BB101
Molecular Cellular Biology

Tutorial 1

What is Life?

We conventionally look for distinctive characteristics exhibited by living organisms

- ✓ Growth
- ✓ Reproduction
- ✓ Ability to sense environment
- ✓ Mount a suitable response

Growth and Development

Increase in mass and increase in number of individuals are twin characteristics of growth.

Plants grow continuously throughout their life span however animal grow up to certain age.

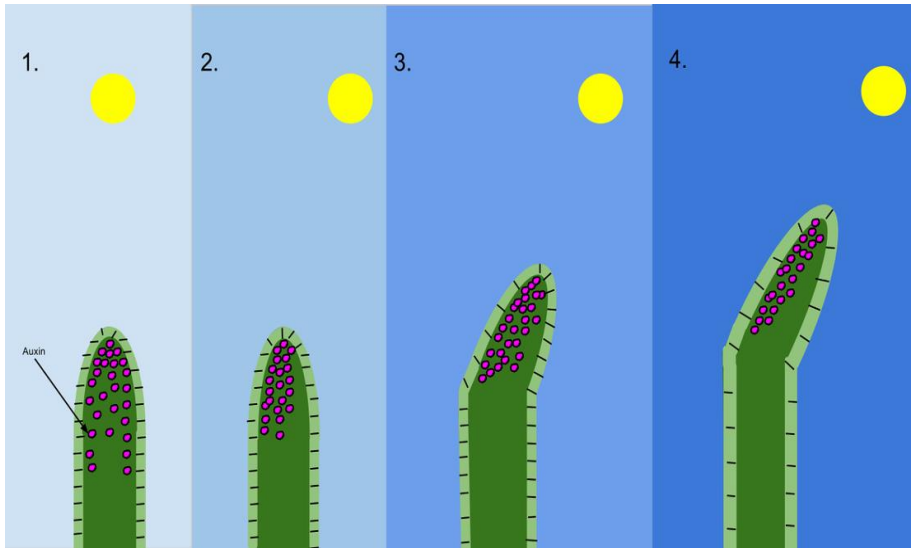
Reproduction

- ✓ Reproduction refers to the production of progeny possessing features more or less similar to those of parents
- ✓ When it comes to unicellular organisms like bacteria, unicellular algae or Amoeba, reproduction is synonymous with growth.
- ✓ There are many organisms which do not reproduce (mules, sterile worker bees, infertile human couples).

Ability to sense environment and mount a suitable response

Living organisms have property to mount specific response to the environment

Mechanical stimulus



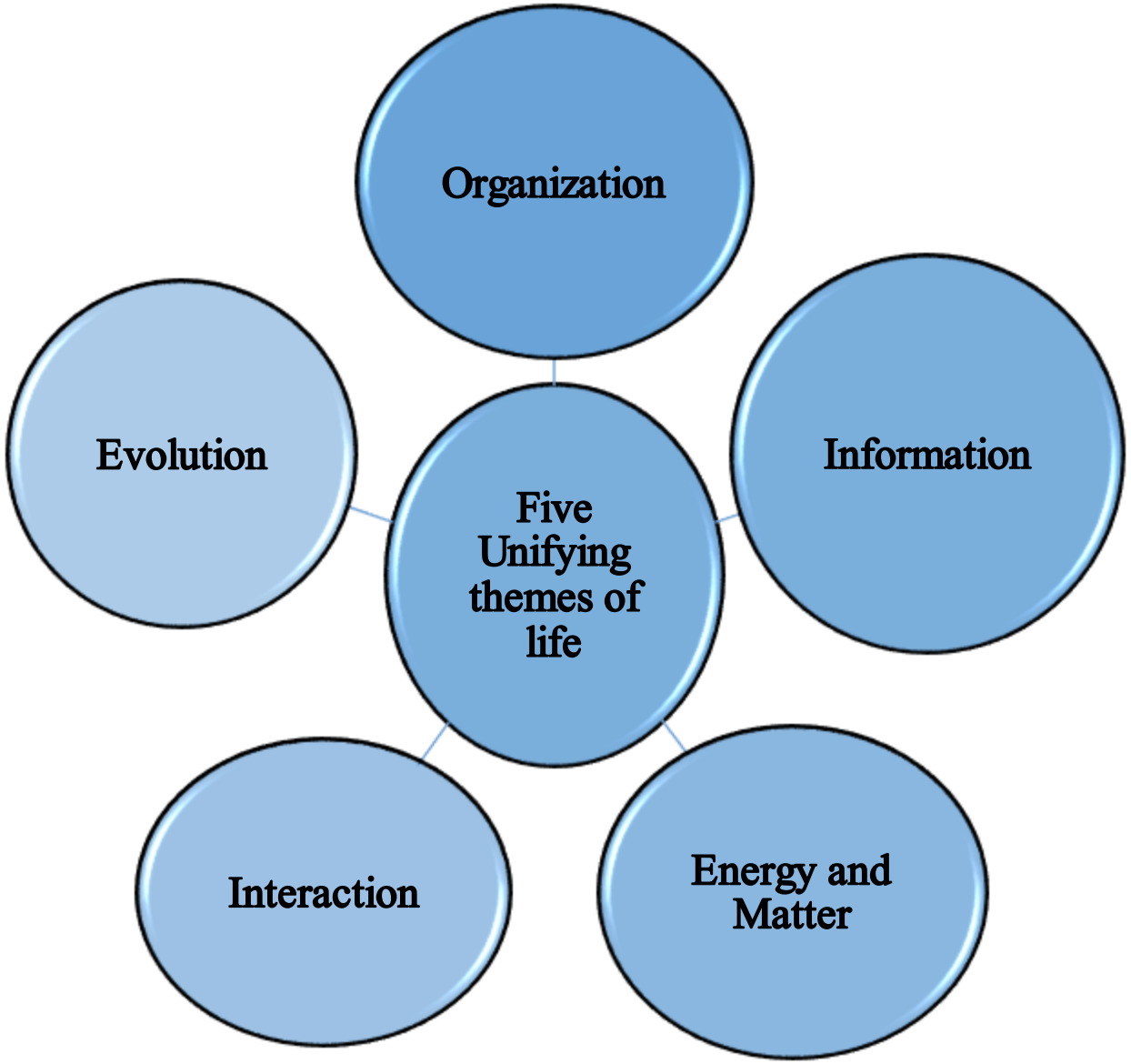
Phototropism

Regulation and Adaptation

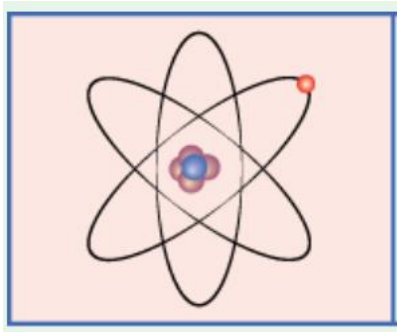
Polar beer have thick layer of fur which protect from cold and desert lizard have spiny body which prevent loss of water.

