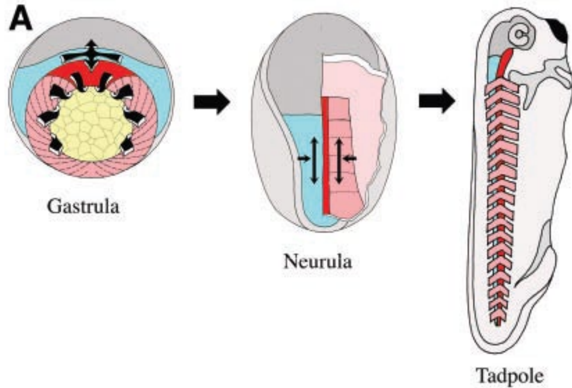
TIRF imaging of Cortical Actin in *Xenopus Laevis* Embryos

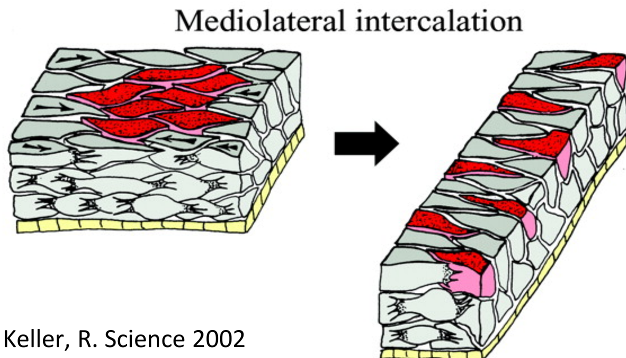
Katherine Pfister
Department of Cell Biology-
MCDB Graduate Program
Keller Lab
BIOL 5070

Background:

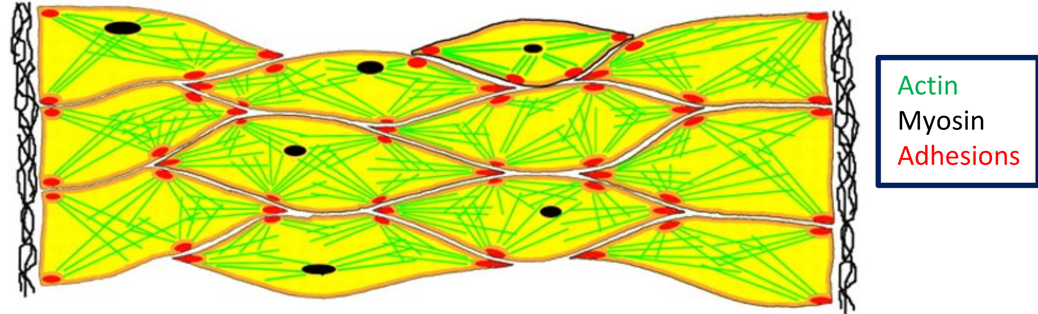
Convergence and Extension



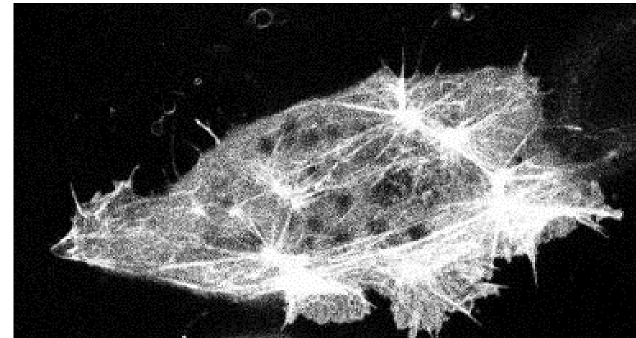
In vertebrates, axial extension takes place through a process called Convergence and Extension, where a field of presumptive axial cells converge in the Medial-Lateral direction and extend in the Anterior-Posterior direction. This process is driven by Mediolateral Intercalation behavior



Keller, R. Science 2002



Mediolateral Intercalation behavior is made possible by reorganization of the cortical actin cytoskeleton, a meshwork several microns thick that lies directly underneath the plasma membrane of these presumptive axial cells. The cortical actin network is reorganized by the activity of myosin motors which exist in node-like structures in the cortex.

