Introduction

All biological phenomena are dynamic. However the concept of acquiring time-lapse (in vivo) images is not new. The idea was hidden in the fact that most of the cells are so transparent and therefore cannot be perceived directly. The adopted solution is to append fluorescent markers to cellular structures. They absorb light of certain wavelengths and then emit light at some other certain wavelengths which is captured after passing through narrow-band filters. This allows the visualization of the green fluorescent protein (GFP) enabled researchers to fuse GFP to many proteins to visualize virtually any cellular structure in the environment of the living cells in recent years.

Live cell studies have revealed an unexpected dynamics of many cellular structures that were previously thought to be of rather stable morphology (i.e. nucleus sub-compartments). Visual inspection of time-lapse movies requires good solution to estimate the velocity with which an organelle moves within the cell. However, studies of live cells do not necessarily allow biologists to discover any underlying mechanisms carried out in live cells. Usually some quantization by automated image processing techniques has to be conducted.

Acquisition of in vivo data

The biological material under interest has to be prepared beforehand. The biologists must stain cellular structures with specific markers. Each type of marker binds with particular type of cellular structure. The goal is to establish which type of structure is currently perceived. The choice of marker is important. One should be aware of the kind of behavior of particular marker. The transformation from top to bottom is in few hundreds of nanometers, in axial direction (z-axis) is in few microns.

The images are therefore preprocessed typically with some low-pass filter. This can be either linear convolution filter such as Gaussian filter, usually joined with blurring effect, or either non-linear one such as median filter. This can help us to distinguish objects from each other better.

After noise removal the most popular step is to segment all image data. The purpose of segmentation is to extract constituent, homogenous and separated parts from an image. The recipe varies from application to application and is non-trivial to conduct. The process of preprocessing and segmentation is focused on the detection of similarities of neighboring pixels. Similar pixels are grouped together and they establish their image segment. The technique of thresholding is among popular ones. Two pixels are considered similar whenever their value is above given threshold.

Local thresholding technique is used for segmenting data presented over here. Object appearance is analyzed to extract edges of objects. This is done by comparing differences between values of neighboring pixels. Unfortunately, edges are high-frequency artifacts which are being suppressed during noise removal step. However, this approach isn’t usually enough in terms of segmentation and technique of boundary refinement have to be employed.

Motion estimation

Motion estimation (or taken from another points of view: objects matching, image registration) is the essence and perhaps also the most demanding step of live cell analysis. The goal is to establish which type of structure is currently perceived. The transformation from top to bottom is in few hundreds of nanometers, in axial direction (z-axis) is in few microns.

Several image statistics are used when performing object matching. Techniques that establish pairing of two sets of points are of particular interest. Points can be small objects themselves or gravity points associated with every point (such as velocity and direction of point heading, the volume or mean gray value). The correspondence of gravity points has to be visualized somehow. The region-based segmentation is focused on the detection of similarities of neighboring pixels. Similar pixels are grouped together and they establish their image segment. The technique of thresholding is among popular ones. Two pixels are considered similar whenever their value is above given threshold.

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