D-FL EPI-FLUORESCENCE ATTACHMENT

Instructions

Thank you for purchasing the Nikon products. This instruction manual is for the users of the Nikon D-FL EPI-FLUORESCENCE ATTACHMENT (abbreviated as "Epi-fl attachment" in this manual).
To ensure correct usage, read this manual carefully before operating the instrument.

- It is prohibited to alter this manual in part or whole without expressed permission.
- The contents of this manual are subject to change without notice.
- Although every effort has been made to ensure the accuracy of this manual, if you note any points that are unclear or incorrect, contact your nearest Nikon representative.
- Be sure to read the manuals for any other products that you are using with this attachment (the microscope, super high-pressure mercury lamp power supply, high-intensity light source, etc.).

Warning/Caution Symbols in This Manual

Though Nikon products are designed to provide you with the utmost safety during use, incorrect usage or disregard of the instructions can cause personal injury or property damage. For your safety, read the instruction manual carefully and thoroughly before usage. Do not discard this manual but keep it near the product for easy reference.

Inside this instruction manual, safety instructions are indicated with the symbols shown below. Be sure to follow the instructions marked with these symbols for your safety.

Symbol	Meaning
	Disregarding instructions marked with this symbol may lead to death or serious injury.
	Disregarding instructions marked with this symbol may lead to injury or property damage.

⚠ WARNING

1. Intended product use

This system should be used only for microscopic observation. Do not use this system for any other purpose.

2. Do not disassemble

Disassembling the system could result in electric shock, exposure to ultraviolet light, or damage to the equipment. Never attempt to disassemble any portion of the microscope or the system unless the procedure is described in this manual. If you have any problems with the system, contact your nearest Nikon representative.

3. Read the instruction manuals carefully

For your safety, carefully read this manual as well as the manual provided with the other equipment you are using with this system. Make certain to heed the warnings and cautions at the beginning of each manual.

- **Cautions regarding the power supply:**
  ⇒ Read the manual for the microscope.
- **Cautions regarding lamp heat:**
  ⇒ Read the manuals for the microscope and the light source (super high-pressure mercury lamp power supply or high-intensity light source).
- **Cautions regarding ultraviolet light produced by the lamp:**
  ⇒ Read the manual for the light source (super high-pressure mercury lamp power supply or high-intensity light source).
- **Cautions regarding lamp bursting and the gas sealed inside the lamp:**
  ⇒ Read the manual for the light source (super high-pressure mercury lamp power supply or high-intensity light source).
- **Cautions regarding the lamp specifications:**
  ⇒ Read the manual for the microscope and the light source (super high-pressure mercury lamp power supply or high-intensity light source).

4. Mercury lamps and xenon lamps

The mercury (or xenon) lamp used with this system requires special handling. To use this system safely and correctly, carefully read the warnings below and beware of the dangers. Also carefully read the manual for the super high-pressure mercury lamp power supply (or high-intensity light source) and the manual (if provided) by the manufacturer of the lamp and follow their instructions.

Hazards of Mercury Lamps and Xenon Lamps

1) Mercury (and Xenon) lamps, when turned on, radiate ultraviolet light that is harmful to the eyes and skin. Direct viewing of the light may result in blindness.

2) Gas is sealed under very high pressure inside the lamps. The pressure increases when the lamp is on. If the lamp is scratched, dirty, subjected to high external pressure or physical impact, or used beyond its operational life, the sealed gas may escape or the lamp may burst. This can result in someone inhaling the gas, injuring themselves on the glass, or other accidents.

3) When the lamp is on, the lamp and its surroundings become extremely hot. Touching the lamp with bare hands could result in burns. Flammable materials placed near the lamp could ignite.

⚠ WARNING

4) Using other than the specified type of lamp could result in an accident, such as burst.

Because safety is a top priority in the design of Nikon products, the hazards described above should not pose any danger as long as you heed all of the warnings and cautions in the manuals and use the system only for its intended purpose.

However, if you fail to heed all of the warnings and cautions in the manuals, if you strike the system, or if you attempt to disassemble the system, the accidents may occur. Be sure to heed all of the warnings and cautions.

5. Always turn the lamp off when changing filter blocks.

When changing filter blocks, always be sure to turn off the lamp connected to the Epi-fl attachment. If the lamp is left on, you might be exposed to ultraviolet light.

6. Leave the D/UV slider in the optical path.

Always leave the D/UV slider, one of the D-ES sliders for reflected illumination, in the optical path when conducting epi-bright-field, epi-dark-field, or epi-DIC microscopy with the mercury lamphouse. Your eyes may be damaged by harmful UV rays if the slider is moved out of the optical path.

⚠ CAUTION

1. Turn off the power when assembling the equipment, connecting or disconnecting cables, or replacing the lamp.

To prevent electric shock and damage to the equipment, always turn off the power switch on the microscope and unplug the power cord before assembling the system, connecting or disconnecting cables, or replacing the lamp.

2. Do not spill liquid on the equipment.

Wetting the microscope or this system may cause a short circuit and the equipment could be damaged or could become extremely hot. If you accidentally spill liquid on the equipment, immediately turn the power switch off and unplug the power cord. Then use a dry cloth to wipe away the moisture. If any liquid gets inside of the equipment, do not attempt to use it; instead, contact your nearest Nikon representative.

3. Caution concerning assembly

Be careful not to pinch your hands or fingers when assembling the equipment.

4. When installed on ECLIPSE ME600P/ME600D microscopes

When this system is installed on ECLIPSE ME600P/ME600D microscope, do not use the excitation light shorter than 400nm. If used, a very small amount of scattered excitation light may enter your eyes since the metallurgical objectives are not designed for UV excitation and thus, they cannot completely block the excitation light shorter than 400nm.

5. Disposing of the microscope

Dispose of the microscope in accordance with the standard procedure used in your laboratory.

Notes on Handling the System

(1) Handle the system gently

This system is a precision optical instrument. Handle the system gently, avoiding any physical shocks.

(2) Handling of filters

- Interference filters (especially excitation filters, which are exposed to strong light) deteriorate with time. Replace them according to the number of hours they have been in use.
- Filter characteristics may change if the filter is exposed to high humidity. To prevent changes in or deterioration of filter characteristics, avoid using or storing the filters under conditions of high humidity or high temperature, and avoid subjecting them to rapid temperature changes. When a filter is not in use, store them in a desiccator or a hermetically sealed container with a drying agent.
- The filters in the nine types of filter blocks listed below offer sharp, high-resolution waveform characteristics in comparison with normal filters. However, because they have sophisticated coatings, they must be handled with extra care. Abrasion caused by cleaning is a special concern. (Follow the procedure described in section "1. Filter and lens cleaning" of chapter "7. Care and Maintenance.")
  Single-band filter blocks: DAPI, FITC, TxRed, GFP
  Multi-band filter blocks: F-R, F-T, D-F, D-F-R, D-F-T

(3) Handling of D-FLD filter block

Two blades are provided at the front of the D-FLD filter block for dark-field illumination to prevent light leaks. To prevent malfunctions, do not apply force to these blades.

(4) Dirty lenses

Do not get dust, fingerprints, etc., on the lenses. Dirt on the lenses, mirrors, etc., will adversely affect the image. If any of the lenses get dirty, clean them as described in chapter "7. Care and Maintenance."

(5) Installation location

To avoid degraded performance and to prevent malfunctions, consider the following requirements when selecting an installation location:

- Install the system in a location with little vibration.
- Avoid installing the system in a location exposed to direct sunlight.
- Avoid installing the system in a dusty location.
- Avoid installing the system in a location subject to high temperatures (40°C or higher) or high humidity (60% or higher). (Such conditions could allow mold or condensation to form on the lenses and filters.)

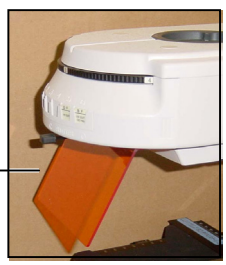
CONTENTS

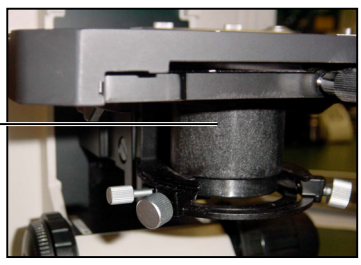
1

Names of Component Parts

See chapter "2. Names of Operational Parts" for the names of the parts that are used to operate the system.
If the system is not yet assembled, see chapter "5. Assembly" first.
For details on the assembly and handling of the microscope and light source, see their respective manuals.

(The illustration depicts a Nikon ECLIPSE E600 microscope with a mercury lamp as the light source.)

