УДК 519.21

Коцур Д.В., аспірант

Терещенко В.М., д.ф-м.н., професор

Застосування моделей активних контурів у задачах трекінгу структур цитоскелету

Київський національний університет імені Тараса Шевченка, 03680, м. Київ, пр-т. Академіка Глушкова 4д,
e-mail: dkotsur@gmail.com, v_ter@ukr.net

D.V. Kotsur, postgraduate

V.M. Tereschenko, Dr.Sci.(Phis.-Math.), Assoc. Prof.

Active contour models for cytoskeletal structures tracking

Taras Shevchenko National University of Kyiv, 03680, Kyiv, Glushkova st., 4d,

e-mail: dkotsur@gmail.com, v_ter@ukr.net

Стаття присвячена дослідженню моделей активних контурів в поєднанні з методом оптичного потоку, і їх застосування в галузі клітинної біології для відстеження окремих компонентів цитоскелета в послідовностях конфокальних зображень. В статті модель активного контуру визначається як двовимірна параметрична крива, що є розв'язком (мінімумом) в задачі мінімізації заданого "енергетичного" функціоналу. Також продемонстровано як інформація з послідовності зображень може бути включена в задачу мінімізації через градієнт зображення, який згодом замінюється на більш стійке векторне поле (градієнтний потік). Зайві деталі зображення відфільтровуються за допомогою запропонованих алгоритмів попередньої обробки в наступній послідовності: згладжування за допомогою анізотропної дифузії, підсилення ребер за допомогою гессіану, адаптивне покращення контрастності зображення з масштабуванням, застосування перетворення з подвійним пороговим значенням, бінаризація. Наводиться повний опис алгоритму трекінгу на основі активних контурів та оптичного потоку, окреслюються сфери його застосування. Результат роботи алгоритму демонструється на прикладі трекінгу окремих мікротрубочок в межах їхньої мережі.

Ключові слова: моделі активних контурів, оптичний потік, градієнтний потік, трекінг

In this paper we investigate active contour models coupled with optical flow method and their application in the field of cell biology for the tracking of individual cytoskeletal components on the time-sequences of confocal images. Firstly, we define active contour model as a parametric curve, which minimizes given "energy" functional. We show how information about the image sequence can be incorporated into the minimization problem by introducing image-based gradient of "external energy", which we replace further by more robust vector field called gradient vector flow. Next, we introduce image pre-processing pipeline, which allows to filter out redundant image features. It consists of the following processing steps: anisotropic diffusion filtering, Hessian ridge enhancement, adaptive contrast enhancement with image scaling, two-level thresholding, binarization. Then, we describe overall tracking procedure. Finally, we outline the applications of algorithm and illustrate an example of the algorithm output for individual microtubules tracking within their network.

Key Words: active contour models, optical flow, gradient vector flow, tracking

1. Introduction

Active contour models or snakes were introduced by Kass et al in the paper [1]. They became a very popular image processing tool and were applied in many diverse fields from industry to the research. The main area of application for active contour models is segmentation of objects defined by their contours on the single image [2], [3]. But this models can be also successfully applied for object tracking. As was shown in paper [4] active contour models can be successfully applied to track individual nonintersecting actin filaments. In [5] the algorithm is even extended to 3D space. The generalization of the snakes for cytoskeleton segmentation and tracking is presented in [6].

In this paper we extend approach [6] and apply active contour models coupled with the optical flow approach in the field of cell biology for tracking of individual cytoskeletal components such as microtubules, actin or keratin cytoskeleton on the time-sequences of confocal images. The significance of this field is determined by the huge role of the cell cytoskeleton in vital processes such as cell migration, wound healing or tumour metastasis formation.

