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M572EN01

Nikon

Upright Motorized Microscope

ECLIPSE

Ni-E

(Focusing Nosepiece System)

Instruction Manual

Operation

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is written for users of the Nikon ECLIPSE Ni-E (Focusing Nosepiece System) 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.
- Training: This product can be used without special training, provided that this manual is read thoroughly before use. Kindly contact your nearest Nikon representative if you have any questions, find any errors, or wish to provide us with your opinion.

Contents of the Manual

The instruction manual for ECLIPSE Ni-E (Focusing Nosepiece System) is provided in two volumes.

♦ **Operation (This manual)**
- Safety Precautions
- Components
- Microscopy Operations
  - Before Microscopy
  - Operation Flowchart
  - IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure
  - Epi-fluorescence Microscopy Procedure
- Individual Operations

♦ **Assembly/Maintenance**
- Assembly
- Troubleshooting
- Maintenance and Storage
- Specifications

Before reading the “Assembly/Maintenance” manual, read the “Safety Precautions” in the “Operation” manual.

Symbols Used in This Manual

The following symbols are used in this manual.

♦ **Symbols for Safety**

⚠ **WARNING** ⚠ **CAUTION**	Highlights important information that should be noted for safety. Read “Safety Precautions” for details.

♦ **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.

Summary of Contents (See the next page for detailed contents.)

Contents

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.

	**Biohazard** This symbol is affixed to the front of the stand of this product, to call your attention to the following: ● 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.
	**Precautions against heat** This symbol is affixed to the lamphouse to call your attention to the following: ● During and immediately after a period of illumination, the lamp and surrounding areas (including the lamphouse) are very hot. ● Risk of burns. Do not touch the lamp or surrounding areas during or immediately after a period of illumination. ● Make sure the lamp and surrounding areas have cooled sufficiently (which may take approx. 30 minutes) before attempting to replace the lamp.
	**Precautions against heat** This symbol is affixed to the top of the arm (both standard and contact), but “Precautions against heat” is not required when using in combination with Ni-E Microscope.

⚠ 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 NI-FLEI 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.

See Chapter 4 “2. Performance Properties” in the “Assembly/Maintenance” instruction manual for designated 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 Heat from the illuminator

During and immediately after a period of illumination, the lamp and surrounding areas (including the lamphouse) are very hot.

- Do not touch the lamp or surrounding areas during or immediately after a period of illumination. There is a risk of burn if you touch the hot area.
- Always attach the lamphouse cover when using this product.
- Make sure the lamp and surrounding areas have cooled sufficiently (which may take approx. 30 minutes) before attempting to replace the lamp.
- To avoid the risk of fire, do not place fabric, paper or highly flammable volatile materials such as gasoline, petroleum benzine, paint thinner, or alcohol near the lamphouse while the lamp is lit or for a period of approximately thirty minutes after the lamp is turned off.

⚠ WARNING

5 Hazards of mercury lamps (when using the NI-FLEI 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.

  Leave the D/UV slider of the D-ES EPI ND slider within the optical path. Removing the D/UV slider from the optical path will cause your eyes to become exposed to ultraviolet light.

- **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, contaminated, 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.

6 Hazardous sample handling

This product is intended primarily for microscopic observations and image capture of samples (biological tissue, brain slice, etc.) in chambers.

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.

⚠ CAUTION

1 Power shutdown

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, replacing lamps, or cleaning this microscope and the objective.

2 Lamp replacement precautions

- To avoid burns, wait at least 30 minutes after the lamp is turned off to give it sufficient time to cool. To avoid electric shock or malfunctions, never attempt to replace the lamp without first turning off the power switches for this product and the peripheral devices (press to the “O” position) and unplugging the power cord from the wall outlet.
- After replacing the lamp, make sure to reattach the lamphouse cover. Do not use the product without the lamphouse cover attached.
- Do not break used lamps. It should be disposed of as industrial waste, in accordance with local regulations and rules.

3 Designated lamp

Using power supplied from control box A to the main body, halogen lamps of up to 12V-100W can be lit. Always use the specified lamp and lamphouse. Using unspecified products may cause malfunctions.

Lamphouse model

- Specified lamp:
  12V 100W halogen lamp PHILIPS 7724 or OSRAM HLX64623
- Specified lamphouse:
  Nikon NI-LH or NI-IRLH precentered lamphouse

Optical fiber model

- Specified lamp:
  12V 100W halogen lamp
- Specified illuminator:
  Sumita Optical Glass’s halogen light source optical fiber illuminator LS-DWL-N

4 Movement of motorized device

This product can be equipped with motorized devices such as motorized epi-fluorescence cube turret and motorized quadrocular tilting tube, which can be controlled from remote controllers and PCs.

To avoid unexpected injuries, note the following in operating motorized devices.

- 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 stage, components and sample on the stage to avoid injuries during operation.

5 Avoid contact with water or chemical solutions.

Never expose this product to water or chemical solutions, and avoid using this product in circumstances where there is risk of exposure to water or chemical solutions. Exposure of electric parts (such as the HG Precentered Fiber Illuminator) to liquids may cause a short circuit, resulting in malfunction or abnormal heating. If water or a chemical solution 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 or a chemical solution enters this product, stop using the product, and contact your nearest Nikon representative.

6 Remove any covers from the product before switching on.

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.

7 Notes on Laying Cables

During a period of illumination, the lamphouse is very hot. Try to lay cables so that they do not contact the lamphouse.

8 Do not place any object on top of the product.

Do not place any object on top of this product.

⚠ CAUTION

9 Cautions on assembling and installing the product

- 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 11 kg. This product is not designed to be portable. When moving the microscope (i.e. to another room within the facility), work in a team of at least two persons, and firmly hold the base of the product.
- Remove all attachments (if mounted) from the microscope before moving the microscope.
- Do not place this product in a locker or cabinet.

10 Cautions on sustained observations

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.

11 Cautions on use, transportation, and storage

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.

- 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)

See Chapter 3 “4 Transportation (Using Fastening Position Mode Switch)” in the “Assembly/ Maintenance” instruction manual for fastening the system during its transportation.

12 Cautions on the disposal of the product

To avoid biohazard risks, dispose of this product as contaminated equipment in accordance with the standard procedure specified for your facility.

Notes on Handling the Product

1 Handling the product carefully

This product is a precision instrument. Avoid subjecting it to sudden impacts and shocks.

Even relatively minor impacts are capable of affecting the precision of the objective.

2 Weak electromagnetic waves

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 Scratches, dirt, and foreign particles 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 3 “2.1 Cleaning Lenses” in the “Assembly/Maintenance” instruction manual.

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. This product is provided with a M6 screw hole on the right and left sides of the recessed area at the lower back of the main body.
- 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 a 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.

6 Protect the ports from dust and extraneous light

Always attach the supplied cap to any ports not in use and slider slot while a tube, epi-fluorescence cube turret or other devices are mounted. Otherwise, extraneous light and dust may be trapped inside the product.

7 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 of use.
- 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 Chapter 3 “2.1 Cleaning Lenses” in the “Assembly/Maintenance” instruction manual.
  Single-band filter cubes: DAPI, FITC, TxRed, GFP
  Multi-band filter cubes: F-R, F-T, D-F, D-F-R, D-F-T

8 Motorized devices

When using motorized devices, do not force the motorized devices to move/stop by hand.

9 Vibration during motorized operation

While the product is designed to minimize the amount of vibration during motorized operation, note that there still may be some effect on the microscopy results.

10 Unpacking and unlocking

1) Check the package contents.
   - ECLIPSE Ni-E x1
   - Hex driver X3
     Hex wrench X2
   - Toolbox X1
   - Accessory sticker X2 types
     (A blinder for the mounting hole for accessory not in use on the top of the arm and nameplate for an additional ND filter to the ND filter cassette)
2) Before turning on the power, remove the fixing screw from the front of the elevating section. (See Chapter 1 “3 Assembly Method - 2 Unfasten the elevating section” in the “Assembly/Maintenance” instruction manual for details.)
3) Do not dispose of the packing materials. They will be reused for transportation.

Chapter

1

Components

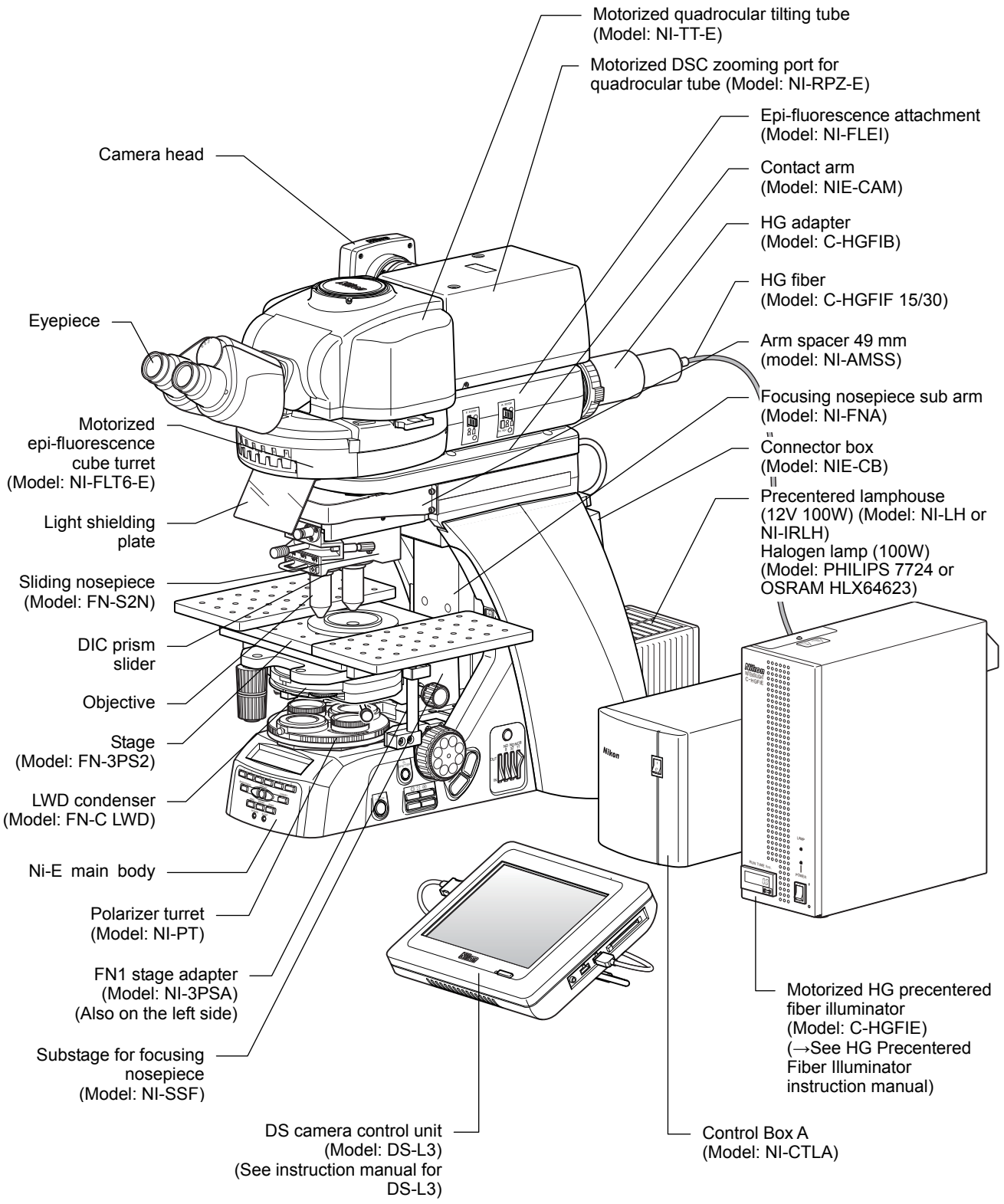
This chapter contains the system configuration diagram of the focusing nosepiece system with name of components and controls.

Figures are examples of the basic system configuration. Devices other than those shown in the figures are also available.

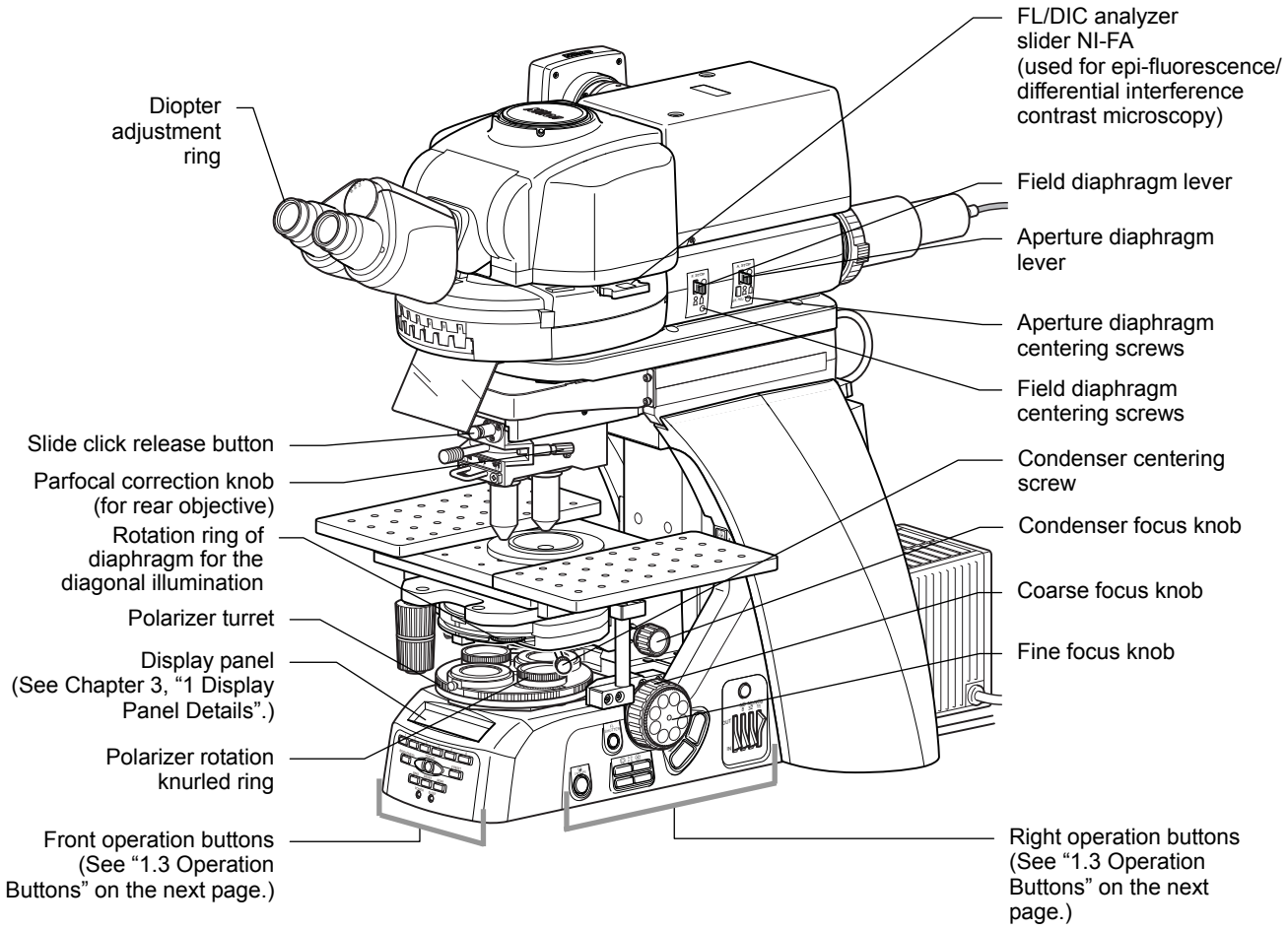
For a list of components used for ECLIPSE Ni-E microscope (focusing nosepiece system), refer to Chapter 1, “2 Components List” in the separately provided “Assembly/Maintenance” instruction manual.

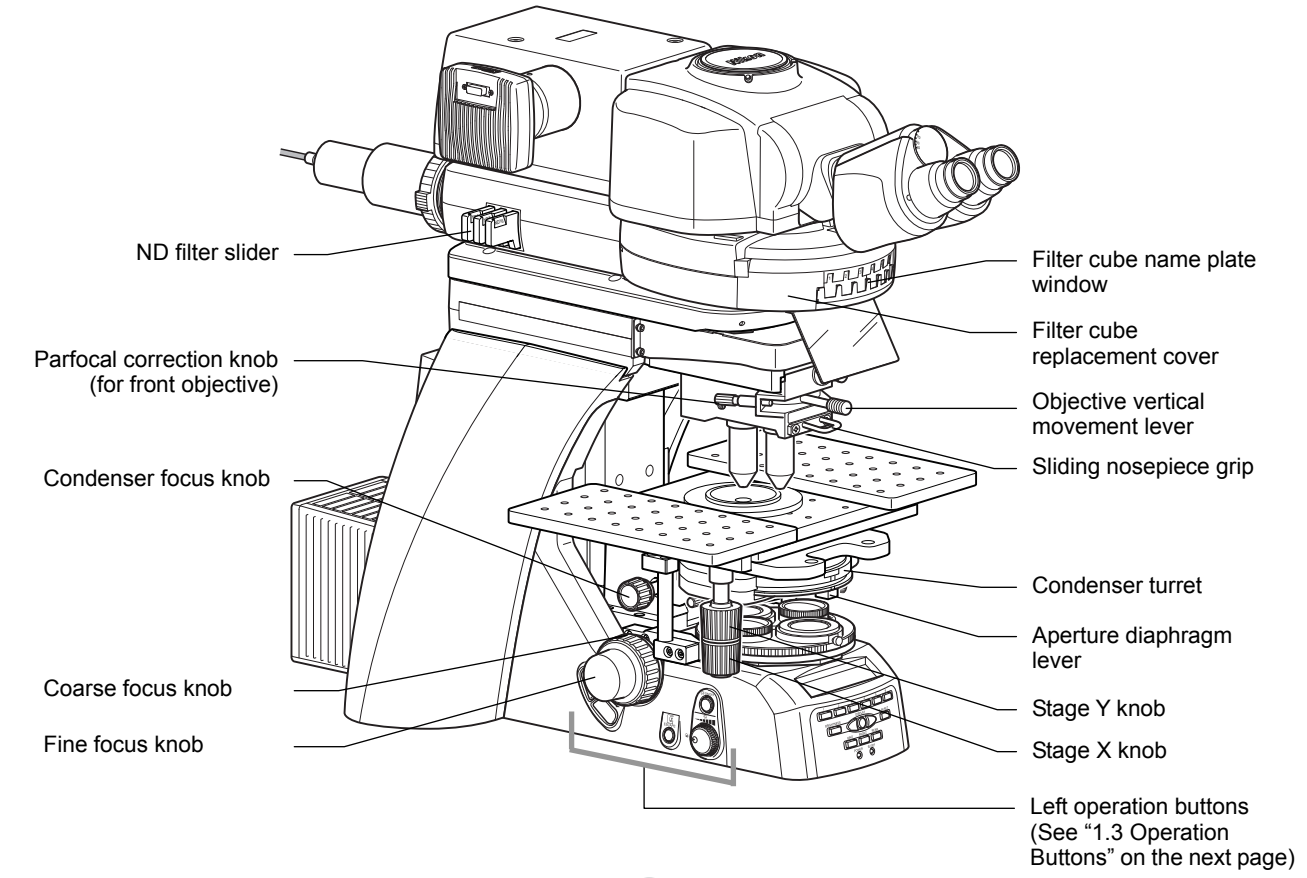
1 Ni-E Focusing Nosepiece System Basic Configuration

1.1 Components

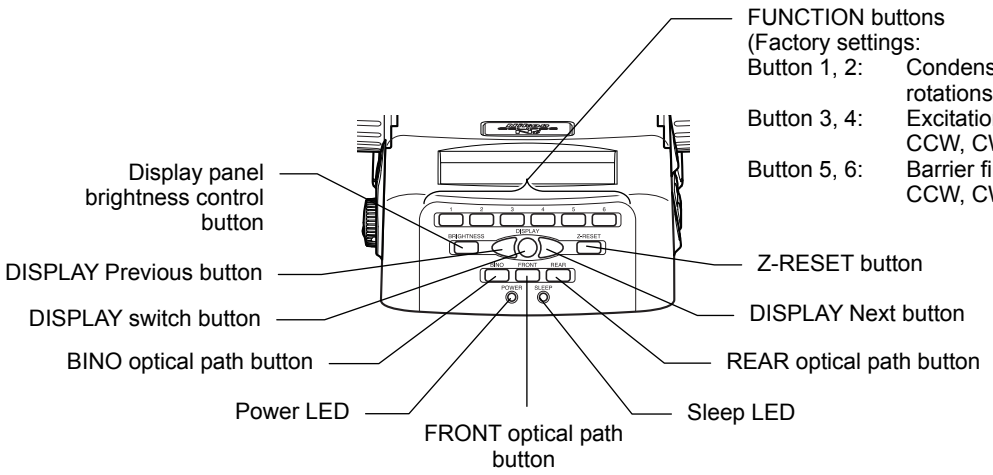


1.2 Controls

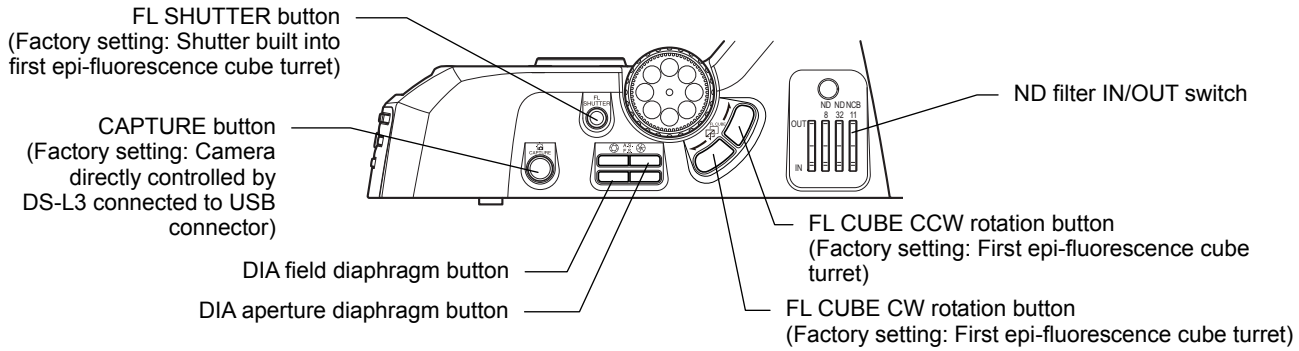

Diopter adjustment ring
FL/DIC analyzer slider NI-FA (used for epi-fluorescence/ differential interference contrast microscopy)
Field diaphragm lever
Aperture diaphragm lever
Aperture diaphragm centering screws
Field diaphragm centering screws
Slide click release button
Parfocal correction knob (for rear objective)
Rotation ring of diaphragm for the diagonal illumination
Polarizer turret
Display panel (See Chapter 3, “1 Display Panel Details”.)
Polarizer rotation knurled ring
Front operation buttons (See “1.3 Operation Buttons” on the next page.)
Condenser centering screw
Condenser focus knob
Coarse focus knob
Fine focus knob
Right operation buttons (See “1.3 Operation Buttons” on the next page.)


ND filter slider
Parfocal correction knob (for front objective)
Condenser focus knob
Coarse focus knob
Fine focus knob
Filter cube name plate window
Filter cube replacement cover
Objective vertical movement lever
Sliding nosepiece grip
Condenser turret
Aperture diaphragm lever
Stage Y knob
Stage X knob
Left operation buttons (See “1.3 Operation Buttons” on the next page)

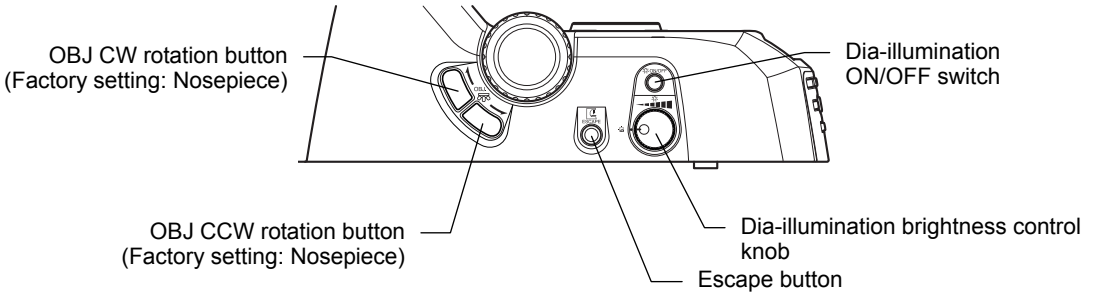
1.3 Operation Buttons



Ni-E Front Operation Buttons



Ni-E Right Operation Buttons



Ni-E Left Operation Buttons

Changing operation button assignments

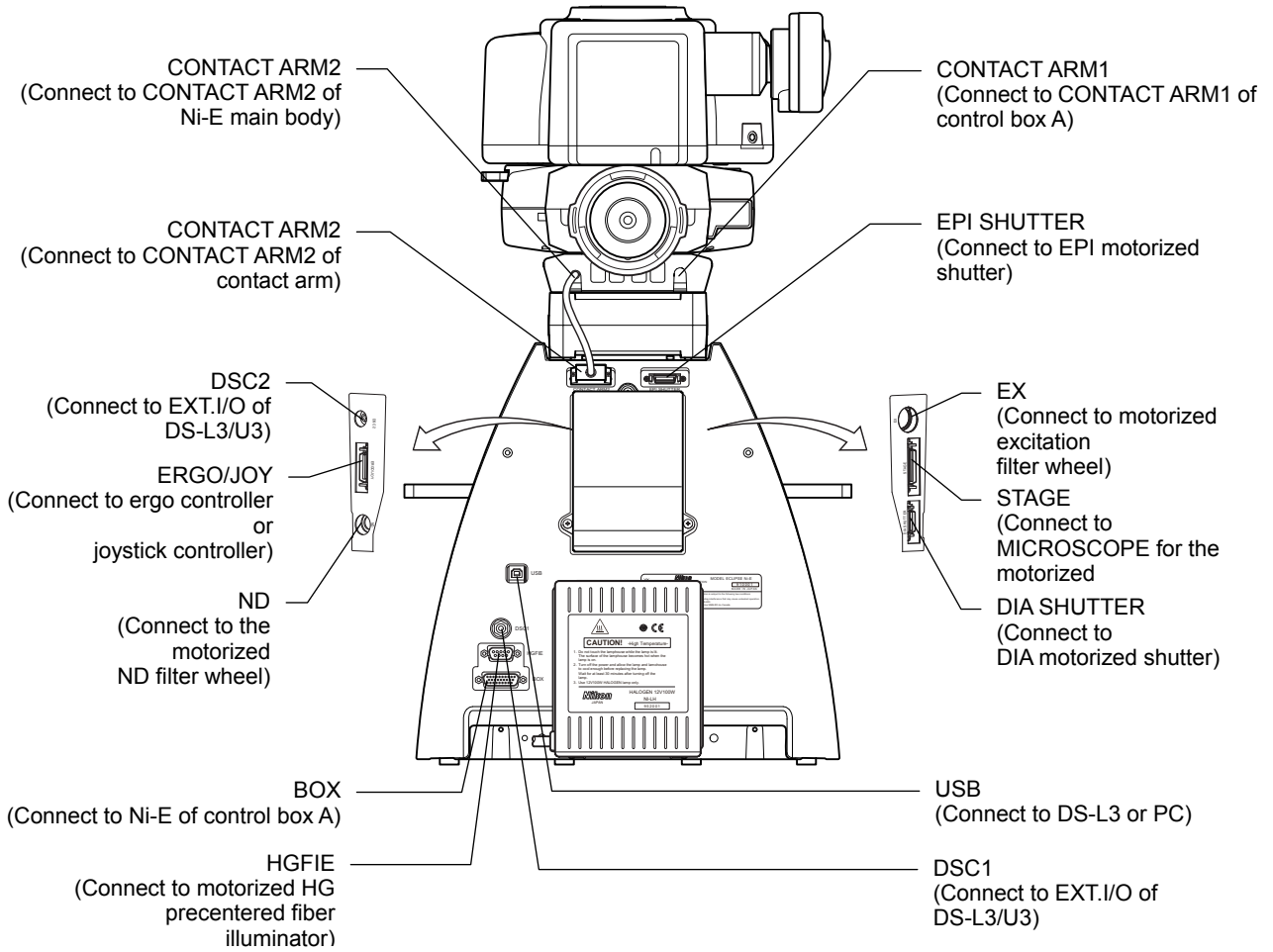
Buttons indicated with “(Factory setting: ...)” can be assigned to different functions.

There are some Ni-E focusing nosepiece systems with target devices that are not motorized at factory shipment. (Example: nosepiece and condenser) Change the function assignment of buttons that are to control the nosepiece and condenser.

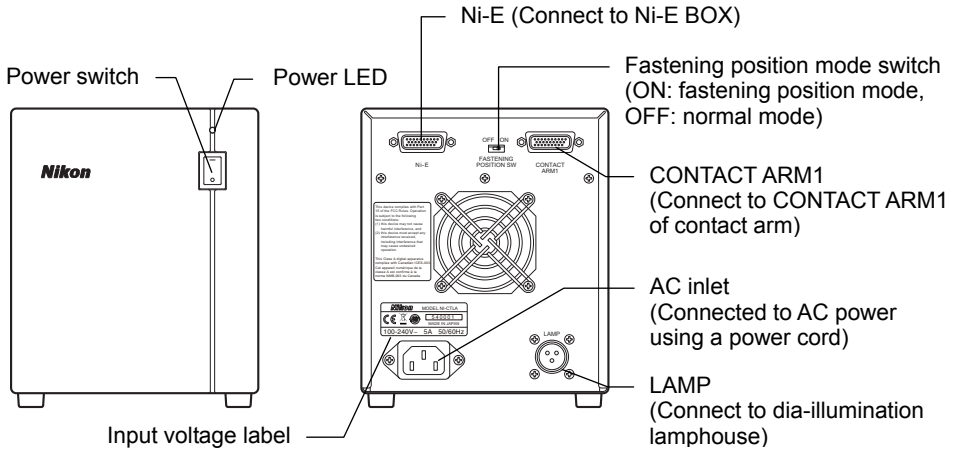
Different functions can be assigned from the DS-L3 DS camera control unit. See Chapter 3, “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3) Configuring the Button Functions - (3-1) Ni-E microscope button”.

In the explanations of operations in this manual, functions of these buttons are assumed to be left as factory settings.

1.4 Connectors



Ni-E (Rear)



Control Box A (Front/Rear)

Chapter

2

Microscopy Operations

This chapter describes the following microscopy operation procedures.

In the examples, the basic system configurations shown in the previous chapter are used.

Operation procedure 1: IR-DIC (Infrared Ray Differential Interference Contrast) microscopy
Operation procedure 2: Epi-fluorescence microscopy (including cautions on switching between or in conjunction with IR-DIC microscopy)

The procedures described herein assume that all required components are attached to the microscope, with all necessary cables properly connected, and that information registration for motorized operation has been completed. The functions of microscope's control buttons are assumed to be set to default (factory setting).

When using the microscope for the first time or changing the motorized settings from the factory default before use, see "1 Before Microscopy" in this chapter and Chapter 3, "20 Operation on DS-L3" - "20.1 Setting Up the Microscope" before the microscopy operation.

If you have yet to complete the assembly of the microscope, see Chapter 1, "3 Assembly Method" in the separately provided "Assembly/Maintenance" instruction manual to complete the assembly.

1 Before Microscopy

Information for motorized operation of the microscope has been set upon shipment. If you start using the microscope with the motorized settings of factory default, turn ON the switch of the Control Box A after the assembly of the microscope to use.

However, information on optical elements such as optional filter cube are not set a factory shipment. It is useful to have the device information configured before the use of the microscope.

You can change the factory default motorized setting information according to your needs.

Configure these settings on the [SETUP MENU] screen of the DS-L3 DS Camera Control Unit.

The table shown below summarizes the contents of the SETUP MENU. Make necessary settings accordingly. (The items marked with "*" are not applicable to the focusing nosepiece system.)

See Chapter 3, “20 Operation on DS-L3” - “20.1 Setting Up the Microscope” in this document for the summary of the setting operation.

