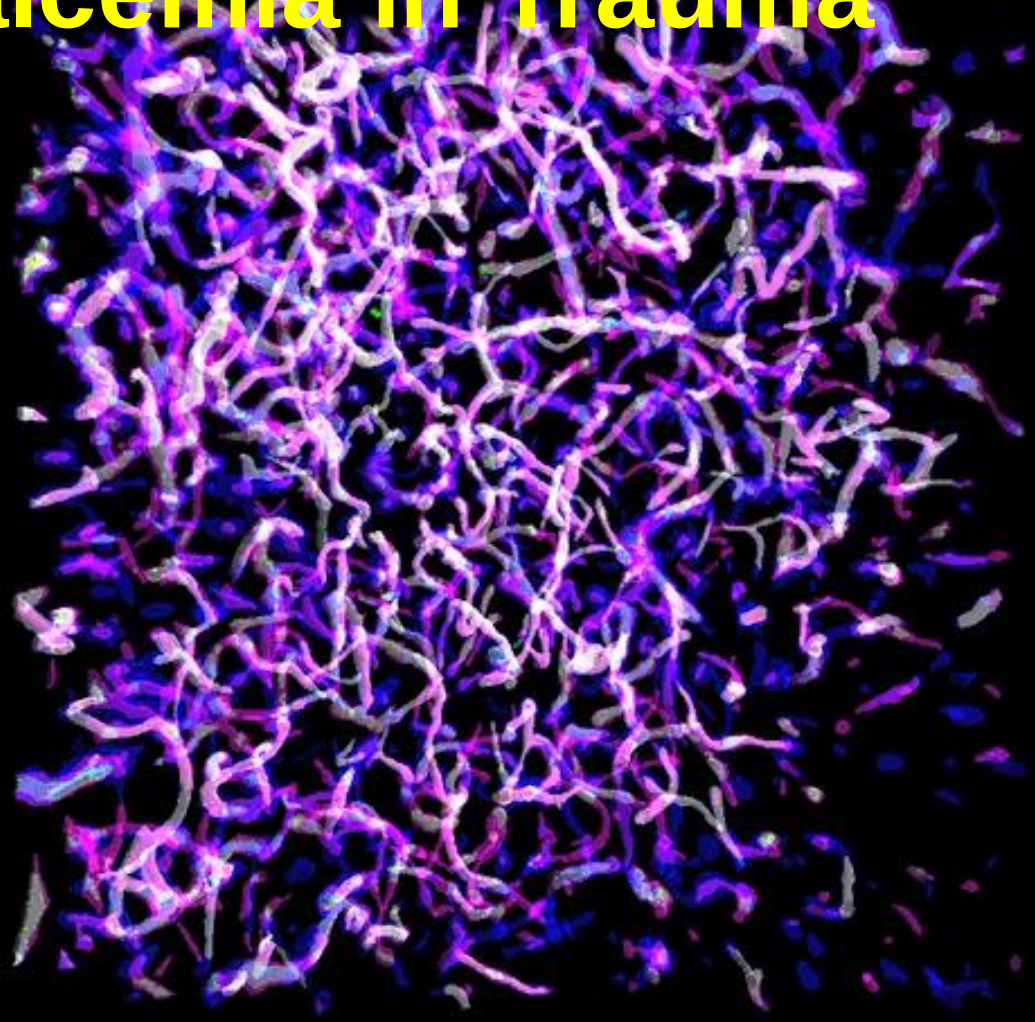
Calcium Signaling and Clinical Implications for Treatment of Hypocalcemia in Trauma



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Associate Professor Department of Emergency Medicine
University of Vermont

In the beginning…

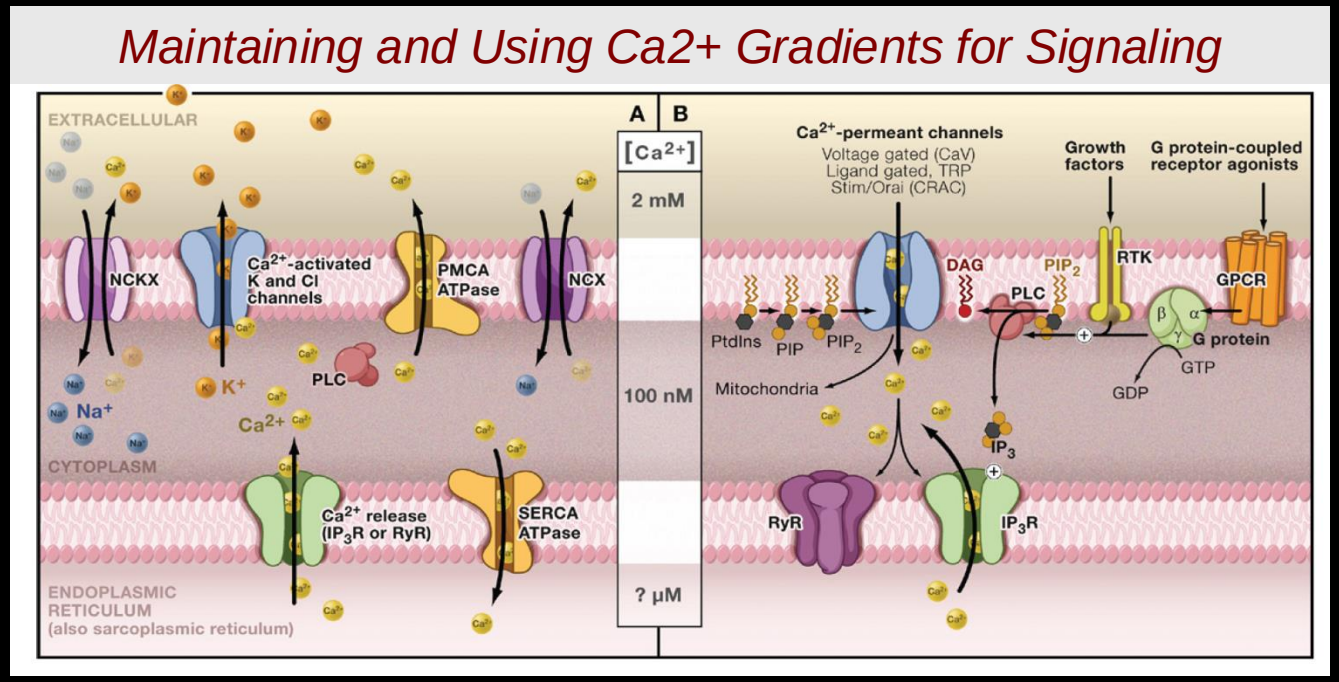
- Calcium ions are primarily created within stars through nuclear fusion processes
- The most significant release of calcium occurred when the first massive stars exploded as supernovae *>13 billion years ago.*
- The majority of calcium in the universe is present as ions (Ca^{2+}) floating between stars

Zhang L, et al. Nature (2022)

Calcium and Phosphate Ions Rule Cell Signaling

- Universal tools for signal transduction: Ca^{2+} and PO^{4-} alter local electrostatic fields and protein conformations thereby controlling protein function
- Unlike Mg^{2+}, Ca^{2+} needs to be maintained at a low level in the cytosol, because it binds water less tightly than and precipitates PO^{4-}.
- Cells invest tremendous energy to maintain a >10,000-fold gradient between their intracellular (100 nM) and extracellular (1.2 mM) calcium concentrations.

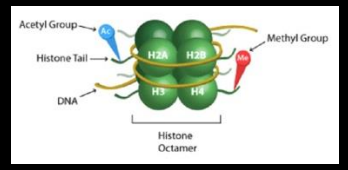
Maintaining and Using Ca2+ Gradients for Signaling

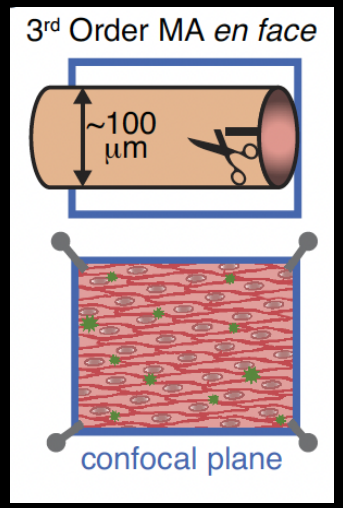


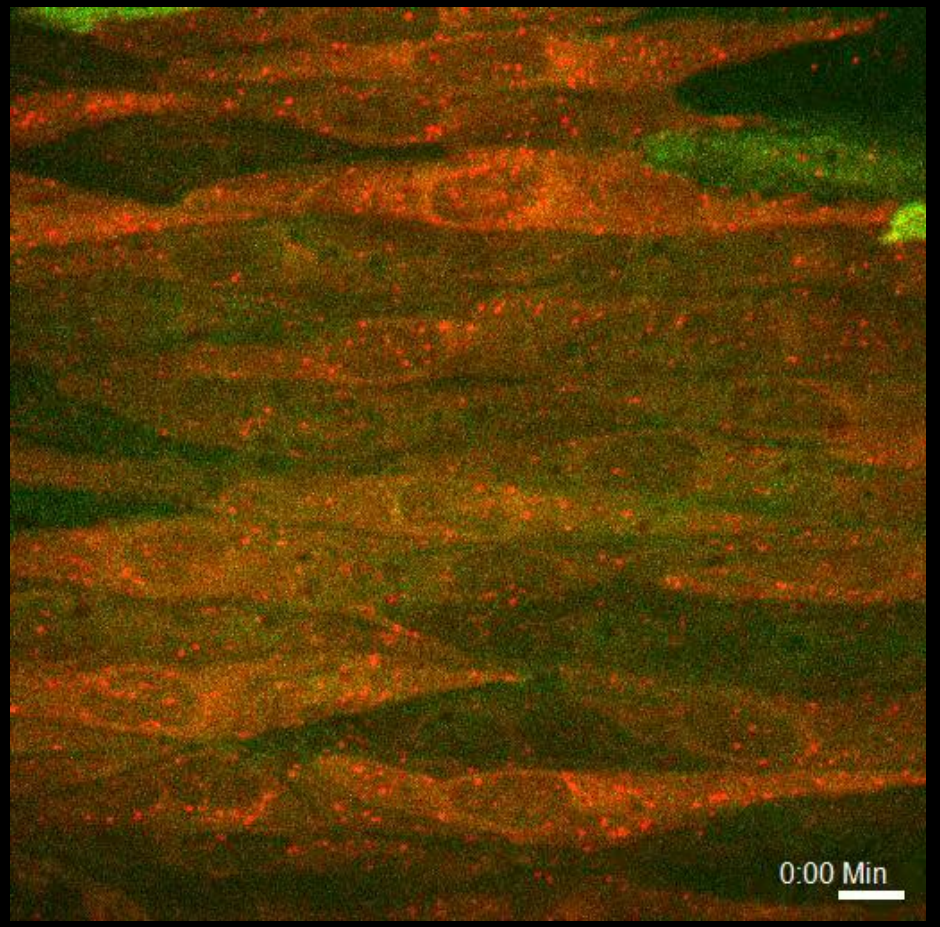
Clapham DE, Calcium Signaling, Cell (2007)

