Balplan*

FLUORESCENCE MICROSCOPE

INSTRUCTION MANUAL

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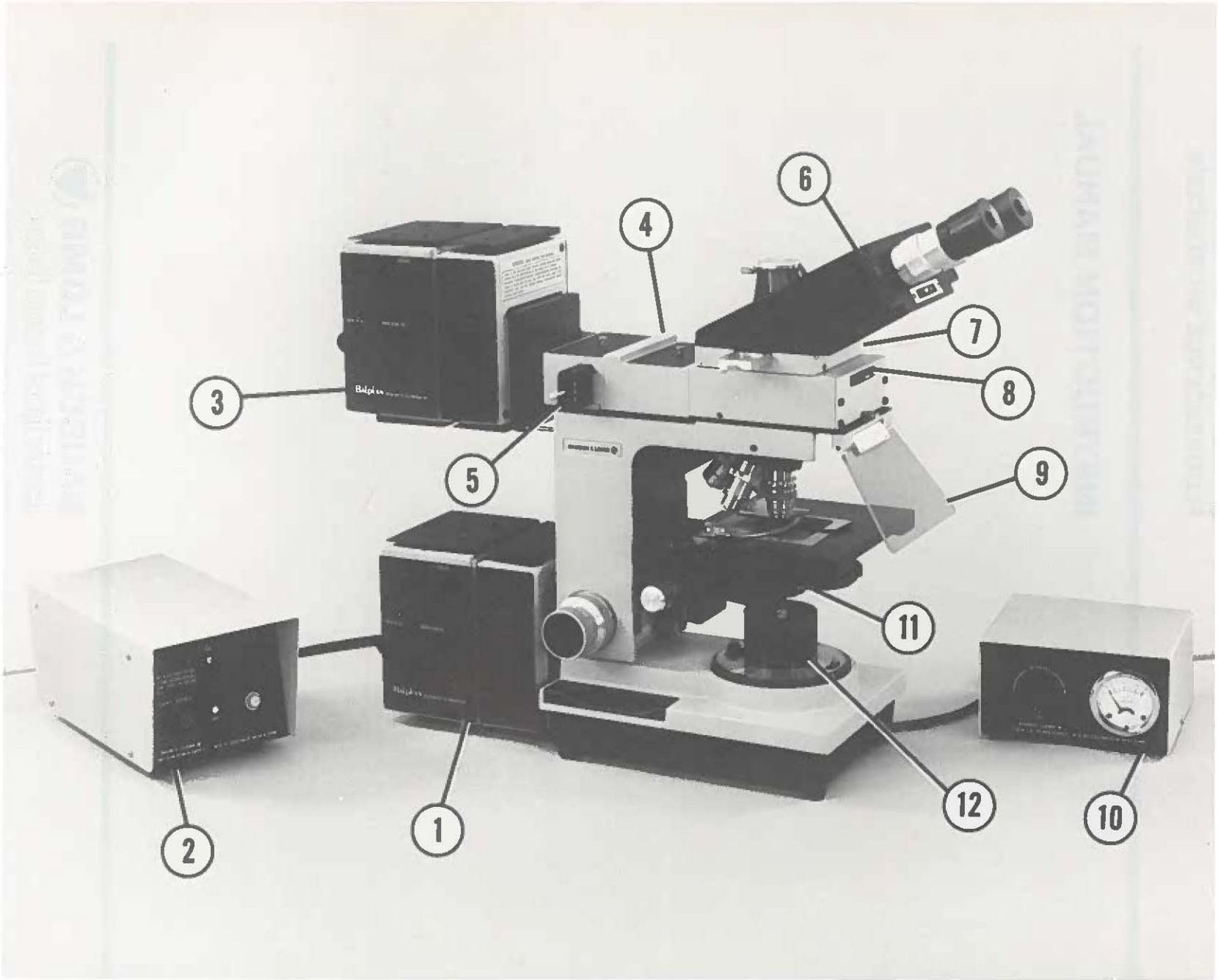

FIGURE 1-1 BALPLAN MICROSCOPE FOR INCIDENT AND TRANSMITTED FLUORESCENCE

1. Lamphouse (HBO)
2. Power Supply for HBO Lamphouse
3. Lamphouse (T-H)
4. Incident-Fluorescence Illuminator
5. Exciter Filter Slider
6. Triocular Head
7. Barrier Filter Slide Attachment with Slider
8. Dichroic Reflector/Barrier Filter Turret
9. Stray-Light Shield
10. Power Supply for T-H Lamphouse
11. Substage Filter Turret
12. Substage Light Shield

Contents

1.0 Warranty

This product is warranted to be free from defects in materials or workmanship. If within one year of its purchase it is found to have any such defects, it will be repaired without charge. Parts (such as lamps) not of Bausch & Lomb manufacture carry the guarantee of their manufacturers.

This warranty does not cover: damage in transit; damage caused by carelessness; improper servicing; modifications made by others than Bausch & Lomb Incorporated; misuse or neglect; unsatisfactory performance as a result of conditions beyond our control. Expendable components such as lamps and fuses carry the manufacturer's warranty only. For further warranty information, contact your dealer.

Registration Card

To register your purchase, please return the enclosed card.

Receipt of Product

Custom designed and tested packaging is provided for all Bausch & Lomb products to protect them during shipment and assure their safe delivery. After the product leaves the factory, responsibility for its safe delivery is assumed by the transportation company handling the shipment.

If your shipment shows evidence of rough handling, request the party making the delivery to note "received in bad order" on your delivery receipt. If you discover "concealed damage" after unpacking the shipment, contact a representative of the transportation company and request that a "Bad Order" report be made out.

In either case, to protect your rights to recovery, notify the transportation company immediately of any damage to your shipment.

Improvements

For better appearance, better performance, greater convenience in using, and longer life, improvements are constantly being made in all Bausch & Lomb products.

Often, the nature of these improvements will be self evident and need not be explained. However, if the improvements affect the use or maintenance of the product, supplementary instructions will be included in your manual or with the instrument.

Service

All optical and mechanical equipment requires periodic servicing to maintain proper performance and compensate for normal wear. Establishing a regular preventive maintenance schedule will help assure long life and maintain peak performance of your instrument. This should include thorough cleaning, checking, and adjustment of the mechanism of your instrument. This will help avoid unexpected trouble and the need for service at inopportune times. The work should be performed only by personnel with the proper training and equipment. Your dealer or Bausch & Lomb can arrange for this maintenance service. For more information, call Bausch & Lomb Product Service, (716) 338-6503.

Unexpected Trouble

If you experience unexpected trouble with your instrument, contact your Bausch & Lomb dealer. He may be able to suggest means for on-site correction of the difficulty.

Outside Service

If you must send your instrument out for service, pack it carefully in a crush resistant carton. Surround it with at least three inches of shock absorbing material to prevent in-transit damage. If a suitable carton is not available, one may be ordered from the factory. Write a letter giving details of the trouble experienced with the instrument and fasten the letter to the instrument. This will expedite prompt and economical repairs. Mark on the shipping container "First Class Letter Enclosed." First class postage will be charged for the letter only; the carton will be accepted at standard package rates.

2.0 Introduction

The excellence of your BALPLAN* Fluorescence Microscope will become obvious as you use it in your investigations. Easy to set up, this Microscope can eliminate unwanted backgrounds without using substage condensers or messy oil-contact techniques. Easy to use, it provides barrier filters, exciter filters, and dichroic reflectors which are carefully matched and plainly identified so you can select the best combination for your specific investigation, even in a darkened room.

The BALPLAN Fluorescence Microscope incorporates the modularity of design and construction which characterizes the BALPLAN family. A wide assortment of precisely engineered and interchangeable heads, multiple viewing adapters, eyepieces, nosepieces, objectives, stages, condensers, illuminators, and accessories is available from stock. As application requirements change, you can economically adapt your BALPLAN Microscope to your exact new need by selecting specific modular elements.

The major BALPLAN modules for fluorescence applications include:

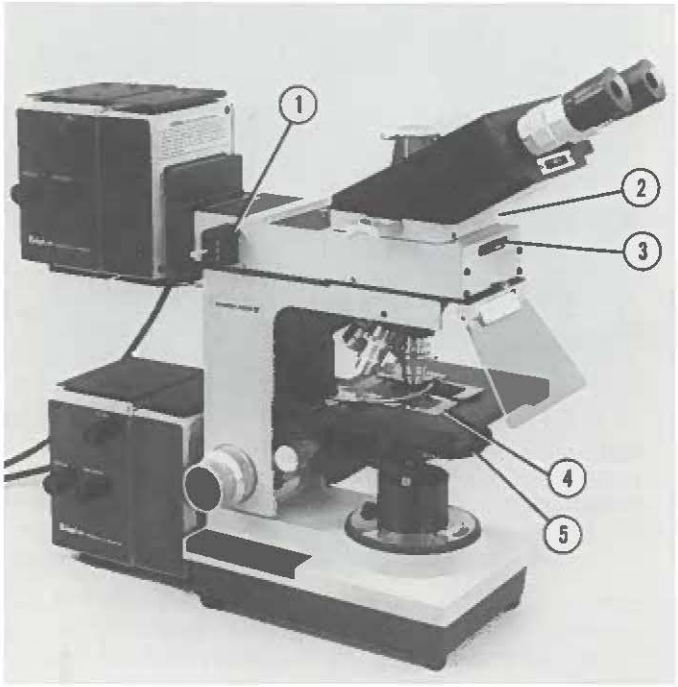

FIGURE 2-1 BALPLAN MICROSCOPE FOR INCIDENT AND TRANSMITTED FLUORESCENCE

1. Exciter Filter Slider
2. Barrier Filter Slide Attachment with Slider
3. Dichroic Reflector/Barrier Filter Turret
4. Elevated Stage Fingers
5. Substage Exciter Filter Turret with Condenser

- Mercury-vapor lamphouse/power supply (50 watt) for near UV and blue-light methods.
- Tungsten-halogen lamphouse/power supply (100 watt) for blue-light examination methods (FITC).
- Incident-fluorescence illuminator for high-efficiency fluorescence, particularly with high-dry and oil-immersion objectives. Matched combinations of dichroic reflectors and barrier filters are turret-mounted.
- Transmitted-light darkfield units with choice of paraboloid or cardoid condensers. Integral filter turret provides quick selection of the appropriate exciter filter. Swing-out occluder protects specimen.
- Broad assortment of exciter and barrier filters.
- Stray-light protection accessories.
- Lamphouses which can be converted (by interchangeable components) for use in either transmitted or incident light modes.

