

# Potential Long-distance Signaling by Blastocoel-traversing Thin Filopodia

Movies:

<http://groups.google.com/group/embryophysics/web/filopodia-7-october-2009?pli=1>

Presented in the Embryo Physics Course <http://www.embryophysics.org>  
October 7, 2009

By

Michael V. Danilchik

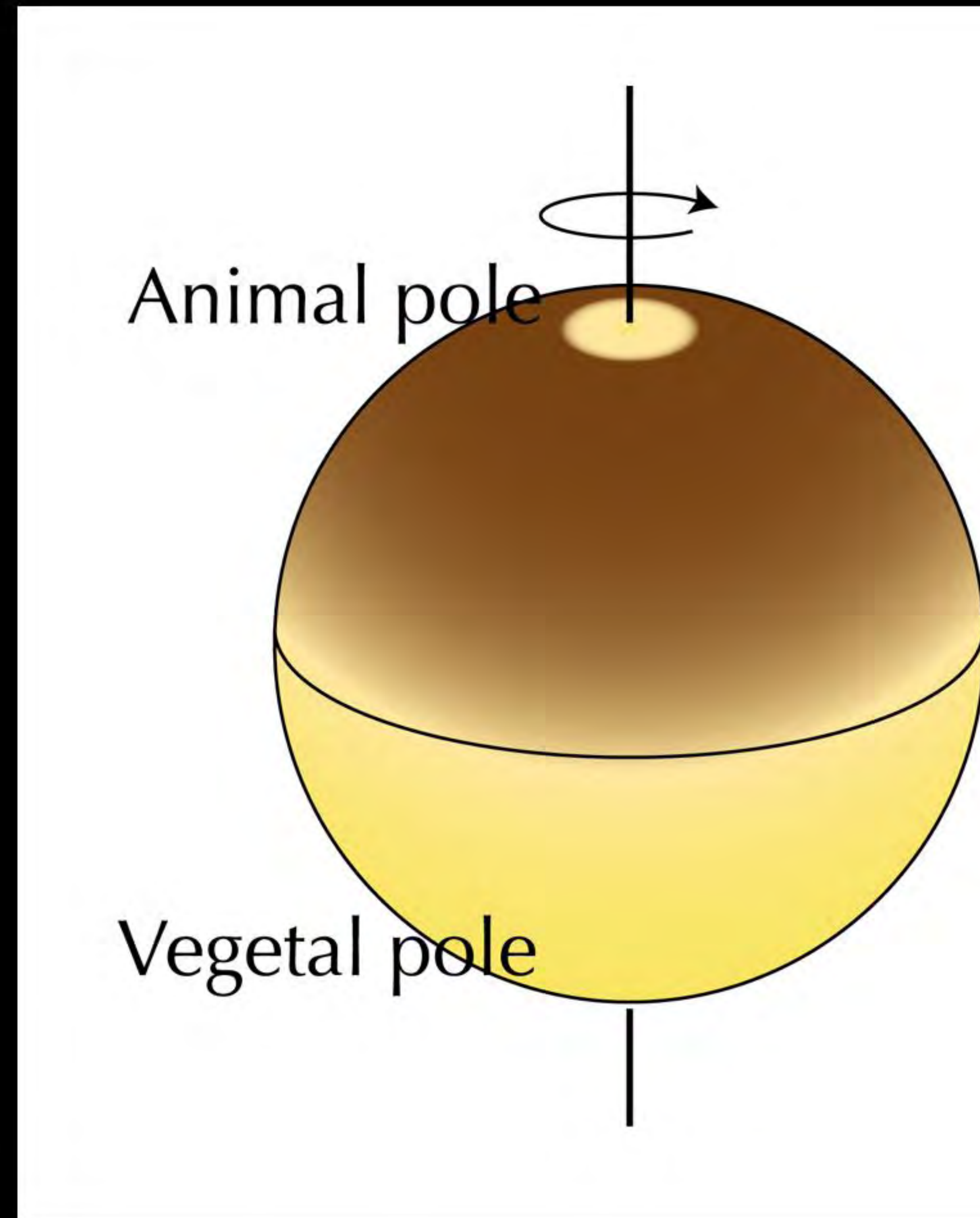
Integrative Biosciences, School of Dentistry  
Oregon Health & Science University

[danilchi@ohsu.edu](mailto:danilchi@ohsu.edu)

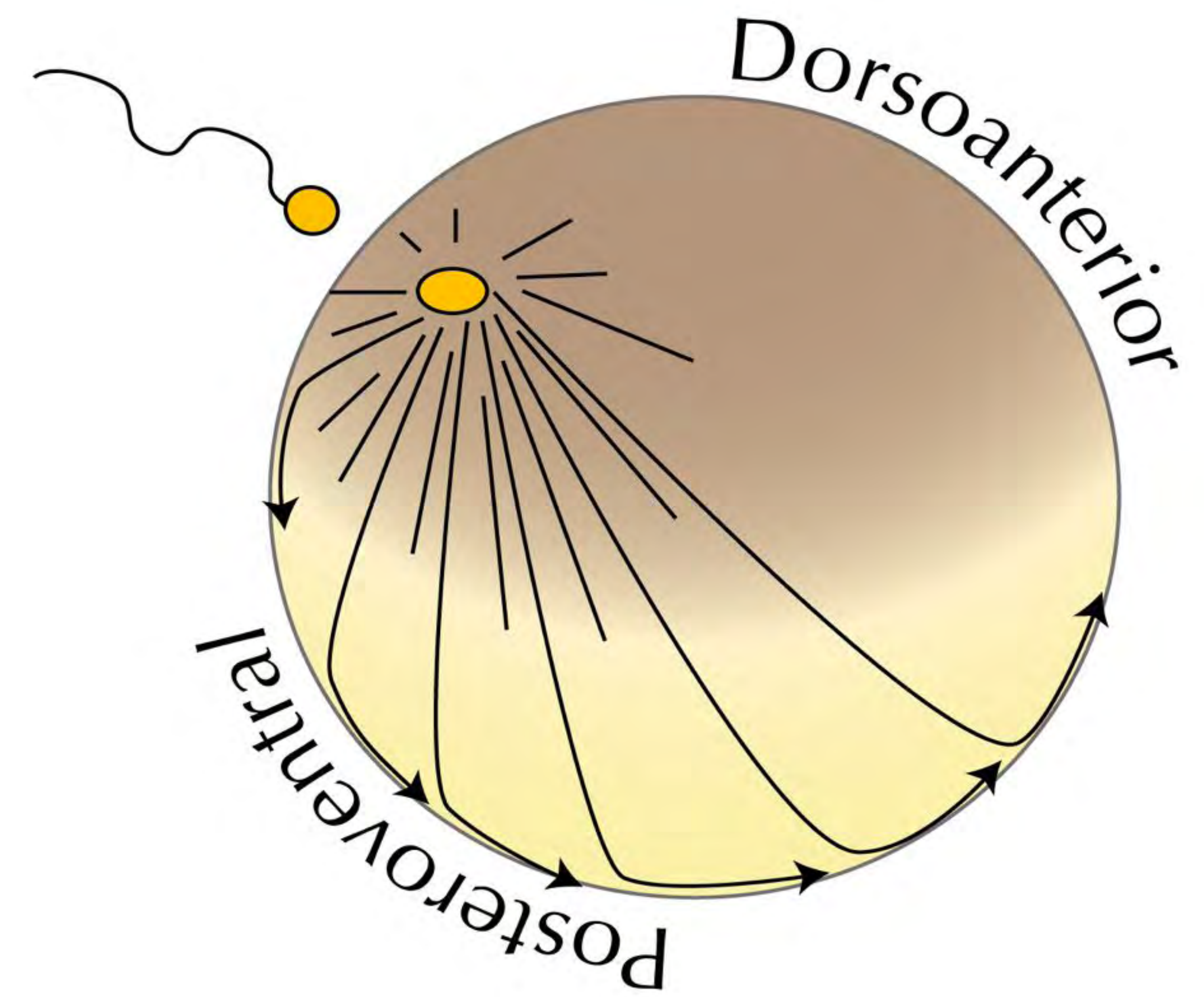


# Potential Long-distance Signaling by Blastocoel-traversing Thin Filopodia

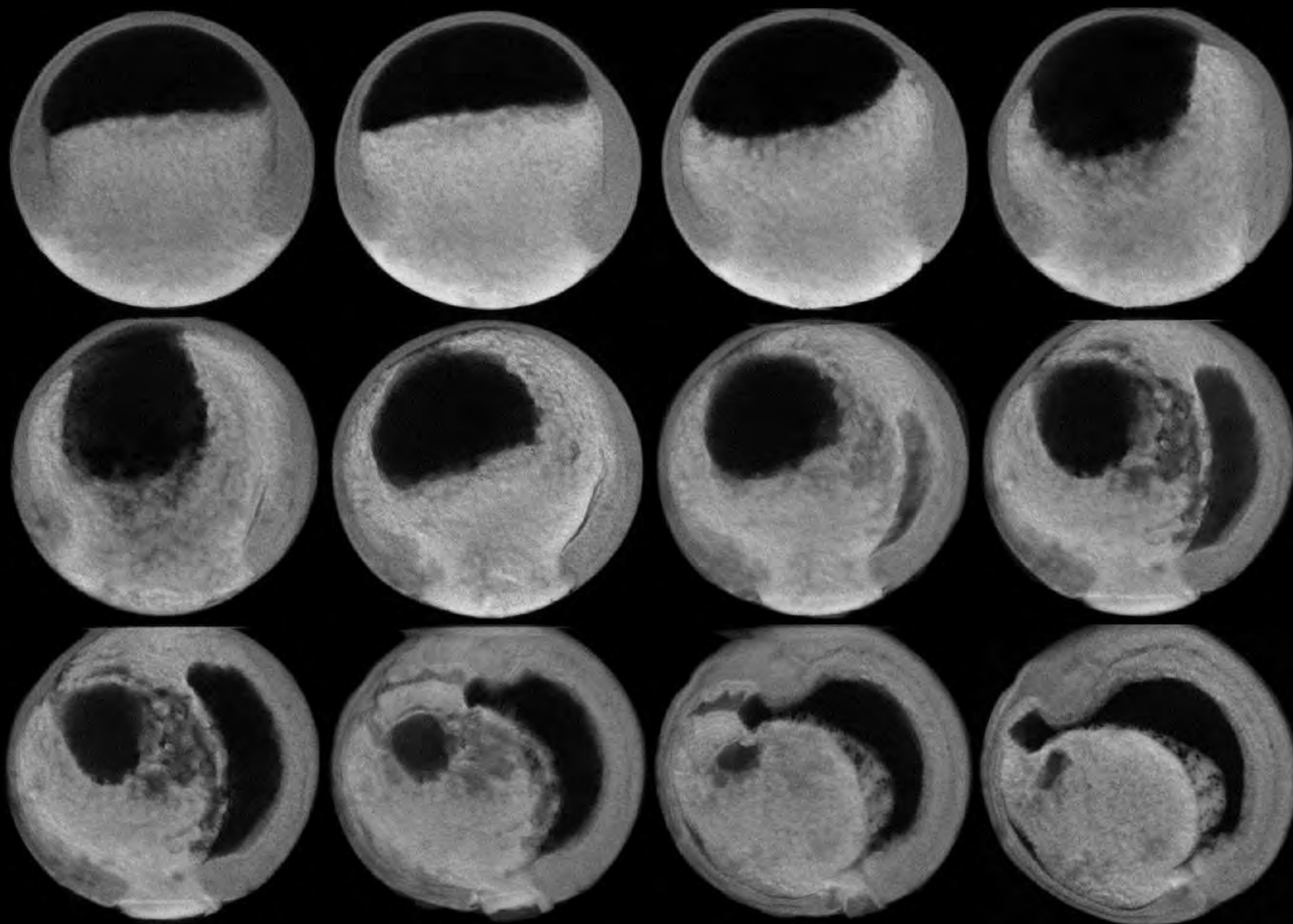
Mike Danilchik  
OHSU, Portland Oregon  
danilchi@ohsu.edu



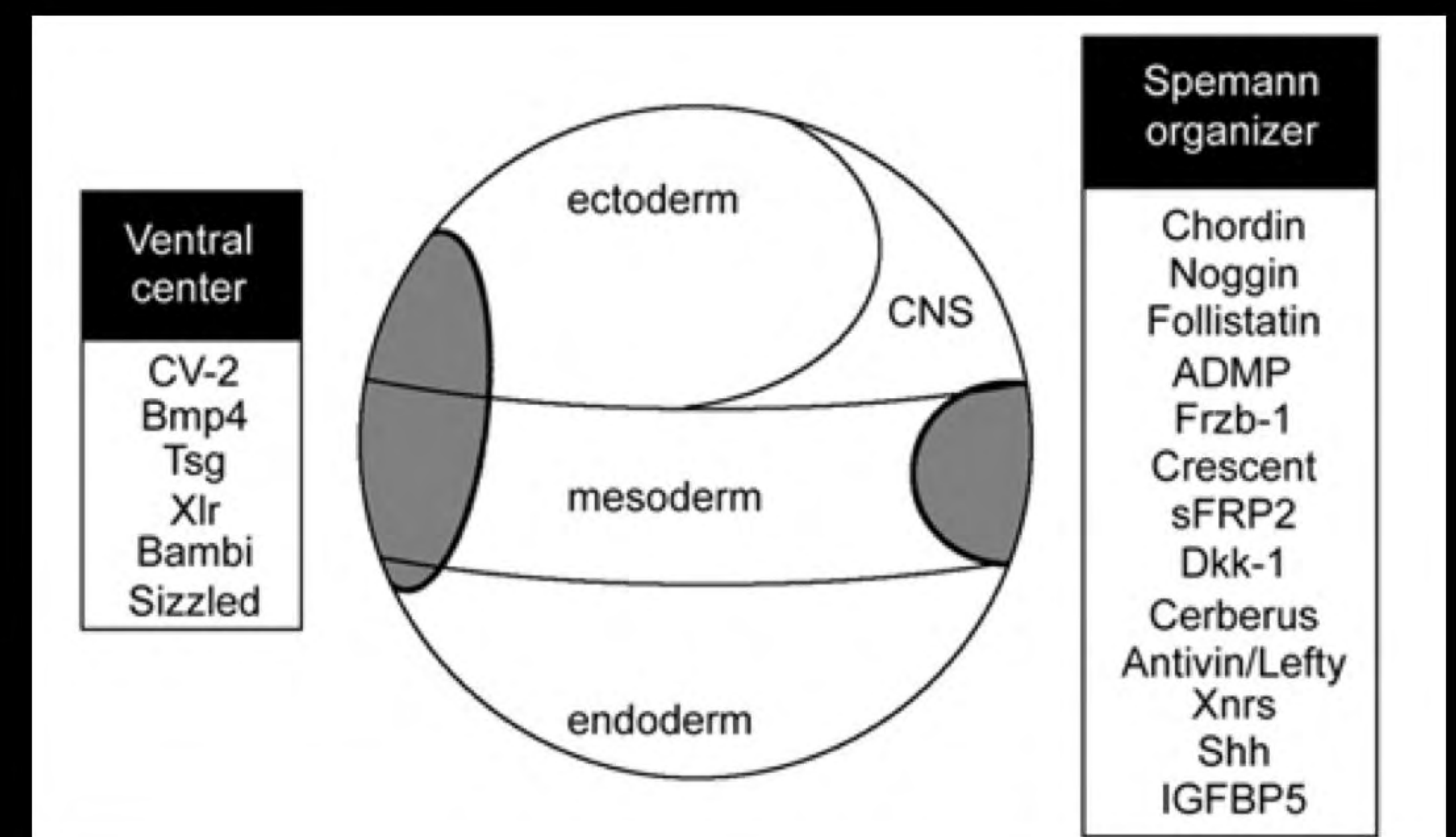
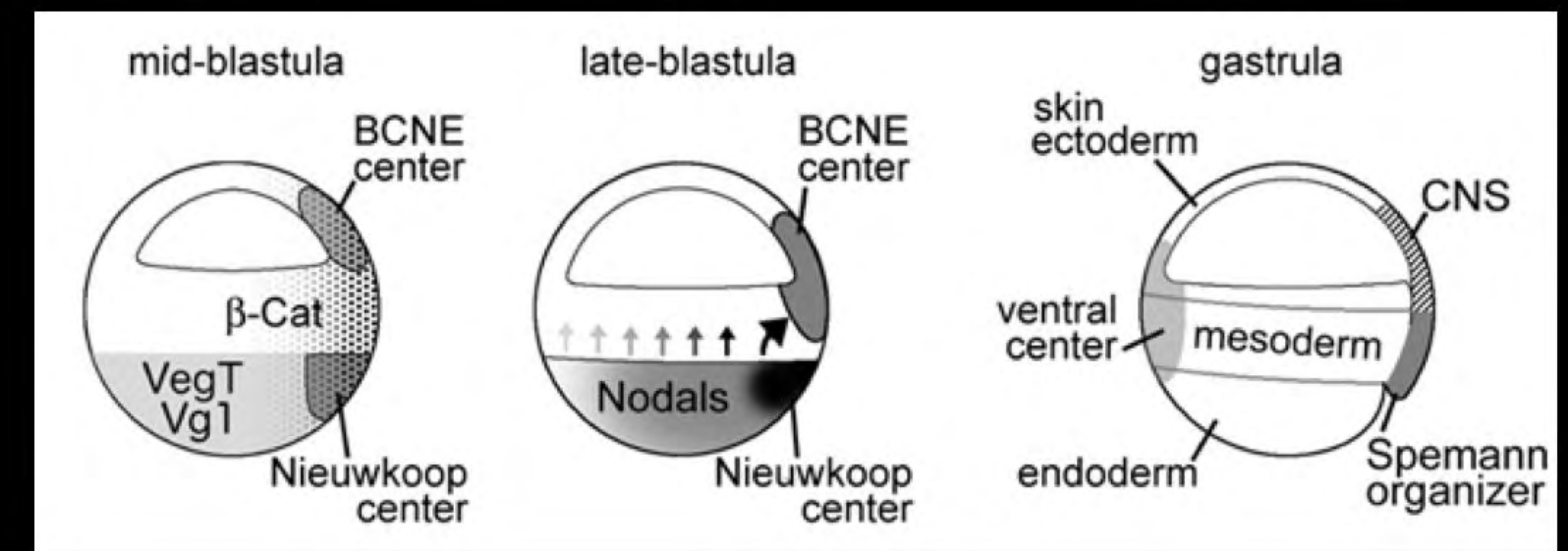
Unfertilized egg is radially symmetric



Dorsal-ventral axis is specified during first cell cycle ...



... the plane of mirror-image (bilateral) symmetry emerges directly from this specification; first manifested during gastrulation.



**TABLE 1. The 17 intercellular signaling pathways\***

Early development and later

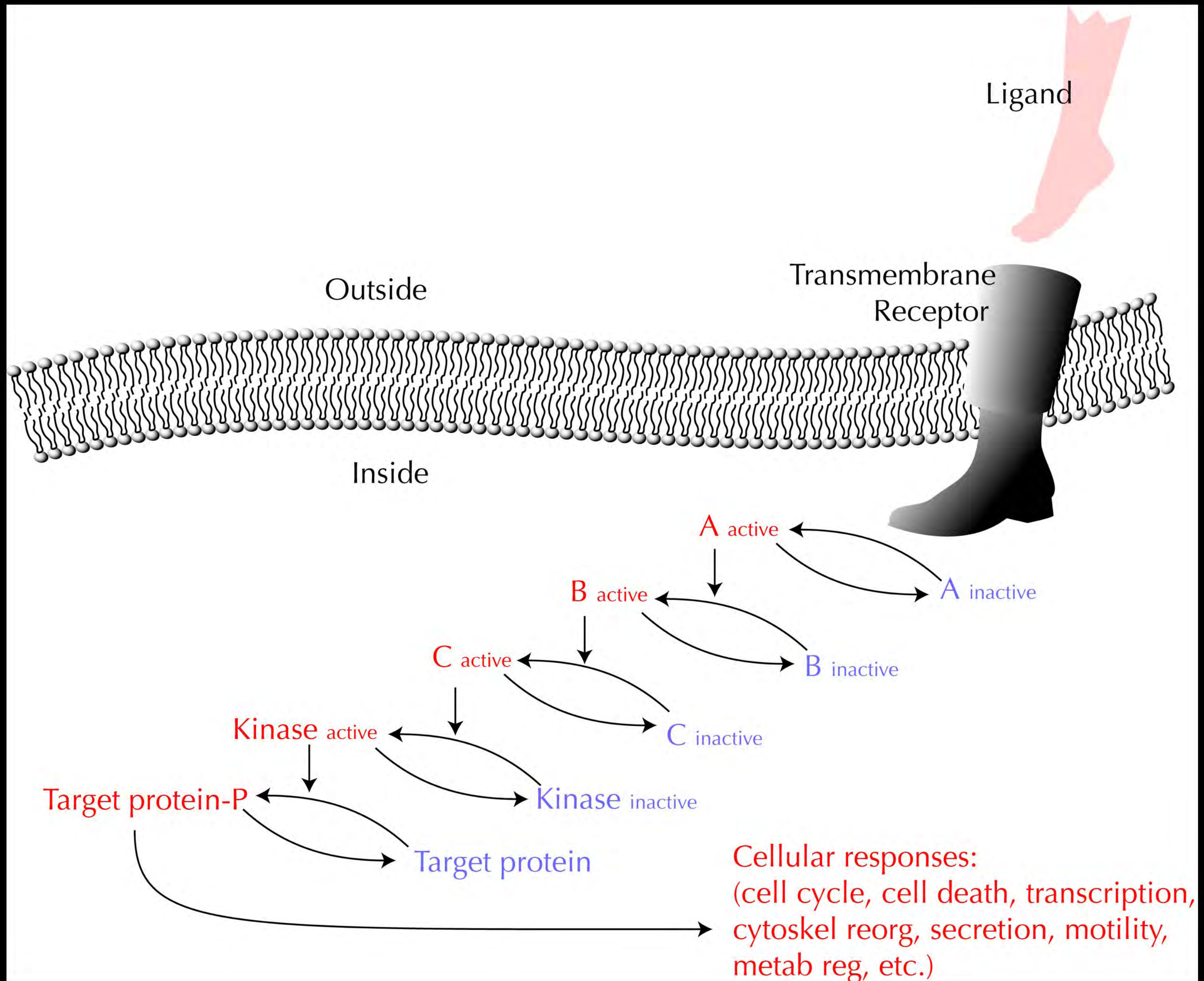
1. Wnt pathway
2. Receptor serine/threonine kinase (TGF $\beta$ ) pathway
3. Hedgehog pathway
4. Receptor tyrosine kinase (small G proteins) pathway
5. Notch/Delta pathway

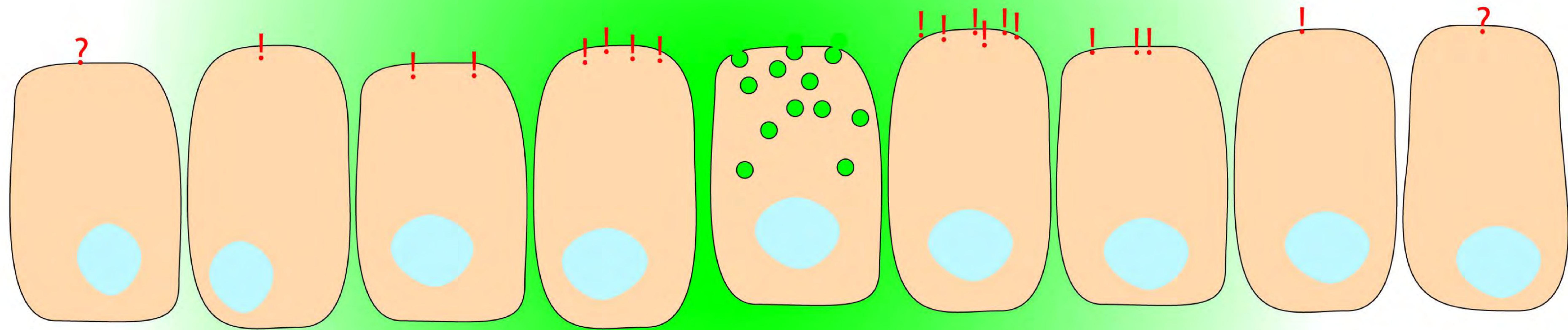
Mid-development and later

6. Cytokine receptor (cytoplasmic tyrosine kinases) pathway
7. IL1/Toll NF $\kappa$ B pathway
8. Nuclear hormone receptor pathway
9. Apoptosis pathway
10. Receptor phosphotyrosine phosphatase pathway

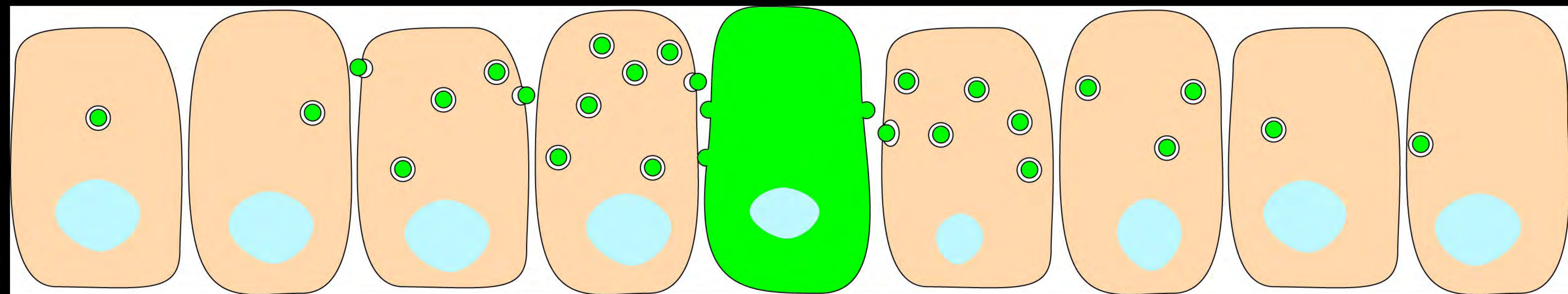
Larval/adult physiology

11. Receptor guanylate cyclase pathway
12. Nitric oxide receptor pathway
13. G-protein coupled receptor (large G proteins) pathway
14. Integrin pathway
15. Cadherin pathway
16. Gap junction pathway
17. Ligand-gated cation channel pathway



