Theme 12. Physics models of optical electromagnetic radiation. Physics concepts of optical instruments structures basing on geometrical optics laws: endoscope; optical microscope. Biological objects optical microscopy

Problem
Biological structures and tissues conditions definitive diagnostic test

Attendance prerequisite
Note! Answer in writing to perform

1. Define or explain: wavelength of a beam of light; light ray; wave front; light pipe; diffuse and specula reflection; angles of incidence, reflection, and refraction; lenses; focal length; lens axis; thin lens formula

Information resources

№	Author(s)	Name of the source (textbook, manual, monograph, etc)	City, publishing house
1	R. M. Berne, M. N. Levy	Physiology	St Louis: Mosby Company, 1983
2	Vander, Sherman, Luciano	Human Physiology The Mechanisms of Body Function	New York: McGraw-Hill Book Company, 1980
3	Vander, Sherman, Luciano	Human Physiology The Mechanisms of Body Function	New York: McGraw-Hill Book Company, 1985
4	N. V. Pronina	Biological Physics The Second Module Lectures	Simferopol, 2006
5	Douglas C. Giancoli	Physics Principles with Applications	Pearson Education Limited; 7th Edition, 2016
6	Martin Hollins	Medical Physics	Tomas Nelson & Sons, 1992
7	I. Tarjan	An Introduction to Physics with Medical Orientation	Akademiai Kiado, Budapest, 1987
8	Joseph W. Kane, Morton M. Sternheim	Physics	John Wiley & Sons Third Edition, 1988
9	John Bullock, Joseph Boyle, Michael Wang	Physiology	Williams & Wilkins Third Edition, 1994

Introduction

The investigation of the human condition using light can hardly be avoided. All patients are seen by their doctors and external appearances are used in many diagnoses. Internal observation could obviously bring much additional information. Specially focused beams enable internal viewing of the eye (with an ophthalmoscope) and the ear (with an auroscope). In most cases a tube (an endoscope) has to be inserted into the orifice, to see inside the body.

The endoscope is basically a tube for looking into the body. The invention of the optical fiber in 1960s came as a real break-through for the science on endoscopy and a great relief to patients.

Reflection of light. Electromagnetic waves of any frequency travel in a vacuum with the same velocity, $\mathbf{c} = 3 \cdot 10^8$ m/s**.** In a material medium, the velocity **v** depends on the frequency of

the wave, but it is never greater than the velocity **c** in the vacuum. The ratio of these velocities is the index of refraction of the medium **n = c/v**.

When a beam of light reaches the boundary separating two media, some light is **transmitted**, some is **absorbed**, and the remainder is **reflected**. The smooth surface of a piece of glass or polished metal reflects light in a particular direction. This is called **specula reflection**. Sometimes the reflection is **diffuse**, and the reflected light travels in all directions. This happens when light strikes a surface such as a sheet of paper or a painted wall with random irregularities that are large compared to a wavelength. Each smooth section of such a surface produces specular reflection, but because of the varying orientations of the sections, the total reflected beam has no unique direction.

In specula reflection (fig.1), the directions of the incident rays relative to the normal to the surface are related very simply:

The reflected light rays are in the same plane as the incident rays and the normal, and make the same angle with the normal.

The intensity $\mathbf{I_r}$ of the reflected wave is determined by the refractive indices $\mathbf{n_1}$ and $\mathbf{n_2}$ of media.

The **reflectance R** is defined as the ratio of reflecting light intensity $\mathbf{I_r}$ to incident light intensity $\mathbf{I_0}$: $\mathbf{R = I_r / I_0}$.

At normal incidence in either direction, the reflectance is $\mathbf{R = (n_2 - n_1)^2 / (n_2 + n_1)^2}$.

This formula is also approximately correct if the angle of incidence is small but not zero. About 4 percent of the light is reflected when light is normally incident from air into glass.