Unifying Themes of Life

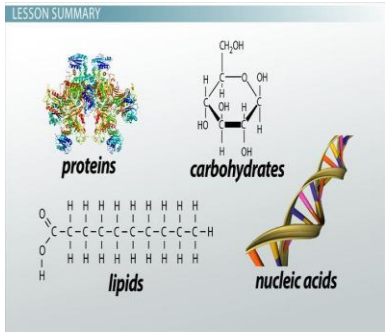
Represents Unity in Diversity



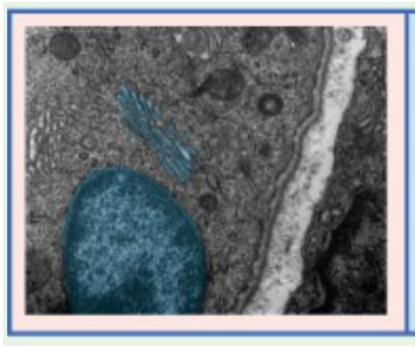
Level of Organization



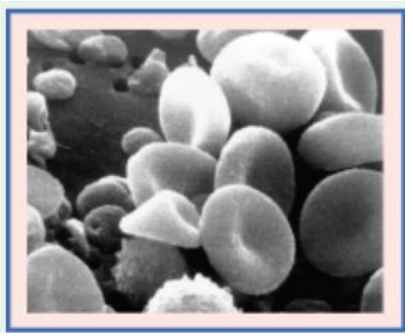
Atoms



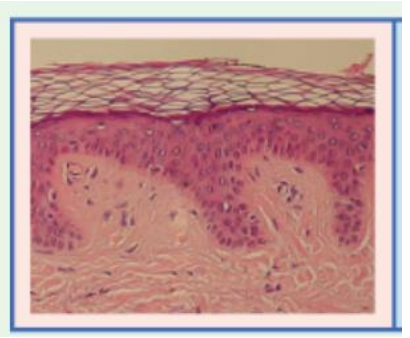
Molecules



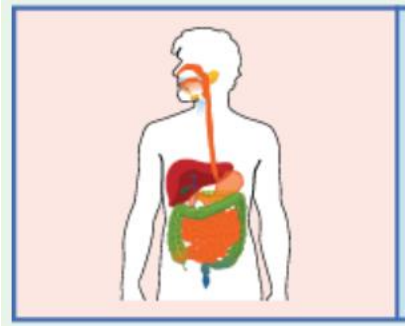
Organelle



Cells



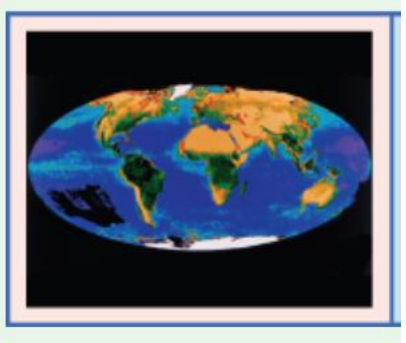
Tissue



Organ system

Organism, Population and Communities

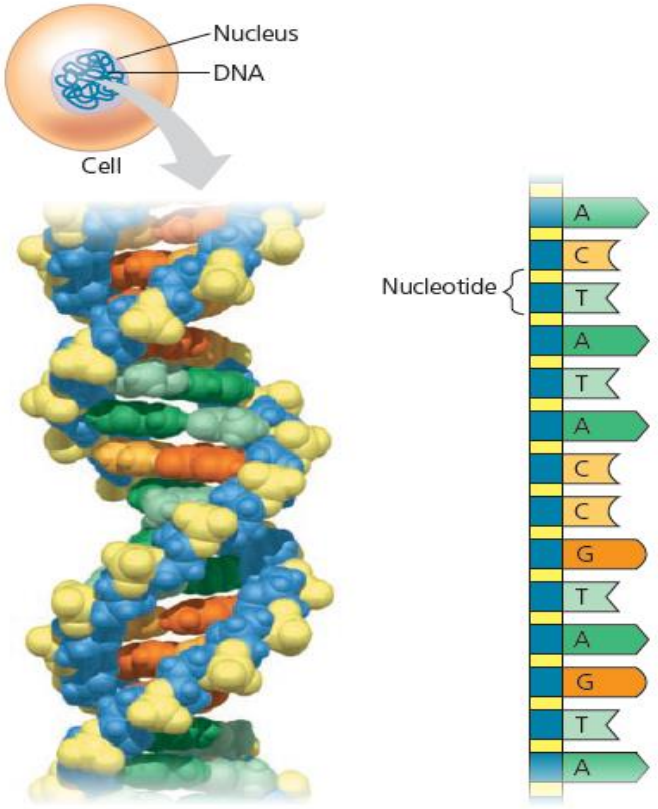
Ecosystem

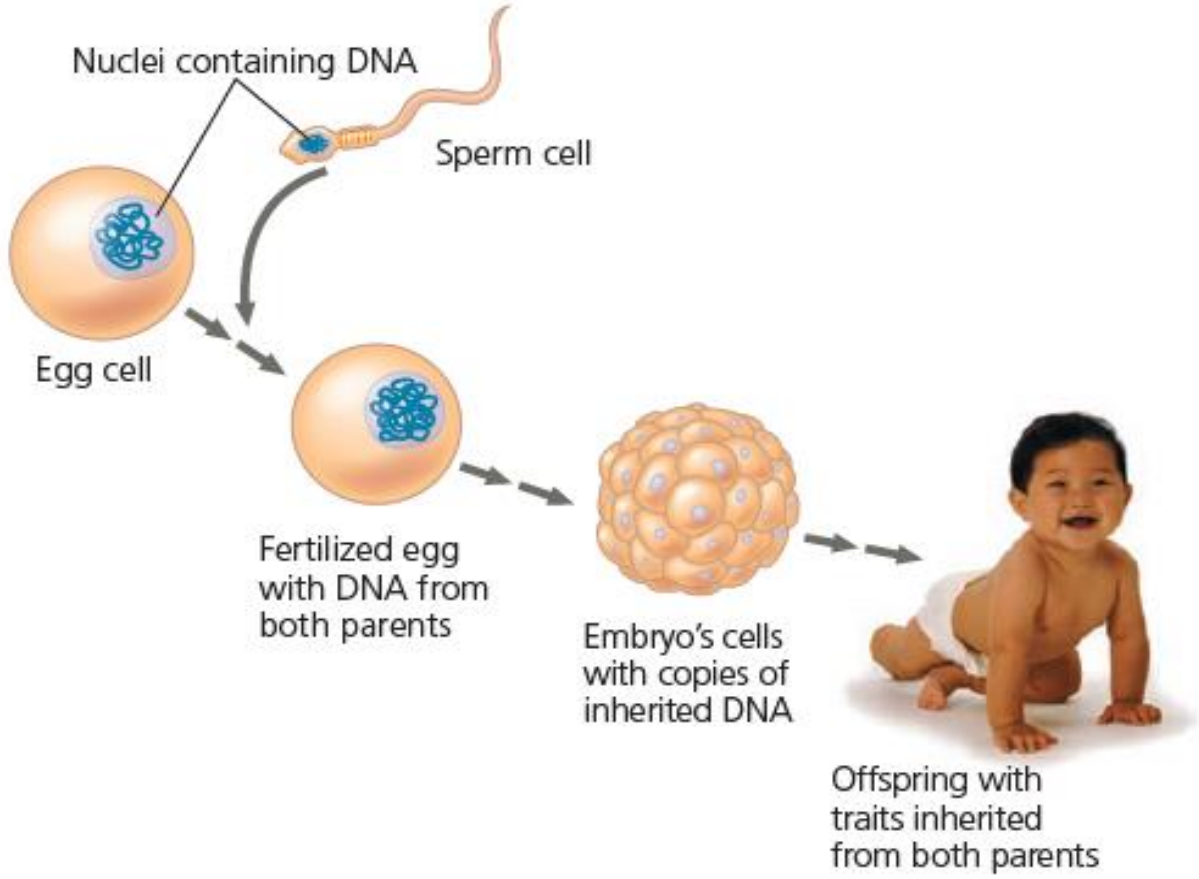


Biosphere

New Properties emerge at successive level of Biological Organization

Information





Genetic Information is encoded in the DNA → **Inherited DNA directs the development of organisms**

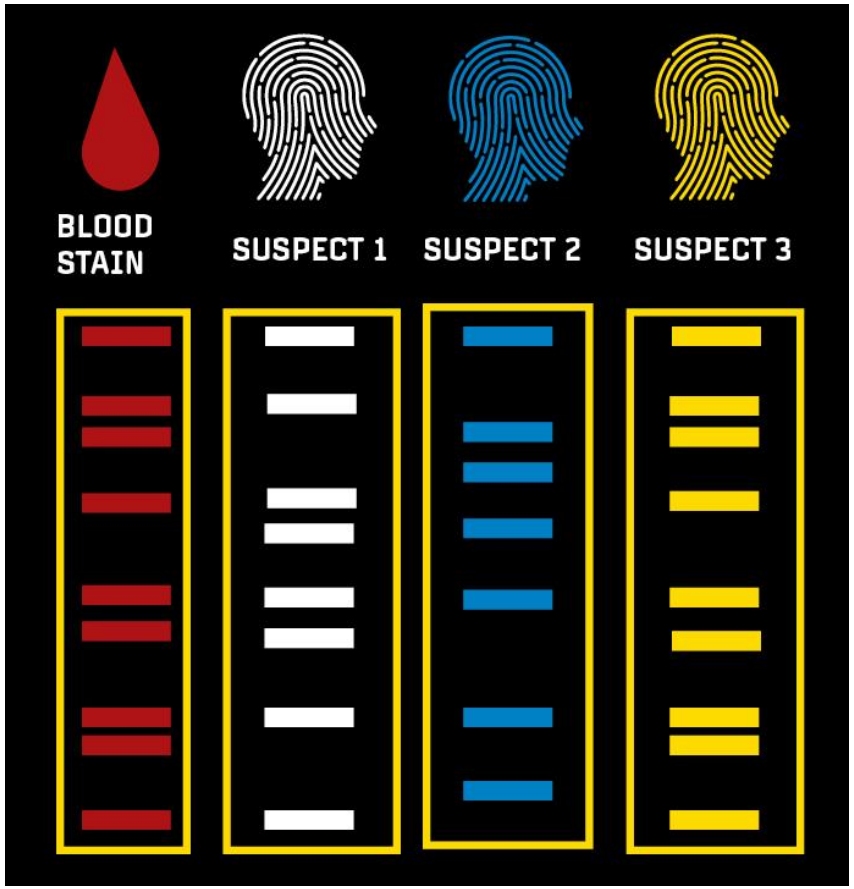
Life processes involve expression and transmission of genetic information

Interesting Fact about DNA

Unrelated people

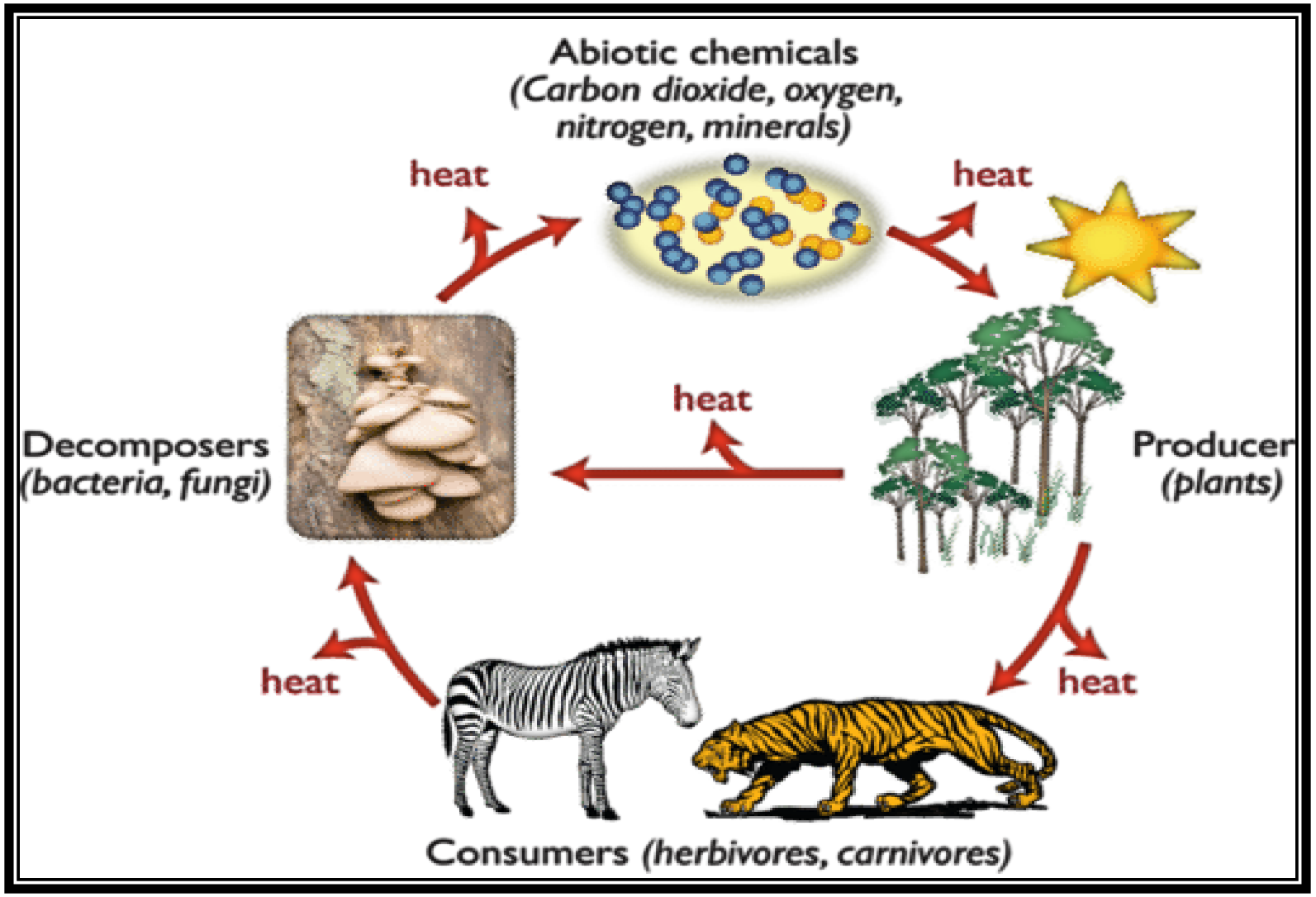
Man and chimpanzee

Parent and offspring



DNA Fingerprinting

Energy and Matter



All living organisms require energy

Sun is the main source of energy in an ecosystem.

