How Multidisciplinarity can Advance Lifescience Understanding

Combining Optics, Image Processing, Computational Simulation to Aid Biological Understanding

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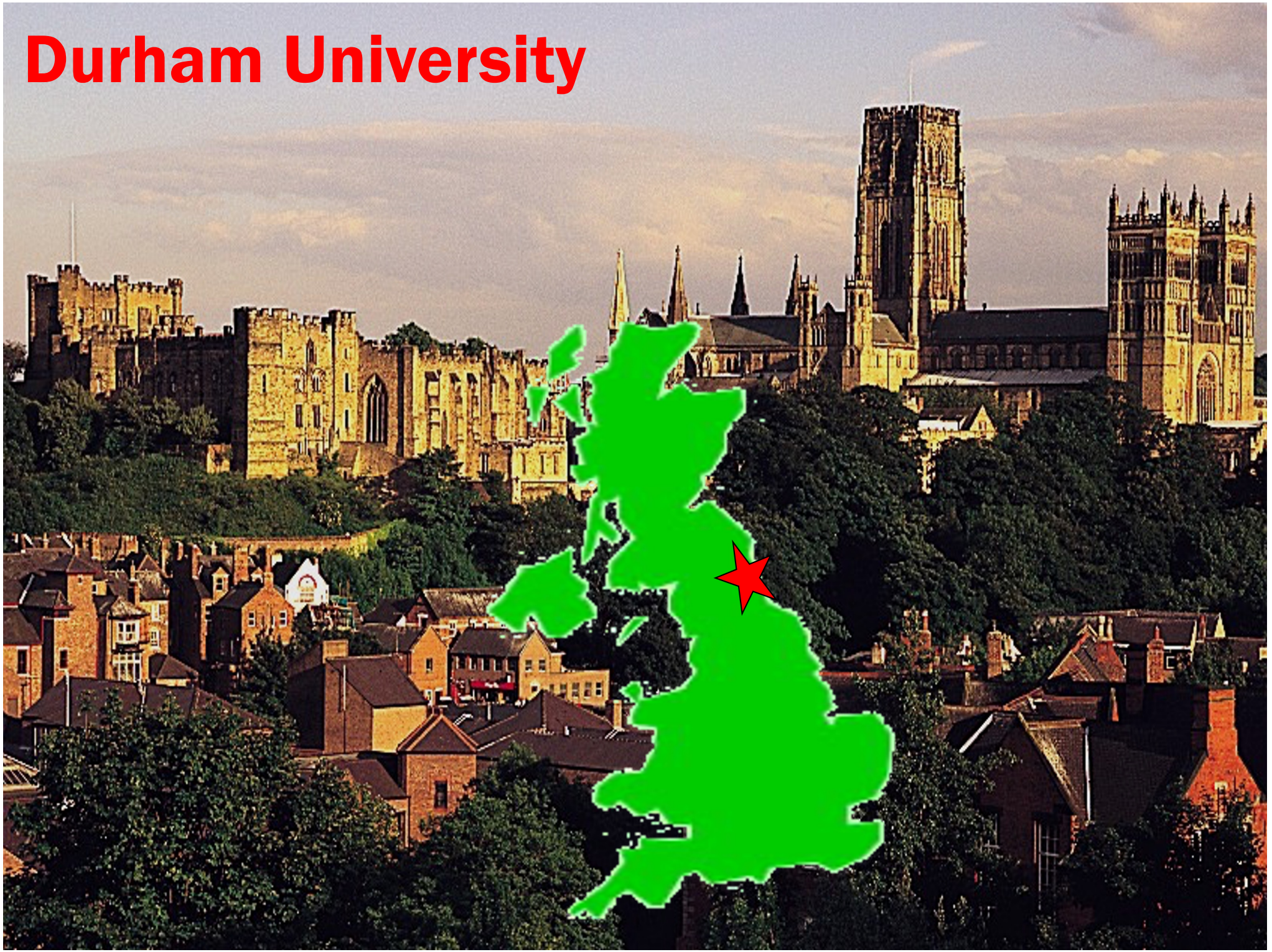

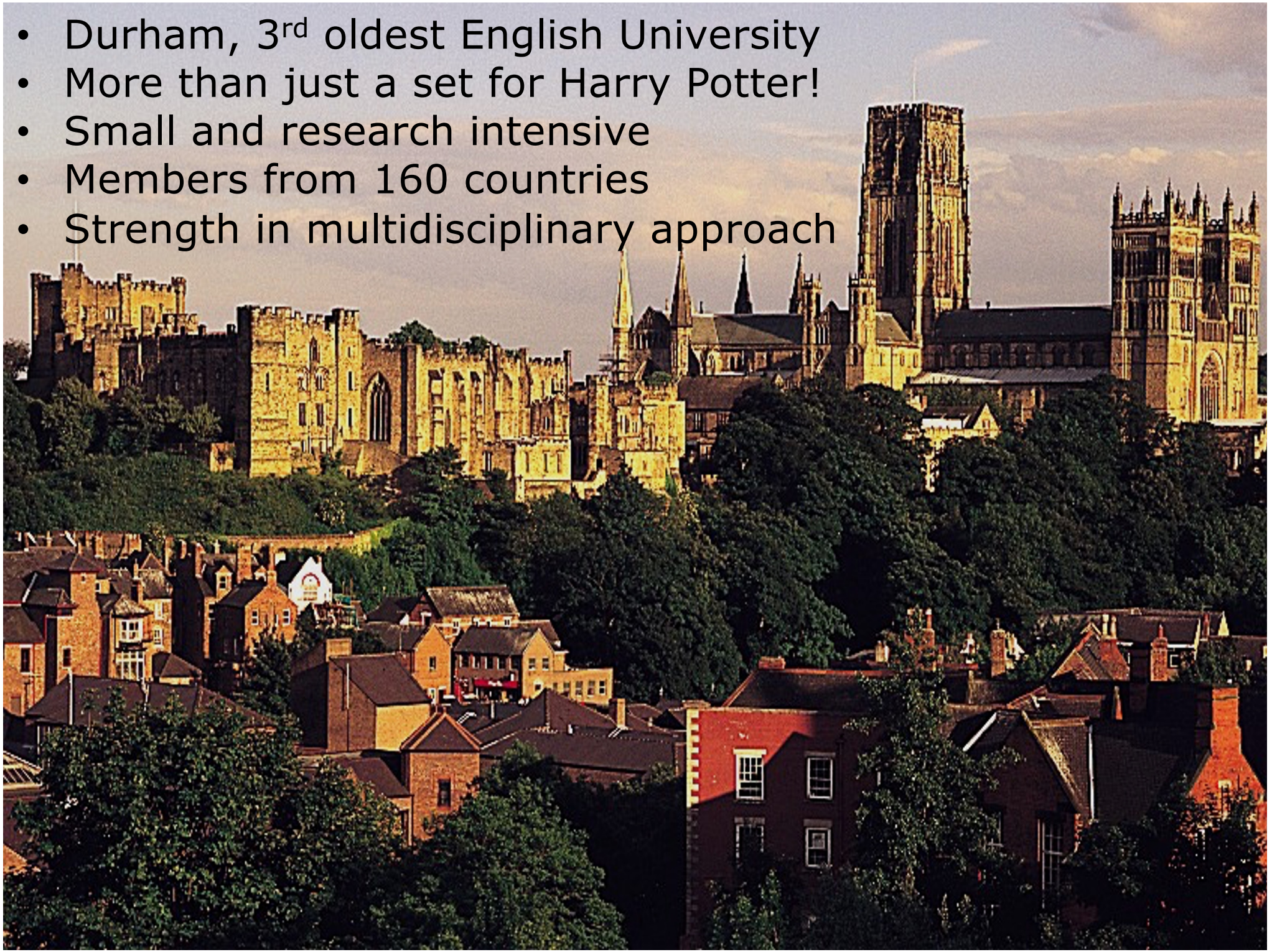
• Durham, 3rd oldest English University
• More than just a set for Harry Potter!
• Small and research intensive
• Members from 160 countries
• Strength in multidisciplinary approach


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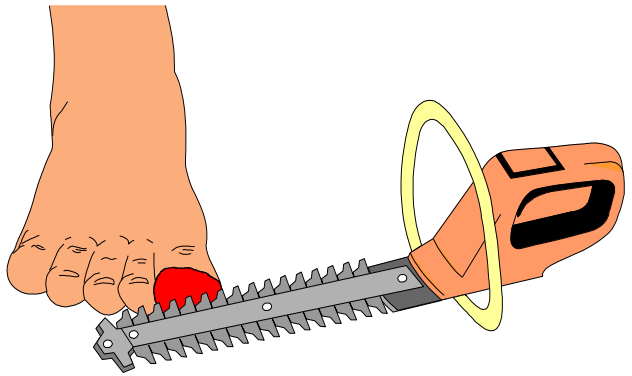
Outline

- Philosophy
- Mechanotransduction by Geometric Modification
- Summary

Multidisciplinarity

- Get the original question right
- Open discussion accepting lack of knowledge- No Stupid questions
- “Transfer” technology/methods
- Choose the right tool for the task in hand
- Don’t over complicate!



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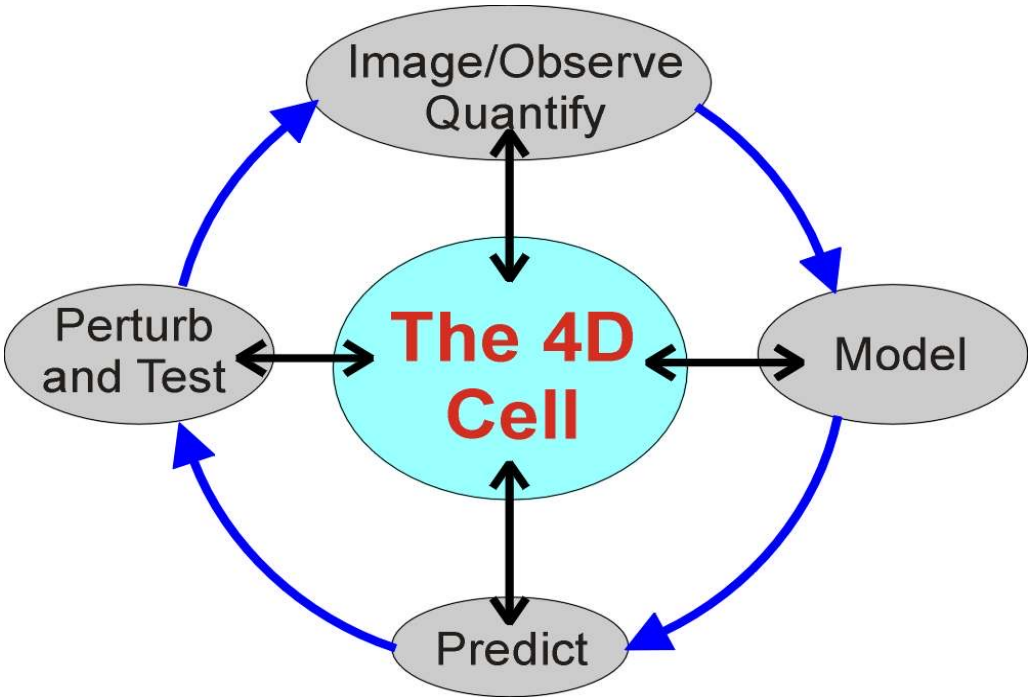
Biology and the Physical Sciences

