

# **Motic<sup>®</sup>**

## **PA53 Bio and PA53 FS6** **Operation Manual**

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**Note**

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

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**This instruction Manual has been prepared for users of the Motic “FS6” Epi-Fluorescence Attachment used in conjunction with the Motic PA53 Upright Microscope.**

**For your own safety, read this manual carefully and thoroughly before using the product. Do not discard this manual. Always keep it near the product for easy reference.**

We are constantly endeavoring to improve our instruments and to adapt them to the requirements of modern research techniques and testing methods. This involves modification to the mechanical structure and optical design of our instruments.

Therefore, all descriptions and illustrations in this instruction manual, including all specifications are subject to change without notice.

## PRINCIPLE

### Fluorescence

The phenomenon that occurs when light absorbed by a material, creates a molecular excitation that causes the material to re-emit energy in the form of light at a different wavelength.

### Fluorescence Microscope for Epi-Fluorescence

The technique of fluorescence microscopy with Epi-illuminators is based on the adaptation of the vertical illuminator used in reflected light microscopy. The radiation emitted by the light source first passes through the exciting filter and is incident to a short-pass filter\* (dichroic beamsplitter). The radiation is then reflected into the objective, which also serves as a condenser. The objective concentrates the exciting radiation in the object field. Fluorescent light emitted from there is collected by the objective and returned in the opposite direction to the short-pass filter, which directs it into the eyepiece through the barrier filter.

\* A filter designed to allow the passage of radiation of wavelengths shorter than a given limit.

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## NOTES FOR SAFETY/SAFETY PRECAUTIONS



To avoid being electrocuted, do not touch the lamp socket while the light is on as a high voltage current is being supplied to the bulb.



Because the bulb produces a large amount of heat, do not touch the housing, socket or bulb when on.



This instruction manual needs to be consulted in all cases where this symbol is used, in order to find out the nature of the potential HAZARD and any actions which have to be taken.



This product has been tested to the requirements of CAN/CSA-C22.2 No. 61010-1, second edition, including Amendment 1, or a later version of the same standard incorporating the same level of testing requirements.



Protective grounding is essential for the use of this product; Ensure the user insert it with good protective earthing socket.



When you place the device, ensure the plug or appliance coupler easy to dial out when the state of emergency.



The equipment should be used on the desk; it cannot be used on the ground.



The MXH-100 starter should be connected with lamp house first, then plug in the cord to an AC outlet with tested ground conductor and connection.



To ensure safety, only use lamp house supplied by Motic.



At all times follow the additional safety advises from Lamp Manual to be aware of lamp or lightsource specific safety warnings.



Do avoid to touch the lamp socket while the light is starter unit is on, as this is a high voltage area and it should be always handled with care!



Because the bulb produces a large amount of heat, do not touch the housing, socket or bulb when it is on or still hot.



Make sure that the switch of voltage range is set to match your available line voltage.



Disconnect the power supply before replacement of fuse.



To avoid the risk, make sure the MXH-100 starter is used with Motic lamp house.



## **WARNING ADDITIONAL SAFETY PRECAUTIONS LUMOS LED/ HBO100 MERCURY LAMP**

- While LED/HBO100 are generally safe illumination systems, precautions should still be taken with this powerful Fluorescence Illumination products.
- When operating these products, please observe the following safety precautions at all times. Failure to do so may result in injuries or damage.
- Strong UV light may be emitted from the products depending on the wavelength selected. Avoid eye and skin exposure at all times. Never look directly into the light output of the Light Source. The emissions could irreversibly damage eyes or skin
- Always ensure that the Light Source is securely attached to the microscope prior to turning on the power.
- Disconnecting the mains power supply is achieved by unplugging the power cord from the power socket. Only plug in the power cable, once the Light Source is attached to the microscope.
- Change of Light bulb: The Exchange of HBO100 Light bulb must strictly follow the dedicated Instruction Manual!
- There are no serviceable parts within the Lumos LED Fluorescence Lamp. Removing any of the screws and covers will result in the safety of being impaired.
- The DC power supply unit should be inspected periodically throughout the lifetime of the system.
- Any electronic equipment connected to this product must comply with the requirements of EN/IEC 60950 and other locally valid safety rules and standards.
- If the light source mercury lamp breaks, the broken glass may be scattered and hurt human beings and/or the instruments. The gas inside the lamp will be released into the air bringing about the danger of inhaling the mercury vapor. So, all the personnel must immediately leave the room so as not to inhale the mercury vapor. Further, thoroughly ventilate the room for at least 30 minutes.

- For changing the bulb of the light source mercury lamp the usage of suitable protection equipment (Solid gloves and Face protection mask) is mandatory
- **WARNING:** UV emitted from this product. Avoid eye and skin exposure to unshielded product.
- **WARNING:** Possibly hazardous optical radiation emitted from this product. Do not look at operating lamp. Eye injury may result.
- **CAUTION:** IR emitted from this product. Avoid eye exposure. Use appropriate shielding or eye protection.

### **Device Safety and EMC**

PA53 FS6 is designed, manufactured and inspected in accordance with the IEC 61010-1:2010/EN 61010-1:2010, GB4793.1-2007, GB4793.9-2013 and YY 0648-2008 safety standards for electrical measurement, control, adjustment and laboratory device.

Electromagnetic is compatible to the standard of IEC 61326-1:2012/ EN 61326-1:2013/ IEC 61326-2-6:2012/ EN 61326-2-6:2013 and EN 55011 level

RS232port shall only be connected to certified computer according to IEC/ANSI/UL 62368-1

### **Transporting, unpacking, storage of the Instrument**

Please observe and follow the safety notes for transportation, unpacking and storage given in this document:

- The microscope is delivered as set, packed into commercial standard plastic and cardboard packaging; Please re-use the original packaging only for any transportation.
- It is advised to keep and use the original packaging for longer storage or return to the manufacturer to avoid losing the warranty.
- At receiving and unpacking the equipment, please verify that all parts specified on the delivery note are present.
- Keep Transport and storage temperatures as specified in this Manual.
- Set the microscope up on a stable worktable with solid and smooth top surface suitable for Instrument use.
- Do not touch optical surfaces.

### **Instrument Disposal**

Please observe the following safety notes for the disposal of the microscope:

Defective Instruments, accessories and consumables should be disposed in compliance with the provisions of the local law.

### **Instrument warranty**

The Instrument should only be used for microcopy applications mentioned and instructed in this operation manual. Please note the following information on the instruments warranty.

- If possible defects Motic must be notified immediately and steps are taken to minimize damage.
- If notified of such a defect Motic will evaluate the defect and if within warrant, rectify it at his discretion, either by repairing the instrument or by delivering a replacement.
- Natural wear, or defects caused by improper use are not covered by the Instrument warranty.
- Motic shall not be liable for damage caused by faulty operation, negligence or tampering with the Instrument.
- Altering or tampering of the instrument shall lead to a forfeit of all warranty claims

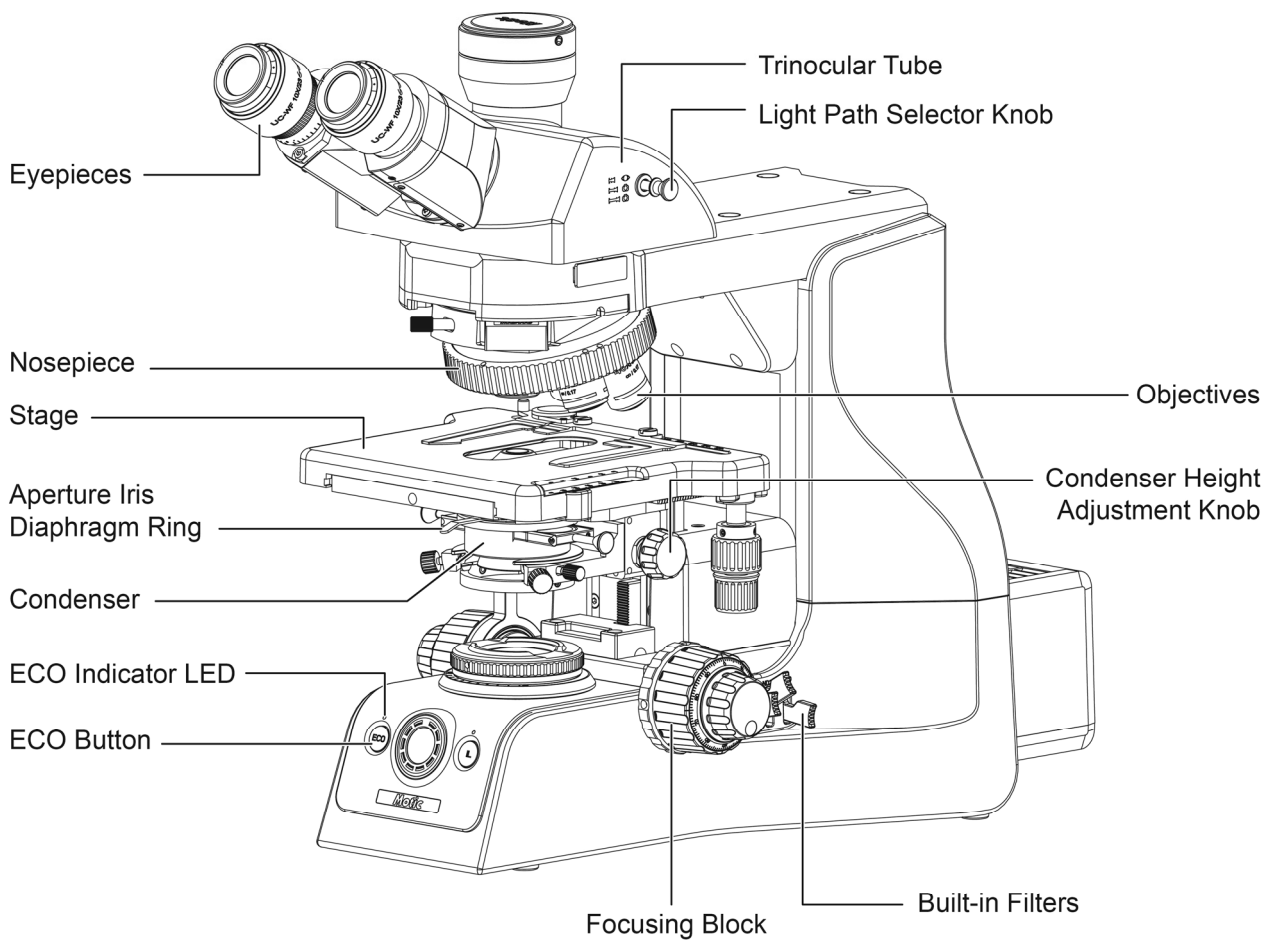
# 1. NOMENCLATURE

## 1.1 Intended use of the Microscope

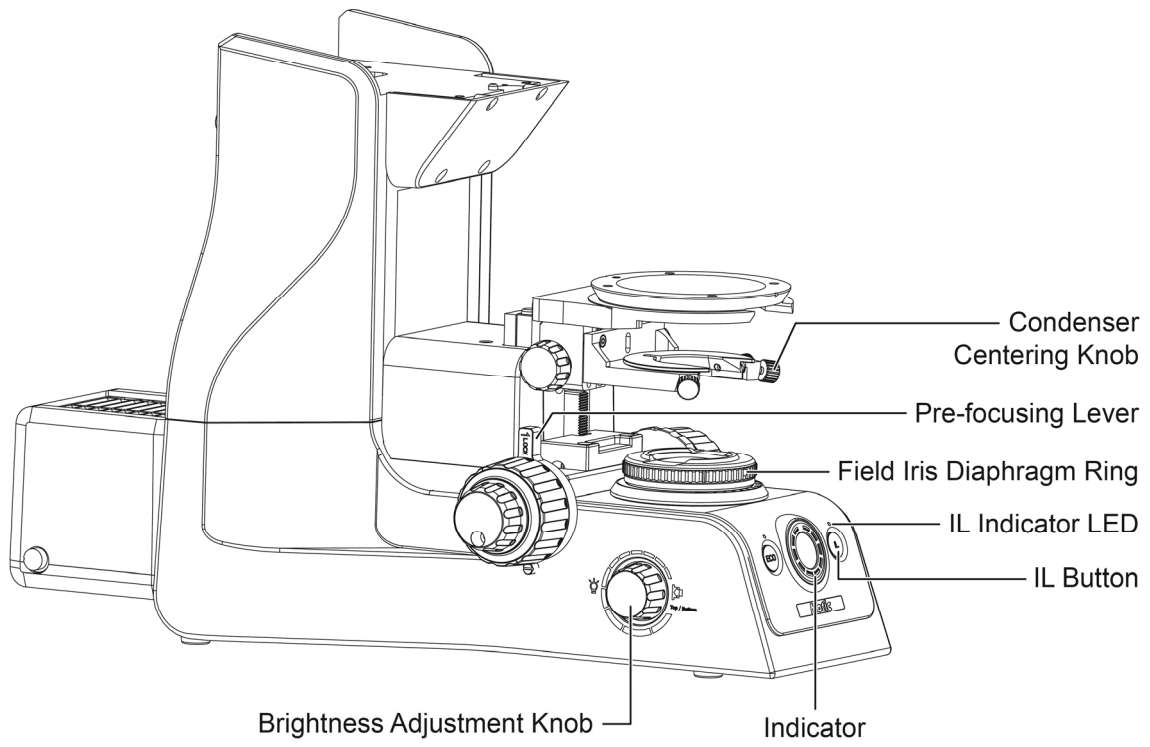
This Microscope is an optical instrument that has been designed to be used to observe and document magnified images of specimens in teaching, routine and research applications.

Do not use this instrument for any other purpose than its intended use.

## 1.2 PA53 Bio

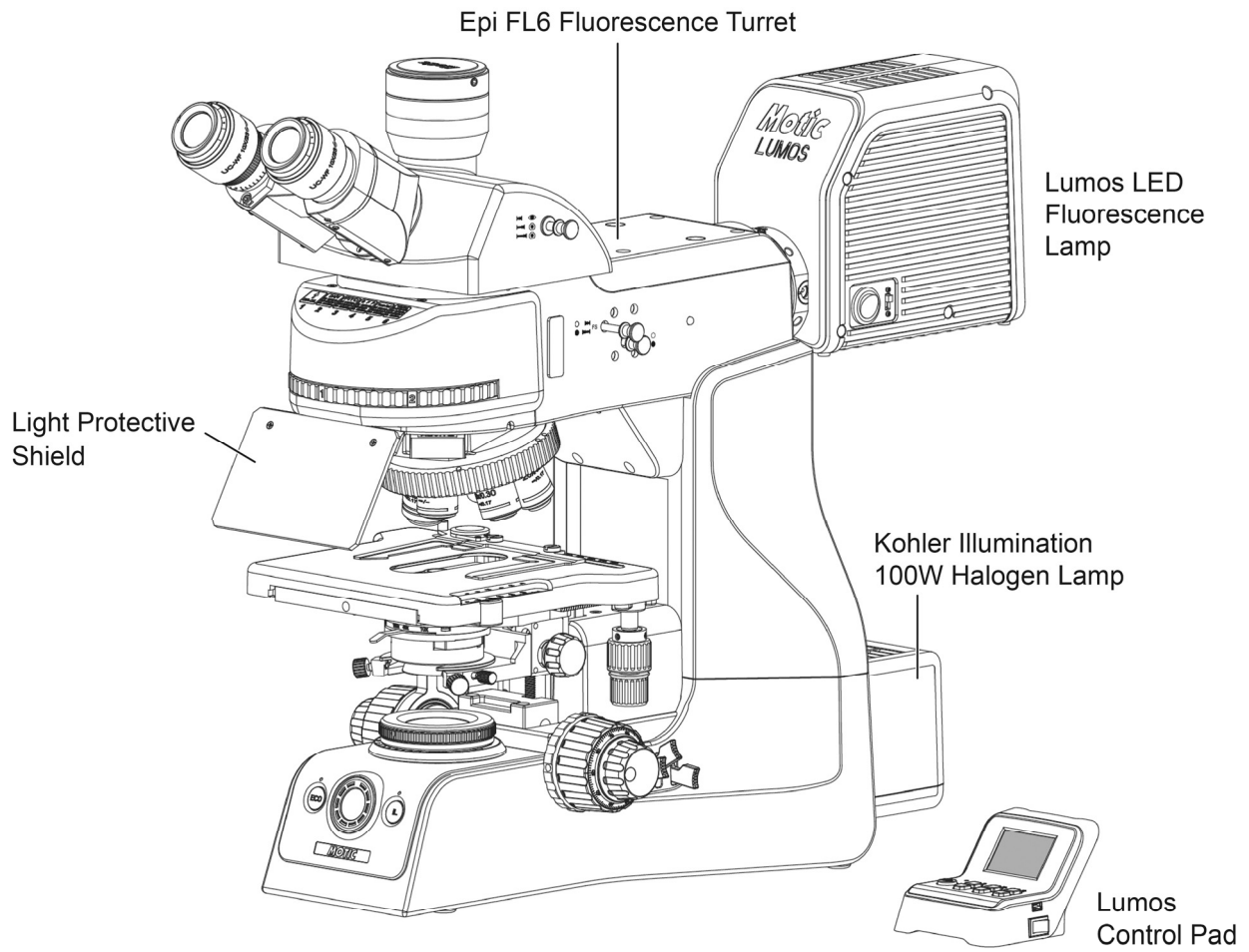


(Fig. 1)



