Oxion Inverso

fluorescence

euromex
microscopes holland

user manual

v.430231

Introduction

With your purchase of this inverted microscope of the Oxion Inverso range you have chosen for a quality product. The Oxion Inverso microscopes are developed for use at universities and laboratories

The maintenance requirement is limited when using the microscope in a decent manner. This manual describes the construction, how to use it and maintenance of the microscope

Contents

1. General safety instructions

Intended use: a non-medical device
This microscope is intended for general observation of cells and tissues, with transmitted/reflected illumination and with the specimen fixed on a slide

1.1 Dangers associated with the operation

- Improper use could result in injury, malfunction or damage to property. It must be ensured that the operator informs every user of existing hazards
- Danger of electrocution. Disconnect the power to the entire lighting system before installing, adding or changing any component
- Not to be used in corrosive or explosive environments
- Avoid direct exposure of eyes to the collimated light beam or direct light from the light guides or fibres
- To avoid a hazard to children, account for all parts and keep all packing materials in a safe place

1.2 Photobiological safety LED, important safety instructions

- Avoid direct eye exposure to any LED light source while switched on
- Before looking through the eyepieces of the microscope, lower the intensity of the LED illumination
- Avoid long and high-intensity exposure to LED light because this may cause acute damage to the retina of the eye

1.3 Photobiological safety instructions fluorescence light sources

- Fluorescent light sources - such as HBO mercury vapor lamps or LED - can be harmful to human eyes, especially ultraviolet and violet light
- Therefore, always mount and use the orange protection shield, supplied with the fluorescence attachments, when applicable
- Operators must close the shutter of the fluorescence attachment, equipped with an HBO mercury vapor illumination, or switch off the LED for fluorescence, when observation of the sample is postponed for a longer time
- Avoid direct exposure of the eye to any fluorescent light source while switched on
- Before looking through the eyepieces of the microscope, lower the intensity of the LED for fluorescence illumination
- Avoid long and high-intensity exposure to LED light because this can cause acute damage to the retina of the eye
- Mercury vapor lamps ***must*** be replaced when reaching a maximum of 200 hours (due to explosion hazard) and properly disposed of, in accordance with local regulation. When replacing the lamp, safety goggles must be used
- Mercury vapor lamps are always under high pressure, even when cool. When turning on a mercury light bulb, it needs to stay on for at least 15 minutes before switching it off. Do not switch it on again for at least 30 minutes, so it has plenty of time to cool down. In the event of a broken bulb, immediately vacate the area for at least 30 minutes before returning

1.4 Prevention of biological and infectious hazards

Infectious, bacterial or viral biohazard substances under observation may be a risk to the health of humans and other living organisms. Special precautions should be taken during in vitro medical procedures:

- **Biological hazards**: keep a logbook of all the biological substances or pathogenic microorganisms that were under observation with the microscope and show it to everybody before they use the microscope or before they do some maintenance work on the microscope! Agents can be bacterial, spores, enveloped or non-enveloped virus particles, fungi or protozoa
- **Contamination hazard:**
  - A sample that is properly enclosed with a cover glass never comes in direct contact with the microscope parts. In that case prevention of contamination lies in the handling of the slides; as long as the slides are decontaminated before use and are undamaged and treated normally, there is virtually zero risk of contamination

- A sample that is mounted on a slide without cover glass, can come in contact with components of the microscope and may be a hazard to humans and/or the environment. Therefore, check the microscope and accessories on possible contaminations. Clean the microscope surfaces and its components as thoroughly as possible. Should you identify a possible contamination, inform the local responsible person in your organisation
- Microscope operators could be contaminated from other activities and cross-contaminate components of the microscope. Therefore, check the microscope and accessories on possible contaminations. Clean the microscope surfaces and its components as thoroughly as possible. Should you identify a possible contamination, inform the local responsible person in your organisation. it is recommended to wear sterile gloves when preparing the slides and handling the microscope in order to reduce contamination by the operator

- **Infection hazard:** direct contact with the focusing knobs, stage adjustments, stage and eyepieces/tubes of the microscope can be a potential source of bacterial and/or viral infections. The risk can be limited by using personal eyeshades or eyepieces. You can also use personal protections such as operation gloves and/or safety goggles, which should be changed frequently to minimize the risk
- **Disinfectant hazards:** before cleaning or disinfecting, check if the room is adequately ventilated. If not, wear respiratory protective gear. Exposure to chemicals and aerosols can harm human eyes, skin and respiratory system. Do not inhale vapours. During disinfection, do not eat, drink or smoke. Used disinfectants must be disposed of according to local or national regulations for health and safety

1.5 Disinfection and decontamination:

- Exterior casing and mechanical surfaces must be wiped with a clean cloth, dampened with a disinfectant
- Soft plastic parts and rubber surfaces can be cleaned by gently wiping a clean cloth, dampened with a disinfectant. Discoloration can occur if alcohol is used
- The front lens of eyepieces and objectives are sensitive to chemicals. We recommend not to use aggressive disinfectants but to use lens paper or a soft fibre-free tissue, damped in cleaning solution. Cotton swabs may also be used. We recommend you use personal eyepieces without eyeshades in order to minimize risk
- Never immerse or dip the eyepiece or objective into a disinfectant liquid! This will damage the component
- Never use abrasive compounds or cleaners that may damage and scratch optical coatings
- Properly clean and disinfect all possible contaminated surfaces of the microscope or contaminated accessories before storing for future use. Disinfection procedures must be effective and appropriate
- Leave the disinfectant on the surface for the required exposure time, as specified by the manufacturer. If the disinfectant evaporates before the full exposure time, reapply disinfectant on the surface
- For disinfection against bacteria, use a 70% aqueous solution of isopropanol (isopropyl alcohol) and apply for at least 30 seconds. Against viruses, we recommend to refer to specific alcohol or non-alcohol based disinfection products for laboratories

Before returning a microscope for repair or maintenance through a Euromex dealer, an RMA (return authorization form) together with a decontamination statement must be filled in! This document - available from Euromex for any reseller- must be shipped together with the microscope at all times

Reference documents:

World Health Organisation:
https://www.who.int/ihr/publications/biosafety-video-series/en/
Robert Koch Institut:
https://link.springer.com/content/pdf/10.1007/s00103-013-1863-6.pdf
US Centre for Disease Control and prevention
https://www.cdc.gov/infectioncontrol/guidelines/disinfection/index.html

Handle with care

- This product is a high quality optical instrument. Delicate handling is required
- Avoid subjecting it to sudden shocks and impacts
- Impacts, even small ones, can affect the precision of the instrument

Handling the LED

Note: Always disconnect the power cord from your microscope before handling the LED bulb and power unit and allow the system to cool down approximately 35 minutes to avoid burns

- Never touch the LED with your bare hands
- Dirt or fingerprints will reduce the life span and can result in uneven illumination, lowering the optical performance
- Use only original Euromex replacement LEDs
- The use of other products may cause malfunctions and will void warranty
- During use of the microscope the power unit will get hot; never touch it while in operation and allow the system to cool down approximately 35 minutes to avoid burns

Dirt on the lenses

- Dirt on or inside the optical components, such as eyepieces, lenses, etc., affects the image quality of your system negatively
- Always try to prevent your microscope from getting dirty by using the dust cover, prevent leaving fingerprints on the lenses and clean the outer surface of the lens regularly
- Cleaning optical components is a delicate matter. Please, read the cleaning instructions further on in this manual

