

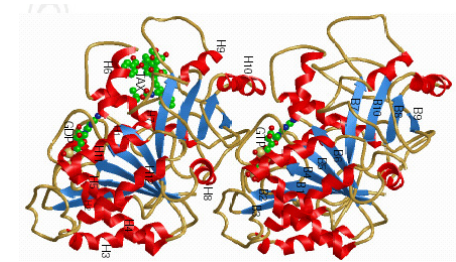
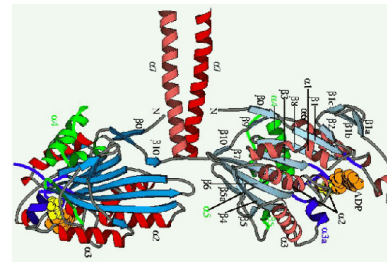
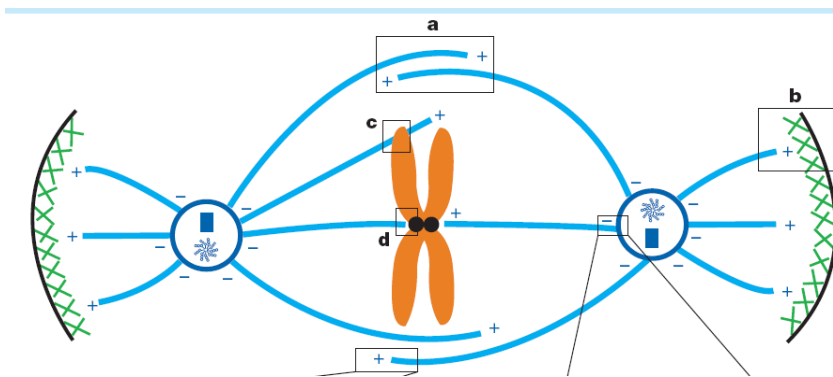


# Lithography Approaches to Artificial Spindle Assembly

**Vivek Verma**

Materials and Metallurgical Engineering  
Indian Institute of Technology, Kanpur

ICTS 2010



# Transport at Macro Scale



**Transport on immobilized tracks**



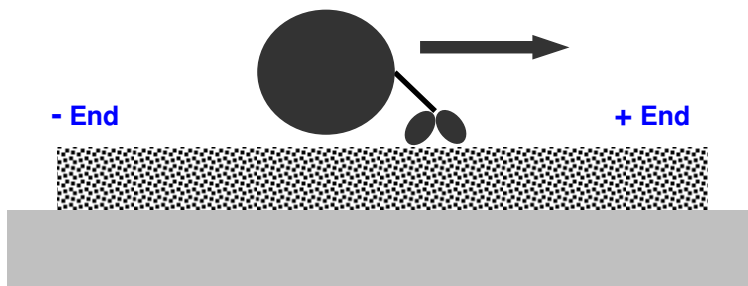
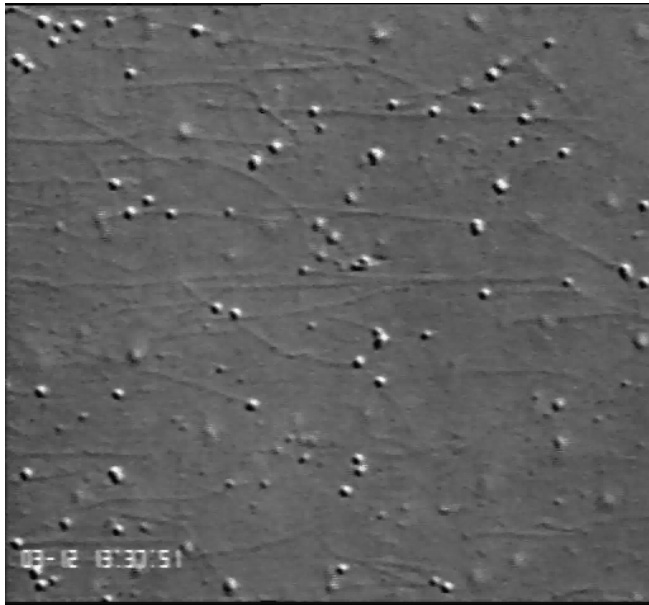
**Transport via moving track**

<http://www.visitbuckinghamshire.org/dbimings/Bucks%20Railway%20Centre.JPG>  
[http://www.conveyco.co.za/images/Home\\_Page/FP003.JPG](http://www.conveyco.co.za/images/Home_Page/FP003.JPG)

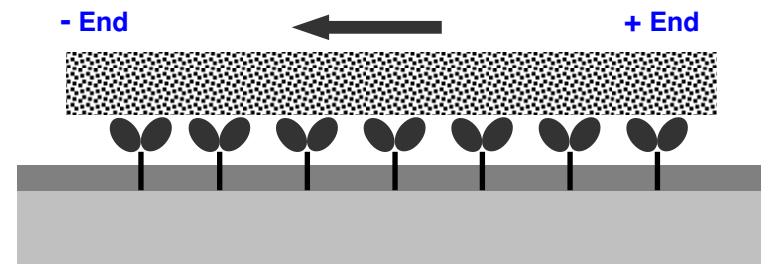
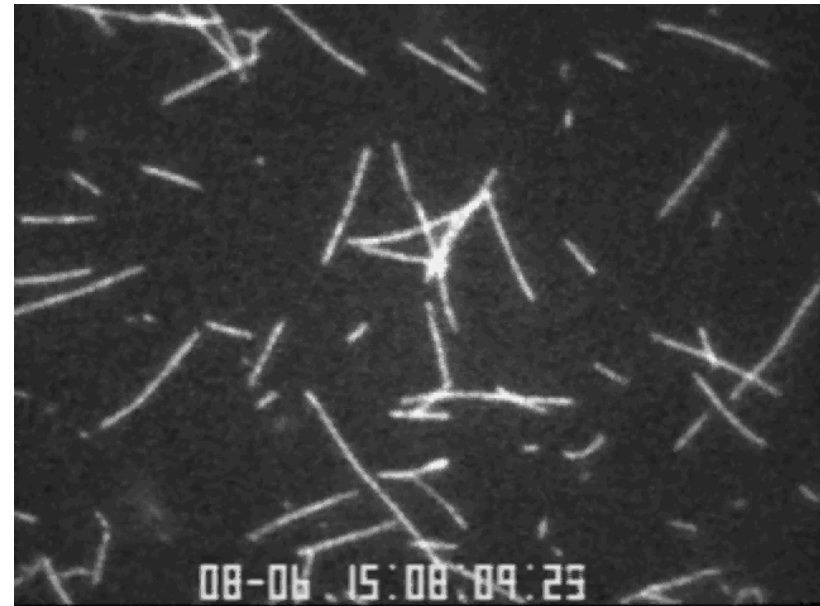


