



Nikon

Upright Motorized Microscope

ECLIPSE

***Ci*-E**

Instruction Manual

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for users of the Nikon ECLIPSE Ci-E microscope. To ensure correct usage, read this manual carefully before operating this product.

- No part of this manual may be reproduced or transmitted in any form without prior written permission from Nikon.
- The contents of this manual are subject to change without notice.
- The equipment described in this manual may differ from the actual product in its appearance.
- 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 equipment described in this manual may not be included in the set you have purchased.
- If you intend to use any other equipment with this product, read the manual for that equipment too.
- If this equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Contents of the Manual

The manual for ECLIPSE Ci-E consists of the following contents. There is also a "Bright-field Microscopy Quick Guide", provided as a separate document.

◆ This manual: Instructions



- Safety Precautions
- Microscopy Procedures
 - Bright-field Microscopy
 - Phase Contrast Microscopy
 - Simple Polarizing Microscopy
 - Sensitive Polarization Microscopy
 - Epi-fluorescence Microscopy
- Individual Operations
- Assembly
- Troubleshooting
- Maintenance and Storage
- Specifications and Safety Standards

◆ Bright-field Microscopy Quick Guide

Symbols Used in This Manual

The following symbols are used in this manual.

◆ Symbols for Safety

- | | |
|----------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
|  WARNING | Highlights important information that should be noted for safety. Read "Safety Precautions" for details. |
|  CAUTION | |

◆ Other Symbols



- | | |
|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
|  | Indicates information you should note or comply with to prevent defects or malfunction of this product. |
|  | Indicates information you should be aware of in using this product, as well as other useful information. |

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Safety Precautions

To ensure correct and safe operation, read this manual before using this product.

WARNING and CAUTION Symbols


Although this product is designed and manufactured to be completely safe during use, incorrect usage or failure to follow the safety instructions provided may cause personal injury or property damage. To ensure correct usage, read this manual carefully before using this product. Do not discard this manual and keep it handy for easy reference.

Safety instructions in this manual are marked with the following symbols to indicate their importance. For your safety, always follow the instructions marked with these symbols.

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

Meaning of Symbols Used on the Product

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

	<p>Biohazard</p> <p>This symbol is affixed to the front of the stand of this product, to call your attention to the following:</p> <ul style="list-style-type: none"> • WARNING: Using this product may constitute a biohazard risk if a sample comes into contact with this product. • To avoid biohazard contamination, do not touch the contaminated portion with bare hands. • Decontaminate the contaminated part in accordance with the standard procedure specified for your laboratory.
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WARNING

1 Do not disassemble.

Disassembling this product may result in electric shock or malfunction. Malfunction and damage due to disassembling or modification are unwarranted.

Do not disassemble parts other than those described in this manual. If you experience problems with this product, contact your nearest Nikon representative.

2 Read the instruction manuals carefully.

To ensure safety, thoroughly read this manual and the manuals for other equipment to be used with this product. Particularly, all warnings and cautions given at the beginning of each manual must be observed.

Safety is a top design priority for Nikon products. Safety is ensured as long as the user observes all of the warnings and cautions given in the manuals, and uses the system only for its intended purpose. However, failure to heed the warnings and cautions given in the manuals, subjecting the system to shock or impact, or attempting to disassemble the system may result in unexpected accidents and injury.

Product with CI-FL or D-FL Epi-fluorescence attachment:

The light source used for Epi-fluorescence microscopy (HG Precentered Fiber Illuminator) requires special care during handling because of its characteristics. Be sure to refer to the manual for the light source being used.

3 Notes on the power cord

Be sure to use the specified power cord. Use of other power cords may result in malfunction or fire. This product is classified as having Class I protection against electric shock. Make sure this product is connected to an appropriate protective earth terminal.

Refer to Chapter 6, "2 Performance Properties" for the specified power cords.

- To prevent electric shock, always turn off the power switch (press to the "O" position) for the microscope before connecting or disconnecting the power cord.

4 Hazards of mercury lamps (when using a CI-FL or D-FL Epi-fluorescence attachment)

The light source used with the epi-fluorescence attachment (HG precentered fiber illuminator) requires special care during handling because of its characteristics. For safe and correct use of this system, carefully read the warnings below. Keep in mind all potential hazards. Additionally, carefully read the manual for the illuminator and the manual from the lamp manufacturer (if provided), then follow the instructions given therein. Failure to heed the warnings and cautions given in the manuals, subjecting the system to shock or impact, or attempting to disassemble the system may result in unexpected accidents and injury.

• **Ultraviolet light**

When lit, mercury lamps radiate ultraviolet light that can damage the eyes and skin. Direct viewing of the light may result in blindness. When changing filter cubes, always turn off the light source of the Epi-fluorescence attachment. Leaving the lamp turned on during filter cube replacement may result in ultraviolet exposure.

• **High-pressure gas**

The lamps contain sealed gas under very high pressure. And the pressure increases when the lamp is on. If the lamp is scratched, fouled, subjected to high external pressure or physical impact, or used beyond its service life, the sealed gas may leak or the lamp may burst, resulting in gas inhalation, injury from glass, or other accidents.

• **Heat**

When the lamp is lit, the lamp and surroundings will become extremely hot. Do not touch the lamp with bare hands or place flammable materials near the lamp. Failure to comply may result in burns or fire.

• **Designated lamp**

Be sure to use the designated lamp. Using other types of lamps may result in accidents, including bursting of the lamp.

5 Hazardous sample handling

This product is intended primarily for microscopic observations and image capture of cells and tissue set on glass slides.

Check to determine whether a sample is hazardous before handling. If sample is hazardous, handle it according to the standard procedure specified for your laboratory. If the sample is potentially infectious, wear rubber gloves and avoid directly touching samples. If such a sample is spilled onto this product, the portion must be decontaminated in a safety manner. Consult your safety supervisor or safety standard of your facility.



<p>1 Power shutdown</p> <p>To prevent electric shock and/or malfunction, always turn off the power switch(es) for this product and the peripheral devices (press to the “O” position) and unplug the power cord from the wall outlet before assembling this product, connecting or disconnecting cables, or cleaning this microscope and the objective.</p> <p>2 Movement of motorized device</p> <p>With this product, the motorized nosepiece can be electronically rotated and the condenser top lens can be electronically swung out. The various motorized components can also be operated by the remote controller or from a PC. Various motorized components can also be operated from remote controllers and PCs.</p> <p>To avoid unexpected injuries, note the following in operating motorized devices.</p> <ul style="list-style-type: none"> ● Before operation, check the state of the entire microscope system to ensure safety when operating the motorized devices. ● Keep your hands and fingers away from the nosepiece, objectives, stage, and components and specimen on the stage to avoid injuries during operation. <p>3 Prevent contact with water.</p> <p>Never allow water to come into contact with this product, and avoid using this product in circumstances where it may be splashed with water. Splashing water onto this product may cause a short circuit, resulting in malfunction or abnormal heating. If water is splashed onto this product, immediately turn off the power switches for this product and the peripheral devices (press to the “O” position) and remove the power cord from the receptacle. Then wipe off moisture with a piece of dry cloth or something similar. If water enters, stop the use of the product and contact your nearest Nikon representative.</p> <p>4 Do not place any object on top of the product.</p> <p>Do not place any object on top of this product.</p>	<p>5 Cautions on assembling, installing, and carrying the product</p> <ul style="list-style-type: none"> ● Take care to avoid pinching your fingers or hands during product assembly and installation. ● Scratches or fouling optical components (such as lens and filters) with fingerprints, etc. will degrade microscope images. Be careful to avoid scratches or direct contact with the lens and filters when assembling. ● The main body weighs approximately 10 kg. When moving it, hold the main body by the grip on the back and the recessed portion at the front bottom side of this product. ● Remove all attachments (if mounted) from the microscope before moving the microscope. ● Do not place this product in a locker or cabinet. <p>6 Cautions on use, transportation, and storage</p> <p>This product must be operated, transported, or stored in accordance with the following conditions. Installing this product in a hot, humid location may result in the formation of mold or condensation on lenses, impairing performance or causing malfunctions.</p> <ul style="list-style-type: none"> ● Operating conditions: temperature: 0 to +40°C, humidity: 60% RH max. (no condensation) ● Transporting/storage conditions: temperature: -20 to +60°C, humidity: 90% RH max. (no condensation) <p>7 Remove any covers from the product before switching on.</p> <p>Do not use this product while it is covered with a piece of cloth, etc., as this will prevent heat release and result in abnormal heat and create a fire hazard. Do not cover this product with a piece of cloth or similar while in use. The system temperature will rise, resulting in a malfunction.</p> <p>8 Cautions on sustained observations</p> <p>To relieve fatigue resulting from long observation sessions, limit continuous observations to one hour. Take at least 10 to 15 minutes breaks between observation sessions. Adjust the layout of other equipment used and the height of your chair.</p> <p>9 Cautions on the disposal of the product</p> <p>To avoid biohazard risks, dispose of this product as contaminated equipment in accordance with the standard procedure specified for your facility.</p>
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Notes on Handling the Product

1 Handle with care

This product is a precision optical instrument and requires gentle handling. Avoid subjecting it to sudden impacts and shocks. Even relatively minor impacts are capable of affecting the precision of the objective.

2 Electromagnetic Environment

Before using this product, Nikon recommends evaluating the electromagnetic environment of the installation site.

Do not use this product close to strong electromagnetic radiation sources (example: unshielded intentional RF sources). They may interfere with the proper operation of this product.

This product emits weak electromagnetic waves. So as to avoid degrading the performance of precision electronic devices, do not install this product near such devices. If TV or radio reception is affected, move the TV or radio further from this product.

3 Dirt on the lens

Scratches or fouling optical components (such as lens and filters) with fingerprints, etc. will degrade microscope images.

If these parts become dirty, clean them as described in Chapter 5, "1.1 Cleaning Lenses".

4 Installation location

This product is a precision instrument. Usage or storage of this product in an inappropriate environment may result in malfunction or a degradation in precision. Consider the following factors when selecting an installation location:

- Select a location free of vibration. Install this product on a level surface.
- Install this product at least 10 cm away from walls.
- Choose a location less exposed to hazards in the event of collisions, earthquakes, or other potential disasters. To keep this product from falling, use a strong rope or other means if necessary to secure it to the working desk or other heavy, stable item.
- Select a layout that allows easy removal of the power cord from the product's AC inlet in the event of an emergency.
- Do not use a desk mat or similar.
- Avoid locations exposed to direct sunlight, locations immediately under room lights, and other bright locations.
- Light from room lights just above this product may enter the objective as extraneous light. If possible, switch off the room lights directly above this product when making observations.
- Select a location with minimal dust.
- To avoid splashes, do not use this product near water.

- Make sure the ambient temperature is 0 to +40°C and humidity is 60% or less. When transporting or storing this product, the ambient temperature must be -20 to +60°C, with the humidity at 90% RH max (with no condensation). Installing this product in a hot, humid location may result in the formation of mold or condensation on lenses, impairing performance or causing malfunctions.
- Do not place this product in a locker or cabinet.

5 Handling of focus knob

- Never turn the focus knobs on the right and left sides of the microscope in opposite directions at the same time. Doing so may damage this product.
- Turning the coarse focus knob past its farthest point will damage this product. Never use undue force when turning the knob.

6 Protect the ports from dust and extraneous light (when the trinocular tube or the ergonomic tube is attached)

To keep out extraneous light and dust, always attach the supplied cap to any port not currently in use.

7 Protect the motorized swing-out condenser connector

When using the microscope without mounting the motorized swing-out condenser, attach the provided cap to prevent dust from entering the connector.

8 Handling of filters (when using the epi-fluorescence attachment)

- Excitation filters inside a filter cube are exposed to strong light and degrade over time. Replace them after the appropriate number of hours.
- Filter characteristics may alter if the filter is exposed to high humidity. To prevent changes or degradation of filter characteristics, avoid using or storing the filters under conditions of high humidity or high temperature. Avoid subjecting filters to rapid temperature changes. When a filter is not in use, store in a desiccator or hermetically sealed container with a drying agent.
- Especially the filters in the nine types of filter cubes listed below offer sharp, high-resolution waveform characteristics superior to normal filters. However, due to their sophisticated coatings, they must be handled with special care. Take care to avoid abrasion from cleaning. Follow the description in "1.1 Cleaning Lenses" in Chapter 5.
Single-band filter cubes: DAPI, FITC, TxRed, GFP
Multi-band filter cubes: F-R, F-T, D-F, D-F-R, D-F-T

9 Motorized devices

Do not force motorized units to move/stop by hand.

1

Microscopy Procedures

1

Bright-field Microscopy

1.1

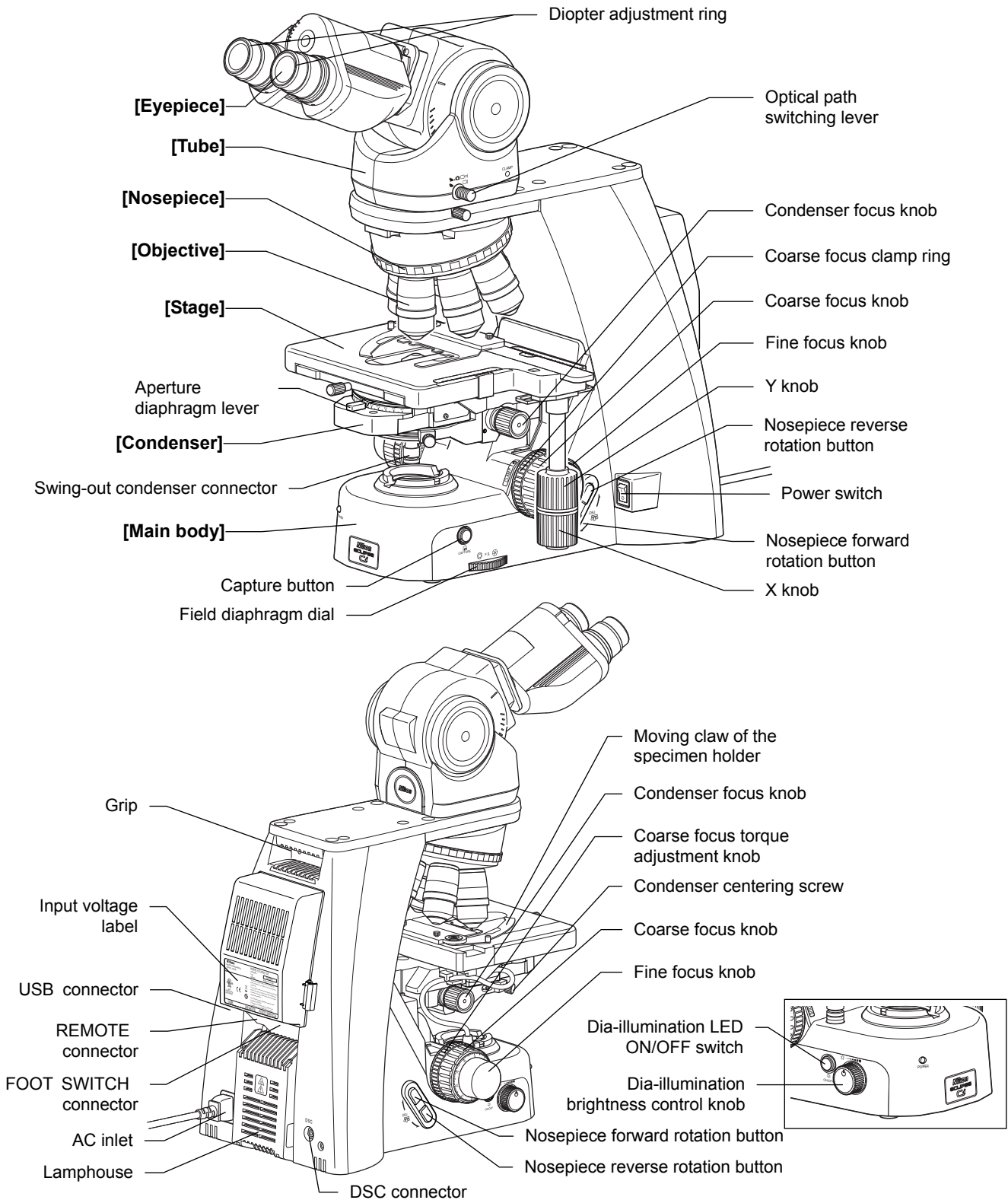
System Configuration and Controls

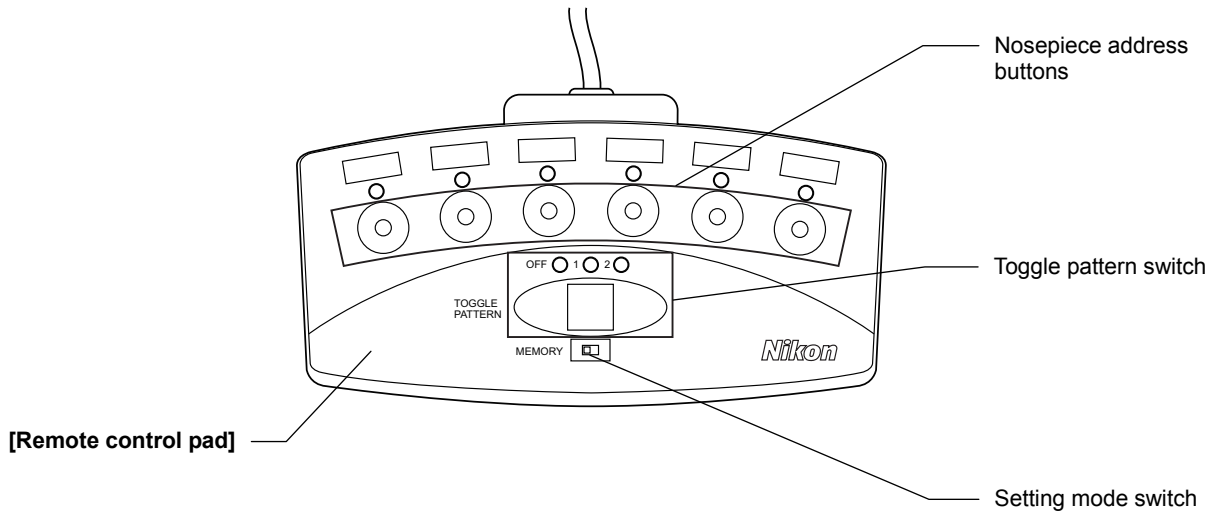
This section explains an example system configuration and the controls required for bright-field microscopy using the ECLIPSE Ci-E.

Names of components are denoted in the following manner: **[Eyepiece]**.

Chap. 1-1

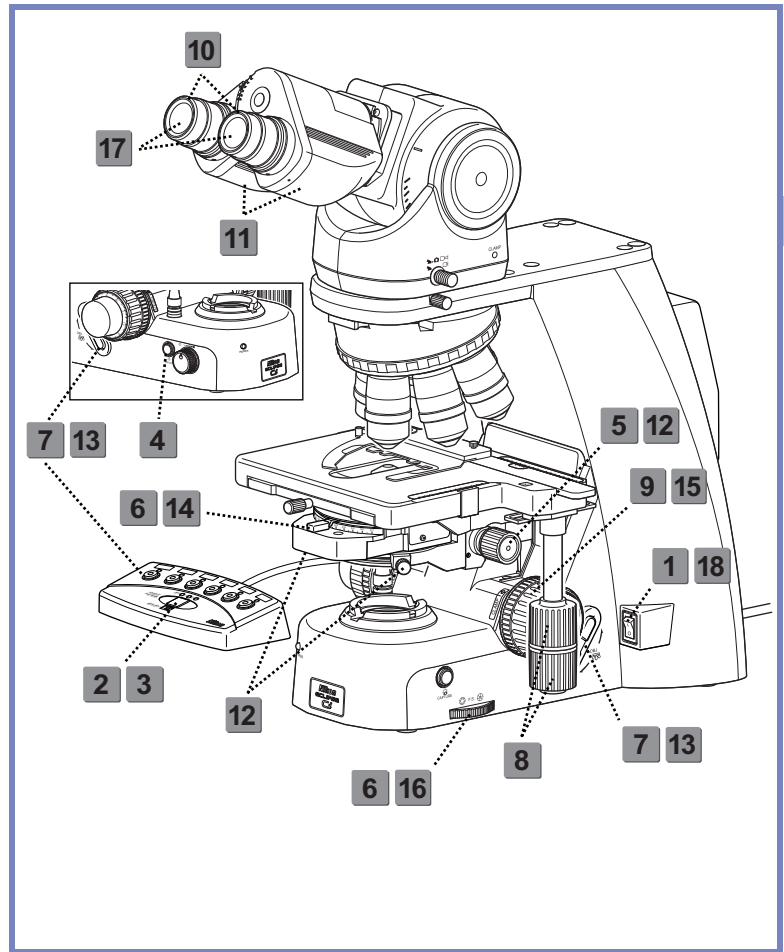
Microscopy Procedures Bright-field Microscopy





1.2 Bright-field Microscopy Procedure

1. Turn on the power.
2. (If using the toggle function) Configure the toggle pattern.
3. Switch to operation mode (left side).
4. Turn ON the dia-illumination LED.
5. Lower condenser slightly from uppermost position.
6. Fully open the field and aperture diaphragms.
7. Bring the 10x objective into the optical path.
8. Bring a specimen into the optical path.
9. Focus on the specimen.
10. Adjust the diopter.
11. Adjust the interpupillary distance.
12. Focus and center the condenser.
13. Bring the desired objective into the optical path.
14. Adjust the aperture diaphragm.
15. Focus on the specimen.
16. Circumscribe the field diaphragm to the field of view.
17. View the specimen.
18. Turn off the power.



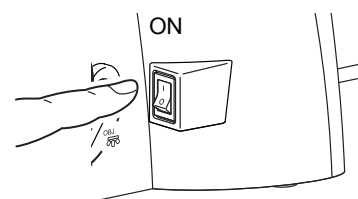
Chap. 1-1

Microscopy Procedures Bright-field Microscopy

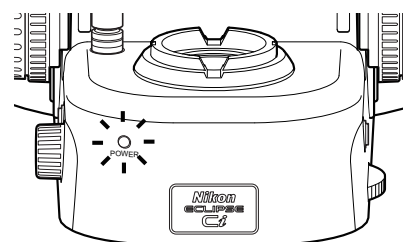
Preparation for microscopy

1 Turn on the power.

Turn on the power switch (press to the "I" position) for the microscope. (The power LED on the front of the main body will light up.)



Power switch ON



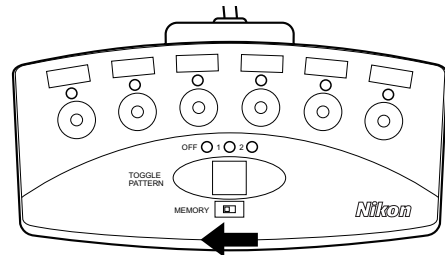
LED Illuminated

2 Select the setup target with the toggle pattern switch, and then switch to the setting mode to configure the motorized functions.

Before using a motorized swing-out condenser for the first time, configure the swing-out of the top lens. (→See Chapter 2, Section 13.1 for details.)
To use the toggle function, configure the toggle pattern. (→See Chapter 2, Section 12.2 for details.)

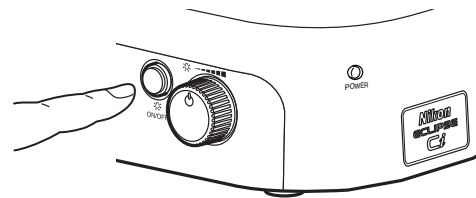
3 Set the setting mode switch to the left side (operation mode).

Set the setting mode switch on the remote control pad to the left side.



4 Turn on the dia-illumination LED.

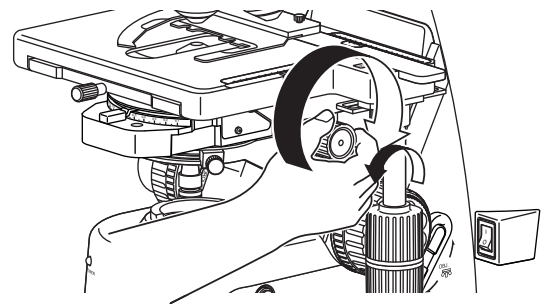
Press the dia-illumination LED ON/OFF switch to turn on the LED.



Dia-illumination LED switch ON

5 Lower the condenser slightly from the uppermost position.

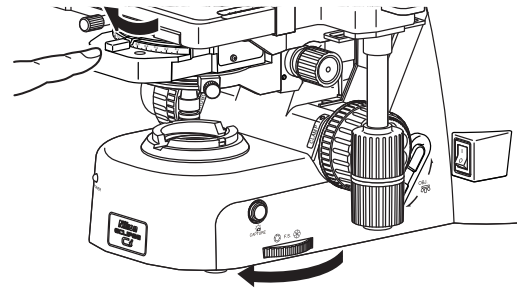
Turn the condenser focus knob until the condenser is positioned at the upper limit (where it clicks to a stop), and then lower it a little.



Lower the condenser from upper limit.

6 Fully open the field diaphragm and aperture diaphragm.

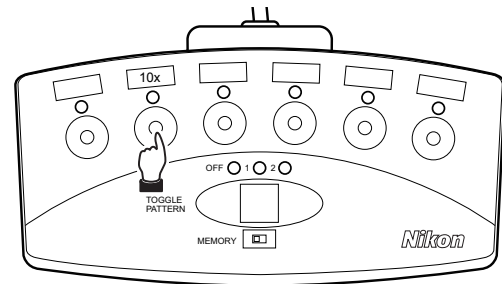
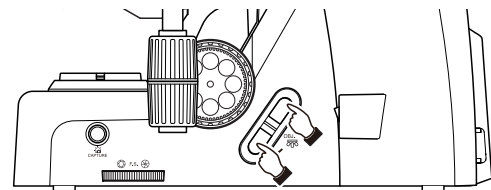
Turn the field diaphragm dial and the aperture diaphragm lever clockwise to open them completely.



Fully opening the field and aperture diaphragms

7 Bring the 10x objective into the optical path.

Press the nosepiece forward/reverse rotation buttons, or the nosepiece address button on the remote control pad to bring the 10x objective into the optical path.

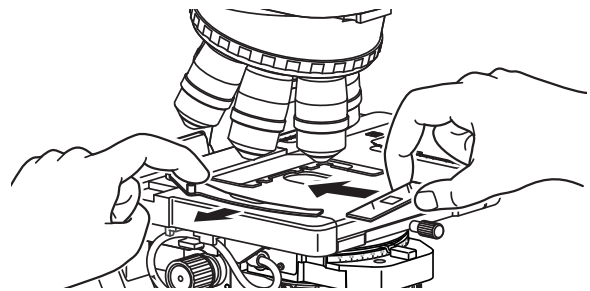


Bringing a 10x objective into the optical path

8 Place a specimen on the stage, and move the stage to bring the target into view.

- (1) Open the claw of the specimen holder's moving part and place the specimen onto the stage, gently stowing the claw back to fix the specimen.

You may use a double swing-claw holder for a single specimen. To set a specimen to a double swing-claw holder, slide and push the specimen into the clearance between the claws.



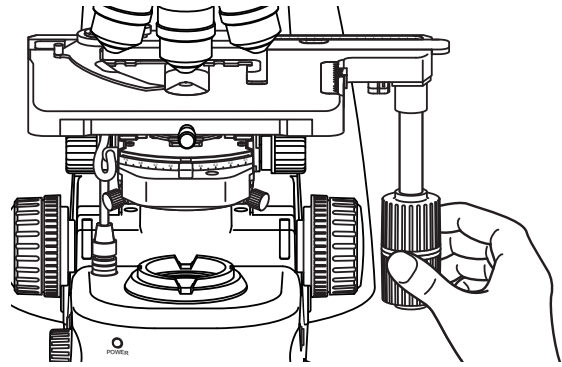
Setting the specimen

⚠ Note on removing specimens

Completely open the movable claw of the specimen holder by sliding it before removing a specimen from the stage.

If a specimen is repeatedly removed with the movable claw closed, the movable claw may be damaged.

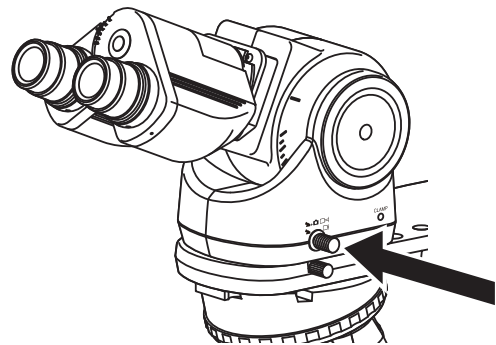
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
(So that the sample sealed under the cover glass will be lighted.)



Bringing the target into the optical path

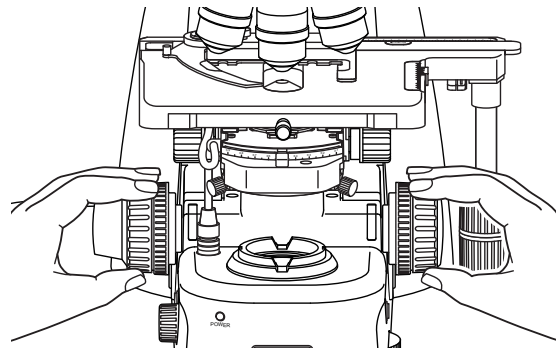
9 Focus on the specimen. (→See Chapter 2 “2 Focusing on the Specimen (Vertical Stage Movement)” for details)

- (1) When using a trinocular tube or ergonomic tube, push in the optical path switching lever to distribute 100% of light to the binocular section.
- (2) Look into the eyepiece and turn the coarse focus knob away to raise the stage to the upper limit. From there, focus on the specimen by lowering the stage.



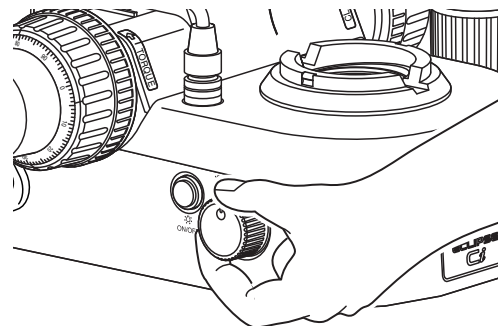
Switching the optical path 100% to the binocular part

- (3) When the focus was roughly adjusted using the coarse focus knob, turn the fine focus knob to accurately adjust the focus.