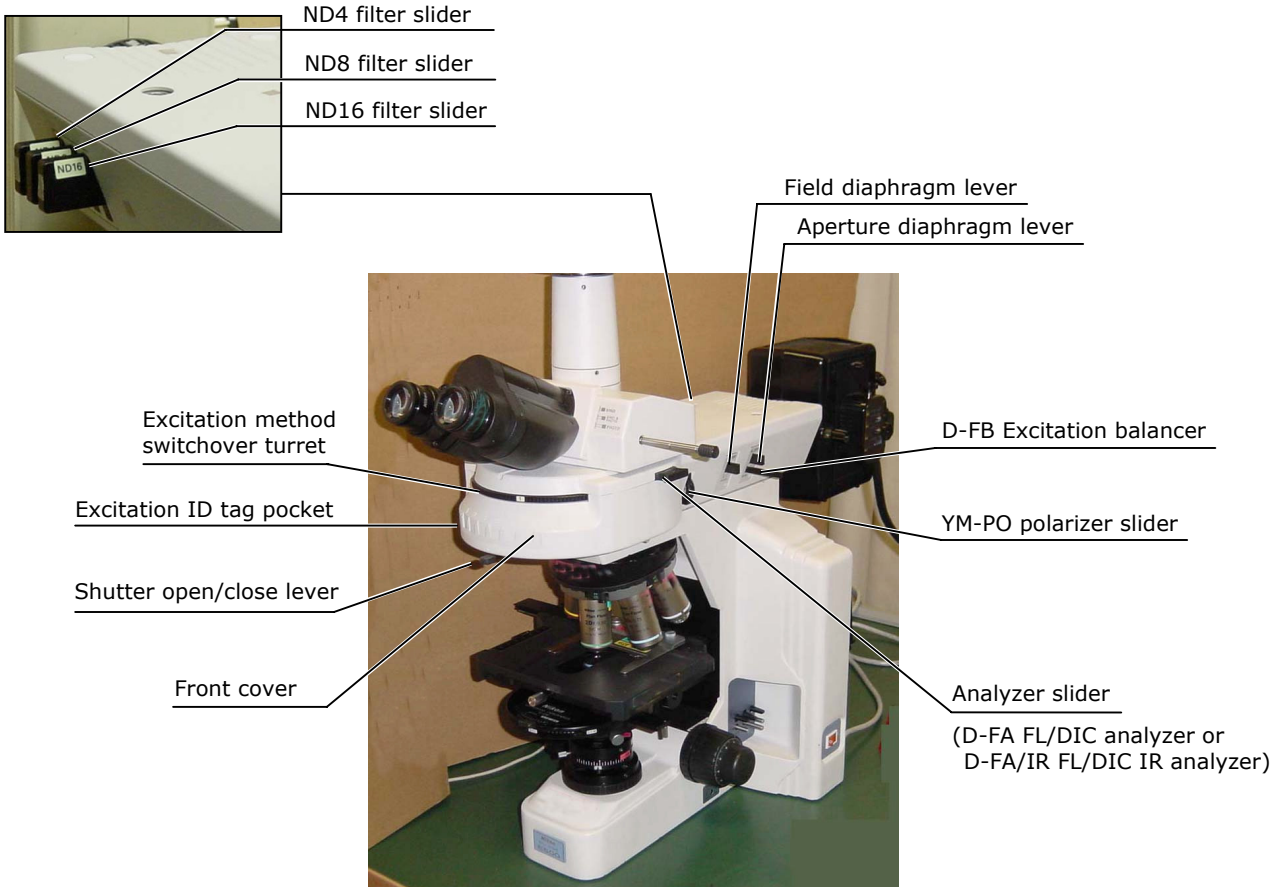




2

Names of Operational Parts

See chapter "1. Names of Component Parts" for the names of the components of the system.
If the system is not yet assembled, see chapter "5. Assembly," first.
For details on the assembly and handling of the microscope and light source, see their respective manuals.
(Not all components may be included in your set.)



3

Microscopy

The general procedure for microscopy is described below.
For details on each step, see the corresponding item in chapter "4. Operation of Each Part."
If the system is not yet assembled, see chapter "5. Assembly" first.
For details on the assembly, handling, and use of the microscope and light source, see their respective manuals.

⚠ WARNING

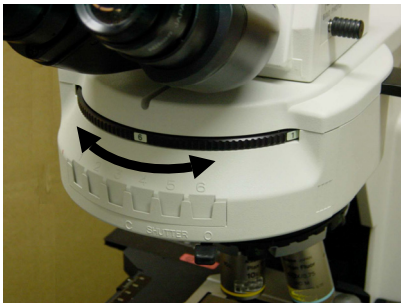
Before using the system, be sure to read the ⚠ WARNING and ⚠ CAUTION sections at the beginning of this manual, and also the section entitled, "Notes on Handling the System." Be certain to heed all of the warnings and cautions.
Also be sure to read the manuals for any other products that you are using with this system (the microscope, super high-pressure mercury lamp power supply, high-intensity light source, etc.), and heed all of the warnings and cautions in those manuals.

Before starting

1. Check the cumulative "lit on" time of the lamp. If the time has exceeded the average operational life for lamps of its kind, replace the lamp.
2. Use a non-fluorescent slide glass.
3. Use a non-fluorescent immersion oil.
4. To prevent fading of the specimen, always close the shutter whenever you are not actually looking through the binocular eyepiece.

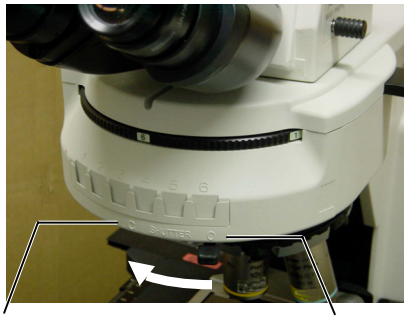
1 Epi-fl microscopy

1 Rotate the excitation method changeover turret to the right or left to bring the filter block of the desired excitation method into the optical path. (p.25)



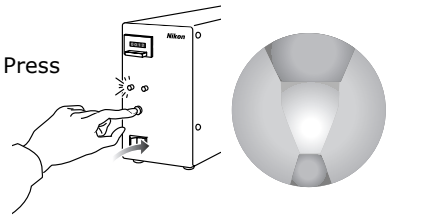
Select the excitation method.

2 Close the shutter so that the optical path is blocked. (p.24)



Close the shutter.

3 Turn the lamp on and center the lamp.
For details, see the manual for the light source (super high-pressure mercury lamp power supply or high-intensity light source).

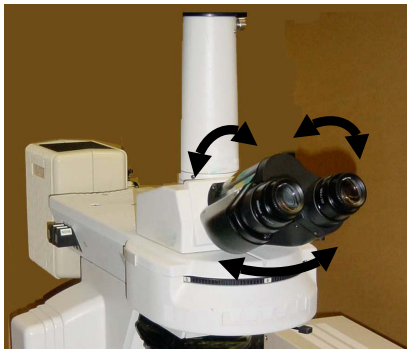


Center the lamp.

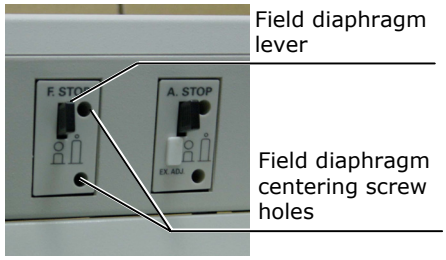
4 Place the specimen on the stage and focus on the specimen with the 10x objective.
For details, see the manual for the microscope.

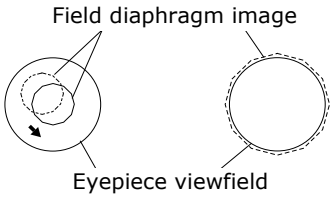
Select the 10x objective.

5 Adjust the diopter and the interpupillary distance.
For details, see the manual for the microscope.



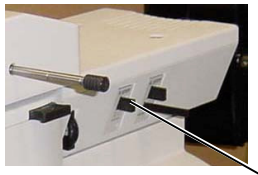
6 Center the field diaphragm. (p. 22)

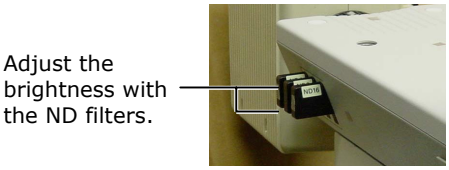




7 Switch to any desired objective and view the specimen.

- Readjust the focus.
- Use ND filters to adjust the brightness. (p. 24)
- Close the field diaphragm so that it is just outside the view field. (p. 22)
- When using an oil-immersion type objective, apply immersion oil between the specimen and the objective. (p. 29)





2 General bright-field microscopy

The microscope with the Epi-fl attachment can be used for general bright-field microscopy in the same way as an ordinary bright-field microscope.

