Bioimage Informatics

Lecture 18, Spring 2012

Bioimage Data Analysis (V)

Single Particle Tracking (part 3)

Outline

- Overview of single particle tracking techniques
- Review: linear assignment based single particle tracking
- Multiple hypothesis tracking
- Application I: fluorescence speckle microscopy

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Overview of Single Particle Tracking Techniques (I)

- Method I: simple nearest neighbor tracking
  - Each particle is assigned to its nearest neighbor
  - Often a search radius is adopted
  - <u>Rarely used</u>; <u>applicable only when particles are well separated</u>

- Method 2: global nearest neighbor tracking
  - Association cost between a pair of particles is their distance

$$\min \sum_{i \in G_k} \sum_{j \in G_{k+1}} a^k(i,j) w^k(i,j)$$

$$st. \quad \sum_i a(i,j) = 1 \quad \sum_j a(i,j) = 1 \quad a(i,j) \in \{0,1\}$$

$$\boxed{w^k(i,j) = \left\| x_j^{k+1} - x_i^k \right\|}$$

Overview of Single Particle Tracking Techniques (II)

- Method 3: global smooth motion tracking

  - Association cost between a pair of particles is their motion smoothness

$$min \sum_{i \in G_k} \sum_{j \in G_{k+1}} a^k(i,j) w^k(i,j)$$

$$st. \quad \sum_i a(i,j) = 1 \quad \sum_j a(i,j) = 1 \quad a(i,j) \in \{0,1\}$$

$$\boxed{c^k(i,j) = w_1 \left[1 - \frac{\left(x_i^k - x_l^{k-1}\right)\left(x_j^{k+1} - x_i^k\right)}{\left\|x_i^k - x_l^{k-1}\right\| \left\|x_j^{k+1} - x_i^k\right\|} \right] + w_2 \left[1 - 2\frac{\sqrt{\left\|x_i^k - x_l^{k-1}\right\| \left\|x_j^{k+1} - x_i^k\right\|}}{\left\|x_i^k - x_l^{k-1}\right\| + \left\|x_j^{k+1} - x_i^k\right\|} \right]}$$

Overview of Single Particle Tracking Techniques (III)

- There are other ways to define the association cost.
  - Example 1: to incorporate particle intensity
- There are a variety of other tracking techniques.
  - Example: joint-probablistic data-association filter (JPDAF)
  - Many of such techniques come from military applications
  - <u>Many of these techniques are based on assumptions that may not necessarily hold in biological applications</u>

Overview of Single Particle Tracking Techniques (IV)

- Distance $\rightarrow$ Global nearest neighbor

$$w^k(i,j) = \left\| x_j^{k+1} - x_i^k \right\|$$

- Smooth motion$\rightarrow$ Global smooth motion

$$w^k(i,j) = w_1\left[1 - \frac{\left(x_i^k - x_l^{k-1}\right)\left(x_j^{k+1} - x_i^k\right)}{\left\|x_i^k - x_l^{k-1}\right\|\left\|x_j^{k+1} - x_i^k\right\|}\right] + w_2\left[1 - 2\frac{\sqrt{\left\|x_i^k - x_l^{k-1}\right\|\left\|x_j^{k+1} - x_i^k\right\|}}{\left\|x_i^k - x_l^{k-1}\right\| + \left\|x_j^{k+1} - x_i^k\right\|}\right]$$

- Mahalanobis distance, where the prediction comes from typically a Kalman filter

$$w^k(i,j) = \left(x_i^k - \hat{x}_j^k\right)^T S\left(x_i^k\right)^{-1}\left(x_i^k - \hat{x}_j^k\right)$$

$$S\left(x_i^k\right) = cov\left(x_i^k - \hat{x}_j^k\right)$$

- Overview of single particle tracking techniques
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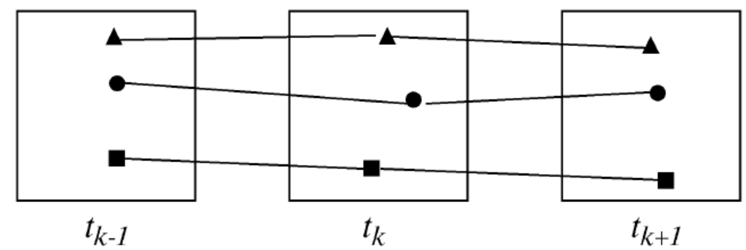
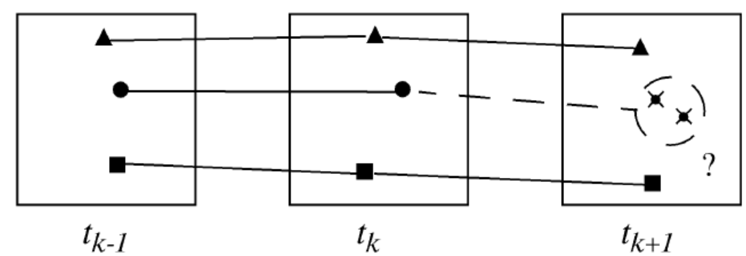
Definition of Particle Tracking (II)

- Different cases
  - - Constant number of features
  - - Feature appearance
  - - Feature disappearance

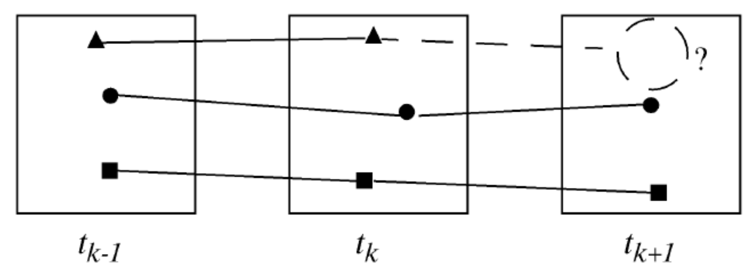


(a)

- Cases of feature appearance & disappearance
  - - Moving in or out of field of view
  - - Moving in or out of the focal plane
  - - Assembly/disassembly
  - - Feature merging/splitting

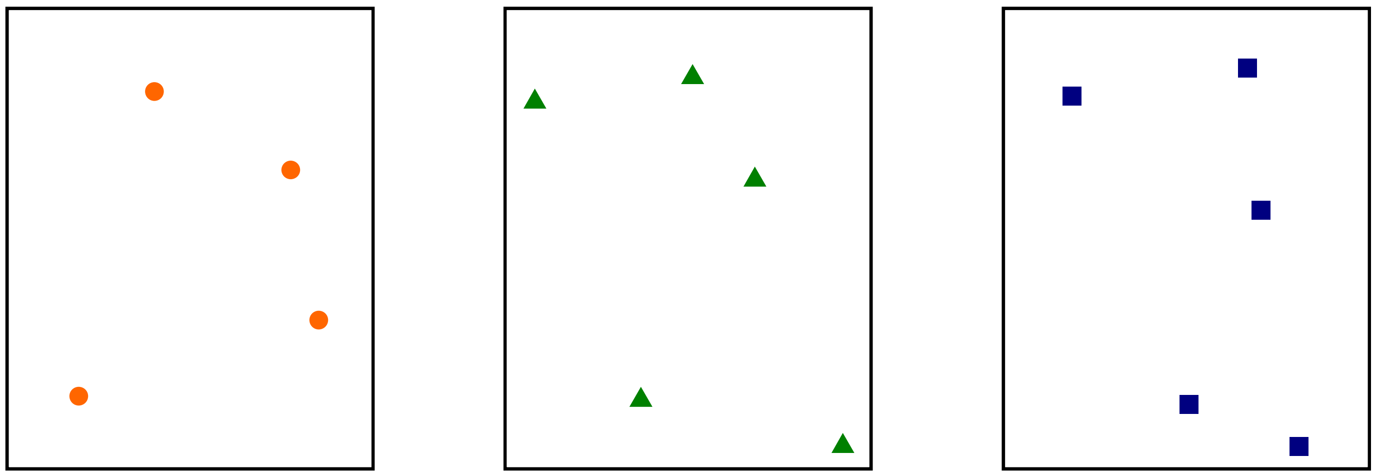


(b)



(c)

Example: Particle Tracking (I)



