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Microscopic and Endoscopic Diagnostics

Laboratory Guide
to the Lab Work No 2

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The aim of the work:

To get acquainted with the principle of action and design of optical microscopes and endoscopes. To learn how to use this equipment for visual observation of microobjects and hard-to-reach places.

Preleminary task

1. Study the principle of operation of compound optical microscope.
2. Read the description of Motic BA310 digital microscope.
3. Familiarize yourself with types of endoscopes and designation.
4. Read the description of video scope Eyoyo NTS200.

Compound Optical Microscope

The optical microscope or light microscope is a type of microscope, which uses visible light and a system of lenses to magnify images of small samples. The optical microscope or light microscope is a type of microscope, which uses visible light and a system of lenses to magnify images of small samples. A compound microscope uses a lens close to the object being viewed to collect light (called the objective lens) which focuses a real image of the object inside the microscope (Fig. 1) A second lens or group of lenses (called the eyepiece) that gives the viewer an enlarged inverted virtual image of the object then magnifies that image. The use of a compound objective/eyepiece combination allows for much higher magnification. Common compound microscopes often feature exchangeable objective lenses, allowing the user to quickly adjust the magnification.

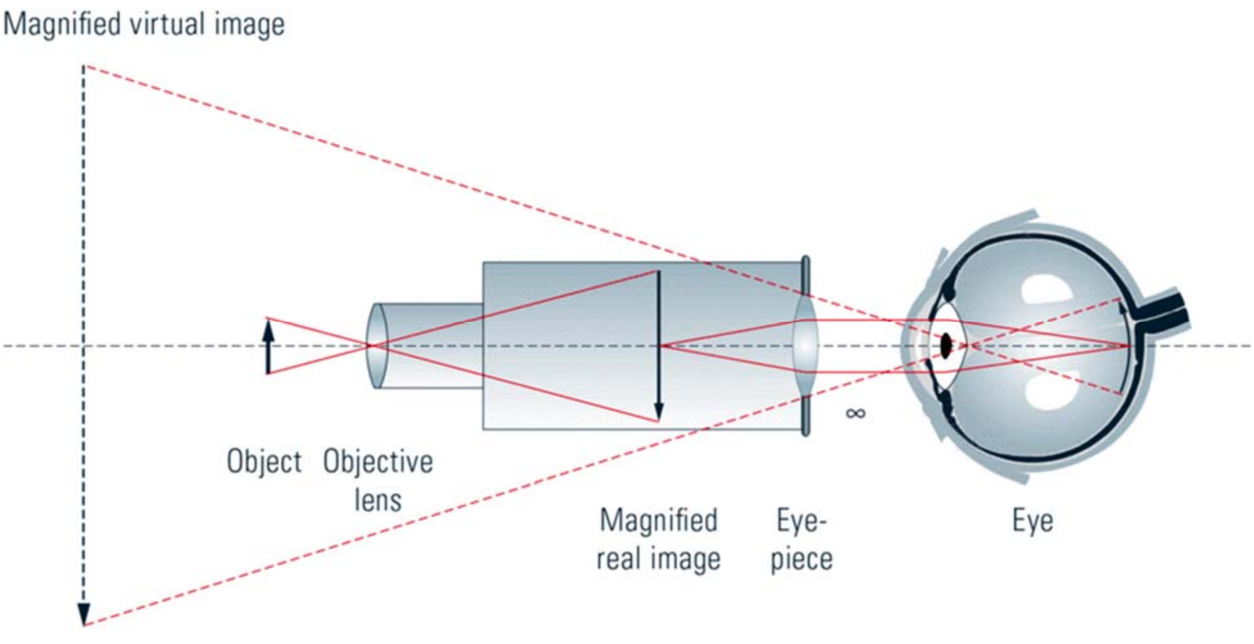

Fig. 1. Image formation in compound microscope

Modern compound microscopes are designed to provide a magnified two-dimensional image that can be focused axially in successive focal planes, thus enabling a thorough examination of specimen fine structural detail in both two and three dimensions. Most microscopes provide a translation mechanism attached to the stage that allows the microscopist to accurately position, orient, and focus the specimen to optimize visualization and recording of images. The intensity of illumination and orientation of light pathways throughout the microscope can be controlled with strategically placed diaphragms, mirrors, prisms, beamsplitters, and other optical elements to achieve the desired degree of brightness and contrast in the specimen.

