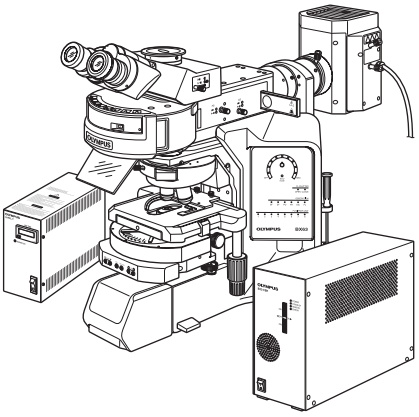


BX3-RFAA
U-LH100HGAPO
U-LH100HG
U-RFL-T
U-AN-2

INSTRUCTIONS

BX3-RFAA

Motorized fluorescence system

Optical Microscope Accessory

To ensure the safety, obtain optimum performance and to familiarize yourself fully with the use of this system, we recommend that you study this manual thoroughly before operating the microscope.

This product is a part of the system product which complies with the CE marking.
Please refer to the instruction manual of your system product for the safety instructions related to the CE marking.

In accordance with European Directive on Waste Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste, but should be collected separately.
Refer to your local our distributor in EU for return and/or collection systems available in your country.

For Korea only

A급 기기 (업무용 방송통신기자재)

이 기기는 업무용(A급) 전자파적합기기로서 판 매자 또는 사용자는 이 점을 주의하시기 바라 며, 가정외의 지역에서 사용하는 것을 목적으로 합니다.

CONTENTS

Correct assembly and adjustments are critical for the motorized reflected fluorescence system to exhibit its full performance. If you are going to assemble the motorized reflected fluorescence system yourself, please read Chapter 8, "ASSEMBLY" (pages 21 to 24) carefully.

IMPORTANT

When the BX3-RFAA motorized fluorescence illuminator is installed on the BX63 motorized microscope. Its motorized operations can be controlled from the BX3-CBH control box as well as various other controllers including the touch panel controller and the U-MCZ controller.
When it is installed on the BX53/BX43 manual microscope, the motorized operations can be controlled from the U-CBM control box, U-HSCBM hand switch.

Motorized operations
- **Switching of mirror unit turret.**
- **Engagement/disengagement of shutter.**

Please also read the separately available instruction manuals listed below to obtain comprehensive understanding of the system operating procedures.

Manual name	Main contents
Motorized Reflected Fluorescence System (This manual)	Reflected fluorescence observation procedures using the BX3-RFAA motorized fluorescence illuminator.
Microscope (BX63, BX53, BX43)	Operating procedures of each microscope.
Control Box/Controller (BX3-CBH/U-MCZ)	The functions of the control box for the BX63 microscope and various operating procedures using the U-MCZ.
Power Supply Unit for Mercury Burner/Xenon Burner (U-RFL-T/U-RX-T)	Information on the power supply unit for the lamp housing for mercury or xenon burner.
Control Box/Hand Switch for CBM (U-CBM/U-HSCBM)	Information on the control of motorized modules installed on a manual microscope.
Motorized Attenuator Wheel (U-AW)	Information on the installation of the U-AW.
75 W Xenon Apo Lamp Housing	Information on the lamp housing for xenon burner. However, the information on the centering of the burner is not applicable to this system.

⚠ SAFETY PRECAUTIONS

1. This system is composed of precision instruments with motorized parts. Handle it with care and avoid subjecting it to sudden or severe impact.
2. The ultrahigh-pressure mercury burner should be the USH-103OL, which is manufactured and supplied by us.
3. Make sure that a mercury burner is attached and that cables are plugged in firmly.
4. The inside of the lamp housing is very hot and hazardous during lighting and for about 10 minutes after turning off. Do not detach or open the lamp housing in this period (page 11).
5. Do not apply excessive force to the stoppers which are provided for some functions. Otherwise, the stopper or equipment may be damaged.
6. Be careful not to have your hand or finger caught by the motorized mechanisms (mirror unit turret, motorized attenuator wheel, etc.).
7. Do not attempt to open, disassemble or modify the power supply unit because it includes high voltage parts inside.
8. Always use the power cord provided by us.
   Before plugging the power cord to the power outlet, make sure that the main switch of the power supply unit is set to " O " (OFF).
9. To ensure safety, be sure to **ground** the power supply unit. Otherwise, we can no longer warrant the electrical safety performance of the system.
10. Before opening the lamp housing for replacement of the burner or any other internal part, set the main switch to " O " (OFF), then unplug the lamp housing connection from the power supply unit, and wait for more than 10 minutes until the lamp housing cools down.
11. The top panel of the lamp housing becomes very hot during operation. To prevent fire hazard, do not block the ventilation through the top panel.
12. The standard service life of the lamp housing is eight (8) years of use or 20,000 hours of total power ON period, whichever is the shorter period.
    For details, see Inspection Sheet on page 25.

Safety Symbols

The following symbols are found on the system.
Study the meaning of the symbols and always use the equipment in the safest possible manner.

Symbol	Explanation
	Indicates the presence of high voltage (1 kV or more). Take caution to guard against electric shock.
	Indicates that the surface becomes hot, and should not be touched with bare hands.
	Indicates a non-specific general hazard. Follow the description given after this symbol or in instruction manual.
I	Indicates that the main switch is ON.
O	Indicates that the main switch is OFF.

Caution indications

Caution indications are placed at parts where special precaution is required when handling and using the System. Always heed the warnings.

Caution indication positions	• Motorized fluorescence illuminator (BX3-RFAA) • Lamp housing for mercury burner (U-LH100HG/U-LH100HGAPO/U-LH75XEAPO) • ND filter slider • Power supply unit (U-RFL-T/U-RX-T)	[Caution against high temperature]  [Caution against high voltage] 

If a caution label is dirty or peeled off, contact us for the replacement or other inquiry.

1 Getting Ready

1. This manual pertains only to the motorized reflected fluorescence system. Before using this system together with the BX63/BX53/BX43 microscope and associated options, make sure that you have carefully read and understood their manuals, and understand how the system should be operated together.
2. The motorized mechanisms cannot be controlled manually. (Applying excessive force to the motorized mechanisms may damage them.)
3. Do not replace modules or connect/disconnect cables while the main switch of the control box (BX3-CBH/U-CBM) is set to " **I** " (ON).
4. The motorized reflected fluorescence system is composed of precision instruments. Handle it with care and avoid subjecting it to sudden or severe impact. Also note that the microscope does not have a waterproof construction.
5. Do not use the system where it is subjected to direct sunlight, high temperature and humidity, dust or vibrations.
6. To allow heat from the unit to dissipate well, reserve clearances of at least 10 cm around the lamp housing and power supply unit.
7. The power cord can also be used to cut the power supply in case of emergency. To make this possible, the power supply unit should be installed so that the power cord connector (on the rear of the power supply unit) or the power outlet is easily accessible for unplugging in case of emergency.
8. Before igniting the burner, make sure that a burner is attached and the cords are connected properly.
9. Avoid turning the lamp on and off repeatedly as this reduces the burner life considerably. The average service life of a mercury burner is about 300 hours (USH103OL) with operating cycles of 2-hour ON and 30-minute OFF.

2 Maintenance and Storage

1. To clean the lenses and other glass components, simply blow dirt away using a commercially available blower and wipe gently using a piece of cleaning paper (or clean gauze).
   If a lens is stained with fingerprints or oil smudges, wipe it with gauze slightly moistened with commercially available absolute alcohol.

CAUTION **Since the absolute alcohol is highly flammable, it must be handled carefully. Be sure to keep it away from open flames or potential sources of electrical sparks — for example, electrical equipment that is being switched on or off.**
Also remember to always use it only in a well-ventilated room.

2. When any part of the system other than glass components gets dirty, do not use organic solvents but wipe it with a clean cloth. If the part is extremely dirty, use a lint-free, soft cloth slightly moistened with a diluted neutral detergent.
3. Do not disassemble any part of the system, as this could result in malfunction or reduced performance.
4. After using the system, set the main switch to " O " (OFF), wait for the lamp housing to cool down sufficiently, and keep it covered with a dust cover during storage.
5. The mercury burner has a service life period of 300 hours. When the hour counter on the power supply unit indicates this value, set the main switch to " O " (OFF) and wait for more than 10 minutes before replacing the mercury burner (page 23). Unlike electric bulbs, the mercury burner seals high-pressure gas inside. If it continues to be used after the service life has expired, the glass tube may eventually explode due to accumulated distortion. When the service life of a mercury burner has expired, it should be treated as an industrial waste. If you cannot dispose of it properly, please contact us.
6. When disposing of the system, always follow your local regulations and rules.