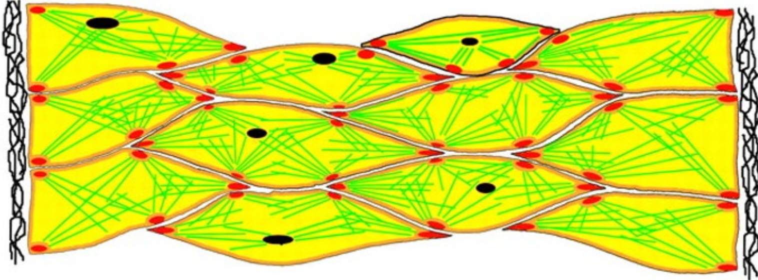


GFP Moesin-labeled Actin, 60x LSCM

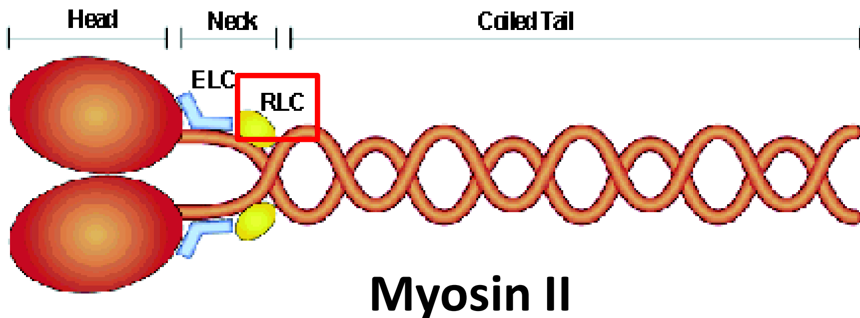
Skoglund et al. Development 2008

Background:

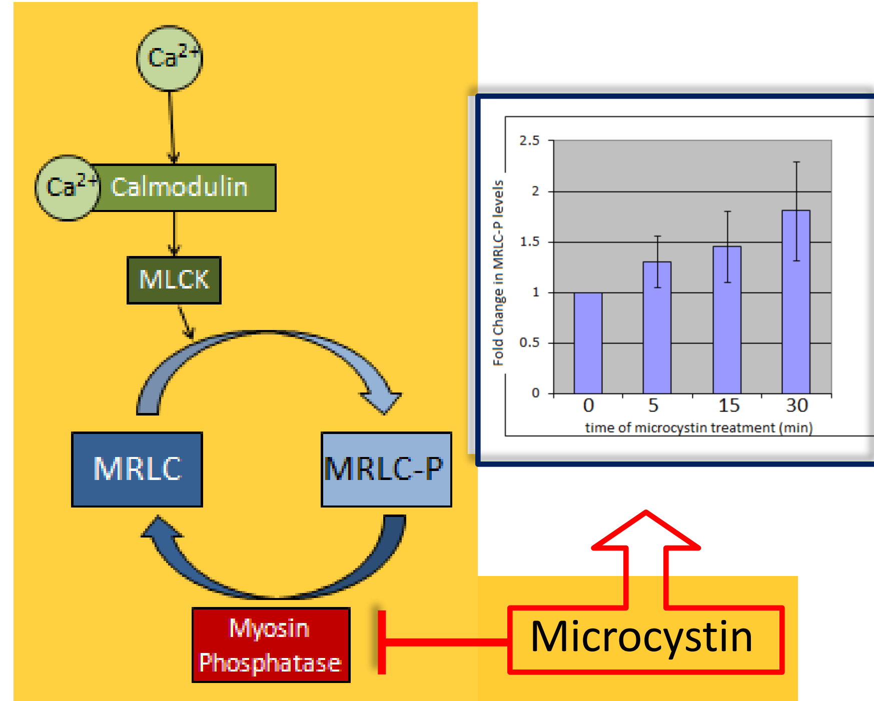
Myosin Motors



Nonmuscle Myosin II B activity crosslinks and allows for contractions of the cortical actin network. Specifically, the activity of the Regulatory Light Chain (through a cycle of phosphorylation and dephosphorylation) enables medial-lateral protrusive activity and mediates Convergence and Extension movements.