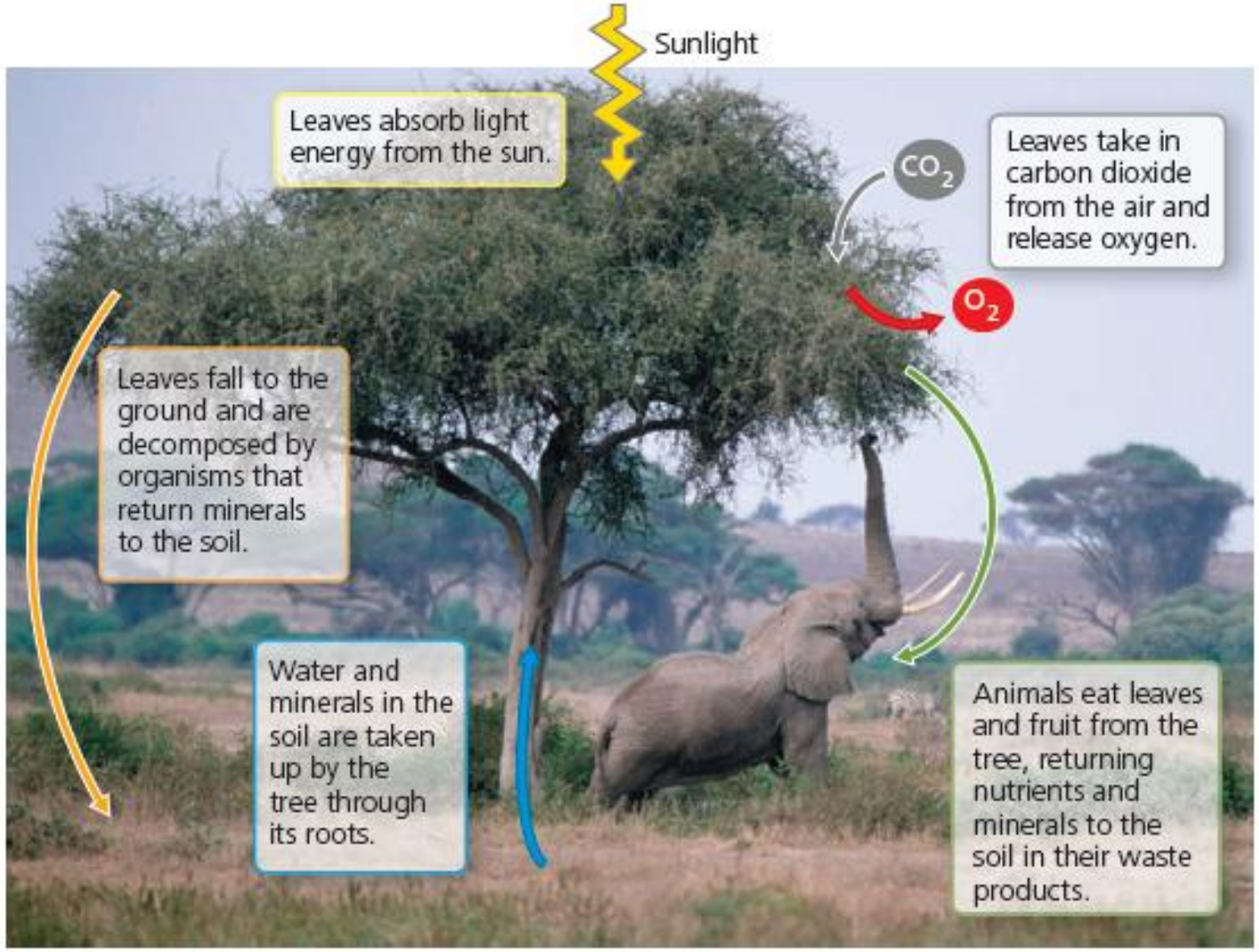
Life requires the transfer and transformation of energy and matter

Interaction

Interaction between organisms is critical for evolution

Social Behaviour: Organisms of same species comes in direct contact

Community dynamics occur when organism of different species come in direct contact



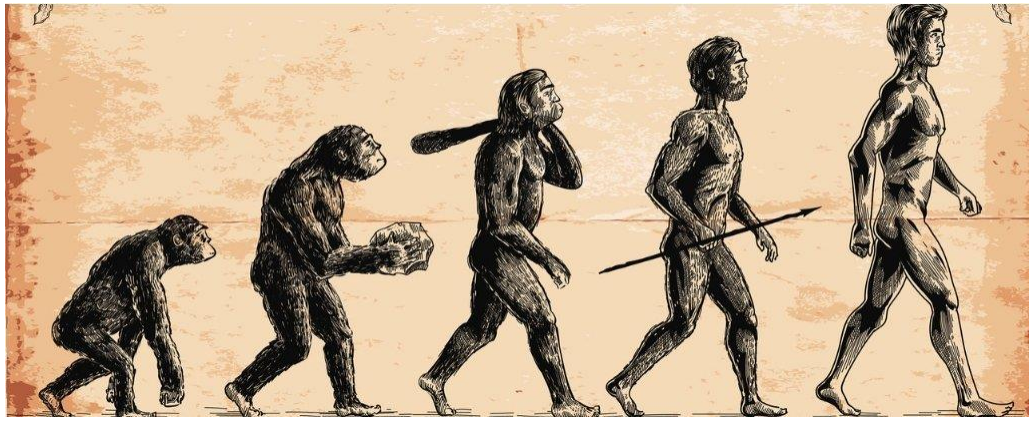
Evolution

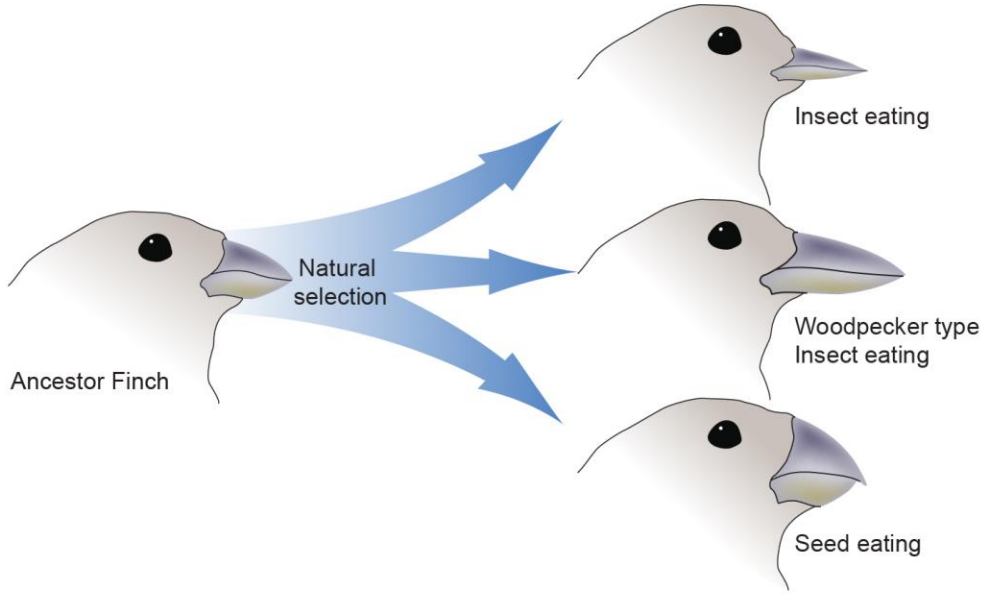
Gene frequency change is a species over time due to following reasons

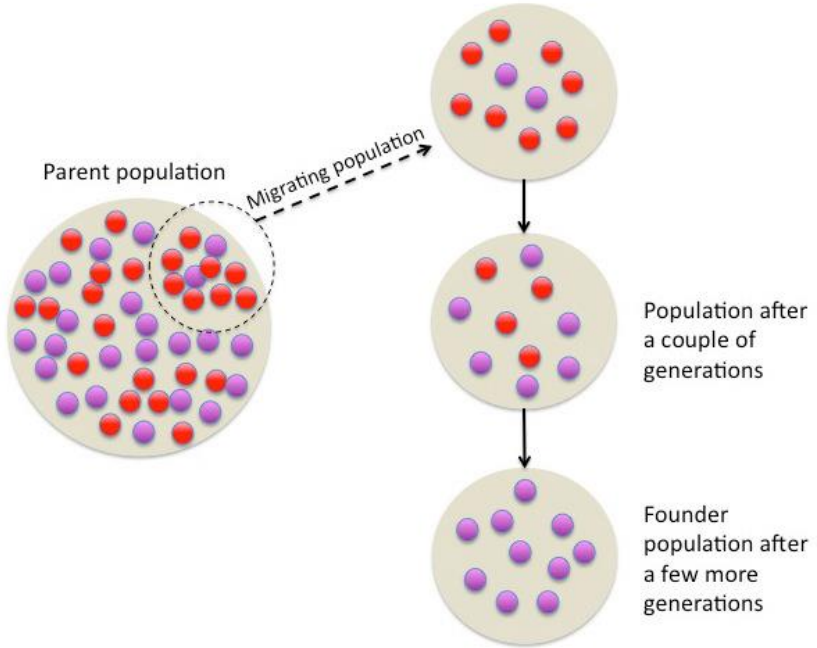
Genetic drift

Natural selection

Founder effects





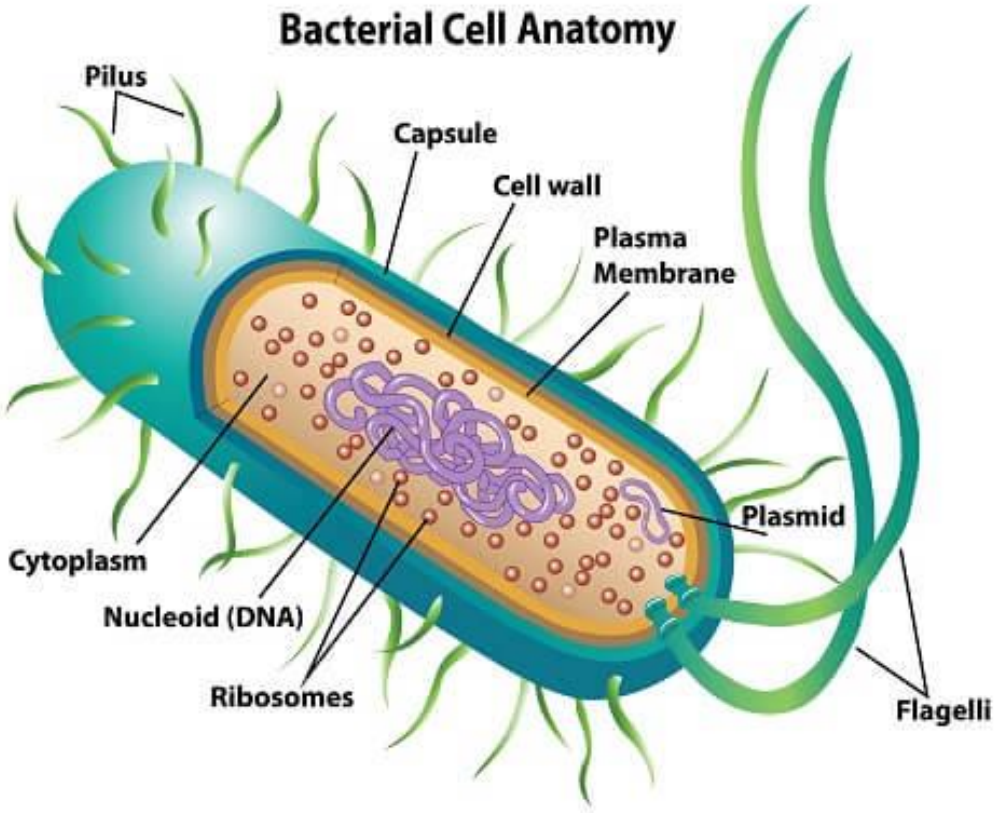


Cell: Basic Unit of Life

- Existing cells divide to form new cells
- Functions of the cells are divided amongst the various organelles present in the cell
- Two main kinds of cells: prokaryotes and eukaryotes
- Prokaryote: pro (before) karyo (nuclei)
- Eukaryote: eu (true) karyote (nuclei)
- Main difference: prokaryote: absence of nuclear membrane

