

# OLYMPUS®

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## INSTRUCTIONS

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# IX81-ZDC2

## FOCUS DRIFT COMPENSATING MICROSCOPE

This instruction manual is for the Olympus Focus Drift Compensating Microscope Model IX81-ZDC2. To ensure the safety, obtain optimum performance and to familiarize yourself fully with the use of this microscope, we recommend that you study this manual thoroughly before operating the microscope. Retain this instruction manual in an easily accessible place near the work desk for future reference.



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## **CE Compliance**

This device complies with the requirements of both directive **2004/108/EC** concerning electromagnetic compatibility and directive **2006/95/EC** concerning low voltage. The CE marking indicates compliance with the above directives.

IEC61326-1 defines two categories according to the location for use.

Class A: Equipment suitable for use in establishments other than domestic, and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

Class B: Equipment for use in domestic establishments, and in establishments directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This system is applied Class A. Some interference may occur if this system is used in domestic location.



In accordance with European Directive 2002/96/EC on Waste Electrical and Electronic Equipment, this symbol indicates that the product must not be disposed of as unsorted municipal waste, but should be collected separately.

Refer to your local Olympus distributor in EU for return and/or collection systems available in your country.

**NOTE:** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications.

Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

**FCC WARNING:** Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

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# IMPORTANT

- ◎ The IX81-ZDC2 microscope features the capability of compensating for the focus drift that poses problems during long hours of time-lapse observation of live cells.  
The microscope has two focus drift compensation modes including the one-shot mode and continuous focus mode, which are to be selected according to the observed specimen.
- ◎ This manual pertains only to the information related to the IX81-ZDC2 (focus drift compensation) facility of the microscope. Please also refer to the instruction manuals for the microscope and associated modules.
- ◎ The IX81-ZDC2 can be controlled with the MetaMorph application software. For the compatible version of the software, consult the respective company.
- ◎ For the applicable modules, contact Olympus or check the latest catalogue for this product.

## Safety Precautions

1. The focus drift compensation function uses a laser diode (wavelength 785 nm) for autofocusing. It is designed to be safe by reducing the laser power (Class 1), it is still not recommended to view the laser light directly by removing the cover, etc. Never remove the warning and caution labels on the microscope.  
The semiconductor laser for autofocusing incorporated in this unit makes it designated as a product of the following class.




**CLASS 1 LASER PRODUCT (IEC60825-1:1993+A1:1997+A2:2001)**

**This product complies with 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No.50, dated (June 24, 2007).**

2. Never attempt to remove the cover using a tool. There is a risk of exposure to the internal strong laser beam as well as other malfunction or failure.  
[Semiconductor laser, wavelength 785 nm, output (max.) 85 mW, equivalent to Class 3B]
3. Always use the power cord provided by Olympus. If the proper power cord is not used, product safety performance cannot be warranted.
4. Always ensure that the **grounding terminal** is properly grounded. If the equipment is not grounded, Olympus can no longer warrant the electrical safety performance of the equipment.
5. Distribute the connection cables at a distance from the lamp housing. If a connection cable contacts the lamp housing or its surroundings, the cable may be melted and cause an electric shock hazard.
6. Never insert metallic objects into the air vents of the microscope as this could result in electrical shock, personal injury and equipment damage.
7. To prevent the microscope system from falling down, do not install modules which cause the microscope system height to exceed 1 meter.
8. Do not use an objective other than those specified to be applicable (see page 4).

### **Safety Symbols**

The following symbols are found on the microscope. Study the meaning of the symbols and always use the equipment in the safest possible manner.

Symbol	Explanation
	Before use, carefully read the instruction manual. Improper use could result in personal injury to the user and/or damage to the equipment.
	Indicates that the main switch is ON.
	Indicates that the main switch is OFF.



## Notes on ZDC (Focus Drift Compensation)

### CAUTION

When using an objective with correction collar, always adjust the collection collar before using the focus drift compensation function. Otherwise, the focus drift compensation will not be available.

Be sure to use an anti-vibration bench to enable the focus drift compensation to function properly.

#### 1. One-shot focus mode

In this mode, the 785 nm laser beam is first focused on the glass bottom dish's cover glass bottom surface (or on the cover glass top surface in the case of an oil- or water-immersion objective) and then moved mechanically from there to the target cell using software to reproduce the desired focus position.

