DIC condenser

Differential Interference Contrast

euromex
microscopes holland

user manual

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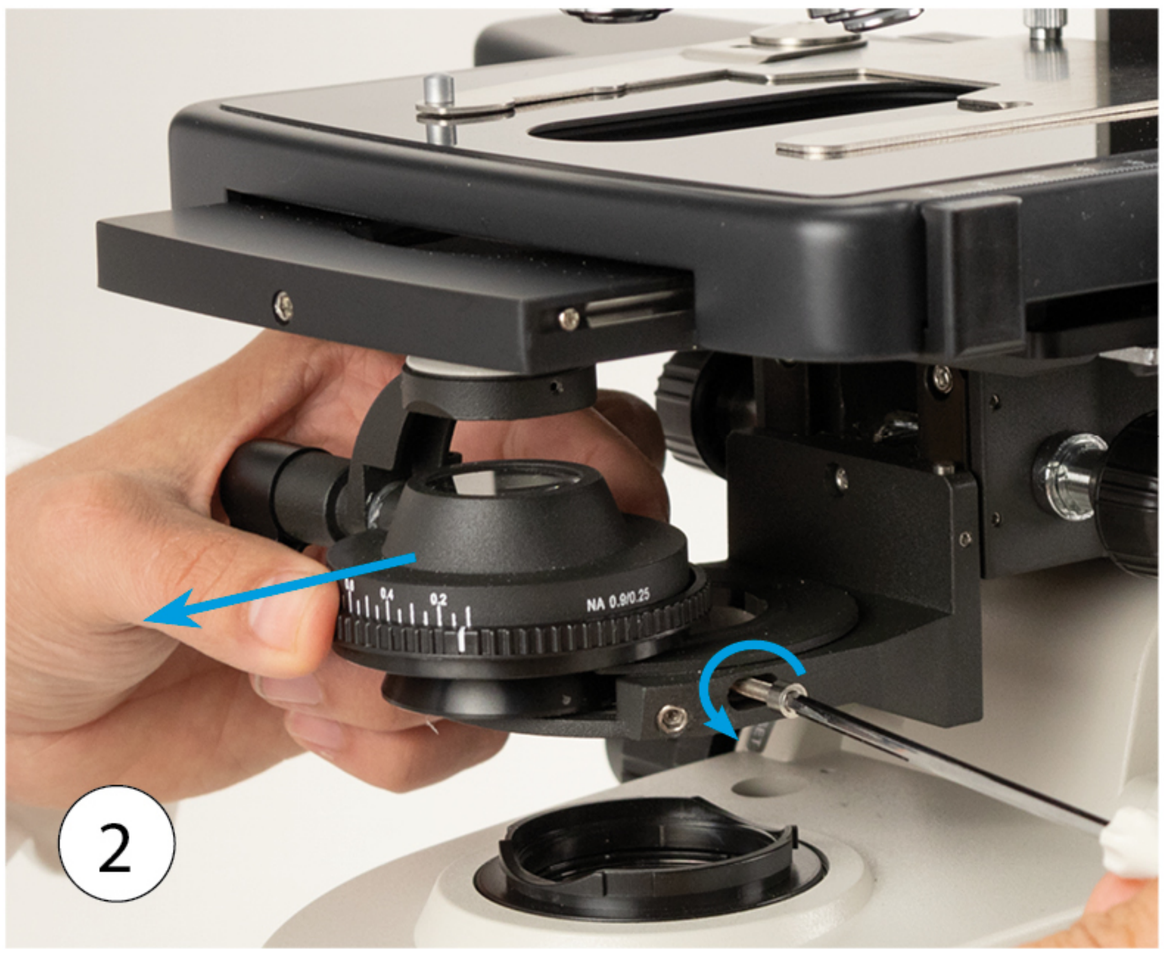
Components

