Chapter 15

Microscope

GMDN Code	**36351**
ECRI Code	**12-536**
Denomination	**Microscopes**

The word *microscope* comes from the fusion of the Greek words *micros* which means *small* and *skopien*, *to see* or *examine*. This chapter presents the care and routine maintenance of microscopes used in clinical practice.

Depending on the contrast system, microscopes are given different names. Among the most common are the following:

- Clear field optical microscope
- Dark field optical microscope
- Fluorescence optical microscope
- Phase contrast optical microscope
- Interference optical microscope
- Polarized light optical microscope
- Inverted optical microscope
- Stereoscopic microscope

PHOTOGRAPHS OF MICROSCOPES

Binocular microscope

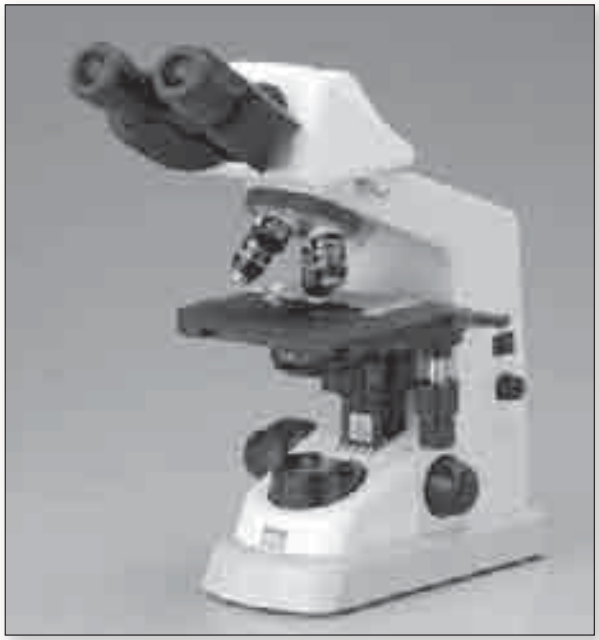


Photo courtesy of Nikon Instruments

▲ This type of microscope uses various systems of lenses and controlled illumination to achieve magnification of an object.

Stereoscopic microscope

Photo courtesy of Olympus

◀ This type of microscope allows tridimensional images or volumes to be appraised by superimposing two single images, one for each eye, over each other.

PURPOSE OF THE EQUIPMENT

The microscope is a precision instrument with optical subsystems (lenses, filters, prisms, condensers); mechanical subsystems controlling the position of the sample in tri-dimensional space X, Y, Z; electrical (transformers and light source) and electronic subsystems (cameras, video, etc.) interacting to amplify and control the image formation of objects which are not detectable to the human eye. To observe samples, it is essential to prepare these according to techniques which emphasize details to be observed.

The microscope constitutes a diagnostic aid of first order in healthcare, in specialties such as haematology, bacteriology, parasitology and in the training of human resources (there are microscopes with specialized additions for students to carry out observations directed by a professor). The technical developments applied to microscopes have allowed the design of numerous specialized models by the industry and academia. These play a key role in developing human knowledge and understanding the workings of nature.

OPERATION PRINCIPLES

The microscope is constructed using the physical properties of lenses interacting with light. A lens is an optical element usually made of glass which can refract light. It is of calculated dimensions and in general has parabolic or spherical surfaces. If light rays reaching one surface of the lens converge in a common point F when exiting it, such lens is known as positive or convergent. If it disperses the light rays crossing it, it is divergent or negative. Positive lenses (convergent) shown in Figure 41 constitute the building blocks of microscopes.

In Figure 41, it is possible to identify the *focus* [F], (the point where the light rays are concentrated) and how light is refracted across the lens. The distance between the lens and the focus is known universally as the *focal distance* [D].

Figure 42 summarizes concepts related to the functioning of lenses applied to the design of microscopes.

Figure 41. Positive (convergent) lens

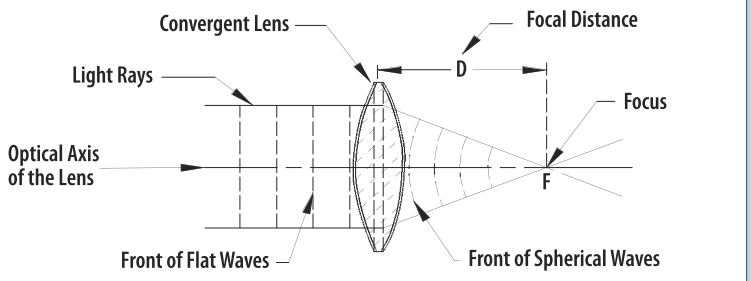
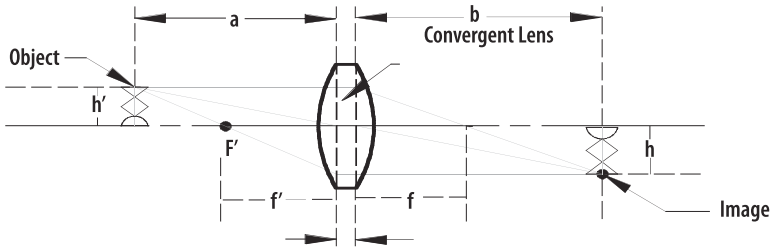


Figure 42. Optics of the convergent lens



Object [h'] located at a distance [a] from the lens produces image [h] at a distance [b] from the lens, where [h] = [h']. The focus [F], where the light waves are concentrated is at the focal distance [f] from the lens. See text for additinal details and related equations.

