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Biomedical Engineering Laboratory I (Fall 2025)

Lab 1 introduction to microscopy

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Abstract

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INTRODUCTION

MATERIALS AND METHODS

An inverted optical microscope (Olympus CKX53) equipped with 4×, 10×, 20×, and 40× objectives, including phase-contrast capabilities and a broadband mercury lamp, was employed throughout the experiments. A digital imaging system was used for recording data. The following specimens and accessories were utilized: transparent rulers, diffraction gratings (70 and 110 lines/mm, Edmund Optics, transmission diffraction grating), pinhole apertures of three diameters (200 μm, 500 μm, and 1000 μm, Thorlabs P200K, P500K and P1000K), microscope slides, MC3T3 cell samples, and fluorescently labeled slides (Invitrogen FluoCells #1 BPAE Cells, with MitoTrackerTM Red CMXRos, Alexa FluorTM 488 phalloidin and DAPI). Two regular ruler (minimum scale 1mm, total length 15cm and 50cm) were used to measure the image distance and the projection. A 90° bent steel ruler was used to measure the object distance.

Four experimental modules were carried out. First, under the 4x objective, the true magnification of the optical system was determined by removing the eyepiece, directly imaging a transparent ruler on the eyepiece tube, measuring the projected scale, and comparing it against the scales on the ruler, while the theoretical magnification was calculated by measuring the object distance and image distance of the microscope system and substitute the figures into the formula . Two results were compared statistically. Second, the numerical aperture of the 10× objective was modified by sequentially placing pinhole apertures of varying sizes above the lens while observing gratings of different line densities, allowing evaluation of diffraction effects and resolution changes. Third, 20x phase contrast imaging was performed by adjusting the microscope’s phase slider, enabling comparison of MC3T3 cell samples under bright-field and phase-contrast conditions. Finally, 40x fluorescence microscopy was conducted by exciting stained slides with specific illumination channels (UV/blue/green from filtered broadband mercury lamp) and capturing images at different emission wavelengths, followed by staining and merging the channels into an RGB composite. The emission colors were identified through eyepieces, including cyan (nucleus excited by UV), green (cytoskeleton excited by blue) and red (mitochondrion excited by green).

All measurements and image acquisitions were conducted under correct focal distance and repeated by multiple group members to ensure consistency, and scale calibration was applied to microscopic images under different magnification (4x, 10x, 20x, 40x) to convert pixel dimensions into physical units.

Acknowledgments

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References

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Annex

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