1. Remove the shielding tube and install a condenser in its place.
2. Close the shutter on the Epi-fl attachment so that the episcopic illumination is blocked.
3. Rotate the excitation method changeover turret to the position where no filter block is set (empty position).
4. Turn on the microscope to turn on the diascopic illumination.
5. Focus on the specimen.
6. Perform the condenser focusing and centering, aperture diaphragm adjustments and field diaphragm adjustment of the microscope. Refer to the instruction manual of the microscope for details.

3 Epi-fl microscopy and DIC (differential interference contrast) microscopy

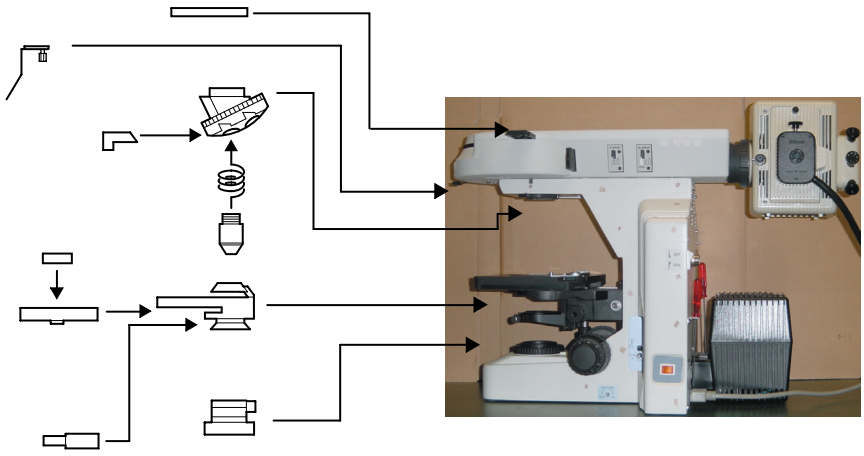
In addition to the Epi-fl microscopy, the differential interference contrast (abbreviated as "DIC" hereunder) microscopy can also be performed by combining certain accessories. The DIC microscopy can be used to search for certain objects instead of Epi-fl microscopy that discolors the specimens. Simultaneously using Epi-fl microscopy with DIC microscopy makes it possible to compensate for the shortcomings of each method.

Refer to the instructions for the C-CU universal system condenser for details on the DIC microscopy.

Accessories required for DIC microscopy:

- DIC sextuple nosepiece
- DIC objectives
- C-CU universal system condenser
  Top lens
  Condenser DIC prisms
  Condenser turret
  Lambda plate
- Objective DIC prisms
- Analyzer slider (D-FA FL/DIC analyzer)
- Polarizer

1 Install the accessories.



For details, refer to the instructions for the C-CU universal system condenser. The special analyzer slider (D-FA FL/DIC analyzer) should be used when combining Epi-fl and DIC microscopy. Remove the cap from the side of Epi-fl attachment and insert the analyzer slider in its place.

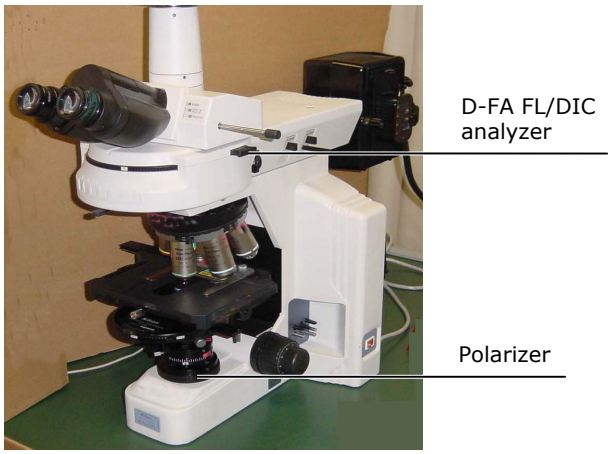
2 Close the shutter on the Epi-fl attachment to block the optical path of the episcopic illumination.

3 Rotate the excitation method changeover turret to the position where no filter block is set (empty position).

4 Turn on the microscope power to turn on the diascopic illumination.

3 Epi-fl microscopy and DIC (differential interference contrast) microscopy

5 Insert the polarizer and analyzer into the optical path.



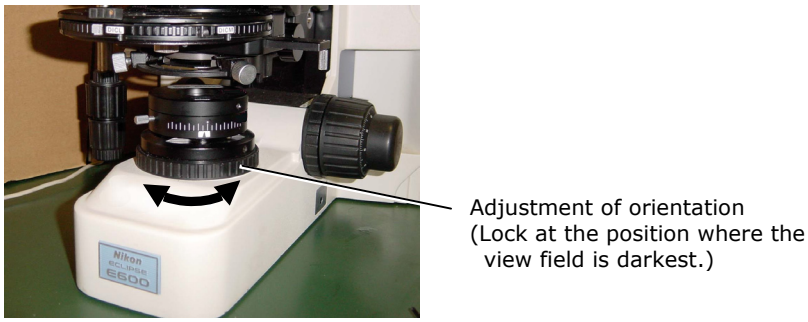
6 Turn the condenser turret so that the indication "A (empty position) " comes to the front.

7 Bring the DIC objective into the optical path.

8 Focus on the specimen.

9 Perform centering and focusing of the system condenser.

10 Adjust the orientation of the polarizer.



11 Turn the condenser turret so that the indication (L, M or H) matches the DIC code of the objective (DIC L, DIC M or DIC H).

12 Adjust the aperture diaphragm and field diaphragm.

When performing Epi-fl and DIC microscopy simultaneously, insert the filter block of the desired excitation method into the optical path and open the shutter of the Epi-fl attachment.
Adjust the ND filters of the Epi-fl attachment, ND filters and the brightness adjuster of the microscope so that the brightness levels of the fluorescent image and the DIC image are balanced.

4 Epi-fl microscopy and phase contrast microscopy

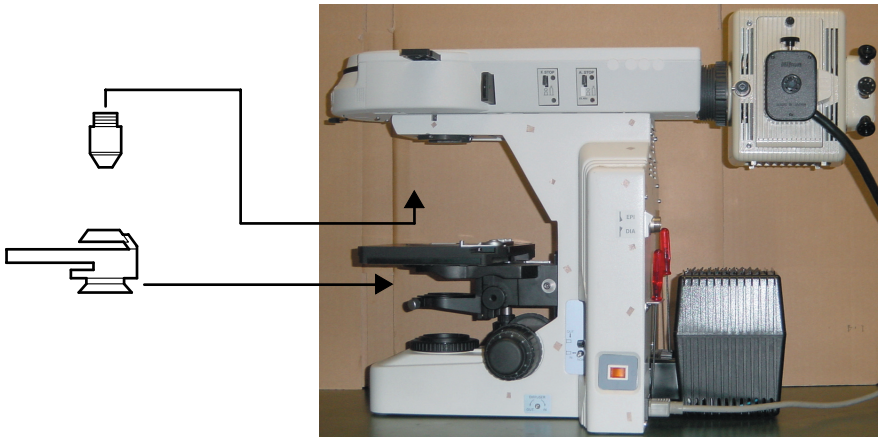
In addition to the Epi-fl microscopy, the phase contrast microscopy can also be performed by combining certain accessories. The phase contrast microscopy can be used to search for certain objects instead of Epi-fl microscopy that discolors the specimens. Simultaneously using Epi-fl microscopy with phase contrast microscopy makes it possible to compensate for the shortcomings of each method.

Refer to the instructions for the C-C Ph condenser (or C-CU universal system condenser) for details on the phase contrast microscopy.

Accessories required for phase contrast microscopy:

- Ph objective
- C-C Ph condenser or C-CU universal system condenser
- Centering telescope

1 Install the accessories.



For details, refer to the instructions for the C-C Ph condenser or C-CU universal system condenser.

2 Close the shutter on the Epi-fl attachment to block the optical path of the episcopic illumination.

3 Rotate the excitation method changeover turret to the position where no filter block is set (empty position).

4 Turn on the microscope power to turn on the diascopic illumination.

5 Bring the Ph objective into the optical path.

6 Turn the condenser turret so that the indication "A (empty position)" comes to the front.

7 Focus on the specimen.

8 Perform centering and focusing of the condenser.

9 Turn the condenser turret so that an indication that matches the Ph code of the objective (Ph1, Ph2 or Ph3) comes to the front.

4 Epi-fl microscopy and phase contrast microscopy

10 Center the Ph annular diaphragm of the condenser.
The centering procedures for the C-C Ph condenser and the C-CU universal system condenser are different. Refer to the instructions of the condenser being used.

11 Adjust the field diaphragm.

When performing Epi-fl and phase contrast microscopy simultaneously, insert the filter block of the desired excitation method into the optical path and open the shutter of the Epi-fl attachment.

Insert and remove the ND filters of the Epi-fl attachment and the microscope so that the brightness levels of the fluorescent image and the phase contrast image are balanced.

