INSTRUCTIONS

BH2-RFC

REFLECTED LIGHT FLUORESCENCE ATTACHMENT

WARNING

This instruction manual applies to the reflected light fluorescence attachment to be used with the Olympus BH2 series microscope. It is recommended that the user read the instruction manual for the BHS or BHT microscope as well, in order to obtain the optimum performance from the integrated use of these instruments.

OLYMPUS®

AX562

BEFORE USE

1 Operation

① This attachment is a precision-manufactured instrument. Always handle it with care, avoiding shock.

② Use the DC-use USH-102D (Ushio Electric) for the mercury burner.

③ Make sure that the bulb is properly installed and electrical cord is connected correctly.

④ To protect eyes from UV radiation exposure, never look directly at the excitation light. Even when handling specimen slides, be sure to look through the UV protective shade, which blocks harmful UV radiation emitted from the mercury burner.

⑤ Do not open the lamp housing when the light is on and for 10 minutes after turning it off.

⑥ The stoppers of the various functions represent the limit of their operational ranges. Never apply undue force to the stoppers.

⑦ High voltage is generated inside the power supply. Do not attempt to disassemble this power supply.

⑧ Ground the power supply unit securely to prevent electric shock.

2 Care and Storage

① Make sure that no dirt, fingerprints, etc. are left on the bulb surface. If it is stained, wipe the bulb surface clean with a small amount of alcohol-ether mixture or benzine.

② Never attempt to disassemble the attachment.

③ When the life time meter of the lighting unit, indicates 200 hours, replace the mercury burner for safety after cooling for at least 10 minutes after switching off.

④ When not in use, the attachment should be covered with the dust cover provided, and kept in areas free from high humidity and rust formation.

⑤ When the dichroic mirror cube is not used, store in the cube case.

CONTENTS

1 ABSTRACT

Model BH2-RFC features the dichroic mirrors which reflect short wavelength radiation towards the objective to illuminate the specimen, while passing longer wavelengths. This application is an refinement over conventional reflected light fluorescence illumination. The illuminator attachment is constructed for use with Olympus BHS and BHT microscopes and permits reflected light fluorescent microscopy not only for biological and medical applications, but also for the chemical and electronic industries.

2 PRINCIPLES

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)

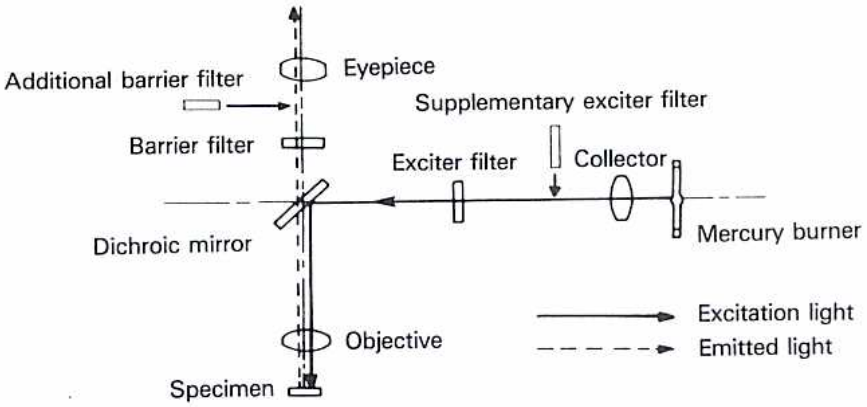


Fig. 1

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 the appropriate 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 other wavelengths of radiation.

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.

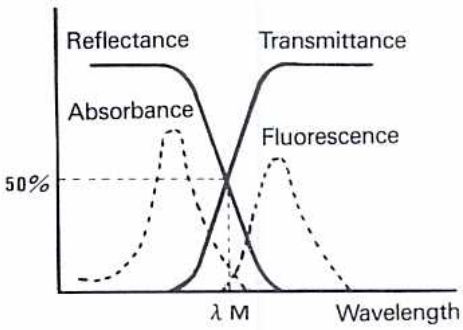
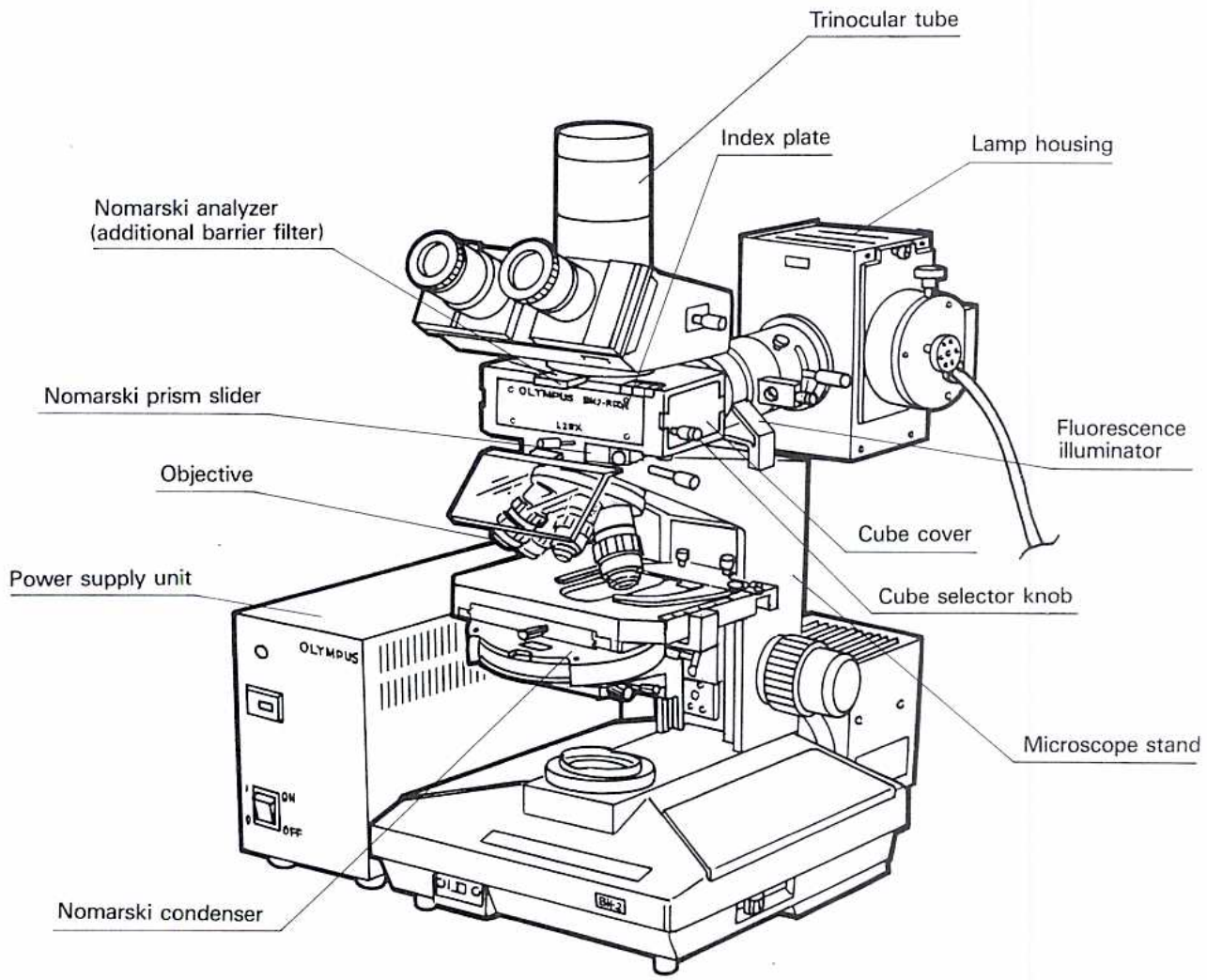


Fig.2

3 STANDARD CONFIGURATIONS



BH2-RFC mounted on Olympus microscope BHS-RFC

■ BH2-RFC Reflected Light Fluorescence Attachment

		Item	BH2-RFC						
			−1	−2	−3	−4	−5	−6	−7
Fluorescence Illuminator, including UV Protective Shield, Immersion Oil 50cc and Dust Cover B071		BH2-RFCA	○	○	○	○	○	○	○
Fluorescence Lamp Hausing		BH2-LSRF-2	○	○	○	○	○	○	
Power Supply		BH2-RFL-T3	○	○	○	○	○	○	
Mercury Burner (2 pcs.)		USH-102D	○	○	○	○	○	○	
Halogen Lamp Housing		BH2-LSRH							○
Transformer		TGH							○
Halogen Bulb (2 pcs.)		JC12V50WHAL-L							○
Power Cord		UYCP	○	○	○	○	○	○	○
Centering Screen		BH2-SGRF	○	○	○	○	○	○	
Dichroic Mirrors Assembly	U Excitation	BH2-DMU	○	○					
	V Excitation	BH2-DMV	○	○					
	BV Excitation	BH2-DMBV	○						
	B Excitation	BH2-DMB		○	○		○	○	○
	G Excitation	BH2-DMG	○	○	○		○		
	IB Excitation	BH2-DMIB	○			○			
Brightfield Cube		BH2-BF	○	○	○	○	○	○	○
Supplementary Exciting filter	B Excitation	20EY455-W22		○	○		○	○	○
	IB Excitation	20EY475-W22	○			○			
	G Excitation	20EO515-W22	○	○	○		○		
		20EO530-W22	○	○	○		○		
	BV Excitation	20EL420-W22	○						
Supplementary Barrier Filter	U Excitation	20L435-W22	○	○					
		20Y455-W22	○	○					
	V Excitation	20Y475-W22	○	○					
	V, BV Excitation	20Y495-W22	○	○					
		20O515-W22	○	○					
	B, IB Excitation	20B460-W22	○	○	○		○	○	○
		20G520-W22	○			○			
		20O530-W22	○	○	○		○	○	○
		20O570-W22	○	○	○		○	○	○
		20O590-W22	○	○	○		○	○	○
	G Excitation	20R610-W22	○	○	○		○		
Objective (for reflected light fluorescence)		DPLAPO10XUV	○	○	○	○			
		DPLAPO20XUV/Oil	○	○	○	○			
		DPLAPO40XUV/ Spring, Iris, Oil	○						
		DPLAPO100XUV/ Spring, Iris, Oil	○						
		DAPO40XUV/ Spring, Iris, Oil		○	○	○			
		DAPO100XUV/ Spring, Iris, Oil		○	○				

Note: o indicates the compatible components for each model.

