OLYMPUS®

INSTRUCTIONS

BX-FLA

REFLECTED LIGHT FLUORESCENCE ATTACHMENT

This instruction manual is for the Olympus BX System Attachment Model BX-FLA. To ensure the safety and obtain optimum performance and to familiarize yourself fully with the use of this attachment, we recommend that you study this manual thoroughly before operating the microscope system. Retain this instruction manual in an easily accessible place near the work desk for future reference.

AX5837

IMPORTANT

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

The universal reflected light fluorescence vertical illuminator features interchangeable cubes to employ excitation light of different wavelengths. If 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 interference contrast.
3. Reflected light fluorescence + transmitted light brightfield or darkfield.

By mounting standard cubes for reflected light observation, the following observation methods become possible.

1. Reflected light brightfield/darkfield observation.
2. Reflected light Nomarski differential interference contrast.
3. Reflected light simple polarized light observation.

This instruction manual consist of two parts: I. Reflected Light Fluorescence Observation and II. Reflected Light Observation Modes. Use these headings to find the relevant page with the instructions for the particular observation mode.

1 Getting Ready

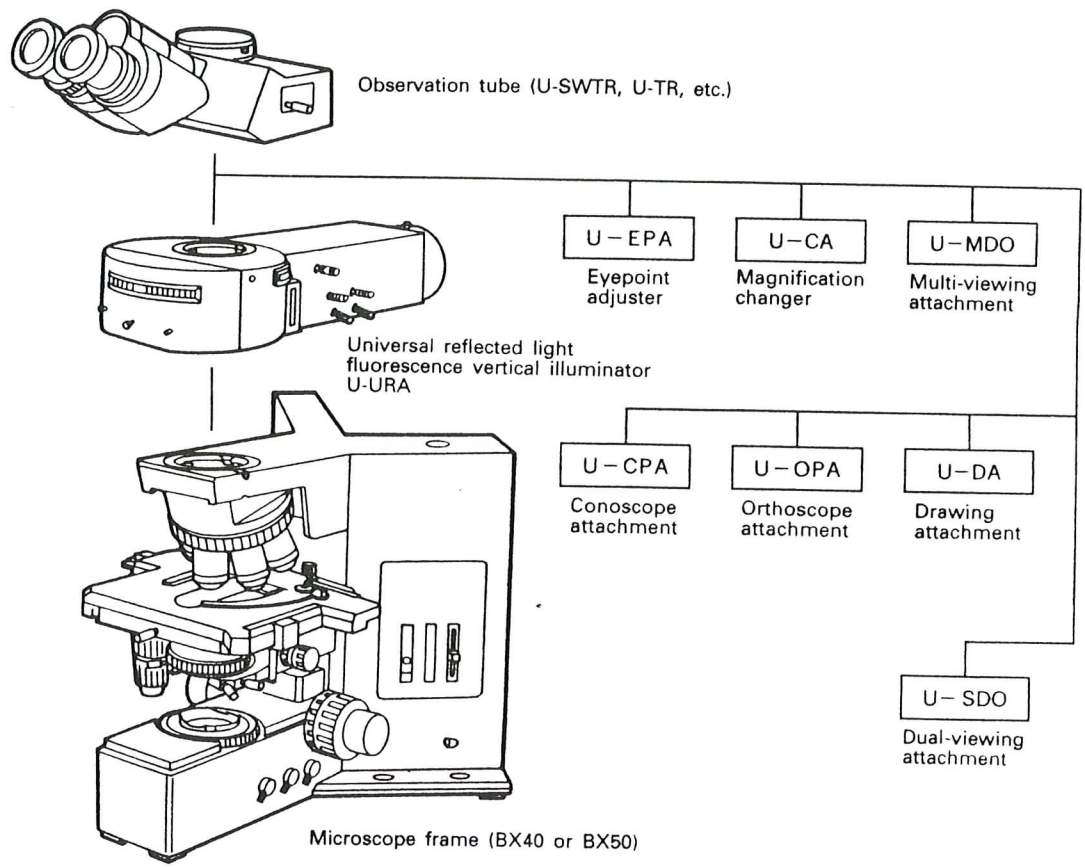
1. A vertical illuminator is delicate. Handle it carefully, and avoid jolts.
2. The high pressure mercury burner used in the unit should be a USH102 (mfd. by Ushio Electric). The halogen lamp used in the unit should be a 12V, 100WHAL halogen bulb (Philips 7724).
3. Verify that the burner is installed 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 open the lamp housing while the burner is turned on or for at least 10 minutes after it is turned off. The lamp housing inside is extremely hot and will cause injury if touched. (See page 11.)
6. The power supply unit contains high voltage components. Do not attempt to disassemble it.
7. Always ground the unit.
8. Before opening the lamp housing for replacement of the burner or other, turn off the main switch and unplug the power output connector from the power unit. Wait for more than 10 minutes for the burner and lamp socket to cool down.
9. Before plugging in the mains power cord, make sure that the main switch on the power supply unit is turned off.

2 Care and Storage

1. Clean all glass components by wiping gently with gauze. To remove fingerprints or oil smudges, wipe with gauze slightly moistened with a mixture of ether (70%) and alcohol (30%) or EE System Cleaner (Olympus EE-6310).
   ⚠ **Since solvents such as ether, alcohol and EE-6310 are highly flammable, they must be handled carefully. Be sure to keep these chemicals 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 these chemicals only in a well-ventilated room.**
2. Do not disassemble any part of the attachment.
3. The burner has a service life period of 200 hours. When the hour counter on the power supply unit indicates 200 hours, replace the burner with a new one. (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 a dichroic mirror cube is not to be used 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 the U-URA. Select an intermediate tube to install on top of the vertical illuminator by referring to the illustration below.



4 Caution

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

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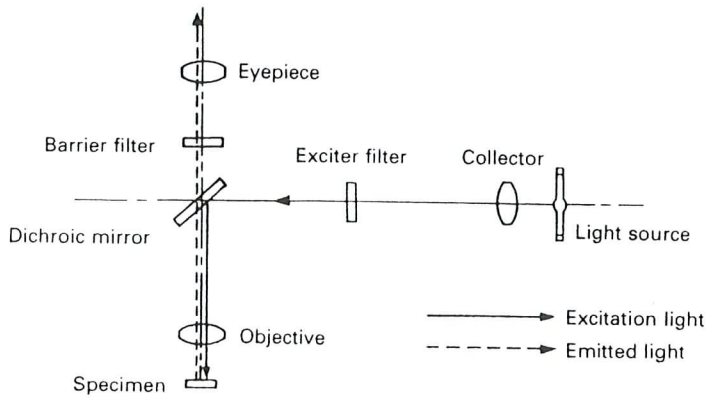
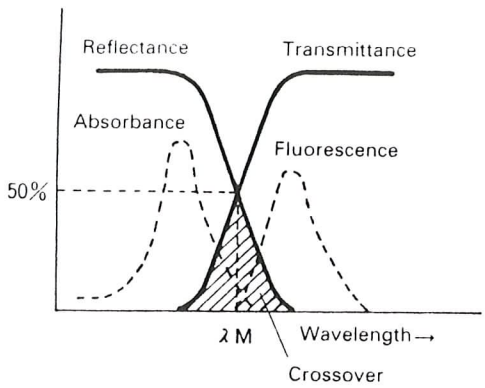
I. REFLECTED LIGHT FLUORESCENCE OBSERVATION

1 PRINCIPLE

I-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)



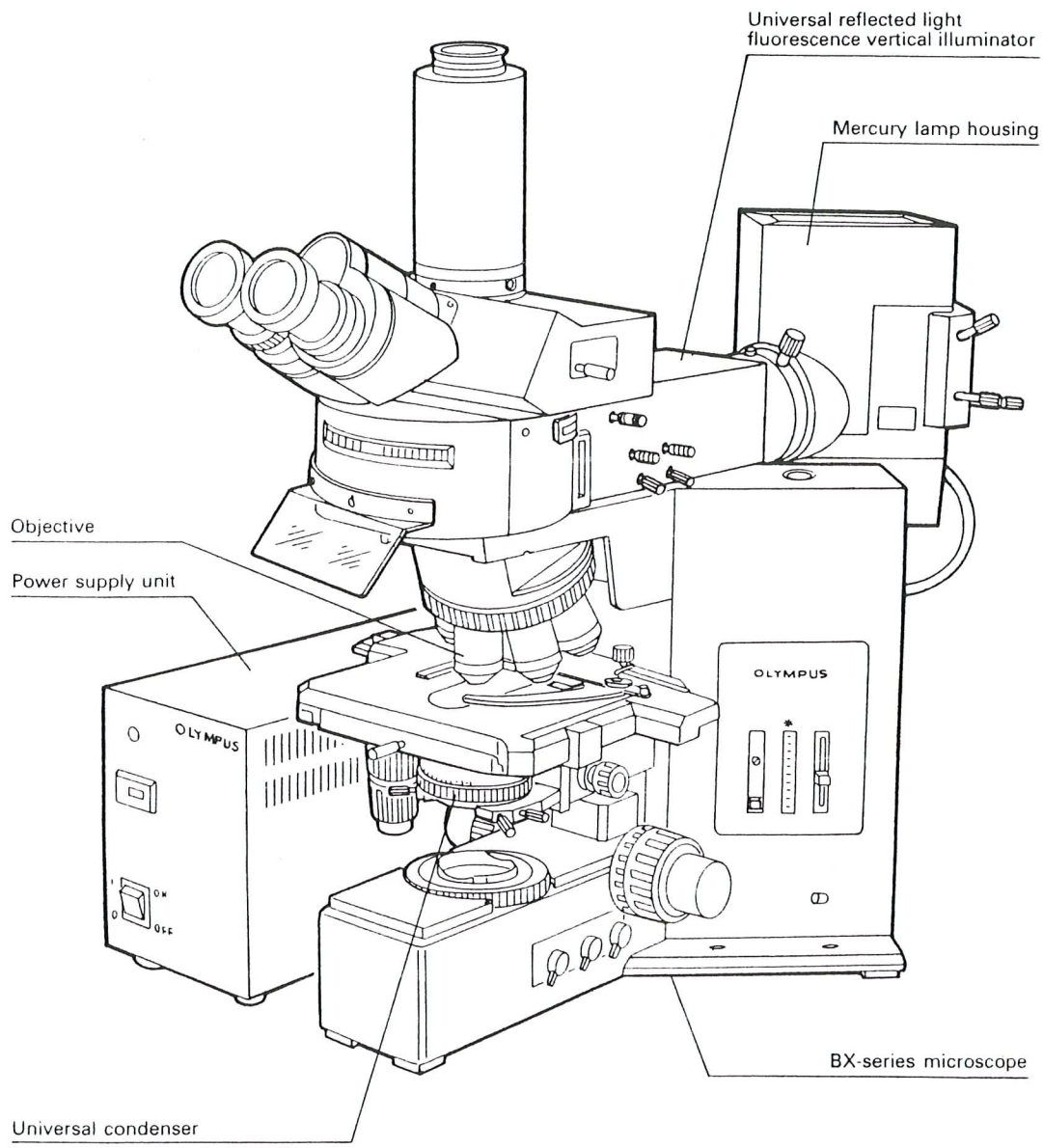


The spectral characteristics of the dichroic mirror when it is positioned at an inclination of 45° to the optical axis of incident light is shown in Fig. 2. Because a cross-over exists between transmittance and reflectance, it is necessary to use an appropirate combination of exciter and barrier filters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

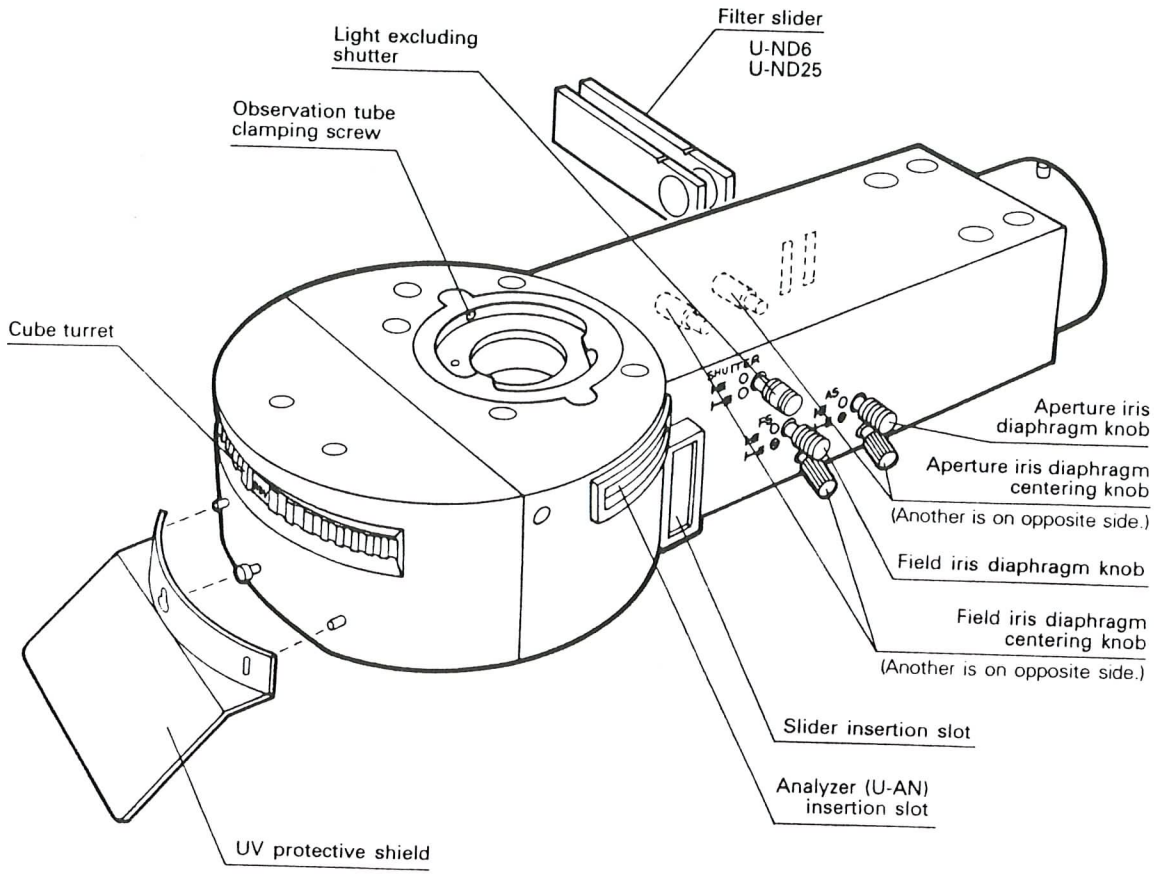
When the dichroic mirror is inclined 45° to the optical axis of incident light, it reflects the excitation light towards the objective, and passes unwanted wavelengths.

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.

