

Nikon

Microscope

ECLIPSE 80i

Instructions

<Reference>

Introduction

Thank you for purchasing this Nikon product.

This instruction manual, which describes basic microscope operations, is intended for users of the Nikon ECLIPSE 80i microscope.



To ensure correct use, please read this manual carefully before operating the product.

- This manual may not be reproduced or transmitted in whole or in part without Nikon's express consent.
- The contents of this manual are subject to change without notice.
- Although every effort has been made to ensure the accuracy of this manual, errors or inconsistencies may remain. If you note any points that are unclear or incorrect, please contact your nearest Nikon representative.
- Some of the products described in this manual may not be included in the set you have purchased.
- Make sure you have read the manuals for any other products attached to or to be used with this microscope (super high-pressure mercury lamp power supply, high-intensity light source, etc.).

Warning/Caution symbols used in this manual



Although Nikon products are designed to provide the utmost safety, ignoring safety precautions or improper use may result in personal injury or property damage, as well as voiding the terms of the warranty. To ensure safe use, please read the instruction manual carefully and thoroughly before trying to operate the instrument. Do not discard this manual. Store in a convenient location near the product for ready reference.

In this manual, safety precautions are indicated by the following symbols. For safe, correct use of the microscope, always follow the instructions indicated by these symbols.

Symbol	Meaning
 WARNING	Disregarding instructions indicated by this symbol may result in death or serious injury.
 CAUTION	Disregarding instructions indicated by this symbol may result in injury or property damage.

Meaning of symbols used on the product

When appearing on the product, the symbols below indicate the need for caution at all times during use. Consult the instruction manual and read the relevant instructions before attempting to use or adjust any part to which the symbol has been affixed.

	<p>Caution! Biohazard</p> <p>This symbol found on the stage indicates the following:0</p> <ul style="list-style-type: none">• WARNING: Contact between sample and microscope may result in biohazard risks.• To avoid biohazard contamination, avoid touching the contaminated portion with bare hands.• Decontaminate the contaminated part according to the standard procedure specified for your laboratory.
	<p>Caution for heat</p> <p>This symbol found on the lamphouse of the ECLIPSE 80i indicates the following:</p> <ul style="list-style-type: none">• The lamp and surrounding areas (including the lamphouse) become very hot during and immediately after a period of illumination.• Risk of burns. Do not touch the lamp or surrounding areas during or immediately after a period of illumination.• Make sure the lamp and surrounding areas have cooled sufficiently before attempting to replace the lamp

Abbreviations Used in The Manual

The product names and abbreviations used in this manual are given below.

The manual uses the following abbreviations:

Name of device	Abbreviation
Microscope ECLIPSE 80i	80i
C-ER Eye Level Riser	Eye Level Riser
C-TE Ergonomic Binocular Tube	Ergonomic Binocular Tube
C-TEP DSC Port for Ergonomic Binocular Tube	DSC Port
D-FL Epi-illumination Attachment	Epi-illumination Attachment
D-DH Digital Imaging Head M	DIH-M
D-CB C-Box	C-Box
D-LH Precentered Lamphouse	Lamphouse
D-NID6 Intelligent Sextuple DIC Nosepiece	Intelligent Nosepiece
C-HS Hand Switch	Hand Switch
DS Camera Head DS-5M	Camera Head
DS Camera Control Unit DS-L1	DS-L1
DS Camera Cable	Camera Cable
Super High-pressure Mercury Lamp Power Supply Unit	Mercury Lamp Power Supply Unit
Super High-pressure Mercury Lamphouse	Mercury Lamphouse

How to use this instruction manual

This instruction manual is composed of two parts, as below:

Manual 1 "Microscopy" describes basic microscope operations that you must follow. Please read this manual carefully before operating the product.

Manual 2 "Reference" describes the operations of each attachment. Please read an appropriate section as necessary.

	Title	Importance	Content
Manual 1	Microscopy	Must be read	Safety precautions Microscopy, Assembly, Troubleshooting, Cleaning and maintenance, Specifications
Manual 2	Reference	As necessary	Detailed operations of attachments

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Individual Operations

Item	Title	Operating sections
1	Power ON/OFF	Power switch, battery, AC adapter
2	Brightness Adjustment	Brightness control knob, preset switch, ND filter, color compensating filter, filter holder
3	Optical Path Switching	Optical path switching knob
4	Vertical Stage Motions	Coarse/fine adjustment knobs, coarse torque adjustment ring, refocusing lever
5	Lateral Stage Motions	X knob, Y knob, XY knob torque adjustment screws
6	Stage Rotation	Stage retaining screw, stage rotation clamp screw
7	Magnification Selection	Revolving nosepiece, DIH-M zoom knob
8	Diopter Adjustment	Diopter adjustment rings
9	Interpupillary Adjustment	Eyepiece sleeve
10	Adjusting Observation Position	Ergonomic binocular tube
11	Adjusting Condenser Position	Condenser focus knob, condenser centering screws
12	Aperture Diaphragm Adjustment	Condenser aperture diaphragm, objective
13	Selection of Condenser	Condenser
14	Field Diaphragm Adjustment	Field diaphragm knob
15	Oil Immersion Operation	Oil immersion objectives, oil immersion condensers
16	Water Immersion Operation	Water immersion objectives, water immersion condensers
17	Fluorescence observation	Fluorescence attachment
18	Fluorescent Filter Selection	Filter cube
19	Image Capture	Camera
20	Accessory Installation	Accessory mounting sections

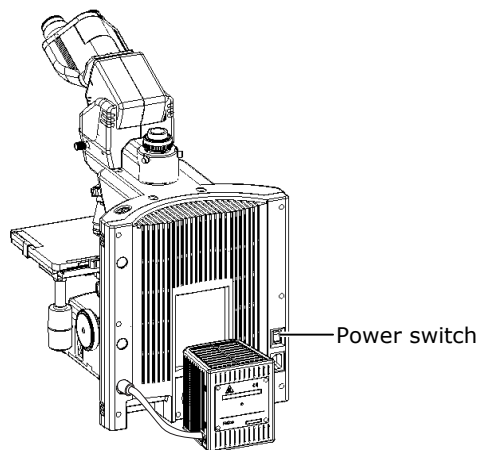
1 Power ON/OFF

1 Power ON/OFF

1.1 Turning on the microscope

To turn on the microscope, press the power switch to the "I" position.

To turn off the microscope, press the power switch to the "O" position.



1.2 DIH-M Power Supply

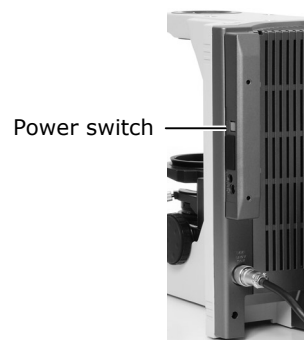
Push in the power switch located on the side the C-Box to switch on power for the DIH-M.

The Power ON indicator on the left side the DIH-M goes on.

The EPI shutter closes automatically and blocks the optical path of the epifluorescent light. (To open the shutter, press the hand switch.)

To turn off power to the DIH-M, press the power switch once again.

The Power ON indicator goes out.



DIH-M Power ON indicator



1.3 Turning on the fluorescence attachment light source (mercury lamp)

Refer to the operating manual provided with the super high-pressure mercury lamp power supply unit.

Always observe all warnings and precautions described in the manual.

