APPENDIX 24: Align DIC—Olympus Transmitted Light Tower (shipped up to October 2009)

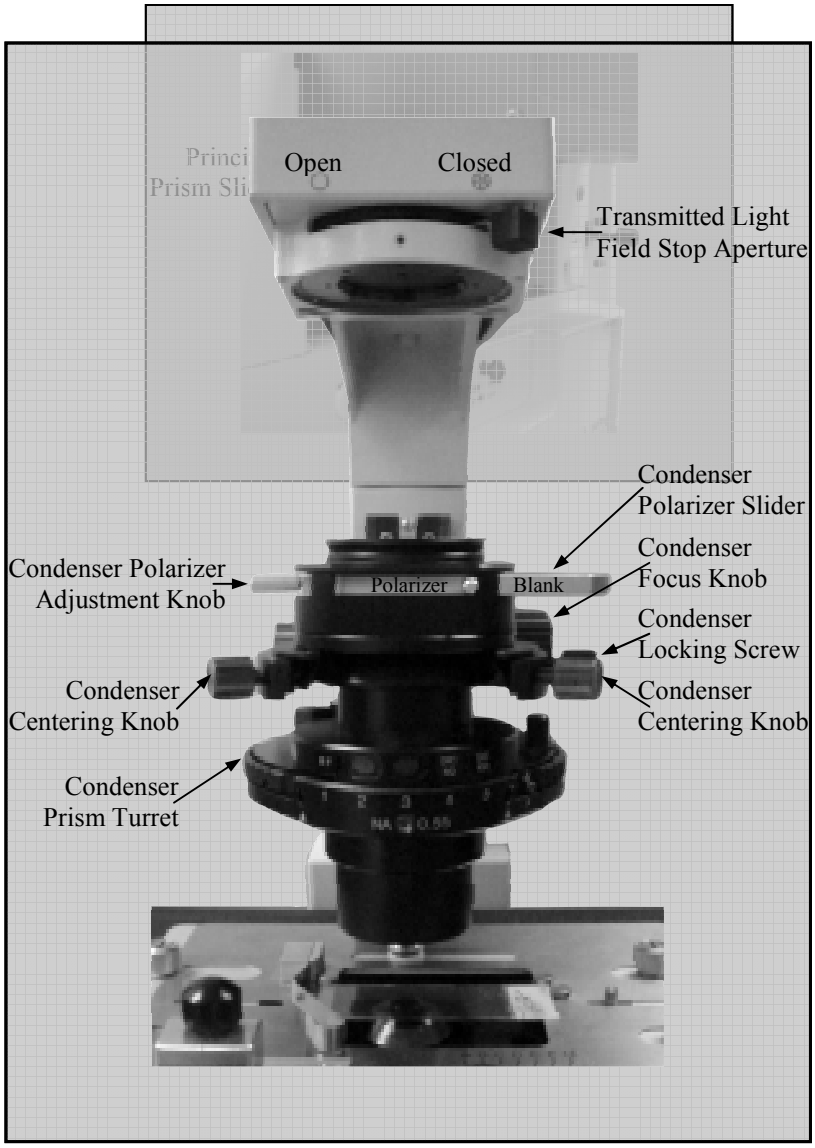


Figure 1. DIC Condenser Assembly.

1. Setting up Köhler Illumination

1.1. Move the Condenser Polarizer Slider into the Blank position.

1.2. Rotate the Condenser Prism Turret into a blank position.

1.3. Remove the Principal Prism Slider (see Figure 2) from the light path.

Figure 2. Principal Prism Slider.

1.4. Using the smallest magnification objective available, mount a specimen slide and focus on the specimen using either brightfield or fluorescence.
NOTE: With higher magnification objectives, 60x or greater, it may be difficult to see the edges of the Transmitted Light Field Stop Aperture.

1.5. Close down the Transmitted Light Field Stop Aperture. Open the Transmitted Light Shutter from the keypad.

1.6. Observe the specimen through the oculars while turning the Condenser Focus Knob (see Figure 1). You will see the Transmitted Light Field Stop Aperture encroaching on the image as you get close to the focal point. Keep focusing the Condenser until the edges of the Transmitted Light Field Stop Aperture (the blades of the iris) are in sharp focus (see Figure 3).

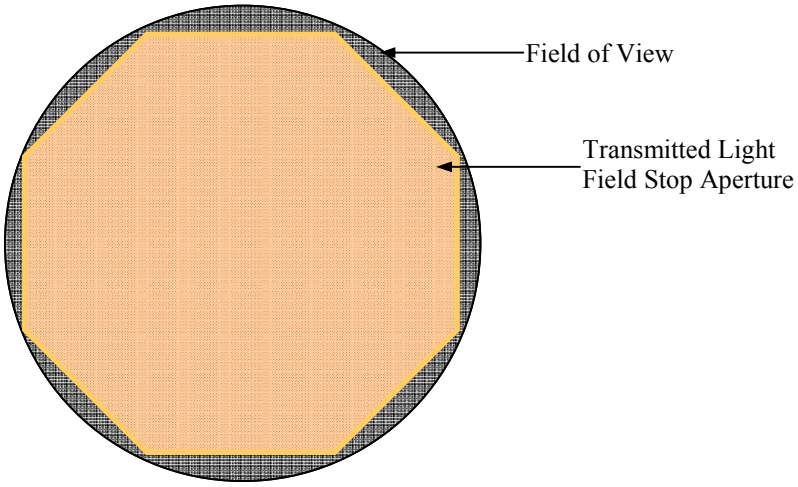


Figure 3. Köhler Illumination Alignment for the Transmitted Light.

NOTE: If you cannot see the iris while moving the Condenser Focus Knob, skip to step 1.7. It may be necessary to alter the centering before you will be able focus on the edges of the iris.

1.7. Center Transmitted Light Field Stop Aperture within the field of view using the two Condenser Centering Knobs (see Figure 1).

1.8. If necessary, repeat steps 1.6 and 1.7 to achieve optimum focus and alignment.

1.9. Once the Condenser has been focused and centered, open the Transmitted Light Field Stop Aperture until it is just outside the field of view. The Condenser will remain in Köhler alignment when different objectives are used but the Transmitted Light Field Stop Aperture

will have to be adjusted (opened or closed to be just outside of the field of view) for each magnification.

NOTE: Köhler Illumination alignment should be checked frequently, especially when the Condenser Assembly is routinely moved.

