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Nonlinear PDE based numerical methods for cell tracking in zebrafish embryogenesis

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ABSTRACT

The paper presents numerical algorithms leading to an automated cell tracking and reconstruction of the cell lineage tree during the first hours of animal embryogenesis. We present results obtained for large-scale 3D+time two-photon laser scanning microscopy images of early stages of zebrafish (Danio rerio) embryo development. Our approach consists of three basic steps – the image filtering, the cell centers detection and the cell trajectories extraction yielding the lineage tree reconstruction. In all three steps we use nonlinear partial differential equations. For the filtering the geodesic mean curvature flow in level set formulation is used, for the cell center detection the motion of level sets by a constant speed regularized by mean curvature flow is used and the solution of the eikonal equation is essential for the cell trajectories extraction. The core of our new tracking method is an original approach to cell trajectories extraction based on finding a continuous centered paths inside the spatio-temporal tree structures representing cell movement and divisions. Such paths are found by using a suitably designed distance function from cell centers detected in all time steps of the 3D+time image sequence and by a backtracking in the steepest descent direction of a potential field based on this distance function. We also present efficient and naturally parallelizable discretizations of the aforementioned nonlinear PDEs and discuss properties and results of our new tracking method on artificial and real 4D data.

1. Introduction

The development of the modern microscopy techniques allows the in vivo imaging of organisms at cell level at very early stages of development without corrupting the cell integrity and normal development of the embryo. The multi-photon laser scanning microscopy is able to deliver 3D+time images of long periods of the embryonic development with a relatively short time step. With such data, one can perform various analyses of the embryogenesis and the development of organisms. Due to a similarity with human in many aspects and due to transparency for the microscope, the zebrafish (Danio rerio) embryogenesis is studied extensively and the results are used both in basic and applied biology and medicine research, e.g. in the anticancer drug design.

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Although a large amount of work has been already done, e.g. by a combination of various image processing techniques and manual inspection, for the zebrafish developmental stages up to about one thousand cells [19], there is a great challenge to study embryogenesis at very complex stages of development with thousands of cells present. In [12,22,18,3] new efficient and robust 3D filtering and segmentation algorithms together with a workflow for performing various analyses of such complex developmental stages were suggested and studied. Among other goals, the image processing algorithms aim to deliver the so-called cell lineage tree, the spatio-temporal branching process giving topological description of movement and division of the cells. This problem is related to tracking of cells during the embryogenesis. Having the tree, one can track the cells by following the links in the tree, either forward or backward. On the other hand, having the good cell tracking algorithm and thus individual cell trajectories, one can reconstruct the tree.

The tracking of cells and construction of the cell lineage tree for such complex stages of embryogenesis is a very difficult problem unsolved satisfactorily yet. A seminal work towards building the cell lineage tree for the complex stages of zebrafish embryo development based on stochastic simulated annealing minimization of a heuristic energy functional has been done in [13]. After the construction of the tree, the individual cells or cell populations are tracked. In this paper we suggest new PDE based cell tracking method for such complex stages of zebrafish embryogenesis. In the present work we continue the effort started in [1,17] devoted to 2D+time and 3D+time cells tracking. In contrast to [13], in our approach we first extract all possible cell trajectories inside the 3D+time data and then the cell lineage tree is reconstructed by finding merging trajectories going backward in time.

Our overall approach to cell tracking consists of three basic steps – the image filtering, the cell centers detection and the cell trajectories extraction yielding the cell lineage tree reconstruction. In all three steps we use suitably designed nonlinear partial differential equations – for filtering the geodesic mean curvature flow in level set formulation is used, for cell center detection the motion of level sets by a constant speed regularized by mean curvature flow is used and solution of the eikonal equation is essential for the cell trajectories extraction. We also present efficient and naturally parallelizable discretizations of the aforementioned nonlinear PDEs and discuss properties and results of our new tracking method.

The first step of our technique is the image filtering. This is an important step because noise is always present in microscopy images and its level increases with the speed of image acquisition. We perform the filtering of 3D images of the 3D+time image sequence by the numerical solution of the so-called geodesic mean curvature flow (GMCF) model [5,11,6] which was chosen from several available methods by careful testing [12].

In the second step of our approach we use the level set center detection (LSCD) method [10] to extract an approximate position of the cell nuclei centers (which we also call the cell identifiers) in every 3D image of the 3D+time image sequence. LSCD method is represented by an evolutionary process based on the motion of level sets of 3D image intensity in normal direction by a constant speed regularized by the mean curvature flow. Following the local maxima of the solution during the evolution one can obtain a reasonable approximation of cell nuclei center positions and its number in every 3D image.

The cell identifiers represent the basic input for the third step of the overall tracking procedure, the cell trajectories extraction and the cell lineage tree reconstruction. The cell trajectories are extracted by finding centered paths inside a specifically constructed 4D spatio-temporal tree structure. Although we call it tree structure, it represents a subset of nonzero measure of the Euclidean space R^4 and is given by a 4D segmentation step. Thus, in the first step of the trajectories extraction we construct a 4D segmentation yielding the desired 4D spatio-temporal tree structure. Then a computation of constrained distance functions inside this 4D segmentation is performed by solving a spatially 4D eikonal equation. By a proper combination of computed distance functions we build a potential field which is backtracked in the steepest descent direction in order to get the cell trajectories. Finally we construct the cell lineage tree by detecting trajectories which merge together when going backward in time indicating mitosis and thus a branching node of the cell lineage tree.

The paper is organized as follows. In the next subsection we present the zebrafish embryo development data acquired by the two-photon laser scanning microscope. Then we present all three steps of our approach, the image filtering, the cell center detection and the cell trajectories extraction. Together with the PDE based models, we outline the numerical approaches used in all three steps of our tracking method. Finally we discuss the numerical experiment devoted to tracking cells in a representative 3D video of the early zebrafish embryogenesis.

1.1. 3D+time = 4D image data of zebrafish embryogenesis

Data for processing comes from two-photon laser scanning microscopy and they are of general interest, so we present some illustrative examples how it looks like. The data we deal with represents first hours of zebrafish embryo development, approximately from the 4th until 14th–20th hour. Usually, the data comes in two channels (two colors), in one channel there is an acquisition of the cell nuclei and in the second channel the acquisition of the cell membranes. The embryo labeling is obtained through RNA injection performed at the one-cell stage to obtain expression of fluorescence proteins staining nuclei and membranes, respectively. The embryo imaging starts about 4 hours post-fertilization and take next 10–15 hours. The 3D images of both channels are obtained by moving the focal plane from the top more deeply inside the embryo and their quality depend on the speed of scanning in one plane. Many various quality datasets are available thanks to EC Embryomics and BioEmergences projects (http://bioemergences.iscpif.fr). The data quality is related to a level of noise depending mainly on the size of the time step between acquisition of subsequent 3D images. The acquisition step ranges from 50 seconds to 5 minutes. A longer time step produces better 3D image quality, one has a good visual impression of what is happening during embryogenesis and such data is well suited for segmentation purposes e.g. for obtaining a shape of cells and other