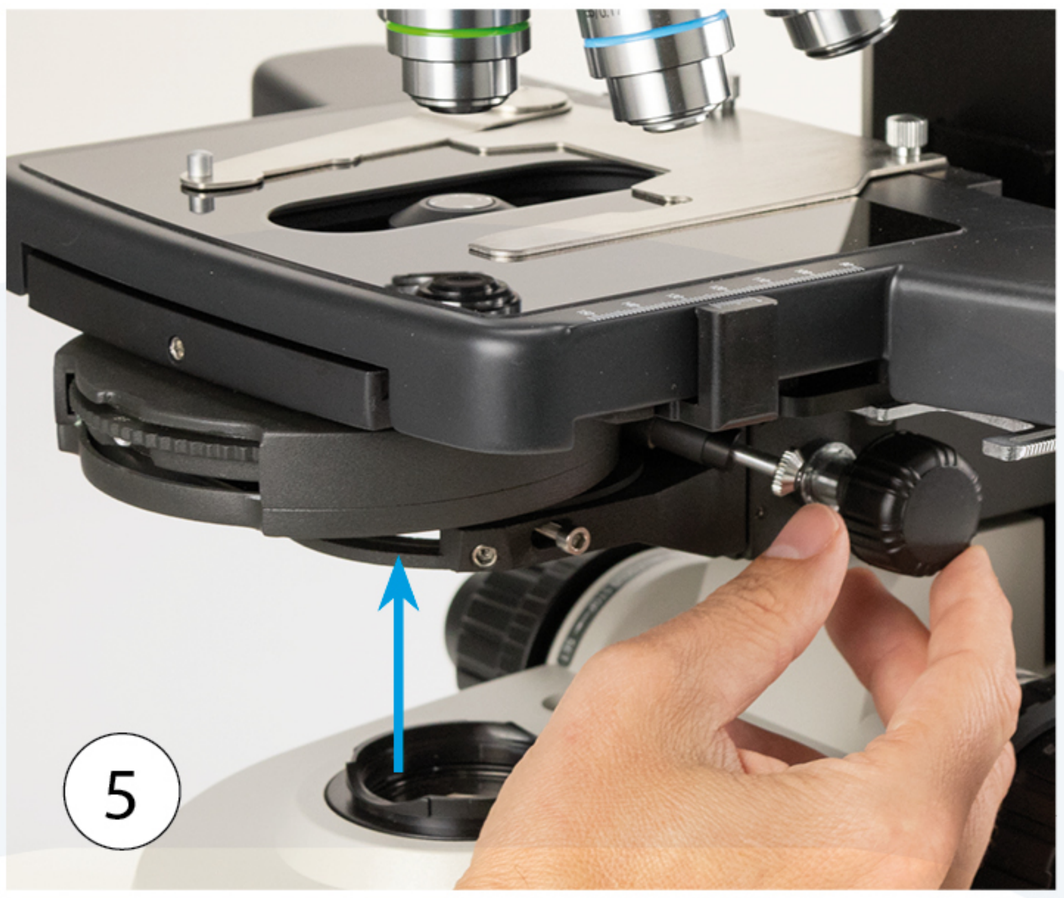
A. DIC condenser (with DIC prisms for 10x, 20x, 40x and 100x oil immersion objectives)
B. Rotatable polarizer
C. DIC analyzer/sliders (with DIC prism; for 10x/20x and 40x/100x)

Installation

Condenser

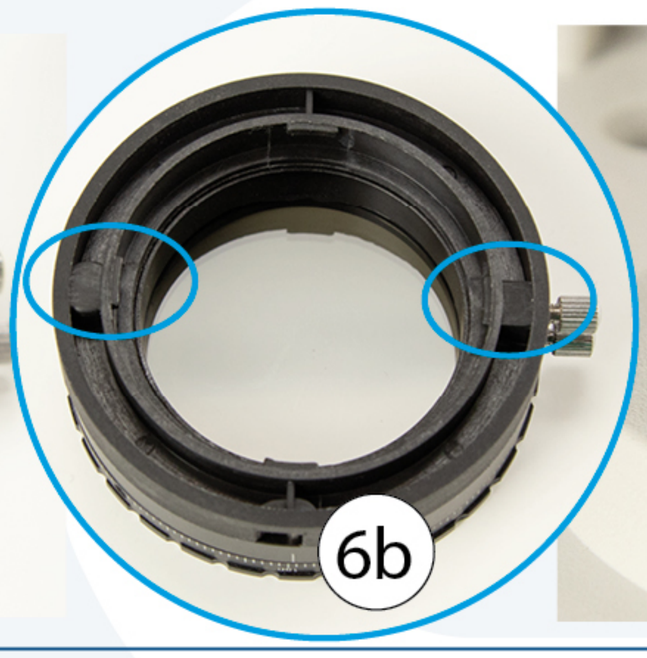
- Lower the condenser until the top lens is below the stage (**1**)
- Using the supplied Allen wrench, loosen the locking screw on the right side of the condenser hanger and pull the existing condenser towards the front of the microscope to remove from the condenser carrier (**2**)
- Slide the DIC condenser into the condenser carrier (**3**)
- Tighten the locking screw (**4**)
- Raise the condenser back to the top of the condenser focus travel (**5**)