BALPLAN Fluorescence Microscopes have the capability for simultaneous or alternate use of incident and transmitted light modes. The incident fluorescence mode can be combined with conventional transmitted light microscopy.

The procedures of fluorescence microscopy normally require subdued lighting for maximum success. You should set up your BALPLAN Fluorescence Microscope in an area which can readily be darkened.

*BALPLAN is a registered trademark of Bausch & Lomb, Inc.

3.0 Mercury Vapor Lamphouse/ Power Supply

The mercury vapor lamphouse/power supply consists of the following three units:

31-32-61	Lamphouse
31-32-56	HBO Lamp Holder/Power Supply
31-31-65	50-watt HBO Mercury Lamp

The lamp holder has been installed in the lamphouse at the factory. For protection during shipment, the lamp is packaged separately and must be installed.

The lamp has a rated life of 100 hours. This assumes that the line voltage is proper and that each burning period is at least 2 hours in duration.

The lamp is guaranteed by its manufacturer. Save the lamp box and the accompanying literature. Should the lamp have an unreasonably short life, return the lamp to the distributor whose name is on the box.

Refer to Figure 8-1 for data regarding the spectral characteristics of the output of the lamp. Replacement lamps may be ordered from Bausch & Lomb, Cat. No. 31-31-65.

Please note and obey the WARNINGS on the front and side of the lamphouse. For your convenience, they are repeated here.

WARNING: *100W T.H. and 50W H.B.O. lamps produce intense radiation. Direct viewing with the unprotected eye may result in eye damage. Disconnect power supply and wait 15 minutes before removing this cover to allow unit to cool and with 50W H.B.O. lamp, avoid possibility that lamp may explode. Replace cover before reconnecting power supply.*

3.1 Installation

3.1.1 Make sure the power supply is disconnected.

3.1.2 Remove the two screws from the front cover (Figure 3-1) and separate the two sections of the lamphouse.

3.1.3 Carefully remove the lamp from its box, handling the metal ends only. It will be marked at its top with the designation L1 or L2 (see Figure 3-2).

3.1.4 Set the L1/L2 selector switch at the back of the power supply (Figure 3-3) at the position which matches the lamp designation.

3.1.5 Loosen the locking screw (Figure 3-4) using the 7/64-inch hexagonal wrench supplied. Insert the lamp in the lampholder (Figure 3-4), taking care that the end with the L1/L2 designation extends upward and the seal-off tip is toward the back of the lamphouse.

FIGURE 3-1 LAMPHOUSE (HBO)
1. Screws

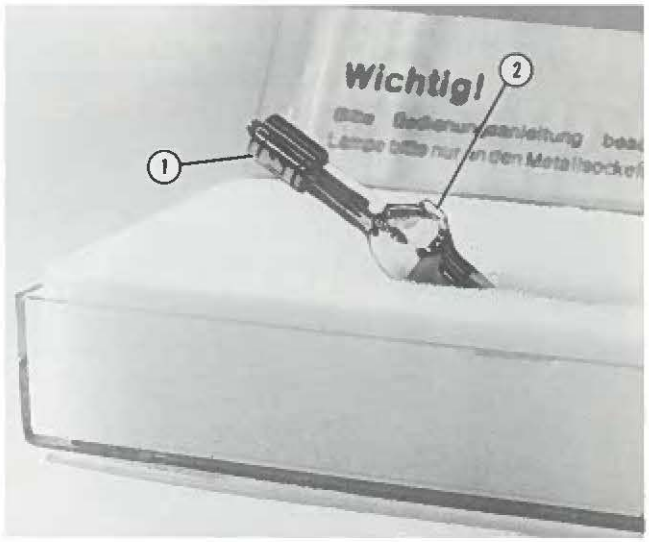

FIGURE 3-2 HBO MERCURY LAMP
1. L2 Designation
2. Seal-off tip

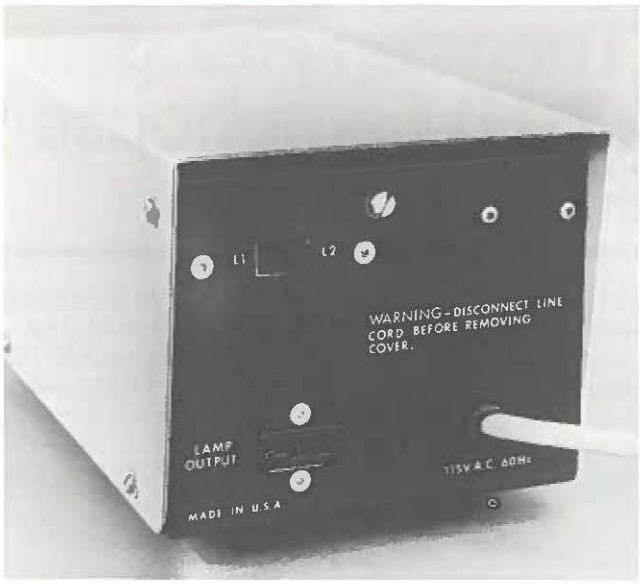

FIGURE 3-3 BACK PANEL OF POWER SUPPLY FOR HBO MERCURY LAMP

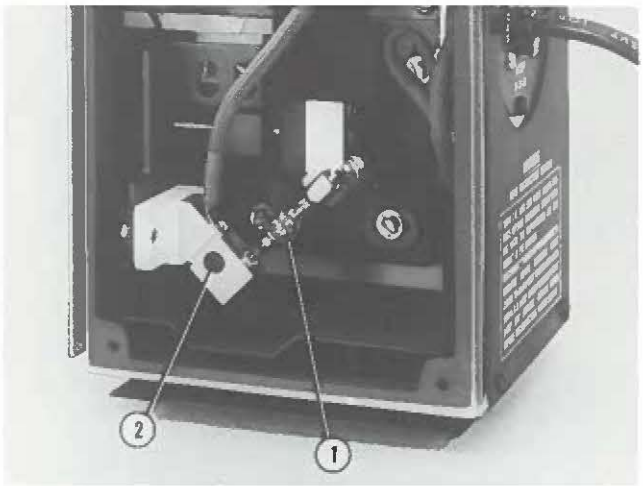

FIGURE 3-4 HBO MERCURY LAMP IN LAMPHOUSE
1. Lamp
2. Locking Screw

3.1.6 Tighten the locking screw with the 7/64-inch hexagonal wrench supplied.

3.1.7 Connect the spring clip top connector to the upper terminal of the lamp by holding the open side of the clip against the side of the lamp terminal with your forefinger behind the clip and your thumb against the side of the lamp terminal, and then gently squeezing the clip onto the terminal. Be very careful; too much force or improper application could fracture the lamp. The cold lamp is not under internal pressure, but the mercury will probably escape if the lamp breaks (see Section 3.2).

3.1.8 If the quartz envelope of the lamp has fingerprints, smudges, etc., clean it using tissue dampened with a solvent such as alcohol or acetone.

3.1.9 Re-connect the two halves of the lamphouse and tighten the two screws.

CAUTION: *Never operate the lamp without the lamphouse completely assembled because potentially harmful UV radiation is emitted.*

3.1.10 Be sure that the line voltage is a nominal 120 volts, 60 Hz. Install the lamphouse by sliding its dovetail slide (Figure 3-5) down over the dovetail slide of the incident-fluorescence illuminator or the research base illuminator.

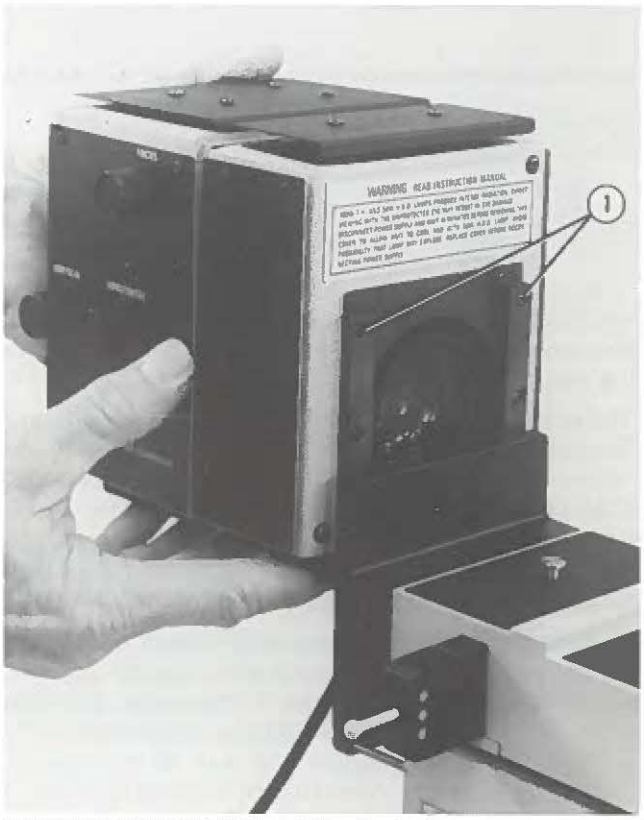

FIGURE 3-5 INSTALLING LAMPHOUSE ON INCIDENT-FLUORESCENCE ILLUMINATOR
1. Dovetail Slides on Lamphouse

3.1.11 Connect the power supply and activate the switch to start. Allow about five minutes for the lamp to reach peak output.

NOTE: *Whenever the lamp is turned off, it must cool for 10-15 minutes before it can be restarted.*

3.2 Lamp Handling Precautions If a hot lamp is cooled too rapidly, explosive failure may occur. The possibility of such failure can be eliminated by never opening the lamphouse without allowing the lamp to cool for at least 15 minutes after turning it off. If the unlikely happens and the lamp envelope breaks allowing mercury to escape, use the clean-up procedure in Section 11.

3.3 Power Supply The power supply is protected by a fuse. Should the fuse blow, replace it with a 5-amp, 125-volt, slow-blow, Type AG fuse. The fuses are available at most electrical supply stores.

4.0 Tungsten-Halogen (T-H) Lamphouse/ Power Supply

The tungsten-halogen lamphouse/power supply consists of:

31-32-61	Lamphouse
31-32-57	T-H Lamp Holder/Power Supply
31-31-58	100-watt T-H Lamp

The lampholder has been installed in the lamphouse at the factory. For protection during shipment, the lamp is packaged in its own container and must be installed.

Refer to Figure 8-2 for date regarding the spectral characteristics of the output of the lamp. Replacement lamps may be ordered from Bausch & Lomb, Cat. No. 31-31-58.

Please note and obey the WARNINGS which are on the front and side of the lamphouse. For your convenience, they are repeated here.