Myosin II



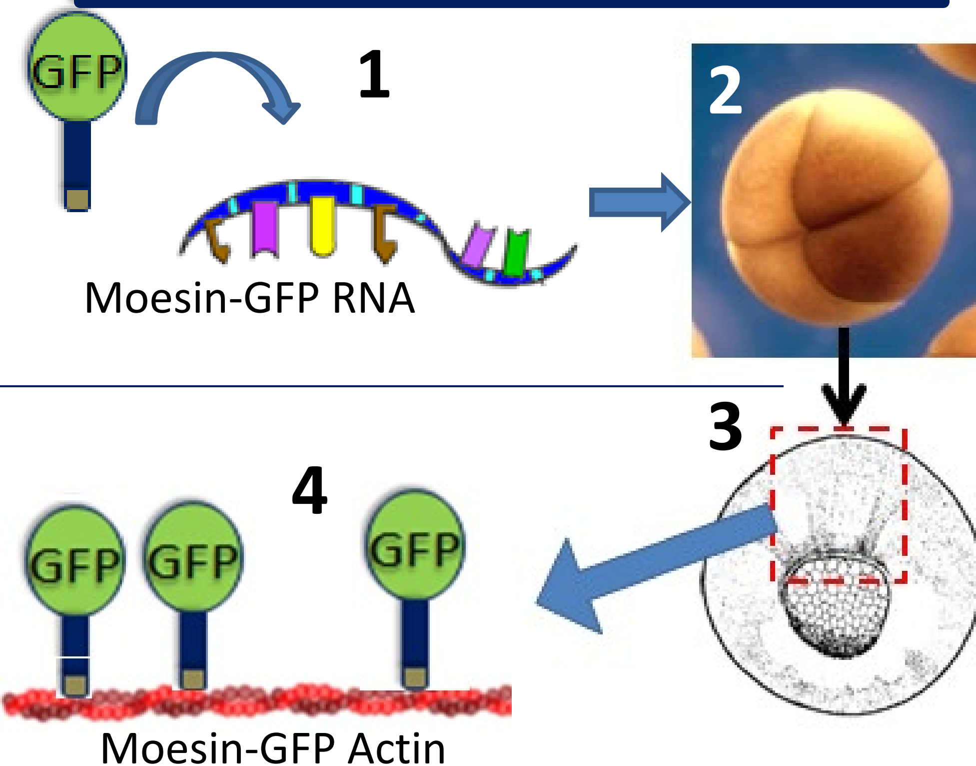
Microcystin is a potent inhibitor of Myosin Phosphatase activity, preventing the proper cycling of phosphorylated and unphosphorylated Regulatory Light Chain that allows for contraction of the cortical actin network

http://edoc.hu-berlin.de/dissertationen/kabaeva-zhyldyz-2002-11-11/HTML/kabaeva-ch1.html

AIM:

To visualize the Cortical Actin Network of Converging and Extending cells to better understand how modifications in actin can affect axis extension

Experimental Design:



1: Moesin GFP RNA construct:
C-terminal Actin binding domain,
N- terminal GFP label

