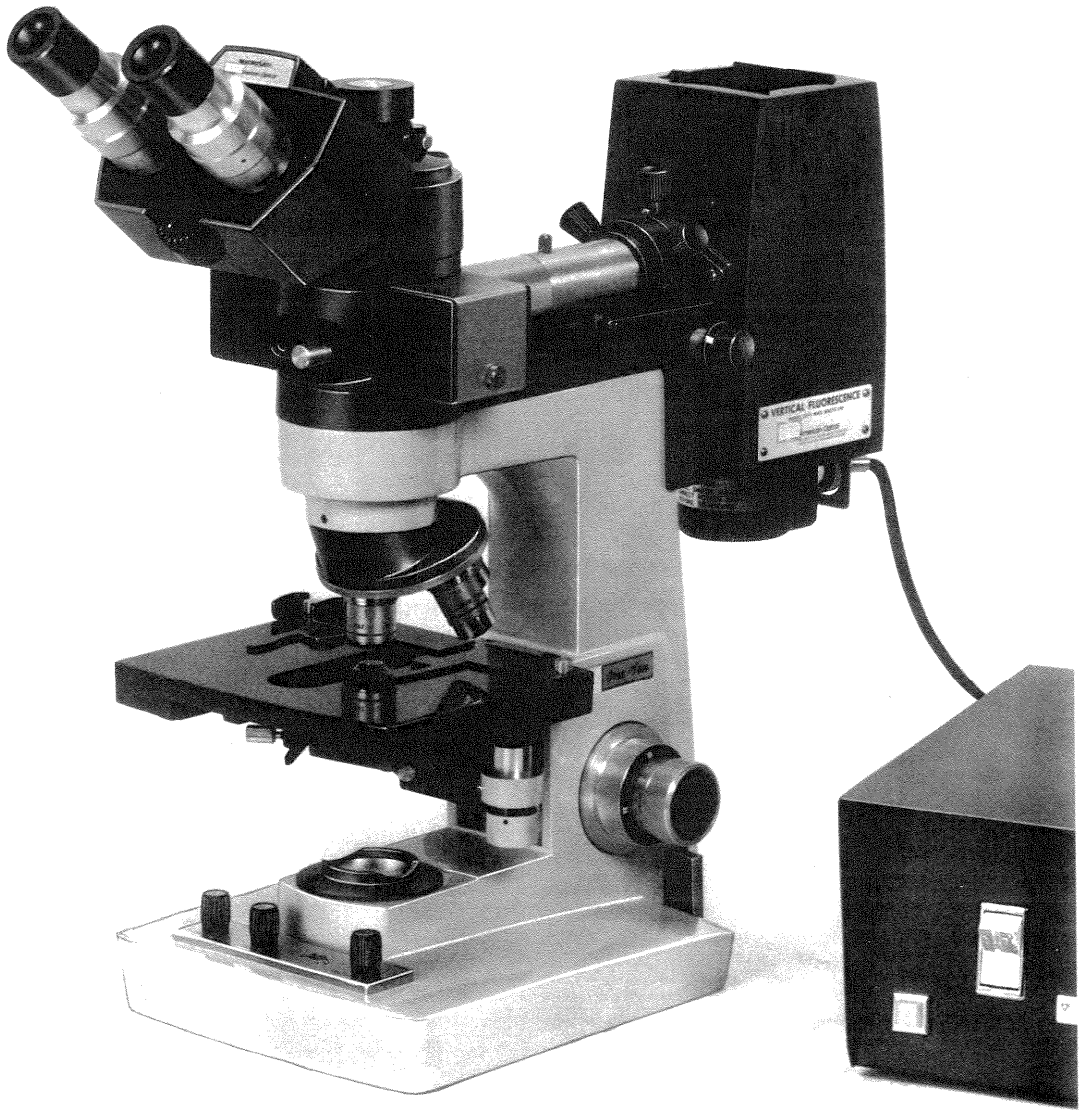
REICHERT VERTICAL ILLUMINATOR FOR INCIDENT LIGHT FLUORESCENCE MICROSCOPY Models 2071M, 2071MP, 2071H, 2071HP

REFERENCE MANUAL



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Division of Warner-Lambert Technologies, Inc.
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I. INTRODUCTION

The Series 2071 Vertical Illuminator module adds convenience and efficiency of incident-light fluorescence microscopy to the Series One-Ten or One-Twenty MICROSTAR® Microscopes. Adaptation is simple, requires no tools or adjustments. The lamphouse prevents leakage of direct radiant energy and is merely warm to the touch. The objective also functions as its own condenser, eliminates alignment complications and utilizes full numerical aperture for excitation and viewing. The full field of view is covered — even for combination of 10X objective and 10X Wide Field eyepiece. Fluorescence intensity is maximized regardless of specimen thickness or density.

The basic illuminator system consists of: a 100 watt Tungsten Halogen Lamp or a 50 watt Mercury Vapor Lamp to provide a rich source of excitation energy; collector lens system and field diaphragm to efficiently control the beam; exciter filters to transmit selected wavelengths; dichroic beam-splitters to selectively reflect and subsequently transmit desirable wavelengths, and barrier filters to bar unwanted wavelengths.

Fundamental to the system is the unique dichroic element. It efficiently reflects excitation energy wavelengths up to a predetermined cut-off point. Longer wavelengths, as emitted by fluorescence energy, pass through the dichroic element and are not deflected from their paths.

