REFLECTED LIGHT FLUORESCENCE ATTACHMENT

MODEL BH2-RFL

INSTRUCTION MANUAL

OLYMPUS

As this instruction manual describes the operation of the reflected light fluorescence attachment only, it is recommended that the user read the instruction manuals for the microscopes BHS or BHT as well, in order to obtain optimum performance from the integrated use of these instruments. Please be sure to use Olympus LB objectives.

Observe the Following Points Carefully

■ For Operation

1. Always handle this attachment with as much care as you would a microscope, avoiding shock.

2. For protection of observer's eyes from UV radiation, never look at excitation light directly. Even when handling the specimen slides, be sure to look through the UV protective shade, which blocks harmful UV radiation emitted from the mercury burner.

3. Do not open the lamp housing when the mercury burner is operating or for about 10 minutes after the burner is switched off.

■ For Maintenance

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. For safety sake, always switch off the power supply unit prior to burner replacement. Replace the burner after about 200 operating hours.

3. Prior to fuse replacement in the power supply unit, disconnect the power cord from the AC outlet.

CONTENTS

I. ABSTRACT

One of the features of the Model BH2-RFL is the dichroic mirrors, originated by Dr. J.S. Ploem, that reflect short wavelength radiation towards the objective to illuminate the specimen, while passing long wavelengths.
The Model BH2-RFL attachment fits the Olympus BHS and BHT microscopes and permits reflected light fluorescent microscopy not only in the biological and medical fields of application but also in the chemical, coal and electronic industries.

II. PRINCIPLE OF REFLECTED LIGHT FLUORESCENCE ILLUMINATOR

The Model BH2-RFL is characterized by an optical system that incorporates dichroic mirrors which direct the excitation beam onto the surface of the objective along the vertical optical axis. Therefore, a bright, clear fluorescent formation against a dark background is ensured. Their radiation does not pass through the specimen, as is the case with transmitted light, so that the full emission intensity of the specimen is used.
As the dichroic mirror is placed at an inclination of 45° to the optical axis of incident light, the spectral characteristics of the mirror is diagramed below:

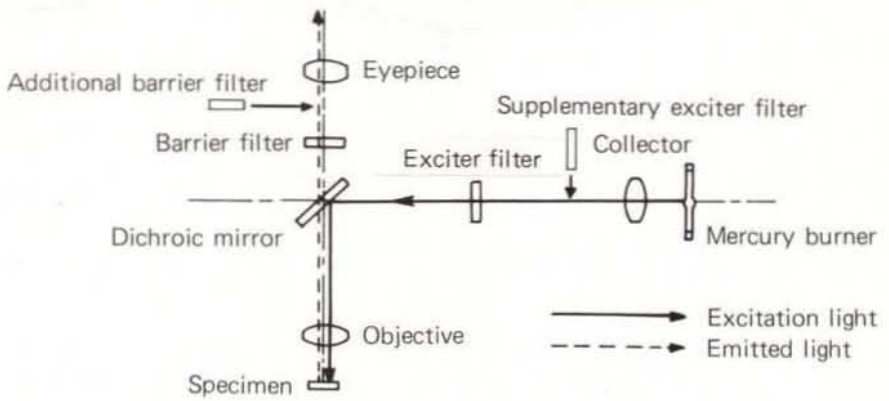


Fig. 1

As there is a cross-over between transmittance and reflectance, it is necessary to use an appropriate combination of exciter filter and barrier filter, in conjunction with a dichroic mirror, so that a good contrast image is obtained by exciting the fluorochrome in the specimen at the desired wavelength.
When the dichroic mirror is inclined 45° to the optical axis of incident light, the mirror reflects the excitation light into the objective, and passes other wavelength radiations.
Upon irradiation with the excitation wavelength, the specimen emits a longer visible wavelength, due to Stokes' law, and the barrier filter between objective and eyepiece blocks out unwanted wavelengths to obtain a black background.