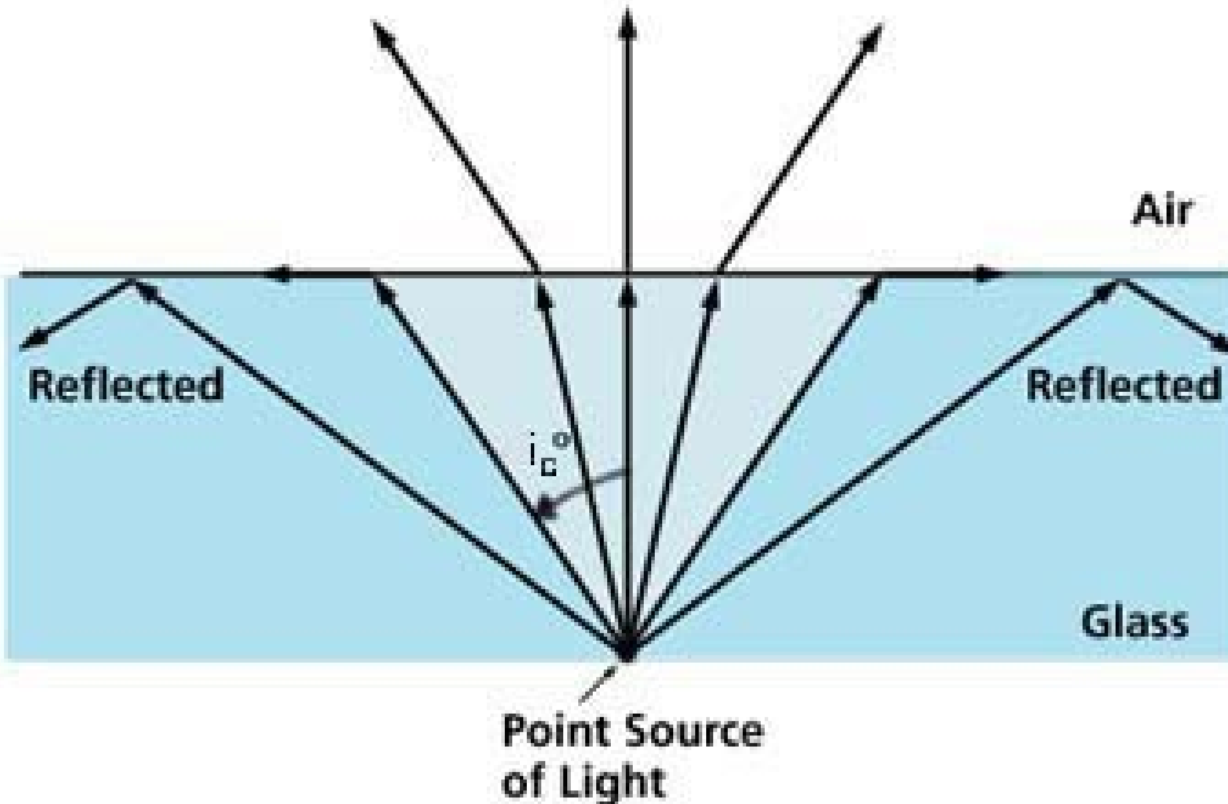
2: inject RNA into early cleavage-stage *Xenopus Laevis* embryos

3: at gastrula stages of development (~10hr after step 2), cut and culture explants of presumptive axial tissue

4: image actin decorated with GFP-Moesin, treat Experimentals with Microcystin during imaging

Microscopy Techniques

TIRFM: Total Internal Reflection Microscopy



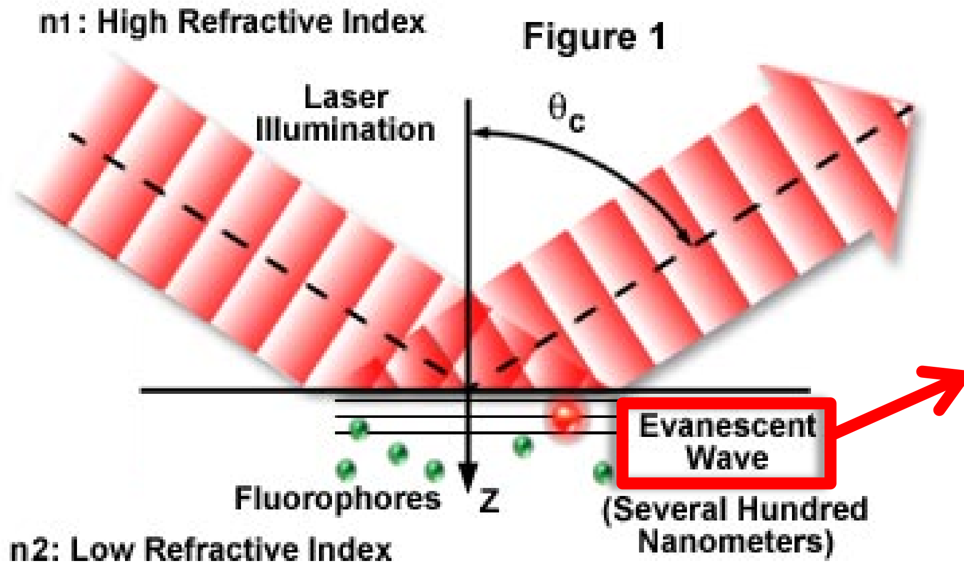
At an interface of Higher Refractive Index→ Lower Refractive Index, light bends **away** from the normal