Excitation energy, which is beamed from the lamp through the exciter filter, is reflected by the dichroic element down through the microscope objective into the specimen. Fluorochromes within the specimen, excited by the energy beam from the objective, emit visible fluorescence energy which returns up through the microscope objective, dichroic element and barrier filters to the microscope eyepiece or camera. See Figure 1.

The unique Fluor Cluster combines exciter filter, dichroic element and barrier filter into one easy-to-use module. Table 1 (page 2) lists the filters and dichroic elements for the various Fluor Clusters, while Table 2 (page 3) shows wavelength transmittance curves.

II. COMPONENTS

Catalog 2071M* Vertical Illuminator includes the following:

	Vertical Illuminator Module
2072	Fluor Cluster
2073	Fluor Cluster
2074	Fluor Cluster
2053	Lampholder for Mecury Vapor Lamp
2054A	Power Supply, 115V, 60 Hz
2055	Mecury Vapor Lamp, 50 W, Osram HBO-50W
6140	Immersion oil, very low fluorescence 1/4 ounce
2071-101	Reference Manual

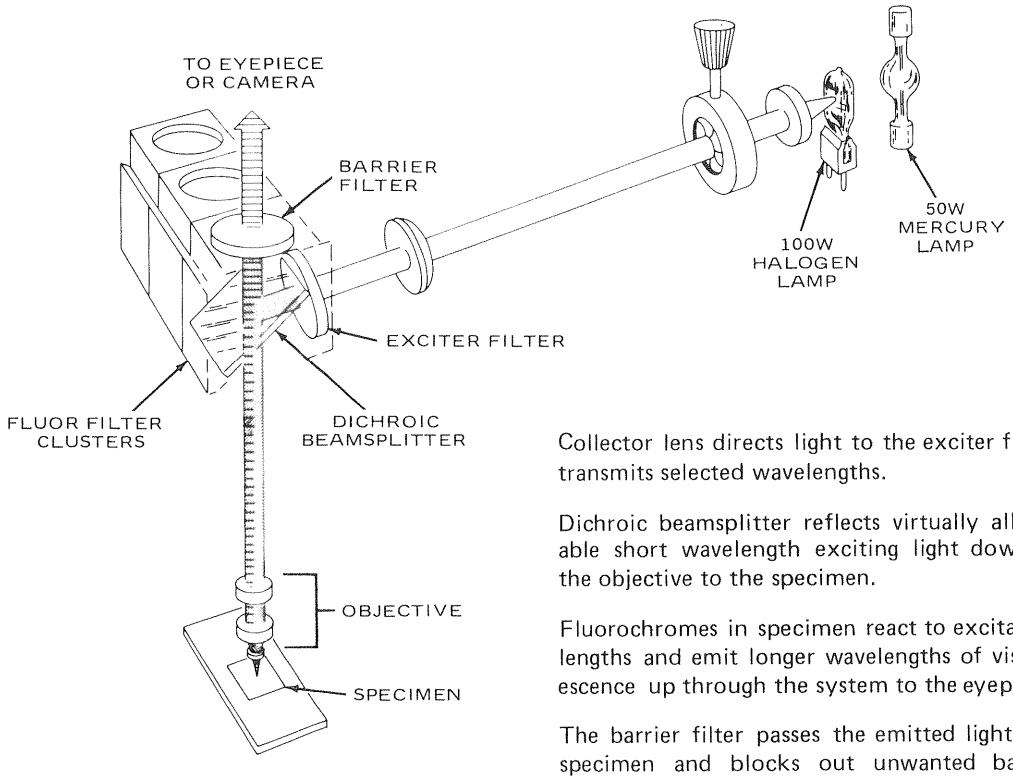
*2071MP includes 2077 Fluor Cluster instead of 2072.

Catalog 2071HP Vertical Illuminator includes:

	Vertical Illuminator Module
2077	Fluor Cluster
2050A	Lampholder for Tungsten Halogen Lamp
1138	Power Supply 115V, 60 Hz
2052	Tungsten Halogen Lamp, 100 W, 12V
6140	Immersion oil, very low fluorescence 1/4 ounce
2071-101	Reference Manual

Figure 1.

PATH OF RADIANT ENERGY THROUGH
MODEL 2071 VERTICAL ILLUMINATOR



Collector lens directs light to the exciter filter, which transmits selected wavelengths.

Dichroic beamsplitter reflects virtually all the desirable short wavelength exciting light down through the objective to the specimen.

Fluorochromes in specimen react to excitation wavelengths and emit longer wavelengths of visible fluorescence up through the system to the eyepiece.

The barrier filter passes the emitted light from the specimen and blocks out unwanted background illumination.

