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How does mitotic spindle assemble in prometaphase?

ICTS TIFR, December 2020

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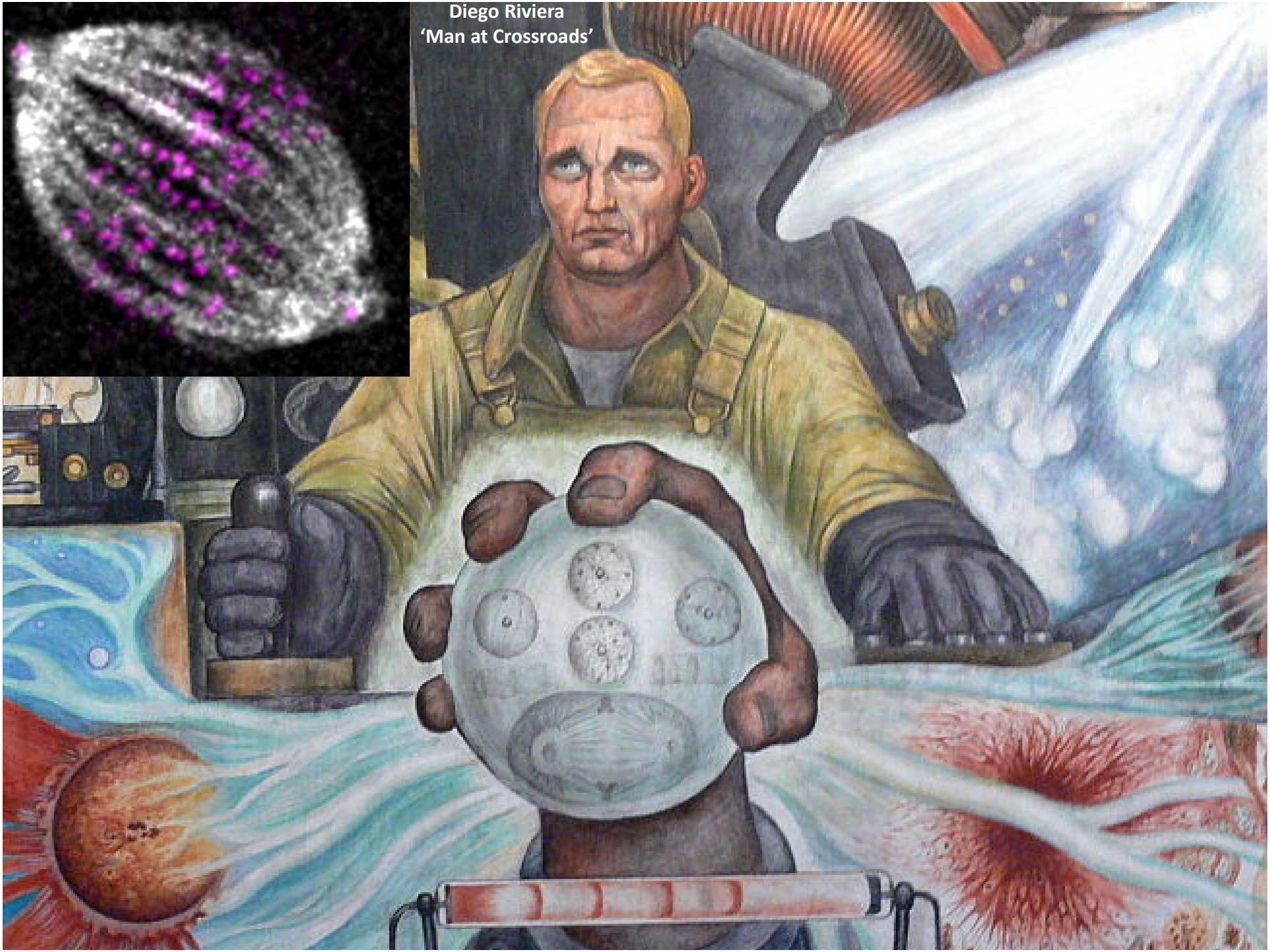
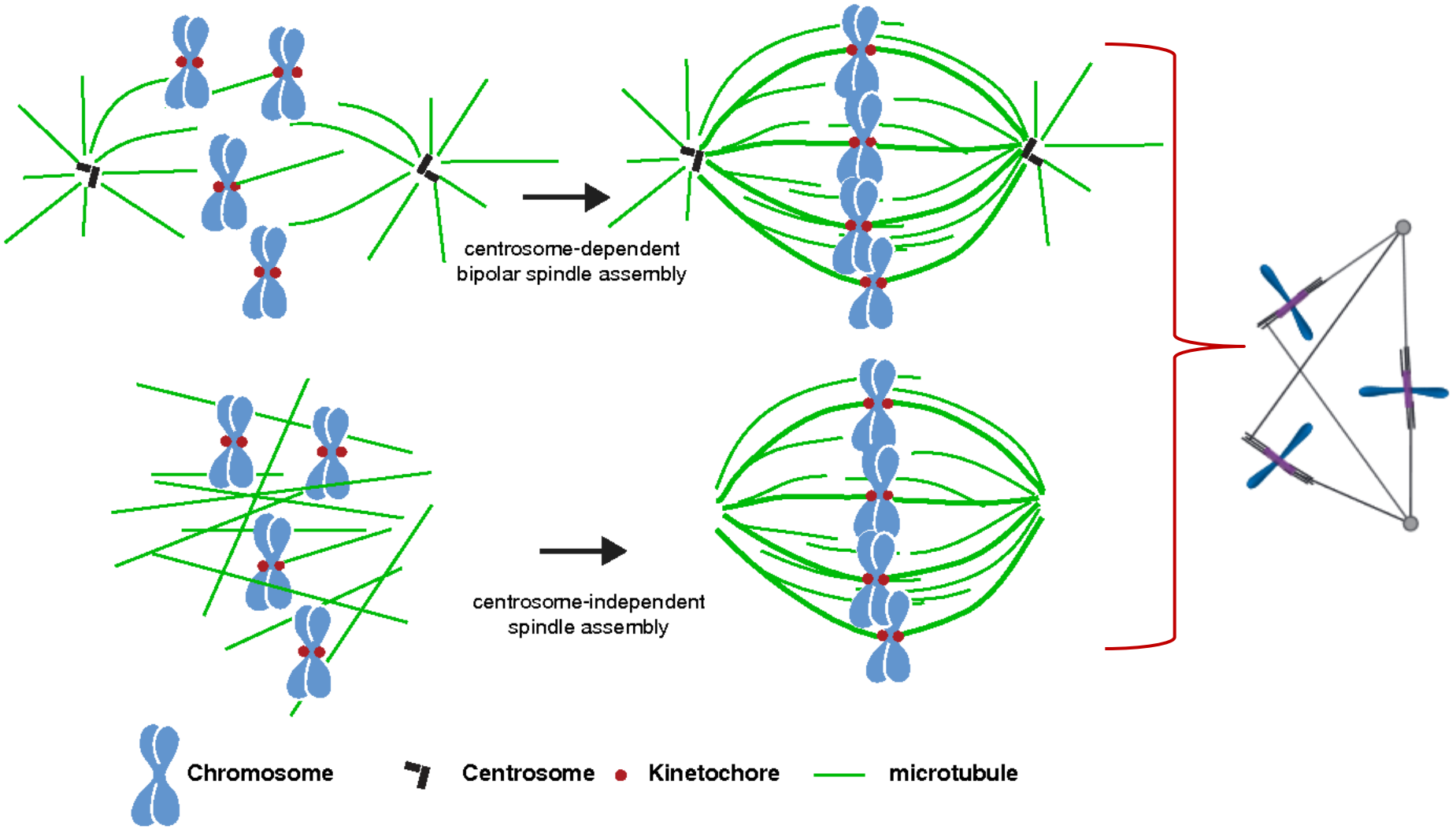
Supported by NSF, NIH

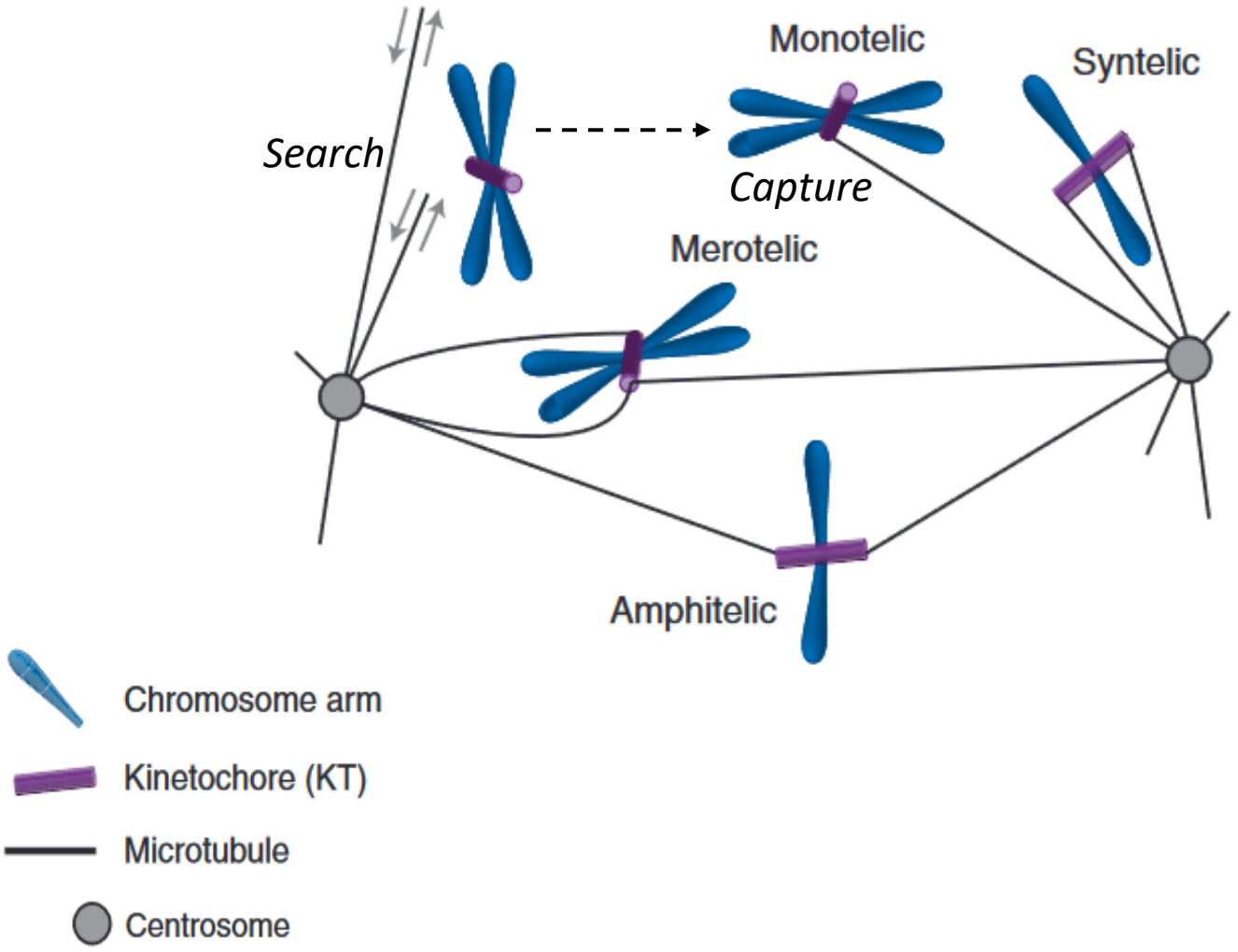

Diego Riviera
'Man at Crossroads'

Spindle self-assembles in prometaphase stochastically, rapidly, accurately. What are the design principles behind this?



