

A guide to Differential Interference Contrast installation on the SliceScope Microscope

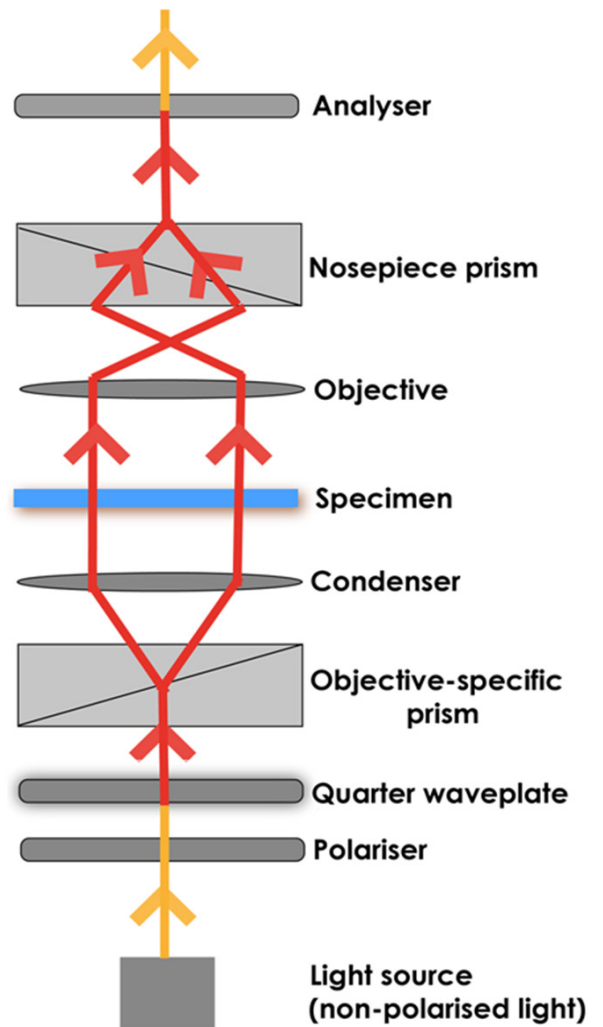
Differential Interference Contrast (DIC) is a microscopy technique that introduces contrast to images of specimens which have little or no contrast when viewed using brightfield microscopy. The images produced using DIC have a pseudo 3D-effect, making the technique ideal for electrophysiology experiments.

How DIC works

In DIC, light emitted from the source is linearly polarised by a polariser. The linearly polarised beam of light enters an objective-specific prism, which splits it into two rays that vibrate perpendicular to each other. The rays are parallel as they pass through a condenser, but as they are vibrating perpendicular to each other, they cannot cause interference.

The split beams pass through the specimen. The specimen's varying thickness and refractive indices alter the wave paths of the beams. They then enter the objective, which focusses them above the rear focal plane. The two beams enter a second prism, in the nosepiece, which combines them. The beams have different lengths, because they passed through different parts of the specimen.

The analyser, which is a second polariser, brings the vibrations of the beams into the same plane and axis, causing destructive and constructive interference to occur. The light then travels to the eyepiece or camera, where a DIC image with differences in intensity and colour can be seen.