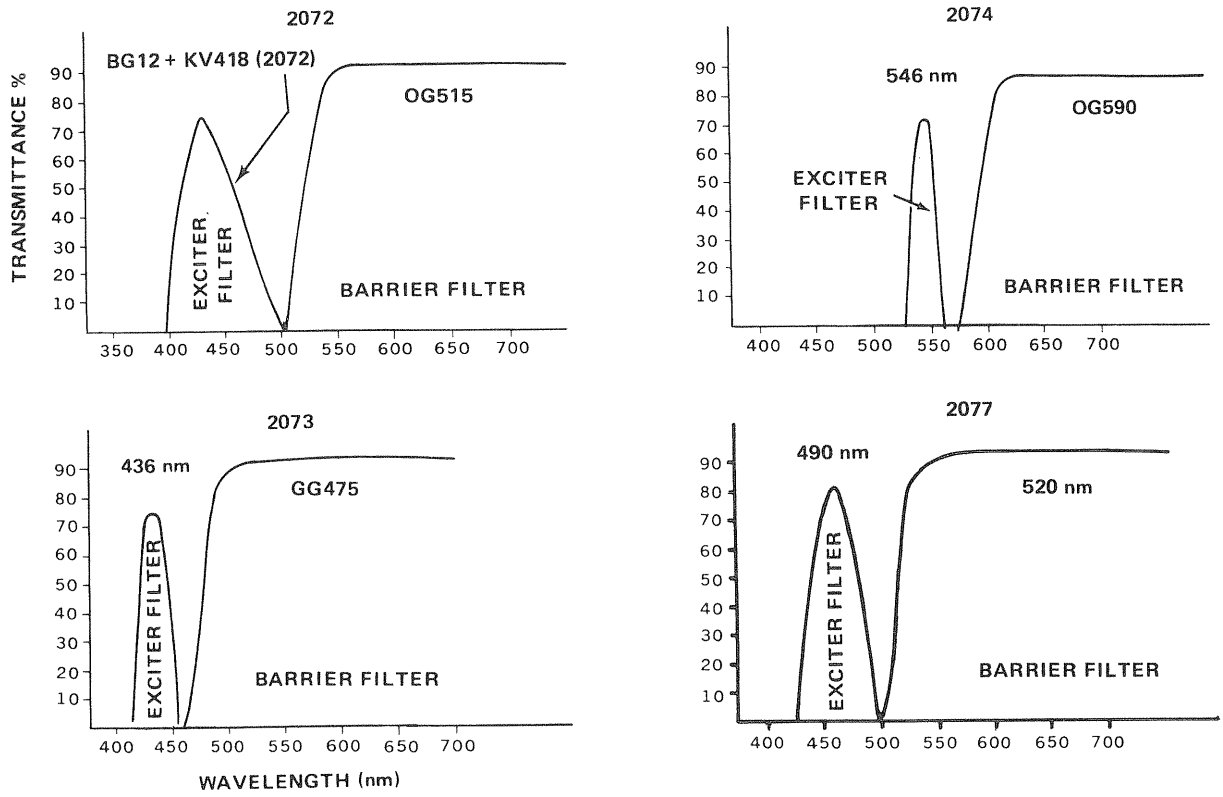
Table 1. Fluor Cluster Data

With the 50 Watt Mercury Vapor Lamp				
Catalog No.	Exciter	Dichroic	Barrier	Fluorochrome
2072	BG12 + KV418 *(2072-601 + 1155-601)	500 nm	OG 515 (2072-603)	FITC, Acridine Orange
2073	436 nm (2073-601)	450 nm	GG475 (2073-603)	Quinacrine Mustard, Auramine
2074	546 nm (2074-601)	560 nm	OG 590 (2074-603)	Rhodamine
With the 50 Watt Mercury Vapor or 100 Watt Halogen Lamp				
2077	490 nm	510 nm	520 nm	FITC, Acridine Orange

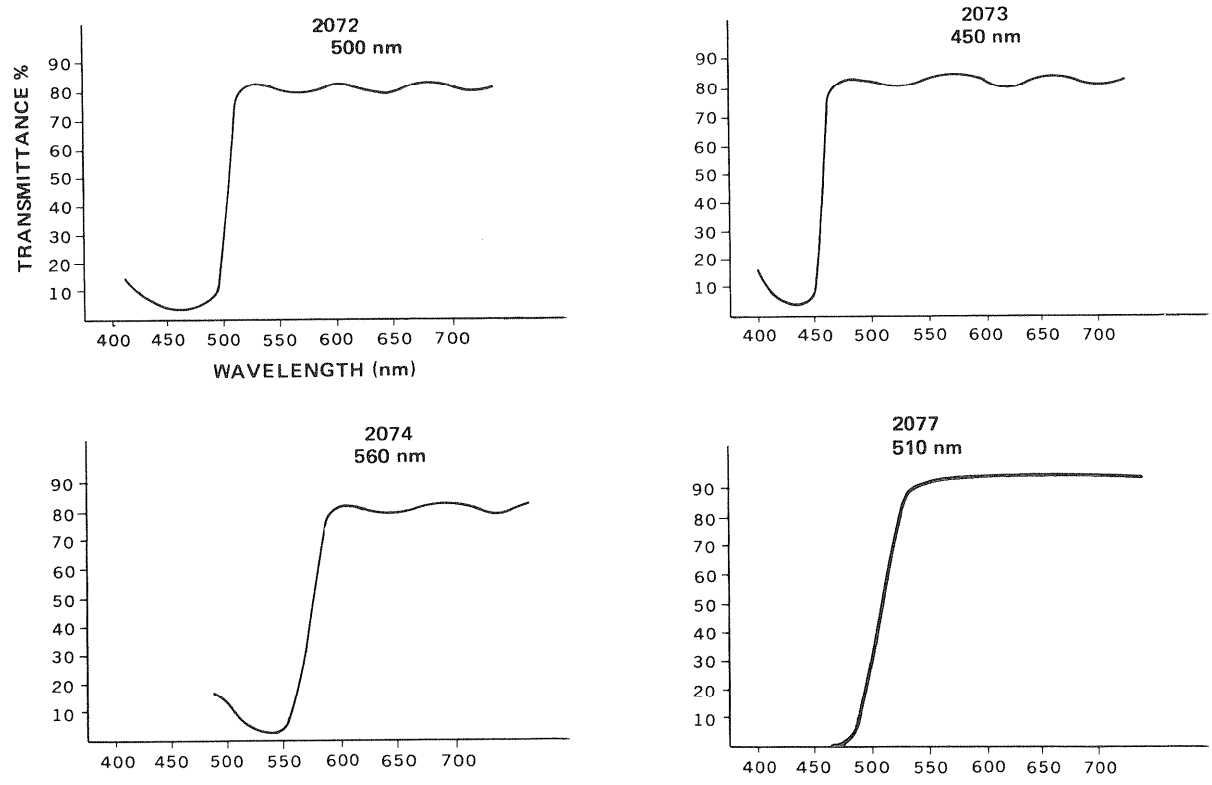
*Numbers in parenthesis are part numbers for filters

