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Motion Flow Analysis in Cell Videos using a Multi-Level Clustering Method

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Abstract— **Analyzing motion flow of cells is an important task for many biomedical applications. It is a challenging problem due to noise in images and uncontrolled motion of cells. In this study, a method to find regions of organized motion and direction of flow is proposed. Since dense optical flow methods might fail due to homogeneous regions and irregular motion patterns, the technique involves analyzing trajectories of strong corner features. Trajectories are clustered to find dominant flow patterns for different regions of the frame, where a multi-level clustering scheme is followed. Experiments show that the technique gives accurate results for detecting region and direction of flow.**

I. INTRODUCTION

Characterizing the motion of cells in tissue and in culture systems is an important task to consider in biomedical research. Some biological applications include the study of cell migration and its variations under different culture conditions or drug actions [2], [12]. The motion patterns can be used as distinguishable features to indicate, for instance, insufficient blood flow, blockages, or even the presence of a tumor.

Analysis of cell motion consists of tracking the location of each cell over entire sequence of images and extracting qualitative and quantitative features. The motion tracks of the cells could provide the raw data necessary to answer questions about the patterns of cell motion and organization. A lot of research has been done on object tracking [11], and many specifically have been working on cell tracking [7], [6]. Some studies are focused on manual or interactive computer-assisted tracking [5]. However in most cases manual tracking is not feasible due to the high number of cells in a single scene and is a tiresome task if dealing with a large number of cells during long periods in order to acquire statistically robust results. Moreover, for some scenarios where cells merge together and form a tissue, it is hard to detect individual cell objects (Figure 1).

Although there is a tremendous amount of work on crowd flow analysis [1], [9], there are few studies on motion flow analysis of cells [8], [3]. Wu et al [9] proposed a method to detect chaotic invariants using a crowd flow representation based on trajectories. Ali et al [1] used dense optical flow and introduced Lagrangian dynamics to perform crowd flow segmentation. Compared to human crowd datasets, cell videos contain more noise and non-organized motion. Due to noise and a large variety of homogeneous regions, optical flow computation might not be robust. Furthermore, some regions on a frame might contain irregular motion patterns, which requires a detailed interpretation of motion flow. Souvenir et al [8] presented a method to estimate the cell flow using Radial Flow Transform filters on videos of natural killer T cells.

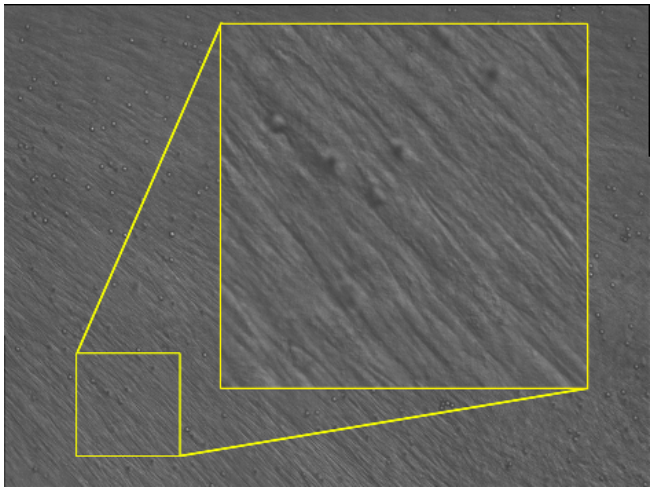

Fig. 1. An example frame from one of the sequences. Bright points are the markers put into the sample. Big yellow square is a zoomed in version of the small patch. Once the cells merge together and form a tissue, it is hard to see individual cells even by eye.

In this study, rather than tracking individual cell objects, we are interested in finding regions of organized motion and direction of flow in cell videos. Our approach involves tracking of strong corner features and clustering trajectories to find different motion patterns. The contribution of the paper is as follows: (i) dominant motion patterns in cell videos are found without detection and tracking of individual cells, (ii) clustering trajectories is performed in a multi-level way by using features such as entropy of direction distribution of displacement vectors and velocity. The experiments show that proposed technique is successful on finding the direction of dominant motion on a given region.

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II. METHOD

Proposed method consists of three stages. First corner points, that are more convenient for tracking are extracted for the first frame and tracked independently throughout the sequence. Computed trajectories are then input to a multi-level clustering scheme. The appropriate clustering method depends on two important observations. First, a sequence might contain irregular motion patterns as well as organized motion flow in a specific direction. Second, there might be different groups of cells moving in different directions. Hence, at the first level of clustering we first classify the trajectories as irregular and organized motion patterns. At the next level, regular trajectories are clustered based on the motion direction. Finally, local flow direction is computed using velocity vectors of the trajectories.

