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Speckle detection and tracking

Image alignment.

Alignment was performed on cropped images of the spindle without segmenting the speckled region. For a given image sequence $I_1, \dots, I_k, \dots, I_N$, the translation and rotation between consecutive frames were calculated using a standard correlation-maximization algorithm (Pratt, 2001). Specifically, if we denote the aligned image of I_{k-1} by I'_{k-1} , spindle motion between I_k and its aligned preceding frame I'_{k-1} was approximated locally using a linear transformation

$$T_k = \begin{bmatrix} R_k & 0 \\ D_k & 1 \end{bmatrix}$$

that maximizes the correlation between I_k and I'_{k-1} , where R_k and D_k denote spindle rotation and translation, respectively.

Speckle detection.

Speckle detection was performed on the aligned images as previously described (Ponti et al., 2003). When out of focus fluorescence was significant, the coefficient in the shot noise model was set to zero in order to avoid the influence of model inaccuracy.

Speckle tracking.

A new particle-tracking algorithm was developed for fluorescent speckle microscopy (Yang, G., A. Matov, and G. Danuser. 2005. Proceedings of the Institute of Electrical and Electronics Engineers International Conference on Computer Vision and Pattern Recognition. 9–17). In brief, speckle tracking was formulated as a modified bipartite graph linear assignment problem and was solved using the Jonker-Volgenant algorithm (Jonker and Volgenant, 1987)

$$\begin{aligned} \hat{a}^k &= \arg \max_{a^k} \sum_{i \in G_k} \sum_{j \in G_{k+1}} a^k(i, j) c^k(i, j) p(i, j, F(i)) \\ \text{st. } \sum_i a^k(i, j) &= 1, \sum_j a^k(i, j) = 1, a^k(i, j) \in \{0, 1\} \end{aligned} \quad (1)$$

where $a^k(i, j)$ denotes the unknown assignment matrix. $a^k(i, j) = 1$ if and only if element i in subgraph G_k is assigned to element j in subgraph G_{k+1} . Subgraph G_k and G_{k+1} consist of all detected speckles in frame k and $k + 1$, respectively. $c^k(i, j)$ denotes the association cost of assigning speckle i to speckle j . This cost is weighed by the probability for linking i to j given the global speckle flux vector field $F(\cdot)$ at speckle i , which is denoted $p(i, j, F(i))$.

Definition of speckle flux vector field.

The vector field of speckle flux is required to solve Eq. 1. For this purpose, a more robust version of the graph algorithm described in Vallotton et al. (2003) was developed (Yang, G., A. Matov, and G. Danuser. 2005. Proceedings of the Institute of Electrical and Electronics Engineers International Conference on Computer Vision and Pattern Recognition. 9–17). This algorithm exploits the speckles in three consecutive frames to establish smooth motion in the flux field. However, because of the small size of PtK1 spindles, the relatively low number of speckles, and the short speckle lifetime, the flux vector field F could not be computed reliably. Instead, the flux field was defined interactively based on spindle geometry. An example (a monastrol-treated monopolar spindle) is shown in Fig. S1 A. After the pole center C_0 (shown in red in Fig. S1 B) was defined, the speckle flux vector at position X_k was computed as

$$F(X_k) = \frac{C_0 - X_k}{\|C_0 - X_k\|}.$$

A deviation tolerance angle, set to be between 45 and 60°, was used to accommodate the inaccuracy of this simplified approximation of spindle geometry and the randomness of speckle motion. For a given track i that consists of the point sequence of $X_{i,0}, X_{i,1}, \dots, X_{i,N}$, its flux rate v_i is defined as its mean velocity; namely, the ratio between total speckle displacement and time (Fig. S1 C):

$$v_i = \frac{\sum_{j=1}^N \|X_{i,j} - X_{i,j-1}\|_2}{N}.$$

Limits of accuracy in tracking speckles in PtK1 spindle fibers produced by the short lifetime of non-KMTs

Because of the short lifetime of nonKMTs, we had to sample one frame every 10 s or faster to guarantee that speckles associated with nonKMTs have a mean speckle lifetime of approximately three frames, which is necessary for reliable tracking. However, this constraint works against the requirements for time sampling imposed by the limited spatial resolution we could afford. Because of the low photon number produced by the few fluorophores per speckle, we were obligated to apply 2×2 pixel binning to capture a fluorescent signal significantly above camera noise. This restricted the effective pixel size to 0.13 μm in object space. Under this condition, 10-s sampling is the fastest rate in which an instantaneous speckle velocity of 0.7 $\mu\text{m}/\text{min}$ can be captured (assuming a speckle localization error of ~ 0.5 pixels). Together, these calculations indicate that qFSM of MT flux in PtK1 spindles reaches the limits of fluorescence imaging and tracking technology.