Presented in Fig. 2 is a typical microscope equipped with a trinocular head and camera system for recording photomicrographs. Illumination is provided by lamp positioned in the lamphouse, which emits light that first passes through a collector lens and then into an optical pathway in the microscope base. Also stationed in the microscope base is a series of filters that condition the light emitted by the incandescent lamp before it is reflected by a mirror and passed through the field diaphragm and into the substage condenser. The condenser forms a cone of illumination that bathes the specimen, located on the microscope stage, and subsequently enters the objective. Light leaving the objective is diverted by a beamsplitter/prism combination either into the eyepieces to form a virtual image, or straight through to the projection lens mounted in the trinocular extension tube, where it can then form a latent image on film housed in the camera system.

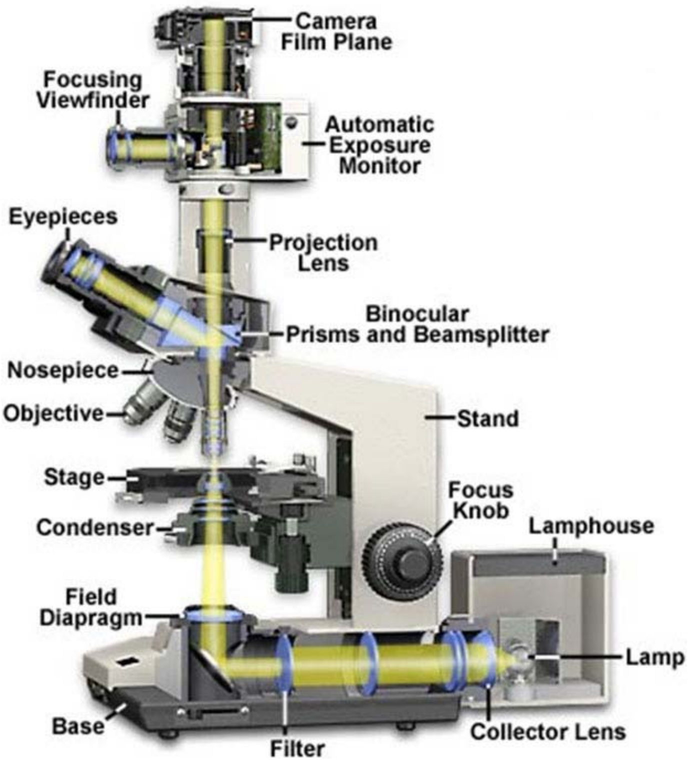

Fig. 2. Scheme of compound microscope [16].

The optical components contained within modern microscopes are mounted on a stable, ergonomically designed base that allows rapid exchange, precision centering, and careful alignment between those assemblies that are optically interdependent. Together, the optical and mechanical components of the microscope, including the mounted specimen on a glass micro slide and coverslip, form an optical train with a central axis that traverses the microscope base and stand. The microscope optical train typically consists of an illuminator (including the light source and collector lens), a substage condenser, specimen, objective, eyepiece, and detector, which is either some form of camera or the observer's eye (Table 1). Research-level microscopes also contain one of several light-conditioning devices that are often positioned between the illuminator and condenser, and a complementary detector or filtering device that is inserted between the objective and the eyepiece or camera. The conditioning device(s) and detector work together to modify image contrast as a function of spatial frequency, phase, polarization, absorption, fluorescence, off-axis illumination, and/or other properties of the specimen and illumination technique. Even without the addition of specific devices to condition illumination and filter image-forming waves, some degree of natural filtering occurs with even the most basic microscope configuration.

While some of the microscope optical components act as image-forming elements, others serve to produce various modifications to illumination of the specimen and have filtering or transforming functions. Components involved in formation of images by the microscope optical train are the collector lens (positioned within or near the illuminator), condenser, objective, eyepiece (or ocular), and the refractive elements of the human eye or the camera lens. Although some of these components are not typically thought of as imaging components, their imaging properties are paramount in determining the final quality of the microscope image.

Table 1. Microscope optical train components

MICROSCOPE COMPONENT	ATTRIBUTES
Illuminator	Light source, collector lens, field diaphragm, heat filters, light balancing filters, diffuser, neutral density filters
Light conditioner	Condenser iris, dark field stop, aperture mask. phase annulus, polarizer, off-center slit aperture, Nomarski prism, fluorescence excitation filter
Condenser	Numerical aperture, focal length. aberrations, light transmission, immersion media. working distance
Specimen	Slide thickness, cover glass thickness, immersion media, absorption, transmission, diffraction, fluorescence, retardation, birefringence
Objective	Magnification, numerical aperture, focal length, immersion media, aberrations, light transmission, optical transfer function, working distance
Image filter	Compensator, analyzer, Nomarski prism, objective iris, phase plate, SSEE filter. modulator plate, light transmission, wavelength selection, fluorescence barrier filter
Eyepiece	Magnification, aberrations, field size, eye point
Detector	Human eye, photographic emulsion, photomultiplier, photodiode array, video camera

