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Visualization and quantitative analyses for mouse embryonic stem cell tracking by manipulating hierarchical data structures using time-lapse confocal microscopy images

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Abstract—**We present a cell tracking method for time-lapse confocal microscopy (3D) images that uses dynamic hierarchical data structures to assist cell and colony segmentation and tracking. During the segmentation, the cell and colony numbers and their geometric data are recorded for each 3D image set. In tracking, the colony correspondences between neighboring frames of time-lapse 3D images are first computed using the recorded colony centers. Then, cell correspondences in the correspondent colonies are computed using the recorded cell centers. The examples show the proposed cell tracking method can achieve high tracking accuracy for time-lapse 3D images of undifferentiated but self-renewing mouse embryonic stem (mES) cells where the number and mobility of ES cells in a cell colony may change suddenly by a colony merging or splitting, and cell proliferation or death. The geometric data in the hierarchical data structures also help the visualization and quantitation of the cell shapes and mobility.**

I. Introduction

Tracking individual cells reveals the historical genealogical relations between cells, differentiated stem cells, or undifferentiated but self-renewing stem cells. Elucidating these cellular spatio-temporal dynamics will help understanding organ development and tissue homeostasis, associated pathologies, as well as understanding of the differentiation potential of mammalian stem cells [1-4]. Recently, 3D time-lapse images have been used for visualization and more accurate quantitative analyses in cell tracking [4-5].

Several methods such as multiple-hypothesis tracking [6], minimum-cost flow tracking [7], neighboring graph, (characterizing spatial distribution of neighboring cells) [8], or Kalman filtering [9] were used to segment and determine the most likely cell correspondence between neighboring frames of time-lapse images. However, the cell-correspondence easily bring errors because the number and mobility of ES cells in a cell-colony may change suddenly caused by a colony merging or splitting, and cell proliferation or death. A cell that corresponded a cell in the same colony may correspond to a cell in different colony. Therefore, prior to the cell tracking, cell colony tracking to a colony should be implemented ahead, and cell number changes caused by proliferation or death should be taken into account.

This study, we use two stage tracking: cell colony tracking then the cell tracking in respective colonies. Four types of dynamical hierarchical data structures are proposed for the tracking: cell colonies segmented out from a 3D volume (time-lapse images) at a frame, cells inside the colonies and image or colony regions included in the colonies and cells. In colony segmentation, the data structures of a colony and its regions record the colony center and cell regions included in every colony region. In cell segmentation, the data structures of a cell and regions record the cell center and region radii. In tracking, colony correspondences between neighboring frames are determined using their centers recorded in the data structures, and classified as either of moving, merging and splitting, and moving into or out the volume. The cell correspondences between correspondent colonies are implemented using the cell centers and classified as moving, proliferating or death.

Time-lapse fluorescence confocal microscopy images for mouse ES cells that undifferentiated but self-renewing were used as example. The result shows the two-stage method can reveal the cell and colony status transition and reduce the cell correspondence errors. The cell numbers and geometric data (centers and radii) recorded in the data structures provide visualization and quantitative analyses for the tracked cells.

II. Materials and Method

A. Microscopy images from mouse ES cell cultures

Mouse ESCs were derived from blastocyst-stage embryos obtained from the transgenic mouse strain carrying the Mvh-Venus reporter gene. Plasmid pPGK-H2B-mCherry-puro, in which the human histone H2B gene was fused with the mCherry gene under the control of the PGK promoter, was introduced by lipofection into the mESC line described above. The plasmid was selected based on puromycin resistance, and mCherry-expressing cells were further purified by fluorescent activated cell sorting. CV-1000 equipped with an EM-CCD camera (Yokogawa Electric Corp., Tokyo, Japan) system was used to obtain the volumes of confocal images: 1024×1024×9 voxels with 266×266×34 μm^3 of mESCs at the early differentiation stage were acquired with 20 min intervals.