# Transport and Nanoscale

**Bead assay:**



**Gliding assay:**



 **Glass Substrate**     **Microtubule**     **Casein**

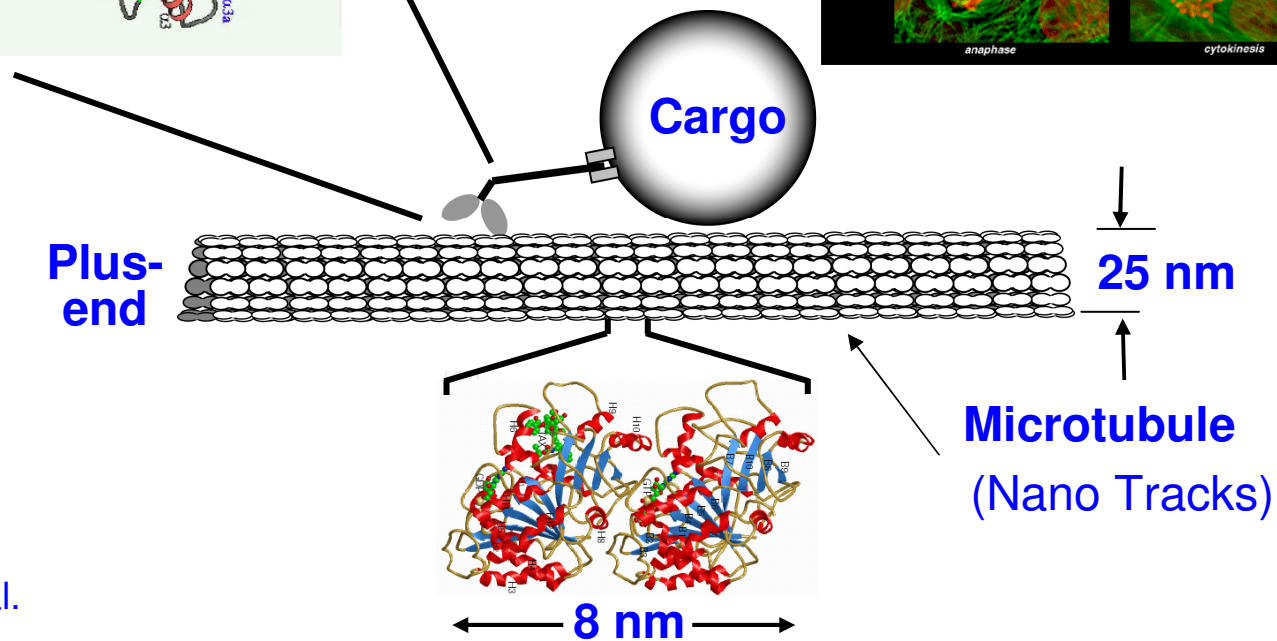
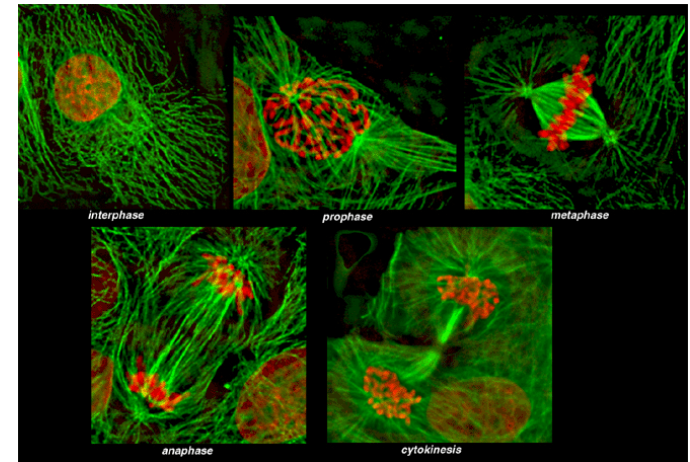
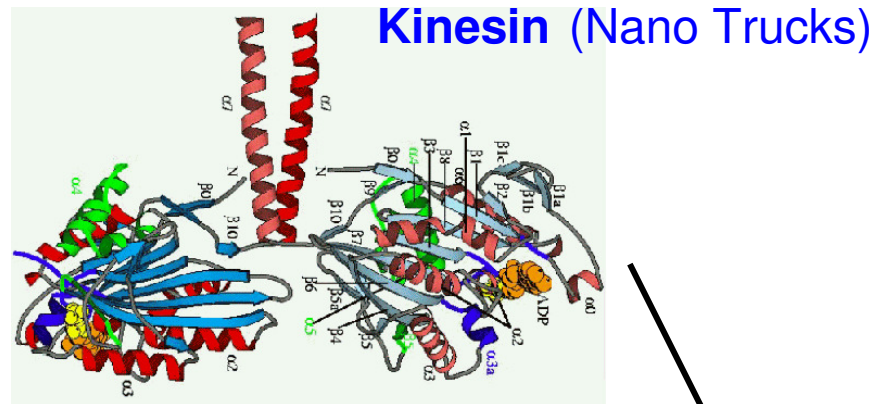
 **Direction of motion**     **Kinesin**     **Cargo**



William Hancock Lab, Penn State



# Kinesin and Microtubule



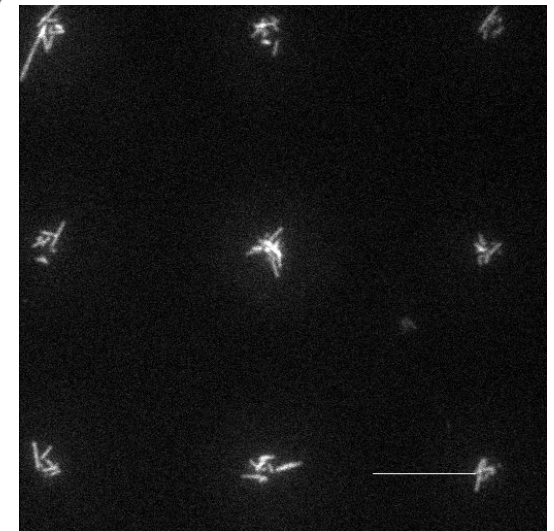
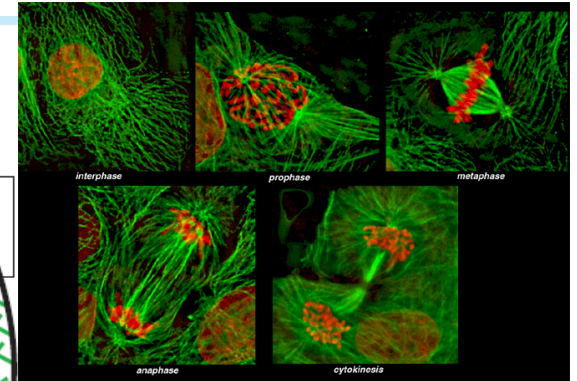
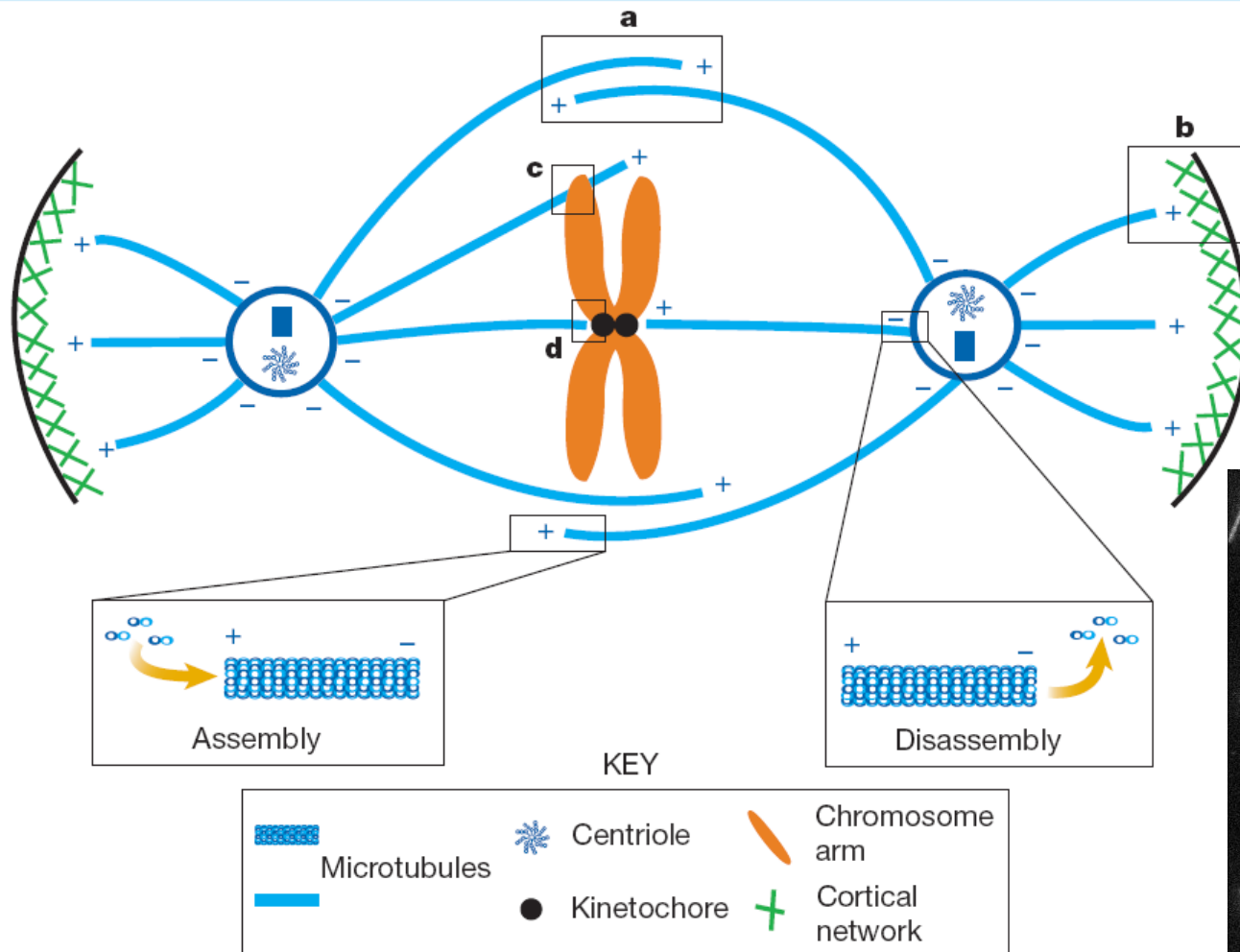
Kozielski et al.

