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A NOVEL APPROACH TO IDENTIFYING MERGING/SPLITTING EVENTS IN TIME-LAPSE MICROSCOPY

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ABSTRACT

This paper investigates the complex motion of particles in the endocytic pathway. We propose a novel tracking method, which identifies merging and splitting events of vesicles, in dual channel fluorescence confocal microscopy. Large amounts of quantitative data are needed for biologists to make sound conclusions about cellular dynamics. Having an automated method also allows biologists to identify rare events, which would otherwise be very time consuming. A co-localisation state is introduced to identify when vesicles are merged, across two channels. The approach is based on a probabilistic association between estimated vesicle states in each channel. We incorporate this into a reversible jump Markov chain Monte Carlo scheme. The approach has been successfully applied to synthetic videos as well as real data.

Index Terms— RJMCMC, Dual channel, Cell, Tracking, Biomedical imaging

1. INTRODUCTION

This work aims to tackle the problem of tracking fluorescently labelled objects in dual channel confocal light microscopy videos. We also propose a novel technique to identify merging and splitting events across the two channels. These events are rare, however are hugely important to biologists. Here we are tracking transferrin and epidermal growth factor (EGF) within a single cell. Transferrin is a protein that is responsible for carrying iron in the blood. The transferrin receptor is recycled along with its ligand; thus transferrin shuttles back and forth between the extracellular fluid and the endosomal compartment. EGF is a small, extracellular signal protein, that stimulates a cell to divide.

Receptor-mediated endocytosis is a process where cells take in specific proteins by invagination (the inward budding of the plasma membrane) to form vesicles containing proteins with receptor sites specific to the molecules being absorbed. Once absorbed they are sorted and follow varying paths. Endocytosed ligands can remain bound to their receptors and thereby share the fate of the receptors. The fates of the receptors (and their ligands) vary: (1) most receptors are recycled

and return to the same plasma membrane domain which they came, (2) some proceed to a different domain of the plasma membrane and (3) some follow the degradative pathway [1].

Interest is increasing in the potential role of EGF-receptor (EGFR) traffic in the response to cancer therapy. Abnormal expression and abnormally regulated intracellular trafficking of the EGFR play a well-recognized role in oncogenesis. Chemoradiotherapy, —the combined treatment of two DNA-damaging agents, ionizing radiation and an alkylating agent—is a common treatment approach for many cancers. Applying a combination of X-rays or chemotherapy and EGFR-targeting drugs may strengthen the effect of local irradiation in destroying cancer cells and/or revert tumour resistance. If used in conjunction with drugs that specifically target the EGFR, controlling EGFR traffic could gain potential benefits in dealing with tumour resistance in conventional chemoradiotherapy [2].

Tracking in fluorescence microscopy is a challenging problem. Along with complex particle motion, high particle densities and low signal-to-noise ratios (SNRs) make tracking difficult. To achieve statistically sound conclusions many vesicles need to be accurately tracked. Despite the challenges faced with fluorescence microscopy videos, probabilistic approaches have been shown to cope well [3, 4]. Methods based on the Kalman filter offer an analytically tractable solution, only in the case of linear systems with Gaussian noise. Tracking methods based on particle filters (PF) are proposed in [5, 6], however degeneracy problems are well established with PFs as well as the high computational complexity [7]. In [8] an approach which uses probabilistic data association, combines the results of the spot enhancement filter and the Kalman filter to track fluorescently labelled HIV-1 particles. A deterministic approach is proposed in [9], where a greedy algorithm is presented to find correspondences using a metric, based on particle size, intensity and trajectory. All the previously mentioned tracking approaches do not offer any solution to identifying vesicle merging and splitting. To detect merging events in virus particles, [10] proposed using the rate of change of intensity within a PF framework. It is worth noting however, that in [10], merging is defined as a fusion with the cell membrane and that intensity variations are not a good indicator in our problem (due to the separate channels).

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In the presented approach, we introduce a reversible jump Markov chain Monte Carlo (RJMCMC) based tracking method for tracking and identifying merging/splitting events. MCMC tracking methods have been established to be effective in high dimensional spaces [7]. We also incorporate a co-localisation strategy to detect merged vesicles, across multiple channels. The remainder of the paper is organised as follows: In Section 2 we lay the foundations for vesicle tracking and merge/split identification. Section 3 details the proposed algorithm. We evaluate the proposed method in Section 4 and conclude in Section 5.