Focusing on the specimen

- (4) Turn the dia-illumination brightness control knob to adjust the brightness of the field of view.

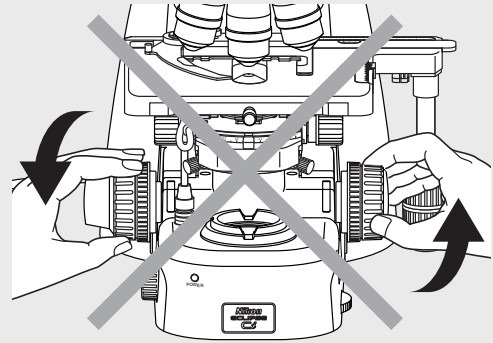


Brightness adjustment

⚠ Notes on controlling the focus knobs

Avoid the following actions, which can cause equipment malfunctions.

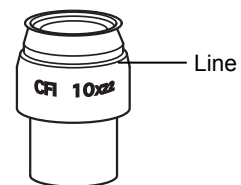
- Rotating the right and left focus knobs in opposite directions.
- Rotating the coarse focus knob past the limit.



Don't rotate the knobs in opposite directions!

10 Adjust the diopter. (→See Chapter 2, “4 Adjusting the Diopter” for details)

- (1) Turn the diopter adjustment ring on the right and left eyepieces to align the end face of the diopter adjustment ring with the line. (This is the diopter adjustment reference position.)
- (2) Focus on the specimen using the 40x objective.
- (3) Bring the 10x (or 4x) objective into the optical path.
- (4) Look into the right eyepiece with your right eye and the left eyepiece with your left eye. Turn the diopter adjustment ring of each eyepiece to focus on the specimen. At this point no focus knobs are used.
- (5) Repeat Steps (2) through (4) to make sure the focus has been adjusted properly.



Reference position for diopter adjustment



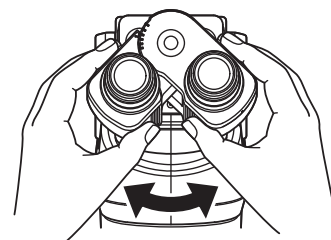
Adjusting the diopter

11 Adjust the interpupillary distance.

Look into both eyepieces and rotate the binocular part to adjust the binocular part's opening until the fields of view for the right and left eyes coincide.

✔ Tip on adjusting the interpupillary distance

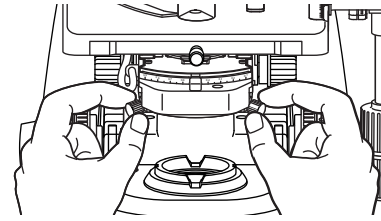
For easy adjustment, look into the eyepiece as if you were looking at a distant object.



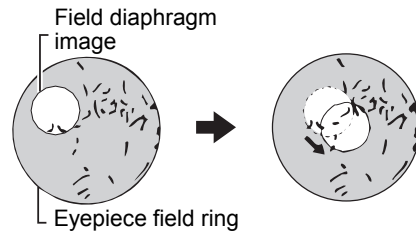
Adjusting interpupillary distance

12 Focus and center the condenser. (→See Chapter 2, “5 Focusing and Centering the Condenser” for details)

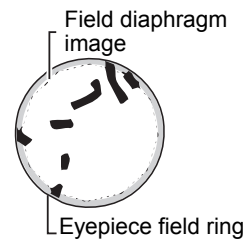
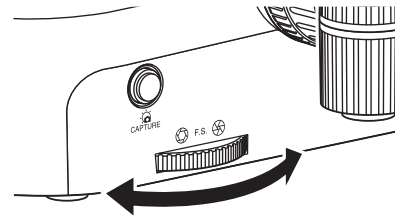
- (1) Look into the eyepiece with the field diaphragm stopped down to the minimum. Focus on the field diaphragm image using the condenser focus knob, then adjust the condenser centering screws to center the diaphragm image within the field of view.



- (2) Bring the 40x objective into the optical path to check the focus and centering of the field diaphragm image. Make adjustments the same way as step (1) as necessary.



- (3) Turn the field diaphragm dial and adjust the field diaphragm image so that its size is almost the same as the field of view.



Focusing and centering the condenser

Chap. 1-1

Microscopy Procedures Bright-field Microscopy

Microscopy operation

13 Select the desired objective.

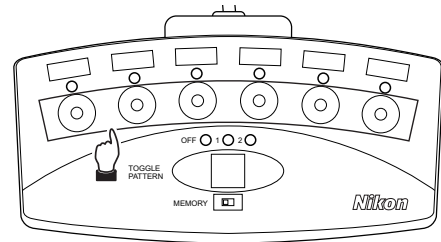
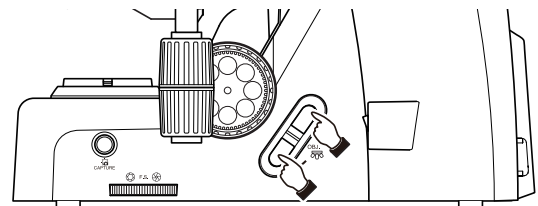
Press the nosepiece forward/reverse rotation buttons, or the nosepiece address button on the remote control pad to move the desired objective into the optical path.

✔ **1-100x swing-out condenser**

Mounting a 1-100x swing-out condenser on the Ci-E main body when using the 1x objective may result in uneven illumination around the field of view.

✔ **Toggle function**

Use the toggle function for easy switching between two objectives. (→Chapter 2, Section 12.2 for details)



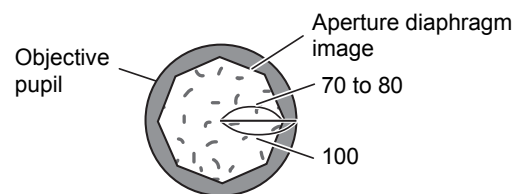
Bringing a desired objective into the optical path

14 Adjust the aperture diaphragm. (→See Chapter 2, “6 Adjusting the Aperture Diaphragm” for details)

Turn the aperture diaphragm lever on the condenser to adjust the aperture diaphragm so that it is set to 70 to 80% of the numerical aperture of the objective used.

✔ **Adjustment timing for the aperture diaphragm**

Be sure to adjust the aperture diaphragm each time you change the objective.
(You can see the aperture diaphragm image with the centering telescope.)



Right size of the aperture diaphragm

15 Focus on the specimen.

- (1) Look into the eyepiece, and adjust the brightness of the field of view by turning the dia-illumination brightness control knob.
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
- (3) If the specimen is not in focus, turn the focus knob to focus on it.

16 Adjust the field diaphragm.

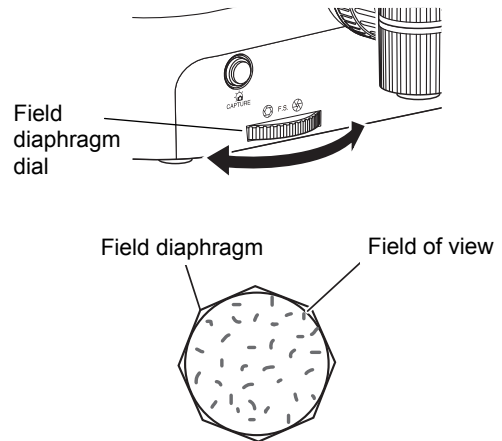
Turn the field diaphragm dial to adjust the field diaphragm so that it almost circumscribes the field of view.

✔ **Size of the field diaphragm**

Normally, adjust the field diaphragm so that it almost circumscribes the field of view. Opening the field diaphragm too much results in an external stray light entering the field of view, generating flare and reducing image contrast. In addition, the specimen will become decolorized over a wider area.

✔ **Adjustment timing for the field diaphragm**

Be sure to adjust the field diaphragm each time you change the objective.



Circumscribe around the field of view

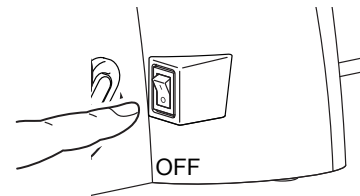
Adjusting the field diaphragm

17 View the specimen.

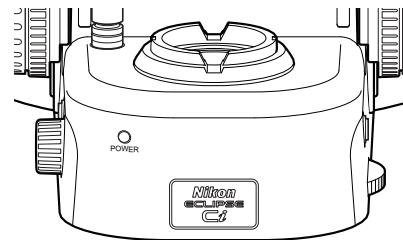
Rotate the stage knob to move the target. If the target is not in focus, use the focus knob to adjust the focus.

18 Turn off the power.

Turn off the power switch (press to the "O" position) for the microscope. (The power LED on the front of the main body will turn off.)



Power off



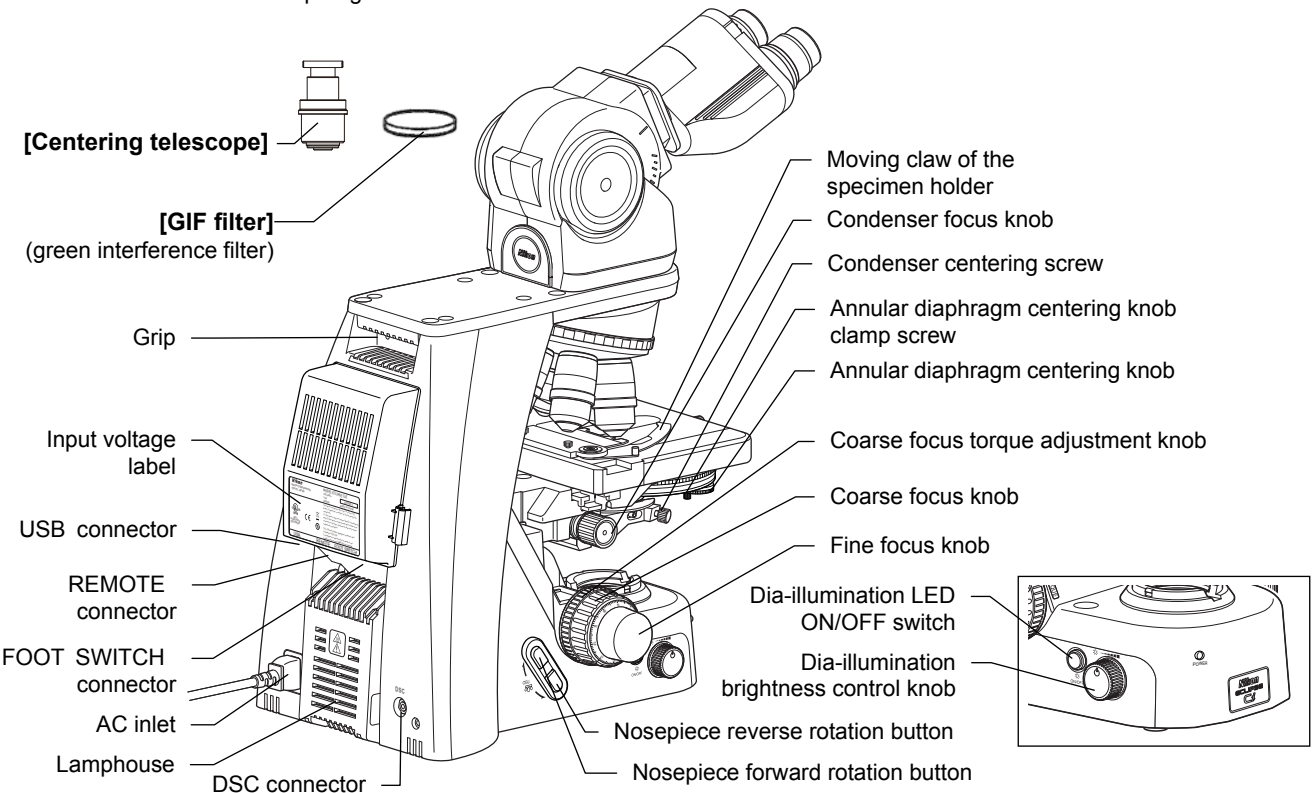
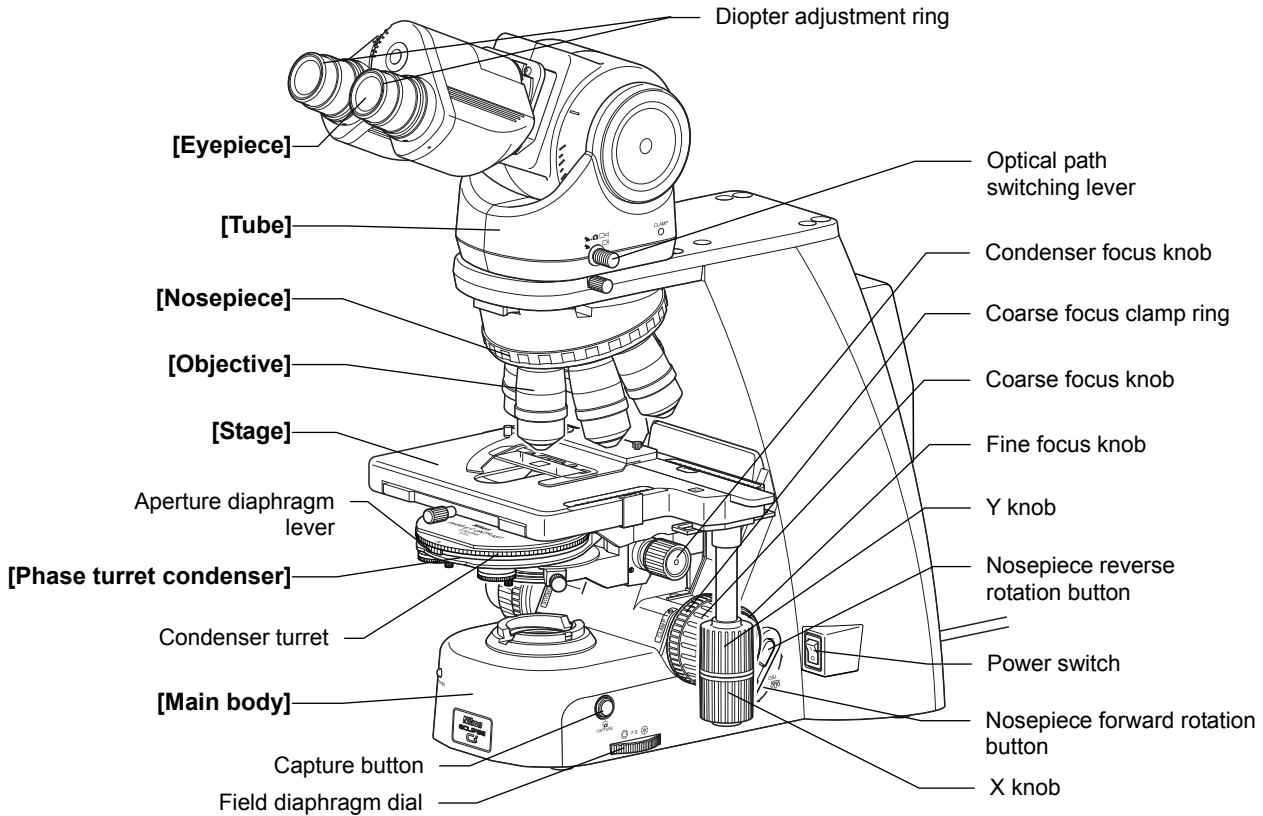
Power LED off

2 Phase Contrast Microscopy

2.1 System Components and Controls

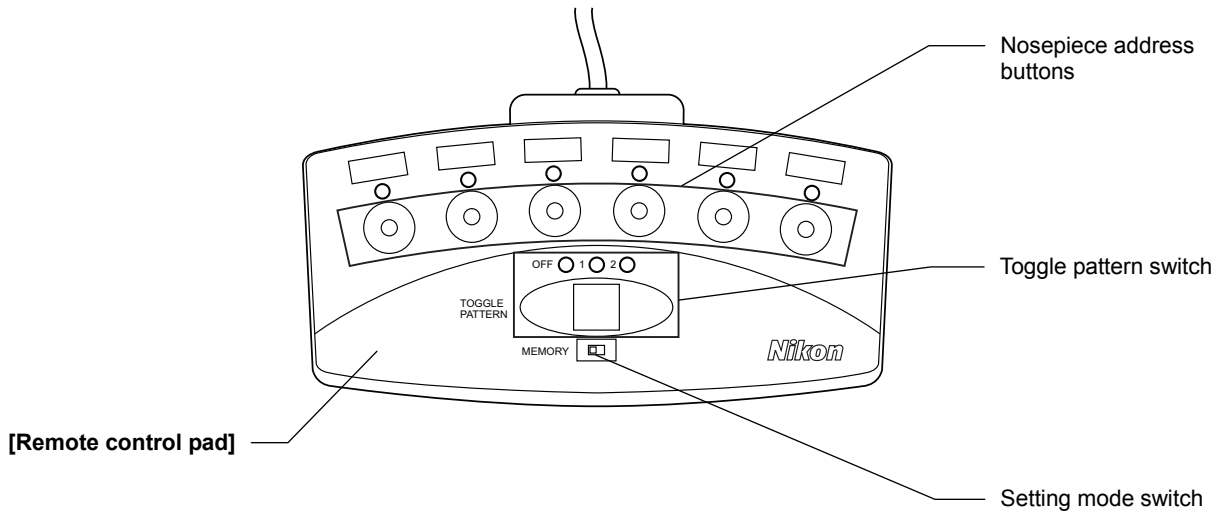
This section explains an example system configuration and the controls required for a phase contrast microscopy using the ECLIPSE Ci-E.

Names of components are denoted in the following manner: **[Phase turret condenser]**.



Chap. 1-2

Microscopy Procedures Phase Contrast Microscopy

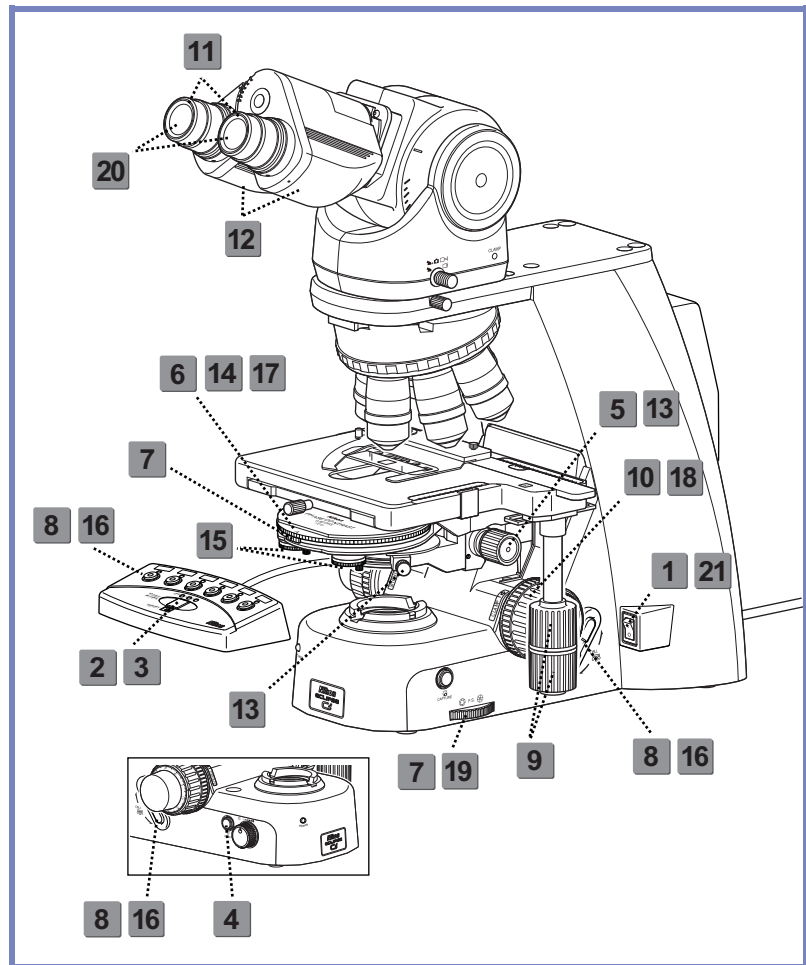


Chap. 1-2

Microscopy Procedures Phase Contrast Microscopy

2.2 Phase Contrast Microscopy Procedure

1. Turn on the power.
2. (If using the toggle function)
Configure the toggle pattern.
3. Switch to operation mode (left side).
4. Turn ON the dia-illumination LED.
5. Lower condenser slightly from uppermost position.
6. Move the turret to the [A: empty] position.
7. Fully open the field and aperture diaphragms.
8. Bring the 10x Ph objective into the optical path.
9. Bring a specimen into the optical path.
10. Focus on the specimen.
11. Adjust the diopter.
12. Adjust the interpupillary distance.
13. Focus and center the condenser.
14. Bring the Ph annular diaphragm [Ph1] position into the optical path.
15. Center the Ph annular diaphragm.
16. Bring the desired Ph objective into the optical path.
17. Match the Ph codes of annular diaphragm and the objective.
18. Focus on the specimen.
19. Circumscribe the field diaphragm to the field of view.
20. View the specimen.
21. Turn off the power.



Chap. 1-2

Microscopy Procedures Phase Contrast Microscopy

Preparation for microscopy

In this procedure, only step titles are shown for operations that are the same as those of bright-field microscopy. See “1.2 Bright-field Microscopy Procedure” for details.

1 Turn on the power.

(The power LED on the front of the main body will light up.)

2 Select the setup target with the toggle pattern switch, and then set the setting mode switch to the right side (setting mode) to configure the motorized functions. (→See Chapter 2, Section 12.2 for details)

3 Set the setting mode switch to the left side (operation mode).

4 Turn on the dia-illumination LED.

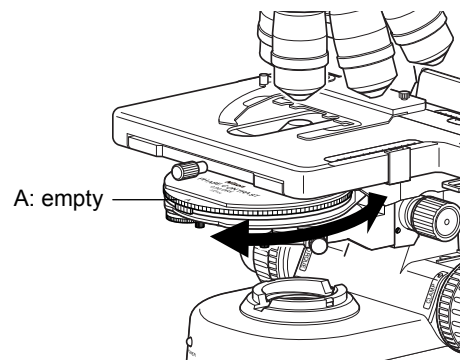
5 Lower the condenser slightly from the uppermost position.

6 Move the condenser turret to the [A: empty] position.

Turn the condenser turret until the [A: empty] symbol comes to the front, and align the hole with the optical path.

✓ **Aperture diaphragm of the condenser**

Positioning the condenser turret at positions other than [A: empty] deviates the aperture diaphragm from the optical path.



Moving the condenser turret to the [A: empty] position

7 Fully open the field diaphragm and aperture diaphragm.

8 Bring the 10x Ph objective (Ph1) into the optical path.

9 Place a specimen on the stage, and move the stage to bring the target into view.

10 Focus on the specimen. (→See Chapter 2 “2 Focusing on the Specimen (Vertical Stage Movement)” for details)

11 Adjust the diopter. (→See Chapter 2, “4 Adjusting the Diopter” for details)

12 Adjust the interpupillary distance.

13 Focus and center the condenser. (→See Chapter 2, “5 Focusing and Centering the Condenser” for details)

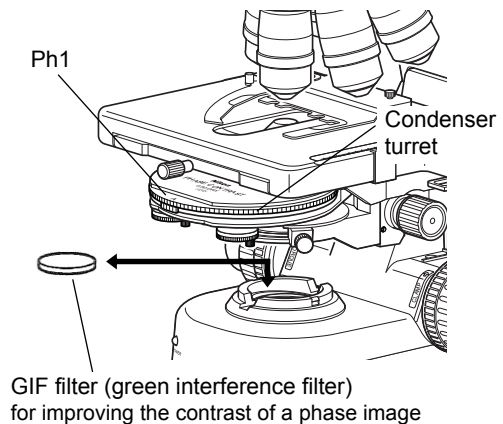
Microscopy operation (→See also: Chapter 2, “16 Tips for Phase Contrast Microscopy”)

14 Bring the Ph annular diaphragm (Ph1) in the condenser turret into the optical path.

Turn the condenser turret until the [Ph1] symbol comes to the front.

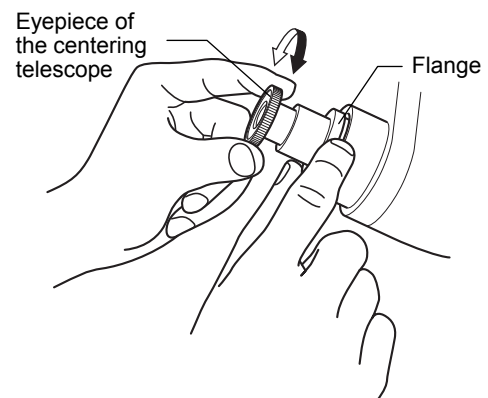
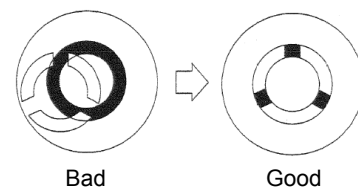
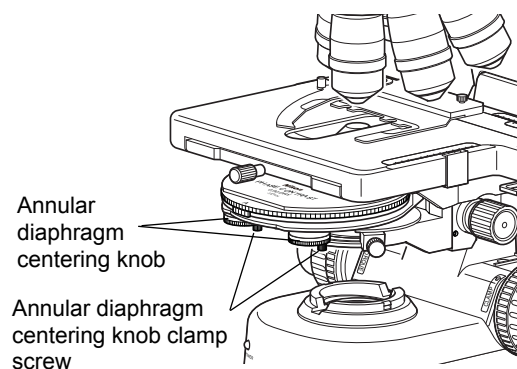
✔ GIF filter

The GIF filter (green interference filter) improves the contrast when placed in the optical path. The filter should be installed on the field lens, or placed inside or on top of the filter cassette holder. Note, however, that it may cause ghosting when mounted inside the filter cassette holder.


Bringing the Ph annular diaphragm into the optical path
15 Center the Ph annular diaphragm.

To optimize the phase effect, it is important to properly overlap the phase plate of the objective with the Ph annular diaphragm image in the condenser.

- (1) Make sure that the 10x objective (Ph1) has been placed into the optical path and that the [Ph1] symbol on the condenser turret is facing the front.
- (2) Rotate the stage knob to move the specimen and bring a portion where there is no sample under the cover glass into the optical path.
- (3) Remove one eyepiece from the tube and attach a centering telescope into the tube.
- (4) Hold the flange of the centering telescope and rotate the eyepiece to focus on the phase plate of the objective.
- (5) If the phase plate of the objective and the annular diaphragm in the condenser are misaligned, loosen the clamp screws of the two annular diaphragm centering knobs before rotating the centering knob to move the entire turret for annular adjustment. Tighten the clamp screws after the completion of the adjustment.
- (6) Remove the centering telescope from the tube, and reattach the eyepiece.


Focusing on the phase ring

Centering the Ph annular diaphragm

16 Bring an arbitrary Ph objective into the optical path.

Press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad to move the desired Ph objective into the optical path.

When using an oil immersion type objective, apply immersion oil between the specimen and the objective.
(→Chapter 2 “14 Oil Immersion” for details)

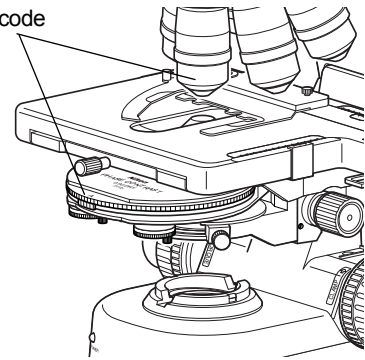
ⓘ Phase turret condenser

The phase turret condenser is intended to be used dry. DO NOT apply immersion oil between the condenser tip's ball and the specimen.

17 Adjust the Ph annular diaphragm in the condenser with the Ph objective to be used.

Turn the condenser turret to bring an annular diaphragm with the same Ph code as the objective into the optical path.

Same Ph code



Matching the Ph codes of Ph annular diaphragm and the Ph objective

✓ Ph code

One of the Ph codes, [Ph1], [Ph2], or [Ph3] is indicated on the Ph objective depending on the size of the phase plate. (Ph codes have nothing to do with the magnification of the objective.) Always use a Ph objective and Ph annular diaphragm with the same Ph code. You cannot expect the phase effect if a different combination of the codes is used.

✓ Centering of the annular diaphragm and the phase plate

The position of each annular diaphragm in the condenser turret has already been adjusted based on the Ph1 annular diaphragm, but the phase image will differ slightly depending on how the annular diaphragm overlaps the phase plate. For a stricter observation or capturing of still images, check whether the annular diaphragm and the phase plate are concentric at each magnification.

18 Focus on the specimen.

- (1) Look into the eyepiece, and adjust the brightness of the field of view by turning the dia-illumination brightness control knob.
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
- (3) If the specimen is not in focus, turn the focus knob to focus on it.

19 Adjust the field diaphragm.

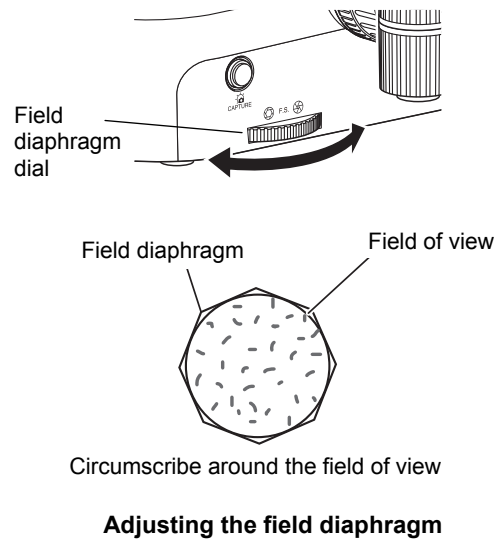
Turn the field diaphragm dial to adjust the field diaphragm so that it almost circumscribes the field of view.

✔ **Size of the field diaphragm**

Normally, adjust the field diaphragm so that it almost circumscribes the field of view. Opening the field diaphragm too much results in an external stray light entering the field of view, generating flare and reducing image contrast. In addition, the specimen will become decolorized over a wider area.

✔ **Adjustment timing for the field diaphragm**

Be sure to adjust the field diaphragm each time you change the objective.



Chap. 1-2

Microscopy Procedures Phase Contrast Microscopy

20 View the specimen.

Rotate the stage knob to move the target. If the target is not in focus, use the focus knob to adjust the focus.