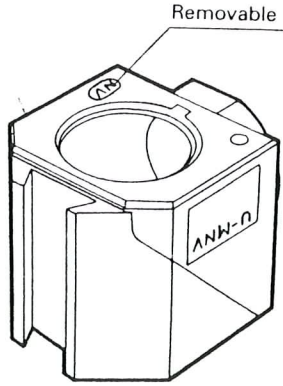
2 NOMENCLATURE



A. Universal Reflected Light Fluorescence Vertical Illuminator



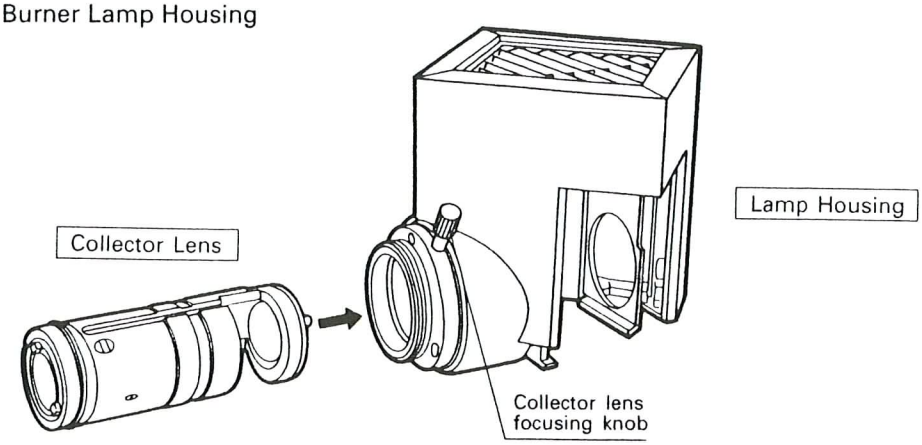
B. Cube

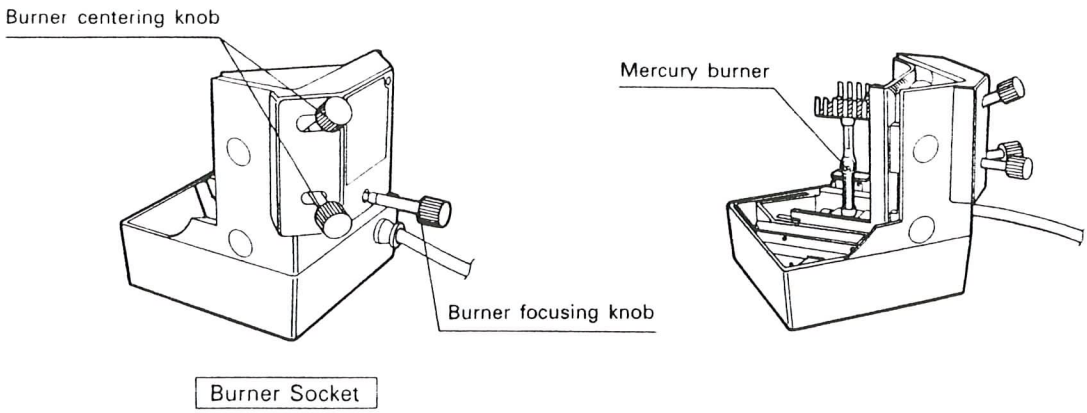


★ Combine with dichroic mirrors, barrier filters and exciter filters as appropriate for the desired excitation method.
Do not disassemble the cube.

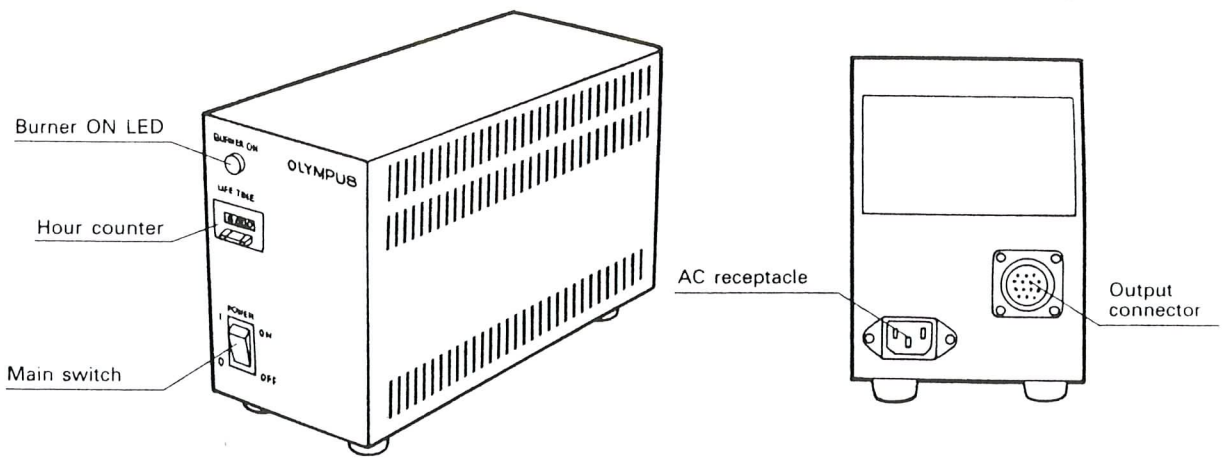
C. Fluorescent Light Source

● Mercury Burner Lamp Housing





● Power Supply Unit

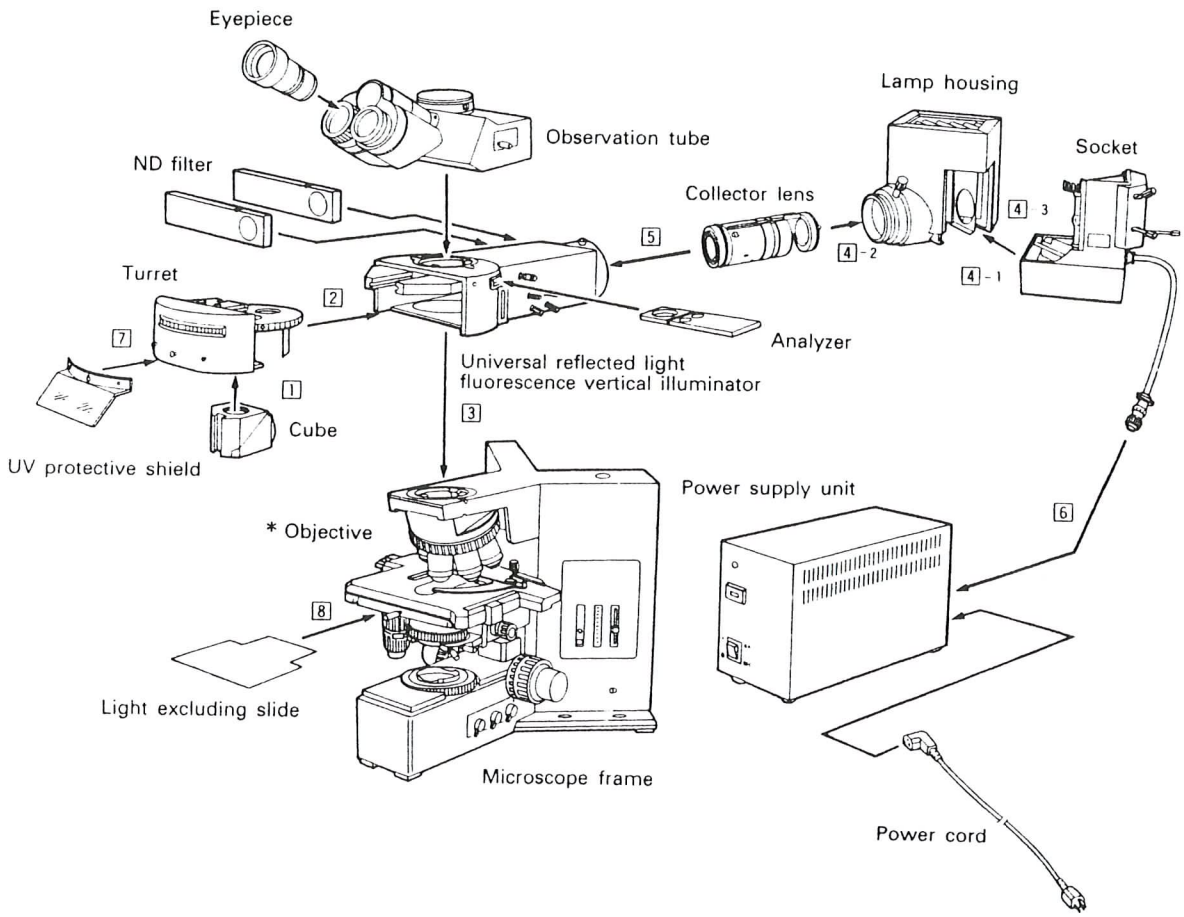


4 ASSEMBLY

4-1 Assembly Diagram

◎ To assemble the BX40 or BX50, consult the instruction manual pertaining to the BX40 or BX50 microscope.

★ When assembling the units, 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 16 regarding objectives to use for different observation methods.

★ For selection of the appropriate cubes for the observation, refer to Section 9 on page 17.

4-2 Assembly Procedure

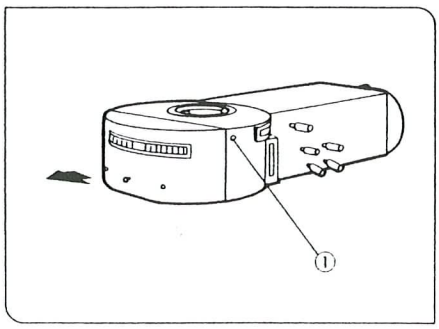


Fig. 1

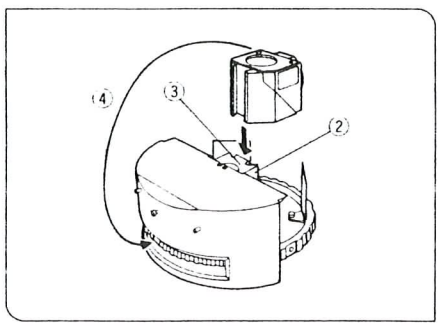


Fig. 2

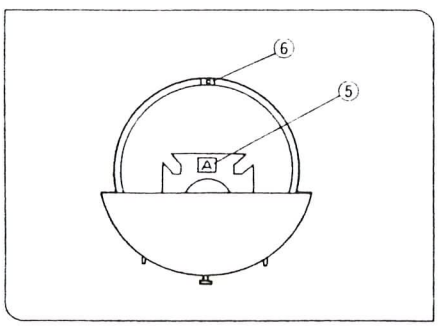


Fig. 3

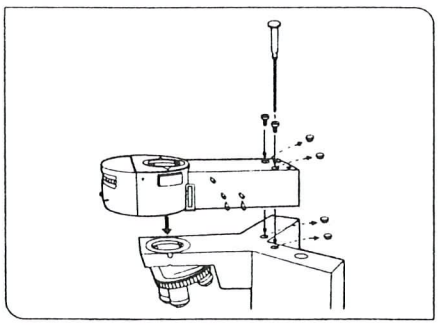
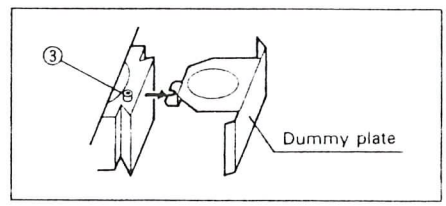


Fig. 4

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

◎ See paragraph **9** on page 17 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 screwdriver provided with the microscope frame. (Fig. 1)
2. Pull out the turret in the direction indicated by the arrow, then invert the turret so that the cube dovetail mounts ② point upward. (Dummy plates are mounted in three of the four cube positions. When you wish to use only one cube, mount it in the empty position. When using two or more cubes, loosen the clamping screw ③ and remove the dummy plate(s) by pulling in the direction indicated by the arrow, and then mount the actual cube(s) instead.)



3. Hold the cube to be mounted with its index side facing upward and slide it all the way onto the dovetail mount. Next, be sure to tighten the cube clamping screw ③ immediately. (Tighten all four cube clamping screws.)
4. Remove the cube's magnetic index sticker ④ and affix it to the corresponding turret position. (Fig. 2)
   - ★ Use a sharp object such as the tip of a ballpoint pen or mechanical pencil to lift the cube's magnetic index sticker.
   - ★ Move the cube's magnetic index sticker (WU, NU, etc.) to the corresponding A, B, C, D position on the turret. The positioning indices ⑤ A, B, C, and D on the cube dovetail mount correspond to the A, B, C and D indices ⑥ on the turret.

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.

★ When performing fluorescence observation, make sure to mount dummy plates in the empty cube positions. For transmitted light observation only, it is unnecessary to mount the dummy plates. However, be sure to tighten the cube clamping screws when no dummy plate is mounted. If the screws are left loose, the screw heads may obstruct turret rotation. Mount the dummy plate by reversing the procedure described in **1**-2.

3 Mounting the Universal 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 screwdriver to clamp the vertical illuminator to the microscope frame (two locations).

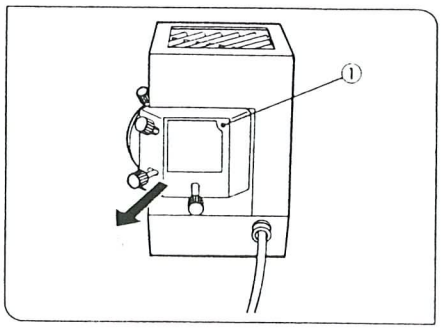


Fig. 5

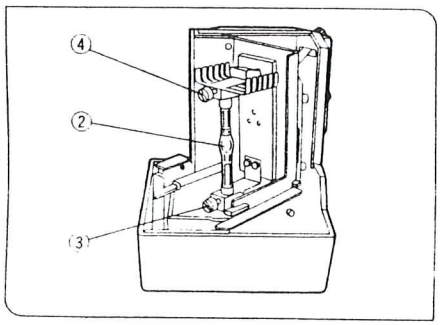


Fig. 6

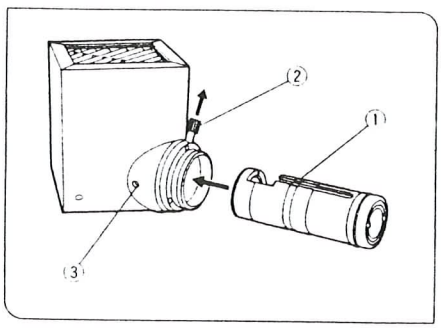


Fig. 7

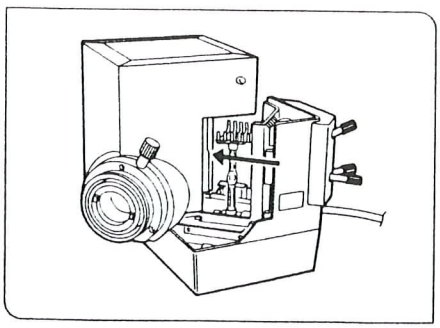


Fig. 8

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 screwdriver provided with the microscope.
2. Remove the socket from the lamp housing as indicated by the arrow. (Fig. 5)
3. Loosen the burner clamping screws ③ and ④ (Fig. 6) and remove the securing post. (For burner replacement, remove the used burner.)
4. With the + pole of the mercury burner ② facing downward, insert the pole into the opening and tighten the burner clamping screw ③. Then loosen the burner clamping screw ④. Insert the pole (marked UP) of the burner into the opening and tighten the burner clamping screw ④. (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 xylene.
   - ★ Dust or other contaminants left on the bulb surface may lead to the appearance of dark spots in the field of view during observation.
   - ★ 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, pull up the collector lens focusing knob ②, and slide the collector lens into the lamp housing as far as it will go. Then, return the collector lens focusing knob ② to its original position. At this point, check that the collector lens can be moved back and forth by turning the focusing knob ②. If not, adjust the position of the collector lens by hand so that it will click into its proper position.
2. Tighten the collector lens retaining screw ③. (Fig. 7)
   Note: If this retaining screw is not tightened firmly, optimum illumination performance will not be obtainable.
3. Reattach the socket to the lamp housing by reversing the procedure outlined in 4-1) above. (Fig. 8)
4. Tighten the socket clamping screw ① with the Allen screwdriver. (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 main 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 main switch back on again.

