Methods for Cytological Examination

1. The photonic microscope

A microscope, whether simple (one lens) or compound (multiple lenses), is an instrument that magnifies an image and allows visualization of greater detail that is possible with the unaided eye. The simplest microscope is a magnifying glass or a pair of reading glasses.

The resolving power of the human eye, the distance by which two objects must be separated to be seen as two objects (0,2 mm) is determined by the spacing of the photoreceptor cells in the retina.

The role of a microscope is to magnify an image to a level at which the retina can resolve the information that would otherwise be below its limit of resolution. Table 1 compares the resolution of the eye to various instruments:

	Distance between Resolvable points
Human eye	0,2 mm
Bright-field microscope	0,2 um
SEM	2,5 nm
TEM	
Theoretical	0,05 nm
Tissue section	1,0 nm

Closely Positioned Objects Resolving Power Is the Ability of a Microscope Lens or Optical System to Produce Separate Images of

The resolution depends not only on the optical system but also on the wavelength of the light source and on other factors, such as specimen thickness, quality of fixation, and staining intensity.

With light whose wavelength is 540 nm, a green-filtered light to which the eye is extremely sensitive, and with appropriate objective and condenser lenses, the greatest attainable resolving power of a bright-field microscope would be about 0,2 um. This is the theoretical resolution and, as already mentioned, depends on all condition being optimal.

The ocular lens magnifies the image produced by the objective, but it cannot increase resolution.

The Microscope Used by Both Students and Researchers Is Referred to as the Bright-field Microscope

The bright-field microscope is the direct descendant of the microscopes that became widely available in the 1800s and opened the first major era of research in histology. The bright-field microscope essentialy consist of:

- The mechanical part
- The optical part
- The light source

1. **The mechanical part**

It is steel made and represents the support for the optical part and the light source

It is composed of:

a) The base - it maintains the stability of the apparatus; inside it there is the light source for illumination of the specimen, the reflecting mirror and the filed diaphragm.
b) The support - it is attached to the base of the microscope by a joint device. It consist of: the mechanical stage, the binocular eyepiece tube and the precision focusing mechanism. The mechanical stage is the device on which the specimen is fixed.

The precision focusing mechanism is necessary to obtain clear images realized by vertical movements (up and down) of the objectives eyepiece system(the tube). This movement is done by two knobs:

- The course focusing knob- for rapid, coarsely movements.
- The fine focusing knob- for slow movements, used to clarify the image.

2. The optical part

It is formed of the eyepiece and the objective system

a) The eyepiece system: It is formed of two lenses assembled in a short metallic tube introduced to the superior extremity of the microscope tube. The ML4 microscope has eyepieces available at 5x, 7x, 10x and 15x magnification.
b) The objective system: It is formed by a lens association, mounted in a metallic tube, screwed up on the revolving nosepiece. The ML4 microscope have dry objectives which magnify the image by 6x, 10x, 20x, 40x and immersion objective lens that magnify the image by 90x and 100x.

3. The Light Source and the Device to Transmit Light

The light source is a 6V bulb that is included in the base.

The reflecting mirror is included in the microscope's base. It is plane and directs the light rays to the microscope optic axis, by the movement of two switches. Between the light source and the mirror there is a field diaphragm.

The light condenser is a lens system mounted on a mobile device that is under the microscope's stage. It focus the beam of light on the specimen.

