

Set Köhler illumination and resolution, contrast and depth of field

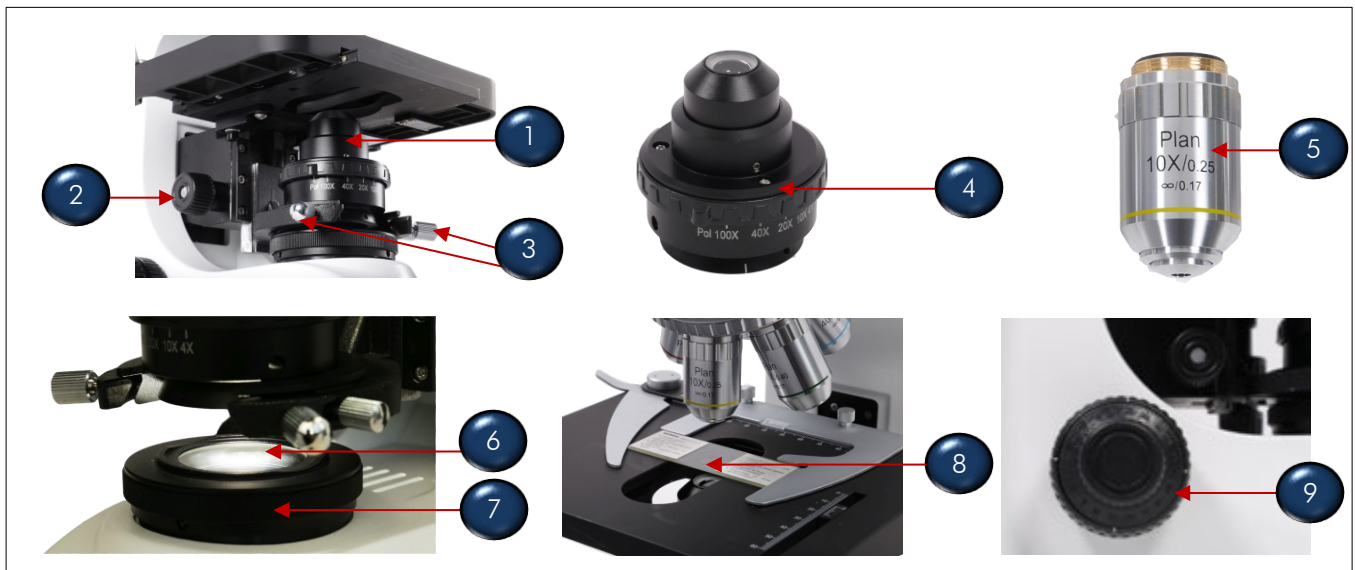


Figure – Köhler illumination: Preparation and adjustment of resolution and contrast

- The following requirements are important for Köhler illumination: In addition to an aperture diaphragm on the condenser, the illumination is equipped with a field diaphragm. It is also possible to adjust the position of the condenser in all three spatial directions.
- For Köhler illumination, the aperture diaphragm and the light field diaphragm must be set correctly. With the adjustment, the microscope image is evenly illuminated, and the best resolution, optimal contrast, and sharpness contrast are set.

⇒ Requirements

1. The **condenser (1)** is employed.
2. The **10X objective (5)** is selected.
3. The **field diaphragm (6)** is fully opened by using the **knurled ring (7)**.
4. A **slide (8)** with specimen is clamped on the mechanical stage for microscopy.
5. The microscope illumination is switched on.

⇒ Procedure

1. The **knurled screw (2)** is located below the mechanical stage on the left side of the microscope. Turn it as far anti-clockwise (away from the user) as possible.
 - ✓ The **condenser (1)** is turned as far up as possible until it stops.
2. Look through the eyepieces and use the **fine- and coarse drive adjustment (9)** of the microscope to bring the image into sharp focus.
3. Turn the **aperture diaphragm (4)** of the condenser to the position **Pol**, thereby fully opening it.
4. Use the **lamp control** to set the brightness to a comfortable level.
5. While looking through the eyepieces, use the **knurled ring (7)** to slowly close the **field diaphragm (6)**.
 - ✓ A blurred and (usually) not centred illuminated ring (the aperture image) can be seen.
6. Move the **condenser (1)** downwards using the **knurled ring (2)** until the **illuminated ring** comes into focus.
- ☞ The illuminated ring is in focus when no halos are visible around the illuminated ring.
 - ✓ The condenser is at the correct height.
 - ✓ The field diaphragm is focussed on the object plane.

