



Nikon

ECLIPSE

Ti Series

TI-TIRF TIRF/Epi-fl Illuminator Unit
TI-TIRF-E Motorized Illuminator Unit

Instructions

Introduction

Thank you for purchasing a Nikon product.

This manual is written for the users of Nikon TIRF System for Ti Series Inverted Microscopes.

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

This product uses lasers for the light source.

Lasers are potentially hazardous and may cause injuries if directed at the eye or skin as a result of improper use. Before using the system, carefully read the precautions concerning correct use provided at the beginning of this manual.

- 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.
- Although every effort has been made to ensure the accuracy of this manual, errors or inconsistencies may remain. If you note any points that are unclear or incorrect, please contact your nearest Nikon representative.
- Some of the equipment described in this manual may not be included in the set you have purchased.
- If you intend to use any other equipment with this product, read the manual for that equipment too.
- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Safety Precautions

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

Warning and Caution Symbols Used in This Manual

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

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

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

Meaning of the Symbols Used on the Product

The symbols marked on the product indicate 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 is marked.

Symbol	Meaning
	<p>Warning label – laser hazard symbol</p> <p>This symbol is displayed on the laser safety cover to warn that the product emits laser light.</p> <p>Your eyes or skin may be exposed to the laser beam emitted from the objective, resulting in injury.</p>
	<p>Precautions against heat</p> <p>This symbol, affixed to the top of the dia pillar illuminator, the rear side of the 12V-100W lamphouse, the optical fiber light source, and the rear side of the super-high pressure mercury lamp (or the xenon lamp house), calls your attention.</p> <ul style="list-style-type: none"> • The lamp and the lamphouse become hot while the lamp is lit and immediately after it is turned off. • Do not touch the lamp and the lamphouse while the lamp is on and immediately after it is turned off to prevent the risk of burns. • Make sure that the lamp and the lamphouse are sufficiently cool before the lamp replacement.
	<p>Biohazard</p> <p>This symbol on the stage calls your attention on the following:</p> <ul style="list-style-type: none"> • If a specimen is spilled onto the product, it may cause the danger of biohazard. • 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 facility.

 **Warning**

Use of controls or adjustments or performance of procedures other than those specified in this manual may result in hazardous radiation exposure.

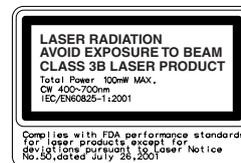
1 Laser safety

This product is designed and built in compliance with the Performance Standards for Light-Emitting Products established by the U.S. FDA and the IEC Laser Product Safety Standard (IEC 60825-1), except for deviations pursuant to Laser Notice No.50. Users are requested to take all appropriate safety measures specified by the preceding standards, in accordance with local laws and regulations.

The laser safety officer (LSO) must take charge of this laser product and users should follow the instructions from the LSO.

FDA Class IIIB Laser Product

IEC Class 3B Laser Product



2 Intended application of the product

This product can be used only for microscopic observations. This product is required to be attached to the specific Nikon microscope, Ti-E, E/B, U, or U/B. Do not use this product for other purpose.

3 Setting up the product

Setup of this product must be handled by trained service personnel.

Other person must not set up this product. If performed, it may result in improper setup, impairing system performance and exposing the eyes or skin to the laser beam, resulting in injury.

4 Prohibition of dismant

This product uses a laser device. Use only in the configuration set up by the Nikon representative. To ensure safety, never attempt to remove any part of the system. Before beginning operations, confirm that all of the parts listed below are in place. Removal of any following part during use may result in unintended exposure of eyes or skin to laser light.

Make sure that all powers to the laser and entire system are turned off when removing an objective.

Eyepiece tube, nosepiece, objective, TIRF/epi-fl illuminator unit, motorized illuminator unit, filter block, stage, optical fiber light source adapter, epi-fl filter turret, TV camera, video camera, or all port caps

5 Do not disassemble.

Disassembling this product may result in electric shock or other hazards.

Never attempt to disassemble any piece of equipment included in this product.

In particular, disassembling any of the components when the laser is on may cause the laser beam to be improperly emitted from this product. If you notice that this product is not functioning properly, immediately stop use, switch off the power switches to all devices, disconnect the power cables from the outlets, and contact your nearest Nikon representative.



Warning

6 Pre-operation check items

Before using the system, always confirm the followings.

If the following conditions are not satisfied, laser light may be irradiated from openings or reflected from reflective objects, and unintended exposure of eyes and skin to laser light may result.

- 1 A cap, a TV camera, or a video camera is attached to each port.
- 2 An objective or a cap is attached to each socket of the nosepiece.
- 3 The optical fiber for laser light is securely fixed.
- 4 The adapter for the optical fiber light source (or the lamphouse) is attached for epi illumination. And the lamphouse is attached for the dia illumination.
- 5 The epi-fl filter turret is attached correctly. (Its cover is attached too.)
- 6 The field diaphragm unit is attached correctly.
- 7 The specimen cover is attached on the stage.
- 8 There is no mirror or other highly reflective piece of metal in the area surrounding the lens aperture.

7 Never attempt to look into the laser light.

When the laser is on, a powerful beam of light is emitted above the stage through the objective. Never look into this beam or reflected beams. When using objectives of 10x or smaller magnification, the nominal ocular hazard distance (NOHD) is 0.9 m or more. If you must approach the laser beam within the NOHD described below, put on laser safety goggles. To prevent reflection of the laser beam emitted from the objectives and resulting inadvertent exposure of eyes or skin to laser light, never place reflective objects on the stage or in the diascope optical path.

- NOHD: 9 m for 1x objective.
- NOHD: 2.3 m for 4x objective.
- NOHD: 0.9 m for 10x objective.

8 Cautions about heat from the light source

The  symbols affixed to the top of the dia pillar illuminator, the rear side of the lamphouse, and the optical fiber light source indicate that the relevant lamp and its surrounding area become very hot while the lamp is lit or right after the lamp is turned off.

Also, the  symbol affixed above the air vent of the optical fiber light source indicates that hot air blows out of the air vent while the lamp is lit.

Follow the cautions below to prevent burn injury and fire.

- Never touch the lamp, its surrounding parts, and the air vents while the lamp is lit or within 30 minutes after the lamp is turned off.
- Keep hands away from the air vents when the lamp is lit. Do not block the airflow from the air vents.
- Make sure that the lamp and its surroundings are sufficiently cool before the lamp replacement.
- Do not allow walls, curtains, or papers to contact the product.
- Do not allow cloths, papers, or highly flammable volatile materials, such as gasoline, benzene, thinner or alcohol, to come near the lamp, its surrounding parts, or the air vents while the lamp is lit or within 30 minutes after the lamp is turned off.
- Do not draw any power cord on the product body.



Warning

9 Mercury lamp and xenon lamp

The mercury lamp (or the xenon lamp) used with this product requires special handling. To use the system safely and correctly, read the warnings below and be aware of potential dangers. Also, carefully read the manuals of the light source (optical fiber light source, super-high pressure mercury lamp power supply, and high-intensity light source) to be used and the manuals published by the lamp manufacturer if provided, and follow their instructions.

Warnings about the mercury lamp and the xenon lamp

- 1 The mercury lamp (or the xenon lamp) radiates ultraviolet light that is harmful to the eyes and skin when the lamp is turned on. Direct viewing of light from these lamps may result in blindness.
- 2 Gas is sealed under very high pressure inside the lamps. The pressure increases when the lamp is on. If the lamp is scratched, dirty, subjected to high external pressure or physical impact, or used beyond its operational life, the sealed gas may escape or the lamp may burst. This can result in someone inhaling the gas, injuring themselves on the glass, or other accidents.
- 3 When the lamp is on, the lamp and its surroundings become extremely hot. Touching the lamp with bare hands could result in burns. Flammable materials placed near the lamp could ignite.
- 4 Using other than the specified type of lamp could result in an accident, such as a burst.

The hazards described above should not pose any danger as long as you heed all of the warnings and cautions in the manuals and use the system only for its intended purpose because safety is a top priority in the design of Nikon products. However, the hazards described above could lead to an accident if you fail to heed all of the warnings and cautions in the manuals, if you strike the system, or if you attempt to disassemble the system. Therefore, always be sure to heed all of the warnings and cautions.

10 Use the ultraviolet light shield

Do not directly see the light irradiated from the objective. Harmful excitation light will be given out from the objective depending on the excitation method used. When you see the part around the objective, be sure to see through the ultraviolet light shield.

11 Notes on handling flammable solvent

The following flammable solvents are used with the product:

- Immersion oil (Nikon Immersion Oil for oil immersion objectives)
- Absolute alcohol (ethyl alcohol or methyl alcohol for cleaning optical parts)
- Petroleum benzine (for wiping the immersion oil)
- Medical alcohol (for disinfecting the microscopy)

Never hold a flame near these solvents. To use solvent, read the instructions provided by the manufacturer and handle the solvent correctly and safely. Note the following precautions to use a solvent with the product.

- Keep solvent from the heat of the lamp, the lamphouse, and the power supply device.
- Do not bring solvent near the product or its surroundings when turning on/off the power switch or plugging/unplugging the power cord.
- Be careful not to spill solvent.

**Caution****1. Precautions on power supply ratings and power cords**

Before connecting the power cords to wall outlets, check your power-line ratings to make sure that the input voltage and current capacity requirements of the products are sufficient. If the device is not running with a rated power supply, an error, a malfunction, or a fire may occur. Always use a power cord that satisfies local safety regulations. Always use three-pole grounded outlets.

For power cord specifications, see Chapter 8, "Specifications and Performance."

2. Use the specific AC adapter only.

The shutter control unit is energized by an AC adapter. Be sure to use the specific AC adapter with the product. Using a wrong AC adapter may result in malfunction, over-heating, or fire. For AC adapter specifications, see Chapter 8, "Specifications and Performance."

- Place the AC adapter in a well-ventilated area to prevent malfunction or fire. If something covers the AC adapter, the ventilation is blocked and abnormal heating may occur.
- Always turn off the power switch of the shutter control unit (press the "ON" side of the power switch) when connecting the AC adapter to prevent malfunction or error.

3. Precautions on connecting cables

Always switch off the power switches to all devices before connecting or disconnecting cables. To prevent wires from breaking, do not pull cables by the sheath; instead, firmly grasp the plug when connecting or disconnecting a cable.

4. Emergency stop

In an emergency, turn off all power switches and unplug the power cords.

5. Do not wet the product.

Do not wet nor spill a liquid on the product to avoid malfunction, over-heating, or electric shock. If a device is subject to contact with water, immediately switch off the power switch to that device and wipe off the water with a dry cloth. If water enters a device, immediately suspend use of the product, unplug the power cord from the outlet, and contact your nearest Nikon representative.

6. Do not place any object on the product.

To prevent deformation, failure, or malfunction, and to avoid injury, never place any objects (heavy ones in particular) on any part of the system.

**Caution****7 Notes on Handling the Product****1. Handle the product carefully.**

The product is a precision optical instrument. Handle the product with care to avoid a physical shock or an impact. Optical-fiber cables, in particular, must not be bent or pulled with excessive force, to prevent failure, malfunction, or wires from breaking.

2. Notes on handling optical parts

Scratches or dirt such as fingerprints on an optical component (such as a lens or a filter) will degrade microscope images. Carefully use the optical parts. Do not damage them. If any part becomes dirty, clean it according to Chapter 7, "Daily Maintenance".

3. Installation location

The product is a precision optical instrument. Therefore, using the product under unsuitable conditions may damage it or may have an adverse effect on its performance. The following conditions must be considered for the installation location.

- To prevent the product from falling and to avoid eventual failure, install each component of the product on a level surface.
- Install the product in a clean place with little dust and dirt. Dust or dirt degrades system performance significantly.
- Use this product at ambient temperatures of between +5 and +35°C and in conditions of humidity of 60% or less (no condensation). Using the product in hot or humid conditions may result in condensation or malfunction.
- Make sure that the product is not subject to strong vibrations, which may degrade image quality. Consult with Nikon representatives about preventive measures you may take when the product is installed.
- To prevent degradation, malfunction, or failure of the laser and the PC, always use power supply that is free from electric noise or sudden voltage fluctuations.
- To prevent failure, do not block or place any obstacles near the cooling fans of the controller and the laser. When the product is installed near a wall, be sure to provide a clearance of 15 cm or more between the wall and the product.
- Install the product at a place where all the power cables can be pulled out easily in an emergency.

4. Cleaning

Use a dry and soft cloth to clean painted, plastic, and printed parts. If necessary, wipe these parts with a cloth moistened with diluted neutral detergent, then wipe off the moisture with a soft and dry cloth. To prevent discoloration or deformation of painted or plastic parts or removal of the printed letters/figures, do not use organic solvents such as paint thinner or alcohol.

5. Other categories

Be sure to follow the instructions specified in this manual. Failure to do so may result in impaired performance, malfunction, failure, or unexpected hazards.

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1

System Configurations and Part Names

1.1 Overview

1.1.1 TIRF/Epi-fl Illuminator Unit and Ti-U System Overview

TIRF/epi-fl illuminator unit and Ti-U system overview (front view)

(Standard configuration to use the TIRF/epi-fl illuminator unit)

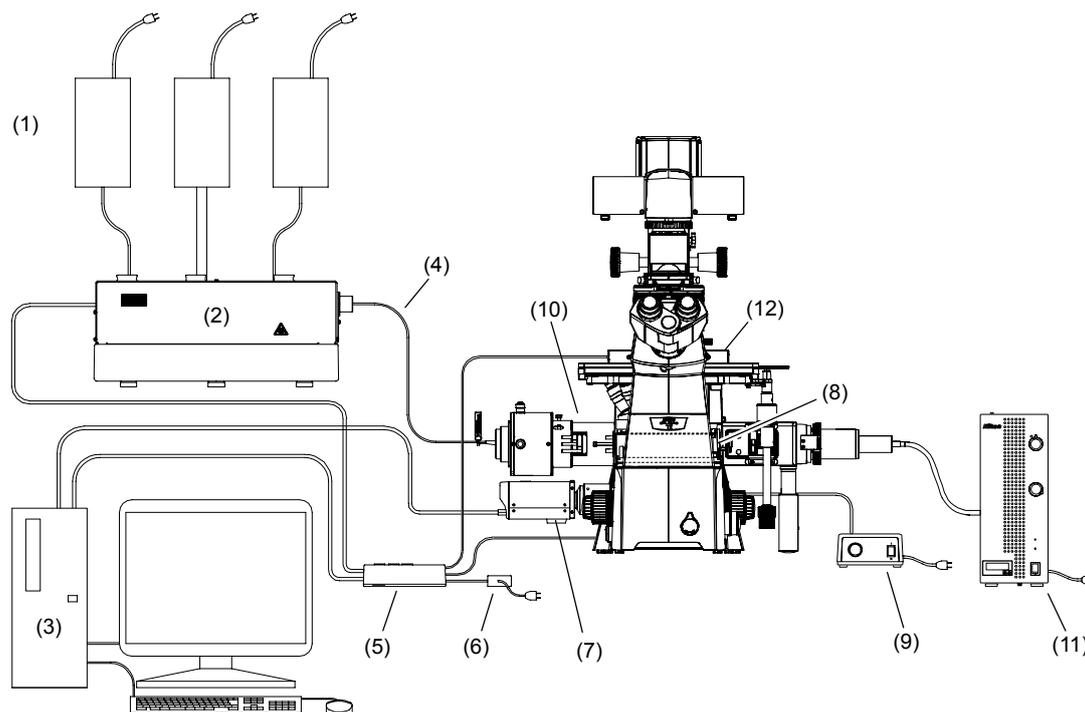
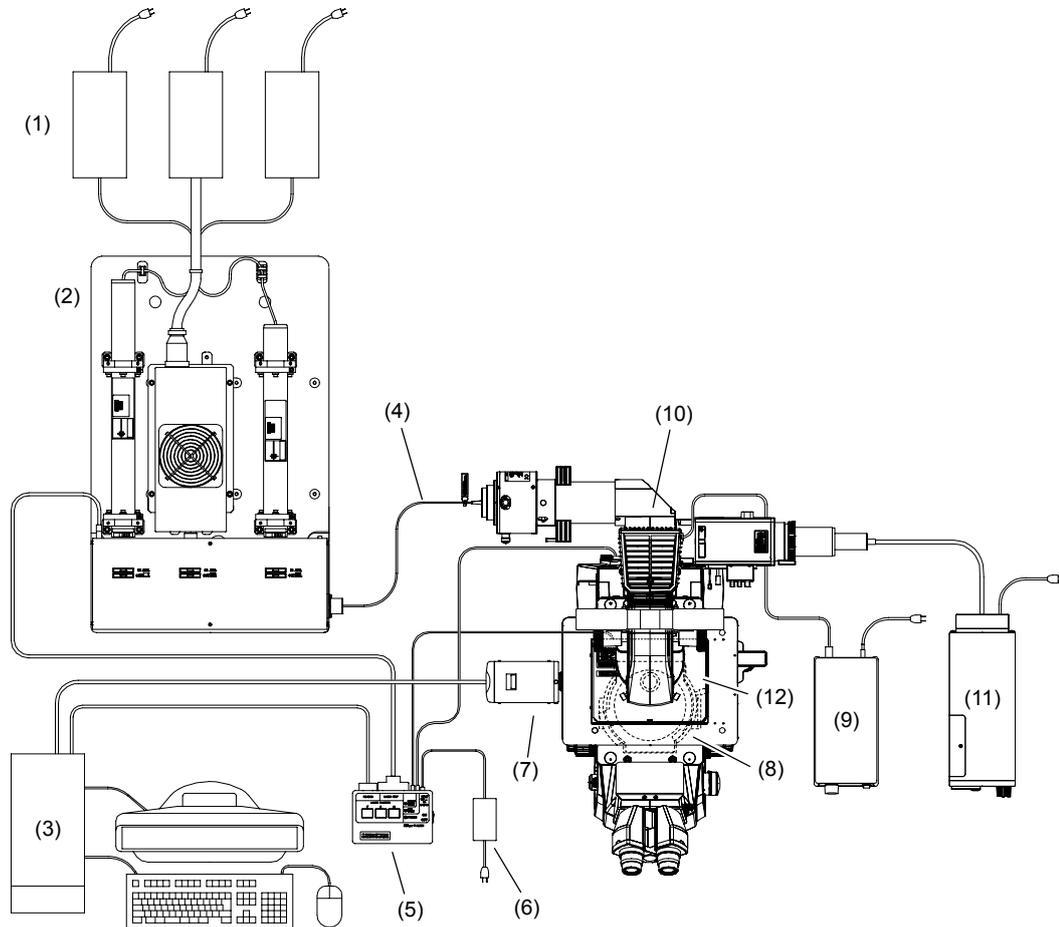


Figure 1.1-1

* For details, see Section 1, "Overall Configuration," in Chapter 8, "Specifications and Performance."

- (1) Power supply devices for the laser devices
- (2) Laser unit
- (3) PC
- (4) Optical fiber
- (5) Shutter control unit
- (6) AC adapter for the shutter control unit
- (7) TV camera (video camera)
- (8) TI-FLC epi-fl filter turret
- (9) Power supply device for the diasopic illumination
- (10) TIRF/epi-fl illuminator unit
- (11) Optical fiber light source
- (12) Laser safety cover

TIRF/epi-fl illuminator unit and Ti-U system overview (top view)**Figure 1.1-2**

* For details, see Section 1, "Overall Configuration," in Chapter 8, "Specifications and Performance."

- (1) Power supply devices for the laser devices
- (2) Laser unit
- (3) PC
- (4) Optical fiber
- (5) Shutter control unit
- (6) AC adapter for the shutter control unit
- (7) TV camera (video camera)
- (8) TI-FLC epi-fl filter turret
- (9) Power supply device for the diascope illumination
- (10) TIRF/epi-fl illuminator unit
- (11) Optical fiber light source
- (12) Laser safety cover

1.1.2 Motorized illuminator unit and Ti-E system overview

Motorized illuminator unit and Ti-E system overview (front view)

(Standard configuration to use the motorized illuminator unit)

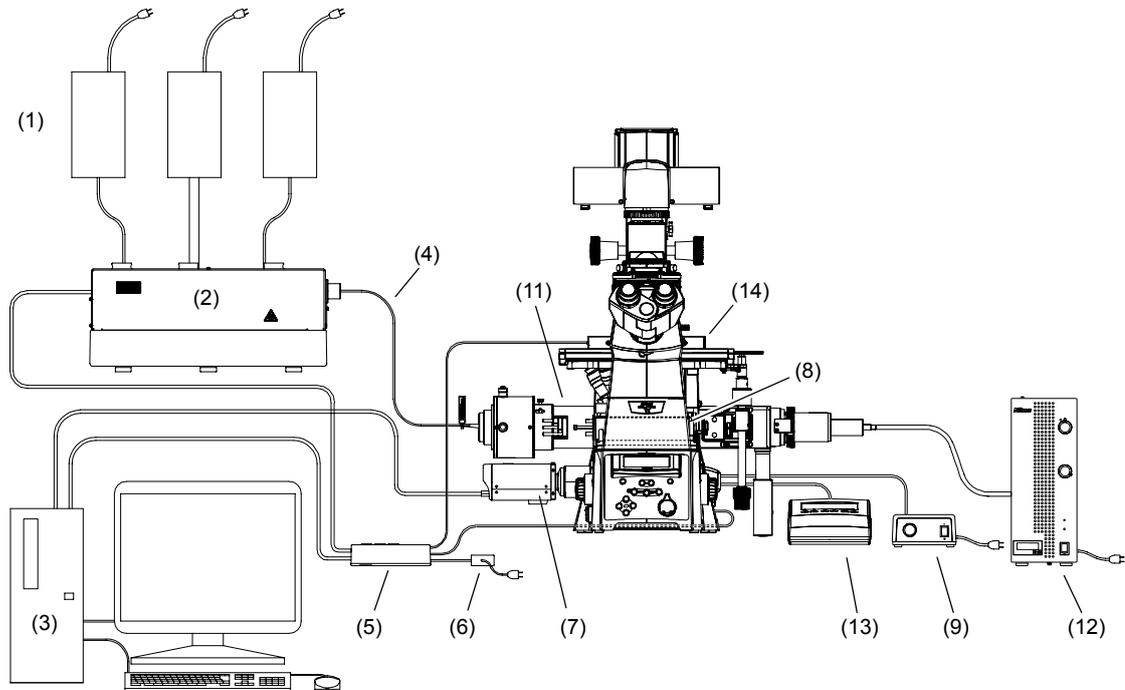
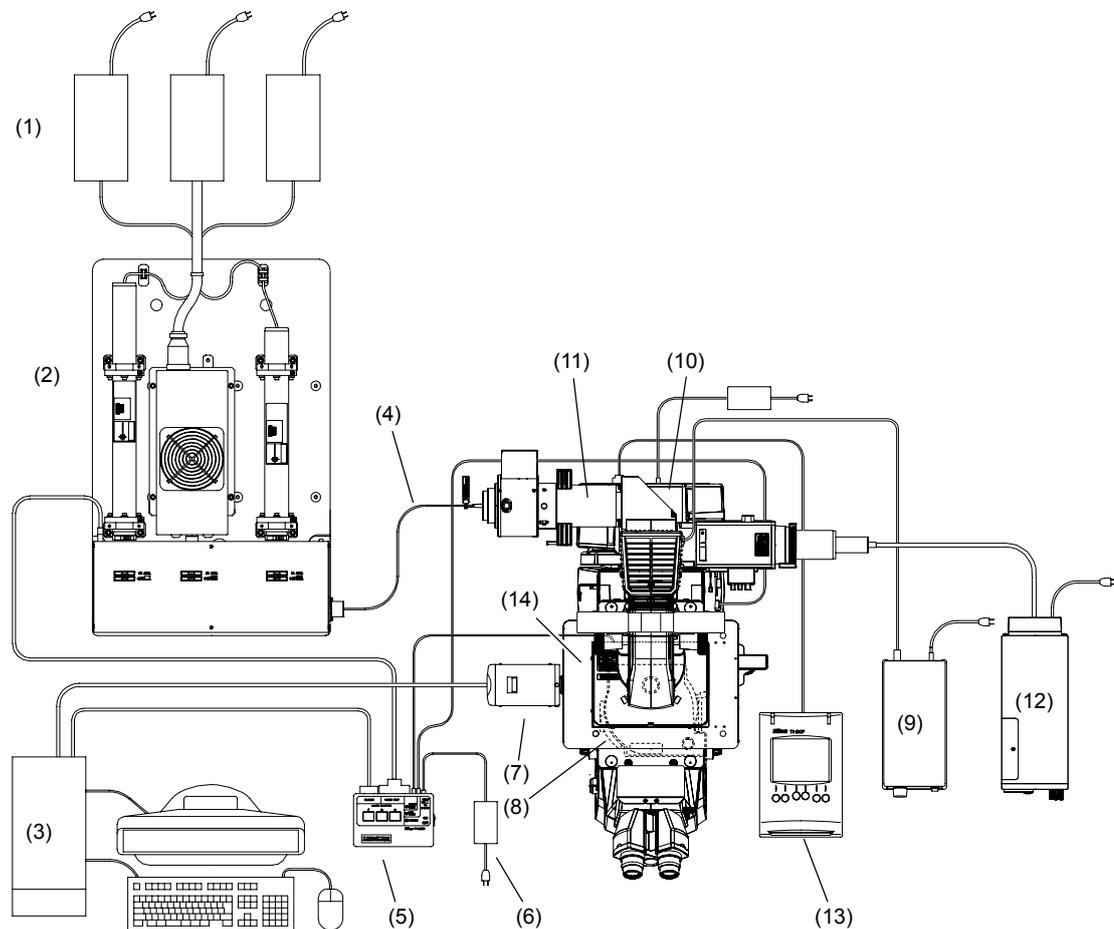


Figure 1.1-3

* For details, see Section 1, "Overall Configuration," in Chapter 8, "Specifications and Performance."

- (1) Power supply devices for the laser devices
- (2) Laser unit
- (3) PC
- (4) Optical fiber
- (5) Shutter control unit
- (6) AC adapter for the shutter control unit
- (7) TV camera (video camera)
- (8) TI-FLC-E/HQ motor epi-fl filter turret
- (9) Power supply device for the diascopic illumination
- (10) Hub Controller A (with AC adapter, behind the microscope main body)
- (11) Motorized illuminator unit
- (12) Optical fiber light source
- (13) Remote control pad
- (14) Laser safety cover

Motorized illuminator unit and Ti-E system overview (top view)**Figure 1.1-4**

* For details, see Section 1, "Overall Configuration," in Chapter 8, "Specifications and Performance."