2 Brightness Adjustment

Image brightness can be adjusted by the following methods:

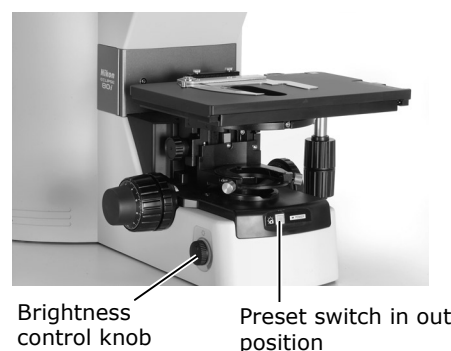
	Method	Operating controls	Explanation
Transmitted image	Adjusting lamp voltage (The 50i is subject to shifts in color temperature.)	Brightness control knob	2.1
		Preset switch	2.2
	ND filter attachment/detachment (for 50i only)	ND filter IN/OUT lever	2.3
Epi-fl image	ND filter	Attaching/removing ND filter for fluorescence attachment	2.4
	Erasure of transmitted image	Microscope power switch	2.5
	Adjustment of aperture diaphragm (for Epi-fluorescent light)	Aperture diaphragm lever (Epi-illumination attachment, DIH-M)	2.6
(Monitor image)	Camera adjustment	Application software for camera control: Display mode, exposure mode, exposure compensation, camera gain adjustment, etc.	2.7

2.1 Adjustment using the brightness control knob

With the preset switch in the out position, rotate the brightness control knob. (The brightness control knob is disabled if the preset switch is depressed.)

Adjusting brightness with the brightness control knob will affect the lamp color temperature and alter the color balance of the image. If accurate color reproduction is critical, set a commercially available color compensation filter on the filter holder to make brightness adjustments.

If accurate color reproduction is critical, do not use the brightness control knob to adjust brightness. Instead, press the preset switch and move the NCB11 filter (color temperature adjustment filter) into the optical path. This will provide optimum color accuracy.



Brightness control knob	Image brightness	Image color
Clockwise turn	Becomes brighter	Color balance shifts to blue
Counterclockwise turn	Becomes darker	Color balance shifts to red

2 Brightness Adjustment

2.2 Adjustment using the preset switch

Pushing in the preset switch switches the lamp voltage to 9V. Push in the preset switch and move the NCB11 filter into the optical path to achieve optimum color reproduction accuracy.

Push the switch to restore it to the out position. This restores brightness to the level previously set with the brightness control knob.



Preset switch

2.3 Adjustment with the ND filter IN/OUT lever

The two forward-located switches of the three closely arranged switches are for ND filters. The ND filters are used to adjust light intensity. Higher ND values correspond to reduced transmissivity and darker images. The color balance is not affected. Push the IN side to move the ND filter into the optical path and darken the image.

ND8: Reduces light intensity to 1/8 previous levels.

ND32: Reduces light intensity to 1/32 previous levels.



ND32
ND8

Brightness	ND8	ND32
1	-	-
1/8	○	-
1/32	-	○
1/256	○	○

2.4 Adjustment with the ND filter for the fluorescence attachment

Pushing in the ND filter attach/detach lever moves the ND filter into the optical path and darkens the fluorescent image.

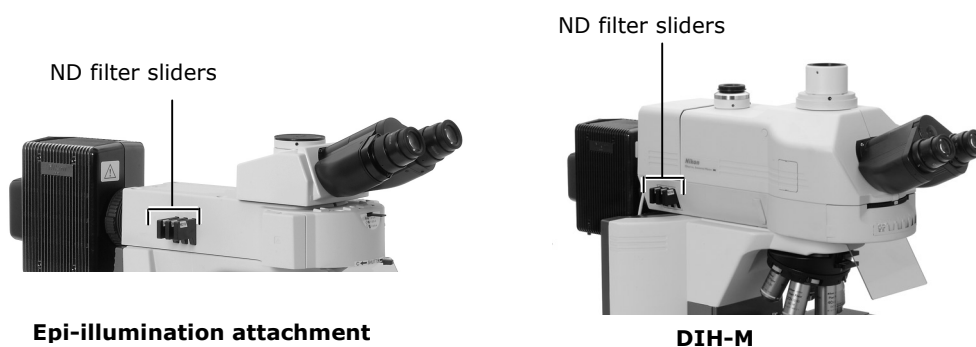
ND filters are used to adjust light intensity. Higher filter numbers correspond to lower transmission rates (i.e., darker images). ND filters do not affect color balance.

ND4: Reduces light intensity by 1/4.

ND8: Reduces light intensity to 1/8 previous levels.

ND16: Reduces light intensity to 1/16 previous levels.

2 Brightness Adjustment



Brightness	ND4	ND8	ND16
1	-	-	-
1/4	○	-	-
1/8	-	○	-
1/16	-	-	○
1/32	○	○	-
1/64	○	-	○
1/128	-	○	○
1/512	○	○	○

2.5 Transmitted image in fluorescence observation

For fluorescence observations, turn off the microscope power switch to cancel the transmitted image.

Bright ambient lights will make it more difficult to view the image. We recommend keeping the room dark during fluorescence observations.

2.6 Adjusting the Epi-fluorescence Aperture Diaphragm

In Epi-fluorescence microscopy, image brightness can be adjusted by varying aperture diaphragm size. Stopping down the aperture diaphragm darkens the image, while opening up the aperture diaphragm makes the image brighter.

Use the aperture diaphragm of the Epi-fluorescence attachment or DIH-M after performing the centering procedures. For a detailed discussion of this topic, refer to "17.5 Epi-fluorescence Aperture Diaphragm."

2.7 Camera adjustment (adjusting the brightness of the image on the monitor)

When observing images captured by the camera and displayed on the monitor, you can adjust brightness by varying camera adjustment parameters, such as display mode, exposure mode, metering mode, exposure compensation, and image level adjustment.

For detailed discussion, refer to the operating manual provided with the camera or camera control software.

3 Optical Path Switching

3.1 Optical path distribution

With the ergonomic binocular tube or trinocular eyepiece tube, the optical path switching lever allows distribution of light to the binocular section and camera port.

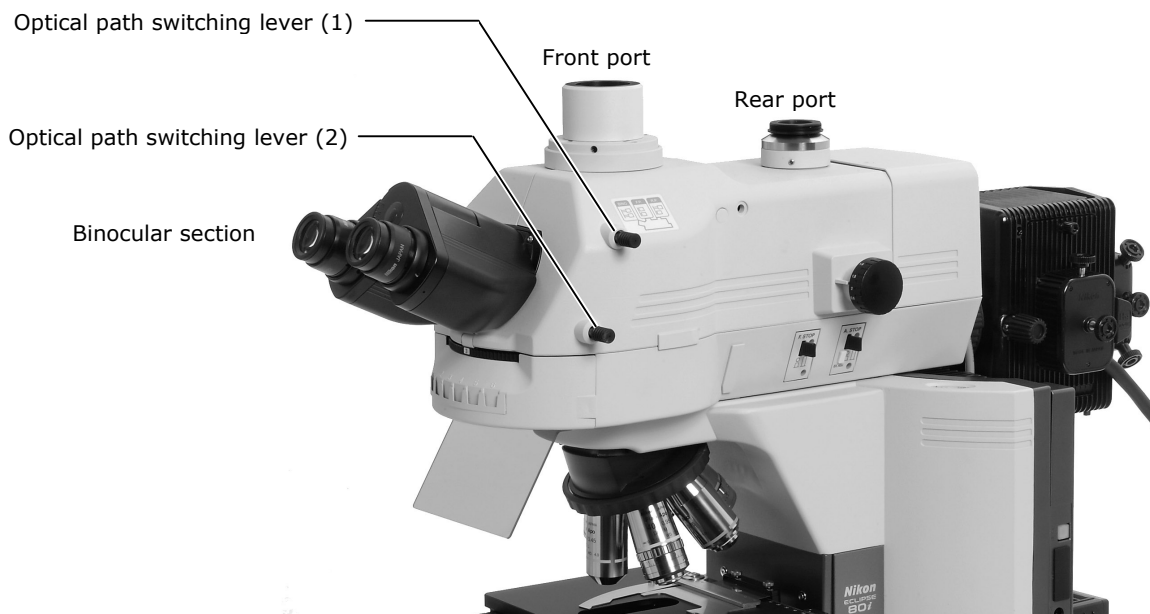
	Position of optical path switching lever	Optical path distribution (%)	
		Binocular section	Camera port
Ergonomic binocular tube	Pushed in	100	0
	Extended	50	50
Trinocular eyepiece tube T	Pushed in	100	0
	Extended by one notch	20	80
	Extended by two notches	0	100
Trinocular eyepiece tube F	Pushed in	100	0
	Extended	0	100