■ Optional Accessories

Fluorescence phase-contrast attachment	BH2-FLPC
Fluorescence phase-contrast objective	DPlanAPO 10× UVPL DPlanAPO 40× oilUVPL DPlanAPO 100× oilUVPL

■ Objectives for Various Observation Techniques

By combining with a BH2-PC, BH2-NCF or BH2-NC condenser, reflected light fluorescence, interference differential contrast and phase contrast microscopy can be performed simultaneously or changed over with one-touch operation. The applicable objectives are listed below.

Observation method / Objective			Reflected light fluorescence						IGS	Phase contrast			Nomarski differential interference contrast	
										Condenser				
			U	V	BV	B	IB	G		PC	NCF	NC	NCF	NC
DplanApo	10× UV	Dry	○	○	○	○	○	○	X	○	○	○	○	X
	20× UV		○	○	○	○	○	○	X	*○	X	*○	○	X
	40× UV		○	○	○	○	○	○	X	–	–	–	X	X
	20× UV	Oil	○	○	○	○	○	○	X	–	–	–	○	X
	40× UV		○	○	○	○	○	○	○	*○	*○	X	○	X
	100× UV		○	○	○	○	○	○	○	○	○	X	○	○
DApo	20× UV	Dry	○	○	○	○	○	○	X	–	–	–	X	X
	40× UV	Oil	○	○	○	○	○	○	○	*○	*○	X	○	X
	100× UV		○	○	○	○	○	○	○	○	○	X	X	X
SPlanApo	10×	Dry	X	X	X	○	○	○	X	–	–	–	X	X
	20×		X	X	X	○	○	○	X	–	–	–	X	X
	40×		X	X	X	○	○	○	X	–	–	–	X	X
	60×	Oil	X	X	X	○	○	○	X	–	–	–	X	X
	100×		X	X	X	○	○	○	X	–	–	–	X	X
SPlan	10×	Dry	X	X	X	△	△	△	X	○	○	○	X	○
	20×		X	X	X	△	△	△	X	○	X	X	X	○
	40×		X	X	X	△	△	△	X	○	X	○	X	○
	100×	Oil	X	X	X	△	△	△	X	○	○	X	○	○

○: Usable X: Not usable –: No corresponding objective

Notes:

- With objectives marked "△" observations is possible but the image will be relatively dark.
- Appropriately adjust the aperture iris diaphragm (AS) and field iris diaphragm (FS) during IGS observation.
- When using the condensers marked with asterisks '*', the magnification number indices will be different from those of the compatible objectives. Ensure the correct compatibility in condenser's instruction manual before using.

■ Optional Accessories

The following listed combinations allow reflected light fluorescence and Nomarski differential interference contrast microscopy, as well as reflected light fluorescence and phase contrast microscopy to be made simultaneously.

Reflected light fluorescence + Nomarski DIC:
BH2-NCF or BH2-NC plus BH2-NAF and BH2-ANF

Reflected light fluorescence + phase contrast:
BH2-NCF, BH2-PC or BH2-NC

■ Dichroic Mirror/Filter Combinations.

Code	Cube	Appli-cation	Dichroic mirror	Exciter filter	Barrier filter	Supplementary exciter filter	Additional barrier filter
U	BH2-DMU	①	DM400	20UG1	17L420		20L435-W22 20Y455-W22
V	-DMV	②	DM455	20BP405	17Y455		20Y475-W22 20Y495-W22 20O515-W22
BV	-DMBV	③	DM455	20BP440	17AFC +17Y475	20EL420-W22	20Y495-W22 20O515-W22
B	-DMB	④	DM500	20BP490	17AFC +17O515	20EY455-W22	20B460-W22 20O530-W22 20O570-W22 20O590-W22
IB*	-DMIB	⑤	DM505	20BP495	18O515IF	20EY475-W22	20B460-W22 20O530-W22 20O570-W22 20O590-W22
G	-GMG	⑥	DM570	20BP545	17O590	20EO515-W22 20EO530-W22	20R610-W22
IGS	-HMIGS	⑦	(Half-mirror)	20L420-W22 + 20PO (Polarizer)	17AN+17DP		
BF	-BF	⑧	(Optical axis adjuster	(Light beam shutter)	(Image position adjuster)	——	——

① For "U"
② For "V"
③ For "BV"
④ For "B"
⑤ For "IB" (interference filter)
⑥ For "G"
⑦ For "IGS" (immuno gold staining method)
⑧ For brightfield or phase contrast, and for Nomarski DIC only

Note:
Supplementary filters do not come with cubes.
★ Dichroic mirror/filter combinations other than listed above should not be used for "IB" excitation.

4 SPECIFICATIONS

Item		Description
Fluorescence illuminator system		Dichroic mirror type reflected light fluorescence, intermediate observation tube magnification: 1.25×
Dichroic mirror		Cube type
		Basic exciter filter/barrier filter built in (detachable)
Supplementary exciter filter		Attached to the cube with a screw (detachable)
Supplementary barrier filter		Attached to the cube by magnet (detachable)
Reflected fluorescence illuminator	Iris diaphragm	Field iris diaphragm and aperture iris diaphragm
	Field iris diaphragm centering	Field iris diaphragm centering mechanism
	Shutter slide	3 positions: empty aperture, 25% ND filter, shutter
	UV protective shade	Orange clear filter (detachable)
Lamp housing for mercury burner	Mercury burner	USH-102D (manufactured by Ushio Inc.) DC-use 100W high pressure mercury burner
	Safety device	Interlock mechanism
	Bulb centering	Vertical and horizontal centering
	Bulb focusing	Collector focusing
Power supply unit	Illumination system	Auto ignition
	Bulb life meter	Accumulated bulb use time (in hours)
	Ratings	100 – 120VAC, 50 – 60Hz continuous use
Nomarski set	Analyser	Slider system with two click stops (empty aperture and analyzer)
	Prism slider	Slider type

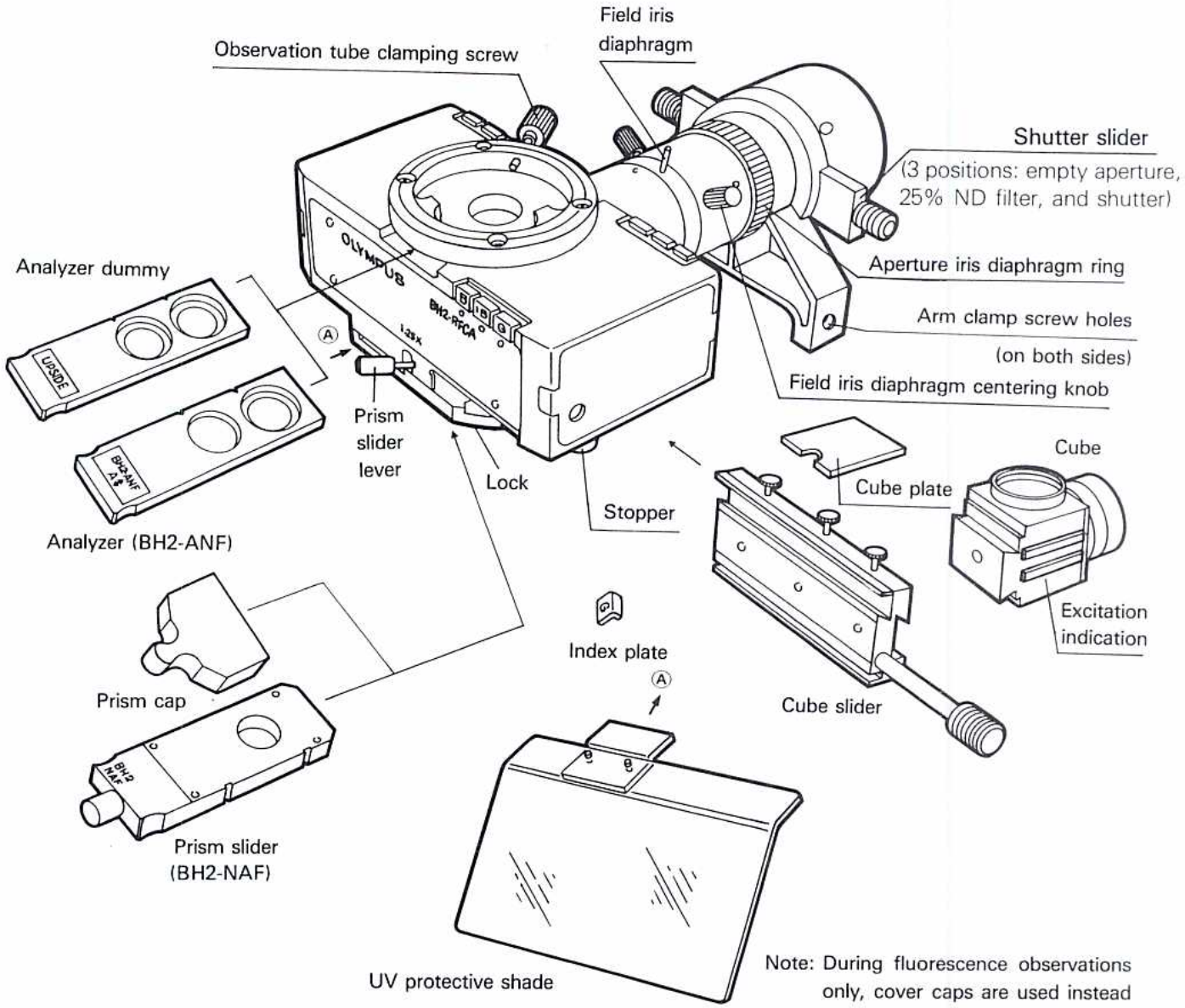
	Lamp housing for mercury burner	Power supply unit
Dimensions	133(W) × 163(D) × 150(H) mm	120(W) × 290(D) × 185(H) mm
Weight	1.4 kg (3.087 lb.)	4 kg (8.82 lb.)

