CELL SEGMENTATION AND TRACKING USING TEXTURE-ADAPTIVE SNAKES

Xiaoxu Wang, Weijun He, Dimitris Metaxas

Department of Computer Science,
Rutgers University, 110 Frelinghuysen Rd,
Piscataway, NJ 08854, USA

Robin Mathew, Eileen White

CABM, Rutgers University,
679 Hoes Lane, Piscataway,
NJ, 08854,USA

ABSTRACT

Identifying cell trajectories is an important step in analyzing physiological events in computerized Video Time-lapse Microcopy. The large variety and transformation of cell shapes and cells' Brownian motion make cell tracking a challenge problem. In this paper we present a cell tracking system, implemented as a particle filter within texture-adaptive active contour formulations. The texture-adaptive weights on the external energy of the active contour model enables snakes to bypass internal psuedo-edges and stop on low-contrast cell boundaries. Using the texture of cells as observation model, we can track cells whose locations follow a multimodal distribution with a particle filter. This system is a novel combination of tracking algorithms and deformable models, and allows for the first time to automatically track non-fluorescence cellular microscopy images. The implemented tracker is tested on both normal and autophagy cell image sequences, to demonstrate the properties of cells in autophagy.

Index Terms— biomedical microscopy, image segmentation, tracking, image texture analysis

1. INTRODUCTION

Time-lapse microscopy images provide critical insight into the fundamental nature of cellular mechanisms for biologists. In this paper, we analyze mobility, proliferation rates and morphology of cells in autophagy, which plays an important role in tumor suppression. Autophagy, also called Self-cannibalism, is a process in which organelles and proteins inside of cells are delivered to lysosomes for proteolysis when cells are deprived of nutrition. During autophagy, cells exhibit different appearances and behaviors causing large interest in biologists [1]. We analyze microscopy videos of cells in autophagy and compare them to normal cells.

High throughput of time-lapse microscopy images call for automatic computer vision tools to assist the quantitative analysis. Most of previous cellular microscopy image analysis are carried on synthetic images or fluorescence images. To make fluorescence images, certain fluorescent proteins are introduced into cells by genetical modification. This procedure is tedious, time-consuming and may interfere with the original proteins under study. Microscopy images without fluorescence are difficult to segment by either edge detection or intensity thresholds due to weak boundary edges and similar intensities between inside areas and outside areas of cell membranes. Most segmentation methods seeking the strongest edges will stop at the boundary of nucleoli or other pseudo-edges inside of cells. Therefore, we need to introduce texture adaptive methods for cell segmentation. Compared to the traditional segmentation methods only using edge information, texture adaptive methods perform better in segmenting those objects with high-contrast inside area and gently dipping boundary. Metamorphs [2] use non-parametric measurements on intensity histograms, which have been coupled with Gabor filters and Random Markov Fields to utilize the spatial information of texture [3]. A SVM classifier is used to separate cells from background based on texture features in [4]. In this paper we use an active contour model which incorporates texture information around the cell membranes and the area constraints to achieve accurate segmentation of cells. The segmentation of individual cells can be used for the observation model of the particle filter in cell tracking.

Geodesic active contours, also called level set methods, are widely used in cell segmentation and tracking because of its topology flexibility [5]. By introducing multiple level set functions ϕ for different cells, the coupled level set methods [6] solve the contour overlapping problem and also increase the complexity. An alternative is to adopt the coupled parametric active contours introduced in [7] which can handle transiently touching objects. The topology change caused by cell division are handled by mitosis detection and snake reinitialization.

Algorithms like Kalman filters and particle filters have been used in cell tracking [8, 5] to utilize the temporal coherency of motion and deformation. [9] present a particle filtering algorithm in geometric active contour framework for tracking moving and deforming objects. Particle filters can deal with the multimodality in the cell location distribution, when it is hard to derive a close-form analysis like calculating the Kalman gain matrix. Particle filters can also outperform other trackers for the cases of nonlinear system dynam-

ics. Here, we use a particle filter incorporate with active contour models to track the movement and deformation of cells.

Section 2 introduces our method for cell segmentation and cell tracking, and presents image processing results. Section 3 describes further results and how the image processing results are used in biology analysis. Section 4 give conclusion and one future improvement of our method.

