

OLYMPUS[®]

INSTRUCTIONS

IX2-ARCEVA

ARC ILLUMINATION TIRF SET

This instruction manual is for the Olympus Arc Illumination TIRF Set. To ensure the safety, obtain optimum performance and to familiarize yourself fully with the use of this system, we recommend that you study this manual thoroughly before operating the system.

As this system employs an arc light source (mercury burner or xenon burner), take special care for safety and please also read the instruction manual for the light source.

Retain this instruction manual in an easily accessible place near the work desk for future reference.



A X 7 3 8 7

CONTENTS

Correct assembly and adjustments are critical for the system to manifest its full performance. If you are going to assemble the system by yourself, please read chapter 3, "ASSEMBLY" (pages. 8 to 11). For the assemblies of the modules for which instruction manuals are available, refer to their instruction manuals.

IMPORTANT – Be sure to read this section for safe use of the equipment. –	1-4
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IMPORTANT

This system has been designed to enable total reflection fluorescence observation using an arc light source (mercury burner or xenon burner).

Total reflection illumination is implemented by limiting the illumination projection area to the area corresponding to the objective pupil NA of 1.39 (because the reflectance of cytoplasm is 1.38).

© This system offers the following advantages compared to the laser-based total reflection illumination system.

- Higher safety, because the laser light is not used.
- Easy handling without the care of interference stripe, because the light source is not a coherent source.
- Easy switching between multiple wavelengths.

Nevertheless, this system is not applicable to molecular fluorescence observation due to low intensity of the illumination. Its applications are limited to high-contrast observations of tissues near cell membranes and physiochemical phenomena.



SAFETY PRECAUTIONS

1. Do not ignite the mercury burner or xenon burner without mounting the lamp housing on the microscope.
The UV light emitted from the burner is hazardous for your eyes. Do not look directly at the light output from the lamp housing.
For details precautions, also refer to the instruction manual for the light source.
2. When centering the burner or replacing the specimen, be sure to look them through the UV cut plate to protect your eyes.

1 Getting Ready

1. This manual pertains only to the handling of the total reflection fluorescence illumination system. Please also read the instruction manuals for the IX2 series (IX81/IX71/IX51) microscope and associated options to understand the comprehensive operating instructions of the microscope system.
2. The total reflection fluorescence illumination system is composed of precision instruments. Handle it with care and avoid subjecting it to sudden or severe impact.
3. Do not use the system where it is subjected to direct sunlight, high temperature and humidity, dust or vibrations.
4. Reserve spaces of 10 cm or more around the lamp housing and power supply to facilitate their ventilation.
5. The fluorescent light illuminator that can be used in combination with this system is only the IX2-RFAL L-shaped reflected light illuminator.
6. The objectives that can be used with total reflection illumination are only the PlanApoN60XO_{TIRFM} and PlanApo60XO_{TIRFM}.
7. The illumination field of total reflection fluorescence observation is limited within the area of field number 11.
8. Since the total reflection light is not point-source light, the excitation light incidence angle on the specimen is not constant and the effusion depth cannot be specified.
9. If an excitation filter is included in the mirror unit during total reflection fluorescence illumination, the repeated reflections cause the leak of direct light to increase and degrade the contrast considerably. To prevent this, remove the excitation filter from the mirror unit and mount in the excitation filter slider.
10. The size of the excitation filter inserted in the filter slot of the excitation slider should be 25 mm in diameter and 6 mm or less in thickness. Only one ND filter with a diameter of 32 mm and thickness of 1 mm or less can be inserted.
11. When ordinary fluorescence observation is performed by inserting a 25 mm excitation filter in the excitation filter slider, the illumination field is limited within the area of field number 20.
12. To prevent light leak, be sure to attach the provided ϕ 32 mm light shields in the unused positions of the excitation filter slider.