Nogales et al.

<http://web.bio.ed.ac.uk/research/groups/earnshaw/mitosisGrL.gif>



# Using Microfabrication to Study Cellular Processes

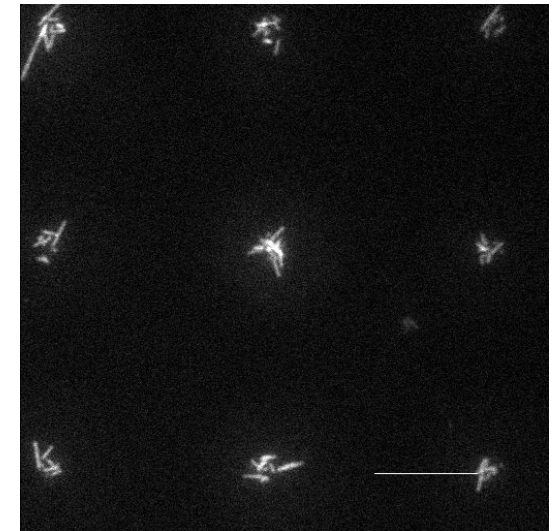
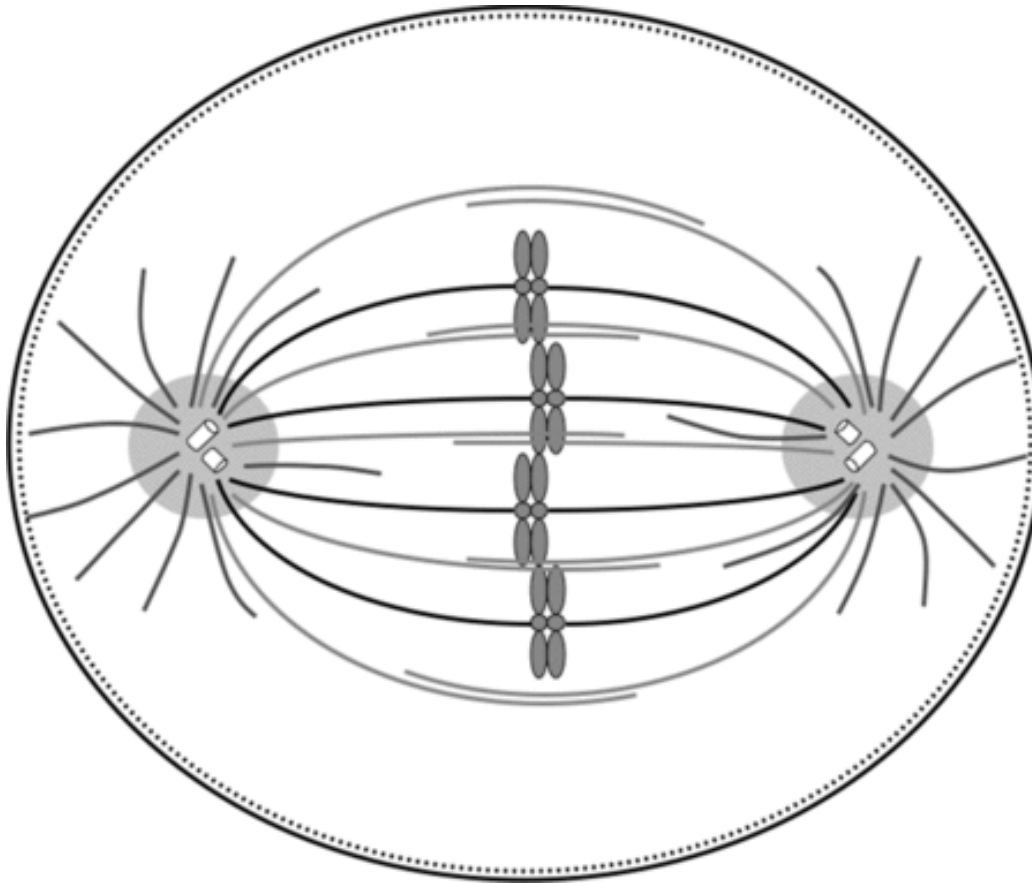


Scale bar: 10  $\mu\text{m}$

Sharp et. al. (2000)  
<http://web.bio.ed.ac.uk/research/groups/earnshaw/mitosisGrL.gif>



# Mimicking Mitotic Spindle

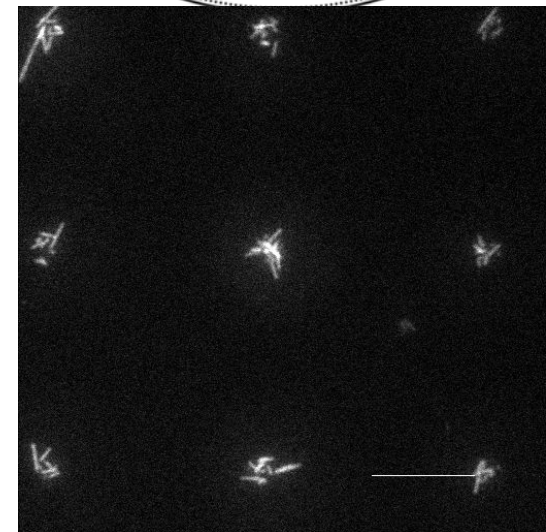
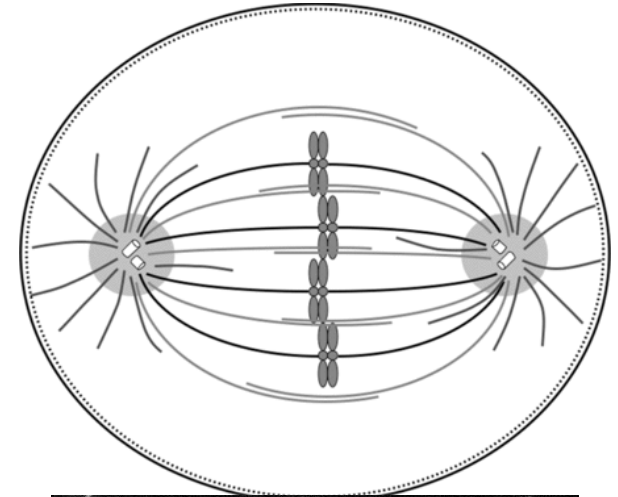
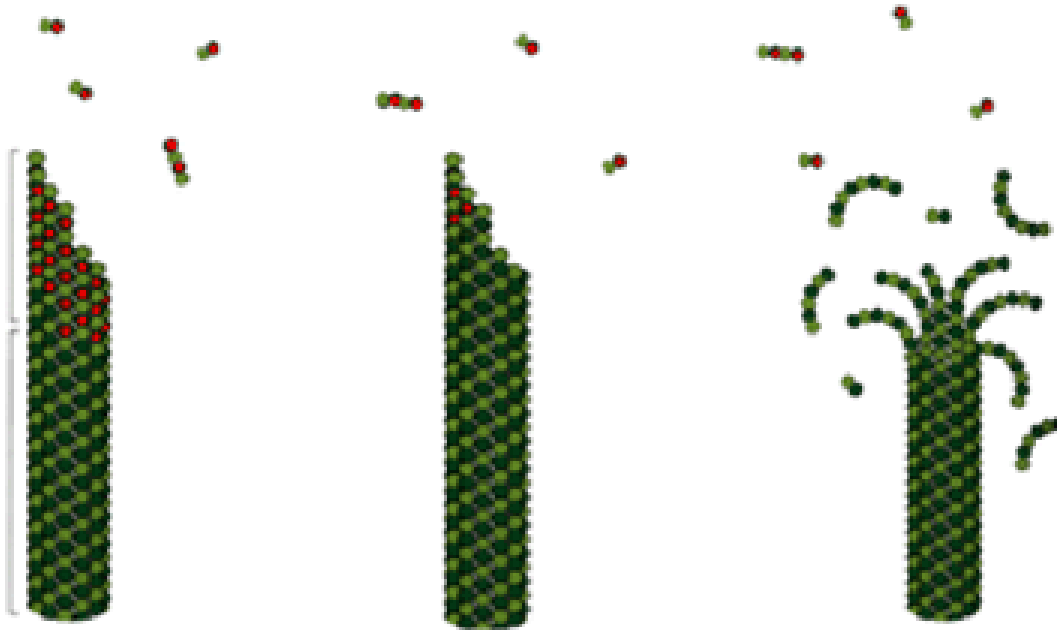