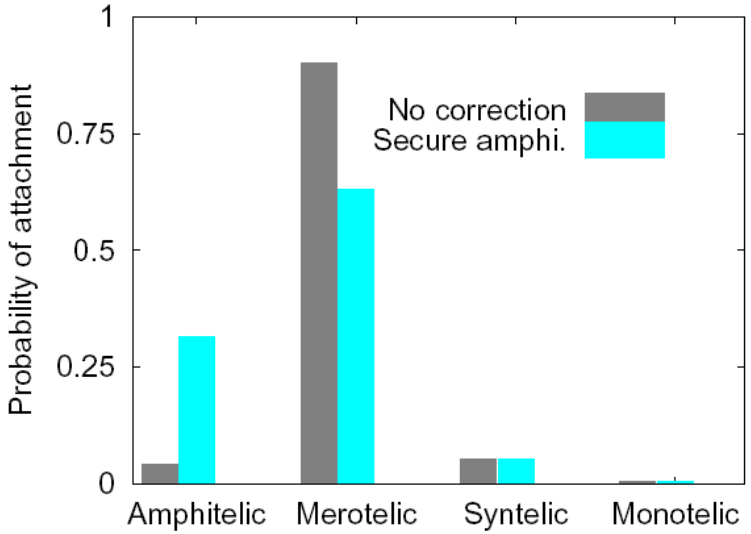
Microtubule dynamic instability and search and capture hypothesis

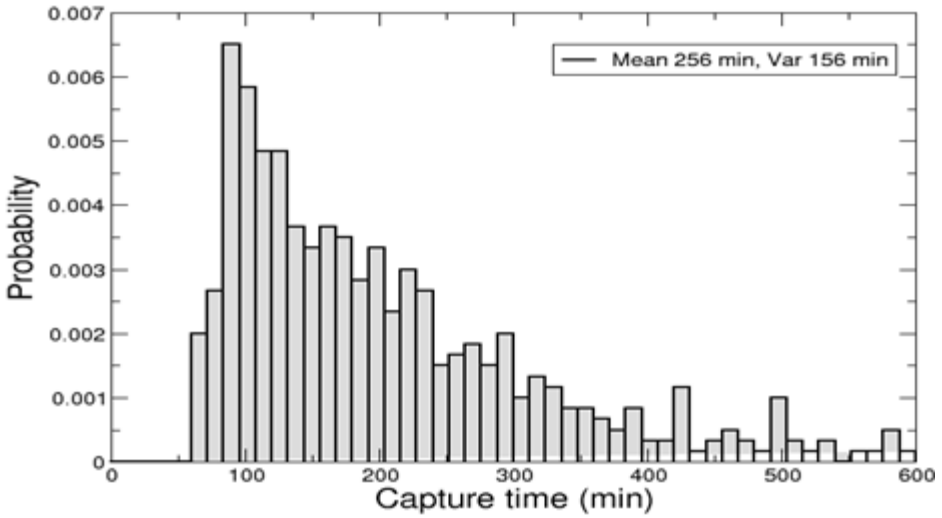
Mitchison & Kirschner, 1984-86
Holy & Leibler, 1994



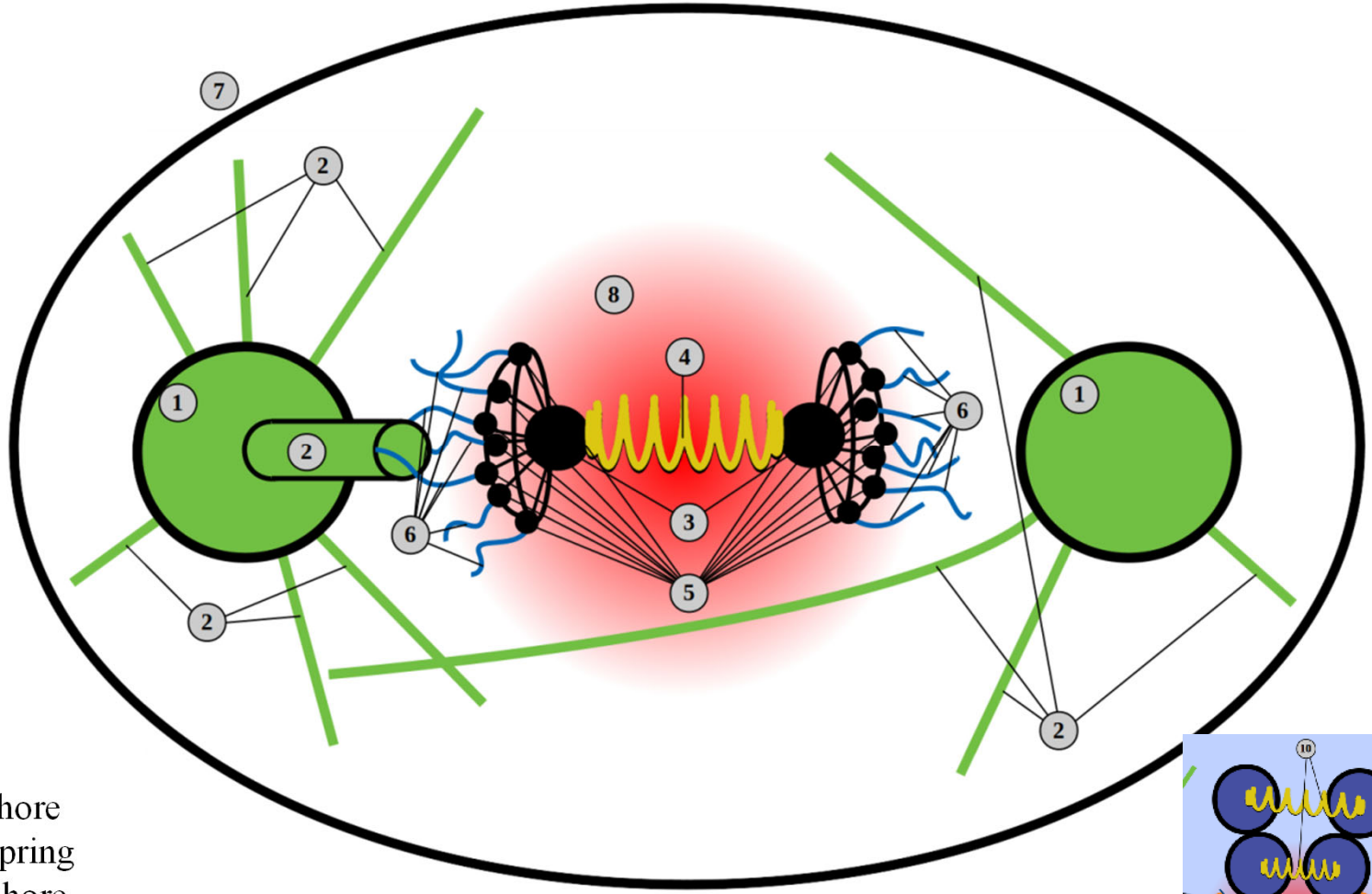
Computer simulations: capture events happen at random times, distributed broadly. Capturing all chromosomes takes a long time, many errors are made.

Wollman et al. Current Biology (2005)
Paul et al, PNAS (2009)
Magidson et al, Cell (2011)
Silkworth et al, Mol Biol Cell (2012)
Magidson et al, Nat Cell Biol (2015)
Renda et al, Open Biology (2020)



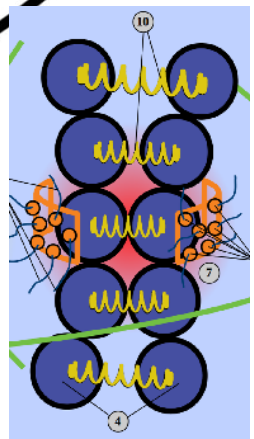


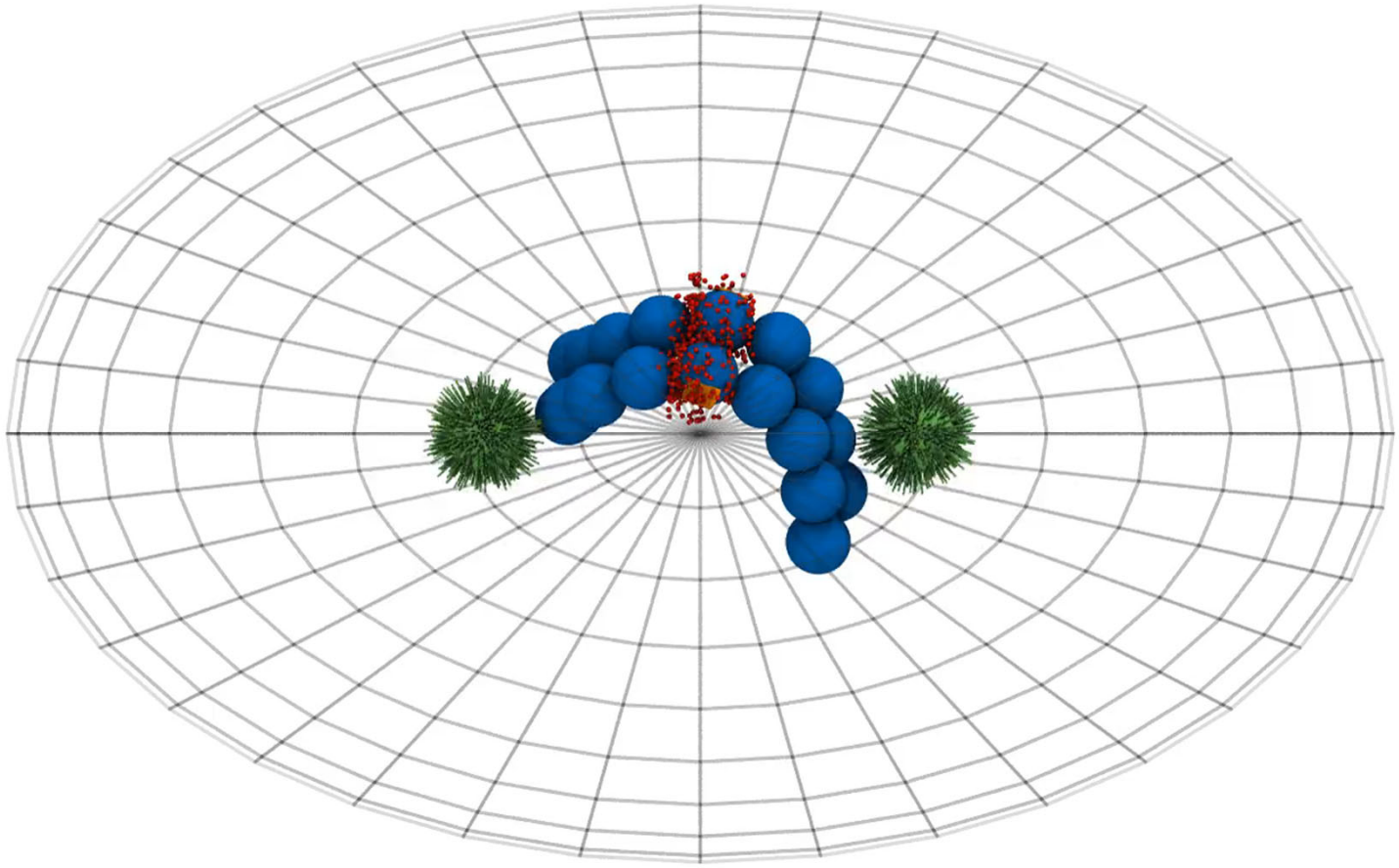
Detailed, geometrically realistic agent-based simulation in 3D with Aurora-B-mediated error correction mechanism (UMass team, in progress)