3 Caution

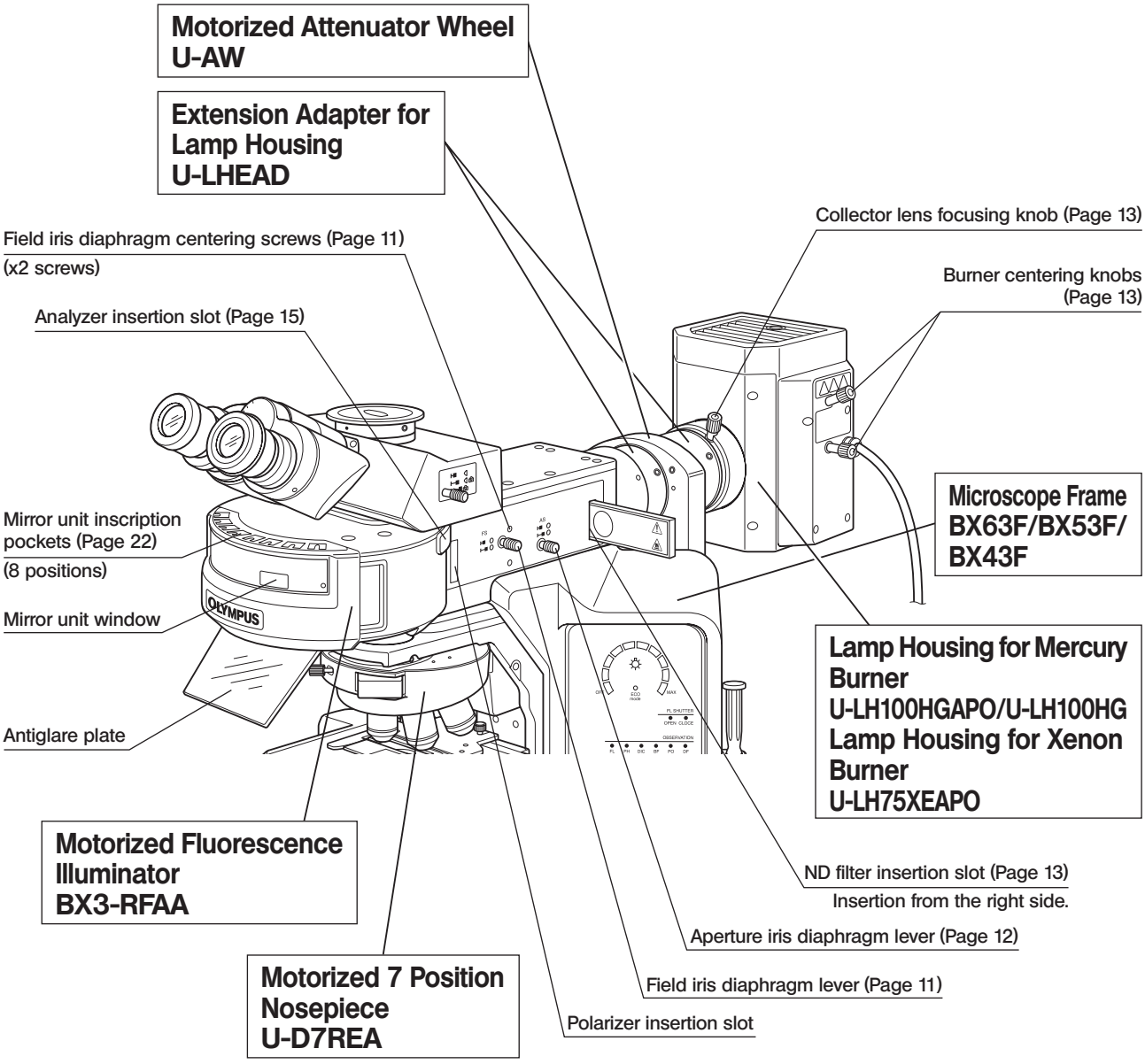
If the system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the system equipment may also be damaged. Always use the system as outlined in this instruction manual.

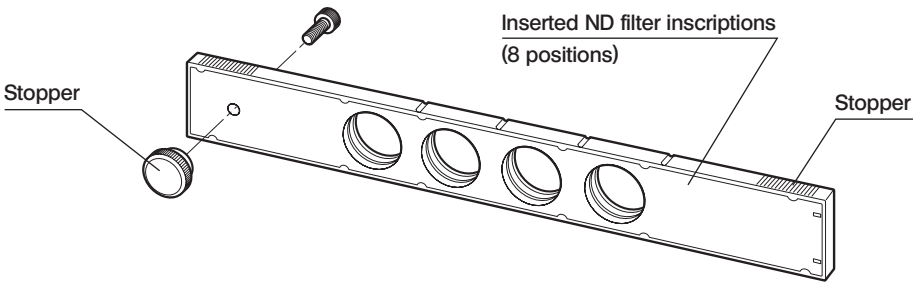
The following symbols are used to set off text in this instruction manual.

CAUTION : Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury or damage to the equipment or other property. It may also be used to alert against unsafe practices.

◎ : Indicates commentary (for ease of operation and maintenance).

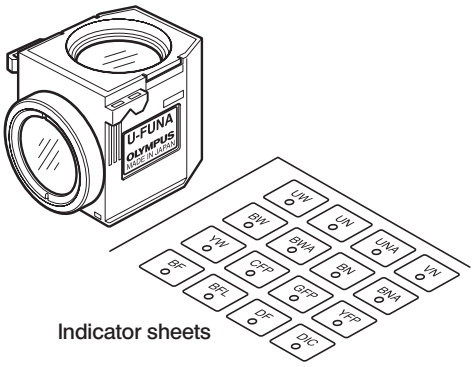
1 NOMENCLATURE





ND filter slider (Provided with BX3-RFAA)

Fluorescence Mirror Units



Indicator sheets

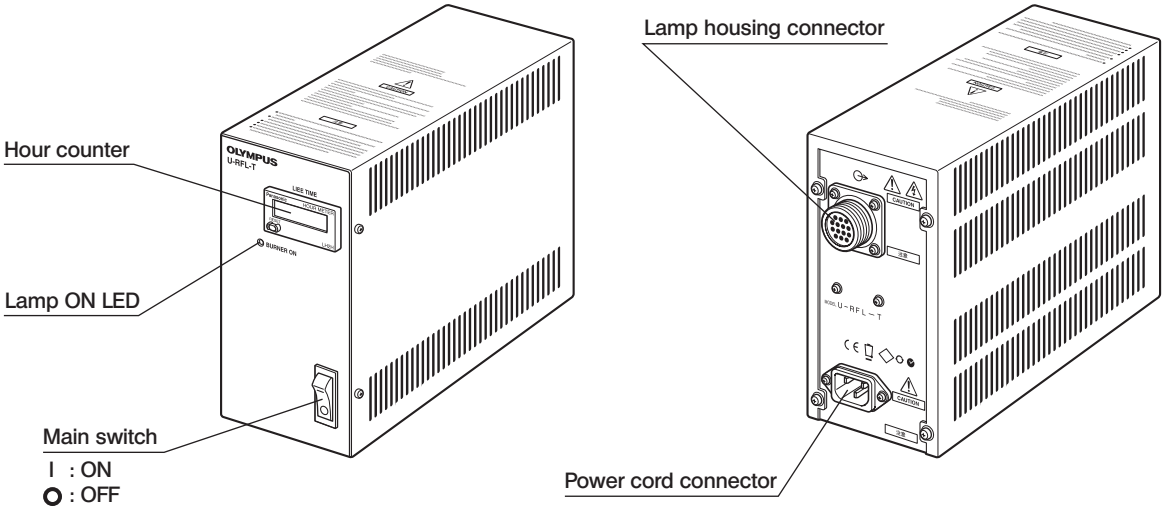
◎Up to eight fluorescence mirror units can be mounted on the BX3-RFAA.

◎When making your original fluorescence mirror unit, use the U-FF dummy mirror unit (which does not contain a filter) (page 24).
Blank indicator sheets provided with the illuminator can be used to write the names of original fluorescence mirror units.

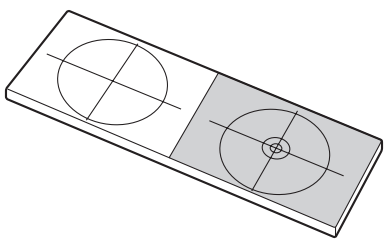
Power Supply Unit U-RFL-T/U-RX-T

◎For details, see the instruction manual provided with the U-RFL-T/U-RX-T.

◎Use the U-RFL-T when the light source is a mercury burner or use the U-RX-T when the light source is a xenon burner.



Centering Target U-CST



2 REFLECTED FLUORESCENCE OBSERVATION PROCEDURE

◎If you need simultaneous observations of reflected fluorescence observation with the phase contrast observation or transmitted light Differential Interference Contrast (DIC) observation, please read Chapter 4, "SIMULTANEOUS FLUORESCENCE OBSERVATIONS" (page 15).

◎"Controls Used" refer to those on the touch panel controller. But the same operations are also available from the U-MCZ controller.

(Controls Used) (Page)

Preparation

- Attach the fluorescence mirror unit and objective matching the observation method. (Pages 9 to 10)
- Center the mercury burner. (Pages 12 to 13)
- Set the main switch ① of the BX3-CBH control box to " **I** " (ON).

Set the main switch of the power supply unit to " I " (ON) and wait for the lamp brightness to stabilize (5 to 10 minutes after ignition). ---- ②Main switch (P.11)

↓

Tap on "FL" (Fluorescence) on the menu screen. ---- ③ **FL** button

↓

Place the specimen on the stage. ---- ④Specimen holder, ⑤X-/Y-axis knobs

↓

Engage the fluorescence mirror unit matching the specimen in the light path. ---- ⑥ **Mirror** tab, ⑦ **Mirror** button

↓

Engage the objective in the light path and focus on the specimen. ---- ⑧ **Objective** button, ⑨ **Focus** button/slider

Engage an ND filter in the light path as required. ---- ⑩ND filter slider (P.14)

↓

Adjust the brightness as required. ---- ⑪Collector lens focusing knob (P.12)

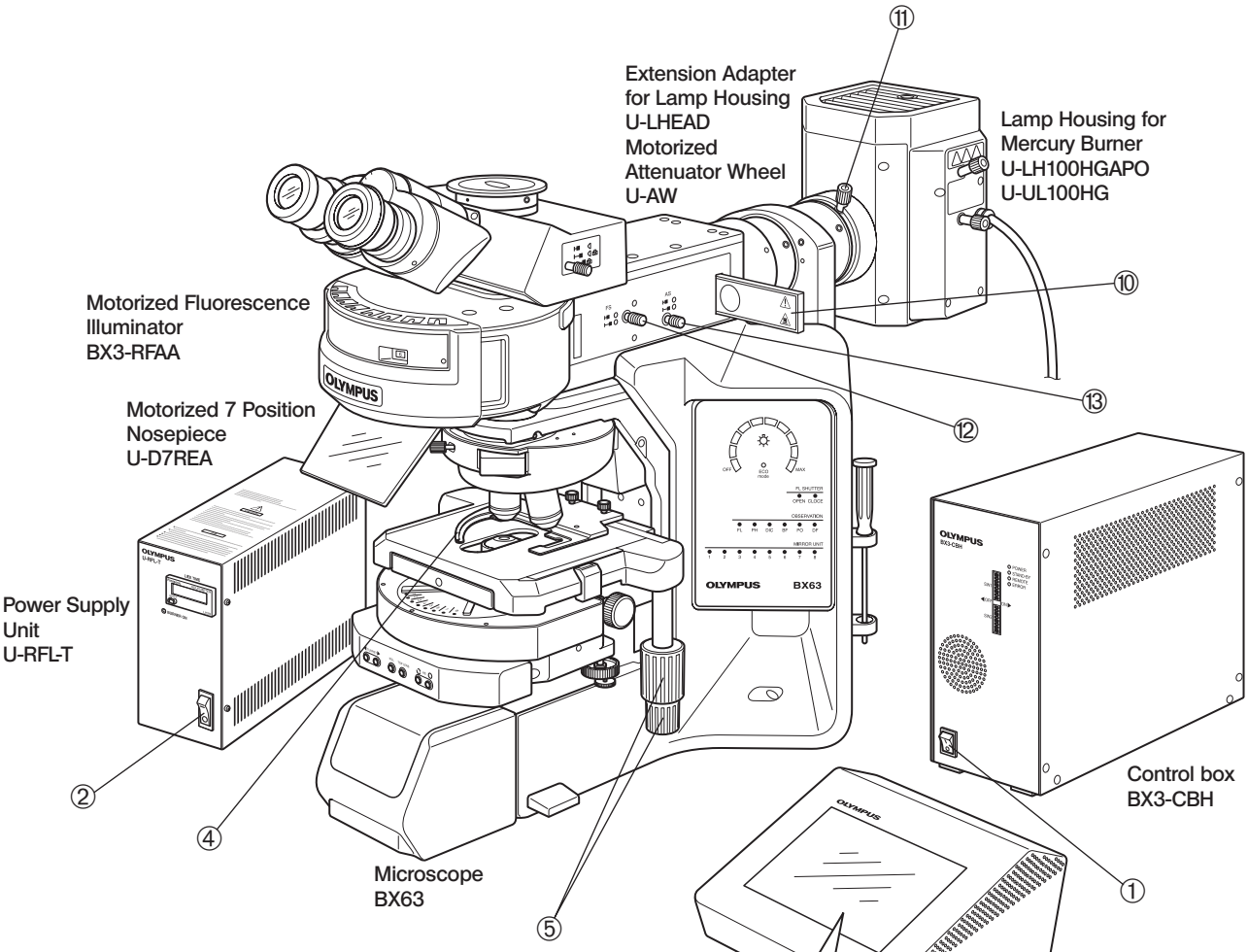
Adjust the field iris diaphragm. ---- ⑫Field iris diaphragm lever (P.11)