Although this mode requires a certain period for moving the focal point, the capability of arbitrary setting of the focus movement range makes it suitable for multi-point time-lapse observation.

#### 2. Continuous focus mode

In this mode, the laser beam is focused continuously on the top surface of the cover glass of the glass bottom dish at the same time as the target cell. During this, the focus is corrected automatically even when the change in environmental temperature due to heat cause defocusing.

The range in which continuous focusing is possible is determined by the objective power, namely 20  $\mu\text{m}$  with the 40X objective and 10  $\mu\text{m}$  with the 60X objective. With the 100X objective, the range varies depending on the model of the objective.

Although this mode enables continuous observation (at the video rate), the fixed focusing range makes it suitable for observation in which the cell position does not vary greatly, such as the TIRF (Total Internal Reflection Fluorescence) observation.

◎Range in which continuous focusing is possible: (Only when using a glass bottom dish)

UAPON 100XO TIRF	4 $\mu\text{m}$ or less
UPSAPO 100XO	3.5 $\mu\text{m}$ or less
UPLFLN 100XO2	3 $\mu\text{m}$ or less
UPLFLN 100XOI2	3 $\mu\text{m}$ or less

3. Applicable objectives:

**CAUTION**

- If objective has an aperture, set it wide open.
- The AF focusing speed is approximately 1 to 5sec. in the focusing range, but slows down depending on the AF search range setting.
- The focus drift compensation is not available with the phase contrast objectives because these incorporate a phase plate.
- The continuous focus mode can be used only with an oil- or water-immersion objective. If an objective other than oil- or water-immersion objective is used, the one-shot focus mode should be used.
- When using a large specimen such as a micro-test plate, focusing may not be achieved due to its inclination.  
The focusing is guaranteed only with a glass bottom dish with a diameter of 35 mm.

Objective Name	NA	WD (mm)
APON 60X O <sub>TIRF</sub>	1.49	0.1
UAPON 100X O <sub>TIRF</sub>	1.49	0.1
PLAPON 60X O	1.42	0.15
UPLSAPO 60X O	1.35	0.15
UPLSAPO 100X O	1.40	0.13
UPLFLN 100X O2	1.30	0.20
UPLFLN 100X OI2	1.30-0.60	0.20
UPLSAPO 20X*	0.75	0.60
UPLSAPO 40X2*	0.95	0.18
UPLSAPO 60X W	1.20	0.28
UPLFLN 20X*	0.50	2.10
UPLFLN 40X*	0.75	0.51
UPLFLN 60X*	0.90	0.20
LUCPLFLN 20X*	0.45	6.60-7.80
LUCPLFLN 40X*	0.60	2.70-4.00
LUCPLFLN 60X*	0.70	1.50-2.20
UAPO 40X OI3/340	1.35-0.65	0.1

\* Compatible with one-shot focus mode only.

©The previous models to the objectives listed above (the objectives having a smaller number or no number at the end of model name) can also be used.

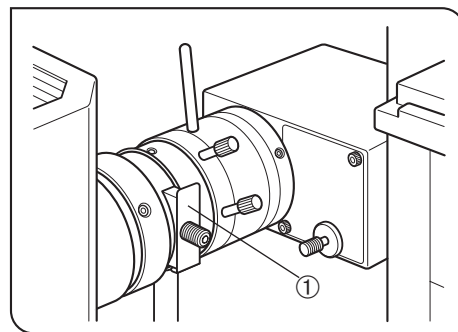
4. With DIC (Differential Interference Contrast) observation, focus compensation is possible only with the gray sensitive color and in the one-shot focus mode. It is neither possible in phase contrast observation.

5. Operating environmental temperatures: 18 to 40°C (64.4 to 104°F)

(Focus compensation is possible under a temperature variation range within 5°C (41°F) and when the microscope is protected against the wind from air conditioners.)