3 Optical Path Switching

With the DIH-M, two optical path switching levers allows distribution of light to the binocular section, front port and rear port.

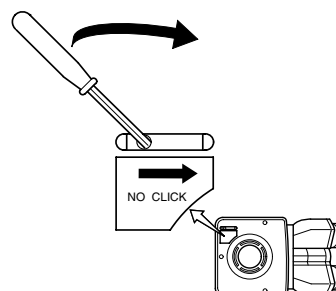
Position of optical path switching lever (1) (top lever)	Position of optical path switching lever (2) (bottom lever)	Optical path distribution (%)		
		Binocular section	Front port	Rear port
Extended	Extended	100	-	-
Pushed in	Pushed in	-	100	-
Extended	Pushed in	-	-	100

It is not possible to have only the optical switching lever (2) extended. Applying excessive force may result in damage.



3.2 Disabling the clicking of the optical path switching lever

The trinocular eyepiece tubes T and F have a "NO CLICK" switch on their tube mounting surfaces. Slide this switch in the direction of the arrow with the tip of a pointed tool to disable clicking for the optical path switching lever. Set the switch to this position if you need to eliminate the slight vibrations resulting from the clicking action.



4 Vertical Stage Motion

4.1 Prohibited actions

Avoid the following actions, which can cause equipment malfunctions.

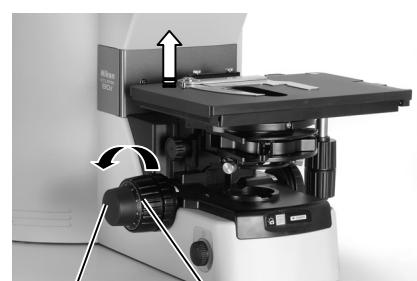
- **Rotating the right and left coarse/fine focus knobs in opposite directions.**
- **Rotating the coarse focus knob past the stopper.**

4.2 Knob rotation direction and stage motion direction

Turn the coarse or fine focus knob to raise or lower the stage and to adjust image focus.

The coarse focus knob is located on either the right side or the left side. A fine focus knob is provided on both sides.

To lower the stage	Turn the knob toward the front.
To raise the stage	Turn the knob toward the back.



Fine focus knob Coarse focus knob

4.3 Number of knob turns and distance of stage travel

No. of knob turns	Distance of stage travel (vertical direction)
Coarse focus knob 1 turn	Approx. 14 mm
Fine focus knob 1 turn	Approx. 0.1 mm
Fine focus knob 1 graduation on scale	1 μm

The vertical motion range (coarse/fine focus stroke) of the stage is from 2 mm above the focal point (reference position) to approximately 25 mm below the focal point.

4 Vertical Stage Motion

4.4

Adjusting the rotating torque of the coarse focus knob

Adjust the rotation torque of the coarse focus knob (rotation resistance) by turning the torque adjustment ring (TORQUE) located at the base of the coarse focus knob. If the torque is too low, the stage may descend under its own weight.

When turned in the direction of arrow	Makes knob harder to turn.
When turned in the direction opposite to arrow	Makes knob easier to turn.



Coarse focus torque adjustment knob

4.5

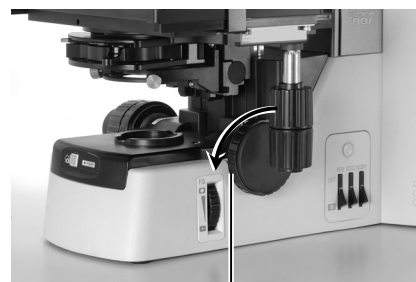
How to Use the Coarse Focus Upper-limit Clamp

The coarse focus upper-limit clamp restricts the movement of the coarse focus knob to maintain the stage at a position where the specimen is in focus. (The clamp does not restrict stage motion adjustments made with the fine focus knob.)

When the coarse focus upper-limit clamp is engaged, the stage cannot be raised with the coarse focus knob. Engaging the coarse focus upper-limit clamp after accurate focus has been achieved lets you return to this precise focus position simply by turning the coarse focus knob until the stage reaches the limit position set by the clamp. This function is intended to save time when several similar specimens are observed in sequence.

[Directions of use]

- (1) With a specimen in focus, rotate the coarse focus upper-limit clamp about 3/4 turns in the direction of the arrow on the microscope base. This engages the coarse focus upper-limit clamp.
- (2) To change specimens, lower the stage using only the coarse focus knob.
- (3) After positioning a new specimen, use the coarse focus knob and gently raise the stage until the stage contacts the clamp. When the stage contacts the clamp, the specimen should be in focus. Use the fine focus knob for precise focusing adjustments.
- (4) When not using the coarse focus upper-limit clamp, loosen the coarse focus upper-limit clamp until it reaches the stopper. (Turn in the direction opposite the direction of the arrow indicated on the microscope base until no farther rotation is possible.)



Engage the coarse focus upper-limit clamp.

5 Lateral Stage Motion

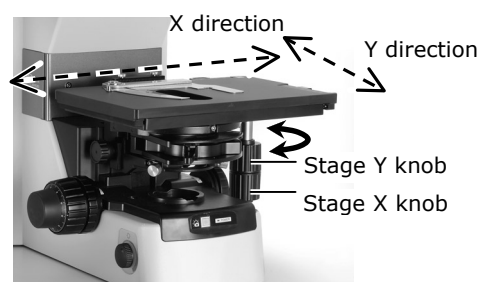
5.1 Prohibited action

Avoid the following actions, which can cause equipment malfunctions.

- **Moving the stage or the specimen holder to the left and right by holding stage or holder directly.**

5.2 Knob rotation direction and stage motion direction

To move the stage in the X or Y direction, rotate the stage X knob or stage Y knob.



5.3 Adjusting the knob heights

The heights (positions) of the X knob and Y knob can be changed. Hold the knob and move it along its vertical axis to the desired height.

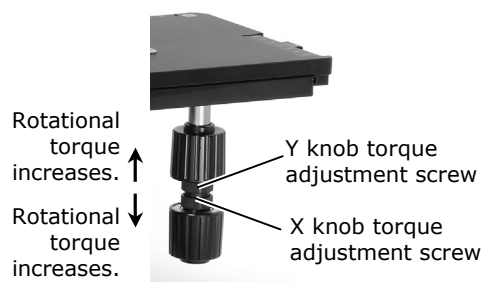
5.4 Adjusting the knob rotation torque

When the X knob and Y knob are moved to the top and bottom positions, the torque adjustment screws can be found between the knobs.