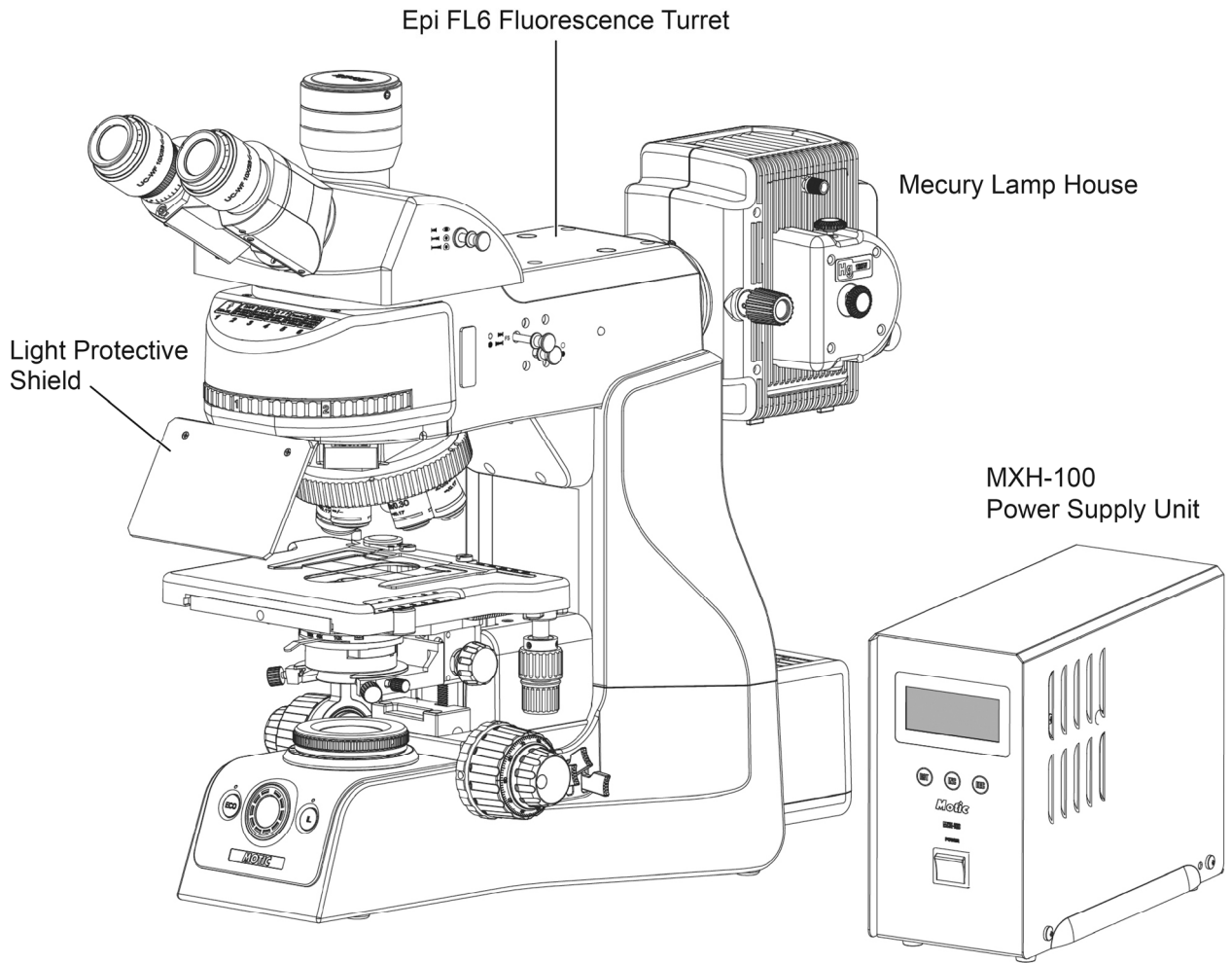
(Fig. 2)

### 1.3 PA53 FS6 with Lumos LED Fluorescence Lamp



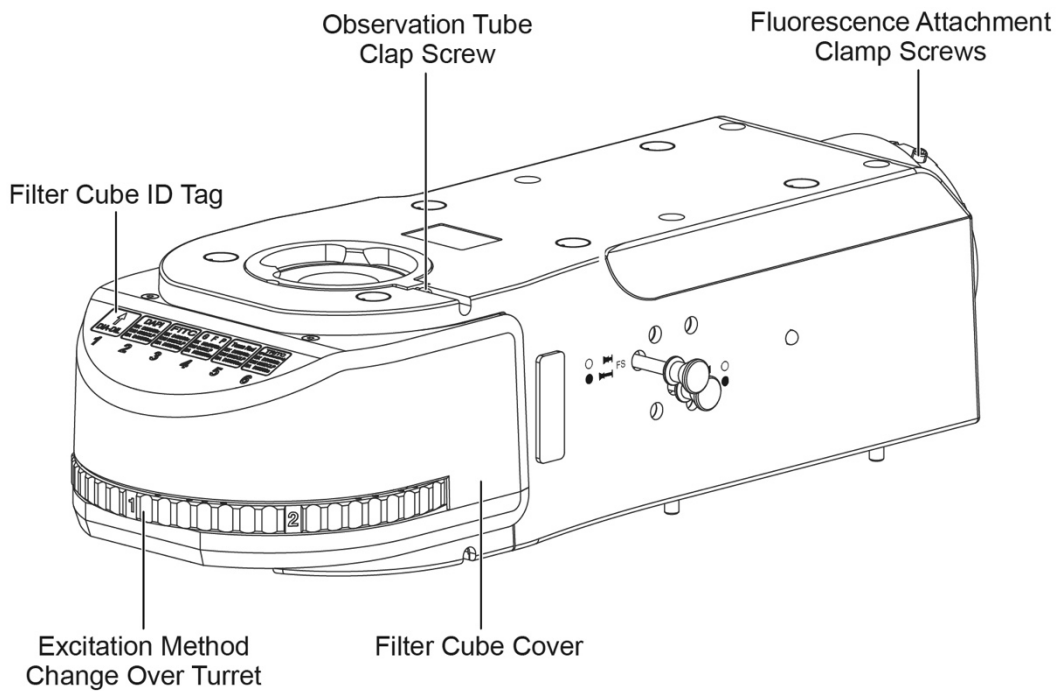
(Fig. 3)

**1.4 PA53 FS6 with HBO100 (Mercury lamp)**



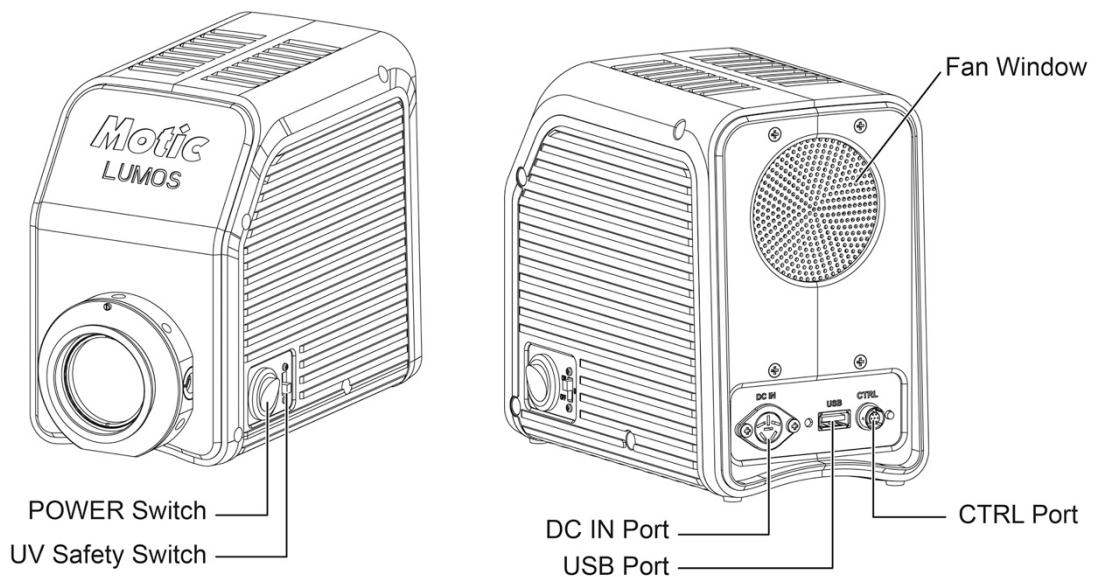
(Fig. 4)

### 1.5 EpiFS6 Fluorescence Turret



(Fig. 5)

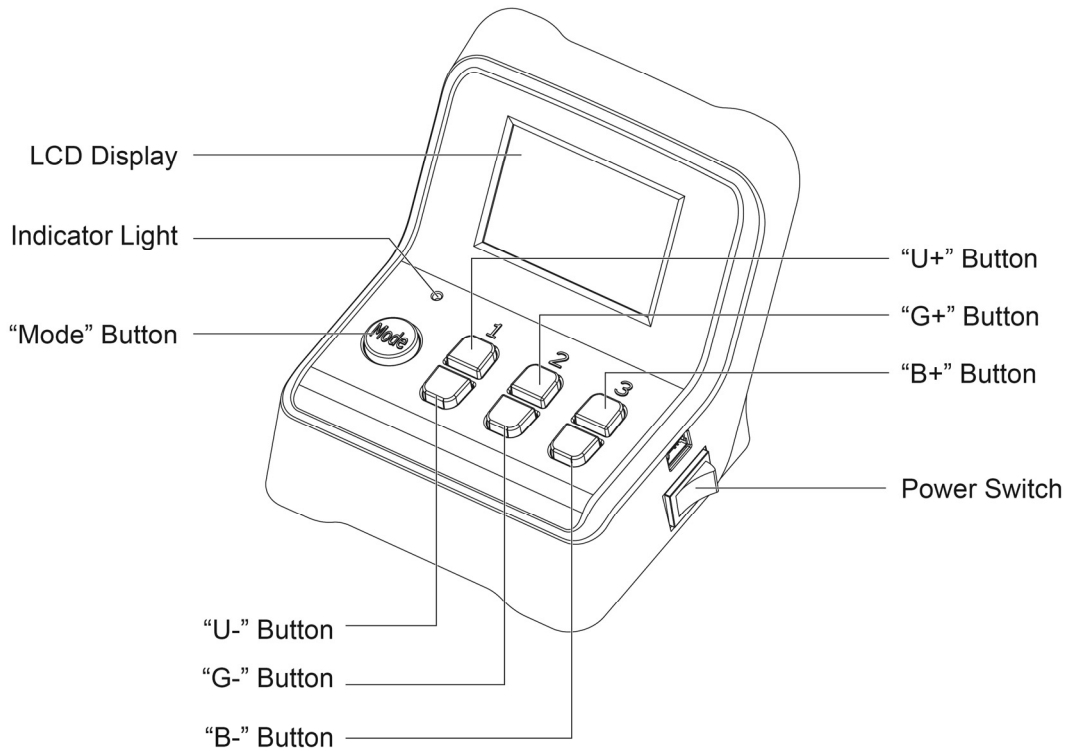
### 1.6 Lumos, LED Fluorescence Lamp



(Fig. 6)

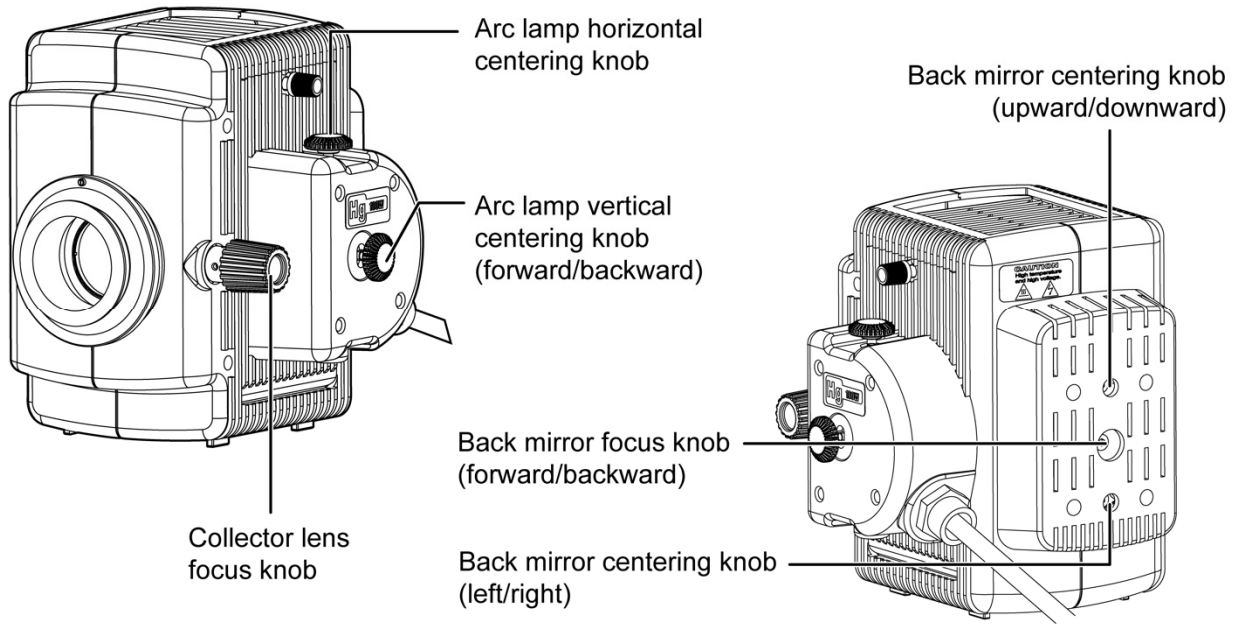


## 1.7 Lumos Control Pad



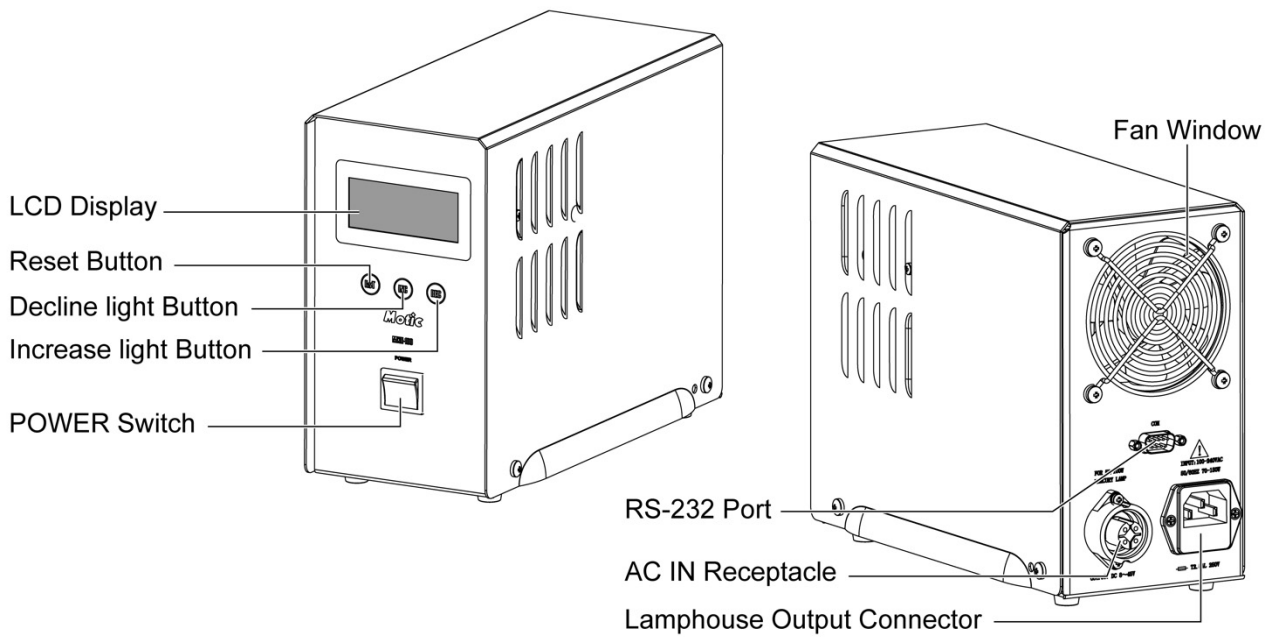
Control Pad  
(Fig. 7)

### 1.8 HBO100 Mercury Lamp



(Fig. 8)

### 1.9 MXH-100 Power supply unit



(Fig. 9)

## 2. SETTING UP THE INSTRUMENT

- Avoid placing the instrument in areas exposed to dust, vibration, high temperature and high humidity and where it is difficult to unplug the power supply cord.
- Avoid locations exposed to direct sunlight, under room lights.
- Select location which allows easy access to power cord from products AC inlet in the event of an emergency.