1.6 Environment, storage and use

- This product is a precision instrument and it should be used in a proper environment for optimal use
- Install your product indoors on a stable, vibration free and level surface in order to prevent this instrument to fall thereby harming the operator
- Do not place the product in direct sunlight
- The ambient temperature should be between 5 to +40°C and humidity should be within 80% and 50%
- Although the system is anti-mold treated, installing this product in a hot, humid location may still result in the formation of mold or condensation on lenses, impairing performance or causing malfunctions
- Never turn the right and left focus knobs in opposite directions at the same time or turn the coarse focus knob past its farthest point as this will damage this product
- Never use undue force when turning the knobs
- Make sure that the microscope system can dissipate its heat (fire hazard)
- Keep the microscope away from walls and obstructions for at least approximately 15 cm
- Never turn the microscope on when the dust cover is in place or when items are placed on the microscope
- Keep flammable fluids, fabric, etc. well out of the way

Disconnect power

Always disconnect your microscope from power before doing any maintenance, cleaning, assembling or replacing LEDs to prevent electric shocks

Prevent contact with water and other fluids

Never allow water or other fluids to come in contact with your microscope, this can cause short circuiting your device, causing malfunction and damage to your system

Moving and assembling

- This microscope is a relatively heavy system, consider this when moving and installing the system
- Always lift the microscope by holding the main body and base of the microscope
- Never lift or move the microscope by its focusing knobs, stage or head
- When needed, move the microscope with two persons instead of one

2. Construction of the microscope

The names of the several parts are listed below:

A. Eyepiece
B. Diopter adjustment
C. Photo/video switch
D. Coaxial coarse- and fine adjustment
E. Photo/video tube
F. UV protection shield
G. Condenser with lamp unit on top
H. Phase slider
I. Filter holder
J. Object stage with mechanical stage
K. HBO Lamp/fluorescence unit
L. X/Y mechanical stage controls
M. On/Off switch
N. Intensity control for brightfield illumination

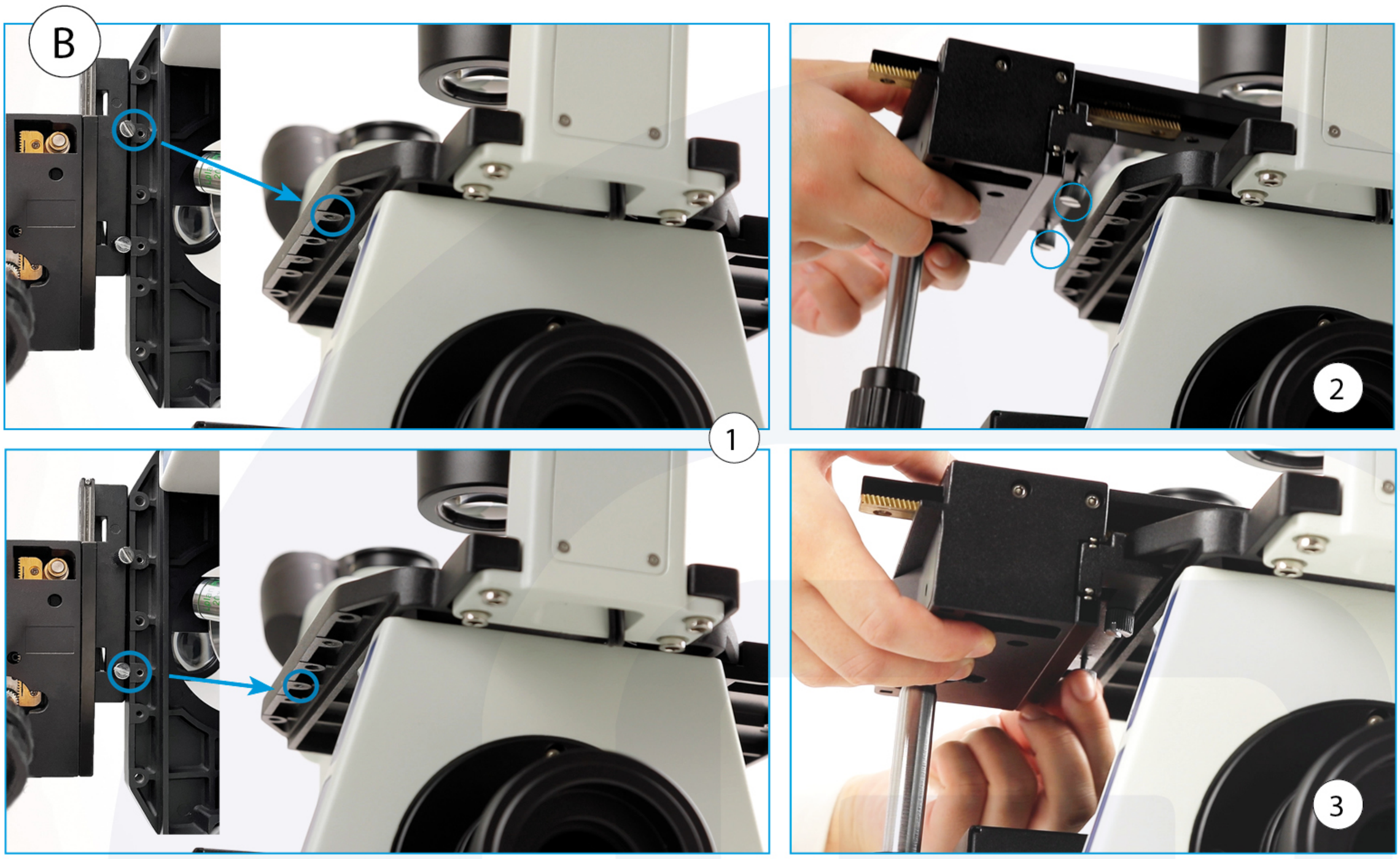
3 Assembling the microscope

3.1 Installing the stage extension

Attach the stage extension plate, using holes **A1** and **A2** underneath the stage

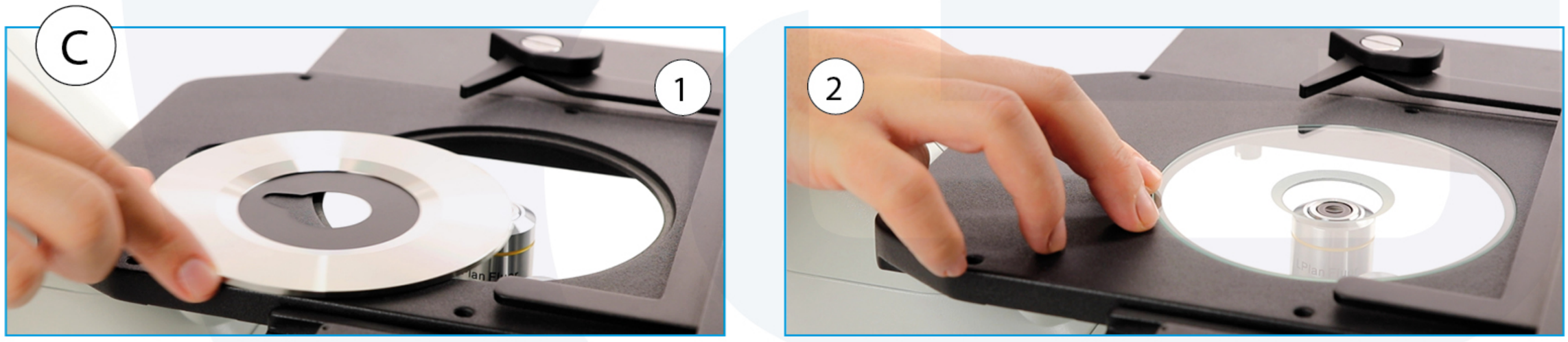
3.2 Installing the x-y stage control

Attach the stage control unit on the other side of the stage as shown below



The fluorescence models are supplied with either a flat object stage (250 x 230 mm), or a stage (250 x 230 mm) with a coaxial mechanical 120 x 78 mm X-Y stage