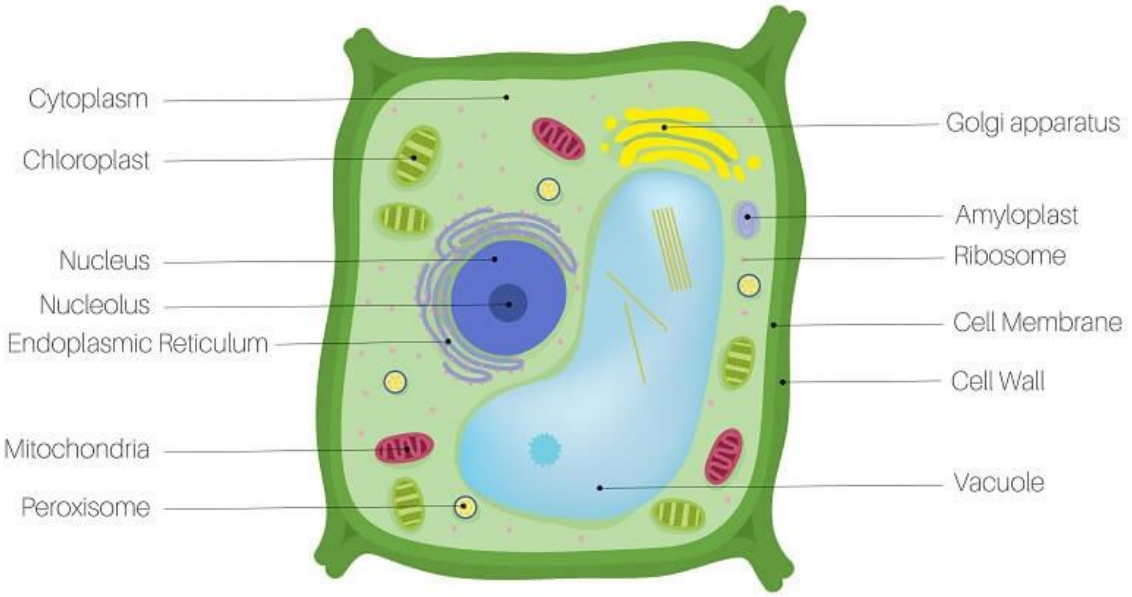
  eukaryote: presence of well defined nuclei
- Prokaryotes: bacteria, algae, mycoplasma
- Eukaryotes: plant, animal cells, fungi

Prokaryotic Cell



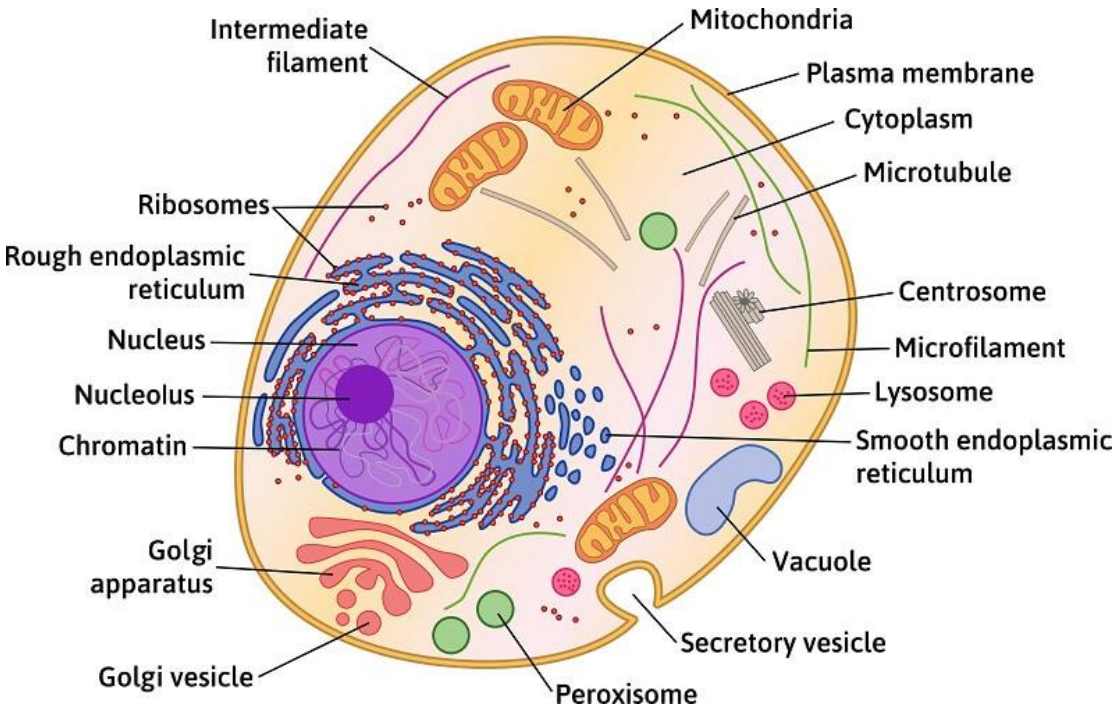
- Nucleoid: cell's DNA; no nuclear membrane
- Cell wall: peptidoglycan; mechanical support and protection
- Cytoplasm: fluid; biochemical reactions of the cell take place
- Ribosomes: protein synthesis
- Plasmids: circular pieces of DNA; replicate independent of chromosomes
- Flagella, cilia: cell motility
- Pilia: cell adhesion to surrounding surfaces

Eukaryotic Cell in Plants



- Cell wall: cellulose; mechanical strength and rigidity
- Nucleus: Cellular DNA
- Nucleolus: site of ribosome synthesis
- Cytoplasm: fluid; biochemical reactions of the cell take place
- Endoplasmic reticulum: Protein modification
- Ribosomes: protein synthesis
- Golgi apparatus: protein folding, sorting and packaging
- Mitochondria: Cellular ATP generation through respiration
- Chloroplast: site of photosynthesis
- Peroxisome: biosynthesis of lipids and detoxification

Eukaryotic Cell in Animals



- Lack cell wall
- Chromatin: complex of DNA and proteins
- Rough ER: protein synthesis; Smooth ER: lipid synthesis
- Vacuole: storage and transport of large molecules
- Plasma membrane: cell membrane; phosphor lipid bilayer with proteins
- Centrosomes: organize DNA during cell division
- Lysosomes: digestive enzymes; cell membrane repair and response to invasion by foreign pathogens
- Cytoskeleton: shape, mechanical strength, anchors organelles within the cell; microfilaments, intermediate filaments, microtubules

How Prokaryotes differ from Eukaryotes ? ?

Similarities: DNA, Plasma membrane, Cytoplasm, Ribosomes

Differences			
Characteristic	**Prokaryon**		**Eukaryote**
	Prokaryote	**Archeabacteria**	
Nucleus	Absent	Absent	Present
Membrane bound organelle	Absent	Absent	Present
Cell Wall	Peptidoglycan	Lack Peptidoglycan	Only in Plants
Cell structure	Unicellular	Unicellular	Mostly multicellular
DNA form	Circular	Linear	Linear
Cellular process	Transcription and translation are coupled	Transcription and translation are not coupled	Transcription and translation are not coupled

Separation of Macromolecules

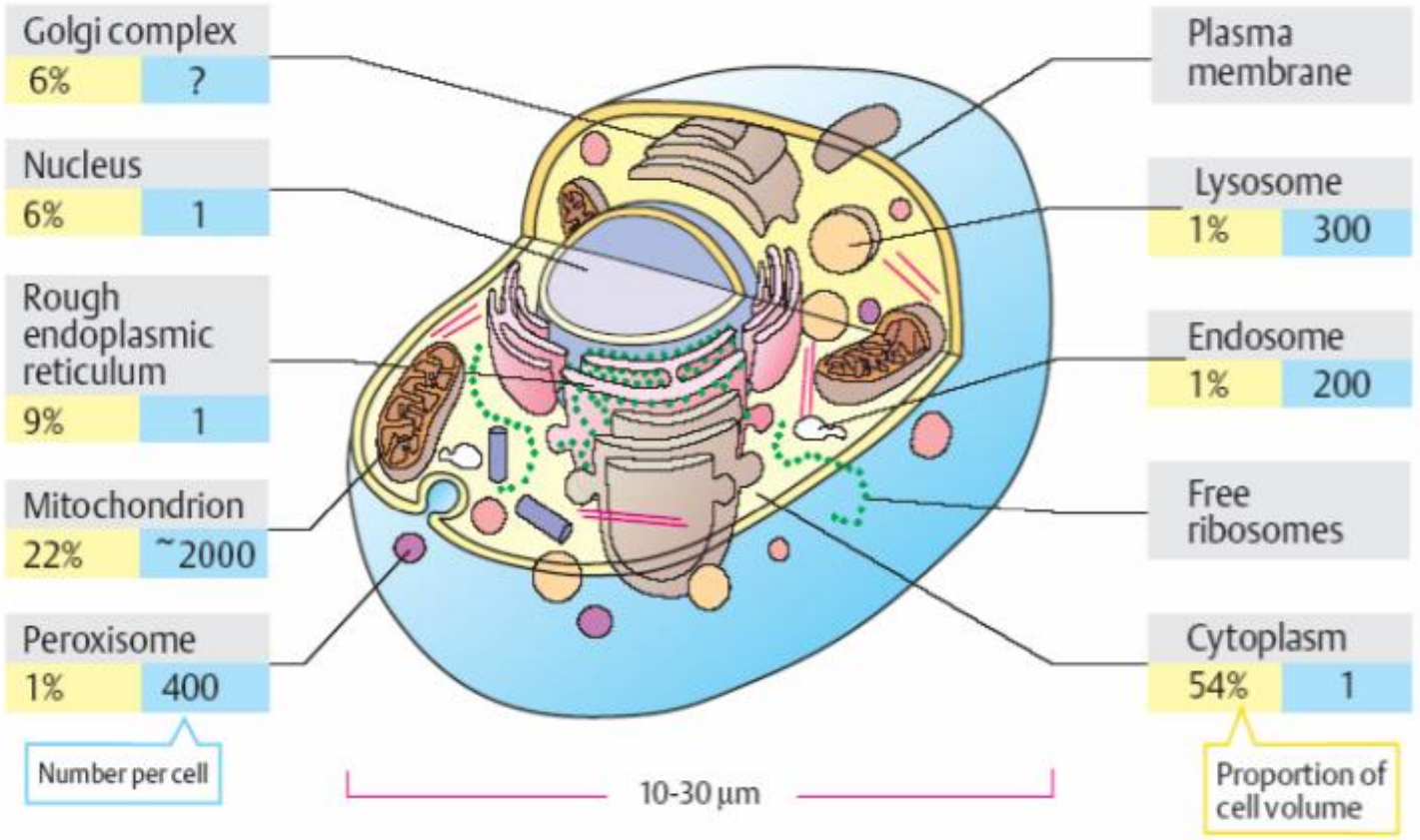


Fig: Structure of Animal Cell

Materials	Density (g/cm3)
Microbial cells	1.05-1.15
Mammalian cells	1.04-1.10
Organelles	1.10-1.60
Proteins	1.30
DNA	1.70
RNA	2.0

Densities of biological materials

What is centrifugation?

- A technique applied for separation of particles from solution or separation of mixtures by applying centrifugal force.