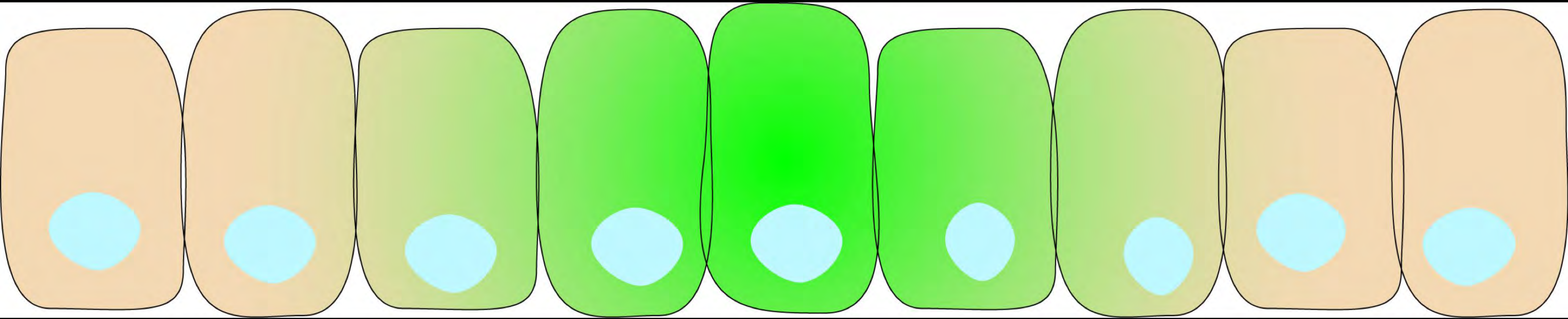


signaling via secreted ligand

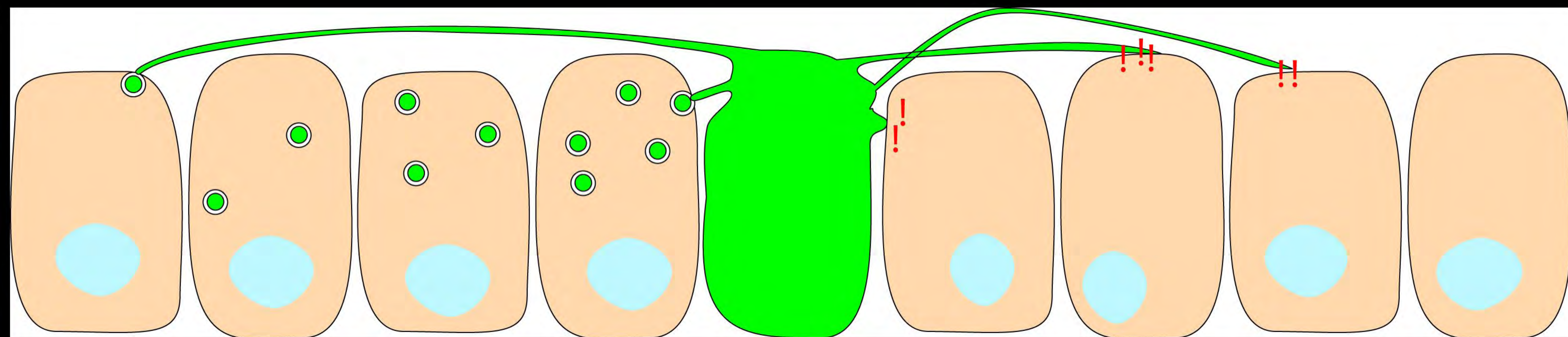


signaling via transcytosis





signaling via gap junctional continuity



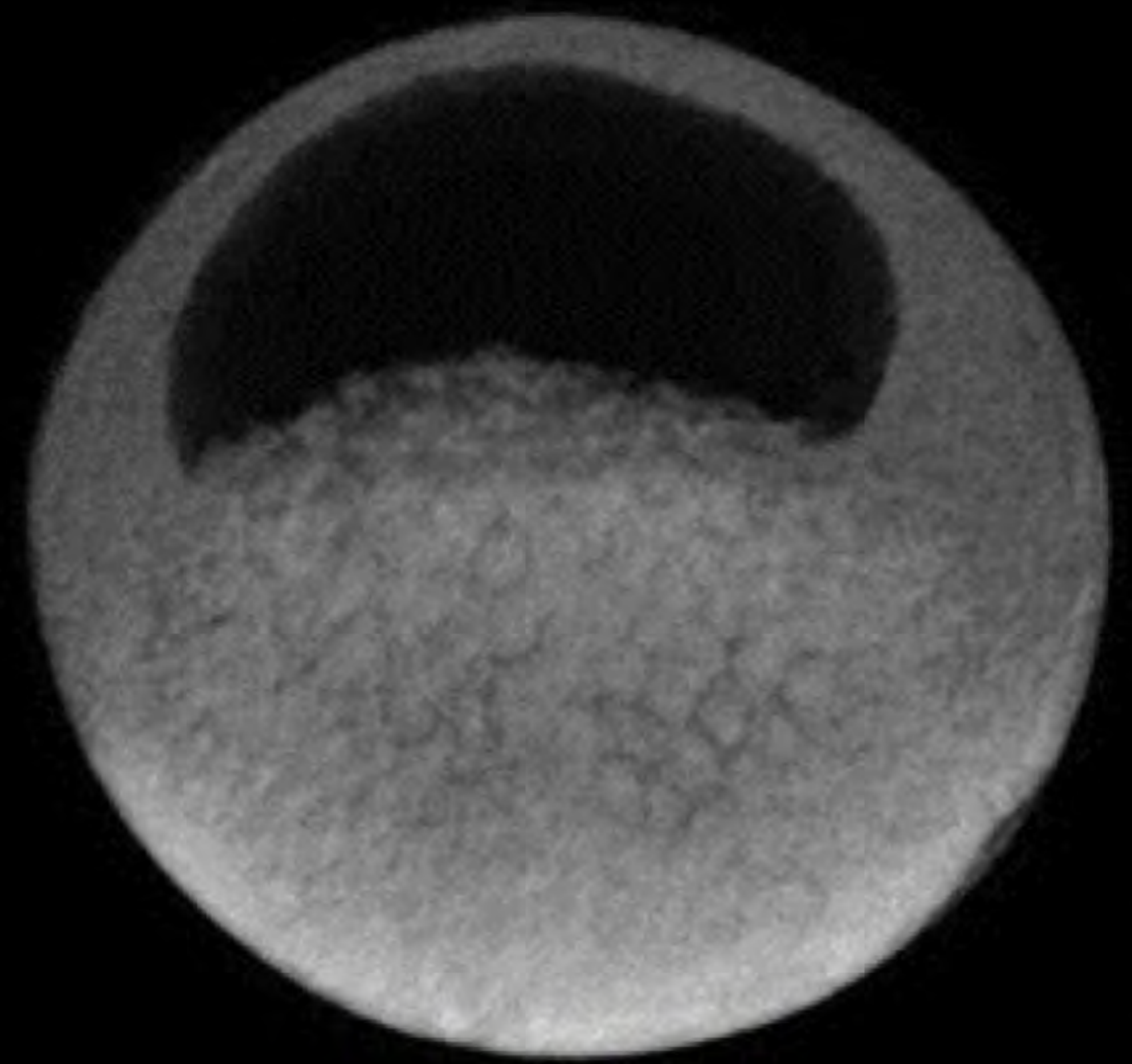
signaling via direct contact

## cleavage, a morphogenetic stage...

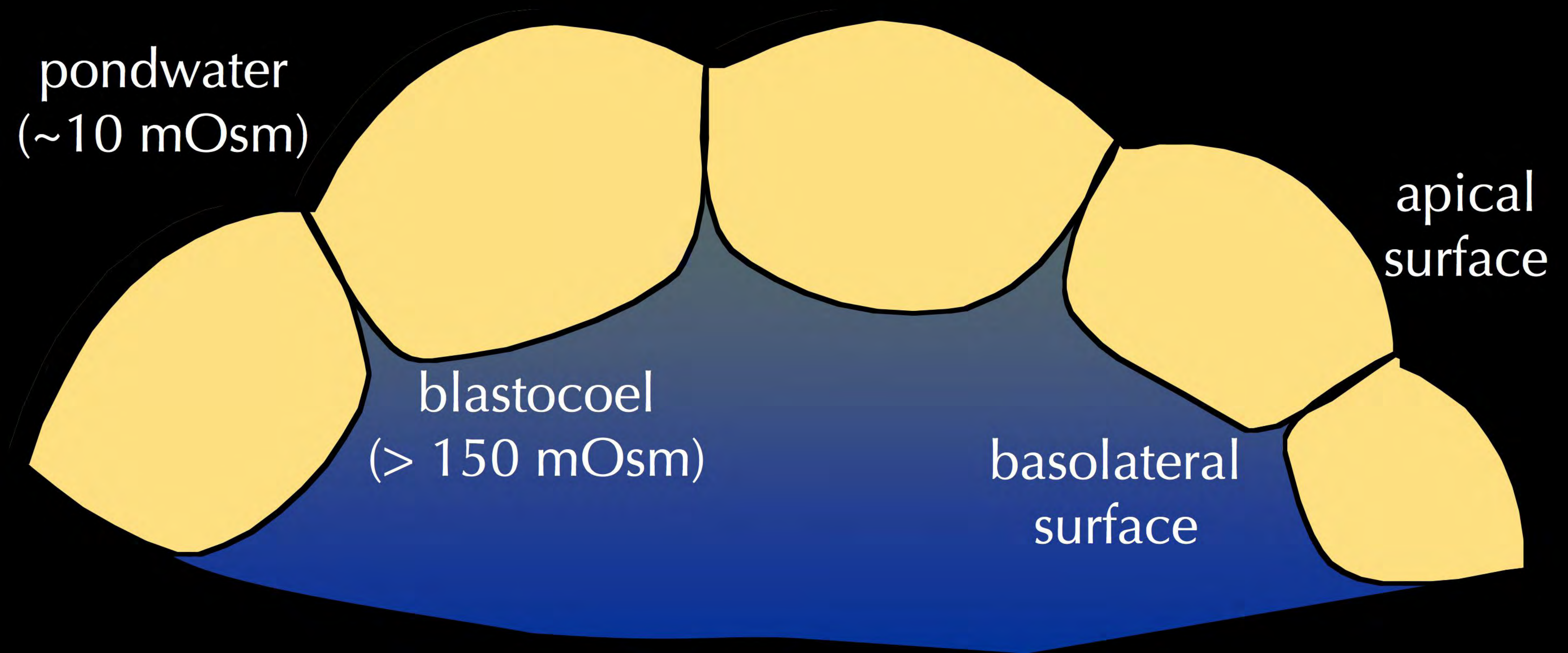
- rapid, ~synchronous cell divisions
- reliance on maternal transcripts and proteins
- cytoplasmic localizations
- membrane specializations (epithelialization)
  - establishment of basolateral domain
  - cell cell adhesion... compaction
- preparations for gastrulation

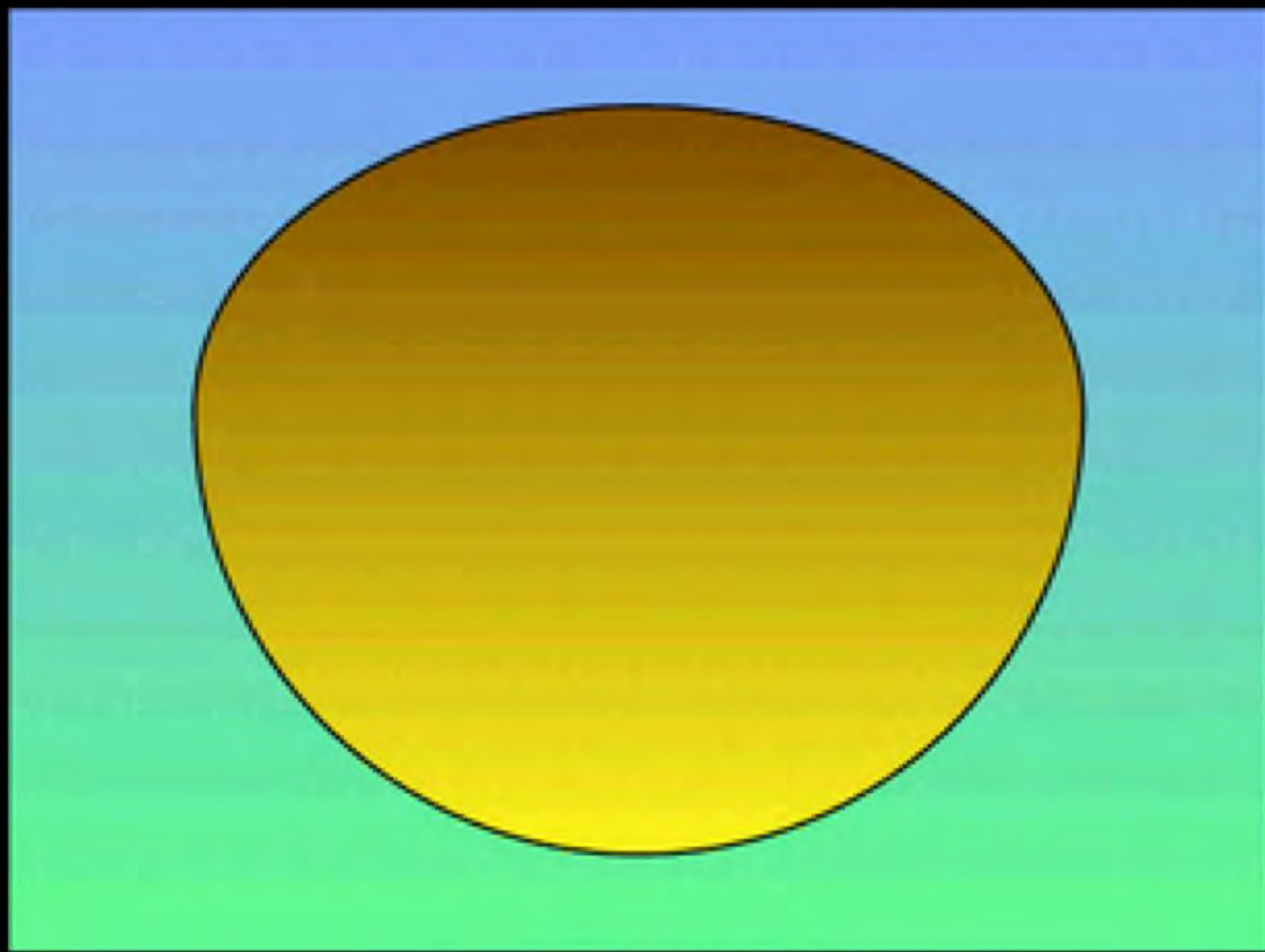


Movie 1



Movie 2

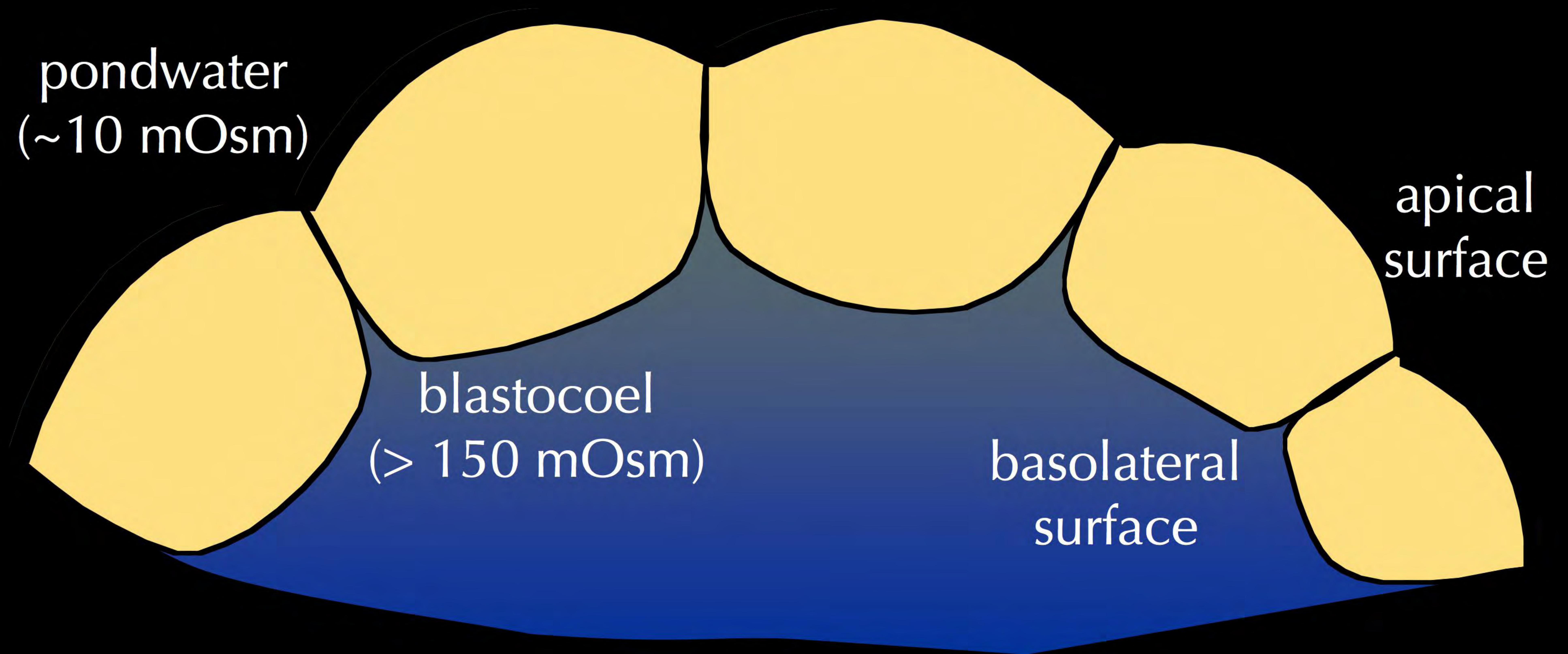


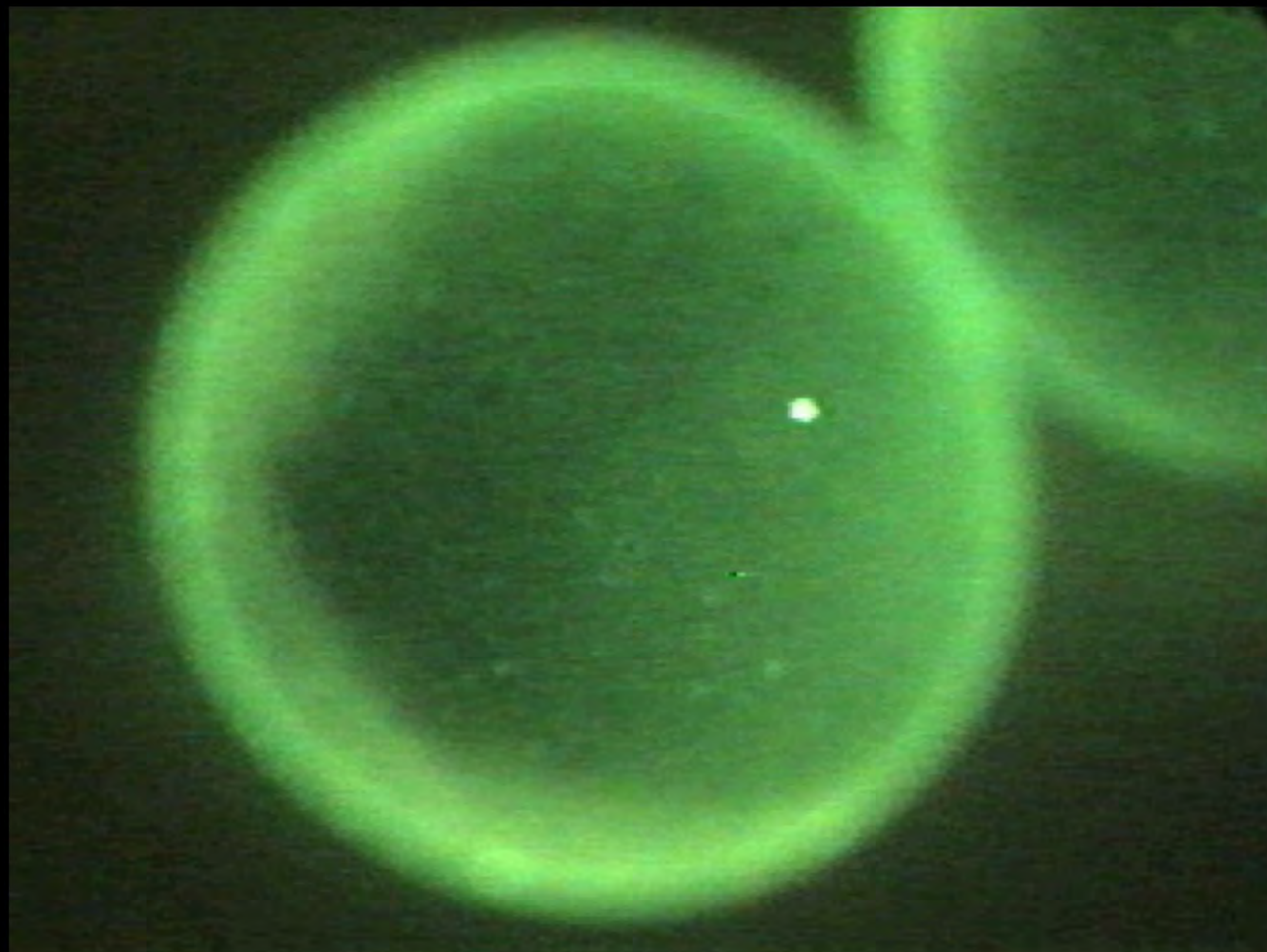


Movie 3



Movie 4





control *Movie 5*

Green = fluorescent lectin  
(binds surface carbohydrates)

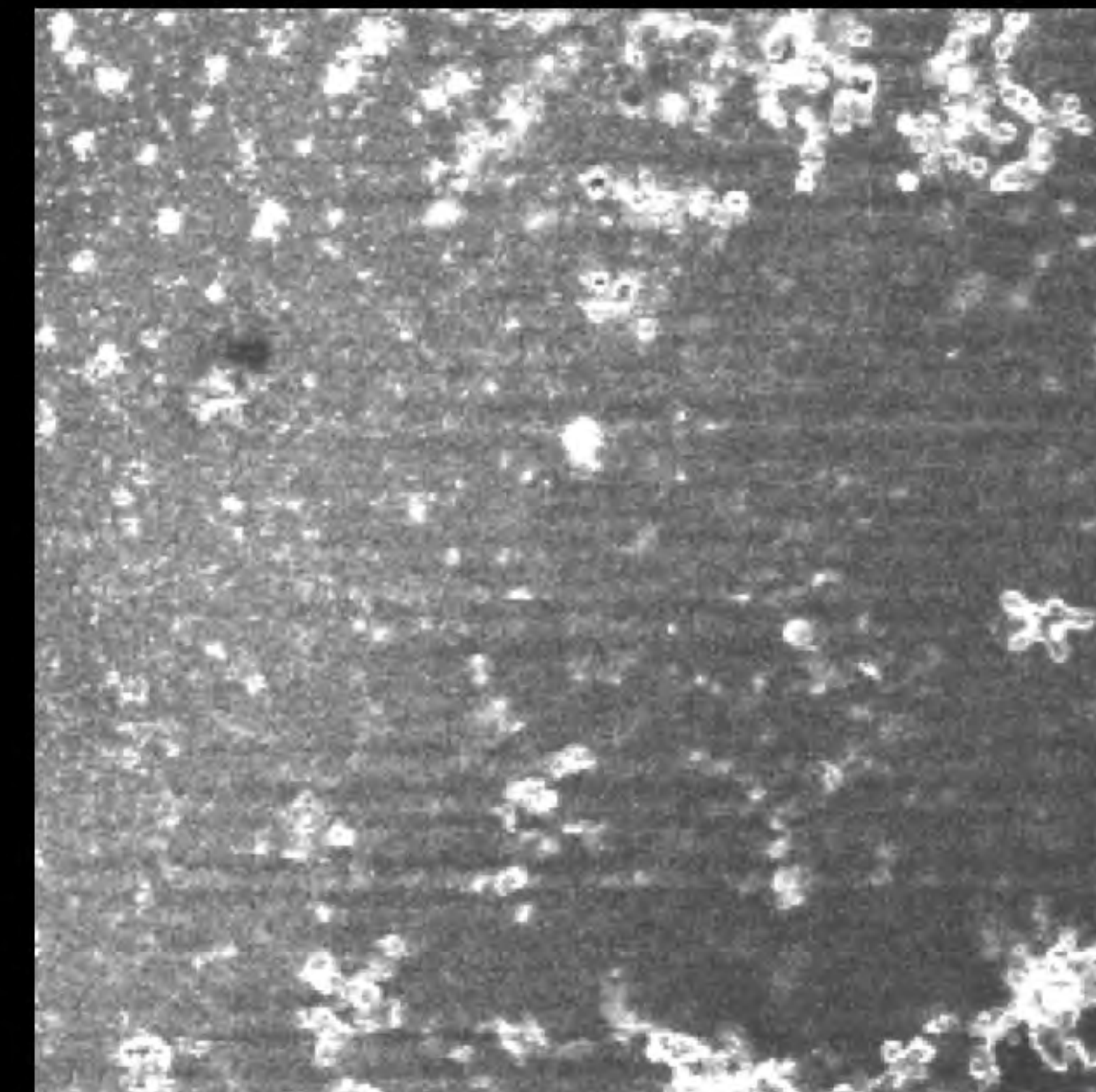
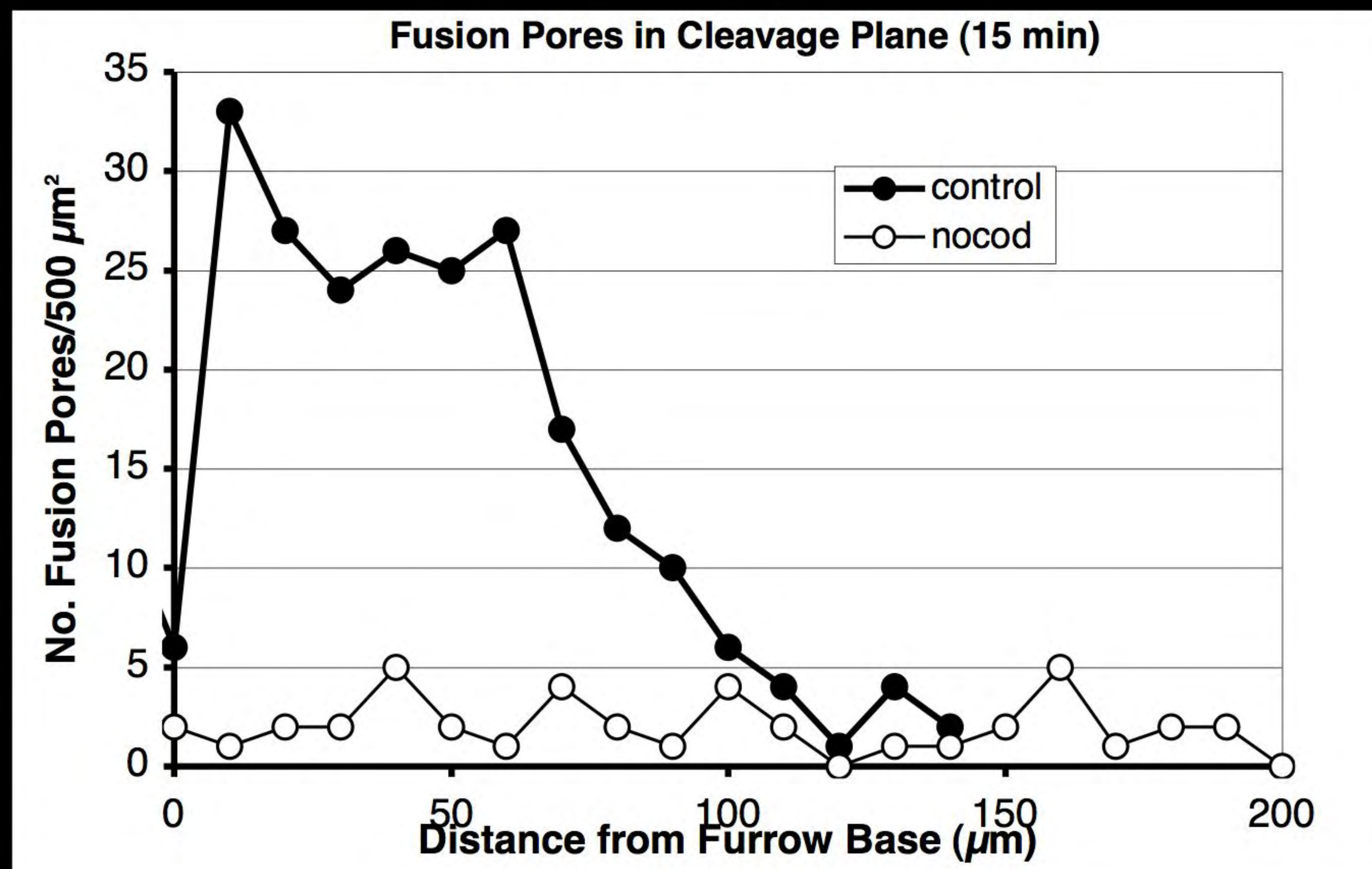
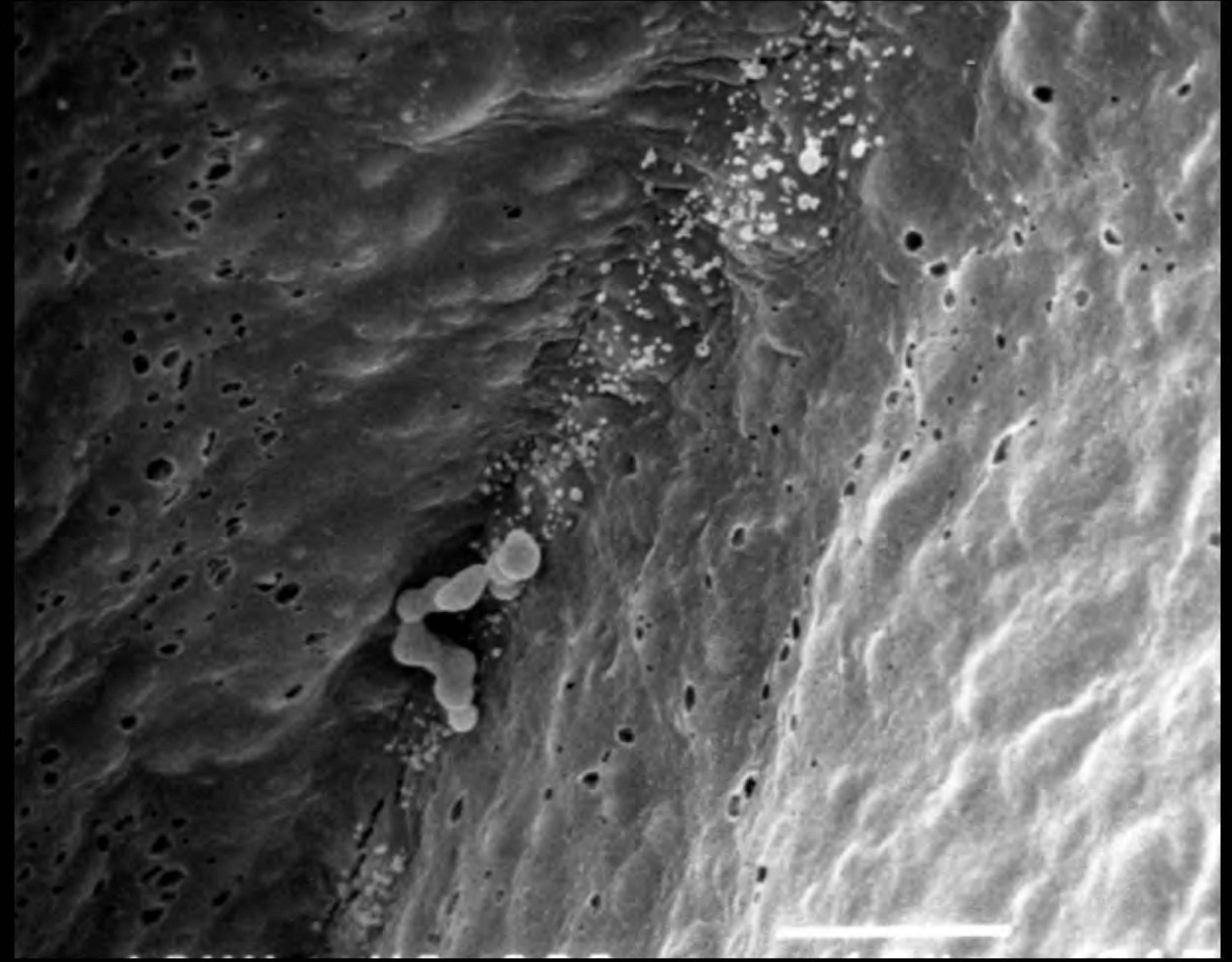
Membrane expansion  
& furrow progression  
both require  
microtubules