See “DS-L3 Microscope Operation” Instruction Manual provided with DS-L3 for details on using DS-L3.

Microscope Setup from DS-L3

Setting	Default setting	Where to see in this manual (DS-L3 for details)
[COMPONENTS]		**→Chapter 3 23.1-(1)**
Objective* (motorized nosepiece/intelligent nosepiece)	None	→DS-L3 Chapter 6 “2.1 Configuring the Objective Information”
DIC module, PH module, etc. (motorized universal condenser)	Address 1: Set to OPEN. Other addresses: None	→DS-L3 Chapter 6 “2.2 Configuring the Condenser Module Information”
Filter cube (motorized/intelligent filter cube on the 1st. and 2nd. layers)	None	→DS-L3 Chapter 6 “2.3 Configuring the Filter Cube Information”
Excitation filter (motorized excitation filter wheel)	None	→DS-L3 Chapter 6 “2.4 Configuring the Excitation Filter/Barrier Filter Information”
Barrier filter (motorized barrier filter wheel)	Address 1: Set to OPEN. Other addresses: None	→DS-L3 Chapter 6 “2.4 Configuring the Excitation Filter/Barrier Filter Information”
[CONNECTION]		**→Chapter 3 23.1-(2)**
Destination camera to output signal from the DSC connector (Connector DSC1: Rear of the microscope) (Connector DSC2: Connector box)	Not connected	→DS-L3 Chapter 6 “3.1 Configuring the Connection of Digital Camera”
Motorized shutter connected to a SHUTTER connector (Connector EPI SHUTTER: Rear of the microscope) (Connector DIA SHUTTER: Connector box)	EPI SHUTTER: Motorized shutter for the epi-illumination DIA SHUTTER: DIA motorized shutter for the dia-illumination	→DS-L3 Chapter 6 “3.2 Configuring the Connection of Motorized Shutter”
[BUTTON FUNC]		**→Chapter 3 23.1-(3)**
Ni-E microscope Functions of six FUNCTION buttons	FUNCTION button 1, 2: Motorized universal condenser Reverse/forward rotation 3, 4: Motorized excitation filter wheel Reverse/forward rotation 5, 6: Motorized barrier filter wheel Reverse/forward rotation	→DS-L3 Chapter 6 “4.2.1 Setting the Function of the Function Buttons”
Ni-E microscope Motorized shutter controlled with the FL SHUTTER button	Shutter built in the motorized epi-fluorescence cube turret on the 1st. layer	→DS-L3 Chapter 6 “4.2.2 Changing the Motorized Shutter Operated with the FL SHUTTER Button”
Ni-E microscope Digital camera controlled with the CAPTURE button	Camera controlled directly with DS-L3 connected to a USB connector	→DS-L3 Chapter 6 “4.2.3 Changing the Digital Camera Operated with the Microscope’s CAPTURE Button”
Ni-E microscope Motorized device operable with the FL CUBE CW/CCW button	Motorized epi-fluorescence cube turret on 1st layer	→DS-L3 Chapter 6 “4.2.4 Changing the Motorized Device to be Operated with CW/CCW Button”
Ni-E microscope Motorized device operable with the OBJ CW/CCW button	Motorized nosepiece	→DS-L3 Chapter 6 “4.2.4 Changing the Motorized Device to be Operated with CW/CCW Button”
Ni-E microscope Enabling/disabling operation buttons	Enabled	→DS-L3 Chapter 6 “4.2.5 Enabling/Disabling the Button Operation”
Ergo controller Functions of operation buttons	Configured (See Chapter 3 “20 Using the Ergo Controller” in this manual.)	→DS-L3 Chapter 6 “4.3 Configuring the Function of the Ergo Controller Buttons”

Setting	Default setting	Where to see in this manual (DS-L3 for details)
DS-L3 Buttons displayed on the [MICROSCOPE CONTROL] screen	Configured (See DS-L3 Chapter 2 “3.1 [MICROSCOPE CONTROL] Screen (Ni-E)”.)	→DS-L3 Chapter 6 “4.1.1 Selecting the Buttons to be Displayed”
DS-L3 Buttons displayed on the [CAM-MIC CONTROL] screen	Configured (See DS-L3 Chapter 2 “3.2 [CAM-MIC CONTROL] Screen (Ni-E)”.)	→DS-L3 Chapter 6 “4.1.1 Selecting the Buttons to be Displayed”
DS-L3 Hiding the [SLEEP] button on the screen	Hidden	→DS-L3 Chapter 6 “4.1.2 Showing/Hiding the [SLEEP] Button”
[MOVEMENT]		**→Chapter 3 23.1-(4)**
Interlocked operation of the objective*	OFF	→DS-L3 Chapter 6 “5.1.1 Configuring the Interlocked Operation with Switching of Objectives” “5.1.2 Changing the Initial Value of the [INTELLIGENT]” “5.1.3 Automatically Switching the Movement Speed of the Microscope’s Elevating Section and Motorized Stage” “5.1.4 Configuring the Parfocal Correction Function (Auto Link Focus)”
Interlocked operation when switching zoom magnification*	OFF	→DS-L3 Chapter 6 “5.1.5 Configuring the Interlocked Operation with Switching of Zoom Magnification”
Interlocking operations with the switching of the optical path*	OFF	→DS-L3 Chapter 6 “5.1.6 Configuring the Interlocked Operation with Switching of Optical Path”
Automatic operation interlocked with capture button operation	OFF	→DS-L3 Chapter 6 “5.1.7 Configuring the Interlocked Operation with Capture Command Sending or Trigger Signal Output”
Retracting amount of the elevating section	Software limit position	→DS-L3 Chapter 6 “5.2 Setting the Retracting Amount of the Elevating Section”
Rotation stop of the nosepiece depending on the position of the elevating section*	No rotation stop	→DS-L3 Chapter 6 “5.3 Disabling the Rotation of the Motorized Nosepiece Depending on the Position of the Elevating Section”
Reverse rotation stop of the nosepiece*	No rotation stop	→DS-L3 Chapter 6 “5.4 Disabling the Reverse Rotation of the Nosepiece”
Toggle rotation of the nosepiece*	Toggle operation OFF	→DS-L3 Chapter 6 “5.5 Configuring Toggle Function (Alternating between Two Objectives)”
[MODE] (Registration of motorized unit for MODE)		**→Chapter 3 23.1-(5)**
Registering a motorized unit with mode (8 modes)	Not registered	→DS-L3 Chapter 5 “2.1 Registering/Changing Target Motorized Devices”
[UTILITY]		**→Chapter 3 23.1-(6)**
Setting the display pattern of the Ni-E front display panel	Pattern 1 (See Chapter 3 “1 Display Panel Details”.)	→DS-L3 Chapter 6 “6.1 Setting the Display of the Ni-E Front Display Panel”
Enabling/disabling the operation of the elevating section	Enabled	→DS-L3 Chapter 6 “6.2 Enabling/Disabling the Operation of the Elevating Section”
Setting the XYZ position software limit	Z-axis: -2000.000 µm X-axis max. value: 34000.000 µm X-axis min. value: -34000.000 µm Y-axis max. value: 27000.000 µm Y-axis min. value: -27000.000 µm	→DS-L3 Chapter 6 “6.4 Setting the Software Limits”
Buzzer that sounds when you press operation buttons	When pressing the button of the Ni-E main body: Buzzer sounds When pressing the button of the ergo controller: No buzzer sound	→DS-L3 Chapter 6 “6.3 Turning ON/OFF the Buzzer”
[MAINTENANCE]		**→Chapter 3 23.1-(7)**
Movement speed of the epi-fluorescence cube turret	Set to “High Speed”	→DS-L3 Chapter 6 “6.5 Setting the Driving Speed of the Epi-Fluorescence Cube Turret”
Resetting data	...	→DS-L3 Chapter 6 “6.6 Restoring the Factory Default Settings”
Display of the program version	...	→DS-L3 Chapter 6 “6.7 Displaying the Program Version”

2 Operation Flowchart

The microscopy operation flow is shown below.
See the procedure for each microscopy operation in the next and subsequent sections for details.

IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure

Preparation

Focus and orientation

Viewing the sample

Epi-fluorescence Microscopy Procedure

Find the target in the specimen with dia-illumination,

Preparation

Focus and Optical System Adjustment

View the specimen

3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure

This section describes the IR-DIC microscopy procedure which is performed using the microscopes controls and buttons.

The microscope can also be controlled from DS-L3 if a DS-L3 DS Camera Control Unit is connected to the microscope. Examples of DS-L3's [MICROSCOPE CONTROL] screen with the used control buttons are shown for procedures that can be performed from DS-L3. The content and layout of buttons on the screens in the figure and the actual [MICROSCOPE CONTROL] screen may differ because the setting of buttons on the [MICROSCOPE CONTROL] screen can be changed as desired.

For details on the procedure for controlling the microscope from DS-L3, see Chapter 3, "20 Operation on DS-L3" - "20.2 Microscope Control" in this manual.

DS-L3 screens in Ni-E (focusing nosepiece system)

The motorized nosepiece, motorized condenser, etc. are not used in the Ni-E focusing nosepiece system although the DS-L3 screens in this manual show the system in which the motorized devices are fully equipped.

The buttons for the unavailable motorized devices and the devices which are not connected to the microscope will be grayed out and cannot be operated in the actual DS-L3 screen.

Preparation

1 Turn on the power.

Press the power switch to [|] to turn on the power.

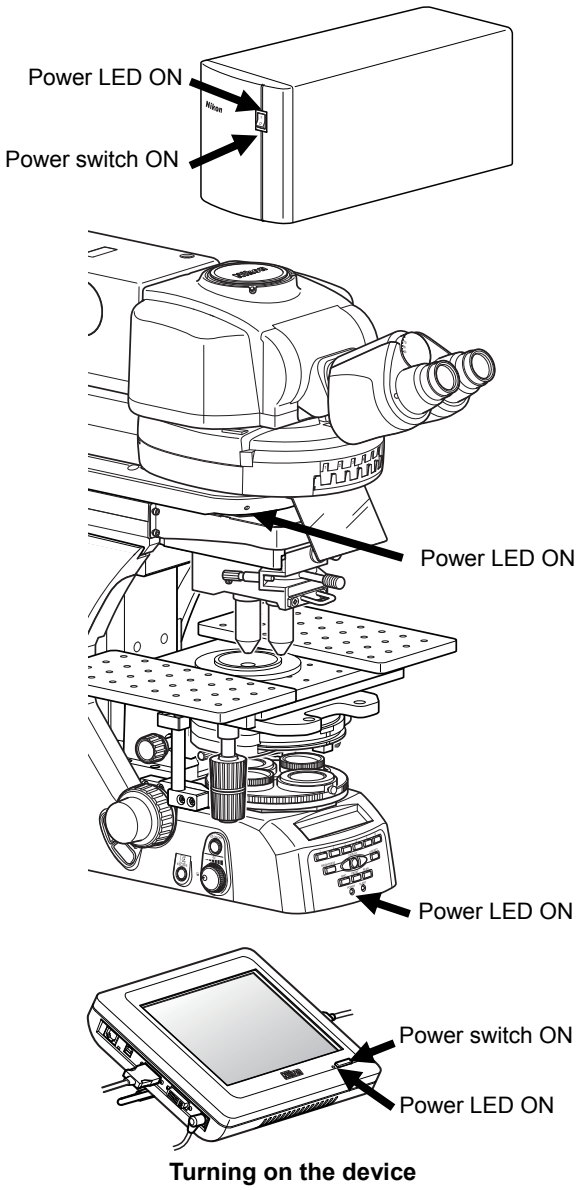
(1) Turn on the power switches for all accessory devices connected. (Except DS-L3)
(The power LED on each device will light up.)

(2) Turn on the power for control box A.
(The power LED on the control box A, front of the microscope, and contact arm will light up.)

(3) Turn on the DS-L3 power switch.

Power on sequence

Turn on the power in the order described above. There is no specific sequence for accessory devices. However, when DS-L3 is connected, turn on control box A before the DS-L3 power switch. This will load data such as microscope's system configuration and settings into DS-L3.



Turning on the device

Display at power on

When the power is turned on, operation progress is displayed on the front display panel of the main body.

When initialization completes, microscope's status appears on the display panel. For details, see Chapter 3, "1 Display Panel Details".

```
 Ni-E Vx.xx_xxxx.xxxx.F1
Data Loading...
```

Top: Model name, firmware version
Bottom: Program startup progress

```
 Ni-E Vx.xx_xxxx.xxxx.F1
Initializing.....
```

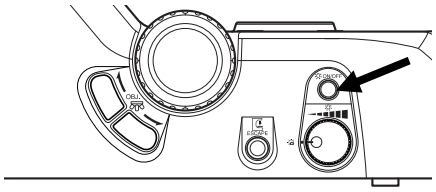
Top: Model name, firmware version
Bottom: Motorized device initialization progress

```
              Z:      0.000um
DIA:ON              FS30.6mm
```

Microscope state display
Display pattern 1: Factory setting example

2 Turn on the dia-illumination lamp.

Press the dia-illumination lamp ON/OFF switch.



Turning on dial-illumination lamp

```
              Z:      0.000um
DIA:ON              FS30.0mm
```

Voltage is not displayed.

Lamp ON, brightness adjustment operation control

The lamp can also be turned on and adjusted from DS-L3 by configuring the lamp control button on the DS-L3's [MICROSCOPE CONTROL] screen or [CAM-MIC] screen. When Ni-E is turned on, control switches on the Ni-E are enabled.

To control from DS-L3, switch the control to the DS-L3 side by pressing the [LAMP CTRL] button configured on DS-L3. When the button is checked, the microscope can be controlled from DS-L3.

DS-L3 [MICROSCOPE CONTROL] top screen

[MIC] button: Switching to MICROSCOPE CONTROL screen

[MICROSCOPE CONTROL] screen

[LAMP CTRL] button: Switch lamp control.

[LAMP ON/OFF] button: Turn on/off the lamp.

[ADJ.] button: Open the sub screen for brightness control.

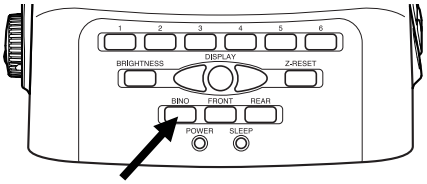
[PHOTO] button (on the sub screen): Photomicrography voltage.

3 Adjust optical path in tube to direct 100% of light to binocular.

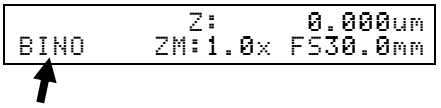
Press the BINO optical path button on the front of the main body.

There are three buttons on the front of the main body for switching the optical path in the tube. The pressed button is lit and that optical path status is set.

- BINO optical path button: 100% to binocular
- FRONT optical path button: 100% to tube adapter
- REAR optical path button: 100% to rear port



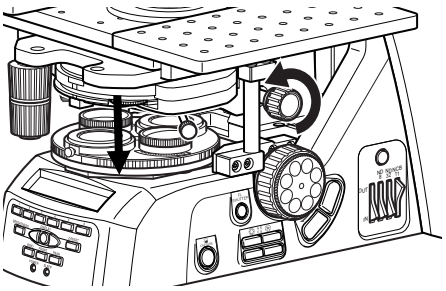
Binocular 100% with [BINO] button



[BINO] button: Binocular optical path 100%

4 Lower the condenser slightly from the uppermost position.

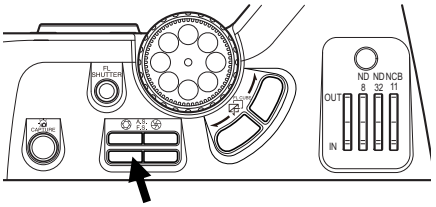
Turn the condenser focus knob until the condenser is positioned at the upper limit (where it clicks to a stop), and then lower it a little.



Lowering the condenser slightly from uppermost position using the condenser focus knob

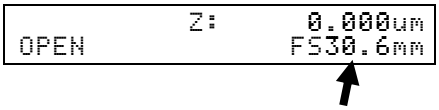
5 Fully open the field diaphragm and aperture diaphragm.

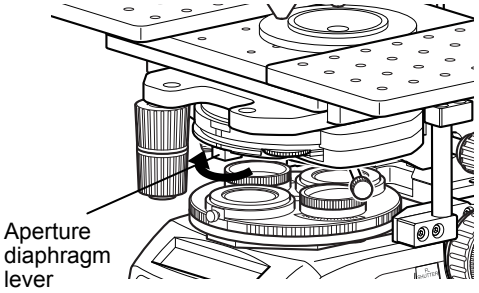
Press the left side of the DIA field diaphragm button (◎ mark side) and fully open the field diaphragm.
Pressing the right side (⊗ mark) will close the diaphragm.
Turn the aperture diaphragm lever fully clockwise to open the aperture diaphragm completely.



DIA field diaphragm button

Field diaphragm fully open



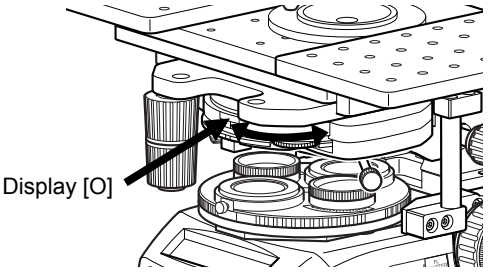


Aperture diaphragm fully open

[F. STOP] button, sub screen: DIA field diaphragm open

6 Set the condenser turret to [O] position (empty: bright-field).

Turn the condenser turret and face the display [O] on the turret to the front. The turret's empty position enters the optical path in this state.



Moving the condenser to the empty position

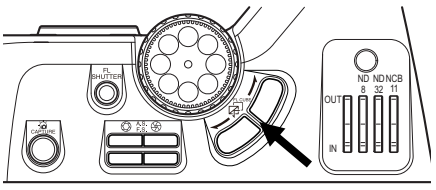
7 Move the epi-fluorescence cube turret to empty position, and remove the DC slider (objective side) and polarizer from the optical path.

Press the FL CUBE CW/CCW button and bring the epi-fluorescence cube turret's [OPEN] (empty position) into the optical path.

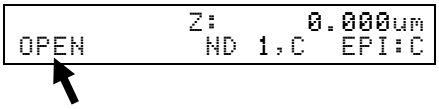
Pressing the FL CUBE CW button turns the turret by one address in the clockwise direction (as viewed from above), while the CCW button turns the turret in the counterclockwise direction.

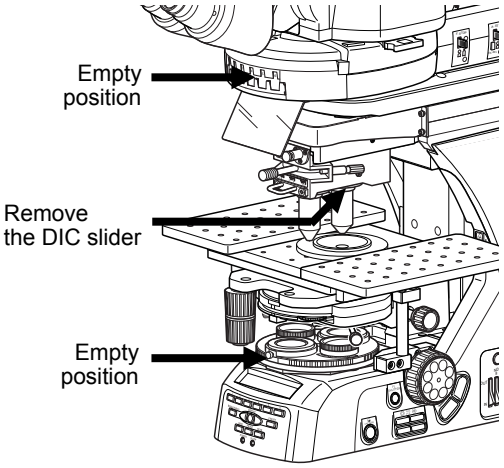
If the DIC slider is in the objective side, pull it out and remove it.

Turn the polarizer turret to bring the empty position into the optical path.



Bringing the [OPEN] position into optical path





Removing the DIC optical element from the optical path

[FL TURRET] button, sub screen: [OPEN]

Intelligent epi-fluorescence cube turret

Switch the intelligent epi-fluorescence cube turret manually because it is not motorized. On DS-L3, only information display is provided.

FL/DIC analyzer slider NI-FA/dummy slider

For IR-DIC microscopy, the FL/DIC analyzer slider inserted in the epi-fluorescence cube turret is not used. If the analyzer slider is inserted, pull it out to the first click-stop position and remove the analyzer from the optical path. If a dummy slider is inserted instead of the analyzer slider, be sure not to insert all the way, but to pull it out by one position.

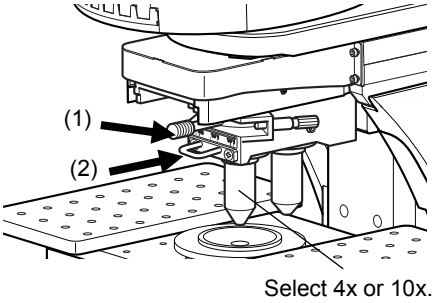
The periphery of field of view may be dark if it is inserted all the way in.

Focus and orientation

8 Bring the 4x or 10x objective into the optical path.

(1) Move the objective vertical movement lever to the center. (to temporarily retract the objective upward)

(2) Push the sliding nosepiece toward the rear, and bring the low magnification objective (4x or 10x) attached to the front into the optical path. (See Chapter 3, “11 Operating the Nosepiece (objective operation”.)



Bringing the 4x or 10x objective into the optical path

Cautions on switching objectives

Be sure to move the objective vertical movement lever to the center before switching objectives (between front and rear).

Objectives cannot be switched when the objective vertical movement lever is at the right or left end. Undue force may result in malfunction.

When using a 4x objective

When using a 4x objective, attach the 4x auxiliary lens to the LWD condenser turret and bring it into the optical path. When using an objective with magnification higher than 4x, remove the 4x auxiliary lens from the optical path.

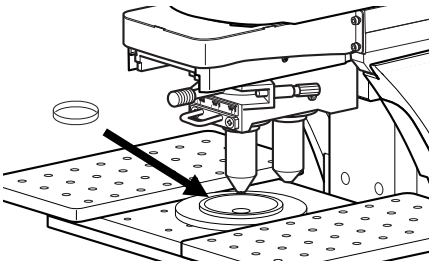
9 Place a sample on the stage, and move the stage to bring the target into view.

(1) Set the sample at the center of the stage.

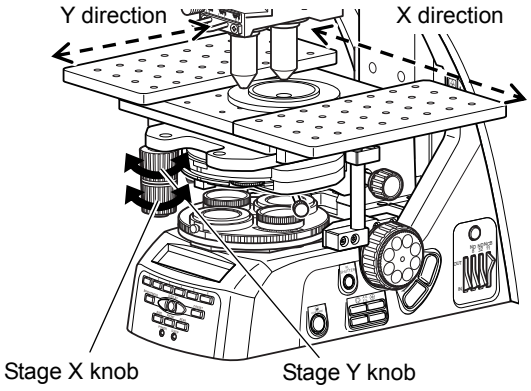
(2) Rotate the stage X/Y knobs to move the stage and bring the target of the sample into the optical path. (See Chapter 3, “5 Bringing the Target into the Optical Path (Horizontal Stage Movement)”.)

Motorized horizontal stage movement

When using a motorized XY stage, use the ergo or joystick controller to control the horizontal stage movement. (See the Ni-E (Focusing Stage System) instruction manual for details on the ergo controller or joystick controller.)



Setting sample

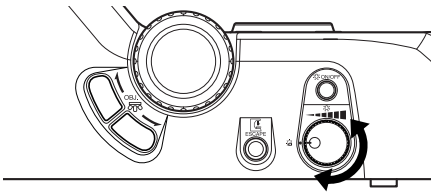


Stage knob operation and stage moving direction

10 Focus on the sample.

(1) Look into the eyepiece, and adjust the brightness appropriately by turning the dia-illumination brightness control knob.

(2) Looking through the eyepiece, focus on the sample by rotating in the order of the coarse focus knob and then the fine focus knob. (See Chapter 3, “4.1 Proper Focusing Procedure”.)



Brightness control

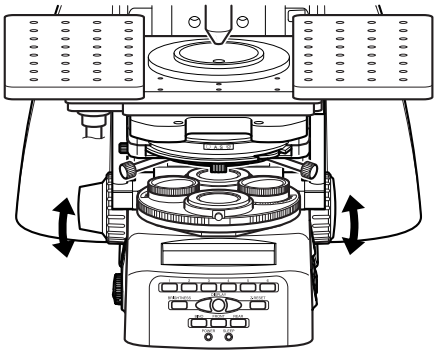
Z-RESET

The Z-axis coordinate value can be reset to zero. This is useful when you wish to use the current position as the reference position in adjusting the focus. (See Chapter 3, “4.3 Resetting the Z-axis Coordinate”.)

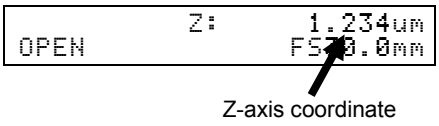
Brightness control

Refocusing

This operation moves the Z-axis position to the retracting position or returns it to the original position. (See Chapter 3, “4.4 Refocusing”.)



Focusing using the focus knob

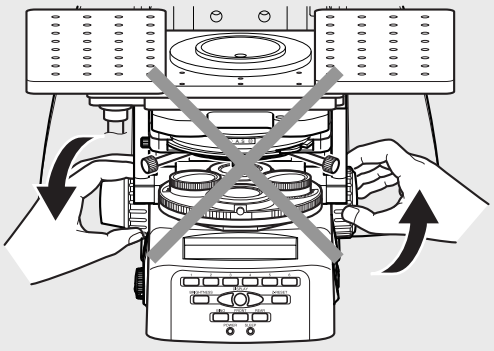


Z-axis coordinate

Note on controlling the focus knobs

Avoid the following action, which can cause equipment malfunction.

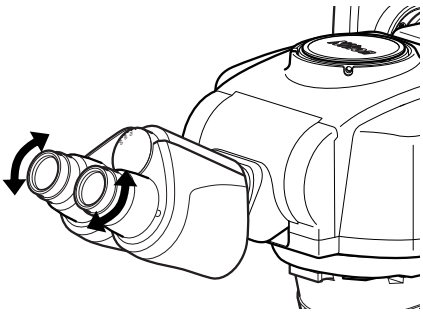
- Rotating the right and left focus knobs in opposite directions.



Do not turn the focus knobs in opposite direction!

11 Perform diopter adjustment.

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. Do not use the focus knobs. (See Chapter 3, “6 Adjusting the Diopter”.)

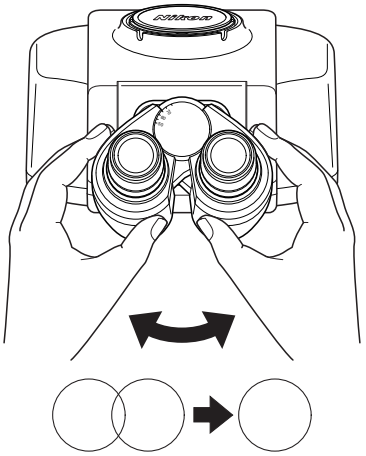


Adjusting diopter

12 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.



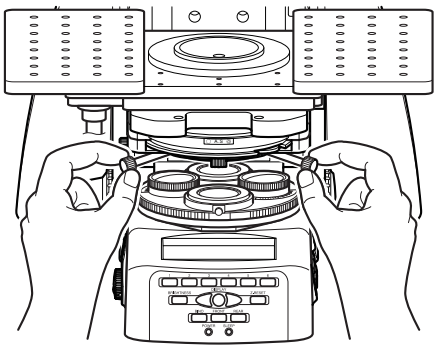
Adjusting the interpupillary distance

13 Focus and center the condenser.

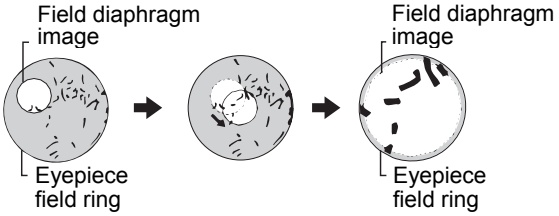
(1) Press the DIA field diaphragm button to close the field diaphragm to a minimum and look into the eyepiece. Focus on the field diaphragm image using the condenser focus knob, and then adjust the condenser centering screws to center the diaphragm image within the field of view.

(2) With the objective vertical movement lever at the center position, pull the sliding nosepiece and bring the rear objective (40x or 60x attached) into the optical path. (The condenser turret remains at the [O] empty position.)

(3) Turn the objective vertical movement lever to the right end, turn the parfocal correction knob on the right side of the sliding nosepiece, and focus on the sample. Turning the knob clockwise raises the objective (focus position rises).
For information on the relationship between the position of the objective vertical movement lever and the position of the objective, see Chapter 3, “11.1 Objective Vertical Movement Lever (objective switching, dipping)”.



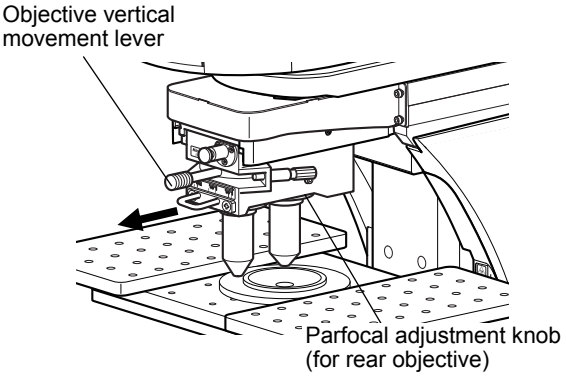
Bringing rear objective into optical path



Focusing and centering the condenser

Cautions when using the parfocal correction knobs

Before using the parfocal correction knobs, make sure that the objective vertical movement lever is at the right or left end. The parfocal correction knob on the left side is for adjusting the front objective, and the parfocal correction knob on the right side is for adjusting the rear objective.



(4) Turn the condenser focus knob to focus on the field diaphragm image. If the image is not at the center of the field, turn the condenser centering screw so that the image is positioned at the center.

(5) Press the DIA field diaphragm button and adjust the field diaphragm image so that its size is close to the field of view.

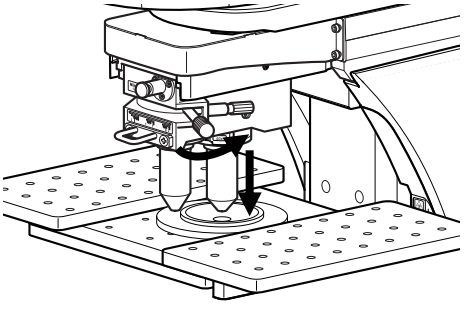
(See Chapter 3, “7 Focusing and Centering the Condenser”.)

14 Water-dip objectives (water immersion).

(1) Slowly move the objective vertical movement lever to the right end, and then press it further right.
This lowers the objective about 1 mm, immersing the tip of the objective in the liquid on the specimen.

(2) Stop pushing the lever and return it to right end.
The objective also returns to the original height.

(See Chapter 3, “11.1 Objective Vertical Movement Lever (objective switching, dipping)”.)



Dipping objectives

15 Adjust the aperture diaphragm.

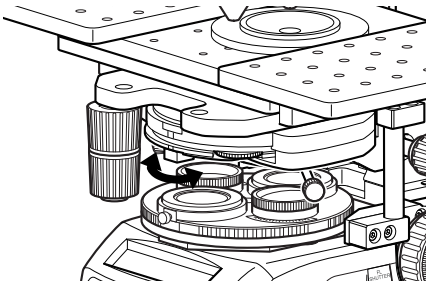
Turn the condenser aperture diaphragm lever to adjust the aperture. The aperture should be adjusted to about 70 to 80% of the numerical aperture of the objective. (See Chapter 3, “8 Adjusting the Aperture Diaphragm”.)

Appropriate size of the aperture diaphragm

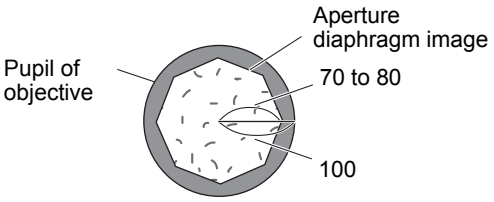
Normally, the appropriate size of the aperture diaphragm is 70 to 80% of the numerical aperture of the objective. Since an excessively small aperture diaphragm opening will degrade the image resolution, Nikon does not recommend setting the aperture diaphragm to less than 60% of the numerical aperture of the objective.

Aperture diaphragm adjustment timing

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



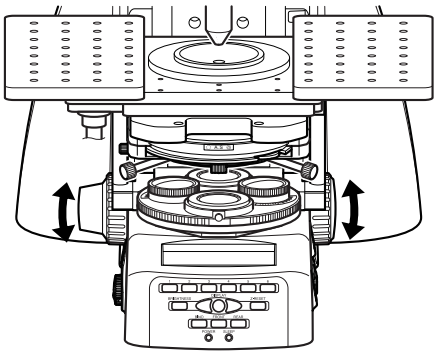
Adjusting aperture diaphragm



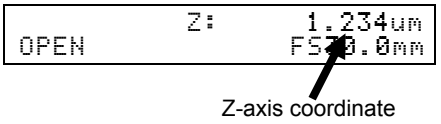
Right size of the aperture diaphragm

16 Focus on the sample.

Focus on the sample by rotating the focus knob. (See Chapter 3, “4.1 Proper Focusing Procedure”.)