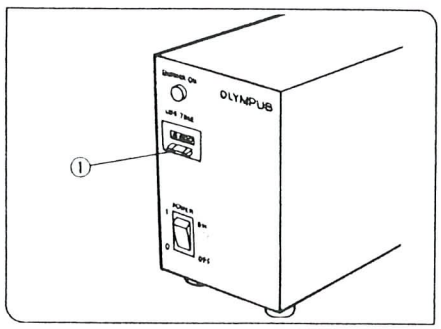


Fig. 9

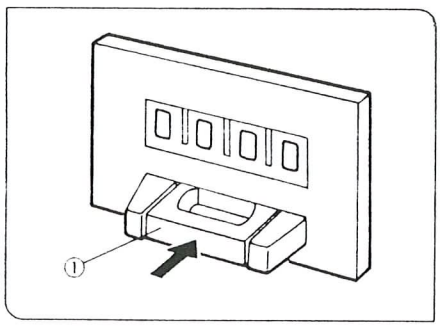


Fig. 10

Resetting the Burner Hour Counter

1. Press the center of the reset button ① (Fig. 10) on the power supply unit's front panel ① (Fig. 9) to reset the burner life indicator to 000.0.

◎ 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 is not properly reset.

Mercury Burner Replacement

1. In order not to impair the safety of the equipment, replace the burner when it has been used for 200.0 hours. The burner may crack if used beyond the specified life time.
2. Before replacing the burner, wait at least 10 minutes after turning the burner off. Before removing the burner, confirm that the main switch on the power supply unit is OFF, and unplug the connecting cord plug from the output connector on the power supply unit. Refer to page 7 for details on replacement procedure.
3. After replacing the burner, reset the burner life time hour counter to "000.0" as outlined above.

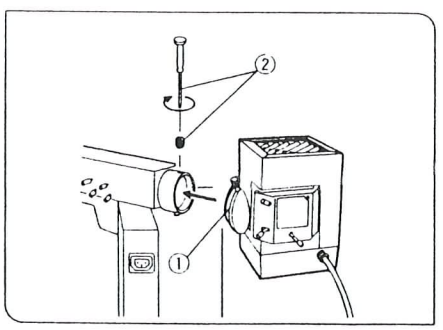


Fig. 11

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 screwdriver.

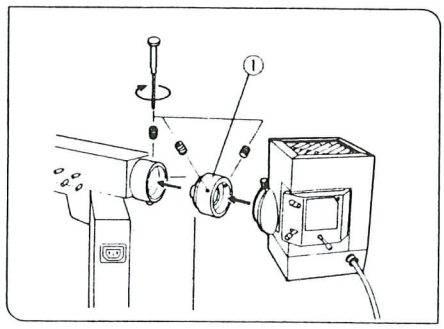


Fig. 12

Install Conversion Lens (option)

When brighter illumination is required, use the optional U-UCV ① conversion lens. To install the conversion 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 super widefield observation (FN26.5).

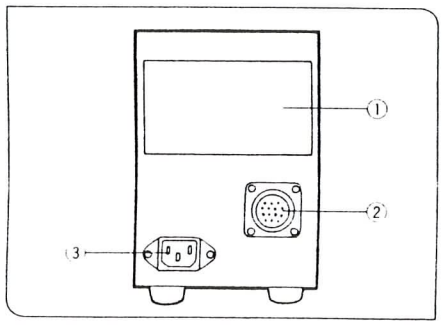


Fig. 13

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 ① 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 connecting cord into the power supply unit's output connector ②.
3. Connect the power cord to the AC receptacle ③ on the power supply unit, then plug the other end of the cord into an AC outlet.

⚠ Connect the power cord correctly and ensure that the ground terminal of the power supply and that of the wall outlet are properly connected. If the equipment is not grounded, Olympus can no longer warrant the electrical safety and performance of the equipment.

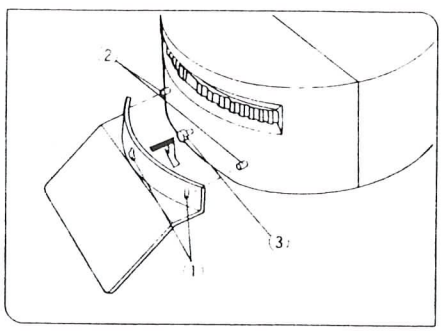


Fig. 14

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.

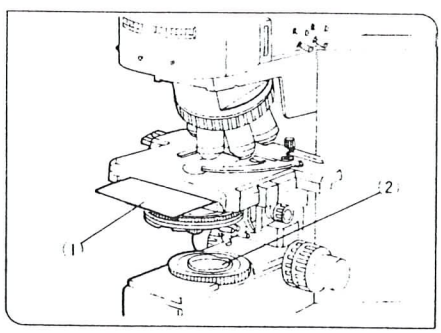


Fig. 15

8 Mounting the Light Excluding Slide (Fig. 15)

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

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

9 Optional Cubes

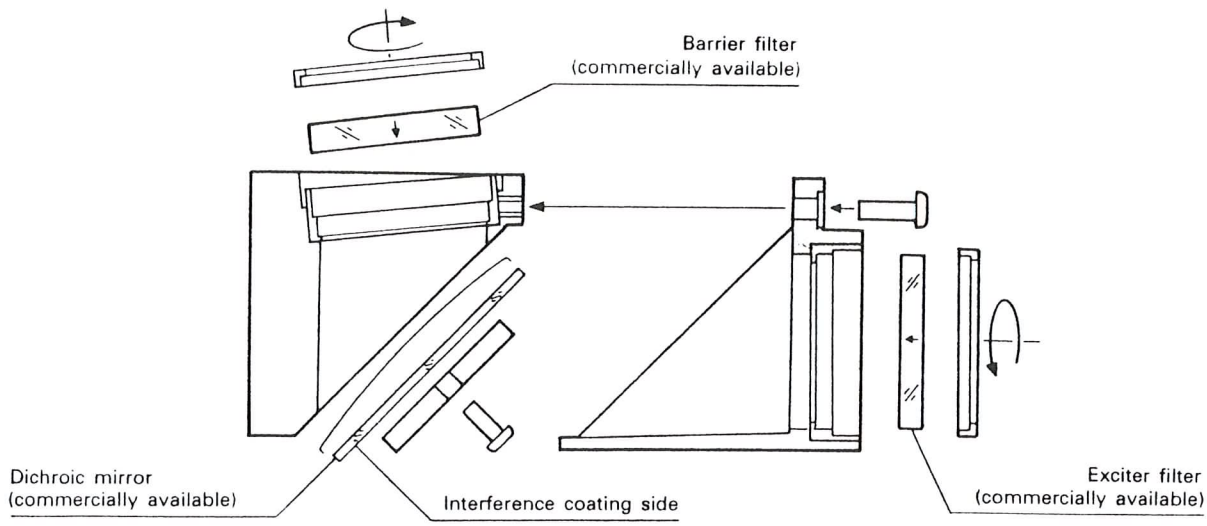
© You can build optional cubes using barrier filters, exciter filters, and dichroic mirrors commercially available.

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.

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. Open the field iris diaphragm so it just circumscribes the field of view. If decentered, use the centering knobs to center it.
5. Always use immersion oil with oil immersion objectives.
6. 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.
7. 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 Supply Unit

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

★ Some mercury burners may not ignite the first time the power is turned on. If the burner does not ignite, turn the main 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 of 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.

★ Even while the burner is turned on, opening the lamp housing causes the safety interlock function to shut off power automatically. In this case, turn off the main switch, and then wait for more than 10 minutes before restarting the burner. Before opening the lamp housing, wait until it becomes sufficiently cool.

★ When resetting the hour counter, be sure to hold down its button until it reads '000.0'.

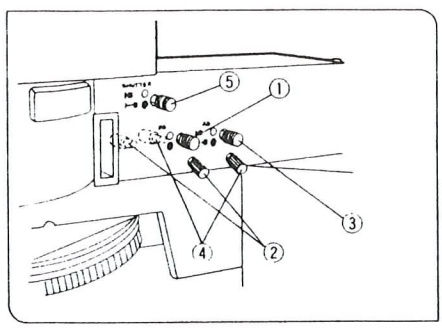


Fig. 16

2 Centering the Field Iris Diaphragm (Fig. 16)

1. Pull out the light excluding shutter ⑤ to 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. Push in the shutter ⑤ to open the light path.
4. 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.
5. Pull out the field iris diaphragm ① on the universal fluorescence vertical illuminator to where the diameter of the diaphragm is at its smallest.
6. Turn the two field iris diaphragm centering knobs ② so that the image of the diaphragm is centered in the field of view.
7. To check centering, open the diaphragm with knob ① until the diaphragm image touches the periphery of the field of view. If the image is not centered precisely, center it again until so.
8. Further enlarge the field iris diaphragm diameter until its image is just outside the field of view.

3 Adjusting the Field Iris Diaphragm (Fig. 16)

To obtain good image contrast, adjust the diameter of the illuminating beam in accordance with the objective in use.
Using the field iris diaphragm knob ① on the vertical illuminator, adjust the diaphragm so that the field of view is circumscribed by the field iris diaphragm in order to exclude stray light.

4 Centering the Aperture Iris Diaphragm (Fig. 16)

1. Pull out the light excluding shutter ⑤ to 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. Push in 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 in the knob ③ in to open the aperture iris diaphragm.

5 Adjusting the Aperture Iris Diaphragm (Fig. 16)

The numerical aperture 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.

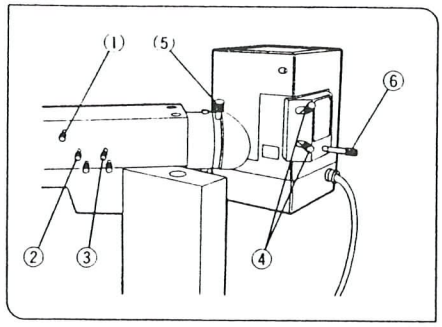


Fig. 17

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

〈When using conversion lens〉

◎ Before attempting the burner centering adjustment, wait for the arc to stabilize (5-10 minutes after turn-on).

◎ Carry out the following procedure after centering the aperture iris diaphragm.

1. Pull out the light excluding shutter knob ① to 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 fluorescent cube into the light path.)

   ★ Do not use the U excitation cube for this adjustment. If it is inevitable to use the U excitation cube, be sure to observe the light through the UV protection shield.
3. Remove the cap of revolving nosepiece (or objective lens), and engage the empty hole in the light path.
4. Pull out the field iris diaphragm knob ② to close this iris, and push in the aperture iris diaphragm knob ③ to fully open this iris.
5. Place white paper on the stage, and push in the light excluding shutter knob ① to open the light path.
6. Screw in the burner focusing knob ⑥ fully.
7. Using the burner centering knobs ④, move the bright image into the visual field.
8. Using the collector lens focusing knob ⑤, converge light. (Fig. 18-A)
9. Using the burner centering knobs ④, set the bright image to the approximate center position. (Fig. 18-A)
10. Loosen the burner focusing knob ⑥ until the size of the bright image is reduced to a minimum. (Fig. 18-B)
11. Using the burner centering knobs ④, split the bright image into two arc images. Move the two arc images as shown in Fig. 18-C.

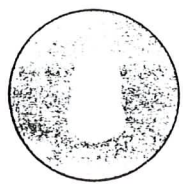


Fig. 18-A

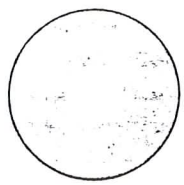


Fig. 18-B

Fig. 18-C

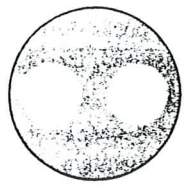


Fig. 18-D

Fig. 18-E

12. Using the burner focusing knob ⑥, make adjustment so that the sizes of two arc images will be almost identical. (Fig. 18-D)
13. Using the burner centering knobs ④, overlay the two arc images. Then, push in the field iris diaphragm knob to open the iris.
14. Adjust the collector lens focusing knob ⑤ so that the arc images will appear as shown in Fig. 18-E. This completes the burner centering adjustment.

    Note that even if the arc images deviate from the center position to some extent, it will not pose any trouble in observation practice.
15. Before proceeding to observation, adjust the collector lens focusing knob ⑤ so that illumination on the visual field will be uniform.

    ★ **To avoid serious injury, never open the lamp housing while the burner is turned on or immediately after it is turned off.**

◎ Recenter the burner each time it is replaced.

〈When not using conversion lens〉

◎ Before attempting the burner centering adjustment, wait for the arc to stabilize (5-10 minutes after turn-on).

1. Pull out the light excluding shutter knob ① to 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 fluorescent 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 in the field iris diaphragm knob ② and the aperture iris diaphragm knob ③ to fully open the iris diaphragms. (Fig. 17)
5. Push in the light excluding shutter knob ① to completely open the light path.
6. Screw in the burner focusing knob ⑥ fully.
7. Using the burner centering knobs ④, make adjustment so that two arc images will be visible as shown in Fig. 18-A'.
8. Using the collector lens focusing knob ⑤, bring either one of the two arc images into focus. (Fig. 18-B')
9. Using the burner focusing knob ⑥, make adjustment so that the sizes of two arc images will be almost identical. (Fig. 18-C')

    ★ **At this point, the two arc images may shift. In this case, adjust the positions of the arc images using the burner centering knobs ④.**
10. While turning the collector lens focusing knob ⑤ repeatedly, check that the degree of blurring of one arc image is equal to that of the other arc image. (That is, when one of two arc images is focused, check that the other arc image is in focus also.)

    If the degree of blurring of one arc image is different from that of the other arc image (the focusing positions of two arc images do not match each other), repeat the above steps 8, 9, 10.

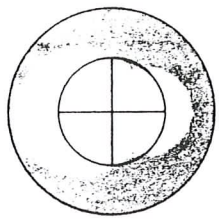


Fig. 18-A'

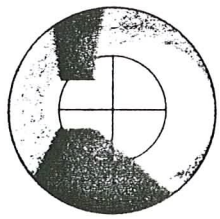


Fig. 18-B'

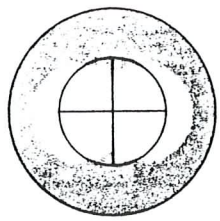


Fig. 18-C'

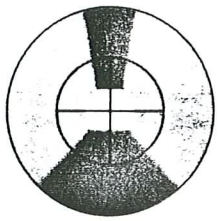


Fig. 18-D'

11. After making sure that the degree of blurring of one arc image is equal to that of the other arc image (the focusing positions of two arc images are identical), overlay the two arc images using the burner centering knobs ④ under in-focus condition.
12. After the two arc images are overlaid, they appear as shown in Fig. 18-D'. This completes the burner centering adjustment.

    Note that even if the arc images deviate from the center position to some extent, it will not pose any trouble in observation practice.
13. Before proceeding to observation, adjust the collector lens focusing knob ⑤ so that illumination on the visual field will be uniform.

    ★ To avoid serious injury, never open the lamp housing while the burner is turned on or immediately after it is turned off.

◎ Recenter the burner each time it is replaced.

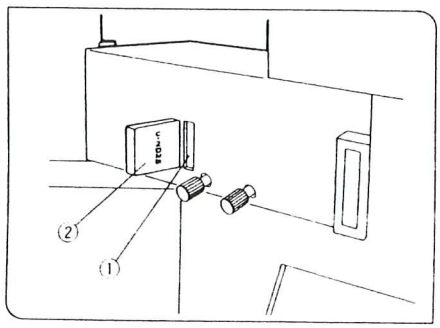


Fig. 19

7 Filter Slots (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.

★ Note that 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 Selecting a Cube

Select the cube which matches the fluorochrome in use.

★ Never mount or use the brightfield cube (U-MBF) together with mirror cubes for fluorescence. The U-MBF brightness is excessive and injury to the eyes could occur. If this type of cube is to be used together with mirror cubes for fluorescence, use the U-MBFL cube equipped with built-in ND filter.