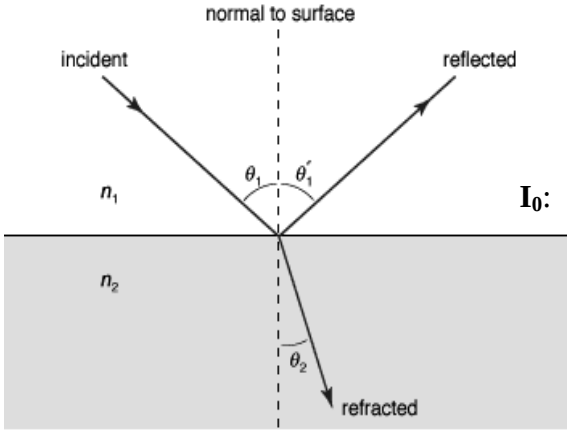

Figure 1

In a microscope or camera with several lenses, about 4 percent of the intensity is lost at each lens surface. Different methods are used to diminish these losses.

Refraction of light. When light rays go from one transparent medium to another with a different index of refraction they are bent or **refracted** (fig.1). As in the case of reflection, it is possible to find a relationship between the two directions. This relationship is called **Snell's law** after Willebrond Snell (1591-1626) who discovered it experimentally. If the indexes of refraction of the media are $\mathbf{n_1}$ and $\mathbf{n_2}$, and angle of incidence, $\mathbf{\theta_1}$, and angle of refraction, $\mathbf{\theta_2}$, are measured relative to the normal direction, then **Snell's law** states: $\mathbf{n_1 \sin\theta_1 = n_2 \sin\theta_2}$.

As in case of reflection, **both rays and the normal are in the same plane.**

Snell's law implies that if **n** increases, then $\sin\theta$ and consequently θ decrease. Thus, a ray bends away from the normal when it enters a rarer medium ($n_2 < n_1$) (fig. 2).

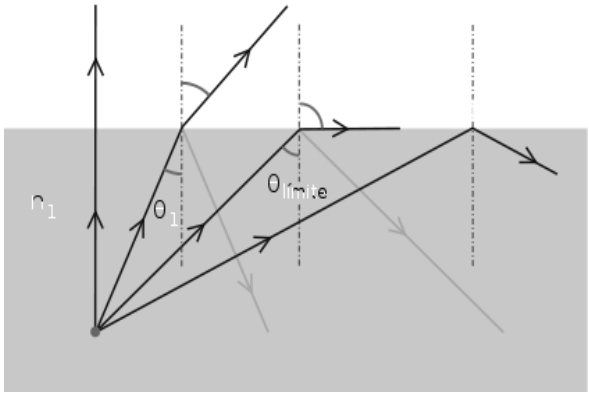

Figure 2

Total internal reflection. Figure 2 shows what happens to rays from a light source inside a tank of water. When the rays reach the surface, some of the light is reflected and some is refracted. As the angle of incidence increases, the intensity of the reflected beam increases. The transmitted beam gradually becomes weaker, and its **intensity diminishes to zero as the angle of refraction reaches $\mathbf{90^0}$**. The corresponding angle of incidence is called the **limite** or **critical angle**, $\mathbf{\theta_c}$. If the angle of incidence exceeds θ_c, no refracted beam is observed and all light is reflected. This is called **total internal reflection**.

The critical angle can be found from Snell's law by setting $\theta_2 = 90^0$ or $\sin\theta_2 = 1$, corresponding to the maximum possible angle of refraction, so then, $n_1 \cdot \sin\theta_c = n_2$, $\sin\theta_c = n_2/n_1$.

If θ_1 larger than θ_c is substituted in Snell's law, $\sin\varphi_2$ turns out to be greater than 1. Since no angle has a sine greater than 1, this implies that there is no refracted beam. This is in agreement with the observation that at any angle equal to or greater than θ_c, the beam is totally reflected.

The fact that no intensity loss occurs in total internal reflection is also the basis for **fiber optics**, a new and rapidly growing branch of optics. The basic principle of fiber optics is illustrated by a long transparent rod of **light pipe** (fig.3).

