Quantitative Comparison of Two Particle Tracking Methods in Fluorescence Microscopy Images

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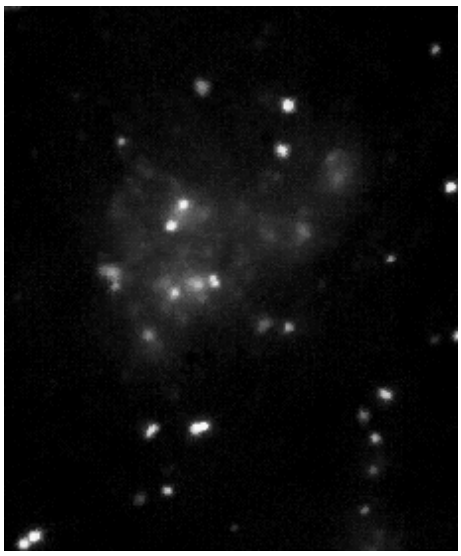
***Abstract*—Tracking of multiple bright particles (spots) in fluorescence microscopy image sequences is seen as a crucial step in understanding complex information in the cell. However, fluorescence microscopy generates high a volume of noisy image data that cannot be analysed efficiently by means of manual analysis. In this study we compare the performance of two computer-based tracking methods for tracking of bright particles in fluorescence microscopy image sequences. The methods under comparison are, Interacting Multiple Model filter and Feature Point Tracking. The performance of the methods is validated using synthetic but realistic image sequences and real images. The results from experiments show that the Interacting Multiple Model filter performed best, under the test conditions.**

I. Introduction

Spot tracking in time-lapsed fluorescence microscopy image sequences is seen as a crucial step in understanding the complex information in the cell. Fluorescence microscopy is a tool used to study and visualize the intracellular processes. Recent advances in fluorescence microscopy provide the ability to image in three dimensions (3D) and record the dynamic processes in the living cells.

The use of fluorescence microscopy and a specific staining method makes biological molecules to appear as bright particles (spots) when viewed through a microscope, as shown in Figure 1. The quantitative analysis of these data requires the tracking of spots in large time-varying image sequences. However, fluorescence microscopy generates a high volume of noisy data that cannot be analysed efficiently by means of manual evaluation, hence there is great demand for automation of the tracking process and it is attracting increased research attention.

The key idea of particle tracking is to establish correspondence between particles in a sequence of images in order to construct their trajectories throughout the time-lapsed sequence. Establishing correspondence between particles in a sequence of frames is complicated by factors such as, for example, a temporary high spot density, high levels of noise, particle disappearance, particle merging, particle splitting and complex motion patterns. Several tracking methods have been proposed to overcome these challenges but often fail to yield satisfactory results in cases of the above mentioned problems [17].



(a)

Fig. 1. (a) Example of real fluorescence image sequence from the study of endosomal ts1 virus labeled with Texas red with bright spots. The image is a single frame from the 2D time lapse studies.

Cheezum et al. [2] quantitatively evaluated several tracking methods for tracking single fluorescent particle. The methods which were compared are not commonly used in biological tracking contexts and these methods were found to fail at signal to noise ratio (SNR) below 4.

Recently, several methods for particle tracking have been proposed [10], [11], [16], which can help to overcome the above mentioned problems.

Sbalzarini et al. [16] presented a 2-D feature tracking method for the automatic detection and analysis of particle trajectories in video imaging. The tracking algorithm requires no assumption about the motion model, it is self initialized, discriminates spurious detections and can handle temporary occlusion as well as particle appearance and disappearance. The algorithm, as presented in [16], starts by finding the particle locations using feature point detection [16] then employs a motion correspondence method for data association based on a graph

theoretical approach to solving the transportation problem [12].

Genovesio et al. [10] presented a method for tracking of multiple moving spot-like particles showing different kinds of dynamics. The method uses the 3-D undecimated wavelet transform to detect spots and the prediction of spot future states is accomplished by the Interacting Multiple Model (IMM) algorithm [1]. Several models corresponding to different biologically realistic movement types are used. Then, association is performed to establish the trajectories based on the maximum likelihood of the innovation among the IMM filters. The last step consists of updating the filters to compute the final estimate.

Godinez et al. [11] developed probabilistic and deterministic approaches for multiple virus tracking in multi-channel fluorescence microscopy images. The deterministic approach is based on a spot-enhancing filter for spot localization, and uses global nearest neighbour for motion correspondences. The probabilistic approaches are based on mixtures of particle filters and on independent particle filters. The probabilistic approaches use particle filters for maintaining and predicting particle state, they employ a motion correspondance algorithm presented in [16]. A total of eight tracking methods were compared in the study and found that the probabilistic methods based on independent particle filters perform best compared to deterministic methods and mixtures of particle filters.

In this work we compare the performance of two tracking methods used for tracking of bright particles in microscopy images using synthetic images.The methods under comparison are interacting multiple models (IMM) [10] and feature point tracking (FPT) [16]. These two methods have been used in biological tracking contexts [9], however no comparison between them has been done.

The layout of the paper is as follows: Section II describes the various algorithms used in the experiments. Section III presents the performance measures and in section IV experiments are discussed. Section V discusses the experimental results, and finally, the conclusions are given in section VI.

II. Tracking Methods

A. Feature Point Tracking (FPT)

In Feature point tracking [16], tracks are represented by associations between sets of detections, where one set is $p_i, i = 1, ...N_k$, in frame k, and the other set is $q_j, j = 1...N_{k+r}$, in frame $k+r$. A set of detections is obtained from the detection algorithm [16], which returns an $N_t \times 2$ matrix $[\tilde{x}_p, \tilde{y}_p]_{p=1}^{N_t}$, where N_t is the total number of particles being detected in frame t. An association matrix $G_r^k(i,j)$ is built for all pairs of detected particles, where the association matrix is given as:

$$G_r^k = g_{ij} = \begin{cases} 1, & \text{if } p_i \text{ and } q_j \text{ match,} \quad (1) \\ 0, & Otherwise \quad (2) \end{cases}$$

Where $r = 1...R$ is the user-defined integer specifying how many frames to be considered.

Each association matrix is augmented by one row and one column for a dummy particle at time step k and $k + r$, respectively. Linking a particle to a dummy means that the particle disappeared from the scene between frames k and $k + r$, and linking a dummy to a particle can also reflect a new particle.

To find the optimal correspondence between links g_{ij}, a cost function based on the transportation problem [12]is defined and the restriction is that it needs to be linear in the association variables g_{ij}, and thus written as:

$$\Phi = \sum_{i=0}^{N_k} \sum_{j=0}^{N_{k+r}} \phi_{ij} g_{ij} \quad (3)$$

Where N_k and N_{k+r} are the number of detections at time k and $k+r$. Symbol ϕ_{ij} represents the cost of associating particle p_i in frame k with particle q_j in frame $k+r$, and includes the Euclidean distance between sets of detections and the intensity moments of order 0 and 2:

$$\begin{aligned} \phi_{ij} &= (\tilde{x}_{pi} - \tilde{x}_{qj})^2 + (\tilde{y}_{pi} - \tilde{y}_{qj})^2 + (m_0(p_i) - m_0(q_j))^2 \\ &\quad + (m_2(p_i) - m_2(q_j))^2 \quad (4) \end{aligned}$$

Where m_0 and m_2 are the intensity moments. Tracks are formed and extended by minimizing this cost function.

B. Interacting Multiple Models (IMM)

The IMM filter is a state estimation algorithm for systems with multiple motion models. Internal model changing is based on a finite state Markovian switching coefficient. The algorithm was first developed for radar tracking systems [1], and introduced to biological applications in [10]. The generic IMM filter using the Kalman filter as proposed in [10] contains several models of linear systems:

$$x_k = A_k^j x_{k-1} + w_{k-1}^j \quad (5)$$

$$z_k = H_k^j x_k + v_k^j \quad (6)$$

Where x_k is the system state at time k, j denotes the index of models, A_k^j is the state transition matrix for model L_k^j at time k and $w_k^j \sim N(0, Q_k)$ is the process noise. z_k is the measurement state, H_k^j is observation matrix for model L_k^j at time k, and $v_k^j \sim N(0, V_k)$ is the measurement noise. The n models of the IMM filter form a discrete set denoted as: $L = \{L^1 ... L^n\}$ and the probability of switching from L_{k-1}^i to L_k^j given as: $\pi_{ij} = P\{L_k^j | L_{k-1}^i\}$.

The track initiation in the IMM filter is accomplished by employing a spot detection method called, Isotropic Undecimated Wavelet Transform (IUWT) [10], [13]. This detection method creates a measurement vector consisting of spot location, volume and the mean intensity. The IMM filter consists of three steps: Interaction/mixing, filtering and combination. The details of each step can be found in [10]. To form the tracks, the maximum likelihood of the innovation among the models of each IMM filter is computed. The likelihood of each model is weghted with the predicted model probability of the IMM filter. Once the association between the measurement and a track is accomplished, the update of each IMM filter is performed.

III. Evaluation

The particle tracking benchmark generator plugin [3], [4] in the ICY software from Institut Pasteur in France [7], [8], was used to create the synthetic image time sequences. The plugin creates realistic $2D/3D$ time fluorescent particles. By adjusting parameters in the plugin configuration text file, different characteristics of particles can be achieved, for example, particle motion type, number of particles, background noise, particle intensity, and dimension and length of sequences.

In order to measure the performance of the two algorithms, we used common measures [15], precision, or true positive measure (TPR), recall rate (RR) defined as:

$$TPR = \frac{N_{TP}}{N_{TP} + N_{FP}}, \tag{7}$$

$$RR = \frac{N_{TP}}{N_{TP} + N_{FN}}. \tag{8}$$

Where N_{TP} is the number of recovered ground truth trajectories, N_{FN} is the number of non-recovered trajectories and N_{FP} is the number of false tracks.

The Jaccard similarity coefficient [4], [5], [15] for tracks is then defined by combining the TPR and RR as:

$$JSC = \frac{N_{TP}}{N_{TP} + N_{FN} + N_{FP}} \tag{9}$$

The JSC takes values in the interval $[0, 1]$. Thus a tracking method with $JSC \rightarrow 1$ is the best while the one with $JSC \rightarrow 0$ is the worst.

IV. Experiments

A. Experiments with synthetic data

The performance of the proposed tracking methods was evaluated using synthetic (with ground truth) image sequences, as shown in Figure 2.

Four types of synthetic image sequences, Seq A, Seq B, Seq C and Seq D, were created using the synthetic data benchmark generator [3]. These synthetic sequences simulated different imaging conditions and different spot movement. Each synthetic sequence was a two dimension plus time $(2D+t)$ image with size of 512×512 pixels containing multiple spots in random locations. The length of the time sequences was fixed at 50.

The first three sequences (Seq A, Seq B and Seq C) contained 10, 50 and 200 spots respectively, moving in linear motion at random directions, with randomly varying velocity between $v_{min} = 2$ and $v_{max} = 4$. Gaussian noise was then added to each sequence resulting in noisy synthetic sequences of signal to noise ratio (SNR) ranging from $\{10, 7, 5, 3, 2, 1\}$. SNR in our experiments was defined as the ratio of spot intensity, I_{max}, divided by the noise standard deviation, σ_{noise}:

$$SNR = \frac{I_{max}}{\sigma_{noise}} \tag{10}$$

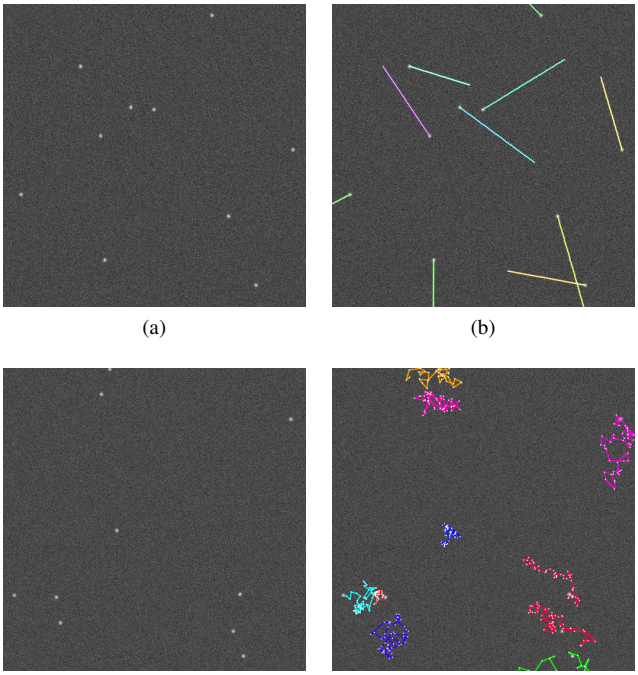


Fig. 2. Examples of synthetic images used in the experiments. (a) Seq A synthetic image with spots at random locations moving in a linear motion. (b) Corresponding ground truth trajectories of Seq A images (c) Seq D synthetic image with spots at random locations moving in Brownian motion (d) corresponding ground truth trajectories

The last sequence type (Seq D) contained ten spots moving in brownian motion with the standard deviation of the motion fixed at $sigma_bMax = 4$ and $sigma_bMin = 2$. Gaussian noise was added to produce SNR ranging from $\{10, 7, 5, 3, 2, 1\}$.

B. Experiments with real data

We also tested the performance of the two tracking methods using a real fluorescence microscopy image sequence from the study of endosomal ts1 virus labeled with Texas red, as shown in Figure 1. This sequences was of $2D+t$ with multiple spots moving in random directions. Since the ground truth of these images was not available, we compared the tracking results with manual inspection. For, manual inspection we used a manual tracking plugin developed by Cordelières [6] in ImageJ [14]. This plugin allows one to follow the movements of spots in time lapsed sequences.

V. Experimental results and discussion

Tables I, II, III and IV show the results of two tracking methods using synthetic images.

The results from Table I indicate that at SNR 10 and 5 the two methods performed well with the same number of $JSC = 1$ for image sequence with 10 spots, however, as SNR decreases to 3 and 2 the performance of the two methods also decreases with FPT having JSC = 0.0415 at SNR of 2, while the IMM has JSC = 0.538. In overall, the IMM has the highest average JSC = 0.633 as compared to FPT with average JSC

= 0.483. These results shows that IMM is more reliable when image noise increases.

Table II and III presents the results of two methods when the density of spots is 50 and 200, respectively. These results show that when the density of spots increases the FPT method performance dropped with an average JSC of 0.327 and 0.275 respectively, while the IMM has the average JSC of 0.574 and 0.571 respectively.

The reason for the poor performance of FPT in Table II and III appears to be that when the density of spots increases, there is an increase in overlapping spots causing the FPT method lose tracks.

Table IV presents the results when particles are moving in Brownian motion. The results indicates that the IMM still has the highest average JSC = 0.541 as compared to FPT with average JSC = 0.367, however, at SNR = 3, the FPT algorithm has the highest JSC = 0.769 than the IMM with JSC = 0.526. The reason for the best performance by FPT at SNR = 3 in Table IV, is that no overlapping of spots occurred at SNR of 3. This shows that when there is no overlapping of spots, the FPT method can perform well.

It may be possible to improve the performance of FPT by adding a post processing step to link track fragments that occur when spots overlap.

The results from real images, Table V, indicates that the IMM performs better with JSC = 0.675 than the FPT with JSC = 0.5.

TABLE I. RESULTS OF SPOT TRACKING METHODS USING SEQ A

SNR		10	5	3	2	1	AVERAGE
IMM	N_{TP}	10	10	10	7	0	
	N_{FP}	0	0	6	3	0	
	N_{FN}	0	0	0	3	0	
	JSC	1	1	0.625	0.538	0	0.633
FPT	N_{TP}	10	10	10	10	6	
	N_{FP}	0	0	17	231	1367	
	N_{FN}	0	0	0	0	4	
	JSC	1	1	0.370	0.0415	0.00436	0.483

TABLE II. RESULTS OF SPOT TRACKING METHODS USING SEQ B

SNR		10	5	3	2	1	AVERAGE
IMM	N_{TP}	50	50	49	29	0	
	N_{FP}	4	3	44	10	0	
	N_{FN}	0	0	1	21	0	
	JSC	0.926	0.943	0.521	0.48	0	0.574
FPT	N_{TP}	48	48	50	49	45	
	N_{FP}	19	45	88	748	4126	
	N_{FN}	2	2	0	1	5	
	JSC	0.696	0.505	0.362	0.0614	0.0108	0.327

TABLE III. RESULTS OF SPOT TRACKING METHODS USING SEQ C

SNR		10	5	3	2	1	AVERAGE
IMM	N_{TP}	198	199	195	104	2	
	N_{FP}	14	24	145	22	0	
	N_{FN}	2	1	5	96	198	
	JSC	0.925	0.889	0.565	0.468	0.01	0.571
FPT	N_{TP}	197	199	199	200	200	
	N_{FP}	149	239	424	1955	15127	
	N_{FN}	3	1	1	0	0	
	JSC	0.564	0.453	0.319	0.0245	0.0131	0.275

VI. CONCLUSION

We compared the performance of two tracking methods, IMM and FPT. In our study we included four types of synthetic images containing realistic image sequences and real images. The results from the experiments indicates that the IMM tracking method performs better than the FPT. The poorer performance of FPT appears to be caused by the overlapping of spots, where an increase in overlapping spots causes the motion correspondence algorithm to deteriorate, which decreases the overall performance of the method.

TABLE IV. RESULTS OF SPOT TRACKING METHODS USING SEQ D

SNR		10	5	3	2	1	AVERAGE
IMM	N_{TP}	10	10	10	7	0	
	N_{FP}	0	4	9	5	1	
	N_{FN}	0	0	0	3	10	
	JSC	1	0.714	0.526	0.467	0.0	0.541
FPT	N_{TP}	10	10	10	10	8	
	N_{FP}	6	16	3	157	1413	
	N_{FN}	0	0	0	0	2	
	JSC	0.625	0.385	0.769	0.05	0.00562	0.367

TABLE V. RESULTS OF SPOT TRACKING METHODS USING REAL DATASET

	Manual	IMM	FPT
N_{TP}	29	27	25
N_{FP}	0	11	21
N_{FN}	0	2	4
JSC	1	0.675	0.5

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