3.3 Placing the stage plates (fig. C1, 2)



3.4 Installing the eyepieces (fig. D)

1. Remove the protection caps
2. Insert the eyepieces
3. Deploy the eyeshades

3.5 Set light source with mercury bulb

- Insert the light source with mercury bulb **(E1)** into fluorescence connector **(E2)**, and rotate to level the top horizontally
- Then tighten the screw **(E3)** with Allen screwdriver
- Use the second Allen screwdriver to tighten the screw on the other side **(E4)**
- Insert the filter holder **(E5)** into the slot **(E6)** after first having removed the screw
- Refasten the screw

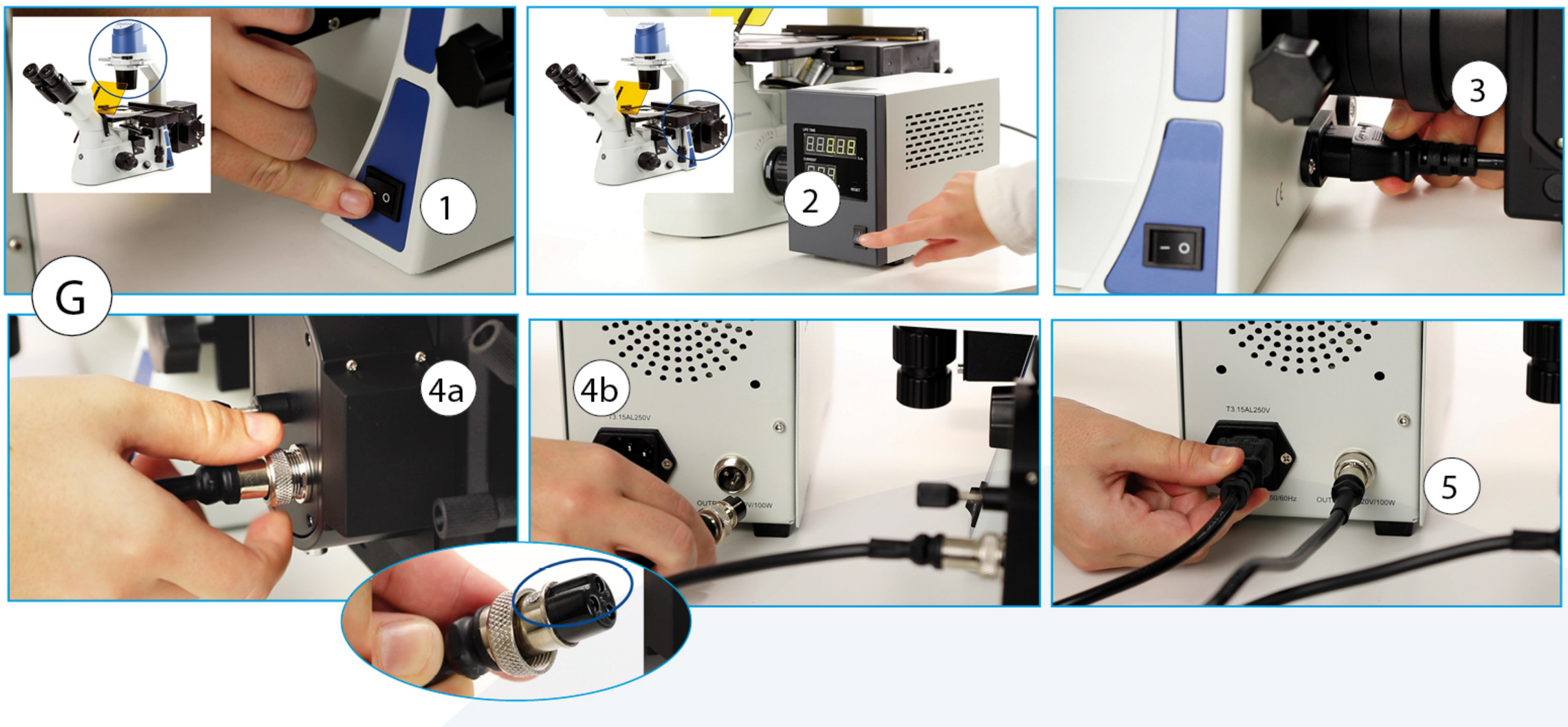
3.6 Putting up the UV absorbtion screen (F)

Two ways to place the UV-absorbtion screen:

3.7 Connecting the power supply

- Ensure the main switch and the mercury lamp power unit switch are on “O”(OFF) (**G1** and **G2**)
- Insert the power cable to the microscope (**G3**)
- Insert the aviation plug to the mercury lamp box on one side (**G4a**) and the external power unit on the other side (**G4b**) and lock both with screw
- Insert the power cable to power unit (**G5**)

The fluorescence power supply is 100-240V



3.8 Inserting the filter slider and the phase contrast slider (H)

3.9 Adjustments

3.9.1 Condenser iris for brightfield (I1)

3.9.2 Light intensity adjustment (transmitted light) (I2)

3.9.3 Selecting the fluorescence filters in epi-illumination (I3)

4 How to use the microscope

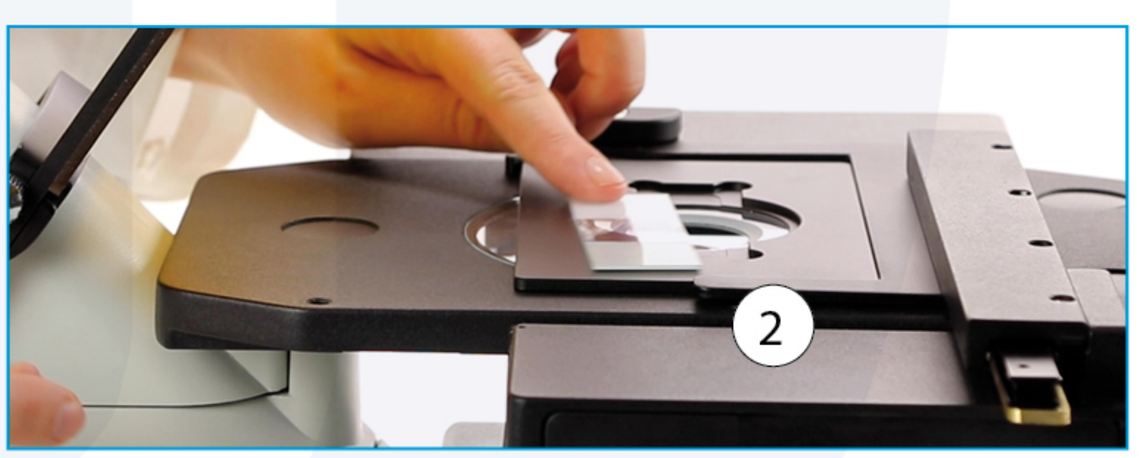
4.1 Switching on

1. Turn on the power for brightfield and phase contrast applications
2. Turn on the power for fluorescence applications. Allow it approx. 5 minutes to reach the correct temperature

Note: There needs to be at least 15 minutes between turning on the HBO bulb (for fluorescence) and turning off, then at least 15 minutes before turning on again

4.2 Using a slide

When using a slide, install the supplied slide and cell culture dish holder (**J1**). Place the slide upside down (**J2**)



4.3 Setting the interpupillary distance (K)

In order to obtain a smooth "compound" image as well as the right interpupillary setting

Caution: The maximum light intensity when using the 4x and 10x can damage the eyes!

