Supplemental Materials

Molecular Biology of the Cell

Masucci *et al*.

Figure S1

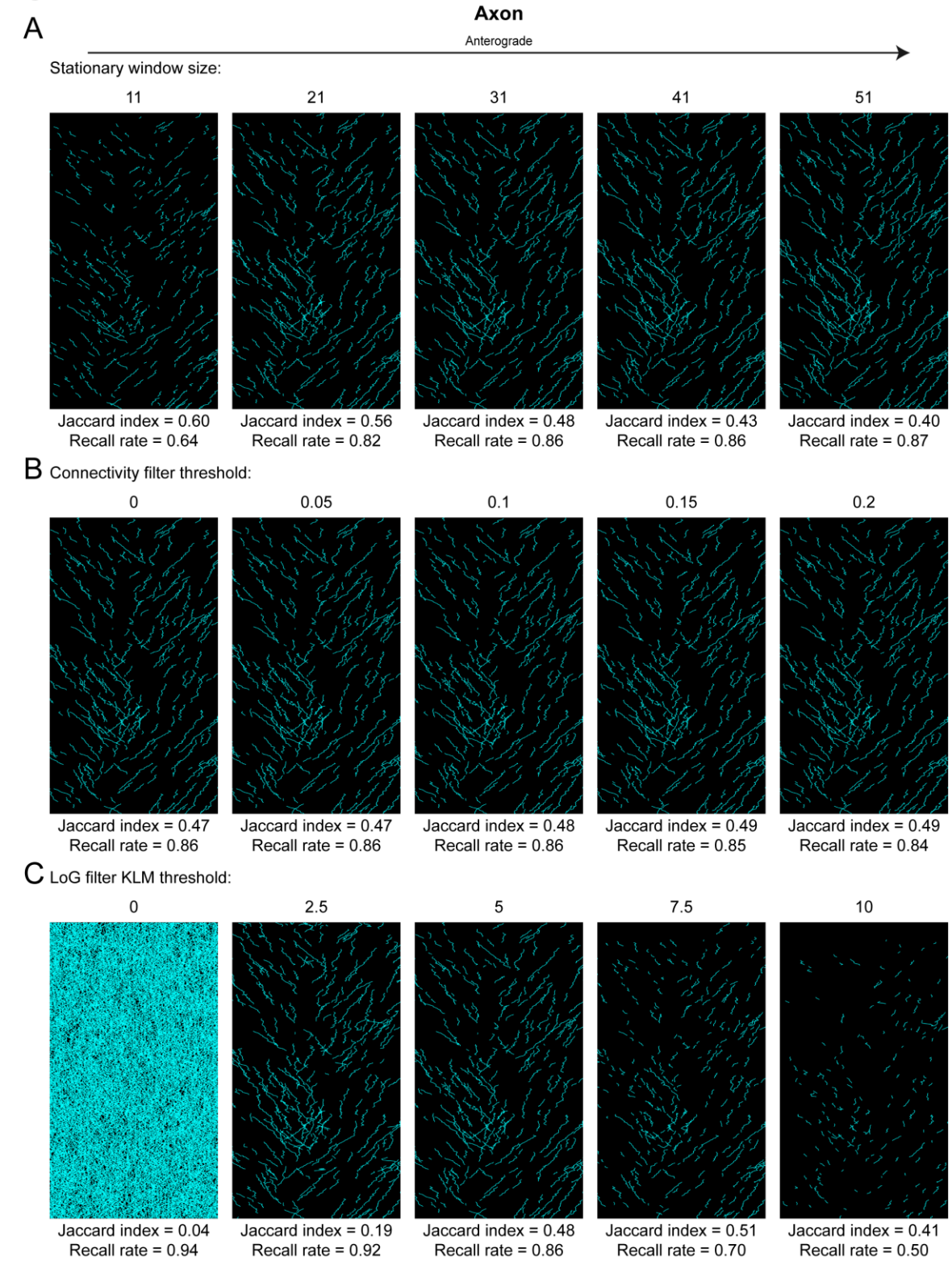
A
Axon
Anterograde
Stationary window size:
11
21
31
41
51
Jaccard index = 0.60
Recall rate = 0.64
Jaccard index = 0.56
Recall rate = 0.82
Jaccard index = 0.48
Recall rate = 0.86
Jaccard index = 0.43
Recall rate = 0.86
Jaccard index = 0.40
Recall rate = 0.87
B Connectivity filter threshold:
0
0.05
0.1
0.15
0.2
Jaccard index = 0.47
Recall rate = 0.86
Jaccard index = 0.47
Recall rate = 0.86
Jaccard index = 0.48
Recall rate = 0.86
Jaccard index = 0.49
Recall rate = 0.85
Jaccard index = 0.49
Recall rate = 0.84
C LoG filter KLM threshold:
0
2.5
5
7.5
10
Jaccard index = 0.04
Recall rate = 0.94
Jaccard index = 0.19
Recall rate = 0.92
Jaccard index = 0.48
Recall rate = 0.86
Jaccard index = 0.51
Recall rate = 0.70
Jaccard index = 0.41
Recall rate = 0.50

