Application of the Integral Optical Flow for Identification of the Cell Population Motion in the Microscopic Images

Olga Nedzvedz, Sergey Ablameyko, Huafeng Chen

***Abstract*—** In this paper, we propose a method to identify the cell population behavior (directional motion, aggregation and dispersion of cells) for microscopic videos on base of the integral optical flow conception. Integral optical flow is form of accumulation of the basic optical flow. Due to the accumulation, displacement vectors of background become small, while those of foreground keep growing. Based on information extracted from integral optical flow, pixel motions are analyzed statistically at all positions for each frame to obtain quantity of pixels and their comprehensive motion at each position. After that, regional motion indicators are defined and computed to describe motions at region-level. Thresholds for motion intensity, quantity and motion direction of pixels are used together to segment regional motion maps and identify cell population behavior. These properties are useful for further analysis of video scene. Experimental results show that our method can identify this behavior effectively.

***Keywords*—** Integral optical flow, image monitoring, motion analysis, segmentation.

I. Introduction

Video microscopic technologies have been used for analyzing of cell population behavior and tracking of cells. Using of microscopy provides opportunity for monitoring intact cells. This technique makes the analysis of a cell population completely label-free and gives possibility to study population of thousands of cells in vitro. Automated cell tracking system shall consider that cell populations have varying and inhomogeneous character. Most interesting questions for investigation include dynamical properties of cells and cellular interactions.

Modern video microscopic systems become more intelligent and they allow not only record and visually analyze images but also start to have software tools for tracking, recognition, identification of cells. However, it is necessary to analyze not only behavior of individual cells, but also analyze, trace and predict cells behavior in population, their aggregation and dispersion. Comparing to individual cells, cell population is often more difficult to track.

Identification of cell population behaviors on videos in traditional way is based on the segmentation of objects of interests from the background and track their movements separately [1, 2, 3]. However, for cell populations this is difficult due to their occlusions [4]. Some works choose to study the population as a single entity, but population of cells is a dynamic structure consisting of interacting dynamic elements.

Methods for detection and tracking of dynamic objects can be divided into tracking-based methods that examine trajectories of objects and tracking-free methods based on examination of visual features such as differences in color or brightness changes [3, 5]. Permanent transformation of cell population like change of size, shape and orientation makes difficult using of this method for cells. Detection and tracking of cell population is a more complicated task compared with tracking of separate cells. For cell population it is difficult to determine the boundaries between cells due to their merging.

Methods like thresholding techniques [6, 7], neural networks and probabilistic object models [8, 9] are used for these objects. One of the most promising methods for motion analysis of

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dynamic objects based on using an optical flow. Optical flow belongs to region-based methods [11]. This method allows to get the distribution of velocities and directions of points of object from shift of these points between two images. Optical flow is widely used for investigation of different types of motion like translation of moving object relative to the static or dynamic background and another moving objects; rotation of the object relative to the axis. However, case of cell population with unstable shape, internal noises and random changes has a great influence on the basic optical flow in video sequence. As a result, the structure of motion vectors can be unclear.

An important aim of the automated systems is detection of spatio-temporal localization of mitosis events. Every mitosis event is the division of cell into two daughter cells, which is always accompanied by a change in size, shape and brightness of the area around cells. Different mitosis detection methods unclude: mean shift algorithms; multiple-object matching methods based on the frame-by-frame segmentation; tracking algorithms based on determination of blob region's characteristics; methods of detection of mitosis based on brightness change [11, 13]. Another classification of approaches to detection of mitosis divides all methods on temporal and spatio-temporal [12]. According to this classification, temporal methods detect moment of time, when one cell divides into two cells. Spatio-temporal methods detect as size, shape, velocity and brightness of cells change.

In this study, we propose a new method to identify cell population behavior based on integral optical flow. Unlike basic optical flow [1, 3, 8] by which foreground is hard to pull apart from background due to random motion of the background, integral optical flow enhance foreground motion and restrict background motion. Therefore, foreground is easy to be obtained. Our method considers three factors to identify motion: motion intensity, quantity and motion direction of pixels moving toward and moving away from certain regions.