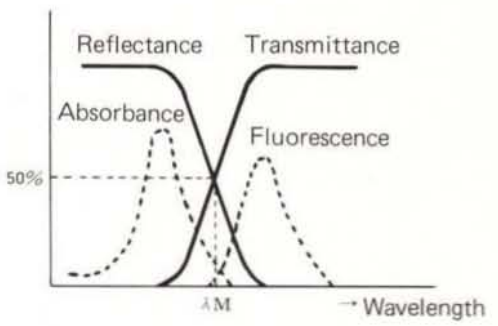


Fig. 2

III. NOMENCLATURE

Model BH2-RFL mounted on Olympus microscope BHS

IV. STANDARD EQUIPMENT

1. Reflected light fluorescence attachment

Component	Model	BH2-RFL					
		1	2	3	4	5	6
Fluorescence illuminator, including UV protective shade, silicon oil 50cc, wooden case and dust cover	BH2-RFA	O	O	O	O	O	O
Fluorescence lamp housing	BH2-LSRF	O	O	O	O	O	
Halogen lamp housing	BH2-LSRH						O
Mercury burner x 2 pcs.	HB0100W/2	O	O	O	O	O	
Halogen bulb x 2 pcs.	JC12V50W HAL-L						O
Power supply	BH2-RFL-T/2	O	O	O	O	O	
Transformer	TGH						O
Power cord	UYCP	O	O	O	O	O	O
Centering screen	BH2-SGRF	O	O	O	O	O	
Dichroic mirror, including exciter filter 20UV-W-2	BH2-DMUV	O	O				
Dichroic mirror, including exciter filter 20UB-W-2	BH2-DMUB			O			
Dichroic mirror, including exciter filter 20BG-W-2	BH2-DMBG	O			O		
Dichroic mirror, including exciter filter 20BO-W-2	BH2-DMBO					O	O
Supplementary exciter filter (for "B")	20EY-455-W23	O		O	O	O	O
Supplementary exciter filter (for "G")	20EY-475-W23	O			O		
Supplementary exciter filter (for "G")	20EO-515-W23	O			O		
Supplementary exciter filter (for "G")	20EO-530-W23	O			O		
Barrier filter (for "U")	20L-435-W	O	O	O			
Barrier filter (for "U")	20Y-455-W	O	O	O			
Barrier filter (for "V")	20Y-475-W	O	O				
Barrier filter (for "V")	20Y-495-W	O	O				
Barrier filter (for "V")	20O-515-W	O	O				
Barrier filter (for "B")	20B-460-W	O		O	O	O	O
Barrier filter (for "B")	20O-530-W	O		O	O	O	O
Barrier filter (for "B")	20O-570-W	O		O	O	O	O
Barrier filter (for "B")	20O-590-W	O		O	O	O	O
Barrier filter (for "G")	20R-610-W	O			O		
Fluorescence free objective 10X (spring-loaded)	UVFL10X/R	O	O	O			
Fluorescence free objective 20X (spring-loaded)	UVFL20X/R	O	O	O			
Fluorescence free objective 40X (spring-loaded, iris, oil)	UVFL40X/Ris	O	O	O			
Fluorescence free objective 100X (spring-loaded, iris, oil)	UVFL100X/Ris	O	O	O			

V. SPECIFICATIONS

1. Fluorescence illuminator: Magnification factor 1.25X

1) Dichroic mirrors (built into insertion holders)

Code	Dichroic mirrors (barrier filters built-in)
U-V	DM-400 (L-420) – DM-455 (Y-455)
U-B	DM-400 (L-420) – DM-500 (O-515)
U-G	DM-400 (L-420) – DM-580 (O-590)
B-G	DM-500 (O-515) – DM-580 (O-590)
B-O	DM-500 (O-515) – Empty

2) Exciter filters: U (UG-1), V (BP-405), B (BP-490), G (BP-545)
Supplementary exciter filters in mounts:
EY-455, EY-475, EO-515, EO-530

3) Barrier filters: L-435, Y-455, Y-475, Y-495, O-515, O-530, O-570, O-590, R-610, B-460

Optional filter: G-520

4) Shutter slider (3 positions: Empty aperture – ND filter – shutter); field iris diaphragm and aperture iris diaphragm provided.

2. Light sources

1) 100W high pressure mercury burner: HBO 100W/2 (manufactured by Osram) or USH 102D (manufactured by Ushio Inc.)

Power supply: Input voltage AC100V, 50/60Hz
(In the U.S.A., power supply specifications are different.)

2) Halogen lamp: 12V 50W

Transformer: TGH, voltage variable O-12V, with voltmeter.

3. Objectives (non-fluorescing) for use with the mercury burner only:

UVFL10X (spring-loaded)
UVFL20X (spring-loaded)
UVFL40X (spring-loaded, iris, silicon immersion)
UVFL100X (spring-loaded, iris, silicon immersion)

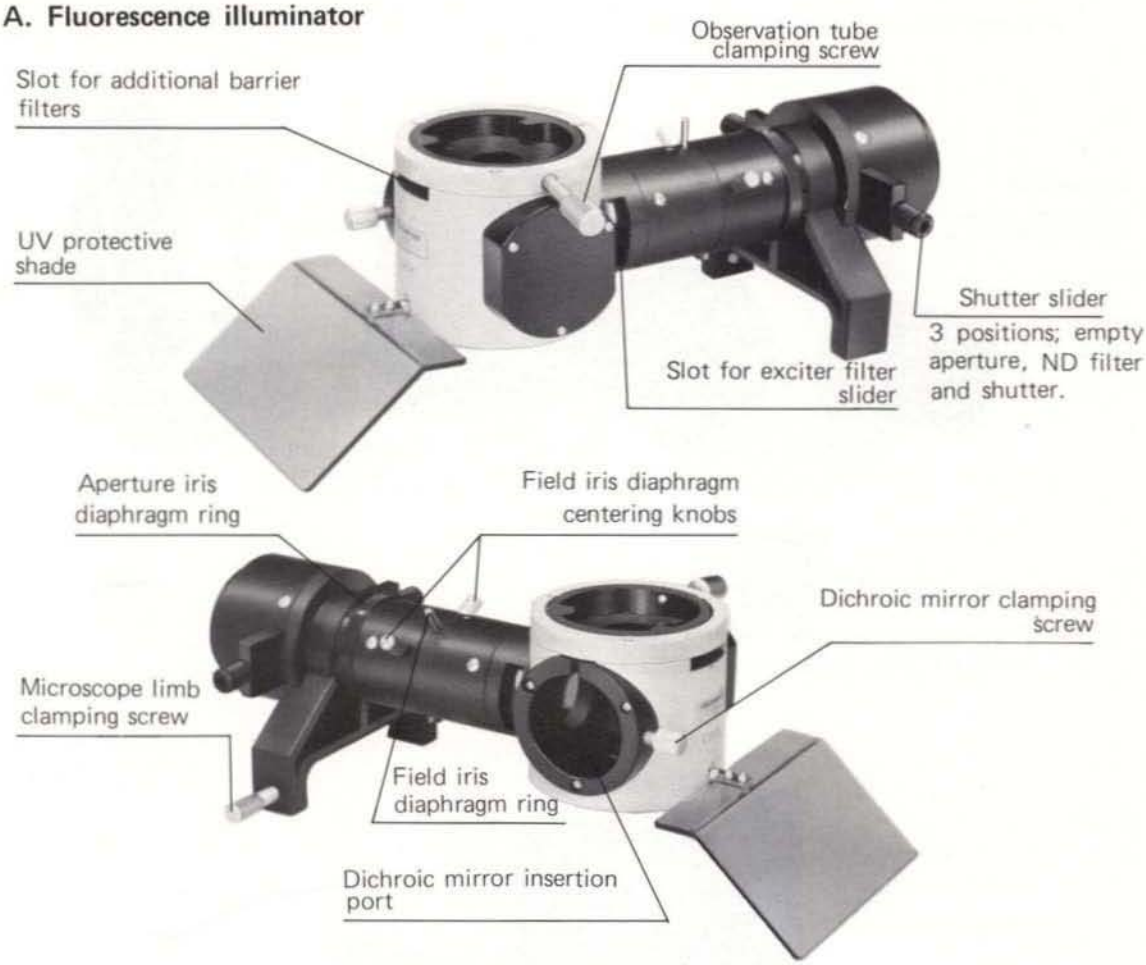
4. Observation tube: Binocular or trinocular.