**Scale bar: 10  $\mu$ m**

<http://www.biochemsoctrans.org/bst/034/0716/bst0340716f01.gif>



# Microtubule Dynamics



**Scale bar: 10  $\mu$ m**



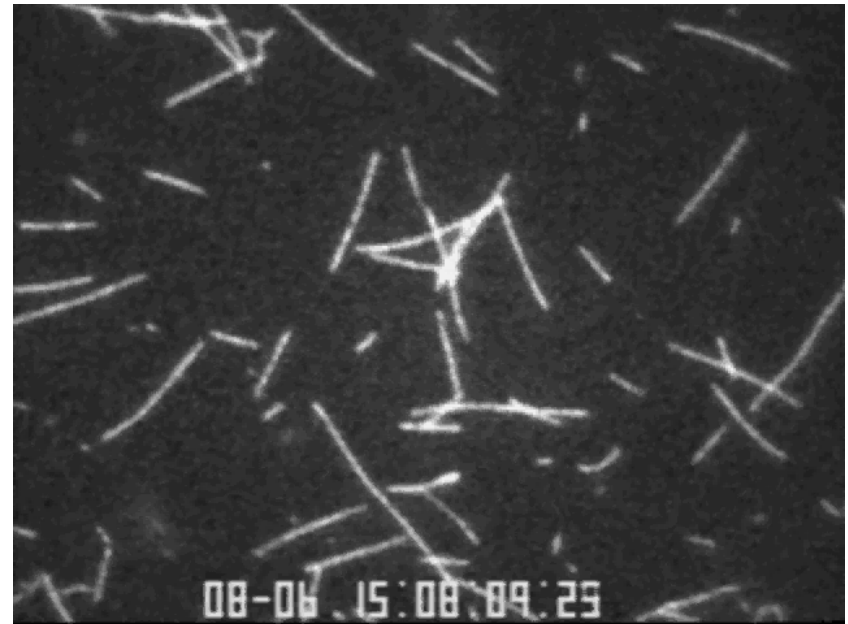
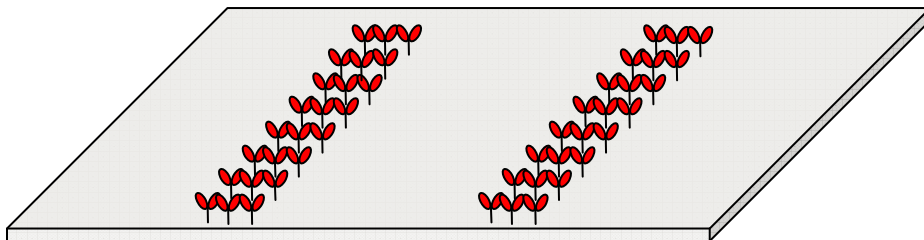
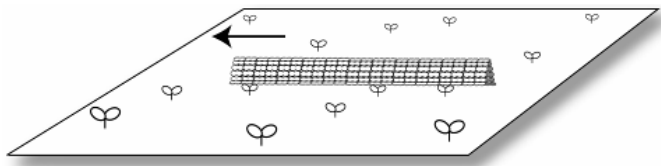
<http://www.biochemsoctrans.org/bst/034/0716/bst0340716f01.gif>

[http://celldynamics.org/celldynamics/events/workshops/archive/2003/cytomod\\_abstracts/HGoodson/images/HGoodson-fig2.gif](http://celldynamics.org/celldynamics/events/workshops/archive/2003/cytomod_abstracts/HGoodson/images/HGoodson-fig2.gif)



# Challenges

- Microtubule motion highly random
- Require patterning kinesin



- Interdisciplinary research

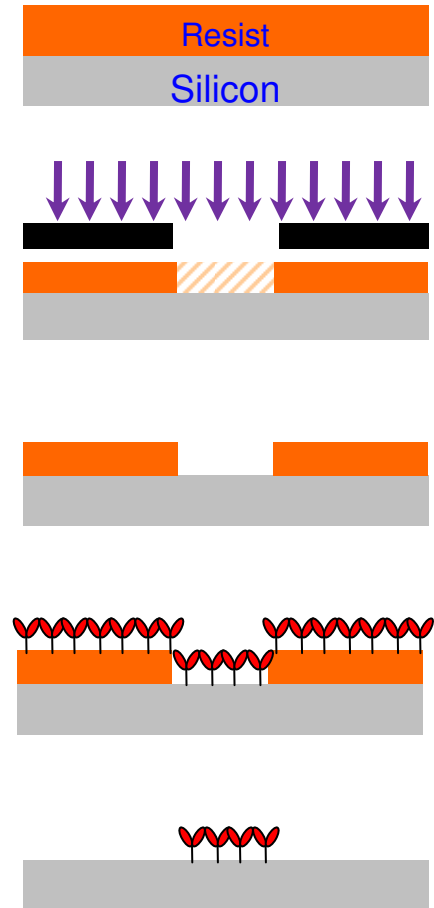


Scale bar: 10  $\mu\text{m}$



# Lithography: Overview

- Spin coat polymer resist, bake (PMMA, UV5)
- Partially expose surface by electron beam (or UV)
- Develop the resist using developers (TMAH, MIBK, IPA)
- Incubate fluorescently labeled casein and kinesin
- Strip resist in solvent (Acetone)

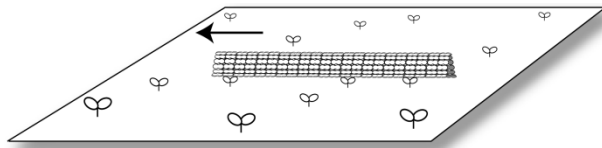


# Objectives of Research

- Study **compatibility** between protein system and micro- and nanofabrication processes
- Develop processes for **patterning** kinesin
- Optimize **motility** performance from surface bound kinesin
- Understand and manipulate biological cell processes



# Establishing Nanofab Design Rules



How do casein, kinesin and microtubules handle nanofabrication processing materials and chemicals?

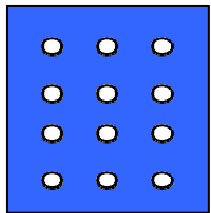
	Casein		Casein + Kinesin		Casein + Kinesin + Microtubules	
	Chemicals	Motility	Chemicals	Motility	Chemicals	Motility
Removers	Acetone	Yes	Acetone	Yes	Acetone*	Yes
	IPA	Yes	IPA	Yes	IPA*	Yes
	Ethanol	Yes	Ethanol	No	Ethanol	No
Developers	MIBK	Yes	MIBK	Yes	MIBK	No
	TMAH	Yes	TMAH	No	TMAH	No
Resists	PMMA	Yes	PMMA	Yes	PMMA	No
	UV 5	Yes	UV 5	No	UV 5	No

**Great**
**Good**
**So-so**

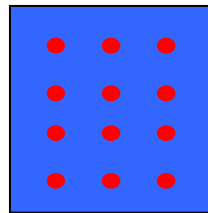
Initiative for biological buffer that work as developer and remover for nanofabrication processes