## 6. Restrictions on combined modules

- The module that can be mounted on the left side port is only a TV adapter that can project the primary image in 1X magnification, that is, the U-TV1X, U-DPCAD or U-SIP. (Optimal AF performance cannot be achieved with other combination.)
- Note on stage  
The IX-SVL2 stage cannot be combined with this microscope.
- Notes on observation tube
  - a) When the U-TBI90 observation tube is used, the image in the peripheral part of eyepiece's visual field may be obscured (about 5%). In addition, no intermediate attachment can be used with the U-TBI90.
  - b) When the U-BI90/BI90CT observation tube is used together with a 4X or 10X objective, the image in the peripheral part of eyepiece's visual field may be obscured. In addition, the IX2-CA2X magnification changer lens unit cannot be used with the U-BI90/BI90CT (it becomes usable by mounting the U-EPA2 eye-point adjuster (occupying only 1 stage) on the U-BI90/U-BI90CT).
- Notes on intermediate attachments
  - a) Only one U-EPA2 eye-point adjuster can be mounted (it cannot be used together with other intermediate attachments).
  - b) Only one U-FWO filter wheel can be mounted (it cannot be used together with other intermediate attachments).
  - c) When the IX-ATU intermediate attachment and GX-SPU side port unit are combined, the image in the peripheral part of eyepiece's visual field may be obscured if the magnification changer knob of the microscope frame is set to 1X.
  - d) The U-CA/U-ECA/U-ECA1.6X magnification changer cannot be used in combination with the microscope frame.
- When the IX-ATU intermediate attachment and U-TR30H trinocular observation tube are combined, the only TV adapter that can be used is the U-TV1X.
- When the GX-SPU side port unit and a TV camera are used, the only TV adapter that can be used is the U-TV1X.
- The FN of the right side port is specified as 16. When a 10X or lower-power objective a CCD camera are used, the brightness in the peripheral area may be slightly insufficient.
- When a 2X, 4X or 10X objective is used with FN 22, the brightness in the peripheral area may be slightly insufficient. (This problem does not occur with FN 16.)
- When a 10X objective is used in transmitted light observation using the IX2-ULWCD condenser, the illumination NA may become insufficient and the resolution may degrade. Also, optimum phase contrast observation is not available using a phase contrast objective (PH1 or PH2).
- When using the IX2-RFAW, remove the light shield ① in the excitation filter slider on the left illuminator to prevent it from coming in the way of operation.  
Some filter sliders are not usable with this microscope.