Calcium signals in endotheliopathy

Histone exposure produces massive aberrant endothelial calcium events

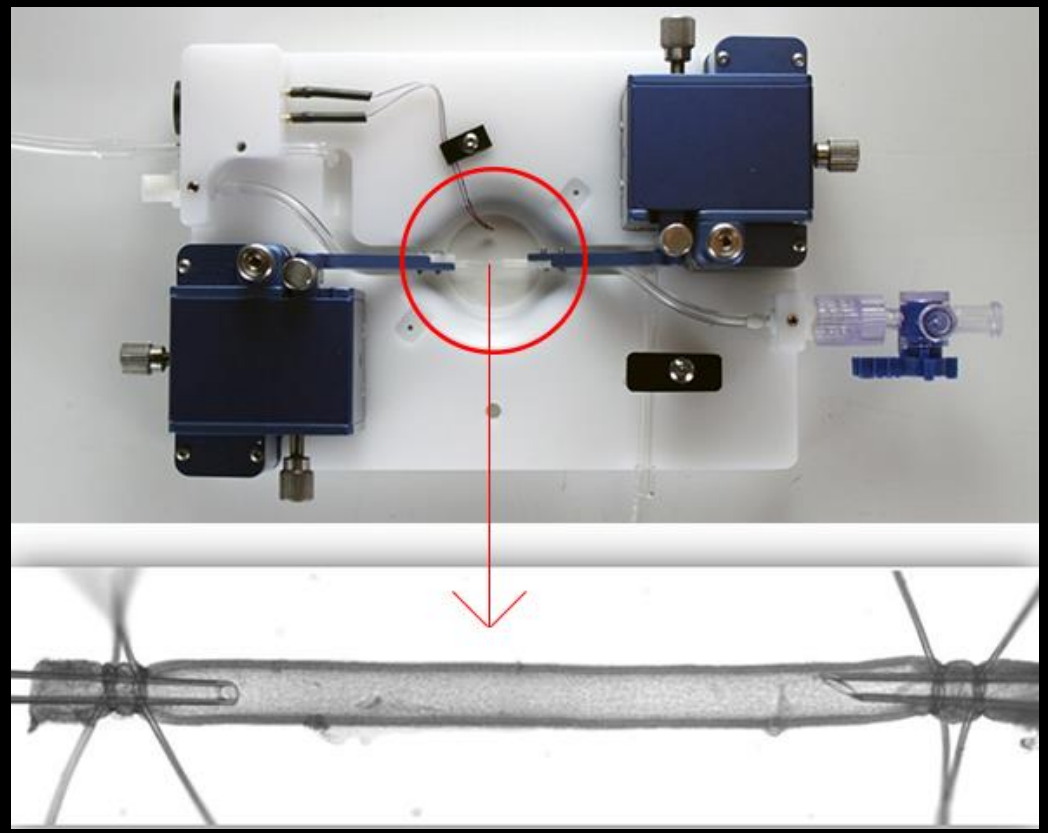


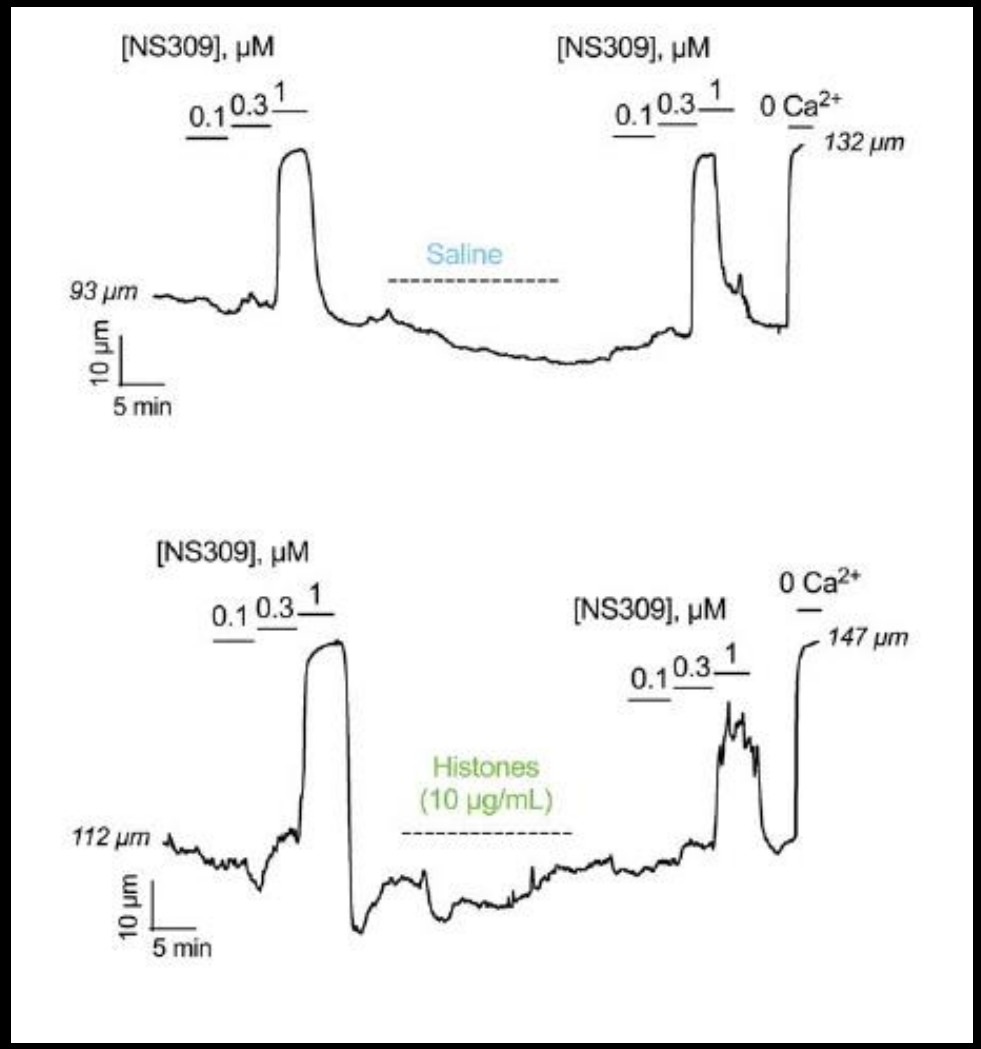




Collier, D. M. *et al.*. *Am. J. Physiol. Heart Circ. Physiol.* **316**, H1309–H1322 (2019)

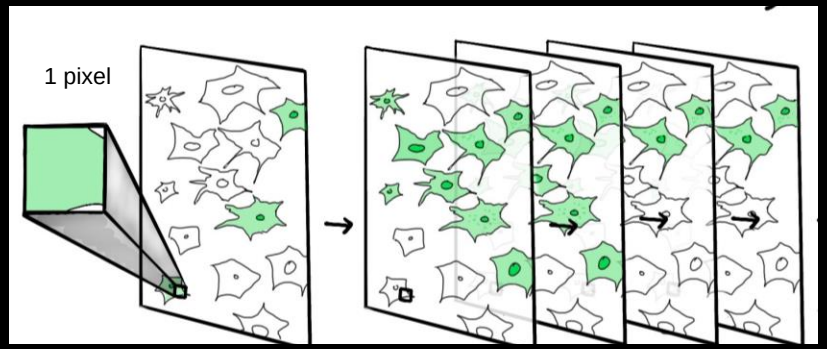
Disruption of vasodilation

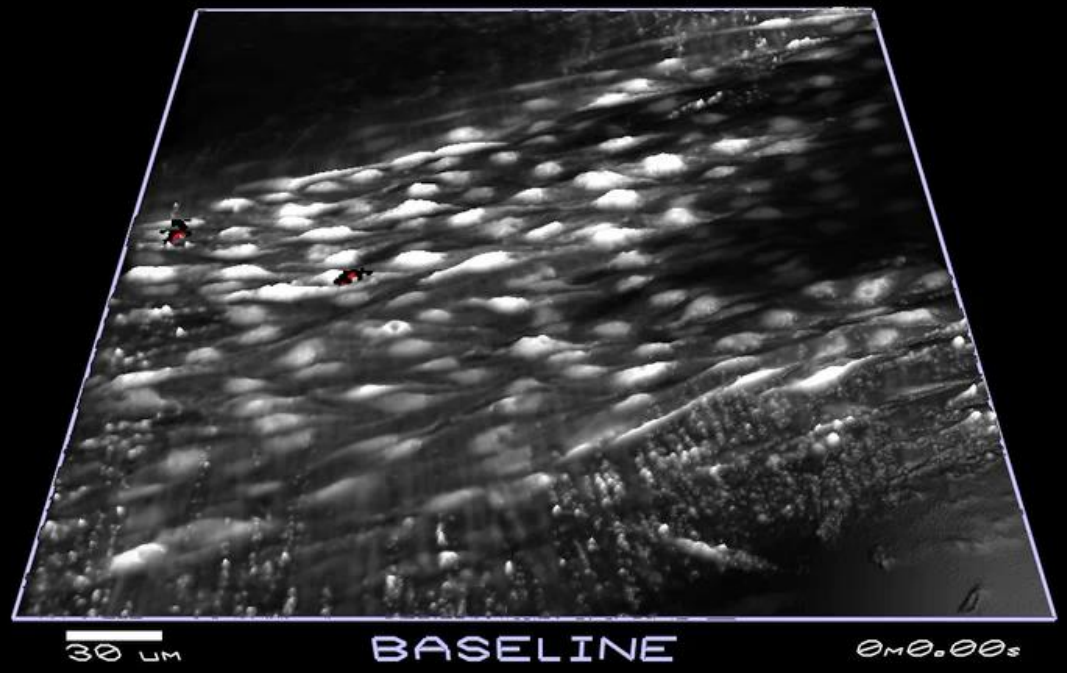




Villalba N, *et al.*. *J. of Immunology* (2023)

SD-based approach to quantify Ca2+ events



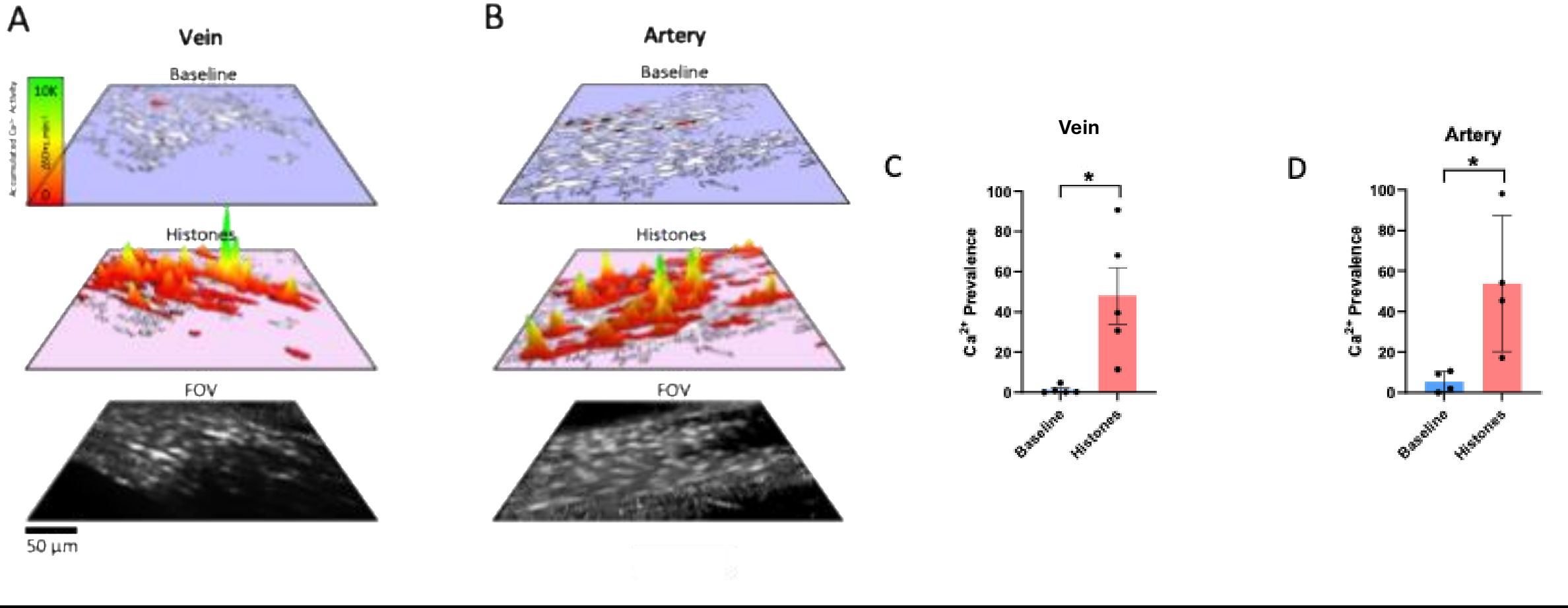


Grant Hennig

Sophie Piffard

Piffard, S.H. et al, *Shock, in press* (2024)

Ca^{2+} signals in veins and arteries are distinct

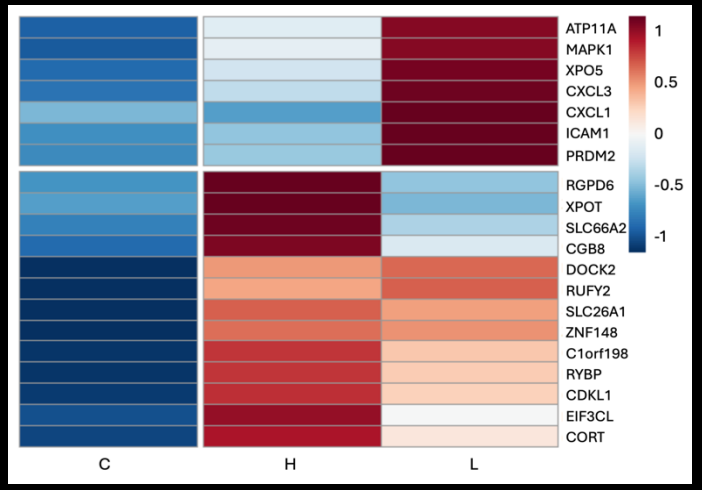


Piffard, S.H. et al, *Shock, in press* (2024)

EC responses to histones and LPS are very different

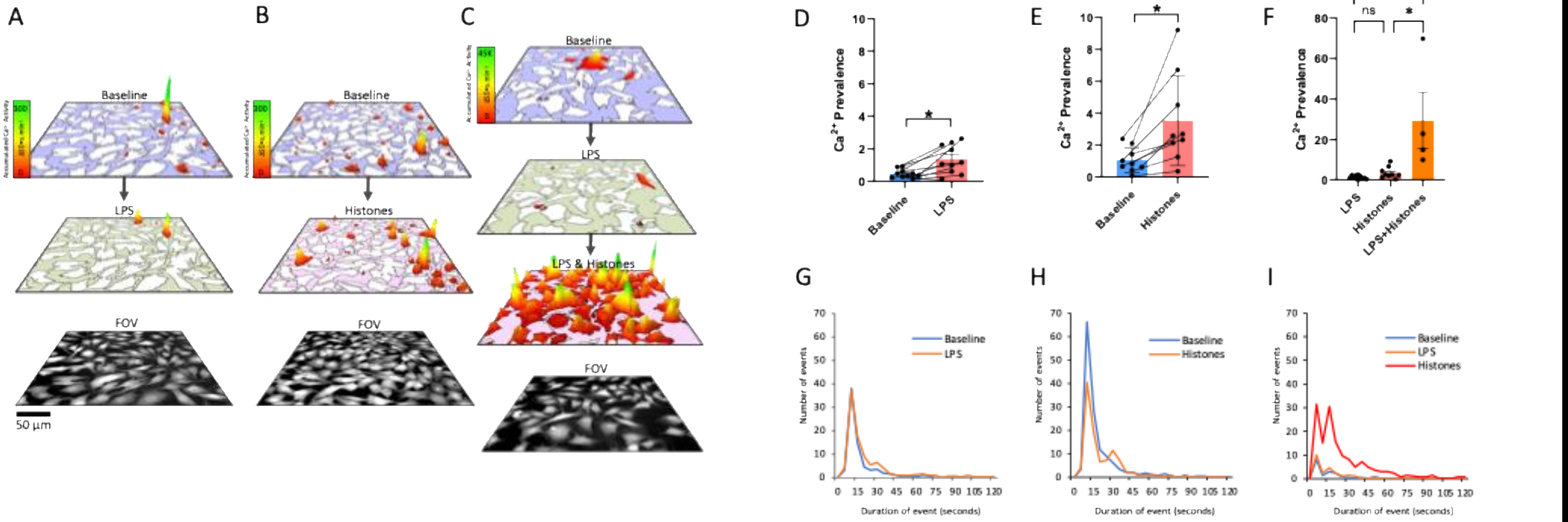
Ca^{2+} events
(5 minutes)

mRNA expression
(4 hours)



