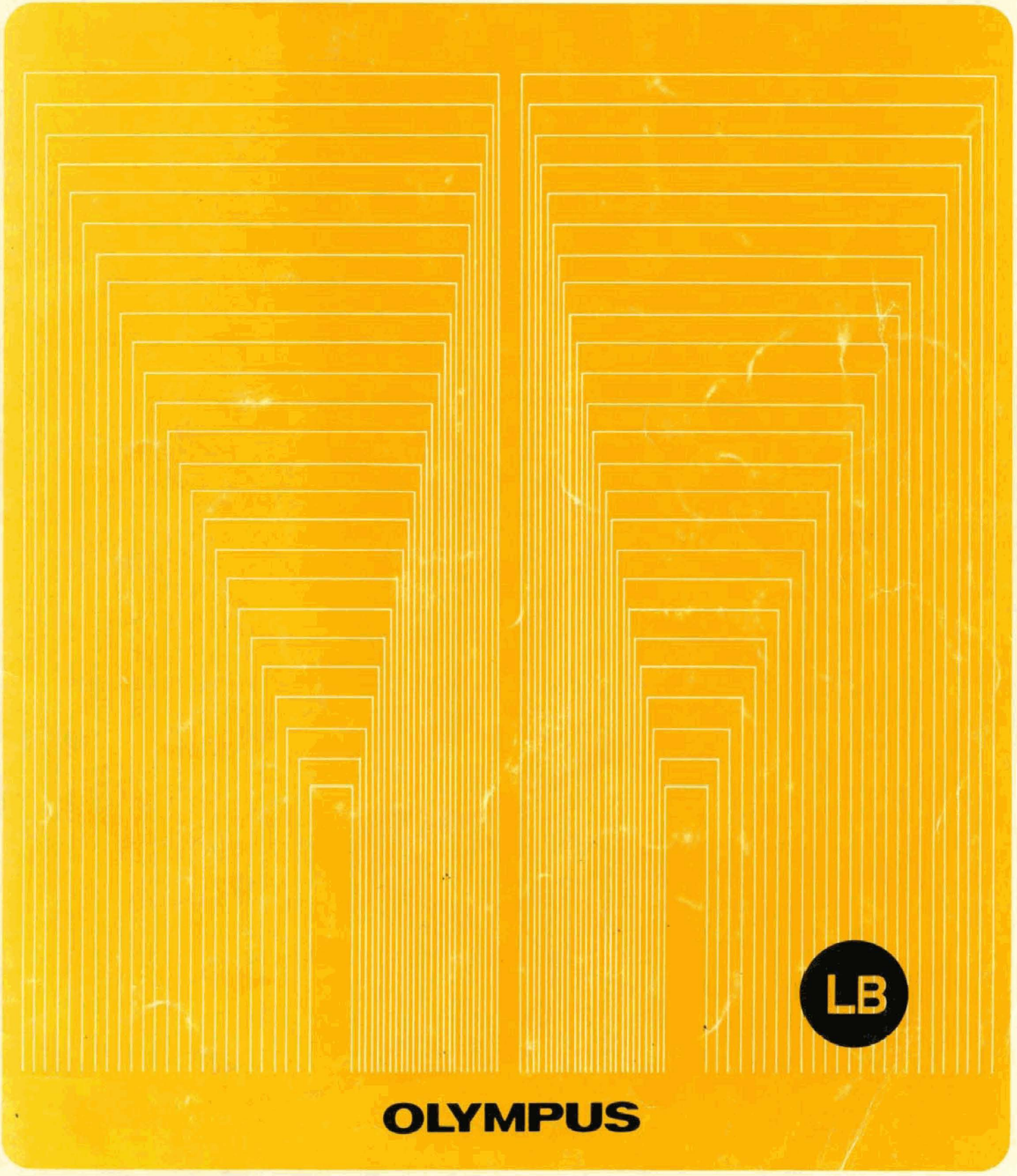
VERTICAL FLUORESCENCE ILLUMINATOR ATTACHMENT

INSTRUCTION MANUAL

MODEL AH-RFL-LB



OLYMPUS

This instruction manual has been written for use of the Vertical Fluorescence Illuminator Model AH-RFL-LB in conjunction with the Universal Research Microscope Model VANOX AHB-LB. Therefore, it is recommended that you read the instruction manual for the VANOX AHB-LB as well as this manual carefully in order to obtain optimum performance from this attachment, in conjunction with the microscope.

