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The most important for research in the field of biology is the possibility of analyzing living cells, which allows a better understanding of their structure and function. The most suitable in such an analysis are different microscopy techniques. Over the centuries light microscopy has been the basic research tool in various disciplines of natural sciences. In the analysis of life processes, the light microscopy techniques are more useful in tracking changes in living cells.

Development of the existing and a creation of the new light microscopy techniques has improved and increased the possibilities of the use of light microscopes to study living cells in greater detail. The former one was a phase contrast technique developed by F. Zernike. Phase contrast is used with an especially good effect by researchers e.g. in biology, and by cell biologists in particular. Living cells belong to the class of phase objects that are difficult to study (without staining) in an ordinary microscope. Phase contrast allows invisible optical path differences or phase shifts occurring in the object plane to be transformed into visible differences of light intensity in the image plane of microscope (Pluta, 1989). The success of the phase contrast microscope has led to a number of subsequent phase imaging methods. Among them, the best known is differential interference contrast (DIC) microscopy patented by G. Nomarski.

Since the construction of the first microscope until now light microscopy has experienced at least twice the hey-day. The first one was at the turn of the 20th century. The return to light microscopy as a useful research technique was because of two reasons: the synthesis of the first fluorochrome, and development of fluorescent microscopy. The use of fluorochromes in the study of living organisms was the milestone in increasing our knowledge of the cell function and the influence of different factors, both internal and external on cell growth and development.

The second hey-day of light microscopy started (and has continued since) with the construction of confocal microscopy and isolation of green fluorescent protein from the jellyfish *Aequoria victoria*. The popularity of the confocal microscopy is evidenced by the increase in the number of confocal systems in use today. The power of this approach lies in its ability to image structures at discrete levels within an intact biological specimen achieving increasingly higher resolutions e.g. resolving power of microscope STORM is 12 nm; (Paterczyk, 2013). The development of light microscopes themselves and their applications have accelerated in last decades, which was possible thanks to the improvements of technology, the use of computer-optimization in the design of optic systems, and computer analysis of microscopic images. The present usage of light microscopy, in particular confocal microscopy and quantum dots, will contribute to a large and rapid increase of information concerning the developmental processes and control mechanisms on the cellular and molecular level.

All mentioned above possibilities of light microscopy techniques and their use in the biological study are not without the limitations, which is obvious to anyone who works in the field of cell biology including tissue cultures. That is why possibilities and limitations of various optical-microscopic techniques related to biological samples, especially the analysis of plant cell structure and function, in particular during *in vitro* cultures, will be discussed.

References

Paterczy B. (2013) *Droga do nanoskopii*. Kosmos, 62 (2): 261-180
Pluta M. (1989) *Advanced light microscopy*. PWN, Warszawa