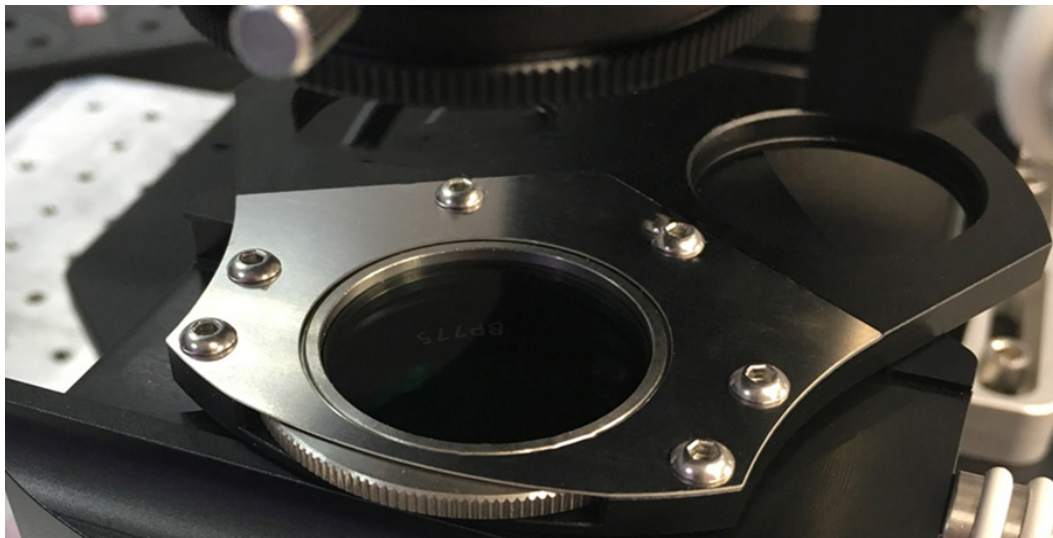
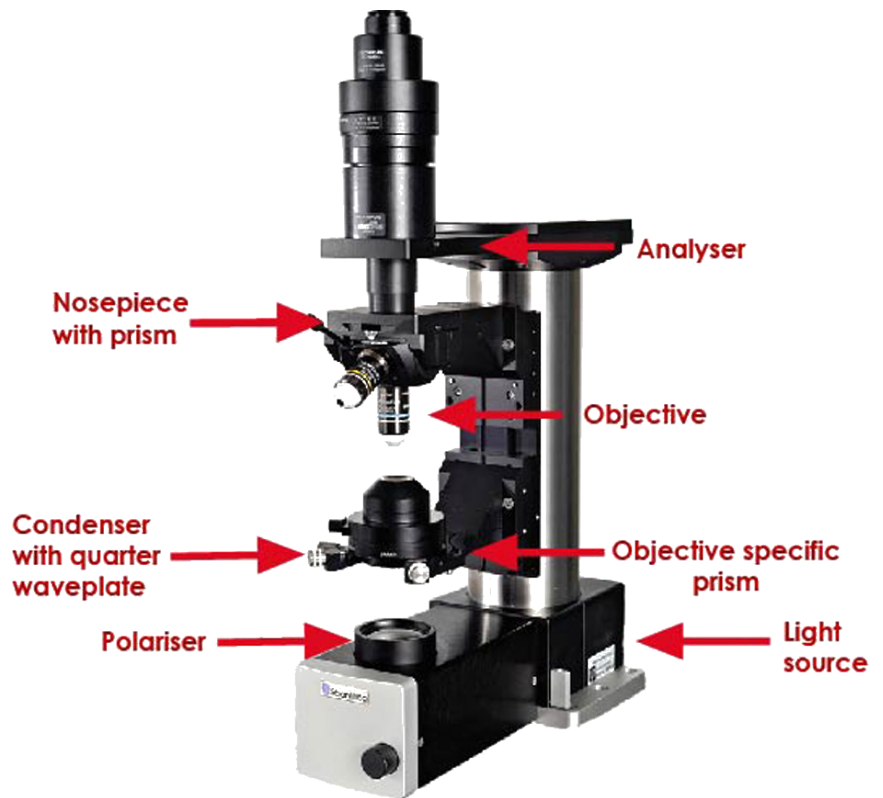


Setting up DIC

DIC can be utilised on virtually any upright or inverted microscope, as long as all components can be installed. In the image below of the Scientifica SliceScope, although not all DIC components are present, their locations are labelled.

Before setting up DIC on a microscope, ensure all components are free of dust and debris, as dirty objective, condenser and lens elements can reduce image quality.