2. THEORETICAL PRELIMINARIES

An object can be represented by a noisy measurement $\mathbf{z}_t$ and state vector $\mathbf{x}_t$. In order to estimate $\mathbf{x}_t$ given a set of measurements $\mathbf{z}_{1:t}$ a statistical approach to finding the posterior probability density function (PDF) $p(\mathbf{x}_t|\mathbf{z}_{1:t})$, is to use Bayesian filtering, which first computes the prior $p(\mathbf{x}_t|\mathbf{z}_{1:t-1}) = \int p(\mathbf{x}_t|\mathbf{x}_{t-1})p(\mathbf{x}_{t-1}|\mathbf{z}_{1:t-1})d\mathbf{x}_{t-1}$ and then update using Bayes' rule:

$$p(\mathbf{x}_t|\mathbf{z}_{1:t}) \propto p(\mathbf{z}_t|\mathbf{x}_t)p(\mathbf{x}_t|\mathbf{z}_{1:t-1}). \quad (1)$$

Here $p(\mathbf{z}_t|\mathbf{x}_t)$ relates the measurement to the state and $p(\mathbf{x}_t|\mathbf{x}_{t-1})$ is the state evolution model.

The MCMC method is able to cope well with high dimensional spaces. MCMC methods work by defining a Markov chain over the space of configurations $\mathbf{x}$, where $\pi(\mathbf{x})$ is the stationary distribution. The Metropolis-Hasting algorithm is a frequently used algorithm for generating samples in $\pi(\mathbf{x})$. Proposal states, $\mathbf{x}'_{kt}$, are generated (according to a proposal distribution) and the state is accepted with a certain probability. Given N samples, $\pi(\mathbf{x})$ is approximated at time, t : $\{\mathbf{x}_t^{(i)}\}_{i=1}^N \approx p(\mathbf{x}_t|\mathbf{z}_t)$. The RJMCMC method [11] takes into account trans-dimensional moves and therefore can increase or decrease the number of objects being tracked. If the total number of states in a frame t is n , then the object specific state can be represented as $\mathbf{x}_{kt}$ where $(k \in \mathbb{Z} : k = 1, \dots, n)$ and $p(k_t, \mathbf{x}_t|\mathbf{z}_t) \propto \{k_t^{(i)}, \mathbf{x}_t^{(i)}\}_{i=1}^N$. A move/jump is selected based on the proposal density, $Q_m(k'_t, \mathbf{x}'_{kt}; k_t^i, \mathbf{x}_{kt})$, where m' is the reverse jump to move m . In our application we consider birth/death and update moves—a birth is a vesicle entering the field of view, coming into the focal plane, or separating from an existing vesicle, the opposite for a death. A move is selected with a certain probability (p_b, p_d, p_u) , respectively. Given the proposal state, the acceptance ratio for that state is expressed as $\alpha = \frac{p(k'_t, \mathbf{x}'_{kt}|\mathbf{z}_t) p_{m'}}{p(k_t, \mathbf{x}_{kt}|\mathbf{z}_t) p_m} \frac{Q_{m'}(k_t, \mathbf{x}_{kt}; k'_t, \mathbf{x}'_{kt})}{Q_m(k'_t, \mathbf{x}'_{kt}; k_t, \mathbf{x}_{kt})}$. For the update move the proposal density is a Gaussian distribution ($\sigma^u = 0.3$). Given k_d as the set of detected objects, an object O_b is added to the identifier set k_t using proposal distribution $Q_b = \frac{1}{|k_d \setminus k_t|}$. Where $(k_d \setminus k_t)$, is the set of objects that have been detected but are not a part of k_t . Given the set of objects $(k_t \cap k_d)$ in k_t that correspond (nearest neighbour) to

more than one detection, in k_d , an object O_d is selected with probability $Q_d = \frac{1}{|k_t \cap k_d|}$. If no new object is detected or all objects are accounted for $Q_{b|d} = 0$ and the move is not taken. We can now define the acceptance ratios for the birth, death and update moves, respectively:

$$\alpha_b = p(\mathbf{z}_t|\mathbf{x}_b) \frac{p(k'_t, \mathbf{x}'_{kt}|\mathbf{z}_{1:t-1}) p_d |k_d \setminus k_t|}{p(k_t, \mathbf{x}_{kt}|\mathbf{z}_{1:t-1}) p_b |k'_t \cap k_d|}, \quad (2)$$

$$\alpha_d = \frac{1}{p(\mathbf{z}_t|\mathbf{x}_d)} \frac{p(k'_t, \mathbf{x}'_{kt}|\mathbf{z}_{1:t-1}) p_b |k_t \cap k_d|}{p(k_t, \mathbf{x}_{kt}|\mathbf{z}_{1:t-1}) p_d |k_d \setminus k'_t|}, \quad (3)$$

$$\alpha_u = \frac{p(\mathbf{z}_t|k'_t, \mathbf{x}'_{kt}) p(k'_t, \mathbf{x}'_{kt}|\mathbf{z}_{1:t-1}) Q_{m'}(k_t, \mathbf{x}_{kt}; k'_t, \mathbf{x}'_{kt})}{p(\mathbf{z}_t|k_t, \mathbf{x}_{kt}) p(k_t, \mathbf{x}_{kt}|\mathbf{z}_{1:t-1}) Q_m(k'_t, \mathbf{x}'_{kt}; k_t, \mathbf{x}_{kt})}. \quad (4)$$