When an illuminated object [h'] is placed at a distance [a] in front of a convergent lens, light rays cross the lens and are refracted. A ray crossing the upper part of the object crosses the optical axis of the lens at the focal point [F']. It is refracted by both surfaces of the lens and exits in one direction, parallel to the optical axis. The ray crossing the upper part of the object in parallel with the optical axis passes through the lens and is refracted. It then travels through the focal point [F] on the image's side until it crosses the first ray at a distance [b] from the lens where the image is formed. In the case shown in Figure 42, the distance [a] is greater than the focal distance [f'], where a real image is formed inverted at a distance [b] behind the lens. The focal distance [f] is related to the distances [a] and [b] in the equation:

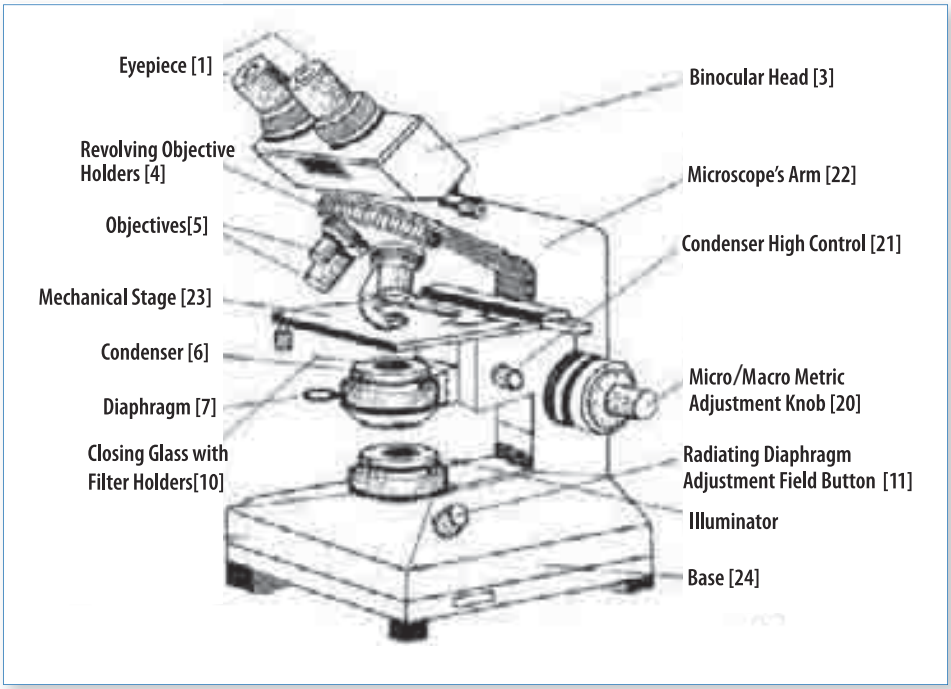
$$\frac{1}{f} \quad \frac{1}{a} \quad \frac{1}{b}$$

The magnification [M] of a lens, defined by the relationship between the size of the object and the size of the image formed is represented by the equation:

$$M \quad \frac{h}{h'} \quad \frac{b}{a}$$

Where:
[h] and [h'] correspond respectively to the dimensions of the image and the object; [a] and [b] to the distances between the lens and the point where the image is formed and between the lens and the point where the object is located.

Figure 43. Diagram of a microscope



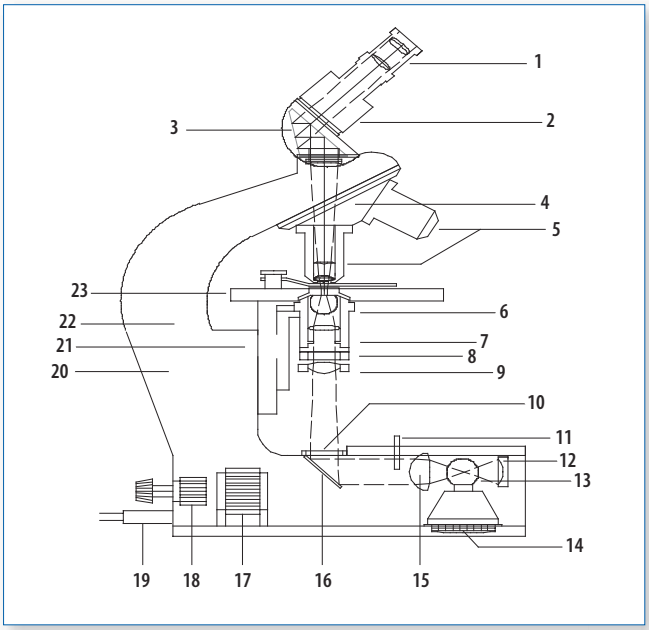
Components

The main components of the microscope subsystems are shown in the table below.

