

# How To Perform DIC Microscopy

Technical Support Note #T-3

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## Introduction

Many biological specimens cannot be effectively visualized using ordinary bright-field microscopy because their images produce very little contrast, rendering them essentially invisible. Differential Interference Contrast (DIC) microscopy is an excellent method for generating contrast by converting specimen optical path gradients into amplitude differences that can be visualized by the human eye. Images produced using DIC optics have a distinct relief-like, shadow-cast appearance giving an illusion of three dimensionality. This tutorial will cover the optical components used in DIC microscopy, the basic principles governing how it works, and the proper alignment of DIC optical components for the Zeiss Axiovert 200M research microscope.

## Principle

DIC microscopy utilizes a system of dual-beam interference optics that transforms local gradients in optical path length in a specimen into regions of contrast in an image. Optical path length is a product of the refractive index and thickness between two points on an optical path and is directly related to the transit time and number of cycles of vibration exhibited by a wave of light traveling between two points. The basic principle of DIC is illustrated in figure 4. Waves of plane polarized light are split into two beams by the condenser prism. These pairs of beams emerge from the condenser lens traveling in parallel to one another and pass through the specimen. The spacing between these paired beams of light varies depending on the prism used, but generally ranges between 0.2-2 $\mu$ m. When the paired beams pass through the specimen, the phase of one beam may be differentially shifted with respect to the other if there is a local gradient in optical path length. DIC optics are designed to convert these phase differences between the two beams of light into amplitude differences which can be visualized by the human eye. This occurs when the beams are recombined by the objective prism and subsequently pass through the analyzer, yielding linear polarized light with a finite amplitude.

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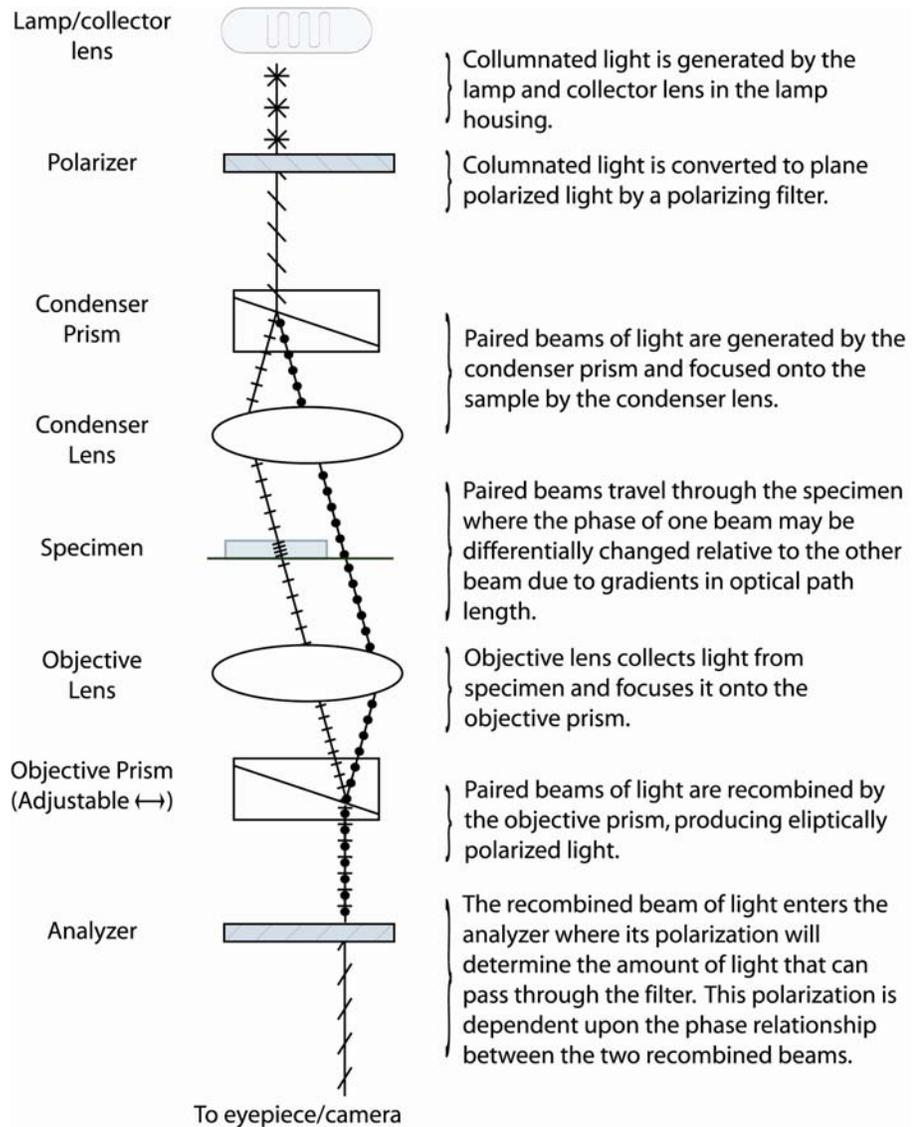


