Supplemental Materials

Molecular Biology of the Cell

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Supplemental methods

Theoretical and technical considerations for determination of imaging interval

From the kymograph in Fig. 1D, we note that spindle movement generates a wavelike pattern in histone and tubulin channels before anaphase onset. In the tubulin channel, the long axis of the spindle often diverges from the line of the kymograph, partially obscuring this wavelike pattern. As these waves appear to have a periodicity of about 1 minute, we elected to begin with single-plane imaging at 5-second intervals.

To assess whether a 5s time step is sufficient to capture spindle dynamics, we first note the Nyquist rate necessary for capturing a sinusoid with a period of 40 seconds (slightly less than the lowest observed oscillatory period) is 20 seconds. However, sampling at the Nyquist rate is insufficient to preserve amplitude of the signal. The minimum fraction preservation of oscillatory peaks can be estimated as the value of a cosine curve that oscillates between 0 and 1, as derived in equation 1 and simplified in equation 2.

\[
(1) \, Preservation = \frac{1}{2} \times \cos(2\pi \times \frac{1 \times \text{timestep}}{\text{period}}) + \frac{1}{2}
\]

\[
(2) \, Preservation = \frac{1}{2} \times \cos(\pi \times \frac{\text{timestep}}{\text{period}}) + \frac{1}{2}
\]

At a time step of 5 seconds, peaks with oscillations of period 40 seconds are preserved within at least 96%.

No traces demonstrated significant high-frequency peaks (Fig. 3L, M), indicating that there were no small-scale dynamics that are missed with this analysis. Additionally, high time resolution imaging was performed, and no apparent fine-scale dynamics were observed. Thus, a period of 5 seconds is optimal to the major spindle dynamics. Under our imaging protocol, samples can be imaged for at least 1000 time points without evidence of phototoxicity, such as lagging chromosomes, dysmorphic nuclei, or cell junction breakdown, and minimal photobleaching is apparent.

We determined that multiple z-plane imaging is not necessary, as both spindle poles generally stay in focus through mitosis. To confirm that oscillatory spindle dynamics were not mirrored in the z-plane, images of spindles in cells expressing GFP-Tub, mChe-H2B, and BFP-CAAX were acquired in 8 different z-planes at 2µm steps, for a total acquisition time of 10 seconds. Brightest point projections were generated in FIJI. Spindle poles were selected manually using the described MatLab GUI for manual pole tracking. Z-stacks were then resliced at every time point along the line connecting the spindle poles. Although much spindle movement and oscillation was apparent in the x-y plane of the brightest point projection movie (Movie 2), such movements were not mirrored in the z-plane (Movie 3). Thus, we deemed that collection of z-series is not necessary for the quantification of spindle dynamics due to increased phototoxicity and decreased temporal resolution.

Adaptation of MEDUSA for detection of cell outlines

The MEDUSA segmentation pipeline (Zulueta-Coarasa et al., 2014) starts with manual initialization of the brightest-point region, followed by refinement by a snakes algorithm and one
pass of subsampling and refinement by LiveWire. Our pipeline does not utilize snakes, as there are often too many bright patches of membrane signal within the cell for snakes to function appropriately. To counteract this, we instead use two passes of subsampling and LiveWire, which ensures that all points in the ring are on the brightest path.

**Elliptical fitting**

In the MatLab regionprops function, the ellipse is fit to match the second-moments of the region. Informally, the ellipse if fit to have the same mass distribution around the centroid as the segmented region. As such, the elliptical fit may not behave in an intuitive manner when the fitted region is hollow or discontinuous. With this in mind, some modification of each segmented region is necessary to ensure that meaningful data are derived from the regions. First off, the segmented cell boundary is an outline. In order for the fitted ellipse to accurately represent the properties of the cell, the outline must be filled beforehand. Proper fitting of tubulin/spindle regions requires careful consideration pre-NEB, when the region consists of two separate, bright centrosomes, and post-Anaphase onset, when the hemi-spindles separate into two distinct regions (much like histone/chromosome regions) (Fig. 7B). In order for an ellipse to accurately capture important properties of these disconnected regions, they must be joined into solid objects. This can be accomplished through calculation of the convex hull, the smallest single region with no concavities that contains all original region (Fig. 7B’’). The convex hull also serves to fill any holes that may exist within the spindle or chromosome regions. The resultant elliptical fitting much more accurately reflects the physical properties of the spindle that we are interested in measuring. This is most clearly reflected in measurement of spindle major axis length.

**Recognition of eccentricity dip at anaphase onset**

To automate recognition of anaphase, we first normalized histone region eccentricity to a maximum of one for each spindle. Starting from the frame denoted as metaphase, we found the first frame where the normalized region eccentricity is below 0.8. By eye, these time points are always post-anaphase onset. We then trace backward from that time point to the first time point where normalized eccentricity drops below 0.95.

**RMS analysis**

The root mean square (RMS) level of spindle rotational acceleration was calculated for 2-minute intervals starting at metaphase, and for 2-minute intervals starting at every frame until 2 minutes prior to anaphase onset. RMS traces were then normalized to a maximum of 1 and interpolated at 100 time points to correspond with progress through metaphase.