5. Optional accessories:

UVFL40X-PL (silicon immersion)
UVFL 100X-PL (silicon immersion)
With these optional objectives, simultaneous reflected light fluorescence and transmitted light phase contrast observations are possible.

VI. IDENTIFICATION OF VARIOUS COMPONENTS

A. Fluorescence illuminator



■ Barrier filter

■ Centering screen for light source

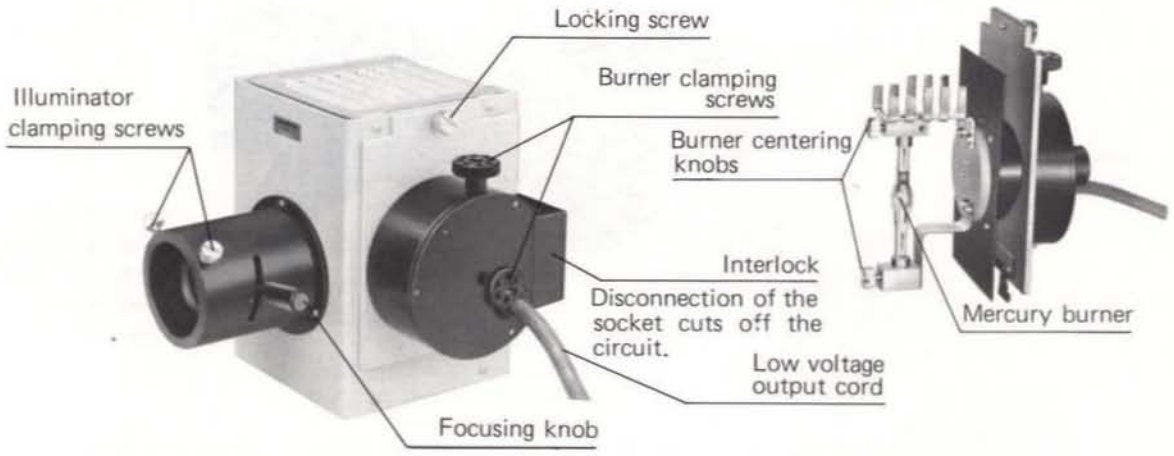
■ Exciter filter slider

■ Supplementary exciter filter

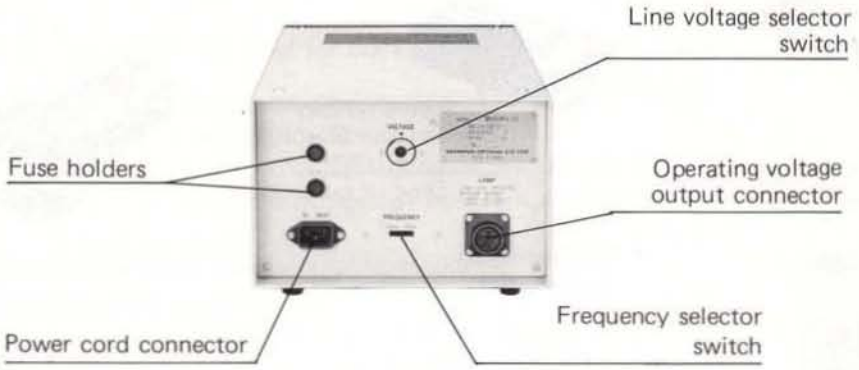
■ Dichroic mirror unit

B. Light sources

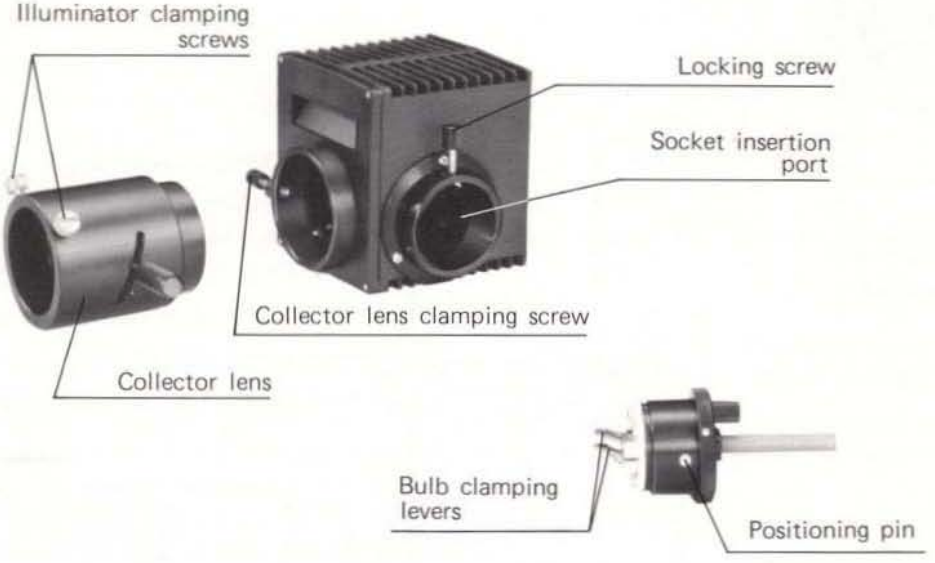
