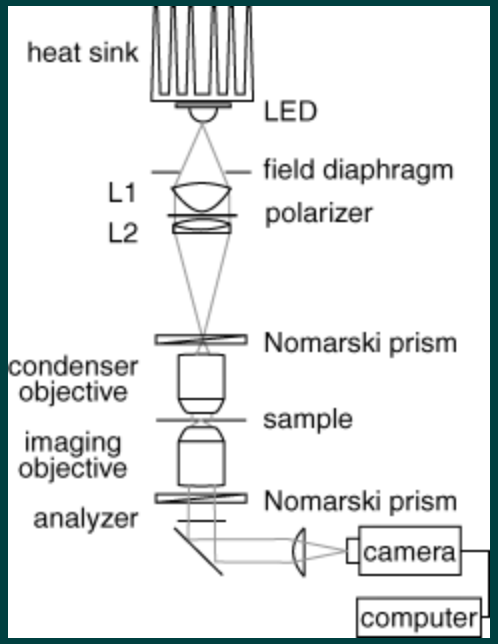
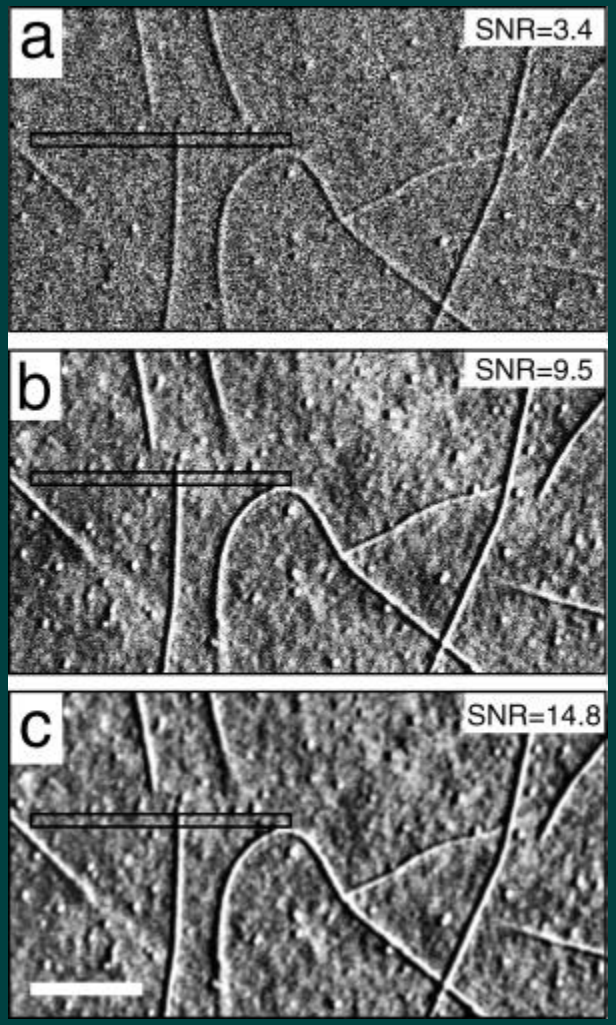
Methods to measure live dynamics, e.g., treadmilling and dynamic instability, of cytoskeletal filaments

