



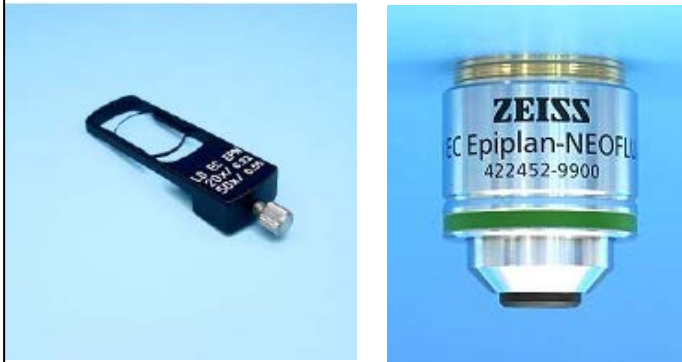
DIC Imaging using Laser Scanning Microscopes (LSMs) on Axio Imager Stands

Differential Interference Contrast (DIC) imaging is a technique used to increase contrast in brightfield images. In confocal systems, DIC images can be acquired simultaneously with fluorescence images and can provide useful morphological information. This manual provides instructions for setting up DIC imaging using a laser scanning microscope (eg. LSM 780, LSM 710, LSM 700, LSM 510, LSM 5 Exciter, LSM 5 Pascal) with the Axio Imager and Axio Imager 2 stands. Please note the system must be equipped with a T-PMT in order to collect DIC images. Any questions or concerns can be directed to the ZEISS Product and Applications Support group at 1-800-509-3905 or Support@zeiss.com

Step 1: Match DIC Slider to Objective

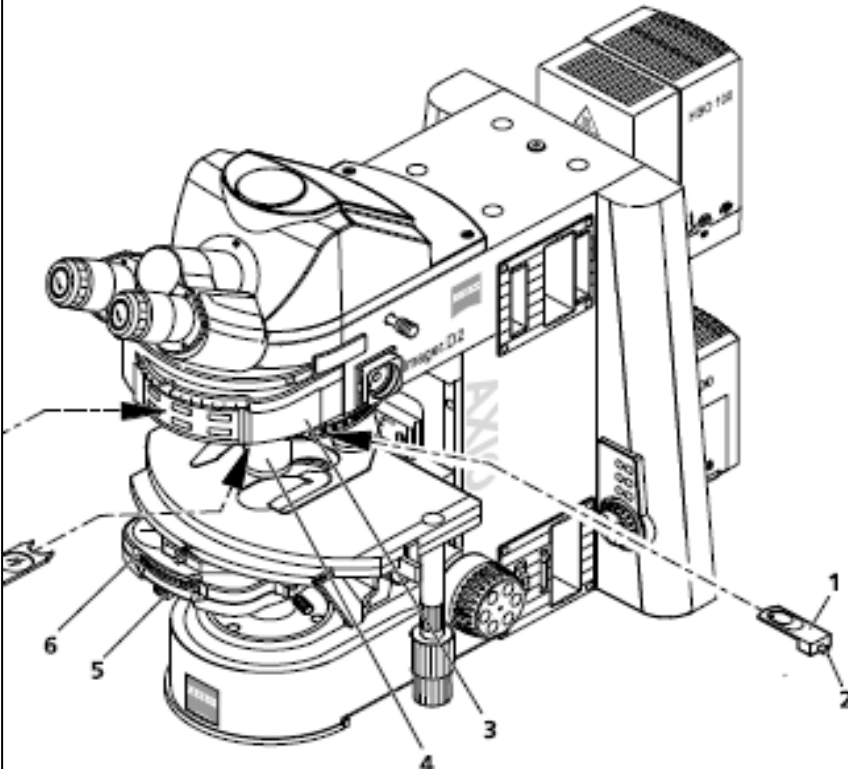
Each lens has a specific DIC slider. Be sure that the lens that is used for imaging has a matching DIC slider inserted.

Figure 1: A typical ZEISS DIC slider (left) and objective lens (right)



The DIC slider fits directly above the objective lens. The location of the DIC slider is shown in Figure 2; the slider is labeled as #1 and the arrow indicates where it should be inserted. Be sure that the DIC slider is fully inserted in the correct orientation. It will only slide fully into position if it is correctly oriented.