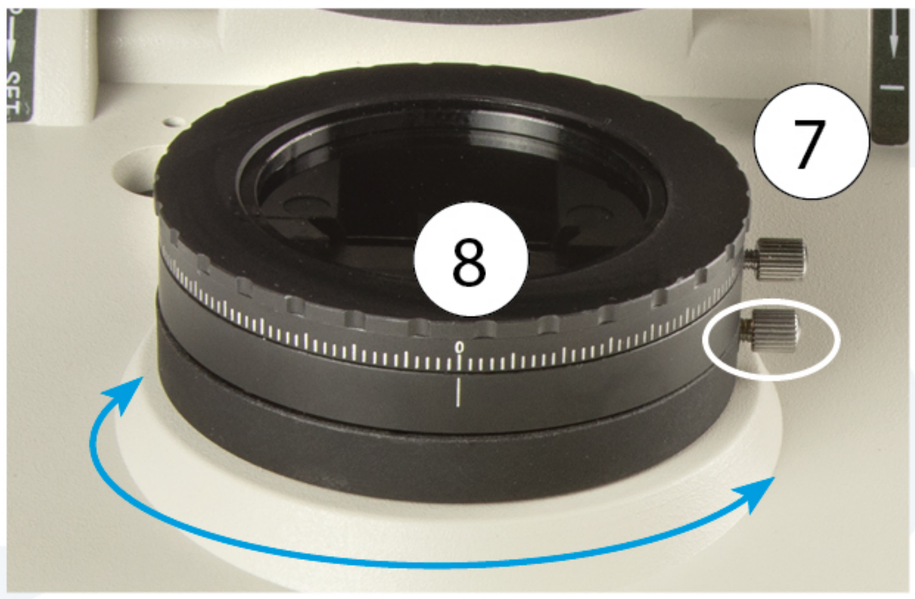


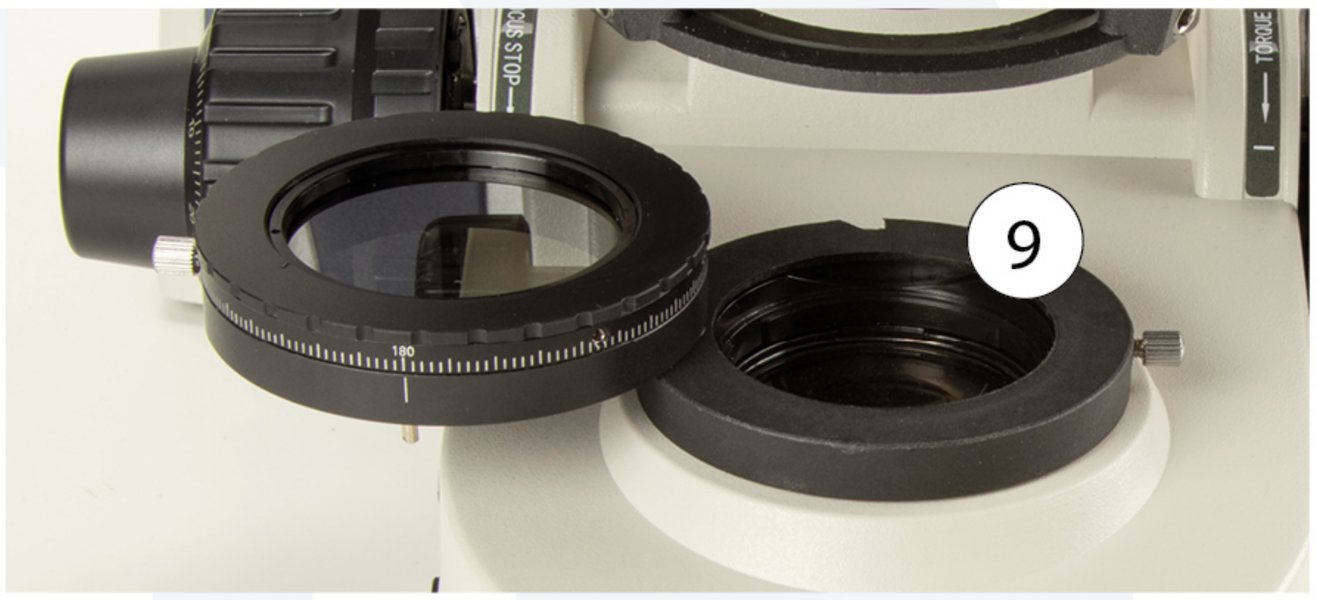


Polarizer

- Place the polarizer attachment (**6a**)on the light well of the microscope base
- The nooks in the bottom of the polarizer (**6b**) coincide with the recesses on the light well (**6c**)
- Turn roughly 45° either way to attach the polarizer (**8**)
- Make sure the locking thumbscrew is oriented to the right side at 3 o'clock position and tighten the lower thumbscrew enough to hold the polarizer attachment to the light well (**7**)
- Set the rotatable polarizer ring to "0" degrees (**6a**)
- The polarizer can be moved into the lightpath and back out again (**9**). When in the light path, the polarizer can be rotated to adjust and optimize the angle of polarization (discussed later)