INSTALLATION REQUIREMENTS

Normally, microscopes use 110 V/60 Hz or 220 V/60 Hz power. Some have a regulated source which allows light intensity adjustments. Other microscopes use a mirror through which light is directed towards the slide located on the platform rather than a lamp. Such microscopes are mostly useful in regions far from urban centres, where there are no electricity lines and are used by health brigades. Certain types of microscopes require special installations; a fluorescence microscope needs a dark cabinet in order for observations to be carried out.

Figure 44. Cross-section of a microscope

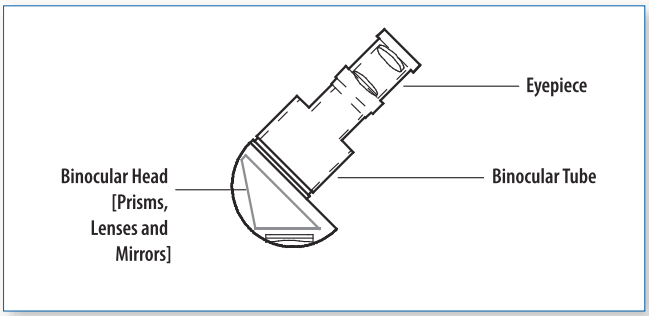


Legend

No.	System	No.	Components
1	Binocular head	1	Eyepiece
		2	Binocular tube
		3	Binocular head
2	Revolving objective holders	4	Revolving objective holders
		5	Objectives
3	Platform, plate or mechanical stage and condenser	6	Condenser
		7	Aperture diaphragm
		8	Filter holders
		9	Wide range lens
		21	Condenser control
		23	Platform, plate or mechanical stage
4	Illuminator	10	Closing glass with filter holders
		11	Settings lever of the diaphragm's light field
		12	Concave mirror
		13	Incandescent light
		14	Light holder with adjustment ring
		15	Collector lens
		16	Mirror
5	Microscope's body	17	Internal transformer
		18	Control rheostat
		19	Feed cable
		20	Macro/micro metric adjustment knob
		22	Microscope's arm
		24	Base

DESCRIPTION OF POTENTIAL PROBLEMS WITH MICROSCOPES

Figure 45. Binocular head



Eyepieces

The most frequent problem affecting eyepieces is the presence of dust and grime, which may be on the external or internal surfaces. Such dust or grime produce shadows interfering with the sample under analysis, especially when high powered lenses are used (40X–100X). If these are external, cleaning the surfaces of the lenses solves the problem. If internal, it is necessary to disassemble the eyepiece, clean the internal surfaces, reassemble and verify the final state.

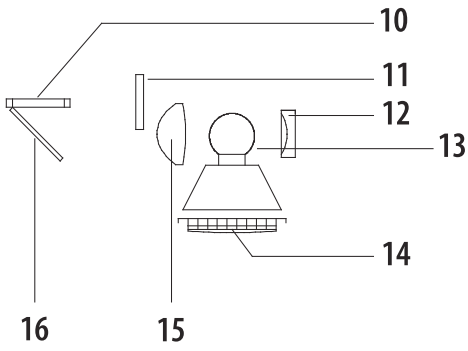
Scratches may be observed on the eyepieces' lenses, especially on those that have been in service for a long time. These are produced by negligence during the cleaning process due to the use of inadequate material for cleaning. Scratches produce cobweb-like shadows in the visual field of the eyepieces. Unfortunately with this type of damage, the eyepieces must be changed. Sometimes the focus mechanisms of the eyepiece stick. To repair, the eyepiece is disassembled; the appropriate solvent is applied to its threading and the focus mechanism is cleaned and reassembled. If the lenses of the eyepiece show ruptures due to abnormal circumstances (marks due to falls, unsuitable use), the eyepieces must be changed.

Binocular head

The state of the binocular head has a direct effect on the quality of the microscope's image. Its most important components are the prisms and mirrors. Grime adhered to the optical components of the head affects the quality of the image. This component can even become dirty due to normal work in the laboratory, such as changing the eyepieces, installing accessories (e.g., cameras) or simply by forgetting to place stoppers when the microscope is not in use.

- ***Prisms.*** These have silver-plated reflective surfaces which can become rusty over time and lose their reflecting capacity. Some prisms have only one coat of reflective paint on their surface through which light enters and leaves. If the reflective surface is damaged, the prism can be removed, cleaned, polished or repainted, installed and aligned in the binoculars head. This kind of maintenance is highly complex and can only be done by specialized laboratories or companies offering this maintenance service. The removal of prisms without training and suitable tools can have a serious impact on the quality of the image and even break the component.
- ***Mirrors.*** These have reflecting surfaces directly exposed and are susceptible to rust. If repair is necessary, the mirror is dismounted and removed from the binocular head and substituted by a new one, cut, cemented and aligned directly where it is being mounted.

Figure 46. Lighting system



10. Closing Glass with Moveable Filters
11. Settings Lever of the Diaphragm's Luminous Field
12. Concave Mirror
13. Incandescent Lamp
14. Moveable Lamp with Adjustment Ring
15. Collector Lens
16. Mirror

This is a fundamental element of the microscope. If the illumination system does not work well, the microscope is out of order as light intensity and contrast are fundamental to observe samples. Several factors may affect the lighting system; the most common ones are grime and deterioration of the mirrors and lenses, defects in the feed voltage, or the use of bulbs other than those recommended by the manufacturers. The anomalies mentioned produce small shadows in the vision field and insufficient light intensity, or a lack of homogeneity in the lighting.