Note: This attachment is specially designed for use with LB series optics, including eyepieces, photo eyepieces, objectives, condensers, etc.; use of optical components other than LB optics will result in unsatisfactory performance of the unit.

Observe the following points carefully:

- Operation

1. Use a D.C. super pressure mercury burner, designated by OLYMPUS, i.e. HB0200W/2 (manufactured by Osram) or USH200MB (manufactured by USHIO INC./Japan).
2. Ascertain that the burner is correctly inserted and clamped and that all electric connections are properly made.
3. Always handle the instrument with the care it deserves, and avoid abrupt motions.
4. Avoid exposure of the instrument to direct sunlight, high humidity and temperature, dust and vibrations.
5. For protection of the observer's eyes from UV radiation, never look at the exciting 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.
6. Do not open the lamp housing while the mercury burner is lighted. Wait for about 10 minutes after the burner is switched off.
7. Make sure that voltage and frequency selector switches are set to conform with the local line voltage and frequency.
8. Note that the Model AH-RFL-LB has a magnification factor of 1.2X, thus the total visual magnification is the product of objective magnification times eyepiece magnification times 1.2.

- Maintenance

1. Be sure that no dirt, fingerprints, etc. are left on the lens and bulb surfaces. If they are soiled, wipe them clean with a cotton gauze. If necessary, use a small amount of alcohol-ether mixture (7:3) or benzine.
2. Never disassemble the attachment for repair by yourself. Only authorized OLYMPUS service personnel should make repairs.
3. Replace the mercury burner after 400 hours of use. Do not touch the burner for about 10 minutes after switching off.
4. Disconnect the line cord from the AC power outlet for fuse replacement.
5. The instrument should be covered after use with the vinyl dust cover provided.

CONTENTS

I. STANDARD EQUIPMENT

Component			Quantity
Vertical fluorescence illuminator (with quintuple revolving nosepiece)		AH-RFL-B-LB	1
Fluorescence lamp housing		AH-RFL-LH-2	1
Power cord		UYCP	1
Power supply unit		BHF-T	1
Barrier filters in slide	L-420, L-435, Y-455, Y-475, Y-495, O-515, O-530, O-570, O-590, R-610		1each
Exciter filters in mount	UG-1, UG-5, IF-405, BG-12		1each
	IF-490		2
Non-fluorescent objectives	UVFL 10X (spring-loaded)		1
	UVFL 20X (spring-loaded)		1
	UVFL 40X (spring-loaded, iris, immersion type)		1
	UVFL 100X (spring-loaded, iris, immersion type)		1
Auxiliary collector lens		A-RFL-AL-2	1
Centering mirror		M-SM	1
Mercury burners (D.C. 200W)			2
Silicone immersion oil (non-fluorescing, 50cc., bottled)			1
Wooden case		AH-RFL-WSC-LB	1

II. SPECIFICATIONS

1. Revolving nosepiece: Quintuple on ball bearing; all 5 positions letter coded for easy identification of objective powers.
2. Exciter filters built in turret:

Code	Exciter filter
U	UG-1
V	BG-3
B	BG-12
G	IF-545 + B-36

1) Dichroic mirrors: Mounted in slide and combined with built-in barrier filters.

Code	Dichroic mirror	Built-in barrier filter
U	DM 400	L-420
V	DM 455	Y-455
B	DM 500	O-515
G	DM 580	O-590

Provided with a slot for insertion of barrier filters, in slide.