1. Centrosomes
2. Microtubules
3. Inner kinetochore
4. Centromere spring
5. Outer kinetochore
6. Ndc80
7. Cell cortex/membrane
8. Aurora B gradient
9. Phosphatase (uniformly distributed)

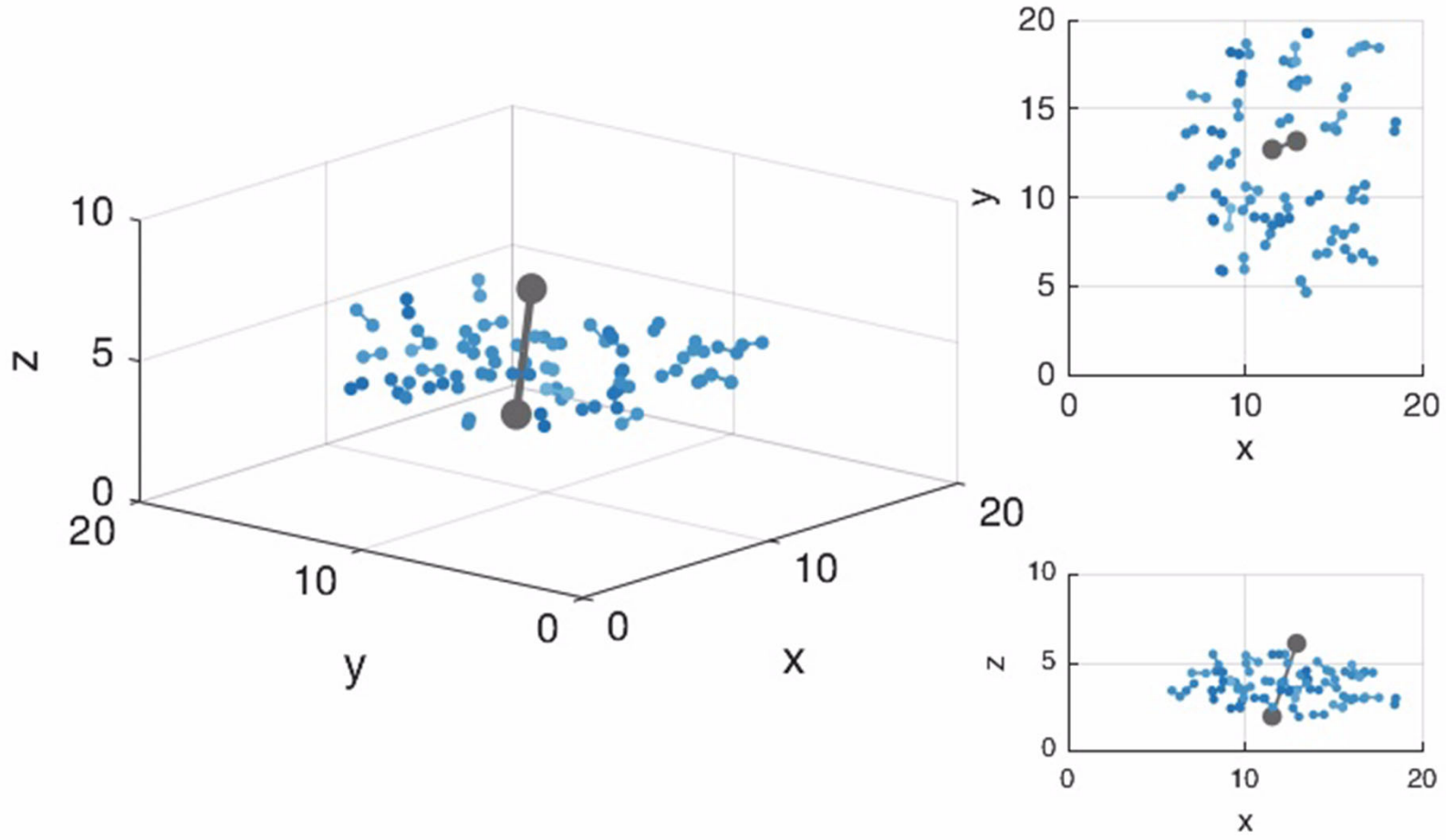
+ chromosome arms



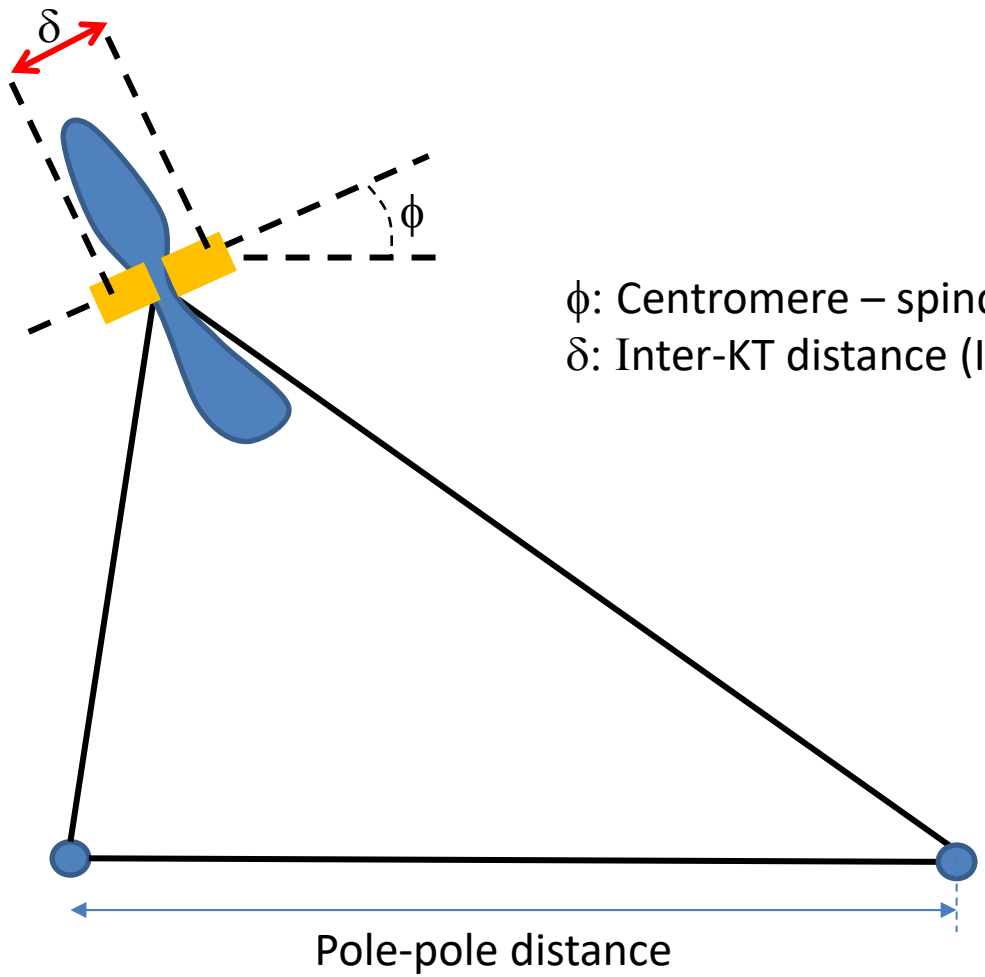


~ 30 minutes of biological time; takes ~ 20 hours of wall-clock time on GeForce GTX 1080.

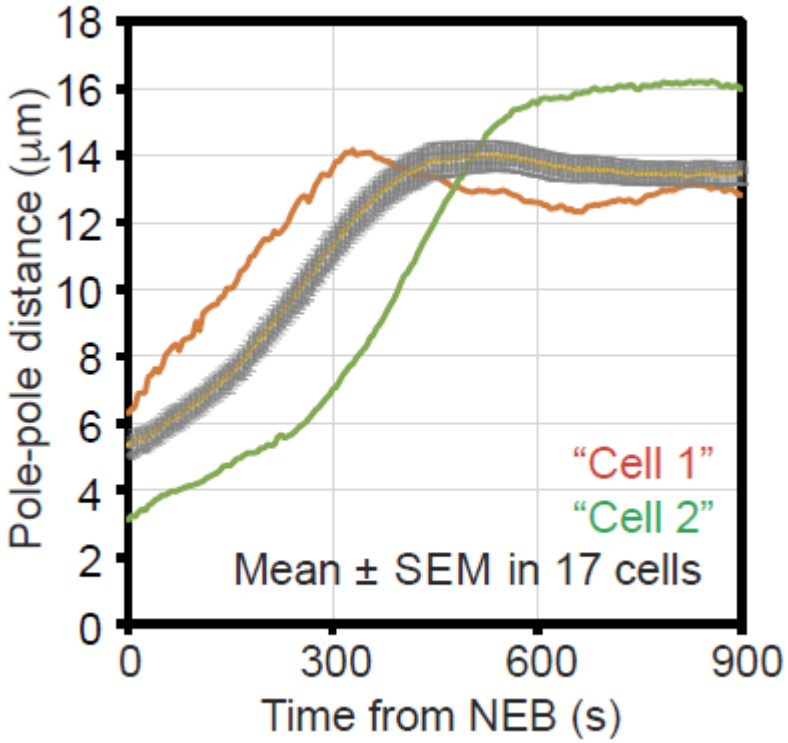
Tracking centrosomes and KTs with high spatial-temporal resolution in RPE-1 cells:



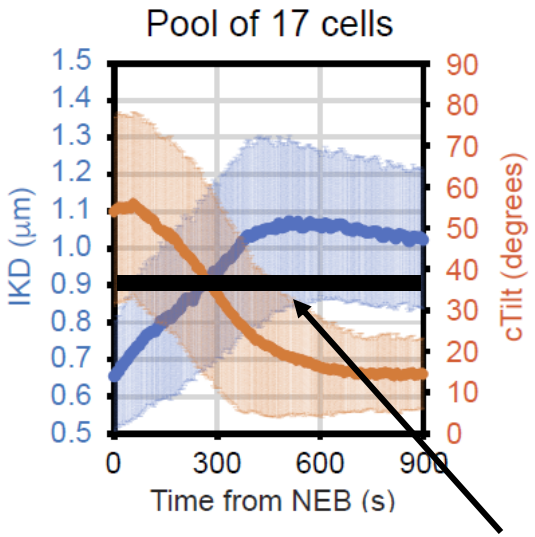
Two important chromosome characteristics:

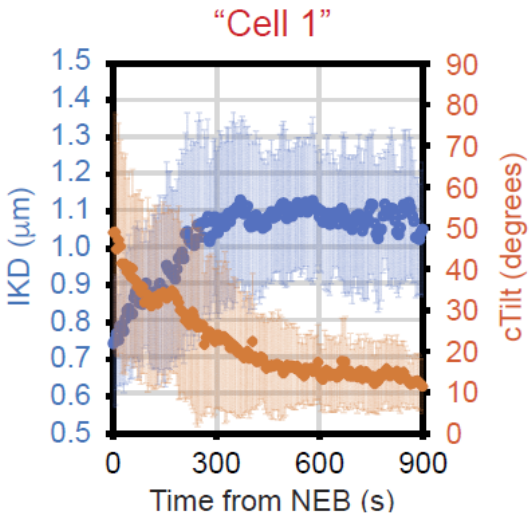


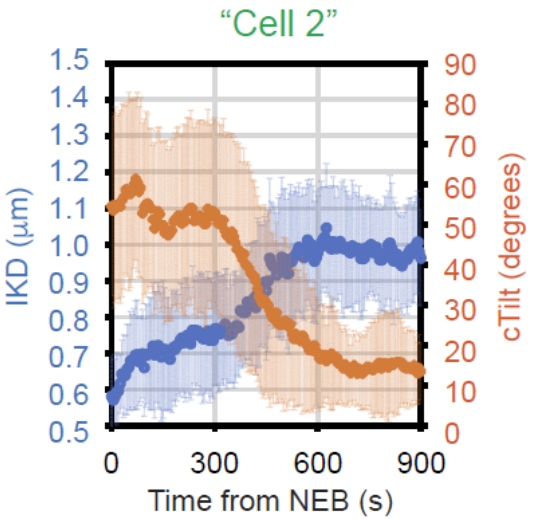
ϕ: Centromere – spindle axis angle (cTilt)
δ: Inter-KT distance (IKD)



Bi-orientation
=
Inter-KT stretch
+
centromere's alignment with spindle axis

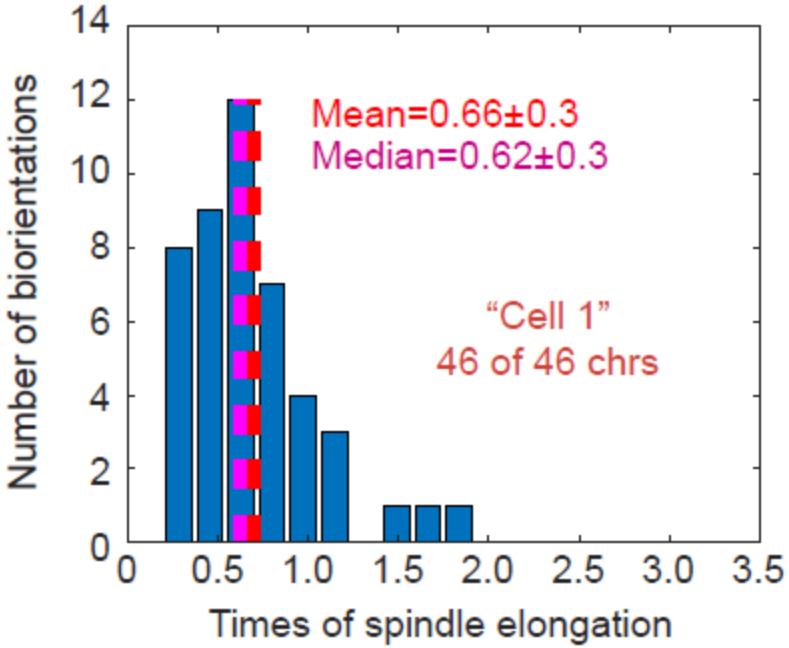


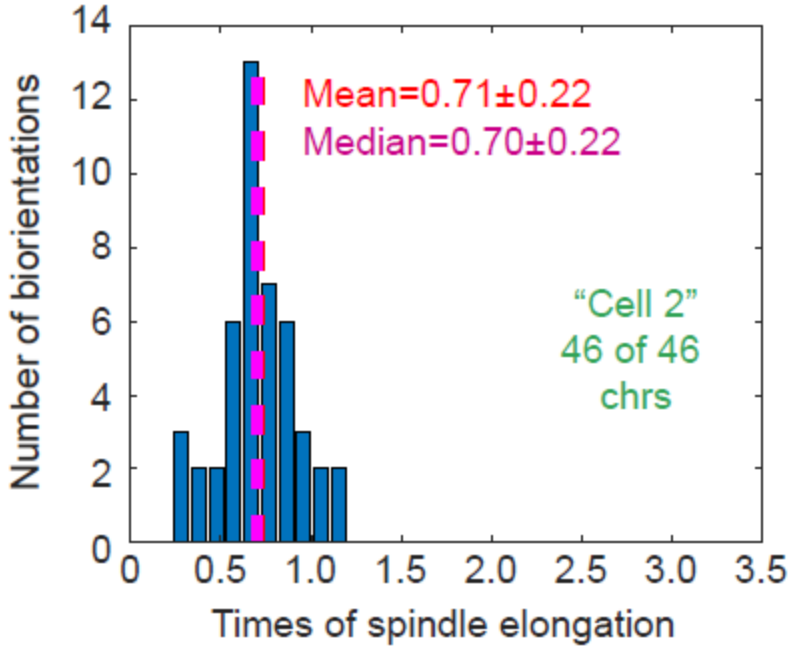




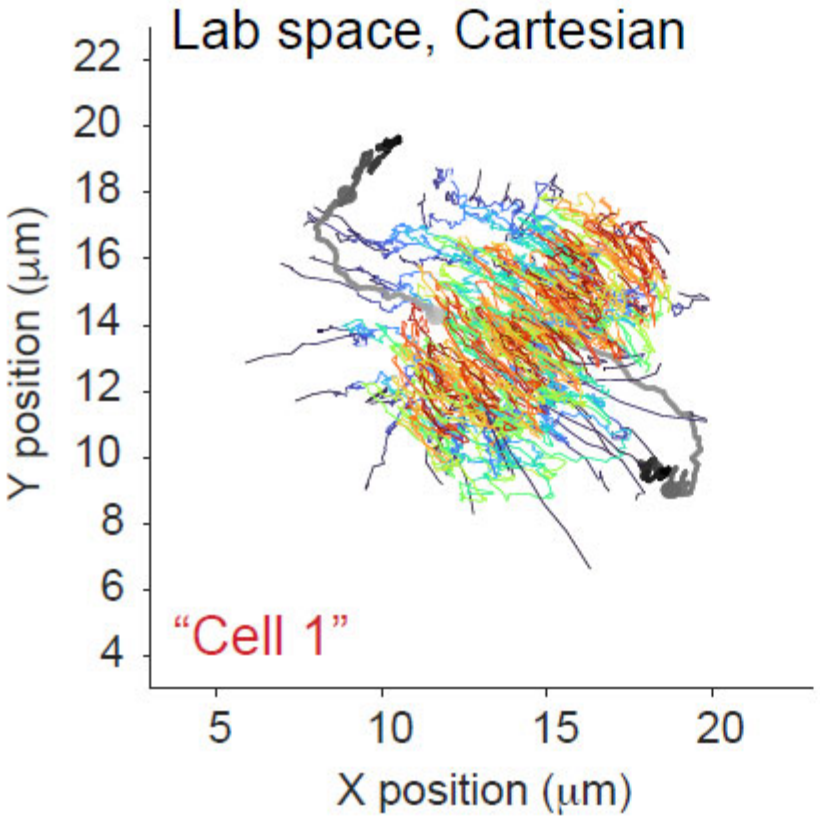
Definition of bi-orientation

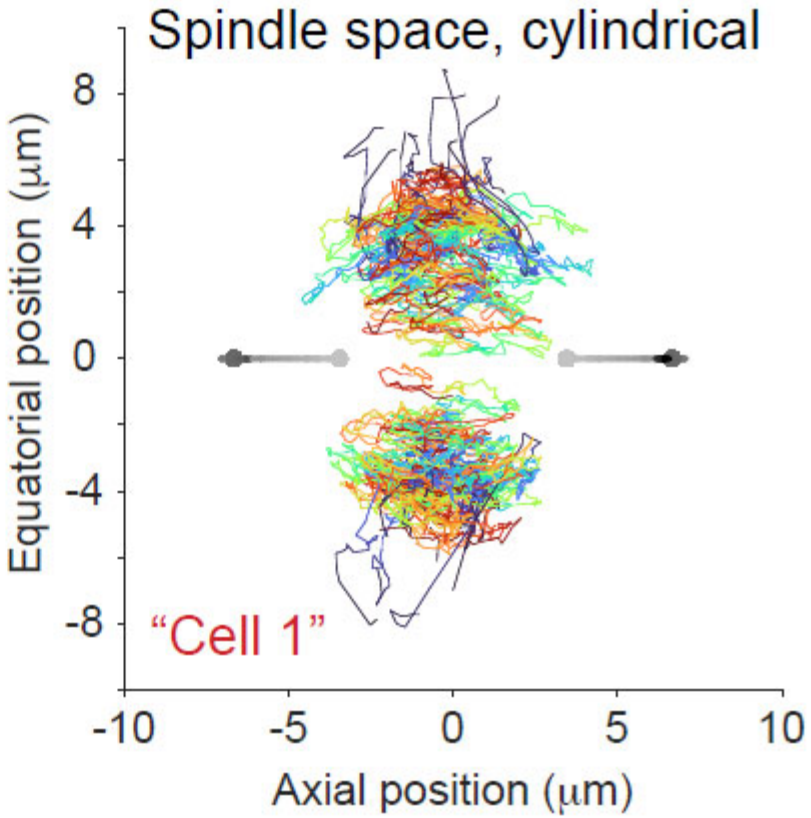
Bi-orientation takes place not very randomly, at certain stage of spindle elongation



