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Lx 400 Fluorescence Attachment
User Manual

To ensure correct use of this instrument and to avoid injury during operation, read this manual completely before use.

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INTRODUCTION

The reflected-light fluorescence microscope uses a dichroic mirror that directs the excitation light through the objective to illuminate the specimen and provide efficient fluorescence observation. This microscope is used to study various cells and organisms that fluoresce when excited by particular wavelengths of incident light.

PRINCIPLE

The dichroic mirror is positioned at an angle of 45° to the optical axis of the incident excitation light. The excitation light is reflected toward the objective, and other, unwanted wavelengths pass through the mirror. When the specimen is illuminated with the excitation wavelength, it emits light of a longer, visible wavelength. The dichroic mirror passes most of this light to the eyepiece, while the barrier filter mounted between the dichroic mirror and the eyepiece blocks unwanted light to provide a black background.

The spectral characteristics of the dichroic mirror positioned at 45° to the optical axis are shown in Fig. B.

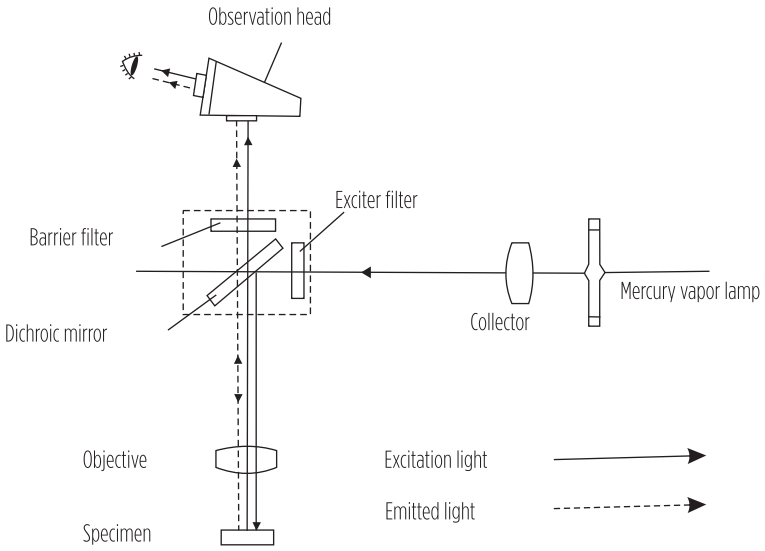


Fig. A

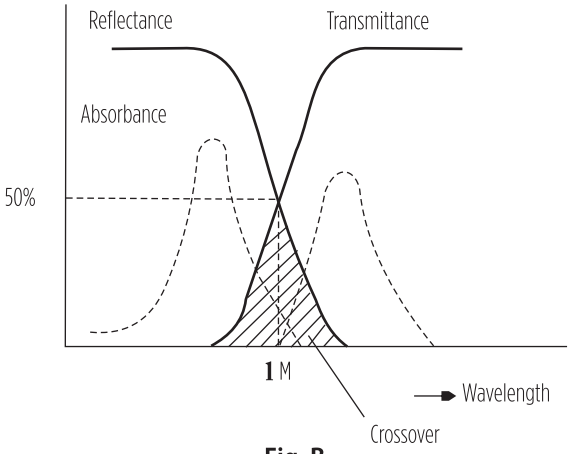


Fig. B

PACKAGING CONTENTS

1. Illumination lamp housing
2. Sliding filter block (blue, green and open)
3. Power supply unit
4. Objective set (4x, 10x, 40x, 100x)
5. Power cable
6. Connecting cable
7. UV shield
8. Immersion oil bottle (for use with the 100x objective)
9. Mercury vapor lamp
10. UV shield fixing screw
11. Allen key
12. The power supply unit may look different depending on your region or country.

SAFETY INSTRUCTIONS

- The fluorescence attachment is manufactured in accordance with CE safety standards and regulations.
- The fluorescence attachment is intended for use only as prescribed in this manual.
- Make sure the main switch on the power supply unit is set to OFF (O) before connecting power to the unit.
- The power supply unit contains high-voltage components. Do not attempt to disassemble the unit when it is turned ON (I).
- To avoid injury, do not remove the lamp housing while the lamp is lit. Also, do not turn the lamp ON while the lamp housing is removed, to avoid damaging the instrument.
- Before opening the lamp housing to replace the lamp, set the main switch to OFF (O) and unplug the lamp housing connecting cord from the output connector on the power supply unit. Wait at least 10 minutes, or until the lamp housing has cooled down, before making any changes.
- Do not open the lamp housing while the system is turned ON, as any contact with internal parts may cause injury.
- A used mercury burner must be disposed of in compliance with the ordinances or regulations of your national or local government.
- The manufacturer will not accept any liability for damage caused by any user following unsafe practices with the instrument.

