A NOVEL MULTIPLE PARTICLE TRACKING ALGORITHM FOR NOISY IN VIVO DATA BY MINIMAL PATH OPTIMIZATION WITHIN THE SPATIO-TEMPORAL VOLUME

Quan Xue[1, 2] *and Mark C.Leake*[1, 2]

[1]Clarendon Laboratory, Oxford Physics, University of Oxford, Parks Road, Oxford, OX1 3PU, UK.
[2]Oxford Centre for Integrative Systems Biology (OCISB), South Parks Road, Oxford, OX1 3QU, UK.

ABSTRACT

Automated tracking of fluorescent particles in living cells is vital for subcellular stoichoimetry analysis [1, 2]. Here, a new automatic tracking algorithm is described to track multiple particles, based on minimal path optimization. After linking feature points frame-by-frame, spatio-temporal data from time-lapse microscopy are combined together to construct a transformed 3D volume. The trajectories are then generated from the minimal energy path as defined by the solution of the time-dependent partial differential equation using a gray weighted distance transform dynamic programming method. Results from simulated and experimental data demonstrate that our novel automatic method gives sub-pixel accuracy even for very noisy images.

Index Terms— fluorescence microscopy, particle tracking, energy function, gray weighted distance transform

1. INTRODUCTION

Tracking fluorescently-tagged nanoparticles in living cells allows monitoring of the dynamics of vital subcellular structures. While Green Fluorescent Proteins (GFP) and its variants offer enormous potential for increasing our understanding of biological processes [3], they also bring challenges for quantitative analysis requiring efficient techniques to evaluate enormous data flow. Manual methods are time consuming and susceptible to user bias; automatic tools for time-lapse microscopy are essential for quantization and systematic study of heterogeneous biological systems, e.g. estimating the diffusion coefficient of proteins [1] or investigating molecular turnover [2] *in vivo*.

Traditional approaches depend on locally correlated information by linking feature points directly. Nearest neighbor methods are simple but very sensitive to noise. Statistical methods use probability density functions for matching a variable number of feature points by integrating of spatial and temporal information. MHT [4] is difficult to be applied directly because NP-hard methods prohibit fast computation. The computational complexity of Particle filter [5] increases very much as well. With the effective prior knowledge about object dynamics, the possibility method such as Bayesian estimation [6] is more effective for tracking accuracy, and generalized likelihood ratio test [7] is shown to provide good tracking result in single-particle tracking. Heuristic methods [8, 9] are deterministic algorithms and can be used to identify putative tracks from qualitative descriptions, but suffer the disadvantage in relying critically on the accuracy in the detection stage and easily fail when ambiguities occur. To overcome errors from failed detection or transient disappearing particles, additional methods are required. Improvements are made in considering the image stack as a 3D volume and transforming the problem into finding the minimal energy path. Typical approaches include fast marching method to resolve the energy function for single-molecule tracking [10]. Only considering the positive force, [11] uses the similar method as [10] in path planning approach to control the zero level set for tracking cells. In [12] a special correlation formulation is described between the spots from continuous frame and then is applied to resolve the energy function. The linear assignment problem solution provides a generalized method based on exacted function to resolve the energy function [13]. These methods are comparatively robust to deal with ambiguities due to object fusion, missing detection and appearance/disappearance of multiple targets in fluorescence particle tracking.

Here, we combine spatial and temporal information by considering the fluorescence image stack as a single 3D volume and propose a new method for viewing particle trajectory as 3D curves within this spatio-temporal volume. The question of particle tracking is then transformed into finding minimal paths in an image-dependent metric from an energy minimization technique. The energy function is defined by the time-dependent and the function is solved using the gray weighted distance transform (GWDT). The tracking system exploits a two-level design: linking and association. The bottom level is responsible for connecting possible spot features by monitoring their displacement into track segments in a successive frame-by-frame manner, resulting in truncated track segments of whole of trajectories. The top level then fuses segments and co-ordinates outputs to obtain the intact full trajectory. Our new approach culminates in similar results to those of earlier studies [10-12] but offers a significant advantage in being shorter in computational complexity and requires far less computer time.