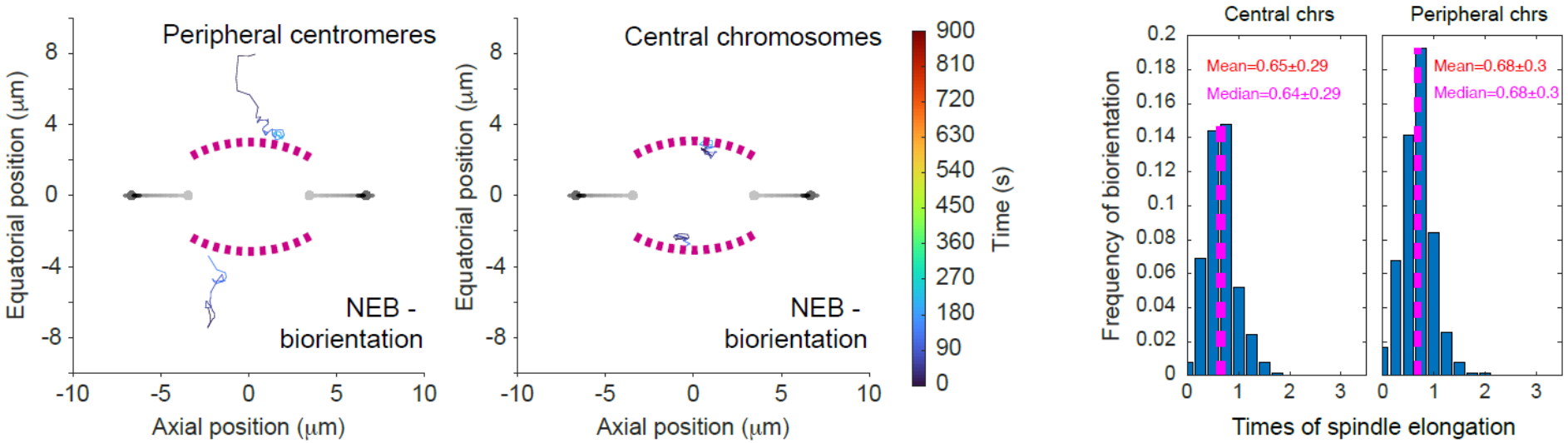


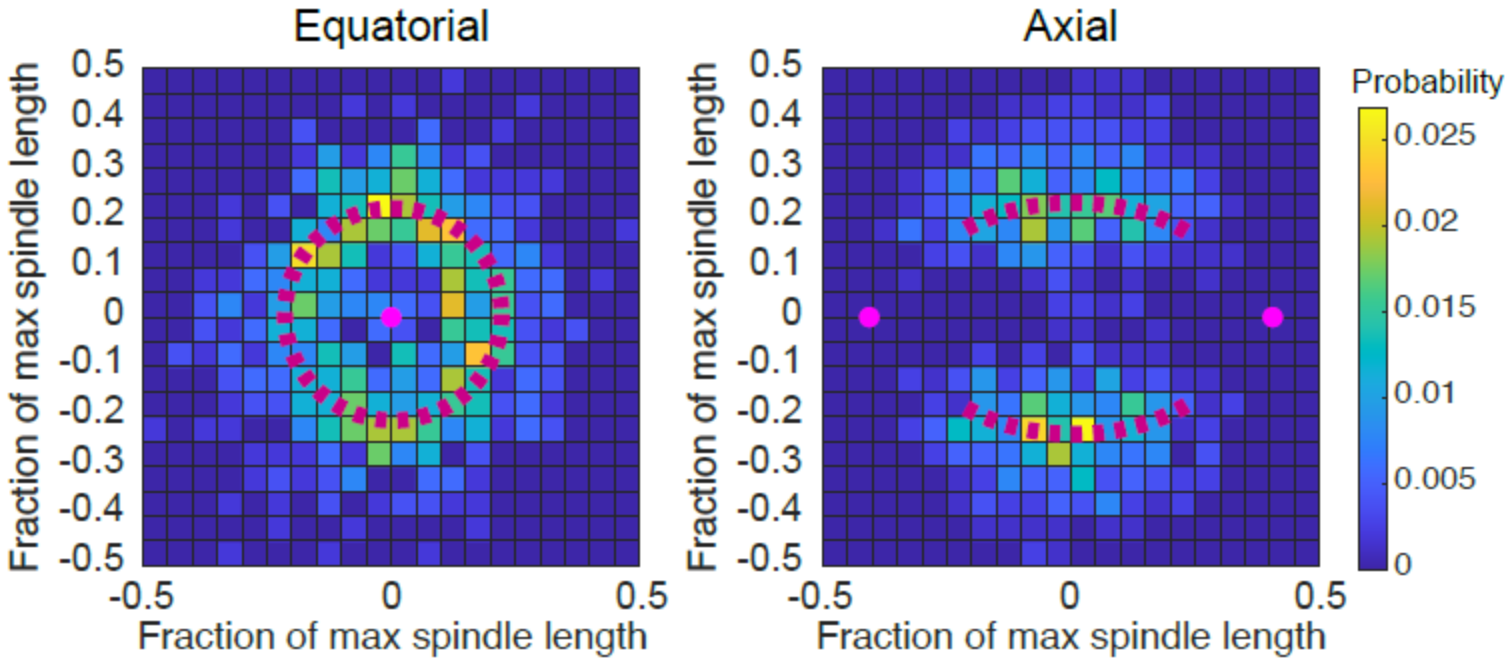
Chromosomes first move centripetally, and then along a barrel-like surface



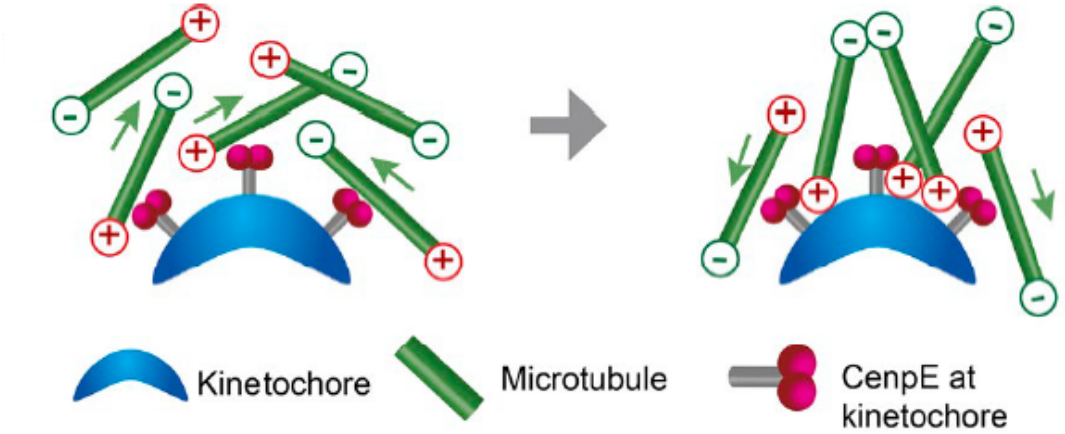


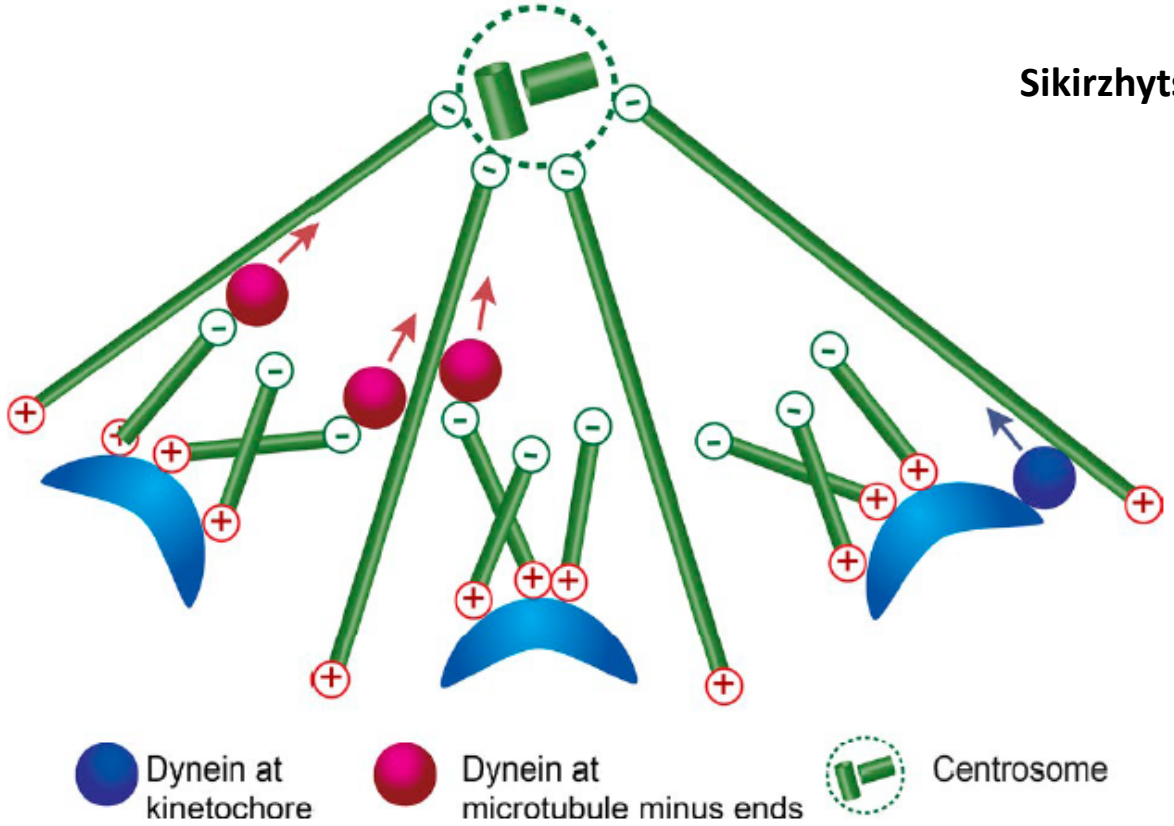
Chromosomes achieve biorientation on a barrel-like surface





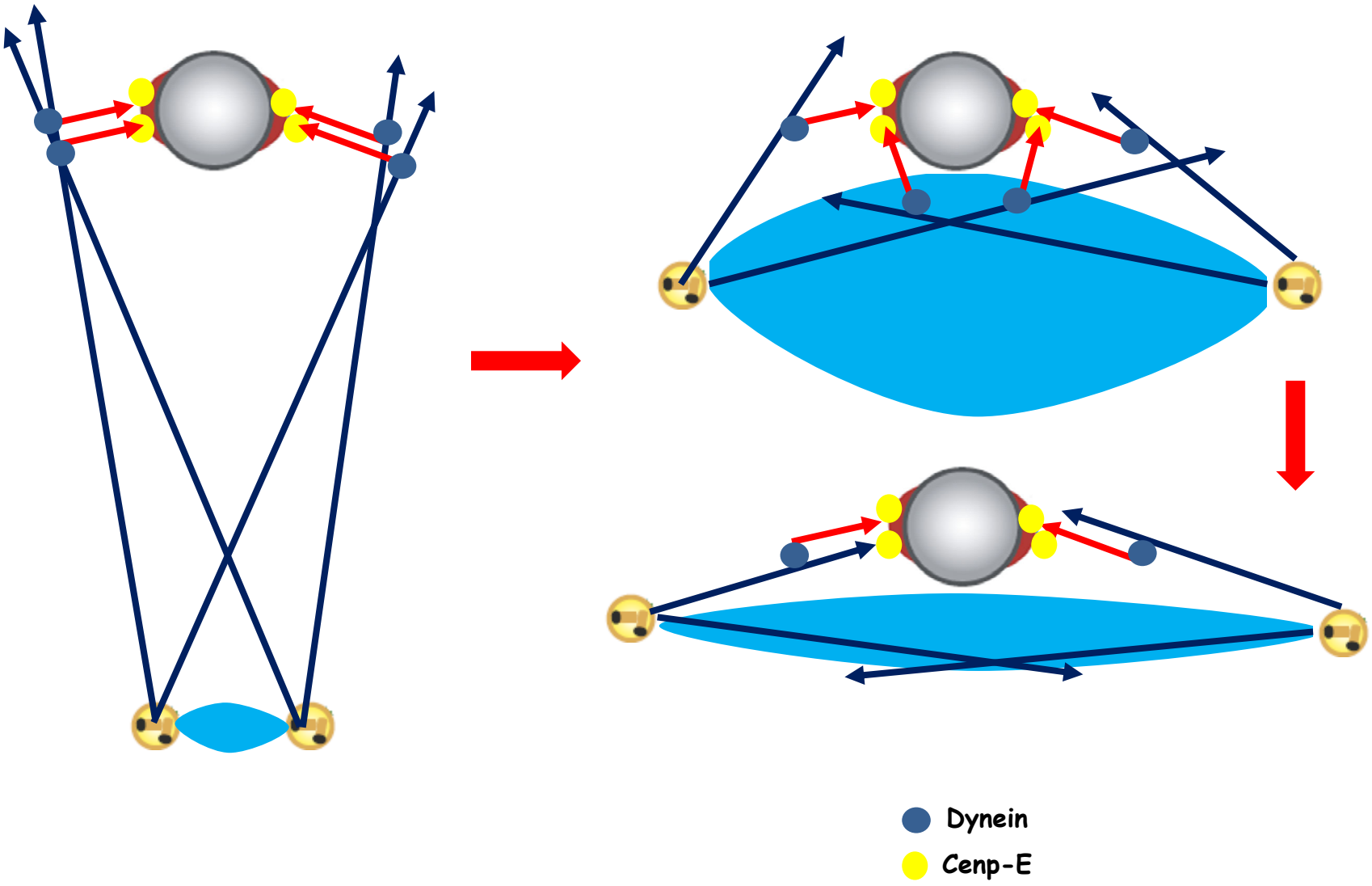
There are short MTs organized by two antagonistic motors on KTs