2. Active contour models

Active contour model is defined as a parametric curve $\mathbf{x}(s) = [x(s), y(s)]$, $s \in [0,1]$. According to Kass et al [1], the position of the contour within one frame in time-sequences is obtained by minimizing "energy" functional:

$$E = \int_0^1 \frac{1}{2} (\alpha |\mathbf{x}'(s)|^2 + \beta |\mathbf{x}''(s)|^2) + E_{ext}(\mathbf{x}(s)) \, ds$$

where α, β are elasticity parameters that controls stretching and bending resistance of the curve correspondingly. This variational problem is solved by reducing to differential equation and applying iterative scheme with artificial time variable t:

$$\mathbf{x}_t(s,t) = \alpha \frac{\partial^2}{\partial \mathrm{x}^2} \mathbf{x}(s,t) + \beta \frac{\partial^4}{\partial \mathrm{x}^4} \mathbf{x}(s,t) - \nabla E_{ext}$$

The impact of "external energy" term E_{ext} or the gradient of "external energy" ∇E_{ext} is crucial in this problem. Normally, it is defined as a function of the input image, which allows to incorporate useful features of the images into the problem statement. For example, in order to obtain convergence towards the edges of the image, "external energy" defined as $E_{ext} = -|\nabla f(x,y)|^2$, where $f(x,y)$ is intensity value of the image. In case if we prefer convergence towards the ridges, we can use the following definition: $E_{ext} = f(x,y)$.

3. Gradient vector flow

In Xu et al. [7] authors propose to replace the gradient of the "external energy" ∇E_{ext} with the vector field $\mathbf{v}(x,y) = [u(x,y), v(x,y)]$, which minimizes functional:

$$\mathcal{E} = \iint \mu(u_x^2 + u_y^2 + v_x^2 + v_y^2) + |\nabla f|^2 |\mathbf{v} - \nabla f|^2 dx dy,$$

where $f(x,y)$ is the intensity of the pixel at the position (x,y), $|\cdot|$ is the Euclidean norm and μ is the regularization (smoothness) parameter. The vector field $\mathbf{v}(x,y)$ is called gradient vector flow (GVF). Here larger values for the regularization parameter leads to smoother resulting vector field.

Comparing to the classical examples of "external energy", the gradient vector flow behaves like more smoothed version of the gradient of input image. It is shown in [7] that it performs better than "classical external energies" in case of concavities and is more stable numerically.

Taking into account gradient vector flow term the evolution of the snake on the single frame can be defined as follows:

$$\mathbf{x}_t(s,t) = \alpha \frac{\partial^2}{\partial \mathrm{x}^2} \mathbf{x}(s,t) + \beta \frac{\partial^4}{\partial \mathrm{x}^4} \mathbf{x}(s,t) - \mathbf{v}(\mathbf{x}(s,t))$$

4. Image preprocessing workflow

Usually input images are corrupted by noise and contain a lot of redundant information (features), which can hinder active contours from the convergence towards desired edges or ridges. This means, that input images require additional preprocessing.

For the tracking problem of cytoskeletal structures, we propose the following image preprocessing pipeline (see also Figure. 1):

1) The input image (see Fig. 1, A) is given as an input for anisotropic diffusion filter.
2) The result of the 1st step (Fig. 1, B) is given as an input for Hessian ridge detector.
3) We apply histogram-based contrast enhancement to the result of the 2nd step:
   - Calculate histogram of the input and choose the most frequent intensity value (denote it by T).
   - Scale intensities of the images using the formula: $I_{new} = (I_{old} - T) * S$, where S is constant (contrast stretching term), I_{new}, I_{old} − new and old intensity values respectively.
4) Then we apply binary threshold to the result of 2nd step (see Fig. 1, C):

$$I_{new} = \begin{cases} I_{old}, & \text{if } T_{min} \leq I_{old} \leq T_{max} \\ T_{min}, & \text{if } I_{old} < T_{min} \\ T_{max}, & \text{if } I_{old} > T_{max} \end{cases}$$

Вісник Київського національного університету
імені Тараса Шевченка
Серія фізико-математичні науки

2016, 4

*Bulletin of Taras Shevchenko
National University of Kyiv
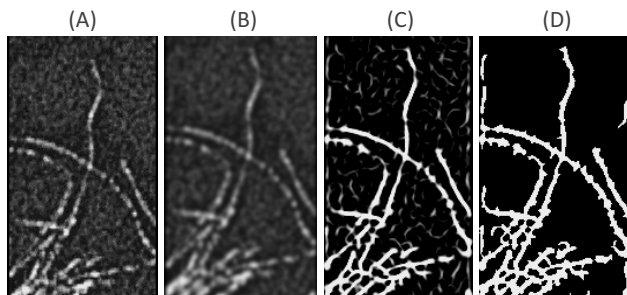Series Physics & Mathematics*

Figure 1. Image preprocessing workflow: (A) – original image, (B) – result of anisotropic diffusion, (C) – Hessian ridge detection, (D) – after applying adaptive binary threshold and filtering small components.

where T_{min}, T_{max} are minimum and maximum constant intensity thresholds.