◎ Use according to excitation wavelength. Many different cube sets are available.
Wide band sets (designated by W) are normally used. There are cases, however where superwide (SW) or narrow (N) band sets are recommended.

1. Extremely weak fluorescence brightness (only B and G excitations)
   Superwide band (SW)
   ◎ With the SWB, strong autofluorescence reduces image contrast.
2. Samples emitting strong autofluorescence
   Narrow band (N)
   ◎ 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 • DAPI: DNA staining • Hoechest 33258, 33342
	U-MNU		BP360-730		
V	U-MNV	DM455	BP400-410	BA455	• Catecholamine • Serotonin • Tetracyline
BV	U-MWBV	DM455	BP400-440	BA475	• Quinacrine, quinacrine, mustard • Thioflavine S • Acriflavine
	U-MNBV		BP420-440		
B	U-MWB	DM500	BP450-480	BA515	• FITC • Acridine orange: DNA, RNA • 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 • 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	For observing only the U excitation stain, when using U excitation stain together with FITC.
IB	U-MWIBA	DM505	BP460-490	BA515-550	For observing only FITC, when using FITC and TRITC or Texas red for double staining.
	U-MNIBA		BP470-490		

GFP Cube

IB	U-MWIB/GFP	DM505	BP460-490	BA510IF	For EGFP, S65T & RSGFP (U-MWIBA/GFP is for color separation.)
	U-MWIBA/GFP			BA510-550	

Cube Name Meaning

U – M N I B A

- U – M: Common to all cubes
- N: Wavelength SW: Superwide band W: Wide band N: Narrow band
- I: Excitation (U, V, BV, B, IB, G, IG, IY)
- B: Excitation (U, V, BV, B, IB, G, IG, IY)
- A: Cube for color separation

9 Objectives for Various Observation Modes

Objective lens		Reflected fluorescence		Transmitted phase contrast	Transmitted Nomarski DIC
		U, V, BV	B, IB, G, IY		
UPlanApo	4X	○	○	—	—
	10X	○	○	○**	○
	20X	○	○	○**	○
	40X	○	○	—	○
	40X OI	○	○	○**	○
	100X OI	○	○	○**	○
PlanApo	1.25X	—	—	—	—
	2X	—	—	—	—
	40X	—	○	—	—
	60X O	○	○	○**	○
	100X O	—	○	—	—
UPlan FI	4X	○*	○*	—	—
	10X	○*	○*	○**	○
	20X	○*	○*	○**	○
	40X	○*	○*	○**	○
	100X O, OI	○	○	○**	○
UApo	20X	○	○	—	○
	40X	○	○	—	○
	40X OI	○	○	—	○

○ : Recommended combination
○* : Usable, but image may be dark depending on NA
— : Not usable, or corresponding objective not available
○** : A phase contrast objective (Ph) is necessary for phase contrast observation.
UPlan FI100XOI phase contrast objective (Ph) is not available.

6 OBSERVATION PROCEDURE

1 Reflected Light Fluorescence Observation

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 provides intense excitation light to enable bright image observation even for a dark fluorescent specimen. Therefore, in observation with a high-magnification objective lens, fading is prone to occur fast and cause degradation in fluorescent image contrast.
To prevent this, reduce the intensity of excitation light to some extent. Thus, fading can be retarded to allow clear fluorescent imaging.
It is advisable to reduce the intensity of excitation light using the ND filter or aperture iris diaphragm wherever applicable to observation. Also, use the shutter so that the specimen will not be exposed to excitation light for a time longer than necessary.
By using a commercially available anti-fading agent (e.g. DABCO), specimen fading can be retarded. For observation at high magnification, it is recommended to use the anti-fading agent also.
★ Note that the anti-fading agent is not applicable to some kinds of specimens.

★ 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 fade rapidly, fading 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 Light Phase Contrast Observation

Phase contrast observation requires a phase contrast condenser (U-PCD) or the universal condenser (U-UCDB) and a phase contrast objective. (See paragraph 8 on page 16.)

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, or the light intensity lever on the microscope frame to adjust the transmitted light intensity.
   ★ For details on using phase contrast observation, see the instructions provided with the phase contrast condenser (BX-PHD) or the universal condenser (BX-UCDB).

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: Universal condenser (U-UCDB), transmitted light DIC slider (U-DICT), analyzer (U-AN), sextuple revolving nosepiece for DIC (U-D6RE).

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

1. Engage the dummy plate.
2. Adjust the polarizer on the universal condenser (U-UCDB).
3. Insert the transmitted light DIC slider (U-DICT) into the slot provided on the nosepiece.
4. Turn the turret on the universal condenser (U-UCDB) 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.
7. 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.
8. Turn the prism movement knob on the transmitted light DIC slider to adjust contrast of the differential interference contrast image.
9. Move the cube corresponding to the desired excitation into the light path and open the light excluding shutter.
10. 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 universal condenser (BX-UCDB).

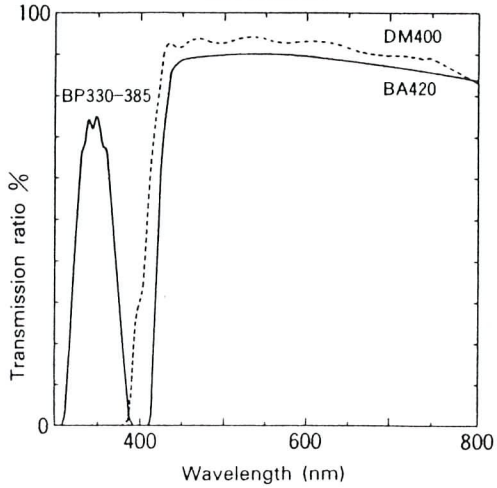
Notes:

- Use the highly wear-resistant U-ANH analyzer-slider instead of the U-AN analyzer when frequently switching between reflected light fluorescence observation and transmitted light Nomarski differential interference contrast observation or when using both modes simultaneously.
- However, if you are frequently switching between reflected light fluorescence observation and transmitted light Nomarski differential interference contrast observation but you do not need to use both simultaneously, then you should use the U-MDICT differential interference contrast cube instead of an analyzer unit (U-AN or U-ANH). This facilitates the switching operation since the analyzer automatically enters the light path when the fluorescent cube is switched to the analyzer cube.

7 TRANSMISSION CURVES OF FILTERS

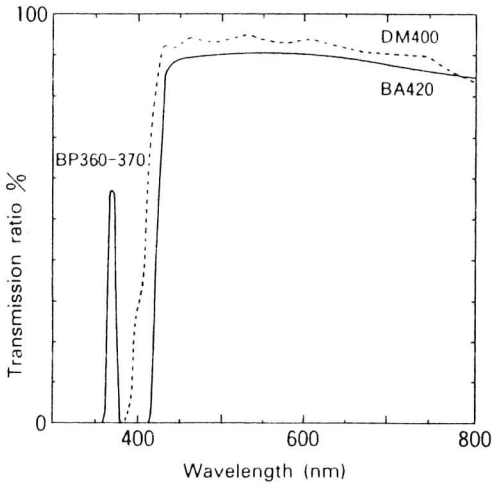
1. U excitation cube (Wide band)

U−MWU



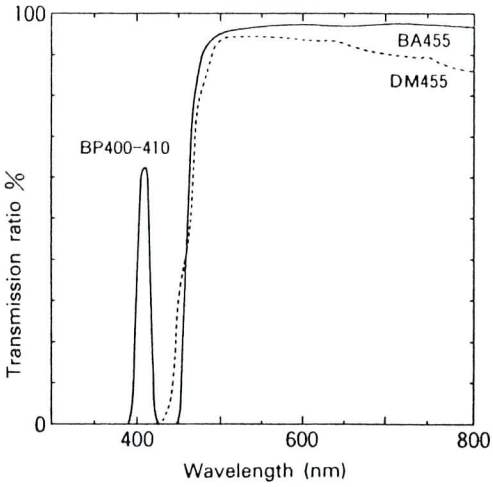
2. U excitation cube (Narrow band)

U−MNU



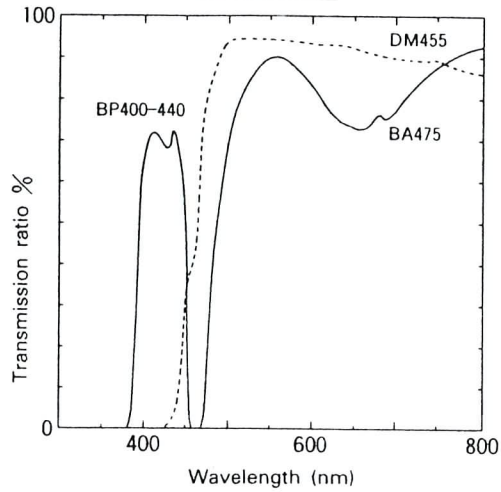
3. V excitation cube (Narrow band)

U−MNV



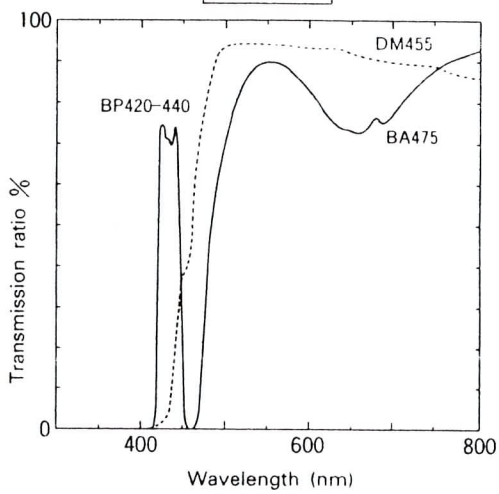
4. BV excitation cube (Wide band)

U−MWBV



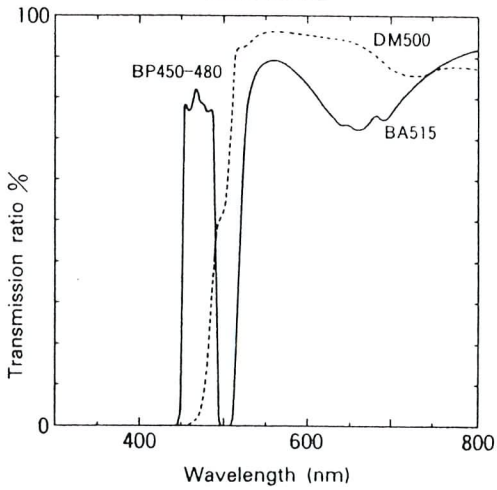
5. BV excitation cube (Narrow band)

U−MNBV



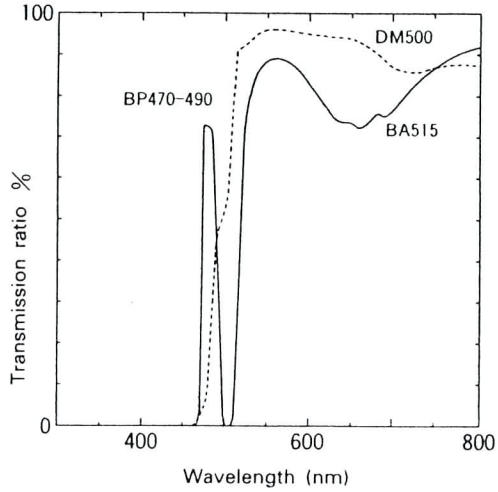
6. B excitation cube (Wide band)

U−MWB



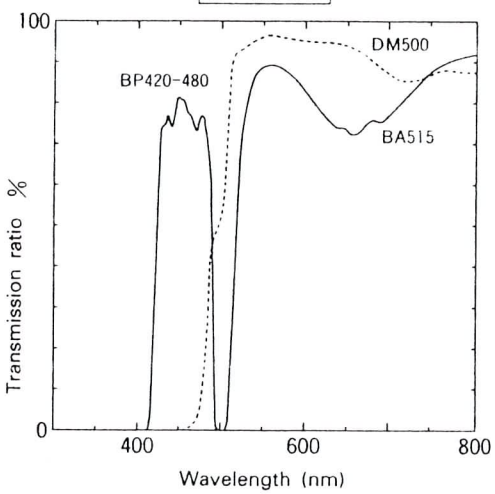
7. B excitation cube (Narrow band)

U−MNB



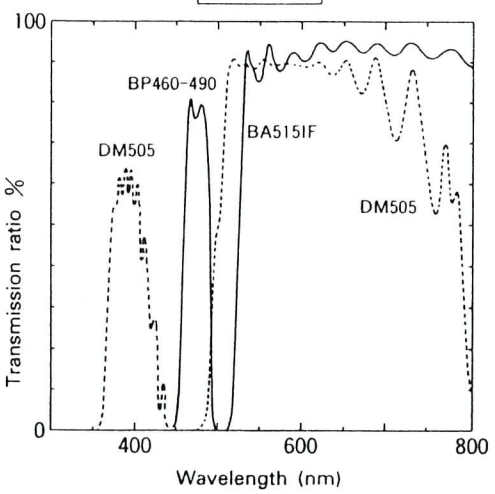
8. B excitation cube (Super wide band)

U−MSWB



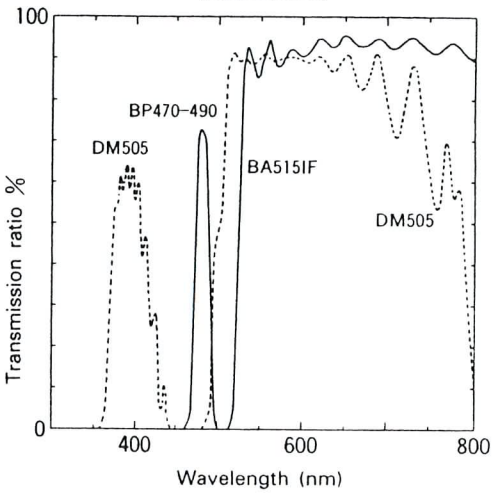
9. IB excitation cube (Wide band)

U−MWIB



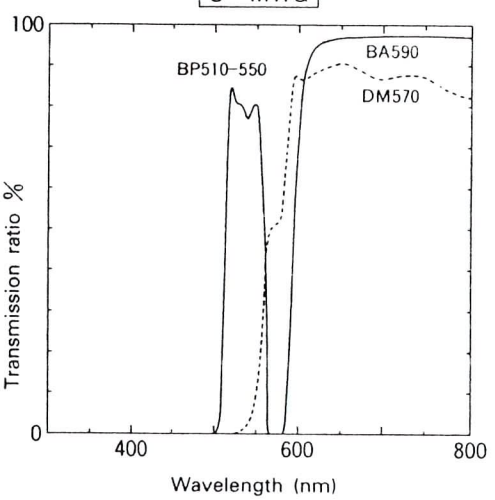
10. IB excitation cube (Narrow band)

U−MNIB



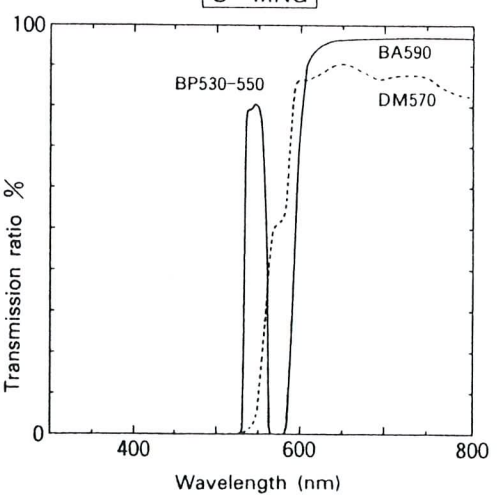
11. G excitation cube (Wide band)