Field of view before adjustment

Field of view after adjustment

4.4 Setting the diopter

In order to compensate for human eye differences, distortion, thickness differences in cover glasses and tune for the best parfocality between objectives, one can use the diopter to do so. Take a good prepared slide for your reference

- Set (both) the diopter adjustments of the eyepieces to "0" (**L1**)
- Select the 10x objective, look for an interesting area on the specimen and focus on this area (**L2, 3**)
- Select the 40x objective and focus on the specimen (**L2, 3**)

Warning:
do not change the coarse and fine adjustment anymore

- With one eye open (close your other eye), rotate the diopter adjustment from "+" to "–" until the selected area gets as sharp as possible
- Take your eyes from the eyepieces and turn the diopter adjustment a few divisions back from "-" to "+", without looking into the eyepieces (**L4**)
- "Reset" your eyes by looking into the distance a few seconds
- Look again into the eyepiece and turn the diopter adjustment from '+' to '-' until the selected area on your specimen gets the optimal sharpness
- Repeat for your other eye

Verification:

- Take your eyes from the eyepieces and look for a few seconds into the distance, in order to "reset" your eyes
- Look again into the eyepieces. If the adjustment is not good, repeat the operation till you reach the same sharpness for the 10x and 40x objective without touching the coarse and micrometric adjustments

This procedure should be followed by each individual user

4.5 Setting the focusing tension (M)

if the nosepiece lowers by itself the tension is too low. Turn the dial clockwise to firm the tension

4.6 Centering the phase annuli

1. Choose the 10x objective
2. Focus on a sample and remove it (N1)
3. Replace an eyepiece for the telescope (N2)
4. Engage the 4x /10x annulus (N3)
5. Close the condenser iris (N4)
6. Focus (N5)
7. Use the rods to position and center the annulus (N6a, 6b)

Note:
Never use both rods at the same time to prevent internal damage

8. Repeat this procedure with the 40x objective and the 20x /40x annulus engaged
9. When finished, replace the telescope for the eyepiece

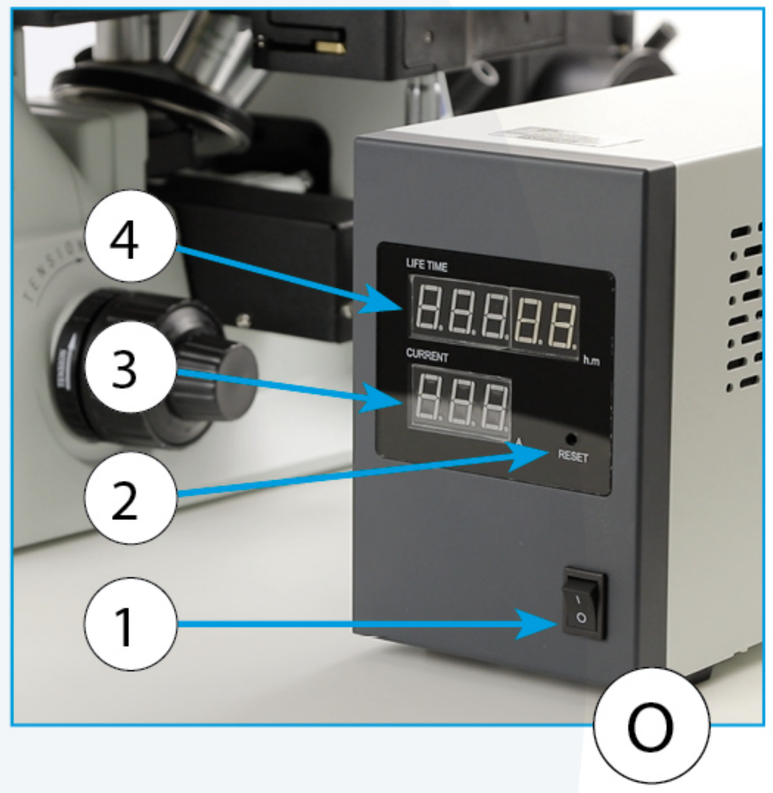
4.7 Reflected fluorescence illumination

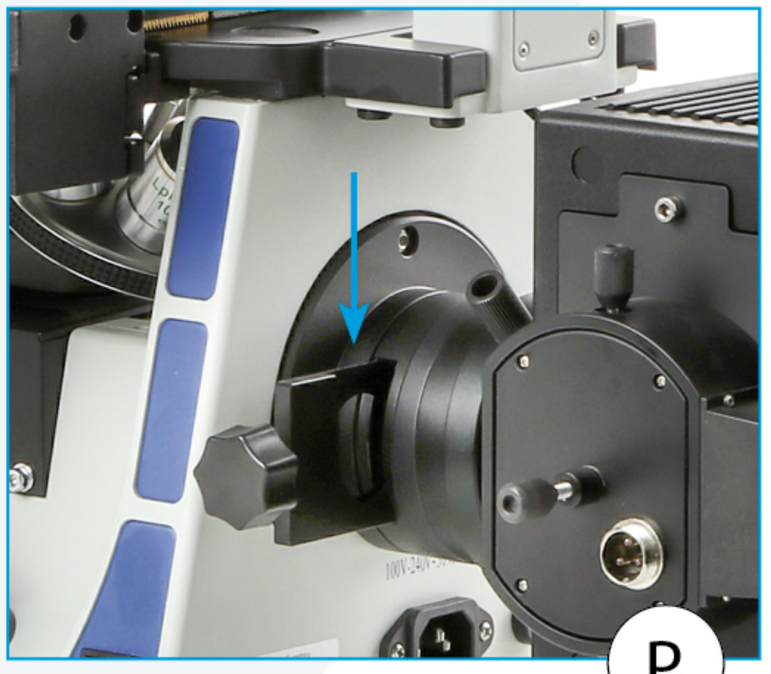
While using transmitted illumination, the way of operation is the same as the inverted biological microscope.

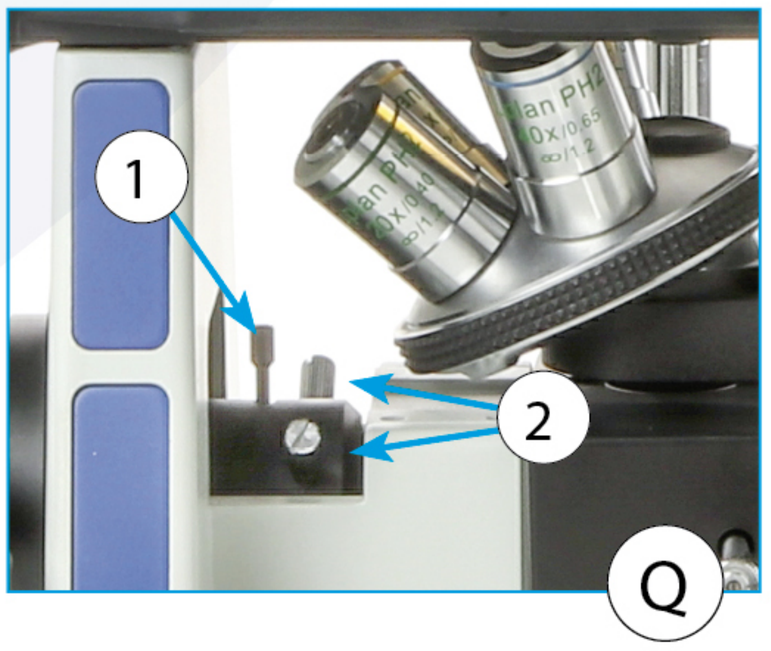
How to operate the reflected fluorescence illumination is shown below
How to center the fluorescence bulb, see **6.4.2 Centering mercury bulb**