- Factors which influenced centrifugation: shape, size density, density, viscosity of the medium , and the speed of the rotor

Principle of centrifugation

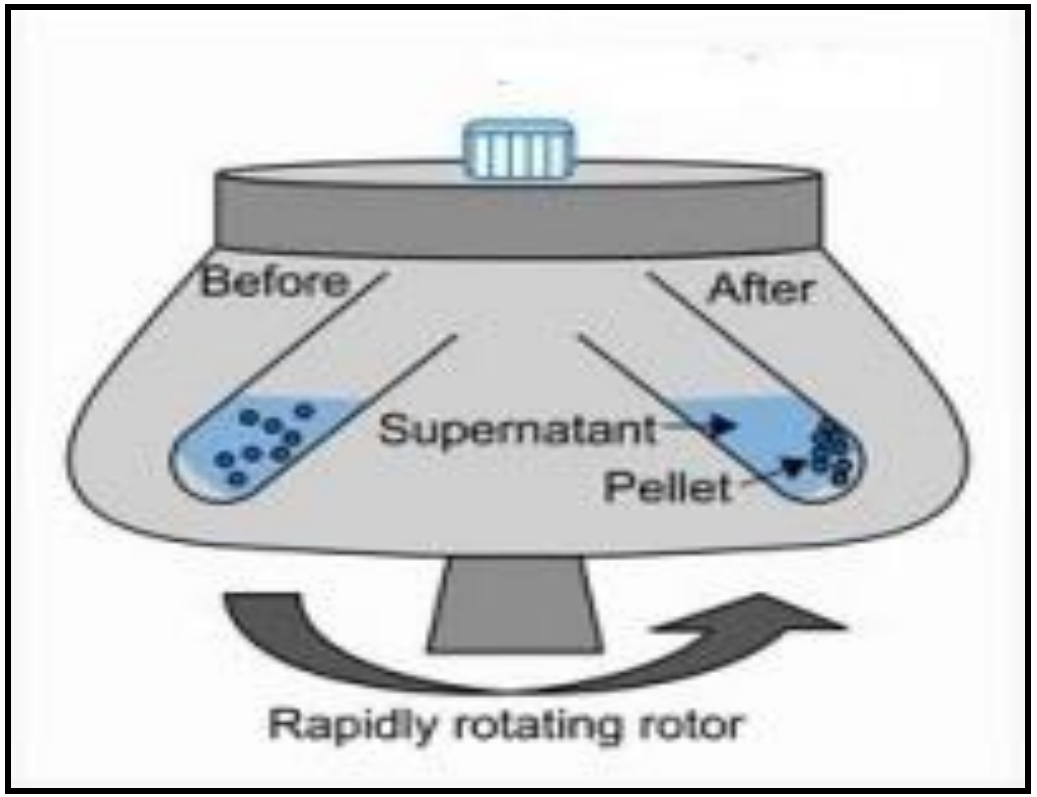
Principle of centrifugation is to separate particles in the liquid media under the influence of a centrifugal force.

Centrifugal force = mw^2r

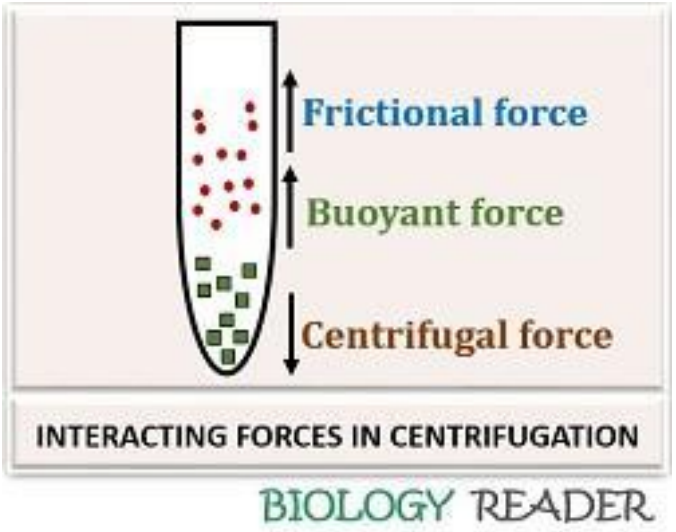
w = angular velocity of rotor

R = radial distance of particle

W = 2π rev min^{-1} / 60 rad/sec



Forces that act on a particle during sedimentation



Buoyant force = $m_o\ w^2\ r$

m_o = mass of fluid displaced by particle

Net force = centrifugal force – buoyant force

= $mw^2r - m_o\ w^2\ r$

At steady state, frictional force is equal to net force and the molecule moves with velocity v downwards

$Vf = mw^2r - m_o\ w^2\ r$

$S = v/w^2r$

Sedimentation coefficient is the ratio of velocity to the centrifugal acceleration.

Relative Centrifugal Force

Relative centrifugal force

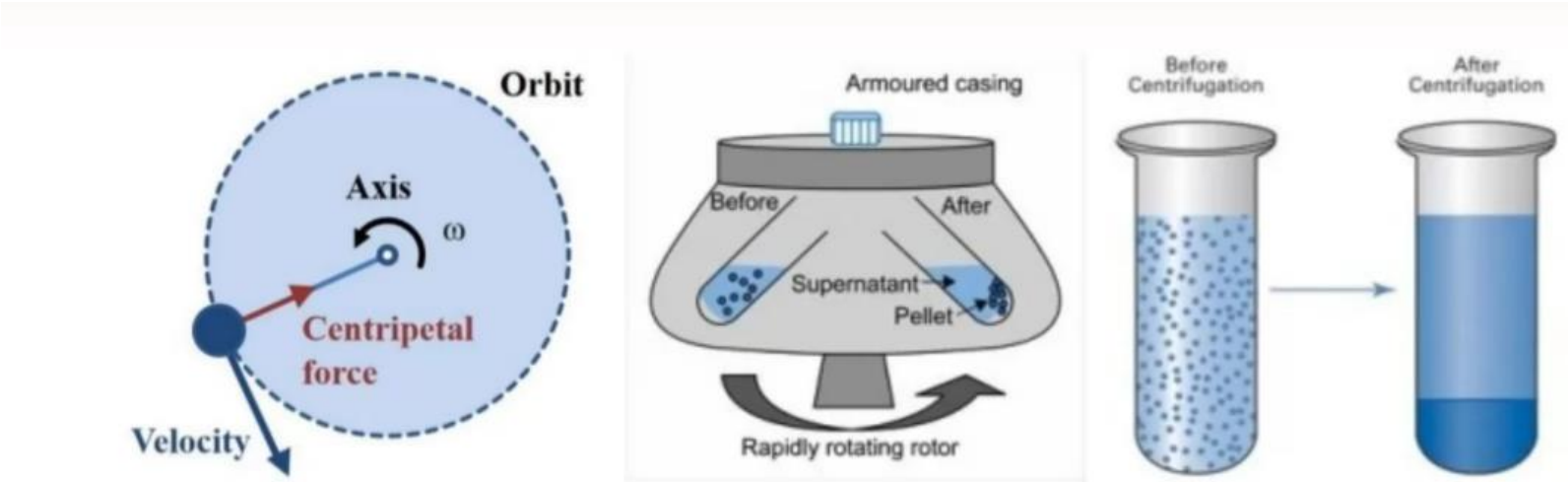
- Force exerted on inside of the rotor by the force of rotor rotation.
- Applied perpendicular to surface of the sample relation to gravity of earth.
- Relative Centrifuge Force (RCF) expressed in xg (multiple of earth gravitational force)

RCF (g Force) = rw^2/g

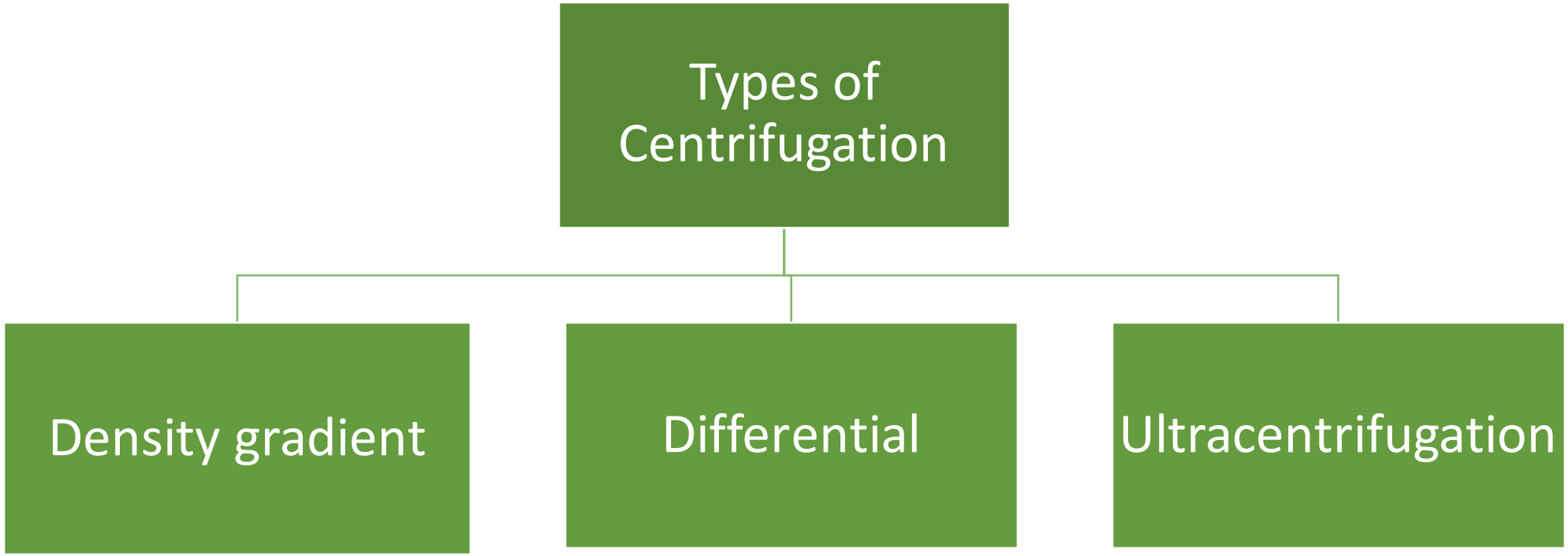
r = radius in cm

g = acceleration due to gravity

w = angular velocity



https://microbiologynote.com/centrifugation/


Types of Centrifugation
Density gradient
Differential
Ultracentrifugation

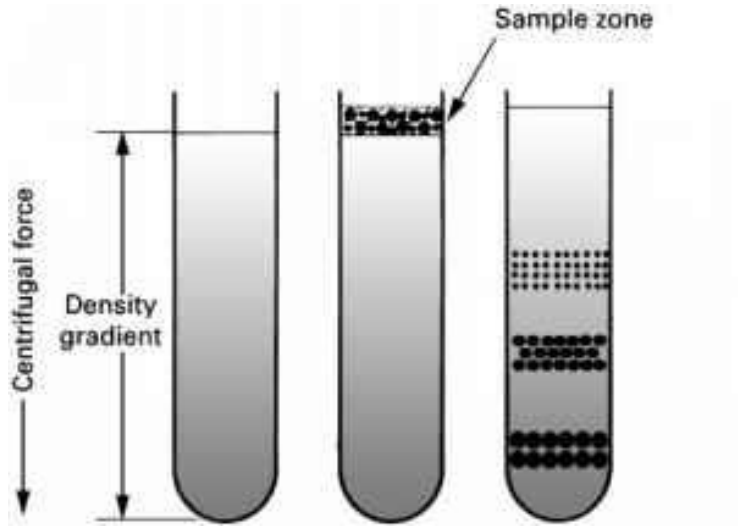
Density gradient centrifugation

Rate Zonal:

The density of the sample is greater than all the layers in the solution

Sample applied on the uppermost layer of the solution

Sedimentation of the particles on the basis of size, shape and density.