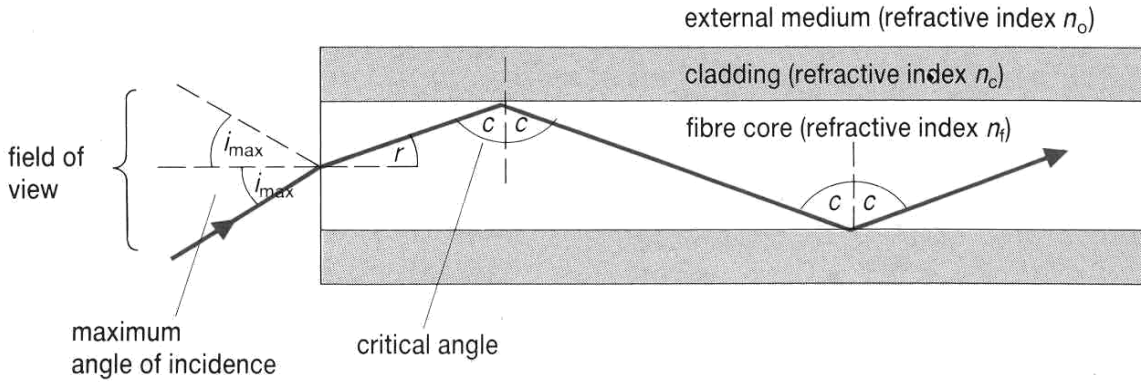

Figure 3

A ray of light entering the pipe is totally reflected if the angle of incidence is large enough when the ray reaches the surface. Even if the pipe curves gradually, light will travel its entire length and arrive non-attenuated after many reflections. Consequently, a single light pipe can transmit light energy quite efficiently. However, the rays from different parts of an object are completely scrambled by the multiple reflections, so a single pipe cannot transmit an image. Images can be transmitted using **bundles of fine glass or plastic fibers** (Figure 26.6), since each fiber transmits rays from a very small region of the object. A bundle of these fibers produces the **fiber optic light guide**. The quality of the image is largely determined by the diameter of the fibers, which can be as small as 10^{-6} m.

The fiber optic endoscope. The instrument is shown in figure 4. The long flexible shaft containing the fibers is usually made of a helical steel band inside steel mash, to prevent the glass fibers from damage whilst permitting the wide range of movements required. This is enclosed in a plastic sheath to provide water proofing, chemical protection, and ease its passage into the body. This shaft is about 10 mm in diameter and up to 2 m long depending on the application. At the far or distal end is a bending section which is fully controllable from the operator's end, to carry out maneuvers and minor operations.

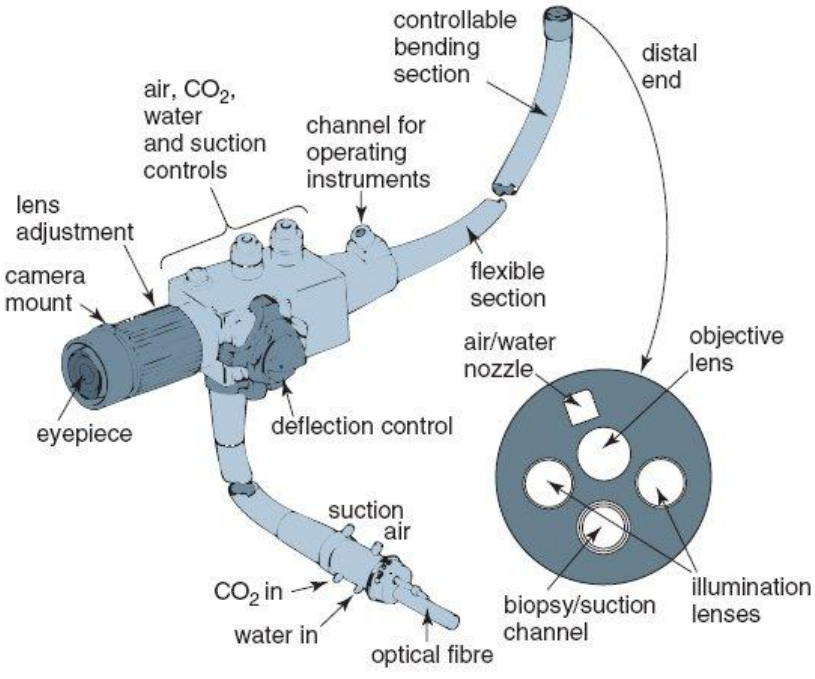

Figure 4

At the distal tip the fibers bringing the light and caring away the image are fitted with lenses. The endoscope shaft contains the following:

- usually two non-coherent fiber optic bundles – the light guides;
- a coherent fiber optic bundle – the image guide;
- a water pipe to wash the distal face of the optical system;
- an operations channel used to insert surgical instruments;
- control cables to operate the end which is adjustable;
- in some cases a channel for suction and one for pumping in air or carbon dioxide gas.

