DIGITAL ANALYSIS OF ACTIN FILAMENTS IMAGES OBTAINED BY FLUORESCENCE MICROSCOPY

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Abstract. The actin cytoskeleton is a basic determinant of cellular morphology and motility. Actin cytoskeleton study is important because a wide spectrum of cellular processes can be monitored through the conformational changes that occur at its level. A template-based method for linear detection was used to process and perform a quantitative analysis of cytoskeleton morphology in various cell types. This approach is fitted for a precise calculation of various parameters such as fiber length and cytoskeleton polarization, and can be used to follow the changes in the morphology of the actin cytoskeleton due to physiological modifications. In our study, several images of four different cell lines: bEnd.3, BJ, MG-63 and OLN-93 were analyzed in control conditions, in order to assess the structural organization of the actin cytoskeleton. A comparative analysis was also performed on the robustness and adaptability of the proposed algorithm, using the images of the four cell lines. The aim of this study was to establish how well the program recognizes the fibers apart from other cellular structures and debris. Following the analysis, criteria were set for the adjustment of computational parameters (e.g., angular resolution, kernel matrix size, etc.). The use of the segmentation algorithm provided consistent quantitative information, revealing the distributions of important morphological features. The adaptive character of the method, due to the manipulation of computational parameters, enables an efficient segmentation of the microscopy images. Thus, a high precision quantitative analysis of the microscopy images was performed, for the assessment fiber morphology and determination of the optimal set of computational parameters used for the digital extraction of the fibers.

Key words: cytoskeleton, actin, microscopy, confocal, digital, analysis.

INTRODUCTION

Many of the most recent advances in cellular biology were possible due to the development of higher resolution microscopy techniques such as confocal microscopy, super-resolution fluorescence microscopy, etc. These increased capabilities facilitate the better study of intracellular structures. However, certain intracellular structures,

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The variation of the computational parameters enabled a precise detection of fiber structures. We adapted the FiberScore base algorithm and optimized the computational parameters in order to analyze different morphologies independently of the pixel intensity.

Among the studies addressing the morphology of the cellular cytoskeleton, it has been reported an extensive use of template-based detection methods for actin fibers. It has been observed that the physiological role of the cells is inducing preferential shapes and orientations in the actin cytoskeleton [6].

Starting from our results, a dynamic analysis of actin fibers conformational changes can be carried out. The algorithm feature that enables the efficient separation between the structures of interest and background proved useful even for images with lower quality. The consistency of the statistical analysis against the different cell types indicates that the algorithm can detect small conformational differences, fact that can be beneficial when studying physiological processes due to different treatments.

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