MOVIE CRUNCHING IN BIOLOGICAL DYNAMIC IMAGING

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Recent advances in biological imaging technologies have enabled the observation of living cells with high resolution during extended periods of time and are impacting biological research in such different areas as high-throughput image-base drug screening, cellular therapies, cell and developmental biology and gene expression studies. Deciphering the complex machinery of cell functions and dysfunction necessitates indeed large-scale multidimensional image-based assays to cover the wide range of highly variable and intricate properties of biological systems. However, understanding the wealth of data generated by multidimensional microscopy depends critically on decoding the visual information contained therein and on the availability of the tools to do so. Innovative automatic techniques to extract quantitative data from image sequences are therefore of major interest. I will present methods we have recently developed to perform the computational analysis of image sequences coming from multidimensional microscopy, with particular emphasis on tracking and motion analysis for 3D+t images sequences using active contours and multiple particle tracking.

1. INTRODUCTION

The advent of multidimensional microscopy (real-time optical sectioning and confocal, TIRF, FRET, FRAP, FLIM) has enabled biologists to visualize cells, tissues and organs in their intrinsic 3D and 3D+t geometry, in contrast to the limited 2D representations that were available until recently. These new technologies are already impacting biological research in such different areas as high-throughput image-base drug screening, cellular therapies, cell and developmental biology and gene expression studies, as they are put-ting at hand the imaging of the inner working of living cells in their natural context. Expectations are high for breakthroughs in areas such as cell response and motility modification by drugs, control of targeted sequence incorporation into the chromatin for cell therapy, spatial-temporal organization of the cell and its changes with time or under infection, assessment of pathogens routing into the cell, interaction between proteins, sanitary control of pathogen evolution, to name but a few. Deciphering the complex machinery of cell functions and dysfunction necessitates large-scale multidimensional image-based assays to cover the wide range of highly variable and intricate properties of biological material. However, understanding the wealth of data generated by multidimensional microscopy depends critically on decoding the visual information contained therein.

Within the wide interdisciplinary field of biological imaging, I will concentrate on work developed in our laboratory on two aspects central to cell biology, particle tracking and cell shape and motility analysis, which have many applications in the important field of infectious diseases.

2. PARTICLE TRACKING

Molecular dynamics in living cells is a central topic in cell biology, as it opens the possibility to study with submicron resolution molecular diffusion, spatio-temporal regulation of gene expression and pathogen motility and interaction with host cells. For example, it is possible, after labelling with specific fluorochromes, to record the movement of organelles like phagosomes or endosomes in the cell,[6] the movement of different mutants of bacteria or parasites[2] or the positioning of telomeres in nuclei (Galy et al., 2000).[3]

I will describe the methods we have developed to perform the detection and the tracking of microscopic spots directly on four dimensional (3D+t) image data.[4, 5] They are able to detect with high accuracy multiple

biological objects moving in three-dimensional space and incorporate the possibility to follow moving spots switching between different types of dynamics. Our methods decouple the detection and the tracking processes and are based on a two step procedure: first, the objects are detected in the image stacks thanks to a procedure based on a three-dimensional wavelet transform; then the tracking is performed within a Bayesian framework where each object is represented by a state vector evolving according to biologically realistic dynamic models.

3. CELL TRACKING

Another important project of our laboratory is motivated by the problem of cell motility. The ability of cells to move and change their shape is important in many important areas of biology, including cancer, development, infection and immunity.[7] We have developed algorithms to automatically segment and track moving cells in dynamic 2D or 3D microscopy.[1, 8] For this purpose, we have adopted the framework of active contours and deformable models that is widely employed in the computer vision community. The segmentation proceeds by evolving the front according to evolution equations that minimize an energy functional (usually by gradient descent). This energy contains both data attachment terms and terms encoding prior information about the boundaries to be extracted, e.g. smoothness constraints. Tracking, i.e. linking segmented objects between time points, is simply achieved by initializing front evolutions using the segmentation result of the previous frame, under the assumption that inter-frame motions are modest. I will describe some of our work on adapting these methods to the needs of cellular imaging in biological research.

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