- The Philosophical Approach



The technology/methodology should help the Biology

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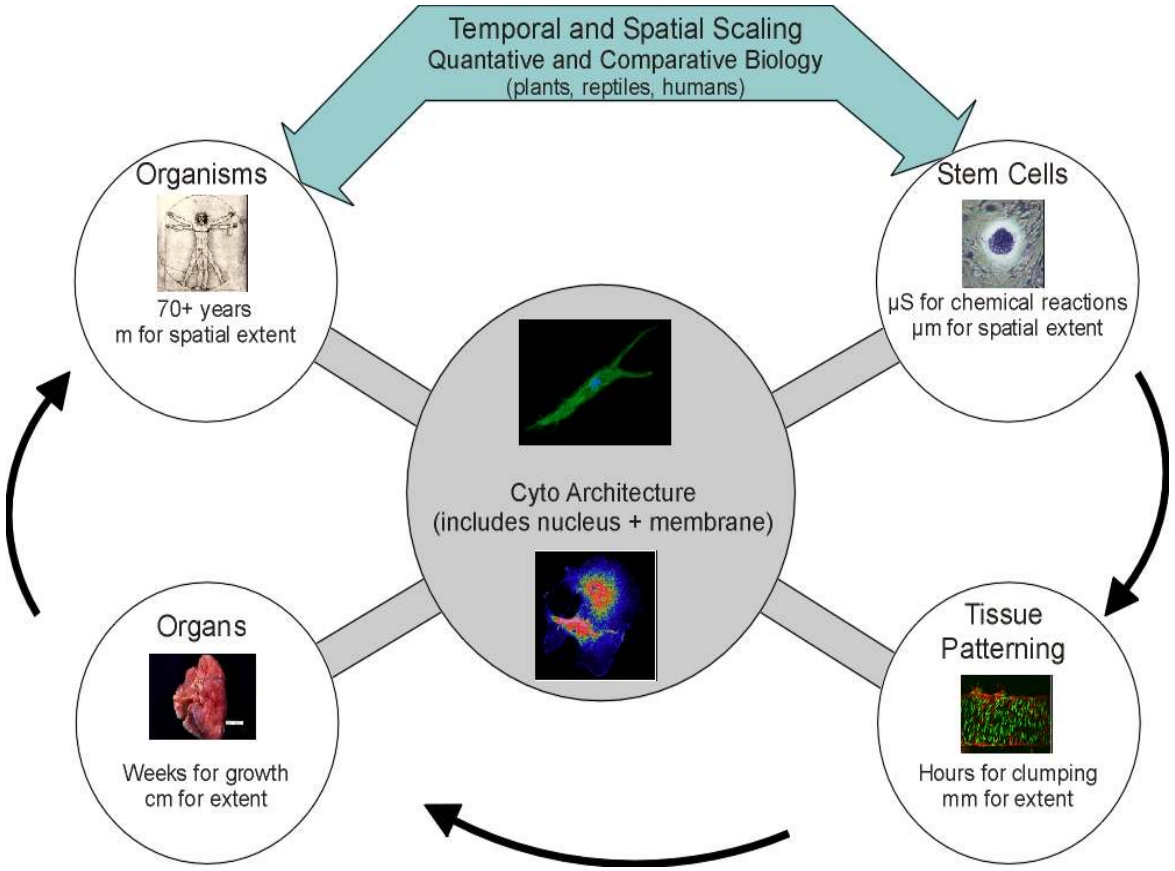
Biology and the Physical Sciences



Multi-scale considerations

1 x 10^{27} in scale

Combining micro-optics, image analysis and computer simulation to understand vascular endothelia sensing

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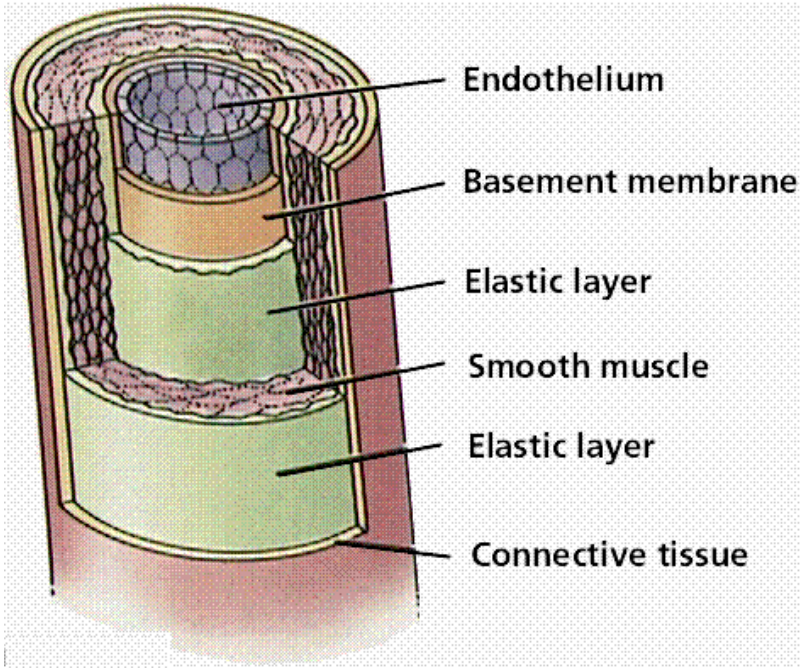
The Endothelium



The Circulatory, Respiratory, and Digestive Systems
http://www.uic.edu/classes/bios/bios100/lecturesf04am/lect20.htm

Monolayer of cells

Innermost layer in all vasculature

Fully connected over whole body

10x more endothelial cells in you than neurones

Controls almost everything…

- Regulates blood vessel formation (angiogenesis)
- Prevents blood clotting
- Regulates vascular permeability
- Determines the extent of smooth muscle proliferation.
- Controls arterial tone (regulate blood flow)

- All without involving the nervous system
  - **How?**

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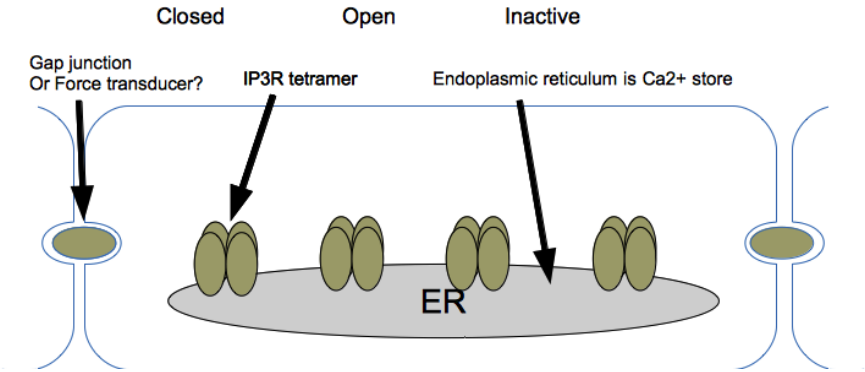
Introduction to IP_3R mediated Calcium waves



ER is a Ca^{2+} reservoir – $[Ca]_{ER}$ ~ 500μM

Cytoslic Ca^{2+} strongly buffered

$[Ca]_{Cyt}$ ~ 70nM

$[B]_{Cyt}$ ~ 1mM

- Locally elevated Ca^{2+} or IP3 opens an IP3R
  - Local source of cytosolic Ca^{2+} with current J_0
  - First IP3R often opened by IP3 from gap junction
  - "Diffusing" Ca^{2+} wavefront triggers a cascade of IP3R openings

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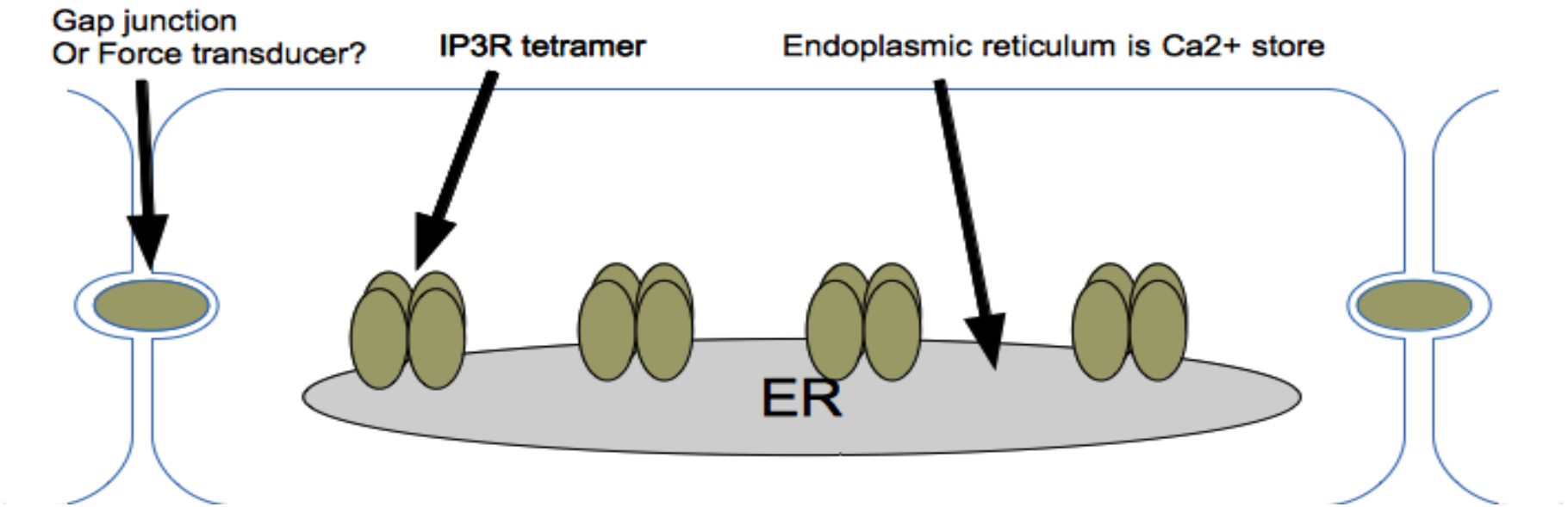
Endothelium



