Validation of a 3D Particle Tracking Tool for Next-Generation Neuroscience Microscopy

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Abstract

Time-lapse, 3D imaging of functional neural networks is a promising approach to gain in-depth understanding of how the central nervous system functions (CNS). These sequences are routinely acquired to study the molecular kinetics and interactions driving development of functional circuits and pathology of neurodegenerative diseases. As the imaging modality continues to improve, a new generation of scientific inquiries, new discoveries, and therapies will be unleashed. However, current methods of 3D image analysis are inadequate for quantifying particle dynamics in these complex models.

We report a 3D particle tracking tool designed for highly sensitive particle detection and tracking. The tool is a general computation framework that dynamically updates matchmaking with a combination of user-definable state profiles and transitions, and track motion energy [1]. We benchmarked our tracking tool against conventional 3D particle tracking methods in real and simulated images. We found that our tool compares well to other methods.

Methods

Simulated Image

Simulated 3D image sequences are created by software for particle tracking tool development and benchmarking.

HIV Image Acquisition

HIV virion are fluorescently labeled with GFP-VPR and imaged on an Olympus IX70 epifluorescent microscope (Applied Precision, Issaquah WA). Images are acquired in z-series on a CCD digital camera (pixel dimension: 0.182 x 0.182 μm^2) and deconvoluted using API Softworks.

Performance Benchmarking

We evaluated the tracking results using the following criteria [2]:

- α: maximum distance (error) of all test tracks from ground truth
- β: qualitative score that describes test tracks without a paired track
- JSC: Jaccard similarity coefficient for track points that describes overall particle detection accuracy
- JSC_θ: Jaccard similarity coefficient for entire tracks

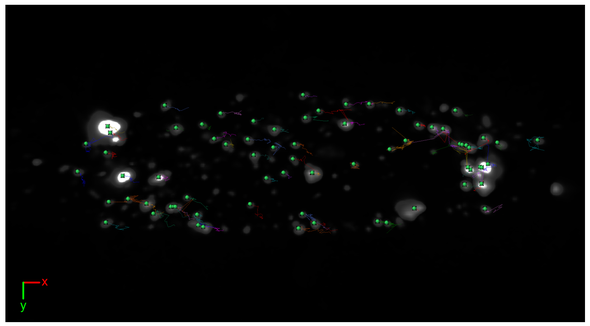

Figure 1. Tracking HIV trafficking in 3D. We applied our reported tracking tool on real images of GFP-labeled HIV virion. Tracks are overlaid on the image in SVCell. Each green circle shows the current track location.

Results

We extended our previously-reported particle tracking tool [1] as basis for the 3D tracking tool. The processing steps for the proposed 3D particle tracking tool is summarized in Figure 2. Pre-processing steps are applied to enhance particle detection accuracy. Matchmaking adopts a modular approach combining bidirectional sort and greedy algorithm [3] for better performance. Matchmaking is further improved by breaking up the matchmaking graph into isolated subgraphs for parallel processing, which results in an up to 40 times speed improvement. Particle tracks are reconciled along their Z-slices and trajectories shorter than 3 frames are removed in the post-processing step.

Figures 1 and 3 (below) shows particle tracking results for HIV trafficking image visualized using SVCell image analysis software (www.svcell.com).

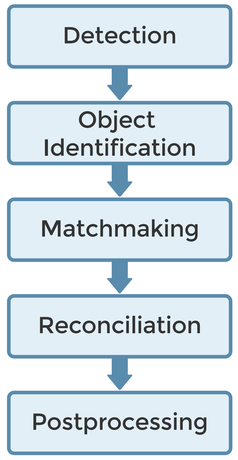

Figure 2. Processing workflow for 3D particle tracking tool

Track Points Matchmaking

After the particles are identified, the particles are moved from the image domain to the data domain for matchmaking. Particles on the current frame (T) is matched to particles on the previous frame (T-1). Each particle pair from frames T and T-1 is placed on a graph, connected by an edge that describes the cost of the pair, determined by the particle's velocity and morphology.