WARNING: *100W T.H and 50W H.B.O. lamps produce intense radiation. Direct viewing with the unprotected eye may result in eye damage. Disconnect power supply and wait 15 minutes before removing this cover to allow unit to cool and with 50W H.B.O. lamp, avoid possibility that lamp may explode. Replace cover before reconnecting power supply.*

4.1 Installation

4.1.1 Make sure the power supply is disconnected.

4.1.2 Remove the two screws from the front cover (Figure 4-1) and separate the two sections of the lamphouse.

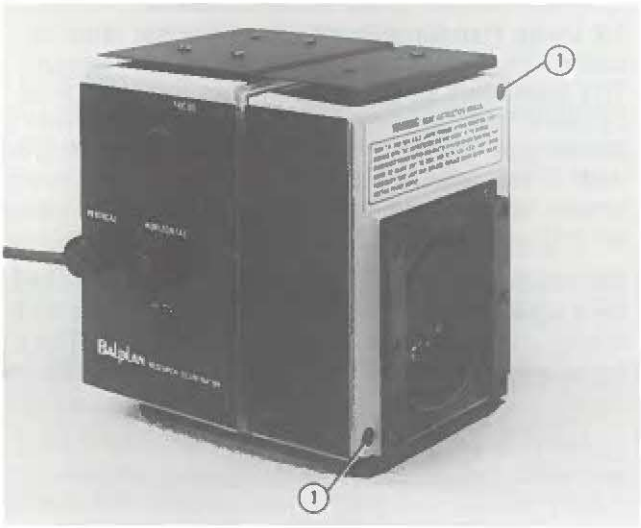

FIGURE 4-1 LAMPHOUSE (T-H)
1. Screws

4.1.3 Carefully remove the lamp from its packaging and insert it in the lampholder (Figure 4-2). The larger diameter pin of the lamp should go into the socket hole which is toward the top of the lamphouse.

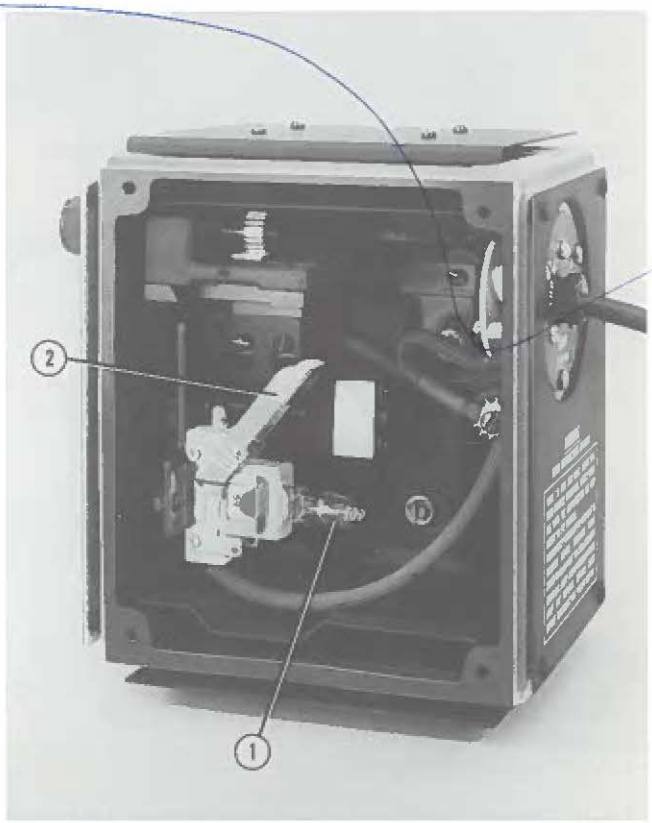

FIGURE 4-2 T-H LAMP IN LAMPHOUSE
1. Lamp
2. Lamp release lever

4.1.4 Remove any fingerprints from the lamp with a tissue dampened with a solvent such as alcohol or acetone. The lamp can be easily removed by means of the lamp release lever (Figure 4-2).

4.1.5 Re-connect the two halves of the lamphouse assembly and tighten the two screws. Because of the high intensity light it emits, the lamp should never be operated unless it is completely enclosed in the lamphouse.

4.1.6 Be sure that the line voltage is a nominal 120 volts, 60 Hz. Install the lamphouse on the dovetail slide of the research base illuminator (Figure 4-3), or the incident-fluorescence illuminator.

4.1.7 Connect the power supply and set the variable switch at the 12-volt position. Most fluorescence work requires a maximum level of energy exciting

3132.32-12 ~~for~~ 220V Allen equip
for 100W Halogen bulb

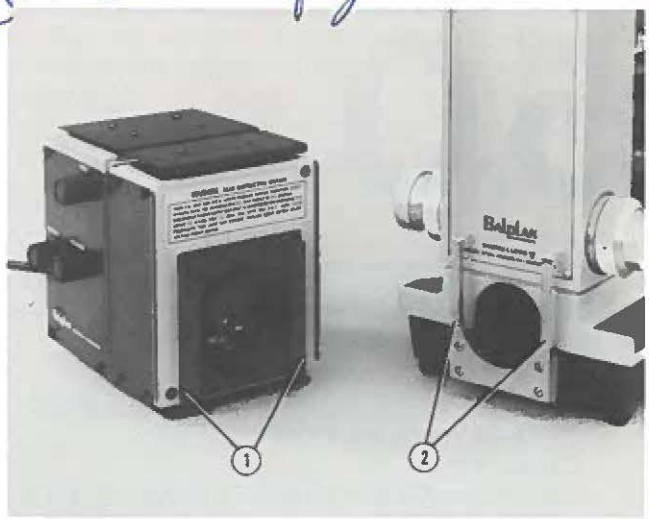

FIGURE 4-3 INSTALLING LAMPHOUSE ON RESEARCH BASE ILLUMINATOR
1. Dovetail Slides on Lamphouse
2. Dovetail Slides on Illuminator

3132-57-103 socket
for the 3132-57-136
Repl lamp 31325
has socket bracket
now per Pat Walsh
RL Hoon 2/8/91

the specimen so the 12-volt setting is the most appropriate. After a period of use, the back of the lamphouse becomes uncomfortably warm. Control knobs and their adjacent surfaces, however, remain comfortable to the touch.

4.2 Power Supply The power supply is protected by a fuse. Should the fuse blow, replace with a 1.5-amp, 125-volt, slow-blow fuse. These fuses are available at most electrical supply stores.

3132 32-12
consists of
311622 green filter
311623 N.D. filter

5.0 Incident-Fluorescence System

The unit which enables your BALPLAN Microscope to be used for incident-fluorescence studies consists of:

31-32-66 Incident-Fluorescence Illuminator
31-32-41 Adapter
Exciter Filters
Lamphouse/Power Supply, 100 Watt (T-H) or 50 Watt (HBO)

The incident-fluorescence illuminator is shipped fully assembled. The lamphouse must be attached as described in Section 3 or Section 4 to make a completely operational system.

5.1 Installation Figure 5-1 shows the BALPLAN microscope with the incident-fluorescence system installed.

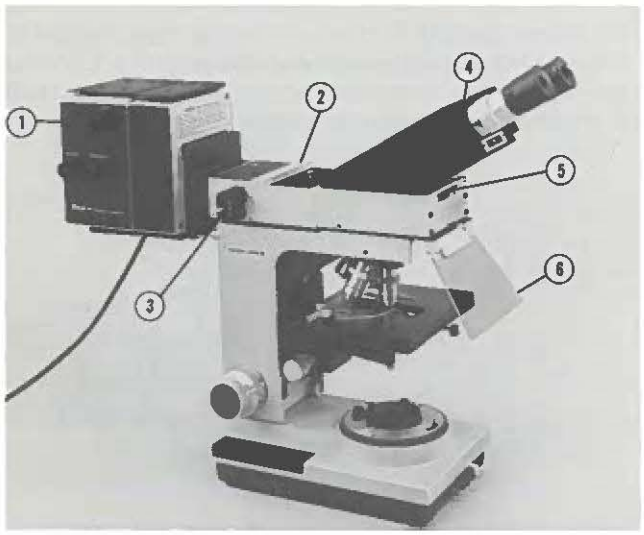

FIGURE 5-1 BALPLAN INCIDENT-FLUORESCENCE MICROSCOPE

1. Lamphouse
2. Incident-Fluorescence Illuminator
3. Exciter Filter Slider
4. Binocular Head
5. Dichroic Reflector/ Barrier Filter Turret
6. Stray-Light Shield

5.1.1 Remove the viewing head from the microscope.

5.1.2 Loosen the knurled clamp screw (Figure 5-2) at the front of the illuminator and engage the illuminator with the mounting ring on the top of the microscope arm. Orient it so the dovetail which will support the lamphouse is at the rear of the arm, and tighten the clamp screw.

5.1.3 Make certain that the lower surface of the illuminator is substantially parallel with the top of the arm.

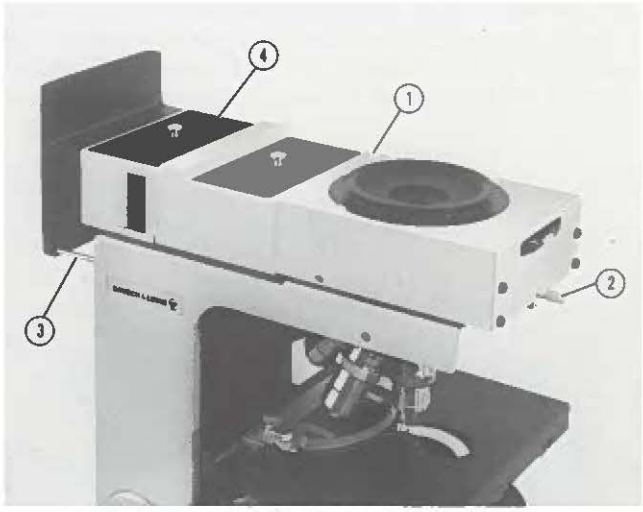

FIGURE 5-2 INCIDENT-FLUORESCENCE ILLUMINATOR INSTALLED ON STAND

1. Incident Fluorescence Illuminator
2. Knurled Clamp Screw
3. Screws
4. Exciter Filter Slider Cover

5.1.4 Remove the cover above the exciter filter slider (Figure 5-2).

5.1.5 There is a jack screw (Figure 5-3) to the rear of the slider and centered between the sides. Adjust this screw so it just touches the top of the microscope arm. This provides increased stability. The fluorescence microscope is unstable unless oriented as shown in Figure 5-2 and described above.

