Microscope image contrast

The source of microscope contrast is the interaction of objects with the illuminating radiation. It arises as a result of differences light intensity at different points of the image.

Contrast can be expressed using the formula: $k = \frac{I_a - I_b}{I_a + I_b}$, where the values of I_a and I_b are the light intensities at two different points. Contrast can also be characterized as the ratio of the difference between the light intensities of the object and the background to the light intensity of this background. For **percentage contrast**: $c = \frac{I_o - I_p}{I_p} \cdot 100$, where I_o denotes the intensity of the object and I_p background intensity. The human eye can distinguish a contrast of a minimum value of 0.02 (2%).

Resulting contrast may be affected

- by diffraction;
- absorption;
- dispersion;
- bending;
- refraction of light;
- by birefringence and light polarization;
- fluorescence.

Contrast enhancement can be achieved using a microscope condenser or special microscopic techniques.

Microscopic techniques

The way we increase the contrast depends on the properties of the object. According to the ability to absorb light, we distinguish between amplitude and phase objects.

Amplitude objects naturally absorb light when observed in a bright field and thereby change its amplitude. A change in the amplitude of light waves is perceived by the human eye as a change in brightness (intensity). Absorption of light by an object can also be achieved through dyeing. The colored sample then selectively absorbs part of the light spectrum.

If the bodies do not absorb light, it is not possible to use bright field microscopy techniques to observe them. We call these bodies **phase objects.** Phase objects change the phase of light. The human eye is unable to detect this change. The contrast increases them using special techniques. In the case of phase objects, we achieve an increase in contrast in two ways − by oblique lighting and detection of phase shifts.

Oblique lighting is used in

- dark field observation;
- Hoffman's modulation contrast.

Phase Shift Detection

- phase microscopy;
- interference microscopy;
- Normansky differential interference contrast;
- polarizing microscopy.

Links

Related articles

- Microscope
- Microscopic methods

External links

- HOFFMAN, Robert – DAVIDSON, Michael. *Contrast in Optical Microscopy* [online]. ©2016. [cit. 2017-01-12]. <http://micro.magnet.fsu.edu/primer/techniques/contrast.html>.
- PLÁŠEK, Jaromír – REISCHIG, Josef. *Kontrast v optické mikroskopii* [online]. Přírodovědecký časopis Vesmír, ©1995. [cit. 2017-01-12]. <http://casopis.vesmir.cz/clanek/kontrast-v-opticke-mikroskopii>.

Source

- NAVRÁTIL, Leoš – ROSINA, Jozef. *Medicínská biofyzika.* 1. edition. Grada, 2005. 524 pp. ISBN 80-247-1152-4.