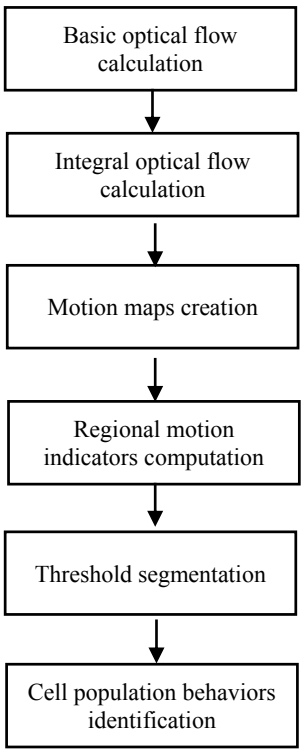


Fig. 1 General scheme of population behaviors identification

In this study, we determine basic structures for three types of motion: directional motion, aggregation of cells and dispersion of cell population. Based on integral optical flow, we

describe motion on the pixel level and at region level. At last, we use threshold segmentation to identify above-mentioned cell conglomerate behaviors. We apply our method on microscopic videos and get good results. General scheme of our method is shown in Fig. 1.

II. Integral Optical Flow

Optical flow describes the movement of pixels between two consecutive frames. The idea of optical flow is based on two assumptions: (1) the pixel intensities of an object do not change between consecutive frames; (2) neighboring pixels have similar motion.

A video frame is a digital image and there is an imaginary underlying grid below it. Each coordinate point of the grid is called a position. Positions are stationary, while pixels may change their positions from frame to frame. Optical flow provides a way letting one study pixel motion at each position at different time.

Basic optical flow only records displacement vector of pixels between two consecutive frames. Taking into account the very short time interval between them, it is hard to distinguish foreground from background due to motion of background. Generally background moves randomly, e.g. back and forth or rotationally. In short time this kind of character doesn't show, but after a long enough time, it will reveal itself thus help identify foreground.

Integral optical flow is an intuitive idea that accumulate optical flow for several consecutive frames. In result of this accumulation, displacement vectors of background become small, while those of foreground keep growing [9].

For description convenience, we use I_t to denote *t-th* frame of video *I*, $I_t(p)$ to denote pixel at position *p* in I_t. Let OF_t denote basic optical flow of I_t. It is a vector field with each vector $OF_t(p)$ represents displacement vector of pixel $I_t(p)$. Assume, $OF_t(p) = \vec{d}$ we can easily determine the position in I_{t+1} at which pixel $I_t(p)$ moves to, which will be $p+\vec{d}$.

Consider optical flows for several consecutive frames have been computed, we can obtain integral optical flow for the first frame of those. Let IOF_t^{itv} denote integral optical flow of I_t, where *itv* is the frame interval parameter used to compute integral optical flow. IOF_t^{itv} is also a vector field which records accumulated displacement information in time period of *itv* frames for all pixels in I_t.

For any pixel $I_t(p)$, its integral optical flow $IOF_t^{itv}(p)$ can be determined as follow:

$$I0F_t^{itv}(p) = \sum_{i=0}^{itv} OF_{t+1}(p_{t+1})$$

where p_{t+1} is the position in I_{t+1} of pixel $I_t(p)$. Note that *x*-component and *y*-component of p_{t+1} should be rounded to the nearest integer, as pixels are at positions with integer coordinates.

Integral optical flow allows to identify foreground or mobile regions by threshold segmentation. Any pixel with a big enough magnitude of its displacement vector will be considered as a foreground pixel.