Adjust the aperture iris diaphragm. ---- ⑬Aperture iris diaphragm lever (P.12)

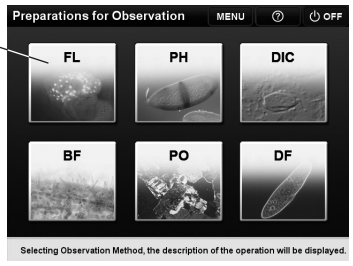
↓

Observation

◎Engage the shutter if you interrupt observation for a short time. ---- ⑭ **Shutter** button

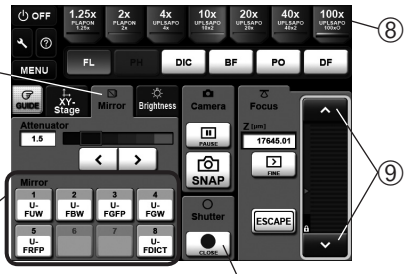


●Preparations for observation



③

●Guidance mode display



⑥ ⑧ ⑨ ⑦ ⑭

©Make a photocopy of the observation procedure pages and post it near your microscope.

3 USING THE CONTROLS

This instruction manual describes the procedures when using BX63 Touch Panel Controller. Same procedures can be applied to U-MCZ. Please see BX3-CBH/U-MCZ Instruction Manual for detail procedures.

1 General Precautions for Observation

1. Make sure that the power cord and connecting cables are plugged in securely.
2. If you perform only transmitted phase contrast or transmitted DIC observations*, leave one position on the turret empty. This allows for transmission of white light and reproduction of original colors.
   * This care is not required when the U-FDICT transmitted DIC mirror unit with built-in analyzer is used.
3. Always use our immersion oil for oil immersion objectives.
4. If you use an objective with correction collar, you can correct contrast degradation due to variation in cover glass thickness by adjusting the correction collar.

   Correction procedure

   If the cover glass thickness is known, match the correction collar to the cover glass thickness using the collar scale provided. If the thickness is not known, turn the collection collar and adjust the fine adjustment knob to where the image contrast is best.
5. Engage the shutter if you interrupt observation for a short time.
6. Color fading of specimens

   This system features high excitation light intensity to ensure bright observation of dark fluorescence specimens.
   In consequence, after long period of observations using high-power objectives, the colors of specimens will fade quicker than usual, causing the view (contrast) of fluorescent images to deteriorate.
   In such a case, slightly reduce the excitation light intensity to slow color fading down and improve the fluorescence images.
   To reduce the excitation light intensity, use ND filters or aperture iris diaphragm as far as the observation is not affected or use the shutter to limit the exposure of specimen to more than necessary light.
   Commercially-marketed color fading protection agent (DABCO, etc.) can also delay fading of specimen colors. The use of fading protection agent is recommended especially when you perform high-magnification observations frequently.

CAUTION **Remember that the fading protection agents cannot be used with certain kinds of specimens.**

2 Selecting the Fluorescence Mirror Unit

Select the fluorescence mirror unit which matches the fluorochrome in use.

◎ Usage according to the excitation light bandwidth:

A set combining excitation filters for different bandwidths can be used according to the type of excitation light. The wide-band (W) set is normally used. However, when the fluorescence emitted from substances other than the fluorescent stain is strong, the Narrow-band (N) set is recommendable (though the fluorescence becomes slightly darker).

Dichroic Mirror and Filter Configurations of Fluorescence Mirror Units

Excitation method	Mirror unit	Dichroic mirror	Excitation filter	Barrier filter	Applications
U	U-FUW	DM410	BP340-390	BA420IF	• Autofluorescence observation • DAPI: DNA staining • Hoechest 33258, 33342: Chromosome
	U-FUN		BP360-370		
V	U-FVN	DM455	BP400-410	BA460IF	• Catecholamine observation • Serotonin observation • Tetracyline: Bones, teeth
BV	U-FBVW	DM455	BP400-440	BA460IF	• Quinacrine, quinacrine mustard: Chromosome • Thioflavine S: Lymphocyte • Acriflavine: Nucleic acid • ECFP
B	U-FBW	DM505	BP460-495	BA510IF	• FITC: Fluorescent antibody • Acridine orange: DNA, RNA • Auramine: Tubercle bacillus • EGFP, S65T, RSGFP
	U-FBN		BP470-495		
G	U-FGW	DM570	BP530-550	BA575IF	• Rhodamine, TRITC:Florescent antibody • Propidium iodide: DNA • RFP
Y	U-FYW	DM600	BP545-580	BA610IF	Texas Red: Fluorescent antibody

Combinations for color separation

U	U-FUNA	DM410	BP360-370	BA420-460	For observing only the U-excitation stain when using U-excitation stain together with FITC.
B	U-FBWA	DM505	BP460-495	BA510-550	For observing only the B-excitation stain when using B-excitation stain with TRITC or Texas Red.
	U-FBNA		BP470-495		
G	U-FGWA	DM570	BP530-550	BA575-625	For observing only the G-excitation stain when using G-excitation stain together with Cy5.
	U-FGNA		BP540-550		

Exclusive combinations for fluorescent proteins

CFP	U-FCFP	DM455	BP425-445	BA460-510	For ECFP.
GFP	U-FGFP	DM490GFP	BP460-480	BA495-540	For EGFP.
YFP	U-FYFP	DM515	BP470-500	BA515-560	For EYFP.
RFP	U-FRFP	DM565HQ	BP535-555HQ	BA570-625HQ	For RFP.
mCherry	U-FMCHE	DM595	BP565-585	BA600-690	For mCherry.

3 Turning the Power Supply Unit On

Set the main switch to " I " (ON). The illumination light will stop flickering and stabilize in 5 to 10 minutes after ignition.

◎The discharge type mercury burner may not be ignited from the beginning on rare occasions due to its characteristics. In this case, set the main switch to " O " (OFF), wait for 5 to 10 seconds, then set it again to " I " (ON).

CAUTION

- **To extend the mercury burner life, do not turn it on and off at short interval. If you want to interrupt observation for a while in less than 2 hours after ignition, do not switch the power supply unit to off but simply close the shutter of the illuminator during the interruption period.**
- **The mercury burner cannot be reignited until the mercury vapor has cooled down and liquefied. Before re-igniting a mercury burner, wait for about 10 minutes after the last time it was turned off.**

◎For the sake of safety, the power supply to the lamp housing is shut down if the lamp housing is opened while the burner is on. If this happens, set the main switch to " O " (OFF), wait for more than 10 minutes, then set it again to " I " (ON). Whenever you want to open the lamp housing, make sure that it has cooled down enough after use.

CAUTION **After replacing the mercury burner, reset the hour counter by holding its reset button till "0.0" is displayed.**

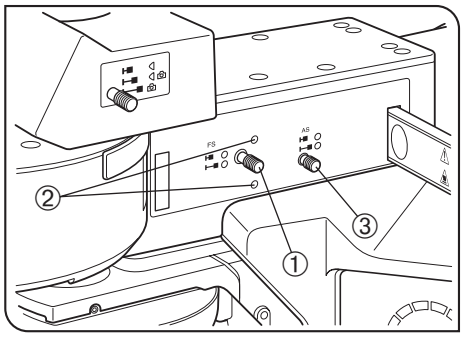
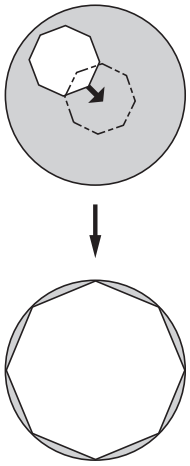


Fig. 1



4 Centering the Field Iris Diaphragm (Fig. 1)

1. Set the FL light path.
   Tap on the Mirror tab to engage a mirror unit other than the ones for U-excitation (U-FUW, U-FUN, U-FUNA) in the light path.

CAUTION **You may use a fluorescence mirror unit for U-excitation if you do not have other mirror units but, in this case, you must always view the images through an anti-glare plate.**

2 Engage the 10X objective in the light path, place the specimen on the stage and bring the image into approximate focus using the Focus buttons or Focus slider.
3. Pull out the field iris diaphragm lever ① of the illuminator to minimize the field iris diameter.
4. Fit the Allen screwdriver provided with the microscope frame in the two field iris centering screws ② and adjust so that the iris image comes on the center of the field of view.
5. While pushing in the field iris diaphragm lever ①, enlarge the field iris diaphragm until the field iris image inscribes the field of view. If eccentricity is found after this, try centering again.
6. Enlarge the field iris diaphragm.

Using the Field Iris Diaphragm

The field iris diaphragm restricts the diameter of the beam of light entering the objective and thus excludes extraneous light, improving image contrast. The field iris diaphragm also functions to prevent color fading of fluorescent light in other part than the observed region.

