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Active contour models for individual keratin filament tracking

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***Abstract*— As a major component of the cytoskeleton, keratin filaments form a branched network, which plays a significant role in the mechanical response, motion and dynamics of the cell. They undergo a complex dynamic lifecycle, which we aim to investigate by tracking individual filaments. In this paper we introduce an active contour-based tracking algorithm to analyze the motion of individual keratin filaments in sequences of confocal images. The algorithm combines parametric active contours (snakes) with Lukas-Kanade's algorithm for optical flow calculation. We define an image preprocessing workflow to compute robustly the external energy of the snake and we impose an additional structural constraint for controlling the length of the contour.**

I. INTRODUCTION

The cytoskeleton plays a main role in cellular motility and dynamics, which in turn is of high relevance for vital and also for pathological processes, such as wound healing and tumor metastasis [5]. As a major component of the cytoskeleton, keratin filaments form a branched network and are essential for the mechanical response to external forces. Biophysical investigation and analysis of different types of keratin filaments requires their localization and the extraction of their motion in the time-sequences of consecutive confocal images. As it was shown previously [7], [3], [4], this problem can be successfully approached for separated individual actin filaments. However, applying this approach to tracking of keratin filaments within a branched network may lead to additional complications and errors, as for example, uncontrolled growth of the snake. In this paper we introduce a tracking algorithm based on stretching open active contours [3] to analyze the global motion features of individual keratin filaments within their network. We define an image preprocessing workflow to calculate robustly the "external energy" of the snake and impose an additional structural constraint for controlling the length of the contour.

II. TRACKING ALGORITHM

In this section, we first define our active contour model as a minimization problem. Then, we introduce an "external energy" based on the image and impose a contour length constraint to control snake growth. Finally, we combine all steps together and present an overall tracking procedure.

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A. Parametric snakes: active contour models

We define a filament as a parametric curve $\mathbf{x}(s) = [x(s), y(s)], s \in [0,1]$. According to [2], the position of the filament within a frame in a time-sequence is obtained by minimizing the following so-called "energy" functional:

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

where α and β are parameters which control the stretching and bending resistance of the curve, correspondingly. This problem is solved by reducing (1) to a differential equation and applying an iterative scheme with an artificial time variable t:

$$\mathbf{x}_t(s,t) = \alpha\mathbf{x}_{ss}(s,t) + \beta\mathbf{x}_{ssss}(s,t) - \nabla E_{ext}(\mathbf{x}(s,t)) \quad (2)$$

The impact of the "external energy" E_{ext} or the gradient of "external energy" ∇E_{ext} is crucial in this problem, because the convergence of a snake considerably depends on this term.

B. External energy and structural constraints

In Xu et al. [6] the gradient of the "external energy" ∇E_{ext} is replaced by the vector field $\mathbf{v}(x,y) = [u(x,y), v(x,y)]$, which minimizes the functional:

$$\mathscr{E} = \int\int \mu\left(u_x^2 + u_y^2 + v_x^2 + v_y^2\right) + |\nabla f|^2|\mathbf{v} - \nabla f|^2 dxdy \quad (3)$$

where $f(x,y)$ is the intensity of the pixel at the position (x,y), $|\bullet|$ is the Euclidean norm and μ is the regularization (smoothness) parameter. The vector field $\mathbf{v}(x,y)$ is called gradient vector flow (GVF). In this case the evolution of the snake on a single frame is defined as follows:

$$\mathbf{x}_t(s,t) = \alpha\mathbf{x}_{ss}(s,t) + \beta\mathbf{x}_{ssss}(s,t) - \mathbf{v}(\mathbf{x}(s,t)) \quad (4)$$

It is shown in [6] that GVF has a larger capture range, compared to the vector field given by ∇E_{ext} defined in [2]. It also improves the snake convergence in case of high concavities. However, the intensity variation along a filament may be high, which leads to additional errors during snake convergence. Therefore, we preprocess images applying the following pipeline of filters: Gaussian smoothing; Hessian ridge enhancement; gamma contrast correction.

The drawback of the snake algorithm itself as defined in [2] is that the open-ended contour (Fig. 1C) tends to shrink over time (Fig. 1D). To overcome this, we use a stretching term for open ends as defined in [7]. However, it may lead to overgrowth of the contour (Fig. 1E). We it this by processing endpoints separately. We define an additional distance-based "energy" potential for the branching and end points of the

