USING PARTICLE FILTER TO TRACK AND MODEL MICROTUBULE DYNAMICS

Koon Yin Kong[1], Adam I. Marcus[2], Paraskevi Giannakakou[4], and May D. Wang[1,2,3]

School of Electrical and Computer Engineering, Georgia Institute of Technology[1]
Winship Cancer Institute, Emory University[2]
Wallace H. Coulter Department of Biomedical Engineering,
Georgia Institute of Technology and Emory University[3]
Weill Cornell Medical College of Cornell University[4]

ABSTRACT

We propose to use particle filter [1], along with active contour [2] to track and model the plus-end tips of microtubules in confocal microscopy. Microtubules are polymers that change between states of growth, shortening, and pause. These events are critical to many cellular functions and are targets for successful cancer chemotherapy agents like Taxol. However, analyses are performed manually by researchers in most cases. Hence there is a need for a rapid and efficient quantification algorithm. In this paper, we propose to uses particle filter to track microtubule dynamics. While there are other algorithms that track microtubule movements, none of them uses inter-frame information. In our system, we use an open active contour to segment individual microtubule in each frame. Particle filter is used to track microtubule movements using information from previous frame. A simple motion and observation model is used to model the motion of microtubule movement. We show some of the results using MCF-7 breast cancer cell lines captured using fluorescent confocal microscopy and conclude that adding particle filter improves the accuracy of the system.

Index Terms— Microtubule Dynamics, Particle Filter, Active Contour, Image Segmentation, Confocal Microscopy

1. INTRODUCTION

The advance of fluorescent microscopy technologies creates a new field for image processing on molecular level. New molecular imaging modalities allow rapid acquisition of dynamics cellular processes with high spatial and temporal resolution. These systems can generate large data set for a small experiment, and create a needed for quantitative and qualitative analyses which current commercial image analysis software cannot fulfill. Here image processing and signal processing techniques can be applied to create a specialized perform to automate the analysis process.

In this paper, we are interested in investigation microtubule dynamics, which refers to the stochastic nature of microtubule movement. Microtubules are tubulin polymers that transition between events of slow growth, rapid shortening, and pause. These events are the result of addition or removal of tubulin. The dynamicity plays important role in cell division and hence they are excellent targets for cancer drugs like paclitaxel.

There are very few algorithms developed to quantify microtubule dynamics. None of them uses temporal information to model and track the tips of microtubules. Saban [3] proposes to use Gaussian kernels to reveal dark microtubules in light backgrounds. Tracking is done using local search window of segmentation result from the previous frame. In [4], microtubule images are enhanced using anisotropic invariant wavelet filtering, three dimensional tube-enhancing, and surface-enhancing filter. Segmentation is done using active shape model. Plus-end tracing is done using a particle filter but it is used to trace microtubule structure instead of its dynamics. Hadjidemetriou [5] preprocesses microtubule images using by matching smoothed ellipse templates. He proposes to track microtubule movement by finding the best fit within a local search window, both intra-frame and inter-frame. In [6], microtubules are segmented using Gaussian kernels and thinning. Matching microtubule tips across frames is done using a graph constructed over the entire video.

We use particle filter to model the dynamics of microtubule across frame. Particle filter is a popular method used in tracking applications. Gustafsson [7] provides a detail review on different particle filter applications. Based on our previous work [8], we employ matched filter to enhance the raw microtubule images. In addition, we combine the two snakes into one to account for growing and shortening, so only one minimization step is needed. Once

the initial frame is segmented using active contour, we use particle filter to predict positions of microtubules tips in subsequent frames. The predicted location microtubule tips are used to initialize the active contour. Fig 1 shows the system diagram.

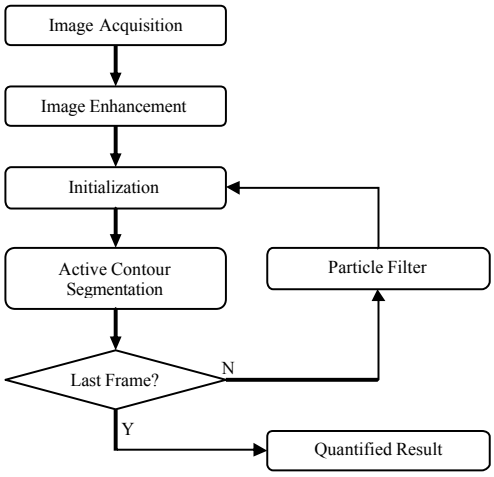


Fig. 1. Microtubules tracking system diagram

The paper is organized as following; section 2 describes how the particle filter is used to model microtubule dynamics, section 3 explains the active contour used to segment individual microtubule dynamics, section 4 provides details on the image acquisition and specimen preparation steps, section 5 contains some of the tracking results, and section 6 provides conclusion and discuss future work.