2. Crossing Emission Polarizer with Condenser Polarizer

2.1. Remove specimen from the stage and clean the objective.

2.2. Set the Eyepiece and Emission Filter Wheels to the Polarizer position.

2.3. Remove the Eyepiece Polarizer from the Eyepiece Filter Wheel:

NOTE: Be careful not to touch the surface of the filter.

2.3.1. Support the White Eyepiece Assembly and use a 3mm hex key to remove it from the Eyepiece Filter Wheel. Make sure to leave the Eyepiece Filter Wheel attached to the microscope base.

2.3.2. Carefully remove the Polarizer from the Eyepiece Filter Wheel and set aside.

2.3.3. Leave the White Eyepiece Assembly off.

2.4. To gain access to the Emission Filter Wheel, push the camera in towards the microscope base and lift the Camera Base off of the Camera Plate.

2.5. Remove the Emission Filter Wheel assembly by unscrewing the Filter Wheel Set Screw using a 3mm hex key.

IMPORTANT: Leave Emission Filter Wheel Motor Cable attached. Be careful not to strain this connection!

2.6. Slide the Condenser Polarizer into the light path. Move the Condenser Polarizer Adjustment Knob into the middle and tighten the knob to secure.

2.7. Verify that the Condenser Prism Turret is in a blank position and that the Principal Prism Slider is not in the light path.

2.8. Switch the Beam Selector to the camera.

2.9. Adjust the %T to 50%. Turn on the Transmitted Light.

2.10. Rotate the Eyepiece Polarizer that was removed in step 2.3 in front of the camera side port until maximum light extinction occurs (see Figure 4). Note the orientation of the dots on the rim of the Polarizer. This will be the proper orientation for the Emission Filter Wheel Polarizer.

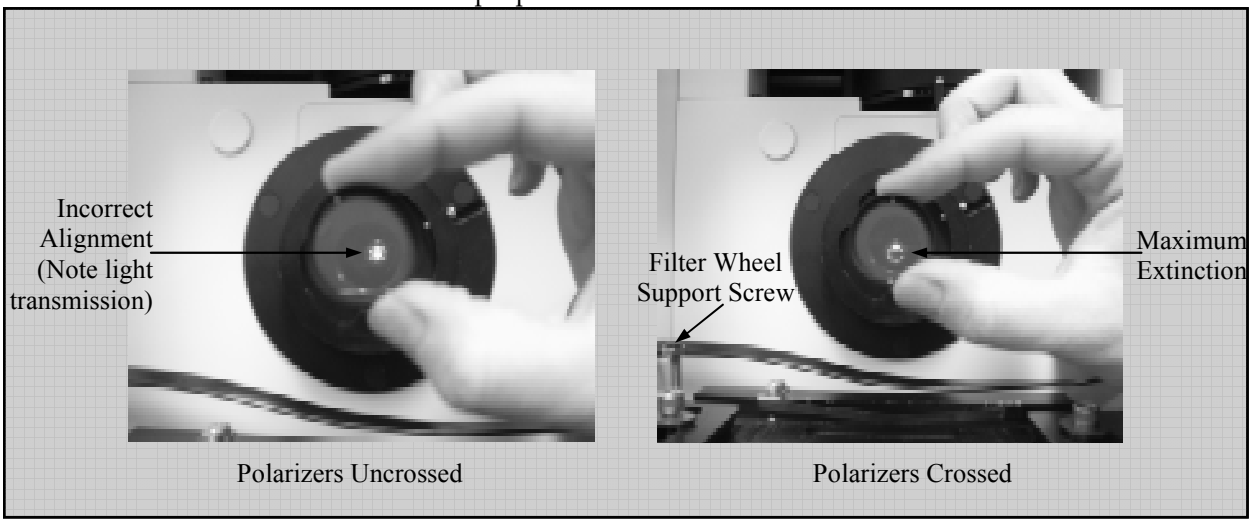


Figure 4. Proper Polarizer alignment.

2.11. Open the Emission Filter Wheel assembly by unscrewing the top plate and rotate the Polarizer until the orientation of the dots matches those you found with the Eyepiece Polarizer.
2.12. Reassemble the Emission Filter Wheel and put it back on the system
2.13. Rotate the Emission Filter Wheel assembly while on the microscope base until you achieve maximum extinction. Adjust Filter Wheel Support Screw so that the Emission Filter Wheel rests on the screw at maximum extinction.
2.14. For additional filter wheels follow steps 2.11 and 2.12 to align the Polarizer identically to that in the primary set.

IMPORTANT: Before unplugging any motor cables, shut the system down.

2.14.1. Check that extinction occurs when resting on Filter Wheel Support Screw.

NOTE: Do NOT adjust Filter Wheel Support Screw to achieve maximum extinction with the additional filter wheels. You MUST rotate the Polarizer within the filter wheel assembly.

2.15. Replace the primary filter wheel assembly.
2.16. Replace the camera.
2.17. Verify extinction with camera:
2.17.1. Verify that the Condenser Polarizer and Emission Polarizer are in the light path.
2.17.2. Acquire an image. Adjust Exposure Time and %T in order to obtain a maximum intensity between 500 and 1500 counts.
2.17.3. Continuously acquire images as you slowly move the Condenser Polarizer Adjustment Knob (see Figure 1) until the lowest maximum intensity is found (this point is maximum extinction). Ideally, this should be close to the middle of its travel and the maximum intensity should increase on either side.

NOTE: If a minimum can not be found, the orientation of the Emission Filter Wheel must be adjusted. Repeat the steps in section 2.

2.17.4. Tighten the Condenser Polarizer Adjustment Knob at maximum extinction.

3. Crossing the Eyepiece Polarizer with the Condenser Polarizer

3.1. Replace the Eyepiece Polarizer with the arrows facing away from you.
3.2. Switch the Beam Selector to the eyepiece.
3.3. Rotate the Eyepiece Polarizer as it sits in its holder until you achieve maximum extinction.
3.4. Secure the White Eyepiece Assembly to the Eyepiece Filter Wheel on the microscope base.

4. Setting Up DIC

4.1. Verify that all three polarizers (Condenser, Emission and Eyepiece) are in the light path.
4.2. Move the Condenser Prism Turret to the correct position for your objective.
4.3. Insert the Principal Prism Slider into the light path under the objective with the writing facing down.

NOTE: This slider has two positions, Normal and BFP-1 (see Figure 5). This corresponds to the type of objective that you are using. If you are unsure, check the objective, it will say BFP-1 if that is the correct position.