- ● Frame i-1
- ▲ Frame i
- ■ Frame $i+1$

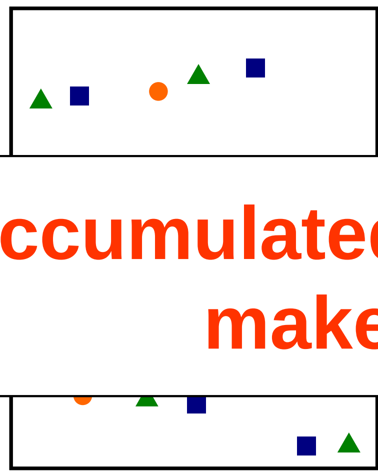
Example: Particle Tracking (II)

● Frame *i*-1

▲ Frame *i*

■ Frame *i*+1

Example of Particle Tracking (III)

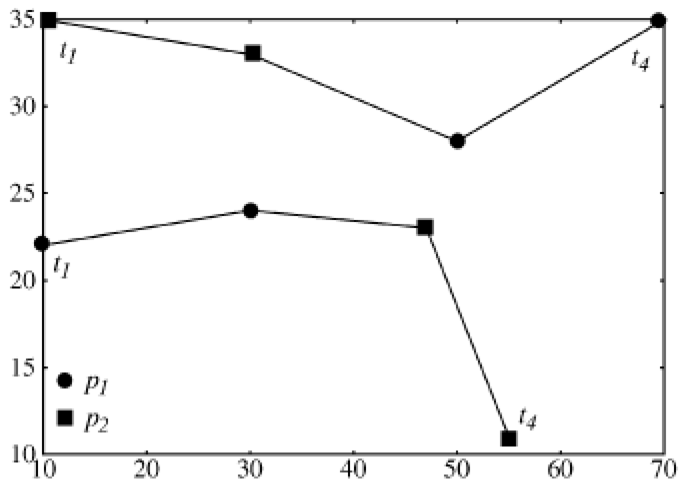


Accumulated evidence from multiple frames makes tracking more reliable.

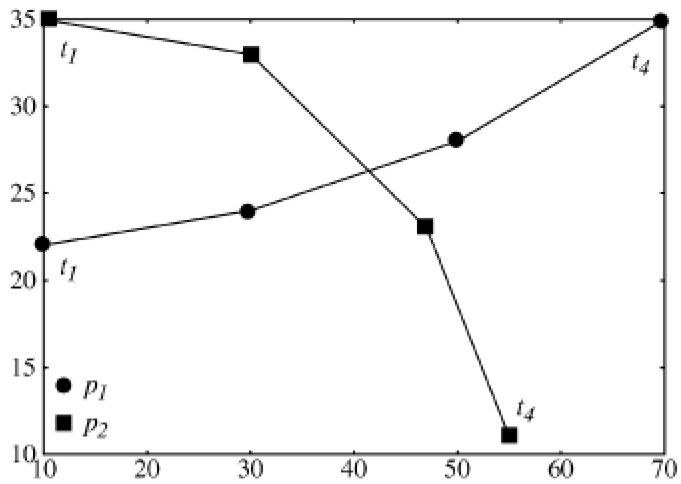
- Frame i-1
- Frame i
- Frame i+1

Particle Tracking Based on Global Linear Assignment (I)

- An optimization strategy is required to resolve conflicts between competing assignments.
- Selection of assignment weight will critically influence outcomes.



(a)



(b)

Veenman PAMI, 2001.

Particle Tracking Based on Global Linear Assignment (II)

- Formulation of the tracking problem as a bipartite graph assignment

$$\min \sum_{i \in G_k} \sum_{j \in G_{k+1}} a^k(i,j) w^k(i,j)$$

$$st. \quad \sum_i a(i,j) = 1 \quad \sum_j a(i,j) = 1 \qquad a(i,j) \in \{0,1\}$$

- There are efficient numerical algorithms to solve large scale assignment problems.

- Why not use a tripartite graph?
  - Optimal assignment of tripartite graph is NP-complete.
  - Difficult to resolve conflicts between two tripartite assignments.

Handling Particle Appearance & Disappearance

- Track appearance and disappearance are handled by introducing virtual points.

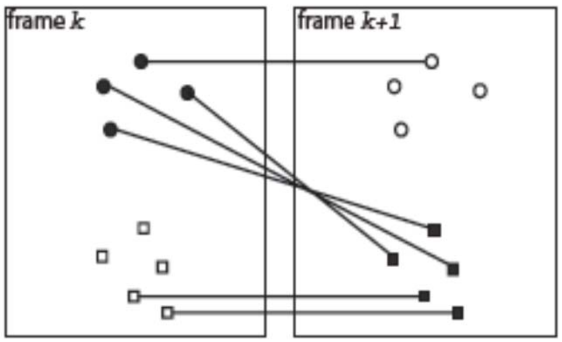


Figure 3. Handling particle appearance and disappearance

G. Yang, A. Matov, G. Danuser, Reliable tracking of large scale dense antiparallel particle motion for fluorescence live cell imaging, *IEEE CVPR, 2005*

References on Linear Assignment

- Burkard R., Cela E., Linear assignment problems and extensions, pp.75-149, in *Handbook of Combinatorial Optimization*, D.-Z. Du & P. M. Pardalos (Eds.), Kluwer Academic Publishers, 1999.

  (Downloadable from class web page)

Assessment of Linear Assignment Based Particle Tracking

- Disadvantage: The algorithm fundamentally only looks for the best solution over two consecutive frames (greedy).
- Disadvantage: It can not handle feature merging and splitting.
- Advantage: It has low computational complexity and can be used to tracking very large number of particles.

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MHT-Based Particle Tracking (I)

- "MHT is a deferred decision logic in which alternative data association hypotheses are formed whenever observation-to track conflict situations occur."

- Multiple competing hypotheses are represented in a tree structure.

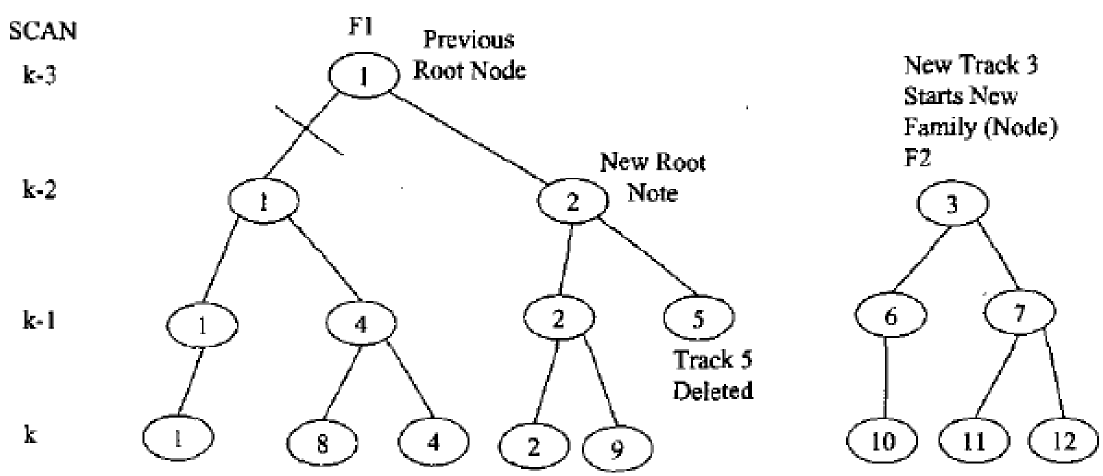


Fig. 4. Family (node) structure with N-scan pruning.

MHT-Based Particle Tracking (II)

- The tree structure provides a flexible way to handle feature appearance/disappearance and merging/splitting.

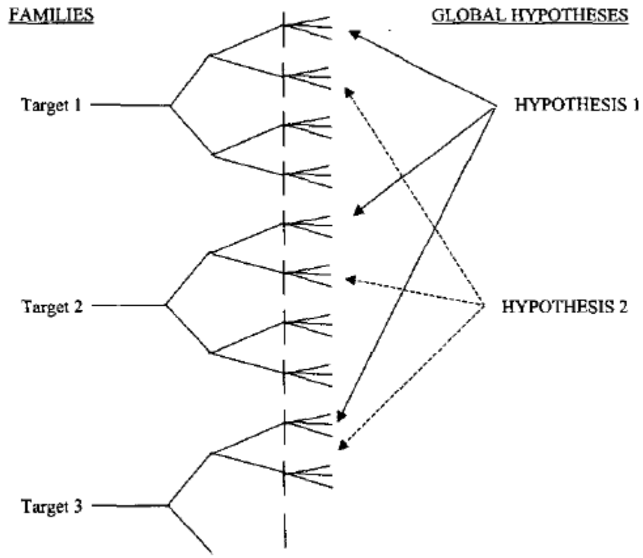


Fig. 5. Formation of hypotheses from tracks in families.

