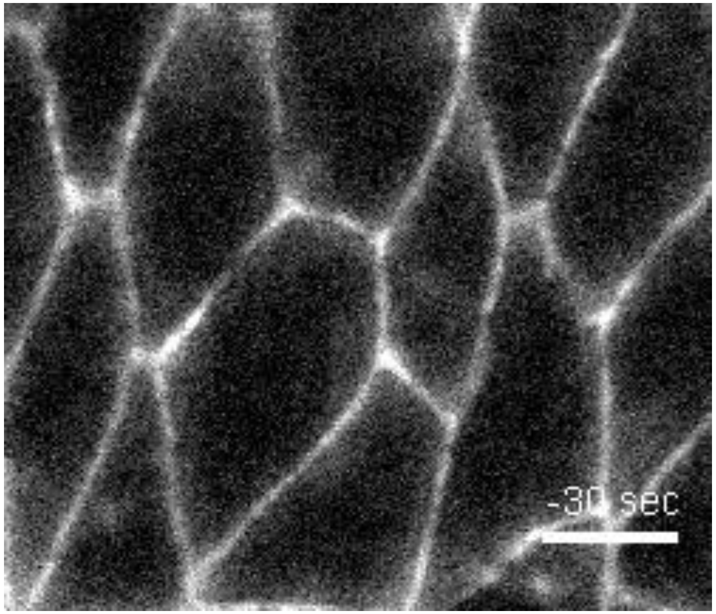
Bangalore, 15th April 2013

Laser Surgery: a powerful tool in BIOLOGY

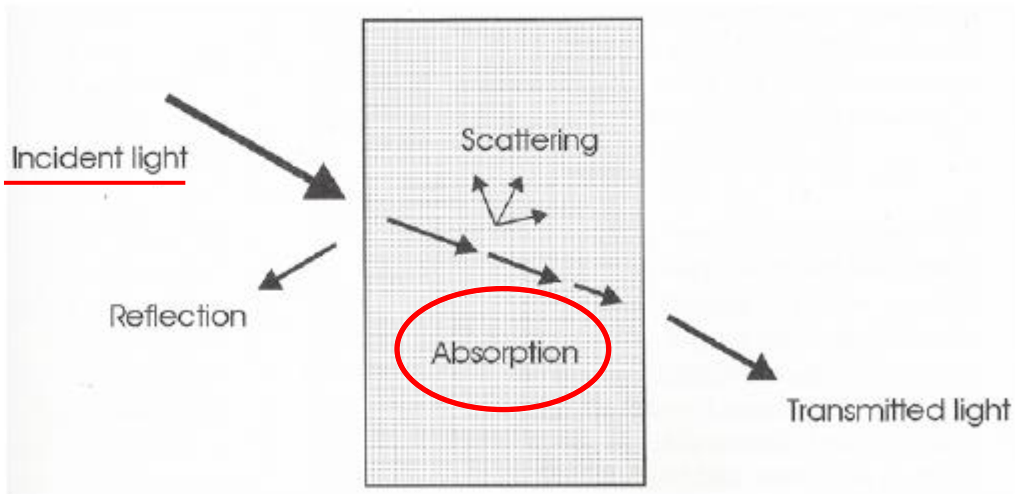
Matteo RAUZI



Rauzi et al. 2007

process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation	1030nm	200fs	50MHz	370mW	3ms	1,2NA water	65%	4nJ	150000
Photo-uncaging	1030nm	200fs	50MHz	40mW	continuous	1,2NA water	65%	0,8nJ	-

Light-matter interaction



Light source: **LASER**

Light Amplification by Stimulated Emission of Radiation

Spatial coherence

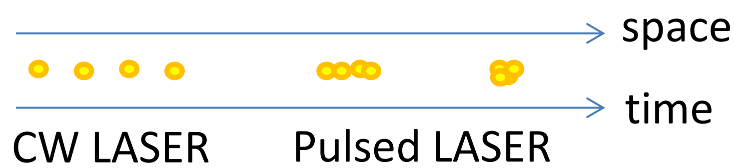
Temporal coherence

Laser-matter interaction depend on **4** main **laser parameters**

1. Power

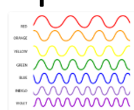
2. Power density

1. Spatial compaction of photons

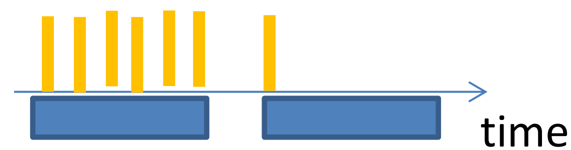


2. Temporal compaction of photons

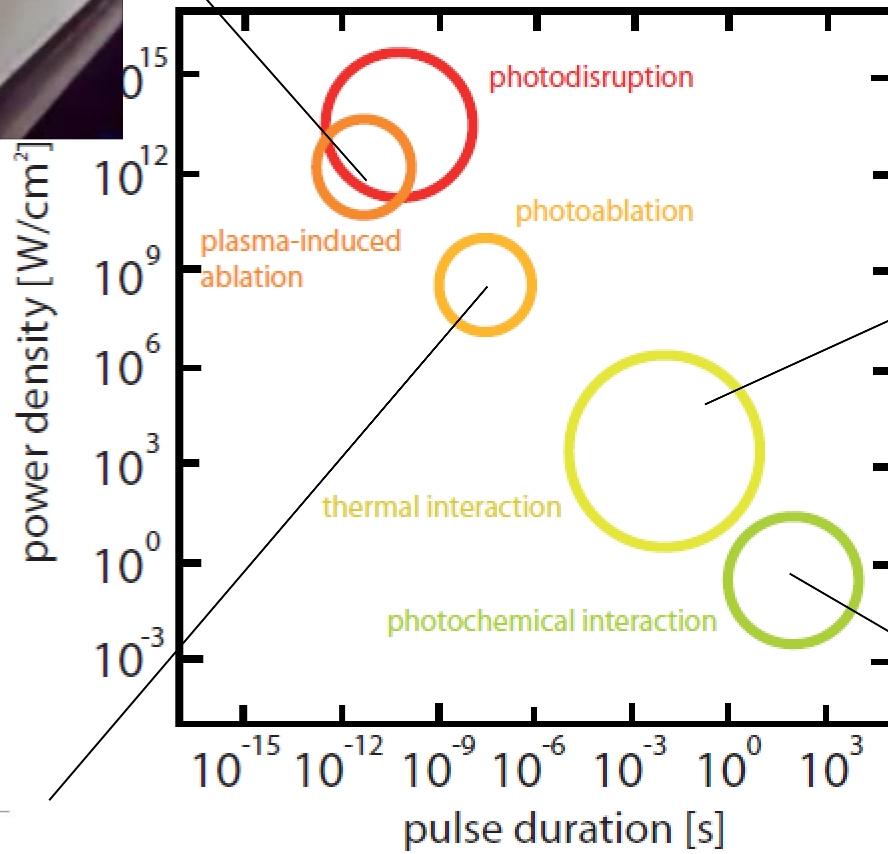
3. Wavelength



4. Pulse repetition rate



Power density and Temporal compaction of photons:
5 categories of laser-tissue interaction



Biological effect
Hyperthermia
Reduction in enzyme activity, cell immobility
Denaturation of proteins and collagen, coagulation
Permeabilization of membranes
Vaporization, thermal decomposition (ablation)
Carbonization
Melting

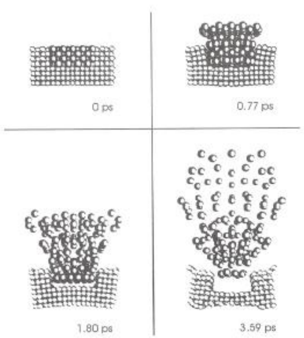


Figure 8.7: Covalent bond breaking between molecules results in an increase in volume and material etching at the surface. Adapted from [118].

Photodisruption

Plasma and Cavitation Dynamics during Pulsed Laser Microsurgery *in vivo*

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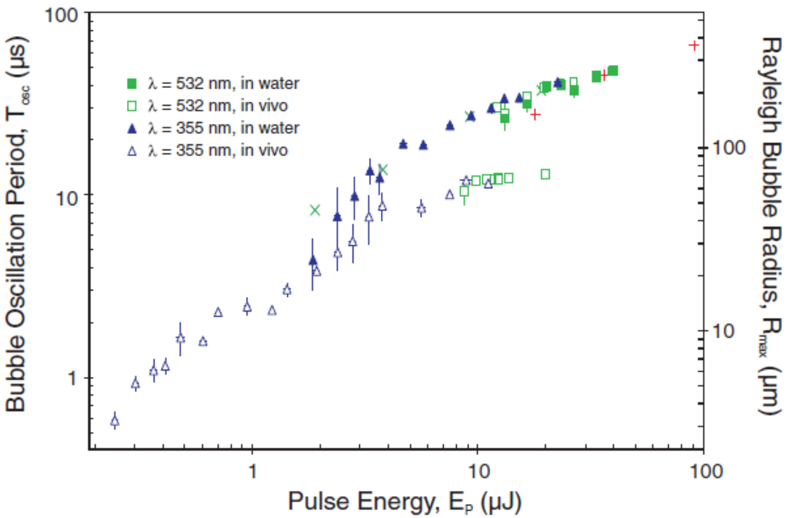
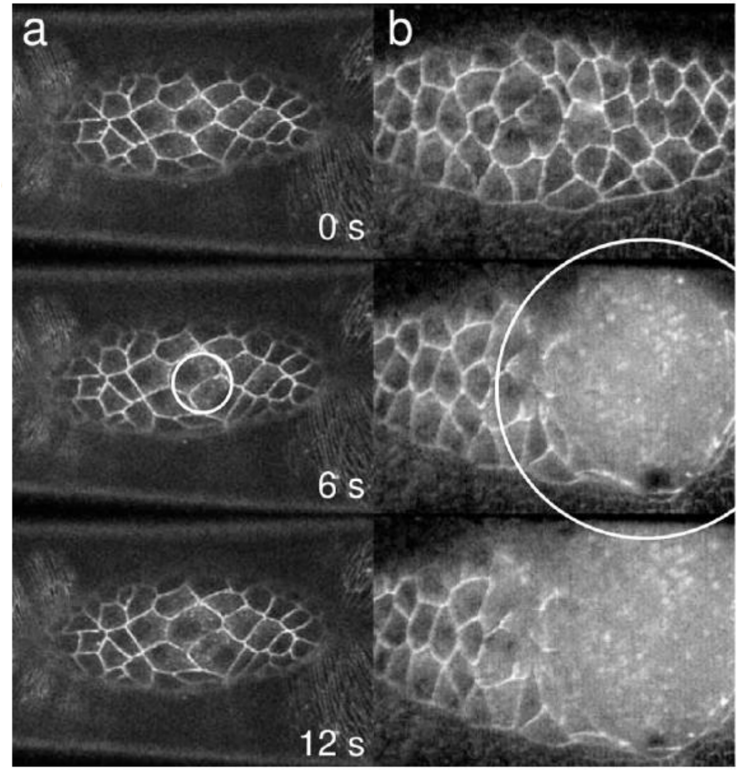
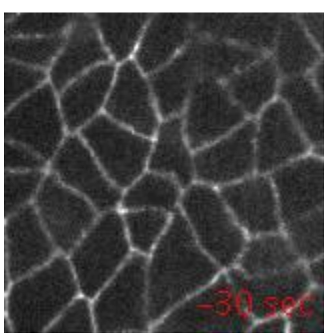
(Received 21 May 2007; published 10 October 2007)



E-cad GFP



process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective	Objective transmission	Pulse energy after the objective	Number of pulses
Ablation a	355nm	4ns	?	?	?	1,3NA oil	?	1,22µJ?	1?
Ablation b	532nm	4ns	?	?	?	1,3NA oil	?	8.26µJ?	1?