**Reconstruction of idealized spindle pole locations for velocity analysis**

Because the threshold for converting spindle images to binary regions is calculated independently for each frame, artifact is introduced in the frame-to-frame measurement of spindle pole location from slight variance in spindle length. To negate the effects of this variation on spindle pole velocity measurements, the major axis length of the elliptical fit to the spindle region was calculated for all frames in a time series. A 10-point running average filter was applied to smooth spindle length. Finally, spindle regions were reconstructed about the histone region centroid using the smoothed spindle length. Pole locations were derived the extrema of the resultant ellipse along the long axis.
Supplemental Figure Legends

Figure S1. Cell outline segmentation

A) Thresholding of median-filtered mTagBFP-CAAX (A’) results in binary images (A’’) that contain intra-cellular noise and discontinuities at dark spots in the image (arrowhead). B) Two-color imaging of mosaicly expressed mChe-CAAX (B’) and mTagBFP-CAAX (B’’) demonstrates peak colocalization in a line scan (C). D) Workflow of our cell boundary detection pipeline. Initial cell boundaries are subsampled and refined 2x with a brightest path approach, and used to initialize subsequent frames.

Figure S2. Segmentation of the mitotic spindle through morphological reconstruction.

A) Mitotic spindle labelled with GFP-Tub. Thresholding of median filtered tubulin image (A’) results in a binary image (A’’) with numerous non-spindle foci of tubulin. B) Line scan of yellow line in 4A’ reveals non-spindle tubulin peaks representing astral bundles. Note the slight trough between the spindle signal and astral signal (arrowhead) C) Workflow of our spindle segmentation pipeline. The manually initialized image is constrained to the previously detected cell boundary and processed with a morphological reconstruction filter to remove non-spindle tubulin peaks. The resultant clean binary region is used to initialize the filter location in next frame of the time series.

Figure S3. Segmentation of chromosomes.

A) A mitotic spindle containing a metaphase plate and a grossly misaligned chromosome in a cell expressing mChe-H2B, GFP-Tub, and BFP-CAAX. B) Workflow of our chromosome segmentation pipeline. Similar to the spindle segmentation, a line is drawn on an initial image. The image is then cropped to the cell boundary and filtered by morphological reconstruction, removing outlying nuclei and misaligned chromosomes from the image. The image is converted to binary at a threshold chosen by Otsu’s method. The same threshold is also applied to the cropped, but non-reconstructed image, highlighting misaligned chromosomes. A line derived from the aligned chromosome region is used to initialize the next frame in the time series.

Figure S4. Derivation of cell and spindle region measurements

A) An elliptical fit to a cell region, indicating measurements derived from the elliptical fit. The same metrics can be obtained through elliptical fitting of tubulin and histone regions. B) Separation of the tubulin region in anaphase spindles results in ill-fitting ellipses (B’). Applying a convex hull to the segmented region improves elliptical fit (B’’). C) Spindle pole location can be estimated as the extrema of the elliptical fit (blue asterisk) or as the extrema of the tubulin region along the major axis of the ellipse (red plus). D) Distance between spindle pole and cell boundary can be measured at the nearest point, at the nearest point along the spindle axis, and at a “target” point (i.e. the cortical point along the anaphase axis of the cell).

Figure S5. Automated selection of NEB, Metaphase and Anaphase.

One hundred and six time series of mitotic cell expressing mChe-H2B, GFP-Tub, and BFP-CAAX were run through our cell, spindle, and chromosome segmentation pipelines. A) A sample trace of average tubulin intensity inside a histone region, normalized to one, plotted against time. Marked points indicate automatically selected (X) and manually selected (O) times of NEB, metaphase, and anaphase, in order. B) A sample trace of histone elliptical fit eccentricity (ratio of inter-foci length to major axis length) over time. X’s and O’s represent automatically and manually selected temporal landmarks as in A. Note the increase in
eccentricity from NEB to metaphase, and the trough beginning at anaphase onset. Time
difference of manually selected frame and automatically selected frame of C) NEB, D)
Metaphase, and E) Anaphase at various thresholds (error bar = standard deviation). NEB
threshold is based on tubulin intensity, as in A, while metaphase and anaphase threshold are
based on normalized histone eccentricity as in B. F) Comparison of interval lengths using
automatically and manually selected time points.
Supplemental Figure 3

A

B

Initialize manually

Crop and Filter

Threshold unfiltered image

Initialize next frame

Reconstruct initializing line

Threshold
Supplemental Figure 4

A

Elliptical Fit

Major Axis

Minor Axis

Orientation

Centroid

Elliptical Fit

Initial Image

Elliptical fit to initial region

Elliptical fit to convex hull region

B

B'

B''

Initial Image

Elliptical fit to initial region

Elliptical fit to convex hull region

C

Tubulin

* Elliptical fit pole estimate

+ Region extrema pole estimate

D

Nearest point

Nearest point along spindle axis

Distance to anaphase axis