MHT-Based Particle Tracking (III)

- Advantages
    - An effective framework to incorporate multi-frame information.
    - A natural way to handle feature appearance and disappearance.
    - A natural way to handle feature merging/splitting.

- Disadvantages
    - Combinatorial explosion if the tree is not pruned.
    - Many variations in implementation.
    - High computation and memory cost.

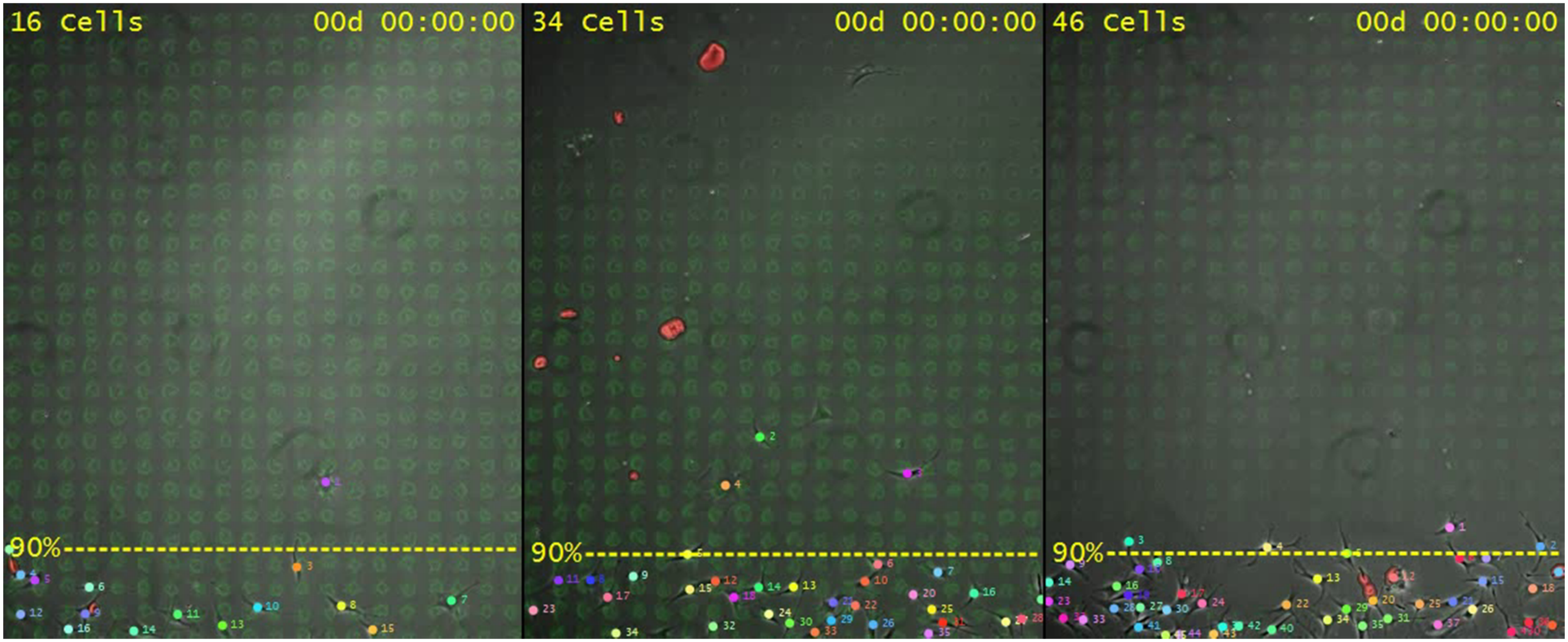
- Overall a very important approach, especially when the number of features to be tracked is small.

Methods to Handling Merging & Splitting

- Tracklet-based approaches
  - Kanade T., Yin Z., Bise R., Huh S., Eom S., "Cell Image Analysis: Algorithms, System and Applications," IEEE Workshop on Applications of Computer Vision (WACV) 2011.
  - http://celltracking.intel-research.net/

- Graph-based approaches
  - Padfield, D, Rittscher, J., Roysam B., "Coupled Minimum-Cost Flow Cell Tracking for High-Throughput Quantitative Analysis," Medical Image Analysis Journal, 2010.
  - http://www.farsight-toolkit.org/wiki/Main_Page

Tracking Migrating Cells



Courtesy of Lee Weiss & Takeo Kanade

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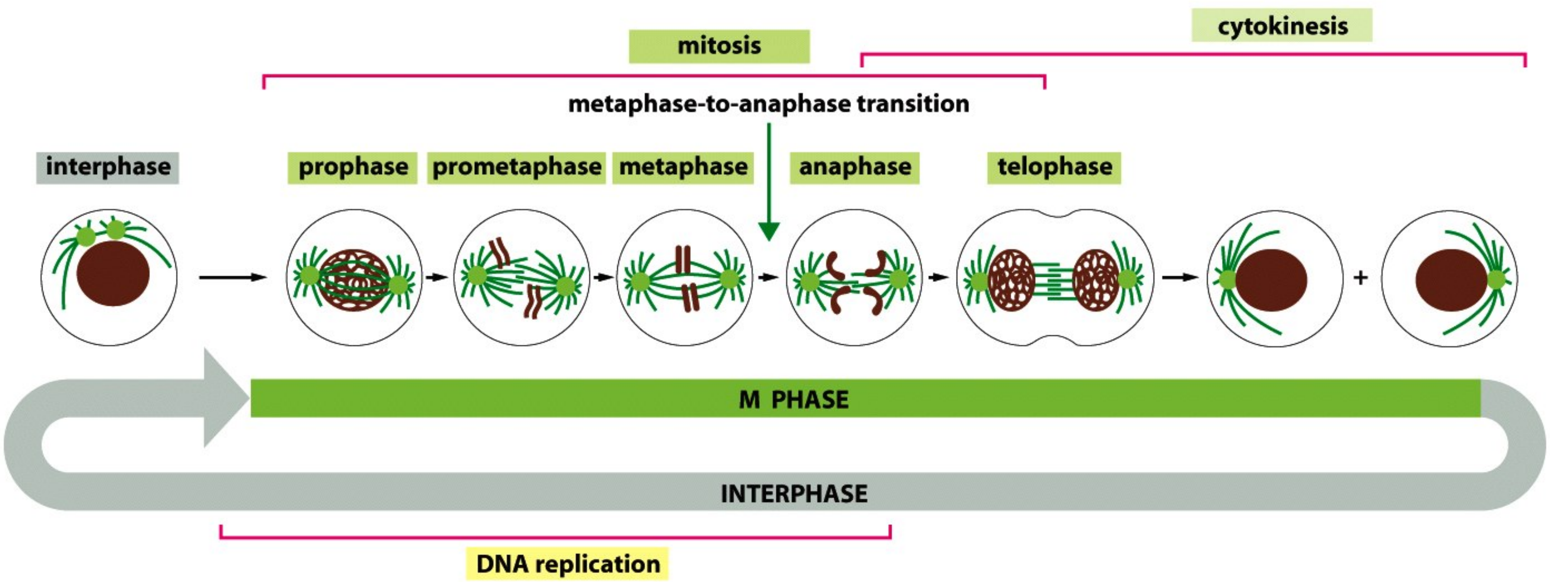
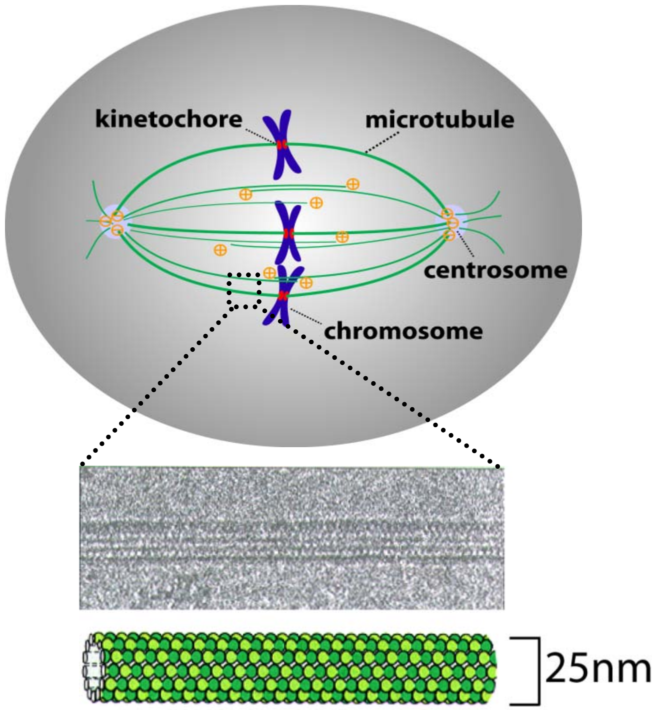
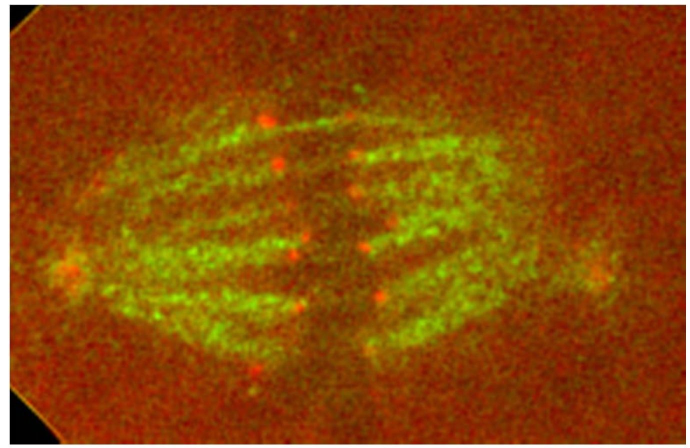
Overview of Cell Cycle