### 2.1 Operating environment

- Indoor use
- Altitude: within 2000m
- Environment temperature: 5°C ~ +40°C
- Humidity: 30% - 75% (No condensation)
- Power supply voltage fluctuation should not be more than  $\pm 10\%$  of nominal voltage;
- Air pressure: 75kPa~106kPa
- Pollution degree II
- Overvoltage category II
- Air pressure of 75kPa to 106kPa
- Avoid frost, dew, percolating water, and rain

## 3. ASSEMBLY INSTRUCTION

### 3.1 PA53 Microscope

Follow the instructions provided in the microscope manual.

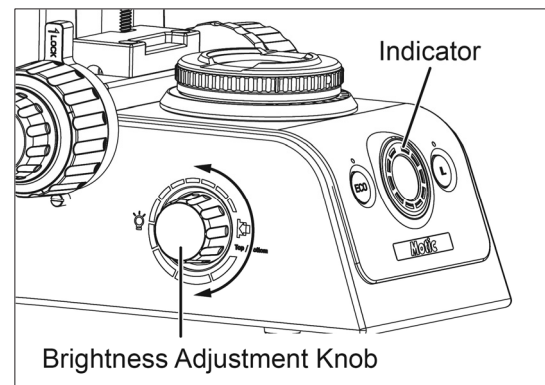
For the purpose of performing simultaneous observation with phase contrast, attach the appropriate phase contrast components referring to the specific instructions provided.

**WARNING: Installation of upper part should be done by service engineer, not by customer!!!**

### 3.2 Base

#### 3.2.1 Voltage Indication and Switch

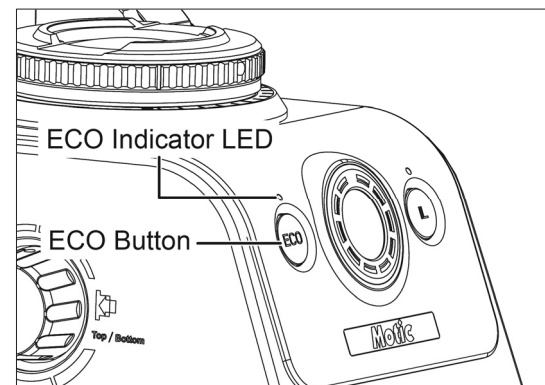
- Push the brightness adjustment knob to switch transmitted / reflected light illumination.
- Turn the brightness adjustment knob counter-clockwise to increase the voltage and make illumination brighter, and vice versa.
- The indicator indicate the voltage.



(Fig. 10)

#### 3.2.2 Turn ON/OFF Power saving function

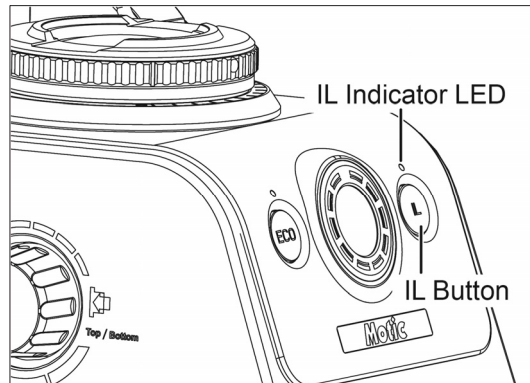
- Push ECO button to toggle power saving function on and off.
- The ECO indicator LED turns on when the power saving function turns on.
- If the power saving function turns on, lamp is automatically turns off after 5 minute when there is nobody sitting in front of microscope.



(Fig. 11)

### 3.2.3 Turn ON/OFF Intelligent Light function

- Push IL button to toggle intelligent light function on and off.
- The IL indicator LED 1 turns on when the intelligent light function turns on.
- If the intelligent light function turns on, the brightness (lamp voltage) automatically changed preset value when the revolving nosepiece is changed.
- To set new preset brightness, press IL button long while IL indicator LED turns on.

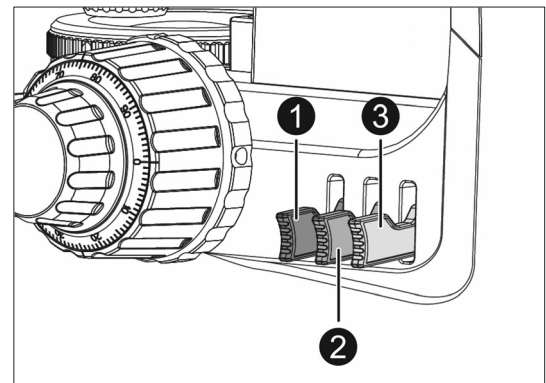


(Fig. 12)

### 3.2.4 Using the built-in Filters

- Each of filter switch can engaged into the light path by push up the switch. Push down the switch disengages the filter from light path.

	<b>Filter Type/Purpose</b>
<b>1</b>	ND6 (Neutral Density Filter for light adjustment, transmittance 6%)
<b>2</b>	ND25 (Neutral Density Filter for light adjustment, transmittance 25%)
<b>3</b>	LB (Light balancing Filter for color balancing)

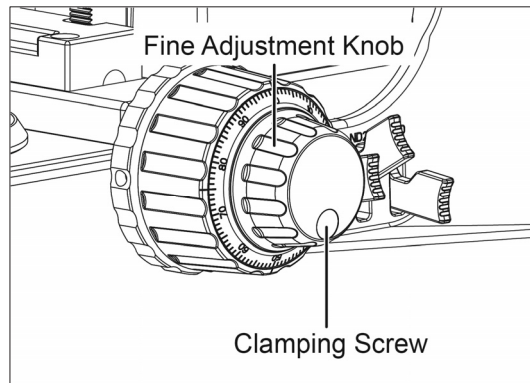


(Fig. 13)

### 3.3 Focusing Block

#### 3.3.1 Replacing the Fine Adjustment Knob

- The fine adjustment knob is designed detachable to prevent interference with hand during manipulation of the fine adjustment knob or XY-axis knob.
- Usually attach the knob on the opposite side to the XY-axis knob.
- Using the Allen screwdriver, loosen the clamping screw and remove the fine adjustment knob.



(Fig. 14)

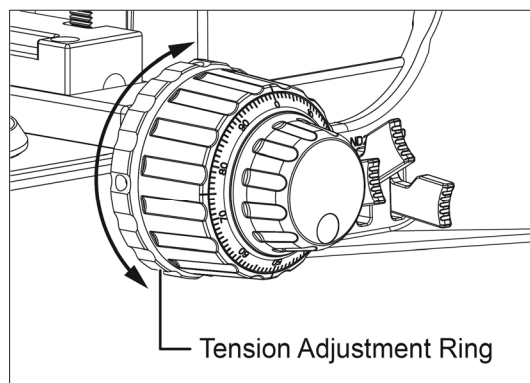
#### 3.3.2 Adjusting the Coarse Adjustment Knob Tension

- If desired, you can change the coarse adjustment tension using the tension adjustment ring.
- Turning the ring clockwise increase tension, and vice versa.

#### NOTE

If the tension is too low, the stage drops by itself and focus is quickly lost after adjustment with the fine adjustment knob.

In this case, turn the ring clockwise to increase tension.



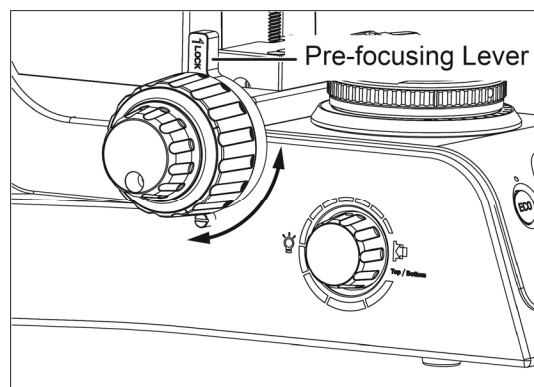
(Fig. 15)

### 3.3.3 Pre-focusing Lever

- The pre-focusing lever ensures that the objectives does not come in contact with the specimen and simplifies focusing.
- After focusing on the specimen with the coarse adjustment knob, turn this lever clockwise and lock. The upper limit on coarse adjustment movement is set at the locked position.
- After changing a specimen, refocusing is easily accomplished by rotating the coarse adjustment knob to reach the pre-focused position, then making fine adjustment with the fine adjustment knob.
- Turn the lever counterclockwise to unlock.

#### NOTE

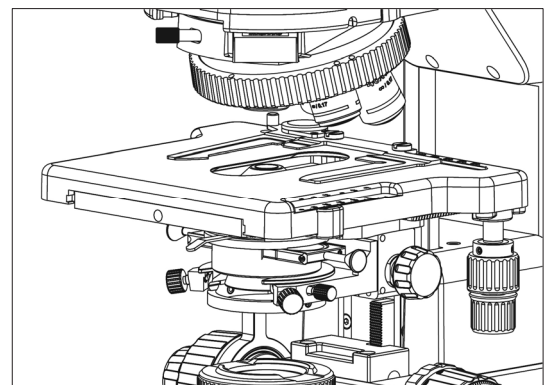
When the pre-focusing lever is locked, a coarse movement stroke is reduced due to the microscope's mechanism, causing the stage not to be able to be lowered to the lower limit. To lower the stage to the lower limit, release the pre-focusing lever.



(Fig. 16)

### 3.4 Rackless Stage

Stage is fitted with a Rackless providing more clearance for the user and avoid the possibility to scratch the users hand.

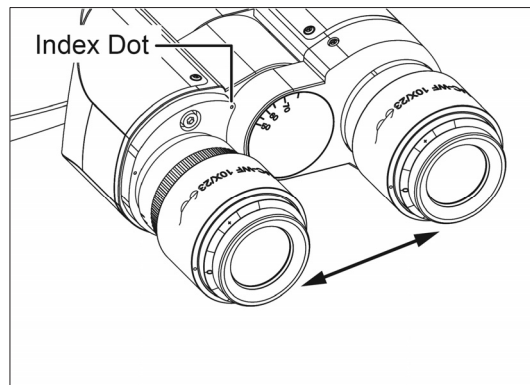


(Fig. 17)

### 3.5 Observation Tube

#### 3.5.1 Adjusting the Interpupillary Distance

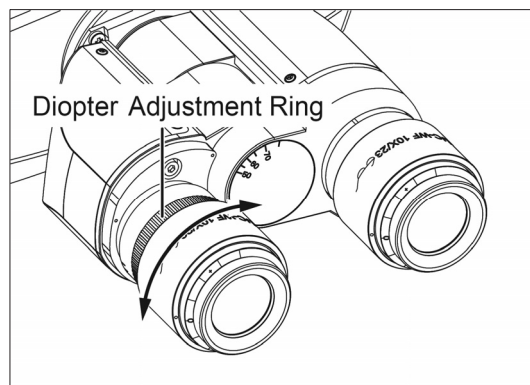
- While looking through the eyepieces, adjust for binocular vision until the left and right fields of view coincide completely.
- The Index dot indicates the interpupillary distance.



(Fig. 18)

#### 3.5.2 Adjusting the Diopter

- The diopter adjustment ring is used when the eyepiece has no function of diopter adjustment.
- Looking through the right eyepiece (there is no diopter adjustment ring), rotate the coarse and fine adjustment knobs to bring the specimen into focus
- Looking through the left eyepiece (there is a diopter adjustment ring), turn only the diopter adjustment ring to focus on the specimens.





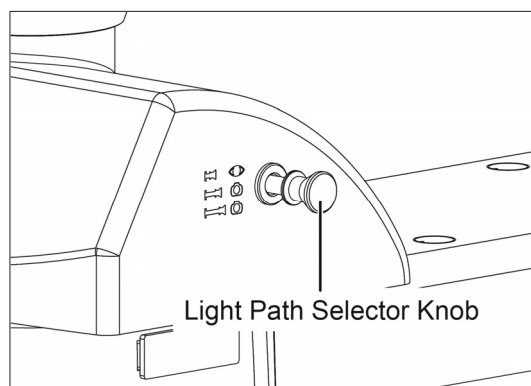
(Fig. 19)



### 3.5.3 Selecting the Light Path of the Trinocular Tube

- Slide the light path selector knob to select the desired light path.

Light Path Selector Knob	Indication	Light Path Ratio
Pushed-in position		Binocular 100%
Pulled-out position		TV & Photo 100%



(Fig. 20)

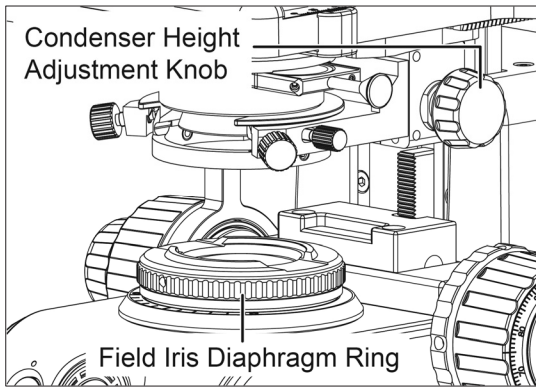
## 3.6 Condenser

### 3.6.1 Centering the Condenser

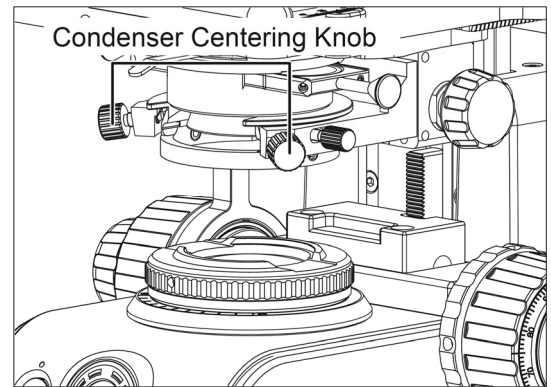
- Turn the condenser height adjustment knob (Fig. 21) to raise the condenser to its upper limit.
- Focus on the specimen using the 10x objectives.
- Rotate the field iris diaphragm ring (Fig. 21) in the direction of the clockwise so that the diaphragm image comes inside the field of view.
- Manipulate the condenser height adjustment knob to focus on the diaphragm image.
- Turn the two condenser centering knob (Fig. 22) to move the iris diaphragm image to the center of the field of view.
- Gradually open the field iris diaphragm. The condenser is properly centered if the iris image is centered and inscribed in the field of view.
- During actual use, open the field diaphragm slightly until its image circumscribes the field of view.

**NOTE:**

The field iris diaphragm restricts the diameter of the beam of light entering the objectives and thus excludes extraneous light, improving image contrast. The diameter of the field iris should be adjusted for objective power to the extent that it just circumscribes the field of view.



(Fig. 21)



(Fig. 22)

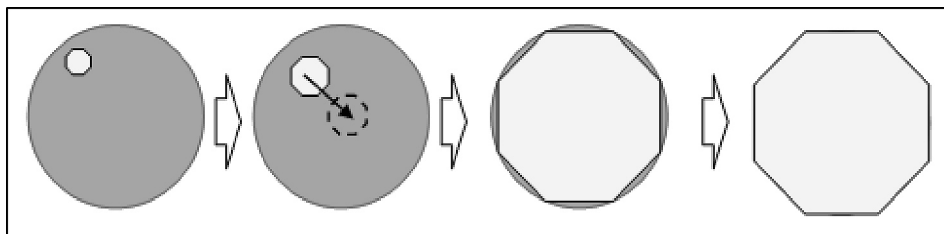


Fig. 23

### 3.6.2 Setting the Aperture Iris Diaphragm

- The aperture iris diaphragm determines the numerical aperture of the illumination system. It has an effect of adjusting image resolution and contrast.
- Since the contrast of microscope specimens is ordinarily low, setting the condenser aperture iris diaphragm to between 70 and 80% of the NA of the objectives in use is usually recommended.
- Remove one of the eyepieces, look into the eyepiece sleeve and adjust the aperture iris diaphragm ring until the image is about 70% of objective's exit pupil.
- You can use the condenser numerical aperture scale to adjust the aperture iris diaphragm.