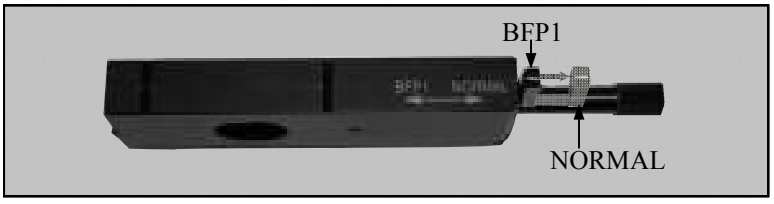


Figure 5. Principal Prism Slider.

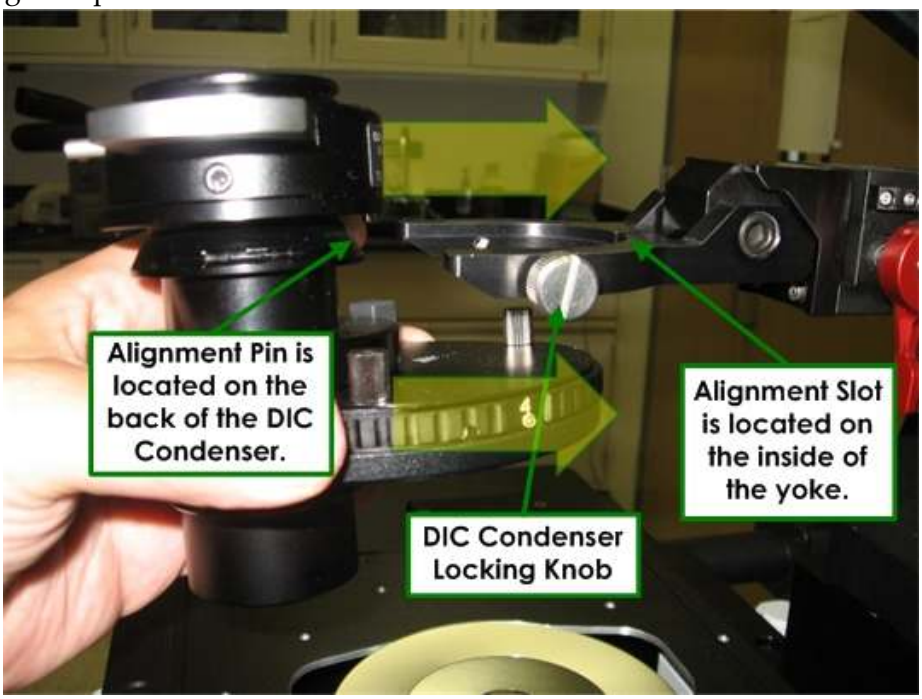
4.4. Direct the Beam Selector to the eyepiece.
4.5. Rotating the knob on the Principal Prism Slider will adjust the contrast seen in the image (i.e. move the shadows from bottom to top and move the black line into or out of the image).

APPENDIX 25: Align DIC—API Transmitted Light Tower (shipped after October 2009)

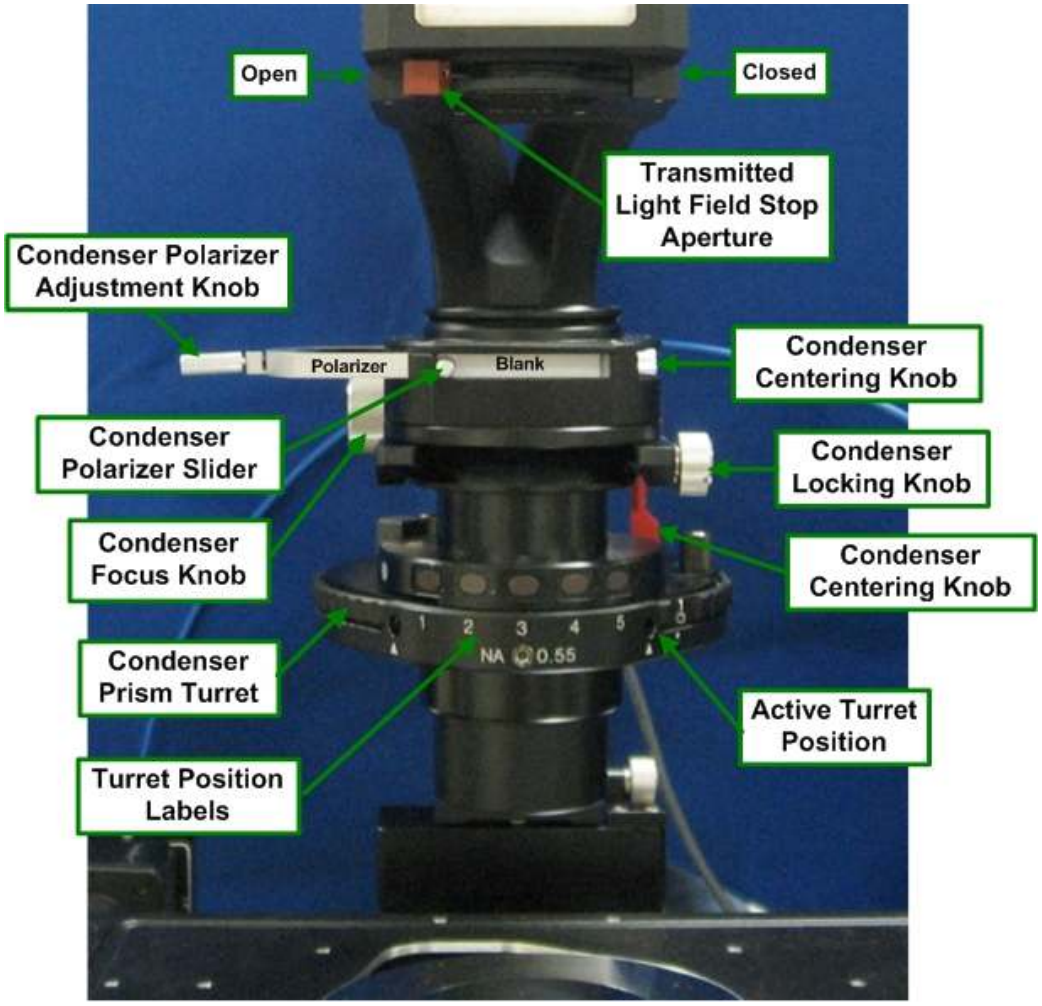
The DIC module is installed into the yoke on the *DeltaVision* system's transmitted light pillar.

