Validation of automated subcellular tracking tool for kinetic image analysis

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Introduction

Timelapse imaging of biological sample at microscopic level generates a wealth of novel, dynamic phenotypes that, if quantitatively analyzed, could provide new insights into biological processes and disease formation. Automated tracking of the subcellular objects is critical to the unbiased and complete quantification of the dynamic phenotypes. A large amount of work is being done in the scientific community to develop particle tracking and characterization tools for data sets composed of massive images. However, these tools are generally difficult to adopt in broad lab settings and are often application specific and limiting. We have reported in last meeting about development of a robust and flexible tracking method called “soft tracking” that can be taught to handle challenging tracking applications.

The objective of this study is to Here we validate the automated subcellular tracking performance of our soft tracking tool[1,2] by comparing with multiple conventional benchmark software including Image J (PTA Plugin), Matlab, Imaris and Metamorph

Teachable Subcellular Tracking Tool

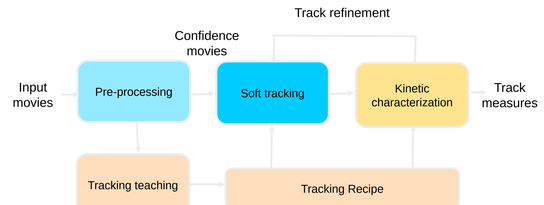

Fig 1. Teachable subcellular tracking architecture consists of a preprocessing step, a soft tracking step and a kinetic characterization step. It is implemented in SVCell™. Input movie(s) are pre-processed to generate confidence movies. The high confidence regions are then detected. The morphology, kinetic 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 tracking teaching step to generate tracking recipe that can be applied to multiple input movies for kinetic high content screening.

Pre-processing

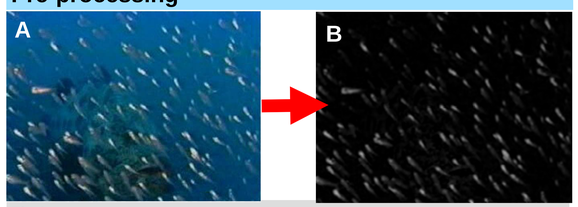

Fig 2. Pre-processing generates a high confidence map using teachable soft matching[3,4] (A) an image frame from the study movie. (B) the confidence map of the frame.

State Based Soft Tracking

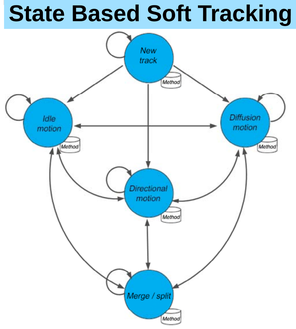

Fig 3. State based soft tracking method applies different tracking models adaptively depending on object states. The track matching between current and next time frame are sequentially performed, prioritized based on states:

- **"Idle"** objects are matched first and excluded from further consideration.
- **“Directional”** motion objects are matched next, reducing the chance that a moving object is confused with a stationary object.
- **“Diffusion motion”** objects are processed last

State Dependent Matching Model

$$Let\ \vec{V}_p = (x_k[t-1]-x_k[t-2], y_k[t-1]-y_k[t-2])$$
$$\vec{V} = (x_i[t]-x_k[t-1], y_i[t]-y_k[t-1])$$

and $\vec{V}_{av}$ is the average of $\vec{V}[t-1]$ for all track in [t-1] frame

The matching scoring model is different with the tracking states

Idle: $TScore_{ik} = Flash(\vec{V};\vec{0},\vec{0},\vec{R}_{idle})$

Directional: $TScore_{ik} = Flash(\vec{V};\vec{V}_p,\vec{V}_{av},\vec{R}_{direction})$

Diffusion: $TScore_{ik} = Flash(\vec{V};\vec{0},\vec{0},\vec{R}_{diffusion})$

Default: $TScore_{ik} = \max\left\{Flash(\vec{V};\vec{V}_p,\vec{V}_{av},\vec{R}_{default}), Flash(\vec{V};\vec{0},\vec{0},\vec{R}_{default})\right\}$