Figure 2: Diagram of the Axio Imager



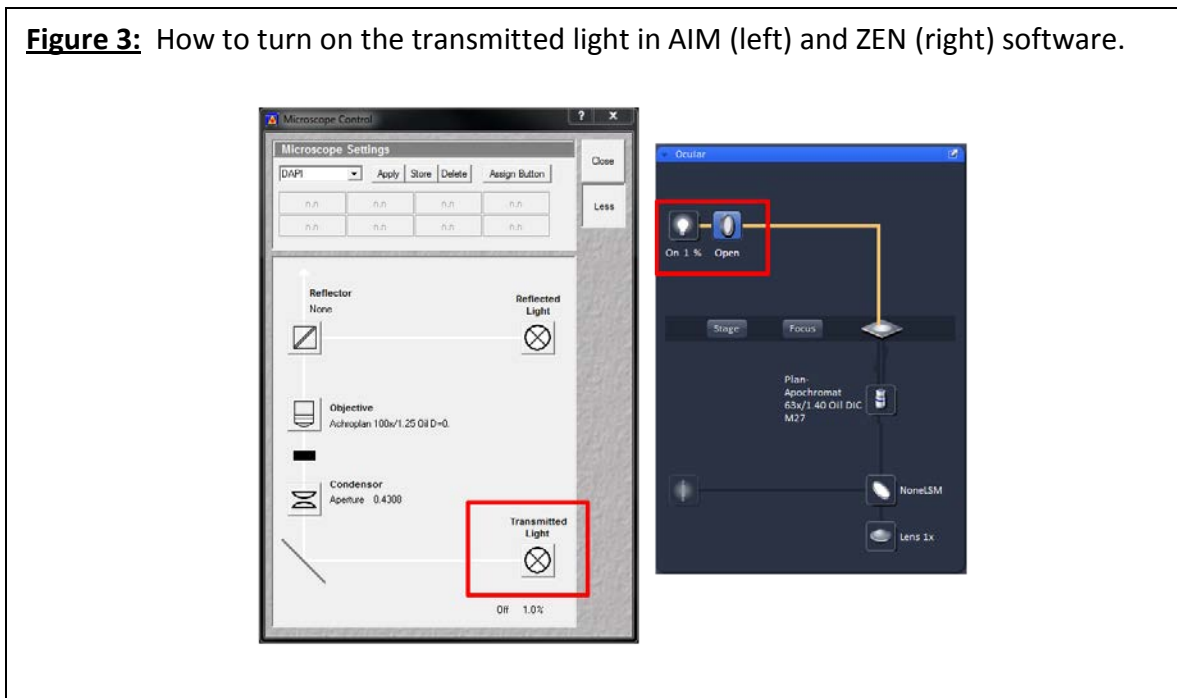
Step 2: Match DIC prism to the DIC slider

The DIC slider will have a Roman numeral I, II, or III printed on it. Select the appropriate I, II, or III prism in the condenser, shown in Figure 2 as #6. The prisms are moved manually unless you have a motorized stand, in which case there will be buttons present on the condenser that you can use to select the appropriate prism.

Step 3: Adjust for Koehler Illumination

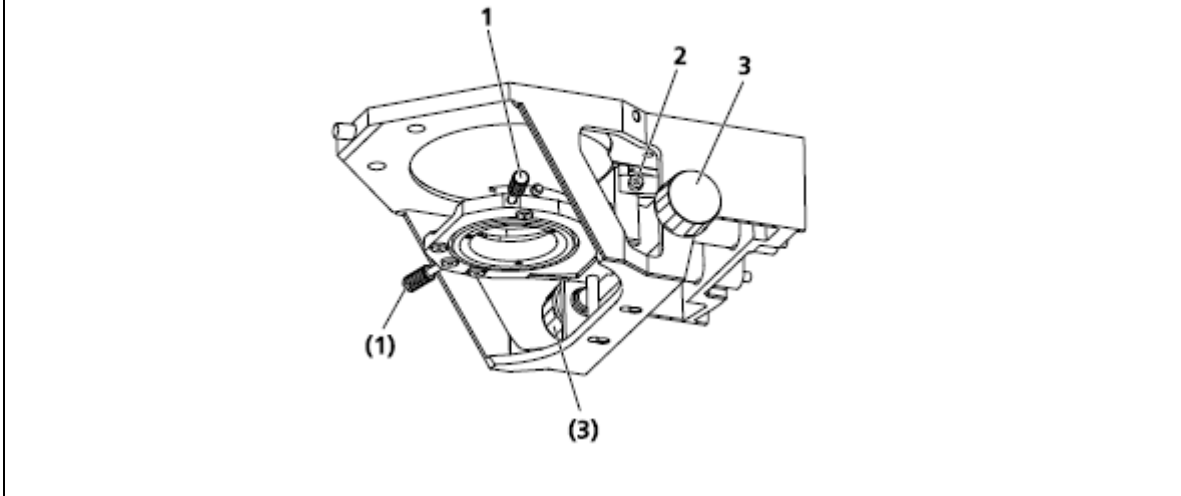
1. Using a low power lens (10x or 20x) and a test sample, such as *Convallaria*, focus on the sample through the eyepieces using transmitted light (halogen lamp illumination).