1. To install a DIC module on a *DeltaVision* system:

1.1 Align the pin on the back of the DIC condenser with the slot on the inside of the yoke.



1.2 Tighten the locking knob on the right to fix the condenser in place.



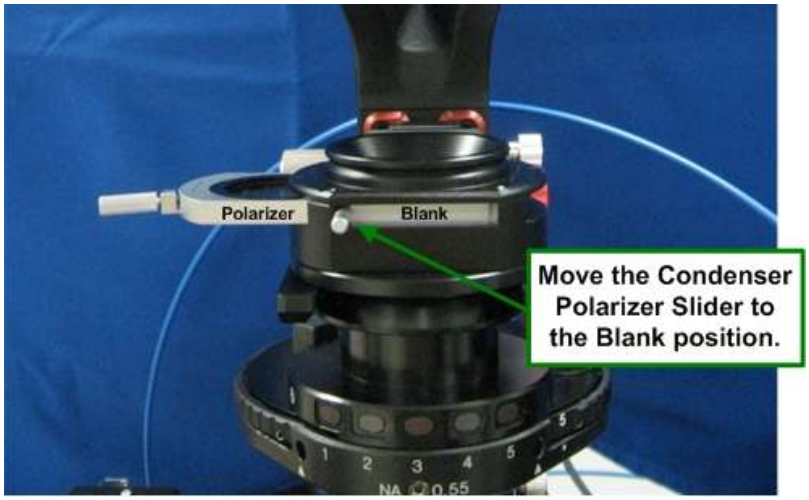
Overview of DIC Controls

Aligning the DIC Module

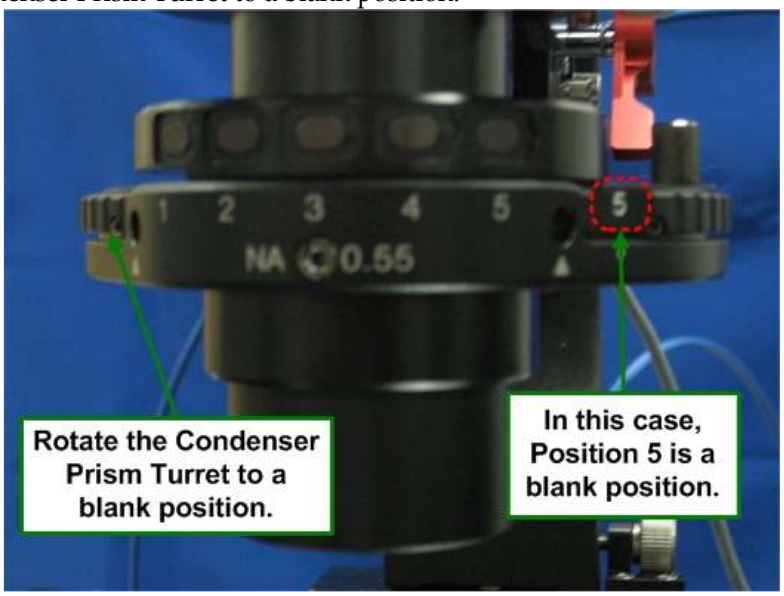
To acquire successful DIC images, it is necessary to properly align all of the DIC components. The alignment process includes setting up Köhler Illumination for the transmitted light source as well as crossing (aligning to 90 degrees) the Condenser Polarizer with both the Emission Polarizer and Eyepiece Polarizer. The Condenser Polarizer is left stationary and the other two polarizers are oriented according to the Condenser Polarizer's position. To accomplish this, you will first remove the Eyepiece Polarizer and use it to find the proper position for the Emission Polarizer. When satisfied with the position of the Emission Polarizer in relation to the Condenser Polarizer, you then replace and cross the Eyepiece Polarizer with the Condenser Polarizer.

2. To set up Köhler Illumination:

Move the Condenser Polarizer slider to the Blank position, as shown.



Rotate the Condenser Prism Turret to a blank position.



From beneath the right underside of the microscope stage, remove the Principal Prism Slider from the light path, as shown.

Principal Prism Slider.

Removing the Principal Prism Slider from the Light Path

> 🕮 **Note** It is not necessary to completely remove the Principal Prism Slider from the system. The slider clicks into place in both the In and Out positions.

Using the smallest magnification objective available, mount a specimen slide and focus on the specimen using either brightfield or fluorescence.

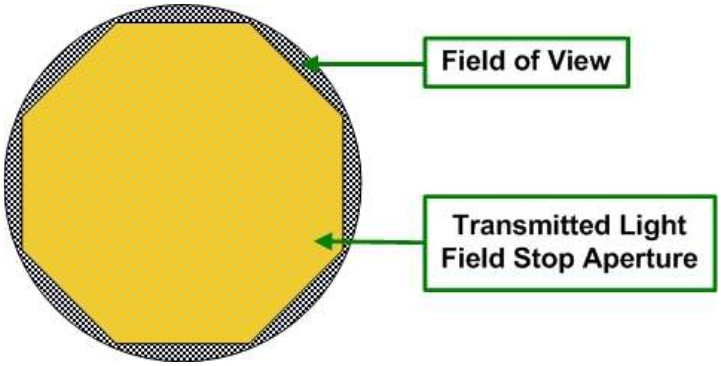
> 🕮 **Note** With higher magnification objectives, 60x or greater, it may be difficult to see the edges of the Transmitted Light Field Stop Aperture.

Move the Transmitted Light Field Stop Aperture to the Closed position.

Use the *DeltaVision* keypad to open the Transmitted Light Shutter.

Observe the specimen through the oculars while turning the Condenser Focus Knob. You will see the Transmitted Light Field Stop Aperture encroaching on the image as you get close to the focal point. Keep focusing the Condenser until the edges of the Transmitted Light Field Stop Aperture (the blades of the iris) are in sharp focus.

> 🕮 **Note** If you lose light as you approach the focal point, you'll probably need to re-center the condenser until the entire field becomes bright, and then continue focusing.



If you cannot see the entire iris while moving the Condenser Focus Knob, it may be necessary to alter the centering before you are able focus on the edges of the iris.

- Center the Transmitted Light Field Stop Aperture within the field of view using the two Condenser Centering Knobs.
- Repeat this step as necessary to achieve optimum focus and alignment.