5 Using the Aperture Iris diaphragm (Fig. 1)

◎Adjust the aperture iris aperture by pulling the aperture iris diaphragm lever ③. This adjusts the brightness and contrast of the observation image.

CAUTION **The aperture iris diaphragm cannot be used as a substitute to the shutter even when it is stopped down to the minimum aperture.**

6 Centering the Mercury Burner (Figs. 2 to 6)

◎The mercury burner emits light by means of discharge produced when a current is supplied across the poles. If the positions of the poles were moved for example during replacement of the burner, the brightness of the light would be irregular. If this happens, it is necessary to adjust the positions of the poles. This operation is called the centering of mercury burner.

◎The mercury burner centering is not required every time before observation, but is recommended after the burner has been replaced or when the brightness of the observation image is irregular.

◎The xenon burner should also be centered in the same way as the mercury burner. (Note that the centering method described in the instruction manual for the xenon burner is invalid with this system.)

◎If the collector lens focusing knob of the lamp housing is hard to access due to the location behind the microscope, insert the U-CLA extension handle (optional) shown on the left into the knob. (Fig. 2)

◎Set the main switch of the power supply unit for the lamp housing for mercury burner to " **I** " (ON) and wait until the illumination light stops flickering and its brightness is stabilized.

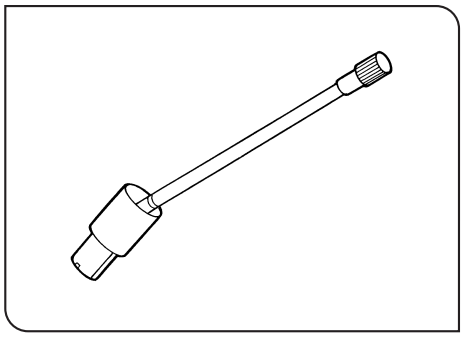


Fig. 2

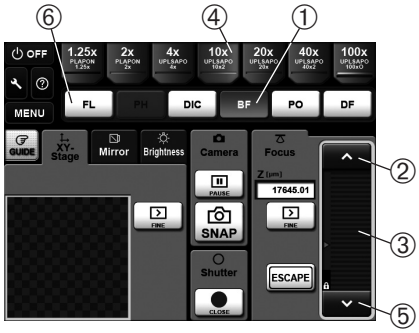


Fig. 3

1. Set the BF light path ①.
2. Move the objectives as apart as possible from the stage using the Focus Up button ② or Focus slider ③.
3. Remove one objective other than the 10X objective from the revolving nosepiece.
4. Engage the 10X objective ④ in the light path and place the U-CST centering target on the specimen holder on the stage.
5. Move the cross lines for reflected light (on the white surface) of the U-CST right below the 10X objective.
6. Look into the eyepieces, focus on the cross lines for reflected light of the U-CST using the Focus Down button ⑤ or Focus slider ③ and move the cross lines on the center of the field.
7. Turn the revolving nosepiece to engage the empty position (the objective cap should be removed) in the light path.
8. Set the FL light path ⑥.
   Tap on the [Mirror] tab to engage a mirror unit other than the ones for U-excitation (U-FUW, U-FUN, U-FUNA) in the light path.

CAUTION **You may use a fluorescence mirror unit for U-excitation if you do not have other mirror units but, in this case, you must always view the images through an anti-glare plate.**

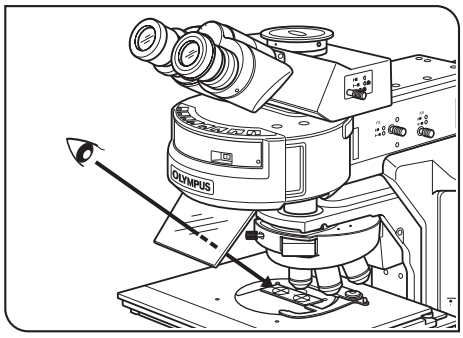


Fig. 4

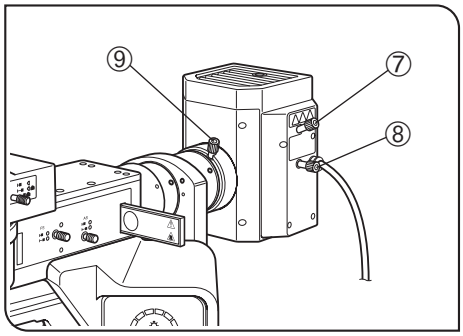


Fig. 5

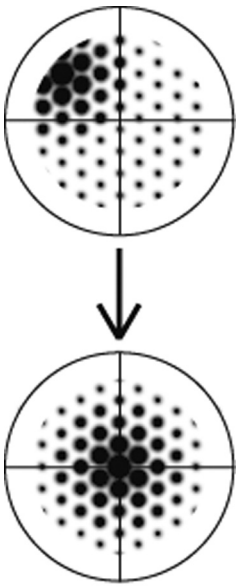


Fig. 6

9. While observing the image on the U-CST directly without through the eyepieces, adjust centering of the mercury burner with the following procedure (Fig. 4).

Mercury burner centering procedure

a) Turn the burner centering knob ⑦ (upper knob) so that the image on the U-CST is as bright as possible.
b) Turn the burner centering knob ⑧ (lower knob) so that the image on the U-CST is brightest.
◎After steps a) and b), the bright position in the image does not have to be located on the center of the U-CST.
c) Turn the collector lens focusing knob ⑨ so that only a part of light is noticeably brighter than other part (Fig. 6).
◎In practice, the bright part is not always located on the top left as shown in Fig. 6.
d) Turn the centering knobs ⑦⑧ so that the part on the center of the cross lines on the U-CST is the brightest.
e) Improve the accuracy of adjustment by repeating steps c) and d).

Now the center of the mercury burner is complete.

10. Engage the 10X objective in the light path and, while looking into the eyepieces, turn the collector lens focusing knob ② so that the image is the brightest.
11. Mount the objective removed in step 3 on the revolving nosepiece.

7 Using the ND Filter Slider (Fig. 7)

◎Specimen color fading can be delayed by reducing the excitation light intensity with ND filters. Use the ND filters as far as they do not hinder observations.
The figure in the model number of each ND filter represents its transmittance.

- ND1.5: Transmittance 1.5%
- ND6: Transmittance 6%
- ND25: Transmittance 25%

CAUTION **Note that the metallic filter frame will be very hot if you leave the filter inserted for a long time while the mercury burner is on. Do not leave the filter insertion positions in other positions than the click positions for a long period of time.**

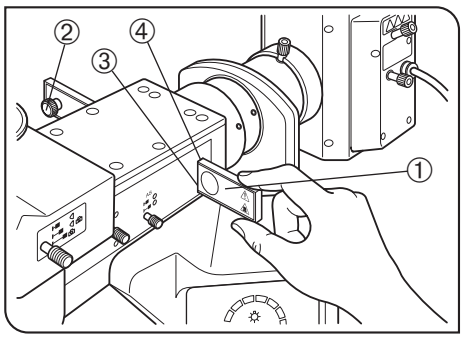


Fig. 7

1. If the stopper ② is attached to the ND filter slider ①, loosen and remove the stopper ② temporarily using the Allen screwdriver.
2. Insert the ND filter slider into the ND filter slider insertion slot ③ on the right side so that the filter name inscriptions face the front.
3. Attach the stopper ② again so that the ND filter slider does not slip out accidentally.
4. The filter positions that can be engaged in the light path include three for the ND1.5/ND6/ND filters and one empty (O) position.
   Each filter can be engaged at the click position.
   The filter being engaged in the light path can be checked at the top positions (left and right) of the insertion slot.
5. When switching the ND filter position by sliding the slider, be careful against burns and pinch the filter frame by the top and bottom.

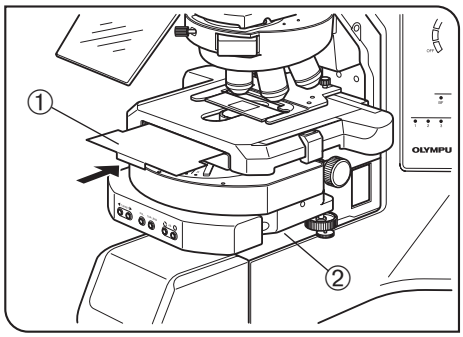


Fig. 8

8 Attaching the Light Shield Sheet (Fig. 8)

◎When a low-power objective is used in fluorescence observation, the fluorescence image may be degraded by the reflected light from the condenser and the area around it. In this case, use one of the following light shield sheets according to the stage.

- BX stage: Light shield sheet ① provided with the BX3-RFAA.
- IX-SVL2 stage: Light shield sheet provided with the BX63F.
- BX3-SSU stage: Same as above.

1. Lower the condenser.
2. Insert the light shield sheet ① into the space on the stage.

◎If you also switch the observation mode to transmitted light observation (phase contrast, DIC, etc.), the light shield sheet ① should simply be placed on the window lens ② only during fluorescence observation.

4 SIMULTANEOUS FLUORESCENCE OBSERVATIONS

◎By properly combining equipment, this system can be used in transmitted light brightfield observation, transmitted phase contrast observation and transmitted DIC observation in addition to the reflected fluorescence observation. With specimens that fade rapidly, fading can be minimized by initially using transmitted phase contrast or transmitted DIC observation for positioning. Reflected fluorescence observation can also be executed simultaneously with phase contrast or DIC observation.

1 Simultaneous Reflected Fluorescence/Phase Contrast Observations

The phase contrast observation requires a phase contrast condenser (U-PCD2) or a universal condenser (U-UCD8/BX3-UCD8A) and a Ph objective.

1. Engage the dummy mirror unit in the light path.
2. Rotate the phase contrast turret to show the same number as the Ph number shown on the objective.
3. Adjust the optical axis between the ring slit and phase plate by centering them.
4. Engage the mirror unit corresponding to the desired excitation into the light path and open the shutter.
5. Adjust the transmitted light for optimum balance of fluorescence and phase contrast brightness, and you are ready for observation.

◎To adjust the brightness of transmitted light, Use ND filters or adjust the transmitted light intensity.

◎For details on the phase contrast observation, refer to the instruction manual for the phase contrast condenser or universal condenser.