A. Feature Detection and Tracking

After cells form a tissue, they move together in an organized way. Although remaining individual cells might appear on the sample, they join the underlying tissue after executing some fast and abrupt movements. Thus, instead of detecting and tracking single cell objects in videos, we detect important feature points and track throughout the sequence. This helps us analyze the flow behavior.

Initially, strong corner points are detected on the first frame. For each pixel (x, y), $n \times n$ window with pixels $\mathbf{p}_1, \mathbf{p}_2, \dots \mathbf{p}_{n^2}$ are considered and minimum eigenvalue of the matrix

$$\mathbf{C}_{x,y} = \begin{bmatrix} \sum_{i=1}^{n^2} I_x^2(\mathbf{p}_i) & \sum_{i=1}^{n^2} I_x(\mathbf{p}_i) I_y(\mathbf{p}_i) \\ \sum_{i=1}^{n^2} I_x(\mathbf{p}_i) I_y(\mathbf{p}_i) & \sum_{i=1}^{n^2} I_y^2(\mathbf{p}_i) \end{bmatrix} \quad (1)$$

is computed where $I_x(\mathbf{p}_i)$ and $I_y(\mathbf{p}_i)$ are the components of the gradient at pixel $\mathbf{p}_i$ in x and y directions respectively. After non-maxima suppression on the obtained eigenvalue map, N points with the largest eigenvalues are chosen. Next, each feature point is tracked independent from the others using Lucas-Kanade (LK) tracker [4]. Since, tracker might have failed for some frames, outlier trajectories are eliminated by thresholding on the displacement between frames.

B. Clustering Trajectories

Based on the main observation, a sequence might contain irregular motion patterns and organized motion in different directions, we follow a multi-level clustering approach. At the first level of the process, we divide the trajectories into two groups, irregular and organized motion patterns. Since we are interested in the transition of organized motion, trajectories with regular motion are clustered according to the direction of motion at the next level.

In order to detect regular motion patterns, we compute the direction histogram of displacement vectors for each trajectory. Assume $\{\mathbf{p}_0^t, \mathbf{p}_1^t, \dots \mathbf{p}_{F-1}^t\}$ is trajectory t consisting of 2D positions $\mathbf{p}_i^t \in \mathbb{R}^2$ for F frames. Displacement vectors are $\mathbf{x}_1^t, \mathbf{x}_2^t, \dots \mathbf{x}_{F-1}^t \in \mathbb{R}^2$ where

$$\mathbf{x}_k^t = \mathbf{p}_k^t - \mathbf{p}_0^t \text{ for } \forall k \in \{1, 2, \dots F-1\} \quad (2)$$

For regular motion, we expect to have the displacement vectors in the same direction. Thus, direction distribution should be peaky. On the other hand, we expect to have sparse distribution for trajectories with irregular motion pattern. Entropy of direction distribution is a good measure for identifying trajectories with organized motion. So, for all trajectories entropy of direction distribution is computed. Trajectories with larger entropy values are classified as irregular.

Let $\angle \mathbf{x}_i^t$ is the direction of i^{th} displacement vector in the trajectory t. Then, a normalized 360 bin histogram $\mathbf{H}^t$ for all directions $\angle \mathbf{x}_1^t, \angle \mathbf{x}_2^t, \dots \angle \mathbf{x}_{F-1}^t$ is computed for the trajectory. Entropy η^t of trajectory t is approximated as

$$\eta^t \approx -\sum_{i=1}^{360} \mathbf{H}_i^t log(\mathbf{H}_i^t) \quad (3)$$

where $\mathbf{H}_i^t$ is the value at i^{th} bin of the histogram $\mathbf{H}^t$.