Endoscope

Endoscopy means looking inside and typically refers to looking inside the body for medical reasons using an endoscope, an instrument used to examine the interior of a hollow organ or cavity of the body. Unlike most other medical imaging techniques, endoscopes are inserted directly into the organ. There are many different types of endoscope, and depending on the site in the body and the type of procedure, a doctor or a surgeon may perform endoscopy, and the patient may be fully conscious or anaesthetised. Most often, the term endoscopy is used to refer to an examination of the upper part of the gastrointestinal tract, known as an esophagogastroduodenoscopy.

For non-medical use, similar instruments are called borescopes. A borescope (occasionally called a boroscope, though this spelling is nonstandard) is an optical device consisting of a rigid or flexible tube with an eyepiece on one end, an objective lens on the other linked together by a relay optical system in between. The optical system in some instances is surrounded by optical fibers used for illumination of the remote object. An internal image of the illuminated object is formed by the objective lens and magnified by the eyepiece which presents it to the viewer's eye. Rigid or flexible borescopes may be fitted with an imaging or video device.

The principle of endoscopic control is simple - the "long eye" allows you to inspect the surface of the internal units of the machine without dismantling and disassembling it - through the technological holes in the casing, with a minimum amount of preparatory work. Remote Visual Inspection, as a kind of visual inspection, is included in the international regulatory documents on non-destructive testing (ND) and in the Guidance Document (RD) 34.10.130-96 (Instruction for the Visual Measurement Control, Ministry of Fuel and Energy of the Russian Federation, 1996.) Necessity The use of endoscopes is reflected in numerous operating manuals, especially aviation equipment. Endoscopy successfully complements known in the industry NDT methods, and in some cases, it is the only possible means of control.

Defined types of defects are different, depending on the purpose of the machine or unit: cracks, pitting, burnout, caverns, foci of corrosion, defects in coatings, wear of friction surfaces, correct location of parts, integrity of internal fasteners, foreign objects in internal cavities, nicks from foreign objects on Internal parts of machines. Flexible fiber-optic endoscopes (fibroscopes) and flexible television endoscopes (video scopes) are produced.

Video scopes quickly gain popularity as universal devices, suitable for almost any task of visual control. The video image is created by a miniature high-resolution video camera located behind the lens at the end of the device. This ensures the highest quality and detail of the endoscopic image. The illumination of the examined area is transmitted from a powerful light source through fiber-optic lightguides located inside the working part of the video camera. To document the results of the survey, digital photography and digital video recording through an endoscope can be performed.

a) b) c)

Fig. 3. Video scope (a), fibroscope (b), and boroscope (c).

Fibroscopes are flexible endoscopes that transmit an image along a fiber bundle with regular (hexagonal) packing. Illumination in fiberoscopes is also transmitted from a powerful light source through fiber-optic light guides (with irregular fiber packing) located inside the working part. When working together with TV cameras or digital cameras, fibroscopes also allow you to document the results of endoscopic examination.

Boroscopes - an ideal tool in the case of direct access to the inspection area. In the rigid working part of the borescope for image transmission, solid-state lenses are used, and the illumination, like that of fibroscopes, is provided by an external light source through a fiberglass rope with irregular packing.

High-speed video recording is a method that allows analyzing trajectories and changing the shape of fast moving bodies by slow playback of a captured image. With the rapid development of high-speed cameras, this method became very popular.

a) b) c)

Fig. 4. High-speed cameras: Olympus i-SPEED (a), Redlake MotionPro X3 (b), Fastec HiSpec 5 (c).

In-lab Equipment

Digital microscope Matic BA310 Digital

The professional digital microscope Matic BA310 Digital is a solution for medical and natural science research, which requires an optical image of high quality. The microscope uses a digital camera with a resolution of 3 megapixels, built into the nozzle. BA310 Digital is a powerful professional digital tool for obtaining high-quality images.

Fig. 5. Motic BA310 Digital microscope

The high-resolution chip and full Keller compliance provide images of excellent contrast. Together with the BA310 Digital software is supplied Motic Images Plus 2.0 Multi Language, which makes it possible to perform an in-depth analysis of the sample.