Photoactivation simulation

To study whether spatial heterogeneity of MT flux can be revealed by photoactivation measurements, we performed a series of simulation experiments in which flux rates of tubulin marks were assumed to follow normal distributions. The results are summarized as follows (see Video 3). (1) Simulation results confirm that the difference in mean MT flux among different fibers is small. The first part of the video PAsimulation_case1 simulates the case when there is no variation in MT flux between different fibers and within each individual fiber (the mean flux rate is set to be $0.65 \mu\text{m}/\text{min}$). In comparison, the next section of the video PAsimulation_case2 simulates the case when there is a SD of $0.27 \mu\text{m}/\text{min}$ in flux rates between different fibers but no variation within a fiber. This simulation shows that if the variations in flux rate were mainly caused by different flux rates in different fibers, the activated fluorescent bar across several fibers would have gradually disintegrated into multiple blobs. It is clear from actual photoactivation videos that the fluorescent bar across different fibers remained largely intact. This indicates that the flux rates along different fibers are similar. (2) Simulation results show that spatial heterogeneity in MT flux cannot be observed from photoactivation videos. The third part of the video, PAsimulation_case3, simulates the case when there is no variation in flux between different fibers but flux varies within a fiber with a SD of $0.27 \mu\text{m}/\text{min}$. No significant differences can be observed between PAsimulation_case1 and PAsimulation_case3. The last section of the video, PAsimulation_case4, simulates the case when there are two modes in MT flux distribution: a total of 75% of the fluorescent marks obey normal velocity distribution of $N(0.65, 0.27)$ and remain active throughout the video, whereas the remaining 25% of fluorescent tubulin marks obey a normal velocity distribution of $N(0.33, 0.27)$ and disappear after the first five frames. Except for the relatively small elongation of the blobs in the first five frames, no difference can be observed between this video and PAsimulation_case3. It would be very difficult to fully recover the two-mode distribution using only photoactivation data because the responses of those short lifetime events of a small population can be easily masked by the rest.

Comparison of flux distribution in monastrol-treated bipolar spindles with monastrol-treated monopolar spindles

We compared the flux distribution of bipolar spindles with monopolar spindles in cells treated with $100 \mu\text{M}$ monastrol by computing their cumulative probability distribution (Fig. S1 D). The cumulative distribution is essentially free of influences from the discrete spatial and temporal sampling observed in this specific group of data (effective pixel size is 0.13 ; frame rate is $10 \text{ s}/\text{frame}$; see inset histogram of monastrol-treated bipole). For each spindle configuration, data from two videos are pooled together to increase the number of samples. The number of tracks used to compute monastrol bipole distribution is 138 (Fig. S1 D, red); the number of tracks used to compute monastrol monopole distribution is 242 (Fig. S1 D, green). The distributions show that there are a higher percentage of tracks with low flux rate in monastrol-treated bipole than in monastrol-treated monopole. By performing a Kolmogorov-Smirnov test of the two distributions, we get a P value of 0.0167 , indicating that the distributions are different. However, the mean flux values are statistically indistinguishable ($0.56 \pm 0.25 \mu\text{m}/\text{min}$ for monastrol-treated bipole; $0.57 \pm 0.35 \mu\text{m}/\text{min}$ for monastrol-treated monopole). We conjecture that the difference between the two configurations originate in the overlap that is still present in the monastrol-treated bipole. Although its ATPase activity is blocked, kinesin 5 may act as a partial cross-linker between oppositely oriented MT fibers and, thus, oppose the pulling-in mechanism in driving poleward flux. Kinesin 5-mediated cross-linking of oppositely oriented MTs is absent in a monopole (compare with Fig. 3 A). This model needs verification in larger spindles where the degree of overlap can be measured accurately.

References

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