13. The aperture iris diaphragm for use in ordinary fluorescence observation is not provided. If it is required to use the aperture iris diaphragm, replace the slit iris diaphragm unit of this system with the aperture iris diaphragm unit of the IX2-RFAL.
14. When the IX2-RFAC, IX2-RFACA and a standard mirror unit are used in combination, it is required to readjust the centering of the slit iris diaphragm unit because, otherwise, the contrast of the total reflection fluorescent light would fluctuate due to variation in the illumination light projection positions between turret positions.
15. The IX2-SFR right-hand knob stage is not recommended because the stage knobs will degrade the operability of the slit iris diaphragm unit considerably. Instead, it is recommended to use the IX-SVL-2.
16. When the IX2-RSPC right-side port adapter is used, some positions of the excitation filter slider would be unusable due to interference with the camera. The following table shows the relationship between the camera size and unusable filter positions.

Height from the center of C-mount	Depth from the center of C-mount	Interference condition
Less than 27 mm	—	None
27 mm to 45 mm exclusive	Less than 34 mm	None
	34 mm to 70 mm exclusive	Deepest (vacant) position is unusable.
	70 mm or more	The two deepest positions are unusable.
45 mm or more	Less than 20 mm	None
	20 mm to 56 mm exclusive	Deepest (vacant) position is unusable.
	56 mm or more	The two deepest positions are unusable.

◎The camera interference can be reduced by using the U-FWR filter wheel or a commercially available motorized filter wheel.

2 Maintenance and Storage

1. Clean all glass components by wiping gently with gauze. To remove fingerprints or oil smudges, wipe with gauze slightly moistened with a mixture of ether (70%) and alcohol (30%).
▲ Since solvents such as ether and alcohol are highly flammable, they must be handled carefully. Be sure to keep these chemicals away from open flames or potential sources of electrical sparks — for example, electrical equipment that is being switched on or off. Also remember to always use these chemicals only in a well-ventilated room.
2. Do not attempt to use organic solvents to clean the non-optical components. To clean them, use a lint-free, soft cloth slightly moistened with a diluted neutral detergent.
3. Never attempt to disassemble any part of the system, for this may degrade the performance.

3 Caution

If the system is used in a manner not specified by this manual, the safety of the user may be imperiled. In addition, the equipment may also be damaged. Always use the equipment as outlined in this instruction manual.

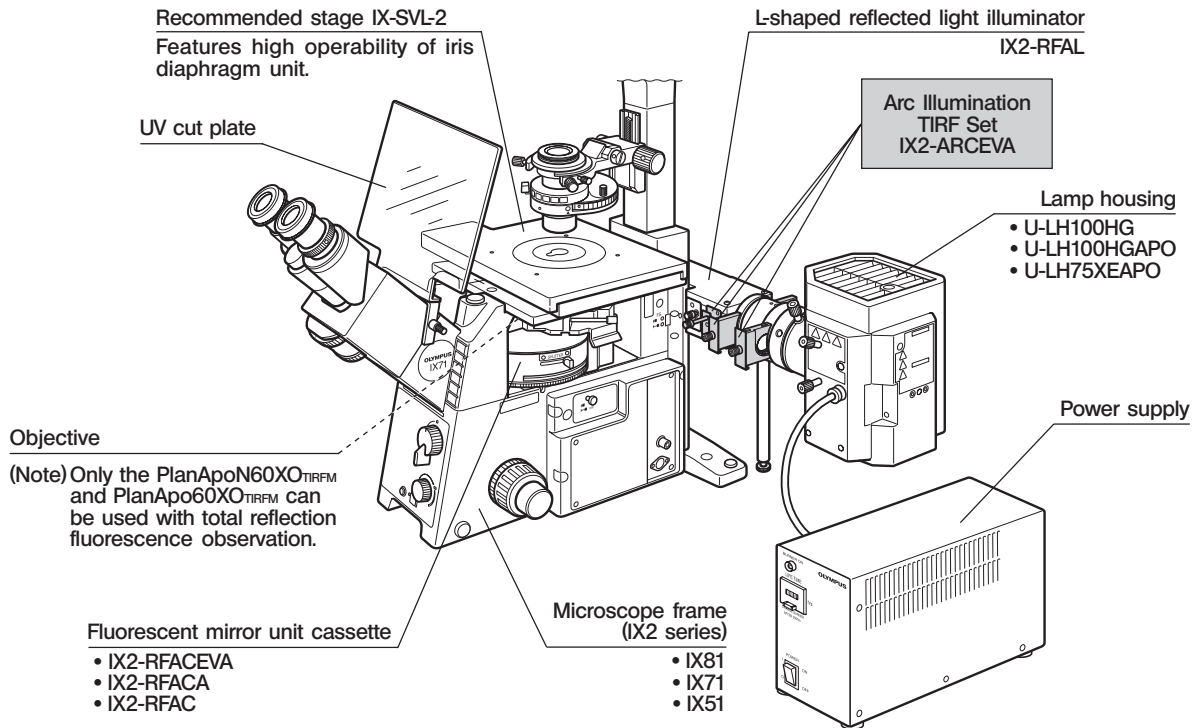
The following symbols are used to set off text in this instruction manual.