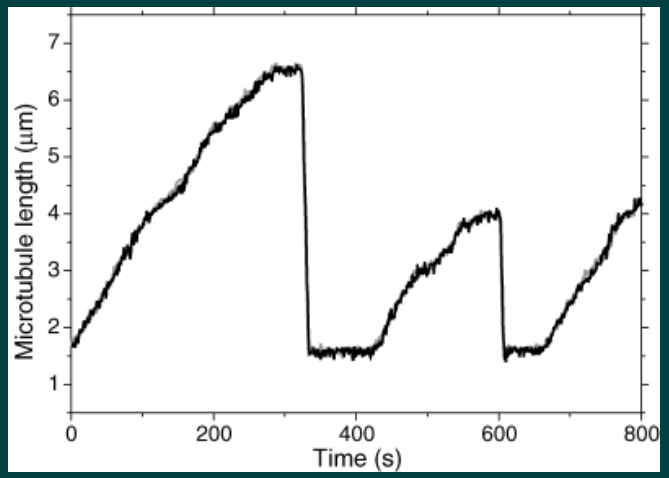
video-enhanced microscopy

fluorescence microscopy with labeled proteins of interest

individual microtubules visualized with video-enhanced DIC



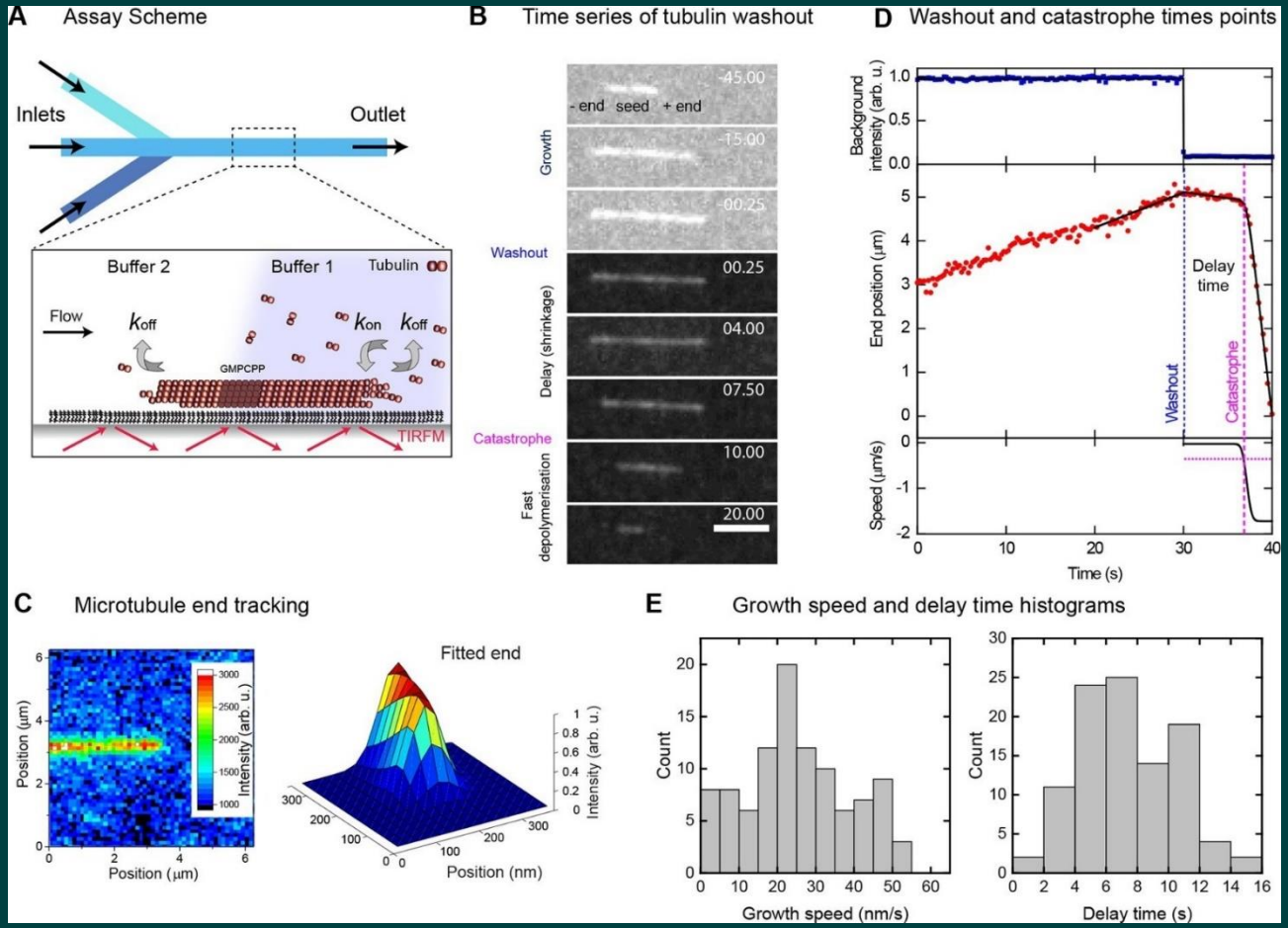


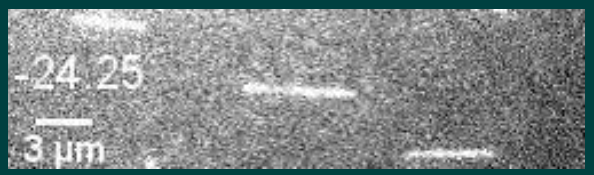


Bormuth et al., 2007

combination of fluorescence with microfluidics to measure GTP-cap at the microtubule end

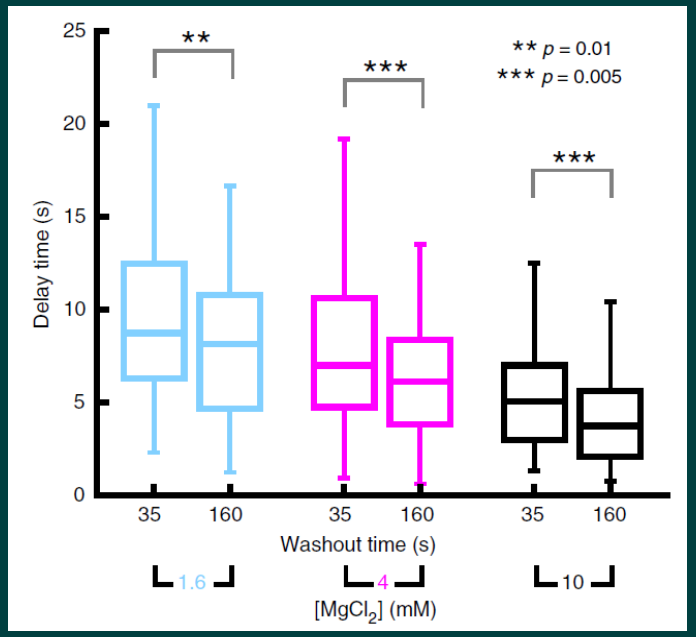
delay before the catastrophe reflects the cap length

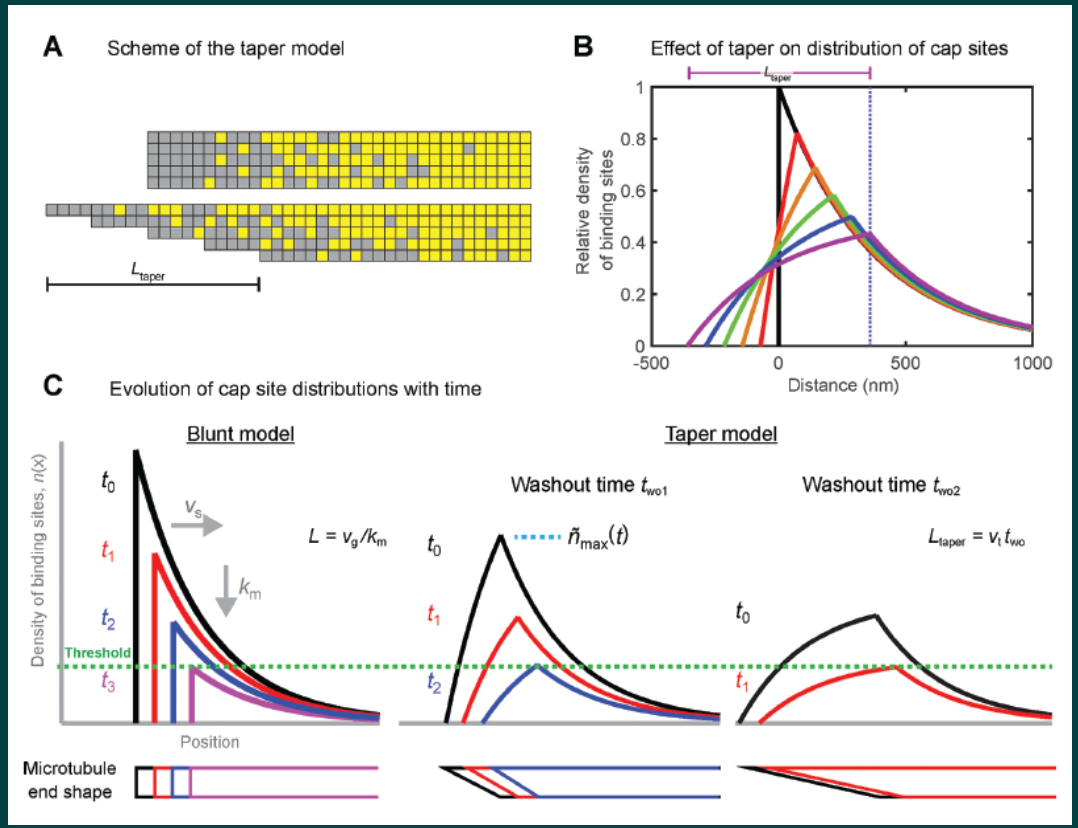




Duellberg et al., eLife, 2016

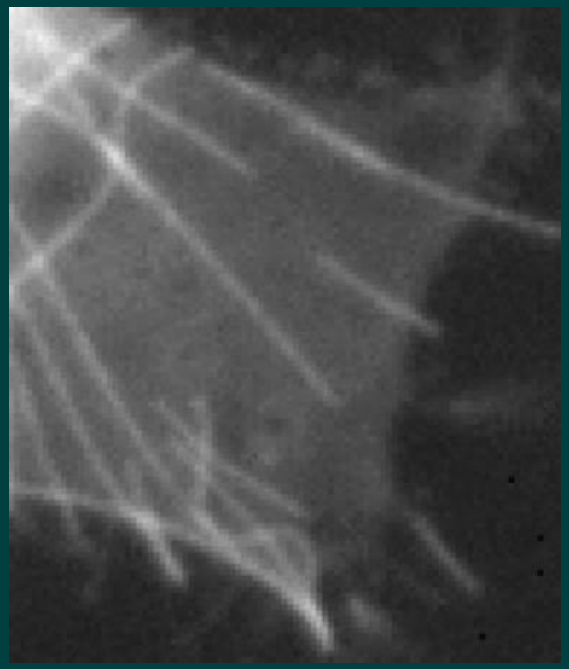
age-dependent delay times explained by a taper model