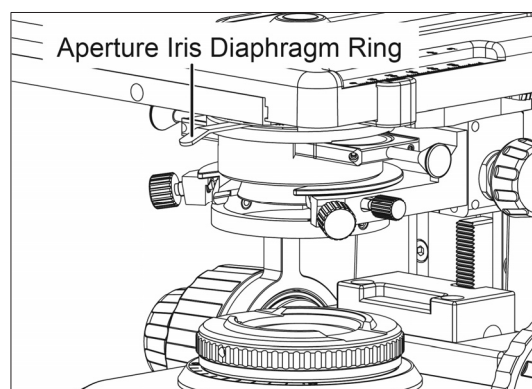


Fig. 24

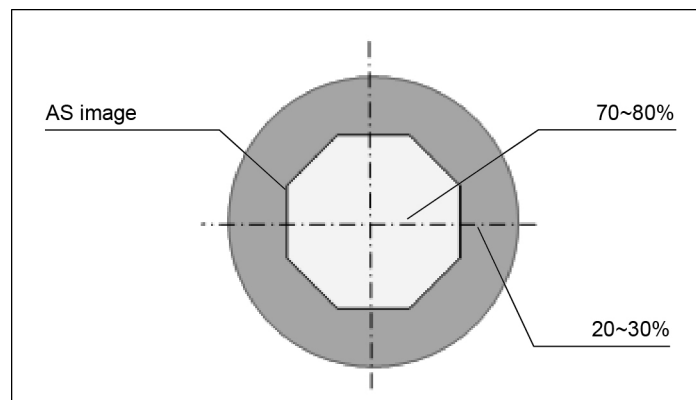


Fig. 25

### 3.7 Installing the Epi-Fluorescence Turret

Please consult the illustrations provided while assembling the attachment



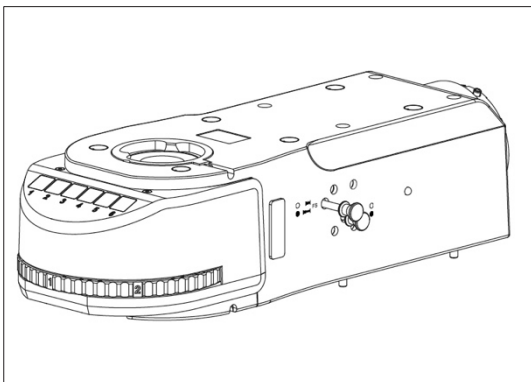
Before Starting: Turn off the microscope power switch and unplug the power cord

### 3.8 Attaching the Fluorescence Illuminator

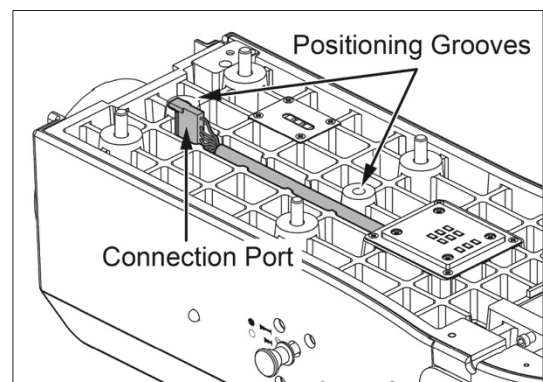
- Fully loosen the four neck cover clamping screws (Fig. 26-3) of the microscope frame with provided 2.5mm Allen wrench. Remove neck cover (Fig. 26-4).
- Place the Fluorescence illuminator on the mounting surface. (Fig. 26-5)  
There are two position pins (Fig. 26-5) on the microscope frame. Insert illuminator so that its positioning grooves (Fig. 26-2) match the pins.
- While maintaining the correct position, tighten the four illuminator clamping screws with provided 4mm Allen wrench. (Fig. 26-6.1)
- Connect the nosepiece signal cable of the illuminator to microscope frame. (Fig. 26-6.2)
- Fix the neck cover. Tighten the four neck cover clamping screws. (Fig. 26-7)

#### Note

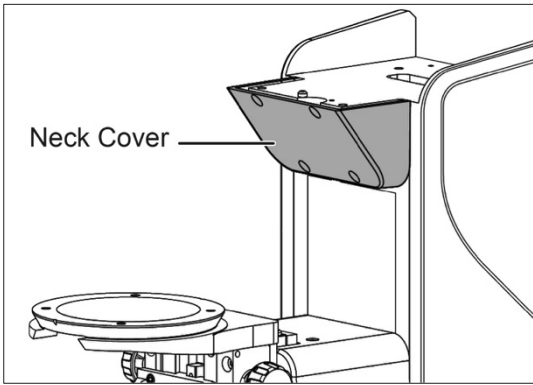
When placing the illuminator, check the nosepiece signal cable is not jammed in the illuminator and microscope frame. The position pin cannot support the weight of illuminator. Grip the illuminator while tightening the four illuminator clamping screws.



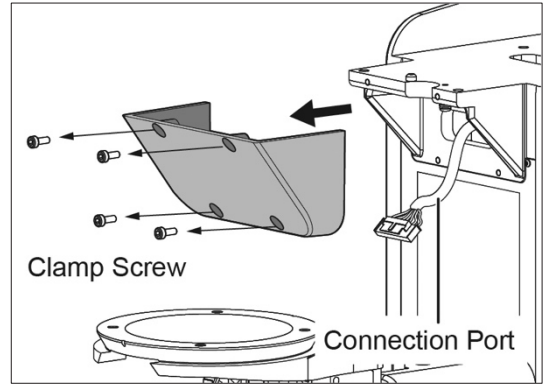
(Fig. 26-1)



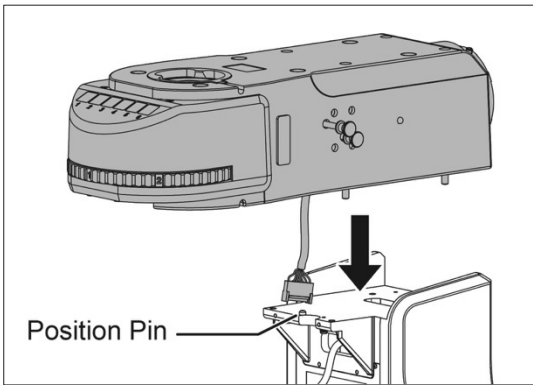
(Fig. 26-2)



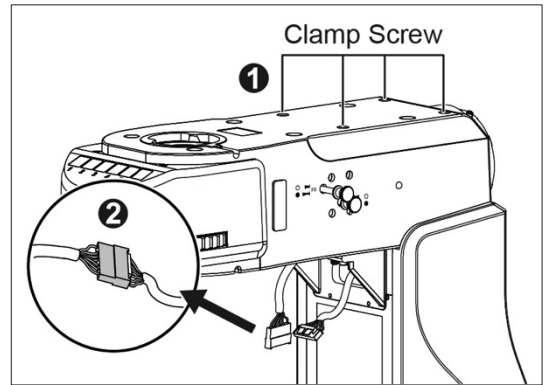
(Fig. 26-3)



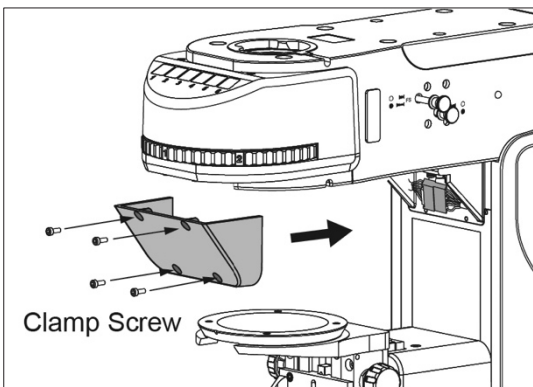
(Fig. 26-4)



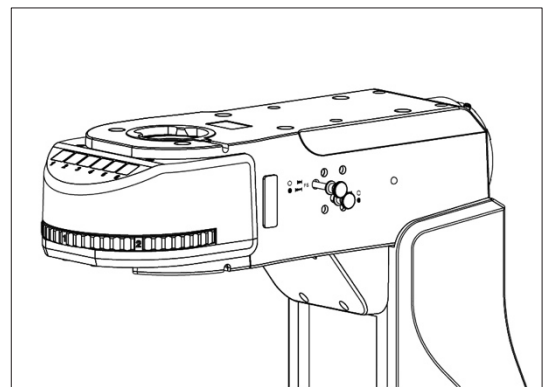
(Fig. 26-5)



(Fig. 26-6)



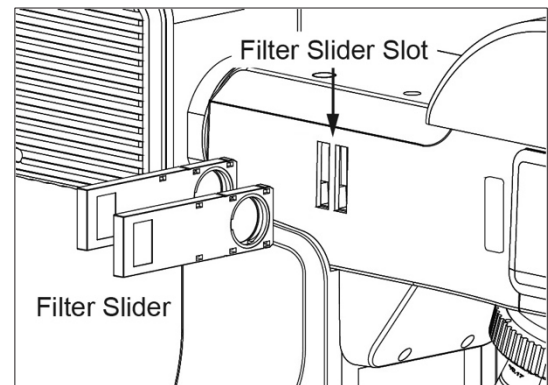
(Fig. 26-7)



(Fig. 26-8)

### 3.9 Installing the slider with the filter holder

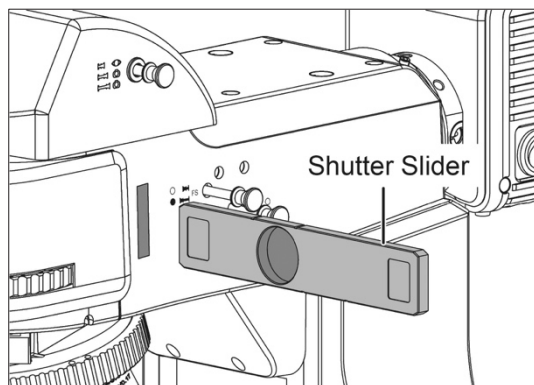
- Insert the slider into the filter slider slot of the fluorescence attachment. (Fig. 27)



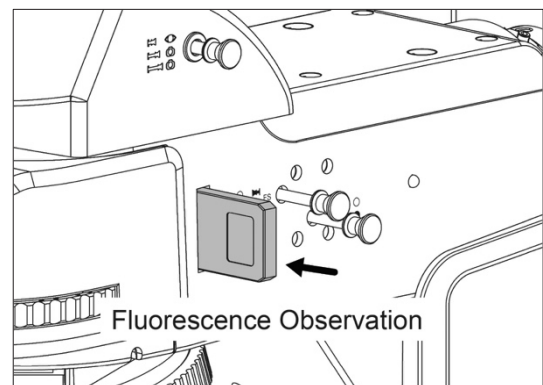
(Fig. 27)

### 3.10 Shutter Slider (Fig. 28-1)

- Blocking the optical path of the Epi-fluorescence by sliding the shutter to the left. (Fig. 28-2)



(Fig. 28-1)



(Fig. 28-2)

### 3.11 Installing the Filter Cubes

**COMMENT: WE HAVE 5 FILTER CUBES AND ONE EMPTY POSITION FOR BF TL**

**CAUTION: TURN OFF THE LAMP BEFORE SWITCHING OR INSERTING NEW FILTER CUBES!**

#### Caution

- Always keep 5 five filter cubes in the turret. Do not leave any of the filter slots empty as strong light may enter and damage the user's eyes when shifting between excitation methods.
- If less than five filter cubes are required for microscopy. Use a "DIA-ILL" dummy cube to ensure that the open space in the turret is filled.

- If the filter turret is not filled with five filter cubes, there is the risk that UV rays will reach the eyes through an empty space, causing serious harm.
- Never perform lamp centering without the UV filter cube in the optical path as harmful UV radiation from the lamp may enter the eyes, possibly resulting in loss of vision.
- During operation with the Mercury lamp always block the reflected light beam path by using the Shutter slider during operating the microscope in transmitted light working mode.

### 3.11.1 Assemble the filter set into the turret

**Note:** Please avoid separating the filter cube itself. As inside dichroic mirror is direction selective, wrong re-assembly can destroy the filter element.

Please remove the filter cube for transportation

**Step 1:** Remove the eyepiece tube by opening the clamp screw and move the eyepiece tube out of its dovetail mount. (Fig. 29-1)

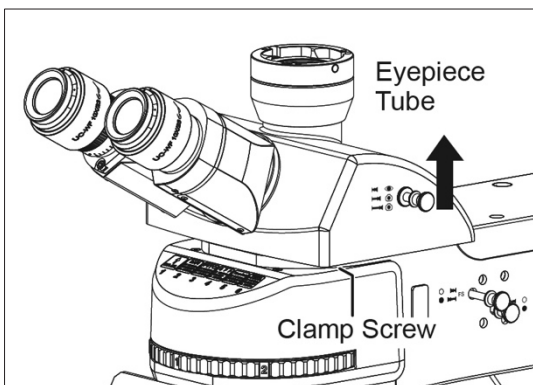
**Step 2:** Open the two clamp screw mounted Filter cube Cover with the screwdriver delivered with Epi FS6 Turret package. (Fig. 29-2)

**Step 3:** Place the filter cube into the dovetail mount of the filter turret. (Fig. 29-3)

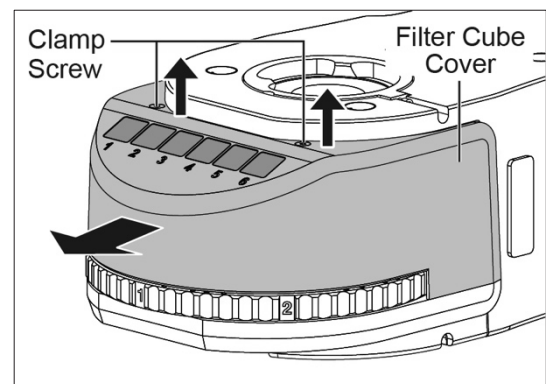
**Step 4:** Insert plastic rivet on both sides, press down with the thumb. (Fig. 29-4)  
The head of the upper part of the rivet has to touch the lower flange.

**Step 5:** Position the remaining filter cubes using the same procedure (Fig. 29-3 and Fig. 29-4) so that there are **\*\*six filter cubes** in the turret. (Fig. 29-5)

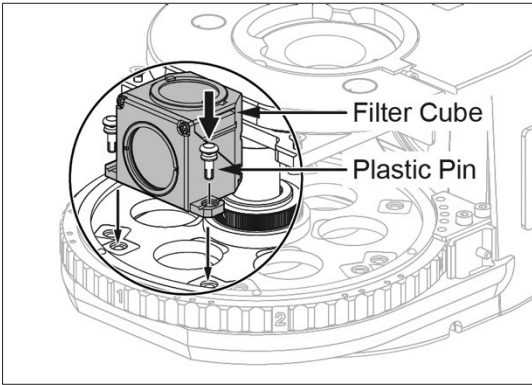
**Step 6:** Replace the filter cube cover and secure with 2 clamp screws. (Fig. 29-6)



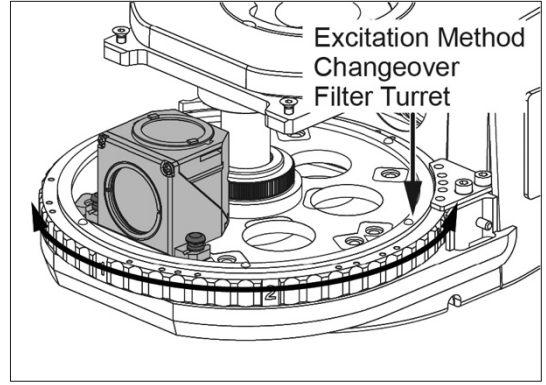
(Fig. 29-1)



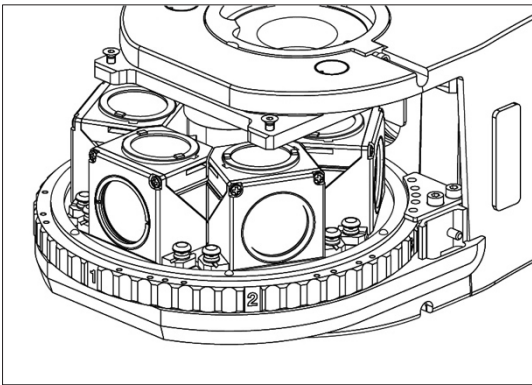
(Fig. 29-2)



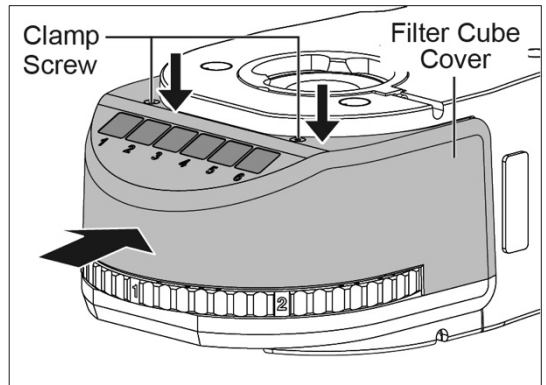
(Fig. 29-3)



(Fig. 29-4)



(Fig. 29-5)



(Fig. 29-6)

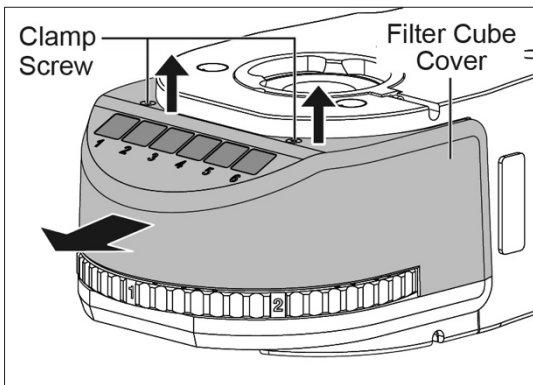


### 3.11.2 Disassemble the filter set into the turret

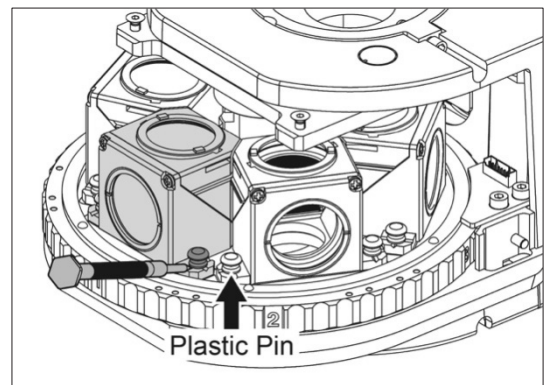
**Step 1:** Open the two clamp screw mounted Filter cube cover with the screwdriver delivered with Epi FS6 Turret package. (Fig. 30-1)