At angles larger than the **Critical Angle**, all of the light is reflected off the interface back in to the Higher Refractive Index Media

http://www.materials.co.uk/refractive.htm

Microscopy Techniques

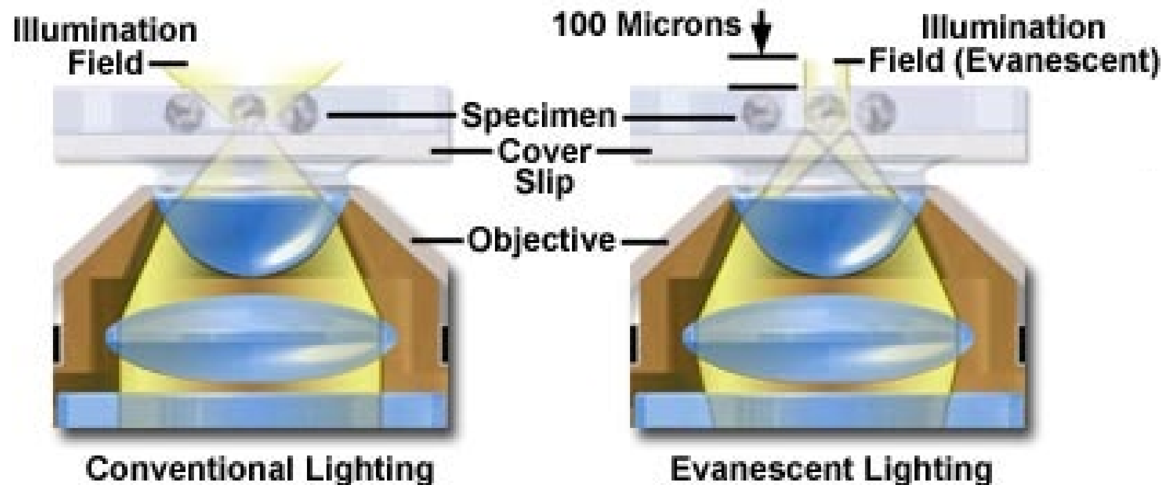
TIRFM: Total Internal Reflection Microscopy



Reflection of laser light off the glass/media interface generates an **Evanescent Wave** from laser energy dissipating at point of reflection

This is what excites the fluorophore molecules of the sample

*Evanescent wave decays exponentially away from point of reflection (~150nm)