■ Lamp housing for mercury burner



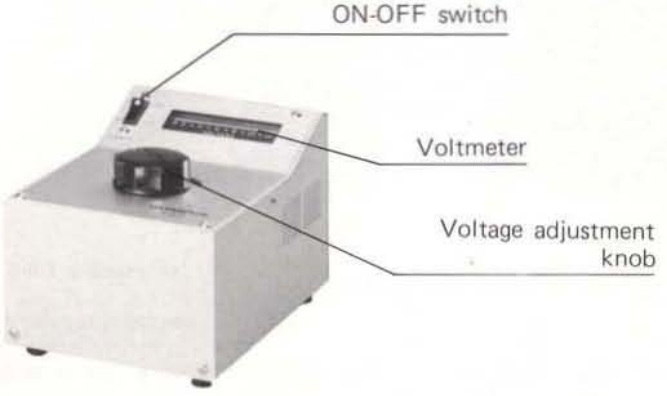
■ Power supply unit for mercury burner
(In the U.S.A., the specifications are different.)



■ Halogen lamp housing

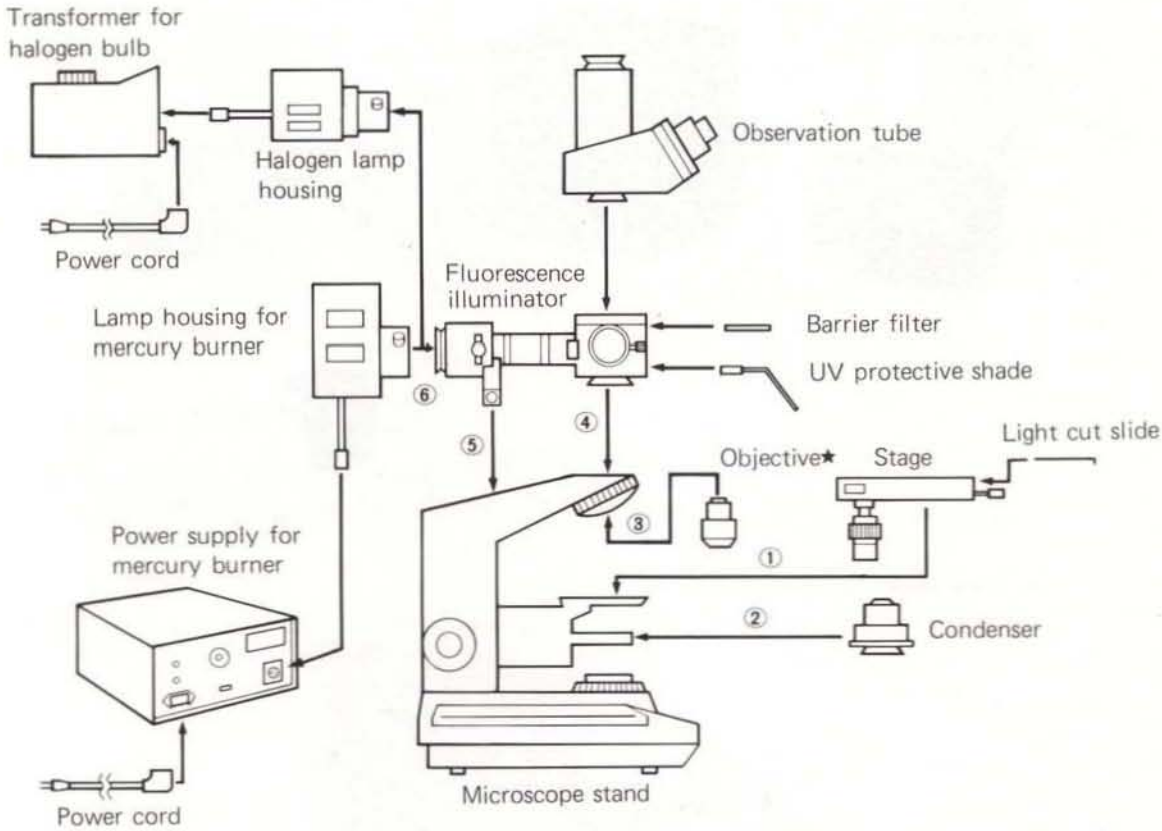


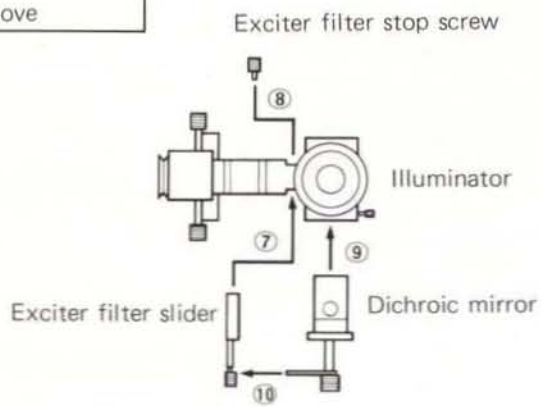
■ Transformer for halogen bulb



VII. ASSEMBLY

The diagram below illustrates the sequential procedure of the assembly of the BH2-RFL attachment. The numbers in circle indicate the assembly order of various components. (Read the instruction manual for the microscope in use, too.)