2.1. Co-localisation State

The objective of our tracking approach is to identify merging/splitting events in dual channel fluorescence microscopy. Red and green coloured fluorescence are used in our experiments. For simplicity we label the red channel, I_r , as a reference channel and its corresponding channel as I_g . The task is to find co-localised vesicles in I_g using the reference channel. We represent the state of a vesicle with the state $\mathbf{x}_t = (x_t, y_t, \sigma_t, \gamma_t)$, where (x_t, y_t, σ_t) are object position and size features. We introduce the co-localisation feature $\gamma_t \in [0, 1]$. A vesicle in I_g is co-localised with a vesicle in I_r if $\gamma_{kt} = 1$. In order to calculate γ we use the states of the objects in $(I_r, \mathbf{x}_{rt})$, and the state of an object in $(I_g, \mathbf{x}_{gkt})$; we determine the probability of merging by using a pairwise Markov random field (MRF) (V, E) , with nodes V and edges E . Edges are formed between two objects using the location vectors $\mathbf{x}_{gk1}$ and $\mathbf{x}_{r1}$, obtained from $\mathbf{x}_{gk}$ and $\mathbf{x}_r$ respectively. $\mathbf{x}_{r1}$ is selected by the nearest neighbours algorithm. The following potential function is employed:

$$\gamma^{prob} = \exp(d(\mathbf{x}_{r1}, \mathbf{x}_{gk1}) - 1) : \mathbf{x}_{r1} \in \mathbf{x}_{rt}. \quad (5)$$

Here d is Dice's coefficient between two circles (radius 15) with centers at points $\mathbf{x}_{r1}$ and $\mathbf{x}_{gk1}$. An object state is set to the merged state, with probability γ^{prob} . Objects which are not closely localised have $\gamma^{prob} = 0$, therefore will have no chance of being labelled as merged. To obtain the final state of γ_t from the Markov chain, we take the mode. Split events are identified when γ changes from 1 to 0.

3. RJMCMC VESICLE TRACKER

Vesicle fluorescence can be difficult to identify in fluorescence microscopy images; photobleaching and autofluorescence add to this [6]. To detect vesicles we do the following steps 1) create a mask by thresholding a frame, by the

98th percentile. 2) Smooth the frame using a Gaussian kernel (with a standard deviation the diameter of a vesicle). 3) Apply the mask to this smoothed image and find the regional maxima to obtain k_d . We model the appearance of a vesicle using Gaussian fitting with parameters, (x, y, σ) . However, it is important to note that for our application we could include the vesicle intensity —intensities are normalized here—in the state but our current approach gave good results. Our observation model is now $p(\mathbf{z}|\mathbf{x}) \propto \exp(D(\mathbf{z}, g(\mathbf{x}))^2)$, where $g(\mathbf{x})$ is the Gaussian function and D is the Euclidean distance. The motion model describes how a state evolves over time; vesicles tend to move randomly, hence we define $p(\mathbf{x}_t|\mathbf{x}_{t-1})$ as a Gaussian function with standard deviations (σ_x) . We assume that vesicle sizes stay the same. We set γ to its previous value and initially we assume that objects are not co-localised. A penalization term, to discourage a tracker from tracking the same object, was proposed in [12]. To model object interactions, a similar approach to Section 2.1 is used, where a MRF is constructed (V, E) , with edges formed between objects which are close to each other. Object interactions are incorporated into the motion model where:

$$p(\mathbf{x}_t|\mathbf{x}_{t-1}) \propto \prod_{j1} p(\mathbf{x}_{j1t}, \mathbf{x}_{j1(t-1)}) \prod_{j1, j2 \in E} \exp(-d(\mathbf{x}_{j1t}, \mathbf{x}_{j2t})).$$

$\mathbf{x}_{j1t}$ and $\mathbf{x}_{j2t}$ represent the position vectors of a pair of objects and $j1 \neq j2$. Here d is defined previously, but with two circles the same size as the corresponding vesicles.

The implementation of our RJMCMC dual channel tracking method is described in the pseudocode. To avoid redundancy we assume that the states from the reference image, $\mathbf{x}_{rt}$, are already estimated. B denotes the number of burn-in iterations, where samples are not stored during this period. Within real microscopy images there may be many spurious detections, due to autofluorescence and vesicles quickly entering and leaving the field of view. These objects do not need to be tracked, so like [6] we propose passing the objects through a buffer, where tracks are only recorded when $(t - t_d) > t_{thresh}$, where t_d is the time of detection.