**Step 2:** Lift head from plastic rivet (both sides of filter cube) (Fig. 30-2)  
Filter cube can be lifted up and removed (Fig. 30-2)

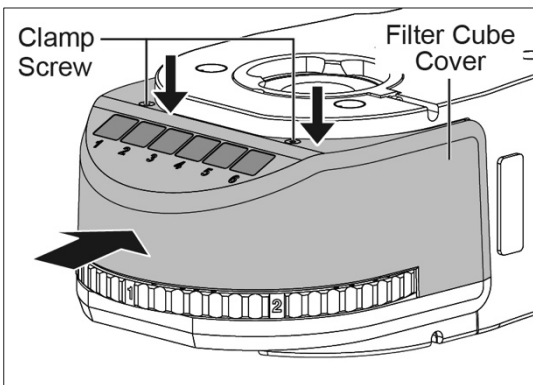
**Step 3:** Replace the filter cube cover and secure with 2 clamp screws. (Fig. 30-3)  
Update filter ID Tag according to equipment



(Fig. 30-1)



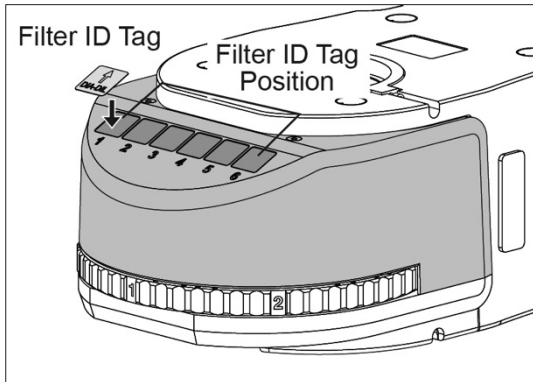
(Fig. 30-2)



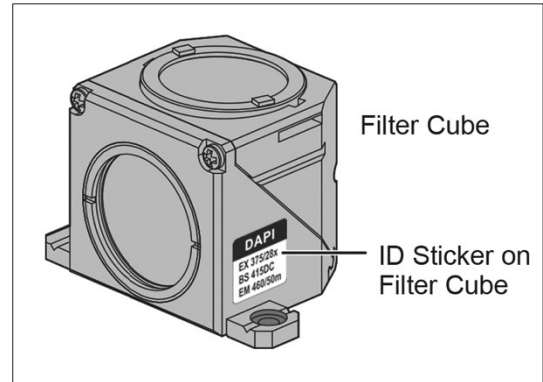
(Fig. 30-3)

### 3.12 Filter cube ID tags

- Place the Filter cube ID tag on the front face of the Epi-fluorescence attachment. (Fig. 31-1)
- Select the ID tags which represent the excitation method cubes in the turret and drop them into corresponding position. (Fig. 31-2)



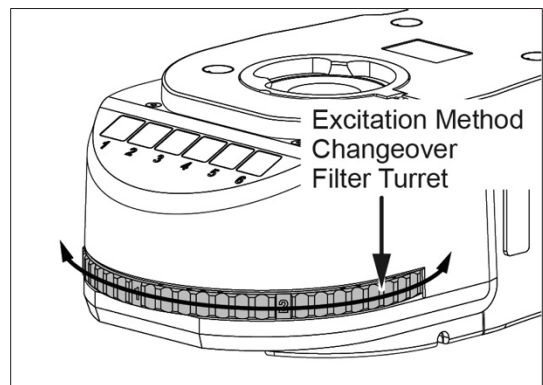
(Fig. 31-1)



(Fig. 31-2)

### 3.13 Arrangement of the Excitation method ID tags

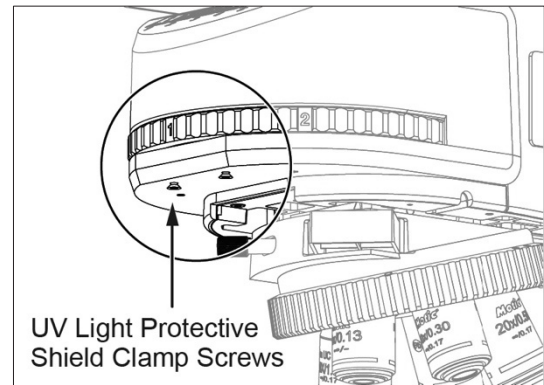
- The position numbers "1", "2", "3", "4", "5", "6" which both on the turn plate and behind the ID tag pocket show the location of the corresponding Filter cube in the filter turret. (Fig. 32)



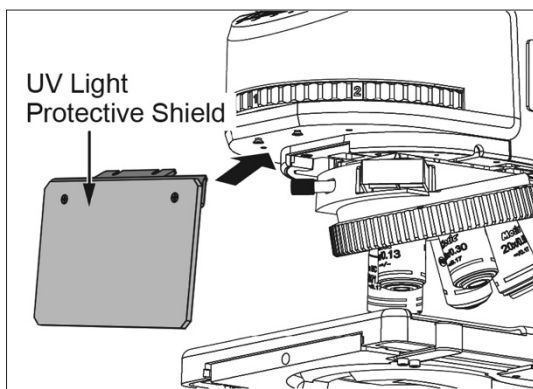
(Fig. 32)

### 3.14 Attaching UV light protective shield

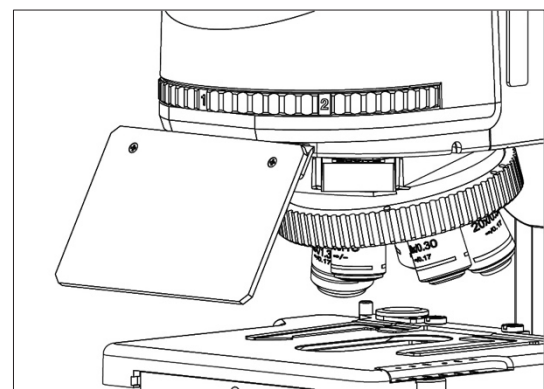
- Loosen UV light protective shield Clamp screws under Epi-fluorescence attachment body. (Fig.33-1)
- Insert the UV light protective Shield – Slots are in the front of shield. (Fig. 33-2)
- Secure in place with the clamp screws. (Fig. 33-3)



(Fig. 33-1)



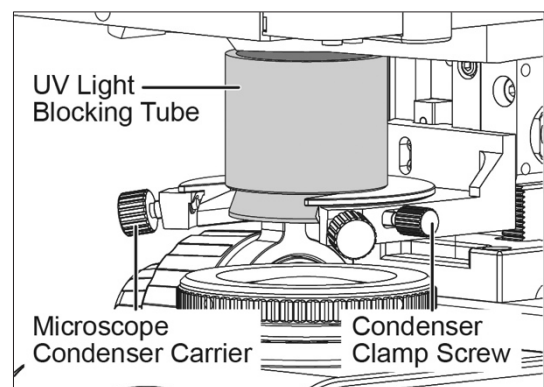
(Fig. 33-2)



(Fig. 33-3)

### 3.15 Mounting UV light blocking tube

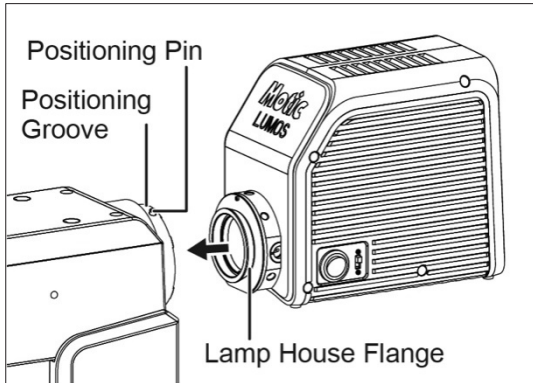
- Mount UV light blocking tube on the microscope condenser carrier and secure in place with condenser clamp screw. (Fig. 34)



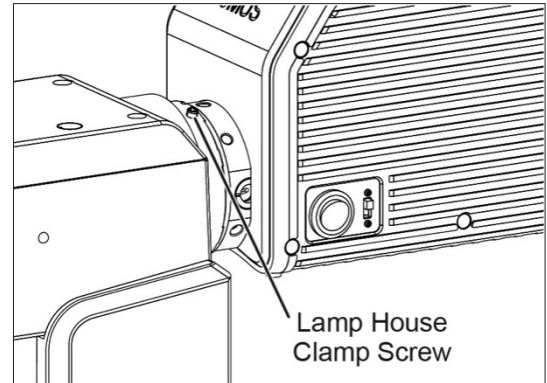
(Fig. 34)

### 3.16 Attaching the Lumos lamp house

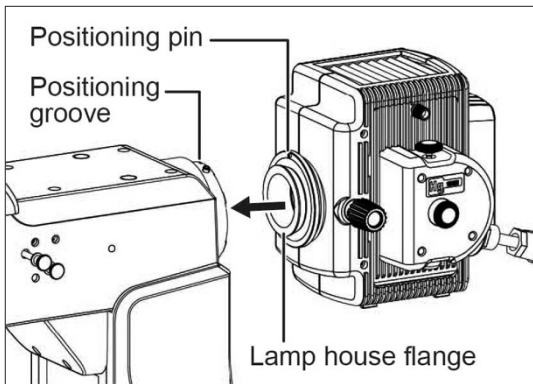
- Attach the Mercury lamp house or Lumos LED lamp to the microscope using the dovetail on the Epi-fluorescence attachment.
- Align the positioning pin on the Mercury lamp house (or Lumos) flange with the positioning groove on the dovetail mount of the fluorescence attachment (Fig. 35-1 / 35-3)
- Secure the dovetail with the provided Allen Key as shown in Fig. 35-2 / 35-4.



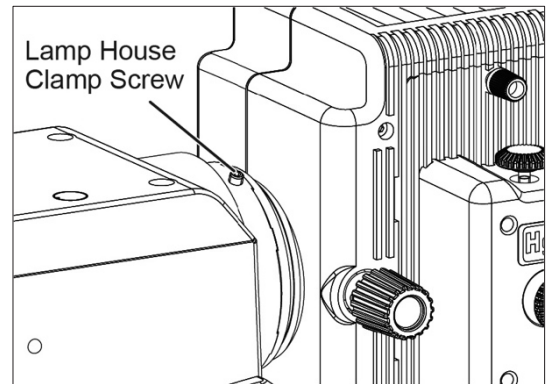
(Fig. 35-1)



(Fig. 35-2)



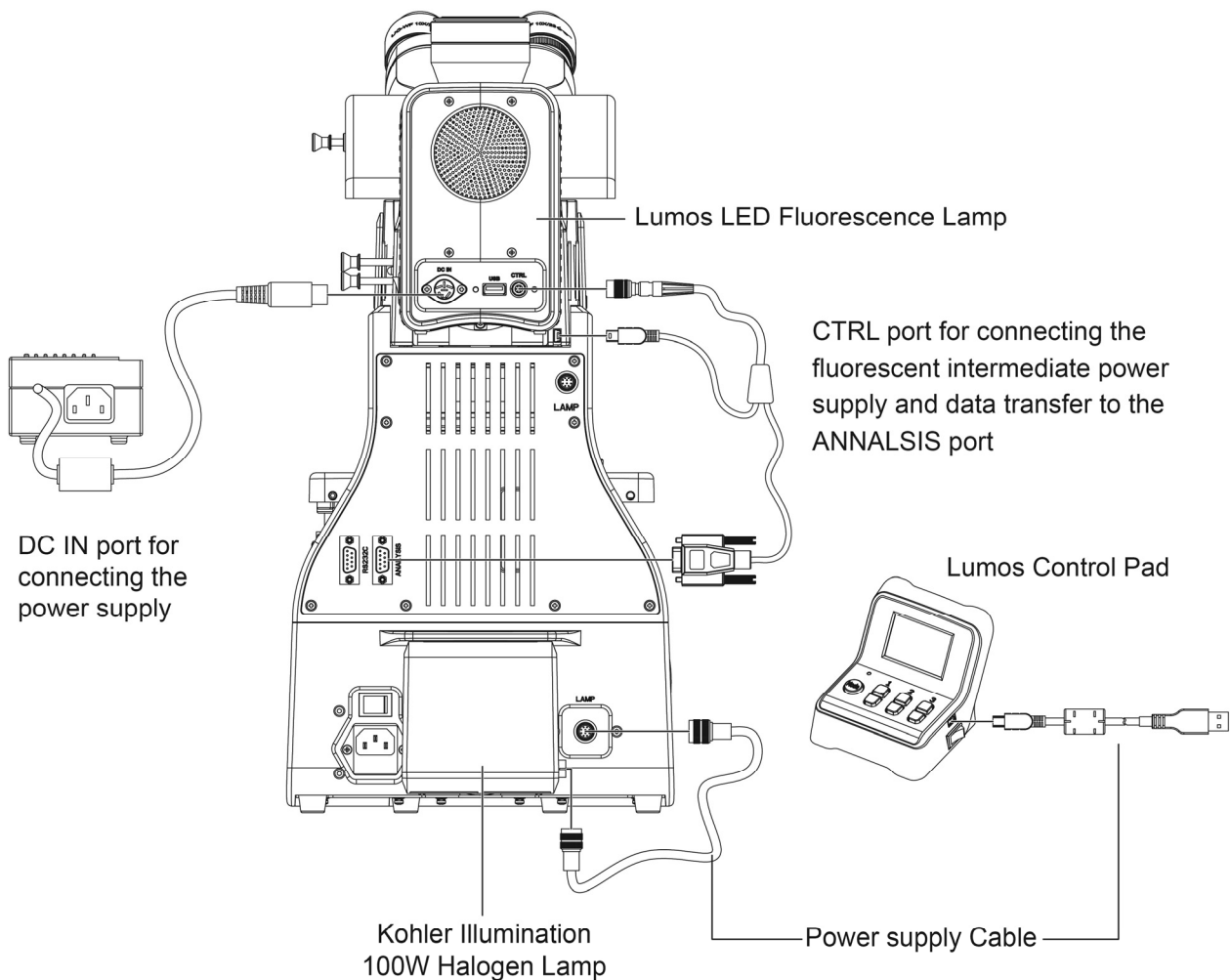
(Fig. 35-3)



(Fig. 35-4)

#### 3.16.1 Connecting cables to the Lumos and use of the ports: (Fig. 36)

1. **DC IN** port for connecting the power supply.
2. **USB** port for connecting the computer.
3. **CTRL** port for connecting the fluorescent intermediate power supply and data transfer to the ANNALSIS port.



(Fig. 36)

### 3.16.2 Setting the reflected light intensity manager

- It is effective when the PA53 FS6 revolving nosepiece with sensor and the LED lamp housing Lumos are used.
  - The desired reflected fluorescent brightness can be set per objective. Once the brightness for the objectives is set, selecting an objective adjusts the Lumos brightness automatically.
1. Switch to reflected fluorescent lighting path, (so that the light intensity indicator on the front of frame is lit in blue).
  2. Engage a low-power objective in the light path, switch the turret to the desired fluorescent channel and bring the specimen in focus.
  3. Press the brightness adjustment button for optimum brightness for observation.

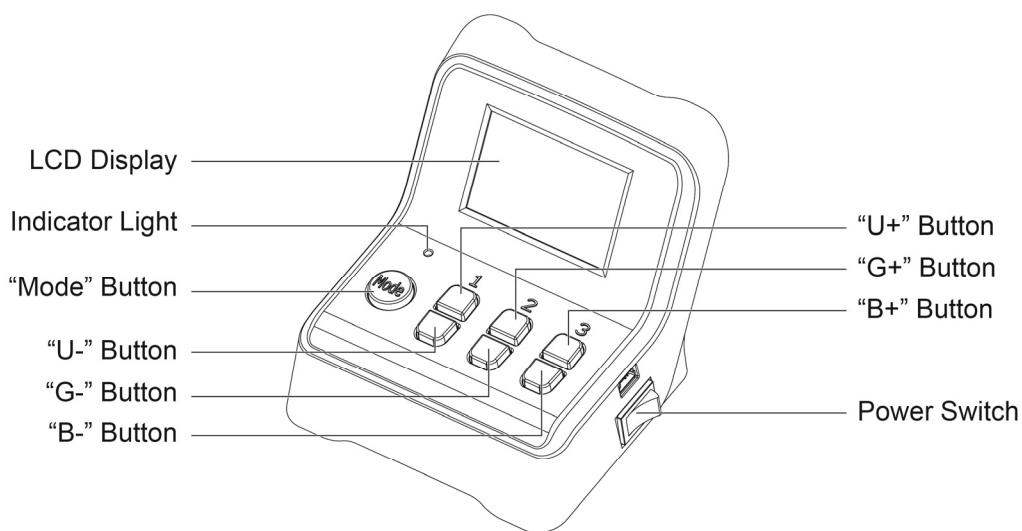
4. After 3 seconds, the microscope automatically memorizes the optimum brightness.
5. Set the optimum brightness of the reflected light path for the mounted objectives by repeating the above for each of them.

