Routine Microscope Care and Adjustments

Your microscope is a high-precision device, and with the proper upkeep and maintenance, you will be rewarded with exceptional results.

Gain a deeper insight into routine caretaking procedures from our microscope experts. Following their advice can help ensure that you continue to enjoy high-quality images with your Olympus microscope. Whether you are working in an imaging core facility, doing research, or educating students—you can benefit from the higher-quality data and fewer sources of error offered by a carefully maintained microscope.

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Imaging Troubleshooting: 3-Minute Microscope Check

Are You Trying to Get an Image But the Screen Is Dark?

In about three minutes, this step-by-step troubleshooting guide can help you identify errors or incorrect settings that may be causing your imaging problems. If at the end of the procedure the problem is not resolved, your system may need to be more thoroughly inspected.

Here is what you should do if you are having imaging problems with your microscope system:

1. Verify the hardware (see Image 1):
   - Follow the illumination light path and check for mechanical barriers (shutters/slides/switches).
   - Check that the excitation light is working (light source on/light coming from the front lens).
   - Ensure that the optical parts (especially the front lens) are clean.
2. Verify the light path (see Image 2):
   - Fluorescence: Check that you have selected the right filters/dichroics.
   - Ensure that the detection light path is correctly guiding the signal (to the oculars or camera/detector).
3. Verify the detection (see Image 3):
   - Using a (known) standard sample or plastic slide, verify that you can acquire a signal.
   - Camera: Make sure that your exposure time is set to a range where you normally detect signals (e.g., 500 ms).
   - FLUOVIEW microscopes: Ensure that the PMT high voltage is at least at 550 V.

Are you getting a signal from the standard sample? Good, the system is working in principle. Try to adapt the settings for the sample you had difficulties with.

Still not getting a signal? Contact the person responsible for the device to do a more thorough inspection or contact a Service Center.


Image 1


Image 2

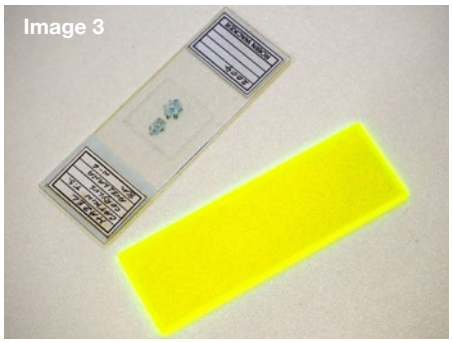


Image 3

Cleaning and Maintenance

Dust and dirt particles can sometimes be seen during observation and may affect image quality. This is particularly frustrating if dust particles are visible when photographing important specimens that cannot be photographed again. To help ensure you get the most from your microscope, cleaning should be a regular part of your maintenance routine. The following basic procedures explain how to clean the microscope and accessories, from the external frame and optical system to specimen slides.

Cleaning the Microscope Frame

When cleaning the frame, avoid touching the lens and using organic solvents that may damage any plastic parts.

Clean stains or dirt off a microscope frame in two steps:

1. First wipe the dirt with a cloth moistened with a small amount of neutral detergent.
2. Remove the dirt completely with a cloth that has been immersed in lukewarm water.

Maintaining the Cleanliness of the Optical System

Keeping the optical system clean is essential for image quality. If dust spots on optical glasses such as lenses, prisms, and filters are left unattended, the dirt can become difficult to remove and may mold. By keeping the optical surface clean, you can avoid many maintenance problems and prolong the life of your microscope.

Important: The following procedures to clean the lens surfaces only apply to exposed areas of objectives, eyepieces, filters, and condensers. If internal or major cleaning becomes necessary, please contact your Olympus microscope dealer.

Cleaning Accessories Such as Filters and Condensers

When cleaning large glass surfaces on both sides of an accessory, such as a filter:

1. Fold a lens tissue soaked in cleaning mixture in two or three layers.
2. Hold the accessory at its edges.
3. Wipe from the center toward the periphery as you slowly rotate it.