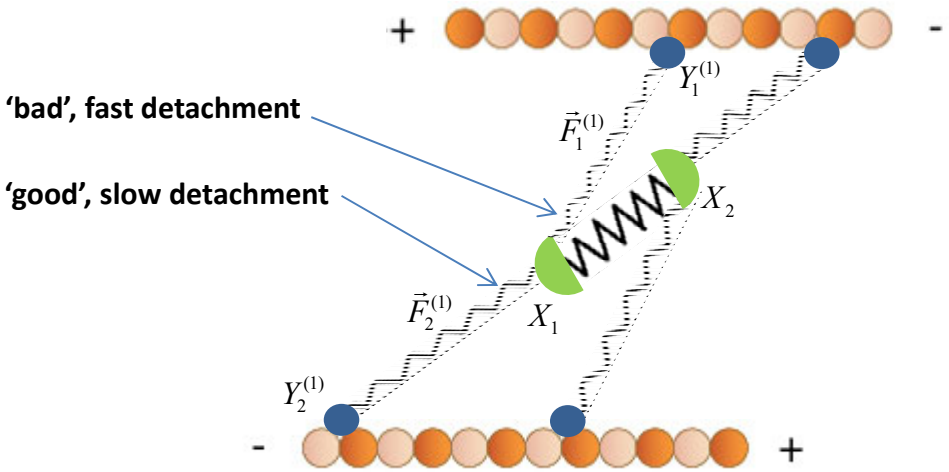


Sikirzhytski et al, 2018

Hypothetical model: geometry and force – dependent polarity sorting



'Tug-of-war' model explains KT-MT polarity sorting



Discrete stochastic model:
centromere is positioned and oriented according to balance of forces and torques from multiple dyneins. Effective detachment rate is higher if angle of pulling relative to KT-KT axis is greater

$$\gamma \frac{\mathrm{d}\boldsymbol{X}_i}{\mathrm{d}t} = \sum_{i \in M_i(t)} \boldsymbol{F}_i + \boldsymbol{F}_{\text{kt-kt}}$$

$$\boldsymbol{F}_{\text{kt-kt}} = \begin{cases} k_{\text{kt-kt}}(\|\boldsymbol{X}_2 - \boldsymbol{X}_1\| - L_{\text{kt-kt}}) \frac{\boldsymbol{X}_1 - \boldsymbol{X}_2}{\|\boldsymbol{X}_1 - \boldsymbol{X}_2\|} & \|\boldsymbol{X}_1 - \boldsymbol{X}_2\| > L_{\text{kt-kt}} \\ 0 & \text{otherwise.} \end{cases}$$

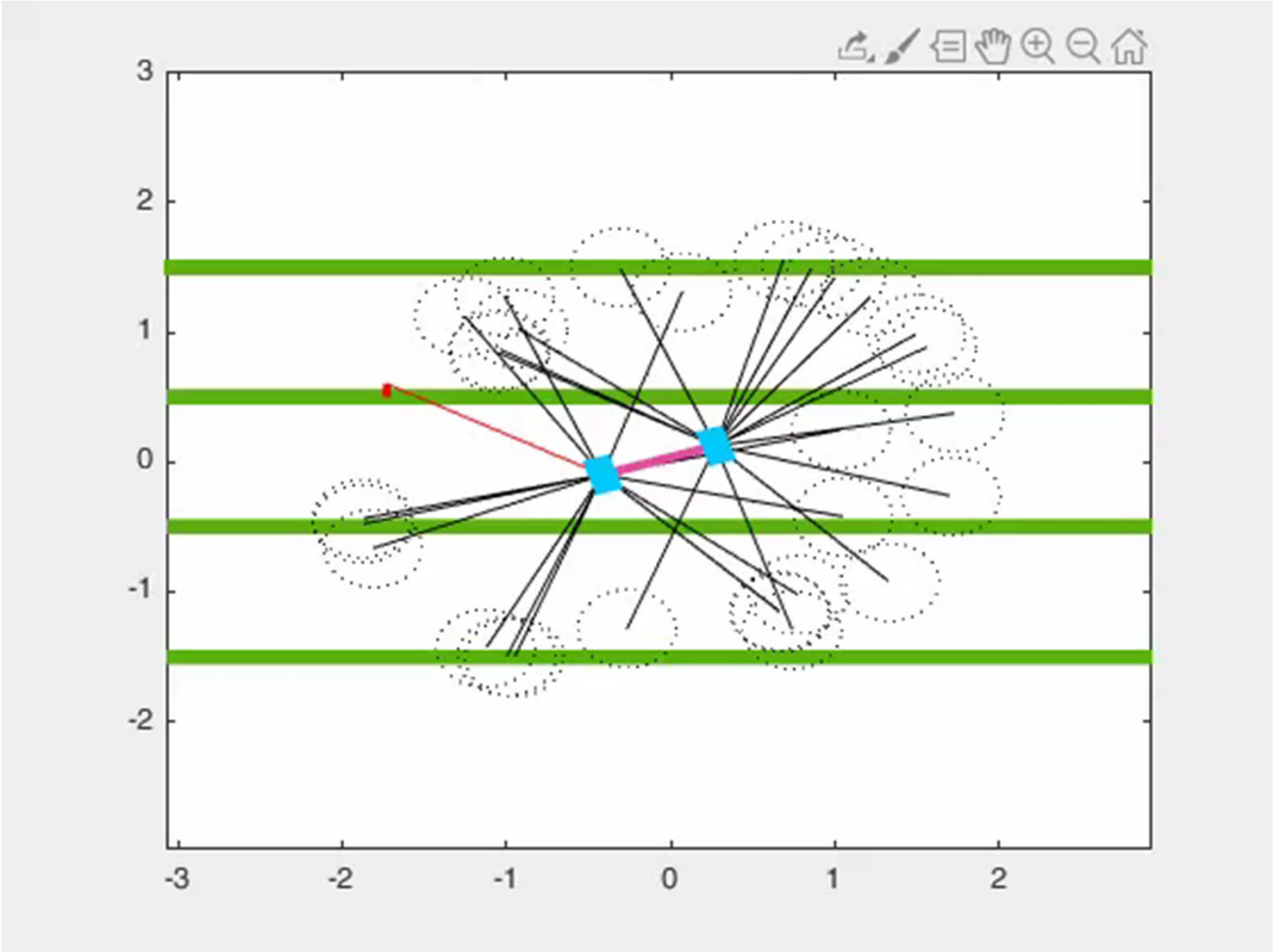
$$\boldsymbol{F}_{\text{i}} = \begin{cases} k_{\text{mot}}(\|\boldsymbol{Y}_i - \boldsymbol{X}_i\| - L_{\text{mot}}) \frac{\boldsymbol{Y}_i - \boldsymbol{X}_i}{\|\boldsymbol{Y}_i - \boldsymbol{X}_i\|} & \|\boldsymbol{Y}_i - \boldsymbol{X}_i\| > L_{\text{mot}} \\ 0 & \text{otherwise.} \end{cases}$$

$$\frac{\mathrm{d}\boldsymbol{Y}_i^j}{\mathrm{d}t} = v_j(\boldsymbol{F}_i) = \begin{cases} \boldsymbol{u}_j v_0 \left(1 - \frac{\|\boldsymbol{F}_i\|}{F_{\text{stall}}}\right) & \boldsymbol{F}_i \cdot \boldsymbol{u}_j \geq 0 \\ \boldsymbol{u}_j v_0 & \boldsymbol{F}_i \cdot \boldsymbol{u}_j < 0 \end{cases}$$

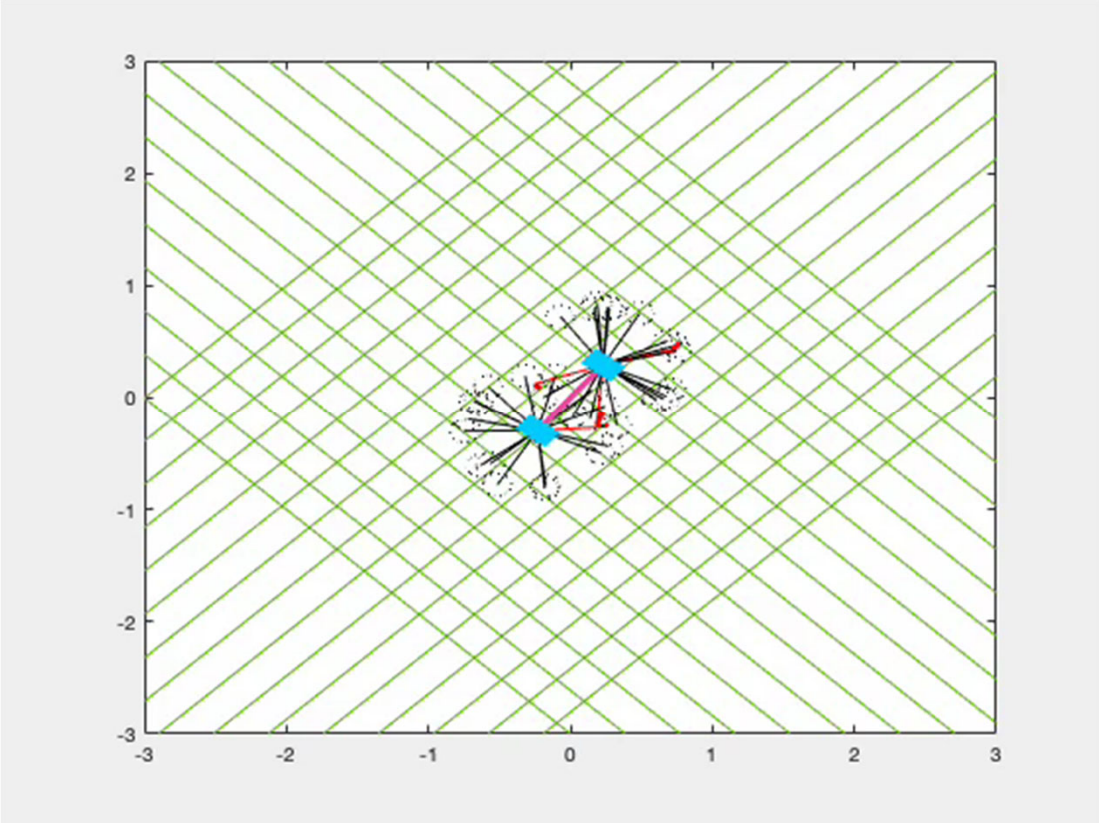
$$k_{\text{off}}(\boldsymbol{F}_i, \boldsymbol{F}_{\text{kt-kt}}) = k_0 \exp\{\eta_1 \|\boldsymbol{F}_i\| - \eta_2 \measuredangle(\boldsymbol{F}_i, \boldsymbol{F}_{\text{kt-kt}})\}$$

$$\measuredangle(\boldsymbol{a}, \boldsymbol{b}) = \frac{\boldsymbol{a} \cdot \boldsymbol{b}}{\|\boldsymbol{a}\| \|\boldsymbol{b}\|}$$