✔ **To switch to bright-field microscopy**

- Turn the condenser turret until the [A: empty] symbol comes to the front.
- Microscopy is possible with an objective of 4x or greater, but UW microscopy with the 4x objective will cause vignetting.
- When the condenser is set to [A: empty], its performance is equivalent to that of the Abbe condenser.

21 Turn off the power.

Turn off the power switch (press to the "O" position) for the microscope. (The power LED on the front of the main body will turn off.)

3 Simple Polarizing Microscopy

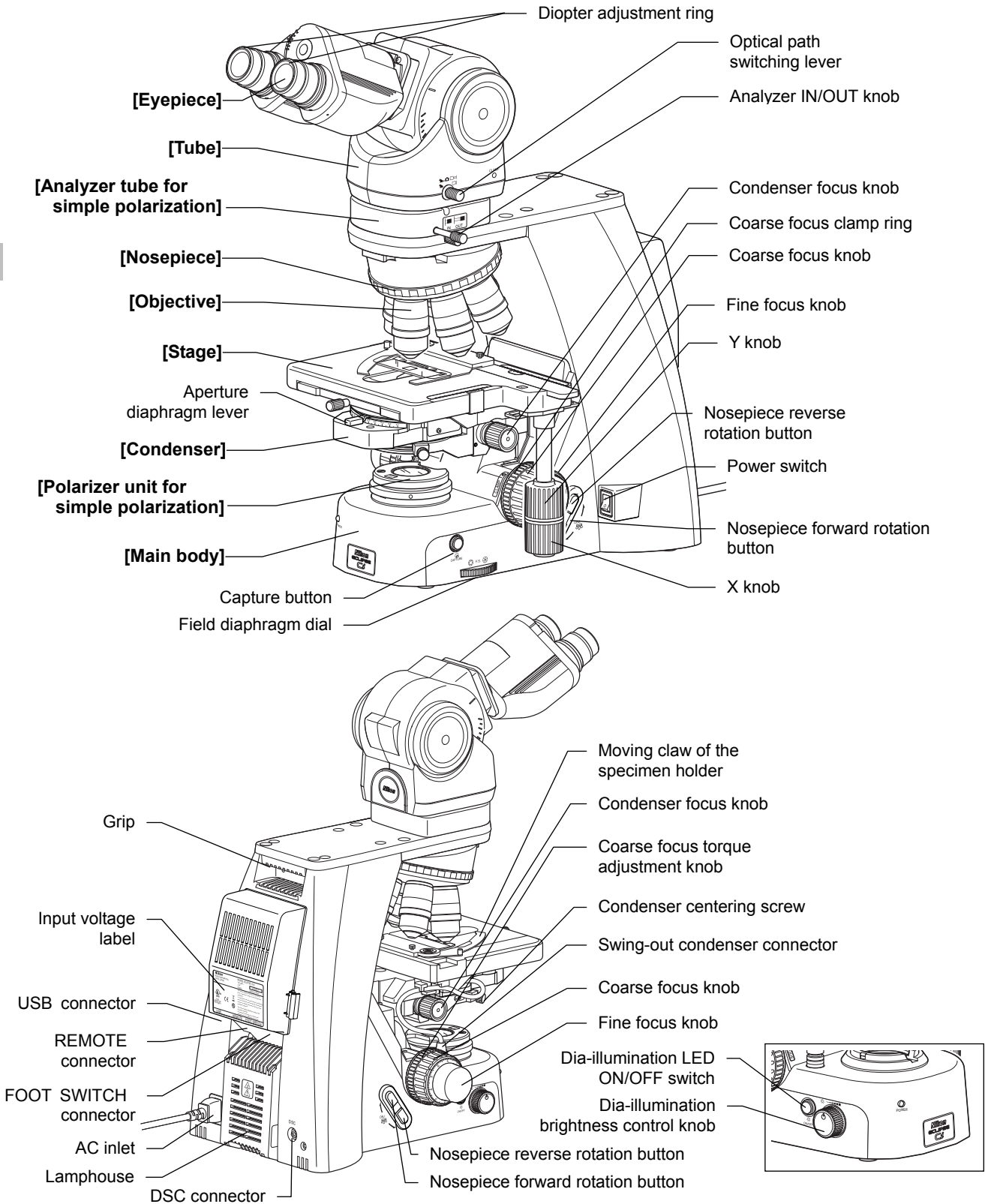
3.1 System Configuration and Controls

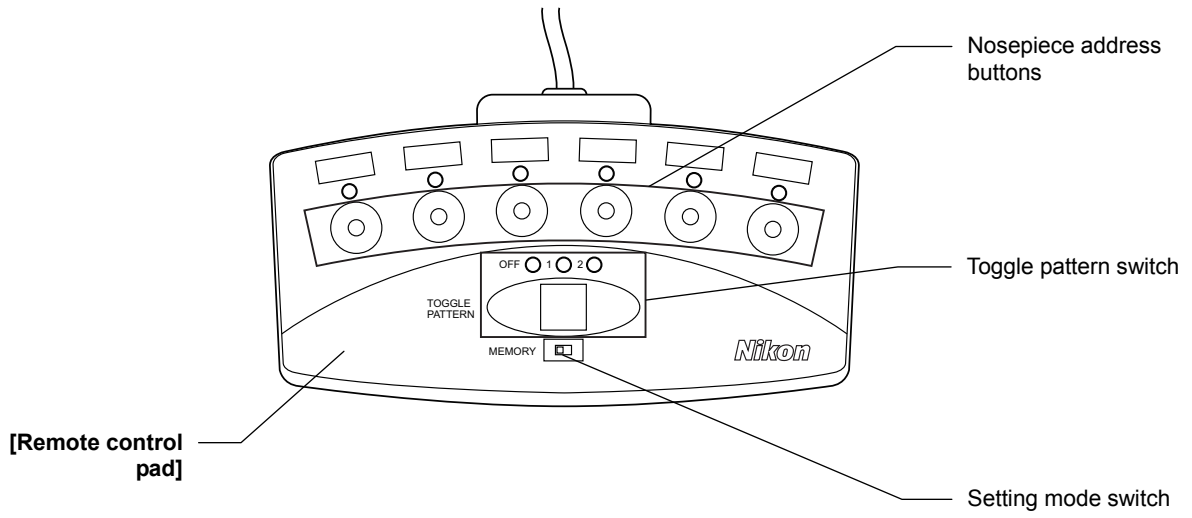
This section explains an example system configuration and the controls required for a simple polarizing microscopy using the ECLIPSE Ci-E.

Names of components are denoted in the following manner: **[Polarizer unit for simple polarization]**.

Chap. 1-3

Microscopy Procedures Simple Polarizing Microscopy

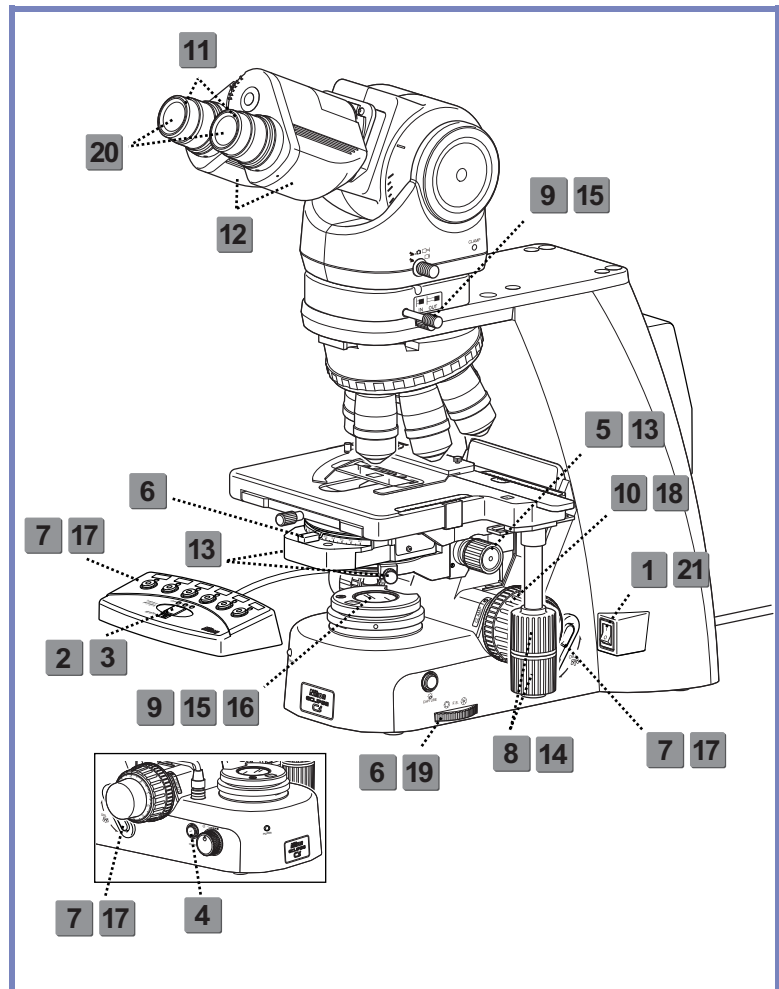




3.2

Simple Polarizing Microscopy Procedure

1. Turn on the power.
2. (If using the toggle function) Configure the toggle pattern.
3. Switch to operation mode (left side).
4. Turn ON the dia-illumination LED.
5. Lower condenser slightly from uppermost position.
6. Fully open the field and aperture diaphragms.
7. Bring the 10x objective into the optical path.
8. Bring a specimen into the optical path.
9. Remove the analyzer from the optical path.
10. Focus on the specimen.
11. Adjust the diopter.
12. Adjust the interpupillary distance.
13. Focus and center the condenser.
14. Bring the portion without sample into the optical path.
15. Set the polarizer unit.
16. Adjust the orientations of analyzer and polarizer.
17. Bring the desired objective into the optical path.
18. Focus on the specimen.
19. Circumscribe the field diaphragm to the field of view.
20. View the specimen.
21. Turn off the power.



Preparation for microscopy

In this procedure, only step titles are shown for operations that are the same as those of bright-field microscopy. See "1.2 Bright-field Microscopy Procedure" for details.

1 Turn on the power.

(The power LED on the front of the main body will light up.)

2 Select the setup target with the toggle pattern switch, and then set the setting mode switch to the right side (setting mode) to configure the motorized functions.

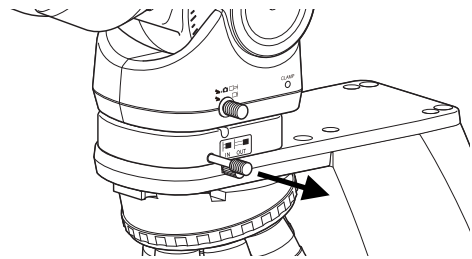
Before using a motorized swing-out condenser for the first time, configure the swing-out of the top lens. (→Chapter 2, Section 13.1 for details)

To use the toggle function, configure the toggle pattern. (→Chapter 2, Section 12.2 for details)

- 3** Set the setting mode switch to the left side (operation mode).
- 4** Turn on the dia-illumination LED.
- 5** Lower the condenser slightly from the uppermost position.
- 6** Fully open the field diaphragm and aperture diaphragm.
- 7** Bring the 10x objective into the optical path.
- 8** Place a specimen on the stage, and move the stage to bring the target into view.
- 9** Remove the analyzer and the polarizer from the optical path.

Pull out the analyzer IN/OUT knob from the analyzer tube for simple polarization to remove the analyzer from the optical path.

The polarizer unit for simple polarization has not yet been installed at this point.



Removing the analyzer from the optical path

- 10** Focus on the specimen. (→See Chapter 2 “2 Focusing on the Specimen (Vertical Stage Movement)” for details)
- 11** Adjust the diopter. (→See Chapter 2, “4 Adjusting the Diopter” for details)
- 12** Adjust the interpupillary distance.
- 13** Focus and center the condenser. (→See Chapter 2, “5 Focusing and Centering the Condenser” for details)

Microscopy operation

14 Bring a portion of the specimen where there is no sample into the optical path.

Rotate the stage knob to move the specimen and bring a portion where there is no sample under the cover glass into the optical path.

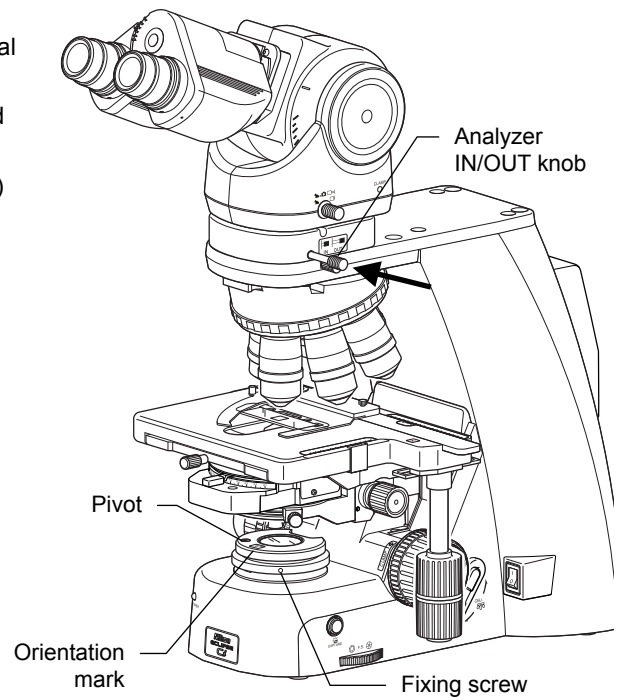
15 Bring the analyzer and the polarizer into the optical path.

Push in the analyzer IN/OUT knob into the analyzer tube for simple polarization to bring the analyzer into the optical path.

Set the polarizer unit for simple polarization over the field lens.

Make sure the orientation mark on the polarizer (roughly) comes to the front at this point.

Keep the fixing screw of the polarizer unit loosened.



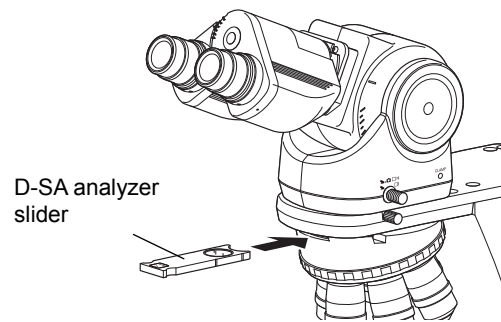
**Bringing the analyzer into the optical path
Installing the polarizer**

✓ Slider-type analyzer for simple polarization

Using the D-SA Analyzer Slider for Simple Polarization instead of an analyzer tube for simple polarization can keep the eye point from rising. To use the D-SA Analyzer Slider for Simple Polarization, insert it into the analyzer slot of the nosepiece. Use the analyzer slider as follows:

Push in: The analyzer goes into the optical path.

Pull out: The analyzer is removed from and the dummy hole goes into the optical path (empty).



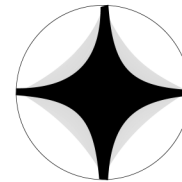
Using D-SA analyzer slider

16 Adjust the orientation of the analyzer and the polarizer.

- (1) Fully open the aperture diaphragm.
- (2) Pull out one eyepiece from the tube.
- (3) Look into the eyepiece sleeve and rotate the whole polarizer unit until you can identify a dark cross. (You will see black stripes that change shape as you rotate the polarizer unit.)
- (4) Tighten the fixing screw of the polarizer unit to fix the polarizer.
- (5) Place the eyepiece back into the tube.

✔ Dark cross (crossed Nicols)

You will see a dark cross when the orientation of the analyzer orthogonally crosses that of the polarizer.



Dark cross

17 Bring a desired objective into the optical path.

Press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad to move the desired objective into the optical path.

18 Focus on the specimen.

- (1) Look into the eyepiece, and adjust the brightness of the field of view by turning the dia-illumination brightness control knob.
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
- (3) If the specimen is not in focus, turn the focus knob to focus on it.

19 Adjust the field diaphragm.

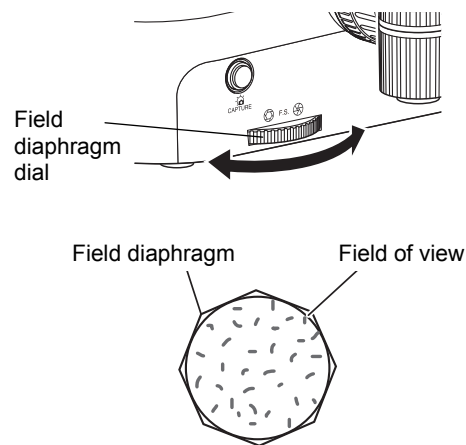
Turn the field diaphragm dial to adjust the field diaphragm so that it almost circumscribes the field of view.

✔ Size of the field diaphragm

Normally, adjust the field diaphragm so that it almost circumscribes the field of view. Opening the field diaphragm too much results in an external stray light entering the field of view, generating flare and reducing image contrast. In addition, the sample will become decolorized over a wider area.

✔ Adjustment timing for the field diaphragm

Be sure to adjust the field diaphragm each time you change the objective.



Circumscribe around the field of view

Adjusting the field diaphragm

20 View the specimen.

Rotate the stage knob to move the target. If the target is not in focus, use the focus knob to adjust the focus.

✔ **Strict polarizing microscopy**

If you need a retardation measurement or stricter polarizing observation, use a dedicated polarizing microscope.

✔ **To switch to bright-field microscopy**

Pull out the analyzer IN/OUT knob to remove the analyzer from the optical path. Remove the polarizer unit from the field lens.

21 Turn off the power.

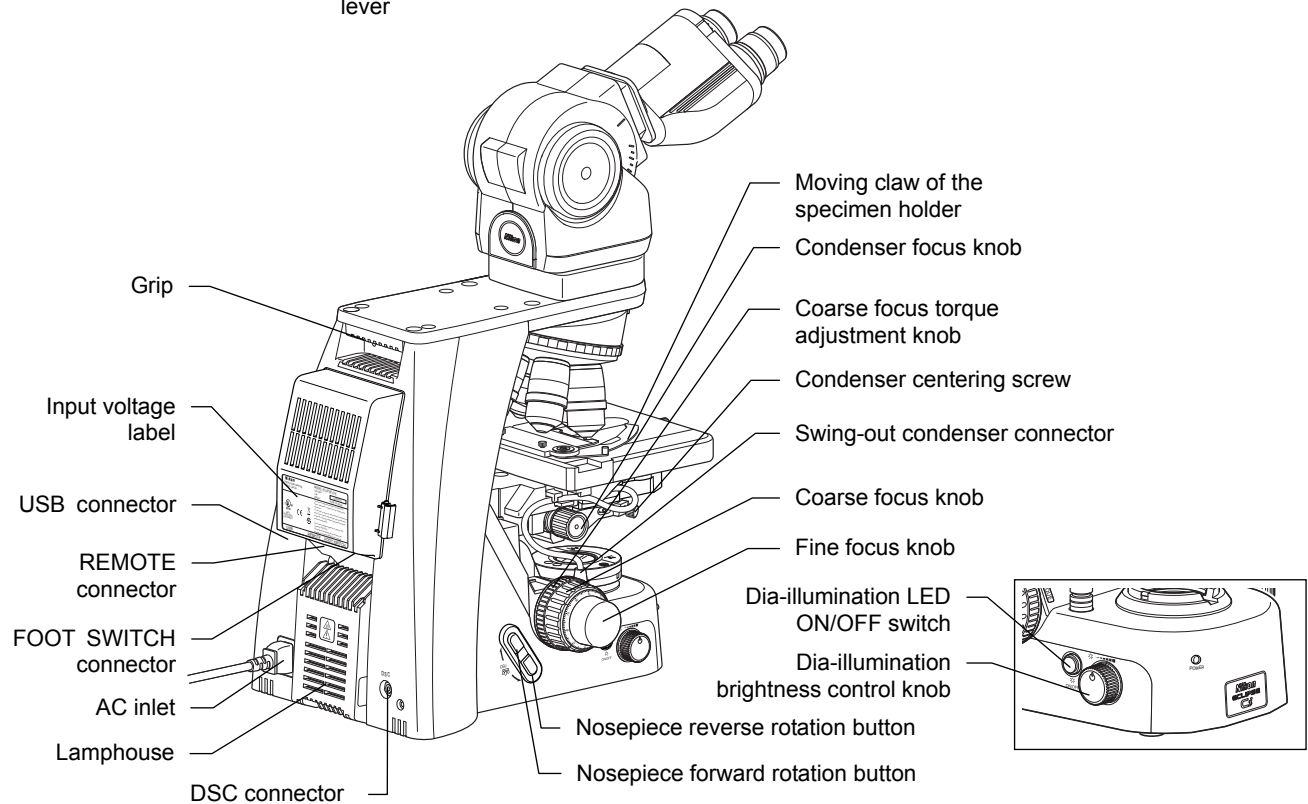
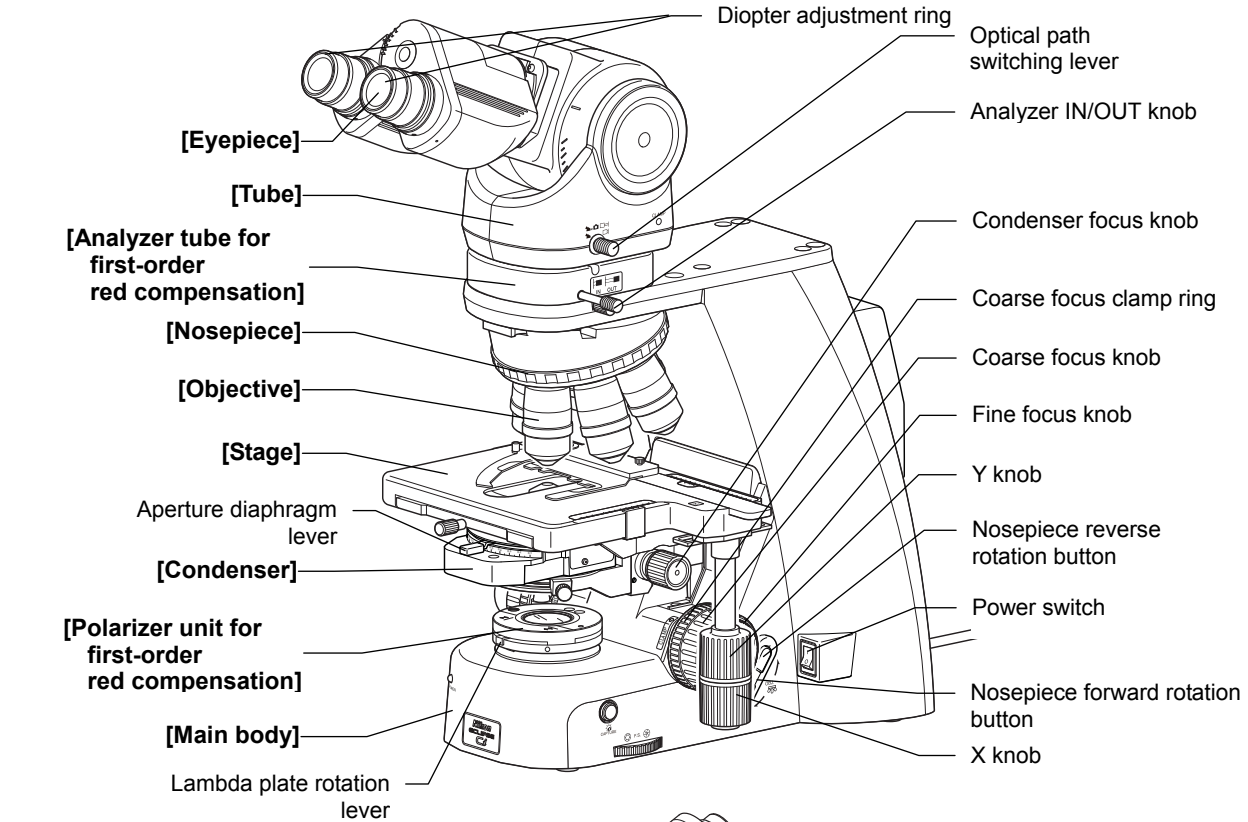
Turn off the power switch (press to the “O” position) for the microscope. (The power LED on the front of the main body will turn off.)

4 Sensitive Polarization Microscopy

4.1 System Configuration and Controls

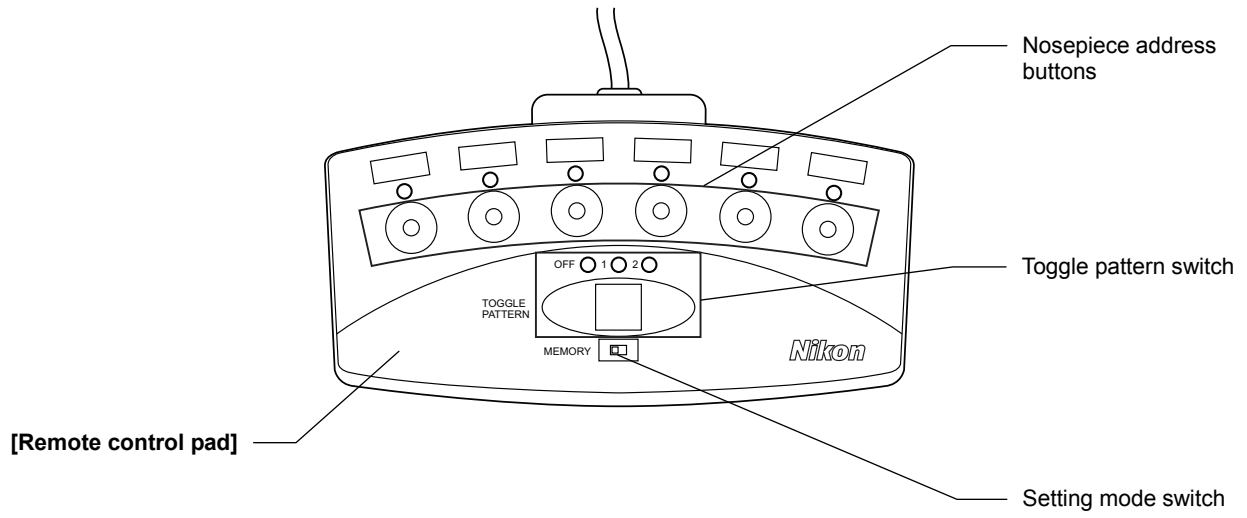
This section explains an example system configuration and the controls required for a sensitive polarization microscopy using the ECLIPSE Ci-E.

Names of components are denoted in the following manner: **[Polarizer unit for first-order red compensation]**.



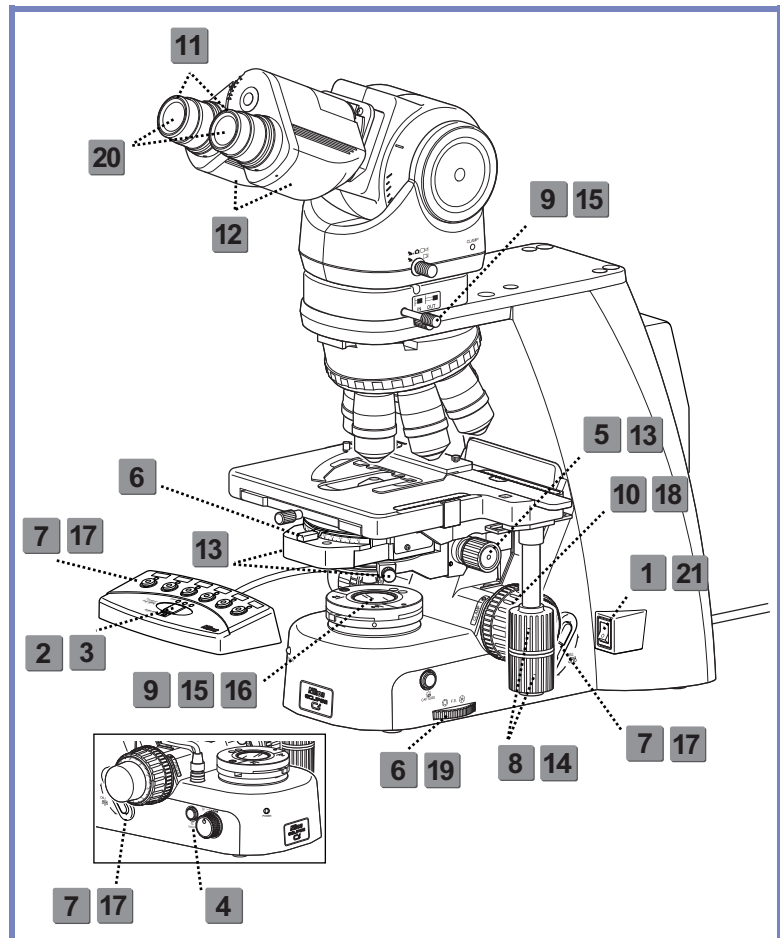
Chap. 1-4

Microscopy Procedures Sensitive Polarization Microscopy



4.2 Sensitive Polarization Microscopy Procedure

1. Turn on the power.
2. Configure the motorized functions.
3. Switch to operation mode (left side).
4. Turn ON the dia-illumination LED.
5. Lower condenser slightly from uppermost position.
6. Fully open the field and aperture diaphragms.
7. Bring the 10x objective into the optical path.
8. Bring a specimen into the optical path.
9. Remove the analyzer from the optical path.
10. Focus on the specimen.
11. Adjust the diopter.
12. Adjust the interpupillary distance.
13. Focus and center the condenser.
14. Bring the portion without sample into the optical path.
15. Set the polarizer unit.
16. Adjust the orientations of analyzer and polarizer.
17. Bring the desired objective into the optical path.
18. Focus on the specimen.
19. Circumscribe the field diaphragm to the field of view.
20. View the specimen.
21. Turn off the power.



Chap. 1-4

Microscopy Procedures Sensitive Polarization Microscopy

Preparation for microscopy

In this procedure, only step titles are shown for operations that are the same as those of bright-field microscopy. See "1.2 Bright-field Microscopy Procedure" for details.

1 Turn on the power.

(The power LED on the front of the main body will light up.)

2 Select the setup target with the toggle pattern switch, and then set the setting mode switch to the right side (setting mode) to configure the motorized functions.