★ Use of objectives

1) Use the UVFL objectives and non-fluorescing immersion oil for UV radiation.
2) LB biological objectives can be used with ordinary immersion oil for blue and green radiation (however, objectives lower than 4X and metallurgical objectives can not be used).

VIII. OPERATION

A. Preparations

1. Install the light sources

■ For the mercury burner

1) Be sure to turn off the main switch.

2) Insert the lower electrode (marked "+") into the bottom terminal and tighten the clamping screws ① securely. (Fig. 1)

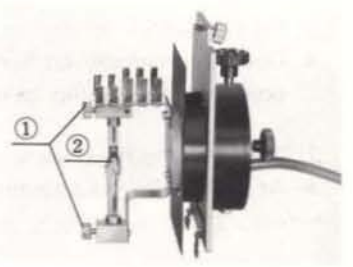


Fig. 1

★ **Ascertain that no dirt, fingerprints, etc. are left on the bulb surface, and when installing, be careful not to touch the bulb portion ②. If the bulb is stained, wipe the bulb surface clean with a small amount of alcohol-ether mixture or benzine.**

■ For the halogen lamp

1) Be sure to turn off the ON-OFF switch of the transformer.

2) Pressing the bulb clamping levers ① against the seat, insert the contact pins of the halogen bulb ③ into the seat holes ②. When the levers are released, they return and the bulb is securely held in the seat. (Fig. 2)

★ **If the bulb is stained with dirt, fingerprints, etc., wipe the bulb surface with a small amount of alcohol-ether mixture or benzine.**

3) Insert the bulb and socket into the halogen lamp housing and clamp with locking screw ③. (Fig. 3)

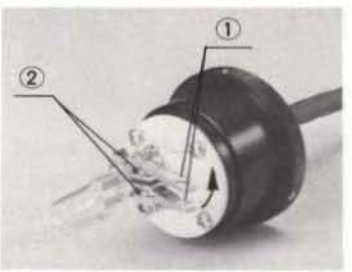


Fig. 2

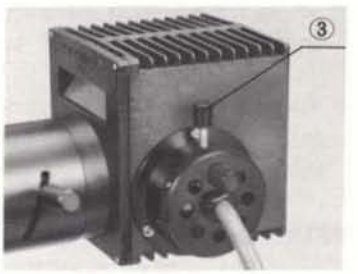


Fig. 3

2. Switching the light sources on

■ Ignition of the mercury burner

1) Make sure that the line voltage selector switch is set to conform with the local mains voltage. (This switch can be turned with a screwdriver, and can be set to the following voltages: 100V-110V-120V or 220V-240V.) (Not relevant for USA power supply units.)

2) Ascertain that the frequency selector switch is set to conform with the local mains frequency. If you find the switch is not correctly positioned, correct it with a screwdriver.

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

4) Turn on the main switch of the power supply unit. At the same time, the switch lamp will light up green.

5) In about 2 or 3 minutes after ignition the arc will be stabilized.

6) 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.**

7) Turn off the main switch to put out the light.

★ **At each bulb replacement, zero the life meter.**

Fuse replacement

To replace a fuse in the power supply unit, remove the fuse holder and replace the fuse. Two fuses are incorporated. Each fuse should be replaced only after disconnecting the power cord from the AC outlet.

■ **Operating the halogen bulb**

1) Ascertain that the line voltage selector switch ① is set to conform with the local mains voltage. (Fig. 4). If not, set it correctly.

2) Ascertain that the voltage adjustment knob ② is set at lowest voltage position, marked with (–), and turn on the ON-OFF switch.

3) Turning the voltage adjustment knob ② towards (+) increases voltage and the voltmeter LED lights up.

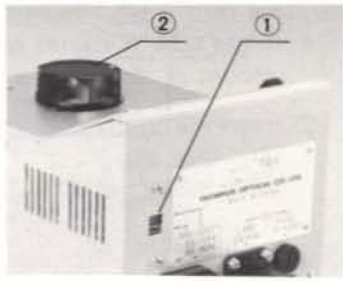


Fig. 4

NOTE: If the input voltage is so high that the max. output voltage exceeds 12V, the LED flashes in warning that further use of the bulb in this condition will greatly reduce the bulb life. To avoid this, lower the voltage.
If you set the voltage adjustment knob at max. without loading the bulb, the LED also flashes. This LED flashing in both cases does not mean any trouble with the transformer.

○ **Fuse replacement of the transformer**

(1) Turn off the ON-OFF switch and disconnect the power cord from the AC outlet.

(2) Pushing in the fuse holder with a screwdriver, rotate it counterclockwise and remove.

(3) Replace the fuse and reset the holder.

3. Centering the light sources

■ For the mercury burner

After the arc has stabilized, center the burner in the following steps:

1) Open the shutter ① by sliding it in the arrow's direction all the way. (Fig. 5)

2) Rotate the field iris diaphragm "F" ② and the aperture iris diaphragm "A" ③ counter-clockwise to the "maximum" position. (Fig. 5)

3) Swing out the objectives from the light path, and remove the dust cap from the nosepiece aperture, so that the light passes through an empty aperture of the nosepiece.