- ▲: Indicates that failure to follow the instructions in the warning could result in bodily harm to the user and/or damage to equipment (including objects in the vicinity of the equipment).
- ★: Indicates that failure to follow the instructions could result in damage to equipment.
- ◎: Indicates commentary (for ease of operation and maintenance).

1 NOMENCLATURE

◎ The modules enclosed in belong to the Arc Illumination TIRF Set.

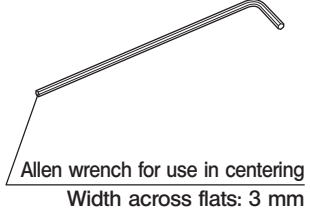
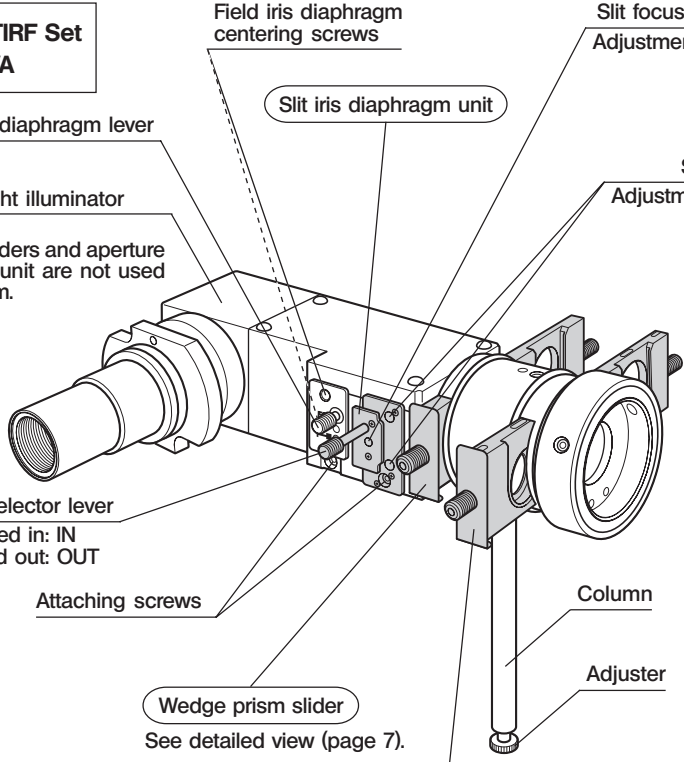
- The illumination field in fluorescence observation is limited to the area of FN 20.
- The illumination field in total reflection fluorescence observation is limited in the area of FN 11.



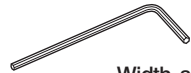
2 CONTROLS

**Arc Illumination TIRF Set
IX2-ARCEVA**

Field iris diaphragm lever
L-shaped reflected light illuminator
IX2-RFAL
(Note) The two filter sliders and aperture
iris diaphragm unit are not used
with this system.



Click additional parts
《 Balls, springs, screws (x 2 each),
and Allen wrench 》



Width across
flats: 2 mm

4-position excitation filter slider

ND filter insertion slots

Filter diameter: 32 mm.
Thickness: 1 mm or less.