Focusing using the focus knob



17 Adjust the field diaphragm.

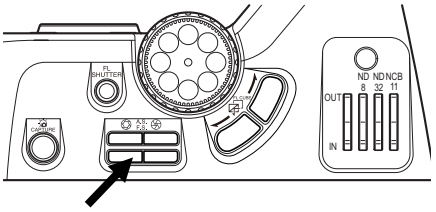
Press the DIA field diaphragm button to adjust the field diaphragm so that it circumscribes the field of view.

Size of the field diaphragm

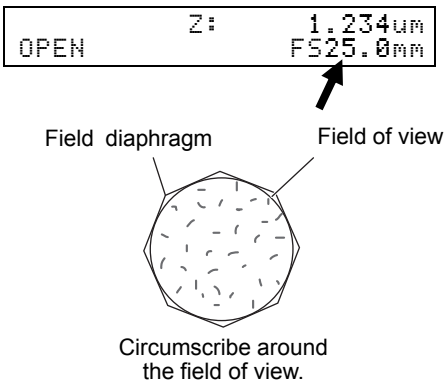
Normally, the field diaphragm should be adjusted so that it circumscribes the field of view. Excessively opening the field diaphragm will result in stray light entering the field of view, generating flare and reducing the image contrast. It will also cause the sample to become decolorized over a wider area.

Field diaphragm adjustment timing

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



Adjusting field diaphragm

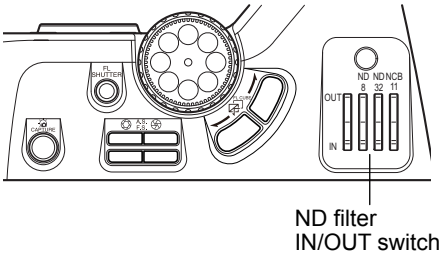


Right Size of the Field Diaphragm

[F. STOP] button, sub screen: DIA field diaphragm adjustment

18 Adjust the brightness.

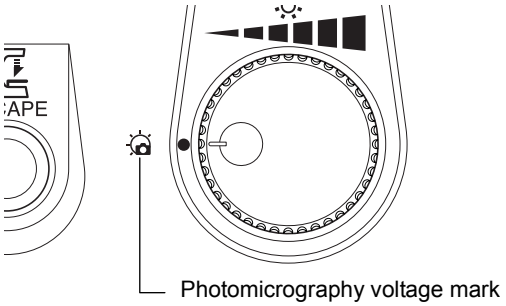
Adjust the brightness of the image using the ND filter. Press the ND filter IN/OUT switch to the [IN] (lower) side to bring the filter into the optical path. Brightness can be adjusted by turning the dia-illumination brightness control knob, but care must be taken because the color of the image will change. (See Chapter 3, “3 Adjusting the Brightness of a Diascopic Image”.)



Brightness adjustment using ND filter

Photomicrography voltage setting: for improved color reproduction

Changing the lamp voltage for brightness adjustment will affect the lamp color temperature and alter the color balance of the image. Increasing the voltage will result in a bluish tint, while reducing the voltage results in a reddish tint. If accurate color reproduction is critical, setting the dia-illumination brightness control knob (on left of the main body) to the mark will adjust the brightness to the photomicrography voltage best suited for image captures. (The photomicrography voltage is adjusted/set at the factory and cannot be changed by the user.) To adjust the brightness further without changing color, bring the NCB11 filter in the filter cassette into the optical path and use the ND filter for adjustment. (See Chapter 3, “3 Adjusting the Brightness of a Diascopic Image”.)



Photomicrography voltage setting

19 Adjust the orientation (vibration direction) of the polarizer and analyzer.

This adjustment is performed to determine the basic performance of differential interference contrast method. Perform it carefully.

(1) Check that the rear of the objective (40x or 60x) is in the optical path and the condenser turret is at [O] (empty position). If they are not at the right position, set 40x (or 60x) and [O] (empty position) accordingly.

(2) Check that the DIC slider is not in the objective position in the optical path.

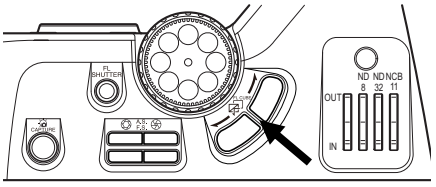
(3) Press the FL CUBE CW/CCW button and bring the DIC analyzer cube IR of the epi-fluorescence cube turret into the optical path.

(4) Turn the polarizer turret to bring the polarizer IR into the optical path.

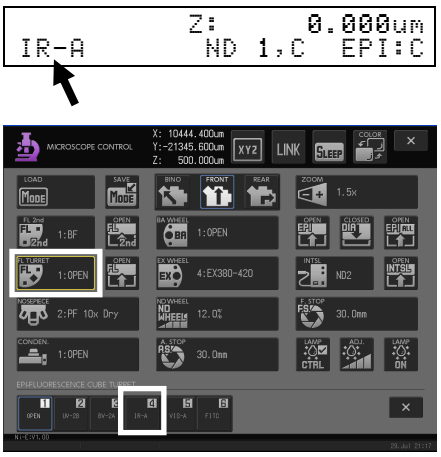
(5) Rotate the stage knob to move the location without sample into the optical path.

(6) Loosen the polarizer fixing screw once, and turn the polarizer rotation knurled ring on the right side of the polarizer turret to adjust the field of view so that the field of view becomes darkest (where dark cross appears). When adjustment is complete, tighten the fixing screw.

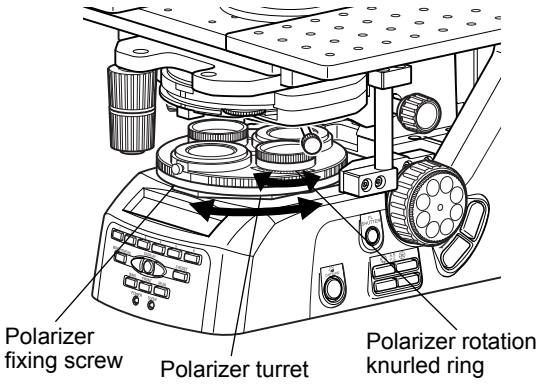
> **For experimentation with IR camera**
>
> During experiments using an IR camera, the orientation cannot be adjusted visually. Adjust the orientation so that the signal intensity becomes minimum (the light passed through the optical system becomes minimum).



Bringing DIC analyzer into optical path



[FL TURRET] button, sub screen: [IR-A]



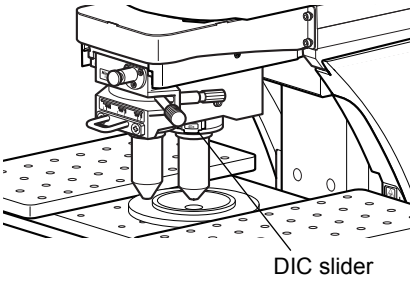
Bringing IR polarizer into optical path adjust position

Dark cross

Viewing the sample

20 Attach the DIC slider (objective side) to the slider nosepiece.

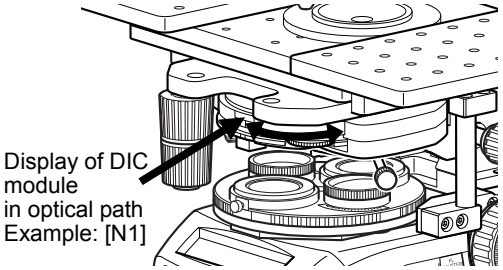
Turn the objective vertical movement lever counterclockwise to lower the objective. You can see the DIC slider insertion slot for the rear objective. Insert the DIC slider into the slot. (If the front objective is in the optical path, turn the objective vertical movement lever clockwise to lower the objective. You can see the DIC slider insertion slot.).
Choose the DIC slider appropriate for the intended microscopy purpose and the objective used.
(See Chapter 3, “16 Differential Interference Contrast Microscopy - 16.2 Using Optical Elements”.)



Attaching the DIC slider on the objective

21 Bring the DIC module (condenser side) into the optical path.

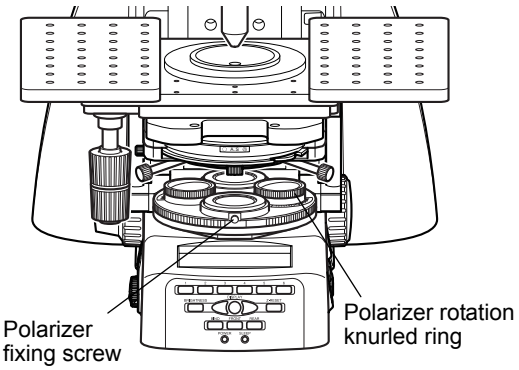
Turn the condenser turret to bring the DIC module suited for the objective into the optical path.
Choose the DIC module appropriate for the intended microscopy purpose and the objective used.
(See Chapter 3, “16 Differential Interference Contrast Microscopy - 16.2 Using Optical Elements”.)



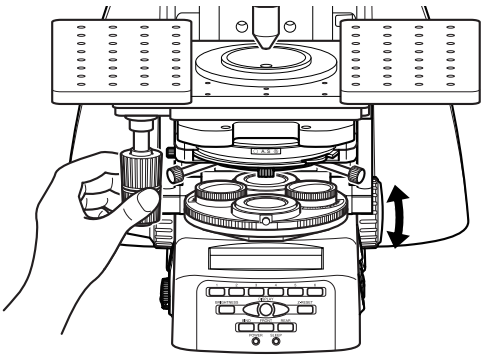
Bringing the DIC module on the condenser into the optical path

22 Observe a sample using a microscope.

- Use ND filters to adjust the image brightness.
- To adjust the contrast, turn the polarizer rotation knurled ring on the right of the polarizer turret to change the direction of the polarizer.
- To observe another part of the sample, rotate the stage knobs to find the position to observe.
- If the sample is not in focus, rotate the focus knobs to adjust the focus.
- To observe with another magnification, attach the desired DIC objective to the sliding nosepiece, adjust the aperture diaphragm and field diaphragm, and follow step 13 and subsequent steps.



Contrast adjustment



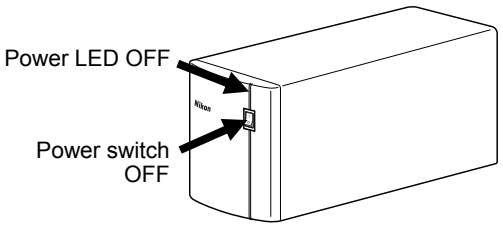
Moving sample and focus

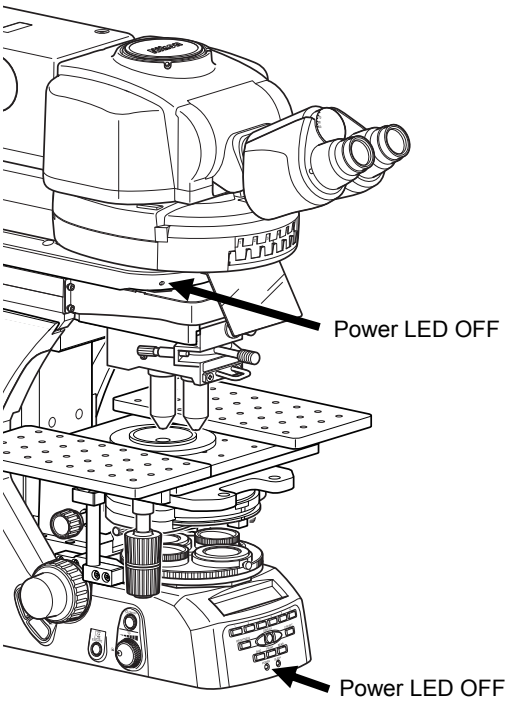
> **Parfocal adjustment of the objective**
>
> Normally, dry objectives and an immersion objectives have different focal lengths. Switching between these two objectives causes misalignment of the focal point. In this case, adjust the focus using the parfocal correction knobs on the sides of the sliding nosepiece.
>
> In the above example procedures, because the objective is currently in focus and doing microscopy using an immersion DIC objective attached to the rear, no parfocal adjustment is required when you attach an immersion type objective at the front side even if its magnification is different.
>
> When you attach a dry objective, perform parfocal adjustment according to Chapter 3, “11.2 Parfocal Correction of Objective”.

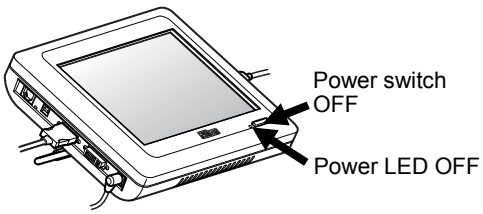
> **FL/DIC analyzer slider NI-FA/dummy slider**
>
> For IR-DIC microscopy, the FL/DIC analyzer slider inserted in the epi-fluorescence cube turret is not used. If the analyzer slider is inserted, pull it out to the first click-stop position and remove the analyzer from the optical path. If a dummy slider is inserted instead of the analyzer slider, be sure not to insert all the way, but to pull it out by one position. The periphery of field of view may be dark if it is inserted all the way in.

23 Turn off the power.

Turn off the power switches (press to the “O” position) on the control box A and connected motorized devices. (Each power LED turns off.)







Turning off the device

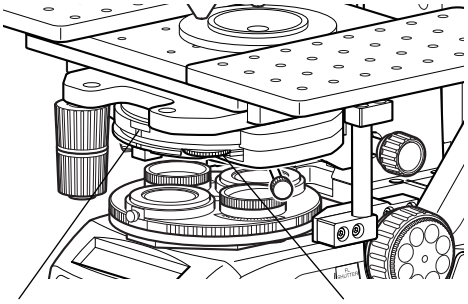
Diagonal illumination

Using diagonal illumination, contrast can be applied to almost transparent samples thus making it possible to observe them. In addition, since the slit of the diaphragm for diagonal illumination can be rotated centering around the optical axis, you can adjust the direction of the shadow on the sample.

1 Bring the diaphragm for diagonal illumination into the optical path.

Attach the diaphragm for diagonal illumination to the turret on the condenser in advance. (See Chapter 1 “3 Assembly Method - 9 Attach the condenser - ■ Attaching the optical module to the condenser turret” in “Assembly/Maintenance”.)

Turn the turret and face the display [OBL] indication to the front. The diagonal illumination diaphragm enters the optical path in this state.



Bringing the diagonal illumination diaphragm into the optical path, and adjusting the direction of the illumination

2 Adjust the direction of the illumination.

Rotate the rotation ring of the diaphragm for diagonal illumination on the condenser turret to adjust the direction of the illumination.

4 Epi-fluorescence Microscopy Procedure

This section describes the epi-fluorescence microscopy procedure which is performed using the microscope's controls and buttons.

Precautions on switching between epi-fluorescence microscopy IR-DIC microscopy are also described.

Find the target in the sample with dia-illumination as described in "3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure" and then prepare for epi-fluorescence microscopy.

The microscope can also be controlled from DS-L3 if a DS-L3 DS Camera Control Unit is connected to the microscope. Examples of DS-L3's [MICROSCOPE CONTROL] screen with the used control buttons are shown for procedures that can be performed from DS-L3. The content and layout of buttons on the screens in the figure and the actual [MICROSCOPE CONTROL] screen may differ because the setting of buttons on the [MICROSCOPE CONTROL] screen can be changed as desired.

For details on the procedure for controlling the microscope from DS-L3, see Chapter 3, "20 Operation on DS-L3" - "20.2 Microscope Control" in this manual.

DS-L3 screens in Ni-E (focusing nosepiece system)

The motorized nosepiece, motorized condenser, etc. are not used in the Ni-E focusing nosepiece system although the DS-L3 screens in this manual show the system in which the motorized devices are fully equipped.
The buttons for the unavailable motorized devices and the devices which are not connected to the microscope will be grayed out and cannot be operated in the actual DS-L3 screen.

⚠ WARNING

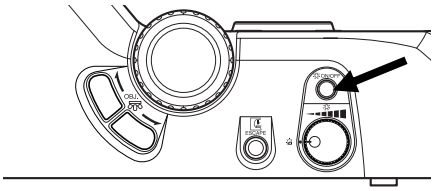
The light source used with the epi-fluorescence attachment (mercury lamp) requires special care during handling because of its characteristics. Make sure you are familiar with and adhere to all warnings and cautions described at the beginning of this instruction manual.

Preparation

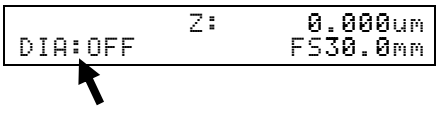
Find the target in the sample with dia-illumination as described in "3 IR-DIC (Infrared Ray (Differential Interference Contrast) Microscopy". (See Chapter 3, "17.7 Other Notes on Epi-fluorescence Microscopy".)

1 Turn off the dia-illumination lamp.

Press the dia-illumination ON/OFF switch.



Turning off dial-illumination lamp

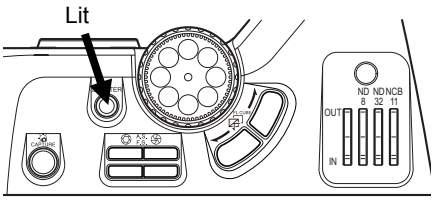


[MICROSCOPE CONTROL] screen

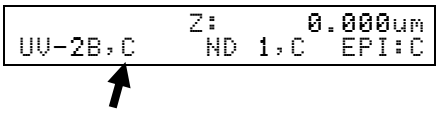
[LAMP ON/OFF] button: Lamp OFF

2 Close the shutter and block the illumination path.

Press the [FL SHUTTER] button and close the shutter in the motorized fluorescence cube turret. The shutter is closed when the shutter button is lit.



Closing shutter



C: Shutter closed

SHUTTER

The shutter blocks illumination.

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

Make sure you closed the shutter when you suspend the microscopy or when pausing epi-fluorescence microscopy to perform microscopy using diascopic light. Be sure to get into the habit of performing this operation.

[SHUTTER FL] button: Open/Close of the shutter in the motorized epi-fluorescence cube turret

The display of the button is switched to indicate the current open/close status.

Display [OPEN]: shutter open, [CLOSED]: shutter closed

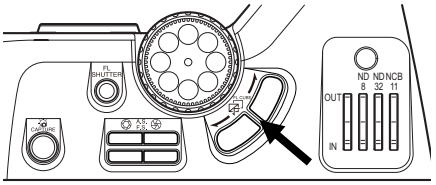
When an intelligent epi-fluorescence cube turret is used, the [SHUTTER FL] button is not displayed. Therefore you cannot check the shutter open/close status on DS-L3.

Shutter blocking epi-fluorescence light

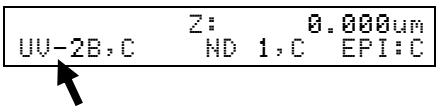
The shutter is built into the epi-fluorescence cube turret and is also equipped on the HG precentered fiber illuminator. Also, an EPI motorized shutter can be attached to the microscope.
Epi-fluorescence illumination can be blocked by closing one of the shutters. (See Chapter 3, “17.3 Protecting the Sample and Preventing It from Decoloration (Using the Shutter)”.)

3 Bring the filter cube into the optical path.

Press the FL CUBE CW/CCW button to bring the desired filter cube into the optical path.
Pressing the FL CUBE CW button turns the turret by one address in the clockwise direction (as viewed from above), while the CCW button turns the turret in the counterclockwise direction.



Bringing the filter cube into the optical path

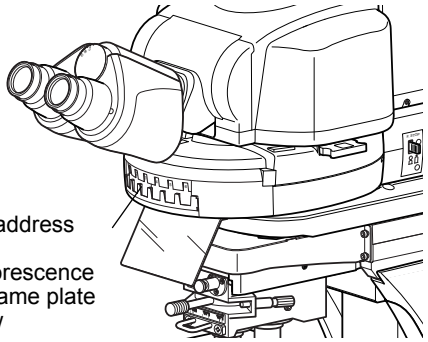


Selecting a filter cube

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 sample and the fluorescence dye. (See Chapter 3, “17.2 Selecting Filters”.)

Intelligent epi-fluorescence cube turret

Switch the intelligent epi-fluorescence cube turret manually because it is not motorized. On DS-L3, only the information display is provided.



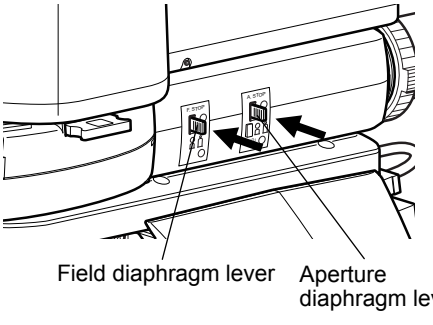
Turret address and epi-fluorescence cube name plate window

[FL TURRET] button: Open the sub screen for switching the filter cube.

Sub screen: Set the filter cube.

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

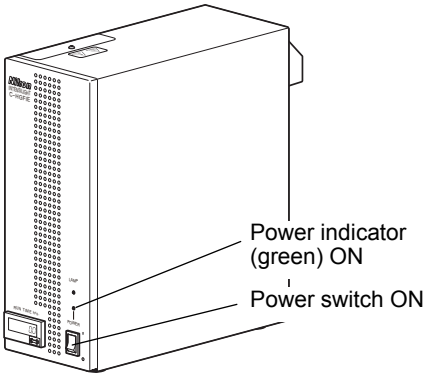
Push in the field diaphragm lever and the aperture diaphragm lever on the epi-fluorescence attachment to fully open the diaphragms.



Fully opening field and aperture diaphragms

5 Turn on the mercury lamp.

See your illuminator's manual for details.



Mercury lamp ON

6 Microscopy with different magnification

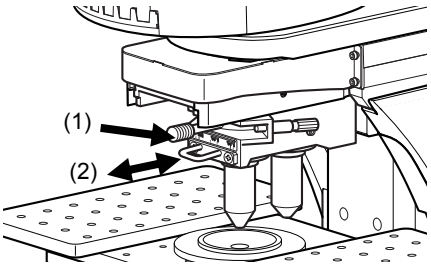
(1) Move the objective vertical movement lever to the center. (To temporarily retract the objective upward.)

(2) Push/pull the sliding nosepiece to bring the objective to be used into the optical path. (See Chapter 3, "11 Operating the Nosepiece (objective operation)".)

Cautions on switching objectives

Be sure to move the objective vertical movement lever to the center before switching objectives (between front and rear).

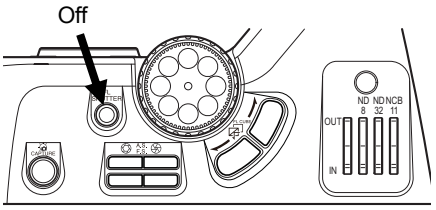
Objectives cannot be switched when the objective vertical movement lever is at the right or left end. Undue force may result in malfunction.



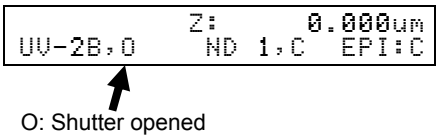
Bringing objective into optical path

7 Open the shutter.

Press the [FL SHUTTER] button and open the shutter in the motorized epi-fluorescence cube turret which is closed in procedure 2.
When the shutter is opened, the button illumination will turn off.



Opening the shutter



O: Shutter opened

[SHUTTER FL] button: Open/Close of the shutter in the motorized epi-fluorescence cube turret

The display of the button is switched to indicate the current open/close status.

Display [OPEN]: shutter open, [CLOSED]: shutter closed

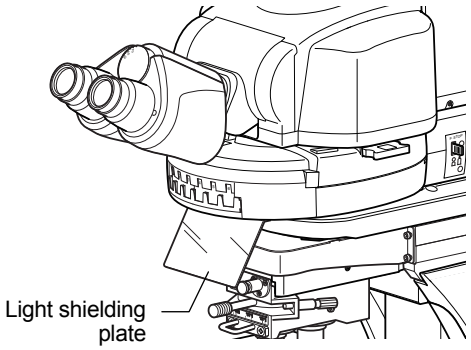
When an intelligent epi-fluorescence cube turret is used, the [SUTTER FL] button is not displayed. Therefore you cannot check the shutter open/close status on DS-L3.

Other shutters

If the shutter of the HG precentered fiber illuminator or EPI motorized shutter is closed, be sure to open these shutters as well.

Light shielding plate on the epi-fluorescence cube turret

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



Focusing and Optical System Adjustment

8 Focus on the sample.

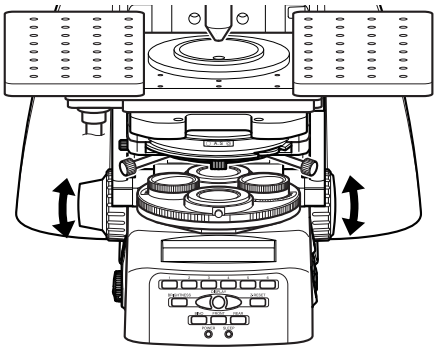
Focus on the sample by rotating the focus knob. (See Chapter 3, “4.1 Proper Focusing Procedure”.)

Z-RESET

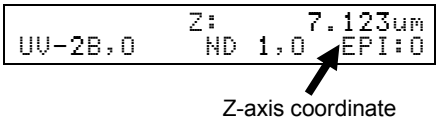
The Z-axis coordinate value can be reset to zero. This is useful when you wish to use the current position as the reference position in adjusting the focus. (See Chapter 3, “4.3 Resetting the Z-axis Coordinate”.)

Refocusing

This operation moves the Z-axis position to the retracting position or returns it to the original position. (See Chapter 3, “4.4 Refocusing”.)



Focusing using the focus knob

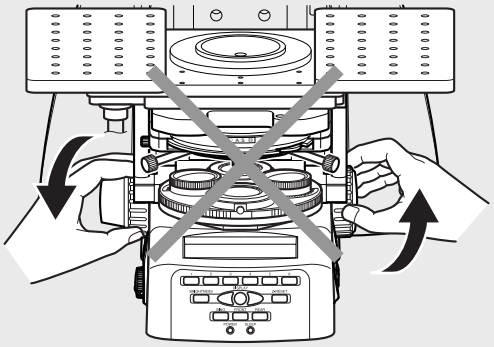


Z-axis coordinate

Note on controlling the focus knobs

Avoid the following action, which can cause equipment malfunction.

- Rotating the right and left focus knobs in opposite directions.



Do not turn the focus knobs in opposite direction!

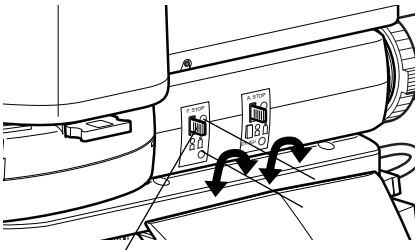
9 Center the field diaphragm of the epi-fluorescence attachment.

(1) Use the field diaphragm lever on the epi-fluorescence attachment to close the field diaphragm, and then move the field diaphragm image to the center of the field of view by turning the field diaphragm centering screw using a hex driver (2 mm across flats).

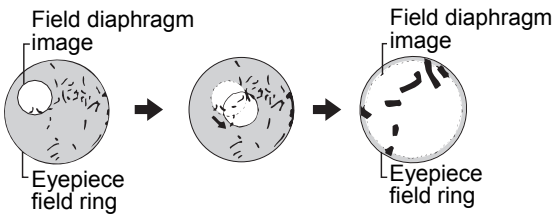
(2) Open the field diaphragm to fit the field of view, and once again, turn the centering screw to move the field diaphragm image to the center of the field of view.

Changing magnification

While it is not absolutely essential to center the field diaphragm each time you change the objective, you should still perform a quick check and make adjustments as necessary.



Field diaphragm lever



Centering the field diaphragm

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

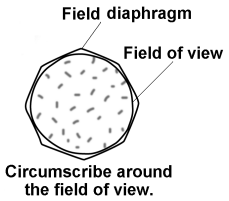
Use the field diaphragm lever of the epi-fluorescence attachment to adjust the field diaphragm so that it circumscribes the field of view.

Size of the field diaphragm

Normally, adjust the field diaphragm so that it 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 sample will become decolorized over a wider area.

Field diaphragm adjustment timing

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

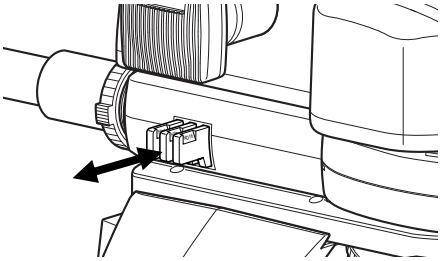


Right Size of the Field Diaphragm

Adjusting the field diaphragm

11 Adjust the brightness.

Use ND filters on the epi-fluorescence attachment to adjust image brightness.
The aperture diaphragm of the epi-fluorescence attachment can also be used for brightness adjustment of the image. Be sure to center the aperture diaphragm before use. (See Chapter 3, “17.4 Adjusting the Brightness of the Fluorescent Image (Using ND Filters and the Aperture Diaphragm)”.)

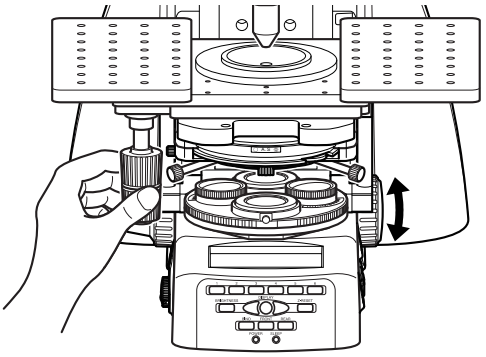


Brightness adjustment using the ND filter

Viewing the sample

12 View the sample.

To observe another part of the sample, rotate the stage knobs to find the position to observe. If the sample is not in focus, rotate the focus knobs to adjust the focus.
To observe under a different magnification, repeat steps 6 and later with another objective.



Diascopic image in fluorescence observation

For fluorescence observations, press the microscope's dia-illumination ON/OFF switch to "OFF" to make the diascopic image disappear.

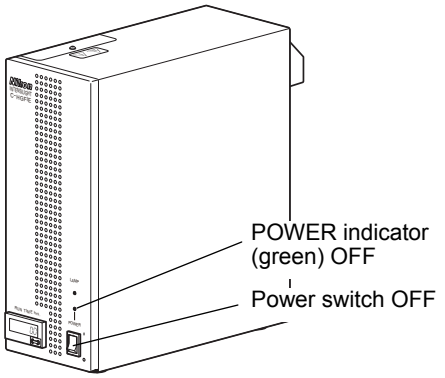
Bright ambient lights will make it difficult to view the image. Nikon recommends keeping the room dark during fluorescence observations.

FL/DIC analyzer slider NI-FA/dummy slider

For epi-fluorescence microscopy, the FL/DIC analyzer slider inserted in the epi-fluorescence cube turret is not used. If the analyzer slider is inserted, pull it out to the first click-stop position and remove the analyzer from the optical path. If a dummy slider is inserted instead of the analyzer slider, be sure not to insert all the way, but to pull it out by one position. The periphery of field of view may be dark if it is inserted all the way in.

13 Turn off the mercury lamp.

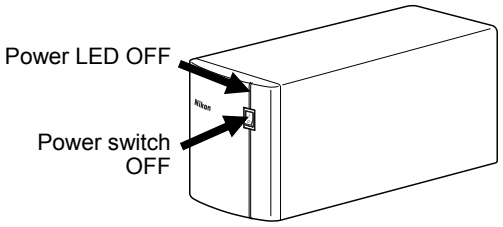
See your illuminator's manual for details.