### 3.16.3 Setting the fluorescence turret channel manager

- It is effective when the PA53 FS6 revolving fluorescence turret with sensor and the LED lamp housing Lumos are used.
- The desired wavelength and intensity can be set per each combination of filter cube (for lower excited light noise or even different wavelength light intensity for multi-band filter cube) and objective lens.

Once the wavelength of Lumos for filter cubes is set, selecting an channel of the turret and objective lens adjusts the optimum Lumos color and intensity automatically.

1. Switch to reflected fluorescent lighting path, (so that the light intensity indicator on the front of frame is lit in blue).
2. Engage a filter cube in the turret and bring the specimen in focus.
3. Press brightness plus and brightness decrease simultaneously to turn off/on the desired wavelength (Lumos pad U channel for UV excite light, B channel for blue excite light, G channel for green excite light).
4. After 3 seconds, the microscope automatically memorizes the optimum wavelength of Lumos for the filter cube.
5. Set the optimum wavelength of Lumos for the mounted filter cubes in turret by repeating the above for each of them.

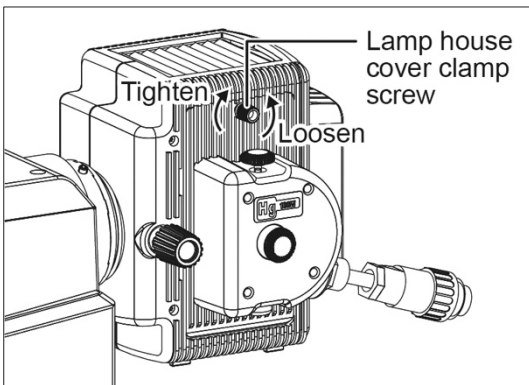


Control Pad  
(Fig. 37)

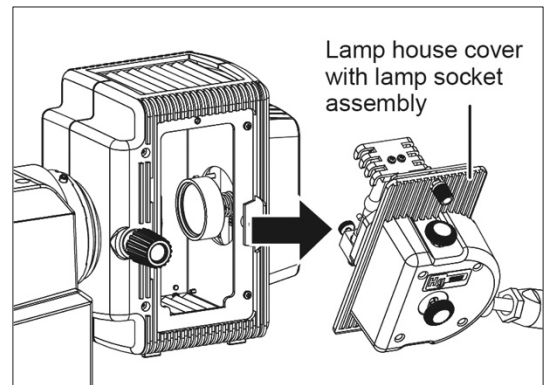
### 3.17 Installing the lamp (Only for Mercury Lamp)

**NOTICE:** Please follow all **NOTES FOR SAFETY / SAFETY PRECAUTIONS** and use personal protection equipment all the time

- In order to prevent electric shock please always make sure to turn the power switch off, and unplug the power cord before installing or replacing the lamp.
- Loosen the Mercury lamp house cover clamp screw (Fig. 38-1 -- Loosen) and remove the cover and the lamp socket assembly. (Fig. 38-2)

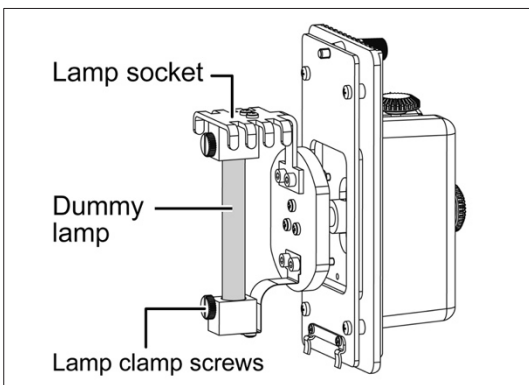


(Fig. 38-1)

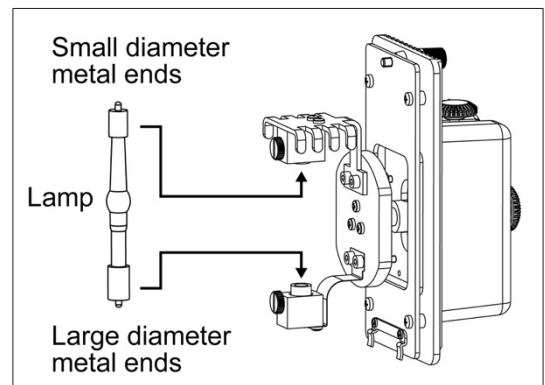


(Fig. 38-2)

- Loosen the lamp clamp screws at both ends of the lamp socket and remove the installed "Dummy lamp". (Fig. 38-3)
- Before handling the lamp, read the handbook supplied with the lamp.
- When installing the lamp, avoid applying force that might cause the lamp to break.
- Match the large and small diameter metal ends on lamp and lamp socket. (Fig. 38-4)

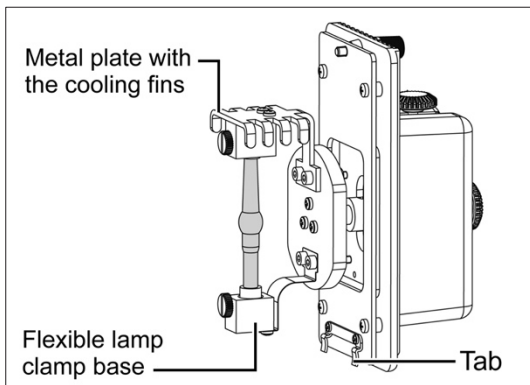


(Fig. 38-3)

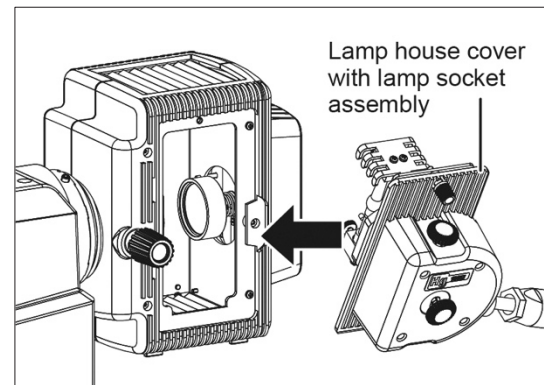


(Fig. 38-4)

- Insert the lamp into the lower flexible lamp clamp base and tighten the clamp screw. (Fig. 38-5)
- Insert the lamp into the upper lamp socket hole supported by the metal plate with the cooling fins and fasten the clamp screw. (Fig. 38-5)
- Insert the bottom tab of lamp house cover over the inner metal surround of the lamp house and pivot the lamp socket cover to close (Fig. 38-6). Secure with the clamp screw.  
(Fig. 38-1 -- Tighten)
- The safety cut-off micro switch will “**click**” as you secure the lamp house cover clamp screw.



(Fig. 38-5)



(Fig. 38-6)

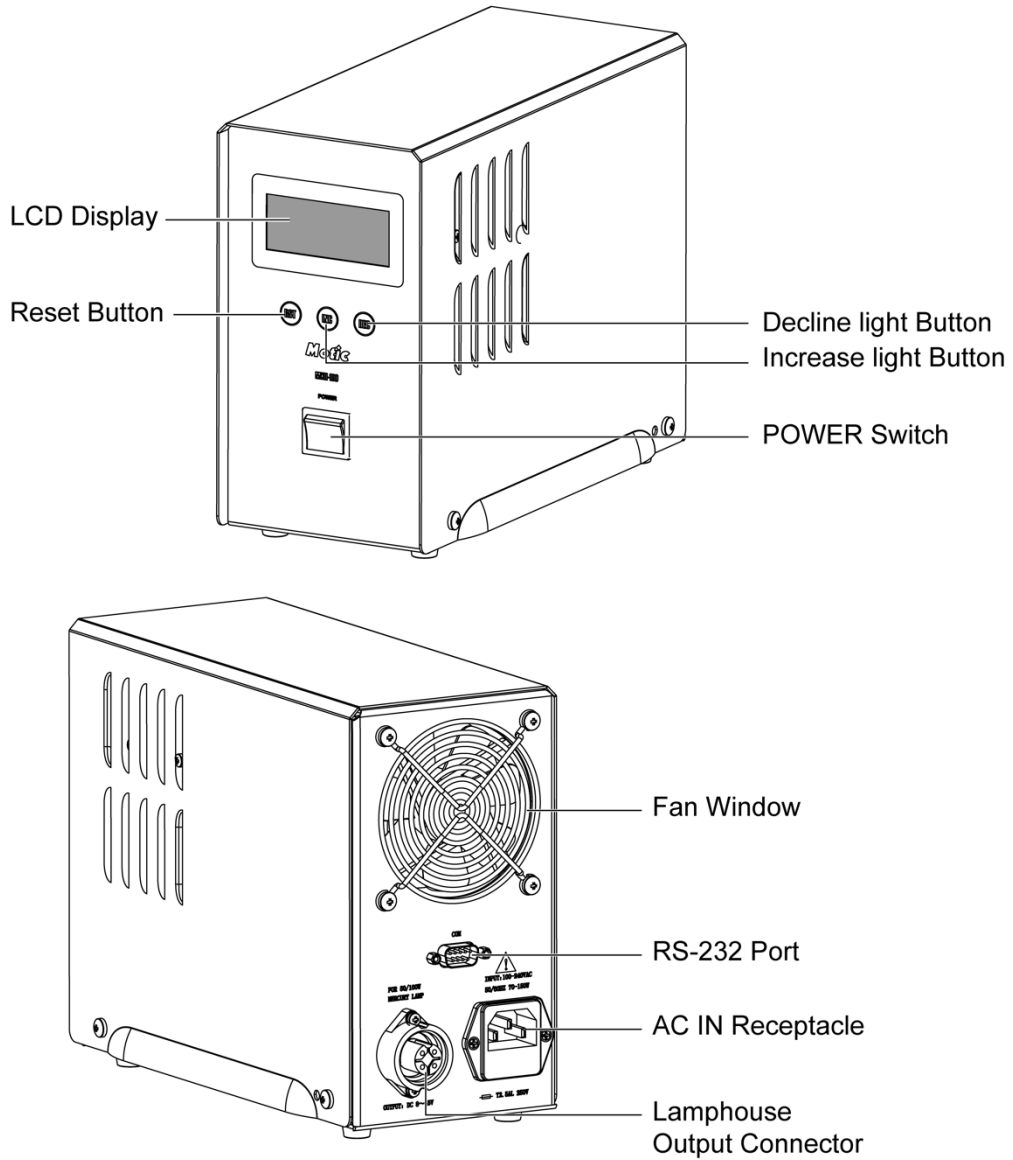


### 3.18 Connecting the \*\*Mercury lamp house to the power supply:

\* Please check the MXH-100 power supply instruction manual for more information.



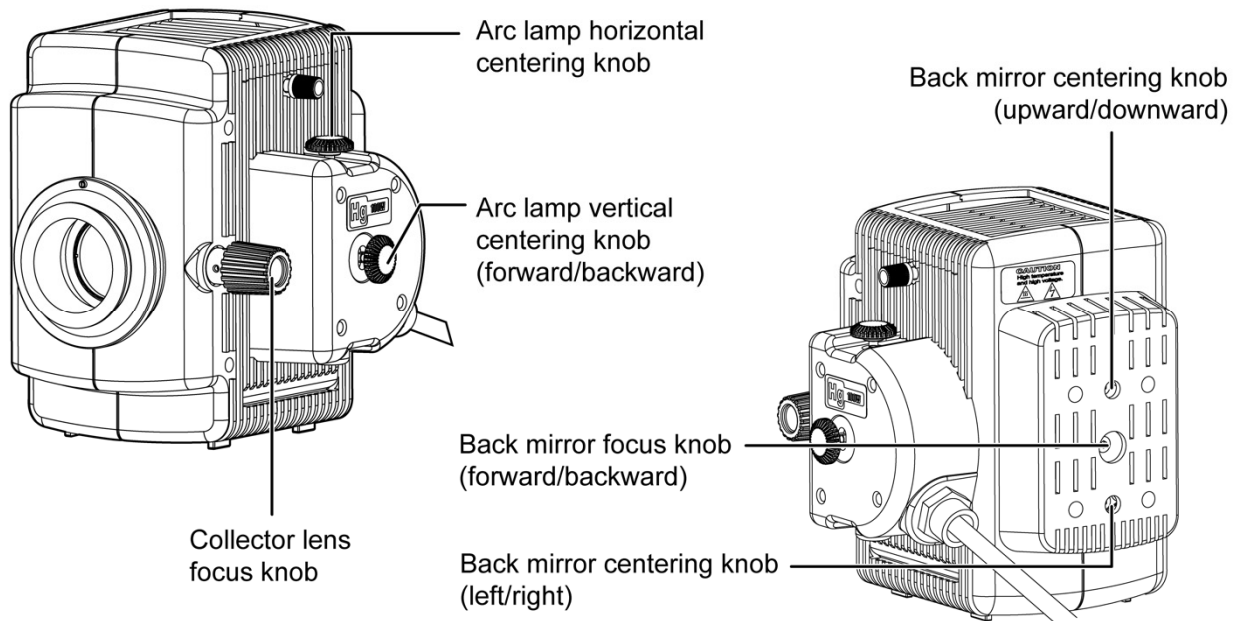
**Turn OFF** the power switch on the power supply.



MXH-100 Power Supply  
(Fig. 39)

### 3.19 Mercury Lamp House\*\*

\* Please check the HBO100 Mercury Lamp instruction manual for more information.



### Lamp Housing

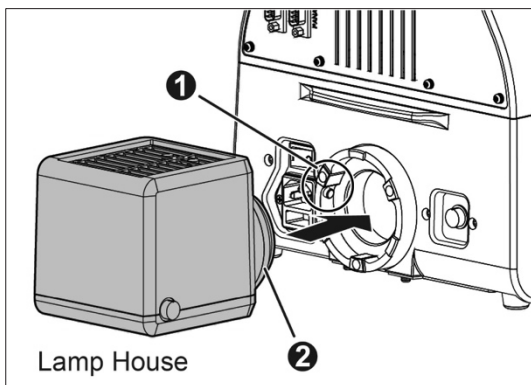
(Fig. 40)

### 3.20 Attaching the Lamp housing

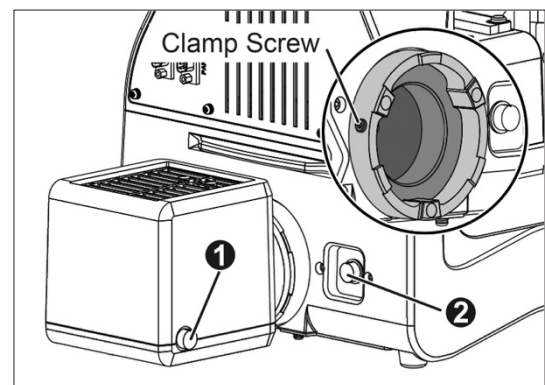
#### WARNING

The bulb, the lamp socket and areas near these will be extremely hot during and right after use. Set the main switch off; disconnect the power cord from the wall outlet, then wait until lamp housing has cooled down before removing the Lamp housing.

- Loosen the clamping screw (Fig. 41-1.1) with provided Allen screwdriver.
- Mount the lamp housing to the connection port (Fig. 41-1.2) on the rear of the illuminator and press the lamphousing as far as it goes.
- Tightened the clamping screw (Fig. 41-1.1) with provided Allen screwdriver.
- Plug the cable coming from the lamp housing (Fig. 41-2.1) into the lamp connector (Fig. 41-2.2) on the rear of the microscope and tighten the ring of the connector to secure the connection.



(Fig. 41-1)



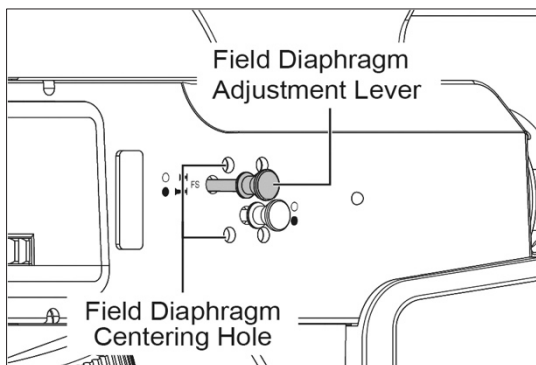
(Fig. 41-2)

### 3.21 Changing the size of the field diaphragm (Fig. 42-1)

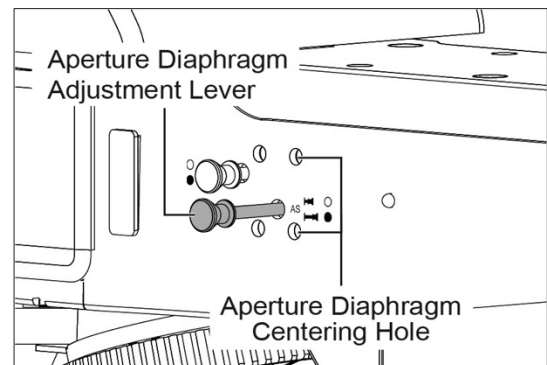
- The field aperture diaphragm determines the illuminated area on the specimen.
- Pull or Push field diaphragm adjustment lever to change the size of the field aperture diaphragm. For normal observation, the diaphragm is set just slightly outside the edge of the field of view.
- If a larger than required area is illuminated, extraneous light will enter the field of view. This will create a flare in the image and lower the contrast.
- Cutting out the excessive light is useful in preventing contrast from being diminished.
- Decreasing excessive illumination is useful in protecting the specimen from fading.
- If the field aperture diaphragm is off the centre of the field of view, centre the field aperture diaphragm with centering hole by Allen key.