5 Epi-bright-field microscopy (Optional)

Attaching the D-FLE filter block for reflected illumination and the D-ES sliders for reflected illumination (both optional) to the attachment enables the episcopic bright-field microscopy (hereunder referred to as epi-bright-field microscopy) with the mercury lamphouse.

Accessories required for epi-bright-field microscopy:

- D-FLE filter block for reflected illumination
- D-ES sliders for reflected illumination

1. Turn off the mercury lamp.
2. Set the attachment shutter to "C."
3. Remove the three ND sliders from the left side of the attachment.
4. Attach three D-ES sliders for reflected illumination to the attachment. Check the nameplate on each slider. Attach the slider marked "ND4" to the rearmost window (lamphouse side), the one marked "ND32" to the center window, and the one marked "D/UV" to the frontmost window.
5. Detach the attachment's front cover and attach the D-FLE filter block for reflected illumination to the turret.
6. Upon completion of the procedure, observe the specimen in the same manner as with Epi-fl microscopy.

⚠ WARNING

Always leave the D/UV slider, one of the D-ES sliders for reflected illumination, in the optical path when conducting epi-bright-field, epi-dark-field, or epi-DIC microscopy with the mercury lamphouse. Your eyes may be damaged by harmful UV rays if the slider is moved out of the optical path.

- If the field of view becomes too bright due to the nature of the specimen, detach the ND4 slider, one of the D-ES sliders for reflected illumination, and attach the supplied ND8 or ND16 filter to the attachment, to adjust the brightness.

6 Epi-dark-field microscopy (Optional)

Attaching the D-FLD filter block for dark-field illumination and the D-ES sliders for reflected illumination (both optional) to the attachment enables the episcopic dark field microscopy (hereunder referred to as epi-dark-field microscopy) with the mercury lamphouse.

Accessories required for epi-dark-field microscopy:

- D-FLD filter block for dark-field illumination
- D-ES sliders for reflected illumination
- Revolving nosepiece for industrial microscopes
  L-NUA U5A nosepiece/L-NU5 U5 nosepiece/ESD/L-NBD5 BD5 nosepiece ESD

1 Turn off the mercury lamp.

2 Set the attachment shutter to "C."

3 Attach the revolving nosepiece for industrial microscopes.

4 Remove the three ND sliders from the left side of the attachment.

5 Attach three D-ES sliders for reflected illumination to the attachment. Check the nameplate on each slider. Attach the slider marked "ND4" to the rearmost window (lamphouse side), the one marked "ND32" to the center window, and the one marked "D/UV" to the frontmost window.

6 Detach the attachment's front cover and attach the D-FLD filter block for dark-field illumination to the turret.

7 Upon completion of the procedure, observe the specimen in the same manner as with Epi-fl microscopy.

- Switching from a dark to a bright field may cause momentary blindness. To avoid this, momentarily close your eyes or do not look into the eyepiece when switching from dark to bright fields.

⚠ WARNING

Always leave the D/UV slider, one of the D-ES sliders for reflected illumination, in the optical path when conducting epi-bright-field, epi-dark-field, or epi-DIC microscopy with the mercury lamphouse. Your eyes may be damaged by harmful UV rays if the slider is moved out of the optical path.

CAUTION

Two blades are provided at the front of the D-FLD filter block for dark-field illumination to prevent light leaks. To prevent malfunctions, do not apply force to these blades.

7 Epi-DIC microscopy (Optional)

Attaching the D-FLE filter block for reflected illumination and the D-ES sliders for reflected illumination (both optional) to the attachment and attaching accessories for epi-DIC microscopy (accessories for industrial microscopes) to the microscope enable epi-DIC microscopy with the mercury lamphouse.

Accessories required for epi-DIC microscopy:

- D-FLE filter block for reflected illumination
- D-ES sliders for reflected illumination
- D-FA FL/DIC analyzer
- Revolving nosepiece for industrial microscopes
  L-NUA U5A nosepiece/ L-NU5 U5 nosepiece/ESD/L-NBD5 BD5 nosepiece ESD
- L-DIC sliders for DIC
- YM-PO polarizer
- Objectives (for industrial microscopes)

1 Turn off the mercury lamp.

2 Set the attachment shutter to "C."

3 Attach the accessories. When attaching D-ES sliders for reflected illumination, make sure the slider marked "ND4" is attached to the rearmost window (lamphouse side), the one marked "ND32" is attached to the center window, and the one marked "D/UV" is attached to the frontmost window.

4 Detach the attachment's front cover and attach the D-FLE filter block for reflected illumination to the turret.

7 Epi-DIC microscopy (Optional)

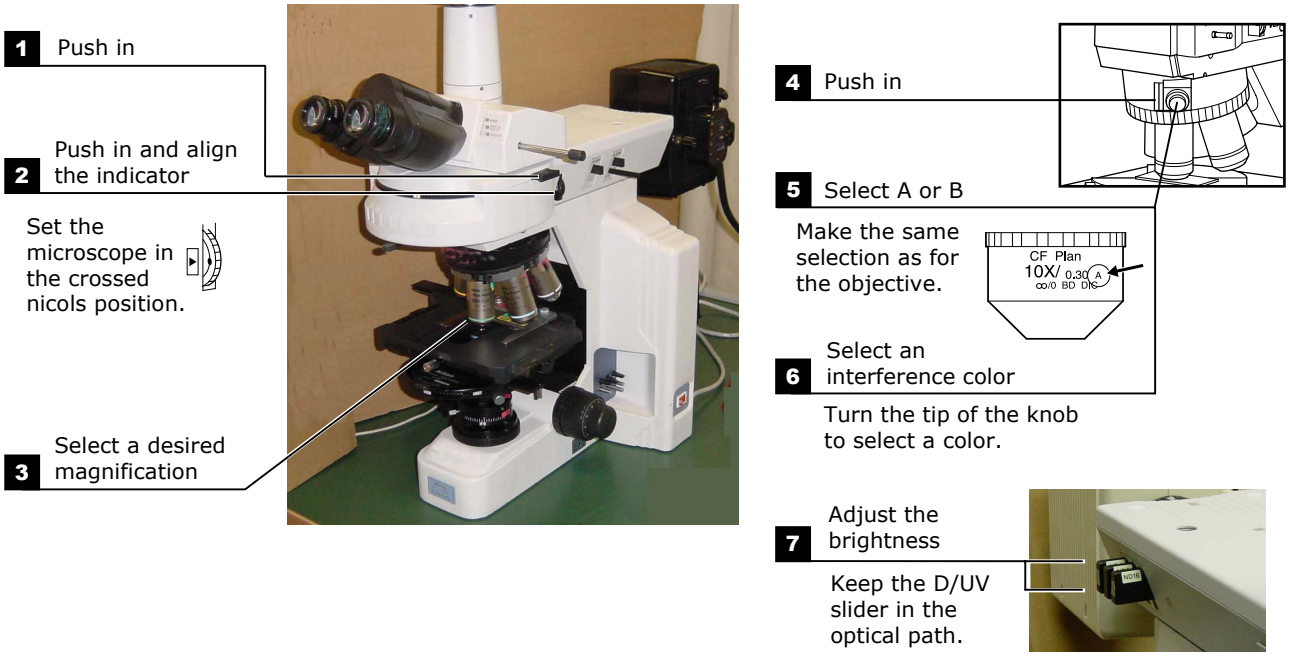
5 Upon completion of the procedure, observe the specimen in the same manner as with Epi-fl microscopy.

WARNING

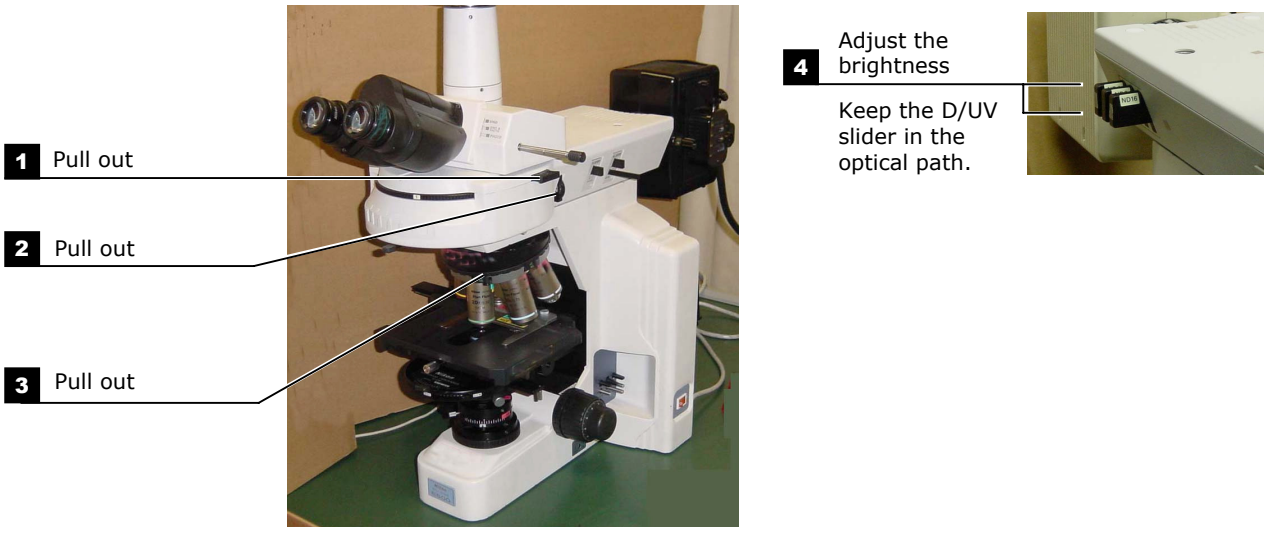
Always leave the D/UV slider, one of the D-ES sliders for reflected illumination, in the optical path when conducting epi-bright-field, epi-dark-field, or epi-DIC microscopy with the mercury lamphouse. Your eyes may be damaged by harmful UV rays if the slider is moved out of the optical path.

