Interference microscopy

Over the past few years the microscopic techniques had faced with some complications with the observation of living specimens as transparency, light and because of that don’t have sufficient contrast.

Thus with the development of these techniques was possible to visualize specimens with adequate contrast.

Interference microscopy or Quantitative interference microscopy

The Interference Microscopy or Quantitative Interference Microscopy is one of these techniques that derive from Phase Contrast Microscopy but is more sensitive than this technique and make possible the easy and clarify viewing of living organisms.

This technique is used by taking light from a condenser and using a prism to separate the light into two beams. Thus, one beam (object beam) goes through the specimen and the objective and the other (reference beam) goes through another objective without touching the specimen. These beams allow a specimen to be seen through the difference in the fields caused by the two beams and the differences of the two images allow details to be seen.

Differential interference contrast microscopy

There is a variation of interference microscopy called Differential Interference Contrast microscopy (DIC), also known as Nomarski Interference Contrast microscopy (NIC) or simply Nomarski microscopy.

This optical microscopy illumination technique used to enhance the contrast in unstained or transparent samples was named after its inventor and also uses two beams produced by a single polarized light.

Initially the polarized light is divided into two rays polarized to each other (sampling and reference rays) when enters in the first Nomarski-modified Wollaston prism. Then this two rays are focused by the condenser for passage through the sample and travel to adjacent areas of the sample, divided by the shear (separation is normally similar to the resolution of the microscope).

After that, they will face different optical way lengths where areas differ in refractive index or thickness which will cause a change in phase of one ray relative to the other according to the delay experienced by the wave in the more optically dense material.

Lastly the rays go through the objective lens and are focused for the second Nomarski-modified Wollaston prism which joins the two rays into one polarized which make an image with a three-dimensional appearance. This final junction of rays leads to interference, brightening or darkening the image at that point according to the optical way difference. These interference techniques have advantages in uses involving living or unstained biological samples, specially their applications in biology, crystallography, mineralogy and chemistry; in standard optical microscopy techniques its resolution and clarity is also visible.

Limitations

On the other hand these techniques also have limitations as its requirement for a transparent sample of similar refractive index. Differential Contrast Microscopy is inadequate for thick samples (tissue slices, pigmented cells) and for most non biological samples because of its polarization dependence.

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