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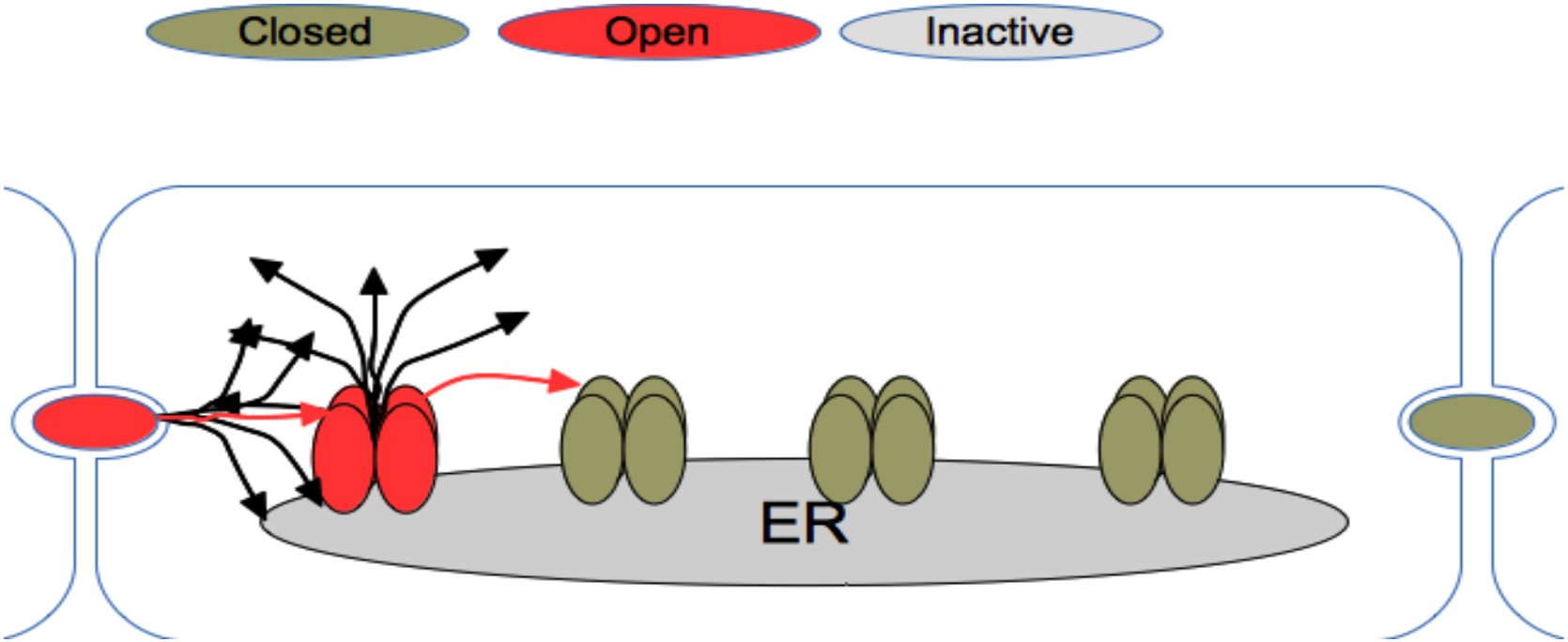
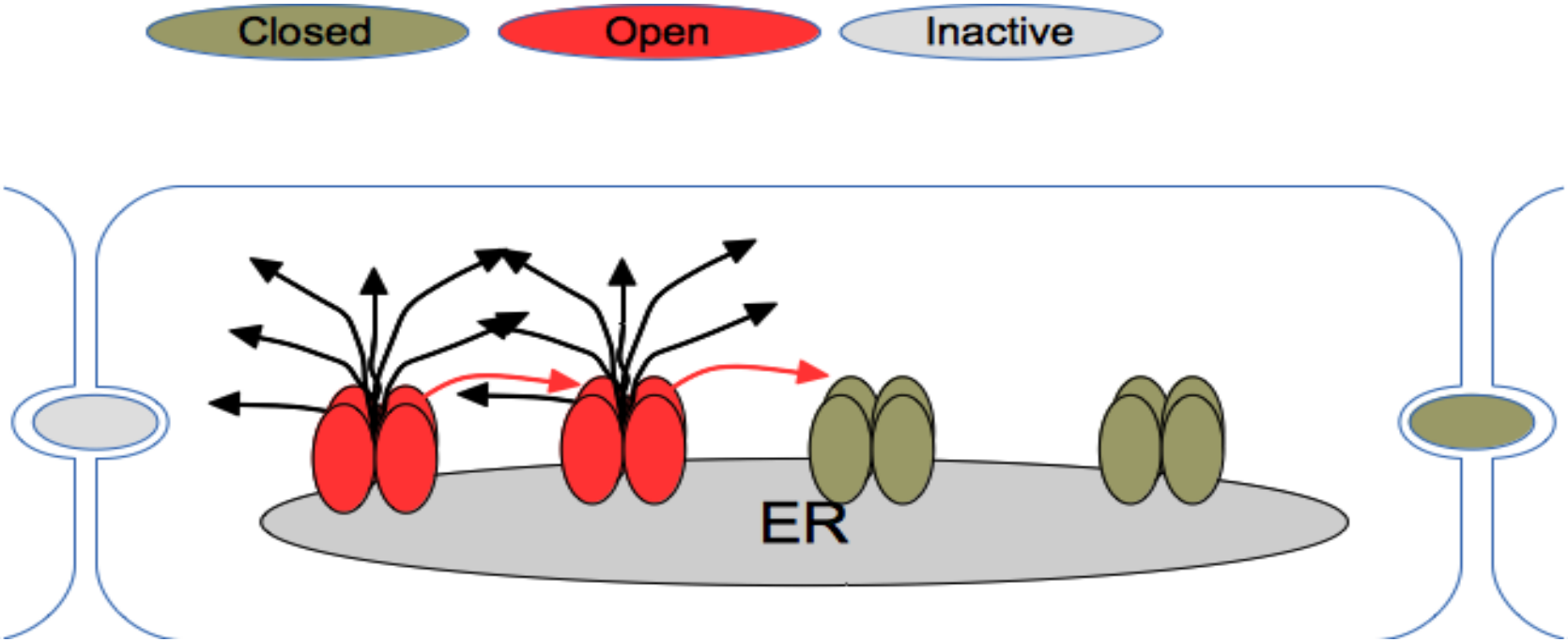
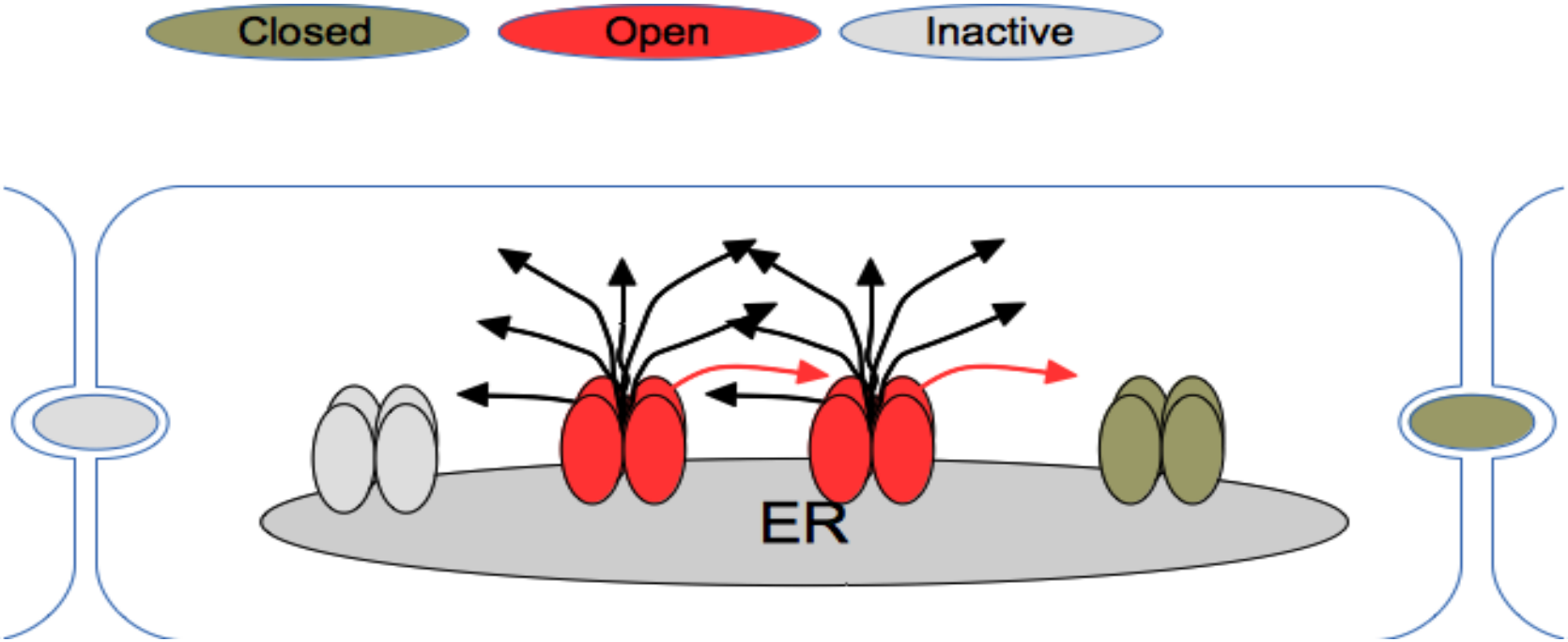
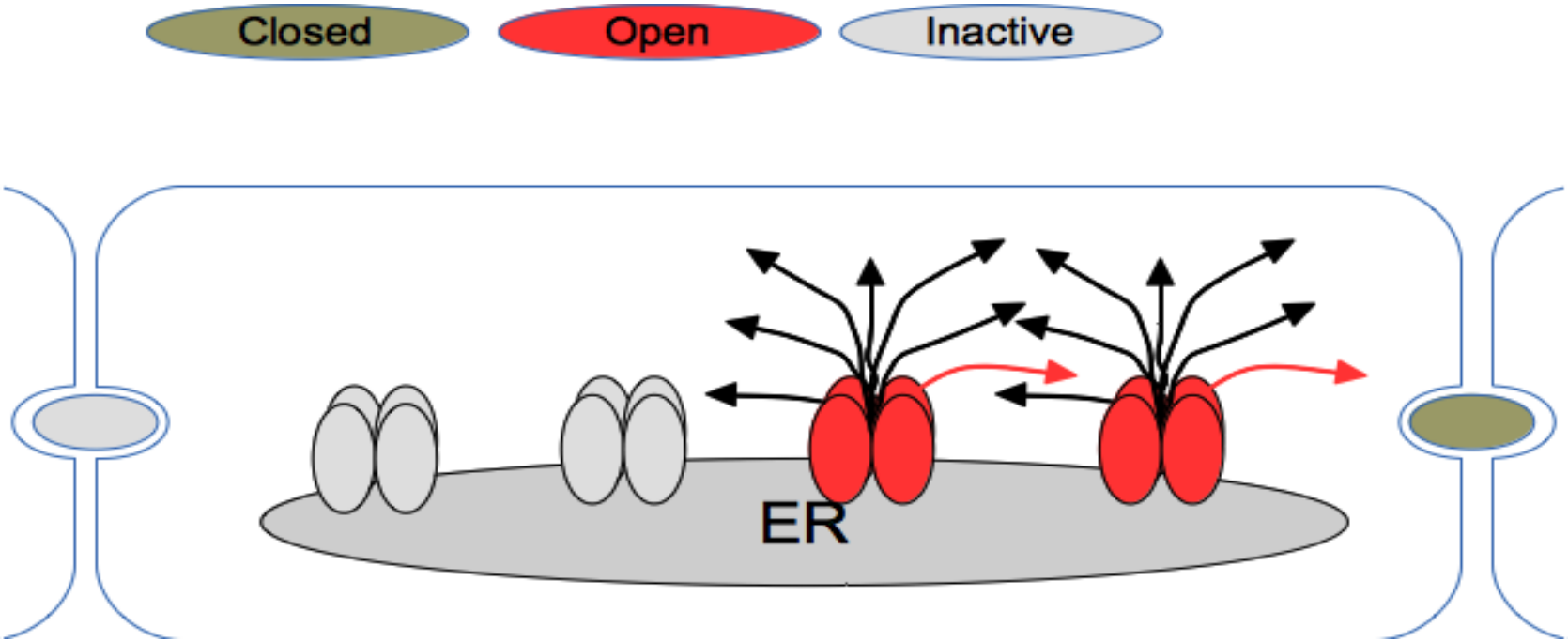
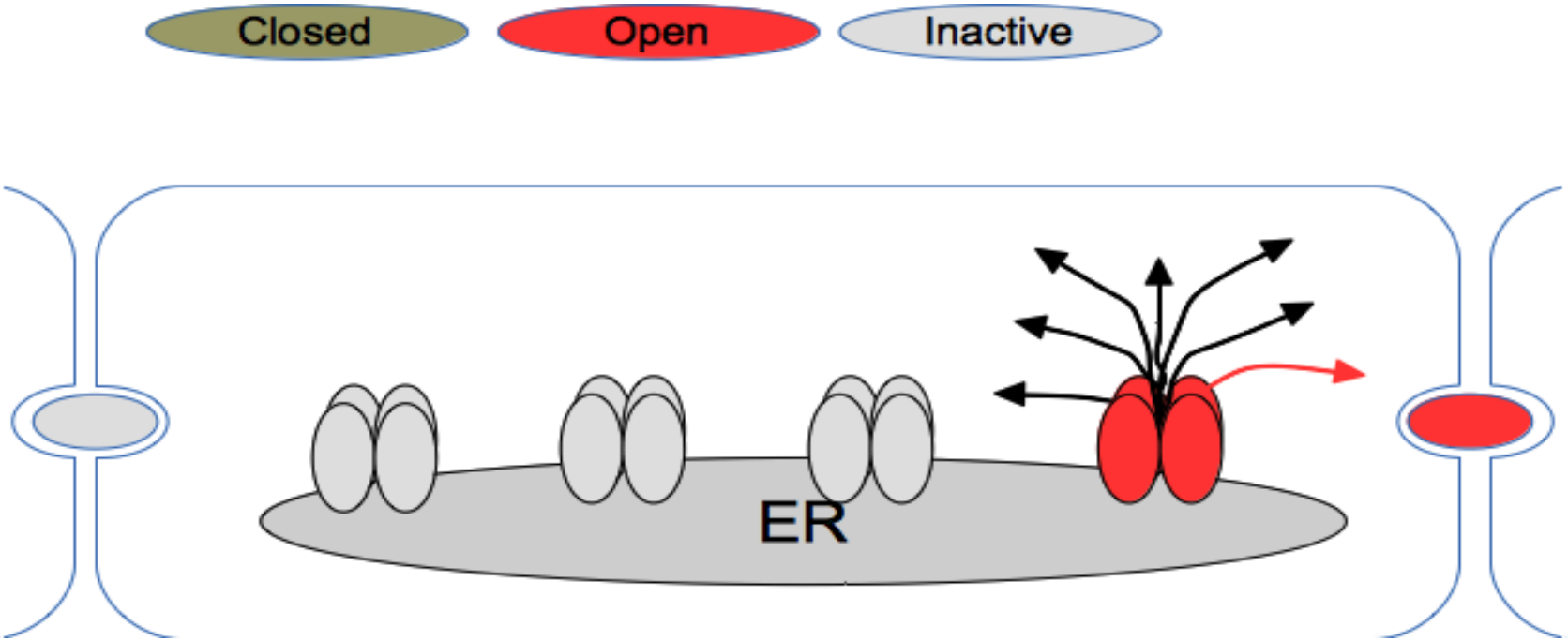








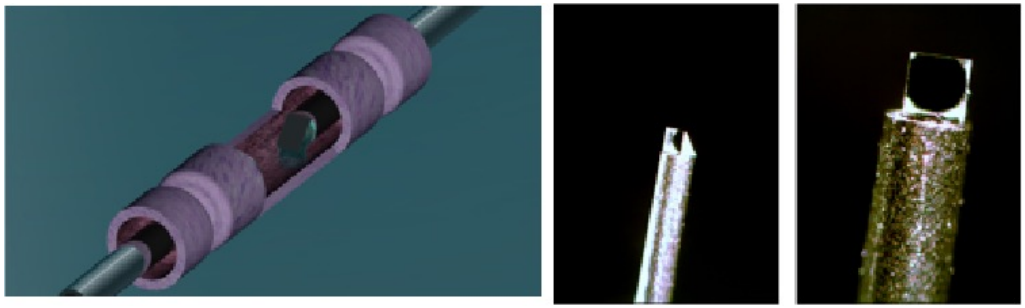


Visualising the Endothelium from inside intact arteries

- Desire
  - camera in a pressurised artery
  - Cellular Resolution on curved surface
  - wide field of view and high speed
  - Operating in fluorescence

"Micro-endoscope for *in vivo* widefield high spatial resolution fluorescent imaging" C D Saunter, S Semprini, C Buckley, J Mullins, J M Girkin, Biomedical Optics Express, 3, 1274, 2012