Internal dust and grime

This occurs when the lighting systems are not sealed to prevent dust and particle infiltration. Dust in the system produces diffusion and a decrease in the quantity of light projected onto the sample. Large particles produce shadows rendering observations difficult. In order to correct the problem, the illuminator is disassembled, its components cleaned, reassembled and realigned.

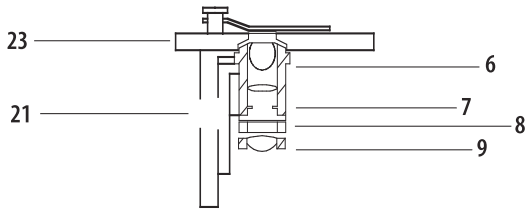
Mirrors

The mirrors have a reflective coating directly on their surface. In recently manufactured microscopes these generally have a protective coat. In older equipment, the reflective coat is exposed to rust.

Incandescent bulb

The bulb is a consumable component with a determined operational life. Its acquisition must be planned ahead to ensure a replacement is always available in the laboratory or in the institution where the equipment is installed. The bulb installation is done according to the manufacturer's instructions. Some equipment, such as the fluorescence optical microscope, uses special bulbs (mercury or xenon light) requiring mounting and calibrating procedures which, although simple, must be carried out according to the manufacturer's recommendations. The voltage supplied to the microscope must correspond to that specified by the manufacturer. Otherwise, unnecessary risks which may affect the quality of lighting are taken. Note that some microscopes use internal or external transformers and voltage regulation systems.

Figure 47. Platform, plate or mechanical stage



6. Condenser
7. Aperture Diaphragm
8. Filter Holders
9. Wide Range Lens
21. Condenser Height Control
23. Plte, Platform or Mechanical Stage

Condenser

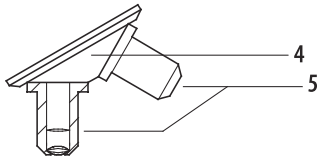
The condenser controls how the light is concentrated on, or contrasted against the sample under observation. It is composed of optical and mechanical elements. The optical elements are the lenses and the mechanical ones those which allow the control of the position of the lenses and the quantity of light reaching the sample through a mechanical diaphragm.

Normally, optical components are affected by the presence of dust. These must be cleaned in a similar manner to lenses, using a fine camel hair brush to remove dust deposited on the surface. The mechanical components require adjustment by tools with special characteristics and each manufacturer has its own designs. The usual routines are focused on cleaning, adjustment and lubrication procedures.

Plate or sample holders

The plate or sample holder comprises a series of components interacting with each other. Their purpose is to control the position of the sample under analysis. The plate has movement capability in the direction X/Y, which the operator controls with independent macro/micrometric buttons. Beside it, the plate has tension devices to allow smooth sliding using "milano tail" type guides, which are normally lubricated. In its upper part, are installed plates or control gripping devices for the specimen slides. Maintenance seeks to keep these mechanisms clean, lubricated and well adjusted.

Figure 48. Revolving, objective holder

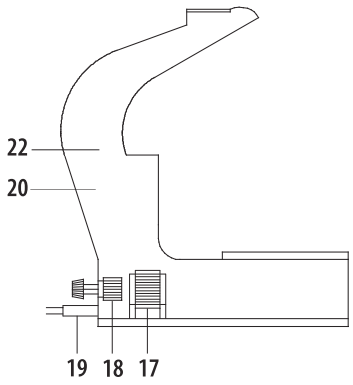


4. Revolving Objective Holder
5. Objectives

The maintenance of the revolving objective holder is simple. It has an internal catch mechanism which allows the objective in use to be aligned with the optical microscope equipment. It simply rotates smoothly until a trip mechanism adjusts the correct position of the next objective. Each manufacturer defines the number of objectives which can be mounted on the revolver. The most common revolvers can hold between three to five objectives. Maintenance seeks to keep the rotating mechanism clean, lubricated and well adjusted.

The objectives should receive routine cleaning of their external optical surfaces. Immersion type objectives require that oil is cleaned off after each use to avoid the objective's internal optical structure from being contaminated with oil through capillarity.

Figure 49. Body of the microscope



17. Internal Transfomer
18. Control Rheostat
19. Feed Cable
20. Micro/Macro Metric Adjustment Knob
22. Microscope Arm

The microscope's body is designed to receive and support the components already described (binocular head, mechanical stage, condenser and revolving objective holder, other components such as the transformer and electrical/electronic elements of the microscope's lighting system).

Maintenance of the microscope's body basically consists in keeping its surface clean, removing grime, dust or elements affecting its presentation and state. It is necessary to take special care with chemical substances that may be corrosive, including dyes used in the laboratories for staining slides.

