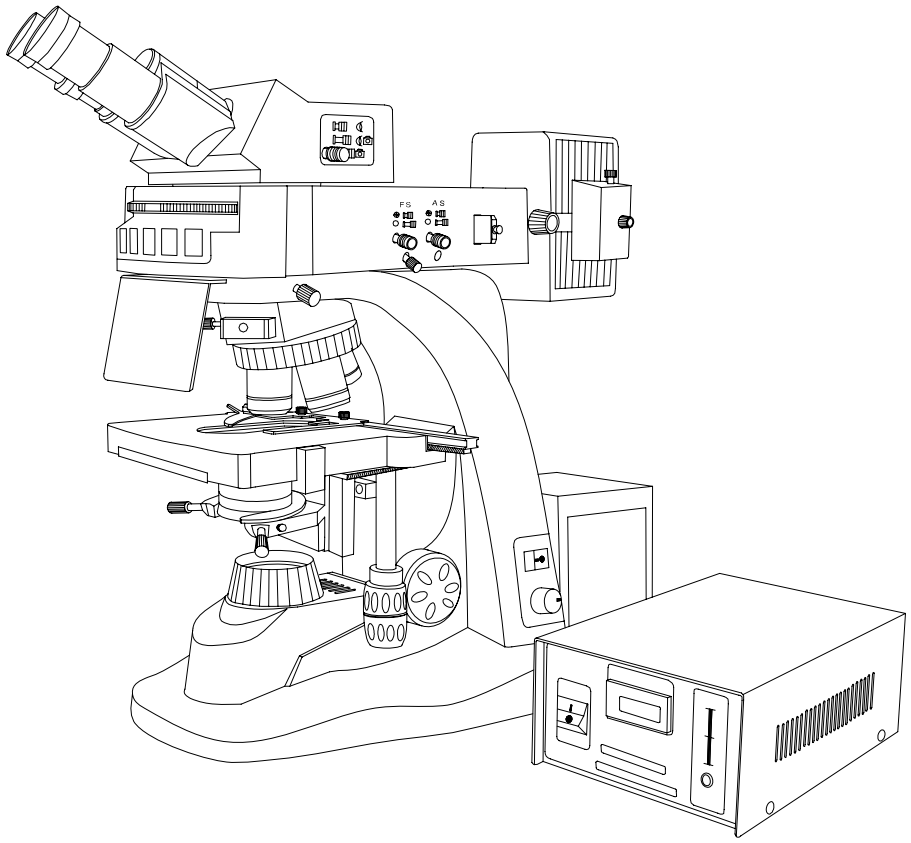
# Epi-fluorescent microscope

# Model 158/358

# Operation Manual




This Operation manual is for 158/358 five wave band Epi-fluorescent microscope. To ensure safety, get the optimal performance and make you fully familiar with the usage of this microscope, we recommend that you should read this handbook carefully before operation.

# Contents

## 1. The basic parameters and specifications of the microscope

- **Eyepieces:**

| Category | Magnification | Visual field diameter(mm) |
|---|---|---|
| Super wide field plan eyepiece | 10X | Φ22 |

- **Objectives：**

| Category | Magnification | Numerical Aperture（NA） | System | Working Distance（mm） |
|---|---|---|---|---|
| Plan infinite big N.A. fluorescent objectives | 4X | 0.15 | dry | 21.6 |
| | 10X | 0.35 | | 2.9 |
| | 40X | 0.75 | | 0.6 |
| | 100X | 0.90 | | 0.62 |

- **Total magnification of microscope：**

| Objective | Eyepiece | Microscope tube | Magnification |
|---|---|---|---|
| 4X | 10X | 1X | 40X |
| 10X | | | 100X |
| 40X | | | 400X |
| 100X | | | 1000X |

- **Mechanical tube length ：** ∞
- **Microscope tube lens：** Focal length (f) = 200 mm
- **Coarse and fine focusing adjustment range：** 25 mm
- **The entire fine focusing adjustment division value：** 0.002 mm
- **Mechanical stage：** Move 76 mm horizontal, 50 mm vertical, graduation 0.1 mm

## 2. Before use

A) Safety note:

- Microscope must be placed on a firm and flat desktop or working table.
- Microscope must not be placed under direct sunlight, keep away from the wall 20 cm
- When connecting microscope with power supply, turn the main switch to "OFF" position, make sure the inlet voltage must be in compliance with label marked on the microscope, then plug into the electrical outlet. Please pay attention to make good earth connection; otherwise, it would influence the electrical safety and instrument performance.
- When replacing the light bulbs, shut off the main switch and pull the power plug out from the electrical socket; never pull power cord directly with hand by force.
- The bulb in light cabin can be replaced after it is cool.
- Microscope is a precision optical instrument, please operate carefully and avoid a sudden sharp shock or impact.
- When moving microscope, you should hold both the arm of main body and base of microscope with 2 hands. Holding the microscope from the viewing head is prohibited.
- The microscope cannot be moved from a low-temperature environment into high-temperature environment suddenly since the optical components would go moldy; that would cause the image not be focused sharply and influence the observation.

## B) Preparation before use:

- Sample specimen: process the collected sample with professional and technical treatment, and make the prepared specimen for microscope observation.
- Preparing a number of materials and appliances: such as alcohol, ether reagents, gauze, cotton, tweezers & pliers, rubber air blower, and so on. Desktop should be clean and tidy, don't put things nothing to do with work.