When cleaning the surfaces of the condenser and the light exit glass:

1. Hold a piece of lens tissue between your middle and index fingers.
2. Fold it, and then wrap it around your index finger.
3. Hold the tissue down with your thumb while wiping the lens surfaces clean.
4. After cleaning, examine the lens surface with a magnifying glass. If color reflected from the lens surface looks uneven, it is an indication that there are still dust specks and dirt on the lens.

   Tip: If you look through the bottom of an eyepiece, it can function as a magnifying glass.

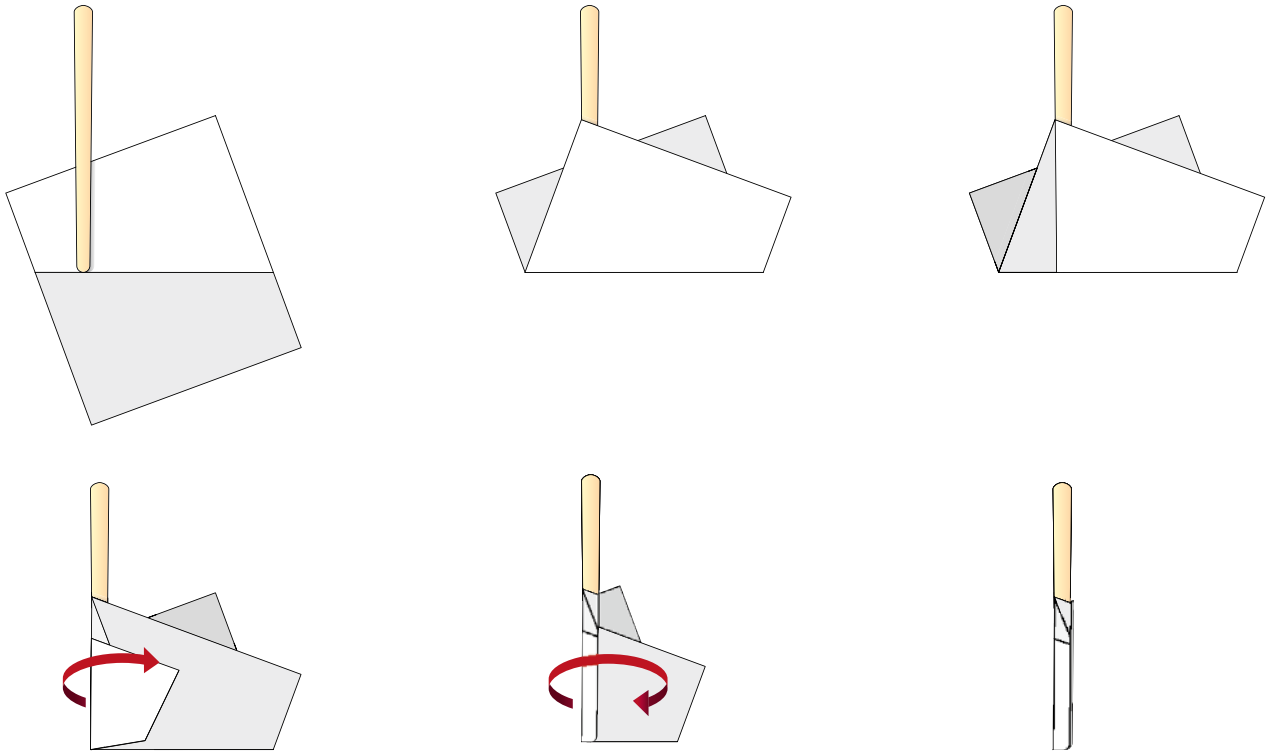
Cleaning Optical Lens Surfaces

1. To help prevent scratches on coatings and optical glass, remove dirt and dust that sticks to their surface with an air gun or blower brush.

2. Wrap the lens tissue around a bamboo stick (see the illustration below).

3. Put a small amount of lens cleaning fluid or cleaning mixture (absolute alcohol) on the tip of the lens tissue, and gently wipe the lens in a circular motion. Discard each lens tissue after a single use.

4. When cleaning a large lens surface, you can also use a lens tissue wrapped around your index finger. Wipe from the center toward the periphery in a circular motion. Always use a clean part of the lens tissue as you rotate your finger.