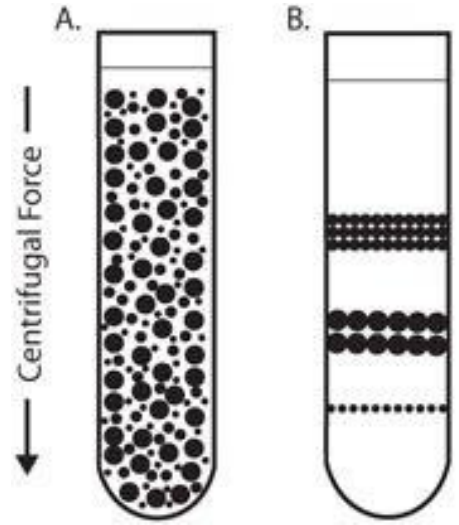


Isopycnic:

Solution contains greater ranges of densities.

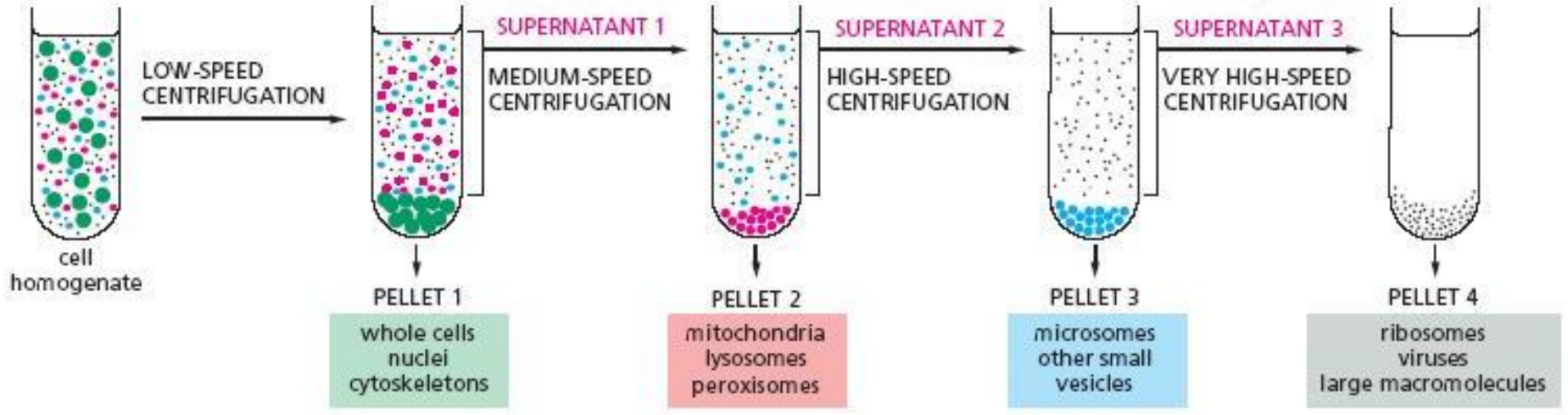
Sedimentation of the sample in accordance with the gradient density similar to the density of the solution.

Separation on the basis of density differences, independence of time.



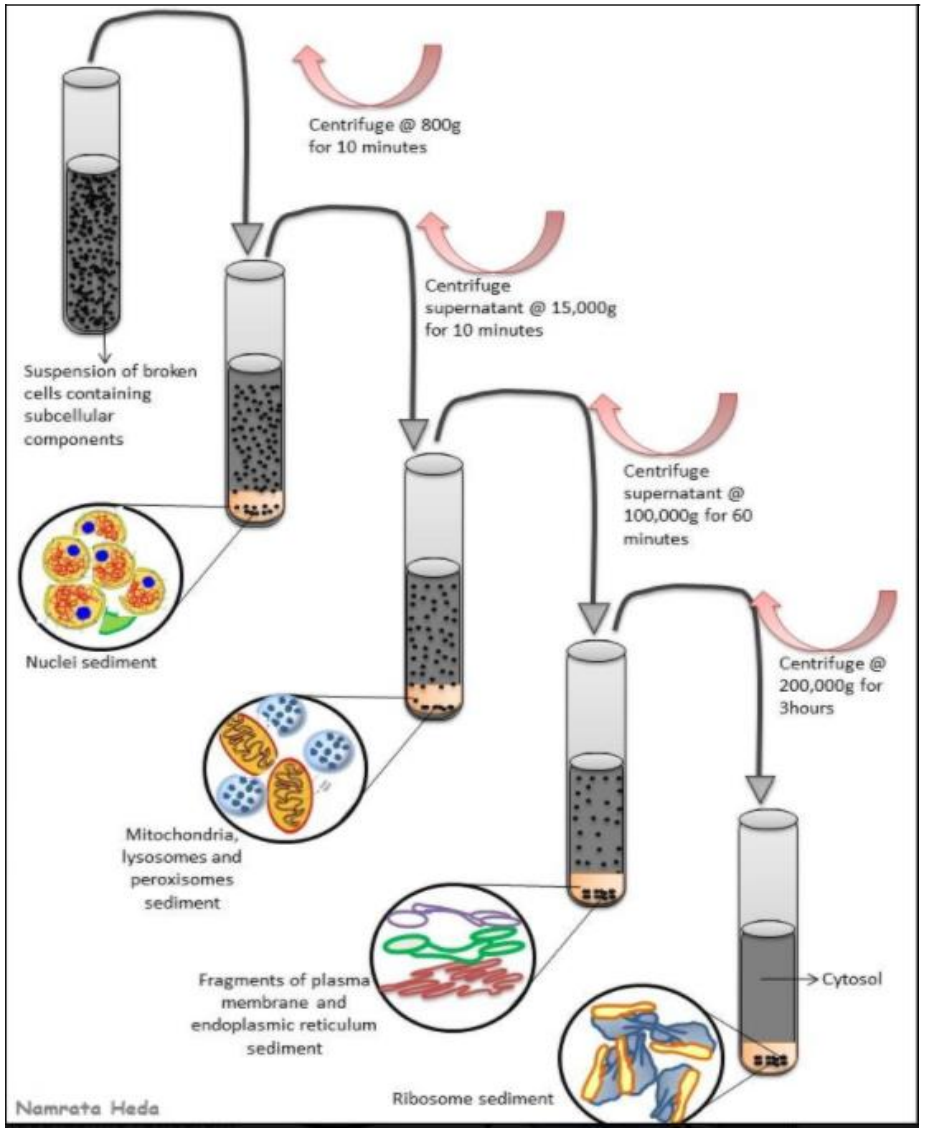
Differential centrifugation

- It separates on the basis of size and density.
- Larger and denser components experience the greatest centrifugal force.
- Greater size particles pellet is formed at the bottom of the tube while smaller ones (less dense) remain in supernatant.



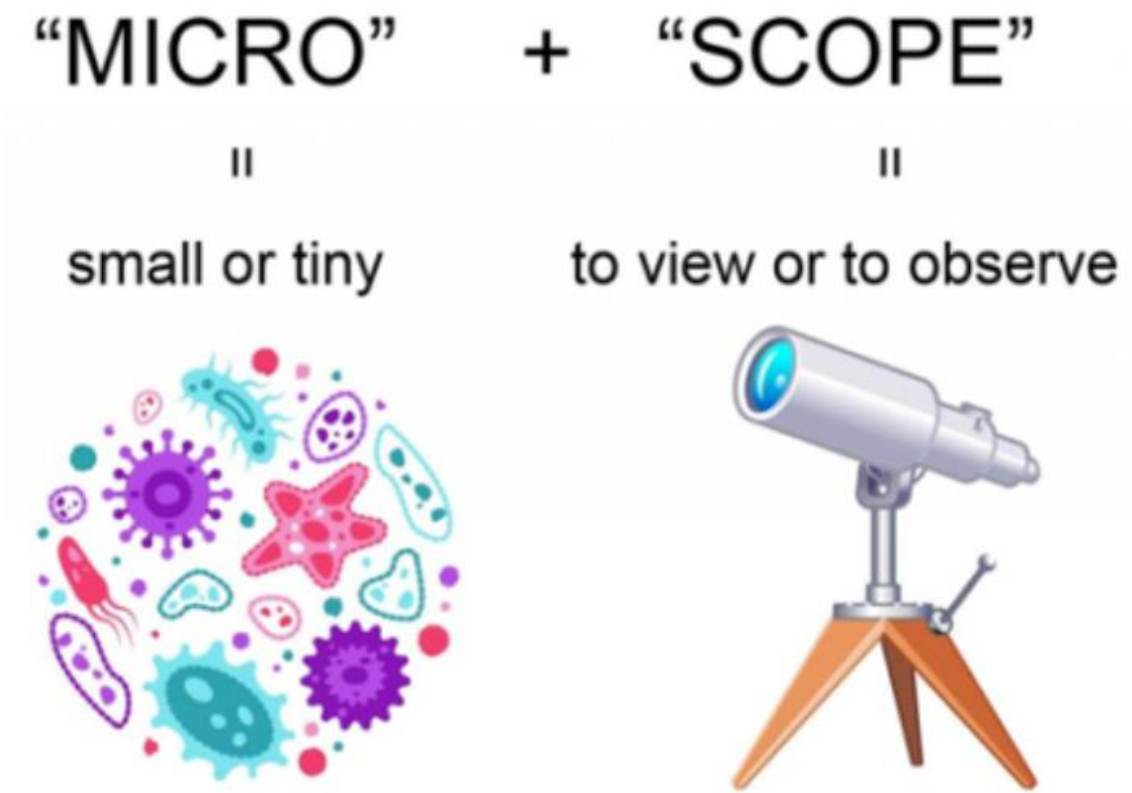
Ultracentrifugation

- Used for separation of smaller molecules like proteins, ribosomes and even viruses.
- It could be run up to 1,50,000 rpm.
- Advanced type of centrifuge and separate particles as less as 10 microns.
- Refrigeration systems are found in these centrifuges to help control the heat that is generated by the spinning.