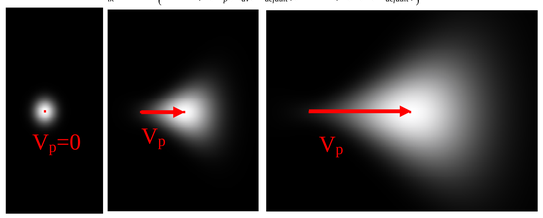

Fig 4. The matching score model Flash(a,b,c,d) is a flash light like distribution which is a function of velocity

Track Refinement

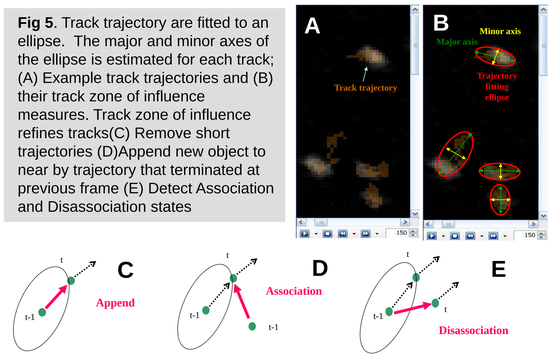

Fig 5. Track trajectory are fitted to an ellipse. The major and minor axes of the ellipse is estimated for each track; (A) Example track trajectories and (B) their track zone of influence measures. Track zone of influence refines tracks(C) Remove short trajectories (D)Append new object to near by trajectory that terminated at previous frame (E) Detect Association and Disassociation states

Study Materials and Methods

Study Data Set

Study data includes three sets of benchmark movies

A. 6 public movies used for benchmark of some specialized particle tracking software[5]. The complete sequences, along with the tracking results, can be accessed at http://www.cs.ucf.edu/~vision/projects/multiframetracking
   - 3 synthetic movies containing different kinetic scenarios (exit and no enter; enter and exit; allowed to exit), generated by a data set generator called Point Set Motion Generator (PSMG)
   - A rotating golf ball (~180 points entering and exiting the scene),
   - Birds (at different altitudes with frequent occlusions),
   - Fishes (a flock of 150+ fishes in the sea);

B. 4 movies of COS7 cell expressing GFP in endosome with systematically reduced temporal sampling rates;

C. A movie containing microtubule (MT) tips transition through “growth”, “pause” and “shrink” phases. It is challenging to track as the MT-tips lose contrast or even disappear during pause phase.

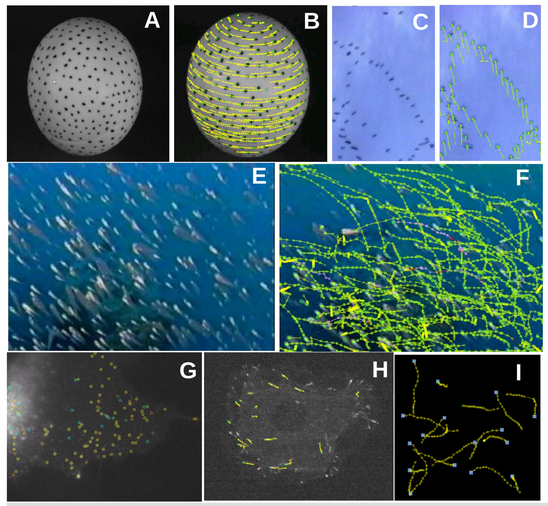

Fig 6. Benchmark movies (A) rotating golf ball and (B) its soft tracking track overlay; (C) Birds and (D) its soft tracking track overlay; (E) Fishes and (F) its soft tracking track overlay; (G) COS7 cell with soft tracking track overlay (different tracking states colored differently); (H) MT tips with soft tracking track overlay; (I) A synthetic movie with soft tracking track overlay.

Study Truth

Study truth tracks were created manually and independently verified.

Benchmark Software

Benchmark software includes

- **Imaris 7.1**,
- **ImageJ plug-in Particle Track and Analysis (PTA),**
- **PolyParticleTracker (Matlab)**
- **Metamorph**

Test Metrics

Test metrics include average tracking sensitivity and track error rate.

