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Nikon

Inverted Research Microscope

ECLIPSE

Ti2-U

Instruction Manual

Introduction

Thank you for purchasing a Nikon product.

This instruction manual is intended for users of the Nikon Inverted Research Microscope ECLIPSE Ti2-U.

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 might differ from the actual product in its appearance.
- Although every effort has been made to ensure the accuracy of this manual, errors or inconsistencies might remain. If you notice any points that are unclear or incorrect, please contact your local 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 the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment might be impaired.
- Products and company names mentioned in this manual are trademarks or registered trademarks of their respective holders.

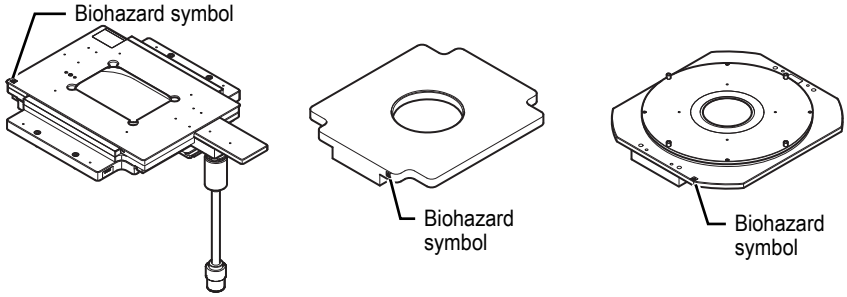
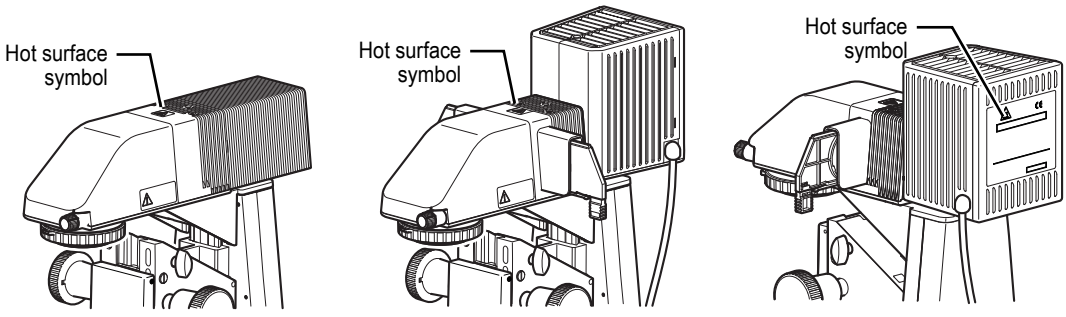
Contents

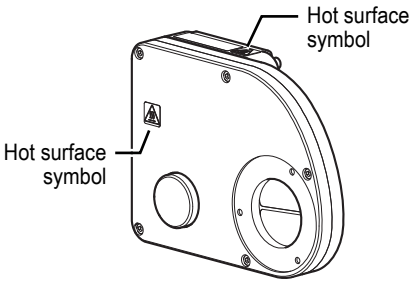
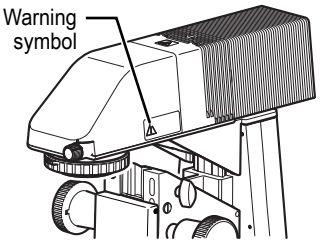
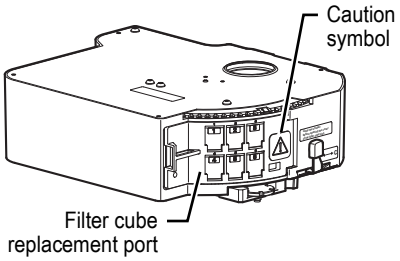
Safety Precautions

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, carefully read this manual and the instruction manuals of products used together before using the product. Be sure to read and observe the safety precautions described at the beginning of each manual. Do not discard this manual and keep it handy for easy reference.

Meaning of Symbols Used on the Product

The symbol appearing on the product indicates the need for caution at all times during use. Always refer to the instruction manual and read the relevant instructions before manipulating any part to which the symbol has been affixed.

Symbol	Description
	**Biohazard** This symbol label is attached on the top or front surface of the stage to remind the user of the following precautions: • Spillage of a specimen from a vessel onto the microscope, presents a biohazard risk. • To avoid biohazard contamination, do not touch the contaminated portion with your bare hands. • Decontaminate the contaminated portion according to the standard procedure of your laboratory.  Manual stage Plain stage Gliding stage
	**Caution for heat** This symbol label is attached on the top surface of the optical path cover on the top of the illumination pillar, on the top surface of the TI2-D-SF filter slider for dia illumination, and on the back surface of the D-LH/LC precentered lamphouse. When the D-LH/LC is used as the dia-illumination source, this symbol reminds the user of the following precautions: • The lamp and lamphouse, the top surface of the optical path cover, and the top surface of the filter slider for dia-illumination become hot while the lamp is lit or immediately after it goes out. • To avoid the risk of burns, do not touch the lamp or lamphouse, the top surface of the optical path cover, or the top surface of the filter slider for dia-illumination while the lamp is lit or immediately after it goes out. • Make sure the lamp, the lamphouse, and the air vents of the HG precentered fiber illuminator have cooled sufficiently before attempting to replace the lamp.  The top surface of the optical path cover The top surface of the TI2-D-SF filter slider for dia illumination Rear surface of the D-LH/LC precentered lamphouse When a TI2-D-LHLED LED lamphouse for dia-illumination is used When the TI2-D-SF filter slider for dia illumination and the D-LH/LC precentered lamphouse are used

Symbol	Description
	**Caution for heat** This symbol label is attached on the front and top surfaces of the motorized shutter unit to remind the user of the following precautions: • The motorized shutter may become hot if closed during a period of illumination. • Burn risk. Do not touch the motorized shutter during and immediately after a period of illumination.  Motorized shutter
	**Warning (photobiological safety)** This symbol label is attached on the side surface of the pillar for dia-illumination to remind the user of the following precautions: • Classification of dia-illumination and fluorescent illumination according to the photobiological risk groups • Do not attempt to view dia-illumination or fluorescent illumination. For details, see “10. Photobiological safety” and “11. Do not attempt to view the illumination section.” under WARNING.  Pillar for dia-illumination (top side surface)
	**Caution (filter cube replacement port)** This symbol label is attached near the filter cube replacement port of the FL turret to remind the user of the following precautions: • Be sure to close the cover of the filter cube replacement port because light including ultraviolet light might leak from the port. • For details, see “12. Cautions on replacing the filter cube” under WARNING.  FL turret

WARNING and CAUTION Symbols Used in This Manual

Safety instructions in this manual are marked with the following symbols to highlight 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.

In addition, the following symbols are used in this manual:

Symbol	Meaning
	This symbol indicates instructions required to avoid malfunction and failure of the product and damage to the equipment or parts.
	This symbol indicates necessary information and tips on the use of this product.

⚠ WARNING

1. Do not disassemble.

Disassembly may cause malfunction and/or electrical shock, and will lead to the forfeiture of all claims against warranty. Do not disassemble any part other than those described in this manual. If you experience any problem with the microscope, contact your local Nikon representative.

2. Confirm the input voltage.

This product is connected to an AC power supply via an AC adapter. The input rating of the AC adapter is 100-240 VAC and 50/60 Hz. Because they are compatible with AC wall outlets worldwide, in normal circumstances, power voltage is not a concern. However, note that you should avoid using this product with an unstable voltage supply.

3. Use the specified AC adapter.

This equipment uses an AC adapter as the power supply. Be sure to use this equipment in combination with the specified AC adapter. Using any other AC adapter might cause problems such as malfunctions, abnormal heat generation, or fire, resulting in a very dangerous situation.

- For details on the specified AC adapter, see “Chapter 8 Specifications.”
- Place this equipment and the AC adapter in a well-ventilated location to prevent malfunctions or fires.
  If this equipment or the AC adapter is covered, or an object is placed on them, heat dissipation will be inhibited, resulting in abnormal heat generation.
- To prevent failures or malfunctions, always turn off the power switch (set it to “o”) of the microscope main body before connecting the AC adapter.

4. Use the specified power cord.

Use only the specified power cord when connecting the AC adapter to the AC power plug. Use of any other power cords could cause malfunction or fire. This product is classified into class 1 in terms of protection against electric shock, and must therefore be connected to a protective grounding terminal.

- For details on the specified power cord, see “Chapter 8 Specifications.”
- To protect from electric shock, always turn off the power switch (set it to “o”) before connecting power cords.

5. Use the specified lamps.

Be sure to use only the specified lamp for the light source. Use of other lamps may cause malfunction or fire.

- The LED of the LED light source cannot be replaced.
- For the specified lamps of the D-LH/LC precentered lamphouse, see “Chapter 8 Specifications.”

6. Cautions on heat from the light source

The halogen lamp and its periphery (including the lamphouse) become extremely hot during and immediately after a period of illumination. Follow the cautions below to prevent burns and fire.

- Do not touch the lamp and the periphery of the lamp while the lamp is on and for approximately 30 minutes after the lamp is turned off. Because they are extremely hot, it may cause burns. When replacing a lamp, wait until the lamp and the periphery of the lamp become sufficiently cool to touch (for approximately 30 minutes).
- To avoid the risk of fire, do not place fabric, paper, or highly flammable materials (i.e. gasoline, petroleum benzine, paint thinner, and alcohol) near the lamphouse while the lamp is on and for about 30 minutes after the lamp is turned off.
- Do not block the air vents on the lamphouse. If any obstacle covers the lamphouse or something is placed on the lamphouse, the heat dissipation is inhibited and the lamphouse becomes abnormally hot.
- The bottom of the TI-PS100W/A power supply becomes hot during use. Do not touch the bottom of the power supply during use, as you may burn yourself. Do not block the air vents on the side of the power supply.

WARNING

7. Fluorescence LED illumination system (for using the epi-fluorescence attachment)

The light source used for epi-fluorescence microscopy requires special care during handling because of its characteristics.

Be sure to use the specified D-LEDI fluorescence LED illumination system. Carefully read the manual supplied with the illumination system before operating the system. Use of other light sources may result in unexpected accidents.

- Ultraviolet light
  When lit, the D-LEDI fluorescence LED illumination system radiates ultraviolet light that can damage the eyes and skin. Do not view light coming from the light source. Direct viewing of the light might result in blindness. When changing filter cubes, always turn off the LED illumination.
- Heat
  When the fluorescence LED illumination system is lit, the LED house of the LED illumination system becomes hot. Do not touch the LED house with bare hands or place flammable materials near the LED house. Doing so might result in burns or fire.

8. Notes on handling flammable solvents

The following flammable solvents are used with this product:

- Immersion oil (Nikon immersion oil for oil immersion lenses)
- Absolute alcohol (ethyl alcohol or methyl alcohol for cleaning optical components)
- Petroleum benzine (for removing immersion oil)
- Medical alcohol (for disinfecting the microscope)

Keep these solvents away from fire. Before using a solvent, carefully read the instructions provided by the manufacturer of the solvent, and handle it correctly and safely. Note the following precautions when using solvents with this product.

- Keep solvents away from any parts that might become hot.
- Keep solvents away from this product and the periphery of the product when turning the power switch on or off, or plugging in or unplugging the power cord.
- Be careful not to spill solvents.

9. Handling of dangerous specimens

This microscope is designed primarily for microscopic observation of living cells and tissue cultures in petri dishes and other vessels.

Before handling a specimen, check to see if it is hazardous. If the specimen is hazardous, follow the standard procedure of your laboratory. When handling specimens that may involve a risk of infection, wear rubber gloves and do not touch the specimen directly. Be careful not to spill the specimen. If a specimen is spilled over this product, or otherwise comes into contact with it, clean the affected area thoroughly following the standard procedure of your laboratory.

⚠ WARNING

10. Photobiological safety

This product is designed and manufactured in accordance with the IEC62471 standard “Photobiological Safety of Lamps and Lamp Systems.”

Light emitted from the dia-illumination section of the Ti2-U main body

The photobiological safety of light emitted from the dia-illumination section (aperture of the condenser lens) is classified into the risk group shown in the table below.

The distance (hazard distance) from the dia-illumination section when the risk group classification is the Exempt Group, which does not cause photobiological hazards, is shown below. This table shows the highest risk when a TI2-D-LHLED LED lamphouse for dia illumination or a D-LH/LC precentered lamphouse is used as the light source.

	Risk group classification	Hazard distance
Infrared radiation hazard to eyes	Risk group 1	0.2 m or more
Retinal blue-light hazard	Risk group 1	0.6 m or more

The emitted light contains ultraviolet radiation, infrared radiation, and blue light. These light sources are potentially hazardous and may cause injury if directed at the eyes or skin. Do not stare at or expose your skin to this light.

Light emitted from the epi-illumination section of the Ti2-U main body

The photobiological safety of light emitted from the epi-illumination section (objective) is classified into the risk groups shown in the table below.

The distance (hazard distance) from the epi-illumination section when the risk group classification is the Exempt Group, which does not cause photobiological hazards, is shown below. This table shows the highest risk when a D-LEDI fluorescence LED illumination system is used as a light source.

	Risk group classification	Hazard distance
UV hazard	Risk group 3	0.9 m or more
Retinal blue-light hazard	Risk group 2	4.1 m or more

The emitted light contains ultraviolet radiation, infrared radiation, and blue light. These light sources are potentially hazardous and may cause injury if directed at the eyes or skin. Do not stare at or expose your skin to this light.

Light emitted from the D-LH/LC precentered lamphouse

The photobiological safety of light directly emitted from the aperture of the D-LH/LC precentered lamphouse is classified into the risk groups shown in the table below.

The following table shows the distance (hazard distance) from the aperture when the risk group classification is the Exempt Group, which does not cause photobiological hazards:

	Risk group classification	Hazard distance
UV hazard	Risk group 2	0.8 m or more
Infrared radiation hazard to eyes	Risk group 2	0.9 m or more
Retinal blue-light hazard	Risk group 1 (Not mentioned on the caution label due to its low-risk characteristic)	3.6 m or more

The emitted light contains ultraviolet radiation, infrared radiation, and blue light. These light sources are potentially hazardous and may cause injury if directed at the eyes or skin. Do not stare at or expose your skin to this light.

When D-LH/LC is not attached to the microscope, remove the lamp cable to prevent the emission of light.

⚠ WARNING

11. Do not attempt to view the illumination section.

Dia-illumination and epi-illumination of the Ti2-U main body

The following warning label indicating the highest risk: Risk group 3 in photobiological safety of the Ti2-U main body is affixed on the side surface of the dia-illumination section to remind the user of the following three precautions. (For the locations of this label, see the figure in “Chapter 1 Names of Parts and Their Functions.”)

Warning label on the Ti2-U main body

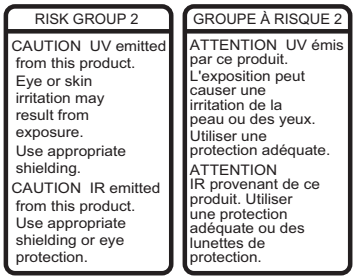
WARNING (Risk Group 3)
UV light is emitted from the objective when epi-illumination is used.
Avoid eye and skin exposure to the UV light. Use the UV light shielding plate.

CAUTION (Risk Group 2)
Possibly hazardous light is emitted from the objective when epi-illumination is used.
Do not stare at emitted light. It may be harmful to the eyes.

NOTICE (Risk Group 1)
IR light is emitted from the aperture of the condenser lens when dia-illumination is used.
Use appropriate shielding or eye protection.

Precentered lamphouse

Caution labels indicating photobiological safety are affixed on the D-LH/LC precentered lamphouse for dia-illumination to remind the user of the precaution below.



Caution label for the D-LH/LC

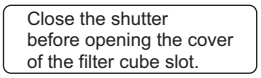
CAUTION (Risk Group 2)
UV light is emitted from the aperture of the lamphouse.
Eye or skin irritation may result from exposure. Use appropriate shielding.

CAUTION (Risk Group 2)
IR light is emitted from the aperture of the lamphouse.
Avoid eye exposure to the IR light. Use appropriate shielding or eye protection.

WARNING

12. Cautions on replacing the filter cube

The following caution label is affixed to the cover of the fluorescence filter cube replacement port of the FL turret. This label reminds you of the following precautions:



Caution label (fluorescence filter cube replacement port cover of the FL turret)

- **Be sure to close the shutter of the FL turret when opening the filter cube replacement port.**
  If the cover is open, illumination including ultraviolet light will leak from the aperture when the cover of the replacement port is opened, possibly disturbing epi-fl microscopy. Leaked light might also be harmful to the eyes.

CAUTION

1. Turn the power off.

To prevent electrical shock or failure, always turn off the power switch (set it to “o”) of the microscope and unplug the power cord before assembling the microscope, connecting/disconnecting cables, replacing parts, or cleaning.

Before plugging in or unplugging the power cord, always turn off the power switch (set it to “o”) of the microscope main body.

2. Do not get the microscope wet or allow foreign matter to get inside it.

Never expose this product to water or chemical solutions. Exposure of the product to liquids may cause a short-circuit, resulting in malfunction or abnormal heating. A short-circuit might also occur if foreign matter gets inside the microscope.

Should you accidentally spill liquid or chemical solutions on the microscope, immediately turn off the power switch of the microscope main body (set it to “o”) and unplug the power cord. (Do not touch the power cord with wet hands.) Then, wipe off any moisture with a dry cloth.

If any liquid or foreign matter gets inside the microscope, stop using the microscope, and contact your local Nikon representative.

There is a higher possibility that liquid is accidentally spilled on the objective and the nosepiece which are positioned immediately below the stage. To prevent liquid from entering the microscope, be sure to attach the caps on the nosepiece holes not in use.

3. Do not place any object on this product.

Do not place anything on the top of this product.

In particular, never place a heavy object on top of the microscope as it might cause deformation, damage, or malfunction of the product, or injury if the object falls.

4. Do not turn on the power when this product is covered.

Do not turn on the power when this product is covered by anything. Doing so might block ventilation of the microscope and cause it to overheat, possibly resulting in fire. Do not cover this product with a cloth or similar material while using it. Doing so will increase the temperature inside the microscope, which might result in failure.

5. Check the state of the microscope when operating the illumination.

When turning on/off the illumination or controlling the light intensity, confirm that the optical path of the entire microscope system is set properly. Because the light source emits very strong light beams, an improper optical path may cause the light to leak into the surrounding area or strong light beams to enter the eyepiece, which could damage the eyes.

6. Cautions on continuous microscopy work

To alleviate fatigue and eye strain, avoid continued use of this product for periods of more than one hour at a time, and take short breaks of 10 to 15 minutes between each work session.

To perform observation in a comfortable position, appropriately arrange the other equipment to be used and adjust the seat height of your chair.

7. Notes on handling the metallic stage ring

The central part of the stage ring is made of a very thin metallic plate. Because of this, the edge of the aperture is very sharp and might cause injury if touched accidentally. Be very careful when handling the stage ring.

CAUTION

8. Notes on cleaning

- Petroleum benzine and absolute alcohol used for cleaning are highly flammable. Handle them with due care, keep them away from fire or sparks, and do not use them when turning the power switch on and off.
- When handling petroleum benzine or absolute alcohol, always follow the instructions provided by the manufacturer.
- Do not use organic solvents (such as alcohol, ether, and thinner) when cleaning the painted, plastic, or printed parts of this product. Using organic solvents might result in discoloration or cause printed text to fade.
- Use petroleum benzine only when wiping immersion oil off the objectives. Do not use petroleum benzine to wipe the incident lens at the bottom of the tube, the prism surface of the tube, or filters.
- When using organic solvents such as absolute alcohol, be sure to wear finger stalls or gloves to avoid direct contact with the solvent.

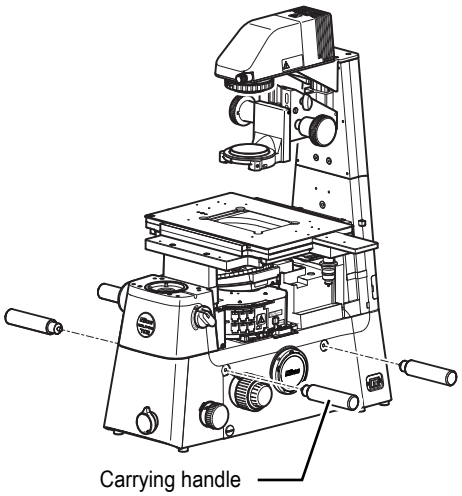
9. Cautions on replacing the lamp

- When replacing the lamp, wait until the lamp and the lamphouse become sufficiently cool (approximately 30 minutes) after the lamp is turned off.
- To prevent electrical shock or failure, always turn off the power switches of the microscope main body and of the power supply (set the POWER switches to “OFF” or to “o”) and unplug the power cord before replacing the lamp.
- After replacement, tightly close the cover of the lamphouse and fix it. Never use the system with the lamphouse cover open.
- Do not break used lamps. Dispose of used lamps according to the regulations or rules of your local government, or ask a licensed industrial waste disposal operator to dispose of it.

10. Cautions on carrying the microscope

When carrying the microscope main body, observe the following precautions to ensure safety.
Failure to observe these precautions might cause the microscope to drop, possibly resulting in damage or serious injury.

- When carrying the microscope a long distance, always use a cart or something similar.
- Be sure to screw the TI-BCH carrying handles all the way in.
  If the carrying handle is not screwed all the way in, it might fail to sustain its rigidity and so could buckle.
- All four TI-BCH carrying handles must be correctly mounted, and two adults must transport the microscope. The two front handles must be held by one person, and the two rear handles must be held by the other person.
  If the TI-BCH carrying handles are not correctly used, the microscope could tip over or fall.



Mounting the carrying handles

- Because the microscope with devices attached to the main body is very heavy, remove as many devices as possible so that the least effort is required to carry the microscope. Using undue effort to lift the microscope could not only hurt your back, but might also cause the microscope to drop, possibly resulting in serious injury. (See below for the product weight.)
  - Ti2-U main body (base): Approximately 20 kg
  - TI2-D-PD pillar for dia illumination: Approximately 4.5 kg
  - TC-S-SR stage: Approximately 3.7 kg
  - TI2-N-ND DIC sextuple nosepiece: : Approximately 0.83 kg

CAUTION

10. Cautions on carrying the microscope (continued)

Before carrying the microscope, always remove the following protruding devices.

- Camera
- TI-BPU back port unit
- Epi-fluorescence attachment (TI2-LA-BM, TI2-LA-BF, TI2-LA-BS)

• Before carrying the microscope, remove detachable cables, and secure moving parts.
- Be sure to clamp the pillar for dia-illumination.
- Do not place any object on the stage.
- Firmly secure or remove the stage.

• While carrying the microscope, do not touch the focus knobs or switches, and do not hold any movable parts such as the tube, the stage, and the pillar for dia illumination. Doing so may cause the microscope to fall or malfunction. Be sure to hold the specified positions when carrying the main body. When carrying only the microscope main body (base) with all the devices removed, firmly hold the microscope by gripping the front bottom and rear recesses of the main body.

11. Cautions on assembly and replacement

Take care not to pinch your fingers or hands in a joint or under a movable part.

Scratches and dirt (such as fingerprints) on optical components such as lenses, filters, and filter cubes will degrade the microscope image. Take care not to scratch these optical components or touch them directly during your work.

12. Cautions on laying cables

The lamphouse becomes very hot while the halogen lamp is lit. Cables must be laid so that they will not contact the lamphouse.

13. Cautions on disposal

To avoid biohazards, dispose of this product as contaminated material, according to the standard procedure of your laboratory.

Handling of This Product

1. Handle with care.

This product is a precision optical instrument. Handle this product carefully to avoid shocks and vibrations. In particular, the precision of the objectives, filter cubes, and LED lamphouse could be affected even by minor shocks.

2. Electromagnetic environment

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

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

This product emits low-level electromagnetic radiation. Do not install this product near precision electronic devices. Otherwise, the performance of such devices might be degraded. If TV or radio reception is affected, move the TV or radio farther away from this product.

This product complies with requirements of EMC directive (2014/30/EU) and IVD directive (98/79/EC).

- Achieve electromagnetic compatibility to ensure satisfactory operation of the product.
  This product is tested and in conformity with FCC regulations (FCC Part15 Subpart B: Radio Frequency Device (commercial and industrial areas)).
- Use this product only in the commercial or industrial area. Using this product in the residential area may affect other devices.

3. Scratches and dirt on optical components

Any scratches or dirt (such as fingerprints) on optical components such as lenses and filters will degrade the microscope image. Take care not to scratch or stain optical components. If they become dirty, clean them according to the procedure in “Chapter 6 Maintenance and Storage of the Device.”

4. Installation location and storage location

This product is a precision optical instrument. Use or storage of this product under inappropriate conditions might result in malfunction or loss of precision. For details on the temperature and humidity at the installation location and storage location, see “8.3 Physical Properties.” The following conditions must also be considered:

- Install and store this product in a splash-, dust-, and vibration-free location.
  Place a cover over this product when storing it, so as to protect it from dust.
- Install and store this product on a level and suitably sturdy desk or table.
  Install this product in a location with minimal exposure to hazards in the event of an earthquake or other potential disaster. If necessary, secure this product to the working desk (or other heavy and stable object) by a strong wire or similar means, so as to prevent this product from tipping over or falling off the desk.
- Install this product in a location where the power cord can be unplugged immediately from the AC inlet of the AC adapter in case of an emergency.
- Avoid placing this product in direct sunlight or immediately under room lights.
  In a bright environment, extraneous light entering the objective will degrade image quality. Extraneous light from a room light immediately above the microscope might also enter the objective. In this case, Nikon recommends turning off the room light immediately above the microscope before use.
- Install this product at least 10 cm away from nearby walls.
- Do not use this product on a desk mat or similar item.
- Do not install this product in a closed space such as a locker or cabinet.

5. Port protection

After mounting the tube, nosepiece, FL turret, and other unit, attach the provided caps to the ports, slider slot, and objective mounting holes not in use.

This prevents extraneous light and dust from entering the product.

6. Nosepiece protection

Always attach the provided caps to the objective mounting hole with no objective mounted and the DIC slider slot with no DIC slider inserted.

Otherwise, extraneous light and dust may enter this product.

Without caps, any liquid accidentally spilled from the specimen may intrude inside the system, which may cause a failure.

7. Objectives

When observing suspended cells in a container, it is necessary to set the objective closer to the container than usual in order to achieve proper focus. If the objective is switched in this state, the tip of the objective might collide with the container.

Likewise, before placing a large container on the stage, make sure that the tip of the objective does not protrude from the stage surface.

8. Oil-immersion microscopy

Use only a minimum quantity of immersion oil. If too much oil is applied, the excess might flow onto the stage and the periphery of the stage, thereby affecting the microscopy work.

After opening an immersion oil container, use oil as early as possible, because oil deteriorates and its refractive index and viscosity change.

When using petroleum benzine or absolute alcohol to wipe off immersion oil or to clean the lenses, follow the instructions provided by the manufacturer of the petroleum benzine or alcohol to be used. Keep these flammable liquids away from fire or sparks.

See “8. Notes on handling flammable solvents” under ⚠WARNING.

9. Fluorescent filter cube

- The excitation filter inside the fluorescence filter cube is subject to very strong light beams, and consequently degrades over time. Replace the filter when its characteristics have changed.
- The characteristics of the filter might change under high humidity. Avoid using and storing fluorescence filter cubes in conditions subject to high temperature, high humidity, or extreme temperature changes, to avoid affecting the characteristics and quality of the filters. When the fluorescence filter cubes are not to be used, Nikon recommends storing them in a desiccator or sealed container with a desiccant.

Chapter 1

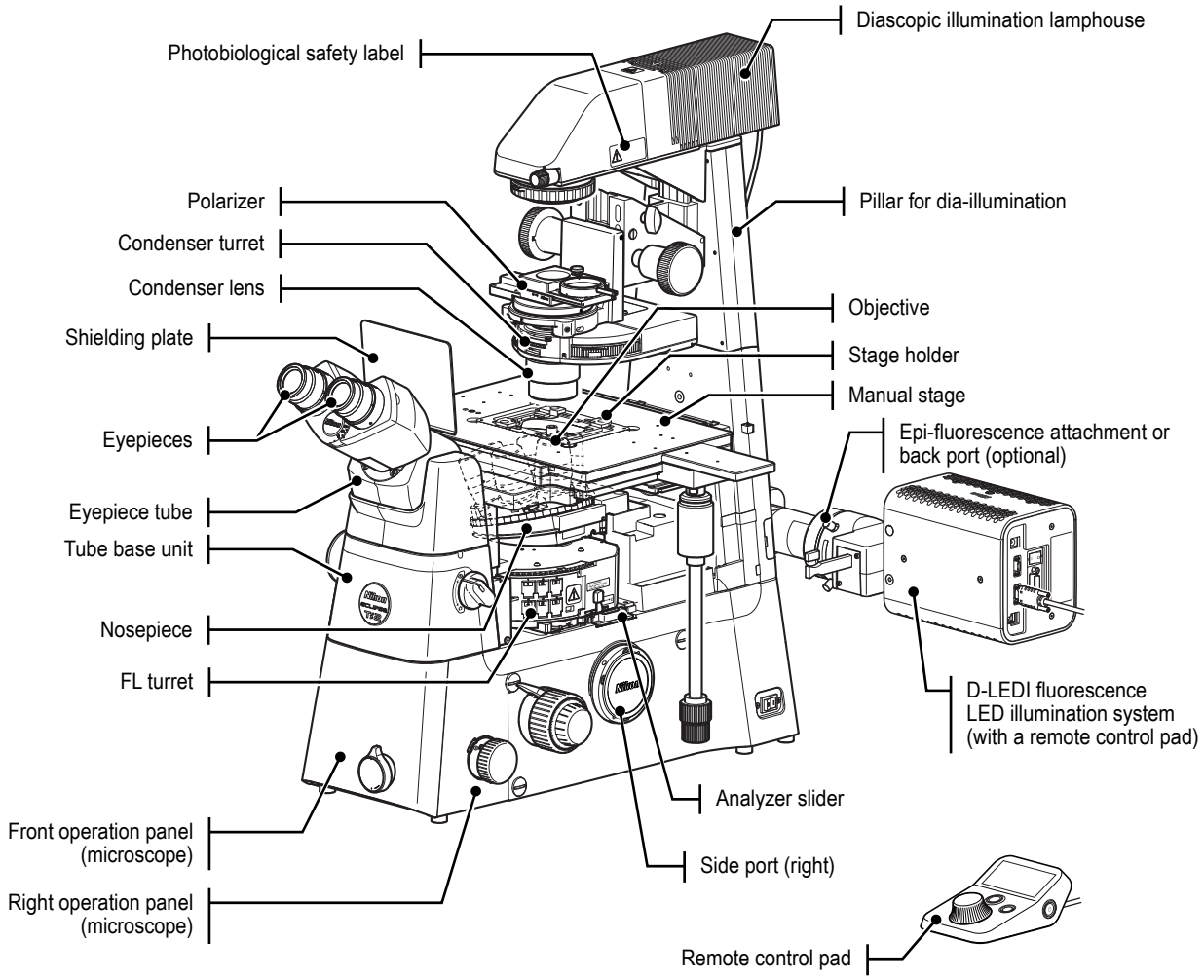
Names of Parts and Their Functions

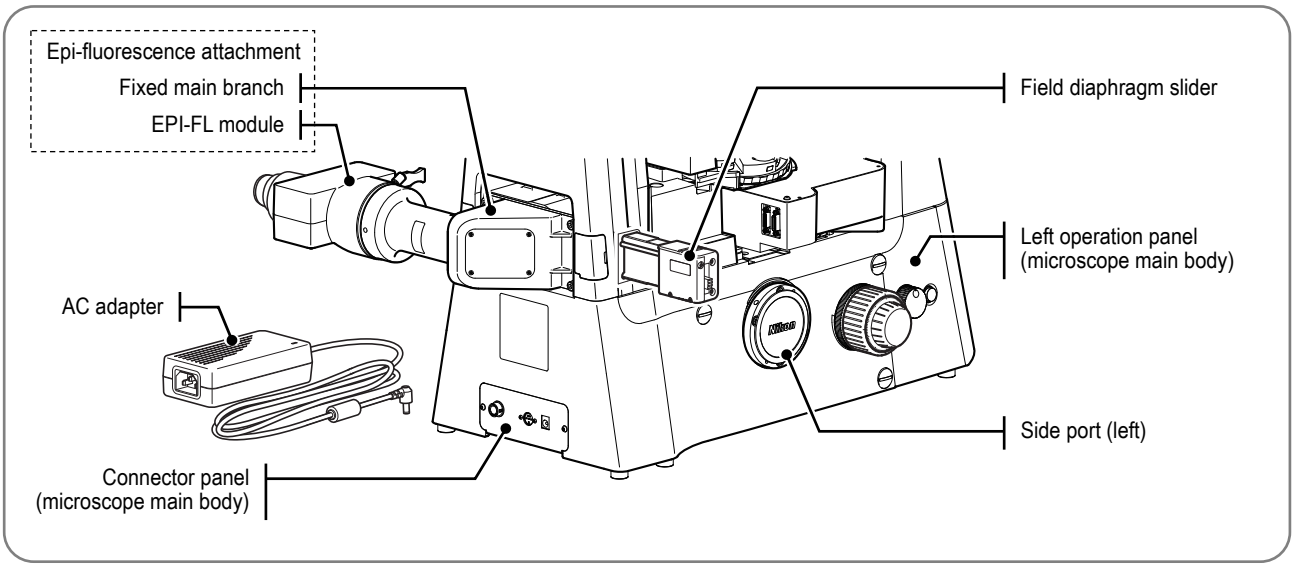
This chapter describes the name and function of each unit and operation section of the microscope system in a basic configuration.

For details on how to use each component, see “Chapter 3 Usage of Components.” For details on the components of the entire microscope system, see “Chapter 7 Assembly of the Devices.”

1.1 System Configuration and Names of Components

This manual describes the basic configuration of the microscope consisting of the following components:





System configuration

1.1 System Configuration and Names of Components (Continued)

The names (generic names) of components of the microscope and the corresponding product names and model numbers are shown in the following table.

In this manual, generic names are used to provide a general description of each device, and product names and model numbers are used to provide product-specific descriptions.

Component name (generic)	Product	Model
Main body	Inverted Research Microscope ECLIPSE Ti2-U	ECLIPSE Ti2-U
Tube	ER tube	TC-T-ER
Tube base unit	Eyepiece tube base unit with port	TI2-T-BC
Eyepiece	Eyepiece	CFI 10X
Manual stage	Stage	TC-S-SR
Stage handle	Long handle	TI2-S-HL
Stage holder	Ring holder set	C-S-HLS
Nosepiece	DIC sextuple nosepiece	TI2-N-ND
Objective	CFI objective	
Port	Direct C mount adapter	
Diascopic illumination lamphouse	Diascopic LED lamphouse	TI2-D-LHLED
Pillar for dia illumination	Pillar for dia illumination	TI2-D-PD
Condenser	Condenser turret	TC-C-TC
	LWD condenser lens	TI-C-LWD
Polarizer	DIC polarizer	TI2-C-DICP
Analyzer slider	Analyzer	TI2-C-DICA
FL turret	Intelligent epi filter turret	TI2-F-FLT-I
Epi-fluorescence attachment	Fixed main branch	TI2-LA-BF
	EPI-FL module	TI2-LA-FL-2
Episcopic field diaphragm slider	Circular field stop slider	TI2-F-FSC
Light source for episcopic illumination	Fluorescence LED illumination system	D-LEDI

Note: For details on how to use the units containing devices other than the above, see “Chapter 3 Usage of Components.”

1.2 Nomenclature and Functions

1.2.1 Dia-illumination Section

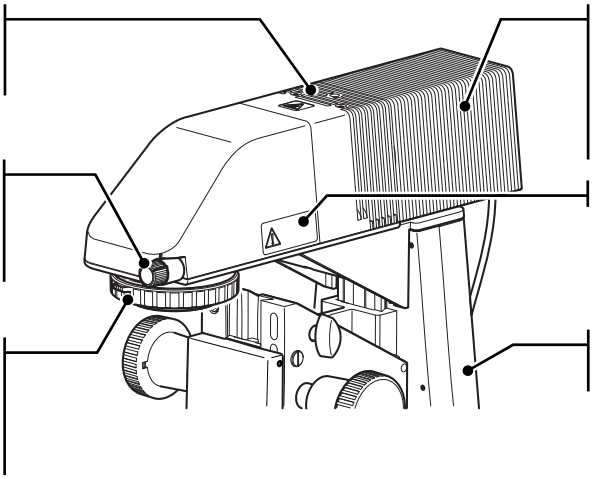
Fixed filter slot
Up to 2 filters can be inserted.
(☞ 3.3.4)

Dia-illumination shutter knob
- C (Close): Close the shutter.
- O (Open): Open the shutter.

(☞ 3.3.1)

Field diaphragm (F.S.) dial
- Turn right: Open the field diaphragm.
- Turn left: Close the field diaphragm.

(☞ 3.3.3)



Lamphouse for Dia illumination
Control this lamphouse by the dia-illumination ON/OFF button and the light control volume.
(☞ 3.3.2 and 3.3.7)

Photobiological safety label

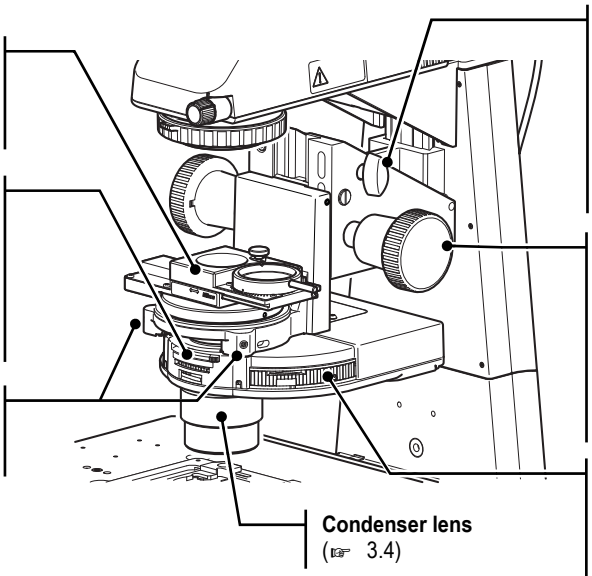
Pillar for dia-illumination
(☞ 3.3.5)

Dia-illumination section

Using the dia-illumination shutter

By using the dia-illumination shutter, illumination can be temporarily blocked while dia-illumination is turned on. By closing the dia-illumination shutter during Epi-FL microscopy, the LED of the dia-illumination section is prevented from emitting autofluorescence due to epi illumination.

1.2.2 Condenser Unit and Polarizer



Polarizer
Change dia-illumination to polarized illumination.
(☞ 3.5.1)

Aperture diaphragm open/close lever
- Turn right (clockwise): Open the aperture.
- Turn left (counterclockwise): Close the aperture.

(☞ 3.4.5)

Condenser centering screws
Adjust the position of the condenser.
(☞ 3.4.3)

Condenser refocusing clamp
Set the lower limit for the condenser's vertical move.
- Tighten the clamp: Set the lower limit at the current position.
- Loosen the clamp: Release the lower limit.

(☞ 3.4.2)

Condenser focus knob (both sides)
Move the condenser vertically.
- Turn backward: The condenser moves up.
- Turn forward: The condenser moves down.

(☞ 3.4.2)

Condenser turret
Turning the turret switches the condenser module.
Through the front window, the address of the module that enters the optical path can be checked.
(☞ 3.4.6)

Condenser lens
(☞ 3.4)

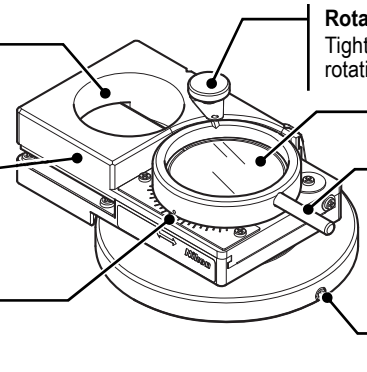
Condenser unit

Polarizer

For microscopy using the DIC, NAMC, or IMSI technique, attach a polarizer to the condenser unit (☞ 3.5.1).

- **DIC polarizer**

  Use this polarizer for DIC microscopy. It can also be used for NAMC or IMSI microscopy.



Hollow hole

Rotational part clamp screw
Tightening this screw fixes the rotational part of the polarizer.

Polarizing plate

Polarizer slider
Use this slider to switch between the polarizing plate and the hollow hole.
(☞ 3.5.1)

Polarizer rotation lever
Use this lever to turn the polarizing plate. Azimuth of the polarizer can be checked by the graduations and guide mark.
(☞ 3.5.1)

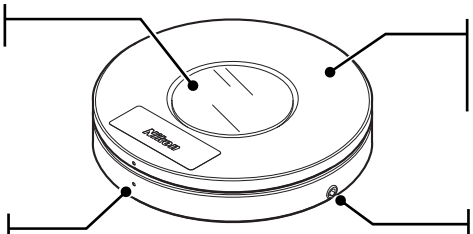
Guide mark
This mark indicates the front side of the polarizer.

Clamp screw
Use this screw to clamp the polarizer to the mount.

DIC polarizer

- **NAMC/IMSI polarizer**

  The NAMC or IMSI polarizer is used for NAMC or IMSI microscopy.



Polarizing plate

Polarizer rotating ring
Use this ring to turn the polarizing plate.
(☞ 3.5.1)

Index mark
This mark indicates the reference position and angle of the polarizer.

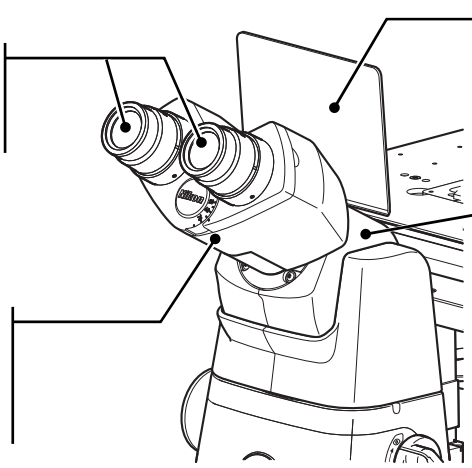
Clamp screw
Use this screw to clamp the polarizer to the mount.
(☞ 3.5.1)

NAMC/IMSI polarizer

1.2.3 Tube and Eyepiece

Eyepiece
Diopter can be adjusted according to the observer's eyesight.
(☞ 3.6.2)

Binocular part
The distance between the eyepieces can be adjusted according to the interpupillary distance of the user.
(☞ 3.6.3)



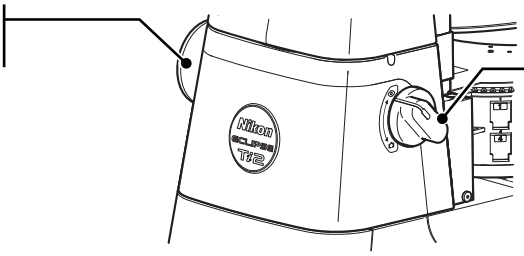
Ultraviolet light shielding plate
This plate prevents ultraviolet light or strong light emitted from the objective from entering your eyes.

Angle adjustment mechanism (ER tube)
The angle of the binocular part can be adjusted in the range of 15 to 45 degrees.
(☞ 3.6.1)

Tube and eyepiece

1.2.4 Tube Base Unit

Tube base unit side port
(☞ 3.7.1)



Tube base optical path changeover knob
- (EYE) Eyepiece observation port
- (CAMERA) Side port

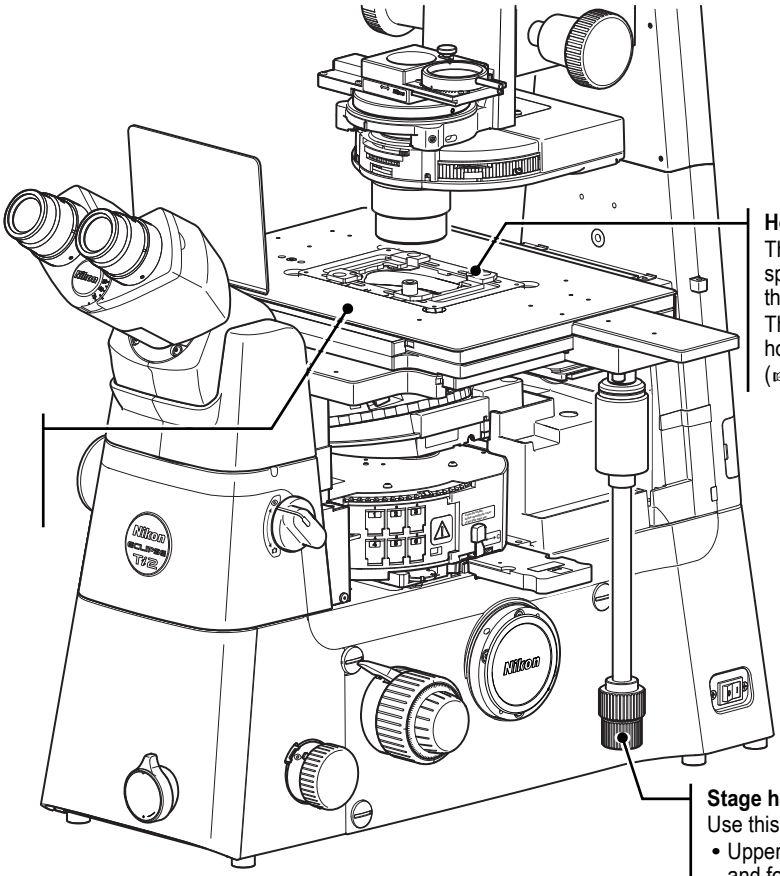
(☞ 3.7.1)

Tube base unit

Side port of the tube base unit

- To mount the camera on the tube base unit side port, the C mount adapter (optional) is required.
- The optical magnification in case where a camera is mounted to the side port depends on the adapter in use.
  Use a required adapter.

1.2.5 Stage



Holder mount
The user can mount a specimen holder suitable for the specimen to be used.
This figure shows a universal holder mounted.
(☞ 3.8.1)

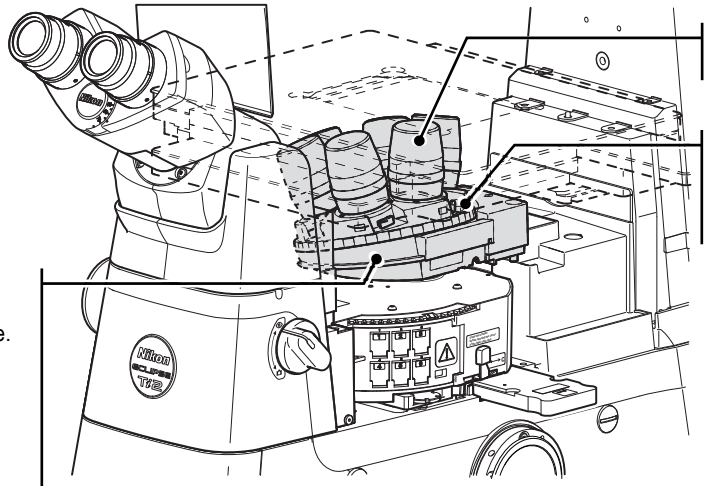
Manual stage
The stage can be moved with the stage handle.
(☞ 3.8.3)

Stage handle
Use this handle to move the stage.
- Upper knob: Move the stage back and forth.
- Lower knob: Move the stage to the right and left.

(☞ 3.8.3)
The stage handle can be selected from three types (long handle, middle handle, and short handle).

Stage

1.2.6 Nosepiece



Objective
(☞ 3.10)

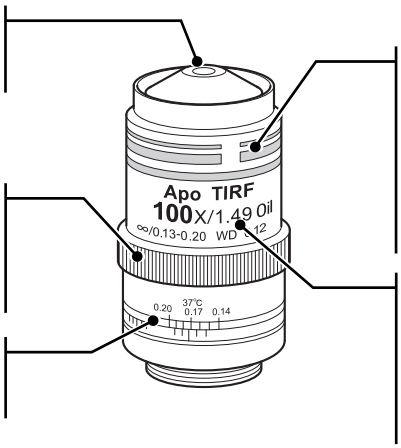
DIC slider slot
Insert the DIC slider in this slot for DIC microscopy.
(☞ 3.9.2)

Nosepiece
When switching the objective, turn the turret on the nosepiece.
- Turning the turret clockwise: Move to the next address on the nosepiece.
- Turning the turret counterclockwise: Move back to the previous address on the nosepiece.

(☞ 3.9.1)

Nosepiece (DIC sextuple nosepiece)

1.2.7 Objectives



Top lens
Lens at the top of the objective

Correction collar
Use this collar to correct the difference in thickness of the cover glass of the culture vessel.

Graduations
Graduations of the correction value

Color code
- Liquid immersion color code
  None: Dry, black: oil, white: water, red: others
- Magnification color code
  black: 1x, gray: 2x, yellow: 10x, green: 20x, light blue: 40x, dark blue: 60x, white: 100x

Specifications
- Type
- Magnification/numerical aperture
- Liquid immersion type
- Mechanical tube length (∞) / Cover glass
- Working distance (WD)

Example of an objective (with correction collar)

Displaying the applicable microscopy

To perform each of Ph, DIC, IMSI, and NAMC microscopy, an objective applicable for the microscopy must be used. An objective intended for a specific microscopy has a code which indicates the relationship of the applicable type of microscopy and optical elements.

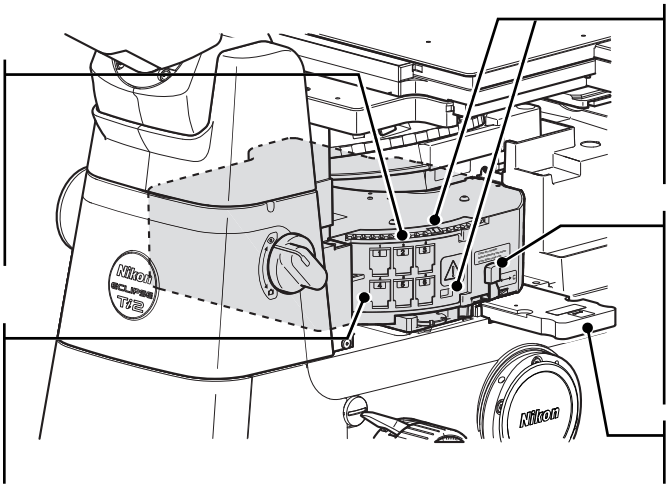
- **Ph objective:** Indicates a Ph code (PhL, Ph1 to Ph4) and the properties of a phase ring (DLL, DL, DM, BM, ADL, and ADH).
- **DIC/IMSI objective:** Indicates the letter of DIC or IMSI and the applicable type of prism (N1, N2, and NR).
- **NAMC objective:** Indicates a NAMC code (NAMC1 to NAMC3).

1.2.8 FL Turret and Analyzer

Turret ring
When switching the filter cube, turn the turret ring until it clicks.
- Turn the ring to the rear: Move to the next address.
- Turn the ring to the front: Move back to the previous address.

(☞ 3.12.1)

Filter cube replacement port
Attach the filter cube to the inside through this port.
The plate holder indicating the type of the attached filter cube is provided.



Turret address indication
This indicates the address of the filter cube currently in the optical path.
The address is displayed on the top and side of the FL turret.
(☞ 3.12.1)

Manual shutter lever
Use this lever to open or close the shutter for blocking epi-illumination.
- "O" side: Open the shutter.
- "C" side: Close the shutter.

(☞ 3.12.2)

Analyzer slider
(☞ 3.5.2)

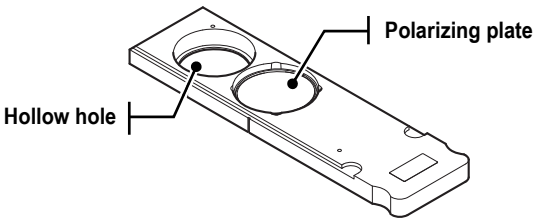
FL turret

Analyzer

The following two types of analyzers are usable in this microscope.

- **Analyzer slider**

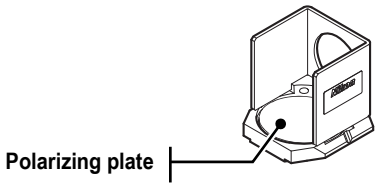
  Insert the analyzer slider into the analyzer slot of the microscope main body. At the first click position, the hollow hole is set, and at the second click, the analyzer enters the optical path.



Analyzer slider

- **Analyzer block / analyzer cube for large field of view**

  Attach this at the specified address inside the FL turret to place the analyzer into the optical path.



Analyzer block

1.2.9 Epi-fluorescence Attachments

Epi-fluorescence attachment described in this manual

This manual describes the following combination of units as the basic configuration.

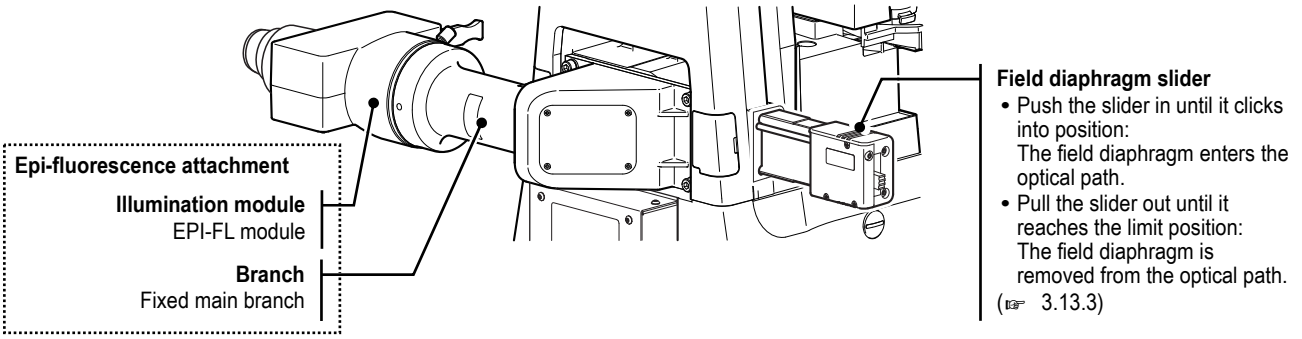
- TI2-LA-BF fixed main branch (Ti2-LAPP system)
- TI2-LA-FL-2 EPI-FL module (Ti2-LAPP system)
- D-LEDI fluorescence LED illumination system

Laser configuration

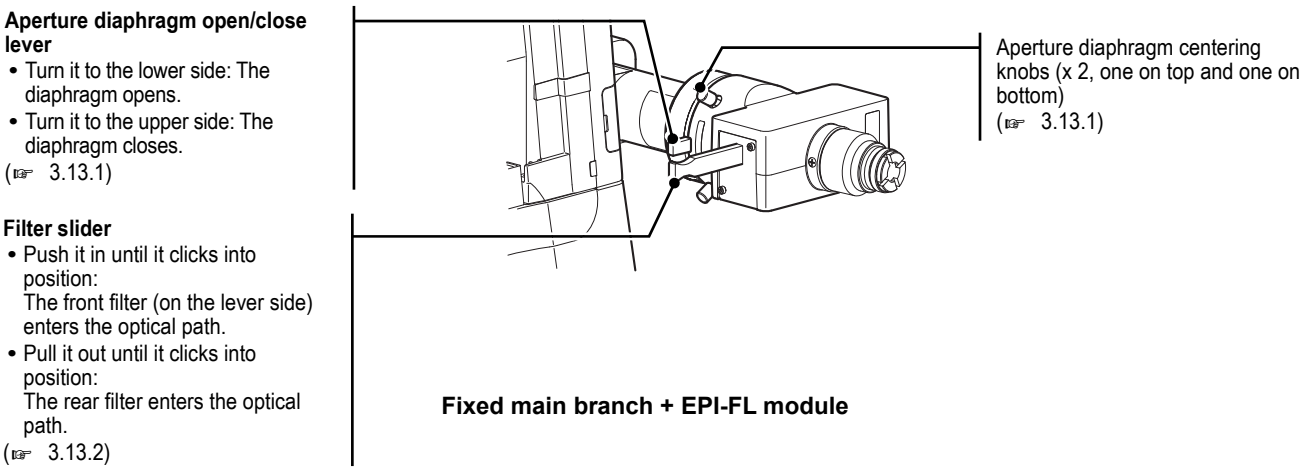
When using other modules of the Ti2-LAPP system or when using a laser as the light source for epi illumination, see the instruction manual for the Ti2-LAPP.

Epi-fluorescence attachment (fixed main branch + EPI-FL module)

■ **Left rear view:**



■ **Right front view:**



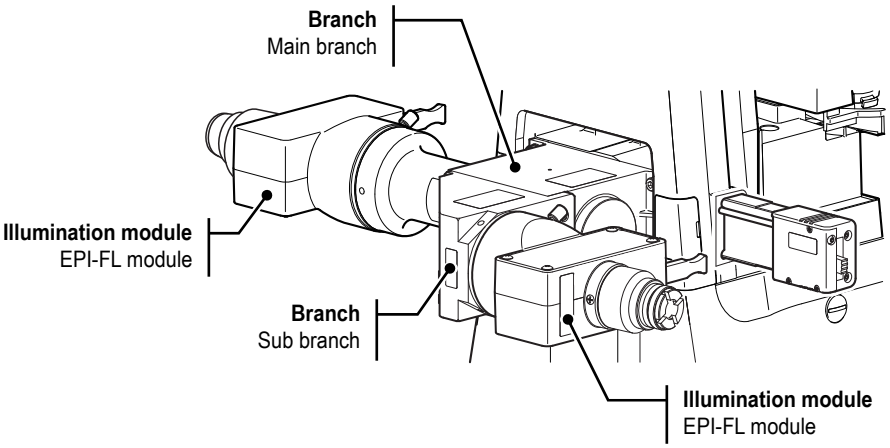
Fixed main branch + EPI-FL module

Epi-fluorescence attachment (combination of a main branch, a sub branch, and two EPI-FL modules)

When a main branch and a sub branch are used, multiple epi-illumination modules can be mounted.

Switching the optical path of the main branch switches between the illumination modules mounted on the main branch and the illumination module mounted on the sub branch.

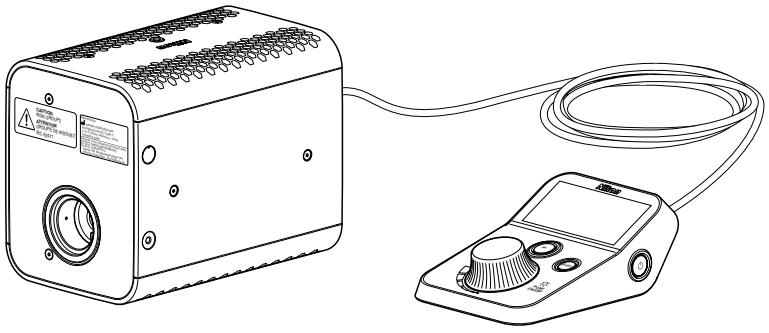
■ **Left rear view:**



Combination of a main branch, sub branch, and two EPI-FL modules

Episcopic light source (D-LEDI fluorescence LED illumination system)

The D-LEDI fluorescence LED illumination system controls respective brightness of four types of LEDs of different wavelengths by turning on or off those LEDs respectively. A dedicated remote control pad is used to control the fluorescence LED light source.