*Movie 6*



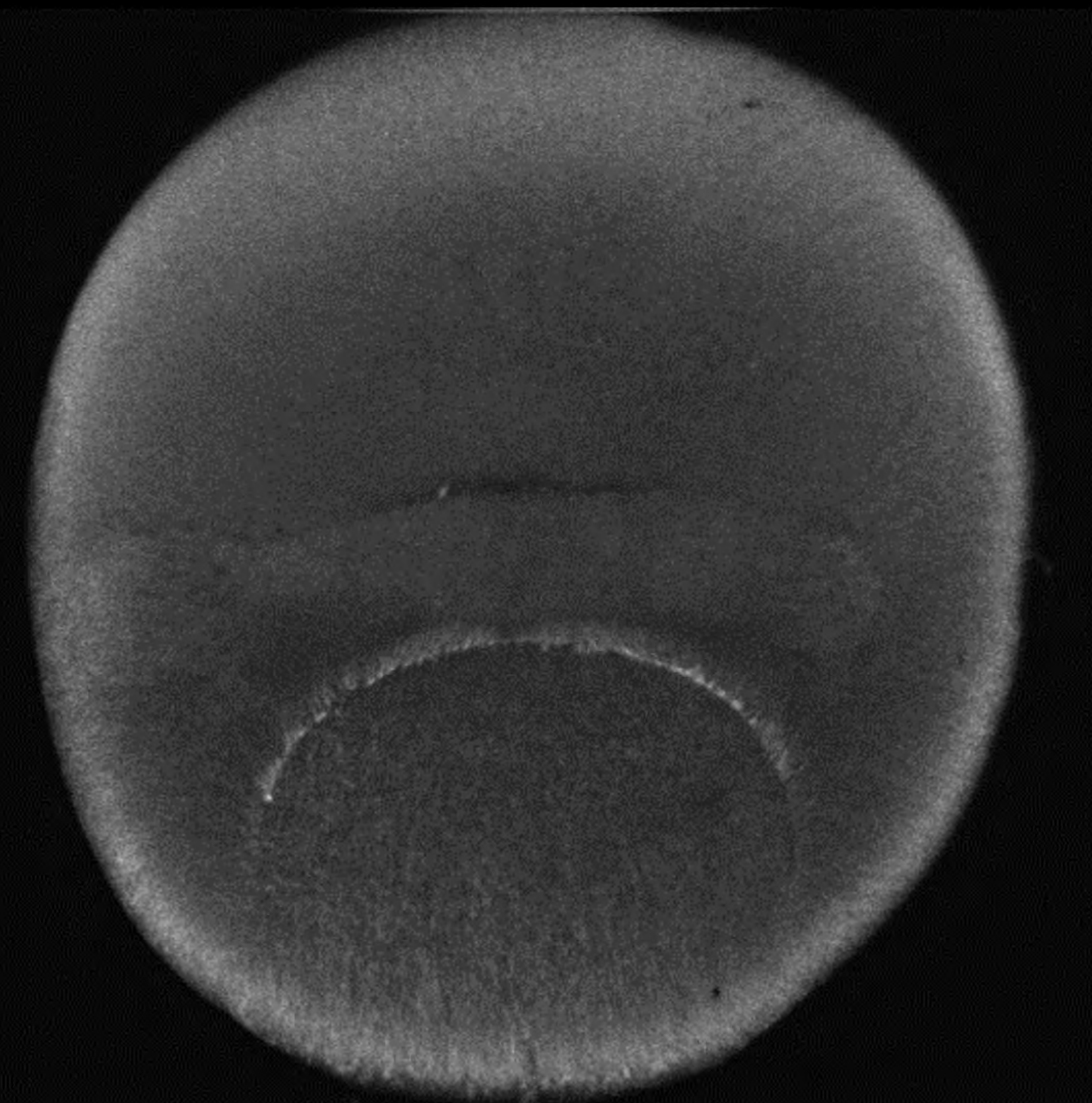
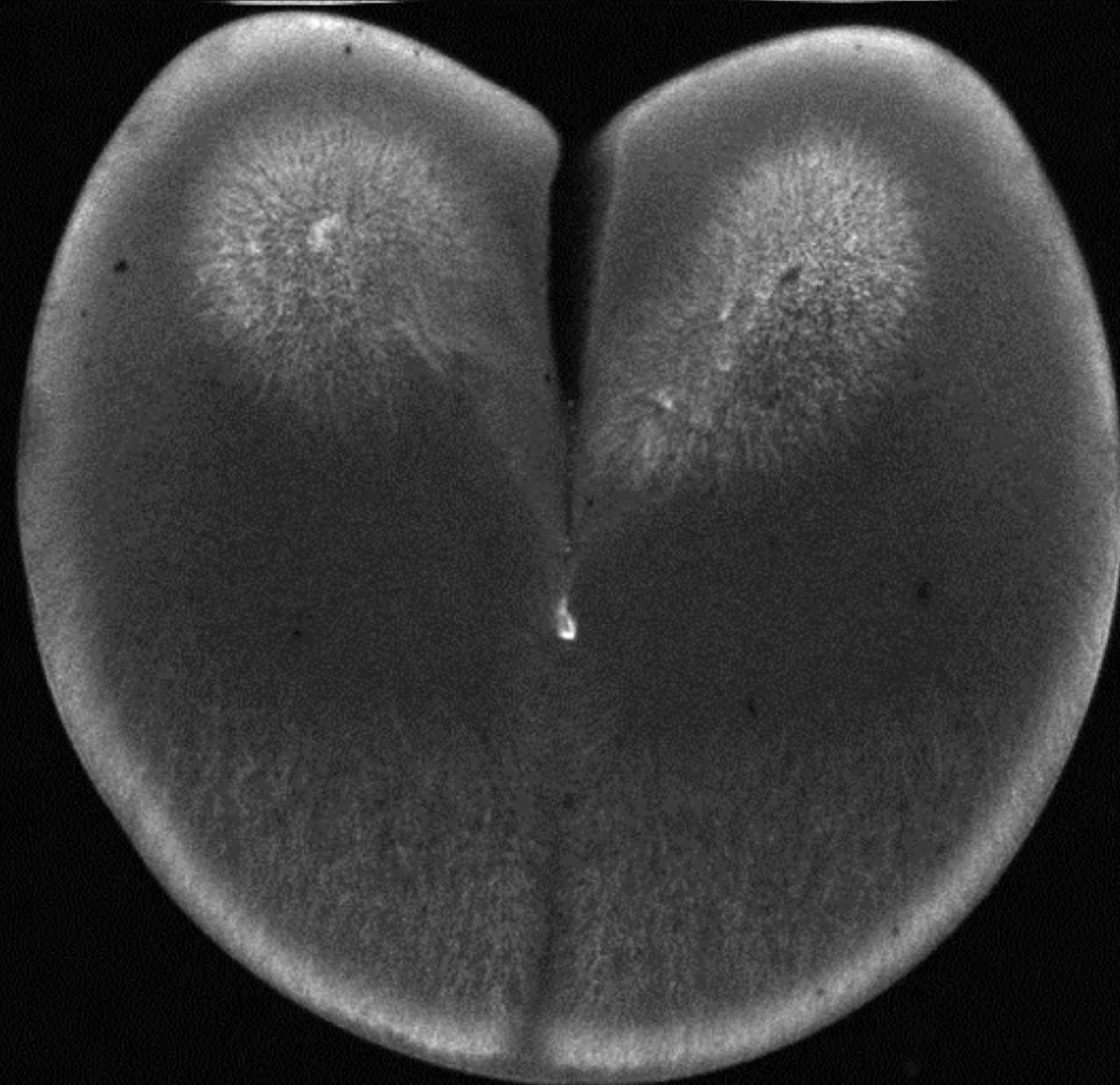
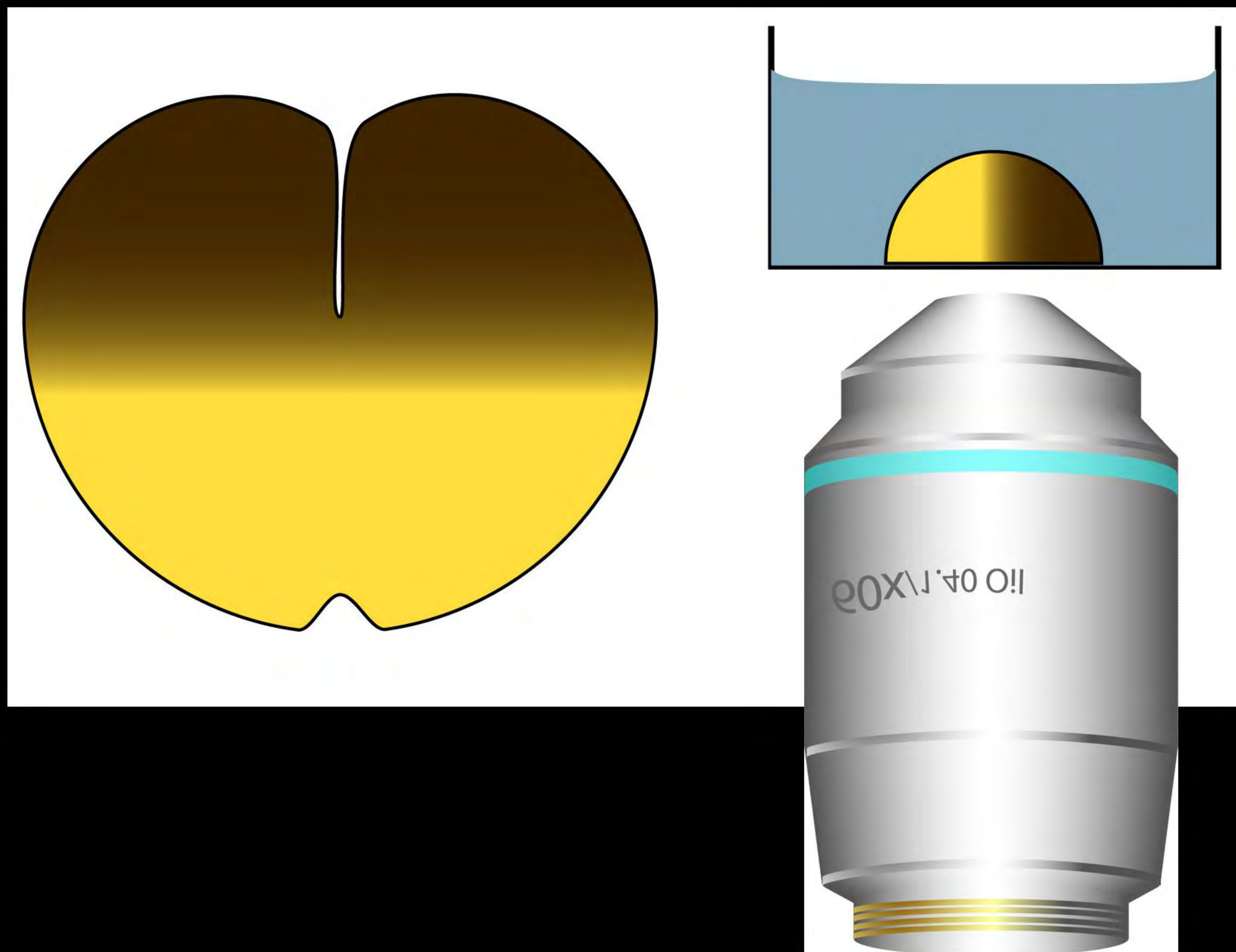
+ nocodazole  
(microtubule inhibitor)

# Localization of exocytotic site depends on furrow microtubules

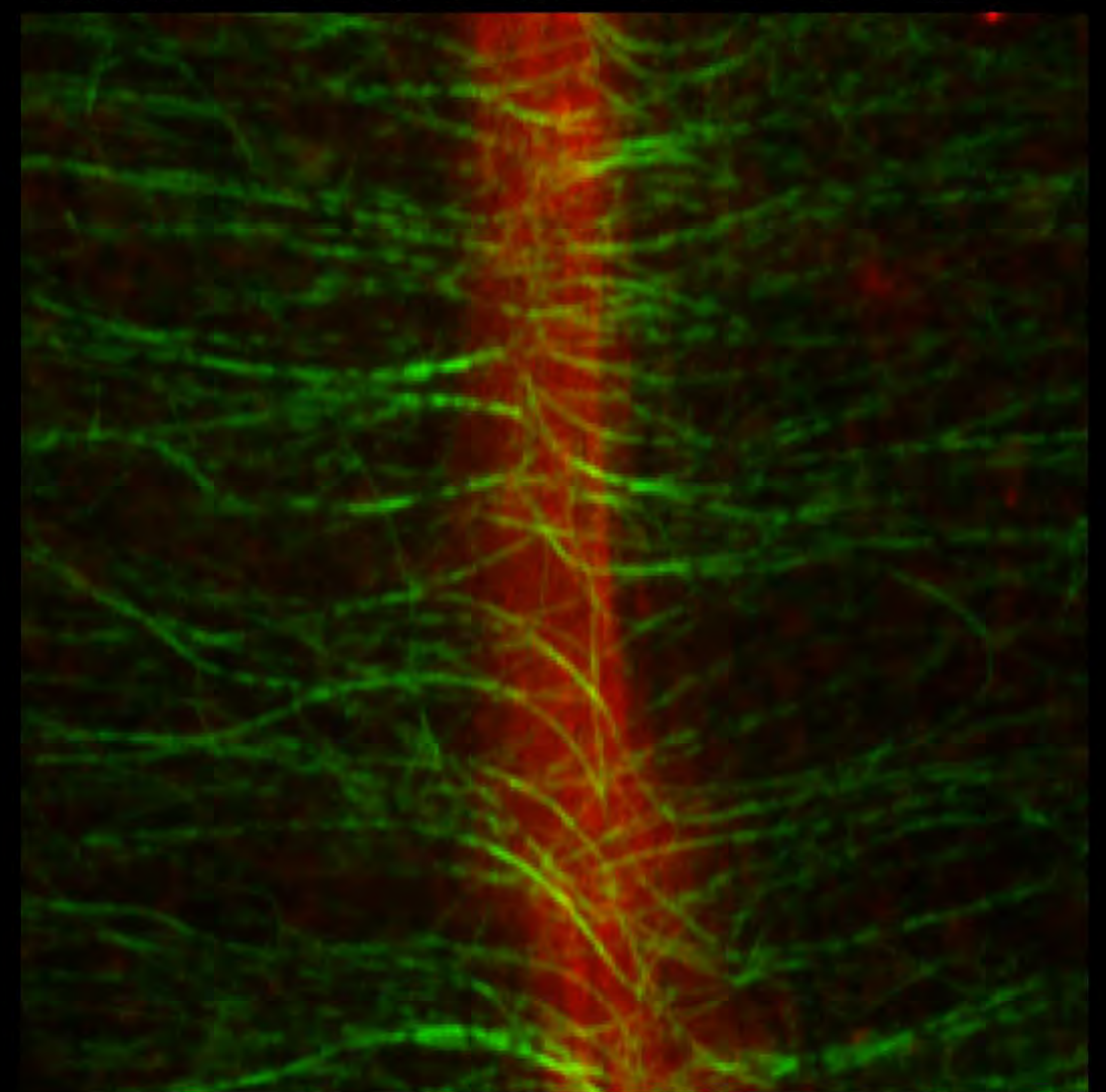
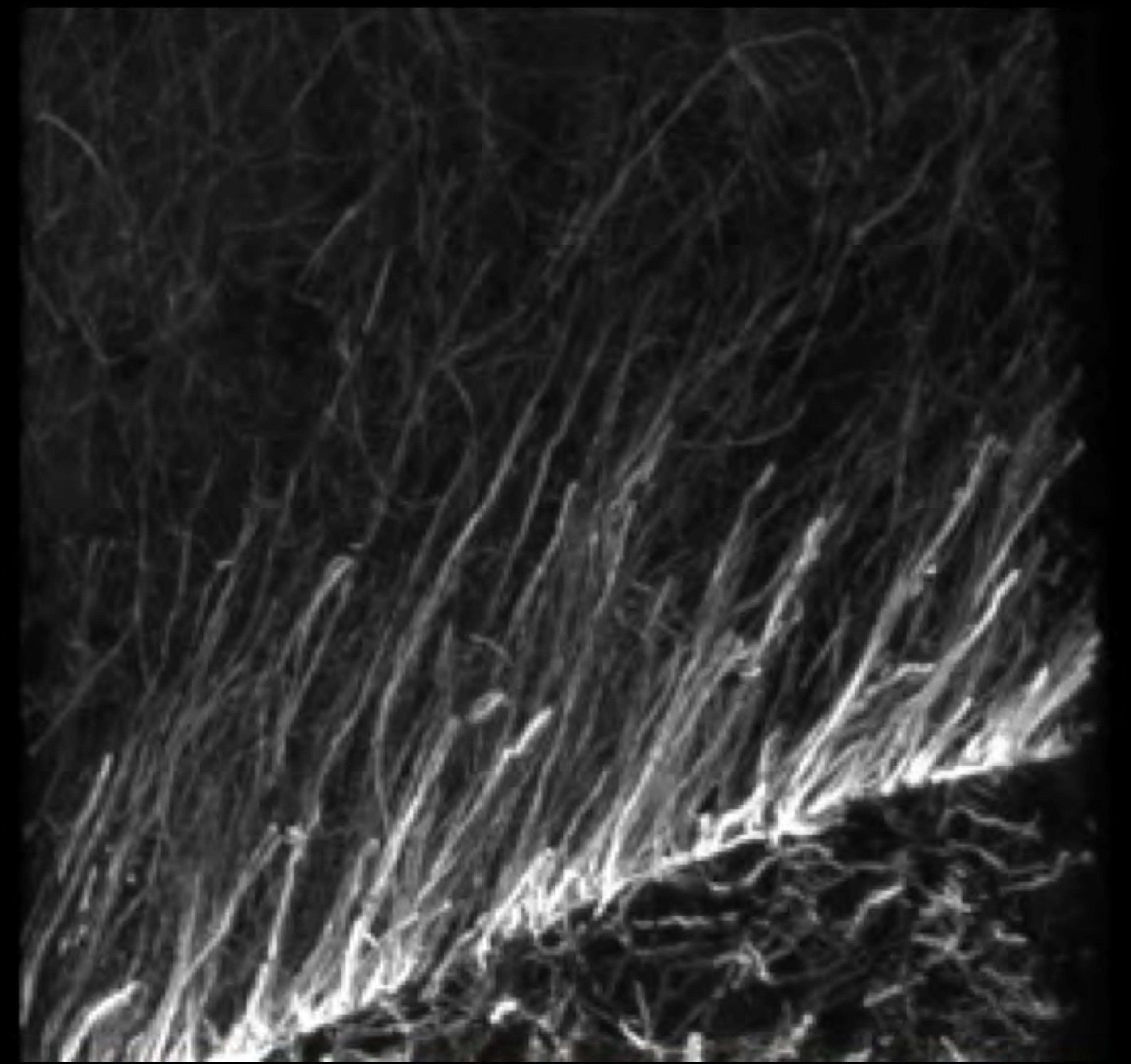
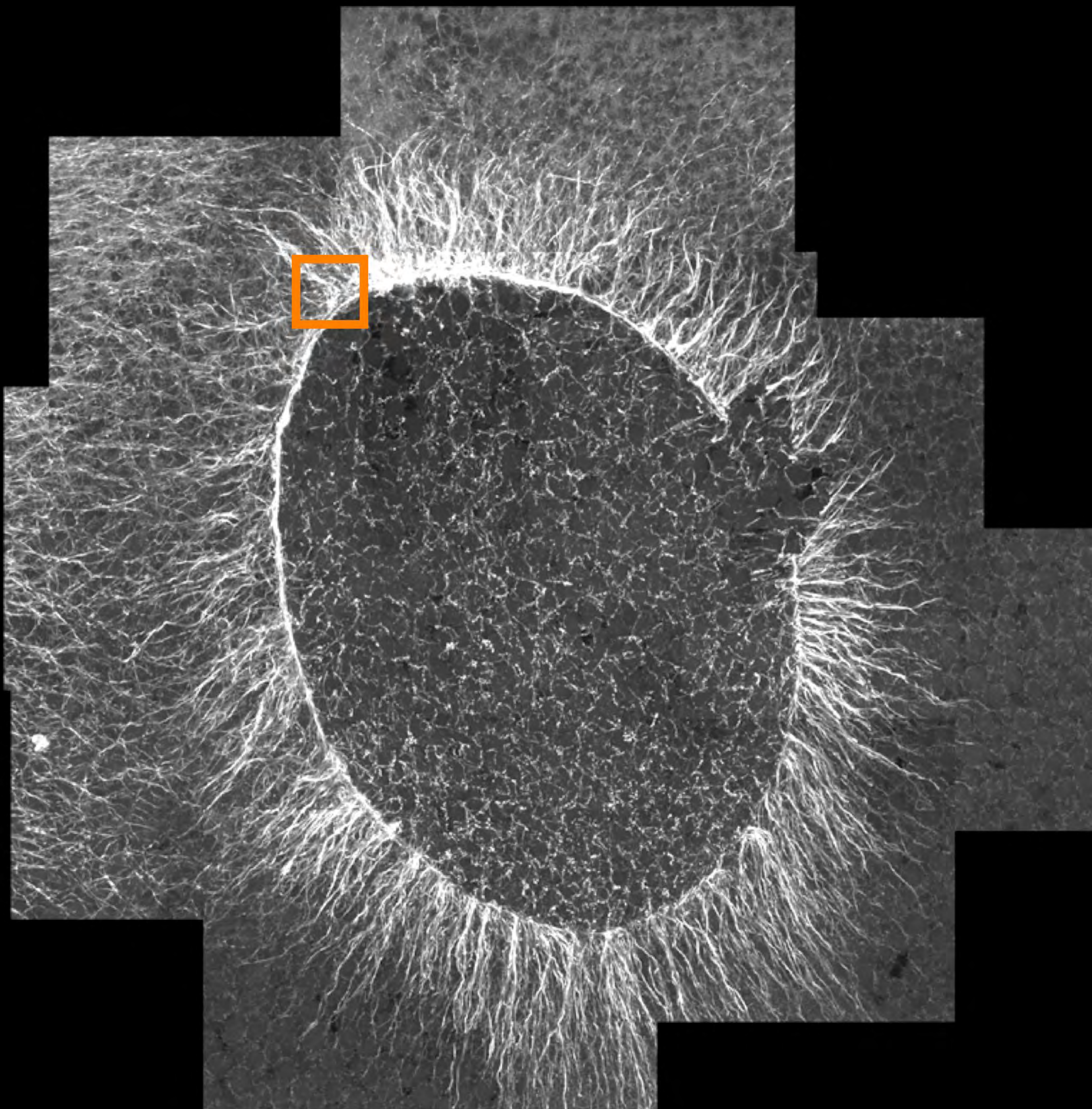


**Movie 7**

FM 1-43 (fluorescent lipid dye)