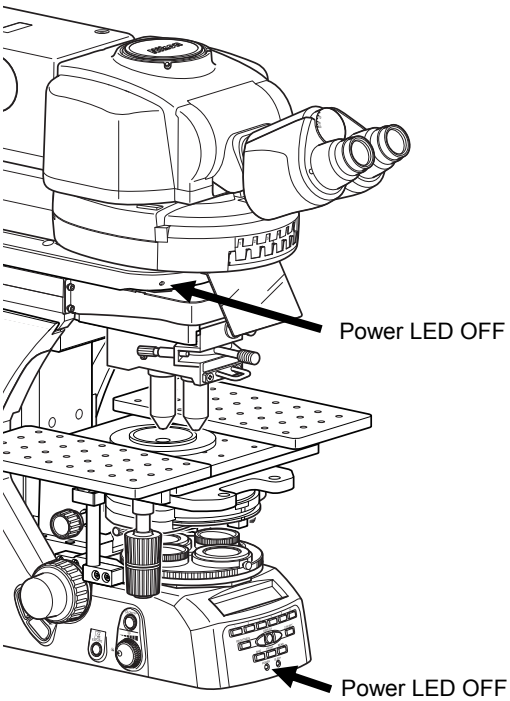


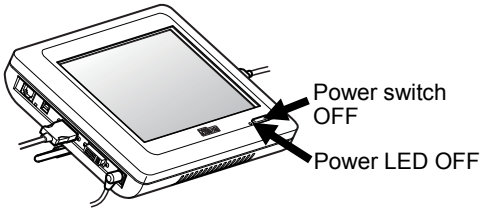
Turning off the mercury lamp

14 Turn off the power.

Turn off the power switches (press to the “O” position) on the control box A and connected motorized devices. (Each power LED turns off.)







Turning off the device

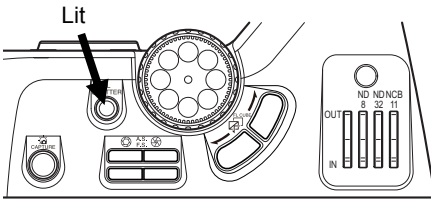
Switching from IR-DIC (infrared ray differential interference contrast) microscopy to epi-fluorescence microscopy

Epi-fluorescence and differential interference contrast methods can be used concurrently to cover the shortcomings of each. For example, when locating the target, differential interference contrast microscopy can be used instead of epi-fluorescence microscopy which tends to cause decoloration of the specimen.
This section provides tips for switching from IR-DIC (infrared ray differential interference contrast) microscopy to epi-fluorescence microscopy when devices required for epi-fluorescence microcopy and IR-DIC (infrared ray differential interference contrast) microscopy are attached. Precautions on concurrent use of epi-fluorescence/differential interference contrast microscopy in the visible light range are also noted. Also see “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure”.

(1) When turning on the power switch at the beginning of “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure”, keep the power of the HG precentered fiber illuminator for epi-fluorescence illumination OFF.

Check that the epi-fluorescence illumination path shutter is closed.

The shutter is closed when the [FL SHUTTER] button is lit.

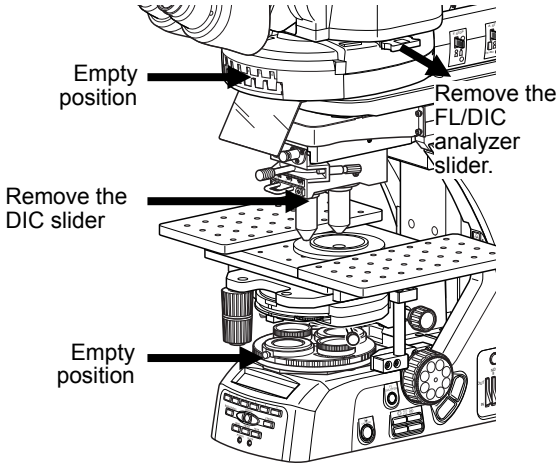


Closing shutter

(2) All optical elements required for differential interference contrast microscopy are once removed from optical path in procedures 6 and 7 of “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure”.

Focus and adjust the optical system by IR-DIC (infrared ray differential interference contrast) microscopy in this state.

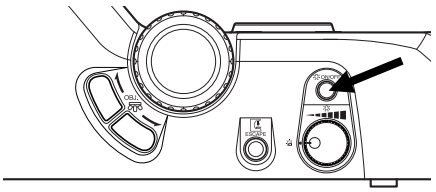
(3) Adjust the orientation of the polarizer and analyzer by procedure 19 of “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure”.



Removing the DIC optical element from the optical path

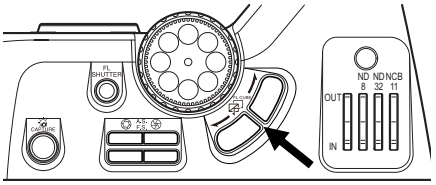
(4) When IR-DIC (infrared ray differential interference contrast) microscopy is finished, go to epi-fluorescence microscopy.

- Press the dia-illumination ON/OFF switch to turn off the lamp and make the diascopic image disappear.



Turning off dial-illumination lamp

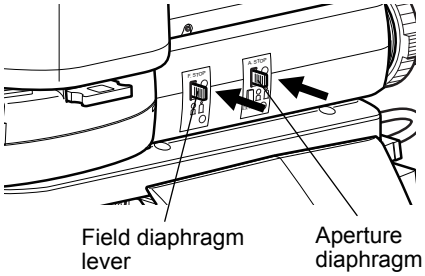
- Rotate the epi-fluorescence cube turret to bring the desired filter cube into the optical path.



Bringing the filter cube into the optical path

- Fully open the field diaphragm and aperture diaphragm of the epi-fluorescence attachment.

(5) Turn on the power of the HG precentered fiber illuminator and continue from procedure 6 of the epi-fluorescence microscopy procedure.

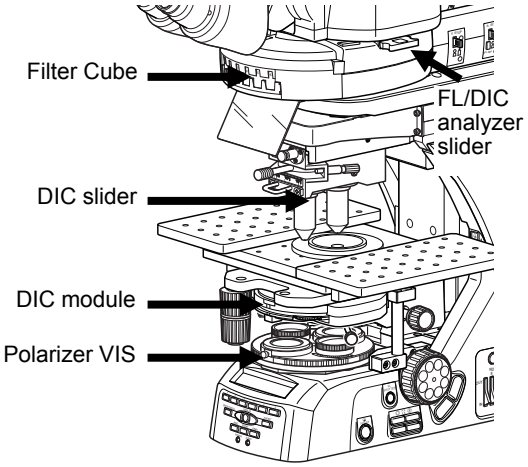


Fully opening field and aperture diaphragms

Performing epi-fluorescence/visible light range differential interference contrast concurrent microscopy

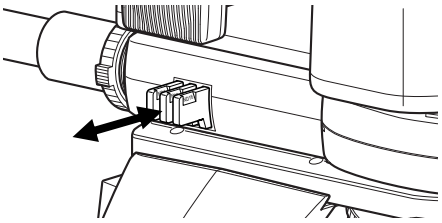
By bringing the following optical elements into the optical path, epi-fluorescence and differential interference contrast in the visible light range can be simultaneously observed by microscope:

Filter cube for the epi-fluorescence cube turret
FL/DIC analyzer slider for the epi-fluorescence cube turret
DIC slider on the objective side for the nosepiece
DIC module for the condenser
Polarizer VIS for the polarizer turret

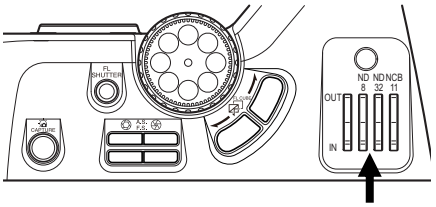


Epi-fluorescence /visible light range differential interference contrast concurrent microscopy

- The analyzer on the FL/DIC analyzer slider enters into the optical path by pushing the slider to the second click-stop position. It is removed from the optical path when the slider is pulled back to the first-click stop position.
- If the diascopic image is not shown under epi-fluorescence microscopy, press the dia-illumination ON/OFF switch to turn on the lamp.
- Adjust the fluorescent image brightness with ND filters in the epi-fluorescence attachment, and differential interference contrast image brightness with ND filters in the main body. During this, dia-illumination should be sufficiently dimmed with the ND filter.



Brightness adjustment of fluorescent image



Brightness adjustment of differential interference contrast image

Chapter

3

Individual Operations

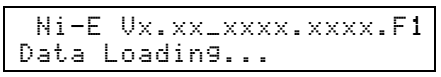
This chapter provides detailed descriptions on how to use the Ni-E microscope display panel, control buttons, each component and each device mainly used with the Ni-E focusing nosepiece system.

1 Display Panel Details

The 2-line, 24-character fluorescent display panel on the front of the main body can display the following information:

(1) Power ON display

When the power is turned on, operation progress is displayed.



Top: Model name, firmware version
Bottom: Program startup progress

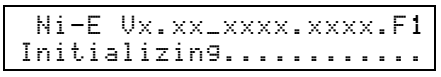
(2) Motorized device initialization display

The twelve dots displayed to the right of “Initializing” indicate the connection status or initialization status of motorized devices.

Motorized device not connected:
Dots displayed

Motorized device connected and initializing:
No dots

Motorized device connected and initialized:
Dots displayed



Top: Model name, firmware version
Bottom: Motorized device initialization progress

Each dot represents a motorized device as shown below.

Initialization indicator dots and corresponding devices

Dot Position	Motorized Device	Dot Position	Motorized Device
1 (leftmost)	Z-axis (main body’s elevating section)	7	Motorized Quadrocular Tilting Tube (optical path switching part)
2	DIA field diaphragm	8	Motorized DSC zooming port for quadrocular tube
3	Motorized XY stage (X)	9	Motorized ND filter wheel
4	Motorized XY stage (Y)	10	Motorized epi-fluorescence cube turret 1 (1st layer)
5	Motorized universal condenser (Not used in the focusing nosepiece system)	11	Motorized epi-fluorescence cube turret 2 (2nd layer)
6	DIA aperture diaphragm (Not used in the focusing nosepiece system)	12	Motorized excitation filter wheel

(3) Initialization completion display

Either “Microscope state” or “FUNCTION button function” can be displayed on the display panel.

After initialization completes, the “Microscope state” display pattern 1 is first displayed.

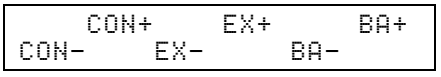
Press a DISPLAY SWITCH button on the front of the main body to select between the “microscope state” display and the “FUNCTION button’s function” display.

■ **“Microscope state” display**

Varies depending on connected motorized devices and has nine patterns.
(See “Microscope state display” on the next page.)

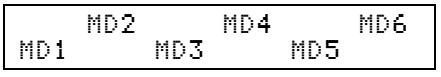
■ **“FUNCTION button’s function” display:**

As factory default, rotation of a condenser, excitation filter wheel, and a barrier filter wheel is set as the FUNCTION button’s function, and thus the display will be as shown on the right.



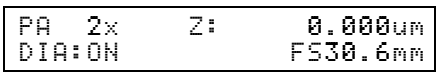
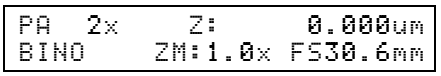
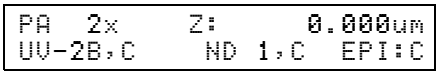
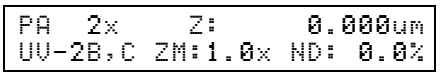
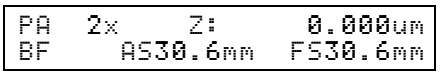
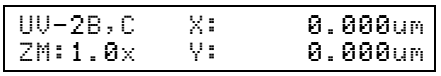
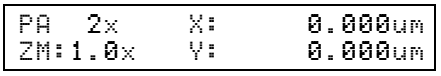
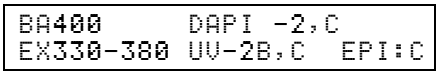
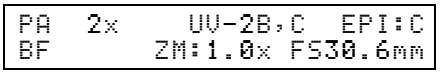
FUNCTION button’s function display (factory default setting)

After a “LOAD MODE” function is set and registered with a FUNCTION button, the registered mode name will be displayed. Mode names, MD1 through MD6 are those set as factory default. Up to four alphanumeric characters can be used for the name and the name can be changed as you wish. When the mode name has been changed, new mode name is displayed.
(See “18.1 Mode Function” for the details on the mode.)



Displaying the mode name when the “LOAD MODE" function has been set to a FUNCTION button

Microscope state display

Pattern 1	**Top** **Bottom**	**Objective, Z-axis position (unit: um)** **Dia-illumination status, field diaphragm status (unit: mm)**	
Pattern 2	**Top** **Bottom**	**Objective, Z-axis position (unit: um)** **Optical path selection, zoom magnification, field diaphragm status (unit: mm)**	
Pattern 3	**Top** **Bottom**	**Objective, Z-axis position (unit: um)** **Status of the epi-fluorescence cube turret (1st layer)/status of epi-fluorescence cube turret's built-in shutter (1st layer), HG precentered fiber illuminator ND status/HG precentered fiber illuminator shutter status, EPI motorized shutter status**	
Pattern 4	**Top** **Bottom**	**Objective, Z-axis position (unit: um)** **Status of the epi-fluorescence cube turret (1st layer)/status of epi-fluorescence cube turret's built-in shutter (1st layer), zoom magnification status, motorized ND filter wheel status (unit: %)**	
Pattern 5	**Top** **Bottom**	**Objective, Z-axis position (unit: um)** **Condenser status, aperture diaphragm status (unit: mm), field diaphragm status (unit: mm)**	
Pattern 6	**Top** **Bottom**	**Status of the epi-fluorescence cube turret (1st layer)/epi-fluorescence cube turret's built-in shutter (1st layer), X-axis position (unit: um)** **Zoom magnification, Y-axis position (unit: um)**	
Pattern 7	**Top** **Bottom**	**Objective, X-axis position (unit: um)** **Zoom magnification, Y-axis position (unit: um)**	
Pattern 8	**Top** **Bottom**	**BA (barrier) filter status, status of the epi-fluorescence cube turret (2nd layer)/status of epi-fluorescence cube turret's built-in shutter (2nd layer)** **EX (excitation) filter status, status of the epi-fluorescence cube turret (1st layer)/status of epi-fluorescence cube turret's built-in shutter (1st layer), EPI motorized shutter status**	
Pattern 9	**Top** **Bottom**	**Objective status, status of epi-fluorescence cube turret (1st layer)/status of epi-fluorescence cube turret's built-in shutter (1st layer), EPI motorized shutter status** **Condenser status, zoom magnification, field diaphragm status (unit: mm)**	

When a motorized device is not connected

The corresponding field will be displayed as blank. Also, if the information for the device (i.e. filter cube) is not set, "-----" is displayed.

DS-L3 MICROSCOPE INFORMATION Screen

When DS-L3 is connected to the microscope, you can check the status of the microscope on the DS-L3 [MICROSCOPE INFORMATION] screen. See Chapter 2, "3.3 MICROSCOPE INFORMATION Screen (Ni-E)" in the DS-L3 instruction manual "Microscope Operation".

Contents of the “microscope state” display are as follows.

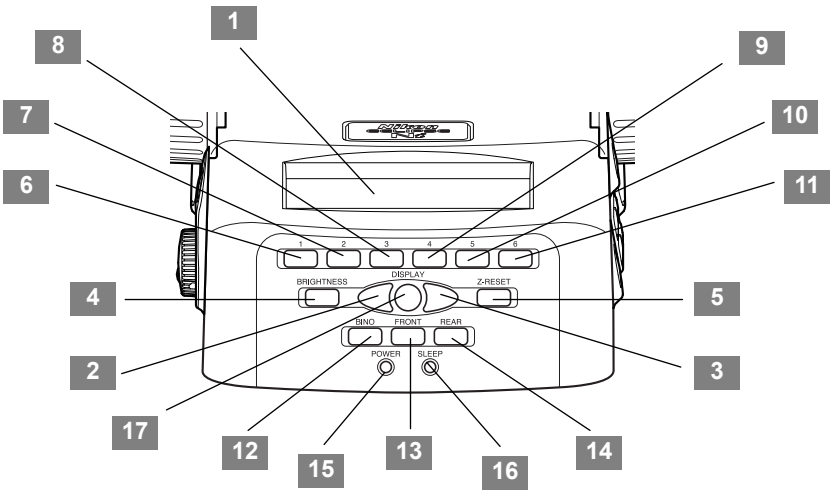
Microscope State Display Contents

Objective	[000x] : Blank: Achromat [AT000x] : Apo TIRF [P 000x] : Plan [PA000x] : Plan Apo(TIRF/VC) [PT000x] : Plan Apo TIRF [PV000x] : Plan Apo VC [PF000x]: Plan Fluor [PU000x]: Plan UW [SF000x]: S Fluor [SP000x]: S Plan Fluor [HM000x]: HMC [OT000x]: Others [F 000x]: Fluor [EP000x]: E Plan [NA000x]: NIR Apo [PI000x]: Plan Apo IR [A 000x]: Apo(λ/LAMBDA)
Optical path switching	[BINO]: Observation port (binocular) [FRONT]: Tube adapter [REAR]: Rear port
Z axis position	[Z: 00000.000um] [Z:-00000.000um] + direction: Objective rises - direction: Objective lowers
Status of the 1st epi-fluorescence cube turret	[UV-2A] Name of the filter cube in the optical path of the motorized/intelligent filter cube (1st layer) (Example)
Status of the 2nd epi-fluorescence cube turret	[UV-2B] Name of the filter cube in the optical path of the motorized/intelligent filter cube (2nd layer) (Example)
Motorized XY stage position	[X: 000000.000um]: Motorized XY stage X coordinate [Y: 000000.000um]: Motorized XY stage Y coordinate
EPI motorized shutter status	[EPI:O]: EPI motorized shutter open (OPEN) [EPI:C]: EPI motorized shutter closed (CLOSE)
Motorized barrier excitation filter wheel	[BA000-000]: Barrier filter name
Motorized excitation filter wheel	[EX000-000]: Excitation filter name
Motorized HG precentered fiber illuminator’s ND status/built-in shutter status	[ND00,O]: ND value, shutter open (OPEN) [ND00,C]: ND value, shutter closed (CLOSE)
Aperture diaphragm status	[AS00.0mm]: Aperture diaphragm diameter
Field diaphragm status	[FS00.0mm]: Field diaphragm diameter
Zoom magnification	[ZM:0.0x]: Zoom magnification
Motorized ND filter wheel status	[ND00.0%]: ND value
Condenser status	[OPEN]: Empty (bright-field) [2/4x]: 2-4x auxiliary lens [N1]: DIC module [N2]: DIC module [NR]: DIC module [Ph1]: PH module [Ph2]: PH module [Ph3]: PH module [DF]: Dark-field module

2 Using Operation Buttons on Ni-E

Various operation buttons are provided on the front, right, and left of the Ni-E main body. The following describes the function of each button (factory default setting):

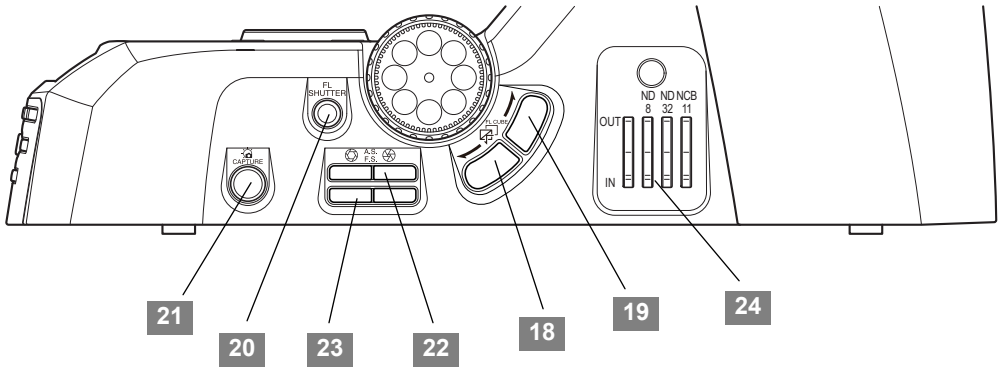
2.1 Front Operation Button



Front Operation Button

No.	Name	Function
1	Display panel	Displays either the “microscope state” or “FUNCTION button’s function”. Use the “DISPLAY SWITCH” button to select the display.
2	DISPLAY Previous button	Switch the microscope state on the display panel to the previous pattern.
3	DISPLAY Next button	Switch the microscope state on the display panel to the next pattern.
4	Brightness Control button	Change the brightness of the display panel. The brightness changes in the following order by pressing the button: Bright → Dark → Off → Bright → ...
5	Z-RESET button	Reset the Z-axis coordinate value on the display panel to 0.000 µm.
6 to 11	FUNCTION button*	Button 1, 2: Rotate the condenser CCW, CW. (The motorized condenser is not used in the focusing nosepiece system. Change the function of the button to a different operation.) Button 3, 4: Rotate the excitation filter wheel CCW, CW. Button 5, 6: Rotate the barrier filter wheel CCW, CW.
12	BINO optical path button	Direct 100% of the optical to the binocular (with status indicator LED, binocular 100% when LED ON, vertical tube or rear port 100% when LED OFF).
13	FRONT optical path button	Direct 100% of the optical to the vertical tube (with status indicator LED, vertical tube 100% when LED ON, binocular or rear port 100% when LED OFF).
14	REAR optical path button	Direct 100% of the optical to the rear port (with status indicator LED, rear port 100% when LED ON, binocular or vertical tube 100% when LED OFF).
15	Power LED	Displays the power ON/OFF status of the main body and control box A. The LED lights up when the power is on.
16	Sleep LED	The LED is on when the device is in sleep mode (where the power supply to motorized devices is suspended to minimize the generation of noise). Turn on/off the sleep mode from DS-L3.
17	DISPLAY switch button	Switch the "microscope state" and "FUNCTION button’s function" display.

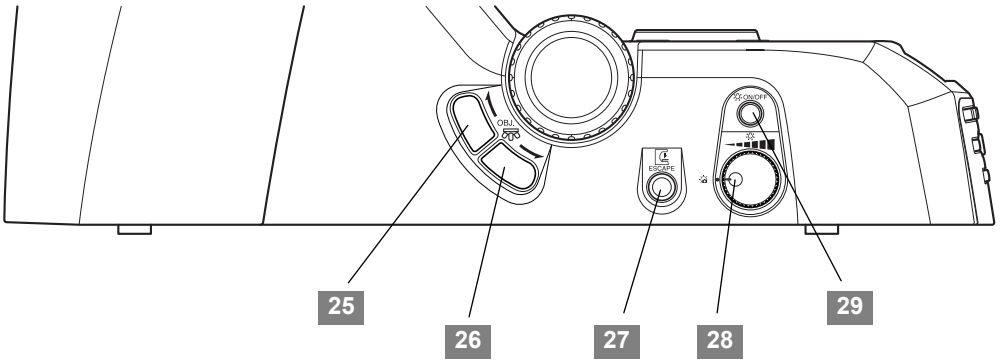
2.2 Right Operation Button



Right Operation Button

No.	Name	Function
18	FL CUBE CW button*	Rotate the 1st layer motorized epi-fluorescence cube turret by one address clockwise (as viewed from above).
19	FL CUBE CCW button*	Rotate the 1st motorized epi-fluorescence cube turret by one address counterclockwise (as viewed from above).
20	FL SHUTTER button*	Open or close the 1st motorized epi-fluorescence cube turret built-in shutter. The shutter is closed when the SHUTTER button is ON.
21	CAPTURE button*	Capture an image using a digital camera connected via USB on the rear of the main body.
22	DIA aperture diaphragm button	Adjust the diameter of the condenser's aperture diaphragm. (The motorized condenser is not used in the focusing nosepiece system.)
23	DIA field diaphragm button	Adjust the diameter of the DIA field diaphragm.
24	ND filter IN/OUT switch	Insert or remove the main body's internal ND filter.

2.3 Left Operation Button



Left Operation Button

No.	Name	Function
25	OBJ CW button*	Rotate the motorized nosepiece by one address clockwise (as viewed from above). (The motorized nosepiece is not used in the focusing nosepiece. Change the function of the button to a different operation.)
26	OBJ CCW button*	Rotate the motorized nosepiece by one address counterclockwise (as viewed from above). (The motorized nosepiece is not used in the focusing nosepiece. Change the function of the button to a different operation.)
27	Escape button	Used for refocusing operation. Pressing the switch raises the objective to the preset position. Press the switch again to restore the objective to the original position. (See “4.4 Refocusing”.)
28	Dia-illumination brightness control knob	Adjust the brightness of the dia-illumination lamp by changing the supplied voltage.
29	Dia-illumination ON/OFF switch	Turn the dia-illumination lamp on or off.

Changing the function of an operation button

For buttons with * appended in the table; [FUNCTION] button, [FL CUBE CW/CCW] button, [OBJ CW/CCW] button, [FL SHUTTER] button and [CAPTURE] button, their functions can be changed. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3) Configuring the Button Functions” for details.)

Enabling/disabling the operation button

You can “disable” the operation button. The buttons can only be configured as a group (front, right, left), and not individually. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3) Configuring the Button Functions” for details.)

3 Adjusting the Brightness of a Diascopic Image

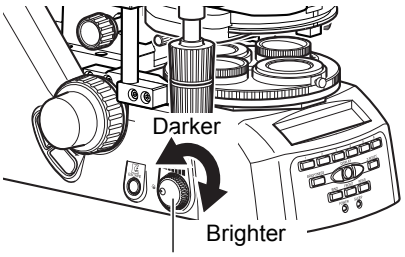
The brightness of a diascopic image can be adjusted by changing the lamp voltage or by using ND filters.

3.1 Adjustment by Lamp Voltage

Turning the dia-illumination brightness control knob changes the voltage of the lamp to change the brightness of the diascopic image.

Brightness Control Knob Rotation and Brightness of the Image

Brightness control knob	Image brightness
Clockwise rotation	Brighter
Counterclockwise rotation	Darker
mark position (Where the knob clicks to a stop after rotating in counterclockwise direction past the point of least brightness)	Brightness with best color reproduction (Photomicrography voltage)

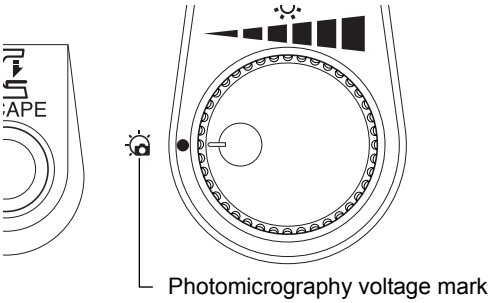


Dia-illumination brightness control knob

Brightness Adjustment

Photomicrography voltage setting: for improved color reproduction

Changing the lamp voltage for brightness adjustment will affect the lamp color temperature and alter the color balance of the image. Increasing the voltage will result in a bluish tint, while reducing the voltage results in a reddish tint. If accurate color reproduction is critical, setting the dia-illumination brightness control knob (on left of the main body) to the mark will adjust the brightness to the Photomicrography voltage best suited for image captures. (The Photomicrography voltage is adjusted/set at the factory and cannot be changed by the user.) To adjust the brightness further without changing color, bring the NCB11 filter in the filter cassette into the optical path and use the ND filter for adjustment.



Photomicrography voltage setting

Control of illumination and brightness adjustments of the lamp

The lamp can also be turned on and adjusted via the DS-L3 once a lamp control button has been set on a [MICROSCOPE CONTROL] or [CAM-MIC CONTROL] screen of the DS-L3. The operation switch of the Ni-E microscope has control when Ni-E microscope has been activated.

To control from DS-L3, switch control to the DS-L3 side by pressing the [LAMP CTRL] button configured on DS-L3. DS-L3 will have control when the button is checked.

[MICROSCOPE CONTROL] Screen

[LAMP CTRL] button: Switch lamp control Sub screen: Brightness adjustment operation

♦Operation on DS-L3 (See "20.2 Microscope Control".)♦

3.2 Adjustment with ND Filters

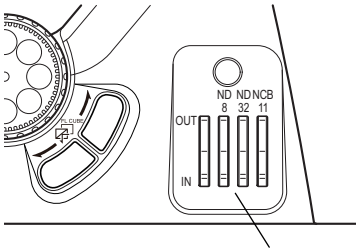
There are two methods for adjusting the brightness with ND filters: bringing/removing the main body's internal ND filters in/from the optical path, or operating the motorized ND filter wheel attached between the main body and dia-illumination.

Using ND filters in the main body

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.

The Ni-E has a built-in filter cassette, with ND8, ND32, and NCB11 filters attached. Pressing the IN/OUT switch for ND8 or ND32 to the [IN] side will bring the corresponding ND filter into the optical path.

ND8:	Reduces light intensity to 1/8.
ND32:	Reduces light intensity to 1/32.
ND8+ND32:	Reduces light intensity to 1/256.



Brightness adjustments with ND filters

Adding or changing ND filters

The filter cassette has an empty filter slot which can be used for an additional ND filter. The filter already attached can also be removed and replaced with another filter. (See Chapter 1 "3 Assembly Method - 19 Replace the ND filter" in the "Assembly/Maintenance" instruction manual.)
Also, up to two φ45 mm filters can be placed on the field lens.

Motorized ND filter wheel

See the Ni-E (Focusing Stage System) instruction manual for details of the motorized ND filter.

4 Focusing on the Sample (Vertical Objective Movement)

Focus adjustment is performed using the focus knobs on the Ni-E main body, ergo controller, or focus knob on joystick. The following instructions assume the use of the focus knobs on the main body. See the Ni-E (Focusing Stage System) instruction manual for information on using the ergo or joystick controller.

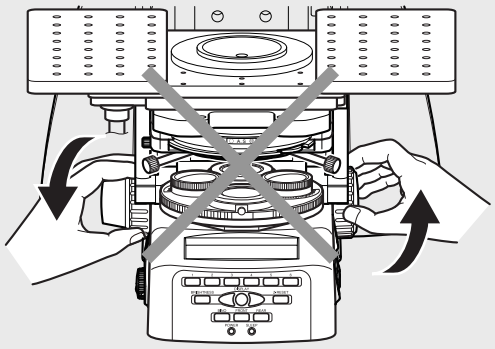
“Resetting the Z-axis coordinate,” “Refocusing,” and other useful functions are available for focus adjustment.

4.1 Proper Focusing Procedure

Note on controlling the focus knobs

When operating the focus knobs on the main body, avoid the following action, which can cause equipment malfunctions.

Rotating the right and left focus knobs in opposite directions.



Do not turn the focus knob!

Using an objective of high magnification may cause the objective to be pushed against the sample, damaging the objective. Focus according to the following procedure:

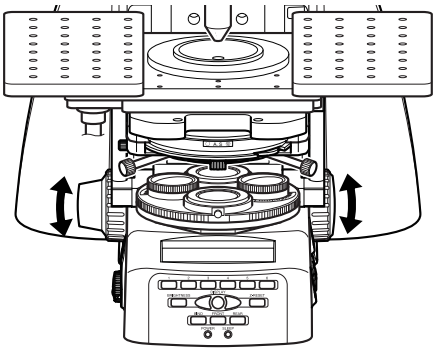
(1) Bring the 10x or 4x objective into the optical path, and turn the coarse focus knob to lower the objective to the lower limit.

(2) Direct 100% of the light to the binocular section.

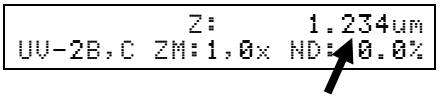
(3) Adjust the brightness of dia-illumination.

(4) Look into the eyepiece and turn the coarse focus knob to raise the objective and focus on the sample.

(5) Turn the fine focus knob to further adjust the focus.



Using the focus knob to focus



The value increases when the objective is raised, and decreases when it is lowered.

Tips for focusing

- When lowering the objective with the coarse focus knob, move your eyes away from the eyepiece and operate the microscope while looking at the microscope from the side.
- When working with the coarse focus knob while looking into the eyepiece, you should only turn the knob in the direction for raising the objective.
- First use an objective of low magnification to adjust the focus, and then switch to an objective of higher magnification.
- Since the 10x or 4x objective has a wider operating distance, the sample will not touch the tip of the objective provided the sample has standard thickness.

Enabling/disabling the operation of the elevating section

You can disable the operation of the elevating section.

As factory default setting, a focus knob is used to drive the elevating section, but you can “disable” the setting when an application software such as NIS-E is used to control the elevating section or you wan to prevent unintended behavior of the elevating section, including an accidental contact of the focus knob of Ni-E microscope or ergo controller (or joystick). (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (6) Configuring Other Functions” for details.)