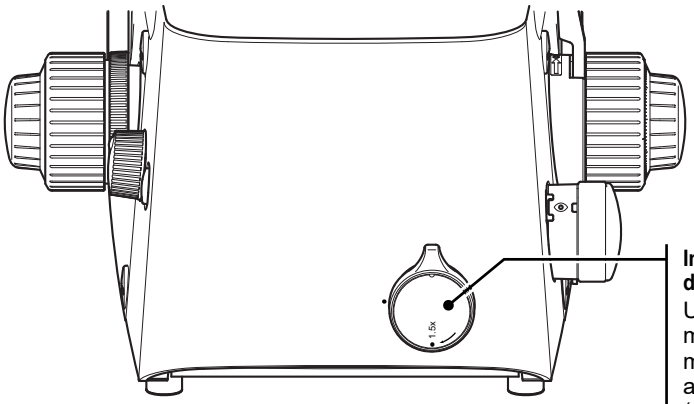
D-LEDI fluorescence LED illumination system

How to operate the light source

For details, see the instruction manual for the light source.

1.3 Operation Sections of the Microscope Main Body

1.3.1 Front Operation Panel



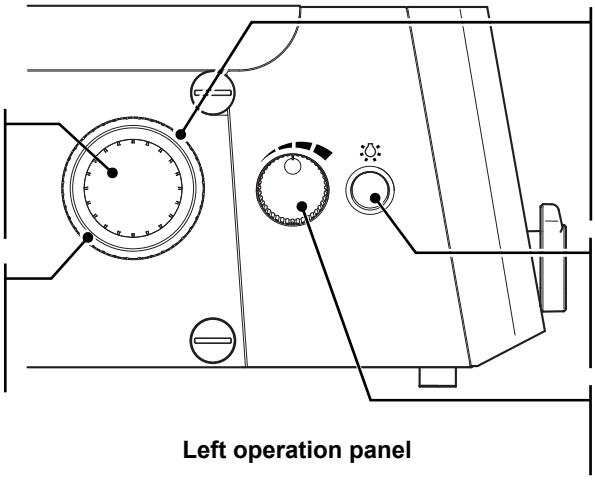
Intermediate magnification dial
Use this dial to switch the magnification of the entire microscope between 1.0x and 1.5x.
(☞ 3.2.2)

Front operation panel

1.3.2 Right and Left Operation Panels

Fine-focus knob (Fine-motion knob)
Use this knob to vertically move the objective (focusing device) in fine mode. (☞ 3.11.1)

Coarse-focus knob (Coarse-motion knob)
Use this knob to vertically move the objective (focusing device) in coarse mode. (☞ 3.11.1)



Coarse-focus knob torque adjustment ring
This ring adjusts the torque of the coarse-focus knob.
(☞ 3.11.2)
Turn the ring counterclockwise to increase the torque or clockwise to reduce the torque.

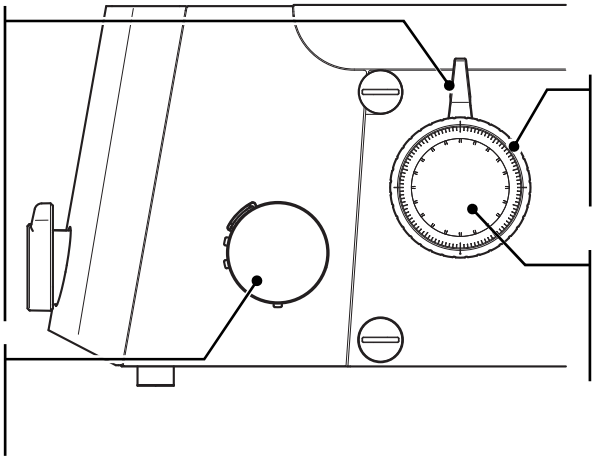
Dia-illumination ON/OFF (☼) button
Use this button to turn on/off the dia-illumination.
(☞ 3.3.2)

Dia-illumination brightness adjuster
Use this knob to adjust brightness of the dia-illumination. (☞ 3.3.7)

Left operation panel

Refocusing lever
Use this lever to set the current position of the coarse-focus knob as the upper limit position. (☞ 3.11.3)
Pushing this lever to the vertical position prevents the nosepiece from moving upward even if the coarse-focus knob is turned (however, the nosepiece will be moved upward by turning the fine-focus knob).

Optical path changeover dial
Use this dial to switch the optical path of the microscope main body. (☞ 3.2.1)



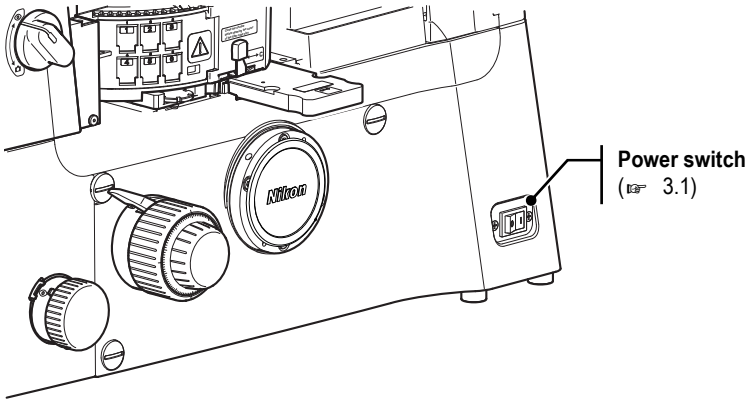
Coarse-focus knob (Coarse-motion knob)
Use this knob to vertically move the objective (focusing device) in coarse mode. (☞ 3.11.1)

Fine-focus knob (Fine-motion knob)
Use this knob to vertically move the objective (focusing device) in fine mode. (☞ 3.11.1)

Right operation panel

1.3.3 Power Switch

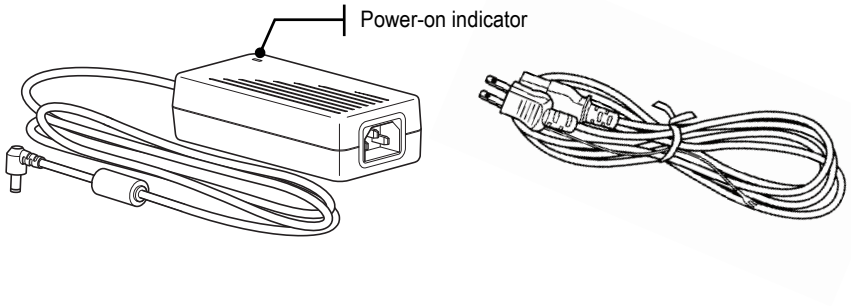
This power switch is for the Ti2-U main body. For details on the procedure for turning on the power, see "3.1 Power-related Operation."



Power switch (for the Ti2-U main body)

1.3.4 AC Adapter and Power Cord

The AC adapter and the power cord are used to supply power to the Ti2-U main body.



AC adapter and power cord

About the AC adapter and power cord

- Be sure to use the AC adapter supplied with this product. (For details on the AC adapter, see “Chapter 8 Specifications.”)
- Be sure to use only the power cord specified in “Chapter 8 Specifications” when connecting the AC adapter to the AC power.

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Chapter 2

Microscopy

This chapter explains the procedures for performing the following microscopy techniques in a standard system:

- **Diascopic bright-field (BF) microscopy (☞ 2.1)**
- **Diascopic phase contrast (Ph) microscopy (☞ 2.2)**
- **Diascopic differential interference contrast (DIC) / IMSI microscopy (☞ 2.3)**
- **Episcopic fluorescence (Epi-FL) microscopy (☞ 2.4)**
- **Diascopic dark-field (DF) microscopy (☞ 2.5)**
- **Nikon Advanced Modulation Contrast (NAMC) microscopy (☞ 2.6)**

System configuration

The microscopy procedure is described based on a microscopy system in the basic configuration described in “Chapter 1 Names of Parts and Their Functions.”
If any of these microscopy techniques require additional parts or components, they are described on the pages corresponding to such techniques.

Setup of the system

The following procedures assume that all the necessary devices are mounted, the cable connections have been completed, and the necessary data for the mounted components is already registered.

If the system has not been assembled yet, assemble the microscope according to the procedure described in “Chapter 7 Assembly of the Devices.”

2.1 Diascopic Bright-field (BF) Microscopy

1 Configure the microscope to the basic state.

1-1 Turn on the power to the system.
[a] Power switch (☞ 3.1)

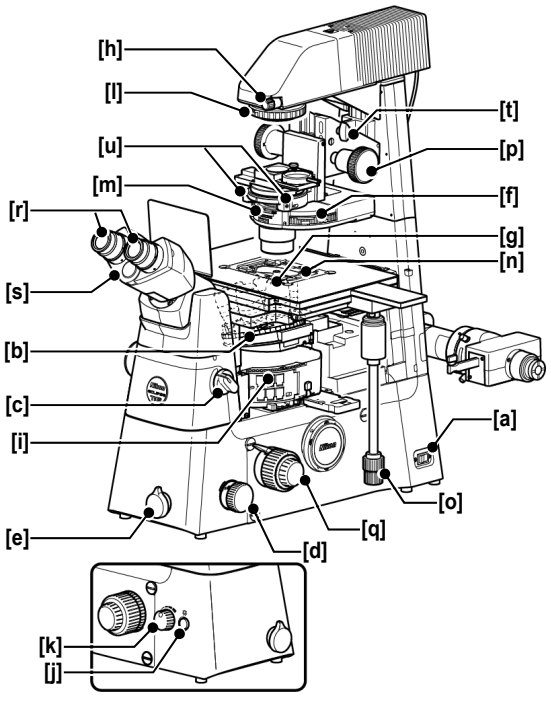
1-2 Place the 10x objective into the optical path.
[b] Nosepiece (☞ 3.9.1)

1-3 Switch the optical path of the tube base unit to 👁 (binocular part).
[c] Tube base optical path changeover knob (☞ 3.7.1)

1-4 Switch the optical path to the required observation port.
[d] Optical path changeover dial (☞ 3.2.1)

1-5 Configure the following controls to the basic state.
[e] Intermediate magnification dial (Set to 1x: ☞ 3.2.2)
[f] Condenser turret (Set to hollow or ND: ☞ 3.4.6)
[g] Objective with a correction collar (Set the correction collar to the reference position: ☞ 3.10.1)

1-6 Remove unnecessary optical elements from the optical path.
- Polarizer (☞ 3.5.1)
- Analyzer (☞ 3.5.2)
- DIC slider (☞ 3.9.2)
- Filter cube (☞ 3.12.1)



Note: Letters within [ ] correspond to those in the procedure.

2 Set a sample and focus on it.

2-1 Open the dia-illumination shutter, and close the FL turret shutter.
[h] Dia-illumination shutter knob (☞ 3.3.1)
[i] FL turret shutter knob (☞ 3.12.2)

2-2 Turn on the dia-illumination LED and adjust the brightness.
[j] Dia-illumination ON/OFF button (☞ 3.3.2)
[k] Dia-illumination brightness adjuster (☞ 3.3.2)
Illumination reaches the observation port.
Use filters if required.

2-3 Fully open the dia field diaphragm.
[l] Field diaphragm dial (☞ 3.3.3)

2-4 Fully open the dia aperture diaphragm.
[m] Aperture diaphragm open/close lever (☞ 3.4.5)

2-5 Place the sample on the stage.
[n] Stage (☞ 3.8.1)
When placing a large specimen on the stage, tilt the pillar for dia-illumination backward to obtain sufficient space (☞ 3.3.5).

2-6 Move the required part of the sample into the center of the field of view.
[o] Stage handle (☞ 3.8.3)

2-7 Move the condenser to the lower limit.
[p] Condenser focus knob (☞ 3.4.2)
Be careful not to allow the tip of the condenser to collide with the specimen. If the condenser cannot be lowered to the limit position, release the refocusing clamp.

2-8 Focus on the sample.
[q] Focus knob (☞ 3.11.1)

2-9 Adjust the diopters and the interpupillary distance if required.
[r] Eyepiece (☞ 3.6.2)
[s] Binocular part (☞ 3.6.3)

2.1 Diascopic Bright-field (BF) Microscopy (Continued)

3 Center the condenser.

3-1 Turn the field diaphragm knob until the dia field diaphragm image becomes visible in the field of view.
[l] Field diaphragm dial (☞ 3.3.3)

3-2 Focus on the dia field diaphragm image.
[p] Condenser focus knob (☞ 3.4.2)
Adjust the focus so that the perimeter of the field diaphragm can be clearly viewed.
If the lower limit of travel range is set, loosen the refocusing clamp.

3-3 Tighten the condenser refocusing clamp.
[t] Condenser refocusing clamp (☞ 3.4.2)
Tightening the condenser refocusing clamp restricts the downward travel range.

3-4 Move the dia field diaphragm image to the center of the field of view and adjust the size of the field diaphragm image to align the image with the circumference of the field of view.
[u] Condenser centering screw (☞ 3.4.3)
[l] Field diaphragm dial (☞ 3.3.3)

3-5 For precise adjustment, change the objective to a higher magnification objective and repeat steps 3-1 to 3-4.

4 Change the objective and observe the specimen.

4-1 Change the objective to the objective with a specified magnification.
[b] Nosepiece (☞ 3.9.1)

4-2 When using a liquid immersion type objective, perform oil or water immersion.
[g] Objective (☞ 3.10.2 and 3.10.3)

4-3 Remove one of the eyepieces, attach the centering telescope, and focus on the pupil of the objective.
[r] Centering telescope (☞ 3.6.4)

4-4 Adjust the size of the dia aperture diaphragm to 70-80% of the size of the numerical aperture of the objective.
[m] Aperture diaphragm open/close lever (☞ 3.4.5)
Adjust the size of the aperture diaphragm image while observing the objective pupil plane and the aperture diaphragm image using the centering telescope.

4-5 Replace the centering telescope with the removed eyepiece.
[r] Centering telescope (☞ 3.6.4)

4-6 Adjust the size of the dia field diaphragm image and move it to the center of the field of view.
[l] Field diaphragm dial (☞ 3.3.3)
[u] Condenser centering screw (☞ 3.4.3)

4-7 When using an objective with a correction collar, adjust the correction collar.
[g] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

4-8 Move the stage and turn the focus knobs to observe the sample.
[o] Stage handle (☞ 3.8.3)
[q] Focus knob (☞ 3.11.1)

2.2 Diascopic Phase Contrast (Ph) Microscopy

1 Focus on the sample with BF microscopy.

1.1 Perform the procedure from step 1 to step 3 in "2.1 Diascopic Bright-field (BF) Microscopy."

2 Set up for Ph microscopy and adjust the phase plate ring and annular diaphragm.

2-1 Change the objective to the objective for Ph microscopy.
[a] Nosepiece (☞ 3.9.1)
The phase plate ring enters the optical path.
Memorize the Ph code of the objective.

2-2 When using a liquid immersion type objective, perform oil or water immersion.
[b] Objective (☞ 3.10.2、3.10.3)

2-3 Focus on the sample.
[c] Focus knob (☞ 3.11.1)

2-4 Place the Ph condenser module into the optical path.
[d] Condenser turret (☞ 3.4.6)
The annular diaphragm enters the optical path.
Select the condenser module having the same Ph code as the objective.

2-5 Remove one of the eyepieces, attach the centering telescope, and focus on the annular diaphragm image.
[e] Centering telescope (☞3.6.4)

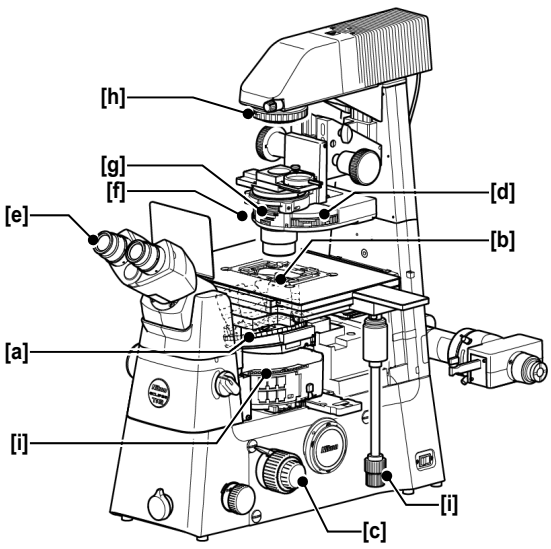
2-6 Overlay the annular diaphragm image on the phase plate ring image.
[f] Annular diaphragm centering screw (☞ 3.4.7)

2-7 Replace the centering telescope with the removed eyepiece.
[e] Centering telescope (☞ 3.6.4)

Configuration of the microscope

In addition to the configuration for BF microscopy, a Ph objective and a condenser module that have the same Ph code are used.

Place a phase ring of the objective and an annular diaphragm of the condenser module into the optical path. This causes diffraction and interference of light which gives contrast to the colorless and transparent image of the sample, enabling microscopy observation.



Note: Letters within [ ] correspond to those in the procedure.

2.2 Diascopic Phase Contrast (Ph) Microscopy (Continued)

3 Perform Ph microscopy.

3-1 Fully open the dia aperture diaphragm.
[g] Aperture diaphragm open/close lever (☞ 3.4.5)

3-2 Adjust the size of the dia field diaphragm image.
[h] Field diaphragm dial (☞ 3.3.3)

3-3 When using an objective with a correction collar, adjust the correction collar.
[b] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

3-4 Move the stage and turn the focus knobs to observe the sample.
[i] Stage handle (☞3.8.3)
[c] Focus knob (☞ 3.11.1)

3-5 When using another objective, repeat the procedure from step 2.

2.3 Diascopic DIC / IMSI Microscopy

1 Focus on the sample with BF microscopy.

1-1 Check that the vessel of the sample is suitable for DIC / IMSI microscopy.
Do not use a plastic container (☞ 4.3.1).

1-2 Follow the procedure from step 1 to step 3 in "2.1 Diascopic Bright-field (BF) Microscopy."

2 Set up for DIC microscopy / IMSI microscopy and adjust the azimuth of the polarizing plate.

2-1 Change the objective to the objective for DIC or IMSI microscopy.
[a] Nosepiece (☞ 3.9.1)

2-2 When using a liquid immersion type objective, perform oil or water immersion.
[b] Objective (☞ 3.10.2、3.10.3)

2-3 Focus on the sample.
[c] Focus knob (☞ 3.11.1)

2-4 Move the field of view to the portion of the specimen where no object is visible (or the portion of a prepared specimen slide where a cover glass is placed).
[d] Stage handle (☞ 3.8.3)

2-5 Remove one of the eyepieces and attach the centering telescope.
[e] Centering telescope (☞ 3.6.4)

2-6 Place the analyzer into the optical path.
[f] FL turret (☞ 3.12.1)
When the analyzer block is attached, turn the FL turret to place the analyzer block into the optical path.
[f] Analyzer slider (☞ 3.5.2)
When the FL turret is not used, insert the analyzer slider into the analyzer slot and push it into the second click position.

2-7 Place the polarizer into the optical path.
[g] Polarizer (☞ 3.5.1)

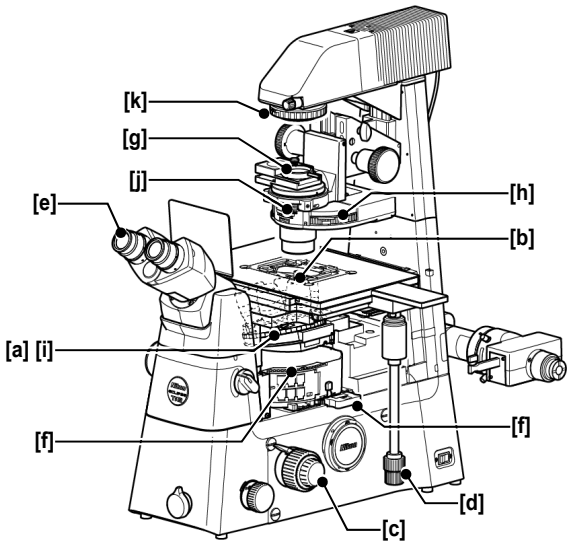
2-8 Align the white indicator on the polarizer with the center of the graduations, and tighten the clamp screw on the polarizer.
[g] Polarizer (☞ 3.5.1)

2-9 Turn the eyepiece section of the centering telescope to focus on the pupil of the objective.
[e] Centering telescope (☞ 3.6.4)

Configuration of the microscope

DIC microscopy is performed by the Senarmont system. A combination of a polarizing plate (polarizer or analyzer) and a DIC prism changes the colorless and transparent sample into different light intensities, which enables microscopy observation.

In addition to the configuration for BF microscopy, a polarizer, analyzer, DIC condenser module (condenser side DIC prism), a DIC objective, and a DIC slider (objective side DIC prism) are used. In the case of IMSI microscopy, a condenser module and a DIC slider, both for IMSI, are used.



Note: Letters within [ ] correspond to those in the procedure.

2.3 Diascopic DIC / IMSI Microscopy (Continued)

2-10 Loosen the clamp screw on the polarizer.
[g] Polarizer (☞ 3.5.1)

2-11 Turn the entire polarizer until a dark cross (Crossed Nicols) appears.
[g] Polarizer (☞ 3.5.1)

2-12 Tighten the clamp screw on the polarizer.
[g] Polarizer (☞ 3.5.1)

2-13 Replace the centering telescope with the removed eyepiece.
[e] Centering telescope in/out dial ((☞ 3.6.4)

3 Perform DIC microscopy.

3-1 Place the condenser module for DIC microscopy or IMSI microscopy and the DIC slider into the optical path.
[h] Condenser turret (☞ 3.4.6)
[i] DIC slider slot (☞ 3.9.2)
Use the condenser module and the DIC slider in the proper combination. For details on combinations, see “4.3 Details of Diascopic DIC and IMSI Microscopies.”

3-2 Focus on the sample.
[c] Focus knob (☞ 3.11.1)

3-3 Remove one of the eyepieces, attach the centering telescope, and focus on the pupil of the objective.
[e] Centering telescope (☞ 3.6.4)

3-4 Adjust the size of the dia aperture diaphragm to 70-80% of the size of the numerical aperture of the objective.
[j] Aperture diaphragm open/close lever (☞ 3.4.5)
Adjust the size of the aperture diaphragm image while observing the objective pupil plane and the aperture diaphragm image using the centering telescope.

3-5 Replace the centering telescope with the removed eyepiece.
[e] Centering telescope (☞ 3.6.4)

3-6 Adjust the size of the dia field diaphragm image.
[k] Field diaphragm dial (☞ 3.3.3)

3-7 When an objective with correction collar is used, adjust the correction collar.
[b] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

3-8 Move the stage and turn the focus knobs to observe the sample.
[d] Stage handle (☞3.8.3)
[c] Focus knob (☞ 3.11.1)

2.4 Episcopic Fluorescence (Epi-FL) Microscopy

1 Configure the microscope to the basic state.

1-1 Turn on the power to the system.
[a] Power switch (☞ 3.1)

1-2 Place the 10x objective into the optical path.
[b] Nosepiece (☞ 3.9.1)

1-3 Switch the optical path of the tube base unit to 👁 (binocular part).
[c] Tube base optical path changeover knob (☞ 3.7.1)

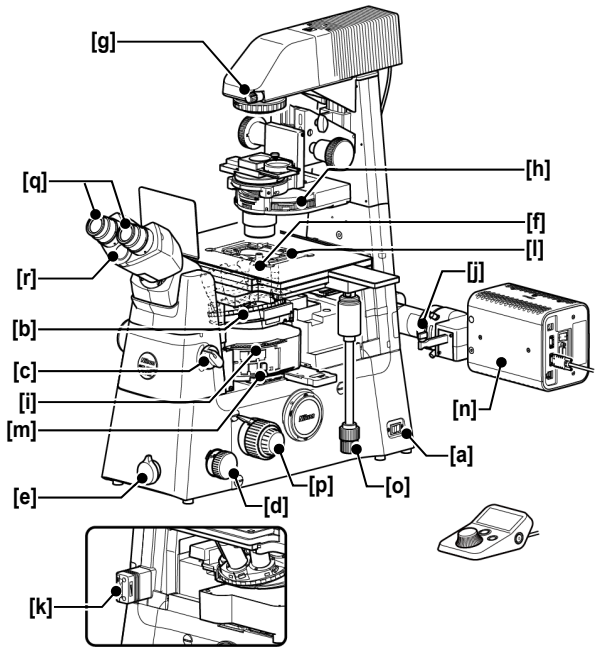
1-4 Switch the optical path to the required observation port.
[d] Optical path changeover dial (☞ 3.2.1)

1-5 Configure the following controls to the basic state.
[e] Intermediate magnification dial (Set to 1x: ☞ 3.2.2)
[f] Objective with a correction collar (Set the correction collar to the reference position: ☞ 3.10.1)

1-6 Remove unnecessary optical elements from the optical path.
- Analyzer slider (☞ 3.5.2)
- DIC slider (☞ 3.9.2)

Configuration of the microscope

For epi-fl microscopy, change the illumination to the epi illumination, arbitrarily select a filter cube, and place it into the optical path.



Note: Letters within [ ] correspond to those in the procedure.

2 Change illumination to epi, set a sample, and focus on it.

2-1 To prevent the dia-illumination LED from emitting autofluorescence due to epi-illumination, close the dia-illumination shutter.
[g] Dia-illumination shutter knob (☞ 3.3.1)
[h] Condenser turret (☞ 3.4.6)

2-2 Specify the FL turret address and place the filter cube suitable for the sample into the optical path.
[i] FL turret (☞ 3.12.1)

2-3 Fully open the epi aperture diaphragm.
[j] Aperture diaphragm open/close lever (☞ 3.13.1)

2-4 Place the field diaphragm slider into the optical path, and fully open the epi field diaphragm.
[k] Field diaphragm slider (☞ 3.13.3)
[k] Field diaphragm open/close lever (☞ 3.13.3)

2-5 Place the sample on the stage.
[l] Stage (☞ 3.8.1)
When placing a large sample on the stage, tilt the pillar for dia-illumination backward to obtain sufficient space. (☞ 3.3.5)

2-6 Open the FL turret shutter.
[m] FL turret shutter knob (☞ 3.12.2)
The epi illumination reaches the observation port.

2-7 Turn on the epi illumination and adjust the brightness.
[n] Light source for episcopic illumination (☞ 3.14.1)

2-8 Move the required part of the sample into the center of the field of view.
[o] Stage handle (☞ 3.8.3)

2-9 Darken the room.
When the contrast shield is used, close it to shield light from the above.
(☞ 3.3.6)

2-10 Focus on the sample.
[p] Focus knob (☞ 3.11.1)

2-11 Adjust the diopters and the interpupillary distance if required.
[q] Eyepiece (☞ 3.6.2)
[r] Binocular part (☞ 3.6.3)

2.4 Episcopic Fluorescence (Epi-FL) Microscopy (Continued)

3 Adjust the epi field diaphragm.

3-1 Turn the epi field diaphragm knob until the field diaphragm image becomes visible in the field of view.
[k] Field diaphragm open/close lever (☞ 3.13.3)

3-2 Move the epi field diaphragm image to the center of the field of view.
[k] Field diaphragm centering screw (☞ 3.13.3)

3-3 Adjust the size of the epi field diaphragm image to closely match the size of the field of view.
[k] Field diaphragm open/close lever (☞ 3.13.3)

3-4 For a fine adjustment, change the objective to a higher magnification objective and repeat the procedure from step 3-1 to step 3-3.

4 Perform Epi-FL microscopy.

4-1 Change the objective to the objective for fluorescence microscopy with a specified magnification.
[b] Nosepiece (☞ 3.9.1)

4-2 When using a liquid immersion type objective, perform oil or water immersion.
[f] Objective (☞ 3.10.2, 3.10.3)

4-3 Adjust the size of the epi field diaphragm image and move it to the center of the field of view.
[k] Field diaphragm open/close lever (☞ 3.13.3)
[k] Field diaphragm centering screw (☞ 3.13.3)

4-4 When an objective with correction collar is used, adjust the correction collar.
[f] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

4-5 Move the stage and turn the focus knobs to observe the sample.
[o] Stage handle (☞ 3.8.3)
[p] Focus knob (☞ 3.11.1)

2.5 Diascopic Dark-field (DF) Microscopy

1 Configure the microscope to the basic state.

1-1 Turn on the power to the system.
[a] Power switch (☞ 3.1)

1-2 Place the 10x objective into the optical path.
[b] Nosepiece (☞ 3.9.1)

1-3 Switch the optical path of the tube base unit to 👁 (binocular part).
[c] Tube base optical path changeover knob (☞ 3.7.1)

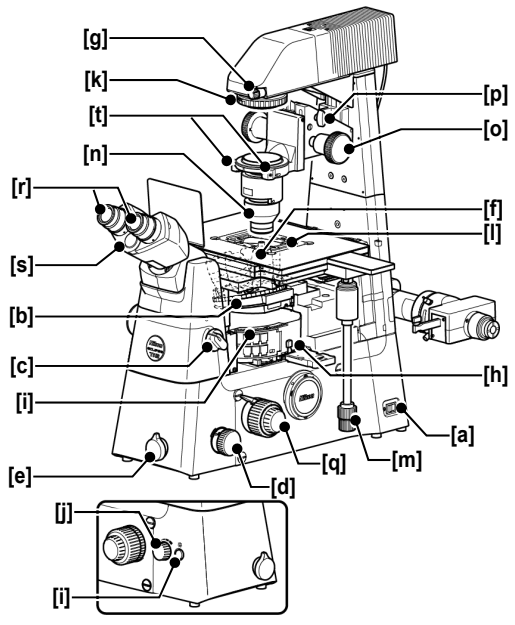
1-4 Switch the optical path to the required observation port.
[d] Optical path changeover dial (☞ 3.2.1)

1-5 Configure the following controls to the basic state.
[e] Intermediate magnification dial (1x: ☞ 3.2.2)
[f] Objective with a correction collar (Set the correction collar to the reference position: ☞ 3.10.1)

1-6 Remove unnecessary optical elements from the optical path.
- Polarizer (☞ 3.5.1)
- Analyzer (☞ 3.5.2)
- DIC slider (☞ 3.9.2)
- Filter cube (☞ 3.12.1)

Configuration of the microscope

Change the condenser from the one for BF microscopy to the one for DF microscopy. Use an objective (excluding 2x and 4x) with smaller NA (NA 0.7 or less for dry condensers, or NA 1.1 or less for oil condensers) than the minimum numerical aperture of the DF condenser.
Use an objective with diaphragm if required.



Note: Letters within [ ] correspond to those in the procedure.

2 Set a sample and focus on it.

2-1 Open the dia-illumination shutter, and close the FL turret shutter.
[g] Dia-illumination shutter knob (☞ 3.3.1)
[h] FL turret shutter knob (☞ 3.12.2)

2-2 Turn on the dia-illumination LED and adjust the brightness.
[i] Dia-illumination ON/OFF button (☞ 3.3.2)
[j] Dia-illumination brightness adjuster (☞ 3.3.2)
Illumination reaches the observation port.
Use filters if required.

2-3 Fully open the dia field diaphragm.
[k] Field diaphragm dial (☞ 3.3.3)

2-4 Place the sample on the stage.
[l] Stage (☞ 3.8.1)
When placing a large specimen on the stage, tilt the pillar for dia-illumination backward to obtain sufficient space (☞ 3.3.5).

2-5 Move the required part of the sample into the center of the field of view.
[m] Stage handle (☞ 3.8.3)

2-6 When using an oil condenser, apply a drop of oil between the sample and the tip of the condenser lens.
[n] DF condenser (☞ 3.4.4)

2-7 Lower the condenser to 1 mm above the sample.
[o] Condenser focus knob (☞ 3.4.2)
Be careful not to allow the tip of the condenser to collide with the specimen. If the condenser cannot be lowered to the required position, release the refocusing clamp.

2-8 Tighten the condenser refocusing clamp.
[p] Condenser refocusing clamp (☞ 3.4.2)
To prevent collision with the sample, be sure to tighten the refocusing clamp at the position after lowering the condenser.

2-9 Focus on the sample.
[q] Focus knob (☞ 3.11.1)

2-10 Adjust the diopters and the interpupillary distance if required.
[r] Eyepiece (☞ 3.6.2)
[s] Binocular part (☞ 3.6.3)

2.5 Diascopic Dark-field (DF) Microscopy (Continued)

3 Adjust the condenser position to set up for DF microscopy.

3-1 Stop down the dia field diaphragm.
[k] Field diaphragm dial (☞ 3.3.3)

3-2 Raise the condenser until a dark shadow appears in the field of view.
[o] Condenser focus knob (☞ 3.4.2)

3-3 Move the black shadow to the center of the field of view.
[t] Condenser centering screw (☞ 3.4.3)

3-4 Adjust the height of the condenser to obtain the highest contrast of the image.
[o] Condenser focus knob (☞ 3.4.2)

4 Perform DF microscopy.

4-1 Change the objective to the objective with a specified magnification.
[b] Nosepiece (☞ 3.9.1)

4-2 When using a liquid immersion type objective, perform oil or water immersion.
[f] Objective (☞ 3.10.2, 3.10.3)

4-3 When using an objective with a diaphragm, align the diaphragm to an optimum position below the minimum numerical aperture of the condenser.
[f] Objective (☞ 3.10.4)

4-4 When an objective with correction collar is used, adjust the correction collar.
[f] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

4-5 Adjust the size of the dia field diaphragm image to the field of view.
[k] Field diaphragm dial (☞ 3.3.3)

4-6 Move the stage and turn the focus knobs to focus on and observe the sample.
[m] Stage handle (☞ 3.8.3)
[q] Focus knob (☞ 3.11.1)

2.6 NAMC Microscopy

1 Focus on the sample with BF microscopy.

1.1 Perform the procedure from step 1 to step 3 in "2.1 Diascopic Bright-field (BF) Microscopy."

2 Set up for NAMC microscopy, and adjust the modulator.

2-1 Place the objective for NAMC into the optical path.
[a] Nosepiece (☞ 3.9.1)

2-2 Focus on the sample.
[b] Focus knob (☞ 3.11.1)

2-3 Place the NAMC condenser module having the same NAMC code as the objective into the optical path.
[c] Condenser turret (☞ 3.4.6)

2-4 Remove one of the eyepieces and attach the centering telescope.
[d] Centering telescope (☞ 3.6.4)

2-5 Check that the polarizer for NAMC/IMSI is in the optical path.
[e] Polarizer for NAMC/IMSI (☞ 3.5.1)

2-6 Turn the eyepiece section of the centering telescope to focus on the modulator of the objective.
[d] Centering telescope (☞ 3.6.4)

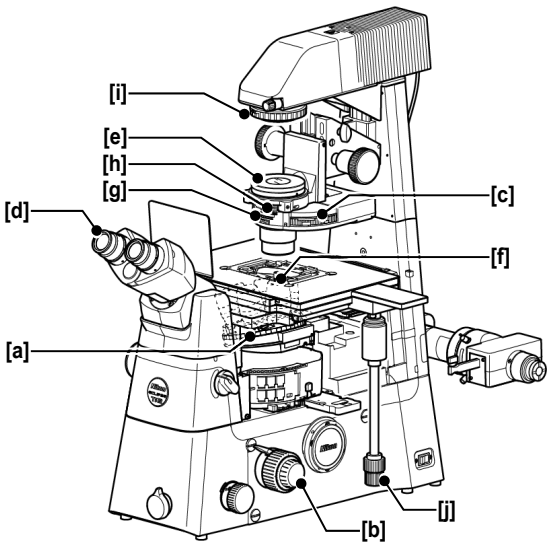
2-7 Adjust the azimuth of the polarizer for NAMC/IMSI so that two slit diaphragms can be seen.
[e] Polarizer for NAMC/IMSI (☞ 3.5.1)

2-8 Adjust the azimuth of the modulator of the objective.
[f] Objective for NAMC (☞ 3.4.8, 4.6.2)
Contrast appears vertically to the azimuth of the modulator.
Adjust the direction of contrast so that the specimen can be observed clearly.

2-9 Adjust the azimuth of the slit diaphragm.
[g] NAMC condenser module (☞ 3.4.8)
Adjust the azimuth so that the longitudinal direction of the slit aperture image becomes parallel to the gray zone in the modulator.

Configuration of the microscope

To conduct Nikon Advanced Modulation Contrast (NAMC) microscopy, a polarizer for NAMC/IMSI, a condenser module (slit diaphragm) for NAMC, and an objective for NAMC are required in addition to the configuration for BF microscopy.
Provide illumination from the side through the polarizing plate and the slit diaphragm, and adjust the azimuth of the modulator in the objective in order to provide contrast to the colorless and transparent image of the sample. This causes different light intensities for microscopy observation.



Note: Letters within [ ] correspond to those in the procedure.

2.6 NAMC Microscopy (Continued)

2-10 Adjust the position of the slit diaphragm.
[g] NAMC condenser module (☞ 3.4.8)
Adjust the position to cause the portion of the slit aperture image that does not change its brightness to overlap with the gray zone in the modulator.
To emphasize the 3D effect, align the inside of the gray zone with the inner side of the aperture diaphragm.
To control decline in contrast due to light leakage, align the inner side of the gray zone with the center of the bridge.

2-11 Replace the centering telescope with the removed eyepiece.
[d] Centering telescope (☞ 3.6.4)

3 Perform NAMC microscopy.

3-1 Fully open the dia aperture diaphragm.
[h] Aperture diaphragm open/close lever (☞ 3.4.5)

3-2 Adjust the size of the dia field diaphragm image.
[i] Field diaphragm dial (☞ 3.3.3)

3-3 When using an objective with a correction collar, adjust the correction collar.
[f] Objective (☞ 3.10.1)
Adjust the correction collar of the objective according to the thickness of the prepared specimen slide or the cover glass of the culture vessel.

3-4 Adjust the azimuth of the polarizer for NAMC/IMSI and adjust the brightness and contrast of the image.
[e] Polarizer for NAMC/IMSI (☞ 3.5.1)

3-5 Move the stage and turn the focus knob to focus on the sample during observation.
[j] Stage handle (☞ 3.8.3)
[b] Focus knob (☞ 3.11.1)

3-6 When using another objective, repeat the procedure from step 2.

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Chapter
3

Usage of Components

This chapter provides details on how to use each module and device in the Ti2-U main body.

CAUTION

- **Before using the product, thoroughly read the “Safety Precautions” at the beginning of this manual, and heed all warnings and cautions written therein.**
- **To use other products together with this product, refer to the respective manuals and heed all warnings and cautions written therein.**

3.1 Power-related Operation

This section describes how to turn on and off the power to the microscope.

Turning on the power to the microscope

When turning on the power to the microscope, follow steps 1 and 2 in order.

(1) Peripheral devices requiring dedicated power supply

- Light source for episcopic illumination
- Power supply for dia-illumination (when a halogen lamp is used)
- Motorized shutter controller
- Digital camera, etc.

▼

(2) Ti2-U microscope main body

Turning off the power to the microscope

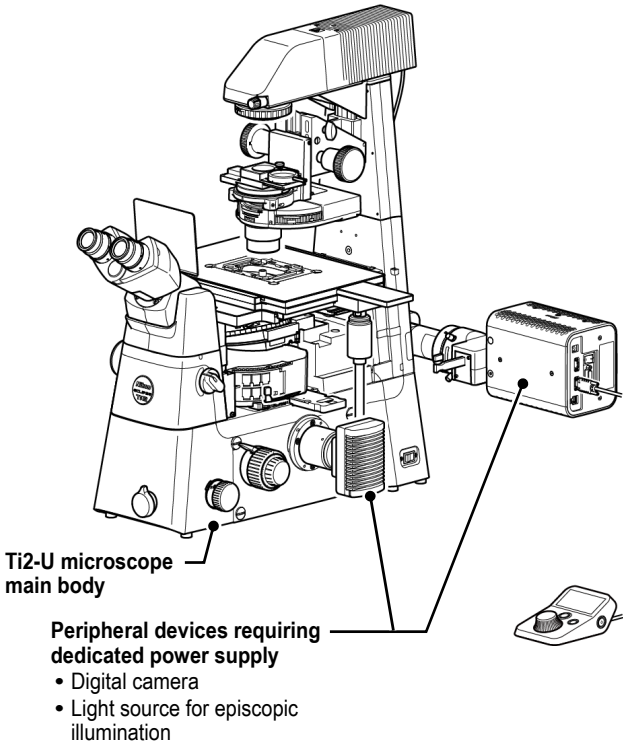
When turning off the power to the microscope, follow steps 1 and 2 in order.

(1) Ti2-U microscope main body

▼

(2) Peripheral devices requiring dedicated power supply

- Light source for episcopic illumination
- Power supply for dia-illumination (when a halogen lamp is used)
- Motorized shutter controller
- Digital camera, etc.



Power-related operation

Power-on order

If the power is not turned on in the correct order, communications between devices might not be correctly performed, thereby preventing the statuses of devices from being recognized correctly. Make sure to turn on the power in the above-stated order.

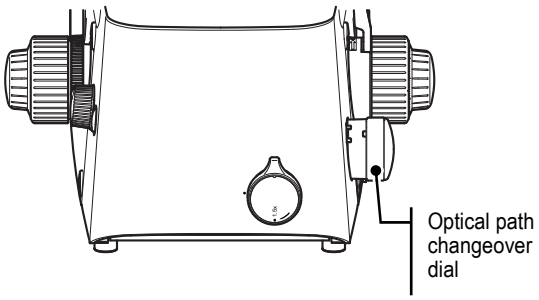
Connecting power supplies and checking the settings of external controls

If a precentered lamphouse is used for dia-illumination, the dia-illumination can be controlled from the Ti2-U main body by connecting the Ti2-U main body to the power supply for dia-illumination. When controlling the power supply from the microscope main body, make sure that the Ti2-U main body and the power supply are correctly connected and that the external control for the power supply is set to ON.

3.2 Optical Parts in the Microscope Main Body

3.2.1 Switching the Output Port

The optical image output port can be switched by setting the optical path changeover dial on the right side of the microscope to the required index mark (two protrusions) position.



Switching the output port

■ **Operation:**

Ti2-U

Optical path changeover dial	Optical path and light amount
👁	Eyepiece observation port 100%
📷 L	Left side port 100%
📷 R	Right side port 100%
AUX	–

Ti2-U (eyepiece:left port = 20:80)

Optical path changeover dial	Optical path and light amount
👁	Eyepiece observation port 100%
📷 L	Left side port 100%
📷 R	Right side port 100%
📷 L + 👁	Eyepiece observation port 20% Left side port 80%

There are protrusions (👁: one protrusion, 📷 L: two protrusions) also on the dials so that the output port can be switched without looking at the dial.

Specifications of the AUX port

The AUX port may be set as follows according to the specification at the time of purchase. There is no output if not specified.

- R80 (Right side port 80%, Eyepiece observation port 20%)

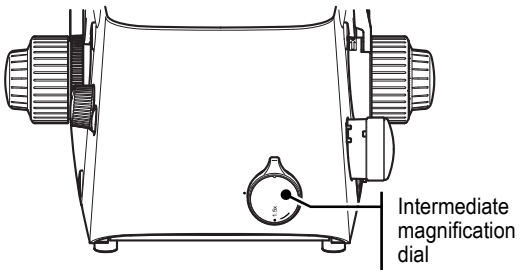
Ti2-U output port

The following output ports are usable in this microscope.

Output port	Description
Eyepiece observation port	An optical output port for visually observing a microscope image through a binocular part of a tube.
Side port (right/left)	An optical output port for a microscope image on both sides of the microscope. A microscopy camera, a confocal head, and a photometer sensor can be attached. Use a selected adapter for attachment.
Tube base unit side port (tube base unit with port)	An optical output port for a microscope image on a side of the tube base. A selected adapter can be used to attach the microscope camera. Turn the optical path changeover dial to "👁" and switch the tube base optical path changeover knob to "📷."
Back port (optional: TI-BPU)	When an FL turret is mounted on the microscope, attaching the TI-BPU back port unit enables an optical image output. Set up FL turrets in a two-tier configuration by using the stage up kit enables simultaneous multi-wavelength microscopy using two cameras.

3.2.2 Switching the Magnification of the Device with the Intermediate Magnification Dial

By removing and inserting the 2nd tube lens inside the microscope, switch the magnification of the device in the microscope between 1.0x (actual magnification of the objective) and 1.5x (magnification of the objective x 1.5).



Device magnification changeover

■ **Operation:**

Intermediate magnification dial	Magnification of the device
Turn in the opposite direction of the arrow (vertical upward)	1x
Turn in the direction of the arrow until “1.5x” aligns with the index mark (sideways)	1.5x

Using the intermediate magnification dial

- The settings of the intermediate magnification dial are valid for all optical image output ports (except the optional back port).
- By replacing the 2nd tube lens inside the microscope, the magnification when the intermediate magnification dial is rotated to 2.0x can be changed. For replacement of the 2nd tube lens, contact your local Nikon representative.

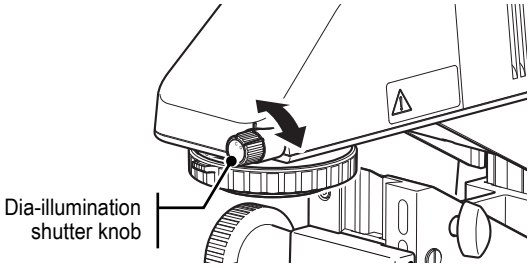
3.3 Using the Dia-illumination Section

3.3.1 Opening/Closing the Dia-Illumination Shutter

The dia-illumination section has a shutter for blocking the optical path.

■ **Operation:**

Dia-illumination shutter knob	Dia-illumination shutter
Turn to the O (Open) side	Open
Turn to the C (Close) side	Close



Opening/closing the dia-illumination shutter

Using the dia-illumination shutter

By using the dia-illumination shutter, illumination can be temporarily blocked while dia-illumination is turned on. By closing the dia-illumination shutter during Epi-FL microscopy, the LED of the dia-illumination section is prevented from emitting autofluorescence due to epi illumination.

3.3.2 Using Diascopic Illumination

Control dia-illumination using the dia-illumination ON/OFF button and brightness adjuster on the left side of the microscope.

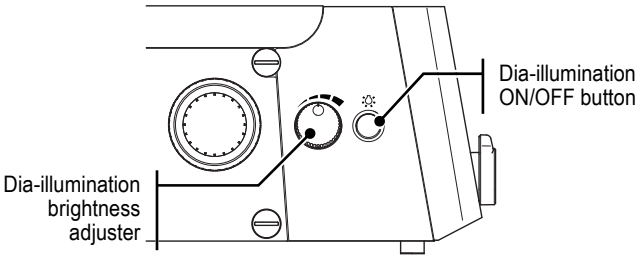
■ **Operation:**

Turning on/off dia-illumination

Dia-illumination ON/OFF button	Dia-illumination
Press the button.	Pressing the button alternates between on and off.

Adjusting dia-illumination

Brightness adjuster	Dia-illumination
Turn it clockwise.	Illumination becomes lighter.
Turn it counterclockwise.	Illumination becomes darker.



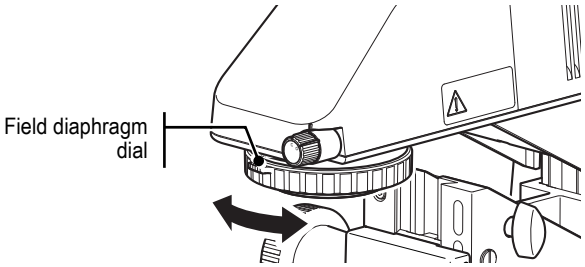
Controlling dia-illumination

Using the brightness adjuster

The brightness adjuster with the upper and the lower limits rotates only within the limits.
The alarm tone does not sound even though the adjuster reaches the limits. Do not forcibly turn the adjuster.

3.3.3 Adjusting the Dia Field Diaphragm

Field diaphragm is intended to restrict the observation range by changing the irradiation range of the illumination. Adjust the field diaphragm using the field diaphragm (F.S.) dial of the dia-illumination section.



Controlling the field diaphragm

■ **Operation:**

Field diaphragm dial	Field diaphragm
Turn it clockwise.	The diameter of the diaphragm increases.
Turn it to the left.	The diameter of the diaphragm decreases.

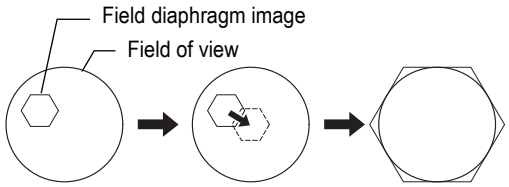
The field diaphragm has an index to indicate the opening degree of the field diaphragm and the following label.

Tips on adjusting the field diaphragm

For normal use, stop down the aperture diaphragm until the illumination range is circumscribed by or inscribed in the field of view.
An illumination range broader than necessary causes stray light to come in from other sources. As a result, a flare occurs and the contrast of the optical image decreases.

Procedure for adjusting the field diaphragm

1. **Place the objective with a specified magnification into the optical path.**
2. **Turn the field diaphragm dial to stop down the field diaphragm until the field diaphragm image is seen in the field of view.**
3. **Turn the condenser focus knob to focus on the field diaphragm image.**
4. **Turn the condenser centering screw to move the field diaphragm image to the center of the field of view.**



Move the field diaphragm image to the center of the field so that the image is about the same as for the field.

Adjusting the field diaphragm

Timing for adjusting the field diaphragm

Adjust the size of the field diaphragm each time the objective is switched.

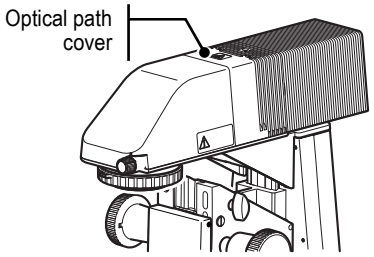
Setting the field diaphragm when photographing using a camera

When photographing an optical image, a good result can be obtained by narrowing the field diaphragm to a range a little broader than the size of the image sensor (frame indicating the shooting range).
Note that if the field diaphragm is narrowed close to the viewing range, vignetting may occur.

3.3.4 Using a Filter for Dia-illumination

Fixed Filter

Up to two filters of any type can be inserted into the fixed filter slot of the dia-illumination section. For details on the procedure for setting the filter, see "7.8.3 Attaching a Fixed Filter for Dia-illumination."



Fixed filter slot (inside the optical path cover)

■ **Operation:**
The filters inserted into the fixed filter slot are always in the optical path. Filters cannot be inserted or removed freely.

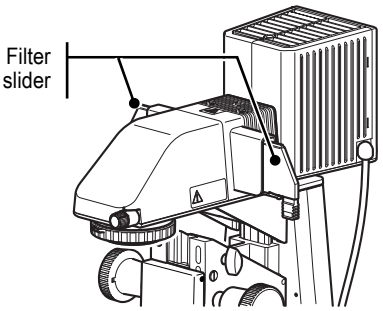
Combining with the LED lamphouse

An ND32 filter is supplied with the LED lamphouse.
When using the LED lamphouse, insert the ND32 filter into the fixed filter slot.

Filter Slider for Dia Illumination

The filter slider for dia illumination has two filter sliders in addition to two fixed filter slots.
Up to two filters can be attached to each filter slider, and they can be placed into the optical path simultaneously.

For details on the procedure for setting the filter, see "7.8.4 Attaching a Filter Slider for Dia Illumination (Optional)."



Filter slider for dia illumination

■ **Operation:**

Filter slider	Filter
Push or pull the slider up to the limit position.	The right or left filter (or no filter) enters the optical path.

Using a fixed filter

Even when the filter slider for dia illumination is in use, the above fixed filter can be used. Up to six filters (two filter sliders for dia-illumination, each with two filters + two fixed filters) can be mounted, and up to four filters can be placed into the optical path simultaneously.

Usable filters

Filter name	Explanation
ND2 **ND4** **ND8** **ND16**	Light absorbing filter (ND: Neutral Density) for adjusting brightness The greater the number, the lower the transmittance and the darker the image becomes. This filter does not affect the color shade of the illumination and is used to adjust the brightness when reproducibility of color is emphasized. ND filters are used to adjust brightness while emphasizing the reproducibility of colors using a halogen lamp. • ND2: Transmittance 50% (light intensity 1/2) • ND4: Transmittance 25% (light intensity 1/4) • ND8: Transmittance 12.5% (light intensity 1/8) • ND16: Transmittance 6.3% (light intensity 1/16)
NCB11	Color temperature correction filter (NCB11: Neutral Color Balance) used for ordinary microscopy or camera photographing For halogen lamps, place this filter into the optical path and adjust the lamp voltage to the rating of the lamp to achieve the best reproducibility of colors. When photographing monochromatic images, remove the filter from the optical path.
GIF	Green interference filter (GIF) Only light with a specified wavelength range such as 530 nm can be transmitted through this filter. This filter enhances contrast when performing PH microscopy, DIC microscopy or microscopy with monochromatic light or photographing monochromatic images.
D	Diffusion filter (D: Diffusion) made of glass for diffusing light This filter is used to make the illumination uniform.

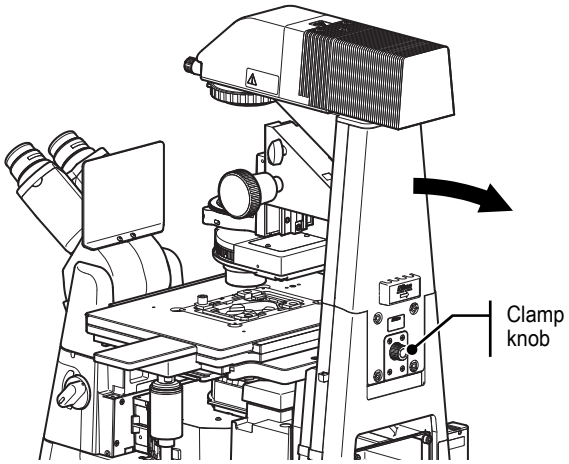
3.3.5 Using the Dia-illumination Pillar

A large space can be maintained by inclining the dia-illumination pillar backward for a large sample replacement.

■ **Operation:**

Clamp knob	Dia-illumination pillar
Loosen the knob.	The dia-illumination pillar is no longer stationary (It can be inclined.)
Tighten the knob.	The dia-illumination pillar is fixed.

Hold the dia-illumination section and the pillar and slowly incline the pillar.



Inclining the dia-illumination pillar

CAUTION

- **When inclining or returning the pillar, take care that your fingers or hands are not pinched in the hinge.**
- **When attaching a relatively heavy component to the upper portion of the pillar, make sure to securely attach it. A loose screw might result in the component falling off when the pillar is inclined. In particular, firmly affix the lamphouse.**
- **Be careful not to touch the lamphouse or its periphery as they might be very hot.**

Tilting clamp knob

Nikon recommends tightening the pillar tilting clamp knob on the rear of the pillar to prevent the pillar from falling unintentionally even though the pillar is usable with the clamp released.

When a motorized shutter or precentered lamphouse for dia illumination is attached, in particular, make sure to use the pillar with the pillar tilting clamp knob tightened.

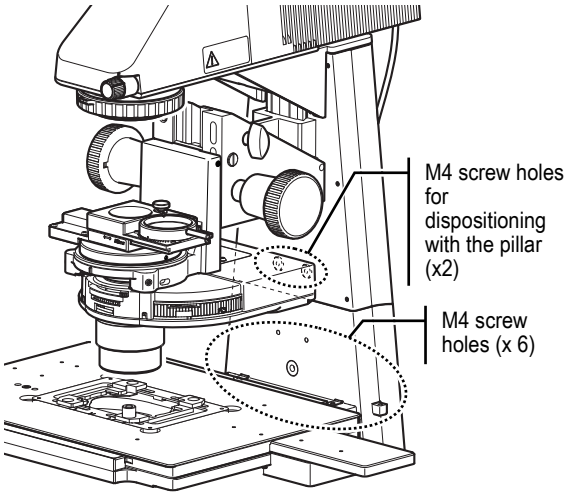
Attach a third party part to the illumination pillar

There are eight M4 screw holes on the front and the side of the pillar for dia-illumination, to which a range of devices, including a manipulator, can be mounted.

The upper two screw holes are used to mount a device which you want to take off the stage by tilting the pillar. The lower six screw holes are used to mount a device always on the stage even when the pillar is tilted.

Adjusting the condenser position and attaching a manipulator

Azimuth of the condenser has no impacts on observation when using a condenser turret but not using a polarizer (BF and Ph microscopy). The space for mounting the manipulator can be ensured by loosening the condenser clamp screw to turn the condenser turret, and securing the condenser turret after turning the condenser to the right and left.



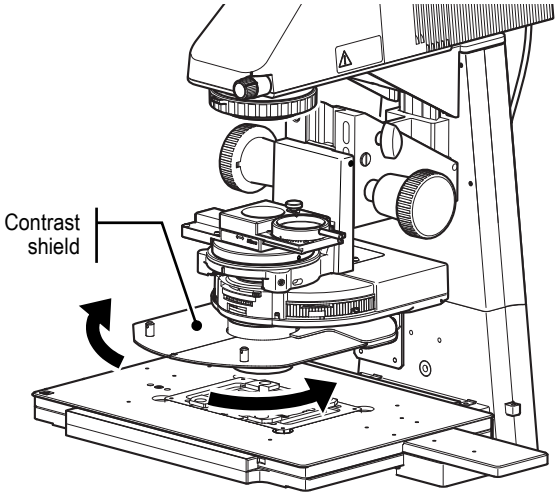
Fixing screw holes

3.3.6 Operating the Contrast Shield (Optional)

The optional contrast shield is a device that blocks the ambient light irradiated from the above. For fluorescence microscopy, close the light shielding plate by operating the open/close knob.

■ **Operation:**

Contrast shield	Ambient light
Open the light shielding plate.	Light is not blocked.
Close the light shielding plate.	Light is blocked.



Operating the contrast shield

Mounting position of the contrast shield

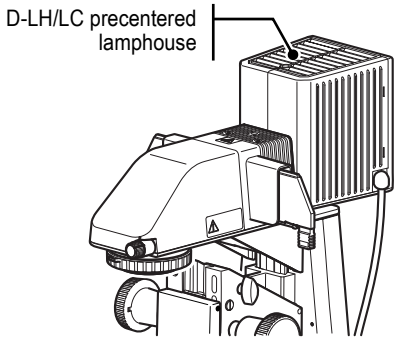
The contrast shied can be mounted at two positions: 60 mm or 30 mm from the stage surface. The shorter the distance from the stage surface, the higher the light shielding performance, but it might become more difficult to manipulate the specimen on the stage. For details on how to mount the contrast shield, see “Chapter 7 Assembly of the Devices.”