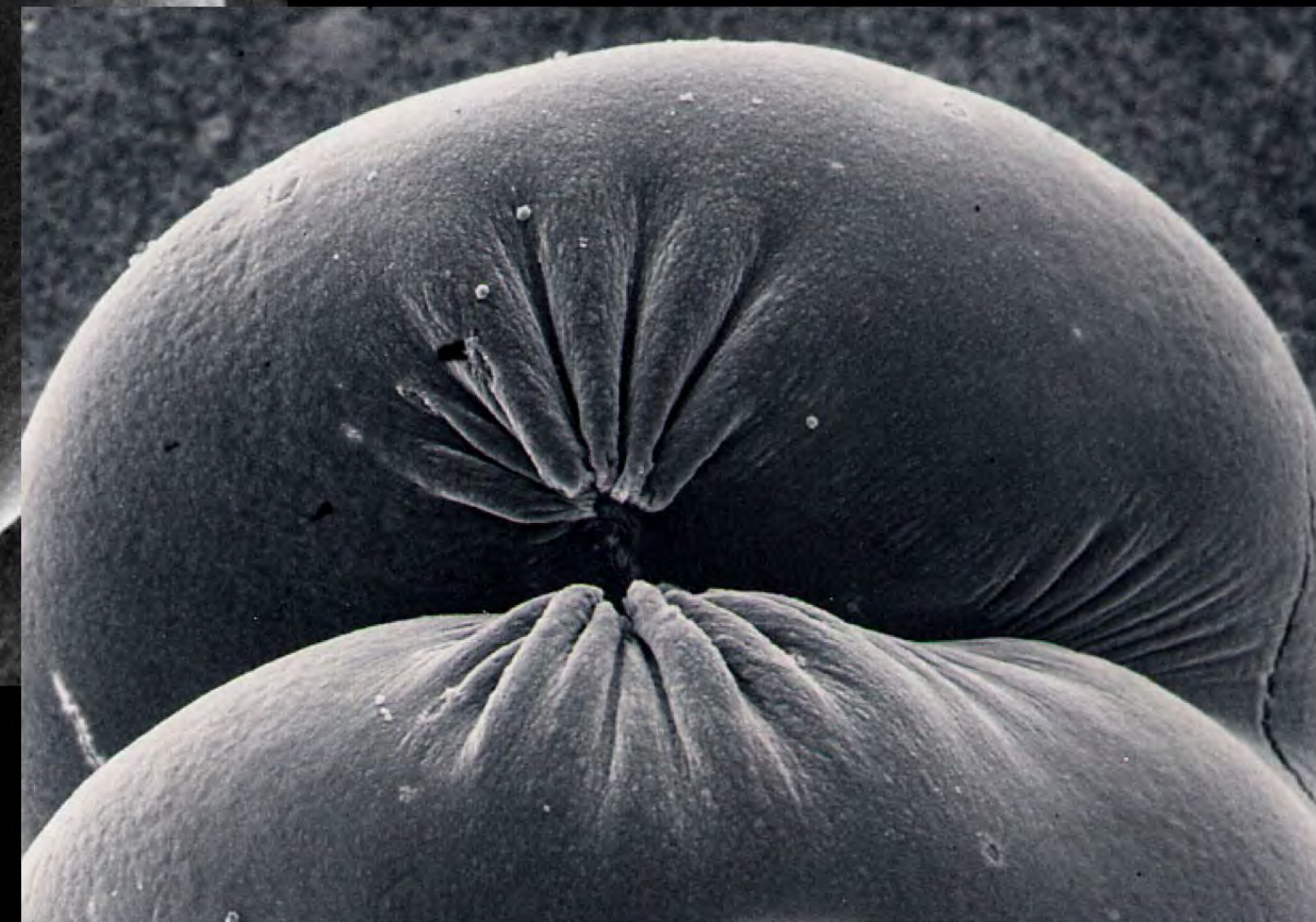
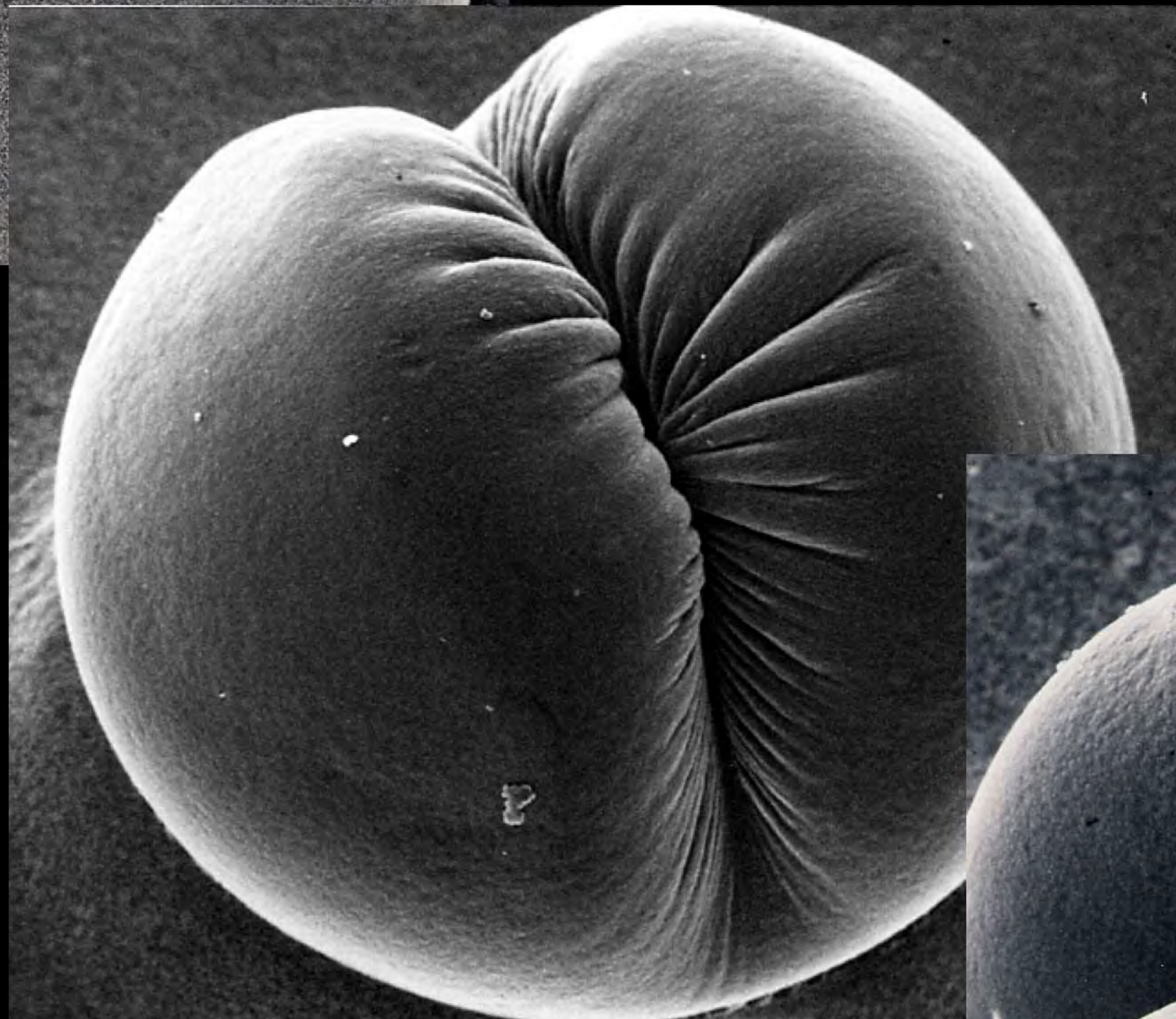
microtubules microfilaments

# Cleavage furrow closure:

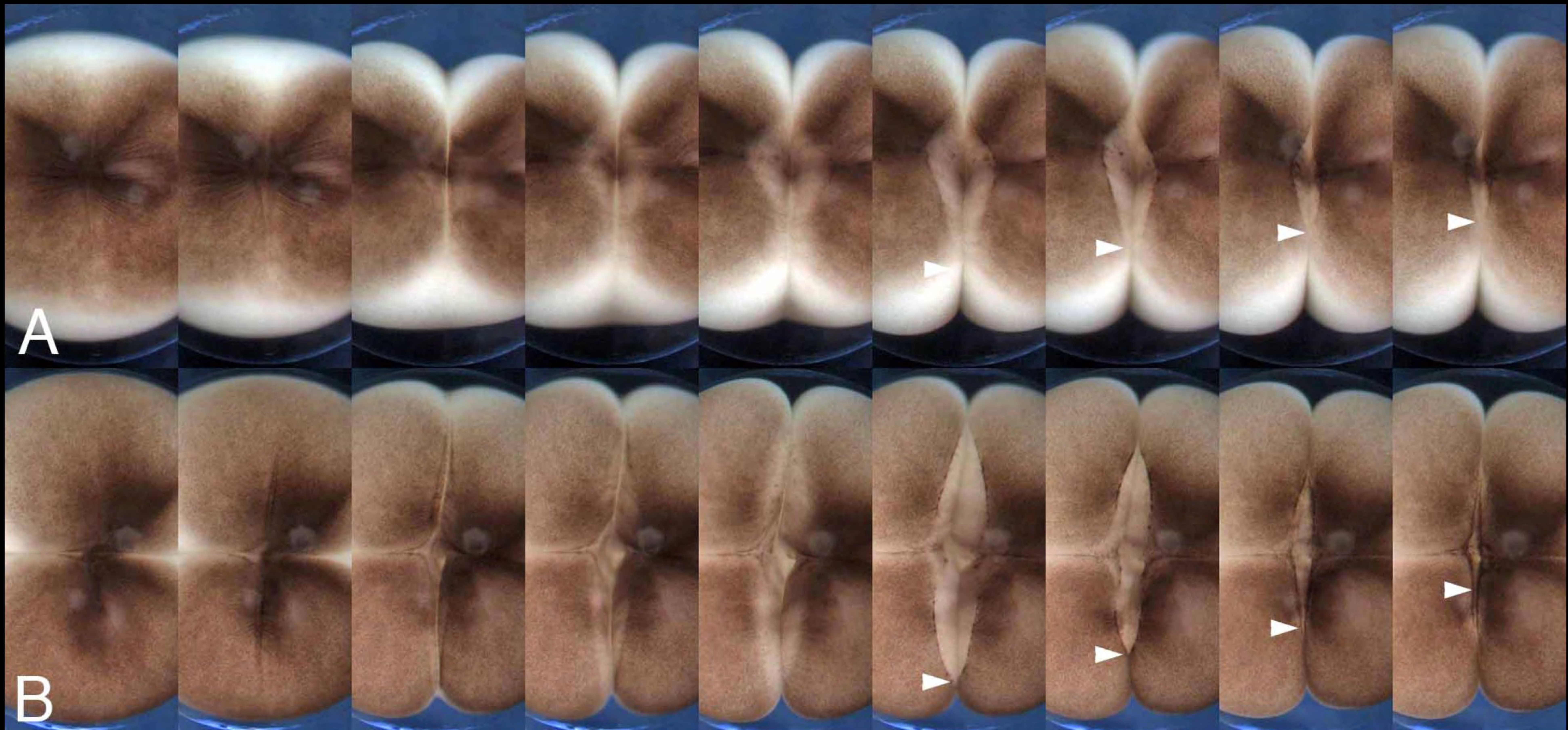
stress folds

marginal protrusive zone

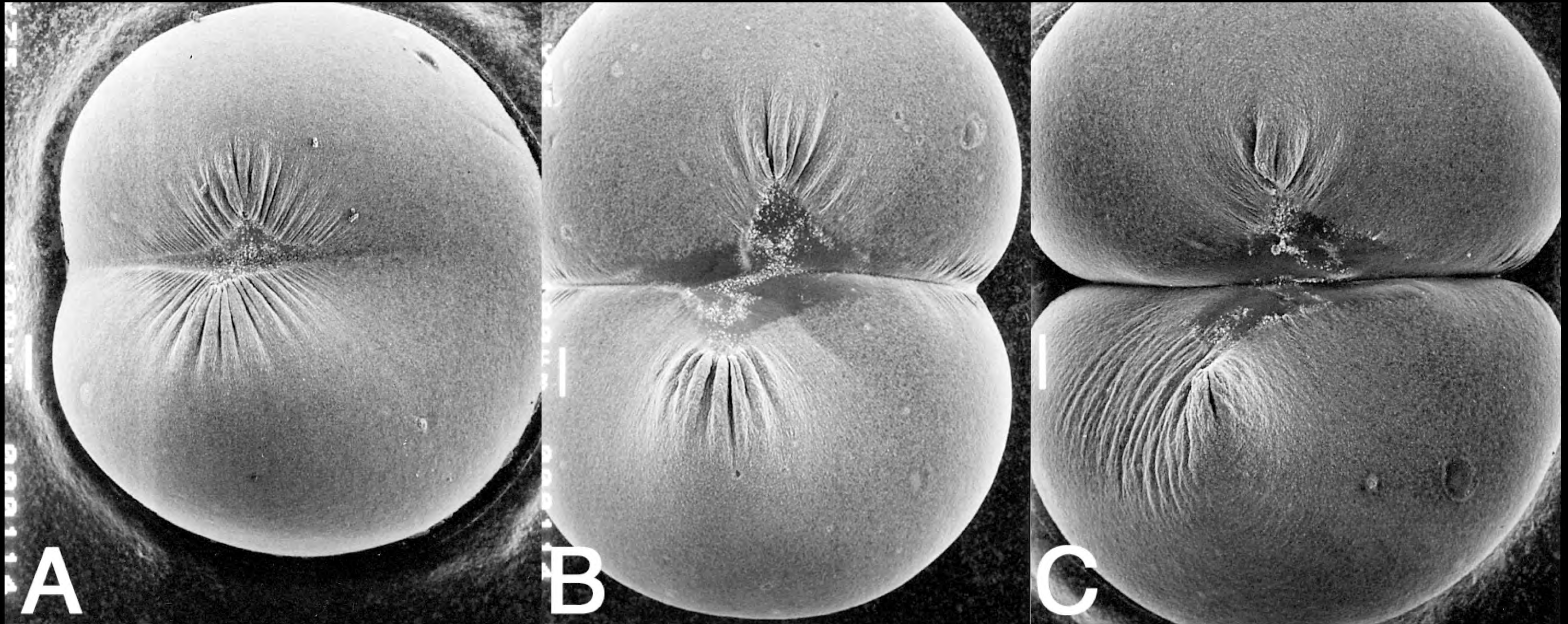
basolateral protrusive zone



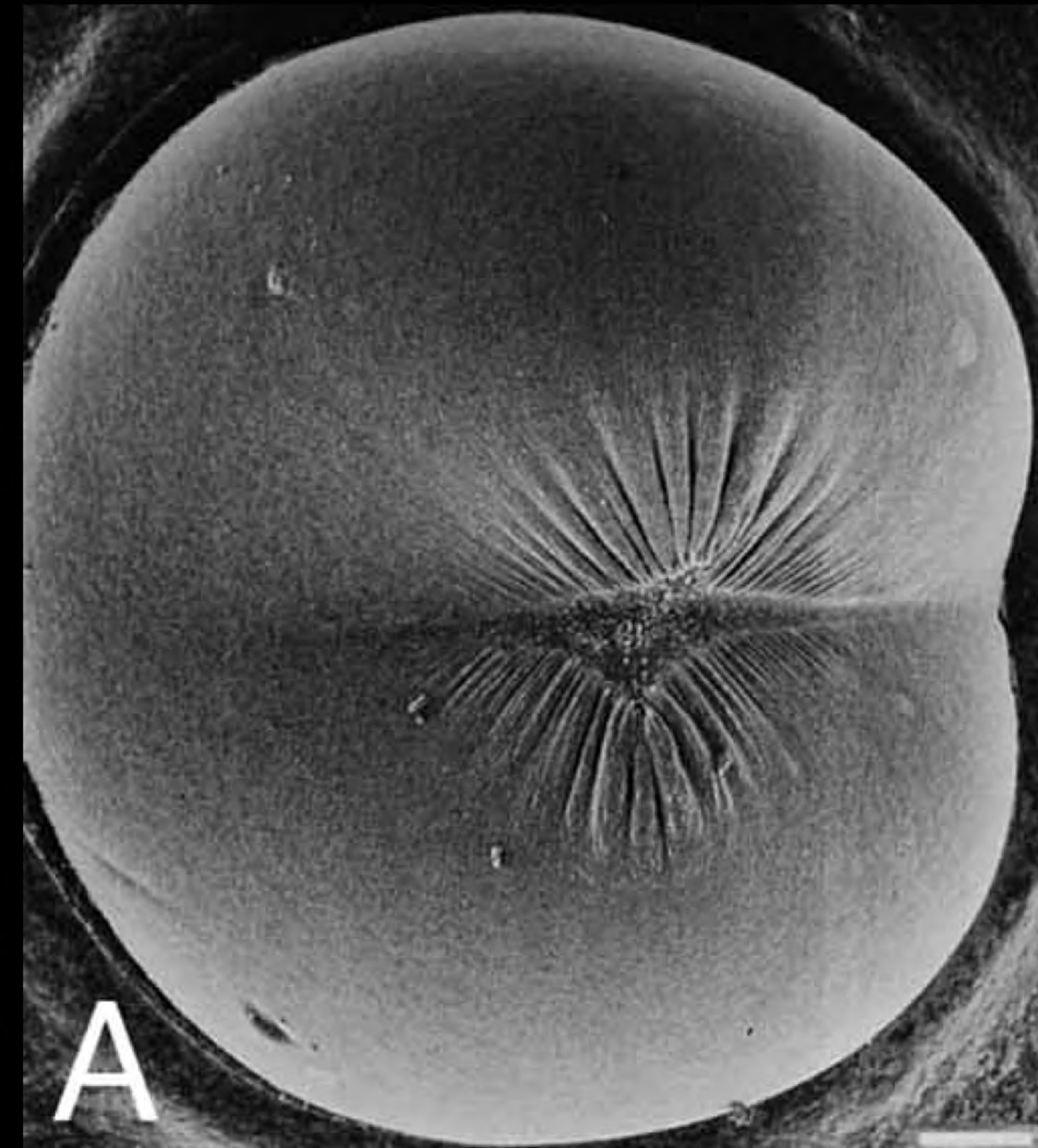
# Cleavage furrow closure via apical zippering & basolateral adhesion

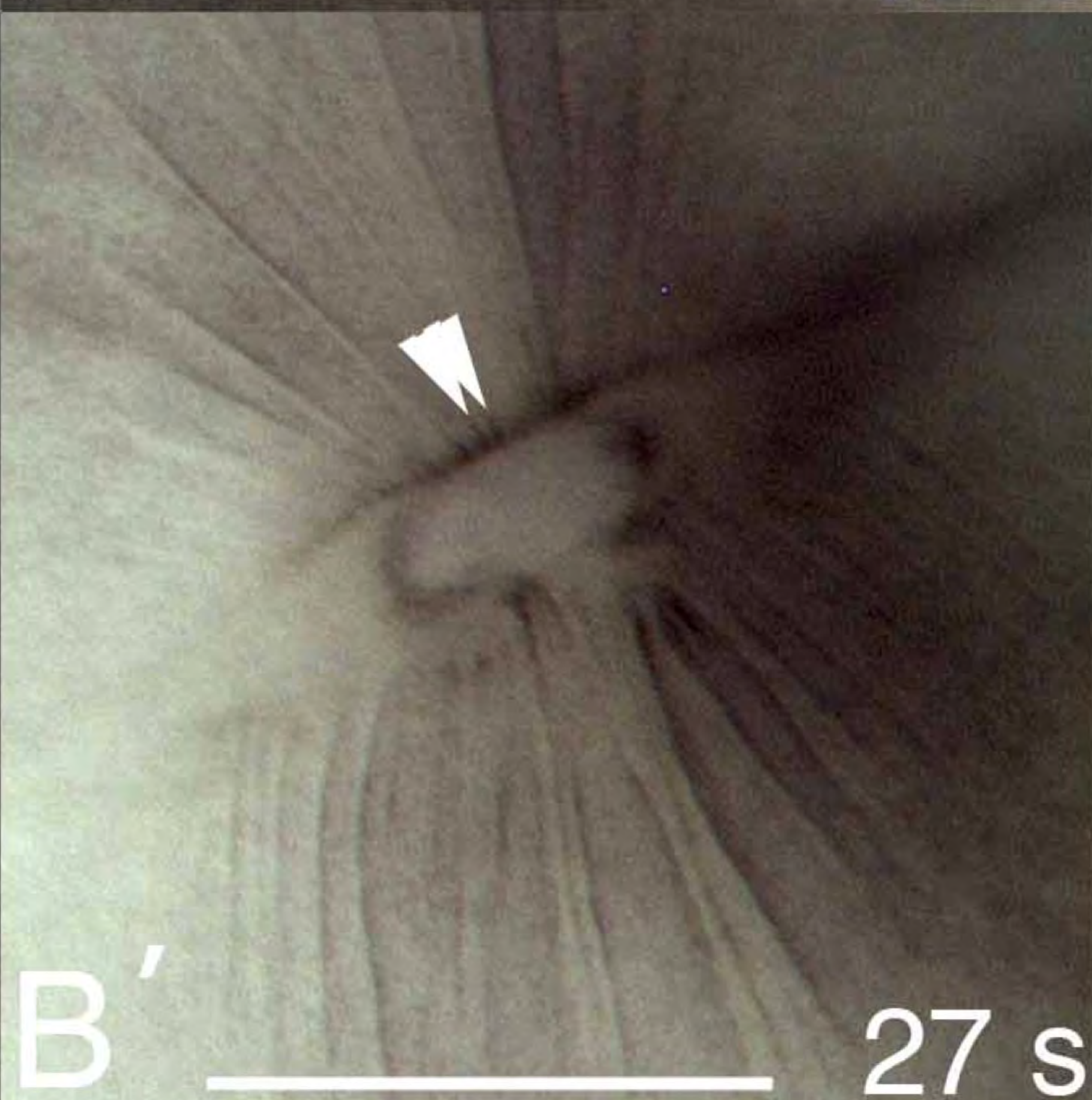
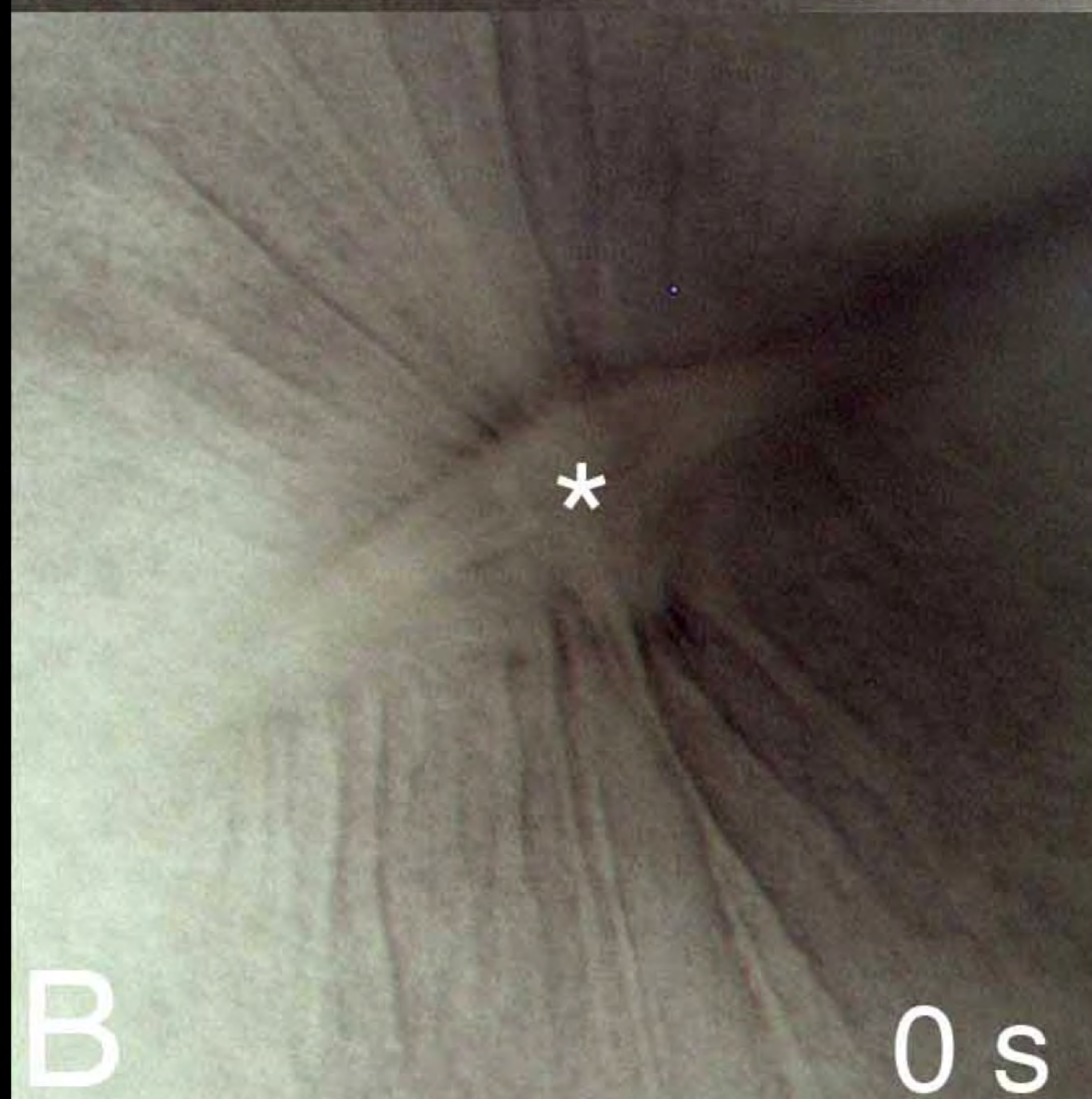
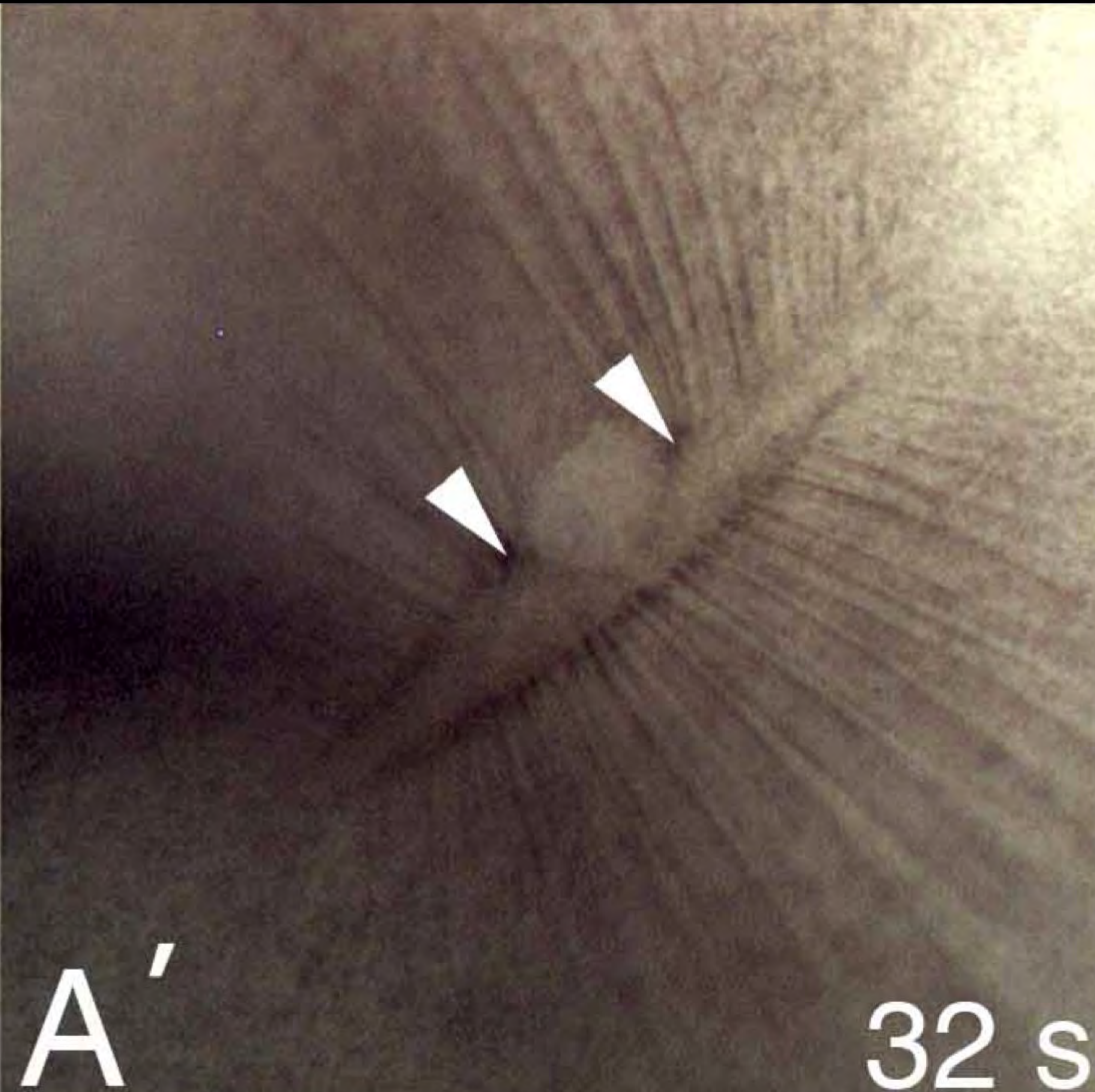
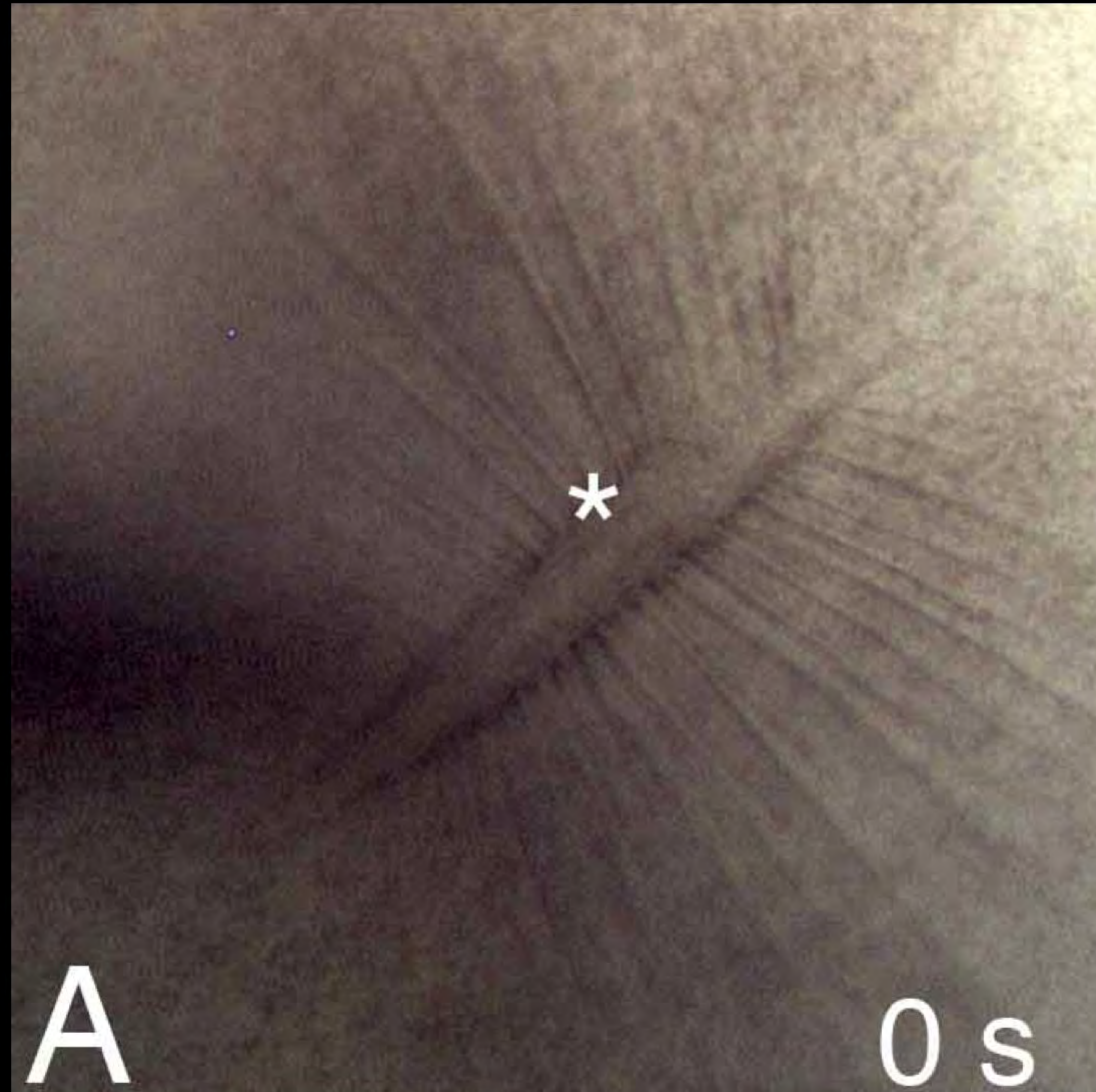