Lower limit of the movement

The default lower limit of the elevating section is set to -2000.000 µm, but this can be changed with software. The lower limit can be set between -2000.000 µm and -250.000 µm. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (6) Configuring Other Functions” for details.)

Changing the upper limit is not available.

Stop position by focus restriction

When operating the elevating section with the focus knob, the actual stop position is up to 20 µm beyond the set upper/lower limit.

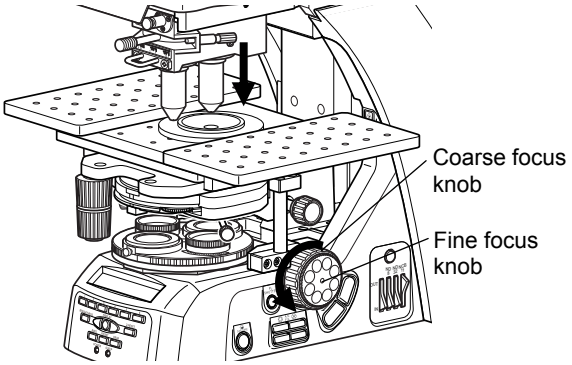
4.2 Focus Knobs on the Main Body

Focus knob rotation and objective 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 between the focus knobs' rotation direction with the objective movement direction.

Focus Knob Rotation direction and Objective Movement direction

Lowers the objective	Turn the knob toward the front.
Raises the objective	Turn the knob toward the back.



Focus knob rotation direction and objective movement direction

Focus knob rotation (turns) and objective movement (distance)

Focus Knob Rotation (turns) and Objective Movement (distance)

No. of knob turns	Objective travel distance (vertical direction)
One rotation of the coarse focus knob	Approx. 2 mm
One rotation of the fine focus knob	Approx. 6 µm

The vertical movement range (coarse/fine focus stroke) of the objective is from about 13 mm above the focal point (reference position) to approximately 2 mm below the focal point.

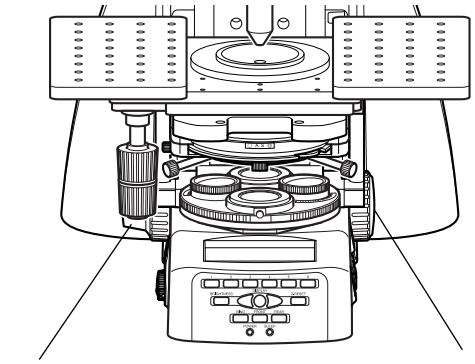
Position exchange of the fine focus knob

The fine focus knobs, located on both sides of the main body, are flat on one end, and convex on the other. The fine focus knob on each side is attached with a magnet, and can easily be swapped.

Position them to best suit your usage.

Removing a flat focus knob

The flat fine focus knob can be easily removed by inserting a hex driver (2 mm across flats) into the notch in the knob.

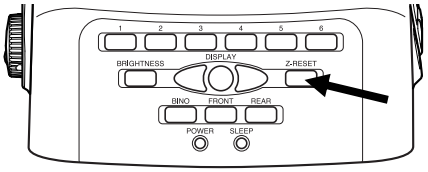


Position exchange of the focus knob

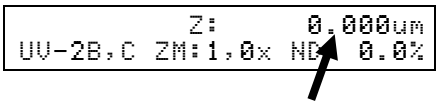
4.3 Resetting the Z-axis Coordinate

The Z-axis coordinate can be reset to zero and used as the reference point for adjusting the focus. To do so, adjust the focus on the sample, then press the Z-RESET button. The Z-axis coordinate value will be reset to 0.000 um.

♦Operation on DS-L3 (See "20.2 Microscope Control".)♦



[Z-RESET] Button used to reset the Z-axis coordinate



4.4 Refocusing

The objective can be raised to a preset retracting position, and then restored back to the original by refocusing operation.

When the Escape button is pressed, the objective is raised to the preset retracting position while saving the current position. Pressing the Escape button once more returns the objective to the original position. This function is useful for temporarily retracting the objective, for example when replacing the sample or when switching the objective.

The Escape button will light up when the objective is “retracted,” and will be turned off otherwise.

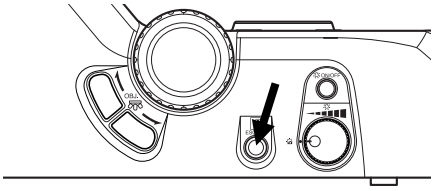
When the stage is retracted, the elevating section cannot be moved by turning the focus knobs.

Retraction Position Range

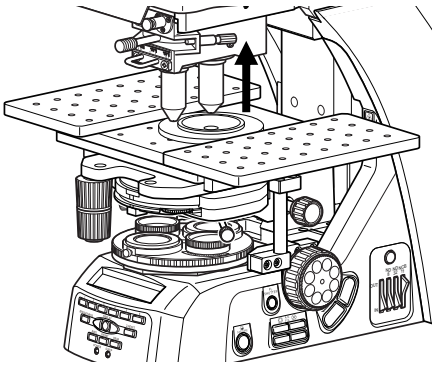
- 5 mm up from current position
- 10 mm up from current position
- Upper limit of the elevating section (software limit)

(See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (4) Configuring the Movement” for details.)

♦Operation on DS-L3 (See "20.2 Microscope Control” .)♦



Retracting/restoring objective with [ESCAPE] button



Focus position is saved and objective retracts

5 Bringing the Target into the Optical Path (Horizontal Stage Movement)

When using the standard stage FN-3PS2, bring the target into optical path using the stage X, Y knobs. (So that the sample is illuminated.)

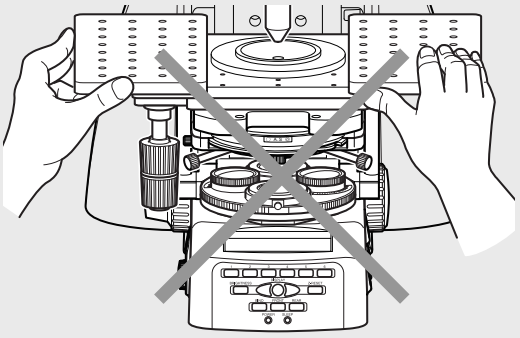
When using a motorized XY stage, use the ergo controller or joystick to control the horizontal stage movement.

5.1 Standard Stage Operation

Notes 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.
- Forcing the stage to move past the X/Y stroke.

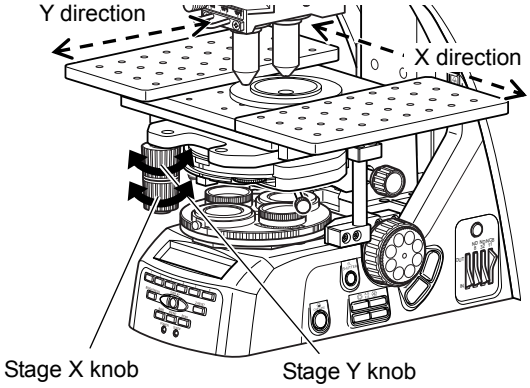


Do not move the stage with a hand.

Knob rotation and horizontal stage movement

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

The stroke of the stage is X: ±29.5 mm, Y: ±29.5 mm.



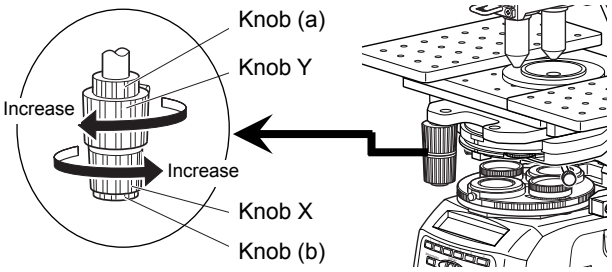
Knob Rotation and Stage Movement

Adjusting the Knob Rotation Torque

The rotation torque of the stage Y movement knob and stage X movement knob can be adjusted. The adjustment task is made easier by bringing the stage to the end of the travel limit.

- Adjusting Y movement torque
  Hold knob (a) and rotate knob Y in the direction indicated by arrow (clockwise) to increase the torque, or rotate it in the opposite direction to decrease the torque.
- Adjusting X movement torque
  Hold knob (b) and rotate knob X in the direction indicated by arrow (counterclockwise) to increase the torque, or rotate it in the opposite direction to decrease the torque. When holding knob (b), prevent it from moving by placing your finger into the hole under knob (b).

Avoid decreasing the torque excessively. If the knobs are too loose, the top surface of the stage may move, even when touched very lightly.



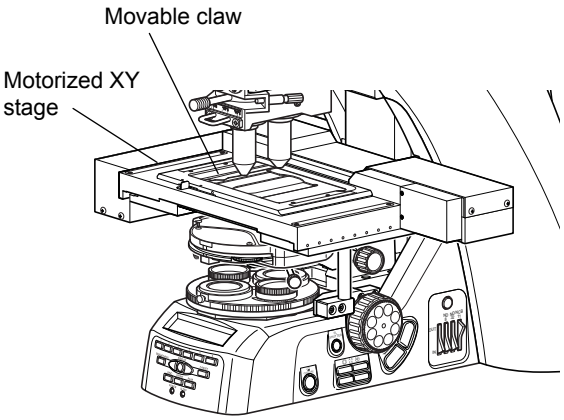
Adjusting the Stage Knob Rotation Torque

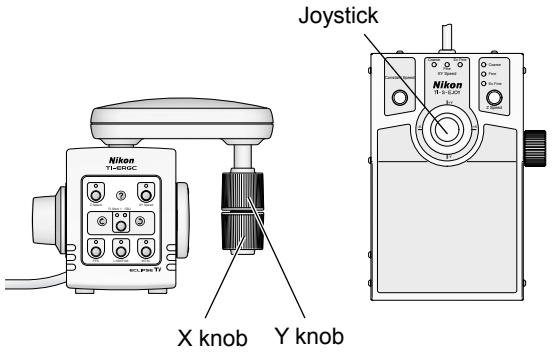
5.2 Operating the Motorized XY Stage

In order to use the motorized XY stage, the motorized XY stage must be connected to the motorized XY stage controller and the motorized XY stage controller must be connected to the connector box.

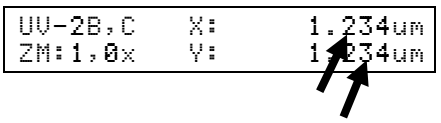
A specimen holder can hold one or two specimens. Open the claw of its moving part and place the specimen onto the specimen holder, gently stowing the claw back to fix the specimen. 35 mm - 60 mm tissue can also be observed by attaching the NI-SH-D Dish Holder.

The motorized XY stage is moved horizontally with the ergo controller or joystick controller. See the Ni-E (Focusing Stage System) instruction manual for details of the ergo and joystick controllers.





Driving the motorized XY stage



Horizontal movement limit position

A limit position of X direction and Y direction movement of the motorized XY stage is set as shown below as default.

X-axis max. value: 34000.000 µm
X-axis min. value: -34000.000 µm
Y-axis max. value: 27000.000 µm
Y-axis min. value: -27000.000 µm

The limit position can be changed programmatically. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (6) Configuring Other Functions” for details.)

Stop position with XY limit

The XY stage slows down and stops after it reached the limit position, therefore, actually the stage stops at a maximum of 800 µm beyond the limit position which has been set.

6 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.

(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 10 in Chapter 2 “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure” to focus on the specimen using the 10x objective.

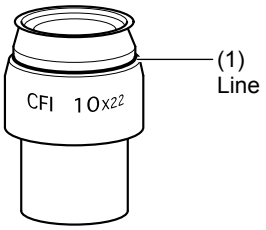
(3) Bring the 40x objective into the optical path, and turn the coarse focus knob and then the fine focus knob to focus on the sample.

(4) Bring the 10x (or 4x) objective into the optical path.

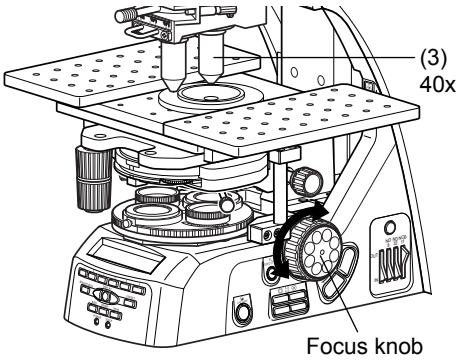
(5) Look into the right eyepiece with your right eye without operating the focus knob, and then focus on the sample by turning the right diopter adjustment ring.

(6) Look into the left eyepiece with your left eye. Without operating the focus knob, focus on sample by turning the left diopter adjustment ring.

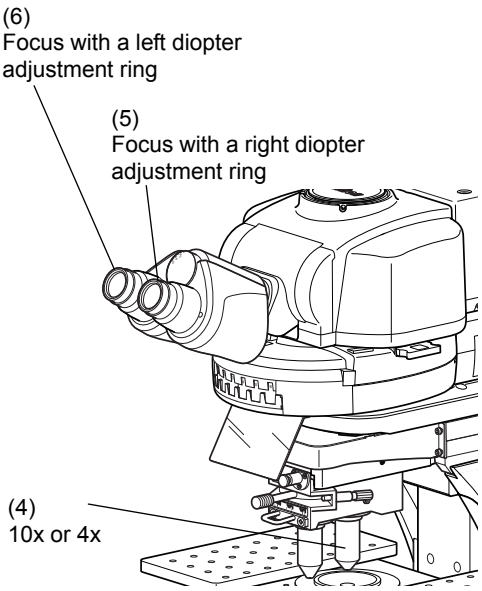
(7) Repeat Steps (3) through (6) to make sure the focus has been adjusted properly.



Aligning the end face of the adjustment ring with the line



Focusing with 40x



Selecting 10x or 4x to focus with right and left diopter adjustment ring

7 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 sample.

(1) Follow Steps 1 through 12 in Chapter 2, “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure” to focus on the sample using the 10x objective.

(2) Press the right side (㊍ mark side) of the DIA field diaphragm button to stop down the field diaphragm to minimum diameter.

(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.

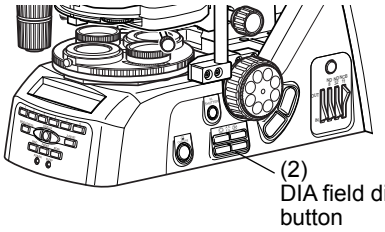
(4) Adjust the condenser centering screws until the field diaphragm image is at the center of the eyepiece field of view.

(5) Bring the 40x or 60x objective into the optical path. Focus on the sample using the parfocal correction knob of the slider nosepiece.

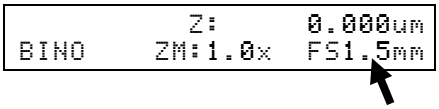
(6) Turn the condenser focus knob to form the field diaphragm image on the sample surface.

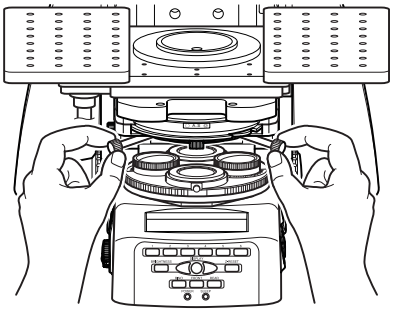
(7) Operate the DIA field diaphragm button to adjust the image so that the field diaphragm image size is almost the same as the field of view.

(8) 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.

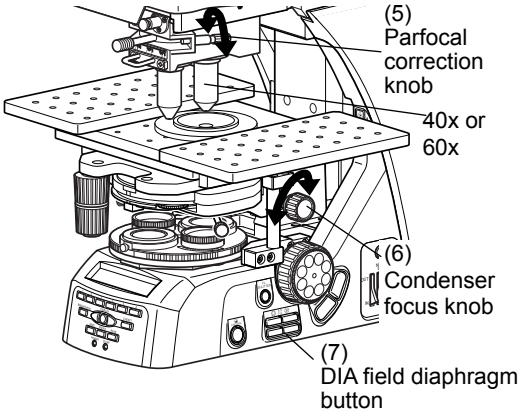


Minimizing the field diaphragm setting

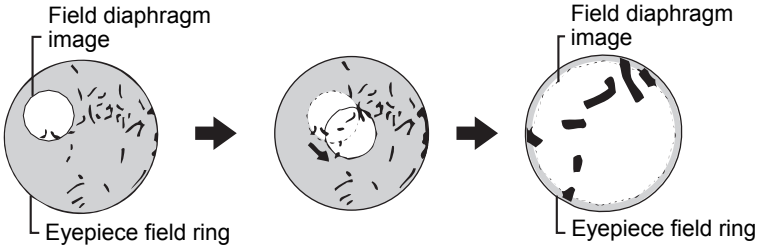




Focusing the field diaphragm image and moving it to the center of the field of view



Focusing the field diaphragm image and opening the field diaphragm wider



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 or 60x objective

A field diaphragm image focused with a 40x or 60x objective cannot be seen as clearly as when the one focused with a 10x objective.

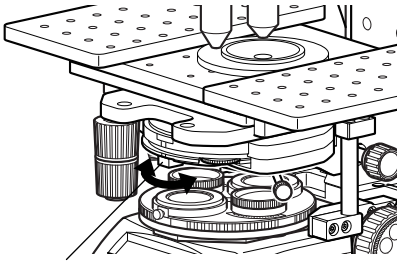
8 Adjusting the Aperture Diaphragm

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.

To adjust the aperture diaphragm, use the aperture diaphragm lever on the condenser. Moving the lever in the clockwise direction will open the diaphragm.

Because the condenser has a scale that indicates the numerical aperture, align the index on the aperture diaphragm lever 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 scale 0.525 to 0.6 on the condenser.
Pull out the eyepiece and look into the tube to view the pupil of the objective (bright area when the aperture diaphragm is fully opened). Adjust the aperture diaphragm lever so that the size of the aperture diaphragm is 70 to 80% of the pupil.



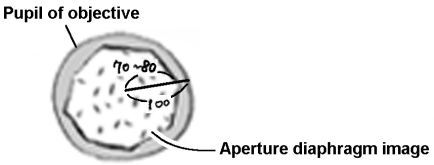
Aperture diaphragm lever

Adjusting the Aperture Diaphragm

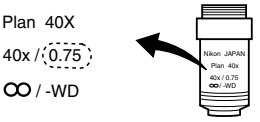
Proper size of the aperture diaphragm

Normally, the appropriate size of the aperture diaphragm is 70 to 80% of the numerical aperture of the objective. It provides satisfactory images with suitable contrast. Since an excessively small aperture diaphragm opening will degrade image resolution, Nikon does not recommend setting the aperture diaphragm to less than 60% of the numerical aperture of the objective.

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.



Right size of the aperture diaphragm



Indication for 40x magnification / numerical aperture 0.75

Proper numerical aperture: 0.75 x 0.7 to 0.8＝0.525 to 0.6

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

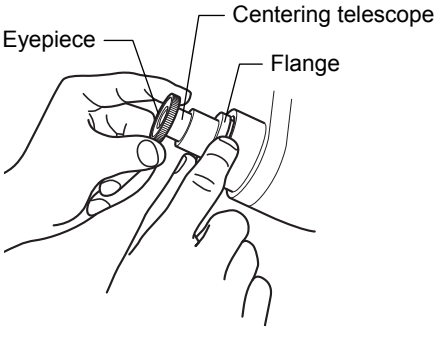
Aperture diaphragm	Resolution	Brightness	Contrast	Focal depth
Stop down	Lower	Darker	Larger	Deeper
Open	Higher	Brighter	Lesser	Shallower

Adjustment timing for the aperture diaphragm

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

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.



Using the centering telescope

9 Using the Condenser

The focusing nosepiece system uses the FN-C LWD condenser appropriate for dia-illumination bright-field microscopy (objective 4x to 100x), dia-illumination differential interference contrast microscopy (infrared ray range, visible range) and diagonal illumination microscopy.

■ **Optical module**

The turret of the LWD condenser has one empty hole and three holes for attaching optical modules. Attach the optical module suitable for each microscopy to the turret. See Chapter 1 “3 Assembly Method - 9 Attach the condenser - ■ Attaching the optical module to the condenser turret” in the “Assembly/Maintenance” instruction manual for details on the attachment procedure.)

- DIC module (required for differential interference contrast microscopy)

  DIC module includes [D-C DIC N1 DRY], [D-C DIC N2 DRY], and [D-C DIC NR DRY].

  Select the DIC module appropriate for the microscopy method and objective. If the combination is not correct, differential interference contrast image cannot be obtained or the contrast decreases significantly. Depending on the purpose of observation, dedicated modules for higher contrast or resolution are available. Note, however, that in principle the contrast contradicts the resolution of the differential interference contrast image (the higher the contrast, the lower the resolution). (See the table in Chapter 1, “3 Assembly Method - 9 Attach the condenser - Combination of DIC Slider on the Objective and DIC Module on the Condenser (when using the FN-C LWD condenser)” in the “Assembly / Maintenance” instruction manual.)

- 4x auxiliary lens (required for low magnification bright-field microscopy)

  When performing 4x bright-field microscopy with a LWD condenser, attach a 4x auxiliary lens to the mount position of DIC module N1. 4x is not indicated on the turret. Remove it when viewing with the objectives greater than 4x magnification.

- Diagonal illumination diaphragm

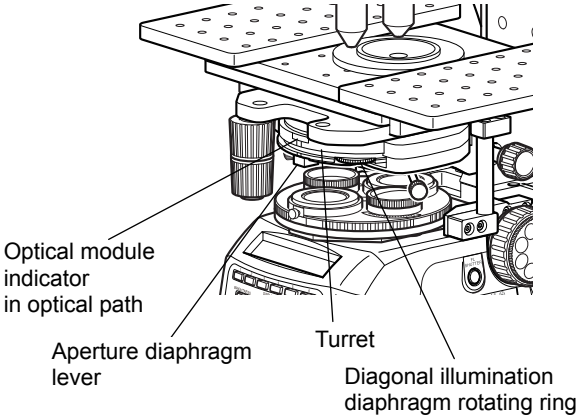
  Attach a diagonal illumination diaphragm to the turret in order to increase the contrast when observing a sample. Using diagonal illumination, contrast can be attained so that even a nearly transparent sample can be observed. You can also adjust the shadow on the sample by turning the diagonal illumination diaphragm rotation ring which rotates the diaphragm around the optical axis.

■ **Switching the optical module (switching the turret)**

Rotate the condenser turret manually to switch. It can be turned either clockwise or counterclockwise. The attached optical module is indicated on the turret side and the optical module indicated at the front of the turret is in the optical path.

Make sure the turret is stopped at a click-stop position when rotating.

Display [N1], [N2]:	DIC module ([N1] when a 4x auxiliary lens is attached.)
Display [OBL]:	Diaphragm for the diagonal illumination
[O]:	Empty hole



LWD condenser

■ **Aperture diaphragm**

A condenser is equipped with an aperture diaphragm to adjust the illumination angular aperture.

Turning the aperture diaphragm lever changes the size of the aperture diaphragm. Turn the diaphragm clockwise to open and counterclockwise to close.

A scale indicating the numerical aperture of the aperture diaphragm of the condenser is displayed at the top of the aperture diaphragm lever. Match the indicator of the aperture diaphragm lever to the scale. (See “8 Adjusting the Aperture Diaphragm”.)

■ **Condenser focus knob**

A condenser focus knob is attached to the right and left sides of the substage.

They are used to 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 sample. (See “7 Focusing and Centering the Condenser”.)

■ **Condenser centering screws**

The condenser holder is equipped with condenser centering screws (x2). They are used to 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 sample. (See “7 Focusing and Centering the Condenser”.)

10 Adjusting the Field Diaphragm

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

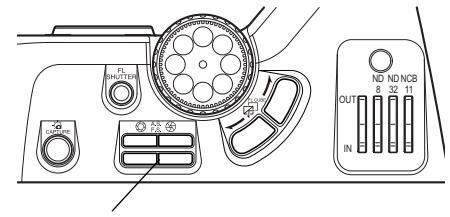
Operate the DIA field diaphragm button to change the size of the field diaphragm.

Press the left side (mark) of the DIA field diaphragm button to open, and the right side (mark) to close the diaphragm.

For Ni-E, the field diaphragm can be adjusted from 1.5 mm to 30.6 mm.

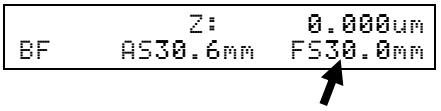
For normal observations, the size of the field diaphragm should almost circumscribe the field diaphragm.

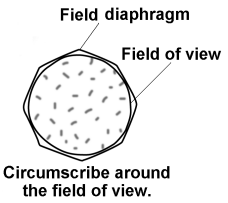
♦Operation on DS-L3 (See “20.2 Microscope Control”.)♦



DIA field diaphragm button

Adjusting the field diaphragm





Right size of 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 sample will become decolorized over a wider area.

Field diaphragm’s adjustment timing

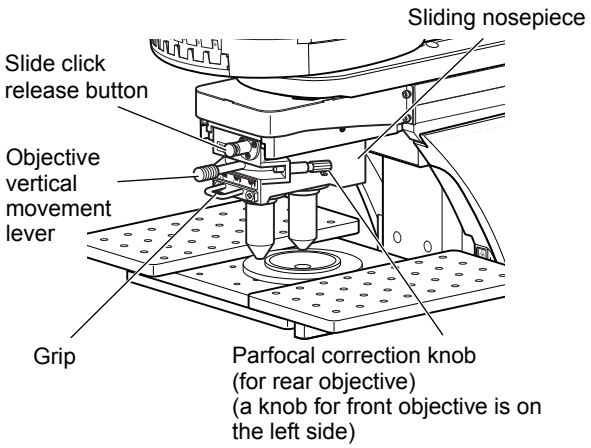
Be sure to adjust the aperture diaphragm each time you change the objective.

11 Operating the Nosepiece (objective operation)

A slider nosepiece that can mount two objectives or a single nosepiece that can mount only single objective can be used with the Ni-E focusing nosepiece system.

To use the sliding nosepiece, attach the objectives to the front and rear and switch them by sliding to the front or rear. Normally, attach a low magnification objective at the front and high magnification objective at the rear.

The sliding nosepiece has levers and knobs to control the objective. Follow the descriptions below to operate them correctly.



Sliding nosepiece controls

11.1 Objective Vertical Movement Lever (objective switching, dipping)

The objective vertical movement lever on the sliding nosepiece is used to retract or dip the objective when switching between the front and rear objective.

The objective retracts when the objective vertical movement lever is moved to the center. By moving the objective vertical movement lever right and left, the objectives can be raised or lowered independently of the vertical movement of the objectives using the focus knobs.

The lever can be used only when an objective is in the optical path.

The stroke of the objective is 15 mm above the focus to allow microscopy using a thick Petri dish.

Relationship between the Position of Objective Vertical Movement Lever and the Position of Objective

Lever position	Position of front objective (when the front objective is in the optical path)	Position of rear objective (when the rear objective is in the optical path)
Right end		Observation point
Center	Retraction position	Retraction position
Left end	Observation point	

Switching Objectives

To switch objectives, follow the procedure below.

The following procedure is described based on switching from the front objective to the rear objective.

Cautions on switching objectives

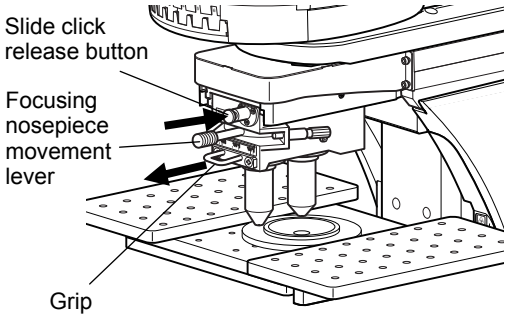
Be sure to move the objective vertical movement lever to the center before pushing or pulling the sliding nosepiece. Objectives can not be switched when the vertical movement lever is at the right or left end. Undue force may result in malfunction.

(1) Move the vertical movement lever at the left end to the center to raise the front objective.

(2) With the front objective raised, hold the grip while pressing the slide click release button and pull the sliding nosepiece to the front.

(3) Gently release the slide click release button and secure the sliding nosepiece at the click-stop position.

(4) Slowly move the vertical movement lever at the center to the right end to lower the rear objective.



Switching objective from front side to rear side

Objective dipping

When the objective vertical movement lever is pushed beyond the right end, the rear (observation point) objective descends 1 mm. When the objective vertical movement lever is pushed beyond the left end, the front (observation point) objective descends 1 mm.

When you stop pressing the lever, it moves back to the right (or left) end position, and the objective moves back to the original height.

Cautions on dipping operation

Do not perform dipping except when performing water immersion microscopy.

Doing so may damage the sample or objective.

11.2 Parfocal Correction of Objective

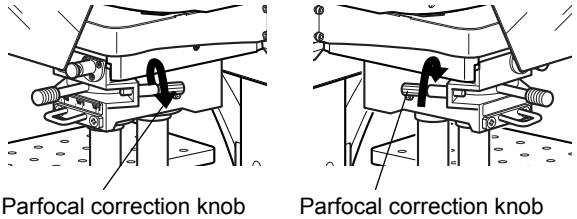
Cautions on parfocal correction

Before performing parfocal correction, make sure that the objective vertical movement lever is at the right or left end.

Normally, a dry objective and an immersion objective have different focal lengths. Switching between these two objectives causes misalignment of the focal point. In this case, adjust the focus using the parfocal correction (focus) knobs on the sides of the sliding nosepiece.

The parfocal correction knob on the left side is for adjusting the front objective, and the parfocal correction knob on the right side is for adjusting the rear objective.

Turning the knob clockwise raises the objective (focus position rises).



Raising focus

Example: When a dry objective is attached at the front and immersion objective is attached at the rear

(1) Move the objective vertical movement lever to the center. (objective retracting position)

(2) Push the sliding nosepiece to bring the dry objective into the optical path.

(3) Move the objective vertical movement lever to the left end. (front objective observation position)

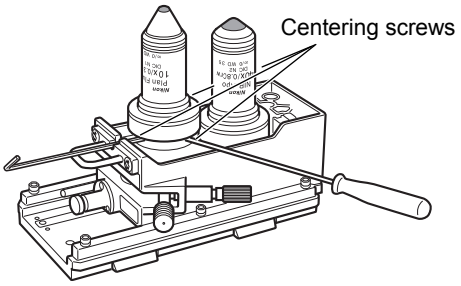
(4) Readjust the focus using the parfocal correction knob on the left sides of the sliding nosepiece. Turning the knob clockwise raises the objective (focus position rises).

11.3 Centering the Objective

With the sliding nosepiece, you can center the objective to minimize the misalignment of the center of the field of view on the front and rear objectives.

Adjust the three centering screws in such a way that the center of the field of view of the rear objective and the center of the field of view of the front objective match. You can more correctly adjust them by using an eyepiece with a crosshair.

When observing smaller samples by using methods such as patch clamping, centering is recommended beforehand.

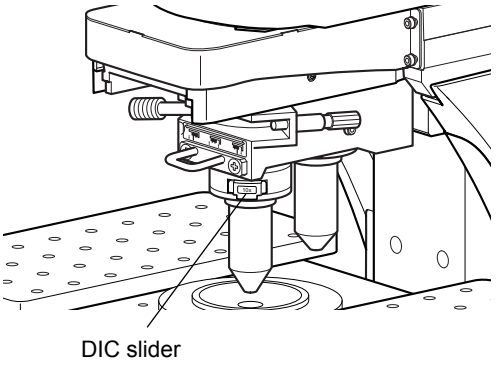


Centering the Objective

11.4 Inserting/Removing the DIC Slider

Insert the DIC slider required for differential interference contrast microscopy in the slot of the objective mount of the slider nosepiece. The slot appears when the objective is lowered.

Make sure the DIC slider is stopped at a click-stop position when inserted.



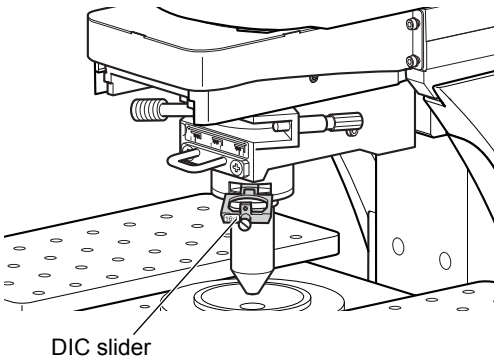
DIC slider

11.5 Single nosepiece

A single nosepiece is used only for the LWD16x or 25x water immersion objective.