Turning the torque adjustment screw to move them closer towards the respective knobs increases rotational torque.

(To increase rotational torque, turn the adjustment screw counterclockwise and clockwise, as viewed from above, for the Y knob and X knob, respectively.)

Avoid loosening these screws excessively. If they are too loose, the top surface of the stage may move, even at a very light touch.



6 Stage rotation

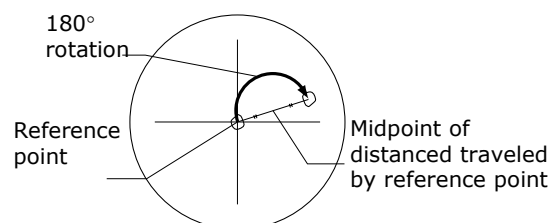
6.1 Stage with Rotating Mechanism

Loosen the rotation clamp screw on the bottom surface of the stage to allow the stage to swivel.



6.2 Centering the Stage

- (1) Move the 10x objective into the optical path and focus on the specimen. Select a specific point on the specimen as a reference point, then move the reference point to the center of the field of view using the stage knobs.
- (2) Loosen the stage rotation clamp screw and rotate the stage approximately 180°.
- (3) If the reference point deviates from the center of the field of view, insert the hexagonal screwdriver into the stage-centering screws* (2 locations) and adjust by moving the stage a distance equal to the distance traveled by the reference point from the center of the field of view.



- (4) Using the stage knobs, return the reference point to the center of the field of view.
- (5) Move the 40x objective into the optical path and repeat steps 2 through 4.

* The stage-centering screws (also used as mounting screws) are located at two points on the back of the elevating section.

7 Selection of Magnification

To change magnification, turn the revolving nosepiece and set the desired objective into the optical path.

If the DIH-M is mounted to the microscope, the zoom knob on the right side allows adjustment of magnification between 0.8x and 2x.

Clockwise rotation of the zoom knob increases magnification. The number at the pointer indicates the effective magnification. The knob clicks at each 0.2x increment.

Total magnification is calculated as follows:

Magnification of eyepiece lens x Magnification of objective x Magnification of zoom knob

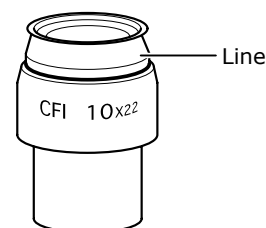


8

Diopter Adjustment

Diopter adjustment compensates for differences in visual acuity between the right and left eyes, improving binocular observation. It also minimizes focal deviations when switching objectives. Adjust diopter settings for both eyepiece lenses.

- (1) Turn the diopter adjustment ring of each eyepiece lens and align the end face of the diopter adjustment ring with the line. (This is the diopter adjustment reference position.)
- (2) Perform steps 1) to 10) in "Bright-Field Microscopy" in the "Microscopy" instruction manual to focus on the specimen with the 10x objective.
- (3) Set the 40x objective in the optical path. Using the coarse/fine focus knobs, focus on the specimen.
- (4) Set the 4x or 10x objective in the optical path.
- (5) Focus on the specimen using the diopter adjustment rings instead of the coarse/fine focus knobs. When making focus adjustments, be sure to look through the right eyepiece with your right eye and the left eyepiece with your left eye.
- (6) Perform steps (3) through (5) twice.



Reference position for diopter adjustment



Use this for focal adjustment.



Set the magnification to 10x and observe with the right eye.

Use this for focal adjustment.



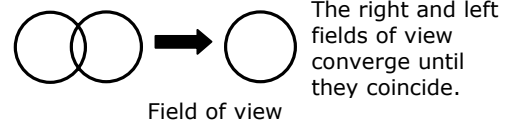
Observe with the left eye.

Use this for focal adjustment.

9 Interpupillary Adjustment

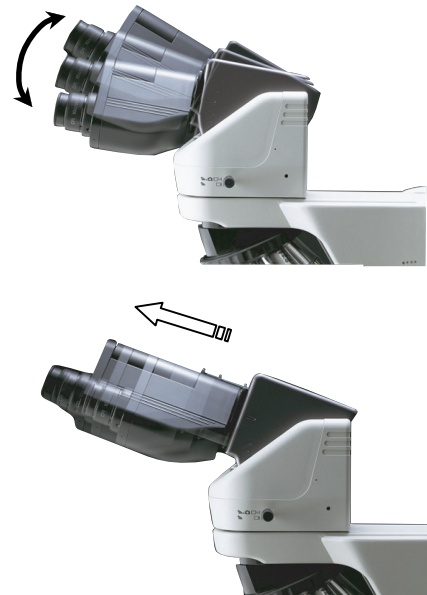
Interpupillary adjustment improves the ease of binocular observation.

Perform steps 1) to 10) in "Bright-Field Microscopy" in the "Microscopy" instruction manual and focus on the specimen using the 10x objective. Then, move the eyepiece sleeve until the fields of view for the right and left eyes coincide.



10 Adjusting the Observation Position

The ergonomic binocular tube makes it possible to extend and tilt the binocular section. Adjust the position of the binocular section for the most comfortable viewing.

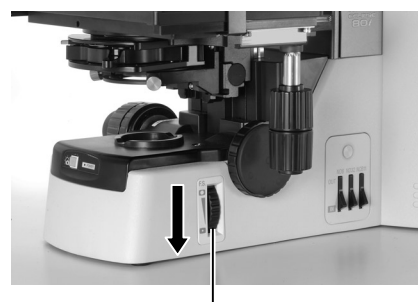


11

Adjusting the Condenser Position

Adjust the condenser (focusing and centering) so that the light passing through the condenser forms an image at the correct location (center of the optical path) on the specimen surface.

- (1) Perform steps 1) to 10) in "Bright-Field Microscopy" in the "Microscopy" instruction manual to focus on the specimen using the 10x objective.
- (2) Stop down the field diaphragm to the minimum setting.
- (3) Turn the condenser focus knob to form the field diaphragm image on the specimen surface.
- (4) Turn the condenser centering screws so that the field diaphragm image is positioned in the center of the field of view.
- (5) Set the 40x objective in the optical path. Turn the coarse/fine focus knobs and focus on the specimen.
- (6) Turn the condenser focus knob to form the field diaphragm image on the specimen surface.
- (7) Adjust the condenser centering screws until the field diaphragm is at the center of the eyepiece field of view. This is easiest if you set the field diaphragm aperture to slightly smaller than the eyepiece field of view.

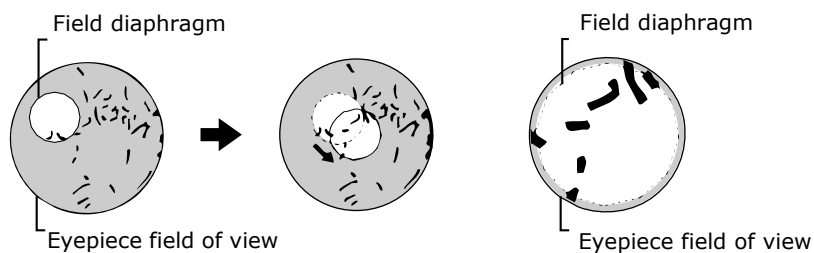


Turn the field diaphragm knob and stop down the field diaphragm to its minimum setting.



Condenser focus knob

Condenser centering screws



12 Adjusting the Aperture Diaphragm

12

Adjusting the Aperture Diaphragm

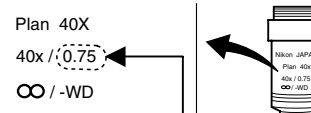
The setting of the aperture diaphragm affects optical image resolution, contrast, depth of field, and brightness. Turning the condenser aperture diaphragm ring (or aperture diaphragm knob) changes the size of the aperture diaphragm.