2. METHODS

When considering two-dimensional fluorescent image $I(x,y)$ changing with the time t in a stack, a single three-dimensional (3D) spatio-temporal volume $V(x,y,t)$ will be constructed, so that the particle trajectories can be viewed as a 3D curve in this representation [10]. As a result, minimal paths can be found by energy minimization [14]. The cost function defined is a function of time so the solution of this equation represents the arrival time from starting point p_c through moving point p to destination point q.

$$u(p_c, q) = \inf_{(p,q)\in V} \{V(x,y,t)\} \tag{1}$$

$u(p_c, q)$ is the minimal energy path, which also implies minimum arrival time along a path $(p_c \cdots p \cdots q)$ inside the spatio-temporal volume $V(x,y,t)$.

When the speed of the front is uniform within the volume, the arrival time is proportional to the minimum distance, GWDT [15] will provide one solution for above equation, which provides a single pass algorithm and whose value at each pixel gives the maximal sum of distances from the light sources weighted by the gray values in the reflective index field.

The grid points in discrete space can be selected as the max-sum difference (u) between neighboring pixels:

$$u(p_c, q) = \max\left\{ w(p_c)\cdot u(p_c, q), \max_{p\in\Omega}\left(w(p)\cdot u(p,q) + w(p_c)\cdot u(p_c,q)\right)\right\} \tag{2}$$

Ω is the connected local region with specified gray weights $w(\cdot)$ for each element p. The sum of local maximal distance between a pixel p and the first pixel element in the grid is determined according to the distance transform image.

Firstly the original gray intensity volume is shown in Fig 1 (a), secondly a distance transform volume is defined, where each pixel is allocated a new distance value. Here, we apply the chessboard distance transform in Fig 1 (b) and this volume will be regarded as the reference marker to point out the potential evolution direction in the following step. By using the gray values as weights and comparing the maximal intensity sum from adjacent voxels, all of the optimal paths from bottom to top layer through the 3D spatio-temporal volume are calculated based on the upwind propagation principle and constructed as a new volume named potential optimal path volume shown Fig 1(c). Lastly the optimal path including the heading and tailing point provides a sequence of points as associated trajectory from the bottom to top

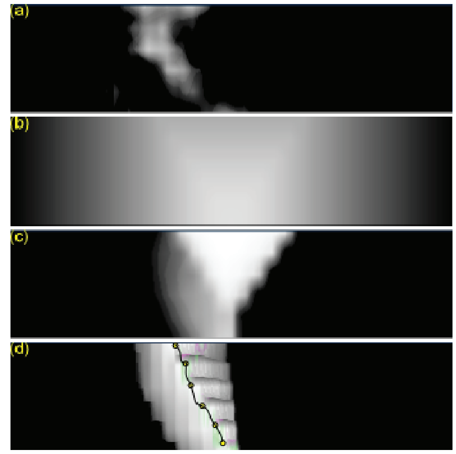

Figure 1. Illustration of optimal path construction during the trajectory association process. Every displayed image is a sectioned view of the stack showing the x-z plane where the motion occurs. (a) Fluorescence image. (b) Distance transforms image. (c) Optimal path image. (d) Reconstructed trajectory by minimal energy path.

A. Initialization phase

1. Intensity volume: reconstructed from time-lapse image stacks.
2. Distance volume: cumulative cost matrix defined by distance transform.
3. Edge volume: all zero volume except the spatial elements, whose position corresponds to the maximal value in distance volume and they have the original gray value.

B. Loop phase

From the non-zero element in edge volume, for each neighboring voxel in 6-connectedness in a 3D volume:

- If the sum of gray intensity of the nearest-neighbor according is larger than existing maximal sum by equation (2), the selected voxels are inspected. The maximal sum of this intensity is saved into the optimal path volume for further consideration.
- If the voxels that do not have maximal sum from nearest-neighbor pixels, they are left unassigned.

