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Investigation of the Microtubule Dynamics with Probabilistic Data Association Filter

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Abstract— **Understanding microtubule dynamics has important implications for establishing nanometer level machines. Object tracking is one of the important issues necessary to elucidate the dynamics of microtubule from video data. In microtubule gliding assays, object tracking becomes non-trivial due to the occurrences of compound objects and high density objects. In this work, we investigate microtubule dynamics focusing on its morphological information, and we developed easy and useful workflow with compound segmentation technique and probabilistic data association filter. Using this workflow, multi-crossing microtubules can be decomposed, and be tracked correctly.**

Keywords—microtubule gliding assay; bio-image informatics; multiple-object tracking; probabilistic data association filter

I. INTRODUCTION

Microtubules and motor proteins are considered the building blocks of artificial bio-machines [1, 2, 3] and molecular robots [4, 5]. They play an important role for the development of actuators in molecules. In order to understand the dynamics of microtubules, microtubule gliding assay (MGA) [6-10] has recently gained increasing interest. In the analysis of MGA video data, microtubule tracking is a highly sought-after approach because of its great benefits over manual tracking.

MGA is a biological experiment observing the dynamics of microtubules driven by motor proteins fixed on a glass surface when ATPs are dosed. In microtubule gilding assay experiments, images taken can be compiled into video data for further investigation. Video data from microtubule gliding assays contain various types of information including density, shapes, locations and velocities of microtubules. Such information can be observed by the human eye; however, the visual quantification of such information is both time-consuming and error-prone, even though it is still commonly analyzed manually. Therefore, computational methodologies that can automatically track the paths in MGA video data are urgently needed.

Automated data analysis plays an important role in collecting statistically significant facts about microtubules dynamics. In order to extract biological information from bio-image data, novel image processing tools including object tracking, data analysis and visualization techniques have been used. Object tracking is of key importance for quantitative analysis of intracellular dynamic processes from time-lapse microscopy image data. Multiple object tracking is a topic of great interest in the field of image processing. There have been substantial works on multiple-particle tracking [11, 12], and tracking algorithm based on Kalman filter [13] and microtubule growth tracks [14, 15]. The first effort for automatic tracking of elongate objects used center-of-mass calculation or cross correlation to track objects in image sequence [16, 17, 18]. However, few studies have focused on object tracking with regards to MGA, especially when dealing with complicated microtubule interactions in diverse morphological and density condition.

In cases with crowded and noisy condition, a probabilistic data association (PDA) filter is often used to track microtubules. On the other hand, in cases with low density conditions, an ad-hoc decomposition algorithm can be applied to extract morphological information and decompose overlying objects. Here, we combined our algorithm with the PDA filter to create a useful tracking and analyzing workflow for both low and high density conditions, and have demonstrated the statistical properties of microtubules such as length, density, group density and shapes. The adopted PDA filter is one of the statistical approaches that probabilistically associate all the validated measurements to the target of interest [19, 20, 21].

The organization of this paper is as follows. Firstly, we start by describing the background of the research issues in MGA. Section 2 describes the general explanation of MGA. Section 3 demonstrates and discusses the results of our computational experiments. Section 4 describes the research method from the viewpoint of image processing.

II. MICROTUBULES, KINESINS AND GLIDING ASSAY

The research objects of interests are microtubules which are long, hollow cylinders made up of polymerized alpha and beta tubulin dimers. The microtubules are approximately 25 nm in diameter. However, due to the effect of fluorescence, the microtubules appear to be approximately 700nm wide.

The *in vitro* gliding assay, also called motility assay, has been widely employed for studying the functions of bio-molecular motor systems and unveiled the mechanisms of actin-myosin and microtubule-kinesin interactions, *in vivo* [22]. The *in vitro* motility assay has also provided us with

valuable insights into important aspects of bio-molecular motor functions. Figure 1 shows an illustration and gliding assay images that produced the video data in this study [9, 10]. Many motor proteins, for example, kinesins, are fixed on the glass surface. The liquid on the surface includes microtubules. Once ATPs are provided, the kinesins bind with the ATPs and then start swinging forward and push the microtubules. Microtubule-kinesin interactions can be observed through the objective lens and recorded as video data. Since the size of microtubules and kinesins is on nano-meter level, the video data tends to be very noisy.