4) Screw the centering screen into the nosepiece aperture so that the arc image of the burner can be projected on the screen.

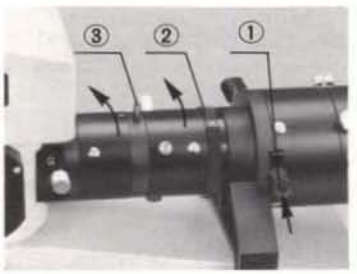
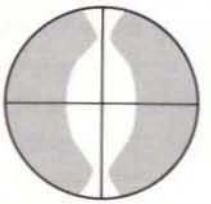


Fig. 5



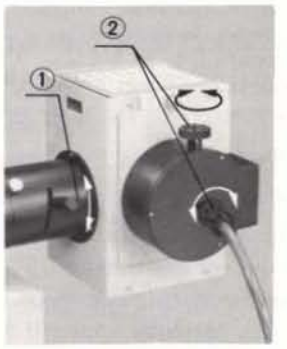


Fig. 6

5) Bring the arc image into focus with the focusing knob ① and center the brightest spot of the arc with the centering knobs ② . (Fig. 6)

★ **Burner centration should be performed each time a burner is replaced.**

★ **Be careful never to open the lamp housing while the burner is on or immediately after switching off.**

■ For the halogen lamp

Take steps 1) through 4) as in case of the mercury burner, then proceed with the following steps:

1) Looking at the filament image projected on the screen, focus it by means of the focusing knob ① . (Fig. 7)

2) Loosen the locking screw ② and, sliding the lamp socket in and out, and rotating in either direction, center the filament image. (Fig. 7)

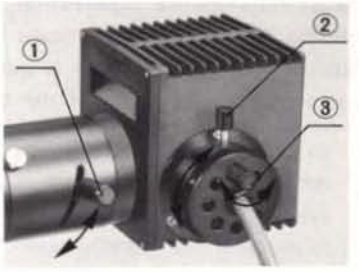


Fig. 7

B. Fluorescence microscopy

Make it practice to use the UV protective shade provided to protect your eyes from fluorescent light.

1) Bring the area of the specimen to be observed into the field of view, and focus with transmitted light emitted from the microscope's tungsten filament bulb. (Refer to the manual for the microscope in use for observation with transmitted light.)
For general microscopy with transmitted light, remove the dichroic mirror. If the B-O dichroic mirror is engaged, however, place the lever at the O position.

■ **Phase contrast observation**

(Use of optional objectives UVFL40X-PL (oil) and UVFL100X-PL (oil) for transmitted light phase contrast and reflected light fluorescence observation)
Transmitted light phase contrast microscopy facilitates observation of a transparent specimen which is difficult to find in reflected fluorescent light. Furthermore it allows visualization of non-fluorescing parts of the specimen. The optional objectives UVFL40X-PL (oil) and UVFL100X-PL (oil) are convenient for this purpose, since they permit alternate observation between reflected light fluorescence and transmitted light phase contrast without changing the objectives.

2) Switch off the tungsten bulb, and insert your selection of dichroic mirror, exciter and barrier filters into the light path in the following steps:

① Stop down the field iris diaphragm "F" until it is within the field of view.

★ **If the diaphragm is decentered, recenter it correctly by means of the two centering knobs.**

② Stop down the aperture iris diaphragm "A" slightly smaller than the field of view to ensure proper contrast.

★ **Use non-fluorescing immersion oil for UVFL40X (oil) and UVFL100X (oil) objectives.**

★ **After use, carefully wipe off the immersion liquid deposited on the lens surfaces with gauze moistened with xylene (no alcohol or ether should be used.)**
Never leave immersion liquid on the lens surfaces after use as remnants of the liquid will seriously impair the performance of the objective.

★ **The objectives UVFL40X (oil) and UVFL100X (oil) are provided with iris diaphragms. It is recommended to stop down the iris diaphragm properly to increase contrast and image definition.**

★ **The objective UVFL40X (dry) is provided with a correction collar which can be moved to spherically correct for a thinner or thicker cover glass as well as a 0.17mm thick cover glass.**
For use of the correction 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, it is good practice to cut off the beam of light by means of the shutter slider rather than to turn off the mercury burner, since fluorescence is quick to fade and repeated on-off switching considerably shortens the useful life of the burner.**

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 quenching specimens, an ND filter is incorporated in the shutter slider of the fluorescence illuminator.
Intensely fluorescing specimens tend to exhibit a ghost image and it is recommended to attenuate the intensity of the exciting light.

■ **Use of the light cut slide**

Use the light cut slide provided, to avoid deterioration of the fluorescence image due to the reflection of the incident light upon the top lens of the substage condenser.
Rack down the condenser and insert the slide ① into the slit in the stage. (Fig. 8)

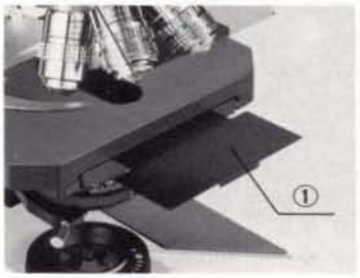


Fig. 8

C. Filter combinations

Excitation region	Exciter filter	Suppl. exciter filter*	Dichroic mirror	Additional barrier filter
U (Ultra-Violet)	U (UG-1)	–	U (DM-400 + L-420)	L-435 and up
V (Violet)	V (BP-405)	–	V (DM-455 + Y-455)	Y-475 and up
B (Blue)	B (BP-490)	EY-455	B (DM-500 + O-515)	O-515 and up, B-460, G-520
G (Green)	G (BP-545)	EY-475, EO-515, EO-530	G (DM-580 + O-590)	R-610

* The supplementary exciter filter is placed behind the exciter filter slider in the suppl. exciter filter slot. (Page 6)

■ **Use of supplementary exciter filters**

The wide-band pass filters BP-490 and BP-545 provided can be used as narrow band pass filters in conjunction with supplementary exciter filters if necessary. Generally speaking, an image to be observed becomes dim in the narrow band, but renders better contrast by restraining autofluorescence and minimizes specimen fading.
Exciter filters EY-455 and EO-530 are particularly recommended for observation of FITC stained specimens in blue irradiation.