2. PARTICLE FILTER

Sequential Monte Carlo method, also known as particle filters, is used as a practical solution to optimal estimation and filtering. The goal is to estimate the states, or hidden parameters, of a system sequentially as new observation becomes available. It can model a more general discrete-time nonlinear, non-Gaussian dynamic system. Mathematically, we can try to find the posterior density $p(X_k|Y_k)$, which tells the probability of a system in a state $X_k = (\vec{x}_k, \vec{x}_{k-1}, \cdots, \vec{x}_1, \vec{x}_0)^T$, given the observed data $Y_k = (\vec{y}_k, \vec{y}_{k-1}, \cdots, \vec{y}_1, \vec{y}_0)^T$. Although confocal microscopy provides good temporal resolution, microtubule dynamics sometimes are too large to be properly captured. This results in large microtubule tip movement between frames. If we simply use the segmentation result from the previous frame as the initialization for the current frame, the initialization could lie outside the capture range of the snake. Even with the capture range extended using gradient vector flow (GVF) [9], microtubule movement could still move out of the extended capture range of the microtubule.

Here we propose a simple model to model the motion of microtubule tips. We define the state as $\vec{x}_k = (x_{1,k}, x_{2,k}, x_{3,k}, x_{4,k})^T$, where $(x_{1,k}, x_{2,k})^T$ is the location of a microtubule tip, $x_{3,k}$ is the velocity in x direction, $x_{4,k}$ is the velocity is y direction, and a_k is random acceleration with uniformly distributed on the interval (-5,5).

$$\begin{bmatrix} x_{1,k} \\ x_{2,k} \\ x_{3,k} \\ x_{4,k} \end{bmatrix} = \begin{bmatrix} 1 & 0 & \Delta t & 0 \\ 0 & 1 & 0 & \Delta t \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x_{1,k-1} \\ x_{2,k-1} \\ x_{3,k-1} \\ x_{4,k-1} \end{bmatrix} + \begin{bmatrix} \frac{\Delta t^2}{2} \\ \frac{\Delta t^2}{2} \\ \Delta t \\ \Delta t \end{bmatrix} a_k \qquad (1)$$

Also we assume that the position of microtubule tip can be observed, hence we model the observed data as $\vec{y}_k = (y_{1,k}, y_{2,k})^T$ and it is corrupted by a random Gaussian noise. We tuned the variance of the measurement noise to be 10.

$$\begin{bmatrix} y_{1,k} \\ y_{2,k} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} x_{1,k} \\ x_{2,k} \\ x_{3,k} \\ x_{4,k} \end{bmatrix} + v_k \qquad (2)$$

We choose a simple model such as (1) because microtubule dynamics are stochastic in nature (hence randomized acceleration). Although we can assume a Gaussian distributed acceleration and solve the system using Kalman filter, we use uniform distribution instead we assume that the microtubules grow or shorten equally likely.

With the assumption and the model in (1), it is not possible to sample directly from the posterior density. So we sample from a known proposal distribution, $q(x_k|Y_k)$. One common choice of proposal is to use the transition prior.

$$q(x_k|X_{k-1}, Y_k) = p(x_k|x_{k-1}) \qquad (3)$$

Once the proposal distribution is defined, we can approximate the state mean by,

$$E[f(x_k)] \approx \sum_{i=1}^{N} \varpi(x_{k,i}) f(x_{k,i}) \qquad (4)$$

where $\varpi(x_{k,i})$ is the normalized importance weight.

$$\varpi(x_{k,i}) = \frac{\omega(x_{k,i})}{\sum_{i=1}^{N} \omega(x_{k,i})} \quad (5)$$

For our algorithm, we set N = 200. We also resample the particle to avoid having algorithm degeneration where most weights have value close to zero.

3. IMAGE SEGMENTATION

Images are first enhanced using matched filter. Matched filtering is a technique used to maximize the signal to noise ratio. We use an inverted second derivative of a Gaussian function in a rectangular mask, described in [8] to model a microtubule segment. Mask pixels that are located along the center line of the rectangle have the highest values and the values decay exponentially away from the center line throughout the mask area. Normalization is used so that the response of a constant intensity area is zero. To account for different orientations, multiple masks are created where each one is the rotated version of the "base" model. These different masks or filters are then convolved with the raw image. The maximum output among those different filters is selected as the output, which represents the best matched microtubule segment at a particular pixel location. Output image is normalized so that the range of value of any pixel is between zero and one. Histogram equalization is performed on the output image to further improve contrast of microtubule.