Figure 3: How to turn on the transmitted light in AIM (left) and ZEN (right) software.



2. Move the condenser to the highest position (towards the sample) using the knob labeled in Figure 4 as #3.

Figure 4: Diagram of the condenser carrier located directly below the sample



3. Open the aperture diaphragm all the way (move the wheel shown in Figure 2 and labeled #5 completely counter-clockwise).
4. Close the luminous-field diaphragm, as shown in Figure 5 and labeled #3, until the edges are visible through the eyepieces. This is illustrated in Figure 6.

Figure 5: Diagram of the Axio Imager

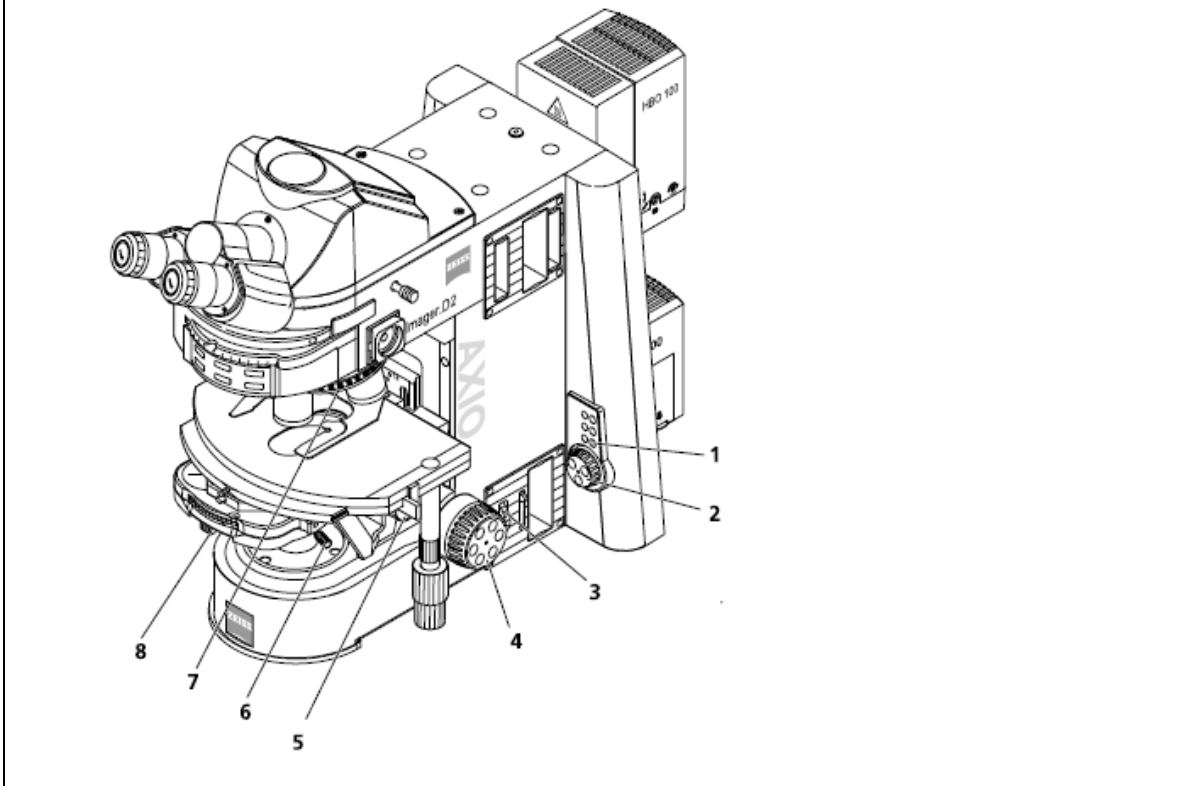
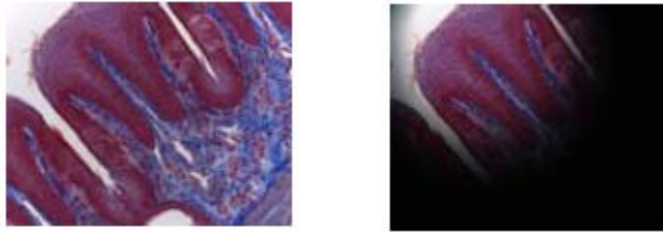
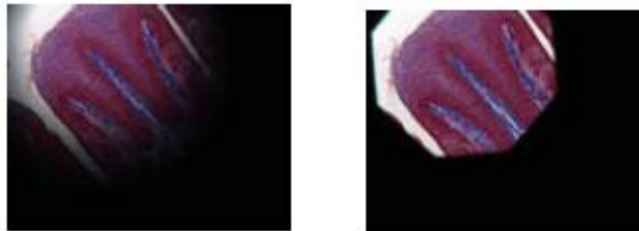


