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OLYMPUS®

INSTRUCTIONS

# BX-FLA

## REFLECTED LIGHT FLUORESCENCE ATTACHMENT

This instruction manual is for use of the Olympus System Microscope Model BX-FLA. We recommend you read this manual carefully in order to familiarize yourself fully with the use of your microscope so that you can obtain optimum performance.

# IMPORTANT

This unit employs a UIS optical system, and should be used only with UIS eyepieces, objectives, and condensers. Less than optimum performance may result if inappropriate accessories and lenses are used.

The reflected light fluorescence vertical illuminator features interchangeable filter cubes to employ excitation light of different wavelengths. It also allows combined or alternating reflected light fluorescence and transmitted white light observations.

1. Reflected light fluorescence + transmitted light phase contrast.
2. Reflected light fluorescence + transmitted light Nomarski differential inteference contrast.
3. Reflected light fluorescence + transmitted light brightfield or darkfield.

## 1 Getting Ready

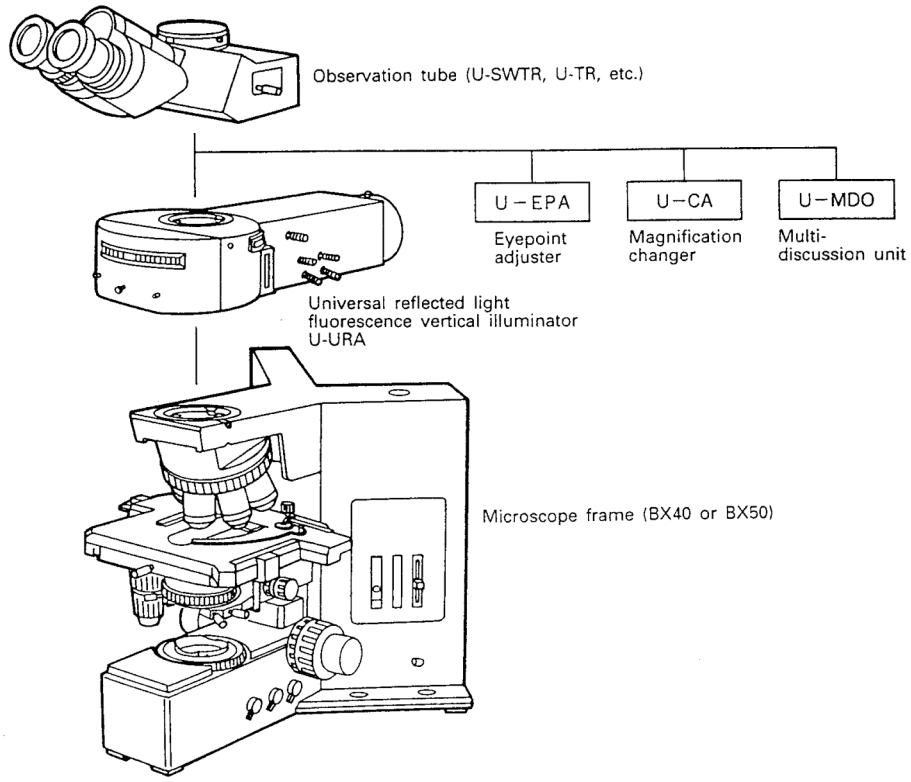
1. The vertical illuminator is delicate. Handle it carefully, and avoid jolts.
2. The high pressure mercury burner used in the unit should be a USH102 (mfd. by Ushio Electric).
3. Verify that the burner is installed correctly and that all cords are properly connected.
4. The ultraviolet rays emitted by the burner are harmful. Be sure to use a UV protective shield with the unit. (See page 9)
5. Do not touch the lamp housing while the burner is in operation, or for at least 10 minutes after turning it off.
6. The power supply unit contains high voltage components. Do not attempt to disassemble it.
7. For safety's sake, be sure to ground the unit.
8. Unplug the mains power cord before replacing the burner.
9. Before plugging in the mains power cord, make sure that the power switch on the power supply unit is turned off.

## 2 Care and Storage

1. Be very careful to avoid getting dirt or fingerprints on the lenses, filters, or the high pressure mercury burner. In case of contamination, clean by wiping lightly with a piece of gauze. To remove fingerprints or other oils, moisten the cloth slightly with a 3 : 7 mixture of alcohol and ether, or with benzine.
   ★ Since ether and alcohol are highly flammable, be careful to keep them away from an open flame or possible sources of electrical sparks, such as power switches.
2. Do not unnecessarily disassemble the unit.
3. For safety's sake, replace the burner when the hour counter on the power supply unit indicates 200 hours. Before replacing the burner, turn off the power switch and wait at least 10 minutes for the burner to cool. (See page 8.)
4. When not using the unit, cover it with the dust cover provided and store it in a dry place to prevent mold formation.
5. If do not use a dichroic mirror cube for a while, place it in its container and store it in a safe place.

## 3 Intermediate Tubes Usable with the Vertical Illuminator

One additional intermediate tube can be used on the BX40 or BX50 together with vertical illuminator. Select an intermediate tube to install on top of the vertical illuminator by referring to the illustration below.

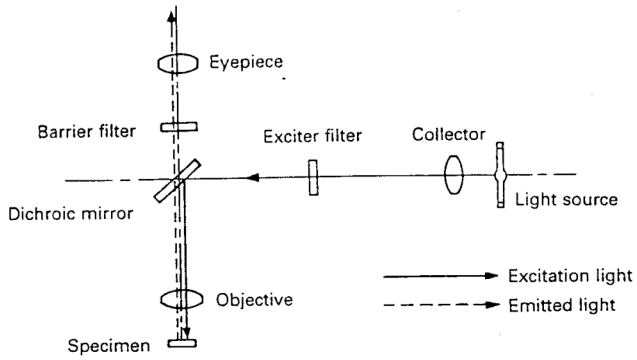


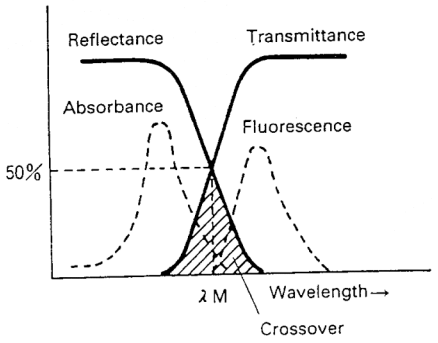
# CONTENTS

# 1 PRINCIPLE



The design of reflected light fluorescence microscopes features dichroic mirrors which direct the excitation light through the objective, to the area of the specimen, thus providing efficient illumination. (Fig. 1)




The spectral characteristics of the dichroic mirror when it is positioned at an inclination of 45° to the optical axis of incident light is shown in Fig. 2. Because a cross-over exists between transmittance and reflectance, it is necessary to use an appropirate comibination of exciter and barrier filters in conjunction with the dichroic mirror. This is necessary to achieve a good contrast image through fluoreochrome excitation in the specimen, at the desired wavelength.

When the dichroic mirror is inclined 45° the optical axis of incident light, it reflects the excitation light towards the objective, and passes unwanted wavelengths.

When the specimen is irradiated by the excitation wavelength, it emits a visible longer wavelength corresponding to Stoke's law. The barrier filter mounted between the objective and eyepiece blocks out unwanted wavelengths providing a black background.