GENERAL MAINTENANCE OF THE MICROSCOPE

Above all, it is necessary to emphasize that the microscope is high precision equipment. The integrity of its optical components, both mechanical and electrical, must be preserved in order to preserve it in the best condition. Each element of the microscope has been developed using the most advanced manufacturing techniques. Its assembly and adjustment are done in the factory using specialized equipment. During this process the required tolerance of the various components of the equipment is highly controlled through advanced measuring techniques. The cleaning of the microscope environment, its installation and careful use are fundamental to achieve a long and operational life. Humidity, dust and bad conditions of the electrical feed, misuse or inadequate installation are counterproductive for its conservation. Microscope maintenance involves a lot of care, patience and dedication. It must only be carried out by trained personnel using specialized tools. General recommendations are presented next. These are required for installing and maintaining a microscope in good working condition.

Installation and storage

1. Ensure that the area where the microscope is installed is protected from dust and humidity. Ideally, there must be an air-conditioning system which guarantees air free from dust or particles, humidity control and permanent temperature control.
2. Verify that the area is secure, having a door with a lock to prevent unauthorized removal.
3. Confirm that the location of the microscope is far from water supplies or where chemical substances are handled in order to avoid spills or splashing. Also, areas with direct sunlight must be avoided.
4. Verify that the area selected has an electrical outlet compatible with the lighting system of the microscope. It must be in good condition with voltage adjusted to the magnitude and frequency of the electric codes and standards. If the microscope uses a mirror, it must be located near a window which allows good illumination, but it should not be directly exposed to sunlight.
5. Install the microscope on a levelled surface of a rigid structure, under which there is sufficient room for the user (the microscopist) to place his/her legs. His or her body should be close to the microscope with the head near the eyepieces without strain of the vertebral column, neck and back.
6. To facilitate the microscopist work position, provide a chair of adjustable height with good back support. If there is no back support; provide support for the feet, placing it at the front of the work space (not on the chair). The purpose of this is for the vertebral column to be as erect as possible and to reduce flexing of the shoulders and neck.
7. Avoid locating microscopes near equipment which produce vibrations such as centrifuges or refrigerators.
8. Try not to move the microscope from its installation position, especially if it is used intensely each day.
9. Cover the microscope with a dust protector if not used for long periods of time, taking precautions so it is not affected by excessive humidity. The dryer the environment, the lower the probability fungi will grow. The protector can be of plastic or cloth of similar quality to that of handkerchiefs which do not deposit lint.
10. In areas of high humidity, keep the microscope in a box or cabinet lit with a bulb of no more than 40 W during the night. This helps keeping the storage area dry and reduces the probability of fungal growth. If this alternative is used, verify that there are some openings permitting ventilation inside.

Cleaning procedures

Cleaning of the microscope is one of the most important routines and must be considered essential. The following materials are required:

1. A piece of clean cloth with a similar texture to that of a handkerchief.
2. A bottle of lens cleaning solution which can be obtained from opticians. Normally, it does not affect the lenses' protective coating nor the adhesives or cements used in their assembly. Among widely used cleaning liquids are ethyl ether, xylene and white gasoline.

Warning: Some manufacturers do not recommend using alcohol or acetone as these can affect (dissolve) the cements and adhesives used for attaching lenses.

3. Lens paper. This can normally be obtained from opticians. If it is not possible to obtain this material, it can be substituted with soft absorbent paper or with medicinal type cotton. Also a piece of soft silk can be used.
4. A piece of very fine chamois. This can be obtained from shoe shops.
5. A rubber (nasal) bulb for blowing air. A device can be made in the laboratory by connecting a Pasteur pipette to the rubber bulb.
6. A plastic cover to protect the microscope from its external environment when not in use. A cloth bag with a texture similar to handkerchief material can also be used.
7. A soft camel hair brush or a fine paint brush. Importantly, the brush's hair should be natural, of uniform length with a very soft texture, dry and free from grease. It is possible to obtain this in photography stores. Also, it is possible to find an equivalent in shops supplying cosmetics.
8. A 250 g packet of desiccant (silica gel). This is used to control the humidity in the microscope's storage box if it is airtight. It changes colour when it is saturated by humidity to detect when it needs to be substituted or renewed. When it is in good condition, the colour is generally blue; when it is saturated with humidity, it is pink.
9. Bulbs and replacement fuses. These should be of the same model as those installed by the manufacturer or of equivalent characteristics.

Note: All required materials for cleaning must be kept clean and stored in containers that protect them from their external environment.

Cleaning of the optical elements

In a microscope, there are two types of optical elements: those external in contact with their outside environment and those internal, inside the body of the microscope and more protected (objectives, eyepieces, mirrors, prisms, condenser, illuminator, etc.). The cleaning procedures, although similar, differ with regard to the care and precautions.