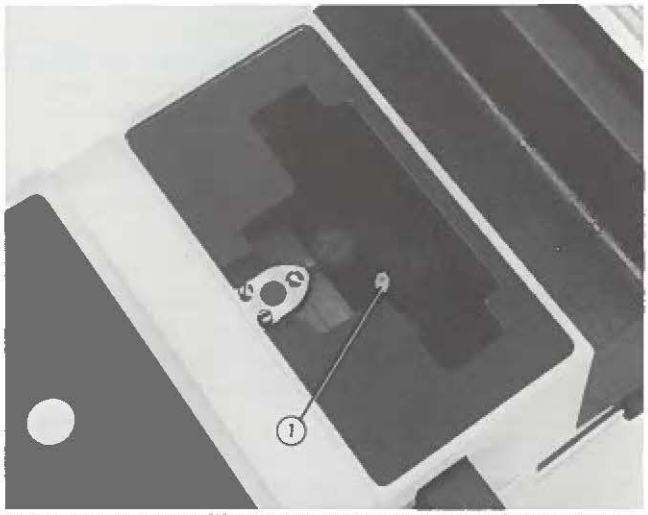

FIGURE 5-3 JACKSCREW ON INCIDENT-FLUORESCENCE ILLUMINATOR

5.1.6 Screw in the two long screws on either side of the dovetail adapter (Figure 5-2) until, with fingertip pressure, they just contact the back of the arm. These screws prevent the lamphouse and illuminator assembly from falling if the front clamp screw is inadvertently loosened.

5.1.7 Install your lamphouse as described in Section 3 or Section 4.

5.2 Dichroic/Barrier Turret The heart of the incident-fluorescence illuminator is the turret at the front of the unit which permits quick and easy selection of dichroic reflector/barrier filter combinations appropriate to the fluorescence technique being used. Dichroics are multi-layer films deposited on glass substrates and are highly efficient in their reflection/transmission characteristics with regard to wavelength. In this instance, the dichroics have been chosen for high reflection of a short wavelength and high transmission of a longer wavelength. The barrier filter associated with a given dichroic reflector filters out the residual unwanted wavelengths which the dichroic might transmit. The turret has four indexed positions—three dichroic reflector/barrier combinations and one open aperture to permit the use of transmitted light techniques without removing the unit. The turret is operated by a knurled wheel. Colored raised dots identify the turret positions (Figure 5-4). The position in the light path can immediately be identified by sight or by touch in a darkened room.

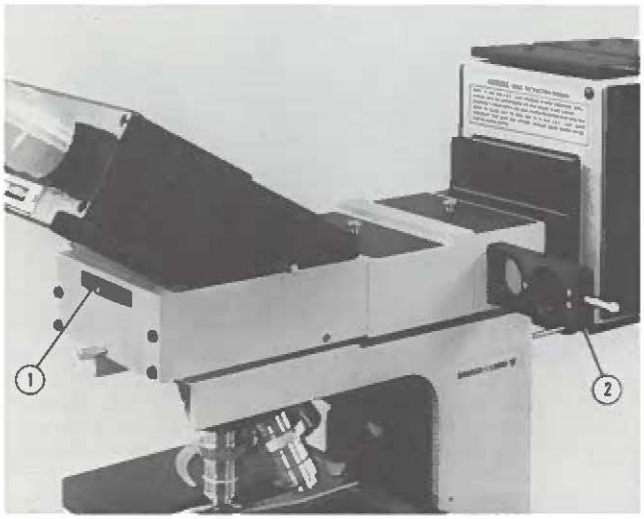

FIGURE 5-4 TURRET AND SLIDER ON INCIDENT-FLUORESCENCE ILLUMINATOR
1. Dichroic Reflector/Barrier Filter Turret
2. Exciter Filter Slider

5.3 Exciter Filters The exciter filter slider (Figure 5-4) has colored dots to identify the exciter filter which is in use. For optimum performance, the number of dots on the exciter filter slider should correspond with the number of dots on the dichroic reflector/barrier filter turret. If two sets of dots on the exciter filter slider are exposed, read the dots closest to the side of the incident-fluorescence illuminator. Figure 5-4 shows the slider and the dichroic reflector/barrier filter turret set at the one-dot position.

The dichroic reflector/barrier filter turret and the exciter filter slider are supplied with the filters installed in the recommended combinations as follows:

T-H Lamphouse/Power Supply

Dot Setting		Filtering		
Exciter Slider	Dichroic/Barrier Turret	Exciter	Dichroic	Barrier
•	•	436nm	450nm	GG-475
••	••	FITC	500nm	OG-515
•••	•••	546nm	560nm	OG-590
••••	Blank	None	None	2A

HBO Lamphouse/Power Supply

Dot Setting		Filtering		
Exciter Filter	Dichroic/Barrier Turret	Exciter	Dichroic	Barrier
•	•	436nm	450nm	GG-475
••	••	BG-12	500nm	OG-515
•••	•••	546nm	560nm	OG-590
••••	Blank	None	None	2A

Many users may prefer other barrier and/or exciter filters to suit their particular application. The "built-in" filters are held in place by means of spring rings and are readily replaceable (see section 5.4). Filters available from Bausch & Lomb are listed on page 22, section 12.0.

5.4 Replacement of Filters All exciter and barrier filters are 1 inch in diameter. The filters are held in their mounts with spring ring retainers (Figure 5-5). You can easily change filters by simply removing the retainer and filter, inserting the new filter, and replacing the retainer. Handle the filters carefully (using lens tissue or a clean cloth) during installation to prevent smudging or fingerprinting the surfaces of the filters.

The dichroic reflectors are cemented into the turret assembly. They should be replaced only by the factory or an authorized service representative.

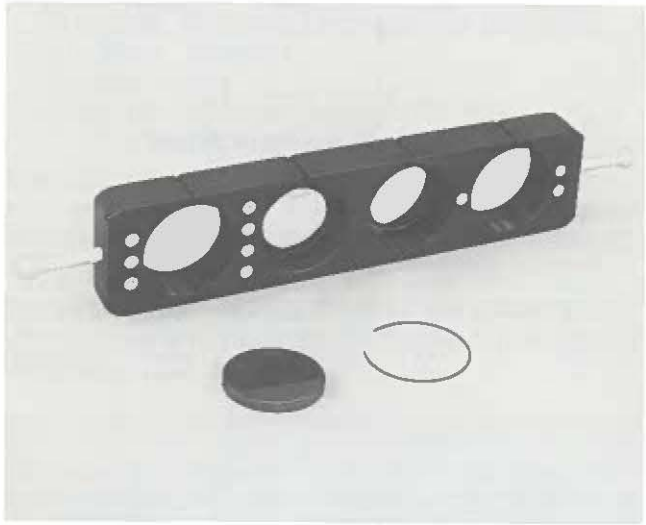

FIGURE 5-5 EXCITER FILTER SLIDER WITH FILTER AND SPRING RING RETAINER REMOVED

5.5 Prevention of Specimen Fading To prevent specimen fading when observation is not in progress, the exciting energy may be blocked off by using the cover over the exciter filter slider as an occluder (See Figure 5-6).

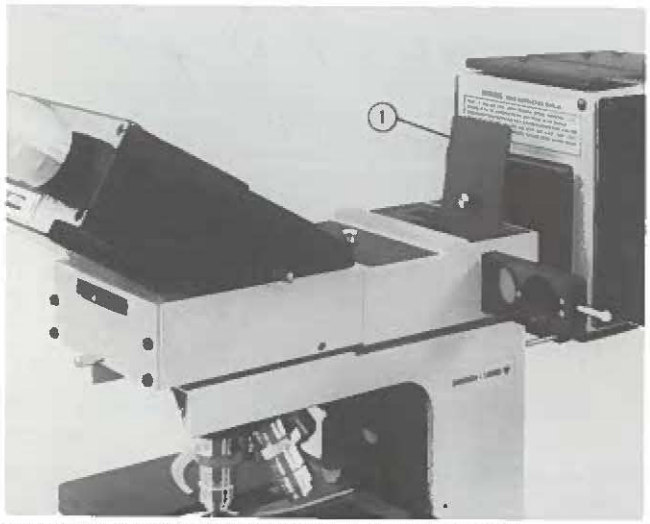

FIGURE 5-6 COVER OF EXCITER FILTER SLIDER IN OCCLUDER POSITION

5.6 Lamp Adjustment After the illuminator has been attached to the microscope and the lamphouse has been installed, adjust the lamp.

5.6.1 Lower the microscope stage about an inch and place a piece of paper on the stage.

5.6.2 Bring a low power (10X) objective into position.

5.6.3 Using the focusing control of the lamphouse (Figures 3-1, 4-1), bring the image of the lamp into focus on the piece of paper.

5.6.4 Using the vertical and horizontal controls of the lamphouse (Figures 3-1, 4-1) center the image of the lamp in the illuminated area.

5.6.5 Focus the lamp using the focus control on the lamphouse to give a generally even area of illumination.

5.6.6 Place a specimen of known fluorescence (a positive control, for example) on the stage of the microscope and bring it into focus, using a 20X or 40X objective. Make any slight horizontal, vertical, and focus adjustments which might be required to obtain an evenly illuminated field of view with maximum fluorescence intensity.

6.0 Transmitted-Fluorescence System

The equipment which comprises this package fits your BALPLAN microscope for transmitted-light fluorescence microscopy. It includes:

31-32-64	Research Base Illuminator
31-32-58	Filter Turret w/Paraboloid Condenser
31-32-59	Filter Turret w/Cardioid Condenser
31-57-92	Slide Attachment
31-35-78	Filter Attachment Slider Lamphouse/Power Supply, 100 Watt (T-H) or 50 Watt (HBO)

6.1 Installation of Research Base Illuminator Figure 6-1 shows the BALPLAN microscope with the transmitted-fluorescence system installed.