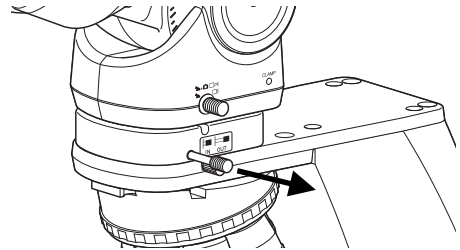
Before using a motorized swing-out condenser for the first time, configure the swing-out of the top lens. (→Chapter 2, Section 13.1 for details)

To use the toggle function, configure the toggle pattern. (→Chapter 2, Section 12.2 for details)

- 3** Set the setting mode switch to the left side (operation mode).
- 4** Turn on the dia-illumination LED.
- 5** Lower the condenser slightly from the uppermost position.
- 6** Fully open the field diaphragm and aperture diaphragm.
- 7** Bring the 10x objective into the optical path.
- 8** Place a specimen on the stage, and move the stage to bring the target into view.
- 9** Remove the analyzer and the polarizer from the optical path.

Pull out the analyzer IN/OUT knob from the analyzer tube for first-order red compensation to remove the analyzer from the optical path.

The polarizer unit for first-order red compensation has not been installed at this point.



Removing the analyzer from the optical path

- 10** Focus on the specimen. (→See Chapter 2 “2 Focusing on the Specimen (Vertical Stage Movement)” for details)
- 11** Adjust the diopter. (→See Chapter 2, “4 Adjusting the Diopter” for details)
- 12** Adjust the interpupillary distance.
- 13** Focus and center the condenser. (→See Chapter 2, “5 Focusing and Centering the Condenser” for details)

Microscopy operation

14 Bring a portion of the specimen where there is no sample into the optical path.

Rotate the stage knob to move the specimen and bring a portion where there is no sample under the cover glass into the optical path.

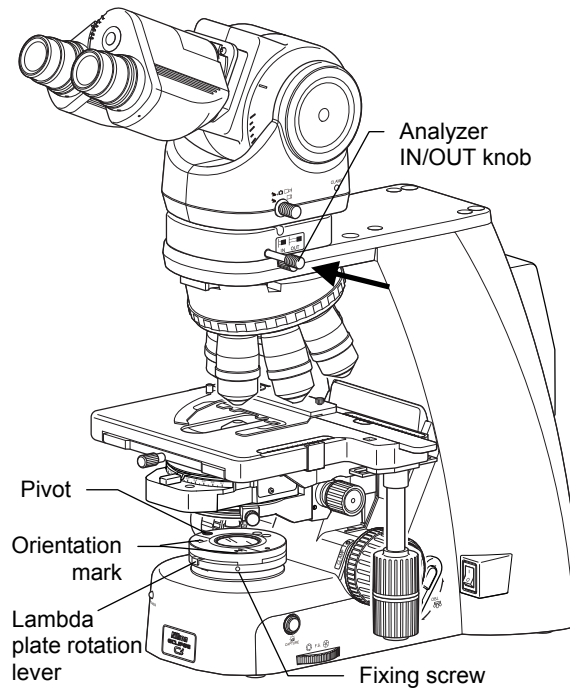
15 Bring the analyzer and the polarizer into the optical path.

Push in the analyzer IN/OUT knob into the analyzer tube for first-order red compensation to bring the analyzer into the optical path.

Set the polarizer unit for first-order red compensation over the field lens.

Make sure the orientation mark on the polarizer (roughly) comes to the front at this point.

Keep the fixing screw of the polarizer unit loosened.



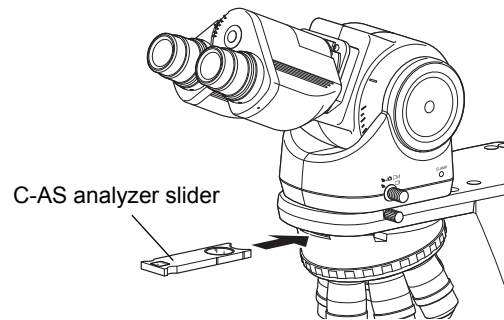
**Bringing the analyzer into the optical path
Installing the polarizer**

✓ Slider-type analyzer for first-order red compensation

Using the C-AS Analyzer Slider for First-order Red Compensation instead of an analyzer tube for first-order red compensation can keep the eye point from rising. To use the C-AS Analyzer Slider for First-order Red Compensation, insert it into the analyzer slot of the nosepiece. Use the analyzer slider as follows:

Push in: The analyzer goes into the optical path.

Pull out: The analyzer is removed from and the dummy hole goes into the optical path (empty).



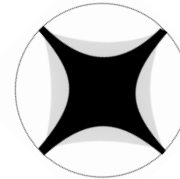
Using D-AS analyzer slider

16 Adjust the orientation of the analyzer and the polarizer.

- (1) Fully open the aperture diaphragm.
- (2) Pull out one eyepiece from the tube.
- (3) Rotate the upper portion of the polarizer unit to remove the lambda plate from the optical path. When doing this, support the polarizer unit for first-order red compensation with hands so that it does not move.
- (4) Look into the eyepiece sleeve and rotate the whole polarizer unit until you can identify a dark cross. (You will see black stripes that change shape as you rotate the polarizer unit.)
- (5) Tighten the fixing screw of the polarizer unit to fix the polarizer.
- (6) Put the eyepiece back to the tube and set the lambda plate back into the optical path.
- (7) Rotate the lambda plate rotation lever to the left/right limit to make sure that the field of view is colored magenta on both sides.

✔ Dark cross (crossed Nicols)

You will see a dark cross when the orientation of the analyzer orthogonally crosses that of the polarizer.



Dark cross

17 Bring a desired objective into the optical path.

Press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad to move the desired objective into the optical path.

18 Focus on the specimen.

- (1) Look into the eyepiece, and adjust the brightness of the field of view by turning the dia-illumination brightness control knob.
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
- (3) If the specimen is not in focus, turn the focus knob to focus on it.

19 Adjust the field diaphragm.

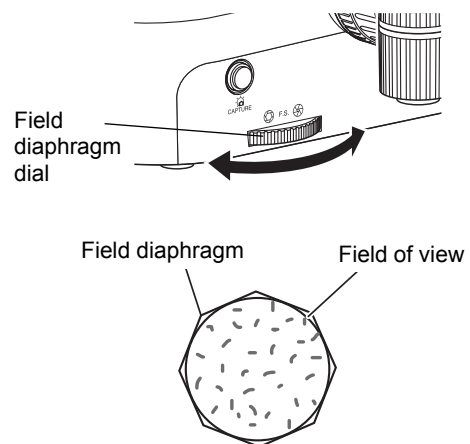
Turn the field diaphragm dial to adjust the field diaphragm so that it almost circumscribes the field of view.

✔ Size of the field diaphragm

Normally, adjust the field diaphragm so that it almost circumscribes the field of view. Opening the field diaphragm too much results in an external stray light entering the field of view, generating flare and reducing image contrast. In addition, the sample will become decolorized over a wider area.

✔ Adjustment timing for the field diaphragm

Be sure to adjust the field diaphragm each time you change the objective.



Circumscribe around the field of view

Adjusting the field diaphragm

20 View the specimen.

- (1) Swing out the lambda plate from the optical path.(The field of view gets darker.)
 - (2) Rotate the stage knob to move the target. If the target is not in focus, use the focus knob to adjust the focus. (The specimen looks brighter in the dark field of view.)
 - (3) Bring the lambda plate back into the optical path.(The background of the field of view will be colored magenta.)
 - (4) Of the needle-like crystals seen in the field of view, check the color of the longitudinal ones.
 - (5) Turn the lambda plate rotation lever from right to left (clockwise) to check the change of color of the crystal being observed.
- Identify the crystal by its change of color.(See the table below.)

Crystal	Position of the lambda plate rotation lever	
	Leftmost	Rightmost
Urate crystal		
Calcium pyrophosphoric acid crystal		

Chap. 1-4

Microscopy Procedures Sensitive Polarization Microscopy

✔ Keep the lambda plate clean

Note that dirt such as dust and fingerprint on the lambda plate can significantly degrade the polarization performance. Keep it clean.

✔ To switch to bright-field microscopy

Pull out the analyzer IN/OUT knob to remove the analyzer from the optical path. Remove the polarizer unit from the field lens.

21 Turn off the power.

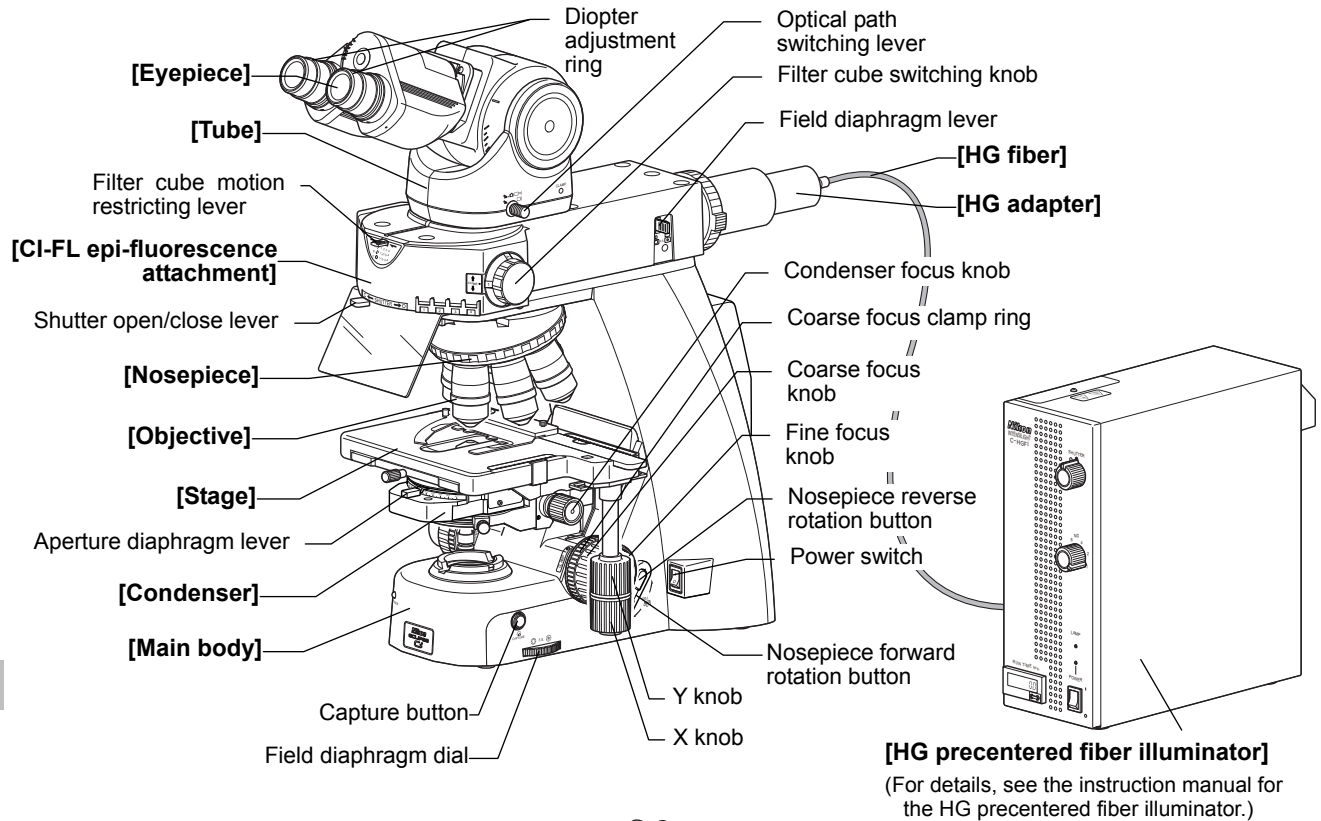
Turn off the power switch (press to the "O" position) for the microscope. (The power LED on the front of the main body will turn off.)

5 Epi-fluorescence Microscopy

5.1 System Configuration and Controls

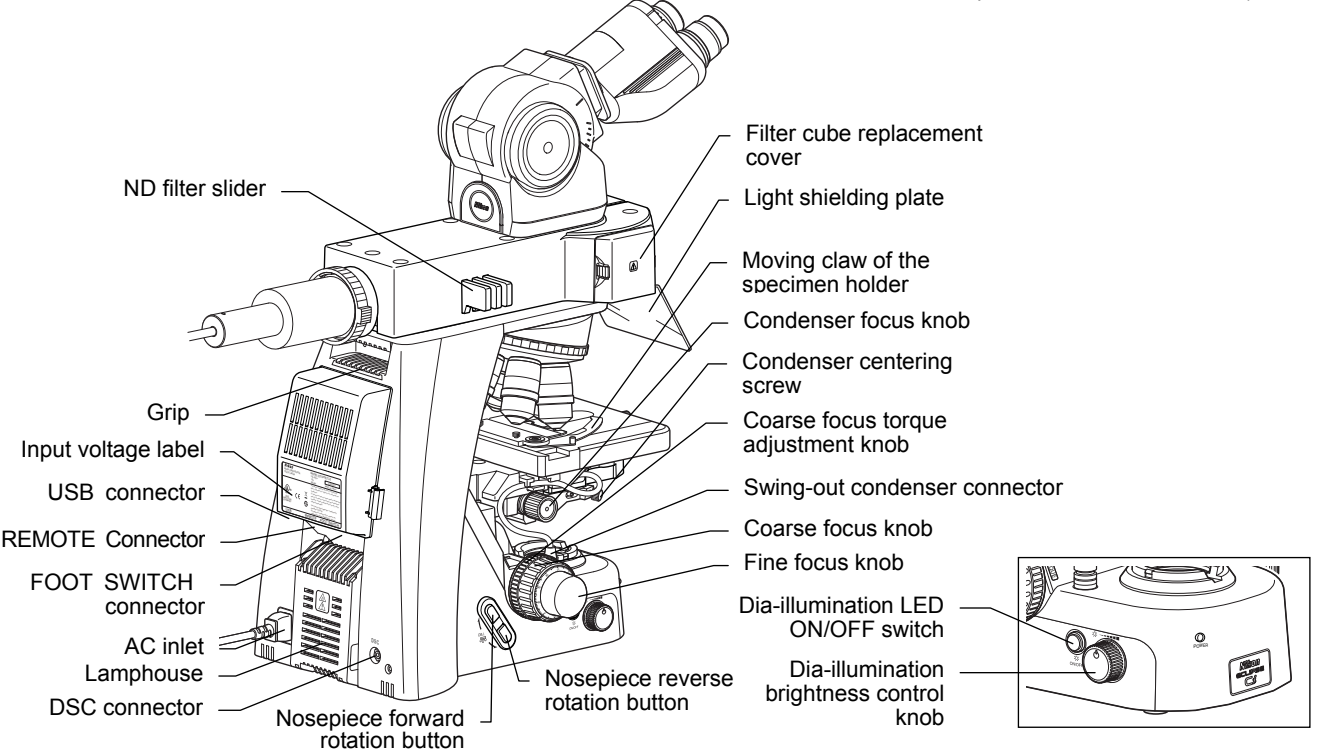
This section explains an example system configuration and the controls required for an epi-fluorescence microscopy using the ECLIPSE Ci-E.

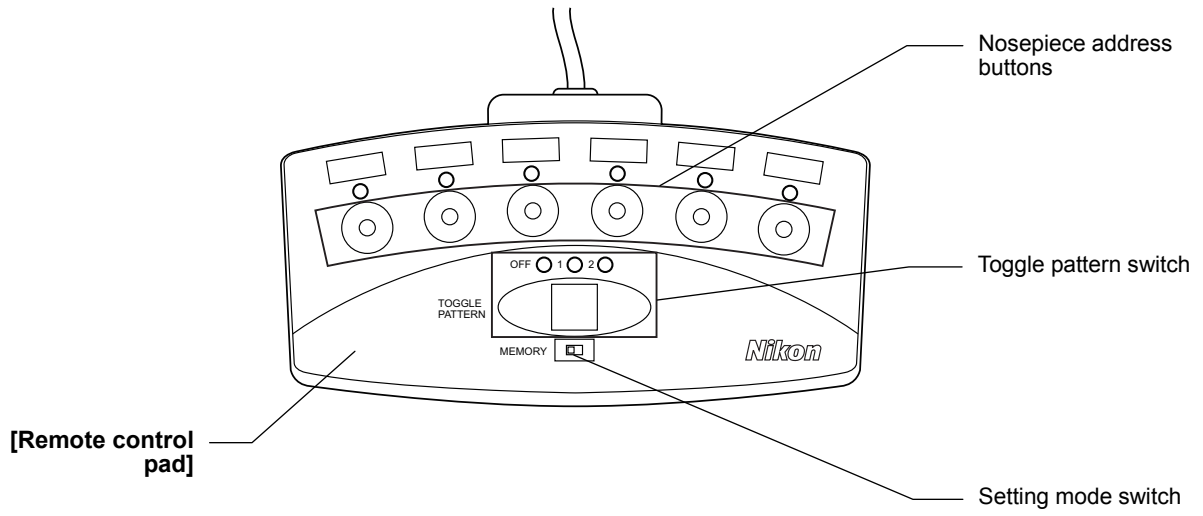
Names of components are denoted in the following manner: **[CI-FL epi-fluorescence attachment]**.



Chap. 1-5

Microscopy Procedures Epi-fluorescence Microscopy





5.2 Epi-fluorescence Microscopy Procedure

! WARNING

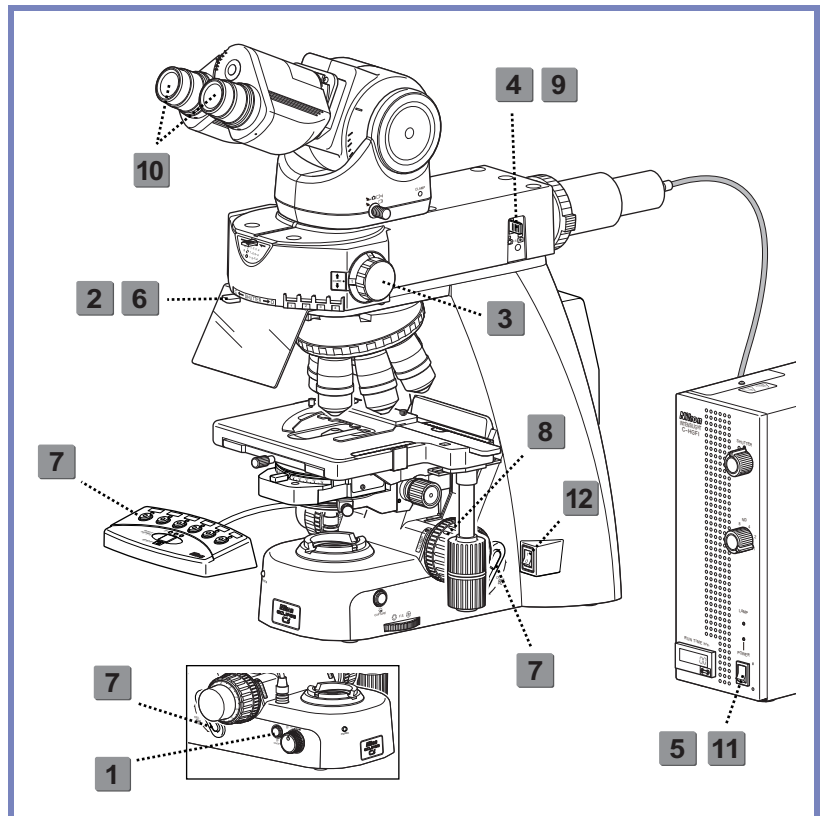
The light source used with the epi-fluorescence attachment (mercury lamp) requires special care during handling because of its characteristics. Make sure you are familiar with and adhere to all warnings and cautions described at the beginning of this instruction manual.

Locate the observation target on the specimen under bright-field microscopy, then proceed to epi-fluorescence microscopy.

(See Chapter 2, "17 Tips for Epi-fluorescence Microscopy" for the tips to locate the observation target on the specimen.)

A CI-FL Epi-fluorescence attachment is used in the following procedure as an example. When using a D-FL Epi-fluorescence attachment, refer to the D-FL Epi-fluorescence attachment Instructions.

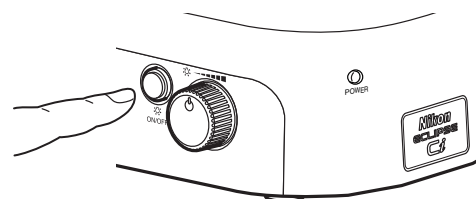
1. Turn ON the dia-illumination LED.
2. Close the shutter.
3. Bring the filter cube into the optical path.
4. Fully open the field diaphragm.
5. Turn on the mercury lamp.
6. Open the shutter.
7. Bring the desired objective into the optical path.
8. Focus on the specimen.
9. Circumscribe the field diaphragm to the field of view.
10. View the specimen.
11. Turn off the mercury lamp.
12. Turn off the power.



Microscopy operation (→See also: Chapter 2, "17 Tips for Epi-fluorescence Microscopy")

1 Turn off the dia-illumination LED.

Press the dia-illumination LED ON/OFF switch to turn off the LED.



Dia-illumination LED switch OFF

Chap. 1-5

Microscopy Procedures Epi-fluorescence Microscopy

2 Close the shutter and block the illumination path.

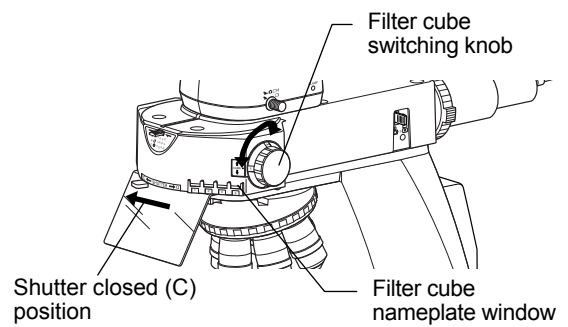
Set the shutter open/close lever of the epi-fluorescence attachment to position “C” to close the shutter and block the optical path.

❗ Shutter of the epi-fluorescence attachment

The shutter blocks illumination.

If the specimen is continuously exposed to the strong light of the mercury lamp, it may become damaged or decolorized.

Be sure to close the shutter when suspending the microscopy or when pausing epi-fluorescence microscopy to perform microscopy with diascopic light. Be sure to get into the habit of performing this operation.



**Closing the shutter
Bringing the filter cube into the optical path**

3 Bring the filter cube into the optical path.

Bring the desired cube into the optical path by turning the filter cube switching knob.

(Confirm the address of the filter cube to be used, and match the switching knob with the number.)

✔ Selecting a filter cube

A filter cube consists of three types of optical components: an excitation filter (EX filter), a barrier filter (BA filter), and a dichroic mirror (DM). Select the filter cube with the appropriate combination of optical components for the characteristics of the specimen and the fluorescence dye.

4 Fully open the field diaphragm of the epi-fluorescence attachment.

Push in the field diaphragm lever of the epi-fluorescence attachment to open the diaphragm fully.

5 Turn on the mercury lamp.

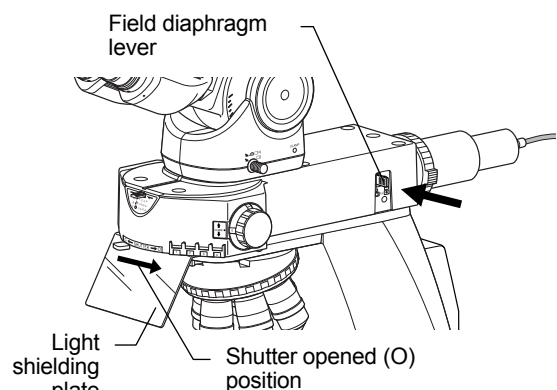
See your illuminator’s manual for details.

6 Open the shutter.

Set the shutter open/close lever of the epi-fluorescence attachment to position “O” to open the shutter.

✔ Light shielding plate of the epi-fluorescence attachment

The light shielding plate protects the observer’s eyes from reflected ultraviolet light, which is originally emitted from the objective at the specimen.



**Fully opening the field diaphragm
Opening the shutter**

7 Bring a desired objective into the optical path.

Press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad to move the desired objective into the optical path.

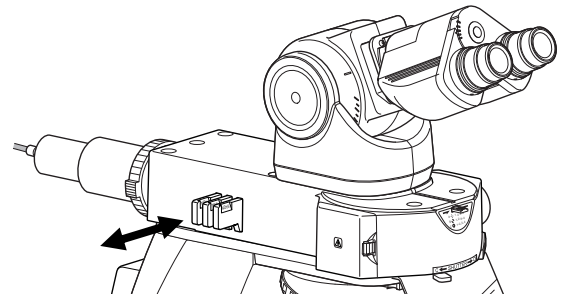
When using an oil immersion type objective, apply immersion oil between the specimen and the objective.
(→Chapter 2 “14 Oil Immersion” for details)

✔ Non-fluorescent immersion oil

Use Nikon designated non-fluorescent immersion oil.

8 Focus on the specimen.

- (1) Look into the eyepiece, and adjust the brightness of the field of view with the ND filter of the epi-fluorescence attachment.
- (2) Rotate the stage knob to move the stage and bring the target into the optical path.
- (3) If the specimen is not in focus, turn the focus knob to focus on it.



Brightness adjustments with ND filters

9 Adjust the field diaphragm of the epi-fluorescence attachment.

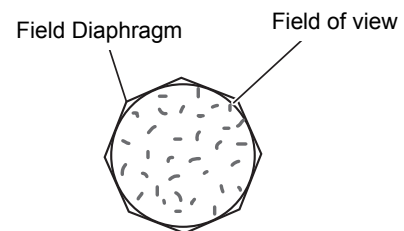
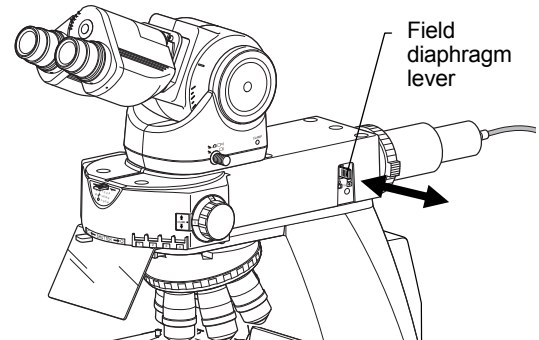
Use the field diaphragm lever of the epi-fluorescence attachment to adjust the field diaphragm so that it almost circumscribes the field of view.

✔ Size of the field diaphragm

Normally, adjust the field diaphragm so that it almost circumscribes the field of view. Excessively opening the field diaphragm will result in stray light entering the field of view, generating flare and reducing the image contrast. In addition, the specimen will become decolorized over a wider area.

✔ Adjustment timing for the field diaphragm

Be sure to adjust the field diaphragm each time you change the objective.



Circumscribe around the field of view

Adjusting the field diaphragm

10 View the specimen.

Rotate the stage knob to move the target. If the target is not in focus, use the focus knob to adjust the focus.

✔ Diascopic image in fluorescence observation

For fluorescence observations, press the dia-illumination LED ON/OFF switch to make the diascopic image disappear.

Bright ambient lights will make it difficult to view the image. Nikon recommends keeping the room dark during fluorescence observations.

✔ To return to bright-field microscopy

- Close the shutter of the epi-fluorescence attachment and block the light emitted by the mercury lamp.
- Press the dia-illumination LED ON/OFF switch to turn on the dia-illumination LED.
- Turn the filter cube switching knob and bring the position where a filter cube is not located into the optical path.

11 Turn off the mercury lamp.

See your illuminator's manual for details.

12 Turn off the power.

Turn off the power switch (press to the "O" position) for the microscope. (The power LED on the front of the main body will turn off.)

2

Individual Operations

1

Adjusting the Brightness of a Diascopic Image

The brightness of a diascopic image can be adjusted by turning the dia-illumination brightness control knob or by removing/inserting ND filters from or into the optical path.

1.1

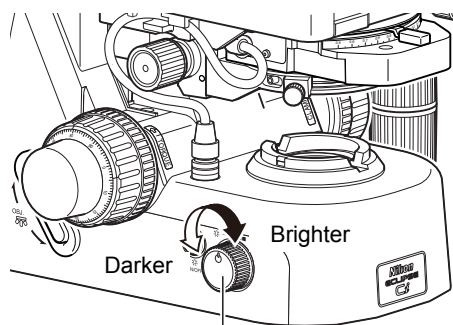
Adjustment with the Dia-illumination Brightness Control Knob

Turning the dia-illumination brightness control knob to change the brightness of an LED allows you to adjust the brightness of a diascopic image. The brightness control knob can be used with click-less operability.

The brightness control knob can be turned without limit, but the brightness of the dia-illumination LED can only be adjusted within the set range. The brightness can be adjusted from minimum to maximum with approximately two rotations of the control knob.

Brightness Control Knob Rotation and Brightness of the Image

Brightness control knob	Image brightness
Clockwise rotation	Brighter
Counterclockwise rotation	Darker



Dia-illumination brightness control knob

Brightness control of dia-illumination

1.2

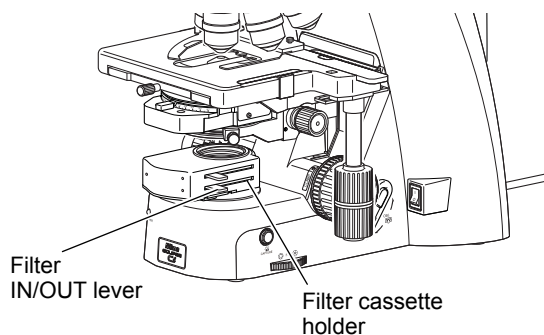
Adjustment with ND Filters

ND filters are used to adjust light intensity. Higher filter numbers correspond to lower transmittance (i.e., darker images). The color balance of the image will not change.

You can adjust the brightness of a diascopic image by adding an ND filter, either by placing the ND filter over the field lens, or by setting the ND filter in the filter cassette holder mounted on the field lens part.

The filter cassette holder can house up to three $\phi 45$ mm filters with a thickness of up to 3 mm. Pushing in the filter IN/OUT lever inserts the ND filters into the optical path. In addition, one filter can be placed on the filter cassette holder.

The filter cassette holder cannot be used with a simple polarizer, polarizer unit for first-order red compensation, or the spacer for the nosepiece.