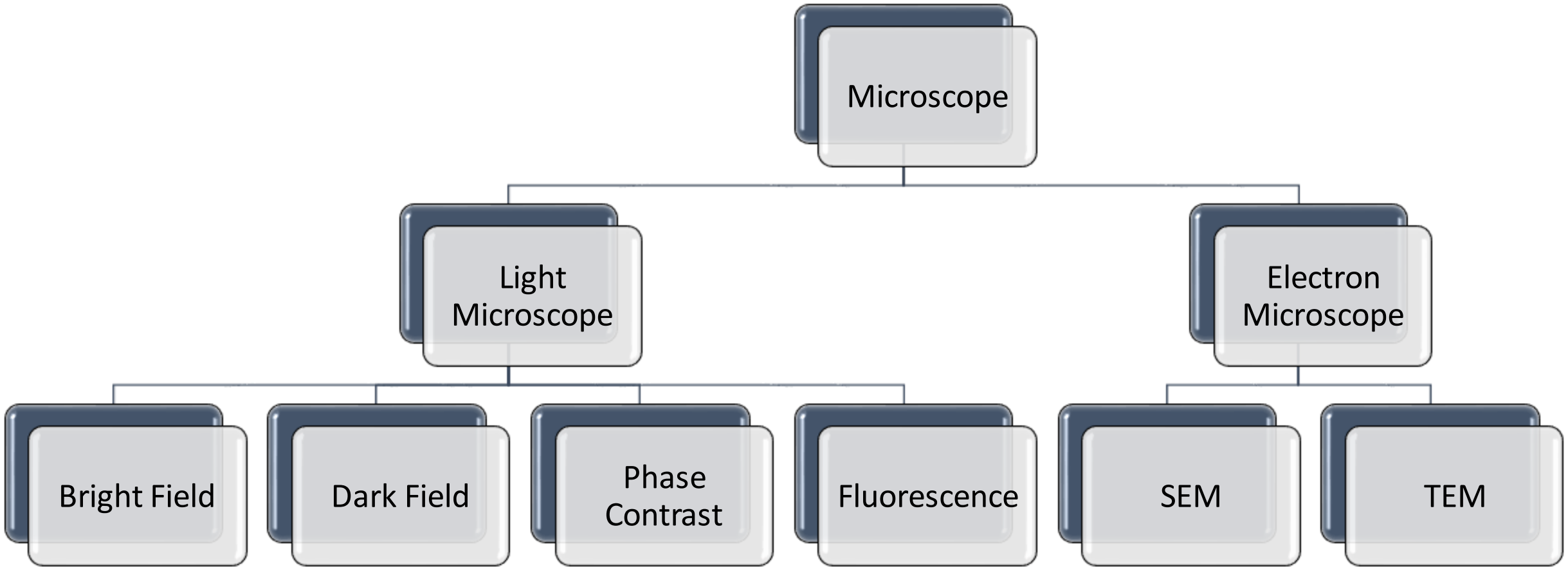
Microscopy

- To see what is not visible to the naked eyes

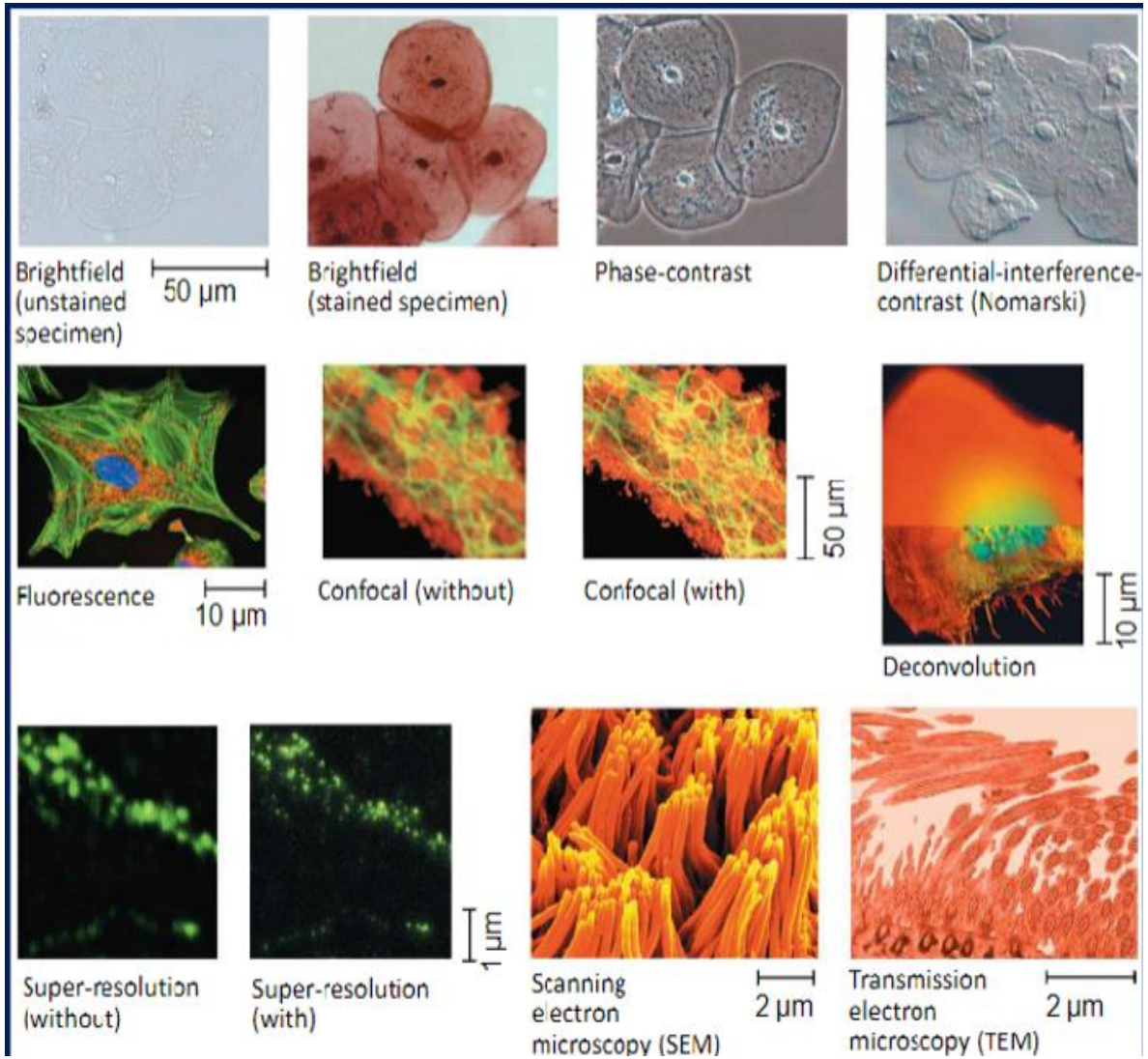


https://www.embibe.com/

Types of Microscopy



Exploring Microscopy



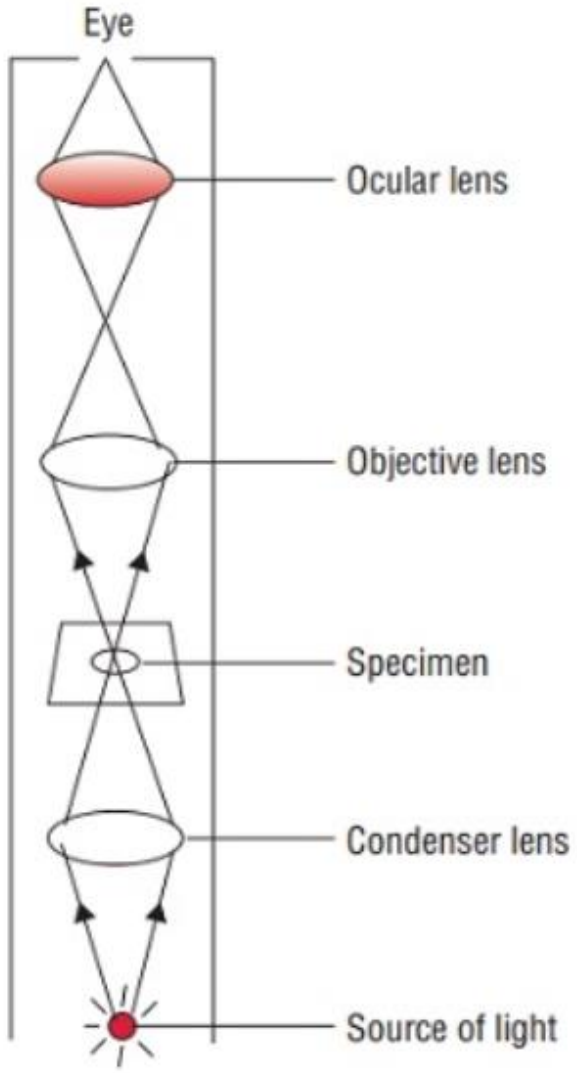
- All are variants of Light microscopy except

  SEM and TEM

- Light Microscopy allows imaging of a live cell

- SEM/TEM: dead cells

Principle of microscopy



Components of a compound light microscope

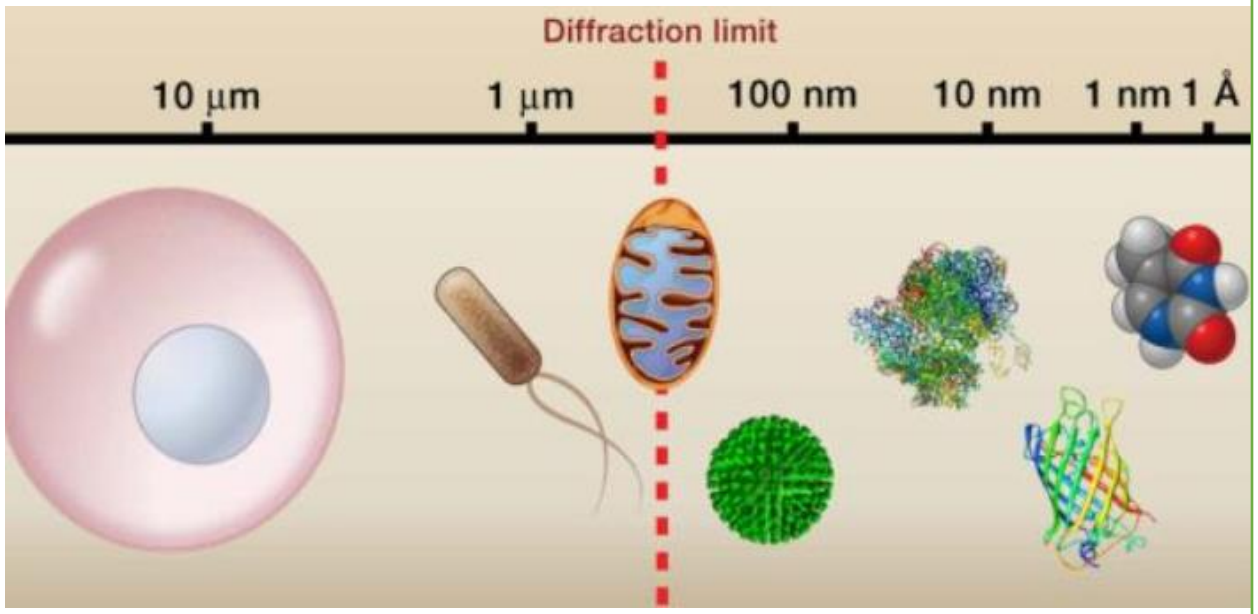
- ✓ **Eyepiece/ocular**: to look through the microscope
- ✓ **Objective lens**: sample visualization; magnification of 40X to 100X
- ✓ **Condenser**: collect and focus the light on to the sample

The Abbe diffraction limit for a microscope

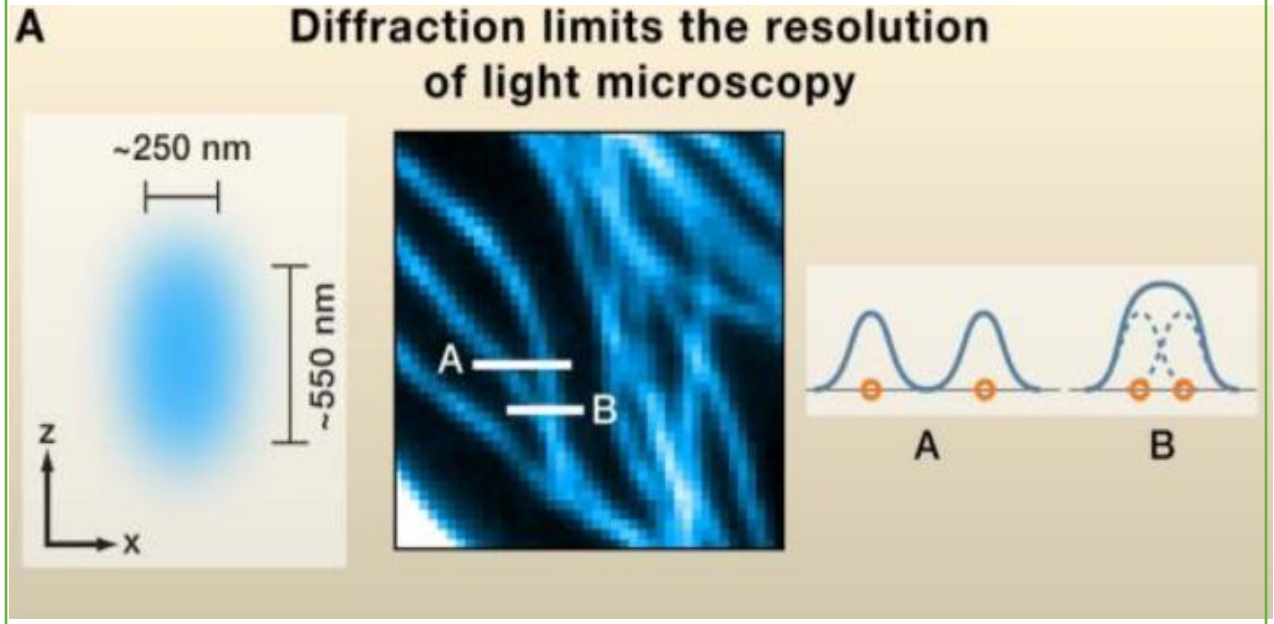
Diffraction $d = \frac{\lambda}{2n \sin\theta} = \frac{\lambda}{2\mathrm{NA}}$ = minimum resolvable distance