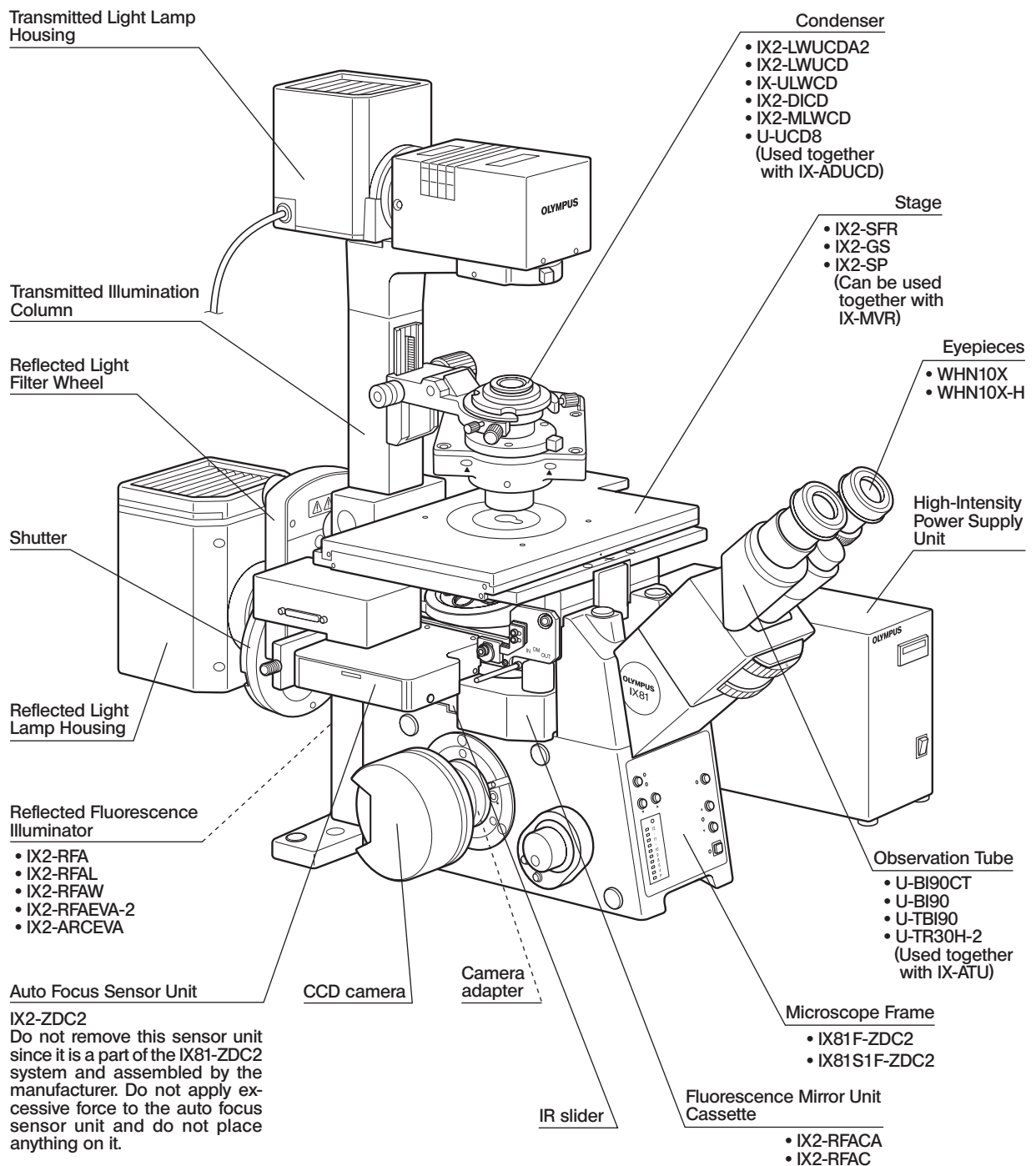


- Optimum phase contrast observation is not available when the IX2-ILL30 transmitted light illumination column and a phase contrast observation (PH1 or PH2) are used.
- AF does not function normally when the U-LH100 transmitted light IR lamp housing is used. To achieve AF, it is required to close the shutter or turn off the light during AF.
- The IBMU/IBML temperature retention box cannot be used because a clearance is produced between the temperature retention box and transmitted light illumination column.
- When performing autofocusing in the continuous focus mode, use the provided IR slider to prevent the IR laser from leaking into the observation light. Note that, when this slider is used, fluorescent dyes with IR-domain absorption wavelengths (700 nm or more) can become unusable or their brightness is reduced.  
When this slider is used, the fluorescence mirror unit used in the DIC observation should be the U-MDICT.
- If the I2-ZDC2PCB control board for exclusive use with IX81-ZDC2 is installed on the IX2-UCB control box, uninstall other focus control boards.

# 1 MODULE NOMENCLATURE

©The IX81-ZDC2 can be combined with the same modules as the IX81 microscope systems, but certain modules are subject to restrictions with regard to the combination with the stages, thermal incubation boxes (IBMU/IBML) and intermediate attachments.

In addition, troubles such as “obscuring” or “insufficient peripheral brightness” may occur depending on combination of modules (see “6. Restrictions on combined modules” on page 5).

