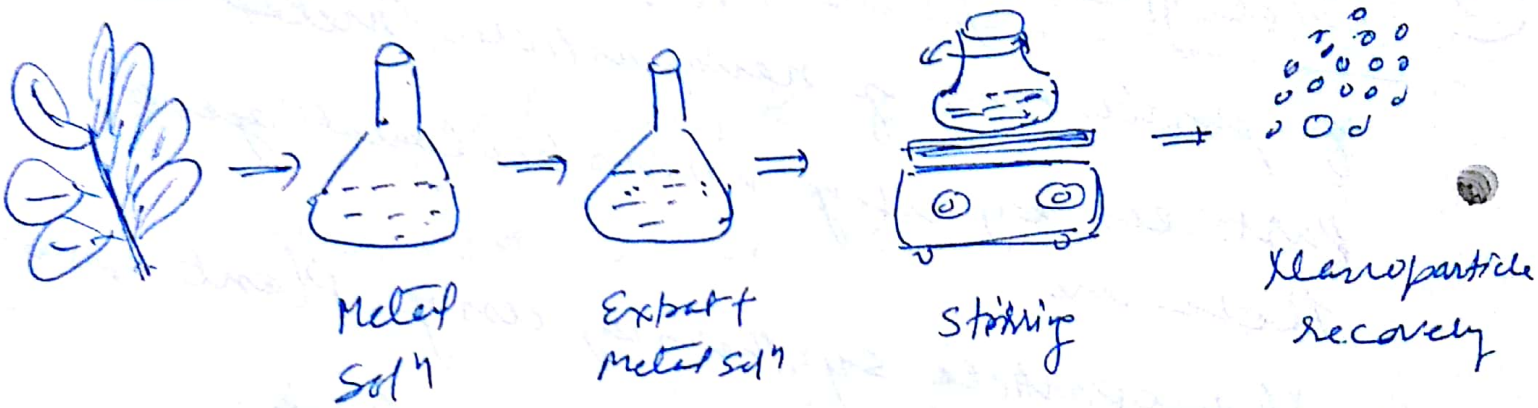
(C) Nanoparticle synthesis by Fungi

(D) Nanoparticle synthesis by yeast

(E) Nanoparticles by other biological particles.

(A) Using Plant Extract :- Plant extracts are used for the production of various metal nano particles. They are the superior option for the synthesis of nanoparticles, as the protocols involving plant sources are free from toxicants.

for the production of gold & silver nanoparticles, Geranium extract, Aloe vera plant extract, sundried Cinnamomum camphora and Azadiracta indica.



Synthesis of nanoparticles from Plant Extract.

(B) Using Bacteria :- Various bacterial species are used for the production of nanoparticles. Bacillus, Pseudomonas, Marinobacter ~~are~~ and Lactobacillus are succesfully used for the production of metal nano particles.

© Using fungi & Biological

Production of nanoparticles by fungi is determined now a days because of their reception towards toxicity, higher bioaccumulation, comparatively economic, effortless synthesis method and simple downstream processing and biomass handling.

Extracellular biosynthesis of silver nanoparticles by Aspergillus niger, Fusarium solani and Aspergillus oryzae are reported to produce silver nanocrystals.

(D) Using yeast:- The extracellular synthesis of nanoparticles in huge quantities by using yeast reported by many scientist. Metal nanoparticles are also synthesized by using Candida glabrata, Schizosaccharomyces pombe. The Marine yeast strain also explored for the production of intracellular metal nanoparticles.

(E) Using other biological particles of

Biological material like viruses, protein, peptides and enzymes could be exploited for biosynthesis of nanoparticles.

for the mineralizations of inorganic materials, Cowpea chlorotic mottle virus and Cowpea mosaic virus have been employed.