Figure 6: In the left image the luminous-field diaphragm is completely open. Close it until you see the edge of the shadow, as shown on the right.



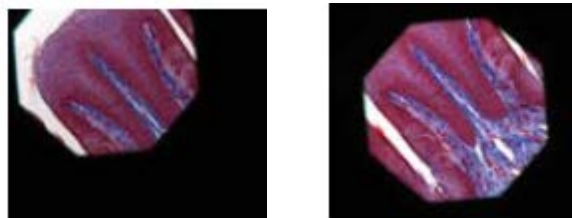
5. Focus the condenser using the knob shown in Figure 4 and labeled #3 until the edges appear sharp. This is illustrated in Figure 7.

Figure 7: These images compare what the image will look like before focusing the condenser (left) and after focusing the condenser (right).



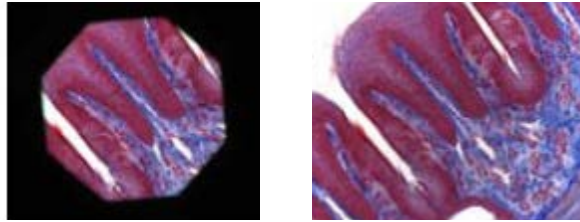
6. Center the image of the luminous-field diaphragm with the centering screws labeled in Figure 4 as #1. This is illustrated in Figure 8.

Figure 8: These images compare what the image will look like before centering the luminous-field diaphragm (left) and after centering the luminous-field diaphragm (right).



7. If you plan on using a higher magnification lens, switch that lens into place. Then open the luminous-field diaphragm, shown in Figure 5 and labeled #3, until the edges are just outside the visible region through the eyepieces. This is illustrated in Figure 9.

Figure 9: These images compare what the image will look like before opening the luminous-field diaphragm (left) and after opening the luminous-field diaphragm (right).



8. Remove one eyepiece to view the back focal plane of the microscope.
9. Close the aperture diaphragm, labeled as #5 in Figure 2, so it illuminates approximately 80% of the field of view.

Figure 10: These images compare what the image will look at before opening the aperture diaphragm (left) and after closing the aperture diaphragm to 80% (right).