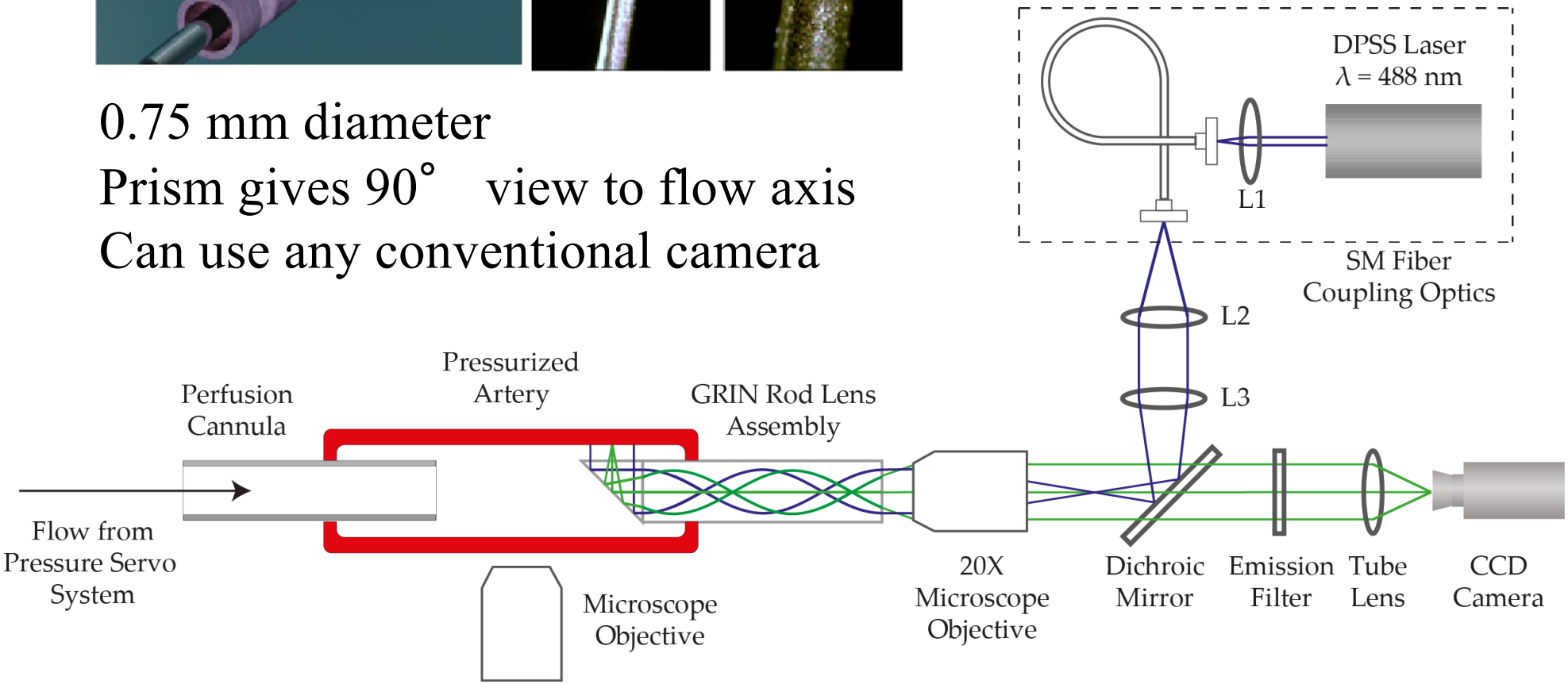
The Technology



0.75 mm diameter

Prism gives 90° view to flow axis

Can use any conventional camera



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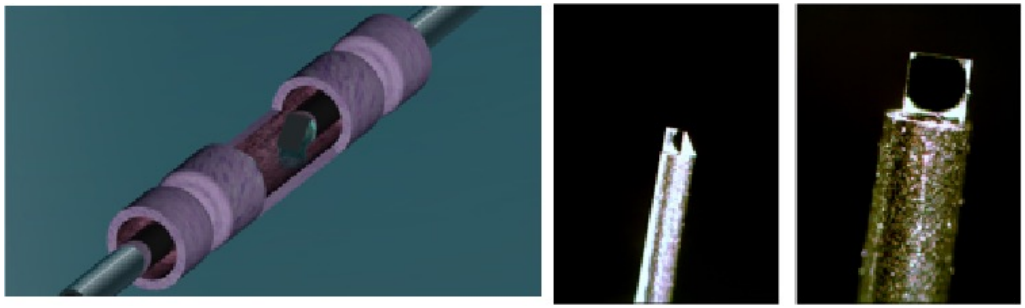
The Technology



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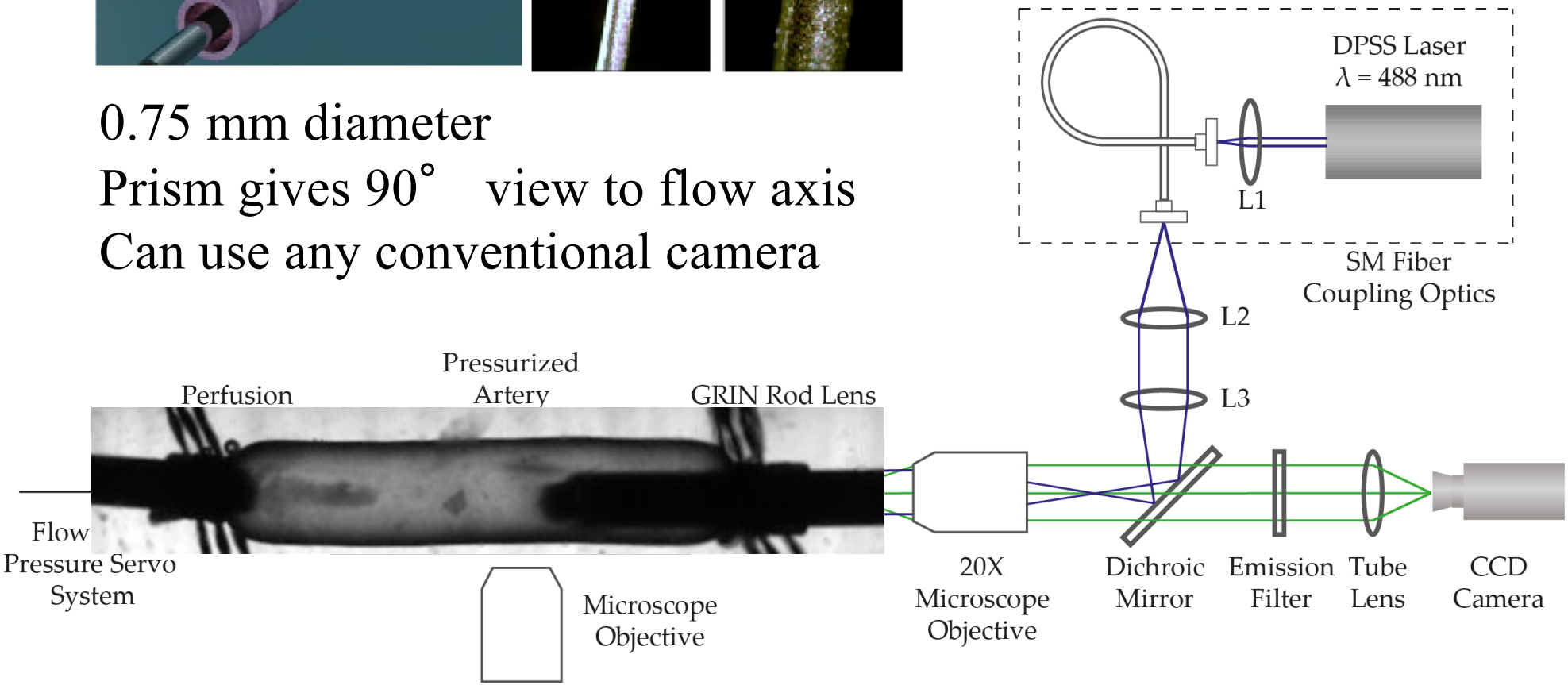

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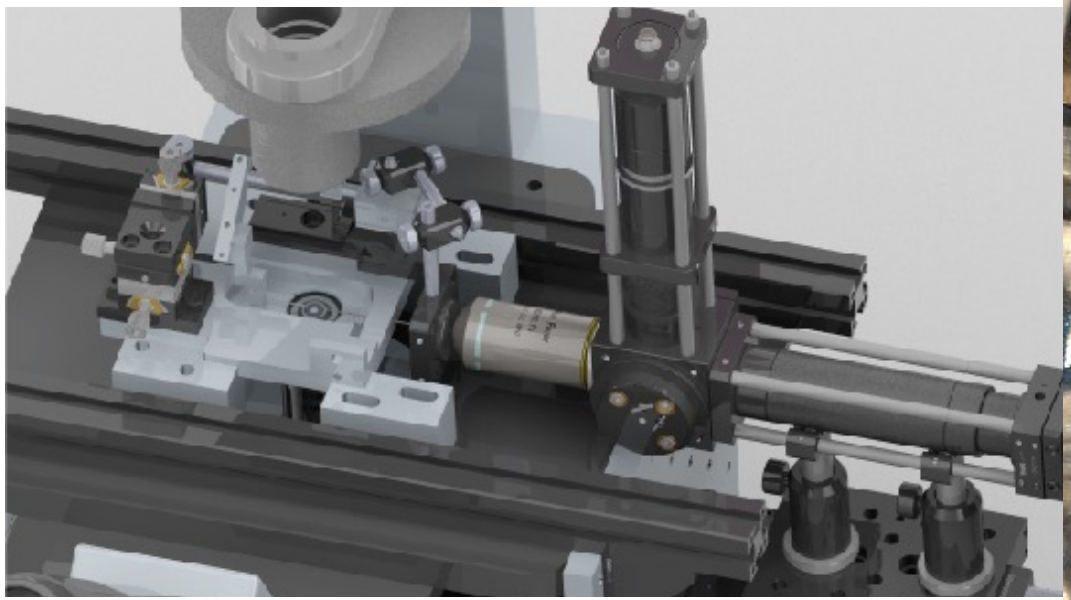
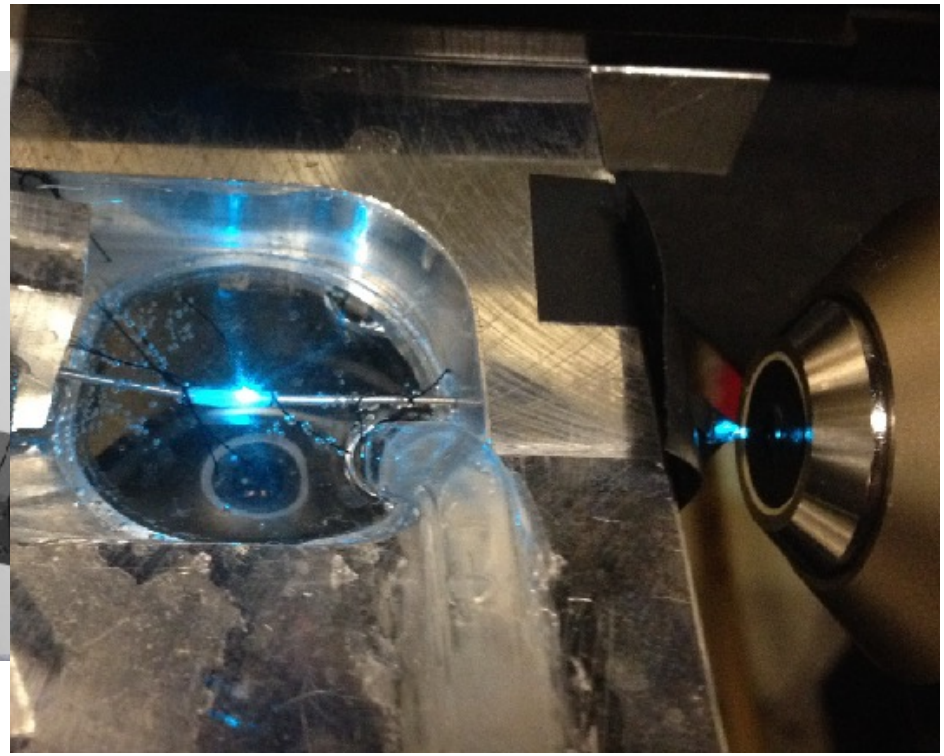
In Use