1) Bring the specimen into focus through bright-field microscopy.

2) Set up the microscope for DIC microscopy.



3) Restore the microscope to bright-field microscopy.



8 Excitation balancer (Optional)

Attaching the optional D-FB excitation balancer to the attachment enables adjustment of excitation light wavelength characteristics. This balancer is used in combination with the Dual-Band filter block.

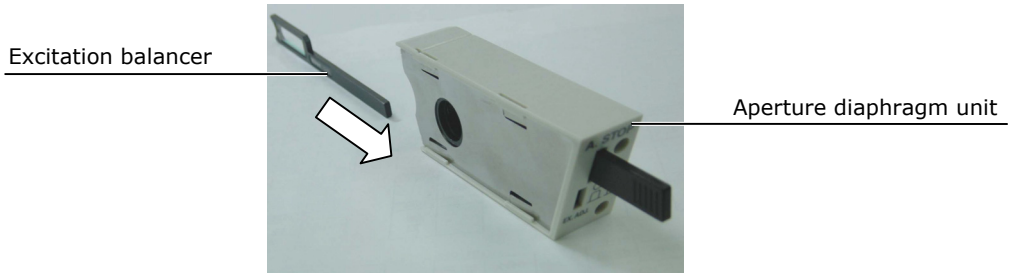
Accessories required:

- D-FB excitation balancer
- Dual-Band filter block

1 Turn off the mercury lamp.

2 Detach the aperture diaphragm unit from the attachment.

3 Insert the D-FB excitation balancer into the aperture diaphragm unit from the rear of the unit, as shown in the figure.



- Be careful not to directly touch the glass surface of the balancer with your finger.

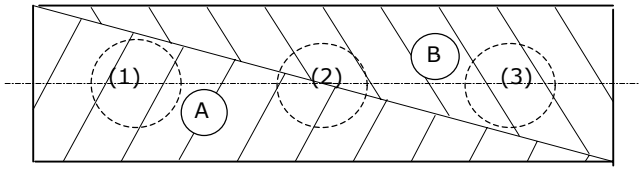
4 Reattach the aperture diaphragm unit to the attachment.

5 Detach the front cover and attach the Dual-Band filter block.

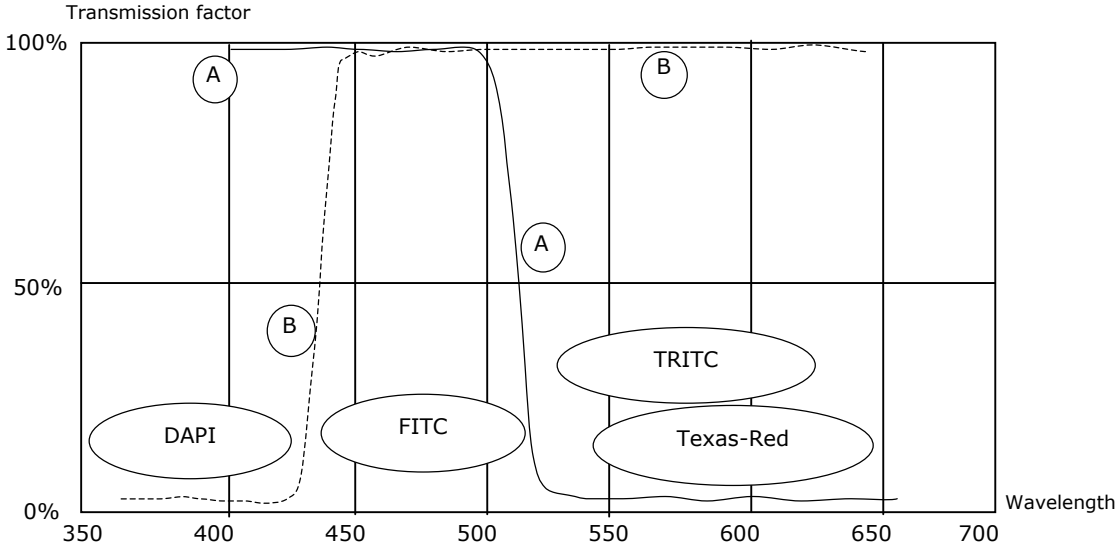
6 Move the excitation balancer horizontally to provide the desired excitation light.

8 Excitation balancer (Optional)

Details of the excitation balancer



Effective diameter of the aperture diaphragm surface



The balancer is set up so that 100% transmission is achieved for FITC, which generally results in dark fluorescence.

Optical path position	DAPI	FITC	TRITC/Texas-Red
(1)	100%	100%	0%
Between (1) & (2)	Variable (100% to 50%)	100%	Variable (0% to 50%)
(2)	50%	100%	50%
Between (2) & (3)	Variable (50% to 0%)	100%	Variable (50% to 100%)
(3)	0%	100%	100%

Objective information

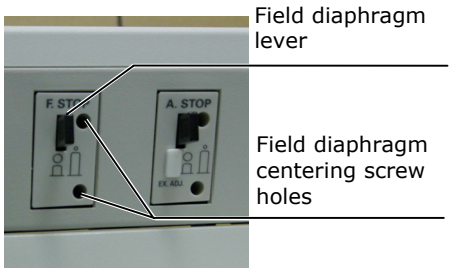
When using this attachment, be sure to select the objectives shown below. Using any other objectives with this attachment may result in unevenness in the field of view.

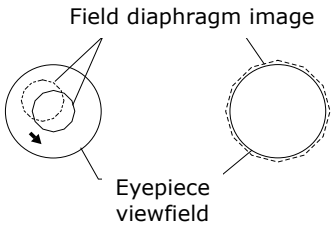
Plan Fluor	40x/0.75	40xH/1.3	100xH/1.3
S Fluor	40x/0.9	40xH/1.3	100xH/1.3
Plan Apo	40x/0.95	60xH/1.4	100xH/1.4

4

Operation of Each Part

1 Field diaphragm





The field diaphragm restricts illumination to the area being viewed. Pushing or pulling the field diaphragm lever changes the size of the field diaphragm. For normal observation, the size of the diaphragm should be such that it is just outside (or inside) the edge of the view field. If a broader area than necessary is illuminated, stray light from outside sources will enter the optical system, creating flaring, reducing the contrast of the optical image, and expanding the area of fading of the specimen.
The operation of the field diaphragm is especially important in photomicrography; generally, the best results are obtained by stopping down the field diaphragm to just slightly larger than the area that will be reproduced on the film, i.e., the size of the picture composition frame.
The field diaphragm of this Epi-fl attachment needs to be centered before usage.

Centering the field diaphragm

1. Perform steps 1 to 5 in "1. Epi-fl microscopy" of chapter "3. Microscopy".
2. Stop down the field diaphragm. (Pull out the field diaphragm lever.)
3. Move the center of the field diaphragm image to the center of the view field. (Turn the field diaphragm centering screws.)
4. Adjust the field diaphragm to roughly the size of the view field. (Push in the field diaphragm lever.)
5. Once again, move the center of the field diaphragm image to the center of the view field. (Turn the field diaphragm centering screws.)