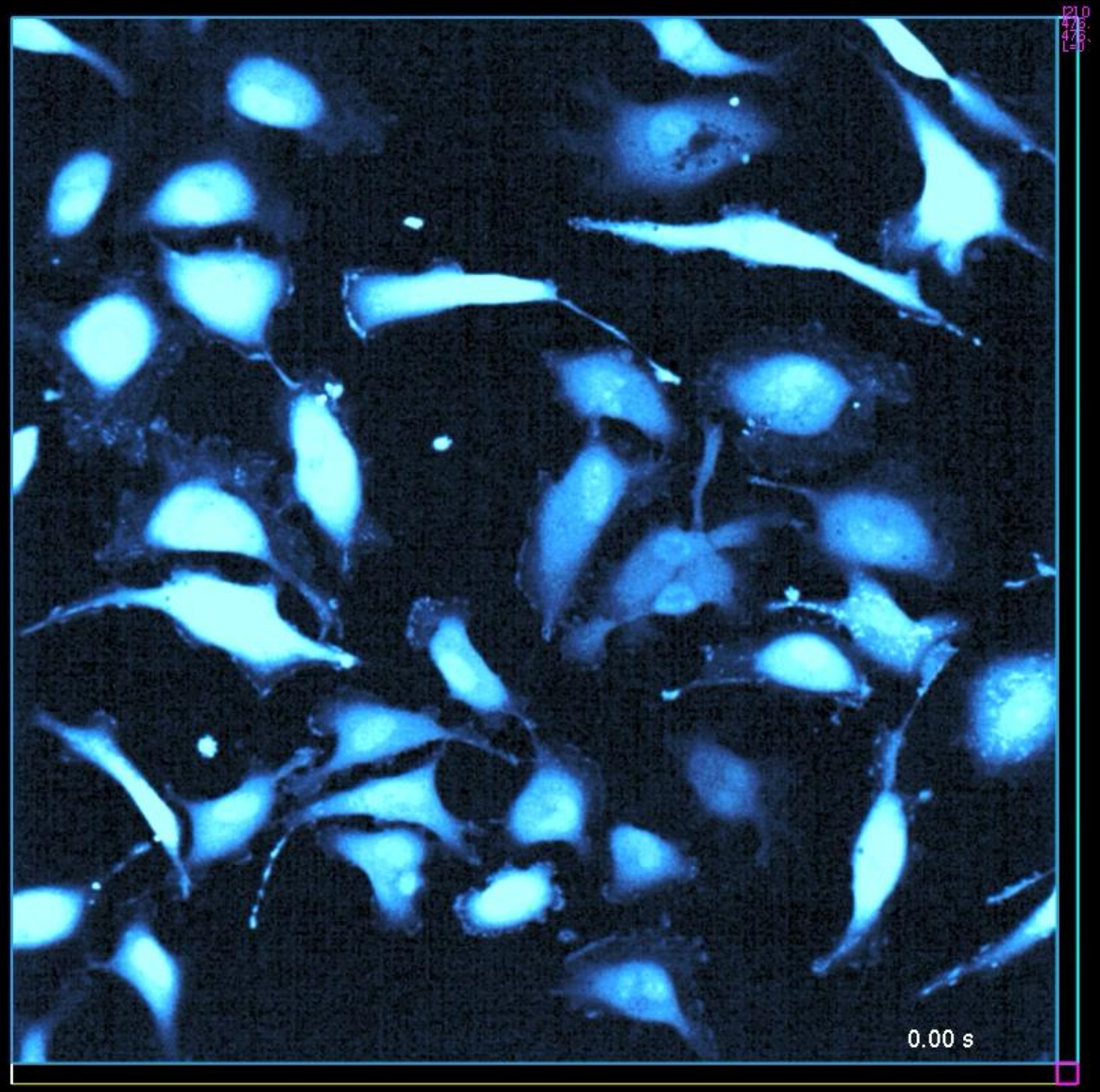
Piffard, S.H. et al, *Shock, in press* (2024)

EC responses to histones and LPS are very different

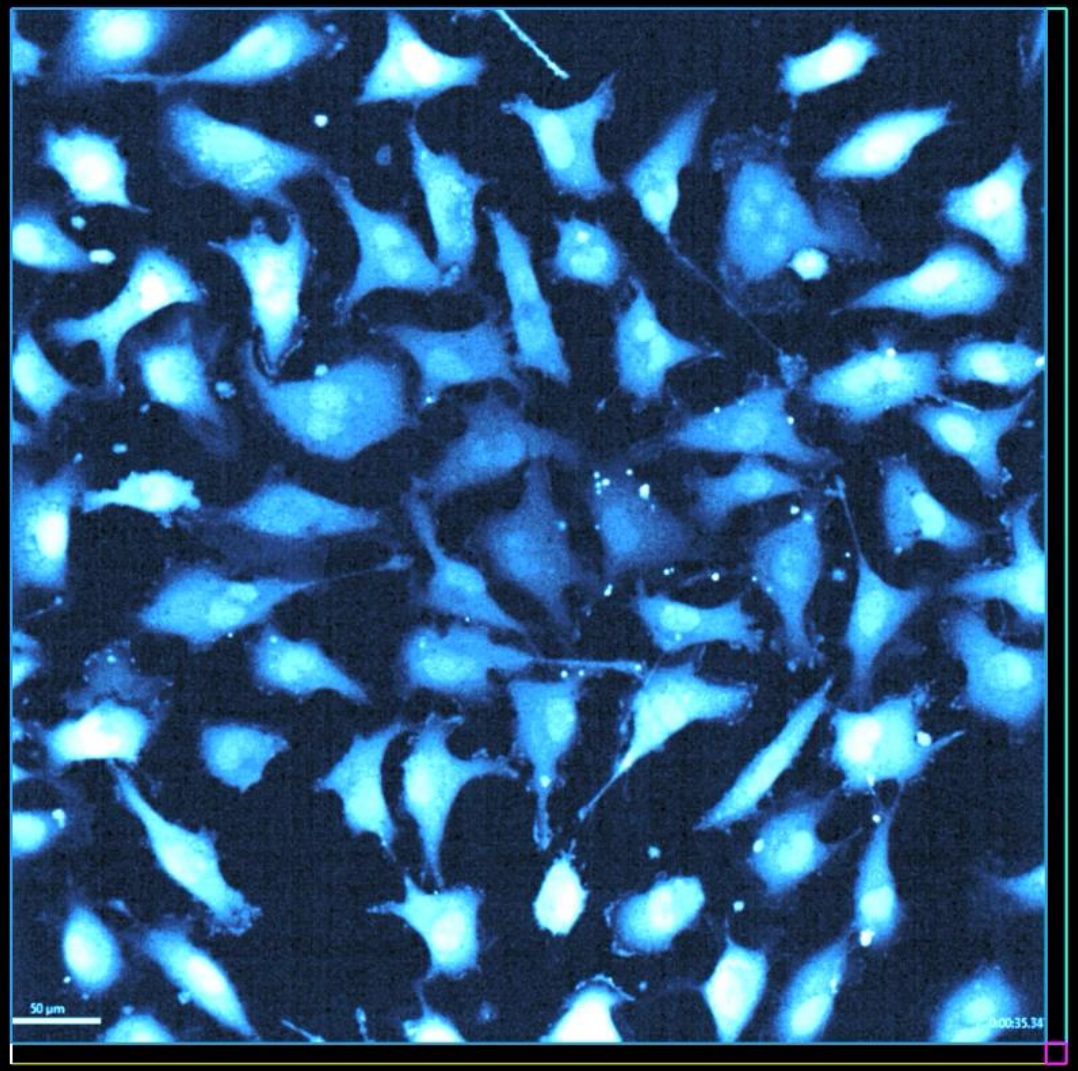


Piffard, S.H. et al, *Shock, in press* (2024)