2. METHODS

2.1. Segmentation using Coupled Snakes with Texture Constraints

The segmentation module in a cell tracking system is crucial since the result serves as the basis for the subsequent analysis. Active contour models that initialize outside of a cell may yield good segmentation results when the background of a microscopic cell image is roughly uniform. It is hard to separate a cell from its nearby neighbors if initializing from the outside because active contour for the cell can be easily attracted to the boundaries of other nearby objects. And dense cell clusters are not uncommon in many cell images. Even with the repulsive forces in [10], cells attached together are still hard to be segmented because boundaries shared by two cells produce both attractive and repulsive forces. Cell nucleus detection can be used for automatic initialization of active contour models inside the cells. The difficulty of initializing active contours from inside lies on the numerous pseudo-edges coming from high-contrast proteins and the weak boundaries between cells. In this paper we develop a novel modification of active contour models that can grow across strong edges inside and stop at the weak boundaries based on a statistical model of cell boundary texture.

Coupled active contours introduced in [7] keep track of multiple cells at the same time, and put penalties on overlapped areas to prevent different contours from crossing each other. Because the texture inside of cells are not homogenous, cell texture and background texture cannot be directly used as external energy as in [2, 7]. Texture likelihoods are used as weights on image energy, the derivatives of intensities. It emphasizes the edge forces around cell membranes and suppresses edge forces in other areas, enabling snakes to bypass internal pseudo-edges and stop on cell boundaries. The energy function of snakes is written as

$$E(C_1,...,C_N) = \sum_{i=1}^{N}(\int_0^1 (E_{int}(C_i(p) + \kappa L_t E_{img}(C_i(p))) + \lambda(1-L_s)E_{ball}(C_i(p)))dp) + \gamma \sum_{i=1}^{N}\sum_{j=1}^{N} Area_{inside(C_i)\cap inside(C_j)} \quad (1)$$

This energy function contains four parts, the internal energy keeping the contour smooth, the external energy coming from the image gradient, the balloon force expanding the contour and the constraint energy preventing different contours from overlapping. We add weights on the external energy and the balloon force to control their influences. L_t is the log likelihood of the area to be around the cell boundaries. The external energy can also be calculated by canny filter and distance map. L_t makes the snake to be affected by the image information most when the contour is around the boundary. L_s is the probability of the object size to be the average cell size. The balloon force decreases when the object size gets closer to the average cell size. Assuming the sizes of cells follow a Gaussian distribution, L_s can be easily calculated by fitting the current cell area to the Gaussian model. The size constraint also helps to keep the active contours growing until they reach the cell membranes. κ and λ are two constant parameters similar to α, β and γ. Different forces are balanced at the cell boundaries by these parameters and make active contours to stop there.

The evolution equation associated with snake i is as the following,

$$\frac{\partial C_i}{\partial t} = \alpha\frac{\partial^2 C_i}{\partial p^2} - \beta\frac{\partial^4 C_i}{\partial p^4} + (\lambda L_{size} - \gamma \vee_{j\neq i} \epsilon_j(C_i))n + \kappa L_t(x,y)|\bigtriangledown[G_\sigma(x,y) * I(x,y)]|^2 \quad (2)$$

where n is the outward normal to contour C_i and $\epsilon_j(C_i)$ is the indicator function on whether point $C_i(p)$ is inside of C_j. In our implementation, if point $C_i(p)$ in any of other contours, they retreat along inward normal direction for one step.

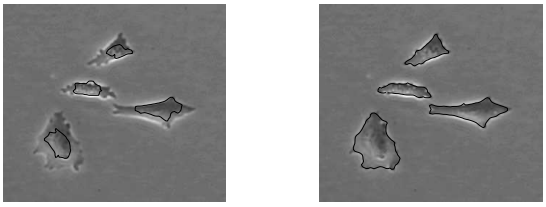

Fig. 1. Comparison between traditional snakes and texture-adaptive snakes: (a) Traditional snakes result, (b) Texture-adaptive snakes result

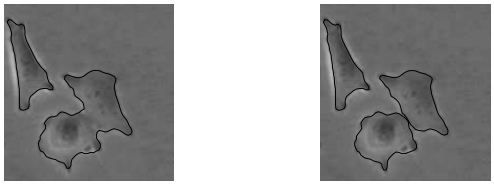

Fig. 2. Comparison between uncoupled snakes and coupled snakes: (a)Uncoupled (b)Coupled

Boundary areas inside of cells and boundary areas outside of cells have different characteristics, and they are taken as two different training sets. Due to illumination and the cell living environment, cell boundaries show different kinds of edges at different angles, edges with high contrast, edges with low contrast and edges between cells. We cluster a training set into several groups with the mixture of Gaussians model using the EM algorithm, and estimate the boundary texture likelihood in snakes by the model. The histogram of gradient magnitudes can be used to distinguish smooth areas separated by a edge and nuclei areas with dotted proteins. Intensity and gradient magnitudes are used as two features of the mixture of Gaussians model. Figure 2 shows that coupled snakes can segment two cells adhering together, whose two nuclei can be clearly observed.

