Supplemental Information

Three-dimensional tracking of plus-tips by lattice light-sheet microscopy permits the quantification of microtubule growth trajectories within the mitotic apparatus

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Conditions for automated tracking and filtering threshold

1. Spot detection
For condition setting for Imaris automated tracking, 20 frames of LLS time-lapse images of a metaphase cell were used [Fig. S1(a)]. MIP and SSP images were used to visualize trajectories of EB1-GFP comets during an observation period (20 frames, 15.100 s).

First, EB1-GFP comets were automatically detected by Local Contrast mode using an estimated object diameter of 0.3 μm, because a single EB1-GFP comet ranged from 3 × 3 pixels in the case of a small comet to approximately 7 × 10 pixels for the largest comets. After detection of candidate objects, true signals were selected by threshold setting for intensity at the center of the spot (Quality). A slope-change point value was useful for selecting positive signals, whereas a larger value failed to detect small comets [Fig. S2(a)].

2. Automated tracking
Automated tracking was executed using an Autoregressive Motion algorithm, which predicts that the spot will move again the same distance and in the same direction. At this step, a limit on travel distance was set on the assumption that microtubule growth rate does not exceed 1 μm/s. The gap-filling option was not used (see also Appendix. Experimental procedures). With these conditions, tracks that match with the actual EB1-GFP trajectories shown in the MIP image in Fig. S1 (a, middle panel) were obtained (b, top left). If the Brownian Motion algorithm developed for randomly moving objects or inappropriate parameters for Autoregressive Motion algorithm was used, each spot was not correctly connected [Fig. S1 (b)].

3. Filtering
To eliminate possible incorrect trajectories, several types of filtering algorithms were used, based on the assumption that microtubule growth is essentially straight with a variable, but limited elongation rate. Examples of filtering effects are shown in Fig. S2 and Fig. S3. Filtering by duration removed short tracks generated by the erroneous detection of background noise [Fig. S3 (b)], whereas filtering by straightness was effective for removing misconnected tracks towards an incorrect direction and wandering tracks that resulted from the detection of
background noise including signal bleed-through from the red channel [Fig. S2 (c)]. Filtering by Max speed removed abnormally long trajectories [Fig. S3 (a)].

Before applying filtering functions, true EB1-GFP trajectories were detected as shown in Fig. S12(c, left) and Movies 15, 16, although high levels of background noises are still present throughout the image. When focusing only on astral microtubules, the detection of comets and tracking was almost perfect. In contrast, the inside of the spindle body spanning the inter-centrosomal space appeared to contain a large quantity of possible pseudo-positive tracks showing random motion. To reduce the background and misconnection mainly attributed to background noise, filtering by max intensity of the spot was effective. However, the detection probability of astral EB1-GFP comets was decreased as the threshold value was elevated [Fig. S3 (b, c); Fig. S3 (a, c)]. This is thought to be due to differences of background levels outside and inside of the spindle. In this study we used a higher intensity threshold to detect EB1-GFP comets in the inside of spindles, at the cost of astral EB1-GFP detection probability.

However, close inspection revealed that tracking errors still occurred inside spindles after filtering processes under high stringency conditions [Fig. S13(b)]. We could not further improve the accuracy by using the filtering functions currently available; therefore, apparent errors were edited manually (deletion, creation, connection, and disconnection).

The above procedures were applied to prometaphase and metaphase cells, but not to anaphase and telophase cells, as described in the main text. Although anaphase/telophase cells generate dense microtubules, in these cells EB1-GFP trajectories were detected with a higher accuracy than for prometaphase/metaphase cells, probably because of the slower microtubule growth rate. It is also possible that lower background levels in anaphase/telophase cells, which could be caused by efficient incorporation of EB1-GFP into the thick microtubule filaments and consequent reduction of diffuse cytoplasmic pool generating background signals, has good effects on automated EB1-GFP comet detection. EB1-GFP tracking in anaphase/telophase cells was fully automated and its accuracy was estimated as shown in Fig. 12 of published main body of the paper.
Supplemental figure legends