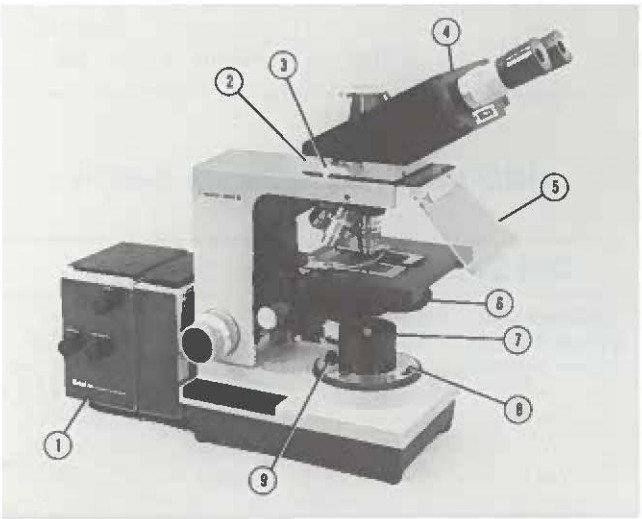

FIGURE 6-1 BALPLAN TRANSMITTED-FLUORESCENCE MICROSCOPE

1. Lamphouse
2. Barrier Filter Slide Attachment
3. Barrier Filter Slider
4. Triocular Head
5. Stray-Light Shield
6. Substage Exciter Turret with Condenser
7. Substage Light Shield
8. Diffuser Control
9. Field Diaphragm Control

6.1.1 Disassemble the illuminator or bottom cover from your BALPLAN microscope by removing the three screws (Figure 6-2).

6.1.2 If the research base illuminator was purchased as a separate item, fasten the cover plate/filter holder to the upper part of the base using the screws and clamps as shown in Figure 6-3.

6.1.3 Insert the two dowels in the bottom of the microscope base (Figure 6-2) into the two holes in the research base illuminator.

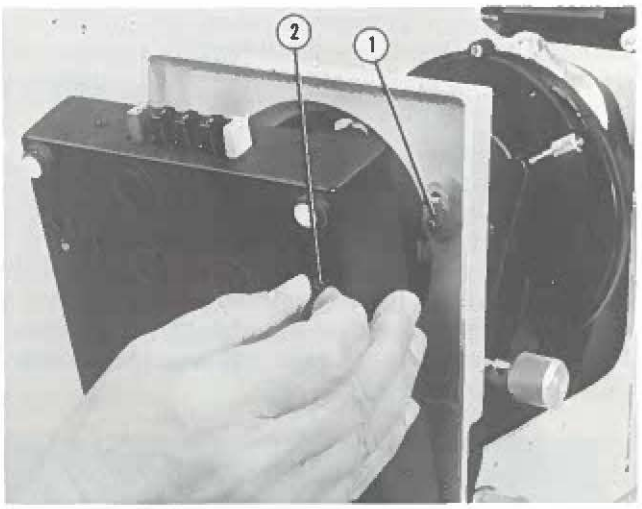

FIGURE 6-2 REMOVING ILLUMINATOR BASE

1. Locating Dowel
2. Screw

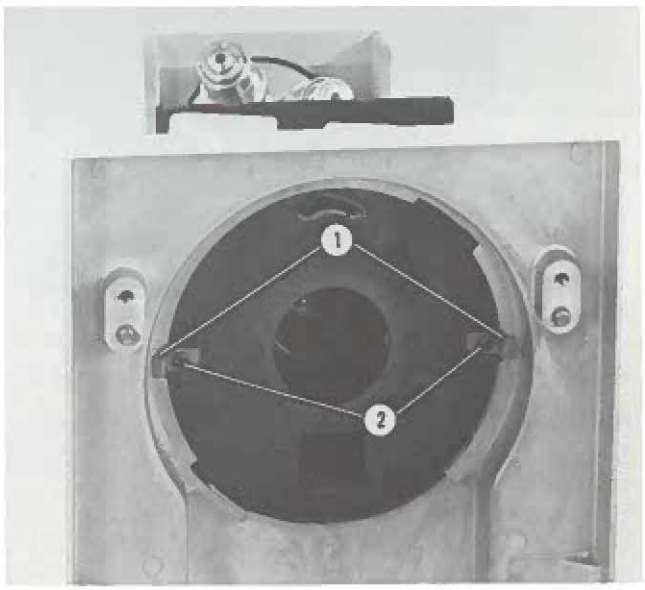

FIGURE 6-3 COVER PLATE/FILTER HOLDER INSTALLED IN MICROSCOPE BASE

1. Clamp
2. Screw

6.1.4 Insert the three mounting screws (Figure 6-2) and tighten them securely using the 9/64-inch hexagonal wrench supplied.

6.1.5 Install your lamphouse as described in Section 3 or Section 4.

6.2 Use of Darkfield Condensers Transmitted-light fluorescence microscopy is almost universally done using a darkfield condenser to attenuate any unwanted light which may slip through the combined filter. A transmission curve for a particular filter may appear to indicate 0% transmission at a particular wavelength; it is not readily apparent that there may be a residual transmission of, say 0.1%. With a high-intensity source, 0.1% transmission of the output is quite noticeable as compared to a true 0% transmission, and conventional brightfield condensers make these small residuals quite evident. They are greatly attenuated when a darkfield condenser is used.

6.3 Exciter Filters The 31-32-58/31-32-59 Filter Turrets with paraboloid/cardioid condensers provide convenient means for the rapid selection of the desired exciter filter. The five filter positions are indexed and are identified by colored raised dots (Figure 6-4) so you can immediately identify by sight or by feel (in a darkened room) which filter is in use. A clear aperture is provided for normal darkfield illumination.

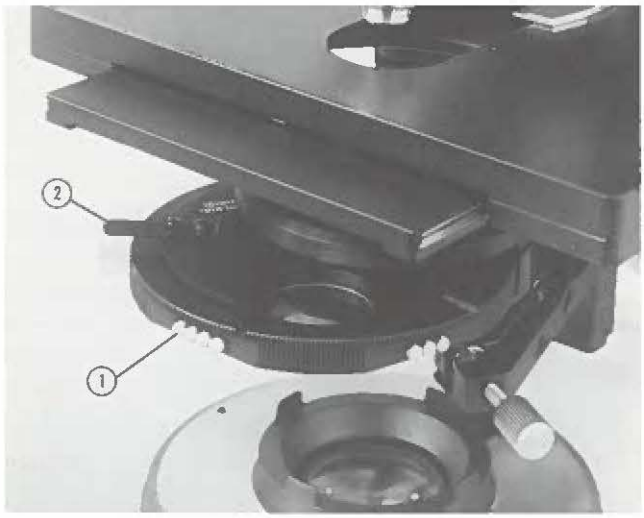

FIGURE 6-4 SUBSTAGE EXCITER FILTER TURRET
1. Raised Identifying Dots
2. Occluder Lever in Left Position

Refer to Section 14 in your BALPLAN Instruction Manual for details regarding paraboloid and cardioid condensers and the mounting procedure. Remember, these condensers must always be coupled to the bottom of the preparation slide using nonfluorescing immersion oil.

6.4 Prevention of Specimen Fading To prevent specimen fading when observation is not in progress, the exciting energy may be blocked off with a convenient occluder. Simply move the lever (Figure 6-4) all the way to the left.

6.5 Dual Filtering Dual filtering (the use of two exciter filters in series) is accomplished by placing the second filter in the filter receptacle at the top of the illuminator base. Two-inch round or square filters are recommended in this instance.

6.6 Barrier Filters The 31-57-92/31-35-78 Slide Attachment/Filter Attachment Slider provide means for rapid interchange of barrier filters. Provision is made for four filters (1 inch in diameter). Each position is indexed and identified by colored dots. The filter in use is identified by the number of dots which are exposed. In situations where two series of dots are exposed, read the series closest to the side of the slide attachment.

For convenience, arrange the filters, insofar as possible, so the number of dots which identify a certain filter correspond to the number of dots of the corresponding barrier filter. The recommended filter combinations are as follows:

T-H Lamphouse/Power Supply

Dot Setting		Filtering	
Condenser Filter Turret	Exciter Slider	Exciter	Barrier
•	•	436nm	GG-475
••	••	FITC	OG-515
•••	•••	546nm	OG-590
••••	Blank	None	2A

HBO Lamphouse/Power Supply

Dot Setting		Filtering	
Condenser Filter Turret	Exciter Slider	Exciter	Barrier
•	•	436nm	GG-475
••	••	BG-12	OG-515
•••	•••	546nm	OG-590
••••	••••	None	2A

Many users may prefer other barrier and/or exciter filters to suit their particular application. The "built-in" filters are held in place by means of spring rings and are readily replaceable (see section 5.4). Filters available from Bausch & Lomb are listed on page 22, section 12.0.

Refer to Section 8 for the spectral transmission characteristics of these filters.

6.7 Replacement of Filters The exciter filters and the barrier filters are 1 inch in diameter and are held in their mounts with a spring ring retainer(Figure 6-5) You can readily change filters by removing the retainer and filter, dropping in the new filter, and replacing the retainer. Handle the filters carefully (using lens tissue or a clean cloth) during installation to prevent smudging or fingerprinting the surfaces of the filters.

6.8 Alignment Procedure Alignment of the system is accomplished easily by the following procedure.

6.8.1 Be sure that the field diaphragm is open and that the diffuser is out of the light path. (Figure 6-1)

6.8.2 Place a piece of paper over the light aperture at the top of the base illuminator.

6.8.3 Use the horizontal, vertical, and focus controls on the lamphouse to obtain an illuminated area which is as even and centered as possible.

6.8.4 Remove the piece of paper.

6.8.5 Place a specimen of known fluorescence (a positive control, for example) on the stage of the microscope.

6.8.6 Choose an appropriate exciter/barrier filter combination for the fluorochrome used on the specimen under examination.

6.8.7 Place a drop of non-fluorescing immersion oil on the top of the condenser. Look directly at the center of the specimen (not through the eyepiece), and raise the condenser until you see a flash of light indicating that oil contact has been made between the slide and the condenser.

6.8.8 Bring the specimen into focus. Use a 10X objective if the specimen characteristics permit.

6.8.9 Using the substage condenser focus control and the condenser centering screws as required, obtain as concentrated and centered a spot of light as possible.

6.8.10 Use the horizontal, vertical and focus controls of the lamphouse to adjust the centering and focusing to obtain maximum fluorescence brightness at the center of the field of view.

7.0 Accessories

31-32-86 Stray Light Shield
31-32-87 Substage Light Shield
31-60-09 Elevated Stage Finger (RH)
31-50-05 Non-Fluorescing Immersion Oil

Fluorescence microscopy is commonly done under lighting conditions which vary from subdued light to complete darkness. In these circumstances, stray light can be very annoying. The 31-32-86 Light Shield accessory (Figure 1-1) filters out any blue light which is incident upon the specimen and not blocked by the mechanical structure of the objective. It fits over the circular mounting ring which is directly on the top of the microscope arm and is hinged to provide easy access for slide insertion and removal.

The 31-32-87 Light Shield accessory (Figure 1-1) serves to block out any stray light which may escape from the base illuminator. It mounts over the filter well on the top of the base.