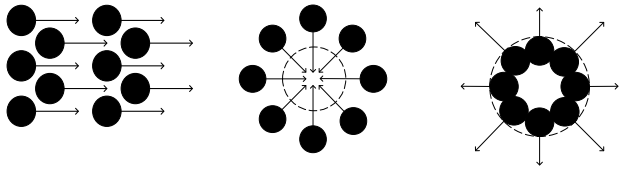
III. Identification of Cell Population Behavior

A. Types Characteristics of Cell Population Motion

We consider three types of cell population motions: directed motion of the cell population, aggregation of cells and dispersion of population.

Directed motion of cells is identified in the case when cells or a cell population moves in the same direction. There are main features of directed motion: some objects move simultaneously from one area of the image to another one; the speed of objects exceeds the speed of chaotic background movement; the direction of movement of cells is the same (Fig. 2a).

Cells aggregation is identified when cells move into a certain region from different directions, this motion can be symmetrical relative to the directions of motion or not. There are main features of aggregation: some objects moves into a certain region from elsewhere; the speed of motion is greater than the speed of chaotic background movement; there are at least two moving directions and they are more or less symmetrical (Fig. 2b).



a b c

Fig 2 Diagram of different types of cells motion: (a) directed motion; (b) aggregation; (c) dispersion. Black solid circles represent cells, arrows show motion directions

Cells dispersion is identified when cells move out of a certain region to different directions like it shown in Fig. 2c. As example, it can be growing of cell population or cell scattering under the influence of some negative factors. Cells dispersion happens when cells move out of a certain region to elsewhere, the speed of motion is greater than the speed of chaotic background movement and their motion is more or less symmetrical.

In general situation it is possible to describe the motion of set of objects with help of a concept of position or a concept of a region, which includes a set of adjacent positions. Basic optical flow and integral optical flow record basic information of motions: starting positions and ending positions of pixels, it allows to determine comprehensive moving directions of pixels.

Motion direction indicates a destination where cells move. In order to determine cells motion direction in a region, we can simply divide [0,2π) into several intervals with equal length and count for each interval number of pixels whose motion direction is in that interval. Interval with most pixels shows main motion direction of cell population.

Motion speed of pixel $I_t(p)$ in time period from I_t to I_{t+1} can be calculated as follow:

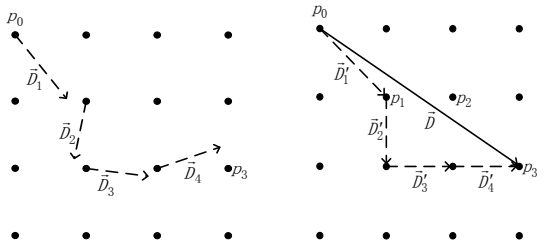
$$s_t^{itv}(p) = \frac{\left|IOF_t^{itv}(p)\right|}{itv}$$

B. Motion Analysis based on the Pixel-level

Integral optical flow allows to determine motion of a pixel in given time period. In Fig. 3a pixel in I_t with original position p_0 moves to position p_3 after four frames in I_{t+4}. Position sequence (p_0, p_3) is simple motion path of the pixel in time period from frame I_t to frame I_{t+4}. In Fig. 3b position sequence (p_0, p_3) is interpolative motion path of the pixel in the same time period. We use an interpolative motion path along with a simple motion path for calculation of integral optical flow.

$\overrightarrow{D_1}$, $\overrightarrow{D_2}$, $\overrightarrow{D_3}$, $\overrightarrow{D_4}$ are displacement vectors of the pixel extracted from basic optical flows $OF_t, OF_{t+1}, OF_{t+2}, OF_{t+3}$, respectively. $\overrightarrow{D_1'}$, $\overrightarrow{D_2'}$, $\overrightarrow{D_3'}$, $\overrightarrow{D_4'}$ in Fig. 3b are their integer versions extracted from integral optical flow $I0F_t^4$:

$$\vec{D} = \overrightarrow{D_1'} + \overrightarrow{D_2'} + \overrightarrow{D_3'} + \overrightarrow{D_4'}$$