Table 2. Reflectance-Transmitter Curves for Series 2071 Vertical Illuminator

TRANSMISSION CURVES FOR EXCITER AND BARRIER FILTER COMBINATIONS



TRANSMISSION CURVES FOR DICHROIC BEAMSPLITTER



III. ASSEMBLY AND ALIGNMENT PROCEDURE

CATALOG 2071M, 2071MP

1. Note whether #2055 Mecury Vapor Lamp is marked either L1 or L2, which designates certain operating voltage characteristics (Figure 2). The symbol L1 or L2 is subsequently referred to in Step 11.

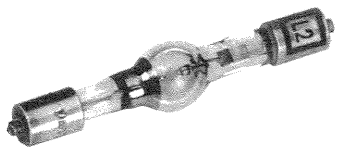


Figure 2.

INSTALLATION OF LAMP AND LAMP HOLDER

WARNING

UNPLUG INSTRUMENT BEFORE PROCEEDING AND DO NOT TOUCH HOT LAMP.

Handle the lamp with care; never hold it by the quartz-envelope, only by the electrodes. If the glass holder has been inadvertently touched, it must be thoroughly cleaned with cloth or tissue and alcohol.

Always allow a waiting period of 2 minutes after turning power supply off before re-use. The lamp must sufficiently be cooled to insure optimum lamp ignition. It is also recommended that the lamp be allowed to cool approximately 5 minutes before lamp replacement. *Never operate lamp when withdrawn from the lamphousing.*

It is also recommended that the line voltage be checked to make sure that it is close to 120V, 60 Hz AC specified. A higher line voltage could conceivably shorten the lamp life and lower lamp voltage might contribute to unsatisfactory lamp operation.

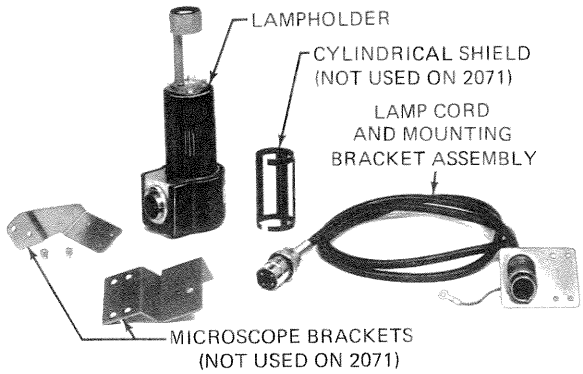


Figure 3. Lampholder Assembly

Figure 4.

2. Remove the cylindrical shield furnished with the #2053 Lampholder (Figure 4). Insert the plain end of the #2055 Lamp into the spring-loaded inner contact of the lampholder as shown in Figure 5A. The other end of the lamp, marked L1 or L2, is now inserted in the hole in the outer support. Orient the evacuation bead (the small exterior bulge on curved surface of lamp envelope) toward the lampholder post. When inserted properly, the silvered portion of the lamp envelope should be at bottom (Figure 5B).

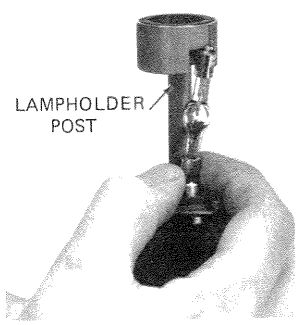
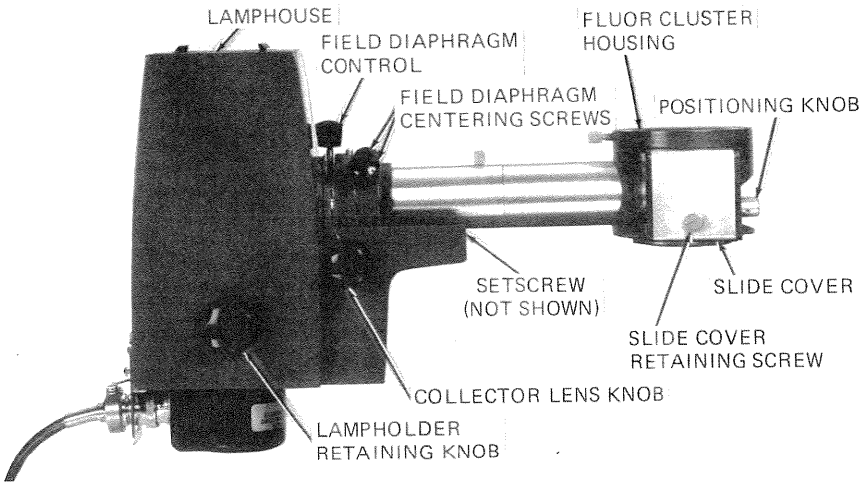


Figure 5A.

Figure 5B.