Cleaning a Specimen Slide with a Cover Glass

Make it a habit to clean each specimen slide both before and after observation. The removal of oil as well as routine cleaning can be done more easily if the specimen slide is removed from the stage.

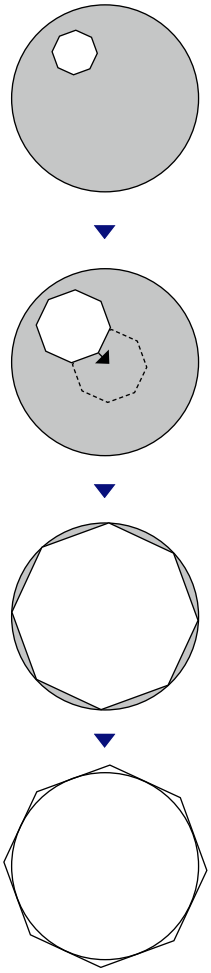
- For routine cleaning: use a soft cloth, gauze, or piece of lens tissue without cleaning liquid to wipe the slide.
  Tip: If the contamination is difficult to remove, try breathing on the slide before wiping it.
- For slides that are particularly soiled with oil: lightly moisten a cloth or cleaning tissue with cleaning mixture, making sure to apply only a small amount, as excessive fluid can seep underneath the cover glass and damage the specimen. If the oil cannot be completely removed with one wipe, continue wiping until the oil film is removed.

Köhler Adjustment—Transmitted Light Microscopy

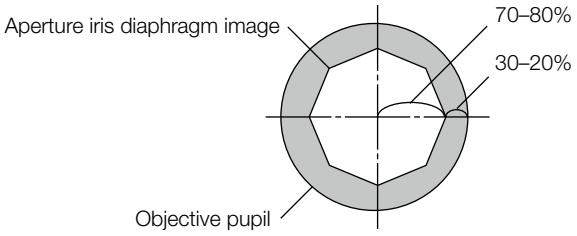
Another important procedure that should be included in your regular maintenance is checking the Köhler illumination. Köhler illumination is an optical alignment method that helps you produce an evenly illuminated visual field with excellent contrast and high sample detail, enabling you to exploit the microscope's full potential.

Upright Microscopes

1. Turn on the microscope's illumination and set the microscope to brightfield mode.
2. Remove unnecessary contrast devices, such as DIC sliders, polarizers, or other optical elements.
3. Engage the 10X objective lens (if available), place the specimen on the microscope stage, and bring it into focus.
4. If available, engage the substage condenser front lens and move it closer to the specimen until it is at the approximate condenser working distance.
5. Close the field stop until its borders become visible when observing the focused microscopic image, and fine-tune the condenser height-adjustment knob to focus the field stop diaphragm image.
6. Using the condenser centering screws, center the field stop diaphragm image.
7. Slowly open the field stop, observing the ring as it approaches the border of the field of view and stopping as soon as the field stop ring disappears.
8. Watching the back focal plane of the objective (observation without eyepiece), open the aperture diaphragm until a considerable change in contrast is noticeable in the image (NA of condenser = 0.7 to 0.8 times the NA of the objective). When 70–80% of the image is bright (see the image on the bottom right), then reinsert the eyepiece.
9. Repeat steps 6 and 7 of this procedure each time you switch to another objective lens.

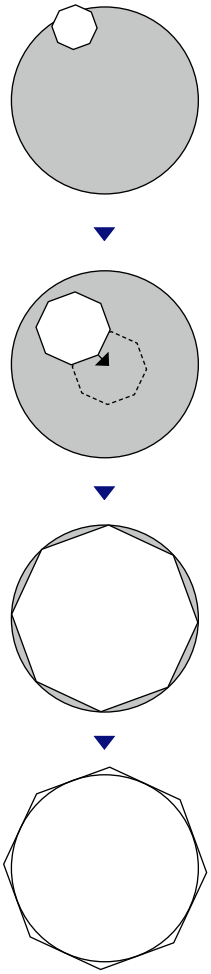


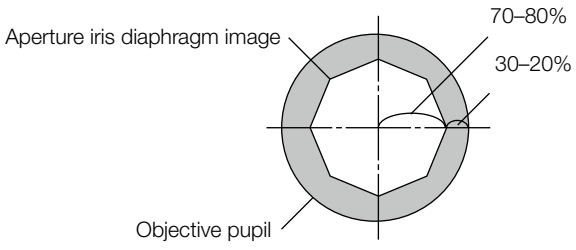
Tip: When using U-SC or UCD8(A) condensers in combination with objective lenses magnifying less than 10X, swing out the top lens of the condenser, open the aperture stop, and use the field stop as an aperture stop.