# 2 NOMENCLATURE

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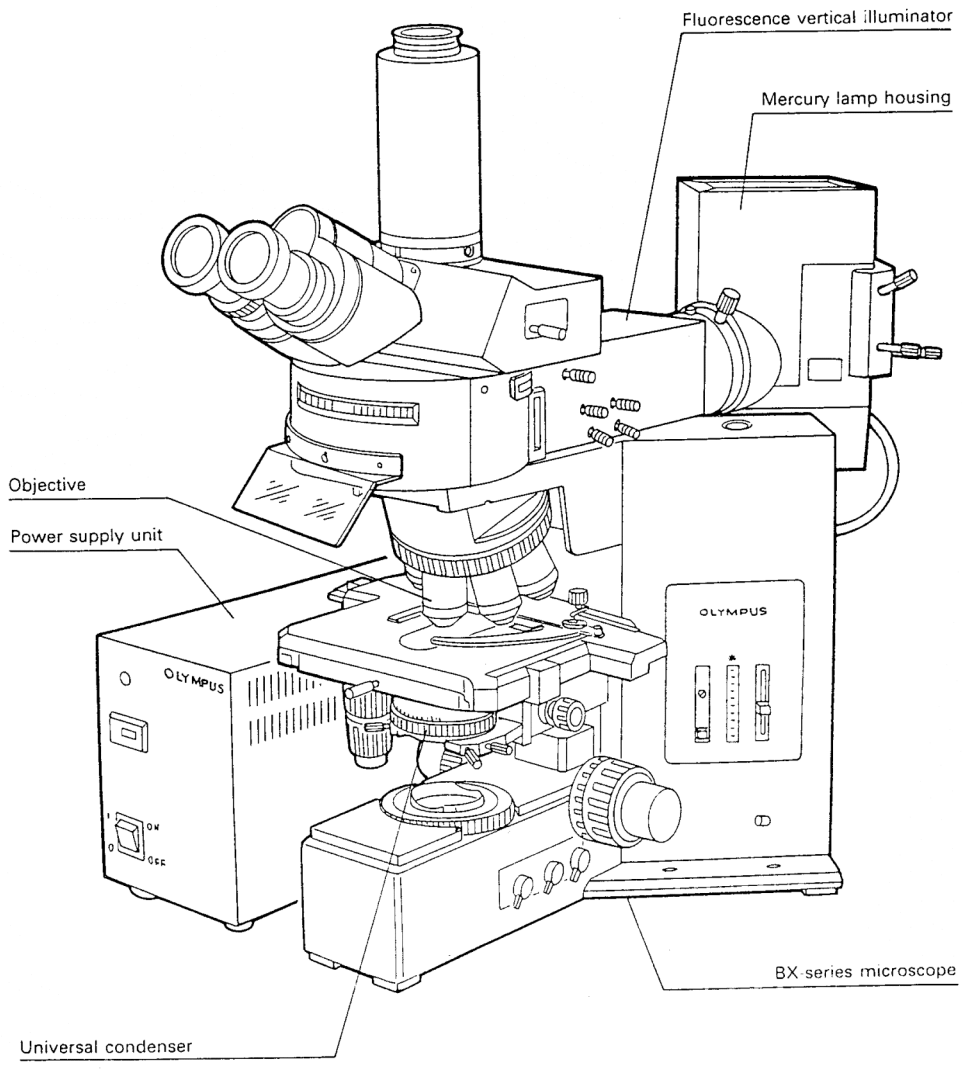
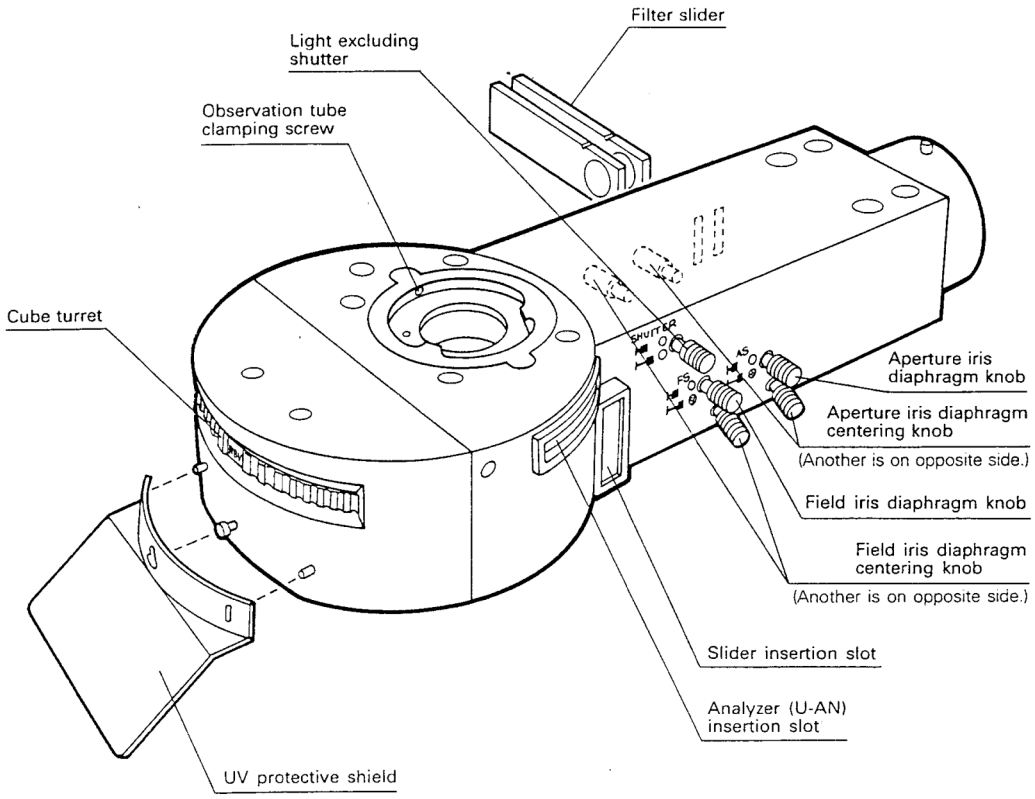
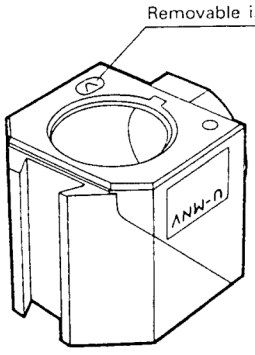


## A. Fluorescence Vertical Illuminator




## B. Cube




★ Combine with dichroic mirrors, barrier filters and exciter filters as appropriate for the desired excitation method.
Do not disassemble the cube.

## C. Fluorescent Light Source

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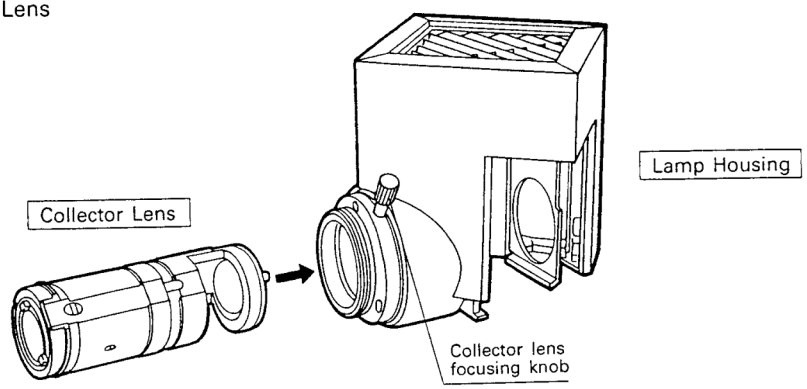
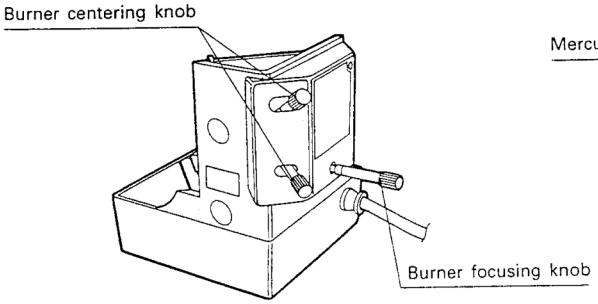
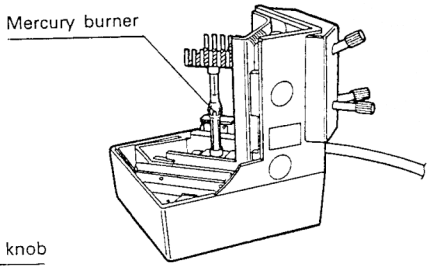
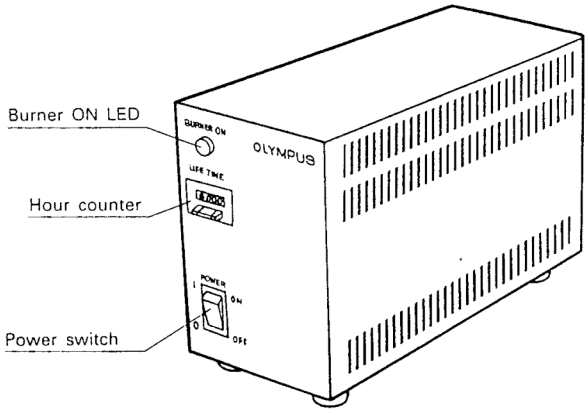
● Collector Lens

