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

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Introduction

A new generation of microscopy and fluorescent probes technologies is enabling the quantitative characterization of the spatial-temporal properties of discrete processes 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 improvable.

We developed a new 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 matching 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 updates. We compared standard correlation-based track candidate matching with our new robust tracking method to provide an evaluation baseline. The results show that our new method is significantly better than a standard correlation, yet we are continuing to improve performance.

For the new 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 parameters that provide consistent detection accuracy for several object types, which include idle, random or linear motion, and isolated, crowded or merged configurations. The results suggest rules for dividing the state space to reduce computational complexity. Additional algorithms can be applied to track both individual and aggregate pocket objects in a single image.

Robust tracking method

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

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

Spatial matching score: The spatial matching score Mn 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 in both current and previous frames. This generate spatial matching score through the correlation method as follows:

\[ M_n = \frac{1}{\sum_{i=1}^{N} \sum_{j=1}^{N} (x_i - \bar{x})(y_j - \bar{y})} \]

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

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In which Vt, i 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 criteria extension.

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

Results

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

Detection accuracy is calculated as the number of detected time points (true correlations, shown in red, within three points of the truth centroids) divided by the number of total time points in the truth track. Only the best matching test track is considered to produce a confidence level (the maximum ratio is limited to 1).

To determine the optimal match test segment, the tracking accuracy for the best match test segment (80.4%), the tracking accuracy for the best match test segment, and the top three match segments combined (90.0%), and that for the top three match segments (92.3%).

Study materials and method

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

Table 1. Error analysis by track states shows that random and idle movement in clusters is a problem good 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. We report the # of missed detection / # 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.

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Next Steps

We will use the state machine approach to apply additional tracking algorithms to improving the real-time tracking accuracy.

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