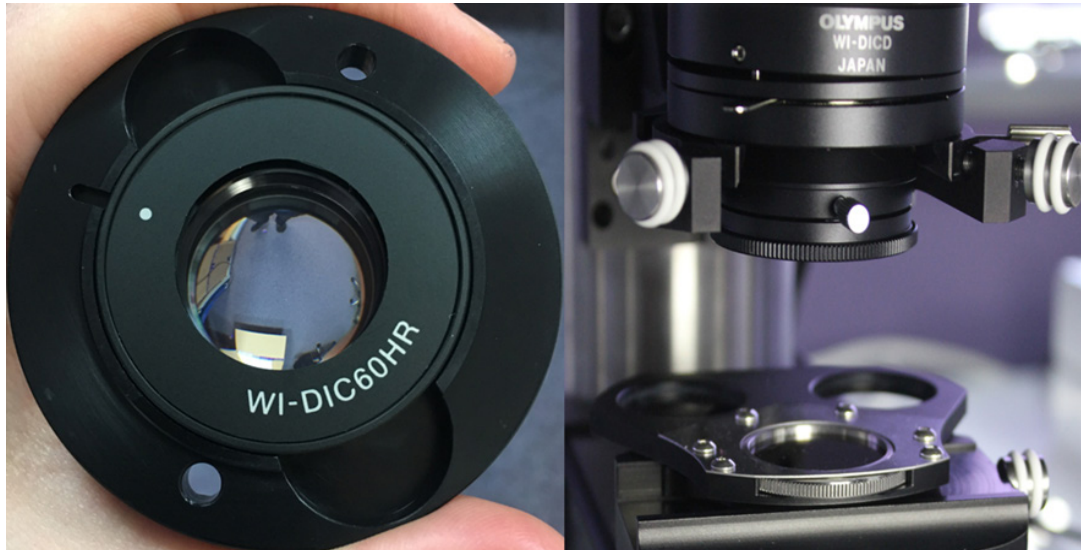
1. If using a white light source, insert an infrared bandpass filter into the slider on the SliceScope filter wheel and secure in place with a locking screw. This ensures only infrared light passes through to the polariser.
2. Insert the polariser into the silver-plated section of the filter wheel, underneath the condenser, shown in the image below. The polariser is on a rotatable mount, enabling it to be rotated when aligning DIC.



Filter wheel with rotatable mount, showing the installed polariser

3. Ensure your condenser does not have a protective covering on it, as this will obscure images produced using DIC.
4. Keeping the condenser facing upwards, use a hex key to unscrew the two screws either side of the condenser to open it. Turn the top section only upside down.

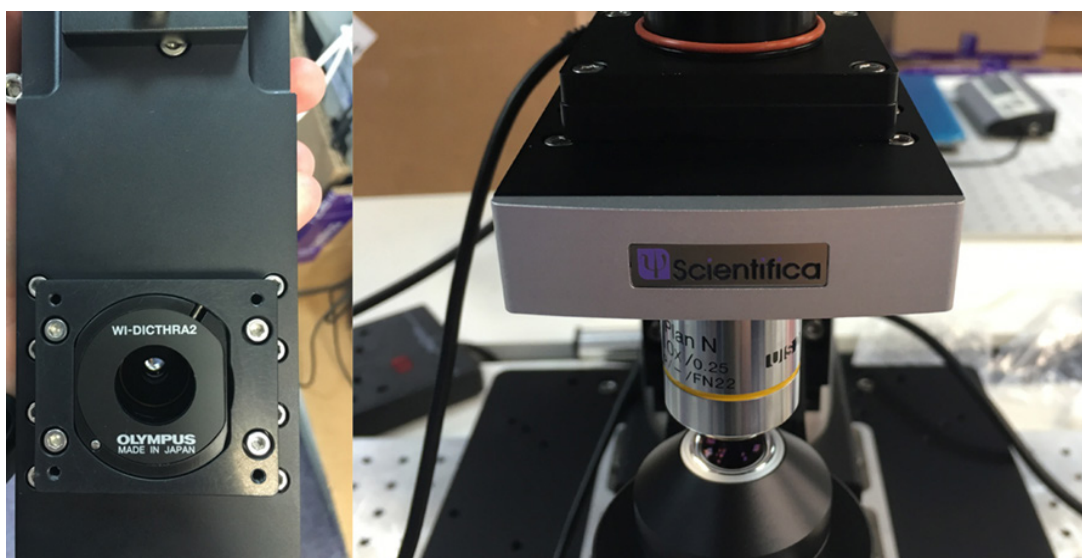
5. Loosen the grub screw on the side of the condenser and remove the spacer ring from the top half, as this is not needed. Insert the objective-specific prism, lining up the metal pin on the prism with the slot in the condenser. Tighten the grub screw and put the condenser back together. Each objective requires a different objective-specific prism, based on the magnification of the objective, for example a 40x objective requires a WI-DIC40HR objective specific prism. Usually, a prism is only required for the high magnification objective you are using.



The left image shows the WI-DIC60HR prism in the condenser. On the right is the condenser setup on the SliceScope with the filter wheel below

6. The second DIC prism (WI-DICT or WI-DICTHRA) is in a fixed mount which is inserted into the nosepiece above the objective. Loosen the grub screw on the right-hand side of the nosepiece arm and slide the nosepiece (WI-SRE) forward. Insert the second DIC prism into the nosepiece and secure it using the small screw at the rear-right corner of the nosepiece.

If using a MOC, pictured below, loosen the four screws to open it and then follow the instructions above.