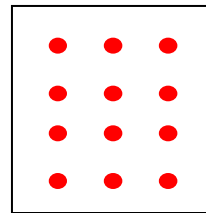
# Kinesin Patterning



Lithography on PMMA resist



Incubate motors



Strip PMMA



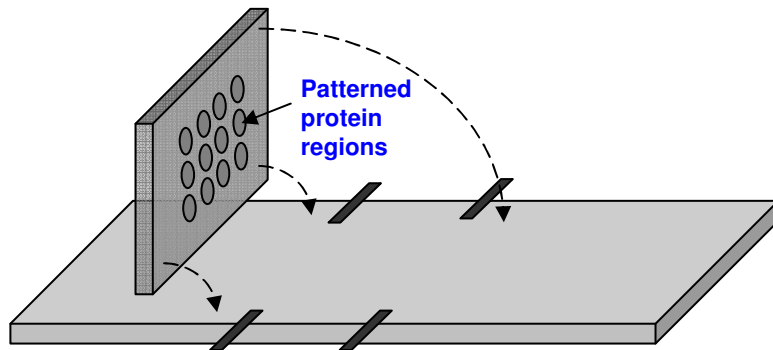
Glass



PMMA



Kinesin



Microscope slide

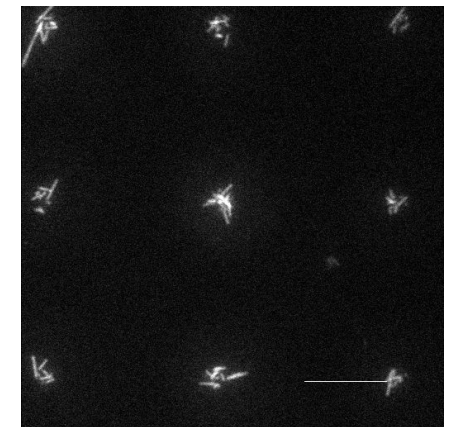
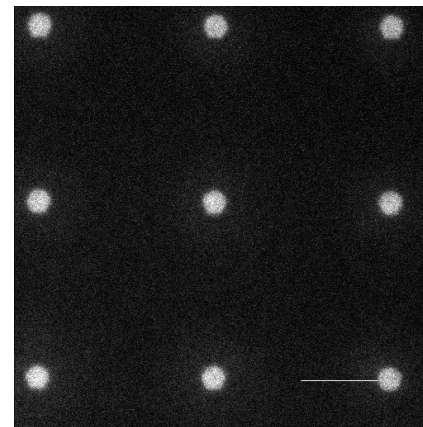
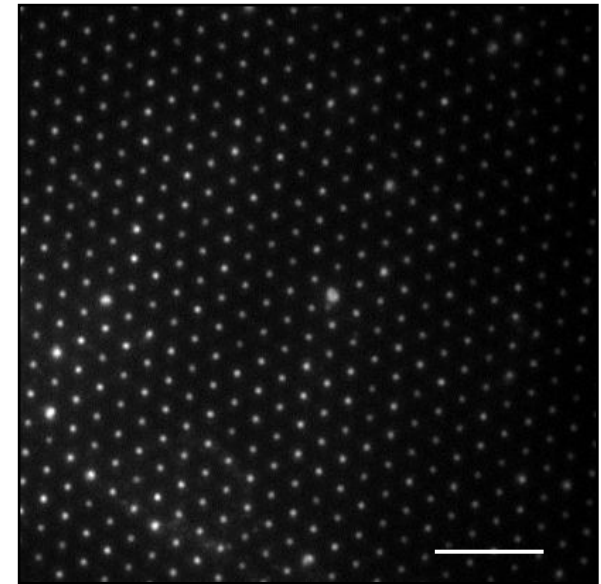


Double sided tape



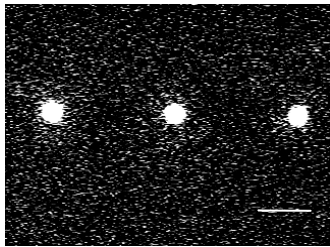
Glass cover slip

Scale bars: 10  $\mu$ m

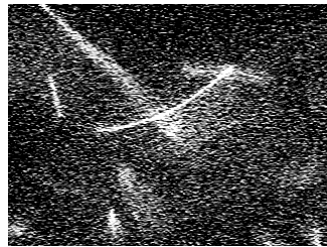




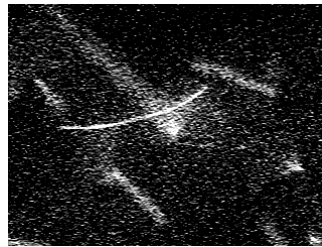
# Kinesin Motors bind to and Move Microtubules



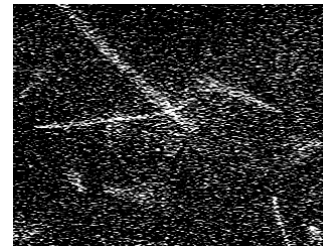
Scale bar 10um



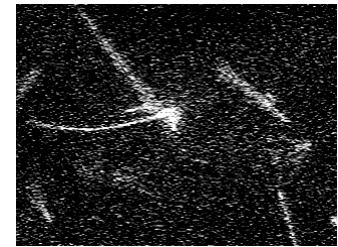
t = 0s



t = 7s



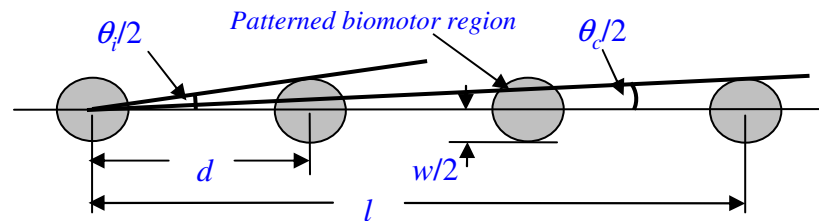
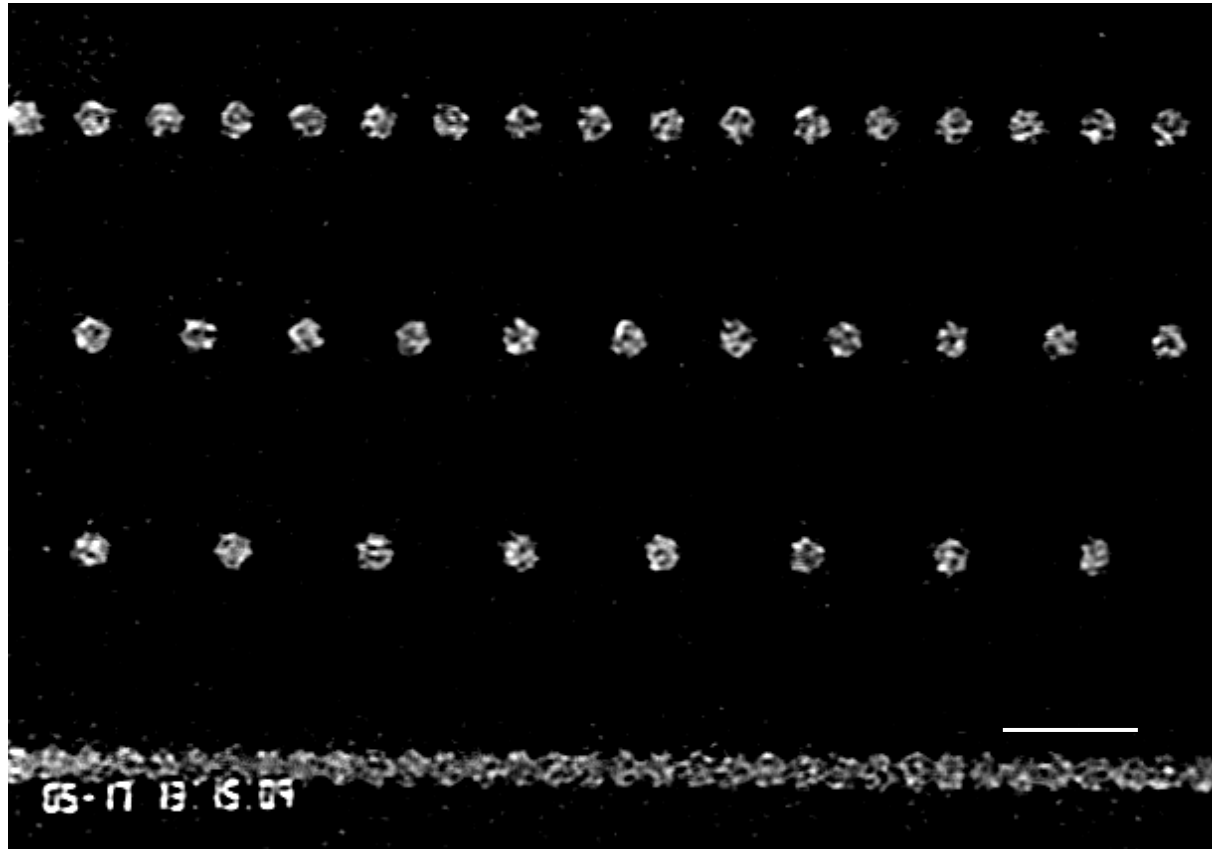
t = 14s