Second level of the clustering method involves more analysis on regular trajectories. Because, we might observe multiple cell flows in different directions in a video, velocity direction of trajectories is used as a feature for clustering. We assume a constant velocity model for each trajectory. So, for trajectory t we have

$$\mathbf{p}_k^t = \mathbf{p}_{k-1}^t + \mathbf{v}^t + \mathbf{w}^t \text{ for } \forall k \in \{1, 2, \dots F\} \quad (4)$$

where $\mathbf{v}^t \in \mathbb{R}^2$ is the constant velocity of trajectory t, and $\mathbf{w}^t \in \mathbb{R}^2$ is Gaussian noise with mean $\mathbf{0}$ and covariance $\mathbf{\Sigma}_t$. Maximum likelihood estimate for velocity of the trajectory is

$$\hat{\mathbf{v}}^t = \frac{1}{F-1} \sum_{k=1}^{F-1} (\mathbf{p}_k^t - \mathbf{p}_{k-1}^t) \quad (5)$$

As a result we will have velocity vectors $\{\hat{\mathbf{v}}^1, \hat{\mathbf{v}}^2, \dots \hat{\mathbf{v}}^T\}$ for all T regular trajectories. We apply medoid-shift algorithm [10] with cosine distance metric to cluster the velocity vectors. Note that, tolerance on the direction difference is input to the medoid-shift algorithm, which affects the number of clusters for the sequence.

C. Finding Flow Direction

In this section, we will explain how we find the flow direction on a region. Among the trajectories in a given region, first the dominant cluster of trajectories that appears the most is found. Let set S contains the indexes of trajectories inside a given region R, and we found k clusters for the sequence. Suppose $S = \bigcup_{i=1}^{k} S_k$ where S_i contains the trajectory indexes that belong to cluster i. Dominant cluster c_R in the region R is $c_R = \underset{i}{argmax} |S_i|$ where $|S|$ indicates the number of elements in the set S. Proportion of trajectories ρ_R that belong to the dominant cluster in the region R is computed as follows

$$\rho_R = \frac{|S_{c_R}|}{|S|} \quad (6)$$

Next mean of the velocities for the trajectories, that are inside the region and belong to dominant cluster is computed and

TABLE I

EXPERIMENT RESULTS

		Percentage	Cosine
Sequence 1	Region 1	94%	0.96
	Region 2	86%	0.89
Sequence 2	Region 1	81%	0.97
Sequence 3	Region 1	100%	0.99
	Region 2	83%	0.91
	Region 3	58%	0.92
Sequence 4	Region 1	90%	0.97
	Region 2	92%	0.99
	Region 3	56%	0.99

assigned as the flow direction μ_R of the region R.

$$\mu_R = \frac{1}{|S_{c_R}|} \sum_{j \in S_{c_R}} \hat{\mathbf{v}}_j \tag{7}$$

III. EXPERIMENTS AND RESULTS

The images of primary Human Corneal Fibroblasts (HPCFs) are acquired with a TE2000-E Nikon inverted microscope. Multiple images are acquired from nine different overlapping regions on the sample and then stitched together to create a 3×3 mosaic[1]. This creates a frame with size 2808 by 3759 pixels. A mosaic image is taken in every six minutes.

The experiments are carried out on 4 video sequences each of them with around 200 frames. Since, it is hard to see the motion patterns for every region of the video by eye, an expert annotated some regions where motion is more visible. For each delineated region, direction of the motion is indicated with a vector. Therefore, the results are evaluated only for the annotated regions. Proportion of trajectories of the dominant cluster (ρ_R) and flow direction (μ_R) are computed for delienated regions. The output direction vector is compared with the hand labeled velocity vector for the region. The results are reported in Table I. Each sequence has multiple annotated regions. Percentage column shows the proportion of trajectories referring to the most popular cluster among all trajectories in the region. The column titled with Cosine indicates the cosine of the angle between manually annotated motion vector and computed flow direction. The results show that even though there are outlier trajectories in a region, it finds the flow direction close to manually labeled direction.

Figure 2 shows the direction histograms for displacement vectors of irregular and organized trajectories. Notice the

[1]This causes noise especially on the overlapping regions.

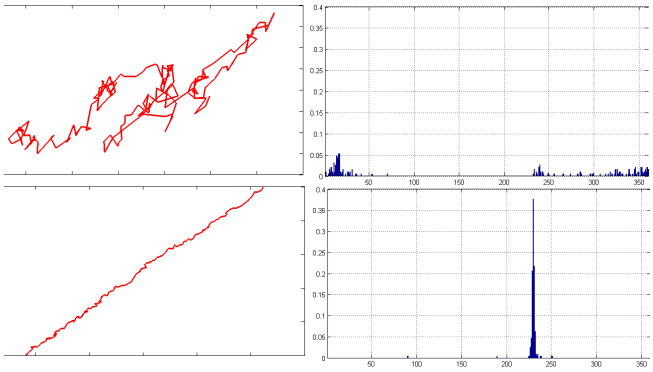

Fig. 2. Direction histograms of displacement vector for irregular (top) and organized (bottom) motion patterns. Entropy values for top and bottom histograms are 3.26 and 0.62 respectively.

sparse histogram for irregular trajectory. Based on entropy values for all histograms, median is selected as the threshold for separating two classes.