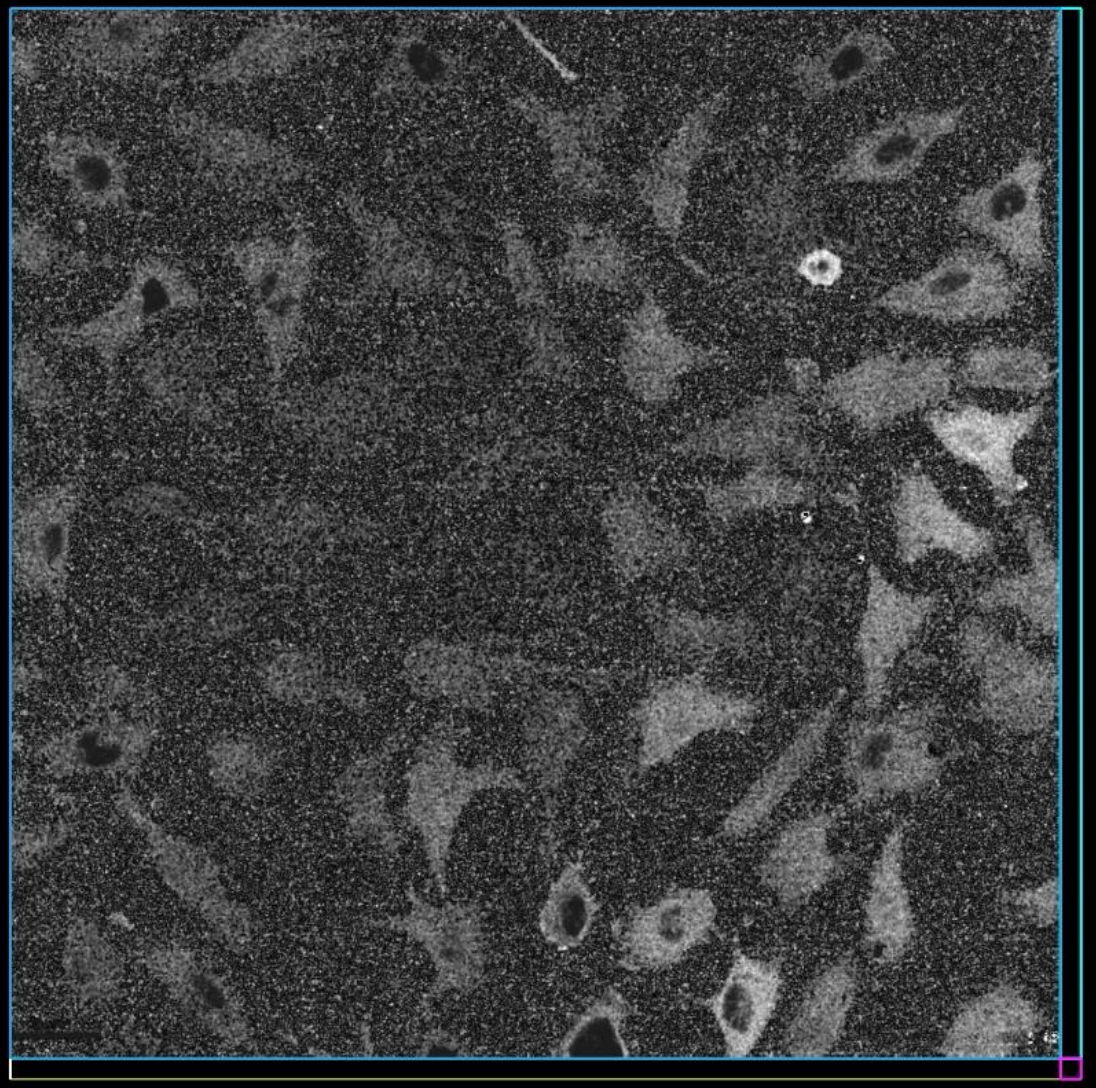
Cell membrane effects of histones



Cell membrane effects of histones



Cell membrane effects of histones



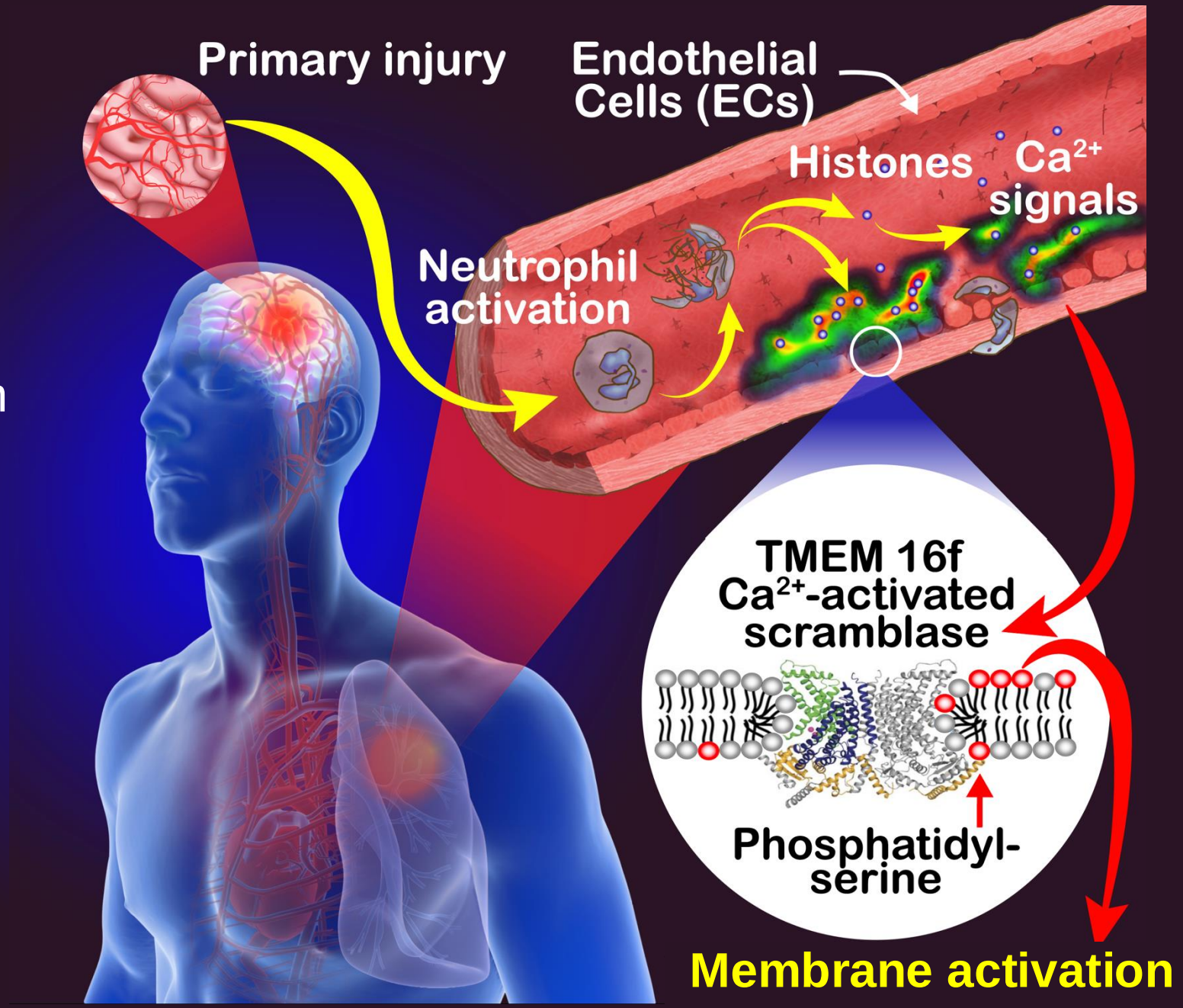
Working Model

- Primary injury (e.g., TBI)
- Trauma factors -> circulation
- Endothelial cell activation

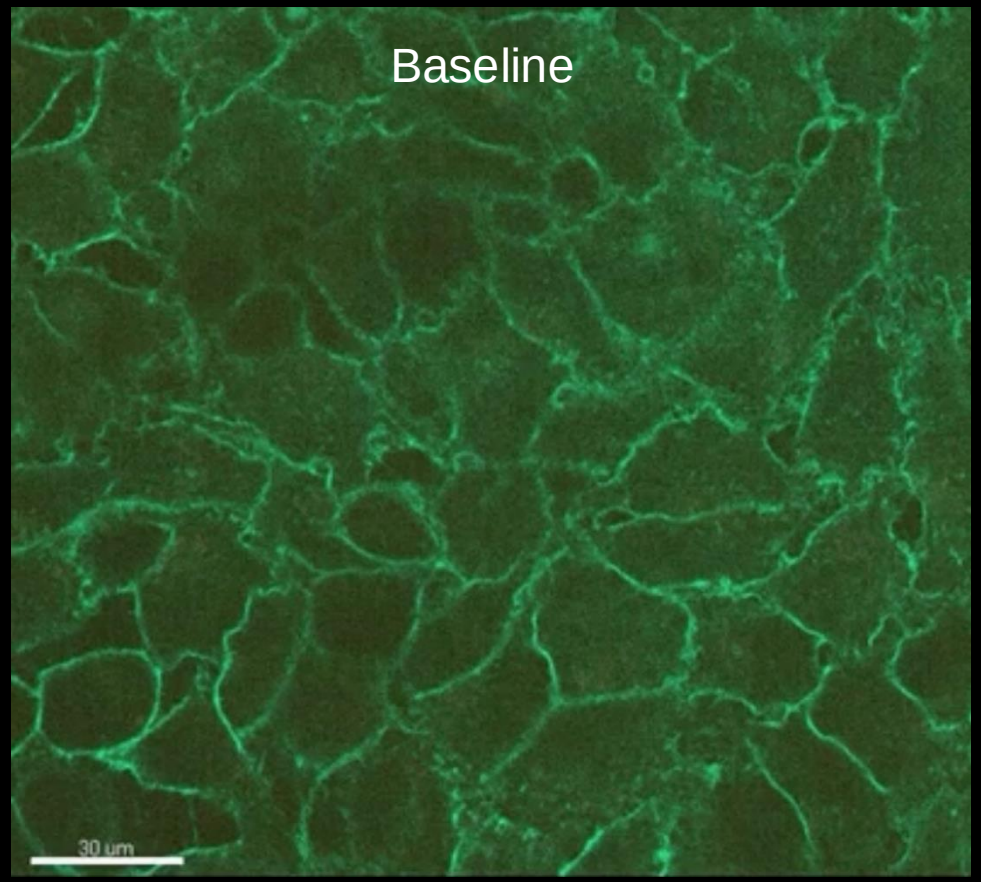
  PS translocation
  Release of membrane particles
  Thromboinflammation

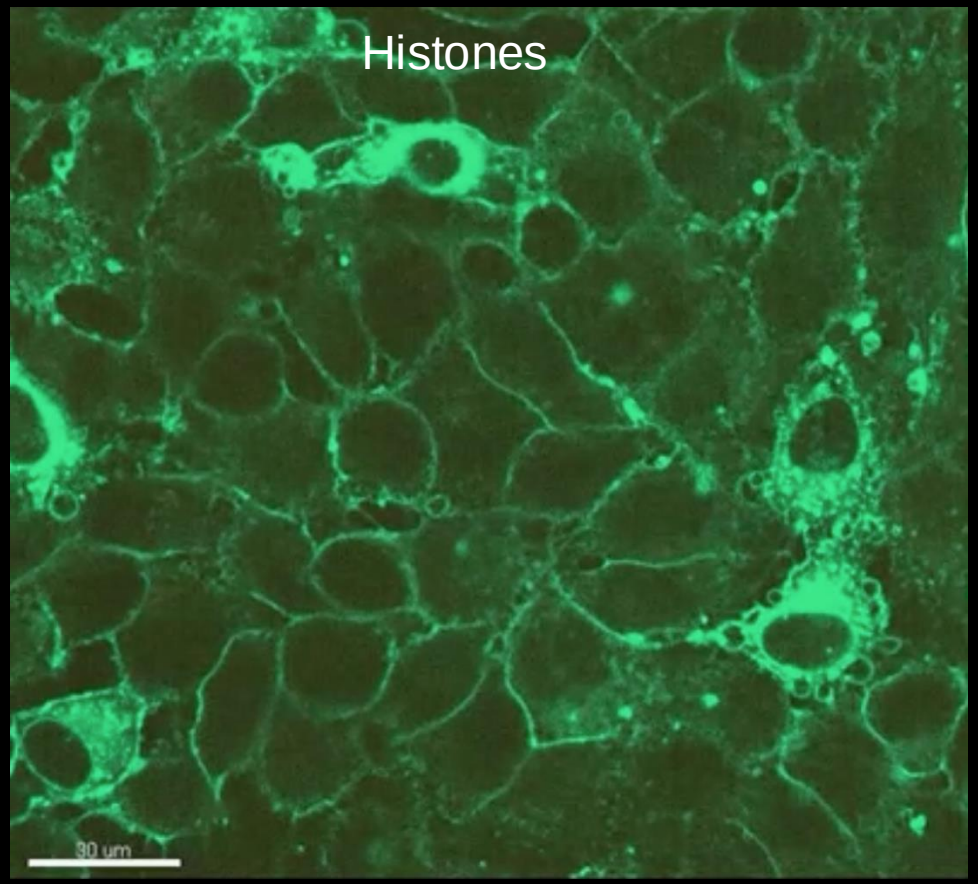
- Endotheliopathy

  Widespread disruption of microvascular vasodilatory and barrier function



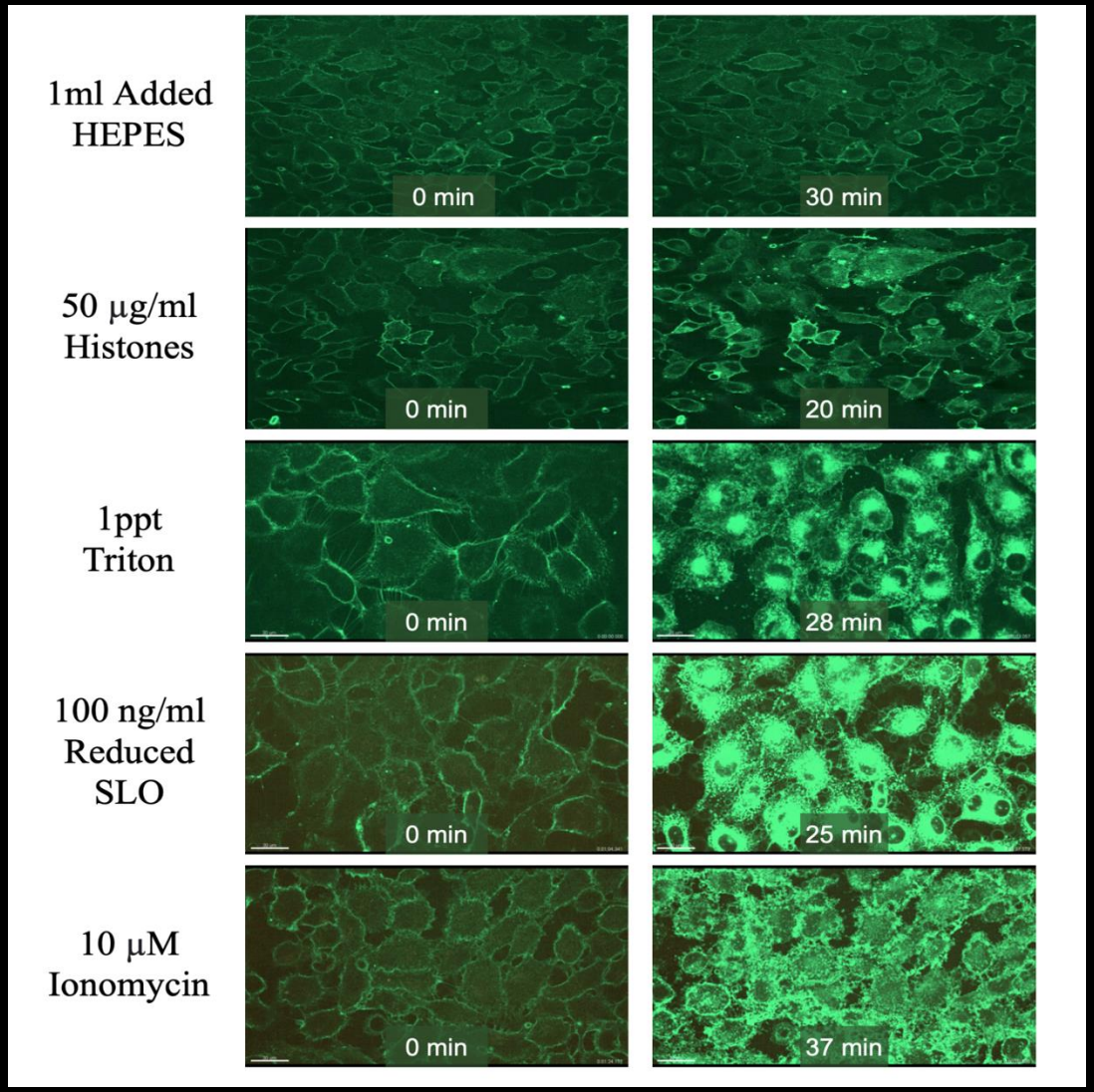
Membrane indicator FM1-43 applied to ECs