3.3.7 Using the D-LH/LC Precentered Lamphouse

In place of the standard dia LED lamphouse, a D-LH/LC precentered lamphouse, which uses a halogen lamp, can be attached.

Connect D-LH/LC with the TI-PS100W/A power supply 100-240V/A before use. Connecting the power supply to the Ti2-U main body and correctly specifying settings enables the lamphouse and the power supply to be operated from the Ti2-U main body.

Use D-LH/LC according to the following notes.



D-LH/LC Precentered Lamphouse

Combined use of the shutter

The precentered lamphouse uses a halogen lamp, in which the tinge of the illumination changes until it is warmed up and at full brightness.
Refrain from turning on and off frequently. Instead, use the dia-illumination shutter to block the illumination.

Attachment of a lamphouse and connection of a power supply

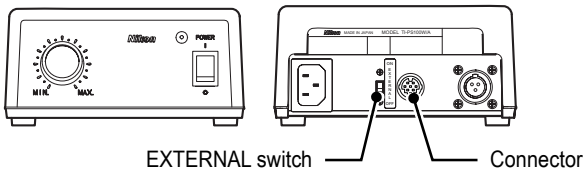
For details on the attachment of a lamphouse and connection of a power supply, see “Chapter 7 Assembly of the Devices.”

Checking the settings of the power supply

When turning on or off or adjusting brightness of the lamp from the main body, check the following settings:

1. **Check the connection between the power supply and the Ti2-U main body.**
2. **Set the EXTERNAL switch on the rear surface of the power supply to ON.**
3. **Flip the POWER switch on the front surface of the power supply to the “I” side.**

   When power is applied, the POWER indicator is lit. To turn off the power, flip the POWER switch to the “O” side.



Connecting power supplies/setting locations

Controlling the lamphouse from the power supply

To adjust the brightness without using the brightness adjuster on the Ti2-U, turn off the EXTERNAL switch to enable the brightness adjuster on the power supply.

Operating the Precentered Lamphouse

Control the precentered lamphouse using the dia-illumination ON/OFF button and brightness adjuster on the left side of the microscope.

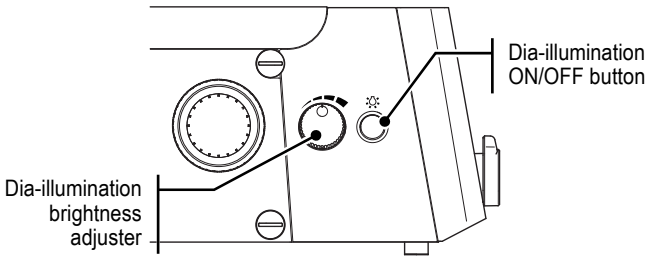
■ **Operation:**

Turning the lamp on or off

Dia-illumination ON/OFF button	Dia-illumination
Press the button.	Pressing the button alternates between on and off.

Brightness adjustment

Brightness adjuster	Dia-illumination
Turn it clockwise.	Illumination becomes lighter.
Turn it counterclockwise.	Illumination becomes darker.



Controlling the dia-illumination (precentered lamphouse)

Controlling from the power supply

When the EXTERNAL switch is set to OFF, the precentered lamphouse can be controlled from the power supply.

■ **Operation:**

Turning the lamp on or off

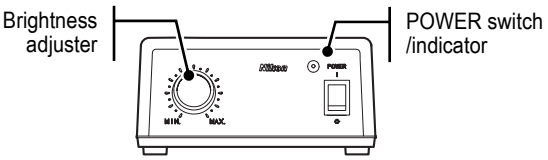
POWER switch	Lamp
Switching between on and off	On: lit, Off: light out

Adjusting the brightness of the lamp

Brightness adjuster	Lamp
Turn it clockwise.	Illumination becomes lighter.
Turn it counterclockwise.	Illumination becomes darker.

■ **Display:**

While dia-illumination is on, the POWER indicator lights. The brightness can be checked by the index of the brightness adjuster.



Operation panel of the power supply

- The dia-illumination ON/OFF button on the left side of the microscope is enabled even when the EXTERNAL switch is turned off.
- Before adjusting the brightness of the lamp from the power supply, turn the brightness adjuster on the left side of the microscope counterclockwise so that the brightness is set to minimum. Otherwise the brightness adjustment range is limited.

Color reproduction with the halogen lamp

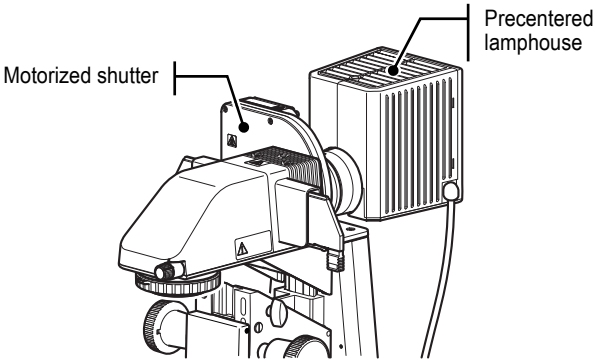
As the D-LH/LC precentered lamphouse uses a halogen lamp, a color temperature of the lamp changes by adjusted brightness using the brightness adjuster and the tinge of the image changes accordingly as shown below.

Voltage	Tinge
High	Bright and bluish light
Low	Dark and reddish light

- To emphasize the reproducibility of colors, set the voltage supplied to the lamp to a value suitable for imaging (photo voltage) using the brightness adjuster.
- To adjust the brightness without changing the tinge of the image, insert the NCB11 filter to the filter slot and adjust using the ND filter.

Using a motorized dia-illumination shutter

When using a precentered lamphouse, an NI-SH-E motorized shutter can be attached to the dia-illumination section. The motorized shutter can be opened and closed with the NI-SH-CON controller for motorized shutter.



Precentered lamphouse and motorized shutter

■ **Operation:**

Motorized shutter controller	Motorized shutter
Control the SHUTTER 1 switch/SHUTTER 2 switch.	The motorized shutter opens or closes

■ **Display:**

The open and closed state of the motorized shutter is indicated by the CLOSE indicator on the front panel of the motorized shutter controller. When the CLOSE indicator is lit, the shutter is closed, and when the CLOSE indicator is unlit, the shutter is open.

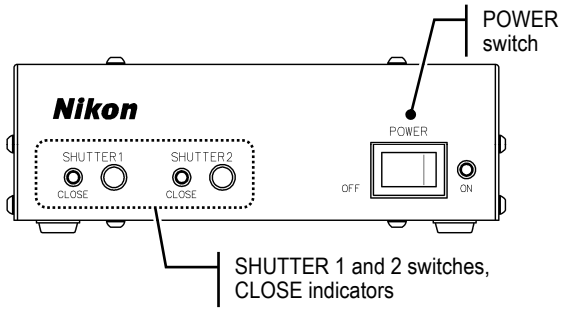
Operating the motorized shutter controller

The motorized shutter controller is equipped with two operation switches (SHUTTER 1 and SHUTTER 2), which enable two motorized shutters to be controlled by a single controller.

■ **Operation:**

Opening and closing the shutter

SHUTTER 1 switch SHUTTER 2 switch	Motorized shutter
Press the switch.	The shutter opens or closes each time the switch is pressed.



Motorized shutter controller

■ **Display:**

The open and closed state of the motorized shutter is indicated by the CLOSE indicator on the front panel.

SHUTTER 1 - CLOSE SHUTTER 2 - CLOSE indicator	Motorized shutter
Lit	Shutter closed
Unlit	Shutter open
Blinking	Error

For details on how to use the motorized shutter controller, see the instruction manual for the motorized shutter controller.

3.4 Using the Condenser Section

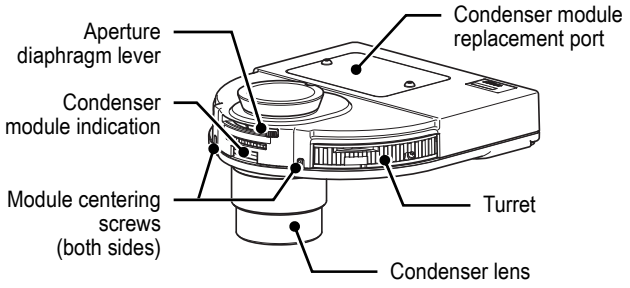
3.4.1 Selecting a Suitable Condenser

The following four types of condensers can be used with this microscope:
Select a condenser suitable for microscopy.

Condenser turret (system condenser)

Up to seven condenser modules can be attached. Microscopy techniques can be switched by turning the turret to change the condenser module.

The condenser lens is replaceable and a range of lenses is available for the applicable microscopy.

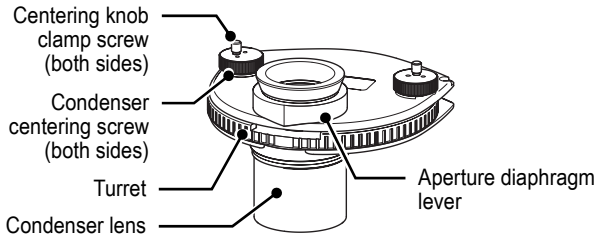


Condenser turret (system condenser)

ELWD-S condenser

This condenser allows BF microscopy and Ph microscopy. Manually turn the turret to select optical elements.

Note: An ELWD condenser lens, which cannot be replaced, is mounted on the ELWD-S condenser.



ELWD-S condenser

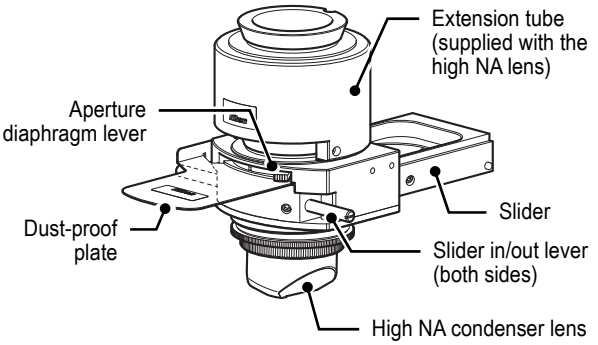
HNA condenser slider

A high-magnification DIC or Ph microscopy (or BF microscopy) is available in combination with a high NA condenser lens. Manually pull in or pull out the slider using the slider in/out levers to select a condenser module.

Two types of high NA condenser lenses can be used:

- TI2-C-LHD HNA dry lens
- TI2-C-LHO HNA oil lens

Note: A high NA lens is required for using the HNA condenser slider.

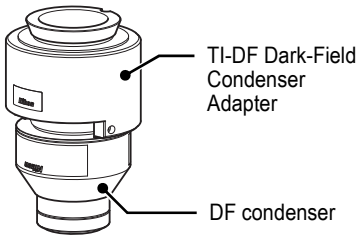


HNA condenser slider

DF condenser

This is a condenser exclusively for DF microscopy. There are two types of condensers and mounted immediately under the TI-DF condenser adapter.

- Darkfield condenser oil
- Darkfield condenser dry



DF condenser

3.4.2 Elevating the Condenser

Turn the condenser focus knob to vertically move the condenser so that the field diaphragm image can be seen clearly in the field. The movement of the condenser can also be limited so that it does not go below the designated position.

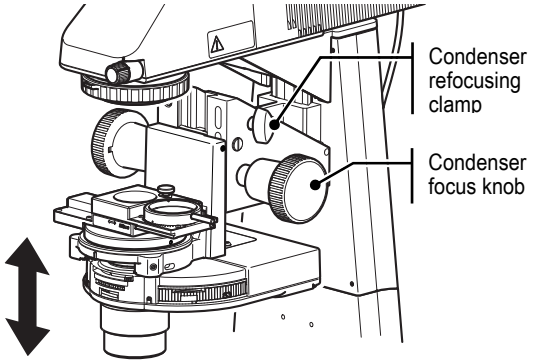
■ **Operation:**

Elevating movement of the condenser

Condenser focus knob	Condenser
Turn it to the rear side.	The condenser goes up.
Turn it to the front side.	The condenser goes down.

Restriction on the lower limit position of the condenser

Condenser refocusing clamp	Condenser
Tighten it.	Set the lower limit position.
Loosen it.	Release the restriction.



Elevating movement of the condenser and position adjustment

Adjusting the rotational torque of the condenser focus knob

The rotational torque of the condenser focus knobs on both sides can be adjusted by turning them in the opposite direction. Turn and tighten the handles to increase the rotational torque and turn and loosen the handles to decrease the torque.

Take care not to decrease the torque too much, as that will cause the condenser to slide down by its own weight.

Using the condenser refocusing clamp

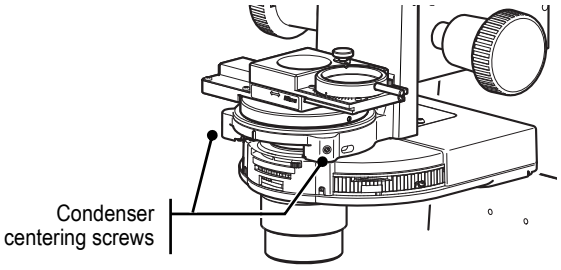
Even when the condenser is moved upward to the escape position to replace samples, the condenser can be returned to the original position by lowering to the limit position. Use this clamp when using a high NA condenser or when the dia-illumination pillar cannot be tilted.

3.4.3 Adjusting the Position of the Condenser (Centering)

Adjust the position of the condenser so that the illumination is at the center of the field. Turn the two condenser centering screws on the front surface of the condenser mount to adjust the position using a 2 mm hexagonal screwdriver.

■ **Operation:**

Condenser centering screw	Position of the condenser
Turn it clockwise (tighten).	The condenser moves to the rear.
Turn it counterclockwise (loosen).	The condenser moves to the front.



Adjusting the position of the condenser

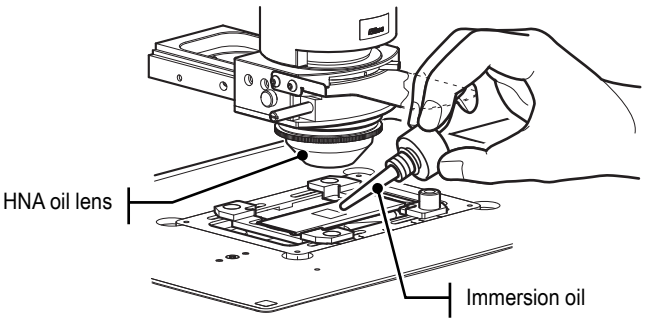
Adjusting the position of the condenser

- Place the 10x objective in the optical path and stop down the field diaphragm so that the field diaphragm image aligns with the center of the field.
- To adjust the position precisely, place a high-magnification objective in the optical path and perform the centering in the same way.

3.4.4 Using an Oil Immersion Condenser Lens

When using the TI2-C-LHO HNA oil lens, use immersion oil to immerse between the prepared specimen slide and the top lens.

Spread immersion oil, about the size of the diameter of the tip of the condenser lens, on the glass slides (or cover glass) of the prepared specimen, and then slowly lower the condenser lens so that the tip of the lens touches the oil.



Oil immersing of the condenser

Notes on using an oil immersion condenser lens

- If air bubbles are mixed in the immersion oil, the quality of the microscope images will decrease. Do not to let air bubbles get mixed in.
- The oil thickness of 1 mm is sufficient. Do not apply too much oil.
- When immersing the condenser in oil, use a clip to firmly hold the specimen in place. Otherwise, the specimen might be drawn to the condenser by the surface tension and the viscosity of the oil.

Focusing on the oil immersion condenser

When using a high-magnification, oil immersion condenser lens, make sure to immerse the area between the prepared specimen slide and the condenser lens before focusing on the condenser.

Using the condenser refocusing clamp

After forming the field aperture image on the specimen plane, tighten the condenser refocusing clamp, and mark the power limit of the condenser. This prevents the tip of the condenser lens from colliding with the specimen and affixes the field aperture image.

Wiping off oil

Oil remaining at the tip of the oil-immersion lens after the wiping or oil adhering to the tip of the dry lens will adversely affect the image. After use, thoroughly wipe off all oil, and make sure that no oil adheres to the tips of other objectives.

- When wiping off oil, use a lens tissue or clean cloth moistened with benzine and gently wipe off a few times. To wipe off oil cleanly, be sure to use the same portion of the tissue or cloth only once. For a clean finish, use absolute alcohol (ethyl alcohol or methyl alcohol) at the end.
- If petroleum benzine is unavailable, use methyl alcohol instead. However, because the cleaning power of methyl alcohol is weak, the user may need to wipe several times (usually three or four times).
- When wiping oil off the specimen, take due care not to damage the specimen.

CAUTION

Take due care when handling absolute alcohol or petroleum benzine, as they are highly inflammable. Do not bring any ignition source or use the power switch near absolute alcohol or petroleum benzine.

3.4.5 Adjusting the Diascopic Aperture Diaphragm

The aperture diaphragm adjusts the numerical aperture of the illumination system. Adjust the aperture diaphragm using the aperture diaphragm open/close lever of the condenser.

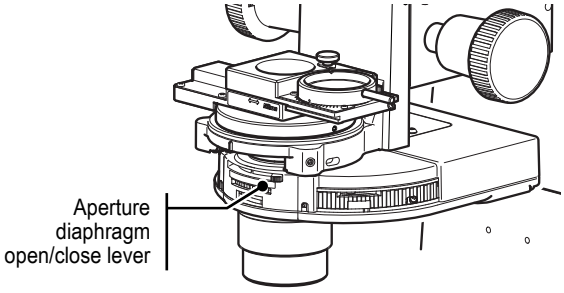
■ **Operation:**

Aperture diaphragm open/close lever	Aperture diaphragm
Turn it towards the right.	The diameter of the diaphragm increases.
Turn it towards the left.	The diameter of the diaphragm decreases.

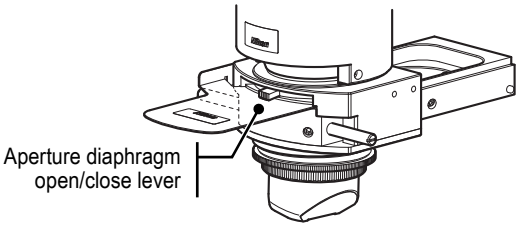
To adjust the aperture diaphragm, observe the pupil surface of the objective by using the centering telescope. (See below.)

■ **Display:**

The following label can be seen on the aperture diaphragm lever of the condenser turret



Adjusting the aperture diaphragm of the condenser turret



Adjusting the aperture diaphragm of the HNA condenser slider

Relation between the aperture diaphragm size and the optical image state

Adjust the aperture diaphragm size to change the resolution, brightness, contrast and the depth of focus of the microscope image.

The resolution and the brightness are decreased and the contrast and the depth of focus are increased by narrowing the aperture diaphragm. They interact and cannot be adjusted individually. It is necessary to adjust the aperture diaphragm size according to specimens to be observed and the purpose of the observation.

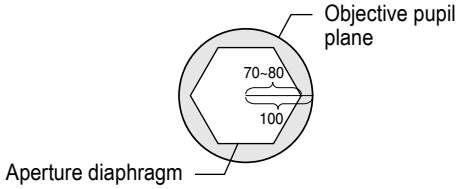
Timing of aperture diaphragm adjustment and tips on adjustment

- Adjust the field diaphragm every time the objective is switched.
- Generally, images with an appropriate contrast can be obtained by narrowing the field aperture to 70% to 80% of the numerical aperture of the objective.
  Adjustment of the aperture diaphragm is important for BF microscopy, DIC microscopy, or image photographing using a camera.
- When performing Ph or NAMC microscopy, fully open the aperture diaphragm. When the aperture diaphragm is narrowed, the optical path is blocked.

Aperture diaphragm adjustment procedure

Adjust the aperture of the diaphragm while observing the actual diaphragm image.

1. **Place the centering telescope into the optical path.**
2. **Look through the centering telescope and turn the eyepiece of the centering telescope to focus the pupil (bright circle) of the objective onto the aperture diaphragm image.**
3. **Adjust the aperture diaphragm so that the size of the aperture diaphragm is 70% to 80% of the size of the pupil of the objective.**



Adjust the field diaphragm so that the size of the field diaphragm image is 70% to 80% that of the pupil plane of the objective.

Adjusting the aperture diaphragm

3.4.6 Switching the Condenser Module

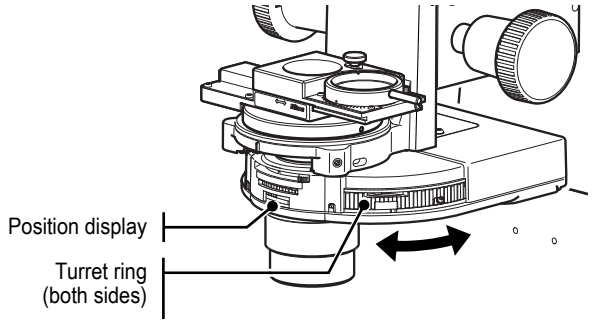
Condenser turret (system condenser)

When switching the condenser module, manually turn the turret ring to place the module into the optical path.

■ **Operation:**

Turret ring	Condenser turret
Turn until it clicks into position.	The front or rear module enters the optical path.

The address of the condenser turret in the optical path can be checked by the position display on the front surface of the turret.



Switching the module on the condenser turret

Shutter addresses on the condenser turret

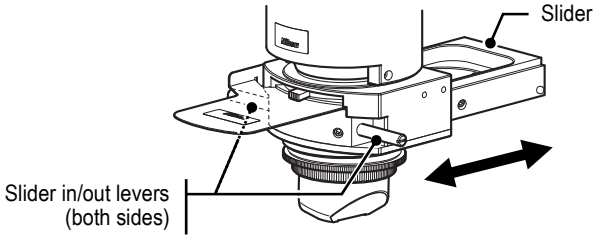
By attaching the light shielding plate module supplied with the condenser turret to the designated address, it can be used as a condenser shutter.

HNA condenser slider

To switch the condenser module, move the HNA condenser slider back and forth.

■ **Operation:**

Slider	Condenser module
Push it in to the rear.	The DIC module enters the optical path.
Pull it out to the front.	The Ph module (or hollow) enters the optical path.



Switching modules by the HNA condenser slider

Usable condenser module list

The following table shows the condenser modules mountable on the condenser turret and the condense slider.

Table Condenser module list

Name	Microscopy technique	Remarks	
ND	BF	ND filter	
PhL	Ph	TC-C-ML-PHL LWD Module TC-C-ME-PHL ELWD Module	
Ph1	Ph	TC-C-ML-PH1 LWD Module TC-C-ME-PH1 ELWD Module	TI2-C-MC-PH1 CLWD Module for PH1
Ph2	Ph	TC-C-ML-PH2 LWD Module TC-C-ME-PH2 ELWD Module	TI2-C-MC-PH2 CLWD Module for PH2
Ph3	Ph	TC-C-ML-PH3 LWD Module	TI2-C-MC-PH3 CLWD module
Ph4	Ph		TI2-C-MC-PH4 CLWD module
DIC	DIC	TC-C-ML-N1D LWD Dry DIC Module TC-C-ML-N2D LWD Dry DIC Module TC-C-ML-NRD LWD Dry DIC Module	TI2-C-MH-N2D High NA Dry DIC Module TI2-C-MH-NRD High NA Dry DIC Module TI2-C-MH-N2O High NA Dry DIC Module TI2-C-MH-NRO High NA Dry DIC Module
NAMC	NAMC	TC-C-MN-10x NAMC 10x Module TC-C-MN-20x NAMC 20x Module TC-C-MN-40x NAMC 40x Module	TC-C-MNL-10x NAMC LWD 10x Module TC-C-MNL-20x NAMC LWD 20x Module TC-C-MNL-40x NAMC LWD 40x Module
IMSI	IMSI	TC-C-MI-N2D IMSI Dry Module TC-C-MI-NRD IMSI Dry Module	

Mounting condenser modules

Condenser turrets have seven module mount positions and condenser sliders have two module mount positions.
There are two positions: the 37 mm-dia. position and the 39 mm-dia. position depending on the purpose.

Table Condenser module mount

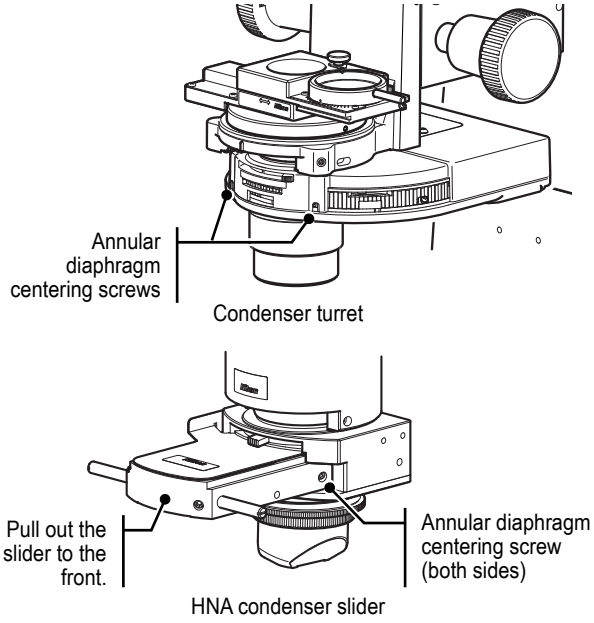
	37 mm-dia. position	39 mm-dia. position
Number of positions	4	3
Centering, rotation adjustment	Allowed	Not allowed
Orientation slit	None	Present
Mountable modules	Ph, NAMC, ND	DIC, IMSI, ND

3.4.7 Adjusting the Ph Module Position

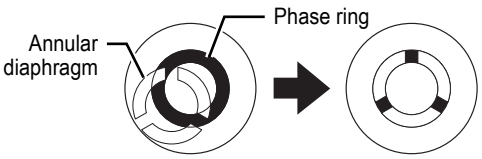
It is necessary to center the annular diaphragm of the Ph module.

Center the annular diaphragm using the centering telescope. Remove one eyepiece, attach the centering telescope, and then focus on the ring diaphragm image by turning the eyepiece section of the centering telescope.

Turn the centering screws of the module using a specified tool to overlay the annular diaphragm image on the phase ring image in the objective.



Annular diaphragm centering screw



Centering of the annular diaphragm

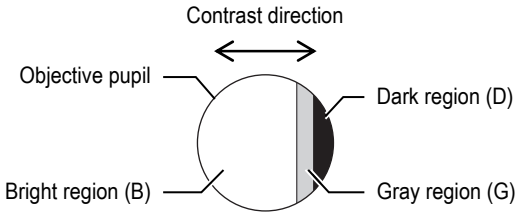
3.4.8 Adjusting the NAMC Module

Before performing NAMC microscopy, adjust the orientation of the modulator of the NAMC objective, and then adjust the position and the orientation of the NAMC module accordingly.

Image quality and adjustment of the modulator of the NAMC objective

NAMC objectives have a modulator with a dark region and gray region pattern as shown on the right.

Contrast appears in the vertical direction (in the direction of the arrow) in relation to the modulator's pattern. Adjust the orientation of the modulator according to the specimen.



Modulator image

Modulator adjustment

The modulator image can be observed using the centering telescope. Turn the modulator ring and adjust the orientation of the modulator so that contrast can be seen in the required direction.

■ **Operation:**

Operation	Polarizing plate
Turn the modulator ring.	The orientation of the modulator is changed.

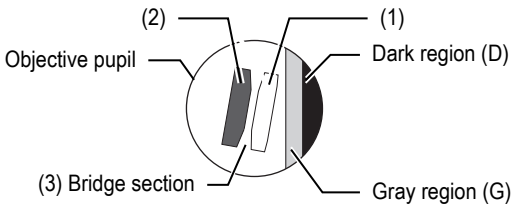
Adjustment by turning the modulator ring

Be careful not to move the correction collar when turning the modulator ring. If the correction collar is turned by mistake, adjust it by following the procedures in “3.10.1 Adjusting the Objective with the Correction Collar.”

NAMC condenser slit image and slit diaphragm adjustment

In the NAMC condenser module, there are two apertures (slit diaphragms) indicated by (1) and (2) in the right figure, and a space called “bridge” indicated by (3) between the apertures.

A polarizing plate is in aperture (2) and the brightness of (2) is changed by changing the azimuth of the polarizer.



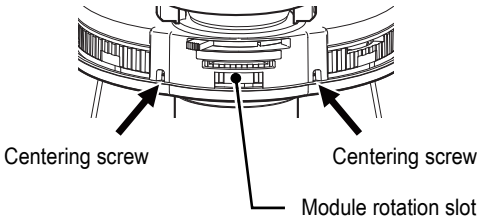
Slit diaphragm image

Slit adjustment

Observe the slit image using the centering telescope.

Adjust the orientation of the slit diaphragm by turning the NAMC condenser module so that apertures (1) and (2) are parallel to gray zone (G) of the modulator.

Adjust the position of the slit to cause the portion of the slit aperture image (1), which does not change its brightness by moving the polarizer, to overlap with the gray zone (G).



Slit diaphragm adjustment

■ **Operation:**

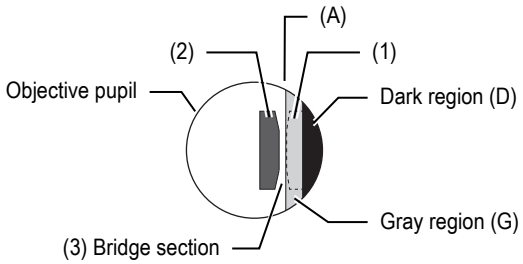
Module adjustment	Polarizing plate
Turn the centering screw.	The position of the slit diaphragm is changed.
Turn the module through the module rotation slot.	The orientation of the slit diaphragm is changed.

Fine adjustment of the NAMC condenser slit diaphragms

Images obtained by NAMC microscopy vary depending on the slit diaphragm adjustment of the NAMC condenser module.

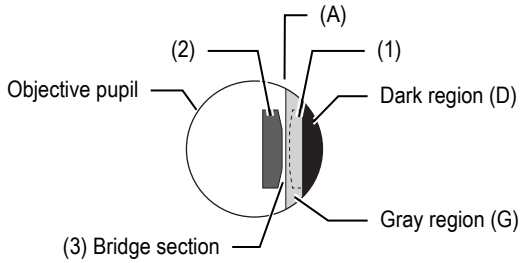
Emphasizing on 3D effects:

Align the inner side plane (A) of the objective's gray zone (G) with the inner plane of aperture (1).



Reducing the contrast degradation due to leakage light:

Align the inner side plane (A) of the objective's gray zone (G) with the center of the bridge section (3).



3.4.9 Using the ELWD-S Condenser

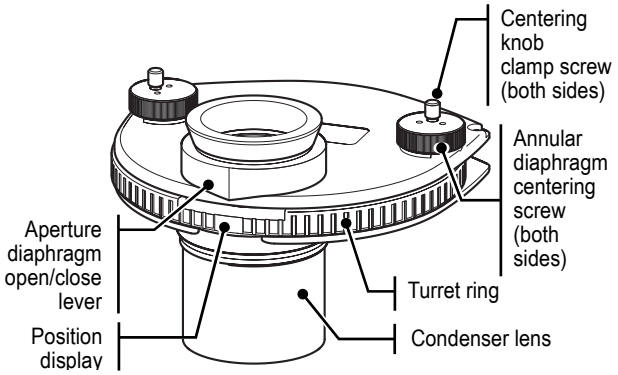
Switching the condenser module

The ELWD-S condenser allows BF microscopy and Ph microscopy. Turn the turret manually to place the module into the optical path.

■ **Operation:**

Turret ring	Condenser
Turn until it clicks into position.	The previous or the next module enters the optical path.

The condenser module in the optical path can be checked through the position display on the front surface of the turret.



Switching modules in the ELWD-S condenser

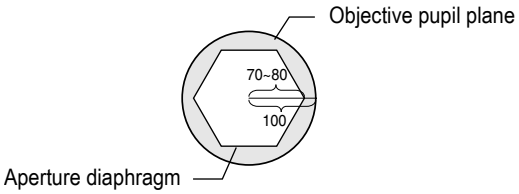
Adjusting the aperture diaphragm

When adjusting the aperture diaphragm with the ELWD-S condenser, operate the aperture diaphragm open/close lever of the condenser.

■ **Operation:**

Aperture diaphragm open/close lever	Aperture diaphragm
Turn it to the right.	The diameter of the diaphragm increases.
Turn it to the left.	The diameter of the diaphragm decreases.

Place the centering telescope into the optical path and adjust the aperture diaphragm so that the size of the aperture diaphragm image is 70% to 80% of the pupil plane of the objective.



Adjust the field diaphragm so that the size of the field diaphragm image is
70% to 80% that of the pupil plane of the objective.

Adjusting the aperture diaphragm

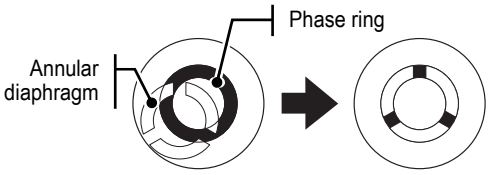
Centering of the annular diaphragm

The ELWD-S condenser has a centering knob to adjust the position of the annular diaphragm used for Ph microscopy.

■ **Operation:**

Annular diaphragm centering screw	Position of the annular diaphragm
Turn it clockwise (tighten).	It moves to the rear.
Turn it counterclockwise (loosen).	It moves to the front.

By centering the annular diaphragm of the lowest Ph code, all annular diaphragms can be centered.



Adjusting the position of the annular diaphragm for Ph microscopy

For this adjustment, use the centering telescope. Place the objective having the lowest Ph code in the optical path, and then, with the ELWD-S condenser, place the annular diaphragm having the same Ph code into the optical path. Loosen the centering knob clamp screw and adjust the centering knob to overlap the image of the phase ring of the objective with the image of the annular diaphragm.

3.5 Using the Polarizer and Analyzer

3.5.1 Using the Polarizer

When performing a DIC microscopy, NAMC microscopy, or IMSI microscopy, attach a polarizer to the condenser mount and place a polarization plate into the optical path. The following two types of polarizers are usable:

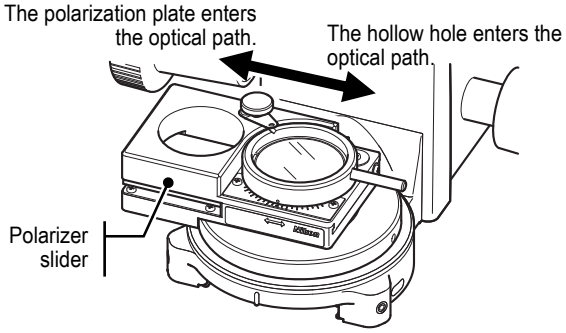
Polarizer	Supported microscopy techniques
TI2-C-DICP DIC Polarizer	DIC
TC-C-DICPNI NAMC/IMSI Polarizer	NAMC, IMSI

Inserting and removing the polarizer (DIC polarizer)

■ **Operation:**

Polarizer slider	Polarizer
Move the slider to the left.	The polarization plate enters the optical path.
Move the slider to the right.	The hollow hole enters the optical path.

The polarizer has a slider for the polarization plate and a hollow hole. Placing the polarization plate in the optical path will change the illumination from dia-illumination to polarized illumination.



Inserting and removing the polarizer

NAMC/IMSI polarizer

The NAMC/IMSI polarizer has no slider. When placing the polarizer into the optical path, attach the polarizer to the condenser mount. When removing the polarizer from the optical path, remove the polarizer from the condenser mount.

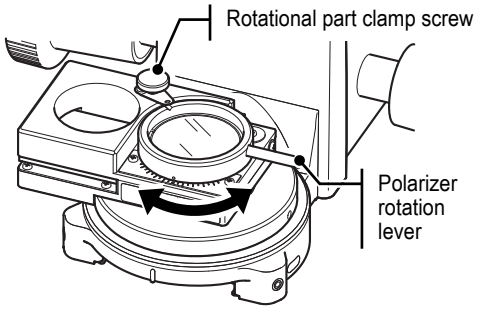
Adjusting the azimuth of the polarizer

Loosen the rotational part clamp screw and adjust the azimuth of the polarizer by moving the lever.

■ **Operation:**

Polarizer rotation lever	Polarizing plate
Turn the lever.	The azimuth changes.

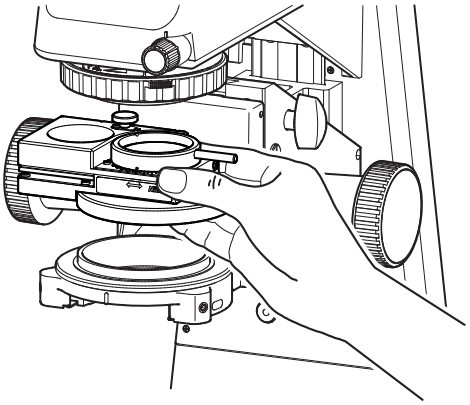
Loosening the clamp screw stops the polarizer from clicking when it is rotated.



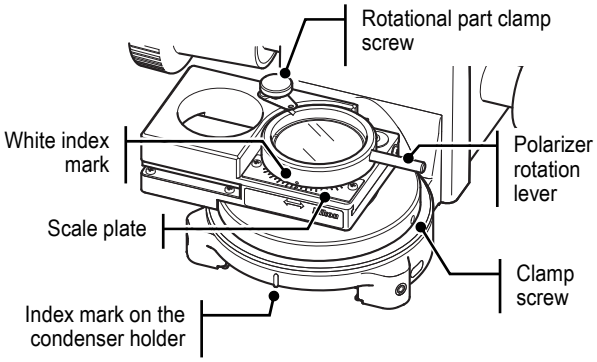
Adjusting the azimuth of the polarizer

Attaching a polarizer and adjusting the azimuth (to form Crossed Nicols)

1. **Place the polarizer on the condenser mount.**
   Do not tighten the clamp screw at this stage.
2. **Remove the DIC condenser module and the DIC slider from the optical path.**
3. **Fully open the aperture diaphragm of the condenser.**
4. **Focus on the specimen, and move the stage to move the field of view to the portion of the specimen where nothing is visible (or the portion of a prepared specimen where a cover glass is placed).**
5. **Place the analyzer and polarizer into the optical path.**
6. **Return the rotational part of the polarizer to the reference position.**
   Aligning the white indicator on the polarizer with the center of the graduations, tighten the rotational part clamp screw.
   Confirm that the steel ball on the tip of the clamp screw is in the groove on the rotational part.
7. **Tighten the clamp screw to fix the rotational part in place.**
8. **Turn the entire polarizer by hand so that the white index mark that was fixed in place in step 6 is lined up with the index mark on the condenser holder.**
9. **Remove one of the eyepieces and attach the centering telescope.**
10. **Turn the entire polarizer until a dark cross appears.**
11. **With the dark cross in view, tighten the clamp screw using a hexagonal screwdriver so that the polarizer is fixed in place.**
12. **Replace the centering telescope with the removed eyepiece.**



Attaching a polarizer



Adjusting and fixing the polarizer

Dark cross on the pupil plane

If no dark cross is observed

- The higher the magnification, the easier the dark cross (Crossed Nicols) is to distinguish. If it is difficult to see a dark cross with a low-magnification objective, replace it with a higher-magnification objective and retry.
- A dark cross may not be visible with some objectives.
  In this case, remove the centering telescope and reinstall the removed eyepiece, increase the illumination, and then observe the specimen plane. Turn the polarizer, and tighten the clamp screw at the position where the field is the darkest.
  The condenser turret can be turned by removing the positioning pin on the condenser turret using a flathead screwdriver. By doing this, the azimuth can be fine-tuned by turning the condenser and polarizer at the same time.

3.5.2 Using the Analyzer

In DIC microscopy and IMSI microscopy, place the polarizer and analyzer in the optical path at the same time, orthogonalize the orientations of both, and conduct observation in the Cross Nicol state.

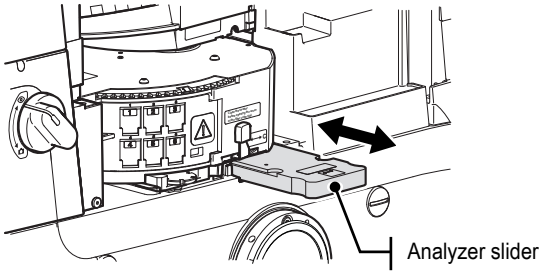
Inserting and removing the analyzer slider

■ **Operation:**

Analyzer slider	Analyzer
Push the slider in to the second click position.	The analyzer enters the optical path.
Return the slider to the first click position.	The analyzer is removed from the optical path.

Inserting and removing the analyzer slider

The analyzer slider van be inserted from either the right or left side of the microscope main body.

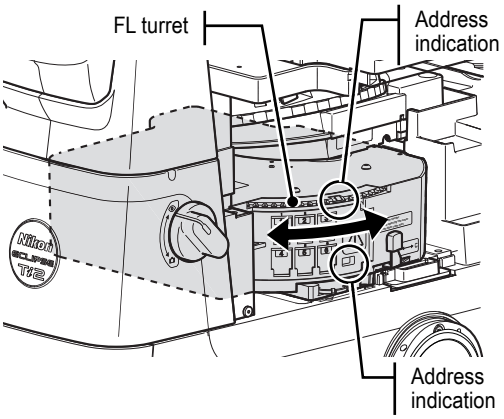


Inserting and removing the analyzer slider

Placing the analyzer block in the optical path

■ **Operation:**

FL turret	Analyzer
Place the address where the analyzer block is attached into the optical path.	The analyzer enters the optical path.
Set it to the A (hollow hole) position or another position.	The hollow hole or another filter cube enters the optical path.



Inserting and removing the analyzer block

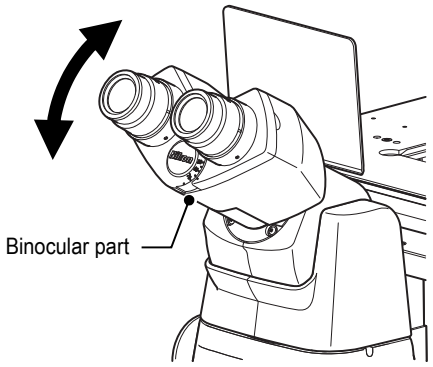
3.6 Using the Tube and the Objective

3.6.1 Adjusting the Angle of the Binocular Part (ER tube)

When using the ER tube (TC-T-ER), the angle of the binocular part is adjustable within the range of 15 to 45 degrees.

■ **Operation:**

Binocular part	Angle of the binocular part
Turn the binocular part upward.	The eyepiece faces upward (max. 45 degrees).
Turn the binocular part downward.	The azimuth of the eyepiece moves closer to horizontal (min. 15 degrees).



Adjusting the angle of the binocular part

Adjust the angle according to your preference for comfortable viewing.

In the case of using an S tube

The S tube (TC-T-TS) has no angle adjustment mechanism for the binocular part.

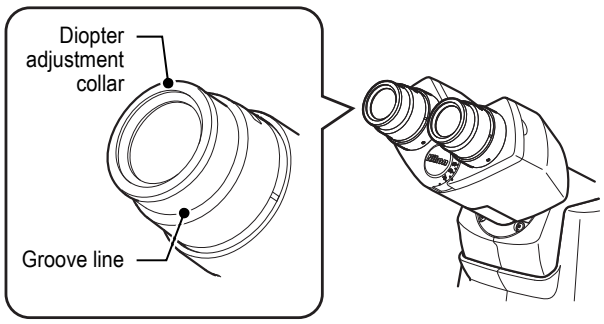
3.6.2 Adjusting the Diopter of the Eyepiece

By turning the diopter correction collar of the eyepiece, the diopter of the eyepiece can be adjusted according to the observer's eyesight.

■ **Operation:**

Diopter adjustment collar	Diopter
Turn it clockwise.	The degree increases.
Turn it counterclockwise.	The degree decreases.

Even if the diopter is adjusted, the tube length remains correct. So the performance of the objective is exerted fully and the focus becomes clearer when the objective is replaced.



Adjusting diopter

Procedure of adjusting the diopter of both eyes

This microscope enables observation through the binocular part using both eyes. By adjusting the diopter for the right and left eye separately with the following procedure, the difference between the right and left eyes are corrected so that observation with both eyes is performed easily.

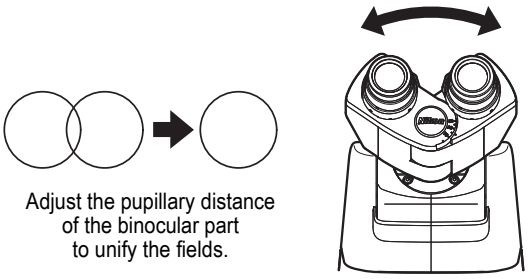
1. **In the BF microscopy status, focus the 10x objective on the specimen.**
2. **Turn the diopter correction collar of the eyepiece for each eye to align the ruling line of the diopter correction collar with the rim of the outer ring of the eyepiece.**
   This position is used as the position of diopter correction “0.”
3. **Place the 40x objective into the optical path.**
4. **While looking through the left eyepiece with your left eye, focus on the specimen by turning the focus knobs.**
5. **Place the 4x or 10x objective into the optical path.**
6. **While looking through the left eyepiece with your left eye, focus on the specimen by turning the diopter correction collar of the left eyepiece.**
   Do not touch the focus knobs of the microscope.
7. **Repeat steps 3 to 6 above twice.**
8. **Adjust the right eyepiece in the same way.**
   Perform steps 3 to 7 for the right eyepiece.

3.6.3 Adjusting Interpupillary Distance

Adjust the distance between the objectives to match the distance between the eyes of the observer. This adjustment makes it easier to perform observation with both eyes.

■ **Operation:**

Binocular part	Distance between eyepieces
Widen the binocular part.	The distance between the eyepieces increases.
Narrow the binocular part.	The distance between the eyepieces decreases.



Adjusting the interpupillary distance

Pupillary distance adjustment

After the diopter adjustment for the eyepieces is complete, place the 10x objective into the optical path, focus on the specimen, and then while looking through the eyepieces with both eyes, adjust the pupillary distance of the binocular part until both fields of view form a single circle.

Use of pupillary distance graduations

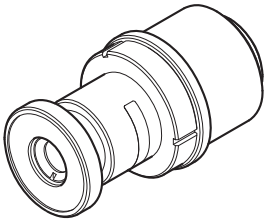
The binocular part has pupillary distance graduations
Remembering the pupillary distance is helpful so that pupillary distance can be easily adjusted the next time.

3.6.4 Operating the Centering Telescope

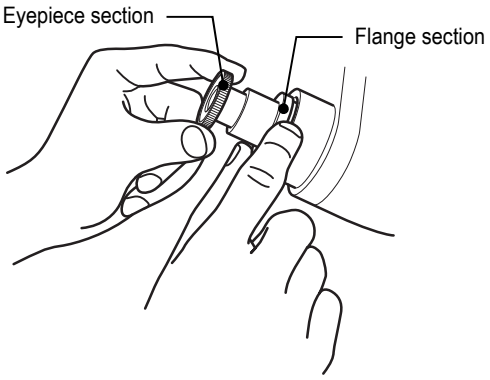
The centering telescope is an optical instrument that is mounted on the binocular part to observe the pupil plane of the objective.

The centering telescope is used for different purposes, such as adjusting the aperture diaphragm, checking for air bubbles during oil-immersion microscopy, adjusting the azimuth of the polarizer, and adjusting the position of the phase ring for Ph microscopy.

To use the centering telescope, remove one of the eyepieces from the binocular part and attach the centering telescope. The focusing position can be adjusted by turning the eyepiece section while holding the flange section on the centering telescope.



C-CT centering telescope



Operating the centering telescope

■ **Operation:**

Centering telescope	Focusing position
While holding the flange section, turn the eyepiece section.	The focusing position of the centering telescope moves.

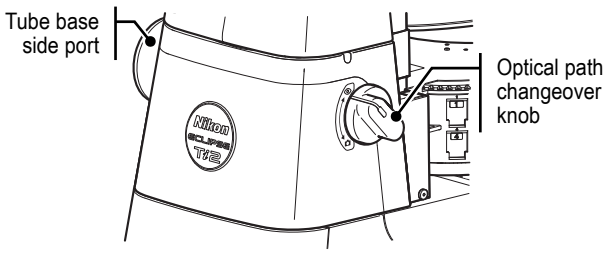
3.7 Using the Tube Base Unit

3.7.1 Switching the Optical Path of the Tube Base Unit (Tube Base Unit with Port)

The eyepiece tube base unit with port has a side port for a camera. The optical path can be switched between the eyepiece section and the side port by using the optical path changeover knob.

■ **Operation:**

Optical path changeover knob	Optical path of the tube base unit
(EYE)	Eyepiece observation port
(CAMERA)	Tube base unit side port



Switching the optical path of the tube base unit

Switching the optical path

To output microscope images from the side port of the tube base unit, select (EYE) using the optical path changeover knob of the microscope main body.

In the case of using an S tube base unit

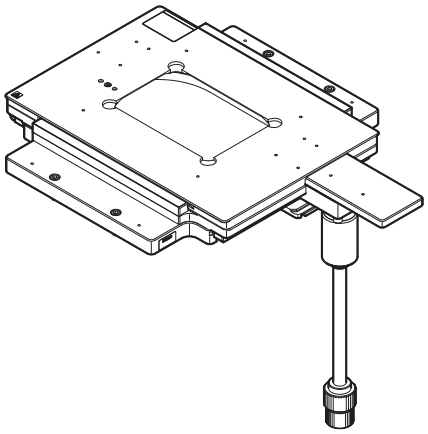
The TI2-T-BS S eyepiece tube base unit has no ports, buttons and knobs.

3.8 Using the Stage

3.8.1 Setting a Sample on the Manual Stage

By attaching a specimen holder to the TC-S-SR manual stage, various types of specimens can be observed. Use a specimen holder appropriate for the specimen to be used.

Product	Model
Terasaki Holder	C-S-HT
Slide Glass Holder	C-S-HS
Universal Holder	C-S-HU
Petridish Holder 35mm	C-S-HP35
Glass Ring Holder	C-S-HG
Acrylic Holder	TC-S-HA
Ring Holder Set	C-S-HLS
Petridish Holder 100mm	C-S-HLP100



TC-S-SR manual stage
(With a long handle mounted)

Tilting the pillar for dia-illumination
When placing a large specimen on the stage, tilt the pillar for dia-illumination as required (☞ 3.3.5).

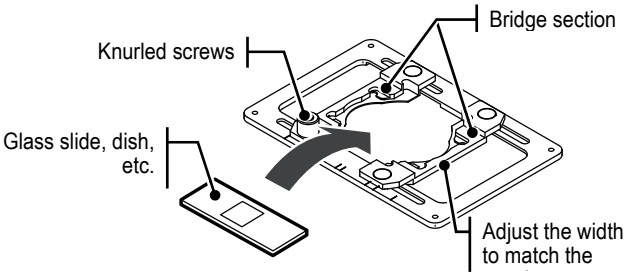
Attaching auxiliary modules
- The optional stage clips can be attached to the screw holes on the upper surface of the specimen holder.
- On the upper surface of the stage, there are screw holes for fixing an auxiliary module such as a manipulator.
- When using a laser light source, attach a laser safety cover.

This section explains how to use the universal holder, petridish holder, and well clamper.

Using the universal holder

The universal holder is used to hold a glass slide (prepared specimen slide) or dish in place.

■ **Operation:**
- Adjust the width of the bridge section to match the specimen, and then put the specimen in place.
- The position of the single-side bridge section can be fixed using a knurled screw.
- Affix the optional stage clips (TS-S-SC), if any, to the two (out of four) screw holes on the specimen holder.



Using the universal holder

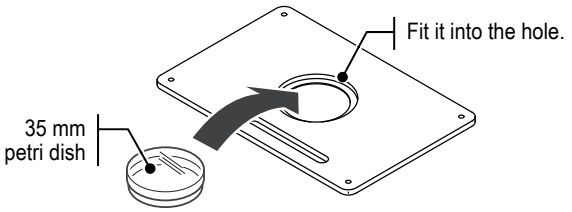
Notes on use of the universal holder
The bridge section of the universal holder can be placed on the objective.
When observing the specimen near the holder section, take sufficient care not to let the objective collide with the holder section.

Using the 35-mm-diameter petri dish holder

This holder is for use with 35-mm-diameter petri dishes.

■ **Operation:**

- Insert the 35 mm petri dish into the central hole.
- Use an optional stage clip to fix the specimen, as required.



Using the 35 mm diameter Petri dish holder

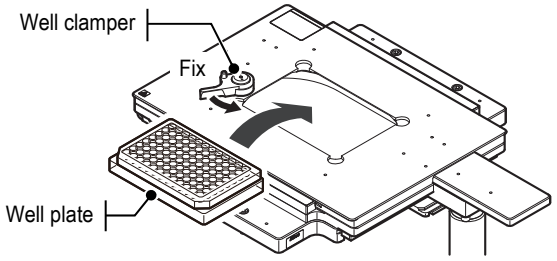
> **Oil and water immersion operation using a petridish holder 35mm**
>
> The petridish holder 35mm has an elongated slot in front through which oil and water immersion of the objective is possible. Hold the nosepiece by hand so that the tip of the objective positions below the hole, and perform oil or water immersion.

Using the well clamper

Place the well plate directly on the holder section of the manual stage, and fix the well plate with the optional well clamper.

■ **Operation:**

- Open the well clamper, correctly orientate the well plate, and place it on the holder section of the stage.
- Close the well clamper gently and fix the well plate.



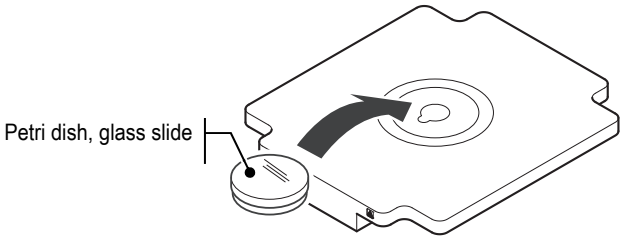
Using the well clamper

3.8.2 Setting a Sample on the Plain Stage/Gliding Stage

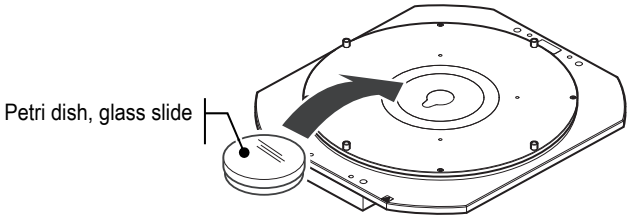
Various types of stage rings can be mounted on the TI-SP Plain Stage and the TC-S-GR Gliding Stage.

■ **Operation:**

- Put a petri dish or a glass slide (prepared specimen slide) on the stage ring.
- When using a gliding stage, use an optional stage clip to fix the specimen, as required.



Setting a sample (plain stage)



Setting a sample (gliding stage)

> **Reapplying the oil when using a stage ring**
>
> If the stage ring has a notch for lubrication, an oil-immersing operation can be performed without taking out a vessel such as a petri dish from the stage.
>
> Set the notch of the stage ring so that the notch matches the rotational direction of the nosepiece. Then, manually affix the nosepiece so that the objective is positioned at the notch, and perform oil-immersion operation.

3.8.3 Moving a Sample on the Stage

Using a manual stage

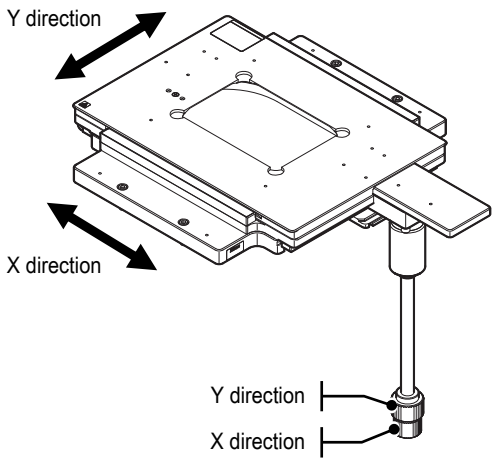
The observation part of the specimen can be moved in the X-axis and Y-axis directions using the stage handles.

■ **Operation:**

Stage handle	Moving direction of the manual stage
Turn the upper handle.	The stage moves in the Y-axis direction.
Turn the lower handle.	The stage moves in the X-axis direction.

The stage can be fixed even when it is rotated 180 degrees. This allows setting the handle position either on the right or left of the microscope.

The length of the stage handle can be selected from long, middle and short at the time of purchase.



Moving the manual stage

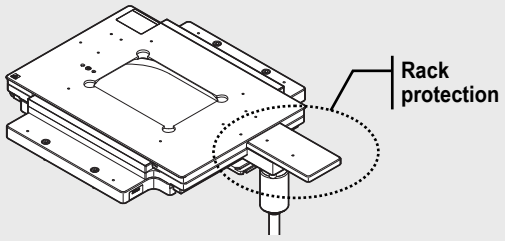
Movement stroke of the manual stage

- The movement stroke of the manual stage is 114 x 73 mm.
- The movement stroke can be restricted to either 75 x 50 mm or 18 x 18 mm using the supplied limit screw.
  For details on the procedure for setting the stroke, see “Chapter 7 Assembly of the Devices.”

Rack protection plate for the manual stage

A protection plate for protecting the rack of the stage is located on the top of the stage handle.

Because the protection plate protrudes from the stage, take due care not to bump your hands or other objects against the plate.

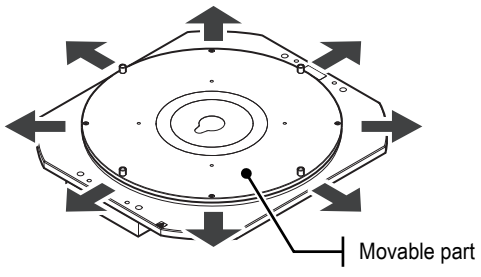


Using a gliding stage

By moving the movable part on the upper surface of the stage, a specimen can be moved to the front/back and right/left.

■ **Operation:**

Movable part	Sample
Move it by hand.	The sample moves in a required direction.



Moving the gliding stage

Movement stroke of the gliding stage

The movement stroke of the gliding stage is 20 mm diameter.

Gliding stage maintenance

If the gliding stage is used beyond its warranty period, the power required to operate it might change. In that case, it is possible to restore to the original status by maintenance work (at a fee). For details, contact your local Nikon representative.

3.9 Using the Nosepiece

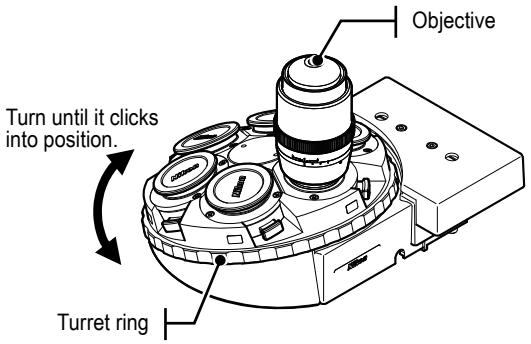
3.9.1 Turning the Nosepiece to Switch the Objective

When switching objectives, manually turn the turret ring of the nosepiece clockwise or counterclockwise until it clicks into position.