Figure 4. Schematic of DIC optical path

### Overview of DIC Optical Components

The DIC system consists of four optical components which must be inserted into the light path of the microscope (the final section of this tutorial will cover the correct positioning and alignment of these components, which is essential in order to generate an image of both high quality and high contrast).

- **Polarizer.** The polarizer is located above the condenser and converts the collimated light emanating from the lamp housing into plane polarized light (figure 1). By convention, the light transmission axis of the polarizer is set in an east-west direction when facing the microscope.

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- Condenser prism. The condenser prism is located within a motorized turret in the condenser housing (figure 1). This turret may also contain several annuli for phase contrast microscopy. Generally, there are two different DIC prisms which are used, DIC II and DIC III, corresponding to the use of either low or high numerical aperture objectives, respectively. The condenser prism functions as a beam-splitter, converting an individual wave of polarized light into two closely spaced waves of light that are then focused onto the specimen by the condenser lens. As will be discussed below, this system of dual beam optics is the basis for the ability of DIC to detect local gradients in optical path length within a specimen.

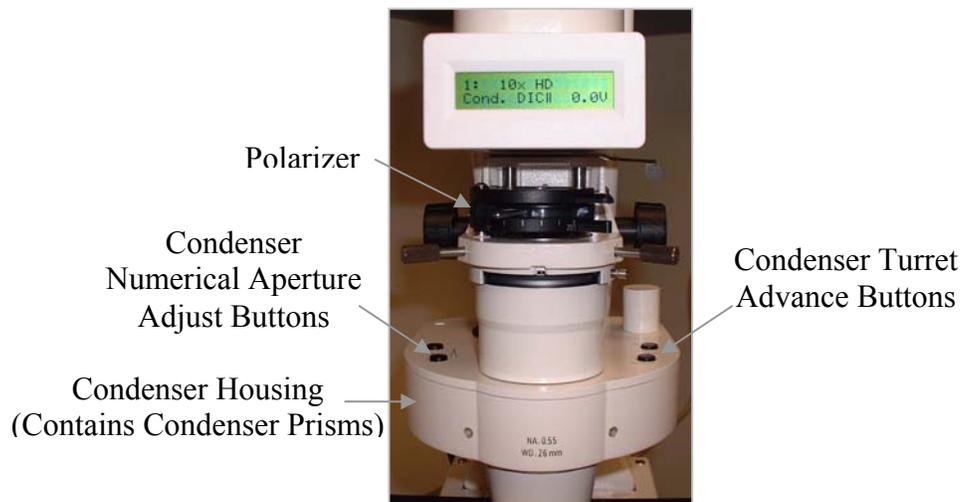


Figure 1. Condensor arm of the Axiovert 200M

- Objective prism. The objective prism is inserted into a slot located directly below the objective. A variety of objective prisms are available which must be matched with the magnification of the objective and the condenser prism (DIC II or DIC III). This information can be found inscribed on the plastic objective prism holder (figure 2). The objective prism functions to recombine the two waves of light produced by the condenser prism after traveling through the specimen and the objective. The objective prism holder has an adjustment screw allowing the position of the prism to be changed in a linear direction. Changing the position of this prism allows adjustment of the shadowing within the image.