In the process of scanning slides which are oil immersed to the condenser, it is an unfortunate fact that the stage surface quickly becomes smeared with oil. This is not only messy, but often the oil film makes it difficult to remove the slide.

Use of the 31-60-09 Elevated Stage Finger (Figure 2-1) essentially eliminates this problem as the lower surface of the slide is located about 0.7mm above the stage surface. A little extra care is needed when inserting a slide to be sure that its bottom surface is properly located on the slide carrying surfaces of the fingers.

8.0 Spectral Transmission Characteristics

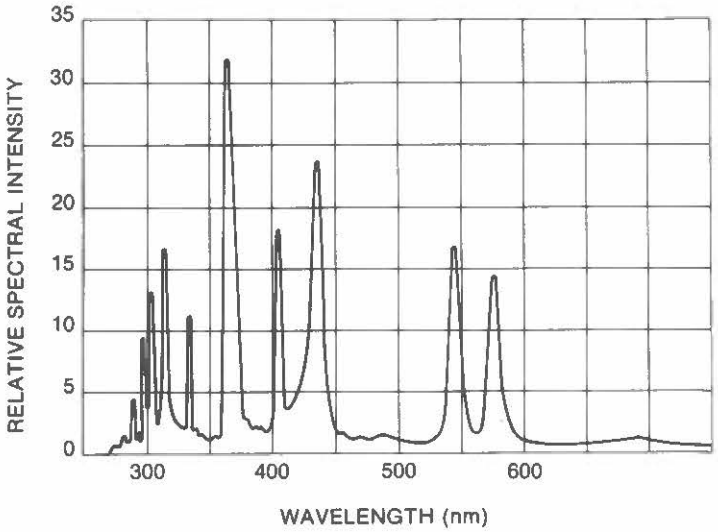

FIGURE 8-1 SPECTRAL CHARACTERISTICS OF THE 50-WATT HBO LAMP

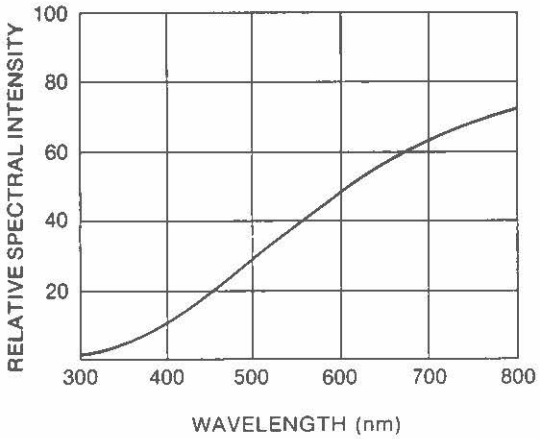