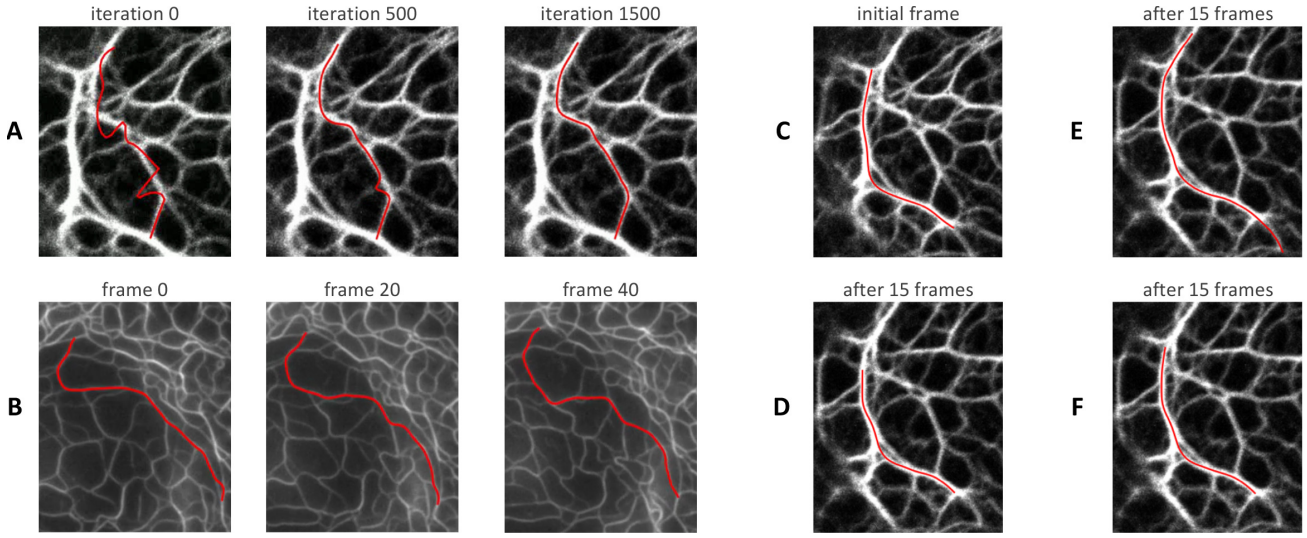

Fig. 1. Life cell imaging of SW13 cells expressing fluorescent HK8-CFP and HK18-YFP proteins (frames were recorded every 30 sec). (A) Snake evolution on a single frame; (B) Tracking result for an individual filament on a time-sequence of 40 frames; (C) Initial position of the snake on the first frame and (D-F) after 15 frames: (D) without stretching term and length constraint; (E) with stretching term only; (F) with stretching term and distance-based potential;

network and allow snake endpoints to be captured by the force field induced by the potential (Fig. 1F).

C. Overall tracking procedure

In our setting, the tracking of individual filaments consists of two main routines: refinement of the position of the filament on the current frame and transition of the filament from the current to the next frame in the sequence. For the second step, we apply pyramidal Lucas-Kanade optical flow computation [1]. It allows to obtain a reasonable fit in case of large deformations of the filament. Incorrect mappings obtained by the optical flow algorithm require the repetition of the refinement step using active contours. Thus, we propose the following tracking procedure (see Fig. 2):

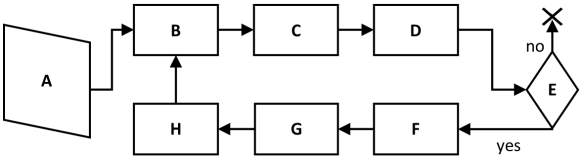

Fig. 2. Block-diagram of the overall tracking algorithm

(A) *Initialization*: The filament is initialized on the first analyzed frame. This can be done manually by user or additional (semi-)automatic segmentation procedures.
(B) *Image preprocessing*: Gaussian smoothing; Hessian ridge detector; gamma contrast correction.
(C) *Calculate the GVF* on the preprocessed image.
(D) *Optimize the position of the snake* on the current image based on the GVF obtained in (C) and take into account a stretching term for open ends [3] and potential for the endpoints.
(E) If the current image isn't the last one in the analyzed sequence, go to the next step. Otherwise, exit the procedure here.
(F) *Calculate the pyramidal optical flow* of the current image with respect to the next image in the time-sequence as described in [1].
(G) Transfer the snake to the next image in the sequence based on the calculated optical flow field.
(H) Select the next image and repeat starting from step (B).

A result obtained by this procedure is depicted in Fig 1. Fig. 1A shows the convergence of the snake on a single frame with an "external energy" as defined above. Fig. 1B shows a filament being tracked in an image sequence of 40 frames.

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REFERENCES

[1] J.-Y. Bouguet, "Pyramidal Implementation of the Lucas Kanade Feature Tracker: Description of the Algorithm," Intel Corporation Microprocessor Research Labs, Tech. Rep., 2000.
[2] M. Kass, A. Witkin, and D. Terzopoulos, "Snakes: Active contour models," *International Journal of Computer Vision*, vol. 1, no. 4, pp. 321–331, 1988.
[3] H. Li, T. Shen, D. Vavylonis, and X. Huang, "Actin filament tracking based on particle filters and stretching open active contour models," *Medical Image Computing and Computer-Assisted Intervention*, vol. 12, no. 2, pp. 673–681, 2009.
[4] H. Li, T. Shen, M. B. Smith, I. Fujiwara, D. Vavylonis, and X. Huang, "Automated actin filament segmentation, tracking and tip elongation measurements based on open active contour models," in *IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2009.
[5] D. M. Toivola, P. Boor, C. Alam, and P. Strnad, "Keratins in health and disease," *Current Opinion in Cell Biology*, vol. 32, pp. 73–81, 2015.
[6] C. Xu and J. L. Prince, "Gradient vector flow: A new external force for snakes," in *IEEE Conference on Computer Vision and Pattern Recognition*, 1997.
[7] T. Xu, H. Li, T. Shen, N. Ojkic, D. Vavylonis, and X. Huang, "Extraction and analysis of actin networks based on open active contour models," in *IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2011, pp. 1334–1340.