2.2. Tracking with Particle Filters

Tracking problems can be taken as the computation of a posteriori distribution $P(X_t|Z_t)$ in a Hidden Markov Model involving a sequence of states X_t and observations Z_t.

$$P(X_t|Z_t) = P(z_t|x_t) * \int P(x_t|x_{t-1})P(x_{t-1}|Z_{t-1})dx_{t-1} \tag{3}$$

.

Without the Gaussian assumption on location distibution, the particle filter [9, 11] uses sampling importance resampling methods, approximating the hidden distribution $p(x_k|y_0, ..., y_k)$ by a weighted set of particles $(w_k^L, x_k^L) : L = 1, ..., P)$, representing P possible locations each frame. Importance weights w_k^L are approximations to the relative posterior probabilities.

The conditional density propagation for visual tracking (Condensation) algorithm [12, 13] gives out all implementation details of a particle filter. The condensation algorithm iterates three steps, a sample, a predict and a measure at each image frame. The tracker is initialized with a set of particles. Each step we predict the location of a particle at the next step by a linear stochastic differential equation and evaluate the result with observation after a stochastic diffusion. After the distribution was built from evaluation, a sample set of new particles are drawn for tracking the location of cells in the next frame.

The observation model in our methods is the similarity of features between the previous contour and the current contour. Here, we use intensity histogram and gradient magnitude histogram as our texture features of contours again. The conditional probability of the observation z_t given state x_t can be evaluated by the following equation. L denotes for the Lth particle.

$$p(z_t|x_t = s_t^L) \propto \sum_{I,|\nabla I|} \frac{1}{1 + exp(d_{EMD})} \tag{4}$$

where d_{EMD} is the Earth Mover's Distance. The EMD between two density distributions is the L^1 distance between their cumulative probability functions(CDF) [4].

In our particle filter, particles are 2D locations of cells in microscopy images. Because the shape of cells can be segmented by active contours, we did not use particle filters to track the cell contour deformation. To get the observation model, the active contour deforms for a small number of iterations, for instance 4. At this step, we did not run the active contour until it converge. If we run it till converge, the results will only depend on the image information and lose the location information provided by predicted particles. After the particle filter predictions are evaluated by observation models, we deform the state $\overline{\pi_i}$ using active contours till convergence.

Tracking results are shown in the following figure,

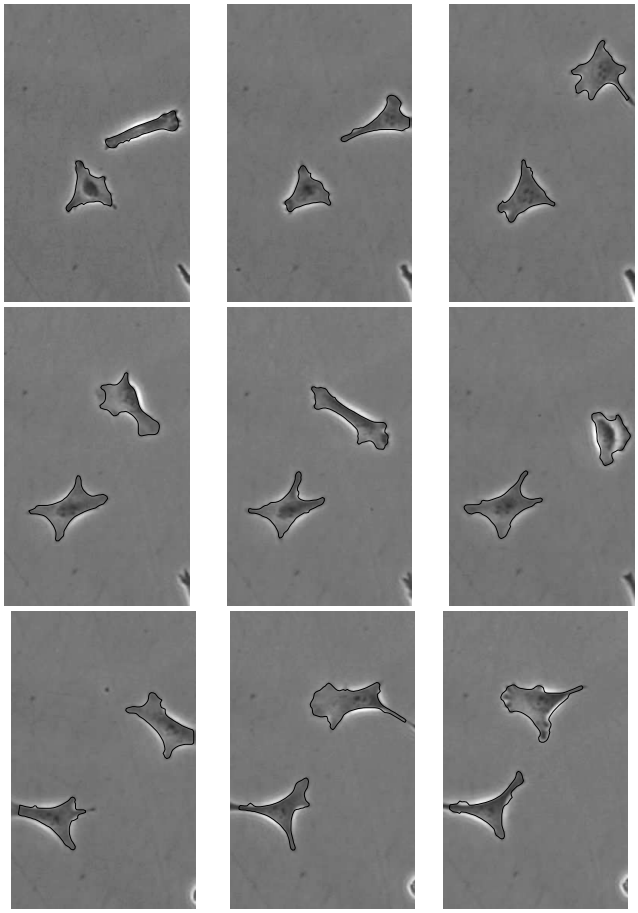

Fig. 3. Tracking results

For the microscopy image sequences with 5 minutes interval, cell motion and deformation between two consecutive frames is very small. Therefore, the tracking results are presented each 8 frames, which have time interval 40 minutes.

3. RESULTS

In the experiments, we compared the motility between a normal cell microscopy image sequence and a sequence for cells in autophagy. Autophagy competent cells were cultured in normal or metabolic stress conditions in the time-lapse chamber equipped with controlled environmental conditions. The time-lapse microscopy system consisted of an Olympus IX71 inverted microscope fitted with temperature, humidity and CO2 controlled environmental chamber (Solent Scientific, UK) and a Coolsnap ES cooled CCD camera. Phase contrast images (10X) at multiple fields were obtained every 10 minutes for 5days.