Objective Prism

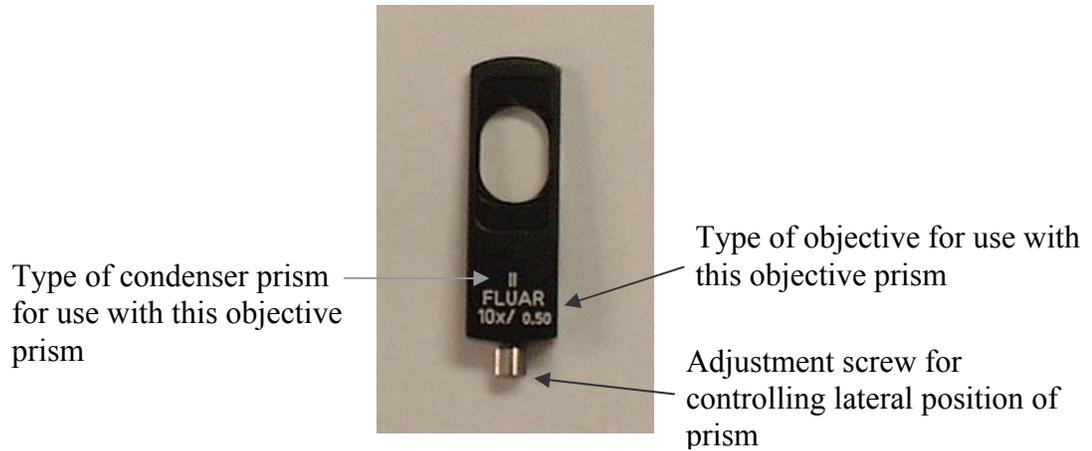


Figure 2. Objective Prism

- Analyzer. The analyzer is simply another polarizing filter located within the motorized filter cube turret below the objective nose-piece of the microscope (figure 3). The analyzer converts the elliptically polarized light emanating from the objective prism into plane polarized light which then forms an image at the image plane. By convention, the light transmission axis of the analyzer is set in a north-south direction when looking at the microscope.



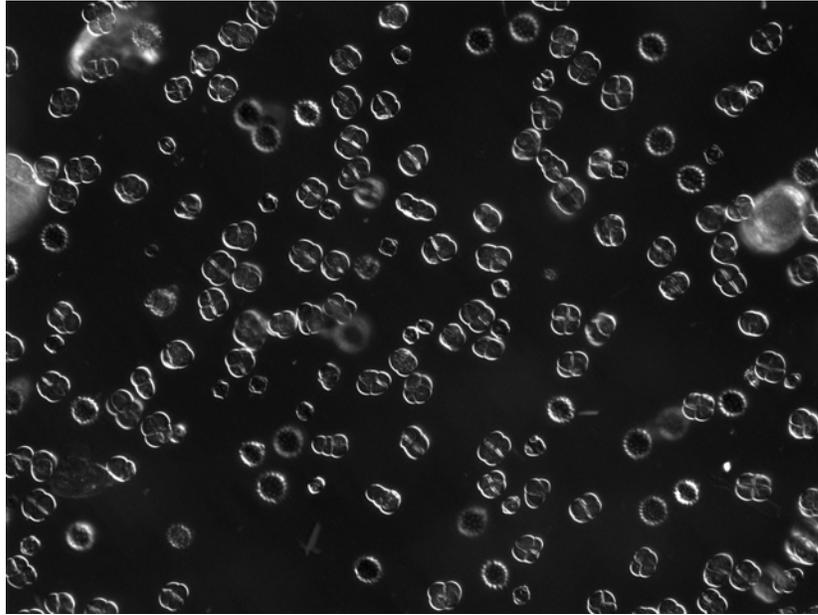
Figure 3. Analyzer cube.