Inverted Microscopes

1. Turn on the microscope's illumination and set the microscope to brightfield mode.
2. Remove unnecessary contrast devices, such as DIC sliders, polarizers, or other optical elements
3. Engage the 10X objective lens (if available), place the specimen on the stage, and bring it into focus.
4. Move the condenser front lens closer to the specimen until it is at the approximate condenser working distance position.
5. Close the field stop until its borders become visible when observing the focused microscopic image, and fine-tune the condenser height-adjustment knob to focus the field stop diaphragm image.
6. Using the condenser centering screws, center the field stop diaphragm image.
7. Slowly open the field stop, observing the ring as it approaches the border of the field of view and stopping as soon as the field stop ring disappears.
8. Watching the back focal plane of the objective (observation without eyepiece), open the aperture diaphragm until a considerable change in contrast is noticeable in the image (NA of condenser = 0.7 to 0.8 times NA objective). When 70–80% of the image is bright (see the image on the bottom right), then reinsert the eyepiece.
9. Repeat steps 6 and 7 of this procedure each time you switch to another objective lens.





Adjusting the Parfocality of a Camera Adaptor

If you're using a camera, you should also keep in mind that you need to adapt the focus and adjust the parfocality. It's usually sufficient to do this once after installation, but if you see focus differences between the eyepiece view and the camera image, it makes sense to check.

Our Adjusting the Parfocality of a Camera Adaptor guide will show you how.

1. Before you begin the camera adaptor's parfocality adjustments, ensure that the microscope is properly adjusted for Köhler illumination.

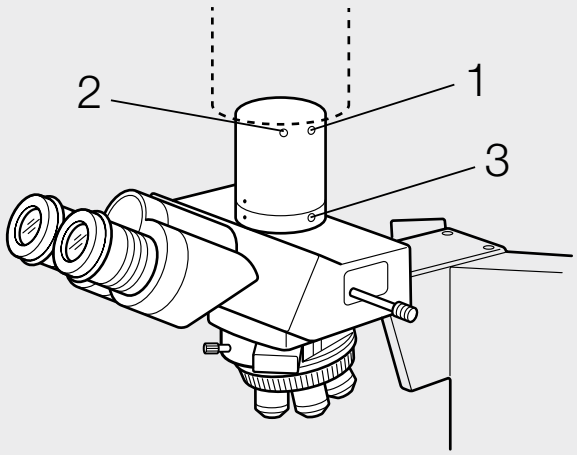
2. Observing through the eyepieces, focus precisely on a sample by adjusting the eyepiece diopters:
   - **a.** Turn the diopters to set the mark at zero.
   - **b.** Set the microscope to the lowest magnification and rough focus on the sample.
   - **c.** Set the microscope to the highest magnification setting and focus using the fine-focus knob until a sharp image is obtained. If your microscope has only one diopter on either the left or right eyepiece, use only one eyepiece without diopter for this fine-focus adjustment.
   - **d.** Without touching the focus handle, change the magnification setting to the lowest setting, and then use the diopter focusing ring to obtain a sharp focus, first for the left eyepiece, and then the right eyepiece.
   - **e.** Repeat steps c and d again to make sure that the focus for the left and right eyepieces have been adjusted for both the highest and lowest magnification settings.

3. Switch to the lowest magnifying objective lens (or lowest zoom position) without touching the focus handle of the microscope.

4. Focus the monitor image by adjusting the parfocality of the camera adaptor:
   - **a.** Loosen the mechanical locking screw on the camera adaptor (see 1 in the image below).
   - **b.** If the adaptor's magnification is 0.35X, 0.5X, or 0.63X, turn the parfocal adjustment screw (see 2 in the image below).