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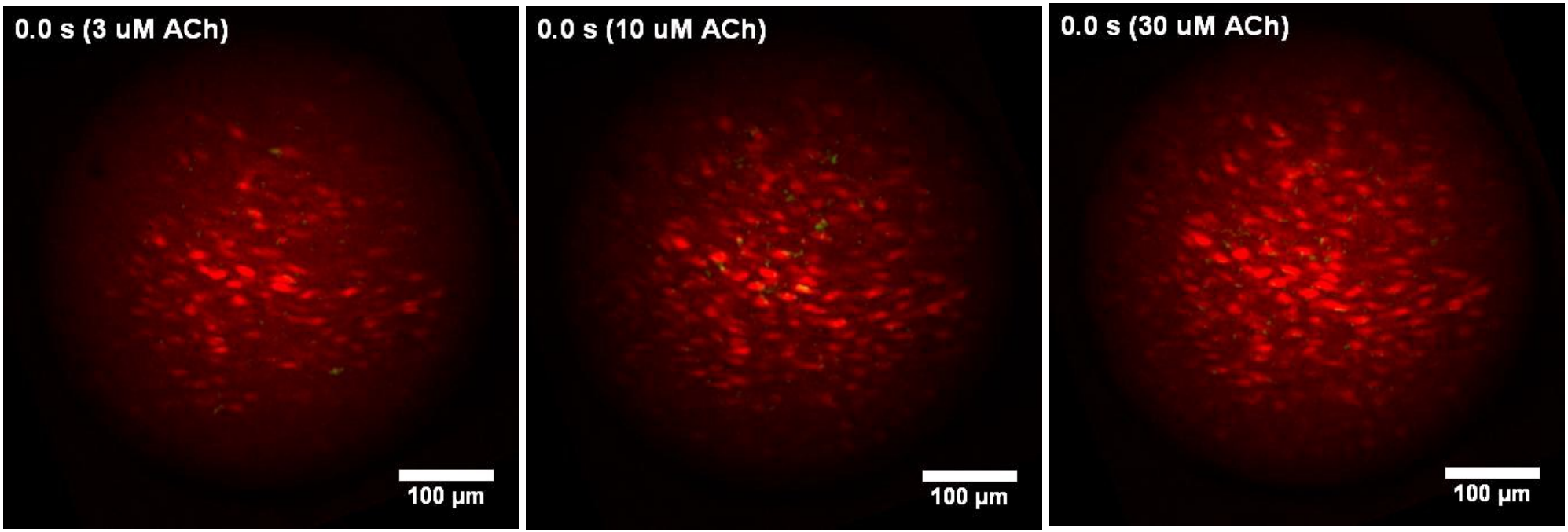
ACh Dose Response

Large field of view (~200 endothelial cells)



"Clusters of specialized detector cells provide sensitive and high fidelity receptor signaling in the intact endothelium, C. Wilson, C. D. Saunter, J. M. Girkin, and J. G. McCarron, " FASEB J., vol. 30, pp. 1–14, 2016.

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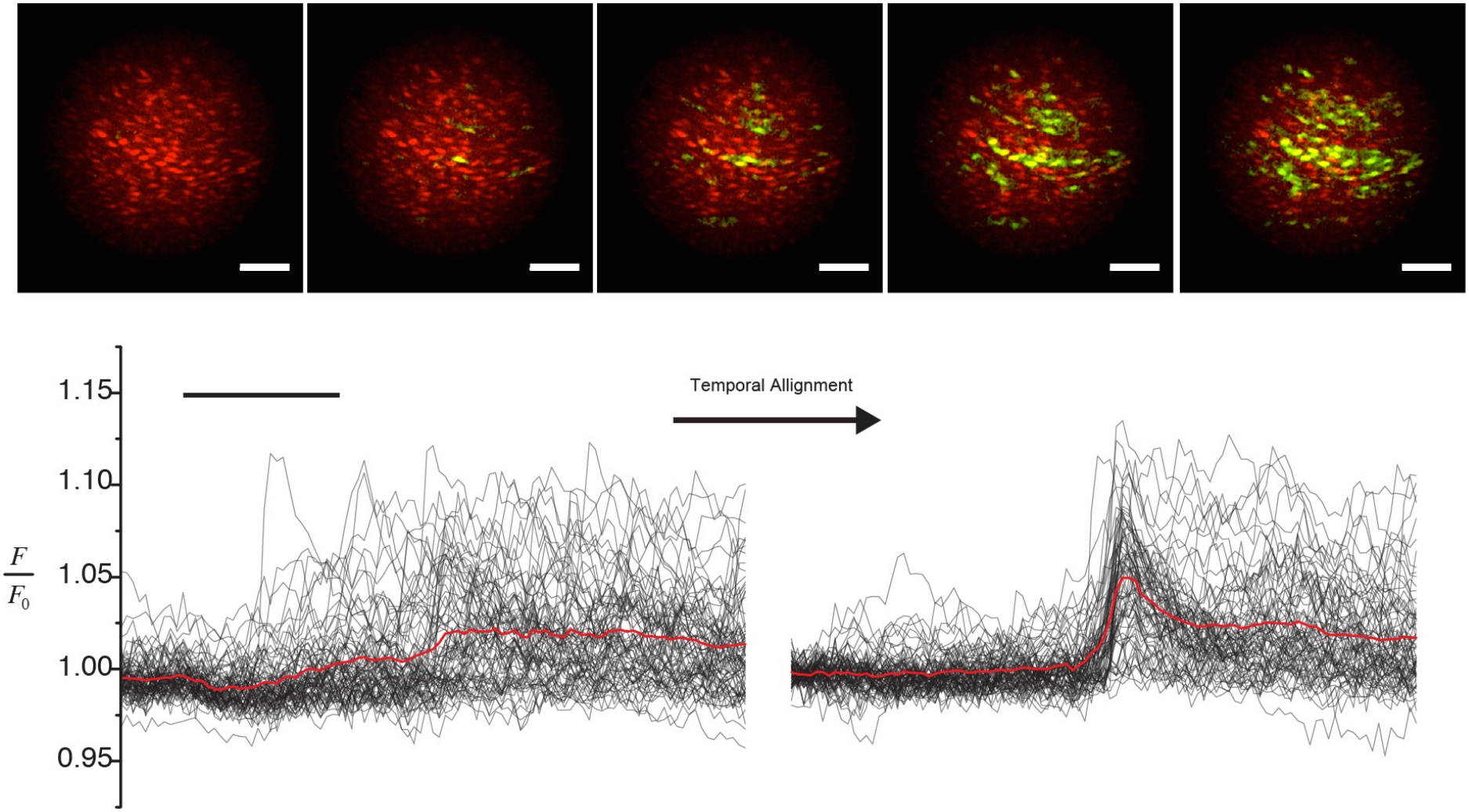
Data Analysis:- Time Synchronisation



Align peak rise of each signal

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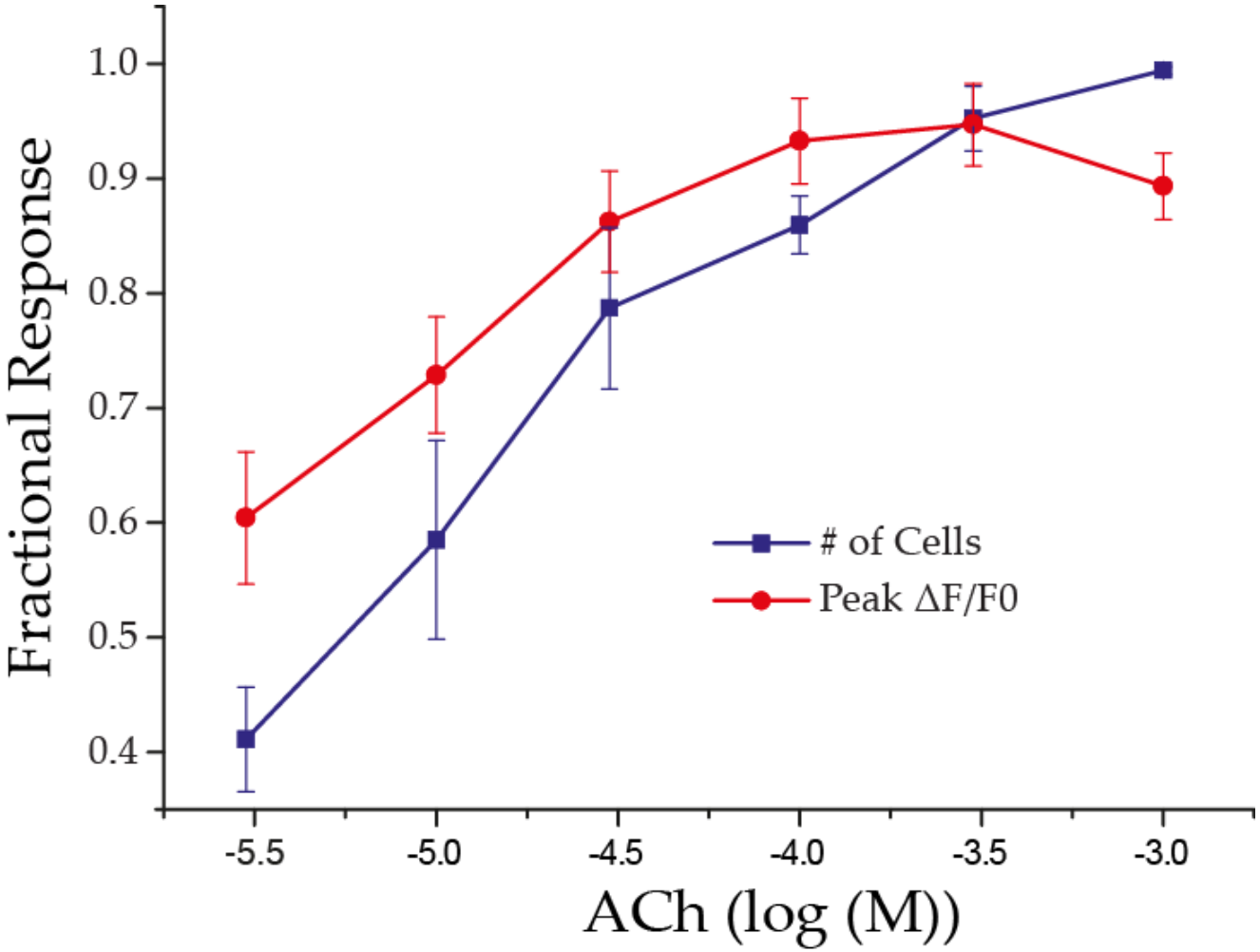
ACh Dose Response: More cells respond & magnitude of response increases



Ca^{2+} rise originated from an IP_3-sensitive Ca^{2+} store as signal went when blocked, caffeine no effect not RyR

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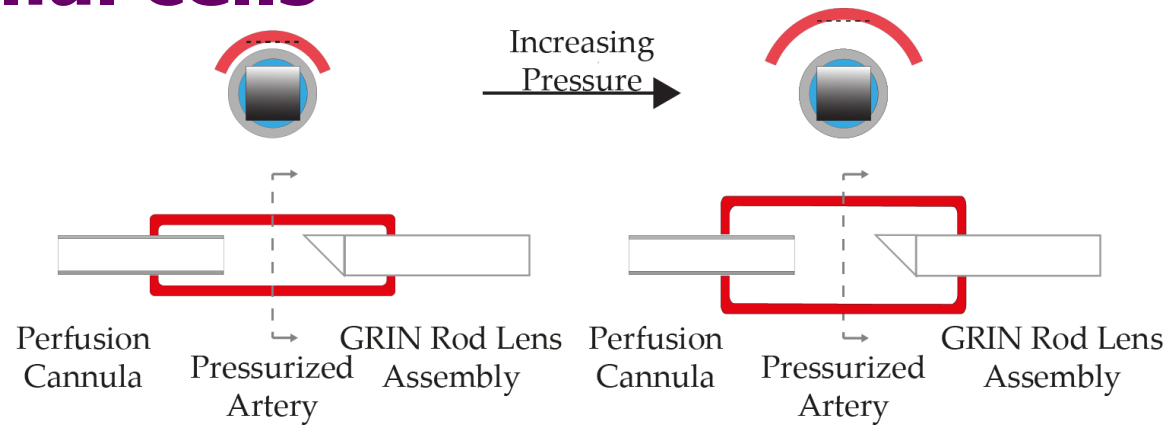
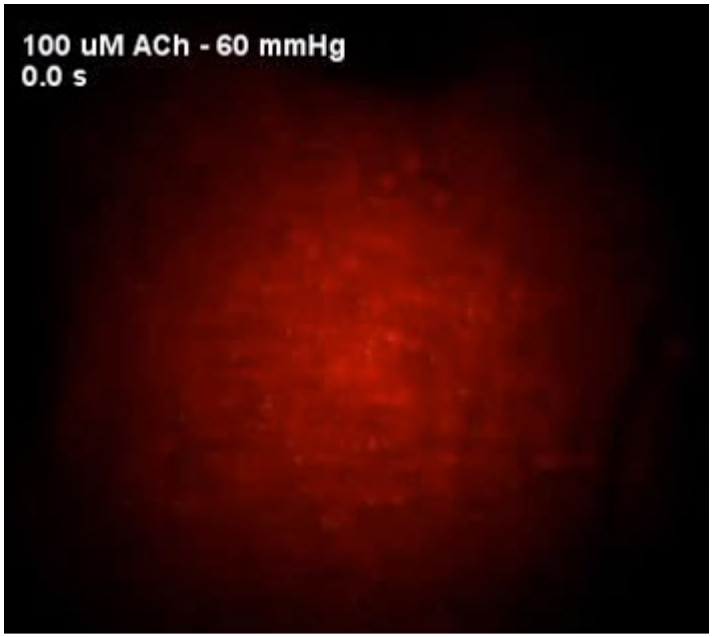
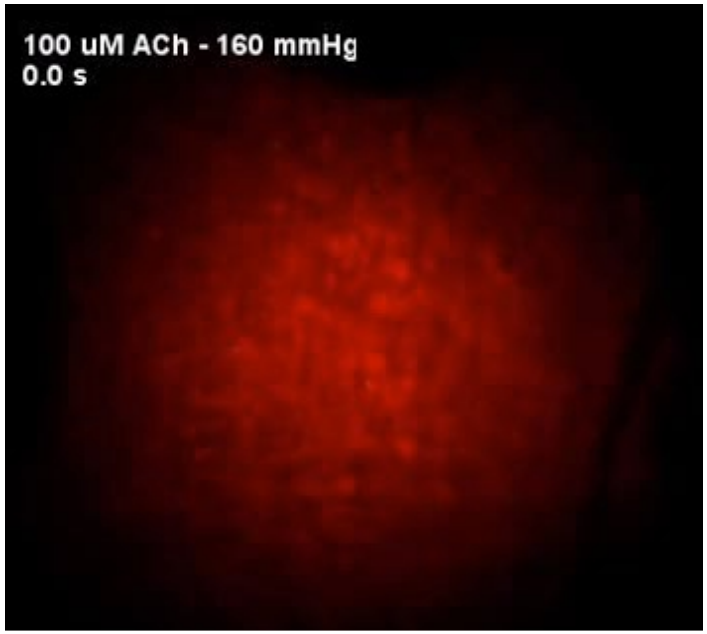
Increased pressure decreases activation of endothelial cells