Final clustering result for two of the sequences is displayed in Figure 3. Irregular trajectories might be due to abrupt motion of single cell objects or resisting flows in opposite directions. A demo video for sequence 1 can be seen at `http://www.youtube.com/watch?v=H_oFo65z4ew`.

Estimated motion directions and annotated vectors are displayed for two sequences in Figure 4. Although there are outlier trajectories in the annotated regions, the technique is able to find the motion direction based on the dominant cluster. Notice also that regular trajectories appear more on the annotated region which shows the strength of our clustering method in computing trajectories.

IV. CONCLUSIONS AND FUTURE WORKS

In this study, a novel method to detect dominant motion patterns in cell videos is proposed. Proposed technique is based on analyzing trajectories and uses a multi-level clustering methodology. The experiments show that, the method is successful in finding regions with regular motion patterns and direction of flow. The cells are less organized at the first days of image acquisition. The researchers are also interested in detecting the time where the cells merge together and show an organized motion. In the future, we want to propose an index, indicating the amount of cell organization. Also, we would like to extend our work to detecting proliferating and merging cells.

REFERENCES

[1] *A Lagrangian Particle Dynamics Approach for Crowd Flow Segmentation and Stability Analysis*, 2007 IEEE Conference on Computer Vision and Pattern Recognition (2007), 1–6.

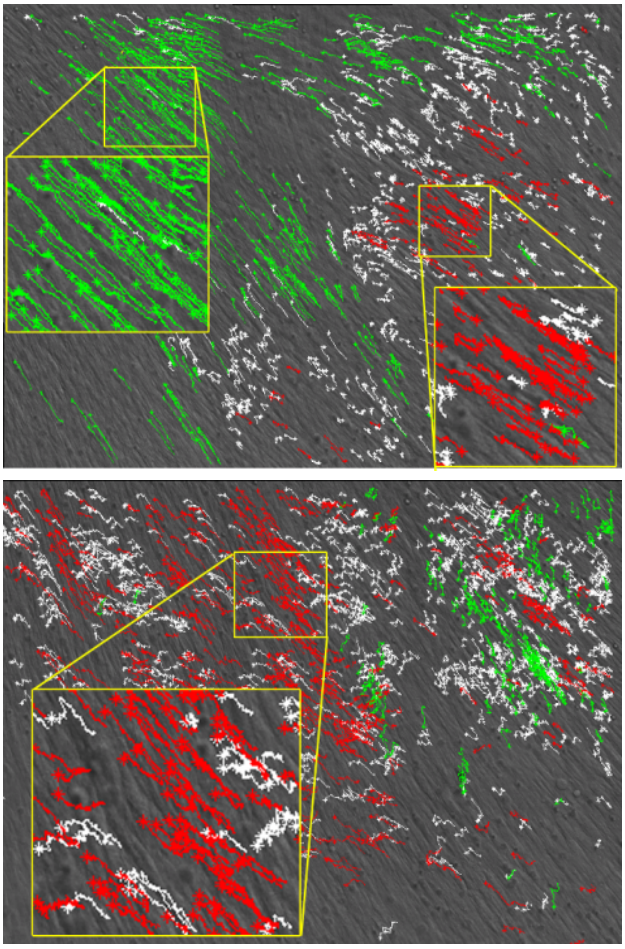

Fig. 3. Clustering results overlayed on the first frame of the sequence for sequence 1 (top), sequence 3 (bottom). Colors represent the clusters. Star on the tip of each trajectory shows the starting position. White trajectories are detected as irregular ones. Notice the opposite motion direction for the regular motion trajectories (red and green) in the top image. Sequence 3 is an early stage of image acquisition where more single cells are visible. Thus irregular trajectories appear in most of the regions of this sequence compared to sequence 1. In the top image, irregular trajectories might be due to resisting flows in opposite directions.

