What is a cell

-Structural and functional unit of organisms.

Non-cellular organism: virus

1665: Robert Hook discovered cell from the piece of cork. He coined the term from the word: cellula (small compartment)

Antonie van Leewenhoek: living cells under microscope (animalcules: little animals)

1839: Schleiden & Schwann: cell theory: all living things are made of cells and cells are the basic structural and functional units of organisms.

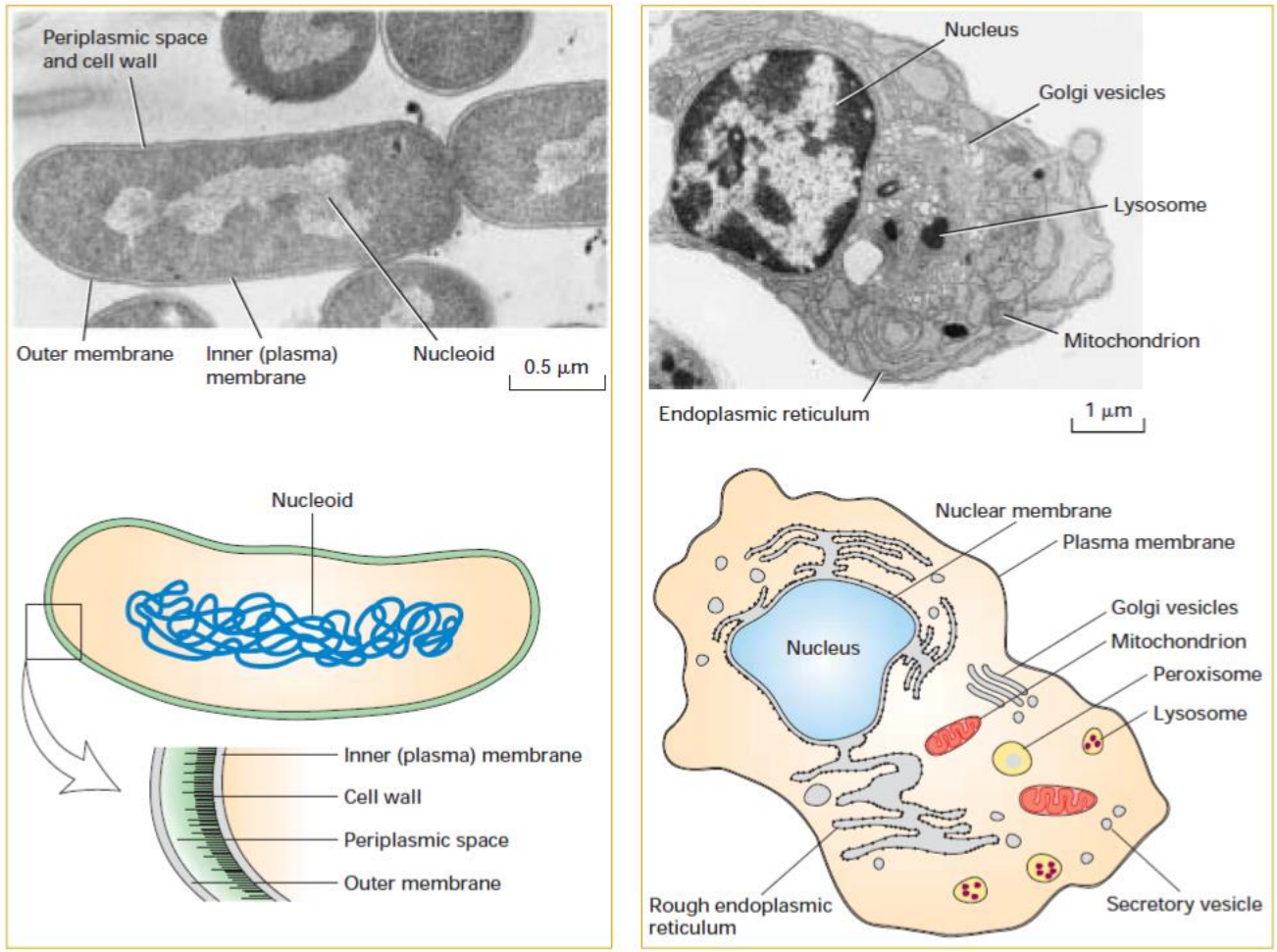
1855: Rudolf Virchow: extension of cell theory: all living cells arise from pre-existing cells

Modern cell theory:

1. All living things are made of cells.
2. Cells are the basic structural and functional units of organisms.
3. All living cells arise from pre-existing cells
4. Energy flow occurs within cells.
5. Cells contain hereditary information (DNA) that passes from cell to cell.
6. All cells have the same chemical composition.