We choose active contour to represent the segmentation result. In particular, the explicit form is selected, which is found by minimizing the following energy term.

$$E_{snake} = \sum_{i=1}^{N} \alpha(s_i) \cdot \dot{v}(s_i) + \beta(s_i) \cdot \ddot{v}(s_i) + E_{ext}(v(s_i)) \quad (6)$$

Compared to implicit form (B-spline, level-set), the explicit form directly minimizes contour energy along each point, which is easier to implement. For a two dimensional curve, the coordinates of each point (x, y) along the curve is parameterized as $v(s_i)$, with i ranging from 0 to N. Active contour converges to microtubules by minimizes under internal, and external constraints. Internal constraints include smoothness and rigidity, which are approximated using first and second derivatives, $\dot{v}(s_i)$ and $\ddot{v}(s_i)$ respectively. We segment an individual microtubule using open contour, so the internal energy terms are adjusted to reflect the topology requirement. Extemal constraints, $E_{\text{int}}(v(s))$, include features that we want the active contour to converge to. In this case, we use the matched filter output as the feature image as well as GVF to extend the capture range of the active contour. Initialization of the first frame is done manually, but subsequent initialization is done by using the predicted value from particle filters.

4. IMAGE ACQUISITION

The dataset we used is MCF-7 breast cancer cell lines stably expressing GFP:tubulin. Cells were grown maintained in RPMI 1640 supplemented with 10% fetal calf serum, nonessential amino acids, 0.1% penicillin/streptomycin, and 40 µg/ml G418 at 37°C in 5% CO2. To image microtubule dynamics we used a Perkin Elmer Ultraview RS spinning disc confocal. The Ultraview is mounted on a Zeiss Axiovert 200m microscope that is enclosed in a heating chamber with heated stage and CO2 perfusion. A 100X Zeiss Plan-Apochromat oil objective was used to image cells which were excited with the 488 nm laser line of an argon ion laser with a dichroic mirror optimized for GFP fluorescence. The emission signal from the GFP:tubulin was detected using a cooled Hamamatsu ORCA-ER with exposure values of 300-500 ms and no binning. The coordinates generated from this tracking feature were used to determine the distance individual microtubule ends changed from a fixed point.

5. RESULT

Each dataset consists of 41 frames and each frame has resolution of 1344 pixels by 1024 pixels. Fig 1 shows the result of the tracking at various points in the video.

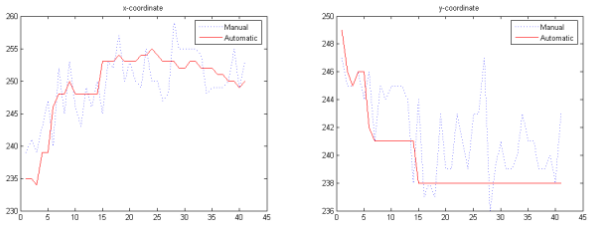
a) Initial Frame b) Frame #10

c) Frame #20 d) Frame #40

Fig. 2. Microtubules tracking result at different frames of the sequence, darker solid lines indicate the tracking.

We only initialize the first frame manual and the algorithm will automatically perform the segmentation and tracking in the subsequent frames. From the figure, we see

that the segmented tip follow the movement of microtubule throughout the video. To illustrate the tip tracking capability further, we compare the tip coordinate between manual tracked and our algorithm. Position of the tip, x and y coordinate is shown in fig 3. Dashed line shows the manual tracked microtubule tip, while solid line shows the tip tracked using our algorithm.



a) x-coordinate b) y-coordinate

Fig. 3. Tracking of Microtubule Tip Using Manual Segmentation and Our Algorithm.Segmentation and Our Algorithm.

We also compare the result with and without particle filter. Using manually segmented microtubule tip as the gold standard, we calculate the error of microtubule tip using particle filter and without using particle filter. We found that the improvement of using particle filter is 5.7%.

6. CONCLUSION AND FUTURE WORK

We have demonstrated how particle filter can be used to track microtubule movement by exploiting temporal information between successive frames. In the future, more work is needed in validation and development of more accurate models for microtubule movements. Our model serves two purposes: first is to provide better microtubule dynamics analysis results, while second and more important one is to allow simulation of microtubule movement. An accurate microtubule model would significantly advance the technology of drug efficacy study and new drug development in silico.

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