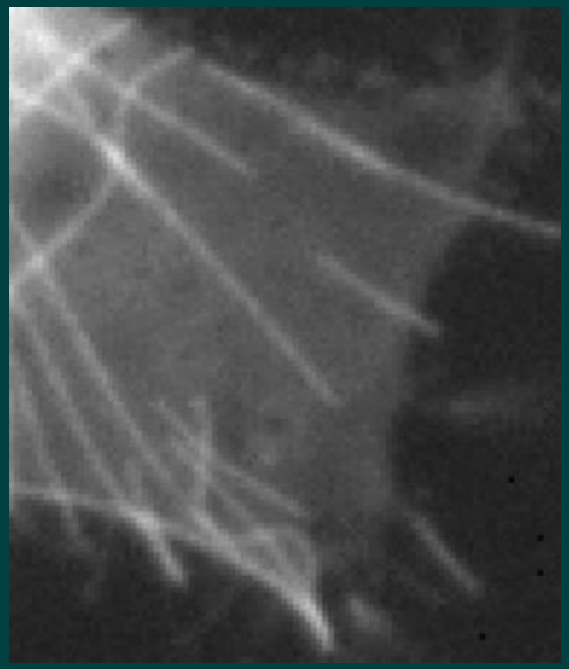
Duellberg et al., MBOC, 2016

Dynamics of individual microtubules in cells



From Rodionov and Borisy, 1997, 1999

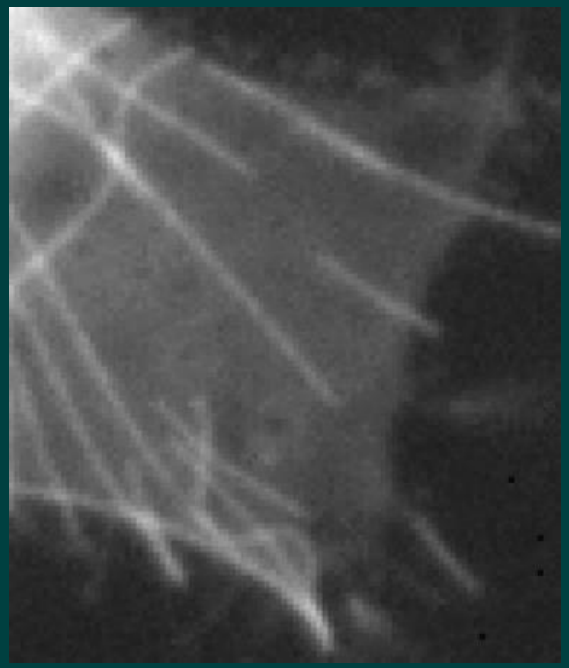
Dynamics of individual microtubules



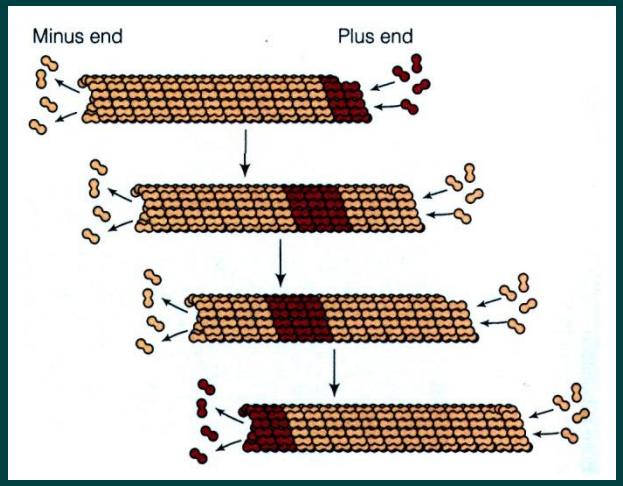
From Rodionov and Borisy, 1997, 1999

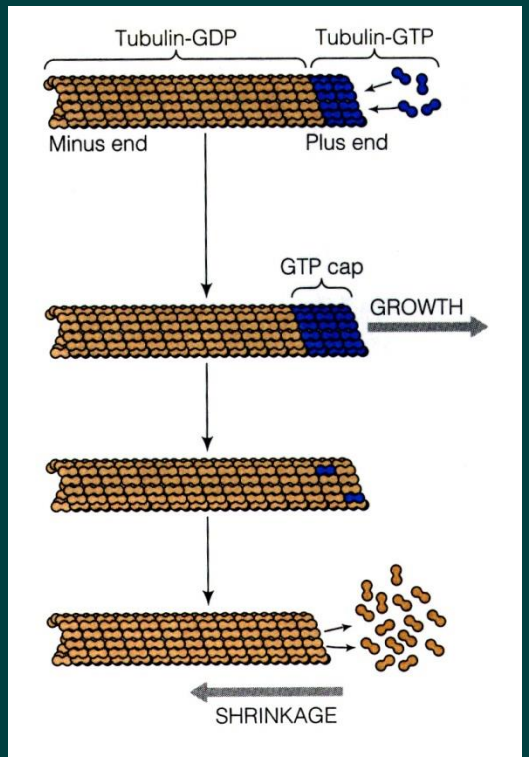
How can we know what is happening?