Evolution of cell

1. Earliest cell: 3.5 billion years ago
2. Rich mixture of organic compounds (primordial soup)
3. Chemoheterotrophs
4. Derive energy from certain compounds & use that energy to synthesize precursor molecules
5. Photosynthetic cells (H_2S – H_2O)
6. Prokaryotic to eukaryotic (1.5 billion years ago)
7. Three changes: 1. More DNA-folding into complexes-equal division into daughter cells (chromosomes)
   2. Intracellular organelles & 3. Cells which were unable to photosynthesize went into symbioses.



▲ FIGURE 1-2 Prokaryotic cells have a simpler internal organization than eukaryotic cells. (a) Electron micrograph of a thin section of *Escherichia coli*, a common intestinal bacterium. The nucleoid, consisting of the bacterial DNA, is not enclosed within a membrane. *E. coli* and some other bacteria are

characteristic of eukaryotic cells is segregation of the cellular DNA within a defined nucleus, which is bounded by a double membrane. The outer nuclear membrane is continuous with the rough endoplasmic reticulum, a factory for assembling proteins. Golgi vesicles process and modify proteins, mitochondria generate

Features	Prokaryotic	Eukaryotic
True membrane bound nucleus	absent	present
DNA complexed with histone	absent	present
No of chromosomes	one	More than one
Mitosis & meiosis	absent	present
Genetic recombination	Partial (unidirectional transfer of DNA)	By crossing over during meiosis
Sterol in plasma membrane	Absent (except in mycoplasma)	present
Ribosome	70S	80S (cytosol) and 70S (organelles)
Unit membrane bound organelles	absent	present
Cell wall	Present; made of peptidoglycan in eubacteria	Made of cellulose in plant and chitin in fungi. Absent in animal cells.

Features (similarity)	Prokaryotic cells	Eukaryotic organelles
Nature of DNA	Ds circular	Ds circular
Histone protein	absent	absent
Ribosome type	70S	70S
Growth	Binary fission	Binary fission

Prokaryotic classification: based on phenotypic characteristics (eg. Morphology, biochemical characteristics)
Eukaryotic classification: evolutionary characteristics (fossil record)
Gene sequence based evolutionary classification: 16S rRNA gene (prokaryots)
Carl Woese: three domain structure (Eukaryots, Eubacteria and Archaea)
: five kingdom structure (Monera, Protista, Fungi, Plantae, Animalia)

Prokaryotic genome	Eukaryotic genome
Single circular chromosome	More than one chromosome
Monoploid	Diploid
One/more plasmids (extrachromosomal DNA: linear/circular)	Plasmids absent.
Transcription and translation occurs simultaneously (due to absence of nuclear membrane)	Separated in space and time (Tc in nucleus while Tl in cytoplasm)
Chromosomes: condensed form; no histone	Chromosomes: membrane-bound nucleus; complexed with histones
Small genome size (E. coli K12: 4.6 Mb)	Higher
Small amounts of non-coding and repetitive DNA	Interrupted with introns

Features	Prokaryotic ribosome	Eukaryotic ribosome
Overall size	70S	80S
Small subunit: No of proteins RNA size	30S 21 16S (1542 bp)	40S 33 18S (2300 bp)
Large subunit No of proteins RNA size	50S 31 23S (2904 bp) 5S (120 bp)	60S 46 28S (4718 bp) 5.8S (160 bp) 5S (120 bp)