Adjusting the brightness with ND filters

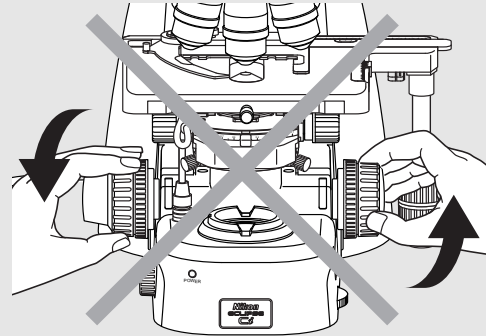
2

Focusing on the Specimen (Vertical Stage Movement)

! Notes on controlling the focus knobs

Avoid the following actions, which can cause equipment malfunctions.

- Rotating the right and left focus knobs in opposite directions.
- Rotating the coarse focus knob past the limit.

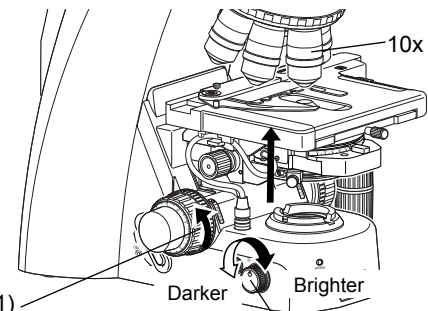


Don't rotate the knobs in opposite directions!

Turn the coarse or fine focus knob to raise or lower the stage and shift the focus onto the specimen.

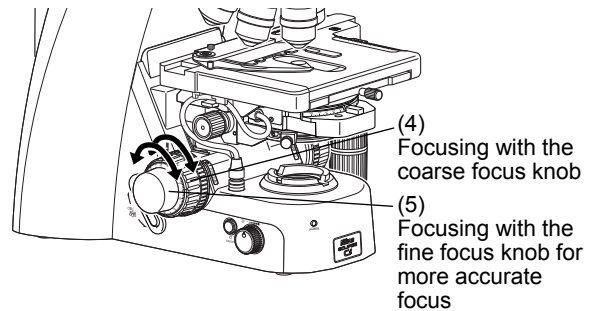
Using an objective of high magnification may cause the specimen to be pushed against the objective, damaging the objective. Follow the procedure below to focus on the specimen to avoid breaking the cover glass or damaging the objective.

- (1) Bring the 10x objective into the optical path and turn the coarse focus knob away to raise the stage to the upper limit.
- (2) When using the trinocular tube or ergonomic tube, push in the optical path switching lever to distribute 100% light to the binocular section.
- (3) Look into the eyepiece, and adjust the brightness of the field of view by turning the dia-illumination brightness control knob.
- (4) Look into the eyepiece and turn the coarse focus knob slowly to lower the stage and focus on the specimen. Release your hand from the coarse focus knob once it has been focused.
- (5) Turn the fine focus knob to adjust the focus more accurately.



- (1) Select the 10x objective and use the coarse focus knob to raise the stage to the upper limit.
- (3) Adjust the brightness of the field of view.

Bringing the 10x objective into the optical path, raising the stage to the upper limit



Using the coarse focus knob to focus → Using the fine focus knob for more accurate focus

✓ Raising the stage with the coarse focus knob

When moving the stage with the coarse focus knob, move your eyes away from the eyepiece and operate the microscope while looking at it from the side.

✓ Performing coarse operation while looking into the eyepiece

When working with the coarse focus knob while looking into the eyepiece, you should only turn the knob in the direction for lowering the stage.

✓ Switching to an objective of higher magnification

First use an objective of low magnification to adjust the focus, then switch to an objective of higher magnification.

✓ Operating distance

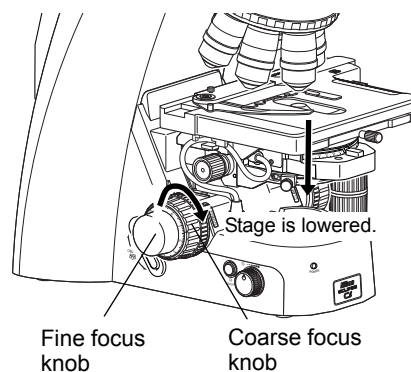
As a 10x or 4x objective has a wider operating distance, the specimen never touches the tip of the objective even if the stage is raised to the upper limit, provided that a slide and a cover glass of standard thickness are used. (Standard thickness being 1.2 mm for the slide glass, and 0.17 mm for the cover glass.)

2.1 Focus Knob Rotation and Stage Movement

Both the coarse focus knob and the fine focus knob are located on both right and left sides on the microscope. The table below shows the relationship of the focus knobs' rotation with the stage movement.

Focus Knob Rotation and Stage Movement

Operations	Stage movement
Turn the knob toward the front.	Stage is lowered.
Turn the knob toward the back.	Stage is raised.



Stage vertical movement

2.2 Number of Focus Knob Turns and Distance of Stage Travel

Number of Focus Knob Turns and Distance of Stage Travel

No. of knob turns	Distance of stage travel (vertical direction)
One rotation of the coarse focus knob	Approx. 9.33 mm
One rotation of the fine focus knob	Approx. 0.1 mm
One scale of the fine focus knob	1 μ m

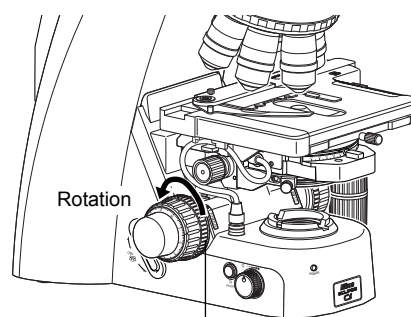
The vertical motion range (coarse/fine focus stroke) of the stage is from approximately 2 mm above the focal point (reference position) to approximately 28 mm below the focal point.

2.3 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 knob (TORQUE) located at the base of the coarse focus knob. If the torque is set too low, the stage may descend under its own weight.

Adjusting the Rotating Torque of the Coarse Focus Knob

Torque adjustment knob operation	Rotating torque
When turned in the direction of the arrow	Rotation torque is increased.
When turned in the direction opposite to the arrow	Makes knob easier to turn.



Coarse focus torque adjustment knob

Adjusting the torque of the focus knobs

2.4 Refocusing

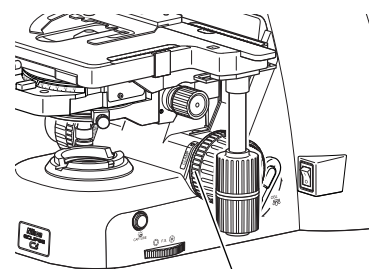
By turning the coarse focus clamp ring after focusing on the specimen, you can prevent the stage from being raised further with the coarse focus knob. The movement of the stage with the fine focus knob will not be locked.

Using this function, you can refocus with ease by simply turning the coarse focus knob to the limit. This is helpful when switching between similar specimens during the observation.

- (1) With the focus set on the specimen, tighten the coarse focus clamp ring by turning it approximately 3/4 of a rotation in the direction of the arrow on the base of the microscope. This will clamp the movement of the coarse focus knob.
- (2) When replacing the specimen, lower the stage by using only the coarse focus knob.
- (3) After replacing the specimen, use only the coarse focus knob to raise the stage slowly until it reaches the upper limit.

At the upper limit, the focus should be more or less on the specimen. Use the fine focus knob for finer adjustment.

If you do not wish to use the refocusing function, be sure to loosen the coarse focus clamp ring to the limit (turn it in the direction opposite to the arrow on the base of the microscope until it hits the limit).



Coarse focus clamp ring

Refocusing

2.5

Position Exchange of the Fine Focus Knob

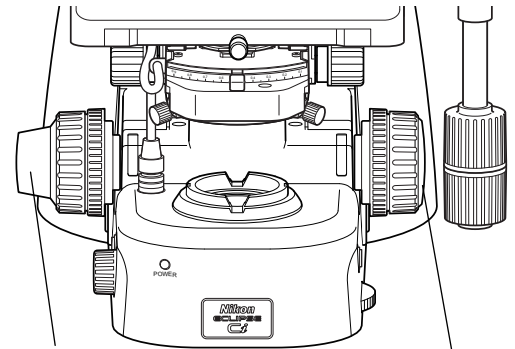
Among left and right fine focus knobs, one is flat and the other is convex. Both fine focus knobs are attached to the coarse focus knobs using magnet, so you can detach the right and left knobs from the coarse focus knobs and swap them.

Position them to best suit your usage.

The flat fine focus knob mounted as a standard component has 2 mm projection. A knob having 7 mm projection is available as an option.

✔ **Removing a flat focus knob**

A flat fine focus knob can be easily removed by inserting a provided tool into the notch in the knob.



Convex fine focus knob

Flat fine focus knob

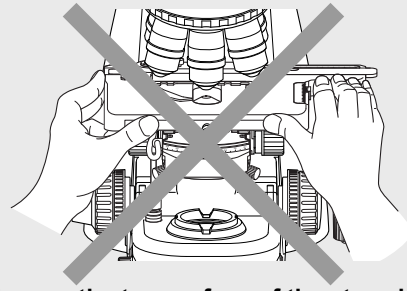
Position exchange of the fine focus knob

3 Bringing the Specimen into the Optical Path (Horizontal Stage Movement)

⚠ Note on moving the stage

Avoid the following actions, which can cause equipment malfunctions.

- Moving the stage to the right and left by holding the top surface of the stage directly.
- Moving the stage past its X/Y stroke.
- Turning the stage X/Y knobs past its limit point.

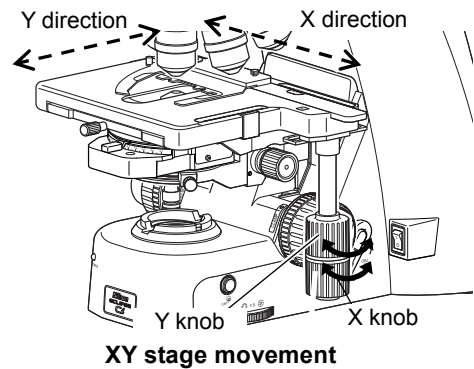


Do not move the top surface of the stage by hand!

Turning the stage knob moves the upper plate of the stage in the X and Y directions so that you can move the stage to bring the target into the optical path. This illuminates the sample sealed under the cover glass.

3.1 Knob Rotation and Stage Movement

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



3.2 Adjusting the Knob Heights

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

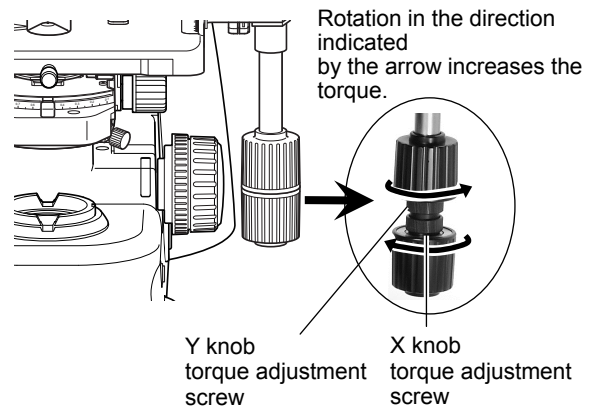
3.3 Adjusting the Knob Rotation Torque

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 it closer to the respective knob increases rotational torque. (To increase the rotational torque, turn the adjustment screw counterclockwise for the Y knob and clockwise for the X knob, as viewed from above.)

Avoid loosening these screws excessively. If they are too loose, the top surface of the stage may move, even when touched very lightly.

⚠ Note on loosening torque adjustment screws

- If the screws are too loose, the top surface of the stage may move, even when touched very lightly.
- If the X and Y knobs are in direct contact with each other, turning one of them may turn them both. Make sure there is space between the X and Y knobs.



4 Adjusting the Diopter

The diopter adjustment ring on an eyepiece can be adjusted to match the eyesight of your right and left eyes.

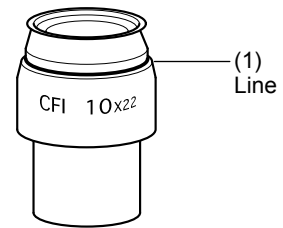
A properly adjusted diopter compensates for differences in visual acuity between the right and left eyes of a person, making binocular observation easier. It also minimizes focal deviations when switching magnification, optimizing the performance of the objective.

Adjust the diopter settings for both eyepieces.

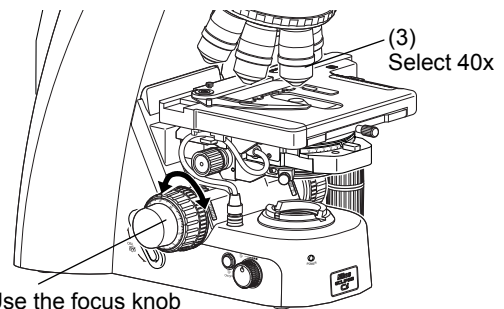
✔ Notch on eyepiece

The eyepiece has a notch to prevent rotation. When attaching, match the notch with the protrusion on the eyepiece sleeve. Otherwise, eyepiece is not attached to the correct position.

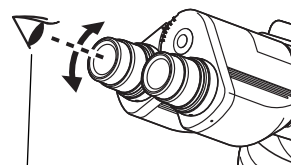
- (1) Turn the diopter adjustment ring on the right and left eyepieces to align the end face of the diopter adjustment ring with the line. (This is the diopter adjustment reference position.)
- (2) Follow Steps 1 through 9 in Chapter 1 “1.2 Bright-field Microscopy Procedure” to focus on the specimen using the 10x objective.
- (3) Press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad to bring the 40x objective into the optical path. Adjust the focus onto the specimen by turning the coarse focus knob and then the fine focus knob.
- (4) Bring the 10x (or 4x) objective into the optical path.
- (5) While looking into the left eyepiece with your left eye without touching the focus knob, focus on the specimen by turning the left diopter adjustment ring.
- (6) While looking into the right eyepiece with your right eye without touching the focus knob, focus on the specimen by turning the right diopter adjustment ring.
- (7) Repeat Steps (3) through (6) to make sure the focus has been adjusted properly.



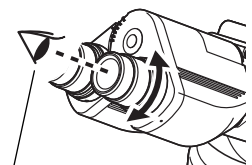
Reference position for diopter adjustment



- (4) Select 10x or 4x.



- (5) Look into the left eyepiece with your left eye to focus with the left diopter adjustment ring.



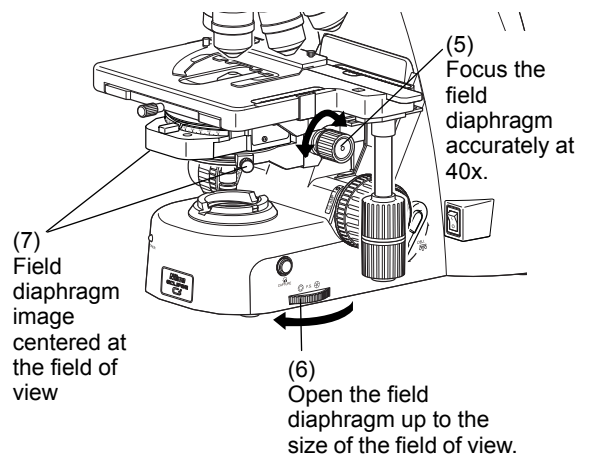
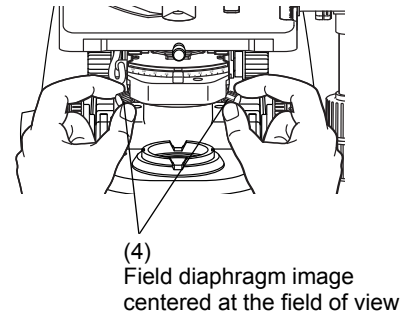
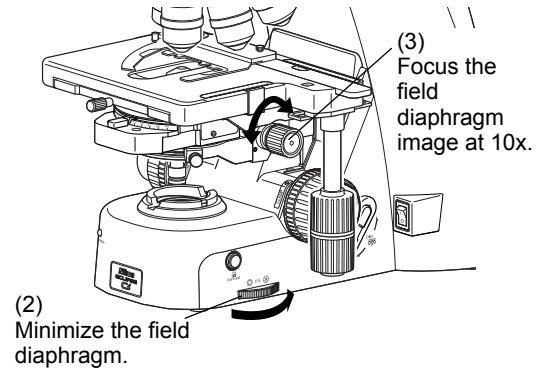
- (6) Also look into the right eyepiece with your right eye to focus with the right diopter adjustment ring.

Adjusting the diopter

5 Focusing and Centering the Condenser

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

- (1) Follow Steps 1 through 9 in Chapter 1 “1.2 Bright-field Microscopy Procedure” to focus on the specimen using the 10x objective.
- (2) Turn the field diaphragm dial counterclockwise fully to minimize the field diaphragm.
- (3) You can see the diaphragm image in the field of view as you look into the eyepiece. Use the condenser focus knob to adjust so that the field diaphragm image can be outlined clearly.
- (4) Adjust the condenser centering screws until the field diaphragm image is at the center of the eyepiece field of view.
- (5) Set the 40x objective into the optical path. Bring the 40x objective into the optical path. As you see the field diaphragm image, if the outline of the image is out of focus, use the condenser focus knob to adjust the focus as much as possible.
- (6) Turn the field diaphragm dial to adjust so that the field diaphragm image size is almost the same as the field of view.
- (7) If the center of the field diaphragm image is not centered, turn the condenser centering screws to move the field diaphragm image to the center of the field of view. This is easiest if you adjust the field diaphragm aperture so that it is slightly smaller than the eyepiece field of view.

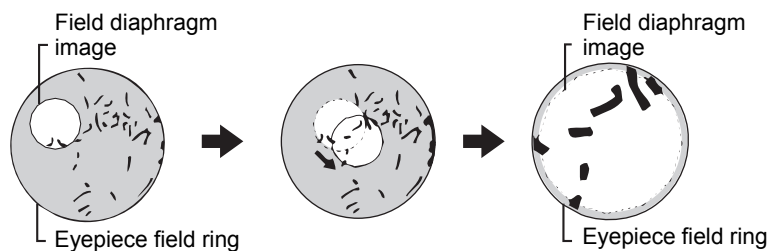


✔ Correct focusing of the field diaphragm image

If the outline of the field diaphragm image is reddish or bluish, you have turned the condenser focus knob too much. When the outline is colorless, focusing is correct.

✔ Field diaphragm image view with the 40x objective

The field diaphragm image that has been focused with the 40x objective can not be seen so clearly as the one focused with the 10x objective.



Focusing and centering the condenser

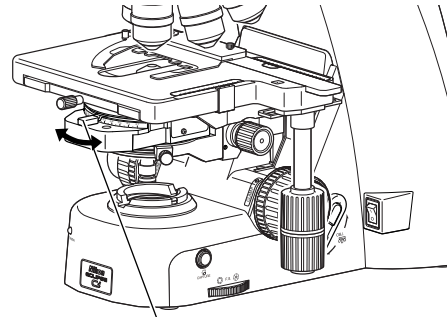
6 Adjusting the Aperture Diaphragm

The aperture diaphragm is used to adjust the illumination angular aperture and is important as it affects the resolution, contrast, focal depth, and brightness of an optical image.

Turning the condenser aperture diaphragm lever changes the size of the aperture diaphragm.

Generally, aperture settings at 70 to 80% of the numerical aperture of the objective will provide satisfactory images with suitable contrast.

A small aperture diaphragm opening reduces resolution and brightness but increases contrast and depth of focus. On the contrary, a large aperture diaphragm size increases resolution and brightness but reduces contrast and depth of focus. These characteristics involve inherent tradeoffs and cannot be optimized independently.



Aperture diaphragm lever

Adjusting the aperture diaphragm

Relationship of the aperture diaphragm size with the optical image's state

Aperture diaphragm	Resolution	Brightness	Contrast	Focal depth
Stop down	Lower	Darker	Larger	Deeper
Open	Higher	Brighter	Lesser	Shallower

✔ Proper size of the aperture diaphragm

Normally, 70 to 80% of the numerical aperture of the objective is the proper size. 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 numerical aperture of the objective.

✔ Adjustment timing for the aperture diaphragm

Be sure to adjust the aperture diaphragm each time you change the objective.

6.1 Adjusting the Aperture Diaphragm Using the Condenser Scale

The scale on the condenser indicates the numerical aperture. The index on the aperture diaphragm lever should be aligned with the scale line that corresponds to 70 to 80% of the numerical aperture of the objective.

The numerical aperture is indicated on the side of the objective.

For a numerical aperture of 0.75, the index should be aligned with the scale on the condenser at 0.525 to 0.6.

Plan 40X
40x / 0.75
∞ / -WD

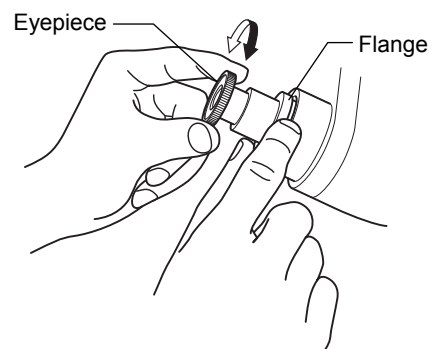


Indication for 40x magnification / numerical aperture 0.75

Proper numerical aperture:
 0.75×0.7 to $0.8 = 0.525$ to 0.6

6.2 Adjusting the Aperture Diaphragm Using the Centering Telescope

- (1) Remove one eyepiece and attach the centering telescope in place.
- (2) Turn the aperture diaphragm lever 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.
- (3) Turn the aperture diaphragm lever to adjust the aperture. Normally, the aperture diaphragm should be adjusted to around 70 to 80% of the size of the field of view.
- (4) Remove the centering telescope and reattach the eyepiece.



Adjustment using the centering telescope

7 Selecting a Condenser

Select a condenser optimal for the magnification of the objective and the microscopy procedure.

Selecting the Magnification of the Objective and Condenser

Objective's magnification	Condenser (⊙: Optimum, ○: Suitable, ×: Not suitable)						
	Achromat aplanatic condenser	Swing-out condenser	Motorized swing-out condenser	Achromat condenser	Abbe condenser	Swing-out condenser 1-100x	Slide achro condenser 2-100x *4
1x	×	×	×	×	×	○*2	×
2x	×	○*2	○*2	×	×	⊙*2	⊙*3
4x	×			○*1	○*1		
10x to 100x	⊙	○	○	○	○	⊙	

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

*2: Swing out the top lens before use.

*3: For objectives of 10x or higher magnification, pull out the slide. For objectives with 2x or 4x magnification, push in the slide to prevent vignetting.

*4: Do not use the 2-100x slide achromat condenser with an achromat objective or a plan achromat objective.

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

For example, when an objective with a numerical aperture of 1.4 is used, the maximum aperture of the swing-out condenser or the Abbe condenser will only be about 65% of the NA of the objective, even when the condenser's aperture diaphragm is wide open.

Refer to Section 16 "Tips for the Phase Contrast Microscopy" for the phase turret condenser.

8 Adjusting the Field Diaphragm

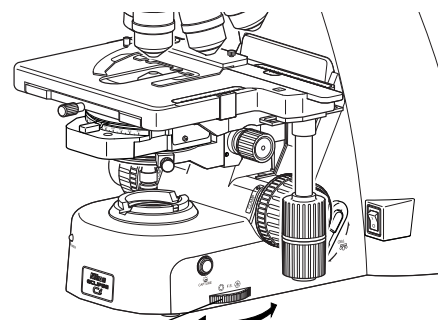
Chap. 2

Individual Operations

The field diaphragm is used to restrict illumination to the area of the specimen being viewed.

Turning the field diaphragm dial changes the size of the field diaphragm.

For normal observations, the size of the diaphragm should almost circumscribe the field of view.



Adjusting the field diaphragm

✓ Proper size of the field diaphragm

Usually, the size is optimal when it almost circumscribes the field of view. Opening the field diaphragm too much and illuminating a broader area than necessary will result in stray light entering the field of view, generating flare and reducing the image contrast. In addition, the specimen will become decolorized over a wider area.

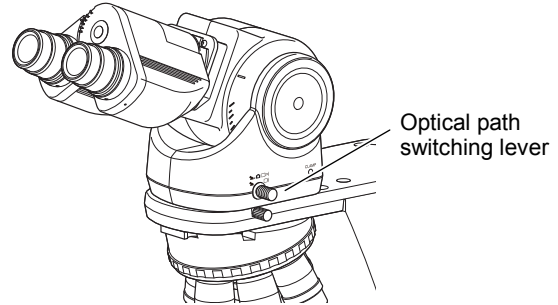
✓ Adjustment timing for the field diaphragm

Be sure to adjust the aperture diaphragm each time you change the objective.

9 Switching the Optical Path of the Tube

9.1 Light 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.



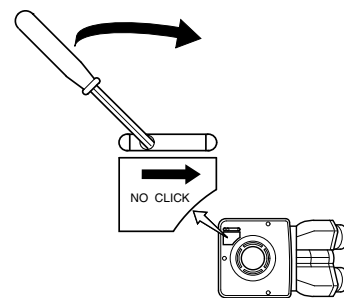
Switching the optical path of the tube

Optical Path Switching Lever and Distribution of Light

	Position of the optical path switching lever	Light distribution (%)	
		Binocular section	Camera port
C-TE2 Ergonomic tube	Pushed in	100	0
	Pulled out	50	50
C-TT trinocular tube T	Pushed in	100	0
	Pulled out by one notch	20	80
	Pulled out by two notches	0	100
C-TF trinocular tube F	Pushed in	100	0
	Pulled out	0	100

9.2 Disabling the Clicking of the Optical Path Switching

C-TT trinocular tube T and C-TF trinocular tube F have a “NO CLICK” switch on their tube attaching surface. 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.

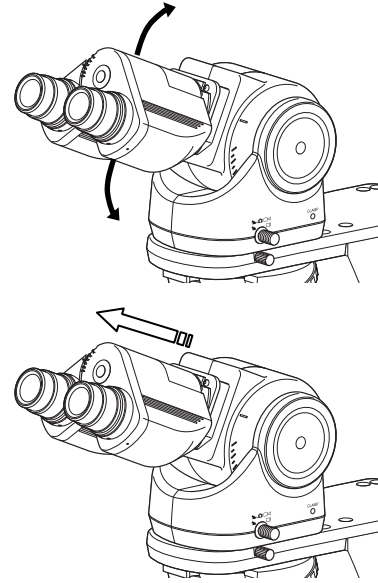


Disabling the clicking of the optical path switching

10 Adjusting the Binocular Section

10.1 Adjusting with the Binocular Section of the Tube

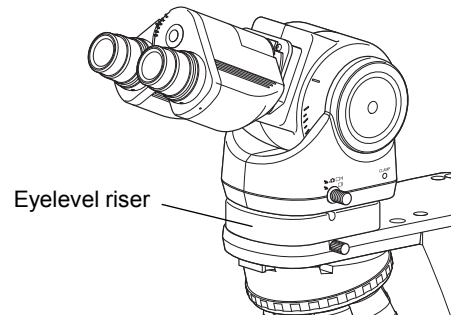
The ergonomic tube makes it possible to tilt and extend the binocular section. Adjust the position of the binocular section for most comfortable viewing.



Adjusting the binocular section

10.2 Using the Eyelevel Riser

Inserting the eyelevel riser between the tube and the microscope arm allows you to bring the eye point 25 mm higher.



Using the eyelevel riser

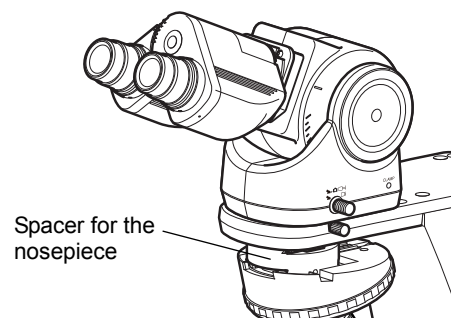
11 Using at Lowered Position of Stage (Spacer for Nosepiece)

Inserting a spacer for the nosepiece between the arm and the nosepiece allows for operation with the stage set 20 mm lower.

A lowered stage facilitates specimen replacement during a cytodiagnostic examination, etc.

✔ Using the filter cassette holder not allowed

The filter cassette holder placed on the field lens is not allowed to use together with the spacer for nosepiece.



Using the spacer for the nosepiece

12 Switching Objectives

12.1 Basic Operation

To switch the objective, press the nosepiece forward/reverse rotation buttons on the main body, or the nosepiece address button on the remote control pad.

✔ **Setting mode switch**

During microscopy, keep this switch set to the left side (operation mode).

✔ **Disabling the reverse rotation from Address 1 to 6**

Normally, the nosepiece rotates in the direction that is the shorter distance to the specified address. However, when the reverse rotation lock is enabled for the rotation from Address 1 to 6, a direct movement from Address 1 to the next Address 6 can be disabled. See Section 13.3 “Reverse Rotation Lock (Rotation from Address 1 to 6)” when using the reverse rotation lock.

✔ **Brightness during nosepiece rotation**

The brightness of the dia-illumination LED degrades during the nosepiece rotation, but it is not a failure.

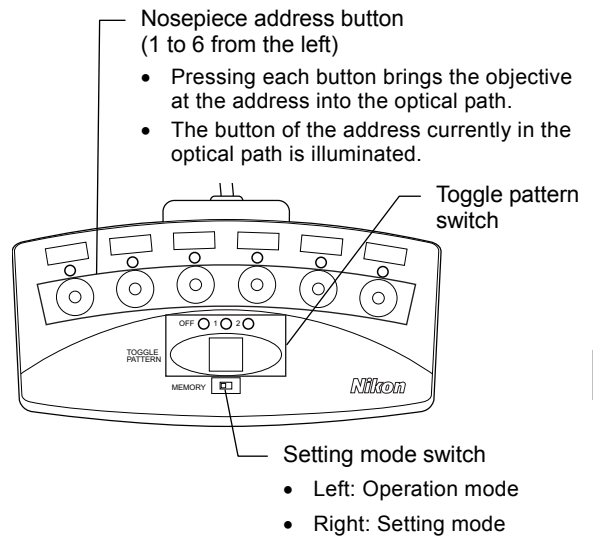
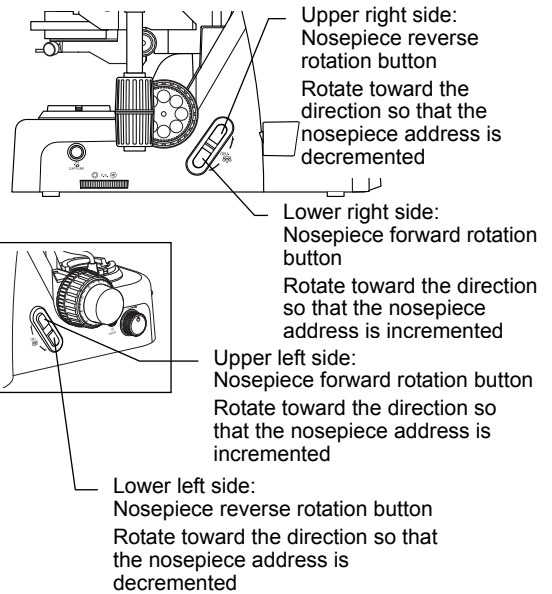
The following is interlocked with the switching of the objective:

• **Brightness of the dia-illumination LED**

The brightness previously set for the address is automatically set. The brightness is reset once the illumination is turned off, and is set to the initial brightness upon next use. For details on setting the default brightness, refer to “13.2 Changing the Initial Brightness of Dia-illumination LED”.