After the DIC Condenser has been focused and centered, move the Transmitted Light Field Stop Aperture toward the Open position until the aperture is just out of view.

The DIC Module will remain in Kohler alignment when different objectives are used, however, you'll need to adjust the Transmitted Light Field Stop Aperture (opened or closed to keep the aperture just outside of the field of view) for each different magnification. Also, focus can change when moving the DIC condenser so it's important to check Köhler illumination frequently.

Before you proceed to crossing polarizers:

If you are able to verify *maximum extinction* as follows, you may not need to perform the procedure for crossing the Emission Polarizer with the Condenser Polarizer:

- Verify that both the Condenser Polarizer and the Emission Polarizer are in the light path.
- Acquire an image. Adjust Exposure Time and %T to obtain maximum intensity between 500 and 1500 counts.
- Acquire images continuously (**File |Continuous Acquire**) as you slowly move the Condenser Polarizer Adjustment knob until the lowest maximum intensity is reached. This point is maximum extinction. This point should be close to the center of the knob's travel, with the maximum intensity increasing on either side.

🕮 **Note** If a minimum intensity (maximum extinction) cannot be found, the orientation of the Emission Filter Wheel must be adjusted as described in the following procedures.

- If you are able to attain maximum extinction, tighten the Condenser Polarizer Adjustment knob to lock the polarizer in place at that position. If this is the case, you will not need to cross the Emission and Condenser Polarizers. Skip the following section and continue with the procedure for crossing the Eyepiece Polarizer with the Condenser Polarizer.

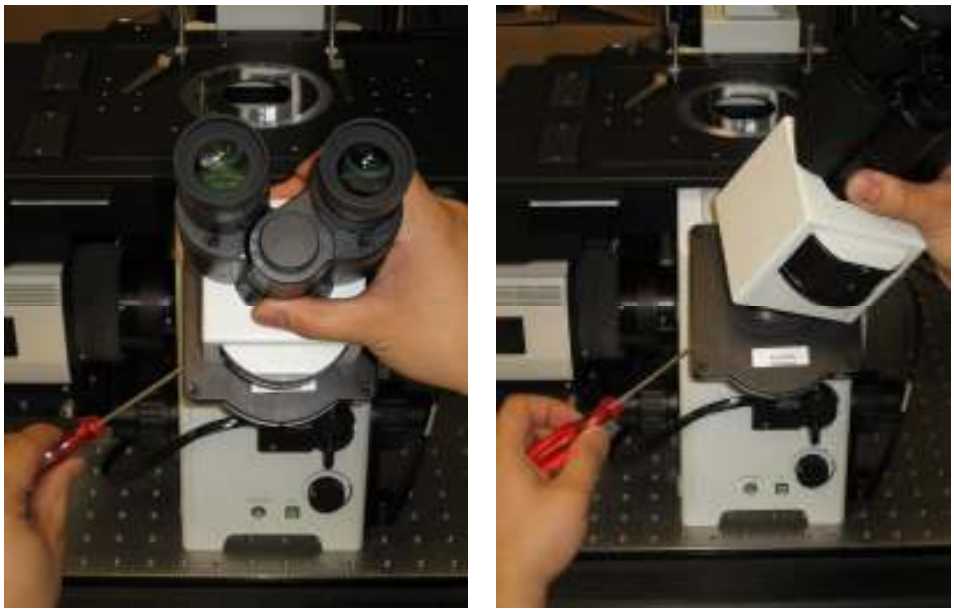
3. To cross the Emission Polarizer with the Condenser Polarizer:

3.1 Remove any specimens from the stage and clean the objective.

3.2 Set the Eyepiece and Emission filter wheels to the Polarizer position.

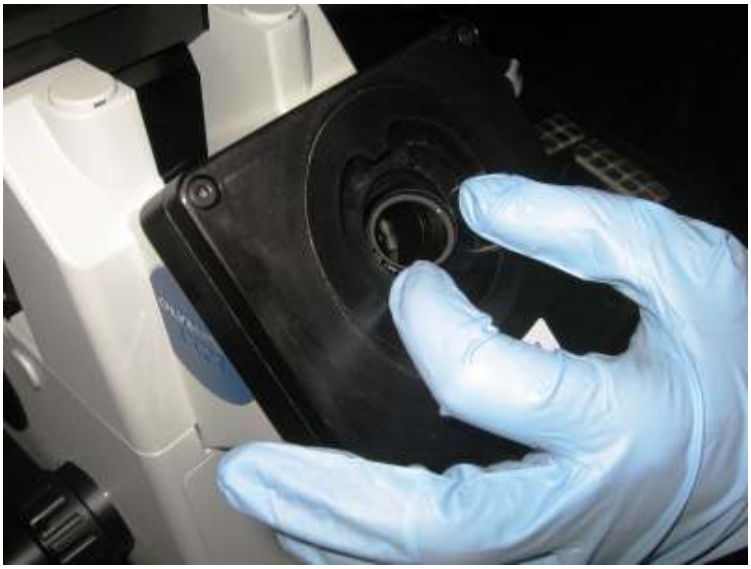
3.2.1 Remove the Eyepiece Polarizer from the Eyepiece filter wheel. (See *Changing Filter Wheel Modules* on Page 136 for illustrated details on removing the Eyepiece assembly.)

3.2.2 Support the white Eyepiece assembly and use a 3mm hex key to remove the Eyepiece assembly from the Eyepiece Filter Wheel. Set the Eyepiece assembly aside. Leave the Eyepiece Filter Wheel attached to the microscope base.



Removing the Eyepiece

- Carefully remove the Polarizer from the Eyepiece Filter Wheel and set it aside. Be careful not to touch the surface of the filter.



Removing the Polarizer from the Eyepiece Filter Wheel

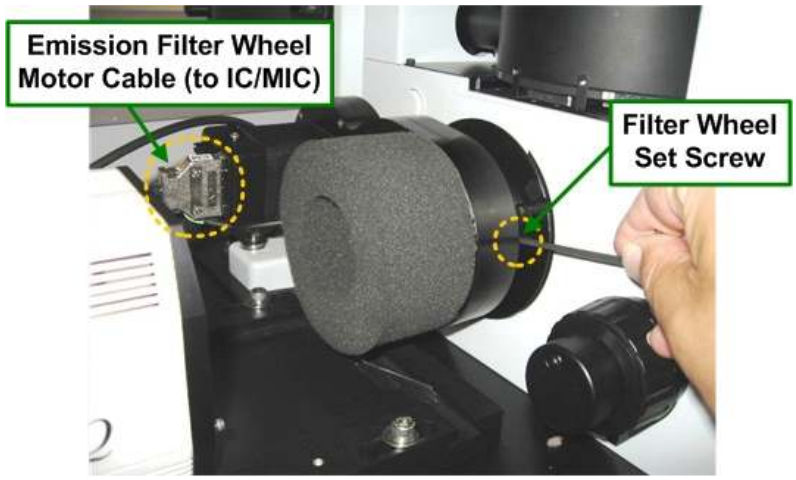
- Leave the white Eyepiece assembly off for now.