1. The external optical elements of eyepieces, objectives, condenser and illuminator are cleaned by gently brushing their surfaces with the camel hair brush. This removes dust particles. The rubber bulb is then used to blow streams of air onto the lenses' surface to ensure that these are free from dust. If dust is found adhered to the optical surface, a piece of very soft clean cloth is used with small circular movements, without exercising too much pressure on the lens. The nasal bulb is used again to blow air on the lens to remove adhered particles. A piece of fine chamois can also be used. If so, place the chamois at the end of a small cylindrical object with a slightly smaller diameter than that of the lens. Without exercising much pressure, rotate gently on the lens surface. Finally, air is blown onto the lens surface with the nasal aspirator. This is sufficient to clean the external surfaces. The piece of chamois can be humidified with distilled water if necessary.
2. Under adequate conditions of installation, interior surfaces of optical elements should not be affected by dust or particles. If for some reason, particles are detected, it is necessary to open them to carry out the cleaning process. An eyepiece or objective must never be opened if there is not a clean environment to carry out the cleaning procedure. Clean with a camel hair brush and with the nasal aspirator according to the procedure explained previously. It is not recommended to dismount the objectives for any reason as this could alter the tolerances achieved by the manufacturer. If dismounted, it would be necessary to realign the elements and this is only feasible if the manufacturer's precise instructions are followed. Cleaning of the objectives will be limited to keeping the front and back lenses clean.
3. If immersion oil residues are detected on the lens surface, remove using lens paper or medicinal type cotton. The lens' surface must be then cleaned with a solution composed of 80 % ether petroleum and 20 % 2-Propanol.

Cleaning of the microscope's body

1. The microscope's body can be cleaned with a detergent solution to remove external filth and cut the grease and oil. This must be applied with a small brush. After the grease and filth have been removed, the microscope's body must be cleaned with a 50/50 solution of distilled water and 95% ethanol.
   Note: This solution is not adequate for cleaning optical surfaces.
2. The parts integrated in adjustment mechanisms for the macro/micrometric (thick and fine) adjustment, the condenser and the stage or platform must be lubricated periodically with refined machine oil to facilitate smooth movement.

Microscope maintenance

Among the most important steps for maintaining a microscope in suitable operation conditions are the following:

1. Verify the adjustment of the mechanical stage. It must move gently in all directions (X-Y) and must stay in the position selected by the microscopist.
2. Test the focus adjustment mechanism. The focus selected by the microscopist must remain stable. The height must not change from that assigned by the microscopist.
3. Verify the functioning of the diaphragm.
4. Clean all the mechanical components.
5. Lubricate the microscope according to the manufacturer's recommendations.
6. Confirm the adjustment of the specimen holder (gripping device).
7. Verify the optical alignment.

Precautions

1. Avoid cleaning optical components with ethanol because it affects the optical elements. Also, do not clean the base of the platform with xylene or acetone.
2. Do not use ordinary paper to clean lenses as it could scratch their surface.
3. To prevent leaving fingerprints, do not touch lenses with bare fingers.
4. Do not clean the eyepieces' lenses or objectives with cloth or paper, because the coating covering the optical elements could deteriorate. Clean these surfaces with a camel hair brush or by blowing air with a nasal aspirator.
5. Avoid leaving the microscope without the eyepieces. Place stoppers on these to prevent dust and particles from entering the binocular head.
6. Do not leave the microscope stored inside a box in humid environments.
7. Avoid pressing the objective against slides as it could damage the thin lamina or its front lens. Adjust the focus slowly and carefully.
8. Keep the platform or mechanical stage clean.
9. Do not disassemble optical components since this can produce misalignments. Optical surfaces must be cleaned first with a camel hair brush and then with a chamois or lens paper.
10. Use both hands for lifting the microscope, one hand supporting the microscope arm and the other supporting its base.
11. Avoid touching the surface of the bulb with fingers when changing it. Fingerprints decrease the light intensity.
12. Verify that the feed voltage is correct in order to prolong the life span of the bulb. Whenever possible, use the lowest light intensity needed for carrying out observations.
13. Connect the microscope to a voltage stabilizer if the feed voltage is not stable.

Special care in warm climates

In warm climates as well as in dry ones, the main problem affecting the microscope is dust since it affects the mechanical and the optical systems. This problem can be controlled by the following steps:

1. Always protect the microscope with a plastic cover when not in use.
2. After use, clean the microscope by blowing air using a nasal aspirator.
3. Clean the lenses with a camel hair brush or with an air brush. If the dust stays adhered to optical surfaces, try to remove it with lens paper. However, rub the surface very gently to avoid scratches.

Special antifungal care in humid climates

In humid and generally warm climates, microscopes can be affected by fungi growing mainly on the surface of lenses, in the grooves of screws and under the protective paint. If the equipment is not adequately protected, it could become useless in a short period of time. The following care instructions will assist in preventing the formation of fungus.

1. At night, store the microscope in a box equipped with an electric light of no more than 40 W. The bulb must be installed in the upper part of the box, near the binocular head and must be kept on during the night. The box must have some openings to allow the air to circulate. The temperature inside the box must not exceed 50 °C so that properties of the microscope's lubricants are not affected.
2. If it is not possible to use a box with electric light, as an alternative, a drying agent such as silicone gel or rice can be used. When a drying agent is used, verify that the microscope is kept in a protected box or under a cover made of fabric similar to that of a handkerchief. Verify that the drying agent is in good condition. If this is not the case, substitute it.
3. Clean the microscope periodically. Use latex gloves if lenses must be touched. This will prevent leaving any fingerprint and decrease the risks of fungal growth.
4. If none of the mentioned alternatives is feasible, put the microscope in a place with good air circulation. When the microscope is not in use, it may be located under direct solar light, for short periods. This reduces the humidity and the risk of fungi growing on the surfaces of the equipment.
5. Air conditioning (temperature and humidity control) significantly prevents fungal growth on microscopes. However, this is not an option for a great number of laboratories. If the air conditioning service is not continuous in the area where the microscope is installed, precautions must be taken to control the humidity.

