Robust tracking of multiple moving objects in subcellular, time-lapse microscopy assays

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Introduction

A new generation of microscope and fluorescent probe technologies is enabling the quantitative characterization of the spatial-temporal properties of discrete proteins or organelles in living cells. The accurate detection and tracking of multiple objects in time-lapse images is challenging because object dynamics can change over time and objects may aggregate, temporarily causing their appearance to change. Existing algorithm performance is inadequate for the vast majority of assays, and much of the characterization is done manually; which is tedious, subjective and irreproducible.

We are developing a robust tracking algorithm incorporating a "headlight" method to reduce the potential number of track match candidates. The "headlight" search region is determined by the temporal state of the object. Track candidate matching is done within the headlight region based on spatial and temporal match characteristics.

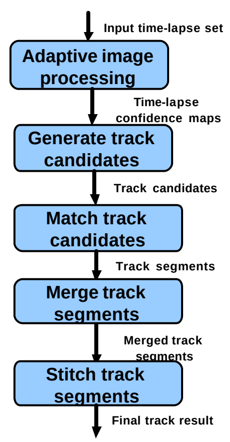
In this study we determine a performance baseline for this preliminary algorithm using two sets of real time-lapse images from vesicle tracking experiments. Algorithm performance is evaluated using a tracking accuracy metric. The tracking "truth" was created manually and validated by independent review and update. We compared standard correlation based track candidate matching with our new robust tracking method to provide an evaluation baseline. The results show that the new method is significantly better than a standard correlation, yet we are continuing to improve performance.

For the next step, we will improve the algorithm with a dynamic, state based controller that can selectively apply algorithm components depending on the state. In this study we have reviewed algorithm performance with respect to track states, which include idle, random or linear motion, and isolated, crowded or merged confluence. This data will help us create rules for the state based selective processing. Additional algorithm components such as bidirectional tracking and iterative track integration will be evaluated.

Robust tracking method

Due to the low signal to noise level of sub-cellular objects, and high segmentation accuracy requirement for a reasonable tracking performance, the conventional approach of segmentation mask generation – object matching approach cannot yield a reasonable automatic tracking result for multiple objection tracing in time-lapse images. Here we present an unique robust tracking algorithm to achieve the automatic tracking goal.

Fig 1. Adaptive spatial temporal detection consists of five steps.
1) Input time-lapse images are adaptively processed to generate confidence maps.
2) The high confidence map regions are then detected, the top 5 best matching score are recorded. The morphology of the detection regions with lower confidence level is considered to produce track candidates.
3) Future and past positions of track candidates are considered to match objects into track segments. Perform iterative match using the match score.
4) Headlight track merge.
5) Track stitching.



The robust tracking is realized by recording the top 5 best matching scores of objects in current frame for each high confidence objects in previous frame. Then find the best matching pairs through the iterative best matching algorithm. Matching score are contributed by two parts: spatial matching score M_s and temporal matching score M_t.

Spatial matching score: the spatial matching score M_s is generated by object confidence values. We used object confidence correlation (method 1) and maximum confidence (method 2) for tracking performance evaluation.

Object confidence correlation is calculated by performing normalized correlation of the pixels above the low confidence level on both current and previous frames. This generates the spatial matching score for the correlation method as follows:

$$M_{s\_Corr} = \frac{(\sum I_{i,t-1} I_{j,t} * n - \sum I_{i,t-1} \sum I_{j,t})^2}{[\sum I_{i,t-1}^2 - (\sum I_{i,t-1})^2] * [\sum I_{j,t}^2 - (\sum I_{j,t})^2]}$$

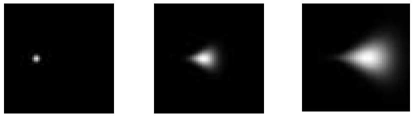
The maximum confidence based method calculates spatial matching score using the ratio of the maximum confidence of the object to the value of the given high confidence level (the maximum ratio is limited to 1).

$$M_{s\_I} = \frac{I_{max}}{I_{highConf}}$$

Temporal matching score: the temporal matching score M_t is generated by the matching candidate location and the predicted location from the trajectory up to the previous frame by the following equation.

$$M_t = e^{\left(-0.5 * \frac{\|V_{i_{t-1},j_t}\|^2 \|V_e\|^2 - (V_{i_{t-1},j_t} \bullet V_e)^2}{\|V_e\|^2}\right)}$$

In which V_e the estimated velocity according to the object i's trajectory history, and $V_{i,j}$ is the velocity between object i in previous (t-1) image frame and object j in current (t) image frame. Furthermore, the algorithm also evaluates the object states, which can be used to determine the parameters and threshold for the best matching search (future extension).



a) Idle object tracking probability region

b) Middle speed tracking probability region

c) Fast speed tracking probability region

Fig 2. The image illustrates the expected probability distribution as temporal matching score in headlight search region when the estimated speed V from previous frames is (a) 0, (b) 80, and (c) 160 in x direction. The highest temporal matching score locations are at $X_{t+1}=X_t+V$, $Y_{t+1}=Y_t$.