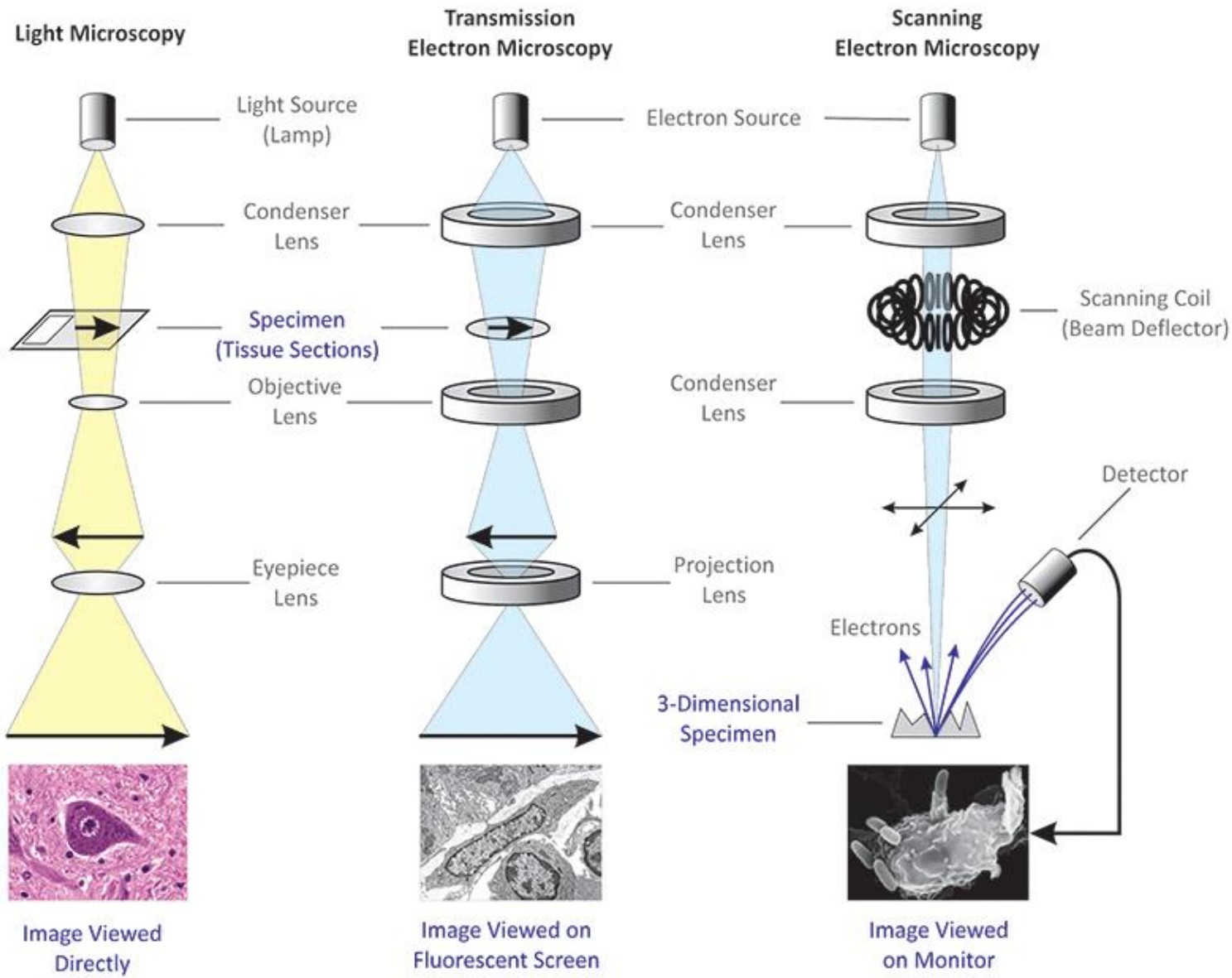
S = Svedberg unit/ sedimentation coefficient = velocity/acceleration
Unit = seconds
$1S = 1x10^{-13}$ s

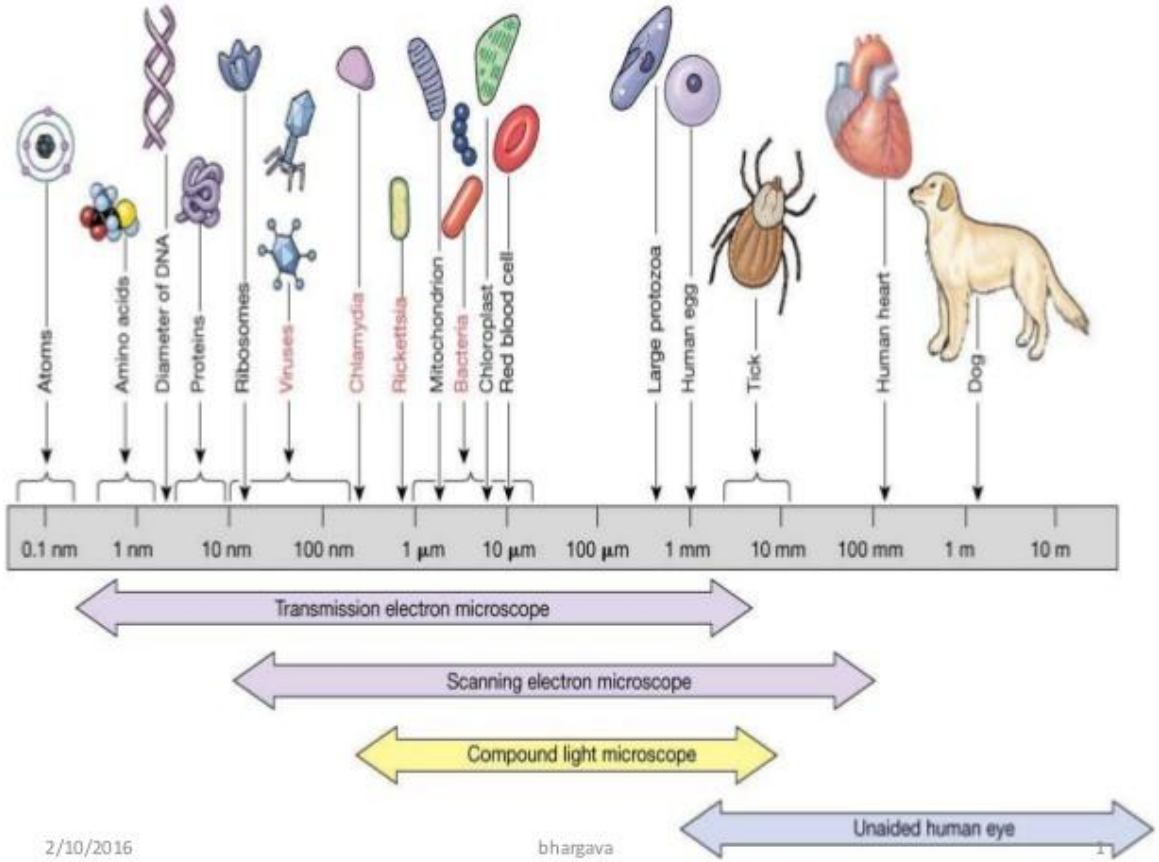
Characteristic	Archaea	Bacteria	Eukarya
Membrane lipids with branched hydrocarbons	✔		
Chromosomes are circular	✔	✔	
Lacks nuclear envelopes	✔	✔	
Lacks membrane bound organelles	✔	✔	
Methionine is the initiator amino acid for protein synthesis	✔		✔
Lack peptidoglycan in the cell wall	✔		✔
Growth not inhibited by streptomycin and chloramphenicol	✔		✔
Histones are associated with DNA	✔		✔
Contains several types of RNA polymerase	✔		✔