Precautions

1) If another intermediate tube is mounted on the BH2-RFCA, its maximum performance may not be exhibited.
2) If a specimen is observed through a 10× or 20× objective in the "U" excitation mode with the condenser attached, a flare might occur depending on the specimen condition. For this condition, either lower the condenser or insert the shutter slider.
3) Olympus guarantees perfect center alignment only when no supplementary filter for the "B" excitation mode is attached or when a BF cube is mounted.
4) Deviation in focus or centering may occur when the Nomarski prism (BH2-NAF) or analyzer (BH2-ANF) is being engaged or disengaged, and when a cube is being changed.
5) When using the attachment for differential interference observation, refer to the instructions for the BH2-NCF and BH2-NAF.
6) It is necessary to attach the supplementary exciter filter attachment ring, even when this filter for flare removal is not in use. Remove the cubes from the reflected light fluorescence attachment before attaching or removing the supplementary exciter filter or barrier filter.
7) To protect the viewer's eyes during microscopic observation, it is most necessary to mount the UV protective shade.
8) To assure original color reproduction when performing only phase contrast or differential interference observation, the use of a BH2-BF cube is recommended.
9) This attachment cannot be used with the Olympus BHSU or BHTU.
10) A superwide field observation tube may not be used due to the insufficient light level at the field periphery.
11) Neither accessory plate nor bertrand lens may be used during polarizing observation.

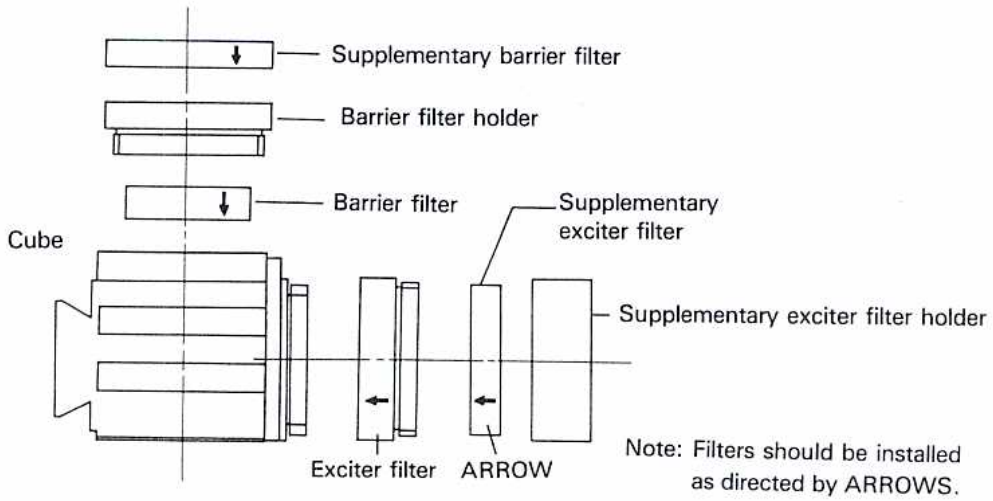
5 NOMENCLATURE

A. Reflected Light Fluorescence Illuminator (for Nomarski DIC)



Note: During fluorescence observations only, cover caps are used instead of the BH2-ANF and BH2-NAF.

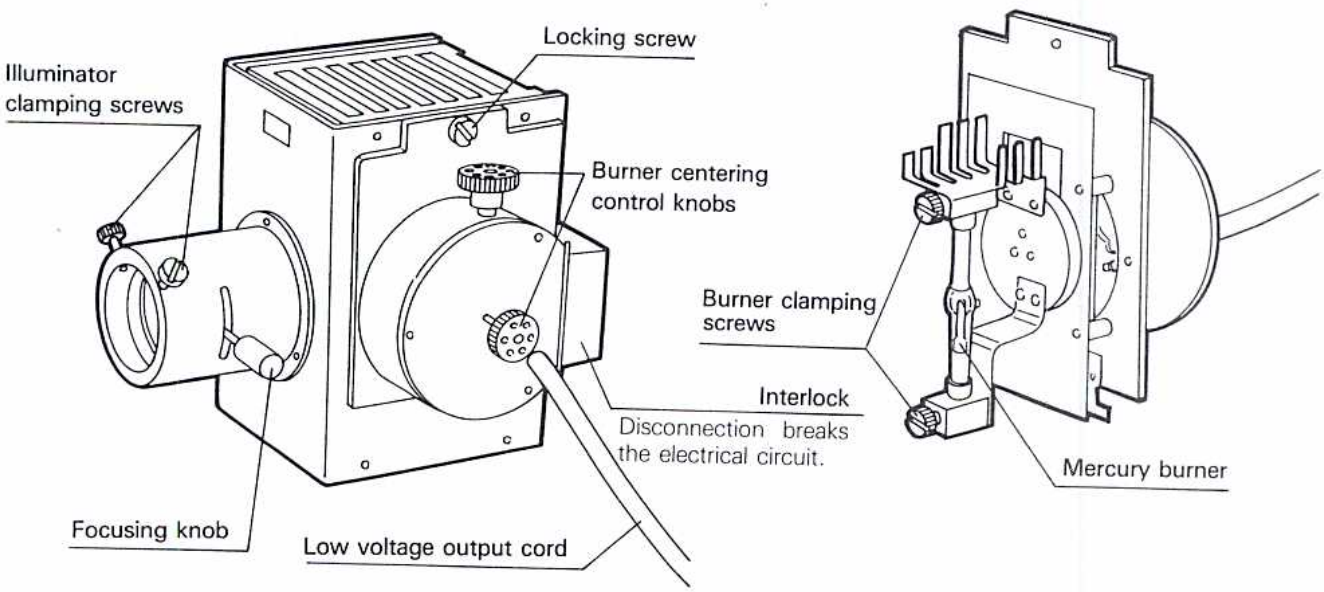
B. Cube



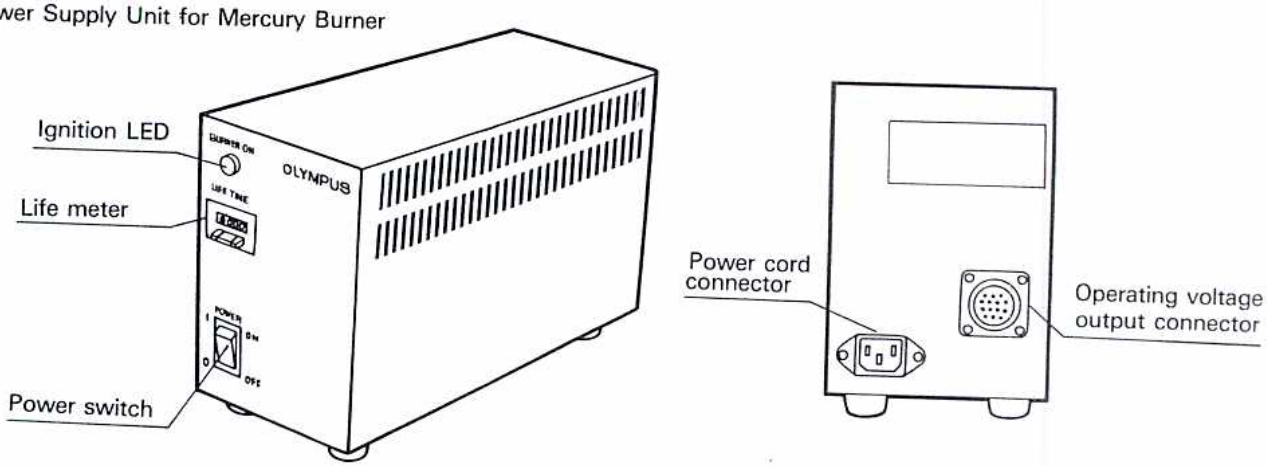
Note: Filters should be installed as directed by ARROWS.

