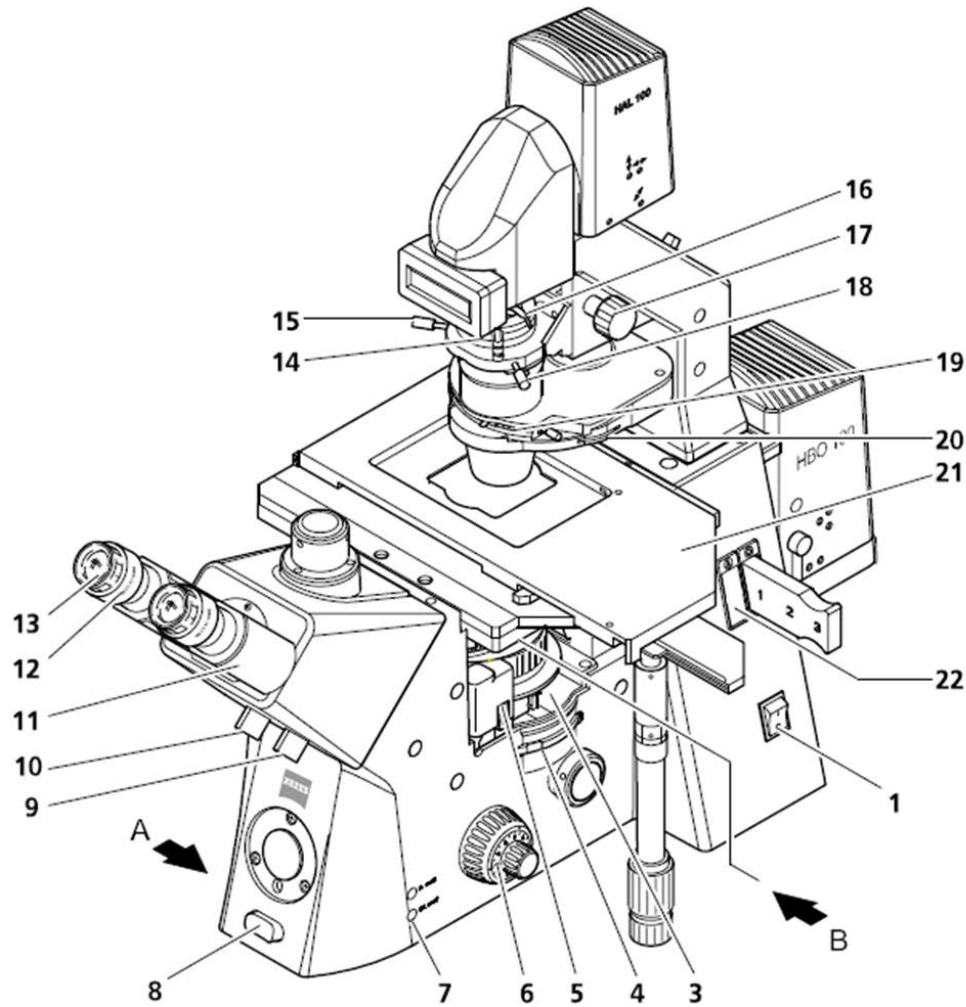




## **DIC Imaging using Laser Scanning Microscopes (LSM) on Inverted Stands**

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 Axiovert 200 or Axio Observer 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](mailto:Support@zeiss.com)

**Figure 1:** Diagram of the AxioObserver



- |  |   |   |
|--|---|---|
| 1 On / Off switch                                    | 10 Rotary or slider knob for Bertrand lens and manual shutter | 18 Centering screw for condenser                    |
| 2 Nosepiece  | 11 Binocular component of the tube                            | 19 Turret of condenser                              |
| 3 Compartment for slider analyzer                    | 12 Setting ring of eyepiece                                   | 20 Setting wheel for aperture stop on the condenser |
| 4 Setting wheel for Optovar turret                   | 13 Eyepiece   | 21 Microscope stage                                 |
| 5 Reflector turret                                   | 14 Polarizer D with 2-position filter changer                 | 22 Compartment for aperture stop slider             |
| 6 Focusing drive coarse / fine                       | 15 Centering screw for condenser                              | 23 Setting wheel for Sideport                       |
| 7 HAL on/off key                                     | 16 Adjusting lever of luminous-field diaphragm                | 24 Setting knob for Frontport / Baseport            |
| 8 Toggle switch for illumination intensity           | 17 Setting knob for vertical adjustment of the condenser      |   |
| 9 Rotary or slider knob for vis / doc beam splitting |   |   |

## Step 1: Match DIC Slider to Objective

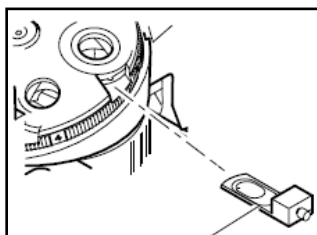
Each lens has a specific DIC slider. Be sure that the lens that is used for imaging has a matching DIC slider inserted.