What is Archaebacteria

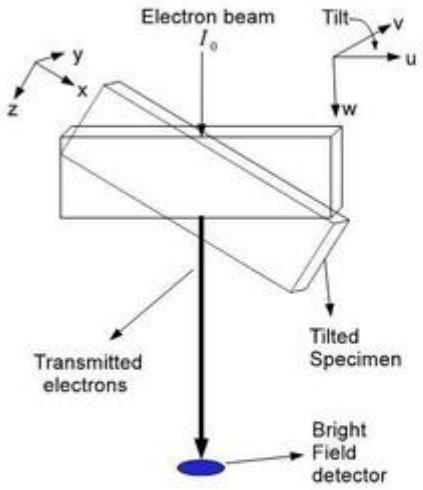
1. Unicellular microorganisms living in extreme environments
2. form a domain of kingdom monera and are found to evolve just after life. Hence, they are called ancient bacteria.
3. Archaebacteria are found in hot springs, salt lakes, oceans, marshlands and soils. They are also found in human skin, oral cavity and colon as well.
4. Archaebacteria play a vital role in carbon cycle and nitrogen cycle. Their pathogenic or parasitic effect is still not observed. Archaebacteria are metabolically diverse, using a variety of substrates as their energy and carbon sources.
5. Asexual reproduction of archaebacteria is identified, occurring by binary fission, budding and fragmentation.
6. Individual archaebacterium is 0.1-15 µmin diameter. Different shapes are, processed by archaebacteria like spheres, rods, plates and spirals. Some cells are flat or square shaped.
7. The cell wall of archaebacteria is made up of pseudopeptidoglycans. The membrane lipids of archaebacteria are ether linked, branched aliphatic chains, containing d-glycerol phosphates. According to the structure of cell wall, archaebacteria are more similar to gram positive bacteria.
8. Archaebacteria genome consists of a single circular chromosome, which exhibits transcription and translation similar to eukaryotes.
9. Three types of archaebacteria are found: methanogens, halophiles and thermophiles. Methanogens are found in oxygen, free environments like marshes, lake sediments and digestive tracts of animals, producing methane gas. Halophiles live in water with high concentrations of salts. Thermophiles live in hot water environments in acid sulfursprings.

Tools of cell biology



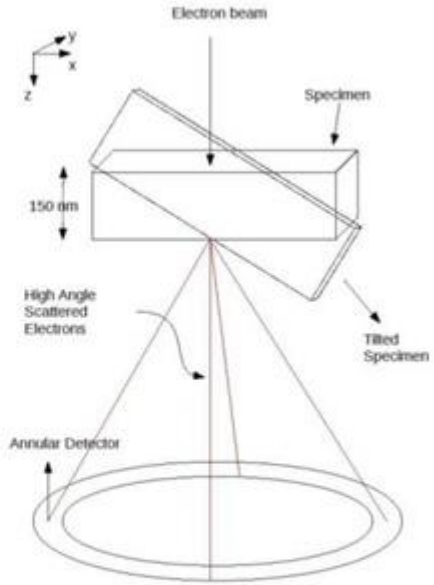


Bright Field (BF) vs. Dark Field Imaging



Bright Field:

Image is **bright** when sample is removed



Dark Field:

Image is **dark** when sample is removed

SIMPLE MICROSCOPE:

Bright-Field Microscopy

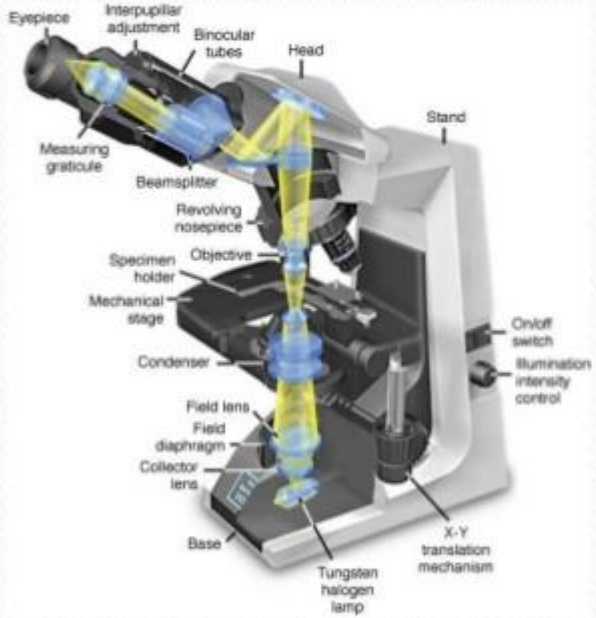
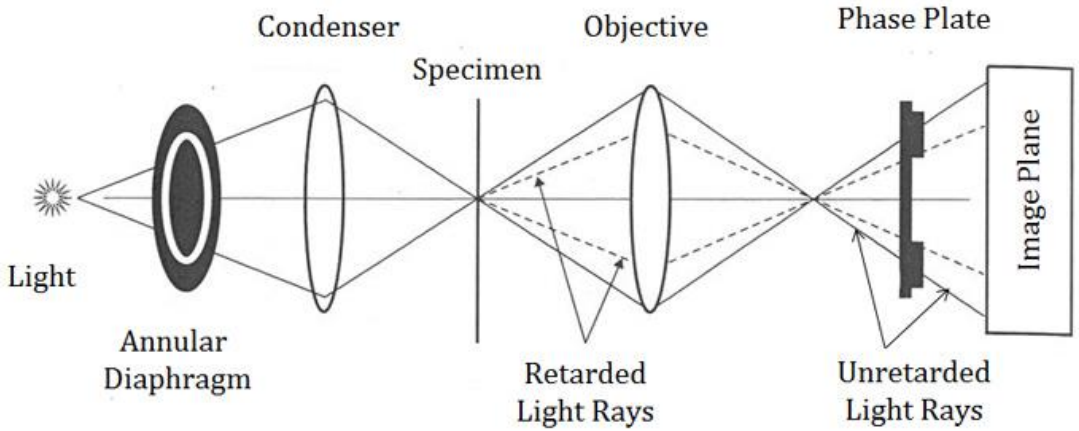
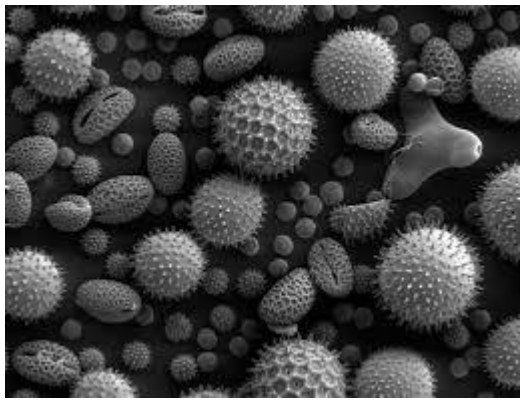