5) Finally, we perform morphological filtering of the components smaller than the given size (see Fig. 1, D).

The result of the step (4) replaces the input image for gradient vector flow. The binary mask (5) will be used later for contour stretching term.

5. Overall tracking algorithm

The tracking of individual contours consists of two main routines: refinement of the position of the contour on the current frame using the procedure described in chapters (2-4) and transition of the contour from current to the next frame of sequence. For the second step, we apply pyramidal Lucas-Kanade optical flow algorithm [8]. It allows to get better fit in case of large deformations of the contour. However, the main assumption of the optical flow algorithm – unchanged image intensity values in the neighborhood of the tracking point, might be violated. This in turn leads to the wrong mapping of the points between the frames. We propose to overcome this issue by repetition of the refinement step using active contours.
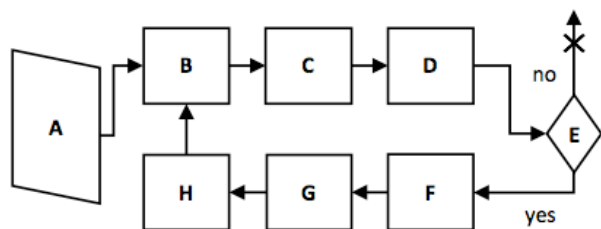

Figure 2. Diagram of overall tracking procedure

Thus, we propose the following tracking procedure (see Figure 2):

A) *Initialization.* We require initialization of the contour on the first analyzed frame. This can be done, for example, manually by user or by additional (semi-)automatic segmentation procedures.

A) *Image preprocessing.* Apply image preprocessing pipeline as described in chapter 4.

B) *Calculate gradient vector flow (GVF)* on the preprocessed image (see chapter 3)

C) *Optimize position of the snake* on the current image based on the obtained GVF and taking into account stretching term for open ends [4] calculated on the binary mask (see step (5) in chapter 4);

D) If current image is last in the analyzed sequence, exit the procedure. Otherwise go to the next step;

E) Calculate pyramidal optical flow from current image to the next image in time-sequence as described in [8]

F) Transfer snake to the next image in the sequence based on the calculated optical flow algorithm.

G) Select next image and repeat starting from step (B);

6. Experimental results and analysis

There are many application of parametric active contours in the field of image processing in particular for cell biology, e.g. cell nucleus and membrane segmentation, cytoskeletal components segmentation [3].

Here we apply active contour models for tracking the dynamics of individual cellular biopolymers, in particular microtubules, which are important structures of the cell cytoskeleton responsible for many vital processes (cell division, molecular transport). The result of the tracking algorithm is shown on the Figure 3. Individual microtubules were tracked on the time-sequence of 91 frame. Here we applied image pre-processing pipeline in the way as defined in chapter (4).

We evaluated tracking algorithm with the proposed image processing pipeline and compared to the approach defined in [6]. The microtubule data for evaluation was taken from free-access Cell Image Library. The dataset contains 5 image sequences, 329 frames and 20 labelled microtubules. Tracked and labelled contours were compared by means of discrete Frechet distance. Correctly tracked data was classified by empirical distance threshold chosen from observations: Th=5.0. The result is the following:

- Our approach (with preprocessing): *82.1%* correct frames.
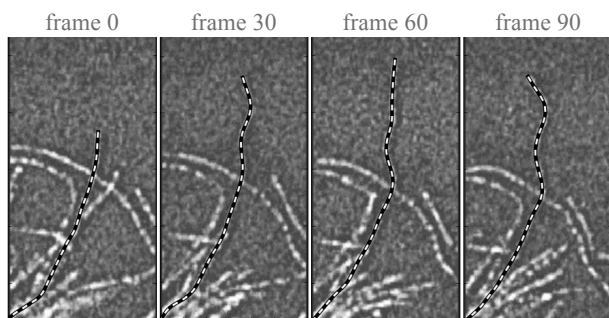- Approach in [6]: *73.6%* correct frames.