Sticker attaching positions

x 6

Light source side

Vacant position

Objective side

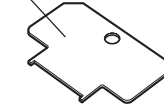
Slider knob

Light leak prevention plate

Excitation filter insertion slots

Filter diameter: 25 mm.
Thickness: 6 mm or less.

Push ring driver



Handwritten indication stickers



Wedge prism slider

ND filter insertion slot

Filter diameter: 32 mm.
Thickness: 1 mm or less.

Slider knob

32 mm light shields (x 4)

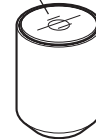
To be inserted in the ND filter insertion slots of the unused positions.

Vacant position

Shutter

Wedge prism

Target



- ▲ The filters and sliders are very hot during and immediately after use. Confirm that they are cooled down enough before replacing them.
- ★ When a commercially available, non-Olympus low reflection ND filter is used, the filter may sometimes be cracked or burnt.

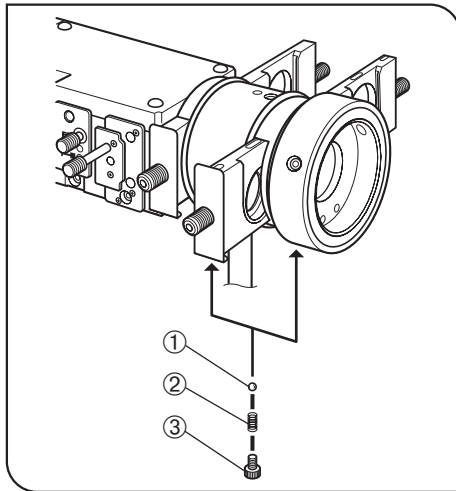


Fig. 1

1 Attaching the Click Additional Parts

(Fig. 1)

1. Using the provided Allen wrench (width across flats 2 mm), remove the screw, which functions as the cap, on the center of the bottom side of the filter insertion slot of the IX2-RFAL.
2. Put the ball ① and spring ② into the center of the bottom side of the filter insertion slot and fix them with the screw ③. Use the provided Allen wrench for use in centering (width across flats: 3 mm) for clamping the screw.

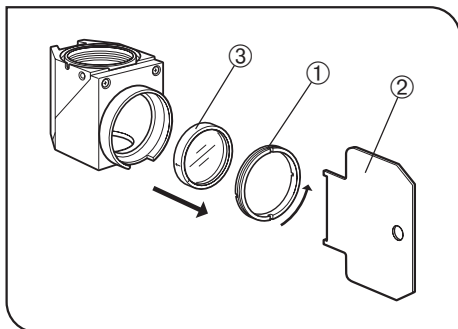


Fig. 2

2 Mounting the Excitation Filter

(Figs. 2 to 4)

- ◎When the IX2-RFACEVA mirror unit cassette is used, the operation in step 1 below is not necessary because no excitation filter is provided with it.
1. Using the push ring driver ②, turn the excitation filter push ring ① of the mirror unit to be mounted in the fluorescence mirror unit counterclockwise and remove the excitation filter ③.
 2. Using the push ring driver ②, turn the push ring ⑤ of the excitation filter slider ④ counterclockwise to remove it.

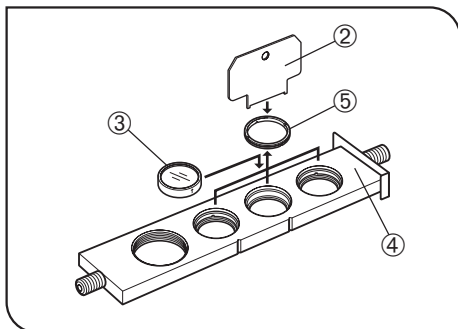


Fig. 3

- ★The excitation filter should be inserted in the specified orientation. Insert it so that the indication arrow on the filter frame points the objective. Ensure the orientation of insertion of the excitation filter slider into the illuminator before assembly.
3. Drop the excitation filter ③ into the excitation filter slider so that the indication arrow on the filter frame faces upward (if the up surface comes on the objective side) and fix with the push ring ⑤.