A small aperture diaphragm opening reduces resolution and brightness but increases contrast and depth of field. A large aperture diaphragm size increases resolution and brightness but reduces contrast and depth of field. These characteristics involve inherent tradeoffs and cannot be optimized independently. Generally, aperture settings that are 70 to 80% of the maximum aperture of the objective will provide satisfactory images with suitable contrast.

Since an excessively small aperture diaphragm opening will degrade image resolution, we do not recommend setting the aperture diaphragm to less than 60% of the objective's maximum aperture.



Aperture diaphragm knob



Condenser aperture diaphragm scale = $0.75 \times 0.7 \sim 0.8 = 0.525 \sim 0.6$

12.1

Adjusting the aperture diaphragm opening using the condenser scale

Since the condenser scale indicates the numerical aperture set, adjust the aperture diaphragm ring according to the scale.

(Normally, the index on the aperture diaphragm ring should align with a scale line that corresponds to 70 to 80% of the maximum aperture of the objective.)

12.2

Adjusting the aperture diaphragm opening using the centering telescope (optional)

Remove one eyepiece lens and mount the centering telescope in place using the optional adapter. Turn the aperture diaphragm ring to stop down to the minimum aperture. While holding down the flange of the centering telescope, turn the eyepiece of the centering telescope and focus on the aperture diaphragm.

Turn the diaphragm ring to adjust the aperture. (Normally, the aperture diaphragm should be adjusted to around 70 to 80% of the size of the field of view.)

After the adjustment, remove the centering telescope and adapter and reinstall the eyepiece.

13 Selecting a Condenser

Objective magnification	Condenser (⊙: Optimum, ○: Suitable, x: Not suitable)			
	Achromatic/aplanat condenser	Achromat condenser	Abbe condenser	1-100x condenser
1x	x	x	x	⊙
2x	x	x	x	
4x	x	○ ^{Note 1}	○ ^{Note 1}	
10x to 100x	⊙	○	○	

Note 1: The entire field of view may not be covered if a UW eyepiece is attached.

Depending on the type of objective, the indicated numerical aperture of the objective may not be achieved.

For example, when an objective with an N.A. of 1.4 is used, the maximum aperture of the Abbe condenser will be only about 65% of the objective's N.A., even when the condenser aperture diaphragm is wide open.

Refer to the condenser operating manual for more information on universal and phase contrast condensers.

14 Adjusting the Field Diaphragm

The field diaphragm controls the amount of illumination falling on the area of the specimen being viewed. Turning the field diaphragm knob changes the size of the field diaphragm. For normal observations, the size of the diaphragm should be slightly wider than the boundary of the field of view. Illuminating a broader area than necessary will result in stray light entering the field of view, generating flare and reducing image contrast. Appropriate field diaphragm settings are particularly important for photomicrography. In general, good results will be obtained by stopping down the field diaphragm to settings slightly wider than the area to be reproduced within the photo frame or monitor display.



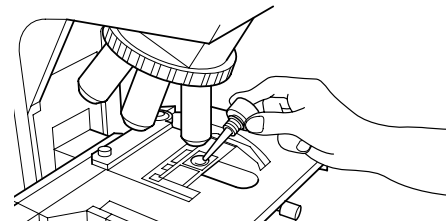
Field diaphragm knob

15

Oil Immersion Operation

Objectives marked "Oil" are oil-immersion objectives. Objectives of this type are used with immersion oil applied between the specimen and the tip of the objective.

For maximum performance, oil-immersion objectives with numerical apertures of 1.0 or higher should be combined with oil-immersion chromatic/aplanat condensers. Oil-immersion condensers are used by applying oil between the specimen and the condenser.



Any bubbles in the immersion oil will degrade image quality. Be careful to prevent bubbles from forming. To check for air bubbles, fully open the field diaphragm and aperture diaphragm, remove the eyepiece, and examine the exit pupil (bright round section) of the objective inside the eyepiece tube. If it is difficult to ascertain the presence of bubbles, mount a centering telescope (optional), then look for air bubbles while turning the eyepiece section of the centering telescope to adjust focus. If you detect bubbles, remove them by one of the following methods:

- Turn the revolving nosepiece slightly to move the oil-immersed objective back and forth once or twice. (In the case of the condenser, gently turn the condenser focus knob to move the condenser up and down slightly.)
- Add oil.
- Remove the oil and apply new oil.

Use as little oil as possible (just enough to fill the space between the tip of the objective and the specimen, or between the tip of the condenser and the specimen). Too much oil will result in excess oil flowing onto the stage and around the condenser.

Any oil remaining on the oil-immersion objective or adhering to the dry-type objective will noticeably degrade image quality. After use, thoroughly wipe off all oil, and make sure that no oil remains on the tips of other objectives. Additionally, carefully wipe off oil from the condenser.

Use petroleum benzene to wipe off immersion oil. For optimum results, we recommend following up petroleum benzene with absolute alcohol (ethyl or methyl alcohol).

If petroleum benzene is unavailable, use methyl alcohol alone. When using just methyl alcohol, note that surfaces will need to be wiped repeatedly to ensure complete removal of immersion oil. Usually, three or four times should be sufficient to clean the lens.

**CAUTION**

When using petroleum benzene or absolute alcohol, always follow the instructions provided by the manufacturer. These liquids are highly flammable and must be kept away from flames and sparks.

16

Water Immersion

Objectives marked "WI" or "W" are water-immersion objectives. These objectives are used with immersion water (distilled water or physiological saline) applied between the specimen and the tip of the objective. Microscopy procedures are the same as for oil-immersion objectives.

Since water evaporates readily, monitor the immersion water during observation. Avoid using too much water, since excess water will flow onto the stage and around the condenser, promoting corrosion.

After use, wipe off water from the tip of the objective and condenser, then follow up by wiping with absolute alcohol.

If you observe water stains, apply a small amount of neutral detergent and wipe gently, then follow up with absolute alcohol.

17 Fluorescence Observation

17.1 Warning

The mercury lamp (or xenon lamp) used with the Epi-illumination attachment or DIH-M requires careful handling. Be sure to read the warnings described in the beginning of this manual and in the operating manual provided by the manufacturers of the super high-pressure mercury light source (or high-intensity light source) and lamp. Observe all the warnings and precautions described in those documents.

17.2 Epi-illumination attachment shutter

The shutter blocks illumination. When suspending microscopy, close the shutter to prevent fading of specimen colors. (Set the shutter lever to the C position to move the shutter into the optical path and block light.) To protect important specimens, make it a habit to use the shutter whenever appropriate.

When pausing Epi-fluorescent microscopy to perform microscopy diascopic light, move the shutter into the optical path to block the Epi-illumination light.

When using the Epi-illumination attachment:

Set the shutter open/close lever to the "C" position to set the shutter into the optical path and to block illumination.

When using the DIH-M:

Press the hand switch to toggle the shutter open or closed. The shutter is automatically kept closed when the DIH-M power switch is turned on.

Shutter lever



Light shielding plate



Hand switch

17.3 Light shielding plate

The light shielding plate protects the observer's eyes from ultraviolet light reflected from the specimen. To remove the plate, loosen the clamp screw and pull it forward.

17.4

Field diaphragm of the Epi-illumination attachment

The field diaphragm controls the illumination on the area of the specimen being viewed. Operating the field diaphragm lever changes the size of the field diaphragm.