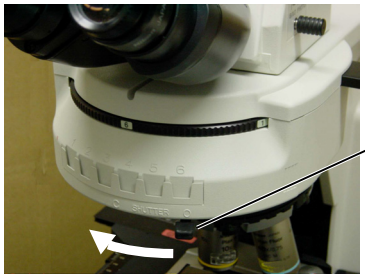
2 Aperture diaphragm

The aperture diaphragm adjusts the numerical aperture of the illumination optical system. During Epi-fl microscopy, the aperture diaphragm is used to adjust the brightness of the image and control the amount of stray light. Pushing or pulling the aperture diaphragm lever changes the size of the aperture diaphragm.
Stopping down the aperture diaphram reduces the amout of the stray light, but the image becomes darker. Conversely, opening the aperture diaphragm increases brightness, but also increases the amout of stray light. During normal Epi-fl microscopy, the aperture diaphragm should be left open and stopped down only to adjust the brightness of the image.
The field diaphragm of this Epi-fl attachment needs to be centered before usage.
(Some sets do not include the centering tool.)

Centering the aperture diaphragm

1. Set the attachment shutter to "C".
2. Remove one of the objectives from the revolving nosepiece and screw in the centering tool in its place.
3. Perform steps 1 to 5 in "1. Epi-fl microscopy" of chapter "3. Microscopy".
4. Move the centering tool into the optical path.
5. Stop down the aperture diaphragm. (Pull out the lever.)
6. Looking straight into the centering tool screen, move the center of the aperture diaphragm image to center of the screen. (Use a hexagonal screwdriver to turn the aperture diaphragm centering screws.)

3 Shutter

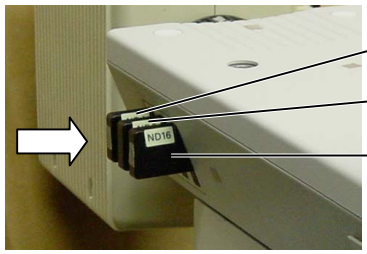


Close the shutter.
(Set the shutter to "C".)

The shutter blocks the light from the light source. To prevent fading of the specimen, always close the shutter when not observing a specimen. (Setting the shutter open/close lever to "C" closes the shutter to block the optical path.) Make closing the shutter a habit to protect important specimens. In addition, when temporarily halting Epi-fl microscopy in favor of microscopy using diascopic light, do not forget to close the shutter and block the episcopic fluorescent light.

4 ND filters

Pushing the ND slider filter sets the filter in the optical path.



An ND filter reduces illumination without changing the color balance of the light. When using strong fluorescent light, or when a specimen is badly faded, adjust brightness by pushing the ND filter sliders to the right to place the ND filters in the optical path. (If the fluorescent light is too strong, the contrast may worsen.)

The chart on the right shows how brightness is affected by different combinations of ND filters.

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

○ : In optical path - : Not in optical path

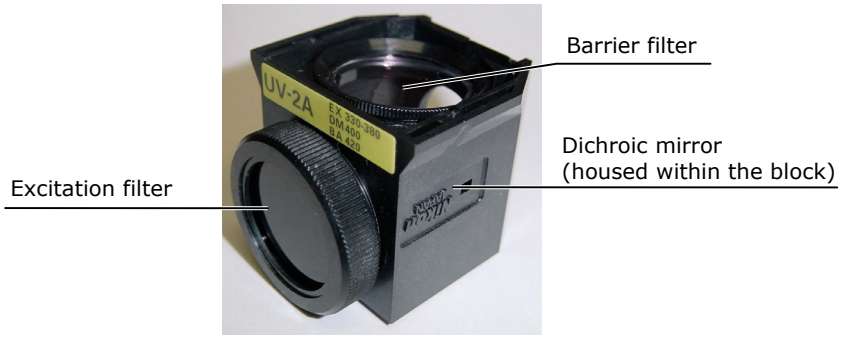
5 Ultraviolet light shielding plate

The ultraviolet light shielding plate prevents the ultraviolet light (irradiated from the objective to the specimen) from bouncing back and entering the observer's eye. To remove this plate, loosen the clamp screws and then pull the plate forward.

6 Excitation method changeover turret

This attachment can hold a maximum of six filter blocks.
Rotate the excitation method changeover turret to the right or left to bring the filter block of the desired excitation method into the optical path.

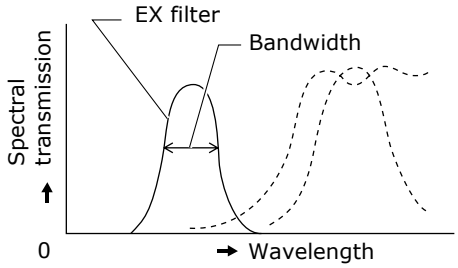
7 Filter blocks



Filter blocks consist of three types of optical components: an excitation filter (EX filter), a barrier filter (BA filter), and a dichroic mirror (DM). Referring to the description that follows, select a combination that best suits your purposes and the characteristics of the specimen and the fluorescent stain.

- You can select different combinations of excitation and barrier filters within the same excitation method.
- Excitation filters, barrier filters, and dichroic mirrors can all be purchased individually.
- Excitation filters will deteriorate over time since they are exposed to intense light. Replace them as necessary.

7.1 Selecting excitation filters (EX filters)



Excitation filters selectively pass the light within a certain range of wavelengths needed to cause the specimen to fluoresce (excitation light) and filter out all other light. The range of wavelengths that a given filter passes is called the "bandwidth" of the filter. An excitation filter's bandwidth determines the brightness of the fluorescent image, the occurrence of self-fluorescence (fluorescence originating from materials other than the fluorescent stain), and the extent of fading. A wide bandwidth allows a high level of excitation light to illuminate the specimen, producing a brighter image. However, a wide bandwidth also leads to a high level of self-fluorescence and severe fading. Conversely, while a narrow bandwidth yields a dark image, since little excitation light reaches the specimen, self-fluorescence and fading are minimal. When self-fluorescence is pronounced, use an excitation filter with a narrow bandwidth. (The resulting fluorescent image will be darker, however.) Excitation filters are likely to deteriorate the more they are used, since they are exposed to intense light. Replace excitation filters as necessary.

	Excitation filter bandwidth	
	Narrow	**Wide**
Brightness of fluorescent image	Dark	Bright
Occurrence of self-fluorescence	Minimal	Pronounced
Extent of fading	Minimal	Pronounced

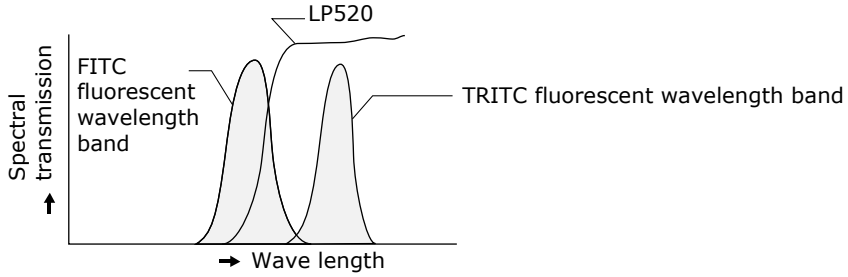
7.2 Selecting barrier filters (BA filters)

A barrier filter allows only fluorescent light generated by the specimen to pass and blocks all other excitation light reflected from the specimen. This filter makes it possible to observe the fluorescent image without unnecessary light (i.e., on a dark background).
There are two types of barrier filters: LP filters (long-pass filters), which block all wavelengths that are shorter than a certain boundary wavelength and allow to pass all wavelengths longer than the boundary wavelength, and BP filters (band-pass filters), which allow only light in a certain range of wavelengths to pass. Use whichever type best suits your purposes.

LP filters (long-pass filters)

LP filters block all wavelengths that are shorter than a certain boundary wavelength and allow to pass all wavelengths longer than the boundary wavelength. The boundary wavelength is called the cut-on wavelength.

1) If your specimen is stained with a fluorescent color having extremely close wavelength bands for its fluorescent light and excitation light, and if you want to observe the fluorescent light most effectively, it is a general rule to chose the barrier filter having the shortest cut-on wavelength, among the barrier filters that suffice the needs.
   Though it is said that the barrier filter with the longer "cut-on" wavelength can generate darker background for the fluorescent image because the separation of the excitation light and the fluorescence light is distinct, it is becoming more common to use barrier filters with shorter "cut-on" wavelengths owing to the improvement of filter performances.
2) To view the fluorescent images of all of the colors in a specimen stained in multiple colors, use an LP filter.

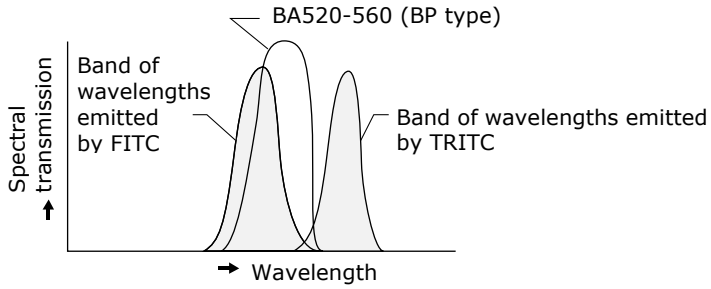


Both the FITC fluorescent image and the TRITC fluorescent image are visible.

