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Supplemental information

u-track3D: Measuring, navigating, and validating dense particle trajectories in three dimensions

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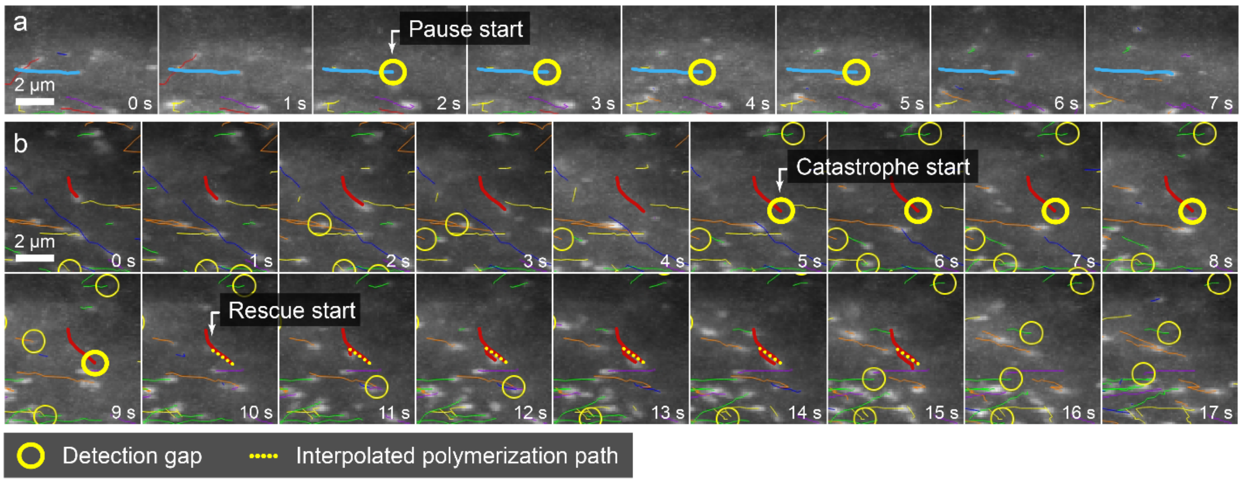
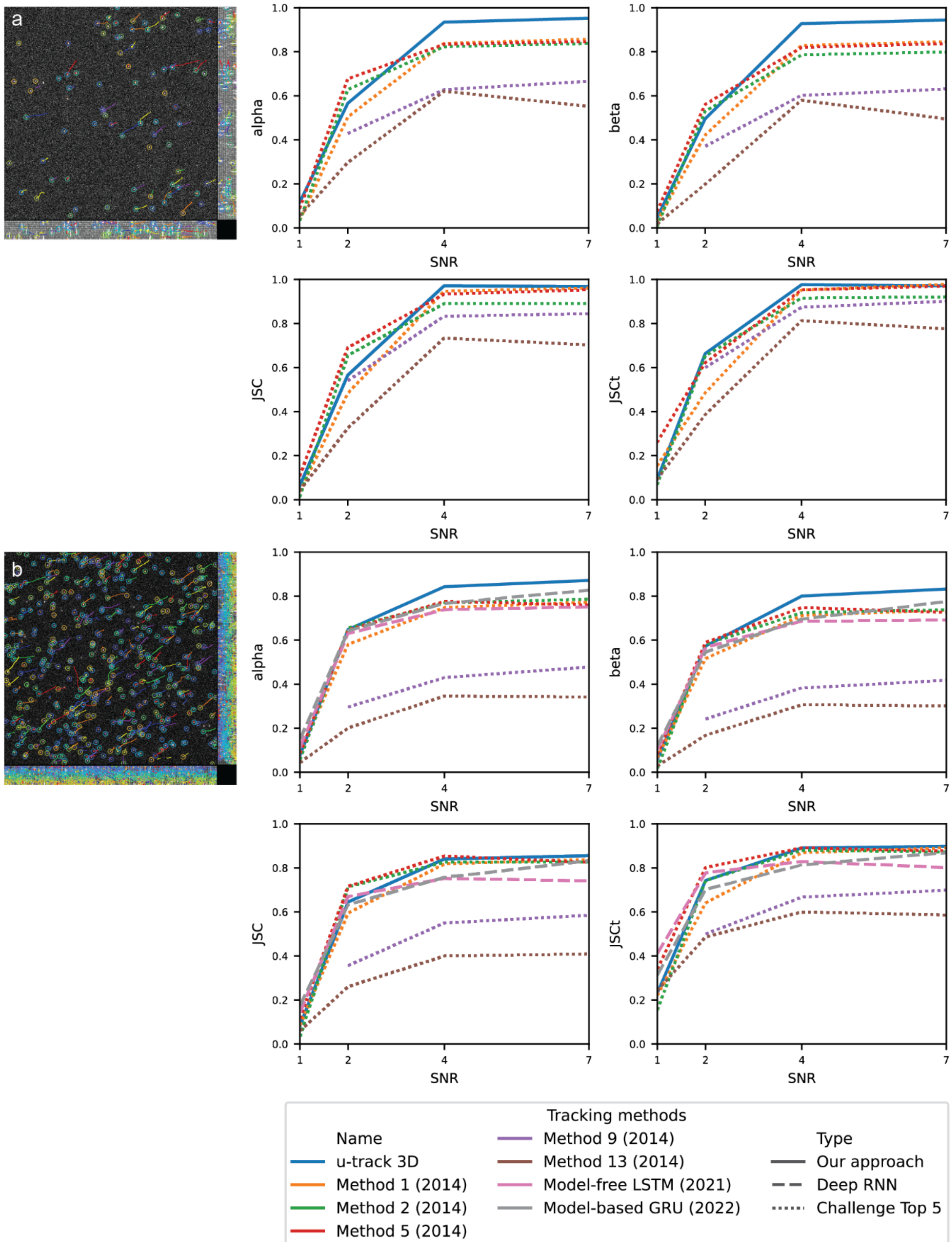
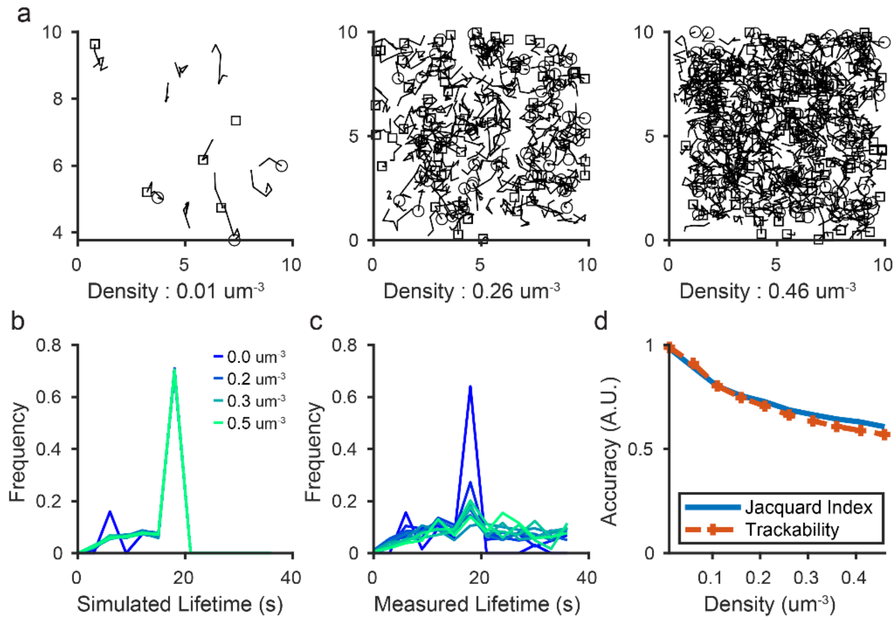
Supplementary Figure 1: u-track3D can recover transient losses in fluorescence caused by microtubule polymerization instability, related to Figure 2. a) Example of a pause in microtubule polymerization detected in HeLa cell in interphase (detail). Yellow circles highlight detection gaps. b) Example of catastrophe and rescue events detected in the same sequence (detail).