The final matching score is the product of spatial matching score and temporal matching score. That is, $M = M_s * M_t$.

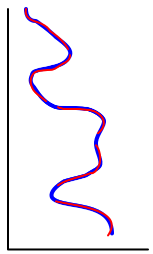


Fig 3. Detection performance is evaluated using the detection accuracy metric which is illustrated in the figure. Detection accuracy is measured for each "true" track shown in blue. The "truth" was created manually using SVCell (SVision LLC / Bellevue, WA) and subject to two rounds of independent review. Detection accuracy is calculated as the number of detected time points (test centroid, shown in red, is within three pixels of the truth centroid) divided by the number of total time points in the truth track. Only the best matching test track is used for the accuracy calculation.

Study materials and method

Detection performance is evaluated using the detection accuracy metric as described in Figure 3

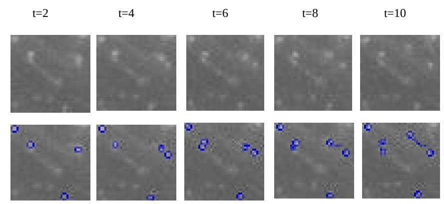


Fig 4. Four time-lapse image sets showing vesicle dynamics in pancreatic duct epithelial cells were used in the study. Two sets of images were from an epi-fluorescence microscope having 160 frames; another two were from a total internal fluorescence microscope having110 frames. The vesicles were labeled with FM-143. The images are captured at one frame per second, with 0.11X0.11 µm pixel size.

The figure shows a representative image sequence with study track segments overlain. It shows stable and merging objects with idle and linear motion states.

Results

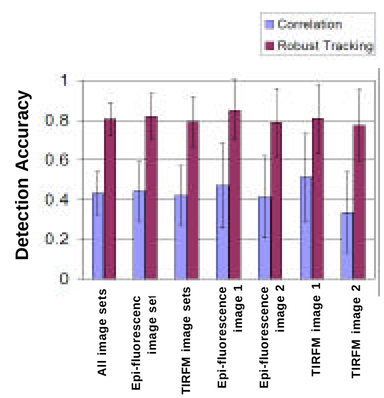


Fig 5. Study results show that the robust tracking method of candidate matching is consistently and significantly better than the correlation method for these image sets. All results are significant at the 95% confidence level (p value < 0.05). Values show the mean detection error comparing the robust tracking method and correlation method. Error bars show the 95% confidence interval.

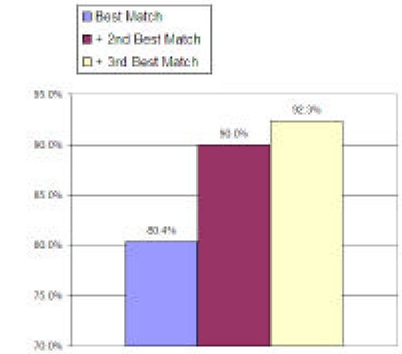


Fig 6. Tracking performance can be improved remarkably if the best matching track segment could be connected to additional matching track segments. The figure shows the tracking accuracy for the best match test segment (80.4%), the tracking accuracy for the best match and second best match segments combined (90.0%), and that for the top three match segments (92.3%).

	Isolated	Cluster	Merge
Idle	26/7740	52/1562	6/6
Linear	1/20	2/8	0/0
Random	2/141	15/67	0/0

Table 1. Error analysis by track states shows that random and idle movement in clusters is a good problem type to address next. We have created an initial state machine that can identify track states such as idle, linear, random movement, and isolated, cluster or merged type confluence. Here we report the # of missed detections / # of correct detections in each state by comparing all test tracks against the truth tracks. In the future we can use the state machine to adaptively apply algorithm components depending on the track state to further improve tracking accuracy.

Next Steps

- We will use the state machine approach to apply new algorithms such as bidirectional tracking and iterative track integration when the track is in a high error (un-reliable) state
- We expect to achieve results similar to the 1st + 2nd Best Match tracking accuracy (90%) using this approach
- We plan to further test our algorithms with additional movies and experiment types including bead and bacteria tracking and virus tracking.

Acknowledgments

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