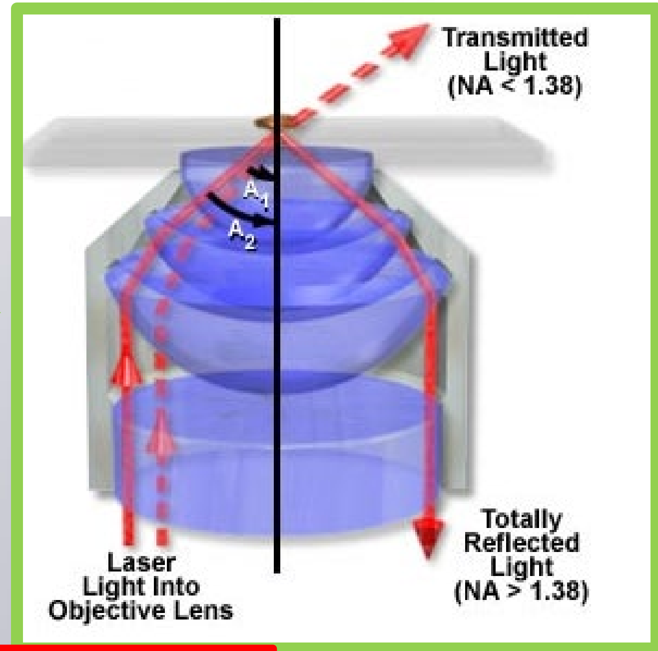
www.olympusmicro.com

Microscopy Techniques

TIRFM: Total Internal Reflection Microscopy

Evanescent wave

Objective



Micrometer

Field stop

Fiber

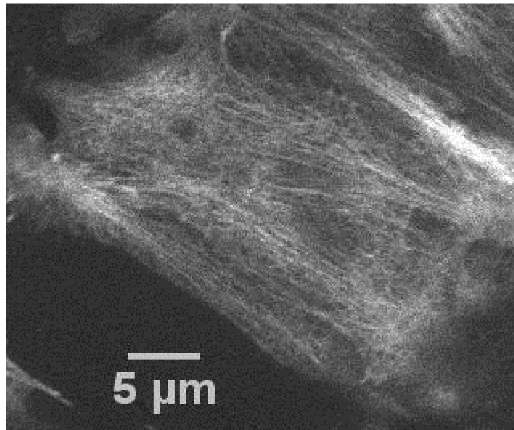
Laser

A **micrometer** mechanically changes the incident angle of laser beam through microscope optics

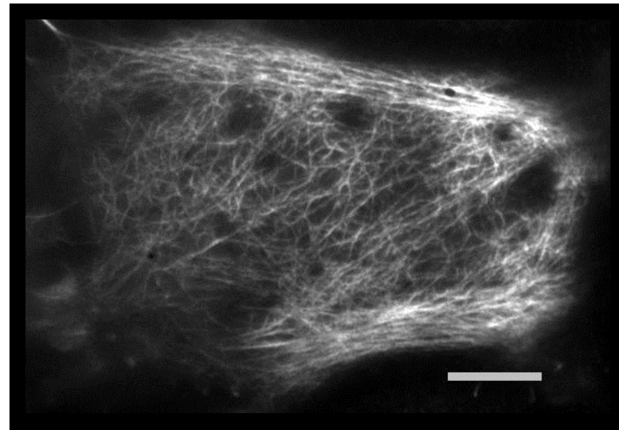
www.olympusmicro.com

Results:

Confocal vs TIRF



LSCM



TIRFM

TIRF only excites fluorophores within the first 100nm of the sample, reducing background; and smaller optical section allows for better resolution than traditional Laser Scanning Confocal Microscopy

Microscope Specifications:

	LSCM	TIRFM
Objective Magnification	60x Plan-Apo	60x Plan-Apo
N.A.	1.4	1.45
Lens Type	Oil-immersion	Oil-immersion: TIRF specific oil
Oil Refractive Index	1.48-1.5	1.515

Results:

Time-Lapse of Untreated Cell-TIRFM

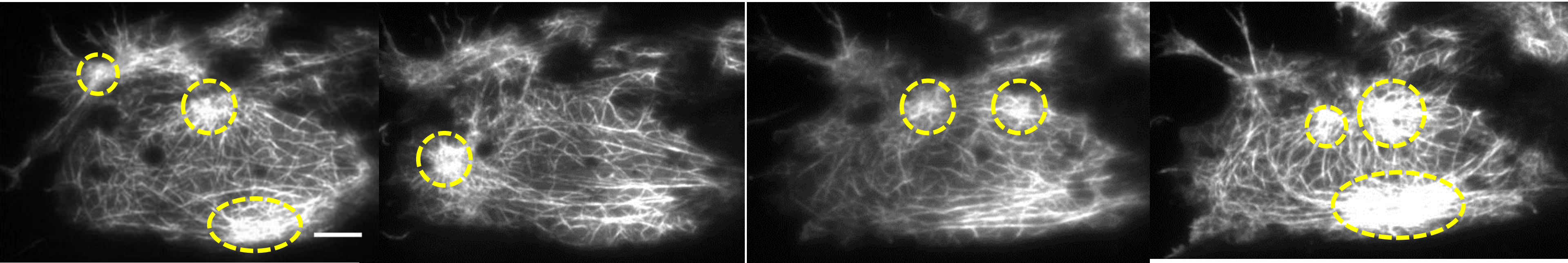
5 µm
0'00" 0'20" 0'40" 1'00" 1'20" 1'40"
2'00" 2'20" 2'40" 3'00" 3'20" 3'40"