- (1) Power supply devices for the laser devices
- (2) Laser unit
- (3) PC
- (4) Optical fiber
- (5) Shutter control unit
- (6) AC adapter for the shutter control unit
- (7) TV camera (video camera)
- (8) TI-FLC-E/HQ motor epi-fl filter turret
- (9) Power supply device for the diascopic illumination
- (10) Hub Controller A (with AC adapter)
- (11) Motorized illuminator unit
- (12) Optical fiber light source
- (13) Remote control pad
- (14) Laser safety cover

1.2 Optical Paths of Lasers

1.2.1 Laser Unit

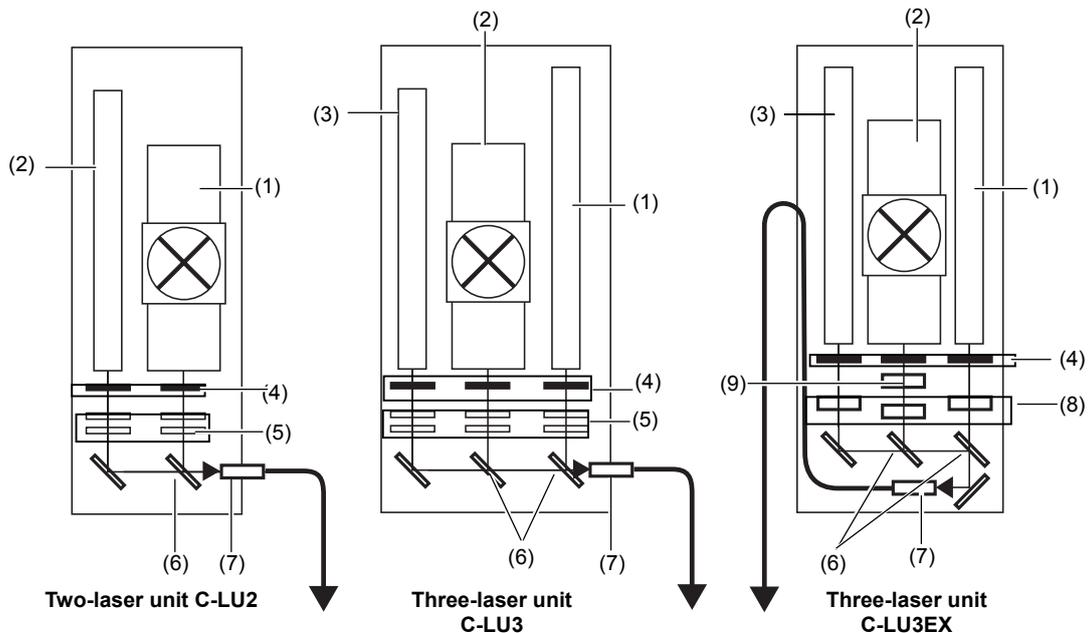


Figure 1.2-1

- | | |
|----------------------------------------------------------|----------------------------------------------|
| (1) Laser mounting position L1
(See the table below.) | (6) Laser coupling mirror |
| (2) Laser mounting position L2
(See the table below.) | (7) Laser outlet (single-mode optical fiber) |
| (3) Laser mounting position L3
(See the table below.) | (8) Continuous brightness adjustment part |
| (4) Motorized shutters | (9) Wavelength selection filter |
| (5) ND filters (ND4 and ND8) | |

Table 1.2-1

Model name	Laser mounting position		
	L1	L2	L3
Two-laser unit C-LU2	405 nm to 514 nm	532 nm to 640 nm	—
Three-laser unit C-LU3	405 nm to 445 nm 594 nm to 640 nm	488 nm to 514 nm	532 nm to 561 nm 633 nm
Three-laser unit C-LU3EX	405 nm to 445 nm 594 nm to 640 nm	488 nm to 514 nm	532 nm to 561 nm 633 nm

1.2.2 Microscope Part (TIRF/Epi-fl Illuminator Unit and Ti-U System)

TIRF/epi-fl illuminator unit and Ti-U system (top view)

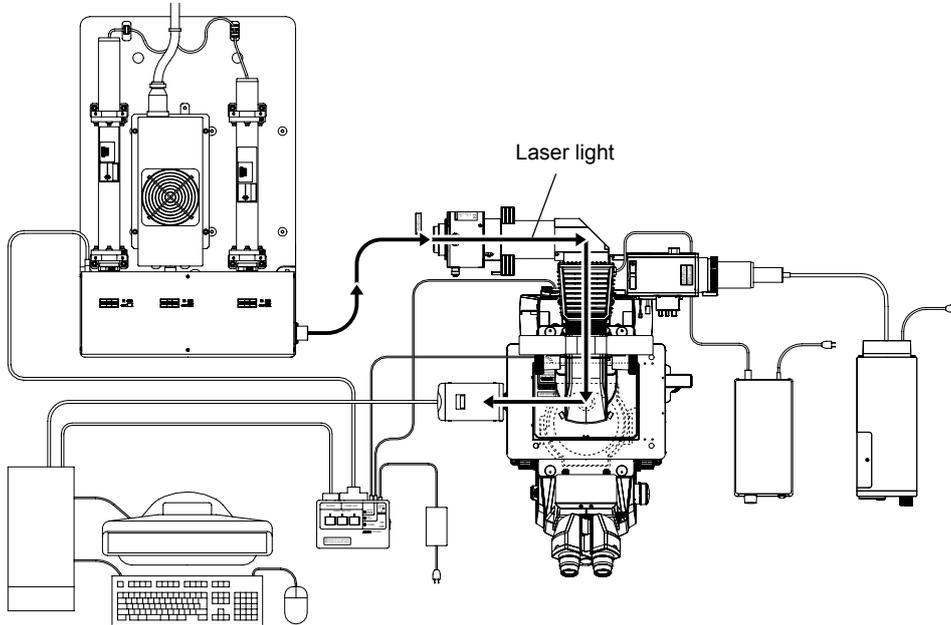


Figure 1.2-2

TIRF/epi-fl illuminator unit and Ti-U system (front left view)

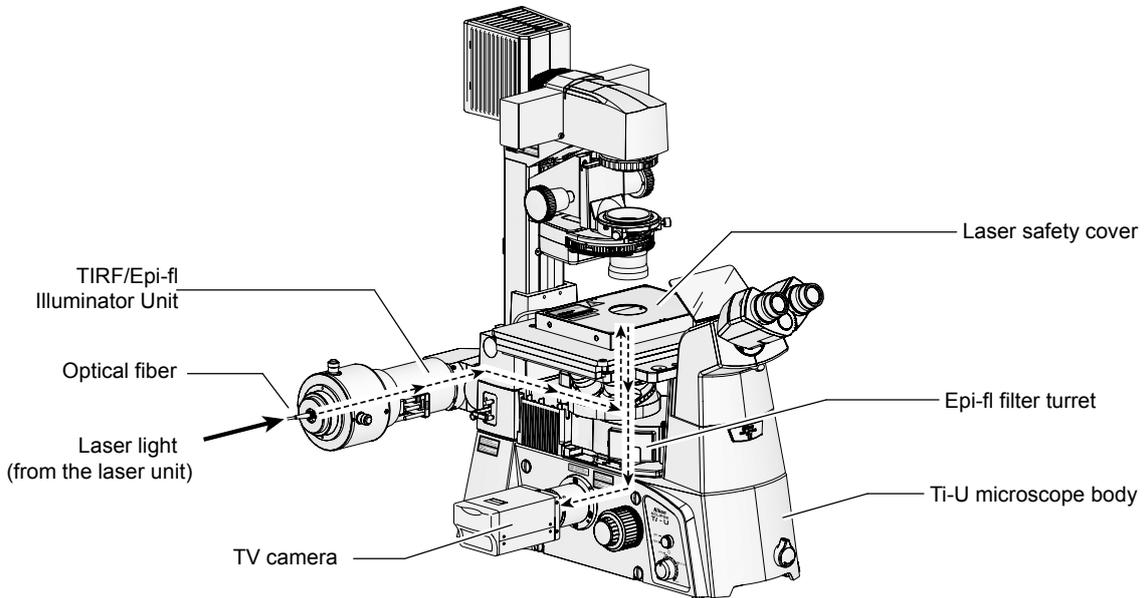


Figure 1.2-3

The TV camera can be installed in a side port (right or left port) or the bottom port (only for Ti-U/B). To change the optical path, use the optical path selector dial on the microscope.

Note that the laser shutter automatically closes when the microscope optical path is changed to the eyepiece. It is not possible to observe images, obtained by irradiating the laser to the specimen, from the binocular part of the microscope.

1.2.3 Microscope Part (Motorized Illuminator Unit and Ti-E System)

Motorized illuminator unit and Ti-E system (top view)

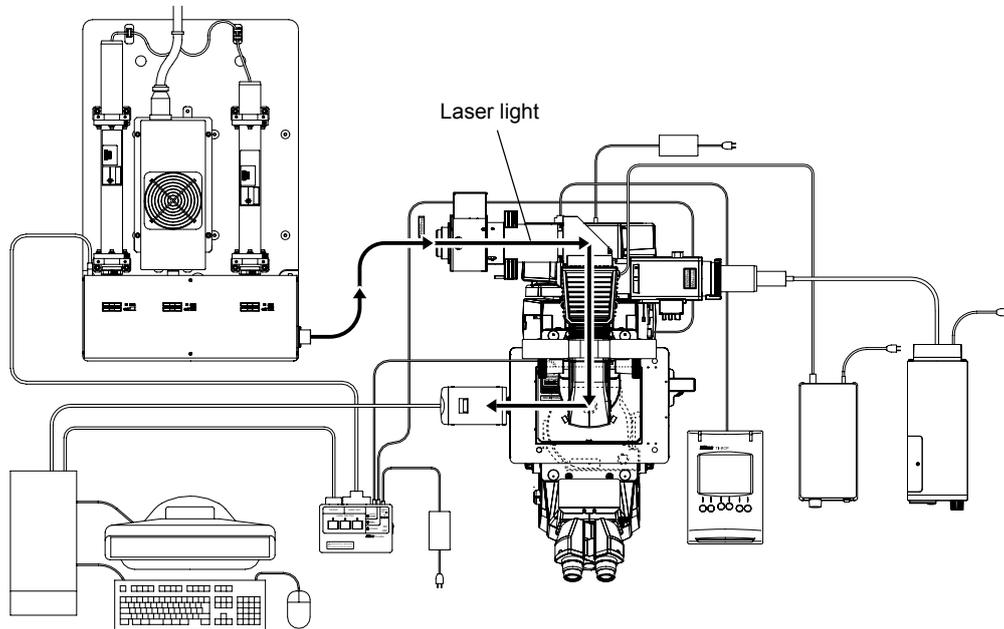


Figure 1.2-4

Motorized illuminator unit and Ti-E system (front left view)

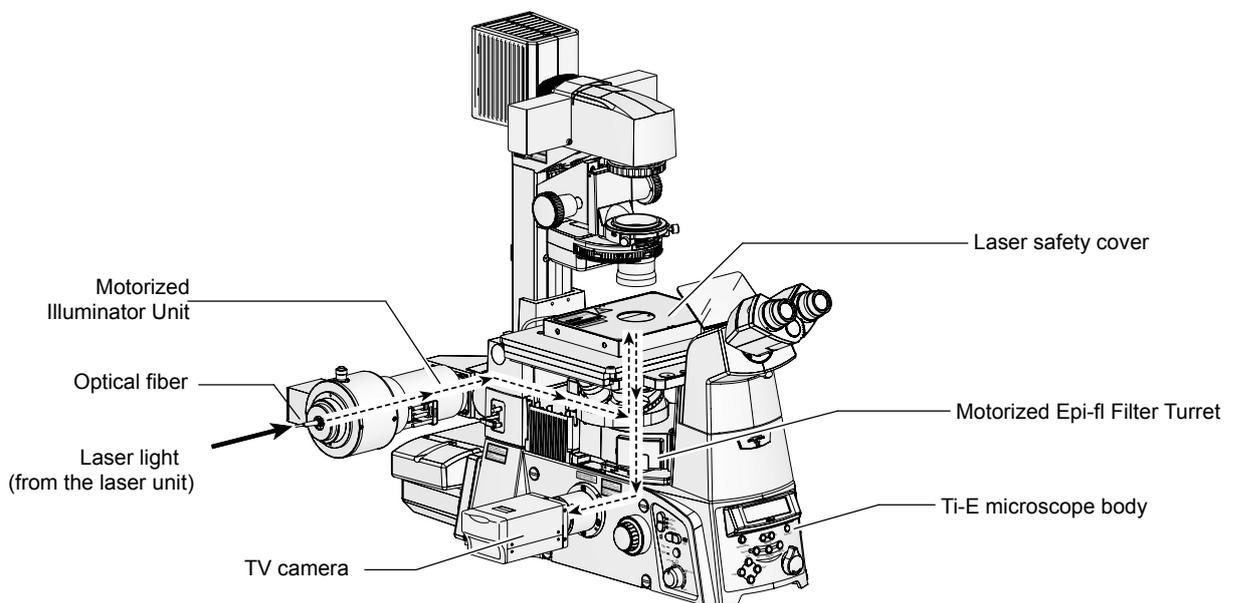


Figure 1.2-5

The TV camera can be installed in a side port (right or left port) or the bottom port (only for Ti-E/B). To change the optical path, use the optical path selector switch on the microscope.

Note that the laser shutter automatically closes when the microscope optical path is changed to the eyepiece. It is not possible to observe images, obtained by irradiating the laser to the specimen, from the binocular part of the microscope.

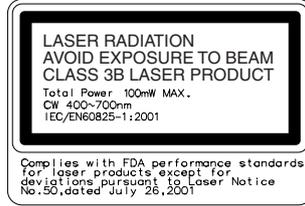
1.3 Safety Labels

1.3 Safety Labels

(1) IEC warning label – laser hazard symbol



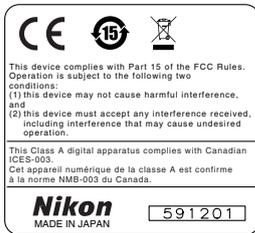
(2) IEC explanatory label – Class 3B laser product



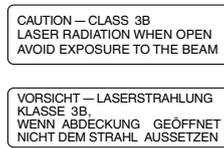
(3) Model name and serial number label



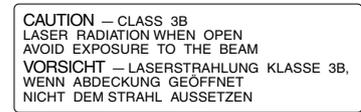
(4) Safety standard label



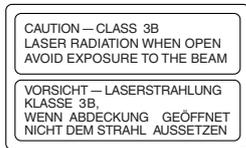
(5) IEC caution label – Class 3B area



(6) IEC caution label – Class 3B area



(7) IEC caution label – Class 3B area



(8) IEC aperture label 1



(9) IEC aperture label 2



(10) TI-TIRF special label

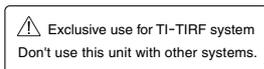


Figure 1.3-1

1.3 Safety Labels

1.3.1 Ti-U Microscope Body

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

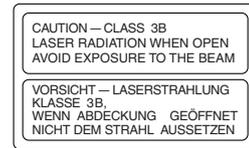
(1) IEC warning label – laser hazard symbol



(2) IEC explanatory label – Class 3B laser product



(7) IEC caution label – Class 3B area



(8) IEC aperture label 1



(9) IEC aperture label 2

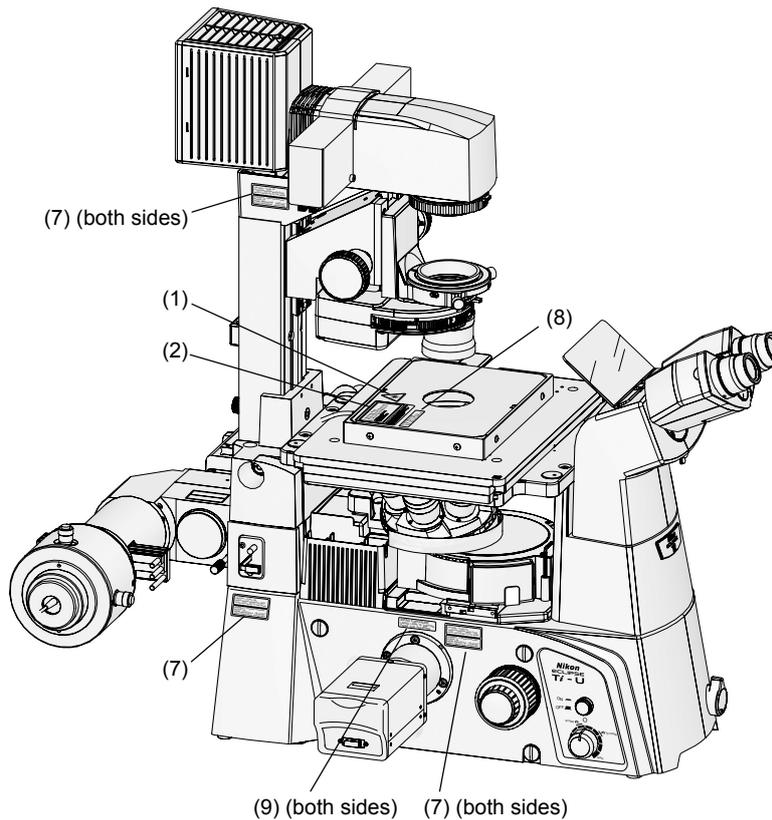


Figure 1.3-2

1.3.2 Ti-E Microscope Body

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

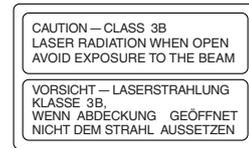
- (1) IEC warning label – laser hazard symbol



- (2) IEC explanatory label – Class 3B laser product



- (7) IEC caution label – Class 3B area



- (8) IEC aperture label 1



- (9) IEC aperture label 2

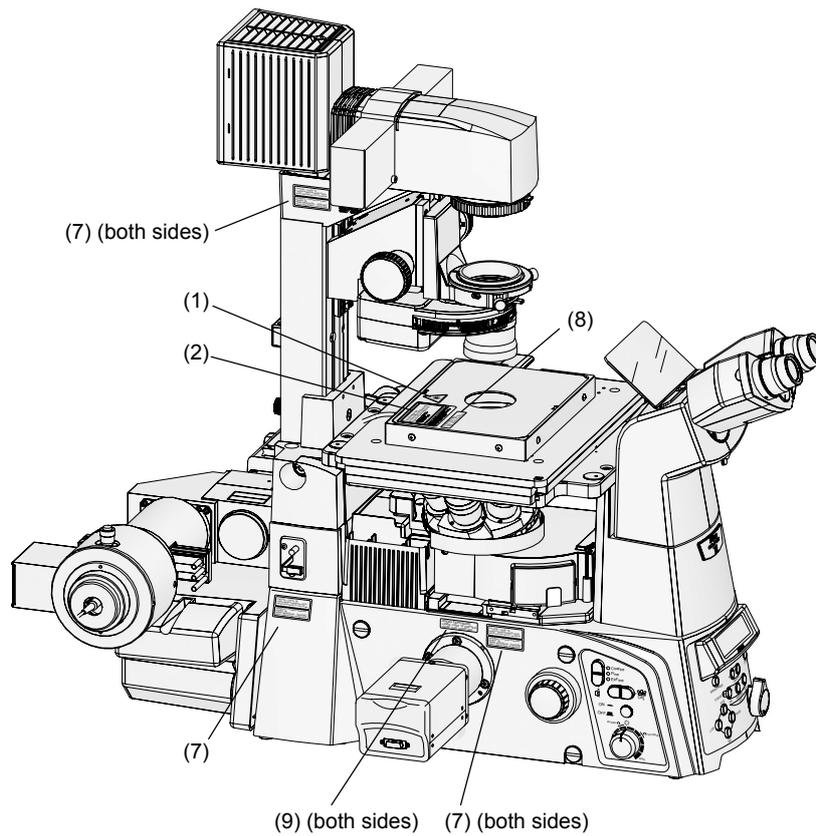


Figure 1.3-3

1.3.3 C-LU2 Two-laser Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

(1) IEC warning label – laser hazard symbol

(6) IEC caution label – Class 3B area



CAUTION – CLASS 3B
 LASER RADIATION WHEN OPEN
 AVOID EXPOSURE TO THE BEAM
 VORSICHT – LASERSTRAHLUNG KLASSE 3B,
 WENN ABDECKUNG GEÖFFNET
 NICHT DEM STRAHL AUSSETZEN

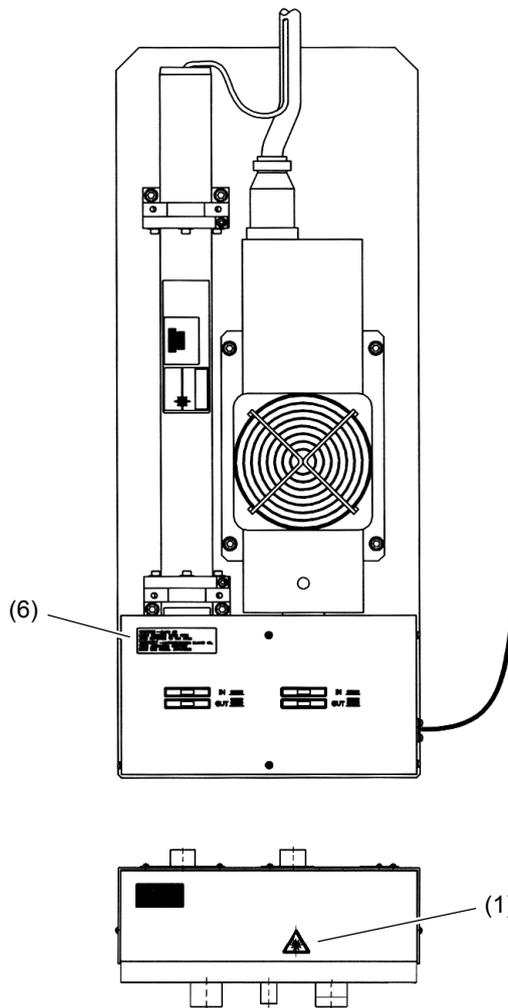


Figure 1.3-4

1.3.4 C-LU3 Three-laser Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

(1) IEC warning label – laser hazard symbol

(6) IEC caution label – Class 3B area



CAUTION – CLASS 3B
LASER RADIATION WHEN OPEN
AVOID EXPOSURE TO THE BEAM
VORSICHT – LASERSTRAHLUNG KLASSE 3B,
WENN ABDECKUNG GEÖFFNET
NICHT DEM STRAHL AUSSETZEN

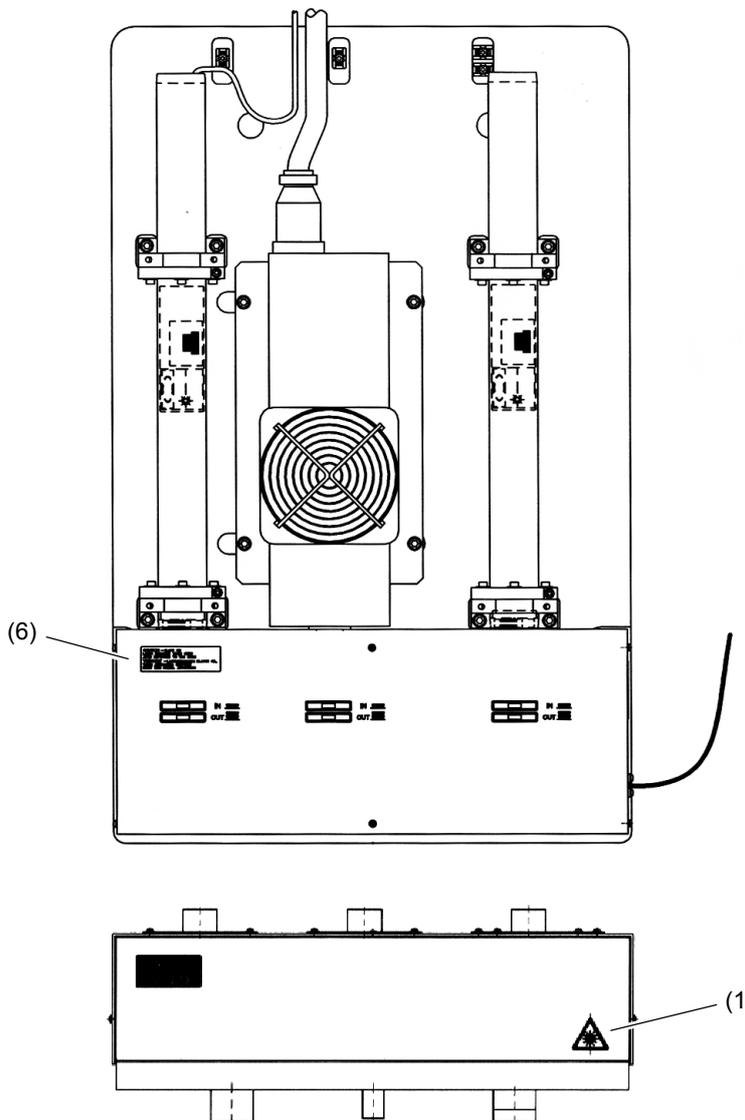


Figure 1.3-5

1.3.5 C-LU3EX Three-laser Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

- (1) IEC warning label – laser hazard symbol (6) IEC caution label – Class 3B area



CAUTION – CLASS 3B
LASER RADIATION WHEN OPEN
AVOID EXPOSURE TO THE BEAM
VORSICHT – LASERSTRAHLUNG KLASSE 3B,
WENN ABDECKUNG GEÖFFNET
NICHT DEM STRAHL AUSSETZEN