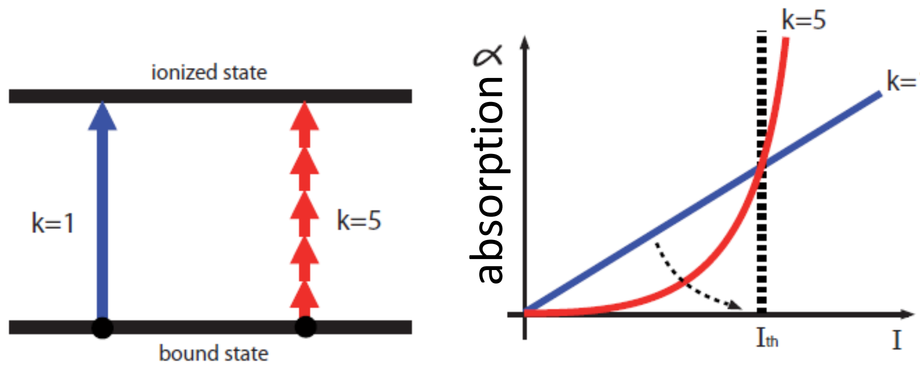
E-cad GFP

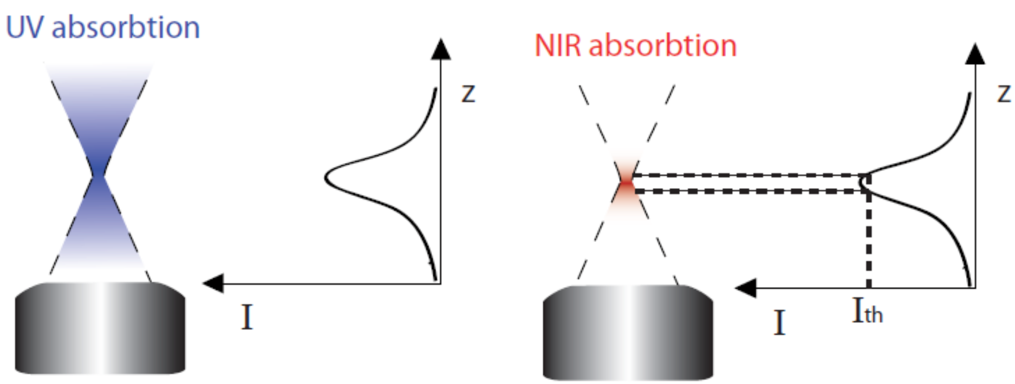
process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective	Objective transmission at 365nm	Pulse energy after the objective	Number of pulses
Ablation	365nm	3-5ns	Catalog 15 Hz	?	Deduced 0,6s	1,4NA oil	?	60µJ?	10

DevCell 2009

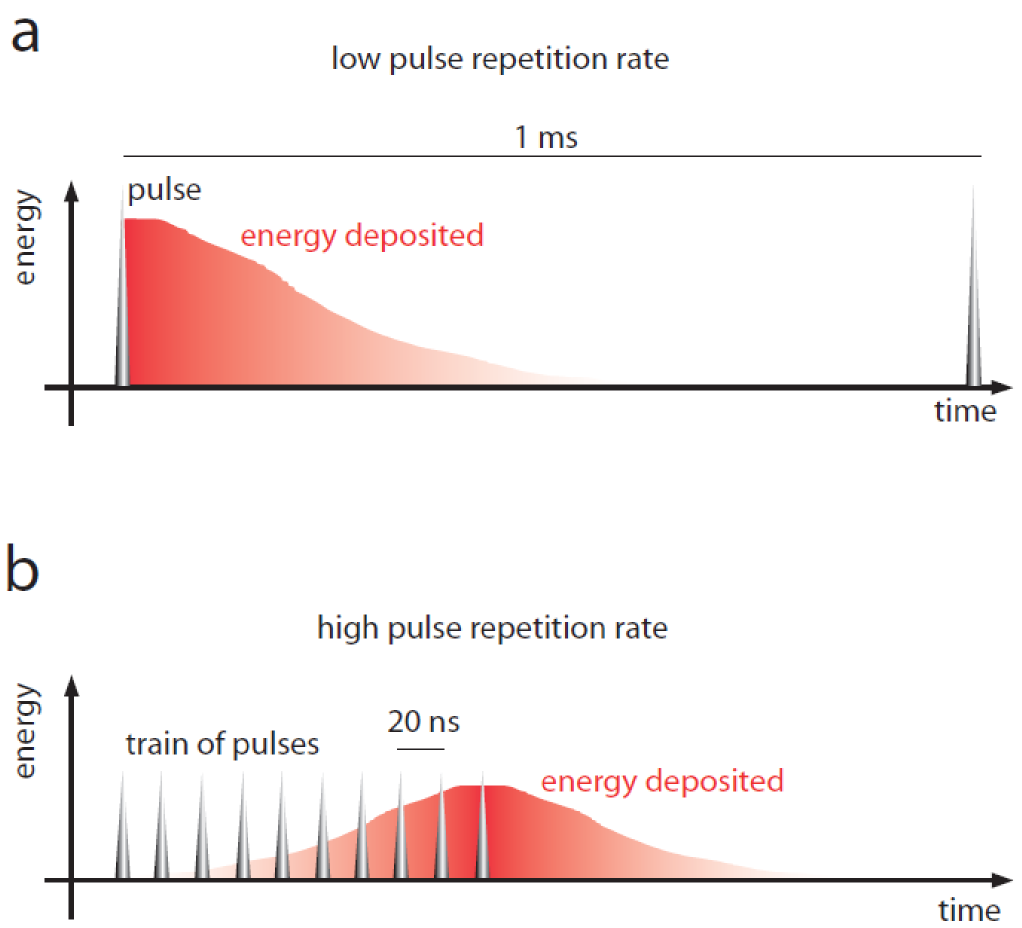
Additional collateral effects: local blackening of the vitelline membrane

UV Vs IR


ionized state
k=1
k=5
bound state
absorption ∝
k=5
k=1
Ith
I


UV absorbtion
z
I
NIR absorbtion
z
I
Ith

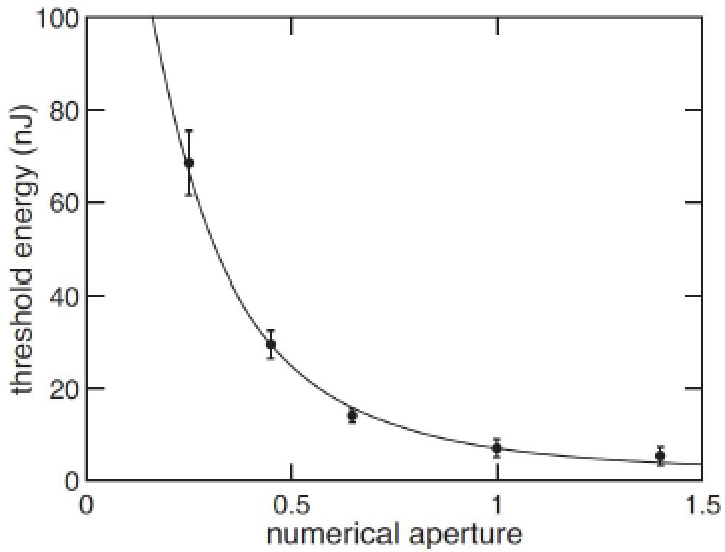
Laser Pulse repetition Rates and tissue interaction



KHz

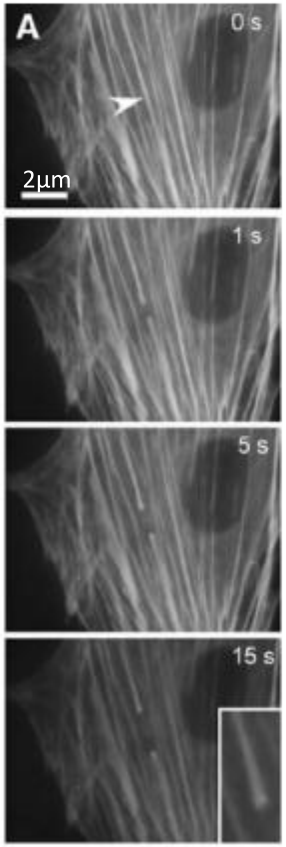
MHz

The objective NA and the **ablation threshold energy** to perform laser ablation



LASER ABLATION & BIOLOGY

Single actin bundle dissection

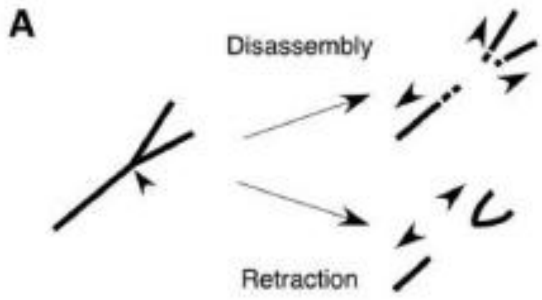


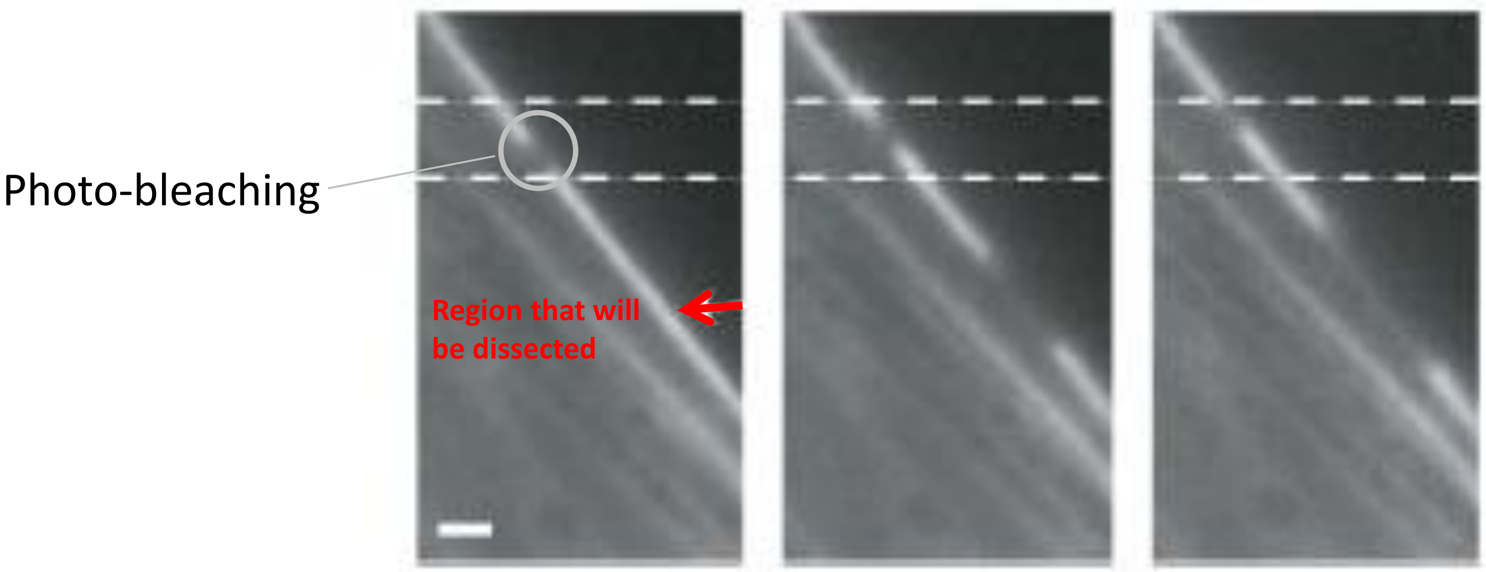
Kumar et al. 2006

Retraction or Disassembly?

process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 790nm	Pulse energy after the objective	Number of pulses
Ablation	790nm	100fs	90MHz	1,5W	170µs	1,4NA oil	?	16nJ	15300