However, when using a normal dichroic mirror, an excitation filter, and an LP filter-type barrier filter in combination, the stain that fluoresces at the longer wavelength (for example, TRITC when the specimen is stained with FITC and TRITC) may not be excited sufficiently, with the result that the fluorescent image created by that stain may appear extremely dark. In this type of situation, the use of a multi-band filter is recommended.

BP filters (band-pass filters)

A BP filter allows only light in a certain range of wavelengths to pass.
This type of filter is used to view the fluorescent image created by a specific stain when a given specimen has multiple stains. (For example, in a specimen with two stains, FITC and TRITC, select BA520-560 to observe the fluorescent image created by FITC.)

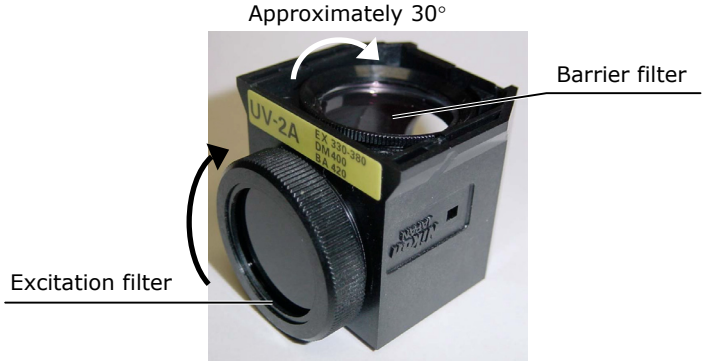


Only the fluorescent image created by FITC is visible

However, you may not be able to distinguish the self-fluorescence from the other fluorescence in the image created by the BP filter since the image will be of only one color (green, in the above example).
It is best to use LP filter if you wish to distinguish the self-fluorescence from the subtle hue.

7.3 Excitation filter and barrier filter replacement

Excitation and barrier filters can be removed from the filter block and replaced with other filters. (The dichroic mirror cannot be removed from the filter block.)
The excitation filter screws into the filter block.
The barrier filter plugs into the filter block. Align the notches on the filter block with the protrusions on the barrier filter, fit the filter into place, and then rotate the filter about 30° in a clockwise direction to secure.



8 Oil-immersion type objectives

Objectives marked "oil" are oil-immersion type objectives. These objectives are used with immersion oil applied between the specimen and the tip of the objective. Always use non-fluorescent oil. (For example, Nikon Immersion Oil DF.) If other kinds of oil is used, fluorescent light from the oil may adversely affect the image.

Bubbles in the oil will adversely affect the viewing of the image. Be careful to prevent the formation of air bubbles. To check for air bubbles, remove the eyepiece, open the field diaphragm and the aperture diaphragm as far as possible, and look at the exit pupil of the objective within the eyepiece tube. (The exit pupil will appear as a bright circle.) If it is difficult to see if there are any bubbles, mount a centering telescope (sold separately) to the eyepiece tube using an adapter (sold separately) (refer to the manual supplied with the microscope) and rotate the eyepiece part of the telescope. If there are bubbles in the oil, remove them by one of the following methods:

- Turn the revolving nosepiece slightly, moving the objective in question back and forth one or two times.
- Add more oil.
- Remove the oil and replace it with new oil.

Use as little oil as possible (just enough to fill the space between the tip of the objective and the specimen). If too much oil is applied, the excess oil will flow onto the stage and around the condenser.

Any oil remaining on oil-immersion type objectives or on the tip of dry-type objectives has a discernible, negative effect on the image. After using oil, wipe all of it away and also make sure that there is no oil on the tips of the other objectives. Oil on the condenser lens should also be wiped away carefully after use.

Use petroleum benzine to wipe away immersion oil. Then, after the oil has been removed, wipe the lens surfaces, etc., with absolute alcohol (ethyl alcohol or methyl alcohol).

If you cannot obtain petroleum benzine, use methyl alcohol. However, because methyl alcohol does not clean as well as petroleum benzine, it will be necessary to wipe the surfaces repeatedly. (Usually, three or four times is sufficient to clean the lenses.)

Use petroleum benzine only to remove immersion oil from the tips of objectives; do not use it for cleaning the fluorescent filters, etc. Use absolute alcohol and petroleum benzine according to the instructions provided by their manufacturers.

9 Fluorescent photomicrography

For the basic procedures and key points of photomicrography, see the manual provided with the photomicrographic equipment. Please note, however, that when using a fluorescent specimen, the fluorescence may fade during exposure. Take the following countermeasures in order to avoid this problem.

(1) Use high-sensitivity film

Use "Tri-X (ISO400)" for monochrome photomicrograph. For color shots, use daylight-type highsensitivity film, such as "Kodak Ektachrome 400 (ISO400)" or "Fujichrome 400 (ISO400)."

(2) Creating a bright optical system combination

Even if the total magnification on the film is the same, the exposure time can vary greatly for different combinations of objectives and projection lenses. Rather than increasing the magnification of the projection lens, increasing the magnification of the objective is recommended. (This is because, in general, the numerical aperture of the objective increases as the magnification increases, and the higher the numerical aperture, the brighter the image.)

(3) Adjusting the excitation light

If the excitation light is too bright, the specimen will fade quickly, making it impossible to get a good shot of the fluorescent image. Therefore, adjust the brightness by inserting ND filters into the optical path.

(4) Specimen

If a faded portion of a specimen is shot, the exposure time increases, the color reproduction is poor, and the resulting photomicrograph will not be satisfactory. Move the specimen and shoot a more vivid portion of the specimen that has not been previously exposed to the excitation light. We recommend using the differential interference contrast method or the phase contrast method to select the portion to be shot, and then switching to the Epi-fl method for shooting the actual photomicrograph.

10 TV monitoring

When monitoring a specimen using a high-sensitivity TV camera, it is sometimes best to insert an infrared (IR) cut filter in front of the camera receptor. Experiment, and use the IR cut filter when needed.

5

Assembly

The procedures for assembling the system are described below.
For details on the assembly, handling, and usage of the microscope, super high-pressure mercury lamp, etc., see their respective manuals.

⚠ WARNING

Before using the system, be sure to read the ⚠WARNING and ⚠CAUTION sections at the beginning of this manual, and also the section entitled, "Notes on Handling the System." Be certain to heed all of the warnings and cautions.
Also be sure to read the manuals for any other products that you are using with this system (the microscope, super high-pressure mercury lamp power supply, high-intensity light source, etc.), and heed all of the warnings and cautions in those manuals. In particular, mishandling a mercury (or xenon) lamp used with this system can lead to a serious accident. Exercise caution.
In order to prevent electric shock, fire, accidents involving ultraviolet light, burns, and other injuries, make sure that the power switches for the microscope and super high-pressure mercury lamp (or high-intensity light source) power supply are turned off before beginning assembly work.

Required tools

- Hexagonal screwdriver: 1 (Provided with the microscope) 1.3mm

See the illustrations while assembling the system.
Scratches or fingerprints on the lenses and filters will adversely affect the image. Handle these components carefully in order to keep them free from scratches and fingerprints.

(1) Installation location

In order to avoid degraded performance and to prevent malfunctions, take the following requirements into consideration when selecting a location to install the system:

- Install the system in a location with little vibration.
- Install the system in a location with little vibration.
- Avoid installing the system in a dusty location.
- Avoid installing the system in a location subject to high temperatures (40°C or higher) or high humidity (60% or higher).

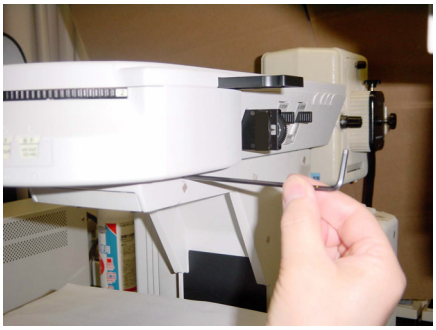
(2) Microscope assembly

Follow the instructions in the microscope manual.

(3) Installing the Epi-fl attachment

1 Place the Epi-fl attachment on the arm of the microscope.

2 Secure by tightening the clamp screw on the right side of the arm.



3 Fasten the Epi-fl attachment to the microscope by tightening the two bolts (provided) with a hexagonal wrench.

4 Cover the bolt-holes with the supplied stickers.

(4) Installing the eyepiece tube

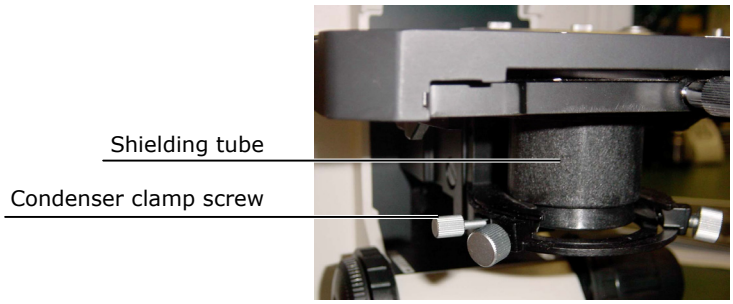
Install the eyepiece tube on the upper mount of the Epi-fl attachment and secure by tightening the clamp screw on the front of the Epi-fl attachment with a hexagonal screwdriver.