■ **Operation:**

Turret ring on the nosepiece	Objective
Turn it clockwise.	The objective with the next address enters the optical path.
Turn it counterclockwise.	The objective with the previous address enters the optical path.

Each address of the nosepiece is marked in the center of the nosepiece and beside the objective mounting hole.



Controlling the manual nosepiece (DIC nosepiece)

Escape operation when the objective is switched

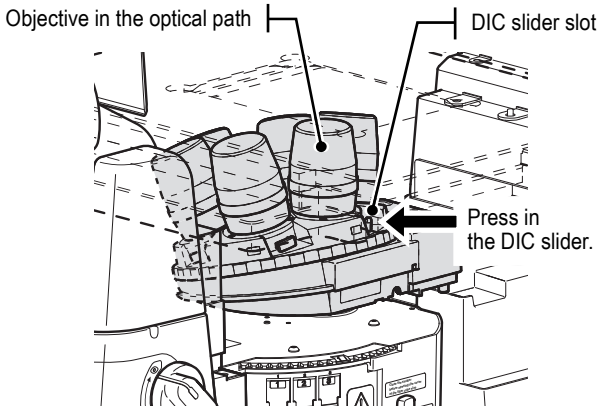
When switching the objective by turning the nosepiece, move the focusing device to lower the objective and then turn the nosepiece so as to prevent the objective from contacting the specimen. (☞ 3.11)

3.9.2 Placing the DIC Prism into the Optical Path

When performing DIC microscopy, insert the DIC slider in the DIC slider slot of the nosepiece.

■ **Operation:**

DIC slider	DIC prism
Press in the slider up to the limit position.	The DIC prism enters the optical path.
Pull out the slider.	The DIC prism is removed from the optical path.



Inserting and removing the DIC slider

Attaching a DIC slider slot cap

If a DIC slider is not in use, attach a DIC slider slot cap to prevent dust and other foreign matter from entering the nosepiece.

3.10 Using the Objective

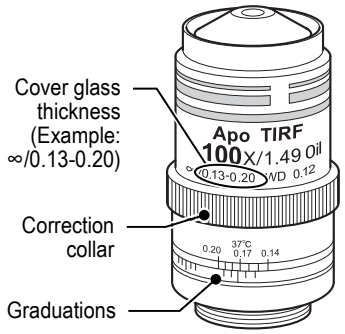
3.10.1 Adjusting the Objective with the Correction Collar

When observing a sample through the bottom plate (cover glass) of a transparent vessel such as a petri dish or a culture bottle, aberration caused by the difference in thickness of the cover glass can be corrected by using an objective with a correction collar.

■ **Operation:**

Correction collar	Appearance of the image
Turning the correction collar	Aberration caused by difference in glass thickness is corrected.

The current correction value can be checked by the graduations and the index mark on the correction collar.



Example of an objective with a correction collar

Correction collar adjustment procedure

1. **Match the graduation of the correction collar with the thickness of the cover glass of the vessel.**

   When measuring the thickness of the glass, either physically measure it or refer to the standard value used by the vessel manufacturer.
2. **Focus on the specimen with the focus knobs.**
3. **If the resolution and contrast of the image are poor, slightly turn the correction collar on the objective in either direction.**

   This will shift the focus slightly. Readjust the focus by turning the fine-focus knob.
4. **If the image becomes clearer, slightly turn the correction collar again in the same direction. If the image becomes less clear, slightly turn the collar in the opposite direction.**
5. **Adjust the focus again, and check the clarity of the image.**
6. **Repeat steps 4 and 5 until the clearest image is obtained.**

Adjustment of the correction collar

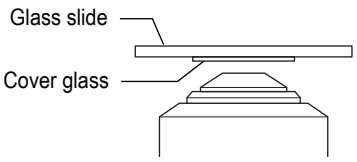
- Nikon recommends writing down the location of the correction collar of the best resolution. That will serve as a reference when a vessel with a different thickness of the cover glass is used.
- The position of 0 mm at the correction collar indicates the position for observation of specimens without cover glass in an inverted microscope.
- Portions with varying thicknesses (such as periphery of the vessel) in the cover glass cannot be corrected. Use the correction function where the thickness of the glass is uniform.

Designated cover-glass thickness

Objectives are labeled with the supported cover glass thickness. For instance, “∞/0.17” indicates that a designated cover glass thickness of 0.17 mm.

■ Glass thickness of 0.17 mm:

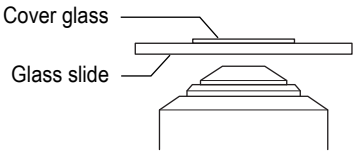
When using the objective labeled “0.17”, use a cover glass with a thickness of 0.17 mm. In this case, face the cover glass downward, and set the specimen so that the cover glass faces the objective.



Glass thickness of 0.17 mm

■ Glass thickness of 1.2 mm:

When using the objective labeled “1.2”, use a normal cover glass with a thickness of 1.2 mm. In this case, face the cover glass upward, and set the specimen so that the glass slide faces the objective.



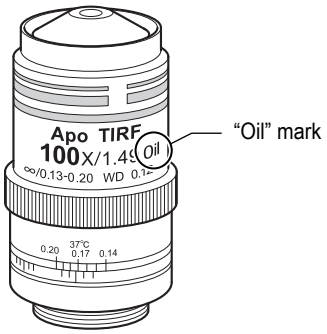
Glass thickness of 1.2 mm

3.10.2 Using Oil Immersion Objectives

Objectives marked with “Oil” are oil immersion objectives.

■ **Operation:**

- When using an oil immersion objective, fill the gap between the tip of the objective and the specimen with oil (Nikon immersion oil). When performing fluorescence microscopy using an oil immersion objective for fluorescence microscopy, use low-fluorescence oil.
- After using an oil immersion objective, wipe the oil off the tip.

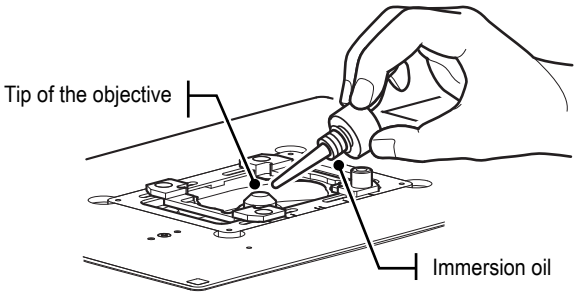


Example of oil immersion objective

Immerse the objective in the oil.

1. **Lower the objective.**
2. **Apply the minimum required amount of oil at the tip of the objective.**

   Take care not to let air bubbles get mixed in. In addition, use as little oil as possible (just enough to fill the gap between the tip of the objective and the specimen), and take care not to allow the oil to adhere to other parts.
3. **Place the specimen on the stage.**
4. **Slowly raise the objective by turning the focus knobs, allowing the oil on the tip of the objective to touch the bottom surface of the specimen.**
5. **Check the oil for bubbles.**
6. **Observe the specimen while operating the stage and the focus knobs.**
7. **After using an oil immersion objective, wipe the oil off the tip.**



Oil immersion

Handling of immersion oil

- If air bubbles are mixed in the immersion oil, the quality of the microscope images will decrease. When using immersion oil, take care not to let air bubbles get mixed in.
- Oil remaining at the tip of the oil-immersion lens after the wiping or oil adhering to the tip of the dry lens will adversely affect the image. After use, thoroughly wipe off all oil, and make sure that no oil adheres to the tips of other objectives.

Wiping off oil

- When wiping off oil, use a lens tissue or clean cloth moistened with benzine and gently wipe off a few times. To wipe off oil cleanly, be sure to use the same portion of the tissue or cloth only once. For a clean finish, use absolute alcohol (ethyl alcohol or methyl alcohol) at the end.
- If petroleum benzine is unavailable, use methyl alcohol instead. However, because the cleaning power of methyl alcohol is weak, the user may need to wipe several times (usually three or four times).
- When wiping oil off the specimen, take due care not to damage the specimen.

Using the refocusing ring

Use the objective refocusing lever to efficiently refocus on the specimen when oil-immersion observation is repeated.

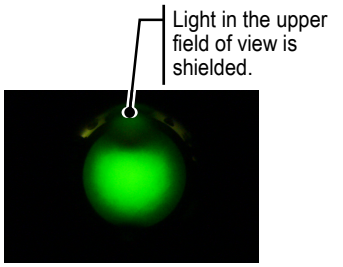
CAUTION

Take due care when handling absolute alcohol or petroleum benzine, as they are highly inflammable. Do not bring any ignition source or use the power switch near absolute alcohol or petroleum benzine.

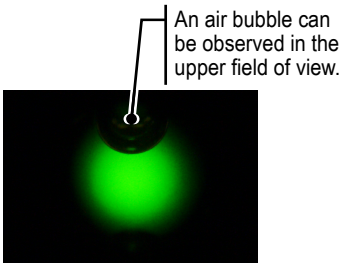
Checking for air bubbles

- Air bubbles can be checked for by observing the pupil plane of the objective. Remove the eyepieces, attach the centering telescope, and focus on the pupil plane of the objective by turning the eyepiece part of the centering telescope.
- If there are air bubbles, slightly turn the nosepiece to move the oil-immersed objective once or twice to remove the air bubbles. If the air bubbles still remain, wipe off the oil and then apply it again.

(1) Objective pupil when the Bertrand lens is placed into the optical path



(2) Field of view after the focus of the Bertrand lens changed from (1)

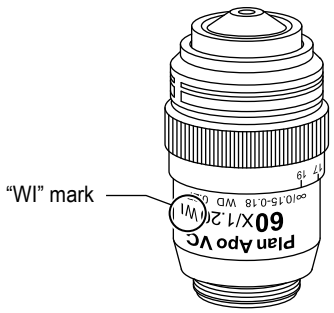


3.10.3 Using a Water Immersion Objective

Objectives marked with “WI” are water immersion objectives. Use water immersion objectives for inverted microscopes.

■ **Operation:**

- When using a water immersion objective, fill the gap between the tip of the objective and the specimen with pure or distilled water.
- After using a water immersion objective, wipe the water off the tip.



Example of a water immersion objective

Handling a water immersion objective

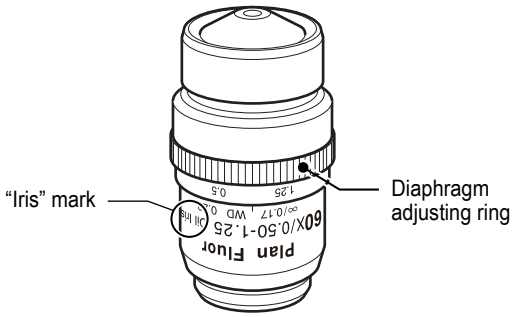
- **When immersing an objective in water, avoid using tap water. There are impurities in tap water that might adhere to the glass surface and harden when the water at the lens tip dries off. This might cause the lens to be scratched or damaged at the time of cleaning.**
- **Plan Apo 60×WI (NA = 1.2) is provided with a correction collar so that the optimal aberration can be obtained for variations of cover glass thickness. Scale display “17” indicates 0.17 mm. For precise correction, measure the thickness of the cover glass with an instrument such as a micrometer and match the thickness with the graduation level of the correction collar, before using the cover glass.**
- **After using the objective, wipe the water off the surface. Otherwise, the metallic portions might become discolored or rusted, or the lens may lose transparency.**
- **After wiping the water off the surface of the objective, use absolute alcohol for a finishing wipe.**
- **If water dries off naturally and a smear-type stain remains, apply a small amount of neutral detergent, gently wipe off the stain, and then use absolute alcohol for a finish wipe.**

3.10.4 Using an Objective with a Diaphragm

When performing DF microscopy, use of an objective with diaphragm allows numerical aperture (NA) adjustment of the objective according to the NA of the DF condenser.

- **Operation:**
  Check the minimum NA of the DF condenser, and then adjust the diaphragm adjusting ring so that the NA of the objective is smaller than the minimum NA.

- **Display:**
  Check the numerical aperture using the graduations on the diaphragm adjusting ring.



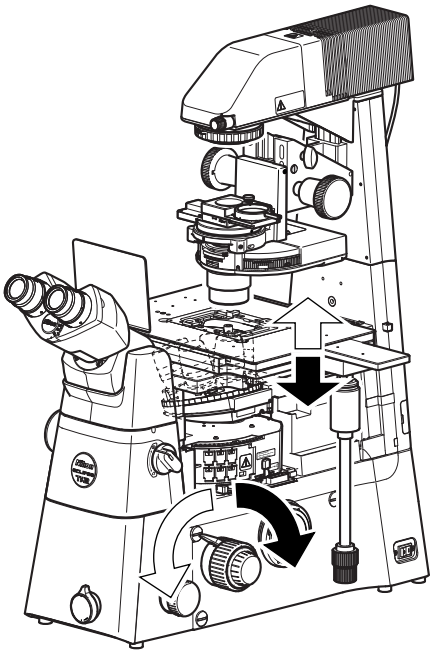
Example of an objective with a diaphragm

3.11 Using the Focusing Device

3.11.1 Focusing on the Sample

Turning the focus knobs on the microscope main body moves the objective vertically, enabling the user to focus on the specimen.

The focus knobs are divided into the following two types. The coarse-focus knob is used to vertically move the objective a large distance, while the fine-focus knob is used to move the objective vertically in small steps.



Elevating movement of the focusing device (objective)

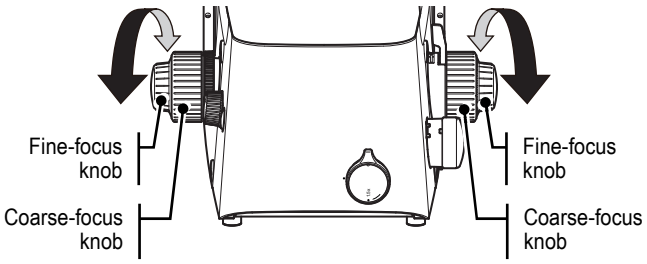
■ **Operation:**

Move the focusing device in fine-motion.

Focus knob	Focusing device
Turn the fine-focus knob to the front (in the direction of the UP arrow).	The focusing device moves upward in fine-motion mode.
Turn the fine-focus knob to the rear (in the opposite direction of the UP arrow).	The focusing device moves downward in fine-motion mode.

Move the focusing device in coarse-motion.

Focus knob	Focusing device
Turn the coarse-focus knob to the front (in the direction of the UP arrow).	The focusing device moves upward in coarse-motion mode.
Turn the coarse-focus knob to the rear (in the opposite direction to the UP arrow).	The focusing device moves downward in coarse-motion mode.



Operating the focus knobs

■ **Degree of objective movement:**

The following table shows the degree of objective movement when the focus knobs are turned.

Degree of knob rotation	Degree of objective movement
One graduation step of the fine-focus knob	1 µm
One turn of the fine-focus knob	0.1 mm
One turn of the coarse-focus knob	5 mm

Movement stroke of the focusing device

The movement stroke of the focusing device is about 8 mm above and about 2 mm below the reference position.

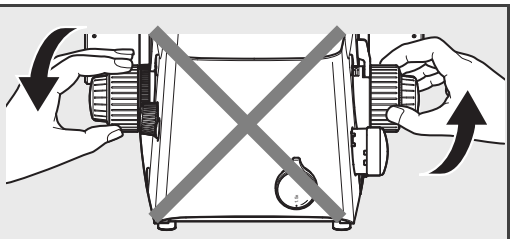
Points on focusing

- When using the coarse-focus knob to move the objective upward, keep your eyes away from the eyepieces, and operate the knob while checking the distance between the specimen and the objective, taking care not to let the objective touch the specimen.
- When moving the objective with the coarse-focus knob while looking through the eyepieces, always move the objective downward.
- Lower magnification objectives are easier to focus. First use a lower magnification objective to focus, and then switch it to a higher magnification objective.

Using the focus knob

To avoid malfunction, never perform the following operations:

- **Rotate the right and left focus knobs in opposite directions.**
- **Attempt to rotate the coarse-focus or fine-focus knob beyond the rotation limit.**



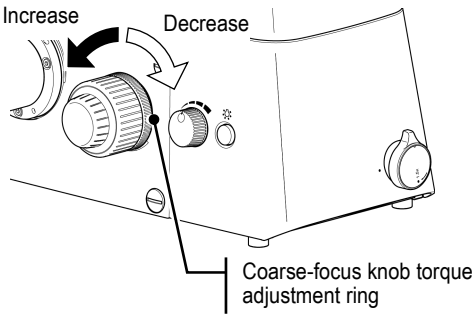
3.11.2 Using the Coarse-Focus Knob Torque Adjustment Ring

The focus knob on the left side of the microscope is equipped with a coarse-focus knob torque adjustment ring for adjusting the torque of the coarse-focus knob.

■ **Operation:**

Coarse-focus knob torque adjustment ring	Torque of the coarse-focus knob
Turn the ring to the rear (counterclockwise)	Increase
Turn the ring to the front (clockwise)	Decrease

Note: The torque of the fine-focus knob is fixed.



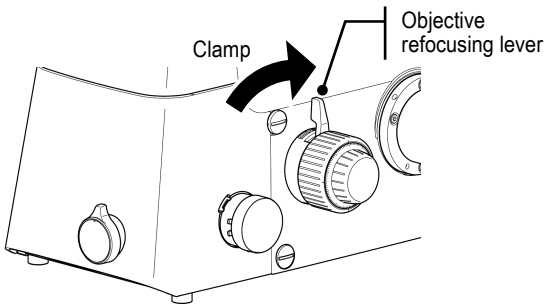
Using the coarse-focus knob torque adjustment ring

Adjusting the coarse-focus knob torque

Excessively loosening the coarse-focus knob torque adjustment ring might lower the nosepiece due to its own weight, causing the specimen to become out of focus during observation.
Adjust the ring to obtain appropriate torque.

3.11.3 Using the Objective Refocusing Lever

The focus knob on the right side of the microscope is equipped with an objective refocusing lever for setting the current objective position as the rotation limit of the coarse-focus knob by turn the lever to the vertical position (CLAMP). This can prevent the objective from touching the specimen when the coarse-focus knob is operated.



Using the objective refocusing lever

■ **Operation:**

Objective refocusing lever	Vertical movement of the nosepiece (by the coarse-focus knob)
Turn the lever to the vertical position (vertical upward) (in the direction of the CLAMP arrow).	The knob is restricted to prevent the objective from moving beyond the current position.
Pull the lever to the front (in the opposite direction to the CLAMP arrow).	Vertical movement restriction is released.

This lever does not influence the movement of the fine-focus knob. This means that the objective can be moved above the limit position by turning the fine-focus knob.

Escaping and restoring the objective with the refocusing lever

When the objective is escaped to change the specimen or switch the objective, using the objective refocusing lever enables the user to restore the focus to its original position easily by simply turning the coarse-focus knob to the limit of its rotation.

3.12 Using the FL turret

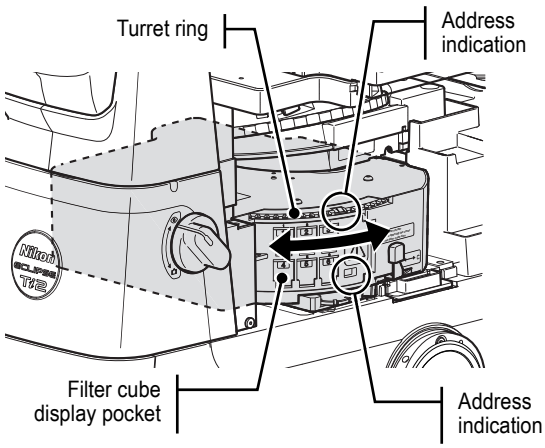
3.12.1 Switching the Filter Cube

When switching the filter cube, turn the turret ring manually to the right or left and stop it at the click position.

■ **Operation:**

Turret ring	Filter cube
Turn the turret ring until it clicks into position.	The filter cube at the preceding or succeeding address enters the optical path.

The filter cube in the optical path can be checked from the address display of the FL turret.



Using the FL turret

Filter cube replacement

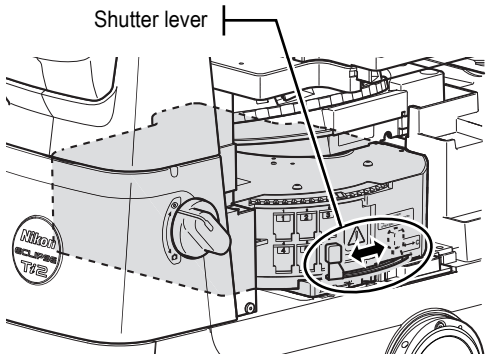
For details on the procedure for replacing filter cubes within the FL turret, see “Chapter 7 Assembly of the Devices.”

3.12.2 Using the Shutter of the FL Turret

The shutter inside the FL turret can be opened and closed using the shutter lever.

■ **Operation:**

Shutter lever	FL turret shutter
Move the lever to the O side (front).	The shutter opens.
Move the lever to the C side (rear).	The shutter closes.



Opening and closing the FL turret shutter

Use of FL turrets in a two-tier configuration

FL turrets can be used in a two-tier configuration by mounting a stage up kit.

When adopting a two-tier configuration, for example, use the lower tier for Epi-FL microscopy by attaching an epi-fluorescence attachment, and use the upper tier for experiments by attaching a photo-activation device. For details on how to use FL turrets in a two-tier configuration, see the instruction manual for the Ti2-LAPP system.

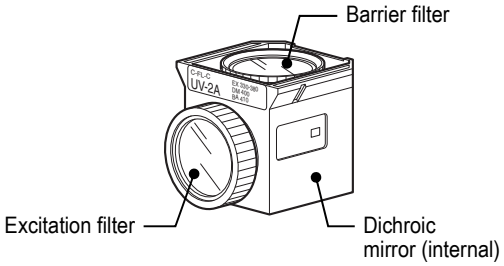
This manual basically describes the FL turret on the first (lower) tier and, also describes the turret on the second (upper) tier as required.

3.12.3 Selecting Filter Cubes

The fluorescent filter cube (mounted on the turret) consists of the three types of optical components: the excitation filter (EX filter), the barrier filter (BA filter), and the dichroic mirror (DM), that are already attached.

Select the filter cube with the combination of suitable optical components according to the characteristics of the specimen and the fluorescence dye.

For details on filter cubes, see “4.4.2 Optical Elements Required for Epi-FL Microscopy.”



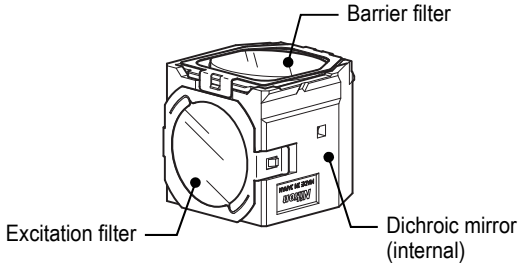
Fluorescent filter cube

> **Replacement of optical elements**
> Excitation filters, barrier filters, and dichroic mirrors can be purchased separately.
> For details on replacing the optical components, see “7.5.3, Replacing an Excitation Filter, a Barrier Filter, and a Dichroic Mirror.”

Filter cube for large FOV

The Ti2-series FL turret supports the filter cubes for large FOV that have the following characteristics:

- **Supporting a large field:** This filter cube supports a filter with an outer diameter of 32 mm (effective diameter of 29 mm), and improves the uniformity of the field of view when a camera with a field number of 22 mm on the imaging surface is used. (Conventional filter cube uses a 25 mm filter.)
- **Applicable microscopy:** The thick dichroic mirror is suitable for applications that require high degree of the dichroic mirror flatness such as TIRF observation.
- **Maintaining mounting compatibility:** The mount part for the FL turret is compatible with the conventional 25 mm-diameter series.



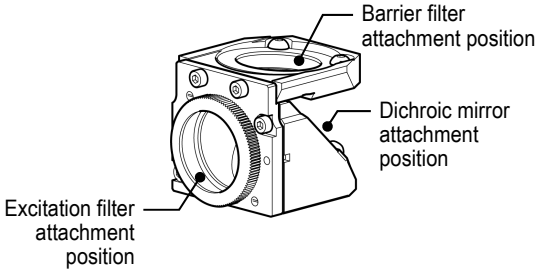
Filter cube for large FOV

> **Replacement of optical elements**
> Excitation filters, barrier filters, and dichroic mirrors can be purchased separately.
> For details on replacing the optical components, see “7.5.3, Replacing an Excitation Filter, a Barrier Filter, and a Dichroic Mirror.”

C-FL-HQ filter cube

Nikon provides a C-FL-HQ filter cube that does not include optical elements. Select a desired combination of three optical elements (barrier filter, excitation filter and dichroic mirror) and attach each element to the C-FL-HQ filter cube for use.

> **Attachment of optical elements**
> For details on how to attach optical elements, see “7.5.4 Attaching Optical Elements to the C-FL-HQ Filter Cube.”



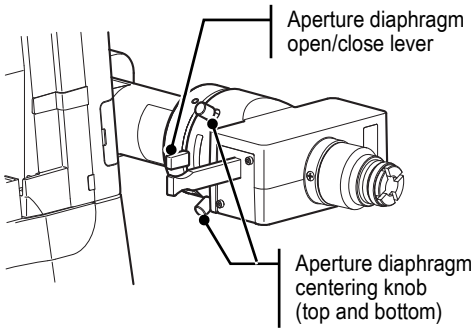
C-FL-HQ filter cube

3.13 Using the Epi-fluorescence Attachment

3.13.1 Adjusting the Aperture Diaphragm of the Epi-fluorescence Attachment

The aperture diaphragm is used to adjust the number of illumination apertures. In an optical system for the epi-fluorescence attachment, the aperture diaphragm adjusts the brightness of the image and the amount of stray light.

The epi-fluorescence attachment modules are equipped with an aperture diaphragm open/close lever and a centering knob, which enable the user to adjust the aperture diaphragm.



Aperture diaphragm open/close lever (EPI-FL module)

■ **Operation:**

Aperture diaphragm open/close lever*	Aperture diaphragm
Turn it to the lower side.	The diameter of the diaphragm increases.
Turn it to the upper side.	The diameter of the diaphragm decreases.

* When the main branch is used in an inverted layout, the direction of operation is the opposite.

The narrower the aperture diaphragm is, the smaller, the amount of stray light becomes, but the darker the image of the specimen becomes. The wider the aperture diaphragm is, the brighter, the image becomes, but the larger the amount of stray light becomes.

By narrowing the aperture diaphragm to the size equal to the pupil diameter of the objective, brightness of the specimen image can be kept and stray light can be removed effectively.

Using the aperture diaphragm open/close lever

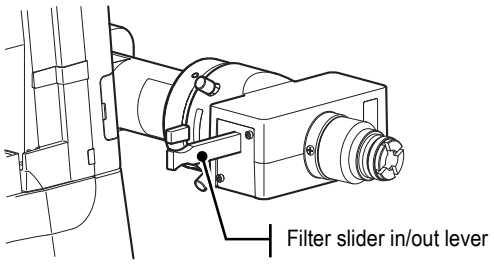
The label shown on the right is affixed next to the aperture diaphragm open/close lever.
Pay attention to the orientation of the label when adjusting the aperture diaphragm of the epi-fluorescence attachment.

3.13.2 Using the Filter Slider

Up to two filters with a diameter of 25 mm and a thickness of up to 5.5 mm can be attached to the filter slider.

■ **Operation:**

Filter slider	Filter
Push in until it clicks into position.	The front filter (on the lever side) enters the optical path.
Pull out until it clicks into position.	The rear filter enters the optical path.



Operating the filter slider

Replacing filters

For details on the procedure for replacing filters, see “Chapter 7 Assembly of the Devices.”

Use of an excitation filter

The excitation filter, which allows specific wavelengths of light from the light source to pass through, is intended for Epi-FL microscopy.

Normally the excitation filter is used by attaching a filter cube to the FL turret. By attaching an excitation filter to the filter slider of the epi-fluorescence attachment, the excitation filter can be switched separately from the filter cube.

Combination of an excitation filter and a barrier filter

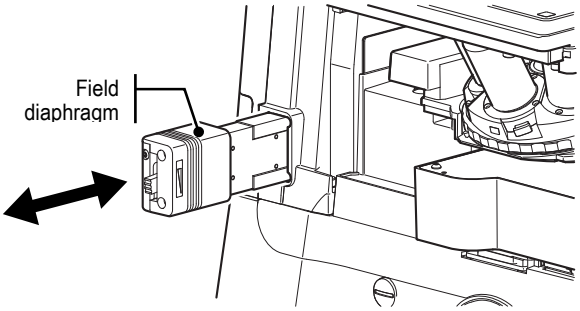
When using an excitation filter mounted in a filter slider, always check the setting status of the barrier filter in the filter cube before starting observation.

Take sufficient care that the combination of the excitation filter and the barrier filter is correct. Incorrect combination might allow very strong light to be transmitted.

3.13.3 Using the Field Diaphragm Slider

Inserting or removing the field diaphragm

The field diaphragm is a diaphragm for restricting the illumination range. The entire field diaphragm can be placed into or removed from the optical path by inserting or removing the field diaphragm slider. Therefore, if the field diaphragm has been adjusted to match the specified optical path beforehand, the field diaphragm status can be restored simply by operating the slider.



Moving the field diaphragm slider

■ **Operation:**

Field diaphragm slider	Field diaphragm
Push in until it clicks into position.	The field diaphragm enters the optical path.
Pull out to the limit position.	The field diaphragm is removed from the optical path.

Using the field diaphragm slider

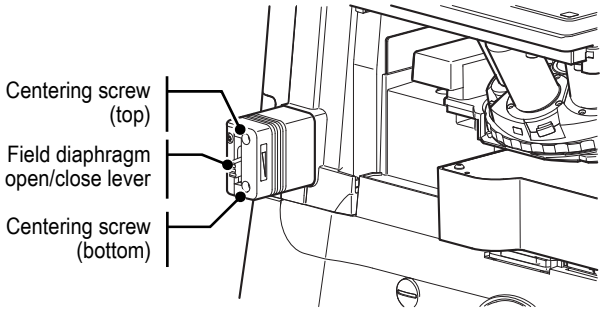
If the field diaphragm has been adjusted to match the diameter of the camera beforehand, a large field of view can be observed through the binocular part just by pulling out the slider. Inserting the slider back in place enables the camera to be used straightaway.

Using the field diaphragm slider during large field-of-view microscopy

To perform microscopy with a large field of view with a field number of equivalent to or more than 22, remove the field diaphragm slider from the optical path.

Adjusting the size of the field diaphragm

Adjust the size of the field diaphragm using the field diaphragm open/close lever of the field diaphragm slider.



Moving the field diaphragm slider

■ **Operation:**

Field diaphragm open/close lever	Field diaphragm
Push it upward.	The diameter of the diaphragm increases.
Push it downward.	The diameter of the diaphragm decreases.

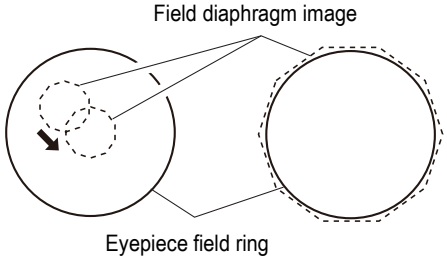
Ordinarily stop down the diaphragm to the degree that it circumscribes (or inscribes) the field. An illumination range wider than necessary causes stray light to come in from other sources. As a result, a flare occurs and the contrast of the optical image decreases, and the specimen might have a wider range of discoloration.

Size of the field diaphragm when capturing images

Adjustment of the field diaphragm is especially important for digital cameras. Generally good results are obtained by narrowing the field diaphragm to a range a little broader than the capturing area (shooting range) of the camera.

Adjust the position of the field diaphragm (centering adjustment)

1. **Focus on the sample in episcopic BF.**
2. **Stop down the field diaphragm using the field diaphragm open/close lever.**
3. **Turn the field diaphragm centering screw using a 2 mm hexagonal screwdriver to move the center of the field diaphragm image to the center of the field.**
4. **Widen the field diaphragm to approximately the same size as that of the field using the field diaphragm open/close lever.**
5. **Turn the field diaphragm centering screw to move the center of the field diaphragm image to the center of the field again.**



Centering and size adjustment for the field diaphragm

Differences by diaphragm shape

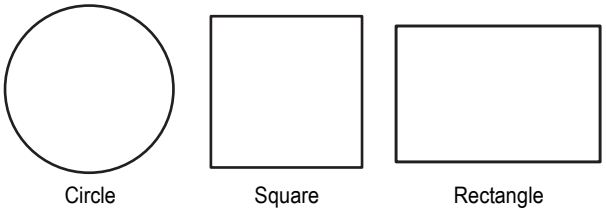
There are three types of field diaphragm sliders: Each type differs in diaphragm shape and has the following characteristics:

- **Circular field stop slider (TI2-F-FSC)**

  Slider with a circular field diaphragm
- **Square field stop slider (TI2-F-FSS)**

  Slider with a square field diaphragm This type is suitable for microscopy with a camera having a square imaging element.
- **Rectangle field stop slider (TI2-F-FSR)**

  Slider with a rectangular (3:2) field diaphragm This type is suitable for microscopy with a camera having a horizontally long imaging element.



Difference by the shape of the field diaphragm

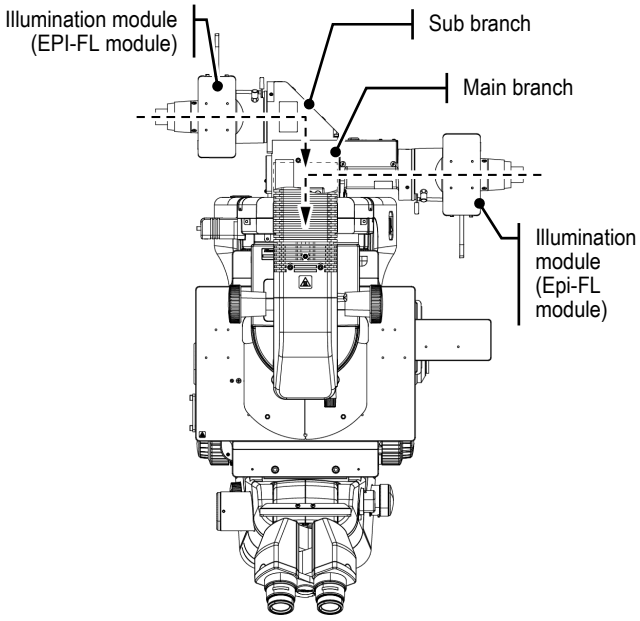
3.13.4 Switching the Optical Path of the Main Branch

To switch the optical path of the main branch, use the optical path changeover lever to switch between insertion and removal of the switching mirror.

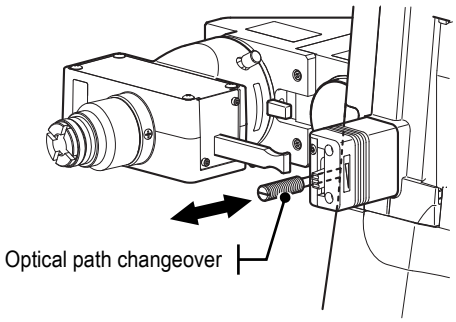
■ **Operation:**

Optical path changeover lever	Switching mirror
Push the lever up to the limit position.	The mirror is switched to the illumination module on the main branch side.
Pull out the lever up to the limit position.	The mirror is switched to the illumination module on the sub branch side.

The changeover status of the optical path of the main branch can be identified as the insertion/removal status of the optical path changeover lever.



Switching the optical path of the main branch



Using the optical path changeover lever

Using the switching mirror

The optical path of the main branch can be switched by inserting or removing the internal switching mirror.

Although only full mirrors and half mirrors are provided by Nikon, a commercially-available dichroic mirror can also be attached according to the microscopy technique.

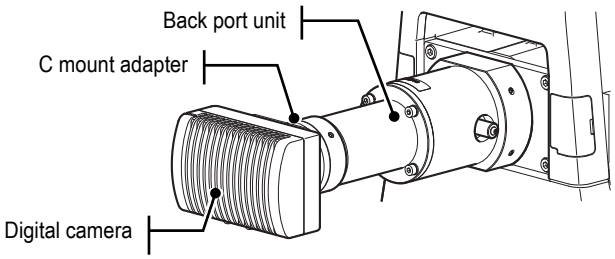
Restrictions for the fixed main branch

Multiple illumination modules cannot be mounted at the same time.

3.14 Using a Back Port Unit (Optional)

An additional camera device can be mounted by attaching a TI-BPU back port unit to the back port on the rear of the illumination pillar.

Because a filter cube (dichroic mirror) in the FL turret is used to split the optical path to the back port, the FL turret can be used to select a wavelength.

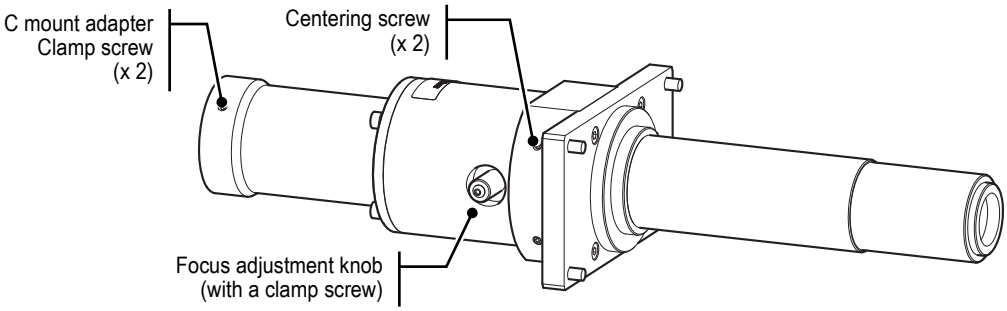


Example of attaching a back port unit

> **Important**
> A dichroic mirror in the filter cube is used to split optical paths to the back port. A poor flatness of the dichroic mirror causes lens effects by curved reflection, resulting in poor imaging status. Use a dichroic mirror with high flatness for splitting optical paths.

Using a back port unit

- **Focusing:**
  Loosening the clamp screws allows turning the focus adjustment knob.
  Turn the focus adjustment knob while watching a camera captured image to align it with an observation image in the eyepieces.
- **Centering:**
  Tightening and loosening two centering screws allows centering a captured image.
  When two cameras are used for a simultaneous capture, make sure that the images captured by these two cameras are centered.



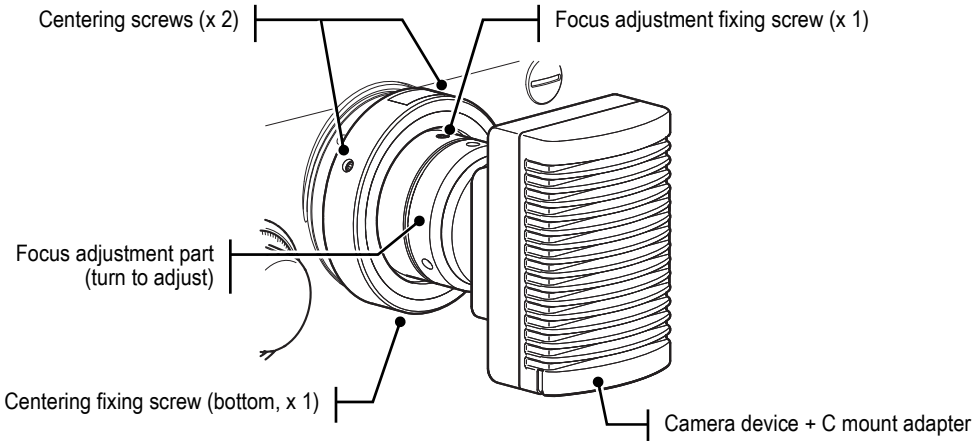
TI-BPU back port unit

Simultaneous Multi-wavelength Microscopy (by Using Stage Up Kit)

If the FL turret is set up in a two-tier (upper and lower) configuration by using the stage up kit, a simultaneous multi-wavelength microscopy is allowed using two cameras by mounting the TI-BPU Back Port Unit to add a camera port.

3.15 Using a Centering C-mount Adapter

Focusing and centering of the camera can be achieved using a centering C-mount adapter, if it is used for attaching the camera device.



Example of attaching a centering C-mount adapter

Adjusting a camera to focus on the specimen

The focus adjustment part can be turned by loosening the focus adjustment fixing screw.

Turn the focus adjustment part while watching the image captured by the camera so that the view of the image becomes equivalent to the image observed through the eyepieces.

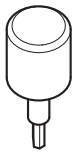
After focus adjustment is complete, tighten the focus adjustment fixing screw to fix the focus adjustment section.

Centering adjustment for the camera device

Tightening and loosening two centering screws allows centering a captured image.

Adjustment tool

When rotating the focus adjustment fixing screw and the centering screw, use the dedicated tool provided with this product.



Dedicated tool

Chapter
4

Microscopy Techniques

This chapter explains the principles of each microscopy, and optical elements required for implementing each microscopy, and combinations of optical elements.

4.1 Details of Diascopic Bright-field (BF) Microscopy

4.1.1 Principles of BF Microscopy

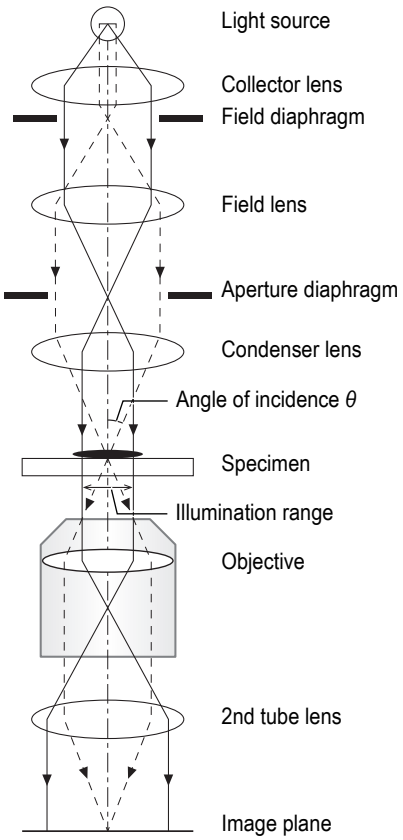
BF microscopy is a method of observing stained specimens, and serves as the basic method for implementing other microscopies.

Illumination method for BF microscopy

There are two types of BF microscopy: critical illumination and Köhler illumination. Critical illumination focuses an image of a light source onto the specimen for bright illumination. On the other hand, Köhler illumination focuses an image of a light source on the aperture diaphragm. This method is featured by its clear field of view, free from illumination unevenness, flare, or ghost, and is indispensable in today's photography. Ordinarily Köhler illumination is used for BF microscopy.

BF microscopy optical system

The optical system of BF microscopy with Köhler illumination is shown in the following figure.



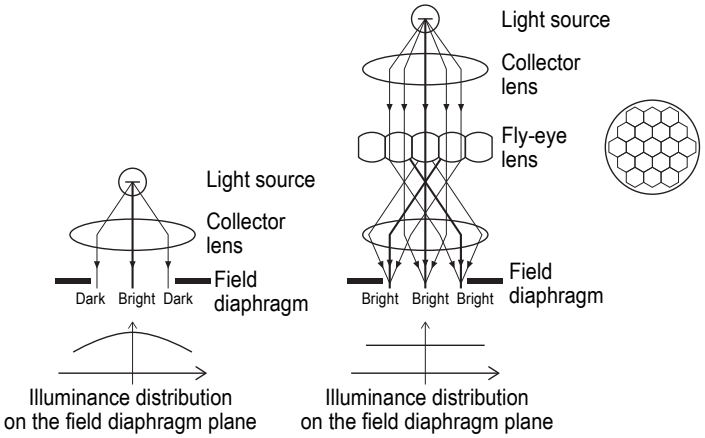
Köhler illumination optical system

Light from the light source is converted by a collector lens into a parallel luminous flux and passes the field diaphragm. Light emitted from the aperture diaphragm is concentrated by a field lens and an image of the light source is formed at the aperture diaphragm.

Light emitted from the aperture diaphragm is converted by the condenser lens into a parallel luminous flux and illuminates the specimen. The field diaphragm can restrict the illumination range of the specimen plane. Unnecessary light can be cut and thus a clear field of view free from flare or ghost can be obtained. The image of the light source can be restricted by the aperture diaphragm. This changes the brightness of the field of view. The incidence angle θ of the illumination also changes. Because of this incidence angle θ, adjustment of the aperture diaphragm causes the contrast resolution and focal depth of images to change.

Use of fly-eye lens to make illumination uniform

The uniformity of the illumination can be further increased using fly-eye lenses for the illumination attachment. A fly-eye lens is an array of small hexagonal lenses. The name “fly-eye lens” derives from the fact that its shape is similar to the compound eye of a fly. The following figure explains the effects of a fly-eye lens.



Use of fly-eye lens to make illumination uniform

Generally speaking, light emitted obliquely from the light source has a lower brightness than light emitted vertically from the light source (light distribution characteristic). If no fly-eye lens is used, light emitted vertically from the light source irradiates the center of the field of view, and light emitted obliquely from the light source irradiates the periphery of the field of view. As a result, light intensity in the periphery of the field of view is lower than in the center of the field even in the case of Köhler illumination. On the other hand, if a fly-eye lens is used, light beams of different emergence angles are intermixed and irradiate their respective position in the field of view. As a result, the field of view can be illuminated with a uniform intensity regardless of the light distribution characteristics of the light source.

Advantages of BF Microscopy

- Because specimens can be illuminated brightly, this microscopy is suitable for stained specimens. This microscopy is also appropriate for focusing or searching for observation objects as a preparatory step for other microscopies.
- The contrast of the specimen can be adjusted by changing the diameter of the aperture diaphragm.

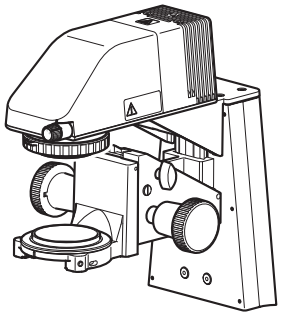
Disadvantages of BF Microscopy

- BF microscopy is not suitable for observing colorless, transparent specimens that do not absorb light.

4.1.2 Optical Elements Required for BF Microscopy

Dia-illumination unit

TI2-D-PD dia-illumination pillar is used.

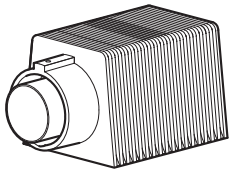


TI2-D-PD Pillar for Dia Illumination

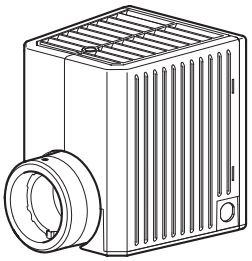
Light source

The following two types are usable as a light source for dia illumination.

- TI2-D-LHLED LED Lamp House for Dia Illumination
- D-LH/LC Precentered Lamphouse



TI2-D-LHLED LED Lamp House for Dia Illumination



D-LH/LC Precentered Lamphouse

Light source for dia-illumination

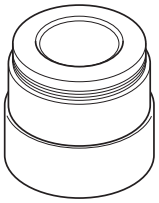
Objective

All CFI-60 objectives for inverted microscope allow BF microscopy.

Condenser lens

Condenser lenses that allow BF microscopy are as follows:

LWD condenser lens


ELWD condenser lens

CLWD condenser lens


HNA condenser lens (dry)


HNA condenser lens (oil)

Usable condenser lens

	LWD condenser lens	ELWD condenser lens	CLWD condenser lens	HNA condenser lens (dry)	HNA condenser lens (oil)
NA	0.52	0.3	0.72	0.85	1.35
Working distance	30 mm	75 mm	13 mm	5 mm	1.9 mm (including the glass slide thickness of 1.2 mm)
Condenser module	OPEN (without module)	OPEN (without module)	OPEN (without module)	OPEN (without module)	OPEN (without module)
Other			The TI2-C-CLWDA CLWD condenser adapter is required.	The T-CHNA High N.A. common condenser lens unit is required.	

TE-C ELWD-S condenser

The ELWD-S condenser is composed of a condenser turret and a dedicated ELWD condenser. Specifications of the ELWD-S condenser are as follows: NA 0.3, WD 65 mm

Notes on the use of the ELWD-S condenser

When using an ELWD-S condenser, be sure to decrease the light intensity before switching the turret from the position of PH microscopy to that of BF microscopy. Otherwise, the field of view rapidly becomes bright.

4.1.3 Combination of Optical Elements

When using a side port of the microscope to perform microscopy with a large field with a field number of equivalent to or more than 22, use the following combination of illumination attachments.

- TI2-D-PD Pillar for Dia Illumination
- TI2-D-LHLED LED Lamp House for Dia Illumination

Large field-of-view microscopy

- Use of a low-magnification lens might cause the field periphery to be too dark.
- When the C mount is used or a stage-up kit is mounted, the maximum field number is 22 on the side port of this microscope.
- Depending on the type of the objective, the field periphery might be out of focus.

4.2 Details of Phase Contrast (Ph) Microscopy

4.2.1 Principles of Ph Microscopy

Ph microscopy is a method of observing colorless, transparent specimens such as living cells in an unstained way using dia-illumination.

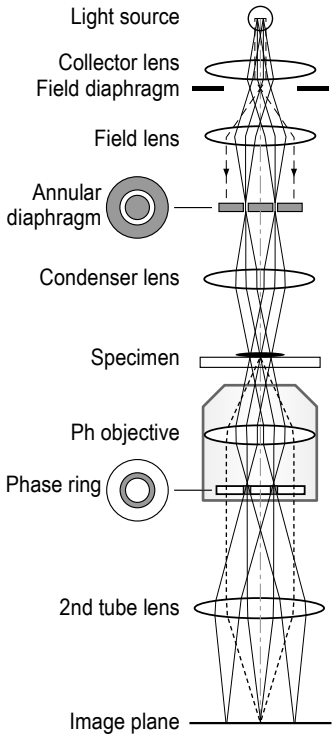
Objects that change the amplitude of light are called amplitude objects (such as transparent objects, or stained objects) and objects that change the phase of light are called phase objects (such as colorless, transparent specimens, or living cells). Ph microscopy visualizes phase objects, which are unrecognizable to the human eye.

Visualizing phase objects

The illumination that has reached the specimen is divided into two types: direct light which is bright, having passed non-phase objects, and diffracted light which has passed phase objects. In case the optical path difference between specimens is very small, the phase of the diffracted light comes approximately 1/4λ later than that of the direct light. In Ph microscopy, the amplitude of the direct light is alleviated with an ND filter, and at the same time, the phase of the direct light is changed with a phase plate by only 1/4 λ. This operation causes the phase contrast of the direct light to be 1/2 λ or 0. Interference between the direct light and optical path allows you to observe the phase change as the contrast of the light.

Ph microscopy optical system

The Ph microscopy optical system is shown in the following figure.



Optical path diagram of Ph microscopy

The light emitted from the light source is annularly narrowed by the annular diaphragm. It passes the condenser lens, and then illuminates the specimen. The illumination is divided as direct light (solid line) which has passed the inside of the specimen and optical path (dotted line) which has passed phase objects and passes the objective. A diffraction phenomenon occurs in a portion with a difference in diffraction ratio. Therefore diffraction light contains shape information of phase objects such as the interface between a living cell and solution or the internal structure of a living cell. Diffraction light and direct light pass a different portion of the phase ring inside the Ph objective, respectively. The phase ring consists of a circular 1/4 wavelength plate and an ND filter through which direct light passes and a transparent material through which most of the diffraction light pass. Direct light and diffraction light reach the image plane and form a Ph microscopy image with a bright and dark contrast.

Dark contrast and bright contrast

If the phase of the direct light is advanced by 1/4 λ so that the phase contrast between the direct light and diffraction light is 1/2 λ, the two lights weaken each other. As a result, the phase object becomes dark and the background becomes brighter (dark contrast). If the phase of the direct light is advanced by 1/4 λ so that the phase contrast between the direct light and diffraction light is 0, the two lights weaken each other. As a result, the phase object becomes dark and the background becomes brighter (bright contrast).

Advantages of Ph microscopy

- Ph microscopy is highly effective in detecting phase contrast. It is said that an optical path phase contrast of up to about 1/1000λ can be detected.
- The image view remains the same regardless of the direction of the specimen placed.
- By using an annular diaphragm for bright field on the condenser side, BF microscopy can be performed without changing the Ph objective.

Disadvantages of Ph microscopy

- If the phase contrast between specimens is too large (as with thick specimens), brightness and darkness might be reversed or image quality might be deteriorated significantly.
- Because of a halo phenomenon, which looks like a shading, occurring at the boundary in a large structure, the fine structure at the periphery might disappear.

> **Objective using an apodization phase plate**
>
> To emphasize the fine structure by reducing halo, use of an objective with an apodization phase plate is recommended.
>
> An apodization phase plate has a special absorption film around the phase film. The feature of the apodization phase plate is to provide low contrast with diffracted light weakened by a large structure of diameter 10 µm or more, while the contrast of fine structure of diameter 10 µm or less is left as is.

4.2.2 Optical Elements Required for Ph Microscopy

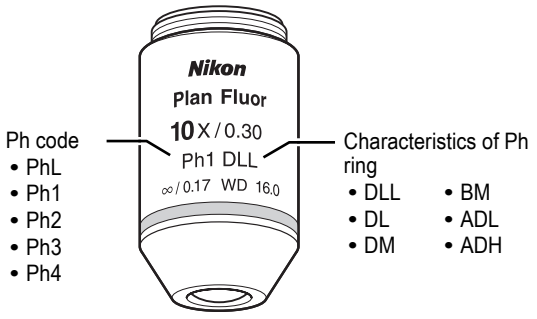
Ph microscopy requires the following components as well as the status of bright field.

- Ph objective (including the phase ring in the figure “Optical path diagram for Ph microscopy”)
- Ph module (equivalent to the annular diaphragm in the figure “Optical path diagram for Ph microscopy”)
- Condenser lens

Ph objective (including the phase ring in the figure “Optical path diagram for Ph microscopy”)

Depending on the color aberration and correction degree of curvature of the image plane, Ph objectives are classified as Achromat, Plan Achromat, Plan Fluor, and Plan Apochromat. In addition, these lenses can also be grouped into several types according to the characteristics of the internal phase ring. Satisfactory microscopy cannot be performed unless the phase contrast of specimens and the characteristics of the phase ring match.

See the following table for use characteristics of phase contrast objectives.



Example of a Ph contrast objective

Characteristics of Ph objectives

Ph objective		View	Contrast		Latitude	Application examples
Dark contrast	**DLL DL**	Generally, objects with a larger phase contrast look dark. Therefore a dark image appears in a relatively bright field, which is similar to an image in BF microscopy.	This is suitable for details observation with a focus on micro contrast.	Half tone (the use range is wide)	Phase contrast and absorbing objects (stained objects) in the low and mid ranges	Spores of fungus, ordinary living cells, thick specimens, fungus, stained specimens, insect eggs, fat globules, crystals, etc.
	DM			Hard tone (the use range is relatively narrow)	Transparent object in the low range	Fungus, protozoa flagellum, fibrin fibril, fine granule, section with a carefully selected mounting agent, ultrathin section, etc.
Bright contrast	**BM**	Generally, objects with larger phase contrast look brighter. Therefore a bright image appears in a relatively dark field, which is similar to an image in DF microscopy.	This microscopy is suitable for observing the form of and detecting and calculating minute fibers or granules with a focus on macro contrast.		Almost all ranges	Fungus, protozoa flagellum, fibrin fibril, fine granule, cytometry, etc.
Dark contrast (apodization)	**ADL**	Generally, objects with a larger phase contrast look darker. Therefore a dark image appears in a relatively bright field, which is similar to an image in BF microscopy. As compared with an ordinary objective for dark contrast, these objectives can enhance the contrast of a fine structure by reducing halo.	This is suitable for details observation with a focus on micro contrast.	Half tone (the use range is wide)	Phase contrast and absorbing objects (stained objects) in the low and mid ranges	Spores of fungus, ordinary living cells, thick specimens, fungus, stained specimens, insect eggs, fat globules, crystals, etc.
	ADH			Hard tone (the use range is relatively narrow)	Transparent object in the low range	Fungus, protozoa flagellum, fibrin fibril, fine granule, section with a carefully selected mounting agent, ultrathin section, etc.

Selection of a proper Ph objective according to use

Plan Fluor Ph objectives can also be used for BF microscopy, DIC microscopy, and Epi-FL microscopy. Plan Apochromat Ph objectives can also be used for BF microscopy, DIC microscopy, and Epi-FL microscopy (excluding UV excitation).

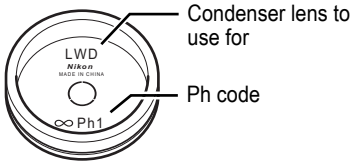
However, both of these objectives have a built-in phase ring (phase plate ring) and thus image view might be different from that with an objective dedicated to each relevant microscopy. When accurate microscopy is required, use the objective dedicated to each microscopy.

Ph module (equivalent to the annular diaphragm in the figure “Optical path diagram for Ph microscopy”)

The Ph module is a ring-shape diaphragm that is attached to the condenser turret.

An ID code called “Ph code” is attached to each of the Ph objectives and Ph modules. There are five codes, PhL and Ph1 to Ph4, depending on the size of the phase ring and annular diaphragm. To perform phase contrast microscopy, it is necessary to place a Ph objective and a Ph module having the same Ph code (Ph codes are unrelated to the magnifications of the objectives) in the optical path.

Make sure to use a combination of a Ph objective and a Ph module having the same code; otherwise the phase contrast effect is not delivered.

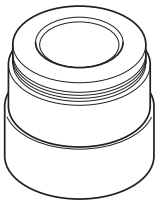


Example of the Ph module

Condenser lens

Condenser lenses that allow Ph microscopy are as follows:

LWD condenser lens


ELWD condenser lens

CLWD condenser lens


HNA condenser lens (dry)


HNA condenser lens (oil)

Specifications and details of condenser lenses

	Condenser turret (system condenser)			HNA condenser slider	
	LWD condenser lens	**ELWD condenser lens**	**CLWD condenser lens**	**HNA condenser lens (dry)**	**HNA condenser lens (oil)**
NA	0.52	0.3	0.72	0.85	1.35
Working distance	30 mm	75 mm	13 mm	5 mm	1.9 mm (including the glass slide thickness of 1.2 mm)
Ph code	PhL, Ph1, Ph2, Ph3	PhL, Ph1, Ph2	Ph1, Ph2, Ph3, Ph4	PhL, Ph1, Ph2, Ph3	Ph1, Ph2, Ph3, Ph4
Other			The TI2-C-CLWDA CLWD condenser adapter is required.	The T-CHNA High N.A. common condenser lens unit is required.	The T-CHNA High N.A. common condenser lens unit is required.

TE-C ELWD-S condenser

Specifications of the ELWD-S condenser are as follows: NA 0.3, WD 65 mm

4.2.3 Combination of Optical Elements

Combinations of the optical elements for Ph microscopy are listed below.