### 3.22 Changing the size of the aperture diaphragm (Fig. 42-2)

- The aperture diaphragm determines the illumination in the back focal plane of the microscope.
- Pull or Push aperture diaphragm adjustment lever to change the size of the field aperture diaphragm. For normal observation, just the aperture diaphragm
- Closing the aperture diaphragm will reduce the resolution but enlarge the depth of focus of the microscope



(Fig. 42-1)



(Fig. 42-2)

### 3.23 Filter Blocks

A cube shaped modules that hold a matched set of fluorescence filters including excitation(EX), barrier-emission (BA) and a dichroic mirror (DM).

The excitation filter allows transmission of light at a wavelength within the absorption spectrum of the dye and rejects light at wavelengths within the emission spectrum of the dye, which could be reflected by the specimen and incorrectly detected as emission energy.

A barrier filter allows the transmission of light at wavelengths within the emission spectrum of the dye and rejects light at wavelengths within the absorption spectrum of the dye, which could be detected as emission energy.

A dichroic mirror is placed at a 45° angle relative to the incoming excitation light; the dichroic mirror performs two functions: it reflects the shorter wavelength exciting radiation light to the specimen and transmits the longer wavelength fluorescence.

## 4. MICROSCOPY

### Epi-Fluorescence Microscopy

#### 4.1 Selecting fluorescence filters:

For best results, select excitation and emission filters with center wavelengths as close to the absorption and emission peaks as possible.

Note: The center wavelength is situated at the midpoint of the bandwidth. It is not necessarily the peak transmission wavelength although with a symmetrical band the center wavelength and the peak wavelength are equal.

#### 4.2 Selecting excitation filters (EX)

Excitation filters selectively pass the light within the range of wavelengths needed to cause the specimen to fluoresce and filter out other light.

To maximize the brightness of the desired fluorescence (the signal) relative to brightness of the background (the noise), one can choose excitation and emission filters with wide bandwidths. However, this may result in unacceptable overlap of the emission signal with the excitation signal, resulting in poor resolution and wide bandwidth which also leads to a high level of self-fluorescence and severe fading.

To minimize spectral overlap, one can instead choose excitation and emission filters that are narrow in bandwidth and are spectrally well separated to increase signal isolation. This will conversely yield a dark image. Since little excitation light reaches the specimen, self-fluorescence and fading are minimal.

#### 4.3 Selecting barrier filters (BA)

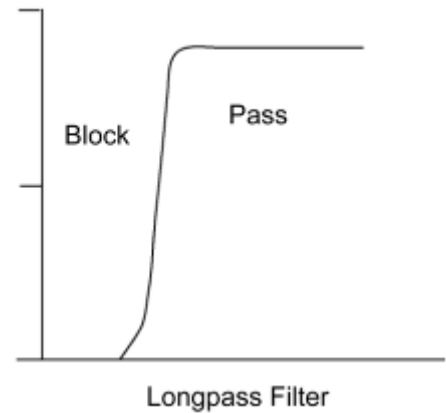
The barrier filter prevents the excitation light from reaching the observers eye. Its transmission should be as low as possible in the range of light used for excitation and as high as possible within the spectral range of the emission from the specimen.

Barrier filters may be Long pass (LP) or Band pass (BP).

#### 4.3.1 Longpass

Longpass filters only allow wavelengths above a certain Wavelength to pass through and will prevent light of lower wavelength from passing through.

Longpass filters should be used when the application requires maximum emission and spectral differentiation is not necessary. This is generally the case for fluorophores that generate a single emitting species in specimens with reasonably low levels of background auto fluorescence.



Fluorophore or fluorescent probe:

Fluorochromes when conjugated to other originally active substances, such as a protein, antibody or nucleic acid, in order to selectively stain targeted substances within the specimen

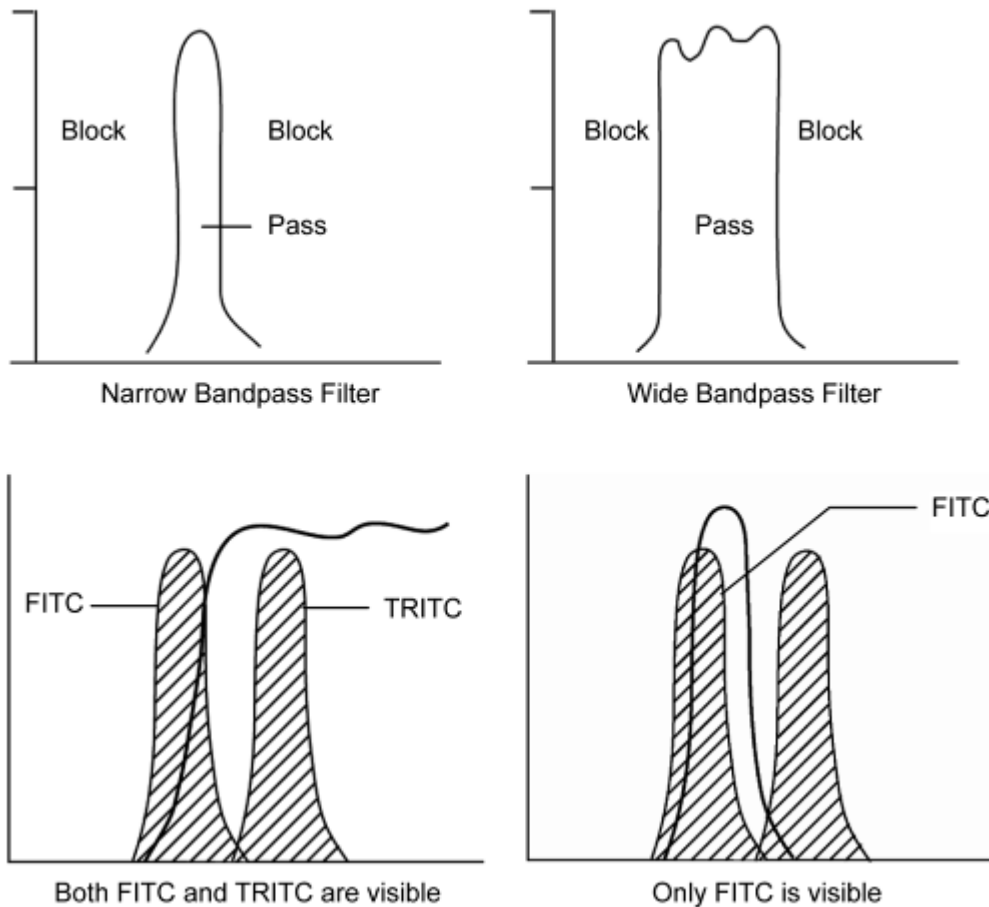
Long pass filters are also useful for observing all the stains on multi-stained specimen such as FITC and TRITC.

#### 4.3.2 Bandpass

Filters that pass light of a certain restricted range of wavelength.

Bandpass filters are useful for observing a certain stain on multi-stained specimen such as when two stains FITC and TRITC are used.

Bandpass filters are designed to maximize the brightness of the desired fluorescence (signal) to brightness of the background (the noise) ratio for applications where discrimination of signal component is more important than overall image brightness.



Selecting fluorescence filters requires a thorough understanding of filter technology. This will enable the user to utilize stain and illumination selection to improve the image quality of the desired fluorescence signal.

Selection of filter combinations also requires knowledge of the excitation and emission spectra of the stain.

#### Epi-Fluorescence and Phase Contrast Microscopy

In addition to Epi-Fluorescence microscopy, phase contrast microscopy can be used to search an organelle or the location of a particular molecule in a cell instead of Epi-Fluorescence microscopy which bleaches the specimen.

Combining Epi-Fluorescence microscopy with phase contrast microscopy makes it possible to compensate for the limitations of each method.

#### **4.4 Using an oil immersion objective**

Oil immersion objectives are labelled with the additional engraving "Oil" and are to be immersed in oil between the specimen and the front of the objective.

The immersion oil supplied by Motic is synthetic, non-fluorescing and non-resining oil, with a refractive index of 1.515

Normally, a cover glass must be used with oil immersion objectives with a few exceptions. Deviations from thickness are not important as a layer of immersion oil acts as compensation above the cover glass.

The small bottle of oil supplied with every immersion objective facilitates application of the oil to the cover slip.

Remove any air bubbles in the nozzle of the oil container before use.

Immersion oil must be used sparingly.

Freedom from air bubbles must be ensured. To check for air bubbles, remove one eyepiece, open the field diaphragm as far as possible and look at the exit pupil of the objective within the eyepiece tube (the exit pupil will appear as a bright circle).

If it is difficult to see if there are any bubbles, use a phase centering telescope and rotate the eyepiece part of the centering telescope to focus on the exit pupil of the objective. Air bubbles in the oil will deteriorate the specimen image. To purge bubbles, swing the immersed objective forward and backwards by rotating the revolving nosepiece, add more oil, or wipe off the oil and apply new oil.

Place a single drop of immersion oil on the cover glass.

Make contact with the cover glass, and focus.

View and wipe clean the objective with a lens cleaning tissue.

Any residual film of oil remaining on immersion type objective, or where it has spread to the surface of dry type objective will have a discernible, negative effect on the image.

To remove a film of oil, moisten a lens tissue or clean cloth with petroleum benzene and lightly wipe the lens surface, wipe the lens surface with absolute alcohol (ethyl alcohol or methyl alcohol).

Petroleum benzene and absolute are highly flammable. Use great care when handling them.



#### **4.5 Fluorescence Photomicrography**

For the basic procedure and key points of photomicrography, see the manuals provided with the photomicrographic equipment

Since the specimen colour may fade effort must be made on minimizing the exposure of the specimen to irradiation both before, and during exposure.

Select the area of interest without using fluorescence, use phase contrast or interference contrast.

Select an appropriate filter combination for the specimen (whether single, dual or triplepass filter sets are used, exposure times for acquiring video or photographic images will increase for dual and triplepass filter sets when compared to singlepass filter sets).

The magnification of the image at the film plane affects the intensity of the image; the latter is inversely proportional to the magnification. To minimize the magnification required to fill the picture with the desired object, the format chosen should be as small as considered practical and hence have shorter exposure times.

Exposure time varies for each objective and projection lens combination even if the total magnifications are the same. The objective with higher numerical aperture (N.A.) should be chosen over increasing the magnification by projection lens. (The numerical aperture of the objective increases with increase in magnification and higher the numerical aperture, the brighter the image).

Excessively bright excitation light will cause the specimen to fade, adjust the brightness by inserting a neutral density filter in the light path as these filters give a constant transmission over a wide range of wavelengths.

Focus collector lens give brighter or more even illumination.

Avoid bleaching of the specimen by blocking the excitation light when not viewing or photographing the specimen.

#### **4.6 Video Fluorescence Microscopy**

Electronic photomicrography utilizes electronic detection devices to acquire images, inserting an IR (infrared) barrier filter in the light path before the detection device may produce better results by avoiding erroneous readings caused by infrared light.

## 5. TROUBLESHOOTING TABLE

As you use your microscope and the Epi-Fluorescence attachment, you may occasionally experience a problem.

The troubleshooting table below contains the majority of frequently encountered problems and the possible causes.

### 5.1 Electrical

Lamp does not light	Power supply not plugged in
	Lamp not installed
	Lamp burnt out
	Fuse is blown
Inadequate brightness	Specified lamp not being used
Lamp blows out immediately	Specified lamp not being used
Lamp flickers	Connectors are not securely connected
	Lamp near end of service life
	Lamp not securely plugged into socket.

### 5.2 Optical

Image not visible	Shutter slider in light path.
	Filter cube unsuitable for specimen.
	Filter cube not completely in light path.
Lamp on but image is unclear or has no contrast	Light source is not centred
	ND filter in optical pat
	Filter cube unsuitable for specimen
	One component of filter cube is missing
	Objective or filters are dirty
	Specimens slide or cover glass dirty
	Slide glass is fluorescing
	Cover glass is missing

	Greasy residue on eyelens
	Room light is too bright
Field of view is partially obscured	Revolving nosepiece not clicked into position.
	Shutter slider in intermediate position
	Filter cube not installed correctly
	Filter cube in intermediate position
	Field diaphragm out of centre
	Field diaphragm is stopped down

## 6. CARE AND MAINTENANCE

### 6.1 Do not disassemble

- Disassembly may significantly affect the performance of the instrument, and may result in electric shock or injury and will void the terms of the warranty.
- Never attempt to dismantle any parts other than described in this manual. If you notice any malfunction, contact your nearest Motic representative.

### 6.2 Lenses and filters

- To clean lens surfaces or filters, first remove dust using an air blower. If dust still persists, use a soft/clean brush or gauze.
- A soft gauze or lens tissue lightly moistened with pure alcohol should only be used to remove grease or fingerprints.
- Use petroleum benzine to clean immersion oil.
- Use petroleum benzine only to remove immersion oil from objective lenses.
- Because petroleum benzine and absolute alcohol are both highly flammable, be careful handling around open flame.
- Do not use the same area of gauze or tissue to wipe lens more than once.

### 6.3 Cleaning or painted or plastic components

- Do not use organic solvents (thinners, alcohol, ether etc.) doing so could result in discoloration or in the peeling of paint.
- For stubborn dirt, moisten a piece of gauze with diluted detergent and wipe clean.
- For plastic components, only moisten a piece of gauze with water and wipe clean.

### 6.4 Disinfecting the Microscope

- Follow the standard procedures for your laboratory.

### 6.5 When not in use

- When not in use, cover the instrument with vinyl dust cover and store in a place low in humidity where mold is unlikely to form.
- Store the objectives, eyepieces and filters in a container or desiccator with drying agent.
- Proper handling of the microscope will ensure years of trouble free service.

**Note:**

- If equipment is used in a manner not specified by the manufacturer, the warranty may be void.
- To avoid getting wet, do not use the microscope near water.

Proper handling of the microscope will ensure years of trouble-free service. If repair becomes necessary, please contact your Motic agency or our Technical Service directly.

**6.6 For MXH-100 Power supply unit**

- Ensure the fan on the back panel of the power supply is kept free of obstructions. Do not cover with cloth or other items that may block the flow of air.
- Ensure air is able to freely flow through the lamp housing. Do not cover with cloth or other items that may block the flow of air as this will affect heat dispersal.
- If there is dust on the outer casing, wipe it off using a dry cloth.
- Do not touch the bulb or collector lens directly with your fingers. Use a dry cloth to wipe the bulb and clean the collector lens.
- When both in use and in storage, always protect the microscope from dust and moisture in order to keep the electrical components running smoothly.
- When cleaning, ensure the power has been turned off and the plug removed from the power supply.
- Turn the power on and off as little as possible to preserve the life of the lamp. If the instrument is damaged, do not take the instrument apart or attempt to repair. Contact your local distributor or a professional to help repair the instrument.
- No liquid is allowed for cleaning any part of this product.
- Disconnect the power supply before replacement of fuse.

## 7. SPECIFICATION

### 7.1 Electrical Specification

- **PA53 FS6:**

INPUT: AC100-240V~, 1.8-1A, 47~63Hz

REFLECTED LAMP: 100W Halogen

- **PA53 FS6 Intermediate:**

INPUT: AC100-240V~, 3.2A, 47~63Hz    OUTPUT: DC 5V/0.5A

LAMP: 10W UV LED and 20W Blue, Green LED

- **PA53 Bio:**

INPUT: AC100-240V~, 1.8-1A, 47~63Hz

REFLECTED LAMP: 100W Halogen

### 7.2 Storage Environment

- Temperature: -20°C ~ +40°C
- Humidity: 10% ~ 90% (No condensation)
- Transportation (equipment stored in the package): Allows the ambient temperature of -40°C to +70°C and a humidity of 10% to 90% (no condensation). PA53 FS6 is a high-precision device, and transport protection such as avoid sun exposure, stay dry, Fragile Handle with care should be taken. If you need to pack the equipment for long-distance transportation, please contact the manufacturer (see back cover) or Motic Distributor.

### 7.3 Replacement of Parts & Accessories

To guarantee product performance, please be sure that components are purchase from Motic authorized distributor;

#### 7.3.1 Standard Accessory List:

- Allen Keys
- Immersion Oil 5ml
- Instruction Manual
- Rivets for install filter cube (for PA53 FS6 only)

#### 7.4 Bulb replacement

The lamp and the lamp house become very hot during and after a period of operation.

