Automated Kinetic Characterization of Intracellular Single-molecule Tracking

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Introduction

Single molecule imaging using GFP-based bright fluorescent markers allows direct visualization of the structural dynamics of proteins of interest while they perform their functions. This enables new tools to investigate the sub-molecular mechanism of intracellular trafficking. Accurate tracking of multiple objects is required to quantitatively characterize the kinetic behavior and interaction of single-molecular "objects" in a movie. This is a challenging task due to complex object dynamics (varying object speeds, directions, contrasts and morphologies) imaging noise and sample obscurations.

We have developed a fully automated, highly robust and flexible tracking method called "soft tracking" generating tracks for our kinetic characterization tool yielding generally accurate tracking results1,2. To further improve the tracking accuracy for multiple interacting fast moving objects, we separated object movements into multiple motion energy channels. Soft tracking performs motion guided self-checking where relative motions derived from inter-frame tracking match pairs are checked with the independently generated motion energy channels to resolve any ambiguous tracks.

Teachable Subcellular Tracking Architecture

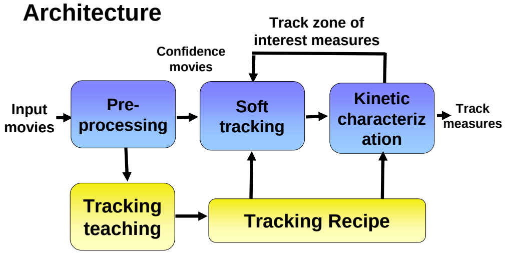


Fig 1. Teachable subcellular tracking architecture consists of a preprocessing step, a soft tracking step and a kinetic characterization step. It is implemented in SVCell™ kinetic module.

Input movie(s) are pre-processed to generate confidence maps. The high confidence map regions are then detected. The morphology, kinetic and object states are considered to produce track candidates and to match objects into track segments.

The soft tracking and kinetic characterization can be taught by a teaching step to generate tracking recipe that can be applied to multiple input movies for kinetic high content screening. Pre-processing is performed by soft matching to generate a high confidence map using teachable structure guided processing[2].

Kinetic characterization is categorized into three movement related states

- **"Idle motion"** objects that is almost stationary.
- **"Directional motion"** objects that moves fast and has direction.
- **"Diffusion motion"** objects that moves fast and random movement.

The soft tracking performs with the order that is idle motion state first, directional motion state, and then diffusion motion state.

Motion Energy (MVE)

Objects in the directional motion state usually have consistent moving directions with varying speeds. The speed variations could cause erroneous tracks. We used motion energy to predict the speed of the object and used the prediction to reduce the effect of the speed variations. We implemented a bi-directional motion energy enhanced tracking scheme as shown in Figure 2.

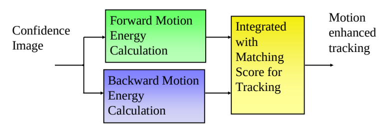


Fig 2. The block diagram of the motion enhanced tracking scheme.

The motion enhanced tracking scheme includes forward and backward motion energy calculation modules that integrate with matching score to enhance tracking accuracy. The motion energy calculation scheme is shown in Figure 3.

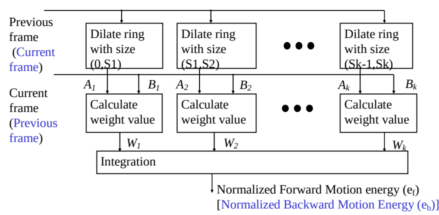


Fig 3. The block diagram of forward (backward) motion energy calculation. The dilate ring with size (a, b) performs dilation with a ring structuring element of the inner radius a and the outer radius b.

In Figure 3, motion confidence weight for each pixel is calculated as

$$W_i = \frac{2*\min[A_i, B_i]}{A_i + B_i}$$

and the integration operation to generate motion energy is calculated by

$$e = \frac{1}{maxSearchRange}\sqrt{\frac{W_1 S_1^2 + W_2(S_1+S_2)^2 + \bullet\bullet\bullet + W_k(S_{k-1}+S_k)^2}{4*(W_1+W_2+\bullet\bullet\bullet+W_k)}} \qquad e=1 \quad \text{if} \quad e>1$$

The maximum search range *maxSearchRange* is learned in teaching stage. The operation sizes are S1=1, S2=2, S3=4, S4=8, S5=16, S6 = 32, and k = 6 The tracking matching score ($Score_{ik}$) is a combination of the morphology score ($MScore_{ik}$) and kinetic score ($TScore_{ik}$) between the kth object in the current frame and ith object in the previous frame. The morphology score is calculated from the similarity measurement such as intensity, shape, relative correlation between the inter-frame objects. The kinetic score is calculated from the movement of the object using flash light like distribution.[1] The two scores are combined to get $Score_{ik}$ as follows:

$$Score_{ik} = \exp[\, factor_{ik} * \ln(MScore_{ik}) + (1 - factor_{ik})\ln(TScore_{ik})]$$

The factor is calculated by

$$factor_{ik} = factor_0 + \frac{e}{10}(1+\cos^2\theta)(9-4e)(factor_E - factor_0)$$

where $factor_E=0.95$, $factor_0=0.2$, $e=\min(e_f, e_b)$,

and θ is angle between two vectors $\vec{v}_p$ and $\vec{v}$, see Figure 4.