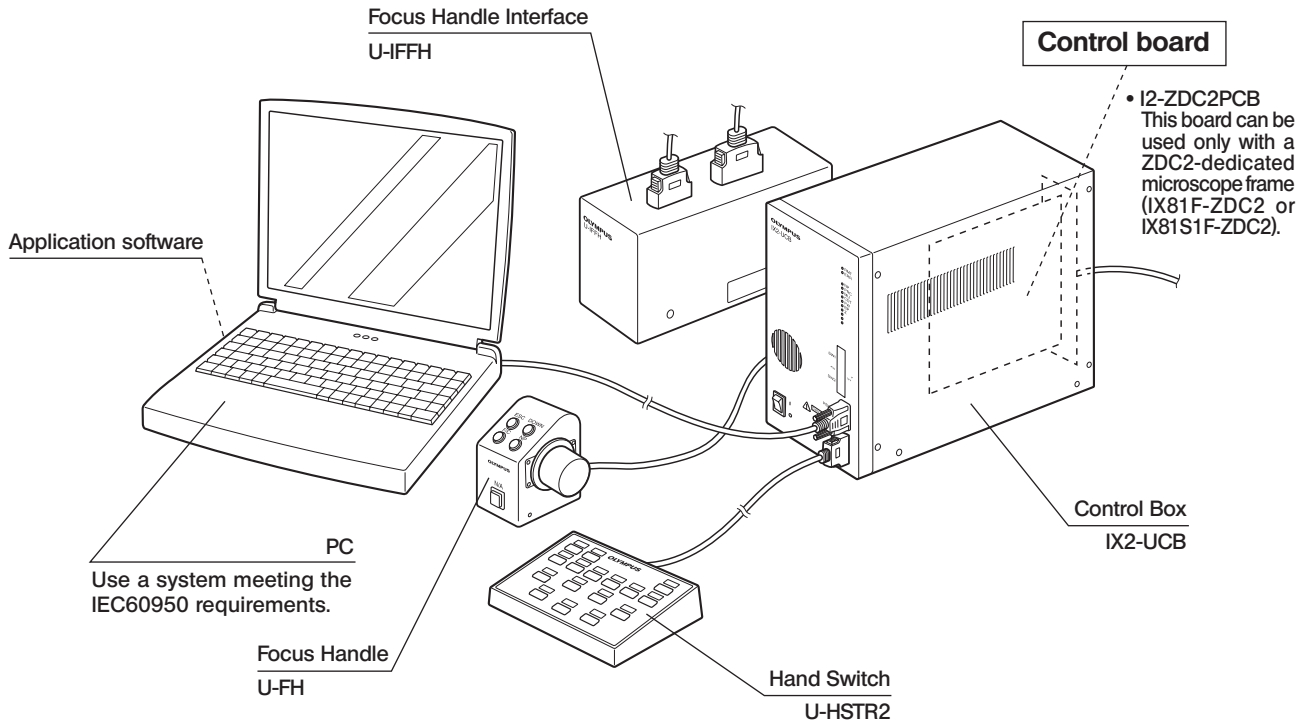
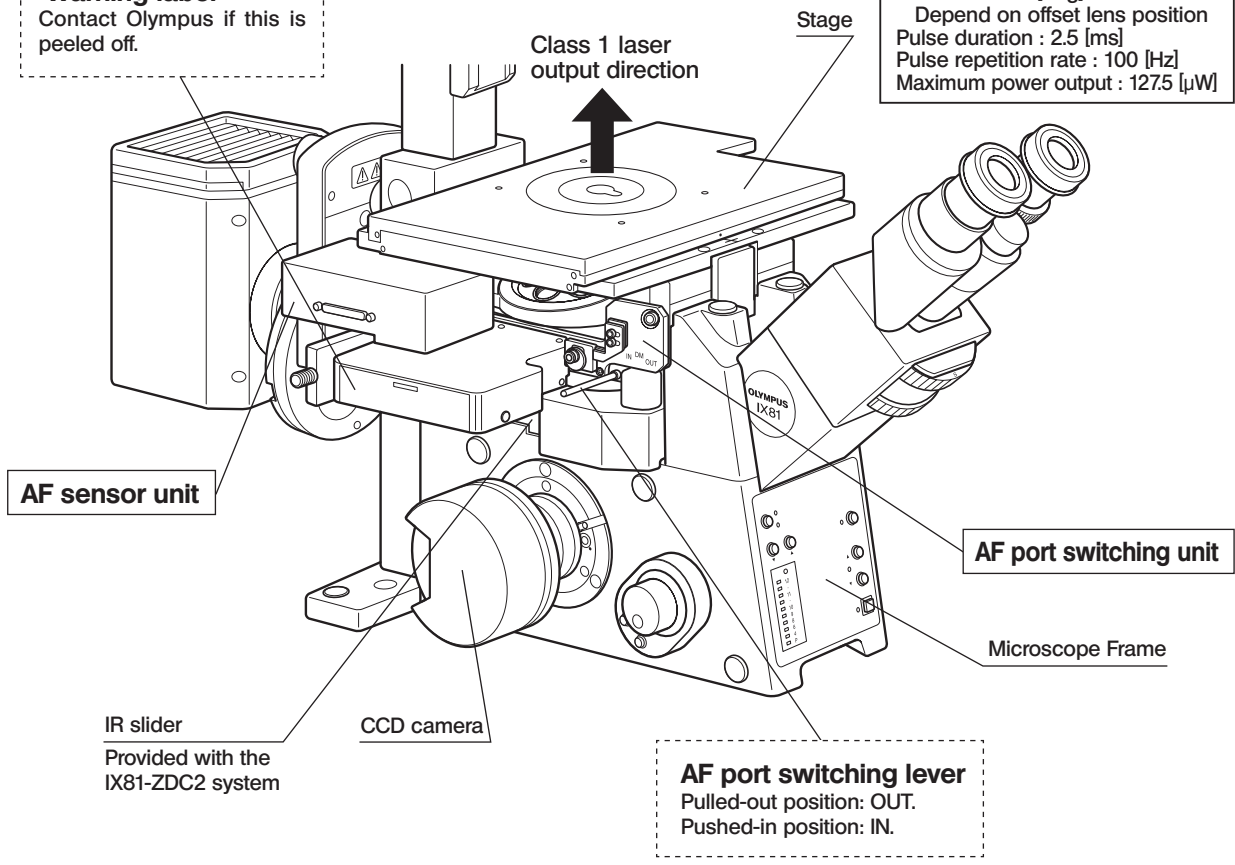


