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plusTipTracker: Quantitative image analysis software for the measurement of microtubule dynamics

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ABSTRACT

Here we introduce plusTipTracker, a Matlab-based open source software package that combines automated tracking, data analysis, and visualization tools for movies of fluorescently-labeled microtubule (MT) plus end binding proteins (+TIPs). Although +TIPs mark only phases of MT growth, the plusTipTracker software allows inference of additional MT dynamics, including phases of pause and shrinkage, by linking collinear, sequential growth tracks. The algorithm underlying the reconstruction of full MT trajectories relies on the spatially and temporally global tracking framework described in Jaqaman et al. (2008). Post-processing of track populations yields a wealth of quantitative phenotypic information about MT network architecture that can be explored using several visualization modalities and bioinformatics tools included in plusTipTracker. Graphical user interfaces enable novice Matlab users to track thousands of MTs in minutes. In this paper, we describe the algorithms used by plusTipTracker and show how the package can be used to study regional differences in the relative proportion of MT subpopulations within a single cell. The strategy of grouping +TIP growth tracks for the analysis of MT dynamics has been introduced before (Matov et al., 2010). The numerical methods and analytical functionality incorporated in plusTipTracker substantially advance this previous work in terms of flexibility and robustness. To illustrate the enhanced performance of the new software we thus compare computer-assembled +TIP-marked trajectories to manually-traced MT trajectories from the same movie used in Matov et al. (2010).

1. Introduction

Microtubules (MTs) are highly dynamic cytoskeletal polymers that stochastically switch between phases of growth, shrinkage, and pause, a behavior known as dynamic instability (Mitchison and Kirschner, 1984). While MTs are best known for their role in segregating chromosomes during cell division (Alberts et al., 2002), they also function in cell polarization and migration (Watanabe et al., 2005; Wittman and Waterman-Storer, 2001), intracellular trafficking (Caviston and Holzbaur, 2006), morphogenesis (Kirschner and Mitchison, 1986), and signaling to adhesions (Kaverina et al., 1998) and other organelles. In addition, MTs are a key drug target for the treatment of cancer (Giannakakou et al., 2000; Risinger et al., 2009) and other pathologies. Thus, quantitative characterization of MT dynamics in different cellular contexts is essential for our understanding of cell physiology and disease.

Researchers have measured MT dynamics in time-lapse images of fluorescently-labeled tubulin injected or expressed in living cells (Goodson and Wadsworth, 2004; Semenova and Rodionov, 2007). Because of the density of MTs in the cell body, this approach allows analysis of MT behavior only at the cell periphery. In recent years, imaging labeled +TIP proteins such as EB1 or EB3, which appear as comets streaking throughout the cell (Fig. 1A), has replaced these experiments as a convenient technique for visualizing MT growth across all phases of the cell cycle (Gatlin et al., 2009; Perez et al., 1999; Piehl et al., 2004; Salaycik et al., 2005; Stepanova et al., 2003; Tirnauer et al., 2002a,b). +TIPs are a subset of MT-associated proteins (MAPs) that bind directly or indirectly at the tips of growing but not shrinking or paused MT plus ends, presumably by recognizing structural or chemical differences between the plus end and the lattice (Fig. 1B) (Akhmanova and Hoogenraad, 2005; Dragestein et al., 2008; Galjart, 2010; Jiang and Akhmanova, 2011; Schuyler and Pellman, 2001). While early studies of +TIPs

Abbreviations: EB3, end-binding protein 3; LAP, linear assignment problem; MT, microtubule; +TIP, MT plus end binding protein; ROI, region of interest.

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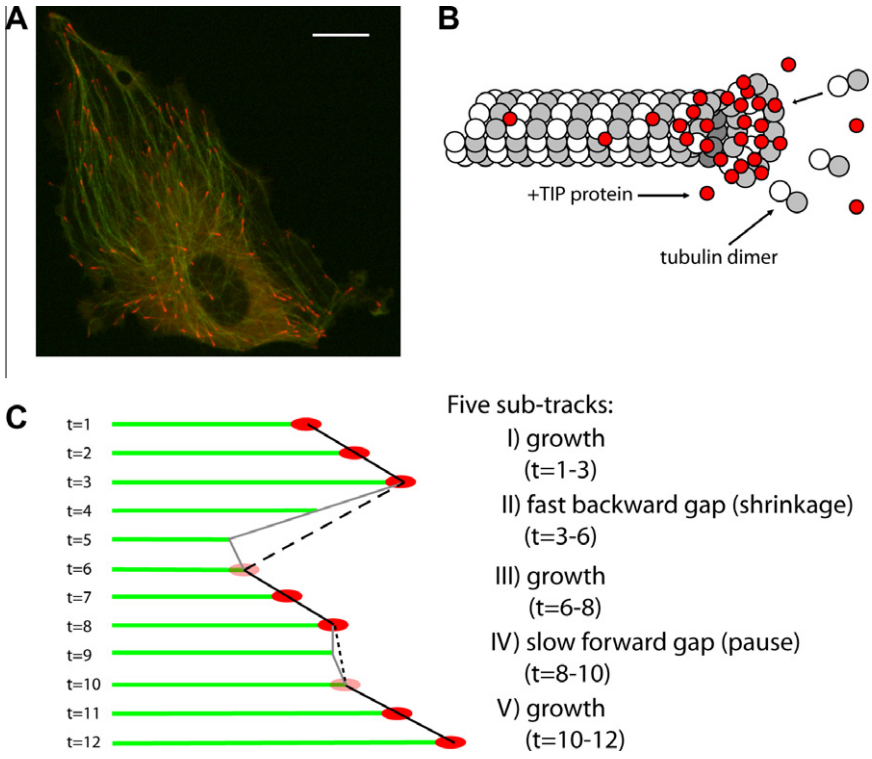