U−MWG



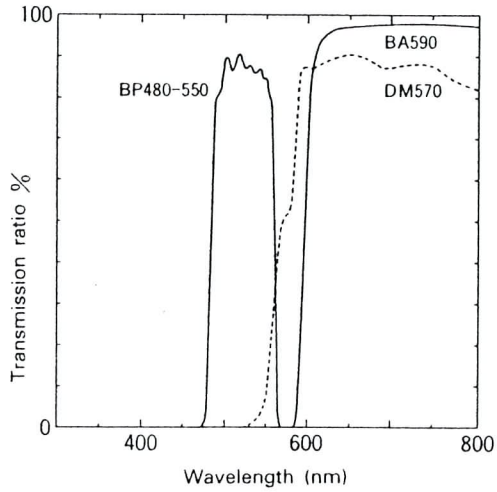
12. G excitation cube (Narrow band)

U−MNG



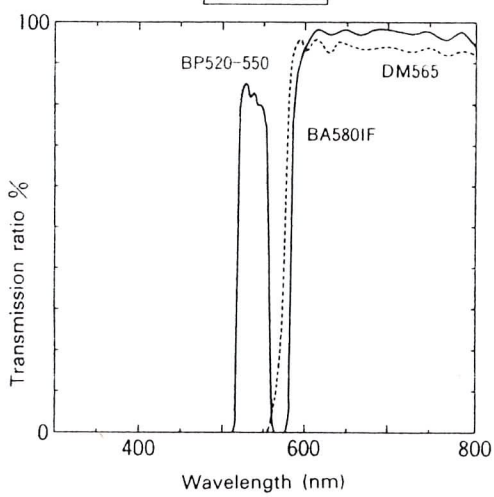
13. G excitation cube (Super wide band)

U-MSWG



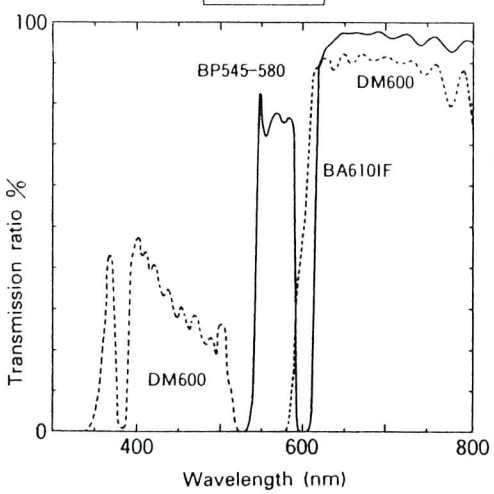
14. IG excitation cube (Wide band)

U-MWIG



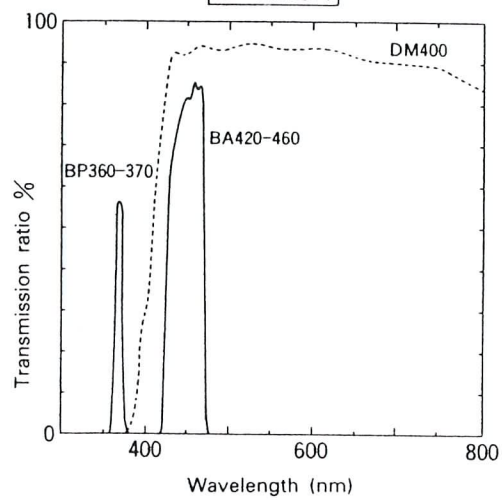
15. IY excitation cube (Wide band)

U-MWIY



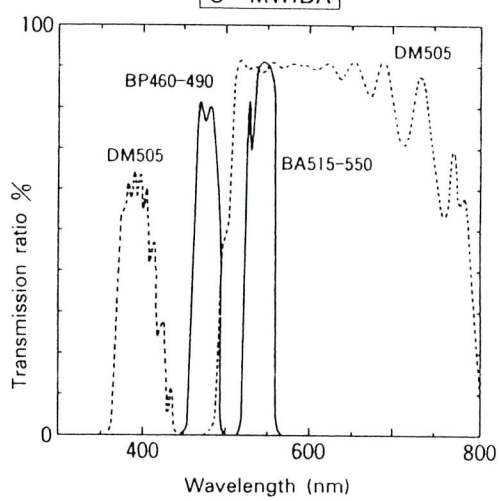
16. U excitation cube for color separation (Narrow band)

U-MNUA



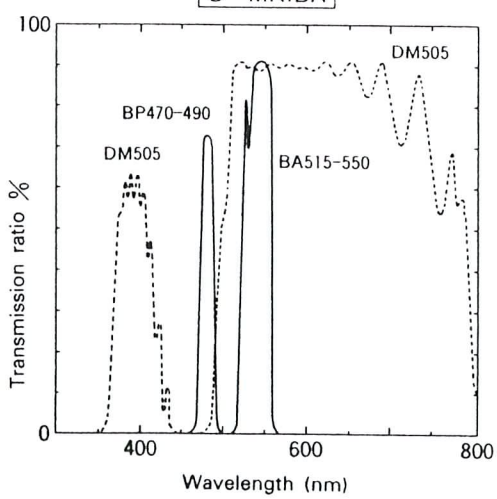
17. IB excitation cube for color separation (Wide band)

U-MWIBA



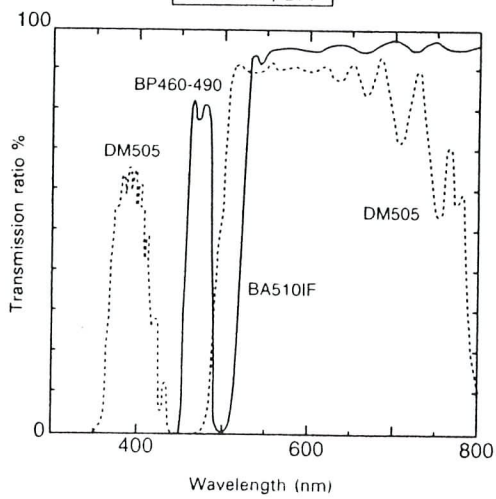
18. IB excitation cube for color separation (Narrow band)

U-MNIBA

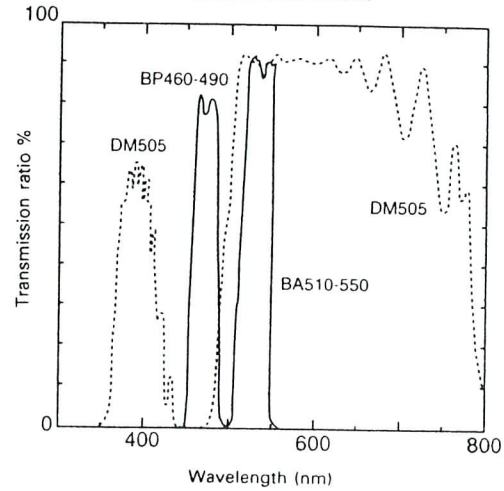


19. IB exitation cube (Wide band)

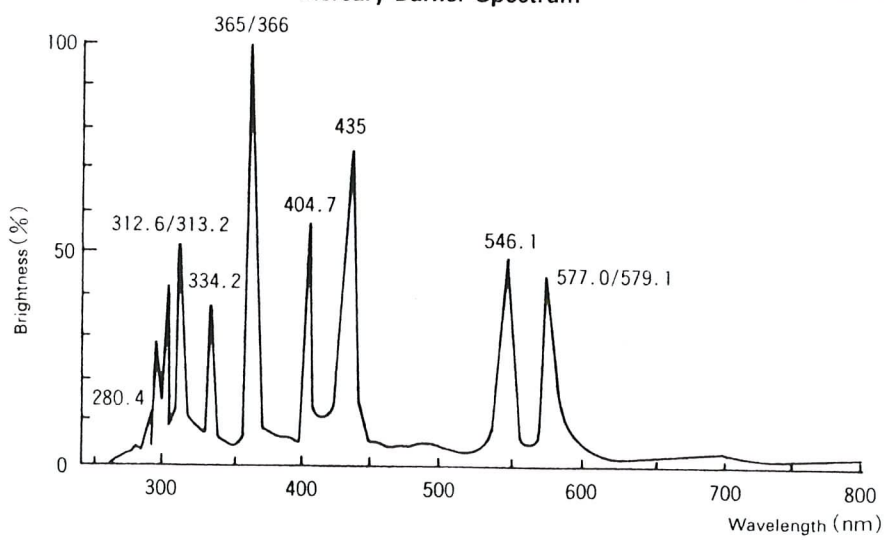
U-MWIB/GFP



20. IB exitation cube for color separation (Wide band) U-MWIBA/GFP



Typical Example of Emission Spectrum of Ultra-High-Vacuum Mercury Burner Spectrum

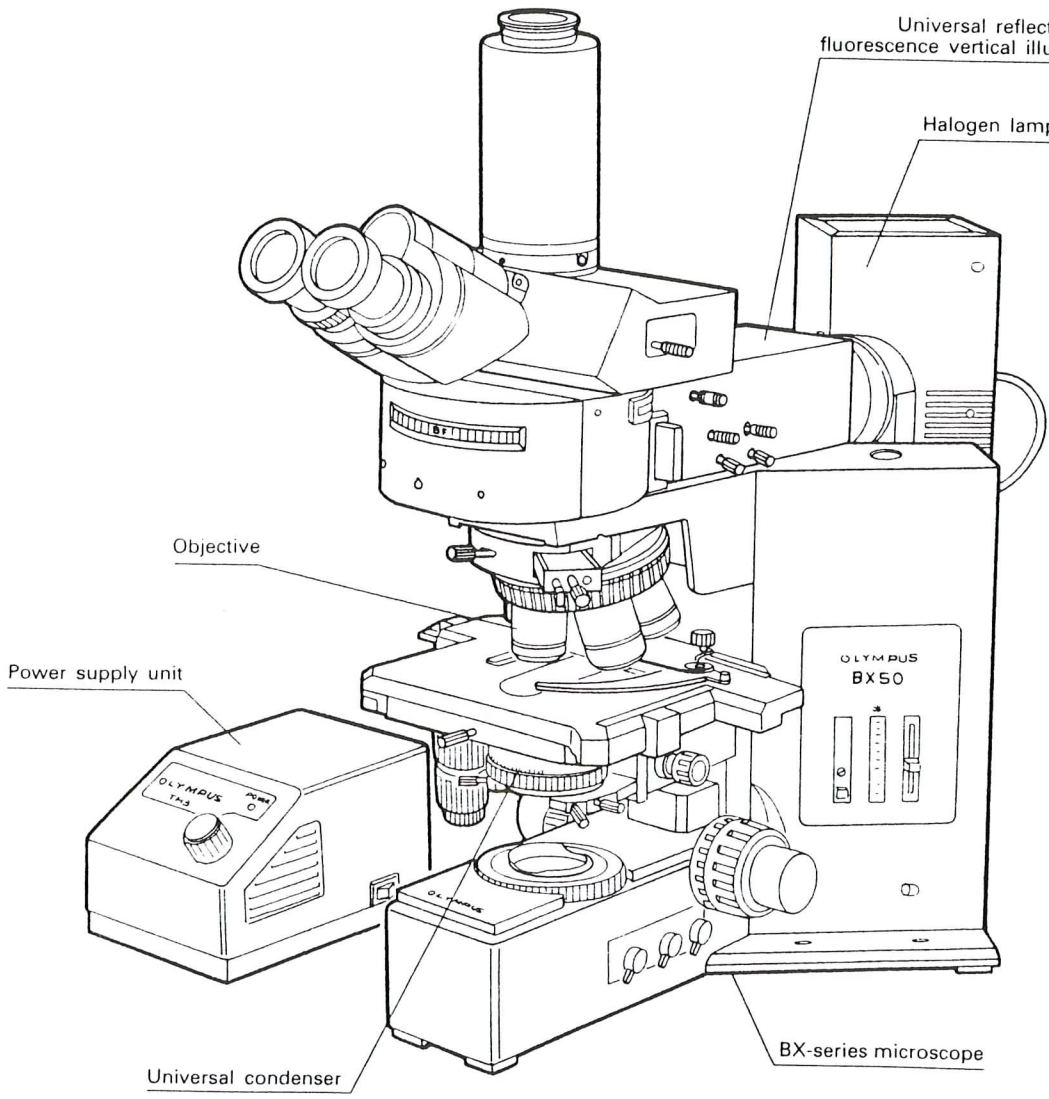


For fluorochrome emission, a light beam having a specific wavelength is selected from a wide spectrum of wavelengths. The five major peaks of luminance are at wavelengths of 365/366, 404.7, 435, 546.1 and 577.0/579.1 nm. In addition, light beams having wavelengths of 334.2 and 490 nm (with rather low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

II. REFLECTED LIGHT OBSERVATION MODES

Reflected light brightfield and darkfield observations become possible by combining the universal reflected light vertical illuminator (U-URA) with metallurgical UIS objectives, brightfield cube (U-MBF), or darkfield cube (U-MDF).

1. NOMENCLATURE



2 PARTS AND NAMES

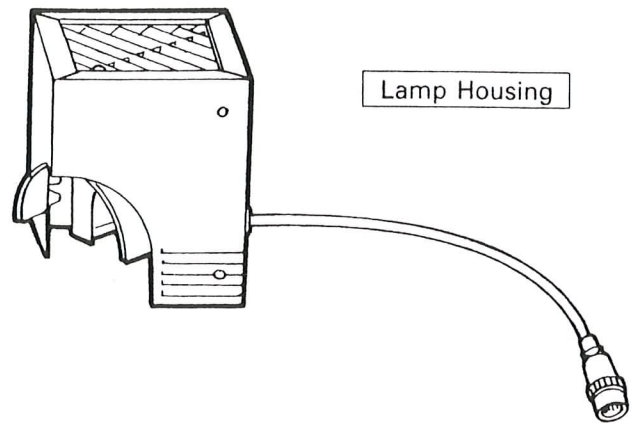
A. Universal Reflected Light Vertical Illuminator

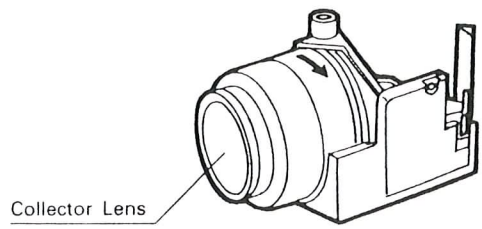
The used illuminator is identical with the Universal Reflected Light Fluorescence Vertical Illuminator shown on page 3.

B. Cube

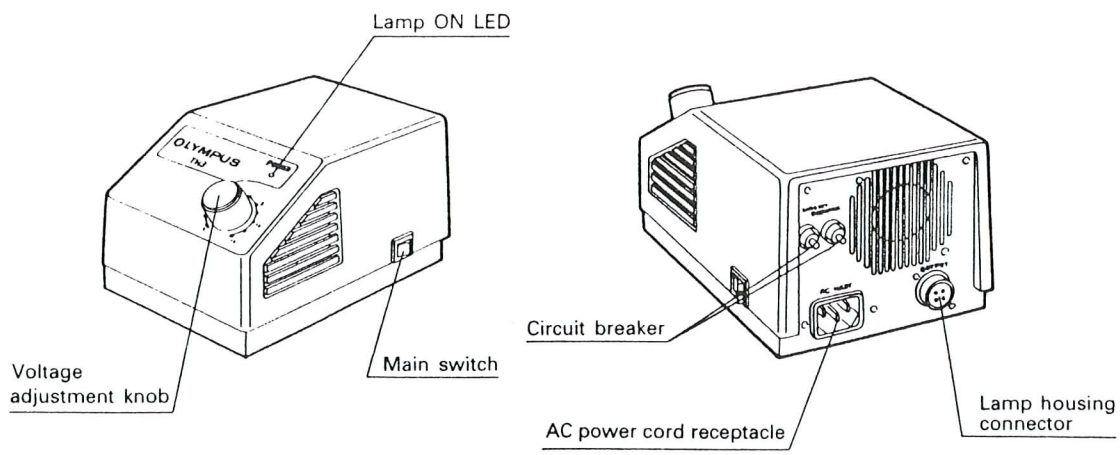
For reflected light observation with white light, use a brightfield cube (U-MBF), a darkfield cube (U-MDF) and a differential interference contrast tube (U-MDIC), etc.

