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A computational framework for particle and whole cell tracking applied to a real biological dataset

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ABSTRACT

Cell tracking is becoming increasingly important in cell biology as it provides a valuable tool for analysing experimental data and hence furthering our understanding of dynamic cellular phenomena. The advent of high-throughput, high-resolution microscopy and imaging techniques means that a wealth of large data is routinely generated in many laboratories. Due to the sheer magnitude of the data involved manual tracking is often cumbersome and the development of computer algorithms for automated cell tracking is thus highly desirable.

In this work, we describe two approaches for automated cell tracking. Firstly, we consider particle tracking. We propose a few segmentation techniques for the detection of cells migrating in a non-uniform background, centroids of the segmented cells are then calculated and linked from frame to frame via a nearest-neighbour approach. Secondly, we consider the problem of whole cell tracking in which one wishes to reconstruct in time whole cell morphologies. Our approach is based on fitting a mathematical model to the experimental imaging data with the goal being that the physics encoded in the model is reflected in the reconstructed data. The resulting mathematical problem involves the optimal control of a phase-field formulation of a geometric evolution law. Efficient approximation of this challenging optimal control problem is achieved via advanced numerical methods for the solution of semilinear parabolic partial differential equations (PDEs) coupled with parallelisation and adaptive resolution techniques.

Along with a detailed description of our algorithms, a number of simulation results are reported on. We focus on illustrating the effectivity of our approaches by applying the algorithms to the tracking of migrating cells in a dataset which reflects many of the challenges typically encountered in microscopy data.

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1. Introduction

Cell migration is an essential part of many normal biological processes and diseases (Friedl and Gilmour, 2009). The dynamics of collective-cell movement, cell-to-cell interactions as well as interactions between cells and the extracellular environment are closely related to the bio-chemical and bio-mechanical properties of a single cell (Friedl and Alexander, 2011; Weigelin et al., 2012; Wolf et al., 2013).

Bio-laboratories nowadays produce a huge amount of data in multi-dimensions (both in space and time) e.g., microscopy images, that is far beyond the capacity of manual analysis in order to make informed decisions about cell shape evolution and migration trajectories (Maska et al., 2014). Hence, one demands computationally fully-automated cell tracking procedures. The focus of this work is to present techniques to solve the challenging problems that arise when one seeks to automate reconstruction of cell shape evolution and cell migration trajectories from static data.

We present two different approaches; the first approach involves an algorithm for single particle tracking that is successively applied for multiple particles in which the most challenging step is detecting cells migrating over a substrate where the intensities of both cells and background, using the microscopy and imaging techniques under consideration, are (spatially) non-uniform and the second approach seeks to address the problem of whole cell tracking in

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which cell shape evolution is reconstructed from static imaging data, with the corresponding recovered data generated by fitting a mathematical model, derived from physical principles, to the data (Croft et al., 2014; Blazakis et al., 2015; Yang et al., 2015). For particle tracking, described in Section 3, we treat each cell as a single object (i.e., a dot) and seek to determine the speed and direction of cell centroid trajectories. The latter approach, illustrated in Section 4, focusses on recovering dynamic cell morphologies and typically is of use to study a single cell or multiple cells in a low density setting. This resulting mathematical problem is formulated as the optimal control of a geometric evolution law (DuChateau and Zachmann, 1989; Rektorys, 1999).

2. Cell culture and microscopy

As mentioned above, to test the performance of our algorithms, we apply them to an experimental dataset generated in the labs of ibidi GmbH (2015). We summarise the details of the experimental protocol used to generate the biological data used in this study.

The human fibrosarcoma cell line HT-1080 (obtained from DSMZ, Germany) was grown in Dulbecco's modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) at 37 °C and 5% CO_2. Cells were grown to 80% confluence, trypsinised, and filled into the chemotaxis chambers of the μ-Slide Chemotaxis ibiTreat (ibidi GmbH, Germany) at a density of 3×106 cells/ml. To perform a migration experiment without chemoattractant both reservoirs and the channel were filled with DMEM with 5% FBS. Video microscopy was performed using a Nikon TiE microscope equipped with a 4x phase-contrast objective and the pixel size 1.66 µm/px. The time-lapse interval was ten minutes over a time period of 24 h.