Figure S1
Automated tracking with different parameters. To examine experiment condition, a time-lapse LLS image containing 20 frames of a metaphase cell was used. (a) The 1st frame image, as well as MIP and SSP images of 20 frames, are shown. Scale bar: 5 μm. (b) Examples of resulting trajectories obtained using different tracking algorithms (Autoregressive motion or Brownian motion) and different parameters (Max distance of instantaneous EB1-GFP movement and possible gap size to be connected). If Brownian Motion algorithms or inappropriate parameters were used, generated trajectory patterns are unmatched with expected pattern, which is suggested by the MIP image.

Figure S2
The effect of different filtering threshold limits. For three types of filter functions shown in the figure, three examples with different threshold limits are displayed. In each condition, a histogram showing the data distribution and resulting image are presented in the top and bottom panels, respectively. In the histogram, the yellow-shaded area indicates selected data. In (a), selected objects at each threshold limit are marked with white dots. Yellow arrowheads in the right panel indicate unselected true signals with higher threshold limit. In (b) and (c), total trajectories (same as Fig. S1(b) top left) and eliminated trajectories are shown in yellow and red, respectively.

Figure S3
Effect of different filtering threshold limits (continued from Fig. S2) and resulting trajectories. (a, b) Same as Fig. S1(b, c). (c) Resulting trajectories obtained using different parameters as shown in the figure.

Movie legends

Movie 1
3D time-lapse image of a HeLa cell (clone A1) stably expressing EB1-GFP (green) and
histone H2B-TagRFP (magenta) acquired using LLS microscopy. Images were acquired at 1.510-s intervals for 45.300-s duration (30 frames). The first frame is rotated vertically before replaying other images in the sequence. Time: h:m:s:ms.

**Movie 2**
A top (apical) view of an anaphase cell and automated tracking of EB1-GFP comets. Detected spot positions are shown in each frame by magenta dots, while generated trajectories are shown with lines persisting for 10 frames with colored time code. Part of an interphase cell comes into sight on the left. Time: h:m:s:ms.

**Movie 3**
A side (lateral) view of an anaphase cell (same cell as Movie 2) and automated tracking of EB1-GFP comets. Detected spot positions are shown in each frame by magenta dots, while generated trajectories are shown with lines persisting for 10 frames with colored time code. Note that many trajectories run along the basal cortex (arrows in Fig. 2 (c)). Time: h:m:s:ms.

**Movie 4**
Effects of drift correction. Random rotation of the spindle was corrected for using the positions of the two centrosomes as a reference. This movie shows original (left) and drift-corrected (right) time-lapse image sequences of a HeLa cell (clone A1) expressing EB1-GFP (green) and histone H2B-TagRFP (magenta) acquired at 1.510-s intervals over a 45.300-s duration (30 frames).

**Movie 5**
Effects of image preprocessing. Input image of a metaphase cell expressing EB1-GFP subjected to drift correction (a) and the subsequent results of intensity equalization (b) followed by top-hat transformation (c). A single slice image (z = 76) is shown.