Bi-orientation on antiparallel MT bundles



Chromosomes must be on the spindle surface to bi-orient



Model predictions:

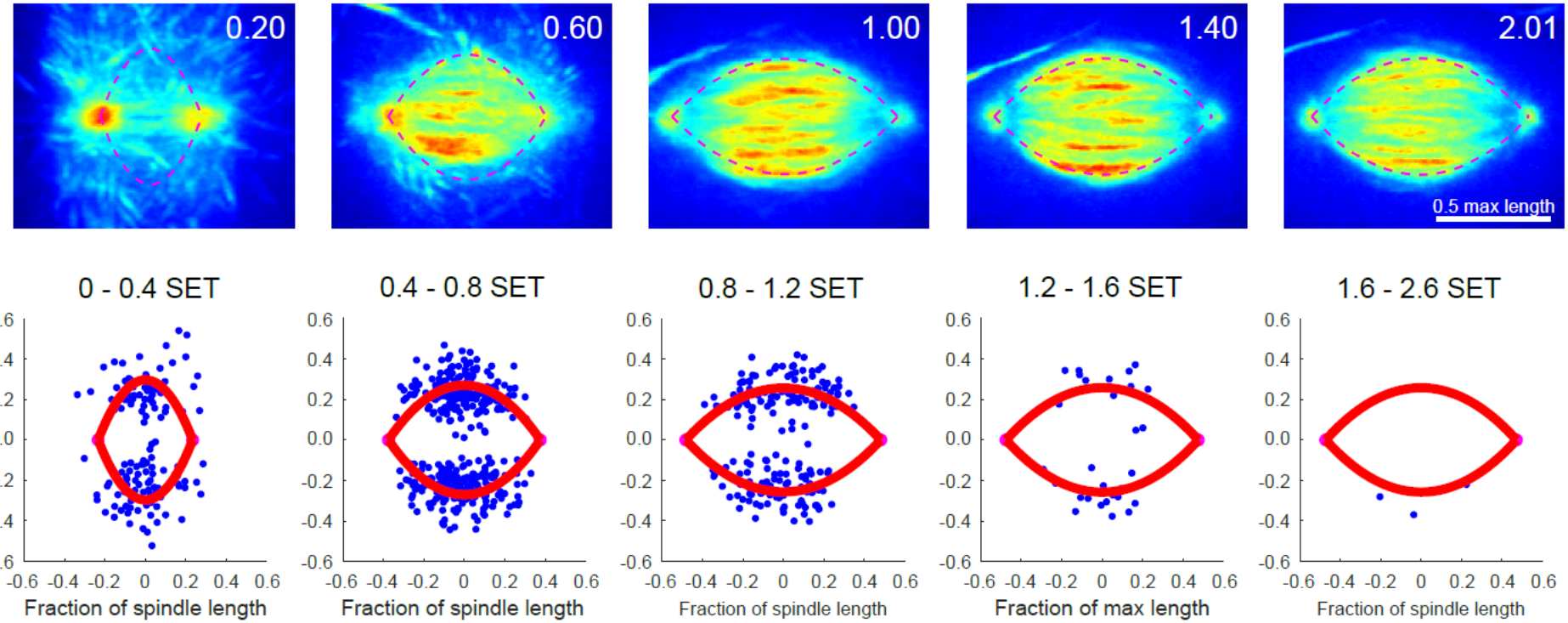
Chromosomes bi-orient upon hitting the spindle

After hitting the spindle, it takes tens of seconds to achieve bi-orientation

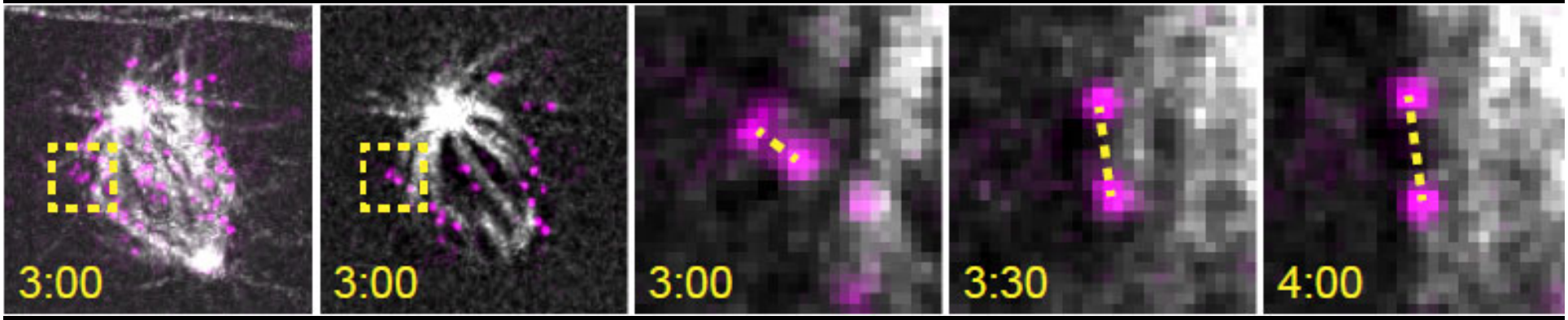
If dynein on KTs is inhibited, chromosomes get to the spindle slower but after hitting the spindle, bi-orient faster

If CENP-E on KTs is inhibited, chromosomes get to the spindle fast, but after hitting the spindle, bi-orient slower

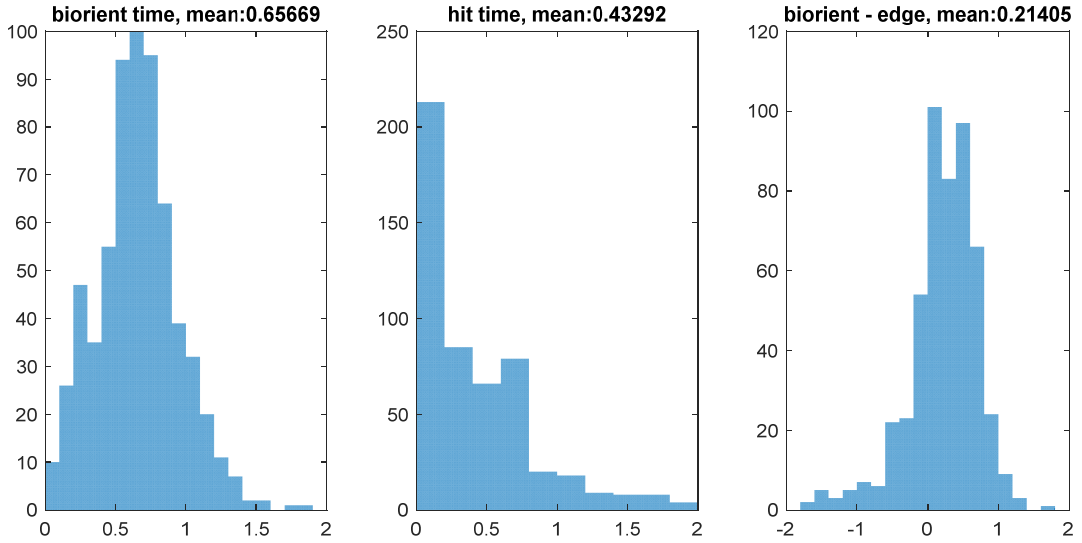
Chromosomes bi-orient upon hitting the spindle



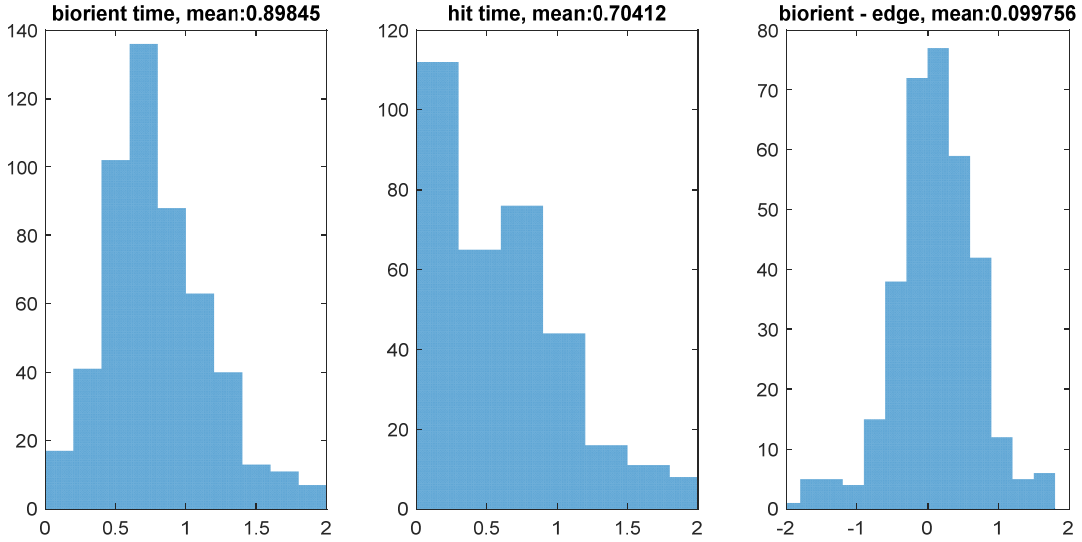
After hitting the spindle, it takes tens of seconds to achieve bi-orientation



If dynein on KTs is inhibited, chromosomes get to the spindle slower but after hitting the spindle, bi-orient faster