The viewing or proximal end of the endoscope contains the controls for all of these functions, an adjustable eyepiece and a connection to the light source and camera. The light source is a high intensity xenon lamp with a lens coupling arrangement to permit wide angle illumination. The camera can be attached to the eyepiece of the image guide. A photocell monitors the brightness and informs the light source so it produces a flash for a suitable exposure. A large variety of accessories and ancillary equipment is available to support the numerous applications of endoscopy.

Lenses. **Image formation**. A lens is a piece of transparent material that can focus a transmitted beam of light so that an image is formed. The lenses in man-made optical instruments are usually manufactured from glass or plastic, while the lens in the human eye is formed by a transparent membrane filled with a clear fluid. For our purposes, it is sufficient to consider **thin, spherical lenses**. These have two spherical surfaces or a spherical and a plane surface and a thickness that is small compared to the radii of the surfaces.

We can categorize all lenses as either **converging** or **diverging**. A converging lens is thicker at its center than at at the edge, while the opposite is true for a diverging lens. A converging lens bends light rays toward its **axis**, the line through its centers of curvature, so that a beam of parallel rays converges at a point. A diverging lens bends rays outward from a axis.

Three of many rays emanating from a point on an object not on the axis have readily predicted paths. Their intersection determines the location of the image. Two rays are actually sufficient to find the image location; the third serves as a check. This ray-tracing procedure is illustrated in figure 5 for converging lens. The lens forms a real image at an image distance $\mathbf{d_i}$. The three numbered rays in the diagram are drawn from the arrowhead as follows:

1. The ray leaving the arrowhead parallel to the axis is deflected by the lens so that it passes through the focal point **F**, in accordance with definition of the focal point.

2. The ray going through the focal point **F** emerges from the lens parallel to the axis.

3. The ray directed at the center of the lens is non-deflected. This happens because at that point the two sides of the lens are almost parallel, so a ray is effectively going through a flat plate. Since the lens is thin, the ray is displaced from its original path by an amount that is negligibly small.

Points below the top of the arrow at the same image distance $\mathbf{d_i}$ will have images at the same image distance $\mathbf{d_i}$. Thus once we have located the image of the top, we can sketch in the entire image of the arrow.

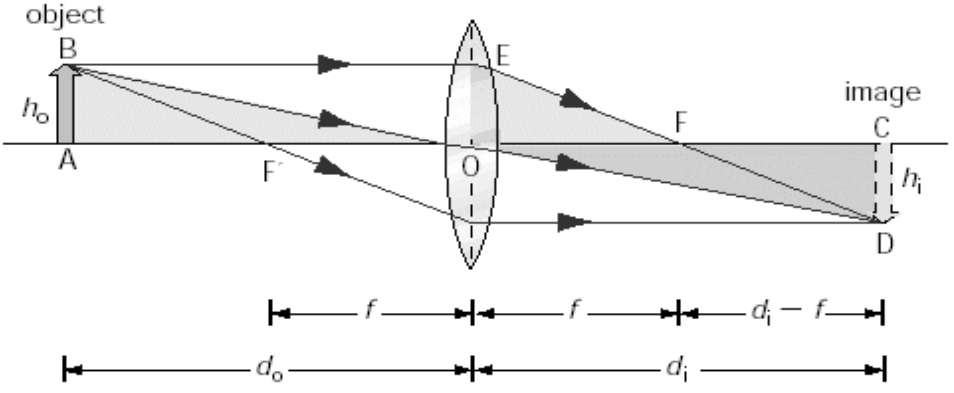

Figure 5

Although ray tracing provides good qualitative insight into the formation of an image by a lens or system of lenses, numerical work is best done algebraic formulas. In order to develop and apply these formulas, we adopt the following sign conventions for the quantities:

1. $\mathbf{d_o}$ is positive for a real object, negative for a virtual object.