C. Termination phase

The optimal path volume is obtained, from which the path connecting starting point and end point are extracted as the associated trajectory.

Figure 2. GWDT algorithm to find the minimal energy path from the spatio-temporal volume for trajectory association.

layer shown in Fig 1(d). The detailed GWDT Algorithm for trajectory association is listed in Figure 2. This algorithm is recursive and belongs to a dynamic programming approach.

Nominally we assume movement smoothness for our tracking method since the particles come from some kind of random diffusion system. As the final detected trajectory is limited within a fairly direction path, this condition prohibits the combinational explosion during volume construction to collect optimal paths in the GWDT. We define a "bounding box" whose center in the bottom layer is the starting point, thus all potential candidates in each frame are considered within this limited box and the investigated paths still provide good approximations. In our applications, we select five pixels as the maximal distance between continuous frames.

3. EXPERIMENTS AND RESULTS

3.1 Evaluation of simulated data

To evaluate the quality of our tracking algorithm and its suitability to biological data, we generated artificial sequences (128pixel×128pixel×30frame). The image was created based on Gaussian profiles for objects and Gaussian background noise with different standard deviations to simulate different levels. The positions of the visual particles are controlled by the random diffusion model, and the spots were made to move randomly and their direction changed randomly at a random time $\langle r^2 \rangle = 4Dt$, where r is the distance from the origin to next point and its position varies with temporal parameter t, and D is the diffusion coefficient. In our experiments, particle velocity is limited to 1-3 pixels/s. It is also important to characterize the adaptation for different fluorescent phenomena such as fluorescent blinking, so spots were allowed to temporally aggregate and cross on several frames.

The level of the noise is characterized by the peak-signal-to-noise ratio (PSNR). We then use the root mean square error (RMSE) to evaluate the accuracy of the tracking. Fig 3 (a) displays the performance of the tracking algorithm when the image is affected by noise. Fig 3 (b) provides the tracking accuracy for five individual trajectories. We find that at PSNR<7dB the accuracy is no longer sub-pixel, the main reason is that the detected particles are not connected correctly during the linking process making it hard to decide whether the segmented trajectories should be connected or not subsequently. Figure 4 displays two tracking results with different intensity profiles of five generated particles. It can be seen that the automatic tracking result is continuous during the whole sequence and can deal with the merging and splitting correctly: since this result stems from tests on simulated data we know that the total number of objects should be fixed, and their trajectories should be continuous from the beginning to the end of frame; "continuous" indicates that the number and length of trajectories is fixed such that any new trajectory and new length observed must indicate an error of the detection algorithm, which comes from either "splitting" (incorrectly generating two apparent trajectories from a single track) or "merging" (incorrectly combining two trajectories into a single track). Figure 5 shows mean-square displacement (MSD) curves corresponding to the movement of particles in Figure 4 and shows that the visual motions are confined in a specified volume consistent with the diffusion coefficient defined in our simulated system.

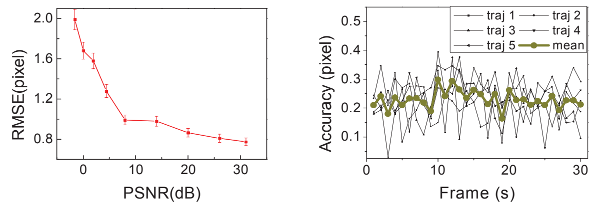

Figure 3. (a) RMSE on the trajectories with the varying PSNR. (b) Tracking error for five simulated trajectories.