Combination of the optical elements for Ph microscopy

Objective Ph code	Condenser lens	Condenser module
PhL	ELWD condenser lens	TC-C-ME-PHL ELWD module
	LWD condenser lens	TC-C-ML-PHL LWD module
Ph1	ELWD condenser lens	TC-C-ME-PH1 ELWD module
	LWD condenser lens	TC-C-ML-PH1 LWD module
	CLWD condenser lens	TI2-C-MC-PH1 CLWD Module for PH1
	HNA condenser lens (dry/oil)	Supplied with the condenser lens
Ph2	ELWD condenser lens	TC-C-ME-PH2 ELWD Module
	LWD condenser lens	TC-C-ML-PH2 LWD module
	CLWD condenser lens	TI2-C-MC-PH2 CLWD Module for PH2
Ph3	LWD condenser lens	TC-C-ML-PH3 LWD Module
	CLWD condenser lens	TI2-C-MC-PH3 CLWD module
Ph4*	CLWD condenser lens	TI2-C-MC-PH4 CLWD module

* Ph4 is used for external Ph microscopy.

4.3 Details of Diascopic DIC and IMSI Microscopies

4.3.1 Principles of DIC Microscopy

DIC microscopy is a method of observing colorless, transparent specimens such as living cells in an unstained way using dia-illumination.

Objects that change the amplitude of light are called amplitude objects (such as transparent objects, or stained objects) and objects that change the phase of light are called phase objects (such as colorless, transparent specimens, or living cells). DIC microscopy visualizes phase objects, which are invisible to the human eye.

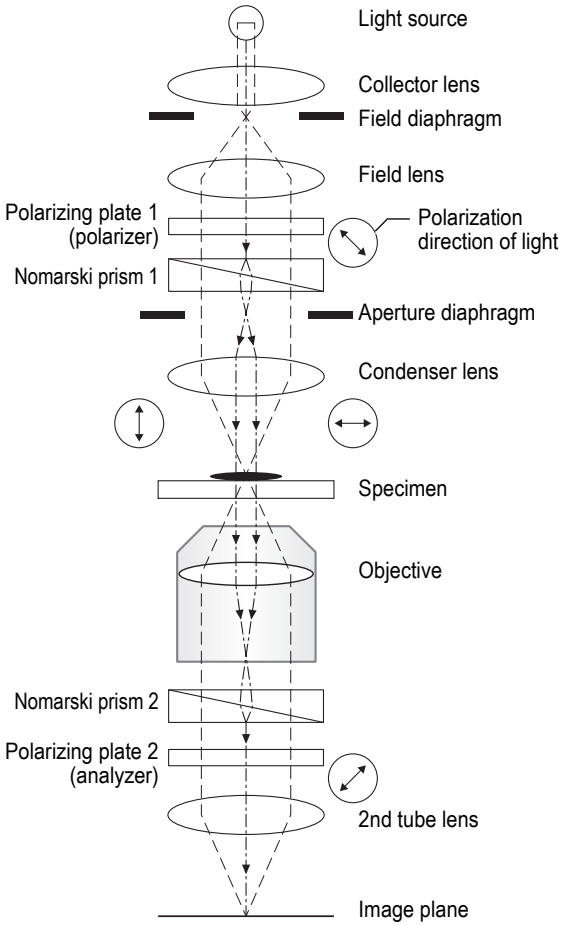
Visualizing phase objects

The illumination that has reached the specimen changes the passing speed (phase) according to the position (refractive index) where it passes the phase object and the thickness (path length). If a polarizer and a Nomarski prism are used, illumination can be divided into two mutually perpendicular polarized beams, each of which passes slightly apart (hundreds of nm) from one another on a phase object. By using a Nomarski prism and an analyzer again to synthesize and cause interference between the two polarized beams with their phase changed according to the passing position, the contrast of adjacent phases on the phase object can be observed as the brightness of light.

DIC microscopy optical system

The DIC microscopy optical system is shown in the figure on the right.

The light emitted from the light source is converted into linear polarization (light that vibrates in only one direction) on polarizing plate 1 (polarizer) and into two beams with their mutually perpendicular polarization planes on Nomarski prism 1. The condenser lens converts the two beams into two parallel beams, spaced slightly apart, and which then pass through the specimen. This separation amount (shear amount) is less than the resolution of the objective, and therefore the two beams do not produce a double image. The two beams are collected onto the rear-side focal plane by the objective and are formed into one beam by Nomarski prism 2. However, if there is no phase contrast in the passing position on the specimen, the beams are blocked by polarizing plate 2 (analyzer) on which polarizing plate 1 and the optical axis intersect at right angles. If there is a phase contrast, a DIC image having a bright and dark contrast due to interference is formed.



Optical path diagram of DIC microscopy

Principles of IMSI Microscopy

IMSI (Intracytoplasmic Morphologically selected Sperm Injection) is a DIC technique for selecting sperms suitable for micro insemination. This microscopy is aimed at achieving improved fertility rate and implantation rate through detailed observation and selection of the head of each sperm using high-magnification DIC images.

Advantages of DIC microscopy

- This microscopy enables observation of the specimens thicker than those for Ph microscopy.
- Unlike Ph microscopy, this microscopy is free from a halo phenomenon in a boundary of a large structure and therefore allow minute structure observation.
- Objectives can be shared with other types of microscopy such as FL microscopy because there is no need for dedicated objectives, unlike Ph microscopy.

Disadvantages of DIC microscopy

- This microscopy is suitable only for specimens whose images have orientation and phase contrast in the direction of shear (direction of the shift of the separated beam)
- It requires the use of objectives with less distortion (with DIC marking) because distortion of polarization deteriorates image quality. For specimens that have polarization property (such as plastic dishes), this microscopy cannot be performed.

4.3.2 Optical Elements Required for DIC Microscopy

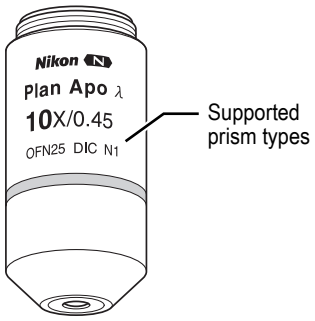
DIC microscopy requires the following components as well as the status of bright field (those enclosed in parentheses are for IMSI observation).

- DIC objective (IMSI objective)
- DIC polarizer (NAMC/IMSI polarizer)
- DIC condenser module (IMSI condenser module)
- Condenser lens
- DIC slider
- Analyzer

DIC objective (IMSI objective)

Use differential interference objectives with less distortion for DIC microscopy.

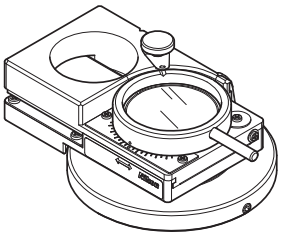
For the types of DIC sliders that support each objective, see the table below.



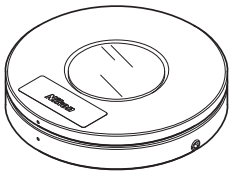
DIC objective

DIC polarizer (NAMC/IMSI polarizer)

Polarizers are classified as DIC polarizers and NAMC/IMSI polarizers.



DIC polarizer



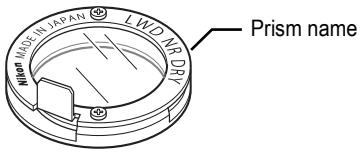
NAMC/IMSI polarizer

Polarizer

DIC condenser module (IMSI condenser module)

Use the DIC condenser module by attaching it to the condenser turret.

The combination of a DIC condenser module and a DIC slider (objective-side DIC prism) depends on objectives used. Use a correct combination.



Example of the DIC condenser module (condenser-side DIC prism)

Condenser lens

Condenser lenses that allow DIC/IMSI microscopy are as follows:

LWD condenser lens

CLWD condenser lens

HNA condenser lens (dry)

HNA condenser lens (oil)

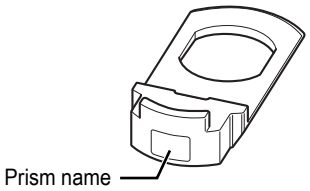
Usable condenser lens

	LWD condenser lens	CLWD condenser lens	HNA condenser lens (dry)	HNA condenser lens (oil)
NA	0.52	0.72	0.85	1.35
Working distance	30 mm	13 mm	5 mm	1.9 mm (including the glass slide thickness of 1.2 mm)
Condenser module	For DIC: N1Dry, N2Dry, NRDry For IMSI: IMSI N2Dry, IMSI NRDry	For DIC: N2Dry, NRDry	For DIC: N2Dry, NRDry	For DIC: N2Oil, NROil
Other		The TI2-C-CLWDA CLWD condenser adapter is required.	The T-CHNA High N.A. common condenser lens unit is required.	

DIC slider

Insert the DIC slider in the DIC slider slot of the nosepiece.

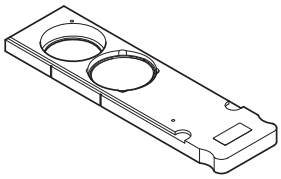
The combination of a DIC condenser module and a DIC slider depends on objectives to be used. Use a correct combination.



DIC slider (DIC prism for objectives)

Analyzer

Analyzers used for DIC microscopy or IMSI microscopy include analyzer slider and analyzer cube.



Analyzer slider

Analyzer block / analyzer cube for large field of view

Analyzer

4.3.3 Combination of Optical Elements

To perform DIC/IMSI microscopy, use a combination of the following optical elements.

Referring to the following table, combine the DIC condenser module and the DIC slider to match the objective and condenser lens in use. In addition to standard combinations, there are combinations focusing on high contrast or high resolution. Select the best combination as required.

> **Contrast and resolution of DIC images**
> In principle, contrast and resolution of the DIC image contradict with each other. (The higher the contrast, the lower the resolution.)
> Select the best combination suitable for the purpose.

■ With the LWD condenser lens

Objective		LWD condenser lens					
		Standard combination		High contrast		High resolution	
		Condenser module	DIC slider	Condenser module	DIC slider	Condenser module	DIC slider
10X	Plan Apo 10XA	N1 Dry (white background + black text)	10x				
	Plan Fluor 10X						
	Plan Apo λ 10X						
	Plan Apo λS 10X						
	Super Fluor 10X						
20X	Plan Fluor 20X	N2 Dry (white background + black text)	20x	N1 Dry (white background + black text)	20x-C		
	Plan Fluor 20XC MI						
	Plan Apo 20X						
	Plan Apo VC 20X						
	Plan Apo λ 20X						
	Super Fluor 20X						
	Apo LWD λS 20XC WI		20x III				
25X	Plan Apo λS 25XC Sil	N2 Dry (white background + black text)	25x II				
40X	Plan Fluor 40X	N2 Dry (white background + black text)	40x I	N1 Dry (white background + black text)	40x I -C		
	Plan Apo 40X						
	Plan Apo λ 40XC						
	Super Fluor 40X						
	Apo LWD λS 40XC WI						
	Plan Fluor 40X Oil		40x II				
	Super Fluor 40X Oil						
	Apo λS 40XC WI						
	Plan Apo 40X Oil		40x III				
	Plan Apo λS 40XC Sil		40x I	N1 Dry (white background + black text)	40x I -C		
60X	Plan Apo 60XA Oil	N2 Dry (white background + black text)	60x I			NR Dry (white background + black text)	60x I -R
	Plan Apo 60XC						
	Plan Apo VC 60X Oil						
	Plan Apo λ 60XC						
	Apo TIRF 60XC Oil						
	Plan Fluor 60XS Oil		60x II				60x II -R
	Plan Apo TIRF 60XC Oil						
	Plan Apo 60XC WI						
1)	Plan Apo VC 60XC WI						
	Plan Apo λ 60X Oil						
	Plan Fluor 60XC						
	Apo λS 60X Oil						
2)	Plan Apo VC 60XA C WI		60x IV				60x IV -R
	Plan Apo IR 60XC WI						
	SR Plan Apo IR 60XAC WI						
100X	Plan Apo VC 100X Oil		100x I				100x I -R
	Plan Apo λ 100X Oil						
	Apo TIRF 100XC Oil						
	SR Apo TIRF 100XC Oil						
	HP Apo TIRF 100XC Oil						
	SR HP Apo TIRF 100XC Oil						
	HP Plan Apo VC 100X Oil						
	SR HP Plan Apo λS 100XC Sil						
	Plan Fluor 100X Oil		100x II				100x II -R
	Plan Fluor 100XS Oil						
	Plan Apo 100X Oil						
	Plan Apo TIRF 100XC						
ELWD	Plan Fluor ELWD 20X C	N1 Dry (white background + black text)	20x I				
	S Plan Fluor ELWD 20XC		20x II				
	S Plan Fluor LWD 20XC						
	Plan Fluor ELWD 40X C S Plan Fluor ELWD 40XC		40x IV				
	Plan Fluor ELWD 60X C S Plan Fluor ELWD 60XC		60x III				

*Description in the parentheses in the Condenser module column indicates the color of the identification label supplied with each module.

*Different types of Plan Apo VC 60XC objectives 1) Magnification display is “60X/1.20.” 2) Magnification display is “60XA/1.20.”

■ With the high NA condenser lens

<table>
<tr><th colspan="2" rowspan="3">Objective</th><th colspan="4">Dry-type high NA condenser lens</th><th colspan="4">Oil-type high NA condenser lens</th></tr>
<tr><th colspan="2">Standard combination</th><th colspan="2">High resolution</th><th colspan="2">Standard combination</th><th colspan="2">High resolution</th></tr>
<tr><th>Condenser module</th><th>DIC slider</th><th>Condenser module</th><th>DIC slider</th><th>Condenser module</th><th>DIC slider</th><th>Condenser module</th><th>DIC slider</th></tr>
<tr><td>10X</td><td>Plan Apo 10XA</td><td rowspan="5"></td><td rowspan="5"></td><td rowspan="38"></td><td rowspan="38"></td><td rowspan="5"></td><td rowspan="5"></td><td rowspan="38"></td><td rowspan="38"></td></tr>
<tr><td></td><td>Plan Fluor 10X</td></tr>
<tr><td></td><td>Plan Apo λ 10X</td></tr>
<tr><td></td><td>Plan Apo λS 10X</td></tr>
<tr><td></td><td>Super Fluor 10X</td></tr>
<tr><td>20X</td><td>Plan Fluor 20X</td><td rowspan="33">N2 Dry
(green background + white text)</td><td rowspan="7">20x</td><td rowspan="33">N2 Oil
(yellow background + black text)</td><td rowspan="7">20x</td></tr>
<tr><td></td><td>Plan Fluor 20XC MI</td></tr>
<tr><td></td><td>Plan Apo 20X</td></tr>
<tr><td></td><td>Plan Apo VC 20X</td></tr>
<tr><td></td><td>Plan Apo λ 20X</td></tr>
<tr><td></td><td>Super Fluor 20X</td></tr>
<tr><td></td><td>Apo LWD λS 20XC WI</td><td>20x III</td><td>20x III</td></tr>
<tr><td>25X</td><td>Plan Apo λS 25XC Sil</td><td>25x II</td><td>25x II</td></tr>
<tr><td>40X</td><td>Plan Fluor 40X</td><td rowspan="5">40x I</td><td rowspan="5">40x I</td></tr>
<tr><td></td><td>Plan Apo 40X</td></tr>
<tr><td></td><td>Plan Apo λ 40XC</td></tr>
<tr><td></td><td>Super Fluor 40X</td></tr>
<tr><td></td><td>Apo LWD λS 40XC WI</td></tr>
<tr><td></td><td>Plan Fluor 40X Oil</td><td rowspan="3">40x II</td><td rowspan="3">40x II</td></tr>
<tr><td></td><td>Super Fluor 40X Oil</td></tr>
<tr><td></td><td>Apo λS 40XC WI</td></tr>
<tr><td></td><td>Plan Apo 40X Oil</td><td>40x III</td><td>40x III</td></tr>
<tr><td></td><td>Plan Apo λS 40XC Sil</td><td>40x I</td><td>40x I</td></tr>
<tr><td>60X</td><td>Plan Apo 60XA Oil</td><td rowspan="5">60x I</td><td rowspan="3">60x I -R</td><td rowspan="5">60x I</td><td rowspan="3">60x I -R</td></tr>
<tr><td></td><td>Plan Apo 60XC</td></tr>
<tr><td></td><td>Plan Apo VC 60X Oil</td></tr>
<tr><td></td><td>Plan Apo λ 60XC</td><td rowspan="13">N2 Dry
(green background + white text)</td></tr>
<tr><td></td><td>Apo TIRF 60XC Oil</td><td rowspan="13">60x II</td><td rowspan="13">60x II -R</td><td rowspan="13">60x II</td><td rowspan="13">60x II -R</td></tr>
<tr><td></td><td>Plan Fluor 60XS Oil</td></tr>
<tr><td></td><td>Plan Apo TIRF 60XC Oil</td></tr>
<tr><td></td><td>Plan Apo 60XC WI</td></tr>
<tr><td>1)</td><td>Plan Apo VC 60XC WI</td></tr>
<tr><td></td><td>Plan Apo λ 60X Oil</td></tr>
<tr><td></td><td>Plan Fluor 60XC</td></tr>
<tr><td></td><td>Apo λS 60X Oil</td></tr>
<tr><td>2)</td><td>Plan Apo VC 60XA C WI</td><td rowspan="3">60x IV</td><td rowspan="3">60x IV -R</td><td rowspan="3">60x IV</td><td rowspan="3">60x IV -R</td></tr>
<tr><td></td><td>Plan Apo IR 60XC WI</td><td rowspan="3">N2 Dry
(green background + white text)</td></tr>
<tr><td></td><td>SR Plan Apo IR 60XC WI</td><td rowspan="3">N2 Oil
(yellow background + black text)</td></tr>
<tr><td>100X</td><td>Plan Apo VC 100X Oil</td><td rowspan="8">100x I</td><td rowspan="8">100x I -R</td><td rowspan="8">100x I</td><td rowspan="8">100x I -R</td></tr>
<tr><td></td><td>Plan Apo λ 100X Oil</td></tr>
<tr><td></td><td>Apo TIRF 100XC Oil</td></tr>
<tr><td></td><td>SR Apo TIRF 100XC Oil</td></tr>
<tr><td></td><td>HP Apo TIRF 100XC Oil</td></tr>
<tr><td></td><td>SR HP Apo TIRF 100XC Oil</td></tr>
<tr><td></td><td>HP Plan Apo VC 100X Oil</td></tr>
<tr><td></td><td>SR HP Plan Apo λS 100XC Sil</td></tr>
<tr><td></td><td>Plan Fluor 100X Oil</td><td rowspan="4">100x II</td><td rowspan="4">100x II -R</td><td rowspan="4">100x II</td><td rowspan="4">100x II -R</td></tr>
<tr><td></td><td>Plan Fluor 100XS Oil</td></tr>
<tr><td></td><td>Plan Apo 100X Oil</td></tr>
<tr><td></td><td>Plan Apo TIRF 100XC</td></tr>
</table>

*Description in the parentheses in the Condenser module column indicates the color of the identification label supplied with each module.

*Different types of Plan Apo VC 60XC objectives 1) Magnification display is “60X/1.20.” 2) Magnification display is “60XA/1.20.”

Combination of IMSI objectives and DIC prisms (condenser side/objective side)

To perform IMSI microscopy, it is necessary to use a correct combination of an IMSI objective, and DIC prisms (condenser side/objective side). Referring to the following table, check the correct combination of the objective, the condenser module and the DIC slider, and then place the optical components of the correct combination in the optical path.

In addition to standard combinations, there are combinations focusing on high contrast and resolution. Select the best combination as required.

Combination of IMSI objectives and DIC prisms

Objective	WD (mm)	Applicable correction collar (mm)	LWD condenser lens (white text)			
			Standard combination (Focus on the contrast)		Focus on the resolution	
			Condenser module	DIC slider	Condenser module	DIC slider
Plan Apo VC 100X Oil	0.13	None	IMSI N2 Dry (white background + black text)	100x I	IMSI NR Dry (white background + black text)	100x I-R
Plan LWD IMSI 100XC	0.95	0.6-1.3	IMSI N2 Dry (white background + black text)	100x III		

Description in the parentheses in the DIC condenser module column indicates the color of the identification label supplied with each module.

Note on performing BF microscopy using IMSI objectives

When performing BF microscopy by using an IMSI objective, remove the DIC slider from the optical path, and then place the polarizer slider and the condenser turret at hollow positions.

4.4 Details of Episcopic Fluorescence (Epi-FL) Microscopy

4.4.1 Principles of Epi-FL Microscopy

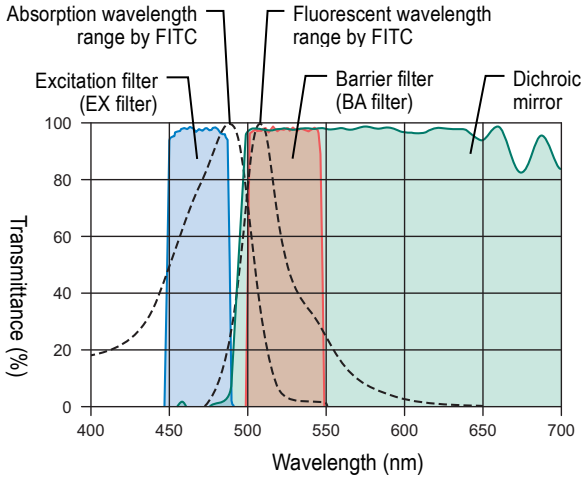
Epi-FL microscopy is a technique of observing specimens labeled with a fluorophore or fluorescent protein using episcopic illumination.

Fluorescent material absorbs light of specific wavelengths (excitation light), and emits light of specific wavelengths (fluorescent light) when it decays from the excited state to the original state (ground state.).

Visualizing fluorescent material

Because the brightness of fluorescent light is very weak when compared with excitation light, excitation light needs to be removed in order to observe only the portions with fluorescent labeling. Therefore, excitation light is removed from the observation optical path using a filter cube (utilizing a characteristic of the fluorescence that has a longer wavelength than excitation light.).

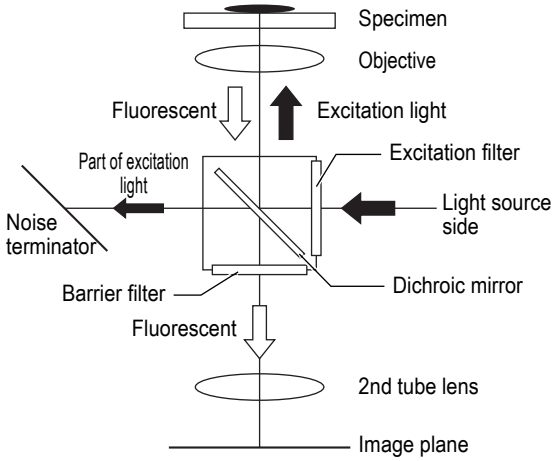
The filter cube is composed of an excitation filter, a dichroic mirror, and a barrier filter. The excitation filter restricts the transmissive wavelength range of excitation light. The dichroic mirror reflects short-wavelength excitation light to irradiate the specimen, and then allows long-wavelength fluorescent light (emitted from fluorescent material) to pass through the dichroic mirror. The barrier filter restricts the transmissive wavelength range of fluorescent light, and removes leaked excitation light and autofluorescence. The wavelength characteristics of optical elements of a filter cube are shown in the following figure.



Wavelength characteristics of fluorescent materials and the excitation filter, the dichroic mirror, and the barrier filter

Epi-FL microscopy optical system

The Epi-FL microscopy optical system is shown in the following figure.



Optical path diagram of Epi-FL microscopy

Excitation light emitted from the light source enters the filter cube from the rear of the microscope, and the excitation light of a specific wavelength is transmitted by the excitation filter. The excitation light is reflected upward by the dichroic mirror and concentrated onto the rear-side focal plane of the objective. The excitation light is irradiated intensively to the field of view, and fluorescent materials in the specimen in the field of view emit fluorescence. The fluorescent light passes through the objective and enters the filter cube from above, and then it passes through the dichroic mirror and the barrier filter to form a fluorescent image.

Noise terminator

Noise terminator—a unique optical system in NIKON fluorescent microscopes—provides high-contrast fluorescent images by practically eliminating excitation light (not reflected by the dichroic mirror) from the observation optical system.

Advantages of Epi-FL microscopy

- Microscopy for only localization and behaviors of interest can be performed by applying fluorescent labeling to specific tissues, cells, or molecules. Use of multiple fluorescent labels enables several objects of interest to be observed simultaneously.
- A single fluorescent molecule can also be observed because only objects of interest with fluorescent labeling are visualized in a very high contrast.

Disadvantages of Epi-FL microscopy

- Irradiation from excitation light or generation of active oxygen might result in specimen phototoxicity.
- Fluorescent material discolors due to irradiation of excitation light, resulting in labeled portions not being visible.
- Visibility of labeled portions depends on the efficiency of fluorophore immunostaining and fluorescent protein generation.

4.4.2 Optical Elements Required for Epi-FL Microscopy

Epi-FL microscopy uses the following components.

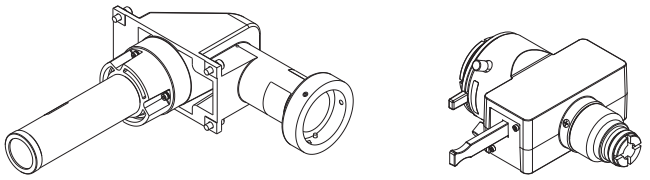
- Objective
- Epi-fluorescence attachment
- Light source
- FL turret + filter cube

> **Notes on using laser light source**
> This manual does not explain microscopy techniques using a laser light source.
> When using a laser, see the instruction manual for Ti2-LAPP.

Epi-fluorescence attachment

The following combination is usable:

- EPI-FL module + assorted main branch



Fixed main branch EPI-FL module

Epi-fluorescence attachment

Light source

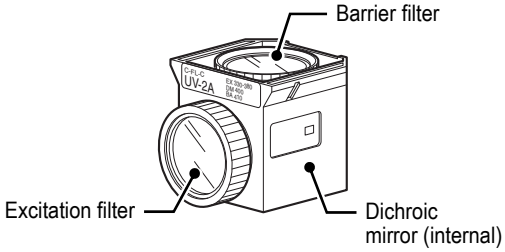
The fluorescence LED illumination system is used for the epi-fluorescence attachment.

For details on wavelength characteristics of the light source, see the instruction manual supplied with the light source.

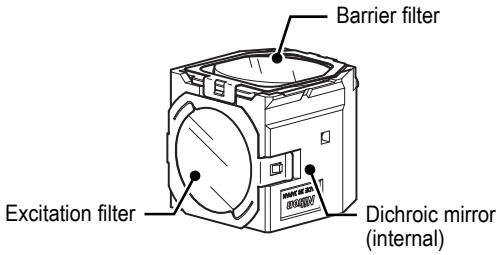
Filter cube

The fluorescent filter cube (mounted on the turret) consists of three types of optical components: the excitation filter (EX filter), the barrier filter (BA filter), and the dichroic mirror (DM).

Select the filter cube with the combination of suitable optical components according to the characteristics of the specimen and the fluorescence dye.



Fluorescent filter cube



Filter cube for large FOV

* For this microscope, the absorption filter should face downward when mounted inside the FL turret. The figure is shown upside down for explanatory purposes.

4.4.3 Combination of Optical Elements

When using a side port of the microscope to perform microscopy with a large field with a field number of equivalent to or more than 22, use the following combination of illumination attachment.

- EPI-FL module + assorted main branch
- Filter cube for large field of view

Large field-of-view microscopy

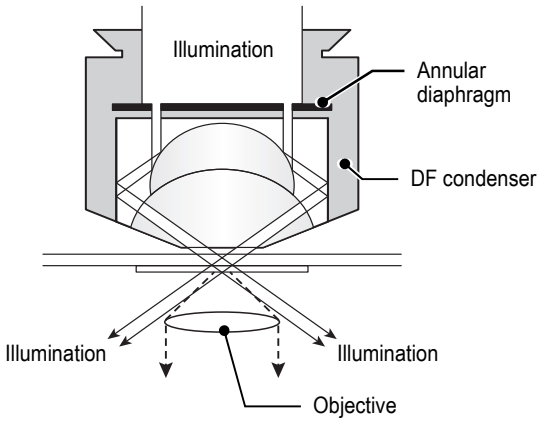
- When using a side port of the microscope to perform microscopy with a large field with a field number of equivalent to or more than 22, remove the epi field diaphragm from the optical path.
- When the C mount is used or a stage-up kit is mounted, the maximum field number is 22 on the side port of this microscope.
- Depending on the type of the objective, the field periphery might be out of focus.

4.5 Details of Diascopic Dark-field (DF) Microscopy

4.5.1 Principles of DF Microscopy

DF microscopy is a technique for improving the contrast of unstained specimens using oblique illumination.

Unlike other diascopic microscopy techniques (Ph or DIC) that form images of dark specimens on a bright background, this microscopy technique enables bright specimens to be visualized in high contrast on a dark background.



Optical path of DF microscopy

Visualizing scattering objects

The illumination that has reached the specimen is divided into two types: bright transmitted light, having passed through non-scattering parts of the specimen, and light scattered and diffracted by the specimen. The intensity of scattering can be observed as brightness/darkness of the light by eliminating bright transmitted light from the optical system and by extracting only scattered and diffracted light.

DF microscopy optical system

The light emitted from the light source is annularly narrowed by the annular diaphragm. It passes through the periphery of the DF condenser lens, and then obliquely illuminates the specimen. DF microscopy uses an objective of lower NA than a DF condenser lens (or objective with diaphragm), to form a DF image utilizing only light scattered from the specimen passing through the objective.

Advantages of DF microscopy

- The DF microscopy enables observation of the low-contrast specimens and micro-objects below the diffraction limit (such as flagellum and gold colloid).

Disadvantages of DF microscopy

- Objectives with a smaller number of apertures are used, so resolution is limited.
- This microscopy technique is not suitable for specimens having multiple scattering elements, such as thick specimens.

4.5.2 Optical Elements Required for DF Microscopy

DF microscopy uses the following components.
Condenser turrets or condenser sliders are not used.

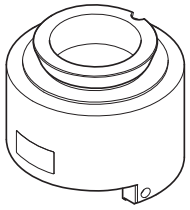
- Objectives with NA smaller than the minimum NA of the condenser, or objectives with a diaphragm (10x or more for the dry-type condenser lens, 20x or more for the oil-type condenser lens)
- Dark-field condenser adapter (TI-DF)
- Darkfield condenser (darkfield condenser Oil or darkfield condenser Dry)
- T-CHNA High N.A. common condenser lens unit (optional)

Objective

DF microscopy requires objectives (excluding 2x and 4x) with NA smaller than the minimum NA of the condenser. Using an objective with a diaphragm, the diaphragm in the objective can be adjusted, enabling DF microscopy.

TI-DF dark-field condenser adapter

Use the TI-DF dark-field condenser adapter to mount a darkfield condenser lens.



TI-DF dark-field condenser adapter

Condenser lens

Condenser lenses that allow DF microscopy are as follows:

Darkfield Condenser Dry

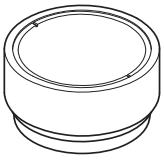
Darkfield Condenser Oil

Usable condenser lens

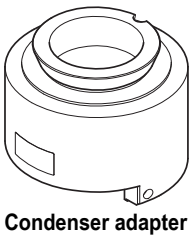
	Darkfield condenser (dry type)	Darkfield condenser (oil type)
NA	0.80 to 0.95	1.2 to 1.43
Working distance	4 mm	1.5 mm
Microscopy technique	DF	DF
Other	The TI-DF dark-field condenser adapter is required.	

T-CHNA High N.A. common condenser lens unit (optional)

Use the T-CHNA High N.A. common condenser lens unit to increase the brightness during DF microscopy.



High NA lens



Condenser adapter

T-CHNA High N.A. common condenser lens unit

Condenser adapter provided with the T-CHNA High N.A. common condenser lens unit

The T-CHNA High N.A. common condenser lens unit is equipped with a dedicated condenser adapter. When using a T-CHNA High N.A. common condenser lens unit, use the provided condenser adapter to mount the condenser lens (TI-DF is unnecessary).

4.5.3 Combination of the Optical Elements Required for DF Microscopy

DF microscopy uses the following components. Condenser turrets or condenser sliders are not used.

- Dark-field condenser adapter (TI-DF)
- Darkfield condenser (darkfield condenser Oil or darkfield condenser Dry)
- Objectives with NA smaller than the minimum NA of the condenser, or objectives with a diaphragm (10x or more for the dry-type condenser lens, 20x or more for the oil-type condenser lens)

4.6 Details of NAMC Microscopy

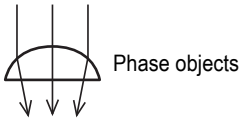
4.6.1 Principles of NAMC Microscopy

NAMC microscopy (Nikon Advanced Modulation Contrast) is a method of observing colorless, transparent specimens such as living cells in an unstained way using dia-illumination.

Objects that change the amplitude of light are called amplitude objects (such as transparent objects, or stained objects) and objects that change the phase of light are called phase objects (such as colorless, transparent specimens, or living cells). NAMC microscopy visualizes phase objects, which are unrecognizable to the human eye.

Visualizing phase objects

The illumination that has reached the specimen changes the refractive direction according to the refractive index and the shape of the phase object. Use of a slit aperture diaphragm and modulator enables optical elements of different transmittance to pass according to the slope of the position in the specimen where the illumination passes. For example, when a trapezoidal phase object is observed, the left side is bright, the top is gray, and the right side is dark and modulated in the obtained image.

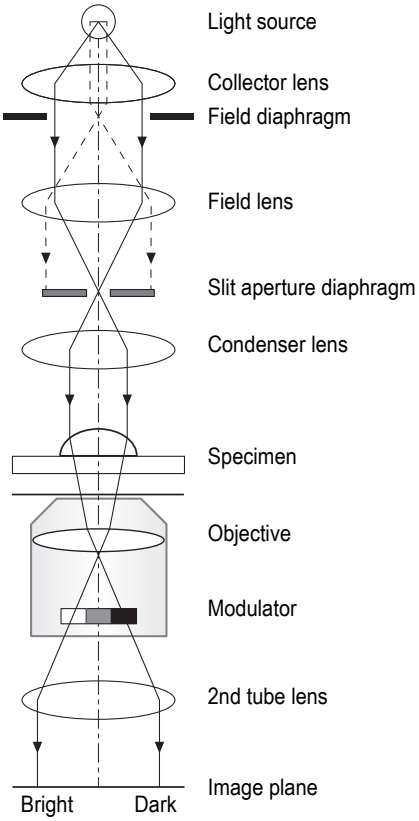


Refraction of beams in a phase object

NAMC microscopy optical system

The NAMC microscopy optical system is shown in the following figure.

The light emitted from the light source is narrowed in a rectangular shape by the slit diaphragm. It passes the condenser lens, and then obliquely illuminates the specimen. The illumination changes the direction of movement according to the shape of the specimen. After passing through the objective for modulation microscopy, the illumination passes through different portions of the modulator in the objective. The modulator is an optical element having three-level transmittance. Depending on the slope of the shape of the specimen through which the beams pass, the modulator provides three levels of brightness. As a result, 3D modulation contrast images similar to those obtained in DIC microscopy are formed.



Optical path diagram of NAMC microscopy

Advantages of NAMC microscopy

- NAMC microscopy enables observation of thick specimens, such as egg cells, usually not suited to Ph microscopy.
- NAMC microscopy does not use polarized beams, unlike DIC microscopy, so even if specimens have polarization properties (such as plastic dishes), contrast is maintained.

Disadvantages of NAMC microscopy

- This microscopy is limited to specimens that have images with orientation (adjustment is possible) and have a slope of shape in the direction of illumination.
- Resolution of this microscopy is poor as compared with Ph microscopy and DIC microscopy.

4.6.2 Optical Elements Required for NAMC Microscopy

NAMC microscopy requires the following components as well as the status of bright field.

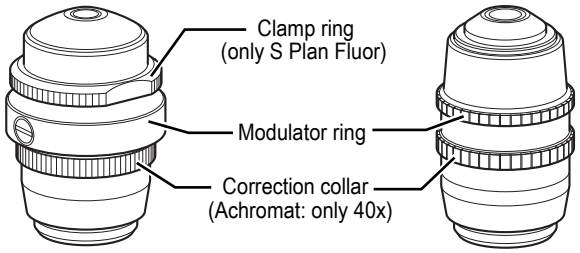
- NAMC objective
- TC-C-DICPNI NAMC/IMSI Polarizer
- NAMC condenser module (to be attached to the slit diaphragm and the condenser turret)
- NAMC condenser lens or LWD condenser lens

NAMC objective

NAMC microscopy requires the use of NAMC objectives with a built-in modulator.

An ID code called “NAMC code” is attached to each of the NAMC objectives and NAMC condenser modules. There are three types of codes: NAMC1 to NAMC3. To perform NAMC microscopy, place a NAMC objective and a NAMC module having the same NAMC code in the optical path.

Combinations of each objective and condenser modules are as listed in the table above.



S Plan Fluor ELWD
20x (NAMC2)
40x (NAMC3)

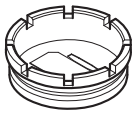
Achromat
10x (NAMC1)
20x (NAMC2)
40x (NAMC3)

NAMC objective

NAMC condenser module

To perform NAMC microscopy, mount a NAMC condenser module, having the same NAMC code as the NAMC objective, on the condenser turret.

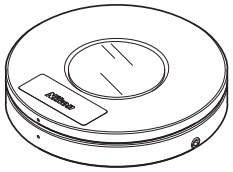
Make sure to use a combination having the same code; otherwise the NAMC effect is not delivered.



NAMC condenser modules (NAMC1, NAMC2, NAMC3)

NAMC/IMSI polarizer

NAMC microscopy requires the use of a NAMC/IMSI polarizer.



NAMC/IMSI polarizer

NAMC condenser lens or LWD condenser lens

The NAMC condenser lens or the LWD condenser lens is required for NAMC microscopy.

NAMC condenser lens

LWD condenser lens

Specifications and details of condenser lenses

	NAMC condenser lens	LWD condenser lens
NA	0.4	0.52
Working distance	44 mm	30 mm
NAMC code	NAMC1, NAMC2, NAMC3	NAMC1, NAMC2, NAMC3

4.6.3 Combination of Optical Elements

When performing NAMC microscopy, use the following combination of objectives, condenser modules, and condenser lenses.

Combination of optical elements

Objective	Condenser module	Condenser lens
NAMC10x (NAMC1)	NAMC1	NAMC condenser lens or LWD condenser lens
NAMC20x (NAMC2)	NAMC2	
NAMC40x (NAMC3)	NAMC3	

Note on performing BF microscopy using NAMC objectives

To perform BF microscopy by using a NAMC objective, place the condenser turret at the BF (hollow) position.

Chapter
5

Troubleshooting

This chapter describes how to solve problems related to the use of this product.

Misuse of this product might adversely affect performance, even if the product is functioning properly. If a problem occurs, be sure to check the relevant tables in this chapter for possible causes before requesting service. When using devices or components not described in this manual, see the appropriate manual for each device or component.

If you detect problems that are not described in this chapter or the problem still persists after measures are taken, turn off the device and contact your local Nikon representative.

5.1 Troubleshooting for Microscopy Techniques

5.1.1 Common Microscopy Troubles

Problem	Check item
The image is invisible.	**Assorted shutters** ☐ Open the dia-illumination shutter. (☞ 3.3.1) ☐ Open the contrast shield. (☞ 3.3.6) ☐ Open the motorized shutter. (☞ 3.3.7) ☐ Adjusting the filed diaphragm and the aperture diaphragm. (☞ 3.3.3, 3.4.5, 3.13.1, 3.13.3) ☐ Remove the light shielding plate module of the condenser from the optical path. (☞ 3.4.6) ☐ Open the FL turret shutter. (☞ 3.12.2)
	Sliders ☐ Move the filter slider (☞ 3.3.4), the analyzer slider (☞ 3.5.2), and the DIC slider (☞ 3.9.2) to the limit position or the click position.
	Nosepiece / objective ☐ Turn the nosepiece until it clicks into position. (☞ 3.9.1) ☐ Attach the nosepiece or the objective again. (☞ 7.6, 7.16)
	Optical path changeover ☐ Correctly select the optical path of the microscope main body. (☞ 3.2.1) ☐ Correctly select the optical path of the tube base unit. (Eyepiece tube base unit with port: ☞ 3.7.1)
	Light source / illumination ☐ Turn on the illumination. (☞ 3.3.2, 3.3.7, 3.14.1) ☐ Adjust the brightness using the brightness adjuster. (☞ 3.3.2, 3.3.7, 3.14.1)
	Specimen / stage ☐ Focusing on the specimen. (Objective: ☞ 3.11.1), condenser lens: ☞ 3.4.2) ☐ Adjust the position of the specimen. (☞ 3.8) ☐ Correctly mount the stage. (☞ 7.7.1)
	Binocular part ☐ Correctly adjust the diopter of the eyepieces. (☞ 3.6.2) ☐ Correctly adjust the interpupillary distance. (☞ 3.6.3)
The field of view is limited. A portion of the field of view is missing.	**Field diaphragm** ☐ Correctly adjust the dia field diaphragm. (☞ 3.3.3)
	Epi-fluorescence attachment-related ☐ Correctly adjust the epi field diaphragm. (☞ 3.13.3) ☐ Turn the FL turret to the filter cube position. (☞ 3.12.1) ☐ Confirm that the filter cube is correctly attached. (☞ 7.5.2) ☐ Confirm that the EPI-FL module is correctly attached. (☞ 7.13)
	Sliders ☐ Move the filter slider (☞ 3.3.4), the analyzer slider (☞ 3.5.2), and the DIC slider (☞ 3.9.2) to the limit position or the click position.
	Camera-related ☐ Confirm that the C mount adapter is correctly attached. (☞ 7.14)

Problem	Check item
The image quality is poor.	**Specimen / stage** ☐ Check the orientation of the cover glass surface of the specimen. (☞ 3.10.1) ☐ Check if the cover glass thickness is appropriate. (☞ 3.10.1) ☐ If uneven blurring occurs, or an out-of-focus condition occurs as a result of stage movement, check whether the specimen is tilted.
	Nosepiece / objective ☐ Check that there is no air bubbles between the specimen and the objective. (Oil immersion objective: ☞ 3.10.2) ☐ Remove the DIC slider from the optical path. (☞ 3.9.2) ☐ Correctly adjust the correction collar of the objective. (☞ 3.10.1) ☐ Use appropriate immersion liquid. (☞ 1.2.7, 3.10.2, 3.10.3) ☐ Clean the tip of the objective. (☞ 6.1.1)
	Condenser ☐ Adjust the height of the condenser. (☞ 3.4.2)
	Filters ☐ Remove the GIF filter if the field of view is green. (☞ 3.3.4) ☐ If the field of view has coloring, remove the filter cube or the barrier filter from the optical path. (☞ 3.12.1)
	Environment ☐ Check if there is ambient light (such as display screen light) entering the image. (☞ 3.3.6) ☐ Correctly select the optical path and eliminate the influence from the ambient light by the split optical path. (☞ 3.2.1)
Dirt or dust is highly visible in the image.	**Maintenance** ☐ Clean the surface of specimens and culture vessels ☐ Clean the objective. (☞ 6.1.1) ☐ Clean the eyepieces. (☞ 6.1.1) ☐ Clean the condenser. (☞ 6.1.1) ☐ If dirt or dust moves when the camera is turned, clean the camera. ☐ Clean the relay lens. (☞ 6.1.1) Check the upper surface of the condenser lens and the lower surface of the objective.
The image is dark.	**Polarizer / Analyzer** ☐ Remove the polarizer from the optical path. (☞ 3.5.1) ☐ Pull out the analyzer slider until it clicks. (☞ 3.5.2) ☐ Remove the analyzer block from the optical path (☞ 3.5.2)
	FL turret ☐ Remove the filter cube from the optical path. Place the required filter cube into the optical path. (☞ 3.12.1)
	Tube base unit ☐ Correctly set the optical path changeover knob. (Eyepiece tube base unit with port: ☞ 3.7.1)
The image is large.	**Intermediate magnification dial** ☐ Return the intermediate magnification dial to 1x. (☞ 3.2.2)
	Camera ☐ Confirm the adapter's variable magnification lens setting. (☞ 7.14)

Problem	Check item
The objective pupil is invisible.	**Assorted shutters** ☐ Open the dia-illumination shutter. (☞ 3.3.1) ☐ Open the contrast shield. (☞ 3.3.6) ☐ Open the motorized shutter. (☞ 3.3.7) ☐ Adjusting the filed diaphragm and the aperture diaphragm. (☞ 3.3.3, 3.4.5, 3.13.2, 3.13.4) ☐ Remove the light shielding plate module from the optical path. (☞3.4.6) ☐ Open the FL turret shutter. (☞ 3.12.2)
	Sliders ☐ Move the filter slider (☞ 3.3.4), the analyzer slider (☞ 3.5.2), and the DIC slider (☞ 3.9.2) to the limit position or the click position.
	Optical path changeover ☐ Correctly select the optical path of the microscope main body. (☞ 3.2.1) ☐ Correctly select the optical path of the tube base unit. (Eyepiece tube base unit with port: ☞ 3.7.1)
	Light source / illumination ☐ Turn on the illumination. (☞ 3.3.2, 3.3.7, 3.14.1) ☐ Adjust the brightness using the brightness adjuster. (☞ 3.3.2, 3.3.7, 3.14.1)
Dirt or dust is highly visible in the pupil.	**Maintenance** ☐ Clean the surface of specimens and culture vessels ☐ Clean the objective. (☞ 6.1.1) ☐ Clean the eyepieces. (☞ 6.1.1) ☐ Clean the condenser. (☞ 6.1.1) ☐ If dirt or dust moves when the camera is turned, clean the camera. ☐ Clean the relay lens. (☞ 6.1.1) Check the upper surface of the condenser lens and the lower surface of the objective.
The image moves.	**Specimen / stage** ☐ Secure the specimen using the stage clip or the well clamper. (☞ 3.8.1) ☐ Secure the specimen holder using the clamp screw. (☞ 7.17)
The focal deviation is high when objectives are switched over.	**Binocular part** ☐ Correctly adjust the diopter of the eyepieces. (☞ 3.6.2)
Binocular images are not integrated as a single image. Eyes are tired during observation.	**Binocular part** ☐ Correctly adjust the diopter of the eyepieces. (☞ 3.6.2) ☐ Correctly adjust the interpupillary distance. (☞ 3.6.3)

5.1.2 Troubles in BF Microscopy

■ Clarity of images

Problem	Check item
The image is dark.	**Light source / illumination** ☐ Adjust the brightness using the brightness adjuster. (☞ 3.3.2, 3.3.7) **Condenser** ☐ Make sure to perform focusing and centering for the condenser precisely. (☞ 3.4.2, 3.4.3) ☐ Adjust the aperture diaphragm to 70% to 80% of the numerical aperture of the objective. (☞ 3.4.5)
Brightness in the field of view is not uniform.	**Condenser** ☐ Check the combination of the objective and the condenser. (☞ 4.1.2) ☐ Focus and center the condenser. (☞ 3.4.2, 3.4.3) ☐ Open the field diaphragm to a size a little larger than that of the field of view. (☞ 3.3.3) ☐ Check the mounting of the part. (☞ 7.11)
Dirt or dust is highly visible in the field of view.	**Condenser** ☐ Focus and center the condenser. (☞ 3.4.2, 3.4.3) ☐ Adjust the aperture diaphragm to an appropriate size. (☞ 3.4.5)
Image quality is poor. Contrast is poor. Resolution is low.	**Condenser** ☐ Focus and center the condenser. (☞ 3.4.2, 3.4.3) ☐ Adjust the aperture diaphragm to an appropriate size. (☞ 3.4.5) **Specimen / stage** ☐ Check whether a cover glass is attached to the prepared specimen slide. (☞ 3.10.1) ☐ Check the thickness of the cover glass of the specimen vessel. (☞ 3.10.1)

■ Brightness and tinge of images

Problem	Check item
The field of view is too bright.	**Light source / illumination** ☐ Adjust the brightness using the brightness adjuster. (☞ 3.3.2, 3.3.7) ☐ Place the ND filter into the optical path. (☞ 3.3.4)
The image is yellowish or bluish.	**Light source / illumination** ☐ When using a Halogen lamp, place the NCB11 filter into the optical path. (☞ 3.3.4) ☐ When using a Halogen lamp, turn the brightness adjuster to match the lamp voltage, and adjust the brightness with a combination of ND filters. (☞ 3.3.4, 3.3.7)
Visually observed image color does not match the color of the image on the monitor.	**Camera** ☐ Set the white balance of the camera.

■ Focusing

Problem	Check item
Out of focus	**Specimen / stage** ☐ Correctly mount the stage. (☞ 7.7) **Focusing** ☐ Release the setting of the refocusing lever of the focusing device. (☞ 3.11.3)
Out of focus with an objective of high magnification	**Specimen / stage** ☐ Check the orientation of the cover glass surface of the specimen. (☞ 3.10.1) ☐ Check if the cover glass thickness is appropriate. (☞ 3.10.1) ☐ Check the thickness of the cover glass of the specimen vessel. (☞ 3.10.1) **Nosepiece / objective** ☐ Check whether the fail-safe device for specimen damage protection of the objective is pushed in. Some objectives have a stopper that maintains the pushed-in state. Push and turn the tip of the object to release the stopper. If the objective does not have a stopper, the tip of the lens cannot be turned. Do not try to forcibly pull it out, and instead contact your local Nikon representative.
One side of the field of view (up, down, right, or left) is not in focus. The image flows (i.e. becomes asymmetrically defocused when moving the focal point).	**Specimen / stage** ☐ Check whether the specimen is tilted. Check the specimen holder for tier difference or other problems. ☐ Correctly mount the stage. (☞ 7.7) **Light source / illumination** ☐ Check the dia-illumination pillar for tilting. (☞ 7.8) **Nosepiece / objective** ☐ Turn the nosepiece to the correct position. (☞ 3.9.1) ☐ Correctly attach the nosepiece. (☞ 7.6)
Difficult to focus.	**Focusing** ☐ Focus on the image using the fine and coarse-focus knobs. (☞ 3.11.1)

5.1.3 Troubles in Ph Microscopy

Problem	Check item
There is no contrast in the phase contrast image.	**Nosepiece / objective** ☐ Place the Ph objective into the optical path. (☞ 3.9.1, 4.2.2) **Condenser module** ☐ Place the Ph module (annular diaphragm) for Ph microscopy into the optical path. (☞ 3.4.6, 4.2.2) ☐ Center the annular diaphragm. (☞ 3.4.7) **Combination of optical elements** ☐ Confirm that the combination of the Ph objective and the annular diaphragm is correct. (☞ 4.2.3)
The contrast in the Ph image is poor.	**Nosepiece / objective** ☐ Change the objective to the Ph objective suitable for the specimen. (☞ 4.2.3) In some cases, for specimens whose contrast is weak in microscopy with a DLL objective, good results might be achieved with a DM objective. **Condenser** ☐ Adjust the height of the condenser. (☞ 3.4.2) **Aperture diaphragm** ☐ Fully open the aperture diaphragm. (☞ 3.4.5)
The quality of the Ph image is poor.	**Specimen / stage** ☐ Prepare a specimen suitable for Ph microscopy. (☞ 4.2.1) Microscopy of specimens that scatter light or that have lens or prism effect will decenter the PH module. In particular, thick live specimens, coarse specimens, and specimens with a microplate are significantly decentered with their lens or prism effect, resulting in image deterioration.
The image is brighter than the background, and therefore cannot be observed.	**Specimen / stage** ☐ Decrease the phase contrast of the specimen. (☞ 4.2.1) When a Ph objective with a dark contrast is used, if the phase contrast of the specimen exceeds the phase contrast allowance (latitude) of the objective, the image cannot be observed because the image is brighter than the background. When a phase contrast specimen is created, the phase contrast can be adjusted by the thickness of the specimen, mounting medium, or refractive index of culture solution.

5.1.4 Troubles in DIC and IMSI Microscopies

Problem	Check item
A portion of the field of view is missing.	**Condenser** ☐ Turn the condenser turret to the regular position. (☞ 3.4.6) ☐ For an HNA condenser slider, move the slider to the limit position. (☞ 3.4.6) **Nosepiece / objective** ☐ Push in the DIC slider until it stops. (☞ 3.9.2) ☐ Correctly attach the nosepiece. (☞ 7.6)
There is no contrast in the DIC or IMSI image.	**Polarizer / Analyzer** ☐ Place the polarizer and the analyzer into the optical path. (☞ 3.5.1, 3.5.2, 4.3.2) **Nosepiece / objective** ☐ Place the DIC/IMSI objective into the optical path. (☞ 3.9.1, 4.3.2) ☐ Place the DIC slider into the optical path. (☞ 3.9.2, 4.3.2) **Condenser module** ☐ Place the DIC/IMSI module into the optical path (☞ 3.4.6, 4.3.2) **Combination of optical elements** ☐ Confirm that the combination of the objective, the DIC slider and the condenser module is correct. (☞ 4.3.3)
The contrast in DIC or IMSI image is poor.	**Polarizer / Analyzer** ☐ Turn the polarizer rotation lever until a dark cross appears. (☞ 3.5.1) **Combination of optical elements** ☐ Select the combination of the optical elements suitable for the specimen. (☞ 4.3.3) **Specimen / stage** ☐ Check whether plastic vessels or lids are used. (☞ 4.3.1) ☐ Use a glass slide and a cover glass free of distortion, dust, or dirt. (☞ 4.3.1) ☐ When a combination of an oil-immersing objective and a heat plate are used for IMSI microscopy, a heat plate with a hole must be used. The total thickness of the heat plate and the cover glass must be within the supporting range (0.6 to 1.3 mm) of the correction collar.

5.1.5 Troubles in Epi-FL Microscopy

Problem	Check item
The image is not a fluorescent image.	**FL turret** ☐ Select an appropriate filter cube. (☞ 3.12.1, 4.4.2)
The contrast in the fluorescent image is poor.	**Light source / illumination** ☐ Close the dia-illumination shutter to prevent the LED from emitting autofluorescence. (☞ 3.3.1) ☐ Check if there is ambient light (such as display screen light) entering the image. ☐ Decrease ambient light, or shield the light from above by attaching an optional contrast shield. (☞ 3.3.6) **Optical path changeover** ☐ Correctly select the optical path and eliminate the influence from the ambient light by the split optical path. (☞ 3.2.1, 3.7.1) **Filter cube** ☐ When using ultraviolet or violet excitation light, use the specified objective. **Nosepiece / objective** ☐ Use low-fluorescent oil (Nikon DF immersion oil). (☞ 3.10.2) **Specimen / stage** ☐ Use a low-fluorescent glass slide and cover glass. **Camera** ☐ If the contrast is degraded by discoloration, reduce the excitation light intensity to increase the camera sensitivity.

5.1.6 Troubles in DF Microscopy

Problem	Check item
The field of view image is invisible.	**Light source / illumination** ☐ Turn on the dia illumination and adjust the brightness. (☞ 3.3.2, 3.3.7) ☐ Decrease ambient light, or shield the light from above by attaching an optional contrast shield. (☞ 3.3.6) **Condenser** ☐ Use the darkfield condenser (☞ 3.4.1, 4.5.2) ☐ Open the aperture diaphragm. (☞ 3.4.5) **Nosepiece / objective** ☐ Place an objective with a smaller NA than the minimum NA of the condenser or an objective with diaphragm into the optical path. (☞ 3.9.1, 3.10.4, 4.5.2)

5.1.7 Troubles in NAMC Microscopy

Problem	Check item
There is no contrast in the NAMC image.	**Polarizer** ☐ Place the polarizer into the optical path. (☞ 3.5.1, 4.6.2) Nosepiece / objective ☐ Place the NAMC objective into the optical path. (☞ 3.9.1, 4.6.2) **Condenser module** ☐ Place the NAMC module into the optical path (☞ 3.4.6, 4.6.2) **Condenser** ☐ Use a NAMC condenser lens. (☞ 3.4.1, 4.6.2) **Combination of optical elements** ☐ Confirm that the combination of the objective and the NAMC module is correct. (☞ 4.6.3) **Modulator adjustment** ☐ Adjust the position of the modulator of the objective. (☞ 3.4.8)
The contrast in the NAMC image is poor.	**Aperture diaphragm** ☐ Fully open the aperture diaphragm. (☞ 3.4.5) **Slit adjustment** ☐ Adjust the slit of the NAMC module according to the modulator position of the objective. (☞ 3.4.8) ☐ If the specimen is of a drop shape, it might have lens effect, so readjust the slit at the desired position. (☞ 3.4.8)
NAMC image quality is poor.	**Condenser module** ☐ Check if there is dust or scratches on the plastic optical system of the NAMC module. (☞ 4.6.2) Wiping resin optical elements with lens tissue might cause a scratch on the surface. Wiping with solvent might harm the surface. Use a simple cleaning method, such as with a blower. **Nosepiece / objective** ☐ Be careful not to turn the correction collar when turning the modulator ring. (☞ 3.4.8) If the correction collar turned by mistake, adjust it again. (☞ 3.10.1)

5.2 Troubleshooting for the Units

5.2.1 Microscope Main Body

Problem	Check item
Power cannot be turned on even when the power switch is pressed.	☐ Correctly connect the AC adapter and the power cord. (☞ 7.15.1)
No instructions on the AUX port	☐ The AUC port might be set to R80 (right side port 80%, eyepiece observation port 20%) or L80 (left side port 80%, eyepiece observation port 20%) according to the specification at the time of purchase. (☞ 3.2.1)
The precentered lamphouse cannot be controlled from the main body.	☐ Correctly connect the cable for the lamphouse. (☞ 7.15.1) ☐ Correctly connect the power supply and the Ti2-U. (☞ 7.15.1) ☐ Turn on the EXTERNAL switch of the power supply. (☞ 3.3.7)

5.2.2 Problems with the Dia-illumination Section

■ LED Lamp House for Dia Illumination

Problem	Check item
When dia-illumination is turned on, illumination does not reach the specimen.	☐ Open the dia-illumination shutter. (☞ 3.3.1) ☐ Remove the shutter of the condenser unit from the optical path. (☞ 3.4.6) ☐ Open the filed diaphragm and the aperture diaphragm. (☞ 3.3.3, 3.4.5)
The LED illumination is too bright.	☐ Insert the ND32 filter supplied with the LED lamphouse into the fixed filter slot. (☞ 7.8.3)

■ D-LH/LC precentered lamphouse

Problem	Check item
Pressing the dia-illumination ON/OFF button does not turn on the dia-illumination.	☐ Correctly connect the cable for the lamphouse. (☞ 7.15.1) ☐ Check whether the lamp is broken. (☞ 7.8.7)
When dia-illumination is turned on, illumination does not reach the specimen.	☐ Open the dia-illumination shutter. (☞ 3.3.1) ☐ Open the motorized shutter if it is in use. (☞ 3.3.7) ☐ Remove the shutter of the condenser unit from the optical path. (☞ 3.4.6)
Brightness of diascopic illumination cannot be adjusted using the dia-illumination brightness adjuster.	☐ Correctly connect the cable for the lamphouse. (☞ 7.15.1) ☐ Turn on the EXTERNAL switch of the power supply. (☞ 3.3.7) ☐ Correctly connect the power supply and the Ti2-U. (☞ 7.15.1)
The lamp is burnt out immediately after dia-illumination is turned on.	☐ Check whether an unsupported lamp is in use.