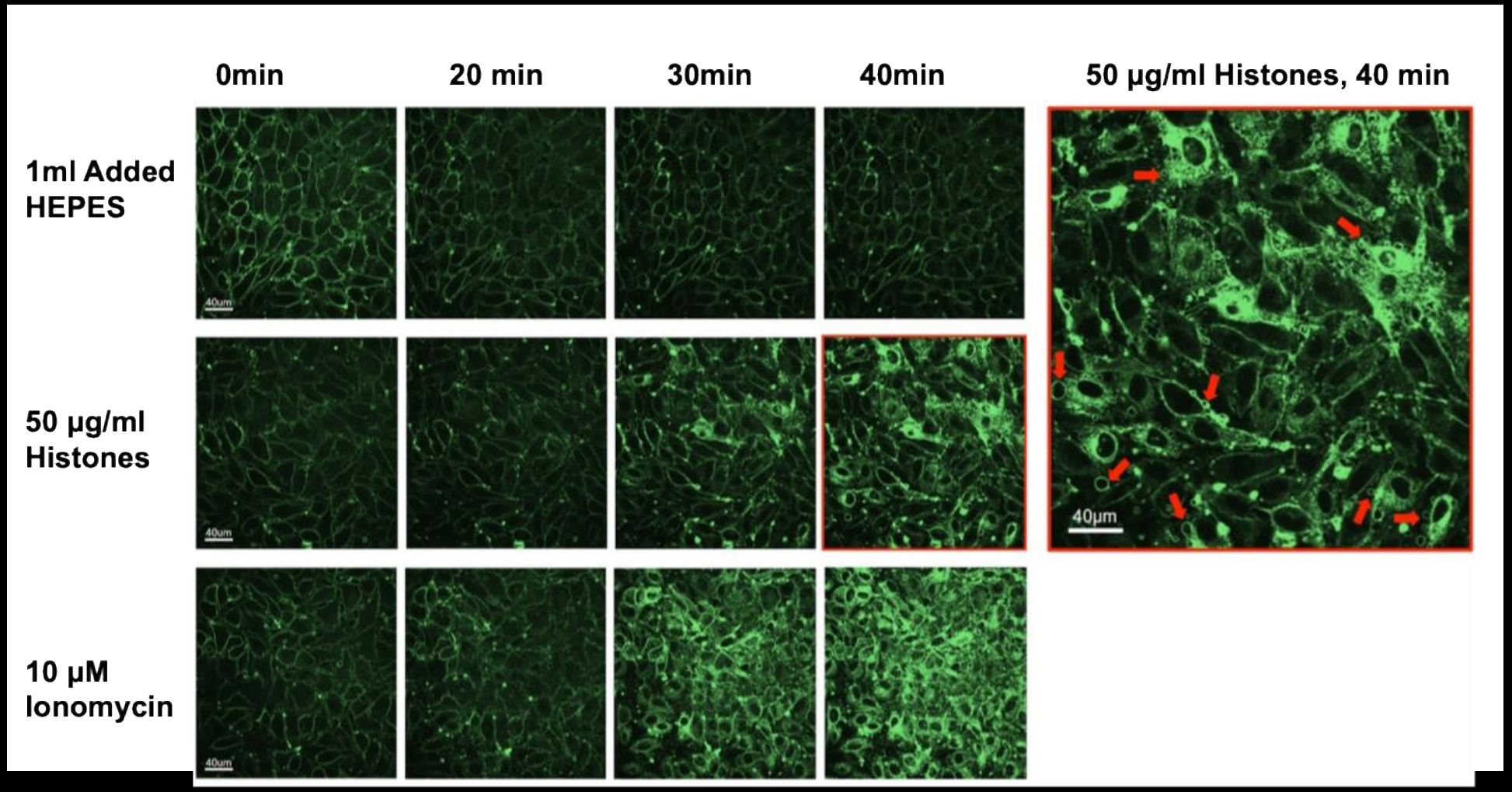
Sophie Piffard & Jade Cleary

Membrane effects of histones and other agents



Jade Cleary (Undergraduate Honors Thesis)

Do responses to histones = ionomycin?



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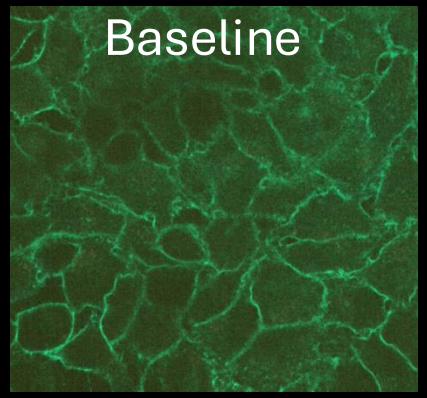
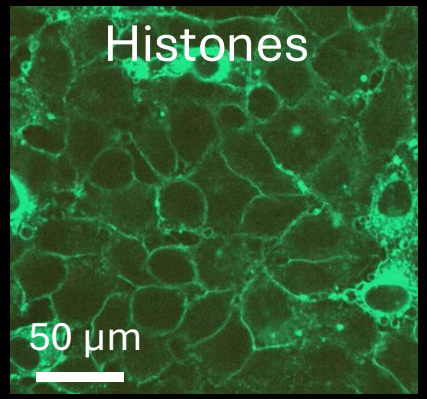
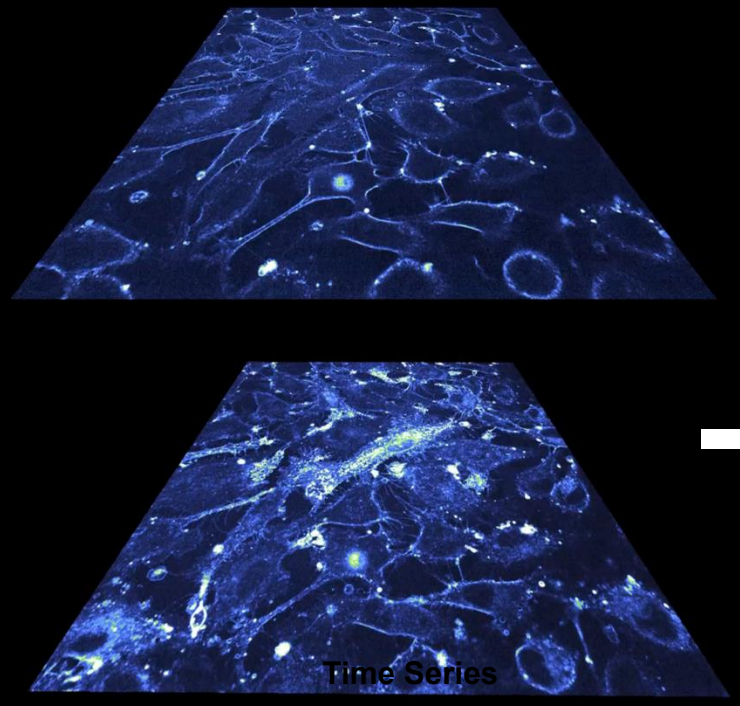
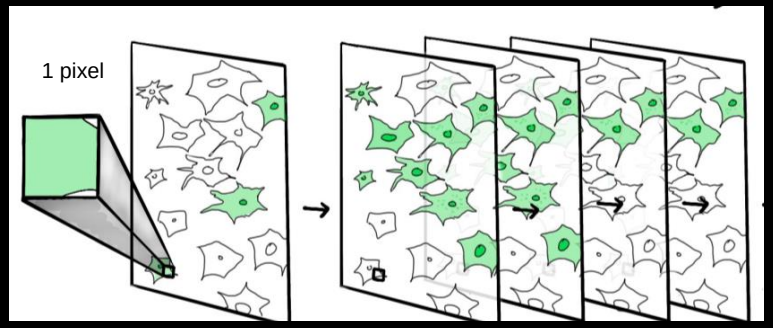
Spatiotemporal analysis of EC membrane activity

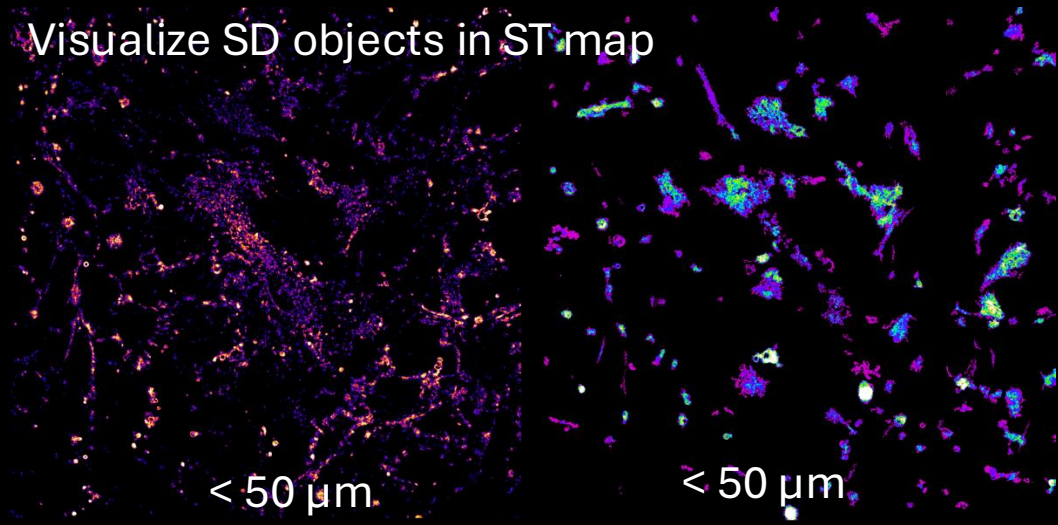


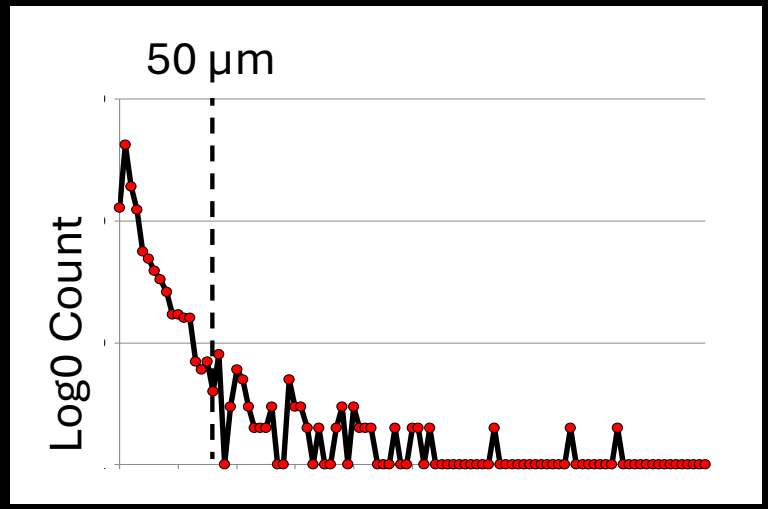






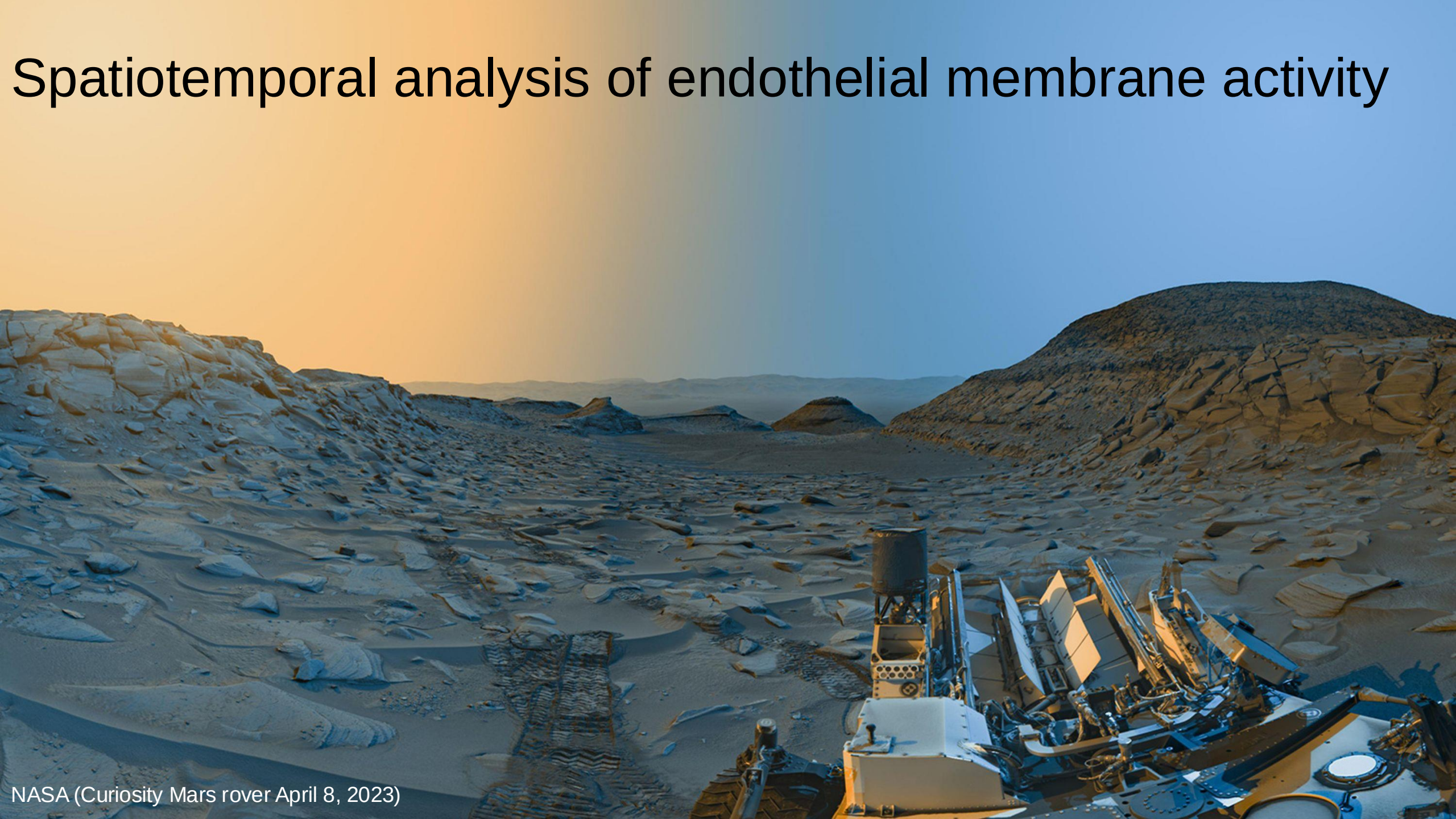
Calculate the SD component of the signal to noise ratio for each of 262,144 pixels in the field of view across the duration of the video