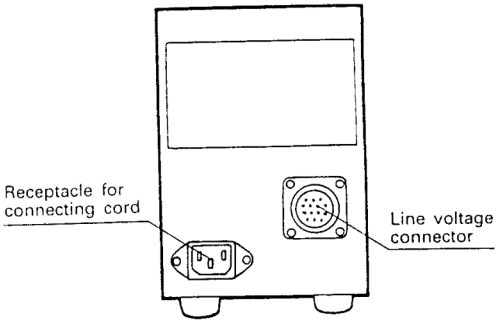



● Power Supply Unit



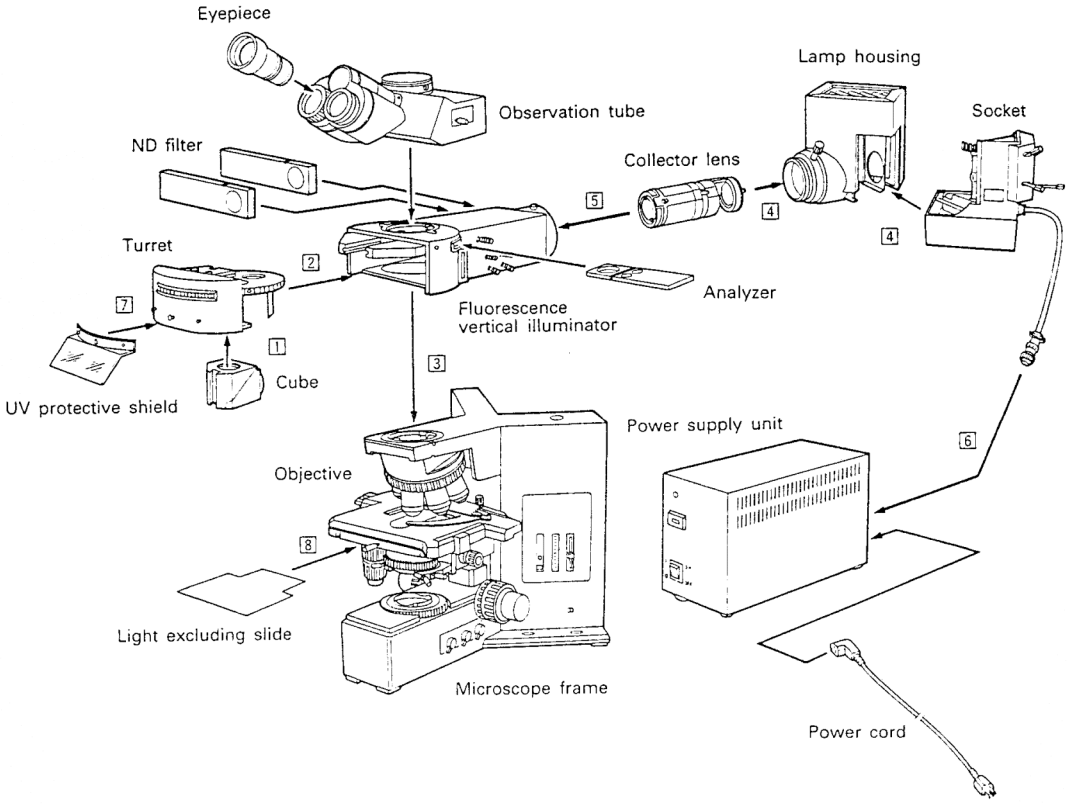
# 3 ASSEMBLY

## 3-1 Assembly Diagram

⊜ Consult the instruction manual provided with the microscope to assemble the BX40 or BX50.

★ When assembling the unit, make sure that all parts are free of dust and dirt, and assemble the parts in the sequence indicated by the numbers in the illustration below.






★ See paragraph 8 on page 17 regarding objectives to use for different observation methods.

## 3-2 Assembly Procedure

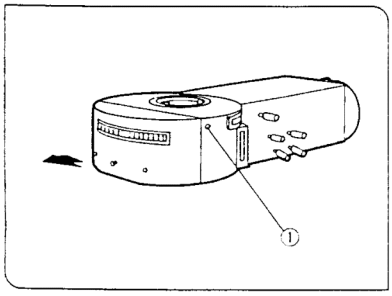



Fig. 1

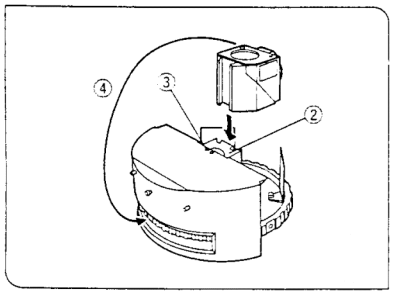



Fig. 2

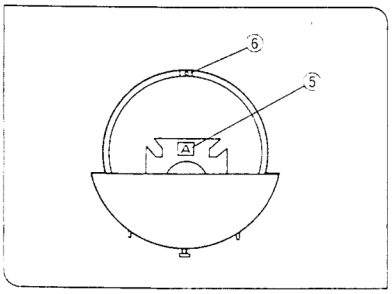



Fig. 3

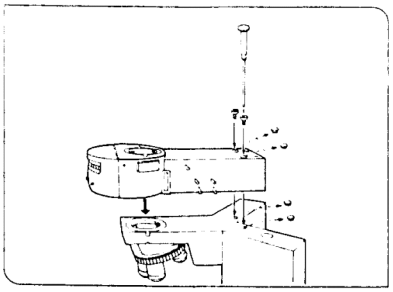
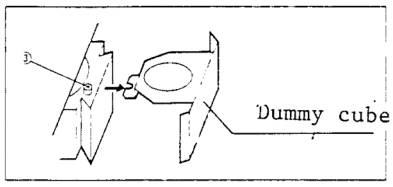


Fig. 4

### 1 Mounting the Cubes (Figs. 1, 2, 3)

☞ See paragraph 9 on page 18 regarding which cubes to use for different observation methods.

1. Loosen the clamping screw ① at the right of the vertical illuminator using the Allen (hex) wrench provided with the microscope frame. (Fig. 1)
2. Pull out the turret in the direction indicated by the arrow, then turn the turret so that the rotating cube dovetail mount ② points upward and loosen the cube clamping screw ③.
   (Dummy cubes are mounted in three of the four cube positions. When you wish to use only one cube, mout it in the remaining empty position. When using two or more cubes, loosen the clamping screw ③ and remove the dummy cube(s) in the direction indicated by the arrow, and then mount the cube(s) in it's place.)




3. Hold the cube to be mounted with the index side facing upward and slide it all the way onto the dovetail mount. Then tighten the cube clamping screw ③. (Tighten all four cube clamping screws.)
4. Remove the cube magnetic index sticker ④ and affix it to the turret (Fig. 2)

★ Use a sharp object such as the tip of a ballpoint pen or mechanical pencil to remove the magnetic cube index sticker.

### 2 Mounting the Turret (Fig. 1)

Insert the turret into the vertical illuminator housing and tighten the lock screw ① while pushing the turret inward as far as it will go.

★ Be sure to tighten the cube clamping screws even when no cube is mounted. If loose, the screw heads will hit the housing when the turret is rotated.

### 3 Mounting the Fluorescence Vertical Illuminator (Fig. 4)

Remove the two plugs from the top of the microscope frame and the vertical illuminator. Then use the provided Allen (hex) wrench to clamp the vertical illuminator to the microscope frame (two locations).