Dynamics of individual microtubules

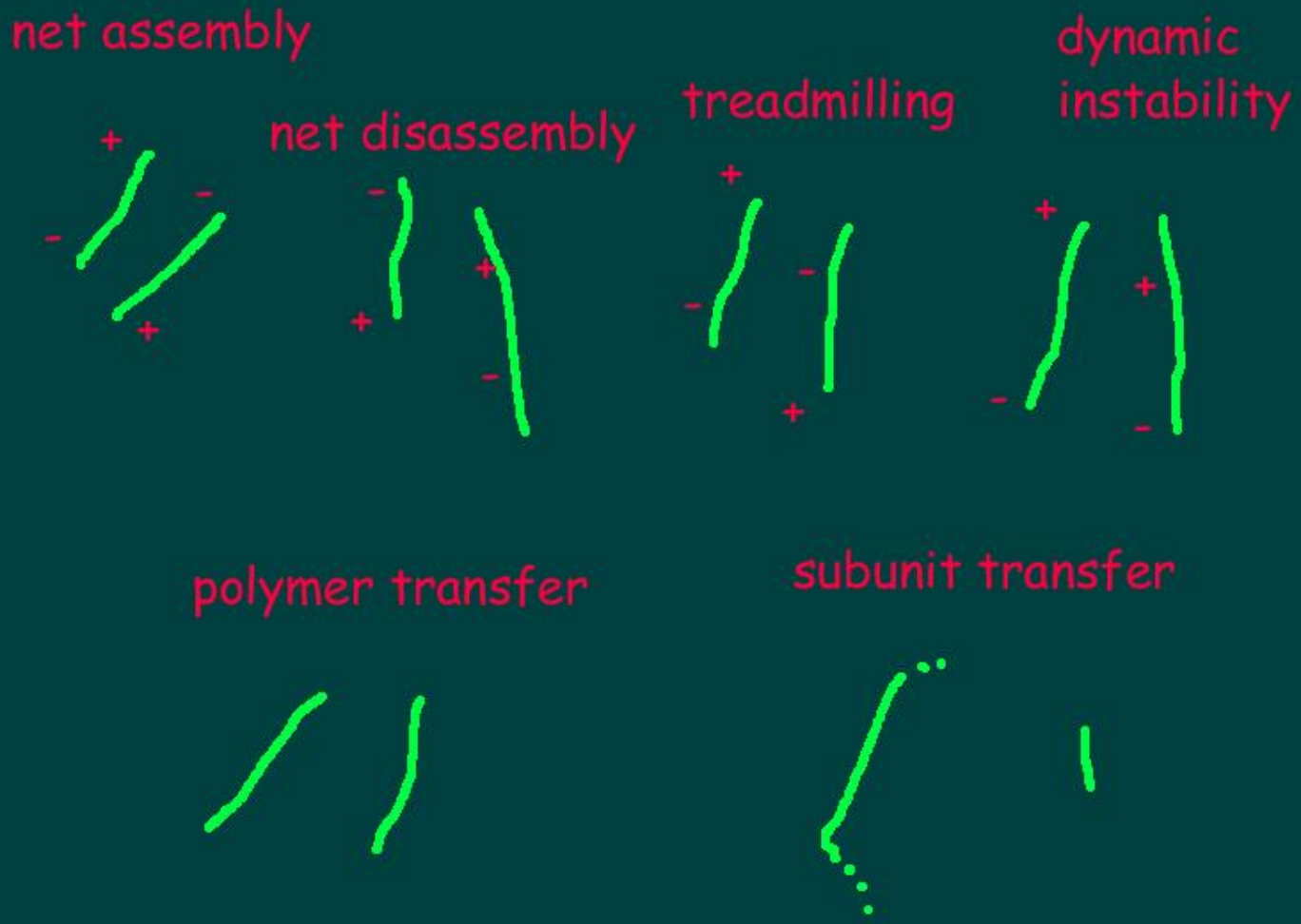


From Rodionov and Borisy, 1997, 1999





types of dynamics to measure



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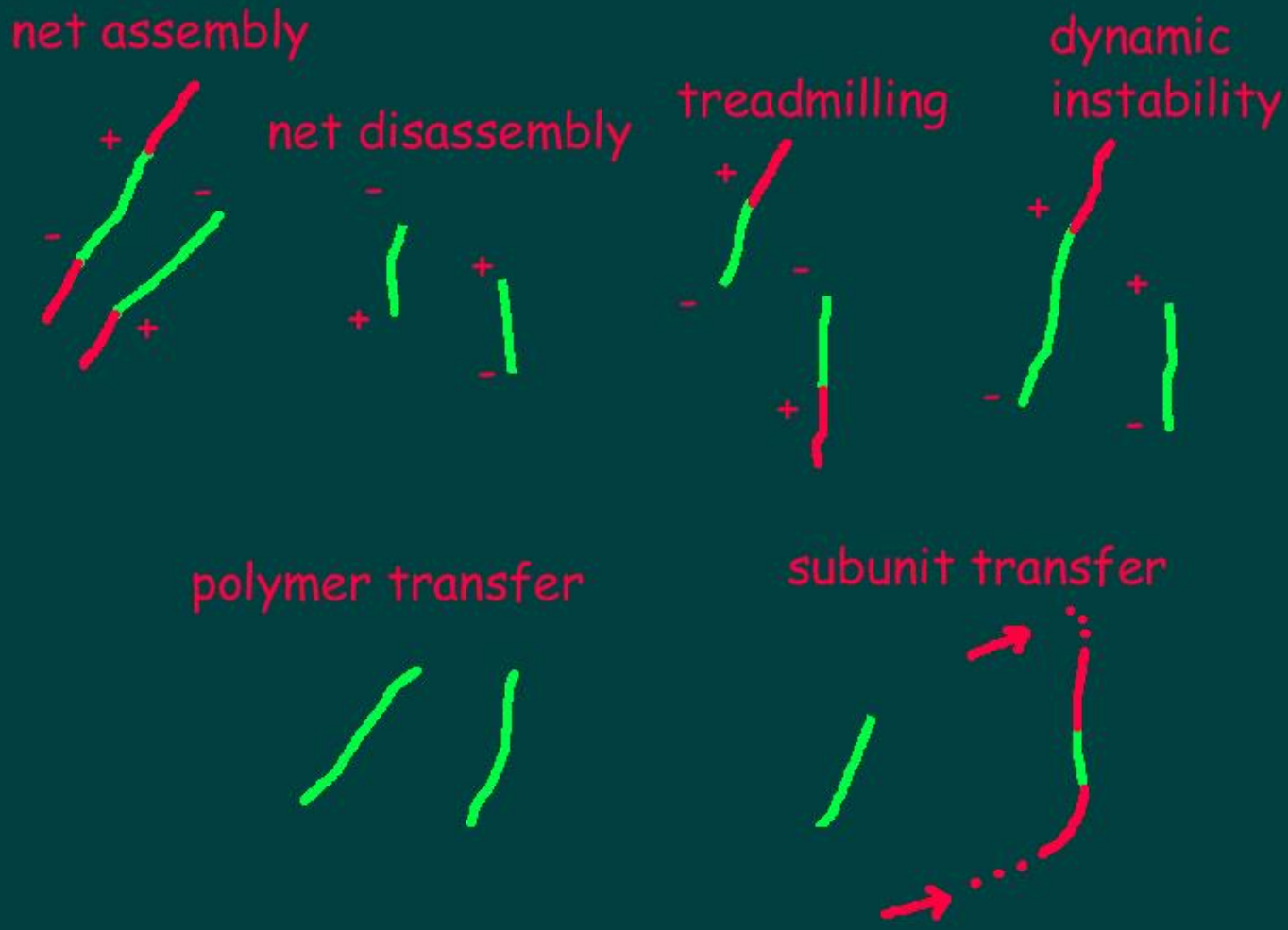
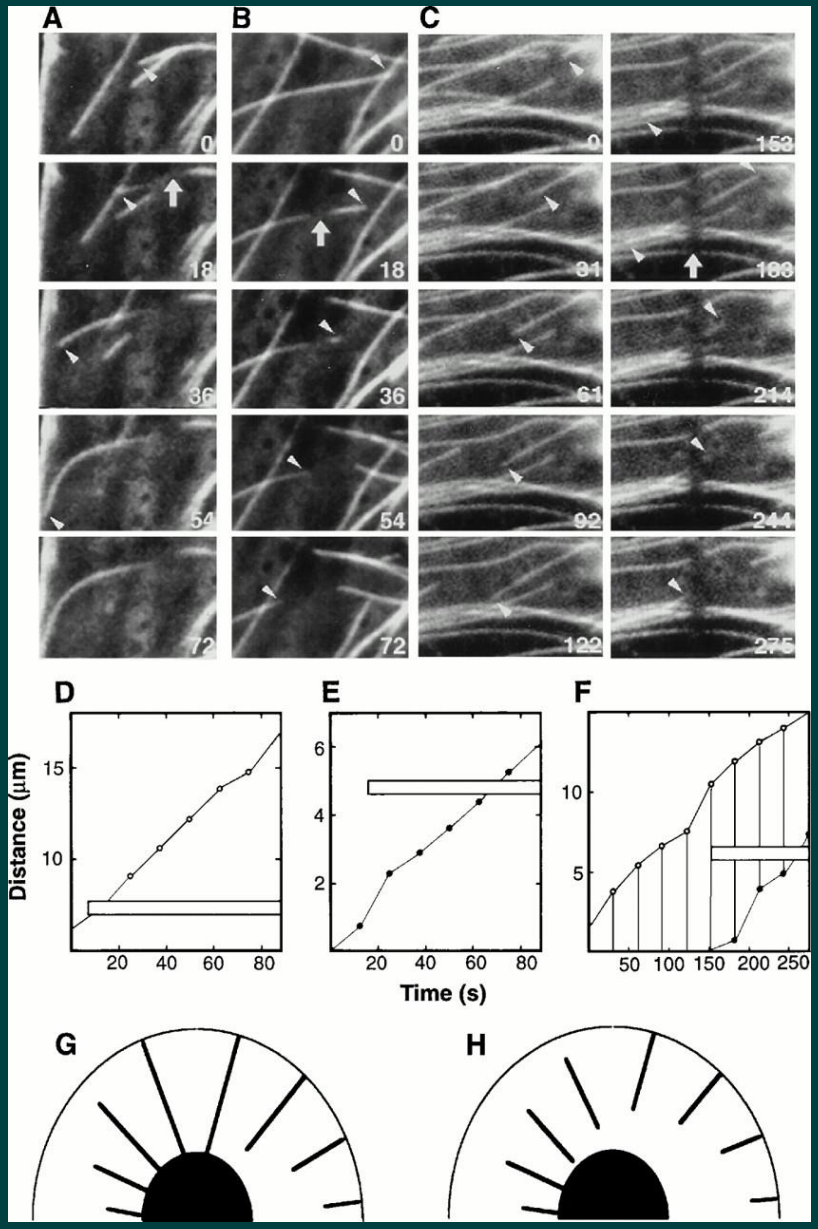


labeling part of the filament for fluorescence microscopy:

photobleacing (FRAP: fluorescenece recovery after photobleaching), photoactivation and their modifications (e.g. FLAP)