FIGURE 8-2 SPECTRAL CHARACTERISTICS OF THE 100-WATT T-H LAMP

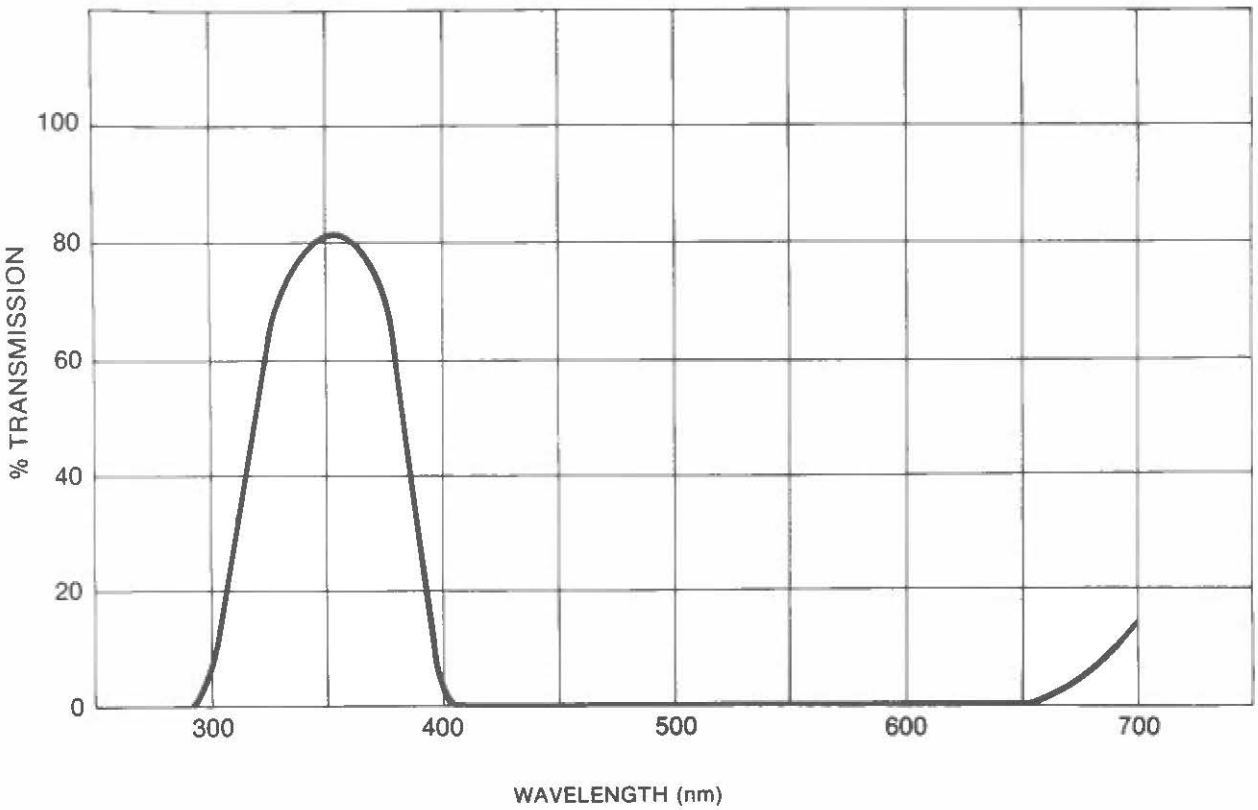

FIGURE 8-3 TRANSMISSION OF THE 31-35-65 EXCITER FILTER; CORNING 5840, 2.3mm THICK

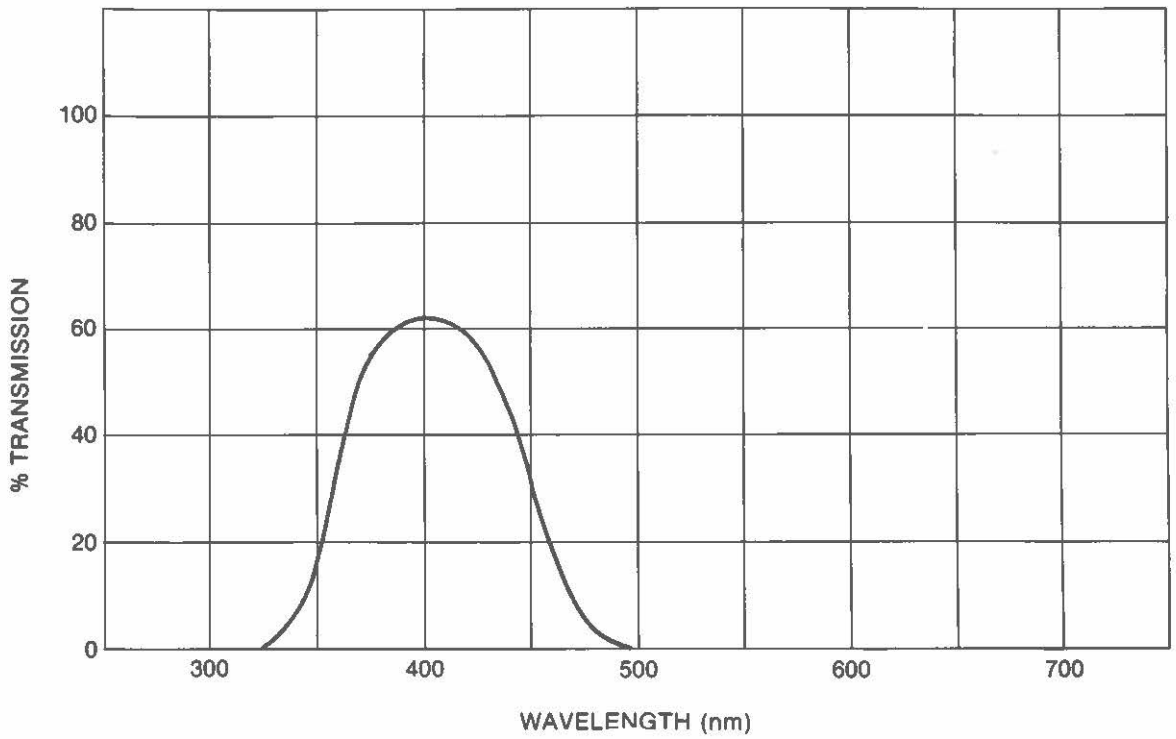

FIGURE 8-4 TRANSMISSION OF THE 31-35-64 EXCITER FILTER; SCHOTT BG-12, 3.0mm THICK

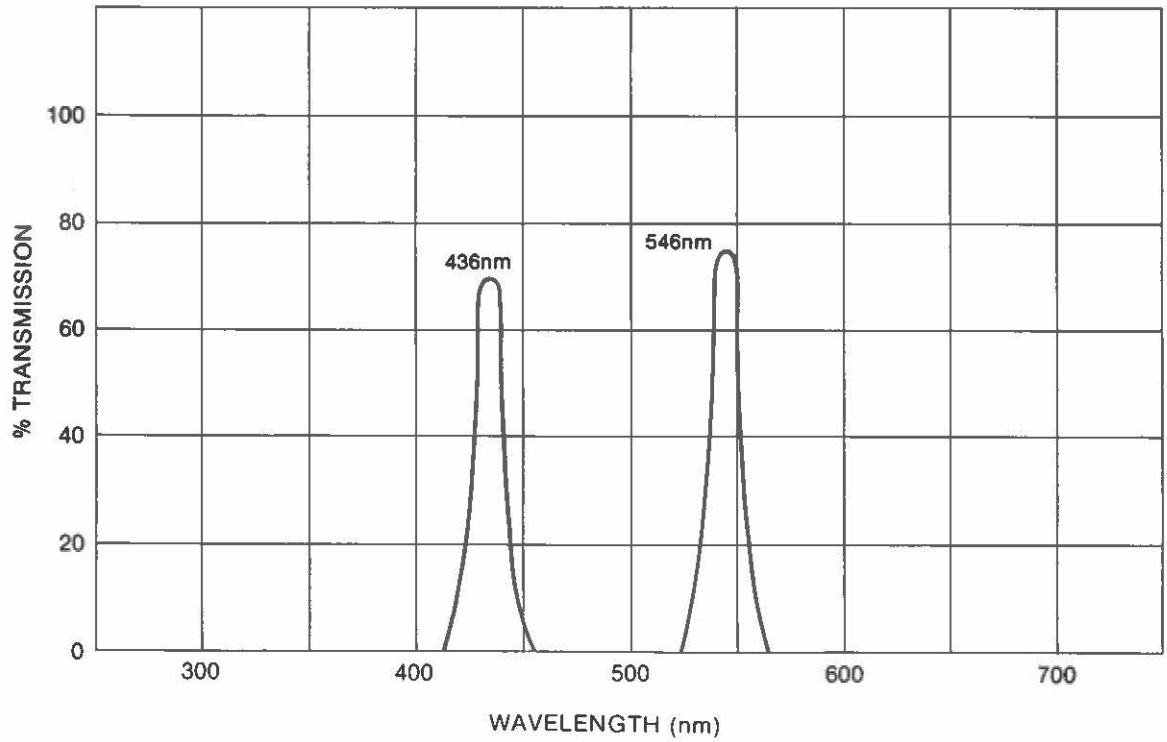

FIGURE 8-5 TRANSMISSIONS OF THE 31-35-66/67 EXCITER FILTERS; 436nm INTERFERENCE, 546nm INTERFERENCE

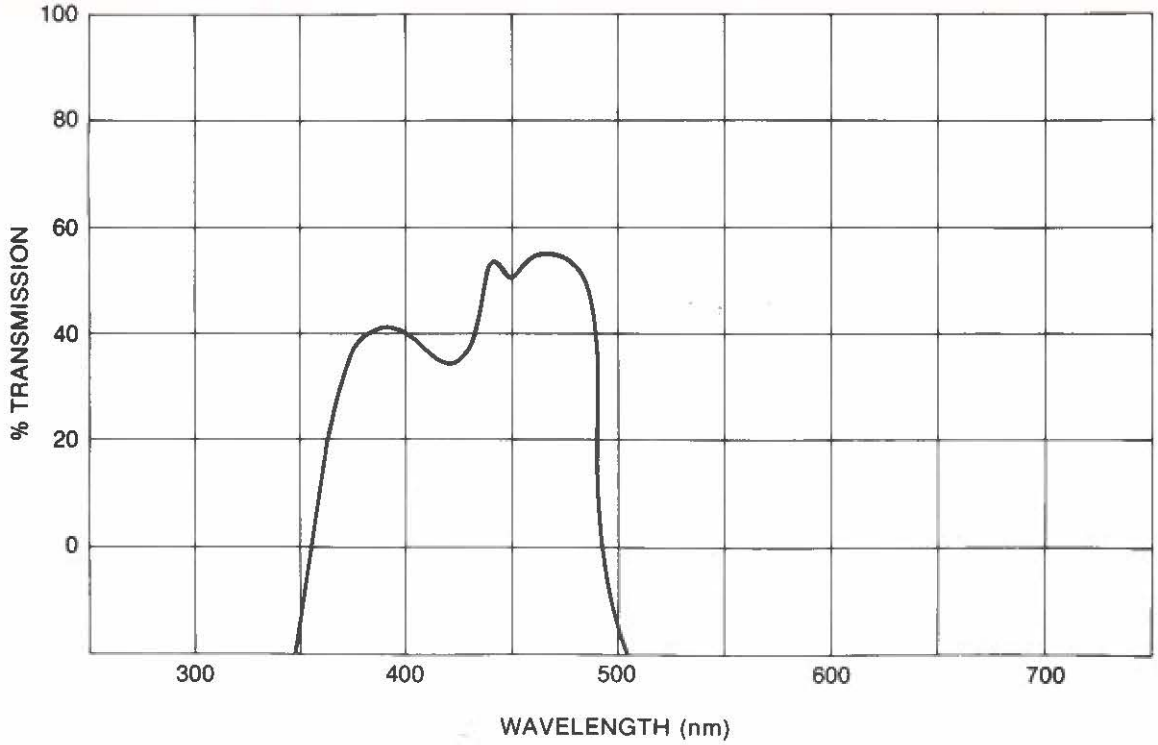

FIGURE 8-6 TRANSMISSION OF THE 31-35-16 EXCITER FILTER; FITC (495nm) INTERFERENCE

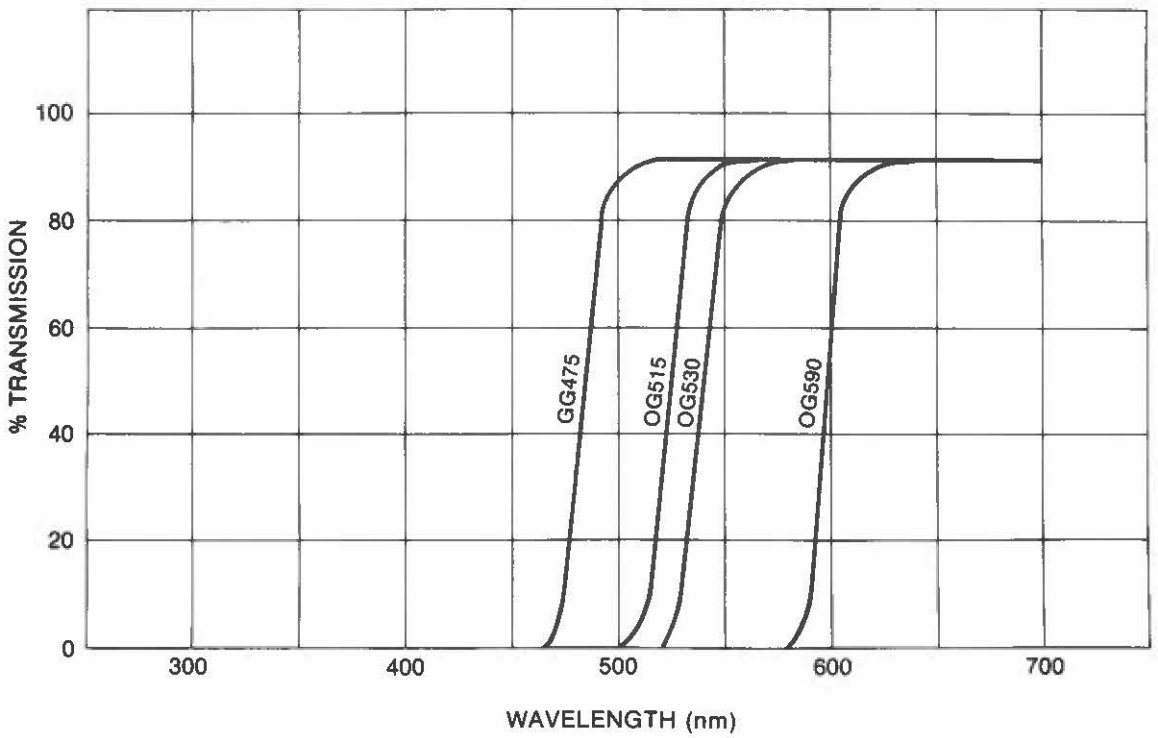

FIGURE 8-7 TRANSMISSIONS OF THE 31-35-24/-60/-82/-15 BARRIER FILTERS; SCHOTT GG475, SCHOTT OG515, SCHOTT OG530, SCHOTT OG590