## C) Safeguard and maintenance:

- When cleaning all glass components, first blow the dust off the surface and then gently clean with gauze; please wipe off oil or fingerprints carefully on the lens surface with a few ether (70%) and alcohol (30%) mixed solution (dip the gauze or absorbent cotton moistened with this organic solvent)

★ **As ether and alcohol are flammable liquids, they must be used with care; pay attention not to make these chemicals close to the fire and EDM sources, such as switch operation in the electronic equipment. Remember to use these chemicals only in a well-ventilated room.**

- To clean the other parts of microscopes use a soft hairless cloth; dip a small amount of neutral cleaning agents and then wipe off the dirt.
- Please don't break down any part of the microscope, which will damage the microscope and lead to low function or performance.

- Please cover microscope with dustcover when it is not in use.
- Operating environment:

Indoor use, the highest elevation is 2 meters.
Ambient temperature: 5 ºC ~ 35 ºC. Relative Humidity: <80%.

## Warning!

**If the microscope is not operated as specified by Operation Manual, it may be hazardous to the safety of the users and may damage the equipment.**
**Please always operate microscope in accordance with this handbook.**

## 3. The Application and Principle of fluorescent microscope

### A) Application：

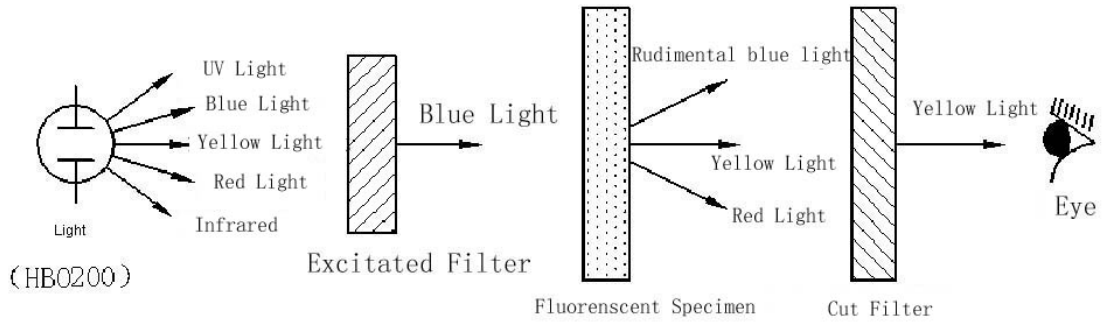
Five wave band Epi-fluorescent microscope model 158/358 is used in fluorescent microscopy techniques, including the fluorescence staining and immunofluorescence. Fluorescence staining method can show the different components inside the cells, especially the two kinds of nucleic acid (DNA and RNA), and it has a stronger specificity, so this technique is treated as a good cytochemical method. Immunofluorescence is a method which is combined with the technique of immunology and fluorescent staining; it has immune specificity and the sensitivity of fluorescence methods. It is widely used in the field of iatrology, pathology and auto-immune disease diagnosis.

### B) The principle of fluorescent microscopy：

There are many substances in nature that emit longer wavelength light (fluorescent light) when they are irradiated by shorter wavelength light (UV, purple, blue or green light); this is called photo-induced fluorescence. After the collected specimen is treated with fluorescent staining, it is excited with short wavelength light to obtain fluorescence, and adapt for the research of collected specimen. Fluorescence dye emits light thus appearing color that can be observed in a dark background; this technique is very sensitive and can be perceived with only a little of light under the microscope. This is why fluorescent dye has become a complex marker; it makes the marked antibody not only have particular activity, but also be seen by naked eye under the fluorescent microscope. Only a extremely diluted pigment of fluorescent staining is required. As a result, it provides a widely use for observing live organs and researching the process and micro-structure of functional organs, and the process of a certain metabolic change.

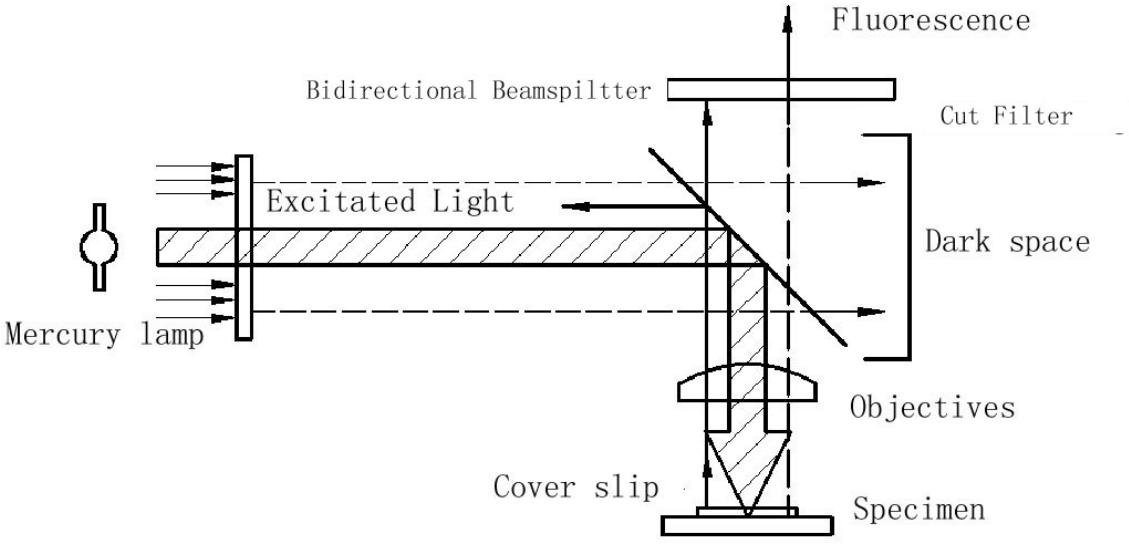
In addition, the fluorescent staining operation is very simple, short time, and easy to make specimen, especially suitable for quick work, such as the rapid diagnosis of infectious diseases.