• **Condenser top lens swing-out**

The condenser top lens is interlocked only when using a motorized swing-out condenser. See “13.1 Configuring the Motorized Swing-out Condenser” for the settings.



12.2 Switching between Two Objectives (Toggle)

Toggle function allows you to view the specimen while alternately switching between two objectives configured in advance. To switch between the objectives, use the nosepiece forward/reverse rotation buttons. Two pairs of objectives can be configured: toggle pattern 1 and toggle pattern 2.

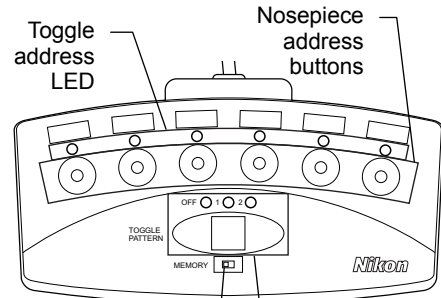
Configuring a toggle pattern

Configure the pair of objectives to be used with the toggle function as a “toggle pattern.”

[Factory setting]

Toggle pattern 1: Address 1 and Address 2

Toggle pattern 2: Address 3 and Address 4

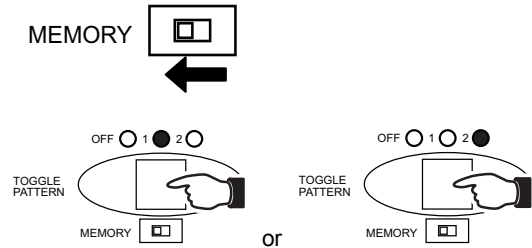


- Setting mode switch
- Left: Operation mode
 - Right: Setting mode
- Toggle pattern switch
- Toggle function OFF
 - Toggle pattern 1
 - Toggle pattern 2

[Procedure]

- (1) With the setting mode switch set to the left side (operation mode), press the toggle pattern switch to select the toggle pattern (1 or 2).

Pressing the toggle pattern switch will switch between “toggle function OFF,” “toggle pattern 1,” and “toggle pattern 2” in order.



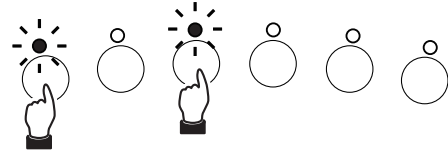
- (2) Set the setting mode switch to the right side to switch to the setting mode.

The toggle address LEDs for the configured toggle pattern will blink.



- (3) Press a nosepiece address button to select the two addresses to be used with the toggle function.

The LED for the specified address will blink. Two addresses can be specified for each toggle pattern. Specifying third address cancels the address you specified first.



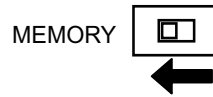
(Example of address 1 and address 3 specified)

✔ When an undesired address is specified

If an undesired address is specified, pressing the toggle pattern switch restores a previous setting.

- (4) **Set the setting mode switch to the left side to operation mode.**

Nosepiece address buttons 1 to 6 light for a few seconds to indicate that setup is complete.



✔ Setup via DS-L4 Microscope Camera Control Unit or a PC

The toggle pattern can also be configured via DS-L4 Microscope Camera Control Unit or Ni Setup Tool. See the respective instruction manual for the details of settings with DS-L4 and Ni Setup Tool.

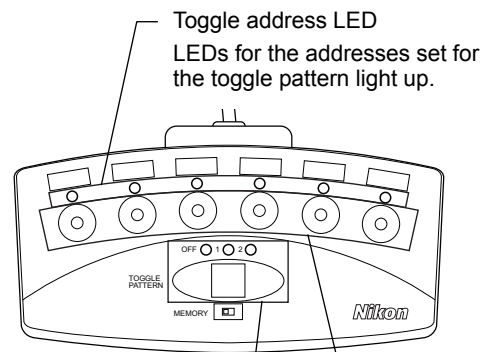
Operation when using the toggle function

To use the toggle function, press the toggle pattern switch to select “toggle pattern 1” or “toggle pattern 2.”

To switch between the two objectives, press the nosepiece forward/reverse rotation buttons (the two buttons are not differentiated when using the toggle function).

✔ Nosepiece address buttons

Pressing a nosepiece address button when using the toggle function allows you to bring a desired address to the optical path.



- Toggle pattern switch
- Toggle function OFF
 - Toggle pattern 1
 - Toggle pattern 2
- Nosepiece address buttons

To stop using the toggle function, press the toggle pattern switch until the toggle function is turned OFF.

Nosepiece Operation

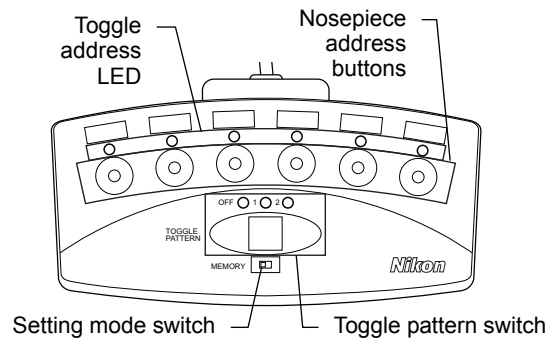
Operation	Microscope main body		Remote control pad
	Nosepiece forward rotation button	Nosepiece reverse rotation button	Nosepiece address buttons
Toggle function OFF	Nosepiece forward rotation (incrementing the address)	Nosepiece reverse rotation (decrementing the address)	Rotate to the specified address
Toggle pattern 1	Switching between two addresses configured in the toggle pattern 1.		
Toggle pattern 2	Switching between two addresses configured in the toggle pattern 2.		

13 Configuring the Motorized Functions

The swing-out operation of the motorized swing-out condenser, initial brightness of the dia-illumination LED, and reverse rotation lock from address 1 to 6 are configured using the remote control pad and buttons on the main body.

✔ Setup via DS-L4 Microscope Camera Control Unit or a PC

Motorized functions can also be configured via DS-L4 Microscope Camera Control Unit or Ni Setup Tool. See the respective instruction manual for the details of settings with DS-L4 and Ni Setup Tool.



Remote control pad buttons used for configuration of motorized functions

13.1 Configuring the Motorized Swing-out Condenser

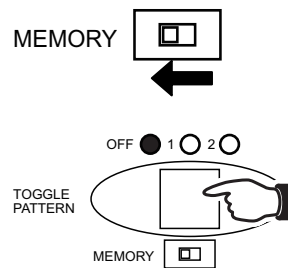
If the motorized swing-out condenser is attached, you can configure the system so that the condenser top lens is automatically swung out when switching to the low-magnification objective.

[Factory setting]

Address 1: Swung out (removed from the optical path)
Address 2 to 6: Brought into the optical path

[Procedure]

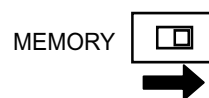
- With the setting mode switch set to the left side (operation mode), press the toggle pattern switch until the toggle function is turned OFF.



- Set the setting mode switch to the right side to switch to the setting mode.

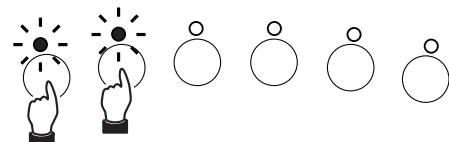
Condenser top lens configuration is indicated by the toggle address LED.

Blinking: Swung out (removed from the optical path)
OFF: Brought into the optical path



- Press a nosepiece address button to configure top lens swing-out for that particular address.

The function is switched between enabled and disabled each time the button is pressed.



(Example of address 1 and address 2 specified)

- Set the setting mode switch to the left side to operation mode.

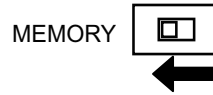
Nosepiece address buttons 1 to 6 light for a few seconds to indicate that setup is complete.



13.2 Changing the Initial Brightness of Dia-illumination LED

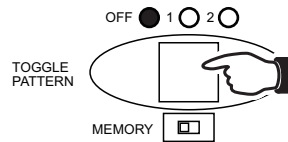
The brightness of the dia-illumination LED is saved for each address of the nosepiece, and the brightness previously set for the address is automatically set if the nosepiece is switched. Note that the brightness is reset once the illumination is turned off, and is set to the configured initial brightness upon next use. The initial brightness can be changed as follows:

- (1) Check that the setting mode switch is set to the left side (operation mode).

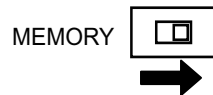


- (2) Bring each of the nosepiece addresses into the optical path and adjust the brightness for all addresses using the dia-illumination LED brightness control knob.

- (3) (If the toggle function is enabled) Press the toggle pattern switch until the toggle function is turned OFF.



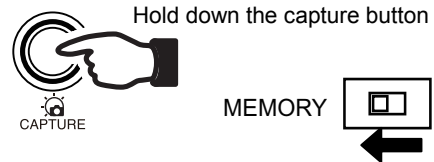
- (4) Set the setting mode switch to the right side to switch to the setting mode.



- (5) Hold down the capture button on the main body, and set the setting mode switch to the left side to go back to the operation mode.

Nosepiece address buttons 1 to 6 light for a few seconds to indicate that setup is complete.

For each of the nosepiece addresses, the brightness set in step (2) is set as the initial brightness.



13.3 Reverse Rotation Lock (Rotation from Address 1 to 6)

Normally, the nosepiece rotates in the direction that is the shorter distance to the specified address. However, when the reverse rotation lock is enabled for the rotation from Address 1 to 6, a direct movement from Address 1 to the next Address 6 can be disabled.

✔ Nosepiece address buttons

Even with the lock function enabled, nosepiece address buttons can be used to directly select an objective and bring it into the optical path. (For example, pressing the button for nosepiece address 6 when address 1 is in the optical path, the nosepiece rotates in the order of 1 → 2 → 3 → 4 → 5 → 6 and address 6 enters into the optical path.)

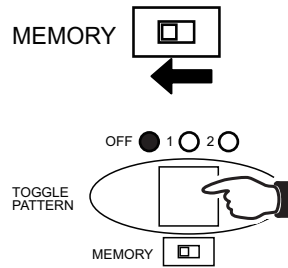
The remote control pad and buttons on the microscope main body are used to set the reverse rotation lock.

[Factory setting]

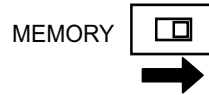
Reverse rotation lock of rotation from Address 1 to 6: Disabled

[Procedure]

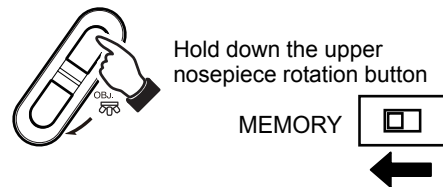
- (1) **With the setting mode switch set to the left side (operation mode), press the toggle pattern switch until the toggle function is turned OFF.**



- (2) **Set the setting mode switch to the right side to switch to the setting mode.**



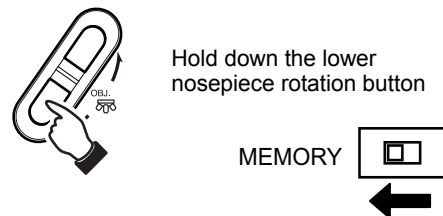
- (3) **To enable the reverse rotation lock from address 1 to 6: Hold down the upper nosepiece rotation button on the main body (nosepiece reverse rotation button on the right or nosepiece forward rotation button on the left), and move the setting mode switch to the left side to go back to the operation mode.**



Nosepiece address buttons 1 to 6 light for a few seconds to indicate that setup is complete.

To disable the reverse rotation lock from address 1 to 6:

Hold down the lower nosepiece rotation button on the main body (nosepiece forward rotation button on the right or nosepiece reverse rotation button on the left), and set the setting mode switch to the left side to go back to the operation mode.



Nosepiece address buttons 1 to 6 light for a few seconds to indicate that setup is complete.

14

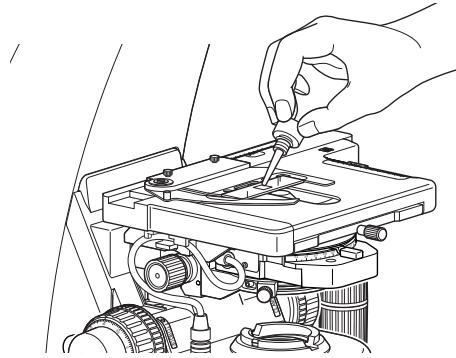
Oil Immersion

! CAUTION

- Petroleum benzene and absolute alcohol are highly flammable. Be careful when handling these materials, especially around open flames and when turning the power switch on or off.
- Follow the instructions provided by the manufacturer when using absolute alcohol.

An objective with an “Oil” marking is an oil-immersion objective. An objective of this type is to be used with designated non-fluorescent immersion oil applied between the sample and the tip of the objective.

For maximum performance, an oil-immersion objective with a numerical aperture of 1.0 or larger should be combined with an oil-immersion achromat aplanatic condenser. The oil-immersion condenser is used by applying a designated non-fluorescent immersion oil between the sample and the condenser.



Oil immersion

! Phase turret condenser

The phase turret condenser is intended to be used dry. Do not apply immersion oil between the condenser and the specimen.

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

✓ Air bubbles in the oil

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, attach a centering telescope, then look for air bubbles while turning the eyepiece section of the centering telescope to adjust the focus.

If you detect bubbles, remove them by one of the following methods:

- Turn the 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 more oil.
- Remove the oil and apply new oil.

✓ Cleaning the oil

Oil remaining on the oil-immersion objective or adhered to the dry-type objective will significantly 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 the oil from the condenser.

Use petroleum benzene to wipe off the immersion oil. For optimum results, Nikon recommends cleaning with absolute alcohol (ethyl or methyl alcohol) after cleaning with petroleum benzene.

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 the immersion oil. (Usually, three or four times should be sufficient to clean the lens.)

15 Water Immersion

An objective with a “WI” or “W” marking is a water-immersion objective. Such an objective is 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.

✔ Cleaning of water

After use, wipe off the 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.

16 Tips for Phase Contrast Microscopy

Phase contrast microscopy is suitable for observation of clear and colorless specimens, undyed or lightly colored specimens, decolored specimens, and ultrathin slices for electron microscopes. The phase contrast method is not suitable for graded or hard dyed specimens.

A phase contrast image appears differently depending on the phase contrast or shape of the specimen, and the properties of the objective. Note the following when you prepare the specimen or select a Ph objective.

Select a specimen for which the center of the Ph annular diaphragm is not misaligned.

The center of the Ph annular diaphragm will be misaligned when observing a specimen that causes scattered light or generates a lens or prism effect. In particular, with live and thick specimens, oversized specimens, and specimens using a microplate, the center will be misaligned due to the lens or prism effect, making the specimen difficult to observe.

Ph objective and specimen

Ph objectives include “achromatic”, “plan achromatic”, “plan flour”, and “plan apochromatic” objectives depending on how much the chroma aberration and field curvature are adjusted. These lenses also are further subdivided into several types depending on the properties of the internal phase plate. For favorable microscopy results, the phase contrast amount of the specimen must match the properties of the phase plate. See the table below for the use properties of Ph objectives.

When using a dark contrast Ph objective, make sure that the phase contrast of the specimen does not exceed the allowed phase contrast amount (latitude). If the phase contrast amount of the specimen is greater than the allowed phase contrast amount, observation is not possible as the image will be illuminated brighter than the background.

You can increase or decrease the phase contrast by the thickness of the specimen, and the refraction index of the mounting agent or culture solution when preparing a phase contrast specimen.

A specimen with weak contrast under a DLL objective may yield better result under a DM objective.

Use Properties of Ph Objectives

Ph contrast objective		Appearance	Contrast		Latitude	Usage example
Dark contrast	DLL DL	Generally, an object with larger phase contrast appears darker. Therefore, the image is shown in black in a relatively brighter field of view, similar to the one observed with bright-field microscopy.	Suitable for detailed observation mainly using micro contrast.	Intermediate (with broader usage)	Phase contrast and absorbing object (stained object) in low and intermediate latitude	Bacteria’s spore, general live cell, slightly thick specimen, bacteria, dyed specimen, egg, fat particle, crystalline, etc.
	DM			High (with relatively narrower usage)		
Bright contrast	BM	Generally, an object with larger phase contrast appears brighter. Therefore, the image is shown brighter in a relatively darker field of view, similar to the one observed with dark-field microscopy.	Suitable for morphology, detection, and calculation of fine fiber and granule mainly using macro contrast.		Almost all areas	Bacteria and protozoal flagellum, fibrin basic fiber, fine granule, blood cell calculation, etc.

Using the GIF filter

The GIF filter (green interference filter) improves the contrast when placed in the optical path. The filter should be installed on the field lens, or placed inside or on top of the filter cassette holder. Note, however, that it may cause ghosting when mounted inside the filter cassette holder.

Phase turret condenser

Phase contrast microscopy requires a phase turret condenser equipped with a Ph annular diaphragm. Matching the Ph annular diaphragm and the phase plate of the Ph objective correctly provides a phase contrast effect.

■ Ph code

One of the Ph codes, [Ph1], [Ph2], or [Ph3] is indicated on the Ph objective depending on the size of the phase plate. (Ph codes have nothing to do with the magnification of the objective.) Always use a Ph objective and Ph annular diaphragm with the same Ph code. You cannot expect the phase effect if a different combination of the codes is used.

■ Centering of the annular diaphragm and the phase plate

The position of each condenser diaphragm in the condenser turret have already been adjusted based on the Ph1 annular diaphragm. Usually, once centered with Ph1, further centering is not required upon switching to another magnification. However, the phase contrast image will differ slightly depending on how the annular diaphragm overlaps the phase plate, thus for a stricter observation or capturing of still images, check whether the annular diaphragm and the phase plate are concentric at each magnification. In addition, slightly decentering the annular diaphragm and the phase plate will produce a shadowing effect, resulting in a stereo image. Use this method as appropriate for the specimen.

Combining with an epi-fluorescence attachment

When the epi-fluorescence and phase contrast attachments are attached to the microscope, it is possible to use epi-fluorescence and phase contrast microscopy in combination. This allows for compensation of each method's shortcomings, for example, by making it possible to find the target using the phase contrast method instead of the epi-fluorescence method that is likely to degrade the color of the specimen or by using both microscopy concurrently.

See Chapter 1 "2.2 Phase Contrast Microscopy Procedure" for the phase contrast microscopy procedure and Chapter 1 "5.2 Epi-fluorescence Microscopy Procedure" for the epi-fluorescence microscopy procedure. When switching between microscopy methods, note the following.

✔ To switch to epi-fluorescence microscopy

- Turn the condenser turret until the [C: Shutter] symbol comes to the front.
- Insert the desired excitation filter cube into the optical path.
- Open the shutter for the epi-fluorescence attachment.

✔ To switch to phase contrast microscopy

- Close the shutter for the epi-fluorescence attachment and block the excitation light from the epi-fluorescence attachment.
- Turn the filter cube switching knob and move the position without a filter cube into the optical path.
- Bring a Ph objective into the optical path.
- Turn the condenser turret to bring an annular diaphragm with the same Ph code as the objective into the optical path.
- Fully open the aperture diaphragm.

✔ To use epi-fluorescence and phase contrast microscopy concurrently

- (1) Use phase contrast microscopy to adjust the focus onto the target.
- (2) Remove the GIF filter, if installed, on the field lens.
- (3) Insert the desired excitation filter cube into the optical path.
- (4) Open the shutter for the epi-fluorescence attachment to focus it again.
- (5) Adjust the brightness of the fluorescent image using the ND filter of the epi-fluorescence attachment.
- (6) Adjust the brightness of the phase contrast image using the dia-illumination brightness control knob.

17 Tips for Epi-fluorescence Microscopy

! WARNING

The light source used with the epi-fluorescence attachment (mercury lamp) requires special care during handling because of its characteristics. Make sure you are familiar with and adhere to all warnings and cautions described at the beginning of this instruction manual.

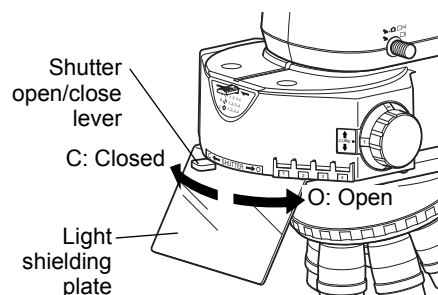
Protecting the specimen and preventing it from decoloration (shutter for the epi-fluorescence attachment)

The shutter blocks illumination.

Set the shutter open/close lever on the epi-fluorescence attachment to position "C" to close the shutter and block the optical path.

If the sample is continuously exposed to the strong light of the mercury lamp, it may become damaged or decolorized.

Be sure to close the shutter when suspending the microscopy or when pausing epi-fluorescence microscopy to perform microscopy with diasopic light. Be sure to get into the habit of performing this operation.



Opening/closing the shutter

Protecting from ultraviolet light (light shielding plate)

The light shielding plate is used to prevent the reflected ultraviolet light from entering the observer's eyes, which is originally emitted through the objective, from the specimen.

Using a non-fluorescent slide, cover glass, and immersion oil

For fluorescence observations, be sure to use a non-fluorescent slide, cover glass and our designated immersion oil for an image with better contrast.

Restricting the illumination to the area of the specimen being viewed (adjusting the field diaphragm)

The field diaphragm is used to restrict illumination to the area of the specimen being viewed.

Turning the field diaphragm lever of the epi-fluorescence attachment changes the size of the field diaphragm.

For normal observations, stop down the diaphragm so that the aperture boundaries circumscribe or inscribe the field of view. Opening the field diaphragm too much and illuminating a broader area than necessary will result in stray light entering the field of view, generating flare and reducing the image contrast. In addition, the specimen will become decolorized over a wider area.

Be sure to adjust the field diaphragm each time you change the objective.

Adjusting the brightness of the fluorescent image (adjusting the ND filter)

■ ND filters in the epi-fluorescence attachment

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

The epi-fluorescence attachment has three ND filters (ND4, ND8, and ND16) built in.

Push in the ND filter IN/OUT levers to insert ND filters into the optical path and darken the fluorescent image.

As shown below, you can combine these three filters to achieve various levels of brightness.

Light Reduction by Combined ND Filters of the Epi-fluorescence Attachment

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

(-: Removed from the optical path, ○: Placed into the optical path)

✔ Improving the S/N ratio (shielding tube)

Exposure to excitation light from the Epi-fl attachment may cause the white LED to light up, resulting in a degradation of the S/N ratio. When performing epi-fluorescence observations solely using epi-fluorescence microscopy, remove the condenser and attach the provided shielding tube to the Epi-fl attachment, and/or place a plate on the field lens.

■ **ND on HG precentered fiber illuminator**

You can also adjust the light intensity using the ND on the HG precentered fiber illuminator. For detailed information, refer to the operating manual provided with the HG precentered fiber illuminator.

Locating a target on the specimen

The standard procedure for epi-fluorescence microscopy is to first locate the target under differential interference contrast or phase contrast microscopy, and then switch to epi-fluorescence microscopy.

To locate the target under the dia-illumination bright-field microscopy, you will need to note the following:

- Under dia-illumination bright-field microscopy, start with a 10x objective, and adequately stop down the condenser.
- Gradually increase the magnification. When the target becomes difficult to locate, switch to epi-fluorescence, and use low excitation light.
- You can also use other techniques, such as using the edge of the cover glass to approximate the position of the target.

17.1 Switching Excitation Methods

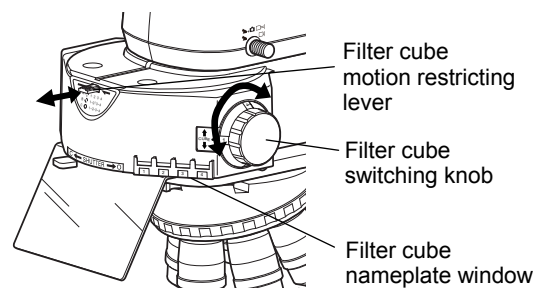
Four filter cubes can be inserted into the CI-FL Epi-fluorescence attachment. (→See Chapter 3, “6 Assembly for Epi-fluorescence Microscopy”)

Move the desired cube into the optical path by turning the filter cube switching knob on the right side of the attachment.

For bright-field observations, leave one cube position empty, and move this empty position into the optical path.

Use the filter cube motion restricting lever located at the upper front section to limit the cube switching operation.

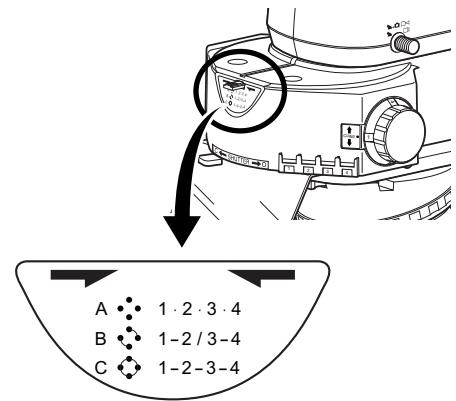
Six filter cubes can be inserted into the D-FL Epi-fluorescence attachment. Refer to the D-FL Epi-fluorescence attachment Instructions for details.



Switching the filter cube

Filter Cube Motion Restricting Lever Functions

Lever position	Filter cube switching
Position A (Push in by two notches)	Locked (filter cubes cannot be switched)
Position B (Push in by one notch)	Switching between positions 1 and 2, or between positions 3 and 4 only. (The position at which the lever is pushed in determines whether switching is for positions 1 and 2 or positions 3 and 4.)
Position C (first click stop position)	Free (switching possible as desired)



Lever position

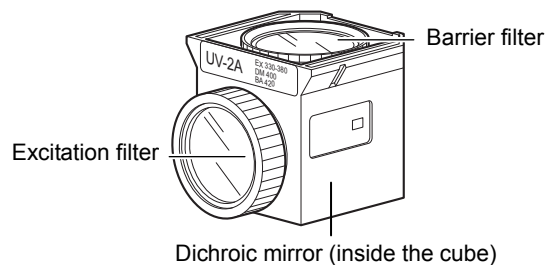
17.2 Selecting Filters

A filter cube consists of three types of optical components: an excitation filter (EX filter), a barrier filter (BA filter), and a dichroic mirror (DM). Select the filter cube with the desired combination of optical components to suit the characteristics of the sample and the fluorophore by referencing the properties of each filter.

You can select a combination of an excitation filter and a barrier filter even if you are using the same excitation method.

Excitation filters, barrier filters, and dichroic mirrors can be purchased separately.

Since excitation filters are exposed to strong light during operations, they will deteriorate over time. Replace the filter at intervals determined by usage.



Filter cube

Spacer inside the filter cube

Some types of filter cubes cannot be inserted directly into the epi-fluorescence attachment.

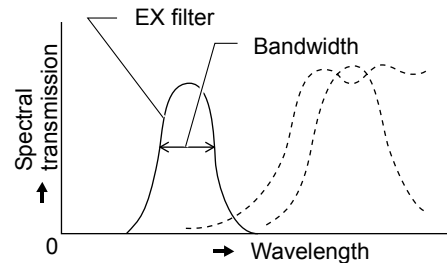
Follow the procedure described in Chapter 3, "6 Assembly for Epi-fluorescence Microscopy - ■ Attaching a filter cube" to remove an internal spacer or reverse the spacer before insertion.

Excitation filter (EX filter)

Excitation filters allow selective transmission of light (excitation light) in the wavelength range 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 the bandwidth.

The bandwidth range of an excitation filter determines the brightness of the fluorescent image, the generation of autofluorescence (fluorescence resulting from substances other than the fluorophores), and degree of fading. The broader the bandwidth, the greater the amount of excitation light irradiated onto the specimen, thereby increasing the brightness. However, this also increases the amount of autofluorescence 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 autofluorescence and fading. For specimens with pronounced auto fluorescence, use excitation filters with a narrow bandwidth. (However, the fluorescent image will be darker.)

Since excitation filters are exposed to strong light during operations, they will deteriorate over time. Replace the filter at intervals determined by usage.



EX filter bandwidth

EX Filter Bandwidth and Fluorescent Image

	Narrow	EX Filter Bandwidth	Wide
Brightness of fluorescent image	Dark		Bright
Generation of autofluorescence	Low		High
Degree of color fading	Low		High

Barrier filter (BA filter)

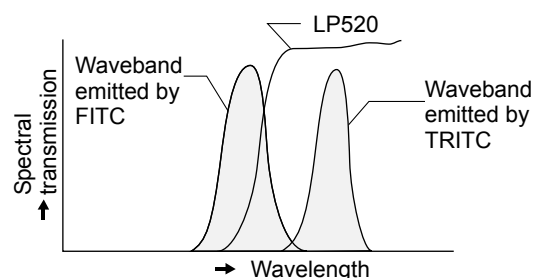
Barrier filters allow only fluorescent light emitted by the specimen to pass, blocking excitation light. This allows the fluorescent image to be viewed without excess illumination (dark background).

There are two types of barrier filters: LP filters that block all light below a certain wavelength but pass all light of longer wavelengths, and BP filters that pass light of a certain waveband and block 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. The border wavelength is called the cut-on wavelength.

- (1) For specimens labeled with a fluorophore in which the fluorescent waveband and excitation waveband (light that the specimen absorbs in order to emit fluorescent light) are very close, selecting a barrier filter with the shortest cut-on wavelength permitted by the performance requirements will result in most 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 developments in filter performance have resulted in increased use of filters of short cut-on wavelengths.
- (2) For multiple-labeled specimens, use an LP filter for microscopy of fluorescent images of all fluorophores. Note that a combination involving an ordinary dichroic mirror, an excitation filter, and an LP-filter-type barrier filter will be incapable of excited fluorophores 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.