Probing single bundle properties with punctual ablation

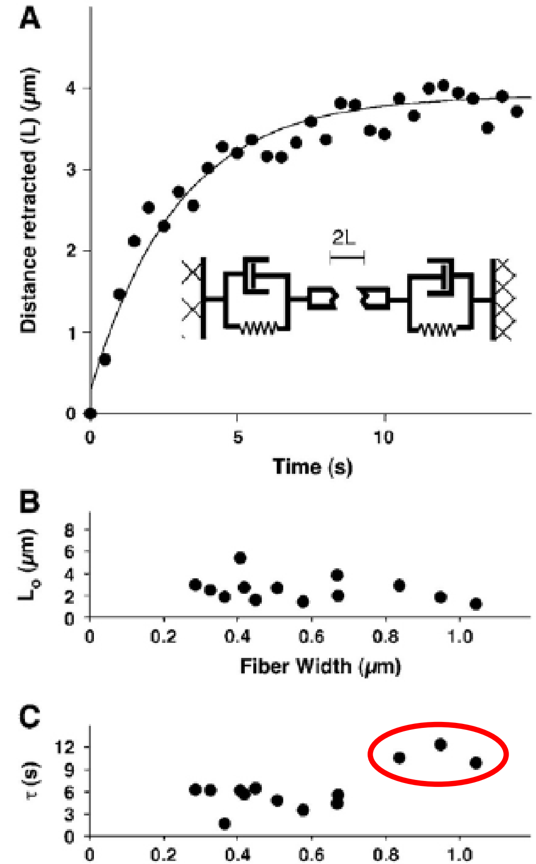


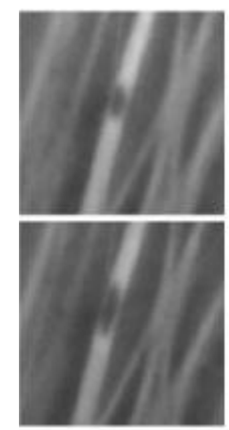


Kumar et al. 2006

Probing single bundle properties with punctual ablation

$$L = L_0(1 - e^{\frac{-t}{\tau}} + D_a)$$





$L = half\ of\ the\ gap\ length$

$L_0 = asymptotic\ length\ of\ the\ gap$

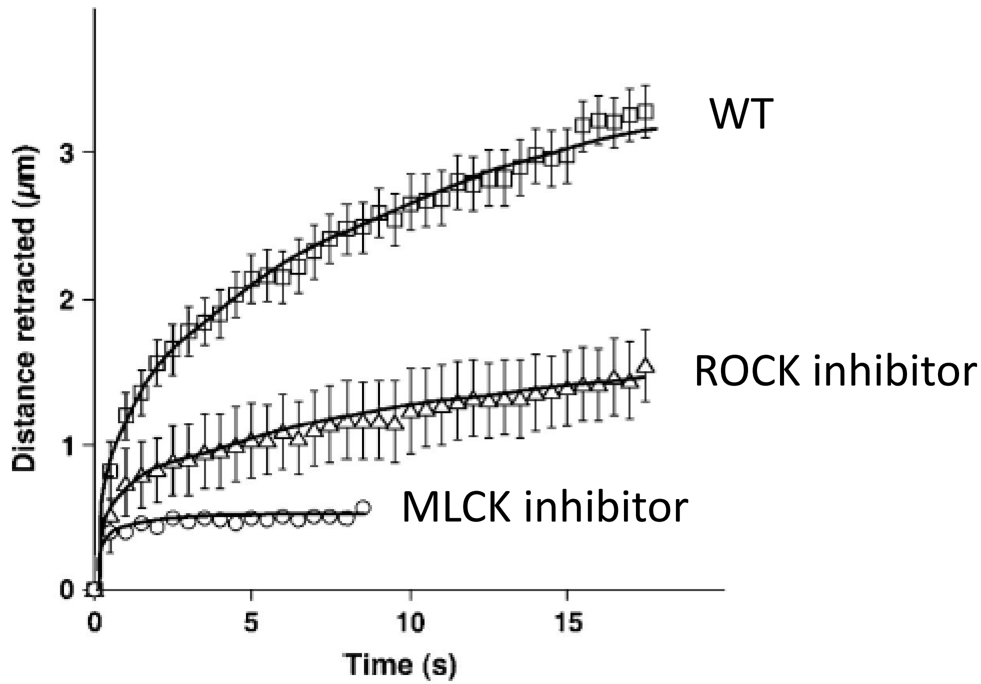
$t = time\ after\ ablation$

$\tau = ratio\ between\ viscous\ and\ elastic\ coefficients\ (Young's\ modulus)$

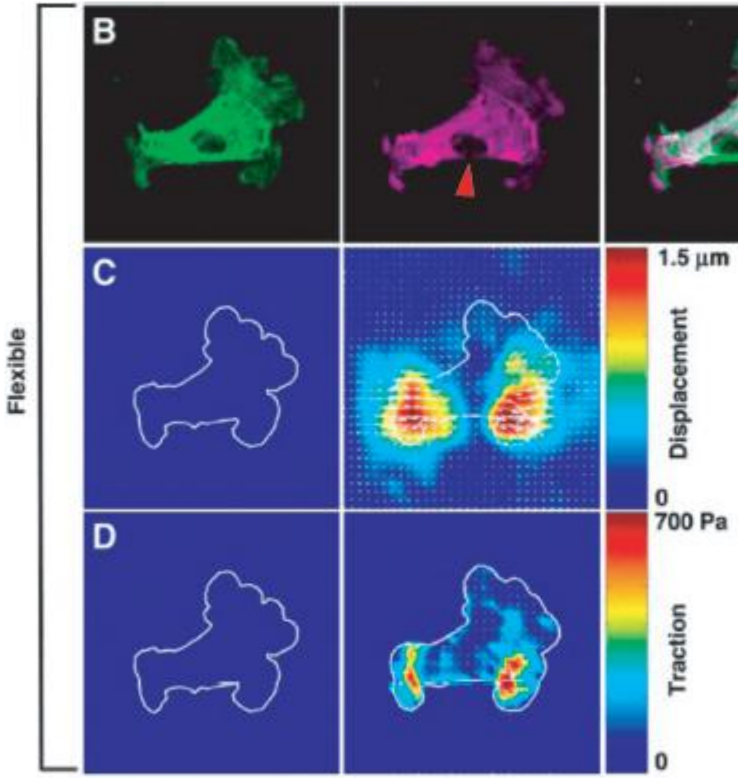
$D_a = length\ of\ the\ fiber\ immediatly\ destroyed\ upon\ irradiation$

Probing single bundle properties with punctual ablation

MyoII inhibition

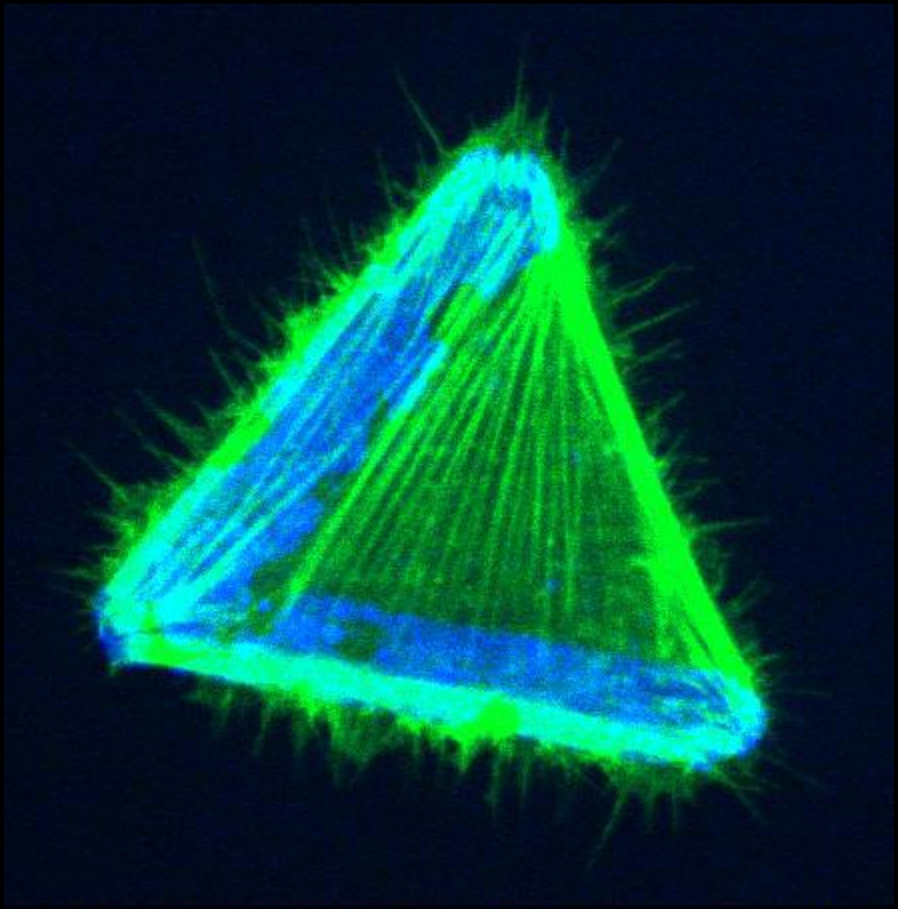


Probing single bundle force contribution to cell shape



IR fs

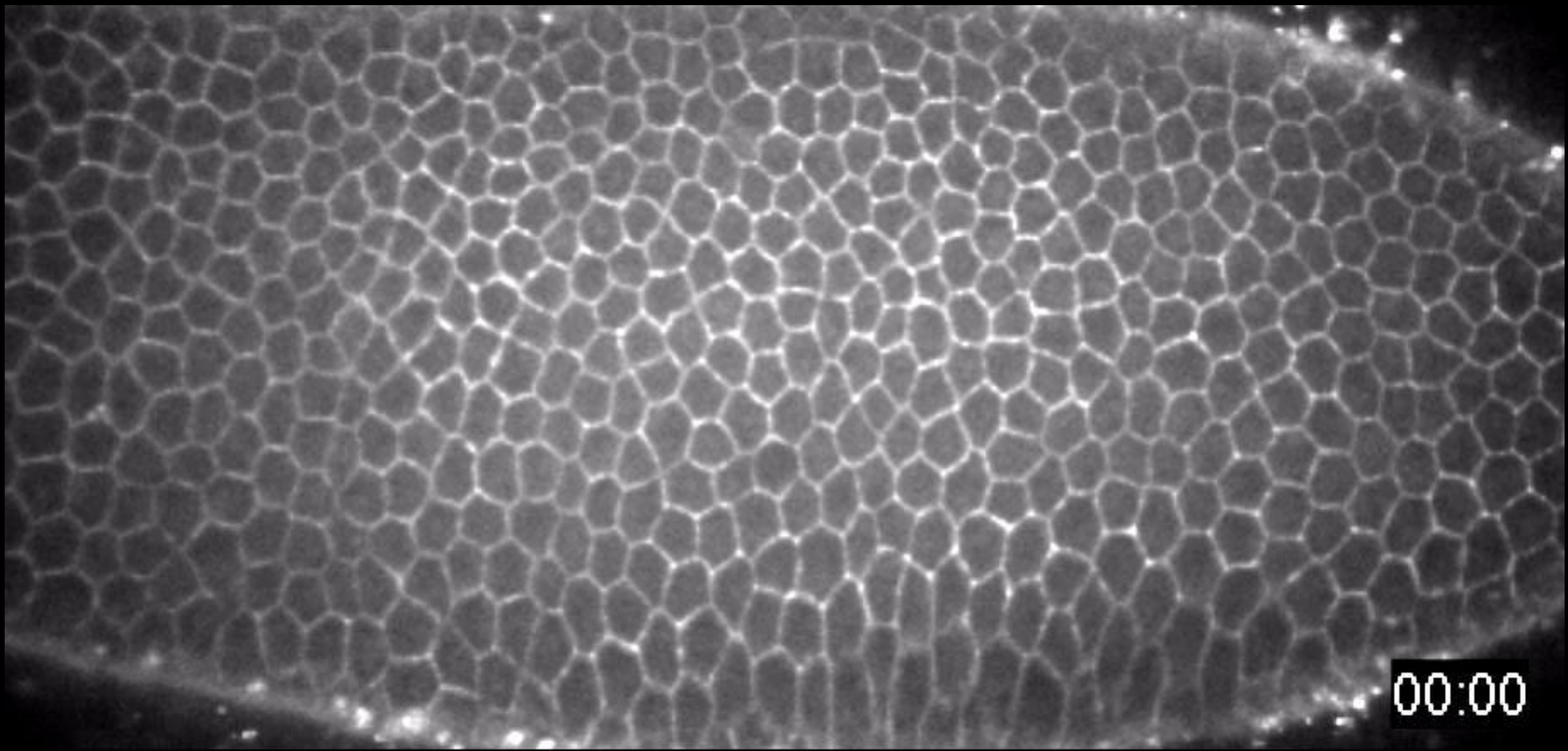
F-actin
fibronectin



in collaboration with ***M. Théry***

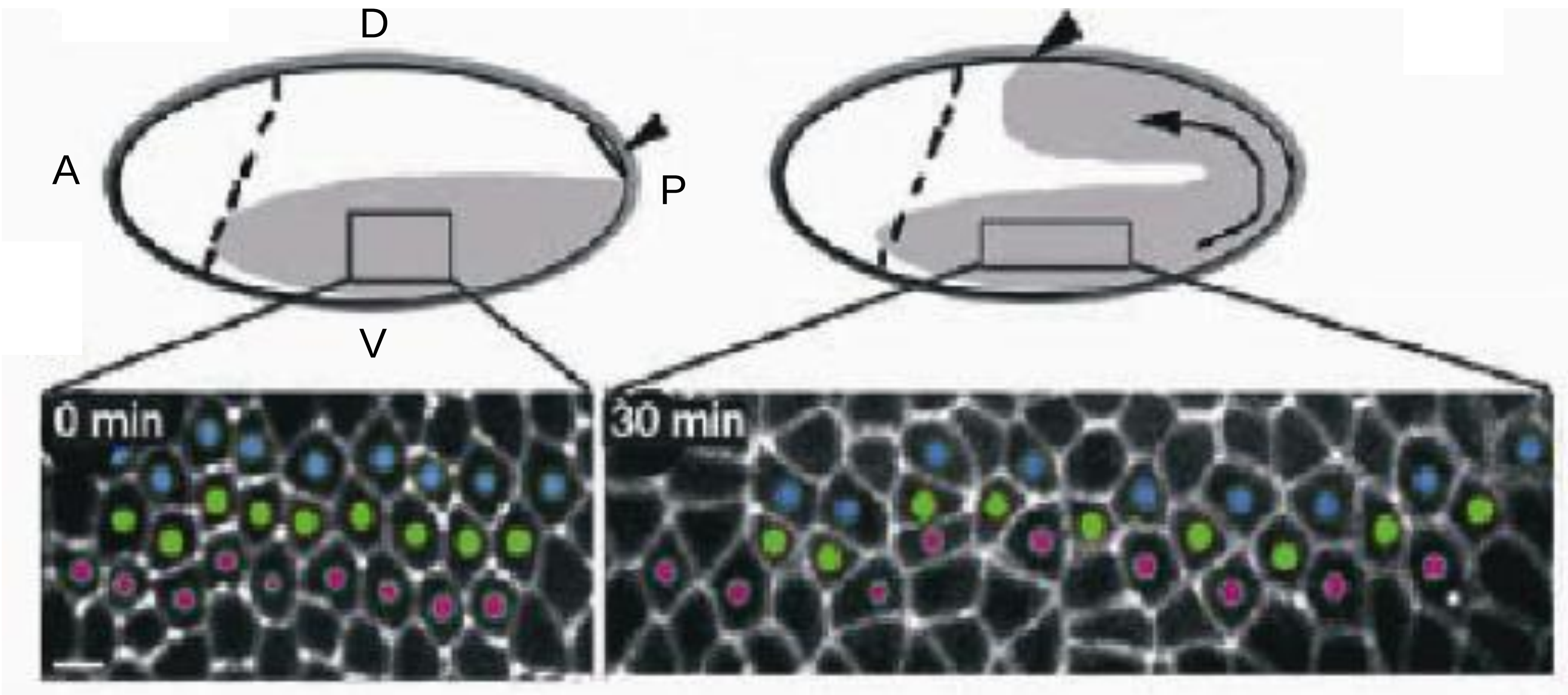
process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation	1030nm	200fs	50MHz	370mW	5ms	1,2NA water	65%	4nJ	250000