   OR If the adaptor's magnification is 1X, loosen the clamping screw (see 3 in the image below), and counterturn its two mechanical sections to adjust the optical pathway length.
   - **c.** Firmly tighten the mechanical locking screw.

5. Loosen the straight phototube clamping screw (see 3 in the image below), rotate the camera to the desired position, and then firmly tighten the straight phototube clamping screw.



Adjusting for Different Observation Methods

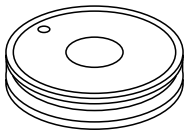
It's true that a well-maintained microscope will help ensure you obtain optimal images. However, it is also important to know how to properly adjust the microscope for your observation method.

The following guides are good references to keep on hand, especially if you do not use these methods on a regular basis.

Differential Interference Contrast (DIC) Adjustment—Transmitted Light Microscopy

1. Ensure that the microscope is properly adjusted for Köhler, set to brightfield illumination mode, and the aperture stop is fully opened.
2. Engage the polarizer into the light path.
3. Engage the analyzer into the light path.
   Turn the polarizer to achieve maximum image darkness (crossed polarized position). This works best without a sample.
4. Engage the nosepiece DIC prism slider positioned inside the objective nosepiece into the beam path (see Image 1), ensuring that no condenser prism is engaged.
   Note: On inverted microscopes, you need to place the DIC slider upside down.
5. Remove the eyepiece and watch the back focal plane of the objective lens (observation without eyepiece).
6. Rotate the DIC prism slider's turning knob until the black fringe is visible.
   The fringe should split the observed image of the back focal plane in half at 45° (see Image 2).
7. Reinsert the eyepiece.
8. Rotate the condenser turret to engage the dedicated prism matching the objective lens used.
9. Fine-tune the DIC prism slider's turning knob to optimize the contrast (positive or negative image topography).

DIC prism (large)



During installation, make sure that the white dot position on top of the prism matches the white dot position of the condenser turret insert. This ensures that the pin on the rear side of the prism fits the notch of the condenser turret's insert.

Note: If your objective has the BFP 1 marking on it, to get the best contrast, you need to use the prism position switch lever to move the prism to the appropriate height.

Image 1

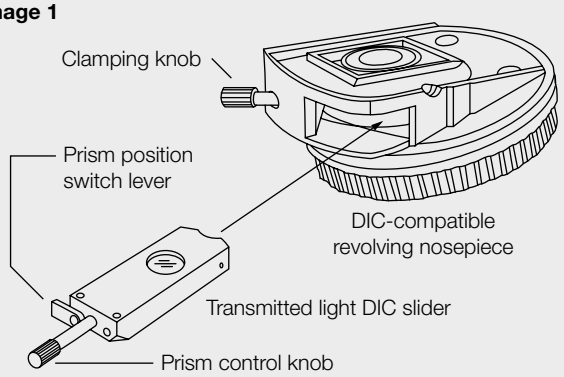
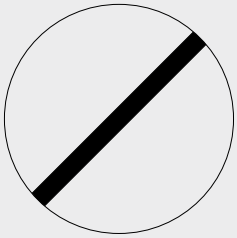


Image 2



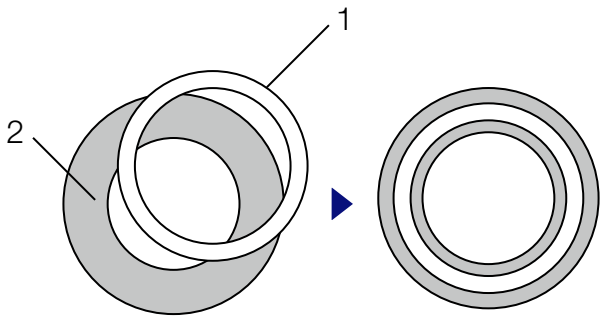
Phase Contrast Adjustment

1. Ensure that the microscope is properly adjusted for Köhler, set to brightfield illumination mode, and the aperture stop is fully opened.

2. For each phase objective, turn the condenser turret to engage the corresponding phase ring (see the inscription on the objective).

3. Remove the eyepiece and insert a centering telescope. Use the centering telescope's focus to bring the back focal plane image into focus so you get a pinsharp image of both the phase ring slit and the phase plate at the same focus position.