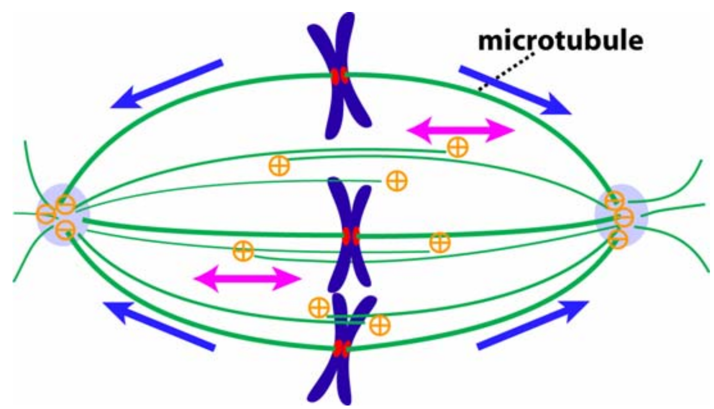
Dynamic Microtubules in the Mitotic Spindle



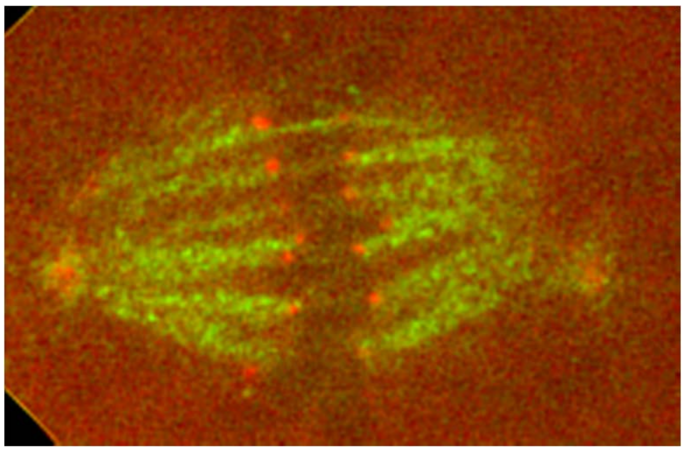
Alberts et al., MBoC5



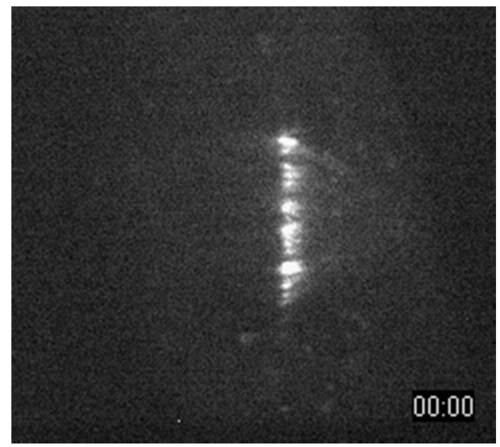
Green: microtubule
Red: kinetochore
5 μm



Confirmation of Poleward Flow of Spindle Microtubules

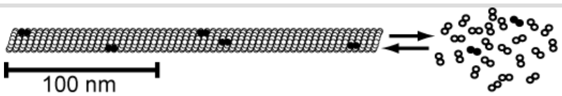


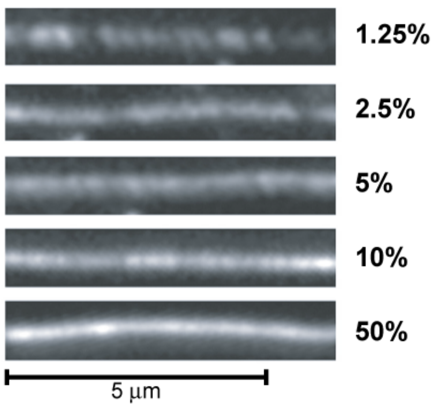
5 μm

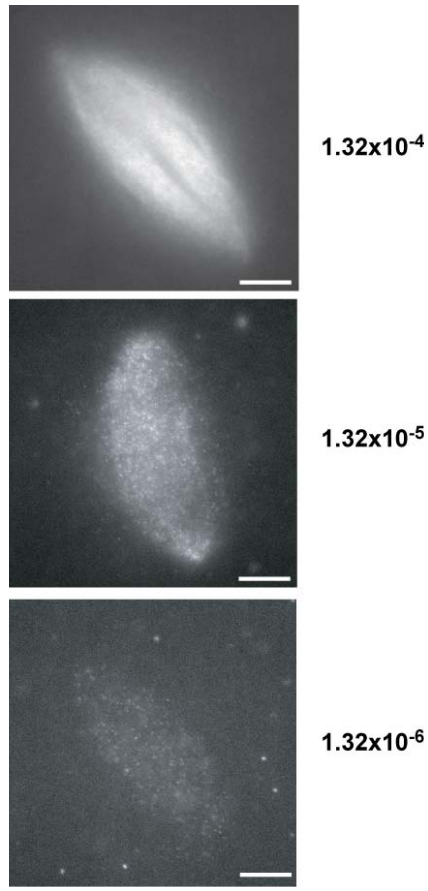


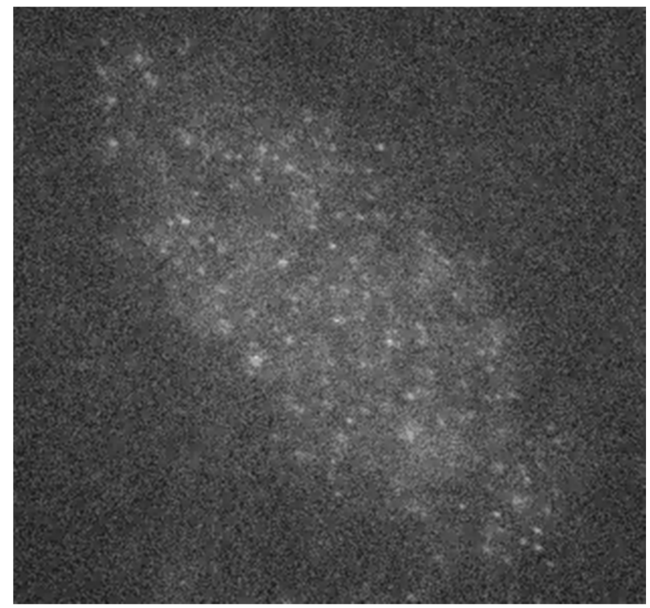
Cameron et al, *JCB*, 173:173-179,2006

Fluorescent Speckle Microscopy (FSM)