2) Magnification factor: 1.2X

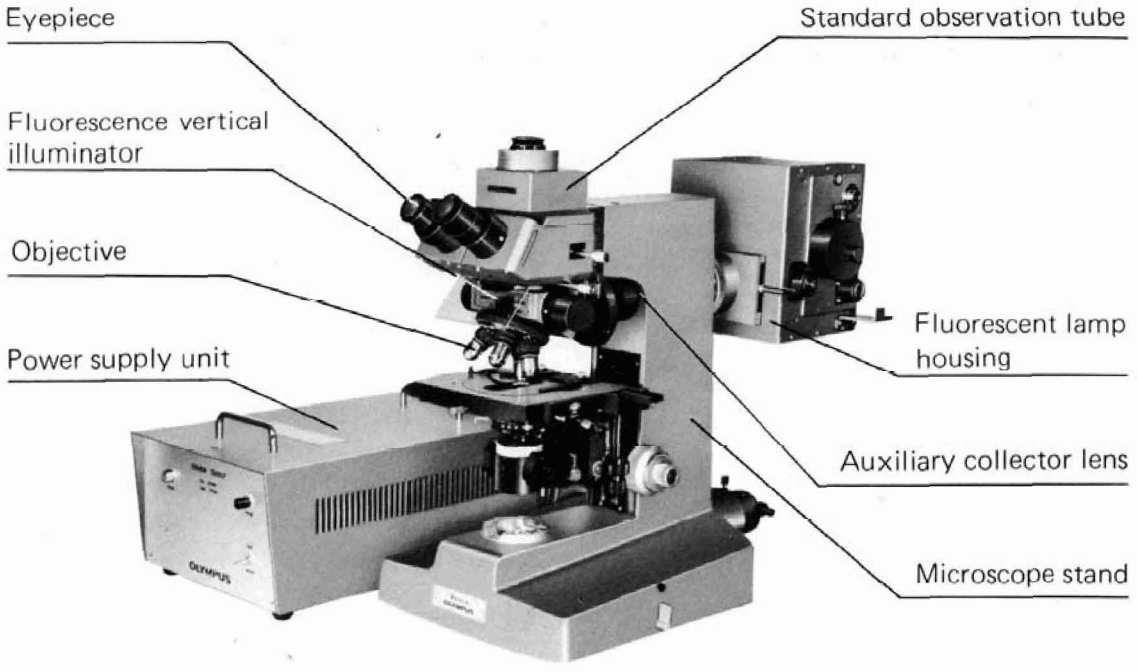
3. Fluorescent light source

1) D.C. super pressure mercury burner: HB0200W/2 (manufactured by Osram) or USH 200MB (manufactured by USHIO INC./Japan).

2) Power supply unit: Input voltage AC 100V-110V-120V or 220V-240V, 50/60Hz. (in the U.S.A., the power supply specifications are different.)

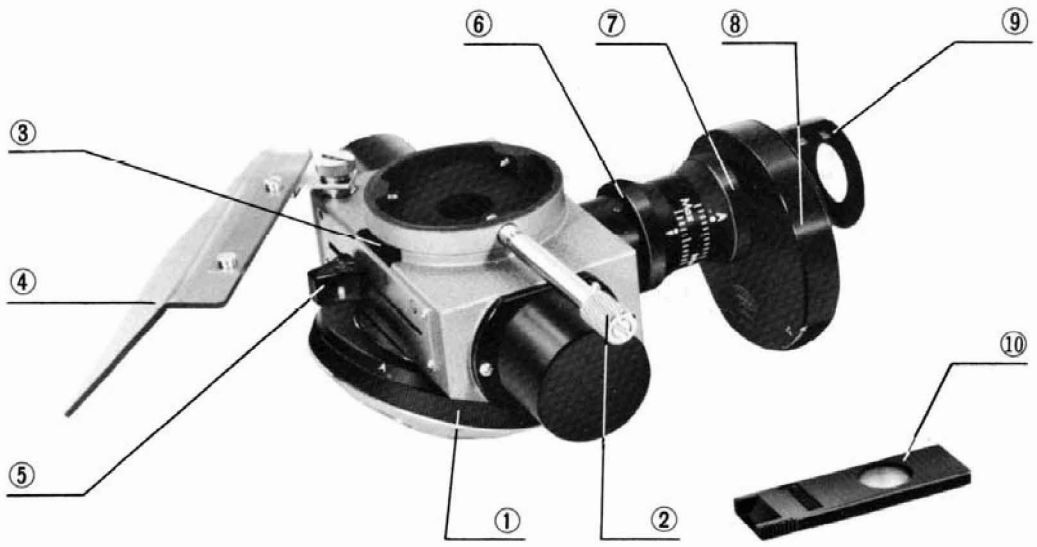
III. NOMENCLATURE

Model AH-RFL-LB on the Universal Research Microscope Model VANOX AHB-LB.