4. Turn the centering screws of the optical element to center the phase ring slit. The phase ring slit is perfectly centered when the bright ring slit image (see 1 in the image below) is concentrically inscribed in the dark image of the lens' phase ring (see 2 in the image below).



Relief Contrast Adjustment

1. Place a stained specimen (preferably a tissue thin section) on the stage and, using the 10X objective (with a modulator installed), align the microscope for proper Köhler illumination. The modulation contrast slit plate should be removed from the condenser for this operation. If the turret condenser has a position for brightfield illumination with an aperture diaphragm, rotate the turret to select this condenser.

2. View the modulator plate in the back focal plane of the objective, using a Bertrand lens, a phase telescope, or by removing an eyepiece and peering down the eye tube. Make certain that the sample is removed from the optical path or that it is moved to a clear area on the microscope slide (see Figure 1).

3. Select the slit aperture plate that corresponds to the 10X objective by moving the appropriate condenser (from the turret) into the optical path. There should be a set of adjustment screws or a lever that enables rotation and translation of the illuminating slit plate within the condenser.

4. Place the circular polarizing filter on the microscope light port beneath the condenser. Rotate this filter while observing the slit image through the Bertrand lens (or phase telescope). Observe that the angle of rotation influences the amount of light (brightness) passing through the polarizer portion of the slit (see Figure 2).

5. Translate the image of the slit so that the opened portion lacking a polarizer is superimposed over the gray region of the modulator plate as illustrated in Figure 3. The portion of the slit containing the polarizing material should be imaged in the clear portion of the modulator just to the right of the gray region.

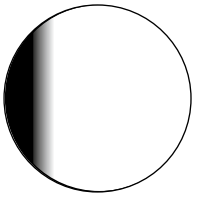
6. Rotate the circular polarizing filter and observe how the region of the slit containing the polarizing material appears and disappears. When the vibration plane of the circular polarizer is perpendicularly aligned with the vibration plane of the polarizer in the slit, the slit size is minimized and maximum contrast is obtained (see Figure 4).

7. Readjust the condenser position by refocusing the field diaphragm to achieve a sharp focus, and open the field iris diaphragm until it is just outside the field of view.

8. Rotate the specimen and/or the circular polarizer at the base of the microscope to achieve optimum contrast. These settings will vary from specimen to specimen.

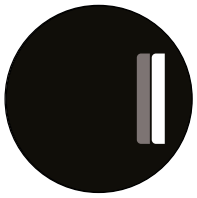
9. Repeat the above steps each time a different magnification is selected for viewing the specimen in modulation contrast.

Figure 1



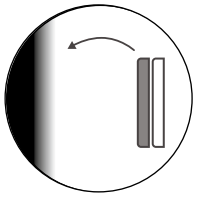
Back focal plane of objective

Figure 2



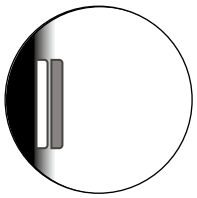
Slit plate

Figure 3



Rotate slit to overlay gradient objective

Figure 4



Final correct positioning

Polarization Adjustment

1. Ensure that the microscope is properly adjusted for Köhler illumination, and set it to brightfield mode.

2. Install the fixed or rotating polarizing element, ensuring that the polarizer is correctly secured into position, either permanently fixed into its holder or placed in the zero position. The polarizer transmission vibration axis must be set to the East-West orientation in this step.

3. Insert the analyzer into the microscope nosepiece or intermediate tube.

4. Rotate the analyzer until the transmission axes of the analyzer and polarizer are crossed at a 90-degree angle. The image should appear as a very dark cross in the field of view (see Figure 1).

5. The final step in polarized-light microscope alignment is to adjust the condenser aperture diaphragm so the bright outer regions of the polarization cross that are visible at the edge of the objective aperture are blocked (see Figure 2).

Figure 1

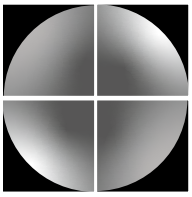
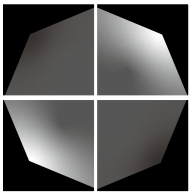


Figure 2



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