2. $\mathbf{d_i}$ is positive for a real image, negative for a virtual image.

3. The object height $\mathbf{h_0}$ is positive if it points above the axis and negative if it points below the axis.

4. The image height $\mathbf{h_i}$ is positive if it points above the axis and negative if it points below the axis.

The **linear magnification m** is the ratio of the image and object heights, $\mathbf{m = h_i/h_o = d_i/d_o}$

The **thin lens formula** is $\mathbf{1/d_o + 1/d_i = 1/f}$. This means that all object points at a distance $\mathbf{d_0}$ from the lens have their images on the same plane.

In discussing lenses, it is often more convenient to deal with reciprocal of the focal length, which is called the **power of the lens P = 1/f**. If the focal length **f** is measured in meters, then **P** is measured in **diopters**: **1 diopter =** $\mathbf{1m^{-1}}$. The power of the pair of lenses is $\mathbf{P = P_1 + P_2}$.

The bright-field light microscope. Although the microscope is one of the oldest and most widely used physical instruments in biology and medicine, new types of microscopes have been developed in recent decades. These make possible more detailed study of cellular structures and sometimes avoid the need for destructive methods in observing living cells.

The figure 6 shows a **bright-field light microscope**, the ordinary microscope found in every biological laboratory. The lenses in the **condenser** focus the incident light on the specimen, and the **diaphragm** regulates the intensity.

The magnification is determined by the focal length of the **objective** and **ocular lenses**. Principle of the microscope is shown in the figure 7. The object under study is placed just beyond the focal point of the objective, so $\mathbf{d_0 \approx f_0}$. Its image is real and inverted, and it is much larger than the object.

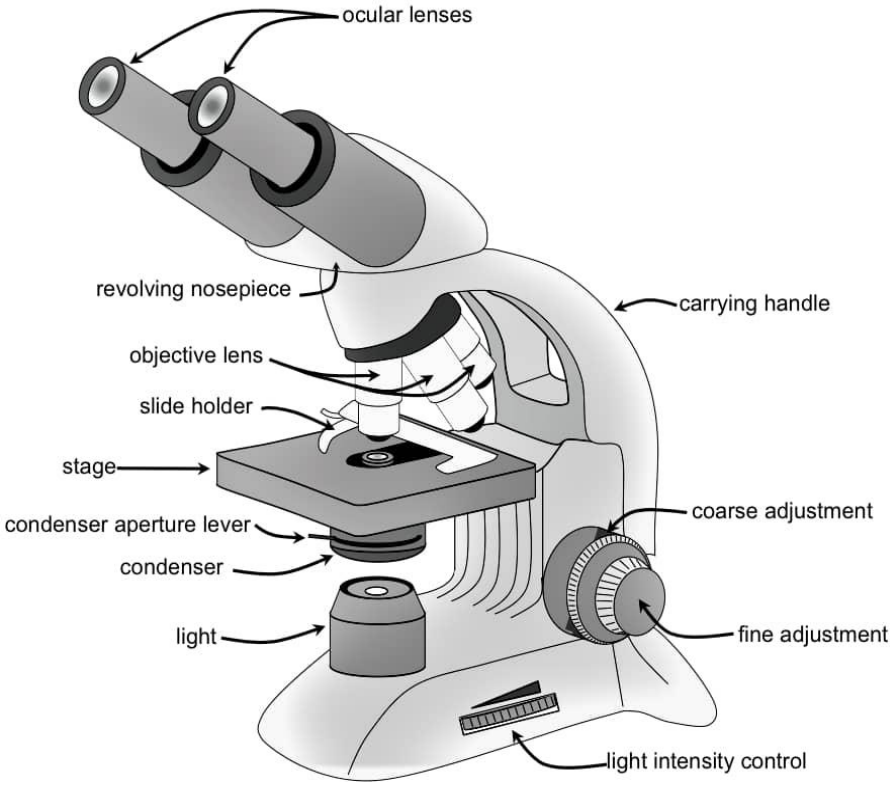

Figure 6

The linear magnification is $\mathbf{m_1 = -h_i/h \approx -d_i/f_0}$, and it is typically around 50. This image then serves as the object for the ocular, which acts as a simple magnifier and provides an enlarged virtual image at a comfortable distance for viewing, $\mathbf{(d_i)' = 0.25\ m}$. Since the angular magnification of the ocular is $\mathbf{M_2 \approx (d_i)'/f_e}$, the overall magnification of the microscope is the product of the two magnifications, $\mathbf{M = m_1M_2 = (-d_i)\cdot(d_i)'/(f_0\cdot f_e)}$.