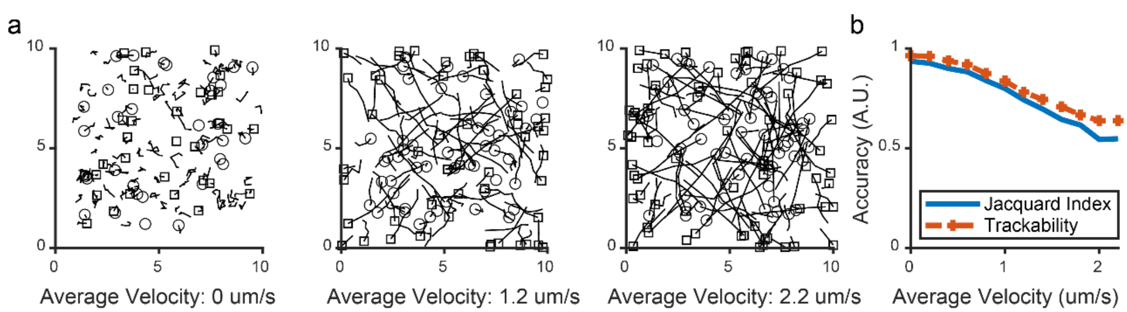
Supplementary Figure 2: u-track 3D performance in comparison to existing methods evaluated on a standard 3D test dataset, related to Figure 3. a) Low particle density. b) Medium particle density scenario.