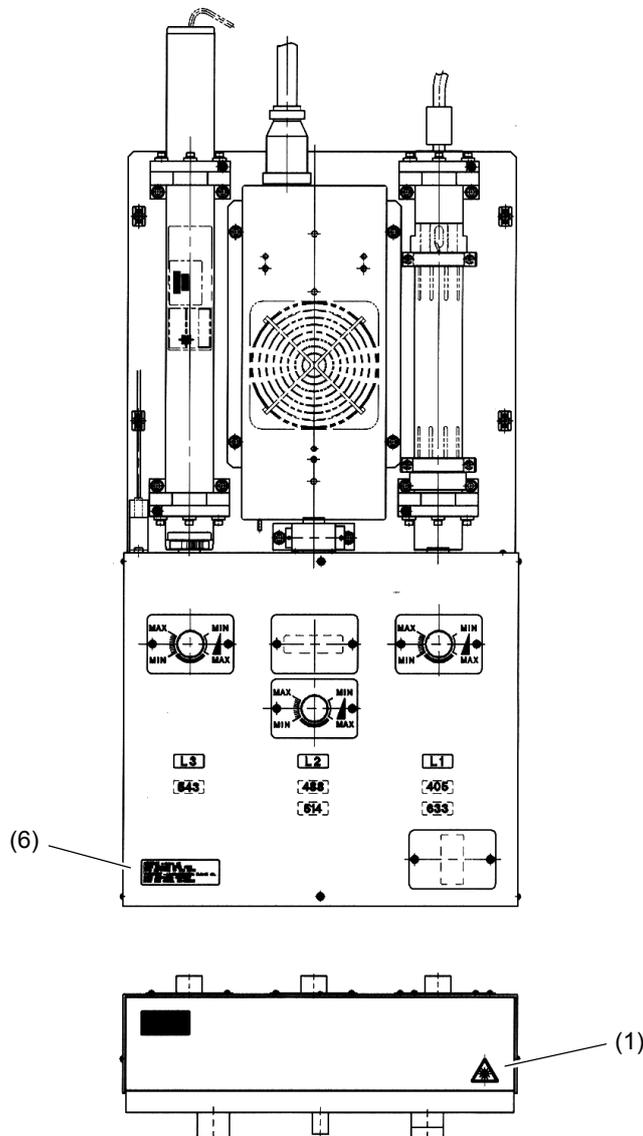


Figure 1.3-6

1.3.6 TI-LUSU Shutter Control Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

(4) Safety standard label

(10) TI-TIRF special label

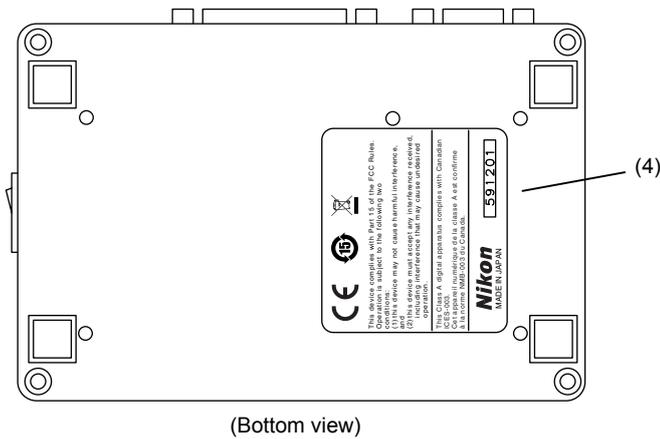
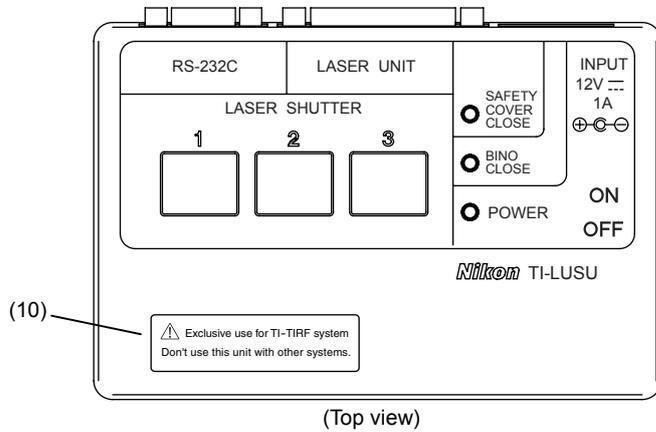
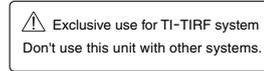
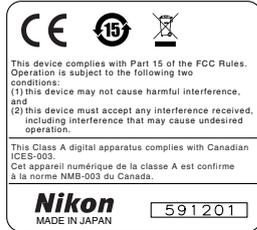


Figure 1.3-7

1.3.7 TIRF/Epi-fl Illuminator Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

(3) Model name and serial number label

(5) IEC caution label – Class 3B area

(7) IEC caution label – Class 3B area

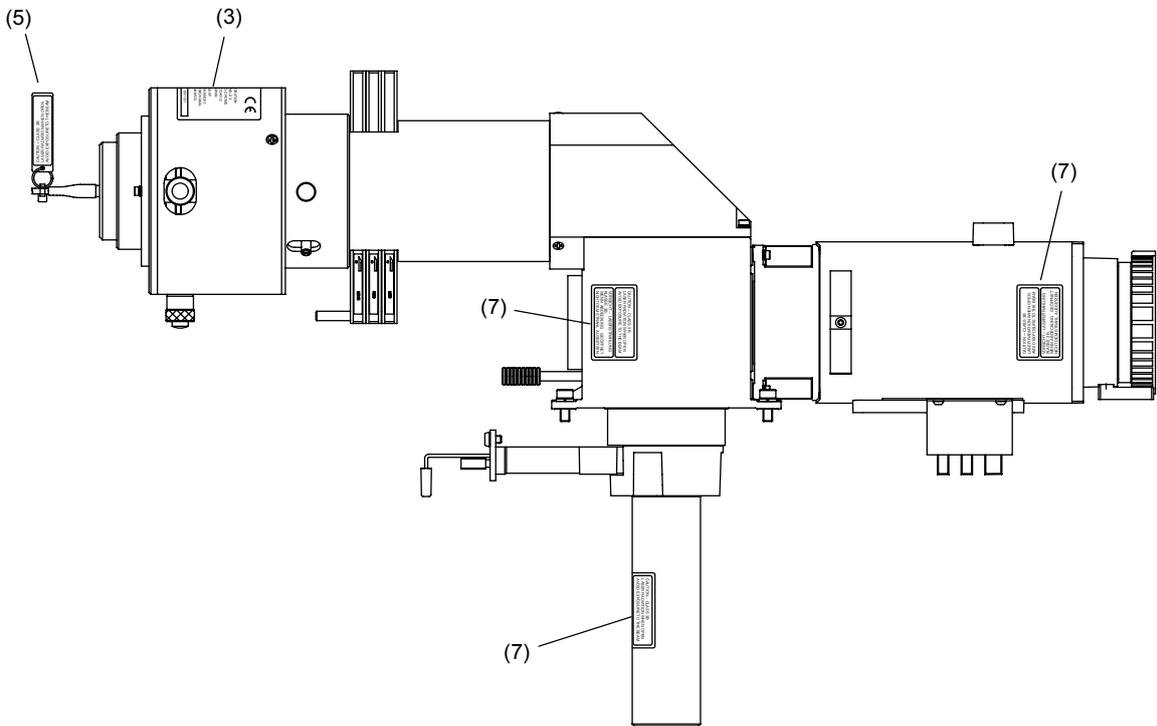
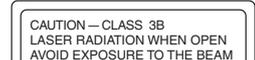
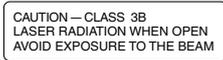


Figure 1.3-8

1.3.8 Motorized Illuminator Unit

The following safety labels are affixed to the product. Before using the product, check that individual labels are affixed at their correct positions.

(3) Model name and serial number label

(5) IEC caution label – Class 3B area

(7) IEC caution label – Class 3B area

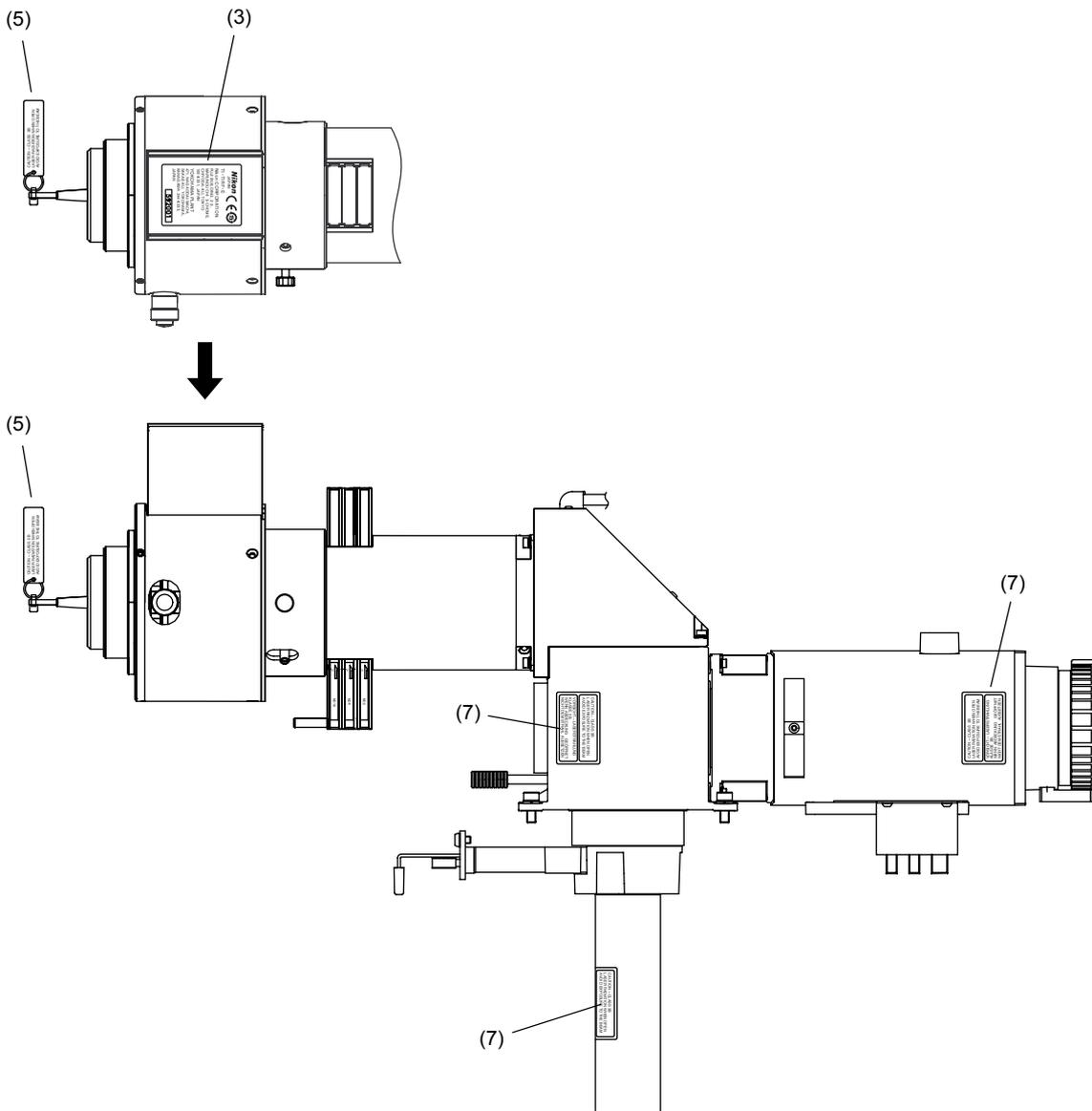
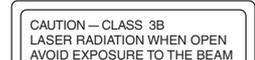
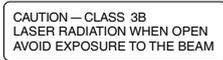


Figure 1.3-9

1.4 Name of Each Part

1.4.1 TIRF/Epi-fl Illuminator Unit and Ti-U System

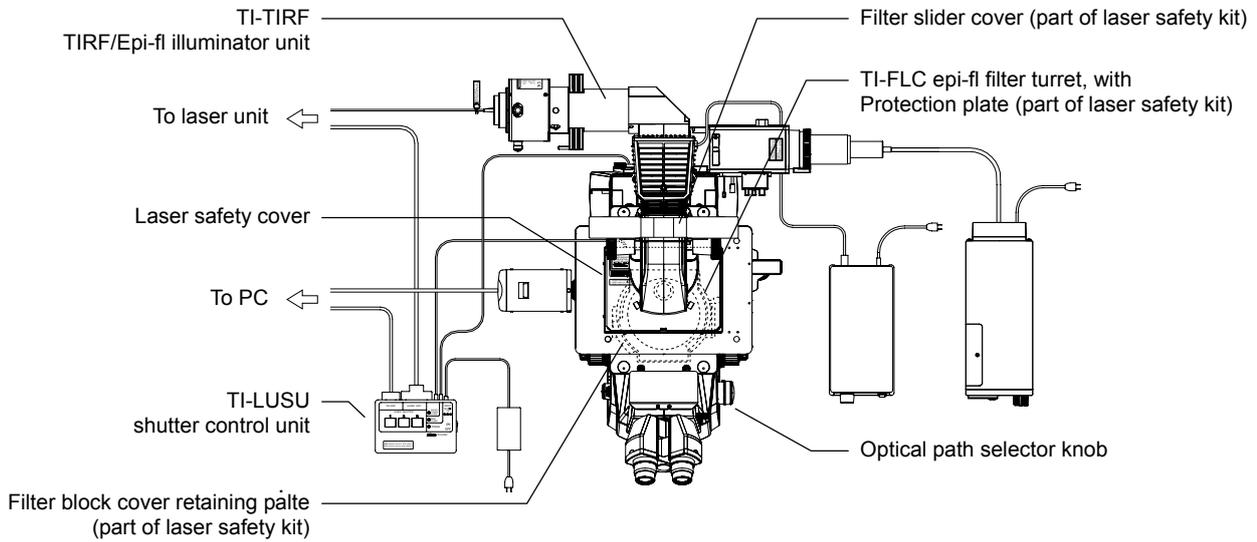
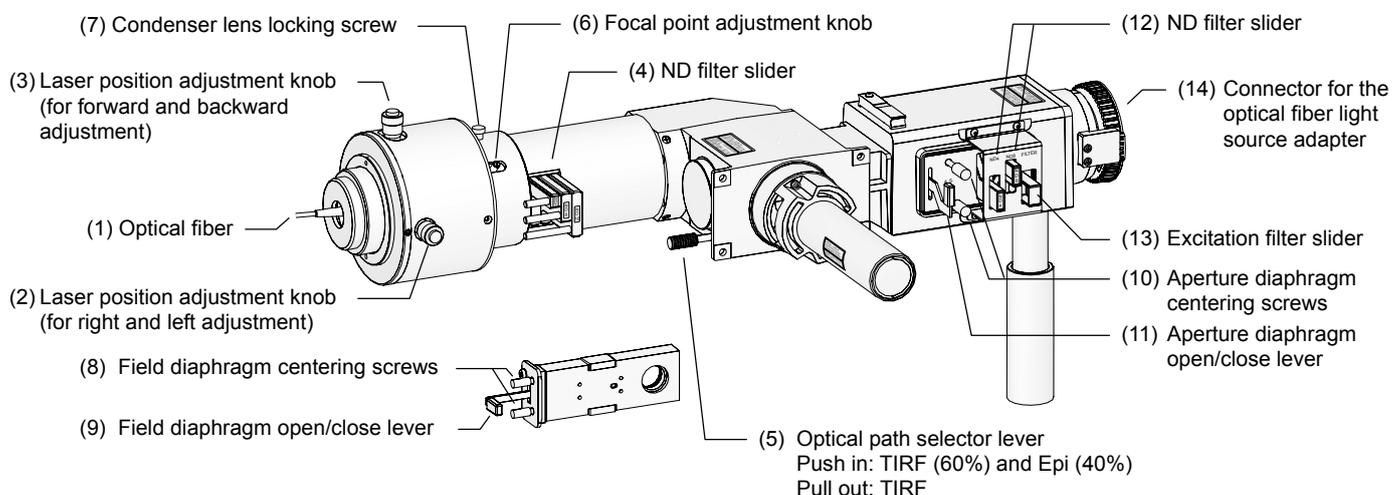


Figure 1.4-1

TI-TIRF TIRF/epi-fl illuminator unit**Figure 1.4-2****Parts used in the TIRF microscopy operation**

- (1) Optical fiber
- (2) Laser position adjustment knob (for right and left adjustment)
- (3) Laser position adjustment knob (for forward and backward adjustment)
- (4) ND filter slider
- (5) Optical path selector lever
Push in: TIRF (60%) and Epi (40%)
Pull out: TIRF
- (6) Focal point adjustment knob
- (7) Condenser lens locking screw

Parts used in the epi-fl microscopy operation

- (8) Field diaphragm centering screws
- (9) Field diaphragm open/close lever
- (10) Aperture diaphragm centering screws
- (11) Aperture diaphragm open/close lever
- (12) ND filter slider
- (13) Excitation filter slider
- (14) Connector for the optical fiber light source adapter

Laser safety cover

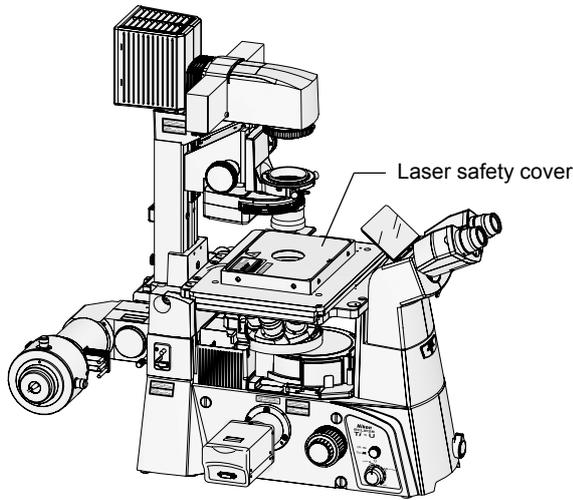


Figure 1.4-3

The laser safety cover prevents laser light from leaking. If the laser safety cover is not attached, the motorized shutters of the laser unit do not open.

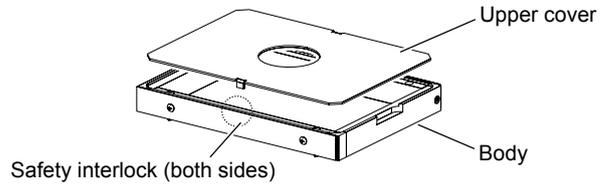


Figure 1.4-4

* If the safety interlock part is touched or gotten wet, misoperation may occur.

Laser safety kit

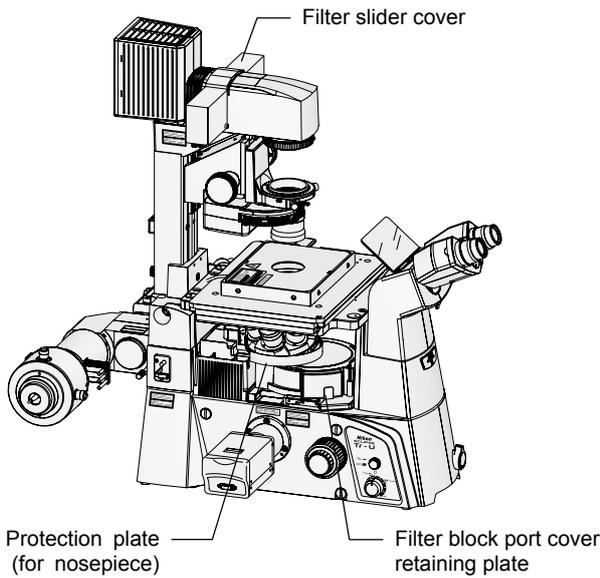


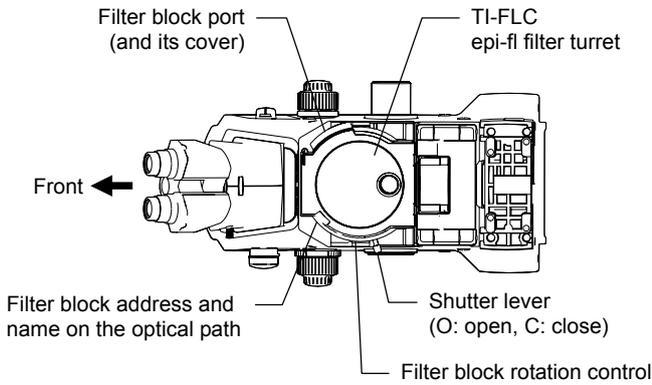
Figure 1.4-5

The laser safety kit is composed of the filter slider cover, protection plate, filter block port fixed plate, and safety labels.

Before using the product, check that the part shown in the left figure is attached.

* For the types and positions of safety labels, see Section 1.3.1, "Ti-U Microscope Body."

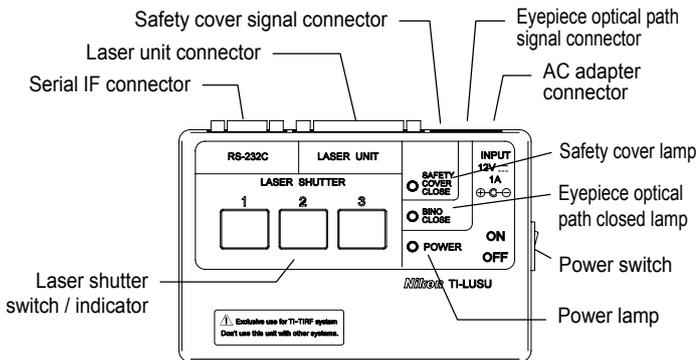
TI-FLC epi-fl filter turret



The epi-fl filter turret is used to select an excitation method by rotating the turret to change filter blocks including a dichroic mirror.

Figure 1.4-6

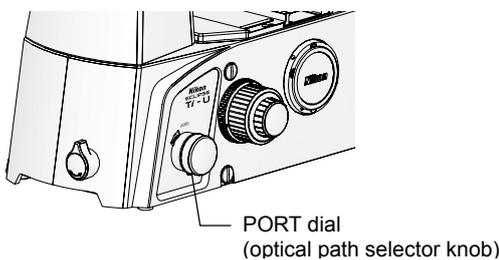
TI-LUSU shutter control unit



The shutter control unit controls motorized shutters in the laser unit.

Figure 1.4-7

Optical path selector knob



The laser shutter does not open when the binocular route of the optical path ("EYE") is selected.

Ti-U

- EYE** Observation port 100%
- R** Right port 100%
- AUX** Auxiliary port
(Visual observation/left port = 20:80)
(Visual observation/right port = 20:80)
- L** Left port 100%

Ti-U/B

- EYE** Observation port 100%
- R** Right port 100%
- B** Bottom port 100%
- L** Left port 100%

1.4.2 Motorized Illuminator Unit and Ti-E System

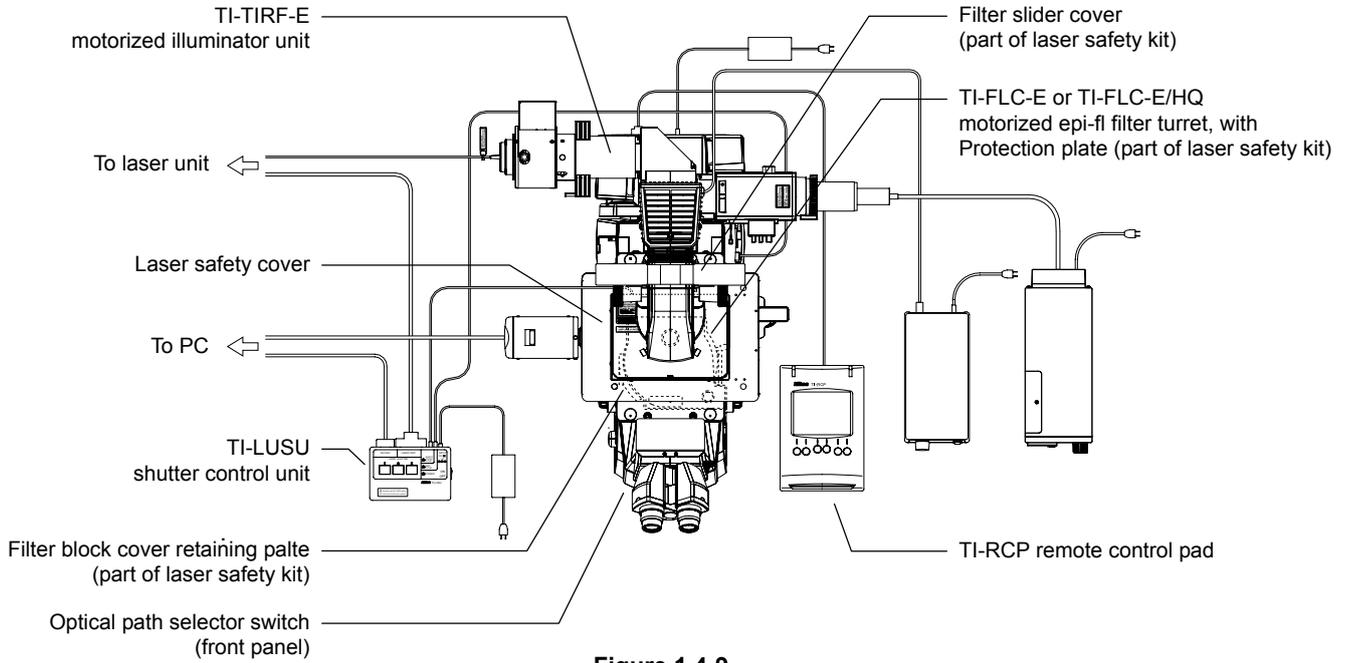


Figure 1.4-9

TI-TIRF-E motorized illuminator unit

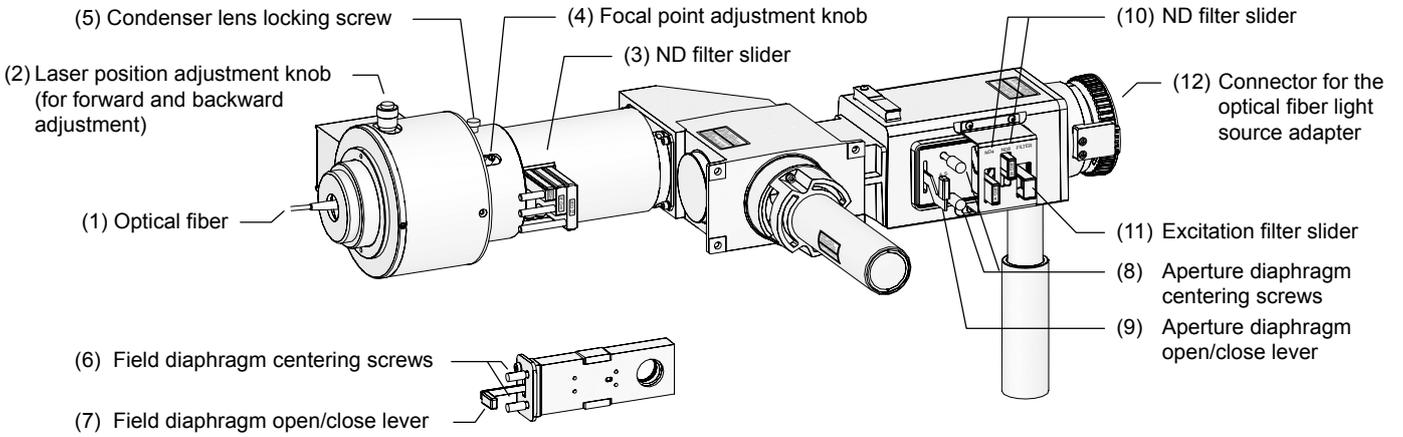


Figure 1.4-10

Parts used in the TIRF microscopy operation

- (1) Optical fiber
- (2) Laser position adjustment knob (for forward and backward adjustment *)
- (3) ND filter slider
- (4) Focal point adjustment knob
- (5) Condenser lens locking screw

Parts used in the epi-fl microscopy operation

- (6) Field diaphragm centering screws
- (7) Field diaphragm open/close lever
- (8) Aperture diaphragm centering screws
- (9) Aperture diaphragm open/close lever
- (10) ND filter slider
- (11) Excitation filter slider
- (12) Connector for the optical fiber light source adapter

The TI-RCP remote control pad (or TI-ERGC ergo controller) allows users to change the optical path between TIRF illumination and episcopic illumination as well as adjust the laser right or left (TIRF illumination angle adjustment).

