EXC-500 Differential Interference Contrast
Installation and Operation

Please read through all these instruction steps before installing and using the EXC500 DIC system. Review the illustrations and photos to familiarize yourself with the location of the components and overall procedure to help assure a successful experience.

Differential Interference Contrast ("DIC") Components

① Rotatable Polarizer

② DIC Condenser (with DIC prisms for 10x, 20x, 40x and 100x oil immersion objectives)

③ DIC Analyzer Sliders (with DIC prism; for 10x/20x and 40x/100x)

Installation

Condenser

1. Lower the condenser until the top lens is below the stage.
2. Using the supplied hex wrench, loosen the locking screw on the right side of the condenser hanger.
3. Pull the existing condenser towards the front of the microscope to remove from the condenser carrier.
4. Slide the DIC condenser into the condenser carrier until the alignment pin sets into the groove in the back of the condenser.
5. Tighten the locking screw.
6. Raise the condenser back to the top of the condenser focus travel.

Polarizer

1. Place the polarizer attachment on the light port of the microscope base with the locking thumbscrew oriented to the left side at 9 o'clock position.

2. Tighten the thumbscrew enough to hold the polarizer attachment to the light well.

3. Set the rotatable polarizer ring to "0" degrees.

The polarizer can be moved into the light path and back out again. When in the light path, the polarizer can be rotated to adjust and optimize the angle of polarization (discussed later).

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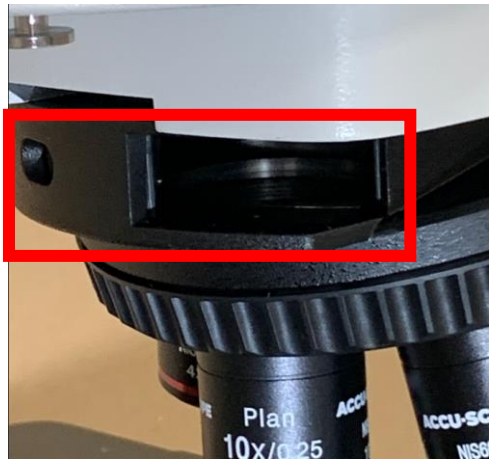
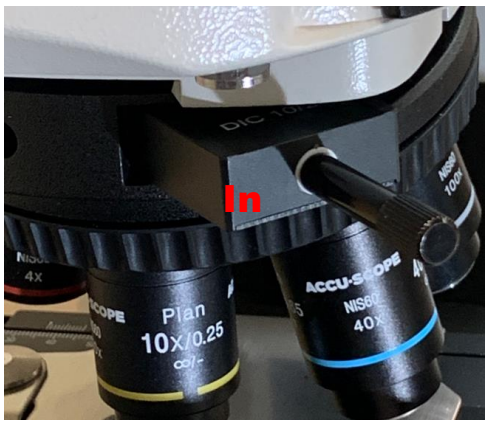
Analyzer



4. Remove the dust plug (red box) from of the nosepiece to open the analyzer slot.



5. With the analyzer 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)

First photo shows analyzer slider in the out position.

Second photo shows the analyzer slider in the “in” or inserted position for DIC observation.

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Note that there are two stop positions on the slider. For DIC observation, push the slider all the way into the slot. For brightfield observation, gently pull the slider out until the detent clicks into place, and this is the "out" position. The slider does not need to be completely removed from the nosepiece for non-DIC observation (e.g., brightfield or fluorescence) and can remain in the out position.

Operation

Alignment and Adjustment

Start with the 10x objective, then repeat for each subsequent objective used for observation.

1. With the polarizer right rotated out of the light path and the DIC analyzer in the "out" position, turn the turret of the condenser to the BF position.
2. Rotate the 10x objective into position, focus on a suitable specimen and perform Köhler alignment according to the instructions for the microscope.

DIC Observation

3. Change to the specimen for DIC observation. DIC specimens often have no staining or inherent coloration.
4. 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.

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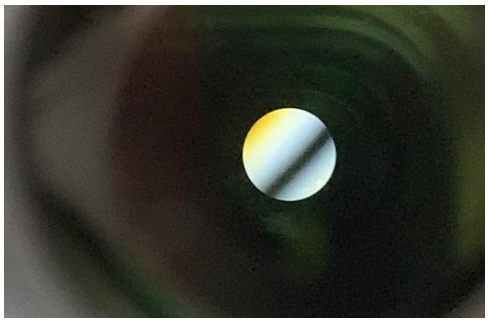
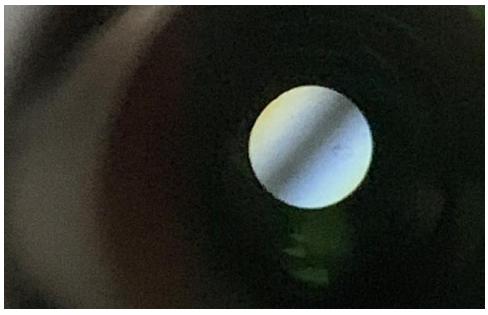
5. Remove one of the eyepieces.

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.

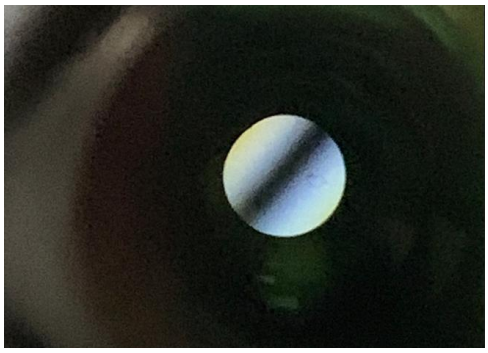
6. Rotate the condenser turret to the BF position.





7. While looking down the eyetube without the eyepiece, turn the Analyzer/Slider control knob 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.

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.



8. Slightly rotate the Polarizer (located on the light port)—seeking to make the shear line as dark as possible.

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9. Rotate the condenser turret to the 10 position 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.

Adjust the DIC Analyzer Slider

10. The final step is to vary the setting of the Analyzer Slider control-knob 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

11. 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.

IMPORTANT: 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).

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