Position of field diaphragm open/close lever	Field diaphragm setting
Pushed in	Open
Pulled	Constricted

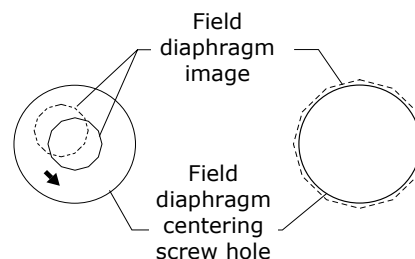
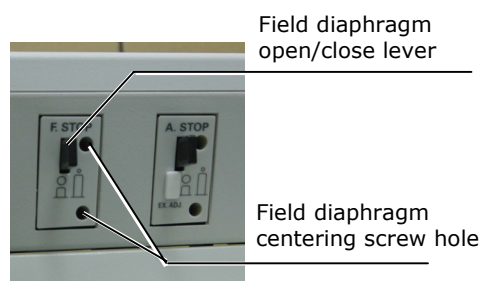
For normal observations, stop down the diaphragm so that the aperture boundaries are just outside (or inside) the field of view. Illuminating an area broader than necessary will result in stray light entering the field of view, generating flare, reducing image contrast, and expanding the area of the specimen subject to fading.

Appropriate field diaphragm settings are particularly important for photomicrography. In general, good results will be obtained by stopping down the field diaphragm to slightly wider than the area to be reproduced within the photo frame or monitor display.

Adjust the field diaphragm of the Epi-illumination attachment or DIH-M after performing the following centering procedures:

Centering the field diaphragm

- (1) Adjust the diopter and interpupillary distance according to the procedures described in "Epi-fluorescence Microscopy" in the separate Microscopy manual.
- (2) Stop down the field diaphragm. (Pull the field diaphragm open/close lever.)
- (3) Move the center of the field diaphragm image to the center of the field of view. (Using the hexagonal screwdriver provided with the microscope, turn the field diaphragm centering screws.)
- (4) Open up the field diaphragm to match the field of view. (Push in the field diaphragm open/close lever.)
- (5) Again, move the center of the field diaphragm image to the center of the field of view. (Turn the field diaphragm centering screws.)



17.5

Epi-illumination Aperture Diaphragm

The aperture diaphragm determines the numerical aperture for illumination and the optical system. In Epi-fluorescence microscopy, it is used to adjust image brightness. Use the aperture diaphragm open/close lever to adjust the size of the opening of the aperture diaphragm.

Position of aperture diaphragm open/close lever	Size of aperture diaphragm
Pushed in	Open
Pulled	Constricted

A narrow aperture diaphragm setting reduces the amount of backlighting and makes the image darker. In contrast, opening up the aperture diaphragm increases backlighting and image brightness. For ordinary Epi-fluorescence microscopic observations, keep the aperture diaphragm open. Stop down the diaphragm only when image brightness needs to be adjusted.

Use the aperture diaphragm of the Epi-illumination attachment or DIH-M after performing centering. (The centering tool may not be included in the set you have purchased.)

Centering the aperture diaphragm

(1) When using the DIH-M, turn on the power switch located on the side of the C-Box.

(2) Close the shutter for the epifluorescent light.

When using the Epi-illumination attachment, set the shutter open/close lever to the "C" position.

With the DIH-M mounted, the shutter closes automatically when power is switched on. Do not touch the hand switch. (If the shutter is opened accidentally, press the hand switch once again to close the shutter.)

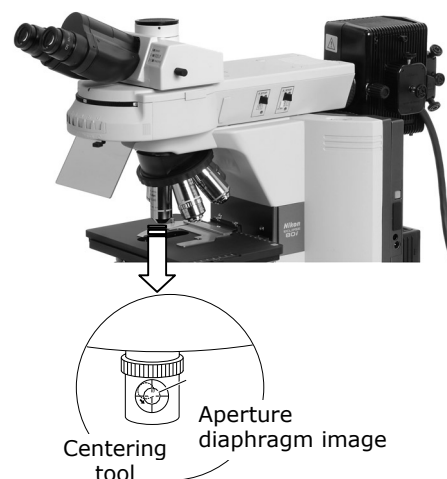
(3) Remove one objective from the revolving nosepiece and insert the centering tool in its place.

(4) Move the centering tool into the optical path.

(5) Turn on the Epi-fluorescent light source and open the shutter.

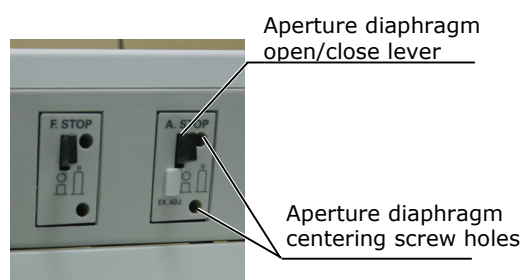
When using the Epi-illumination attachment, move the shutter open/close lever to the "O" position.

When using the DIH-M, press the hand switch.



17 Fluorescence Observation

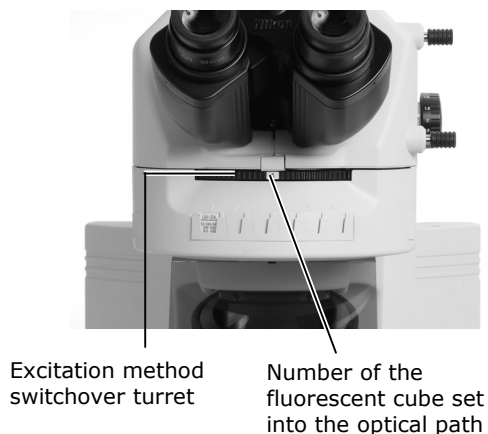
- (6) Stop down the aperture diaphragm. (Pull the aperture diaphragm lever.)
- (7) While observing the centering tool screen, move the center of the aperture diaphragm image to the center of the screen. (Using the hexagonal screwdriver provided with the microscope, turn the field diaphragm centering screws.) Be sure to view the centering tool screen squarely, from the front.
- (8) After centering is completed, close the shutter, remove the centering tool, and reinstall the objective.



17.6 Switching Excitation Methods

Up to six filter cubes each can be mounted to the Epi-illumination attachment and to the DIH-M. Rotate the excitation method switchover turret on the front of the equipment to move the desired cube into the optical path.

The number on the front section of the turret indicates the filter cube set into the optical path.



17.7

Epifluorescent ND Filters

Pushing in an ND filter slider sets the respective ND filter into the optical path and darkens the fluorescent image.

ND filters reduce light intensity without altering the color balance of the illumination. Higher filter numbers correspond to lower transmission rates (i.e., darker images). If fluorescence is too strong or if specimen colors fade too rapidly, insert ND filters into the optical path to adjust brightness. Excessively strong fluorescence may result in poor image contrast.

ND4: Reduces light intensity to 1/4 previous levels.

ND8: Reduces light intensity to 1/8 previous levels.

ND16: Reduces light intensity to 1/16 previous levels.

You can combine these three filters to achieve various levels of brightness.