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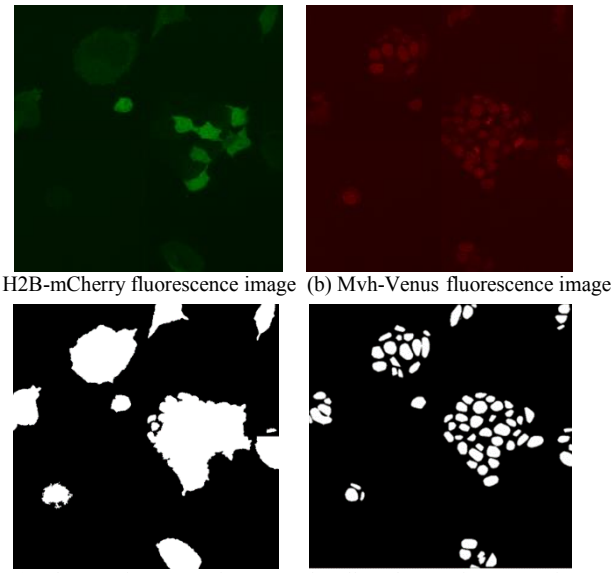


(a) H2B-mCherry fluorescence image (b) Mvh-Venus fluorescence image

(c) colony regions on an image (d) cell nucleus regions on an image

Figure 1. Cell and colony ranges in a confocal image

B. Image processing of cell colony region extraction

Bilateral filtering to reduce high noise-to-signal ratios of all H2B-mCherry and Mvh-Venus fluorescence images (Fig. 1(a) and 1(b)) and thus promote the effects of the following mean-shift filtering and adaptive thresholding. Mean-shift filtering followed by an OR operation of every pair of the filtered H2B-mCherry and Mvh-Venus fluorescence images to extract approximate regions of cell colonies (Fig. 1(c)). Adaptive thresholding and convex-hull algorithm on every H2B-mCherry fluorescence image were implemented to remove noise outside the colonies and inside cell regions to obtain cell regions (Fig. 1(d)). Then, using a series of segmentation algorithms and dynamic cell region and solid structures (Fig. 2(a) and (b)), convex cell regions and solids with reasonable sizes can be labelled, segmented and reconstructed. [4]

C. Dynamic data structures of colony regions and colonies

A data structure (as in Fig. 2(c)) is dynamically allocated for every labeled colony region when the 4-connectivity component labeling computation is used to label colony region (Fig. 1(c)). The structure includes a region ID, a pointer pointing to the next region of the same colony, the image number which image this region belongs to, and a colony ID that will be obtained from colony segmentation in a volume. All regions of a colony can be accessed by the pointers to the next colony region by this segmentation, thus the region ID is also incremented to represent which number of this region is in the colony.

Meanwhile, a data structure is dynamically allocated when a new 3D colony segmented (Fig. 2(d)). The structure includes two pointer matrices to record the colony regions and cell solid included in the colony. The colony structure also includes the center, the tracked colonies at the previous (P) and next (N) frames (P and N IDs) and a pair of distances for tracking calculation. After finishing the 3D colony segmentation for a volume, total colonies in the volume can be accessed by the colony pointers pointing to their respective next colonies.