To find the optimal solution for the particle pairs, we first divide the graph into sub-graphs composed of only valid connections based on maximum particle pair distances. Each sub-graph is independent and can be solved in parallel by the Hungarian algorithm [3]. Our approach can process track points matchmaking much faster with no loss in tracking accuracy compared to applying the algorithm on the full graph.

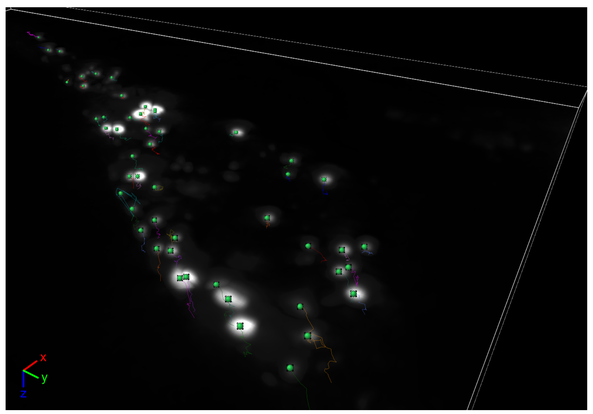

Figure 3. Tracking HIV trafficking in 3D. Result tracks are overlaid on the image. 3D volume rendering from SVCell is used to visualize the virus particles in 3D.

Validation

Table 1 shows a summary of the performance benchmarks of our 3D particle tracking tool on the simulated images sorted by SNR and density.

Table 1. Summary of particle tracking tool performance

SNR	Density	α	β	JSC	JSC_θ
4	Low	0.845	0.829	0.897	0.914
4	Medium	0.699	0.655	0.685	0.819
7	Low	0.871	0.859	0.927	0.961
7	Medium	0.724	0.683	0.705	0.876

Figure 4 shows the benchmark results for each tracking analysis score for our particle tracking tool (SVCell) and other conventional tools.

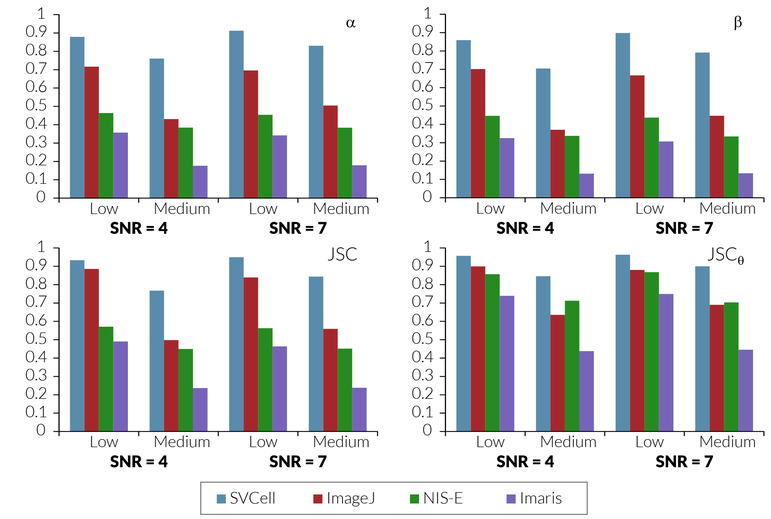

Figure 4. Benchmark comparison of four particle tracking tools.

References

[1] Lee JSJ, et al. Automated kinetic characterization of intracellular single-molecule tracking. Poster presented at: ASCB 49th Annual Meeting; 2009 Dec 5-9; San Diego, CA.

[2] Chenouard N, et al. Objective comparison of particle tracking methods. Nat Methods. 2014 Mar; 11(3):281-9.

[3] Kalaidzidis Y. Multiple object tracking in fluroescence microscopy. J Math Biol. 2009 Jan; 58(1-2):57-80.

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