IV. IDENTIFICATION AND FUNCTION OF VARIOUS COMPONENTS

A. Vertical Fluorescence Illuminator



① Revolving nosepiece: Provided with 5 threaded apertures to accept objectives.

② Observation tube clamping screw: This illuminator is attached to the circular dovetail of the observation tube and clamped with this screw.

③ Barrier filter insertion slot: Place the dust slide ⑩ into this slot when no barrier filter is used.

④ UV protective shade: Make it a rule to use this shade to protect your eyes from fluorescent light. Can be swung out for insertion of a barrier filter in mount or when operating the dichroic mirror selector lever.

⑤ Dichroic mirror selector lever: Dichroic mirrors combined with built-in barrier filters can be selected with this lever. (*See NOTE below.)

⑥ Field iris diaphragm ring:

⑦ Aperture iris diaphragm ring:

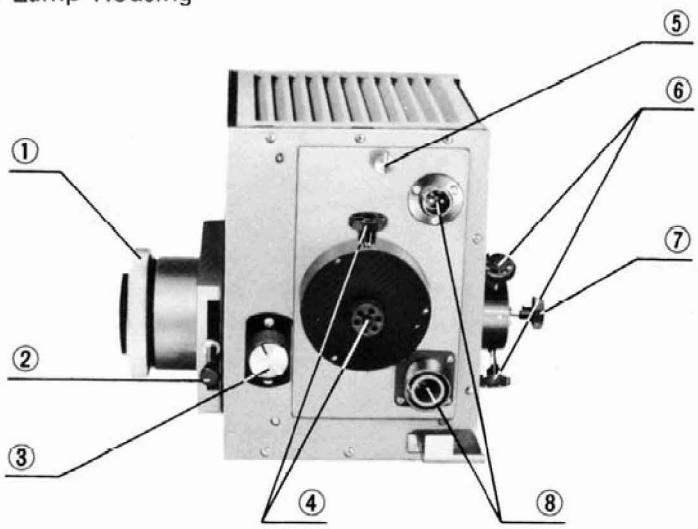
⑧ Exciter filter turret: Incorporates filters UG-1 (coded U for ultraviolet), BG-3 (V for violet), BG-12 (B for blue violet) and IF-545 + BG-36 (G for green violet), with an additional aperture (O).

⑨ Filter mount: Accepts two exciter filters in mount.

⑩ Dust slide

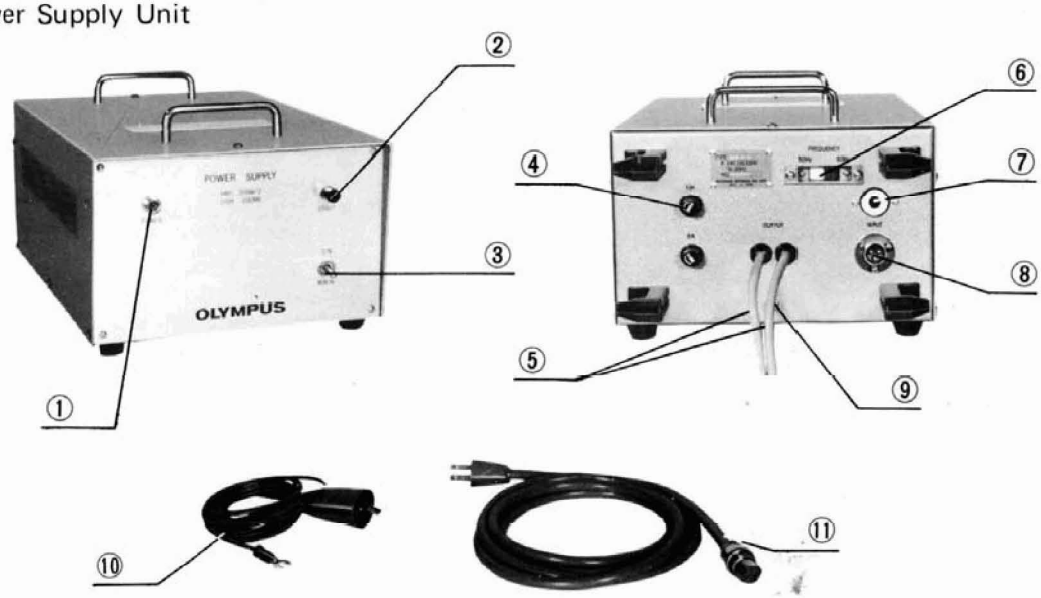
* **NOTE:** The Model AH-RFL-LB incorporates dichroic mirrors, which reflect short wavelength radiation towards the objective to illuminate the specimen, while passing long wavelength.

