New methods in histology

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Abstract

Many light-microscope techniques are available for observing cells. Cells that have been fixed and stained can be studied in a conventional light microscope, whereas antibodies coupled to fluorescent dyes can be used to locate specific molecules in cells in a fluorescence microscope. Living cells can be seen with phase-contrast, differential-interference-contrast, dark-field, or bright-field microscopes. All forms of light microscopy are facilitated by digital image-processing techniques, which enhance sensitivity and refine the image. Confocal microscopy and image deconvolution both provide thin optical sections and can be used to reconstruct three- dimensional images. Techniques are now available for detecting, measuring, and following almost any desired molecule in a living cell. Fluorescent indicator dyes can be introduced to measure the concentrations of specific ions in individual cells or in different parts of a cell. Virtually any protein of interest can be genetically engineered as a fluorescent fusion protein, and then imaged in living cells by fluorescence microscopy. The dynamic behaviour and interactions of many molecules can be followed in living cells by variations on the use of fluorescent protein tags, in some cases at the level of single molecules. Various super resolution techniques can circumvent the diffraction limit and resolve molecules separated by distances as small as 20 nm. Discovering the detailed structure of membranes and organelles requires the higher resolution attainable in a transmission electron microscope. Specific macromolecules can be localized after being labeled with colloidal gold linked to antibodies. Three- dimensional views of the surfaces of cells and tissues are obtained by scanning electron microscopy. The shapes of isolated molecules can be readily determined by electron microscopy techniques involving fast freezing or negative staining. Electron tomography and single-particle reconstruction use computational manipulations of data obtained from multiple images and multiple viewing angles to produce detailed reconstructions of macromolecules and molecular complexes. The resolution obtained with these methods means that atomic structures of individual macromolecules can often be “fitted” to the images derived by electron microscopy. In this way, the TEM is increasingly able to bridge the gap between structures discovered by x-ray crystallography and those discovered with the light microscope.

About the lecturer

The main research interests of Prof. Mirosław Sopel are focused on studying biological and molecular mechanisms during the treatment of difficult-to-heal wounds using innovative physical methods such as radial shock waves. Currently, the research continues in the clinical area, as well as it includes immunocytochemical studies on patient wound sections and in vitro wound model and emphases on the importance of the effect of mechanical forces on the proliferative activity and gene expression profile of shock wave-treated cells.