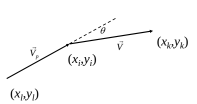


Fig 4. The directional change angle of the track.

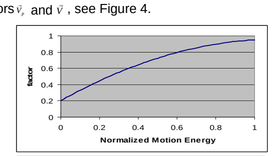
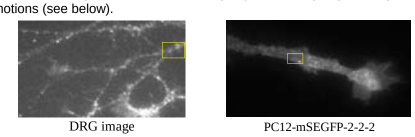


Fig 5. factor as a function of Normalized Motion Energy value (angle θ is 0).

Study Materials and Methods

Our hypotheses are (1) motion energy enhancement (MVE) can improve the tracking accuracy in fast moving objects interacting with multiple surrounding objects; (2) MVE has no adverse side effect on other tracked objects. The truth are created manually and verified independently. We evaluate the fast moving object tracking improvement by case studies (hypothesis 1) and comparing the tracking performance with and without MVE using tracking accuracy metrics (hypothesis 2). The tests are conducted using two movies containing objects undergoing heterogeneous motions (see below).



Motion Energy Example

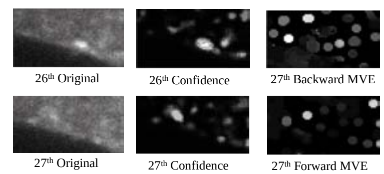


Fig 6. Motion energy examples in yellow box of PC12-mSEGFP-2-2-2 movie.

Tracking Result Comparison

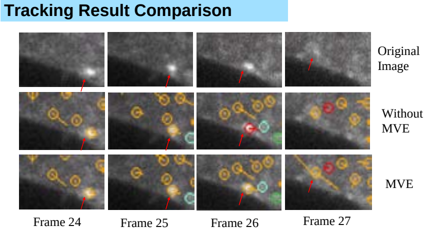


Fig 7. The tracking examples in yellow box of PC12-mSEGFP-2-2-2 image.

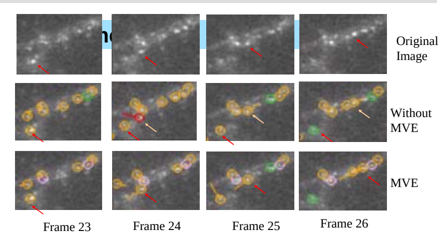


Fig 8. The tracking examples in yellow box of DRG image.

Tracking Accuracy Metrics

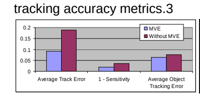
Average track error: No. of tracks having ≥ 10% incorrect tracking time points over the entire time divided by the total number of tracks

Average object tracking error: No. of incorrect tracking time points over the entire time divided by the total number of time points

Average matching tracking sensitivity: For each truth trajectories, no. of objects in the detected tracks having ≥ 10% overlay with the truth trajectories divided by all objects in the truth trajectories

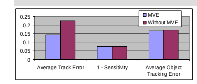
Results

We used manual tracks as the truth and evaluated the tracking performance using the tracking accuracy metrics. Since the manual tracks are subject to human drawing errors and the automatic detection also introduce additional errors, we used 'radial limit' of 5 pixels in both x and y locations for applying tracking accuracy metrics.3



	MVE	Without MVE
Average Tracking Error	0.091±0.060	0.190±0.086
Tracking Sensitivity	0.982±0.035	0.964±0.049
Tracking object Error	0.063±0.010	0.076±0.011

Fig 9. The plots of tracking accuracy metrics for the motion enhanced tracking and without motion enhanced tracking for DRG image. (B) Table of the numerical values of the metrics.



	MVE	Without MVE
Average Tracking Error	0.143±0.116	0.225±0.129
Tracking Sensitivity	0.925±0.063	0.925±0.063
Tracking object Error	0.166±0.028	0.170±0.028

Fig 10. The plots of tracking accuracy metrics for the motion enhanced tracking and without motion enhanced tracking for PC12-mSEGFP-2-2-2 image. (B) Table of the numerical values of the metrics.

Study results show that (1) MVE can improve the tracking accuracy in fast moving objects interacting with multiple surrounding objects; (2) MVE has no adverse side effect on other tracked objects yielding an overall improvement in tracking accuracy. We believe the technologies have broad applications, and are working to validate them on a number of live cell assays.

Future Efforts

- We will continue to test these methods using different types of experimental images from additional assays
- We will create simulated movies containing systematically perturbed object morphological and kinetic parameters to further characterize and benchmark the MVE soft tracking performance. This would provide the directions for future improvements.
- We will further improve motion energy to increase the tracking accuracy.

Literature cited

1. **Lee JSJ**. etal., 2008 Automatic quantitative characterization of rapid protein dynamics in live cell microscopy assays. Poster presentation at the 2008 American Society of Cell Biology conference in San Francisco, CA.
2. **Lee JSJ.** 2002. Structure-guided image processing and image feature enhancement. United States patent number 6,463,175 .
3. **Lee JSJ. 2009**. Automatic quantitative characterization of kinetic events during exocytosis . Poster presented at the 2009 Society for Neuroscience conference in Chicago, IL.

Acknowledgments

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