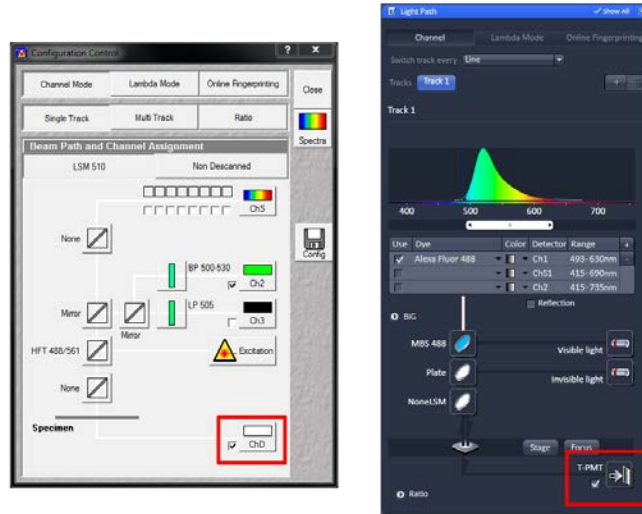
10. Replace the eyepiece.
11. Load your experimental sample.

****The field size and the objective aperture are different for each lens. If a different lens is selected for imaging, these must be adjusted accordingly****

Step 4: Configuring the Transmitted Light Channel in AIM or ZEN

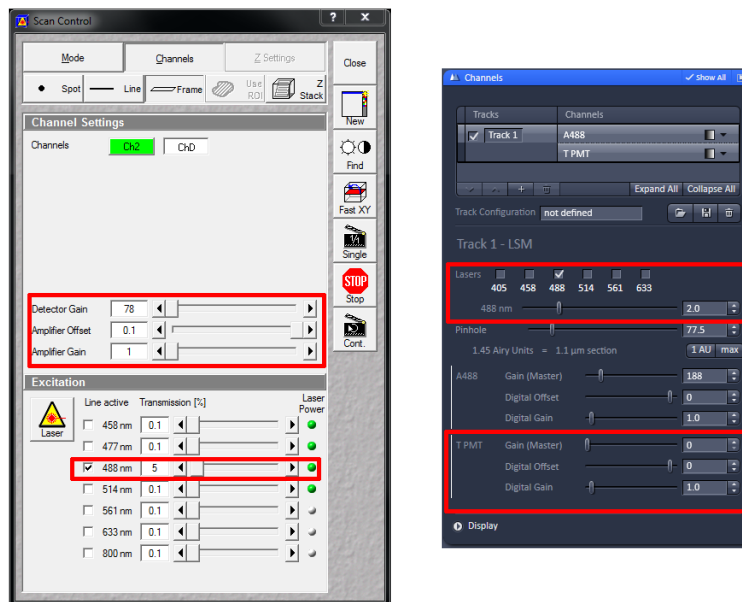
DIC images can be simultaneously collected with a fluorescent image using any laser line. Select Channel D or T-PMT in the Configuration Control or Light Path menu to acquire a DIC image, this is illustrated in Figure 11. Be sure to select an excitation laser; any laser is suitable except for the 405.

Figure 11: DIC images are collected in the detector labeled ChD in AIM (shown on the left) or T-PMT in ZEN (shown on the right).



Once the transmitted light channel has been selected in the Configuration Control / Light Path menu, the gain and offset can be set similarly to a fluorescent channel. Increase the gain until the channel image just turns white and then decrease the offset to bring out the structures. Laser power can also be adjusted to increase or decrease intensity. These controls are shown in Figure 12.

Figure 12: Detector gain, amplifier offset and laser power are used to balance the DIC image in AIM (left) or ZEN (right).

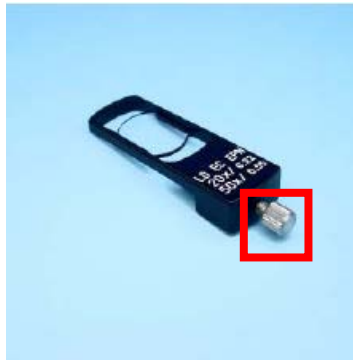


Step 5: Final Touches

There are two final adjustments that can be made to qualitatively optimize the image by increasing/decreasing contrast and resolution.

Adjust the shear on the Wollaston prism by turning the screw in the DIC slider either clockwise or counterclockwise. This screw is shown in Figure 13. This is performed while scanning an image and qualitatively assessing what looks best.

Figure 13: This image indicates the screw that, when turned, adjusts the Wollaston prism. You can turn it while the DIC slider is in place above the objective and the system is scanning an image.



Adjust the rotatable polarizer, shown in Figure 14 as #1, until the intensity of the image is lowest to create a proper DIC image.

Figure 13: Diagram of where a rotatable polarizer would sit below the condenser carrier