Long-pass filter

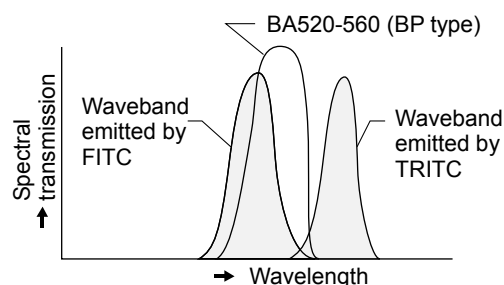
(Both FITC and TRITC images are visible.)

BP filter (bandpass filter)

Bandpass filters pass only light of a certain wavelength range, blocking all other light.

BP filters are used for microscopy of fluorescent images involving a specific fluorophore in multiple-labeled specimens. (For example, in a double-labeled specimen of FITC and TRITC, the BA520-560 filter enables microscopy of just the FITC fluorescent image.)

However, BP filters will not separate autofluorescence, if any (because the fluorescent image in the above combination is green only). LP filters are better suited for making fine separation of autofluorescence based on slight color differences.



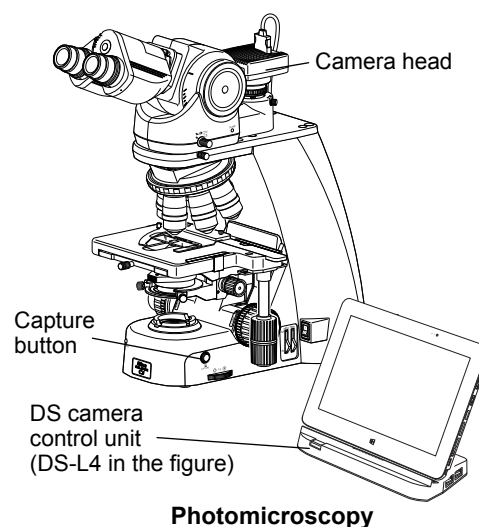
Band-pass filter

(Only the FITC fluorescent image is visible.)

18 Capturing Images

When a DS camera head or the DS-U3 DS Digital Camera Control Unit is connected to the microscope, you can use the Capture button on the base of the microscope to capture digital images with ease.

When using a tube that allows for light distribution to the binocular section and the camera port, images can also be captured while in an observation posture from the binocular section.



18.1 Photomicroscopy

The photomicroscopy procedure is described below. Also refer to the instruction manual of the DS-U3, DS-L4, or the camera's control software for the details including the camera settings.

In addition, when using the DS-L4, you must configure at least the following information:

- Folder for data storage.
- File name for saving the file
- File format and file size.
- Date and destination of data

- (1) **Adjust the illumination of the microscope correctly, and adjust the focus onto the specimen image.**
- (2) **Adjust the installation position of the camera head based on the stage movement direction.**

Loosen the attachment guide fixing screw on the C mount, and adjust the camera position and rotation. The movement on the monitor should be in the opposite direction of the stage. (When the stage is moved in the direction from left to right, the image on the monitor should move from right to left.) After making the appropriate adjustments, tighten the screws firmly.

- (3) **Focus the image.**

If the image viewed through the eyepiece appears to be in focus but the image on the monitor is out of focus, turn the camera fine focus adjustment ring on the C mount until the image on the monitor is in focus. Note that such out of focus situations may also indicate incorrect diopter adjustment. Make sure you have made diopter adjustments as well. (→Chapter 2 "4 Adjusting the Diopter")

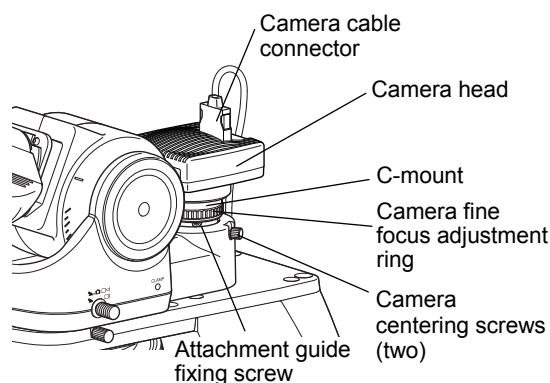
- (4) **Center the camera.**

Turn the right and left camera centering screws to align the image through the eyepiece with the image on the monitor.

- (5) **Select the camera scene mode suitable for the microscopy method.**

- (6) **Adjust the camera's white balance.**

To adjust the white balance, press the WB button while capturing an image of a clear section of the specimen slide. (For fluorescent photomicrography, it is recommended that you adjust the white balance under normal bright-field microscopy conditions before capturing images.)



Adjusting the camera head attachment position

- (7) **Align the specimen.**
- (8) **Readjust the focus onto the target.**
- (9) **Adjust the image brightness using the camera exposure compensation function.**
- (10) **Check the image using the [FREEZE] button.**
- (11) **If the image is acceptable, press the Capture button to save the image.**

(The operating procedure differs if the DF/FL scene mode is selected. For details, refer to the instruction manual provided with the camera.)

✔ Number of images captured

Seriography is not available because the Capture button is designed to capture only one image.

18.2

Tips on Microscope Settings for Photomicroscopy

Adjusting the light intensity

LED: Adjust the brightness using the brightness control knob.

Filter: Place a commercially available color compensation filter on the field lens on the microscope base, inside or on the filter cassette holder as necessary.

Adjusting the condenser

- Always focus and center the condenser.
- For phase contrast microscopy, center the annular diaphragm.
- The diaphragm aperture should generally be adjusted to 70 to 80% of the numerical aperture of the objective.

Confirming the photomicrographic range

The image on the monitor represents the photomicrographic range.

Confirming the focus

Check the focus both through the eyepiece and on the monitor. If the focal positions for the two images differ, adjust the camera fine focus adjustment ring at the camera port.

Adjusting 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 piece of cloth or similar.

Protecting fluorescent images from decoloration

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

■ **Use a brighter optical system 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. Nikon recommends increasing the magnification of the objective rather than of the zoom. (Generally, the numerical aperture of the objective increases with magnification. The larger the numerical aperture, the brighter the resulting image.)

■ **Adjusting the excitation light**

Excessively bright excitation light will accelerate the decoloration of the specimen, making it more difficult to acquire suitable fluorescent images. Insert ND filters into the optical path to adjust the brightness.

■ Specimen

Photomicrography of faded specimen sections requires prolonged exposure time 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. For best results, use the phase contrast method to select a specimen section for photomicrography, and then switch to the fluorescent method to capture images.

Adjusting the brightness of the image on the monitor

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

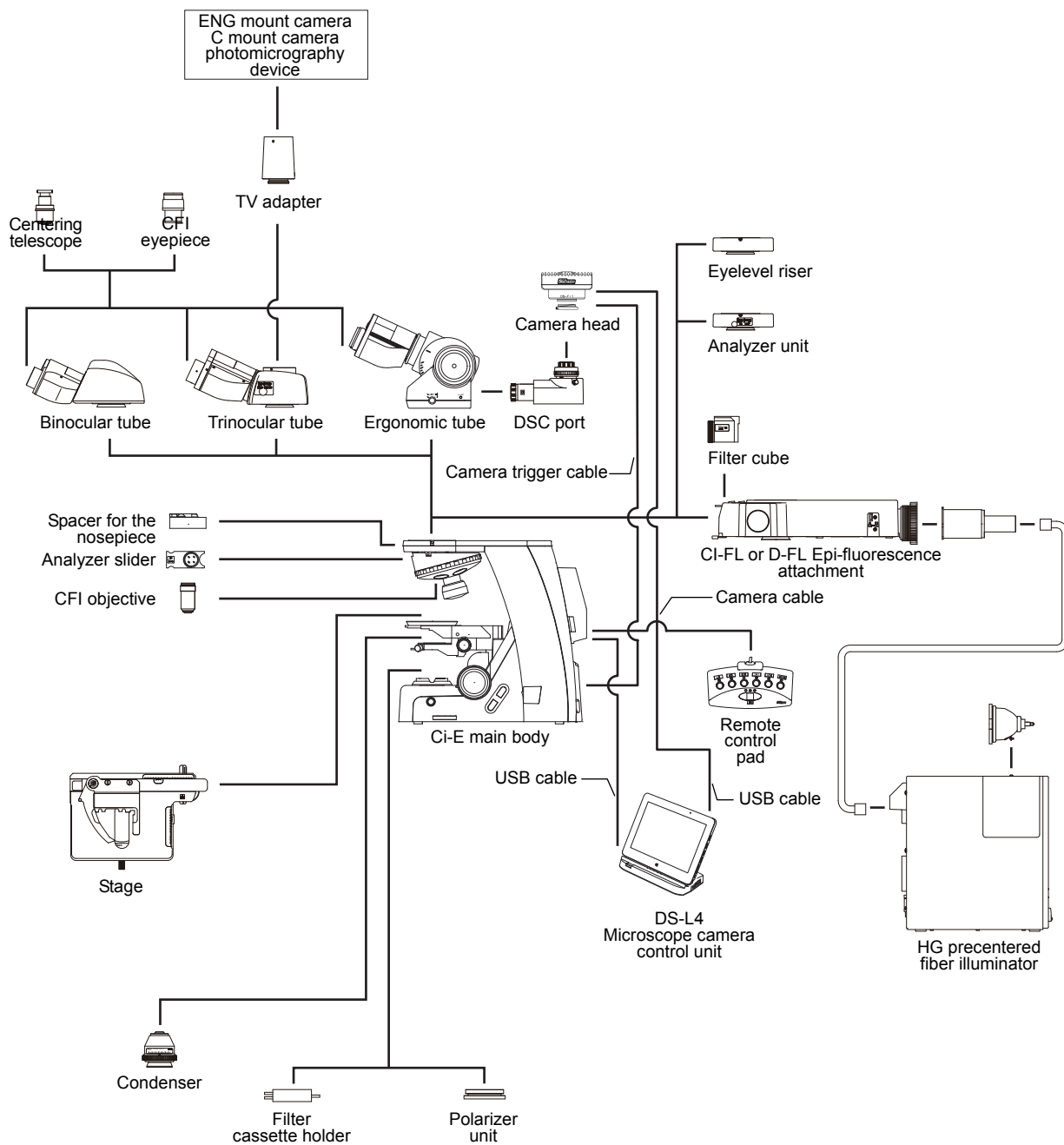
See the instruction manual of the DS-U3, DS-L4, or the camera's control software for details.

3

Assembly

1

ECLIPSE Ci-E System Configuration



2 Assembly for Bright-field Microscopy

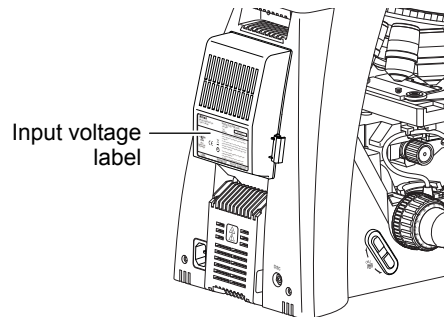
[Tool for assembly: Hex driver]

1 Check the input voltage.

Check the input voltage indicated on the back of the microscope. Use the microscope only if the indicated input voltage matches the power supply voltage for the area in which the microscope will be used.

⚠ WARNING

If the indicated voltage and the supplied voltage differ, do not attempt to use the microscope. Contact your nearest Nikon representative for advice.

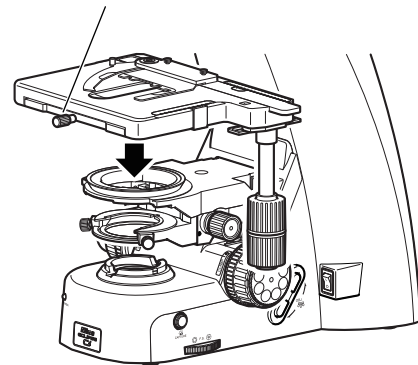


Checking the input voltage

2 Attach the stage.

- (1) Turn the coarse focus knob to remove the cushioning material from the substage section.
- (2) Turn the coarse focus knob until the elevating section is brought to the lowermost position.
- (3) Place the stage on the substage and fix into place with the stage clamp screw.

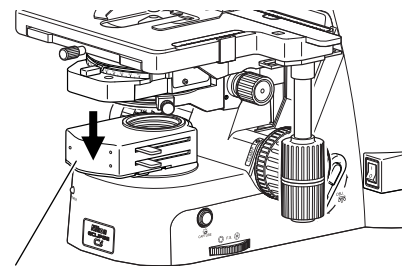
Stage clamp screw



Attaching the stage

✔ Using the filter cassette holder

Insert the filter cassette holder on the field lens of the microscope after the stage has been raised up to the upper limit.



Filter cassette holder

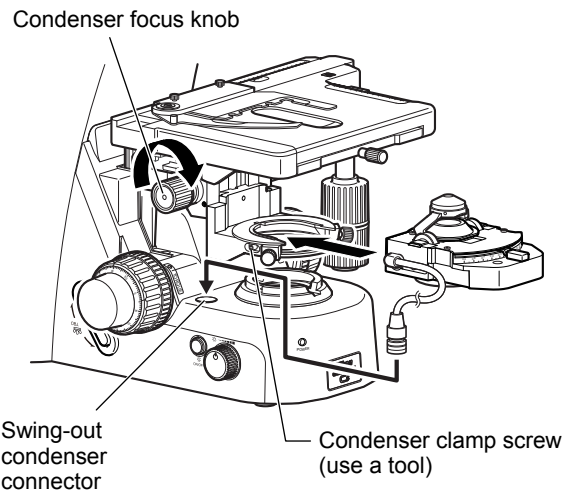
Attaching a filter cassette holder

✔ Exclusivity of the filter cassette holder and the spacer for the nosepiece

The filter cassette holder cannot be used simultaneously with the spacer for the nosepiece.

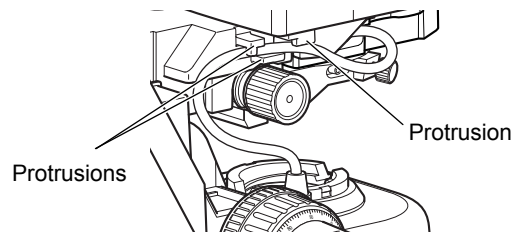
3 Attach the condenser.

- (1) Turn the coarse focus knob until the elevating section is brought to the upper limit.
- (2) Turn the condenser focus knob until the condenser holder reaches the bottom.
- (3) Insert the condenser, adjust it to face the front, then secure it in place with the tool.
- (4) Turn the condenser focus knob until the condenser holder reaches the top.



Attaching the condenser

When using the motorized swing-out condenser, secure the condenser cable using the three protrusions (as shown), and then connect the cable to the swing-out condenser connector on the main body.

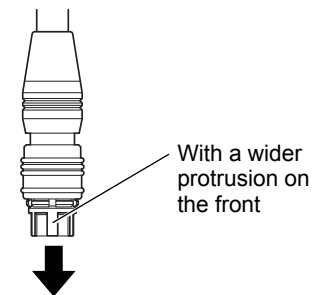


Motorized swing-out condenser cable

⚠ CAUTION

Make sure that the power to the microscope is turned OFF before connecting the condenser cable.

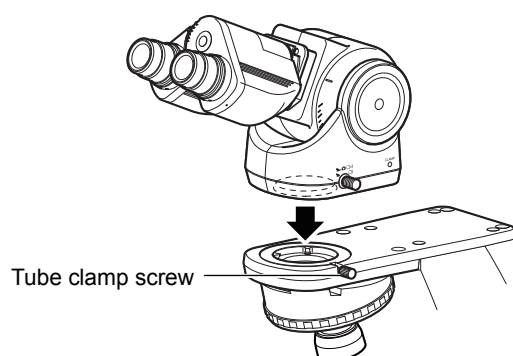
Connect the motorized swing-out condenser cable to the microscope main body so that a wider protrusion among those at the end of the plug faces the front (facing the observer).



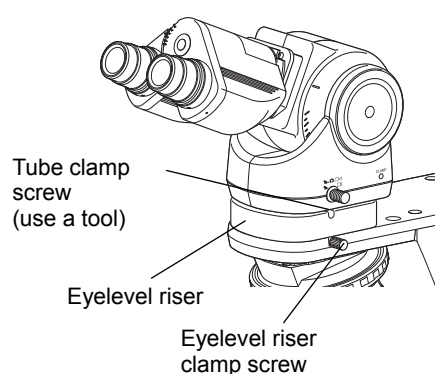
Motorized swing-out condenser

4 Attach the tube.

Place the tube on the microscope arm and secure it by tightening the clamp screw on the arm.

**Attaching the tube****✔ Using the eyelevel riser**

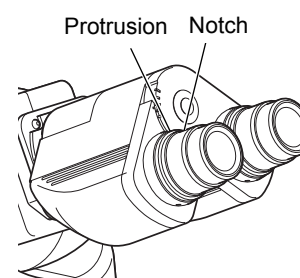
Place the eyelevel riser on the microscope arm and secure it by tightening the clamp screw on the arm. Attach the tube on the eyelevel riser and secure it by tightening the clamp screw on the eyelevel riser using a tool.

**Attaching the eyelevel riser****5 Attach eyepieces.**

Make sure the notch on the eyepiece side and the protrusion of the eyepiece sleeve are aligned, then insert the eyepieces.

✔ Notch on eyepiece

The eyepiece has a notch to prevent rotation. When attaching, match the notch with the protrusion on the eyepiece sleeve. Otherwise, eyepiece is not attached to the correct position.

**Attaching eyepieces****6 Mounting the spacer for the nosepiece (only when using the spacer for the nosepiece)**

Contact your nearest Nikon representative when the spacer for the nosepiece needs to be mounted or removed.

7 Attach the objective.

Screw the objective into the nosepiece. Select the nosepiece address so that the magnification of the objectives increases as you go from address 1 to 6.

8 Connect connectors.

⚠ CAUTION

Make sure that the power is turned OFF before you connect connectors shown below.

Connection of the remote control pad

Connect the remote control pad cable to a remote control pad connector on the rear of the microscope.

Connection of DS-L4 or a PC

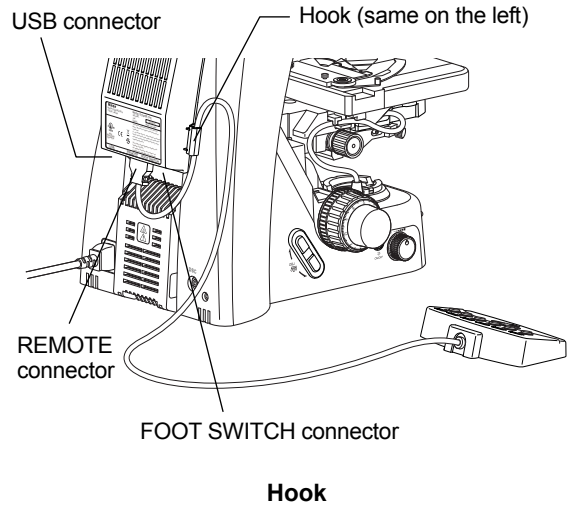
✔ Ni Setup Tool

To connect a PC, Ni Setup Tool software must have been installed in advance. For details on the installation procedure, see the software manual for “Ni Setup Tool”.

Using a USB cable, connect the microscope to a USB connector on the DS-L4 or the PC.

Refer to the “Ni Setup Tool” software manual or the DS-L4 instruction manual to perform the necessary configuration before use.

The microscope has two hooks on its rear, which can be used to secure the remote control pad cable and the USB cable.

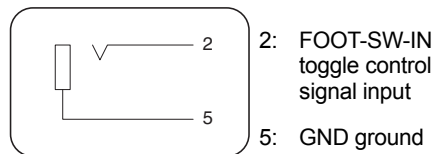


✔ Toggle control by external signals (Connecting a foot switch)

The Ci-E has a connector (FOOT SWITCH connector) for controlling the toggle function with an external device. Connecting this connector to an external switch box, a toggle operation of the objectives is possible. See the following for connection using the foot switch connector.

■ Connector pin layout

A 3.5 mm diameter mono mini jack (for audio cables) is used for the connector on the device. Connect using the figure on the right.



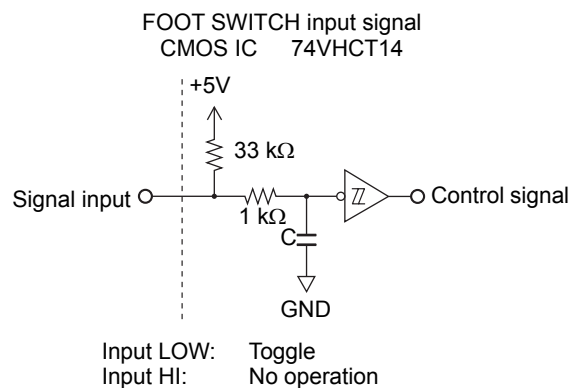
■ Toggle operation

To perform the toggle operation, press the toggle pattern switch to select “toggle pattern 1” or “toggle pattern 2”.

Under this condition, shorting Pin No. 2 on this connector (FOOT-SW-IN) and Pin No. 5 (GND) or inputting a LOW signal to Pin No. 2 allows the microscope to perform the toggle (The toggle does not repeat if shorting or signal input continued).

If a momentary type switch is used, for example, two nosepiece addresses set in toggle pattern 1 or 2 can be switched alternately each time the switch is pressed.

Connector circuit diagram



9 Connect the power cord.

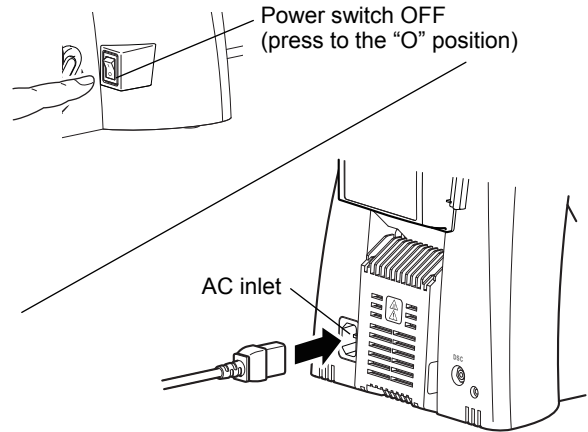
WARNING

Be sure to use the specified power cord. Use of other power cords may result in malfunction or fire. This product is classified as having Class I protection against electric shock. Make sure this product is connected to an appropriate protective earth terminal.

Refer to Chapter 6, “2 Performance Properties” for the specified power cords.

To prevent electric shock, always turn off the power switch (press to the “O” position) for the microscope before connecting or disconnecting the power cord.

- (1) Check that the microscope power switch is OFF.
- (2) Plug the power cord into the AC inlet at the back of the microscope.
- (3) Plug the other end of the power cord into a wall outlet.



Connecting the power cord

Assembly of the system configuration required for bright-field microscopy is now completed.

3

Assembly for Phase Contrast Microscopy

Follow the procedure described in Section 2 “Assembly for Bright-field Microscopy” to perform assembly.

Note the following:

- **Attaching a condenser for phase contrast microscopy**

Use a condenser for the phase contrast microscopy in the step 3, “Attach the condenser.”

The procedure is the same.

- **Attaching a Ph objective**

A Ph objective must be attached in the step 7, “Attach the objective”.

The procedure is the same.

4

Assembly for the Simple Polarizing Microscopy

Follow the procedure described in Section 2 “Assembly for Bright-field Microscopy” to perform assembly.

Note the following:

- **Attaching an analyzer tube for simple polarization**

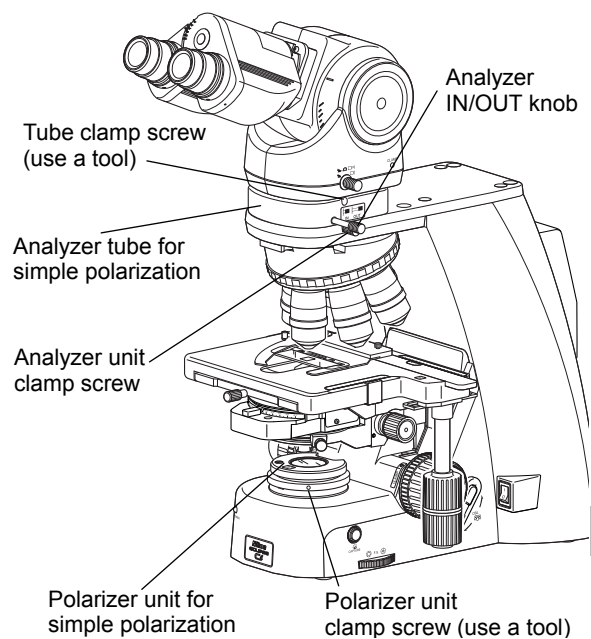
Attach an analyzer tube for simple polarization to the microscope arm prior to the step 4, “Attach the tube.”

Place the analyzer unit (analyzer tube for simple polarization) on the arm so that the analyzer IN/OUT knob is positioned to the right, and secure the analyzer unit in place with the analyzer unit clamp screw.

Place the tube on the analyzer unit and tighten the tube clamp screws of the analyzer unit using the tool provided with the microscope to secure the tube.

- ✓ **Slider-type analyzer for simple polarization**

When using the D-SA Analyzer Slider for Simple Polarization instead of an analyzer tube for simple polarization, insert it into the analyzer slot of the nosepiece. (To use the D-SA Analyzer Slider for Simple Polarization, the C-NA sextuple nosepiece with analyzer slot is required.)



Attaching an analyzer tube for simple polarization, polarizer unit for simple polarization

- **Attaching a polarizer unit for simple polarization**

Set the polarizer unit for simple polarization over the field lens on the base of the microscope. Make sure the orientation mark on the polarizer comes to the front at this point and tighten the polarizer unit clamp screw.

The actual securing will take place after the orientation of the analyzer and polarizer has been adjusted in Step 16 of the simple polarizing microscopy procedure.

5 Assembly for Sensitive Polarization Microscopy

Follow the procedure described in Section 2 “Assembly for Bright-field Microscopy” to perform assembly.

Note the following:

■ Attaching an analyzer tube for first-order red compensation

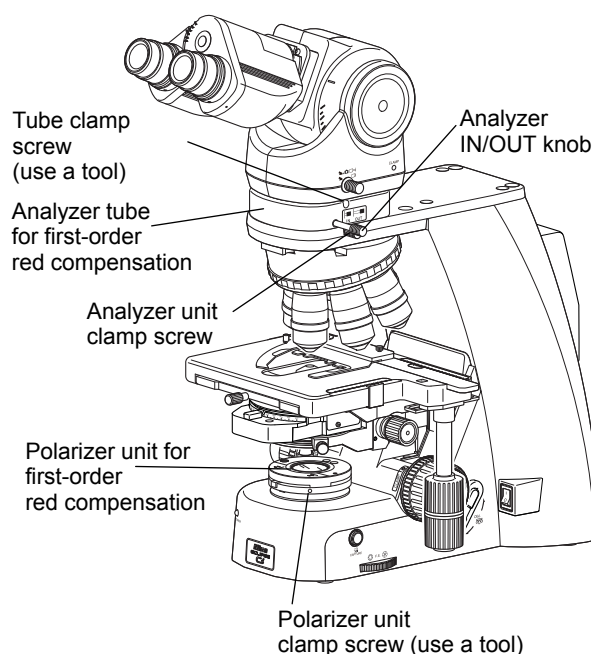
Attach an analyzer tube for first-order red compensation to the microscope arm prior to the step 4, “Attach the tube.”

Place the analyzer unit (analyzer tube for first-order red compensation) on the arm so that the analyzer IN/OUT knob is positioned to the right, and secure the analyzer unit in place with the analyzer unit clamp screw.

Place the tube on the analyzer unit and tighten the tube clamp screws of the analyzer unit using the tool provided with the microscope to secure the tube.

✓ Slider-type analyzer for first-order red compensation

When using the C-AS Analyzer Slider for First-order Red Compensation instead of an analyzer tube for first-order red compensation, insert it into the analyzer slot of the nosepiece.



Attaching an analyzer tube for first-order red compensation, and a polarizer unit for first-order red compensation

■ Attaching a polarizer unit for first-order red compensation

Set the polarizer unit for first-order red compensation over the field lens on the base of the microscope. Make sure the orientation mark on the polarizer comes to the front at this point and tighten the polarizer unit clamp screw.

The actual securing will take place after the orientation of the analyzer and polarizer has been adjusted in Step 16 of the sensitive polarization microscopy procedure.

6 Assembly for Epi-fluorescence Microscopy

Follow the procedure described in Section 2 “Assembly for Bright-field Microscopy” to perform assembly.

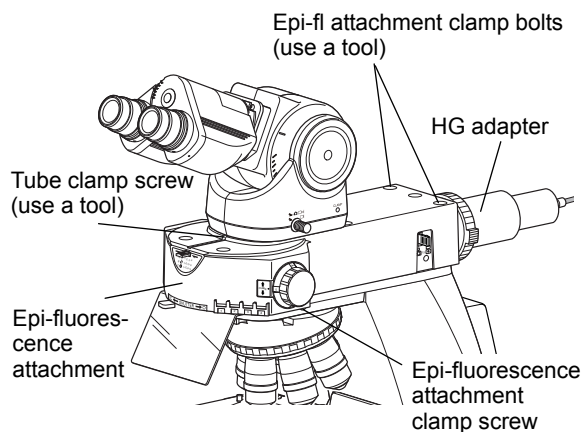
Note the following:

(For details on attaching a D-FL Epi-fluorescence attachment, refer to the instruction manual provided with the D-FL Epi-fluorescence attachment.)

■ Attaching the epi-fluorescence attachment

Attach the CI-FL Epi-fluorescence attachment to the microscope arm prior to the step 4, “Attach the tube.”

- (1) Place the epi-fluorescence attachment on the microscope arm.
- (2) Secure it by tightening the clamp screw on the arm and two fixing bolts at the rear of the epi-fluorescence attachment. Use a hex wrench provided with the epi-fluorescence attachment to tighten the fixing bolts.
- (3) Attach the HG adapter of the mercury lamp illuminator to be used to the bayonet mount on the back. (For detailed information, refer to the instruction manual provided with the mercury lamp illuminator.)
- (4) Place the tube on the epi-fluorescence attachment and tighten the tube clamp screws of the epi-fluorescence attachment using the tool provided with the microscope to secure the tube.