- The principle of excitation fluorescence:



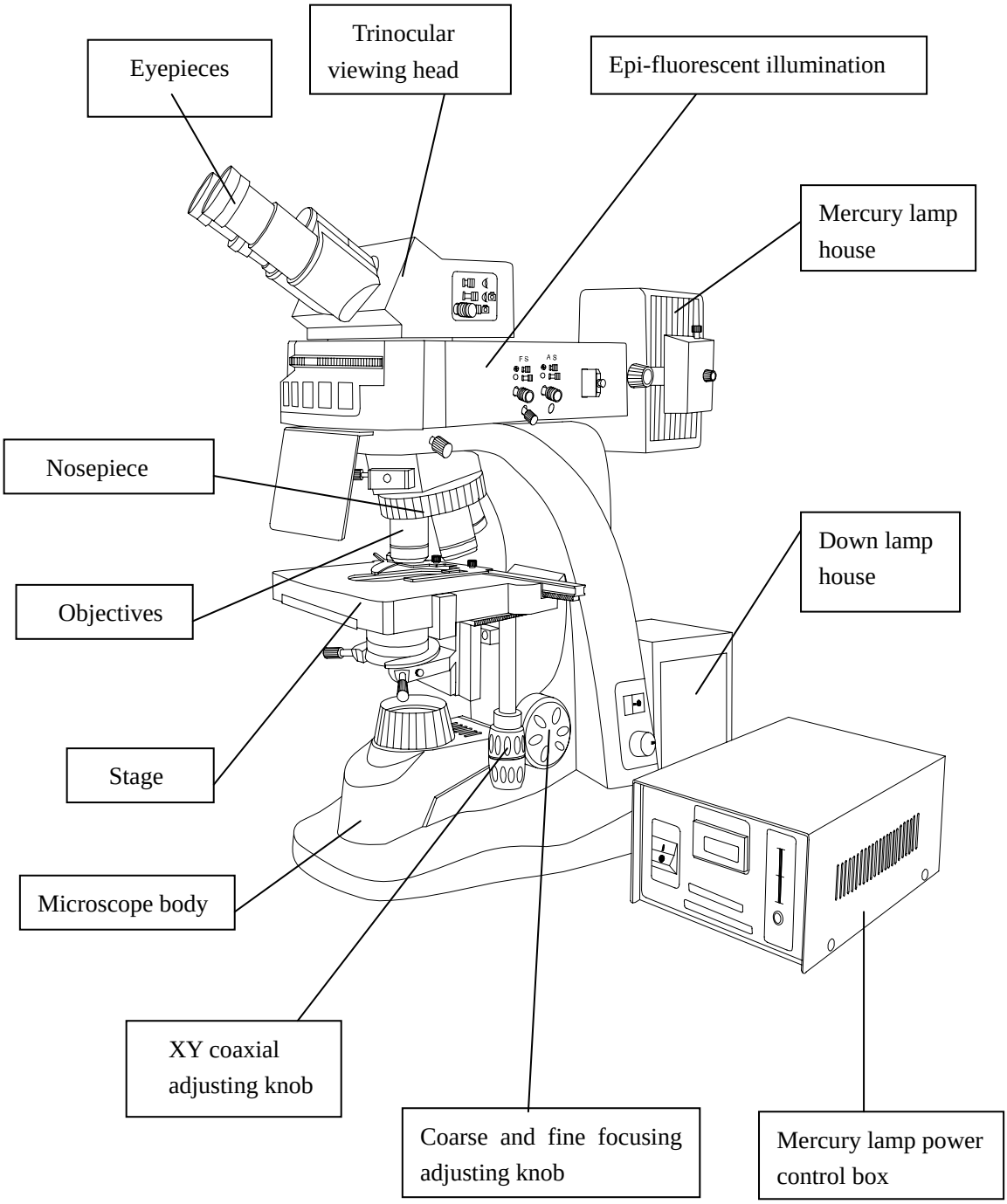

Light Source: Super high pressure Mercury lamp which provides strong enough exciting light (shorter wavelength light) for excitation of fluorescent specimen.
Exciting filter: only pass the excitation light of corresponding specimen, the rest of light is cut off.
Fluorescent specimen: the specimen dyed with the fluorescent pigment
Cut filter: blocks the excitation lights through the specimen and make observation easier.