# 2 NOMENCLATURE

◎ The module names enclosed in    are involved in focus drift compensation.  
 The assembly and adjustments are to be done by Olympus.  
 Also when the system is going to be transported and reinstalled, consult Olympus.

Wavelength : 785 [nm]  
 Beam divergence :  
 ① Objectives attached  
 0 to 1.49 [NA] \*  
 Depend on objective lens  
 ② Objectives removed  
 -0.14 to 0.03 [deg]  
 Depend on offset lens position  
 Pulse duration : 2.5 [ms]  
 Pulse repetition rate : 100 [Hz]  
 Maximum power output : 127.5 [ $\mu$ W]

**Warning label**  
 Contact Olympus if this is  
 peeled off.



# 3 OPERATION

## 3-1 Operating Precautions

The Class 1 laser beam that is output from the objective is not hazardous (but direct staring for an extended period is still inhibited), and the laser beam intensity will not increase even in the case of malfunction. However, to prevent an incident, make sure not to tilt the glass bottom dish so that the reflected laser beam will not be scattered around.

## 3-2 Focus Drift Compensation Procedure

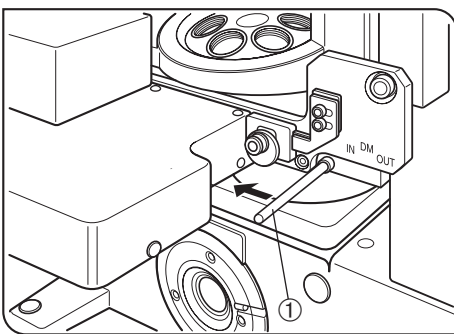


Fig. 1

### 1 Preparation

(Fig. 1)

1. Set the power switches of the IX2-UCB control box and PC to "I" (ON).
2. Set the AF port switching lever ① of the AF sensor unit to the pushed-in position (IN).

Ⓞ This operation engages the dichroic mirror (DM) in the light path of the AF sensor unit, making AF detection possible.

If AF detection is not necessary, setting the lever ① to the OUT position makes it possible to eliminate the small loss of the observation light. This is also effective in observation with IR light (785 nm or higher wavelengths).

### 2 Adjusting the Correction Collar

If you use an objective with collection collar, the following adjustment is necessary.

1. How to perform correction for bottom thickness of the glass-bottom dish:
 

When the thickness of the bottom of the glass-bottom dish is known, match the scale reading of the correction collar to the thickness of the glass bottom.
2. How to find the optimum position based on image resolution and contrast:
  - If the thickness of the glass-bottom dish is unknown, the optimum position for the collection collar can be obtained by judging the image resolution and contrast. When a satisfactory image is not obtained after focusing, rotate the correction collar to the left and right, refocus each time and compare the images at both sides. Then rotate the collar in the direction yielding a better image, and rotate the collection collar to the left and right, refocus each time and compare the images. Repeat this cycle until the position with the optimum image is found.

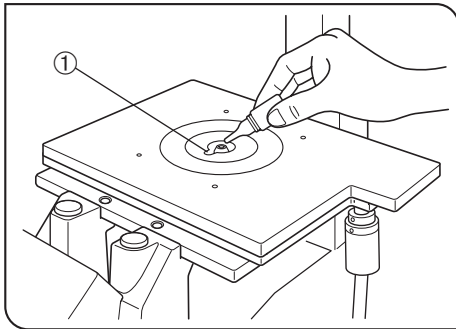


Fig. 2

### 3 Using Oil- or Water-Immersion Objective (Fig. 2)

☉ If you use an oil-immersion objective, use immersion oil as described below.