Analyzer

- Loosen the dust plug (10b) with the screw (10a) and remove it from of the nosepiece to open the analyzer slot (**10**)
- With the analyzer (**C** on p.2) facing up and the metal knob oriented out, slide the analyzer into the slot. The analyzer attaches to the slider with magnets and uses a pin that fits in a slot on the slider to ensure correct orientation (and not a 180° rotation)

Note that there are two positions on the slider

- For DIC observation, push the slider all the way into the slot (**11a**)
- For brightfield observation, gently pull the slider out until it clicks into place. (out, **11b**)
  The slider does not need to be removed completely from the nosepiece for non-DIC observation (e.g., brightfield or fluorescence) and can remain in the out position

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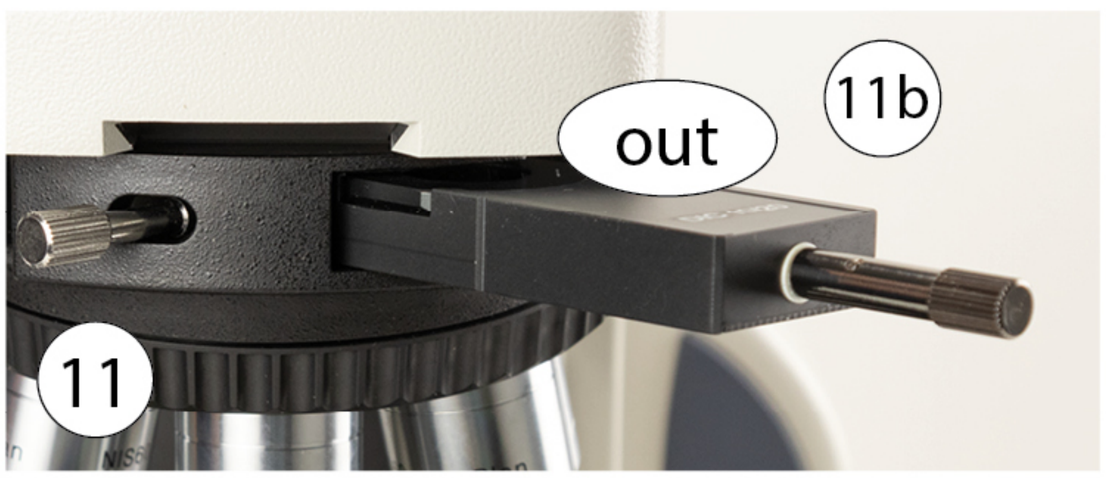


Operation

Alignment and adjustment

- Start with the 10x objective, then repeat for each subsequent objective used for observation
- With the polarizer right rotated out of the light path (**9**) and the DIC analyzer in the "out" position (**11b**), turn the turret of the condenser to the BF position (**12**)
- Rotate the 10x objective into position, focus on a suitable specimen and perform Köhler alignment according to the instructions for the microscope

DIC Observation

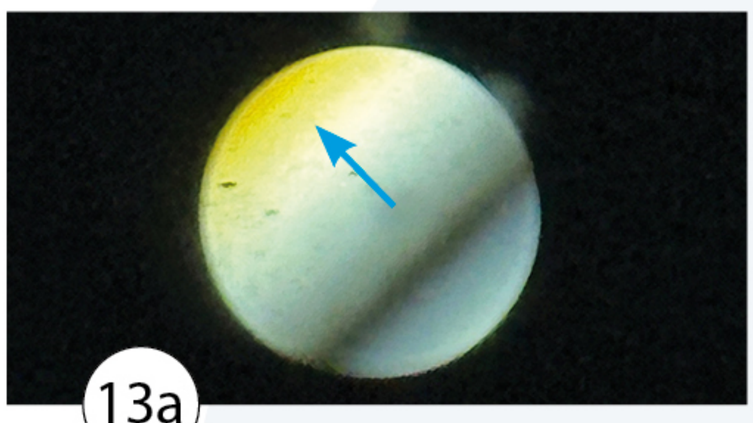
- Change to the specimen for DIC observation. DIC specimens often have no staining or inherent coloration
- Focus the 10x objective on the new specimen