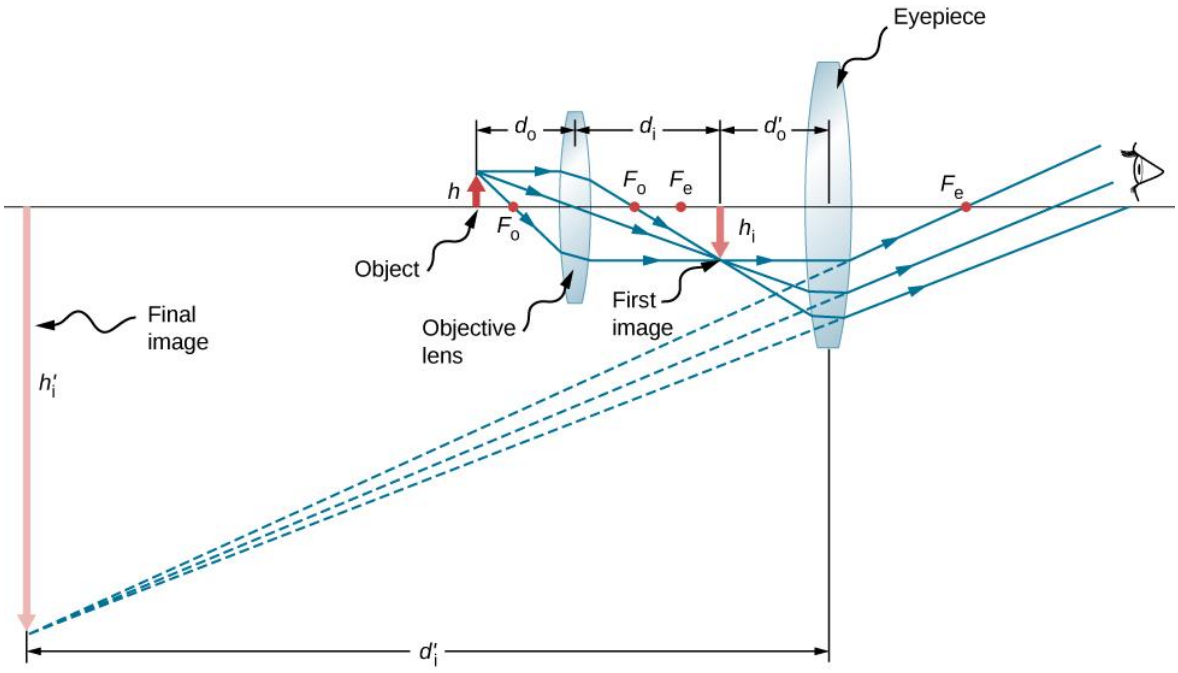

Figure 7

Resolution. When the separation between two points in a microscopic specimen is comparable to the wavelength **λ** of the light, we would expect diffraction effects to be important. A detailed analysis shows that the minimum separation **d** that can be resolved by a microscope is **$d = \lambda/(2 \cdot n \cdot \sin\beta)$**. Here, **λ** is the light wavelength in air, **n** is the refractive index of the medium between the objective lens and the object under study, and **β** is the angle subtended by the objective lens (fig. 8).