3. Segmentation and particle tracking

In this setting, the first step is to individually represent each cell by a single dot (typically the centre of the segmented cell), this is achieved in a two-part process, first the cells are segmented from background and noise effects, then each individual cell is detected and labelled. The second step is to determine the correspondence between cells from one frame to the other, this is typically done by linking the corresponding dots between imaging frames. After a brief review of some existing algorithms for segmentation, we describe our algorithms for each step and illustrate their effectiveness on the experimental dataset under consideration in this work.

3.1. A review of segmentation techniques

In phase-contrast microscopy, phase shifts of the specimen are transformed into amplitude (intensity) shifts, thus permitting objects that are usually almost invisible (e.g. cells), to be optically visible. Furthermore, this also results in possible background inhomogeneities and various noise effects also becoming more prominent. Certain techniques are necessary to help identify cells and image segmentation, so that a common approach can be employed. Image segmentation is defined as a process of partitioning an image into homogeneous groups such that each region is homogeneous but that no union of two adjacent regions is homogeneous (Pal and Pal, 1993). In this section, we describe some widely used segmentation techniques from the literature.

For completeness, there is an alternative, namely fluorescent microscopy, that has been commonly used to study processes in the physiological context of intact living cells (Pepperkok and Ellenberg, 2006). The basic idea is to bind some fluorescent stains with the DNA of the targeted cellular components, for example, the nucleus of the cell. Since the light from the fluorescent stains have specific wavelength (Grynkiewicz et al., 1985), with correct imaging techniques, it is possible to only capture these illuminated components, in turn helping identify the positions of cells. In principle, it is possible to label cells in fluorescent microscopy with no or little image segmentation. On the other hand, comparing to phase-contrast microscopy, disadvantages of fluorescent microscopy include that the extra staining requires further manual input thereby prolonging the whole process and that, the fluorescence can only illuminate for a certain length of time, namely its bleach rate (Denk et al., 1990), thus is unfavourable for longer experiments. Moreover, advanced segmentation techniques are still required if the illuminated components collide or overlap.

We illustrate a typical phase-contrast image from the biological dataset used in this work in Fig. 1 and the full length video (*raw_data.avi*) is included in the supplementary material.

Depending on the objective lens, the phase-contrast technique works in a certain range of phase shifts only. If the shift is too big, artefacts are created. The halo effect is a common phase shift artefact. For example, when a cell rounds up (such as when undergoing cell division), a bright halo is often visible around the cell. If cells or cellular structures like filopodia are less thick and flat on the substrate they are optically dark.

In Fig. 1, we identify (by arrows in the figure) three main sets of distinct features representing the cells:

(a) cells can be clearly observed, they have little or no halo artefacts and the centre generally is the brightest with the highest intensity value;

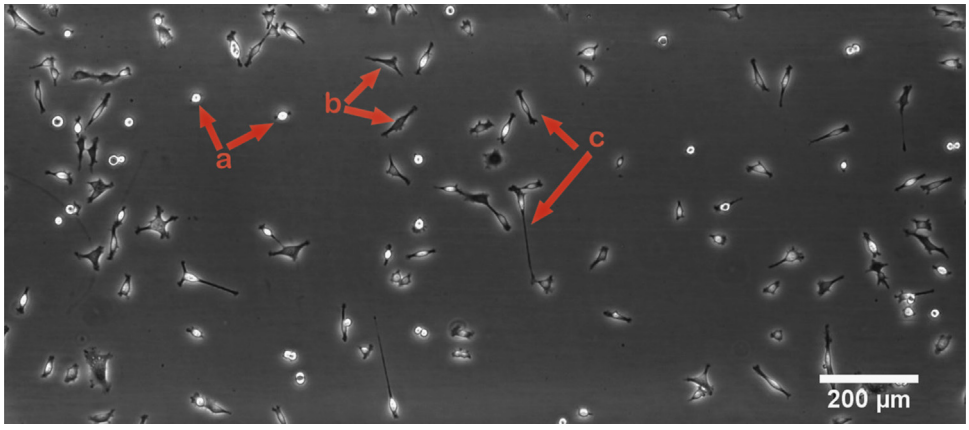

Fig. 1. A typical phase-contrast image from the biological dataset. Arrows and three letters are used to identify cells with distinct features.

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