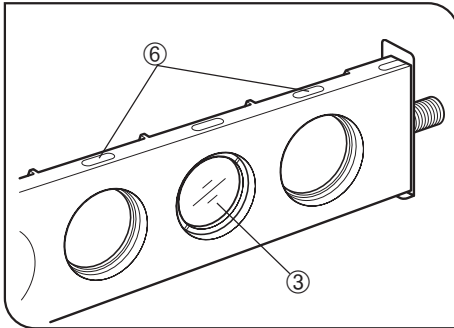


Fig. 4

4. Write the name (or abbreviation) of the mounted filter on one of the provided handwritten indication stickers and attach it to one of the sticker attaching positions ⑥ (position on the left or right of the position in which the filter is mounted).

In the above case, attach the sticker to the position on the left of the filter position because the left side comes near the slider inlet when the slider is set on the illuminator.

3 Replacing the Modules Provided with the IX2-RFAL

⑥ Replace the following modules as shown in the illustration on page 6.

- Aperture iris diaphragm: Remove the attaching screw and replace with the slit iris diaphragm unit.
- Filter slider (aperture iris diaphragm side): Remove the slider knob and replace with the wedge prism slider.
- Filter slide (lamp housing side): Remove the slider knob and replace with the excitation filter slider.

4 Mounting the IX2-RFAL

5 Mounting the UV Cut Plate

☉ Refer to the instruction manual for the IX2 reflected fluorescence system.

6 Mounting the ND Filter

☉ The ND filter attenuates the excitation light and helps delaying color fading of the specimen.

Insert the ND filter gently into the ND filter insertion slot of the slider.

4 OPERATION

4-1 Preparation

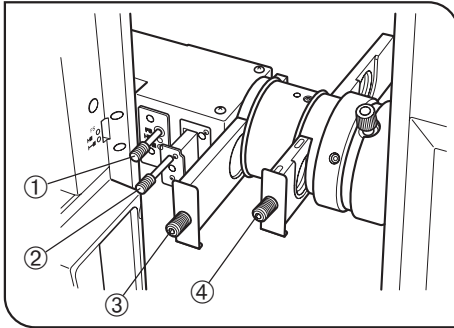


Fig. 5

1 Centering the Burner

(Fig. 5)

⊙ The following settings are the original settings required for this system. The details may be variable according to the instruction manual for each lamp housing.

- ① Field iris diaphragm: Pulled-out position (Closed)
- ② Slit: Pulled-out position (Slit OUT)
- ③ Wedge prism: Nearest position (Vacant position)
- ④ Excitation filter: B or IB excitation filter engaged. Also the B or IB mirror unit should be engaged in the light path.

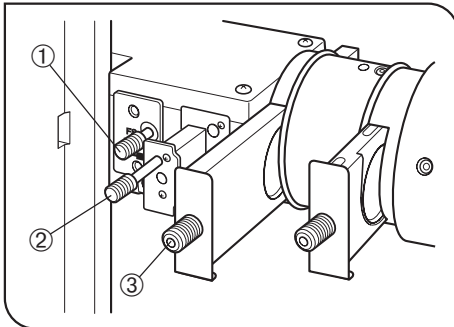


Fig. 6

2 Adjusting the Slit

(Figs. 6 to 8)

⊙ Open the field iris diaphragm ①, set the slit selector lever ② to the OUT position and set the wedge prism ③ to the vacant position before the following adjustment. (Fig. 6)

1. Engage the PlanApoN60XO_{TIRFM} or PlanApo60XO_{TIRFM} objective (which requires immersion oil) in the light path.
2. Place a fluorescent specimen on the stage and bring the specimen into focus using general fluorescent light of B or IB.
3. Remove the specimen, rotate the revolving nosepiece and screw the target into an objective position.