C. Light Source for Reflected Light



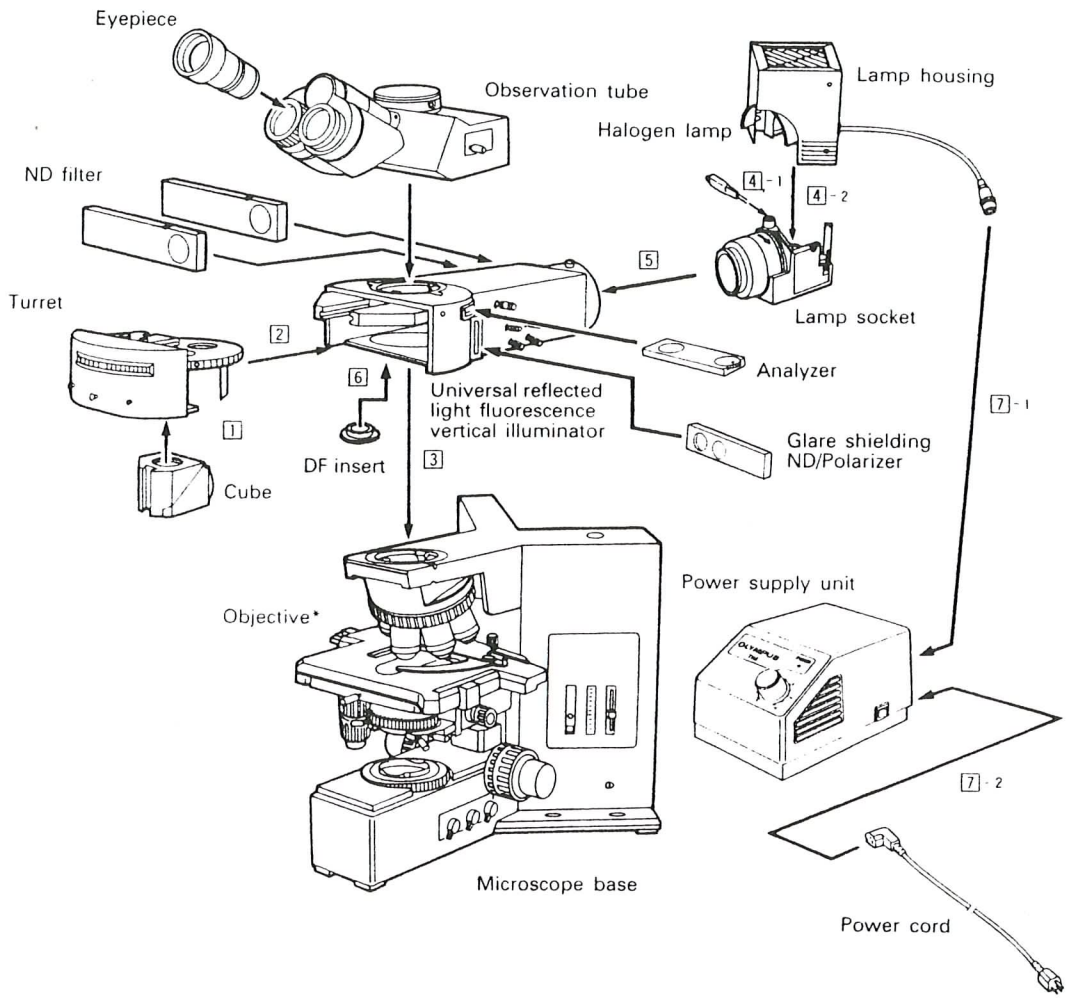


● Power Supply Unit



3 ASSEMBLY

3-1 Assembly Diagram



*When MPlan-BD objectives are used in combination with the U-ULH lamp housing (mercury burner and xenon burner) for darkfield observation, illumination at the periphery may be slightly insufficient depending on the specimen.

★ If the brightfield cube (U-MBF) is engaged while the mercury burner or xenon burner is used, the brightness will be excessive and injury to the eyes could occur. To prevent this, use the U-MBF in combination with an ND filter or use the U-MBFL cube equipped with built-in ND filter.

3-2 Detailed Assembly Diagram

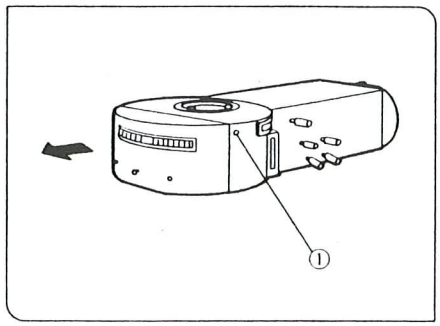


Fig. 1

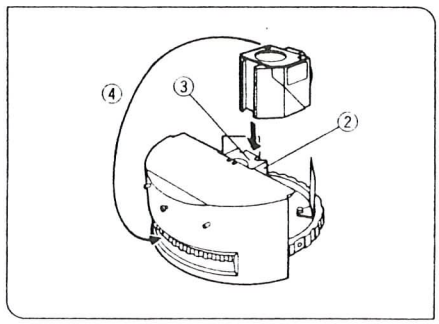


Fig. 2

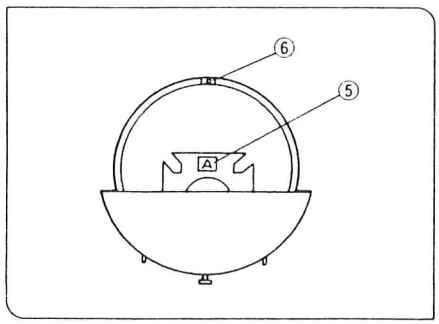


Fig. 3

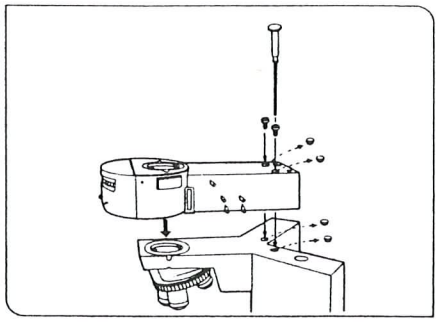
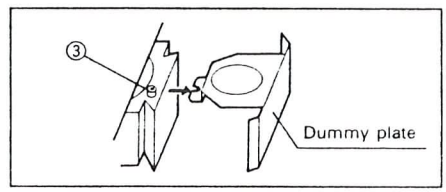


Fig. 4

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

◎ See page 25 regarding which cubes to use for different observation methods.

1. Using the Allen screwdriver provided with the microscope, loosen the clamping screw ① at the right side of the vertical illuminator. (Fig. 1)
2. Pull out the turret in the direction indicated by the arrow, then invert the turret so that the cube dovetail mounts ② point upward. (Dummy plates are mounted in three of the four cube positions. When you wish to use only one cube, mount it in the empty position. When using two or more cubes, loosen the clamping screw ③ and remove the dummy plate(s) by pulling in the direction indicated by the arrow, and then mount the actual cube(s) instead.)



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 immediately. (Tighten all four cube clamping screws.)
4. Remove the cube's 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 lift the cube's magnetic index sticker.

★ Move the magnetic cube index sticker (BF, DF, etc.) to the corresponding A, B, C, D position on the turret. The positioning indexes ⑤ A, B, C, and D on the cube dovetail mount correspond to the A, B, C and D indexes ⑥ on the turret.

2 Mounting the Turret (Fig. 1)

Insert the turret into the vertical illuminator housing and tighten the clamping screw ①.

★ When performing fluorescence observation, make sure to mount dummy plates in the empty cube positions. For transmitted light observation only, it is unnecessary to mount the dummy plates. However, be sure to tighten the cube clamping screws when no dummy plate is mounted. If the screws are left loose, the protruding screw heads may obstruct turret rotation. Mount the dummy plate by reversing the procedure described in 1-2.

3 Mounting the Universal 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 screwdriver to clamp the vertical illuminator to the microscope frame (two locations).

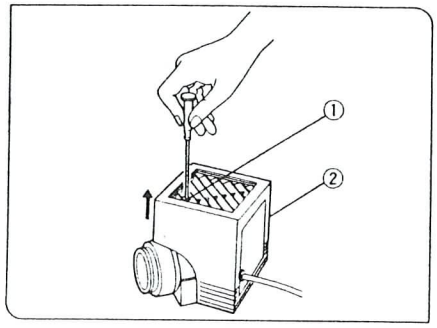


Fig. 5

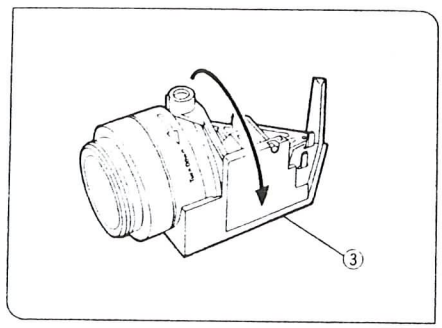


Fig. 6

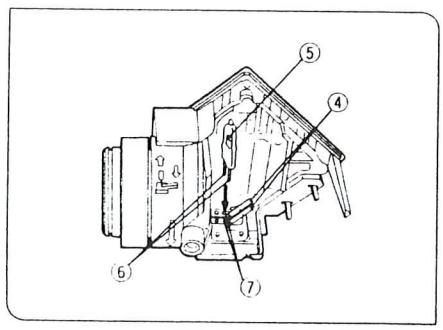


Fig. 7

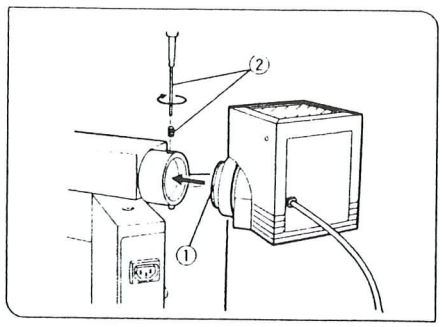
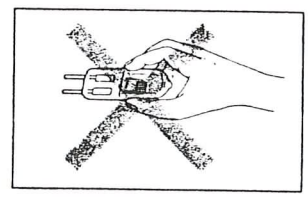


Fig. 8

4 Installing the Halogen Bulb (Figs. 5, 6, 7)

◎ The applicable bulb is a 12V, 100WHAL halogen bulb (Philips 7724).

1. Fully loosen the lamp housing clamping screw ① on top of the lamp housing cover with the provided Allen screwdriver. (Fig. 5)
2. Lift the lamp housing cover ② upward to remove it.
3. Turn the lamp socket ③ 90° in the direction indicated by the arrow. (Fig. 6)
4. Holding the bulb ⑤ with gloves or a piece of gauze, depress the bulb clamping levers ④ and insert the bulb pins ⑥ fully into the pin holes ⑦. Gently release the bulb clamping levers ④ to their original positions to secure the bulb. (Figs. 6, 7)



★ Do not touch the bulb with bare hands. If fingerprints or other smears are accidentally left on the bulb, wipe it clean with a piece of soft cloth.

5. Slide the lamp housing cover onto the housing base from above. Tighten the clamping screw ① while pressing downward on the cover. (Fig. 5)

★ Whenever you replace the bulb, first turn OFF the main switch and wait for bulb, lamp socket and lamp house to cool.

5 Mounting the Lamp Housing (Fig. 8)

1. Insert the collector lens portion ① of the 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 screwdriver.

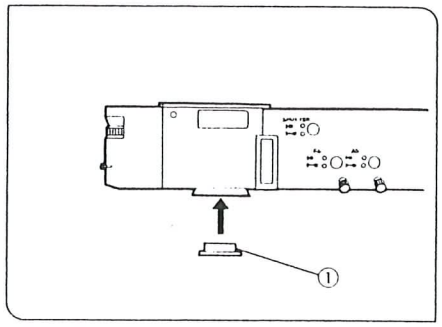


Fig. 9

6 Mounting the DF Insert (Fig. 9)

1. Remove the revolving nosepiece.
2. Mount the DF insert ① making sure it is held securely in place by the magnet on the underside of the vertical illuminator.
3. Reattach the revolving nosepiece.

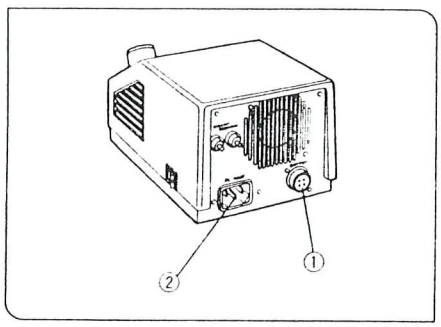


Fig. 10

7 Connecting the Lamp Housing Cord (Fig. 10)

1. Connect the lamp housing cord connector to the lamp housing connector ① on the power supply unit.
2. Connect one end of the power cord to the AC power cord receptacle ② and the other end to an AC outlet.

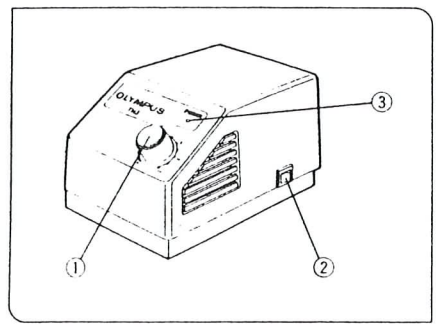


Fig. 11

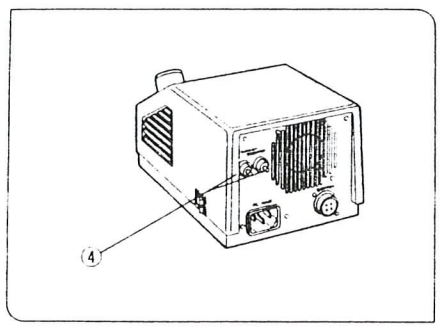


Fig. 12

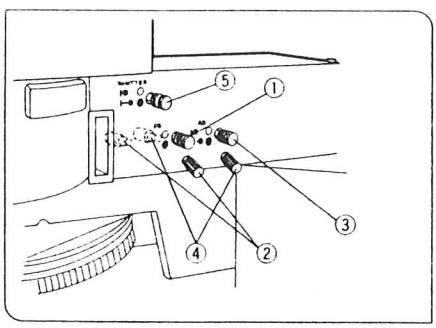
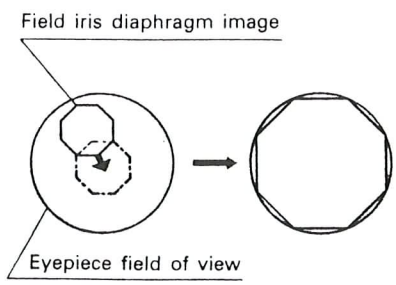


Fig. 13



1 Adjusting the Illumination Brightness (Figs. 11,12)

1. After making sure the transformer's voltage adjustment knob ① is turned to the "2" side (low voltage side), turn ON the main switch ②.
   - At this point, the operating indicator LED ③ (green) goes on.
2. Rotating the voltage adjustment knob ① clockwise (toward "12" on the scale) will raise the voltage. (Fig. 11)
3. Rotate the voltage adjustment knob to adjust the brightness to a level suitable for the observation.
   - To keep the color temperature of the light source uniform during photomicrography, adjust with an ND filter.