2 Simultaneous Reflected Fluorescence/Transmitted Light Differential Interference Contrast (DIC) Observations

The transmitted DIC observation requires the following accessories;
① universal condenser (U-UCD8/BX3-UCD8A); ② transmitted DIC slider (U-DICT, U-DICTS, U-DICTHR or U-DICTHC); ③ analyzer (U-AN-2); ④ revolving nosepiece for DIC or motorized revolving nosepiece.

◎In order for reflected fluorescence to be effective in the simultaneous observation, insert the U-AN-2 analyzer into the analyzer insertion slot above the dichroic mirror on the illuminator.

CAUTION **If the analyzer (U-ANT) is mounted in the transmitted DIC slider, the fluorescence observation image may become dark and/or the analyzer may be burnt.**

1. Engage the dummy mirror unit in the light path.
2. Adjust the polarizer on the universal condenser to the “crossed Nicol” status.
3. Insert the transmitted DIC slider into the position provided on the nosepiece.
4. Rotate the turret on the universal condenser to select the Nomarski prism matching the objective to be used for observation.
5. Engage the objective to be used in the light path.
6. Place the specimen on the stage and focus on the specimen.
7. Adjust the field iris diaphragm of the transmitted light illumination unit (built into the microscope frame) and the aperture iris diaphragm of the universal condenser.
8. Turn the prism movement knob on the transmitted DIC slider to adjust contrast of the DIC image.
9. Engage the mirror unit corresponding to the desired excitation in the light path and open the shutter.
10. Adjust the transmitted light intensity for optimum fluorescence and DIC image brightness.

◎For details on the transmitted DIC observation, refer to the instruction manual for the universal condenser (U-UCD8 or BX3-UCD8A).

◎if you are frequency switching between reflected fluorescence observation and transmitted DIC observation but you do not need to use both simultaneously, then it will be more convenient for you to use the DIC mirror unit (U-FDICT) instead of an analyzer (U-AN-2). This facilitates the switching operation because the analyzer simultaneously enters the light path when the fluorescence mirror unit is switched to the DIC mirror unit.

5 TROUBLESHOOTING GUIDE

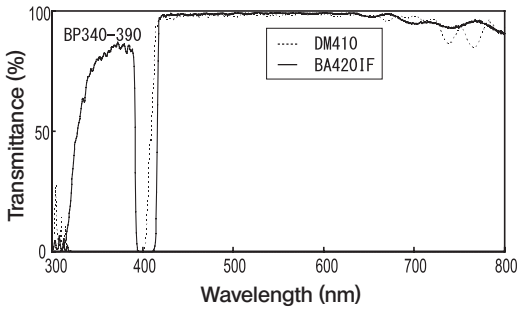
Under certain conditions, performance of the system may be adversely affected by factors other than defects. If problems occur, please review the following list and take remedial action as needed. If you cannot solve the problem after checking the entire list, please contact us for assistance.

Problem	Cause	Remedy	Page
1. Optical system			
a) Burner is ON but nothing can be seen from eyepieces or light is dark.	Shutter is closed.	Open the shutter.	7
	ND filters are engaged in light path.	Remove ND filters as required.	13
	Fluorescence mirror unit is improperly engaged in light path.	Engage it correctly.	12
	Aperture and field iris diaphragms are not fully enlarged.	Enlarge aperture iris diaphragm and field iris diaphragm sufficiently.	11,12
	Fluorescence mirror unit does not match specimen.	Use fluorescence mirror unit matching specimen.	10
	The burner is decentered.	Center the mercury burner.	12
b) Image is low quality, not sharp or poor in contrast.	Dirt/dust on objective or filter.	Clean thoroughly.	4
	Fluorescence mirror unit does not match specimen.	Use fluorescence mirror unit matching specimen.	10
c) Field of view is obscured or not evenly illuminated	Objective is not correctly engaged in light path.	Make sure that revolving nosepiece clicks properly into place.	–
	Fluorescence mirror unit is not correctly engaged in light path.	Engage fluorescence mirror unit correctly in light path.	–
	Field iris diaphragm is set too small.	Enlarge field iris diaphragm sufficiently.	11
	ND slider is not stopped at click position.	Make sure that ND slider clicks properly into place.	13
	Burner is deviated from optical axis.	Center mercury burner.	12
2. Electrical system			
a) Main switch cannot turn system ON.	Power cord is not connected properly.	Connect firmly.	–
b) Setting main switch to " I " (ON) cannot ignite mercury burner.	Connectors are not connected properly.	Connect firmly.	–
	Mercury burner is not attached.	Attach mercury burner.	23
	Safety device in lamp housing is active.	Set up the lamp socket correctly.	23
	Auto ignition is malfunctioning.	Set main switch of power supply unit to " O " (OFF) then " I " (ON) again. (OFF/ON can be repeated.)	11
c) Mercury burner flickers or is dark.	It is soon after ignition.	Wait for 10 minutes or more after ignition.	11
	Burner life has expired.	If hour counter indicates 300 hours (USH-103OL), replace mercury burner.	23
	Burner is deviated from optical axis.	Center mercury burner.	12
d) Motorized mechanisms will not move.	Connectors are not connected properly.	Set the main switch of the control box (BX3-CBH/U-CBM) to " O " (OFF) and reconnect the connectors firmly.	–
e) Shutter is opened from the touch panel controller but the light is absent or obscured.	Shutter is moved by external vibrations.	Retry opening the shutter.	–
f) Turret will not rotate (or stops in the middle of movement).	Fluorescence mirror unit is half dropped out of the turret.	Insert the fluorescence mirror unit correctly in position.	22

6 SPECTRAL CHARACTERISTICS OF FILTERS

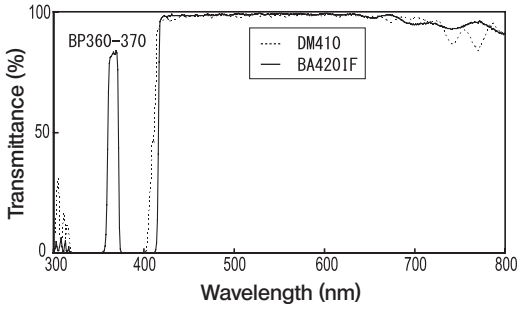
1. U-excitation (Wide band)

U-FUW



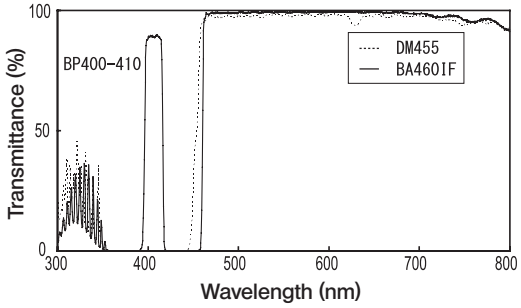
2. U-excitation (Narrow band)

U-FUN



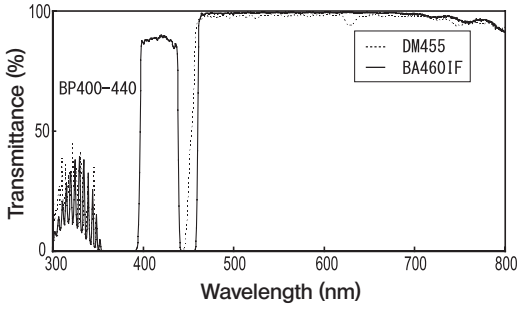
3. V-excitation (Narrow band)

U-FVN



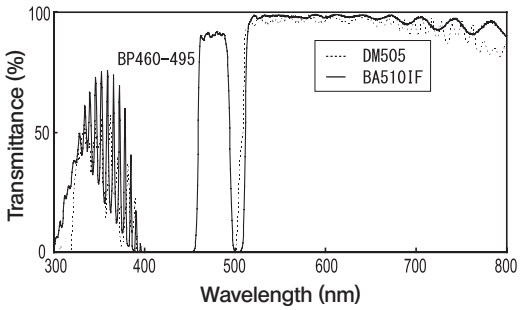
4. BV-excitation (Wide band)

U-FBVW



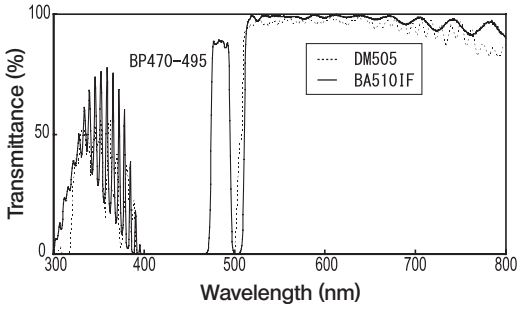
5. B-excitation (Wide band)

U-FBW



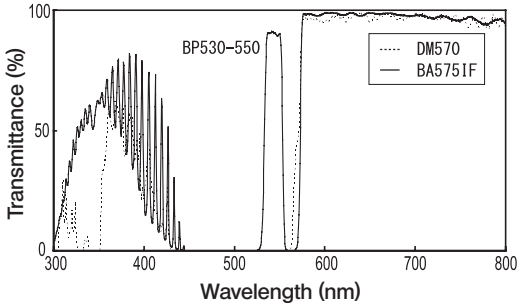
6. B-excitation (Narrow band)

U-FBN



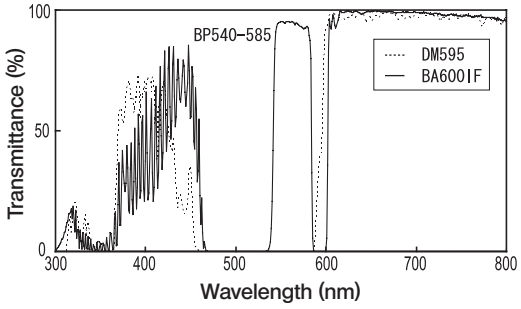
7. G-excitation (Wide band)

U-FGW



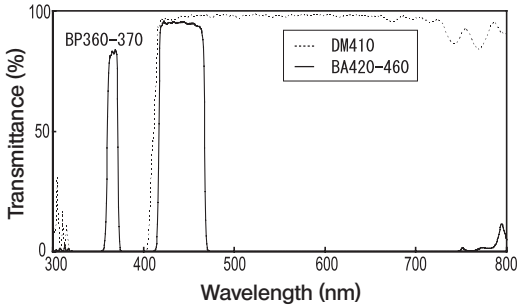
8. Y-excitation (Wide band)