5.2.3 Pillar for Dia Illumination and Condenser

Problem	Check item
The field diaphragm image is invisible even though the field diaphragm is stopped down.	☐ Switch the objective to a lower-magnification objective. (☞ 3.9.1) ☐ Focus on the condenser. (☞ 3.4.2)
The condenser does not move down.	☐ Release the condenser refocusing clamp. (☞ 3.4.2) ☐ Check the installation position of the condenser elevating section. (☞ 7.8.2)
The condenser cannot be centered.	☐ Check if the centering screw is missing. (☞ 3.4.3) ☐ Correctly adjust the centering screw. (☞ 3.4.3) Do not turn the condenser module centering screw.
The condenser moves down.	☐ Turn the condenser focus knob on both sides in the tightening direction to increase the rotational torque. (☞ 3.4.2)
The polarizer rotation lever does not turn.	☐ Loosen the rotational part clamp screw. (☞ 3.5.1)
The contrast shield cannot be attached.	☐ Select a contrast shield (for LWD or ELWD) according to the condenser to be used. (☞ 7.9) ☐ Select a position suitable for the contrast shield from the two positions. (☞ 7.9)

5.2.4 Stage

Problem	Check item
The drive range of the stage is small.	☐ Release the stroke limit. (☞ 7.7.4)
The position of the specimen is unintentionally changed.	☐ Secure the specimen using the stage clip or the well clamper. (☞ 3.8)

5.2.5 Nosepiece and Focusing Device

Problem	Check item
The image is split.	☐ Remove the DIC slider. (☞ 3.9.2)
The objective contacts the specimen.	☐ Set the objective refocusing lever. (☞ 3.11.3)
The objective does not move up.	☐ Release the objective refocusing lever. (☞ 3.11.3)
The rotational torque of the focus knobs is large or small.	☐ Adjust the rotational torque by turning the coarse-focus knob torque adjustment ring. (☞ 3.11.2)
The objective contacts the specimen or the specimen holder during nosepiece rotation.	☐ Move down the objective, and then turn the nosepiece. (☞ 3.9.1)

5.2.6 Epi-fluorescence Attachment

Problem	Check item
When epi-illumination is turned on, illumination does not reach the specimen.	☐ Open the FL turret shutter. (☞ 3.12.2)

Chapter
6

Maintenance and Storage of the Device

This chapter describes how to maintain and store the device.

Maintenance work done incorrectly could affect the performance of the product.
When performing maintenance work on the product, follow the instructions in this chapter.

When performing maintenance work for components not described in this manual, see the appropriate manual for each component.

6.1 Cleaning the Device

Clean or disinfect the lenses and other components according to the following instructions.

Cleaning tools

- Blower
- Soft brush
- Soft cotton cloth, lens cleaning tissue, gauze, etc.
- Absolute alcohol (ethyl alcohol or methyl alcohol), medical alcohol
- Petroleum benzine (only for wiping off immersion oil)
- Finger cots, gloves

CAUTION

- **Petroleum benzine and absolute alcohol used for cleaning are highly flammable. Handle them with due care, particularly around open flames or when turning the power switch on and off.**
- **Always follow the instructions provided by the manufacturer when using petroleum benzine or absolute alcohol.**
- **Do not use organic solvents (such as alcohol, ether, and thinner) when cleaning the painted, plastic, or printed parts of this product. Using organic solvents might result in discoloration or cause printed text to fade.**
- **Use petroleum benzine only when wiping immersion oil off the objectives or condenser lenses. Do not use petroleum benzine for any other purpose.**
- **When using organic solvents such as pure alcohol, be sure to wear finger cods or gloves to avoid direct contact with the solvent.**

6.1.1 Cleaning the Lenses

Keep the lenses free of dust, fingerprints, and other dirt. Any dirt on lenses and filters will degrade the image quality. If the lenses become dirty, clean them according to the following procedure.

Cleaning off minor dirt (such as dust)

(1) Blow dust off using an air blower.

(2) If this is insufficient, brush away dust with a soft brush or wipe away gently with a piece of gauze.

Cleaning off heavy dirt (such as fingerprints or oil stains)

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

> **Tips on cleaning lenses**
> Do not reuse cotton cloth, lens cleaning tissue, or gauze that has already been used.

> **Cleaning the NAMC condenser module**
> The NAMC condenser module has built-in plastic optical elements. When cleaning this module, simply clean it with a blower.
> Wiping plastic optical elements with lens tissue might cause a scratch on the surface and wiping with solvent might damage the surface. At the time of assembly, take care that the plastic part is free of dirt; especially avoid touching this part by hand.

6.1.2 Cleaning Parts other than Lenses

Cleaning off minor dirt (such as dust)

Wipe off using a silicone cloth.

Cleaning off heavy dirt (such as fingerprints or oil stains)

Lightly moisten a piece of gauze with a neutral detergent and wipe the dirt gently.

6.1.3 Cleaning Off Immersion Oil

(1) Wipe off using petroleum benzine.

(2) Then, finish off the cleaning with absolute alcohol (ethyl or methyl alcohol.)

> **If petroleum benzine is unavailable**
> If petroleum benzine is unavailable, use methyl alcohol.
> Note that methyl alcohol is less effective, and requires more wipes.

6.1.4 Decontaminating the Product

Use 70% medical alcohol for routine disinfection of the product.

Use of organic solvents on plastic parts may result in discoloration.

Never use any form of hydrogen peroxide (H_2O_2) to decontaminate this product, because it might cause various precision components in the microscope to deteriorate.

> **Handling a hazardous specimen**
> If a specimen comes into contact with this product, check if the specimen is hazardous.
> If the specimen is hazardous, follow the standard procedure of your laboratory.

6.2 Storing the Product

Note the following precautions when storing this product.

- Store this product in a dry location where mold is unlikely to form.
- Store this product in a temperature range of -20 to +60°C with a relative humidity of up to 90% (no condensation).
- Store objectives and eyepieces in a desiccator or other vessel that contains a desiccant.
- Place a dust cover over this product to protect it from dust.
- Do not cover this product unless the power switch on the main body of the microscope has been turned off (set it to “o”) and the lamphouse becomes sufficiently cool to touch (for approximately 30 minutes).

6.3 Regular Inspection (Charged)

To maintain the performance of this product, Nikon recommends periodic inspections (chargeable service).

Chapter 7

Assembly of the Devices

This chapter presents the system configuration diagram and components list for the Ti2-U microscope, and also explains how to assemble devices.

WARNING

- Before assembling or connecting devices, thoroughly read “Safety Precautions” at the beginning of this manual, and heed all warnings and cautions written therein.
- To prevent electric shock, fire, and product damage, be sure to turn off the power switches on all devices, and unplug the power cords beforehand.

CAUTION

- Take care not to pinch your fingers or hands.
- When mounting or demounting optical components (such as lenses and filters), make sure that the light source is turned off.
- Scratches and dirt on optical components (such as lenses and filters) will degrade the microscope image. Keep them free of scratches, dust, fingerprints, and other dirt.

Notes on handling

- Be sure to put the supplied cap on any unused port to prevent the intrusion of extraneous light and dust.
- This product is a precision optical instrument. Handle this product with due care, and avoid subjecting it to physical shocks. In particular, the accuracy of objectives might be lost by even mild physical shocks.
- Some devices require cable connections.
  Be sure to turn off the power to the microscope and peripheral units before connecting a cable.

Cable connection

- Nikon recommends connecting cables at the end of the assembly.
  For details on the connector positions, see “7.15 Connection of Each Cable.”

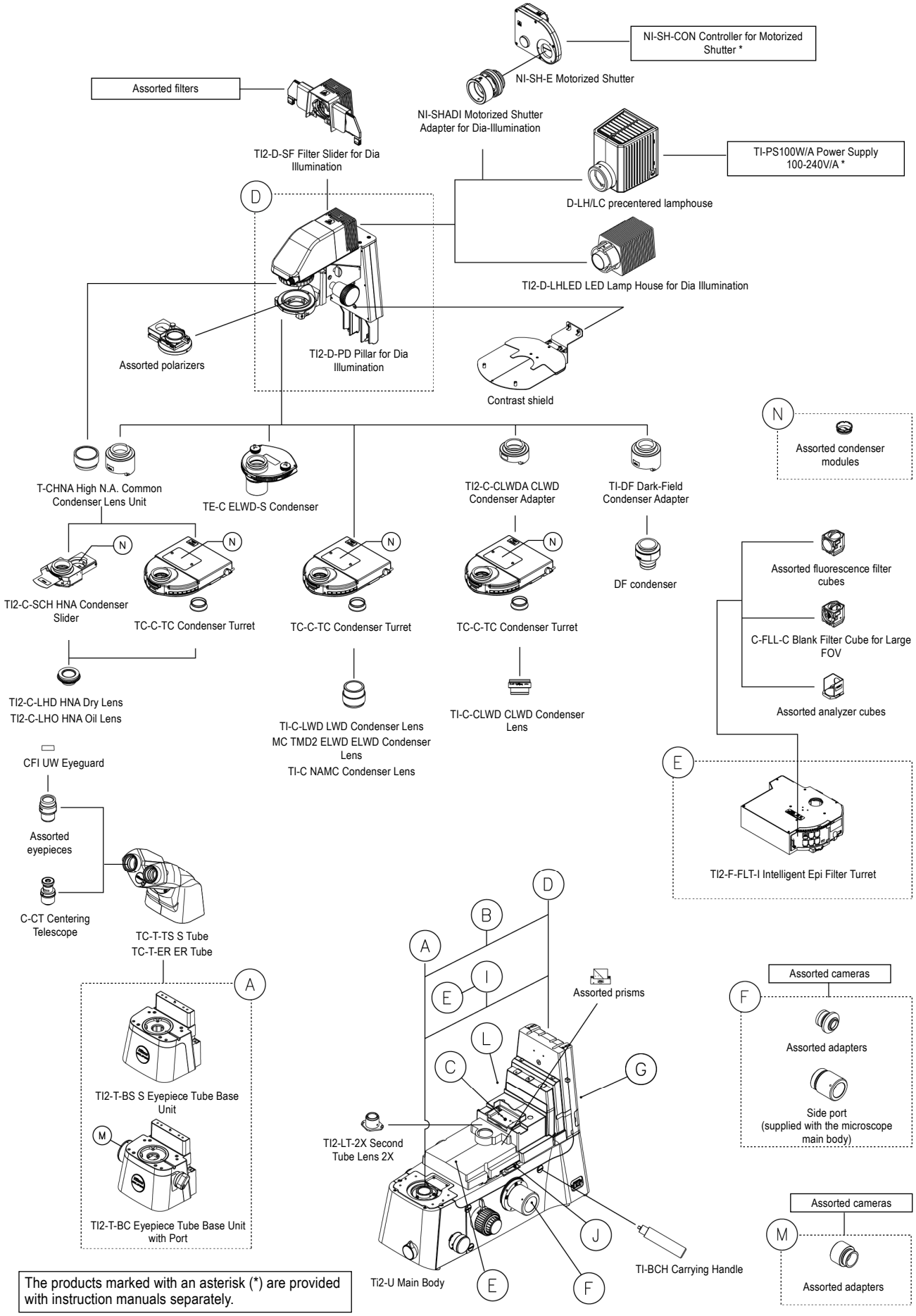
Installation location

Select an appropriate place for installation after reading “Notes on Handling the Product” at the front of this manual.

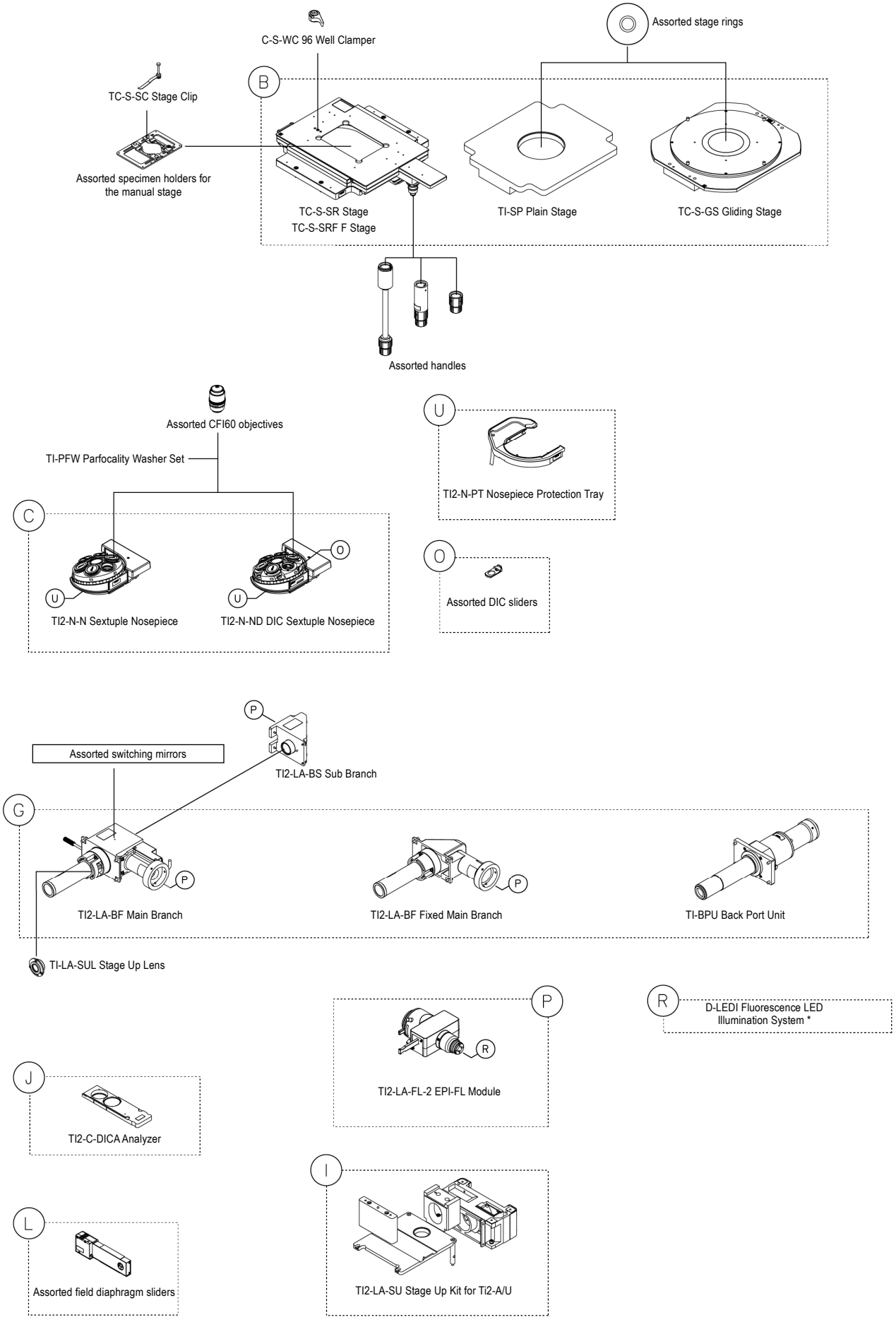
Assembly tools supplied with the microscope

- 4 mm hex wrench (ball point): x 1
- 3 mm hex wrench: x 1
- 2 mm hex screwdriver: x 2

7.1 Ti2-U System Configuration



The products marked with an asterisk (*) are provided with instruction manuals separately.


C-S-WC 96 Well Clamper
Assorted stage rings
TC-S-SC Stage Clip
B
Assorted specimen holders for the manual stage
TC-S-SR Stage
TC-S-SRF F Stage
TI-SP Plain Stage
TC-S-GS Gliding Stage
Assorted handles
Assorted CFI60 objectives
TI-PFW Parfocality Washer Set
U
TI2-N-PT Nosepiece Protection Tray
C
O
TI2-N-N Sextuple Nosepiece
TI2-N-ND DIC Sextuple Nosepiece
Assorted DIC sliders
P
Assorted switching mirrors
TI2-LA-BS Sub Branch
G
TI2-LA-BF Main Branch
TI2-LA-BF Fixed Main Branch
TI-BPU Back Port Unit
TI-LA-SUL Stage Up Lens
R
D-LEDI Fluorescence LED Illumination System *
TI2-LA-FL-2 EPI-FL Module
J
TI2-C-DICA Analyzer
I
L
Assorted field diaphragm sliders
TI2-LA-SU Stage Up Kit for Ti2-A/U

7.2 List of Components

The following table shows the components of the ECLIPSE Ti2-U microscope.
Some components may be unavailable, depending on when you purchased this product. For details, contact your local Nikon representative.

Device	Description	Model	Remarks
Main body	ECLIPSE Ti2-U main body	ECLIPSE Ti2-U	
	AC adapter	FSP040-RHAN2/ FSP040-RHAN3	For the microscope main body
Tube base unit	Eyepiece Tube Base Unit	TI2-T-BS	
	Eyepiece tube base unit with port	TI2-T-BC	
Tube	S tube	TC-T-TS	
	ER tube	TC-T-ER	
Eyepiece	CFI eyepiece	CFI series	10x, 12.5x, 15x For details, contact your local Nikon representative.
	Centering telescope	C-CT	
FL turret	Intelligent epi filter turret	TI2-F-FLT-I	
	Fluorescent filter cube	Assorted models	
	Fluorescent filter cube for large FOV	C-FLL series	
	Blank filter cube for large FOV	C-FLL-C	
Nosepiece	Sextuple nosepiece	TI2-N-N	
	DIC sextuple nosepiece	TI2-N-ND	
	Nosepiece protection tray	TI2-N-PT	A combination of water tray and protection plate (optional)
Objective-side DIC prism	DIC sliders	Assorted models	See §**3.9**.
Objective	CFI objective	CFI series	
	Parfocality washer set	TI-PFW	For parfocal adjustment of objectives without correction collar
Stage	Stage	TC-S-SR	For right-rear/left-front handle
	F Stage	TC-S-SRF	For right-front/left-rear handle
	Plain stage	TI-SP	
	Gliding Stage	TC-S-GS	
Stage handle	Long handle	TI2-S-HL	
	Middle handle	TC-S-HM	
	Short handle	TC-S-HS	
Specimen holder	Specimen holder	Assorted models	See §**3.8.1**.
	96 well clamper	C-S-WC	For manual stage
	Stage clip	TC-S-SC	Stage clip used as a specimen holder
Stage ring	Glass stage ring 32		
	TE acrylic stage ring		
Pillar for dia-illumination	Pillar for dia-illumination	TI2-D-PD	
Diascopic illumination lamphouse	Diascopic LED lamphouse	TI2-D-LHLED	LED lamp
	Precentered lamphouse	D-LH/LC	Halogen lamp
	Power Supply 100-240V/A	TI-PS100W/A	Power supply for precentered lamphouse
	12 V 100 W halogen lamp	(Commercially available product)	Lamp for precentered lamphouse For the specified lamps, See §**8.2**.
	Remote cable for 100 W lamphouse	S-TI2-100WRC	For precentered lamphouse

Device	Description	Model	Remarks
Diascopic illumination filter	Filter slider for dia illumination	TI2-D-SF	Recommended when a D-LH/LC precentered lamphouse is used for dia-illumination. Usable filters: • TI2-D-D 45 Diffuser • MF45 GIF • MF45 NCB11 • MF45 ND2A • MF45 ND8A • MF45 ND16A
	Diascopic filter (for fixed filter)	Assorted models	Usable filters: • ND2 • ND4 • ND8 • ND16 • NCB11 • GIF • D
Contrast shield	Contrast shield ELWD	TS2-LS	For ELWD condenser lens
	Contrast shield	TS2R-LS	
Analyzer	Analyzer	TI2-C-DICA	Mounted in analyzer slider slot
	Analyzer block	TI-A	Mounted on the FL turret
	Analyzer cube for large FOV	TI2-C-DICACL	Mounted on the FL turret
Polarizer	DIC polarizer	TC-C-DICP	
	NAMC/IMSI polarizer	TC-C-DICPNI	
Condenser	Condenser turret	TC-C-TC	System condenser
	ELWD-S condenser	TE-C	Condenser for ELWD
	HNA condenser slider	TI2-C-SCH	Condenser for HNA lens T-CHNA High N.A. common condenser lens unit is required.
	High N.A. common condenser lens unit	T-CHNA	For HNA lens
	DF condenser (oil)		The TI-DF dark-field condenser adapter is required.
	DF condenser (dry)		The TI-DF dark-field condenser adapter is required.
	HNA dry lens	TI2-C-LHD	Condenser lens for high NA objectives
	HNA oil lens	TI2-C-LHO	Condenser lens for high NA objectives
	LWD condenser lens	TI-C-LWD	Condenser lens for long-working-distance objectives
	ELWD condenser lens	MC TMD2 ELWD	Condenser lens for long-working-distance objectives
	NAMC condenser lens	TI-C NAMC	NAMC condenser lens
	CLWD condenser lens	TI-C-CLWD	Condenser lens for high NA long-working-distance objectives The TI2-C-CLWDA CLWD condenser adapter is required.
	CLWD condenser adapter	TI2-C-CLWDA	For CLWD condenser lens
	Condenser adapter	TI-DF	For dark-filed condenser
Condenser module for Ph microscopy	CLWD PH module	TI2-C-MC series	For CLWD condensers
	LWD PH module	TC-C-ML series	For LWD condensers
	ELWD PH module	TC-C-ME series	For ELWD condensers
Condenser module for DIC microscopy	LWD dry DIC module	TC-C-ML series	For LWD condensers
	IMSI dry DIC module	TC-C-MI series	IMSI for LWD condenser
	HNA dry DIC module	TI2-C-MH series	For HNA dry lens
	HNA oil DIC module	TI2-C-MH series	For HNA oil lens

Device	Description	Model	Remarks
Condenser module for NAMC microscopy	NAMC module	TC-C-MN series	For NAMC condensers
	NAMC LWD module	TC-C-MNL series	For LWD condensers
Motorized shutter	Motorized shutter	NI-SH-E	Diascopic illumination Mountable only when a precentered lamphouse is used. Mounted by Nikon personnel.
	Controller for motorized shutter	NI-SH-CON	
	Motorized shutter cable long	S-TI2-SHCLL	For connecting the motorized shutter controller
	Motorized shutter cable long	NI-SHCL	For connecting the motorized shutter controller
	Motorized shutter adapter for dia-illumination	NI-SHADI	
	Motorized shutter adapter for inverted epi-fl	TI-SHAEP-I	
Epi-fluorescence attachment	Fixed main branch	TI2-LA-BF	A branch used for connecting the EPI-FL module to the microscope
	Main branch	TI2-LA-BM	A branch used for connecting the EPI-FL module to the microscope
	Sub branch	TI2-LA-BS	In combination with the TI2-LA-BM main branch, two EPI-FL modules can be attached.
	Switching mirror	TI-LA-SWM	For switching the optical path of the TI2-LA-BM main branch Select either 50:50 or 100:0 (total reflection).
	Stage-up lens	TI-LA-SUL	For the lower-tier main branch when a stage-up kit is used For details, contact your local Nikon representative.
	EPI-FL module	TI2-LA-FL-2	Required for a combination of each branch and an LED light source
Light source for episcopic illumination	Fluorescence LED illumination system	D-LEDI	Fluorescence LED light source
Episcopic field diaphragm slider	Circular field stop slider	TI2-F-FSC	
	Square field stop slider	TI2-F-FSS	
	Rectangle field stop slider	TI2-F-FSR	Aspect ratio: 2:3
Stage-up	Stage-up kit for Ti2-A/U	TI2-LA-SU	For details, contact your local Nikon representative.
Port	Back port unit	TI-BPU	Port for the second camera device
	Side port adapter		Supplied with the microscope main body (x 2)
	C mount adapter with centering tool	TI2-P-CCA	

Device	Description	Model	Remarks
Other	Camera adapter	Assorted models	For mounting assorted camera models
	Carrying handles	TI-BCH	Microscope carry handle
	Fix plate for CF	TI2-FP	For fixing base plates. For details, contact your local Nikon representative.
	T-BP/E20L80 prism		For splitting optical paths. For details, contact your local Nikon representative.
	T-BP/E20R80 prism		For splitting optical paths. For details, contact your local Nikon representative.
	Second tube lens 2X	TI2-LT-2X	Built into the microscope main body in place of the standard 1.5x intermediate variable magnification lens. For details, contact your local Nikon representative.

7.3 Installing the Microscope Main Body (Base)

Notes on installation work

As this product is heavy, make sure at least two people are available when performing installation work.
When lifting the microscope main body (base), firmly hold it by gripping the recess in the front bottom part and the handhold position in the rear part.

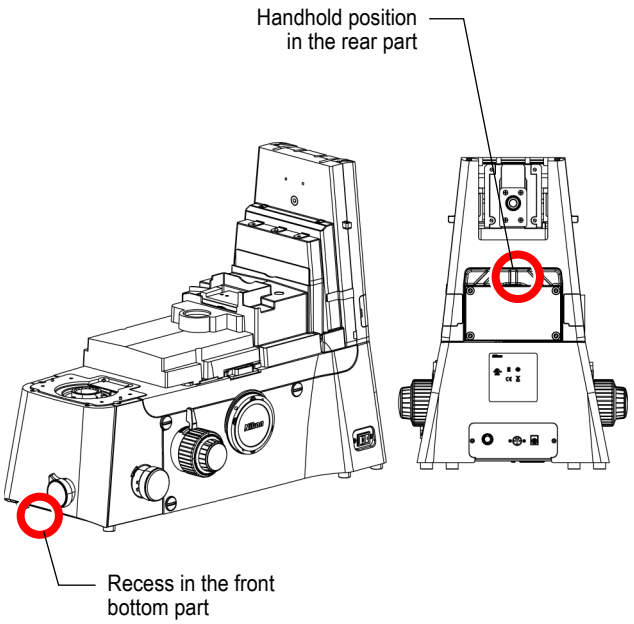
7.3.1 Removal and Installation

Install the microscope main body in an appropriate location.

1. **Select the installation location.**

   For information about installation locations, see “4. Installation location and storage location” under “Notes on Handling This Product” at the beginning of this manual.

2. **Take out the microscope main body (base) from the packing box, and install it in a stable location.**



Microscope main body (base)

Notes on moving the microscope

Before moving the microscope, thoroughly read ⚠ CAUTION “10. Cautions on carrying the microscope” in “Safety Precautions” at the beginning of this manual and “7.18 Moving the Microscope”, and always heed all warnings and cautions written therein.

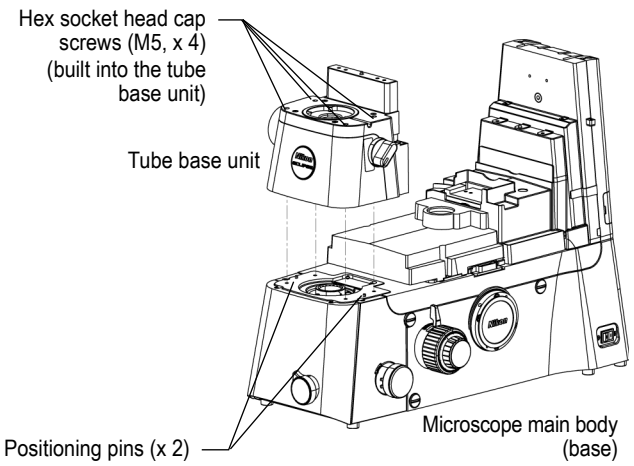
7.4 Mounting the Tube Base Unit

Mount the eyepiece tube base unit (TI2-T-BC or TI2-T-BC) on the microscope main body (base). (Tool: 4 mm hex wrench, supplied with the microscope main body)

1. **Place the tube base unit on the front part of the microscope main body (base) while keeping the eyepiece tube mounting position of the tube base unit facing frontwards.**

   Two positioning holes are provided on the bottom of the tube base unit. Place the tube base unit by aligning them with the positioning pins of the microscope base.

2. **Insert the hex wrench into the tube base unit from above, and affix the unit by tightening the four M5 hex socket head cap screws built into the stage.**



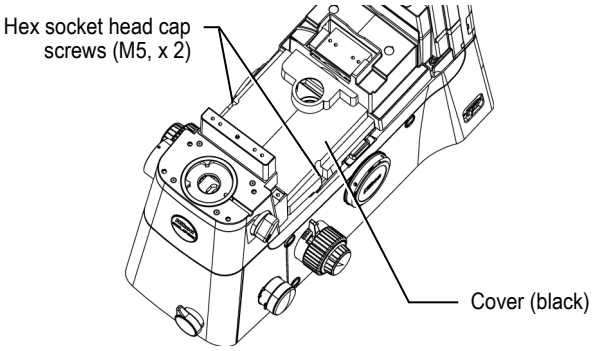
7.5 FL Turret (Required for Epi-FL Microscopy)

An FL turret can be attached to the microscope main body (base).

7.5.1 Attaching an FL Turret

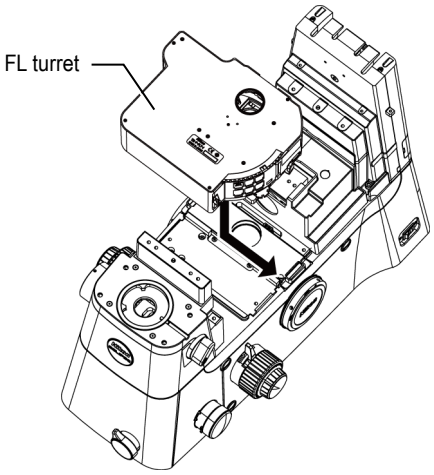
1. Remove the cover (black) from the nosepiece mount.

Remove the two M5 hex socket head cap screws using a hex wrench.
(Tool: 4 mm hex wrench, supplied with the microscope main body)



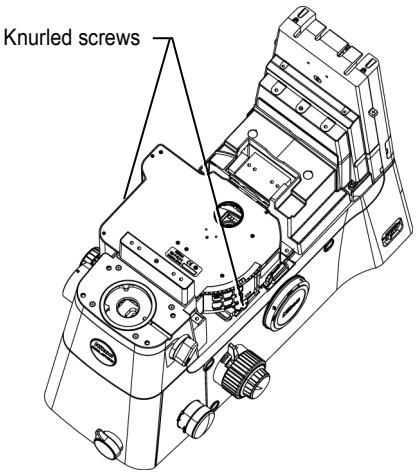
Microscope main body (base)

2. Place the FL turret on the microscope main body (base), and press the turret toward the right side as far as it will go.



3. Affix the knurled screws at the bottom of both sides of the FL turret by turning them manually.

To fix the screws more firmly, insert a hexagonal screwdriver into the grooves around the knurled screws and turn and fasten the knurled screws.
(Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)



> **Setting up the FL turret in a two-tier configuration**
>
> The FL turret can be set up in a two-tier (upper and lower) configuration by using the TI2-LA-SU stage-up kit for the Ti2-A/U. In this case, the lower tier is the first tier (FL1), and the upper tier is the second tier (FL2). As with the case in which only the first tier is used, mount and connect the turret and register filter cubes.
>
> Stage-up kits must be mounted by Nikon personnel. For details, contact your local Nikon representative.

7.5.2 Mounting a Filter Cube

Mount a filter cube on the FL turret.

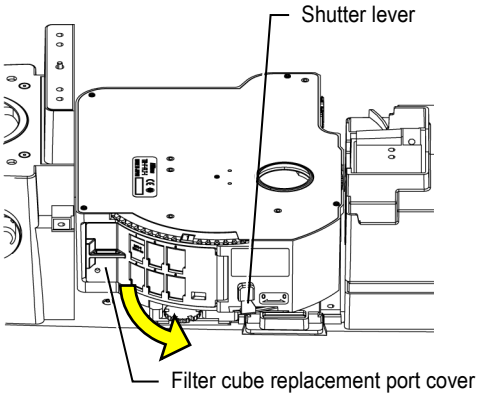
Precautions on mounting or removing a filter cube

- Before mounting or removing a filter cube, make sure that the assembly of other devices has been completed.
- Before mounting or removing a cube, make sure that the light source is turned off.
- When mounting a cube, make sure that the power switch of the microscope main body is turned off, and then mount the cube by turning the cube switching turret manually.

Filter cube component replacement

For details on replacing the optical components, see “7.5.3 Replacing an Excitation Filter, a Barrier Filter, and a Dichroic Mirror.”

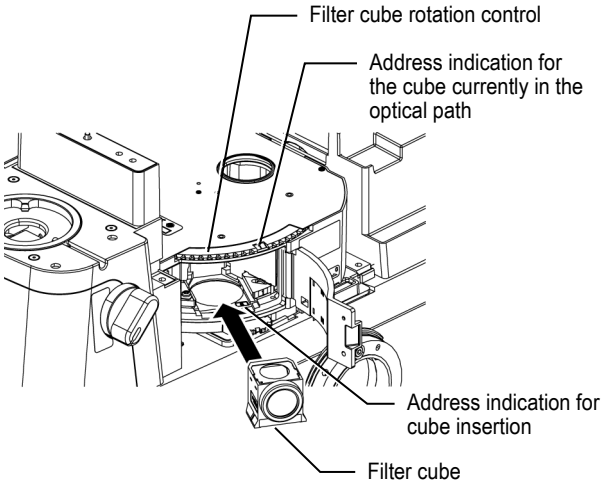
1. **Close the shutter of the FL turret by setting the shutter lever to the C side (rear).**
2. **Open the filter cube replacement port cover.**
3. **Check the position display (address) of the turret inside (on the right side of) the replacement port, and manually turn the turret so that the required address moves to the aperture.**



FL turret

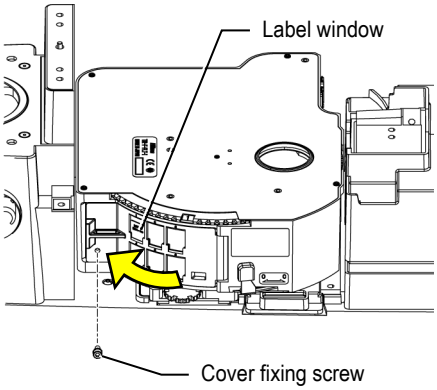
4. **Insert the filter cube into the dovetail of the turret, and press the filter cube in until it reaches the limit position.**
5. **Insert the filter cube label into the label window of the replacement port cover.**

   Insert the filter cube label into the label window of the same address as that shown in the address indication for cube insertion.
6. **Repeat steps 3 to 5 to attach all the required filter cubes.**



7. **Close the replacement port cover.**

   The replacement port cover is attached by magnet, and therefore usually it does not need to be fixed using a screw. The replacement port cover can be secured using a cover fixing screw (M3 screw supplied with the FL turret) so that it does not open, if required.
   (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)



7.5.3 Replacing an Excitation Filter, a Barrier Filter, and a Dichroic Mirror

The excitation filter, barrier filter, and dichroic mirror in the filter cube can be removed and replaced with other filters or mirrors.

> **Handling optical elements**
> When handling optical elements, wear gloves if possible, and do not touch the surface of the filter or the mirror with your bare hands. Keep the optical elements free of dust, fingerprints, and stains.

Replacement of the fluorescent filter cube components

Follow the procedure below to replace the fluorescent filter cube components.

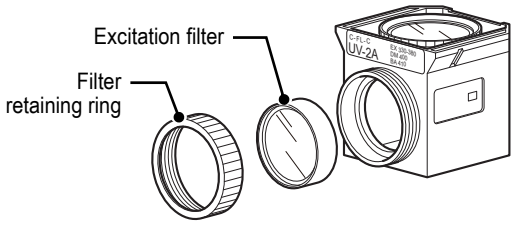
Replacing the excitation filter

The excitation filter is fixed to the filter cube with the screw-in type filter retaining ring.

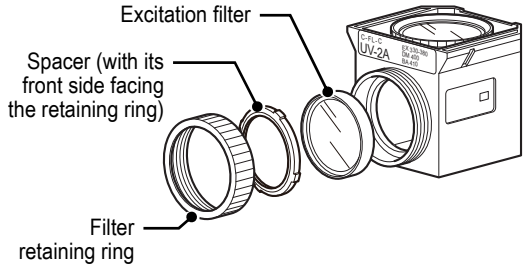
When attaching a thin excitation filter, the spacer is required to be placed between the excitation filter and the filter retaining ring.

- When thickness of filter is ≥6 mm and ≤7.2 mm: Spacer not required
- When thickness of filter is ≥4.5 mm and <6 mm: Spacer required
- When thickness of filter is ≥3.5 mm and <4.5 mm: Spacer required

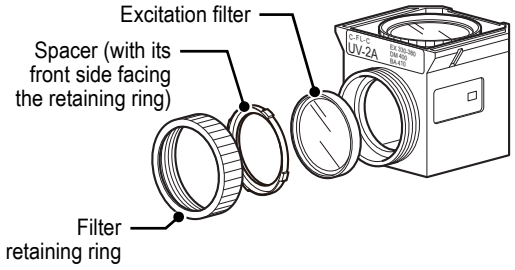
1. **Turn the excitation filter retaining ring counterclockwise and remove it.**
2. **Replace the excitation filter with the desired one. If the spacer is required, place the spacer checking its attaching direction. Then, fix the excitation filter (and the spacer if required) by screwing the filter retaining ring.**



Replacement of the excitation filter
(Thickness of filter is ≥6 mm and ≤7.2 mm)



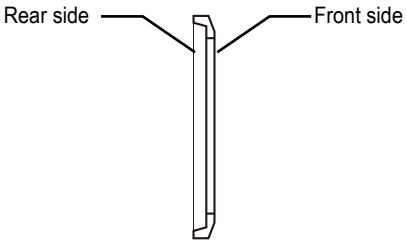
Replacement of the excitation filter
(Thickness of filter is ≥4.5 mm and <6 mm)



Replacement of the excitation filter
(Thickness of filter is ≥3.5 mm and <4.5 mm)

> **Attaching direction of the excitation filter**
> When attaching the excitation filter, check that the arrow on the filter ring points the dichroic mirror.

> **Attaching direction of the spacer**
> The attaching direction of the spacer differs depending on the thickness of the excitation filter.
>
> If the direction of the spacer is wrong, the filter cube may collide with other components when attached.
>
> - When thickness of filter is ≥4.5 mm and <6 mm
> Place the spacer as its front side faces the retaining ring.
> - When thickness of filter is ≥3.5 mm and <4.5 mm
> Place the spacer as its rear side faces the retaining ring.



Cross section of the spacer

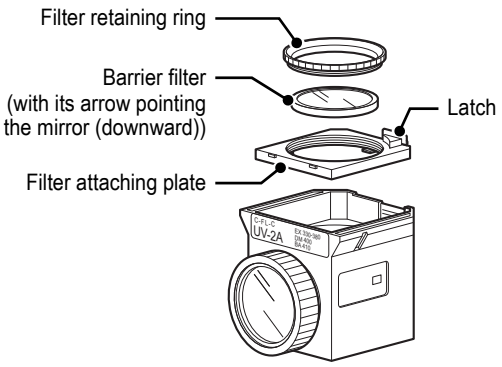
* When attaching a non-Nikon excitation filter, ask the manufacturer the attaching direction in advance.

Replacing the barrier filter

The barrier filter is fixed to the filter attaching plate at the upper part of the filter cube with the screw-in type filter retaining ring.

1. **Press the latch inward and remove the barrier filter with the filter attaching plate.**
2. **Turn the filter retaining ring and remove it from the filter attaching plate.**
3. **Replace the barrier filter with the desired one and fix it by reversing the steps shown above.**

> **Attaching direction of the barrier filter**
> When attaching the barrier filter, check that the arrow on the filter ring points the dichroic mirror (downward).



Replacement of the barrier filter

* When attaching a non-Nikon barrier filter, ask the manufacturer the attaching direction in advance.

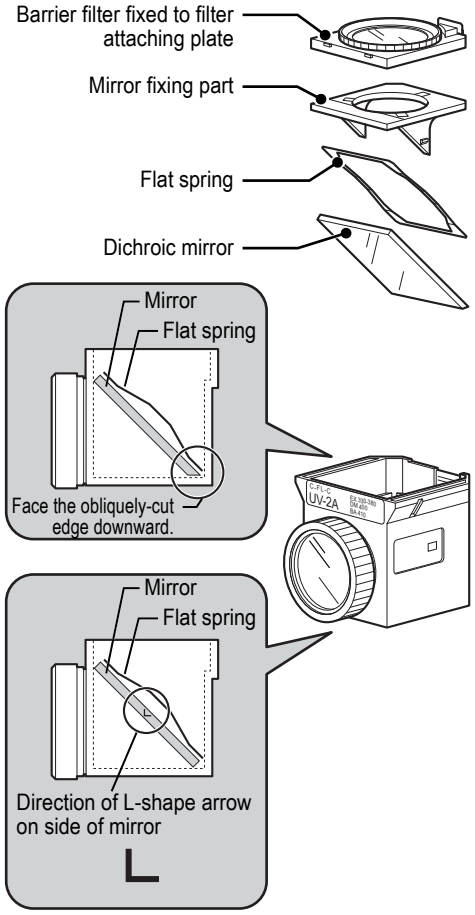
Replacing the dichroic mirror

The dichroic mirror is fixed in the filter cube with the flat spring and mirror fixing part.

1. **Remove the barrier filter with the filter attaching plate.**
2. **The mirror fixing part is fixed to the cube with the latch on both sides. Lift the mirror fixing part upward to remove it.**
3. **Remove the flat spring and the dichroic mirror.**
4. **Checking the attaching direction, place the desired dichroic mirror as the original mirror has been.**

> **Attaching direction of the dichroic mirror**
> To identify the reflection face, a dichroic mirror has either of the following features: The one edge of the dichroic mirror is obliquely cut, or an L-shape arrow is marked on the side of the dichroic mirror.
> - **Dichroic mirror with obliquely-cut edge**
> Place the mirror as the obliquely-cut edge faces downward so that the obliquely-cut edge matches the bottom surface of the filter cube.
> - **Dichroic mirror with L-shape arrow**
> Place the mirror as the L-shape arrow points the bottom side (opposite side from the surface that contacts with the flat spring).

5. **Attach the flat spring in the direction it holds both of the edges of the dichroic mirror.**
6. **Attach the mirror fixing part and the barrier filter as they have been.**



Replacement of the dichroic mirror

* When attaching a non-Nikon dichroic mirror, ask the manufacturer the attaching direction in advance.

Replacement of the fluorescent filter cube for large FOV components

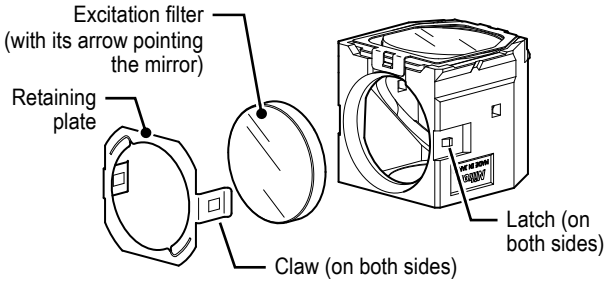
Follow the procedure below to replace the components of the fluorescent filter cube for large FOV.

Replacing the excitation filter

The excitation filter is fixed to the mount hole of the filter cube with the retaining plate.

1. **Open the claws of the retaining plate to release the latches and remove the retaining plate from the filter cube.**
2. **Replace the excitation filter and fix it with the retaining plate.**

   To securely attach the retaining plate back to the original position, push the retaining plate so that its claws engage with the latches.



Replacement of the excitation filter

> **Attaching direction of the excitation filter**
>
> When attaching the excitation filter, check that the arrow on the filter ring points inside the filter cube (dichroic mirror side.)

* When attaching a non-Nikon barrier filter, ask the manufacturer the attaching direction in advance.

Replacing the barrier filter

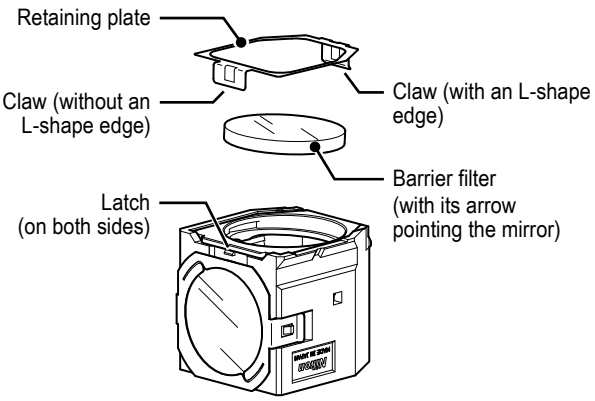
The barrier filter is fixed to the mount hole of the dichroic mirror fixing part with the retaining plate.

1. **Open the claws of the retaining plate to release the latches and remove the retaining plate from the filter cube.**

   The rear claw of the retaining plate has an L-shape edge for smooth opening.
2. **Replace the barrier filter and fix it with the retaining plate.**

   First hook the front claw and then the rear claw to mount the retaining plate.

   To securely attach the retaining plate back to the original position, push the retaining plate so that its claws engage with the latches.



Replacement of the barrier filter

> **Attaching direction of the barrier filter**
>
> When attaching the barrier filter, check that the arrow on its outer periphery points inside the filter cube (dichroic mirror side.)

* When attaching a non-Nikon barrier filter, ask the manufacturer the attaching direction in advance.

Replacing the dichroic mirror

The dichroic mirror is fixed inside the filter cube with the flat spring and the mirror fixing part.

1. **Remove the barrier filter and the retaining plate.**
2. **Lift the dichroic mirror fixing part upward to remove it.**

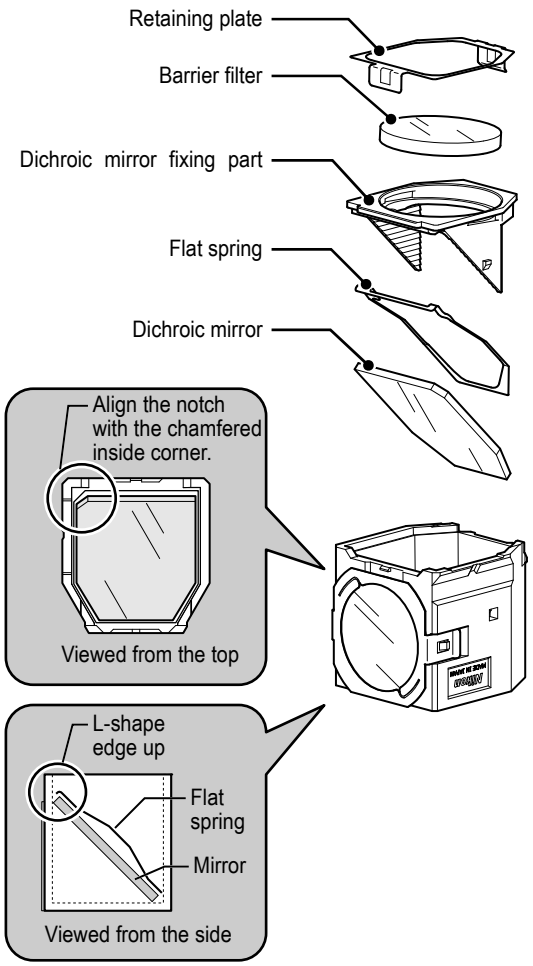
   The mirror fixing part is fixed to the cube with the latch on both sides of the cube.
3. **Remove the flat spring and the dichroic mirror.**
4. **Checking the attaching direction, place the desired dichroic mirror as the original mirror has been.**

> **Attaching direction of the dichroic mirror**
>
> The dichroic mirror has one notched (chamfered) corner, and the filter cube has one chamfered inside corner to match with the mirror notch, which allows the mirror installation only in the correct direction.
>
> To attach the dichroic mirror, align the mirror notch with the chamfered inside corner of the filter cube.

5. **Attach the flat spring on the dichroic mirror.**

   Place the flat spring with the L-shape edge up and the outer arc faces the dichroic mirror as shown in the figure on the right.
6. **Attach the dichroic mirror fixing part back to the original position.**

   Push it in until it is securely fixed to the filter cube with the latch on both sides.
7. **Attach the barrier filter and the retaining plate back to the original position.**



Replacement of the dichroic mirror

* When attaching a non-Nikon dichroic mirror, ask the manufacturer the attaching direction in advance.

7.5.4 Attaching Optical Elements to the C-FL-HQ Filter Cube

An excitation filter, a barrier filter and a dichroic mirror can be attached to the C-FL-HQ filter cube

Usable optical elements

The C-FL-HQ filter cube can be used with the following optical elements:

- Excitation filter Ø25 mm, t = 6 mm
- Barrier filter Ø25 mm, t = 3.5 mm
- Dichroic mirror 36 x 25.7 mm, t = 1 mm

Handling optical elements

When handling optical elements, wear gloves if possible, and do not touch the surface of the filter or mirror with your bare hands. Keep the optical elements free of dust, fingerprints, and stains.

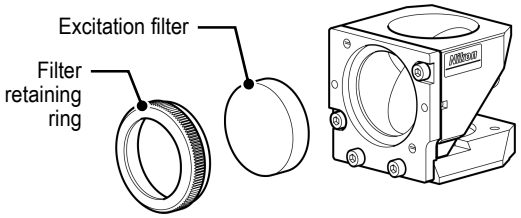
Excitation filter attachment

The excitation filter is fixed to the filter cube with the screw-in type filter retaining ring.

1. **Turn the excitation filter retaining ring counterclockwise and remove it.**
2. **Attach the excitation filter in place.**
3. **Put the retaining ring back to the original position and fix the filter.**

Attachment direction of the excitation filter

When attaching an excitation filter, check that the arrow on the filter ring points the dichroic mirror (inside the filter cube.)



Attaching the excitation filter

* When attaching a non-Nikon excitation filter, ask the manufacturer the attaching direction in advance.

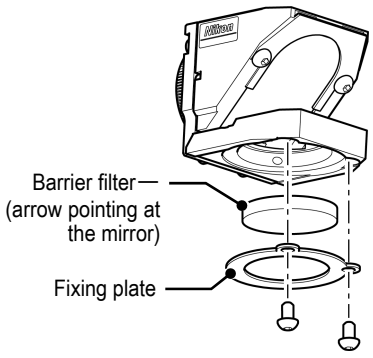
Barrier filter attachment

Attach the barrier filter to the bottom of the filter cube. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Remove the two fixing screws to remove the fixing plate.**
2. **Attach the barrier filter in place.**
3. **Put the fixing plate back to the original position, and tighten the two fixing screws to fix the plate.**

Attachment direction of the barrier filter

When attaching a barrier filter, check that the arrow on the filter ring points the dichroic mirror (upward.)



Attaching the barrier filter

* When attaching a non-Nikon barrier filter, ask the manufacturer the attaching direction in advance.

Dichroic mirror attachment

Attach the dichroic mirror to the inclined plane of the filter cube. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Loosen the two fixing screws to remove the fixing plate.**
2. **Remove the flat spring.**
3. **Attach the dichroic mirror to the filter cube in a correct direction.**

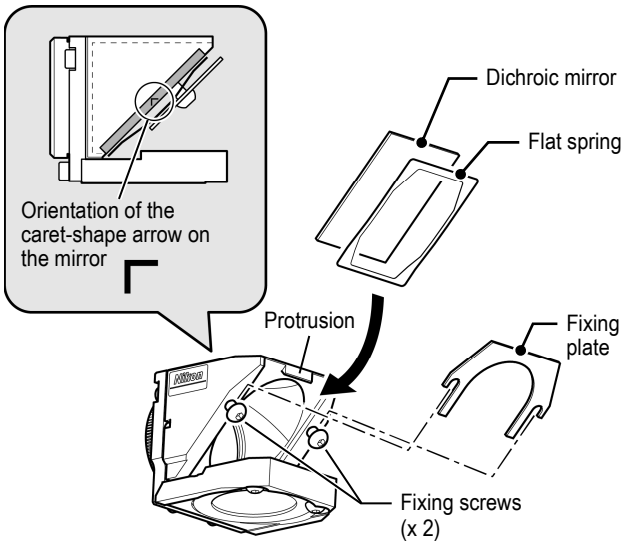
   Align the mirror with the protrusion on the filter cube.

   > **Attachment direction of the dichroic mirror**
   > To identify the reflection face, a caret-shape arrow is marked on the side of the dichroic mirror.
   > Attach the mirror so that the caret-shape arrow points the mirror seating surface (opposite side of the flat spring.)

4. **Attach the flat spring so that it holds both ends of the dichroic mirror.**

   Align the flat spring with the protrusion on the filter cube.

5. **Put the fixing plate back to the original position, and tighten the two fixing screws to fix the plate.**



Attaching the dichroic mirror

* When attaching a non-Nikon dichroic mirror, ask the manufacturer the attaching direction in advance.

7.6 Attaching a Nosepiece

Attach the nosepiece.

The basic mounting procedure is common to all manual nosepieces.

Attaching an objective
Before attaching an objective, make sure that the stage has been mounted.

Mounting a protection plate (optional)
The TI2-N-PT nosepiece protection tray (water tray and protection tray) can also be attached between the nosepiece and the FL turret. The nosepiece protection tray should be mounted by Nikon personnel. For details, contact your local Nikon representative.

1. **Remove the drip-proof cover from the top of the nosepiece.**

   (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

   Remove the cover mount screw using a hexagonal screwdriver, and then remove the drip-proof cover from the nosepiece.

2. **Attach the nosepiece to the microscope main body in a correct direction.**

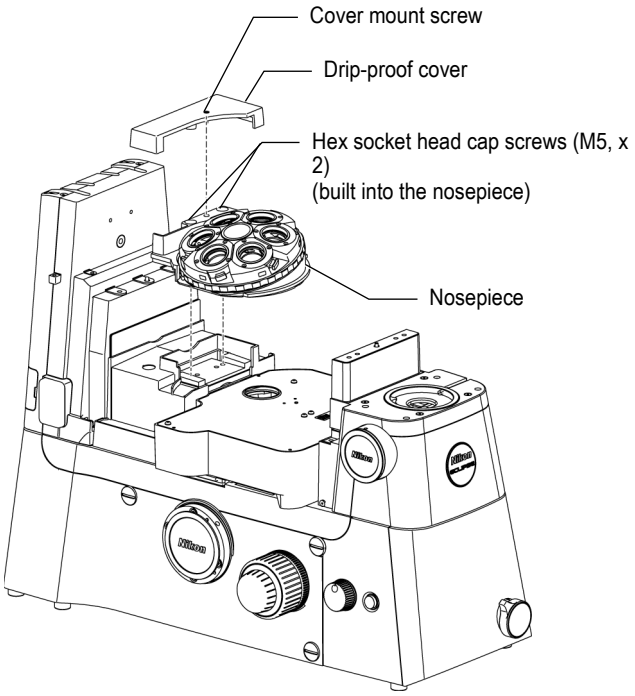
   Place the nosepiece on the mounting surface of the elevating junction unit, and then press the nosepiece from the front of the microscope toward the rear, until it reaches the limit position.

3. **Use a hex wrench to tighten the two M5 hex socket head cap screws that are incorporated into the nosepiece.**

   (Tool: 4 mm hex wrench, supplied with the microscope main body)

4. **Attach the drip-proof cover on the nosepiece.**

   Place the drip-proof cover on the nosepiece, and then affix the cover mount screw removed in step 1 by tightening it using a hexagonal screwdriver.



Attaching a nosepiece

7.7 Mounting a Stage

Mount the stage to the microscope main body and remove the fixing tools from the stage.

7.7.1 Mounting the Stage

Mount the stage to the microscope main body.

Notes on handling

- If objectives are already attached to the nosepiece, remove them before performing the following procedure.
- When handling a stage, make sure to hold the base plate at the bottom.
  If you hold other portions, the precision of the instrument might be affected and could result in failure.

Using a manual stage

- Nikon recommends attaching stage handles before mounting the stage on the microscope main body. (See §**7.7.2**.)
- To restrict the stage strokes, remove the fixing tools of the stage beforehand. (See §**7.7.4**.)

The basic mounting procedure is common to all stages. (Tool: 4 mm hex wrench, supplied with the microscope main body)

1. **Place the stage on the mounts of the tube base unit and microscope main body.**

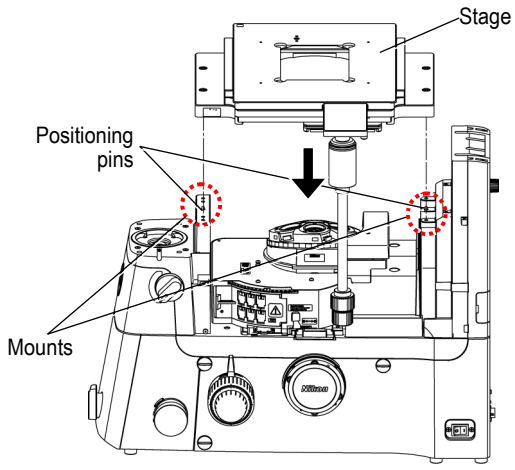
   Positioning pins are located on the mounts of the illumination pillar. Align the holes on the bottom of the stage with these pins.

   Mount orientation of the manual stage

   The azimuth of the mounted manual stages (TC-S-SR and TC-S-SRF) is changeable by 180 degrees.

   Change the azimuth of the stage in accordance with the required handle position.

2. **Using a hex wrench, tighten the four M5 hex socket head cap screws built into the stage to affix the stage.**



Mounting the stage

7.7.2 Attaching a Stage Handle (Manual Stage Only)

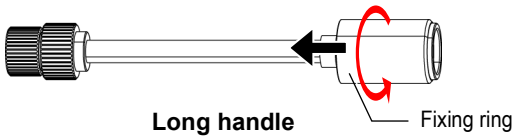
Attach the stage handle to the manual stage.
Nikon recommends attaching stage handles before mounting the stage to the microscope main body.

There are three types of stage handles: the TI2-S-HL long handle, the TC-S-HM middle handle, and the TC-S-HS short handle.

Using a TI2-S-HL long handle

Four M4 set screws are supplied with the long handle. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Loosen the fixing ring of the long handle by turning it.**



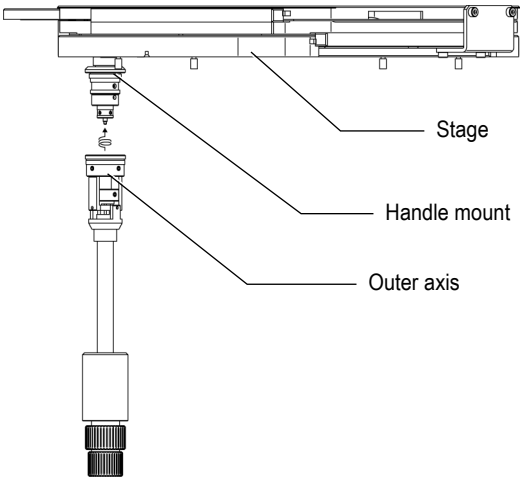
2. **Place the stage at the end of the desk.**

   Place the stage a little bit away from the desk so that the handle mount does not touch the desk.