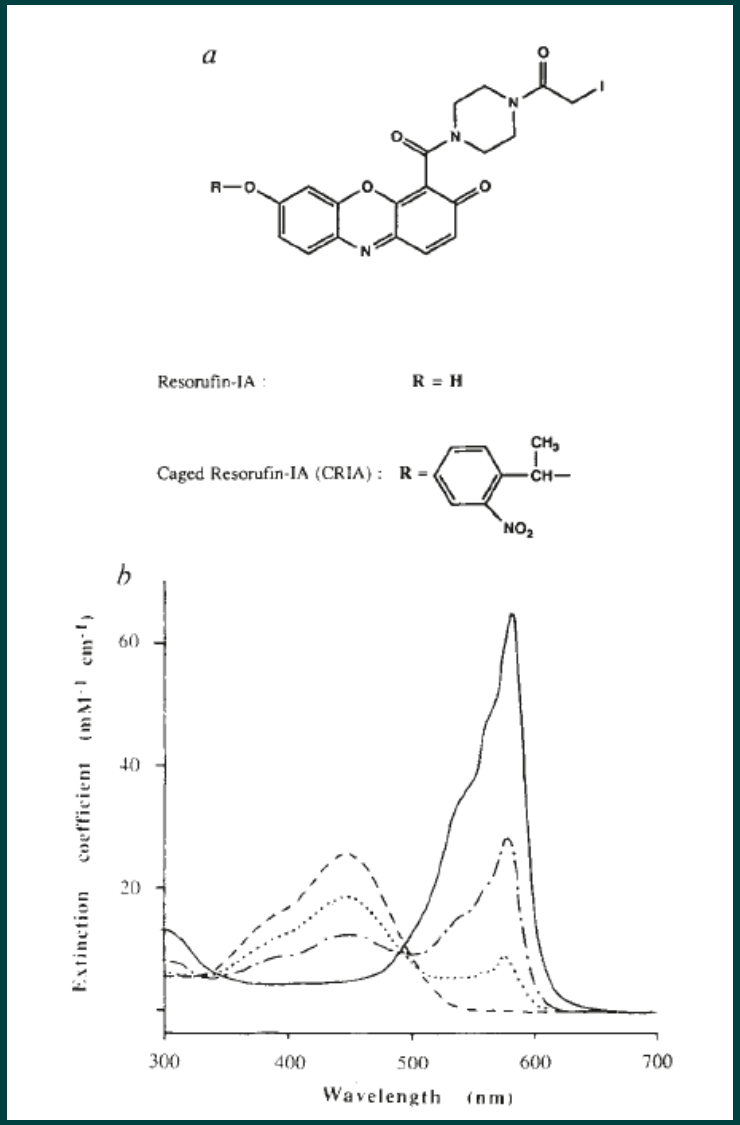
fluorescent speckle microscopy (FSM) including single molecule speckling

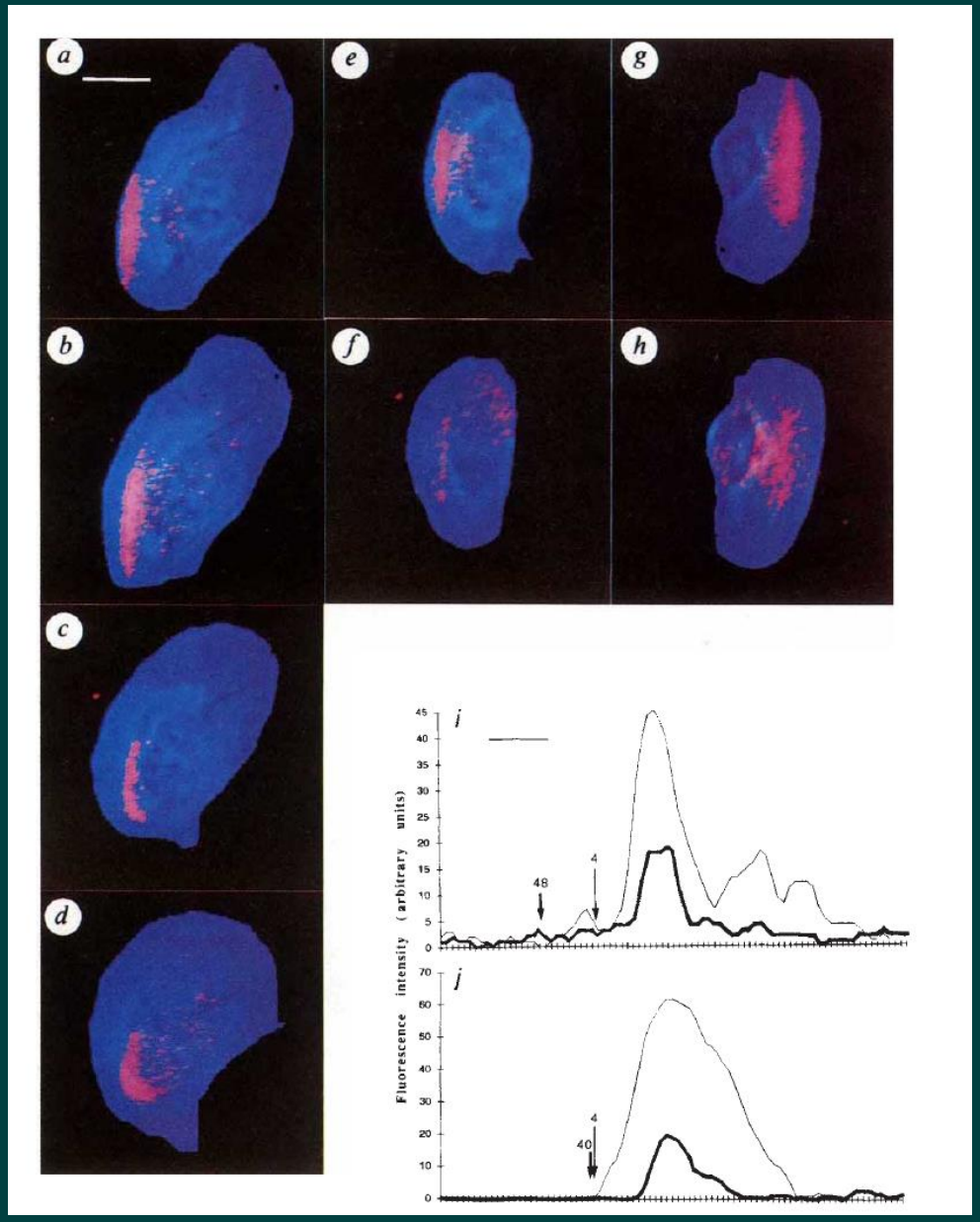
Microtubule treadmilling demonstrated with photobleaching