TI-RCP remote control pad

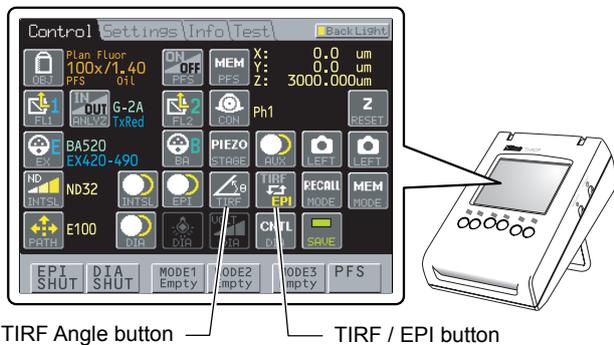


Figure 1.4-11

User can perform the following operations using the remote control pad:

- **TIRF/EPI button:**
The optical path is changed between TIRF illumination (laser light) and episcopic illumination.
- **TIRF Angle button:**
TIRF illumination position (illumination angle) adjustment *

* The laser is adjusted right or left and the illumination angle of TIRF illumination (total reflection illumination) is adjusted.

Laser Safety Cover

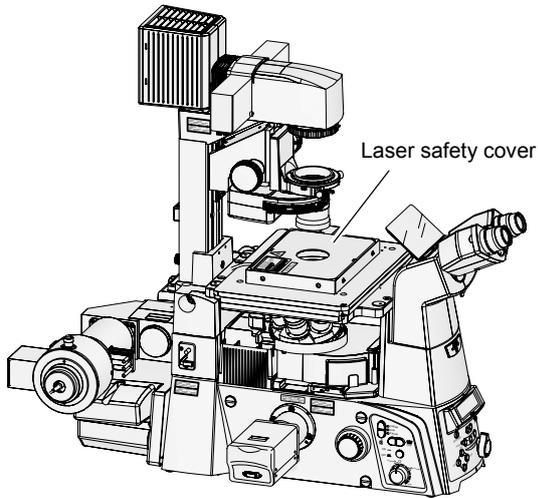


Figure 1.4-12

The cover prevents laser light from leaking. If the laser safety cover is not attached, the motorized shutters of the laser unit do not open.

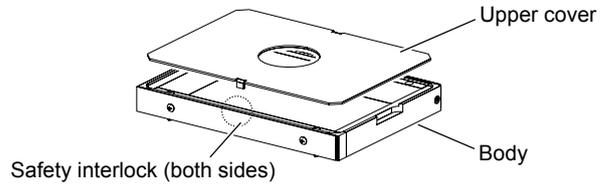


Figure 1.4-13

* If the safety interlock part is touched or gotten wet, misoperation may occur.

Laser safety kit

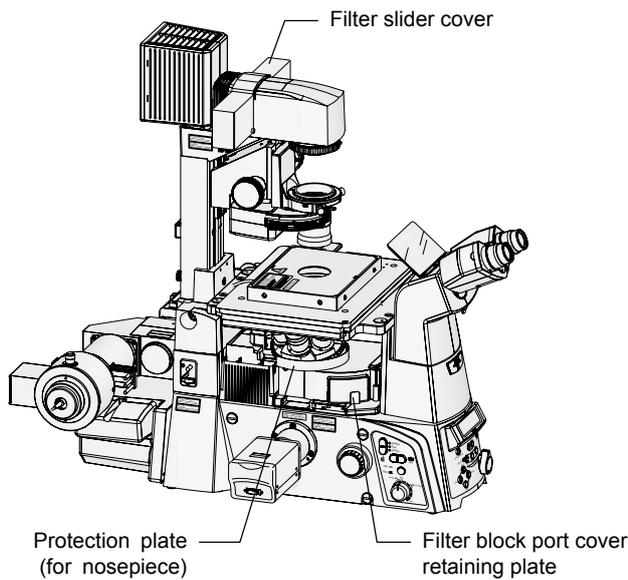


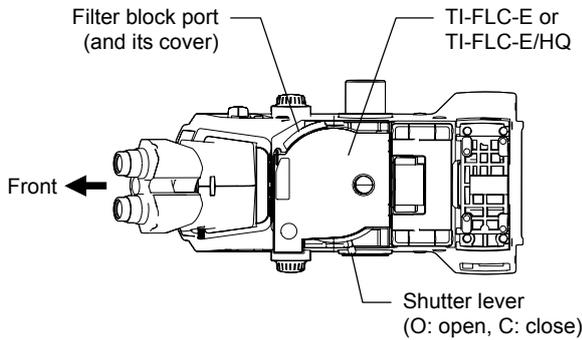
Figure 1.4-14

The laser safety kit is composed of the filter slider cover, protection plate, filter block port fixed plate, and safety labels.

Before using the product, check that the part shown in the left figure is attached.

* For the types and positions of safety labels, see Section 1.3.2, "Ti-E Microscope Body."

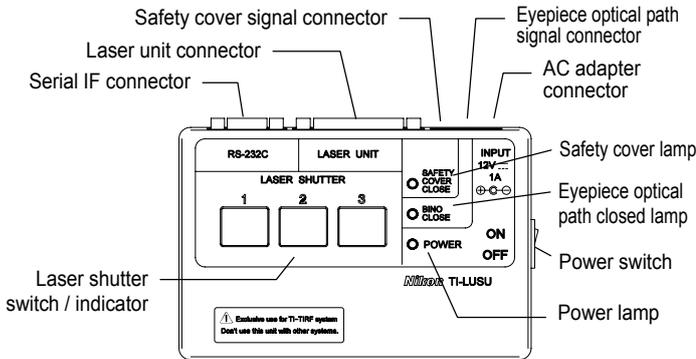
TI-FLC-E Motorized Epi-fl Filter Turret or TI-FLC-E/HQ Motor Epi-fl Filter Turret



The epi-fl filter turret is used to select an excitation method by rotating the turret to change filter blocks including a dichroic mirror.

Figure 1.4-15

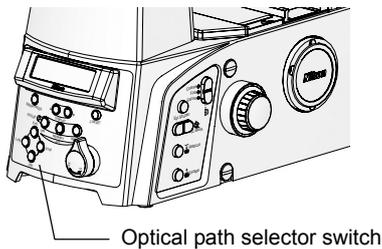
TI-LUSU shutter control unit



The shutter control unit controls motorized shutters in the laser unit.

Figure 1.4-16

Optical path selector switch



The laser shutter does not open when the binocular route of the optical path ("EYE") is selected.

Ti-E

- EYE** Observation port 100%
- L100** Left port 100%
- L80** Left port 80% and observation port 20%
- R100** Right port 100%

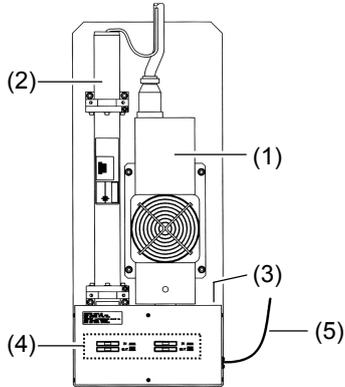
Ti-E/B

- EYE** Observation port 100%
- L100** Left port 100%
- L80** Left port 80% and observation port 20%
- B100** Bottom port 100%

Figure 1.4-17

1.4.3 Laser Unit

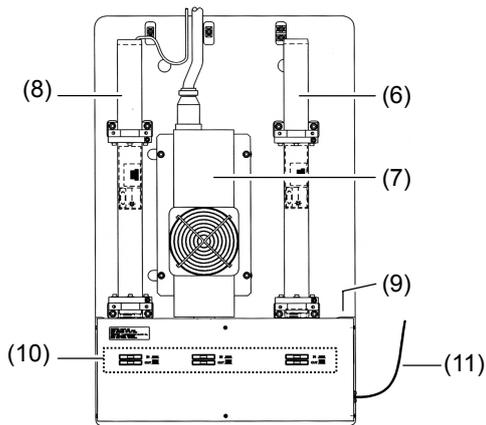
Two-laser Unit (C-LU2)



- (1) Laser mounting position L1
- (2) Laser mounting position L2
- (3) Shutter control unit connector
- (4) ND filter slider
- (5) Single-mode fiber

Figure 1.4-18

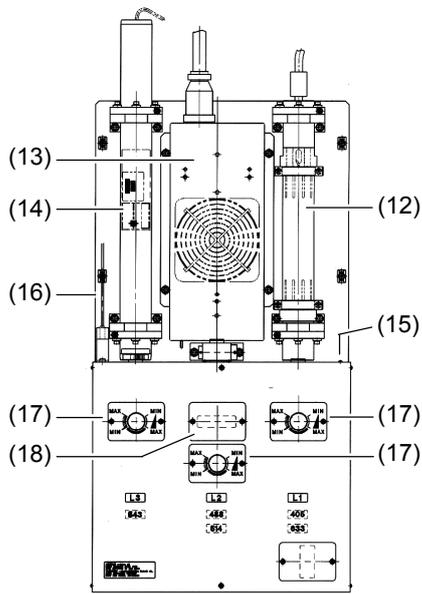
Three-laser unit (C-LU3)



- (6) Laser mounting position L1
- (7) Laser mounting position L2
- (8) Laser mounting position L3
- (9) Shutter control unit connector
- (10) ND filter slider
- (11) Single-mode fiber

Figure 1.4-19

Three-laser unit EX (C-LU3EX)



- (12) Laser mounting position L1
- (13) Laser mounting position L2
- (14) Laser mounting position L3
- (15) Shutter control unit connector
- (16) Single-mode fiber
- (17) Brightness control knob
- (18) Ar wavelength selector (option)

Figure 1.4-20

* For details of available lasers for each position of laser units, see the table, "Mountable Lasers," in Section 1, "Overall Configuration" in Chapter 8, "Specifications and Performance."

2

Microscopy Procedure Overview

Table 2-1

1	Preparation	Attach a TIRF objective. (For information about TIRF objectives, refer to Section 1, "Overall Configuration" in Chapter 8, "Specifications and Performance." Adjust the diopters and the interpupillary distance.
2	Safety Check	Check the following: <ul style="list-style-type: none"> • A TV camera, a video camera, or a dust-proof cap is attached to each port of the microscope. • An objective or a cap is attached to each socket of the nosepiece. • The field diaphragm unit is attached correctly. • The optical fiber for laser light is securely fixed. • The adapter for the optical fiber light source or the lamp house is attached correctly. • The diasopic illumination lamp house is correctly attached to the 100W dia pillar illuminator. • The filter slider cover is correctly attached to the 100W dia pillar illuminator. • The epi-fl filter turret is attached correctly. • The filter block port cover of the FL turret is closed so that it cannot be opened. • The protection plate is inserted between the FL turret and the nosepiece (or PFS nosepiece). • The laser safety cover is attached on the stage.
3	Start-up Operation	Start up the laser devices. Turn on devices such as the microscope, the optical fiber light source, the shutter control unit, and the camera.
4	Epi-fl Microscopy (Focusing)	Set up the optical path for epi-fl microscopy. Select an excitation method. Open the shutter for the epi illumination. Focus on the specimen. Center the field diaphragm and adjust the diaphragm size. Adjust the brightness with the ND filters. Close the shutter for the epi illumination.
(Reference)	Simple Surface Reflection Interference Microscopy (Condition check on the contact of the specimen and the cover glass)	Change the excitation method settings. (Place the filter block, which has settings of "G excitation filter", "UV dichroic mirror", and "No barrier filter", into the optical path.) Reduce the size of the aperture diaphragm fully. Adjust the field diaphragm opening to a smallest size for the observation. Close the shutter for the epi illumination.
5	TIRF Microscopy	Set up the optical path for TIRF microscopy. Select an excitation method. Open the shutter for the laser. Adjust the laser position with the focal point adjustment knob. Adjust the laser position to provide TIRF illumination. (When magnification is changed, repeat above steps from the laser light adjustment.)
6	Termination Procedure	Turn off the laser devices. Turn off the optical fiber light source. Turn off other devices.

3

Detailed Microscopy Procedure

3.1 Preparation

3.1.1 Attaching a TIRF Objective

Attach a TIRF objective to the socket of the nosepiece. (For information about TIRF objectives, refer to Section 1, “Overall Configuration” in Chapter 8, “Specifications and Performance.”)

3.1.2 Diopter Adjustment and Interpupillary Distance Adjustment

See the instruction manual for the relevant microscope to adjust the diopter and the interpupillary distance.

3.2 Safety Check

This product uses class IIIB lasers in the FDA expression (class 3B lasers in the IEC expression).

Laser is harmful light. This product has openings at the parts other than the objective and the analyzer.

If laser light is irradiated to eyes or skin through any of these openings, it may cause injury or other problems.

Be sure to perform the following safety check every time before energizing a laser device:

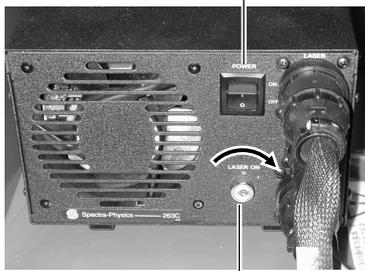
- A TV camera, a video camera, or a dust-proof cap is attached to each port of the microscope.
- An objective or a cap is attached to each socket of the nosepiece.
- The field diaphragm unit is attached correctly.
- The optical fiber for laser light is securely fixed.
- The adapter for the optical fiber light source or the lamp house is attached correctly.
- The diascopic illumination lamp house is correctly attached to the 100W dia pillar illuminator.
- The filter slider cover is correctly attached to the 100W dia pillar illuminator.
- The epi-fl filter turret is attached correctly.
- The filter block port cover of the FL turret is closed so that it cannot be opened.
- The protection plate is inserted between the FL turret and the nosepiece (or PFS nosepiece).
- The laser safety cover is attached on the stage.

3.3 Start-up Operation

3.3.1 Starting Up the Laser Devices

1 Air-cooled Ar laser

(a) Switch on the power switch



(b) Turn the key switch clockwise

Figure 3.3-1

1. On the air-cooled argon (Ar) laser device, switch on the power switch (a) and turn the key switch clockwise (b).



(d) Select the Run mode. (c) Turn the key switch clockwise.

Figure 3.3-2

2. On the remote controller of the air-cooled Ar laser, turn the key switch clockwise (c). Check that the Run mode is selected (d).

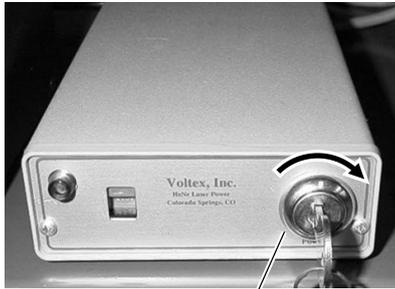
Three lamps on the remote controller lights up green in approximately one minute. The laser output stabilizes in approximately 15 minutes.



Caution

- Be sure to read the instruction manual supplied with the air-cooled argon laser.
- Always select Run mode when using the air-cooled Ar laser. Image quality may be degraded if Run mode is not selected.
- The output power is adjustable with the adjustment knob, but it is recommended that the laser be used with the standard output power. Particularly, if the laser is running with low power, the laser emission becomes unstable, and image quality may be degraded.

2 Other lasers



Turn the key switch clockwise.

Figure 3.3-3
Power supply device for the He-Ne laser

On the power supply device for the laser, turn the key switch clockwise to turn on it. When the three-laser unit is used, turn on another power supply device, too.

The laser light turns on in approximately 30 seconds, and the laser output stabilizes in approximately 15 minutes.

* For the He-Ne laser



Caution

Be sure to read the instruction manual supplied with the lasers.

3 ND filter setting

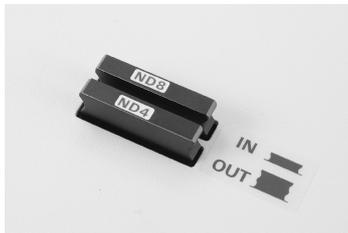


Figure 3.3-4 ND filter

To prevent the specimen from degrading with the intense laser light, place an ND filter (ND4 or ND8) into the optical path before exposing laser light.

3.3.2 Turning on the Power Switches for Other Devices

Turn on the devices such as the microscope, the optical fiber light source, the shutter control unit, and the camera.

3.4 Epi-fl Microscopy (Focusing)

Before performing TIRF microscopy, find out the object with epi-fl microscopy, and then bring the object into focus.

1 Optical path settings

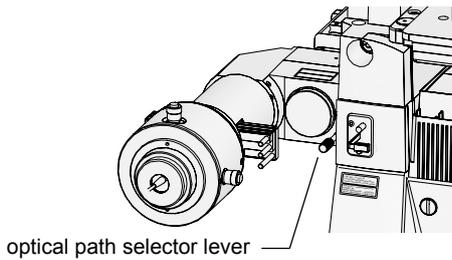


Figure 3.4-1

1. Set up the epi-fl microscopy.

For the TIRF/epi-fl illuminator unit:
Push in the optical path selector lever.
(See Figure 3.4-1.)

For the motorized illuminator unit:
Press the TIRF/EPI optical path selector button on the remote control pad to select the epi illumination. (See Figure 3.4-2.)



Figure 3.4-2

TIRF / EPI button

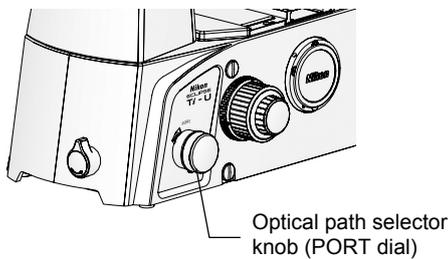


Figure 3.4-3

2. Set up the optical path of the microscope to "Observation 100%."

Ti-U, Ti-U/B: Use the optical path selector knob on the microscope. (See Figure 3.4-3.)

Ti-E, Ti-E/B: Use the optical path switch selector switch on the microscope body or the Optical Path (PATH) button on the remote control pad. (See Figure 3.4-4 and 3.4-5.)

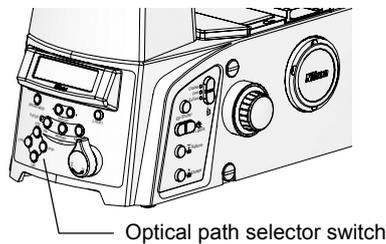


Figure 3.4-4



Figure 3.4-5

Optical path (PATH) button

2 Selecting an excitation method.

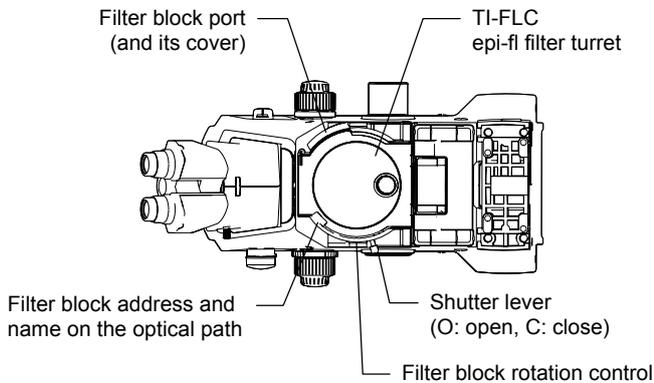


Figure 3.4-6

Rotate the filter block rotation control on the epi-fl filter turret to place the filter block and push/pull the excitation filter slider on the illuminator to place the excitation filter to perform the desired excitation method.

3 Opening the shutter for the epi illumination

Rotate the filter block rotation control on the epi-fl filter turret to place the filter block and push/pull the excitation filter slider on the illuminator to place the excitation filter to perform the desired excitation method.

4 Focusing

Place the specimen on the stage. Rotate the focus knob of the microscope to focus on the specimen.

5 Centering and adjusting the field diaphragm

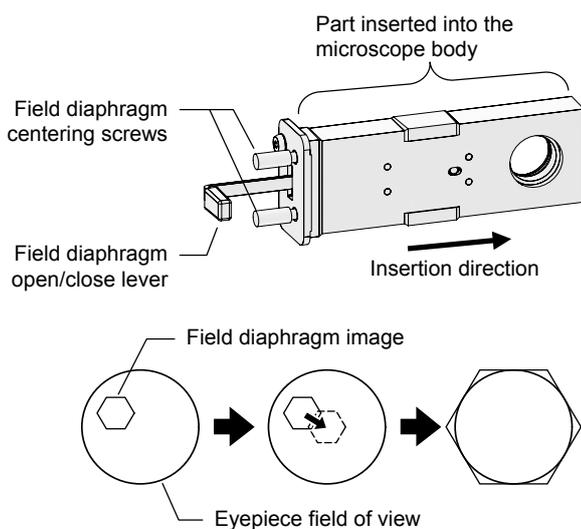


Figure 3.4-7

1. **Reduce the field diaphragm opening.**
Push in the field diaphragm open/close lever.
2. **Move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.
3. **Expand the field diaphragm opening as wide as the field of view.**
Adjust the field diaphragm open/close lever.
4. **Once again, move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.
5. **When the field diaphragm image comes to the center, adjust the field diaphragm open/close lever so that the diaphragm image circumscribes the field of view.**

6 Brightness adjustment with ND filters

Table 3.4-1

ND4	ND8	Brightness
OUT	OUT	1
IN	OUT	1/4
OUT	IN	1/8
IN	IN	1/32

IN: Place the component into the optical path.

OUT: Remove the component from the optical path.

When the ND filter slider, which is located near the lamphouse, is pushed into backward, an ND filter is placed in the optical path, thus reducing the brightness of the image.

7 Closing the shutter for the epi illumination

In order to prevent fading of the specimen, always close the shutter in the epi-fl filter turret when not actually performing the epi-fl microscopy.

Reference Simple Surface Reflection Interference Microscopy**~ Condition Check on the Contact of the Specimen and the Cover Glass ~**

In theory, it is possible to make high-contrast observations of areas in contact with the cover glass under the TIRF illumination. In practice, it is difficult to determine from images under the TIRF illumination alone whether specimens such as cell cultures are actually in contact with the cover glass. Simple surface reflection interference microscopy with the TIRF/epi-fl illuminator unit easily allow the observer to determine which parts of the specimen are in contact with the cover glass, because of the presence of interference fringes.

If an air gap exists between the cover glass and the specimen (such as cell-culture cell membrane), interference fringes caused by light reflected from the surfaces can be observed with the following optical settings. Before performing TIRF microscopy, find out the object with epi-fl microscopy, and then bring the object into focus.

Settings

1. Place the filter block, which has settings of “G excitation filter”, “UV dichroic mirror”, and “No barrier filter”, into the optical path.
2. Reduce the opening of the aperture diaphragm to a smallest size.
3. Adjust the field diaphragm opening to a smallest size for the observation.

Explanation

With these settings, interference fringe components in the light from the specimen image will be increased by the following effects.

- Using the G excitation restricts the interference fringe wavelengths and allows observations with more highly visible wavelengths.
- The characteristics of the UV dichroic mirror ensure high transmissivity with low reflectivity of visible light, giving an image in which interference fringe intensity is not reduced.
- Reducing the aperture diaphragm opening enables illumination by near-parallel light rays. If the aperture is matched to the objective N.A., illumination angles of incidence will be large corresponding to the N.A.. The resulting wide range of optical path lengths will reduce interference fringe contrast.
- Reducing the field-diaphragm aperture blocks unnecessary light, increasing image contrast.

3.5 TIRF Microscopy

3.5.1 TIRF Microscopy

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

When the laser is moved to the edge of the pupil, the laser ray tilts. When the angle becomes the critical angle or larger relative to the specimen, the illumination becomes a total reflection condition.

In contrast, if the laser rays are not parallel rays, non-parallel rays will pass through the cover glass even when the angle of the laser optical axis becomes the critical angle or larger. It can cause degrade image quality and damage the specimen. Therefore, the laser focal position must be adjusted each time the objective is changed.

In TIRF microscopy, images are captured by a TV camera (video camera) and observed on the monitor.

1 Optical path settings

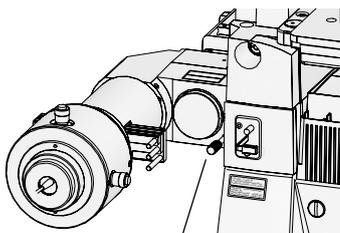


Figure 3.5-1

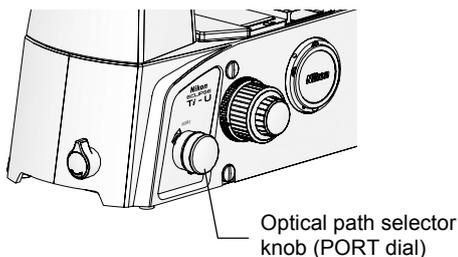


Figure 3.5-2

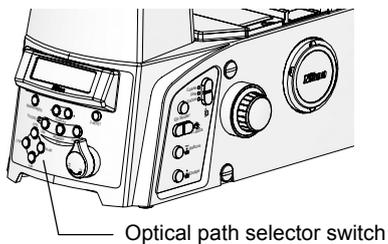


Figure 3.5-3

1. Set up for TIRF microscopy.

Pull the optical path selector lever on the TIRF/epi-fl illuminator unit.

2. Select the optical path of the microscope to the camera port.

Ti-U, Ti-U/B: Use the optical path selector knob on the microscope. (See Figure 3.5-2.)

Ti-E, Ti-E/B: Use the optical path switch selector switch on the microscope body or the Optical Path (PATH) button on the remote control pad. (See Figure 3.5-3 and 3.5-4.)



Figure 3.5-4

NOTE: The laser shutter does not open when the binocular route of the optical path is selected.

2 Selecting an excitation method

Rotate the filter block rotation control on the epi-filter turret to insert the filter block of the desired excitation method for the specimen into the optical path.

3 Open the shutter for the laser.

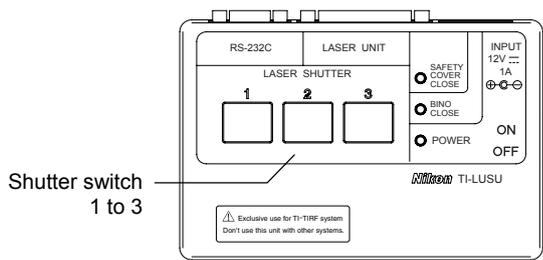


Figure 3.5-5

Press the shutter button on the shutter control unit to open the desired optical path of the laser.