Fig.1. +TIPs mark growing MT plus ends. (A) Dual-channel image of tdTomato-EB3 (red) and GFP-tubulin (green) taken with a spinning-disk confocal microscope (courtesy of Ken Myers, NIH/NHLBI). Bar, 10 μm. (B) +TIPs have a higher binding affinity for growing MT plus ends than for the MT lattice, leading to the comet-like appearance observed in fluorescence images like (A). (C) plusTipTracker first tracks +TIP comets (red ovals) to form growth sub-tracks (I, III, V; solid black lines) and subsequently groups sub-tracks inferred to have come from the same MT to reconstruct shrinkage (II; dashed black line) and pause (IV; dashed black line) behavior. Transparent red ovals indicate newly-formed comets. The time lag between MT rescue and reappearance of a detectable comet can lead to underestimation of shrinkage speed and positional drift during pause. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

focused on qualitative observations, kymograph analysis and recently-developed automated particle tracking methods have enabled the estimation of nucleation rates (Piehl et al., 2004; Salaycik et al., 2005; Srayko et al., 2005) and the systematic measurement of growth speeds (Gatlin et al., 2009; Houghtaling et al., 2009; Kelly et al., 2010; Sironi et al., 2011; Smal et al., 2008).

The disadvantage of tracking MT dynamics by +TIP imaging is that comets reveal directly only the phases of MT growth. However, many of the MT-associated functions are determined by the dynamic switching between phases of growth, pause, and shrinkage. In principle, these transitions can be recovered from the growth trajectories: where two MT growth events are collinear and separated by a short time lag, it is likely that they belong to the same MT. By linking them together, parameters like shrinkage velocity or pause duration can be inferred (Fig. 1C). Matov et al. showed proof of concept for this approach (Matov et al., 2010), but a globally optimal solution, integrated into a software package, is necessary for application of the method by the broader cytoskeleton community.

Here we describe plusTipTracker, a Matlab-based open source software package enabling +TIP comet detection, track reconstruction, track visualization, sub-cellular regional analysis, and MT subpopulation analysis. The package is available from http://lccb.hms.harvard.edu and runs on either Windows or Linux operating systems. Graphical user interfaces and several stand-alone support functions allow novice Matlab users to track and visualize thousands of MTs in minutes—a vast improvement for cytoskeleton biologists who have for many years painstakingly tracked MTs by hand. In addition, batch processing and bioinformatics tools make possible the development of cell-based screens from time-lapse images, enabling diverse applications from drug discovery to mechanistic cell biology.

2. Materials and methods

2.1. Imaging and software

plusTipTracker has been tested on a wide range of +TIP live-cell data and performs optimally on image series filmed at 60× or 100× magnification with a frame rate ranging from 0.5 to 2.0 s. The software was developed and tested using Matlab R2008a. It can be assumed that the package runs on newer Matlab versions and the website http://lccb.hms.harvard.edu will feature regular updates.

Project setup, particle detection, tracking, and track post-processing are controlled by the plusTipGetTracks panel (Fig. 2A), while visualization and some analysis tools are accessed via the plusTipSeeTracks panel (Fig. 2B). Choice of optimal tracking parameter settings may be performed on a representative movie with the help of the plusTipParamSweepGUI tool (not shown). A full description of the software, along with a reference guide to all relevant functions, is available in Applegate and Danuser (2010), which is included with package download.

2.2. +TIP comet detection

2.2.1. Watershed segmentation

Fluorescently-labeled +TIPs appear as near-resolution-sized comets, hereafter referred to as particles, that vary in size, shape, and intensity over time and across regions of the cell. The image background signal also varies regionally and temporally, and due to the generally low signal-to-noise ratio (SNR), particles are often difficult to discern relative to the background. Because of particle heterogeneity and poor signal quality, application of a global