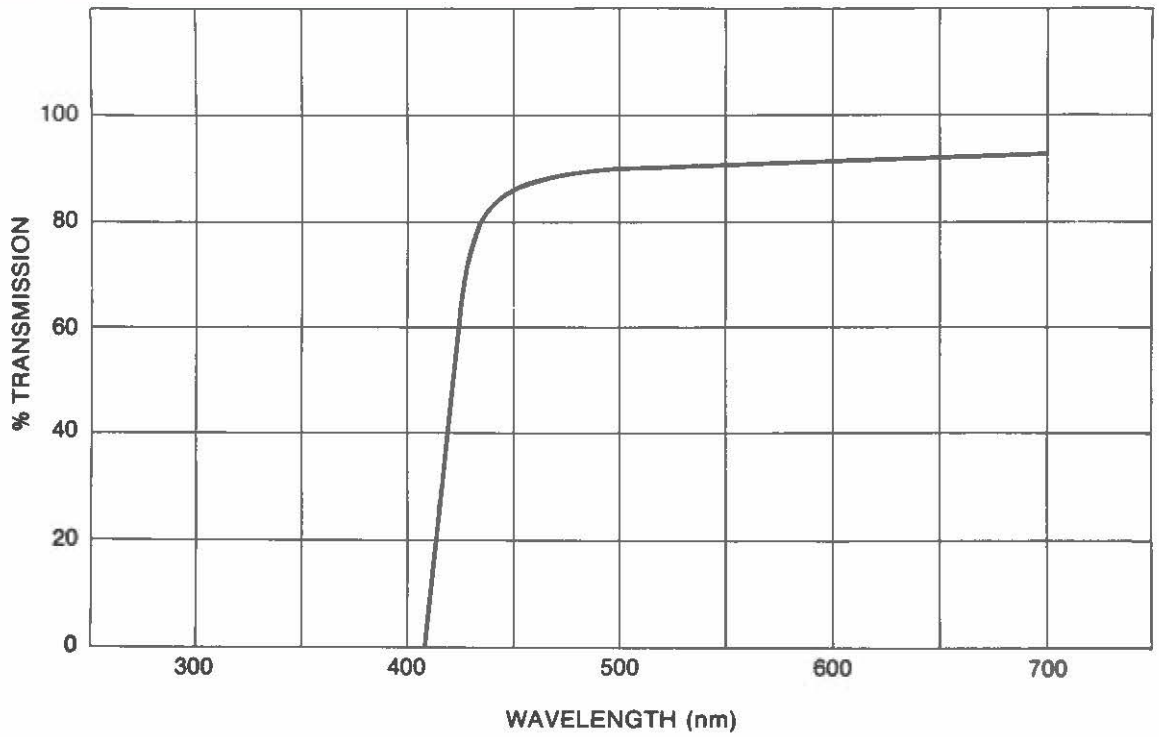

FIGURE 8-8 TRANSMISSION OF THE 31-35-23 BARRIER FILTER; 2A

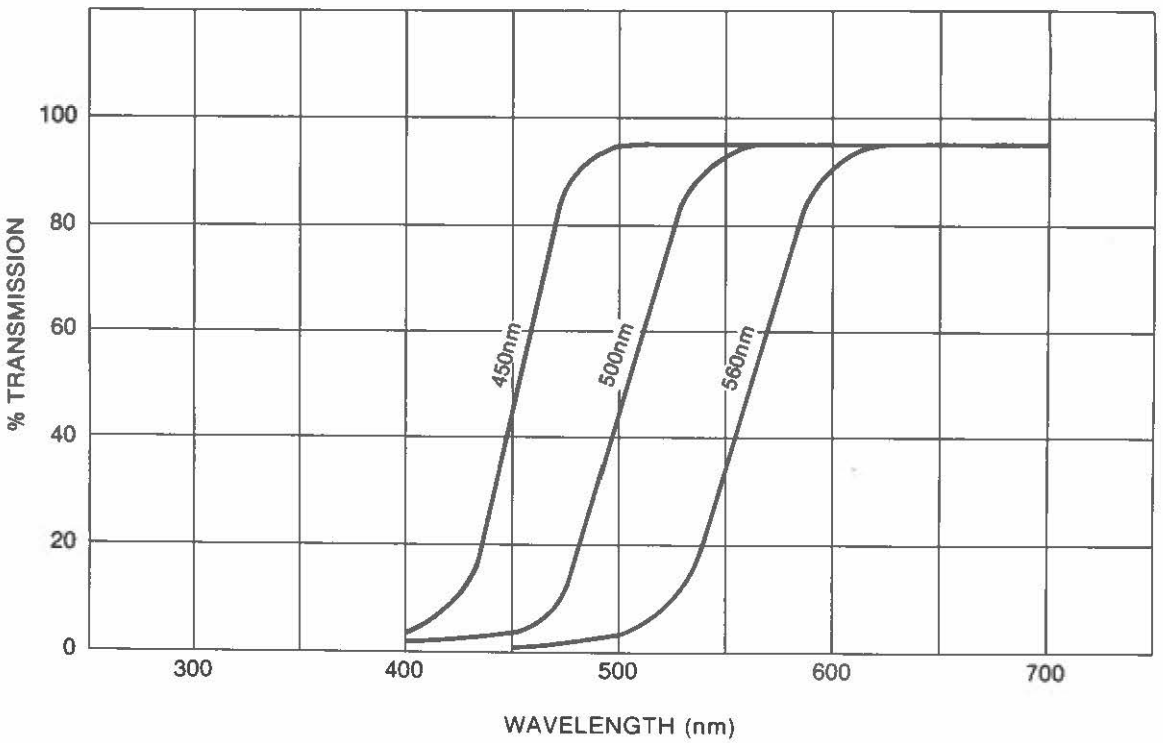

FIGURE 8-9 TRANSMISSION CURVES OF THE 450, 500, 560nm DICHROIC REFLECTORS

9.0 Typical Applications

9.1 Use of Filters

9.1.1 Many microscopists prefer the use of the OG530 Barrier Filter when using the BG-12 or FITC Exciter Filters because a blacker background is obtained. Others prefer the lighter background produced by the OG515 Barrier because it is easier to identify the relationship of the fluorescing particle with respect to its matrix.

9.1.2 Use the 2A Barrier Filter to completely absorb any possible UV radiation even if no exciter filter is in the light path.

9.2 Fluorescence Microscopy Techniques The techniques employed in fluorescence microscopy are continually changing. Many studies are in progress to determine the optimum combination of staining/conjugation techniques, choice of illuminant (HBO/T-H), method of illumination (incident/transmitted), and choice of exciter/barrier filter combinations to obtain the maximum results for a particular procedure. A study of the literature indicates that there is a wide diversity of opinion as to the best combinations. The choices of a particular microscopist are undoubtedly influenced by the equipment available. There are, however, three primary areas of investigation which are evident:

A. UV excitation, with fluorescence emission in the visible blue and longer wavelengths.

B. Blue-light excitation, with fluorescence emission in the green-yellow and longer wavelengths.

C. Green-light excitation, with fluorescence emission in the orange and longer wavelengths.

The most prevalent area of usage in clinical fluorescence microscopy at this time is that of blue light—use of an exciter filter which has a peak transmission or sharp cut-off in the region 400-500nm. Some of the tests involved in this area are:

E. Coli
Streptococcus (B. Hemolytic, Group A)
B. Pertussis
Acid Fast—Tuberculosis
Anit-Nuclear Antibodies (ANA)—Lupus Erythematosus
Anti-Mitochondria
Syphilis
Toxoplasmosis
Gonorrhea

Most of these tests use fluorescein isothiocyanate (FITC) as the conjugate.

The following is a listing of wavelength region/fluorochrome, with respect to exciter and barrier filter combinations (suitable dichroic reflectors are combined with the barrier filters for incident-illumination equipment).

Wavelength Region	Fluorochrome	Exciter Filter	Barrier Filter
UV-365nm	DNAS	5840	2A
Blue-436nm	Quinacrine Mustard Auramine	436nm	GG475
Blue-400 to 500nm	FITC Acridine Orange	FITC BG-12	OG515 OG530
Green-546nm	Rhodamine TRITC	546nm	OG590

This instruction manual is not intended to be a text on fluorescence microscopy procedures or techniques; a substantial literature already exists.

Suggested references which contain extensive bibliographies are:

Emmel, V.M. and Cowdry, E.V., *Laboratory Techniques in Biology,* 4th ed (1964), pp 158-167, Williams & Wilkins.
Oster, G. and Pollister, A.W., *Physical Techniques in Biological Research,* Vol. III (1956), pp 91-148, Academic Press.
Nairn, R.C., *Fluorescent Protein Tracing,* 3rd ed (1969), Williams & Wilkins.

10.0 High-Intensity Brightfield Illuminator

In addition to their fluorescence applications, the 31-32-64 research base illuminator and the tungsten-halogen illuminator make an excellent high-intensity illuminator for the BALPLAN Microscope. This combination is well suited for high-power phase-contrast applications, darkfield microscopy of colloidal suspensions, use with multiple-head conference microscopes, etc. Even microprojection can be done with a conventional brightfield condenser and a viewing screen attachment (42-12-20).

10.1 Installation For maximum protection during transportation, the base illuminator is shipped unassembled to the microscope stand. To simplify assembly, lay the microscope stand on its back.

10.1.1 Remove the base currently installed on your BALPLAN microscope.

10.1.2 If the base illuminator was purchased as a separate item, fasten the cover plate/filter holder to the upper part of the base using the screws and clamps as shown in Figure 6-3.

10.1.3 Insert the two dowels in the bottom of the microscope base (Figure 6-2) into the two holes in the base illuminator.

10.1.4 Insert the three mounting screws (Figure 6-2) and tighten them securely using the 9/64-inch hexagonal wrench supplied.

10.1.5 If your microscope has a multiple viewing attachment, a 31-35-40 transformer and a 31-32-75 adapter cord should be used to illuminate the pointer. Plug the adapter cord into the adapter.

10.1.6 Install the tungsten-halogen illuminator using the procedure in Section 4.1.

CAUTION: *This is a high intensity source. Although there is no ultraviolet radiation emitted, the sheer amount of white light energy could be harmful to the eyes. Approach the eyepieces with caution and use filtration as required.*

Refer to the BALPLAN Microscope Instruction Manual for general comments about the use of the BALPLAN Microscope equipment.

The flip-in/flip-out diffuser (Figure 6-1) will be useful in situations where it is desirable to even the field or aperture illumination, particularly when using low power (2.5X, 4X) objectives.

11.0 Cleanup Procedure For Spilled Mercury

The possibility of the HBO mercury lamp breaking and spilling mercury is extremely remote if the handling precautions in Section 3.2 are observed. This cleanup procedure is suggested for use in the unlikely event of mercury spills.

11.1 Leave the immediate vicinity of the spilled mercury. Do not begin cleanup operations until any concentration of mercury vapor in the air has had time to dissipate by air circulation.

11.2 Pick up all visible droplets of mercury with an eye dropper or a vacuum pick.

11.3 Convert the mercury to a less harmful compound by mixing it with a material such as HgX* and seal the compound in a poly bag.

NOTE: *A complete mercury spill clean-up kit can be purchased from J.T. Baker, Phillipsburg, N.J. 08865*

11.4 Dust the area of the spill with HgX, sweep up carefully and completely, and seal the sweepings in a poly bag.

11.5 Dispose of the poly bags using proper precautions.

11.6 If the lamp broke inside the lamphouse, replace it. Place the lamphouse in a ventilated hood, turn on the lamp and leave it on (in the hood) for four hours.

**HgX is a registered trademark of, and is available from: Acton Associates, 100 Thompson Street, Pittston, PA. 18640*

12.0 Parts List

Cat. No.	Description
Exciter Filters	
31-35-16	FITC (Interference-495nm), 25.4 mm diameter
31-35-64	BG-12 (Schott-3.0 mm), 25.4 mm diameter
31-35-65	5840 (Corning-2.3 mm), 25.4 mm diameter
31-35-66	436nm (Interference), 25.4 mm diameter
31-35-67	546nm (Interference), 25.4 mm diameter
Barrier Filters	
31-35-15	OG-590 (Schott-3.0 mm), 25.4 mm diameter
31-35-23	2A, 25.4 mm diameter
31-35-24	OG-475 (Schott-3.0 mm), 25.4 mm diameter
31-35-60	OG-515 (Schott-3.0 mm), 25.4 mm diameter
31-35-82	OG-530 (Schott-3.0 mm), 25.4 mm diameter
Illuminators & Accessories	
31-32-41	Lamp Bracket (for 31-32-66)
31-32-56	50 watt HBO lamphouse/power supply (120 volt)
31-32-73	50 watt HBO lamphouse/power supply (220 volt)
31-32-57	100 watt T-H lamphouse/power supply (120 volt)
31-32-74	100 Watt T-H lamphouse/power supply (220 volt)
31-32-61	Lamphouse
31-32-64	Research base illuminator
31-32-75	Adapter cord
31-32-66	Incident-fluorescence illuminator (includes dichroic reflectors/barrier filters in turret and exciter filter slider only)
31-32-58	Paraboloid condenser with filter turret
31-32-59	Cardioid condenser with filter turret
31-32-86	Stray-light shield
31-32-87	Substage light shield
31-35-40	Transformer
31-35-78	Filter attachment slider
31-57-92	Slide attachment
31-66-09	Elevated stage finger attachment
31-50-05	Non-fluorescing immersion oil
Replacement Lamps	
31-31-65	50 watt HBO lamp
31-31-58	100 watt T-H lamp