Online Methods:

Software

The ClusterTrack software described in this paper is available as **Supplementary Software 1** and at lccb.hms.harvard.edu; a user-friendly software package with additional functionality for track visualization and statistical processing, not described here, is also available at lccb.hms.harvard.edu.

Cell Culture and Microscopy

HaCaT keratinocytes stably expressing EB1-EGFP were cultured in DME medium, 10% fetal bovine serum and 250 μg ml^{-1} geneticin at 37 °C, and 5% CO_2. mCherry-tagged tubulin was introduced into cells by infection with a recombinant adenovirus. Cells were treated with nocodazole, taxol, or Trichostatin A for ~60 min before imaging. For microscopy, cells were grown on #1.5 coverslips, and the medium was supplemented with 10 mM K-HEPES pH 7.0. Cells were imaged at 37 °C with a 100x NA 1.49 or a 60x NA 1.45 objective lens (CFI APO TIRF; Nikon) on an inverted microscope system (TE2000 Perfect Focus System; Nikon) equipped with a spinning-disk confocal unit (CSU10; Yokogawa) with 200 mW, 488 nm, and 561 nm solid-state lasers (LMM5; Spectral Applied Research), electronic shutters, a cooled charge-coupled device camera (Cool-SNAP HQ2; Photometrics), and controlled by NIS-Elements software (Nikon).

Image Analysis

All programs for EB1-EGFP comet detection, tracking, and geometrical clustering were written in MATLAB (Mathworks, Nalick, MA) and C++. Hand-tracking of microtubule ends in

the dual-wavelength validation movie was done using the tracking function in NIS-Elements (Nikon). Movies were generated with MetaMorph (Molecular Dynamics) and QuickTime Pro (Apple).

Statistical comparison of parameters of microtubule dynamics

None of the parameters of microtubule dynamics (rates of growth and shrinkage, duration of growth, shrinkage, and pauses) were normally distributed. To determine the statistical significance of differences in the mean values between parameters under different experimental conditions we applied a permutation t-test. In brief, for specific parameters 400 values were bootstrap-sampled from the data of different experimental conditions. In agreement with the central limit-theorem the two distributions of the means were always normal and thus could be analyzed for differences using regular Student t-test statistics.

Supplementary Material

Supplementary Figure 1	Flow diagram of EB1-EGFP comet detection, tracking, and geometrical clustering.
Supplementary Figure 2	Detection in widefield epifluorescence images.
Supplementary Figure 3	Detection performance in response to high frequency pixel noise.
Supplementary Figure 4	Magnification dependence of detection and tracking.
Supplementary Figure 5	Frame rate dependence of detection and tracking.
Supplementary Figure 6	Demonstration of tracking fidelity in the cell interior.
Supplementary Table 1	Control parameters for comet detection, tracking, and clustering
Supplementary Table 2	Quantification of false positive events assigned by growth track clustering.
Supplementary Note 1	Difference of Gaussian transformation and σ_1 and σ_2 selection
Supplementary Note 2	Unimodal thresholding to select EB1-EGFP pixels
Supplementary Note 3	Selection of EB1-EGFP comets based on average comet shape
Supplementary Note 4	Estimation of growth rate measurement error
Supplementary Note 5	EB1-EGFP Comet Tracking
Supplementary Note 6	Latency of EB1-EGFP comet formation
Supplementary Note 7	Adjustments to hand-tracked trajectories
Supplementary Note 8	Determination of thresholds for the selection of significant growth and shortening events in computer-generated growth tracks and backward gaps
Supplementary Note 9	Practical guidelines for applying the method
Supplementary Video 1	Overlay of computer-generated growth tracks (yellow) onto a EB1-EGFP time-lapse sequence. Total length is 30 s (75 frames). Images were acquired every 0.4 s. Plays at 15 frames s^{-1}, and is thus accelerated 6x.

Supplementary Video 2	Dual-wavelength time-lapse sequence of mCherry-tubulin and EB1-EGFP used for validation of the clustering algorithm. Hand-tracked microtubule ends are indicated by blue crosses. Total length is 60 s (97 frames). Images were acquired every 0.62 s. Plays at 15 frames s^{-1}, and is thus accelerated 9.3x.
Supplementary Video 3	Same sequence as Video 2 with automatically detected EB1-EGFP comets highlighted by bright green dots.
Supplementary Video 4-6	Regions of the dual-wavelength time-lapse sequence in Video 2 showing examples of microtubule trajectories inferred by the clustering algorithm. Upper left: Microtubule channel; Lower left: EB1 channel; Upper right: Microtubule and EB1 color overlay: Lower right: Microtubule trajectory overlaid on the EB1 channel. Green indicates growth tracks, red are backward and blue are forward gaps. These videos were used for the visual validation documented in Table 2. Video 4 and 5 display trajectories without false positive events. Video 6 displays a trajectory where the second forward link (in blue) is erroneous. This event was counted as false positive.
Supplementary Software 1	ClusterTrack software for detection, tracking and growth track clustering of plus end-labeled microtubules in the analysis of microtubule dynamics.