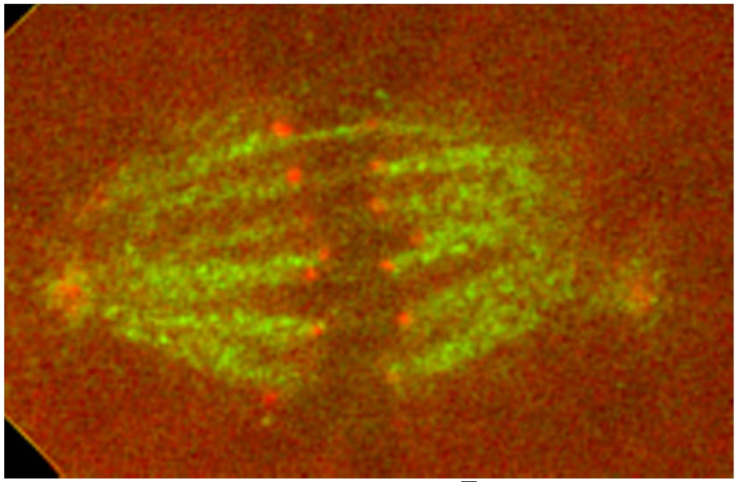






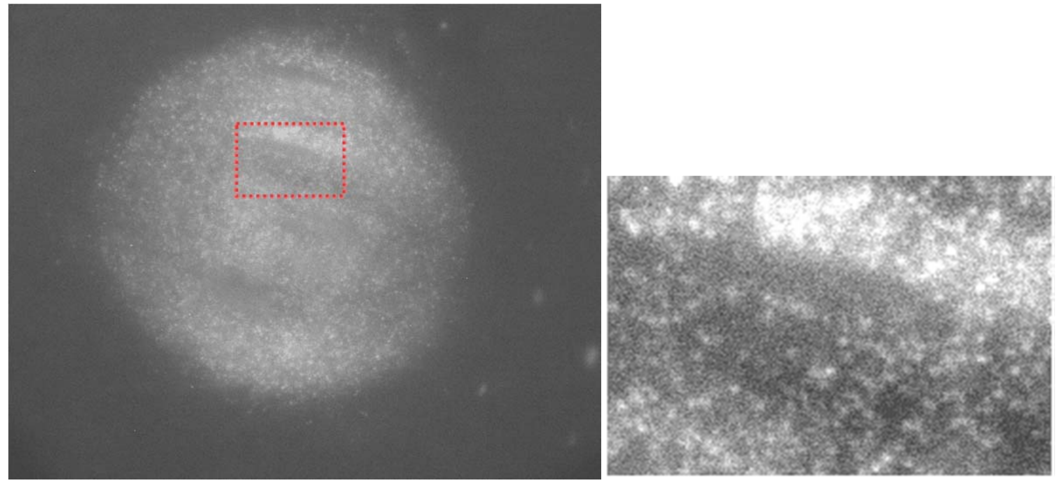


FSM of Dynamic Spindle Architecture



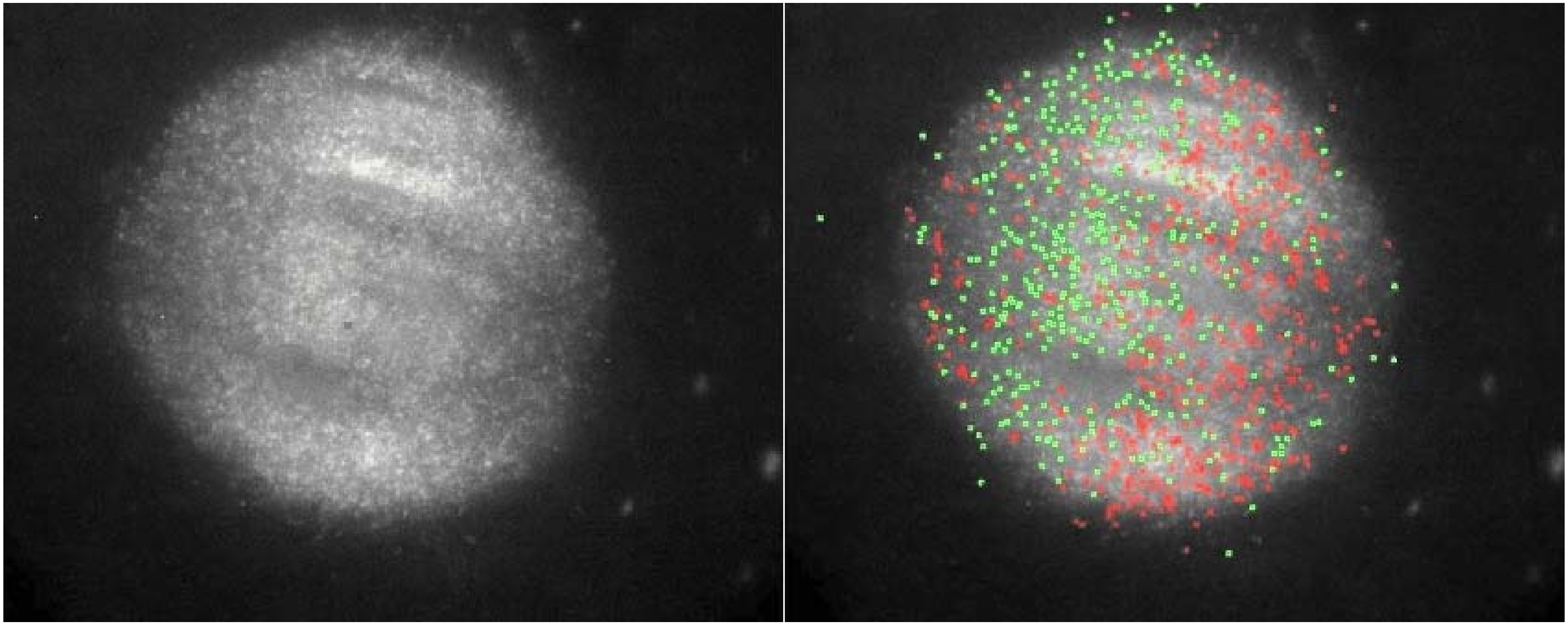
5 µm

Fluorescent speckle microscopy



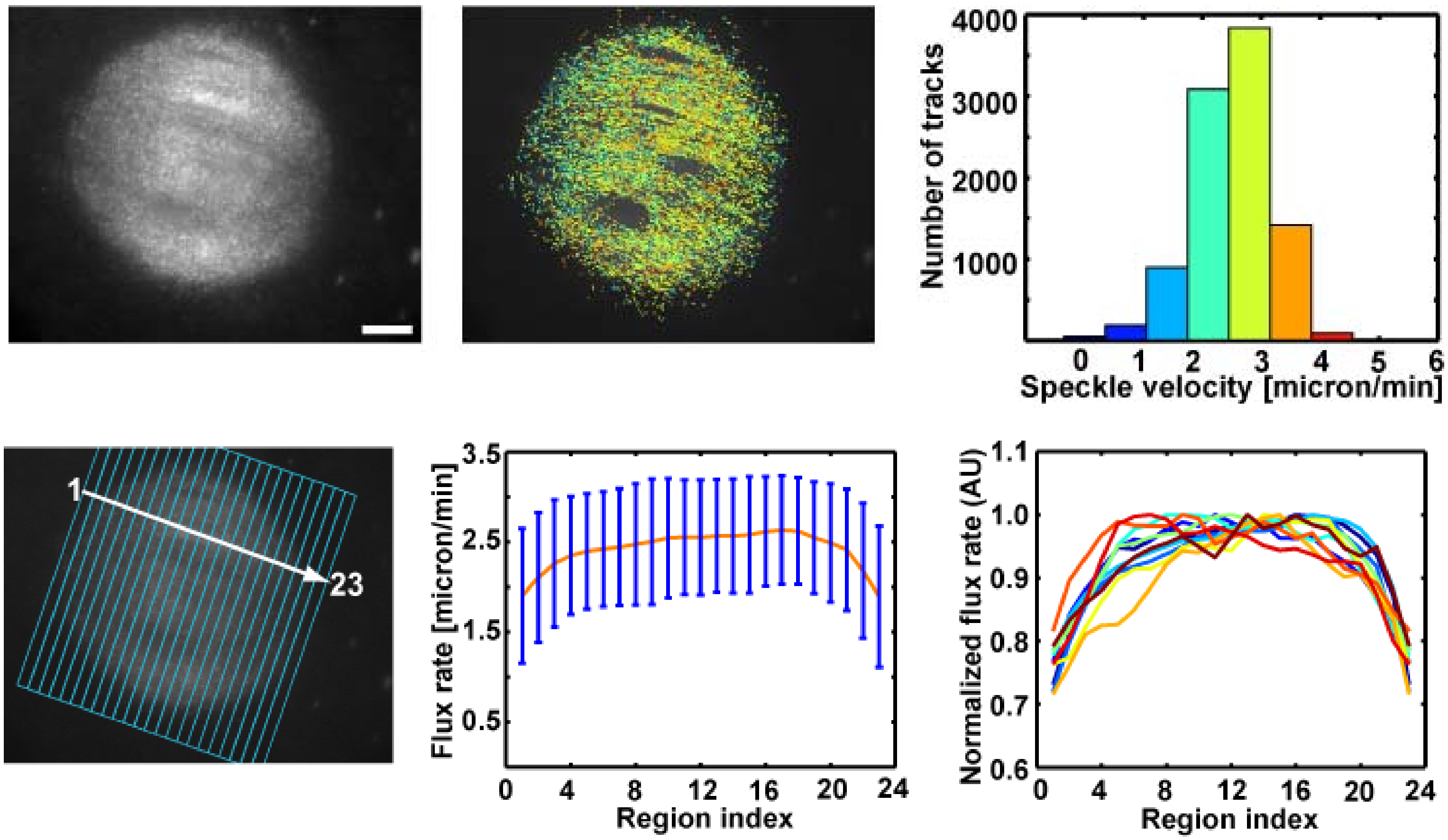
10 µm

Quantitative Mapping of Spatial-Temporal Spindle Dynamics



Yang et al., *J. Cell Biol.,* 182:631-639, 2008

Regional Variations of Microtubule Flux



Yang et al., *J. Cell Biol.,* 182:631-639, 2008

Questions?

Movement of a Free Molecule (I)

- The average kinetic energy of a particle of mass *m* and velocity v_x is

Boltzmann constant=1.381×10^{-23} J/K

1 Joule = 1 N·m

$t_K = t_C + 273.15$

$$\left\langle \frac{1}{2} m v_x^2 \right\rangle = \frac{kT}{2}$$

where k is Boltzmann's constant and T is absolute temperature (Einstein 1905).

- Principle of equipartition of energy

$$\left\langle \frac{1}{2} m v^2 \right\rangle = \frac{3 \cdot kT}{2}$$

Howard Berg, Random walks in biology, Princeton University Press, 1993

Movement of a Free Molecule (II)

- Molecular mass of GFP is 27 kDa. One atomic mass unit (Da) is 1.6606×10^{-24}g. So the mass of one GFP molecule is 4.4836×10^{-20}g.

  At 27 degree C, kT is $4.1451\times10^{-14}\text{g}\cdot\text{cm}^2/\text{sec}^2$.

$$\sqrt{\langle v_x^2 \rangle} = \sqrt{\frac{kT}{m}} = 961.51\ \text{cm/sec}$$

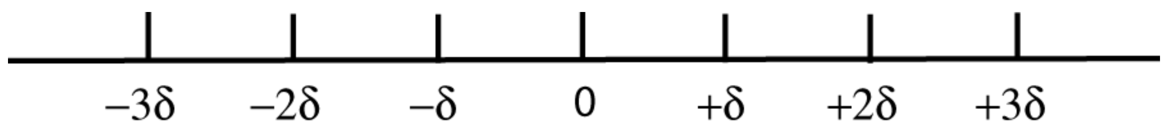
Howard Berg, Random walks in biology, Princeton University Press, 1993

1D Random Walk in Solution (I)

- Assumptions: *consider an ensemble of N particles*,