U-FYW



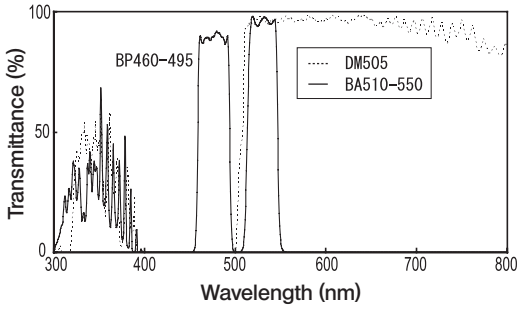
9. U-excitation, color separation (Narrow band)

U-FUNA



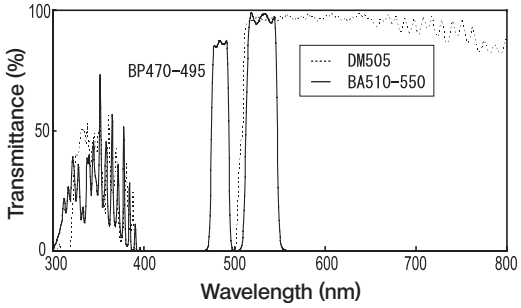
10. IB excitation, color separation (Wide band)

U-FBWA



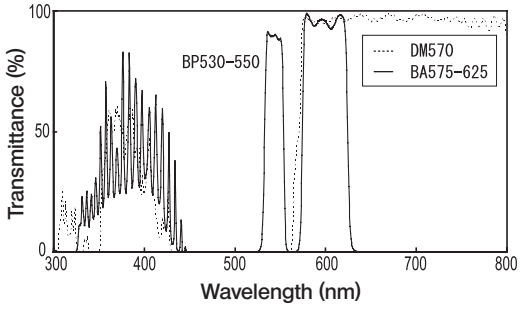
11. IB excitation, color separation (Narrow band)

U-FBNA



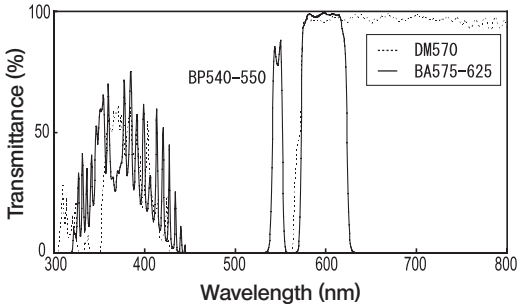
12. G-excitation, color separation (Wide band)

U-FGWA



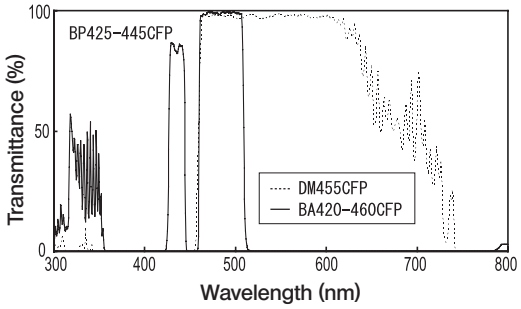
13. G-excitation, color separation (Narrow band)

U-FGNA



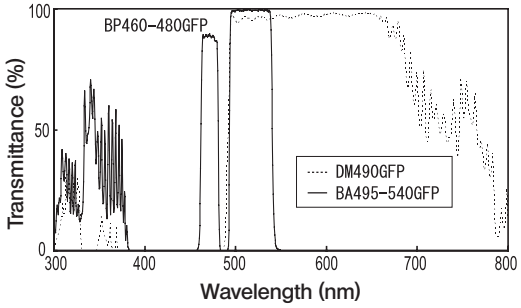
14. For cyan fluorescent protein (CFP)

U-FCFP



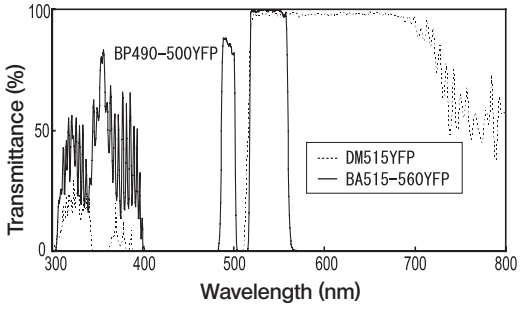
15. For green fluorescent protein (GFP)

U-FGFP



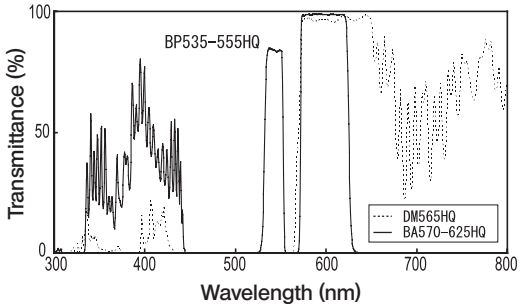
16. For yellow fluorescent protein (YFP)

U-FYFP



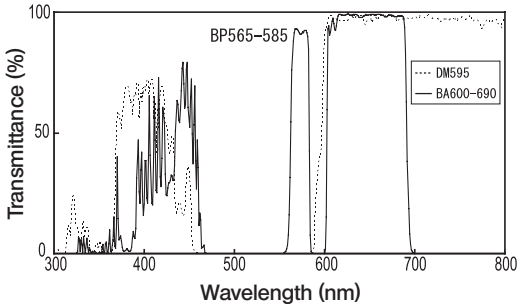
17. For red fluorescent protein (RFP)

U-FRFP

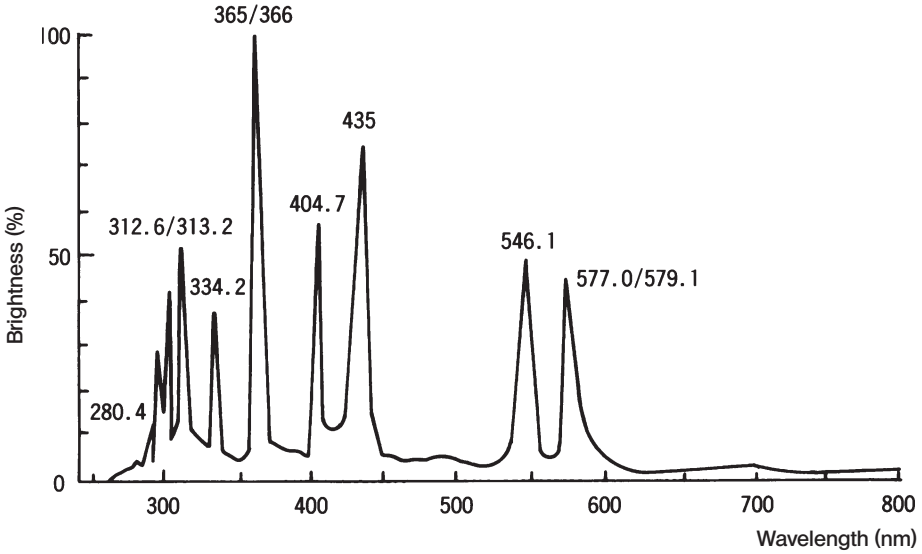


18. For mCherry

U-FMCHE



Typical Example of Emission Spectrum of Ultrahigh-Vacuum Mercury Burner Spectrum



For fluorochrome emission, a light beam having a specific wavelength is selected from a wide spectrum of wavelengths. The five major peaks of luminance are at wavelengths of 365/366, 404.7, 435, 546.1 and 577.0/579.1 nm. In addition, light beams having wavelengths of 334.2 and 490 nm (at rather low luminance) are also applicable to fluorochrome emission.

7 SPECIFICATIONS

Item	Specification
Motorized Fluorescence Illuminator BX3-RFAA	• UIS2 optical system • Illuminator magnification: 1X (FN 22) • Observation switching: Motorized mirror unit turret (switching time approx. 0.5 sec.). Number of mirror unit positions: 8. • Aperture iris diaphragm and field iris diaphragm • Shutter provided (motorized engagement/ disengagement, switching time 0.2 sec.)
	• Slider insertion slots ① Analyzer (U-AN-2) ② ND filter slider
	• Available observation modes ① Reflected fluorescence ② Transmitted DIC ③ Transmitted phase contrast ④ Transmitted polarized light ⑤ Transmitted light brightfield ⑥ Transmitted light darkfield • Applicable microscopes BX63, BX53, BX43
Lamp housing for mercury burner	• Lamp housing for 100 W mercury burner U-LH100HG • Apo lamp housing for 100 W mercury burner U-LH100HGAPO • Mercury burner*: USH-103OL • Power supply unit U-RFL-T
Lamp housing for xenon burner	• Lamp housing for 75 W xenon burner U-LH75XEAPO • Xenon burner: UXL-75XB • Power supply unit U-RX-T
Operating environment	• Indoor use. • Altitude: Max. 2000 meters • Ambient temperature: 5° to 40°C (41° to 104° F) • Maximum relative humidity: 80% for temperatures up to 31°C (88°F), decreasing linearly through 70% at 34°C (93°F), 60% at 37°C (99°F), to 50% relative humidity at 40°C (104°F). • Supply voltage fluctuations: Not to exceed ±10% of the normal voltage. • Pollution degree: 2 (in accordance with IEC60664-1) • Installation/Overvoltage category: II (in accordance with IEC60664-1)
Storage environment	• Temperature: Min. -25°C, Max. 65°C • Humidity: Min. 0%, Max. 90%

* The USH-102D (mfd. by USHIO) or HBO103W/2 (mfd. by OSRAM) are also usable, but the performance is not guaranteed.