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

Average matching tracking sensitivity: For each truth trajectories, no. of objects in the detected tracks having ≥ 80% overlay with the truth trajectories divided by all objects in the truth trajectories

Benchmark Software Parameters

The parameters for each benchmark software are adjusted to optimize their

Image Name	Synthetic 1	Synthetic 2	Synthetic 3	Ball/Fish/Bird	Endosome	MT tips
[Algorithm]						
Enable Region Of Interest	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
Enable Region Growing	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
Enable Tracking	TRUE	TRUE	TRUE	TRUE	TRUE	TRUE
[Source Channel]						
Source Channel Index	1	1	1	1	1	1
Estimated Diameter	5	5	5	8	5	5
Background Subtraction	TRUE	TRUE	TRUE	TRUE	TRUE	True
[Classify Spots]						
"Quality" above	Automatic Threshold	Automatic Threshold	38	6	4	4
[Tracking]						
Algorithm Name	Autoregressive Motion (AM)	AM	AM	AM	AM	AM
MaxDistance	10	10	10	20	6	10
MaxGapSize	3	3	3	3	3	3

Tab 1. Parameters to reproduce Imaris results. Note: Imaris could not run original Bird and Fish files. We had to create the confidence image in which the Background is dark and the object of interest is bright

Image Name	thres hold	RoiSize	Min Intesty	Nearest Particle Range	Min size
Ball	25	17	15	0.5	5
Fish	50	25	15	0.5	5
birds	25	17	15	0.5	5
synthetic	25	10	100	0.8	5
MT tip	61	20	35	0.5	5
Cos Cell	98	17	35	0.5	5

Tab 2. Parameters to Reproduce PTA results. Note that Ball, Bird and Fish were tracked using SVCell confidence image

Image Name	Threshold	Object Region Width	Object Region Height	Search Region Width	Search Region Height
Ball	35	5	5	17	17
Fish	45	5	5	17	17
birds	35	5	5	17	17
synthetic	35	5	5	17	17
MT tip	300	5	5	17	17
Cos Cell	100	5	5	17	17

Tab 3. Parameters used for Metamorph

Image Name	Brightness maximum	Minimum track length	Search new particles in N frames
Synthetics	255	2	5
Fish	255	2	5
Birds	255	2	5
Ball	255	2	5

Tab 4. Parameters used for PolyParticleTracker in Matlab

Results

Validation Study Results

The results in (sensitivity, error) show our soft tracking tool had superior performance overall (0.718±0.033, 0.052±0.003), followed by Imaris (0.475±0.037, 0.154±0.006), and ImageJ PTA (0.398±0.037, 0.171±0.006).

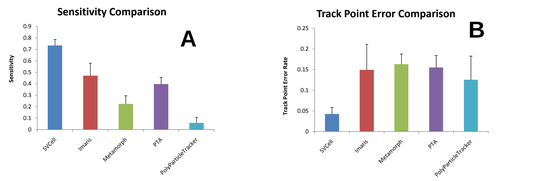

Fig 7. Bar charts showing (A) tracking sensitivity and (B) tracking errors for SVCell (soft tracking) and the benchmark software

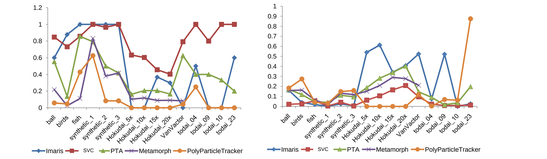

Fig 8. Individual movie performance plots for (A) tracking sensitivity and (B) tracking errors for SVCell (soft tracking) and the benchmark software

Conclusion

The results indicate that our soft tracking tool could support subcellular tracking for comprehensive quantification of biological events.

Literature cited

1. **Lee JSJ,et al.** Automatic quantitative characterization of kinetic events during exocytosis. Poster 2009 Society for Neuroscience conference in Chicago, IL.
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Acknowledgments

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