**Movie 6**
Effects of image preprocessing. Input image of a telophase cell expressing EB1-GFP subjected to drift correction (a), and the subsequent results of intensity equalization (b) followed by top-hat transformation (c). A single slice image (z = 58) is shown.
**Movie 7**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of a prometaphase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 8**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of a metaphase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 9**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of an anaphase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 10**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of a late anaphase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet
center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 11**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of a telophase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 12**
3D-tracking of EB1-GFP motion generated from single-color time-lapse images of a late telophase cell acquired with an LLS microscope at 0.755-s intervals. Results from a subsequence of the first 40 frames are shown with white dots indicating EB1-GFP comet center position and colored lines indicating instantaneous speed, which is persisting for 8 frames. The last image shows all entire trajectories initiated in the 1st to 40th frame with lines while white dots indicate EB1-GFP comet center position in the first frame. The upper color bar indicates the instantaneous speed range for EB1-GFP tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 13**
EB1-GFP comet tracking in a metaphase cell after automated tracking but before the filtering process. Detected spots are shown by magenta dots. Note that most astral EB1-GFP comets are detected accurately. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 14**
EB1-GFP comet tracking in a metaphase cell after automated tracking but before the filtering process (same as Movie 13). Detected spots and generated trajectories are shown with white
dots and colored lines persisting for four frames indicating instantaneous speed, respectively. The color bar indicates the instantaneous speed range for tracks (0.3–0.6 μm/s). Note that most astral EB1-GFP comets are tracked accurately, while in inside spindles, possible misconnections are often observed. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 15**
EB1-GFP comet tracking in a metaphase cell after automated tracking and filtering processes (Max spot intensity ≥ 2000). Detected spots are shown by magenta dots. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 16**
EB1-GFP comet tracking in a metaphase cell after automated tracking and filtering processes (Max spot intensity ≥ 2000) (same as Movie 15). Detected spots and generated trajectories are shown by white dots and colored lines persisting for four frames indicating instantaneous speed, respectively. The color bar indicates the instantaneous speed range for tracks (0.3–0.6 μm/s). Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 17**
EB1-GFP comet tracking in a metaphase cell after automated tracking and filtering processes (Max spot intensity ≥ 4000). Detected spots are shown by magenta dots. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 18**
EB1-GFP comet tracking in a metaphase cell after automated tracking and filtering processes (Max spot intensity ≥ 4000) (same as Movie 17). Detected spots and generated trajectories are shown by white dots and colored lines persisting for four frames indicating instantaneous speed, respectively. The upper color bar indicates the instantaneous speed range for tracks (0.3–0.6 μm/s). Although several true EB1-GFP tracks were lost especially in the aster, most apparent misdetections/misconnections were reduced for further analysis. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 19**
Computational reslicing of the whole cell volume of an anaphase cell. Using the Imaris plane
clipping function, the upper and lower part of the cell was made invisible, so that an approximately 4-μm segment spanning two centrosomes remained. Rotation of the segment allows top-view observation. Time: h:m:s:ms (time is not indicated with color code in the images).

**Movie 20**

Tracking results of the segment shown in Movie 19. Detected spot positions are shown in each frame by magenta dots, while generated trajectories are shown by white lines persisting for four frames. Note that some of dots/trajectories lie astride the outside of the segment are not visible. Time: h:m:s:ms (time is not indicated with color code in the images).
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(a) 1st frame image  | MIP image of 20 frames  | SSP image of 20 frames

(b) Autoregressive motion
Max distance: 0.5 μm
Max gap size: 0

Brownian motion
Max distance: 0.5 μm
Max gap size: 0

Brownian motion
Max distance: 2 μm
Max gap size: 0

Autoregressive motion
Max distance: 0.5 μm
Max gap size: 3

Autoregressive motion
Max distance: 2 μm
Max gap size: 0

Autoregressive motion
Max distance: 2 μm
Max gap size: 3
(a) Quality (intensity at the center of the spot)

- ≥ 0
- ≥ 900
- ≥ 1800

(b) Track duration

- ≥ 2 frames
- ≥ 3 frames
- ≥ 4 frames

(c) Track straightness

- 0.5 - 1.0
- 0.7 - 1.0
- 0.9 - 1.0
(a) Max speed
- 0.0-0.6 μm/s
- 0.0-0.8 μm/s
- 0.0-1.0 μm/s

(b) Max intensity
- ≥ 5000
- ≥ 10000
- ≥ 20000

(c) Result image
- Befoer filtering
  - Track Duration: ≥ 2 frames
  - Track straightness: 0.7-1.0
  - Max speed: 0.0-0.6 μm/s
  - Max intensity: ≥ 9000

- Track Duration: ≥ 2 frames
  - Track straightness: 0.7-1.0
  - Max speed: 0.0-0.6 μm/s
  - Max intensity: ≥ 20000