Removal of fungal hair

1. Check and clean the microscope frequently using the procedures mentioned in this chapter. Control the humidity conditions where the microscope is stored. If adequate ventilation is maintained, it decreases the possibility of fungal growth on the microscope.
2. If fungal growth is detected, use a small piece of cotton dampened in an antifungal solution, normally ether or xylol (xylene). Rub gently making circular motions on the entire surface of the lens. An oscillatory movement can also be used, towards the front and back or left-right-left, exercising a very moderate pressure on the surface of the lens. If necessary, repeat the procedure with a new piece of cotton.
3. When removal of the fungal hair is completed, clean with a small piece of clean cotton.

Microscope care

Frequency: Daily (after use)

1. Clean the immersion oil off from the 100X objective. Use lens paper or, if not available, use medicinal type cotton.
2. Clean the sample holders.
3. Clean the condenser.
4. Place the light intensity control rheostat in the lowest position and then turn off the lighting system completely.
5. Cover the microscope with a protective cover (of plastic or cloth). Ensure that it is kept in a well ventilated place where the humidity and temperature are controlled. If it has a ventilated storage box equipped with a light bulb for humidity control, place the microscope inside, turn on the light and close the box.

Frequency: Each month

1. Remove dust particles from the microscope's body. Use a piece of cloth dampened with distilled water.
2. Remove dust particles from the eyepieces, objectives and condenser. Use a rubber bulb for blowing air. Next, clean the lenses' surface with lens cleaning solution. Do not apply this solution to lenses directly, but on lens paper and then rub their surfaces gently with the wet paper.
3. Remove the slide holder mechanism, clean carefully and reinstall.

Frequency: Every six months

As a complement to the monthly maintenance routines, the following are recommended:

1. Carry out a general visual inspection of the microscope. Verify that each component is in good condition, clean and mechanically well adjusted.
2. Verify that good ventilation conditions, temperature and humidity control are maintained in the place of installation.
3. Test the quality of the electric system that feeds the microscope. Verify the integrity of the connectors, fuses and of the incandescent light.

TROUBLESHOOTING TABLE

Lighting system

PROBLEM	PROBABLE CAUSE	SOLUTION
The lighting system does not come on.	The electrical feed cable is disconnected.	Connect the electrical feed system.
	The protection fuse is burnt out.	Replace the protection fuse.
	The bulb is burnt out.	Replace the light bulb. Ensure it is well aligned.
	The lighting switch is defective.	Replace the switch.
The lighting system is not producing uniform light.	The electrical system shows voltage errors.	Check and repair the electrical system. Connect the microscope through a voltage stabilizer.
	The microscope's connector to the wall outlet is slack.	Connect the plug to the outlet. If any of the elements are defective, replace it.
	The bulb is badly installed and is not making good contact.	Reinstall the bulb.
	There are metal or black specks on the bulb's surface.	Replace the light bulb.
The sample is not illuminated in a uniform manner.	The light source is not centred.	Rectify the alignment of the condenser.
	The objective is not well centred.	Slowly turn the revolving objective holder until the adjustment catch sound.
The sample is poorly illuminated.	The diaphragm's iris is almost closed.	Open the diaphragm's iris until the lighting is adequate.
	The condenser is very far (very low).	Bring the condenser closer.
	The condenser's lenses show dust or fungal growth.	Clean the condenser. Remove the dust with a brush. Remove the fungi with a lens cleaning solution.
There is excessive contrast in the image.	The diaphragm's iris of the condenser is almost closed.	Open the iris of the diaphragm slightly.
The image is slightly too clear and shiny.	The diaphragm's iris of the condenser is very open.	Close the diaphragm's iris slightly.

Optical/mechanical system

PROBLEM	PROBABLE CAUSE	SOLUTION
The mechanical stage does not stay in position and the image is continually going out of focus.	The adjustment tension of the mechanical stage is slack.	Adjust the tension mechanism of the mechanical stage.
The mechanical stage cannot be raised to its higher limit.	The mechanical stage is locked very low.	Loosen the locking mechanism of the mechanical stage. Adjust to the desired height. Readjust the locking mechanism.
There is poor quality of the image with objective 40X.	The lenses show fungi.	Remove the fungi using a cleaning solution. Follow the manufacturer's instructions regarding the device.
	The lenses are damaged.	Check the objective. Verify if the lenses show scratches, punctures or nicks. Replace the objective.
	The lenses are accidentally smeared with immersion oil.	Remove the oil carefully with lens paper.
The immersion objective does not give clear images.	The objective is being used without immersion oil.	Place immersion oil on the slide.
	The immersion oil is of a low refraction index.	Use good quality oil.
	There is immersion oil in the interior of the objective.	Clean the lenses with lens paper. If cleaning the outside is not the solution, send the objective to a specialized laboratory for repair. (Dismount the lenses, clean, change the seals, cement, realign and assemble).
Dust or visible dirt is in the field of vision.	Dust present on the collector lens of the light source.	Remove particles of dust with a camel hair brush.
	Dust present on the upper lens of the condenser.	Remove the dust particles with a camel hair brush.
	There is dust on the eyepiece.	Remove the particles of dust with a camel hair brush.