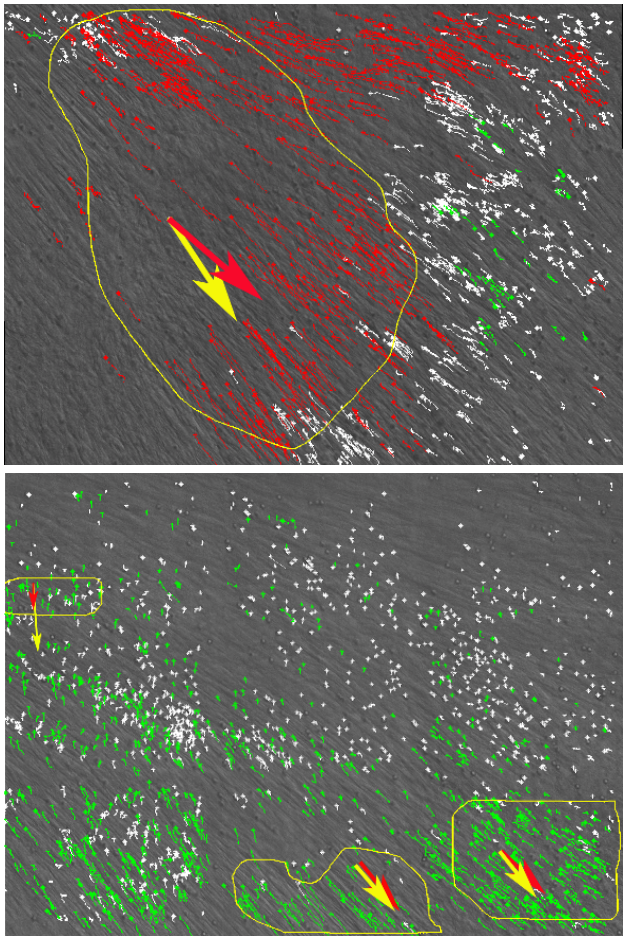

Fig. 4. Annotated regions (yellow boundaries), motion vectors (yellow arrows) and estimated motion directions (red arrows) are displayed on the selected regions for sequence 2 (top) and sequence 4 (bottom).

[2] Joseph B, Darro F, Behard A, Lesur B, Collignon F, Decaestecker C, Frydman A, Guillaumet G, and Kiss R, *3-aryl-2-quinolone derivatives: synthesis and characterization of in vitro and in vivo antitumor effects with emphasis on a new therapeutical target connected with cell migration*, J Med Chem **45** (2002), no. 12, 2543–2555.

[3] Ali Bashashati and Ryan R. Brinkman, *A survey of flow cytometry data analysis methods*, Adv. Bioinformatics (2009).

[4] Jean-Yves Bouguet, *Pyramidal implementation of the lucas-kanade feature tracker description of the algorithm*, 2000.

[5] Concita Cantarella, Leandra Sepe, Francesca Fioretti, Maria Carla Ferrari, and Giovanni Paolella, *Analysis and modelling of motility of cell populations with motocell.*, BMC Bioinformatics (2009), –1–1.

[6] Kang Li, Mei Chen, and Takeo Kanade, *Cell population tracking and lineage construction with spatiotemporal context*, Proceedings of the 10th International Conference on Medical Image Computing and Computer-Assisted Intervention (MICCAI), 2007, pp. 295 – 302.

[7] Dipti Prasad Mukherjee, Nilanjan Ray, and Scott T. Acton, *Level set analysis for leukocyte detection and tracking.*, IEEE Transactions on Image Processing (2004), 562–572.

[8] Richard Souvenir, Jerrod P. Kraftchick, Sangho Lee, Mark G. Clemens, and Min C. Shin, *Cell motion analysis without explicit tracking*, Computer Vision and Pattern Recognition, 2008.

[9] Shandong Wu and Brian E Moore, *Chaotic Invariants of Lagrangian Particle Trajectories for Anomaly Detection in Crowded Scenes*, International Conference on Computer Vision and Pattern Recognition(CVPR) (2010).

[10] Takeo Kanade Yaser Ajmal Sheikh, Erum Arif Khan, *Mode-seeking via medoidshifts*, (2007).

[11] Alper Yilmaz, Omar Javed, and Mubarak Shah, *Object tracking: A survey*, ACM Comput. Surv. **38** (2006).

[12] Christophe Zimmer, Elisabeth Labruyere, Vannary Meas-Yedid, Nancy Guillen, and Jean-Christophe Olivo-Marin, *Segmentation and tracking of migrating cells in videomicroscopy with parametric active contours: A tool for cell-based drug testing*, IEEE Trans. Med. Imaging **21** (2002), no. 10, 1212–1221.