60 mmHg **160 mmHg**

"Pressure-dependent regulation of Ca 2+ signaling in the vascular endothelium," C. Wilson, C. D. Saunter, J. M. Girkin, and J. G. McCarron, J. Physiol., vol. 593, no. 24, pp. 5231–5253, 2015.

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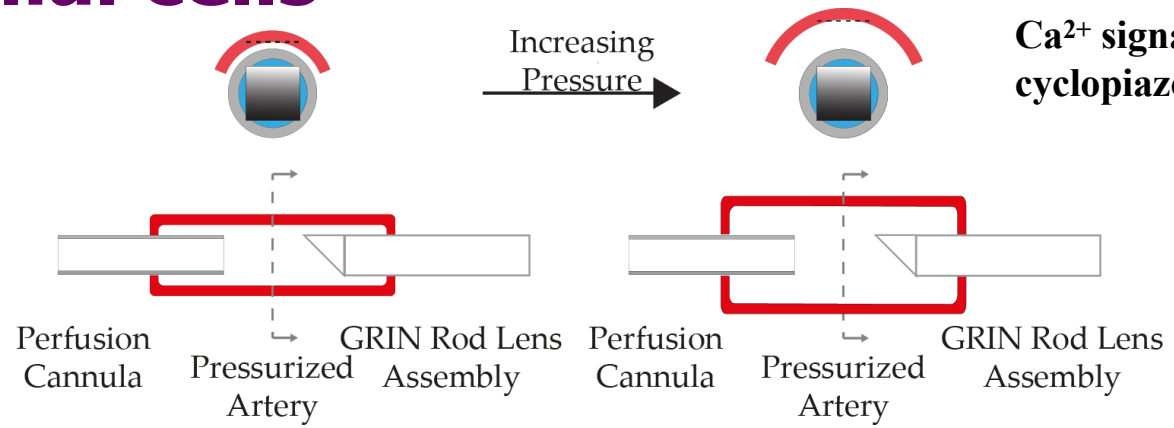
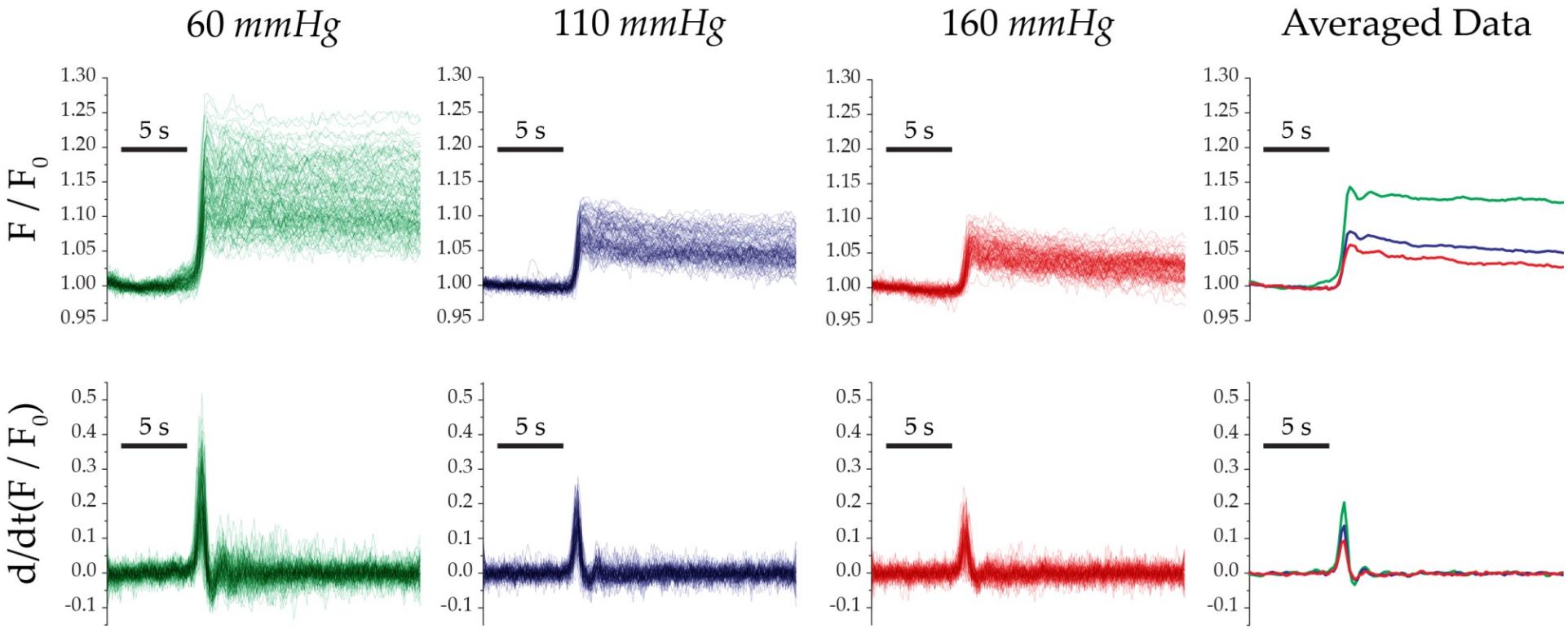


Ca^{2+} signals are abolished by cyclopiazonic acid and 2-APB



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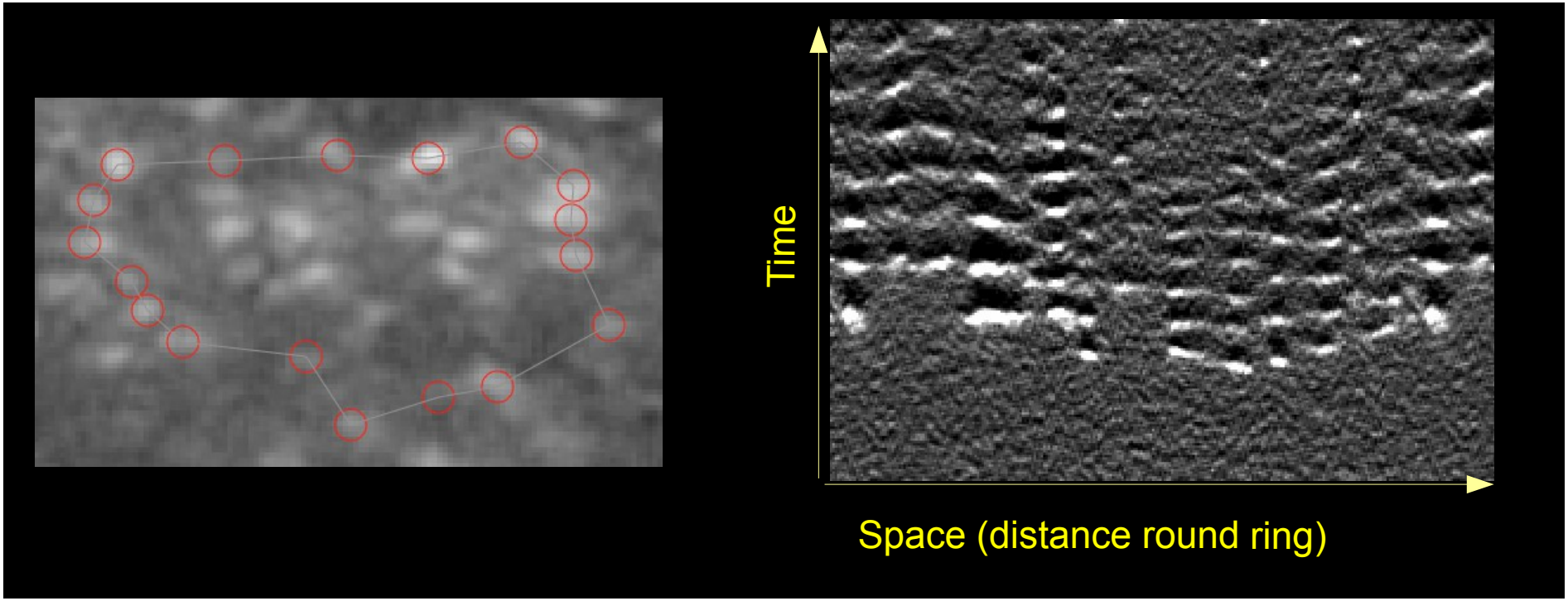
Data Analysis:
Signally via Spacing of Receptors?

- Complex but repeatable patterns seen
- Calcium computing?



