Microtubules Paper

November 21, 2012

Abstract

This will be our abstract. Fundamental task(s) which the software accomplishes, examples of biological insights from the use of the software, details of availability, including where to download the most recent source code, the license, any operating system dependencies, and support mailing lists.

% Please keep the abstract between 250 and 300 words

Author Summary

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Introduction

A description of the problem addressed by the software and of its novelty and exceptional nature in addressing that problem.

Design and Implementation

Details of the algorithms used by the software, how those algorithms have been instantiated, including dependencies. Details of the supplied test data and how to install and run the software should be detailed in the supplementary material.

EB1-Tracking

Placeholder txt.

The 'EB1 view' is obtained from the EB1 marker. Starting with the spots seen in inter-frame difference images, Paul Southam labels chains. Potential chains start from every spot in every frame. From each, the next frame is searched for a spot which could be part of the same growing microtubule (about the right distance away, etc). This continues from frame to frame until no further

spots can be added. This process generates a lot of chains (>100,000) most of which are short segments of longer chains that are removed as a final step.

The marker, EB1, reports the positions of growing microtubule tips. It moves relatively fast and can be distinguished from the tubulin marker by taking the difference image between successive frames (Fig. P1 first panel). The purpose of our Matlab software package, MicrotubuleAnalysis, is to track these spots over time to allow a quantitative analysis of microtubule dynamics. It uses four algorithms. (P1) Finding spots, (P2) finding sequences of spots that form a coherent chains, (P3) refining the set of chains by removing duplicates and identifying branches from what appear to be the straightest chains. At the output of MicrotubuleAnalysis each chain is associated with a set of features that may be used by a classifier to distinguish microtubules from noise with the help of data from tubulin labelled microtubules.

Spot Finding

Illustrates the steps used to find and characterise EB1 spots. The steps: 1. 2D median filter image (remove fine scale image noise while preserving edge boundaries for spots). 2. Intensity normalise image (max min normalise) to get image intensities within range 0 to 1. 3. Threshold the image removing the lowest 5% intensity values. 4. Perform Otsu threshold (computes the optimum threshold for the image). Method assumes that each the image contains a bi-modal histogram (two classes of pixels) and calculates the optimum threshold for separating those two classes by minimising intra class variances. 5. Matlab function imfill to fill in any spot holes. 6. Sieve to a low scale to remove very small spot. 7. Use Matlab regionprops to calculate spot WeightedCentroids, Orientation, MajorAxisLength (green lines).

Chain Finding

Fig. PJ2 Steps to finding the sequence of spot movements (chains). Chains are started at every spot in every frame. This is a makes a lot of chains, most of which get pruned out at the end. In this example (A-E, G-H) the starting point yields seven chains, one for each finishing point. These are pruned to three candidate microtubule chains. The starting spot is labelled red, others yellow except the spot visible in the current frame which is white.

A) Frame 1 pick a starting spot, here shown in red with a white centre. The remaining spots will be invoked frame by frame. B) Frame 2 find all spots in the current frame, in all directions and within the expected range. Here just one (white centre) and create a chain (here length 2, edge shown in dark blue). For each edge compute a velocity and place a Gaussian probability distribution (PG orange) centered at the expected position of the next spot . C) Frame 3 find all spots in the current frame with $PG > 0.05$. Here just one, white centre. Create a new chain that finishes at this spot (lighter blue) and repeat step (B) to predict the next spot. There are now two chains starting at the red spot. D) Frame 4 repeat steps (B) and (C). In this case one spot is too unlikely given the