# Stress folds ("tension lines")

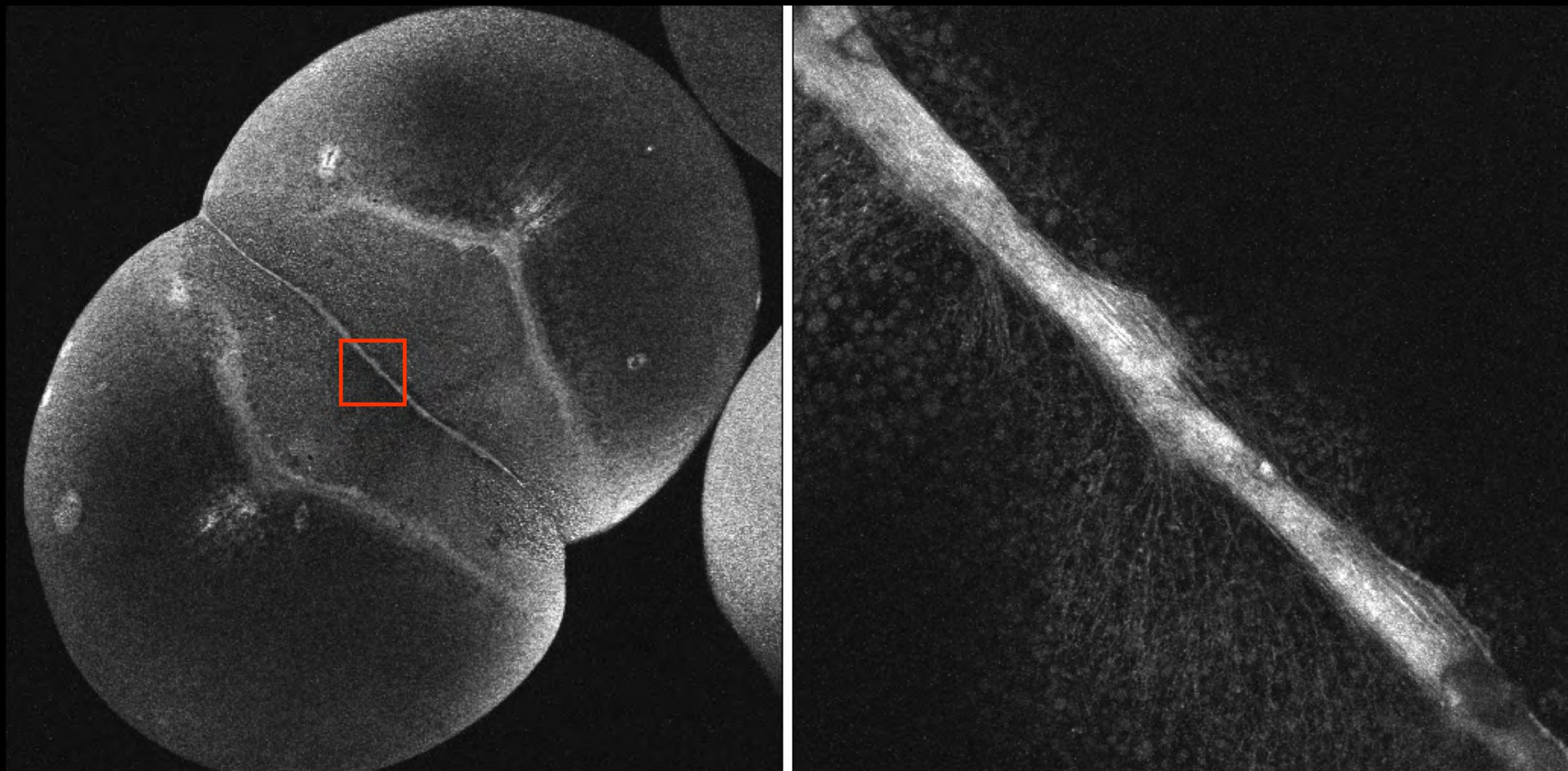


Marginal contractility isn't a function  
of contractile ring



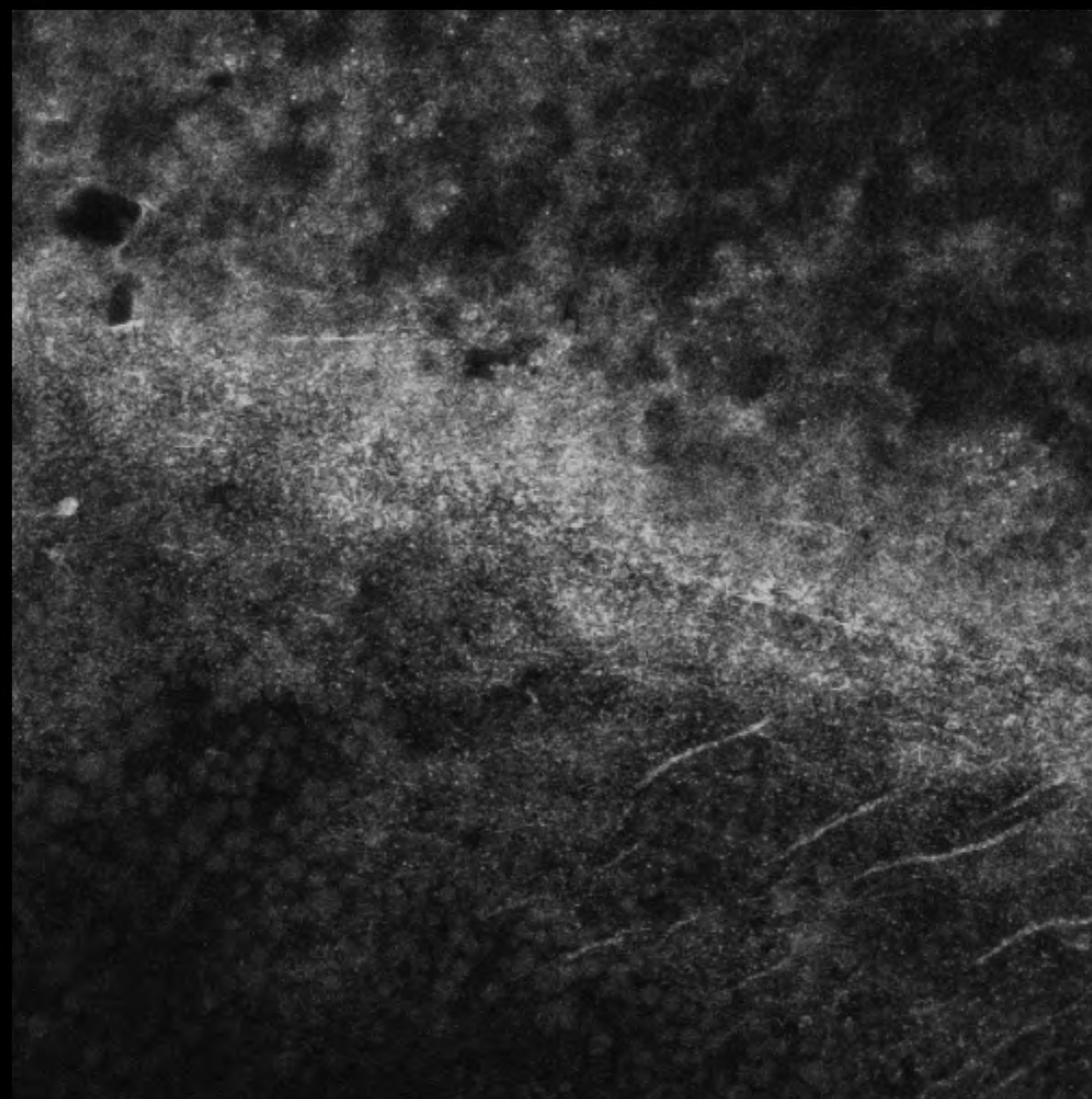
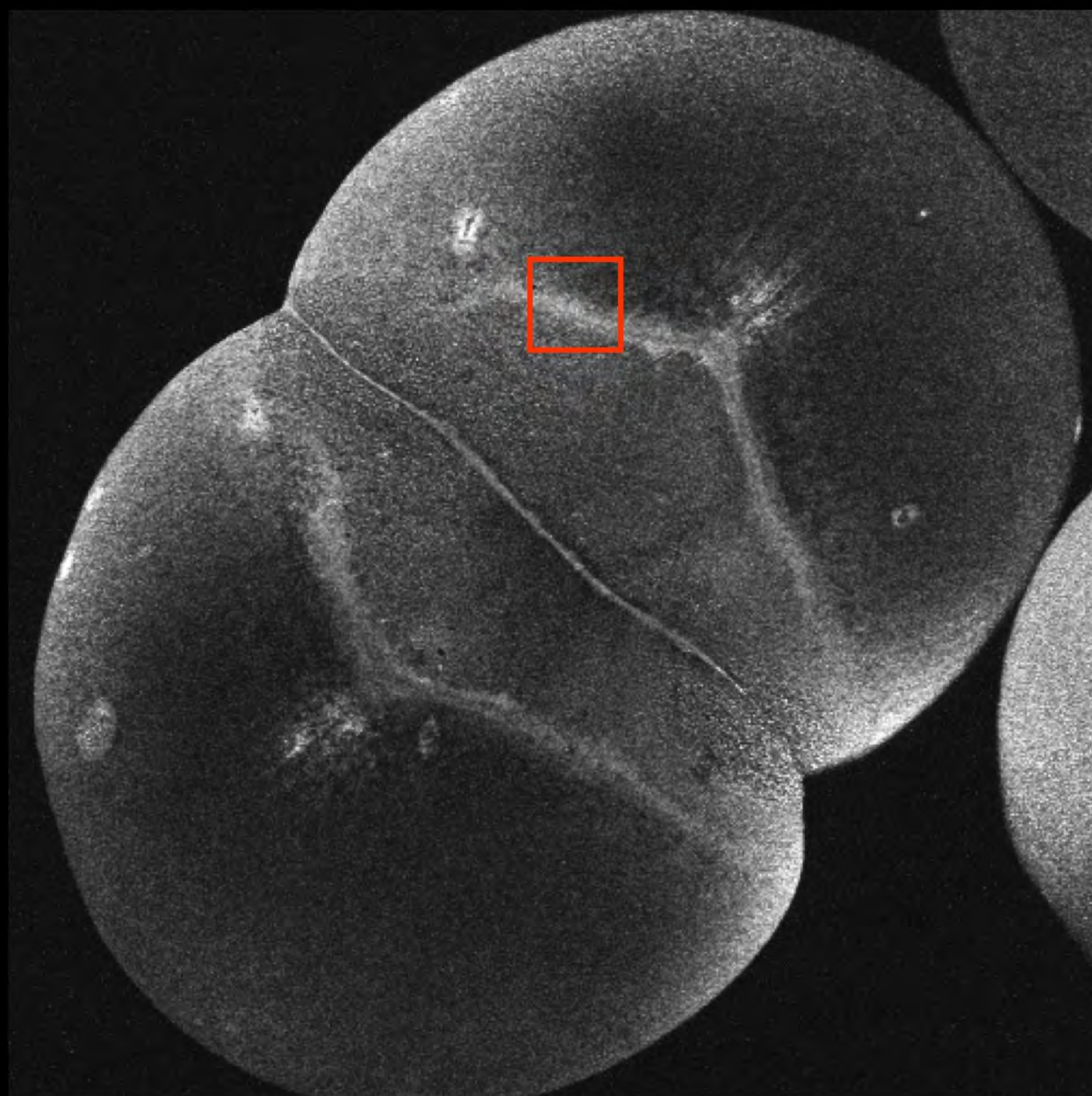


# Parallel bundles of contractile f-actin



Rh-phalloidin

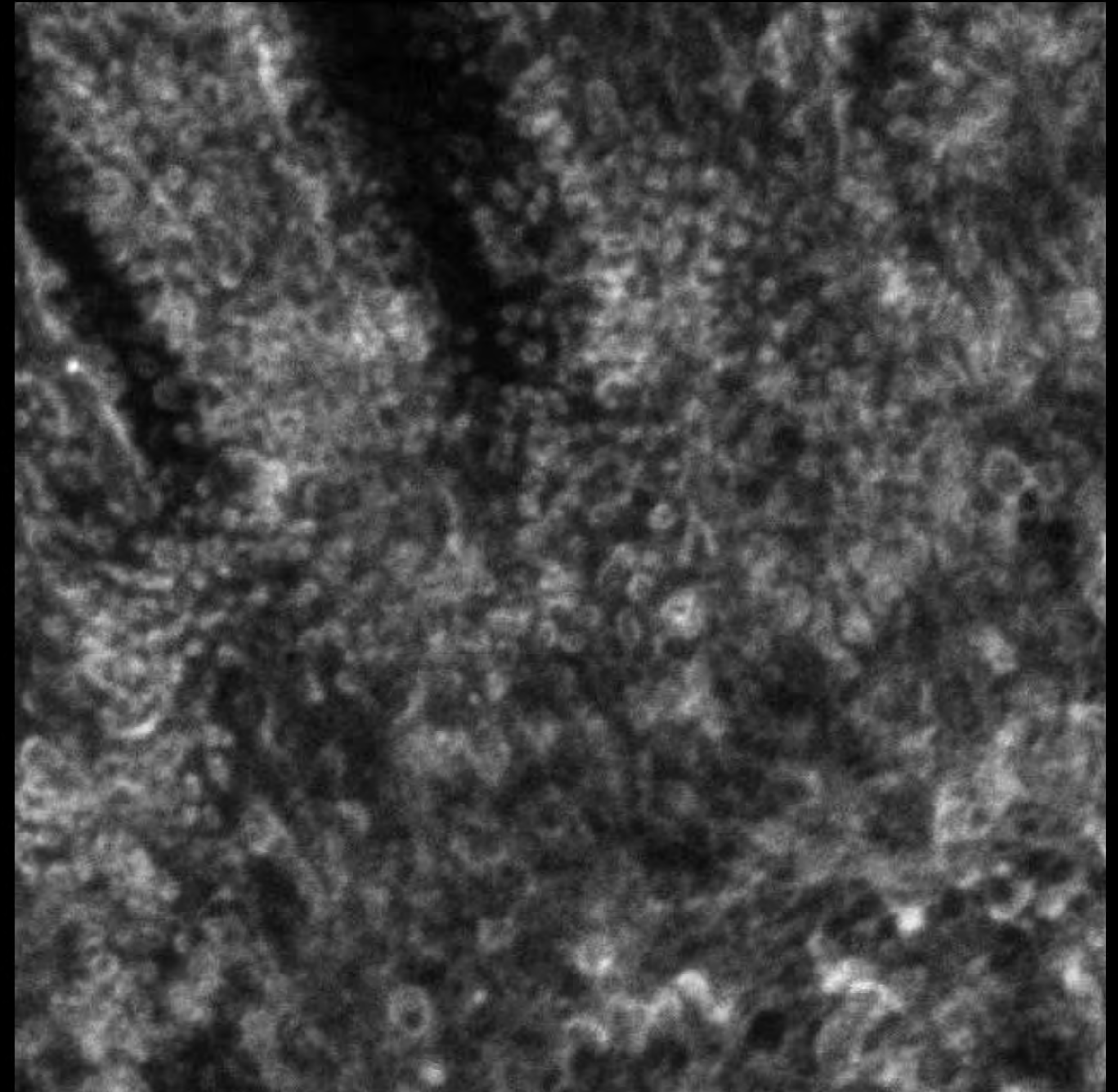
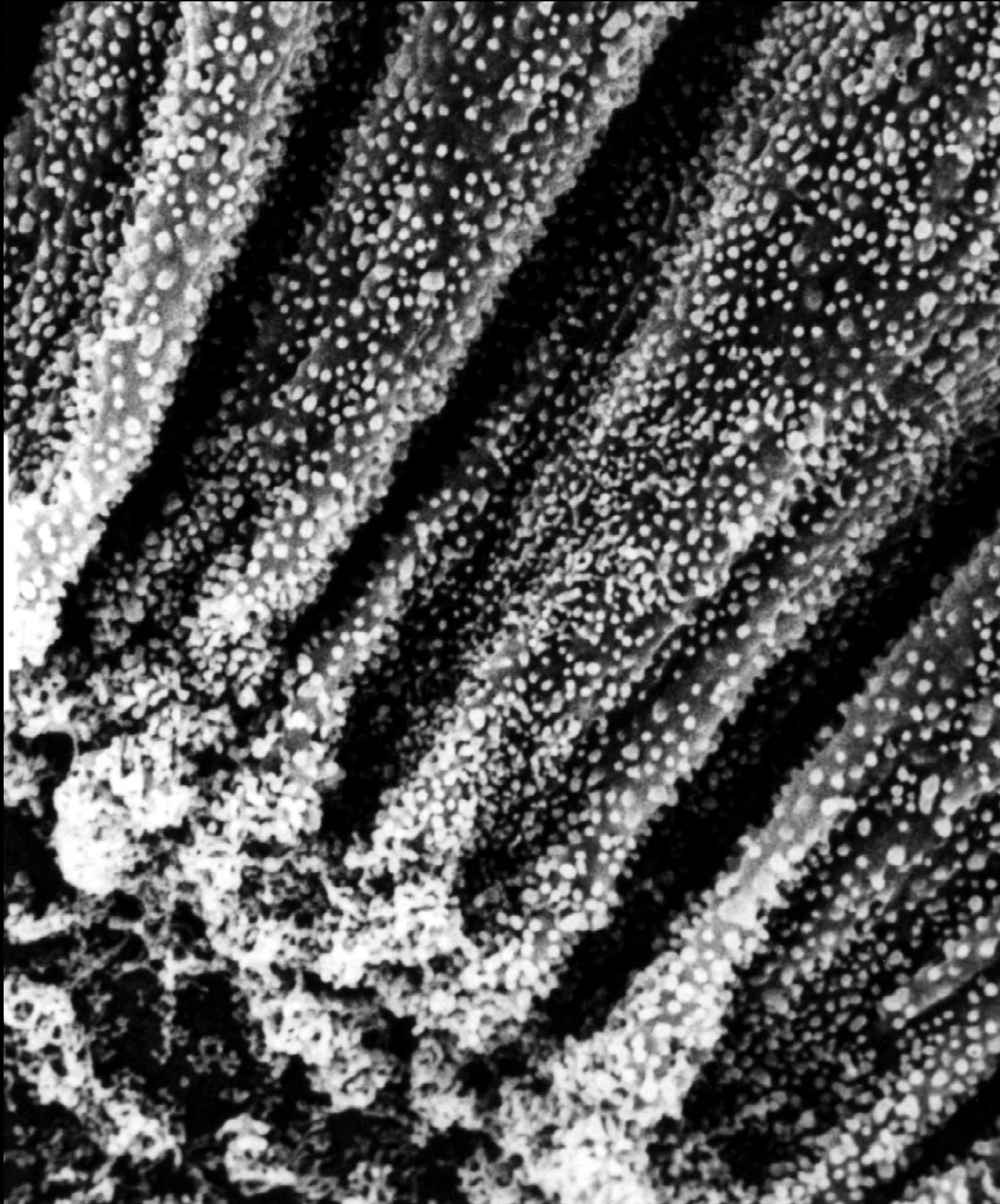
# Amorphous band of contractile f-actin