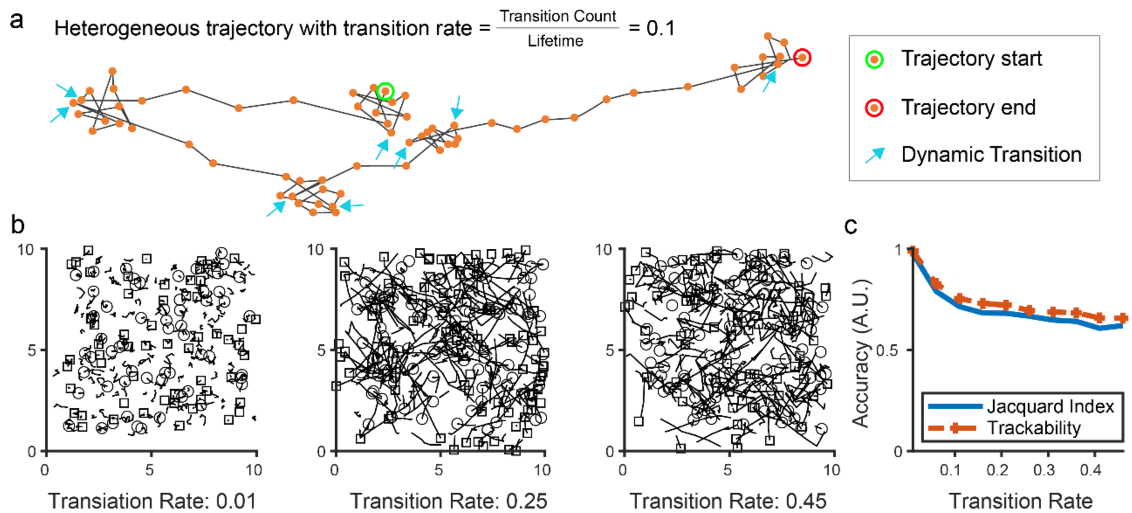
Supplementary Figure 3: The trackability score predicts the performance decrease associated to particle density, related to Figure 6. a) Examples of simulated trajectories with particle density ranging from 0.01 to 0.5 um^{-3} with a fixed diffusion coefficient of 0.3 um^2/s. Visualization is limited to five consecutive frames to reduce clutter. b) Lifetime of simulated trajectories. c) Lifetime distribution measured through tracking. d) Accuracy measured through the Jaccard coefficient on the ground truth and estimated with the trackability score using the detection set.



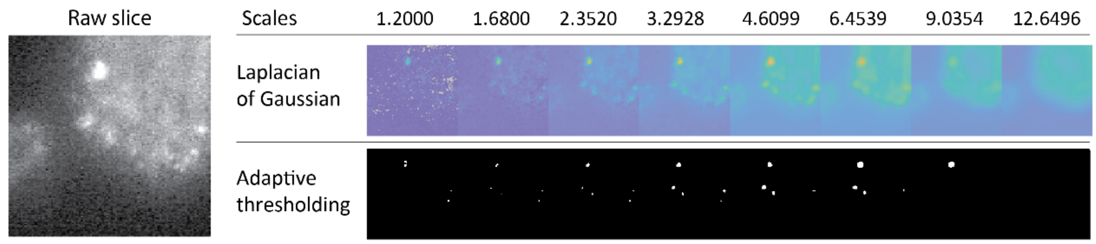
Supplementary Figure 4: The trackability score predicts the decrease in performance associated to particle velocity, related to Figure 6. a) Examples of simulated trajectories presenting directed motions described by velocities ranging from 0 to 2.2 um/s with a fixed diffusion component coefficient of 0.15 um^2/s and density set to 0.1 um^{-3}. Visualization is limited to five consecutive frames to reduce clutter. b) Accuracy measured through the Jaccard coefficient on the ground truth and estimated with the trackability score using the detection set.