B. Fluorescent Lamp Housing



① Knurled ring: Attaches the lamp housing to the microscope stand.
② Shutter lever: Pull out the shutter lever to block the light.
③ Collector lens focusing knob
④ Burner centering knobs
⑤ Socket clamping screw
⑥ Reflector centering knobs
⑦ Reflector focusing knob
⑧ Connectors: Accept the plugs of the connecting cord.

C. Power Supply Unit



① Pilot lamp
② Start button
③ Main switch
④ Fuse holder
⑤ Connecting cords
⑥ Frequency selector switch
⑦ Line voltage selector switch
⑧ Power cord connector
⑨ Grounding terminal
⑩ Grounding cord
⑪ Power cord

D. Auxiliary Collector Lens

E. Exciter Filters in Mount (6 pcs.)

F. Barrier Filters in Slide (10 pcs.)

G. Centering Mirror

V. ASSEMBLY

Prior to assembly, remove all dust caps and be careful not to touch the optical elements with your fingers.

1. Attach the lamp housing. (Fig. 1)

   Place the lamp housing next to the flange of the opening provided on the microscope limb, with positioning groove aligned with positioning pin, and lock by turning the knurled ring clockwise.

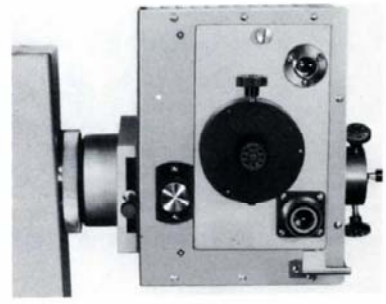


Fig. 1

2. Mount the auxiliary collector lens. (Fig. 2)

   Insert the lens into the front of the opening on the microscope limb.

Fig. 2

3. Attach the observation tube.

   1) Remove the standard observation tube from the microscope stand, and turn the selector turret on top of the microscope stand to position "M.P.". (Fig. 3)

   2) Check that the two clamping levers on the right hand side of the dovetail mount are unclamped (levers pointing upwards).

   3) Insert the tube dovetail slide into the dovetail mount on the microscope stand and lower the tube as far as possible.

   4) Firmly lock the tube with the upper clamping lever.

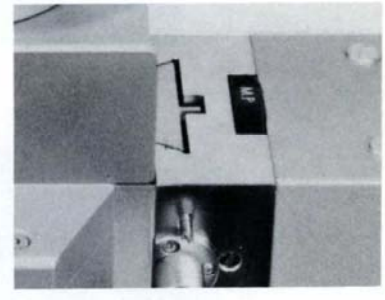


Fig. 3

4. Attach the vertical fluorescence illuminator.

   1) Clamp the UV protective shade ① to the upper edge ② of the vertical illuminator. (Fig. 4)

   2) Clamp the vertical illuminator to the observation tube in the same manner as the standard revolving nosepiece.

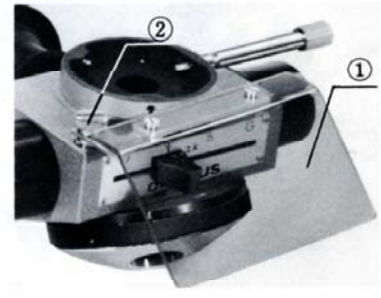


Fig. 4

5. Mount objectives and eyepieces.

6. Install the mercury burner.

Wipe the surface of the mercury burner clean with an alcohol-ether mixture, benzine, etc. Use great care to make sure that no dirt, fingerprints, etc., are left on the bulb surface, and when installing, be carefull not to touch the bulb portion.

1) Loosen the socket clamping screw ①, and lift up the socket as shown by the arrow in Fig. 5. At this time, pay attention to the following:
   (1) Be sure to use a DC type mercury burner (HB0200W/2 or USH200MB).
   (2) Note that the connecting cord prevents socket removal unless it is unplugged.

2) Remove the retainer of the socket terminals, used for transportation.