Insert the DIC slider required for differential interference contrast microscopy in the slot of the objective mount of the single nosepiece.

Make sure the DIC slider until is stopped at a click-stop position when inserted.



Single nosepiece

12 Using the Polarizer Turret

The polarizer turret is attached to the field lens and used for differential interference contrast microscopy. The turret has four holes with the FN-P polarizer for visible range (λ=430 nm to 770 nm) and the FN-IRP polarizer for infrared ray (λ=850 nm to 950 nm) attached. The remaining two holes are empty and ϕ33 filter can be attached.

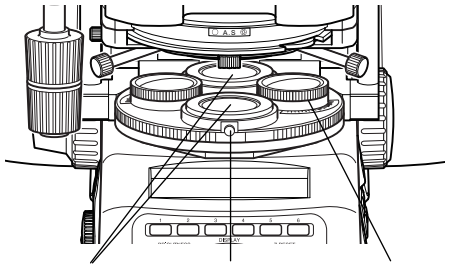
In the standard configuration, an NCB filter is attached.

See Chapter 1 “3 Assembly Method – 7 Attach the polarizer turret” in the “Assembly/Maintenance” instruction manual for details on how to attach the polarizer.

Turn the turret and bring the polarizer into the optical path for differential interference contrast microscopy. Make sure the turret is stopped at a click-stop position when switched.

To adjust the contrast, turn the knurled ring on the right of the polarizer turret to change the direction of the polarizer.

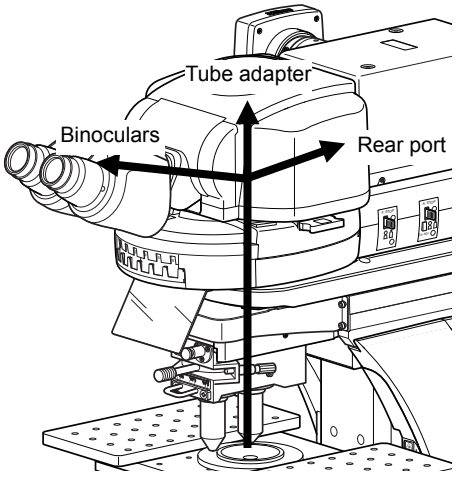
Provided adjustment tool for the 1/4 lambda plate orientation is used for orientation adjustment when the polarizer is attached. (See Chapter 1 “3 Assembly Method – 7 Attach the polarizer turret” in the “Assembly/Maintenance” instruction manual.)



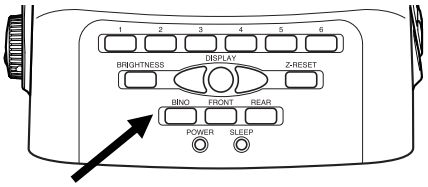
Polarizer turret

13 Switching the Optical Path of the Tube

The motorized quadrocular tilting tube has three optical paths, directed to the binocular, tube adapter, and rear port. Pressing an optical path button directs 100% of the light to the corresponding optical path. When a button is pressed, it will light up to indicate the selected optical path. The B/F/R optical path switch on the ergo controller can also be used to switch the optical path. See the Ni-E (Focusing Stage System) instruction manual for details of the ergo controller.

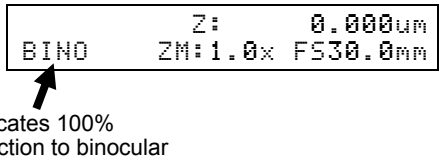


- BINO optical path button: 100% tube binocular
- FRONT optical path button: 100% to tube adapter
- REAR optical path button: 100% to rear port (to DSC zoom port NI-RPZ/NI-RPZ-E)



From the left: BINO, FRONT, and REAR buttons

Motorized optical path switching



Indicates 100% direction to binocular

♦Operation on DS-L3 (See "20.2 Microscope Control”.)♦

Interlocked operation of optical path switching

The optical path switching of the motorized quadrocular tilting tube can be interlocked when pressing the [CAPTURE] button to capture an image. See “18.2 Interlocking Function” for conditions for interlocking and interlocked operations.

Manual-type tube

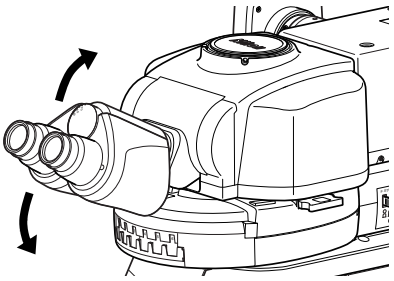
A manual-type tube can also be used for Ni-E (focusing stage system). See the Ni-E (Focusing Stage System) instruction manual for details on switching the optical path for each tube.

14 Adjusting the Binocular Section

With the quadrocular tilting tube, the angle of the binocular can be changed. Adjust the position of the binocular section for most comfortable viewing.

Adjustment range: 15° to 35°

The observation position can be adjusted also when the ergonomic binocular tube is used. See the Ni-E (Focusing Stage System) instruction manual.



Adjusting the Binocular Section

15 Water Immersion

An objective with a "WI" or "W" marking is a water-immersion objective that can focus on the sample at an operating distance under water. This objective is used by filling the chamber with water (distilled water or physiological saline) and dipping the tip of the objective into the water.

When observing a position slightly deeper than the surface (about 0.2 mm, depending on the state of the specimen) of a specimen such as a brain slice, Nikon recommends using infrared ray microscopy or a water immersion objective with a correction ring (a Plan100xW).

Since water evaporates readily, monitor the immersion water during observation. Avoid filling the chamber with too much water or spilling the water, since excess water will flow onto the stage and around the condenser, promoting corrosion. In such cases, immediately wipe off the water.

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.

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.

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

If petroleum benzine 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.)

⚠ CAUTION

- Absolute alcohol used for cleaning off the immersion water and petroleum benzene used for cleaning immersion oil 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 absolute alcohol.

16 Differential Interference Contrast Microscopy

An analyzer, a polarizer, an object lens side DIC slider, and a condenser side DIC module are required for differential interference contrast microscopy, which observes clear colorless specimen or undyed specimen at high contrast.

An Ni-E focusing nosepiece system can be used for differential interference contrast microscopy in the infrared region (λ=850 nm to 950 nm) and in the visible light region (λ=430 nm to 770 nm).

When performing IR-DIC (infrared differential interference contrast) microscopy, attach a DIC analyzer cube IR to the epi-fluorescence turret and a polarizer IR to the polarizer turret.

When performing differential interference contrast microscopy in the visible light region, attach a DIC analyzer cube VIS to the epi-fluorescence cube turret and a polarizer VIS to the polarizer turret.

When performing differential interference contrast microscopy in the visible light region, differential/epi-fluorescence microscopy can be performed simultaneously by inserting an FL/DIC analyzer slider in the slot at the right top of the epi-fluorescence cube turret instead of using the analyzer cube.

The objective side DIC slider is inserted in the slot on the object mount for both the slider nosepiece and single nosepiece and the condenser side module is attached to the turret of the LWD condenser.

The polarizer is attached to the polarizer turret mounted on the field lens.

16.1 Tips for Differential Interference Contrast Microscopy

Critical optical adjustments

Especially, in the differential interference contrast microscopy, the following two optical adjustments are critical. Inappropriate adjustment will result in unclear images. Operate with due care.

- **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 sample. Properly focus on the field diaphragm image, and make adjustment so that its center matches the center of the field of view and its size is close to the field of view. (See Chapter 3, Section 7 “Focusing and Centering the Condenser”.)

- **Adjusting the orientation of the polarizer and analyzer**

  The basic performance of the differential interference contrast method can be determined by this adjustment.

  Adjust the orientation of the analyzer in the epi-fluorescence cube turret and the polarizer on the field lens. The orientation of the analyzer is fixed in Ni-E. Adjust the orientation of the polarizer to find a position where field of view is darkest. (See Chapter 2 “3 IR-DIC (Infrared Ray Differential Interference Contrast) Microscopy Procedure - 19 Adjust the orientation (vibration direction) of the polarizer and analyzer”.)

Combination of DIC slider on the objective and DIC module on the condenser

The differential interference contrast microscopy requires a DIC slider and a DIC module on the objective and condenser sides respectively. A combination of the DIC slider on the objective side and the DIC module on the condenser side varies depending on the objectives used. Note that an inappropriate combination may result in an inability to produce an interference contrast image, or a significant degradation of contrast.

A dedicated DIC module is designed to provide higher contrast or resolution for particular microscopy purposes. Note, however, that in principle contrast and resolution compete under differential interference contrast microscopy (i.e. increasing the contrast will degrade the resolution, and vice versa), so select the combination accordingly. For details, see the table “Combination of DIC Slider on the Objective and DIC Module on the Condenser” in Chapter 1 “3. Assembly Method - 9 Attach the condenser” in the “Assembly/Maintenance” instruction manual.

16.2 Using Optical Elements

■ **DIC module for the condenser**

There are three types of DIC modules to be attached to the LWD condenser: [D-C DIC N1 DRY], [D-C DIC N2 DRY] and [D-C DIC NR DRY]. Note that an inappropriate combination may result in an inability to produce an interference contrast image, or a significant degradation of contrast. Select a DIC module appropriate for the objective to be used or suitable for your microscopy purpose. For details, see the table “Combinations for the DIC Slider for the Objective and the DIC Module for the Condenser” in Chapter 1 “3. Assembly Method – 9 Attach the condenser” in the “Assembly/Maintenance” instruction manual..

See also “9 Using the Condenser” for details.

■ **DIC slider for the objective**

Insert a DIC slider to a slot where the objective of the nosepiece is set.

Be sure to insert it properly to the limit.

See also “11.4 Inserting/Removing the DIC Slider” and “11.5 Single nosepiece”.

■ **Analyzer cube**

The analyzer cube is attached to the turret section of the epi-fluorescence cube turret. There are two types of analyzer cubes: [IR-A] for differential interference contrast microscopy in the infrared ray range and [VIS-A] for differential interference contrast microscopy in the visible light range. Turn the turret to bring the analyzer cube to be used into the optical path.

See also “17.1 Epi-fluorescence Cube Turret Operation”.

■ **Polarizer**

There are two types of polarizer: polarizer for the infrared ray range and polarizer for the visible light range. Attach it to the polarizer turret which is mounted on the field lens and turn the turret to bring the polarizer into the optical path. See also “12 Using the Polarizer Turret”.

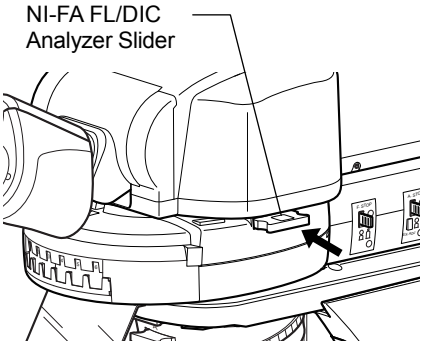
■ **FL/DIC analyzer slider**

This is an optical element required for switching the epi-fluorescence microscopy and differential interference contrast microscopy in the visible light range or using both microscopy methods concurrently.

Insert it into a slot on the upper lateral right of the epi-fluorescence cube turret.

The slider has two click positions. The slider will click once after insertion into the slot. At this point, the analyzer is not in the optical path. To bring the analyzer into the optical path, push the slider further to the second click-stop position. Insert it properly to the limit.

The analyzer slider attached to the nosepiece is not required when using the FL/DIC analyzer slider.



Inserting/removing the FL/DIC analyzer slider

17 Epi-fluorescence Microscopy

An epi-fluorescence microscopy used for the observation of a fluorescence image requires a brighter illuminator including such an optical element as a filter or mercury lamp.
Ni-E uses an epi-fluorescence attachment with a fluorescence cube turret to which a filter cube is attached (motorized, intelligent, or manual), HG precentered fiber illuminator using a mercury lamp as an illuminator (motorized or manual), ND filters, an aperture diaphragm and a field diaphragm.

⚠ 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.

17.1 Epi-fluorescence Cube Turret Operation (Switching Excitation Methods)

The epi-fluorescence cube turret has six slots. Attach the epi-fluorescence cube to a slot and bring it into the optical path. For bright-field microscopy, be sure to set Address 1 to empty [OPEN] and bring it into the optical path.

DIC analyzer cube

Attach a DIC analyzer cube to the epi-fluorescence cube turret to perform differential interference contrast microscopy with the Ni-E focusing nosepiece system. (See “16 Differential Interference Contrast Microscopy”.)

Motorized epi-fluorescence cube turret operation

Use a FL CUBE CW/CCW button to switch the epi-fluorescence cube turret. The turret rotates to the adjacent address each time the button is pressed.

The attach information on the filter cube has not been set as default.

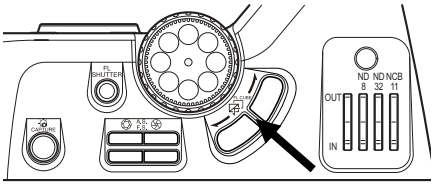
Information on the filter cube on the optical path is displayed on the display panel after setting the attach information on the filter cube to the epi-fluorescence cube turret before switching.

In addition, the front slot cover for a filter cube replacement has a window for inserting a filter cube nameplate. It is also useful if you keep inserting the nameplate into the window at the address to which the filter cube was attached.

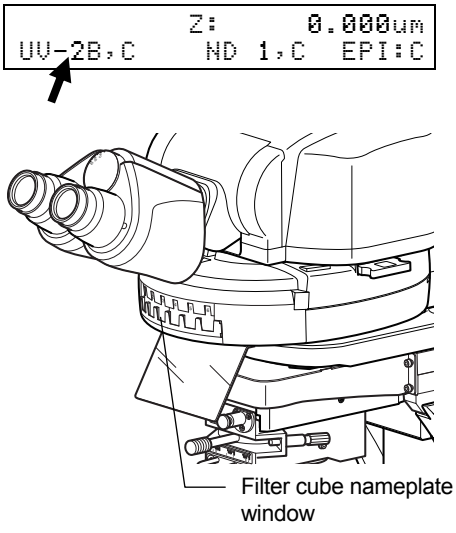
The setting of the attach information is performed from the DS-L3. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (1) Setting Optical Elements Installation Information” for details.)

See Chapter 1, “3 Assembly Method - 14 Attach the epi-fluorescence cube turret and epi-fluorescence attachment” - ■ Attach the filter cube/analyzer cube” in the “Assembly/Maintenance” instruction manual for information on how to attach the filter cube to the turret.

♦Operation on DS-L3 (See “20.2 Microscope Control”.)♦



Switching of the epi-fluorescence cube turret



Inserting the nameplate into the address window

Using double layered epi-fluorescence cube turrets

Two epi-fluorescence cube turrets can be used by layering them on top of each other.

Attach an attachment using a laser as an illuminator on the 1st layer, which should be used for light stimulation when using a double-decked turret. On the 2nd layer is attached an epi-fluorescence attachment used for the epi-fluorescence microscopy. This double-decked configuration is supplied as a separate system, so this document does not mention the description on that system.

Basically this document describes the epi-fluorescence cube turret on the 1st layer but mentions the one on the 2nd layer as needed. The front display panel indicates “-2” on the back of the filter cube indication to indicate the epi-fluorescence cube turret on the 2nd layer.

The switching of the second layer epi-fluorescence cube turret can be assigned to the FUNCTION button. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3-1) Ni-E microscope button” for details.)

Configuring the speed of the epi-fluorescence cube turret

Both epi-fluorescence cube turrets on the 1st and 2nd layers are configured for “high speed” operation upon shipment, but can also be configured for “low speed” operation for use with thick dichroic mirrors. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (7-1) FL turret drive speed” for details.)

Interlocked operation of the epi-fluorescence cube turret

The switching of the motorized epi-fluorescence cube turret can be interlocked when pressing the [CAPTURE] button to capture an image. See “18.2 Interlocking Function” for conditions for interlocking and interlocked operations.

Intelligent epi-fluorescence cube turret/manual epi-fluorescence cube turret

See the Ni-E (Focusing Stage System) instruction manual for details of the intelligent epi-fluorescence cube turret/manual epi-fluorescence cube turret.

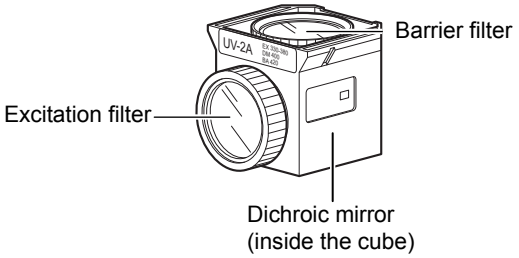
17.2 Selecting Filters

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, and attach the filter cube to the epi-fluorescence cube turret.

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.



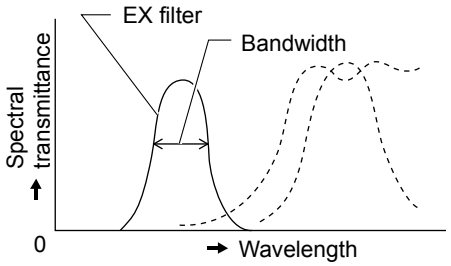
Filter cube

Excitation filter (EX filter)

Excitation filters allow selective transmission of light (excitation light) in the wavelength range required for fluorescent light emissions from the sample, 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 sample, 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 sample and causes the image to appear darker, but reduces autofluorescence and fading. For samples with pronounced autofluorescence, use excitation filters with a narrow bandwidth. (Note that this will make the fluorescent image 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 sample to pass, blocking excitation light. This allows the fluorescent image to be viewed without excess illumination (dark background).

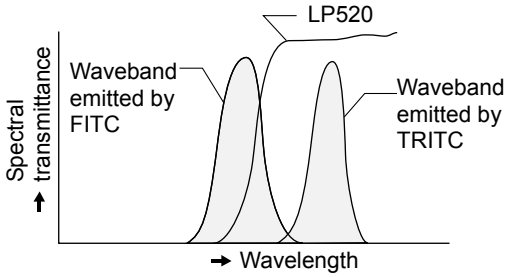
There are two types of barrier filters: LP filters that block all light below a certain wavelength but pass all light of longer wavelengths, and BP filters that pass light of a certain waveband and block all other light. Use the filter type appropriate for your intended purpose.

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 samples labeled with a fluorophore in which the fluorescent waveband and excitation waveband (light that the sample 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 samples, 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, Nikon recommends using multiband filters.



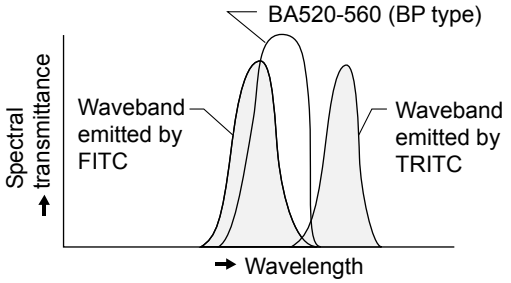
Long-pass filter
(Both FITC and TRITC images are visible.)

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 samples. (For example, in a double-labeled sample 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.



Bandpass filter
(Only the FITC fluorescent image is visible)

17.3 Protecting the Sample and Preventing It from Decoloration (Using the Shutter)

If the sample is continuously exposed to the strong light of the mercury lamp used for the epi-fluorescence attachment, it may become damaged or decolorized. Be sure to close the shutter when suspending microscopy or when pausing epi-fluorescence microscopy to perform microscopy with diascopic light. Be sure to get into the habit of performing this operation.

Any of these shutters can be used to open/close the optical path as shutters for blocking the epi-fluorescence illumination are provided in the following attachments:

- Manual/intelligent/motorized epi-fluorescence cube turret
- Manual/motorized HG precentered fiber illuminator (Intensilight)
- EPI motorized shutter

Opening and closing the shutter of a motorized unit

■ **Motorized epi-fluorescence cube turret**

Press the FL SHUTTER button to open/close the shutter of a motorized epi-fluorescence cube turret. “The shutter is closed” when the button is ON.

The FL SHUTTER button function is set to “open/close the shutter built in the epi-fluorescence cube turret on the 1st layer” as default. Changing the setting of the button function allows you to use the FL SHUTTER button for opening/closing the motorized epi-fluorescence cube turret on the 2nd layer”.

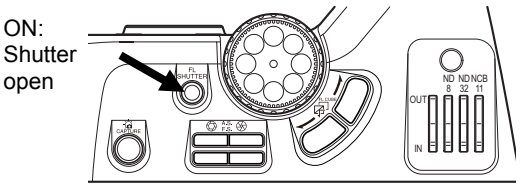
■ **Motorized shutter**

For Ni-E, the motorized shutter can be attached to the epi-fluorescence side or dia-illumination side to provide a fast opening/closing of an epi-fluorescence illumination path or dia-illumination path.

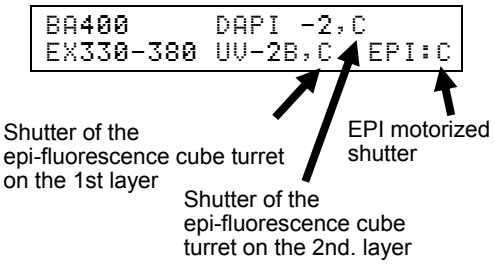
On the epi-fluorescence side, attach the shutter between the epi-fluorescence attachment and the HG adapter of the HG precentered fiber illuminator. On the dia-illumination side, it is attached between the lamphouse mount at the rear of the microscope base and the dia-illumination lamphouse.

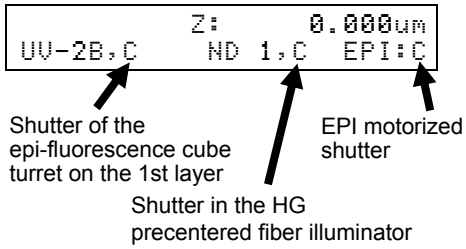
One motorized shutter can be attached on the epi-fluorescence and dia-illumination side respectively for use if two shutters are provided. This allows for immediate blocking of the diascopic light, and is useful when switching between epi-fluorescence and differential interference contrast methods. (A different attachment adapter is used for EPI and DIA sides.)

Press the FL SHUTTER button to open/close the motorized shutter. Change the FL SHUTTER button function to the opening/closing operation function of an EPI motorized shutter or DIA motorized shutter. “The shutter is closed” when the button is ON.

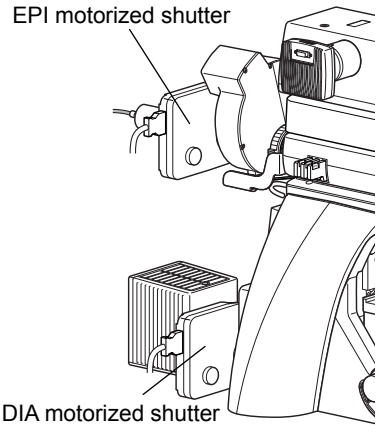


Opening/closing the shutter





C: shutter closed
O: shutter open



Motorized shutter

Motorized Shutter Connector

There are two connectors for connecting the motorized shutter cable: “EPI SHUTTER” on the rear of the main body and “DIA SHUTTER” on the connector box. Connect the EPI motorized shutter on the epi-fluorescence side to the “EPI SHUTTER” connector and the DIA motorized shutter on the dia-illumination side to the “DIA SHUTTER” connector.

These are set by factory default. Change the setting if you intend to use the motorized shutter for AUX (other than EPI/DIA).

Change the configuration on the DS-L3. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (2) Configuring the Device Connection Information” for details.)

■ **Motorized HG precentered fiber illuminator**

Press the FL SHUTTER button to open/close the motorized HG precentered fiber illuminator. Change the FL SHUTTER button function to the opening/closing operation function of the motorized HG precentered fiber illuminator. “The shutter is closed” when the button is ON.

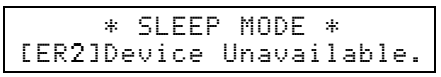
Change the FL SHUTTER button function on the DS-L3. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3-1) Ni-E microscope button” for details.)

Grouped operation of EPI side shutters

Three types of shutters on the EPI side (including the shutter in the epi-fluorescence cube turret in the 2nd layer, if attached) can be opened and closed all at once. This is performed with the ergo controller’s EPI all switch. See the Ni-E (Focusing Stage System) instruction manual for details of the ergo controller.

Grouped operation of EPI side shutters is not available in sleep mode. A message showing “Sleep mode is active. Drive/operation not available” is displayed and you hear a buzz. (Beep) The warning disappears automatically approximately after 3 seconds. Reset the sleep mode before you continue the operation.

(See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3-3) Buttons on the Screens on DS-L3 - ■ SLEEP button” for details.)



While in sleep mode, drive operation of motorized units not available

♦Operation on DS-L3 (See “20.2 Microscope Control”.)♦

Intelligent/manual epi-fluorescence cube turret shutter

See the Ni-E (Focusing Stage System) instruction manual for details on the intelligent/manual epi-fluorescence cube turret shutter operation.

17.4 Adjusting the Brightness of the Fluorescent Image (Using ND Filters and the Aperture Diaphragm)

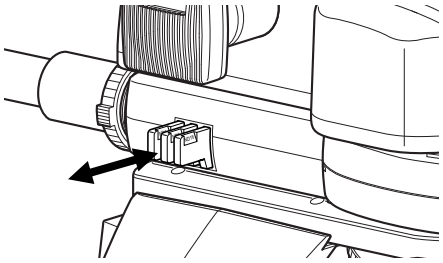
The brightness of the epi-fluorescence image can be adjusted by using the ND filters in the epi-fluorescence attachment and the motorized HG precentered fiber illuminator. ND filters are used to adjust light intensity. The higher filter number, the lower transmittance, and darker images are produced when placed in the optical path. ND filters do not affect the color balance. Image brightness can also be adjusted by using the aperture diaphragm on the epi-fluorescence attachment.

ND filters in the epi-fluorescence attachment

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

If the fluorescence is too strong, or if there is too much decoloration of the specimen, push the ND filter slider into the right side to bring the ND filter into the optical path and adjust the brightness. (Excessive fluorescence may degrade the contrast of the image.)

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



Inserting/removing the ND filter

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)

ND filter in the motorized HG precentered fiber illuminator

See the Ni-E (Focusing Stage System) instruction manual for details of the ND value adjustment of the motorized HG precentered fiber illuminator.

Aperture diaphragm

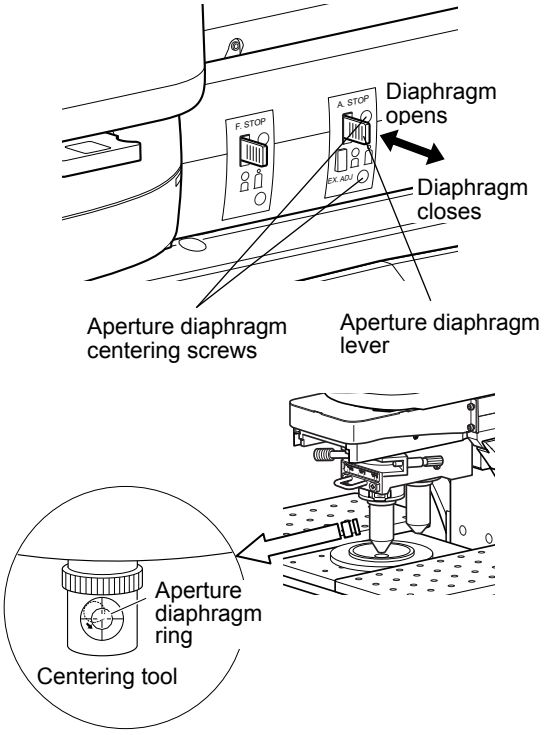
The aperture diaphragm is used to adjust the numerical aperture of the illumination system. Under epi-fluorescence microscopy, it is also used to adjust the brightness of the image, as well as the size of stray light.

Turning the aperture diaphragm lever of the epi-fluorescence attachment changes the size of the aperture diaphragm. Pushing in the lever opens the aperture diaphragm while pulling it out closes it. Stopping down the aperture diaphragm reduces stray light, but will also make the image darker. Conversely, a larger aperture diaphragm size increases the brightness, but may also increase the amount of stray light. For standard epi-fluorescence microscopy, keep the diaphragm open, and stop down as necessary to adjust the image brightness.

Be sure to center the aperture diaphragm of the epi-fluorescence attachment as follows before use.

■ **Centering the aperture diaphragm**

(1) Close the shutter of the epi-fluorescence cube turret.
(2) Replace one objective on the nosepiece with the centering tool.
(3) Under epi-fluorescence microscopy, focus on the sample.
(4) Bring the centering tool into the optical path.
(5) Pull out the aperture diaphragm lever to stop down the diaphragm.
(6) While watching the screen on the centering tool, turn the aperture diaphragm centering screw with the provided hex wrench (opposite side distance: 2) to move the aperture diaphragm image to the center of the screen. Be sure to look straight at the screen.



Adjusting and centering the aperture diaphragm

17.5 Restricting the Illumination to the Area of the Sample Being Viewed (Centering and Adjusting the Field Diaphragm)

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

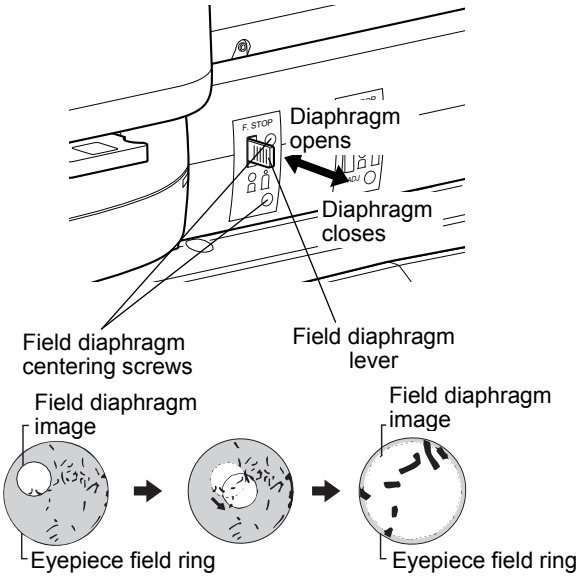
Turning the field diaphragm lever of the epi-fluorescence attachment changes the size of the field diaphragm. Pushing in the lever opens the field diaphragm while pulling it out closes it.

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 sample will become decolorized over a wider area.

Be sure to center the field diaphragm of the epi-fluorescence attachment as follows before use.

■ **Center the field diaphragm**

(1) Pull out the field diaphragm lever on the epi-fluorescence attachment to stop down the field diaphragm.
(2) Turn the field diaphragm centering screw with the provided hex wrench for M4 setscrew to move the field diaphragm image to the center of the field of view.
(3) Push in the field diaphragm lever and open the field diaphragm to match the size of the field of view.
(4) Turn the field diaphragm centering screw once again to align the center of the field diaphragm image with the center of the field of view.



Adjusting the field diaphragm

Field diaphragm adjustment timing

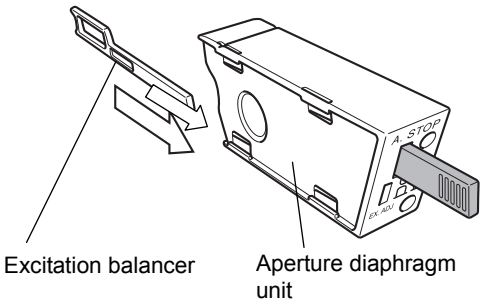
Be sure to adjust the size of field diaphragm each time you change the objective. While it is not absolutely essential to center the field diaphragm each time you change the objective, you should still perform a quick check and make adjustments as necessary.

17.6 Changing the Waveform Characteristics of the Excitation Light (D-FB Excitation Balancer)

By attaching the D-FB Excitation Balancer to the epi-fluorescence attachment, the waveform characteristics of the excitation light can be changed arbitrarily. The balancer is to be used together with a dual-band filter cube.

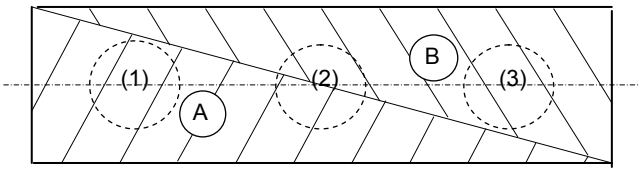
■ **Attaching and using the excitation balancer**

(1) Turn off the power for the epi-fluorescence light source.
(2) Remove the aperture diaphragm unit from the epi-fluorescence attachment.
(3) Insert the D-FB Excitation Balancer into the rear of the aperture diaphragm unit, as shown in the figure. Be careful not to touch the glass surface of the excitation balancer.
(4) Attach the aperture diaphragm unit back onto the epi-fluorescence attachment.
(5) Attach a dual-band filter cube onto the epi-fluorescence attachment.
(6) Adjust the excitation balancer horizontally to attain the desired excitation light.