- Diagram of Epi-fluorescence:



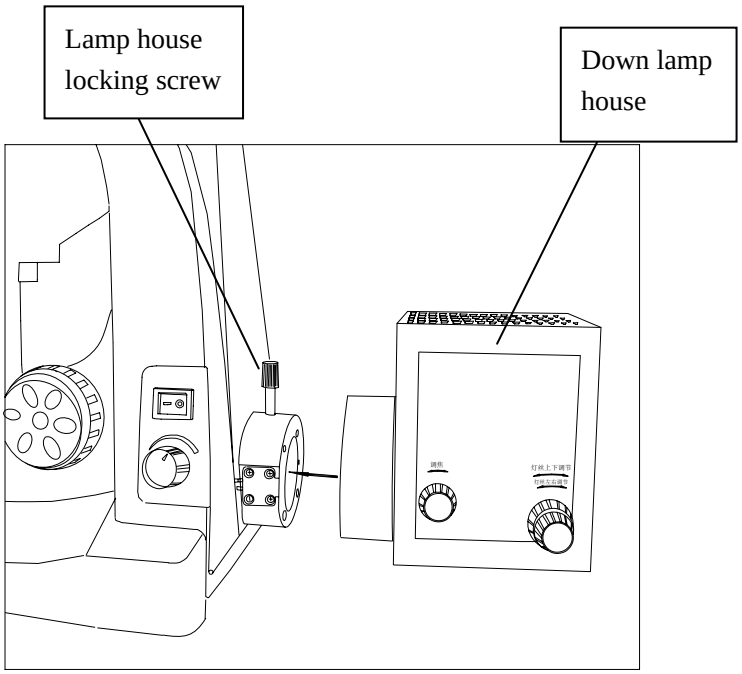
## 4. Installation and use of microscope

A) Description of each part of microscope



B) Installation:

1. Installation of down lamp house



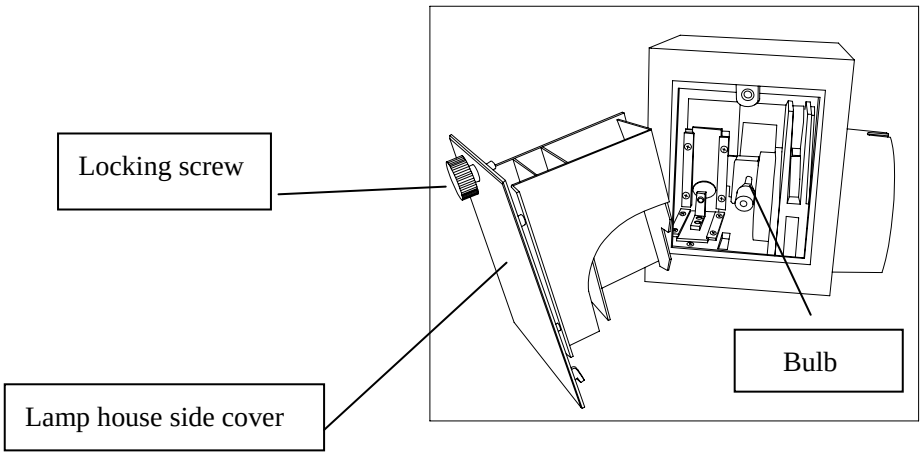

① Release the Lamp house locking screw.
② Insert into lamp house as per the direction of the arrow.
③ Lock and tighten the locking screw.

2. Installation of halogen lamps

The down illumination light source of this microscope is 12V/50W bulb.

**★ Warning: (special attention)**

Whenever replacing the bulb, firstly turn off the main switch and wait for complete cool of light bulb, the lamp socket and house.



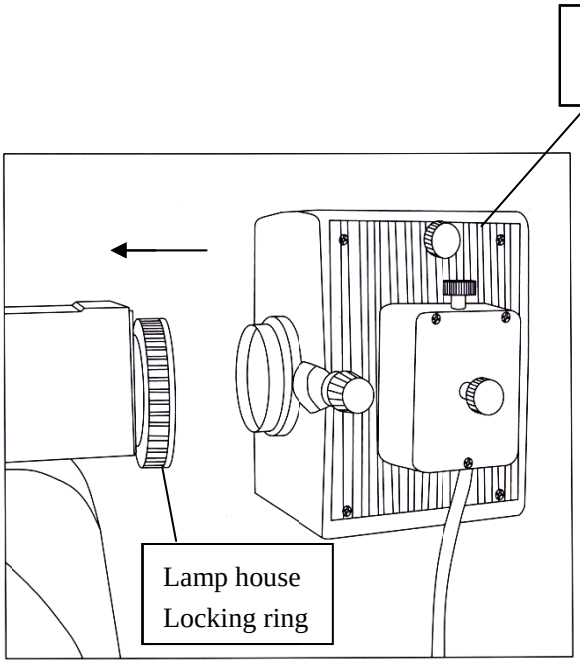

① Release the locking screw.
② Remove the lamp house side cover.

③ Wear gloves or a piece of gauze to hold bulbs; insert the bulb pins into lamp holders and make sure the pins are inserted completely.

**Do not touch bulb with your fingers; if occasionally fingerprint is left on the light bulb, use a soft cloth to clean.**

④ Install the side cover of the lamp house.
⑤ Tighten the locking screw.