Figure S1. Parameter sweep of Cega on simulated data using axonal background. Simulated data using axonal background signal was used where mean photon emissions were set to 200 photons, which corresponds to a SNR of 2.8. A) Kymographs of tracks determined from simulated particles within axonal compartments using Cega with a stationary model sliding window median of 11 to 51 frames. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. Shorter windows resulted in the removal of longer duration tracks. B) Kymographs of tracks determined from simulated particles within axonal compartments using Cega with a connectivity filter threshold of 0 to 0.2. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. Changing the connectivity filter threshold did not dramatically influence signal detection and tracking. C) Kymographs of tracks determined from simulated particles within axonal compartments using Cega with a LoG threshold of 0 to 10. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. These values were used to threshold the KL divergence score of LoG filtered determined coordinates. Smaller LoG threshold values failed to remove the coordinates of spurious signal present in the KL divergence movie, whereas large thresholds eliminated the signal of moving motors in the KL divergence model.

Figure S2

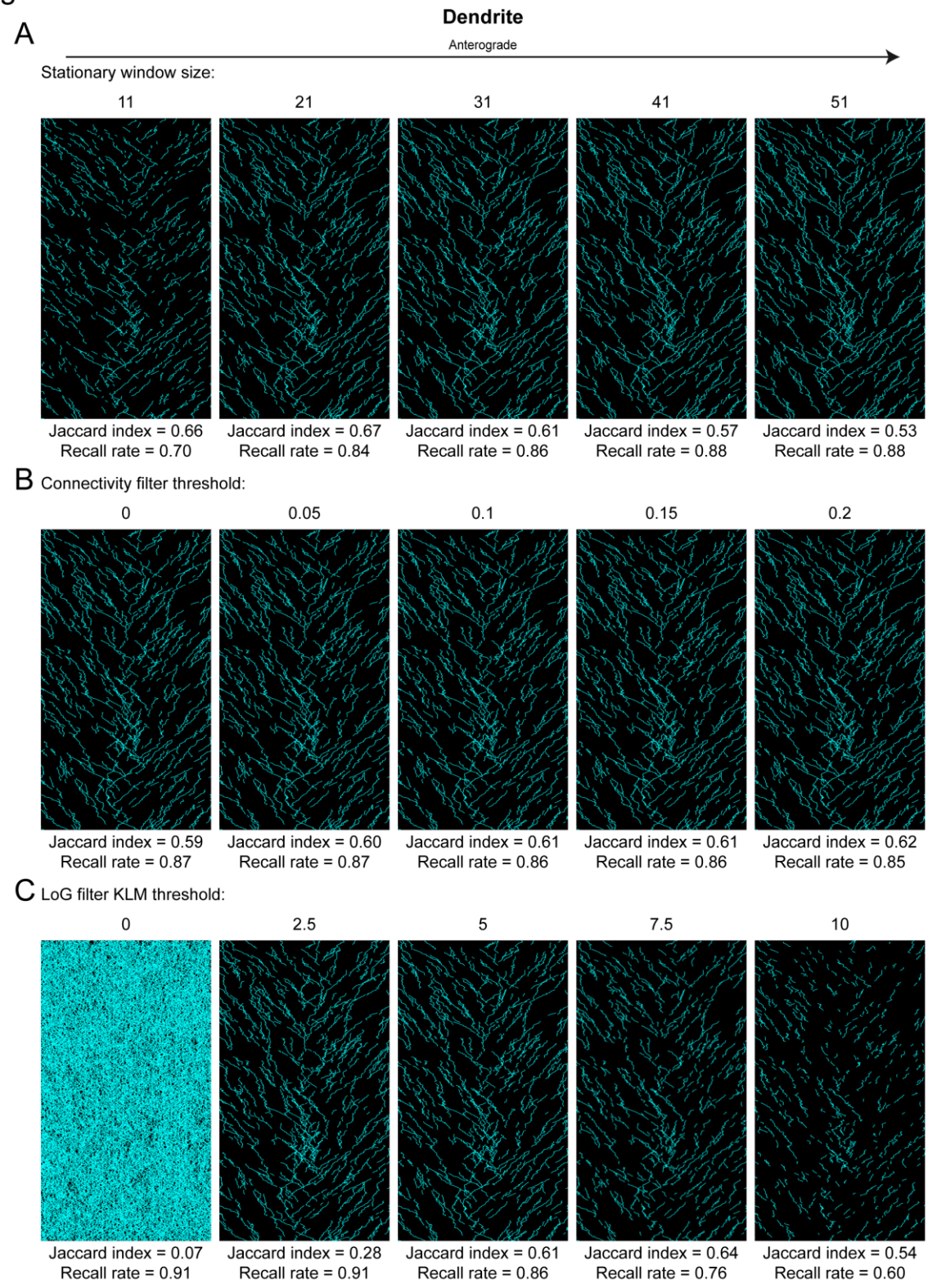
A
Dendrite
Anterograde
Stationary window size:
11
21
31
41
51
Jaccard index = 0.66
Recall rate = 0.70
Jaccard index = 0.67
Recall rate = 0.84
Jaccard index = 0.61
Recall rate = 0.86
Jaccard index = 0.57
Recall rate = 0.88
Jaccard index = 0.53
Recall rate = 0.88
B Connectivity filter threshold:
0
0.05
0.1
0.15
0.2
Jaccard index = 0.59
Recall rate = 0.87
Jaccard index = 0.60
Recall rate = 0.87
Jaccard index = 0.61
Recall rate = 0.86
Jaccard index = 0.61
Recall rate = 0.86
Jaccard index = 0.62
Recall rate = 0.85
C LoG filter KLM threshold:
0
2.5
5
7.5
10
Jaccard index = 0.07
Recall rate = 0.91
Jaccard index = 0.28
Recall rate = 0.91
Jaccard index = 0.61
Recall rate = 0.86
Jaccard index = 0.64
Recall rate = 0.76
Jaccard index = 0.54
Recall rate = 0.60