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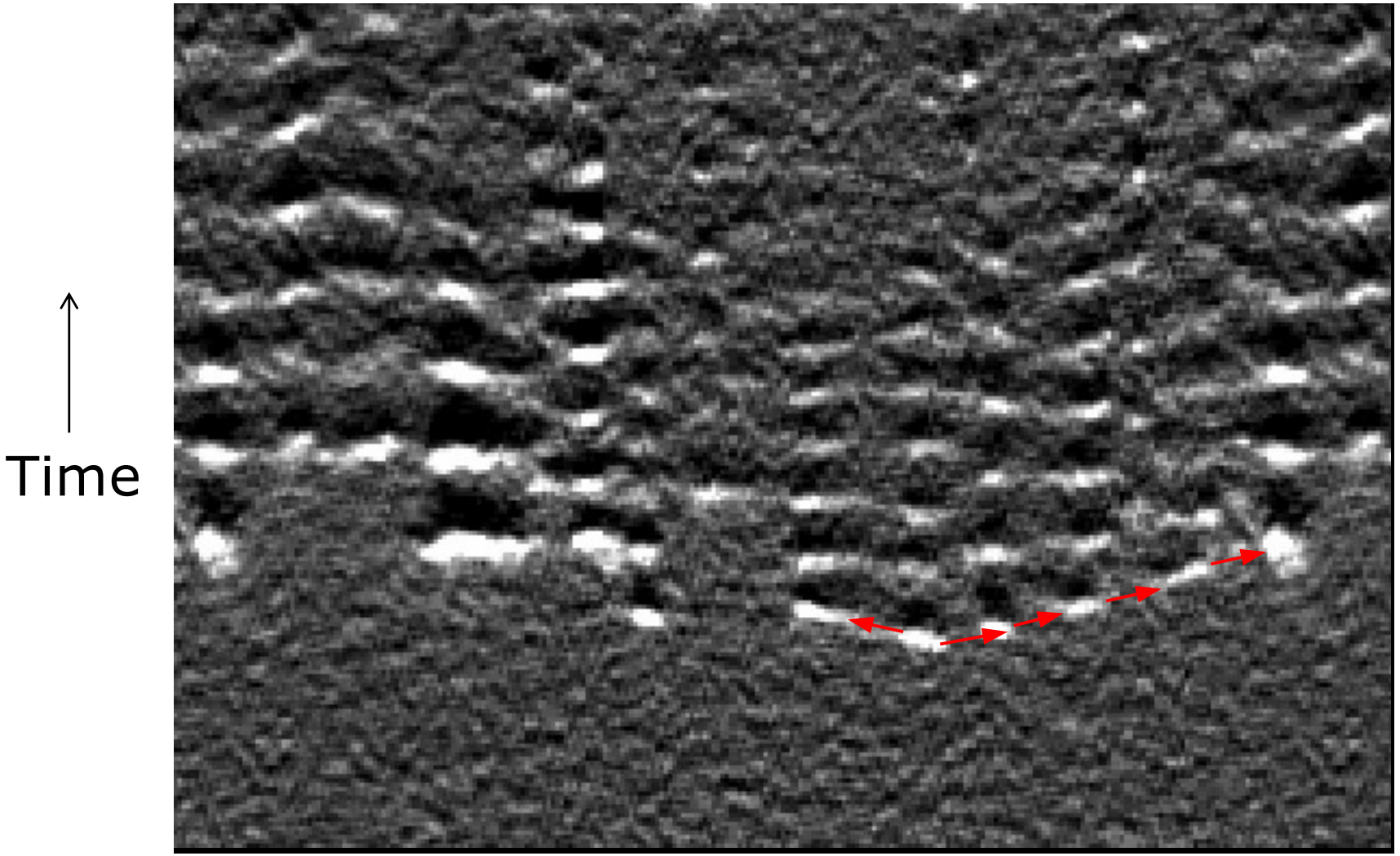
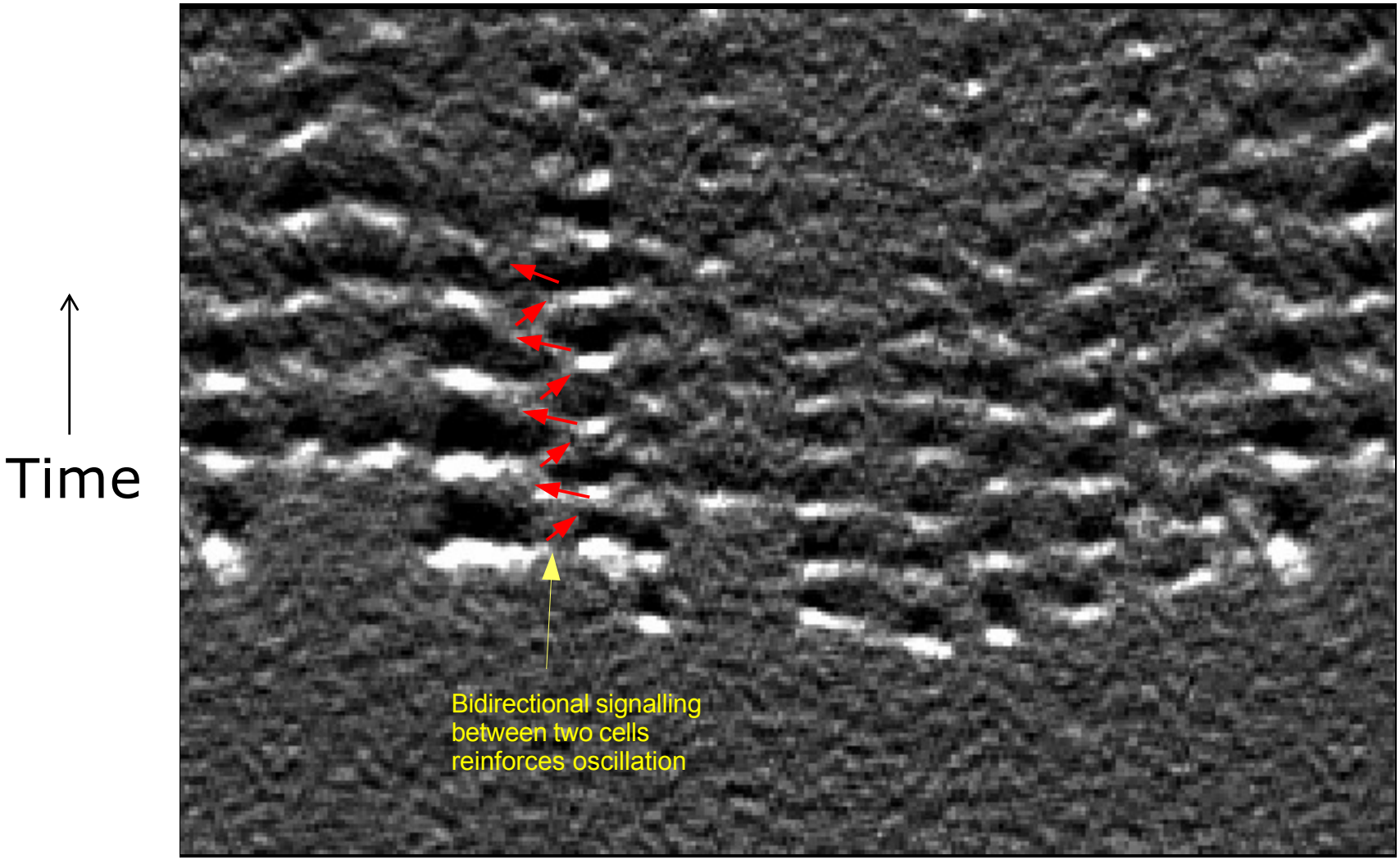
Simple Signal Pathway



Ring Oscillator = Clock or Memory?



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How does the cell sense pressure?

- Calcium release from ER to cytosol by IP_3R is reduced at higher lumen pressures

- The IP_3R receptor…
  - Is tiny - tetramer of ~300 kDa molecules
    - Pressure differential small
    - Thermal noise large
  - Is deeply embedded within a cell
  - Has no known mechano-sensor

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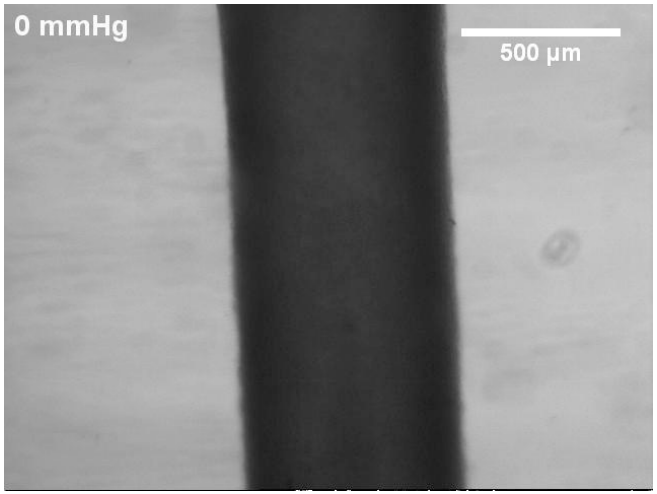
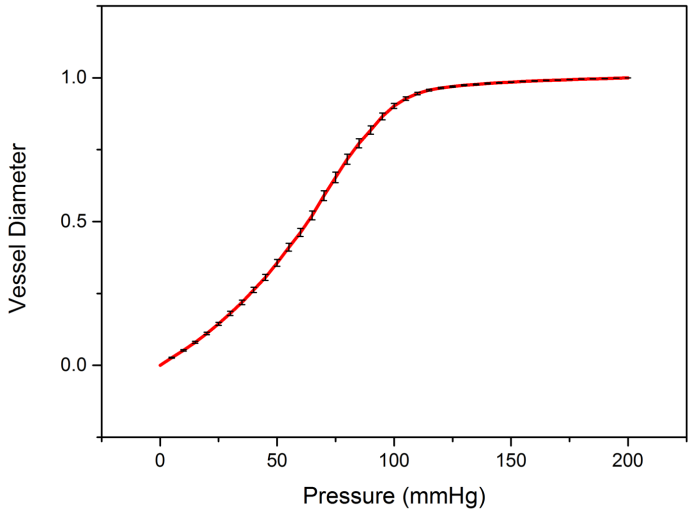
Working hypothesis

- Artery expands with pressure
- Cells stretched in width
- Conservation of volume
  - Cell must thin in radial direction
- Thin cells limit Ca^{2+} diffusion within IP_3R microdomains
  - Increased [Ca^{2+}]
  - Decreased conc. gradient from store
  - Decreased injection current





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Lawton, P. F. *et al.* VasoTracker, a Low-Cost and Open Source Pressure Myograph System for Vascular Physiology. *Front. Physiol.* 10, 99 (2019).

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Computer Modelling

- Diffusion model of Ca^{2+} and buffer
- Used literature figures
- Solved by simulation of coupled equations
- Model single IP_3R and micro-domain cluster
- Change height of ER to maintain constant volume

$$c(r) = c_\infty + \frac{J_{Ca}}{2\pi D_{ca}} \frac{e^{-r/\lambda}}{r} \qquad \frac{\partial C_s}{\partial t} = D_s \nabla^2 C_s + \phi_s + J_s$$

$$J_{Ca,cyt} = J_{leakage,er} + J_{SERCO,er} + J_{IP^3R,er} + J_{leakage,pm}$$

$$\phi_{Ca,cyt} = -K_{on}.C_{Ca,cyt}C_{B,cyt} + K_{off}C_{CaB,cyt}$$

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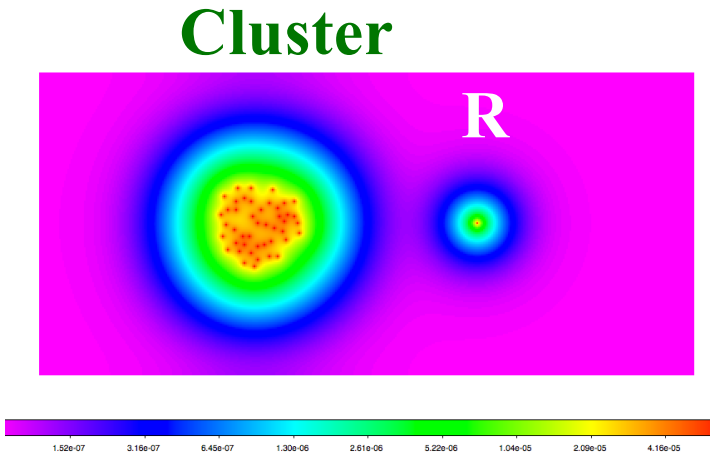
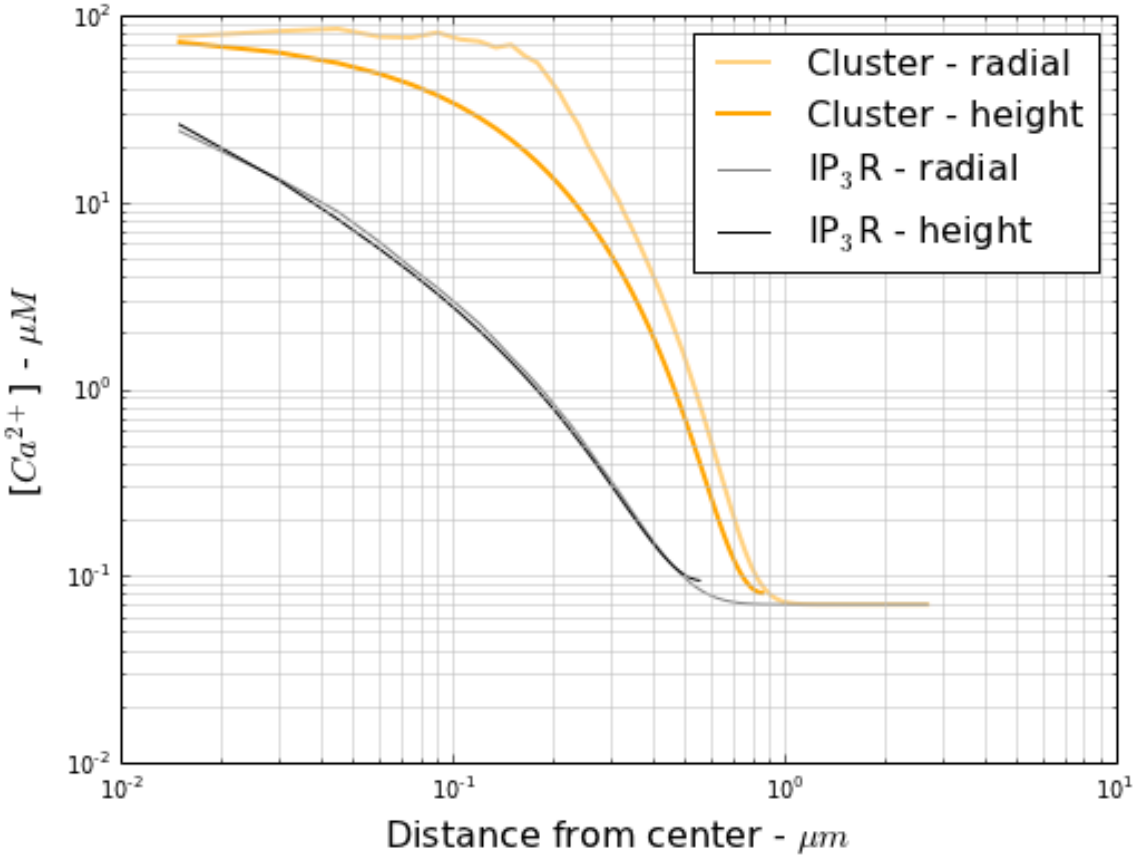
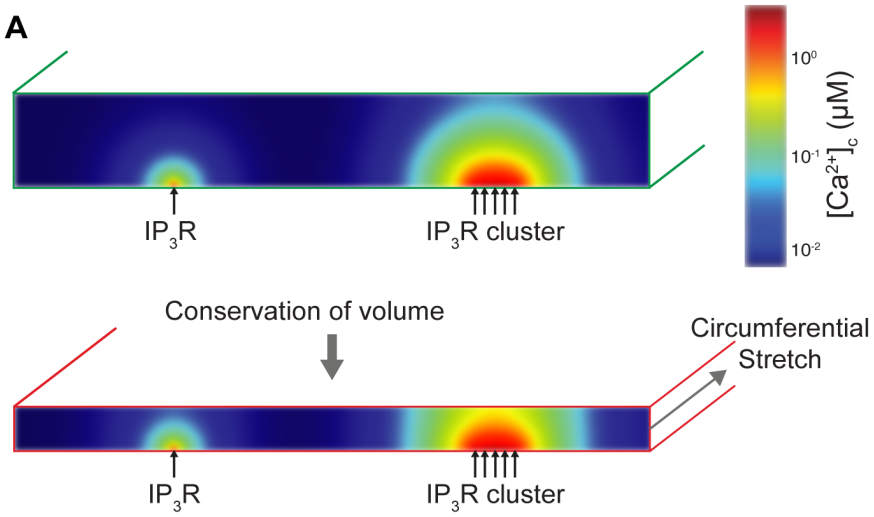
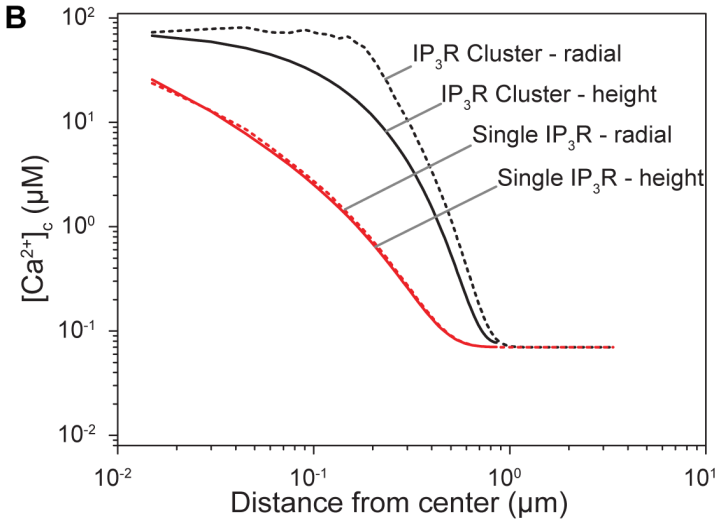
Individual vs clustered IP_3R