Attaching the epi-fluorescence attachment

■ Attaching a filter cube

⚠ WARNING

Always turn off the mercury lamp before inserting or removing a filter cube.

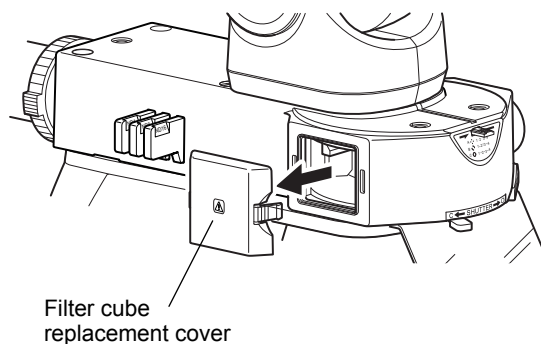
- (1) Remove the filter cube replacement cover on the left of the epi-fluorescence attachment.
- (2) Insert a filter cube.
The filter cubes listed below cannot be inserted directly into the filter bay of the epi-fluorescence attachment. Remove an internal spacer or reverse the spacer before insertion.

<Filter cubes requiring spacer removal>

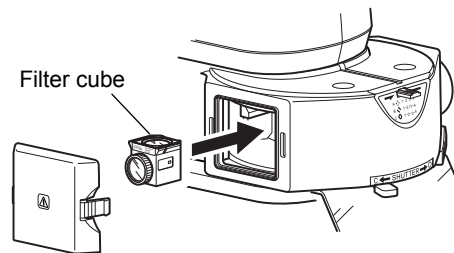
- UV-2A
- UV-2B

<Filter cubes requiring spacer reversal>

- DAPI
- FITC
- GFP-L
- GFP-B
- TRITC
- Tx-Red



Filter cube replacement cover

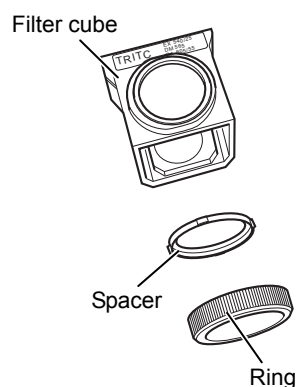


Filter cube

Attaching a filter cube

● Removing/attaching the internal spacer ●

- 1) Place the filter cube on a work table with the excitation filter facing up.
- 2) Unscrew the ring retaining the excitation filter. (Be careful to avoid dropping the filter.)
- 3) Remove the spacer inside the removed ring.
- 4) Remove or reverse the spacer as appropriate for the particular filter cube type before its insertion.
- 5) Reattach the ring.



Attaching/removing the internal spacer

- (3) Insert a nameplate into the position with the same address as the one indicated on the filter cube switching knob on the right side of the microscope.
- (4) Turn the filter cube switching knob and insert other filter cubes into the remaining open bays.
- (5) Restore the slot cover back to its original position.

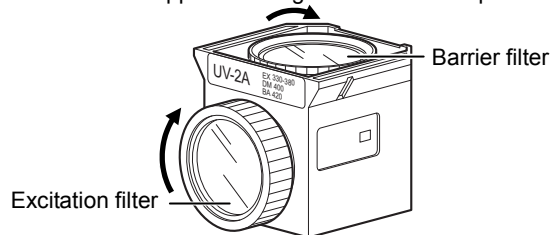
■ **Replacing excitation and barrier filters**

The excitation filter, barrier filter, and dichroic mirror can be removed from the cube for replacement.

Excitation filters are screw-in filters, while barrier filters are slide-in filters.

Fit the projection on the barrier filter in the groove on the filter cube and turn clockwise by approximately 30 degrees to secure it in place.

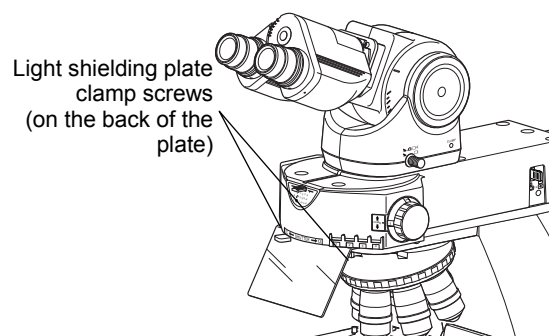
Turn approx. 30 degrees to secure in place.



Replacing the excitation and barrier filters for the filter cube

■ **Attaching a light shielding plate**

Secure a light shielding plate in place with the fixing screws on the lower front of the epi-fluorescence attachment. To remove the plate, loosen the fixing screws and pull it forward.



Attaching a light shielding plate

■ **Attaching a shielding tube**

Attach a shielding tube using the same procedure for attaching a condenser to the condenser holder.

7

Attaching a Camera

■ **When attaching a camera head to the ergonomic tube**

- (1) Screw the camera head into the C mount on the DSC port.
- (2) Remove the rear cover of the ergonomic tube and insert the DSC port.
- (3) Secure the DSC port in place with the tool provided with the microscope.
- (4) Connect the camera head and a camera control unit.
When using the DS-L4 microscope camera control unit: First, connect the DSC connector on the microscope and the EXT./O connector of the DS camera head with a camera trigger cable.
Next, connect the USB connector on the microscope and the USB 2.0 connector on the DS-L4 with a USB cable, and the USB 3.0 connector on the DS-L4 and the USB (D) connector on the DS camera head with a USB cable.

When using the DS-U3 digital camera control unit:
First, connect the DSC connector on the microscope and the EXT./O connector on the DS-U3 with a camera trigger cable
Next, connect the CAMERA 1 connector or CAMERA 2 connector of the DS-U3 and the CAMERA OUT connector on the DS camera head with a DS camera cable. Connect the DS-U3 to a PC to control the camera on the application software.

For more information, read the instruction manual of the camera and the camera control unit to be used.

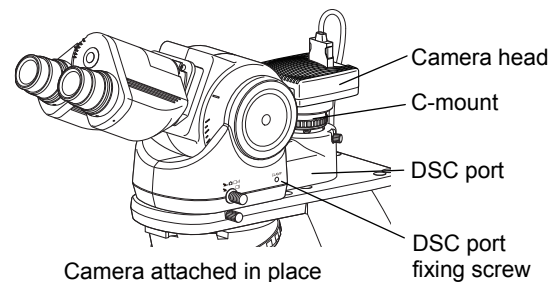
✔ **Notes when connecting cables**

When connecting a camera trigger cable to the DSC connector, insert it to the end.

Prior to photomicrography, adjust the camera position as appropriate. (See Chapter 2, “18.1 Photomicroscopy”.)

■ **Installing a camera head to the trinocular tube**

Attach a C mount camera, ENG mount camera and a photomicrography device via an adapter to the trinocular tube.



Attaching a camera head

4

Troubleshooting

Misuse of this product may adversely affect performance, even if this product is properly functional. If any of the following problems occurs, be sure to check the following table for possible causes before requesting service.

If you detect problems that are not listed below or the problem still persists after measures are taken, turn off the device and contact your nearest Nikon representative.

1

Optical System and Operation

1.1

General

Problem	Cause	Measure
A dirty or dusty field of view when looking into the eyepiece.	Dirt or dust rotates when the eyepiece is turned. ➔ The eyepiece is dirty.	Clean the eyepiece. (→Chapter 5 “1.1 Cleaning Lenses”)
	Dirt or dust does not rotate when the eyepiece is turned ➔ (1) to (5) (1) The specimen is dirty if dirt or dust moves when the specimen is moved on stage. (2) The tip of the condenser lens is dirty if dirt or dust goes in and out of view when the condenser is moved up and down while using a low magnification objective. (3) The objective is dirty if dirt or dust disappears when the objective is switched. (4) A field diaphragm image is not focused on the specimen surface. (Condenser adjustment is incorrect) (5) An aperture diaphragm is stopped down too far.	(1) Clean the specimen. (2) Clean the condenser. (→Chapter 5 “1.1 Cleaning Lenses”) (3) Clean the objective. (→Chapter 5 “1.1 Cleaning Lenses”) (4) Make sure the condenser is focused and centered. (→Chapter 2, “5 Focusing and Centering the Condenser”.) (5) Open it to the proper size. (→Chapter 2 “6 Adjusting the Aperture Diaphragm”)
Dirt or dust appears on the monitor.	Dirt or dust on the monitor moves when the camera is turned ➔ Lenses or specimen are dirty or dusty.	Check and clean them in accordance with “Dirt or dust does not rotate when the eyepiece is turned” of “A dirty or dusty field of view when looking into the eyepiece.”
	Dirt or dust on the monitor does not move when the camera is turned ➔ The camera is dirty.	Detach the camera and clean it by following the instruction manual of the camera.
The image quality is poor. The contrast or the resolution is poor.	No cover glass is attached.	Attach a cover glass of the specified thickness (0.17 mm). (However, no cover glass is required for an NCG objective.)
	The thickness of the cover glass is inadequate.	
	The objective correction ring does not match the thickness of the cover glass. (For the objective with a correction ring)	Correct the ring as appropriate.

Problem	Cause	Measure
The image quality is poor. The contrast or the resolution is poor.	The lens and specimen are dirty or dusty.	Check and clean them in accordance with “Dirt or dust does not rotate when the eyepiece is turned” of “A dirty or dusty field of view when looking into the eyepiece.”
	An aperture diaphragm is stopped down too far. Otherwise, it is open too much.	Open it to the proper size. (→Chapter 2 “6 Adjusting the Aperture Diaphragm”)
	The field diaphragm image is not focused on the specimen surface. (A condenser adjustment is incorrect.)	Make sure the condenser is focused and centered. (→Chapter 2, “5 Focusing and Centering the Condenser”.)
	No immersion oil is applied to the tip of an oil-immersion objective.	Apply our designated non-fluorescent immersion oil.
	The designated immersion oil is not used.	(→Chapter 2 “14 Oil Immersion”)
	The immersion oil contains air bubbles.	Remove the air bubbles. (→Chapter 2 “14 Oil Immersion”)
The field of view is too bright.	The immersion oil adheres to the tip of the dry-type objective.	Clean it as appropriate. (→Chapter 2 “14 Oil Immersion”)
	The brightness of the dia-illumination LED is not adjusted appropriately.	Adjust the brightness using the brightness control knob to attain a suitable brightness. (→Chapter 2, “1 Adjusting the Brightness of a Diascopic Image”)
The field of view is too dark.	The initial brightness setting of the dia-illumination LED is not appropriate.	Change the initial brightness. (→Chapter 2, “13.2 Changing the Initial Brightness of Dia-illumination LED”.)
	The brightness of the dia-illumination LED is not adjusted appropriately.	Adjust the brightness using the brightness control knob to attain a suitable brightness. (→Chapter 2, “1 Adjusting the Brightness of a Diascopic Image”)
	The aperture diaphragm is stopped down too far.	This should normally be adjusted to 70 to 80% of numerical aperture of the objective. (→Chapter 2 “6 Adjusting the Aperture Diaphragm”)
	A field diaphragm image is not focused on the specimen surface.	Make sure the condenser is focused and centered. (→Chapter 2, “5 Focusing and Centering the Condenser”.)
	The optical path is not switched to binocular 100%.	Set to binocular 100%. (→Chapter 2, “9 Switching the Optical Path of the Tube”)
Color of the image visible to the naked eye is different from that of the image displayed on the monitor.	The initial brightness setting of the dia-illumination LED is not appropriate.	Change the initial brightness. (→Chapter 2, “13.2 Changing the Initial Brightness of Dia-illumination LED”.)
	White balance of the camera is not set correctly.	Set the white balance in accordance with the camera’s instruction manual.
The entire field of view is bluish or yellowish.	Filter cube is in the optical path when non epi-fluorescence observation.	Remove the filter cube from the optical path.

Problem	Cause	Measure
Lack of visibility around periphery of field of view. Illumination is uneven across the field of view. The field of view is not visible.	Parts are attached incorrectly.	Confirm that parts (nosepiece, condenser, etc.) are correctly attached. (→Chapter 3, “2 Assembly for Bright-field Microscopy”)
	Movable parts are not seated correctly.	Correctly set the optical path switching lever, nosepiece, filter cube switchover turret, condenser turret, and slider, etc. (Move the part until it clicks.)
	A field diaphragm image is not focused on the specimen surface.	Make sure the condenser is focused and centered. (→Chapter 2, “5 Focusing and Centering the Condenser”.)
Lack of visibility around periphery of field of view. Illumination is uneven across the field of view. The field of view is not visible.	The field diaphragm is stopped down too far.	Open the field diaphragm slightly wider than the field of view. (→Chapter 2 “8 Adjusting the Field Diaphragm”)
	Incorrect combination of the objective with the condenser.	Adopt an appropriate combination. (→Chapter 2 “7 Selecting a Condenser”)
	In low magnification microscopy, required condenser operation was not performed.	Perform the operation such as swinging out or sliding the condenser.
	The lens and specimen are dirty or dusty.	Clean them as appropriate. (→Chapter 5 “1.1 Cleaning Lenses”)
Out of focus with an objective of high magnification.	The specimen is upside down.	Turn up the cover glass and attach it to the stage. (→Chapter 1 “1.2 Bright-field Microscopy Procedure - 8 Place a specimen on the stage, and move the stage to bring the target into view”)
	The thickness of the cover glass is inadequate.	Attach a cover glass of the specified thickness (0.17 mm).
	A fail-safe device for specimen damage protection of the objective is pushed in.	Some objective has a stopper to keep the pushed in state. Turn the tip of the object to release. If the objective does not have a stopper, do not pull because the tip cannot be turned. In this case, contact your nearest Nikon representative.
A focal deviation is high when switching over objectives.	The objective lens is attached incorrectly.	Screw the objective all the way in. (→Chapter 3, “2 Assembly for Bright-field Microscopy - 7 Attach the objective”.)
	Diopter adjustment has not been performed.	Perform diopter adjustment. (→Chapter 2 “4 Adjusting the Diopter”)
The image is not in focus although the stage is raised to the highest position.	The stage is attached incorrectly.	Attach it correctly. (→Chapter 3, “2 Assembly for Bright-field Microscopy - 2 Attach the stage”.)
	The refocusing position is set lower than the focusing position.	Check and reset the setting. (→Chapter 2, “2.4 Refocusing”.)

Chapter 4 Troubleshooting

Problem	Cause	Measure
One side of the field of view (up, down, right, or left) is not focused. The image flows (i.e. becomes asymmetrically defocused when moving the focal point)	The specimen is tilted relative to the stage surface.	Position the specimen in place on the stage. (→Chapter 1 “1.2 Bright-field Microscopy Procedure - 8 Place a specimen on the stage, and move the stage to bring the target into view”)
	The stage is tilted.	Attach the stage correctly. (→Chapter 3, “2 Assembly for Bright-field Microscopy - 2 Attach the stage”.)
Images in left and right eyepieces are not coincident.	Interpupillary adjustment has not been performed.	Perform interpupillary adjustment. (→Chapter 1, “1.2 Bright-field Microscopy Procedure - 11 Adjust the interpupillary distance”.)
	Diopter adjustment has not been performed.	Perform diopter adjustment. (→Chapter 2 “4 Adjusting the Diopter”)
Eyes become fatigued.	Diopter adjustment has not been performed.	Perform diopter adjustment. (→Chapter 2 “4 Adjusting the Diopter”)
	Brightness is inadequate.	Adjust the brightness using the brightness control knob to attain a suitable brightness. (→Chapter 2, “1 Adjusting the Brightness of a Diascopic Image”)
The specimen does not move smoothly.	The specimen holder is not securely-fixed to the stage.	Fix the holder securely. (→Chapter 1 “1.2 Bright-field Microscopy Procedure - 8 Place a specimen on the stage, and move the stage to bring the target into view”)
	Rotating torque of the stage knob is set too heavy.	Adjust to the appropriate torque weight. (→Chapter 2, “3.3 Adjusting the Knob Rotation Torque”.)

1.2 Epi-fluorescence Microscopy

Problem	Cause	Measure
Lack of visibility around periphery of field of view. Illumination is uneven across the field of view. The field of view is not visible.	The filter cube is misaligned.	Push the cube in to the limit. (→Chapter 3, “6 Assembly for Epi-fluorescence Microscopy - ■ Attaching a filter cube”.)
A fluorescent image is not visible (when the lamp is ON).	The shutter is closed.	Open the shutter. (→Chapter 2, “17 Tips for Epi-fluorescence Microscopy - Protecting the specimen and preventing it from decoloration (shutter for the epi-fluorescence attachment)”.)
	The selection of the filter cube is incorrect.	Use a correct filter cube. (→Chapter 2, “17.2 Selecting Filters”.)
The fluorescent image is very dark (when the lamp is ON).	The ND filters of the epi-fluorescence attachment are in the optical path.	Remove the ND filters from the optical path as necessary. (→Chapter 2, “17 Tips for Epi-fluorescence Microscopy - Adjusting the brightness of the fluorescent image (adjusting the ND filters) - ■ND filters in the epi-fluorescence attachment”.)
	ND filters in the HG precentered fiber illuminator are used to suppress the brightness too much.	Adjust the brightness. (→Check your illuminator’s manual.)
	A halogen light source is used for a dark specimen.	Change the light source to a mercury lamp.
	A mercury lamp in the HG precentered fiber illuminator has reached the end of its product life.	Replace the lamp. (→Check your illuminator’s manual.)
	A designated objective is not used at UV or V excitation.	Use a designated objective.
	The room is bright.	Make it darker.
	The optical path switching lever is not set to 100% of light distribution for the binocular section.	Switch the lever position to 100% of light distribution for the binocular section. (→Chapter 2, “9 Switching the Optical Path of the Tube”)
	The dia-illumination LED is on.	Turn off the dia-illumination LED.
The fluorescent image quality is poor.	The filter cube being used is not suitable for the specimen.	Use a filter cube suitable for the specimen. (→Chapter 2, “17.2 Selecting Filters”.)
The contrast of the fluorescent image is poor.	The objective or cover glass is dirty.	Clean it as appropriate. (→Chapter 5 “1.1 Cleaning Lenses”)
	The immersion oil is fluorescent.	Use the non-fluorescent immersion oil designated by Nikon. (→Chapter 2, “17 Tips for Epi-fluorescence Microscopy”.)
	The slide glass is fluorescent.	Use a non-fluorescent slide glass. (→Chapter 2, “17 Tips for Epi-fluorescence Microscopy”.)
	Stray light is entering from the condenser.	Lower the condenser, or remove the condenser and attach a shielding tube.

1.3

Phase Contrast Microscopy

Problem	Cause	Measure
Poor contrast.	The Ph annular diaphragm of the condenser does not match the phase plate image of the objective.	Adjust so that they match. (→Chapter 1, “2.2 Phase Contrast Microscopy Procedure - 15 Center the Ph annular diaphragm”.)
	The Ph annular diaphragm of the condenser and the objective selected do not match.	Put the Ph annular diaphragm with the same Ph code as the objective into the optical path. (→Chapter 1, “2.2 Phase Contrast Microscopy Procedure - 17 Adjust the Ph annular diaphragm in the condenser with the Ph objective to be used”.)
	The phase contrast of the specimen is too large.	Change the mounting agent or thickness of the sample when preparing the specimen. (→Chapter 2, “16 Tips for Phase Contrast Microscopy”.)
	The type of the Ph objective is not suitable for phase contrast of the specimen.	Use a Ph objective suitable for the specimen. (→Chapter 2, “16 Tips for Phase Contrast Microscopy”.)

2

Electrical System

2.1

General

■ Power supply

Problem	Cause	Measure
There is no power even though the power switch is on.	The power cord is not connected, or is connected improperly.	Connect the cable properly. (→Chapter 3, “2 Assembly for Bright-field Microscopy - 9 Connect the power cord”.)

■ Illumination

Problem	Cause	Measure
The dia-illumination LED is not illuminated.	There is no power supplied.	Plug in the power cord. (→Chapter 3, “2 Assembly for Bright-field Microscopy - 9 Connect the power cord”.)
The dia-illumination LED cannot be turned ON/OFF with the ON/OFF switch.	The microscope is set to the setting mode.	Set the setting mode switch on the remote control pad to the left side to switch to the operation mode.
	A control is given to DS-L4 or Ni Setup Tool. Otherwise, an operation has been disabled due to DS-L4 or Ni Setup Tool settings.	Change the setting with DS-L4 or Ni Setup Tool.
A brightness cannot be adjusted with the dia-illumination LED brightness control knob.	A control is given to DS-L4 or Ni Setup Tool. Otherwise, an operation has been disabled due to DS-L4 or Ni Setup Tool settings.	Change the setting with DS-L4 or Ni Setup Tool.

■ Motorized nosepiece

Problem	Cause	Measure
The motorized nosepiece does not move.	The microscope is set to the setting mode.	Set the setting mode switch on the remote control pad to the left side to switch to the operation mode.
	An operation has been disabled due to DS-L4 or Ni Setup Tool settings.	Change the setting with DS-L4 or Ni Setup Tool.
The motorized nosepiece skips addresses.	The toggle function is ON.	Press the toggle pattern switch on the remote control pad until the toggle function is turned OFF. (→Chapter 2, “12.2 Switching between Two Objectives (Toggle)”.)

■ Motorized swing-out condenser

Problem	Cause	Measure
The motorized swing-out condenser does not move.	The motorized swing-out condenser cable is not connected.	Connect the cable properly. (→Chapter 3, “2 Assembly for Bright-field Microscopy”.)
The motorized swing-out condenser's top lens is not in the designated position.	The top lens has been misaligned due to external force.	Turn the power OFF, and then turn it ON again.
Operation of the motorized swing-out condenser is not linked to the rotation of the nosepiece.	Interlocking is not configured.	Configure interlocking. (→Chapter 2, “13.1 Configuring the Motorized Swing-out Condenser”.)

■ Capture button

Problem	Cause	Measure
The capture button does not function.	A camera trigger cable is not properly connected.	Attach it correctly. (→Chapter 3, “7 Attaching a Camera”.)
	A destination of a capture trigger signal has not been set correctly.	Change the setting with DS-L4 or Ni Setup Tool.

2.2

Epi-fluorescence Microscopy

Problem	Cause	Measure
The mercury lamp does not work.	There is no power supplied.	Plug in the power cord. (→Check your illuminator’s manual.)
	The lamp has burned out.	Replace the lamp with the specified type. (→Check your illuminator’s manual.)
	The mercury lamp is not attached.	Attach a designated lamp. (→Check your illuminator’s manual.)
	The mercury lamp’s connector is not connected to the illuminator.	Connect it to the illuminator. (→Check your illuminator’s manual.)
The mercury lamp burns out soon after it is turned on.	The lamp type is incorrect. The lamp is at end of its life.	Replace the lamp with the specified type. (→Check your illuminator’s manual.) Contact your nearest Nikon representative for advice if a new lamp will burn out soon after replacement.

Clean and decontaminate the lenses and other parts in accordance with the following procedure.

■ **Tools used for cleaning**

- Blower
- Soft brush
- Soft cotton cloth, lens tissue, gauze, etc.
- Absolute alcohol (ethyl or methyl alcohol), medical alcohol
- Petroleum benzene (use only for cleaning immersion oil)

 **CAUTION**

- Petroleum benzene and absolute alcohol used for cleaning are highly flammable. Be careful when handling these materials, particularly around open flames or when turning the power switch on or off.
- Follow the instructions provided by the manufacturer when using petroleum benzene or absolute alcohol.
- When cleaning the product, do not use organic solvents (alcohol, ether, thinner, etc.) for coated, plastic, or printed areas. It will result in discoloration or peeling of printed characters.
- Petroleum benzene should be used only to wipe off immersion oil from the objective, and never to clean the entrance lens at the bottom of the eyepiece tube, prism surface of the eyepiece tube, or the filters.


Keep the lens free of dust and fingerprints. If there is contamination on the lenses or filters, image quality decreases. If any of the lenses become dirty, clean them by the procedure given below.

■ **Cleaning light dirt (dust)**

- (1) Blow dust off using an air blower.
- (2) If this is insufficient, brush away dust with a soft brush or wipe away gently with a piece of gauze.

■ **Cleaning tough dirt (fingerprint or grease)**

Moisten lightly a piece of soft, clean cotton cloth, lens tissue, or gauze with absolute alcohol (ethyl or methyl alcohol) and wipe the dirt off.

 **Tips on wiping**

Do not reuse cotton cloth, lens tissue, or gauze that have already been used.

■ **Cleaning light dirt (dust)**

Wipe with a silicon cloth.

■ **Cleaning tough dirt (fingerprint or grease)**

Dampen a piece of gauze a little with neutral detergent and wipe the dirt gently.

1.3 Cleaning Immersion Oil

- (1) Wipe with petroleum benzene.
- (2) Finish off the cleaning with absolute alcohol (ethyl or methyl alcohol) after cleaning with petroleum benzene.

✔ If petroleum benzene is not available

If petroleum benzene is unavailable, use methyl alcohol alone. However, typically wipe three or four times because the detergency is weak.

1.4 Decontaminating the Product

For routine disinfection of this product, Nikon recommends using 70% medical alcohol. Use of organic solvents on plastic parts may result in discoloration.

✔ Cautions on disposal

If contact occurs between a sample and this product, determine whether the sample is hazardous. If the sample is hazardous, follow the standard procedures for your facility.

2 Storage

- Store this product in a dry location where mold is unlikely to form. Storage conditions are as follows: temperature (-20°C to +60°C), humidity (90% RH max., no condensation)
- Store the objectives and eyepieces in a desiccator or similar container with a drying agent.
- Place a cover over this product to protect it from dust.
- Switch off the microscope (press the switch to the “O” position) and wait for the lamphouse to cool before covering this product with a cover.

3 Regular Inspections (Charged)

To maintain the performance of this product, Nikon recommends periodic inspections (chargeable service). Contact your nearest Nikon representative for details.

1

Microscopy (Principles)

Use objectives and eyepieces of the microscope to magnify minute cells and tissue optically, and manipulate levers and knobs of the microscope unit to adjust the focus or move the observation point. Then observe or take photographs of the sample fixed on the slide.

■ **Intended use of the product (for medical care)**

This microscope is intended for use in microscopic experiment and diagnostics of cells and tissues at hospitals or by doctors in private practice in the field of pathology, anatomy, and cytology.

The microscopy with diasopic/episcopic illuminations is used to observe a sample fixed on the slide (cells and tissue) as the specimen.

The product is classified as an in-vitro diagnostic medical device. **IVD**

This product is not intended for use for measurement.

The scale on the focus knob and stage is an indicator to reproduce the position and does not guarantee the value of the thickness or length of a sample measured using this scale.

■ **Intended user**

It is intended for the medical professional and those who work on experimentations in the field of pathology and cytology.

2

Performance Properties

■ **ECLIPSE Ci-E Nikon Microscope**

Model	ECLIPSE Ci-E
Optical system	Infinity-corrected CF optical system Objective: CF160 Eyepiece: Field number 22 (with ergonomic tube/binocular tube), 25 (with trinocular tube T/F) Nosepiece: Motorized sextuple
Focus up/down motion	Drive system: Manual coarse/fine motion (calibration markings for fine motion: 1 μm /marking) Stroke: 2 mm upward, 28 mm downward With refocusing mechanism
Light source for dia-illumination	White LED
Control power	12 V 75 W integrated (100 to 240 V)
Input ratings	100-240 VAC \pm 10%, 50/60 Hz, 1.0 A
Power consumption (nominal)	13 W

Power cord	<ul style="list-style-type: none"> • When used in 100-120 V region, outside Japan UL listed detachable power cord set, 3 conductor grounding (3 conductor grounding Type SVT, No.18 AWG, 3 m long maximum, rated at 125 VAC minimum) • When used in 220-240 V region Detachabale power cord set approved according to EU/EN standard, 3 conductor grounding (3 conductor grounding Type H05VV-F 1 mm², 3 m long maximum, rated at 250 VAC minimum) • When used inside Japan PSE approved detachable power cord set, 3 conductor grounding (3 conductor grounding Type VCTF 3 x 0.75 mm², 3 m long maximum, rated at 125 VAC minimum)
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■ **CI-FL Epi-fluorescence Attachment for Nikon Microscope**

Model	CI-FL Epi-fluorescence attachment
Optical system	Infinity-corrected CF optical system Variable intermediate magnification: 1x
Fluorescent turret	Manual quadruple turret
Field diaphragm	Manual adjustment
ND filter	Manual, 3 filters (slider type; ND4, ND8, ND16)
Shutter	Manual (using a lever at the bottom of the turret)
Supported illuminator	HG precentered fiber illuminator

3 Physical Properties

■ **ECLIPSE Ci-E Nikon Microscope**

Model	ECLIPSE Ci-E	
Operating conditions	Temperature:	0°C to +40°C
	Humidity:	60% RH max. (no condensation)
	Altitude:	2000 m max.
	Pollution degree:	Degree 2
	Overvoltage category:	Category II
	Electrical shock protection class:	Class I
	Indoor use only	
Transport/storage conditions	Temperature:	-20°C to +60°C
	Humidity:	90% RH max. (no condensation)
External dimensions and mass (Main body only)	External dimensions:	223 (W) x 331.5 (H) x 331 (D) mm (excluding projections)
	Mass (reference):	Approx. 10 kg

<p>Safety standards</p> <p>When using with the CI-C-E Motorized Swing-out Condenser</p>	<ul style="list-style-type: none"> • C-UL-US Listed • FCC Part 15 Subpart B Class A Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. • CAN ICES-003(A) / NMB-003(A) • Australian EMI (AS/NZS CISPR11) <p>CE Marking</p> <ul style="list-style-type: none"> • IVD Directive This equipment complies with the emission and immunity requirements of IEC/EN 61326-2-6. • Low Voltage Directive • EMC Directive
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■ **CI-FL Epi-fluorescence Attachment for Nikon Microscope**

Model	CI-FL Epi-fluorescence attachment	
Operating conditions	Temperature:	0°C to +40°C
	Humidity:	60% RH max. (no condensation)
	Altitude:	2000 m max.
	Pollution degree:	Degree 2
	Overvoltage category:	Category II
	Electrical shock protection class:	Class I
	Indoor use only	
Transport/storage conditions	Temperature:	-20°C to +60°C
	Humidity:	90% RH max. (no condensation)
Mass (reference)	Approx. 2 kg	