a b

Fig 3 Pixel motion path: (a) simple motion path; (b) interpolative motion path

Consider a vector $\overrightarrow{p_0p_{n-1}}$ represents an integral optical flow for the effective pixel motion path starting from p_0 and ending at p_{n-1} where где $n \geq 2$. In order to compute contributions of pixel quantity and comprehensive motion of this path for every position in it, we use the normalized vector of integral optical flow, because it carries information of both pixel number and motion direction. The normalized vector of $\overrightarrow{p_0p_{n-1}}$ can be computed as follow:

$$\overrightarrow{v_{norm}} = \frac{\overrightarrow{p_0p_{n-1}}}{|\overrightarrow{p_0p_{n-1}}|}$$

where $\overrightarrow{v_{norm}}$ is the normalized vector, $|\overrightarrow{p_0p_{n-1}}|$is the magnitude of $\overrightarrow{p_0p_{n-1}}$.

Vector $\overrightarrow{v_{norm}} = 1$ indicates pixel number for a single effective pixel motion path, four values can be computed for any position p_i $(0 \leq i < n)$ in the path:

$$s_{in} = w_{in} \cdot |\overrightarrow{v_{norm}}|;$$
$$s_{out} = w_{out} \cdot |\overrightarrow{v_{norm}}|;$$
$$\overrightarrow{v_{in}} = w_{in} \cdot |\overrightarrow{v_{norm}}|;$$
$$\overrightarrow{v_{out}} = w_{out} \cdot |\overrightarrow{v_{norm}}|;$$

where w_{in}, w_{out} are weighting coefficients for determining percentage of incoming and outgoing pixels at the position and $w_{in}+w_{out}=1$.

For statistical motion analysis, only pixels, which actually move, should be considered. Thus for each position, only pixel motion path whose starting position is different from its ending position should be taken into account.

Description of cell motion and determination of the type of motion can be performed with help of motion maps. In this paper, several types of motion maps are proposed. Suppose that in-pixel quantity map (*IQ-map*) is a map with a scalar value at each position indicating number of pixels moving toward the corresponding position. Out-pixel quantity map (*OQ-map*) is a map with a scalar value at each position indicating number of pixels moving away from the corresponding position. In-pixel comprehensive motion map (*ICM-map*) is a map with a vector at each position indicating comprehensive motion of pixels moving toward the corresponding position. Out-pixel comprehensive motion map (*OCM-map*) is a map with a vector at each position indicating comprehensive motion of pixels moving away from the corresponding position.

IQ-map, *OQ-map*, *ICM-map* and *OCM-map* of I_t can be determined as follows to formulas:

$$IQ_t(p) = \sum_{a \in S_t(p)} s_{in}(a, p)$$
$$OQ_t(p) = \sum_{a \in S_t(p)} s_{out}(a, p)$$
$$ICM_t(p) = \sum_{a \in S_t(p)} \overrightarrow{v_{in}}(a, p)$$

$$OCM_t(p) = \sum_{a \in S_t(p)} \overrightarrow{v_{out}}(a, p)$$

where p is value of position on appropriate map, $S_t(p)$ is set of motion paths (simple or interpolative depending on which type of motion path is used for motion analysis).

According to created motion maps for time of I_t , important characteristics of pixel motions at that time will be determined: positions with bigger value on *IQ-map* are positions to which more pixels move; positions with bigger value on *OQ-map* are positions from which more pixels leave; positions with smaller vector magnitude on *ICM-map* are positions to which pixels move in more symmetrical directions; positions with smaller vector magnitudes on *OCM-map* are positions from which pixels leave in more symmetrical directions. Based on this, one can conclude that positions with big values on *IQ-map* and small vector magnitudes on *ICM-map* are positions at which pixels tend to aggregate; positions with big values on *OQ-map* and small vector magnitudes on *OCM-map* are positions at which pixels tend to disperse.

C. Motion Analysis based on Region-level

Description of the cell population motion one can perform at the region-level based on the study of the displacement of regions of interest. Motion intensity is a major factor for detecting different events in cell population. Any region with a high enough regional motion intensity is considered an intensive motion region. It is appropriate to use average displacement of pixels to represent motion intensity in a region.