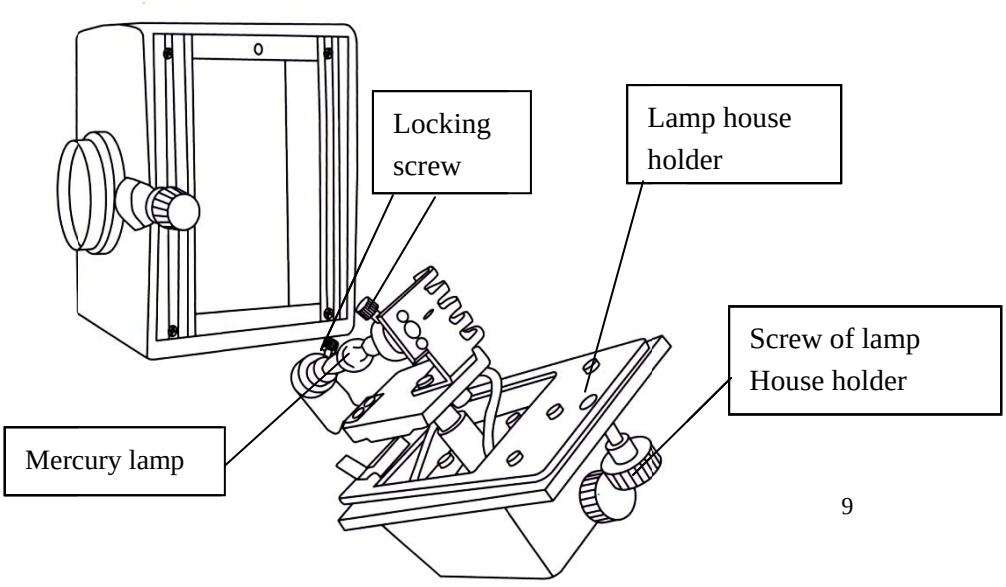
## 3. Installation of mercury lamp house




① Release the Lamp house locking ring.
② Insert into lamp house as per the direction of the arrow.
③ Lock and tighten the locking ring.

## 4. Installation of mercury lamp

Release the screw of lamp house holder and remove the lamp house holder carefully, release the locking screw with screwdriver and put into the lamp tube (please note the positive and negative pole of lamp tube), tighten the locking screw, then put the lamp house holder into the lamp house and tighten the screw of lamp house holder. Please be careful in operation and don't damage the lamp tube.

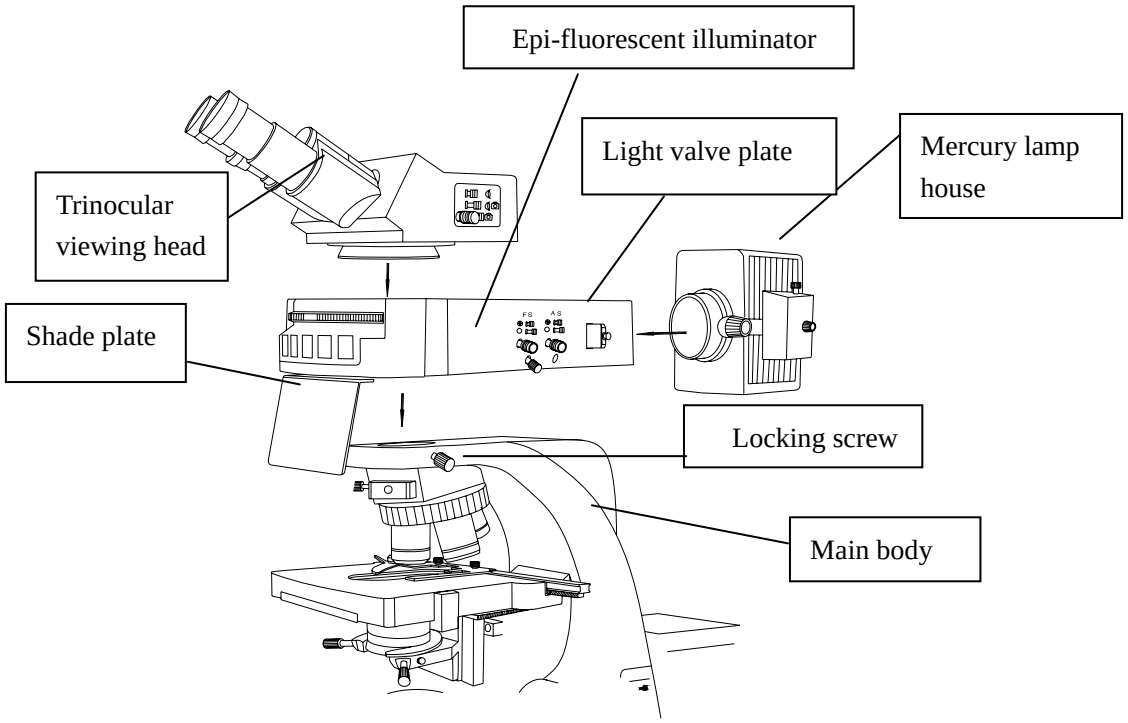


★ **Do not touch Mercury lamp tube with bare fingers when replacing the mercury lamp** (**cover the whole glass tube with a cotton cloth or wear gloves when changing the mercury lamp).**

## ★ Warning: (special attention)

Whenever replacing the Mercury lamp tube , you have to wait for the complete cool of lamp house and pull out the power plug

## 5. Installation of epi-fluorescent illuminator




① Release the locking screw
② Install the epi-fluorescent illuminator
③ Tighten the locking screw

## 6. Installation of microscope head

① Fully release the fixing screw of microscope head.
② Rotate observation tubes of microscope toward front.
③ Tighten the locking screw to fix the microscope head.