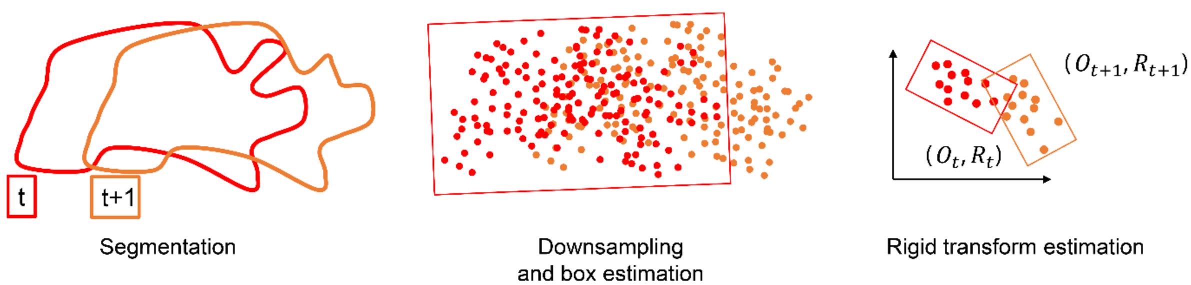
Supplementary Figure 5: The trackability score predicts the decrease in performance associated to the heterogeneity of motion types in a single trajectory, related to Figure 6. a) Illustration of the transition rate used to simulate a dataset with increasing heterogeneity. b) Examples of simulated trajectories with diffusive motion described by a coefficient set to 0.1 um^2/s, and directed motion set to 1.5 um/s with a diffusive component of 0.1 um^2/s. Density is set to 0.2 um^{-3}. Visualization is limited to five consecutive frames to reduce clutter. c) Accuracy measured through the Jaccard coefficient on the ground truth and estimated with the trackability score using the detection set.



Supplementary Figure 6: Principle of multiscale Laplacian-of-Gaussian filtering (top) and multiscale adaptive thresholding approach (bottom) demonstrated on a slice of a volumetric imaging of cellular adhesions (detail), related to Section "Multiscale particle detector" in STAR Methods.



Supplementary Figure 7*:* Principle of generic point cloud tracking applied to cell Dynamic region of interest estimation for the cell, related to Section "Dynamic Region of Interest estimation" in STAR Methods.



Supplementary Table 1: Parameters used to simulate the trajectories, related to Figure 6 and Supplementary Figures 3, 4 and 5.

Varying parameter	Diffusion	Density	Velocity	Transition Rate
Figure	5.f-i	Supp 2	Supp 3	Supp 4
Diffusion Coef. (um^2/s)	0.1 – 1.0	0.3	0.15	0.1
Density (um^{-3})	0.1	0.01 – 0.5	0.1	0.2
Velocity (um/s)	0	0	0 – 2.2	1.5
Transition Rate	0	0	0	0 – 0.5
Volume size (um^3)	1000	1000 um^3	1000 um^3	1000 um^3
Frame rate (Hz)	1	1	1	1
Movie Length (s)	150	150	150	150
Trajectory lifetime (s)	20	20	20	20
Standard error on detection	0.005	0.005	0.005	0.005

Supplementary Table 2: Tracking parameters used to evaluate the trackability score, related to Figure 6 and Supplementary Figures 3, 4 and 5. Note that the gap closing parameters are disabled for this experiment.

Varying parameter	Diffusion	Density	Velocity	Transition Rate
Figure	5.f-i	Supp 2	Supp 3	Supp 4
LinearMotion	0	0	1	0
minSearchRadius	3	3	3	3
maxSearchRadius	5	5	9	9
brownStdMult	3	3	3	3
useLocalDensity	0	0	0	0
localDensityWindow	NA	NA	NA	NA
searchRadiusFirstIteration	6	6	9	9