Circuit Breaker Reset (Fig. 12)

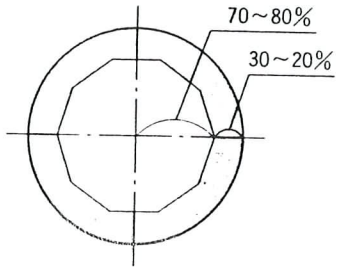
The circuit breakers ④ will activate in case of brightness adjustment circuit malfunction (short circuit, etc.) or in case of overcurrent. When they activate, the center portion of the breaker will pop up and the power will be cut off.
To reset the breakers, press in the center portion. If a breaker activates again, unplug the power cord from the AC outlet and contact your Olympus representative.

2 Centering the Field Iris Diaphragm (Fig. 13)

1. Rotate the revolving nosepiece to engage the 10X objective, then place a specimen on the stage and bring the image into approximate focus.
2. Pull out the field iris diaphragm knob 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 adjust so that the image of the diaphragm is centered in the field of view.
4. To check centering, open the diaphragm by pushing in the field iris diaphragm knob ① until the diaphragm image touches the perimeter of the field of view. If the image is not centered precisely, center it again.
5. Further enlarge the field iris diaphragm diameter until its image just circumscribes the field of view.

3 Adjusting the Field Iris Diaphragm (Fig. 13)

- **Reflected light brightfield observation**
  To obtain good image contrast, adjust the diameter of the illuminating beam in accordance with the objective in use. Using the field iris diaphragm knob ① on the vertical illuminator, adjust the diaphragm so that the field of view is circumscribed by the field iris diaphragm in order to exclude stray light.
- **Reflected light darkfield observation**
  Alway keep the field iris diaphragm knob ① pushed in to leave the diaphragm open.



4 Centering the Aperture Iris Diaphragm (Fig. 13)

1. Rotate the revolving nosepiece to engage the 10X objective, then place a specimen on the stage and bring the image into approximate focus.
2. Remove the eyepieces. Looking at the objective pupil inside the observation tube, pull out the aperture iris diaphragm knob ③ to leave the diaphragm stopped down to approximately 70-80% of the objective pupil.
3. At this point, if the diaphragm is not centered precisely, center it with the objective pupil by manipulating the two aperture iris diaphragm centering knobs ④.

◎ Using the centering telescope U-CT30 facilitates centering.
◎ When using over 100X objectives, use the centering telescope for the centering. Centering may also be done by observing through the eyepiece and centering until the optimal specimen contrast condition is obtained.

5 Adjusting the Field Iris Diaphragm (Fig. 13)

- Reflected light brightfield observation
  Adjust the numerical aperture of the illumination system to control the depth of focus, contrast, resolution, etc.
- Reflected light darkfield observation
  Always keep the aperture iris diaphragm knob ③ pushed in to leave the diaphragm open.

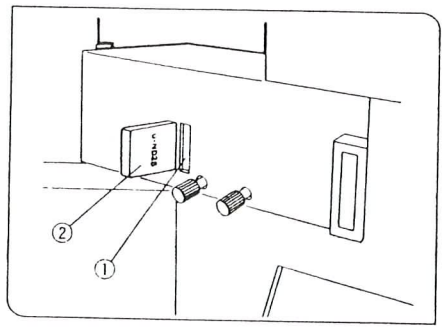


Fig. 14

6 Filter Slots (Fig. 14)

As necessary, up to two filters may be individually inserted into the filter slots ① and ②. Insert the filter with the marked side facing toward the observer.
As you insert the filter, you will hear two clicks. At the first, the filter is in the empty position, and at the second the filter is engaged in the light path.

	Usabel filters	Application
①	U-FR (Frost filter)	To eliminate uneven illumination.
②	U-LBD (Color temperature conversion filter)	To convert the color temperature of the source to the color temperature of daylight. Used for comfortable observation and when taking color photographs.
	U-IF550 (Green filter)	To increase contrast during monochrome observation. Used when taking monochrome photographs.
	U-ND25 (Neutral density filter)	To adjust illumination brightness. (Transmission ratio 25%)
	U-ND6 (Neutral density filter)	To adjust illumination brightness. (Transmission ratio 6%)

5 OBSERVATION METHODS

5-1 Reflected Light Brightfield/Darkfield Observation

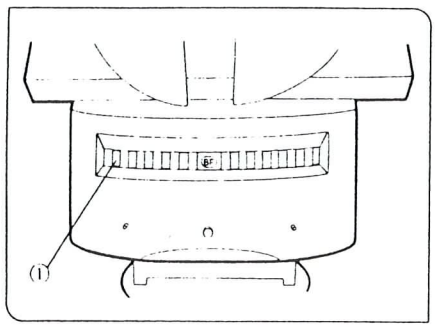


Fig. 15

1 Selecting the Light Path for Observation (Fig. 15)

Rotate the cube tube turret to engage either the BF or DF cube ① according to the desired observation method.

	Cube symbol	Field iris diaphragm	Aperture iris diaphragm	Glare shielding ND
Reflected brightfield	BF	Adjust as necessary		IN
Reflected darkfield	DF	Open		

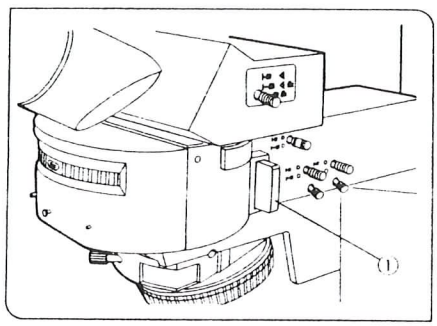


Fig. 16

2 Glare Shielding ND Filter (U-DND) (Fig. 16)

1. Insert the glare shielding ND slider ① into the filter slot on the right side of the vertical illuminator.
2. As you insert the filter, you will hear two clicks. At the first, the ND filter is in the empty position, and at the second the filter is in the light path.
3. Ordinarily, if the ND filter is in the light path, it will prevent the glare effect otherwise noticeable when switching from darkfield to brightfield.

◎ When the illumination is too low during brightfield observation, or if needed to shorten the exposure time during photomicrography, or to brighten the field of view during darkfield observation, remove the filter from the light path.

5-2 Reflected Light Nomarski Differential Interference Contrast Observation

★ When using the DIC prism U-DICRH for observation with sensitive color, use it in combination with the polarizer U-POTP.

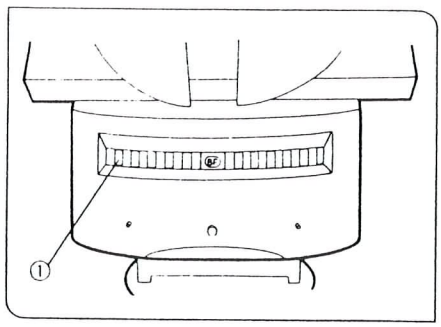


Fig. 17

1 Selecting the Light Path (Fig. 17)

1. Rotate the cube turret to engage the BF cube ① into the light path.

◎ When the U-MDIC differential interference contrast cube is inserted in the cube cassette, engage this cube in the light path. The analyzer and polarizer are set to the "crossed Nicol" position (complete extinction) so adjustment is not required.

2. Insert the analyzer (U-AN360) and the polarizer (U-PO) to engage them both into the light path.
3. Rotate the analyzer dial until complete extinction is obtained ("crossed Nicol" position).

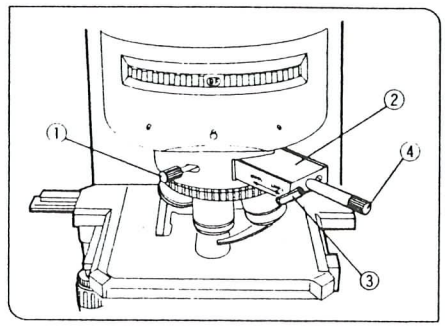


Fig. 18

2 Installing the Nomarski Prism (Fig. 18)

1. Loosen the DIC clamping screw ① at the front of the revolving nosepiece, and insert the U-DICR differential interference contrast prism ② with the inscription facing upward. Tighten the clamping screw to secure the prism.
2. If a UMPlan objective is used, push in the selector lever ③. If an LMPlan objective is used, pull out the selector lever.

★ Since the U-DICRH is not provided with a selector lever, it cannot be used with the LMPlanFl series and LMPlanApo series objectives.

3 Observation Method (Fig. 18)

U-DICR

1. Rotate the prism control knob ④ of the DIC prism slider to select the interference color of the background, and to achieve the maximum contrast depending on the specimen under observation, as outlined below. (Fig. 18)
2. Rotating the prism control knob of the U-DICR slider will continuously change the interference color of the background from gray to magenta (–100 - 600 nm).

- If the background color is black, (0-order fringe), darkfield-like observation is possible.
- If the background color is gray, a three-dimensional looking image with maximum contrast can be obtained.
- If the background color is magenta, even a minor optical retardation can be observed as a color change.

U-DICRH

1. Rotate the prism control knob ④ of the DIC prism slider to select the interference color of the background, and to achieve the maximum contrast depending on the specimen under observation, as outlined below. (Fig. 18)
2. Rotating the prism control knob of the U-DICRH slider will continuously change the interference color of the background from –100 to 100 nm. Select the retardation offering optimum contrast.

- If the background color is black, (0-order fringe), darkfield-like observation is possible.
- If the background color is gray, a three-dimensional looking image with maximum contrast can be obtained.
- If the background color is magenta, even a minor optical retardation can be observed as a color change.

  To use the magenta sensitive color, use the polarizer U-POTP. Position the polarizer so that the λ symbol can be seen from the front when the polarizer is inserted into the slot.

  ★ Care should be taken to keep the specimen surface clean, as even a small amount of contamination on the surface may show up due to the exceptionally high sensitivity of the differential interference contrast method.

◎ Stopping down the aperture iris diaphragm may increase the contrast somewhat.

4 Switching Between Brightfield and Darkfield Observation (Fig. 18)

1. Loosen the DIC clamping screw ① at the front of the revolving nosepiece, and gently pull the U-DICR differential interference contrast prism ② outward until a click is heard. Tighten the clamping screw again.
2. Rotate the turret to disengage the U-MDIC differential interference contrast cube.

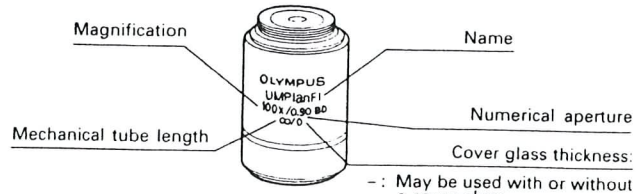
5-3 Reflected Light Simple Polarized Light Observation

◎ To prepare for simple polarized light observation using the vertical illuminator, perform paragraph 1 in Section 5-2, Reflected Light Nomarski Differential Interference Contrast Observation outlined on page 32

1 Observation

1. Place the specimen on the stage and move the stage to bring the specimen into focus. Simple polarized light observation is now possible.
2. Adjust the field iris diaphragm until the diaphragm opening circumscribes the field of view.
3. Stopping down the aperture iris diaphragm may increase the contrast somewhat.

6 OPTICAL CHARACTERISTICS



–: May be used with or without a cover glass
O: Used without a cover glass

Optical character / Objectives	Mag.	N.A.	W.D. (mm)	Cover glass thickness	Resolution (μm)	Eyepiece						Remarks
						WH10X (FN22)			WH15X (FN14)			
						Total mag.	Depth of focus (μm)	Field of view	Total mag.	Depth of focus (μm)	Field of view	
MPlan Plan Achromat (FN22)	5X 10X 20X 50X 100X	0.10 0.25 0.40 0.75 0.90	19.6 10.6 1.3 0.38 0.21	— — 0 0 0	3.36 1.34 0.84 0.45 0.37	50X 100X 200X 500X 1000X	97.5 18.4 6.09 1.42 0.73	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	74.2 13.7 4.64 1.11 0.60	2.8 1.4 0.7 0.28 0.14	
MPlan-BD* Plan Achromat for brightfield/ darkfield (FN22)	5X 10X 20X 50X 100X	0.10 0.25 0.40 0.75 0.90	12.0 6.5 1.3 0.38 0.21	— — 0 0 0	3.36 1.34 0.84 0.45 0.37	50X 100X 200X 500X 1000X	97.5 18.4 6.09 1.42 0.73	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	74.2 13.7 4.64 1.11 0.60	2.8 1.4 0.7 0.28 0.14	
UMPlan FI Universal Plan Semi Apochromat (FN26.5)	5X 10X 20X 40X 50X 100X	0.15 0.30 0.46 0.75 0.80 0.95	20.0 10.1 3.1 0.63 0.66 0.31	— — 0 0 0 0	2.24 1.12 0.73 0.45 0.42 0.35	50X 100X 200X 400X 500X 1000X	58.9 14.7 5.10 1.66 1.30 0.67	4.4 2.2 1.1 0.55 0.44 0.22	75X 150X 300X 600X 750X 1500X	43.3 10.8 3.84 1.27 1.01 0.55	2.8 1.4 0.7 0.35 0.28 0.14	
UMPlan FI-BD Universal Plan Semi Achromat for brightfield/ darkfield (FN26.5)	5X 10X 20X 50X 100X	0.15 0.30 0.46 0.80 0.90	12.0 6.5 3.0 0.66 0.31	— — 0 0 0	2.24 1.12 0.73 0.45 0.37	50X 100X 200X 500X 1000X	58.9 14.7 5.10 1.30 0.73	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	43.3 10.8 3.84 1.01 0.60	2.8 1.4 0.7 0.28 0.14	
UMPlan FI-BDP Universal Plan Semi Apochromat for reflected light polarized light (FN26.5)	5X 10X 20X 50X 100X	0.15 0.25 0.40 0.75 0.90	12.0 6.5 3.0 0.66 0.31	— — 0 0 0	2.24 1.34 0.84 0.45 0.37	50X 100X 200X 500X 1000X	58.9 18.4 6.09 1.42 0.73	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	43.3 13.7 4.64 1.11 0.60	2.8 1.4 0.7 0.28 0.14	
LMPlan FI Long working distance Plan Semi Apochromat (FN26.5)	5X 10X 20X 50X 100X	0.13 0.25 0.40 0.50 0.80	22.5 21.0 12.0 10.6 3.4	— — 0 0 0	2.58 1.34 0.84 0.67 0.42	50X 100X 200X 500X 1000X	70.1 18.4 6.09 2.50 0.87	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	52.2 13.7 4.64 2.03 0.72	2.8 1.4 0.7 0.28 0.14	
LMPlan FI-BD Long working distance Plan Semi Apochromat for brightfield/darkfield (FN26.5)	5X 10X 20X 50X 100X	0.13 0.25 0.40 0.50 0.80	15.0 10.0 12.0 10.6 3.3	— — 0 0 0	2.58 1.34 0.84 0.67 0.42	50X 100X 200X 500X 1000X	70.1 18.4 6.09 2.50 0.87	4.4 2.2 1.1 0.44 0.22	75X 150X 300X 750X 1500X	52.2 13.7 4.64 2.03 0.72	2.8 1.4 0.7 0.28 0.14	
MPLan Apo Plan Apochromat (FN26.5)	20X 50X 100X 100XO	0.60 0.95 0.95 1.40	0.9 0.3 0.35 0.08	0 0 0 0	0.56 0.35 0.35 0.24	200X 500X 1000X 1000X	3.68 1.04 0.67 0.59	1.1 0.44 0.22 0.22	300X 750X 1500X 1500X	2.71 0.80 0.55 0.47	0.7 0.28 0.14 0.14	O: Immersion
MPlan Apo BD Plan Apochromat for brightfield/darkfield (FN26.5)	100X	0.90	0.31	0	0.37	1000X	0.73	0.22	1500X	0.60	0.14	
SLMPlan Ultra long working distance Plan Apochromat (FN26.5)	20X 50X	0.35 0.45	21.0 15.0	0 0	0.96 0.75	200X 500X	7.24 2.91	1.1 0.44	300X 750X	3.47 2.40	0.7 0.28	
LMPlan Apo Long working distance Plan Semi Apochromat (FN26.5)	150X 250X	0.9 0.9	1.0 0.80	0 0	0.37 0.37	1500X 2500X	0.60 0.50	0.15 0.09	2250X 3750X	0.51 0.44	0.09 0.06	
LMPlan Apo-BD Long working distance Plan Apochromat (FN26.5)	150X 250X	0.9 0.9	1.0 0.80	0 0	0.37 0.37	1500X 2500X	0.60 0.50	0.15 0.09	2250X 3750X	0.51 0.44	0.09 0.06	

*When MPlan-BD objectives are used in combination with the U-ULH-lamp housing (mercury and xenon sockets) for darkfield observation, the peripheral field of view may be insufficiently illuminated

III. TROUBLESHOOTING GUIDE

Fluorescence Observation

Trouble	Cause	Remedy
1. Optical system		
a. The bulb is on, but image cannot be seen or is dark.	The shutter is closed or the ND filter is engaged.	Open the shutter, or remove ND filter from optical path.
	The cube is not engaged correctly.	Engage the cube correctly into the light path.
	The aperture iris diaphragm and field iris diaphragm are not sufficiently opened.	Open the aperture iris diaphragm sufficiently. 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 engaged correctly.	Engage the cube correctly into light path.
	The field iris diaphragm is stopped down too far.	Open the field iris diaphragm sufficiently.
	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.
d. Dark spots in the field of view.	Dust or other contaminants on bulb or bulb facing collector lens side.	Clean carefully.
2. Electrical system		
a. Main switch indicator does not light up.	The power cord is connected incorrectly.	Connect correctly.
b. Main switch indicator lights, but mercury burner does not ignite.	Connectors are connected incorrectly.	Connect correctly.
	The burner has not been installed.	Install the burner.
	The lamp housing interlock is operating.	Tighten the burner socket locking screw securely.
	Auto ignition is not operating as intended.	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.

