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Label free cell-tracking and division detection based on 2D time-lapse images for lineage analysis of early embryo development

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ABSTRACT

In this paper we report a database and a series of techniques related to the problem of tracking cells, and detecting their divisions, in time-lapse movies of mammalian embryos. Our contributions are (1) a method for counting embryos in a well, and cropping each individual embryo across frames, to create individual movies for cell tracking; (2) a semi-automated method for cell tracking that works up to the 8-cell stage, along with a software implementation available to the public (this software was used to build the reported database); (3) an algorithm for automatic tracking up to the 4-cell stage, based on histograms of mirror symmetry coefficients captured using wavelets; (4) a cell-tracking database containing 100 annotated examples of mammalian embryos up to the 8-cell stage; and (5) statistical analysis of various timing distributions obtained from those examples.

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1. Introduction

In recent years, a number of studies related to time-lapse imaging of early embryos have been published ([20,12,18,25,19,16,6,5,24,8]).

For human *in vitro fertilized* (IVF) embryos, certain measurements relating to cell division timing have been shown to correlate with embryonic viability in a clinical setting ([25,16,24,8]). The relevance of cell cycle timing statistics stems from the fact that embryonic development depends on the proper coordination of many cellular events in space and time. In model organisms, the contribution of different genes to early developmental events can be studied by silencing gene activity using RNA interference (RNAi) and analyzing any resulting changes in cellular behavior (including cell cycle timing) in early embryos (e.g. [23]).

These applications motivated us to study the problem of cell tracking and division detection in time-lapse images of early mouse embryos. The input is a series of images of a well containing about ten embryos, from the first cell until after the blastocyst cavitation phase. In this paper, we report algorithms aiming to

1. detect, in the first frame, the locations of the embryos, track each embryo for the duration of the movie, and create cropped movies displaying one particular embryo in the center of the frames; and
2. for each embryo, track individual cells, and detect when they divide (up to the 8-cell stage[2]).

It is possible to capture timing information without tracking cells. In [16], for instance, the sum of absolute differences between pixels for consecutive frames is used to detect cell division events. This approach allows the duration of first and second generation cells to be evaluated, under the assumption that all 2nd-generation cells divide before any 3rd-generation cell does. However, evaluating the timing of 3rd generation cells requires knowledge of which 2nd-generation cell was their progenitor.[3] Thus, we are interested in building a lineage tree of cells (Fig. 1), which requires cell tracking in addition to detection of cell division times. As a result, we can measure individual cell duration times as well as gather information about the synchronicity of divisions for cells of the same generation.

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[2] Here, "stage" refers to the number of cells in an embryo at a given time. For instance, an embryo at the 4-cell stage contains precisely four cells. On the other hand, "generation" refers to the depth of a cell in the lineage tree of cells. Thus, a 2nd-generation cell is one of the two daughters produced by the first cell division in the embryo (c00 and c01 in Fig. 1 are 2nd generation cells).

[3] Suppose there are four 3rd-generation cells and one of them – let's call it *C* – divides. To determine the duration of this cell's existence, it is necessary to know when it first appeared, and therefore which 2nd generation cell is the mother of *C*. This is not possible if the only data available are the timepoints at which divisions occurred in the 2nd generation, because there are two 2nd-generation cells and these may not divide synchronously.

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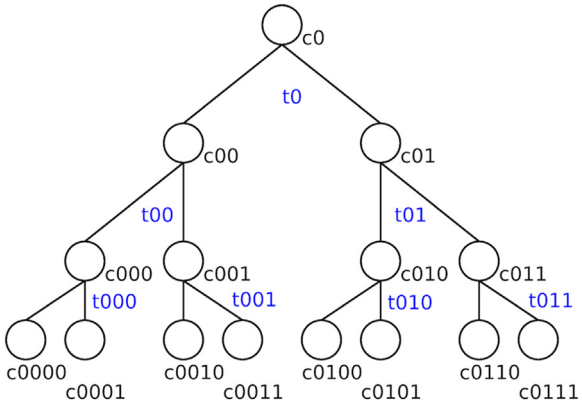

Fig. 1. Our main goal is to capture spatio-temporal information related to the lineage tree of each embryo, rather than just the times when a cell division event occurs. This allows gathering statistics of cell duration for different generations of cells, as well as measuring synchronicity of cell divisions for cells that are "siblings."

In this spirit, our approach resembles more that of [25], in which cell tracking is considered. Our method differs in two main directions. First, we do not use a brute force approach for the automated tracker.[4] Rather, we analyze cell division based on circularity information, using histograms of centers that are captured using a bank of Morlet wavelets [3]. Second, our semi-automated tracker works for one additional generation, allowing timing analysis up to the 8-cell stage.