4 Collimate the laser beam (perform each time the objective is changed).



Warning

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the focal point adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

Never look into the objective during this work.
It is dangerous because accidental exposure of eyes or skin to the laser beam may occur.

3.5 TIRF Microscopy

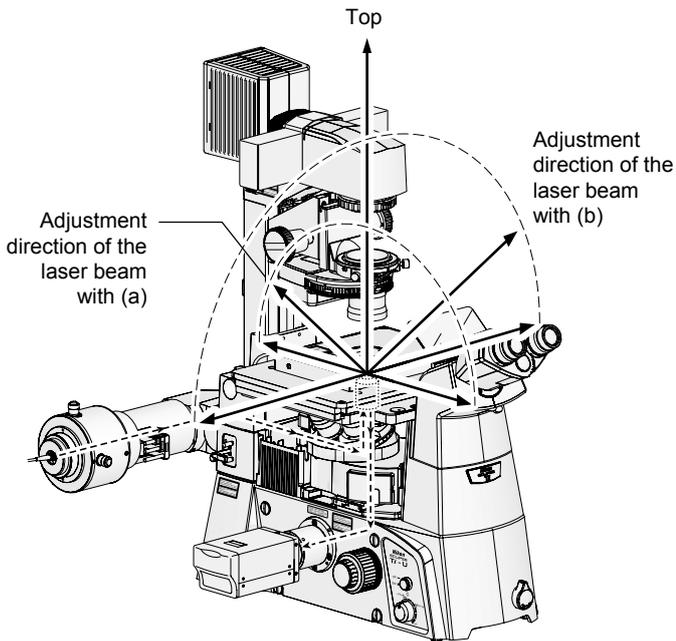


Figure 3.5-6

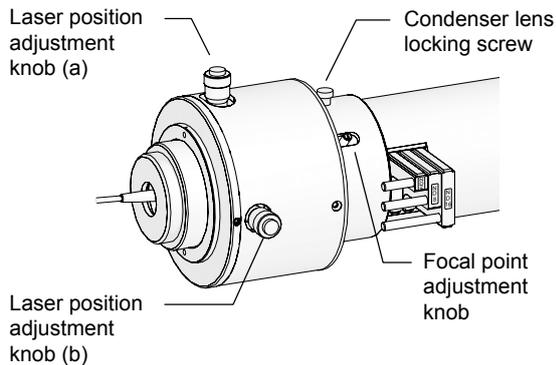


Figure 3.5-7

1. **Turn the laser position adjustment knob to move the laser beam just above the objective.**

For future reference, note the corresponding tick mark on the laser position adjustment knob at which the laser beam passes straight through the objective. Return the knob to this position to ensure that the beam is directed properly.

2. **Loosen the condenser lens locking screw.**
3. **Move the focal point adjustment knob back and forth to focus the laser on the objective pupil plane.**

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

4. **When adjustment is complete, tighten the condenser lens locking screw.**
5. **Adjust the laser position to provide TIRF illumination.**

Turn the laser position adjustment knob to make the laser beam focus on the edge of the pupil plane, thus providing TIRF illumination.

When viewed from the front of the microscope,

- To move the focal point to forward or backward, turn the laser position adjustment knob (a) (knob located in vertical direction).
- To move the focal point to left or right, turn the laser position adjustment knob (b) (knob located in horizontal direction).

3.5.2 Motorized TIRF Microscopy

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

When the laser is moved to the edge of the pupil, the laser ray tilts. When the angle becomes the critical angle or larger relative to the specimen, the illumination becomes a total reflection condition.

In contrast, if the laser rays are not parallel rays, non-parallel rays will pass through the cover glass even when the angle of the laser optical axis becomes the critical angle or larger. It can cause degrade image quality and damage the specimen. Therefore, the laser focal position must be adjusted each time the objective is changed.

In TIRF microscopy, images are captured by a TV camera (video camera) and observed on the monitor.

1 Optical path settings



Figure 3.5-8

TIRF / EPI button

1. Press the TIRF/EPI optical path selector button on the remote control pad to select the TIRF illumination.



Figure 3.5-9

Optical path (PATH) button

2. Press the Optical path button on the remote control pad to select the camera port.

NOTE: The laser shutter does not open when the binocular route of the optical path is selected.

2 Selecting an excitation method

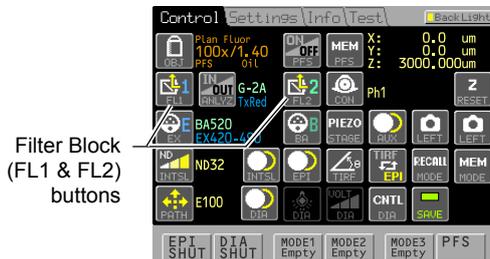


Figure 3.5-10

Press the Filter Block button on the remote control pad to place the specific high-precision filter cube suitable for the specimen into the optical path.

3 Open the shutter for the laser.

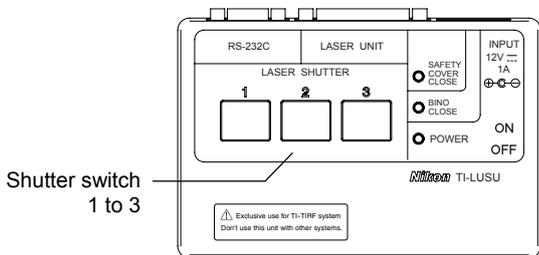


Figure 3.5-11

Press the shutter button on the shutter control unit to open the desired optical path of the laser.

4 Collimate the laser beam (perform each time the objective is changed).

Warning

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the focal point adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

**Never look into the objective during this work.
It is dangerous because accidental exposure of eyes or skin to the laser beam may occur.**

3.5 TIRF Microscopy

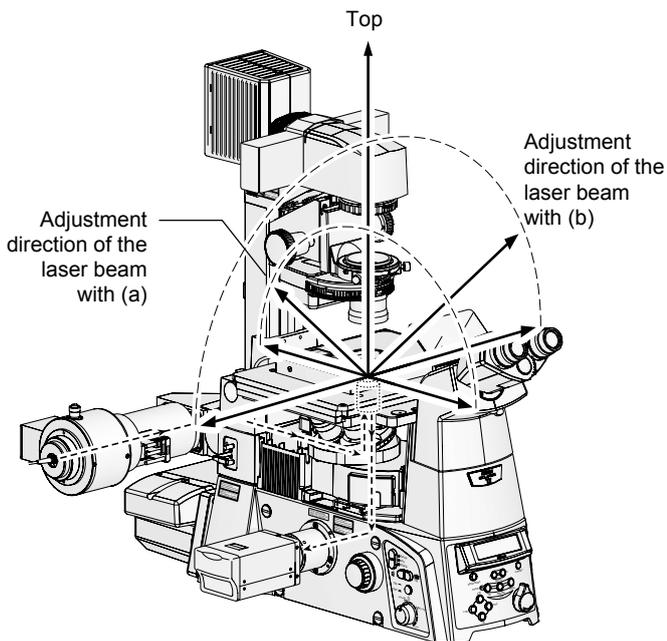


Figure 3.5-12

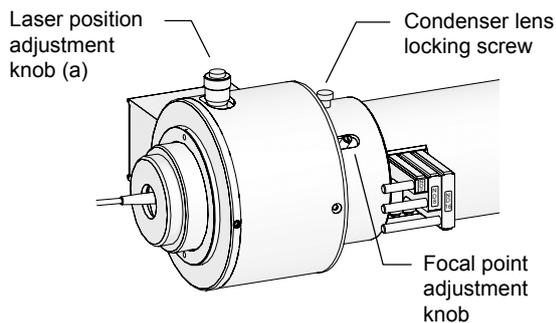


Figure 3.5-13

1. **Press the laser position adjustment button to move the laser beam just above the objective.**

For future reference, keep the record of the laser position value displayed on the remote control pad at which the laser beam passes straight through the objective.

2. **Loosen the condenser lens locking screw.**
3. **Move the focal point adjustment knob back and forth to focus the laser on the objective pupil plane.**

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

4. **When adjustment is complete, tighten the condenser lens locking screw.**

5. **Adjust the laser position to provide TIRF illumination.**

Press the laser position adjustment button to make the laser beam focus on the edge of the pupil plane, thus providing TIRF illumination.

When viewed from the front of the microscope,

- To move the focal point to forward or backward, turn the laser position adjustment knob (a) (knob located in vertical direction).
- To move the focal point to left or right, press the laser position adjustment (TIRF Angle) button (b).



TIRF Angle button (b)

Figure 3.5-14

3.6 Termination Procedure

First of all, turn off all laser devices.

1. Turn off the laser devices.

Refer to the instruction manual supplied with the lasers. Turn off all laser devices. And then, unplug the power cords from the wall outlets.

- **For the air-cooled Ar laser**

Switch off the key switch on the remote controller, and leave it for approximately 15 minutes to sufficiently cool down the laser head. After checking that the laser is sufficiently cool, turn off the key switch and the power switch, which are on the power supply device.

2. Turn off the optical fiber light source.

The light source stays very hot because of lighting. If you want to put a cover on the lamp, be sure to wait at least 30 minutes after the lamp goes off.

3. Turn off other devices.

Turn off the microscope and the shutter control unit, and then, unplug the power cords.

4

Operation (Detailed Explanation)

4.1 TIRF/Epi-fl Illuminator Unit

The TIRF/epi-fl illuminator unit is the main optical unit of this system. Excitation light from a laser device is guided into this unit through an optical-fiber cable. Excitation light emitted from the TIRF/epi-fl illuminator unit forms an image on the objective pupil plane.

Besides, TIRF (100%) and TIRF (60%) - Epi-fl (40%) are selectable with the optical path selector lever.



Caution

To protect your eyes and skin from exposure to the laser, do not detach the TIRF/epi-fl illuminator unit from the microscope.

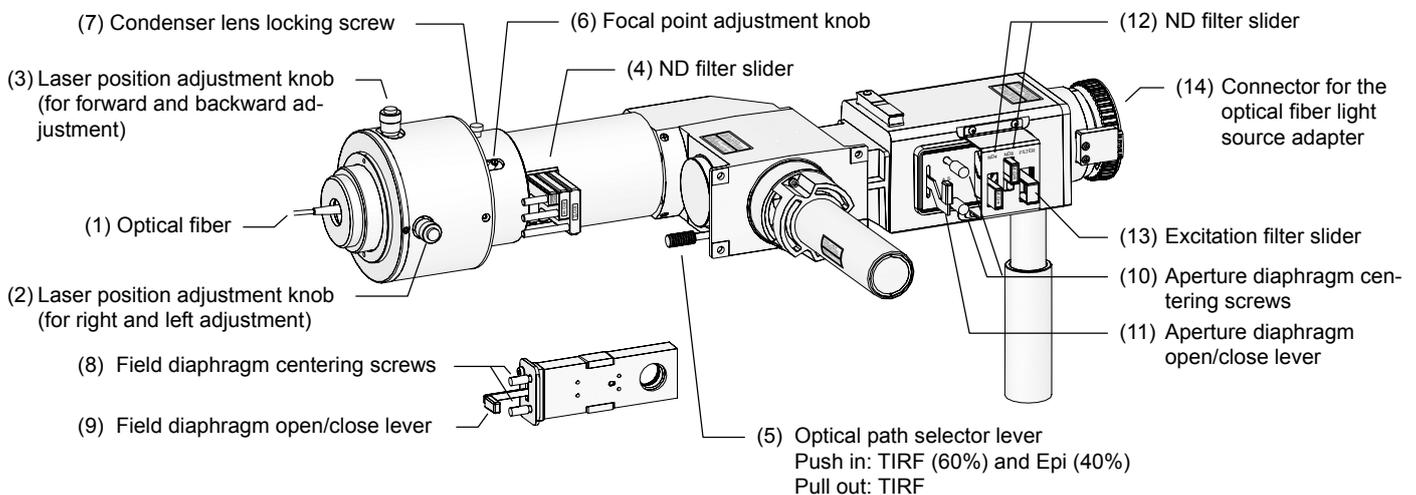


Figure 4.1-1

Parts used in the TIRF microscopy operation

- (1) Optical fiber
- (2) Laser position adjustment knob (for right and left adjustment)
- (3) Laser position adjustment knob (for forward and backward adjustment)
- (4) ND filter slider
- (5) Optical path selector lever
Push in: TIRF (60%) and Epi (40%)
Pull out: TIRF
- (6) Focal point adjustment knob
- (7) Condenser lens locking screw

Parts used in the epi-fl microscopy operation

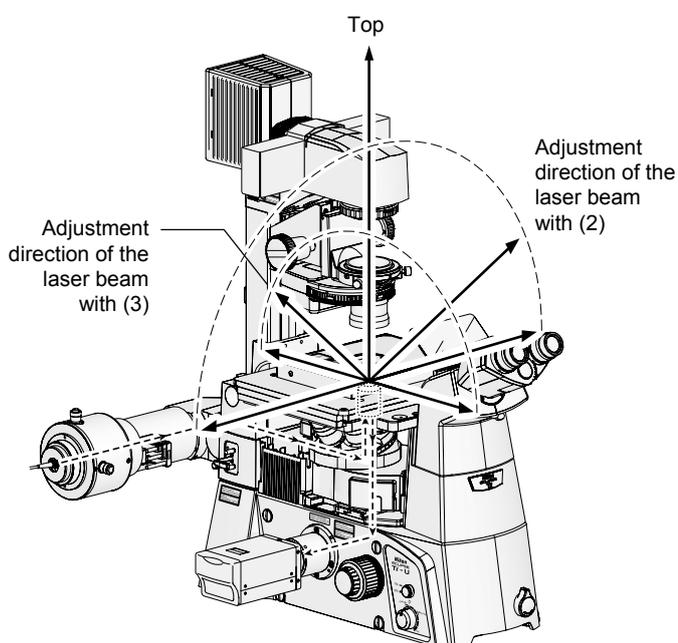
- (8) Field diaphragm centering screws
- (9) Field diaphragm open/close lever
- (10) Aperture diaphragm centering screws
- (11) Aperture diaphragm open/close lever
- (12) ND filter slider
- (13) Excitation filter slider
- (14) Connector for the optical fiber light source adapter

(1) Optical fiber

The optical fiber conducts the laser beam from the laser unit to the TIRF/e-pi-fl illuminator unit

**Caution**

- To maintain the system performance and to protect your eyes and skin from exposure to the laser, do not detach the optical fiber from the TIRF/e-pi-fl illuminator unit.
- To prevent damage to the optical fiber, do not bend the optical fiber cable or pull it with excessive force.

(2) Laser position adjustment knob (for right and left adjustment)**(3) Laser position adjustment knob (for forward and backward adjustment)**

The horizontally-mounted knob is rotated to move the laser beam focal point across the objective pupil plane. The focal point moves right and left as you face the microscope. The vertically-mounted knob is rotated to move the laser beam back and forth. As the laser beam moves on the objective pupil plane, the light projected from the objective changes as follows:

- The light is passed straight through upward when the laser beam strikes the center of the objective.
- The angle of the laser beam tilts gradually when the beam moves toward the edge of the pupil plane.

For future reference, note the corresponding tick mark on the laser position adjustment knob at which the laser beam passes straight through the objective. Return the knob to this position to ensure that the beam is directed properly.

Figure 4.1-2

**Warning**

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the laser position adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

(4) ND filter slider

Table 4.1-1

ND2	ND8	ND16	Brightness
OUT	OUT	OUT	1
IN	OUT	OUT	1/2
OUT	IN	OUT	1/8
OUT	OUT	IN	1/16
IN	IN	OUT	
IN	OUT	IN	1/32
OUT	IN	IN	1/128
IN	IN	IN	1/256

IN: Place the component into the optical path.

OUT: Remove the component from the optical path.

These sliders are used to place ND filters into the optical path of the laser light. To set an ND filter, push in the slider. To set an opening, pull the slider. When using strong fluorescent light, or when a specimen is badly faded, adjust the brightness by pushing in an ND filter slider to place an ND filter into the optical path. (If the fluorescent light is too strong, the contrast becomes worse.)

The table on the left shows the brightness adjustment with ND filter combinations.

(5) Optical path selector lever

Operate the lever to change the optical path between the TIRF illumination and the epi-fl illumination.

- **Push in:** TIRF (60%) and epi (40%)
- **Pull out:** TIRF (100%)

(6) Focal point adjustment knob**(7) Condenser lens locking screw**

The objective optical axis direction focal point of the laser light can be changed by moving the focal point adjustment knob to and from.

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays. If the focal point is shifted from the pupil plane, the laser rays are not parallel rays. Therefore, even when the angle of the laser optical axis becomes the critical angle or larger, non-parallel rays will pass through the cover glass. It can cause degrade image quality and damage the specimen.

Adjust the focal point of the laser light each time when objectives are switched.

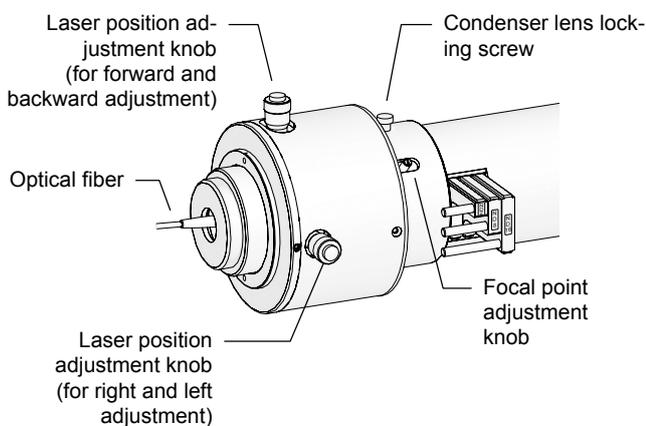


Figure 4.1-3

Focal point adjustment

1. **Turn the laser position adjustment knob to move the laser beam just above the objective.**
2. **Loosen the condenser lens locking screw.**
3. **Move the focal point adjustment knob back and forth to focus the laser on the objective pupil plane.**

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

4. **When adjustment is complete, tighten the condenser lens locking screw.**

**Warning**

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the laser position adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

(8) (9) Field diaphragm (field diaphragm centering screws, field diaphragm open/close lever)

The field diaphragm can be adjusted with the field diaphragm centering screws and the field diaphragm open/close lever. The field diaphragm restricts the illuminated area on the specimen to an observation area. Operating the field diaphragm open/close lever changes the size of the field diaphragm.

Usually the opening of the field diaphragm is reduced to a size that circum-scribes (or inscribes) the field of view. If a broader area than necessary is illuminated, stray light will enter the optical system, creating flaring, reducing the contrast of the optical image, and expanding the area of color fading of the specimen.

The operation of the field diaphragm is especially important for video cameras and digital cameras. Generally, reduce the opening to an area that is slight larger than the picture composition frame, that is, the image capture area. The field diaphragm of this system needs to be centered before usage.

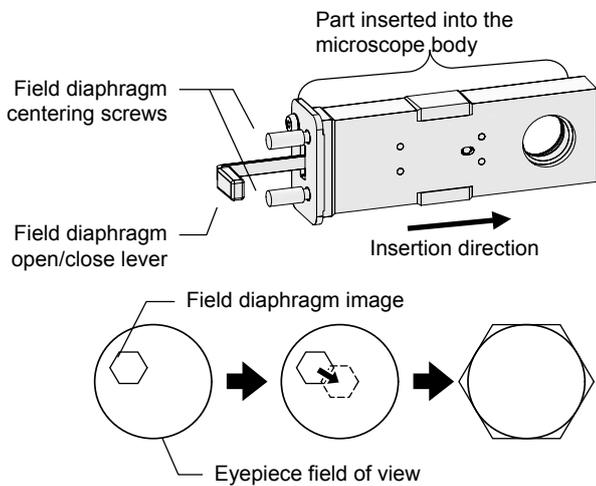


Figure 4.1-4

Centering the field diaphragm

1. **Perform steps 1 to 5 in Section 3, “Epi-fl Microscopy” in Chapter 3, “Detailed Microscopy Procedure.”**
2. **Reduce the field diaphragm opening.**
Push in the field diaphragm open/close lever.
3. **Move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.
4. **Expand the field diaphragm opening as wide as the field of view.**
Adjust the field diaphragm open/close lever.
5. **Once again, move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.

(10) (11) Aperture diaphragm (aperture diaphragm centering screws, aperture diaphragm open/close lever)

The aperture diaphragm can be adjusted with the aperture diaphragm centering screws and the aperture diaphragm open/close lever.

The aperture diaphragm is used to adjust the numerical aperture of the optical system of an illumination. When using an epi-fl illumination optical system, the aperture diaphragm functions to adjust the brightness of the image and the size of stray light.

When the aperture diaphragm size is reduced, the amount of stray light decreases but the image of the specimen also becomes darker. When the aperture diaphragm size is increased, the image becomes brighter but the stray light increases. It is possible to effectively eliminate stray light and secure a bright specimen image by reducing the aperture diaphragm size to an area equivalent to the diameter of the objective pupil.

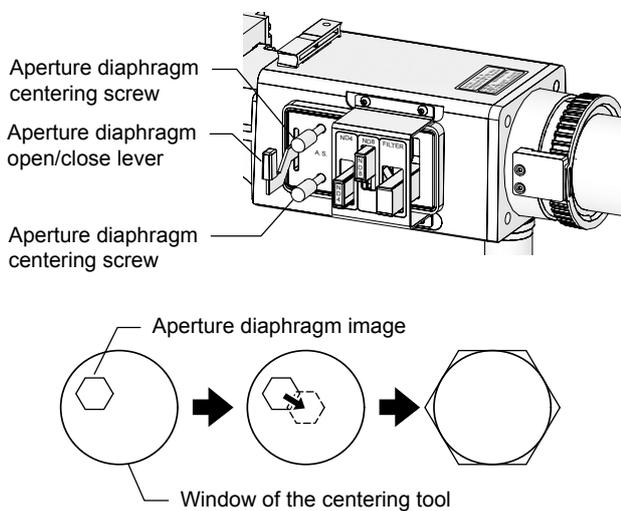


Figure 4.1-5

Centering the aperture diaphragm

1. **Perform steps 1 to 5 in Section 3, “Epi-fl Microscopy” in Chapter 3, “Detailed Microscopy Procedure.”**
2. **Reduce the aperture diaphragm opening.**
Push in the aperture diaphragm open/close lever.
3. **Move the center of the aperture diaphragm image to the center of the window of the centering tool.**
Adjust the aperture diaphragm centering screws.
4. **Expand the aperture diaphragm opening as wide as the window of the centering tool.**
Adjust the aperture diaphragm open/close lever.
5. **Once again, move the center of the aperture diaphragm image to the center of the window of the centering tool.**
Adjust the aperture diaphragm centering screws.

(12) ND filter slider

Table 4.1-2

ND4	ND8	Brightness
OUT	OUT	1
IN	OUT	1/4
OUT	IN	1/8
IN	IN	1/32

IN: Place the component into the optical path.
OUT: Remove the component from the optical path.

These sliders are used to place ND filters into the optical path of the episcopic illumination. When using strong fluorescent light, or when a specimen is badly faded, adjust the brightness by pushing in an ND filter slider to place an ND filter into the optical path. (If the fluorescent light is too strong, the contrast becomes worse.)

The table on the left shows the brightness adjustment with ND filter combinations.

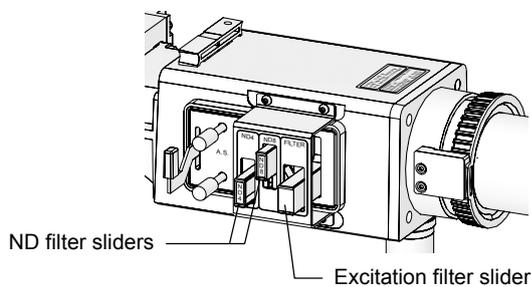
(13) Excitation filter slider

Figure 4.1-6

Up to three filters of 25 mm in diameter and 5.5 mm in maximum thickness can be attached to the excitation filter slider and can be selected handily.

Thanks to the excitation filter location, not on the filter block but on the slider, the laser light does not pass through the excitation filter for TIRF microscopy.

**Caution**

Whenever using an excitation filter on the excitation filter slider to perform microscopy, be sure to check the barrier filter settings.

If the combination of the excitation filter and the barrier filter is not correct, very strong light may pass through. Great care should be taken about filter combinations.

(14) Connector for the optical fiber light source adapter

The optical fiber light source is attached here.

4.2 Motorized Illuminator Unit

4.2.1 Motorized Illuminator Unit

The motorized illuminator unit is the main optical unit of this system. Excitation light from a laser device is guided into this illuminator unit through an optical-fiber cable. Excitation light emitted from this illuminator unit forms an image on the objective pupil plane.



Caution

To protect your eyes and skin from exposure to the laser, do not detach the motorized illuminator unit from the microscope.

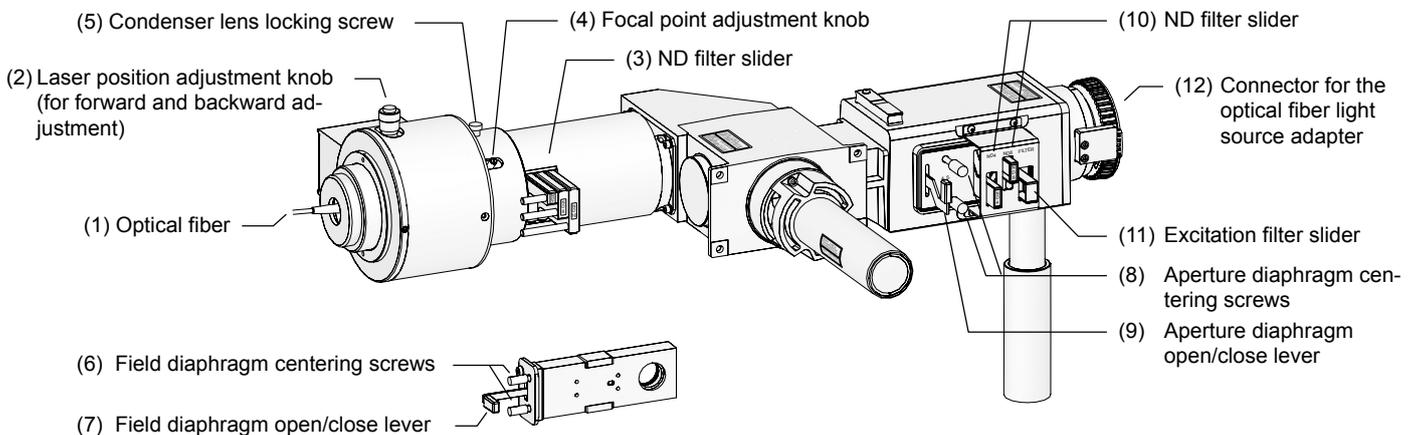


Figure 4.2-1

Parts used in the TIRF microscopy operation

- (1) Optical fiber
- (2) Laser position adjustment knob (for forward and backward adjustment *)
- (3) ND filter slider
- (4) Focal point adjustment knob
- (5) Condenser lens locking screw

Parts used in the epi-fl microscopy operation

- (6) Field diaphragm centering screws
- (7) Field diaphragm open/close lever
- (8) Aperture diaphragm centering screws
- (9) Aperture diaphragm open/close lever
- (10) ND filter slider
- (11) Excitation filter slider
- (12) Connector for the optical fiber light source adapter

(1) Optical fiber

The optical fiber conducts the laser beam from the laser unit to the TIRF/epi-fl illuminator unit

**Caution**

- To maintain the system performance and to protect your eyes and skin from exposure to the laser, do not detach the optical fiber from the TIRF/epi-fl illuminator unit.
- To prevent damage to the optical fiber, do not bend the optical fiber cable or pull it with excessive force.