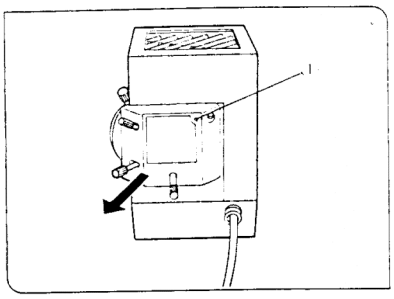



Fig. 5

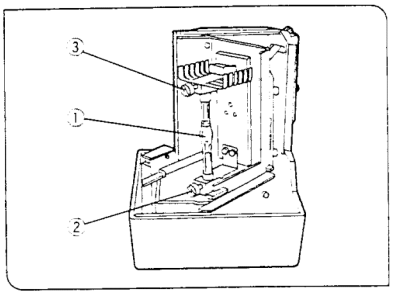



Fig. 6

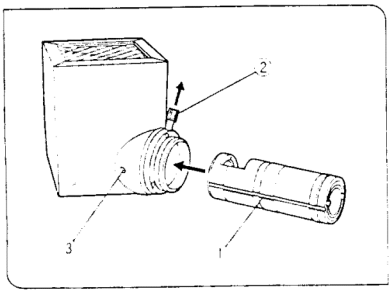



Fig. 7

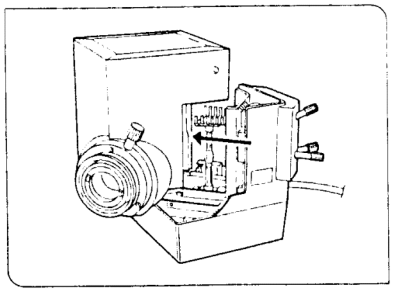


Fig. 8

## 4 Assembling the Lamp Housing for the Mercury Burner (Figs. 5, 6, 7, 8, 9, 10, 11)



### Mounting the Mercury Burner

1. Remove the burner socket clamping screw ① using the Allen (hex) wrench provided with the microscope.
2. Remove the socket from the lamp housing indicated by the arrow. (Fig. 5)
3. Loosen the burner clamping screws ② and ③ (Fig. 5) and remove the securing post. (For burner replacement, remove the used burner.)
4. Insert the + pole of the mercury burner ① into the + terminal and tighten the + clamping burner screw ②. Then insert the – pole of the buner into the – terminal, and tighten the – screw ③. (Fig. 6)
   - ★ Use only a USH102 burner (mfd. by Ushio Electric).
   - ★ Be careful to avoid getting fingerprints or dirt on the burner. To remove fingerprints or other oils, moisten the cloth slightly with a 3 : 7 mixture of alcohol and ether, or with benzine.
   - ★ At this point, mount the collector lens.
   - ★ In order to avoid possible damage to the burner, the collector lens can only be installed or removed while the socket and lamp housing are removed.

### Mounting the Collector Lens

1. Align the collector lens positioning groove ① with the pin inside the lamp housing, then the collector lens focusing knob ② to slide the collector lens into the lamp housing as far as it will go. Then tighten the collector lens retaining screw ③. (Fig. 7)
2. Reattach the socket to the lamp housing by reversing the procedure given in 4-1) above. (Fig. 8)
3. Tighten the socket clamping screw ① with the Allen (hex) wrench. (Fig. 5)
   - ★ A click is heard, when the clamping screw ① is tightened. This sound indicates that the safety interlock switch is functioning properly.
   - ★ If you accidentally loosen the clamping screw while the burner is operating, the interlock switch turns off the burner. In order to restart the burner, you must turn off the power switch on the power supply unit. Then pull out the power cord (as a safety precaution if you open the lamp housing) and wait for about 10 minutes, then retighten the clamping screw and turn the power switch back on again.

3

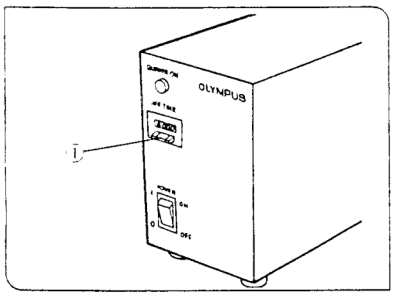



Fig. 9

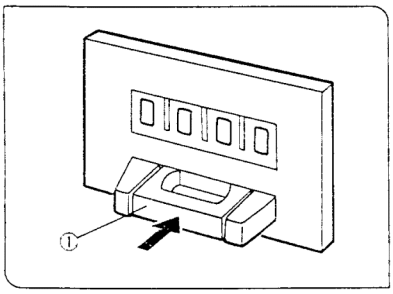



Fig. 10

### Resetting the Burner Hour Counter

1. Press the center of the reset button ① (Fig. 9) on the power supply unit's front panel to reset the burner life indicator to 000.0.

2. The indicator shows elapsed time in hours. For safety's sake, replace the burner when the indicator counts 200.0 hours.
   ★ Make sure that the indicator is properly reset to 000.0. The burner may not start if the indicator not properly reset.

### Mercury Burner Replacement

1. For safety's sake, replace the mercury burner when it has been used for 200 hours.
2. Before replacing the burner, for wait at least 10 minutes after shutting off, verify that the power switch is turned off and unplug the power cord before removing the burner. See Figs. 5, 6 for the replacement procedure
3. After replacing the burner reset the counter as shown in Fig. 10.

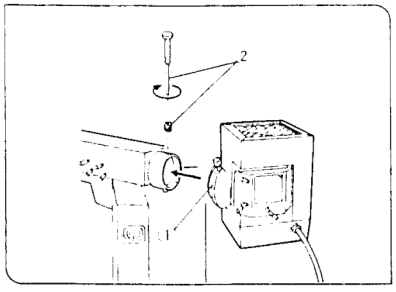



Fig. 11

## 5 Mounting the Lamp Housing (Fig. 11)

1. Insert the collector lens portion ① of the mercury lamp housing into the vertical illuminator and push inward until it clicks in place.
2. Tighten the collector lens clamping screw ② with the Allen (hex) wrench

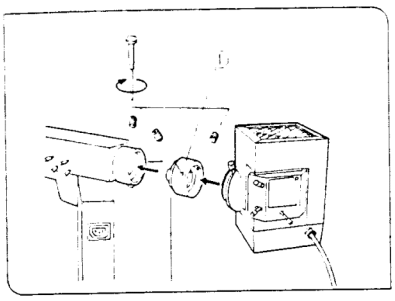


Fig. 12

### Install Conversion Lens(option)

When more illumination is required, use the optional U-UCV ① converter lens. To install the covertor lens, insert it between the vertical illuminator and the lamp housing and fasten it with the two clamping screws. (Fig. 12)

★ Light intensity in the periphery of the viewfield may be slightly reduced in superwide field observation(FN26.5).



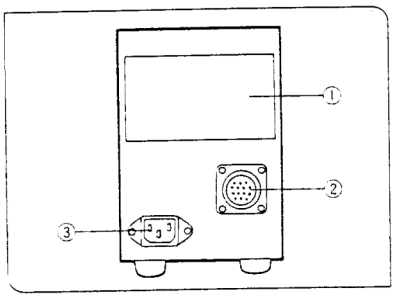



Fig. 13

## 6 Connecting the Power Supply Unit (Fig. 13)

1. Verify that the voltage and frequency of the mains outlet match the requirements indicated on the name plate a on the power supply unit. (100V systems can be used with voltages in the 100-120V range and frequencies of 50-60 Hz.
2. Plug the connecing cord into the power supply unit's secondary connector ②.
3. Connect the power cord to the input connector ③ on the power supply unit, then plug the other end of the cord into an outlet.

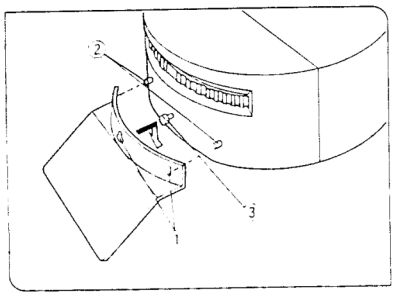



Fig. 14

## 7 Mounting the UV Protective Shield (Fig. 14)

Align the UV protective shield's key holes ① over the guide pins ② and the mounting pin ③ and lower the shield into place.

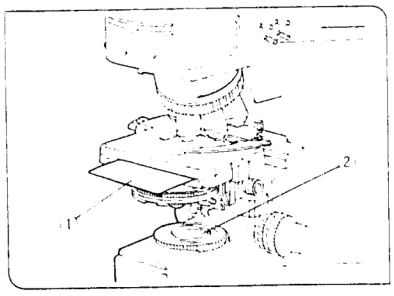



Fig. 15

## 8 Mounting the Light Excluding Slide (Fig. 15)

When performing fluorescence observation with a low-power objective, image clarity may be reduced by light reflecting condenser. If this happens, use the light excluding slide.