Reflected Light Observation Modes

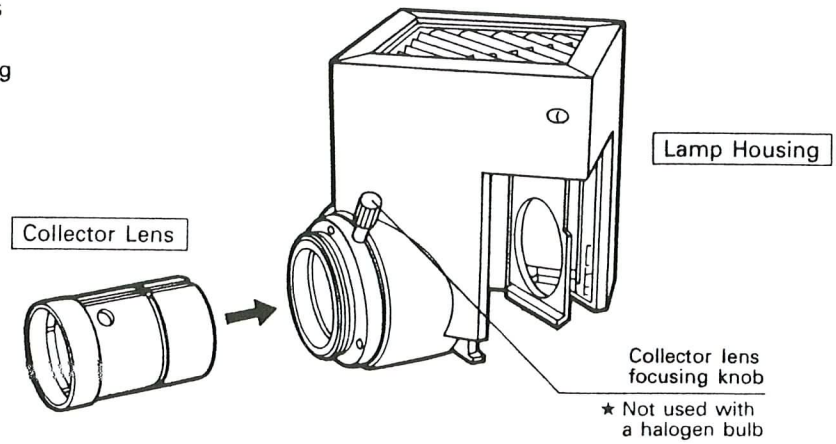
Trouble	Cause	Remedy
a. Lamp lights, but field of view remains dark.	Halogen bulb for reflected light is not ignited.	Ignite the bulb.
	The aperture and field iris diaphragms are stopped down during reflected light darkfield observation.	Open the aperture and field iris diaphragm.
	Cube is not mounted.	Mount cube.
	The cube is not engaged correctly.	Engage the cube correctly in the light path.
	Correct cube for the observation mode is not engaged.	Mount correct cube for the observation mode in turret and engage in light path.
b. Field of view is obscured, or field of view is not evenly illuminated.	The field iris diaphragm is not properly centered.	Center the field iris diaphragm correctly.
	The field iris diaphragm is stopped down too far.	Open the field iris diaphragm sufficiently.
	The halogen bulb is not centered correctly.	Adjust the centering correctly.
	Frost slider is not engaged.	Engage frost slider in light path.
	Filter(s) stopped at intermediate position.	Set filter(s) at click-stop positions.
c. The image shows diffraction.	The aperture iris diaphragm is stopped down too far.	Open the aperture iris diaphragm sufficiently.
d. Visibility is poor. • Image is not sharp. • Contrast is poor. • Details are indistinct.	A non-UIS series objective used.	Use only UIS series objectives with this unit.
	The front lens of the objective is dirty.	Clean the objective.
	Immersion oil is not being used with an oil immersion objective.	Use immersion oil.
	Recommended immersion oil not used.	Use the provided immersion oil.
	DF insert not mounted.	Mount DF insert.
e. Part of the image is blurred.	The specimen is placed in a tilted position on the stage.	Place the specimen correctly on top of the stage and secure it with the specimen holder.
	The revolving nosepiece is not properly mounted.	Mount the nosepiece correctly.
	The objective is not correctly engaged in the light path.	Make sure that the revolving nosepiece clicks into place correctly.

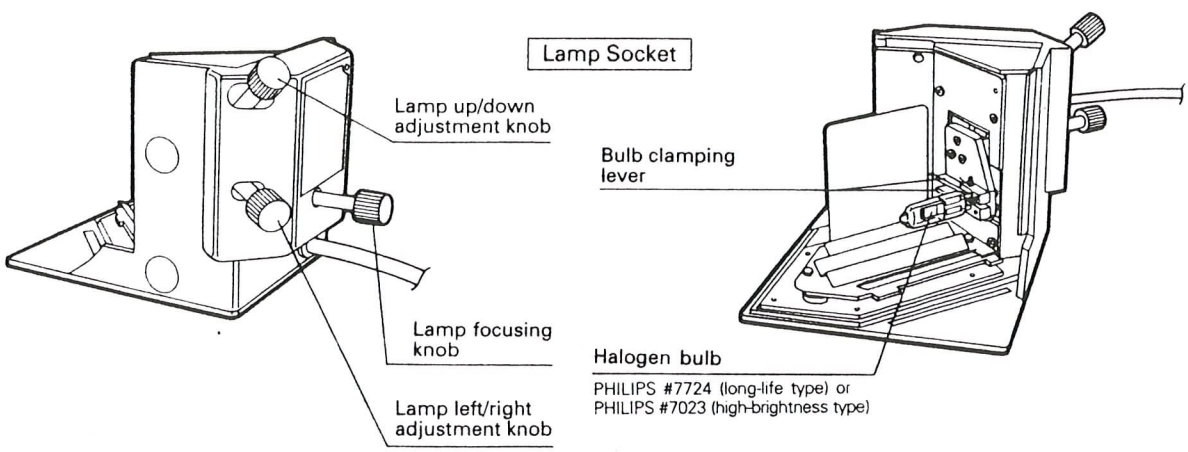
APPENDIX

USING HALOGEN LAMP HOUSING

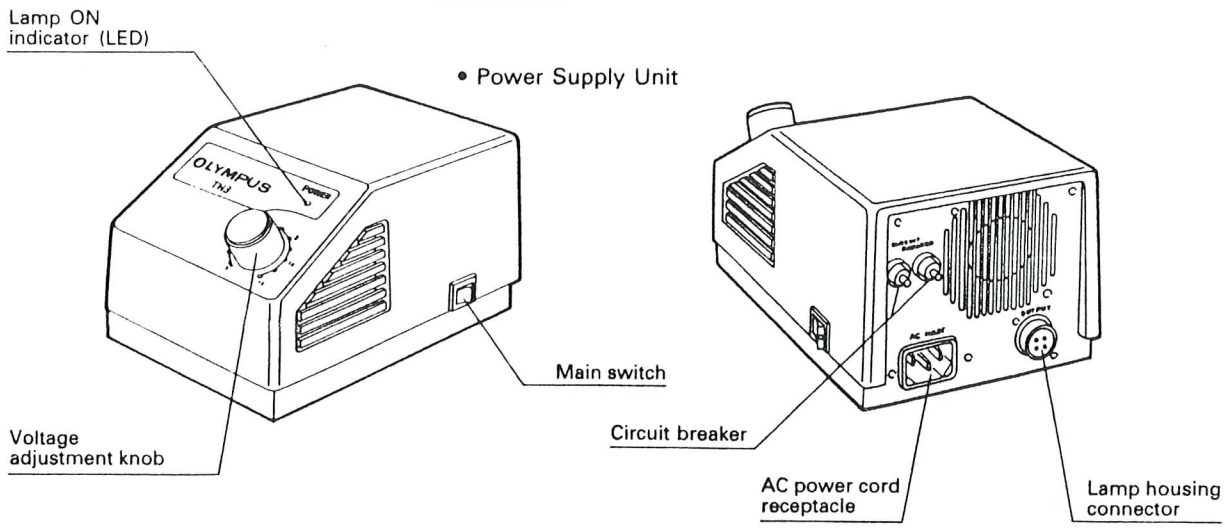
1. Parts and Names

• Halogen Lamp Housing





• Power Supply Unit



2. Assembling the Halogen Lamp Housing

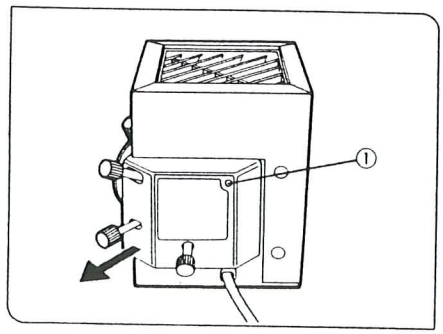


Fig. 20

Mounting the Halogen Bulb (Figs. 20, 21)

1. Remove the socket clamping screw ① using the Allen screwdriver provided with the microscope frame. (Fig. 20)
2. Remove the socket from the lamp housing as indicated by the arrow.

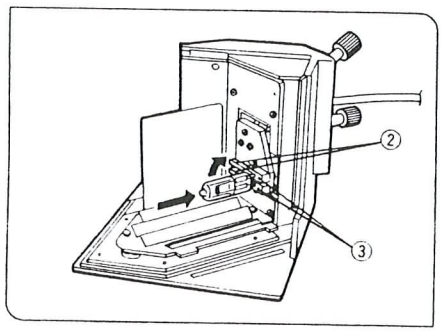


Fig. 21

3. Holding the bulb with gloves or a piece of gauze, depress the bulb clamping levers ②, and insert the bulb pins fully into the pinholes ③. Gently release the bulb clamping levers ② to their original positions to secure the bulb. Whenever you replace the bulb, first turn off the main switch and wait for bulb, lamp socket and lamp house to cool down. (Fig. 21)

★ Use only the 12V 100W halogen lamp (PHILIPS #7724 or #7023).
★ Do not touch the halogen bulb with bare hands. If the bulb surface is contaminated with fingerprints or dust, wipe the bulb with a soft cloth or gauze.

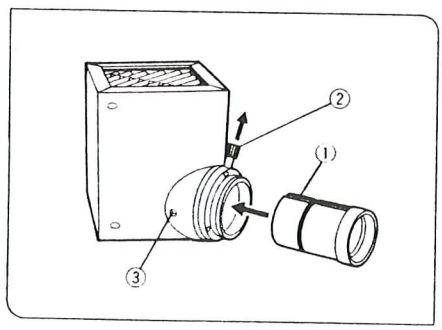


Fig. 22

Mounting the Collector Lens and Socket (Figs. 22, 23)

1. Align the collector lens positioning groove ① with the pin inside the lamp mount. Then, pull up the collector lens focusing knob ②, and insert the collector lens until it comes to the end. (Fig. 22)
2. Securely fasten the collector lens with the clamping screw ③.

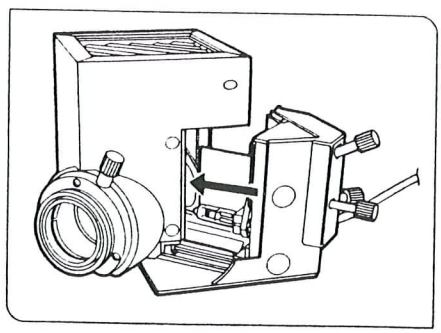


Fig. 23

3. Remount the lamp socket in the reverse order of removal. (Fig. 23)
4. Tighten the socket clamping screw (① in Fig. 20) using the Allen screwdriver.

3. Preparing the Power Supply Unit

◎ When the transmitted light source is used simultaneously, the built-in power supply of the BX microscope is not usable. The TH3 power supply unit is required in this case.
When only the vertical illumination is used, the built-in power supply of the BX50 microscope can be used. (Connect the LH connector to the microscope connector.)

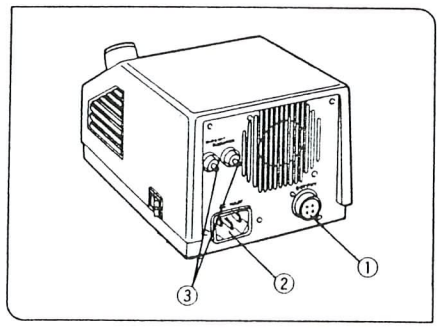


Fig. 24

Cord Connections (Fig. 24)

1. Connect the lamp housing cord connector to the output connector ① on the power supply unit. (Fig. 24)
2. Connect one end of power cord to the AC power cord receptacle ②, and the other end to an AC outlet.

Circuit Breaker Reset (Fig. 24)

The circuit breakers ③ will activate in case of brightness adjustment circuit malfunction (short circuit, etc.) or in case of overcurrent. When they activate, the center portion of the breaker will pop up and the power will be cut off.
To reset the breakers, press in the center portion. If a breaker activates again, unplug the power cord from the AC outlet and contact your Olympus representative.

4. Centering the Halogen Lamp

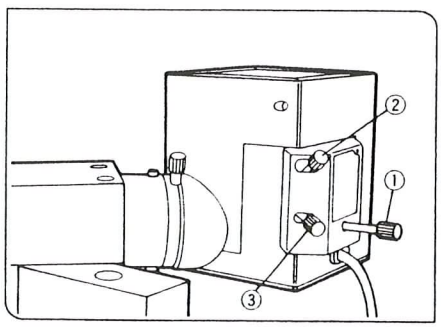
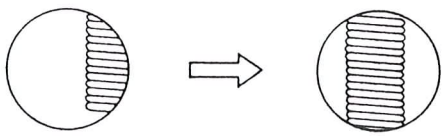


Fig. 25



◎ Before proceeding to the following procedure, remove the analyzer, polarizer and filters from the light path.

1. Engage the 10X objective.
2. Place a highly reflective specimen (e.g. mirror) on the stage, and bring the image into approximate focus.
3. Remove one of the eyepieces, and make adjustment so that the filament image in microscope sleeve will be positioned at center.

   Focusing of filament image:
   Turn the bulb focusing knob ①.
   Vertical adjustment:
   Turn the bulb up/down adjustment knob ②.
   Horizontal adjustment:
   Turn the bulb left/right adjustment knob ③.

4. Return the removed analyzer, polarizer and filters to their original positions.

OLYMPUS

OLYMPUS OPTICAL CO., LTD.
43-2, Hatagaya 2-chome, Shibuya-ku, Tokyo Japan

OLYMPUS OPTICAL CO. (EUROPA) GMBH
(Premises/Goods delivery) Wendenstrasse 14-16, D-20097 Hamburg, Germany
(Letters) Postfach 10 49 08, 20034 Hamburg, Germany

OLYMPUS AMERICA INC.
2 Corporate Center Drive, Melville, N.Y. 11747-3157, U.S.A.

OLYMPUS OPTICAL CO. (U.K.) LTD.
2-8 Honduras Street, London EC1Y OTX, United Kingdom

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