3.3 To gain access to the Emission Filter Wheel, remove the camera by pushing in and pulling up on the camera end as shown. Refer to *Changing Cameras* on Page 133 for removal details.

Removing the Camera

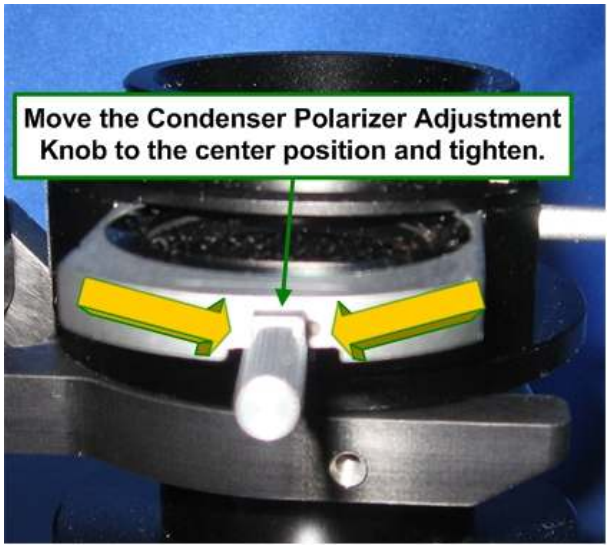
3.4 Using a 3mm hex key, loosen the set screw to remove the Emission Filter Wheel assembly.

> 🕮 **Note** Leave the Emission Filter Wheel Motor Cable connected, but use extreme care not to strain this connection.



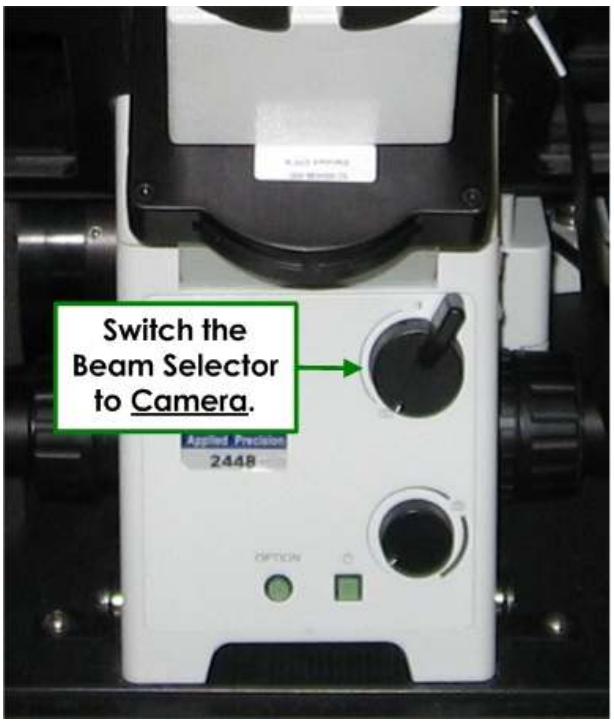
Removing the Emission Filter Wheel

3.5 Using the Condenser Polarizer Slider, move the Condenser Polarizer into the light path. Move the Condenser Polarizer Adjustment Knob into the center of its travel and tighten the knob to secure it.



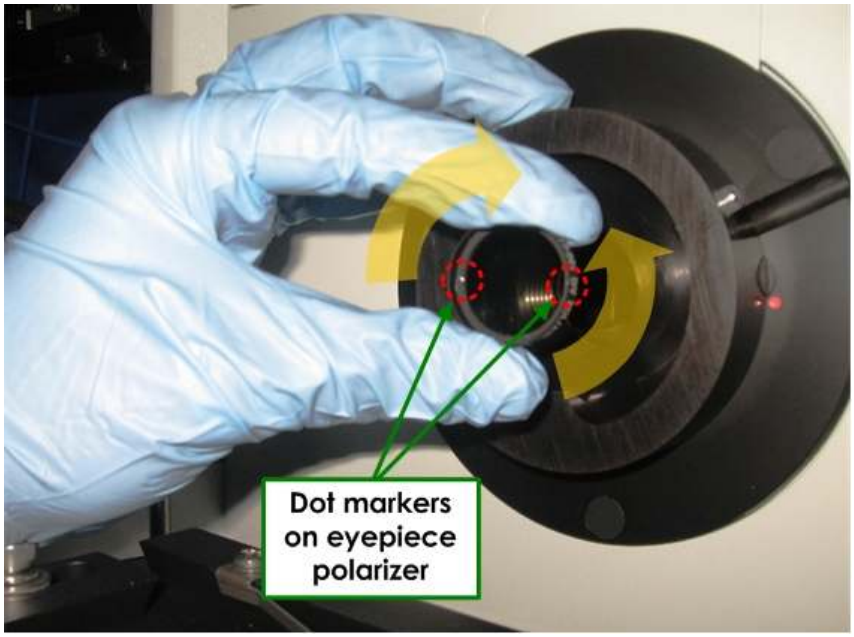
3.6 Verify that the Condenser Prism Turret is in a blank position and that the Principal Prism Slider is not in the light path.