GERMBAND ELONGATION



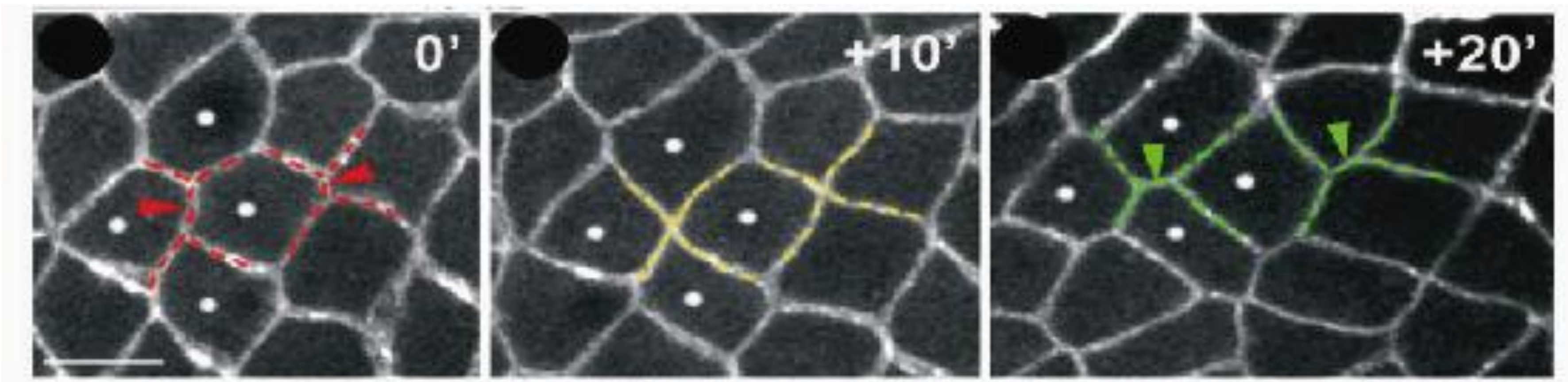
by Thomas Lecuit

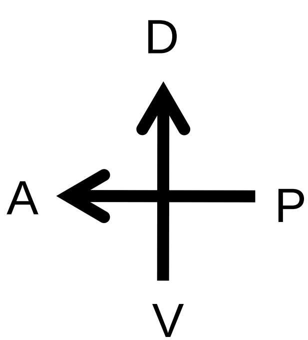
Cell intercalation is responsible for tissue elongation



Bertet et al. (2004)

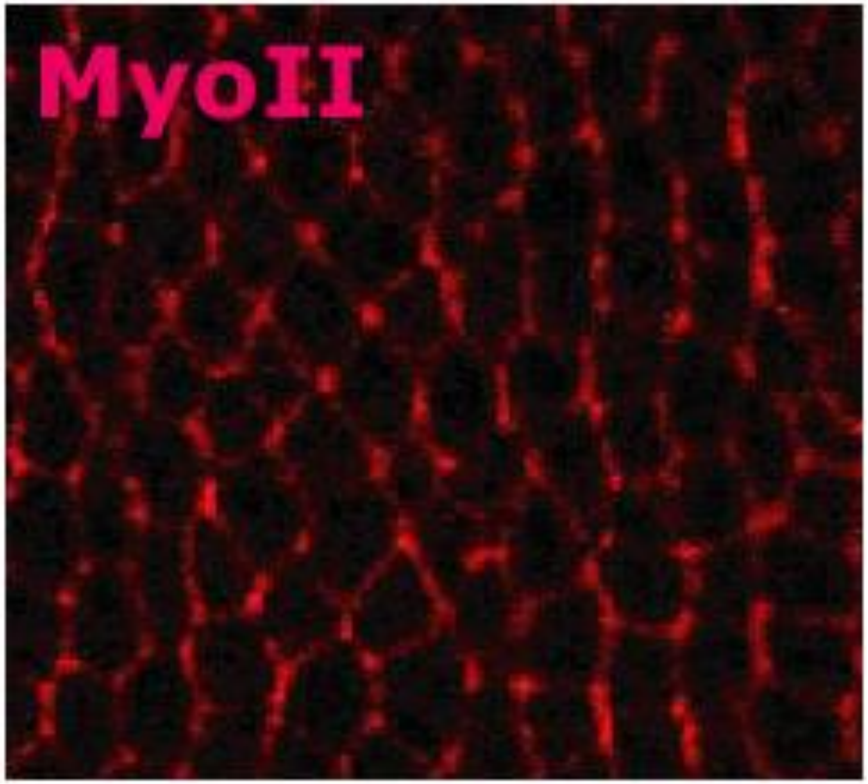
Polarized junction remodeling drives intercalation

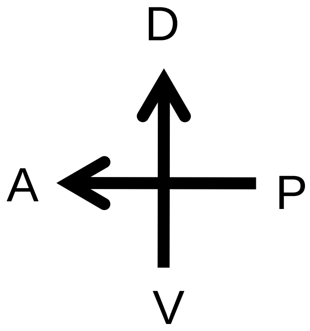




Bertet et al. (2004)

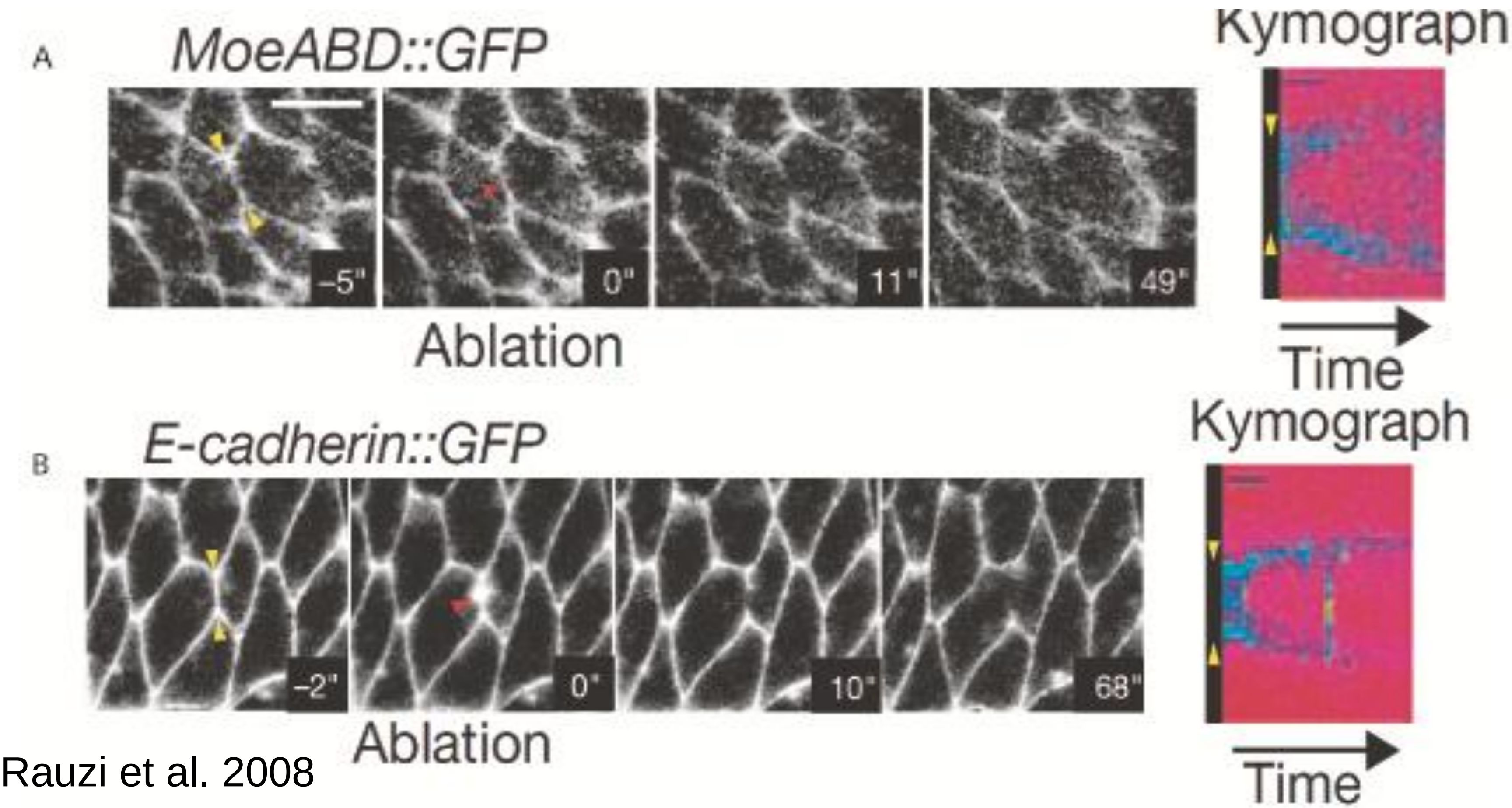
Myo-II, a key effector of intercalation, is planar polarized





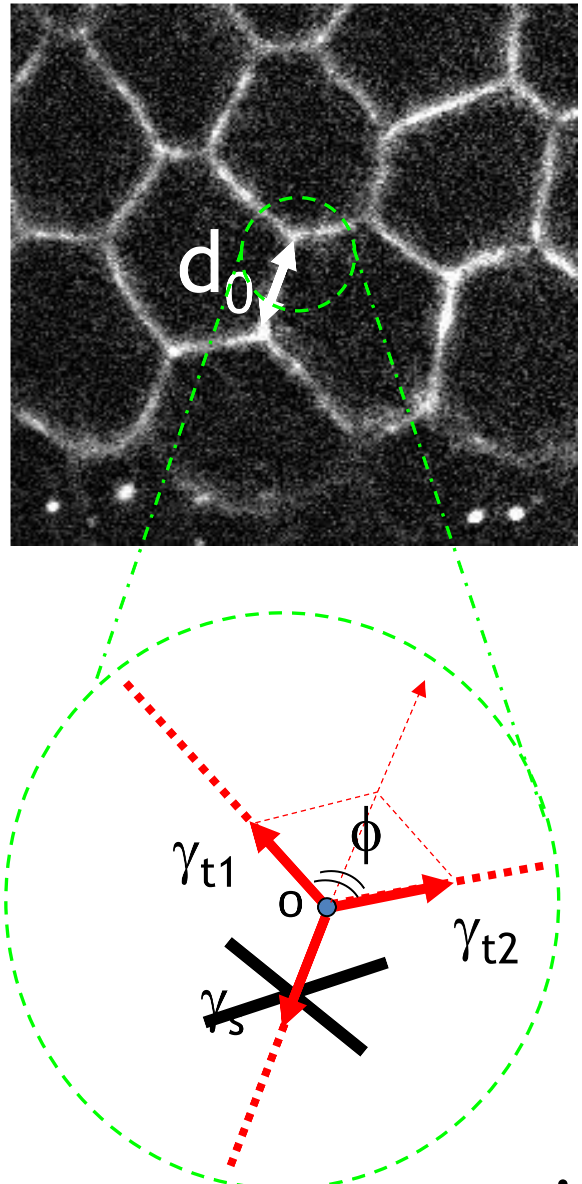
Bertet et al. (2004)

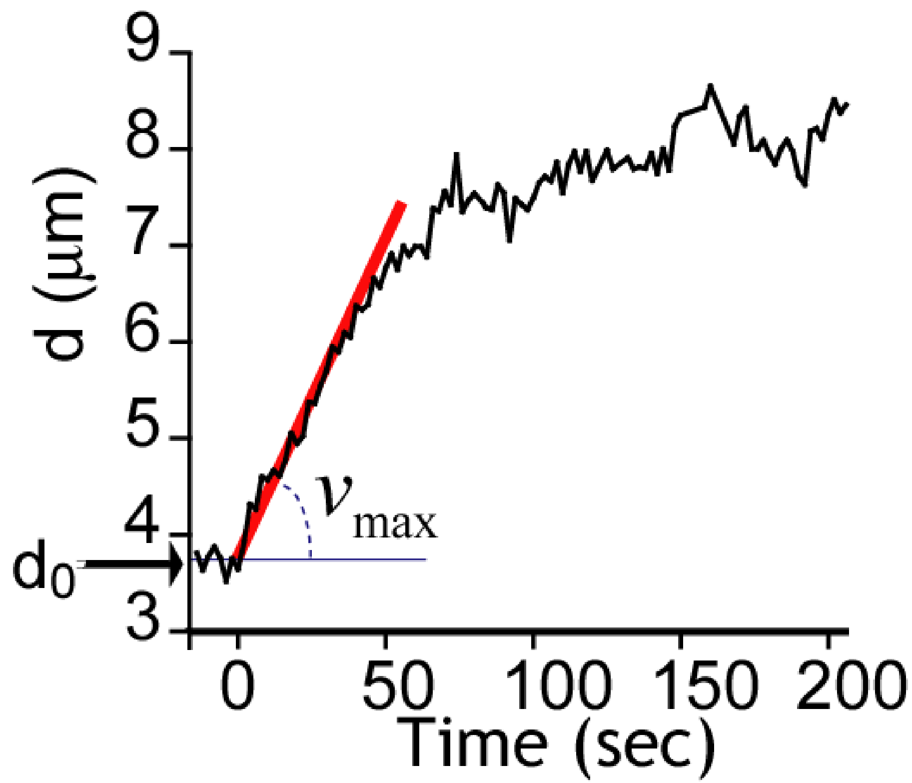
F-actin ablation and depletion of E-cadherin



Rauzi et al. 2008

process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation	1030nm	200fs	50MHz	370mW	3ms	1,2NA water	65%	4nJ	150000