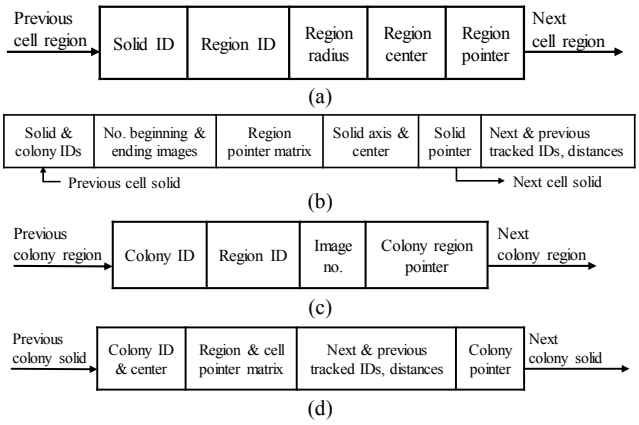


Figure 2. Dynamic data structures of cell and colony regions and solids

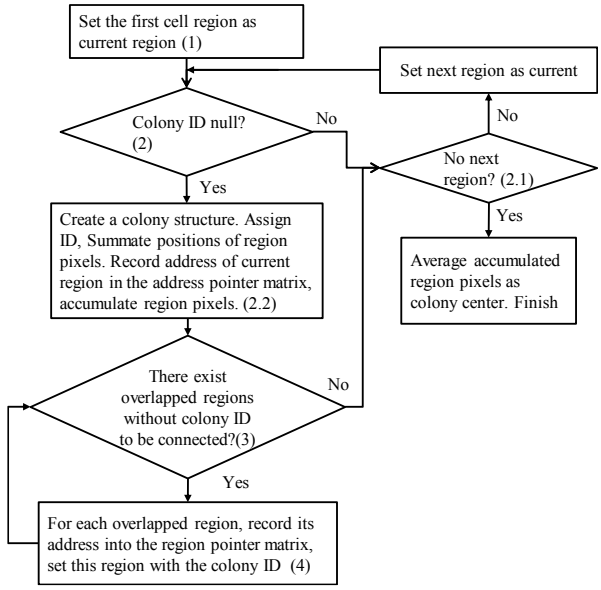


Figure 3. Flow chart of segmenting cell colonies in a volume

D. Segmentation of cell colonies in a volume

Colonies in a volume are segmented as follows (Fig. 3):

1) Set the first colony region (from the uppermost image) as the current processed region.
2) Check if the colony ID of the current region is null?
   2.1) If null, create a new colony dynamic structure, assign an incremental colony ID, and record the address of the current region structure to the region pointer matrix of the colony structure, and accumulate region pixels.
   2.2) If not null, set the next region as the current region and repeat the procedures from Step 2 if there exist next regions. Otherwise, average accumulated region pixels as colony center and finish the colony segmentation.
3) For each overlapped region, record its address into the region pointer matrix, set this region with the colony ID, and accumulate region pixels. Repeat from Step 2.2, after processing all overlapped regions.

Overlapping of two cell (or colony) regions in neighboring images is the presence of more than 10% of pixels in either region with the same image coordinates as the pixels in the other region.

E. Classification of cell solids to every colony

Cell-solids in a volume are classified to either cell colony in the volume by setting the cell-solid of the cell-regions included in every colony-region of the colony as belonging to the colony, and record the addresses of these cell-solids into the cell pointer matrix of the colony.

F. colony tracking

Colony correspondences between neighboring frames are implemented as follows (Fig. 4).

1) Set the first cell colony at the *P* frame as the current processed colony.
2) Set the current colony as the tracking colony.
3) Use the Kalman filter and the recorded distance (colony center to center) to the *P* frame (*P* distance) of the tracking colony to find the *N* tracked colony with the shortest distance [9].
4) Set the *N* tracked ID of the tracking colony as the tracked colony and *N* distance as the shortest distance.

   Check if this *N* colony is already tracked (with *P* tracked ID)

   4.1) If not, set the *P* tracked ID of the tracked colony as the ID of the tracking colony,

   4.2) If tracked, check if this shortest distance is smaller than the recorded distance of the tracked colony.

   4.2.1) If it is, set *P* Track ID to the tracked colony and set the replaced colony as the tracking colony. Repeat the step from Step 3.

   4.2.2) If not, exclude the tracked colony and repeat from Step 3 if existing any next *N* colony, and repeat from Step 5 if existing no *N* colonies
5) If there is the next colony (to the current) to process?

   5.1) If yes, set the next colony as the current colony and repeat the procedures from Step 2.

   5.2) If no, for every colony without *P* ID at the *N* frame, calculate and record its shortest distance and *P* ID.

Most colony correspondences are one-to-one, indicating the *P* tracked ID of the *N* colony and the *N* tracked ID of the *P* colony are the same and also the distance. Meanwhile, a *P* colony possesses no correspondent *N* colony, indicating its recorded *N* tracked colony does not record it. This *P* colony is considered as merging into its recorded colony together with a one-to-one correspondent *P* colony, if the sum of solid numbers of this colony with the one-to-one correspondent *P* colony is near their recorded *N* colony. Otherwise, it is a disappeared (moved outside the volume) colony. Conversely, a *N* colony without its one-to-one correspondent *P* colony, it is considered as splitting from its recorded *P* colony together with the one-to-one correspondent colony of its recorded *P* colony if the sum of solid numbers of them is near with its recorded *P* colony. Otherwise, it is an appeared (moved in) one. A one-to-one correspondence without merging (or disappearing), splitting (or appearing) is a moving colony.