Regional motion intensity is an average of displacement vector magnitudes extracted from integral optical flow for pixels in a certain region. It can be described according to formula:

$$MI_t(r) = \frac{1}{n} \sum_{p \in r} \left| IOF_t^{itv}(p) \right|$$

where $MI_t(r)$ is the regional motion intensity of region r at the time of I_t, n is position number in r, p is one position in r, $IOF_t^{itv}(p)$ is the displacement vector of $I_t(p)$ extracted from integral optical flow.

For any region regional in-pixel relative quantity $IRQ_t(r)$ of certain region r at the time of I_t can be determined as follow:

$$IRQ_t(r) = \frac{1}{n} \sum_{p \in r} IQ_t(p),$$

where r is certain region, $IQ_t(p))$ is value at position p on *IQ-map* at the time of I_t.

Regional out-pixel relative quantity $ORQ_t(r)$), which is an average of values on *OQ-map* at positions in a certain region, can be determined by formula:

$$ORQ_t(r) = \frac{1}{n} \sum_{p \in r} OQ_t(p)$$

where $OQ_t(p)$ is value at position p on *OQ-map* at the time of I_t.

By comparing $IRQ_t(r)$ with $ORQ_t(r)$), one can know whether more pixels move toward a certain region than pixels move away from it or vice versa. Identification of cells aggregation or cells dispersion can be performed by calculation regional in/out indicator IOI_t:

$$IOI_t = \frac{IRQ_t(r)}{ORQ_t(r)}$$

In this formula $IOI_t(r) > 1$ means more pixels move into r, while $IOI_t(r) < 1$ means more pixels move out of r.

There are two additional features to describe the nature of the motion of a cell population. Regional in-pixel symmetry $IS_t(r)$ is the ratio of regional in-pixel relative quantity of a certain region to magnitude of average vector of that region on *ICM-map*:

$$IS_t(r) = \frac{IRQ_t(r)}{\left|\frac{1}{n}\sum_{p\in r} ICM_t(p)\right|}$$

where n is position number in r, $IRQ_t(r)$, $ORQ_t(r)$) are values for region r on *IRQ-map* and *ORQ-map* at the time of I_t, respectively, $ICM_t(p)$, $OCM_t(p)$ are values at position p on *ICM-map* and *OCM-map* at the time of I_t, respectively.

Regional out-pixel symmetry $OS_t(r)$ can be determined by the formula respectively:

$$OS_t(r) = \frac{ORQ_t(r)}{\left|\frac{1}{n}\sum_{p\in r} OCM_t(p)\right|}$$

The bigger $IS_t(r)$ or $OS_t(r)$ is, the more symmetrically corresponding pixels move.

IV. Identification Cell Population Behavior Using Motion Maps

The identification of the behavior of cell population in the region r can be performed on base of the following characteristics: regional motion intensity $MI_t(r)$, regional out-pixel relative quantity $ORQ_t(r)$ and regional out-pixel symmetry $OS_t(r)$. Lower limits of region size and regional motion intensity should be properly determined for this identification according to specific properties of cells.

Directed motion occurs if $MIt(r)$, $ORQ_t(r)$ and $OS_t(r)$ equal to the thresholds or exceed them. Thereby, the directional motion is going to happen in region r at the time of I_t if the following conditions are met:

$$MI_t(r) \geq t_{11};\ ORQ_t(r) \geq t_{12};\ (3)\ OS_t(r) \geq t_{13}.$$

where t_{11} is the threshold for *MI-map*, t_{12} is the threshold for *ORQ-map*, t_{13} is the threshold for *OS-map*.