C. Fluorescent Light Source

★ Lamp Housing for Mercury Burner



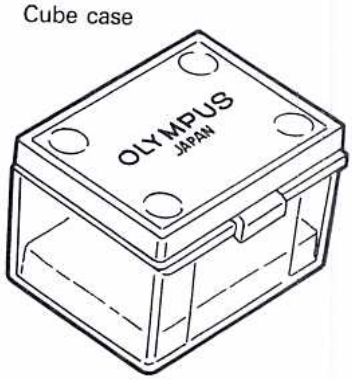
★ Power Supply Unit for Mercury Burner



D. Other Equipment

Centering screen

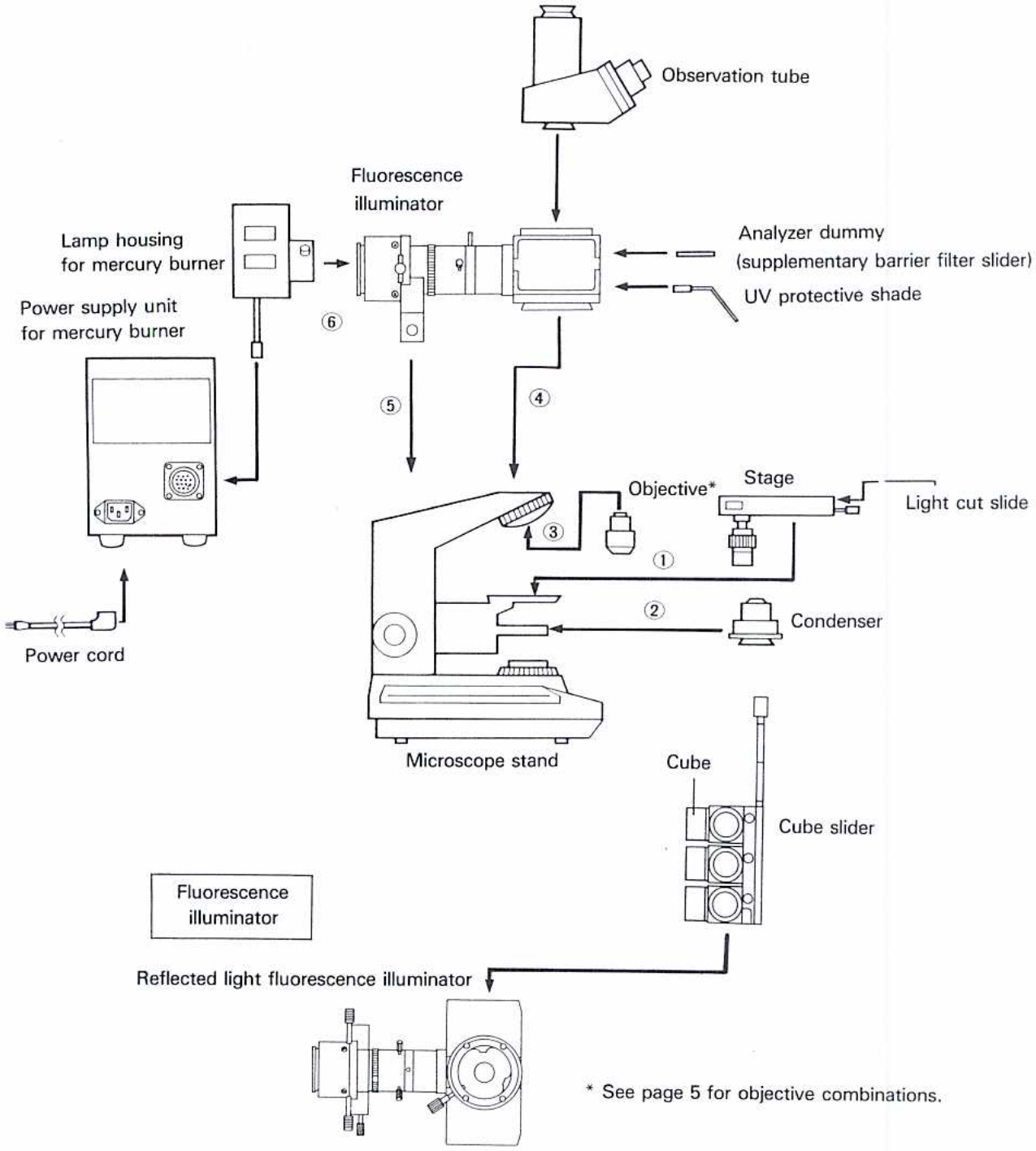
Cube case



6 ASSEMBLY

6-1 Assembly Outline

The diagram below illustrates the sequential procedure for assembling the BH2-RFC attachment. The numbers in circle indicate the assembly order of various components. (Also read the instruction manual for the microscope in use.)



* See page 5 for objective combinations.

6-2 Assembly of Mercury Burner

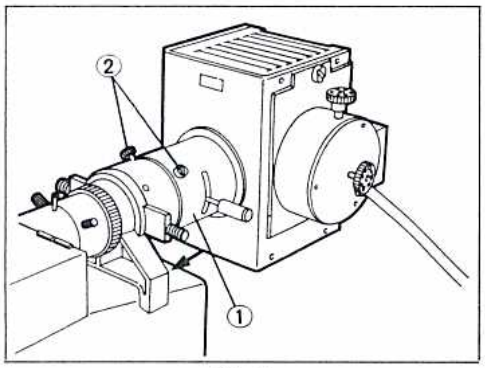


Fig. 1

START BY:

A. Gently insert the sleeve ① of the mercury burner's lamp housing into the collector adapter.

1 Mounting the Mercury Burner (Fig. 1)

(1) After aligning the lamp housing, tighten the two illuminator clamping screws ②.

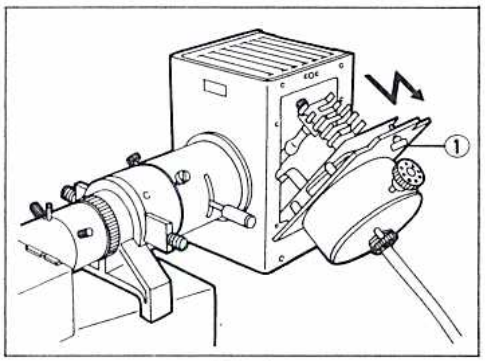


Fig. 2

2 Mounting the High Pressure Mercury Burner

(1) Loosen the locking screw ① and remove the bulb socket by tilting it forward and lifting up. (Fig. 2)

(2) Remove the bulb clamping screws ② and ③ (Fig. 3) to detach the transferral bulb seating retainer. (Remove the old bulb when replacing the bulb.)

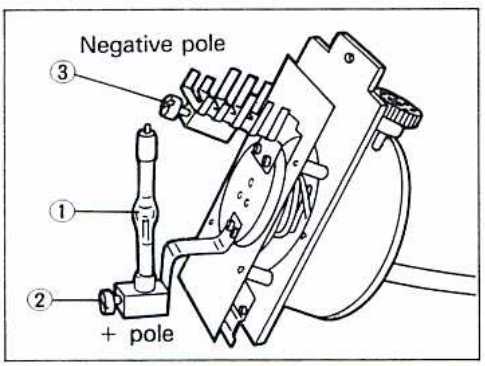


Fig. 3

(3) Placing the positive (+) pole of mercury burner ① downward, clamp the (+) pole with the screw ②. Next, loosen the screw ③ for the negative (−) pole (with "UP" indicator) and insert the bulb's (−) pole into the (−) pole insertion slot. Securely tighten the screw ③. (Fig. 3)

★ Be sure to use a USH-102D or HBO100W/2 bulb.

★ Take care not to leave fingerprints or dirt on the bulb. If the bulb surface becomes dirty, wipe it clean with gauze that has been soaked with alcohol-ether mixture or benzene.

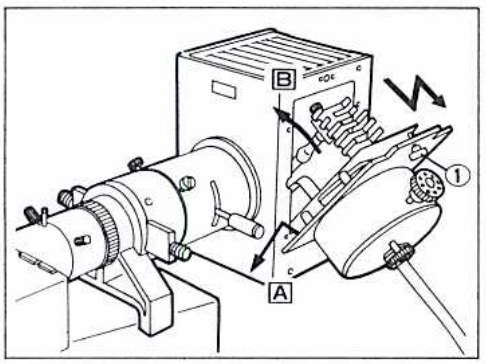


Fig. 4

(4) Align the mounting leg of the bulb socket with the cutout of the lamp housing (A) and securely tighten the locking screw ① (B). (Fig. 4)

★ When the locking screw ① is securely tightened, a click sound will be heard. This indicates that the interlock is operating normally. (Fig. 4)

★ If the locking screw ① is loosened by mistake while the bulb is being lit, the interlock mechanism will start to operate, thus turning off the bulb. When lighting the bulb again, turn off the power switch on the power supply unit and wait for 10 minutes. Afterwards, securely tighten the locking screw ① before turning the power switch ON. (Fig. 4)

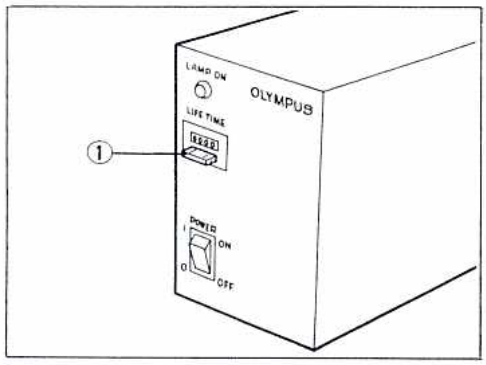


Fig. 5

(5) Press the reset button ① to reset the life meter to "000.0." (Fig. 6)

★ The units on the life meter "000.0" represent hours. When the life meter reads "200.0," replace the bulb.