The difficulties involved with microtubule tracking mainly result from the similarity of microtubule structures,
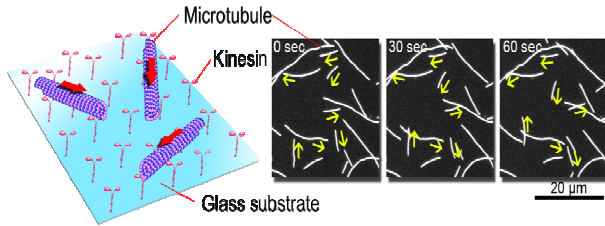

Fig. 1 Schematic illustration of microtubule gliding assay.

and regions of high density where individual microtubules cannot be distinguished easily. They look very similar in general and change their curves from time to time. Sometimes, it becomes difficult to track them even by the human eyes when the number of microtubule becomes large. Also, some microtubules can suddenly appear or disappear in video data, which is mainly due to the vertical movement on the glass surface.

III. RESULTS AND DISCUSSION

A. Microtubule path tracking examples

Figure 2 shows the results of multiple microtubule tracking by the PDA filter on high density video data (a) and low density (b) video data; about 1200 microtubules and 55 microtubules, respectively. These two videos will be used for further analysis throughout this paper.

Tracking paths were normalized and superimposed at their origins; this allows quick visual comparison of the different types of motion. Figure 2c shows the 1200 normalized paths of high density microtubules videos. In this figure, most of the paths tend to go in a smooth direction and smooth curve. On the other hand, figure 2d shows that the 55 normalized paths of the low density microtubules videos. In this figure, many flexible paths as well as strait paths also can be observed.

Tracking accuracy is strongly depends on the microtubule lengths and density. Figure 2e, f, and g show 521, 308 and 101 microtubule tracks length filtered by 10, 30, and 70 pixels in the high density video. Their tracking accuracy is 76%, 89% and 86%. As for the density, the tracking accuracy is 97% and 95% for 8 and 55 microtubules in low density videos with 479*658 and 480*636 pixels,

respectively. The accuracy is 84% and 60% for 240 and about 1200 microtubules in high-density video with 2160*2560 pixels, respectively. Although it is too early to conclude anything about microtubule tracking, tracking accuracy becomes lower when increasing the number of microtubules mainly due to the occurrences of compound objects described in IV C.

B. Microtubule mobility property

Microtubule tracking data provides us with useful information about microtubule dynamics. From the tracking data, many kinds of features can be estimated such as
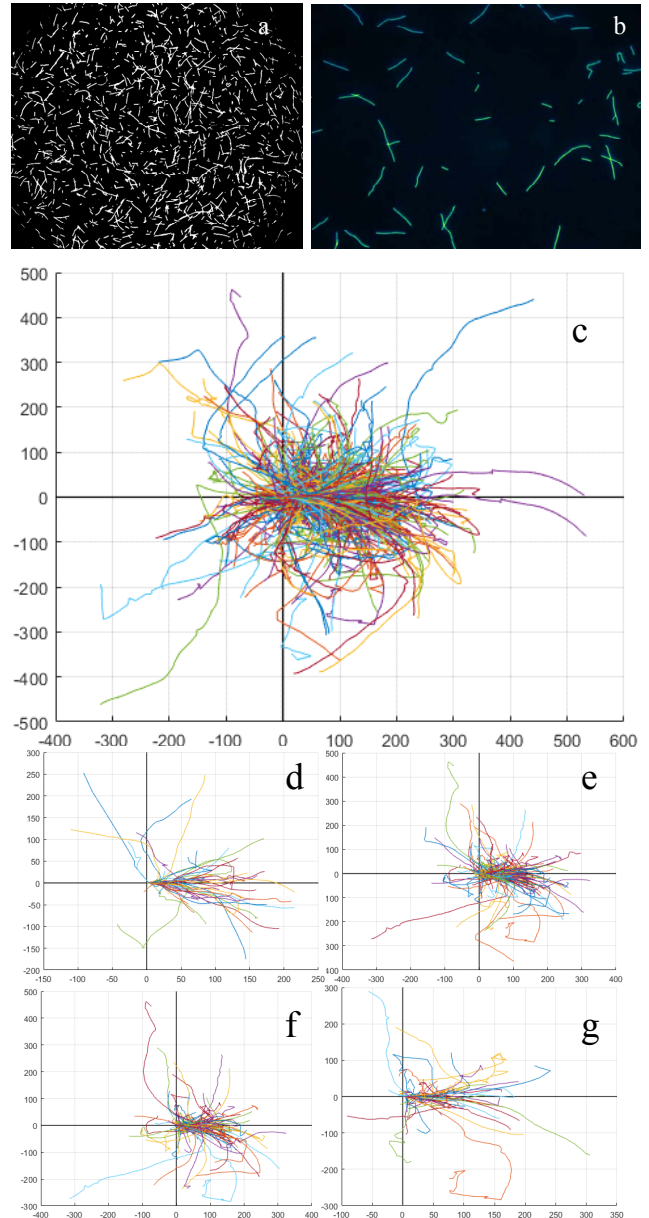