The identification of cells aggregation can be performed on base of the following characteristics: regional motion intensity $MI_t(r)$, regional in-pixel relative quantity $IRQ_t(r)$, regional in/out indicator $IOI_t(r)$, regional in-pixel symmetry $IS_t(r)$. Aggregation occurs if $MI_t(r)$, $IRQ_t(r)$, $IOI_t(r)$, $IS_t(r)$ meet thresholds:

$$MI_t(r) \geq t_{21};\ IRQ_t(r) \geq t_{22};\ IOI_t(r) \geq t_{23};\ IS_t(r) \geq t_{24},$$

where t_{21} is the threshold for *MI-map*, t_{22} is the threshold for *IRQ-map*, t_{23} is the threshold for *IOI-map*, t_{24} is the threshold for *IS-map*.

Cells dispersion is identified in region r if $MI_t(r)$, $ORQ_t(r)$, $IOI_t(r)$ and $OS_t(r)$ meet thresholds:

$$MI_t(r) \geq t_{31};\ ORQ_t(r) \geq t_{32};\ IOI_t(r) \geq t_{33};\ OS_t(r) \geq t_{34},$$

where t_{31} is the threshold for *MI-map*, t_{32} is the threshold for *ORQ-map*, t_{33} is the threshold for *IOI-map*, t_{34} is the threshold for *OS-map*.

Threshold for motion intensity can be determined with help of the frame interval parameter. With bigger frame interval parameter, more intensive motion field will be obtained, thus threshold for motion intensity should be bigger for the same situation monitoring application. It can be determine as:

$$t_s = \alpha \cdot itv$$

where t_s is threshold for motion intensity, itv is frame interval parameter, α is a constant for the specific task.

Region size is another parameter that affects determination of threshold. If bigger region is used, threshold for pixel quantity should be smaller:

$$t_q = \frac{\beta}{s}$$

where t_q is threshold for pixel quantity, s is area of region, β is a constant for the specific task.

V. Stages of the Cell Cycle Detection

The method based on the integral optical flow allows detecting of spatio-temporal localization of cell cycle stages. We consider four main cell cycle stages: normal stage, mitosis, apoptosis and intermediate stage. The information about cell stage is significantly important for the monitoring of cell population evolution. Every change in the stage of cell cycle is accompanied by a change in size, shape and brightness of the area around cells. Processes of mitosis or apoptosis are going from intermediate stage. Vectors of integral optical flow going from center of any event have structure like a star. During the intermediate stage of evolution cell is growing and vectors of optical flow have a star structure and after that cell can be destroyed (in stage of apoptosis) or divided into two new cells (in stage of mitosis). In case of apoptosis, vectors of optical flow have various random directions. In case of mitosis, they have two opposite directions.

Determination of cell cycle stages can be performed with help of additional motion map called regional out-pixel comprehensive motion map (*ROCM-map*), can also be created to show how symmetrically pixels move away from a region. Let $ROCM_t$ denote ROCM map, then

$$ROCM_t(c) = ROCM_t(r) = \frac{1}{n}\sum_{p \in r} OCM_t(p),$$

where n is position number in r, c is the center of cell, which coincides with center of the region.

In general, the bigger $ROCM_t\ (c)$ is, the more symmetrically corresponding pixels move. For mitosis stage symmetry on the *ROCM-map* is larger than the threshold value, for apoptosis stage it is very low.

VI. Conclusion

We have presented a method for cell population behaviors identification. Our method mainly consists of the following steps: integral optical flow computation, position-level motion analysis, region-level motion analysis and threshold segmentation. The accumulative effect of integral optical flow allows to obtain steady motion regions, which are usually regions of interest. Pixel motion intensity, quantity and motion direction are together used to describe motions and identify cell population behaviors. The effectiveness of our method has been demonstrated and confirmed by our experimental results. Due to the combination of simple solutions, this algorithm can be easy realized on base of many computer systems of image analysis.

Although we can get good results for jittering videos through changing thresholds in some cases, but that is not universal, thus our method should be applied to stable videos generally. In different applications thresholds for motion intensity, quantity and motion direction of pixels should be set depending on the scene, camera setup and specific purposes.

Acknowledgment

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