**CAUTION** Always use immersion oil supplied by Olympus.

☉ If the objective in use can accommodate the oil-proof cap, be sure to mount the cap.

1. Using a low-power objective, bring the specimen into focus.
2. Rotate the revolving nosepiece to engage the oil immersion objective.
3. Remove the specimen and move the stage insert cut-out ① close to the objective front lens. Apply a drop of the provided immersion oil to the objective front lens. Place the specimen and rotate the fine adjustment knob to bring the specimen into focus.

**CAUTION** • Use as little oil as possible. After gently wiping off the oil on the oil-proof cap, remove the cap. Then clean the tip of the objective and areas around it as well as the cap.

• If the oil contains air bubbles, the image will be degraded. Make sure the oil is free of air bubbles.

a) To check for air bubbles, remove the eyepieces, completely open the field iris diaphragm and aperture iris diaphragm, and look at the objective exit pupil (looking like a bright circle) in the observation tube. Any air bubbles can be seen in this way.

b) To remove air bubbles, slightly rock the revolving nosepiece manually to engage and disengage the oil immersion objective once or twice.

4. After use, remove immersion oil from the objective front lens by wiping with gauze slightly moistened with absolute alcohol.

☉ The same procedure is applicable when using a water immersion objective.

**CAUTION** Caution on using the immersion oil:

If immersion oil comes into contact with your eye or skin, immediately take the following action.

For eye: Rinse with clean water (for more than 15 minutes).

For skin: Wash with soap and water.

If the appearance of the eye or skin changes or pain continues, immediately consult your doctor.

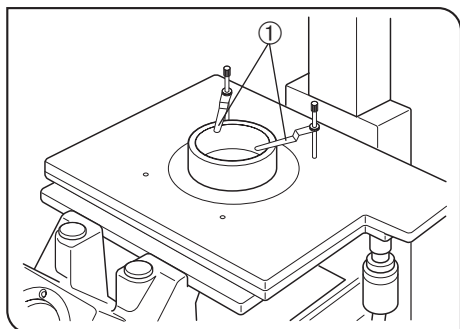


Fig. 3

#### 4 Placing the Specimen (Fig. 3)

Put the specimen in the glass-bottom dish (with cover glass thickness of 0.16 to 0.18 mm) and place the dish on the center of the stage.

- Ⓞ If the dish is prone to slide on the stage, attach the stage clips (IX-SCL) ① or use the provided weight.

#### 5 Setting the Focus Drift Compensation Function

1. Run the "Application Software for PC."
2. Set up the AF (autofocusing) functions.

The following parameters should be set to start AF.

##### In one-shot mode

- Offset : Setup of the amount of compensation between the cover glass top surface and target position.
- AF zone : AF search zone (AF center, width and range)

##### In continuous focus mode

Confirm that the cover glass top surface is detected in the one-shot mode before switching to the continuous focus mode.

Ⓞ For details, refer to the instruction manual and Help of the application.

#### CAUTION

When using the MetaMorph application software, install IX2-BSW Ver. 1.7 (in the CD provided with this product) in your PC in advance.

