Methods 115 (2017) 91–99

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

Mathematical imaging methods for mitosis analysis in live-cell phase contrast microscopy

Joana Sarah Grah [a,*], Jennifer Alison Harrington [b], Siang Boon Koh [b,1], Jeremy Andrew Pike [b], Alexander Schreiner [b,2], Martin Burger [c], Carola-Bibiane Schönlieb [a], Stefanie Reichelt [b]

[a] University of Cambridge, Department of Applied Mathematics and Theoretical Physics, Centre for Mathematical Sciences, Wilberforce Road, Cambridge CB3 0WA, United Kingdom
[b] University of Cambridge, Cancer Research UK Cambridge Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, United Kingdom
[c] Westfälische Wilhelms-Universität Münster, Institute for Computational and Applied Mathematics, Einsteinstrasse 62, 48149 Münster, Germany

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form 4 February 2017
Accepted 6 February 2017
Available online 9 February 2017

Keywords:
Phase contrast microscopy
Mitosis analysis
Circular Hough transform
Cell tracking
Variational methods
Level-set methods

ABSTRACT

In this paper we propose a workflow to detect and track mitotic cells in time-lapse microscopy image sequences. In order to avoid the requirement for cell lines expressing fluorescent markers and the associated phototoxicity, phase contrast microscopy is often preferred over fluorescence microscopy in live-cell imaging. However, common specific image characteristics complicate image processing and impede use of standard methods. Nevertheless, automated analysis is desirable due to manual analysis being subjective, biased and extremely time-consuming for large data sets. Here, we present the following workflow based on mathematical imaging methods. In the first step, mitosis detection is performed by means of the circular Hough transform. The obtained circular contour subsequently serves as an initialisation for the tracking algorithm based on variational methods. It is sub-divided into two parts: in order to determine the beginning of the whole mitosis cycle, a backwards tracking procedure is performed. After that, the cell is tracked forwards in time until the end of mitosis. As a result, the average of mitosis duration and ratios of different cell fates (cell death, no division, division into two or more daughter cells) can be measured and statistics on cell morphologies can be obtained. All of the tools are featured in the user-friendly MATLAB®Graphical User Interface *MitosisAnalyser*.

1. Introduction

Mathematical image analysis techniques have recently become enormously important in biomedical research, which increasingly needs to rely on information obtained from images. Applications range from sparse sampling methods to enhance image acquisition through structure-preserving image reconstruction to automated analysis for objective interpretation of the data [1]. In cancer research, observation of cell cultures in live-cell imaging experiments by means of sophisticated light microscopy is a key technique for quality assessment of anti-cancer drugs [2,3]. In this context, analysis of the mitotic phase plays a crucial role. The balance between mitosis and apoptosis is normally carefully regulated, but many types of cancerous cells have evolved to allow uncontrolled cell division. Hence drugs targeting mitosis are used extensively during cancer chemotherapy. In order to evaluate the effects of a given drug on mitosis, it is desirable to measure average mitosis durations and distribution of possible outcomes such as regular division into two daughter cells, apoptosis, division into an abnormal number of daughter cells (one or $\geqslant 3$) and no division at all [4,5].

Since performance of technical equipment such as microscopes and associated hardware is constantly improving and large amounts of data can be acquired in very short periods of time, automated image processing tools are frequently favoured over manual analysis, which is expensive and prone to error and bias. Generally, experiments might last several days and images are taken in a magnitude of minutes and from different positions. This leads to a sampling frequency of hundreds of images per sequence with an approximate size of 1000^2 pixels.

1.1. Image characteristics in phase contrast microscopy

In live-cell imaging experiments for anti-cancer drug assessment, the imaging modality plays a key role. Observation of cell

* Corresponding author.
E-mail address: jg704@cam.ac.uk (J.S. Grah).
[1] Present address: Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA 02114, USA.
[2] Present address: PerkinElmer, Schnackenburgallee 114, 22525 Hamburg, Germany.

http://dx.doi.org/10.1016/j.ymeth.2017.02.001
1046-2023/

cultures originating from specific cell lines under the microscope requires a particular setting ensuring that the cells do not die during image acquisition and that they behave as naturally as possible [6]. Here, phase contrast is often preferred to fluorescence microscopy because the latter requires labelling or transgenic expression of fluorescent markers, both causing phototoxicity and possibly changes of cell behaviour [7–9]. As opposed to this, cells do not need to be stained for phase contrast microscopy. Moreover, phase shifts facilitate visualisation of even transparent specimens as opposed to highlighting of individual specific cellular components in fluorescence microscopy. We believe that one main advantage of our proposed framework is that it can be applied to data acquired with any standard phase-contrast microscope, which are prevalent in many laboratories and more widespread than for instance recently established quantitative phase imaging devices (e.g. Q-Phase by Tescan).

