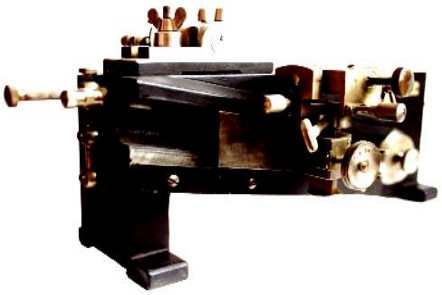
Seminar: Microscope, histological technique.
Practical class: Various cell types.



- **fibroblasts (slide # 97),**
- **isolated cells from smooth muscles (slide # 19),**
- **nerve cells impregnated with silver nitrate (slide # 112),**
- **proper use of the light microscope (text # 21),**

Text # 21.

PROPER USE OF THE LIGHT MICROSCOPE

This brief introduction to the proper use of the light microscope is directed to those students who will be utilizing the microscope for the routine examination of tissues. If the following comments appear elementary, it is only because so frequently many users of the microscope fail to use it to its fullest advantage. Despite the availability of today's fine equipment and its widespread use, there is relatively little formal instruction on the use of the light microscope.

Expensive and highly corrected optics can perform optimally only when the illumination and observation beam paths are centered and correctly adjusted. The use of proper settings and proper alignment of the optic pathway will con-tribute substantially to the recognition of minute details in the specimen and to the faithful display of color for the visual image and for photomicrography.

Kohler illumination is one key to good microscopy and is incorporated in the design of practically all modern laboratory and research microscopes. Figure 1.9 shows the two light paths and all the controls for alignment on a modem laboratory microscope and should be referred to in following the instructions given below to provide appropriate illumination in your microscope.-

The alignment steps necessary to achieve good Kohler illumination are few and simple. They are:

1. Focus the specimen.
2. Close the field diaphragm.
3. Focus the condenser by moving it up or down until the outline of its field diaphragm appears in sharp focus.
4. Center the field diaphragm with the centering controls on the (condenser) substage. Then open the field diaphragm until it covers the full field observed.
5. Remove the eyepiece (or use a centering telescope or a phase telescope accessory if available) and observe the exit pupil of the objective. You will see an illuminated circular field, the radlus of which is directly proportional to the numerical aperture of the objective. As you close the condenser diaphragm, its outline will appear in this circular field. For most stained materials, set the condenser diaphragm to cover approximately two-thirds of the objective aperture. This setting results in the best compromise between resolution and contrast.

By using only these five simple steps, the image obtained will be as good as the optics allow. Now let us find out why.

Principles of bright-field microscopy. First, why do we adjust the field diaphragm to cover only the field observed? Illuminating a larger field than the optics can "see" only leads to internal reflections or stray light, resulting in more "noise" or a decrease in Image contrast.

Second, why is there emphasis on the setting of the condenser diaphragm or, in other words, the illuminating aperture? This diaphragm greatly influences the resolution and the contrast with which specimen detail can be observed.

For most practical applications the resolution is determined by

$$d = \frac{\lambda}{NA_{objectiv} \quad e + NA_{condense} \quad r}$$

where d = point-to-point distance of resolved detail (in nm)
λ = wavelength of light used (green =540 nm)
NA = numerical aperture or sine of half angle picked up by the objective (condenser) of a central specimen point multiplied by the refractive index of the medium between objective (condenser) and specimen

How do wavelength and numerical aperture directly influence resolution? Specimen structures diffract light. The diffraction angle is directly proportional to the wavelength and inversely proportional to the spacing of the structures. According to Ernst Abbe, a given structural spacing can be resolved only when the observing optical system (objective) can see some of the diffracted light produced by the spacing. The larger the objective's aperture, the more diffracted light participates in the image formation, resulting in resolution of smaller detail and sharper Images.

Our simple formula, however, shows that the condenser aperture. Is just as important as the objective's aperture. This is only logical when you consider the diffraction angle for an oblique beam or one of higher aperture. This angle remains essentially constant, but is represented to the objective in such a fashion that it can be picked up easily

How does the aperture setting affect the contrast (contrast simply being the Intensity difference between dark and light areas in the specimen)? The closest to the real contrast transfer from object to image theoretically would be obtained by the interaction (interference) between nondiffracted and all the diffracted wavefronts.

For the transfer of contrast between full transmission and complete absorption in a specimen, the intensity relationship between diffracted and nondiffracted light would have to be 1:1 in order to get full destructive Interference (black) or full constructive Interference (bright). When the condenser aperture matches the objective aperture the nondiffracted light enters the objective with full Intensity, but only part of the diffracted light can enter, resulting in decreased contrast. In other words, closing the aperture of the condenser to two-thirds of the objective aperture brings the Intensity relationship between diffracted and nondiffracted light close to 1:1 and thereby optimizes the contrast. Closing the condenser aperture (or lowering the condenser) beyond this equilibrium will produce Interference phenomena or Image artifacts such as diffraction rings or lines around specimen structures. Most microscope techniques used for the enhancement of contrast, such as dark field, oblique illumination, phase contrast, or modulation contrast, are based on the same principle, that is, they suppress or reduce the intensity of the nondiffracted light to improve an inherently low specimen contrast.

By observing the steps outlined above and maintaining clean lenses, the quality and fidelity of visual images will vary only with the performance capability of the optical system.

M. H. Ross, L. J. Romrell *HISTOLOGY - Williams & Wilkins, Baltimore 1989*

Obligatory literature:

1. Junqueira's Basic Histology: Text and Atlas, last edition
2. Gartner L. P., “Textbook of Histology”, Elsevier, last edition.
3. Sadler T. W. “Langman’s Medical Embryology”, 2015, Wolters Kluwer Health, thirteenth edition.
4. Daniel J. Chiego, Jr.: “Essentials of Oral Histology and Embryology”: A Clinical Approach, Elsevier 4th edition, 2014

Supplementary literature:

1. Stevens A., Lowe J. “Human Histology” 2005, Elsevier Mosby, third ed.
2. Ross M.H., Pawlina W. “Histology: A text and atlas”, 2011, Lippincott Williams & Wilkins, sixth ed.
3. Schoenwolf, Bleyl, Brauer, Francis-West “Larsen's Human Embryology” 5th Ed.
4. Nanci A. “Ten Cate’s - Oral Histology”, 2008, Elsevier, seventh edition or newer