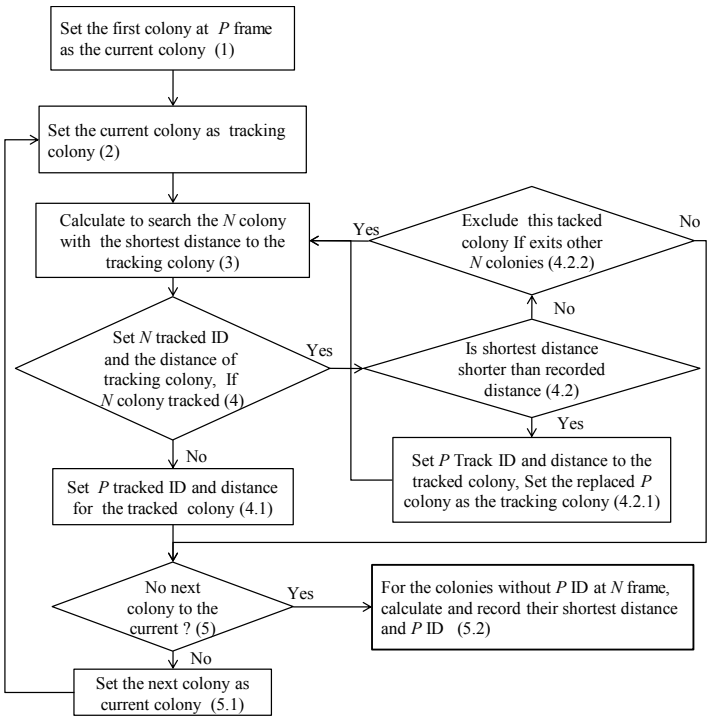


Figure 4. Flow chart of colony correspondence calculation

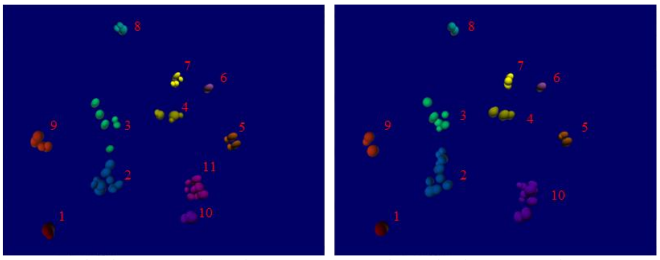
G. Cell tracking

The cell correspondences between frames is implemented between cell solids in the correspondent colonies. For a moving colony correspondence, the cells at a *P* colony are used to make correspondence with the cells in an *N* colony. For a merging correspondence, the solids included in multiple *P* colonies are used to make correspondence with the cells in the correspondent *N* colony. For a splitting correspondence, the cells in a *P* colony are used to make correspondences with the cells in its correspondent *N* colonies. The cell correspondence computations are based on the cell centers recorded in the dynamic data structures.

Most cell correspondences are one-to-one, indicating the *P* tracked ID of the *N* cell solid and the *N* tracked ID of the *P* cell are the same and also the distance. Meanwhile, a *P* cell possesses no correspondent *N* cell, indicating its recorded *N* tracked cell does not record it. This *P* cell is considered as a death cell, or a disappeared cell if the *P* cell is near the boundary of the volume. Conversely, a *N* cell without its one-to-one correspondent *P* cell, it is a proliferated cell from its recorded *P* cell. However, it is an appeared (moved to outside) one, if near to the volume boundary. A one-to-one correspondence without death, proliferation, appearance or disappearance is a moving cell.