4.7.1 Illumination

- Turn on the power supply, and switch on the mercury lamp unit. Wait for approx. five minutes for the excitation of the mercury lamp to become stable. (**O1**)
- Timer (LIFE TIME) (**O4**) displays from left to right as follows: **hour, minute,** and **seconds**. When more than five, the first five digits will be shown
- If you want to see the hidden minutes and seconds: press timer reset button (RESET) (**O2**) for five seconds. After 6 seconds it will return to the former state
- If you need to reset timer (LIFE TIME) (**O4**), please long-press the timer reset button (**O2**). After 5 seconds the timer will be cleared
- Am-meter (CURRENT) (**O3**) shows the scope from 0 ~ 9.99 A
  - ⚠ Do not cut off power supply within 15 minutes after mercury bulb light has been turned on to avoid damaging it
  - ⚠ In order to prolong the lifespan of the mercury bulb, please do not re-light it within 15 minutes after it has been turned off
  - When the timer (**O4**) indicates "200.00", it means the mercury bulb has burnt for 200 hours, the end of its estimated lifespan. Therefor replacement is due
  - ⚠ Do not stare at fluorescence light directly
  - Put the filter slider (**P1**) in the middle position for fluorescence light
  - If filter slider is pulled to far right position an extra filter can be inserted to be used in combination with the fluorescence light
  - Push the filter slider to far left position in order to protect the slide from long time exposure to fluorescence light





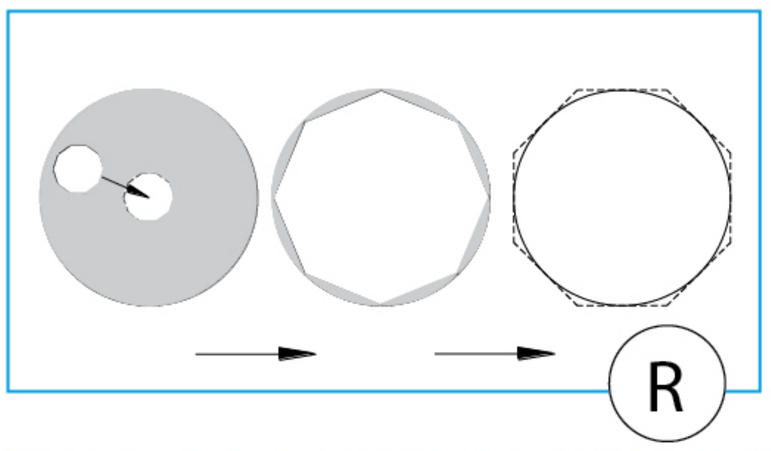


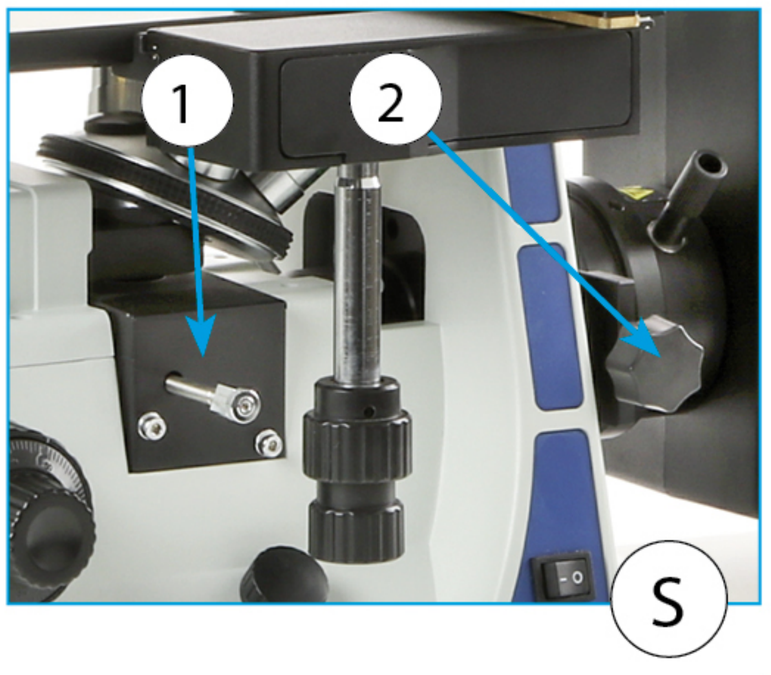
4.7.2 Field of view diaphragm

Field of view diaphragm limits the diameter of the light beam entering the condenser, thus eliminating stray light. This enhances the image contrast. When field of view diaphragm image is right at edge of the field of view, the performance of the objective is optimal, and the image is clearest

- Turn adjusting rod (**Q1**) for field of view diaphragm clockwise to open the field of view diaphragm, anti-clockwise to close it
- Observe through eyepiece to find image of field of view diaphragm
- Adjust two screws (**Q2**) at both sides of field of view diaphragm to center the image
- Open the field diaphragm gradually. If the image of field diaphragm matches the position of the field of view, it means the diaphragm is centered correctly (**R**)
- During observation, open the field diaphragm a little to have the edge fall right outside the field of view in order to obtain optimal imaging

⚠ ***Note:*** In order to prevent the fluorescence specimen to deteriorate, do not expose the same position of the specimen for a long time





4.7.3 Select fluorescence filter block

The filter block should be in accordance with specimen.
Standard filter blocks are blue, green and UV

- Pull the filter slider (**S2**) to the far right position
- Use filter exchanging rod (**S1**) to select required filter block

Note: For transmitted illumination, please pull both rods out (for OX.22 models) or pull the rod to outer position (for OX.24 models)

4.8 Magnifications

The total magnification of your microscope can be calculated by multiplying the magnification of the eyepiece with the magnification of the objective. The magnifications are displayed in the table below:

Eyepiece	Objective	Magnification
10x	4x	40x
10x	10x	100x
10x	20x	200x
10x	40x	400x
10x	60x	600x

4.9 Illumination

Halogen illumination: adjustable 50 W halogen, 90-240 V mains
Fluorescence illumination: 100 W mercury vapor

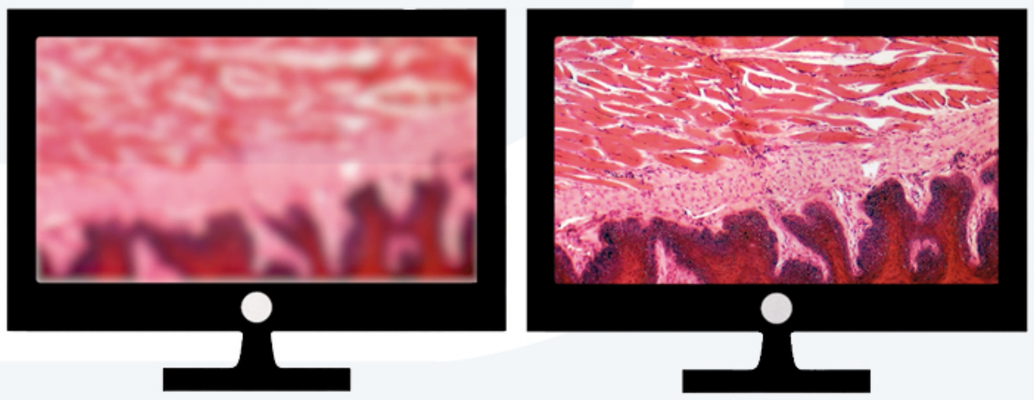
5. Photo and video

- The models can be equipped with a vertical photo tube or C-mount adapter, on which a photo or video CCD or CMOS camera can be attached
- By switching the photo/video switch (**C, p. 6**) below the eyepieces, 80% of the light is directed to the photo tube. The image remains visible through both eyepieces, with a capacity of 20%