(1) A particle *i* has equal probabilities to walk to the left and to the right.
(2) Particle movement at consecutive time points are independent.
(3) Movement of different particles are independent.
(4) Each particle moves at a average step size of $\delta = v_x \cdot \tau$

$$x_i(n) = x_i(n-1) \pm \delta$$



$$\langle x(n) \rangle = \frac{1}{N}\sum_{i=1}^{N} x_i(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i(n-1) \pm \delta\right]$$

$$= \frac{1}{N}\sum_{i=1}^{N} x_i(n-1) = \langle x(n-1) \rangle$$

- Property 1: The mean position of an ensemble of particles undergoing random walk remains unchanged.

Howard Berg, Random walks in biology, Princeton University Press, 1993

1D Random Walk in Solution (II)

- Property 2: The mean square displacement of a particle undergoing random walk increases linearly w.r.t. time.

$$\left\langle x^2(n)\right\rangle = \frac{1}{N}\sum_{i=1}^{N} x_i^2(n) = \frac{1}{N}\sum_{i=1}^{N}\left[x_i^2(n-1) \pm 2\delta x_i(n-1) + \delta^2\right]$$
$$= \left\langle x^2(n-1)\right\rangle + \delta^2$$

$$\left\langle x^2(n)\right\rangle = n\delta^2 = \frac{t}{\tau}\delta^2 = 2Dt \qquad \left\langle r^2(n)\right\rangle = \left\langle x^2(n) + y^2(n)\right\rangle = 4Dt$$

$$\left\langle r^2(n)\right\rangle = \left\langle x^2(n) + y^2(n) + z^2(n)\right\rangle = 6Dt$$

Howard Berg, *Random walks in biology*,
Princeton University Press, 1993

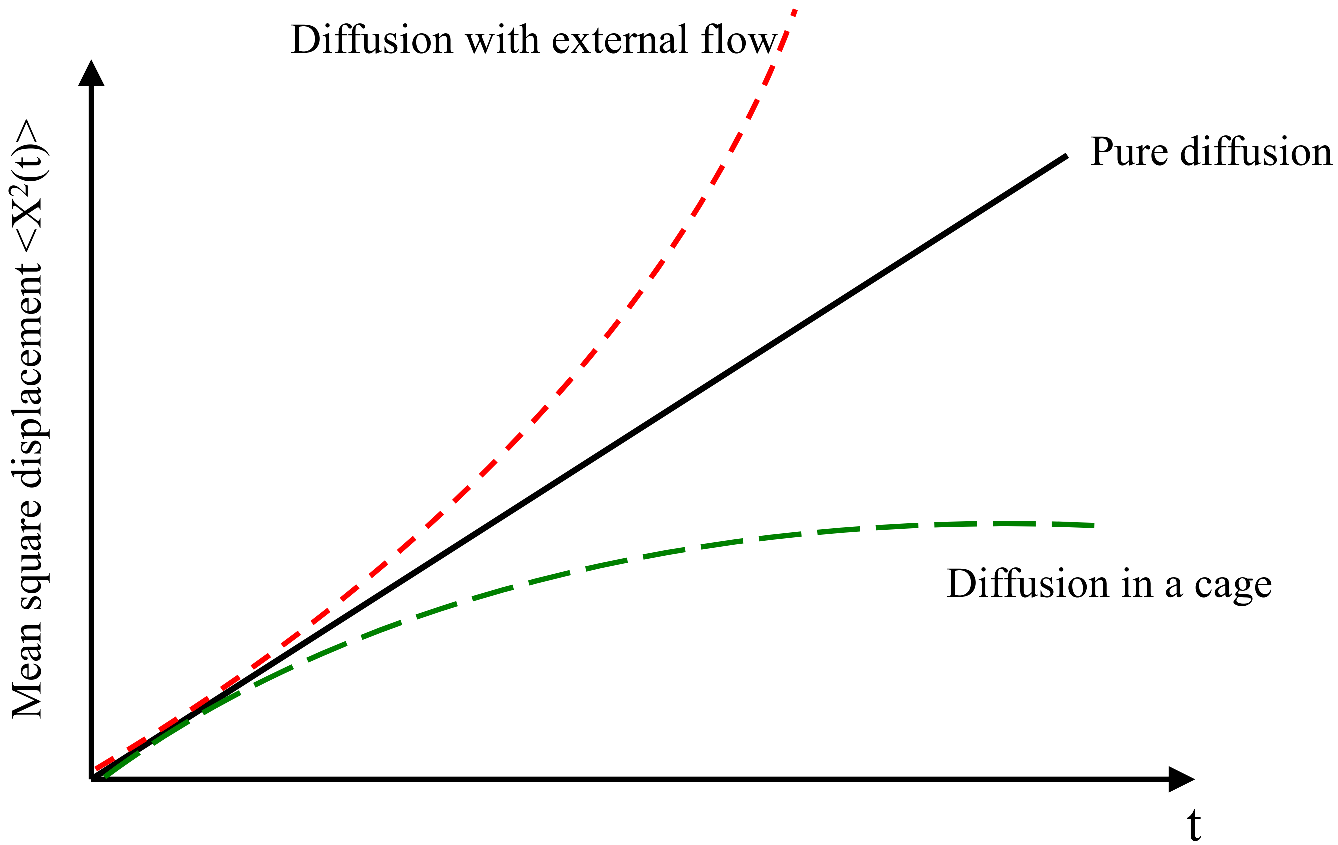
Application of the Microscopic Theory (I)