## Methods

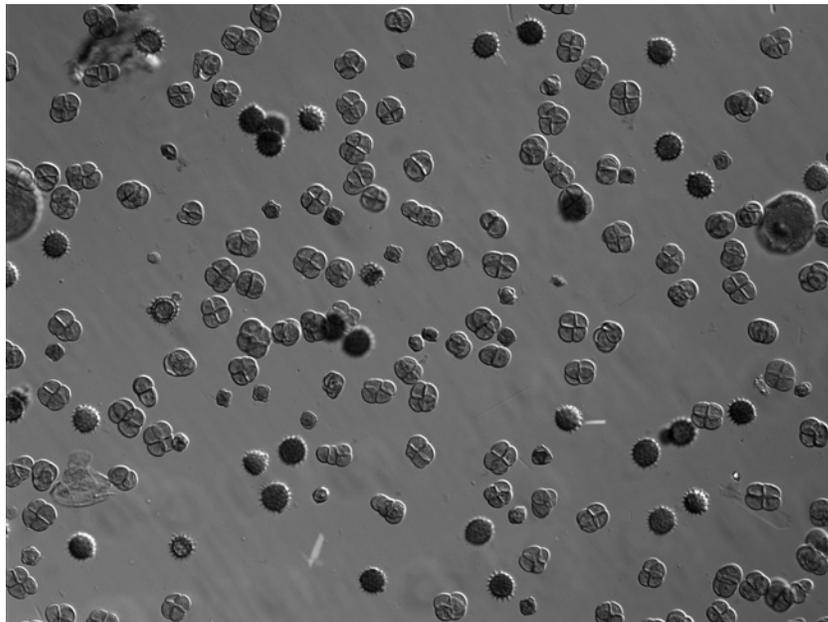
1. With no DIC optics in place, roughly set Koehler illumination so that ample light is traveling from the condenser to the objective.
2. The two polarizing filters (the polarizer above the condenser and the analyzer within the filter cube turret) must be adjusted so that they are orientated  $90^{\circ}$  with respect to one another. To do this, move the analyzer into the light path by

selecting the motorized internal filter turret position containing the analyzer cube. The transmission axis of the analyzer in the filter cube turret is permanently fixed in a north-south orientation. Place the polarizer into the light path by swinging the plastic filter holder located above the condenser into place. The polarizer needs to be rotated until it is orientated at  $90^{\circ}$  with respect to the analyzer. To do this, look through the eyepieces with the lamp on and rotate the polarizer using its adjustment lever. As the adjustment lever is moved, the field of view should change from bright to dark. The polarizer should be positioned at a point where the field of view is maximally dark, with very little light traveling through to the eyepiece. When making this adjustment ensure there are no other DIC optics in place aside from the polarizer and the analyzer.

3. Put both DIC prisms into the light path.
  - a. For the condenser prism this is accomplished by pressing one of the position advance buttons on the right of the condenser turret until the microscope status display reads DIC II or DIC III. The use of either DIC II or DIC III will depend on the objective being used. For low numerical aperture air objectives the DIC II prism is used, while for high numerical aperture oil immersion objectives the DIC III prism is used. The correct condenser prism for use with a particular objective will be printed on the objective prism.
  - b. To put the objective prism in place, rotate the desired objective to a side position so that it is accessible, remove the plastic spacer occupying the slot beneath the objective and slide the prism into this slot. In the proper position, the plastic prism holder will be flush with the objective nosepiece of the microscope.
4. Mount and focus a specimen on the stage and reset Koehler illumination. Precise adjustment of Koehler illumination is critical for the proper function of DIC microscopy.
5. Adjust the lateral position of the objective prism until the desired amount of shadowing is obtained. This is accomplished by turning the positioning screw located on the objective prism holder. The positioning screw should be adjusted until the image exhibits the shadow-cast, three dimensional appearance that is the distinguishing feature of DIC microscopy. Figure 5 shows examples of how objective prism adjustment can affect the DIC image.
6. Finally, the numerical aperture of the condenser should be adjusted to improve contrast. This can be done using the two buttons located on the left side of the condenser housing (figure 1). In practice, the condenser diaphragm position can be set based on empirical observation of image quality.



Poor objective prism adjustment.



Good objective prism adjustment

Figure 5. Demonstration of objective prism adjustment.

### References

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