5.1 Installation camera and parfocality

- Depending on the adapter (OX.9833 is used in illustrations), attach the adapter to the camera (**T1),** goes also for the standard adapter AE.5120-2). With some models the adapter is attached to the microscope while the separable ring is attached to the camera (Not shown in illustraions)
- Attach camera with adapter to microscope and fixate with screw (**T2**)

- Focus while looking through the eyepieces (**T3**)
- Adjust the adapter until the digital image is in focus (**T4a** OX.9833, **T4b** AE.5120-2. Fixate height of the adapter)



6. Maintenance and cleaning

Always use the dustcover over the microscope after use. Always keep the eyepieces and objectives mounted on the microscope to avoid dust entering the instrument

6.1 Cleaning the optics

When the eyepiece lens or front lens of the objectives are dirty they can be cleaned by wiping a piece of lens paper over the surface (circular movements). When this does not help put a drop of alcohol on the lens paper

Caution:
Never put isopropanol or alcohol directly on the lens!

Please note that Euromex offers a special microscope cleaning kit: PB.5275. It is not necessary – and not recommended – to clean the lens surfaces at the inner side of the objectives. Sometimes dust can be removed with high pressured air. There will never be dust in the objectives if the objectives are not removed from the revolving nosepiece

Caution:
Cleaning cloths containing plastic fibres can damage the coating of the lenses!

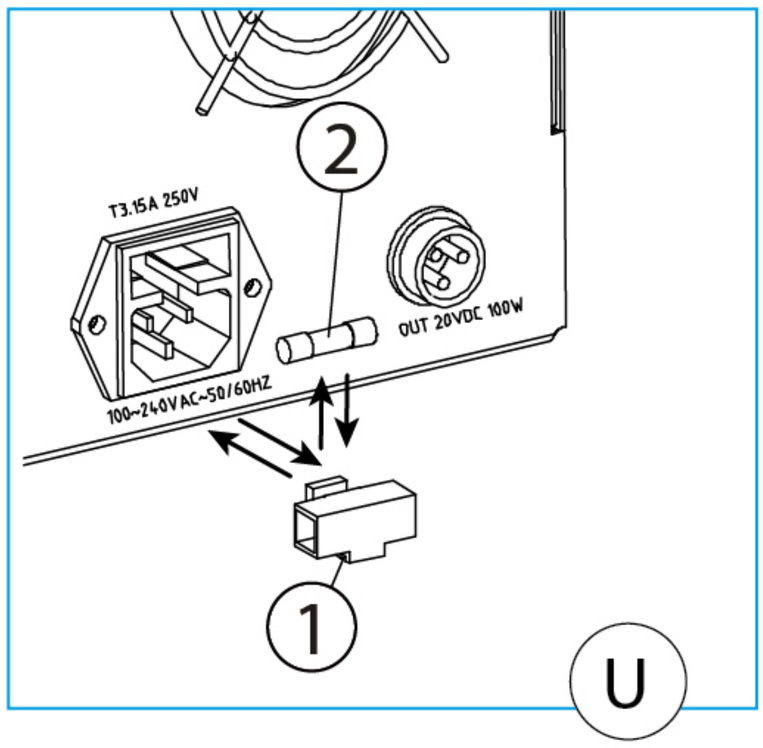
6.2 Maintenance of the stand

- Dust can be removed with a brush. In case the stand or table is really dirty the surface can be cleaned with a non-aggressive cleaning product.
- All moving parts like the height adjustment or the coaxial course and fine adjustment contain ball bearings that are not dust sensitive. With a drop of sewing-machine oil the bearing can be lubricated

6.3 Changing the fuse

Caution: Always remove the power cable from the mains supply before changing the fuse!

- Ease the fuse support (**U1**) out of the holder using a straight screwdriver.
- Insert a new fuse (**U2**) in the support, then ease the support back into the holder



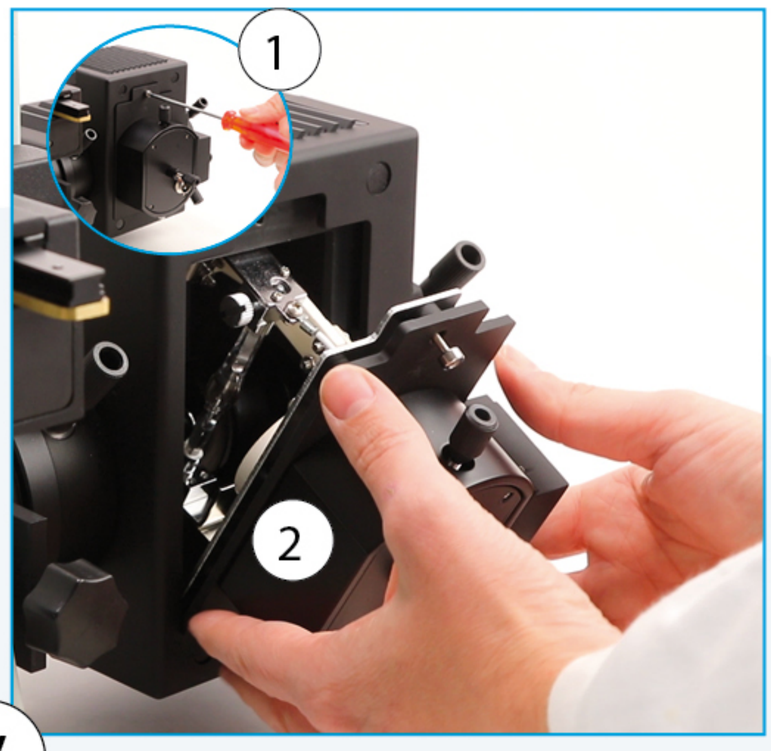
6.4 Changing and centering the mercury bulb

Visit Euromex.academy for complete document and/or video tutorial

Caution: Always remove the power cable from the mains supply before changing the bulb!