Fig. 2 (a) high density video includes 1200 objects, (b) low density video includes 55 objects, (c) and (d): Centralized and normalized path of microtubules on fig. 2(a) and (b), respectively; 521, 308 and 101 length filtered microtubule tracks are shown in (e), (f), (g) , respectively. (Unit is pixel).

instantaneous displacement, speeds, turning angles, direction autocorrelation, total and net distances, directionality ratio, lengths, area and mean square displacement. For example, Figure 3a shows that the angles of aggregation are between -1.5 (-86 degrees) and +1.5 (86 degrees) approximately. Figure 3b shows that the average velocity of microtubules is approximately 20~25 pixel/s (about 100 ~125 nm/s) for the microtubules whose lengths range from 10 to 80 nanometers in the low density video, and approximately 6~8 pixel/s in the high density video.

Distribution of instantaneous microtubule velocities from our experiment video, at a high density and public data [24], at a low density, are shown in Figure 4a and 4b, respectively.

Fig.3 Relations of instantaneous velocity, direction and length of microtubules in low density video (Red, 55 microtubules) and high density video (Blue, 521 length filtered microtubules). Pixels were used as a unit to measure length, then the speed was found by measuring pixels traveled per second, then the direction was measured in radians.

Interestingly, the distributions are not similar as their densities are different. The distribution of the low density video formed a peak and gathered at this peak, however the distribution of the high density video spreads across a wide region, and this may be caused by the interaction of microtubules. The distribution may result from various speed of microtubules.

Figure 5 shows the direction autocorrelation (DA) of the three microtubules randomly selected. Autocorrelation is a member of the spatial autocorrelation family [25], which is used to measure how it correlates with itself over different scales [26]. One of the major reasons that directional autocorrelation analysis is used is that it maintains directional persistence influenced by speed. For each of the consecutive displacement vectors, autocorrelation coefficients are calculated as cosine of their angle subtraction.

It yields a value between -1 and 1; the values 1, 0, -1 mean that displacement vectors are parallel, orthogonal and antiparallel, respectively. In this figure, the DA value of these microtubules goes in nearly straight direction, except for one that turned on their track. The corresponding path of these can be observed in Figure 5(a). To calculate the direction autocorrelation, the following formula is used:

$$DA = \cos(\alpha_{i+1} - \alpha_i) \qquad (1)$$

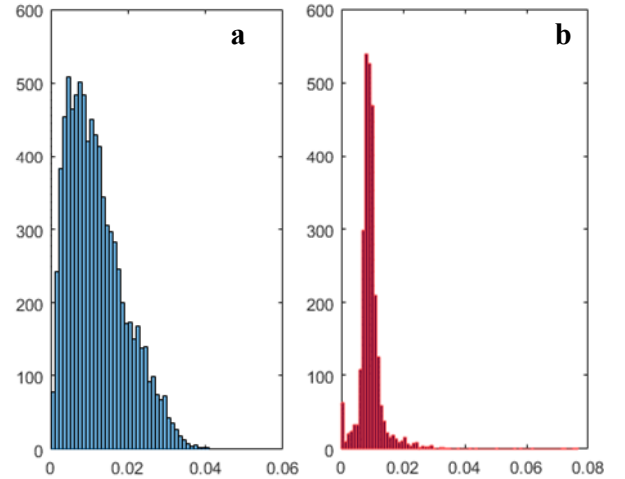$$\alpha_i = \arctan(\ (y_{i+1} - y_i) / (x_{i+1} - x_i)\ ) \qquad (2)$$

Fig.4 Distribution of microtubule velocities (a) High density, (b) Low density. (Unit is normalized pixel).