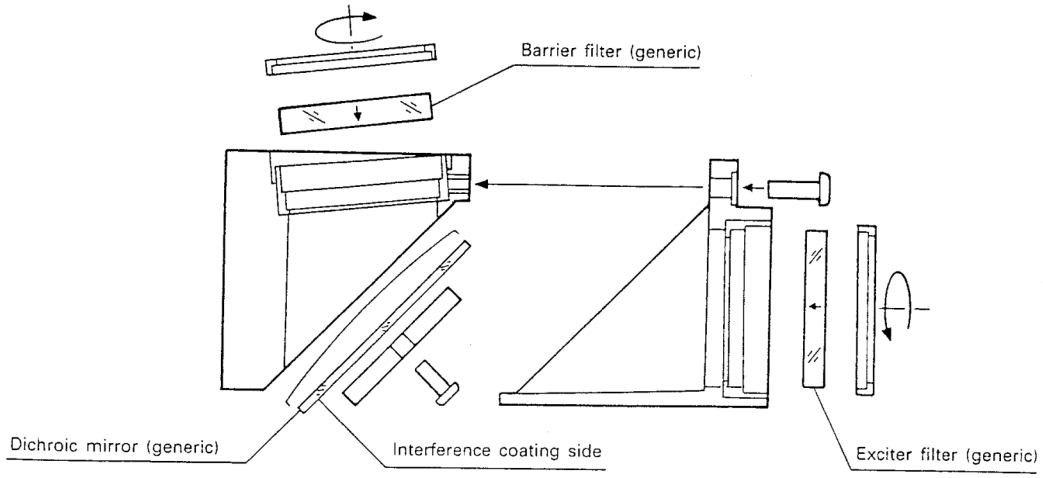
To mount the ① insert it into the space underneath the stage. When switching between transmitted unit observation methods (phase contrast observation, Nomarski observation, etc.), set the slide on top of the light exit window ②.

## 9 Optional Cubes

You can build optional cubes using generic burner filters, exciter filters, and dichroic mirrors available in the market.

### Dimensions of Optical Components for Cubes

- Barrier filter
- Exciter filter $25^{-0.1}_{-0.2}$ mm dia, 6 mm max thickness
- Dichroic mirrors $26^{-0.1}_{-0.3}$ mm X $38^{-0.1}_{-0.3}$ mm, 1 ± 0.05 mm thickness




★ When changing dichroic mirrors, be extremely careful to avoid contamination by fingerprints, etc.

# 4 OPERATION

Overall precautions for observation

1. Verify that the power supply voltage and frequency match the requirements indicated on the name plate.
2. Make sure that the power cord and connecting cord are plugged in securely.
3. If you perform only transmitted light phase contrast or transmitted light differential interference contrast observations, leave one cube position on the turret empty. This allows for transmission of white light.
4. Always use immersion oil with oil immersion objectives.
5. If you use an objective with correction collar such as the UPlanApo40X, you can correct variations in cover glass thickness by adjusting the correction collar.

   Correction procedure:
   Turn the correction collar and adjust the fine focus knob to where the image is as sharp as possible. Cover glass thicknesses for which correction is possible are from 0.11 to 0.23 mm.
6. Engage the shutter if you interrupt observation for a short time.

   (Turning the mercury burner On and Off repeatedly will significantly shorten the life span of the burner.)



## 1 Turning On the Power

Turn on the power switch. Between 5 and 10 minutes are required for the arc to stabilize after the burner is ignited.

★ Some mercury burner may not ignite the first time the power is turned on. If the burner does not ignite, turn the power switch off once, then repeat after 5 or 10 seconds.

★ To avoid shortening the life of the burner, do not turn the burner off within 15 minutes ignition.

★ After turning the burner off, it cannot be re-ignited until the mercury vapor cools and condenses to liquid. Wait for about 10 minutes before restarting the burner.

★ A safety interlock automatically shuts off the burner if the lamp housing is opened. If this happens, turn off the power switch, then wait about 3 minutes before restarting the burner. Before opening the lamp housing, wait about 10 minutes for it to cool.

★ After replacing the burner, be sure to reset the hour counter.

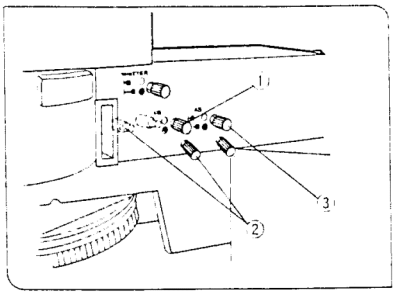



Fig. 16

## 2 Centering the Field Iris Diaphragm (Fig. 16)

1. Rotate the revolving nosepiece to bring the 10X objective into the light path, then place a specimen onto the stage and bring the image into approximate focus.
2. Pull out the field iris diaphragm ① on the universal fluorescence vertical illuminator to where the diameter of the diaphragm is at its smallest.
3. Turn the two field iris diaphragm centering knobs ② to where the image of the diaphragm is centered in the field of view.
4. Check centration by opening the diaphragm with knob ① until the diaphragm image touches the perimeter of the field of view. If the image is not centered precisely, recenter it.
5. Further enlarge the field iris diaphragm diameter until it is just outside the field of view.

## 3 Adjusting the Field Iris Diaphragm (Fig. 16)

Use the field iris diaphragm to adjust the diameter of the illuminated area. The field iris diaphragm also helps to prevent stray light from areas outside the field of view from affecting image contrast.

## 4 Centering the Aperture Iris Diaphragm (Fig. 16)

1. Engage the light excluding shutter ⑤ and close the light path.
2. Move the B or IB cube into the light path.
   (If neither of these cubes is available, move some other fluorescence cube into the light path.)
3. Screw the centering screen (BH2-SGRF) into the revolving nosepiece and move it into the light path.
4. Disengage the shutter ⑤ to open the light path.
5. Pull out the aperture iris diaphragm knob ③ to bring the shadow of the aperture iris diaphragm into the centering screen (BH2-SGRF).
6. Adjust the two aperture iris diaphragm centering knobs ④ to bring the diaphragm shadow into the center of the centering screen.
7. Push the knob ③ in to open the aperture iris diaphragm.

## 5 Adjusting the Aperture Iris Diaphragm (Fig. 16)

The aperture number of the illumination system affects the brightness of the observed image.
With normal fluorescence observation, push the aperture iris diaphragm knob ③ all the way in to completely open aperture.
If the specimen bleaches too quickly, because the excitation light is too strong, use an ND filter to attenuate the light. If the light is still too strong, stop down the aperture iris diaphragm. However, do not stop down the aperture diaphragm unnecessarily, and do not use the aperture iris diaphragm as a shutter.

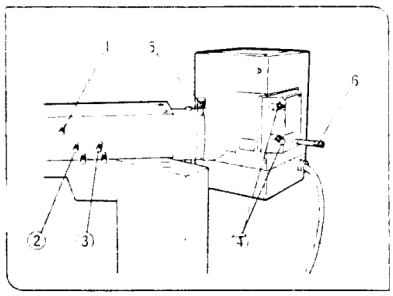



Fig. 17

## 6 Centering the Mercury Burner (Figs. 17, 18)

**When not using conversion lens**

O Wait for the arc to stabilize (5 – 10 minutes) before centering the burner.

1. Engage out the light excluding shutter ① and block the light path.
2. Move the B or IB cube into the light path. (If neither of these cubes is available, move some other cube into the light path.)
3. Screw the centering screen (BH2-SGRF) into the revolving nosepiece and move it into the light path.
4. Push into the field iris diaphragm knob ② and the aperture iris diaphragm knob ③ to completely open light path.
5. Push light excluding shutter knob ① and completely open the light path.

**Align the Image of the Arc**

6. Using the burner centering knobs ④, adjust so that two arc images are visible side by side in the field of view. (Figs. 17, 18-A, B)

**Adjusting the Arc Focus**

7. Adjust focusing of the direct arc image using the collector lens focusing knob ⑤. (Figs, 17, 18-C)
8. The burner focusing knob ⑥ changes the image as follows depending on the direction in which it is turned. (Fig. 17)
   (1) As you turn the knob, the blurred reflected arc image becomes gradually clearer, and the formerly focused direct image becomes blurred.
   (2) Turning the knob in the opposite direction causes both images to become blurred.

