

# Phase contrast microscopy

## Characteristics

Most light microscopes, which are used for examining not only biological specimens, allow the observation of only such objects that absorb the passing light to varying degrees - thus changing the amplitude of the passing electromagnetic wave. These objects are called **amplitude**.

However, there are many objects that provide very little or no difference in absorption of light radiation after the passage of light and are therefore unsuitable for observation in transmitted light. However, due to the different refractive index these preparations change the phase of the passing light wave at different points. The rays passing through the preparation therefore differ in the phase of the light wave. We call such objects **phase**. These include most unstained biological preparations.

However, our eye is not able to distinguish small phase differences, which is why phase objects appear to us as structureless and transparent. Therefore, an ordinary microscope is not suitable for observing them. For their observation, the so-called **phase microscope**, was invented, which is capable of converting phase changes into amplitude changes, and thus into changes observable by the human eye. It thus enables the observation of many cell structures, which before the invention of the phase microscope could only be observed on dead and stained cells.

The phase shift depends on the wavelength of the radiation of the source, the refractive index of the observed object, the length of the optical path of the light in the observed object (thickness of the object).

## Function Principle

The image of the object in the image focus is created by the interference of two waves. A **direct wave** that passes through the specimen unchanged, and a **diffraction wave**, that is phase-shifted by the object. However, the phase shift of these two waves is relatively small.

We can separate direct and diffractive waves by placing a ring-shaped **phase plate** in the image focus of the lens. This shifts the phase of the direct wave by a value corresponding to a quarter of the wavelength.

Depending on the phase plate used, we can get two interference images.

- **Positive phase contrast** – a phase plate with an optical thickness of  $3/4$  wavelength shifts the phase of the direct wave by  $+ 90^\circ$ . Thicker parts of the specimen will appear *dark*, while thinner parts will appear *light*.
- **Negative phase contrast** – a phase plate with an optical thickness of  $1/4$  wavelength shifts the phase of the direct wave by  $- 90^\circ$ . Thicker parts of preparation will appear *light*, while thinner parts will appear *dark*.

## Meaning

It is used to observe unstained objects, especially structures in living cells such as the **the nucleus, nucleolus, chromosomes and vacuoles**. It can significantly increase the contrast even in weakly colored and poorly distinguishable structures of histological preparations.

## History

The first phase microscope was invented by the Dutch physicist Frederik Zernike in 1935 and used the phase contrast method.

## Links

### Source

- [http://www.sci.muni.cz/kfar/html/fazovy\\_kontrast.pdf](http://www.sci.muni.cz/kfar/html/fazovy_kontrast.pdf)
- <http://xarquon.jcu.cz/edu/zbb/fazovyk.pdf>
- [https://en.wikipedia.org/wiki/Phase\\_contrast\\_microscopy](https://en.wikipedia.org/wiki/Phase_contrast_microscopy)

### References

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