Since the direction of each vector is defined by its start and end points on two frames, it is plausible that low and high frame rates may cause the incorrect recording of the true vectors. Another factor that may affect the accuracy of the direction autocorrelation is the resolution of the original image or the pixel grid effect.

Figure 6 shows the directionality ratio of the microtubule paths. This parameter, also called straightness, results from the value of the straight-line length between the start point and the endpoint of the trajectory divided by the length of the trajectory. This ratio is equal to 1 for a straight microtubule trajectory and approaches 0 for a highly curved irregular trajectory. Thus, the directionality ratio is easy to understand and compute. We define that the trajectory may close to straight if the directionality ratio is greater than 0.8 in this paper. We found that more than 71% microtubules in low density motion video move in nearly straight direction.

The applied methods of analysis above each show different details depicting microtubule dynamics. The directionality ratio reports a single value for each microtubule showing the uniformity of movement across all frames. The DA value is capable of showing detailed information concerning changes in the path of individual microtubule from frame to frame.

IV. METHODS

A. Microtubule tracking

Movies of microtubules were captured by fluorescence microscopy [23], and we have developed our algorithm in MATLAB R2015a using an image processing toolbox. Microtubule tracking is performed by a workflow of object detection, object decomposition and tracking path estimation. There are two choices in solving a tracking problem: on-line tracking and off-line tracking. We adopted the latter one since on-line tracking is more prone to failure at the beginning of the tracking process. In the process, object decomposition process may pass if video data includes crowded and non-tube-like microtubules.

B. Object detection
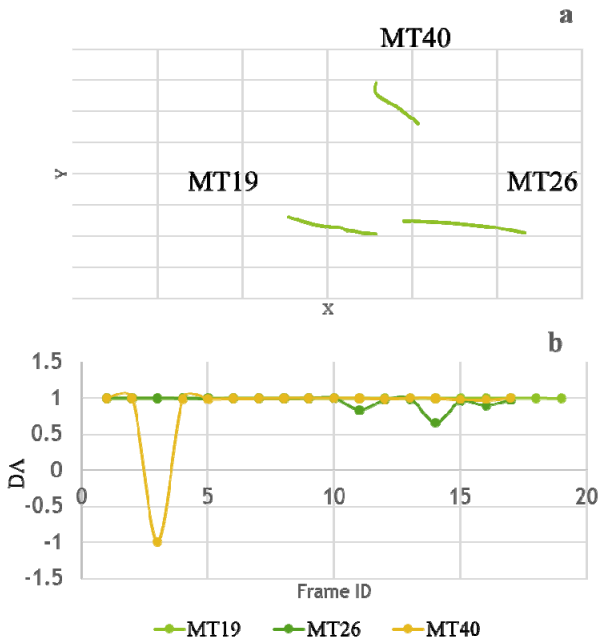
The workflow of object detection as follow:

Fig.5 (a) Three microtubule tracking paths randomly selected in high density video, (b) Corresponding direction autocorrelation of the microtubules.
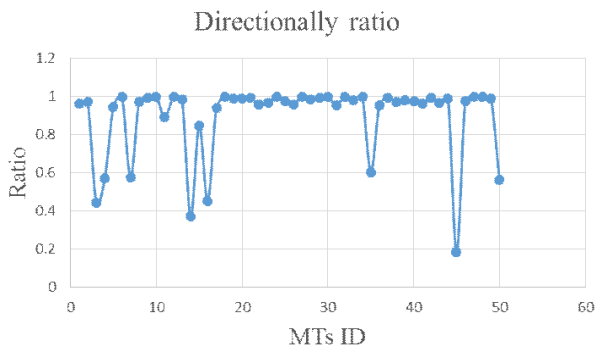

Fig.6 Directionality ratio of a microtubule path in high density video.

Step 1: Conversion from video data to a series of image frames.

Step 2: Conversion of an image frame to binary image followed by noise elimination with regards to object length information. Here we define a threshold ξ and remove a microtubule when its length is smaller than ξ for further analysis.

Step 3: Object smoothing with MATLAB morphological operations, which transforms each microtubule into a one pixel wide microtubule. The bwmorph() function (image processing toolbox) is used for the thinning algorithm.
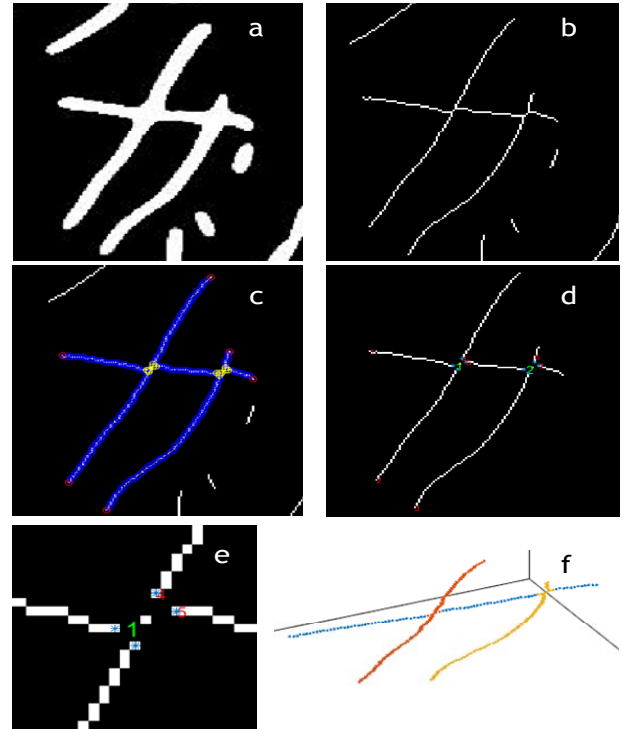

Fig.7 Illustration of decomposing process: (a) Binarized target region, (b) Thinning, (c) Detection of the heads (red), crossing points (yellow) and pixel information of each part (blue), (d) Grouping the parts surrounding a same crossing point, (e) Highlighting the four parts surrounding crossing point 1, (f) Decomposed and colored microtubules. High density video is used for this illustration.

Step 4: For tracking extract the head points from subtraction image of two consecutive images. The head point is a plus end of microtubule which is the head on the moving direction. As defined in Section D of Methods, we assumed that body of microtubule always follows the track of the head, therefore, the remaining of the subtraction of binarized two consecutive images would include the head position of microtubule. Using same method it is possible to determine the end point of microtubule. For analyzing the shape information such as length and elasticity, single and crossing microtubules are classified and decomposed using the ad-hoc method described in Section C.

From the video data, the shapes of the microtubules are firstly obtained with the image processing, including noise elimination, background filtering and grey or binary scale conversion as described in [27, 28]. After this process, all objects are numbered and microtubule tracking paths are detected. The estimation algorithm is described in Section D. The tracking paths are used to estimate microtubule motion

characteristics such as head movement tracks, microtubule direction distribution, movement speed distribution, direction autocorrelation, and so on.

C. Object decomposition

A compound object consists of more than two microtubules. In order to track the same microtubule, it is sometimes necessary to decompose a compound object into a collection of single microtubules. Object decomposition is not trivial because there are many cases forming the same compound structure. The main steps of the ad-hoc algorithm of the decomposing process are as follow: As shown in Figure 7, after adopting the object detection process which is described in Section B of Methods, first, detect the heads of each part, crossing points and pixel information of each part from the binary image, α, β, γ respectively. Secondly, find the part sets Γ surrounding a same crossing point. If $\overrightarrow{\alpha_i\beta_j} \leq \epsilon, \alpha_i \in \alpha, \beta_j \in \beta$, then α_i is a member of part set Γ_i. Then estimate corresponding parts which surround a crossing point. If $\overrightarrow{\gamma_x\gamma_y} \leq \theta, \gamma_x, \gamma_y \in \Gamma$ then the γ_x, γ_y are recognized as the same MT body. By running this process each individual microtubule can be detected.

D. Probabilistic data association

A track is a sequence of objects in sequential frames that belong to the same physical entity [13]. Detection of the same entity in different frames is a key of object tracking. In case of microtubule tracking, the same entity may change its shape and topology due to the random movement and mutual interaction of microtubules.

A microtubule tracking path can be defined by a sequence of objects selected from each frame of the video data. An object is null if there is no occurrence of the microtubule in a frame. A microtubule tracking path is unique if all objects in the path are exclusive from other microtubule tracking paths. Two tracking paths have a conflict if the paths share the same object in the same frame. The objective of microtubule tracking path estimation is to extract a number of unique tracking paths as long as possible and as often as possible, while maintaining the accuracy of the tracking path.

In our study, we assumed the following conditions with respect to the microtubule movement:
- A microtubule does not change its length except for overlapping, disconnection, sudden appearance and disappearance of the microtubule.
- The head of microtubule may change its direction randomly but the body of the microtubule always follows the track of the head.

Assuming the above conditions, we can define the target microtubule detection rule as follows. Objects in successive frames can be considered as the same microtubule if the objects have similar estimated and measured location at the direction of movement. In other words, the superposition pattern of the objects forms a tracking path of a microtubule head. The detection of the microtubule of interest becomes a non-trivial issue when dealing with complicated microtubule interactions such as crossing. Another issue in tracking path detection comes from sudden appearance and disappearance of microtubules in addition to entering-from and passing-to outside of the frame. This indicates that the number of objects in a video frame can vary, and no identical entity is guaranteed to be found in the next frame. Therefore, a probabilistic approach is necessary for the tracking path detection.

The PDA filter consists of two main steps: data association and microtubule track update. Suppose the state and measurement vectors are x and z, and the predicted state and state covariance are $\hat{x}$, P respectively, then the simplified description of the approach may be presented by:

$$x(k) = F(k-1)x(k-1) + v(k-1) \quad (3)$$

$$z_i(k) = H(k)x(k) \quad (4)$$

Where F is the system transition matrix, H is the measurement matrix, R and Q is the covariance. Simply it can be defined that state is the coordinate vector of the microtubule's head position. If the target was detected and the corresponding measurement was in the validation region, by using (4), one of the most appropriate validated measurements can be selected as a target originated.
The state vector, the measurement and state covariance matrices can be predicted in time from k-1 to k using by (5), (6), and (7):

$$\hat{x}(k|k-1) = F\hat{x}(k-1|k-1) \quad (5)$$

$$P(k|k-1) = FP(k-1|k-1)F' + Q \quad (6)$$

$$\hat{z}(k|k-1) = H\hat{x}(k|k-1) \quad (7)$$

The innovation covariance corresponding to the correct measurement is:

$$S(k) = R + HP(k|k-1)H' \quad (8)$$

The state update equation of the PDA filter is:

$$\hat{x}(k|k) = \hat{x}(k|k-1) + W \quad (9)$$

$$W = P(k|k-1)H'S^{-1} \quad (10)$$

where W is a Kalman gain matrix. The state updating process is done separately for each microtubule head.

A true measurement is $\theta^i(k)$ in validation region estimated by:

$$\theta^i(k) = ((C'S'C') < d) > 0 \quad (11)$$

$$C = (z_i(k) - \hat{z}(k|k-1)) \quad (12)$$

where d is the threshold corresponding to the gate probability. It is set up for each time step to pick the measurement for association to the target of interest. In the PDA filter the association probability to the object being tracked is estimated after measurement prediction and innovation calculation for each time step.

V. Conclusions

Microtubule tracking path analysis of mobility properties such as the relationship between speed, length and direction, direction autocorrelation and directionality ratio are demonstrated for studying the microtubule gliding dynamics. The PDA filter gives a reasonable tracking accuracy in the low density microtubule video and length limited microtubules in the high density video. Complicated compound objects may cause incorrect tracking estimation. Further study is necessary to improve the tracking accuracy with regards to image noise elimination and compound objects to detect true microtubule head positions and data associations.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions.

B.M. wrote the manuscript, designed research, performed research, and analyzed data. D.I. and A. Kakugo wrote the manuscript, designed research and performed research with regards to experimental data. A. Konagaya wrote the manuscript, designed research, and analyzed data. All authors read and approved the final manuscript.

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