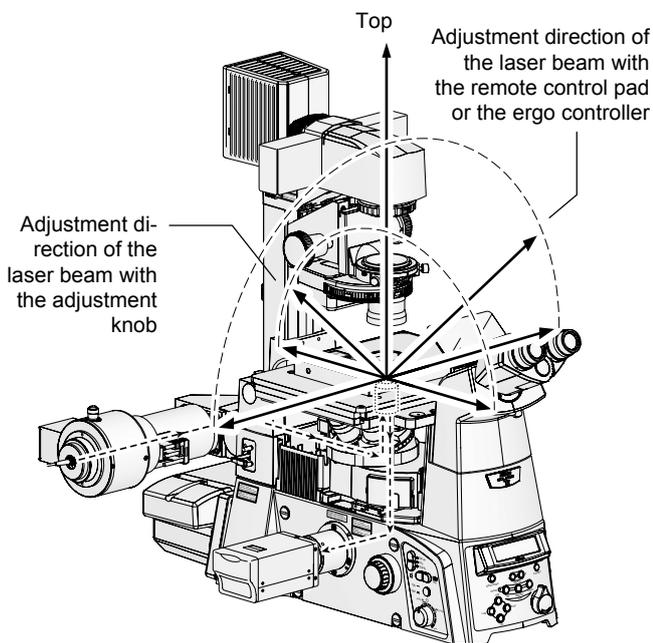
(2) Laser position adjustment knob (for forward and backward adjustment)

Figure 4.2-2

The laser position adjustment knob is rotated to move the laser beam back and forth. As the laser beam moves on the objective pupil plane, the light projected from the objective changes as follows:

- The light is passed straight through upward when the laser beam strikes the center of the objective.
- The angle of the laser beam tilts gradually when the beam moves toward the edge of the pupil plane.

For future reference, note the corresponding tick mark on the laser position adjustment knob at which the laser beam passes straight through the objective. Return the knob to this position to ensure that the beam is directed properly.

To use TI-TIRF-E in combination with Ti-U or Ti-U/B

To use the motorized TI-TIRF-E in combination with Ti-U or Ti-U/B, the TI-HUBC/B HUB controller B must be attached to the rear side of the microscope.

In such cases, the ergo controller allows users to change the optical path between TIRF illumination and episcopic illumination as well as adjust the laser right or left (TIRF illumination angle adjustment).

**Warning**

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the laser position adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

(3) ND filter slider

Table 4.2-1

ND2	ND8	ND16	Brightness
OUT	OUT	OUT	1
IN	OUT	OUT	1/2
OUT	IN	OUT	1/8
OUT	OUT	IN	1/16
IN	IN	OUT	
IN	OUT	IN	1/32
OUT	IN	IN	1/128
IN	IN	IN	1/256

IN: Place the component into the optical path.
OUT: Remove the component from the optical path.

These sliders are used to place ND filters into the optical path of the laser light. To set an ND filter, push in the slider. To set an opening, pull the slider. When using strong fluorescent light, or when a specimen is badly faded, adjust the brightness by pushing in an ND filter slider to place an ND filter into the optical path. (If the fluorescent light is too strong, the contrast becomes worse.)

The table on the left shows the brightness adjustment with ND filter combinations.

(4) Focal point adjustment knob**(5) Condenser lens locking screw**

The objective optical axis direction focal point of the laser light can be changed by moving the focal point adjustment knob to and from.

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays. If the focal point is shifted from the pupil plane, the laser rays are not parallel rays. Therefore, even when the angle of the laser optical axis becomes the critical angle or larger, non-parallel rays will pass through the cover glass. It can cause degrade image quality and damage the specimen.

Adjust the focal point of the laser light each time when objectives are switched.

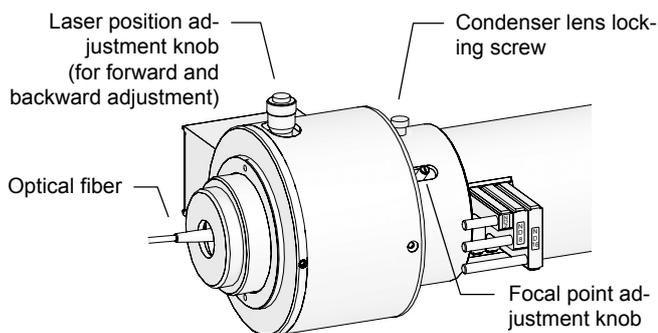


Figure 4.2-3

Focal point adjustment

1. Turn the laser position adjustment knob to move the laser beam just above the objective.
2. Loosen the condenser lens locking screw.
3. Move the focal point adjustment knob back and forth to focus the laser on the objective pupil plane.

When the laser beam is focused on the objective pupil plane, the laser beam projected from the objective forms parallel rays.

4. When adjustment is complete, tighten the condenser lens locking screw.

**Warning**

Never look into the laser beam. (FDA CDRH: Class IIIB, EN60825-1: Class 3B, IEC60825-1: Class 3B) When adjusting the laser position adjustment knob, never look into the objective and the areas near the objective from above or from the stage bottom because intense laser rays are projected upward and downward from the objective. And never place or mount any reflective materials on the stage, in the room, or on the ceiling of the room or the chamber to protect your eyes and skin from exposure to illumination light reflections from the objective.

(6) (7) Field diaphragm (field diaphragm centering screws, field diaphragm open/close lever)

The field diaphragm can be adjusted with the field diaphragm centering screws and the field diaphragm open/close lever.

The field diaphragm restricts the illuminated area on the specimen to an observation area. Operating the field diaphragm open/close lever changes the size of the field diaphragm. Usually the opening of the field diaphragm is reduced to a size that circumscribes (or inscribes) the field of view. If a broader area than necessary is illuminated, stray light will enter the optical system, creating flaring, reducing the contrast of the optical image, and expanding the area of color fading of the specimen.

The operation of the field diaphragm is especially important for video cameras and digital cameras. Generally, reduce the opening to an area that is slight larger than the picture composition frame, that is, the image capture area.

The field diaphragm of this system needs to be centered before usage.

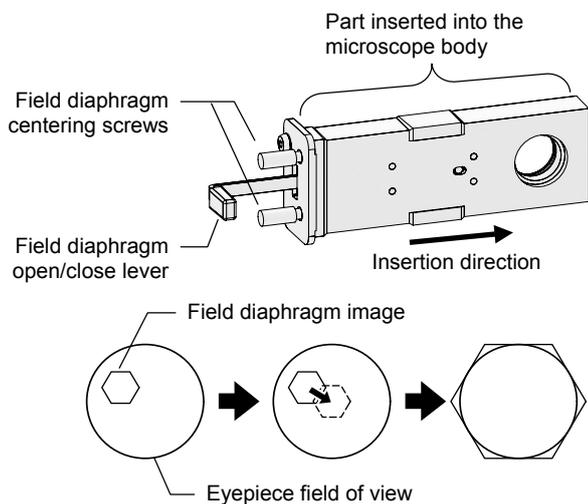


Figure 4.2-4

Centering the field diaphragm

1. **Perform steps 1 to 5 in Section 3, “Epi-fl Microscopy” in Chapter 3, “Detailed Microscopy Procedure.”**
2. **Reduce the field diaphragm opening.**
Push in the field diaphragm open/close lever.
3. **Move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.
4. **Expand the field diaphragm opening as wide as the field of view.**
Adjust the field diaphragm open/close lever.
5. **Once again, move the center of the field diaphragm image to the center of the field of view.**
Adjust the field diaphragm centering screws.

(8) (9) Aperture diaphragm (aperture diaphragm centering screws, aperture diaphragm open/close lever)

The aperture diaphragm can be adjusted with the aperture diaphragm centering screws and the aperture diaphragm open/close lever.

The aperture diaphragm is used to adjust the numerical aperture of the optical system of an illumination. When using an epi-fl illumination optical system, the aperture diaphragm functions to adjust the brightness of the image and the size of stray light.

When the aperture diaphragm size is reduced, the amount of stray light decreases but the image of the specimen also becomes darker. When the aperture diaphragm size is increased, the image becomes brighter but the stray light increases. It is possible to effectively eliminate stray light and secure a bright specimen image by reducing the aperture diaphragm size to an area equivalent to the diameter of the objective pupil.

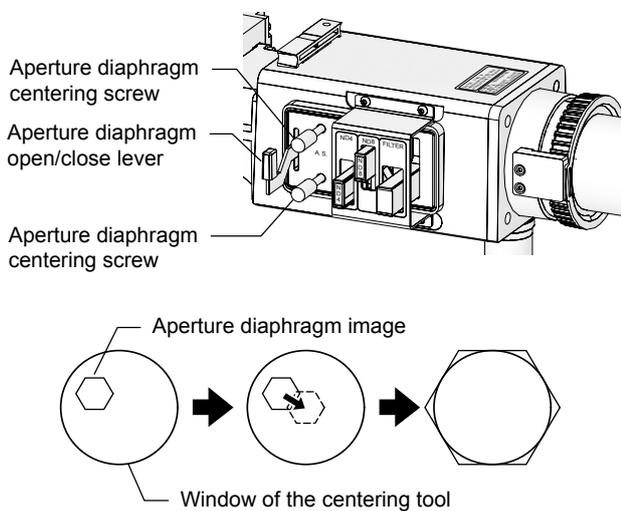


Figure 4.2-5

Centering the aperture diaphragm

1. **Perform steps 1 to 5 in Section 3, “Epi-fl Microscopy” in Chapter 3, “Detailed Microscopy Procedure.”**
2. **Reduce the aperture diaphragm opening.**
Push in the aperture diaphragm open/close lever.
3. **Move the center of the aperture diaphragm image to the center of the window of the centering tool.**
Adjust the aperture diaphragm centering screws.
4. **Expand the aperture diaphragm opening as wide as the window of the centering tool.**
Adjust the aperture diaphragm open/close lever.
5. **Once again, move the center of the aperture diaphragm image to the center of the window of the centering tool.**
Adjust the aperture diaphragm centering screws.

(10) ND filter slider

Table 4.2-2

ND4	ND8	Brightness
OUT	OUT	1
IN	OUT	1/4
OUT	IN	1/8
IN	IN	1/32

IN: Place the component into the optical path.
OUT: Remove the component from the optical path.

These sliders are used to place ND filters into the optical path of the episcopic illumination. When using strong fluorescent light, or when a specimen is badly faded, adjust the brightness by pushing in an ND filter slider to place an ND filter into the optical path. (If the fluorescent light is too strong, the contrast becomes worse.)

The table on the left shows the brightness adjustment with ND filter combinations.

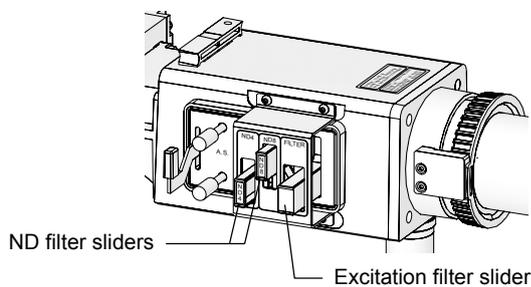
(11) Excitation filter slider

Figure 4.2-6

Up to three filters of 25mm in diameter and 5.5mm in maximum thickness can be attached to the excitation filter slider and can be selected handily.

Thanks to the excitation filter location, not on the filter block but on the slider, the laser light does not pass through the excitation filter for TIRF microscopy.

**Caution**

Whenever using an excitation filter on the excitation filter slider to perform microscopy, be sure to check the barrier filter settings.

If the combination of the excitation filter and the barrier filter is not correct, very strong light may pass through. Great care should be taken about filter combinations.

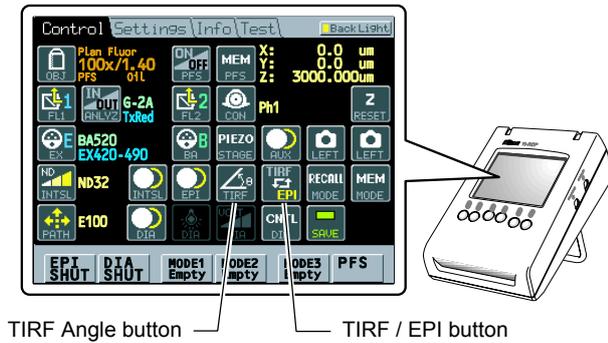
(12) Connector for the optical fiber light source adapter

The optical fiber light source is attached here

4.2.2 Remote Control Pad

With the remote control pad, the optical path can be changed between the TIRF (100%) and the TIRF (60%)-Epi-fl (40%), and the laser positions can be adjusted.

Switching the optical path between the TIRF illumination and the epi-fl illumination



TIRF/EPI button (Optical path selector button)

Operate the TIRF/EPI optical path selector button to change the optical path between the TIRF illumination and the epi-fl illumination.

- TIRF (60%) and Epi (40%)
- TIRF (100%)

Figure 4.2-7

Laser position adjustment (for right and left adjustment)



Figure 4.2-8

TIRF/Angle button (Laser position adjustment button)

When the laser position adjustment (**TIRF Angle**) button on the LCD touch panel (see Figure 4.2-7) is pressed, the second screen appears (see Figure 4.2-8), from which the position of the laser beam focal point on the objective pupil plane is controlled.

The laser illumination position can be moved right and left with the [+/-] buttons on the second screen on the LCD touch panel.

When using the TI-ERGC Ergo controller, when the [TI-ERGC] button on the second screen is ON, adjustment of the laser position can be performed using the laser position adjustment knob of the ergo controller.

As the laser beam moves on the objective pupil plane, the light projected from the objective changes as follows:

- The light is passed straight through upward when the laser beam strikes the center of the objective.
- The angle of the laser beam tilts gradually when the beam moves toward the edge of the pupil plane.

The laser positions are displayed on the remote control pad. For future reference, note the laser position at which the laser beam passed straight upward.

* For details on the laser adjustment procedure, refer to “(Reference) Laser Position Adjustment and Incident Light Angle Calculation” on the following page.

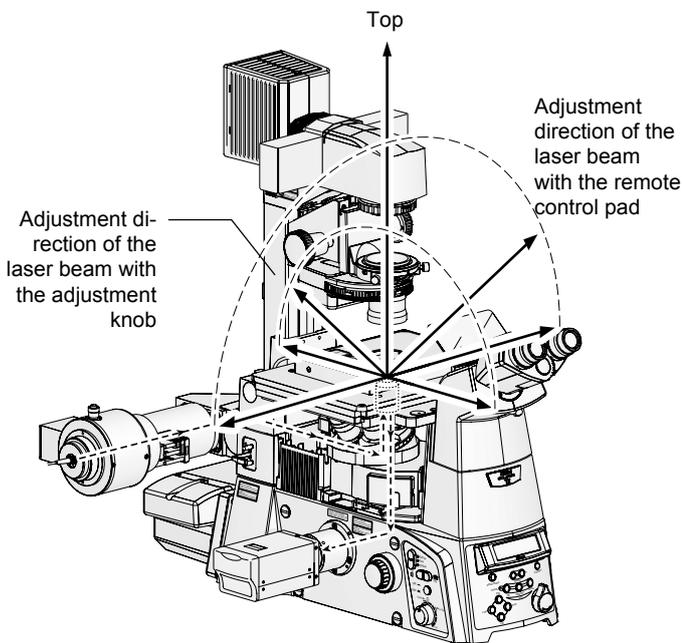


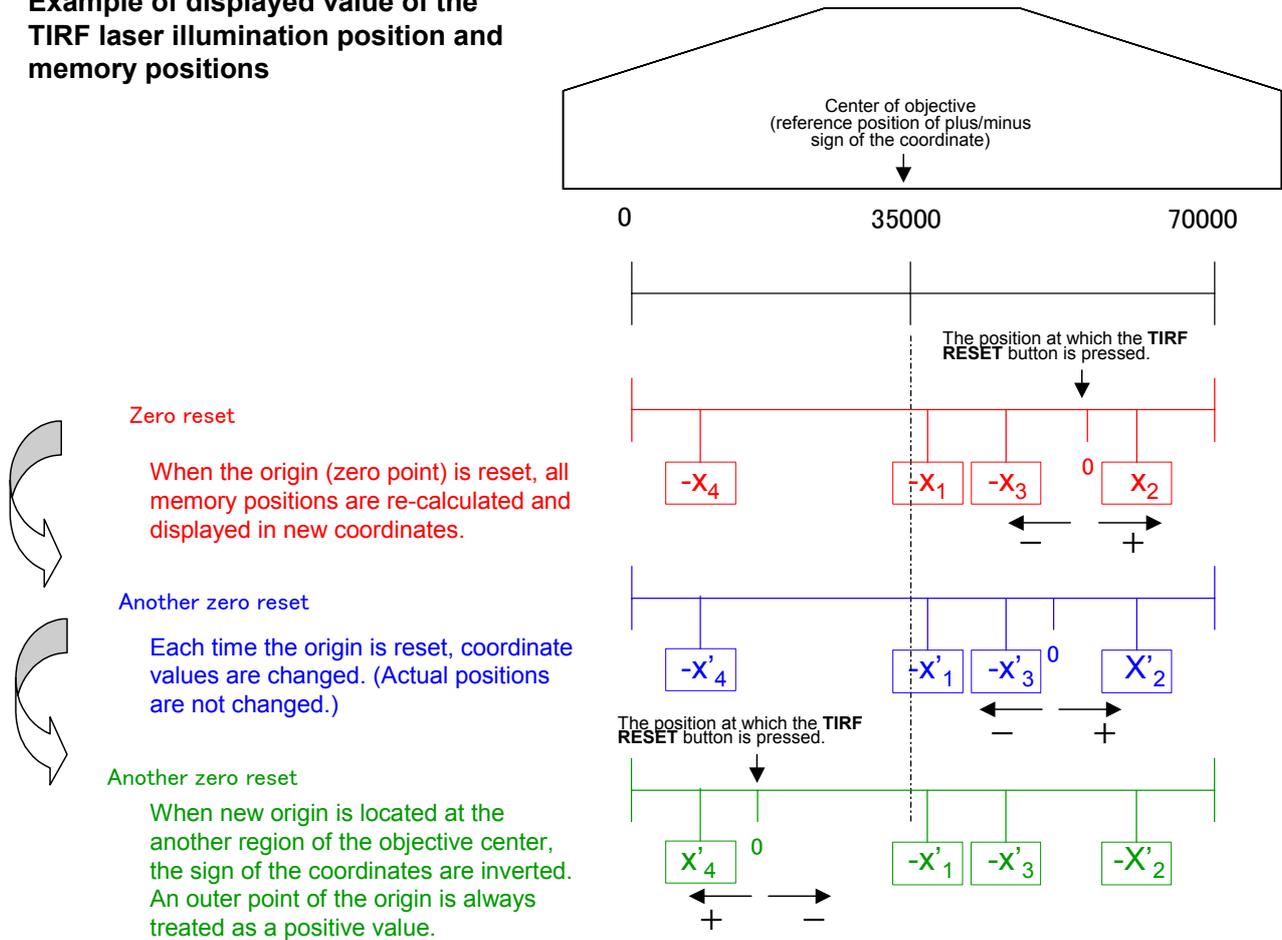
Figure 4.2-9

Reference Laser Position Adjustment and Incident Light Angle Calculation

Laser Position Adjustment

The value displayed on the second screen indicates the current position of the laser. (Same coordinates are used for all objectives.) Four laser illumination positions can be saved in memory. Besides, the origin (zero point) can be reset to any position with the [TIRF RESET] button. When the origin is reset, an outer point of the origin is treated as a positive value (the TIRF laser light incident angle increases) and an inner point of the origin is treated as a negative value (the TIRF laser light incident angle decreases). (See the figure below.)

Example of displayed value of the TIRF laser illumination position and memory positions



Zero reset

When the origin (zero point) is reset, all memory positions are re-calculated and displayed in new coordinates.

Another zero reset

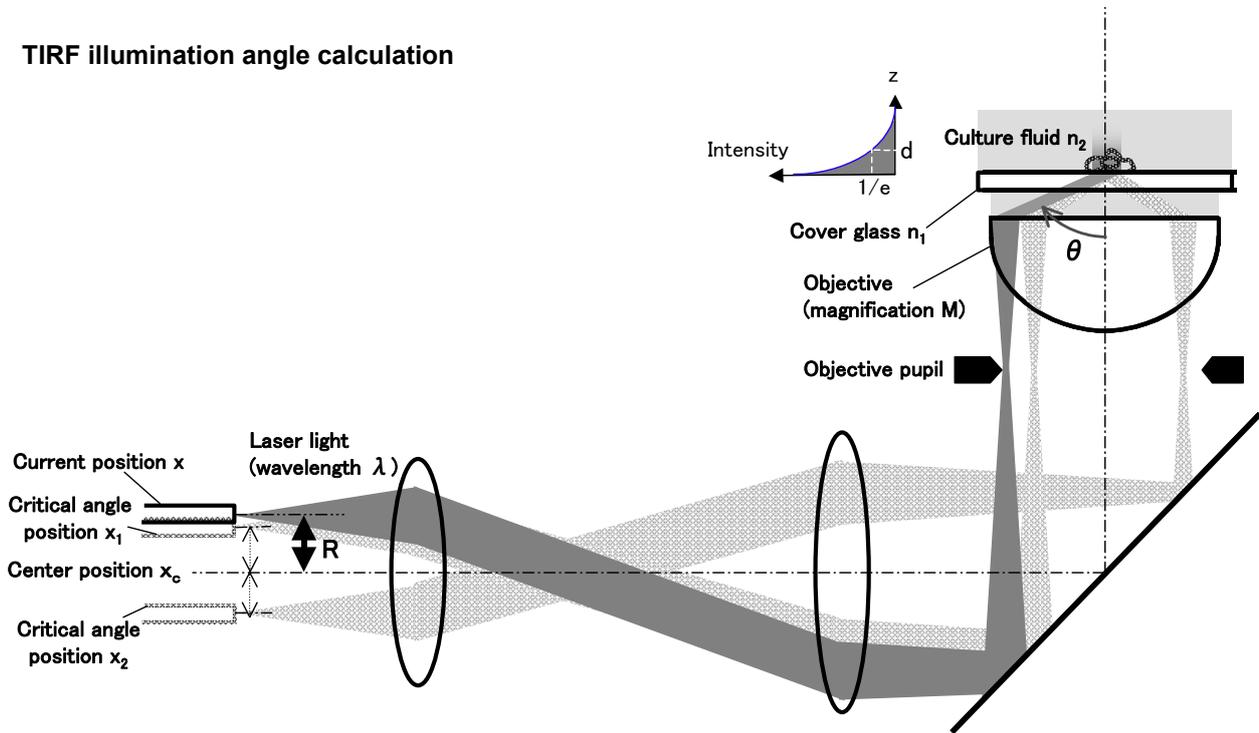
Each time the origin is reset, coordinate values are changed. (Actual positions are not changed.)

Another zero reset

When new origin is located at the another region of the objective center, the sign of the coordinates are inverted. An outer point of the origin is always treated as a positive value.

Incident light angle calculation

TIRF illumination angle calculation



The following is an example to obtain the laser light incident angle to the boundary surface between the specimen and the cover glass and to obtain the depth of light energy into the specimen.

(1) Incident angle

This section describes a simple method to detect the laser light position from the center of the optical axis.

1. Detect the center of the optical axis.
Move the laser illumination position from end to end to detect critical angle positions. (At a critical angle position, total reflection of light begins.) You can detect two critical angle positions, x_1 and x_2 . The center, x_c , of the optical axis can be obtained using the following formula (a).

Formula (a)

$$\text{Center: } x_c = (x_1 + x_2) / 2$$

2. Read the laser illumination position, x , you want to know the incident angle. When the distance of the laser illumination position from the center, x_c , is R [μm], they are expressed as the following formula. $R = x - x_c$
- Then, calculate the incident angle θ using the following formula (b): Reset the origin (zero point) at the center position, x_c . R can be read directly on the display.

Formula (b)

$$\sin\theta = 2 \times R \times M / (200000 \times n_1)$$

R : distance of the laser illumination position from the center x_c , [μm]

M : magnification of the objective

n_1 : refractive index of the cover glass

(Example: $n_1 = 1.522$ for white plate, wavelength 546 nm, and temperature 23°C)

Note:

- Actual refractive index varies depending on cover glass component, temperature, laser wavelength, and beam spot size. Besides, critical angle positions may have slight measurement errors. Therefore, the angle obtained in this step should be treated as a guideline value.
- The coefficient, 2, in the formula (b) is the optical coefficient from the laser outlet to the pupil. The coefficient, 200000, is the conversion factor to get a micron value for the second objective of $f = 200$ mm (focal length).

(2) Light energy into the specimen

The depth, d , of the light energy is expressed using the following formula (c). Where, the light energy is $1/e$. (The "e" is the base of the natural logarithm.)

Formula (c)

$$d = \lambda / \{ 4\pi \cdot [n_1^2 \times \sin^2\theta - n_2^2]^{1/2} \}$$

λ : laser wavelength

θ : incident light angle to the boundary surface between the cover glass and the culture fluid

where $\theta > \theta_c$ (θ_c is the critical angle, $\sin\theta_c = n_2 / n_1$)

n_1 : refractive index of the cover glass

n_2 : refractive index of the culture fluid

(Example: $n_2 = 1.333$ for pure water, wavelength 546 nm, and temperature 23°C)

Calculation example:

When n_1 is 1.522 and n_2 is 1.333, the critical angle θ_c is 61.1°. And, when a larger angle θ , 64°, is entered into the formula (3), d is 0.26λ . Therefore, when the laser wavelength λ is 488 nm, the depth is 127 nm.