Object	Distance diffused			
	1 μm	**100 μm**	**1 cm**	**1 m**
K^+	0.25ms	2.5s	2.5×10^4s (7 hrs)	2.5×10^8s (8 yrs)
Protein	5ms	50s	5.0×10^5s (6 days)	5.0×10^9s (150 yrs)
Organelle	1s	10^4s (3 hrs)	10^8s (3 yrs)	10^{12}s (31710 yers)

K+: Radius = 0.1nm, viscosity = $1\text{mPa}\cdot\text{s}^{-1}$; T = 25°C; D=2000 μm^2/sec
Protein: Radius = 3nm, viscosity = $0.6915\text{mPa}\cdot\text{s}^{-1}$; T = 37; D = 100 μm^2/sec
Organelle: Radis = 500nm, viscosity = $0.8904\text{mPa}\cdot\text{s}^{-1}$; T = 25°C; D = 0.5 μm^2/sec

Jonathon Howard, *Mechanics of motor proteins and the cytoskeleton*, Sinauer, 2001

Application of the Microscopic Theory (II)



H. Qian, M. P. Sheetz, E. L. Elson, *Single particle tracking: analysis of diffusion and flow in two-dimensional systems*, Biophysical Journal, 60(4):910-921, 1991.

Application of the Microscopic Theory (III)

- Calculation of diffusion coefficient (Einstein-Stokes equation)

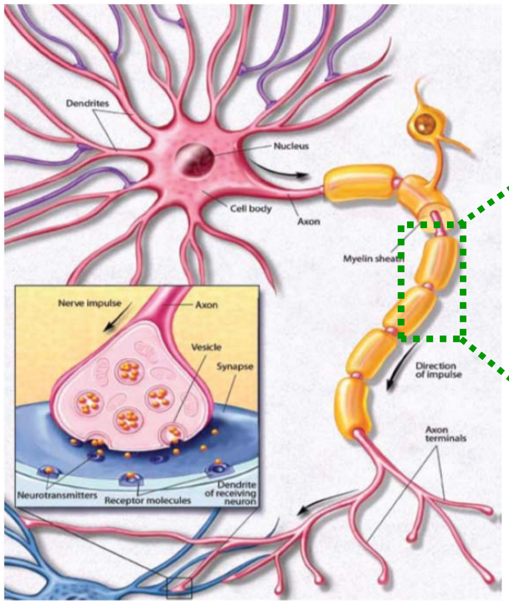
  - diffusion of spherical particles through liquid in which viscous force dominates

$$D = \frac{kT}{6\pi\eta r}$$

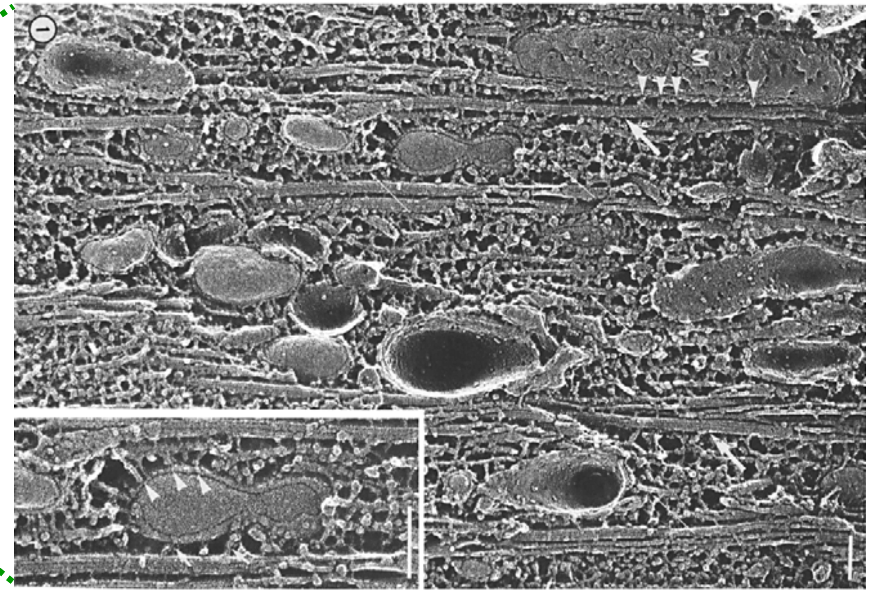
- Boltzmann constant: $k = 1.381 \times 10^{-23}$ J/k $= 1.381 \times 10^{-17}$ N·μm/k
- Absolute temperature: T = 273.15 + 25
- Viscosity: $\eta = 0.8904$ mPa·s $= 0.8904 \times 10^{-3} \times 10^{-12}$ N·μm^{-2}·s
- Sphere radius: r = 500nm = 0.5μm
- Calculated diffusion coefficient: D = 0.5 μm^2/s

Howard Berg, *Random walks in biology*,
Princeton University Press, 1993

An Overview of Axonal Transport



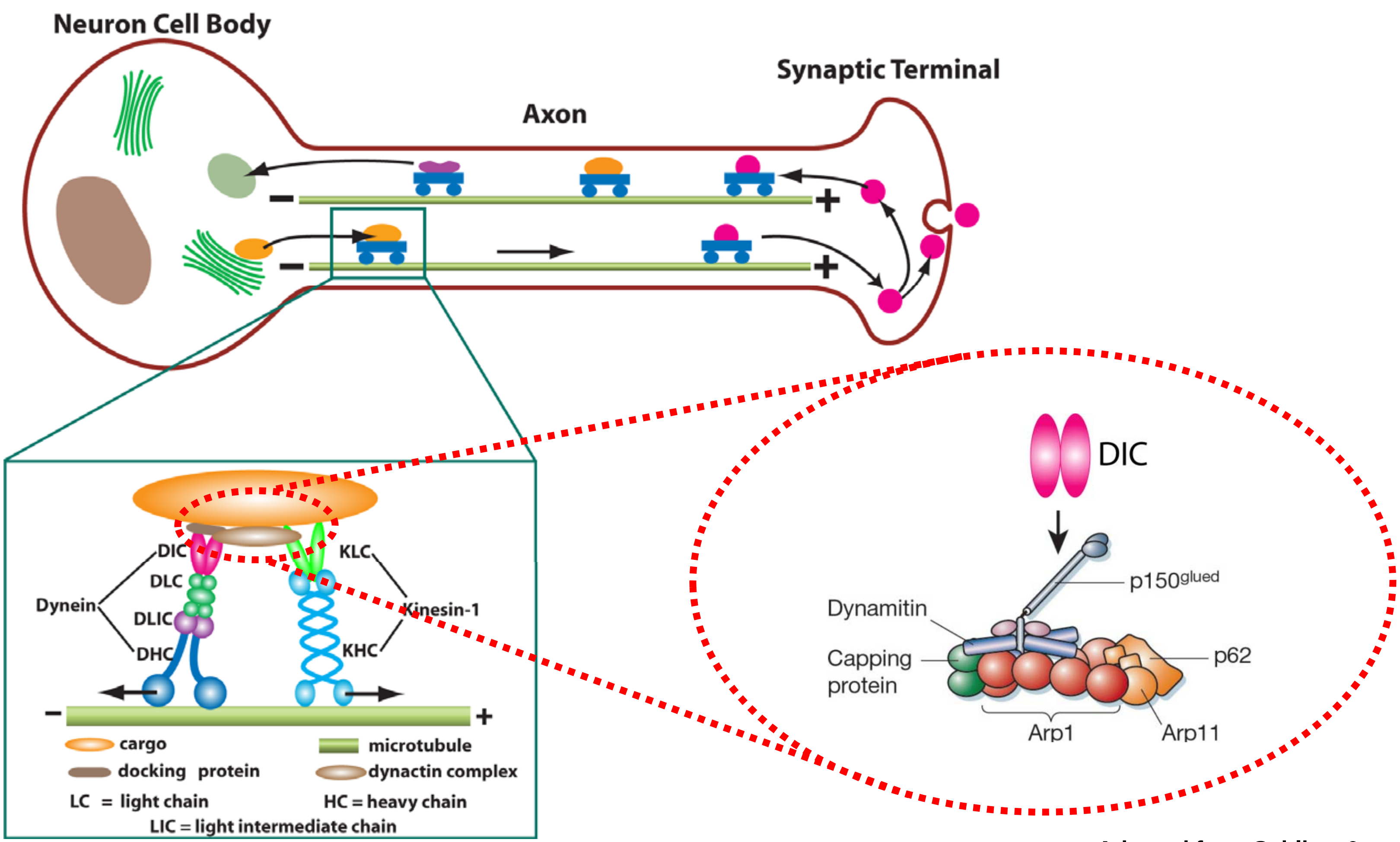
From *Brain Facts*, Society for Neuroscience



