DIGITAL OPTICAL AND SCANNING PROBE MICROSCOPY FOR INSPECTION AND MANIPULATION OF BIOCELLS

SERGEI CHIZHIK, LIZAVETA DROZD[c], NIKITA FOMIN

[1]Luikov Heat and Mass Transfer Institute, Minsk, 220072, Belarus
[c]Corresponding author: Tel.: +375297651531; Fax: +375172922513; Email: lizadr@tut.by; drozd.elizaveta@gmail.com

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ABSTRACT: As biomedical interest has progressed to the study of biological tissues and single cells investigations of the different biomaterials involved require very careful use of fine-scale measurement methods, Atomic Force Microscopy (AFM) and Dynamic Laser Speckle (DLS) being reported here. AFM and DLS are complex experimental systems with the functions of scanning probe and optical microscopy. A special optical system makes it possible to visualize the objects and position the probe within microscale dimensions. AFM is used both for visualization and identification of the local adhesion and viscoelastic properties of biological cells, and for manipulation of the cell by means of varying the load being applied to it. Additional information about cellular activity could be obtained by laser probing via DLS of the living tissues being studied. These techniques, AFM and DLS, greatly enhance the potential for measurements and open a new field of experiments in cell biology. The purpose of this chapter is to show the application of AFM and DLS to studies of biological cells, namely measurement of the motility of general cells in living tissues and the elastic modulus of a single cell membrane, as well as identifying the forces causing membrane damage. The time-space cross-correlation analysis of the temporal evaluation of the dynamic bio-speckle patterns is shown to be a means of real time flow visualization of the microcirculation of blood in living tissue. Digital processing of bio-speckle pattern records yields 2D maps exhibiting the temporal and spatial variations in subskin blood flow. This could be used for bio-medical diagnostic purposes, e.g. for detecting micro-scale deviations from the normal case. Three methods of evaluating dynamic speckle patterns are described. Both decorrelation and auto-correlation analyses have been realized in real time mode, when a total digital specklegram treatment was performed during the time interval between successive frames (40 ms). Results in the form of 2D maps of subskin blood flux were visualized on the PC monitor with a frequency of 25 Hz.

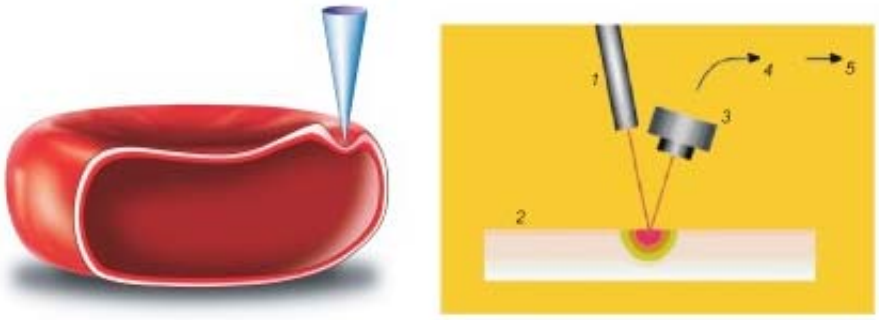

Fig. 1 Schematic illustration of AFM tip probing of single erythrocyte in-vitro (left) and laser inspection of erythrocytes motility in a living tissue in-vivo by DLS (right): 1 - laser; 2 - tissue under study; 3 - CCD camera; 4 - digital frame grabber; 5 - PC.