λ = wavelength of the light, n= refractive index
NA= numerical aperture of the lens

For eg., Green light around 500 nm, NA = 1,
the Abbe limit is roughly $d = \lambda/2$ = 250 nm (0.25 µm),

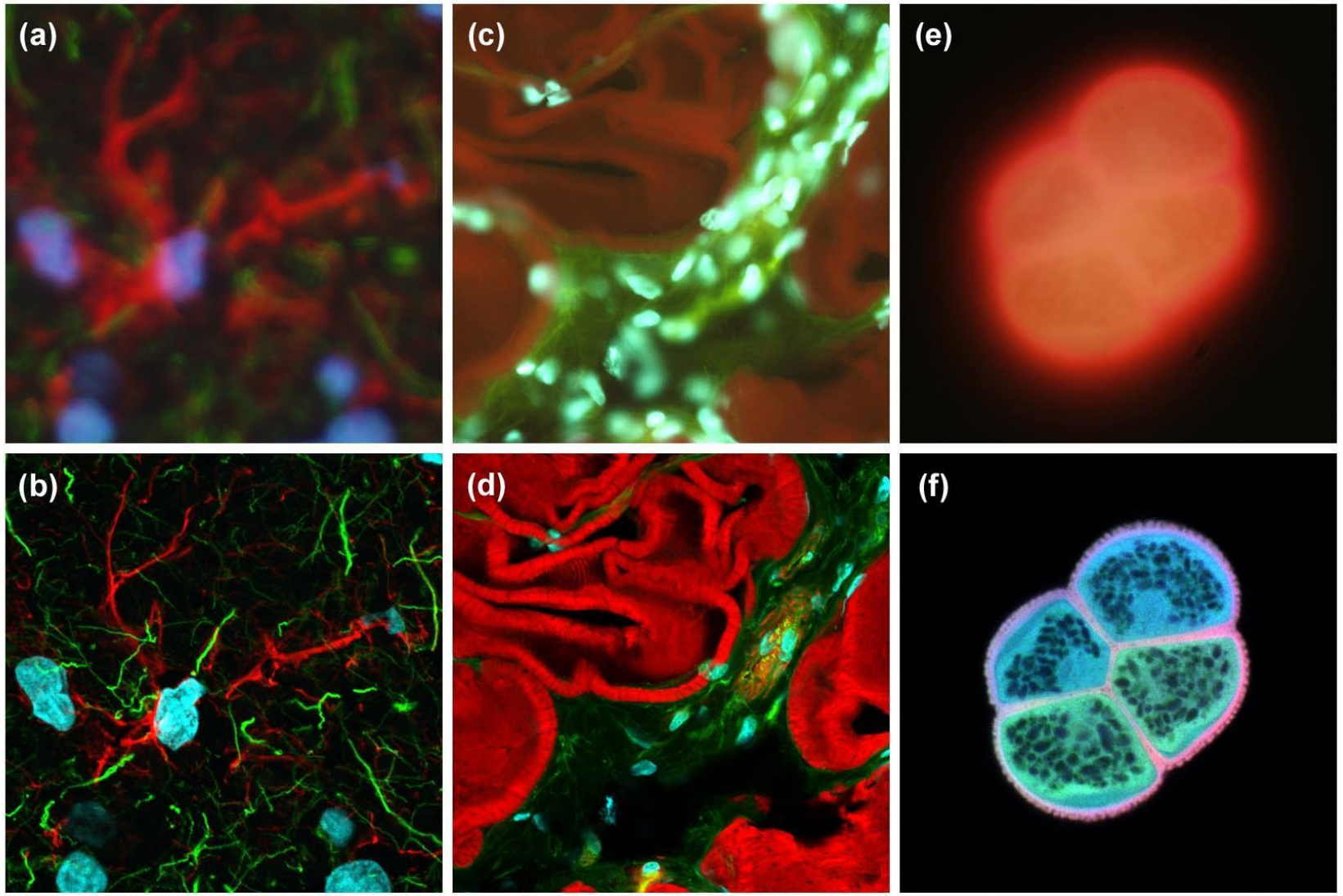


Two objects separated by a distance smaller than this resolution limit appear as single entities



Huang et al, Cell. 2010

Why Prefer Confocal Imaging ?



Widefield

Out of focus light ‘blurs’ image

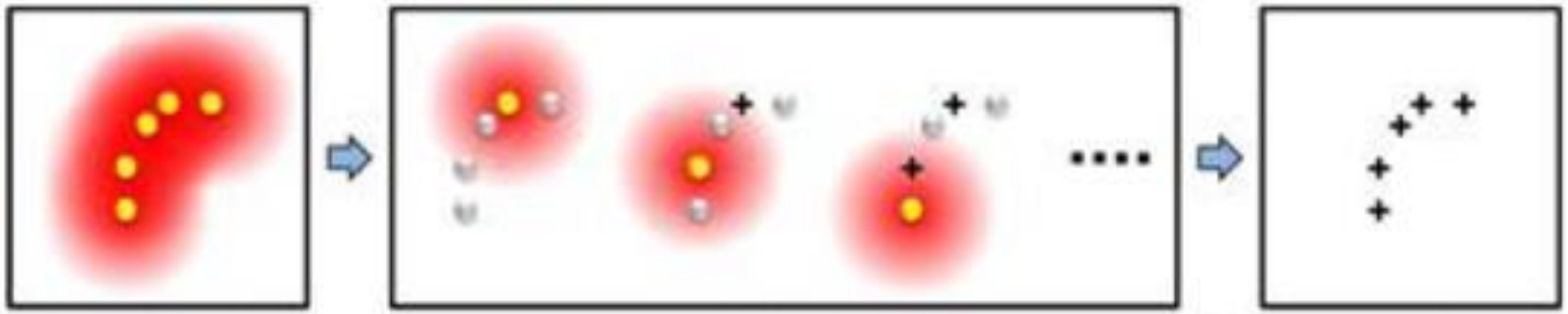
Confocal

Out of focus light is blocked

Super-Resolution microscopy

Umbrella term for various methodologies developed to **beat diffraction-limit**

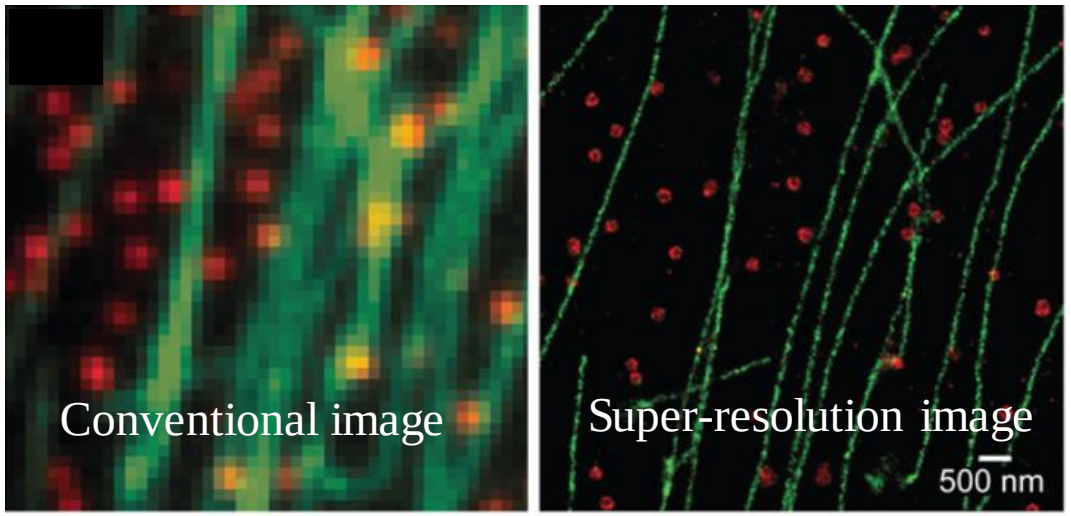
- **A physical or chemical property of the fluorophore is used to maintain neighboring molecules in different states (i.e., "on" and "off"), enabling them to be resolved from each other**



Fluorophores too close to resolve

Stochastic activation and localization of individual molecules

Super-resolution image reconstructed from localizations

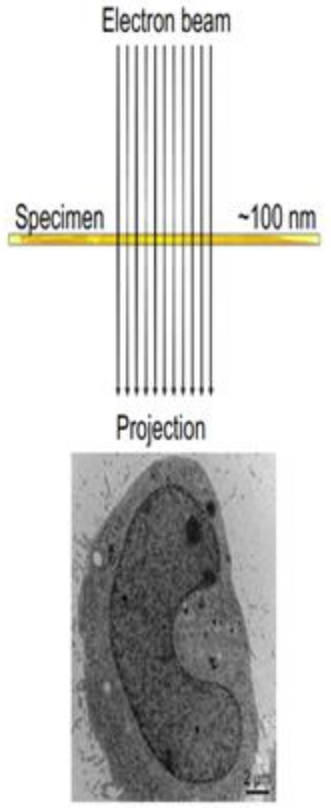


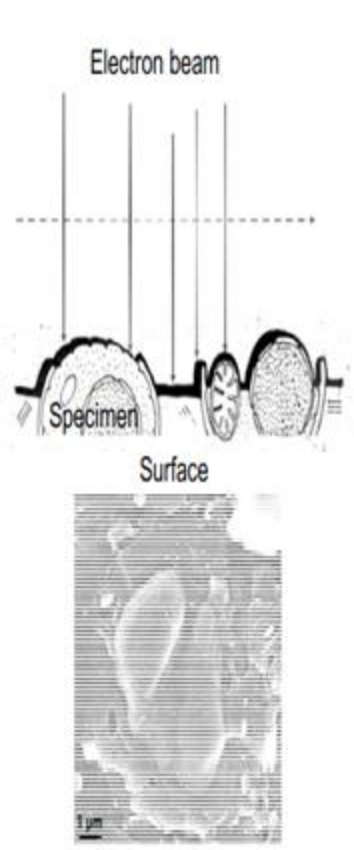
Immuno-stained microtubule (*green*) and clathrin-coated pits (*red*) *Huang et al., 2009*

Electron Microscopy

Transmission electron microscopy

Scanning electron microscopy





In the electron microscope, the light is substituted with electrons and the glass lenses are substituted with electromagnetic/electrostatic lenses.

Based on the sample being considered, SEM or TEM are used {Reference:https://www.zmb.uzh.ch/static/bio407/assets/Script_AK_2014.pdf}


THANK
YOU !