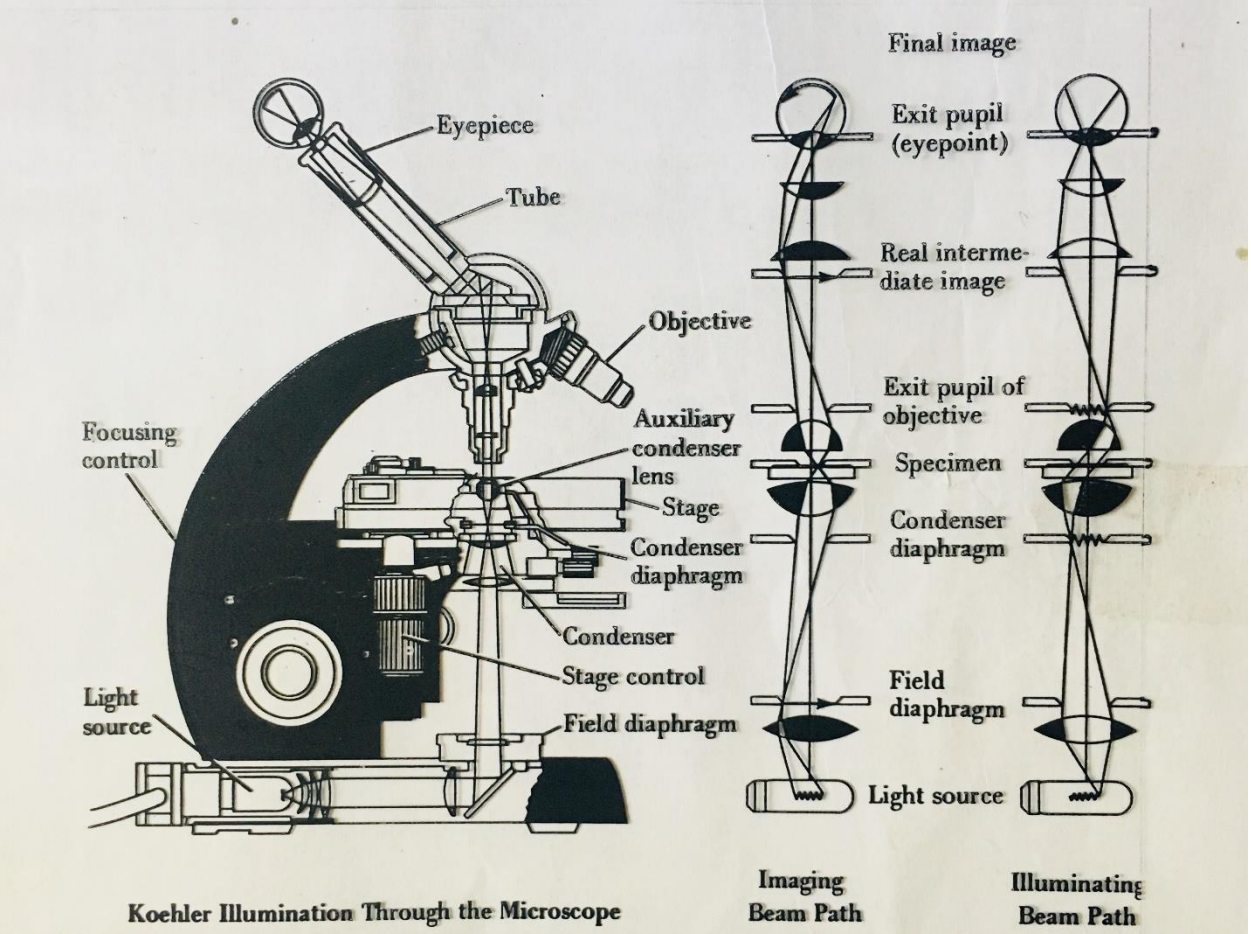

Figure 1 Diagram of a modern light microscope showing a cross-sectional view of its operating components and light path. (Courtesy of Carl Zeiss, Inc., Thornwood, NY.)

WORK TECHNIQUES

- We connect the microscop to 220V electric source
- By revealing rotation, we put the 10x objective lens in the microscope optic axis. We move down the condenser until we obtain an uniform light with high intensity.
- We put the specimen on the stage, positioning it with the spring-loaded clamps, with the coarse focusing focal distance (for the 10x objective that’s 16mm).
- We fix the examination interpupilar distance by approaching or moving off the two ocular tubes

- Looking through the eyepiece tubes we move up the coarse focusing knob, until the image appears in the microscope field; if we don't observe anything, it means that the objective isn't in the objective axis, the specimen isn't in the microscope field or we moved up too fast optic system. By moving the fine focusing knob, we obtain the clear image.
- The use of the immersion objective requires the condenser maximum moving up and the aperture diaphragm completely opened on the specimen, we put a drop of cedar oil. Rotating the revolving head, we bring the immersion objective in the optic axis. Looking laterally, we descend the tube with the coarse oil and it is in the field of the specimen.
- After the examination, we move up the microscope's tube with 1-2 cm, we take off the specimen and we clean the specimen with xilol or benzen impregnate wadding.
- After we finish using it, we disconnect the microscope from the electric main and cover it.

A specimen to be examined with the bright-field microscope must be sufficiently thin for light to pass through it. Although some light is absorbed while passing through the specimen, the optical system of the bright-field microscope does not produce a useful level of contrast in the unstained specimen. For this reason, the various staining methods discussed earlier are employed. Other optical system, described below, may be used to enhance the contrast without staining.

2. The Phase Contrast Microscope Enables 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 od 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 portion of the specimen, light portions of the image correspond to less dense portions of the specimen.

He 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 um) section of plastic embedded tissue.

3. 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 dark background on which small particles in the specimen that reflect some light into the 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.

4. **The Polarizing Microscope Utilizes the Fact That 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 objectives lens and the viewer.

Both the polarizer and the analyze 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 or paracrystalline array to rotate the plane of the plane of polarized light is called birefringence (double refraction). Striated muscle and the crystalloid inclusion in the testicular interstitial cells (Leydig cells), among other common structures, exhibit birefringence.

We can study:

- Structures with linear shape – collagen fibers, myelin, muscle fibers
- Structures with radial symmetry – proteic granules, cholesterol
- Biological membranes

5. **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 fluorescence microscope is used to display naturally occurring fluorescent (autofluorescent) 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 objective allows only the narrow band of wavelength of the fluorescence to reach the eye or to reach a photographic emulsion or other analytic processor.

With primarily fluorescence aid we can identify:

- Pigments – porphyrins (red fluorescence), lipofushine (red-brown fluorescence)
- Amined acids – tyrosine (blue fluorescence)
- Viruses and Koch bacillus (green fluorescence)
- Biogene amines: adrenaline, noradrenaline, serotonine
- Natural tooth is autofluorescent

Sustained by fluorochroming acridine orange made we can identify:

- Nucleic acid – RNA (red fluorescence) and DNA (yellow green fluorescence)
- Elastic and reticular collagen fibers (green fluorescence)
- The nucleus of leukocytes (green fluorescence)
- 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 fluorochrome; by induced fluorescence it can by identify the localization of the antigens into the cells.

6. 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 200 nm. Thus it may achieve a resolution of 0,1 um.

In principle, UV microscopy in 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 aminoacids. Using specific illuminiating 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.