t = 21s



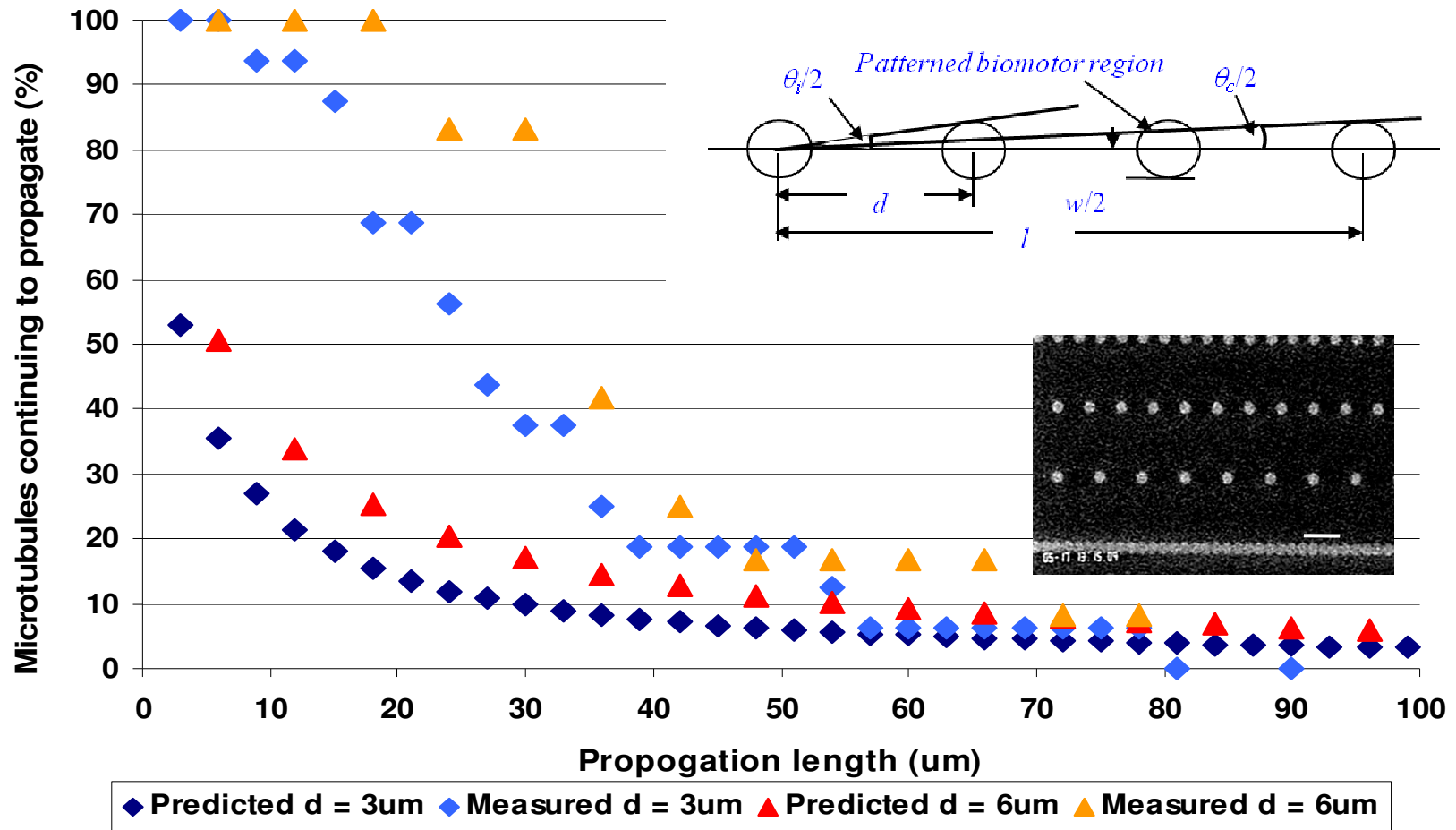
# Kinesin Arrays



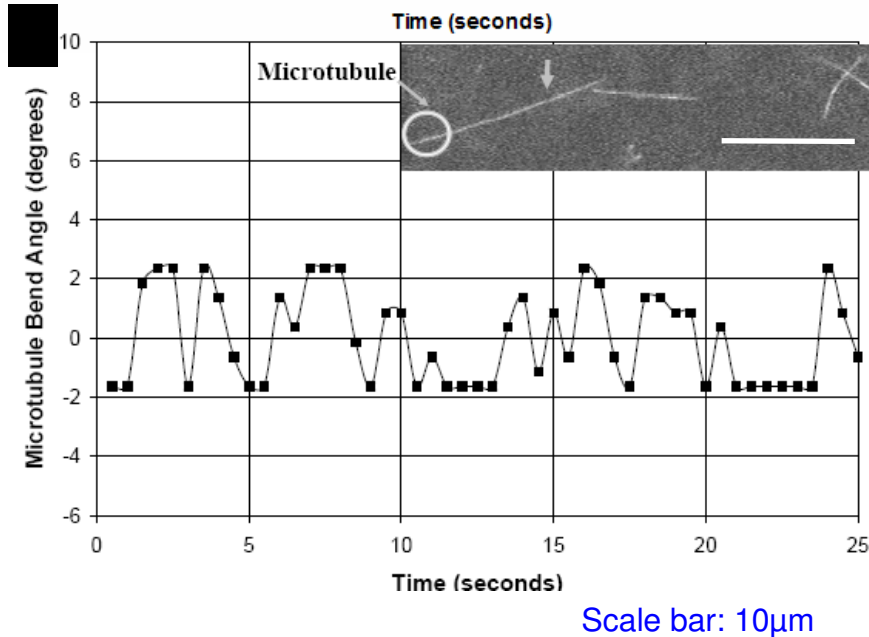
Scale bar 10um



# Microtubule are Guided via Patterned Kinesin Dots



# Microtubule Rigidity and Dynamics of its Interaction with Kinesin Motors



- Microtubule was partially bound to kinesin
- Tip displacement of free end of microtubule was measured
- Flexural rigidity of microtubule was calculated

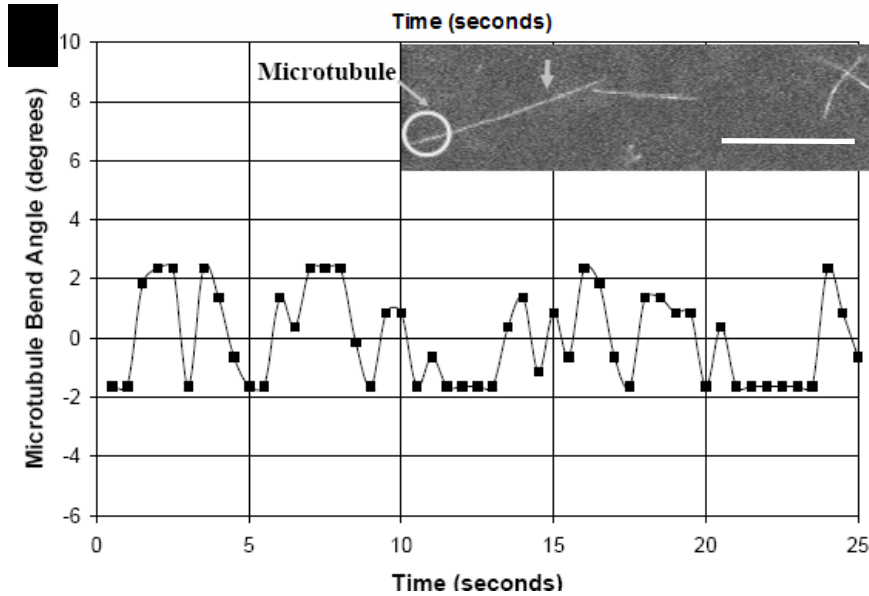
$$EI = \frac{k_B TL^3}{3\langle y^2 \rangle}$$

Cassimeris *J Cell Sci*, (2001)