ND filter sliders

Epi-illumination attachment



ND filter sliders

DIH-M

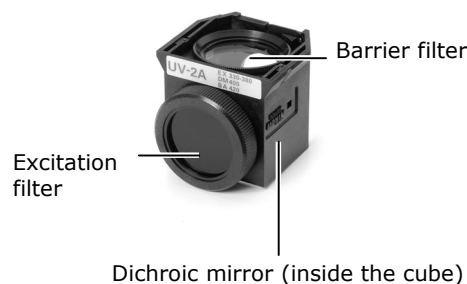
Brightness	ND4	ND8	ND16
1	-	-	-
1/4	○	-	-
1/8	-	○	-
1/16	-	-	○
1/32	○	○	-
1/64	○	-	○
1/128	-	○	○
1/512	○	○	○

(-: Outside optical path, ○: In optical path)

18 Selecting Fluorescent Filters

A filter cube is comprised of the following three optical components: an excitation filter (EX filter), a barrier filter (BA filter), and a dichroic mirror (DM). Select a combination of filter cubes appropriate for the specimen characteristics, fluorescent pigment, and the purpose intended. Keep in mind the following:

- Different combinations of excitation and barrier filters may be selected for the same excitation method.
- Other types of excitation filters, barrier filters, and dichroic mirrors can be purchased separately.
- Excitation filters are exposed to strong light during operations and tend to age rapidly. Replace the excitation filter at frequent intervals.

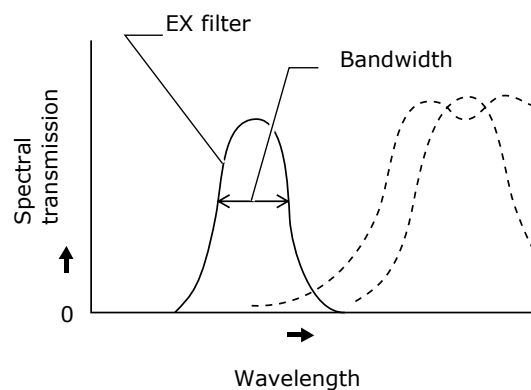


18.1 Selecting excitation filters (EX filters)

Excitation filters allow selective transmission of light (excitation light) in the waveband required for fluorescent light emissions from the specimen, blocking light of all other wavelengths. The range of wavelengths allowed to pass through a filter is referred to as bandwidth.

The range of the bandwidth of the excitation filter determines the brightness of the fluorescent image, the generation of self-fluorescence (fluorescence resulting from substances other than fluorescent pigments), and degree of fading. The broader the bandwidth, the greater the amount of excitation light irradiated onto the specimen, increasing brightness. However, this also increases the amount of self-fluorescence and causes faster color fading. Narrow bandwidth reduces the amount of excitation light striking the specimen and causes the image to appear darker, but reduces self-fluorescence and fading. For specimens with pronounced self-fluorescence, use excitation filters that pass a narrow bandwidth (note that this will make the fluorescent image darker).

Excitation filters are exposed to strong light during operations and tend to age rapidly. Replace the filter at intervals determined by usage.



18 Selecting Fluorescent Filters

	Narrow	EX filter bandwidth	Wide
Brightness of fluorescent image	Dark		Light
Generation of self-fluorescence	Low		High
Degree of color fading	Low		High

18.2**Selection of barrier filter (BA filter)**

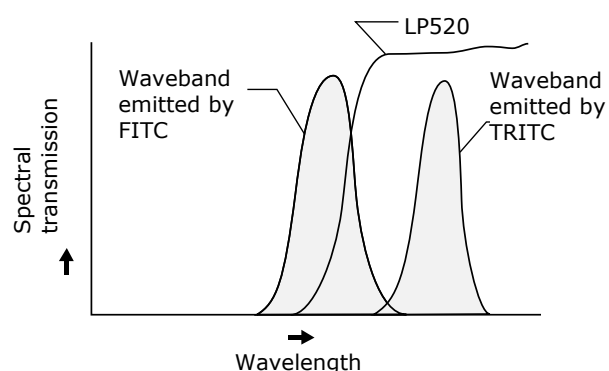
The barrier filter allows only fluorescent light emitted by the specimen to pass, blocking excitation light. This allows viewing of a fluorescent image without excess illumination (dark background).

There are two types of barrier filters: LP filters block all light below a certain wavelength but pass all light of longer wavelengths. BP filters pass only light of a certain waveband, blocking all other light. Use the filter type appropriate for your intended purpose.

LP filter (long-pass filter)

LP filters block all light below a certain wavelength but pass all light of longer wavelengths. This border wavelength is called a cut-on wavelength.

- (1) For specimens dyed with a fluorescent pigment in which the fluorescent waveband and excitation waveband (light that the specimen absorbs in order to emit fluorescent light) are very close, select a barrier filter with the shortest cut-on wavelength permitted by performance requirements for efficient fluorescent microscopy. If the cut-on wavelength is long, excitation light and fluorescent light will be entirely distinct, tending to darken the background of fluorescent images. However, recent advances in filter performance have resulted in increased use of filters of short cut-on wavelengths.
- (2) For multiple-dye specimens, use an LP filter for microscopy of fluorescent images of all dyes. Note that a combination involving an ordinary dichroic mirror, an excitation filter, and an LP-filter-type barrier filter will be incapable of exciting dyes that emit long-wavelength fluorescent light – for example, TRITC in the case of FITC and TRITC. This will result in very dark TRITC fluorescent images. For such cases, we recommend using multiband filters.



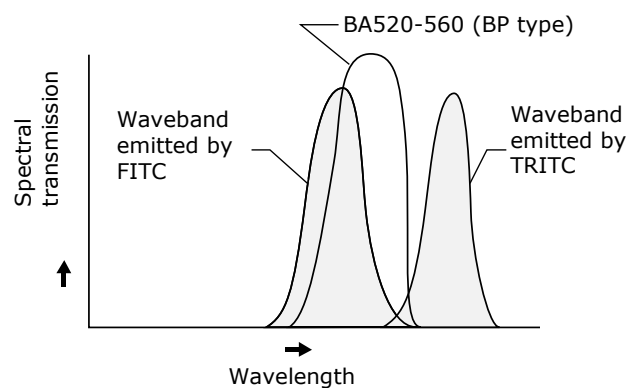
Both the FITC and TRITC images are visible.

18 Selecting Fluorescent Filters

BP filter (bandpass filter)

The bandpass filter passes only light of a certain wavelength, blocking all other wavelengths. BP filters are used for microscopy of fluorescent images involving a specific dye in multiple-dye specimens. (For example, in a double-dye specimen of FITC and TRITC, the BA520-560 filter enables microscopy of just the FITC fluorescent image.)

However, BP filters will not indicate self-fluorescence, if any (because the fluorescent image in the above combination is green only). LP filters are better suited for making fine distinctions in self-fluorescence based on slight color differences.

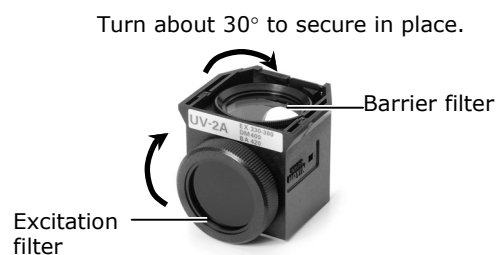


Only the FITC fluorescent image is visible.

18.3**Replacing excitation and barrier filters**

Excitation and barrier filters can be removed from the filter cube and replaced. (Dichroic mirrors cannot be dismantled from the filter cube.) Excitation filters are screw-in filters.

Barrier filters are slide-in filters. Align the projection on the barrier filter with the groove on the filter cube and turn clockwise approximately 30 degrees to secure in place.