3) Insert the lower electrode (marked with "+") into the bottom terminal and tighten with clamping nut ②, then insert the upper electrode into the slot of the upper mounting terminal, and lock with clamping nut ③. Be sure to turn the pearl on the burner envelope ① 90° away from the optical axis. (Fig. 6)

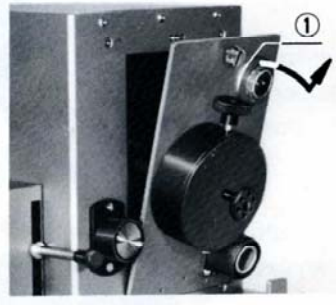


Fig. 5

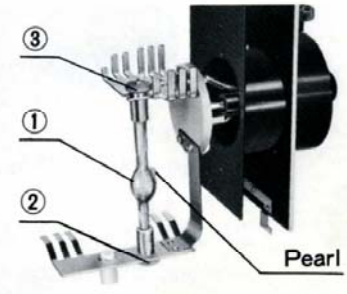


Fig. 6

VI. OPERATION

A. Ignition of the Burner

1) Ascertain that the line voltage selector switch on the power supply unit 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.)

2) Ascertain that the freqnency selector switch on the power supply unit is set to conform with the local mains frequency (50Hz or 60Hz). If you find the switch is not correctly positioned, unscrew the transparent cover and set the switch correctly.

3) Check complete electric connection.

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

5) Press the start button, and the burner will ignite.

★ If the line voltage is lower than 10% of the rated voltage, the arc will sometimes flicker.

6) Do not switch off the burner within 15 minutes after ignition.

★ Repeated on-off switching considerably shortens the burner life. After the burner is switched off, do not re-ignite for 3 minutes or more in order to give it time to cool.

Fuse replacement:
Disconnect the power cord from the AC outlet, and remove the fuse holder from the power supply unit.

B. Burner Centration

After the arc has stabilized (2~4min.), center the burner as follows:

1) Rotate the exciter filter turret until the turret click stops at the "G" position.
2) Open the shutter by pushing the shutter knob all the way.
3) Rotate the knurled rings "F" ① (for the field iris diaphragm) and "A" ② (for the aperture iris diaphragm) to the MAX. position. (Fig. 7)
4) Swing the UV protective shade to the left.
5) Slide the dichroic mirror selector lever to the "B" position.

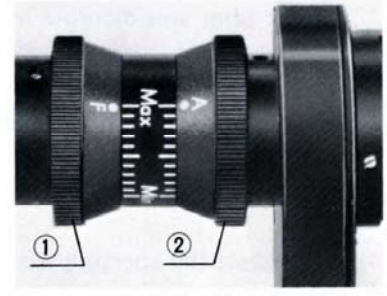


Fig. 7

6) Insert the barrier filter O-570 into the slot of the vertical illuminator.
7) Place the burner centering mirror on the stage, and focus on it with the 10X objective.
8) Pull out the light path selector lever built-in the observation tube up to the CV position (yellow-green line).
9) Remove the cap from the photo eyepiece attachment hole on top of the observation tube. As you look through the opening, you can see the arc images of the burner (spots of brightness) at the back of the objective.

NOTE: If the burner is out of center, four spots of brightness can be seen as the real and reflected images. The reflected image can be identified from the real image, by manipulating the reflector centering knobs, because the reflected image moves as you operate the reflector centering knobs, while the real image does not.

a) Bring the real image into focus with the collector lens focusing knob.
b) Bring the real image to position (Fig. 8b) by means of the burner centering knobs.
c) Bring the real and reflected images into symmertrical positions with each other by means of the reflector centering knobs. (Fig. 8c)
d) Equalize the sizes of the reflected and real images with the reflector focusing knobs.
e) Superimpose the real and reflected images.