Our contributions are

1. a method for counting embryos in a well, and cropping each individual embryo across frames, to create individual movies for cell tracking – Section 3.1;
2. a semi-automated method for cell tracking that works up to the 8-cell stage, along with a software implementation available to the public – Section 3.2;
3. an algorithm for automatic tracking up to the 4-cell stage, based on histograms of mirror symmetry coefficients captured using wavelets – Section 3.3;
4. a cell-tracking database containing 100 annotated examples of mouse embryos up to the 8-cell stage, to be publicly available for other researchers – Section 4;
5. statistical analysis of various timing distributions obtained from those examples – Section 5.

Regarding item 5 above, more specifically we provide (1) statistics of cell duration for 1st-, 2nd- and 3rd-generation cells; (2) statistics of synchronicity of division for 2nd- and 3rd- generation cells; (3) statistics of cell radii per generation, and total volume of the embryo, assuming the cells are spheres of the measured radii. In summary, our measurements show that for mouse embryos, under standard laboratory conditions:

1. 1st-generation cells divide about 1 h:38 min after pronuclear envelope breakdown[5];
2. The duration of 2nd-generation cells is about 19 h:29 min;
3. The duration of 3rd-generation cells is about 11 h:26 min;
4. 2nd-generation sibling cells divide about 42 min apart in time, and 3rd-generation siblings about 35 min apart;
5. The radii of 1st-, 2nd-, and 3rd-generation cells are about 36.09 μm, 27.41 μm, and 21.14 μm, respectively;
6. There is an apparent reduction in the total volume of cells up to the 8-cell stage: in the 2nd generation it is 88% that of the 1st generation, and total volume is 92% in the 3rd generation relative to the 2nd generation.

The last observation was surprising, since intuitively we expected total cell volume to be conserved in each generation. This apparent reduction in volume most likely arises from changes in osmotic balance during cell culture (since cells adjust their volume in response to the tonicity of their environment), but could in part reflect the depletion of stored maternal products, which are metabolized throughout embryogenesis both to produce energy for cellular processes (e.g. cell division) and to synthesize new cellular components (e.g. membrane and chromatin).

Although our methods relate to a specific problem in biology, the technique we introduce for event detection in a sequence of frames (Section 3.3) is of general interest in Computer Vision. The algorithm consists of monitoring the output of a shape descriptor (in our case, a descriptor of circularity), looking for points in the time-series where the evidence for the existence of that shape reduces drastically. This idea can be used in other tracking scenarios to infer time points at which a target object disappears from the field of view. In our case the target object is a cell, but it could in principle be any shape (e.g., face, car, and hand), as long as its descriptor is relatively invariant between successive frames.[6]

Furthermore, by making our cell-tracking database available, we not only facilitate reproducibility, but we also contribute to other researchers working on techniques such as tracking and shape analysis. We are already using the database ourselves to test novel methods in basic Computer Vision problems such as circle and ellipse detection [7].

1.1. On related research

While the literature on cell tracking for mammalian embryos is relatively small, a large body of publications is available for cell tracking in general, dealing with model organisms such as zebrafish, *Drosophila*, and *C. elegans* (e.g., [1,11,15,4,17,2,13]).

Many of these techniques require "labeling" cells (that is, marking them with dyes or fluorophores) and/or reconstructing 3D geometry using stacks of images in different focal planes (z-stacks). The methods discussed in this paper are "label-free" and are designed for images that are grayscale and bi-dimensional.

In terms of algorithms, the main difference between this paper and our previous work [6] is in how we handle cell division. Previously, we looked at pixel variances at the image and cell levels, and we computed likelihoods of cell presence from the accumulator space of a wavelet-based circular Hough transform [6]. In Section 3.2 we report a strategy in which human interaction determines the frames of cell division with 100 percent accuracy. In Section 3.3 we introduce an improved automatic method for division detection, based on mirror symmetry between pairs of pixels with tangents.

Technically, the methods of this paper aim at the same problem and similar input data as discussed in [25,16]. [25] also perform cell tracking by looking at cell boundaries, but use particle filters

[4] The method in [25] is a particle filter, which can be considered "brute force" since at every time point all local parameter variations are treated as valid possibilities and are compared to the input data for validation. Their model also incorporates the testing of the cell-division hypothesis for every cell in every frame.

[5] Upon fertilization, the maternal and paternal chromosomes are contained in separate pronuclear compartments surrounded by membranes, which must be disassembled prior to the first mitotic division in the zygote to enable mixing of their genetic material during all subsequent divisions in the developing embryo.

[6] Many event detection techniques in Computer Vision fall in the class of background subtraction algorithms ([21]), which are not usually designed to work at the local level of individual objects. To the best of our knowledge, our method for event detection at the level of a moving object is novel, but we do not discuss event detection in detail here, as the focus of the paper is on the Biology application.

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