4.2.3 Ergo Controller

When the motorized illuminator unit is attached on the Ti-U or Ti-U/B, laser position adjustment and optical path alteration are performed from the TI-ERGC Ergo controller. The Ergo controller is connected to the hub controller B that is mounted on the back of the microscope.

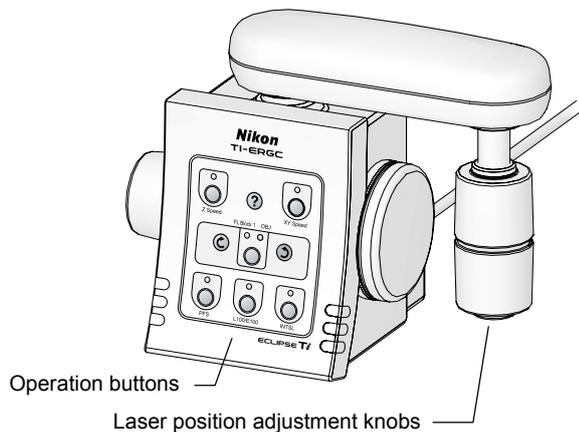


Figure 4.2-10

Optical path selector button

Any button on the ergo controller can be assigned for changing the optical path between the TIRF illumination and the epi-fl illumination.

- TIRF (60%) and Epi (40%)
- TIRF (100%)

Laser position adjustment (for right and left adjustment)

Turn the laser position adjustment knobs on the ergo controller to move the laser beam focal position on the objective pupil plane. These knobs move the laser beam right and left.

As the laser beam moves on the objective pupil plane, the light projected from the objective changes as follows:

- The light is passed straight through upward when the laser beam strikes the center of the objective.
- The angle of the laser beam tilts gradually when the beam moves toward the edge of the pupil plane.

Ergo controller operation

The above operation is allowed when the ergo controller button settings are in the default state (factory default). If the ergo controller button settings have already been changed, be sure to check the current settings.

4.3 Laser Safety Cover

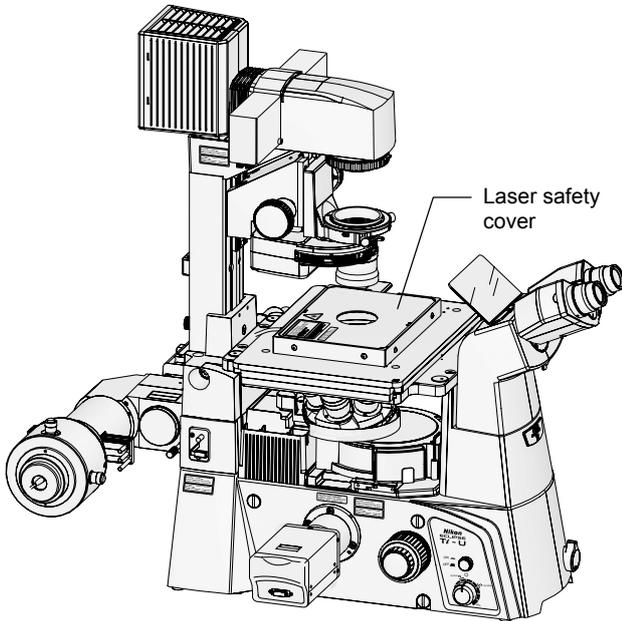


Figure 4.3-1

The laser safety cover is connected to the shutter control unit.

The upper plate of the laser safety cover is required to be attached securely to open the shutter of the laser unit.

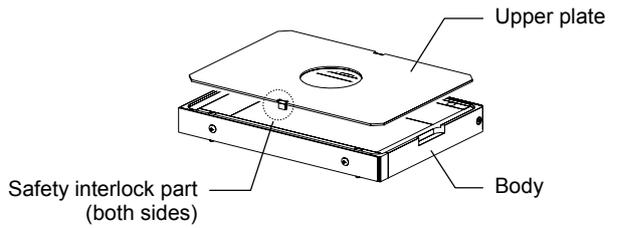


Figure 4.3-2

* If the safety interlock part is touched or gotten wet, misoperation may occur.

4.4 Shutter Control Unit (TI-LUSU)

The shutter control unit controls motorized shutters in the laser unit.

Although the unit can open or close up to three channels of motorized shutters, operation of motorized shutters in the factory default conditions is conducted through mutually exclusive control, as a result of which only one shutter can be opened at a time.

Confirm that the cables from the AC adapter, eyepiece tube optical path signal (microscope), laser safety cover, and the laser unit are connected to the proper positions.

* If you want to operate two or three shutters at a time, contact your nearest Nikon representative.



Caution

To prevent malfunctions, always turn off the system power before attaching or detaching any of the cables.

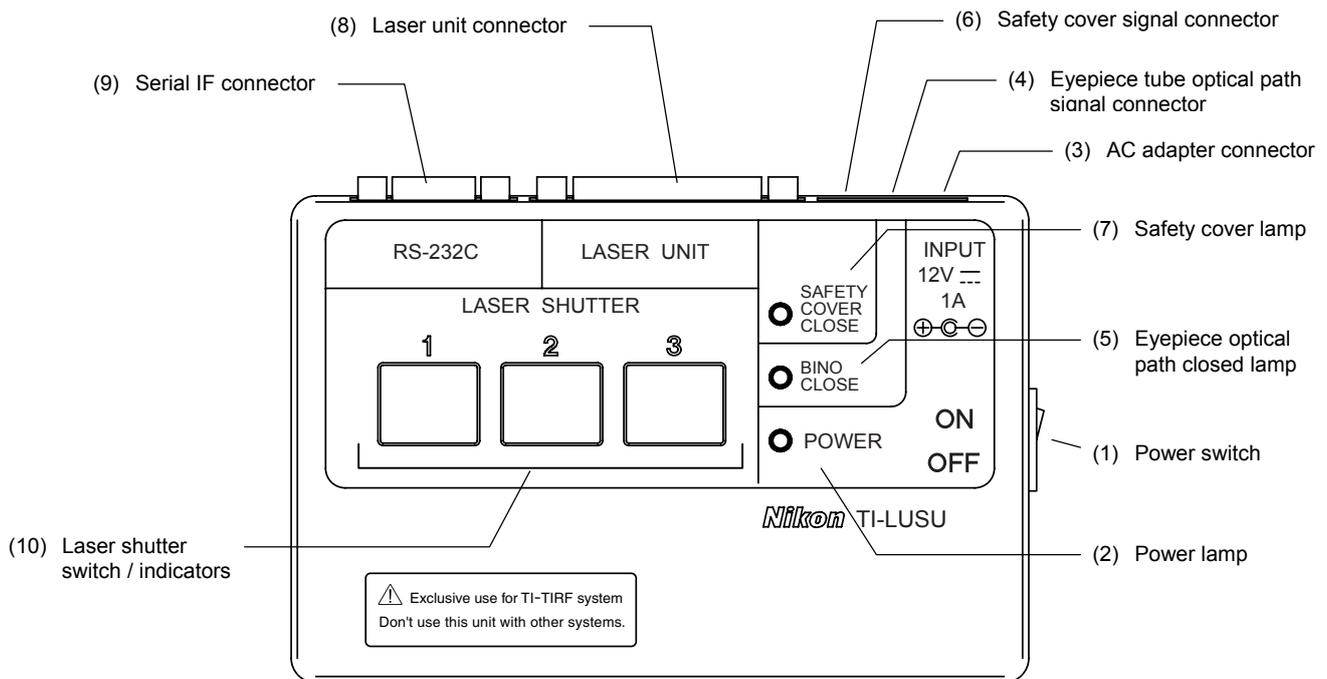


Figure 4.4-1

(1) Power switch

Use this switch to turn on/off the 12VDC power supply from the AC adapter.

(2) Power lamp

The lamp lights up green when the power is turned on.

(3) AC adapter connector

The output from the AC adapter is connected here.

(4) Eyepiece tube optical path signal connector

The connector for the optical path signal to the eyepiece tube is connected here.

(5) Eyepiece optical path closed lamp

The lamp lights up yellow when the eyepiece optical path is closed.

When the lamp is not lit, the eyepiece optical path is open and the motorized shutters in the laser unit are closed.

(6) Safety cover signal connector

The connector for the safety cover signal is connected here.

(7) Safety cover lamp

The lamp lights up yellow when the safety cover is closed.

(8) Laser unit connector

The cable with D-sub 25-pin connectors for the laser unit is connected here.

The cable comes with the laser unit.

(9) Serial IF connector

To control the system with a PC, connect the provided RS-232C cable here.

You can open or close the motorized shutters from your PC.

The following additional functions are available when issuing commands from the PC:

- **Timer operation for the motorized shutters**

Each of the motorized shutters can be timer-programmed so that it remains open for a preset period (0.1 to 100 seconds).

The shutters are switched to timer-set operation mode even when operating the shutters with the shutter switch.

- **Disabling shutter switch**

A command can be issued to disable shutter operation with the shutter switch.

(10) Laser shutter switch/indicator

The switch controls the motorized shutters on the laser unit and the indicators show their conditions.

The motorized shutters in the laser unit can be opened with this switch only when the optical path in the eyepiece tube is selected to the non-binocular route and the safety cover is closed.

If the binocular route is selected or the safety cover is open, no motorized shutter can be opened even if the switch is pressed.

When the non-binocular route is selected, the eyepiece optical path closed lamp lights up yellow.

When the safety cover is closed, the safety cover lamp lights up yellow.

If the eyepiece tube optical path signal connector or the safety cover signal connector is not connected, no motorized shutter can be opened.

When a motorized shutter is open, the lamp in the laser shutter switch lights up yellow.

When the timer mode is activated on the TI-LUSU shutter controller and a motorized shutter is open, the laser shutter switch blinks yellow. To activate the timer mode, settings such as the shutter time must be set up on the PC.

4.5 TI-FLC Epi-fl Filter Turret

The epi-fl filter turret reflects the illumination light from the epi-fl illuminator and directs the light to the objective. The epi-fl filter turret can hold up to six filter blocks that have an excitation filter, a barrier filter, and a dichroic mirror. Filter blocks can be changed by rotating the filter block rotation control.

The product can be used with any of the following three epi-fl filter turrets.

Table 4.5-1

TI-FLC epi-fl filter turret	This is a manual operation type device.
TI-FLC-E motorized epi-fl filter turret	This is a motorized operation type device. The TI-HUBC HUB controller is required to use the device.
TI-FLC-E/HQ motor epi-fl filter turret	This is a motorized operation type device. Three special high-precision filter blocks are attached. The TI-HUBC HUB controller is required to use the device.

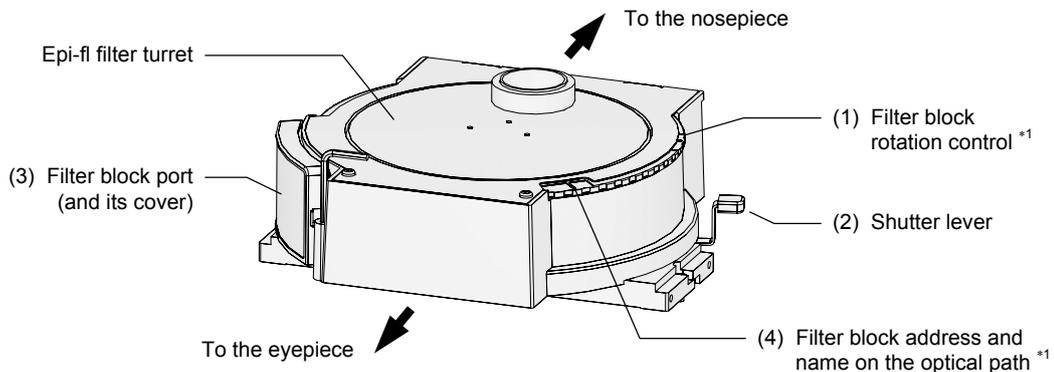


Figure 4.5-1

*1 The part with this symbol is not attached to the TI-FLC-E motorized epi-fl filter turret and the TI-FLC-E/HQ motor epi-fl filter turret.

(1) Filter block rotation control

The filter block rotation control is used to change the filter block positions.

(2) Shutter lever

The shutter lever is used to close the shutter and block the light by moving it clockwise.

(3) Filter block port and its cover

To change a filter block, detach the cover.

(4) Filter block address and name on the optical path

The filter block address and the name of it are shown here.

4.6 Filter Block

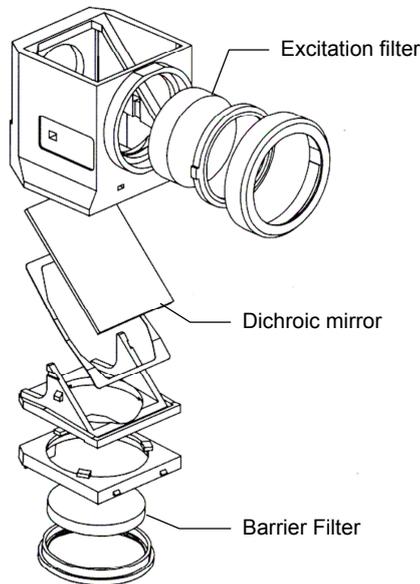


Figure 4.6-1

A filter block consists of three optical elements, an excitation filter (EX filter), a barrier filter (BA filter), and a dichroic mirror (DM). Read the following articles to select a combination of optical elements suitable for your purpose and to the characteristics of the specimen and fluorophore.

- You can select a different combination of an excitation filter and a barrier filter for an excitation method.
- Excitation filters, barrier filters, and dichroic mirrors can be purchased individually.
- Excitation filters will deteriorate over time because they are exposed to intense light. Replace them as necessary.
- Three special high-precision filter blocks are attached to the TI-FLC-E/HQ Motor Epi-fl filter turret. The special high-precision filter blocks are not equipped with any filter and dichroic mirror. Use a filter and a dichroic mirror suitable for your purpose.

Important

The special high-precision filter blocks, which are attached to the TI-FLC-E/HQ motor epi-fl filter turret, are adjusted to match the turret addresses. Be sure to attach them to the addresses shown on the filter block bodies.

(1) Excitation filter (EX filter) selection

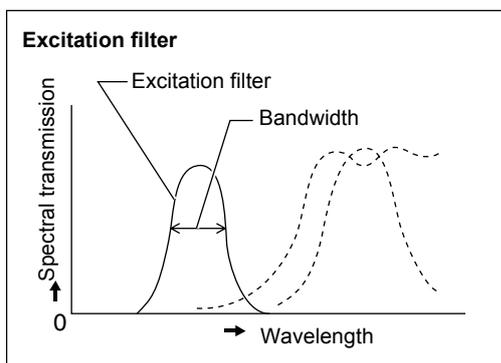


Figure 4.6-2

An excitation light filter transmits light selectively and blocks other light. The transmitted light is called excitation light. It is used to excite the fluorophore in the specimen and fluorescent light is emitted from the specimen.

The wavelength range of light that can pass through the filter is called the bandwidth.

The bandwidth of the excitation filter determines the brightness of the fluorescent image, the occurrence of autofluorescence (fluorescence resulting from substances other than the fluorophores), and degree of fading. When the filter has a wide bandwidth, a large amount of excitation light will be irradiated on the specimen. In this case, the image becomes bright but the amount of autofluorescence becomes large and fading of the specimen occurs soon. On the contrary, when the filter has a narrow bandwidth, a small amount of excitation light will be irradiated on the specimen. In this case, the image becomes dark but the amount of autofluorescence becomes small and fading of the specimen occurs late.

For specimens with pronounced autofluorescence, use an excitation light filter with a narrow bandwidth. (The resulting fluorescent image will be darker, however.)

Excitation filters will deteriorate over time because they are exposed to intense light. Replace them depending on their total operating hours.

Table 4.6-1

	Excitation filter bandwidth	
	Narrow	Wide
Brightness of the fluorescence image	Dark	Bright
Occurrence of autofluorescence	Minimal pronounced	Pronounced
Extent of fading	Minimal pronounced	Pronounced

(2) Barrier filter (BA filter) selection

A barrier filter transmits only fluorescent light emitted by the specimen but blocks the excitation light.

This filter makes it possible to observe the fluorescent image without unnecessary light (that is, on a dark background).

There are two types of barrier filters: LP filters (long-pass filters), which block all light that is shorter than a certain boundary wavelength and allow all light to pass that is longer than the boundary wavelength, and BP filters (band-pass filters), which allow only light in a certain bandwidth to pass. Use a proper filter depending on the purpose.

LP filter (long-pass filter)

A long pass filter transmits light that have longer wavelength than a certain wavelength but blocks light that have shorter wavelength. The boundary wavelength is called the cut-on wavelength.

- 1) Excitation light is light that is irradiated to the specimen. The fluorophore in the specimen absorbs the excitation light energy. As a result, fluorescent light is emitted from the fluorophore instead. When a specimen is labeled with a fluorophore that emits fluorescent light of very close wavelengths to the excitation light, select a barrier filter with the shortest cut-on wavelength permitted by performance requirements for efficient fluorescent microscopy.

A longer cut-on wavelength tends to result in a more complete separation between excitation light and fluorescent light, rendering a darker background of the fluorescent image. With the recent advancement in filter performance, however, filters with shorter cut-on wavelengths can be used for this purpose and they are used more often than before.

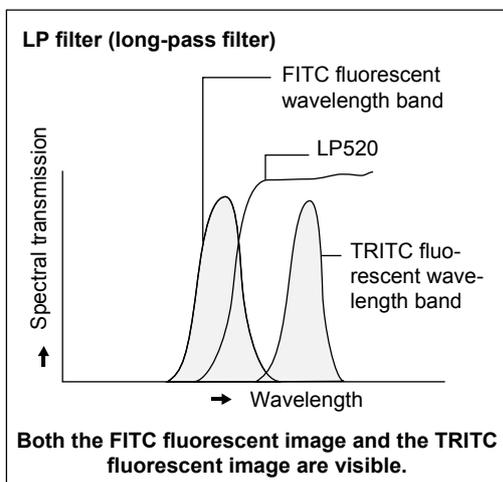


Figure 4.6-3

- 2) An LP filter is used for a specimen labeled with multiple wavelengths where fluorescent images for all wavelengths are desired.

However, the usual combination of a dichroic mirror, an excitation light filter, and a barrier filter of LP filter type, may not be sufficient to excite a fluorophore that emits fluorescent light of longer wavelength (for example, the TRITC when the specimen is labeled with the FITC and the TRITC), making the fluorescent image for the TRITC very dark. In a case like this, a multi-band filter is recommended.

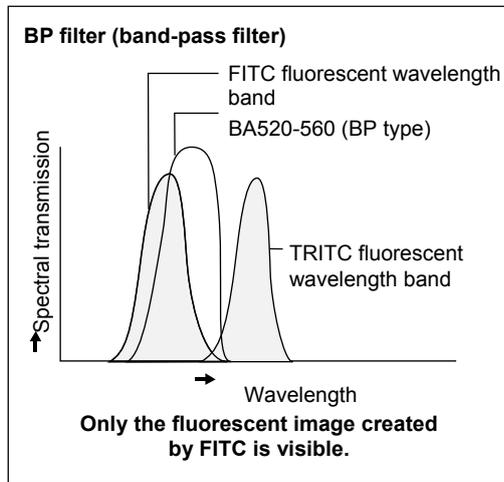
BP filter (band-pass filter)

Figure 4.6-4

A BP filter transmits light of a certain bandwidth.

This type of filter is used to observe a fluorescent image only emitted by a certain fluorophore when the specimen is labeled with multiple fluorophore. (For example, when a specimen is labeled with the FITC and the TRITC and you wish to observe a fluorescent image only emitted by the FITC, use a filter of BA520-560.)

However, you cannot distinguish the autofluorescence from the other fluorescences in the image transmitted through the BP filter because the image will only be of one color (green, in the above example).

When you wish to distinguish the autofluorescence by a subtle difference of hue, an LP filter is more useful.

(3) Excitation filter and barrier filter replacement

The excitation light filter and the barrier filter in the filter block can be replaced with other filter. (The filters are screwed into the filter cube.)

For the special high-precision filter blocks, the excitation filter is screwed into the filter block and the barrier filter is fastened to the filter block by screws. When changing or attaching a filter, check the direction of the filter.

4.7 Laser Unit

A laser unit is a base device to mount laser devices. It introduces laser beam to the photo activation illuminator unit through a single-mode optical fiber. One two-laser unit and two three-laser units are available. Use any laser unit with an optical fiber.

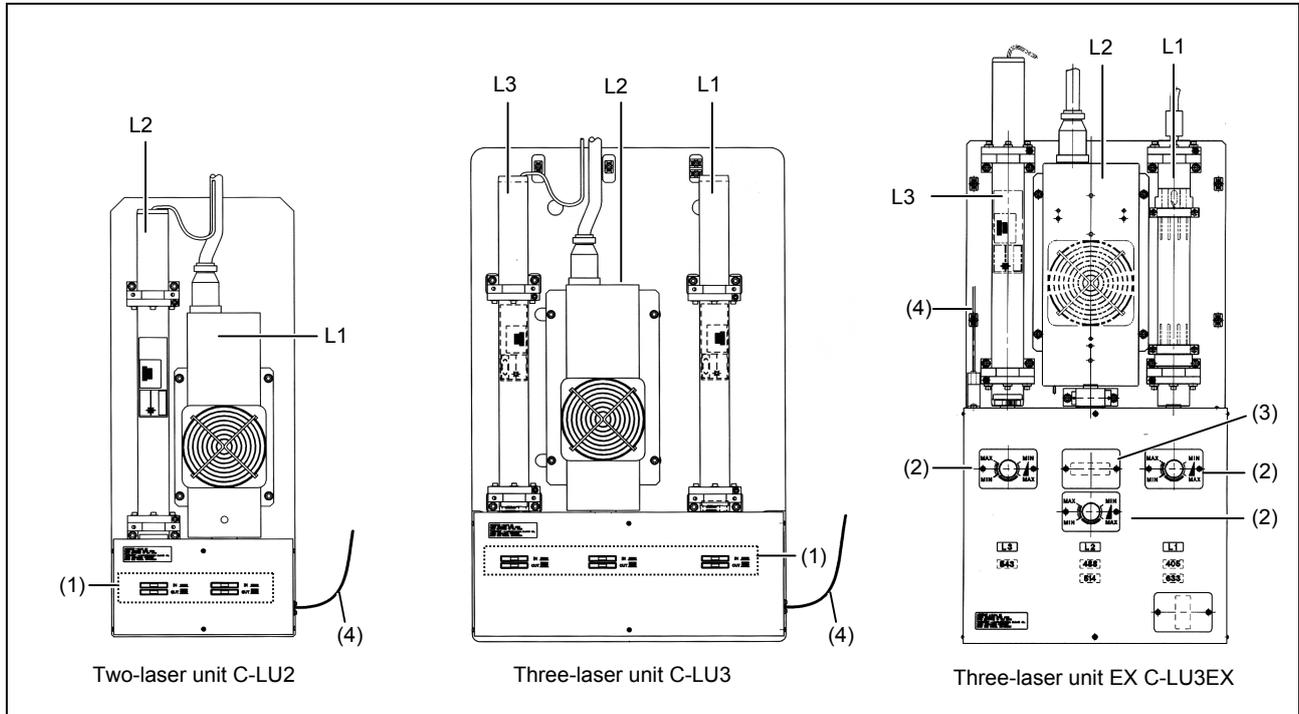


Figure 4.7-1

Refer to the table 4.7-3 for available lasers (L1, L2, and L3) for each unit.

To control laser intensity for the two-laser unit C-LU2 and the three-laser unit C-LU3, insert or remove the two ND filters listed below in front of each laser head. For the three-laser unit C-LU3 EX, use the brightness control knob in front of each laser head.

A motorized shutter is provided for each laser and can be controlled on the shutter control unit, TI-LUSU.



Caution

The laser head and the optical fiber of this unit are precisely aligned. Avoid subjecting the laser head or the optical fiber to vibration or shock. If you need to remove the laser head from the unit due to laser malfunction or for other reasons, contact your nearest Nikon representative.

(1) ND filter slider (C-LU2, C-LU3)

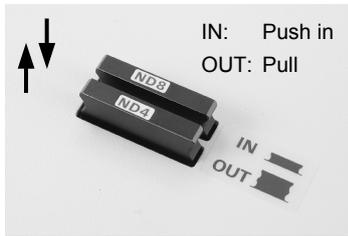


Figure 4.7-2

Table 4.7-1

ND4	ND8	Brightness
OUT	OUT	1 (100%)
IN	OUT	1/4 (25%)
OUT	IN	1/8 (12.5%)
IN	IN	1/32 (3.1%)

IN: Place the component into the optical path.
 OUT: Remove the component from the optical path

(2) Brightness control knob (only for the C-LU3EX)

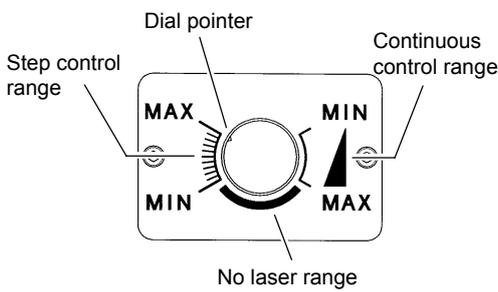


Figure 4.7-3

This knob controls light intensity in 11 discrete steps or continuously. The following shows approximate transmittance in each step when controlling the light intensity of Ar laser. In the no laser area, the laser beam is not emitted.

Table 4.7-2

Transmittance (%)	100	80	50	25	10	3.5	1.3	0.5	0.15	0.05	0.02
-------------------	-----	----	----	----	----	-----	-----	-----	------	------	------

(3) Ar wavelength selector (only for the C-LU3EX, option)

Select one of two Ar laser wavelengths (488 nm or 514 nm).

(4) Single-mode fiber inlet

The single-mode fiber is connected here.