Hirokawa N., *JCB*, 94:129, 1982 Bars: 0.1μm

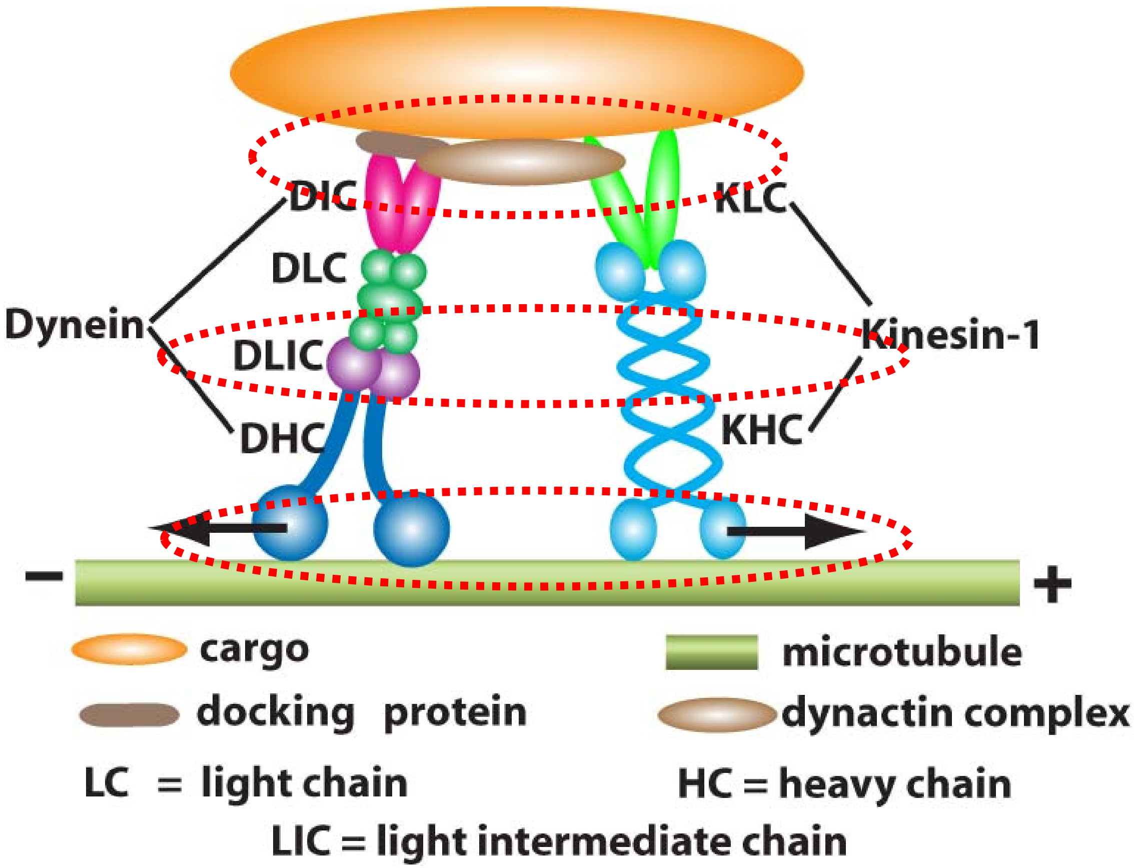
- Axonal transport is critical to survival and function of neurons.
- Axonal transport is a powerful model of intracellular transport.
- Axonal transport may be a good model to study spatiotemporal cell signaling.
- Many mitotic motors also drive axonal transport.

Molecular Motor Machinery of Axonal Transport

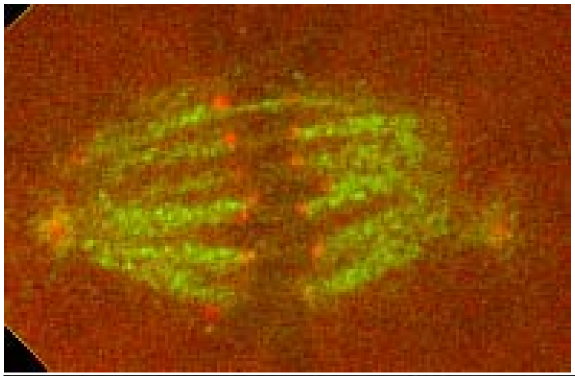


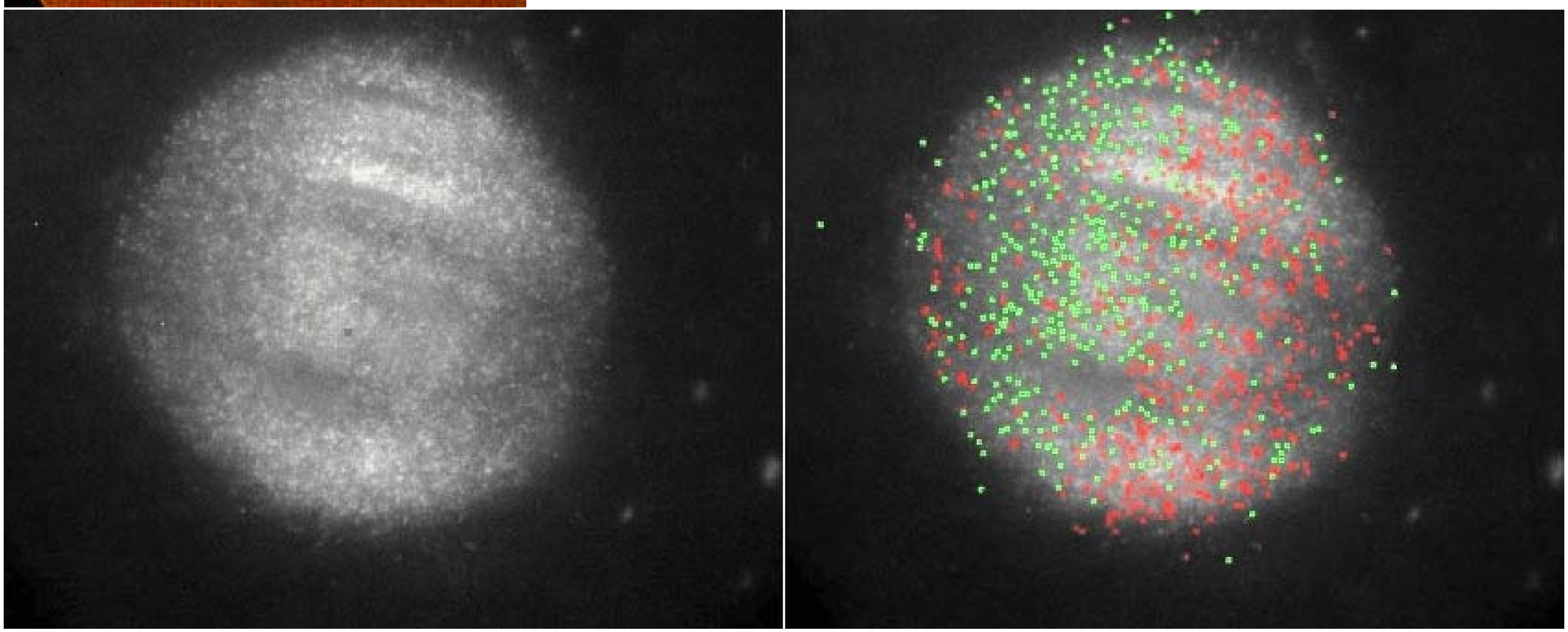
Adapted from Schliwa & Woehlke, *Nature*, 422:759, 2003

Potential Mechanisms of Axonal Transport Defects



Tracking Results Demo





Yang G., Cameron L.A., Danuser G., and Salmon E.D. (2008) Regional variation of microtubule flux reveals microtubule organization in Xenopus extract meiotic spindles, Journal of Cell Biology, vol. 182, pp. 631-639.