   Adjust by turning the burner focusing knob ⑥ in the direction that results in condition (1).

direct arc image

reflected arc image

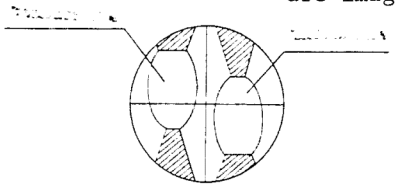


Fig. 18-A

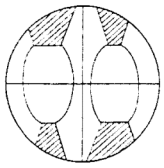


Fig. 18-B

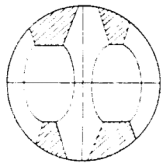


Fig. 18-C

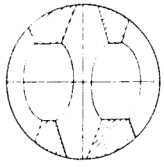


Fig. 18-D

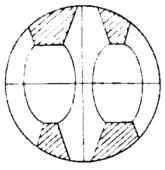


Fig. 18-E

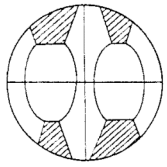


Fig. 18-F

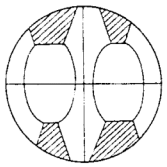


Fig. 18-G

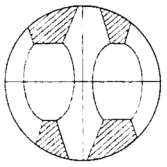


Fig. 18-H

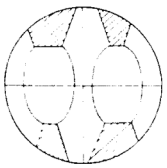


Fig. 18-I

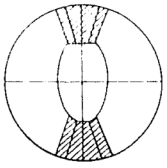


Fig. 18-J

9. Refocus the direct arc image using the collector lens focusing knob ⑤. (Figs. 17, 18-C)
10. Turn the burner focusing knob in direction (1) so that both arc images are blurred to the same degree. (Figs. 17, 18-F)
11. Refocus the direct arc image using the collector lens focusing knob ⑤. (Figs. 17, 18-G)
12. Fine-adjust the burner focusing knob ⑥ so that both arc images blur at the same time when you turn the collector lens focusing knob ⑤. (Figs. 17, 18-H)
13. Turn the collector lens focusing knob ⑤ so that the brightness of the two arc images is greatest.
14. Turn the burner centering knobs so that the direct arc image and the reflected arc image overlap. (Figs, 17, 18-J)

★ Since it is extremely dangerous, never open the lamp housing while the burner is on or immediately after switching it off.

◎ Recenter the burner after each burner replacement.

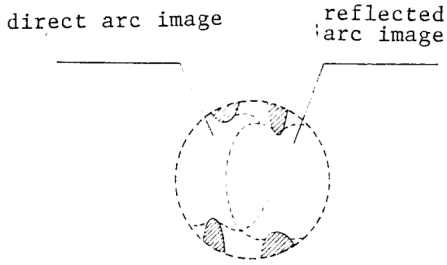



Fig. 18-A'

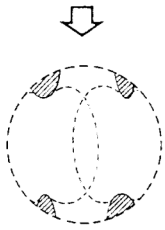


Fig. 18-B'

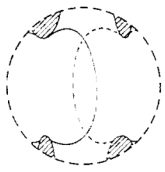


Fig. 18-C'

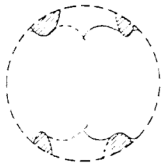


Fig. 18-D'

## When using conversion lens

Wait for the arc to stabilize (5 – 10 minutes) before centering the burner.

Do the following procedures after centering aperture iris diaphragm.

1. Engage out the light excluding shutter ① and block the light path.
2. Move the B or IB cube into the light path. (If neither of these cubes is available, move some other cube except U excitation cube into the light path.)

   ★ Do not use U excitation cube for safety. In case you use U excitation unavoidably, make sure to see the light through UV protection shield.
3. Move empty hole of revolving nosepiece into light path.
4. Push into the field iris diaphragm knob ② and the aperture iris diaphragm knob ③ to completely open light path.
5. Place the white paper on the stage.
6. Push light excluding shutter knob ① and completely open the light path.

### Align the Image of the Arc

7. Using the burner centering knobs ④, adjust so that two arc images are visible side by side in the field of view. (Figs. 17, 18-A', B')

### Adjusting the Arc Focus

8. Adjust focusing of the direct arc image using the collector lens focusing knob ⑤. (Figs. 17, 18-C')
9. The burner focusing knob ⑥ changes the image as follows depending on the direction in which it is turned. (Fig. 17)

   (1) As you turn the knob, the blurred reflected arc image becomes gradually clearer, and the formerly focused direct image becomes blurred.

   (2) Turning the knob in the opposite direction causes both images to become blurred.

   Adjust by turning the burner focusing knob ⑥ in the direction that results in condition (1). (Figs. 17, 18-D')

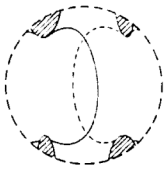


Fig. 18-E'

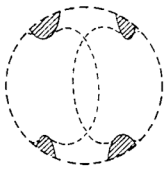


Fig. 18-F'

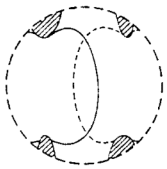


Fig. 18-G'

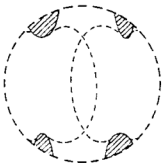


Fig. 18-H'

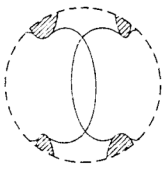


Fig. 18-I'

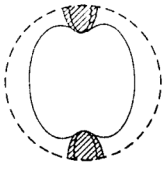


Fig. 18-J'

10. Refocus the direct arc image using the collector lens focusing knob ⑤. (Figs. 17, 18-E')
11. Turn the burner focusing knob in direction (1) so that both arc images are blurred to the same degree. (Figs. 17, 18-F')
12. Refocus the direct arc image using the collector lens focusing knob ⑤. (Figs. 17, 18-G')
13. Fine-adjust the burner focusing knob ⑥ so that both arc images blur at the same time when you turn the collector lens focusing knob ⑤. (Figs. 17, 18-H')
14. Turn the collector lens focusing knob ⑤ so that the brightness of the two arc images is greatest.
15. Turn the burner centering knobs so that the direct arc image and the reflected arc image overlap. (Figs, 17, 18-J')

★ **Since it is extremely dangerous, never open the lamp housing while the burner is On or immediately after switching it Off.**

© Recenter the burner after each burner replacement.

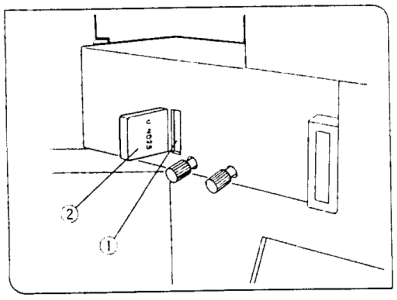



Fig. 19

## 7 Filter Slot (Fig. 19)

As necessary, up to two filters (ND6 or ND25) may be individually inserted into filters slots ① and ②. Insert the filters with the marked side facing toward the observer.

As you insert a filter, you will hear two clicks. At the first, the filter is at the empty position, and at the second it enters the light path.

★ Take note of the metal filter frame will become very hot if you leave the filter inserted for a long time while the mercury burner is on.



## 8 Objective for Various Observation Techniques

| Objective lens | | Reflected fluorescence | | Transmitted phase contrast | Transmitted DIC |
|---|---|---|---|---|---|
| | | U, V, BV | B, IB, G, IY | | |
| UPlanApo | 4X | ○ | ○ | — | — |
| | 10X | ○ | ○ | ○ | ○ |
| | 20X | ○ | ○ | ○ | ○ |
| | 40X | ○ | ○ | — | ○ |
| | 40XOI | ○ | ○ | ○ | ○ |
| | 100XOI | ○ | ○ | ○ | ○ |
| PlanApo | 1.25X | — | — | — | — |
| | 2X | — | — | — | — |
| | 40XO | — | ○ | — | — |
| | 100X | — | ○ | — | — |
| UPlan FI | 4X | ○* | ○* | — | — |
| | 10X | ○* | ○* | ○ | ○ |
| | 20X | ○* | ○* | ○ | ○ |
| | 40X | ○* | ○* | ○ | ○ |
| | 100XO, OI | ○ | ○ | ○ | ○ |

○ Recommended combination
○* Usable, but image may be dark depending on NA
— Not usable, or combination without corresponding objective
★ A phase contrast objective (PH) is necessary for phase contrast observation

## 9 Selecting a Cube

Selecte the cube which matches the fluorechrome in use.