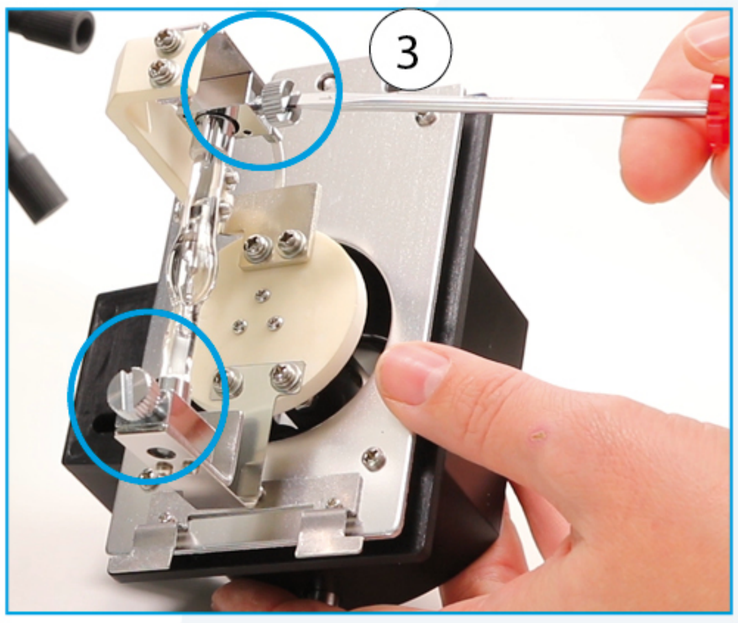
6.4.1 Installing mercury bulb

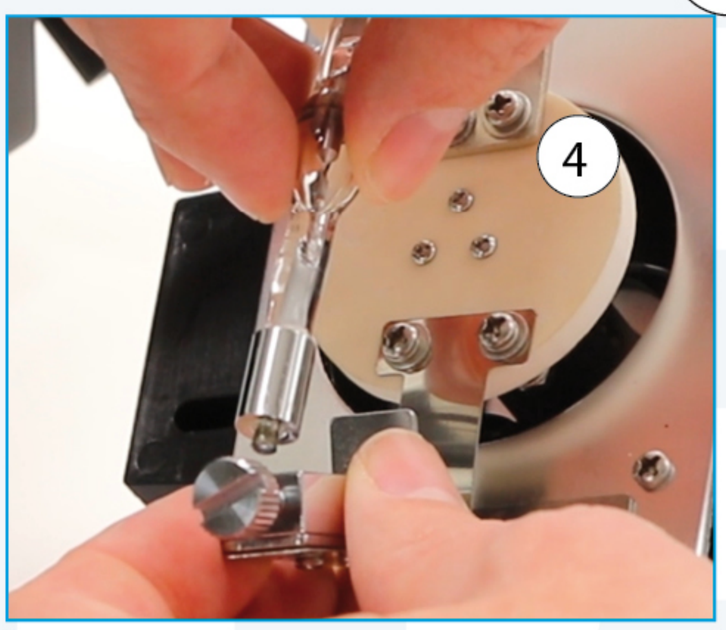
- Loosen the lock-screw (**V1**) completely (Allen tool) and take off the bulb holder completely (**V2**)
- Loosen the lock screws (**V3**) for mercury bulb and take it out (**V4**)
- Place the bulb while keeping the small and large side of the bulb in mind, then tighten the screw (**V5**)
- Put the bulb holder into its housing (**V2**) and tighten the screw (**V1**)
- Use these steps also to replace the bulb
- Replace bulb during or after operation of max. 250 hours

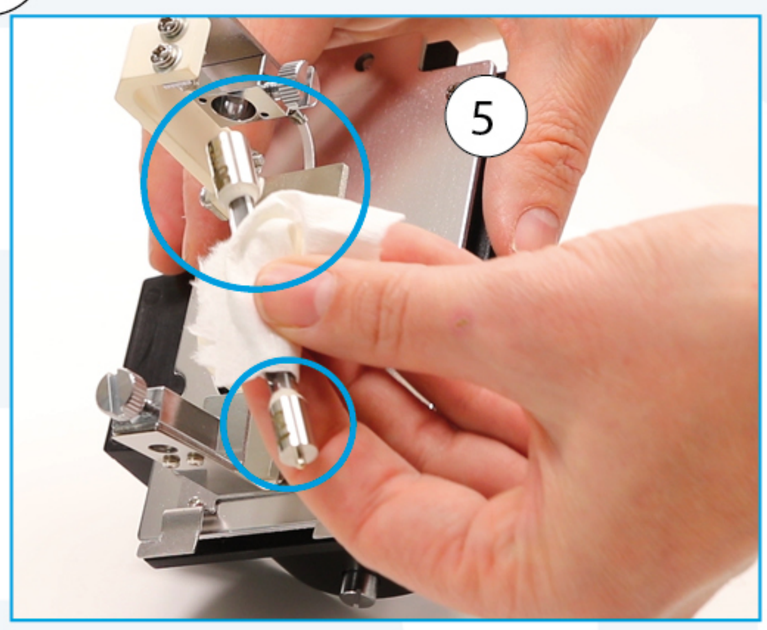


V

Note: During or just after operation, the bulb, bulb house and surrounding space may be very hot. Before replacing the bulb, please set the power supply for fluorescence at "O" (OFF) and remove power plug from the socket. After allowing the instrument to cool down, replace bulb. After replacing the bulb, please note the timer on power supply will be set at zero, by pushing the timer reset button. (**O2**, p 12)







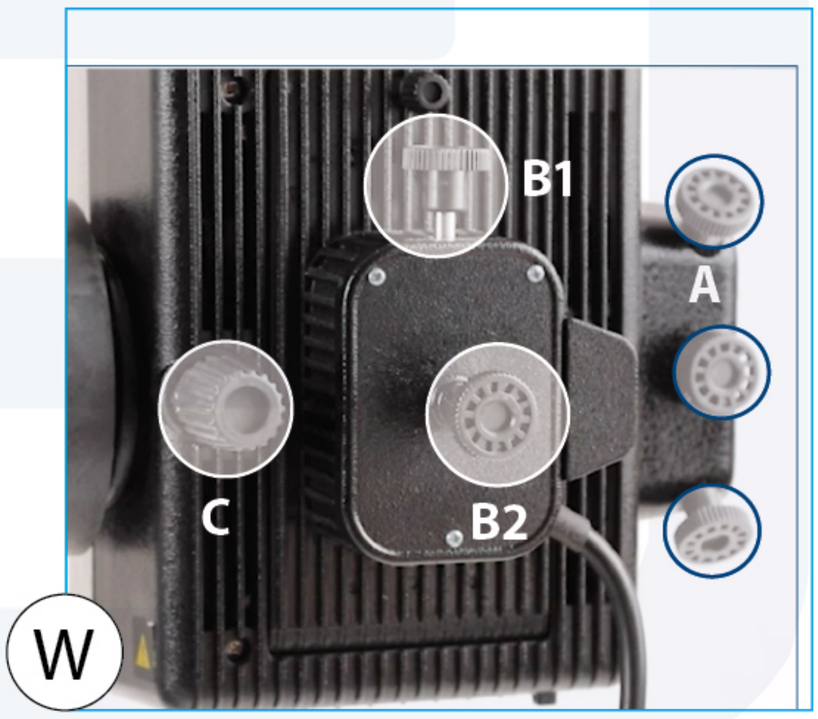
6.4.2 Centering mercury bulb

Excerpt from "alignment fluorescence"

- Place a piece of white paper with a cross painted on it over the stage so that it is in the center of the observation
- Rotate the nosepiece to a free position (without objective) to allow the beam of light to pass through its interior and reflect on the white piece of paper

fig. W

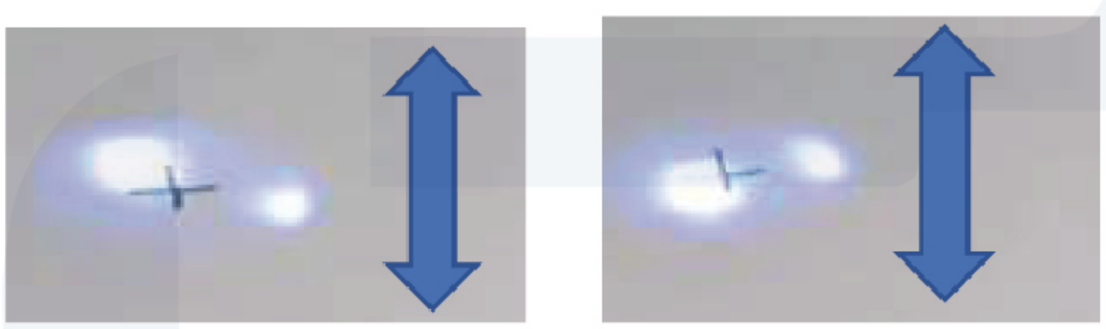
- **A.** 3 screws at the back of the lamp house, to control the position of the reflected beam
- **B.** 2 screws at the side of the lamp house, to control the position of the direct beam
- **C.** 1 screw controls the focusing