expected velocity and new chains are formed that finish on the two acceptable spots. E) Frame 5 repeat the process over successive frames until there are no more likely spots. F) A probability Pm is associated with every edge. It accounts for spot eccentricity (spots are often streaked in the direction along which the microtubule is growing). (A) to (F) is repeated starting at every spot in every frame. The result of this exhaustive trawl is many duplicate chains that are subsets of the others and have to be pruned out. G) Chains that are subsets of others are removed and a straight line is fitted to each remaining chain (here three chains) to give the probability that each arose by chance, PS. We assume that the straightest , where PS is low are most likely to be microtubules. The others may include branches and other false positives. H) Truncate chains that include branches (here c=2 and c=3) by forming new start points and refitting lines. Repeat step (G). Chains of length <=2 are trivial and Ps is set to 1 (for these there is no evidence from the EB1 data to conclude that they are microtubules – such chains are retained however because evidence from the tubulin signal might be available). H) Truncate chains that include branches (here c=2 and c=3) by forming new start points and refitting lines. Repeat step (G). Chains of length <=2 are trivial and Ps is set to 1 (for these there is no evidence from the EB1 data to conclude that they are microtubules – such chains are retained however because evidence from the tubulin signal might be available).

Chain Refining

Orientation Texture Tool

Tubulin Segmentation

Jake puts stuff here.

To characterise the microtubule properties and their emergent behaviour, we have written MTanalyse (G14). Jacob Newman, identified microtubules (usually bundles of microtubules) within cells from the tubulin marker. He used shape models to precisely track the cell shape, steerable Gabor filters to find long thin objects represented by the tubulin fluorescence signal, a 'per pixel' K-nearest neighbour (KNN) classifier to find pixels associated with the long-thin objects and a custom algorithm to link the regions identified in individual frames over time. The result is a set of labelled regions which are candidates for microtubules or microtubule bundles: the 'tubulin view'.

MT Classifier

Jake puts stuff here.

The information from the two markers is pooled in a classifier. The two training sets are: microtubules in which we see the EB1 chain elongating and laying down a tubulin marker trail and, secondly, EB1 chains for which there is no correlated tubulin marker. The result from Cell 2, for example, is ~5000

microtubules from a movie sequence 340*7.5=2550 s. Fig. 4 shows example microtubules and their dynamics and Fig. 5 summarises some of the features

Results

%Examples of biological problems solved using the software, including results obtained with the deposited test data and associated parameters.

Our continued analysis of microtubule behaviour in the growing leaf confirms our preliminary result shown in the last report that a majority of the microtubules starting at the edges of the cell are to be found on the long edges, even when the microtubules are not transverse (Fig. JPJ 1). We also observe that the distribution of angles of nucleating microtubules is similar throughout the cell face, suggesting that there is no particular importance attributable to those nucleating at the edge (Jake has figure). These results are inconsistent with the published view that reorientation is caused by a switching-wall mechanism where the position from which microtubules emerge shifts between adjacent edges of the cell [Allard et al., 2010 (Mol Biol Cell 21, 278-86); Dhonukshe et al., 2012 (Cell, 149, 383-96)].

Feature Microtubule property Pause Grow + end Rate ~ 0.09 µ.s-1 Grow - end too few to estimate Shrink - end Rate ~ 0.03 µ.s-1 Catastrophe Rate is too fast to estimate, instead we estimate the degradation rate ~ 0.1 µ2.s-1 frequency* Sever Estimated as a degradation rate * Nucleate Spontaneous in cytoplasm ~ 0.00012 µ-2.s-1 Branch, axial nucleation, rescue Difficult to distinguish between these. Proportion of new microtubules (blue) nucleating from existing (black) all angles (including co-axially and rescue) ~ 4669/4880=96% measured over all frames Interactions between microtubules Cross ~70% of inter microtubule interactions result a crossing Collide into tubulin ~16% of inter microtubule interactions result in either pausing or catastrophe (annihilation) of blue MT

Collide head on (EB1 s) ~14% of inter microtubule interactions result in either pausing or catastrophe (annihilation) of both Zipper Fig. JPJ 5 Examples of microtubule properties and behaviour obtained from MTanalyse. Arrows show direction of change of length, blue shows the active microtubule.

Availability and Future Direction

Where the software has been deposited. Any future work planned to be carried out by the authors, how others might extend the software.

Acknowledgments

BBSRC?