TIME-LAPSE IMAGING AND 3D CELL TRACKING BY COMPUTER-CONTROLLED VIDEO MICROSCOPY. APPLICATIONS TO CHEMOTAXIS.

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KEYWORDS: Time-lapse, 3D cell tracking, living cells, chemotaxis.

In this work, we investigate cell migration in 3D reconstituted collagen gel substrata under anisotropic conditions, such as chemotaxis and contact guidance. Collagen solutions seeded with cells are loaded in especially designed two-well chambers and incubated at 37°C to allow gel formation. The samples are then transferred to a video microscopy workstation equipped with an incubating system and motor units for remote operation of focus drive and x-y stage. Time-lapse imaging is carried out by computer-controlled, iterative focusing of selected regions of the samples followed by image acquisition and storage. A dedicated software is used to control system operation, including sample scanning, optical sectioning and temperature control. Image analysis techniques are used to extract cell contours from the archived images and reconstruct cell trajectories. The motility parameters of the cell populations under investigations, which include Jurkat cells and lymphocytes, are presented both for the isotropic case and in presence of a concentration gradient (chemotaxis). The data are discussed and compared to previous studies [1], including contact guidance experiments [2].