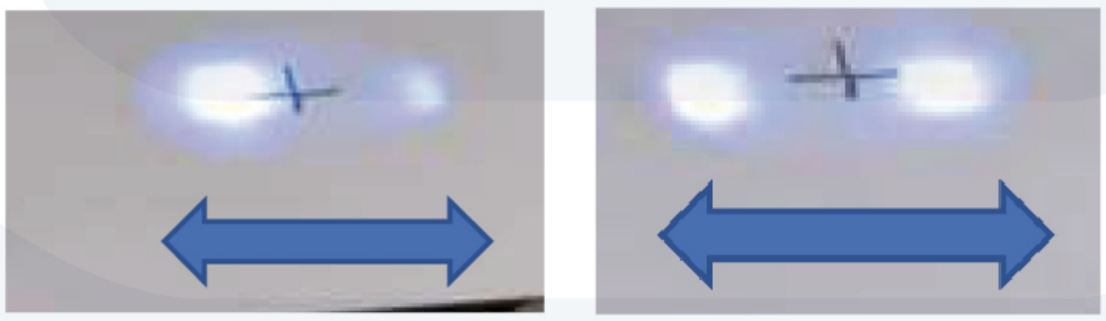
W

Normally you should see two beams: direct and reflected beams

To move the direct beam with the horizontal and vertical adjustment knobs on the side of the lamp house **(B1 and B2)**

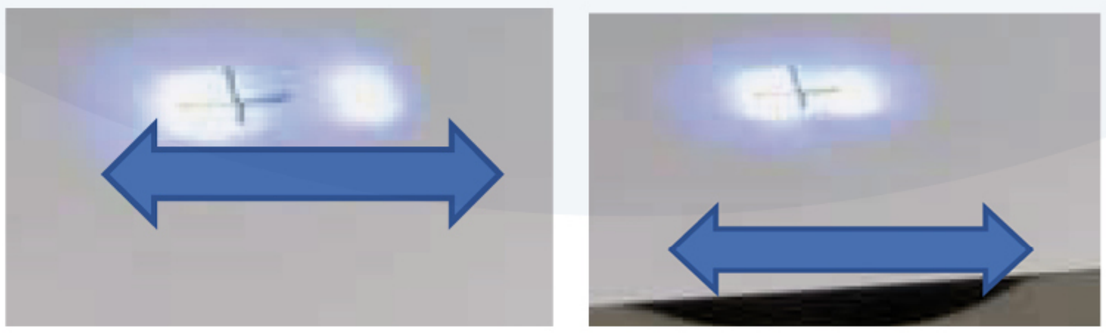


Vertical movement

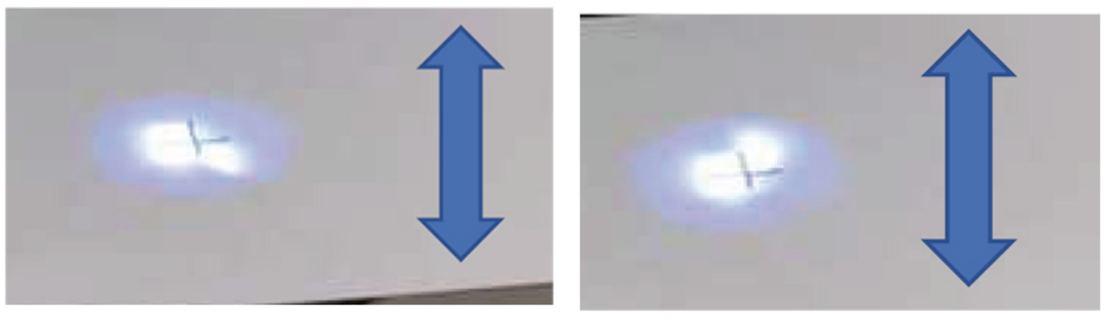


Horizontal movement

- To move the reflected beam horizontally and vertically, use the adjustment knobs at the back of the microscope **(A, if available**)



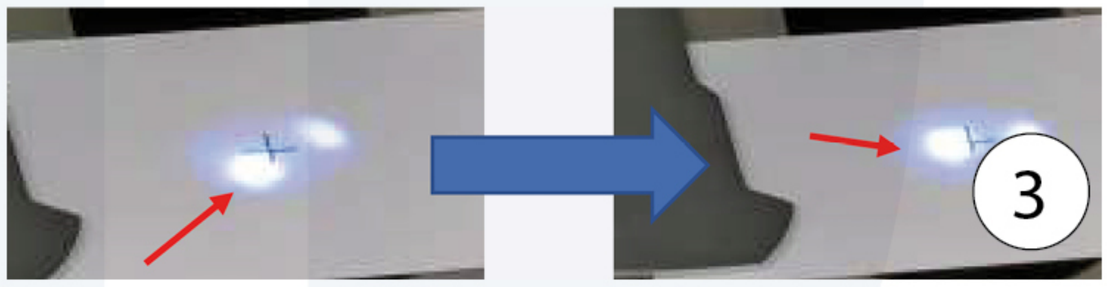
Horizontal movement



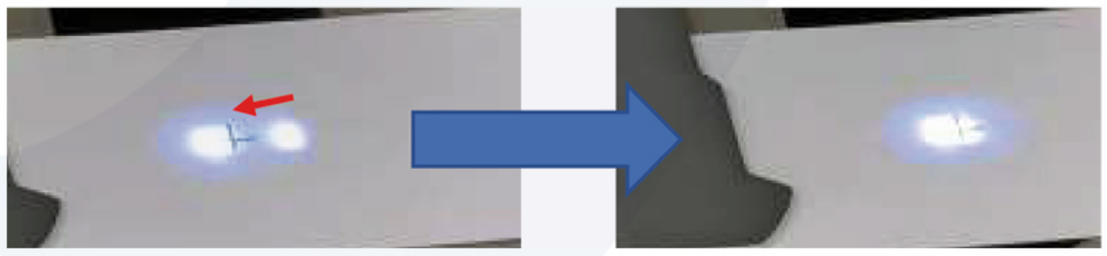
Vertical movement

To focus the reflected beam, use the middle knob at the back of the lamp house **(A, middle knob)**

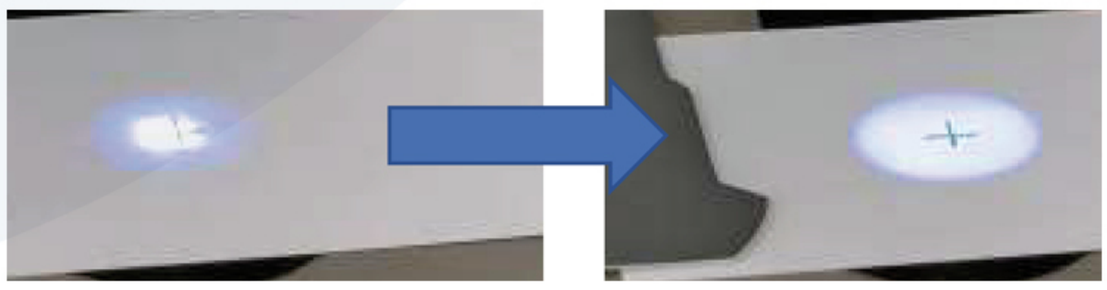
Use the knobs at the side of the lamp house to move the direct beam slightly off the center of the cross **(B1 ad B2)**



Align now both - the direct and reflected - beams so that they are in a symmetrical position, just besides each other but not completely overlapping in the middle



Then defocus with the focusing adjustment knob **(C)**



Position an objective to check the focused beam.
To focus the reflected beam, use the middle knob at the back of the lamp house **(A, middle knob)**