⚠ Avoid using the lamp beyond its service life. The mercury lamp contains high-pressure gases. If the lamp is used beyond its service life, stress may accumulate inside the lamp and, in the worst but very rare case, the lamp may burst.

INSTALLATION

Unpack all parts from the original packaging and check that all items in the packing list are included. We recommend keeping the original packaging for storing the attachment parts when they are not in use.

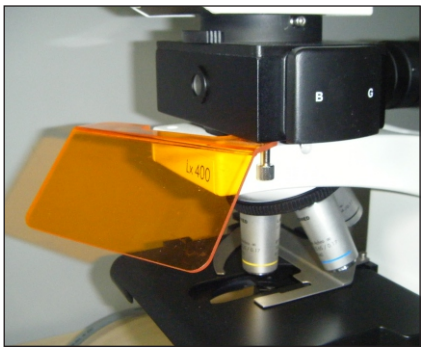


Fig. 1

1. Remove the UV shield from its packing and attach it to the base of the sliding filter block with the thumbscrew provided in the kit.

   Fix the UV shield by tightening the thumbscrew clockwise, as shown in Fig. 1.

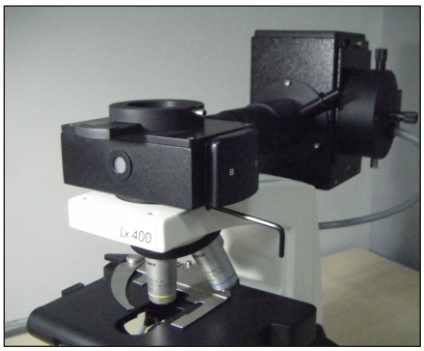


Fig. 2

2. Remove the observation head from the dovetail adapter and fit the sliding filter block in place of the observation head by tightening the screw (an Allen key is provided in the kit), as shown in Fig. 2.

   Make sure that the attachment fits firmly in the dovetail adapter, with no tilt or play.

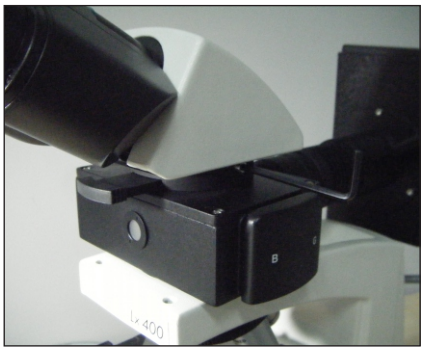


Fig. 3

3. Place the observation head on top of the filter block and secure it by tightening the thumbscrew, as shown in Fig. 3.

   Check that the observation head fits correctly in the dovetail adapter.

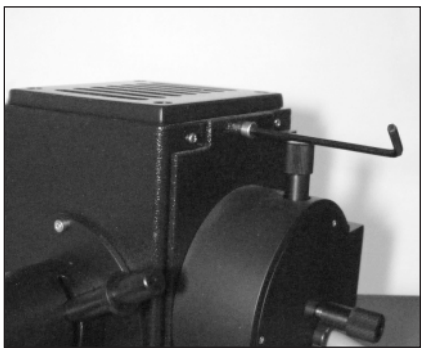
INSTALLATION

4. Remove the power supply unit from its packaging and make sure there is no physical damage to the connecting terminals or switches.

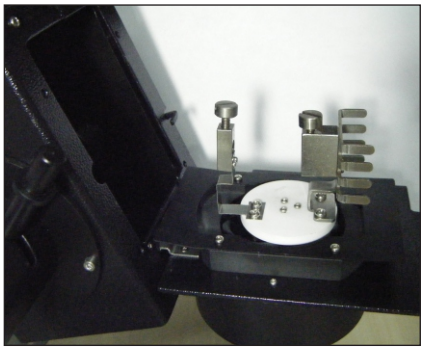


Fig. 4

5. Loosen the thumbscrew indicated by the arrow in Fig. 5 and gently pull the lamp door out of the lamp housing.


Fig. 5

6. To prevent damage to the mercury vapor lamp, a plastic rod is fitted in its place during transit. Remove this rod by loosening the two screws indicated by the two arrows in Fig. 6.