**Figure 2:** A typical ZEISS lens (left) and DIC slider (right)



The DIC slider fits directly below the objective lens. 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 (see Figure 3).

**Figure 3:** The DIC slider fits under the lens and must be in the correct orientation.



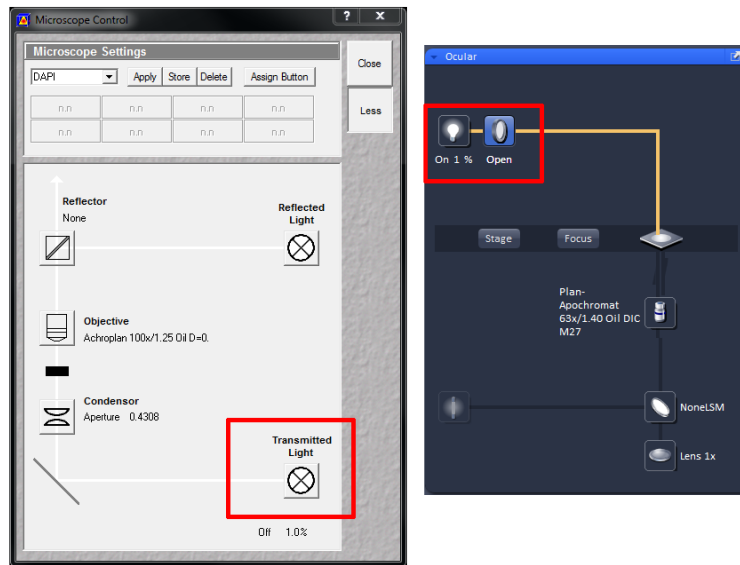
## Step 2: Match DIC prism to the DIC slider

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 (Figure 1, #19). 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

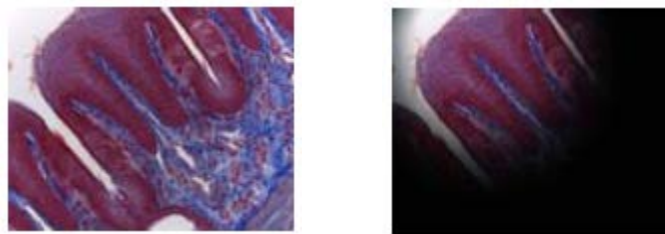
1. Focus a low power lens (10x or 20x magnification) on a test sample, such as *Convallaria*, using the transmitted light (halogen lamp illumination).

**Figure 4:** How to turn on the transmitted light in AIM (left) and ZEN (right) software. Be sure that you have selected for Ocular mode to view the sample through the eyepieces.



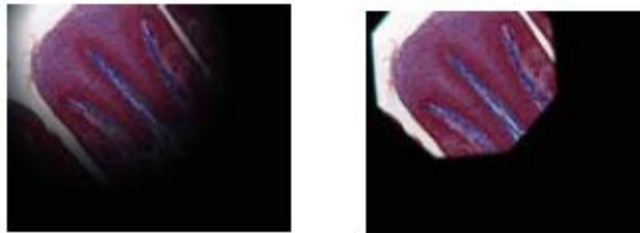
2. Move the condenser to the lowest position (towards the sample) using the knob shown in Figure 1 and labeled as #17.
3. Open the aperture diaphragm all the way (move the wheel shown in Figure 1 and labeled #20 completely counter-clockwise).
4. Close the luminous-field diaphragm (shown in Figure 1 and labeled #16) until the edges are visible, as exemplified in Figure 5.

**Figure 5:** 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 1 and labeled #17 until the edges appear sharp. See Figure 6 for an example of this.

**Figure 6:** These images compare what the image will look like before focusing the condenser (left) and after focusing the condenser (right).