Risk of burn – Do not touch the lamp during or immediately after period of operation.

Make sure the lamp has cooled sufficiently before attempting to replace the lamp.

- In order to prevent electric shock always turn the power switch off and unplug the power cord before installing or replacing the bulb.

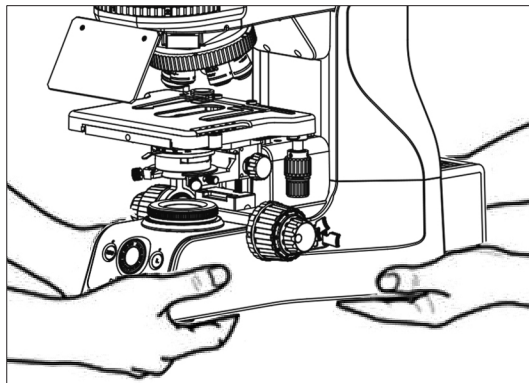
#### 7.5 Shifting of Instrument

##### Caution:

To avoid the risk of electric shock, this equipment must only be connected to a supply main with protective earth.

The protection provided by the equipment may be impaired if the equipment is used in a manner not specified by the manufacturer.

- When moving the microscope, grasp rear handle and the base with more than two peoples as shown below.



(Fig. 43)

## 7.6 Miscellaneous

- Automatic voltage selection device is applicable to worldwide voltage configuration, but it is recommended to use the power cable matches with the rated voltage of your area. Incorrect use of power cable may cause fire, or damage to the device
- For external power cable, power cable with ground protection must be used.
- To prevent electric shock, make sure the power switch is turned off before connecting the power cable.
- Please use standard slide, other slides are not allowed to be loaded into the device.
- The product should not be placed in the place with direct sunlight, dust, mechanical vibrations, high temperature or high humidity.  
(Please reference to Working Environment and Storage Environment)
- Grounding: Please make sure the device is connected to a wall outlet grounding Terminal, if the device is not grounded, our company no longer guarantee the safety of electrical equipment and EMC performance.
- If an emergency occurs, remove the power cord. Please install the device in a place where you can disconnect the power cable connector, or the socket is placed in the place where you can remove the power cord.
- Please keep this device in the environment with good ventilation.
- Please do not place the device in position which is difficult to disconnect power cable connector.
- Please do not put non-relevant stuff on the device.



## 7.7 Basic Specification

Model	PA53 BIO	PA53 FS6
Optical System	Scalable Infinity-Corrected Optical System (CCIS®)	
Illumination	Transmitted Illumination System	Transmitted / Reflected Fs Illumination System
Transmitted Illumination	Kohler illumination 100W long life halogen lamp	
Reflected Flour Illumination	/	High-power LED Flour Light source
Observation Tube	30° Trinocular (100:0, 20:80, 0:100)	
Eyepieces	Wide-Field (FN23) Eyepiece 10X/23mm, Diopter adjustable	
Nosepiece	Coded Quintuple (5) revolving nosepiece	
Recommended Objective	<b>Plan UC Achromat Series</b>	<b>Plan UC Fluor Series</b>
	Plan UC 4X/0.1, W.D=30.5mm, CG=0.17	Plan UC Fluor 4X/0.13, W.D=17.3mm, CG=0.17
	Plan UC 10X/0.25, W.D=17.4mm, CG=0.17	Plan UC Fluor 10X/0.3, W.D=11.7mm, CG=0.17
	Plan UC 20X/0.45, W.D=0.8mm, CG=0.17 (Optional)	Plan UC Fluor 20X/0.5, W.D=2.2mm, CG=0.17
	Plan UC 40X/0.65, W.D=0.6mm, CG=0.17	Plan UC Fluor 40X/0.75, W.D=0.7mm, CG=0.17
	Plan UC 60X/0.8, W.D=0.35mm, CG=0.17 (Optional)	Plan UC Fluor 100X/1.3 oil, W.D=0.1mm, CG=0.17
	Plan UC 100X/1.25 Oil, W.D=0.16mm, CG=0.17	
	Plan UC 100X/0.8, W.D=2mm, CG=0.17	
Plan Achromat Series	Plan APOCHROMAT 4X/0.15 W.D. =20mm, CG=0.17	Not recommend for Professional Flour Application
	Plan APOCHROMAT 10X/0.35 W.D. =4.2mm, CG=0.17	
	Plan APOCHROMAT 20X/0.75 W.D. =0.55mm, CG=0.17	
	Plan APOCHROMAT 40X/0.95 W.D. =0.18mm, CG=0.17	
	Plan APOCHROMAT 100X/1.3 oil. W.D. =0.25mm, CG=0.17	

HPlan SAPO Series	HPlan SAPO 4X/0.13, W.D=17.3mm, CG=0.17	Not recommend for Professional Flour Application
	HPlan SAPO 10X/0.3, W.D=11.7mm, CG=0.17	
	HPlan SAPO 20X/0.5, W.D=2.2mm, CG=0.17	
	HPlan SAPO 40X/0.75, W.D=0.7mm, CG=0.17	
	HPlan SAPO 100X/1.3 oil, W.D=0.1mm, CG=0.17	
Plan UC Phase Contract Series	Plan UC PH 10X/0.25, W.D=17.4mm, CG=0.17, Ph1	
	Plan UC PH 20X/0.45, W.D=0.8mm, CG=0.17, Ph2	
	Plan UC PH 40X/0.65, W.D=0.6mm, CG=0.17, Ph2	
	Plan UC PH 100X/1.25 Oil, W.D=0.16mm, CG=0.17, Ph3	
Focusing Mechanism	Stage Z range: 29.5 mm, Coarse and Fine focusing knob Coarse focusing: 17.7mm/turn; fine focusing: 0.1/turn, Upper limit stopper, Torque adjustment ring	
Stage	Rackless stage	
Flour Filter Cubes	/	DAPI / Hoechst / AlexaFluor 350
	/	EGFP / FITC / Cy2 / AlexaFluor 48
	/	TRITC / Cy3 / TagRFP / AlexaFluor 546
	/	DAPI / Green / Orange #1 FISH

## **FLUORESCENCE FILTER CUBES (Ø25mm Series)**

The fluorescence vertical illuminator can accommodate three filter blocks and a DIA-Filter dummy block (devoid of filters) that enables normal brightfield observation. Filter blocks are mounted on a slider inside the Epi-fluorescence attachment and can be inserted into the optical path by means of the excitation method change over knob. Each block has an accompanying ID tag that can be inserted into a pocket on the illuminator exterior housing in sequential order to enable the operator to easily select the proper block for fluorescence observation.

The excitation filters deteriorate with time due to exposure to heat and intense light. Replace when necessary.

Handle Multi-Band filters with great care as the very complex coatings wear easily. Special attention should be given to moisture. When filter is not in use, store in a desiccator.

## STANDARD FLUORESCENCE FILTER SETS

AT39002 EGFP/FITC/Cy2/AlexaFluor 488

Exciter AT480/30x

Dichroic AT505DC

Emitter AT535/40m

AT39004 TRITC/Cy3/TagRFP/AlexaFluor 546

Exciter AT540/25x

Dichroic AT565DC

Emitter AT605/55m

AT39010 Texas Red/mCherry/AlexaFluor 594

Exciter AT560/40x (EX)

AT600DC (BS)

Emitter AT635/60m (EM)

AT39000 DAPI/Hoechst/AlexaFluor 350

Exciter :AT375/28x

Dichroic :AT415DC

Emitter:AT460/50m

The emission at these wavelengths will be better detected by camera than by the unaided eye. Only a small percentage of humans can detect these wavelengths.

AT39001 ECFP/Cerulean

Exciter AT435/20x(EX)

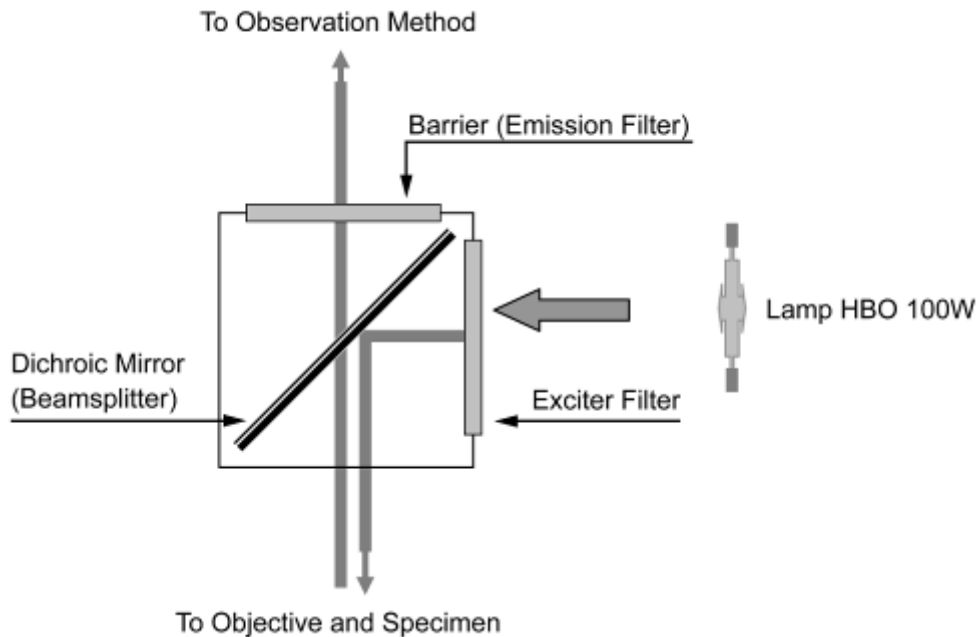
AT455DC(BS)

Emitter AT480/30m(EM)

This set will typically exclude the signal from YFP but not from GFP.

## ARRANGEMENT OF FILTERS IN A FLUORESCENCE CUBE

### Upright Microscope



It is often the case that specific combinations of excitation filter, emission filter and dichroic mirror are needed to visualize and/or quantitate the fluorescence emission from a particular fluorescent species.

In newer models of fluorescence microscopes, manufacturers have provided a means to change these optical elements in a convenient manner by arranging a set of four or more filter cubes in a circular (or linear) turret under the objective. With a turret arrangement, a specific filter cube can be selected in a manner similar to that of selecting a specific objective.

## NOMENCLATURE

### **Excitation Filter - EX**

A filter used in fluorescence microscopy designed to pass only those wavelengths, which excite fluorescence.

### **Excitation Filter D350/50x**

The center wavelength is at 350nm; full bandwidth is 50nm [=  $\pm 25$ nm].

In some cases when the bandwidth is not specified, the letter "x" is used to define the filter as an exciter filter and is generally used for narrow band UV excitation filters, e.g. D350x.

### **Dichroic Mirror - DM**

The dichroic mirror is the optical component that separates the excitation light from the fluorescence. Dichroic mirror is designed to reflect selectively the shorter wavelength exciting radiation and transmit the longer wavelength fluorescence.

Dichroic mirror is placed in a 45° incidence angle to light, allowing the mirrors to act as a precise discriminator of excitation and fluorescence wavelengths.

Light passing through the excitation filter is reflected 90° toward the objective and the specimen. Finally, light emanating from the specimen is passed through and directed toward the emission filter and observation method.

Dichroic multi-layer thin-film coatings makeup typically consists of one of the following design types: short wave pass, long wave pass, or bandpass filter. These design types comprise the basis of color determination and color separation and this are determined by the transmittance and/or reflectance of a band of wavelengths.

### **Dichroic Beam splitter 505DCLP**

The cut-on wavelength is approximately 505nm for this dichroic longpass application.

### **Emission Filter - EM (Barrier filter)**

Emission filter is the last component in a fluorescence cube, which transmits fluorescence emission wavelengths while blocking excitation wavelengths. Emissions filters are coloured glass or interference filters that have transmission properties of a bandpass or longpass filters.

Most interference are mounted at a slight angle to allow for better imaging by suppressing ghost images.

### **Emission filter D460/50m\***

The center wavelength is at 460nm; full band is 50nm [=  $\pm 25$ nm].

\*m –emission

## DEFINITIONS OF FILTER TERMINOLOGY

### **Bandpass Filters**

Bandpass filters transmit a band of wavelengths and block all light above and below the specified transmission range. These filters are characterized with respect to optical performance by their centre wavelength (CWL) and bandwidth, also referred to as the full width at half of maximum transmission (FWHM). (See Figure Below)

### **CWL**

Center wavelength

For optical bandpass filters, the arithmetic means of the cut-on and cut-off wavelength at 50% of peak transmission.

### **DCLP**

Dichroic Longpass.

### **DCXR**

Dichroic Longpass, extended reflection including the UV.

### **FWHM**

Bandpass filters are usually named by their filters center wavelength (the arithmetic means of the wavelengths at 50% of center peak transmission) and by range of wavelengths (bandwidth) of light they transmit at 50% peak transmittance (full width half maximum, FWHM). Example D350/50x is an interference filter with maximal transmission at 350nm and transmits light from 325 and 375nm. (See Figure Below)

### **GFP**

The Green Fluorescent Protein (GFP) from the jellyfish *Aequoreavictoriais* used as a fluorescent indicator for monitoring gene expression in a variety of cellular systems, including living organisms and fixed tissues.

The wild type GFP has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm which is in the lower green portion of the visible spectrum.

Due to this widespread usage different mutants of GFP have been engineered over the last few years: Color mutants have been obtained from the GFP gene as well: in particular the cyan fluorescent protein (CFP) and the yellow fluorescent protein (YFP) are two colour variants employed for fluorescence resonance energy transfer (FRET) experiments.

**GG**

Green Glass – Longpass absorption glass from Schott Glassworks with cut-on wavelengths in the violet and blue-green regions

**HQ**

A designation for high-performance filters with narrow cuton and cutoff wavelengths

**LP**

Longpass Filters

Longpass filters transmit a wide spectral band of long wavelength radiation while blocking short wavelength radiation. (See Figure Below)

**Narrowband Filters**

Narrowband filters with a very narrow band, typically 1 to 3nm wide. They can be used successfully only with high intensity light sources because of the narrow bandwidth.

**ND**

Neutral density filters are designed to reduce transmission evenly across a portion of the spectrum.

**OG**

Orange Glass - Longpass absorption glass from Schott Glassworks with cut-on wavelengths in the green, yellow and orange regions

**PC**

Polychromatic mirrors or beam splitters.

These beam splitters reflect and transmit more than two bands of light. Dual-band or Triple-band filter sets include three carefully balanced combinations that contain dual or triple bandpass excitation and emission filters, these dedicated filter sets also incorporate polychromatic mirrors or beam splitters with multiple bandpass characteristics, having transmission and reflection regions that are matching to the specific excitation and emission filters employed.

**RG**

Red Glass - Longpass absorption glass from Schott Glassworks with cut-on wavelengths in the red and far red regions



## SP

### Shortpass Filters

Shortpass filters transmit a wide spectral band of short wavelength radiation and block long wave radiation. (See Figure Below)

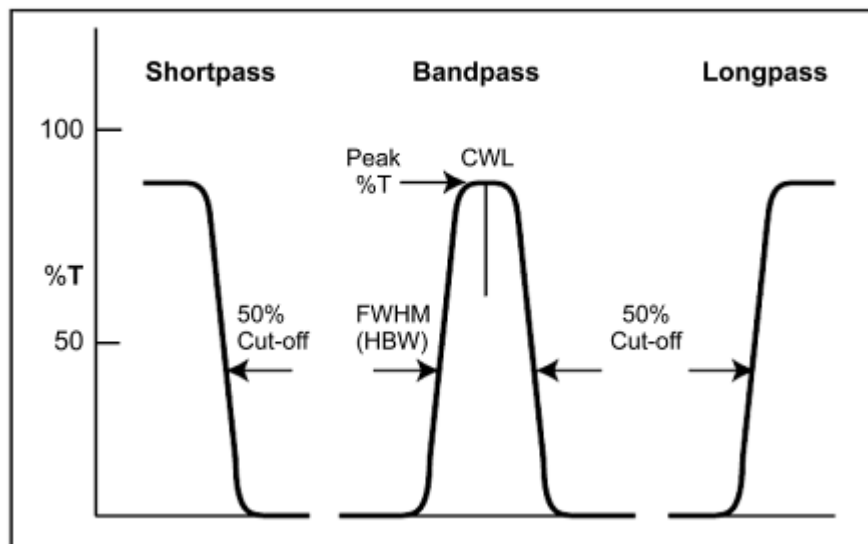
## S/N

### Signal to Noise Ratio

Ratio of intensity of signal to that of the background.

## Stokes shift

The distance between the peak absorption and emission of a dye, usually in nm.



### Filters for isolating the wavelength of illumination:

Shortpass and longpass filters, block or transmit wavelengths at specific cut-off wavelengths.

Bandpass filters exhibit broadband or short band transmission centered on a particular band of wavelengths. Filters performance is defined by the central wavelength (CWL) and by the full width at half maximum (FWHM).



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