NOTE: Lampholder retaining knob has a safety detent spring which must be pushed in when turning to engage locking screws.

Figure 6.

The cylindrical shield is **not** used in the #2071M or MP Vertical Illuminator. It is used with the lampholder only in the Model V120 Microscope.

3. Turn the collector lens knob to move the collector lens forward toward the filter housing. Loosen the lampholder retaining knob (Figure 6).

4. Attach lamp cord mounting bracket with the 4 screws supplied to the rear of the lamphouse using a 5/64"Allen wrench (supplied). Ground wire must be attached as shown (Figure 7).

NOTE: The separate brackets are not used with #2071 Illuminator.

5. Insert lampholder into lamphouse with lamp forward (Figure 8).

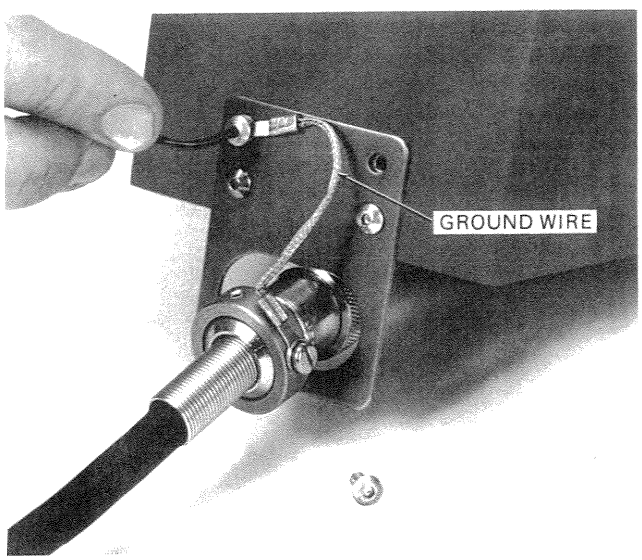


Figure 7.

6. Position lampholder receptacle opposite cable connector and tighten lampholder retaining knob (Figure 9).

NOTE: Lampholder retaining knob has a safety detent spring which must be pushed in when turning to engage locking screw.

7. Insert cable connector into lampholder receptacle and tighten threaded connector ring finger tight (Figure 9).

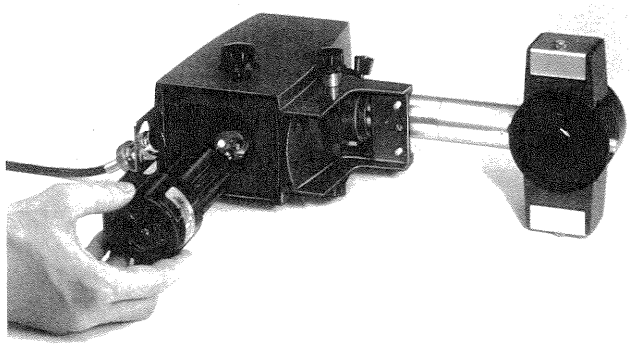


Figure 8.

8. Place the assembled #2071 Vertical Illuminator module on your Series One-Ten or One-Twenty Microscope in place of the binocular or trinocular body. Make sure the assembled unit is seated firmly, and then tighten the locking screw at the top of the microscope arm (Figure 10). Tighten set screws until they touch microscope arm (Figure 6).

Fasten the body on top of the Vertical Illuminator filter housing. See Cover.

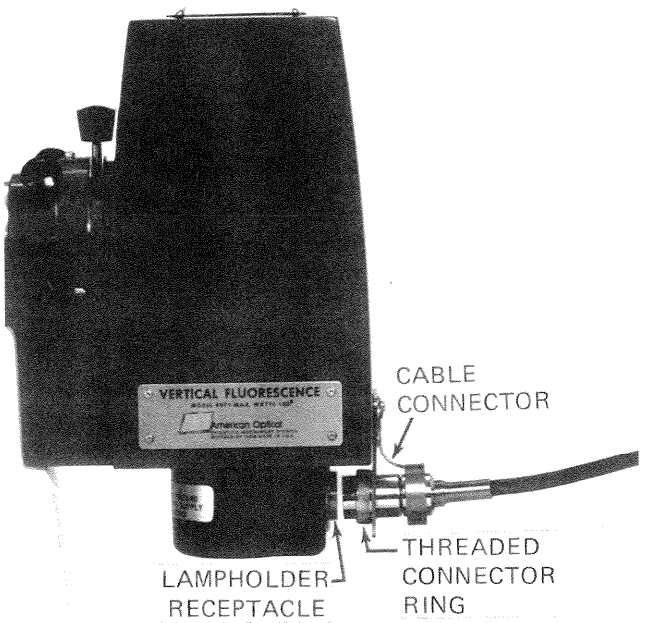


Figure 9. Attaching Lamp Cord

9. Remove the two slide covers from the Illuminator body by loosening the retaining screw on each (Figure 6).

10. The 2071M and MP outfits include three Fluor Clusters. (See Table 1.) Two Fluor Clusters can be mounted in the Illuminator at a given time. DO NOT TOUCH THE OPTICAL SURFACES OF THE FLUOR CLUSTERS (Figure 11). To install, move positioning knob to either side of its excursion. See Figure 12. With the retaining lever swung out of the way (Figure 13), slide desired Fluor Cluster into position on locating rods (Figure 14). Swing retaining lever horizontally to secure Fluor Cluster (Figure 15). Move positioning knob to opposite excursion and repeat for second Fluor Cluster. Replace slide covers.