# 4 SPECIFICATIONS

Item	Specification
Applicable microscope frame	IX81F-ZDC2, IX81S1F-ZDC2
Applicable observation tubes	U-BI90-CT, U-BI90, U-TBI90, U-TR30H (in combination with the IX-ATU)
Applicable control box	IX2-UCB (Control board and Application Software for PC (see page 1) are required.)
Controller	PC
Focus detection method	Pupil-division reflection active autofocus using a laser diode and 2-division detector. Laser diode: 785 nm (Class 1: IEC60825-1, Class I : CDRH) Laser pulse duration: 0.5 to 2.5 ms. Frequency: 100 Hz. Momentary maximum output power; 1275 [ $\mu$ W]
Applicable objectives	Refer to the list of applicable objectives (page 4).
Observation method	Reflected light fluorescence observation is recommended. TIRF. DIC (compatible only with one-shot focus) *The focus drift compensation function is not applicable to the phase contrast and polarized light observations.
Field number	22
AF focusing speed (One-shot focus mode only)	Approximately 1 to 5 sec. from the focusing range (Excluding the offset drive time) (Note: The AF speed may slow down depending on the PC setup.)
AF repeatability	<ul style="list-style-type: none"> <li>• 20X and 40X objectives: <ul style="list-style-type: none"> <li><math>\pm 0.1 \mu\text{m}</math> (with constant temperature),</li> <li><math>\pm 0.17 \mu\text{m}</math> (with less than 5°C temperature change)</li> </ul> </li> <li>• 60X objective or 40X/60X/100X oil-/water-immersion objectives: <ul style="list-style-type: none"> <li><math>\pm 0.07 \mu\text{m}</math> (with constant temperature),</li> <li><math>\pm 0.1 \mu\text{m}</math> (with less than 5°C temperature change)</li> </ul> </li> </ul>
Dimensions & weight	IX81-ZDC2 system : 303 (W) x 313 (H) x 434 (D) mm., 19.2 kg (42.2 lb) ( AF sensor unit : 76 (W) x 76 (H) x 201 (D) mm., 0.9 kg (2 lb) ) (* Image-forming lens section/excluding the connector )
Operating Environment	<ul style="list-style-type: none"> <li>• Indoor use.</li> <li>• Altitude: Max. 2000 meters.</li> <li>• Ambient temperature: 18 to 40°C (64.4 to 104°F)</li> <li>• Maximum relative humidity: 80% for temperatures up to 31°C (88°F), decreasing linearly through 70% at 34°C (93°F), 60% at 37°C (99°F), to 50% relative humidity at 40°C (104°F).</li> <li>• Supply voltage fluctuations: <math>\pm 10\%</math>.</li> <li>• Pollution degree: 2 (in accordance with IEC60664)</li> <li>• Installation (overvoltage) category: II (in accordance with IEC60664)</li> </ul>

# 5 TROUBLESHOOTING GUIDE

Under certain conditions, performance of the microscope 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, please contact Olympus for assistance.

Problem	Cause	Remedy	Page
a) Autofocusing is impossible.	The AF port switching lever is not set to IN.	Set it to IN.	8
	Bubbles are present in the oil or water.	Remove the bubbles.	-
	An objective other than specified is in use.	Use another objective or use manual focusing.	4
	The objective is mounted improperly.	Screw in the objective firmly.	-
	The transmitted light bulb generates infrared light.	Close the shutter or turn off the light bulb during AF.	5
	A plastic bottom dish is in use.	Use a glass bottom dish.	3
	Electrical noise is interfering with operation.	Ground the IX2-UCB. Be also sure to ground the ancillary equipment.	1
	The glass bottom dish has a large scratch.	Use another glass bottom dish or use manual focusing.	-
	A phase contrast objective is in use.	Use the specified objective.	4
	Immersion oil (or water) of oil- or water-Immersion objective is dried.	Supply immersion oil (or water).	-
	A water-immersion objective is used and the interface between the water and glass bottom dish is focused.	Set the AF zone so that its center is located on the interface between the culture fluid and glass bottom dish and the AF zone width is $\pm 10 \mu\text{m}$ .	10
b) Autofocusing takes long time or fails.	AF is set improperly.	Set up AF properly. (For the setting method, refer to the instruction manual for the application software.)	10
	Electrical noise is interfering with operation.	Ground the IX2-UCB. Be also sure to ground the ancillary equipment.	1
	There is significant vibration in the in the installed environment, or an anti-vibration bench is not used.	Be sure to use an anti-vibration bench.	-
	The specimen is vibrating.	Lock the specimen firmly. Also confirm that the stage center plate is free of abnormality.	-
c) AF is applied to a position deviated from the target position.	The offset value is input improperly.	Enter the correct offset value. (For the setting method, refer to the instruction manual for the application software.)	10
	Immersion oil (or water) of oil- or water-Immersion objective is dried.	Supply immersion oil (or water).	-
	A water-immersion objective is used and the interface between the water and glass bottom dish is focused.	Set the AF zone so that its center is located on the interface between the culture fluid and glass bottom dish and the AF zone width is $\pm 80 \mu\text{m}$ .	10
d) The specimen moves in the X-Y directions during autofocusing.	The specimen is not locked.	Lock the specimen by placing a weight on the glass bottom dish, for example. Also confirm that the stage center plate is free of abnormality.	-

# **OLYMPUS®**

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**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, Center Valley, Pennsylvania 18034-0610, 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, Melbourne, Australia

**OLYMPUS LATIN AMERICA, INC.**

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