★ **As a rule, this procedure is required only after burner replacement.**

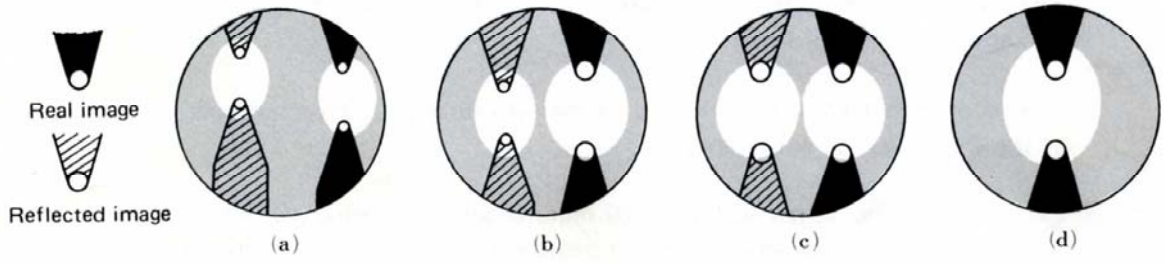


Fig. 8

C. Fluorescence Microscopy

1) Bring the area of the specimen to be observed into the field of view, and focus with transmitted light, emitted from the halogen or tungsten filament bulb. (See the instruction manual for the Universal Research Microscope Model VANOX-AHB-LB.

2) Switch off the transmitted light source, and select the most suitable combination of exciter filter and dichroic mirror for your specimen.

NOTE: Additional exciter filters in mount and barrier filters in slide, both provided, can be used as desired. For details read E. "Use of Filters".

3) Stop down the field iris diaphragm ("F") until it is within the field of view.

4) Stop down the aperture iris diaphragm ("A") to ensure proper contrast.

★ **Use non-fluorescing silicone immersion oil for UVFL40X (immersion type) and UVFL 100X (immersion type) objectives.**

5) 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.

6) The objectives UVFL40X (immersion type) and UVFL100X (immersion type) are provided with iris diaphragms. It is recommended to stop down the iris diaphragm slightly to increase contrast and image definition.

7) The objective UVFL40X (dry) is provided with a correction collar which can be set 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 in best definition.

8) When fluorescence observation is to be interrupted briefly (for about 30 minutes or less), it is good practice to cut off the beam of light by means of the opaque shutter rather than to turn off the mercury burner, since repeated on-off switching considerably shortens the useful life of the burner.

D. Burner Replacement

1) The average life of burner is about 400 hours, provided that each lighting duration should be kept longer than 2 hours.

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

3) Do not touch the burner for about 10 minutes after switching off. Then, ascertain that the main switch is off, and disconnect the lower connecting cord from the lamp housing.

E. Use of Filters

Excitation method	Exciter filter			Dichroic mirror selector knob	Barrier filter in mount
	Spectral band	Exciter filter turret	Exciter filter in mount		
Ultraviolet	Wide	U (UG-1)	None	U (DM 400 + L-420)	L-420 and up
	Narrow		UG-1		
Violet	Wide	V (BG-3)	UG-5	V (DM 455 + Y-455)	Y-475 and up
	Narrow		IF-405		
Blue	Wide	B (BG-12)	BG-12	B (DM 500 + O-515)	O-530 and up
	Narrow	None	IF-490 (2 pcs.)		
Green	Narrow	G (IF-545 + BG-36)	None	G (DM 580 + O-590)	R-610

Excitation methods and applications

1) Ultraviolets: The line spectrum at bright lines 334nm and 365nm.

- Fluorescence antibody method (FITC)
- Congo red test

2) Violet: The line spectrum at bright lines 405nm and 435nm.

- Catecholamine

3) Blue: The line spectrum at bright lines 405nm and 435nm, and continuous spectrum at 490nm.

- Fluorescence antibody method (FITC)
- Acridine yellow and acridine orange
- Auramine
- Tetracycline

4) Green: The line spectrum at bright line 546nm.

- Fluorescence antibody (TRITC)
- Feulgen
- Rhodamine B
- Fuchsin

VII. OPTICAL DATA

Objective / Eyepiece	Type	UVFL				
	Magnification	10X	20X	**40X	40X (immersion type)	100X (immersion type)
	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
	*Resolving power (μ)	0.84	0.52	0.395	0.26	0.26
	Remarks			Collection collar	Iris diaphragm	Iris diaphragm
NK5X (Field No. 21)	Total magnif.	50X	100X	200X	200X	500X
	Focal depth (μ)	28.83	9.05	3.66	2.26	1.05
	Field of view (mm)	2.1	1.05	0.53	0.53	0.21
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

* The resolving power is obtained with fully opened aperture diaphragm.