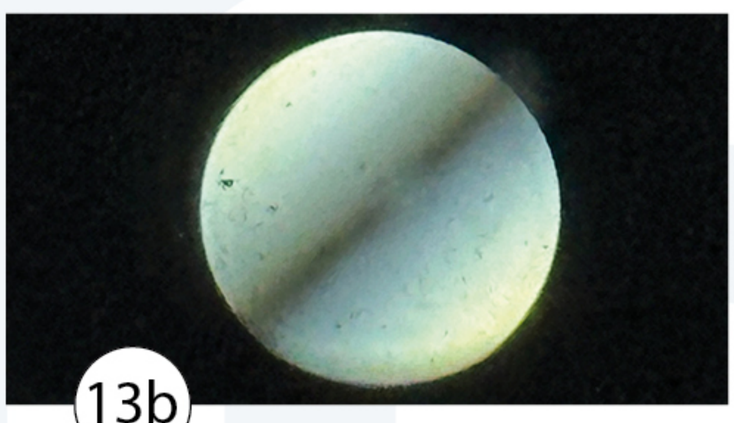
Note: it may be helpful to temporarily close the field diaphragm to help focus the objective. Once the 10x is focused, be sure to fully open the field diaphragm

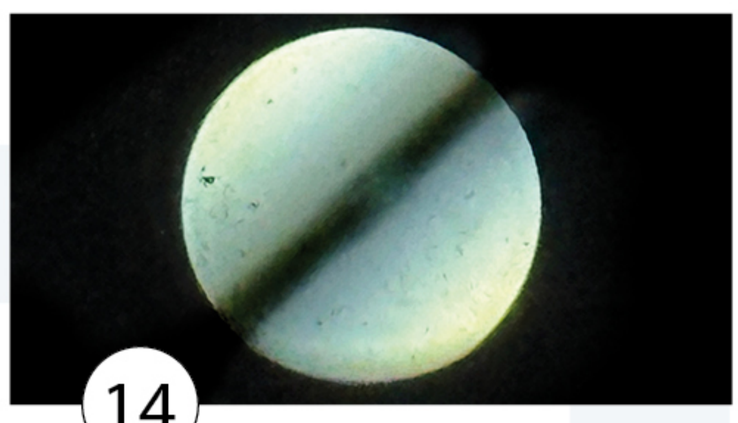
- Remove one of the eyepieces

Note: The DIC alignment procedure includes both observing through an eyepiece AND looking down an empty eyetube at the rear focal plane of the objective being aligned

- Start with the condenser turret in the BF position (**12**)
- Remove one eyepiece
- While looking down the eyetube without the eyepiece, turn the analyzer/slider control knob (**16**) until a dark line is observed in the rear of the objective. The line will be at a slight angle, running from southwest-to-northeast (**13a, 13b**)


13a


13b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Note: For best results, make sure this dark line (known as a shear line) passes through the center of the rear of the objective, adjusting the analyzer/slider control knob as needed

- Slightly rotate the polarizer (located on the light port), in order to make the shear line as dark as possible (**14**)
- Rotate the condenser turret to the 10 position (**15**) and observe the specimen through the remaining eyepiece. A preliminary DIC image should be seen through the scope's eyepiece. The second eyepiece can now be reinstalled

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Adjust the DIC analyzer/slider

- The final step is to vary the setting of the analyzer/slider control-knob (**16**) to attain the "best" DIC image – this is subjective to the user, and a "sensitive gray" image is often considered to be ideal. Fine tuning optimizes the image for "3D" effect and the "evenness" of background of field of view

Alignment for DIC Observation with Other Objectives

- Repeat the procedure described for 10x DIC alignment, selecting, in turn, the appropriate analyzer/slider and condenser turret position for the DIC objective being aligned

Note: The condenser/turret has individual positions for BF, 10 (DIC), 20/40 (DIC), 100 (DIC) and an FL position that can be used during EPI fluorescence observation. There are two DIC analyzer/sliders; one for 10/20 objectives and another for 40/100 objectives

TECHNICAL NOTE:

- The parameter affecting the 3D effect in a DIC image is the "phase gradients" in the specimen
- Phase gradients are a result of differences in the refractive indices of the mounting medium, features in the specimen, and curvature of specimen features
- In the case of living specimens, the "freshness" of the specimen impacts the quality of the contrast generated by DIC. As a fresh preparation ages, the weight of the cover glass gradually compresses the specimen, reducing phase gradients, thereby reducing the DIC image contrast and pseudo-3D effect

Note: The DIC 10x image may not exhibit uniform evenness across the field of view. Field evenness is typically better at higher magnifications (20x, 40x, 100x)