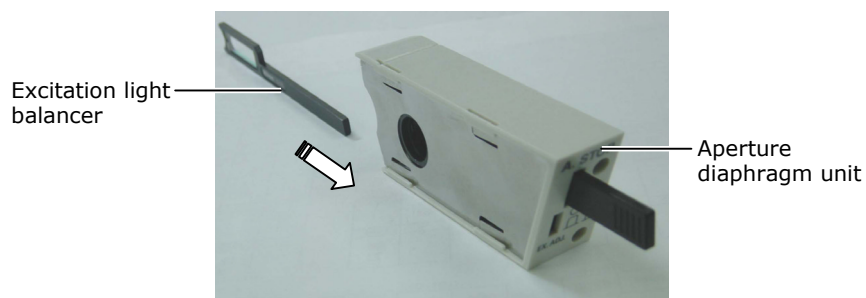
19**Excitation Light Balancer**

Mount the optional D-FB excitation light balancer to the Epi-illumination attachment or DIH-M to adjust the wavelength characteristic of the excitation light. The excitation light balancer is used in combination with a dual-band filter cube.

Required accessories

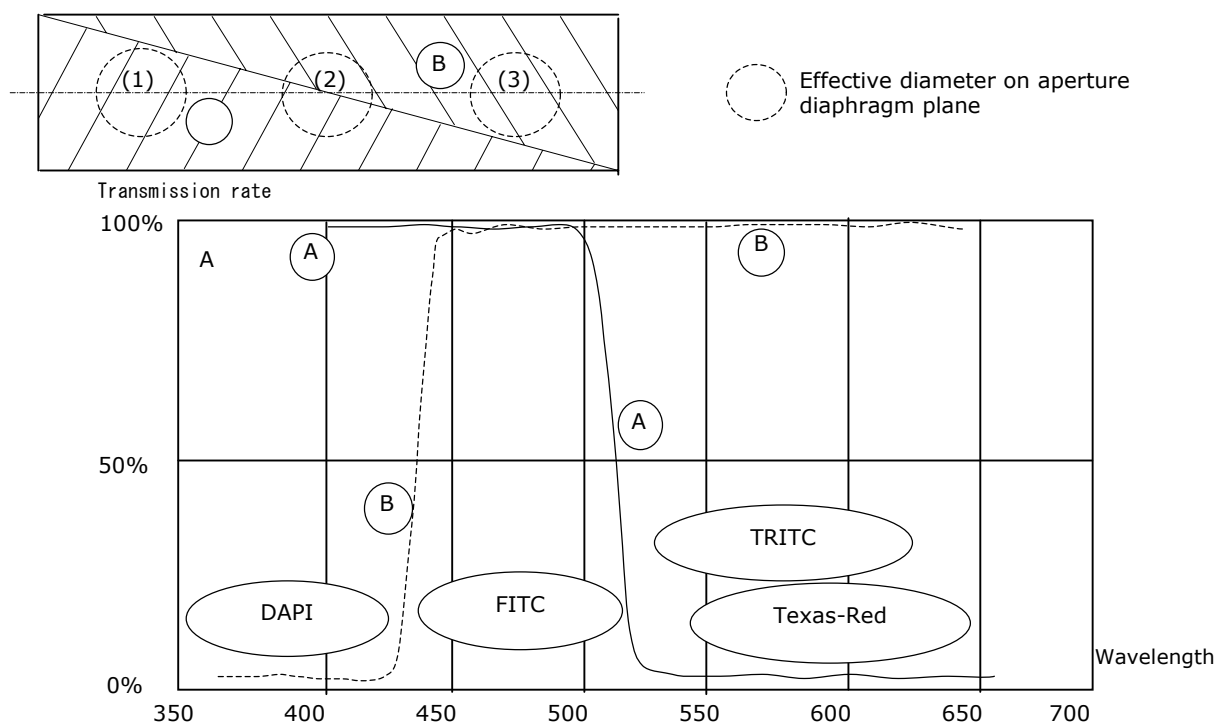
- D-FB excitation light balancer
- Dual-band filter cube

- (1) Switch off the light source for the Epi-illumination attachment.
- (2) Detach the aperture diaphragm unit from the Epi-illumination attachment or DIH-M.
- (3) Insert the D-FB excitation light balancer from the back end of the aperture diaphragm unit to install it.



- Take care not to touch the glass surface of the excitation light with your bare hands or fingers.
- (4) Reattach the aperture diaphragm unit to the Epi-illumination attachment or DIH-M.
 - (5) Mount a filter cube to the Epi-illumination attachment or DIH-M.
 - (6) Move the excitation light balancer laterally to adjust the excitation light.

Detailed view of excitation light balancer



The excitation light balancer is set so that the transmission rate remains at about 100% for typical dark fluorescent FITC.

Position of optical path	DAPI	FITC	TRITC/Texas-Red
(1)	100%	100%	0%
Between (1) and (2)	Variable (100% - 50%)	100%	Variable (0% - 50%)
(2)	50%	100%	50%
Between (2) and (3)	Variable (50% - 0%)	100%	Variable (50% - 100%)
(3)	0%	100%	100%

About objectives

Lamp voltage: If accurate color reproduction is critical, push in the preset switch on the microscope, set the NCB11 filter into the optical path, and use ND filters to make brightness adjustments.

Plan Fluor	40x/0.75	40xH/1.3	100xH/1.3
S Fluor	40x/0.9	40xH/1.3	100xH/1.3
Plan Apo	40x/0.95	60xH/1.4	100xH/1.4

20 Image Capture

Images can be captured by mounting a camera head to the ergonomic binocular tube or trinocular eyepiece tube.

For more detailed discussion of this topic, refer to the operating manual provided with the camera head or camera control software.

Proper adjustment of light intensity and focus on the microscope side are important for obtaining clear images. Listed below are key considerations in capturing clear images.

20.1 Adjusting light intensity

Lamp voltage: When the 50i is used in applications for which accurate color reproduction is critical, set the brightness control knob at a midpoint and use ND filters to make brightness adjustments.

Filter: Place a commercially available color compensation filter on the filter holder at the microscope base, as necessary.

20.2 Adjusting the condenser

- Always focus and center the condenser.
- Center the annular diaphragm for phase contrast microscopy.
- For normal operations, set the diaphragm aperture to 70 to 80% of the N.A. of the objective.

20.3 Confirming the photomicrographic range

The image on the monitor represents the photomicrographic range.

20.4 Confirming focus

Check focus by viewing through the eyepiece and viewing the monitor. If the focal positions for the two images differ, adjust the focal position adjustment screw at the camera port.

20.5 Making adjustments to keep out extraneous light

Field diaphragm: Stop down the diaphragm to a setting just slightly wider than the area shown on the monitor.

Eyepiece: Cover the eyepiece with a cloth.

20.6

Fluorescence photomicrography

The fluorescence of fluorescent specimens may fade during exposure. To prevent this, do the following:

- (1) Select a brighter optical combination.
Even if the overall magnification is the same on the monitor, the combination of objective and camera zoom can result in significant variations in exposure time. We recommend increasing the magnification with the objective rather than the zoom. (Generally, the aperture of the objective increases with magnification. The larger the numerical aperture, the brighter the resulting image.)
- (2) Adjusting the excitation light
Excessively bright excitation light will accelerate specimen fading while making it more difficult to acquire suitable fluorescent images. Use ND filters to adjust brightness.
- (3) Specimen
Photomicrography of faded specimen sections requires prolonged exposure times and results in poor color reproduction and low-quality images. Move the specimen to obtain images from a fresh section of the specimen previously unexposed to excitation light. Use the differential interference contrast or phase contrast methods to select a specimen section for photomicrography. For best results, switch to the fluorescent method to capture images.
- (4) Supersensitive TV camera
Under certain conditions, it may help to place an IR (infrared ray) blocking filter in front of the supersensitive TV camera sensor. Experiment with the IR blocking filter to determine its characteristics.