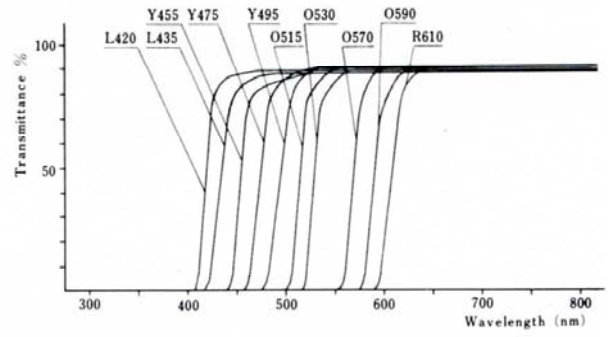
** Optionally available.

Technical terms:

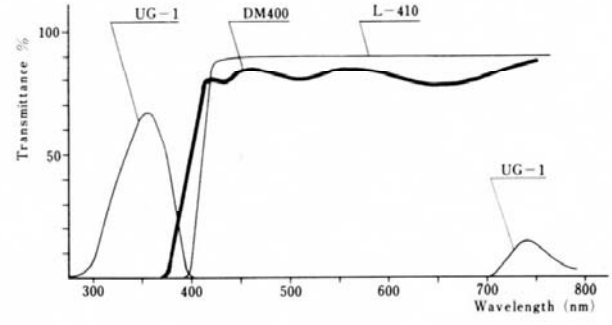
- Working distance: (W. D) — The distance from cover glass to the closest point of the objective when focused on the speciman.
- Numerical aperture: (N. A.) — The N. A. represents a performance number which can be compared to the relative aperture (f-number) of a camera lens. The N. A. values can be used for directly comparing the resolutions of all types of objectives. The larger the N. A., the higher the resolving power.
- Resolving power: — The ability of a lens to register small details. The resolving power 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.

VIII. SPECTRAL CHARACTERISTICS OF FILTERS

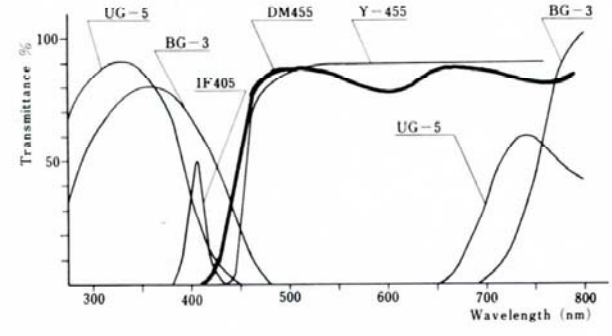
1. Barrier filters in slide



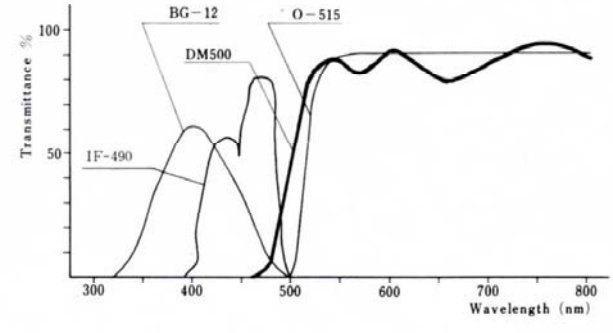
2. Ultraviolet exciter filters



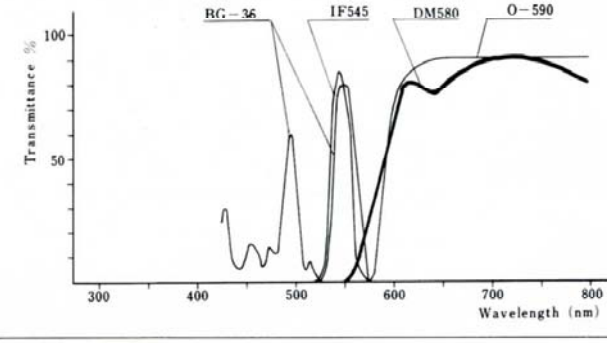
3. Violet exciter filters



4. Blue exciter filters



5. Green exciter filters



OLYMPUS OPTICAL CO., LTD.

OLYMPUS TOKYO

22-2, NISHISHINJUKU 1-CHOME,
SHINJUKU-KU TOKYO, JAPAN.

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