The average cell moving speeds are calculated every half day for each sequence. From Figure 4, we can observe that cells show higher mobility when they are provided enough nutrition. When the cells are deprived of nutrition, their motilities decrease gradually.

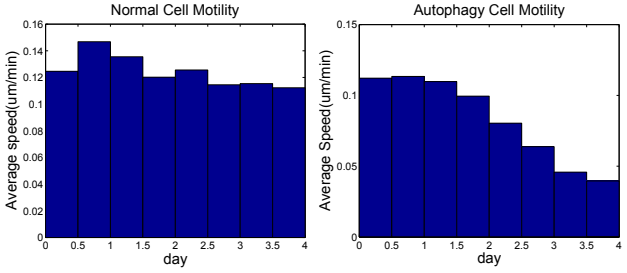

Fig. 4. Motility comparison between normal cells and autophagy cells

4. CONCLUSION AND FUTURE WORK

The cell tracking system we implemented fully exploits temporal information in cell motion, and yields robust cell trajectories with high time efficiency. The low-contrast boundaries showed by cell membranes can be accurately segmented in cell clusters with texture-adaptive active contours. The tracking results provide useful information on the research of autophagy cell mechanism. The cell tracking system also provides a basis for future microscopy image processing dedicated to cancer cell research.

We can further improve our tracking system by using alternative methods like Markov chain Monte Carlo (MCMC) to solve the mixture of Gaussians model, which used as the texture representation. The MCMC method solves this problem by building a bayesian network, in which the weights of different Gaussians are drawn from a binomial distribution.

5. REFERENCES

[1] K. Degenhardt, R. Mathew, B. Beaudoin, K. Bray, D. Anderson, G. Chen nd C. Mukherjee, Y. Shi, C. Gelinas, Y. Fan, D. A. Nelson, S. Jin, and E. White, "Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis," *Cancer Cell*, vol. 1, pp. 51–64, 2006.

[2] X. Huang, D. N. Metaxas, and T. Chen, "Metamorphs: Deformable shape and texture models," *Computer Vison and Pattern Recognition*, , no. 1, pp. 496–503, 2004.

[3] X. Huang, R. Huang Z. Qian, and D. N. Metaxas, "Deformable-model based textured object segmentation," *Energy Minimization Methods in Computer Vision and Pattern Recognition*, pp. 119–135, 2005.

[4] W.He, X.Wang, D.Metaxas, R.Mathew, and E.White, "Cell segmentation for division rate estimation in computerizedvideotime-lapse miscroscopy," *First MICCAI Workshop on Microscopic Image Analysis with Applications in Biology*, 2006.

[5] F.Yang, M. A. Mackey, F. Ianzini, G. Gallardo, and M. Sonka, "Cell segmentation, tracking, and mitosis detection using temporal context," *Medical Image Computing and Computer-Assisted Intervention*, pp. 302–309, 2005.

[6] S. K. Nath, K. Palaniappan, and F. Bunyak, "Cell segmentation using coupled level sets and graph-vertex coloring.," , no. 1, pp. 101–108, 2006.

[7] C. Zimmer and J.-C. Olivo-Marin, "Coupled parametric active contours," *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 27, no. 11, pp. 1838–1842, 2005.

[8] B. Olivo-Marin J.-C. Genovesio, A. Zhang, "Tracking of multiple fluorescent biological objects in three dimensional video microscopy," *International Conference onImage Processing*, vol. 1, pp. 14–17, 2003.

[9] Y. Rathi, N. Vaswani, A. Tannenbaum, and Yezzi A, "Particle filtering for geometric active contours with application to tracking moving and deforming objects," *Computer Vision and Pattern Recognition*, , no. 2, pp. 2–9, 2005.

[10] H. Cai, X. Xu, J. Lu, J. Lichtman, and S. P. Yung, "Shape-constrained repulsive snake method to segment and track neurons in 3d microscopy images," *International Symposium on Biomedical Imaging*, 2006.

[11] I. Smal, W. Niessen, and E. Meijering, "Bayesian tracking for fluorescence microscopic imaging," *International Symposium on Biomedical Imaging*, 2006.

[12] M. Isard and A. Blake, "Condensation – conditional density propagation for visual tracking," *International Journal of Computer Vision*, vol. 29, no. 1, pp. 5–28, 1998.

[13] J. H. Kotecha and P. M. Djurić, "Gaussian particle filtering," *IEEE SPM*, vol. 20, pp. 19–38, 2003.