★ After each bulb replacement, accurately reset the life meter to "000.0". Unless set correctly, the bulb may not light.

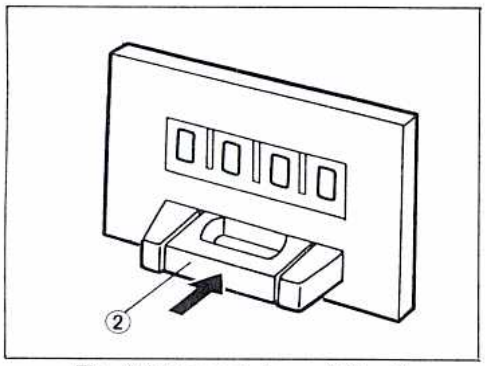


Fig. 6 (Enlarged view of Fig. 5)

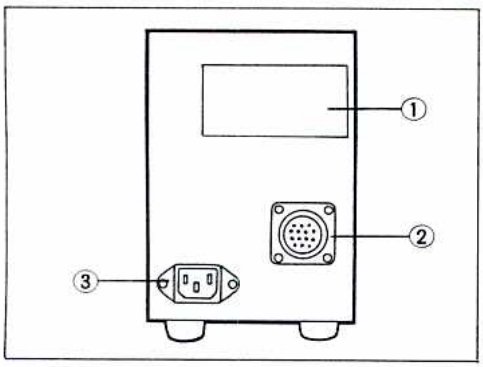


Fig. 7

3 Setting the Power Supply Unit (Fig. 7)

(1) Confirm that the local input voltage and frequency are within the ratings listed on the name plate ①.
(100 – 120V, 50 – 60Hz/220 – 240V, 50 – 60Hz are usable for 100W unit.)

(2) Connect the line cord to the operating voltage output connector ② of the power supply unit correctly.

(3) Connect the power cord to the power cord connector ③ of the power supply unit and insert the power plug to the AC outlet.

4 Replacement of Mercury Burner

(1) For safety purposes, replace the burner with a new one after it has been used over 200 hours.

(2) For safety, confirm that 10 minutes or longer has passed after the power switch has been turned OFF, before removing the burner. For replacement procedure, refer to section 2 .

(3) After the burner is replaced, reset the life meter in the same manner as discussed in STEP (5) of section 2 .

5 Cube Mounting Procedure

(1) Loosen the locking screw ② on the bottom of the attachment and release the lock.

(2) Pull the knob ⑥ out from the side of the attachment. Although the cover ⑤ comes out simultaneously, it does not interfere with mounting a cube onto the cube slider. If it interferes with operation, turn the knob ⑥ to remove it from the slider ④. Then pull out the cover ⑤ from the slider ④.

(3) Hold the cube slider ③ by hoiding the top and bottom finger rests ⑧.

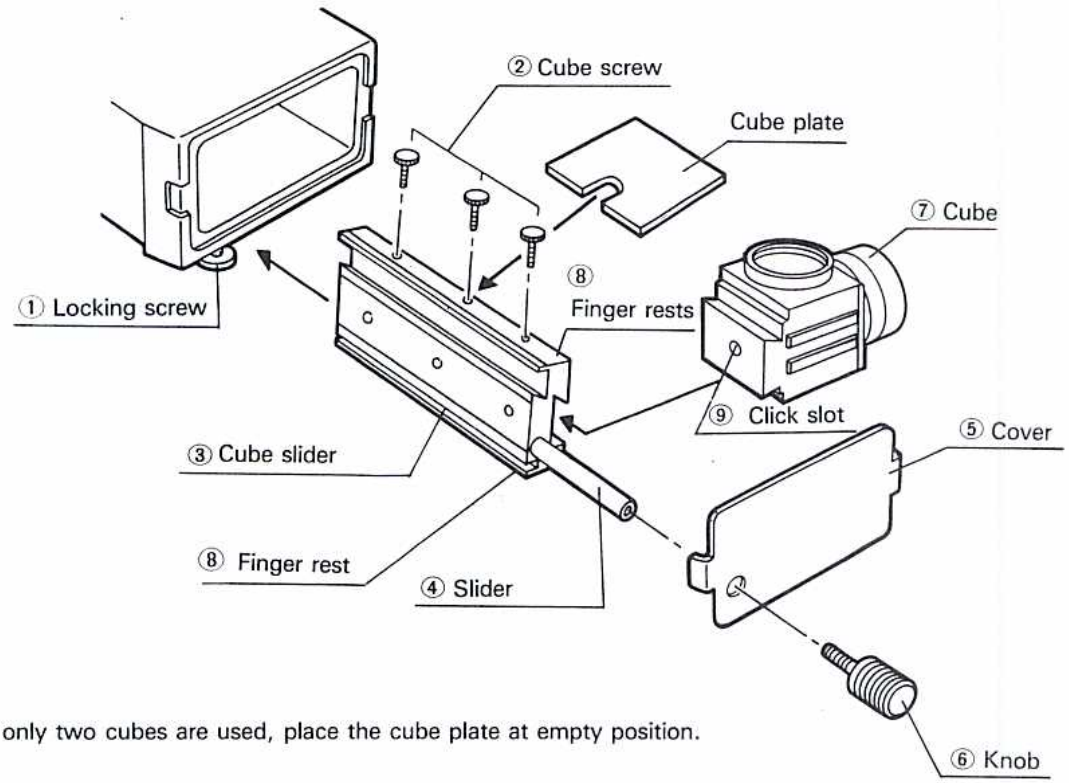
(4) Loosen the cube screw ② and release the lock.

(5) Insert the desired cube ⑦ into the dovetailed slider and push in until a click is heard. Tighten three cube screws ②. (slider ④ acceps three cubes at maximum.)

(6) Insert the slider with three cubes attached into the cube slider.

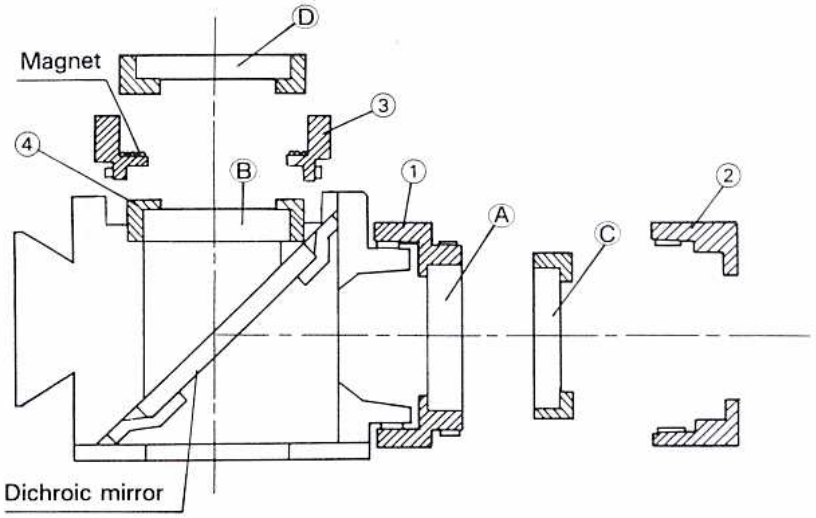
(7) Push in until the slider makes contact with the stopper, and screw in the locking screw ①.

(8) Replace the cover ⑤ in position (magnet latched). If the cover is removed in STEP (2) above, tighten the locking screw ①, attach the cover ⑤, and then screw the knob ⑥ into the slider ④.



Note: If only two cubes are used, place the cube plate at empty position.

6 Filter Mounting Procedure



END BY:

A. Mount the filter in accordance with the following.

■Attaching and Removing Filters

(1) Exciter filter Ⓐ

This filter comes attached to the frame ①. Mount the frame ① to the cube using the screw.

(2) Barrier filter Ⓑ

This filter comes attached to the frame ④. Detach the frame ③ to remove the barrier filter.

(3) Supplementary exciter filter Ⓒ

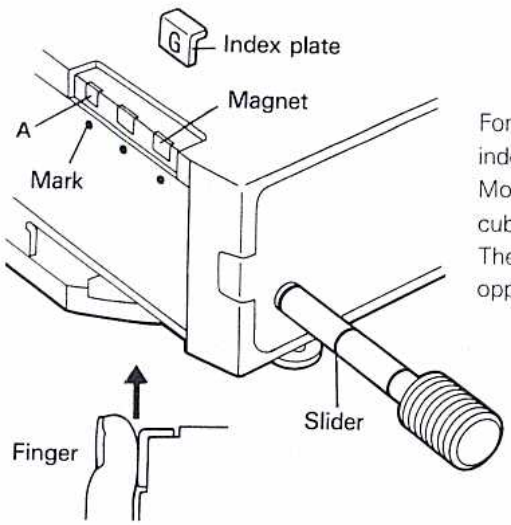
This filter comes attached to the frame ②. Screw in the frame ② to secure the filter.

(4) Supplementary filter Ⓓ

This filter comes attached to the frame. By pushing the filter into the frame ③, it is secured by the magnet.

