Some other types of microscopy

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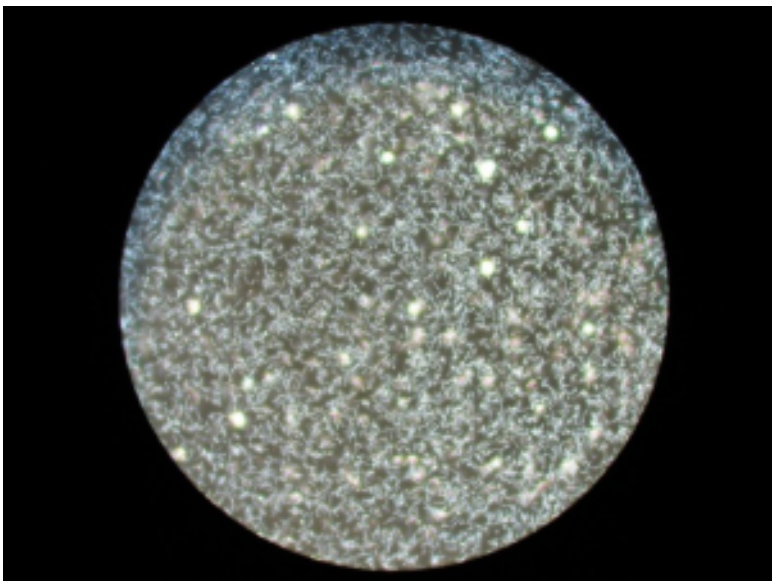
Dark field microscopy: Radiance Against a Dark Background

Dark-field microscopy is ideally used to illuminate unstained samples causing them to appear brightly lit against a dark background. This type of microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. Rather than illuminating the sample with a filled cone of light, the condenser is designed to form a hollow cone of light. The light at the apex of the cone is focused at the plane of the specimen; as this light moves past the specimen plane it spreads again into a hollow cone. The objective lens sits in the dark hollow of this cone; although the light travels around and past the objective lens, no rays enter it.

The entire field appears dark when there is no sample on the microscope stage; thus the name dark-field microscopy. When a sample is on the stage, the light at the apex of the cone strikes it. The rays scattered by the sample and captured in the objective lens thus make the image.



Dark field microscopy visualisation of live bacteria: Spirochetes bacteria observed under dark field microscopy

Samples observed under dark-field microscopy should be carefully prepared since dust and other particles also scatter the light and are easily detected. Glass slides need to be thoroughly cleaned of extraneous dust and dirt. It may be necessary to filter sample media (agar, water, saline) to exclude confusing contaminants. Sample materials need to be spread thinly; too much material on the slide creates many overlapping layers and edges, making it difficult to interpret structures.

Phase contrast microscopy: Visualizing differences in refractive indexes of different parts of a specimen relative to unaltered light

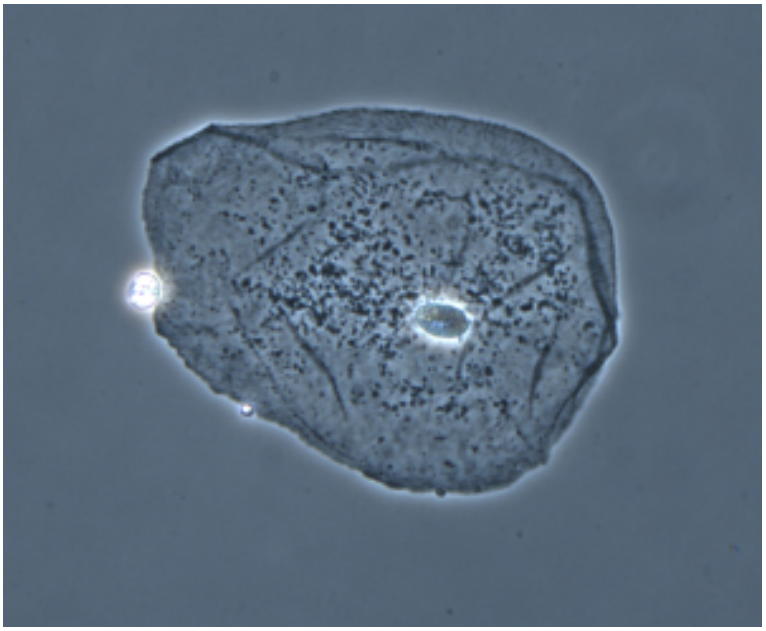
Phase-contrast microscopy is a method of manipulating light paths through the use of

strategically placed rings in order to illuminate transparent objects.