Features of Motic BA310 Digital microscope:

- Siedentopf binocular nozzle with a 30° inclination, built-in CMOS chip ½ inch for digital camera
- 3MP - 2048×1536 pixels with USB 2.0 output
- Wide-angle eyepieces N-WF10X / 20 mm with dioptric correction in both eyepieces
- Reverse turret with 4 lenses
- Achromatic lenses CCIS® EF-N PL with magnification 4X, 10X, 40X S, 100X S-oil

- Coaxial mechanism for coarse and fine focusing with tension adjustment
- Built-in mechanical coaxial stage with low level (right-hand control)
- Focused and centered condenser with iris and filter slot
- Keller's 6V / 30W quartz halogen lamp with brightness adjustment
- Motic Images Plus 2.0 for PC and Macintosh.

Image Analysis Software Motic Images Plus 2.0

Multilingual image analysis software Motic Images Plus 2.0 - your ticket to the digital world of microscopy. Many years ago this program was created to receive images and record them in digital format. Currently, Motic Images Plus 2.0 - is no longer just a "basic" set. Thanks to the support of users of digital microscopes Motic worldwide, this program has become the most perfect tool for image analysis and has the following functions:

- Image Capture
- Automatic image capture
- Video recording
- Measurements
- Automatic counting
- Creating reports
- Image comparison
- Merge images

Eyoyo NTS200 Endoscope parameters

Fig. 6. Eyoyo NTS200 Endoscope Inspection Camera

Features of Eyoyo NTS200 Endoscope

- High Definition video recording with sound
- Built-in 1W LED flash light by CREE
- Large 3.5" color LCD monitor with high resolution and visibility

- Detachable LCD receiver for remote viewing with cable
- 4X zoom and 360° image rotation & flip over
- Capture images (JPG) up to 3 megapixels or videos (AVI) up to 720p HD
- Menu in 7 languages (English, Chinese, French, German, Spanish, Russian, Japanese)
- Waterproof probe and camera head
- Slim 8.2 mm camera head with 6 pcs of adjustable LEDs
- Conveniently save data in Micro SD card
- View saved data in computer via USB cable
- Output to TV, supports NTSC & PAL
- Selectable Colored / Black & White in viewing and recording
- Ergonomic and robust designs & one-hand operation
- Flexible, portable, easy to operate and stable performance
- Adjustable handle angle
- High performance camera module with crystal clear output
- Users can control all the keys with one hand
- Adjustable handle offers extra comfort
- Stainless steel shield and overall reinforced structures ensures sturdiness and heavy duty quality
- LED Flash light : 1W CREE LED
- Operating temperature: 0° to 45°C
- Power Source : 4*AA battery
- Max battery life: 4~5 hours
- Weight: 445 g (excluding the probe)
- Dimension (Grip): 260(L)x95(W)x80(H) mm (excluding the probe)
- Probe size: 8.2 mm diameter, 1 m tube length.
- Supported Operating Systems: Mac os, Windows 8, Windows XP, Windows 7, Windows Vista, Windows 98.

Monitor

- Screen Type: 3.5 in. (88.9mm) Color LCD
- Resolution: 320*240 pixels (QVGA)
- Rotation: 360° rotation
- Zoom: 4x digital zoom
- Video recording resolution: 720*480, 1280*720 pixels
- Image capturing resolution: 720*480, 1600*1200, 2048*1536 pixels
- Menu languages : English, Chinese, French, German, Spanish, Russian and Japanese
- Remote connection port : AV-out, USB, TF card slot
- Data storage media: supports up to 32GB.

Camera

- Connectivity: Closed System/CCTV Wired
- Sensor: CMOS
- High definition: 480P(SD)
- Diameter: 8.2 mm
- Shaft diameter: 7.6 mm
- Shaft length: 100 cm
- Viewing angle: 60°
- Depth of field: 80 mm - infinity
- Light source: 6 adjustable high-intensity LEDs.

Fig. 7. Probe

In-lab task

1. Familiarize yourself with Motic BA310 digital microscope. Switch on the light; connect to PC; open software.
2. Observe using microscope the sample of biotissues (sample 1). Adjust sharpness of the image, set the appropriate color correction of the camera.
3. Capture 6-8 images of different areas of the object and save then on the PC.
4. Familiarize yourself with video scope Eyoyo NTS200.
5. Observe the surface of sample 2 (integrated circuit) using video scope. Make records.
6. Observe the inner surface of sample 3 (tube) using video scope. Make records.

Control questions:

1. Enumerate the methods for visual diagnostics.
2. What are the similarities and differences of compound microscope, loupe and telescope?
3. Please, name the basic parts of compound microscope.
4. How to change the magnification of microscope?
5. Please draw the ray path in a microscope.
6. What types of endoscopes do you know?
7. What is the difference between fiberscope and video scope?
8. What are the main advantages of endoscopic operations?