8 ASSEMBLY

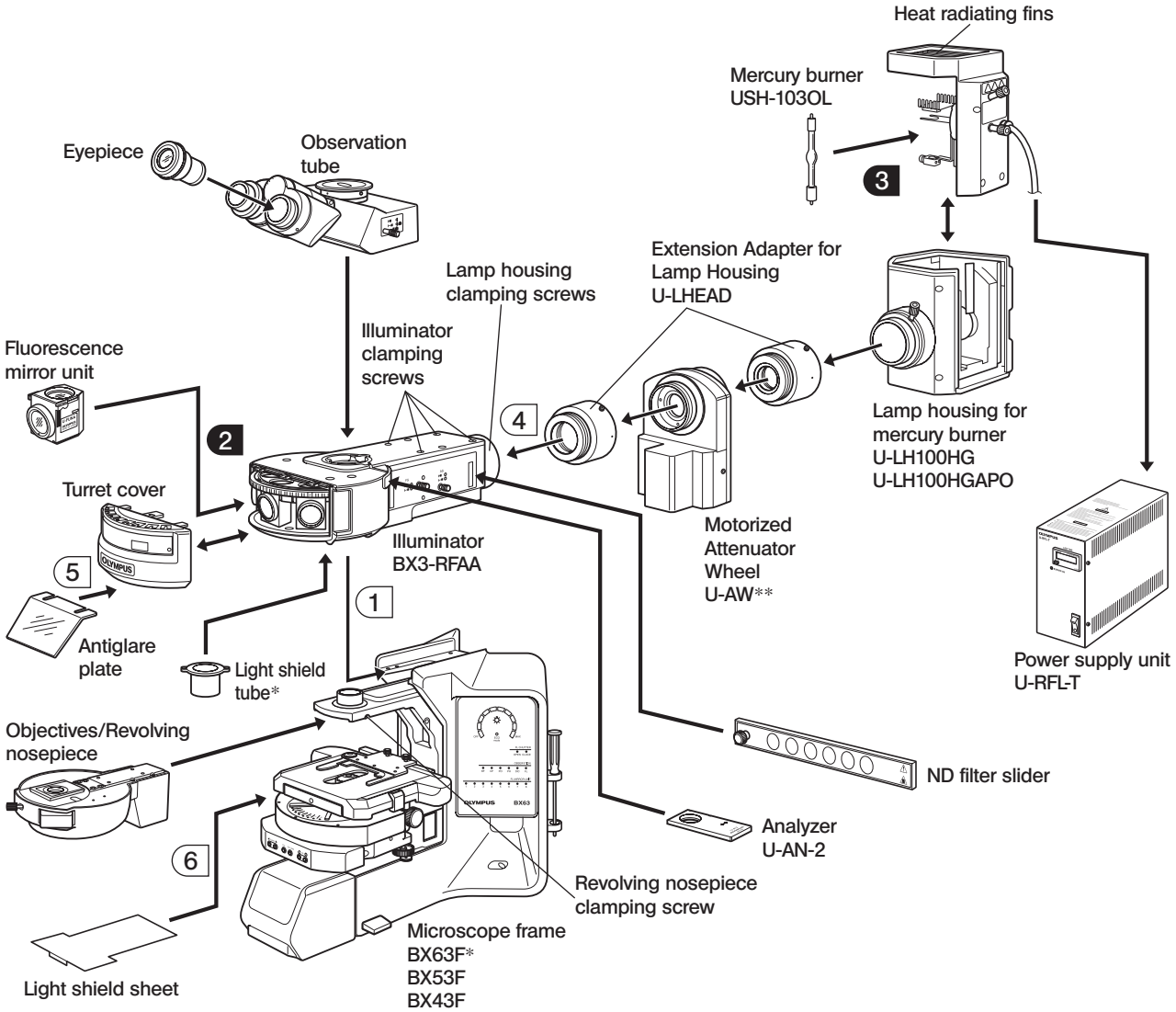
8-1 Assembly Diagram

The diagram below shows the sequence of assembly of the various modules. The numbers indicate the order of assembly.
The module numbers shown in the following diagram are merely the typical examples. For the modules with which the module numbers are not given, please consult us or the catalogues.

CAUTION **When assembling the microscope, make sure that all parts are free of dust and dirt, and avoid scratching any parts or touching glass surfaces.**

The assembly steps of the fluorescence mirror units will be detailed on the subsequent pages.
◎All assembly operations are possible by using the Allen screwdriver ( ) provided with the microscope. However, the reflected fluorescence illuminator (including the light shield tube) should be installed by us in order to retain the performance.

CAUTION
- **Be sure to insert the sliders in the orientations shown in the diagram. Otherwise, they cannot be fitted in click positions and engaged correctly in the light path.**
- **To prevent fire, install the lamp housing with the heat radiating fins positioned on the top and with sufficient space around the housing.**



* If your system is based on the BX63F, remove the light leak prevention ring from the illuminator before installing the light shield tube. The light shield tube is not required if your system uses the BX53 or BX43.

** When the U-AW motorized attenuator wheel is installed, the brightness may drop unless the U-LHEAD extension adapter for lamp housing is used.

8-2 Detailed Assembly Procedures

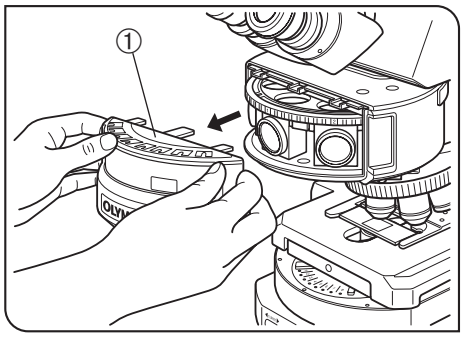


Fig. 9

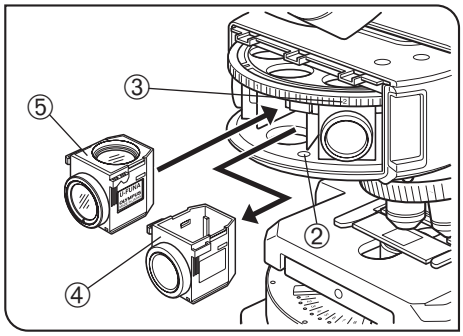


Fig. 10

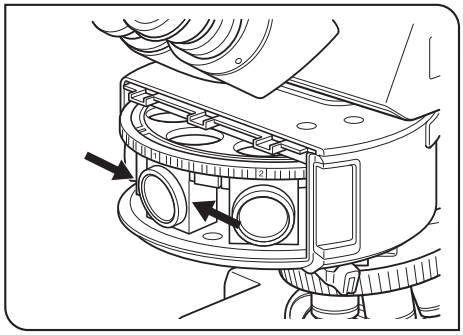


Fig. 11

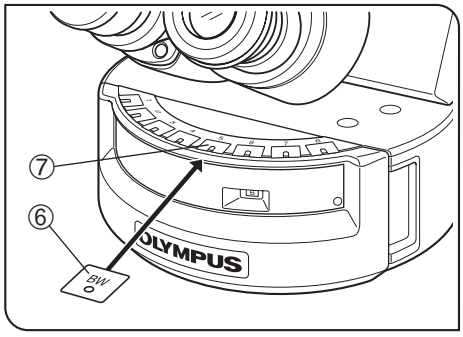


Fig. 12

2 Mounting the Fluorescence Mirror Units (Figs. 9 to 12)

CAUTION **Before mounting the fluorescence mirror units, be sure to engage the shutter in the light path for safety. Be also sure to always use the motorized control to rotate the turret.**

1. Engage the shutter in the light path.
2. Hold the left and right sides of the cover ① on the front of the illuminator and remove the cover by pulling it toward you.
3. Rotate the turret ③ with the motorized control so that the turret position ② you want to mount the fluorescence mirror unit comes on the front.

CAUTION **Be careful not to have your finger caught by the mechanism.**

4. Hold the dummy mirror unit ④ by its sides, pull it out and push in the fluorescence mirror unit ⑤ into the position until it clicks.
   Push the mounted fluorescence mirror unit with a light force alternately from the left and right sides so that it is not inclined.
5. Insert the indicator sheet ⑥ for the fluorescence mirror unit mounted in each turret position ② under the film of the corresponding mirror unit name inscription plate pocket ⑦.
6. Mount other necessary fluorescence mirror units in the same way as above.

CAUTION **Do not remove the dummy mirror units from the turret positions you do not want to mount fluorescence mirror units (in order to prevent like leakage).**

7. Attach the cover ① in the original position.

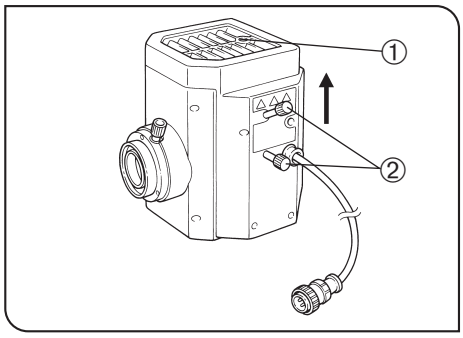


Fig. 13

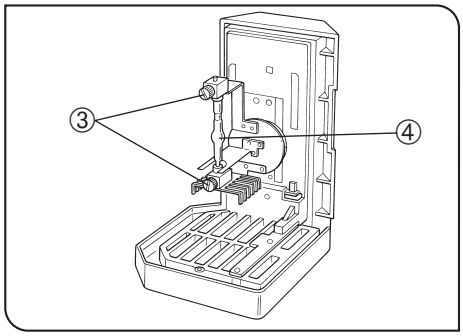


Fig. 14

3 Attaching the Mercury Burner and Lamp Housing (Figs. 13 & 14)

◎If your system uses the xenon burner, attach the burner, set the hour counter and connect the system by referring to the instruction manuals for the lamp housing and power supply unit.

1. Loosen the socket clamping screw ① using the Allen screwdriver.
2. Hold the upper section of lamp housing and pull it upward to remove the socket section.

CAUTION **To prevent malfunctions, do not hold the lamp housing by the centering knobs ②.**

3. Place the socket section upside down as shown in Fig. 14.

◎The lamp housing is equipped with the holder for transportation in the factory shipment condition, or with an old burner when the burner is replaced. Remove the holder or old burner by loosening the two burner holding screws ③.

4. Attach the + (positive) pole of the mercury burner ④ to the fixed mount on the upper side, then the - (negative) pole to the mount on the lower side.

CAUTION
- **Be sure to use the USH-103OL (mfd, by us) burner.**
- **Be careful and avoid leaving fingerprints or contaminants on the mercury burner. Otherwise, there is a danger of explosion due to distortion of glass caused by the stains. If the burner is contaminated, clean it by wiping gently with gauze slightly moistened with absolute alcohol.**

5. Attach the socket section with burner to the original position and tighten the clamping screw ①.

CAUTION
- **Align the external edges of the lamp housing with those on the socket section, and push the lamp housing straight downward.**
- **Attach the lamp housing on the lamp housing installation position of the microscope system so that the heat radiating fins face upward. To prevent a fire hazard, reserve ample spaces above, below and on the rear of the lamp housing.**
- **Do not light the mercury burner while it is not mounted on the microscope because the UV rays in its light are harmful to your eyes.**
- **The UV rays in the light of the mercury burner may damage the specimen if this is sensitive to UV rays.**

Burner Service Life

USH-103OL: 300 hours

◎ This value assumes light cycles composed of 2 hours of lighting and 30 minutes of extinction. Do not turn it on and off at a shorter cycle than the above, for this will shorten the service life of the burner.