(5) Mounting the shielding tube

Mount the shielding tube on the microscope's substage (where the condenser is normally mounted) and secure it in place with the condenser clamp screw.

CAUTION

Failure to mount the shielding tube could allow ultraviolet light that passes through the specimen to leak out. (Ultraviolet light is not visible to the human eye.) Be certain to mount the shielding tube.



(6) Mounting the ultraviolet light shielding plate

Mount the ultraviolet light shielding plate under the Epi-fl attachment and secure it in place with the locking screws.

⚠ CAUTION

Failure to mount the ultraviolet light shielding plates could allow ultraviolet light to reflect off of the specimen and back into the observer's eye. (Ultraviolet light is not visible to the human eye.) Be certain to mount the ultraviolet light shielding plate.

(7) Installing the light source

Install the light source to the Epi-fl attachment. (For details, see the manual provided for either the super high pressure mercury lamp power supply or the high-intensity light source.)

⚠ WARNING

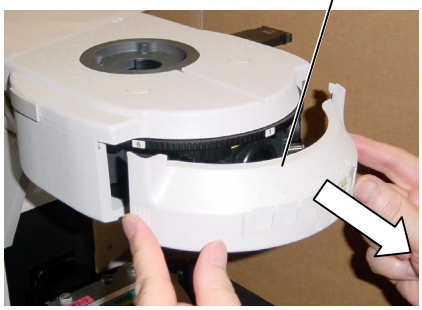
A mercury lamp (or xenon lamp) requires careful handling. Be sure to heed all of the warnings and cautions in the manual for the light source.

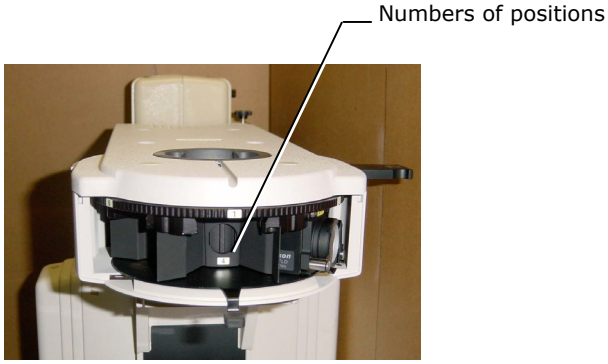
(8) Installing the filter blocks

Do not touch the surfaces of the filters.

Six filter blocks can be attached Epi-fl atttachement. When performing diascopic microscopy in addition to Epi-fl microscopy, attach the dia-filter block "DIA ILL(A)" to at least one position of the mounts.

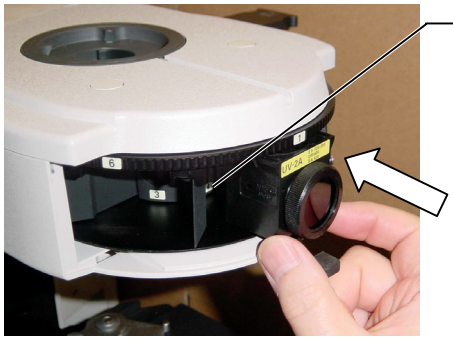
1 Pull the front cover toward you to detach.





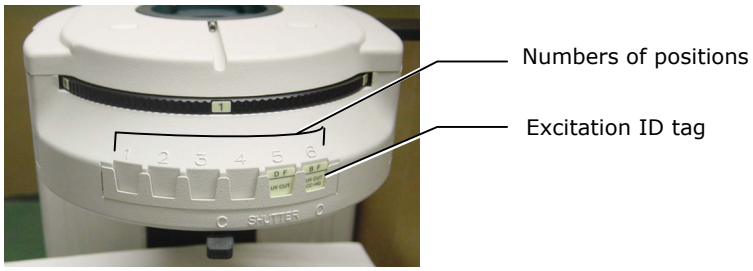
View without the front cover

2 Insert the filter block into the turret groove.



3 Reattach the front cover.

4 Insert the excitation ID tags for each filter block installed into the excitation ID tag pocket indicating the corresponding number of positions.



This completes the assembly procedure.

6

Troubleshooting Tables

Improper use of the microscope may adversely affect performance, even if the microscope is not damaged.

Problem	Cause	Countermeasure
Lamp does not light.	The power is not supplied.	Connect the power cord.
	The lamp connector is not connected to the power supply.	Connect the lamp connector to the power supply.
	The lamp has reached the end of its operational life.	Replace the lamp.
	The fuse is blown.	If the fuse can be replaced, replace it. Otherwise, contact your nearest Nikon representative.
Even though the lamp is on, the image is not visible.	The shutter is closed.	Open the shutter.
	The filter block selection is incorrect.	Select the correct filter block.
Even though the lamp is on, the image is extremely dark.	The light source is not centered properly.	Center the lamp. Especially when using a 100x objective, recenter the lamp while observing the fluorescent image.
	ND filters are in the optical path.	If necessary, remove the ND filters from the optical path.
	A halogen lamp is being used with a dark specimen.	Change the light source to a mercury lamp.
	A designated objective is not being used with UV or V excitation.	Use the designated objective.
	The room is too bright.	Darken the room.

Problem	Cause	Countermeasure
Contrast is poor.	The objective or cover glass is dirty.	Clean the objective or cover glass.
	The immersion oil is fluorescing.	Use non-fluorescent immersion oil (Nikon Immersion Oil DF).
	The slide glass is fluorescing.	Use a non-fluorescent slide glass.
Viewing is poor.	There is no cover glass in place.	Use a cover glass. (However, no cover glass is required when using an NCG objective.)
	No immersion oil has been applied to the tip of an immersion-oil type objective.	Apply Nikon Immersion Oil DF.
	The specified immersion oil is not being used.	Apply Nikon Immersion Oil DF.
	The filter block being used is not suited for the specimen.	Use a filter block suited for the specimen.
The view field is vignetting.	The field diaphragm has been stopped down too far.	Open the field diaphragm so that it is just outside of the view field.
	The ND filter slider has stopped at an intermediate position.	Pull out or push in the slider all of the way.
	The installed position of a filter block has deviated from the prescribed position.	Push in till the limit.

7

Care and Maintenance

1 Filter and lens cleaning

Do not get dust, fingerprints, etc., on the lenses or filters. Dirt on the lenses, filters, etc., will adversely affect the image. If any of the lenses or filters get dirty, clean them as described below.

- Use an air blower to blow away dust. If that does not suffice, brush away the dust with a soft brush, or wipe it away gently with gauze.
- Only if there are fingerprints or grease on a lens or filter, dampen a piece of soft, clean cotton cloth, lens tissue, or gauze with absolute alcohol (ethyl alcohol or methyl alcohol) and wipe away the dirt. However, do not use the same area of the cloth, etc., to wipe more than once.
- Use petroleum benzine to clean off immersion oil. Wiping with absolute alcohol (ethyl alcohol or methyl alcohol) after the oil has been removed finishes the clean up process.
  If you cannot obtain petroleum benzine, use methyl alcohol. However, because methyl alcohol does not clean as well as petroleum benzine, it will be necessary to wipe the surfaces repeatedly. (Usually, three or four times is sufficient to clean lenses or filters.)
- Use petroleum benzine only to remove immersion oil from objectives; do not use petroleum benzine for cleaning the entrance lens on the eyepiece tube, filters, etc.
- Absolute alcohol and petroleum benzine are both highly flammable. Be careful when handling them, when around open flames, when turning the power switch on or off, etc.
- Use absolute alcohol and petroleum benzine according to the instructions provided by their manufacturers.

2 Cleaning of painted components

Do not use organic solvents (such as alcohol, ether, or paint thinner) on painted components, plastic components, or printed components. Doing so could result in discoloration or in the peeling of printed characters. For persistent dirt, dampen a piece of gauze with diluted detergent and wipe lightly.

3 Storage

Store the system under conditions of low humidity where mold is not likely to form.
Store the objectives, eyepieces, filter blocks, etc., in a desiccator or similar container with a drying agent.
Put the vinyl cover over the system to protect it from dust.
Before putting on the vinyl cover, turn off the power switches for the microscope and the Epi-fl attachment light source, and wait until the lamphouse is cool.

4 Regular inspections

Regular inspections of this system are recommended in order to maintain peak performance.
Contact your nearest Nikon representative for details about regular inspections.