From Rodionov and Borisy, 1997, 1999

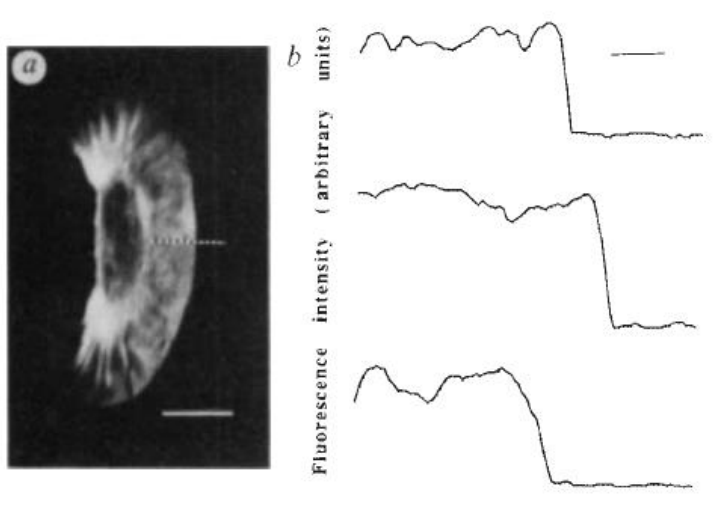
Photoactivation – actin stationary as the cell is moving





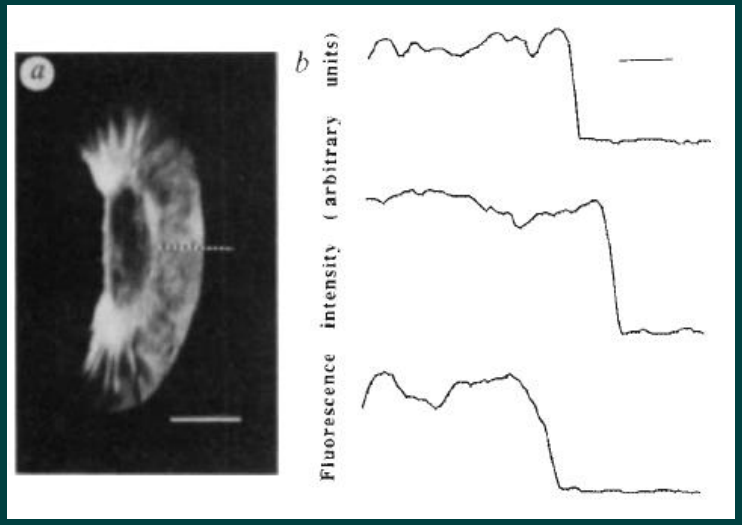
Theriot and Mitchison, Nature, 1991

Where is the assembly happennig?



Theriot and Mitchison, 1991:
actin concentration profile is flat,
disassembly is everywhere
(photoactivated mark disappears),
consequently…

Where is the assembly happennig?



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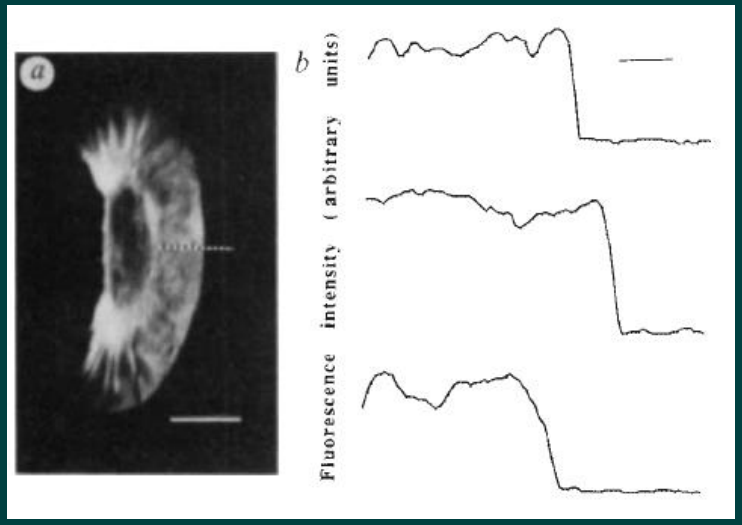

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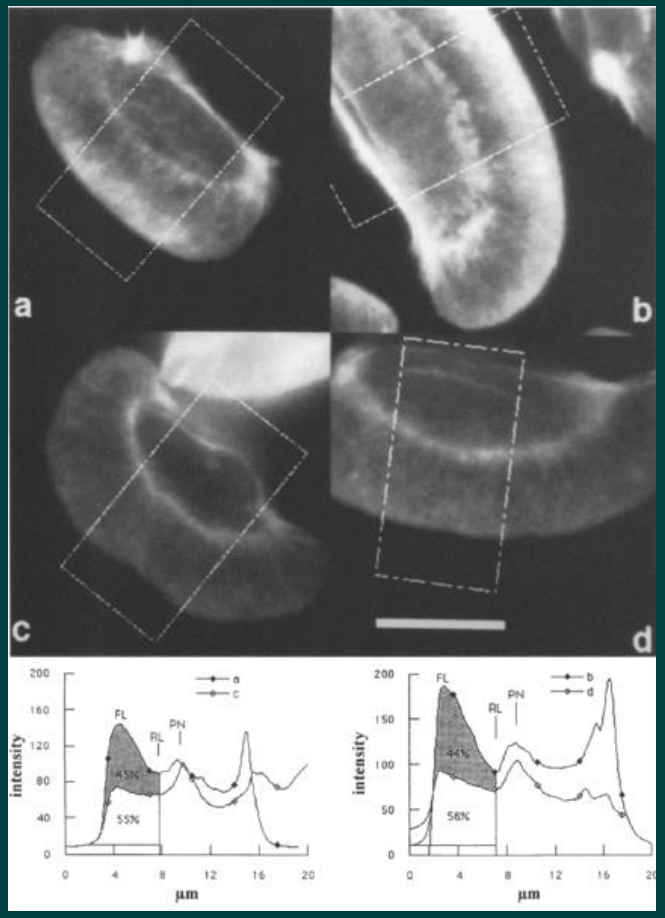
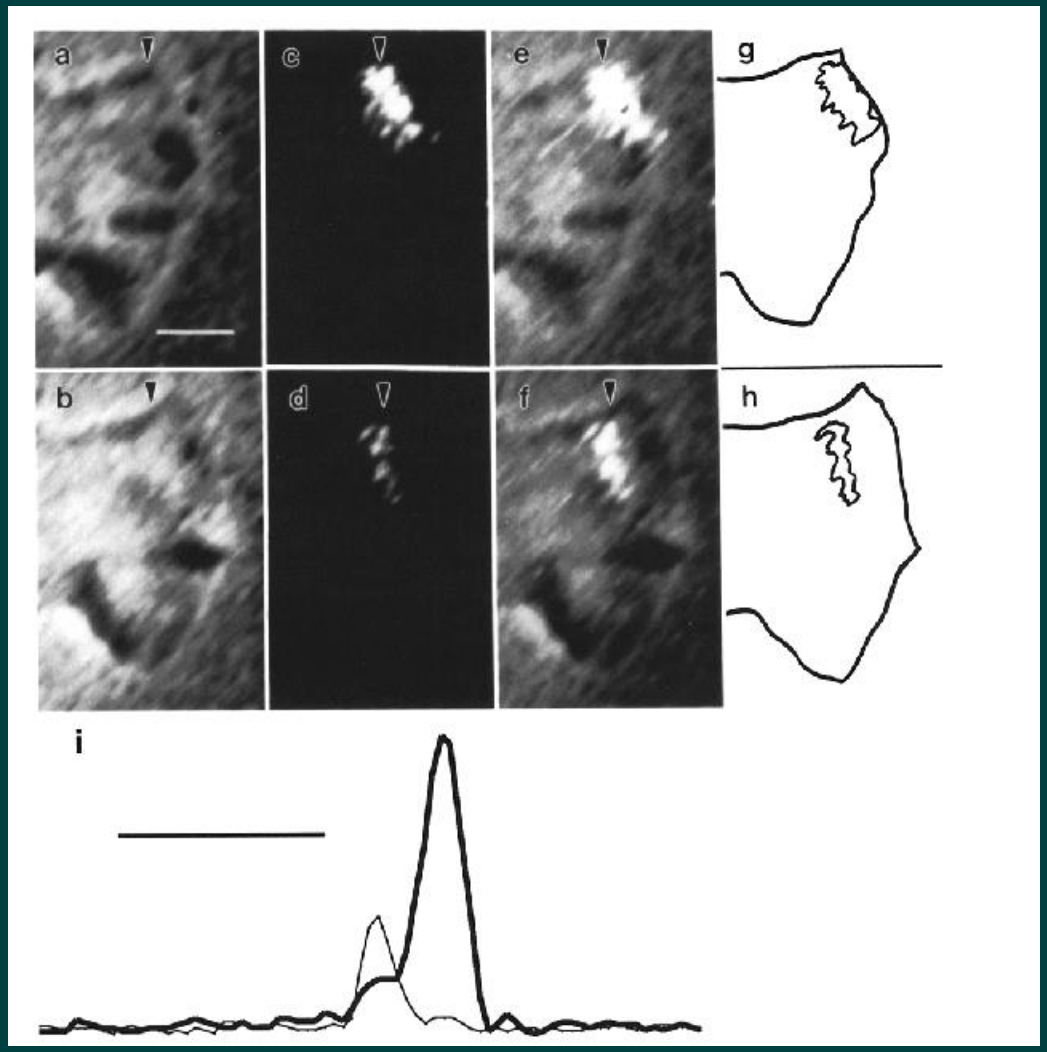