Fig. 6

7. Mount the mercury vapor lamp in place of the plastic rod and tighten the screws to secure the lamp, as shown in Fig. 7. Insert the thicker end of the mercury lamp into terminal A and the thinner end into terminal B, as shown in Fig. 7.

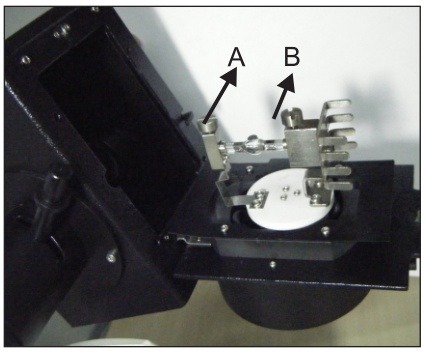


Fig. 7

8. Do not touch the lamp, to avoid leaving fingerprints on the glass. If there is any residue on the surface of the lamp, gently wipe it off with soft tissue paper.

INSTALLATION

9. To connect the fluorescence attachment, remove the connecting cable from its packaging and insert the three-pin female connector into the input terminal of the lamp housing door, as shown in Fig. 8.



Fig. 8

10. Next, connect the other female end of the connecting cable to the male output terminal on the rear panel of the power supply unit, as shown in Fig. 9.



Fig. 9

11. Finally, connect the power cable from the appropriate power outlet (either 110V or 220V) to the AC inlet socket on the rear panel of the power supply, as shown in Fig. 10.



Fig. 10

MAINTAINING OPERATING LIFE

- Make sure that the power supply unit is connected to the correct supply voltage and line frequency.
- Set the main switch on the power supply unit to (I). After the lamp has ignited, allow at least 5 minutes for the light to stabilize.
- To avoid shortening the operating life of the power supply unit, do not turn the power ON while the lamp is not mounted.
- Avoid turning the power ON and OFF at short intervals, as this may reduce the working life of the power supply.
- After the lamp has been turned OFF, do not re-ignite it before the mercury vapor has cooled and condensed. Wait approximately 10 minutes before turning the fluorescence attachment ON again.

CENTERING THE LIGHT BEAM

First allow the mercury vapor lamp at least five minutes to reach full brightness (it is very important to give the system enough time to ignite fully, to obtain a fully illuminated centering beam). When the lamp is fully lit, a beam of light is visible through the beam centering window (arrow 1, Fig. 11) on the front of the sliding filter block.

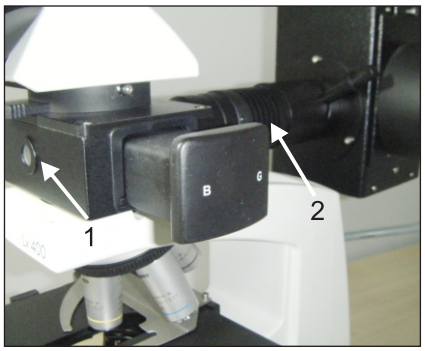
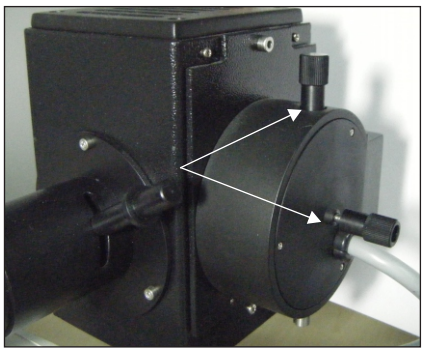


Fig. 11

Adjust the sharpness of this beam with the condenser knob (arrow 2, Fig. 11) until a focused beam appears in the window.

To center this beam of light in the window, use the two centering knobs on the lamp housing, indicated by the two arrows in Fig. 12. Adjusting these two knobs shifts the focused beam. Move the beam to the center of the window, as shown in Fig. 12.