↪ Continuation - Köhler illumination

↳ Continuation - Köhler illumination

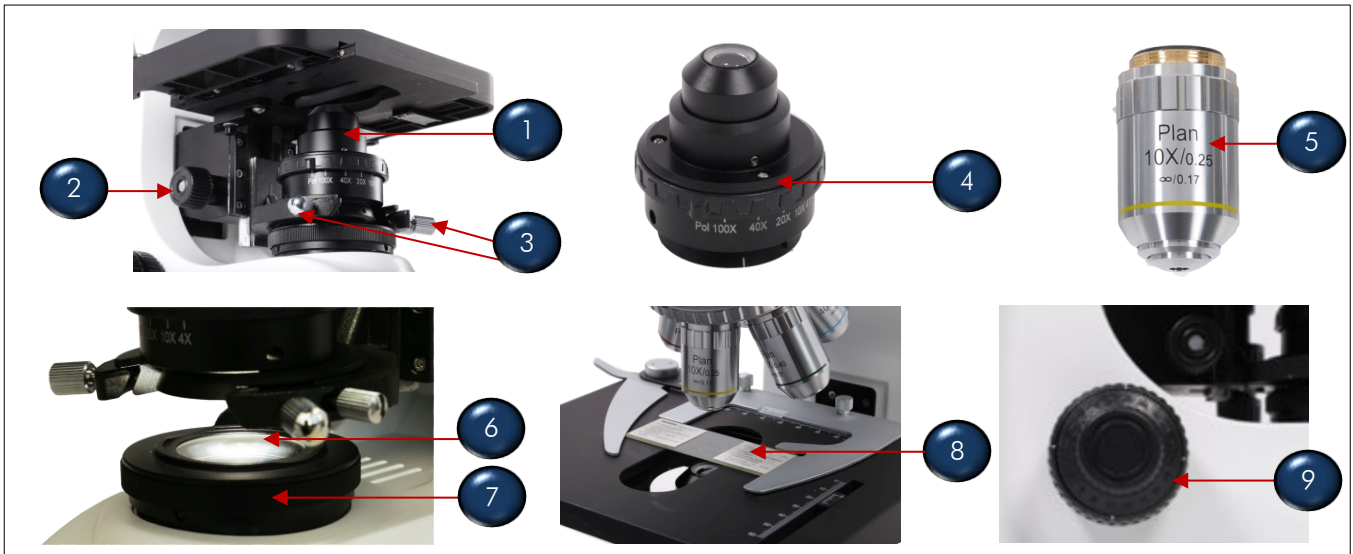


Figure – Köhler illumination: Setting the resolution, contrast and depth of field

7. Use the **adjustment screws (3)** on the condenser mount to centre the illuminated ring in relation to the field of view.
8. Using the **knurled ring (7)** slowly open the **field diaphragm (6)** again until the field of view is fully illuminated.
 - ✓ The field diaphragm is correctly opened wide.
 - ✓ Interfering stray light is minimised to the maximum level.
9. Turn the **aperture diaphragm (4)** of the condenser to the **10X position**.
10. Look through the eyepieces and slightly open or close the **aperture diaphragm (4)**. Adjust until the subjectively best setting for resolution, contrast, and depth of field is found.
 - The aperture diaphragm of the condenser has orientation markings for the various magnifications of the objectives, indicated by positions (Pol, 100X, 40X, 20X, 10X, 4X).
 - ① Since the optimum setting for resolution, contrast, and depth of field is subjective and sample-dependent, the best position may deviate from the marking.
 - ✓ The aperture diaphragm is correctly opened wide.
 - ✓ Resolution, contrast, and depth of field are correctly set.

⇒ Result

The Köhler illumination and resolution, contrast, and depth of field are correctly adjusted for the 10X objective magnification.

- When switching objectives, note that small adjustments are necessary: adjust the height of the condenser, the field diaphragm, and the centring of the aperture image.
- The field of view becomes smaller with greater magnification, so the aperture diaphragm needs to be opened wider with increasing magnification. This helps maintain the best possible contrast and depth of field.
- An aperture diaphragm opening of 60% - 80% (between positions 100X and 40X) can serve as a rough compromise between proper adjustment and the need for adjustment. Consequence: there may be significant compromises in the microscope image quality.
- Please ensure that the aperture diaphragm is not closed too far, as this can distort the edges of the objects.
- The setting of the aperture diaphragm also depends on the specimen. Therefore, we recommend experimenting with the aperture diaphragm to find the best setting for the current specimen.
- When using a microscope camera, adjust the aperture diaphragm based on the camera image. This is done because the camera sensor does not rely on subjective human perception.
- Never use the aperture diaphragm or field diaphragm for brightness control, but only the lamp control.