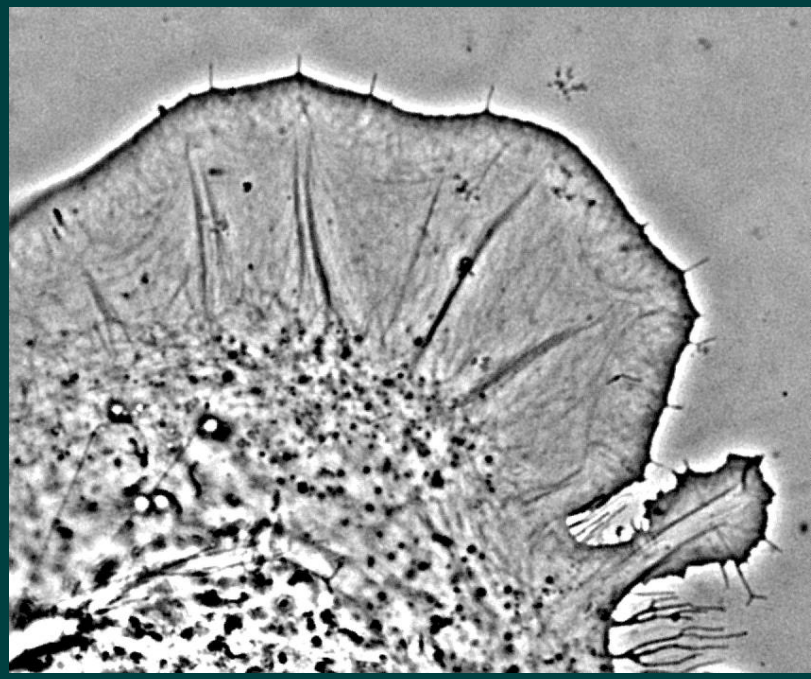
Small et al., 1995:
wrong, actin profile is not flat,
but steeply decreasing from the edge