- Parallel beams of light are passed through objects of different densities.
- The microscope contains special condensers that throw light "out of phase" causing it to pass through the object at different speeds.
- Internal details and organelles of live, unstained organisms (e.g. mitochondria, lysosomes, and the Golgi body) can be seen clearly with this microscope.
- A phase ring in condenser allows a cylinder of light to pass through it while still in phase. Unaltered light hits the phase ring in the lens and is excluded. Light that is slightly altered by passing through a different refractive index is allowed to pass through.
- Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium. Elements of lower refractive index advance the wave.

- Much of the background light is removed and light that constructively or destructively interfered is let through with enhanced contrast.

Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrastand high resolution . This tool works best with a thin specimen and is not ideal for a thick specimen. Phase-contrast images have a characteristic grey background with light and dark features found across the sample. One disadvantage of phase-contrast microscopy is halo formation called halo-light ring.



Phase contrast micrscopy of cheek epilthelial cell

Interference Microscopy: a variation of phase-contrast microscopy that uses a prism to split a light beam in two.

Stereo Light Source

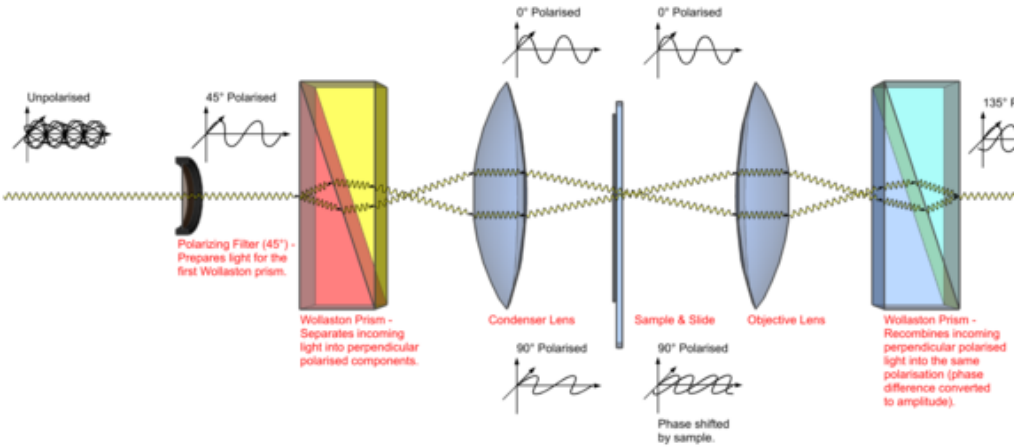
Interference microscopy uses a prism to split light into two slightly diverging beams that then pass through the specimen. It is thus based on measuring the differences in refractive index upon recombining the two beams. Interference occurs when a light beam is retarded or advanced relative to the other.

There are three types of interference microscopy: classical, differential contrast, and fluorescence contrast. Since its introduction in the late 1960s differential interference contrast microscopy (DIC) has been popular in biomedical research because it produces high-resolution images of fine structures by enhancing the contrasted interfaces. The image produced is of a thin optical section and appears three-dimensional, with a shadow around it. This creates a contrast across the specimen that is bright on one side and darker on the other.

The Interference Microscope

The microscope is a bright field light microscope with the addition of the following elements: a polarizer between the light source and the condenser, a DIC beam-splitting prism, a DIC beam-combining prism, and an analyzer .

Manipulating the prism changes the beam separation, which alters the contrast of the image. When the two beams pass through the same material across the specimen they produce no interference. When the two beams pass through different material across the specimen such as on the edges, they produce alteration when combined.



Path of light in differential interference contrast microscopy (DIC). Two parallel light beams pass through the specimen and combine to produce an image

Fluorescence differential interference contrast (FLIC) microscopy was developed by combining fluorescence microscopy with DIC to minimize the effects of photobleaching on fluorochromes bound to the stained specimen. The same microscope is equipped to simulataneously image a specimen using DIC and fluorescence illumination.

Source: Boundless. “Microscopy.” *Boundless Microbiology*. Boundless, 27 Jun. 2015. Retrieved 29 Jun. 2015 from https://www.boundless.com/microbiology/textbooks/boundless-microbiology-textbook/microscopy-3/light-microscopy-29/