Note: The dichroic mirror cannot be replaced.

Index Plate Replacement



For easy identification of cube position, the color marks located under the index plate of the illuminator correspond to the color bands on slider. Mount the appropriate index plate on section "A" in the order of the cubes mounted. Push up on the index plate with a finger to remove it. The removed index plate should be mounted to the receptacle on the opposite side to eliminate misplacement.

7 OPERATION

7-1 Preparations

(1) Make sure that the input voltage and frequency are within the ranges indicated on the name plate.

(2) Ascertain that the operating voltage output cord and the power cord are connected correctly.

(3) Switch ON the power supply unit's power. The arc will stabilize 5 or 10 minutes after the light is lit.

- ★ The mercury burner sometimes may not ignite during the first power ON switching, due to electrode condition, etc. If your burner does not ignite, repeat turning off and on the power switch as many times as necessary but with 5 to 10 seconds intervals.
- ★ Do not switch OFF the burner within 15 minutes after the ignition. Once the mercury burner is switched off, do not re-ignite it for 3 minutes or more in order to give it time to cool.
- ★ When the burner is reignited, it takes time for the mercury vapor to cool off and liquify.
- ★ If the lamp housing is opened during operation, the burner will shut off for safety purposes. In this situation, switch the power OFF after 3 minutes. Open the lamp housing after it has cooled down.
- ★ After each burner replacement, reset the bulb life meter.
- ★ If the power switch is on and off in a short period of time, this will reduce buner life considerably. Igniting time for over 30 minutes and extinguishing time for over 10 minutes is recommended for maximum life of the burner.

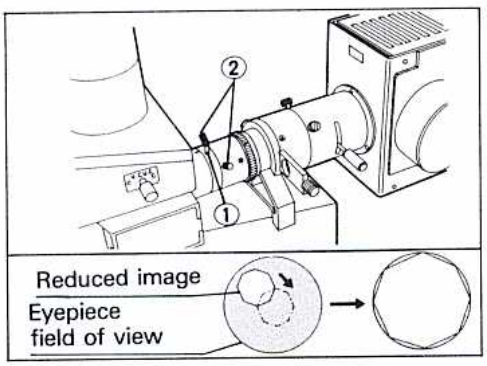


Fig. 8

1 Field Iris Diaphragm Centering (Fig. 8)

(1) Rotate the revolving nosepiece to use the 10× objective. Place the specimen on the stage, and bring it approximately into focus.

(2) Rotate the field iris diaphragm ring ① of the reflected light fluorescence illuminator clockwise to minimize diaphragm's opening.

(3) Rotate the two field iris diaphragm centering knobs ② to bring the reduced diaphragm image into the center of the view field.

(4) Open the field iris diaphragm, by rotating the ring ① counterclockwise until the polygonal diaphragm image is superimposed upon the circle indicating the field of view. If the image is off center, repeat the field iris diaphragm centering operation.

(5) Continue opening the diaphragm until the reduced image circumscribes the field of view.

2 Field Iris Diaphragm Adjustment (Fig. 8)

This proces adjusts the illumination area to achieve optimum image contrast.

Depending on the objective in use, rotate the field iris diaphragm ring ① of the reflected light fluorescence illuminator to reduce the diaphragm opening, until the reduced image circumscribes the field of view, thus shutting out unnecessary light.

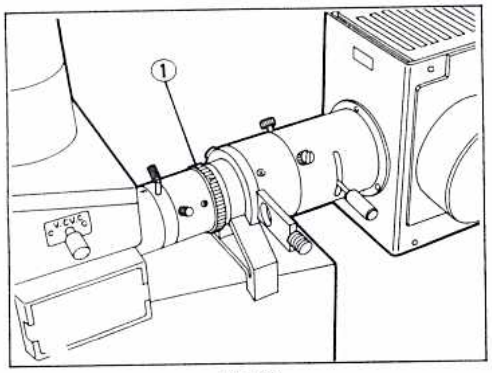


Fig. 9

3 Adjustment of the Aperture Iris Diaphragm (Fig. 9)

The aperture iris diaphragm adjusts the numerical aperture of the illuminator which regulates the resolution, contrast, and focal depth of the observed image.

For normal fluorescence microscopy, turn the aperture iris diaphragm ring ① counterclockwise to open the aperture iris diaphragm.

★ **Should discoloration of the specimen occur, reduce the light level with the ND filter. If this problems remains, close the aperture iris diaphragm opening.**

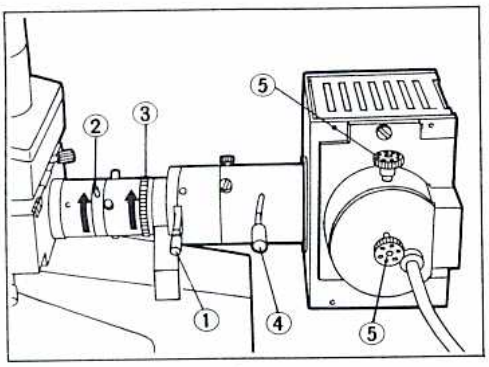


Fig. 10

4 Centering of the Mercury Burner (Figs. 10, 11)

After the arc has been stabilized, center the burner as follows:

(1) Slide the shutter ① until the empty aperture is selected.

(2) Turn the field iris diaphragm ring ② and aperture iris diaphragm ring ③ counterclockwise to open the diaphragm openings.

(3) Withdraw the objectives from the light path and remove the dust cap from the revolving nosepiece.

(4) Screw the centering screen (BH2-SGRF) into the nosepiece aperture so that the arc image of the burner can be projected onto the screen.

(5) Focus the arc image with the focusing knob ④ and center the brightest spot of the arc image on the screen ⑥ with the centering knobs ⑤.

★ **Do not open the lamp housing during or immediately after operation of the burner.**

★ **Burner should be recentered every time the burner is replaced.**

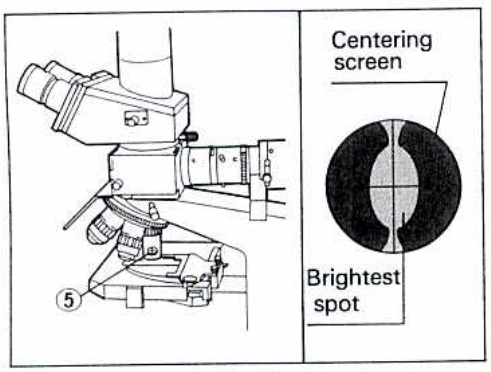


Fig. 11

5 Cube/Filter Combinations

There are many kinds of cubes and filters for different observation purpose. The following table shows the wavelength characteristics and major applications of various excitation methods.

■ Wavelength Characteristics and Major Applications of Various Excitation Methods

Excitation	Wavelength (high pressure mercury burner (Hg))	Applications
U	Bright lines at 334 and 365nm	Observation of autofluorescence DAPI stain for DNA study FITC stain for fluorescent antibody method
V	Bright lines at 405nm	Observation of catecholamine Observation of serotonin Tetracycline stain for studies of teeth, bone, etc.
BV	Bright lines at 405 and 435nm	Quinacrine/quinacrine mustard stain for chromosome study Thiofravine S for limphocite study Acriflavine for nucleic acid
B	Bright lines at 435nm and spectrum regions near 490nm	FITC stain for fluorescent antibody method Acridine orange stain for nucleus study Auramine stain for tubercle bacillus test
IB	Spectrum regions near 490nm	
G	Bright lines at 546nm	RB200 stain for fluorescent antibody method TRITC stain for fluorescent antibody method Feulgen stain for DNA study

Use of supplementary exciter filters

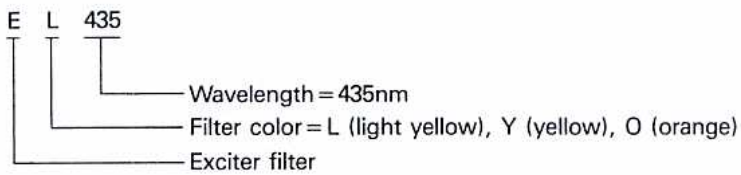
The BP490 and BP545 exciter filters for blue or green excitation utilize extremely wide bands, allowing them to be used for exciting a range of fluorescent materials with different absorption characteristics. This enables a brighter fluorescent image to be observed. However, flare is increased in the wide band, causing certain problems such as a lowering of contrast and increased nonspecific fluorescence. The optimum excitation band differs according to type of specimen, color of fluorescence, and purpose of observation. Supplementary exciter filters that are provided for blue and green excitation are designed to narrow this band for optimum observation.

Although the image appears dimmed in the narrow band, it provides better contrast by restraining autofluorescence and minimizes specimen fading. The EY455 exciter filter is particularly effective for the observation of FITC-stained specimens in blue irradiation. Use of IB irradiation gives better contrast and a brighter fluorescent image. EO515 and EO530 exciter filters are recommended for observation of rhodamine-stained specimens in green irradiation.

★ **EY455 is not effective when using a halogen light source.**

The lower three digits of the numbers indicated on the supplementary exciter filter give the wavelengths in nm. The filters block out the light at wavelengths shorter than that indicated.

Example of supplementary exciter filter coding:



Use of supplementary barrier filters

In addition to the barrier filter built into each cube, supplementary barrier filters can be fitted as required to block unnecessary fluorescence and to control color.
The lower three digits of the numbers indicated on the filters, excluding the B460 and G520, give respective wavelengths in nm. These filters are used for transmitting light at wavelengths longer than that indicated, while blocking out those shorter than the indication.