Figure 1-3: Bright-Field Microscope

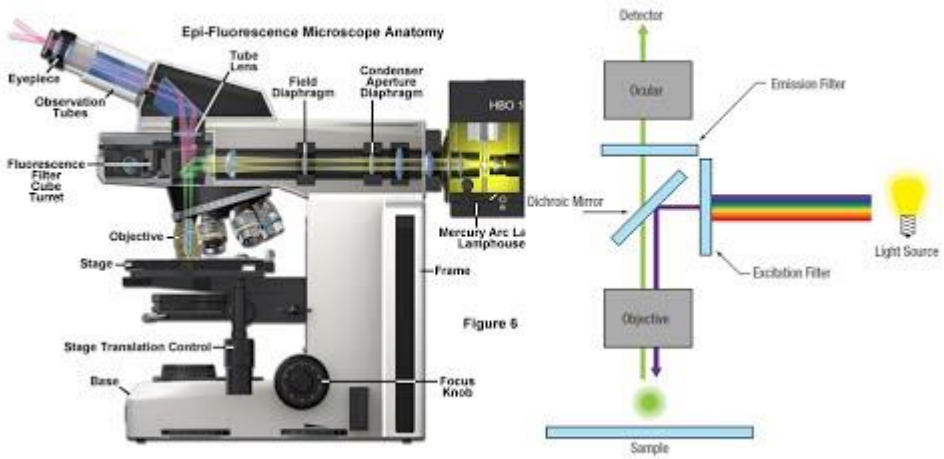
- **Objective lenses** enlarge & project the illuminated image of the object in the direction of the eyepiece.
  - For routine histological studies objectives having 3 different magnifications are generally used :
  - **X4** for low magnification observations of a large area (field) of the tissue.
  - **X10** for medium magnification of a smaller field.
  - **X40** for high magnification of more detailed areas.

