Z3 - Advanced methods of optical microscopy for examination of biological samples

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The main goal of the Z3 exercise is an observation of biological systems using advanced methods of optical microscopy using Olympus IX71 fluorescence microscope and the dedicated software. The activities are divided into three blocks. Firstly, students learn the basic concepts of optical microscopy and the construction of the microscope. Using white light microscopy to observe biological samples they learn how to operate the microscope and use the software. Secondly, students get to know two advanced methods of improving contrast in unstained biological specimens - differential interference contrast (DIC) and phase contrast microscopy by observation of fish keratocyte migration. Finally, students prepare samples with fluorescently labelled cells and examine them using fluorescence microscopy.

Preparatory questions

- Basic optical phenomena: diffraction, interference, polarization, fluorescence [1, 2, 3, 4, 5].
- Construction and working principle of bright field microscope [6, 7, 8].
- Construction and working principle of phase contrast microscope [9, 10].
- Construction and working principle of differential interference contrast (DIC) microscope [11, 12].
- Construction and working principle of fluorescence microscope [13, 14, 15].
- Structure of cell [16, 17, 18].
- Migration of cells [19, 20, 21, 22, 23].

Computational assignments

Give the total magnification for a microscope if the following lenses are used:

OCULAR	OBJECTIVE	MAGNIFICATION
x5	x10	
x10	x4	
x20	x20	

Calculate the high power field of view of a microscope (ocular x5, objective x40) when the low power magnification (ocular x5, objective x10) gives a field of view of 1.2 mm.

Apparatus and materials

- Olympus fluorescence microscope IX71 with digital camera.
- Power suppliers.
- PC with software.
- Chemical reagents.

Experiment

- Observation of biological specimens using bright field microscopy
- Observation of biological specimens using phase contrast microscopy
- Observation of biological specimens using differential interference contrast microscopy
- Preparation of biological specimens for fluorescence microscopy
- Observation of biological specimens using fluorescence microscopy
- Observation of fish keratocyte migration

Data analysis

Data analysis include:

- The comparison of images recorded for biological specimens using different techniques of optical microscopy and the assessment of their adventages/disadventages.
- Estimation of the velocity of migrating fish keranocytes.
- Examination of proliferation process observed using fluorescence microscopy, i.e. number of cells vs. time of incubation should be plotted.

Safety rules

Use of gloves and other protective clothing to prevent skin contact is recommended when working with biological and chemical reagents.

Rysunek 1: Construction and working principle of bright field microscope: 1. ocular lens; 2. revolver (to hold multiple objective lenses); 3. objective lenses; 4. focus knob - coarse adjustment; 5. focus knob - fine adjustment; 6. stage; 7. light source; 8. condenser; 9. mechanical stage

Literatura

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