## 7. Objective installation

Mount into the nosepiece the objective lenses from low magnification to high magnification, in clockwise direction; make sure that the mounted objective lenses are firmly tightened.

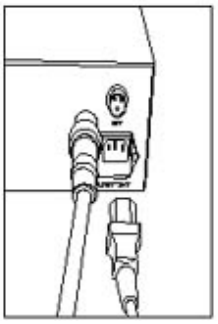
## 8. Eyepiece installation

Take off dustproof covers from eyepieces and insert eyepieces into the eyepiece tube; make sure the eyepieces are properly inserted.

## 9. Connecting the power cable

**Winding and bending easily damage power cable, do not pull strongly by force.**

① Make sure that the main switch is off.
② Connect Power control box and mercury lamp house.
③ Insert one plug of power cable into the socket.
④ Then insert the other plug of power cable into the power outlet.

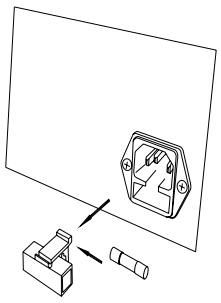


Note: Connect the power cable properly and ensure that the earth cable is connected correctly between power supply and instrument.

## 10. Replacing the fuse

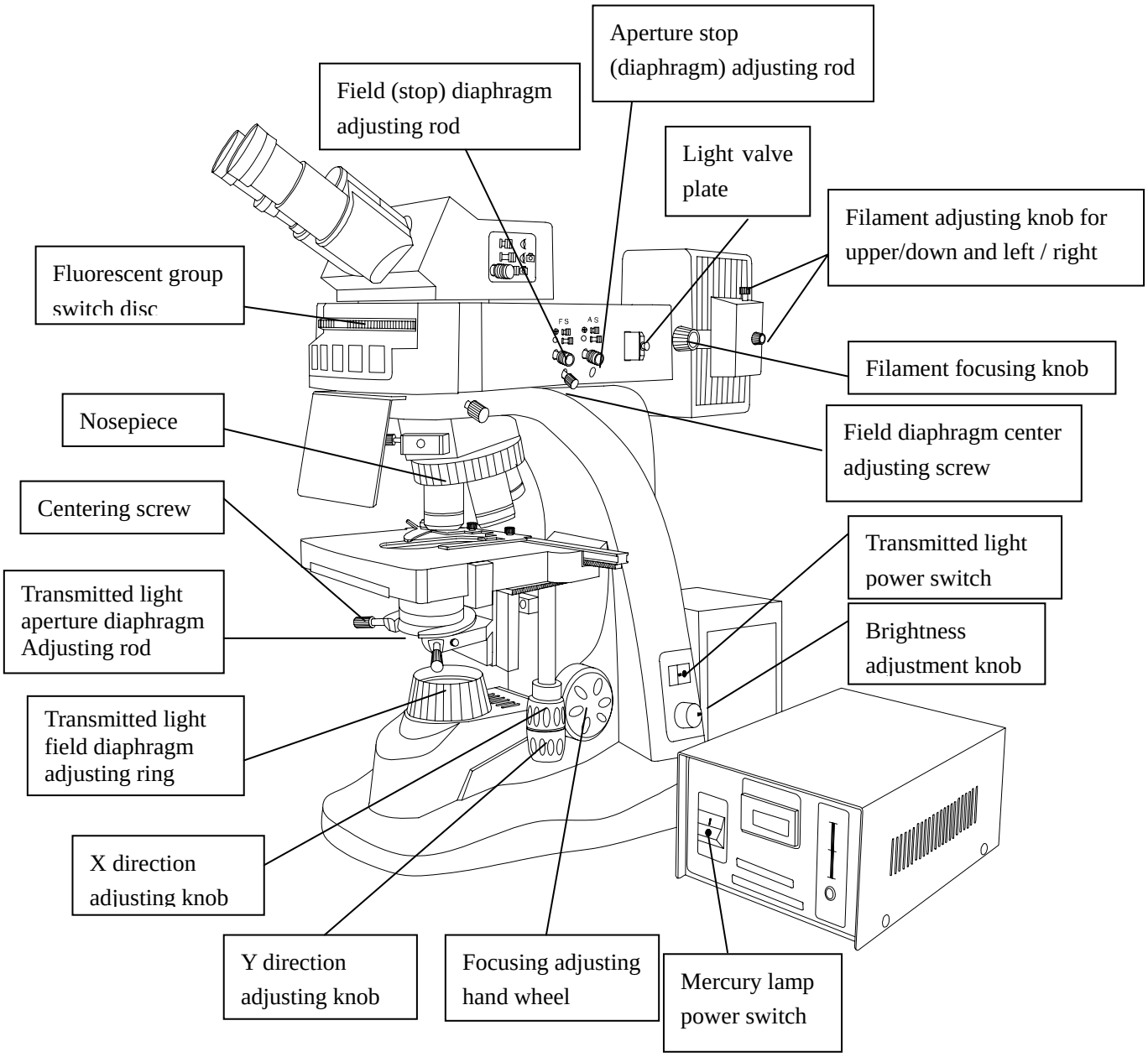
Before replacing the fuse, turn off the main switch and unplug the power cables.