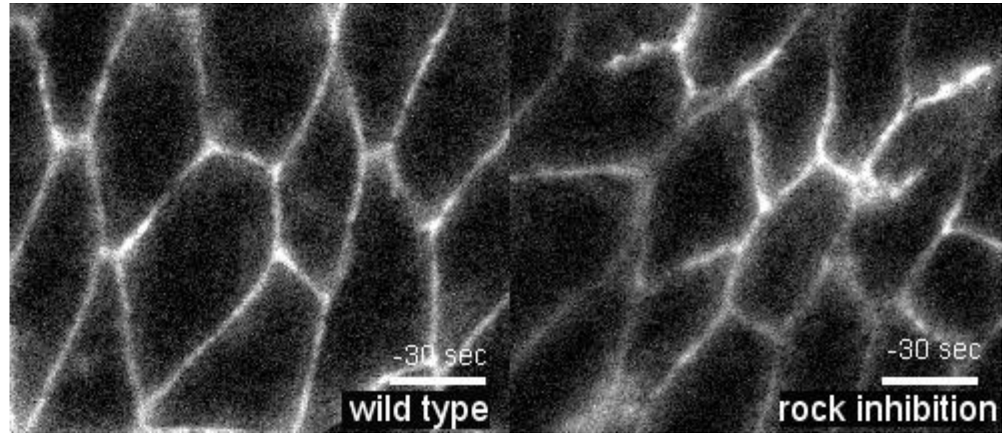


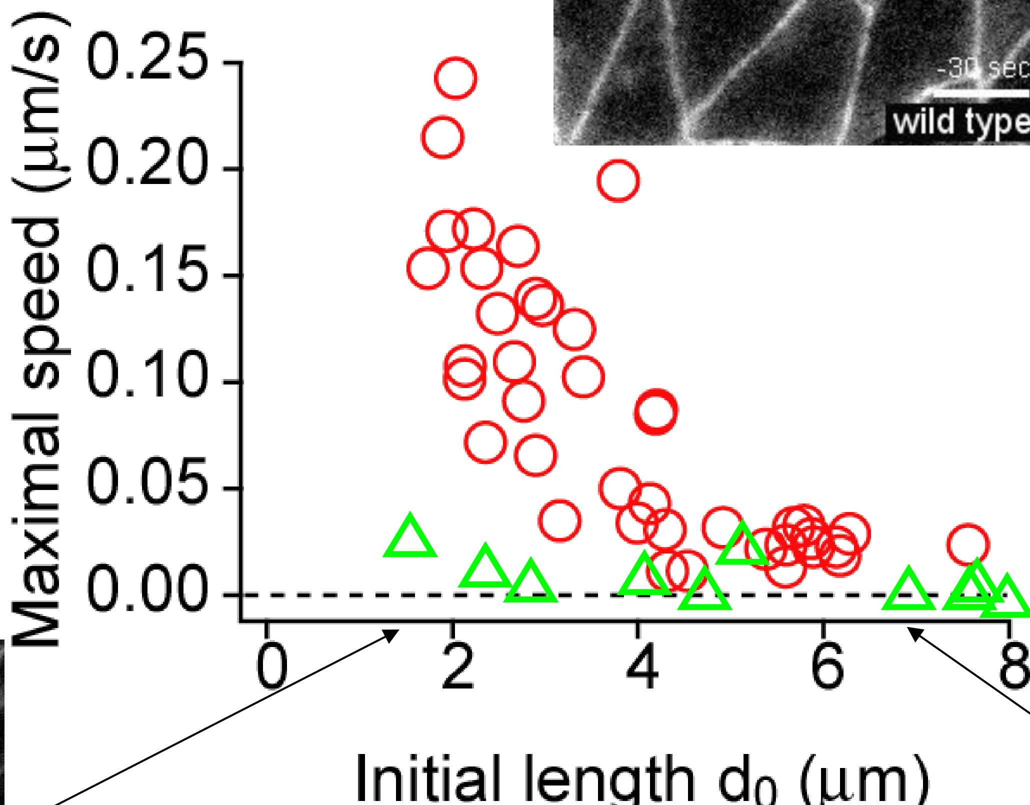
$$v_{max} = \frac{|\overrightarrow{\gamma_{t1}} + \overrightarrow{\gamma_{t2}}|(t = 0^{+})}{\mu} = \frac{|\overrightarrow{\gamma_{s}}|(t = 0^{-})}{\mu}$$

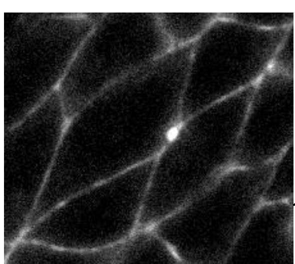
μ: friction coefficient

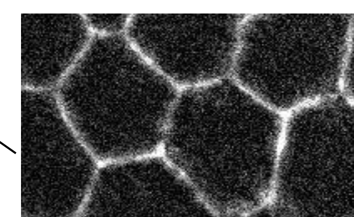
Tension of ‘vertical’ junctions

Inhibition of Myo-II activity

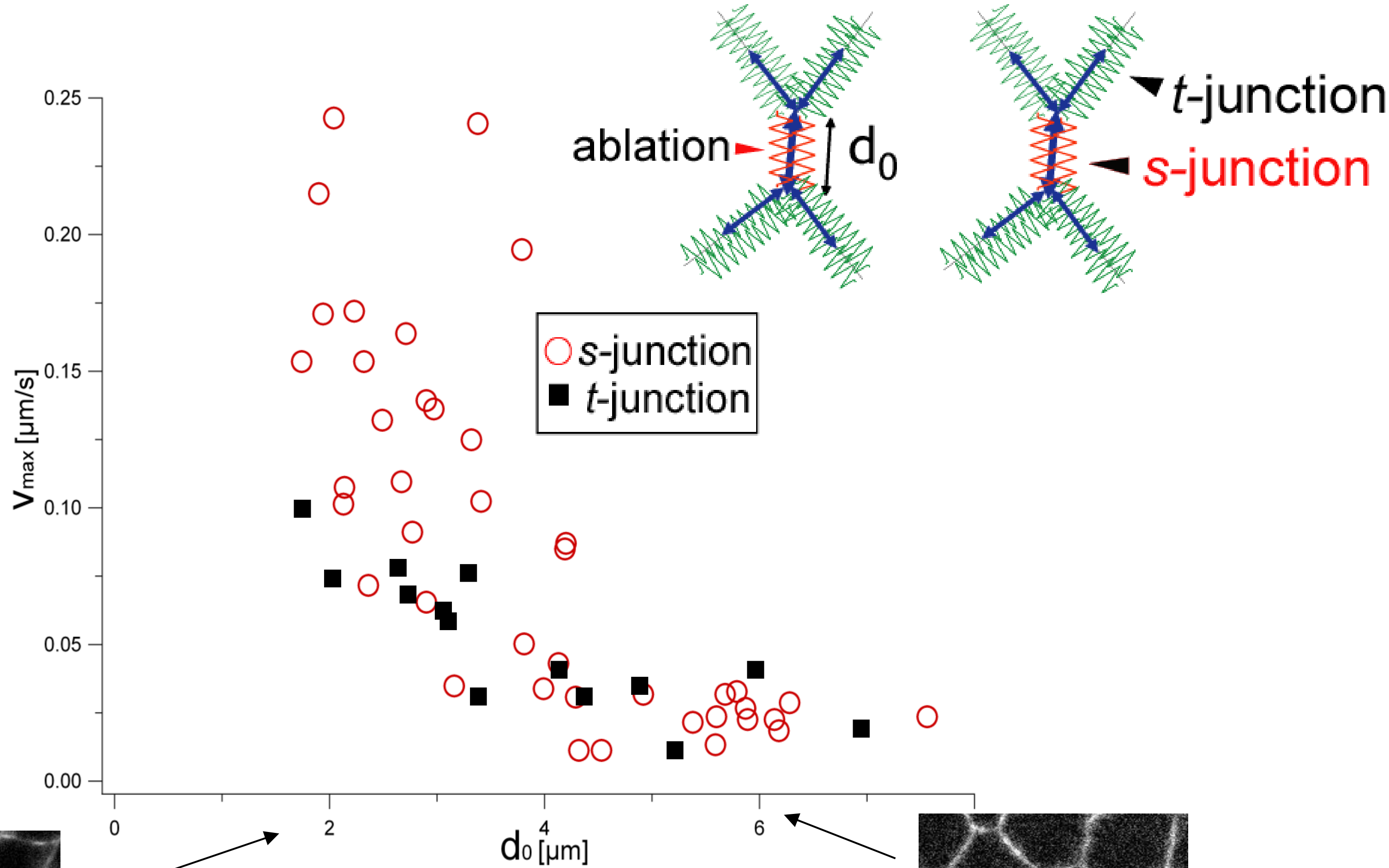


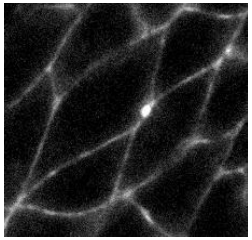


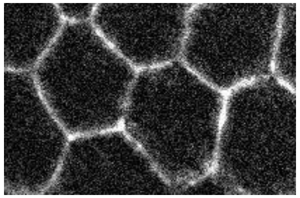




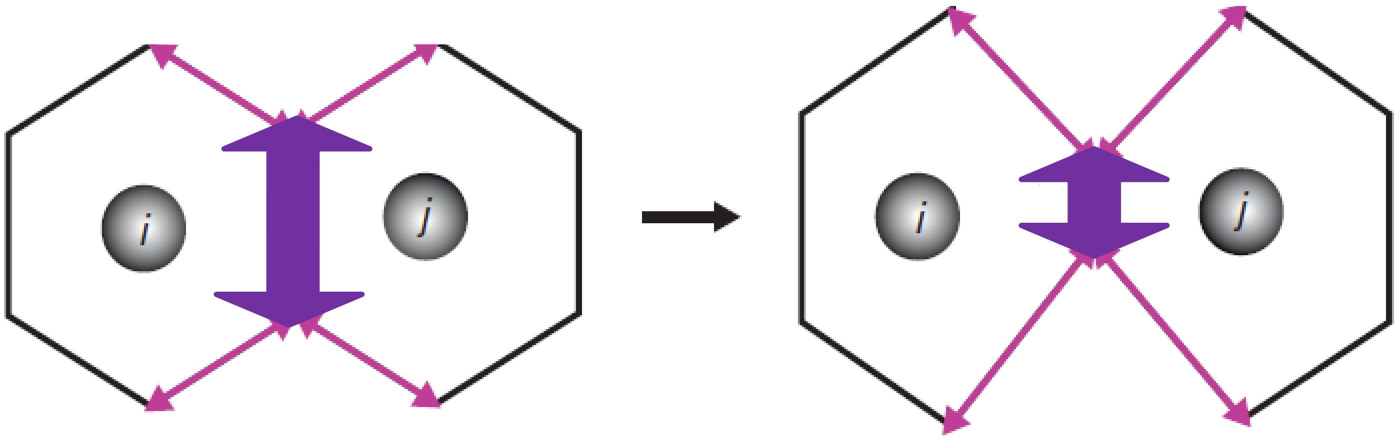
Tension comparison between 'vertical' and 'transverse' junctions







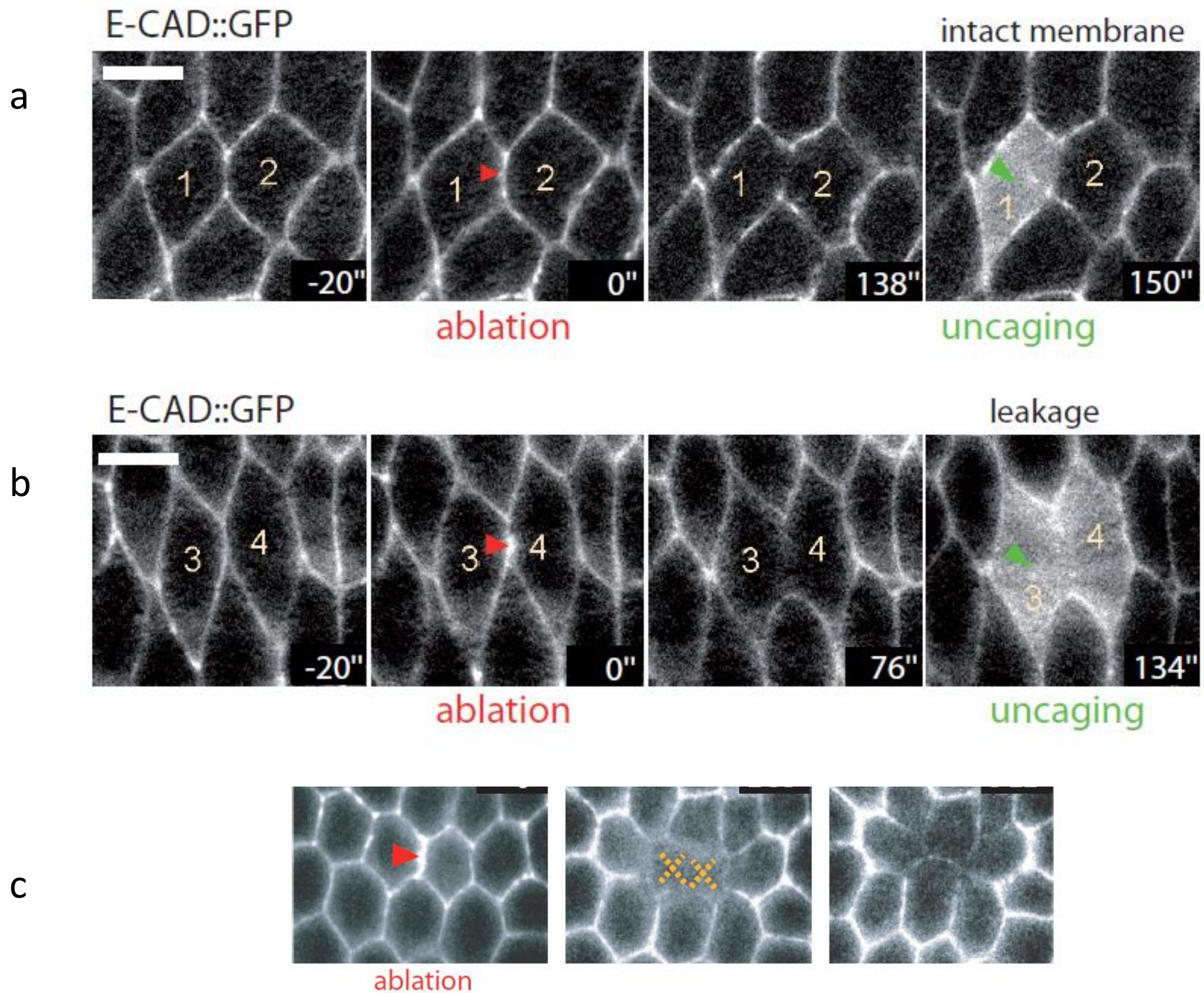
a CORTICAL model



- Local subcellular tensions at the CORTEX drive intercalation
- Such forces are MyoII dependent