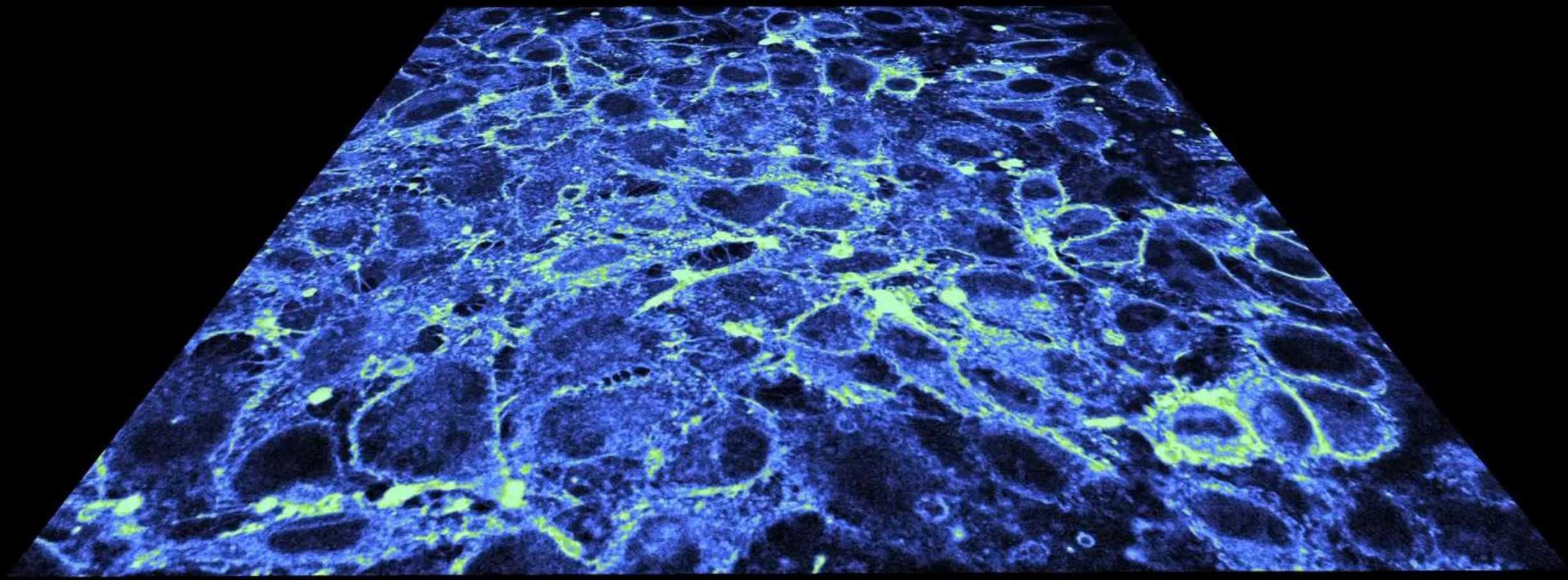
Histogram shows number of SD objects

Spatiotemporal analysis of endothelial membrane activity



NASA (Curiosity Mars rover April 8, 2023)

Endothelial cell activation with ionomycin



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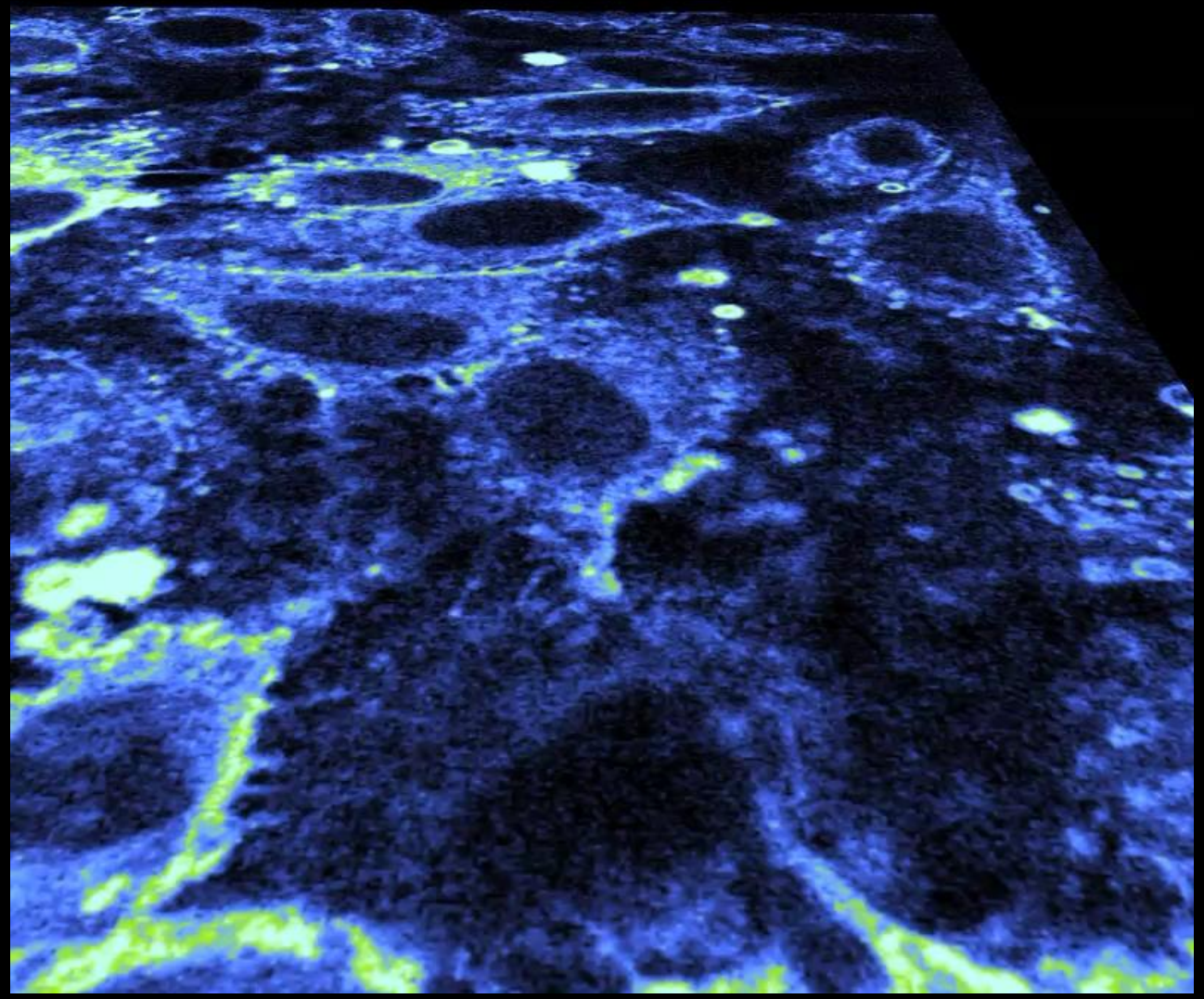
Calcium ionomycin – exosomes



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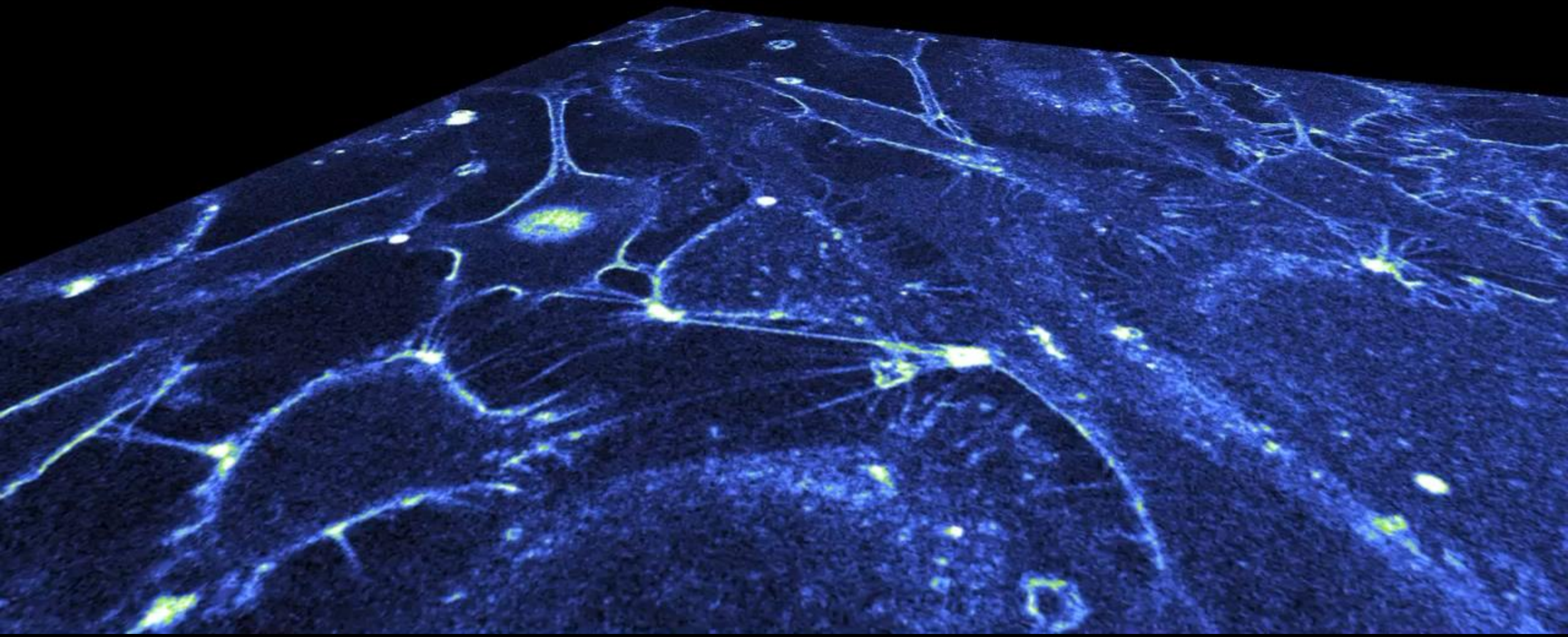
Histones – membrane ruffling



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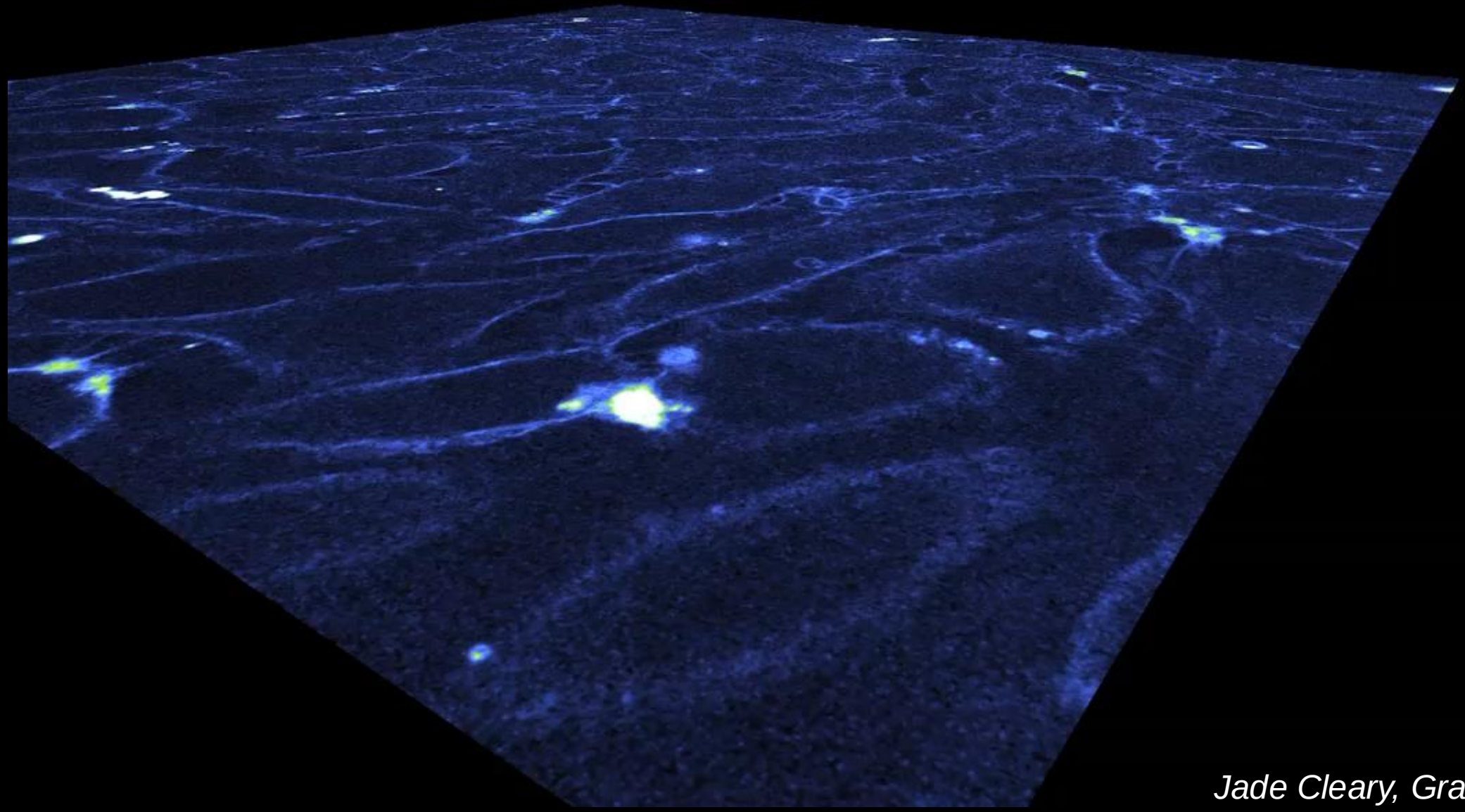
Histones – bleb city