Rh-phalloidin

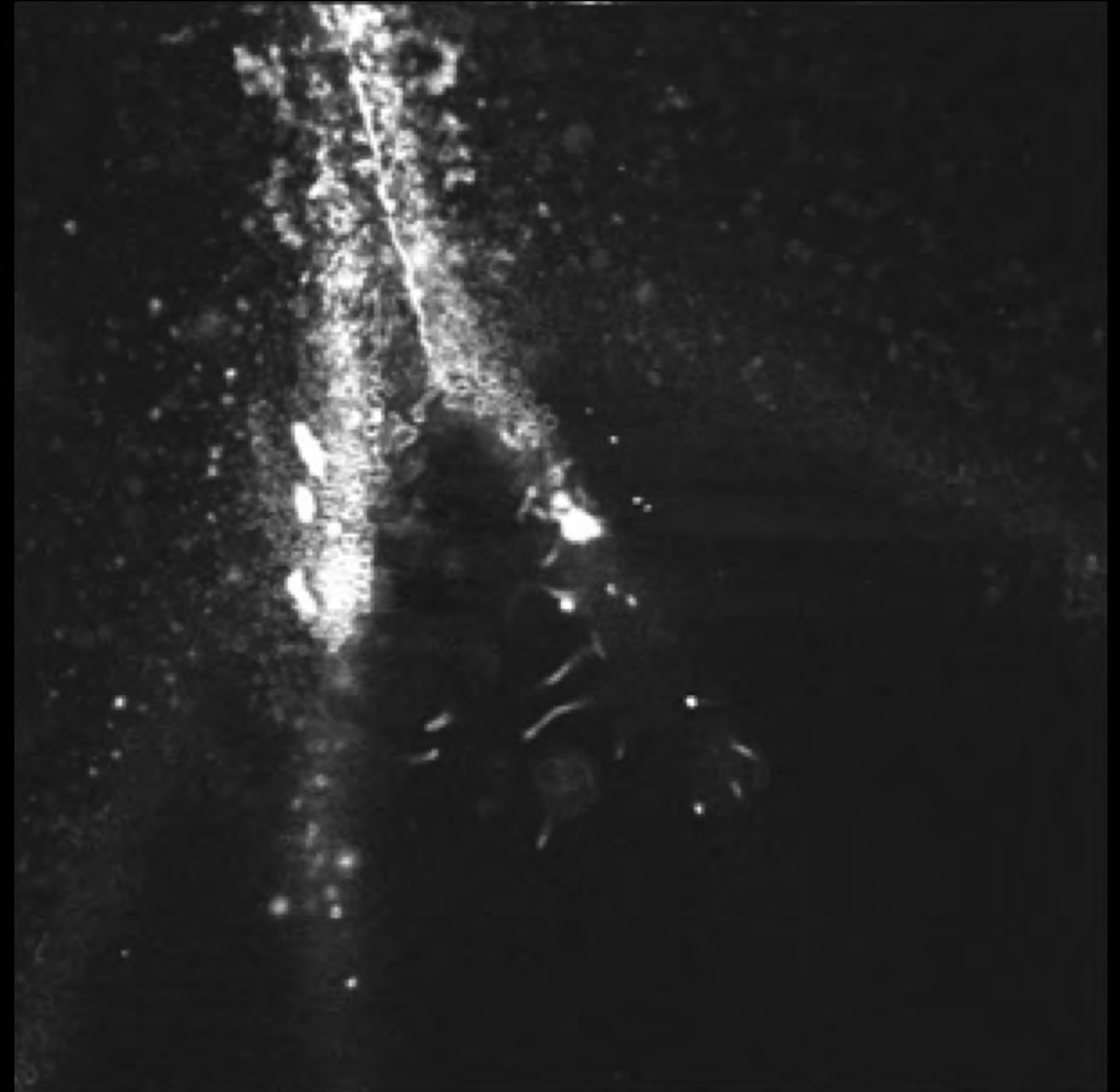
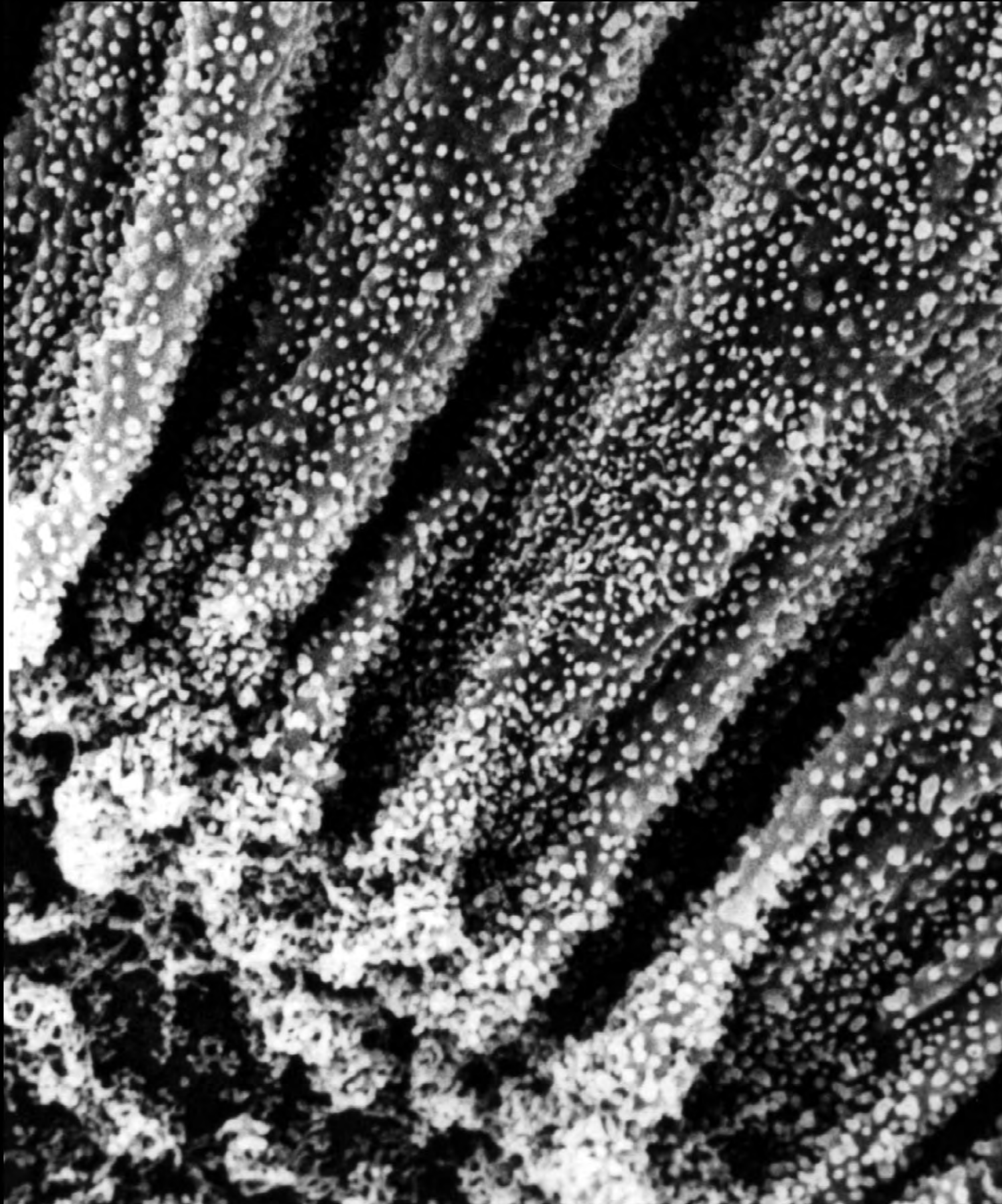