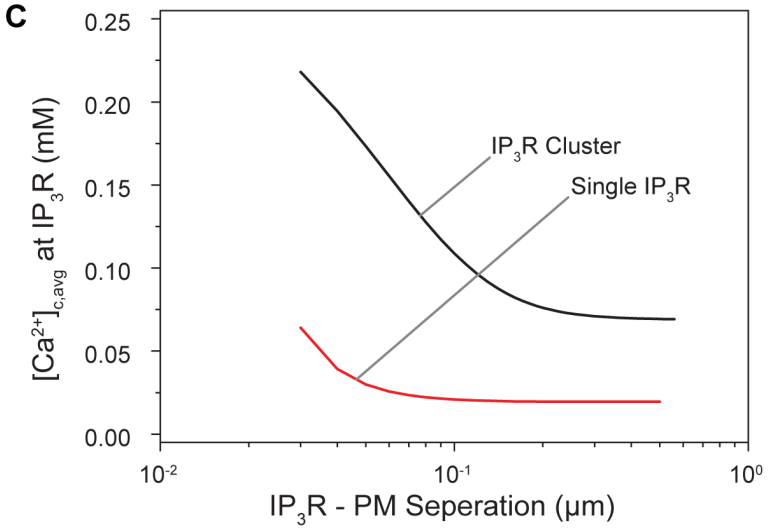


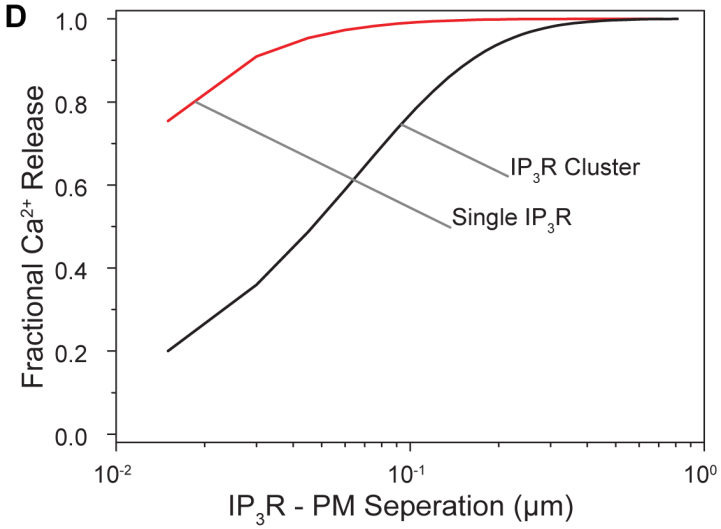


Modelling Results









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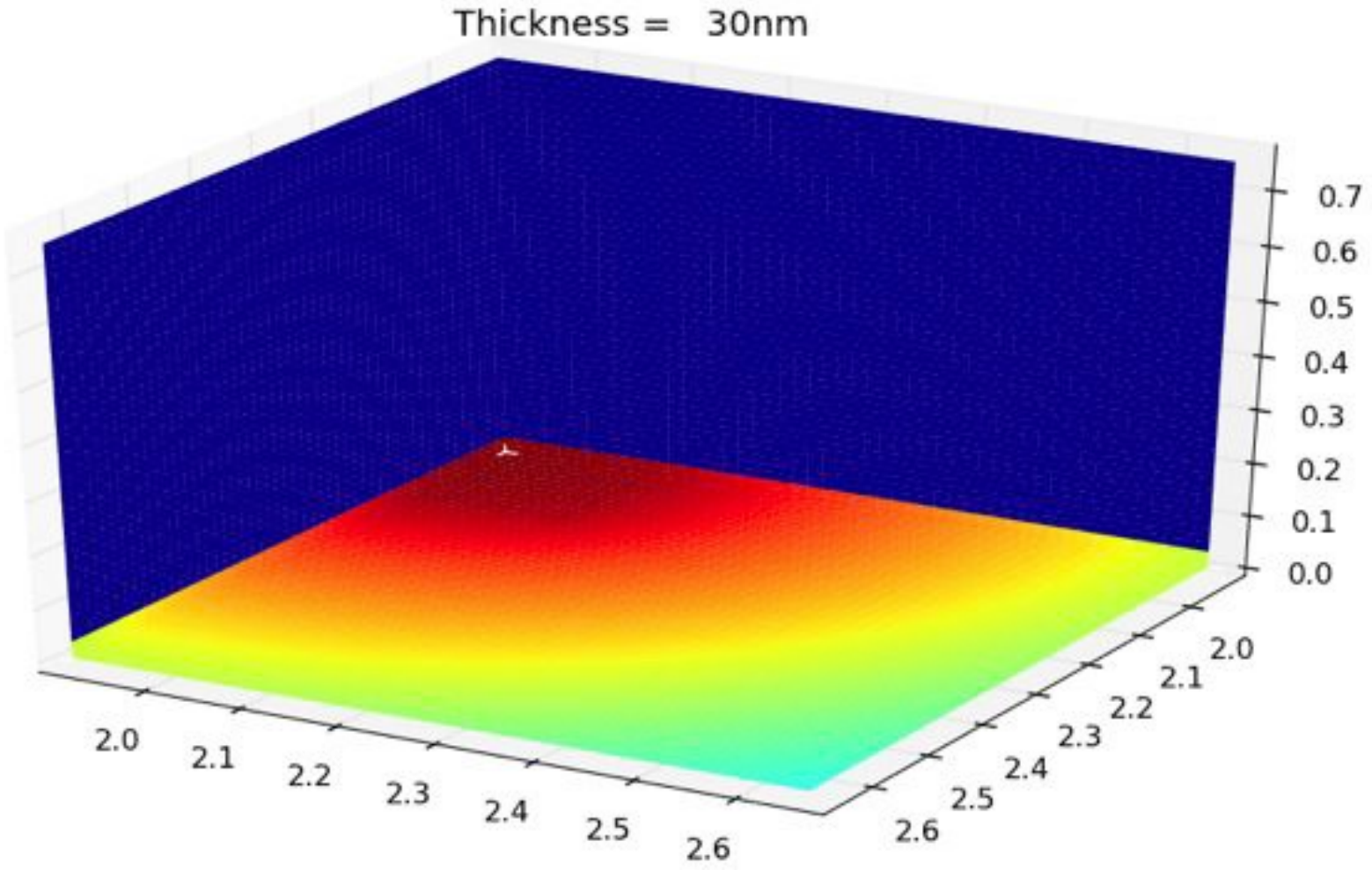
[Ca2+] at a cluster under thinning



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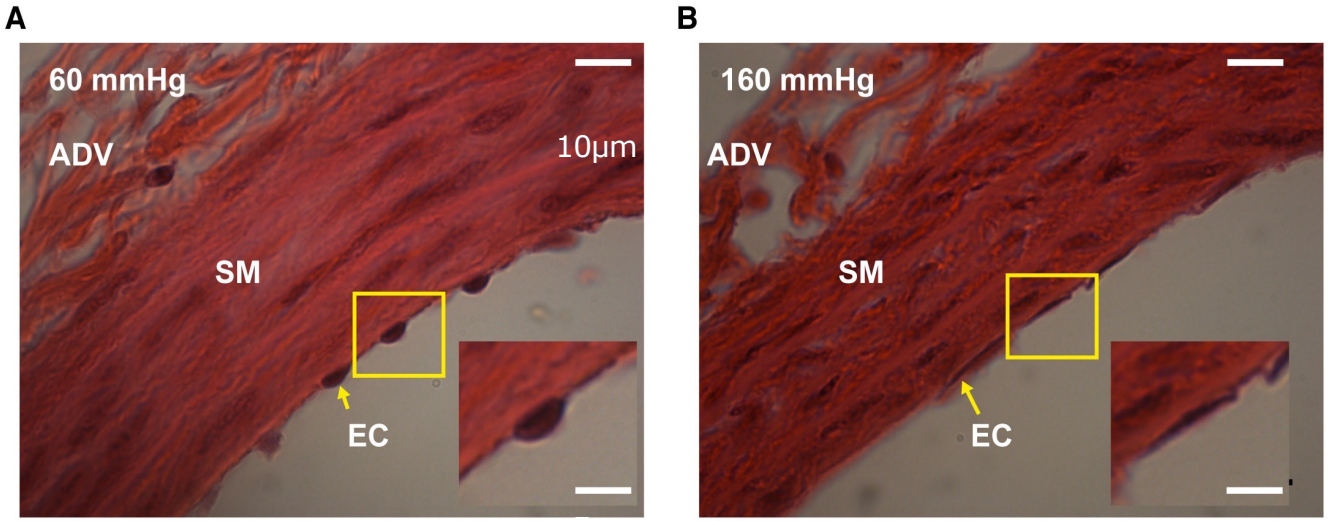
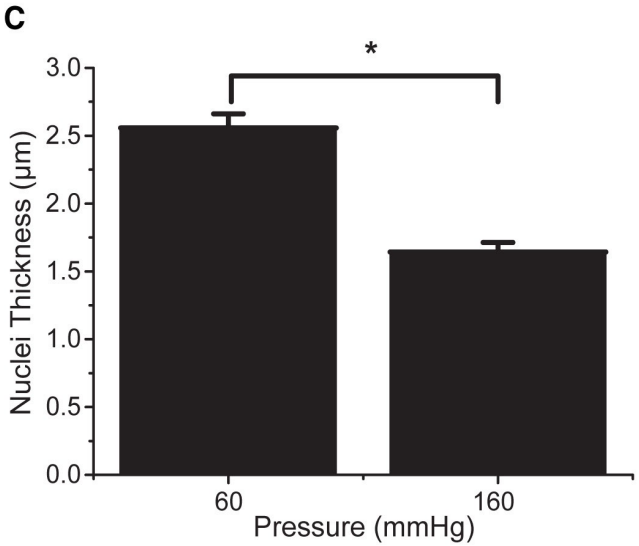
Fixed Tissue Imaging: Supporting Evidence





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Summary

- Now looking at signally through to smooth muscle cells
- Remember your core science skills
- Ask questions and discuss openly
- Vascular Pressure Sensing possible without direct sensors
- Simple Models often work

Lawton, P. F. *et al.* Multi-plane remote refocusing epifluorescence microscopy to image dynamic Ca 2 + events. *Biomed. Opt. Express* 10, 5611–5624 (2019).

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- **University of Durham**
  - Dr Chris Saunter, Dr Penny Lawton

EPSRC

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Thank You for Your Attention

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