Figure 11.

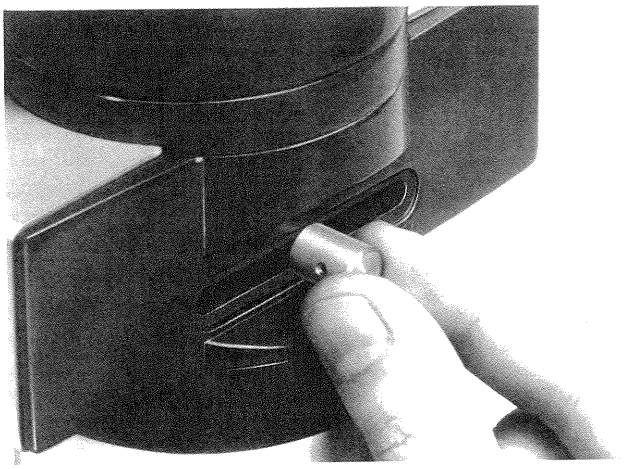


Figure 12.

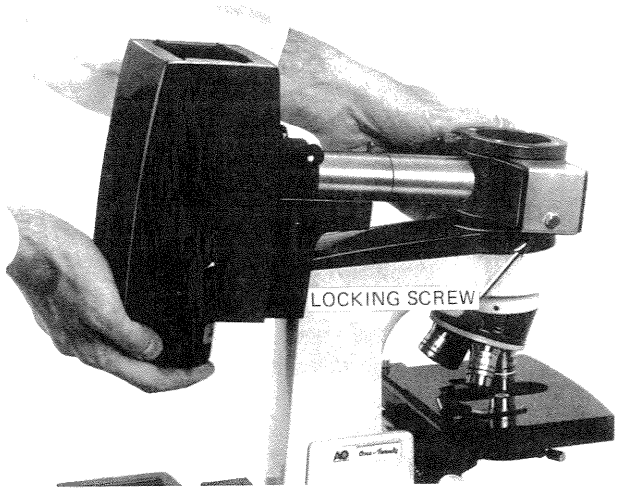


Figure 10. Attaching Lampholder

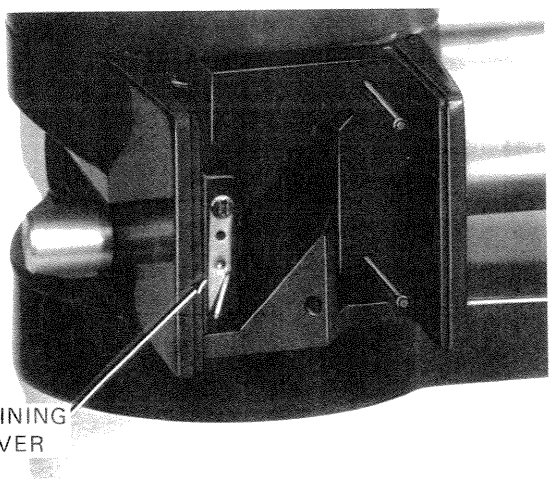


Figure 13. Fluor Cluster Locating Rods

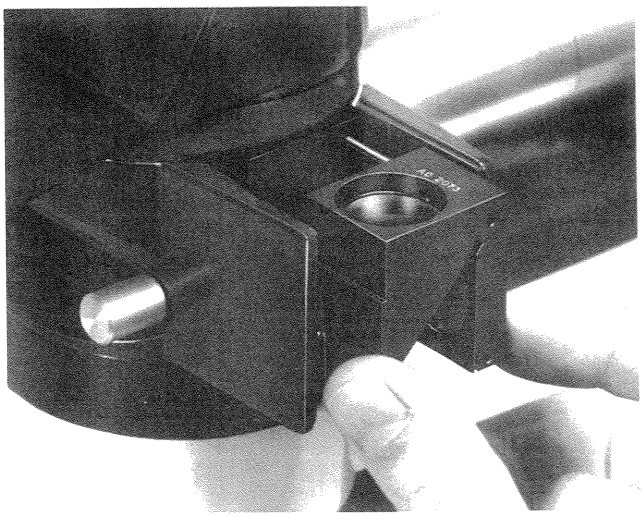


Figure 14.

11. Make sure power supply is not connected to wall outlet. Connect the cord from the lampholder to the outlet on the back of the power supply to correspond with L1 or L2 found on the lamp (Step 1) (Figure 16).

NOTE: Never operate the lamp when withdrawn from the lamphouse.

12. Plug the power supply into an electrical outlet, 115V, 60 Hz. Press power switch on the front of the power supply (note pilot light) and allow about 5 minutes for the Mercury Vapor Lamp to develop full brightness.

13. Place desired Fluor Cluster into optical path by use of positioning knob (Figure 12).

    Place the field diaphragm control at the middle of its excursion (Figure 6).

    Turn the collector lens knob to bring the collector lens fully forward, and then back about one-quarter turn.

14. Remove an objective from the microscope nosepiece and turn the clear opening into the optical path.

15. Place a piece of white paper on the microscope stage, under the nosepiece opening, to reflect the light. Loosen the lampholder retaining knob, and adjust the lampholder to center the bright image of the arc as it reflects from the white paper (Figure 17A). Then tighten the lampholder retaining knob.

16. Remove the paper and return the objective to the nosepiece.

17. Place a vivid fluorescent test slide on the microscope stage and focus on it with the 10X objective. The field should show brightly fluorescing particles against a dark background.