4.7 Laser Unit

Table 4.7-3

Product name	Mountable position and adapter			Remarks
	Two-laser unit C-LU2	Three-laser unit C-LU3	Three-laser unit C-LU3EX	
Manufacturer: Spectra-physics Model: 163-C02 (488-514)	L1	L2	L2	The Ar wavelength selector device is required.
Manufacturer: Coherent Model: Radius 405 (405)	L1 (D)	L1 (E)	L1 (E)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: 56 ICS 425 (408)	L1 (A)	L1 (A)	L1 (F)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: 56 ICS 426 (440-445)	L1 (A)	L1 (A)	L1 (F)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: IMA101040ALS (457-514)	L1	L2	L2 (only for 488 nm and 514 nm)	
Manufacturer: Melles Griot Model: IMA101065ALS (457-514)	L1	L2	L2 (only for 488 nm and 514 nm)	
Manufacturer: Spectra-physics Model: 163-C12 (488)	L1	L2	L2	
Manufacturer: Spectra-physics Model: 161C-030 (488)	L1	L2	L2	
Manufacturer: Coherent Model: Sapphire 488-10/20/25/30/50/75 CDRH (488)	L1 (C)	L2 (C)	L2 (C)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: BCD010/020/030 (488)	L1 (C)	L2 (C)	L2 (C)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: 58GCB020 (532)	L2 (G)	L3 (G)	L3 (G)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: 05LGP 193 (543)	L2	L3	L3	
Manufacturer: Melles Griot Model: 85YCA010/020/025 (561)	L2 (B)	L3 (B)	L3 (B)	A laser adapter is required for use.
Manufacturer: JDS Uniphase Model: 1677P (594)	L2	L1	L1	
Manufacturer: JDS Uniphase Model: 1125P (633)	L2	L1 or L3	L1, L3	When mounted on the L3 position, the transmittance is 20% to 30%.
Manufacturer: Melles Griot Model: 56 ICS 272 (635)	L2 (A)	L1 (A)	L1 (F)	A laser adapter is required for use.
Manufacturer: Melles Griot Model: 56 ICS 264 (640)	L2 (A)	L1 (A)	L1 (F)	A laser adapter is required for use.

Table 4.7-4

Adapter list
(A) MXA22068 (C-LAR/LD laser adapter)
(B) MXA22069 (C-LAR532/561 laser adapter)
(C) MXA22070 (C-LAR488 laser adapter)
(D) MXA22071 (C-LAR405 laser adapter)
(E) MXA22043 (C1-LAR408 laser adapter R)
(F) MXA22044 (C1-LAS408 laser adapter S)
(G) MXA22098 (C-LAR/532 laser adapter)

5

Shutter Control Unit Communications Commands

5.1 Communication-Controlled Operation

You can control this system from a PC or another device through the RS-232C interface. Create your own control program based on the information given in this manual.

5.1.1 Communications Method

Start-stop serial communication

5.1.2 Connector Specifications

The following signals are used for the serial communication connector on the product:

Table 5.1-1 D-sub 9-pin connector (male)

Pin number	Signal	I/O
1	Not used	-
2	RxD	Input
3	TxD	Output
4	Not used	-
5	GND	GND
6	Not used	-
7	Not used	-
8	Not used	-
9	Not used	-

5.1.3 Cable Specifications

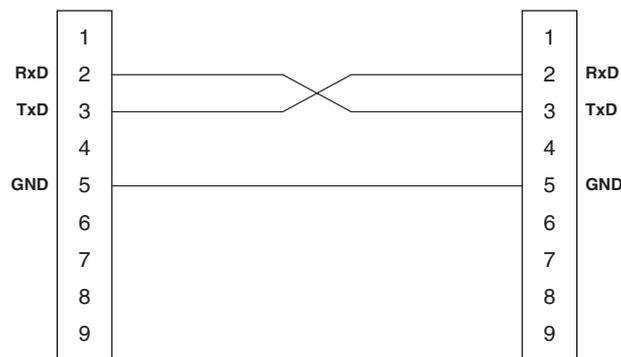


Figure 5.1-1

To ensure trouble-free operation, use the supplied RS-232C cable for connecting a PC.

5.1.4 Communications Parameters

Table 5.1-2

Baud rate	9600 bps (fixed)
Data length	8 bits (fixed)
Start bits	1 bit (fixed)
Stop bits	1 bit (fixed)
Parity bits	None

5.1.5 Communications Format

The data format used when the system receives data from external equipment is referred to as “command format,” while the data format used when the system sends data is called “response format.” In the following description, “[“ and ”]” indicate delimiters and are not actual data (characters) that are sent or received.

Command format

[A][B B B][C.C][C R]

[A] Command identifier (one lower-case alphabet character, ASCII code, one byte)

Identifier	Description
c	Operation, control, and data setting commands
r	Data request commands

[BBB] Command name (three upper-case alphabet characters, ASCII code, three bytes)

[C...C] Parameter (ASCII code)

[CR] Carriage return (0x0D)

Response format

[D][B B B][E.E][C R][L F]

[D] Response identifier

Identifier	Description
o	Response given when command processing is normally terminated
n	Response given if an error occurs during command processing
a	Response given to an r command

[BBB] Command name (three upper-case alphabet characters, ASCII code, three bytes)

[E...E] Data read or error code (ASCII code)

[CR] Carriage return (0x0D), [LF] Line feed (0x0A)

[Error codes]

Code	Type	Description
1	Command error	Wrong command identifier or wrong command name
2	Parameter error	Improper parameter
4	Receive buffer overflow	Received data exceeds the limit (32 bytes)
6	Command overflow	Number of commands that can be processed is exceeded during continuous transmission of commands (five commands)
7	Processing inhibit error	Processing is inhibited.
8	Processing stop error	Command processing is stopped.

5.1.6 Communications Sequence

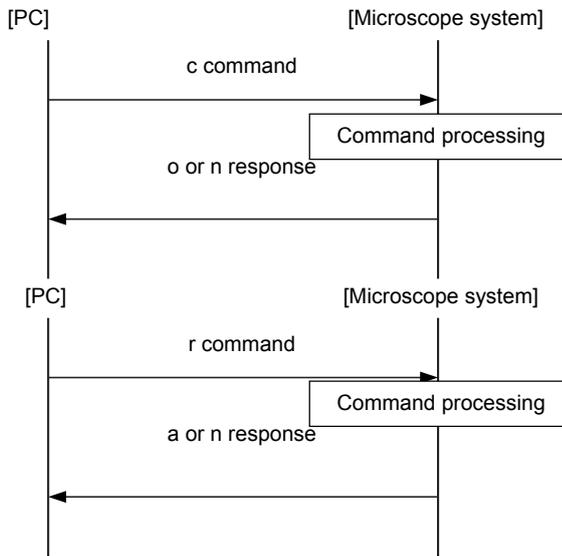


Figure 5.1-2

5.1.7 Commands

Standard command

The following are factory default commands and can be used in the standard condition.

The shutter control unit controls laser shutters so that only one shutter opens at a time in the standard condition.

1 c command: Operation, control, and data setting command

c command	o: Normal response n: Error response	Description
cTSO(1)	oTSO nTSO(6)	The command opens the shutter of the argument and closes any other shutters. If an open time period has been specified, the shutter will close when the specified period elapses. If the binocular route is selected on the microscope, no shutter opens. (1): [1-3] Shutter number (6): Error code
cTSC	oTSC nTSC(6)	The command closes all shutters.
cTTS(2):(3)	oTTS nTTS(6)	The command sets up the shutter open time. (2): [1-3] Shutter number (3): [0000-1000] Time setting * [0000] is used to open the shutter continuously. The value is specified in steps of 0.1 seconds. (100.0 seconds maximum) Example: 0001 for 0.1 seconds (6): Error code
cTSW(4)	oTSW nTSW(6)	The command enables/disables the laser open/close switch on the control panel. (4): [1] Enabled, [0] Disabled (6): Error code

2 r command: Data request command

r command	a: Normal response n: Error response	Description
rTSR	aTSR(1) nTSR(6)	The command reads the shutter condition. (1): [0] means all shutters are closed. [1] to [3] means the shutter of the number is open. (6): Error code * The rTSR command is disabled in the shutter independent control mode.
rTTS(1)	aTTS(2) nTTS(6)	The command reads the shutter open time setting. (1): [1-3] Shutter number (2): [0000-1000] Time setting (6): Error code
rTES	aTES(3) nTES(6)	The command reads the open/close conditions of the eyepiece tube optical path. (3): [1] open, [0] closed (6): Error code
rTSW	aTSW(4) nTSW(6)	The command reads the setting for the laser open/close switch on the control panel. (4): [1] Enabled, [0] Disabled (6): Error code
rVER	aVER(5) nVER(6)	The command reads the program version. (5): V*.* (Five characters. "*" is a numeral. Example: V1.00) (6): Error code
rPNM	aPNM(5) nPNM(6)	The command reads the program name. (5): TI-LUSU (6): Error code

Shutter independent control commands

The following commands are enabled when the shutter control unit is set to the shutter independent control mode. Two or more shutters can be opened at a time.

The shutter independent control commands cannot be used in the standard settings. If you want to use the commands, contact your nearest Nikon representative.

1 c command: Operation, control, and data setting command

c command	o: Normal response n: Error response	Description
cTSD(2)	oTSD nTSD(6)	The command opens/closes the shutter 1st, 2nd, and 3rd at a time independently. (2): [0]: 1st shutter closed, 2nd shutter closed, 3rd shutter closed [1]: 1st shutter open, 2nd shutter closed, 3rd shutter closed [2]: 1st shutter closed, 2nd shutter open, 3rd shutter closed [3]: 1st shutter open, 2nd shutter open, 3rd shutter closed [4]: 1st shutter closed, 2nd shutter closed, 3rd shutter open [5]: 1st shutter open, 2nd shutter closed, 3rd shutter closed [6]: 1st shutter closed, 2nd shutter open, 3rd shutter open [7]: 1st shutter open, 2nd shutter open, 3rd shutter open (6): Error code
cTIO(3)	oTIO nTIO(6)	The command opens the shutter of the argument. (3): [1-3] Shutter number (6): Error code
cTIC(4)	oTIC nTIC(6)	The command closes the shutter of the argument. (3): [1-3] Shutter number (6): Error code

2 r command: Data request command

r command	a: Normal response n: Error response	Description
rTEX	aTEX(1) nTEX(6)	The command reads the condition of the shutter independent control mode. (1): [1] Enabled, [0] Disabled (standard condition) (6): Error code
rTSD	aTSD(2) nTSD(6)	The command reads the shutter condition. (2): [0]: 1st shutter closed, 2nd shutter closed, 3rd shutter closed [1]: 1st shutter open, 2nd shutter closed, 3rd shutter closed [2]: 1st shutter closed, 2nd shutter open, 3rd shutter closed [3]: 1st shutter open, 2nd shutter open, 3rd shutter closed [4]: 1st shutter closed, 2nd shutter closed, 3rd shutter open [5]: 1st shutter open, 2nd shutter closed, 3rd shutter closed [6]: 1st shutter closed, 2nd shutter open, 3rd shutter open [7]: 1st shutter open, 2nd shutter open, 3rd shutter open (6): Error code

6

Troubleshooting

Misuse of the product may adversely affect the product performance, even if the product is not damaged. If any of the following troubles occurs, be sure to check the following table for possible causes before requesting a service. Also refer to the instruction manual supplied with the microscope, the light source and the DIC attachment when it is used.

For problems not listed below and in case the problem persists, disconnect the device and contact your local Nikon representative.

6.1 Epi-fl Microscopy

Table 6-1

Trouble	Cause	Countermeasure
The lamp does not light up.	No power is supplied to the product.	Plug the power cord into a wall outlet.
	The lamp connector is not connected to the power supply device.	Connect it correctly.
	The lamp is at the end of its life.	Replace the lamp with a specific one.
Even though the lamp is lit, the image is not visible.	A shutter is closed.	Open the shutter.
	A filter block is stopped at an intermediate position.	Rotate the filter block rotation control to a clickstop position.
	The filter cube selection is wrong.	Use the correct filter block.
	The combination of excitation filter, barrier filter and dichroic mirror is inappropriate, or one of these components is missing.	Use a filter block of a correct combination.
	The system is set up for TIRF microscopy.	Push in the optical path selector lever to select the epi-fl microscopy.
	The optical path of the microscope is not set up for binocular observation.	Check the optical path selector knob, the Optical Path button, or such to set up the optical path for binocular observation.
Even though the lamp is lit, the image is too dark.	A ND filter is placed in the optical path.	Remove the ND filter from the optical path as necessary.
	The combination of excitation filter, barrier filter and dichroic mirror is not suitable for the specimen.	Use a filter block that has a suitable combination for the specimen.
	The objective is not suitable for observation in UV or V excitation.	Use the designated objective for UV excitation.
The excitation light is leaking over to the observation side.	The filter block is shifted from its correct position.	Insert the filter block until it touches the back of the turret.
The contrast is poor.	The objective or cover glass is dirty.	Clean the lens and optical elements.
	The immersion oil is fluorescing.	Use non-fluorescent immersion oil (Nikon Immersion Oil DF).
	The glass slide is fluorescing.	Use a non-fluorescent glass slide.
	The room is too bright.	Darken the room.

6.1 Epi-fl Microscopy

Trouble	Cause	Countermeasure
The image quality is poor.	No cover glass is used.	Use a cover glass. (However, no cover glass is necessary when using an NCG objective.)
	No immersion oil is applied to the tip of an oil immersion objective.	Apply Nikon Immersion Oil DF.
	The specific immersion oil is not used for the objective.	Apply Nikon Immersion Oil DF.
	The filter block is not suited for the specimen.	Use a filter block suitable for the specimen.
The field of view is vignetted.	The field diaphragm size is reduced too far.	Open the field diaphragm so that it circumscribes the field of view.
	The ND filter slider or the shutter slider is stopped at an intermediate position.	Push or pull the slider to a click-stop position or to a limit.
	The filter block is shifted from its correct position.	Insert the filter block until it touches the back of the turret.

6.2 TIRF Microscopy

Table 6-2

Trouble	Cause	Countermeasure
The system does not start up.	The power cord is not connected to an wall outlet.	Plug the power cord into an wall outlet.
No TIRF image can be seen.	The laser is not lit.	Refer to Section 2, "Start-up Operation" in Chapter 3, "Detailed Microscopy Procedure" and turn on the laser.
	The motorized shutter of the laser unit is closed.	Set up the optical path for the TIRF illumination. Check that a laser safety cover is attached correctly. Operate the shutter switch on the shutter control unit to open the shutter.
	The optical path on the microscope is not set for the TV camera (video camera).	Set up the optical path for the camera port.
The TIRF image is too dark.	Wavelength specifications of filters such as the dichroic mirror and the barrier filter or a laser are not suitable for the specimen.	Use filters and a laser suitable for the fluorophore.
	The laser power is not enough.	Adjust the ND filters of the laser unit and the TIRF/epi-fl illuminator unit.
	The filter block in the optical path is not appropriate.	Use a filter block suitable for the specimen.
	The position of the laser light is shifted from the objective pupil.	Adjust the laser position.
	The optical path is set to TIRF (60%) - Epi (40%).	Pull out the optical path selector lever to use TIRF (100%).
The contrast of the TIRF image is poor.	The position of the laser light is too close to the center of the objective pupil.	Adjust the laser position.
	The laser light projected from the objective is not parallel.	Adjust the position of the condenser lens.
	The light from the Epi-illumination lamp gets into the optical path.	Close the shutter for the epi illumination.

7

Daily Maintenance



Warning

Do not remove any components or parts on the system during operation. Never attempt to remove the objective, the filter block, the optical fiber, the lamphouse, the eyepiece, and any other parts during operation. Removing any one of them may cause the laser beam to be projected through that portion, resulting in exposure of eyes or skin to the laser. It is very dangerous. Before performing maintenance, be sure to turn off all power switches including the lasers, and unplug the power cords from the wall outlets.

7.1

Replacing the Objective and the Filter Block

Before replacing the objective and the filter block, be sure to turn off all power switches including the lasers, and unplug the power cords from the wall outlets.

Never attempt to remove the objective and the filter block during operation.

7.2

Replacing lamps

Before replacing the lamp, be sure to turn off all power switches including the lasers, and unplug the power cords from the wall outlets.

Never attempt to remove the adapter for the optical fiber light source during operation.

For details about replacing the diascopic illumination lamp, see the instruction manual provided with the microscope.

For replacing the Epi-illumination lamp, see the instruction manual provided with the epi-fl illuminator light source. The lamp and nearby parts are extremely hot when the lamp is lit or immediately after it has been turned off. When replacing the lamp, wait at least 30 minutes after the lamp is turned off.

7.3

Cleaning the filters and lenses

Do not let dust, fingerprint, or so on get on the lenses and the filters. Dirt on the lenses, filters, or so on will adversely affect the view of images. Especially in TIRF microscopy, if the tip of the objective and the cover glass are dirty, it can substantially reduce contrast. If any of the optical components get dirty, clean them as described below.

- Brush away dust with a soft brush, or gently wipe them off with gauze.
- Only if there are fingerprints or grease, dampen a piece of soft, clean cotton cloth, lens tissue, or gauze lightly with absolute alcohol (ethyl or methyl) and gently wipe off the dirt.
- When removing the immersion oil off from the objective, use only petroleum benzine. After wiping off with petroleum benzine, wipe with absolute alcohol (ethyl or methyl) to make the surface clearer. If petroleum benzine is unavailable, use methyl alcohol. However, methyl alcohol does not clean as well as petroleum benzine, it will be necessary to wipe the surface repeatedly. (Usually, three- or four-times wipe is sufficient to clean the lenses.)
- Never use petroleum benzine to clean the entrance lens of the eyepiece tube or prism surface of the eyepiece tube.
- Absolute alcohol and petroleum benzine are highly flammable. Be careful when handling around open flames and when turning on/off the power switches or so on. A spark may ignite a flame.
- Follow the instructions provided by the manufacturer when using absolute alcohol or petroleum benzine.

7.4 Cleaning the Product

- We recommend that you use a silicon cloth to clean the product.
- For persistent dirt, dampen a piece of gauze with neutral detergent and wipe gently.
- Use of organic solvent could result in discoloration of the plastic parts.

7.5 Disinfecting the Product

- We recommend that you use 70% medical alcohol for disinfecting the product.
- If a specimen is spilled onto the microscope, check to determine whether the specimen is hazardous. If the specimen is hazardous, follow your standard facility procedures.
- Use of organic solvent could result in discoloration of the plastic parts.

7.6 Storage

- Store the product in a dry place where mold is not likely to form.
- Store the objectives and eyepieces in a desiccator or similar container with a drying agent.
- Put the dust-proof cover over the product to protect it from dust.
- Before putting on the dust-proof cover, turn off all power switches of components including the lasers (push them to the "O" side) and wait until the lamphouse gets cool sufficiently.

7.7 Moving and relocating the system (charged)

The following tasks should be performed by trained service personnel. If performed, it may result in improper setup, impairing system performance and exposing the eyes or skin to the laser beam, resulting in injury. If you need to move or relocate this product, contact your nearest Nikon representative.

- To detach a laser device from the laser unit.
- To detach the optical fiber from the laser unit.
- To detach the optical fiber from the TIRF/epi fl illuminator unit or the motorized illuminator unit.
- To detach the TIRF/epi fl illuminator unit or the motorized illuminator unit from the microscope.
- To detach the epi-fl filter turret from the microscope.

7.8 Periodical inspection (charged)

Periodical inspections (expenses charged) are recommended in order to maintain the performance of the product. For details, contact your nearest Nikon representative.

8

Specifications and Performance

8.1 Overall Configuration

Table 8.1-1

Unit	Device	Model name	Remarks
Microscope and accessories	Ti	Ti-E Ti-E/B Ti-U Ti-U/B	Use the TI-DH Dia Pillar Illuminator 100W.
	Objective for TIRF microscopy	Apo TIRF 60xH/1.49	Select the appropriate objective for observations.
		Apo TIRF 100xH/1.49	
		Plan Apo TIRF 60xH/1.45	
		Plan Apo TIRF 100xH/1.45	
		Plan Apo VC 60xH/1.4	
	Plan Apo VC 100xH/1.4		
	Shutter control unit	TI-LUSU	The unit can be connected to a PC.
	AC adapter	AD-1260B	For the shutter control unit.
	Epi-fl filter turret	TI-FLC TI-FLC-E/HQ	The TI-FLC-E/HQ comes with three special high-precision filter blocks.
Epi illumination lamp and power supply device		Make sure to read the instruction manuals for the products.	
Stage ring (concentric ring)	26 32	Model 32 is made of glass.	
TIRF illuminator	TIRF	TI-TIRF TI-TIRF-E	Two illumination modes are available, TIRF (100%) and TIRF (60%)-Epi-fl (40%).
	Optical fiber	C1 single mode fiber	A straight fiber can be used when used with the straight fiber adapter.
	Straight fiber adapter	TIRF2 S	The adapter is attached to the illuminator to use an optical fiber with a straight tip.
	Stage-up lens	TI-TIRF Stage-up Lens	Use the stage-up lens when a stage riser is installed and a TIRF illuminator is attached to the lower tier. The TI-BSUK70 70 mm Stage Up Kit is also required.
TIRF image observation devices	PC	Commercially available	
	Monitor	Commercially available	
	TV camera (video camera)	Commercially available	An adapter is required to connect to the microscope port.
Laser unit	Two-laser unit	C-LU2	See table 1 for mountable lasers.
	Three-laser unit	C-LU3	
	Three-laser unit EX	C-LU3EX	See table 1 for mountable lasers.

8.1 Overall Configuration

Mountable lasers

Table 8.1-2

Color	Product name	Specifications	Mountable unit and position	Remarks
Multi Lines	Manufacturer: Spectra-physics Head: 163-C02 Power supply: 263-C04	488-514nm/40mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	The Ar wavelength selector device is required.
Violet	Manufacturer: Coherent Head: RADIUS 405 Power supply: CDRH control box	405nm/25mW	C-LU2(L1) C-LU3(L1) C-LU3EX(L1)	A laser adapter is required for use.
	Manufacturer: Melles Griot Head: 56 ICS 425 Power supply: 56IMA016/018/019	408nm/38mW	C-LU2(L1) C-LU3(L1) C-LU3EX(L1)	A laser adapter is required for use.
	Manufacturer: Melles Griot Head: 56 ICS 426 Power supply: 56IMA016/018/19	440-445nm/15mW	C-LU2(L1) C-LU3(L1) C-LU3EX(L1)	A laser adapter is required for use.
Blue	Manufacturer: Melles Griot Head: IMA101040ALS Power supply: Model175B/300 series	457, 477, 488, 514nm /40mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	
	Manufacturer: Melles Griot Head: IMA101065ALS Power supply: Model175B/300 series	457, 477, 488, 514nm /65mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	
	Manufacturer: Spectra-physics Head: 163-C12 Power supply: 263-C04	488nm/25mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	
	Manufacturer: Spectra-physics Head: 161C-030 Power supply: 263-C04	488nm/10mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	
	Manufacturer: Coherent Head: Sapphire 488-10/20/25/30/50/75 CDRH Power supply: CDRH control box	488nm/10, 20, 25, 30, 50, 75mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	A laser adapter is required for use.
	Manufacturer: Melles Griot Head: 85BCD010/020/030 Power supply: 85BCD series Laser controller	488nm /10, 20, 30mW	C-LU2(L1) C-LU3(L2) C-LU3EX(L2)	A laser adapter is required for use.
Green	Manufacturer: Melles Griot Head: 58GCB020 Power supply: 56IMA017	532nm/20mW	C-LU2(L2) C-LU3(L3) C-LU3EX(L3)	A laser adapter is required for use.
Green	Manufacturer: Melles Griot Head: 05LGP 193 Power supply: 05LPL951-065/100V, 05LPL951-065/115-230V	543nm/1mW	C-LU2(L2) C-LU3(L3) C-LU3EX(L3)	

8.1 Overall Configuration

Color	Product name	Specifications	Mountable unit and position	Remarks
Yellow	Manufacturer: Melles Griot Head: 85YCA010/020/025 Power supply: 85YCA series control box	561nm /10, 20, 25mW	C-LU2(L2) C-LU3(L3) C-LU3EX(L3)	A laser adapter is required for use.
	Manufacturer: JDS Uniphase Head: 1677P Power supply: 1207-3/100V 1207-1/115V 1207-2/230V	594nm/2mW	C-LU2(L2) C-LU3(L1) C-LU3EX(L1)	
Red	Manufacturer: JDS Uniphase Head: 1125P Power supply: 1202-3/100V 1202-1/115V 1202-2/230V	633nm/5mW	C-LU2(L2) C-LU3(L1, L3) C-LU3EX(L1, L3)	When mounted on the L3 position, the transmittance is 20% to 30%.
	Manufacturer: Melles Griot Head: 56 ICS 272 Power supply: 56 IMA 015/018/019	635nm/3mW	C-LU2(L2) C-LU3(L1) C-LU3EX(L1)	A laser adapter is required for use.
	Manufacturer: Melles Griot Head: 56 ICS 264 Power supply: 56 IMA 015/018/019	640nm/12mW	C-LU2(L2) C-LU3(L1) C-LU3EX(L1)	A laser adapter is required for use.

8.1 Overall Configuration

8.1.1 Shutter Control Unit**Table 8.1-3**

Model name	TI-LUSU
Input ratings	12VDC 1A

8.1.2 AC Adapter for the Shutter Control Unit**Table 8.1-4**

Manufacturer	Medi-Power Electronics Inc. (Taiwan)
Model name	AD-1260B
Input voltage ratings	100-240VAC, 1.2A, 50/60Hz
Input voltage fluctuation	±10%
Output voltage ratings	12VDC
Output current ratings	5A
Remarks	UL listed, GS approved, CE satisfied, and PSE approved product

8.1.3 Power Cord for the AC Adapter of the Shutter Control Unit

Be sure to use one of the following power cords.

In Japan, use a PSE certified power cord.

Table 8.1-5

For Japan	Detachable cord set with a PSE mark, 3 conductor grounding Type VCTF 3 x 0.75 mm ² , 3 m long maximum, rated at 125 VAC minimum
For countries where the power supply is 100 to 120 VAC	UL Listed detachable cord set , 3 conductor grounding Type SVT, No. 18 AWG, 3 m long maximum, rated at 125 VAC minimum
For countries where the power supply is 220 to 240 VAC	Power cord set approved according to EU/EN standards, 3 conductor grounding Type HO5VV-F, 3 m long maximum, rated at 250 VAC minimum

8.1.4 Environmental Conditions**Operating conditions****Table 8.1-6**

Temperature	5 to 35°C
Relative humidity	60% RH maximum with no condensation
Altitude	2,000 m maximum
Pollution degree	Degree 2
Installation category	Category II
Electric shock protection class	Class I Indoor use only

Storage conditions**Table 8.1-7**

Temperature	-20 to 60°C
Relative humidity	90% RH maximum with no condensation

8.1.5 Safety Standards Compliance

Table 8.1-8

Laser safety	<ul style="list-style-type: none"> • This product complies with IEC60825-1 Class 3B requirements. • This product complies with FDA Class IIIB requirements.
CE marking	<ul style="list-style-type: none"> • This product meets EU Low Voltage Directive requirements. • This product meets EU EMC Directive requirements. (EN61326) <div style="text-align: center;">  </div>
Other categories	<ul style="list-style-type: none"> • This product complies with FCC15B Class A requirements. This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. • This product complies with Canadian EMC standards. (ICES-003 Class A) Cet appareil numérique de classe A est conforme à la norme NMB-003 du Canada. • This product complies with Australian EMC standards. (AS/NZS2064 Group 1 Class B)