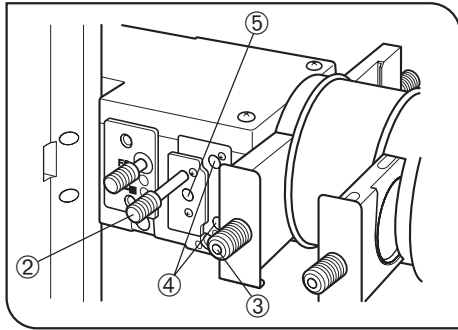


Fig. 7

4. Push in the slit selector lever ② (IN position) and set the wedge prism slider ③ to the intermediate click position to engage the slit and wedge prism in the light path. (Fig. 7)
5. Using the Allen wrench (width across flats 3 mm), turn the two centering screws ④ alternately so that a crescent slit is visible on the target. Adjust so that the slit is located as close as possible to the perimeter of the target.
6. If the slit is not sharply visible, adjust the focus adjustment screw ⑤ using the Allen wrench (width across flats 3 mm).

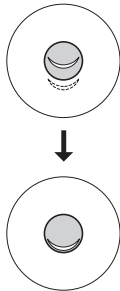


Fig. 8

4-2 *Total Reflection Fluorescence Observation*

- The only objective that can be used in total reflection fluorescence observation is the PlanApoN60XO_{TIRFM} or PlanApo60XO_{TIRFM}.
 - Since the illumination intensity is lower than with the laser light source, this system is not applicable to molecular fluorescence observation. Its applications are limited to high-contrast observations of tissues near cell membranes and physiochemical phenomena.
1. Set the slit to the OUT position and the wedge prism to the pulled out vacant position.
 2. Using an objective other than the PlanApoN60XO_{TIRFM} or PlanApo60XO_{TIRFM}, set up the microscope condition for general fluorescence observation.
 3. Push in the field iris diaphragm lever to the open condition and engage PlanApoN60XO_{TIRFM} or PlanApo60XO_{TIRFM} objective in the light path.
 4. Focus on the lowest position of the specimen (position closest to the cover glass).
 5. Set the wedge prism in the intermediate click position and push in the slit to the IN position.

Now the microscope is ready for total reflection fluorescence observation.

The effusion depth can be fine adjusted by slightly rotating the lower slit centering screw as described below.

- Clockwise: This moves the slit inward, increasing the effusion depth.
- Counterclockwise: This moves the slit outward, decreasing the effusion depth.

5 TROUBLESHOOTING GUIDE

Under certain conditions, performance of the system may be adversely affected by factors other than defects. If problems occur, please review the following list and take remedial action as needed.

If you cannot solve the problem after checking the entire list, contact Olympus.

Phenomenon	Cause	Treatment	Page
a. Total reflection fluorescence cannot be obtained.	The PlanApoN60XO _{TIRFM} or PlanApo60XO _{TIRFM} objective is not engaged in the light path.	Engage them in the light path.	14
	The wedge prism and slit are not engaged in the light path.		
	The slit positioning is incorrect.	Adjust using the target.	13
	An excitation filter is left in the mirror cassette unit.	Remove it.	9
b. The field of view is irregular during total reflection fluorescence observation.	The lamp housing is not centered properly.	Center it correctly. If the field is still irregular after adjustment, the irregularities may sometimes be eliminated by decentering the lamp housing slightly.	12

OLYMPUS®



OLYMPUS CORPORATION

Shinjuku Monolith, 3-1, Nishi Shinjuku 2-chome, Shinjuku-ku, Tokyo, Japan

OLYMPUS EUROPA HOLDING GMBH

Wendenstraße 14-18, 20097 Hamburg, Germany

OLYMPUS AMERICA INC.

3500 Corporate Parkway, P.O. Box 610, Center Valley, PA 18034-0610, U.S.A.

OLYMPUS SURGICAL & INDUSTRIAL AMERICA INC.

One Corporate Drive, Orangeburg, NY 10962, U.S.A.

OLYMPUS SINGAPORE PTE LTD.

491B River Valley Road, #12-01/04 Valley Point Office Tower, Singapore 248373

OLYMPUS AUSTRALIA PTY. LTD.

31 Gilby Road, Mount Waverley, VIC. 3149, Australia

OLYMPUS LATIN AMERICA, INC.

5301 Blue Lagoon Drive, Suite 290 Miami, FL 33126, U.S.A.