3.7 Switch the Beam Selector on the *DeltaVision* to the **Camera** icon.

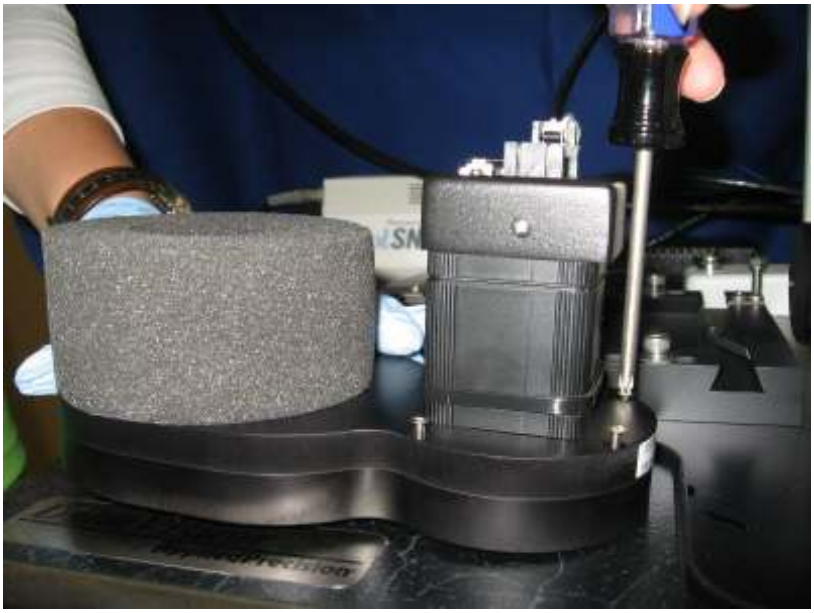


3.8 Adjust the T% to 50% and turn on the transmitted light.

3.9 Hold the Eyepiece Polarizer you removed in Step 3 in front of the camera side port and rotate the polarizer until maximum light extinction occurs. Note the orientation of the dots on the rim of the polarizer. This is the correct orientation for the Emission Filter Wheel Polarizer.



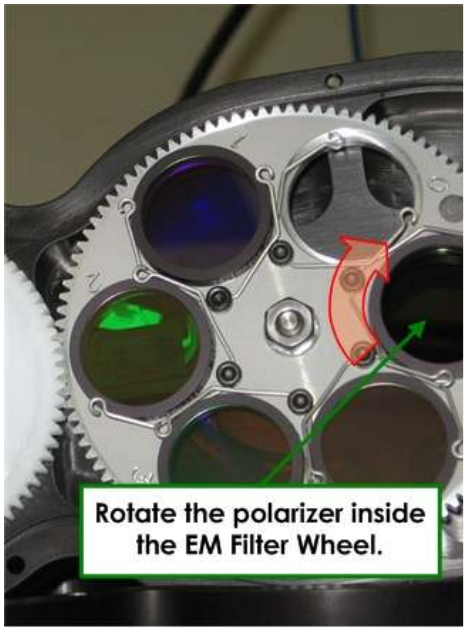
3.10 Use a Phillips screwdriver to remove the top plate from the Emission Filter Wheel assembly.

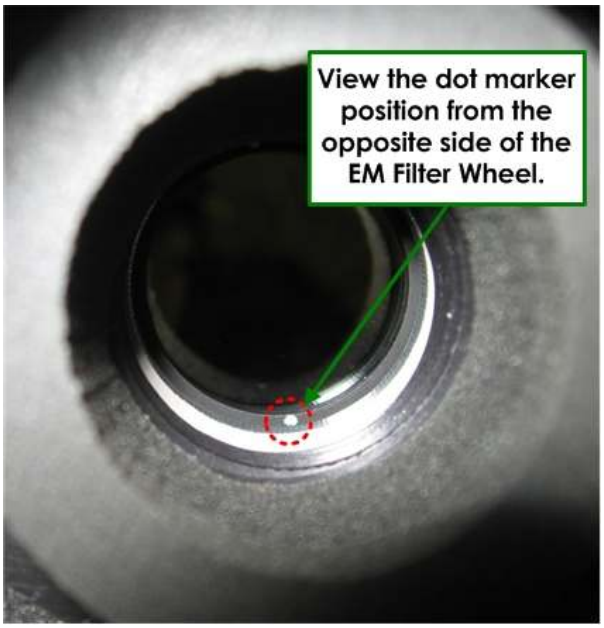


Removing the Emission Filter Wheel Top Plate

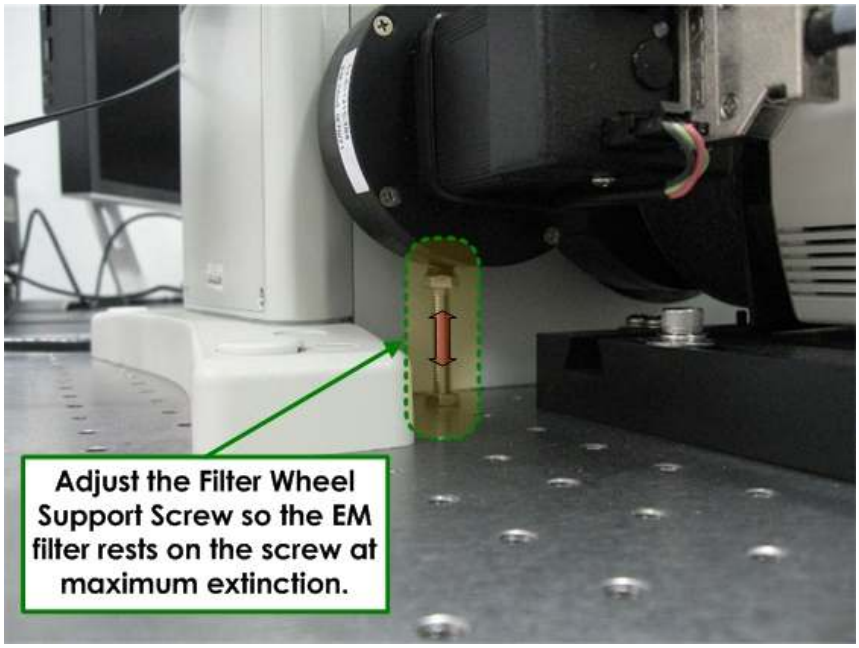
🕮 **Note** Eight Phillips screws hold the EM filter cover in place. Some of the screws are located beneath the foam collar.

3.11 Rotate the Polarizer within the Emission Filter Wheel until the orientation of the dots matches those determined for the Eyepiece Polarizer.





3.12 Reassemble the Emission Filter Wheel and replace the unit back onto the *DeltaVision* system. Adjust the Filter Wheel Support Screw so that the Emission Filter Wheel rests on the screw at maximum extinction.



3.13 Repeat Steps 10 – 13 to align any additional filter wheels.

> 🕮 **Note** When aligning additional filter wheels, make sure the filter wheel rests on the Filter Wheel Support Screw, but **Do NOT** adjust the Filter Wheel Support Screw to achieve maximum extinction. You must rotate the Polarizer within each filter wheel assembly.