CAUTION **After replacing the burner, reset the hour counter of the power supply unit to "0.0."**

6. Connect the connector of the lamp housing to the U-RFL-T power supply unit.
7. Connect the power cord to the power supply unit and its power plug to the power outlet.

Making an Optional Fluorescence Mirror Unit

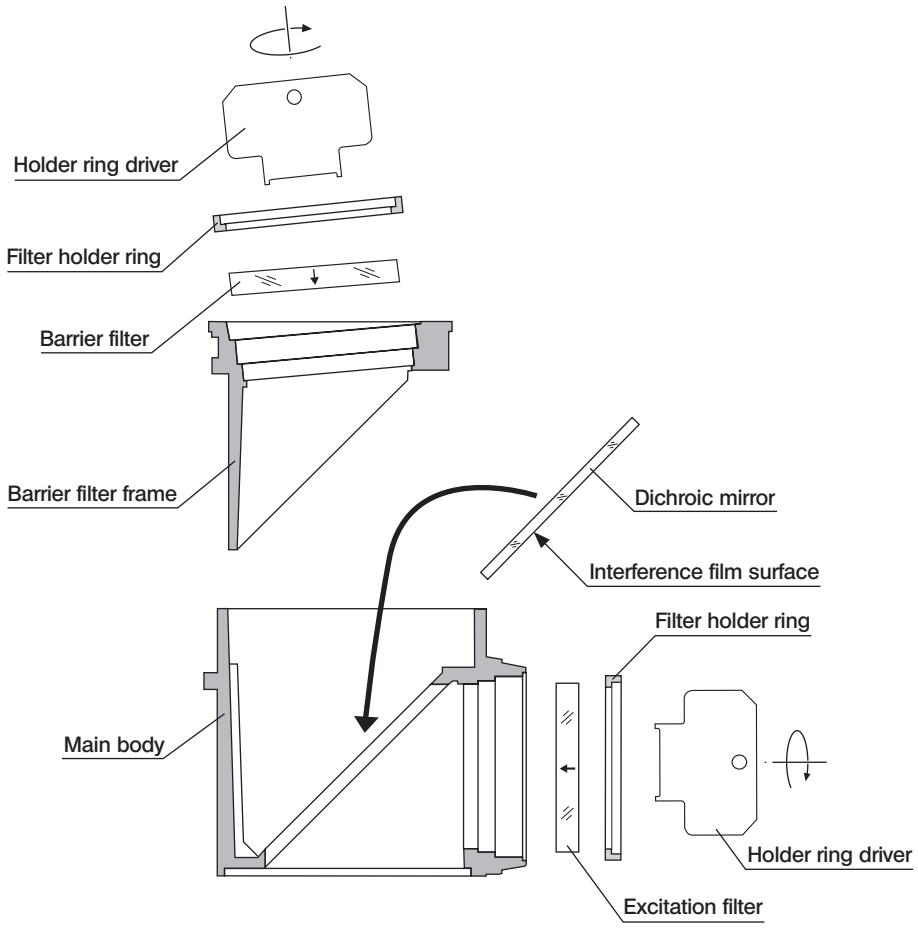
◎You can also fabricate original fluorescence mirror units by fitting a commercially available barrier filter, excitation filter or dichroic mirror in the U-FF empty mirror unit frame.

Dimensions of Optical Parts

- Barrier filter
- Excitation filter

} Diameter ϕ25 -0.1/-0.2 mm, max. thickness 6 mm.

- Dichroic mirror — 26 -0.1/-0.3 x 38 -0.1/-0.3 mm, thickness 1 ±0.05 mm



CAUTION **When disassembling and assembling the fluorescence mirror unit, place it so that the barrier filter frame comes on the top (as shown above) and handle it by taking care not to stain it with fingerprints, etc.**
If the fluorescence mirror unit is disassembled with the filter frame downward, Dichroic mirror may fall in some cases.

1. Disassemble the fluorescence mirror unit by lifting up the absorption filter frame.
2. Fix the absorption filer in the absorption filter frame and the excitation filter in the main body respectively. Place each filter by aligning the directions of the arrows located on the side surfaces toward the absorption filter frame and the main body respectively.
   Fit the holder ring driver into the groove of the holder ring driver, and fix it by rotating the filter holder ring.
   ◎If the filter holder rings may exceed the absorption filter frame or the main body depending on the filter thickness, re-attach the filter by placing the filter holder ring upside down.
3. Place the main body on the table as shown in the figure, and place the dichroic mirror on it gently.
4. Move the absorption filter frame gently back to the original position.

9 LAMP HOUSING INSPECTION SHEET

- Study the instruction manual for the lamp housing before inspection.
- For safe use of the lamp housing, we recommend performing the following inspection periodically (every time you replace the mercury burner and at least every 6 months).
- The table below identifies the check items to be observed. Put (—) if not applicable or (✓) if applicable.
- If there is any (✓) mark noted, immediately stop use of the product, and contact us for detailed inspections or replace the lamp housing.
- If you detect an abnormality other than that listed below or with other our product, also stop the use of the product and contact us for detailed inspections.
- Note that the service, replacement and detailed inspections are charged after expiration of the warranty period.

If you have any questions, please contact us.

	Check results (Date)			
Check items	/	/	/	/
1. More than 8 years have passed since original purchase or the total power ON time has exceeded 20,000 hours.				
2. Illumination flickers when you move the lamp cable or lamp housing.				
3. Lamp cable is unusually hot to the touch.				
4. Scorching or burning odor is produced during use.				
5. Deformation, backlash, or looseness, etc. when you assemble the lamp housing. (Impossibility of removing the top section of lamp housing when you attempt to replace the lamp bulb, etc.)				
6. Discoloration, deformation or cracking of the lamp housing.				
7. Melting, crack, deformation or solidification of the lamp cable or a wiring part.				
8. Increased frequency of servicing compared to similar devices put into use at the same time as the lamp housing.				

* When the Check Result columns become insufficient, copy this sheet.

PROPER SELECTION OF THE POWER SUPPLY CORD

If no power supply cord is provided, please select the proper power supply cord for the equipment by referring to " Specifications " and " Certified Cord " below:

CAUTION: In case you use a non-approved power supply cord for our products, we can no longer warrant the electrical safety of the equipment.

Specifications

Voltage Rating	125V AC (for 100-120V AC area) or, 250V AC (for 220-240V AC area)
Current Rating	6A minimum
Temperature Rating	60˚C minimum
Length	3.05 m maximum
Fittings Configuration	Grounding type attachment plug cap. Opposite terminates in molded-on IEC configuration appliance coupling.

Table 1 Certified Cord

A power supply cord should be certified by one of the agencies listed in Table 1 , or comprised of cordage marked with an agency marking per Table 1 or marked per Table 2. The fittings are to be marked with at least one of agencies listed in Table 1. In case you are unable to buy locally in your country the power supply cord which is approved by one of the agencies mentioned in Table 1, please use replacements approved by any other equivalent and authorized agencies in your country.

Country	Agency	Certification Mark	Country	Agency	Certification Mark
Argentina	IRAM		Italy	IMQ	
Australia	SAA		Japan	JET, JQA, TÜV, UL-APEX/MITI	,
Austria	ÖVE		Netherlands	KEMA	
Belgium	CEBEC		Norway	NEMKO	
Canada	CSA		Spain	AEE	
Denmark	DEMKO		Sweden	SEMKO	
Finland	FEI		Switzerland	SEV	
France	UTE		United Kingdom	ASTA BSI	,
Germany	VDE		U.S.A.	UL	
Ireland	NSAI				

Table 2 HAR Flexible Cord

APPROVAL ORGANIZATIONS AND CORDAGE HARMONIZATION MARKING METHODS

Approval Organization	Printed or Embossed Harmonization Marking (May be located on jacket or insulation of internal wiring)		Alternative Marking Utilizing Black-Red-Yellow Thread (Length of color section in mm)		
			Black	Red	Yellow
Comite Electrotechnique Belge (CEBEC)	CEBEC	‹HAR›	10	30	10
Verband Deutscher Elektrotechniker (VDE) e.V. Prüstelle	‹VDE›	‹HAR›	30	10	10
Union Technique de l´Electricite´ (UTE)	USE	‹HAR›	30	10	30
Instituto Italiano del Marchio di Qualita´ (IMQ)	IEMMEQU	‹HAR›	10	30	50
British Approvals Service for Electric Cables (BASEC)	BASEC	‹HAR›	10	10	30
N.V. KEMA	KEMA-KEUR	‹HAR›	10	30	30
SEMKO AB Svenska Elektriska Materielkontrollanstalter	SEMKO	‹HAR›	10	10	50
Österreichischer Verband für Elektrotechnik (ÖVE)	‹ÖVE›	‹HAR›	30	10	50
Danmarks Elektriske Materialkontroll (DEMKO)	‹DEMKO›	‹HAR›	30	10	30
National Standards Authority of Ireland (NSAI)	‹NSAI›	‹HAR›	30	30	50
Norges Elektriske Materiellkontroll (NEMKO)	NEMKO	‹HAR›	10	10	70
Asociacion Electrotecnica Y Electronica Espanola (AEE)	‹UNED›	‹HAR›	30	10	70
Hellenic Organization for Standardization (ELOT)	ELOT	‹HAR›	30	30	70
Instituto Portages da Qualidade (IPQ)	np	‹HAR›	10	10	90
Schweizerischer Elektro Technischer Verein (SEV)	SEV	‹HAR›	10	30	90
Elektriska Inspektoratet	SETI	‹HAR›	10	30	90

Underwriters Laboratories Inc. (UL)	SV, SVT, SJ or SJT, 3 X 18AWG
Canadian Standards Association (CSA)	SV, SVT, SJ or SJT, 3 X 18AWG

This product is manufactured by **EVIDENT CORPORATION** effective as of Apr. 1, 2022.
Please contact our "Service Center" through the following website for any inquiries or issues related to this product.

EVIDENT CORPORATION

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(Life science solutions)

Service Center

https://www.olympus-lifescience.com/support/service/

(Life science solutions)

Our Website

https://www.olympus-lifescience.com

(Industrial solutions)

Service Center

https://www.olympus-ims.com/service-and-support/service-centers/

(Industrial solutions)

Our Website

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