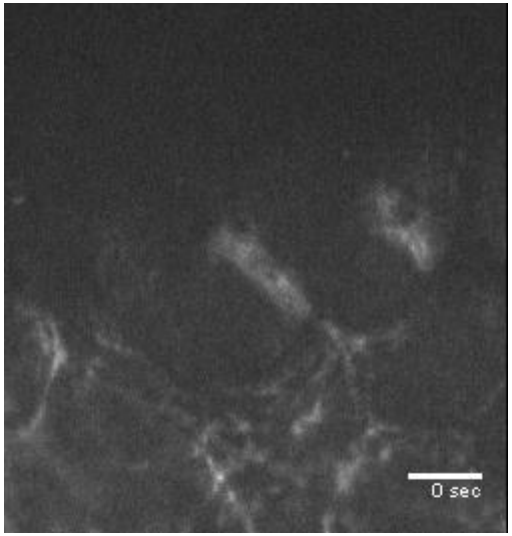
Rauzi et al. 2008

Probing membrane integrity after ablation



process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation a	1030nm	200fs	50MHz	370mW	3ms	1,2NA water	65%	4nJ	150000

Measuring the ablation spot size



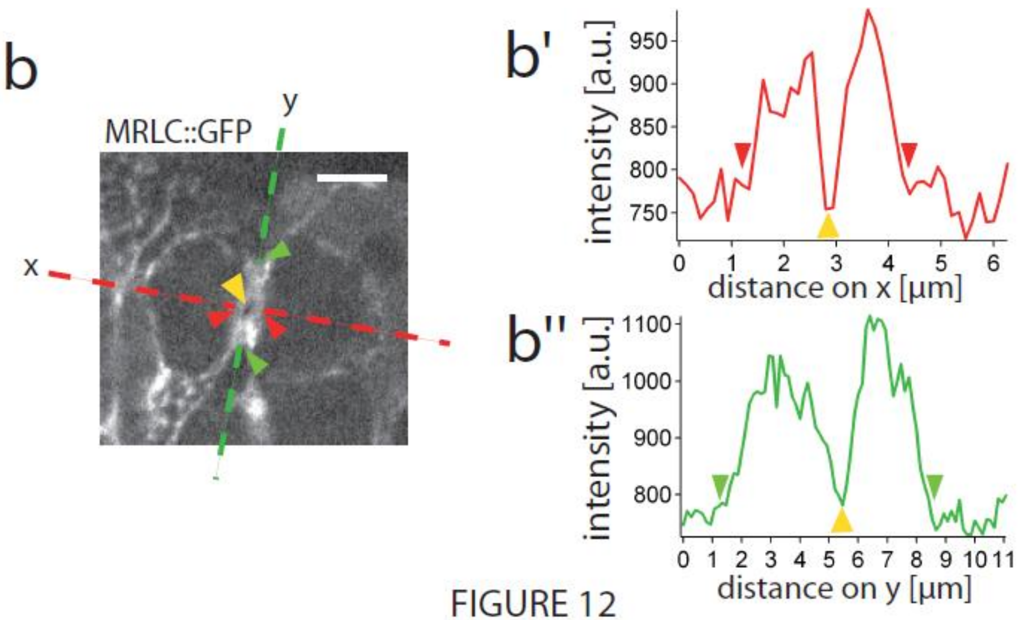
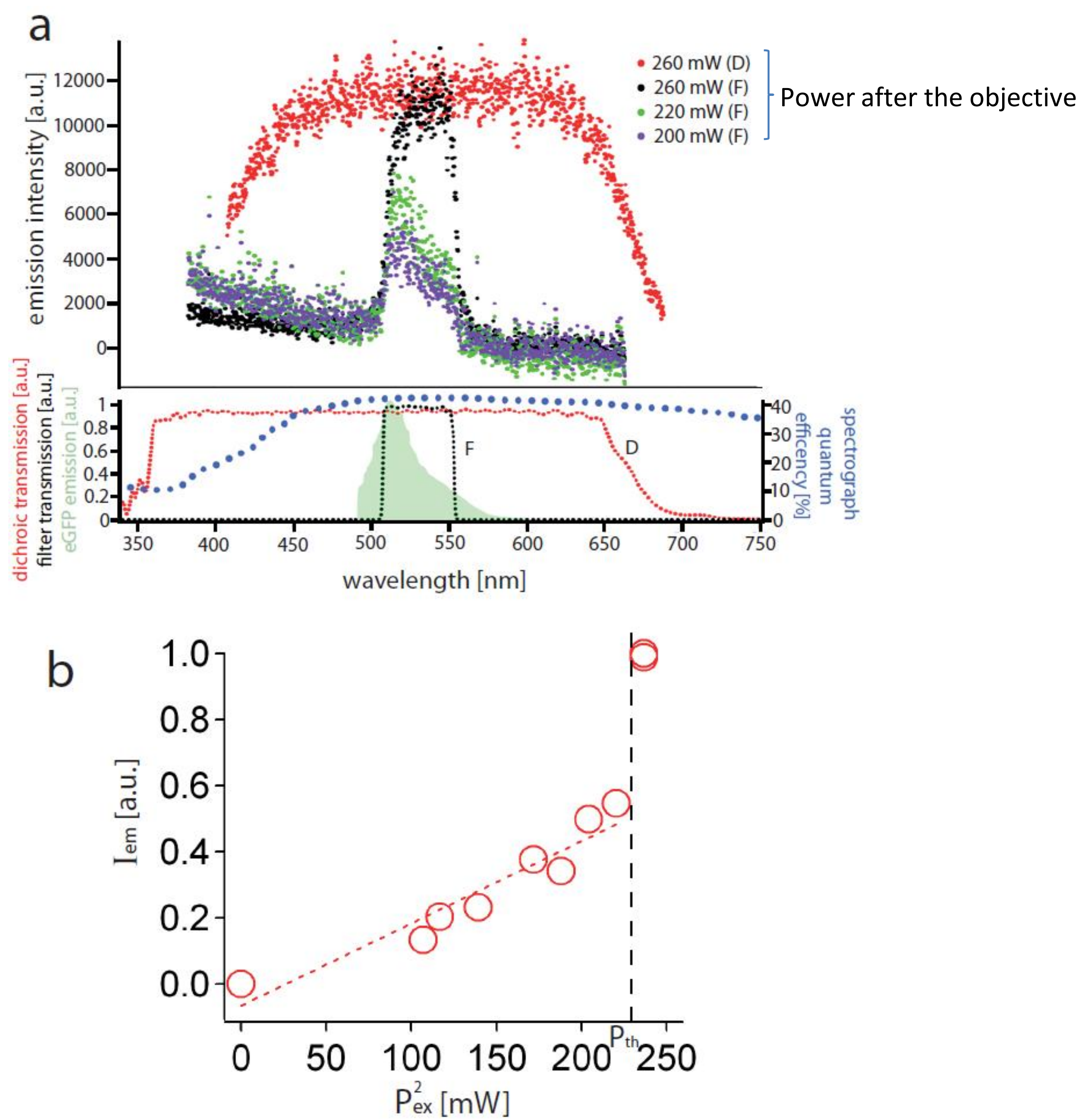


FIGURE 12

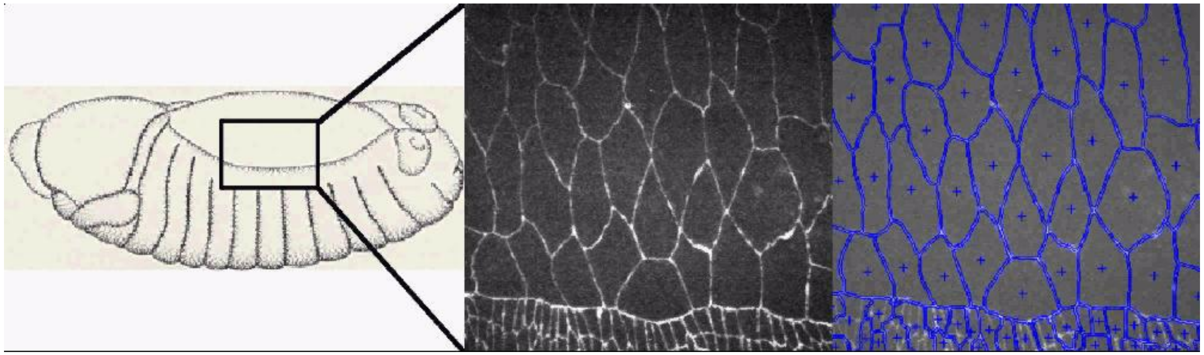
process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation a	1030nm	200fs	50MHz	370mW	3ms	1,2NA water	65%	4nJ	150000

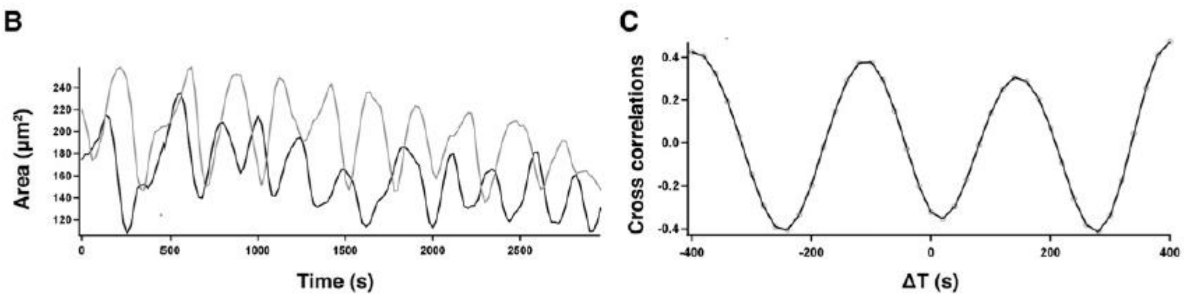
Testing plasma formation during junction ablation

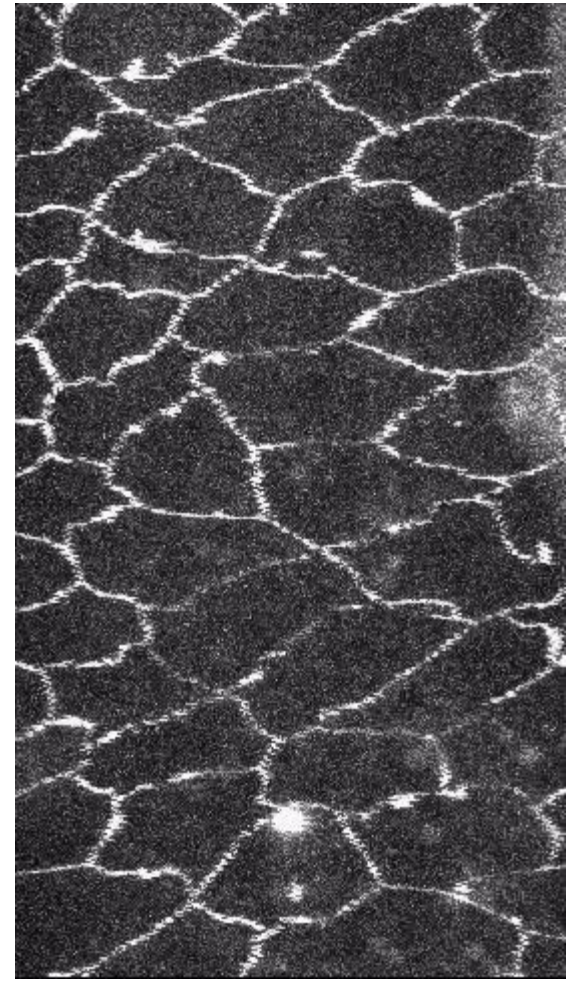


process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation a	1030nm	200fs	50MHz	370mW	3ms	1,2NA water	65%	4nJ	150000