There are two common image characteristics occurring in phase contrast imaging (cf. Fig. 1). Both visual effects highly impede image processing and standard algorithms are not applicable in a straightforward manner. The shade-off effect leads to similar intensities inside the cells and in the background. As a result, edges are only weakly pronounced and imaging methods such as segmentation relying on intensity gradient information (cf. Section 2.2.2) often fail. Moreover, region-based methods assuming that average intensities of object and background differ from one another (cf. Section 2.2.3) are not applicable either. Secondly, the halo effect is characterised by areas of high intensity surrounding cell membranes. The brightness levels increase significantly immediately before cells enter mitosis due to the fact that they round up, form a nearly spherically-shaped volume and therefore the amount of diffracted light increases. In addition, both effects prohibit application of basic image pre-processing tools like for example thresholding or histogram equalisation (cf. [10]).

1.2. Brief literature review

Over the past few years a lot of cell tracking frameworks have been established (cf. [11]) and some publications also feature mitosis detection. In [12], a two-step cell tracking algorithm for phase contrast images is presented, where the second step involves a level-set-based variational method. However, analysis of the mitotic phase is not included in this framework. Another tracking method based on extended mean-shift processes [13] is able to incorporate cell divisions, but does not provide cell membrane segmentation. In [14] an automated mitosis detection algorithm based on a probabilistic model is presented, but it is not linked to cell tracking. A combined mitosis detection and tracking framework is established in [15], although cell outline segmentation is not included. Li et al. [16] provide a comprehensive framework facilitating both tracking and lineage reconstruction of cells in phase contrast image sequences. Moreover, they are able to distinguish between mitotic and apoptotic events.

In addition, a number of commercial software packages for semi- or fully automated analysis of microscopy images exist, for example *Volocity*, *Columbus* (both PerkinElmer), *Imaris* (Bitplane), *ImageJ/Fiji* [17] and *Icy* [18] (also cf. [19]). The last two are open source platforms and the latter supports graphical protocols while the former incorporates a macro language, allowing for individualisation and extension of integrated tools. However, the majority of plugins and software packages are limited to analysis of fluorescence data.

A framework, which significantly influenced development of our methods and served as a basis for our tracking algorithm, was published in 2014 by Möller et al. [20]. It incorporates a MATLAB®Graphical User Interface that enables semi-automated tracking of cells in phase contrast microscopy time-series. The user has to manually segment the cells of interest in the first frame of the image sequence and can subsequently execute an automatic tracking procedure consisting of two rough and refined segmentation steps. In the following section, the required theoretical foundations of mathematical imaging methods are discussed, starting with the concept of the circular Hough transform and continuing with a review of segmentation and tracking methods leading to a more detailed description of the above-mentioned framework. For a more detailed discussion, we refer the interested reader to [10] and the references therein.

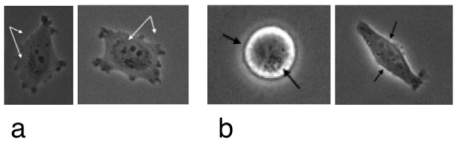

Fig. 1. Common image characteristics in phase contrast microscopy: shade-off effect (a) and halo effect (b) (HeLa DMSO control cells).

2. Mathematical background

2.1. The circular Hough transform

The Hough transform is a method for automated straight line recognition in images patented by Paul Hough in 1962 [21]. It was further developed and generalised by Duda and Hart in 1972 [22]. More specifically, they extended the Hough transform to different types of parametrised curves and in particular, they applied it to circle detection.

The common strategy is to transform points lying on straight line segments or curves in the underlying image into a parameter space. Its dimension depends on the number of variables required in order to parametrise the sought-after curve. For the parametric representation of a circle, which can be written as

$$r^2 = (x - c_1)^2 + (y - c_2)^2, \tag{1}$$

the radius r as well as two centre coordinates (c_1, c_2) are required. Hence, the corresponding parameter space is three-dimensional. Each point (x, y) in the original image satisfying the above equation for fixed r, c_1 and c_2 coincides with a cone in the parameter space. Then, edge points of circular objects in the original image correspond to intersecting cones and from detecting those intersections in the parameter space one can again gather circles in the image space.

For simplification, we fix the radius and consider the two-dimensional case in Fig. 2. On the left, we have the image space, i.e. the x–y-plane, and a circle in light blue with five arbitrary points located on its edge highlighted in dark blue. All points fulfil Eq. (1) for fixed centre coordinates (c_1, c_2). On the other hand, fixing those specific values for c_1 and c_2 in the parameter space, i.e. c_1-c_2-plane, on the right, and keeping x and y in (1) arbitrary, leads to the dashed orange circles, where the corresponding edge points are drawn in grey for orientation. All of the orange circles intersect in one point, which exactly corresponds to the circle centre in the original image. Hence, from intersections in the parameter space one can reference back to circular objects in the image space.

A discussion on how the circular Hough transform is embedded and implemented in *MitosisAnalyser* can be found in Section 3.1.

2.2. Image segmentation and tracking

In the following, we would like to introduce variational methods (cf. e.g. [23,24]) for imaging problems. The main aim is minimi-