① Pull out the fuse holder and take out the used fuse.
② Insert the new fuse.



Note: Use only specified rated fuse.

## C) Control


Aperture stop (diaphragm) adjusting rod
Field (stop) diaphragm adjusting rod
Light valve plate
Filament adjusting knob for upper/down and left / right
Fluorescent group switch disc
Filament focusing knob
Nosepiece
Field diaphragm center adjusting screw
Centering screw
Transmitted light power switch
Transmitted light aperture diaphragm Adjusting rod
Brightness adjustment knob
Transmitted light field diaphragm adjusting ring
X direction adjusting knob
Y direction adjusting knob
Focusing adjusting hand wheel
Mercury lamp power switch

## D) Observation steps

## 1. The use of fluorescent microscope

① Shut off the microscope transmitted light source, turn on the switch of mercury lamp power control box

② Wait for Mercury lamp shining after starting, then close the light valve plate

③ When the Mercury lamp shines fully, open the light valve plate

④ Place the fluorescent specimen (which is dyed by fluorescent pigment) on the stage, then adjust focus to get image of fluorescent specimen by using the objective 4X or 10X first.

⑤ As required depending on the fluorescent specimen pigment, choose the corresponding fluorescent light filter group (rotating fluorescent group switch disc) and adjust fine focusing knob to get the image of fluorescent specimen in dark background through eyepieces.

**Note: Please close the light valve when you don't need to observe fluorescence; the specimen should not be irradiated for long time by short wavelength light, otherwise, the fluorescence will be faded.**

**Note: Please don't turn on and shut off the Mercury lamp in short time, the lamp must be fully shined for a while after starting. After use and shut off the power supply, please don't turn the lamp on again immediately, wait at least until the lamp is completely cooled. When it is in use, open or close the mercury lamp light source by inserting and removing the light valve**

⑥ Adjusting the field diaphragm

Adjusting the field diaphragm center adjusting screw makes the field diaphragm image move to the field center. Pull the field diaphragm adjusting rod to make the field diaphragm a little bigger than field of view.

⑦ Adjusting the aperture diaphragm

Take off one of the eyepieces and cover with a paper the top of the eyepiece tube; make a small hole in the center and observe through the hole with the eye, the size of aperture diaphragm (Polygon) can be seen inside. Move the aperture diaphragm adjusting rod to regulate it, the most appropriate size is about 3/4 de diameter of the back focusing ground of objective lens (circular hole). At this time, it does not diminish the resolution of objective lens, but you can increase the contrast, and make brightness of eyeshot suitable for observation of eyes.

## NOTES:

1. When using, don't touch the mercury lamp house to avoid burning skin.
2. In operation, The anti-UV shade plate must be installed, so as to avoid damage in eyes.
3. Interrupt the operation, close light valve plate immediately.
4. To insert and pull the power cable, it must be held by the plug, don't pull the power cable.
5. Start observing once the mercury lamp is fully shined**.** If the power supply is shut off, please don't turn on the lamp again immediately; wait until it is completely cooled.

## 2. The use of down illumination (normal use of microscope):

① Close the diaphragm plate or shut off the mercury lamp power, turn the transmitted light power switch on, and adjust the brightness through light intensity adjusting knob.
② Set the Fluorescent group switch disc at the neutral gear position "O".
③ Rotate nosepiece and place 10X objective in the light path.
④ Place the samples on the stage.
⑤ Rotate XY coaxial hand wheel to move the sample into the light path.
⑥ Observe with the right eye through the right eyepiece and turn coarse focusing knob to focus the sample; then adjust fine focusing knob to get a clear and sharp image.
⑦ Observe with the left eye through the left eyepiece and move the diopter adjusting ring to make image clear in the left eyepiece. At this time, the image in both eyepieces is in focus and clear.
⑧ Regulate the eyepiece interpupillary distance.
⑨ Regulate filament center: remove one eyepiece and adjust filament focusing knobs and filament upper/down, left/right adjusting knob until a clear filament image is observed in the middle of the back focusing ground of objective lens.
⑩ Observe with different magnification objectives and adjust the light intensity to the required level. Since objectives are guaranteed the parfocal accuracy, to get a clear image it is just necessary to adjust the fine focusing knob.
⑪ Insert the selected light filter into light path
⑫ Regulate field diaphragm on the base until the field diaphragm is a little bigger than the field of view.
⑬ Regulate aperture diaphragm under condenser: take off one of the eyepieces and cover with a paper the top of the eyepiece tube; make a small hole in the center and observe through the hole with the eye, the size of aperture diaphragm (Polygon) can be seen inside. Move the aperture diaphragm adjusting rod to regulate it, the most appropriate size is about 3/4 de diameter of the back focusing ground of objective lens (circular hole). At this time, it does not diminish the resolution of objective lens, but you can increase the contrast, and make brightness of eyeshot suitable for observation of eyes.