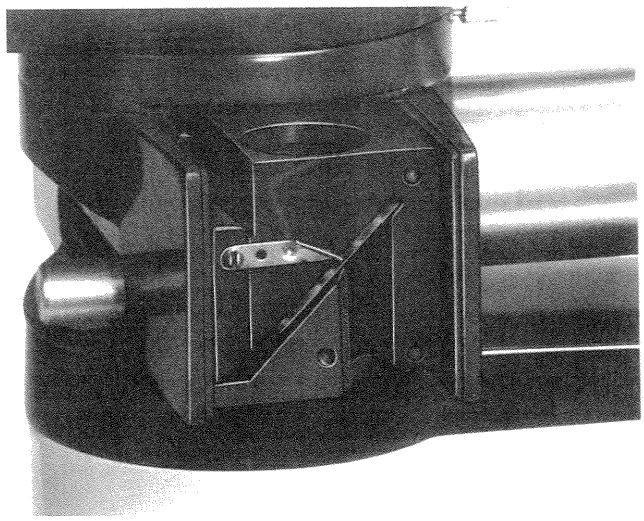


Figure 15. Locking Lever

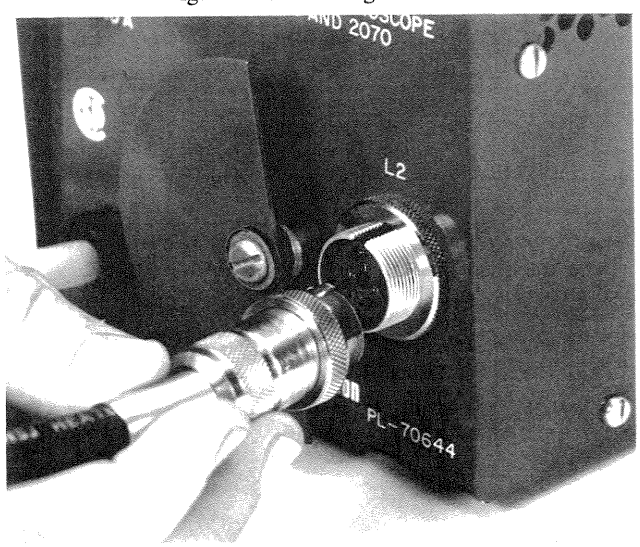


Figure 16.

18. Open the field diaphragm until it slightly encroaches into the field of view. Then adjust the two field diaphragm centering screws (Figure 6) to make the diaphragm image concentric with the field of view (Figure 17B). Open field diaphragm until it is just out of the field of view.

19. Focus the collector lens to obtain even illumination of the field.

The Vertical Illuminator is now ready for use. No further adjustment to settings of the collector lens and field diaphragm are needed when changing from one objective to another. However, you may have to recenter the field diaphragm when changing Fluor Clusters.

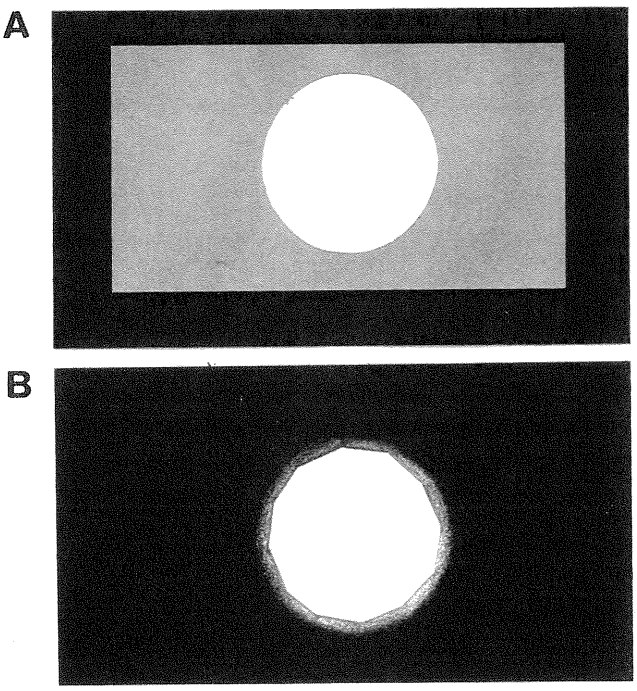


Figure 17.

Specimens should be prepared in the manner normally used for fluorescence observations. Specimen particles which do not fluoresce will not be seen by incident illumination. Such particles can be best observed using ordinary illumination from below the stage in combination with a darkfield condenser.

20. For transmitted light work, positioning knob should be placed in center position.

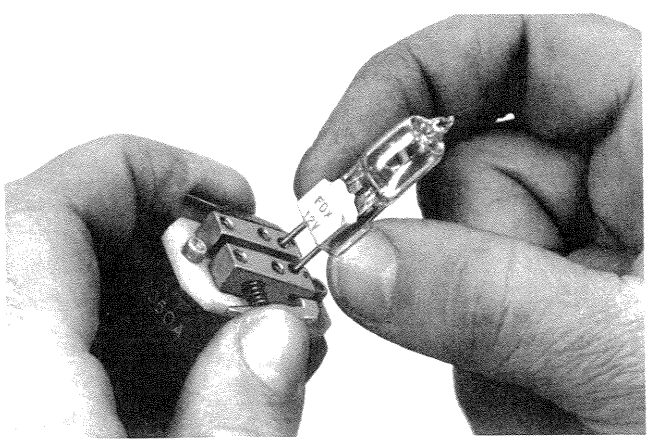


Figure 18.