★ **EY-455 is not effective when using a halogen light source.**

■ Use of additional barrier filters

In addition to the barrier filter built in each dichroic mirror unit, additional barrier filters can be applied if necessary, mainly for the purposes of blocking unnecessary fluorescence and controlling color rendition.
Especially, the additional barrier filter B-460 is capable of rendering the FITC fluorescent light in blue excitation more natural, while the G-520 adds further capabilities of blocking or reducing the fluorescent light other than the FITC, so that it can be used for contrast enhancement or showing up only the FITC image out of a double stained image (e.g. FITC + Rhodamine).
Other barrier filters are also capable of blocking wavelengths shorter than indicated in the respective filter designations. Particularly in B excitation, the O-515 and O-530 filters are sometimes effective to enhance the contrast.

■ Coding of the exciter filters

Ex. R – 6 1 0

Wavelength = 610nm

Filter color = R (red) [Other colors: O (orange), Y (yellow) and L (light yellow)]

NOTE: In some cases, application of additional barrier filters may affect the color of the emitted light, e.g. the normally apple green fluorescence of FITC will become yellow-green when an O-515 barrier filter is added.

Application of excitation regions

Excitation	Wavelength (high pressure mercury burner (Hg)	Application
UV	334nm 365nm	• FITC stain for general pathology, bacterial specimens, etc. • Observation of autofluorescence. • Fluorescence antibody method for general observation.
V	405nm 435nm	• Observation of catecholamine, serotonin (5-HT) • Quinacrine mustard stain for chromosome photometry and study. • Tetracycline stain for studies of teeth, bone, etc. • Propidium iodite for DNA study.
B	Bright lines 405nm, 435nm, and continuous spectrum regions near 490nm	• Fluorescent antibody method (FITC), double stained for lens protein synthesis and photometry. • Acridine orange (yellow) stain for observation of cancer cells, red blood cells, roundworms, etc. • Auramine stain for tubercle bacillus test. • Quinacrine mustard (QM) stain for chromosome photometry and study.
G	546nm	• Fluorescence antibody method for stained and cultured specimens. • RB-200 stain. • Feulgen stain for quantitative photometry of DNA contained in cells. • Ethidium bromide for DNA study.

D. Burner replacement

1) The average life of a mercury burner is about 200 hours.

2) It is recommended to keep a record of the burning time of each burner, and replace it at the end of its life expectancy.

3) For safety's sake, do not replace the burner for about 10 minutes after switching off.

Fig. 9

4) After burner replacement, zero the life meter, pressing the reset button ① by means of a screwdriver. To avoid accidental manipulation of the reset button, lock it with the lock lever provided. (Fig. 9)

IX. TROUBLESHOOTING

Troubles	Causes	Remedies
a) The lamp is on, but nothing can be seen through the eyepieces.	The shutter slider is closed.	Move the shutter to open aperture.
	The combination of exciter filter and barrier filter is not correct.	Follow the filter combinations as listed at page 14.
b) Blurred or soft images.	Objectives designed for the mechanical tube length 160mm are not used.	Use the biological objectives designed for the mechanical tube length 160mm, with this illuminator.
	A cover glass is not used on the specimen.	Put a cover glass on the specimen.
	Objectives other than LB series are used.	Use Olympus LB series objectives.
c) Insufficient contrast.	The objectives fluoresce.	Use non-fluorescing objectives for UV radiation.
	The immersion oil fluoresces.	Use non-fluorescing immersion oil.
	The surfaces of objectives or filters are dirty.	Clean them.
d) Irregular illumination.	The lamp is not centered.	Adjust their positions correctly.
	The collector is not correctly positioned.	

X. OPTICAL DATA

Objective / Eyepiece	Type	UVFL-				
	Magnification	10X	20X	40X	*40X (oil)	*100X (oil)
	N. A.	0.4	0.65	0.85	1.30	1.30
	W. D. (mm)	1.16	1.03	0.25	0.11	0.14
	Focal length (mm)	15.84	8.11	4.59	4.56	1.91
	**Resolution (μ)	0.84	0.52	0.40	0.26	0.26
	Remarks			Correction collar	Iris diaphragm	Iris diaphragm
WHK8X (Field No. 20)	Total magnif.	80X	160X	320X	320X	800X
	Focal depth (μ)	18.98	6.02	2.50	1.51	0.75
	Field of view (mm)	2.0	1.0	0.5	0.5	0.2
WHK10X (20)	Total magnif.	100X	200X	400X	400X	1,000X
	Focal depth (μ)	15.7	5.02	2.12	1.25	0.65
	Field of view (mm)	2.0	1.0	0.5	0.5	0.2
WHK15X (14)	Total magnif.	150X	300X	600X	600X	1,500X
	Focal depth (μ)	11.33	3.67	1.60	0.92	0.51
	Field of view (mm)	1.4	0.7	0.35	0.35	0.14

* Immersion objectives.