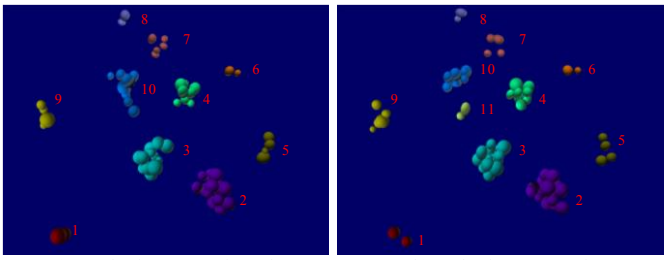
III. Results and Discussion

The prototype system was implemented on a PC with Intel Core i7-8700, 64GB Ram and an Asus dual-RTX2080 graphics card. A set of time-lapse confocal microscopy images of live mESCs taken over two days was used to test the effectiveness of the proposed methods and system.



(a) Colonies at previous frame (b) Colonies at next frame

Figure 5. Colony and cell tracking with a colony merging



(a) Colonies at previous frame (b) Colonies at next frame

Figure 6. Colony and cell tracking with a colony splitting

TABLE I. COLONY AND CELL TRACKING WITH A COLONY MERGING

Colony No.	P (fr.)	Mov.	Ap.	Dis.	Pro.	Death	N (fr.)
No.1	2	1	0	0	0	1	1
No.2	11	9	1	0	1	0	11
No.3	7	6	0	0	0	1	6
No.4	5	4	0	0	0	1	4
No.5	4	3	0	1	0	0	3
No.6	1	1	0	0	0	0	1
No.7	4	3	0	0	0	1	3
No.8	3	2	0	0	0	1	2
No.9	4	3	0	1	0	0	3
No.10	3	3	0	0	0	0	14
No.11	9	9	0	0	2	0	--

fr.: frame, Mov.: Moving, Ap.: appearing, Dis.: disappearing, Pro.: proliferation.

TABLE II. COLONY AND CELL TRACKING WITH A COLONY SPLITTING

Colony No.	P (fr.)	Mov.	Ap.	Dis.	Pro.	Death	N (fr.)
No.1	2	2	0	0	0	0	2
No.2	18	18	0	0	1	0	19
No.3	15	15	0	0	2	0	17
No.4	8	8	0	0	1	0	9
No.5	4	4	0	0	0	0	4
No.6	2	2	0	0	0	0	2
No.7	5	4	0	0	0	1	4
No.8	2	2	0	0	0	0	2
No.9	4	4	0	0	1	0	5
No.10	11	9	0	0	0	2	10
No.11	--	2	0	0	0	0	2

fr.: frame, Mov.: Moving, Ap.: appearing, Dis.: disappearing, Pro.: proliferation.

Figure 5 and Table I show the cell and colony tracking result between two neighboring frame, in which two (No. 10 and No. 11 at the *P* frame) colonies merged into one (No. 10 at the *N* frame). Figure 5 shows the cells labelled as belonging to the same colony are assigned as the same color. Table I shows the cell number of every colony at the *P* frame. The cells might move, appear, disappear, die or proliferate to be the cells of the *N* frame. Instead of reconstructing a cell using its images regions, the cell was reconstructed as a sphere with 0.32 times of the radius by averaging the radii of their image regions. The cells in different colonies can be assigned respective colors for visualization. From the recorded center positions, the cell mobility can be quantified.

Through the two stage tracking (the colony prior to the cell correspondence), 3 errors were reduced comparing only using the cell correspondence. For example, a cell, that belonged to Colony 3 but was near to Colony 2 at the *P* frame, was computed to correspond to a cell in the same colony (Colony 3) at the *N* fame but not a near cell in Colony 2 by the two stage correspondences and hierarchical data structures.

Figure 6 and Table II show the cell and colony tracking result between two neighboring frame, in which one colony (No. 10 at the *P* frame) divided to two (No. 10 and No. 11 at the *N* frame). Table II shows the cell number and thus the moving, appearing, disappearing proliferating, and death cells of every colony at the *P* frame and *N* frame, respectively. Through the colony prior to the cell correspondences, 2 cell tracking errors can be reduced comparing only using the cell correspondence.

IV. CONCLUSION

We presented dynamic hierarchical data structures to record the segmentation results of cell solids and cell colonies. The data in the structures are then used for colony and cell correspondences, and quantitative analyses and visualization. Experimental results show that the tracking method uses the proposed two stage tracking and hierarchical data structures can achieve high tracking accuracy, analyze the cell and colony status changes, and visualize the cell shapes and mobility.

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