Вісник Київського національного університету   **2016, 4**   *Bulletin of Taras Shevchenko*
імені Тараса Шевченка   *National University of Kyiv*
Серія фізико-математичні науки   *Series Physics & Mathematics*

frame 0   frame 30   frame 60   frame 90

Figure 3. Tracking individual microtubule
dynamics (dashed line) for 91 frames.

7. Conclusions and outlooks

In this work we presented tracking algorithm based on parametric active contour models coupled with pyramidal optical flow and applied it for the problem of individual cytoskeletal components tracking on the time-sequences of confocal images. We introduced image preprocessing pipeline for filtering redundant image features, which improves the stability of active contour models. The algorithm output was demonstrated for individual microtubules tracking within their network.

Список використаних джерел

1. Kass M. Snakes: Active contour models / M. Kass, A. Witkin, D. Terzopoulos. // International Journal of Computer Vision. – 1988. – №4. – P. 321–331.
2. Zhang R. A Novel Gradient Vector Flow Snake Model Based on Convex Function for Infrared Image Segmentation / R. Zhang, S. Zhu, Q. Zhou. // Sensors. – 2016. – №10. – P. 1756–1773.
3. Connecting Markov random fields and active contour models: application to gland segmentation and classification / J.Xu, J. P. Monaco, R. Sparks, A. Madabhushi. // Journal of Medical Imaging. – 2017. – №2. – P. 132–143.
4. Automated actin filament segmentation, tracking and tip elongation measurements based on open active contour models / [H. Li, S. Tian, M. B. Smith et al.]. // Proceedings of the IEEE International Symposium on Biomedical Imaging: From Nano to Macro. – 2009. – P. 1302–1305.
5. Xu T. 3D actin network centerline extraction with multiple active contours / T. Xu, D. Vavylonis, X. Huang. // Medical Image Analysis. – 2014. – №2. – P. 272–284.
6. Smith M. B. Segmentation and tracking of cytoskeletal filaments using open active contours / M. B. Smith, H. Li, T. Shen. // Cytoskeleton. – 2010. – №11. – P. 693–705.
7. Xu C. Gradient Vector Flow: A New External Force for Snakes / C. Xu, J. L. Prince. // Proceedings of the IEEE Computer Society Conference on Computer Vision and Pattern Recognition. – 1997. – P. 66–71.
8. Sharmin N. Optimal Filter Estimation for Lucas-Kanade Optical Flow / N. Sharmin, R. Brad. // Sensors. – 2012. – №12. – P. 12694–12709.

References

1. KASS, M., WITKIN, A. and TERZOPOULOS, D. (1988). Snakes: Active contour models. International Journal of Computer Vision, 1(4), p. 321-331.
2. ZHANG, R., ZHU, S. and ZHOU, Q. (2016). A Novel Gradient Vector Flow Snake Model Based on Convex Function for Infrared Image Segmentation. Sensors, 16(10), p. 1756–1773.
3. XU, J., MONACO, J., SPARKS, R. and MADABHUSHI, A. (2017). Connecting Markov random fields and active contour models: application to gland segmentation and classification. Journal of Medical Imaging, 4(2), p. 132-143.
4. LI, H., SHEN, T., SMITH, M., FUJIWARA, I., VAVYLONIS, D. and HUANG, X. (2009). Automated actin filament segmentation, tracking and tip elongation measurements based on open active contour models. IEEE International Symposium on Biomedical Imaging: From Nano to Macro, p.1302–1305.
5. XU, T., VAVYLONIS, D. and HUANG, X. (2014). 3D actin network centerline extraction with multiple active contours. Medical Image Analysis, 18(2), p.272-284.
6. SMITH, M., LI, H., SHEN, T., HUANG, X., YUSUF, E. and VAVYLONIS, D. (2010). Segmentation and tracking of cytoskeletal filaments using open active contours. Cytoskeleton, 67(11), p. 693-705.
7. XU, C. and PRINCE, J. (1997). Gradient vector flow: a new external force for snakes. Proceedings of IEEE Computer Society Conference on Computer Vision and Pattern Recognition, p. 66-71.
8. SHARMIN, N. and BRAD, R. (2012). Optimal Filter Estimation for Lucas-Kanade Optical Flow. Sensors, 12(12), p. 12694-12709.