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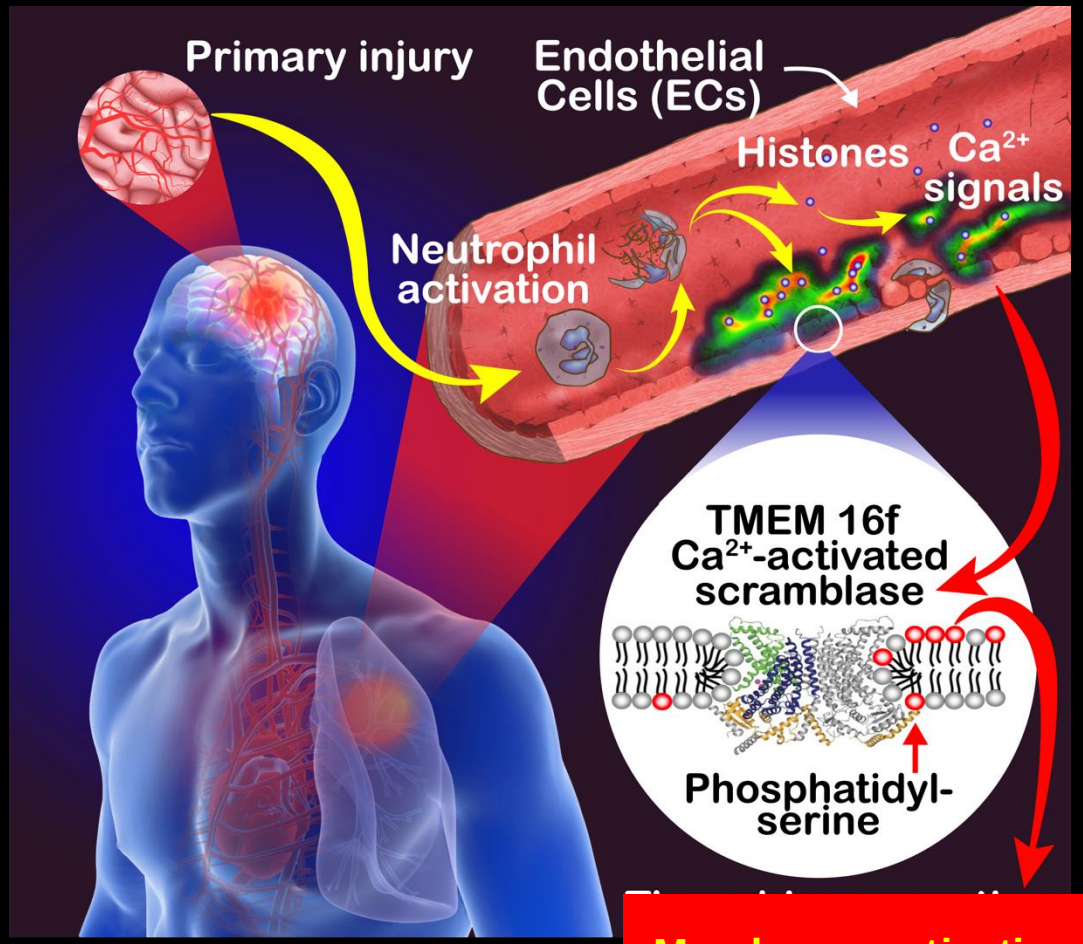
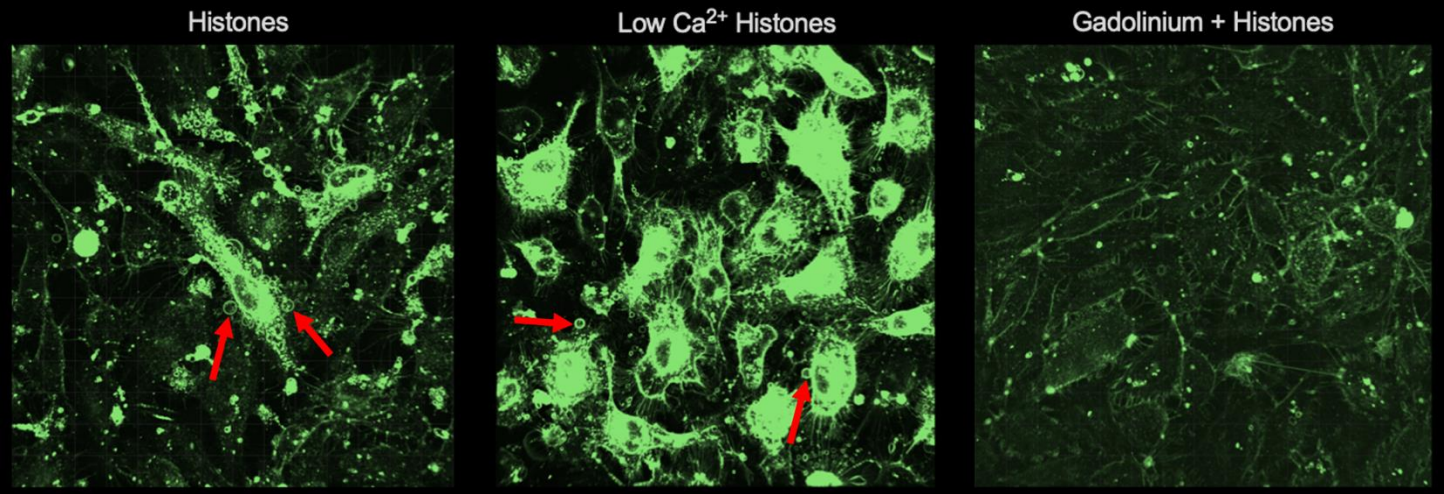
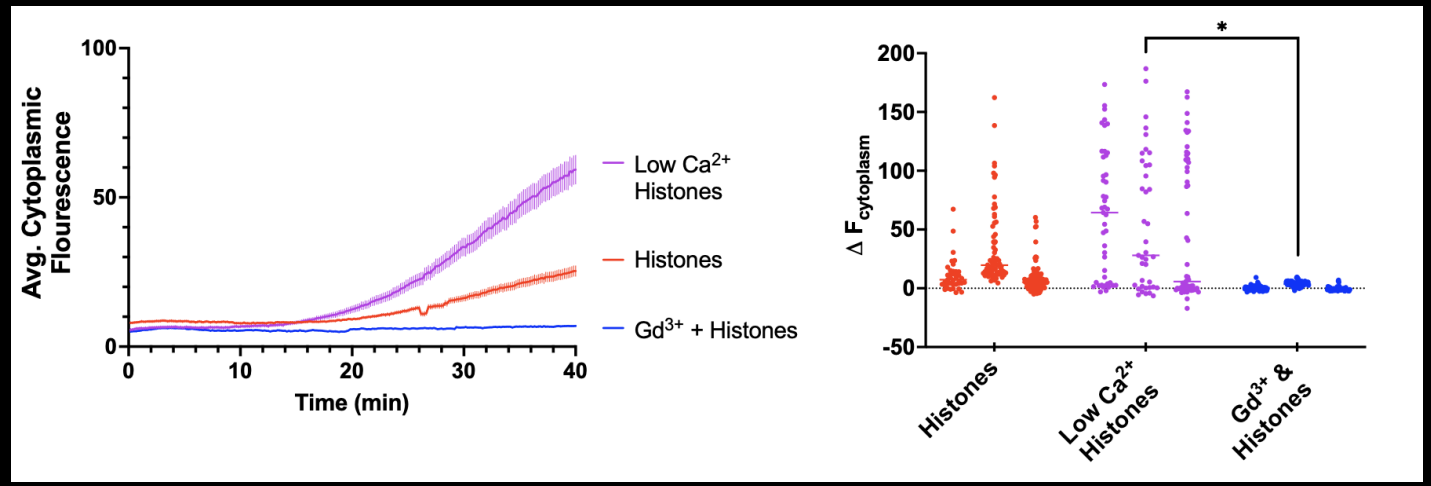
The “killer question"

- We don’t see Ca^{2+} entry into ECs when we remove extracellular Ca^{2+}
- So, we hypothesize that Ca^{2+} into ECs drives membrane effects.
- Does removal extracellular Ca^{2+} prevent membrane responses?



Membrane effects of histones *exacerbated* by low Ca^{2+}





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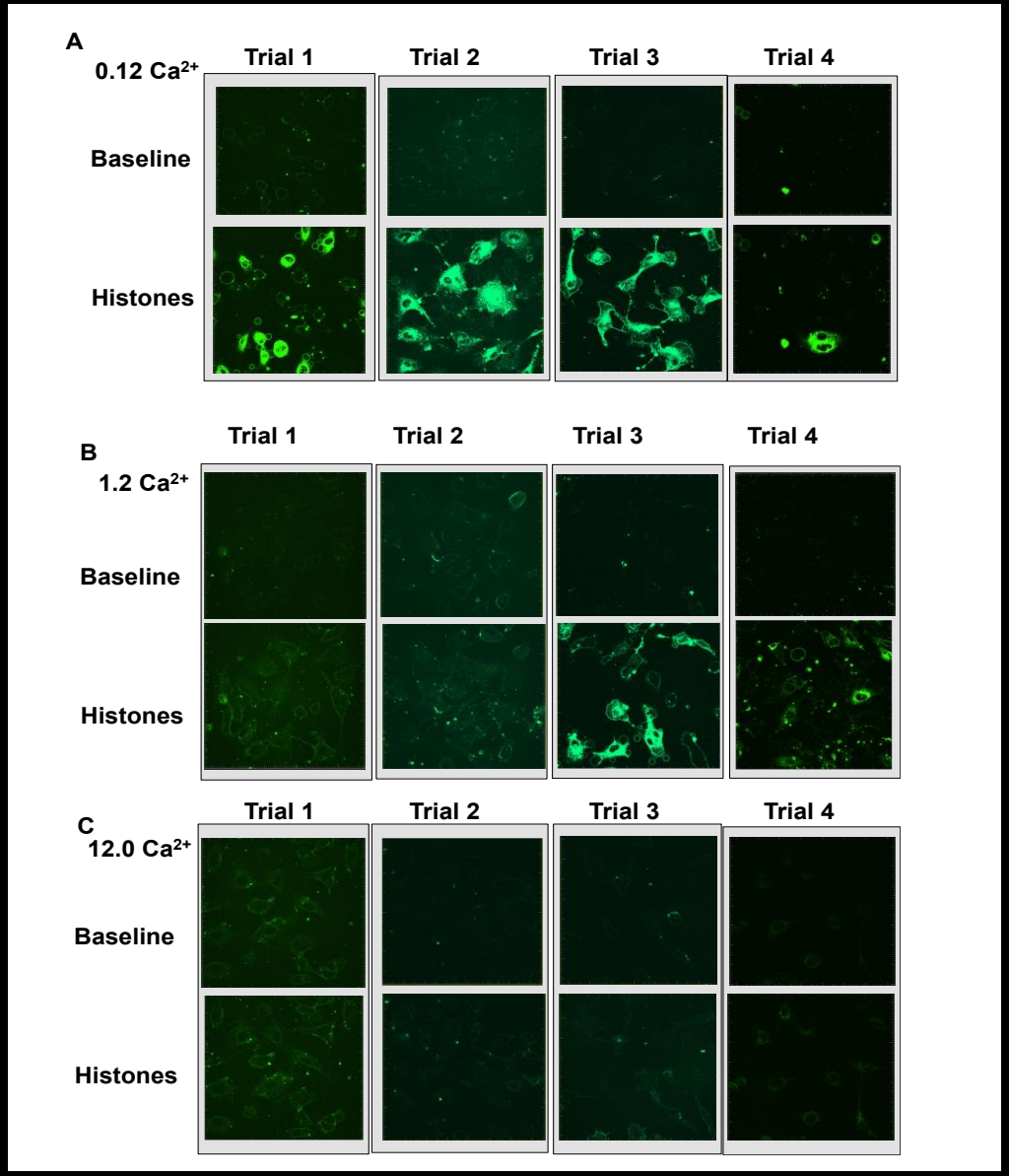
Membrane effects are *blocked* by elevated Ca^{2+}



Clinical implications

- Administration of citrated RBCs decreases [Ca^{2+}] to an extent which depends on total dose of citrate and rate of infusion.
- Ca^{2+} replacement therapy during massive blood transfusion is common, but thresholds at which Ca^{2+} should be administered are unknown.
- We show that increased Ca^{2+} protects endothelial membranes from circulating trauma factors such as histones (likely due to surface charge effects).
- This supports aggressive measures to replete Ca^{2+} (*)

**During trauma resuscitation hypercalcemia is also associated with increased mortality, increased blood product use, and greater hospital resource consumption. Hypercalcemia also provokes RBC thromboinflammation.*

DeBot M… Moore EE, *Transfusion* (2022)
MacKay EJ, … Cannon JW, *Anesthesia Analgesia* (2017)
Goodman M, *THOR meeting* (2024)

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Bio-Electric Phenomena as an Etiologic Factor in Intravascular Thrombosis[1]

PHILIP N. SAWYER[2] AND JAMES W. PATE[3]

From the Naval Medical Research Institute, and Tissue Bank, Naval Medical School, National Naval Medical Center, Bethesda, Maryland

IN A PREVIOUS PAPER (1) it was reported that abnormal electric potentials in the aortic wall seemed to be related to intravascular thrombus formation. In a series of dogs receiving aortic grafts, it was observed that in most of the animals a positively polarized intimal potential appeared in the vessel at the time of operation. In four of the dogs this positive potential persisted, and in these dogs complete spontaneous thrombosis occurred.