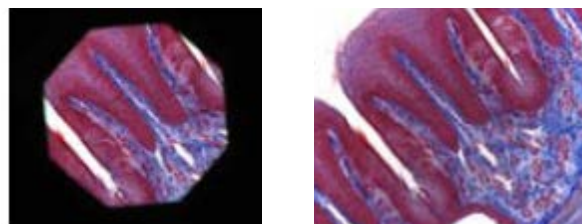
6. Center the image of the luminous-field diaphragm with the centering screws, shown in Figure 1 as #15 and #18. An example of this is shown in Figure 7.

**Figure 7:** 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, move that lens into place. Then open the luminous-field diaphragm (shown in Figure 1 and labeled #16) until the edges are just outside the visible region through the eyepieces. This is illustrated in Figure 8.

**Figure 8:** 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 (shown in Figure 1 as #13) to view the back focal plane of the microscope.
9. Close the aperture diaphragm (shown in Figure 1 as #20) so it illuminates approximately 80% of the field of view. Figure 9 illustrates this.

**Figure 9:** 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.

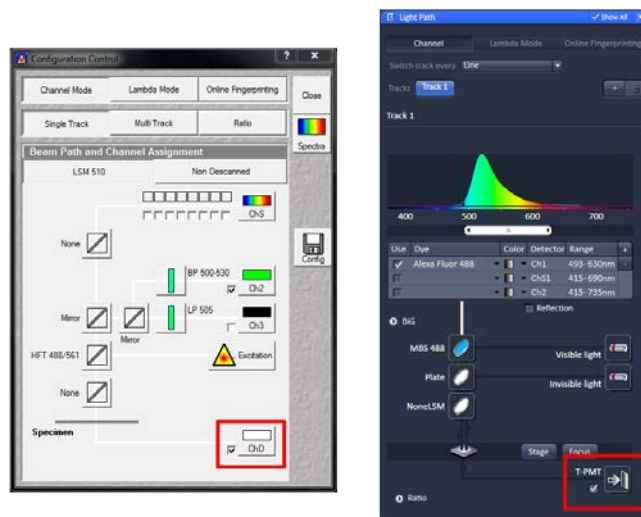
11. Load your experimental sample.

\*\*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

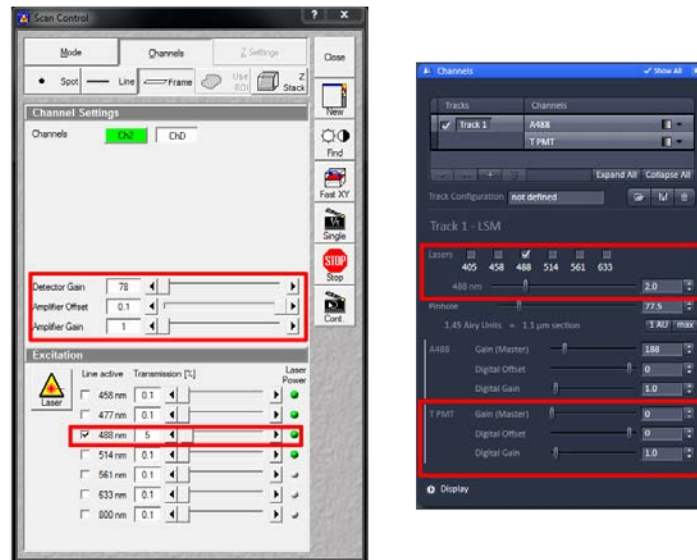
DIC images can be simultaneously collected with a fluorescent image using any laser line. Select Channel D or T-PMT in the Configuration Control or Light Path menu to acquire a DIC image. Be sure to select an excitation laser.

**Figure 10:** 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, 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 adjustments are controlled in the software, as shown in Figure 11.

**Figure 11:** Detector gain, amplifier offset and laser power are used to balance the DIC image in AIM (left) or ZEN (right).

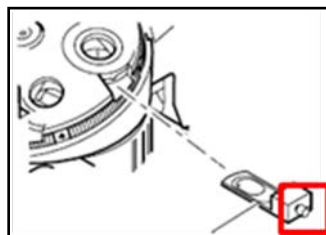


## Step 5: Final Touches

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

The shear on the Wollaston prism can be adjusted by turning the screw in the DIC slider either clockwise or counterclockwise. This is performed while scanning an image and qualitatively assessing the resulting image.

**Figure 12:** Turn the screw in the DIC slider to adjust the shear on the Wollaston prism.



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