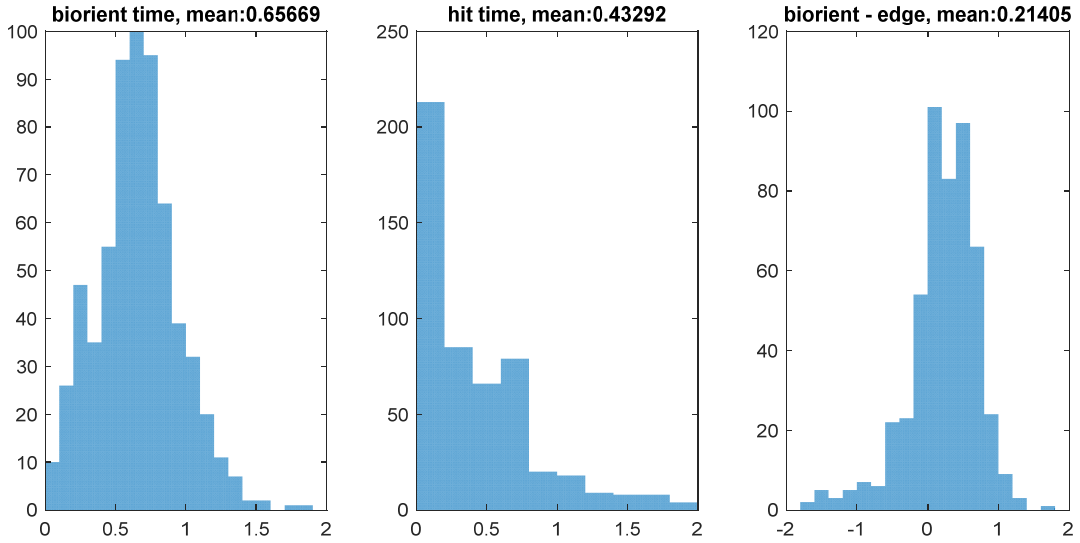
Control



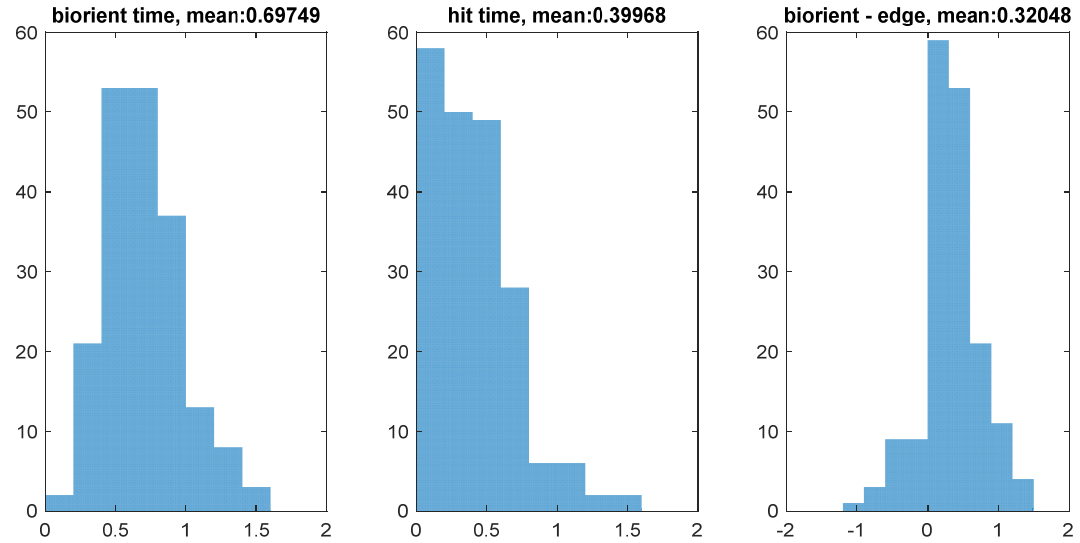
Dynein inhibited

Normalized time

If CENP-E on KTs is inhibited, chromosomes get to the spindle fast, but after hitting the spindle, bi-orient slower

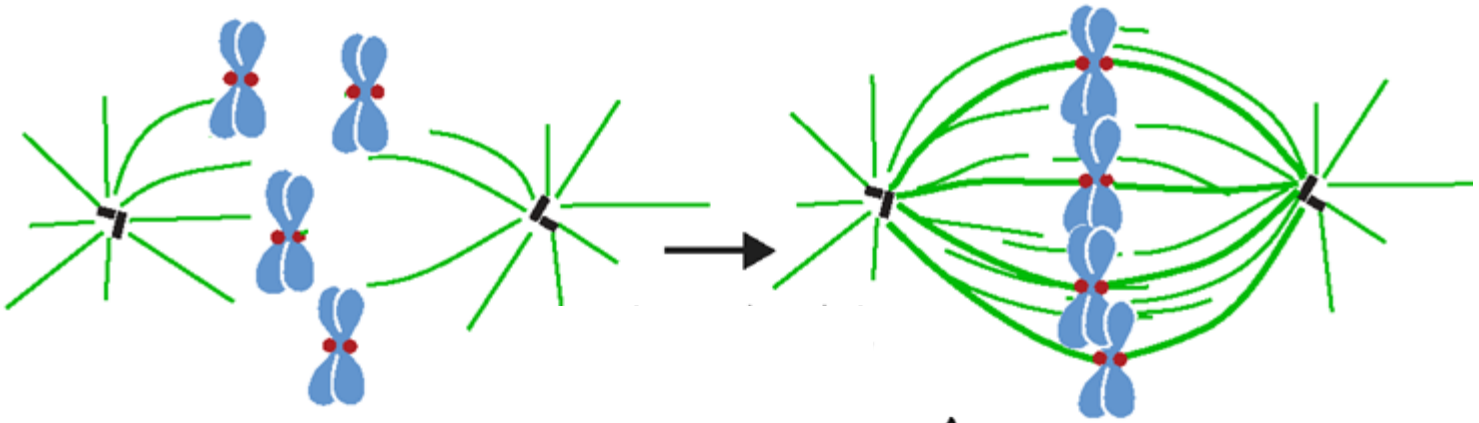


Control



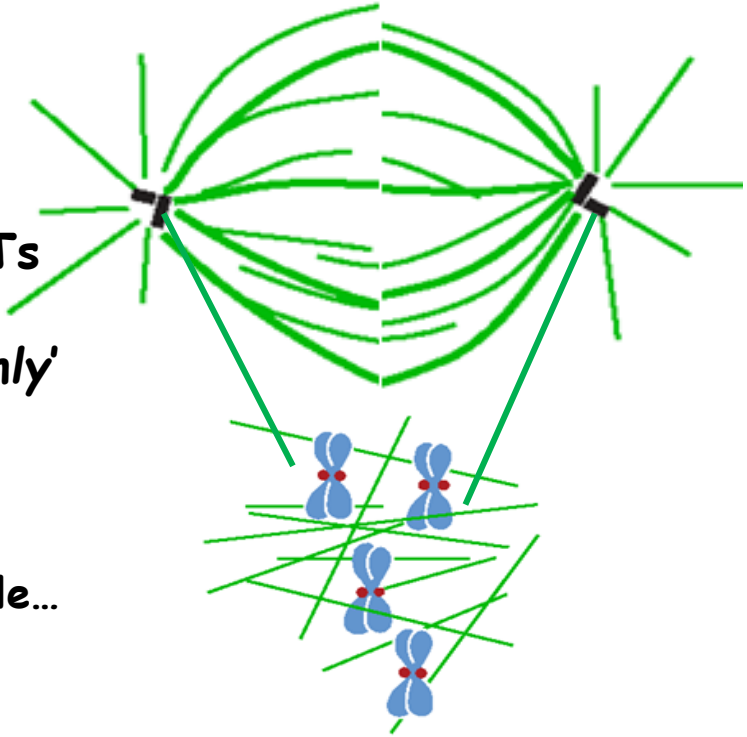
CENP-E inhibited

Normalized time



From: 'Spindle is made by *random* Search-and-Capture by two MT asters'

To:



'Nascent spindle, astral MTs, short MTs and chromosomes interact *randomly* to build the mature spindle *non-randomly*'

Many puzzles remain:

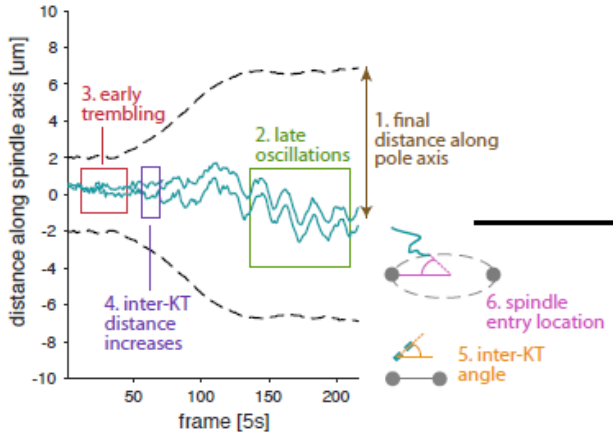
Rapid synchronous convergence to the spindle…

Proper end-on attachment…

Etc…

Quantifying and clustering chromosome/KT trajectories:

individual trajectories



compute barcode

oscillations? | inter-KT distance increases? | enters spindle near pole?

0 1 1 0 1 1

ended far from center? | early trembling? | inter-KT angle aligns with spindle?

1 = yes 0 = no

assemble data

data

trajectory 1
trajectory 2
⋮

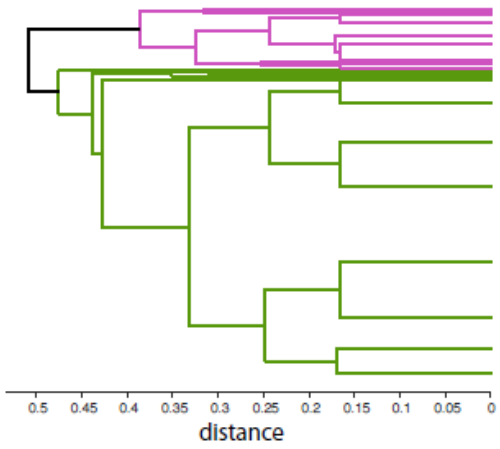
$$\begin{bmatrix} 0\ 1\ 1\ 0\ 0\ 1 \\ 1\ 0\ 1\ 0\ 0\ 1 \\ \vdots \end{bmatrix}$$

hierarchical clustering

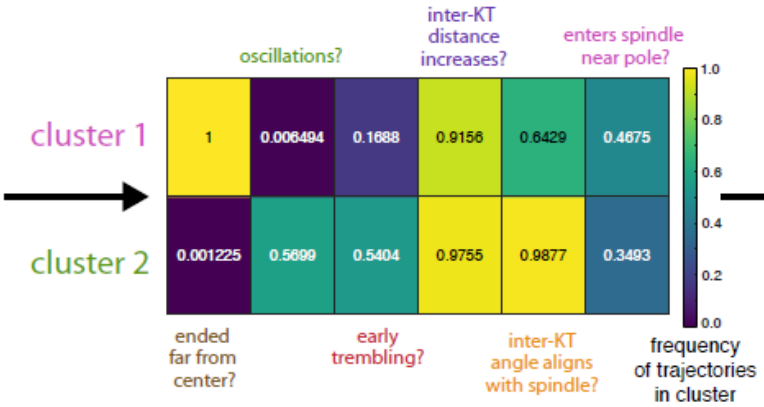
Hamming distance

$$\text{dist}\left(\begin{bmatrix}0\ 1\ 1\ 0\ 0\ 1\end{bmatrix}, \begin{bmatrix}1\ 0\ 1\ 0\ 0\ 1\end{bmatrix}\right) = 2/6$$

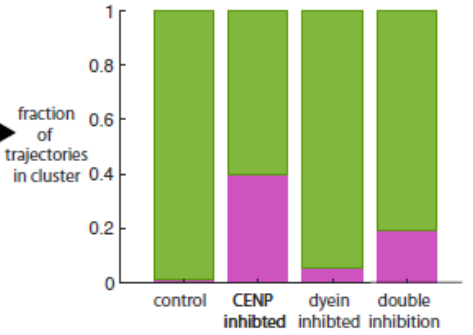
(% of differing components)



cluster composition



clusters per cell type



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Evgenii Kliuchnikov

+ Kenneth Marx

Supported by NSF, NIH