Fig. 12

OBSERVING A SPECIMEN

- Turn the power supply ON. Allow the lamp at least 5 minutes to reach full brightness.
- Place the specimen slide on the stage. Select the excitation light with the sliding filter block as follows:

  For G excitation: pull the sliding filter block to its outermost position.

  For B excitation: push the sliding filter block to its innermost position.

  For O light (non-excited light): set the sliding filter block to its middle position.
- Observe the specimen slide through the eyepieces of the microscope.
- When fluorescent light is not required, turn the power supply of the attachment OFF and set the sliding filter block to its middle (neutral) position. To view the specimen normally with the microscope illumination, turn the microscope ON.


Fig. 13

TROUBLESHOOTING

	Problem	Cause	Remedy
	Optical system		
1)	The mercury lamp lights up, but the field of view remains dark	The filter slider is not engaged properly in the light path.	Set the filter to the required position. This procedure is shown on page 7.
2)	The image is unclear, blurred or has poor contrast.	a) Objectives and/or filters are dirty. b) The field iris diaphragm is not fully open.	Clean them with tissue or lens paper. Adjust the field iris diaphragm so that the image circumscribes the field of view.
3)	The field of view is vignetted or not evenly illuminated.	a) The objective is not engaged properly in the light path.	When rotating the nosepiece, make sure that the objective clicks into position.
		b) The filter slider is not engaged properly in the light path.	Engage the required filter properly in the light path.
		c) The mercury lamp is not centered properly.	Center the mercury lamp correctly. This procedure is shown on page 7.
		d) The beam focus deviates from the correct position.	Adjust the condenser knob. This procedure is shown on page 7.
4)	The mercury lamp does not light after the power supply is switched ON.	Some mercury lamps may not ignite the first time the power supply is turned ON.	Press the Trigger button on the front panel of the power supply unit and wait for the lamp to ignite.
	Electrical system		
1)	The lamp housing or main switch indicator does not light up.	a) The power cord is not connected properly.	Make sure the power cord is firmly in place.
		b) The fuse has blown.	Replace it with a fuse of the same type and rating.
2)	The main switch indicator lights up, but the mercury lamp remains OFF.	a) The connecting cable is not engaged properly.	Make sure the connection is secure.
		b) The mercury lamp is not mounted.	Install the mercury lamp as described on page 5.
3)	The light flickers intermittently.	a) The lamp has not been given enough time to reach full brightness.	Allow at least 5 minutes after turning the power supply ON for the lamp to reach full brightness. If the flickering persists, press the Trigger button.
		b) The service life of the lamp has expired.	Replace the mercury lamp (part no. 3127010).

TECHNICAL SPECIFICATIONS

Type	:	Reflected-light fluorescence illumination with sliding dichroic mirror cubes
Observation modes	:	B excitation, G excitation and open field (O)
Lamp type	:	HBO 50 W high-pressure mercury vapor lamp
Supply voltage	:	110V AC, 60 Hz / 220V AC, 50 Hz
Operating environment	:	Indoor use Ambient temperature 5°C to 40°C Maximum relative humidity 80% at 40°C

FILTER SPECIFICATIONS

B excitation

Excitation filter	:	Transmission 90% at wavelengths of 420 to 480 nm. Transmission 0% at wavelengths from 485 nm.
Dichroic mirror	:	Transmission 93% at wavelengths from 540 nm. Transmission 0% at wavelengths of 400 to 530 nm.
Barrier filter	:	Transmission 95% at wavelengths from 535 nm. Transmission 0% at wavelengths of 400 to 500 nm.
Recommended fluorescent dye	:	Acridine orange, both DNA and RNA
Alternative fluorophores	:	5-Carboxyfluorescein (5-FAM) 5-Carboxytetramethylrhodamine (5-TAMRA) 5-FAM (5-Carboxyfluorescein)

G excitation

Excitation filter	:	Transmission 85% at wavelengths of 490 to 525 nm. Transmission 0% at wavelengths from 530 nm.
Dichroic mirror	:	Transmission 90% at wavelengths from 600 nm. Transmission 0% at wavelengths of 400 to 595 nm.
Barrier filter	:	Transmission 92% at wavelengths from 615 nm. Transmission 0% at wavelengths of 400 to 575 nm.
Recommended fluorescent dye	:	Acridine Red FM™ 5-95
Alternative fluorophores	:	Ethidium bromide LDS 751 (DNA) Nitrobenzoxadiazole

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