3.14 Move the camera back into its operating position on the *DeltaVision* system and verify extinction as follows:

- Verify that both the Condenser Polarizer and the Emission Polarizer are in the light path.
- Acquire an image. Adjust Exposure Time and %T to obtain maximum intensity between 500 and 1500 counts.
- Acquire images continuously (**File** | **Continuous Acquire**) as you slowly move the Condenser Polarizer Adjustment knob until the lowest maximum intensity is reached. This point is maximum extinction. This point should be close to the center of the knob's travel, with the maximum intensity increasing on either side.

🕮 **Note** If a minimum intensity cannot be found, the orientation of the Emission Filter Wheel must be adjusted.

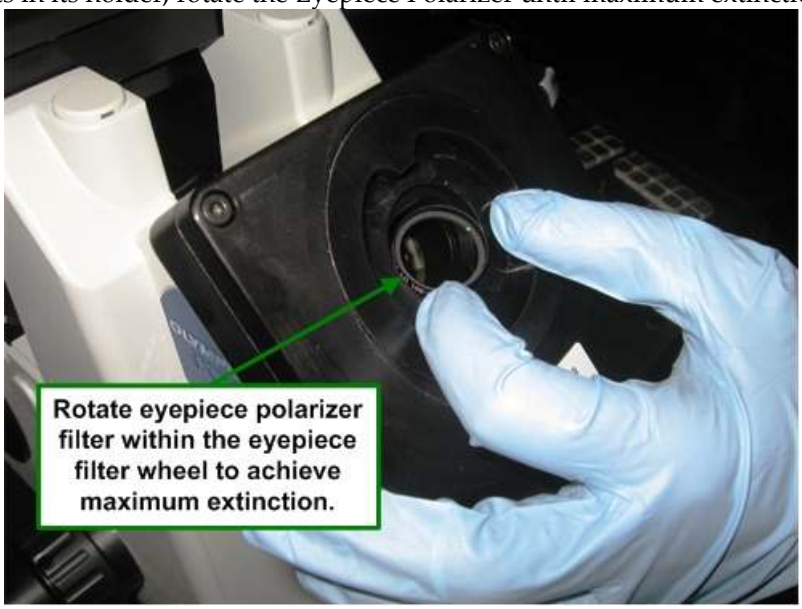
- Tighten the Condenser Polarizer Adjustment knob at maximum extinction.

4. To cross the Eyepiece Polarizer with the Condenser Polarizer:

4.1 Return the Eyepiece Polarizer to the Eyepiece Filter Wheel with the arrows facing away from you.

4.2 Switch the Beam Selector on the front of the microscope to the **Eyepiece** icon.

4.3 As it rests in its holder, rotate the Eyepiece Polarizer until maximum extinction is achieved.



4.4 Return the white Eyepiece assembly to the Eyepiece Filter Wheel on the microscope base and tighten with a 3mm hex key.

Reconnecting the Eyepiece

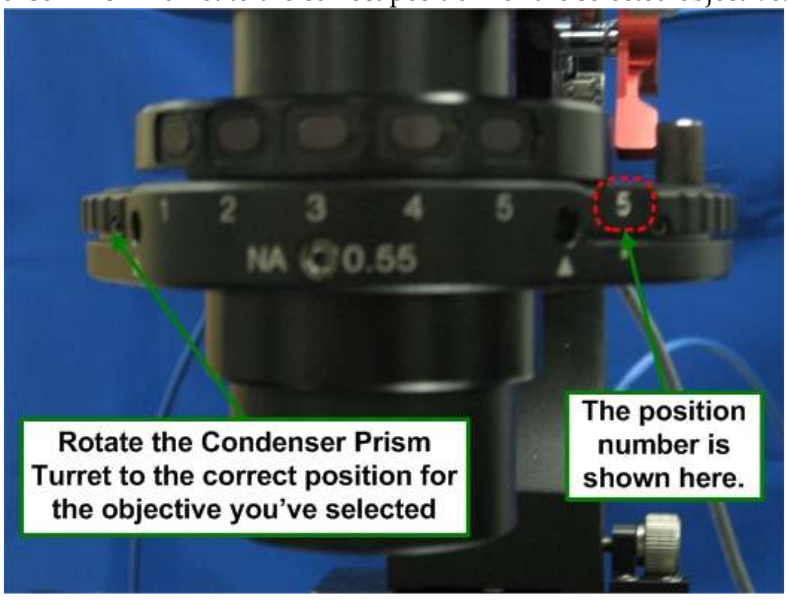
Setting Up DIC

After you have completed all of the DIC alignment tasks described previously, you are ready to perform the DIC setup procedure.

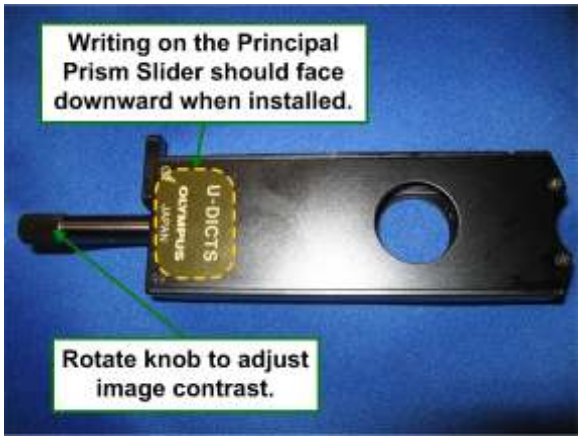
5. To set up DIC:

Verify that all three of the polarizers (Condenser, Emission, and Eyepiece) are in the light path.

Move the Condenser Prism Turret to the correct position for the selected objective.



Move the Principal Prism Slider into the light path under the objective (it will click into place). For proper orientation, be sure that the writing on the slider is facing down.



The Principal Prism Slider has two positions: **Normal** and **BFP1** (as shown below). The selected position will depend on the type of objective being used. If you are unsure, check the objective. It will say **BFP1** if that is the correct position.

Switch the Beam Selector to the **Eyepiece** icon.

Return the sample to the stage to begin imaging.

Switch the Beam Selector to the **Camera** icon.

Acquire images continuously (**File | Continuous Acquire**) as you rotate the knob on the Principal Prism Slider to adjust the contrast displayed in the images. For example, move the shadows from bottom to top and move the black line into or out of the image.