Time-Lapse of Microcystin treated Cell-TIRFM

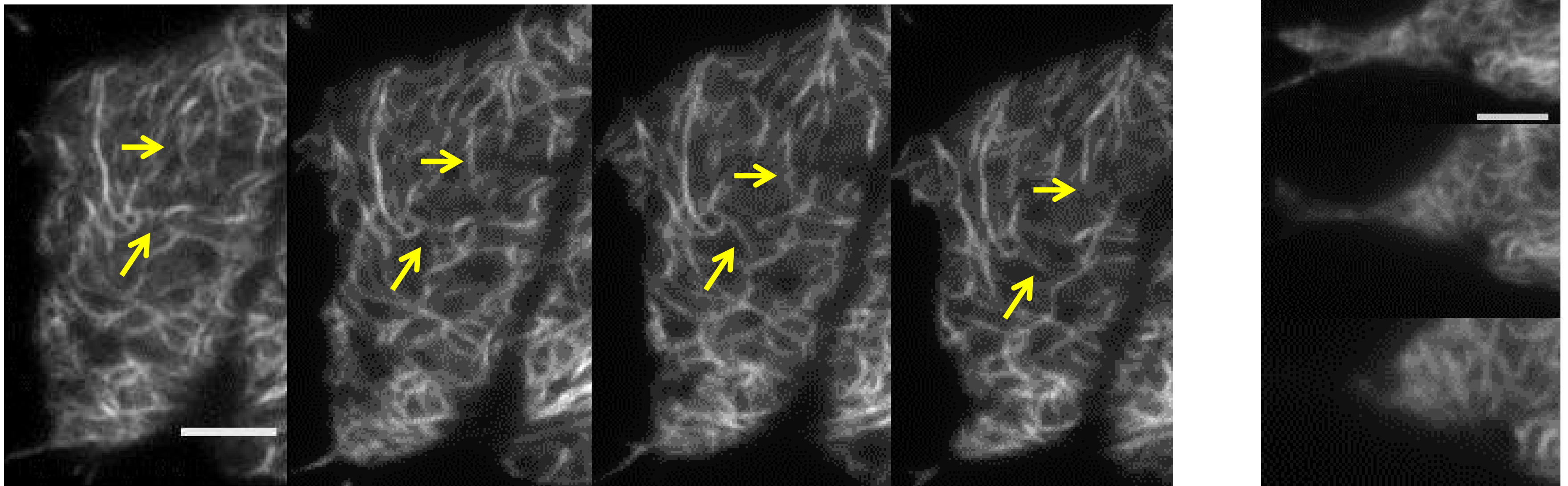
5 µm
0'00" 0'20" 0'40" 1'00" 1'20" 1'40"
2'00" 2'20" 2'40" 3'00" 3'20" 3'40"

Results:

Close-up: **Control Cells** exhibit dynamic Node and Cable structure in Cortical Actin Network



Close-up: **Experimental Cells** exhibit breaking of actin cables and loss of tension in Cortical Actin Network



Discussion:

-TIRFM provides a system for higher resolution imaging of cortical actin dynamics in Converging and Extending axial tissue, as compared to traditional Laser Scanning Confocal Microscopy

-Proper cycling of phosphorylated/unphosphorylated Myosin Regulatory Light Chain is necessary for maintaining the dynamic node and cable meshwork of the cortical actin network

-when this cycling is disturbed by a Myosin Phosphatase inhibitor (microcystin), the cortical actin network loses tension and the cell contracts, preventing normal axial elongation

Acknowledgements:

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References:

R. Keller, “Shaping the Vertebrate Body Plan by Polarized Embryonic Cell Movements”, *Science, Vol. 298 pp. 1950-1954, 6 December 2002*

P. Skoglund, A. Rolo, X. Chen, B. Gumbiner, R. Keller. “Convergence and extension at gastrulation require a myosin IIB-dependent cortical actin network”, *Development vol. 135, pp. 2435-2444, 15 July 2008*