Figure S2. Parameter sweep of Cega on simulated data using dendritic background. Simulated data using axonal background signal was used where mean photon emissions were set to 200 photons, which corresponds to a SNR of 2.8. A) Kymographs of tracks determined from simulated particles within dendritic compartments using Cega with a stationary model sliding window median of 11 to 51 frames. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. Shorter windows resulted in the removal of longer duration tracks. B) Kymographs of tracks determined from simulated particles within axonal compartments using Cega with a connectivity filter threshold of 0 to 0.2. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. Changing the connectivity filter threshold did not dramatically influence signal detection and tracking. C) Kymographs of tracks determined from simulated particles within axonal compartments using Cega with a LoG threshold of 0 to 10. Jaccard indices and recall rates for tracks determined after Cega filtering are listed below each corresponding kymograph. These values were used to threshold the KL divergence score of LoG filtered determined coordinates. Smaller LoG threshold values failed to remove the coordinates of spurious signal present in the KL divergence movie, whereas large thresholds eliminated the signal of moving motors in the KL divergence model.

Figure S3

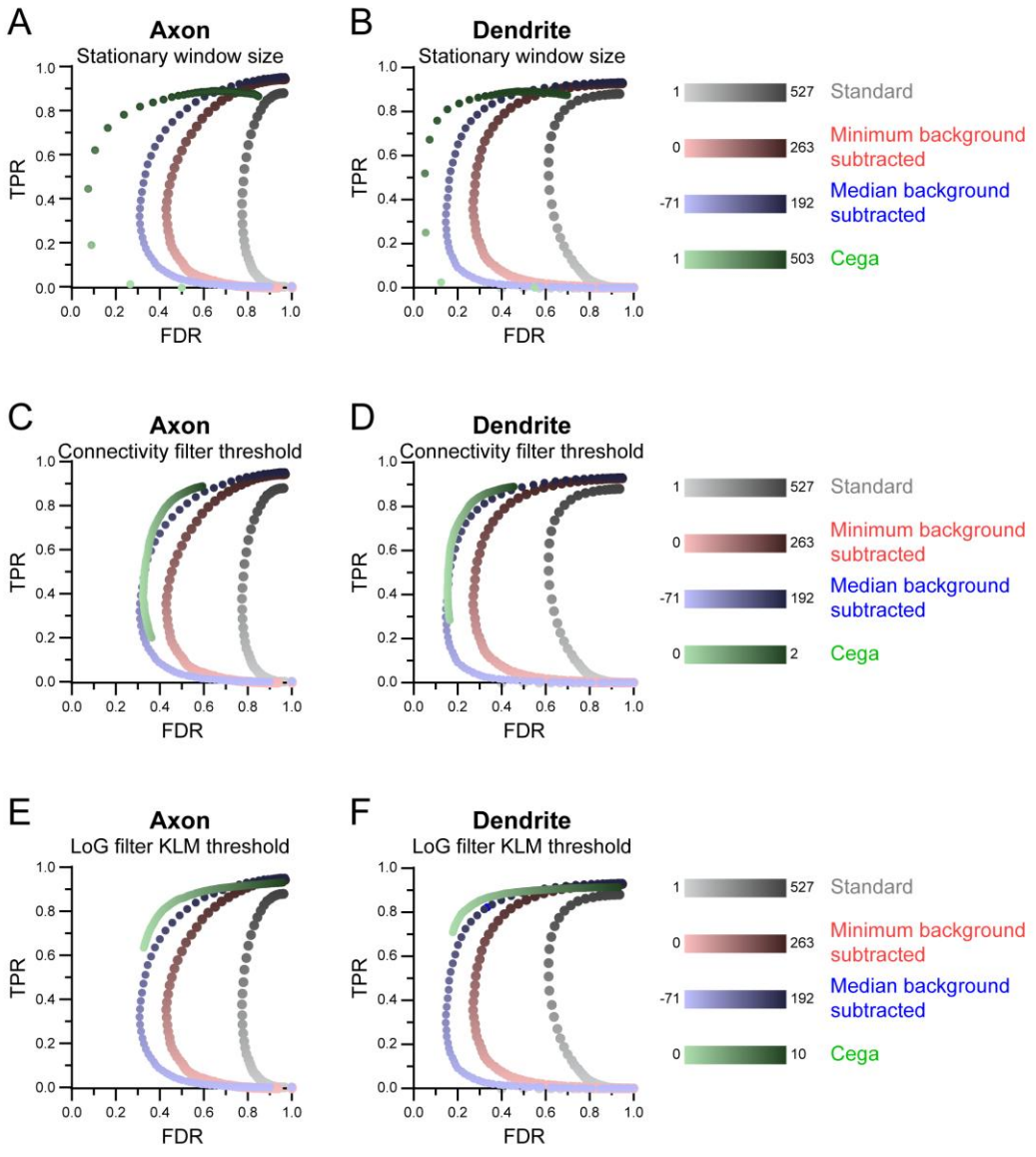
A
Axon
Stationary window size
B
Dendrite
Stationary window size
C
Axon
Connectivity filter threshold
D
Dendrite
Connectivity filter threshold
E
Axon
LoG filter KLM threshold
F
Dendrite
LoG filter KLM threshold
TPR
FDR
1
527
Standard
0
263
Minimum background subtracted
-71
192
Median background subtracted
1
503
Cega
0
2
Cega
0
10
Cega

Figure S3. Cega parameter sweep and performance test on simulated data. ROC plots for Cega detection on simulated data using axonal and dendritic background signal, where mean photon emissions were set to 200 photons, which corresponds to a SNR of 2.8. A-B) Cega performance against median or minimum background subtraction methods, or standard methods. Cega's stationary model sliding window median was changed from 1 to the full frame length of our data (503 frames). In contrast to Cega, the other methods produced a horseshoe shaped ROC curve due to the high number of false positives present when the thresholding is too low and the majority of signal detected is background, and too high and the majority of signal detected is from background signal brighter than the signal of simulated particles. C-D) Cega performance against other detection methods as the connectivity filter threshold was changed from 0 to 2 and the threshold of the other methods was varied. E-F) Cega performance against other detection methods as the LoG filter KLM threshold was changed from 0 to 10, and the thresholds for the other methods were swept.