Laser severing: a tool to probe cell oscillation coupling



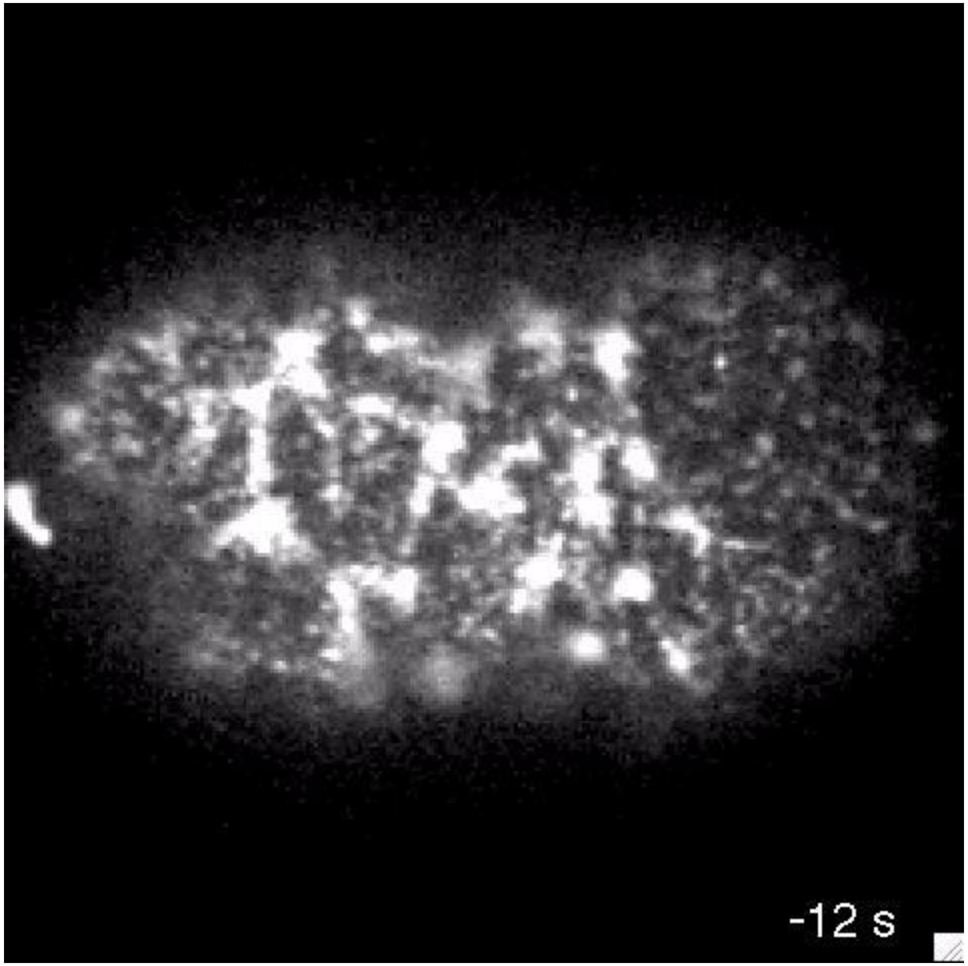


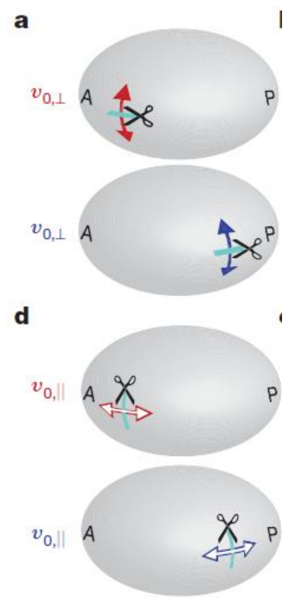


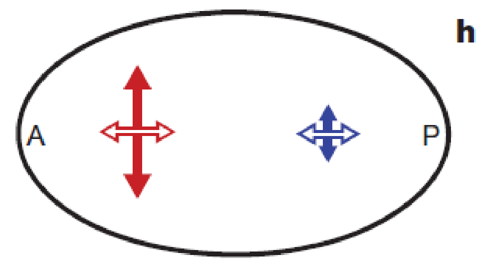
Solon et al. 2009

process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 355nm	Pulse energy after the objective	Number of pulses
Ablation	355nm	470ps	1KHz	?	?	1,2NA water	?	100-200nJ?	5

testing Cortical Tension Anisotropies producing Cortical Flows with laser dissection



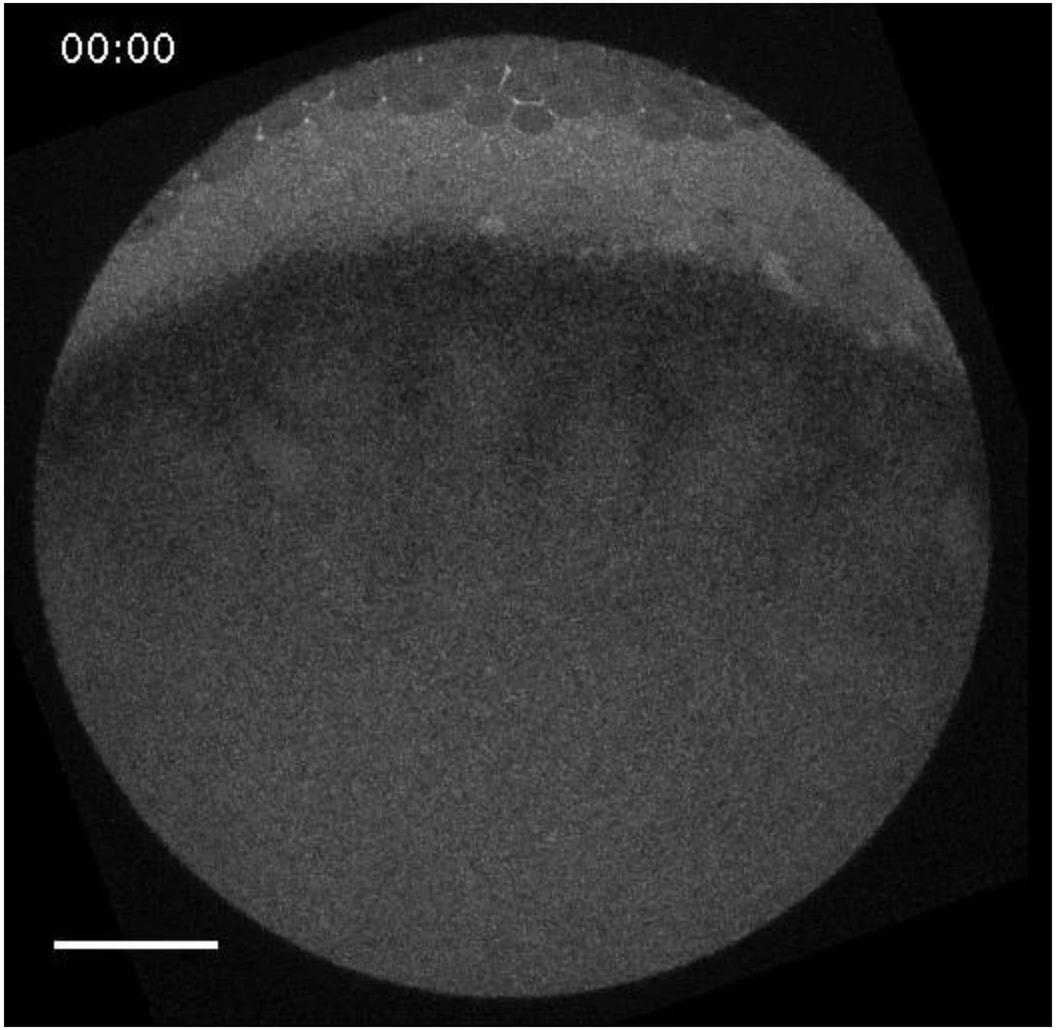


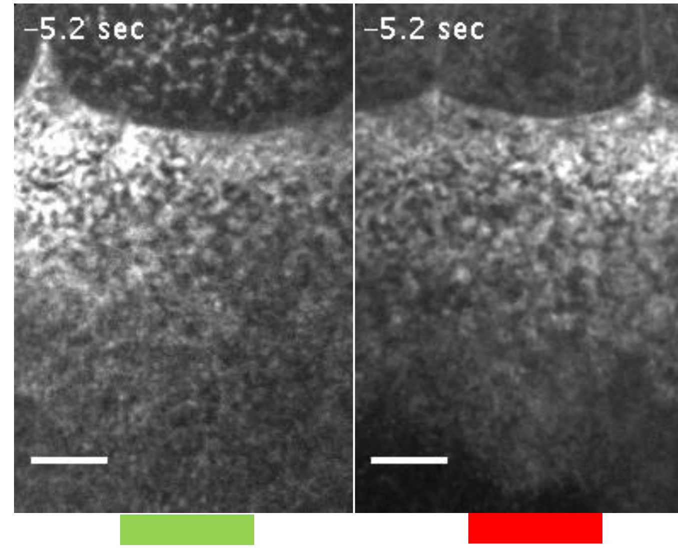


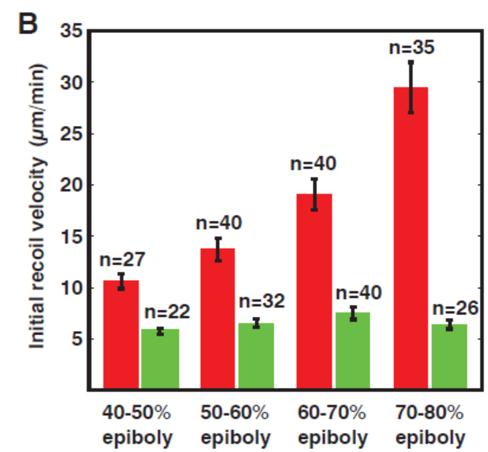
Mayer et al. 2010

process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 337nm	Pulse energy after the objective	Number of pulses
Ablation	337nm	?	500Hz	?	5sec for 6µm	?	?	?	50

Laser surgery: a tool to rule out force contributions in epithelial spreading in Zebrafish gastrulation



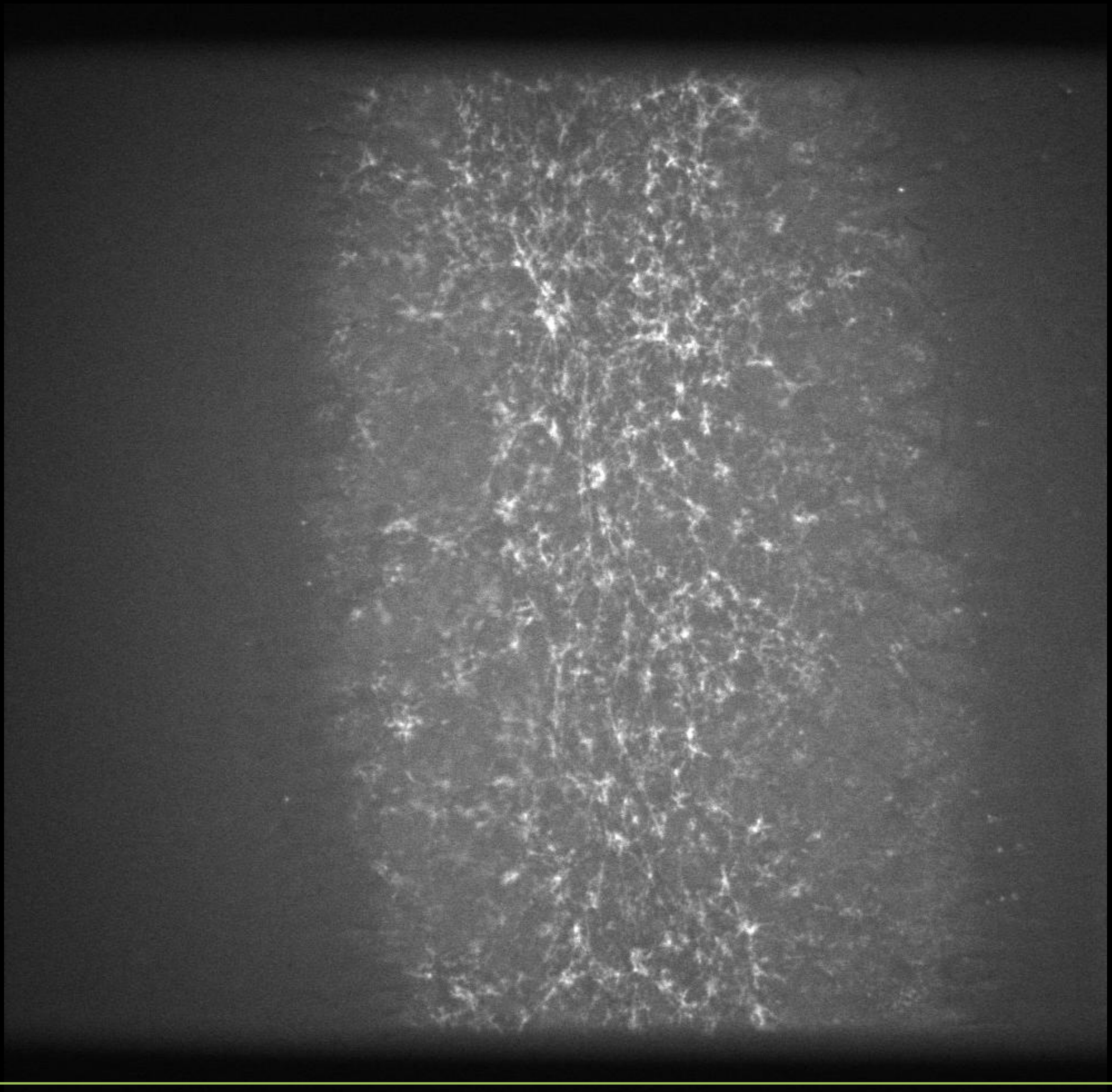




Behrndt et al. 2012

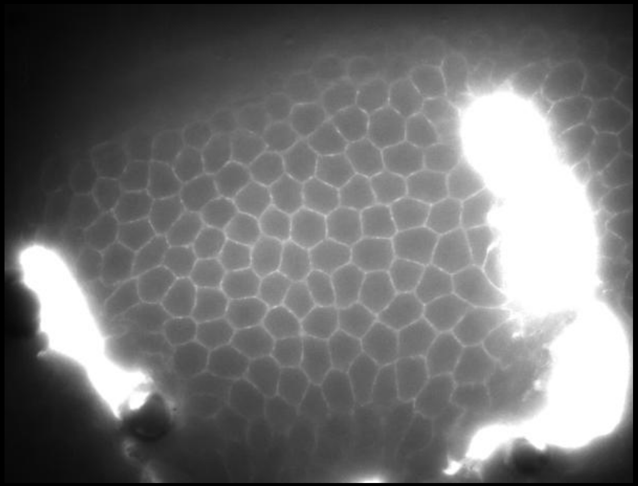
process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 355nm	Pulse energy after the objective	Number of pulses
Ablation	355nm	350ps	1KHz	0,75mW?	1,2s for 20µm	1,2NA water	?	Deduced 750nJ?	25