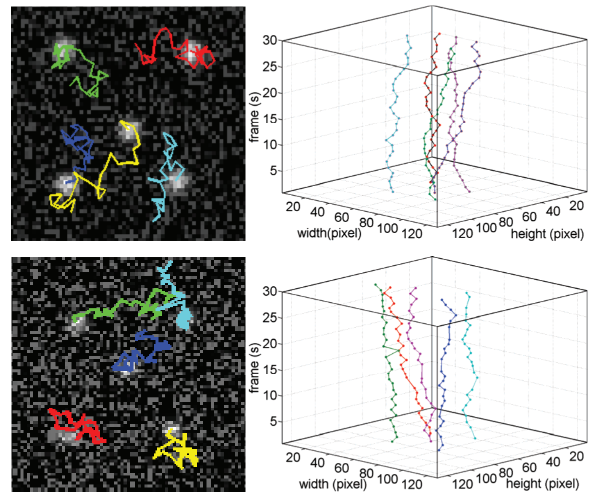

Figure 4. Tracking of simulated particle sequences with different intensity profile and noise levels. Overlayed on 2D original image (left) and its 3D trajectories (right).

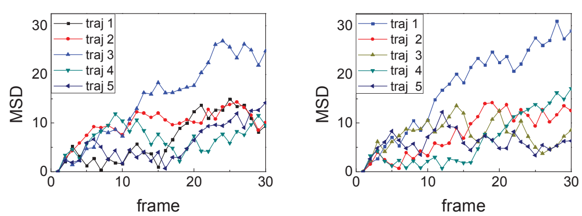

Figure 5. Average MSD for five simulated particles. By doing a linear fit to these traces we found that the predicted and assigned diffusion coefficients always agreed to within better than ~10%.

3.2 Evaluation of experimental data

We applied our methodology to dynamic analysis in real data from video sequences which contain ~100 living bacterial cells (*E. coli* strain WX103 labeled with fluorescent proteins TetR-YFP and LacI-CFP) using image sequences with dimension 512×512×30. Results are shown in Figures 6 and 7. It can be seen from these that the fluorescent blinking can be overcome effectively even with skips of several frames. The integrated and continuous trajectories can be obtained using our algorithm, to a similar standard as earlier methods [10-12].

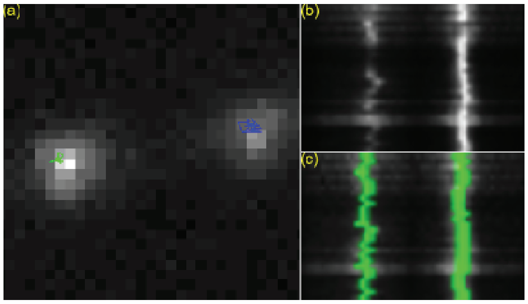

Figure 6. Visualization of the results in a 2D image with 2 tracks (cropped from real data). (a) Trajectories on x-y view frame (b) original trajectories on x-z view. (c) Corresponding trajectories (volume rendering) in x-z view.

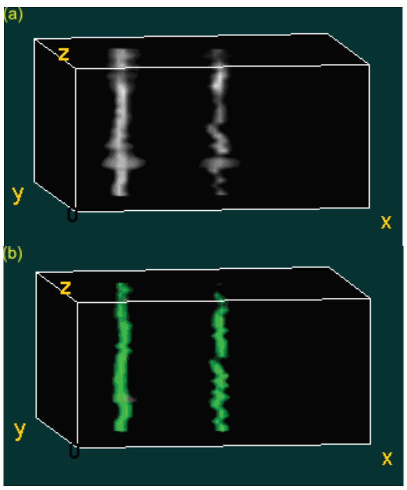

Figure 7. (a) 2D+T particle movement representation in a 3D spatio-temporal volume. (b) 2D+T tracking detected result (volume rendering) using our technique.

4. CONCLUSION

We have demonstrated the applicability of multiple particle tracking based on GWDT dynamic programming for a minimal energy path within a single spatio-temporal volume. Our results demonstrate that the automated method produces sub-pixel accuracy for PSNR>7dB. We are validating this method now for other biological applications in order to enhance the performance over existing automated tracking software for subcellular dynamics.

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