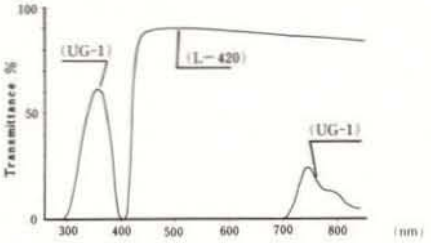
** The resolution is obtained with fully opened aperture diaphragm.

Technical terms:

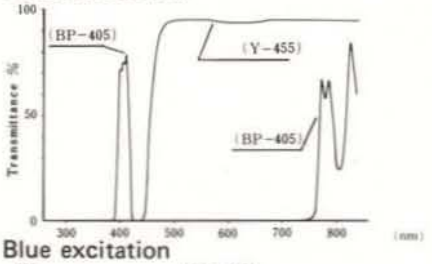
- Working distance: (W. D.) — The distance from the cover glass to the nearest point of the objective.
- Numerical aperture: (N. A.) — The N.A. represents a performance number which can be compared to the relative aperture (f-number) of a camera lens. The N.A. values can be used for directly comparing the resolutions of all types of objectives. The larger the N.A., the higher resolving power.
- Resolution: — The ability of a lens to register small details. The resolution of a lens is measured by its ability to separate two points.
- Focal depth: — The distance between the upper and lower limits of sharpness in the image formed by an optical system. As you stop down the aperture iris diaphragm, the focal depth becomes larger. The larger the N.A. of an objective, the shallower the focal depth.
- Field number: — A number that represents the diameter in mm of the image of the field diaphragm that is formed by the lens in front of it.
- Field of view diameter: — The actual size of the field of view in mm on the object surface.

XI. CHARACTERISTIC CURVES OF FILTERS

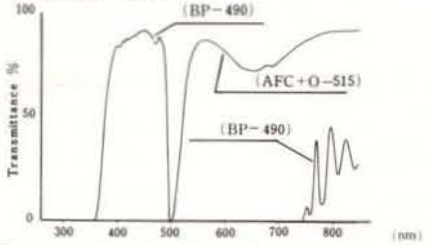
1. Ultra violet excitation



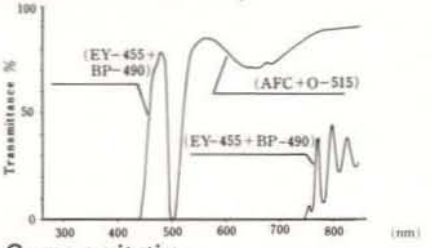
2. Violet excitation



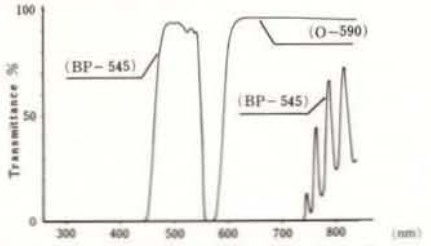
3. Blue excitation



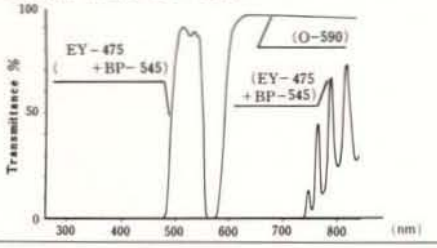
4. Blue excitation (including suppl. exciter filter EY-455)



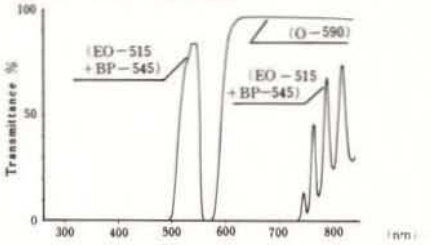
5. Green excitation



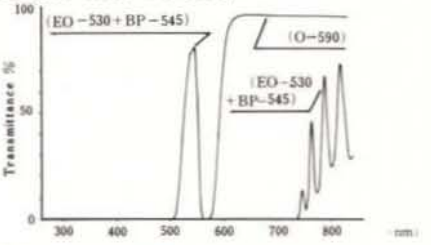
6. Green excitation (including suppl. exciter filter EY-475)



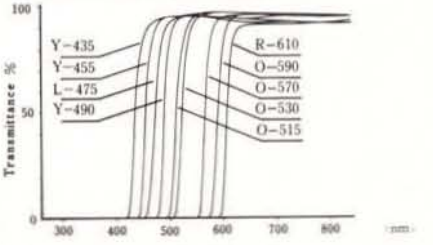
7. Green excitation (including suppl. exciter filter EO-515)



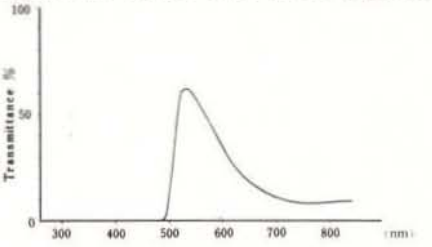
8. Green excitation (including suppl. exciter filter EO-530)



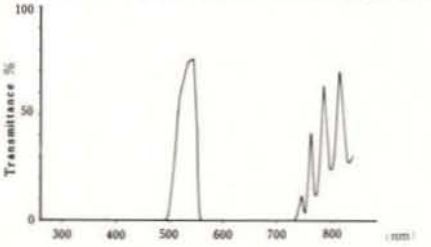
9. Additional barrier filters



10. Additional barrier filter B-460 (optional)**



11. Additional barrier filter G-520 (optional)**



* AFC (Auto fluorescence cut filter)

** Barrier filters for blue excitation (with AFC + O-515)

MEMO

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