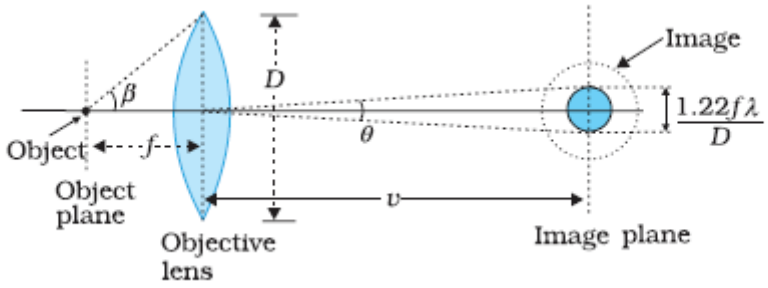

Figure 8

The product **$(n \cdot \sin\beta)$** is called the **numerical aperture** and is sometimes marked on the instrument. If two points in a specimen are separated by less than **d**, their diffraction patterns overlap so much that their images cannot be distinguished.

Diffraction cannot be ignored since it independently limits the resolution of any light microscope to about 250 nm. Consequently, the useful magnification of the ordinary light microscope is limited to roughly 400. Larger magnifications may make viewing more comfortable, but they reveal no additional details. Since a typical bacterial cell has a diameter of about 1000 nm, it is not possible to make very detailed studies of bacterial structures with light microscopes.

Equation for **d** suggests two ways to improve the resolution of microscope: use shorter wavelength light and media with larger indices of refraction. With **oil immersion objectives**, some improvement is obtained by immersing the object in a medium such as oil of cedar, which has n = 1.4. **Ultraviolet microscopes** use light with wavelengths somewhat shorter than visible light. In addition to having a smaller minimum resolvable separation, ultraviolet microscopes are

useful because substances such as nucleic acids and proteins strongly absorb ultraviolet light. This leads to very good contrast, which is also necessary to obtain good resolution.

Contrast. To distinguish an object from its surroundings, there must be sufficient contrast or variation in light intensity. Without good contrast, the actual resolution achieved will be much less than that implied by the design of the microscope. **Staining** with dyes that are absorbed differently in various parts of an object is sometimes used to improve contrast. Other dyes are used to make a sample **fluoresce**. In this case the sample is illuminated with intense ultraviolet light, and the fluorescent constituents emit light at a longer wavelength. This light is detected after the ultraviolet light is removed from the beam by a filter.

Lab test protocol
NOTE! Copy lab test protocol and bring

Lab test
Measurement of ocular scales for different ocular and objective linear magnifications. Observation of the blood specimen.

Problem.
What are that biological specimens would be reasonable to be investigated by a bright-field light microscope?

Equipment.
The bright-field light microscope

Procedure.
1. Use oculars $6^{\times}$ with objectives $20^{\times}$ and Gorjaev camera to determine ocular ruler skale. Put data at the table 1.
2. Observation and measurement of the blood erythrocyte dimension. Put data at the table 2.

Observations
1. Estimate the accuracy of measurement provided by the bright-field light microscope
2. How is the accuracy is determined by parameters of the objective and ocular?

Analysis and conclusions
1. Give examples of the biological specimens for investigations by the bright-field light microscope

Table 1

Ocular magnification	Objective magnification	Gorjaev camera division, a, mm	Gorjaev camera boxes number, n	Ocular ruler divisions number, m	Scale factor S = an/m, mm	The mean of scale factor $S_{mean} = (S_1 + S_2)/2$, mm
$6^{\times}$	$20^{\times}$	0.2	1	33	S_1 =	
		0.05	5	41	S_2 =	

Table 2

Ocular magnification	Objective magnification	Number of erythrocytes, l	Ocular ruler divisions number, k	Erythrocyte size, L = kS/l, mm	Erythrocyte size mean $L_{mean}=(L_1+L_2+L_3+L_4)/4$, mm	Absolute error $\Delta L=L-L_{mean}$	Mean of absolute error $\Delta L_{mean} = (\Delta L_1+ \Delta L_2+ \Delta L_3+ \Delta L_4)/4$	Relative error $\varepsilon=(\Delta L_m/L_{mean})100\%$
$6^{\times}$	$20^{\times}$	4	5	L_1 =	L_{mean} =	ΔL_1 =	ΔL_{mean} =	ε =
		6	9	L_2 =		ΔL_2 =		
		2	3	L_3 =		ΔL_3 =		
		3	5	L_4 =		ΔL_4 =		