The image on the left shows the objective prism in the MOC. The image on the right is the nosepiece, where the objective prism is located

7. If fluorescence is being used, insert the analyser (U-AN) into the fluorescence turret, as shown below. If fluorescence isn't being used, insert the analyser (U-ANT) into the U-KPA.
8. The U-KPA can then be secured on the SliceScope under the imaging lens or trinocular head.
9. The polariser and analyser need to be aligned so that their transmission pathways cross at a 90 degree angle to one another. This can be achieved by rotating the polariser in the filter wheel. As this can be rotated 360 degrees, the analyser and U-KPA can be mounted in any orientation.



The image on the left is a U-AN analyser, and the image on the right shows the U-AN analyser inserted into a fluorescent turret on the SliceScope.

10. For DIC microscopy to be performed, Koehler illumination must be first set-up. Once this has been set-up, DIC will need to be aligned.
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Aligning DIC

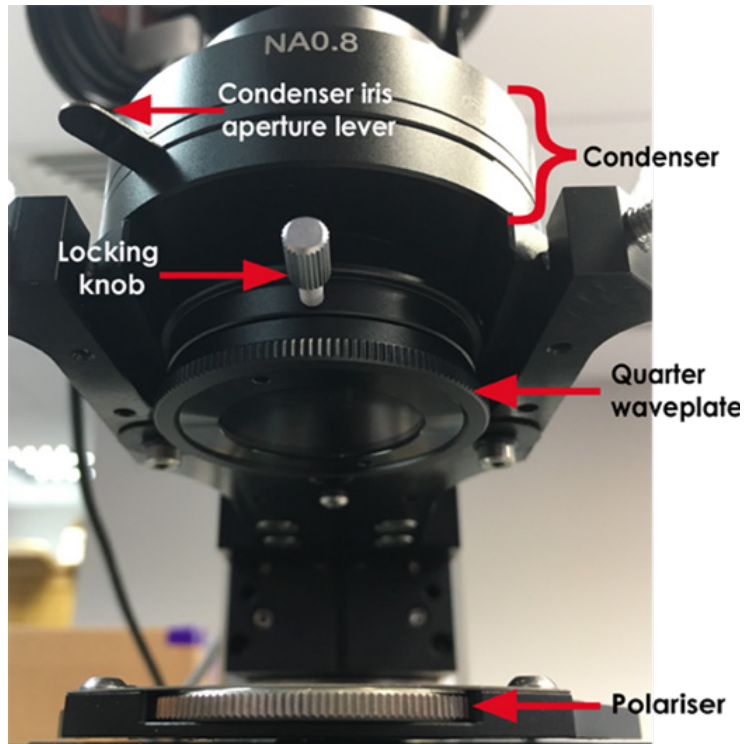
1. Focus on a blank sample plate using either a 4x or 10x objective.
2. Move the DIC slider with the U-AN analyser into the light path. The U-ANT is not on a slider and is in a fixed position which is always in the light path.
3. If using a trinocular head, remove one eyepiece and view the sample directly down the trinocular head.
- 4.a. When using eyepieces: rotate the polariser until there is a dark stripe through the centre of the field of view. This indicates that the polariser and analyser are aligned at 90 degrees to each other.
- 4.b. When using a camera: rotate the polariser until the image is at its darkest.

5. If the condenser and objectives were removed, put them back at this point. Double-check that your condenser is still set-up for Koehler illumination.
6. Use your DIC objective to bring your sample into focus.
7. Add a specimen so that contrast can be optimised for the sample you are viewing.
8. As the SliceScope uses de Sénarmont DIC, a quarter waveplate is required. Adjusting the quarter wave plate changes whether structures appear concave or convex. Unscrew the locking knob at the front of the image below and adjust the quarter wave plate as desired.

9. Lock the quarter waveplate in position using the locking knob.

10. The lever in the image above controls the condenser iris aperture. If using eyepieces, remove one eyepiece and look down so you can see the internal condenser iris. Adjust the lever until the condenser iris just disappears from the field of view. When using a camera, adjust the lever until the image is at its sharpest.

11. As DIC is a function of specimen orientation, rotating the sample can improve or change the image contrast.



You should now have a clear DIC image! An example of a DIC image is below; the image has good contrast and the *C.elegans* has a 3D effect.



An adult Caenorhabditis elegans nematode imaged using DIC by Bob Goldstein

If you have any questions or require further guidance, please contact us at info@scientifica.uk.com.