BASIC DEFINITIONS

Acetone. This is a colourless, flammable liquid with an excellent capacity to mix with water; a solvent used for a great number of organic substances. Boiling point: 56 °C. Chemical formula:
$CH_3 – CO – CH_3$

Diaphragm. This is a device which controls the flow of light through the microscope. There are two types of diaphragms: the aperture diaphragm which adjusts the angle of the aperture in the microscope, and the field diaphragm which regulates the size of the image. The purpose of the diaphragms in optical microscopes is to prevent rays of light with severe aberrations from reaching the image formation levels and to ensure an adequate distribution of light in the sample as well as in the image's space.

Ethanol. This is a colourless liquid also known as ethylene alcohol. A widely used industrial solvent, for example in the pharmaceutical industry. Its density is 0.806 g/cm^3, boiling point 78.3 °C and chemical formula:
$CH_3 – CH_2OH$

Ether. This is a liquid substance derived from alcohol by eliminating one molecule of water between two molecules of alcohol. It is an excellent solvent which is not very soluble in water and is very volatile and flammable. Its boiling point is 35 °C and chemical formula:
$CH_3 – CH_2 – O – CH_2 – CH_3$

Eyepiece. Set of lenses through which the microscopist observes the image (real or virtual image depending on the relationship that exists with other sets of microscope lenses).

Field depth. The specimen or sample's compactness which is reasonably clear at a determined level of focus.

Field of vision. The surface area seen when looking through the microscope. The area decreases with increasing power of magnification. The diameter of the field of vision is measured in millimetres (mm) on the intermediate plane of the image. The field of vision in an optical microscope at a particular magnification is expressed as its diameter in mm or simply as a number.

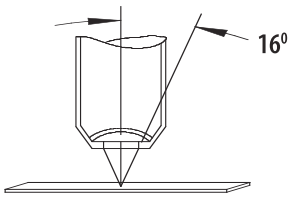
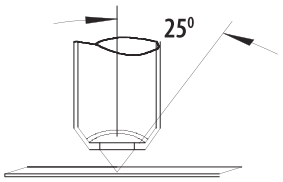
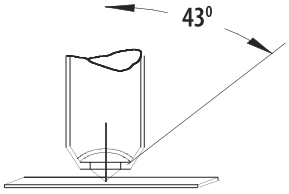
Focus. The point where, as a result of the light's refraction, the light rays passing through a lens are concentrated. If the light rays converge in one point, the lens is positive and the focus is real; if the light rays diverge, the lens is negative and the focus virtual.

Focus depth. A range at which the image plane can be moved maintaining clarity.

Numerical aperture. This is a measurement of the capacity of an objective to concentrate light and distinguish minute details of an object. Normally, the value of the numerical aperture is recorded on the side of the objective's body. Greater values of numerical aperture allow a greater number of oblique rays of light to pass through the objective's front lenses, producing a higher resolution of the image. It is expressed mathematically as:

$$NA = n\sin(\phi)$$

Where:
NA = numerical aperture
n = refraction index (n = 1 air; n = 1.52 immersion oil)
Φ = aperture angle. At a greater the angle, a greater thenumerical aperture, a greater resolution

Numerical aperture	Mathematical expression
	NA = n x Sin Φ 0.27 = 1 x Sin (16°) Magnification approx. 10X
	NA = n x Sin Φ 0.42 = 1 x Sin (25°) Magnification approx. 20X
	NA = n x Sin Φ 0.68 = 1 x Sin (43°) Magnification approx. 40X

Propanol. Also known as isopropyl alcohol and prepared by the hydration of propylene. It is used as a solvent as well as in the preparation of acetone. Its boiling point is 83 °C and chemical formula:
$CH_3 – CHOH – CH_3$

Range of useful magnification. [RUM] of an objective/eyepiece combination is defined by the numerical aperture of the system. For perceiving the details of an image, a minimum magnification traditionally between 500 and 1000 times the numerical aperture [NA] of the objective is required. {Acceptable from RUM = (500) x [NA] to (1 000) x [NA]}.

Refraction index. Value calculated by comparing the speed of light in space and in a second medium of greater density. It is normally represented by the letter [n] or [n´] in technical literature or in mathematical equations.

Resolution. The ability to distinguish the finest details from a slide or particular sample. Among factors which most influence achieving a good resolution are the numerical aperture, the type of sample, the lighting, the aberration correction and the type of contrast used. It is one of the most important characteristics of the microscope.

Revolving objective holder. Mechanical device designed for mounting the objectives and allowing rapid interchange by means of a rotational movement. Its capacity depends on the type of microscope. In general, it varies between three and five objectives.

Xylene. Ethyl benzene isomer obtained from coal. It is used as a solvent and also in the preparation of dyes and lacquers. Its boiling point is 138 °C / 144 °C and chemical formula: