Probabilistic Tracking of Virus Particles in Fluorescence Microscopy Image Sequences

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Abstract. Fluorescence time-lapse microscopy is a powerful technique for observing the spatial-temporal behavior of viruses. To quantitatively analyze the exhibited dynamical relationships, tracking of viruses over time is required. We developed probabilistic approaches based on particle filters for tracking multiple virus particles in time-lapse fluorescence microscopy images. We employed a mixture of particle filters as well as independent particle filters. For the latter, we have developed a penalization strategy to maintain the identity of the tracked objects in cases where objects are in close proximity. We have applied the approaches to synthetic images and quantified their performance. We have also successfully applied the approaches to real microscopy images of HIV-1 particles and have compared the tracking results with ground truth from manual tracking.

1 Introduction

The aim of our work is to study the dynamic behavior of the human immunodeficiency virus (HIV) based on live cell microscopy using fluorescently labelled virus particles. Tracking single virus particles yields quantitative information that contributes to the understanding of viral processes (e.g., cell entry). To obtain statistically sound conclusions, many individual particles must be automatically tracked. However, tracking virus particles is challenging. Problems are due to the small size of viruses as well as their complex motion behavior. Also, one has to cope with the large number of virus particles, the relatively high level of cell autofluorescence, as well as a relatively low signal-to-noise ratio.

In previous work on *virus tracking*, a *deterministic* two-step paradigm encompassing virus localization and motion correspondence has been typically employed. For localization, most approaches employ a maximum intensity search strategy, where intensity maxima are associated with virus particles (e.g., [1]). For motion correspondence, approaches that consider the motion of all viruses via graph-theoretical algorithms have been used (e.g., [2]). In contrast to the deterministic schemes, *probabilistic* approaches additionally include a spatial-temporal filter. An approach using a pool of Kalman filters has been presented in [3]. However, the steps of object localization and spatial-temporal filtering are

uncoupled. This entails that temporal information is not used for localization, and analogously image information is not directly used by the filter to estimate the position of an object. In contrast to the Kalman filter, the particle filter, which has been introduced to the field of computer vision in [4], exploits more efficiently the image and temporal information encoded in an image sequence. An approach using a mixture of particle filters for virus tracking has been presented in [5]. There, however, only a fixed number of objects could be tracked. Generally, the number of objects changes over time (e.g., objects enter the field of view).

In this contribution, we introduce probabilistic approaches for tracking multiple viruses in fluorescence microscopy time-lapse images. We have developed two approaches based on particle filters, namely an approach using a *mixture of particle filters* and an approach using *independent particle filters*. In contrast to the former scheme, the latter approach can track a variable number of objects. We address the problem of filter merging that arises when using independent particle filters via a penalization mechanism based on a deterministic motion correspondence algorithm. The developed approaches are fully automatic and have been successfully applied to synthetic image sequences as well as to real microscopy image sequences displaying HIV-1 particles.

2 Materials and Methods

In our approaches, tracking is formulated as a Bayesian sequential estimation problem. At time step t, the aim is to estimate the state $\mathbf{x}_t$ of a virus given a sequence of measurements $\mathbf{y}_{1:t}$. By modeling the temporal behavior using a *dynamical model* $p(\mathbf{x}_t|\mathbf{x}_{t-1})$ and incorporating image information via a *measurement model* $p(\mathbf{y}_t|\mathbf{x}_t)$, a Bayesian filter estimates the *posterior* distribution $p(\mathbf{x}_t|\mathbf{y}_{1:t})$ via stochastic propagation and Bayes' theorem:

$$p(\mathbf{x}_t|\mathbf{y}_{1:t}) \propto p(\mathbf{y}_t|\mathbf{x}_t) \int p(\mathbf{x}_t|\mathbf{x}_{t-1}) p(\mathbf{x}_{t-1}|\mathbf{y}_{1:t-1}) d\mathbf{x}_{t-1}$$

An estimate of $\mathbf{x}_t$ can be obtained from the posterior $p(\mathbf{x}_t|\mathbf{y}_{1:t})$, which, in our case, is estimated using a particle filter. This algorithm approximates the posterior with a set $\{\mathbf{x}_t^i; w_t^i\}_{i=1}^{N_s}$ of N_s random samples $\mathbf{x}_t^i$ (the 'particles') that are associated with importance weights w_t^i. In the case of tracking multiple objects that have a similar appearance, multiple modes arise in the posterior distribution. Although a particle filter can in principle handle such a distribution, in practice the filter cannot maintain the multimodality over several time steps [4, 6]. To address this problem, one may model the posterior $p(\mathbf{x}_t|\mathbf{y}_{1:t})$ as a non-parametric M-component *mixture model*:

$$p(\mathbf{x}_t|\mathbf{y}_{1:t}) = \sum_{m=1}^{M} \pi_{m,t} p_m(\mathbf{x}_t|\mathbf{y}_{1:t})$$

where $\pi_{m,t}$ denotes the weight of the *m-th* component [6]. Each $p_m(\mathbf{x}_t|\mathbf{y}_{1:t})$ is approximated using a set of particles $\{\mathbf{x}_t^i; w_t^i\}_{i \in \tau_m}$, where τ_m is the set of indices

indicating which particles belong to component m, and particles are allocated using a clustering mechanism. Note that $\sum_{m=1}^{M} |\tau_m| = N_s$, where $|\cdot|$ denotes the set size operator. The estimation performance deteriorates as the number of objects increases, since N_s remains constant, i.e., fewer particles are allocated to each component.

Alternatively, one may track multiple objects by instantiating one *independent particle filter* per object. In this case, for each filter, an independent set of particles of size N_s is used. Failures arise when objects are in close proximity, since the filters converge towards the object with the best likelihood $p(\mathbf{y}_t|\mathbf{x}_t)$. To address this problem, we propose a novel penalization strategy comprising three steps: first, the approach determines objects that are in close proximity. This reduces to finding cliques in an undirected graph, where the vertices are given by the filtered position estimates of the objects, and an edge is said to join two vertices if the distance between the positions of two objects is below a certain value. The second step determines the most plausible position $\hat{\mathbf{x}}_t$ for each object in each clique by seeking modes in the probability density function that is induced by merging all particles of all filters of a clique. The plausible positions are assigned to each object via a global nearest neighbor approach [1]. In some cases (e.g., when objects merge), a plausible position might not be found for an object. In this case, this object is not further considered in the penalization scheme; the filter may merge temporarily with another filter. In the third step, the weights of particles that are relatively distant to the most plausible position $\hat{\mathbf{x}}_t$ of an object are assigned lower values via a Gaussian function; given the lower weights, the resampling step of the particle filter might discard the penalized particles.

3 Results

We have applied our approaches to synthetic as well as real microscopy image sequences. To automatically detect virus particles, we employ either the spot-enhancing filter (SEF) [7] or 2D Gaussian fitting (GaussFit) and combine them with the particle filter approaches. The approaches using a mixture of particle filters (MPF) can only track objects that enter the field of view at time step $t = 0$, while those using independent particle filters (IPF) can track objects that enter the field of view at any time step. To measure the performance, we used the tracking accuracy defined as $P_{\text{track}} = \frac{n_{\text{track,correct}}}{n_{\text{track,total}}}$, which reflects the ratio between the number of correctly computed trajectories $n_{\text{track,correct}}$ and the number of true trajectories $n_{\text{track,total}}$. The value for $n_{\text{track,correct}}$ is computed as the weighted sum of the percentage of tracked time steps $r_{\text{tracked},i}$ for each *i-th* true trajectory: $n_{\text{track,correct}} = \sum_{i=1}^{n_{\text{track,total}}} w_i r_{\text{tracked},i}$, where the weight w_i is given by a Gaussian function, which takes as its argument the number of correctly computed trajectories $n_{\text{track},i}$. The weighting scheme is introduced to penalize computed trajectories that are broken. Note that $P_{\text{track}} \in [0, 1]$.

We validated the approaches based on several synthetic image sequences. Here, we describe the experimental results obtained for one image sequence. This

Table 1. Description of real microscopy image sequences

	Dimensions	No. of time steps	No. of objects
Seq. 1	256 × 256	250	23
Seq. 2	512 × 512	200	15
Seq. 3	512 × 512	400	43

Table 2. Tracking performance P_{track} for real microscopy image sequences

	SEF&MPF	GaussFit&MPF	SEF&IPF	GaussFit&IPF
Seq. 1	84.82%	81.95%	86.73%	82.61%
Seq. 2	84.64%	85.64%	93.54%	93.54%
Seq. 3	50.62%	49.04%	74.64%	67.76%

sequence consists of 100 images (256×256 pixels, 16-bit) displaying 20 synthetic particles. The SNR level is 4.55 and the noise model was assumed to be Poisson distributed. The quantitative experimental results are as follows: SEF&MPF achieves 79.28%, GaussFit&MPF attains 80.00%, SEF&IPF yields 85.65%, and GaussFit&IPF achieves 90.13%.

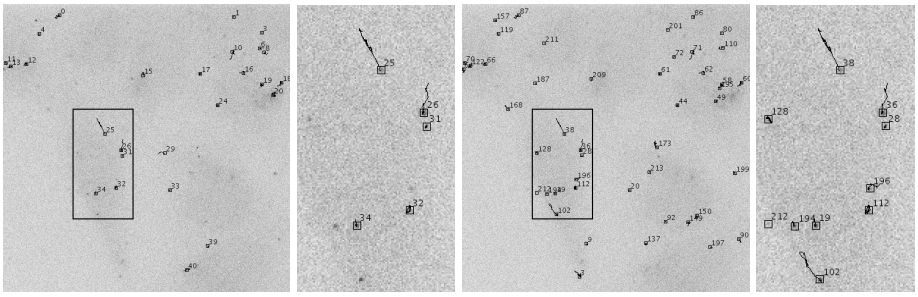
We also validated the algorithms using real microscopy image sequences. In these sequences, fluorescently labeled HIV-1 particles were imaged using a fluorescence wide-field microscope. Fluorophores were excited with their respective excitation wavelengths and movies were recorded with a frequency of 10Hz [8]. Ground truth for the virus positions was obtained by manual tracking using the commercial software MetaMorph. The quantitative experimental results for three sequences are presented in Table 2. Each sequence consists of 200 up to 400 frames (Table 1). Sample images of tracking results for the real sequence "Seq. 3" are shown in Fig. 1. Analogously to the experiments using synthetic data, it turns out that the approaches using IPF yield a higher tracking accuracy than those using MPF.

4 Discussion

Our experimental results suggest that the approaches based on independent particle filters (IPF) outperform those using a mixture of particle filters (MPF). The reason for this is twofold. First, since the MPF allocates a different number of particles to each object via a clustering mechanism, poor estimation results are obtained for those objects with few allocated particles. The clustering mechanism may generate non-compact clusters, which lead to inaccurate position estimates, in particular, for objects that lie in close proximity. In contrast, the IPF not only uses a constant number of particles for each object, but also copes with problems induced by the proximity of objects via our penalization scheme. Second, since the MPF cannot track a variable number of objects, its performance is reduced. In contrast, the IPF is able to handle a variable number of objects.

In summary, we have developed probabilistic approaches based on particle filters for tracking multiple viruses in microscopy image sequences. Our quantita-

Fig. 1. Tracking results for two approaches for the real microscopy image sequence "Seq. 3" (time step $t = 140$). For both approaches, an enlarged section delineated with a black rectangle is shown next to the original image



SEF&MPF: Original image and section SEF&IPF: Original image and section

tive experimental results based on synthetic and real microscopy image sequences show that the approaches yield a good tracking performance. The superior performance of our approach using IPF in combination with the novel penalization scheme suggests that this approach is well-suited for solving the problem of multiple object tracking.

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