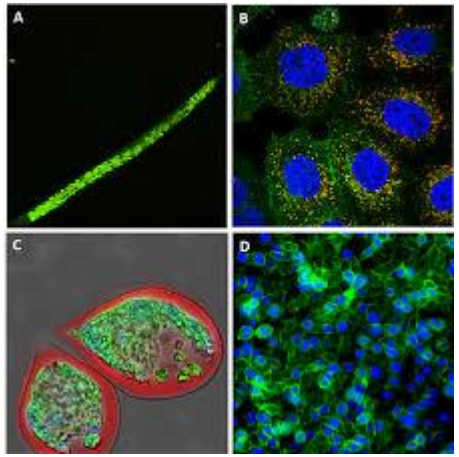
Phase Contrast Microscope

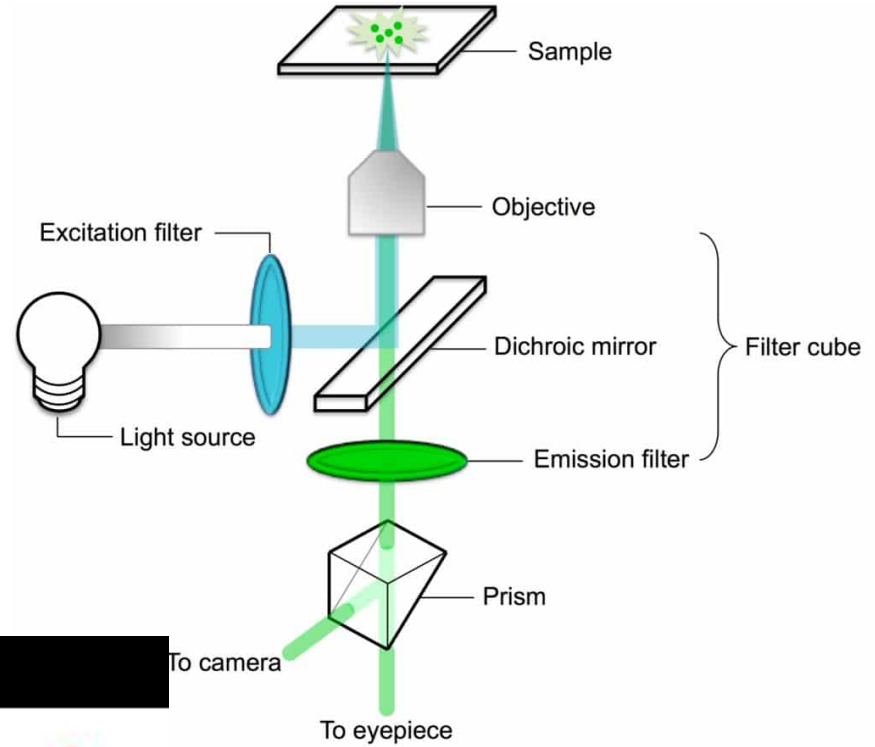




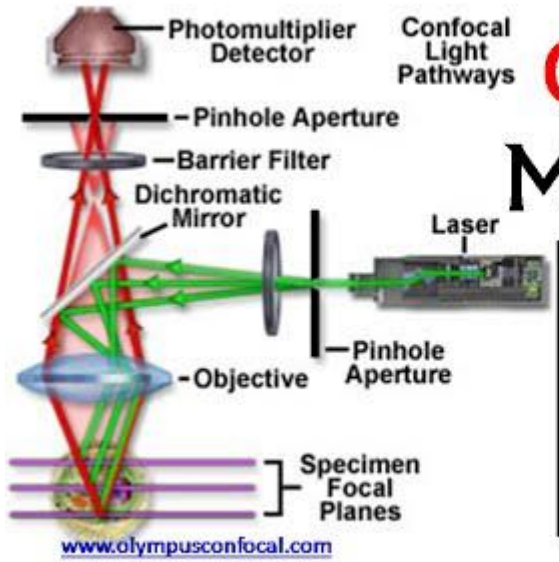
Fluorescence Microscopy

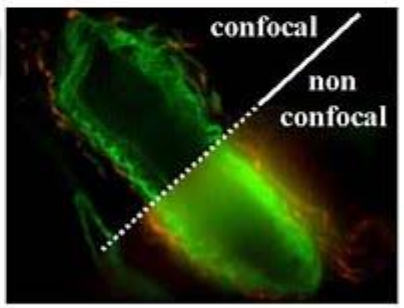






Confocal Microscopy





Cell fractionation is the process used to separate cellular components while preserving individual functions of each component.[1] This is a method that was originally used to demonstrate the cellular location of various biochemical processes. Other uses of subcellular fractionation is to provide an enriched source of a protein for further purification, and facilitate the diagnosis of various disease states.

(1) Homogenation: Tissue is typically homogenized in a buffer solution that is isotonic to stop osmotic damage. Mechanisms for homogenization include grinding, mincing, chopping, pressure changes, osmotic shock, freeze-thawing, and ultra-sound. The samples are then kept cold to prevent enzymatic damage. It is the formation of homogenous mass of cells (cell homogenate or cell suspension). It involves grinding of cells in a suitable medium in the presence of certain enzymes with correct pH, ionic composition, and temperature. For example, pectinase which digests middle lamella among plant cells.

(2) Filtration: This step may not be necessary depending on the source of the cells. Animal tissue however is likely to yield connective tissue which must be removed. Commonly, filtration is achieved either by pouring through gauze or with a suction filter and the relevant grade ceramic filter.

(3) Purification is achieved by differential centrifugation – the sequential increase in gravitational force results in the sequential separation of organelles according to their density.

Differential centrifugation (also **differential velocity centrifugation**) is a common procedure in biochemistry and cell biology used to separate organelles and other sub-cellular particles on the basis of sedimentation rate. Although often applied in biological analysis, differential centrifugation is a general technique also suitable for crude purification of non-living suspended particles (e.g. nanoparticles, colloidal particles, viruses).

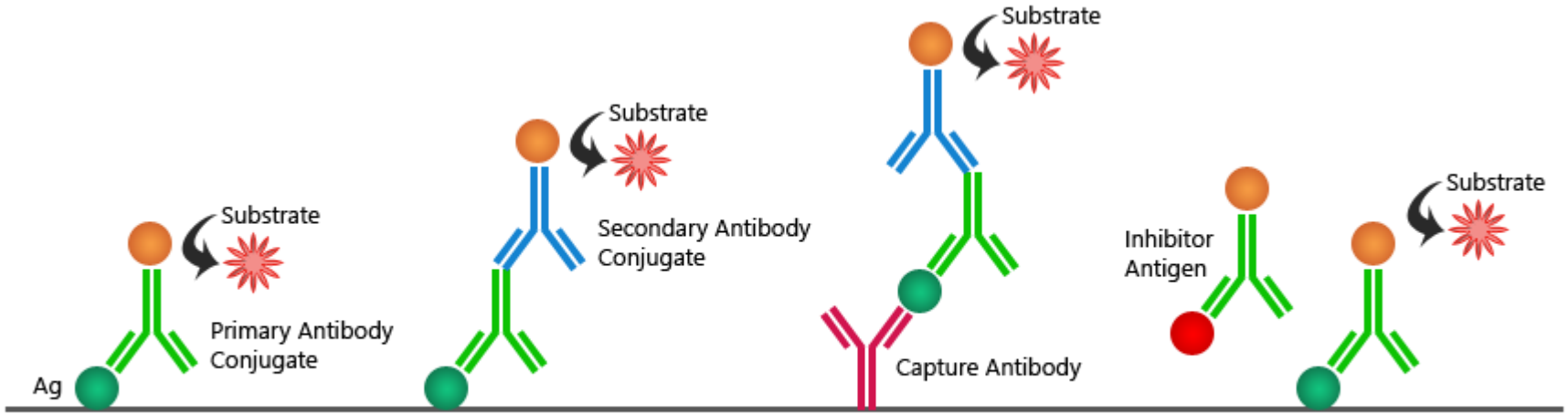
In a typical case where differential centrifugation is used to analyze cell-biological phenomena (e.g. organelle distribution), a tissue sample is first lysed to break the cell membranes and release the organelles and cytosol. The lysate is then subjected to repeated centrifugations, where particles that sediment sufficiently quickly at a given centrifugation force for a given time form a compact "pellet" at the bottom of the centrifugation tube. After each centrifugation, the *supernatant* (non-pelleted solution) is removed from the tube and re-centrifuged at an increased centrifugal force and/or time. Differential centrifugation is suitable for crude separations on the basis of sedimintation rate, but more fine grained purifications may be done on the basis of density through equilibrium density-gradient centrifugation