It has been demonstrated previously that the intima of the normal aorta is polarized negatively with respect to the adventitia (1). However, trauma can reverse this polarity so that the intima becomes positive with respect to the adventitia. Because of these findings, an extensive investigation was undertaken in order to determine if the reversal of the normal polarity of the vessel wall was related to the formation of intravascular thrombi.

EXPERIMENTATION WITH IN VITRO CLOTTING OF HEPARINIZED AND CITRATED BLOOD BY THE PASSAGE OF AN ELECTRIC CURRENT

Technique

Several 30-cc samples of blood were drawn from one dog either heparinized with 1 cc (10 γ) of heparin for each 5 cc of blood or citrated with 40 mg of sodium citrate/cc of blood. Aliquots of this blood were placed in Kahn tubes. A pair of platinum electrodes of known weight separated by lucite rings inserted into the 30 minutes. The electrode pairs were then removed from the remaining blood in the tube and all unprecipitated blood elements carefully blotted and removed from the electrodes (fig. 1*B*). The electrodes and precipitated blood elements were then carefully weighed and the net weight of the precipitated blood elements was calculated.

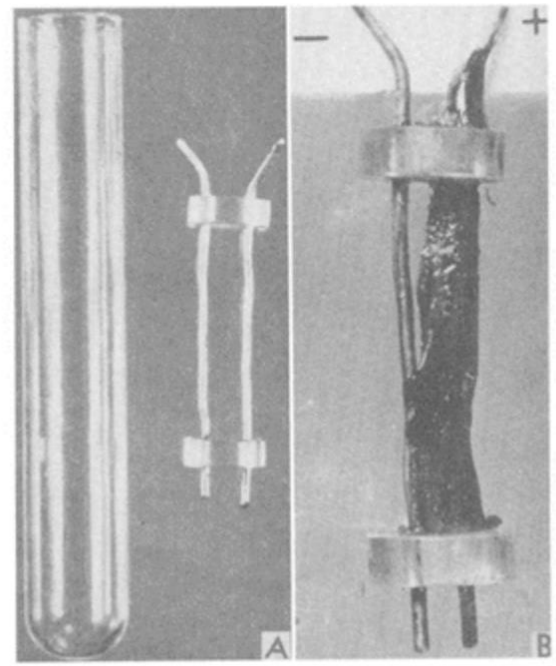

FIG. 1. *A*: Platinum electrode pair and tube used to precipitate blood elements *in vitro*. *B*: Appearance of electrode pair after passing current for 30 minutes in a test tube containing heparinized blood.

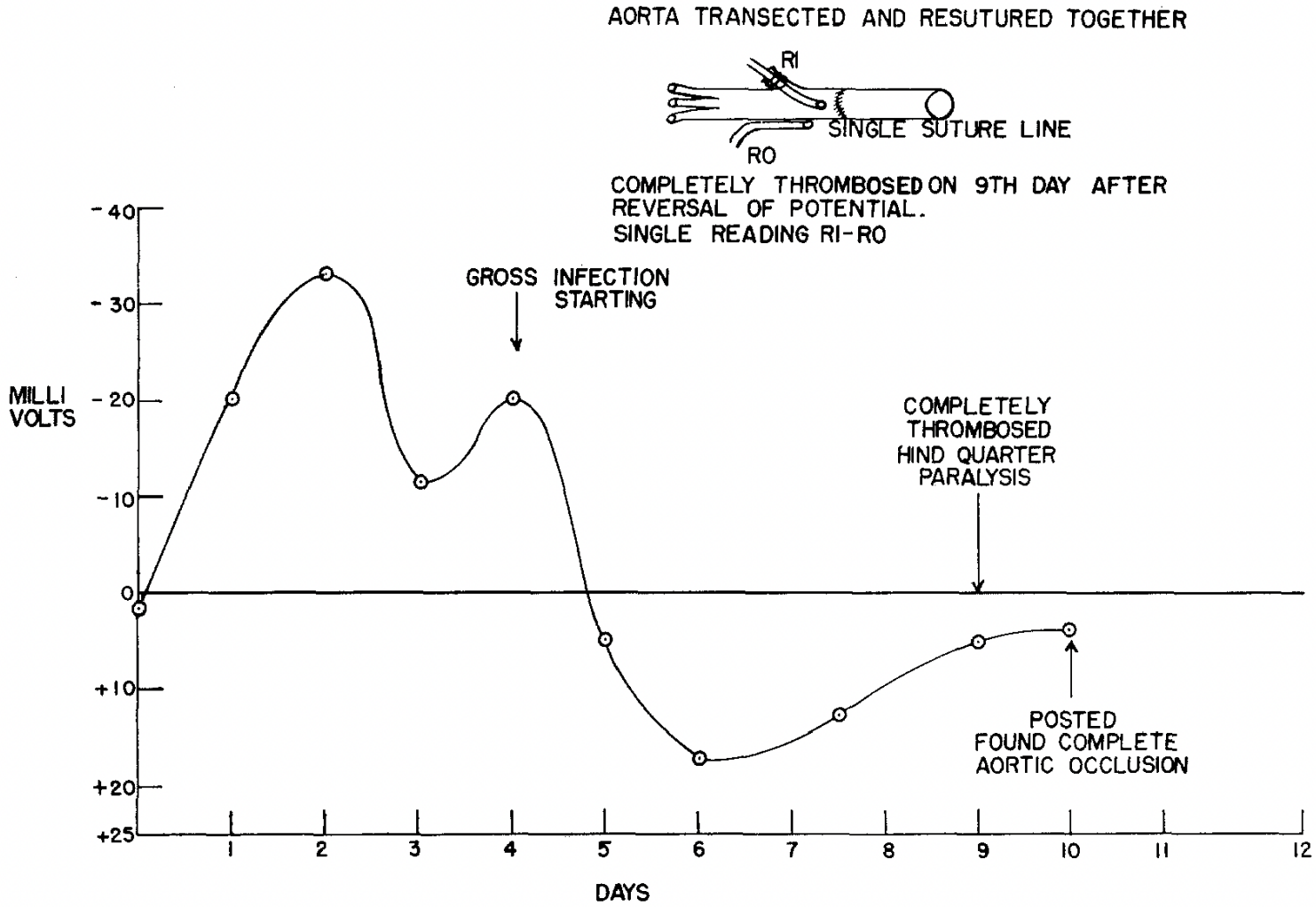

FIG. 1. Transmural potential across canine aorta as a function of time (9).

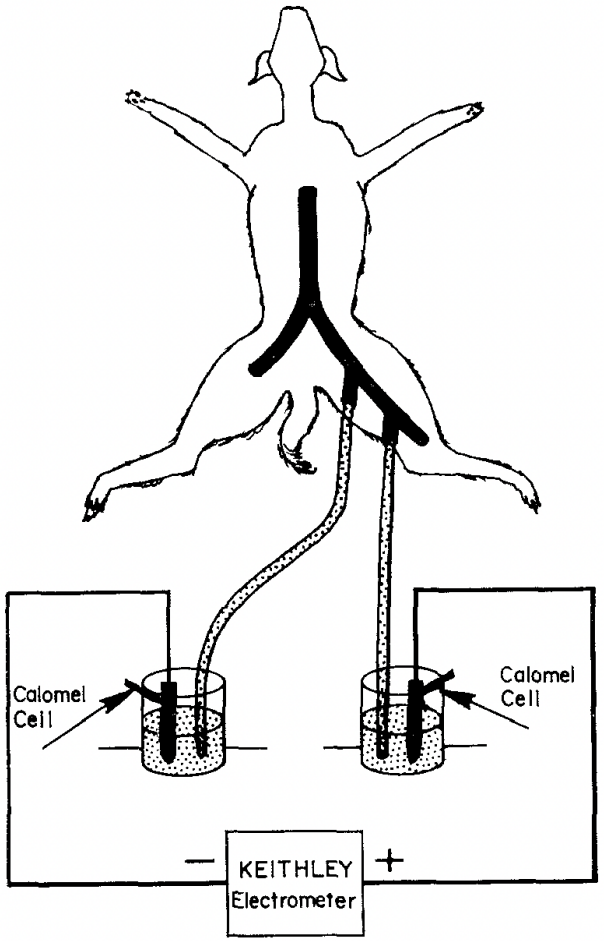

FIG. 6. Experimental arrangement for measurements of streaming potentials in canine blood vessels (e.g., femoral artery) *in vivo* (13).

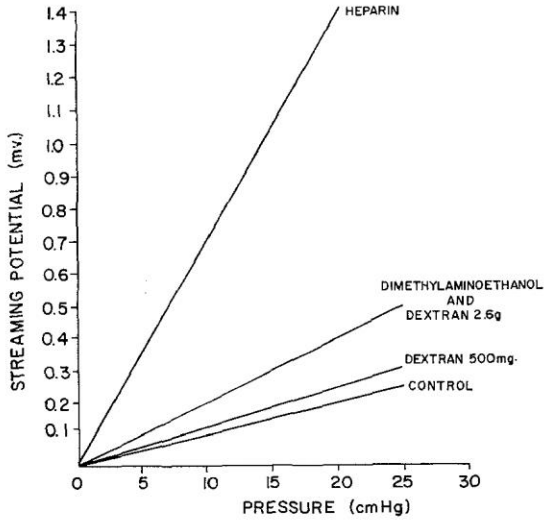

FIG. 4. Effect of some anticoagulants on streaming potentials between ends of canine blood vessels as a function of pressure (13).

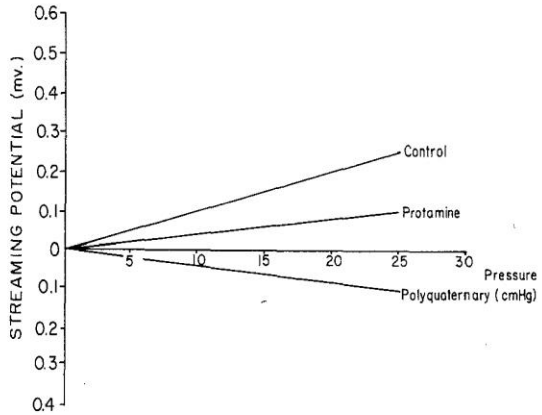

FIG. 5. Effect of some coagulants on streaming potentials across canine blood vessels as a function of pressure (13).

Calcium block of Na+ channels and its effect on closing rate
C. M. Armstrong and Gabriel Cota PNAS March 30, 1999, 96 (7) 4154-4157

The effects of calcium on gating kinetics and open probability, as described by Frankenhaeuser and Hodgkin (6), are usually explained by *the surface charge hypothesis*. This hypothesis holds that calcium alters gating by neutralizing negative charge at the membrane surface, thus changing the local field near the voltage-sensing parts of the Na+ or K+ channels (5). The permeation-blocking effects are regarded as separate phenomena, having no effect on kinetics and open probability. An obvious alternative not ruled out by existing data is that calcium's effects on gating are associated with its ability to occupy and block Na+ channels, and that it is calcium occupancy rather than a surface charge mechanism that stabilizes the closed state when external calcium concentration is increased. In support of this idea, we show here that calcium block and closing rate of Na+ channels are closely related, and that Na+ channels close freely, and perhaps preferentially, when calcium occupied. The following paper (9) shows that calcium has large effects on gating only in cases where it is free to enter and leave the channel, and that calcium seems to be essential for channel closing.

5 B Hille *Ionic Channels of Excitable Membranes* (Sinauer, Sunderland, MA, 1992).

6 B Frankenhaeuser, A L Hodgkin *J Physiol (London)* **137**, 218–244 (1957).