Photoactivation: actin transport and disassembly in fibroblasts

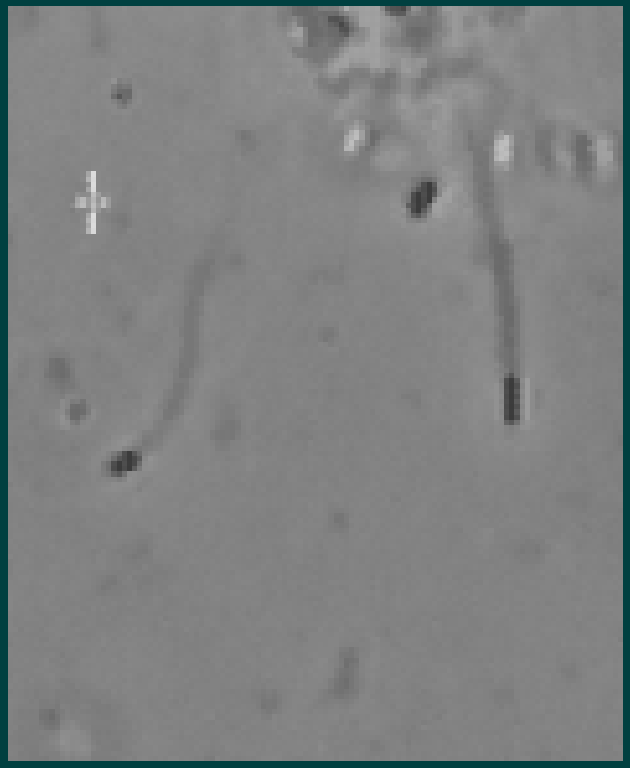


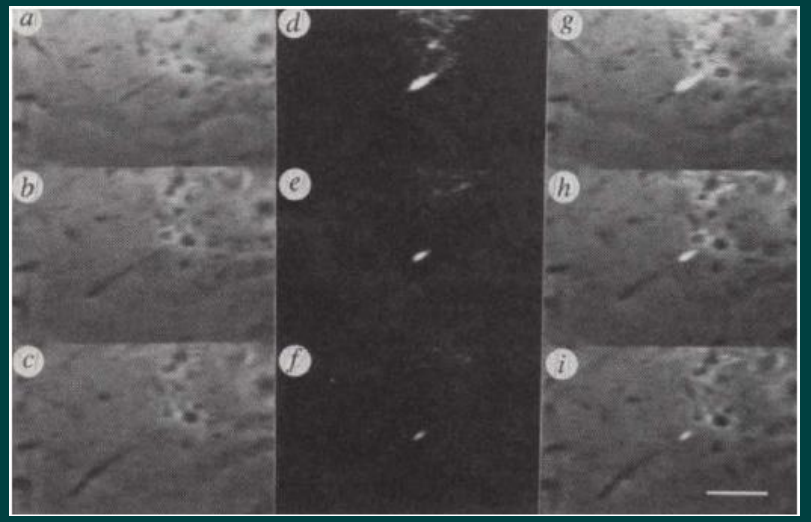
Theriot and Mitchison, 1992

Enhanced phase contrast



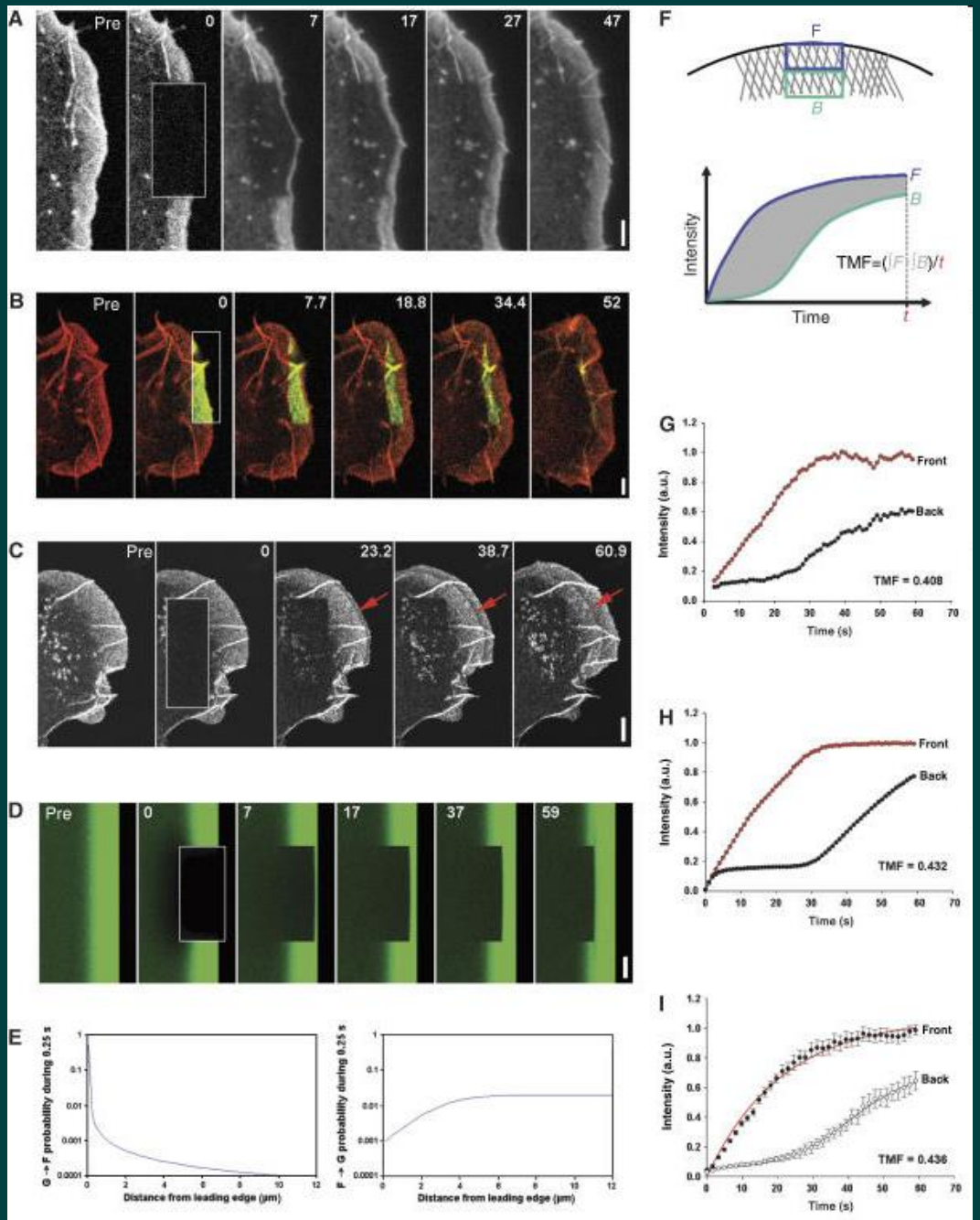
Actin tails of intracellular pathogen Listeria:
actin is stationary as the tail grows (similar to the leading edge)





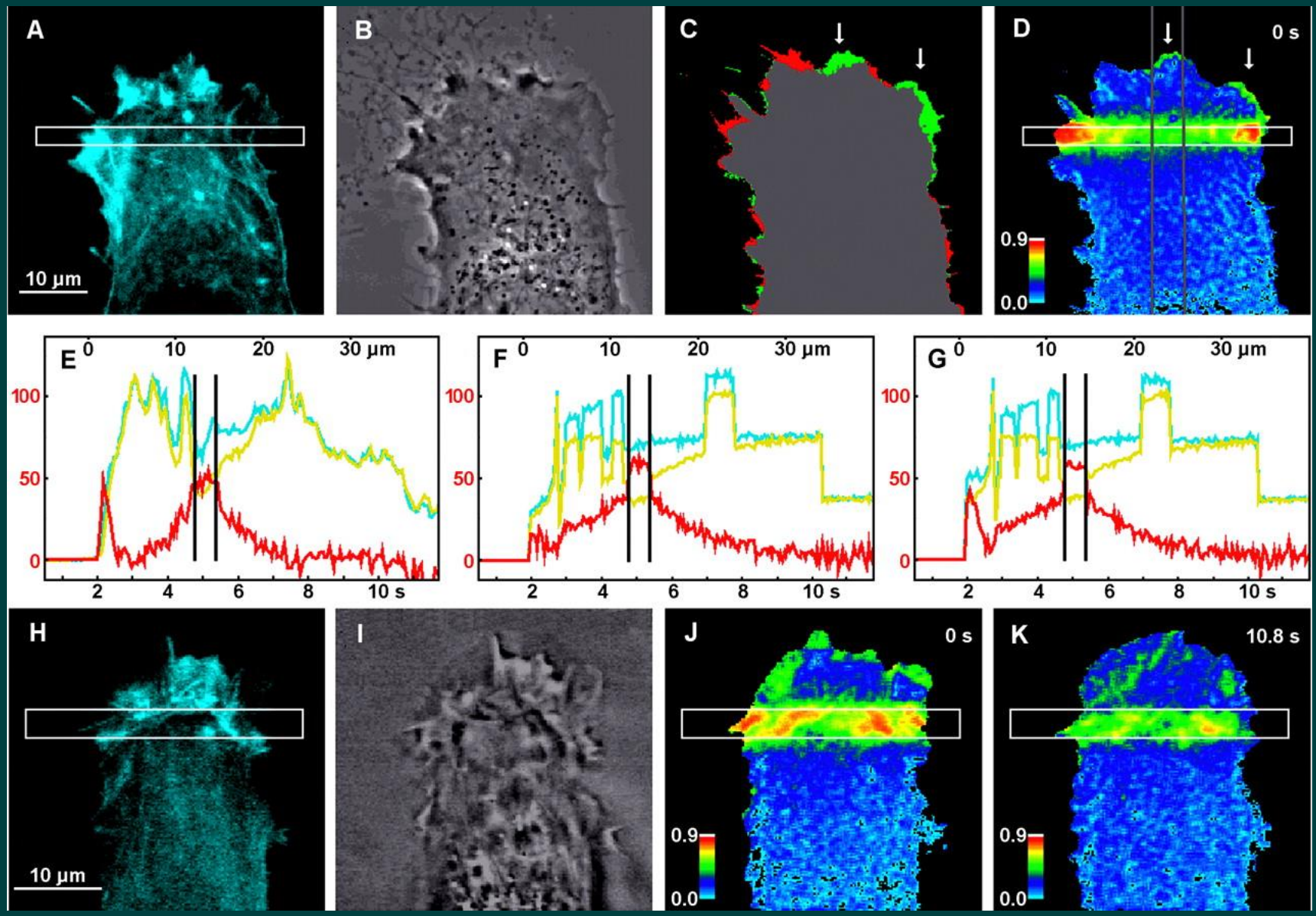
Photoactivation, Theriot et al., 1992

Quantification of FRAP shows predominant assembly at the edge



Lai et al., 2008

Fluorescence localization after photobleaching



Zicha et al., 2003

next time:

fluorescence speckling microscopy

evidence that actin assembly drives cell protrusion

mechanism of actin assembly at the cell edge: how does it go as fast?