## 3. Specifications of the fluorescent device

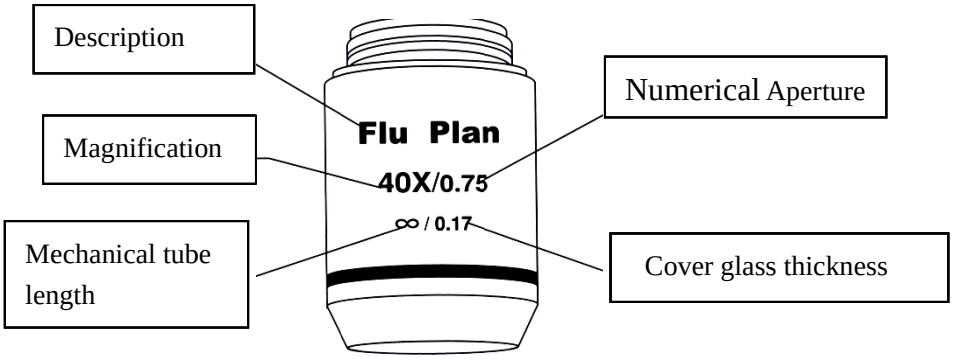
1). The magnification of device system is 1X
2). Fluorescent modules:

| G | B | BV | V | U |
|---|---|---|---|---|
| EX510--560<br>DM575<br>BA590 | EX450--490<br>DM505<br>BA520 | EX400--440<br>DM455<br>BA470 | EX380--420<br>DM430<br>BA450 | EX330--380<br>DM400<br>BA420 |

3). Short wavelength global mercury lamp GCQ100 20V/100W.
4). Input voltage of power supply box: 220 V / AC.

## 5. Optical performance and characteristics:

Structural features and performance parameters marked on the objective lens, see below.




The optical characteristics combination of Eyepiece and Objectives listed in the following table

| **Optical characteristics** | | **4X** | **10X** | **40X** | **100X** | **Remark** |
|---|---|---|---|---|---|---|
| **Numerical Aperture** | | 0.15 | 0.35 | 0.75 | 0.90 | |
| **Mechanical tube length** | | ∞ | ∞ | ∞ | ∞ | |
| **Thickness of cover slip** | | 0.17 | 0.17 | 0.17 | 0.17 | mm |
| **Color circle mark** | | Red | Yellow | Blue | White | |
| **System** | | Dry | Dry | Dry | Dry | |
| **Working distance** | | 21.6 | 2.9 | 0.6 | 0.62 | mm |
| **Viewing field diameter** | | Φ6 | Φ2.4 | Φ0.60 | Φ0.24 | mm |
| **Resolution** | | 2.24 | 0.96 | 0.45 | 0.37 | micron |
| **10X Eyepiece** | **viewing field diameter** | Φ22 | Φ22 | Φ22 | Φ22 | mm |
| | **Total magnification** | 40 | 100 | 400 | 1000 | times |

## 6. Troubleshooting guide：

According to different operation method, though it is not a fault, but will not give full performance of the microscope. After the issue is occurred, please find out the reason further.

| Phenomenon | Causation | Disposal |
|---|---|---|
| **No illumination** | Bulb burn out | Change bulb |
| | Fuse burn out | Change fuse |
| | power cable not connected | Check the power connector |
| **There's Light, but viewing field is dark** | Brightness is not adjusted | Adjust light intensity knob |
| | Aperture diaphragm shrink too small | Enlarge the aperture diaphragm |
| | Viewing field diaphragm shrink too small | Enlarge the viewing field Stop (diaphragm) |
| **See dirt or dust in the viewing field** | Some dirt and dust on the eyepieces and specimen | Clean all |
| **Poor visibility :<br>Image is not obvious<br>The contrast is bad<br>Resolution is bad** | Incorrect objective position in the optical path | To ensure the objective position is correct |
| | Aperture diaphragm shrink too small | Enlarge the aperture stop (diaphragm) |
| | Group of objective dirty | Clean objectives |
| | Dirt and dust on the specimen | Clean it |
| **Image illegible** | Sample didn't level up | Level up the sample |
| | Incorrect objective position in the optical path | To sure the objective position correct |
| | Light source center deflected | Adjust center of light source |
| **Light intensity turns to high level, brightness field of view little changed, uneven lighting** | Light source center deflected | Adjust center of light source |
| **Bulb Sometimes lighting, sometimes dark** | Light bulb about to burn out | Change bulb |
| | Pins of bulb not connected | Check connecting |
| **Light bulb burned immediately** | Use wrong bulb | Use specified bulb |
| **Stage automatically decline or after fine adjusting, rapidly defocus** | Coarse focusing adjusting tension is not enough | Adjusting tension hand wheel |
| **Images not focused to sharp position and when coarse focusing, the stage can not be adjusted upward** | Coarse focusing stop is inaccurate | Coarse focusing stop position regulating |
| **Field of view is observed differently under left and right eye** | Pupillary distance adjusting incorrectly | Adjust the pupillary distance |
| | Diopter adjusting incorrectly | Adjust diopter |
| | Eyepiece of left and right side is in different magnification | Use the same rate eyepiece |
| | You have not accommodated to the microscope's observation yet | Observation from the eyepiece, look at the entire field of view prior to concentrated in specimen area. Before observe, looking upward or beyond would also be useful. |