   Take care not to drop the stage during the work.

3. **Place the joint of the outer axis of the long handle to the handle mount of the stage, and turn the handle to tighten it.**

   Firmly hold the handle mount by one hand and then turn the handle.

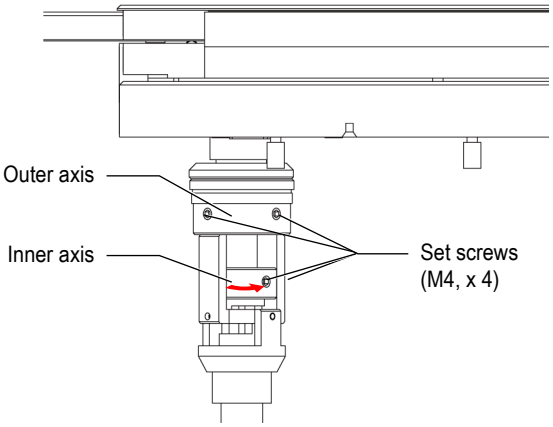


4. **Tighten the inner axis of the long handle by turning it.**

   Firmly hold the outer axis of the handle by one hand and then turn the inner axis.

5. **Firmly affix the handle using four M4 set screws.**

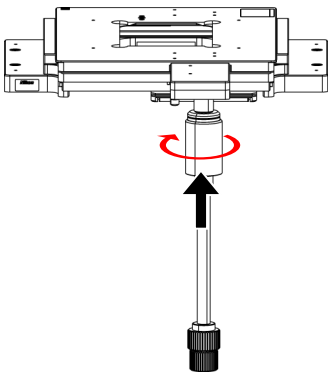
   Affix a set screw at the tip of the hexagonal screwdriver and screw it into each hole (four in total) on the side of the long handle to affix the handle.



6. **Return the fixing ring to the original position and screw it in.**

7. **Mount the manual stage to the microscope main body.**

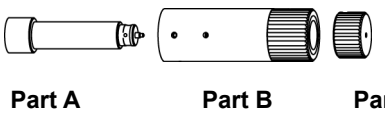
   For details on the procedure for mounting the stage, see “7.7.1 Mounting the Stage.”



Using a TC-S-HM middle handle

Six M4 set screws are supplied with the middle handle. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Remove each of the middle handle parts by turning them, and divide them into three parts.**

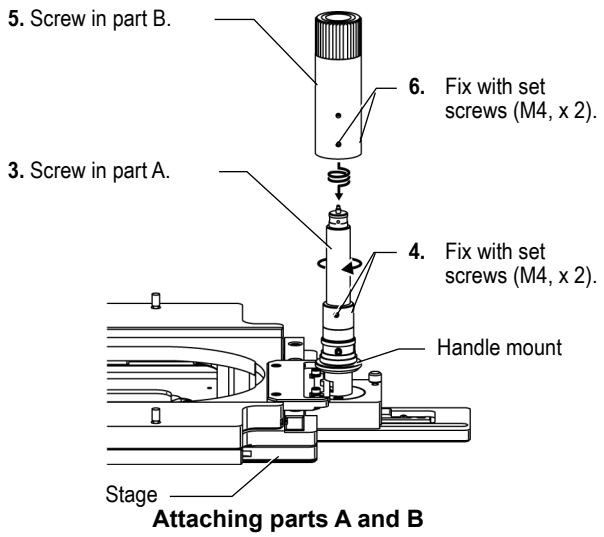


2. **Place the stage with its top surface facing downward while making sure that the top surface is not damaged.**
3. **Screw in part A to the handle mount while firmly holding the handle mount by one hand.**
4. **Firmly affix the attached part A using the two M4 set screws.**

   Attach a set screw to the tip of the hexagonal screwdriver and screw it into each hole (2 in total) at the lower part of the side of part A to affix it.
5. **Cover part A with part B and screw it in.**

   When screwing in, use one hand to firmly hold the root of the handle mount.
6. **Firmly affix part B using two M4 set screws.**

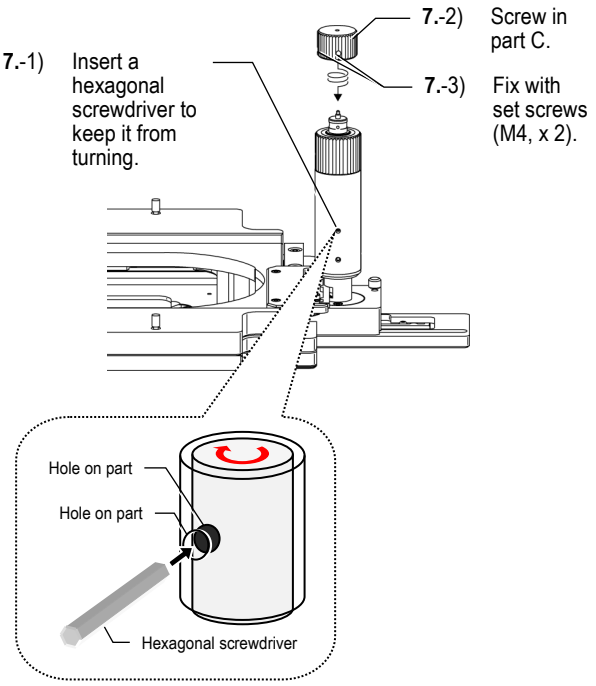
   Affix a set screw to the tip of the hexagonal screwdriver and screw it into each hole (2 in total) at the lower part of the side of part B.
7. **While using a hexagonal screwdriver to keep part A from turning, screw in part C, and then firmly affix it using set screws.**



Attaching parts A and B

> **Notes on mounting**
> When screwing in part C, make sure to keep part A from turning using a hexagonal screwdriver to avoid co-rotation. If part C is screwed in while part A is free to rotate, the rack of the stage might be damaged.

   1) Insert the hexagonal screwdriver into a hole on the side of part B, and with the screwdriver pressed in lightly, turn part A inside. When the hexagonal screwdriver reaches the position of the hole on part A, insert the screwdriver further.
   2) While keeping the hexagonal screwdriver inserted into the hole, screw in part C to part A.
   3) Affix a set screw to the tip of the hexagonal screwdriver and screw it into each hole (2 in total) on the side of part C.



Attaching part C

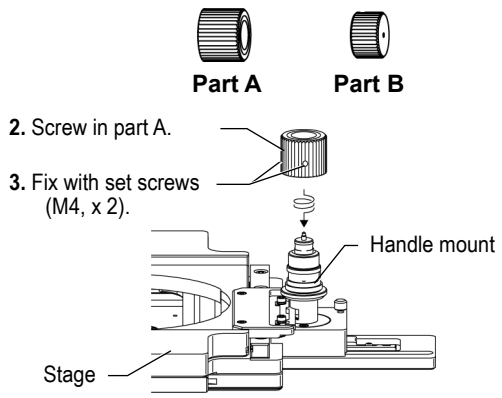
8. **Mount the manual stage on the microscope main body (See §7.7.1.)**

Using a TC-S-HS short handle

Four M4 set screws are supplied with the short handle. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Place the stage with its top surface facing downward while making sure that the top surface is not damaged.**
2. **Screw in part A to the handle mount while firmly holding the handle mount by one hand.**
3. **Firmly affix the attached part A using the two M4 set screws.**

   Attach a set screw to the tip of the hexagonal screwdriver and screw it into each hole (2 in total) at the lower part of the side of part A to affix it.

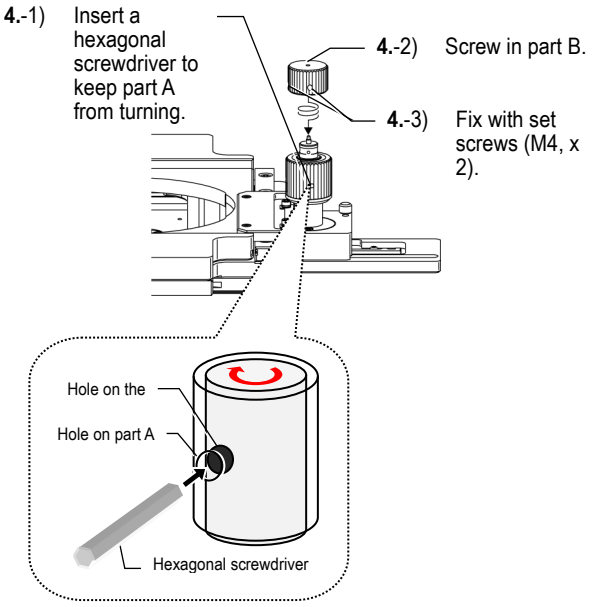


Attaching part A

4. **While using a hexagonal screwdriver to keep the inner shaft from turning, screw in part B and then firmly mount it using set screws.**

> **Notes on mounting**
> When screwing in part B, make sure to keep part A from turning using a hexagonal screwdriver to avoid co-rotation. If part B is screwed in while part A is free to rotate, the rack of the stage might be damaged.

   1) Insert the hexagonal screwdriver into a hole on the side of part A.
   2) With the hexagonal screwdriver pressed in lightly, turn part B inside.
   3) Affix a set screw to the tip of the hexagonal screwdriver and screw it into each hole (2 in total) on the side of part B.



Attaching part B

5. **Mount the manual stage to the microscope main body. (See §7.7.1.)**

7.7.3 Removing a Fixing Tool of the Stage

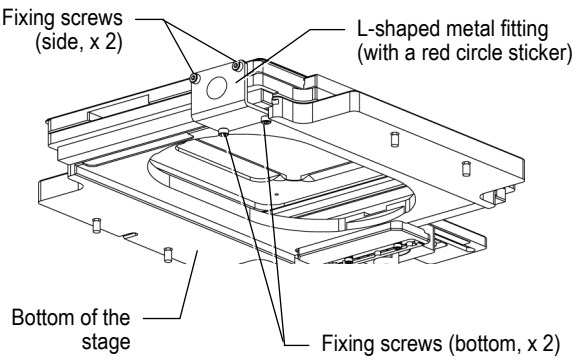
A fixing tool (L-shaped metal fitting) is attached to the stage and is used to affix the stage plate. Before removing the fixing tool, attach the stage to the microscope main body. (Tool for manual stage: 3 mm hex wrench, supplied with the microscope main body)

1. **Loosen and remove the fixing screws on the bottom of the L-shaped metal fitting.**

   For identification, a red circle sticker is affixed on the L-shaped metal fitting. Remove the two fixing screws by loosening them from the lower part of the stage using a hex wrench.

2. **Remove the L-shaped metal fitting by loosening the two fixing screws on the side of the fitting.**

   Remove the L-shaped metal fitting from the side of the stage by loosening the two fixing screws using a hex wrench.
   When removing the L-shaped metal fitting, hold it by hand to prevent it from dropping.



7.7.4 Restriction on Stage Strokes (Manual Stage Only)

The maximum movable range (stroke) of the manual stage is 114 mm (X direction) x 73 mm (Y direction) and this can be restricted.

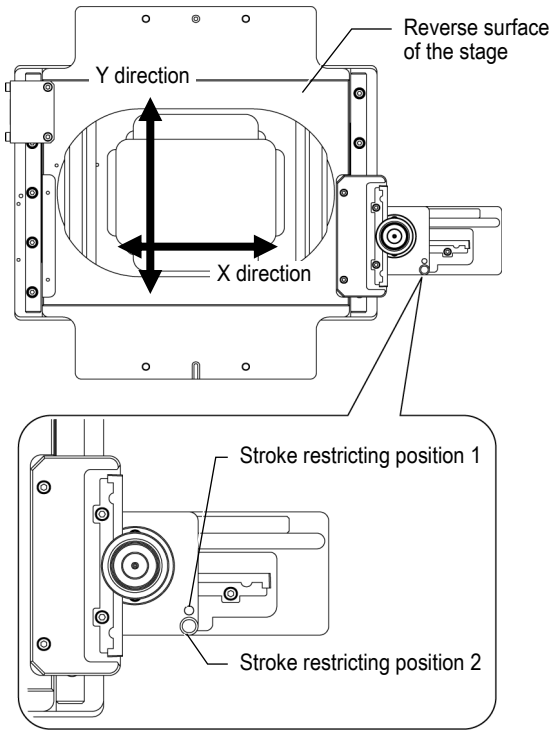
Two types of the stroke limit screws are supplied with the manual stage.

1. **Attach the stroke limit screw (knurled screw) at the restricting position of the X stroke on the reverse surface of the stage.**

   Attaching the knurled screw at stroke restricting position 1 restricts the movable range in the X direction to 75 mm.

   Attaching the knurled screw at stroke restricting position 2 restricts the movable range in the X direction to 18 mm.

> **Attaching the X stroke limit screw**
> In the case of stroke restriction in the X-direction, the stroke limit screw can be attached before mounting the stage on the microscope main body.



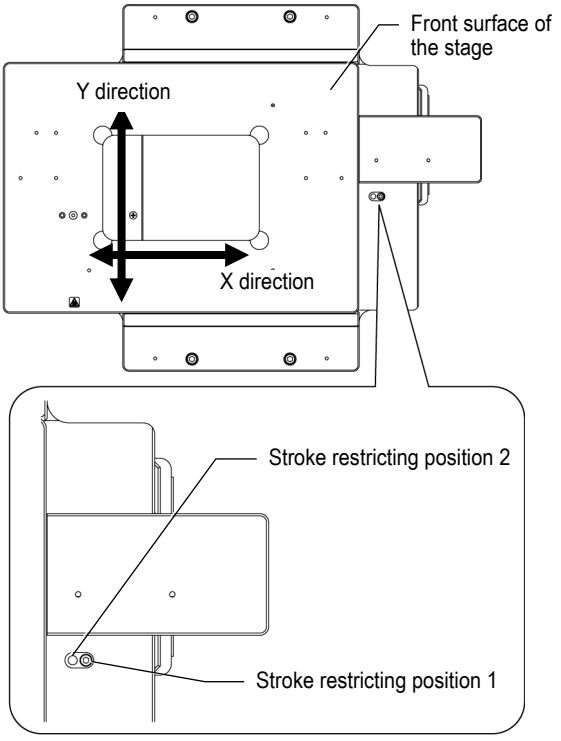
Restricting the X stroke

2. **Move the stage up to the position where the screw hole for Y-stroke restriction can be seen.**
3. **Attach the stroke limit screw (hex socket head cap screws) at the Y-stroke restricting position on the front surface of the stage using a hexagonal screwdriver.**

   (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

   Attaching the knurled screw at stroke restricting position 1 restricts the movable range in the Y direction to 50 mm.

   Attaching the knurled screw at stroke restricting position 2 restricts the movable range in the Y direction to 18 mm.



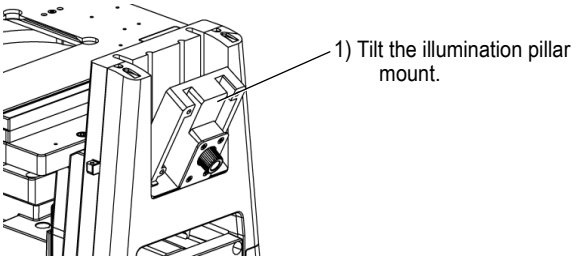
Restricting the Y stroke

7.8 Mounting a Diascopic Illumination Pillar and a Lamphouse

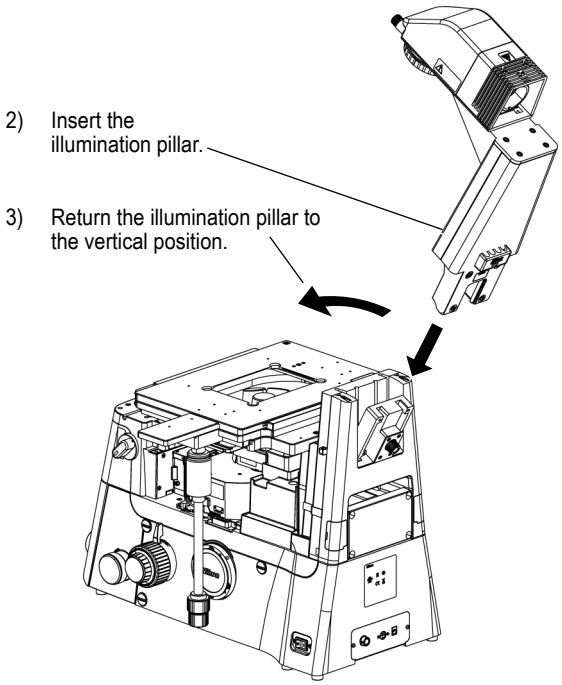
7.8.1 Mounting an Illumination Pillar

Mount the TI2-D-PD pillar for dia illumination on the microscope main body. (Tool: 4 mm hex wrench, supplied with the microscope main body)

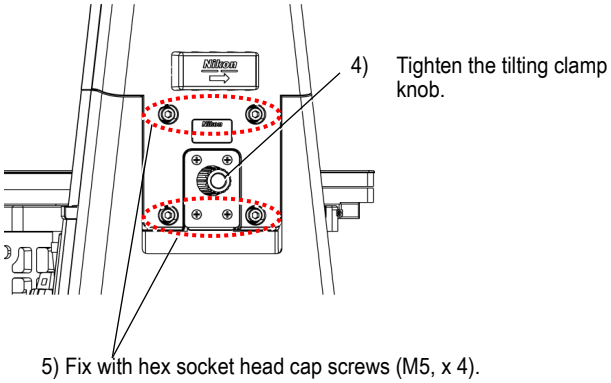
1. **Loosen the tilting clamp knob on the microscope main body to tilt the illumination pillar mount to the front.**



2. **Insert the illumination pillar along the grooves of the tilted illumination pillar mount.**
3. **Return the illumination pillar to the vertical position.**



4. **Tighten the tilting clamp knob so that the pillar does not tilt.**
5. **From the rear surface of the illumination pillar, tighten the four M5 hex socket head cap screws supplied with the illumination pillar to affix the illumination pillar.**

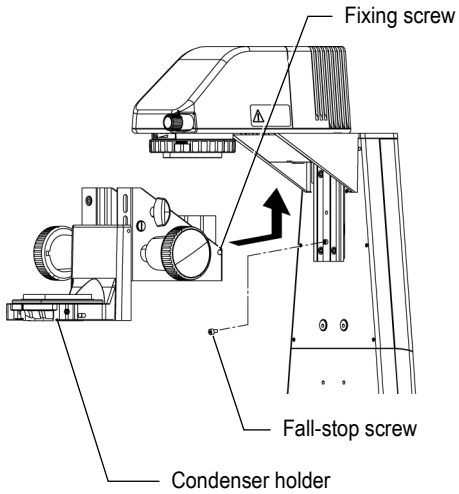


7.8.2 Attaching the Condenser Holder

Attach the condenser holder to the illumination pillar. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Remove the fall-stop screw (hex socket head cap screws) using a hexagonal screw-driver.**
2. **Attach the condenser holder upward along the dovetail grooves of the illumination pillar.**

   Slide the condenser holder upward as far as it goes and affix it provisionally using the fixing screw on the right side.
3. **Affix fall-stop screw on the illumination pillar side.**

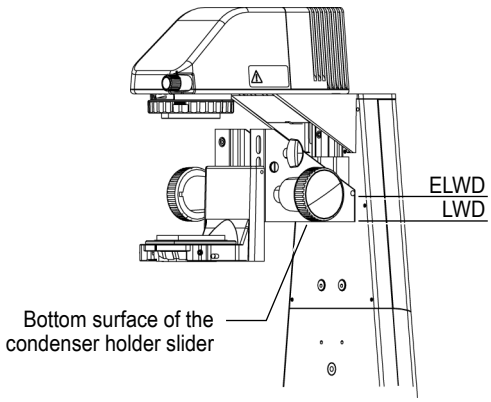


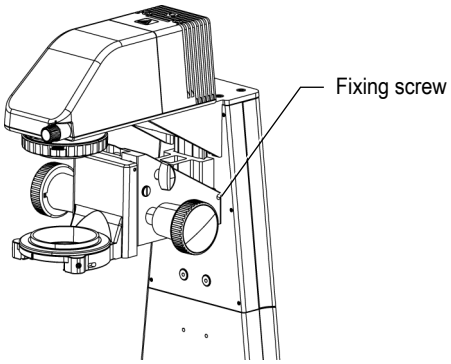
4. **Loosen the provisionally tightened fixing screw, slide the slider of the condenser holder to the default position, and firmly tighten the fixing screw using a hexagonal screwdriver.**

   The default position of the condenser holder is as follows:

   ELWD and ELWD-S condensers:
   Before affixing, slide the condenser holder so that the bottom surface of the condenser holder slider matches the index position marked “ELWD” on the illumination pillar.

   Condenser lens other than the above:
   Before affixing, slide the condenser holder so that the bottom surface of the condenser holder slider matches the index position marked “LWD” on the illumination pillar.



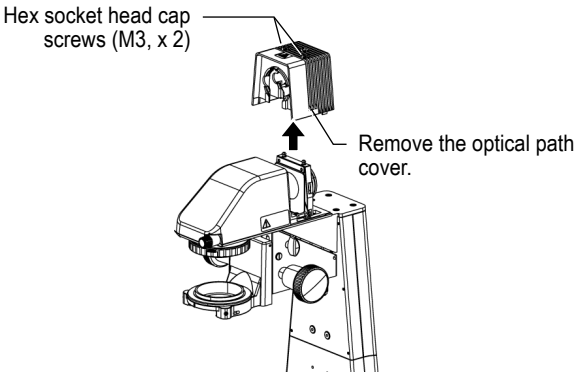


7.8.3 Attaching a Fixed Filter for Dia-illumination

Desired filters can be attached to the optical path cover for dia-illumination. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

Handling of the optical components
Do not touch the surface of optical components such as the filter with bare hands.

1. Insert a hexagonal screwdriver into the hole on the top of the illumination pillar, and remove the optical path cover by loosening the two M3 hex socket head cap screws.



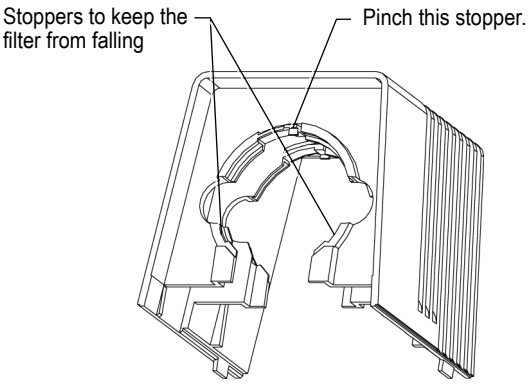
Removing the optical path cover

2. Attach required filers inside the optical path cover.

Two filters can be attached.

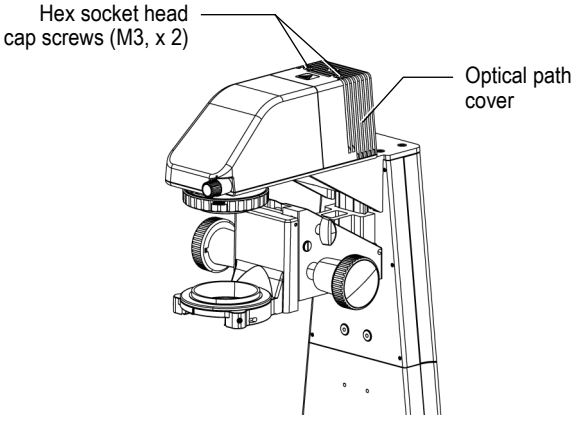
The filter mounting hole inside the optical path cover has three stoppers to keep the filter from falling.

Combining with the LED lamphouse
Attach the ND filter (ND32) supplied with the LED lamphouse.



Attaching a filter

3. Return the optical path cover to the original position, and affix the cover by tightening the two M3 hex socket head cap screws on the top of the optical path cover.



Attaching an optical path cover

7.8.4 Attaching a Filter Slider for Dia Illumination (Optional)

Desired filters can be attached to the TI2-D-SF filter slider for dia illumination. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

The filter slider for dia-illumination is an option that is recommended when a D-LH/LC precentered lamphouse is used for dia-illumination.

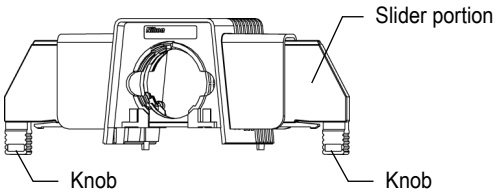
CAUTION

Precautions against heat:
Do not touch the precentered lamphouse while the lamp is lit and for approximately 30 minutes after it has been turned off.

Handling of the optical components
Do not touch the surface of optical components such as the filter with bare hands.

1. Remove the slider portion from the filter slider for dia illumination.

The stoppers at both ends are used to limit the sliding movement of the slider portion.
Press the stopper on the side opposite to the knob to release the lock to remove the slider portion from the slot. Pulling the slider portion forcibly might damage the stopper.

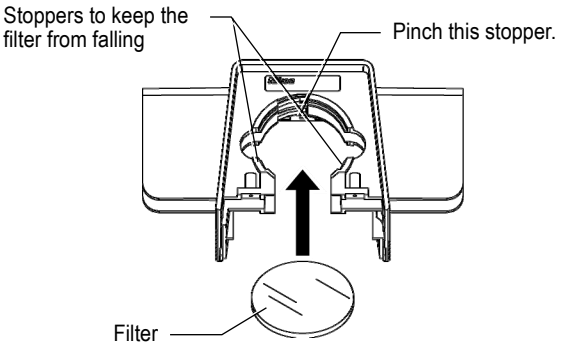


Removing the slider portion

2. Attach fixed filters inside the cover.

Two filters can be attached inside the cover.

The filter mounting hole inside the cover has three stoppers to keep the filter from falling. Attach the filter while pinching the movable stopper on the upper side.



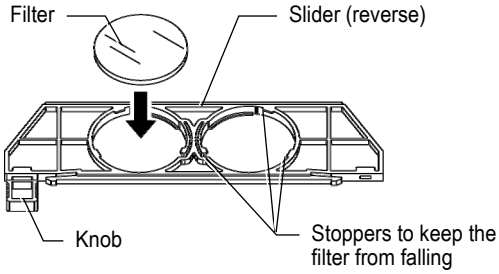
Attaching a fixed filter

3. Attach a required filter to the slider portion.

Attach the slider portion starting with its reverse surface.

The mounting hole has three stoppers to keep the filter from falling, and one of them can be shifted sideways. Shift this stopper to attach the filter.

4. Affix the label indicating the filter type on the knob of the slider portion.



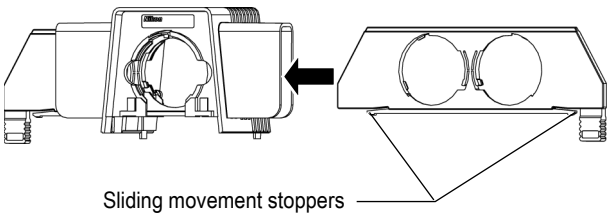
Attaching a filter

5. **Insert the slider portion into the slot of the filter slider for dia illumination.**

   The slider portion has stoppers that define the limit position of the sliding movement. Insert the slider portion into the slot by pressing the stoppers upward.

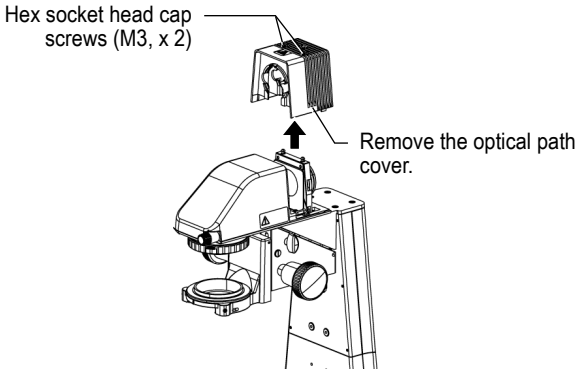
   Two slider portions can be inserted (four filters in all) into the filter slider for dia illumination.

   Although the slider portions can be inserted from both sides, be sure to insert one from each side to prevent the knobs from being on the same side. Inserting the slider portions from the same side will cause a knob conflict disabling the sliding movement.



Inserting the slider portion

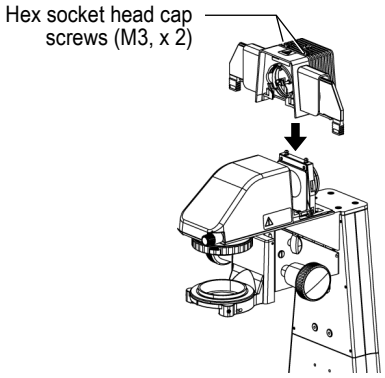
6. **Insert a hexagonal screwdriver into the hole on the top of the illumination pillar and remove the optical path cover by loosening the two M3 hex socket button head screws.**



Removing the optical path cover

7. **Place the filter slider for dia illumination on the top of the illumination pillar.**
8. **Insert a hexagonal screwdriver into the hole on the top of the illumination pillar, and firmly affix the filter slider for dia illumination to the pillar by tightening the two M3 hex socket button head screws.**
9. **Mount a lamphouse.**

   For details on attaching a lamphouse, see “7.8.6 Mounting a Lamphouse for Dia-illumination.”



Attaching a filter slider for dia illumination

7.8.5 Attaching a Motorized Dia-Illumination Shutter (Optional)

The NI-SH-E motorized shutter can be attached through the NI-SHADI motorized dia-Illumination shutter adapter only when a precentered lamphouse is used.

Handling of the motorized shutter
The shutter vanes are very thin and fragile. If the shutter vanes deform as a result of accidental contact, the shutter might malfunction or fail to achieve desired performance.
Take due care not to touch the shutter vanes when handling the motorized shutter.

Mounting and removing the motorized shutter
Motorized shutters must be mounted by Nikon personnel. If the motorized shutter needs to be mounted or removed, contact your local Nikon representative.

7.8.6 Mounting a Lamphouse for Dia-illumination

The TI2-D-LHLED LED lamphouse for dia illumination or the D-LH/LC precentered lamphouse to the dia illumination pillar can be attached. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

CAUTION

- Attachment/removal of a lamphouse
  Before attaching or removing a lamphouse, make sure that the light source is turned off.
- **Precautions against heat:**
  When a precentered lamphouse is used, do not touch the lamphouse while the lamp is lit and for approximately 30 minutes after it has been turned off.

The basic mounting procedure is common to all dia-illumination lamphouses.

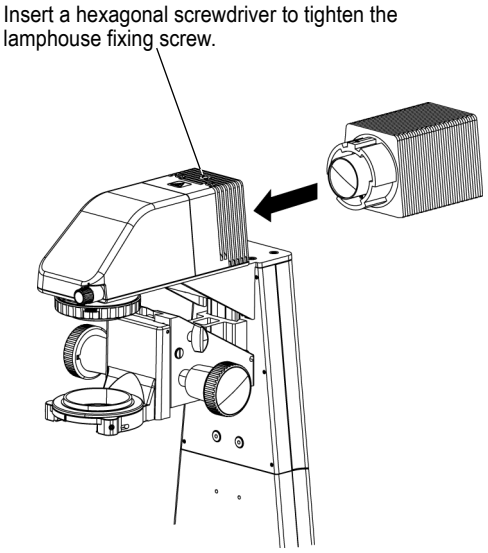
1. **Insert the lamphouse into the lamphouse mount on the top of the illumination pillar.**

   Insert the lamphouse by matching the grooves of the lamphouse cylinder with the pillar-side pins.
2. **Insert a hexagonal screwdriver from the hole on the top of the illumination pillar, and firmly affix the lamphouse by tightening lamphouse fixing screw.**
3. **Affix the lamphouse cable by using the cable clamp on the rear of the illumination pillar.**

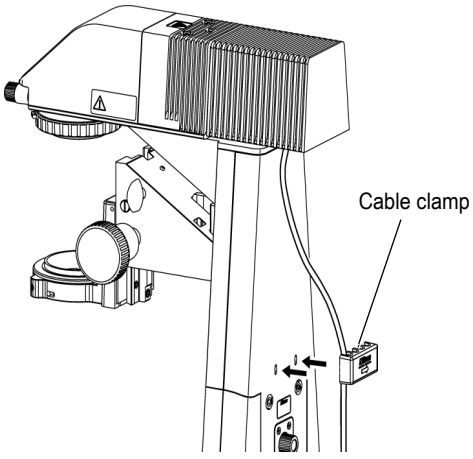
   The cable clamp is affixed to the holes of the illumination pillar through the stoppers at both ends of the clamp. The cable clamp can be removed by squeezing the stoppers on both sides to remove them.

   Up to four cables can be routed through the cable clamp.

> **Cable connection**
> Nikon recommends connecting all cables together at the end of the assembly. For details on the connector positions, see “7.15 Connection of Each Cable.”



Attaching a lamphouse



Affixing a cable

7.8.7 Replacing a Dia-Illumination Lamp (Precentered Lamphouse only)

WARNING

- There are predefined combinations of lamps, illumination pillars, and power supplies. Use the lamps, illumination pillars, and power supplies in correct combinations referring to “Diascopic illuminator” in “8.2 Performance Properties.” Be sure to use the designated lamps.

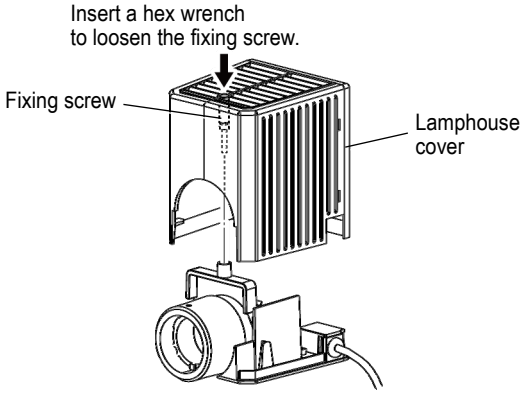
CAUTION

- When replacing a lamp, make sure the power is turned off and that the power cords are removed.
- **Precautions against heat**
  While the lamp is on and immediately after the power for the lamp is turned off, the lamp and its periphery are very hot. Before replacing a lamp, turn the power off and wait about 30 minutes for the lamp to cool off sufficiently.
- After replacing a lamp, firmly close the cover of the lamphouse. Never turn on the lamp with the cover removed.

Notes on Handling a Lamp
Do not touch the lamp glass with your bare hands. Fingerprints and other dirt on the lamp may result in uneven illumination and reduce the service life of the lamp. Wear gloves or use cloth when handling the lamp.

1. **Insert a hex wrench into the hole on the top of the lamphouse cover, and loosen the fixing screw to remove the lamphouse cover.**

   (Tool: 3 mm hex wrench, supplied with the microscope main body)

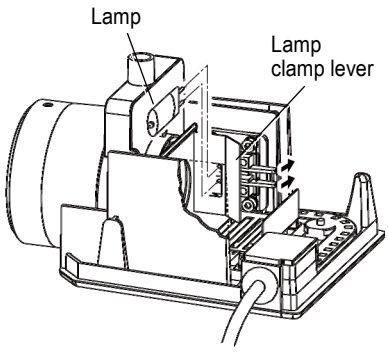


Removing a lamphouse cover

2. **Push in the lamp clamp lever and remove the used lamp from the socket.**
3. **Insert a new lamp into the socket.**

   Insert the lamp electrode (pin) into the socket pin hole as far as it goes while pushing in the lamp clamp lever, and then release the lamp clamp lever.

   - Be sure to use the designated lamp.
   - Do not touch the lamp glass with your bare hands.
   - When releasing the lamp clamp lever, take care that the lamp does not tilt.
4. **Securely attach the lamphouse cover at the original position by tightening the fixing screw.**



Pushing in the lamp clamp lever opens the pin hole of the socket. Remove the used lamp by pushing in the lever and then attach a new lamp.

Replacing a lamp

7.9 Attaching a Contrast Shield (Optional)

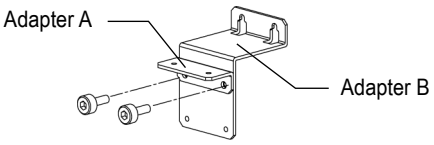
Attach a contrast shield to prevent light from entering the objective when performing Epi-FL microscopy or DF microscopy. (Tool: 3 mm hex wrench, supplied with the microscope main body)

If an LWD condenser is mounted, use a TS2R-LS contrast shield. If an ELWD condenser is mounted, use a TS2-LS contrast shield ELWD.

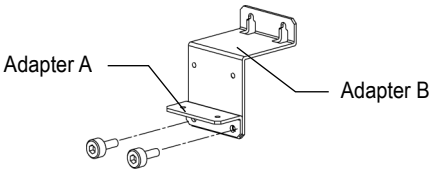
1. **Mount adapter A on adapter B, and tighten the screws using a hex wrench, as shown in the figure to the right.**

   A contrast shied can be mounted at two positions. (60 mm or 30 mm from the stage surface)

   To emphasize the light shielding performance, mount the contrast shield 30 mm from the stage surface. For ease of handling, mount the specimen 60 mm from the stage surface.

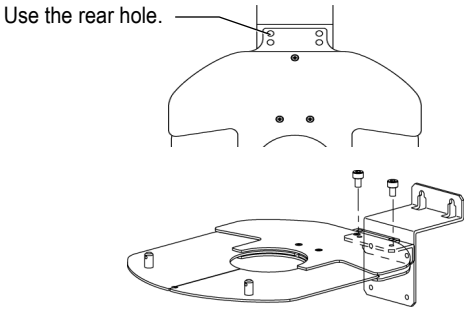


60 mm from the stage surface

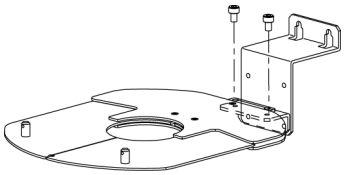


30 mm from the stage surface

2. **Attach the contrast shield to adapter A, and secure the shield by tightening the screws using a hex wrench.**



60 mm from the stage surface



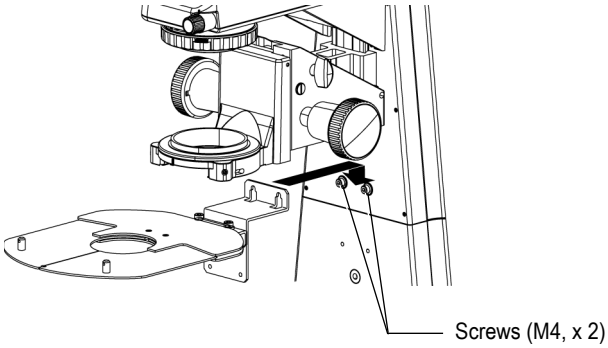
30 mm from the stage surface

3. **Screw the two M4 screws into the illumination pillar.**

   Do not fully tighten the screws.

4. **Hook the contrast shield with an adapter on the illumination pillar, and affix it by tightening the screws using a hex wrench.**

> **Attaching a contrast shield and a condenser**
> Attach the condenser with the contrast shield kept open.



Attaching a contrast shield

7.10 Attaching an Analyzer and a Polarizer (Required for DIC Microscopy)

7.10.1 Attaching an Analyzer

Attach an analyzer to the microscope main body.

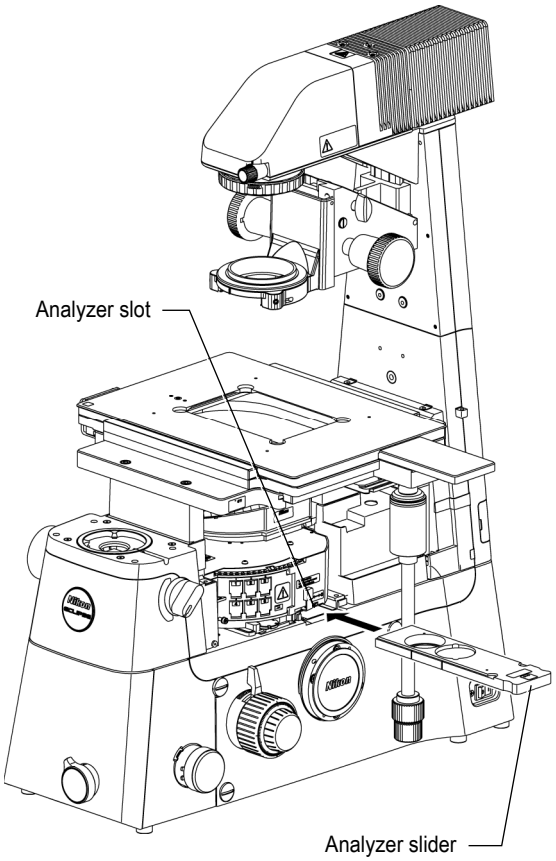
Alternatively, an analyzer cube can be attached to the FL turret.
The attachment procedure for analyzer cubes is the same for fluorescent filter cubes. For details, see “7.5.2 Mounting a Filter Cube.”

1. **Remove the dust-proof slider from the microscope main body.**
2. **Insert the TI2-C-DICA analyzer.**

   The analyzer slider can be inserted from both sides of the microscope main body.

   Setting the slider to second click position will place the analyzer into the optical path.

   Returning to the first click position will remove the analyzer from the optical path, with a hollow hole placed in the optical path instead.



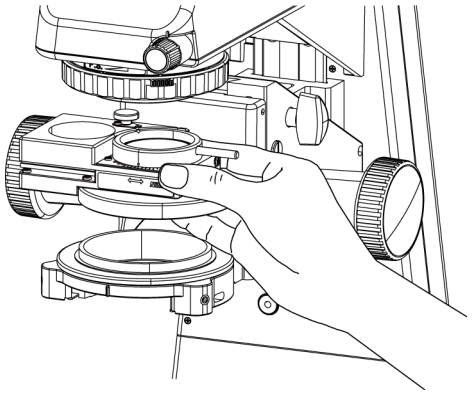
7.10.2 Attaching a Polarizer

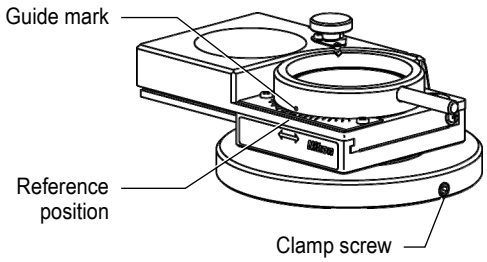
Attach a polarizer on the upper side of the mount of the condenser holder attached to the illumination pillar. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. Put the polarizer on the condenser mount and adjust the azimuth.

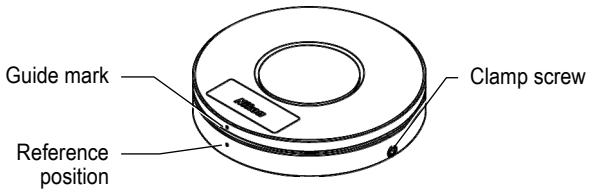
> **Polarizer attachment and azimuth adjustment**
> The polarizer needs to be attached while adjusting the azimuth. For details on the procedure for mounting the polarizer see "Attaching a polarizer and adjusting the azimuth (to form Crossed Nicols)" of "3.5.1 Using the Polarizer."

2. After adjusting the azimuth, tighten the clamp screw using a hexagonal screwdriver.





Position of the clamp screw for the DIC polarizer



Position of the clamp screw for the NAMC/IMSI polarizer

7.11 Attaching a Condenser

This section describes the procedures for mounting condenser modules, a condenser, and a condenser lens.

Components to be attached for each microscopy

Performing NAMC with DIC microscopy:

- Use a polarizer, a condenser module (slit diaphragm), and an objective that support NAMC.
- Attach an NAMC objective for and an NAMC condenser module, both of which must have the same NAMC code.

Performing IMSI with DIC microscopy:

- Use a polarizer, an analyzer, a condenser module, an objective, and a DIC slider that support IMSI.
- Attach an IMSI objective and an IMSI condenser module, both of which must have the same IMSI code.

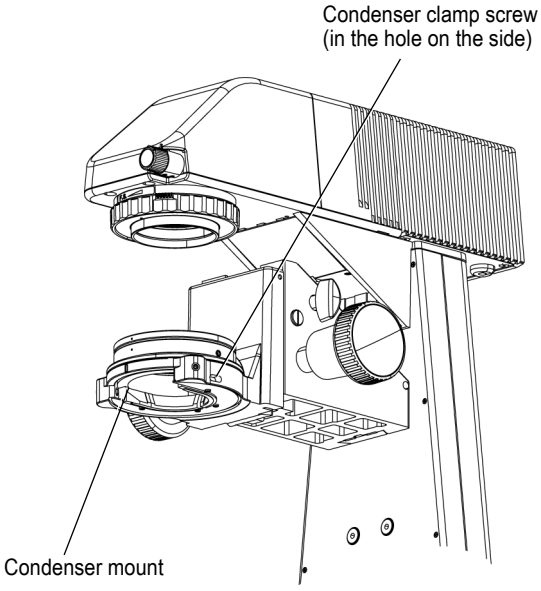
Performing a Ph Microscopy:

- Use an objective suitable for Ph microscopy and a condenser module for Ph microscopy suitable for the condenser used.

7.11.1 Preparation for Attaching a Condenser

1. **Loosen the condenser clamp screw (hex socket head cap screw) on the right side of the condenser holder using a hexagonal screwdriver.**

   (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)



Condenser holder

2. **Attach a condenser or a condenser turret to the condenser mount.**

   For condenser: See §**7.11.2**.

   ELWD condenser: See §**7.11.3**.

   DF condenser: See §**7.11.4**.

   High NA condenser: See §**7.11.5**.

7.11.2 Attaching a Condenser Turret (System Condenser)

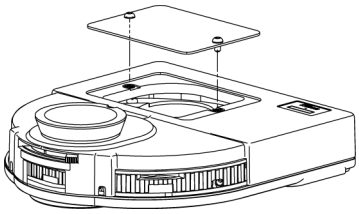
Attach a TC-C-TC condenser turret to the condenser mount of the illumination pillar.
(Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

Assembling a system condenser

Assemble a system condenser by inserting a condenser module into the condenser turret and a condenser lens on the bottom of the turret.

> **Combination of a condenser lens and a condenser module**
> Attach a condenser module suited to the purpose.
> For combinations of condenser lenses and condenser modules that can be attached to condensers, see “3.4 Using the Condenser Section.”

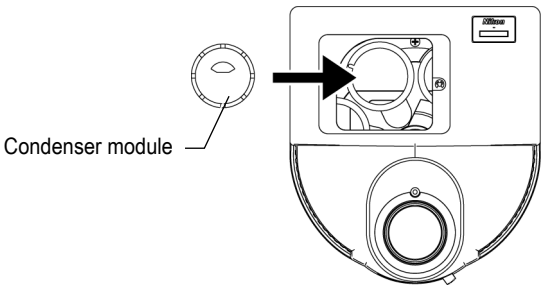
1. Loosen the two lid fixing screws using a hexagonal screwdriver to remove the lid from the top of the condenser turret.



2. Put the condenser module into the turret inside so that it fits the turret. (Maximum of seven modules)

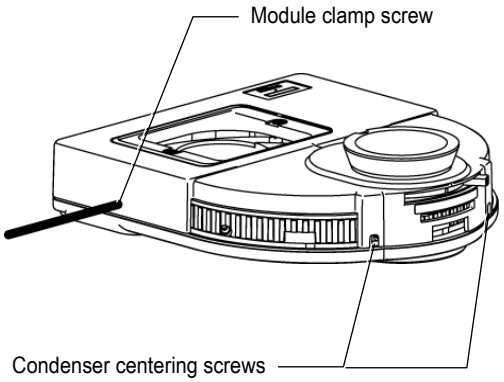
There are two different sizes of the condenser modules: the 37 mm diameter module and the 39 mm diameter module. Mount each module in an appropriate position for the size of the module.

Insert the module into the module mount so that the numbers in NA and Ph code displayed on the module increase when the turret is turned clockwise (as seen from above).



Module for DIC microscopy/IMSI microscopy

Modules for DIC microscopy and IMSI microscopy have a positioning pin.
Align the pin on the reverse side of the module with the notch of the turret, and insert the module into the module. Insert a hexagonal screwdriver into a hole on the left side of the condenser turret, and tighten the clamp screw.



ND filter for BF microscopy (module)

When performing BF microscopy, mount the ND filter for BF microscopy. ND filter for BF microscopy can be mounted on both 37 mm-dia. position and 39 mm-dia. position by reversing the ND filter.

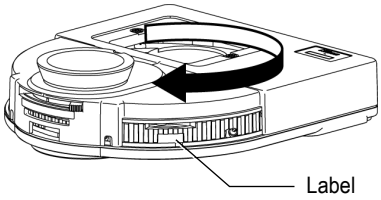
To mount the ND filter on the 37 mm-dia. position, push away the protrusion at the mount position and insert the ND filter. The tilted filter is not easily inserted. Be sure not tilt the filter and push it all the way into the turret. After inserting the filter, lightly tighten the centering screws.

After inserting the ND filter on the 39 mm-dia. position, insert a hexagonal screwdriver into a hole on the left side of the condenser turret and tighten the clamp screw.

3. **Affix the label of the mounted module to the condenser turret.**

   In the position for the BF microscopy module, affix an [ND] or a [O] label, or an appropriately named label.

   Turn the turret until the position at which the module is mounted aligns with the opening on the right or left side, and then affix a label to the turret.

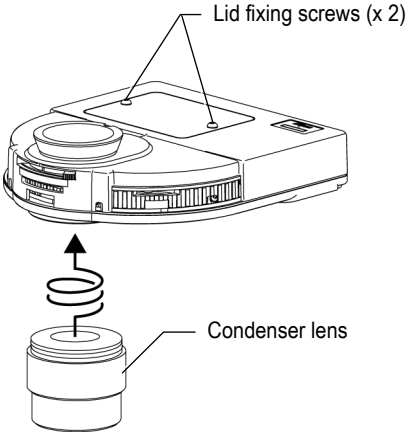


4. **Firmly attach the lid to the upper part of the condenser module by tightening the two lid fixing screws using a hexagonal screwdriver.**

5. **Screw in the condenser lens to the bottom of the turret.**

Attaching a CLWD condenser lens

To mount the CLWD condenser lens, first attach the TI2-C-CLWDA CLWD condenser adapter to the bottom of the turret, and then screw in the CLWD condenser lens.

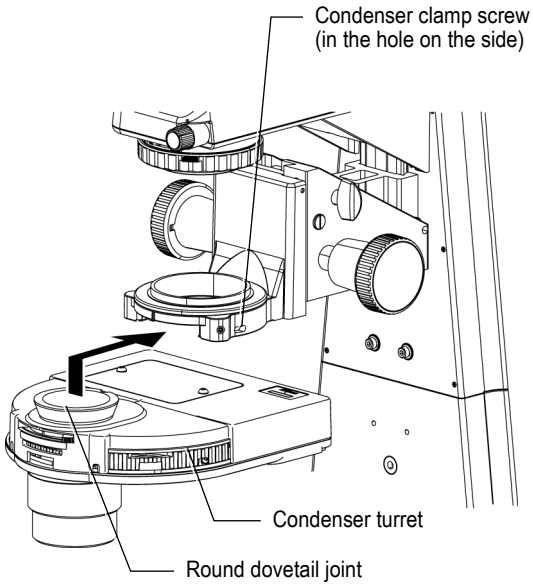


Attaching the system condenser

Attach the assembled system condenser to the condenser mount of the illumination pillar.

1. **Insert the round dovetail joint of the condenser turret into the bottom of the condenser holder, and affix the joint by tightening the condenser clamp screw using a hexagonal screwdriver.**

   Attach the condenser turret by sliding it in from the front.



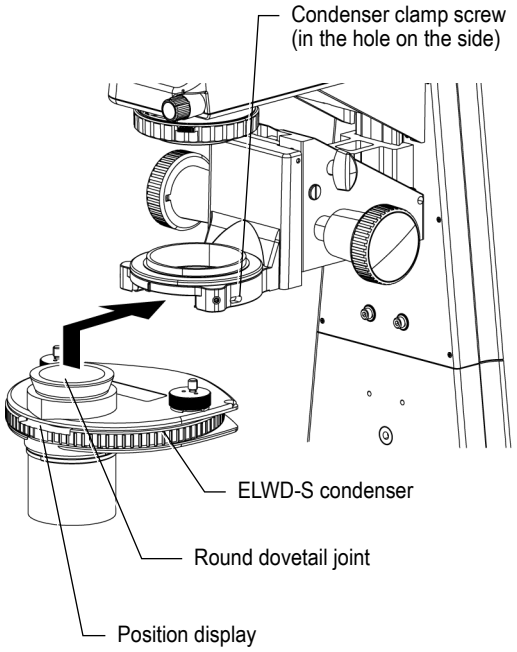
Attaching a condenser turret

7.11.3 Attaching an ELWD-S Condenser

Attach an ELWD-S condenser TE-C to the condenser mount of the illumination pillar. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Insert the round dovetail of the ELWD-S condenser into the bottom of the condenser holder, and affix it by tightening the condenser clamp screw using a hexagonal screwdriver.**

   Slide the condenser turret from the front to mount the turret so that the position display of the TE-C ELWD-S condenser faces the front (toward the observer).



7.11.4 Attaching a DF Condenser

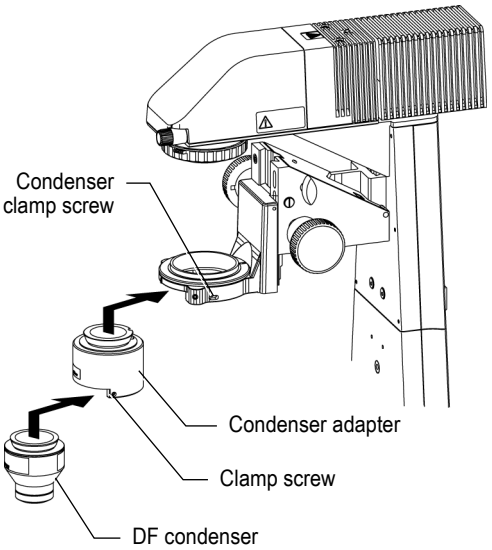
Attach a TI-DF dark-field condenser adapter and a DF condenser to the condenser mount of the illumination pillar. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Insert the round dovetail joint of the condenser adapter into the bottom of the condenser holder, and affix the joint by tightening the condenser clamp screw using a hexagonal screwdriver.**

   Attach the condenser adapter by sliding it in from the front.

2. **Insert the round dovetail of the DF condenser into the bottom of the condenser adapter, and affix it by tightening the clamp screw using a hexagonal screwdriver.**

   Attach the DF condenser by sliding it in from the front.



7.11.5 Attaching a High NA Condenser

Attach a T-CHNA High N.A. common condenser lens unit and a manual condenser (TC-C-TC condenser turret or TI2-C-SCH HNA condenser slider), and a high NA condenser lens to the illumination pillar and the condenser mount. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

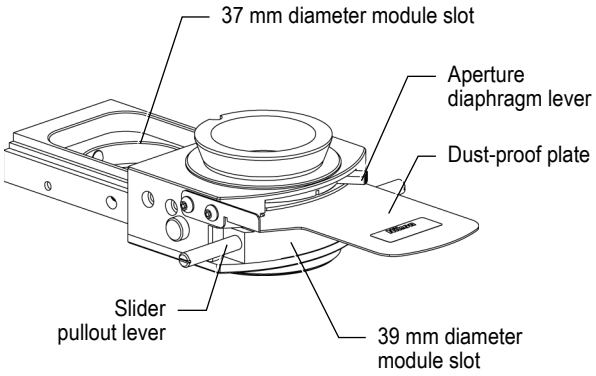
The TI2-C-SCH HNA condenser slider is used as an example here.

1. Attach the condenser module to the manual condenser.

2. When the HNA condenser slider is in use, insert the module into the module slot of the slider, and tighten the two hex socket head cap screws using a hexagonal screwdriver.

When the condenser turret is in use, see "7.11.2 Attaching a Condenser Turret (System Condenser)."

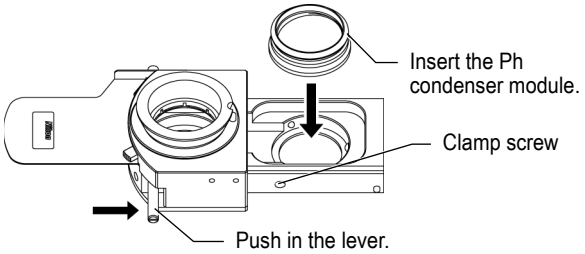
> **Combination of condenser modules**
> Attach a condenser module suited to the purpose.
> For details on combinations of condenser lenses and condenser modules that can be attached to condensers, see "3.4 Using the Condenser Section."



HNA condenser slider

■ **Mounting the Ph condenser module**

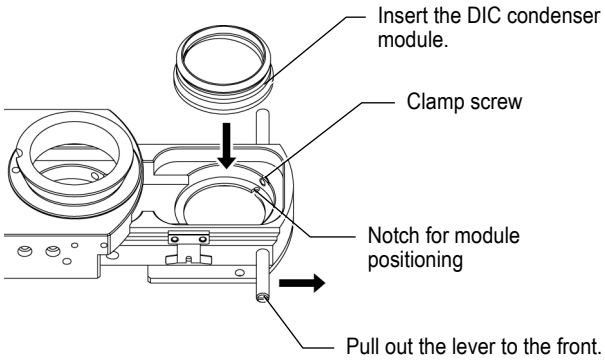
Push in the slider in/out lever to set the Ph condenser module (37 mm diameter) in the 37 mm diameter-module insertion slot.
Then, insert a hexagonal screwdriver into a hole on the side of the slider, and tighten the clamp screw.



■ **Mounting the DIC condenser module**

Pull out the slider in/out lever to set the DIC condenser module (39 mm diameter) in the 39 mm diameter-module insertion slot.

The DIC condenser module has a positioning pin. Align the pin on the reverse side of the module with the notch of the slider, and insert the module into the slider.
Then, insert a hexagonal screwdriver into a hole on the front right of the slider, and tighten the clamp screw.



■ **Mounting the ND filter for BF microscopy (module)**

When performing BF microscopy, mount the ND filter for BF microscopy. ND filter for BF microscopy can be mounted on both 37 mm-dia. and 39 mm-dia. slots by reversing the ND filter.

To mount the ND filter on the 37 mm-dia. position, push away the protrusion at the mount position and insert the ND filter. The tilted filter is not easily inserted. Be sure not tilt the filter and push it all the way into the turret.
Then, insert a hexagonal screwdriver into a hole on the side of the slider, and tighten the clamp screw.