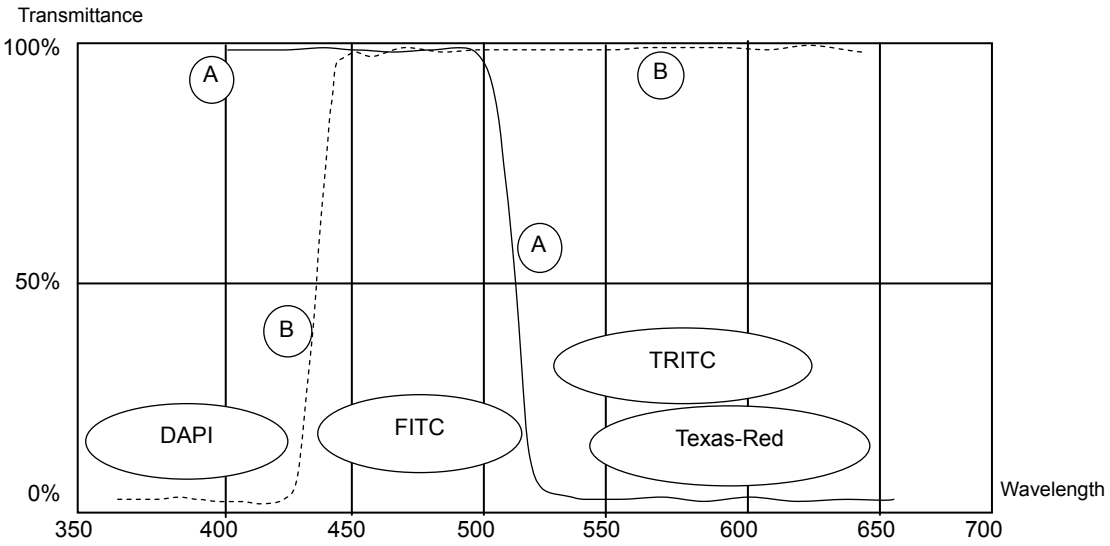


Attaching the excitation balancer

■ **Excitation balancer details**



: Effective diameter on the aperture diaphragm surface



The excitation balancer is configured to achieve almost 100% transmittance at all times for FITC, which generally has low fluorescence.

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

■ **Objectives**

The excitation balancer should be used in combination with the following objectives. Use of other objectives may result in unevenness in the field of view.

Plan fluor	40x/0.75	40xH/1.3	100xH/1.3
S fluor	40x/0.9	40xH/1.3	100xH/1.3
Plan apochromat	40x/0.95	60xH/1.4	100xH/1.4

Motorized excitation filter wheel, motorized barrier filter wheel

Motorized excitation filter wheel and motorized barrier filter wheel can be attached on the epi-fluorescence side. See the Ni-E (Focusing Stage System) instruction manual for details.

17.7 Other Notes on Epi-fluorescence Microscopy

■ **Locating a target in the sample**

The standard procedure for epi-fluorescence microscopy is to first locate the target under dia-illumination differential interference contrast microscopy, and then switch to epi-fluorescence microscopy. To locate the target under 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 sample to determine the approximate position of the target.

■ **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 sample.

18 Useful Functions

18.1 Mode Function

“MODE” saves and loads a “microscopy state” (position and status of motorized accessories) and is useful for an observation when switching between different microscopy state.

To use this function, you must have registered a set of motorized units for loading in each mode. More than one motorized unit can be registered for one mode with up to 8 modes available for setting.

No motorized units are registered for any mode as default.

Ex.: Switching between the differential interference contrast and epi-fluorescence microcopy in observation

MODE1: Register the field diaphragm, elevating section, lamp brightness adjustment
MODE2: Register the epi-fluorescence turret, FL shutter, and Intensilight

Up to four alphanumeric characters can be used to name a file in mode. Here, MODE1 is named as “mdic” and MODE2 as “mfl” for registration.

1 Register the mode.

Start from the DS-L3 [MICROSCOPE SETUP] screen.

[SETUP MENU]→[MODE]

(1) Specify MODE1.

For the initial registration of the mode, factory setting mode names [MD1] to [MD8] are displayed on the buttons.

The mode name is displayed on the button for which a mode has been registered.

(1) Specify MODE1

(2) Add a mode.

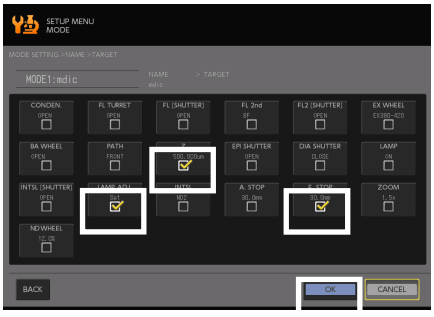
Specify a mode number to open a sub menu. An [ADD] button appears when registering for the first time.

A [MODIFY] and [DELETE] button appear when updating. Enter the mode name (up to 4 alphanumeric characters) when prompted and then specify the target motorized device.

The mode name changes to [MD1] when you [DELETE] a mode.

(2) Add a mode [YES]

(3) Enter [mdic] on the screen for entering the mode name, and use ► [NEXT] button to navigate to a device selection screen. Select a device to specify.



(3) Specify a motorized unit and click [OK]

(4) Now MODE1 has been registered and the registered mode name is displayed on the [MODE1] button. [SAVE MODE1] button is shown on the right of the mode name button.
Repeat the same procedure for MODE2 registration.

(4) MODE1 and MODE2 have been registered

2 Save the mode.

Save the mode on the [MICROSCOPE SETUP] screen. Alternatively, you can use the [MICROSCOPE CONTROL] screen.

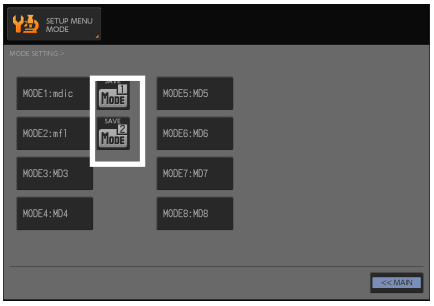
Operation on the [MICROSCOPE SETUP] screen

(1) Perform the differential interference contrast microscopy to get ready for save.

(2) Press the [SAVE MODE1] button displayed on the right of the registered MODE [mdic] on a mode registration complete screen.
The current state of the registered device will be saved.

(3) Perform the epi-fluorescence microscopy to get ready for save.

(4) Press the [SAVE MODE2] button for MODE [mfl].
The current state of the registered device will be saved.



Saving a mode

Operation on the [MICROSCOPE CONTROL] screen

(1) Perform the differential interference contrast microscopy to get ready for save.

(2) Press the [SAVE MODE] button.

(3) Press [mdic] when a sub screen is opened.
The current state of the registered device will be saved.
Save MODE2 [mfl] as well in the same way.

Each save updates the status of what was saved.
If the device is suspended properly during save, the device will be excluded from the target for the save automatically.

[MICROSCOPE CONTROL] screen

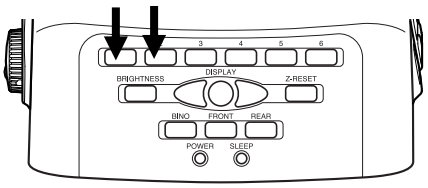
Saving a mode [mdic]

3 Load the mode.

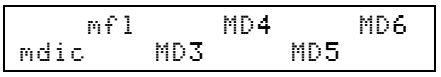
(1) Press [FUNCTION1] and [FUNCTION2] buttons alternately.

This assumes that a load function for MODE 1 and MODE 2 is allocated to FUNCTION 1 button and FUNCTION 2 button respectively. The MODE load function is not set to a FUNCTION button in Ni-E as default. See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3-1) Ni-E microscope button” to change the function assignment.

(2) Saved differential interference contrast microscopy and epi-fluorescence microscopy will be loaded alternately.



Loading a mode



Switched to the display of the FUNCTION button function

You can work with on the DS-L3 [MICROSCOPE CONTROL] screen.

Press the [LOAD MODE] button. Press [mdic] and [mfl] alternately when a sub screen of the MODE selection (load) opened.

Saved differential interference contrast microscopy and epi-fluorescence microscopy will be loaded alternately.

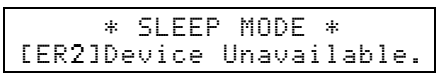
[MICROSCOPE CONTROL] screen

Loading a mode

Loading a mode in Sleep mode

Loading a mode is not available in Sleep mode. A message showing “Sleep mode is active. Drive/operation not available” is displayed and you hear a buzz. (Beep) The warning disappears automatically approximately after 3 seconds. Reset the sleep mode before you continue the operation.

(See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (3-3) Buttons on the Screens on DS-L3 -■ SLEEP button” for details.)



While in sleep mode, drive/operation of motorized units not available

Motorized Unit that Save/Loads the State with MODE Function

Setting items	Stored Information
CONDEN.	Motorized universal condenser's condenser module currently in the optical path (not supported for focusing nosepiece) (The motorized condenser is not used in the focusing nosepiece system.)
FL TURRET	Filter cube of the motorized epi-fluorescence cube turret in the optical path
FL [SHUTTER]	Open/closed state of the motorized epi-fluorescence cube turret's built-in shutter
FL 2nd	Filter cube of the motorized epi-fluorescence cube turret on the 2nd layer that is brought in the optical path
FL2 [SHUTTER]	Opening/closing of the built-in shutter of the motorized epi-fluorescence cube turret on the 2nd layer
EX WHEEL	Motorized excitation filter wheel's excitation filter currently in the optical path
BA WHEEL	Motorized barrier filter wheel's barrier filter currently in the optical path
PATH	Optical path in the motorized quadrocular tilting tube
Z	Position of the elevating section*1
EPI SHUTTER	Opening/closing of the EPI motorized shutter
DIA SHUTTER	Opening/closing of the DIA motorized shutter
LAMP ADJ.	Brightness of the dia-illumination lamp
LAMP	Turns the dia-illumination lamp on or off.
INTSL [SHUTTER]	Opening/closing of the motorized HG precentered fiber illuminator's built-in shutter
INTSL	ND filters in the motorized HG precentered fiber illuminator
A. STOP	Aperture diaphragm diameter of the motorized universal condenser (not supported for focusing nosepiece) (The motorized condenser is not used in the focusing nosepiece system.)
F. STOP	Field diaphragm diameter
ZOOM	Zoom magnification of the motorized DSC zoom port
ND WHEEL	ND filter transmittance of the motorized ND filter wheel

*1 The elevating section of the Ni-E is automatically excluded from the target of mode setting upon recycling the power on the microscope main body, so as to avoid contact between the sample and the objective.

18.2 Interlocking Function

With the Ni-E focusing nosepiece system, the rotation of the motorized quadrocular tilting tube and the motorized epi-fluorescence cube turret can be set to interlock when pressing the [CAPTURE] button to capture an image.

For interlocking settings, see "20 Operation on DS-L3" - "20.1 Setting Up the Microscope - (4) Configuring the Movement" or Chapter 6 "5 Configuring the Movement of Motorized Devices ([MOVEMENT] Screen)" in the "Microscope Control" manual bundled with DS-L3.

Setting items	Interlocking	Interlock condition
PATH (Optical path of the motorized quadrocular tilting tube)	• FRONT (tube adapter) • REAR (rear port) • OFF (Do not interlock as factory setting)	Motorized quadrocular tilting tube must be connected
FL TURRET FL 2nd (cube inside motorized epi-fluorescence cube turret)	• Bring the specified filter cube into the optical path. • OFF (Do not interlock as factory setting)	Motorized epi-fluorescence cube turret must be connected

Interlocking in Sleep mode

No interlocking takes place in Sleep mode.

19 Capturing Images

A microscope image can be captured with a DS camera head attached to a tube or zooming port.

Use DS-L3 or DS-U3 DS Camera Control Unit.

DS-L3 is connected to the USB connector, DSC1 connector at the rear of the microscope or the DSC2 connector of the connector box to control the camera.

DS-U3 is connected the DSC1 or DSC2 connector to control the camera.

Up to two cameras can be connected.

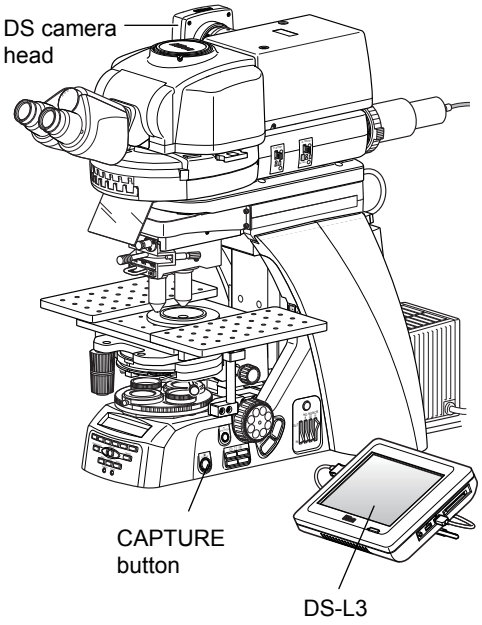
You must have set the position and the manufacturer name of the camera at the end of the DSC when capturing an image with the DSC. Configuration is performed on the DS-L3. (See “20 Operation on DS-L3” - “20.1 Setting Up the Microscope - (2) Configuring the Device Connection Information” for details.)

This setting is not required when capturing an image with a camera cable-connected to DS-L3 controlled with the USB.

CAPTURE button commands the image capture.

The CAPTURE button function is set to capture an image using a camera connected to the USB as default.

♦Operation on DS-L3 (See “20.2 Microscope Control”.)♦



Interlocking setting caused by [CAPTURE]

“Interlock/Do not interlock” setting is available for the optical path switching of the motorized quadrocular tilting tube or motorized epi-fluorescence cube turret rotation when pressing the [CAPTURE] button to capture an image.

See “18.2 Interlocking Function” for conditions for interlocking and interlocked operations.

Notes on the [CAPTURE] function using a trigger signal

When the CAPTURE button is set to connect to DSC, an unintended capture may occur when the microscope is turned ON/OFF. To avoid this, set to connect to the USB.

In addition, only DS-L3 is supported for a capture with the USB.

Photomicroscopy

The photomicroscopy procedure is described below. Also see the instruction manual provided with the DS-U3, DS-L3, or the microscope’s setup/control software “Ni Setup Tool” instruction manual for the details including the camera settings.

Using DS-L3

You must choose at least the following information:

- Folder for data storage.
- Name of the file to be saved. (Or select “Auto”.)
- 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 DS camera head attachment position.

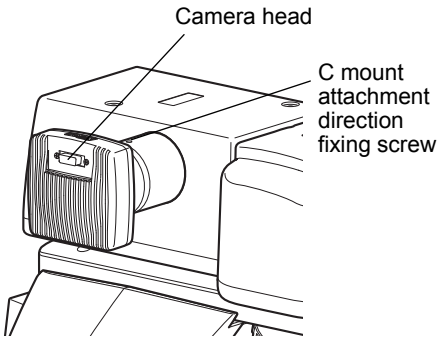
For details on the camera head attachment, see Chapter 1 “3 Assembly Method - 18 Attach the camera and DS camera control unit” in the “Assembly/Maintenance” instruction manual.

■DSC zooming port for quadrocular tube

Loosen the attachment direction fixing screw for the C mount so that an image moves in the reverse direction when the stage is moved right and left, adjust the camera position, and then tighten the screw to fix it.

■DSC port for ergonomic binocular tube

See the Ni-E (Focusing Stage System) instruction manual for details.



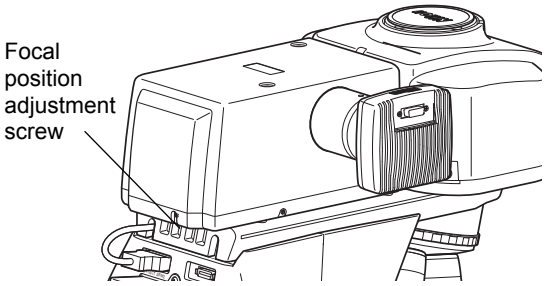
DSC zooming port camera position adjustment

(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, make adjustment.

■DSC zooming port for quadrocular tube

Adjust the position of the internal lenses from the rear of the zooming port by rotating the focal position adjustment screw using a hex wrench to focus it.



Focusing the image using a zooming port

■DSC port for ergonomic binocular tube

See the Ni-E (Focusing Stage System) instruction manual for details.

Note that, in both ports, such out of focus situations may also indicate incorrect diopter adjustment. Make sure you have made diopter adjustments as well. (See Chapter 3 “6 Adjusting the Diopter”.)

(4) Select the camera scene mode suitable for the microscopy method.

(5) 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, Nikon recommends adjusting the white balance under normal bright-field microscopy conditions before capturing images.)

NCB filter

The Ni-E's filter cassette is equipped with an NCB filter for color temperature conversion.

(6) Align the specimen.

(7) Readjust the focus onto the target.

(8) Adjust the image brightness using the camera exposure compensation function.

(9) Check the image using the DS-L3 [FREEZE] button.

(10) If the image is acceptable, press the CAPTURE button to save the image.

(For details, see 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.

ZOOM

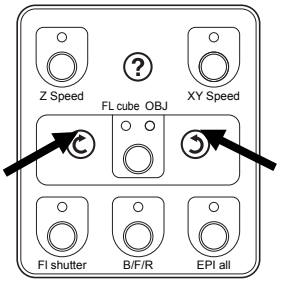
Attaching a camera to the zooming port allows to zoom in an image.

■ **Motorized DSC Zooming Port**

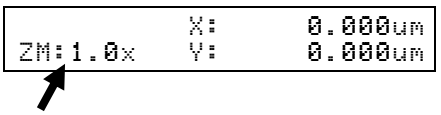
The final magnification of the motorized DSC zooming port can be from 0.6x up to 2.0x.

Use the CW/CCW button of the ergo controller for a zoom operation. A zoom magnification is displayed in 0.1x increments. See the Ni-E (Focusing Stage System) instruction manual for details of the ergo controller.

♦Operation on DS-L3 (See "20.2 Microscope Control".)♦



Zoom operation



Limited zoom range

Note that the zoom range may be limited depending on capture conditions or type of the camera when using a DS camera made by other manufacturers.

Limb darkening at the zoom port

- When performing bright field microscopy with 2-layer epi-fluorescence cube turret, the edge of the field of view may be dark at low zoom magnification.
- When performing epi-fluorescence microscopy with 2-layer epi-fluorescence cube turret, the edge of the field of view may be dark at low zoom magnification.

Manual DSC zoom port

See the Ni-E (Focusing Stage System) instruction manual for details of the manual DSC zoom port.

Tips on Microscope Settings for Photomicroscopy

■ **Adjusting the light intensity**

Lamp voltage: When accurate color reproduction is critical, turn the brightness control knob to the mark, bring the NCB11 filter into the optical path, and use the ND filters to adjust the brightness.

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

■ **Adjusting the condenser**

- Always focus and center the condenser.
- For differential interference contrast microscopy, align the orientation of the polarizer and analyzer.
- 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 samples 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 sample, making it more difficult to acquire suitable fluorescent images. Insert ND filters into the optical path to adjust the brightness.

- **Sample**

  Photomicrography of faded sample sections requires prolonged exposure time and results in poor color reproduction and low-quality images. Move the sample to obtain images from a fresh section of the sample previously unexposed to excitation light. For best results, use the differential interference contrast microscopy method to select a sample 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 provided with the DS-U3, DS-L3, or the microscope's setup/control software "Ni Setup Tool" for details.

20 Operation on DS-L3

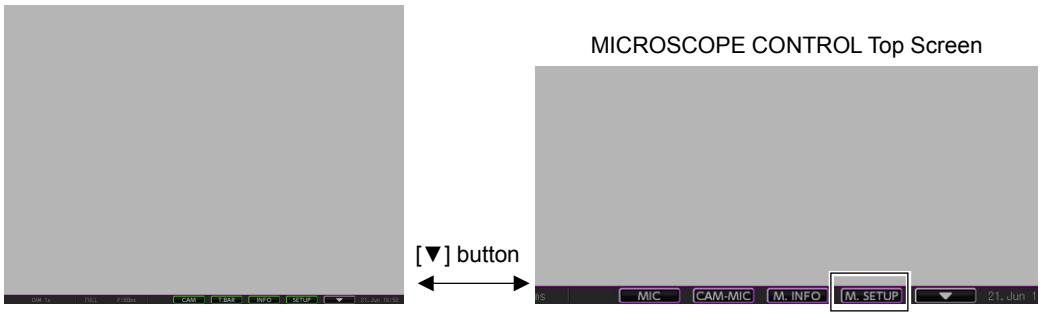
20.1 Setting Up the Microscope

If you use the microscope as it is set up as default, a specific setup is not required, however, if you want to change the setting to meet your needs, you want to use useful functions, or you want to set the information on optical elements, use DS-L3 for the operation below. References are provided so that the instruction manual bundled with DS-L3 “Microscope Operation” for detailed explanation can be used. In addition, to restore all settings to the default setting, see (7) Maintenance “✔ Restoring the factory default settings for microscope data (from the microscope)”.

1 Display the [MICROSCOPE CONTROL] top screen.

Turning ON the DS-L3 displays the top screen of the camera control at first.
Use the [▼] button to navigate to the MICROSCOPE CONTROL top screen.

Camera Control top screen

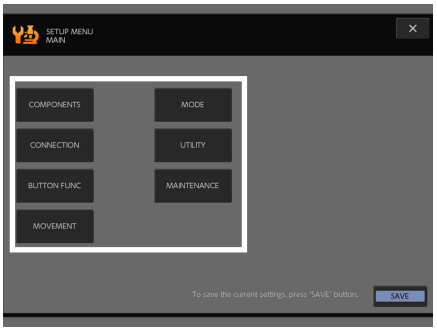


MICROSCOPE CONTROL Top Screen

Using [M. SETUP] button to navigate to [MICROSCOPE SETUP MENU] screen

2 Display the [MICROSCOPE SETUP MENU] screen.

MICROSCOPE SETUP MENU screen



Selecting a Setup Menu

3 Select a setup menu.

Press your desired menu button on the [MICROSCOPE SETUP MENU] screen.

[COMPONENTS] → (1) Setting Optical Elements Installation Information
[CONNECTION] → (2) Configuring the Device Connection Information
[BUTTON FUNC] → (3) Configuring the Button Functions
[MOVEMENT] → (4) Configuring the Movement
[MODE] → (5) Setting MODE Function
[UTILITY] → (6) Configuring Other Functions
[MAINTENANCE] → (7) Maintenance

✔ DS-L3 screen

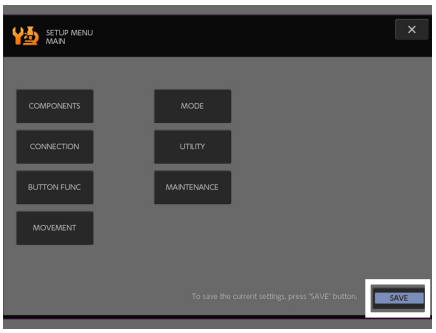
The content of the DS-L3 screen will differ depending on the configuration and settings of your microscope.

Buttons of unavailable function are grayed out and cannot be used.

4 Shown below is the actual settings. See (1) through (7).

Saving the setting

After completing the setting, be sure to press the [SAVE] button on the [MICROSCOPE SETUP MENU] screen to save the settings. If the DS-L3 is turned off without saving settings, previous values are restored. See Chapter 6 “1 Bulk Saving of Settings” in the DS-L3 instruction manual “Microscope Operation” for the details of saving.



Saving the setting

(1) Setting Optical Elements Installation Information (See DS-L3 Chapter 6 “2 Configuring Optical Elements Information”.)

Configure optical elements used for the following motorized units:

<Default settings>

Address 1 of the motorized universal condenser and the motorized barrier filter wheel is set to [OPEN] by default and cannot be changed. The rest of all has not yet been set. [OPEN] setting is available only for Address 1 in a motorized/intelligent epi-fluorescence cube turret.

Device icons on the top of the screen are as shown below from left.

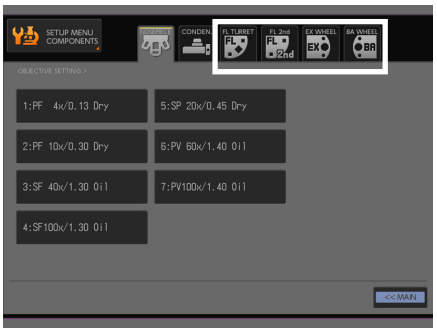
- Motorized nosepiece/intelligent nosepiece: Objective settings (not used in the focusing nosepiece system)
- Motorized condenser: DIC module, PH module settings (not used in the focusing nosepiece system)
- Motorized/intelligent epi-fluorescence cube turret (on the 1st layer): Filter cube settings
- Motorized/intelligent epi-fluorescence cube turret (on the 2nd layer): Filter cube settings
- Motorized excitation filter wheel: Excitation filter settings
- Motorized barrier filter wheel: Barrier filter settings

Press a device icon button, then press the address button of an optical element to select the optical element from a list of registered elements.

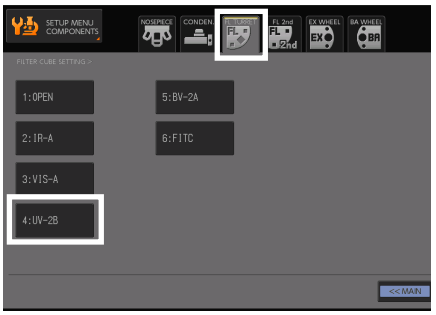
If you cannot find an optical element you want to set in the list, pressing the [OPTIONAL] button allows you to add up to 10 optical element information. Follow the instruction on the screen during the operation. Use the same procedure to set it again when an optical element attachment has changed.

You can use the same procedure for any devices to set the optical element information.

[SETUP MENU] → [COMPONENTS]



Device selection



Example: Epi-fluorescence cube turret, address 4 setting

Selecting a filter cube

(2) Configuring the Device Connection Information (See DS-L3 Chapter 6 “3 Setting the Connections of Motorized Units”.)

(2-1) Connecting the digital camera

(See DS-L3 Chapter 6 “3.1 Configuring the Connection of Digital Camera”.)

Perform this configuration when a camera control unit is connected to the main body using a trigger cable to capture an image by sending trigger signal from the DSC connector on the main body.

This configuration is unnecessary if you are capturing from a camera directly connected to this DS-L3 by pressing the [CAPTURE] button on the camera control screen on DS-L3.

<Default setting>

NOT-CONNECTED

(2-2) Connecting the motorized shutter

(See DS-L3 Chapter 6 “3.2 Configuring the Connection of Motorized Shutter”.)

Perform this configuration when changing the objective of using the motorized shutter to AUX (other than EPI/DIA).

<Default setting>

EPI SHUTTER: EPI motorized shutter, DIA SHUTTER: DIA motorized shutter

(3) Configuring the Button Functions (See DS-L3 Chapter 6 “4 Configuring the Functions of Buttons”.)

Change the function of buttons equipped with the Ni-E microscope and the ergo controller.

The function of buttons located on the DS-L3 screen can be selected as desired here.

(3-1) Ni-E microscope button

(See DS-L3 Chapter 6 “4.2 Configuring Buttons on the Microscope”.)

Change the function of buttons equipped with the Ni-E microscope.

[SETUP MENU] → [BUTTON FUNC]

Configuring button functions

■ **FUNCTION button**

(See DS-L3 Chapter 6 “4.2.1 Setting the Function of the Function Buttons”.)

<Factory setting>

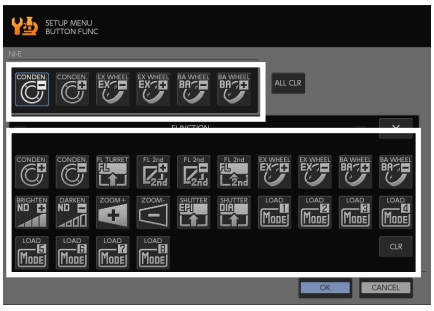
Button 1 and 2: Rotate the condenser counterclockwise and clockwise (The motorized condenser is not used in the focusing nosepiece system.)

Buttons 3 and 4: Rotate the motorized excitation filter wheel counterclockwise and clockwise.

Buttons 5 and 6: Rotate the motorized barrier filter wheel counterclockwise and clockwise.

Press the [FUNC SET] button under [FUNCTION BUTTON] to specify the button number and to select a function.

[FUNC SET] button under [FUNCTION BUTTON]



Selecting the FUNCTION button function (motorized accessory)

■ **FL SHUTTER, CAPTURE and CW/CCW buttons**

(See DS-L3 Chapter 6 “4.2.2 Changing the Motorized Shutter Operated with the FL SHUTTER Button”.)

(See DS-L3 Chapter 6 “4.2.3 Changing the Digital Camera Operated with the Microscope’s CAPTURE Button”.)

(See DS-L3 Chapter 6 “4.2.4 Changing the Motorized Device to be Operated with CW/CCW Button”.)

Function setting of FL SHUTTER, CAPTURE, and CW/CCW buttons

Units Supported for Buttons

Operation Button	Target Unit
FL SHUTTER Button	[INTSL] Shutter built in the HG precentered fiber illuminator
	[FL TURRET] Shutter built in the epi-fluorescence cube turret on the 1st layer (default setting)
	[FL 2nd] Shutter built in the epi-fluorescence cube turret on the 2nd layer
	[EPI/DIA/AUX] Motorized shutter connected to the EPI SHUTTER connector at the position set in “(2) Configuring the Device Connection Information”
	[EPI/DIA/AUX] Motorized shutter connected to the DIA SHUTTER connector at the position set in “(2) Configuring the Device Connection Information”
CAPTURE Button	[OFF] Inactive
	[USB] Camera directly controlled by DS-L3 connected to the USB connector (default setting)
	[NOT-CONNECTED/FRONT/LEFT/AUX] Camera connected to DSC1 at the position set in “(2) Configuring the Device Connection Information”
	[NOT-CONNECTED/FRONT/LEFT/AUX] Camera connected to DSC2 at the position set in “(2) Configuring the Device Connection Information”
CW/CCW Button	[L: FL / R: OBJ.] OBJ CW/CCW button (left button) is for 1st layer fluorescence cube turret, FL CUBE CW/CCW button (right button) is for motorized nosepiece (The motorized nosepiece is not used in the focusing nosepiece system)
	[L: OBJ. / R: FL] OBJ CW/CCW button(left button) is for motorized nosepiece, FL CUBE CW/CCW button (right button) is for 1st layer epi-fluorescence cube turret (default setting) (The motorized nosepiece is not used in the focusing nosepiece system)
	[L: OBJ. / R: OBJ.] OBJ CW/CCW button (left button) and FL CUBE CW/CCW button (right button) are for motorized nosepiece (The motorized nosepiece is not used in the focusing nosepiece system)

■ **Enabling operation button**

→(See DS-L3 Chapter 6 “4.2.5 Enabling/Disabling the Button Operation”.)

Enable/disable the buttons located on the front and lateral right/left on the base of the Ni-E microscope.

The buttons can only be configured as a group (front, lateral left, lateral right) not individually.

<Factory setting>

All “enabled”

Enabling/Disabling Ni-E buttons

(3-2) Operation Buttons on Ergo Controller

(See DS-L3 Chapter 6 “4.3 Configuring the Function of the Ergo Controller Buttons”.)

Change the function of buttons equipped with the ergo controller.

See the Ni-E (Focusing Stage System) instruction manual for details of the ergo controller.

(3-3) Buttons on the Screens on DS-L3

(See DS-L3 Chapter 6 “4.1 Configuring the Screen Buttons for DS-L3”.)

Configure the buttons on DS-L3 screens.

■ **Function Setting on the [MICROSCOPE CONTROL」/[CAM-MIC CONTROL] Screen**

(See DS-L3 Chapter 6 “4.1.1 Selecting the Buttons to be Displayed”.)

Up to 15 buttons can be aligned on the [MICROSCOPE CONTROL] screen and up to 12 on the [CAM-MIC CONTROL] screen.