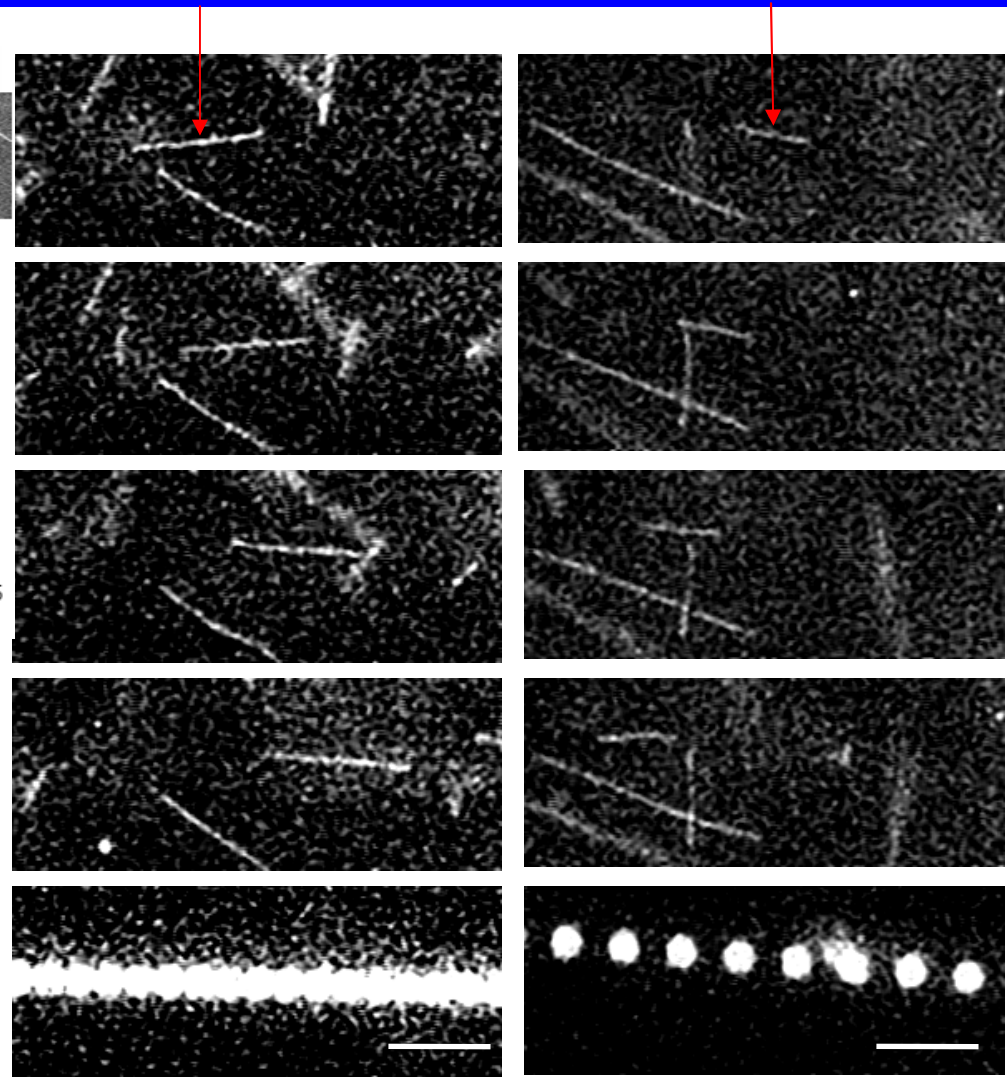
# Microtubule Rigidity and Dynamics of its Interaction with Kinesin Motors



Scale bars: 10 $\mu$ m

$$EI = \frac{k_B TL^3}{3 \langle y^2 \rangle}$$

Cassimeris *J Cell Sci*, (2001)



t = 0, 3.5, 7, 10.5s

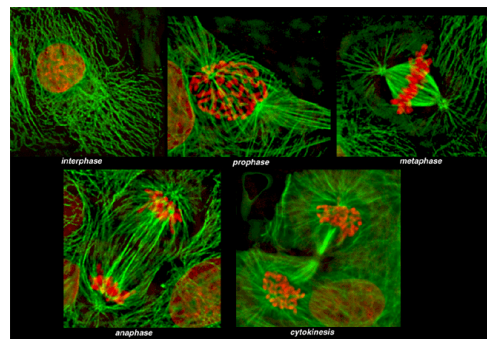
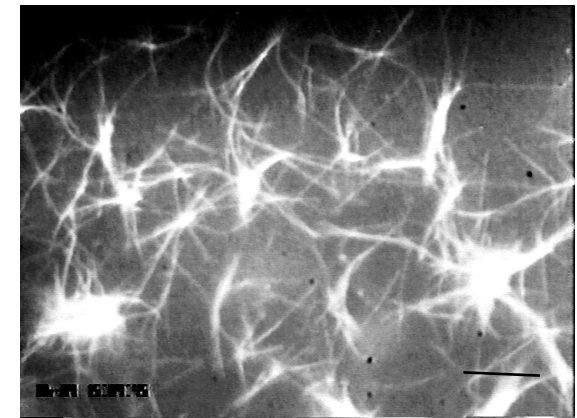
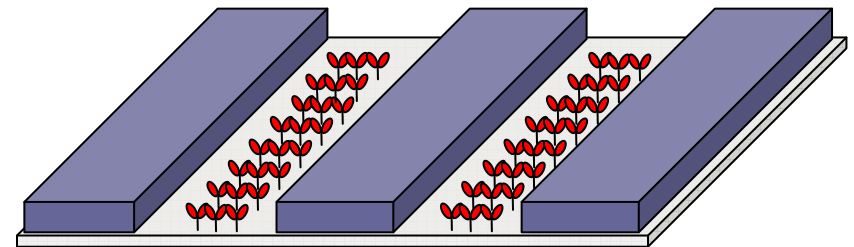
t = 0, 5, 9, 13s



Verma et. al, *Biomed Microdev* (2008), Verma et. al., *IBE St Louis* (2007)

# Summary and Future Directions

- Established compatibility between lithography chemicals and proteins
- Patterned kinesin and studied microtubule dynamics
- Hybrid synthetic devices
- Study more fundamental areas

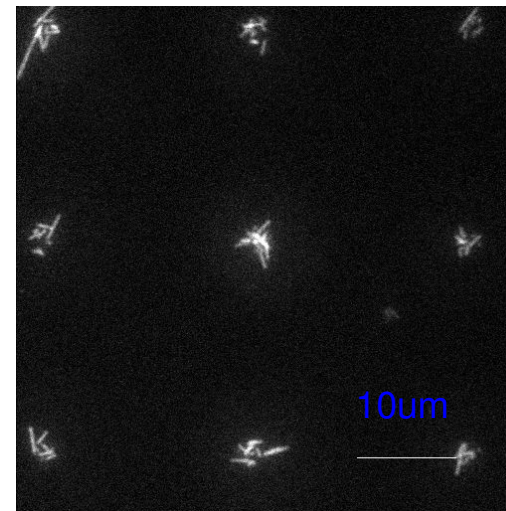
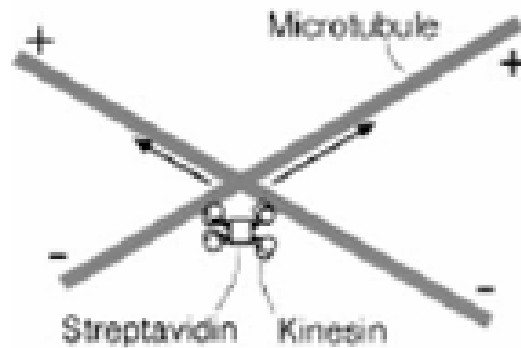
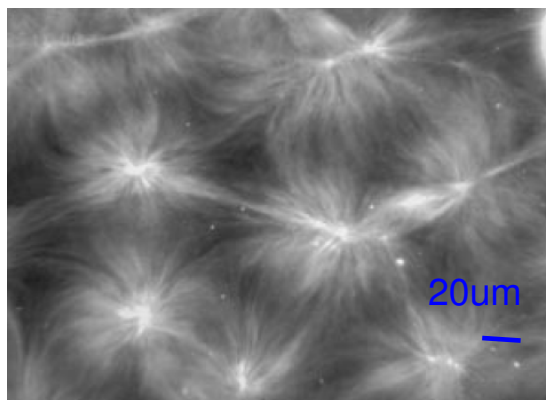
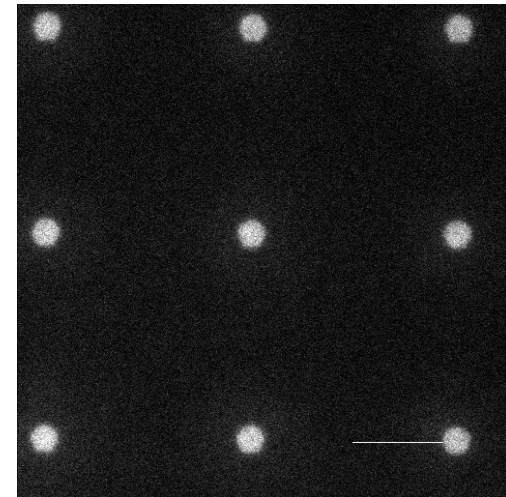
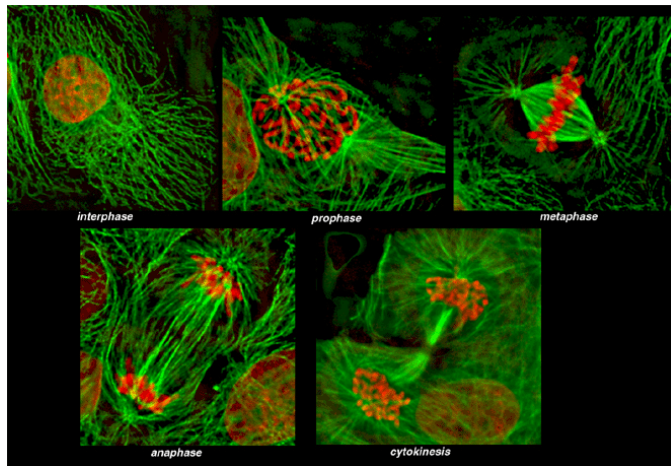