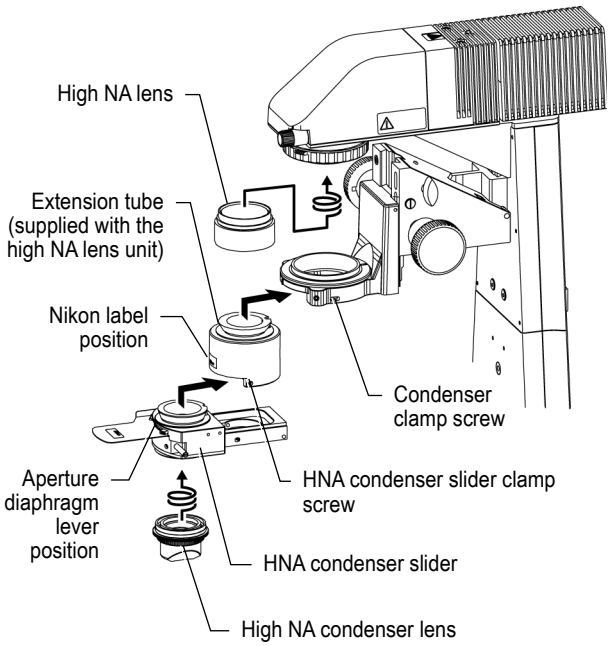
After inserting the ND filter on the 39 mm-dia. position, insert a hexagonal screwdriver into a hole on the front right of the slider and tighten the clamp screw.

2. Screw in to attach a high NA lens to the bottom of the field diaphragm dial of the illumination pillar.

3. Attach the extension tube of the high NA lens unit to the bottom of the condenser mount.

1. Insert the extension tube of the high NA lens unit into the bottom of the condenser mount so that the Nikon nameplate faces the front (towards the observer), and tighten the condenser clamp screw using a hexagonal screwdriver.

> **Order of attachment**
> Alternatively, the HNA condenser slider can be attached to the extension tube first, which can be then attached to the condenser mount.

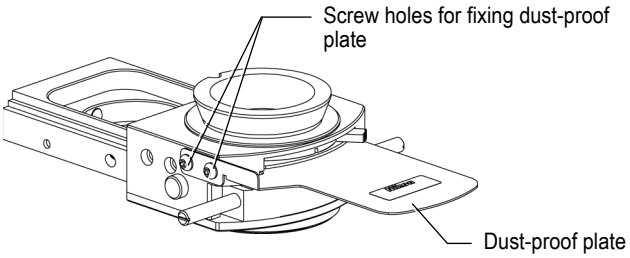


4. Attach the HNA condenser slider to the bottom of the extension tube.

3. Insert the aperture diaphragm lever of the HNA condenser slider into the bottom of the extension tube so that the lever faces the front (towards the observer), and tighten the condenser clamp screw using a hexagonal screwdriver.

5. Screw in to attach a high NA condenser lens to the bottom of the HNA condenser slider.

> **Dust-proof plate of the HNA condenser slider**
> The dust-proof plate is affixed to the HNA condenser slider with two screws.
> Use the screw holes on the opposite side to mount the dust-proof plate at the reverse position. This is useful if more work space is required in front of the microscope.



Dust-proof plate

7.12 Attaching the Tube and Eyepieces

Attach an eyepiece tube to the tube base unit and eyepieces to the binocular part of the eyepiece tube. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

The basic mounting procedure is common to all eyepiece tubes and eyepieces.

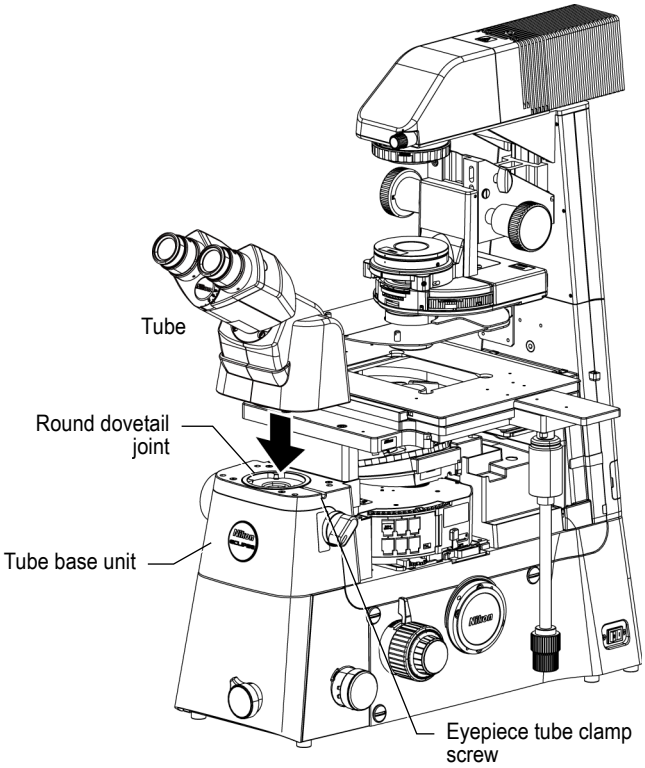
1. **Loosen the eyepiece tube clamp screw on the top of the tube base unit using a hexagonal screwdriver.**

   Leave the hexagonal screwdriver inserted into the tube clamp screw.

2. **Place the eyepiece tube on the tube base unit, and insert the round dovetail joint (male) on the bottom of the eyepiece tube into the round dovetail joint (female) on the tube base unit.**

3. **Securely hold the tube base unit and tighten the tube clamp screw using the hexagonal screwdriver.**

   Confirm that there is no deviation or backlash between the tube and the tube base unit.

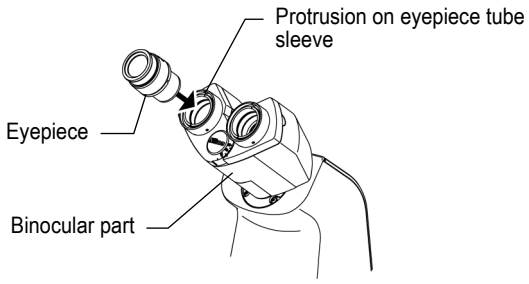


Attaching an eyepiece tube

4. **Attach the eyepieces to the binocular part of the eyepiece tube.**

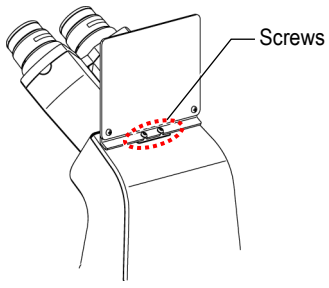
   When inserting an eyepiece, align any one of the three grooves on the eyepiece with the one protrusion on the eyepiece tube sleeve. Make sure that the right and left eyepieces have the same magnification.

   Attach rubber eye guards (optional) to the eyepieces, if required.



Attaching an eyepiece

5. **Securely attach the ultraviolet light shielding plate to the tube using the hexagonal screwdriver.**



Attaching the ultraviolet light shielding plate

7.13 Attaching an Epi-fluorescence Attachment

To perform epi-fl microscopy, attach an FL turret, an epi-fluorescence attachment, and a light source for episcopic illumination to the microscope main body.

Mounting FL turrets

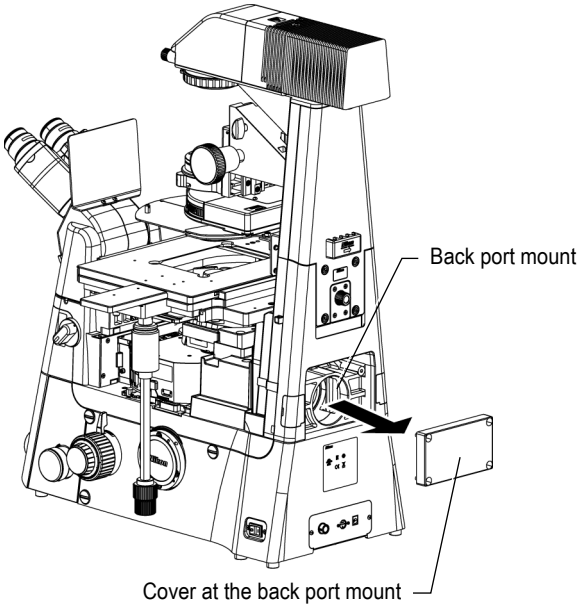
For details on the procedure for mounting the FL turret, see “7.5.1 Attaching an FL Turret.”

7.13.1 Mounting an EPI-FL Module (TI2-LA-FL-2)

1. Remove the cover from the back port mount (guide section) on the rear surface of the microscope main body.

Loosen and remove the four hex socket head cap screws (M5) securing the cover by using a hex wrench.

(Tool: 4 mm hex wrench, supplied with the microscope main body)

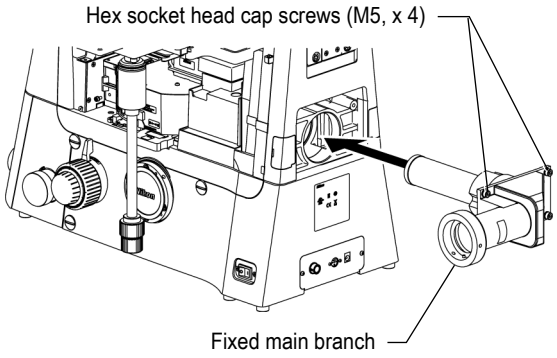


2. Attach a branch.

■ Using a TI2-LA-BF fixed main branch

(1) Insert a fixed main branch into the back port mount (guide section) on the rear of the microscope main body, and tighten the four M5 hex socket head cap screws using a hex wrench.

(Tool: 4 mm hex wrench, supplied with the microscope main body)



Attaching the fixed main branch

■ Using a TI2-LA-BM main branch

Attach the main branch to the rear of the microscope main body.

Attaching a TI2-LA-BS sub branch to the main branch enables two illumination modules to be mounted.

Using a stage-up kit to form a main branch in a two-tier configuration

If the FL turret is set up in a two-tier (upper and lower) configuration by using the TI2-LA-SU stage up kit for Ti2-A/U, the main branch can be mounted in a two-tier configuration.

If an illumination module is mounted in the lower tier, the distance to the objective will lengthen, causing the convergence position to be misaligned. To prevent this problem, a TI-LA-SUL stage-up lens needs to be inserted into the main branch.

Stage-up kits must be mounted by Nikon personnel. For details, contact your local Nikon representative.

Cable connection

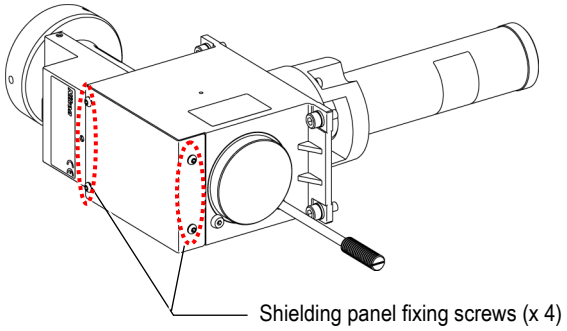
Nikon recommends connecting all cables together at the end of the assembly. For details on the connector positions, see “7.15 Connection of Each Cable.”

(1) Prepare for attaching a main branch.

A shielding panel is mounted on the main branch.

Remove the four shielding panel fixing screws using a hex wrench, and then remove the shielding panel.

(Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)



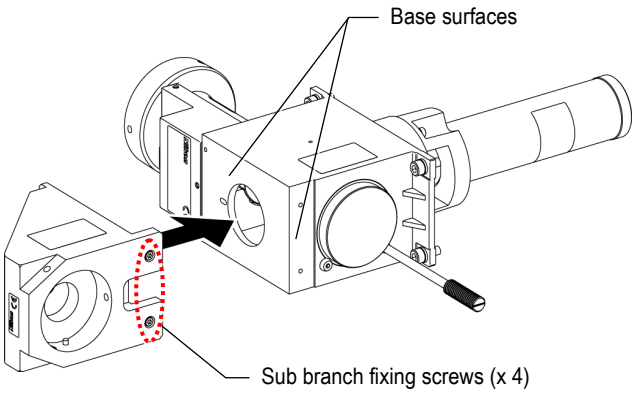
Removing the shielding panel from the main branch

Using a sub branch in combination with the main branch

Mount a sub branch at the back of the main branch, and secure the sub branch by tightening the four sub branch fixing screws using a hex wrench.

When mounting the sub branch, correctly place it on the base surfaces of the main branch, and then secure it.

(Tool: 2.5 mm hex wrench, supplied with the main branch)



Attaching a sub branch

(2) Mount a switching mirror on the main branch.

Mount the mirror (or dichroic mirror) to be used in the mirror unit provided, and then mount the mirror unit on the branch body.

(Tool: 2.5 mm hex wrench, supplied with the main branch)

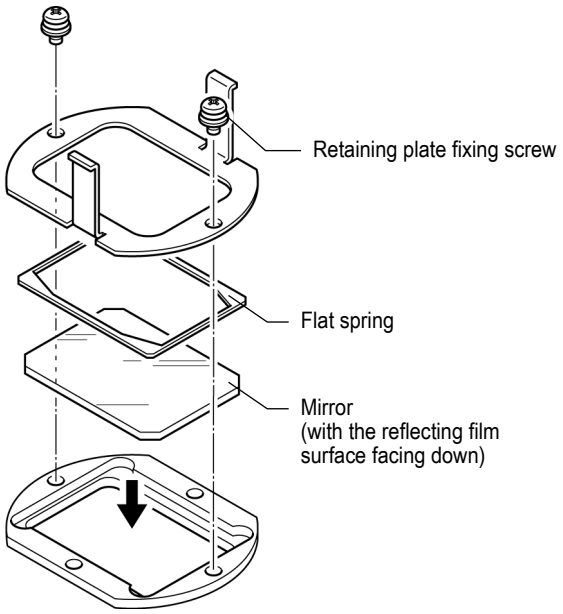
1) Remove the two retaining plate fixing screws with a Phillips head screwdriver, and then remove the retaining plate and the flat spring.
2) Mount the mirror with the reflecting film surface facing down.
(Mirror size: 36 x 25.7 x 2)
Note that the thickness is only 2 mm.
3) Mount the flat spring and the retaining plate, and firmly secure them with two retaining plate fixing screws.
Take care not to pinch the flat spring.
4) Remove the screw fixing the mirror replacement window cap by using a hex wrench, and then remove the cap by turning it counterclockwise.
5) Positioning pin holes are located in the mirror unit. When mounting the mirror unit on the branch body, correctly align the pin holes with the pins on the branch body. Be sure to orientate the mirror unit as shown in the figure when mounting it. If the mirror unit is not orientated correctly when mounted, the stoppers on the mirror unit will interfere with the parts when the mirror is switched.

> **Attaching a mirror unit**
> The stoppers in the mirror unit are slightly deviated from the center of the unit. Be sure to mount the mirror unit with its shorter side facing the microscope side.

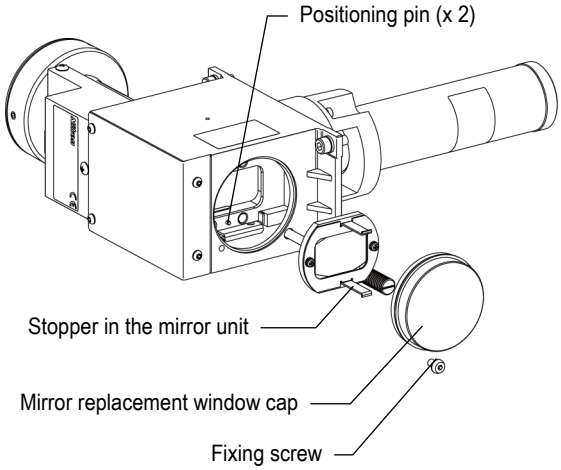
6) Screw the mirror replacement window cap into the mirror replacement window.
7) Tighten the fixing screws using a hex wrench to secure the mirror replacement window cap.

(3) Insert a fixed main branch into the back port mount (guide section) on the rear of the microscope main body, and tighten the four hex socket head cap screws (M5) using a hex wrench.

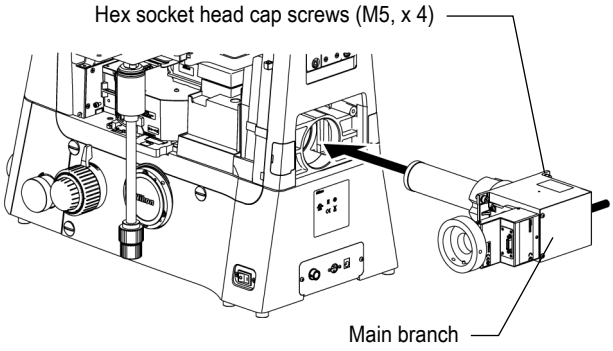
(Tool: 4 mm hex wrench, supplied with the microscope main body)



Mounting a mirror



Mounting the switching mirror



Attaching the main branch

3. Attach an EPI-FL module to the illumination module mount of the branch.

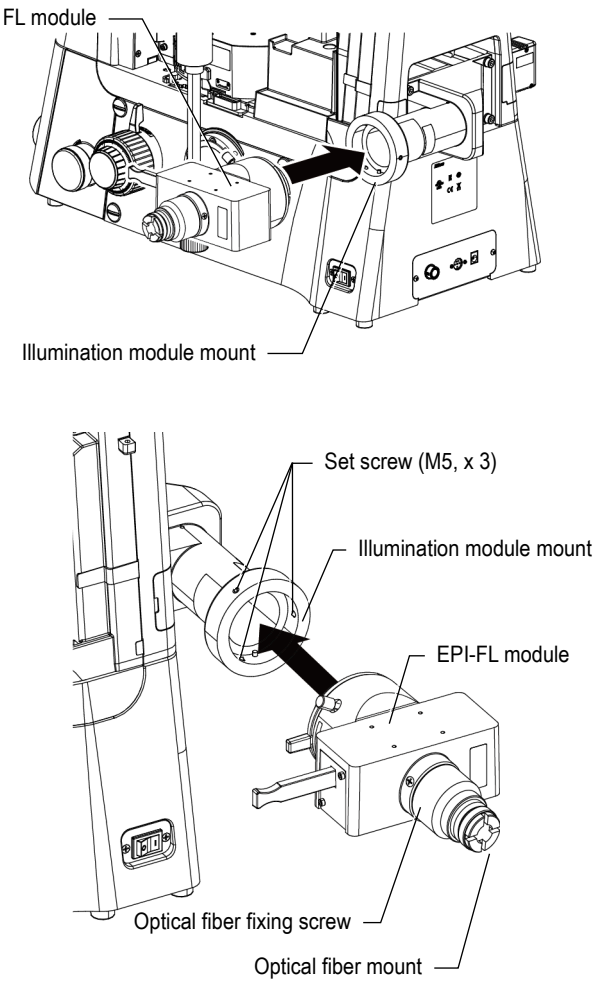
Align the groove with the positioning pin of the illumination module mount, insert the EPI-FL module, and then tighten the three M5 set screws using a hexagonal screwdriver.

(Tool: 2.5 mm hex wrench, supplied with the main branch)

Notes on mounting
Be sure to attach at least one EPI-FL module, and cover all illumination module mounts of unused FL modules with caps supplied with branches.

4. Attach a fluorescence LED illumination system to the EPI-FL module.

For details on how to attach a fluorescence LED illumination system, see the instruction manual for the D-LEDI Fluorescence Illumination System.

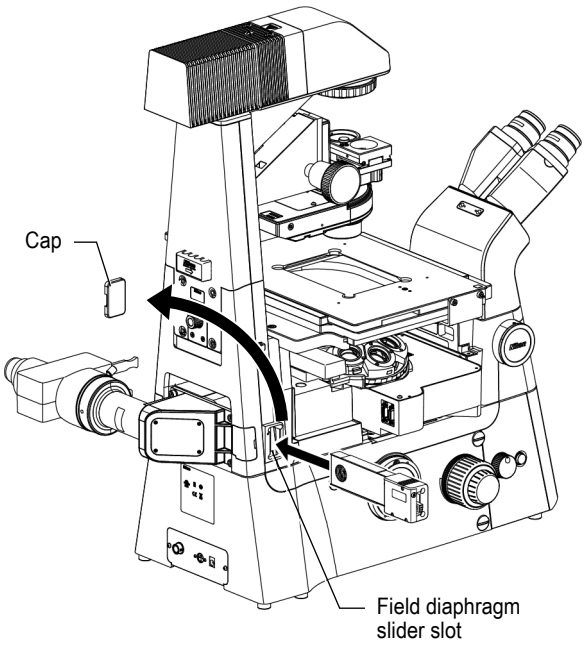


Attaching an EPI-FL module

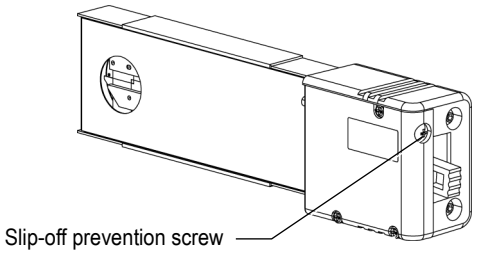
7.13.2 Attaching a Field Diaphragm Slider

Attach an Epi-FL field diaphragm slider (TI2-F-FSC circular field stop slider, TI2-F-FSS square field stop slider, or TI2-F-FSR rectangle field stop slider) to the dedicated slot of the microscope main body. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

1. **Remove the cap attached to the field diaphragm slider slot on the left rear side of the microscope main body.**
2. **Insert the field diaphragm slider into the slot.**
3. **Tighten the slider slip-off prevention screw using a hexagonal screwdriver.**



Rear of the microscope main body



Field diaphragm slider

7.14 Attaching a Camera Device

A camera device can be attached to the right or left side port on the microscope main body or to the back port of the microscope main body.

Removing a camera device
- To remove the camera device, loosen the camera device adapter fixing screws while firmly holding the camera device.
- Loosening the screws without firmly holding a camera device may allow it to fall. When loosening the screws that affix each port adapter or the camera device adapter fixing screws, make sure to securely hold the camera device.

7.14.1 Attaching a Camera Device to the Side Port (Microscope Main Body)

Attach the camera device to the side port on the side of the microscope main body (base). (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

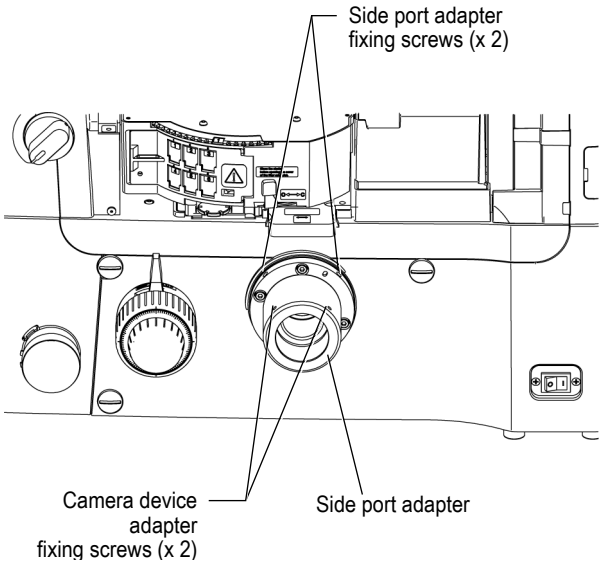
Side port
- First attach an adapter to the camera device, and then attach the camera device together with the adapter to the side port adapter.
- Be sure to put a cap on the side port to protect, it if it is not in use.

Adapters

Attaching a camera device requires an adapter and a side port adapter compatible with the camera device. Two side port adapters are supplied with the microscope main body for the right and left ports.

Note that when the centering C-mount adapter is used, the adapter suitable for the camera device is required while the side port adapter is not required.

1. **Loosen the two side port adapter fixing screws on the side of the microscope main body (base) using a hexagonal screwdriver, and remove the plastic cap attached to the side port.**
2. **Insert the side port adapter supplied with the microscope main body into the side port, and tighten the two side port adapter fixing screws using a hexagonal screwdriver.**
3. **Attach various types of adapters to the camera device.**
4. **Insert the adapter attached to the camera device into the side port adapter, and affix the adapter by tightening the two camera device adapter fixing screws using a hexagonal screwdriver.**



■ **Example: Attaching a C mount TV camera to the C mount direct adapter (optional)**

(1) Firmly screw in the C mount direct adapter to the C mount of the TV camera.

(2) Insert the C mount direct adapter into the side port adapter, and affix it by tightening the camera device adapter fixing screws.

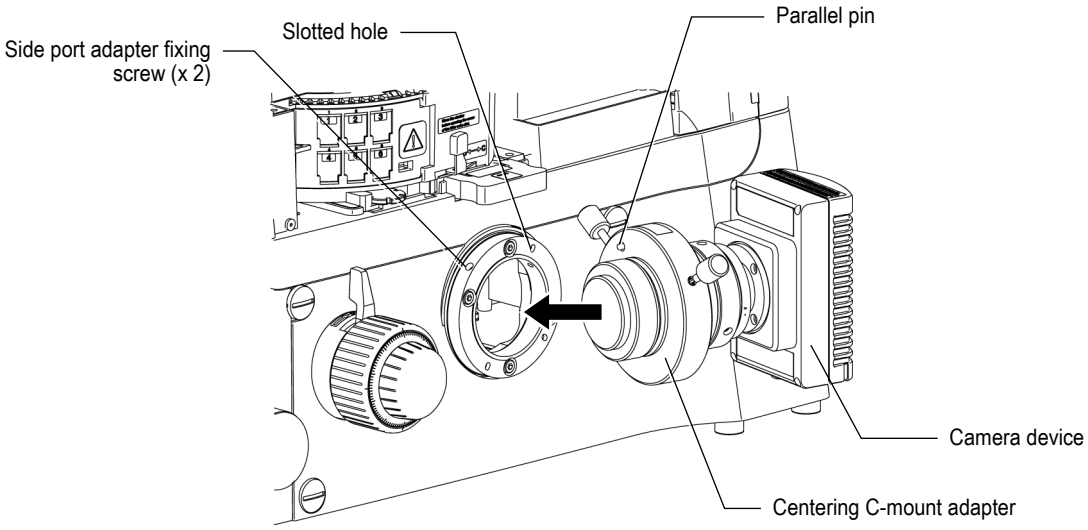
■ **Example: Mounting a camera device on a centering C-mount adapter (optional)**

(1) Firmly screw in the C-mount adapter suitable for the camera device to the C mount of the camera device.

(2) Insert the centering C-mount adapter into the C-mount adapter attached to the camera device, and affix it by tightening the three fixing screws supplied with the centering C-mount adapter.

(3) Insert the centering C-mount adapter into the side port of the microscope main body.
Align the parallel pin of the centering C-mount adapter with the slotted hole of the side port (shown in the figure below) when inserting the adapter to the side port.

(4) Affix the centering C-mount adapter by tightening the side port adapter fixing screws.



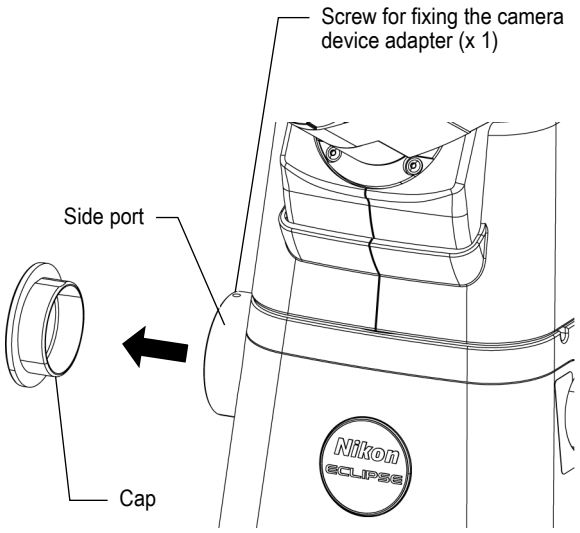
7.14.2 Attaching a Camera Device to the Side Port on the Tube Base Unit

Attach the camera device to the side port on the side of the TI2-T-BC tube base unit. (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)

Side port of the tube base unit
- First attach an adapter to the camera device, and then attach the camera device together with the adapter to the side port of the tube base unit.
- Be sure to put a cap on the side port to protect it, if it is not in use.

Adapters
Attaching the camera device to the side port requires an adapter compatible with the camera device.

1. **Loosen the camera device adapter fixing screw of the tube base unit using a hexagonal screwdriver, and remove the plastic cap from the side port.**
2. **Attach various types of adapters to the camera device.**
3. **Insert the adapter attached to the camera device into the side port of the tube base unit, and affix the adapter by tightening the camera device fixing screw using a hexagonal screwdriver.**



■ **Example: Attaching a C mount TV camera to a DS relay lens 0.55x (optional)**

(1) Firmly screw the DS relay lens 0.55x into the C mount of the TV camera.

(2) Insert the DS relay lens 0.55x into the side port adapter, and securely affix the lens by tightening the camera device adapter fixing screw.

7.14.3 Attaching the Back Port Unit (Optional)

As the second port for the camera device, attach the TI-BPU back port unit to the back port of the rear of the microscope main body, and attach the camera device.

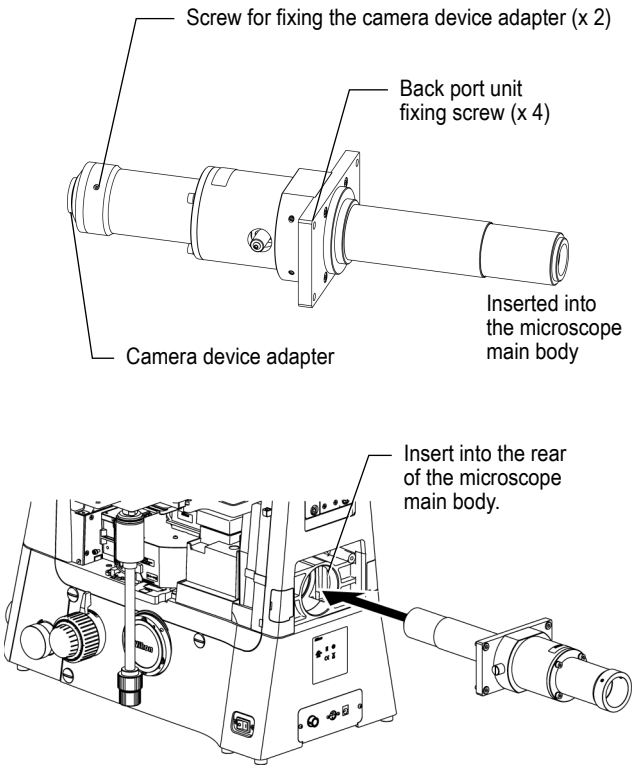
1. **Insert the back port unit into the back port on the rear of the microscope main body, and then secure it by tightening the four fixing screws using a hex wrench.**

   (Tool: 4 mm hex wrench, supplied with the microscope main body)

2. **Attach various types of adapters to the camera device.**

3. **Insert the adapter attached to the camera device into the back port unit, and secure the adapter by tightening the two camera device adapter fixing screws using a hexagonal screwdriver.**

   (Tool: 2 mm hexagonal screwdriver, supplied with the microscope main body)



Attaching a back port unit

■ **Example: Attaching a C mount TV camera to the C mount direct adapter**

(1) Firmly screw in the C mount direct adapter to the C mount of the TV camera.

(2) Insert the C mount direct adapter into the back port unit and securely affix it by tightening the camera device adapter fixing screw.

7.15 Connection of Each Cable

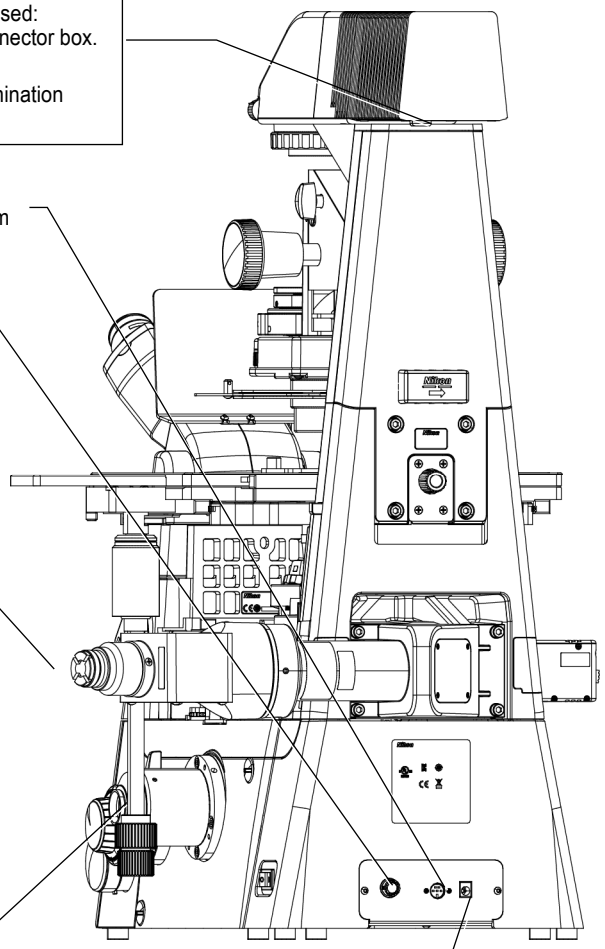
Connect the cable for each device.

Cautions for connecting cables
- Be sure to turn off the power to the microscope and peripheral units before connecting any cables.
- Fully and securely insert the end of the cables to the connectors to connect the cables.

7.15.1 Position of Each Connector

The position of each connector is as follows:

Diascopic illumination-related connectors
- When a TI2-D-LHLED LED lamphouse for dia-illumination is used: Connect to the LAMP CTRL connector of the rear surface connector box.
- When a D-LH/LC precentered lamphouse is used: Connect to the 12 VDC output connector of the diascopic illumination power supply.



Mounting a fluorescence LED illumination system

For details on how to mount a fluorescence LED illumination system, see the instruction manual for the D-LEDI Fluorescence LED Illumination System.

Mounting a power supply for dia-illumination

For details on how to mount a power supply for dia-illumination when using a D-LH/LC precentered lamphouse, see the instruction manual for the TI-PS100W/A power supply unit.

7.15.2 Clamping Cables

Attaching a provided cable clamp appropriately helps to bind wiring cables.

At the shipment of the product, there are no cable clamps attached. Attach them as required.

Notes on attaching the cable clamp

- When binding cables connected to the movable devices, clamp them loosely enough for the cable clamps not to be pulled tight when the devices are moved.
  Make sure that the cables are not pulled especially when the illumination pillar is tilted or the stage is moved to its movable range limit.
- A cable may not reach a connector depending on where the cable clamps are installed.
  Make sure that the cables can reach the relevant connector before attaching the clamps.

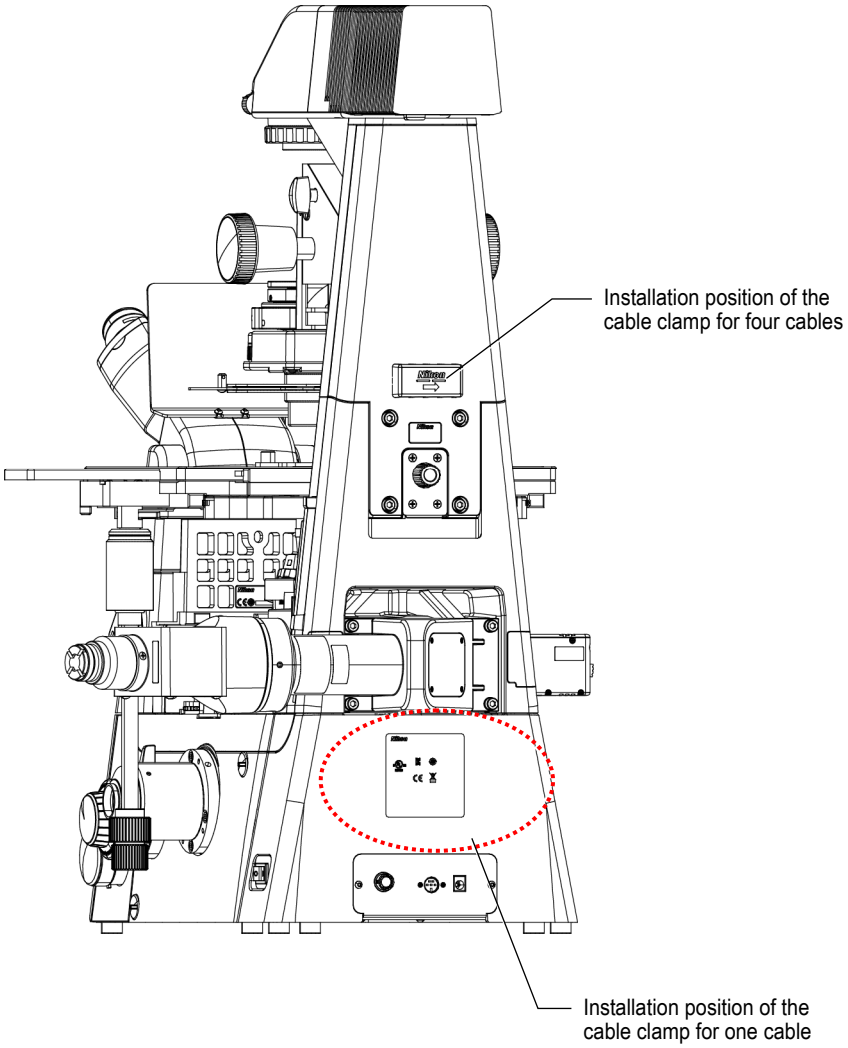
■ Type of supplied cable clamps

- Cable clamp for four cables (x1)···supplied with the illumination pillar
- Cable clamp for one cable (x2)···supplied with the microscope main body. Can be attached anywhere.

■ Installation of cable clamp

Peel a paper liner on the back of a cable clamp for one cable and attach it to any required position.

The standard installation position of the cable clamp is shown below.



7.16 Attaching Objectives

Attach an objective to the nosepiece.

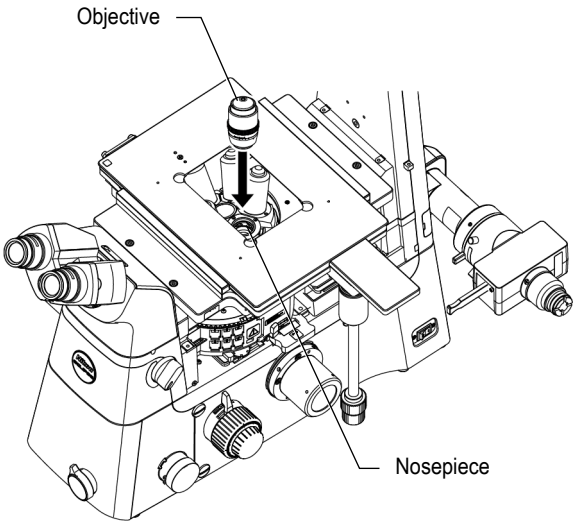
Notes on attaching an objective

- If no stage is attached, attach one.
- Nikon recommends attaching objectives after assembling all devices. However, remove stage rings, holders, and the like from the stage.
- To prevent objectives from impacting the stage, turn the focus knobs to lower the nosepiece to the bottom position before attaching the objectives.
- To prevent incidence of extraneous light and intrusion of dust and solutions (which might cause failure if spilled inside), be sure to put supplied objective screw caps on objective mount holes with no objective attached, and supplied DIC slider slot caps on DIC slider slots without a DIC slider attached.
- To perform parfocal adjustment with an objective without correction collar, a parfocal washer set must be attached between the objective and the nosepiece. (See §**7.16.2**.)

7.16.1 Attaching CFI60 Objectives

1. **Screw in an objective to the objective mount hole of the nosepiece through the stage hole.**

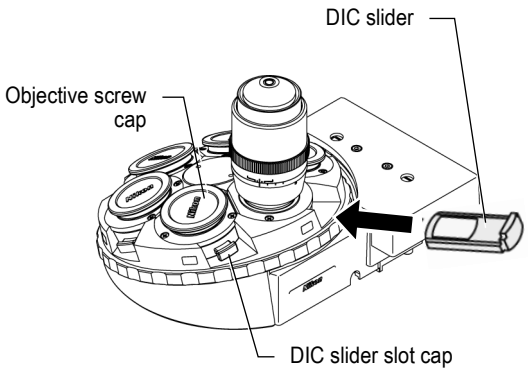
   Screw in the objective to the nosepiece in such a way that when turning the nosepiece clockwise (as seen from above), the magnification of the objective increases.



Attaching an objective

2. **Be sure to put an objective screw cap over the unused objective mount hole of the nosepiece.**
3. **To perform DIC microscopy, attach a DIC slider.**

   Remove the DIC slider slot cap immediately under the DIC objective that is attached to the nosepiece, and insert a DIC slider suitable for the DIC objective.
   Note that the type of the DIC slider to be used depends on the objective.
   For details on the combination of objectives and DIC sliders, see “4.4.3 Combination of Optical Elements.”



Attaching an objective screw cap and a DIC slider

7.16.2 Parfocal Adjustment of Objectives without Correction Collar

If parfocal adjustment is required for an objective without correction collar, use a TI-PFW parfocality washer set.

While observing and adjusting the thickness of glass or the like through an objective correction collar, the parfocal position for each objective might differ and then the image might be blurry and out of focus when switching several objectives in a nosepiece.
Adjusting the parfocal position for each objective using a parfocal washer set at the time of setup will decrease the difference in focal position when switching the objective to a different magnification, and will prevent the loss of the object being observed.

> **Parfocal washer set**
> The parfocal washer set consists of four types of washers with different thicknesses (t): 0.02 mm, 0.03 mm, 0.05 mm, and 0.1 mm.
> For identification purposes, t = 0.03 and t = 0.05 have one and two D cuts, respectively.

1. **Set up the microscope with an IMSI microscopy set.**
2. **When attaching an objective to be used, first install the parfocal washer and then attach the objective to the nosepiece.**

   Install a parfocal washer of 0.02 mm thick to the bearing surface of the nosepiece of each objective.
3. **In diascopic BF microscopy, adjust the position of the correction collar of each objective, and correct the glass thickness of the heat plate.**

   When an objective correction collar is moved, the focal position is also moved, so the specimen needs to be in focus again.
   Use a specimen with a focal point that is easily found.
4. **Check the difference in focal position for each objective in reference to the objective having the highest magnification.**

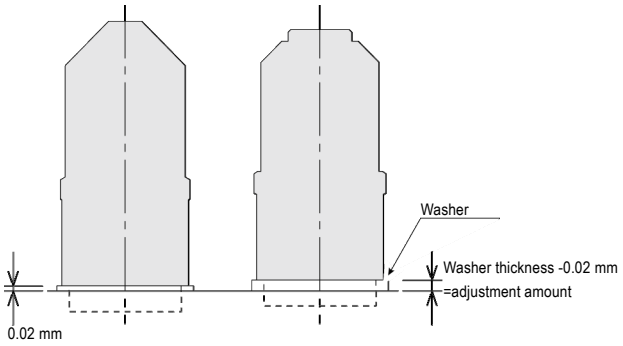
   1) Focus on the specimen with the highest-magnification objective.
   2) Read the scale of the fine-focus knob and write down the values.
   3) Switch to an objective having another magnification by turning the nosepiece.
   4) If the observation image is blurry, adjust it by turning the fine-focus knob up to the position where the image is in focus.
   5) Read the scale of the fine-focus knob, compare the values with those in step 4-2), and record the difference between the top and bottom positions.

> **Fine-focus knob**
> The scale of the fine-focus knob is in increments of 1 µm. Pay attention to the rotational direction (vertical direction of the objective) when using the fine-focus knob.

5. **From the results on each objective obtained in step 4-5), find the objective with the longest distance to the focal point (the objective that can be moved to the lowest position while staying in focus). Then using that objective as the new reference, raise the level by installing some washers having the thickness equal to the difference between the objective and the focal point to the nosepiece screw bearing surface for the other objectives.**

   Combine four washers of different thickness to get the required thickness. Because the objective used as the reference has a 0.02 mm thick washer in step 2, the adjustment amount is the total washer thickness minus 0.02 mm. (See the figure on the right.)

6. **Adjust washers for all objectives other than the reference objective, and adjust the parfocal point for each objective as much as possible.**



Adjustment of the parfocal point

Notes on Assembly

When moving the correction collar, the focal position for the objective also moves. Do not touch the correction collar while it is in use.

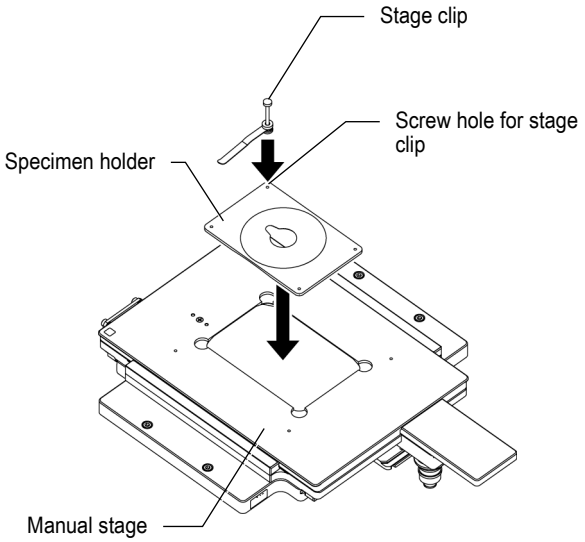
7.17 Attaching a Specimen Holder

Attach a specimen holder to the stage.

7.17.1 Attaching a Specimen Holder for the Manual Stage

The basic mounting procedure is common to all specimen holders for the manual stage.

1. **Place a specimen holder on the holder mount of the manual stage.**
2. **When using a TC-S-SC stage clip (optional), screw in the stage clip pillar into a screw hole (any of the 4 holes) on the specimen holder.**



7.17.2 Attaching a Stage Ring

Attach the stage ring at the center of the TI-SP plain stage, the TC-S-GS Gliding Stage, or the TI2-S-HJ stage ring holder.

CAUTION

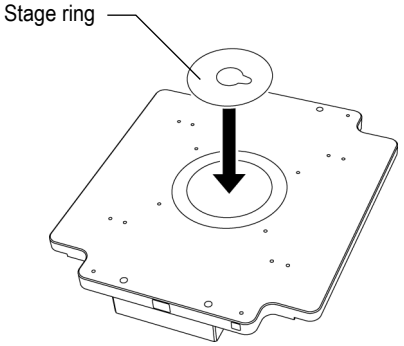
Metallic stage ring

The central part of the stage ring is made of a very thin metallic plate. Because of this, the edge of the aperture is very sharp and might cause injury if touched accidentally.

Be very careful when handling the stage ring.

Attaching a stage ring

Attach a stage ring after performing the procedure described in “Section 7.16 Attaching Objectives.”



7.18 Moving the Microscope

When moving the microscope, attach the TI-BCH carrying handles.

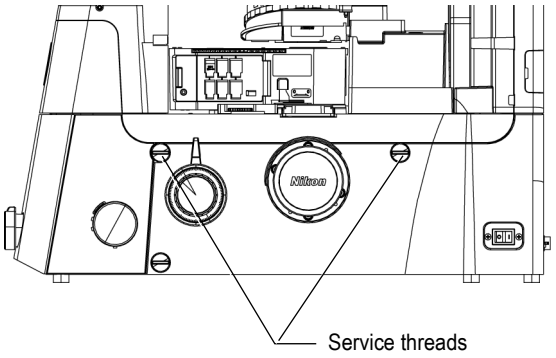
The TI-BCH carrying handles are support handles that are used to move the microscope a short distance, such as when changing the room layout or when placing the microscope into a cart.

Notes on moving the microscope

Before moving the microscope, thoroughly read ⚠ CAUTION “10. Cautions on carrying the microscope” in “Safety Precautions” at the beginning of this manual, and always heed all warnings and cautions written therein.

1. **Remove all devices and cables that can be removed from the microscope.**
2. **Remove the caps from the two M6 upper service threads out of four on each side of the microscope main body (base).**

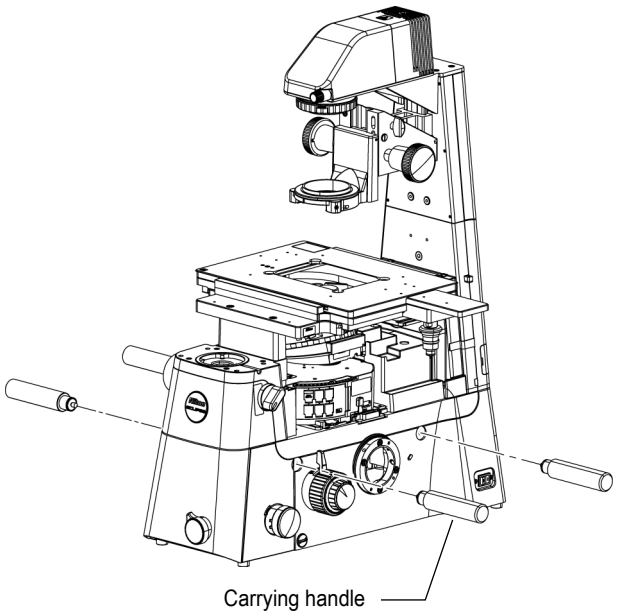
   Service threads are located on the right and left side of the main body. Remove the two caps from each side.



3. **Mount the four carrying handles into the service threads on the microscope main body (base).**

   Two service threads are located on the right and left side of the main body. Mount carrying handles on both service threads on each side.

   Be sure to screw the TI-BCH carrying handles all the way in.



Mounting the carrying handles

Chapter
8

Specifications

This chapter describes the specifications and ratings of the Ti2-U microscope.

8.1 Principles of Operation

Use the objectives and eyepieces of the microscope to optically magnify the details of cells and tissues, and manipulate the levers and knobs of the microscope to adjust the focus, move the observation position or change the magnification, and observe or capture an image of a specimen on a glass slide or in a vessel (such as a petri dish or microplate).

Intended purpose

This microscope and accessories are optical instruments used to enlarge images of specimens, preparations, and cultures for medical purposes.

Intended users

This product is intended for researchers, medical professionals and those who work on experimentations in the field of genetics, immunology, physiology, pharmacology, neurology, cellular biology and molecular biology.

8.2 Performance Properties

Ti2-U microscope main body

Model	ECLIPSE Ti2-U
Optical system	Infinity-corrected CFI60 optical system Observation optical system: Inverted observation; field number: 22 Device magnification: 1.0x/1.5x manual switching system (effective for all ports) Changeable to 1.0x / 2.0x by using an optional lens. Objective: CFI60 Output port: Observation port, left side port, right side port Optical path changeover: Four-position, manual changeover system
Focusing unit	Objective elevation system: Drive method: Manual Focus mechanism: Stroke approximately 10 mm (approximately 8 mm (upward), approximately 2 mm (downward)) Refocusing mechanism: Manual; the escape position is 0.5 mm above the lower limit position.
Pillar tilting section	Backward tilting up to 25 degrees is enabled; the clamp mechanism is provided.

AC adapter

Model	3-AC (Model name by the manufacturer: FSP040-RHAN2) 4-AC (Model name by the manufacturer: FSP040-RHAN3)
Manufacturer	FSP Group Inc.
Input rating	100-240 VAC±10%, 1.5 A, 50-60 Hz
Output rating	12 VDC, 3.33 A max.
Power cord	When used in a 100-120 VAC region, outside Japan: UL listed detachable power cord set, 3-conductor grounding (3-conductor grounding Type SVT, NO.18 AWG, 3 m long maximum, rated at 125 VAC minimum) When used in a 220-240 VAC region: EU/EN listed detachable power cord set, 3-conductor grounding (3 conductor grounding Type H05VV-F 1 mm^2, 3m long maximum, rated at 250 VAC minimum) When used in Japan: PSE-approved detachable power cord set, 3-conductor grounding (3-conductor grounding Type VCTF 3 x 0.75 mm^2, 3 m long maximum, rated at 125 VAC minimum)

* For details, see the instruction manual supplied with the AC adapter.

Diascopic illuminator

TI2-D-PD **Pillar for Dia Illumination**	Illumination method:	Köhler illumination
	Magnification of the light source:	LED1.3x, 1.7x (used with a high NA lens) Halogen lamp: 4.5x, 5.8x (used with a high NA lens)
	Field diaphragm:	Diameter of 34 or more to diameter of 1.2 or less
	Shutter:	Manual
Condenser holder	Condenser vertical stroke:	66 mm, with the refocusing mechanism
	Condenser fixing section:	Round dovetail mount, with the centering mechanism
	Polarizer fixing section:	Diameter 63.8 mm, joint
Fixed filter slot	Number of slots:	2-Position, fixed
	Supported filters:	Diameter 45 mm, thickness 3 mm or less
TI2-D-LHLED **LED Lamp House for Dia Illumination**	LED used:	White LED
	Power source for illumination:	Supplied from the Ti2-U main body
TI2-D-SF **Filter Slider for Dia Illumination**	Holder type:	Two-hole filter slider (x 2), slide system
	Fixed filter slot:	Two positions
	Supported filters:	Diameter 45 mm, thickness 3 mm or less
D-LH/LC **Precentered Lamphouse**	Input rating:	12 VDC 100 W
	Lamp rating:	12 V 100 W Halogen lamp
	Designated lamp:	Halogen lamp (OSRAM HLX 64623 or PHILIPS 7724I)

Condenser

TC-C-TC **Condenser turret**	Number of modules:	7
	Module mount holes:	Small opening (diameter 37 mm), 4 (for Ph, NAMC, and ND) Large opening (diameter 39 mm), 3 (DIC, IMSI, and ND)
	Turret drive method:	Manual
	Detection of turret position signals:	None
	Lens mount:	M48 x 0.75
	Supported condenser lenses:	LWD, ELWD, CLWD, and NAMC condensers
	Aperture diaphragm:	Diameter 34 to diameter 2 or less
	Shutter:	Shutter addresses using a shielding plate can be set.
TI2-C-SCH **HNA condenser slider**	Number of modules:	2
	Module mount holes:	Small opening (diameter 37 mm), 1 (for Ph, and ND) Large opening (diameter 39 mm), 1 (for DIC and ND)
	Slider drive method:	Manual
	Lens mount:	M48 x 0.75
	Supported condenser lenses:	HNA dry lens, HNA oil lens
	Aperture diaphragm:	Diameter 34 to diameter 2
TE-C **ELWD-S Condenser**	Condenser lenses:	NA0.3, OD65
	Annular diaphragm:	PhL, Ph1, Ph2
	Lens mount:	M48 x 0.75
	Aperture diaphragm:	Diameter 27 to diameter 2 or less

Tube base unit

TI2-T-BC **Eyepiece Tube Base Unit with Port**	Output port: Optical path changeover:	Left side port, field number 16 Manual, lever changeover system
TI2-T-BS **S Eyepiece Tube Base Unit**	Output port:	Observation port

Eyepiece tube

TC-T-TS **S Tube**	Binocular type: Depression angle: Eyepiece tube magnification: 1x Field number:	Siedentopf type 35 degrees (fixed) 22
TC-T-ER **ER Tube**	Binocular type: Depression angle: Eyepiece tube magnification: 1x Field number:	Siedentopf type 15 to 45 degrees, continuous variable 22

Stage

TC-S-SR **Stage** **TC-S-SRF** **F Stage**	Stroke: Holder mount: External dimensions:	X axis: ±57 mm, Y axis: ±36.5 mm (full stroke) X axis: ±36.5 mm, Y axis: ±25.0 mm (limit position 1) X axis: ±9.0 mm, Y axis: ±9.0 mm (limit position 2) 128 x 86 mm 290 (W) x 38 (H) x 300 (D) mm (excluding knobs and racks)
TI-SP **Plain Stage**	External dimensions:	300 (W) x 35 (H) x 260 (D) mm (excluding mounting screws)
TC-S-GS Gliding Stage	Stroke: External dimensions:	Ø20 mm 272 (W) x 35 (H) x 300 (D) mm (excluding mounting screws)

Nosepiece

TI2-N-ND **DIC Sextuple Nosepiece**	Objective mount hole: Nosepiece drive method: Nosepiece position detection: Nomarski prism slots: Nomarski prism detection:	6 Manual None Manual None
TI2-N-N **Sextuple Nosepiece**	Objective mount hole: Nosepiece drive method: Nosepiece position detection: Nomarski prism:	6 Manual None None

FL turret

TI2-F-FLT-I **Intelligent Epi Filter Turret**	Number of filter cubes retained: Turret drive method: Shutter drive method:	6 (large-field filter cubes are supported) Manual (without the position detecting function) Manual (without the position status detecting function)

Epi-fluorescence attachment

TI2-LA-BF **Fixed main branch**	Illumination module mount: Diameter 50 fitting, M5 fixing screw (x 3)	
	Field diaphragm:	Supported with a field diaphragm slider (optional)
TI2-LA-BF **Main Branch**	Illumination module mount: Diameter 50 fitting, M5 fixing screw (x 3)	
	Sub branch mount:	Contact with the base surface, diameter 3 fitting, M3 hex socket head cap screw (x 4)
	Field diaphragm:	Supported with a field diaphragm slider (optional)
TI2-LA-BS **Sub Branch**	Illumination module mount: Diameter 50 fitting, M5 fixing screw (x 3)	
	Field diaphragm:	Supported with a field diaphragm slider (optional)
TI2-LA-FL-2 **EPI-FL module**	Supported light source:	Fluorescence LED illumination system
	Filter slider:	2-position, slide system
	Supported filters:	Diameter 25 mm; thickness 6 mm or less
	IR cut filter:	Attachable to a branch connection
	Aperture diaphragm:	Diameter 1.2 to 8.0 mm, with centering mechanism
	Accessories:	Shielding plate for eyepiece tube

8.3 Physical Properties

Model	ECLIPSE Ti2-U
Electrical shock protection class	Class I Class I (AC adapter)
Environmental conditions	Operation: Temperature: 0 to 40°C Humidity: 60% RH max. (at +40°C, no condensation) Storage and Transport: Temperature: -20 to +60°C Humidity: 90% RH max. (no condensation) Altitude: 2,000 m max. Pollution: Degree 2 Overvoltage category: Category II Indoor use only
External dimensions and mass	**Ti2-U microscope system** (Device combinations: Ti2-U, TI2-D-PD, TI2-D-LHLED, TI2-C-TC, TI2-T-BC, TC-T-ER, TC-S-SR, TI2-N-ND-I, TI2-F-FLT-I, TI2-LA-BF, TI2-LA-FL-2) External dimensions: 390 (W) x 668 (H) x 626 (D) mm (excluding protruding parts) Mass (reference): Approximately 37.6 kg **Ti2-U microscope main body** External dimensions: 240 (W) x 378 (H) x 527.5 (D) mm (excluding protruding parts) Mass (reference): Approximately 20 kg

Safety standards
Configuration of microscope:
• Ti2-U
• TI2-D-PD
• TI2-D-LHLED
• TI2-C-DICP
• TC-C-TC
• TI2-T-BC
• TI2-N-ND
• TI2-F-FLT-I
• TI2-LA-BF
• TI2-LA-FL-2
• TI2-LA-SU
	• CE Marking
	  • IVD Directive
	  This equipment complies with the emission and immunity requirements of IEC/EN 61326-2-6.
	  • Low Voltage Directive
	  • EMC Directive
	• C-UL-US Listed
	• FCC Part 15 Subpart B Class A
	Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules.
	These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.
	This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications.
	Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at the user's expense.
	• CAN ICES-003(A) / NMB-003(A)
	• Australian EMI (AS/NZS CISPR11)