4. EXPERIMENTS AND RESULTS

We have applied our approach to synthetic and real microscopy time lapse videos. We used two sets of synthetic videos to verify our approach. The first demonstrates the overall tracking accuracy of our approach. We also compare our results to the well-established microscopy tracking method u-track [13]. We set the following parameters as: $\sigma_x = 0.3$, $p_b = 0.08$, $p_d = 0.08$, and $t_{thresh} = 5$. We also do 40 iterations in our MCMC method, where the first 20% of samples were discarded as the burn-in. In our synthetic images we simulated 40 vesicles, modelled as a Gaussian with $\sigma = 1.5$. The sequence lasted 100 frames where each frame was (512×512) . They varied in intensity and exhibited a random motion. Vesicles were also allowed to leave/enter the scene or enter/leave the focal plane. We added vary-

Algorithm 1: Vesicle tracking with merge/split identification

Generate samples $(k_{gt}^i, \mathbf{x}_{gt}^i)_{i=1}^n$.

for $k = 1, \dots, n$ **do**

• Initialize sampler where target $(k_{gt-1}, \mathbf{x}_{gt-1})$ is updated according to the motion model, and used as the initial sample in the Markov Chain.

for $i = 1, \dots, (N + B)$ **do**

• Select a move type m , where

$$p_u = (1 - (p_b + p_d)).$$

• Obtain the new $(k'_t, \mathbf{x}'_t)$, based on the move selected from Q_m .

• Determine γ'_t from γ'^{prob} given $\mathbf{x}_r$ and $\mathbf{x}'_{kt}$, as outlined in (5), if states are within 4 pixels.

• Calculate the acceptance ratio

$$\alpha = \min(1, \alpha), \text{ based on the move type from: } ((2), (3), (4)).$$

• Accept the proposed move with probability α i.e. $(k_{gt}^i, \mathbf{x}_{gt}^i) = (k'_t, \mathbf{x}'_t)$; otherwise set as previous sample.

• If a move type that increases dimensionality is selected: $(k_{gt}, \mathbf{x}_{gkt}) \cup (O_b, \mathbf{x}_b)$.

• If a move type that decreases dimensionality is selected: $(k_{gt}, \mathbf{x}_{gkt}) \setminus (O_d, \mathbf{x}_d)$.

end

end

ing levels of Poisson noise, with five equally spaced SNRs ($Seq_{1-5} = 1.91 - 7.68$). Here $SNR = \frac{I_{max} - I_b}{\sqrt{I_{max}}}$ (as described in [3]), where I_{max} is the expected peak intensity and I_b is the expected background intensity.

We test our merge/split approach in our second set of tests. Here we create two channels (with the same levels of noise) and compare our detected merged tracks to ground truth tracks of the merged vesicles. Sequences are created as previously described but with higher particle density. Frames were 150×150 pixels. 75% of vesicles were merged; also vesicles had the possibility of splitting. Vesicles in separate channels that were within 6 pixels of each other were set to be co-localised.

In Fig. 1 we show the average root mean square error (RMSE) on the vertical axis. We calculate the RMSE as the Euclidean distance between corresponding tracked and ground truth vesicles. It is labelled as ‘proposed’ in Fig. 1. The same set of test videos were tracked using u-track. Our second set of experiments are labelled as ‘proposed-merged’. Here split events (the end of a merged track) were correctly identified; however, areas of improvement could be focused on dealing with temporary vesicle disappearance. We also demonstrate our method on real microscopy videos. The first channel is transferrin bound to Alexa Fluor 488 and the second is EGF bound to Alexa Fluor 647. In Fig. 2 an image

Fig. 1. RMSE of our tracking method ‘proposed’ and u-track. RMSE of co-localised tracks labelled ‘proposed-merged’.

Fig. 2. Transferrin vesicles (top). EGF vesicles tracks (bottom). At frames 5, 20 and 50, left to right respectively.

section 150×150 pixels is shown. Detected vesicles are circled in red, while merged vesicles are shown with a green arrow. The blue lines represent the paths of the merged vesicles. While manual tracks for these videos are difficult to obtain results were verified by one co-author, Paul Verkade a cell biology expert.

5. CONCLUSION

In this work we have presented a RJMCMC approach for tracking and identifying merging and splitting events in dual channel fluorescence microscopy videos. The two channels represent the transport of Transferrin and EGF within a cell. Identifying merging and splitting events can help biologists understand how the cell sorts, recycles and degrades these molecules; this can lead to a better understanding of cell proliferation and cancer. In our approach we introduce a probability based co-localisation state as a robust way to decide whether vesicles are merged or not. We evaluate our vesicle tracking method on realistic synthetic and real videos. Our method also identified and tracked merged synthetic vesicles with a high degree of accuracy.

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