Scale bar: 10  $\mu\text{m}$

<http://web.bio.ed.ac.uk/research/groups/earns-haw/mitosisGrL.gif>



# Mimicking Cells: Asters



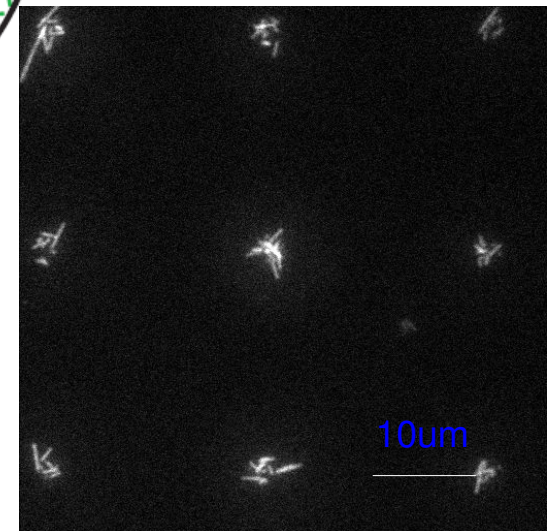
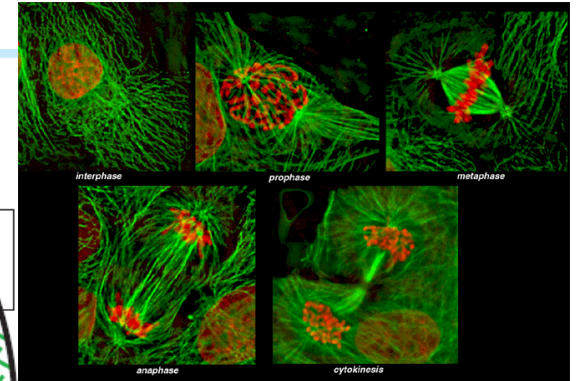
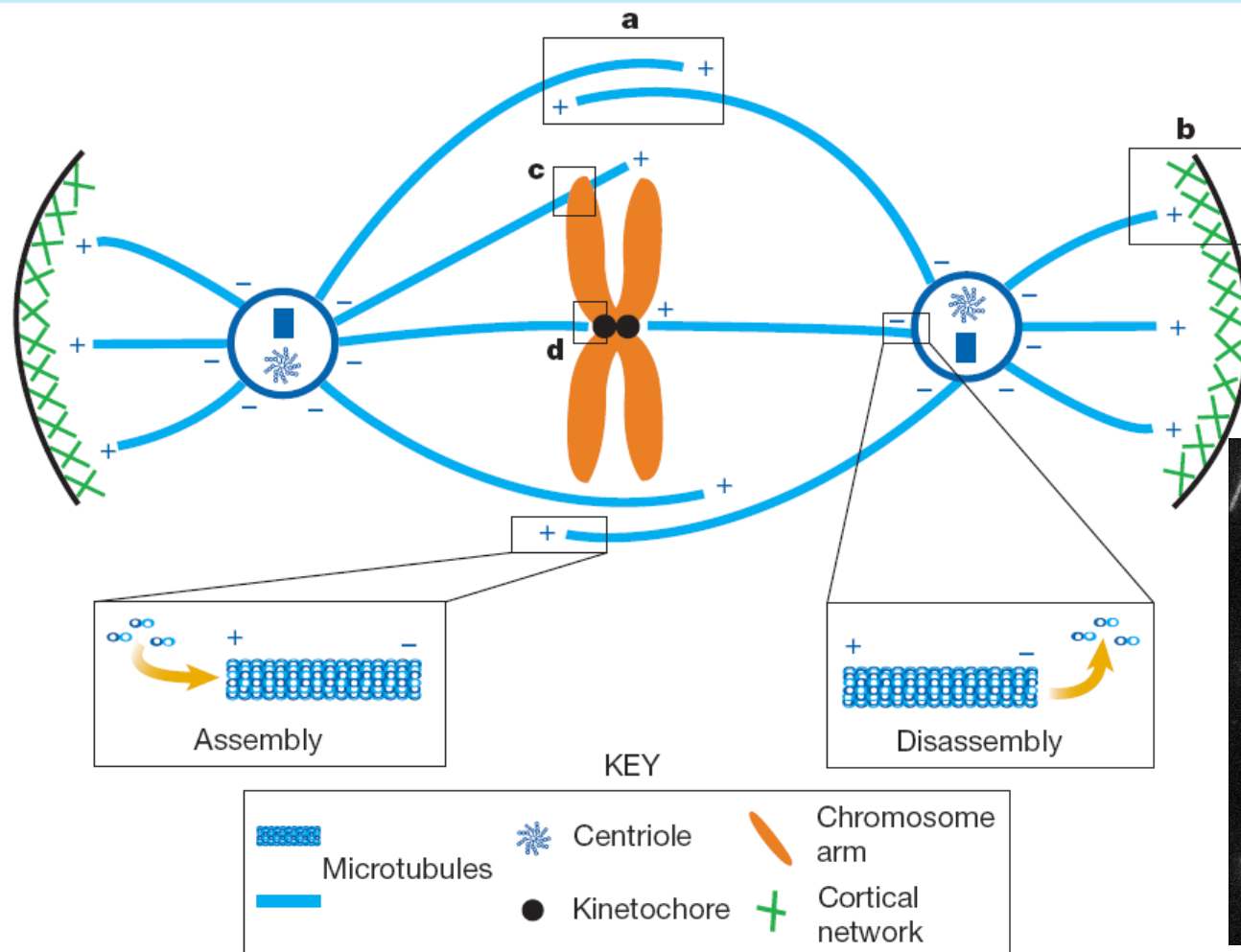
Multi-head motor assembly containing 4 kinesin linking two microtubules.



<http://web.bio.ed.ac.uk/research/groups/earnshaw/mitosisGrL.gif>  
Nédélec et al. (1997)



# Integrating Proteins and Fabrication to Study Cellular Processes



Sharp et. al. (2000)  
<http://web.bio.ed.ac.uk/research/groups/earnshaw/mitosisGrL.gif>



# Conclusions

- First to establish compatibility between biological motors and lithography chemicals
- Integrated nanofabrication process directly implementing biological motor proteins
  - Compatibility
  - Patterning
- New approaches for assessing kinesin and microtubule mechanics
- Tools for direct integration of microtubules to understand and manipulate spindle assembly



# Acknowledgements: Mentors



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Prof. Will Hancock



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- Dr. Catchmark's lab members
- Dr. Ed Basgall, Dr. Khalid Eid
- Penn State Nanofab staff



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- Haythornthwaite Foundation
  - American Academy of Mechanics Founder's Prize and Grant
- Pennsylvania State University



Thank you!