Example of supplementary barrier filter coding:

R 610
- Wavelength = 610nm
- Filter color = R (red), O (orange), Y (yellow), L (light yellow)

The supplementary barrier filters, B460 and G520, transfer light at the wavelength (nm) close to that indicated by the lower three-digit number on the filter designation. When combined with other filters, they can be used to control the color of fluorescent image.
The B460 blue filter is used to control the color of the fluorescent image in blue excitation which exhibits a strongly yellowish tone. However, the orange and red fluorescence remain dim.
The G520 filter blocks out wavelengths longer than 540nm, namely orange and red fluorescence, allowing green light to pass through.
In addition to controlling the color of the fluorescent image in the same way as the B460, the G520 filter also blocks or reduces fluorescent light other than FITC. This allows it to enhance contrast, and means that it shows only the FITC image of a double stained image (e.g. FITC + Rhodamine).

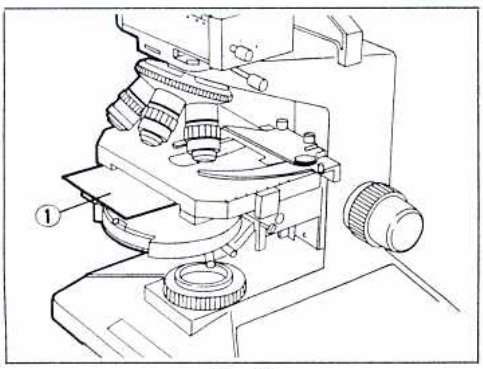


Fig. 12

6 Use of the Light Cut Slide (Fig. 12)

- Use the light cut slide to avoid deterioration of fluorescent image owing to reflection of the incident light upon the top lens of the substage condenser.
- Lower the condenser and insert the slide ① into the slit in the stand.

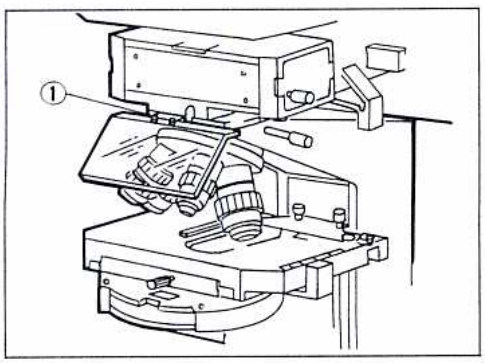


Fig. 13

7 Mounting the UV Protective Shade

Insert the UV protective shade into the slit ① on the illuminator.

7-2 Microscopy Procedures

1 Fluorescence Microscopy

(1) Insert the BF cube into the light path and bring the area of the specimen to be observed into the field of view with phase contrast or Nomarski differential interference contrast microscopy.

(2) Switch OFF the transmission light source and observe the specimen with the exciter cube suitable for the specimen inserted into the light path.

★ Precautions during observation.

- Stop down the field iris diaphragm until the opening circumscribes the field of view.
  If the field iris diaphragm becomes decentered, recenter it correctly by means of the field iris diaphragm centering knob.
- Use immersion oil for the oil immersion objective. After use, carefully wipe off the immersion oil deposited on the lens surface with gauze moistened with pylene, alcohol or ether.
- The objective DPlan Apo 40× UV (Dry) is provided with a corrective collar which can be moved to correct lowered resolution owing to thicker or thinner glass (0.11 – 0.23mm or 0.004 – 0.009in.), as well as a 0.17mm (0.007in.) thick cover glass. For use of the corrective collar, set it at 0.17mm and then turn it in either direction while looking through the microscope and focusing on the specimen until the image can be seen most sharply.
- When fluorescence observation is to be interrupted briefly, shut the shutter slider and keep the burner on. (This makes the burner's life last much longer.) In order to obtain sufficient emission intensity even from a weakly fluorescing specimen, this instrument is provided with an enhanced optical and illuminating system. Therefore, if it is necessary to reduce the irradiation intensity of the illuminator for rapidly discoloring or strongly fluorescing specimens, a 25% ND filter is incorporated in the shutter slider of the fluorescence illuminator.

> Specimens that display intense fluorescence tend to be subject to ghosting. To prevent this, it is recommended that the light intensity be reduced.

★ Reflected light fluorescence mode with transmitted brightfield, transmitted light phase contrast and transmitted light differential interference contrast mode may be used in combination. The initial positioning of a transparent specimen, in particular, during either transmitted phase contrast or transmitted interference contrast microscopy can minimize specimen fading. Reflected light fluorescence and phase contrast observations, and reflected light fluorescence and differential interference contrast observations may be conducted simultaneously. This provides a sufficiently clear image for the fluorescing parts to be viewed clearly.

2 Simultaneous Observation of Reflected Light Fluorescence and Light Phase Contrast Modes

★ To perform phase contrast microscopy, both the condenser and the objective for phase contrast observation should be used.

(1) Insert the BF cube in the light path and set the condenser for phase contrast observation. (For alignment of the condenser for phase contrast observation, refer to its instructions.)

(2) Insert the exciter cube into the light path and open the shutter slider.

(3) Adjust the transmitted light intensity to attain optimum balance between the brightness of the fluorescent image and that of the phase contrast image.

★ Use the aperture iris diaphram (AS) in combination with the ND (neutral density) filter to adjust transmitted light intensity.

3 Simultaneous Observation of the Fluorescence and the Nomarski Differential Interference Contrast Modes

★ The Nomarski attachment is required for Nomarski differential interference contrast microscopy.

(1) Insert the BF cube into the light path and attach the polarizer and Nomarski prism. (Refer to the instructions provided for the Nomarski attachment for the BH2-RFC). The following steps are a repeat of those for the phase contrast microscopy, starting at (2) of the previous page.

Note:

① A BF cube should be used when either phase contrast or Nomarski differential interference contract microscopy is performed separately.

② When these two microscopy modes are combined for simultaneous observation, the fluorescence may appear dimmed.

③ Specimens that display intense fluorescence tend to be subject to ghosting. To prevent this, it is recommended that the light cut slide be used.

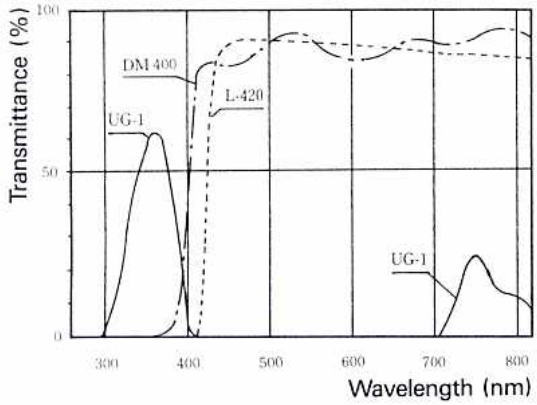
8 TROUBLESHOOTING GUIDE

If you are unfamiliar with any aspects of the use of this model or feel that performance is less than 100%, check the items on the following list.

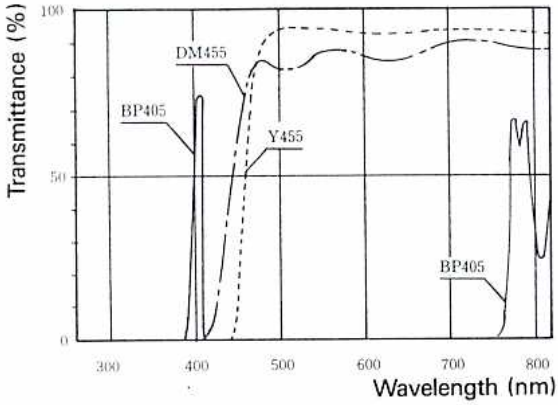
Trouble	Cause	Remedy
1 Optical system		
a. The bulb is on, but image cannot be seen or is dark.	The shutter slider is closed or the 25% ND filter is in use.	Move the shutter to open aperture.
	The cube is not inserted into light path correctly.	Insert the cube into the light path correctly.
	The exciter filter and barrier filter are incorrectly combined.	Follow the filter combinations as listed on page 6.
	The aperture iris diaphragm, field iris diaphragm, or objective's rainbow iris diaphragm opening is not completely opened.	Completely open the aperture iris diaphragm and rainbow iris diaphragm openings, and open the field iris diaphragm opening 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 exciter filter and barrier filter are incorrectly combined.	Follow the filter combinations as listed on page 6.
	The aperture iris diaphragm or field iris diaphragm opening is not opened correctly.	Open the aperture iris diaphragm opening 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 a 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 opening is closed excessively.	Open the field iris diaphragm opening as required.
	The shutter slider is not inserted far enough.	Insert the shutter slider until it clicks.
	The mercury burner is not centered correctly, or focus adjustment has not been completed.	Center the mercury burner or adjust the focus.
d. Excessive glaring.	Either exciter filter or barrier filter has not been inserted.	Insert required filter.
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.	Connectors are connected incorrectly.	Connect correctly.
	The burner has not been not installed.	Install the burner.
	The lamp housing interlock is operating.	Tighten the bulb 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 life meter reads over 200 hours.