© The band width differs depending on the excitation . There are many filter sets offered. Wide band sets (designated by W) are normally used, however there are cases where ultra-wide (SW) or narrow (N) band sets are recommended.

1. Extremely weak fluorescence brightness (only B and G excitations)
   Ultra-wide band (SW) is recommended.
   © With the SWB, strong autofluorescence reduces image contrast.
2. Samples emitting strong autofluorescence
   Narrow band (N) is recommended
   © Brightness is somewhat reduced.

### Filter Cube Configurations

| Excitation | Cube | Dichroic mirror | Exciter filter | Barrier filter | Fluorochrome |
|---|---|---|---|---|---|
| U | U-MWU | DM400 | BP330-385 | BA420 | • Auto fluorescence<br>• DAPI: DNA staining<br>• Hoechest 33258, 33342 |
| | U-MNU | | BP360-730 | | |
| V | U-MNV | DM455 | BP400-410 | BA455 | • Catecholamine<br>• Serotonin<br>• Tetracyline |
| BV | U-MWBV | DM455 | BP400-440 | BA475 | • Quinacrine, quinacrine, mustard |
| | U-MNBV | | BP420-440 | | • Thioflavine S<br>• Acriflavine |
| B | U-MWB | DM500 | BP450-480 | BA515 | • FITC<br>• Acridine orange: DNA, RNA<br>• Auramine |
| | U-MNB | | BP470-490 | | |
| | U-MSWB | | BP420-480 | | |
| IB | U-MWIB | DM505 | BP460-490 | BA515IF | |
| | U-MNIB | | BP470-490 | | |
| G | U-MWG | DM570 | BP510-550 | BA590 | • Rhodamine, TRITC<br>• Propidium iodide: DNA |
| | U-MNG | | BP530-550 | | |
| | U-MSWG | | BP480-550 | | |
| IG | U-MWIG | DM565 | BP520-550 | BA580IF | |
| IY | U-MWIY | DM600 | BP545-580 | BA610IF | Texas red |

Band Pass Barrier Filter Combinations

| | | | | |
|---|---|---|---|---|
| U | U-MNUA | DM400 | BP360-370 | BA420-460 |
| IB | U-MWIBA | DM505 | BP460-490 | BA515-550 |
| | U-MNIBA | | BP470-490 | |

# 5 OBSERVATION PROCEDURE

## 1 Observation with the Reflected Light Vertical Illuminator

1. Bring a suitable cube into the light path.
2. Bring the desired objective into the light path
3. Open the shutter and focus the specimen.
4. Adjust the collector lens focusing knob to where brightness and evenness of illumination in the field of view are at maximum.

This unit's optics and light source are designed to be bright so that strong fluorescence can be obtained even from weakly fluorescing specimens. If the specimen bleaches rapidly or is strongly fluorescing, use an ND slider (6 or 25) to reduce the brightness of the light source.
Use the shutter to avoid overexposing the specimen to the light source. Since contrast may be reduced if the specimen fluoresces too strongly, reduce the excitation light by use of ND filter.

★ This unit can be used in combination with transmitted light brightfield observation, transmitted light phase contrast observation, and transmitted light differential interference contrast observation as well as reflected light fluorescence observation.
With specimens that bleach rapidly, bleaching can be minimized by initially using transmitted light phase contrast observation or transmitted light differential interference contrast observation for positioning. Reflected light fluorescence can also be used in combination with phase contrast or differential interference contrast observation, making it easy to tell which portion of the specimen is fluorescing.



## 2 Using Simultaneous Reflected Light Fluorescence and Transmitted Phase Contrast Observation

Phase contrast observation requires a phase contrast condenser (U-PCD) or the universal condenser (U-UCD) and a phase contrast objective. (See section 8 on page 17.)

1. Bring an empty position on the cube turret into the light path.
2. Rotate the phase contrast turret to show the same number as the ph number (ph 1 - 3) shown on objective lens.
3. Adjust optical axis between the ring slit and phase plate by centering knobs.
4. Bring the cube corresponding to the desired excitation into the light path and open the shutter.
5. Adjust the transmitted light for the best balance of fluorescence and phase contrast brightness and you are ready for observation.
   ★ Use ND filters to adjust the transmitted light intensity.
   ★ For details on using phase contrast observation, see the instructions provided with the phase contrast condenser (BX-PC) or the universal condenser (BX-UCD).

## 3 Using Simultaneous Reflected Light Fluorescence and Transmitted Nomarski Differential Interference Contrast Observation

The following accessories are required for transmitted light Nomarski differential interference contrast observation:

◎ In order for reflected light fluorescence to be effective in the simultaneous observation mode, insert the analyzer (U-AN) into the slot in the vertical illuminator.

1. Adjust the polarizer on the universal condenser (U-UCD).
2. Insert the transmitted light DIC slider (U-DICT) into the slot provided on the nosepiece. (U-D6RE)
3. Turn the upper turret on the universal condenser (U-UCD) to the Nomarski prism whose turret number matches the objective to be used for observation.
4. Rotate the objective to be used into the light path.
5. Place the specimen on the stage and focus.
6. Adjust the field iris diaphragm of the transmitted light illumination unit (built into the microscope base) and the aperture iris diaphragm of the universal condenser.
7. Turn the prism movement knob on the transmitted light DIC slider to adjust contrast of the differential interference contrast image.
8. Move the cube corresponding to the desired excitation into the light path and open the light excluding shutter.
9. Adjust the transmitted light for optimum fluorescence and differential interference image brightness.

★ For details on using transmitted light differential interference contrast observation, see the instructions provided with the universl condenser (BX-UCD).

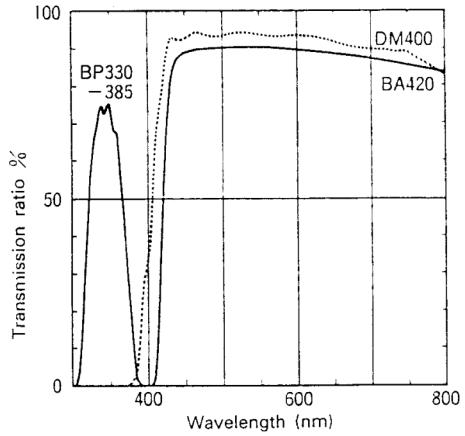
# 6 TROUBLESHOOTING GUIDE

| Trouble | Cause | Remedy |
|---|---|---|
| 1. Optical system | | |
| a. The bulb is on, but image cannot be seen or is dark. | The shutter slider is closed or the ND filter is engaged. | Move the shutter to open aperture, or remove ND filter from optical path. |
| | The cube is not brought into light path correctly. | Bring the cube into the light path correctly. |
| | The aperture iris diaphragm, field iris diaphragm, or the objective's iris diaphragm opening are not completely opened. | Completely open the aperture iris diaphragm and objective iris diaphragm, and open the field iris diaphragm until the image circumscribes the field of view. |
| | A cube unsuitable for the specimen is used. | Change to a suitable cube. |
| b. Image is unclear, blurred or has insufficient contrast. | Objectives or filters are dirty. | Wipe them clean. |
| | The aperture iris diaphragm or field iris diaphragm are not opened correctly. | Open the aperture iris diaphragm completely, and open the field iris diaphragm until the image circumscribes the field of view. |
| | A cube unsuitable for the specimen is used. | Change to suitable cube. |
| c. Image is partially obscured or unevenly illuminated. | The objectives are not inserted into the light path correctly. | Rotate the revolving nosepiece until it clicks. |
| | The cube is not inserted into light path correctly. | Insert the cube into light path correctly. |
| | The field iris diaphragm is closed excessively. | Open the field iris diaphragm as required. |
| | The ND slider is not in click position. | Push the ND slider until it clicks properly. |
| | The mercury burner is not centered correctly, or focus adjustment has not been completed. | Center the mercury burner or adjust the focus. |
| 2. Electrical system | | |
| a. Power switch indicator does not light up. | The power cord is connected incorrectly. | Connect correctly. |
| b. Power switch indicator lights, but mercury burner does not start. | Connectors are connected incorrectly. | Connect correctly. |
| | The burner has not been not installed. | Install the burner. |
| | The lamp housing interlock is operating. | Tighten the burner socket locking screw securely. |
| | Auto ignition is not operating as required. | Turn off the power of the power supply unit, switch on again. (Repeat as necessary.) |
| c. The bulb flickers or is dark. | Insufficient time has elapsed since the burner was turned on. | Wait for 10 minutes after turning on the burner. |
| | The bulb life has expired. | Replace the mercury burner if the hour counter reads over 200 hours. |