Marginal protrusions  
are actin-filled



Rh-phalloidin, 2-cell

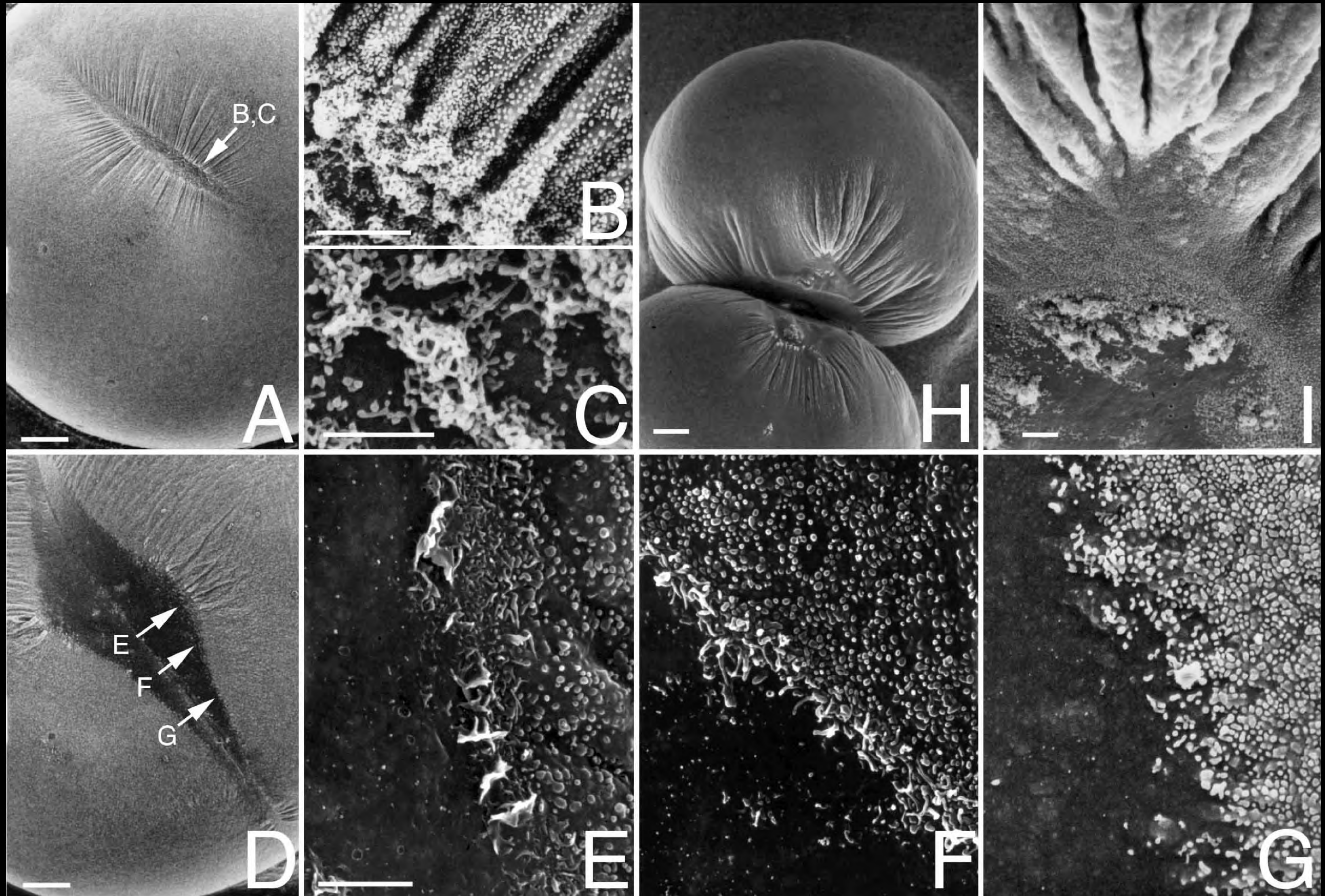
... and motile



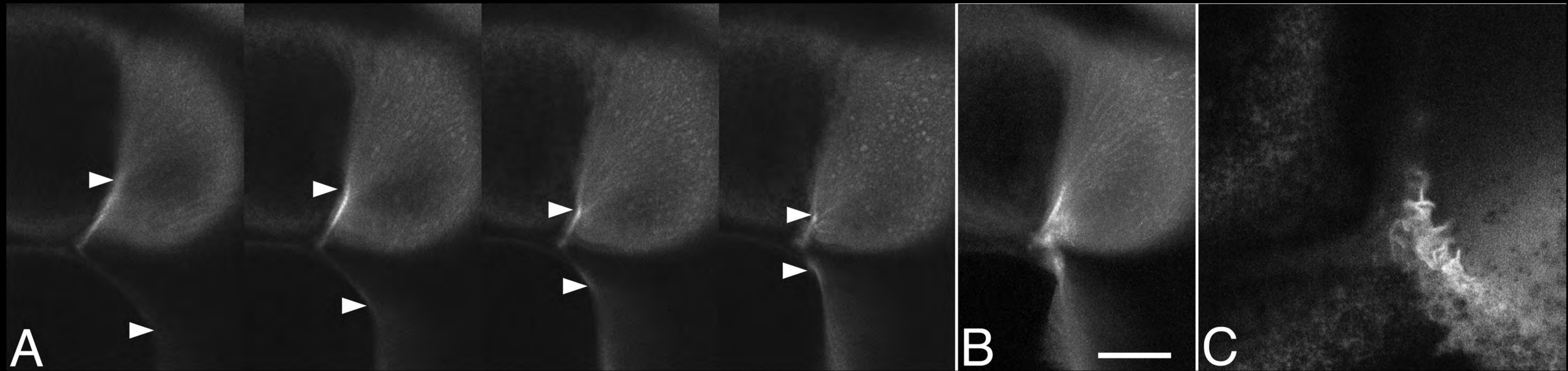
FM1-43

Movie 8

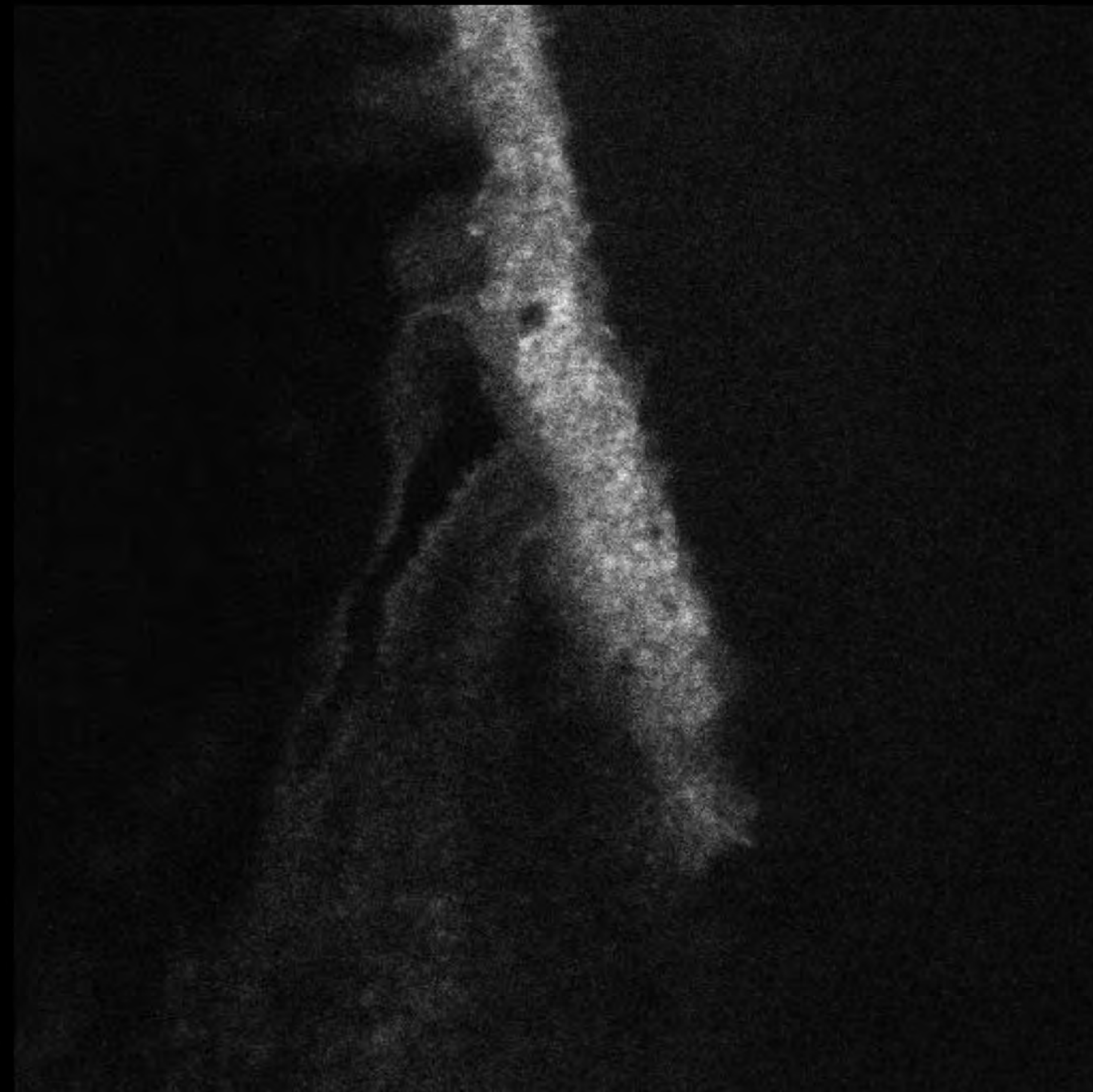
# Marginal Protrusive Zone



# Protrusions generate tension along marginal zone

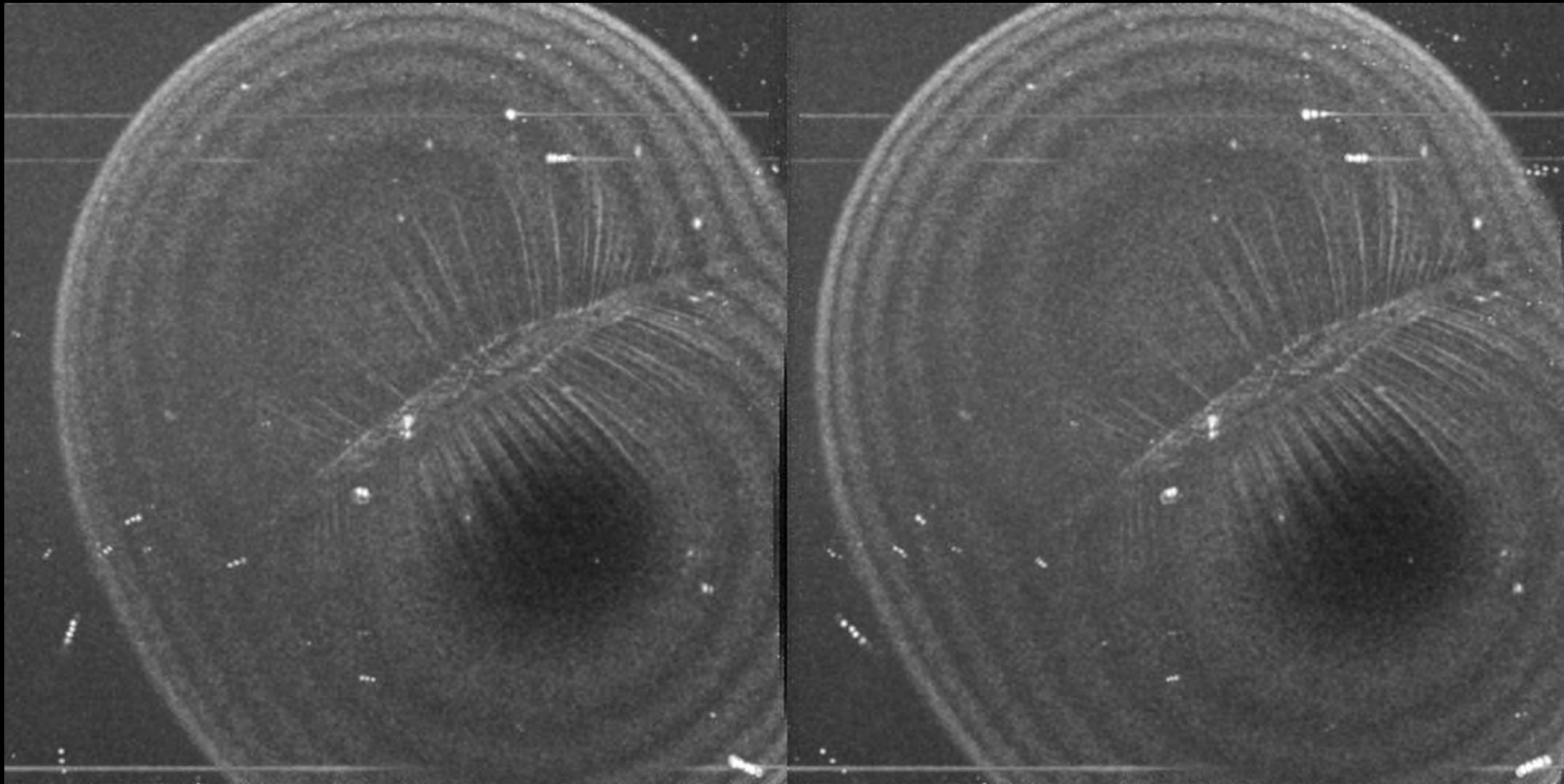


eGFP-rGBD



Movie 9

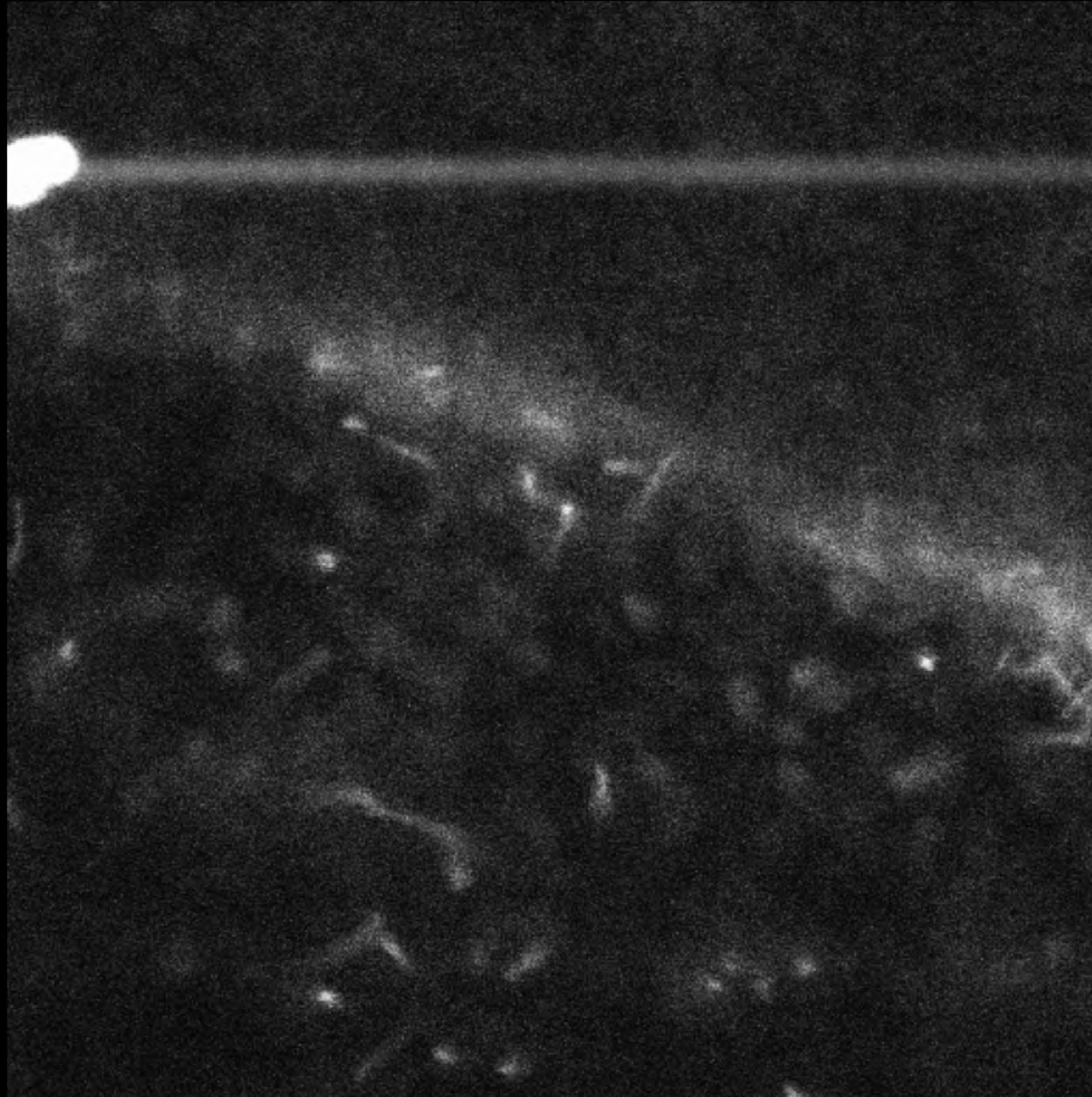
# Basolateral Protrusive Zone



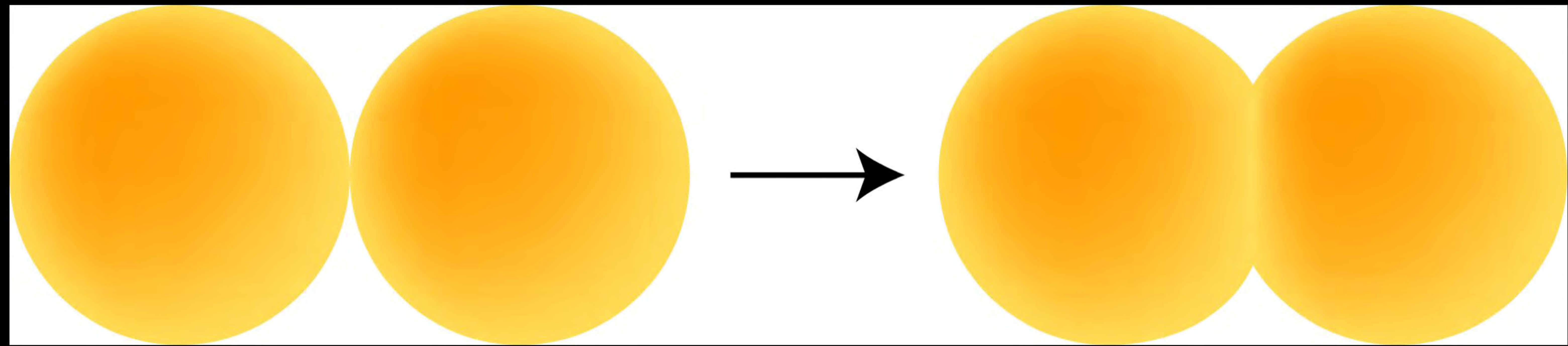
Movie 10

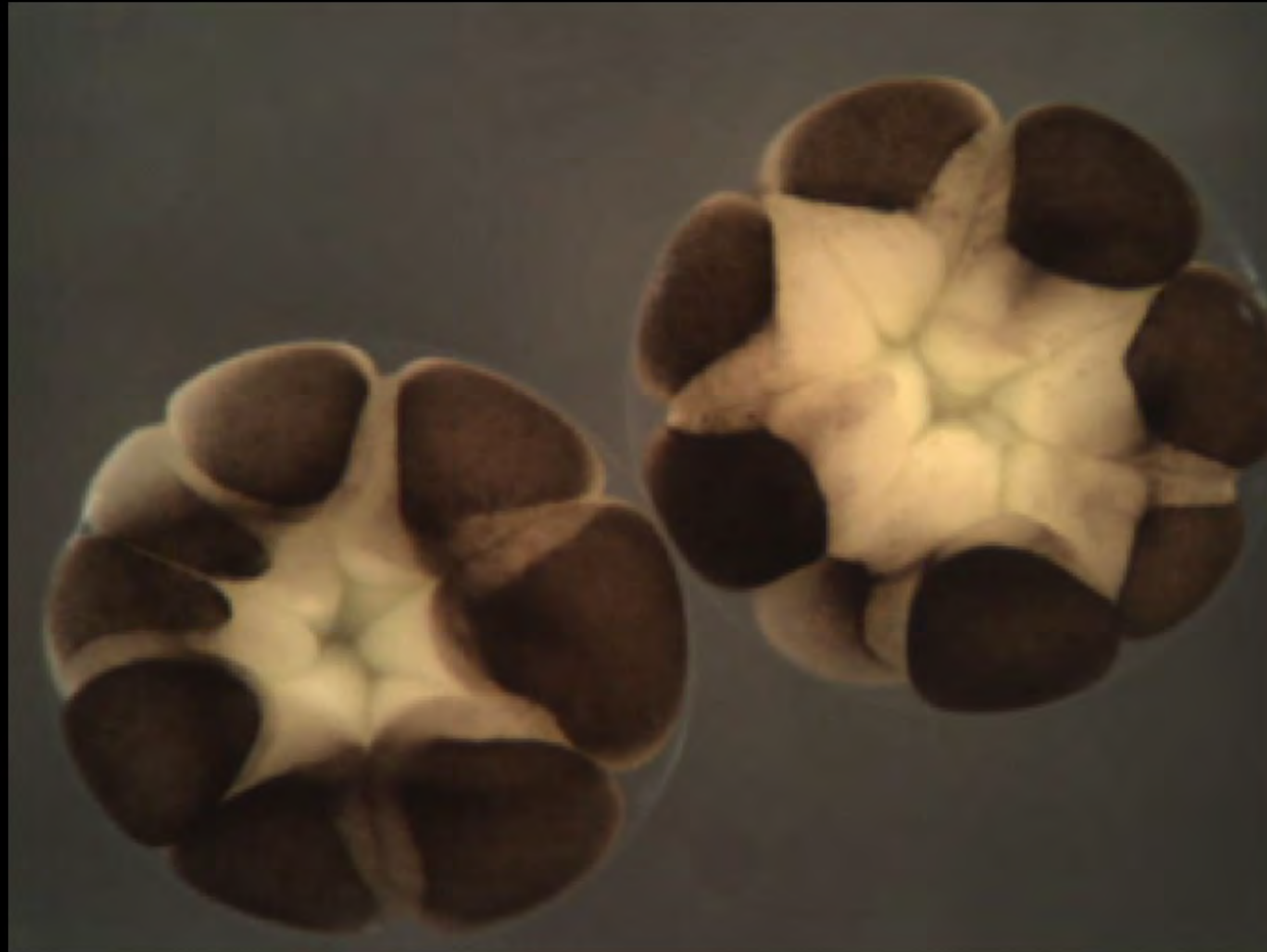
FM1-43

# Basolateral Protrusive Zone



Movie 11

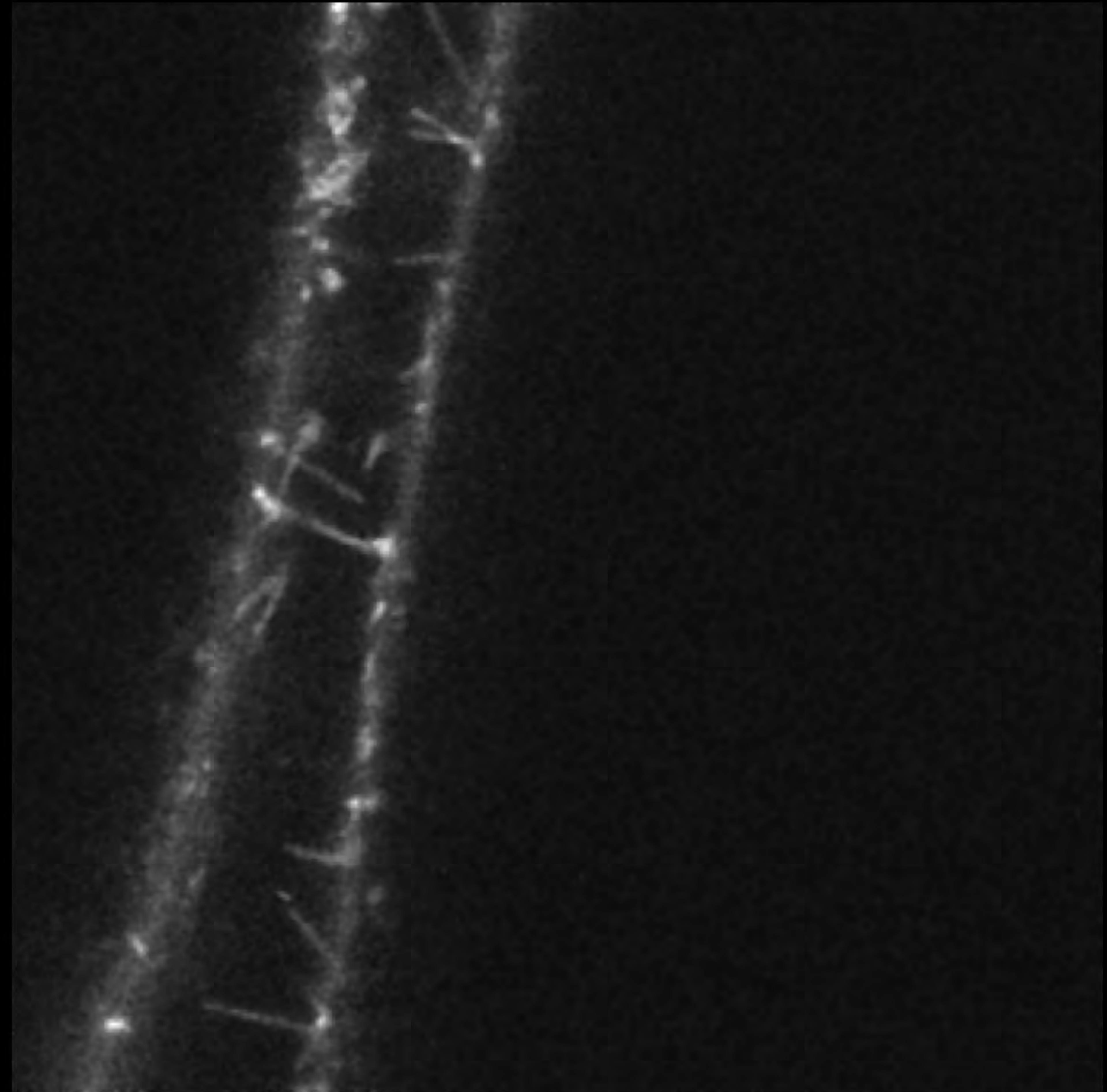
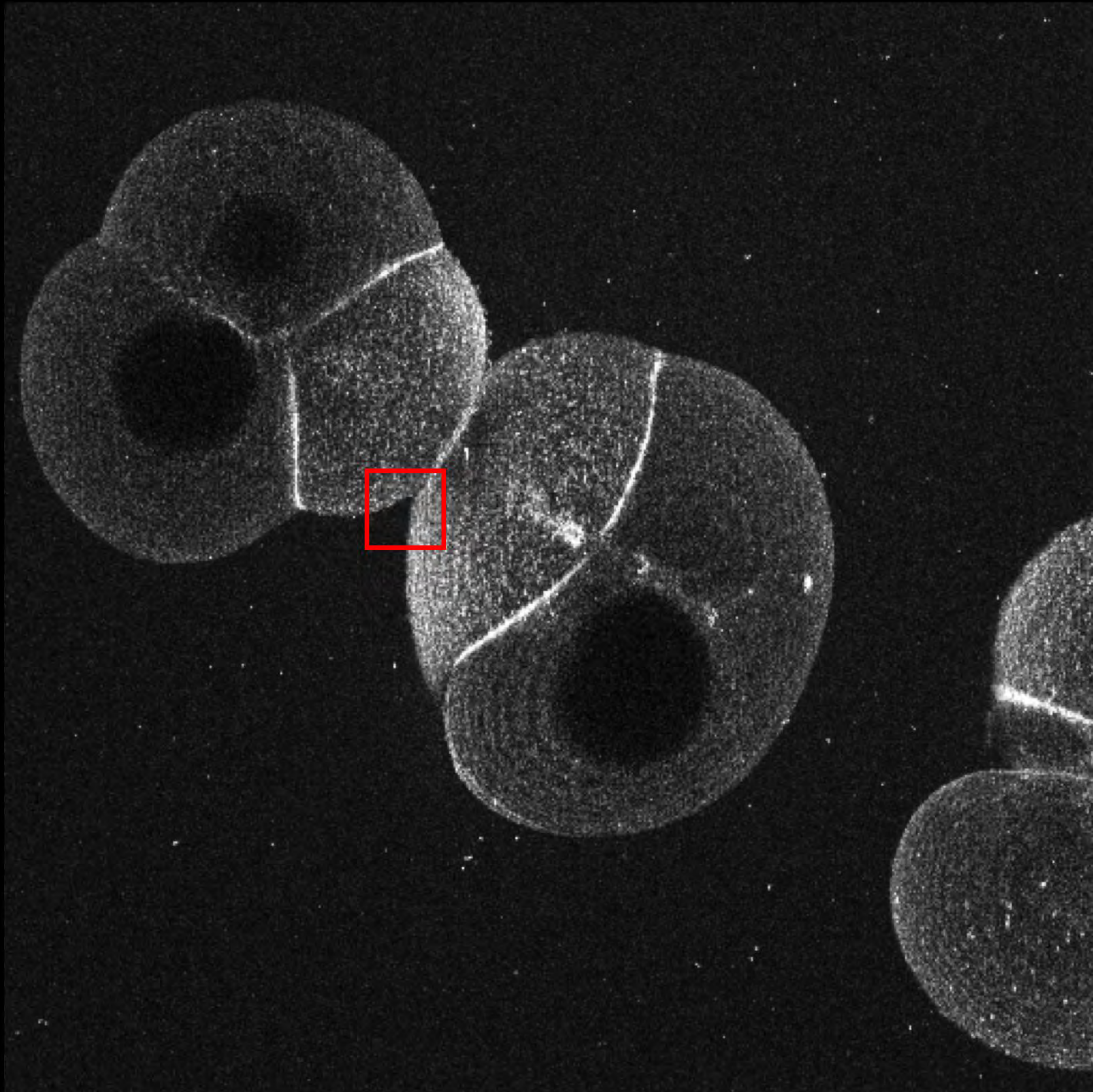




## Movie 12

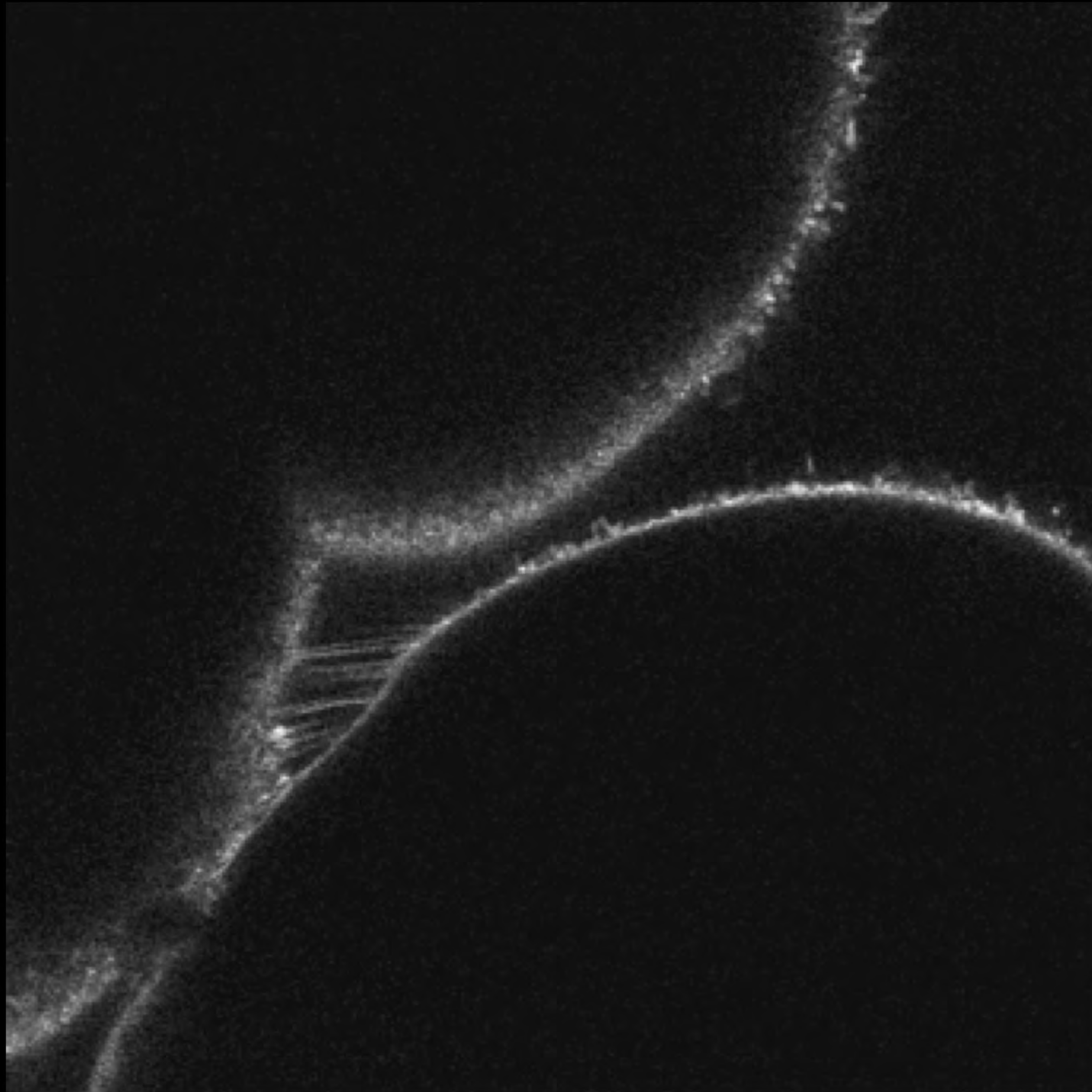
Recovery after low  $[Ca^{2+}]$  dissociation



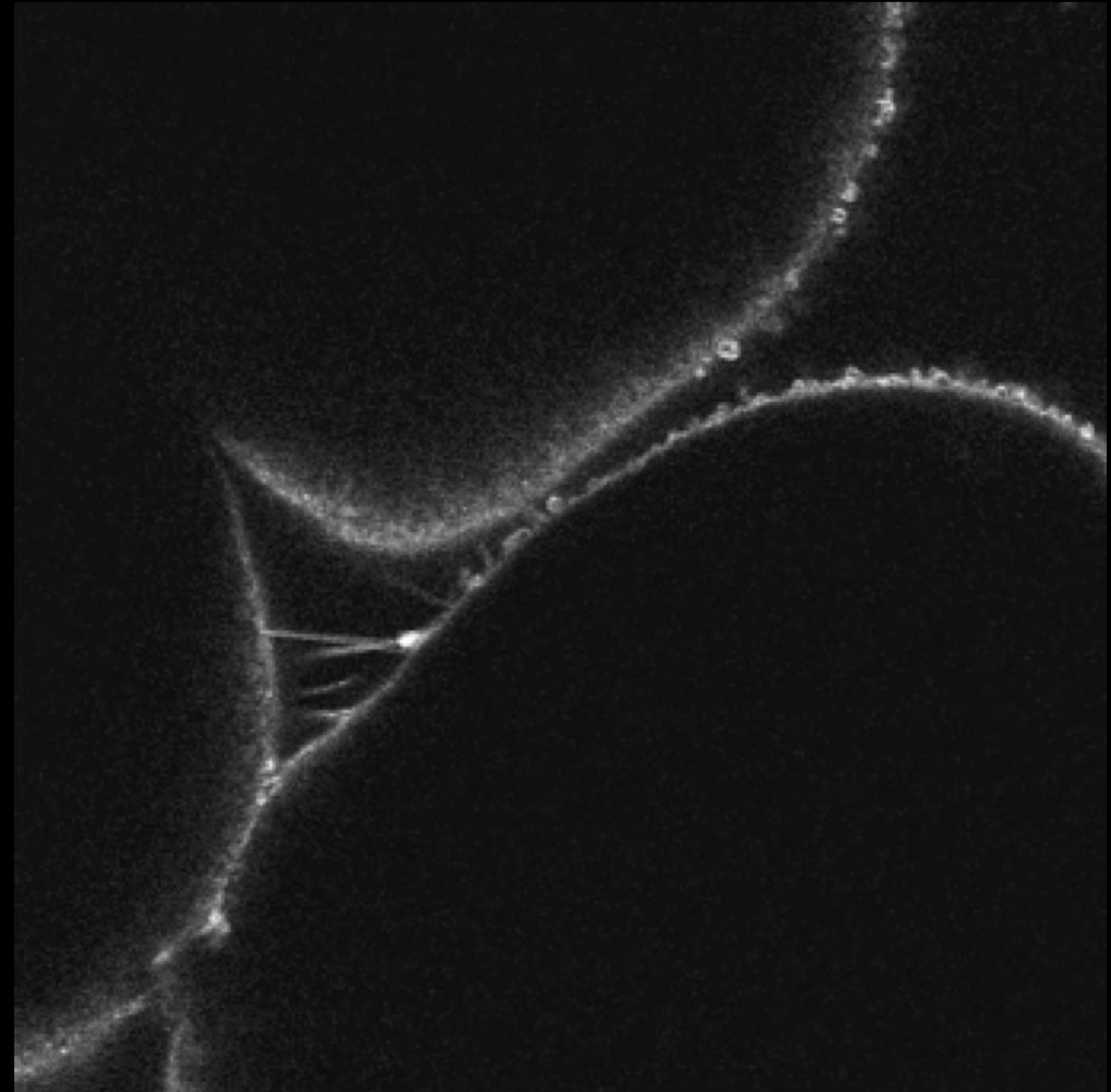


Movie 13

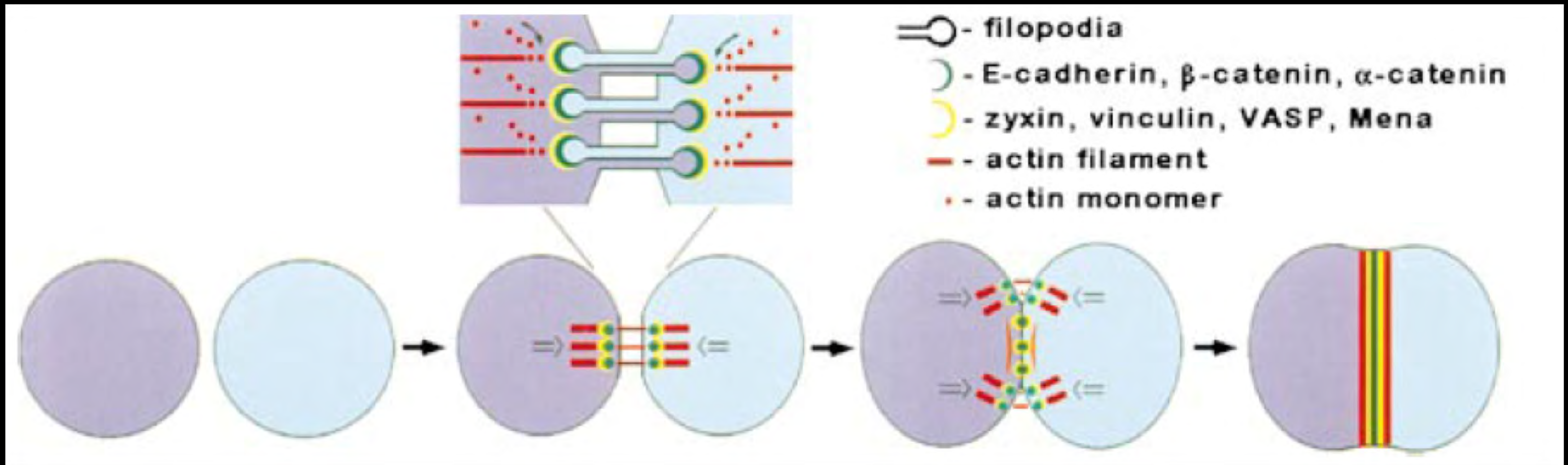
Recovery after low  $[Ca^{2+}]$  dissociation

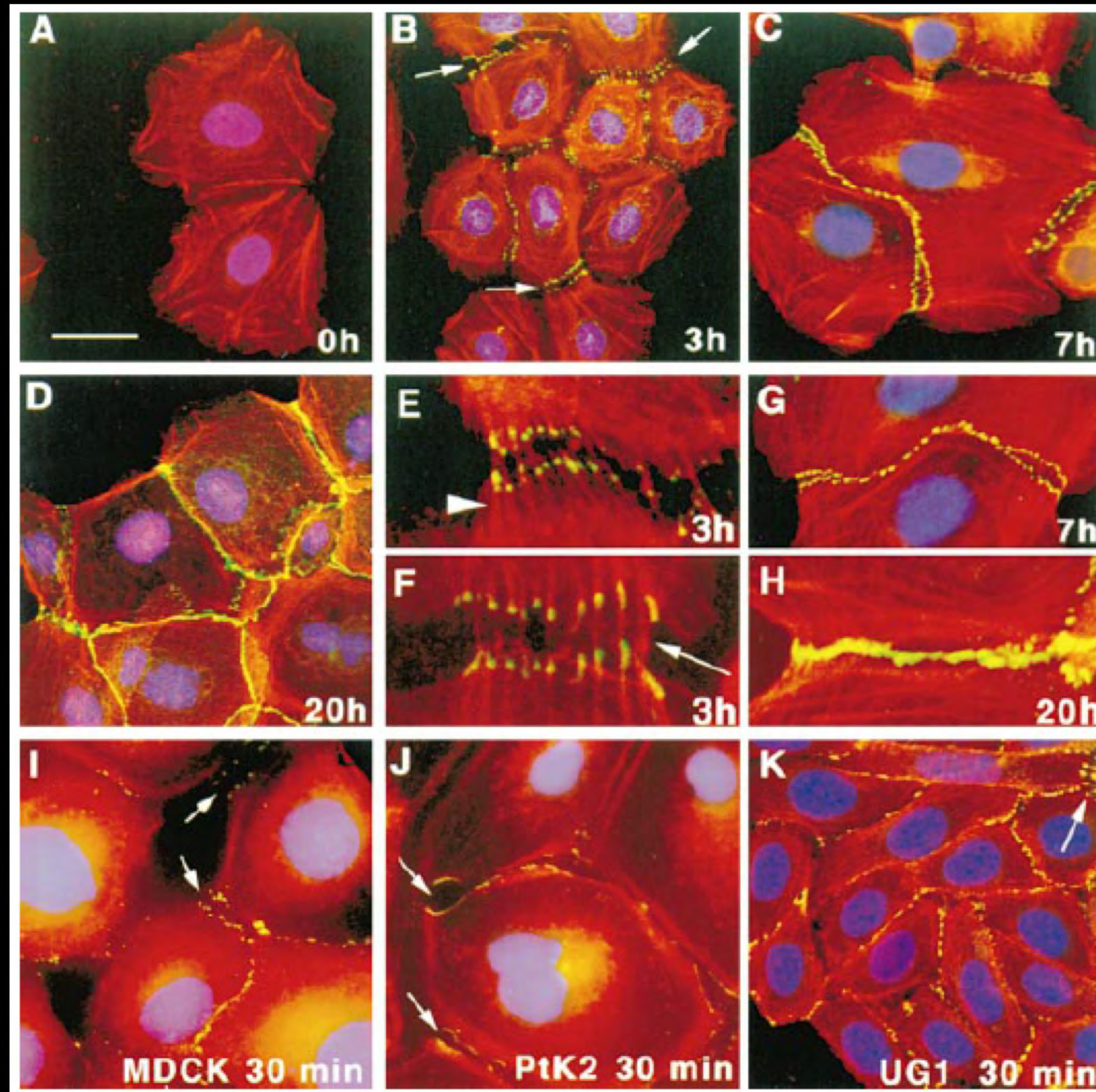


Movie 14



Recovery after low  $[Ca^{2+}]$  dissociation





# Marginal Protrusive Zone

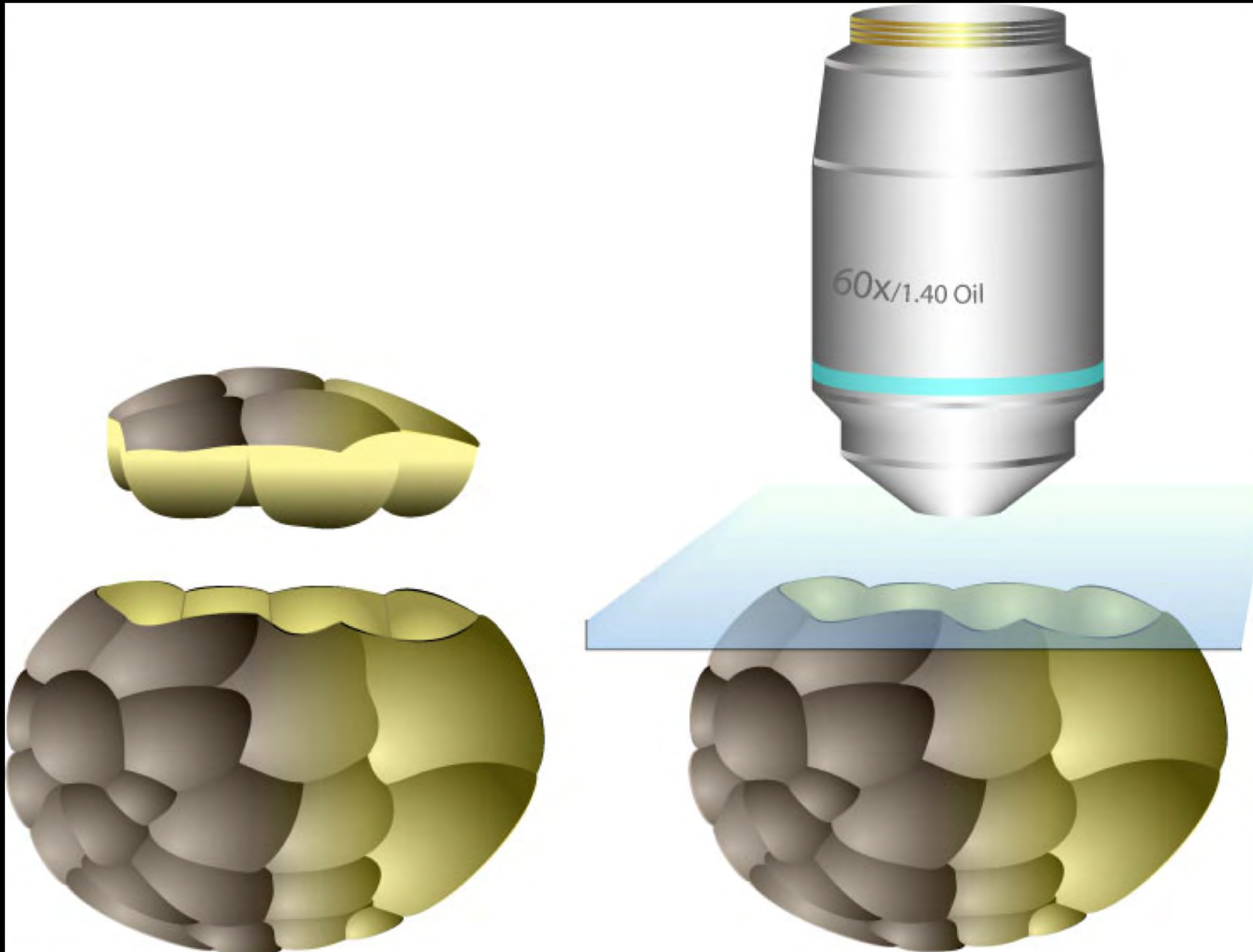
dynamic!

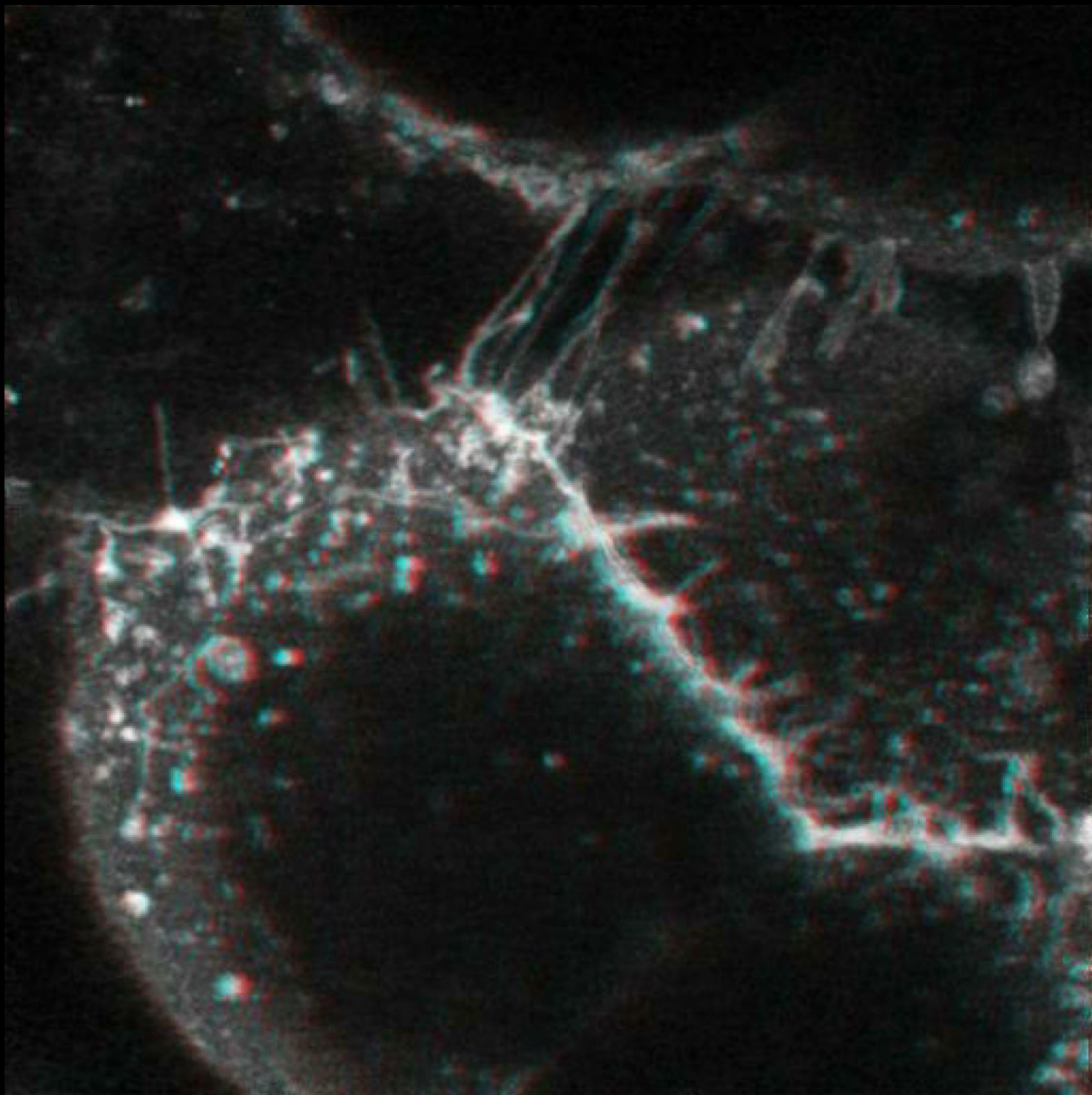
localized actin-based protrusive activity:  
tension at apical/basolateral boundary  
& at stress folds

# Basolateral Protrusive Zone

dynamic!