<Factory setting>

For information on [MICROSCOPE CONTROL] screen, see DS-L3 Chapter 2 “3.1 MICROSCOPE CONTROL Screen (Ni-E)”.

For information on [CAM-MIC CONTROL] screen, see DS-L3 Chapter 2 “3.2 CAM-MIC CONTROL Screen (Ni-E)”.

DS-L3 [MICROSCOPE CONTROL] screen control button configuration

(Example of specifying Epi-fluorescence cube turret → Filter cube)

■ **SLEEP button**

(See DS-L3 Chapter 6 “4.1.2 Showing/Hiding the [SLEEP] Button”.)

Set to show or hide the [SLEEP] button on the [MICROSCOPE CONTROL] screen to turn ON/OFF the sleep mode on the DS-L3. The sleep state is the state where the power supply to motorized devices is stopped to minimize the generation of noise.

<Factory setting>

Set to “Hide”.

Showing/Hiding the Sleep button

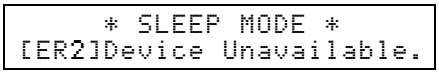
Driving the following motorized units is not available in Sleep mode:

- Motorized nosepiece (not used in the focusing nosepiece system)
- Motorized universal condenser (not used in the focusing nosepiece system)
- Motorized epi-fluorescence cube turret 1, motorized epi-fluorescence cube turret 2
- Motorized barrier filter wheel
- Motorized excitation filter wheel
- Motorized quadrocular tilting tube
- Motorized shutter (DIA and EPI), motorized epi-fluorescence cube turrets 1 and 2 and built-in shutters
- Motorized universal condenser built-in motorized aperture diaphragm (not used in the focusing nosepiece system)
- Motorized DSC zooming port
- Motorized ND filter wheel

The following operation is not available in Sleep mode:

- Loading MODE
- Grouped operation of EPI side shutters

The message “Sleep mode is active. Drive/operation not available” as shown in the figure on the right side is displayed on the Ni-E front display panel and you hear a buzz (beep) from the microscope if the motorized units listed above were not driven even if they were commanded so or manipulated in Sleep mode. The warning disappears automatically approximately after 3 seconds. Reset the sleep mode before you continue the operation.



While in sleep mode, drive/operation of motorized units not available

> **Driving units after switching to SLEEP OFF**
>
> After the mode switches from Sleep ON to OFF, an initial unit drive may be enabled (initialization) to detect an origin.
>
> In that case, drive it to your desired position again.

(4) Configuring the Movement (See DS-L3 Chapter 6 “5 Configuring the Movement of Motorized Devices”.)

Set the movement of the Ni-E microscope and motorized accessories.

(4-1) Configuring Interlocking

(See DS-L3 Chapter 6 “5.1 Configuring Interlocked Operation”.)

See Chapter 3 “18.2 Interlocking Function” in this document for the details of interlocking.

■ **Capture interlock**

(See DS-L3 Chapter 6 “5.1.7 Configuring the Interlocked Operation with Capture Command Sending or Trigger Signal Output”.)

Specify to interlock the optical path of a motorized quadrocular tilting tube and a filter cube with an image capture using the [CAPTURE] button for the microscope main body.

Specify an optical path or filter cube to set the interlocking.

[SETUP MENU] → [MOVEMENT]

Specifying target to interlock with image capture

Interlock setting (Example: epi-fluorescence cube turret)

(4-2) Escape distance

(See DS-L3 Chapter 6 “5.2 Setting the Retracting Amount of the Elevating Section”.)

Set the distance the objective rises from the current position when the microscope’s [ESCAPE] button is pressed.

<Factory setting>

“SOFTWARE LIMIT”

For software limit, see “(6) Configuring Other Functions - (6-4) Software Limit” for details.

[ESCAPE DISTANCE] setting

Escape amount selection

(5) Setting Mode Function (See DS-L3 Chapter 5 “2 Using the MODE Function”.)

In order to use the MODE function, “register” the motorized devices for the modes and “save” the status of the registered motorized device. “Load” the saved mode on the [MICROSCOPE CONTROL] screen.

See Chapter 3 “18.1 Mode Function” in this document for the details on the MODE function.

(5-1) Registering Mode (Add/Modify/Delete)

(See DS-L3 Chapter 5 “2.1 Registering/Changing Target Motorized Devices
(See DS-L3 Chapter 5 “2.4 Deleting a Mode”.)

<Factory setting>

Mode name [MD1] to [MD8]: Not registered

Specify a mode number to open a sub-menu. [Add] button is displayed for an initial registration and [MODIFY] and [DELETE] buttons for a registration update, so follow the instruction on the screen to enter a mode name (up to four alphanumeric characters) and specify a target motorized unit.

When you “delete” the mode, its mode name will change to the factory default name (MD1 to MD4).

[SETUP MENU] → [MODE]

Specifying mode NO. → Adding/modifying/deleting mode

(5-2) Saving a mode

(See DS-L3 Chapter 5 “2.2 Saving/updating a Mode (State of Motorized Devices)”.)

After the mode has been registered, the mode name registered for the [MODE (No.)] button is displayed and the [SAVE MODE] button is displayed on the right of the button. Let the microscope enter your desired microscopy state here and press the [SAVE MODE] button, then the current position and state (mode) of the registered motorized unit will be saved.

You can also save the mode on the [MICROSCOPE CONTROL] screen. See the following figure (5-3). Press the [SAVE MODE] button to open the submenu. Set the desired microscopy state using the microscope and press the corresponding mode number button to save the current microscopy state for the registered motorized device.

**Registered mode
(Example: MODE1 and MODE2 registered)**

(5-3) Loading a mode

(See DS-L3 Chapter 5 “2.3 Loading a Mode”.)

The saved mode can be recalled on the [MICROSCOPE CONTROL] screen.

Press the [LOAD MODE] button to open the submenu. Specify the number of the mode to be loaded.

[MICROSCOPE CONTROL] screen

[LOAD MODE] button [SAVE MODE] button

**Saving a mode: [SAVE MODE] button
→ Press the [MODE No.] button for the mode to be saved in the submenu.
Loading a mode: [LOAD MODE] button
→ Press the [MODE No.] button for the mode to be loaded in the submenu.**

(6) Configuring Other Functions (See DS-L3 Chapter 6 “6 Configuring Other Functions”.)

(6-1) Ni-E display panel

(See DS-L3 Chapter 6 “6.1 Setting the Display of the Ni-E Front Display Panel”.)

Set a display pattern to be displayed on the display panel of Ni-E microscope at the system startup.

The display pattern depends on the type of the motorized unit connected and is inclusive of 9 patterns. (See Chapter 3 “1 Display Panel Details - (1) Power ON display”.)

<Factory setting>

“PATTERN 1”

[SETUP MENU] → [UTILITY]

Pressing the [DISPLAY PATTERN SET] button brings up a sub-screen prompting you to select a display pattern, therefore, select a pattern by pressing the DISPLAY buttons on the Ni-E main body then press the [OK] button.

Setting display pattern

> **Display switching between “microscope state” and “FUNCTION button’s function”**
>
> Pressing the DISPLAY switch button switches the status display and the function display of the FUNCTION button.

(6-2) Enabling elevating section operation

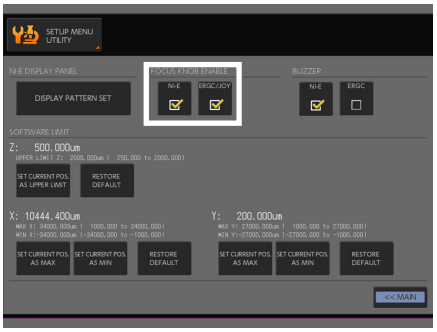
(See DS-L3 Chapter 6 “6.2 Enabling/Disabling the Operation of the Elevating Section”.)

Enable/disable the focus knob function that drives the elevating section of the microscope.

<Factory setting>

“Enabled”

It is set to “Enabled” when the button is checked.



Enabling/Disabling Z Control

(6-3) BUZZER

(See DS-L3 Chapter 6 “6.3 Turning ON/OFF the Buzzer”.)

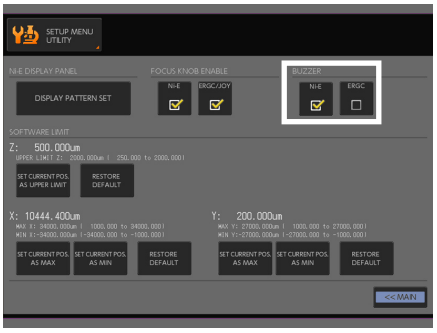
Set the buzzer ON/OFF when pressing a button of the microscope or the ergo controller.

<Factory setting>

Ni-E main body: “ON”

Ergo controller: “OFF”

It is set to “BUZZ” when the button is checked.



Buzzer ON/OFF

(6-4) Software limit

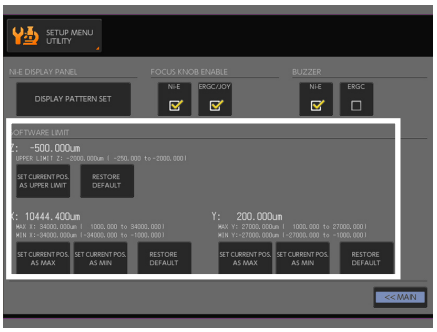
(See DS-L3 Chapter 6 “6.4 Setting the Software Limits”.)

Programmatically set the limit position of the microscope’s elevating section and the motorized XY stage movement.

In order to set the limit in XY direction, the motorized XY stage must be connected.

Setting items	Default setting	Configurable range
Z-axis lower limit	-2000.000 µm	-2000.000 µm to -250.000 µm
X-axis maximum value	34000.000 µm	10000.000 µm to 34000.000 µm
X-axis minimum value	-34000.000 µm	-34000.000 µm to -1000.000 µm
Y-axis maximum value	27000.000 µm	1000.000 µm to 27000.000 µm
Y-axis minimum value	-27000.000 µm	-27000.000 µm to -1000.000 µm

Drive the unit to where you want it to be the limit and press an appropriate button to set the limit position.



Z, X, and Y value settings

Restriction of elevating section

- **Stop position during actual operation**

  When operating the elevating section with the focus knob, the actual stop position is up to 20 µm beyond the set upper/lower limit.

- **Set upper/lower limit position**

  For focusing stage system, the upper limit is set 0.5µm above the current position and for focusing nosepiece system, the lower limit is set 0.5 µm below the current position.

- **If the elevating section is currently at the limit position, it cannot be returned to default (factory setting).**

  In this case, move the elevating section below the limit position (above the limit position for focusing nosepiece system) and then press the [RESTORE DEFAULT] button once more.

XY movement restriction

- **Stop position during actual operation**

  When controlling the stage in the XY direction with the ergo controller or joystick controller, the actual stop position of the stage is up to 800 µm beyond the set limit.

- **Maximum/minimum value to be set**

  When setting the maximum or minimum value, a value 0.5 µm greater or less than the current position is set (the front, back, left, and right limits are also the current value added or deducted by 0.5 µm).

- **If the stage is currently at the limit position in the XY direction, it cannot be returned to default (factory setting).**

  In this case, move the stage within the limit position and then press the [RESTORE DEFAULT] button once more.

(7) Maintenance

(7-1) FL turret drive speed

(See DS-L3 Chapter 6 “6.5 Setting the Driving Speed of the Epi-Fluorescence Cube Turret”.)

Set the drive speed of the epi-fluorescence cube turret to High or Low.

When a thick dichroic mirror is used for a filter cube, set the drive speed to “Low”.

If you are using two layered epi-fluorescence cube turrets, configure 1st and 2nd layers separately.

In order to perform this configuration, the motorized epi-fluorescence cube turret must be attached.

<Factory setting>

High for both the 1st and 2nd layers

(7-2) Resetting data

(See DS-L3 Chapter 6 “6.6 Restoring the Factory Default Settings”.)

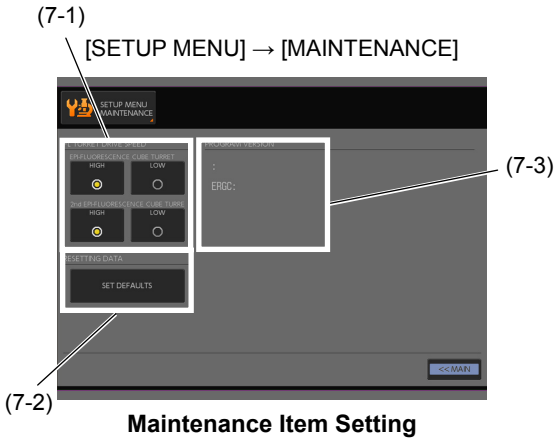
Restore all settings made on the [MICROSCOPE SETUP MENU] to the default settings.

(7-3) Program version

You can check the program version of the microscope system.

When you are using an ergo controller, the program version of the ergo controller is displayed after [ERGC:].

When you are using the joystick controller, the program version of the joystick controller is displayed after [JOY:] .



Maintenance Item Setting

Restoring the factory default settings for microscope data (from the microscope)

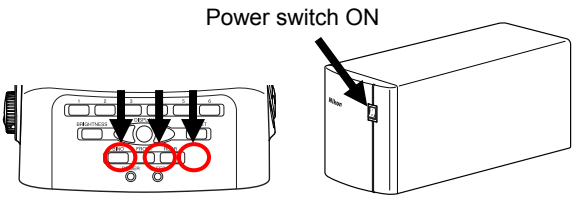
You can restore the setting data to the default setting with the button operation of the Ni-E microscope..

Use this method to restore objective device information to factory default.

<Procedure>

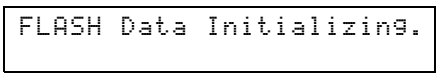
Turn on the power while holding down the following three buttons on the front of the main body.

- DISPLAY Previous button
- DISPLAY Next button
- Z-RESET button



Turning on the power switch while holding down three buttons

When successful, the display shown on the right side appears and data is restored to factory default.



Restoration to factory default settings completed

20.2 Microscope Control

The microscope can be controlled from DS-L3.

See “Chapter 5 Individual Operations” in “DS-L3 Microscope Operation” Instruction Manual for the details of usage.

(1) What in Ni-E can be controlled by DS-L3

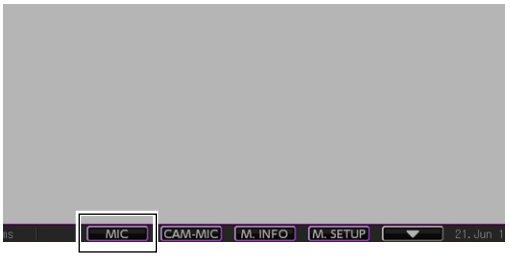
Device	Operation available on DS-L3	Required setting (Reference in DS-L3 Instruction Manual)
ECLIPSE Ni-E main body	ON/OFF and lamp brightness control of dia-illumination Adjustment of DIA field diaphragm Retraction and restoration[*2] of elevating section[*1]	None
	Parfocal correction	Focus position (See Chapter 6, “5.1.4 Configuring the Parfocal Correction Function (Auto Link Focus)”.)
	Output of capture trigger signal from DSC connector	Information on camera connected to DSC connector (See Chapter 6, “3.1 Configuring the Connection of Digital Camera”.)
NI-TT-E motorized quadrocular tilting tube	Optical path switching	None
NI-RPZ-E motorized DSC zooming port for quadrocular tube	Adjustment of zoom magnification	None
NI-N7-E motorized septuple nosepiece	Switching of objective	Information on attached objectives (See chapter 6, “2.1 Configuring the Objective Information”.)
NI-ND6-E motorized sextuple DIC nosepiece		
NI-N7-I intelligent septuple nosepiece	Address detection for nosepiece in optical path	
NI-ND6-I intelligent sextuple DIC nosepiece		
NI-FLT6-E motorized epi-fluorescence cube turret[*3]	Switching of filter cube, opening/closing of internal shutter	Information about the attached filter cube (See Chapter 6, “2.3 Configuring the Filter Cube Information“.)
NI-FLT6-I intelligent epi-fluorescence cube turret[*3]	Address detection for turret in optical path	
NI-EXW-E motorized excitation filter wheel	Switching of excitation filter	Information of attached filters (See Chapter 6, Section 2.2 “Configuring the Excitation/Barrier Filter Information”.)
NI-BAW-E motorized barrier filter wheel	Switching of barrier filter	
C-HGFIE motorized HG precentered fiber illuminator	Switching built-in ND filters, opening/closing the built-in shutter	None
NI-ND-E motorized ND filter wheel	Adjustment of ND filter transmittance	None
NI-SH-E motorized shutter[*4]	Opening/closing of EPI/DIA/AUX motorized shutter	None[*5]
NI-CUD-E motorized universal condenser dry	Switching of module Adjustment of DIA aperture diaphragm	Information of attached condenser modules (See Chapter 6, “2.2 Configuring the Condenser Module Information”.)
NI-S-E motorized XY stage	Movement to specimen replacement position[*2]	None

*1: The microscope’s elevating section lowers.
*2: DS-L3 cannot be used to move the elevating section or the motorized XY stage to an arbitrary position.
*3: Motorized/intelligent epi-fluorescence cube turrets may be used in a single layer, or in two overlapping layers.
*4: Two motorized shutters can be operated.
*5: For details on changing the motorized shutter connection to AUX (other than EPI/DIA), see DS-L3 Chapter 6 “3.2 Configuring the Connection of Motorized Shutter”.

(2) Buttons on the [MICROSCOPE CONTROL] screen

In order to control the microscope from DS-L3, press the [MIC] or [CAM-MIC] button on the [MICROSCOPE CONTROL] top screen to display the [MICROSCOPE CONTROL] or [CAM-MIC CONTROL] screen. The same operation is available from either screen, but the [MICROSCOPE CONTROL] screen is used as an example to summarize the operation in this document.

The [MICROSCOPE CONTROL] screen displays the buttons as shown below. The button alignment is set to the default setting. When you want to use a function that is not shown, see “20.1 Setting Up the Microscope - (3) Configuring the Button Functions - (3-3) Buttons on the Screens on DS-L3” to display your desired button.



MICROSCOPE CONTROL top screen

X: X coordinate of the motorized XY stage
Y: Y coordinate of the motorized XY stage
Z: Z coordinate of the motorized XY stage

Moves to the [SETUP MENU] - [MOVEMENT] screen.

Closes the [MICROSCOPE CONTROL] screen.

Operation buttons (optical path, etc.)
Press a button to operate the corresponding motorized device.
The selected button is displayed with a blue frame.

A button for opening an operation sub screen (FL turret, etc.)
Press the button to open a sub screen at the bottom of the screen.

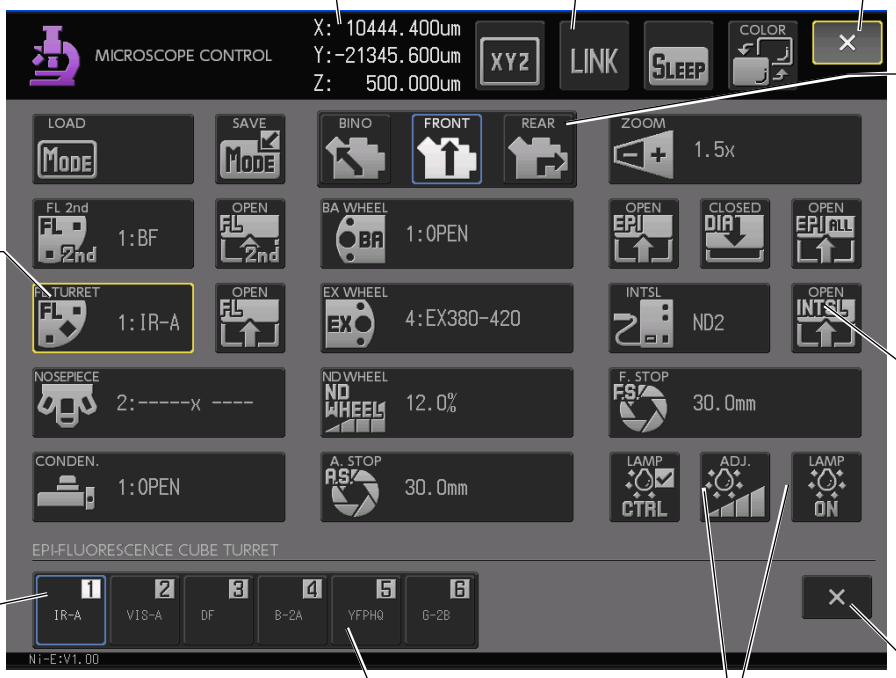
ON/OFF or open/close button (lamp, motorized shutter, etc.)
Each time the button is pressed, the status switches and the corresponding motorized device operates.

Sub screen

Closes the sub screen.

Operation button
Press a button to operate the corresponding motorized device. The selected button is displayed with a blue frame.

These buttons are effective when DS-L3 has lamp control.

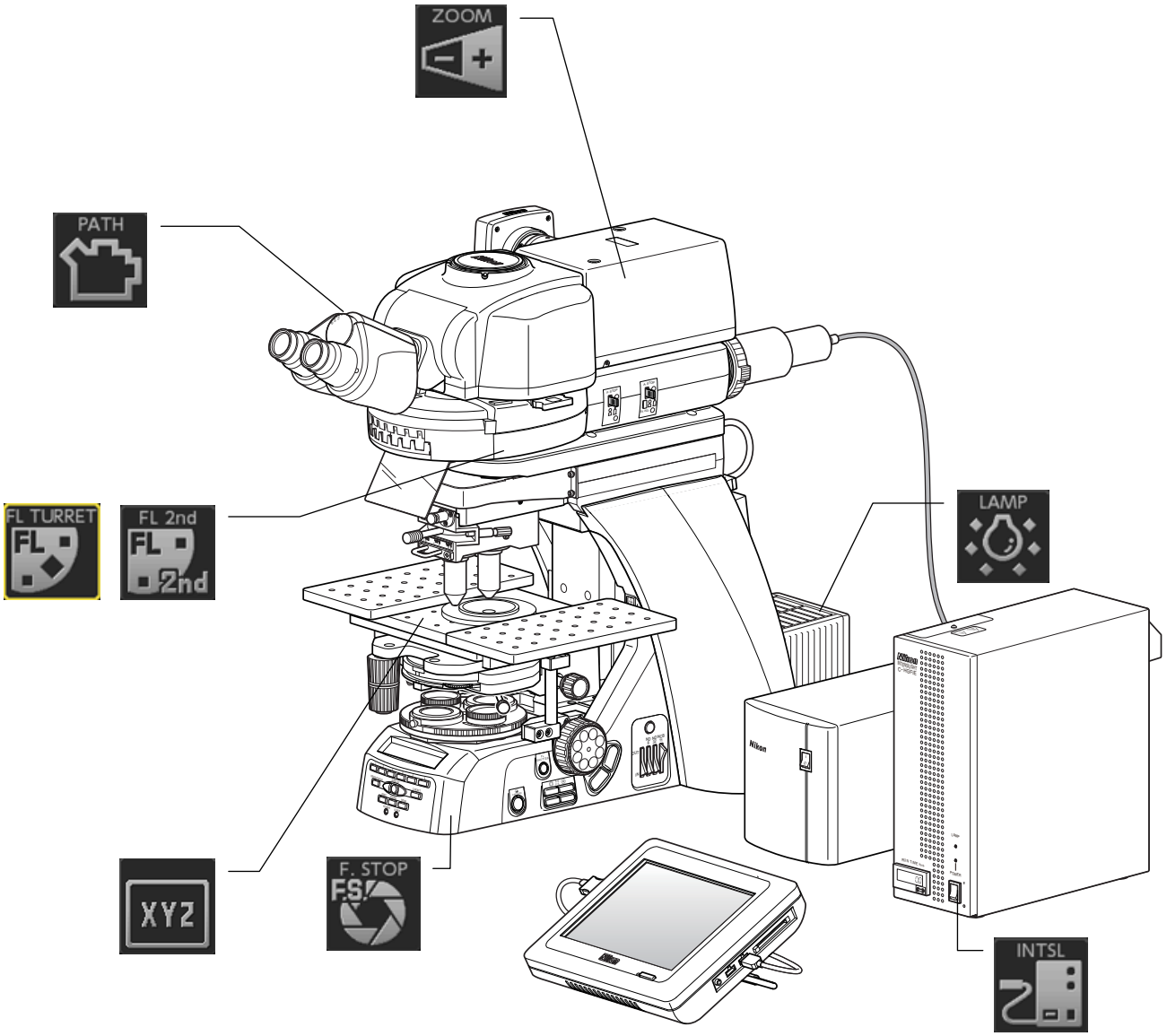


Example of a sub screen with a slider (Lamp adjustment)

Slider
Available operations:
- Dragging the yellow marker being pressed
- Press the [◄] or [►] button.
- Pressing the area to the left or right of the yellow marker (for greater movement than when using the [◄] or [►] button)

(3) Controlling Ni-E with buttons

Pressing a button allows you to control the following parts of Ni-E or motorized accessories:
See the list for the function and descriptions on the buttons.



In the above figure, the motorized devices to be controlled by the following buttons are not attached.
Connect the target motorized device to Ni-E when controlling these devices.

Reference for Buttons with their Functions and Operation Procedures

Operation Button		Function	Operation Reference in DS-L3 Instruction Manual
 [NOSEPIECE]	 [Objective (Address)]	Switch the objective.	Chapter 5, “1.1 Switching the Objective (Motorized Nosepiece)”
 [PATH]	 [BINO]	Switch the optical path of the motorized quadrocular tilting tube to the binocular section.	Chapter 5 “1.2 Switching the Optical Path (Motorized Quadrocular Tilting Tube)”
	 [FRONT]	Switch the optical path of the motorized quadrocular tilting tube to the tube adapter.	
	 [REAR]	Switch the optical path of the motorized quadrocular tilting tube to the rear port.	
	 [ZOOM]	Adjust the zoom magnification of the DSC zooming port.	Chapter 5 “1.3 Adjusting the Zoom Magnification (Motorized DSC Zooming Port)”
 [FL TURRET]	 [Filter Cube (Address)]	Switch the filter cube of the motorized epi-fluorescence cube turret (1st layer).	Chapter 5, “1.4 Switching the Filter Cube (Motorized Epi-fluorescence Cube Turret)”
	 [SHUTTER FL]	Open/close the motorized epi-fluorescence cube turret’s built-in shutter (1st layer).	Chapter 5, “1.5 Opening/Closing the Motorized Epi-fluorescence Cube Turret’s Built-in Shutter”
 [FL 2nd]	 [Filter Cube 2nd (Address)]	Switch the filter cube of the motorized epi-fluorescence cube turret (2nd layer).	Chapter 5, “1.4 Switching the Filter Cube (Motorized Epi-fluorescence Cube Turret)”
	 [SHUTTER FL 2nd]	Open/close the motorized epi-fluorescence cube turret’s built-in shutter (2nd layer).	Chapter 5, “1.5 Opening/Closing the Motorized Epi-fluorescence Cube Turret’s Built-in Shutter”
 [EX WHEEL]	 [Excitation Filter (Address)]	Switch the excitation filter.	Chapter 5 “1.7 Switching the Excitation Filter (Motorized Excitation Filter Wheel)”
 [BA WHEEL]	 [Barrier Filter (Address)]	Switch the barrier filter.	Chapter 5 “1.8 Switching the Barrier Filter (Motorized Barrier Filter Wheel)”
 [CONDEN.]	 [Condenser Module (Address)]	Switch the condenser module.	Chapter 5 “1.9 Switching the Condenser Module (Motorized Universal Condenser)”

Operation Button		Function	Operation Reference in DS-L3 Instruction Manual
 [A. STOP]		Adjust the aperture diaphragm diameter of a motorized universal condenser.	Chapter 5 “1.10 Adjusting the DIA Aperture Diaphragm (Motorized Universal Condenser)”
 [INTSL]	 [Intensilight (ND number)]	Switch the ND of the HG precentered fiber illuminator.	Chapter 5 “1.12 Operating the Motorized HG Precentered Fiber Illuminator (Intensilight)”
	 [SHUTTER INTSL]	Open/close the built-in shutter in the HG precentered fiber illuminator.	
 [ND WHEEL]		Adjust the ND filter transmittance of the motorized ND filter wheel.	Chapter 5, “1.13 Adjusting the ND Filter for Dia-illumination (Motorized ND Filter Wheel)”
 [LAMP]	 [LAMP CTRL]	Transfer the control of the dia-illumination lamp between the microscope and DS-L3.	Chapter 5, “1.14.1 Transferring the Control of the Dia-illumination Lamp/LED”
	 [ADJ.]	Adjust the brightness of the dia-illumination lamp.	Chapter 5, “1.14.3 Adjusting the Brightness of the Dia-illumination Lamp/LED”
	 [PHOTO]	Adjust the dia-illumination lamp to the brightness that offers optimal color reproduction.	
	 [LAMP ON/OFF]	Turn the dia-illumination lamp ON/OFF.	Chapter 5, “1.14.2 Turning the Dia-illumination Lamp/LED ON/OFF”
 [F. STOP]		Adjust the diameter of the DIA field diaphragm.	Chapter 5, “1.15 Adjusting the DIA Field Diaphragm”
 [SHUTTER]	 [SHUTTER EPI ALL]	Open/close all HG precentered fiber illuminator’s built-in shutter, EPI motorized shutter, epi-fluorescence cube turret’s built-in shutter.	Chapter 5, “1.6 Opening/Closing All Shutters for Epi-illumination”
	 [SHUTTER FL]	Open/close the motorized epi-fluorescence cube turret’s built-in shutter (1st layer). (Equivalent to the [SHUTTER FL] button on the sub screen of the [FL TURRET] button.)	Chapter 5, “1.5 Opening/Closing the Motorized Epi-fluorescence Cube Turret’s Built-in Shutter”
	 [SHUTTER FL 2nd]	Open/close the motorized epi-fluorescence cube turret’s built-in shutter (2nd layer). (Equivalent to the [SHUTTER FL 2nd] button on the [FL 2nd] sub screen.)	

Operation Button		Function	Operation Reference in DS-L3 Instruction Manual
[SHUTTER]	[SHUTTER INTSL]	Open/close the built-in shutter in the HG precentered fiber illuminator. (Equivalent to the [SHUTTER INTSL] button on the [INTSL] sub screen.)	Chapter 5 “1.12.1 Opening/Closing the Motorized HG Precentered Fiber Illuminator’s Built-in Shutter”
	 [SHUTTER EPI]	Open/close the EPI motorized shutter.	Chapter 5, “1.16 Opening/Closing the EPI/DIA/AUX Motorized Shutter”
	 [SHUTTER DIA]	Open/close the DIA motorized shutter.	
	 [SHUTTER AUX]	Open/close the AUX motorized shutter.	
 [CAPTURE]	 [CAPTURE FRONT]	Output the capture trigger signal to the digital camera connected to the tube adapter.	Chapter 5, “1.17 Outputting Capture Trigger Signals from the Microscope”
	 [CAPTURE LEFT]	Output the capture trigger signal to the digital camera connected to the DSC zooming port.	
	 [CAPTURE AUX]	Output a capture trigger signal to a digital camera connected to a position other than the above.	
 [SAVE]		Save the current microscopy state as a MODE.	Chapter 5, “2.2 Saving/Updating a Mode (State of Motorized Devices)”
 [LOAD]	 [LOAD (MODE number)]	Load a saved MODE.	Chapter 5, “2.3 Loading a MODE”
 [XYZ]	 [Z-axis RESET]	Reset the Z-axis coordinate displayed on DS-L3 to zero (0).	Chapter 5, “1.18.1 Zero-resetting the Z-axis Coordinate”
	 [ESCAPE]	Move the microscope’s elevating section to the retracting position. Press the button again to restore it to the original position.	Chapter 5, “1.18.2 Retracting the Elevating Section”
	 [Specimen Removal Position]	Move the microscope’s elevating section to the retracting position, and the motorized XY stage to the sample removal position (front). When you press this button again, only the motorized XY stage returns to its original position.	Chapter 5, “1.18.3 Retracting the Elevating Section and Moving the Stage to the Specimen Removal Position”

Operation Button	Function	Operation Reference in DS-L3 Instruction Manual
 [SLEEP]	Enter the sleep state to reduce noise.	Chapter 5, “3 Entering the Sleep State (Noise Reduction)”
 [COLOR]	Change the background color for [MICROSCOPE CONTROL].	Chapter 5 “4 Changing the Background Color of the [MICROSCOPE CONTROL] Screen”