# 7 TRANSMISSION CURVES OF FILTERS

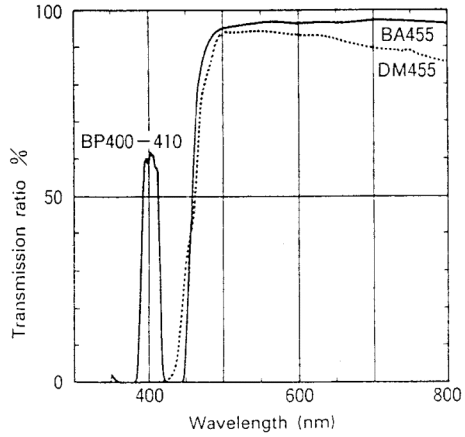
1. U excitation cube (Wide band)

U−MWU



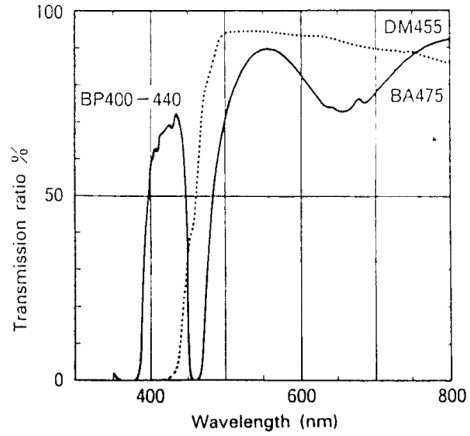

2. V excitation cube (Narrow band)

U−MNV



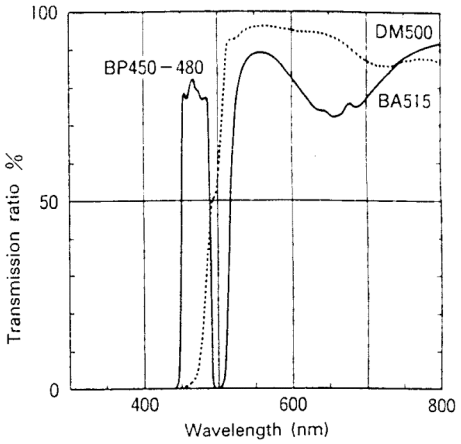

3. BV excitation cube (Wide band)

U−MWBV



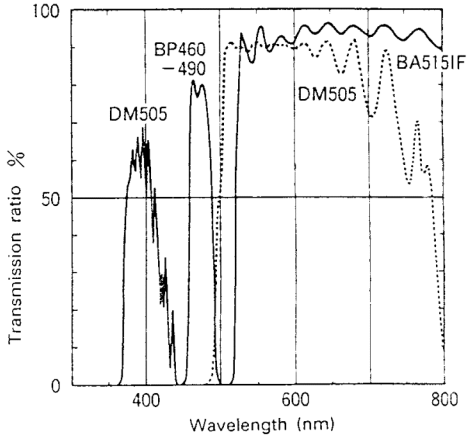

4. B excitation cube (Wide band)

U−MWB



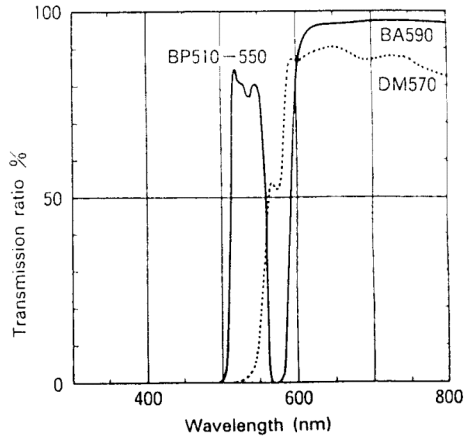

5. IB excitation cube (Wide band)

U−MWIB



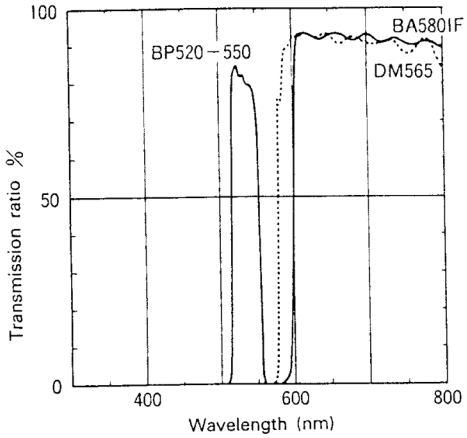

6. G excitation cube (Wide band)

U−MWG



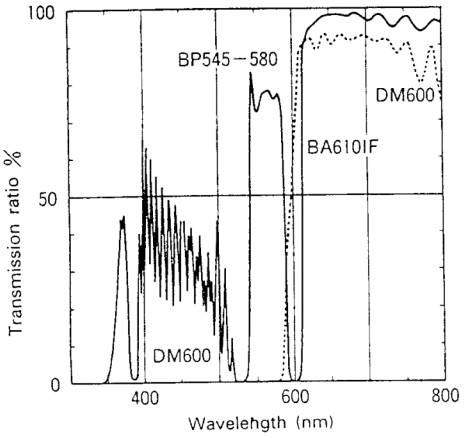
7. IG excitation cube (Wide band)

U-MWIG

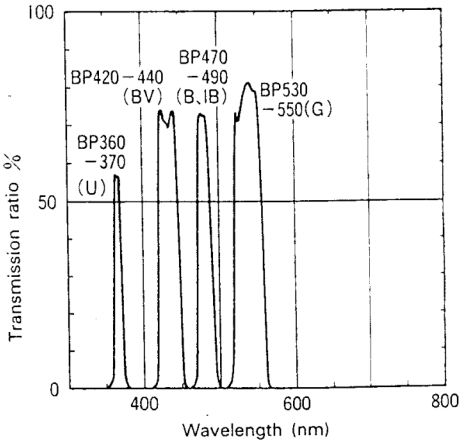



8. IY excitation cube (Wide band)

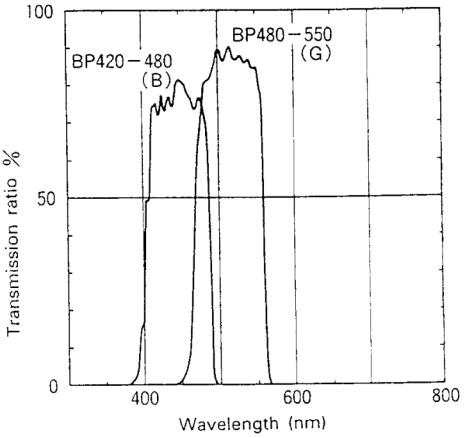
U-MWIY



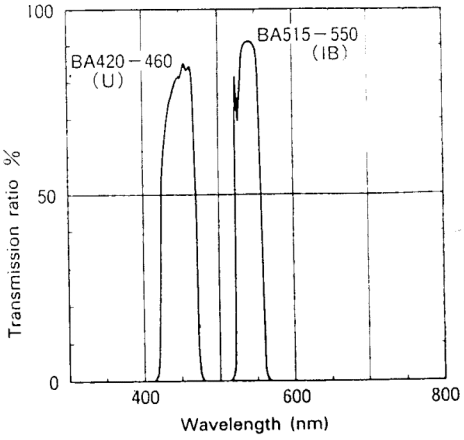

9. Narrow band exciter filters (excluding V excitation)




10. Super wide band exciter filters




11. Band pass barrier filters



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