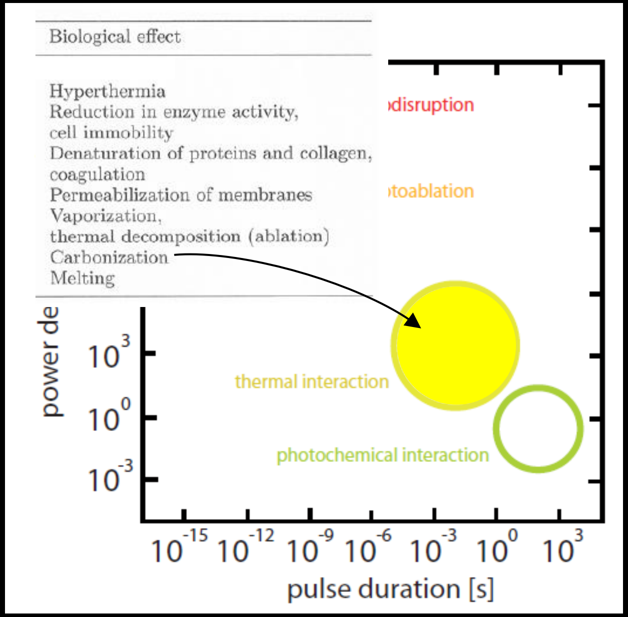
Dissection of the acto-myosin meshwork during *Drosophila* embryo gastrulation



process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation	1030nm	200fs	50MHz	180mW	9sec for 100µm	1,2NA water	65%	2nJ	500M

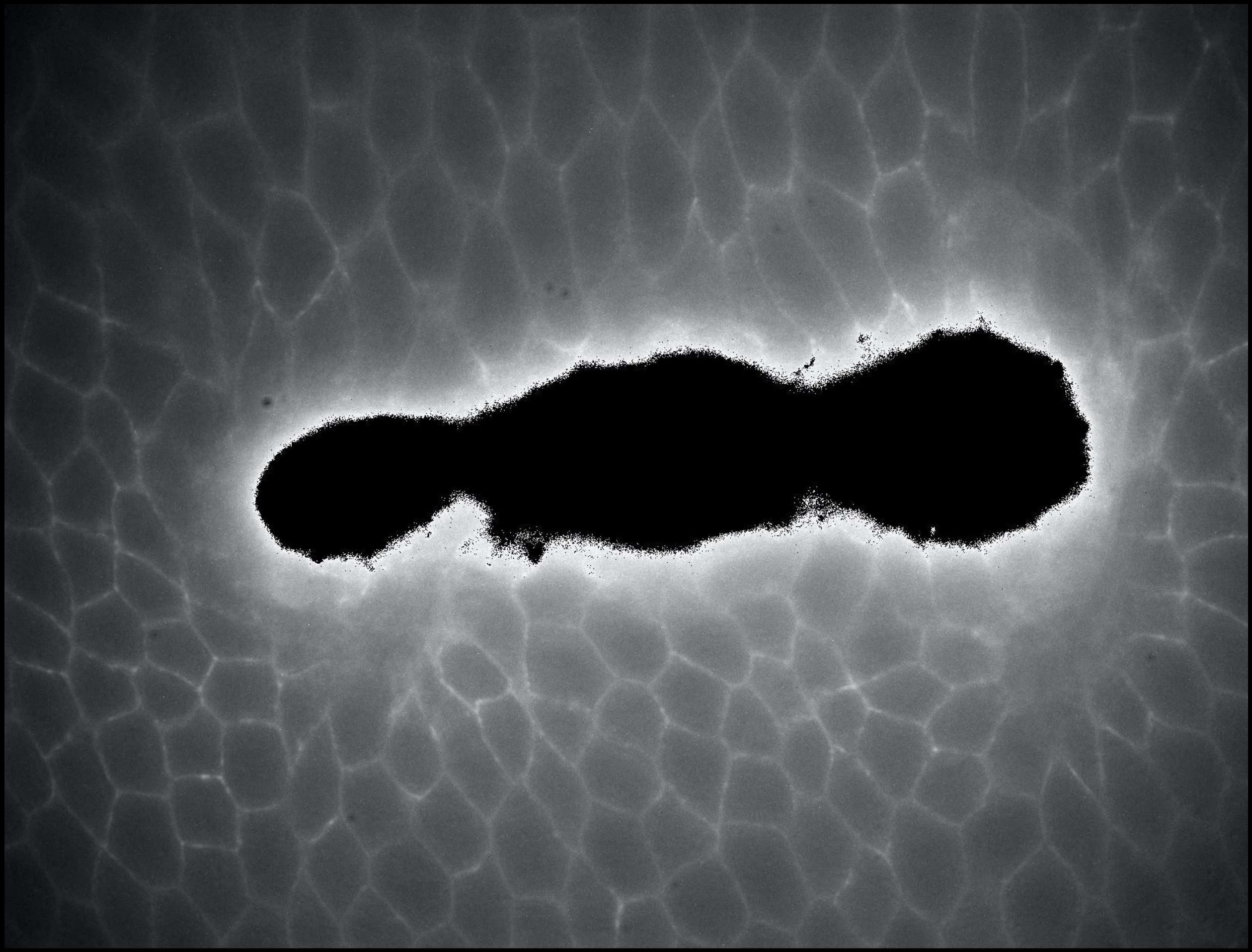
IR laser CARBONIZATION in the *Drosophila* embryo

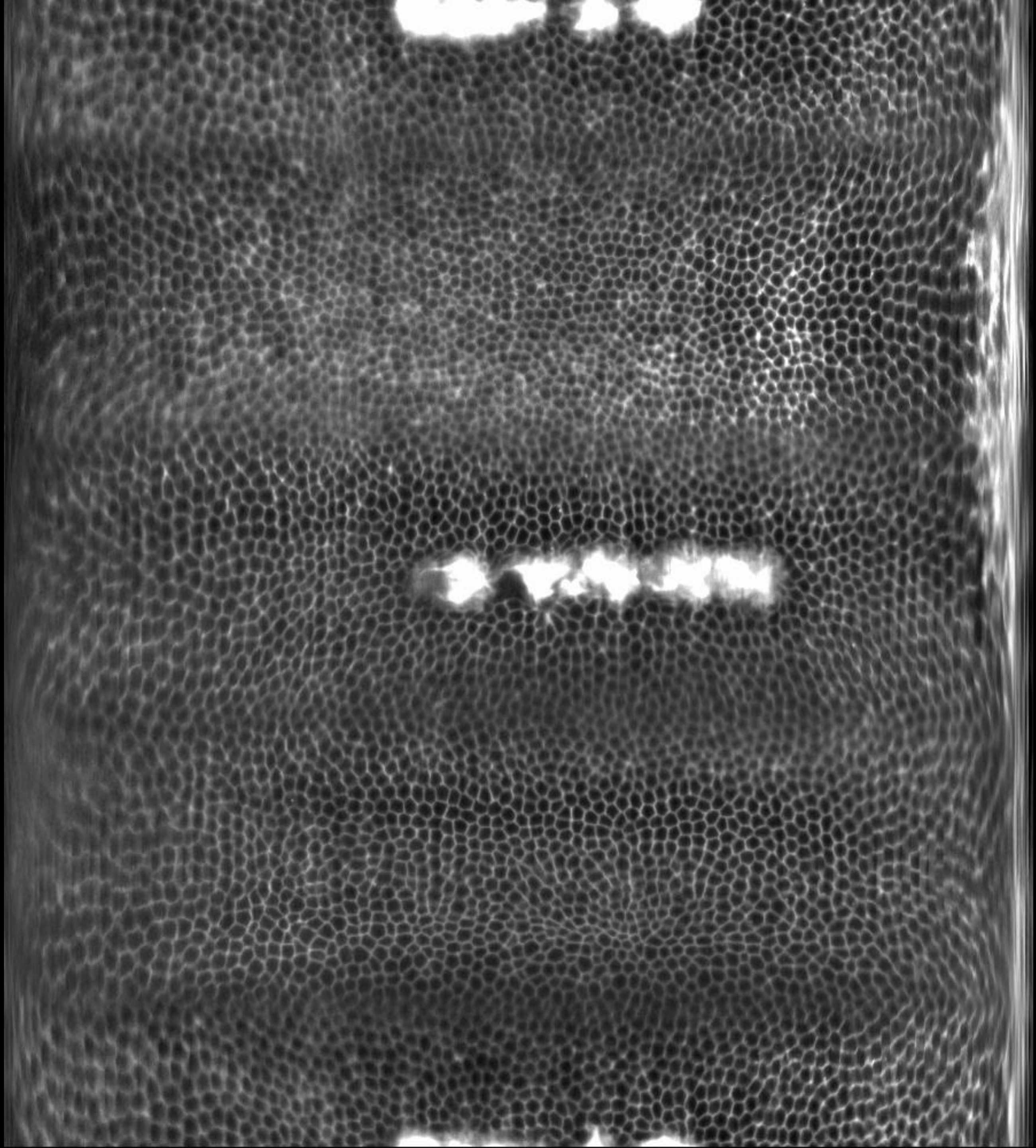




process	wavelength	pulse duration	Pulse repetition rate	Average power at the back aperture	Exposure time	Objective NA and immersion	Objective transmission at 1030nm	Pulse energy after the objective	Number of pulses
Ablation	1030nm	200fs	50MHz	180mW	25sec for 280µm	1,2NA water	65%	2nJ	500M

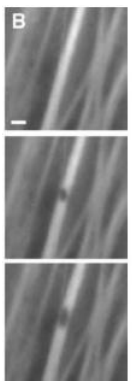
IR laser CARBONIZATION in the *Drosophila* embryo

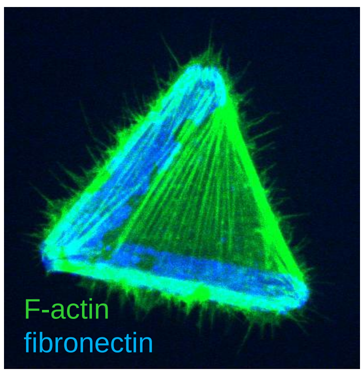


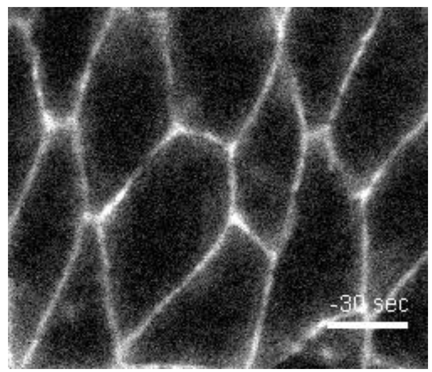

LR
D
LL
V
LR

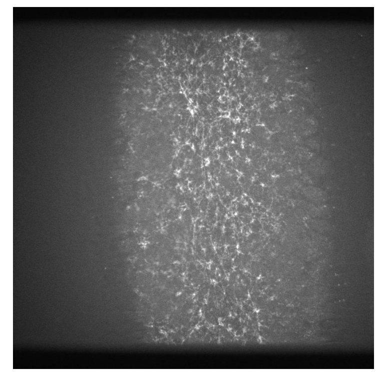
Laser dissection:

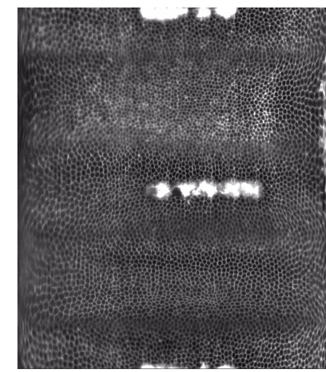
A tool highly **<u>spatial and temporal specific</u>**
that can be applied at **<u>different scales</u>**





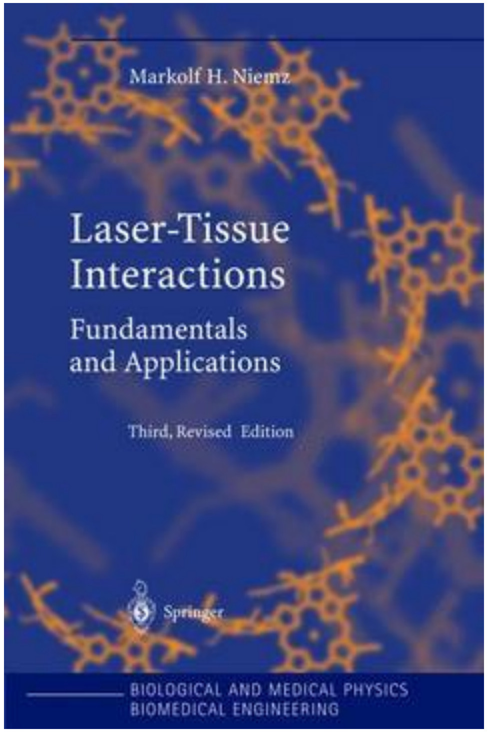






actin fiber → *in toto Drosophila* embryo

Interesting books



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Laser ablation
Micro-pillars