1. The Phase Contrast Microscope Enable the Examination of Unstained Cells and Tissues and Is Especially Useful for Living Cells

- The phase contrast microscope takes advantage of the fact that there are small differences in the index of refraction in different parts of a cell and in different parts of a tissue sample.
- Light passing through areas of relatively high refractive index (denser areas) is deflected and becomes out of phase with the rest of the beam of light that has passed through the specimen.
- By adding other induced-out-of-phase wavelengths by the use of a series of optical rings in the condenser and objective lenses, the phase contrast microscope essentially abolishes the amplitude of the initially deflected portion of the beam and produces a useful amount of contrast in the image. Dark portions of the image correspond to dense portions of the specimen, light portion of the image correspond to less dense portions of the specimen.
- The phase contrast microscope is, therefore, used to examine living cells and tissues, such as cells in tissue culture, and is used extensively to examine unstained semithin (approximately 0,5 μm) section of plastic embedded tissue.

2. In Dark-field Microscopy, NO Direct Light From the Light Source Is Gathered by the Objective Lens

- In dark-field microscopy, only light that has been scattered or diffracted by structures in the specimen reaches the objective.
- To achieve this, the dark-fields microscope is equipped with a special condenser that illuminates the specimen with strong, oblique light. Thus the field of view appears as a dark background on which small particles in the specimen that reflect some light into objective appear bright.
- The effect is similar to dust particles that are seen in the light beam emanating from a slide projector in a darkened room. The reflected light from the dust particles reaches the retina of the eye, thus making the particles visible.
- The resolution of the dark-field microscope cannot be better than that of the bright-field microscope, using as it does, the same wavelength source. Smaller individual particles can be detected in dark-field images, however, because of the enhanced contrast that is created.
- The dark-field microscope is useful in examining autoradiographs, in which the developed silver grains appear white in a dark background. Clinically, it is useful in examining urine for crystals, such as those of uric acid and oxalate, and in demonstrating spirochetes, particularly Treponema pallidum, the organism that causes syphilis, a sexually transmitted disease.

3. The Polarizing Microscope Utilizes the Fact Highly Ordered Molecules or Arrays of Molecules Can Rotate the Angle of the Plane of Polarized Light

- The polarizing microscope is a simple modification of the light microscope in which a polarizing filter, called the polarizer, is located between the light source and the specimen and a second polarizer, called the analyzer, is located between the objective lens and the viewer.
- Both the polarizer and the analyzer can be rotated; the difference between their angles of rotation is used to determine the degree by which a structure affects the beam of polarized light.
- The ability of a crystal of para-crystalline array to rotate the plane of polarized light is called birefringence (double refraction). Striated muscle and the crystalloid inclusions in the testicular interstitial cells (Leydig cells), among other common structures, exhibit birefringence.
- We can study:
    a) Structures with linear shape – collagen, fibers, myelin, muscle fibers;
    b) Structures with radial symmetry – protein granules, cholesterol
    c) Biological membranes

4. The Fluorescence Microscope Utilizes the Fact That Certain Molecules Fluoresce Under Ultraviolet Light

- A molecule that fluoresces emits light of wavelengths in the visible range when exposed to an ultraviolet (UV) source. The fluoresces microscope is used to display naturally occurring fluorescents (auto-fluorescent) molecules, such as vitamin A and some neurotransmitters.
- Various filters are inserted between the UV light source and the specimen to produce monochromatic or near-monochromatic light. A second set of filters inserted between the specimen and the fluorescence to reach the eye or to reach a photographic emulsion or other analytic processor.
- With primarily fluorescence aid we can identify:

a) Pigments – porphyrins (red fluorescence),
- lipofuscin (red-brown fluorescence)

b) amino acids – tyrosine (blue fluorescence)
c) viruses and Koch bacillus (green fluorescence)
d) biogenic amine; adrenaline, noradrenaline, serotonin
e) natural tooth is auto fluorescence

- Sustained by fluorochroming acridine orange made we can identify:

a) Nucleic acid – RNA (red fluorescence) and DNA (yellow green fluorescence)
b) Elastic and reticular collagen fibers (green fluorescence)
c) The nucleus of leukocytes (green fluorescence)
d) Mucins (green fluorescence)

- Acridin orange staining is used in precocious cancer cytodiagnosis

!!! The most important application is immunofluorescence that is based on the coupling of an acid with a green fluorescence; by induced fluorescence it can by identify the localization of the antigens into the cells.

5. **The Ultraviolet Microscope Uses Quartz Lenses With an Ultraviolet Light Source**

- The image in the UV microscope is dependent on the absorption of UV light by molecules in the specimen. The UV source has a wavelength of approximately 200nm. Thus it may achieve a resolution of 0,1 µm.
- In principle, UV microscopy is not unlike the workings of a spectrophotometer; the results are usually recorded photographically. The specimen cannot be inspected directly through an ocular because the UV light is not visible and is injurious to the eye.
- The method is useful in detecting nucleic acids, specifically the purine and pyrimidine bases of the nucleotide. It is also useful for detecting proteins that contain certain amino acids. Using specific illuminating wavelengths, UV spectrophotometric measurements are commonly made through the UV microscope to determine quantitatively the amount of DNA and RNA in individual cells. It is used clinically to evaluate the degree of ploidy (multiples of normal DNA quantity) in section of tumors.