9 CHARACTERISTIC CURVES OF FILTERS

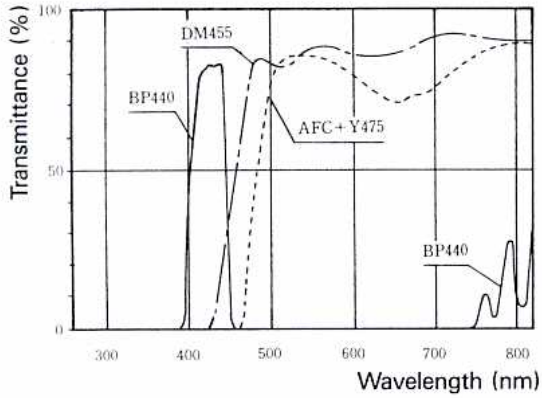
1. Ultra-violet excitation (wide band)



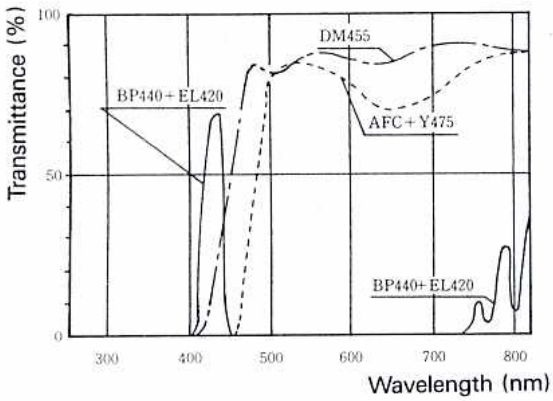
2. Violet excitation (narrow band)



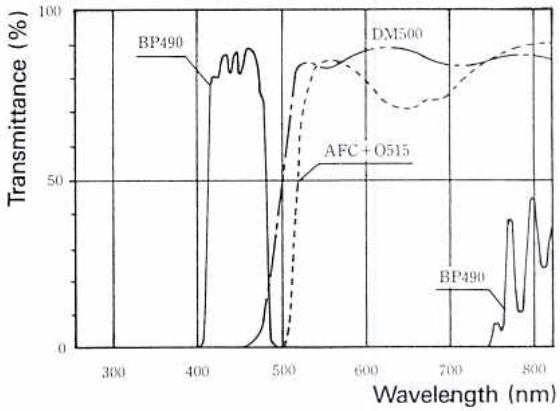
3. Blue violet excitation (wide band)



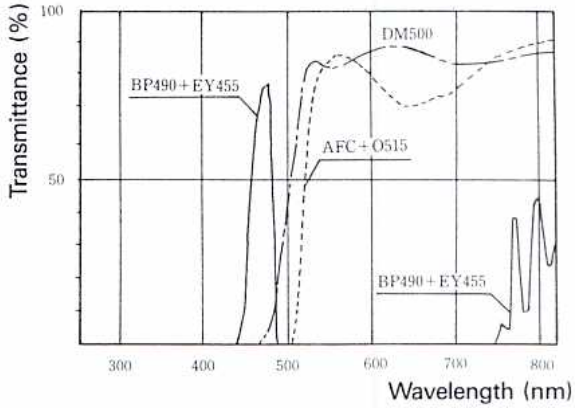
4. Blue violet excitation (narrow band)



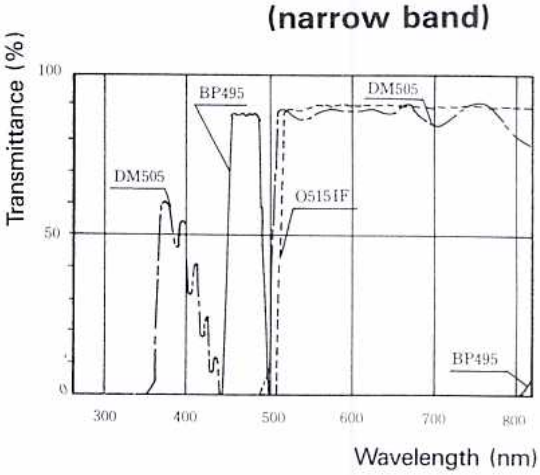
5. Blue excitation (wide band)

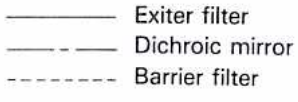


6. Blue excitation (narrow band)

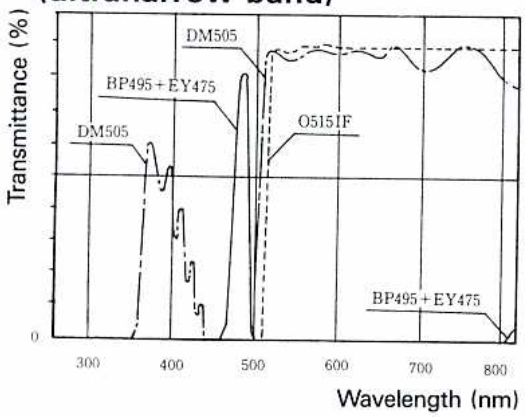


7. Interference blue excitation (narrow band)

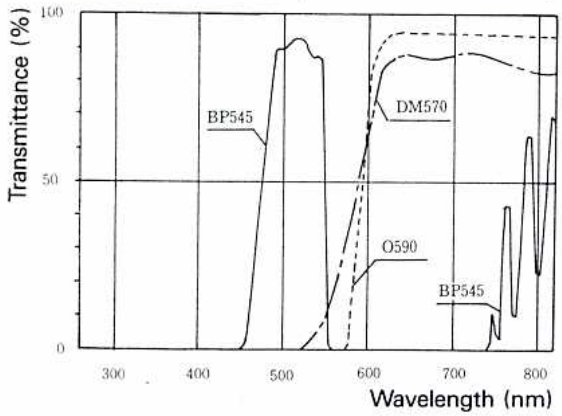




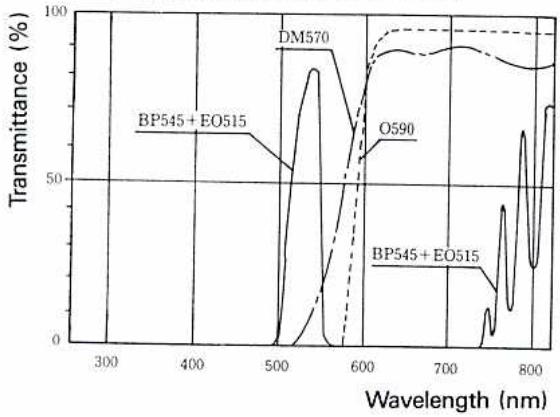
8. Interference blue excitation (ultranarrow band)



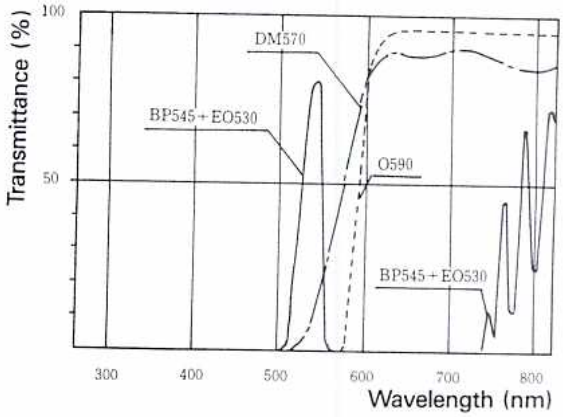
9. Green excitation (ultrawide band)



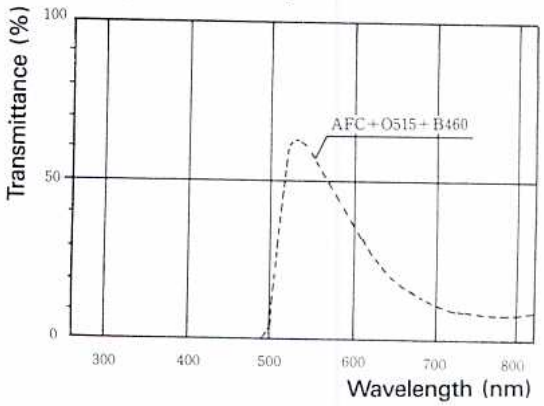
10. Green excitation (wide band)



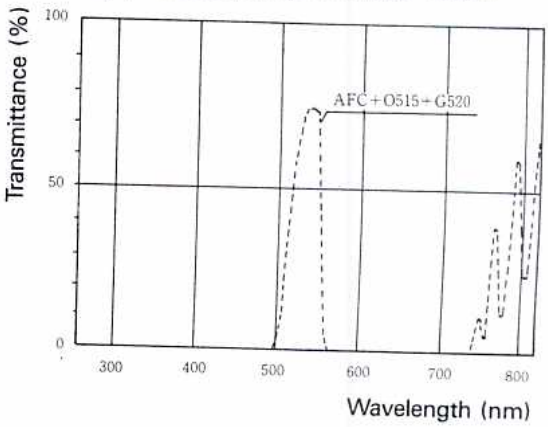
11. Green excitation (narrow band)



12. Supplementary barrier filter



13. Supplementary barrier filter



10 USE OF IGS CUBE

The IGS cube is used to observe immuno-gold stained specimens.

For observation, use the following items:

★ IGS cube

BH2-HMIGS

★ Objectives

D Plan Apo 40× UV (oil)
D Plan Apo 100× UV (oil)
D Apo 40× UV (oil)
D Apo 100× UV (oil)

Note:
Flare may become prominent during observation. To prevent this, close the reflected light fluorescence illuminator's aperture iris diaphragm and field iris diaphragm openings as required.

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Printed in Japan 9203 M 01