The **enzyme-linked immunosorbent assay** (**ELISA**) is a commonly used analytical biochemistry assay, first described by Engvall and Perlmann in 1972.[1] The assay uses a solid-phase **enzyme immunoassay** (**EIA**) to detect the presence of a ligand (commonly a protein) in a liquid sample using antibodies directed against the protein to be measured. ELISA has been used as a diagnostic tool in medicine, plant pathology, and biotechnology, as well as a quality control check in various industries.
In the most simple form of an ELISA, antigens from the sample are attached to a surface. Then, a matching antibody is applied over the surface so it can bind to the antigen. This antibody is linked to an enzyme, and in the final step, a substance containing the enzyme's substrate is added. The subsequent reaction produces a detectable signal, most commonly a color change.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are non-specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

The steps of **direct ELISA** follows the mechanism below:
•A buffered solution of the antigen to be tested for is added to each well (usually 96-well plates) of a microtiter plate, where it is given time to adhere to the plastic through charge interactions.
•A solution of nonreacting protein, such as bovine serum albumin or casein, is added to each well in order to cover any plastic surface in the well which remains uncoated by the antigen.
•The primary antibody with an attached (conjugated) enzyme is added, which binds specifically to the test antigen coating the well.
•A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme.
•The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength.

A "sandwich" ELISA is used to detect sample antigen.
The steps are:
1.A surface is prepared to which a known quantity of capture antibody is bound.
2.Any nonspecific binding sites on the surface are blocked.
3.The antigen-containing sample is applied to the plate, and captured by antibody.
4.The plate is washed to remove unbound antigen.
5.A specific antibody is added, and binds to antigen (hence the 'sandwich': the antigen is stuck between two antibodies). This primary antibody could also be in the serum of a donor to be tested for reactivity towards the antigen.
6.Enzyme-linked secondary antibodies are applied as detection antibodies that also bind specifically to the antibody's Fc region (nonspecific).
7.The plate is washed to remove the unbound antibody-enzyme conjugates.
8.A chemical is added to be converted by the enzyme into a color or fluorescent or electrochemical signal.
9.The absorbance or fluorescence or electrochemical signal (e.g., current) of the plate wells is measured to determine the presence and quantity of antigen.



DIRECT ELISA **INDIRECT ELISA** **SANDWICH ELISA** **COMPETITIVE ELISA**

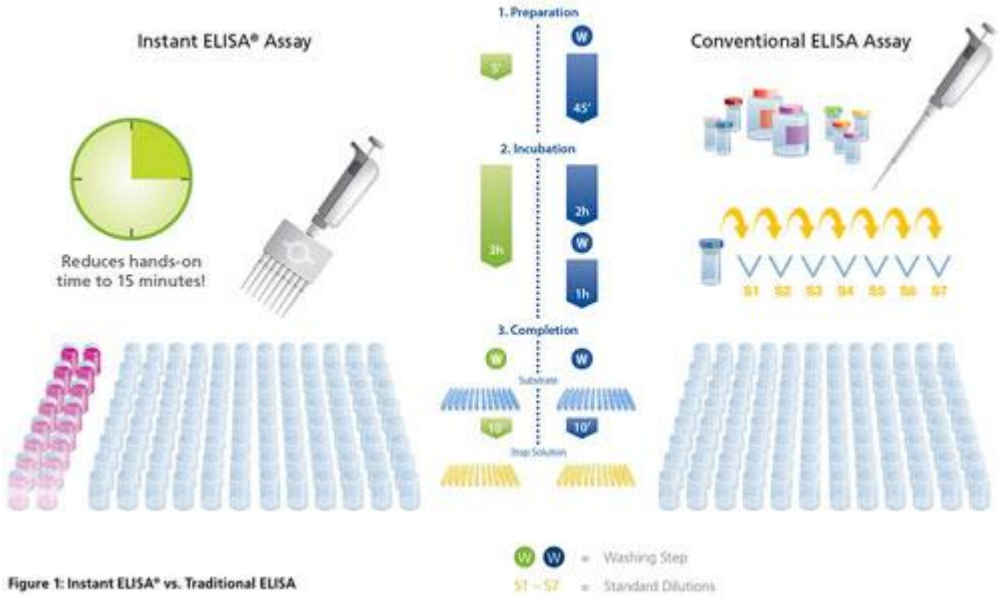


Figure 1: Instant ELISA® vs. Traditional ELISA