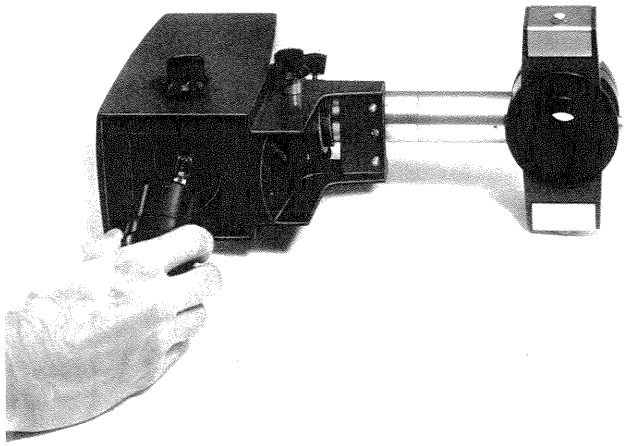


Figure 19.

CATALOG 2071HP

1. Insert #2052 Tungsten Halogen Lamp into the lampholder by pressing pinch grip fingers on socket (Figure 18). Insert lamp pins into socket holes and release fingers. Care should be taken when handling the Tungsten Halogen Lamp. Fingerprints and stains will etch the glass and should be removed with cleaning tissue and alcohol or acetone before using the lamp.

2. Turn the collector lens knob to move the collector lens forward toward the filter housing. Loosen the lampholder retaining knob (Figure 6).

3. Insert the lampholder into the lamphouse with lamp forward (Figure 19). Leave about an inch of the lampholder protruding and tighten the retaining knob.

4. Place the assembled #2071 Vertical Illuminator module on your Series One-Ten or One-Twenty Microscope in place of the binocular or trinocular body. Make sure the assembled unit is seated firmly, and then tighten the locking screw at the top of the microscope arm (Figure 9). Tighten set screws until they touch microscope arm (Figure 6).

   Fasten the body on top of the Vertical Illuminator filter housing.

5. Remove the two slide covers from the Illuminator body by loosening the retaining screw on each (Figure 6).

6. The 2071HP outfit includes the 2077 Fluor Cluster. (See Table 1.) DO NOT TOUCH OPTICAL SURFACES OF FLUOR CLUSTER (Figure 11). To install, move positioning knob to either side of its excursion. With the retaining lever swung out of the way (Figure 13), slide Fluor Cluster into position on locating rods (Figure 14). Swing retaining lever horizontally to secure Fluor Cluster (Figure 15). Replace slide covers.

7. Check to see that the #1138 Variable Transformer is turned off and insert the 2-prong plug of the lampholder cord into the receptacle on the back of the transformer.

8. Plug the transformer cord into a 115V, 60 cycle outlet. Turn on transformer to 12 volt setting.

9. Place Fluor Cluster into optical path by use of positioning knob.

   Place the field diaphragm control at the middle of its excursion (Figure 6).

   Turn the collector lens knob to bring the collector lens fully forward, and then back about one-quarter turn.

10. Follow Steps 14 through 19 under Catalog 2071M. Instead of centering the image of the arc, center the image of the Tungsten Halogen Lamp filament.

11. For transmitted light work the positioning knob should be placed in either the center or occluder position.

NOTE: BECAUSE OF THE CRITICAL ALIGNMENT OF THE COMPONENTS, SERVICE AND REPLACEMENT OF MAJOR PARTS IS BEST ACCOMPLISHED AT THE FACTORY.

COMMON TERMS USED IN FLUORESCENCE MICROSCOPY

BARRIER FILTER — *Filter located between the specimen and eyepiece to remove all but the desired visible wavelengths of light.*

DICHROIC BEAMSPLITTER — *A highly efficient beamsplitter that selectively reflects virtually all the desirable short wavelength exciting light to the specimen. And it selectively transmits the longer wavelength fluorescence emitted from the specimen for further treatment by the barrier filter.*

EXCITER FILTER — *Filter located between the light source and specimen slide to screen out undesired wavelengths of light and allow a select band of excitation radiation to pass through to the specimen.*

FITC FILTER — *A multilayer filter with high transmittance at 495 nm and shorter wavelengths and no transmittance at 500 nm and higher wavelengths.*

FITC — *Fluorescein Isothiocyanate - A stable, yellow-green fluorochrome used in antibody-antigen testing for labeling tissue. This organic dye, like most fluorochromes, has a high affinity to be absorbed by high protein concentration areas. It absorbs light of wavelengths 480-490 nm and emits at 520-530 nm. The emitted color is bright apple green.*

FLUORESCENCE — *Occurs when a substance absorbs light energy at one wavelength and quickly emits light of another, longer, wavelength.*

FLUOROCHROME — *An organic dye that, when placed on a specimen, emits light of longer visible wavelengths than the existing wavelengths.*

NOTE

ON HBO50 MERCURY VAPOR LAMPS

It is suggested that you retain instruction material enclosed with HBO50 Mercury Vapor Lamps.

In the event of lamp malfunction, fill out questionnaire included in lamp manufacturer's instructions. Direct it, with defective lamp, to the lamp distributor generally cited on back page of questionnaire booklet. Principal domestic distributors of Osram lamps:

G. W. GATES
P.O. Box 216
Hempstead Turnpike & Lucille Ave.
Franklin Square
Long Island, NY 11010

MACBETH SALES CORP.
Jeanne Drive
S & G Industrial Park
Newburgh, NY 12550

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