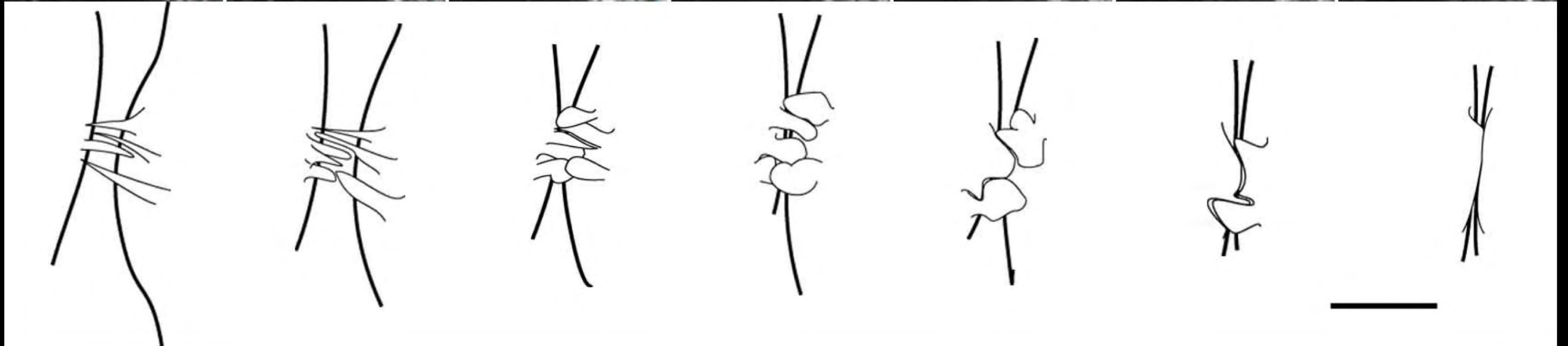
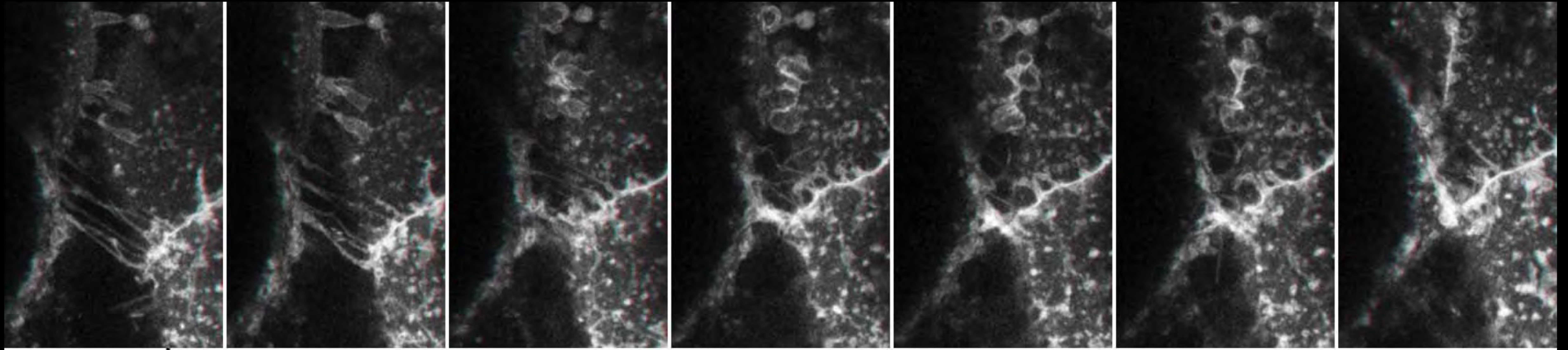
ad hoc reactive to local contact  
highly adhesive





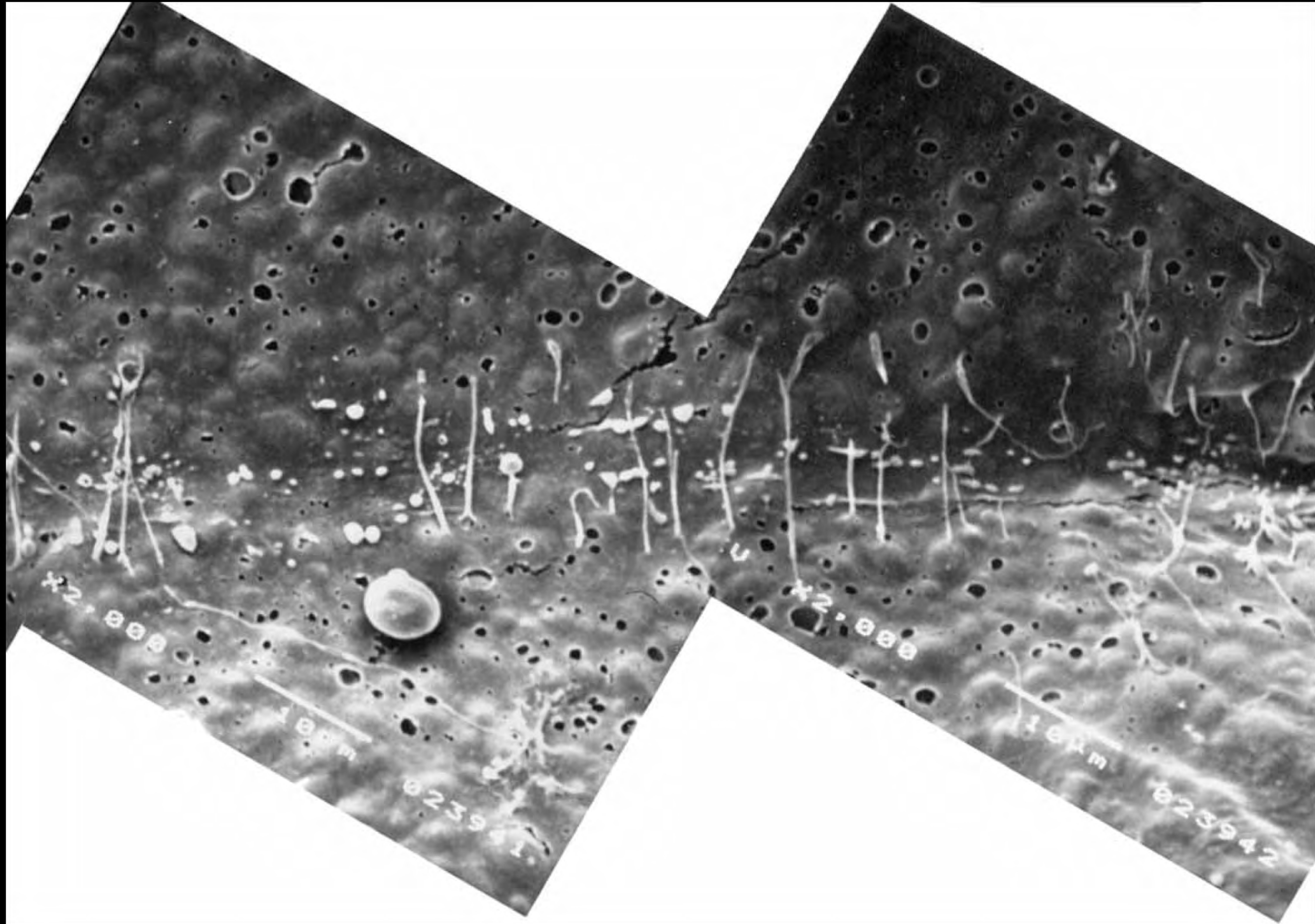
Movie 15

eGFP-mem  
Blastocoel buffer  
256-cell

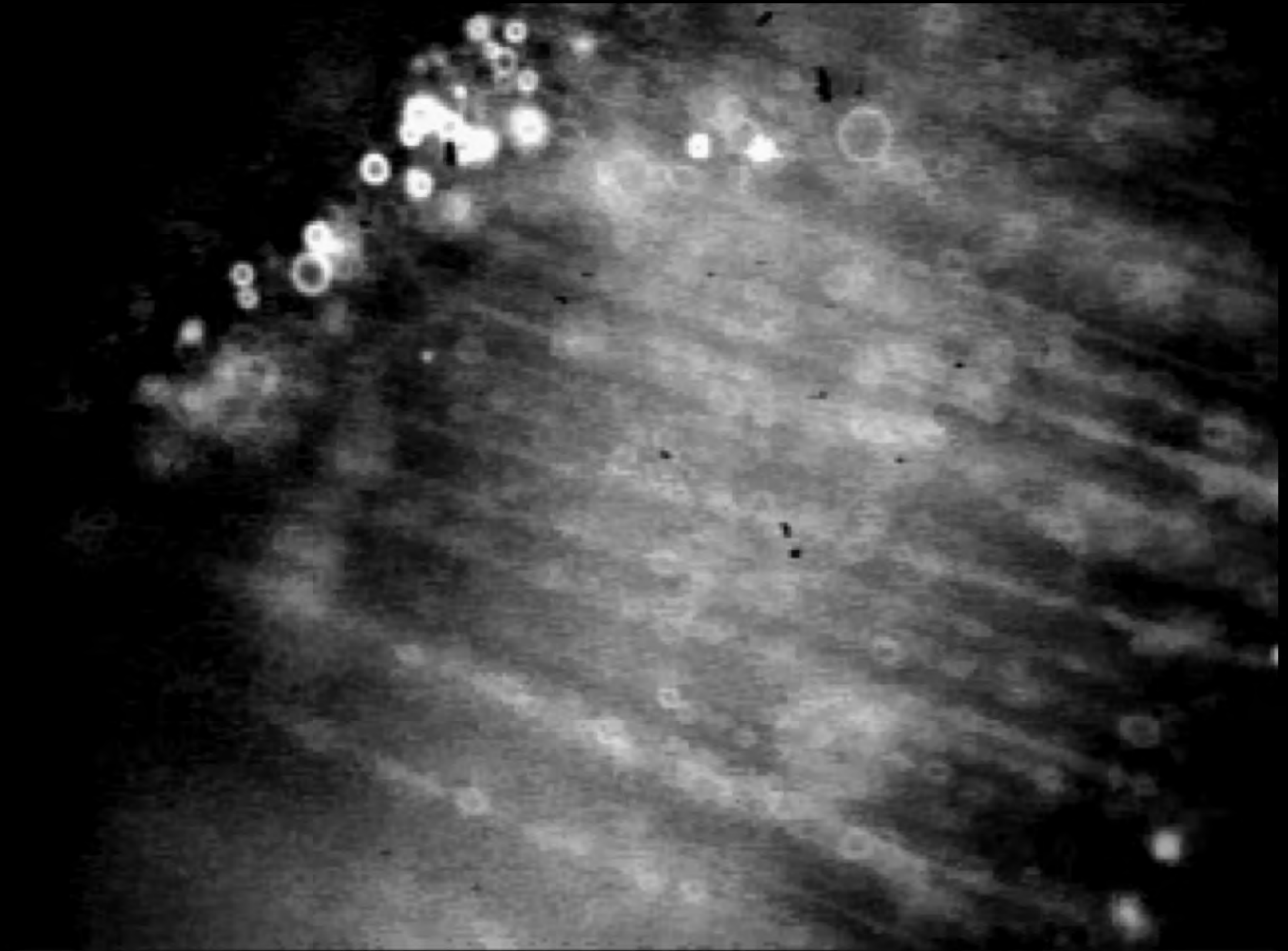
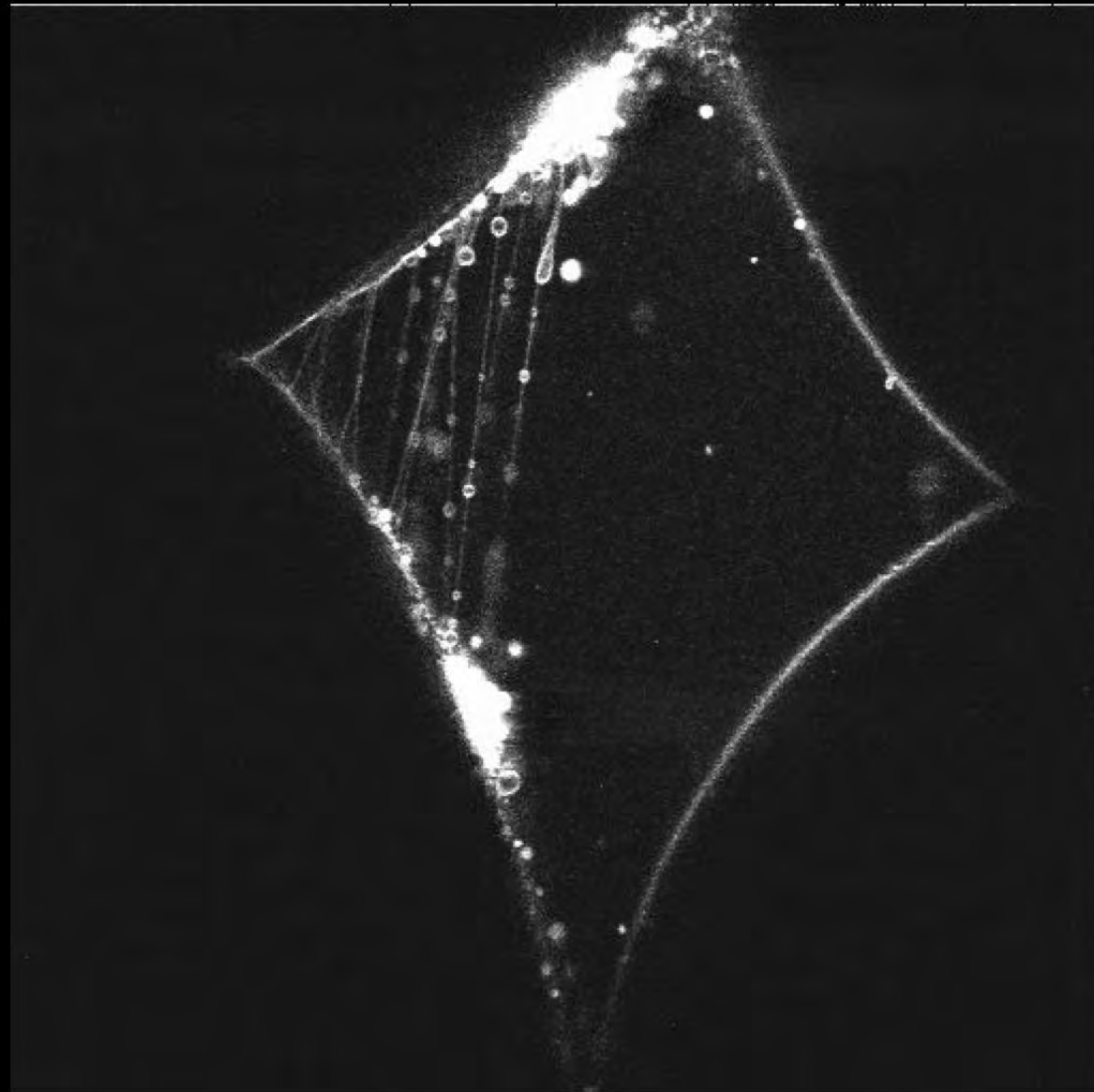




# Traversing filopodia

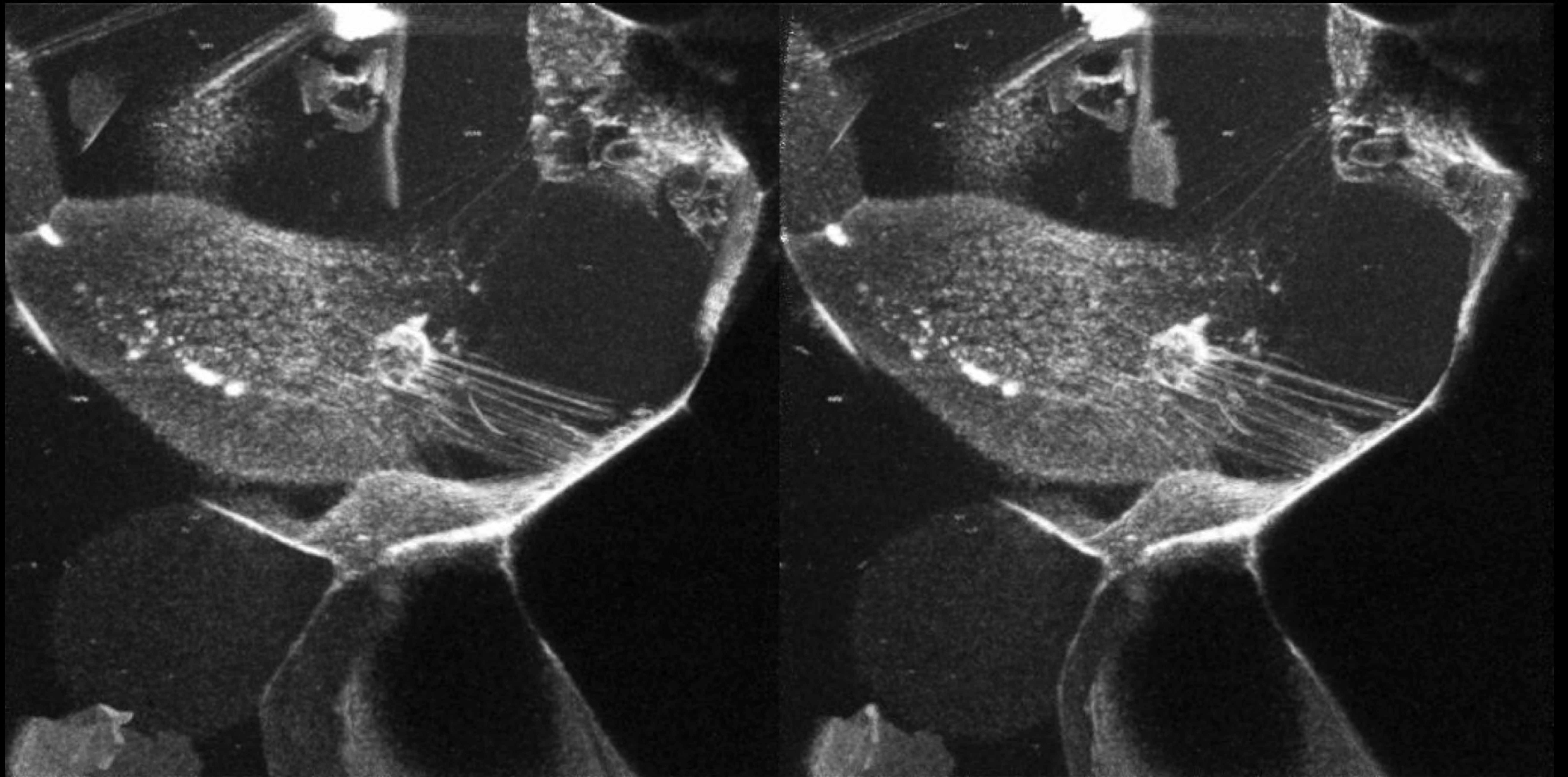


# Traversing filopodia



Movie 16

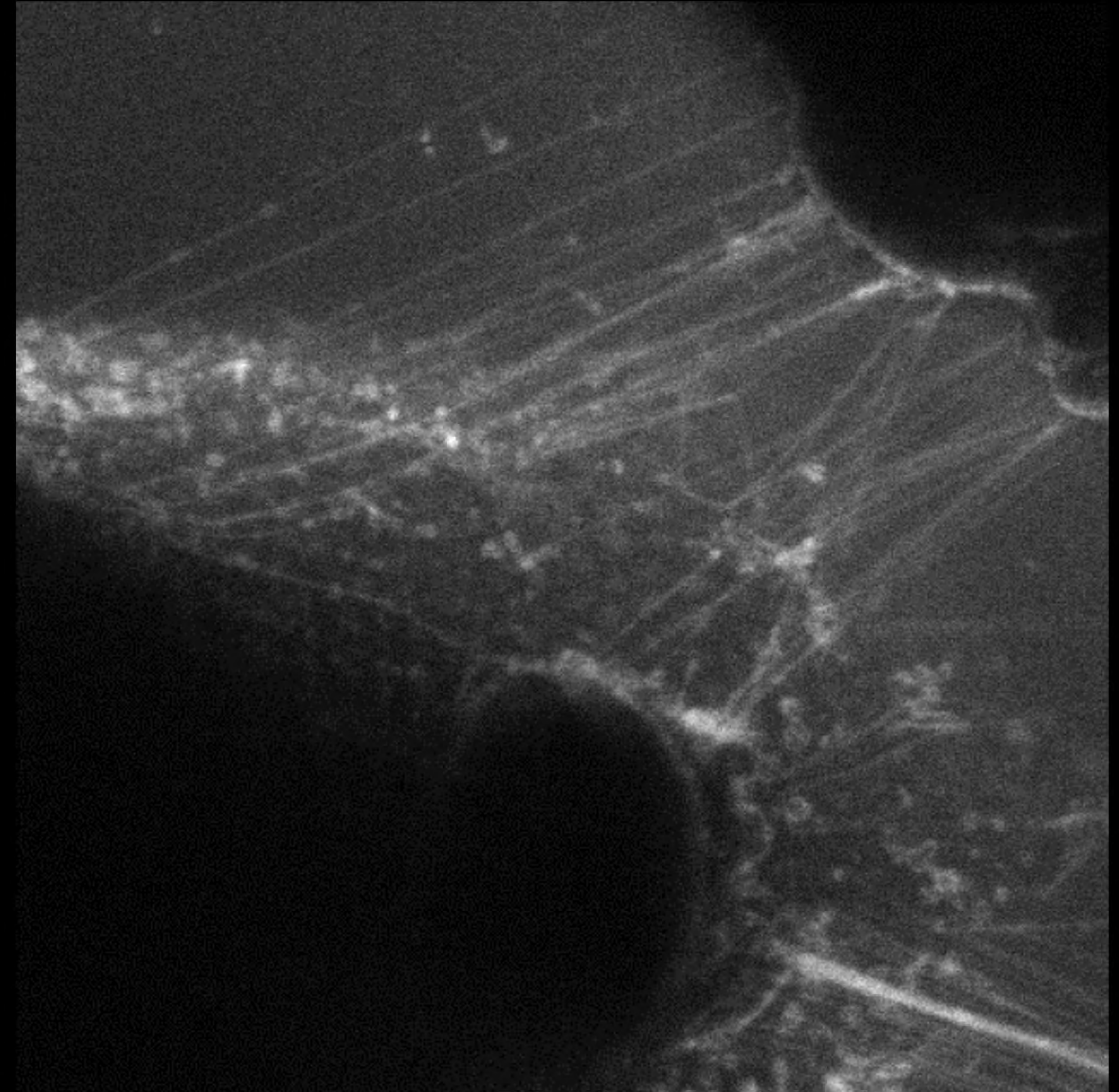
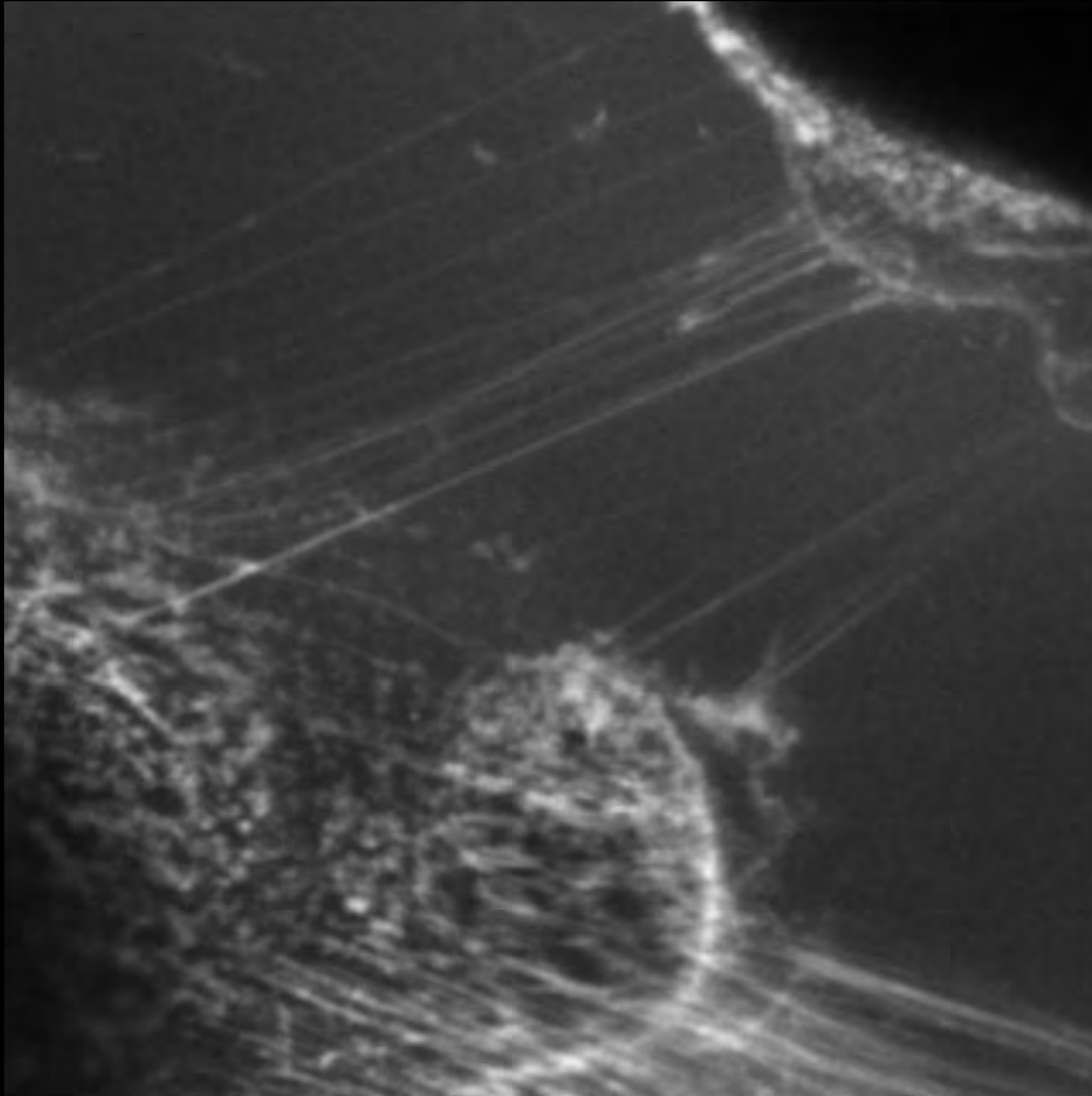
# Traversing filopodia



Movie 17

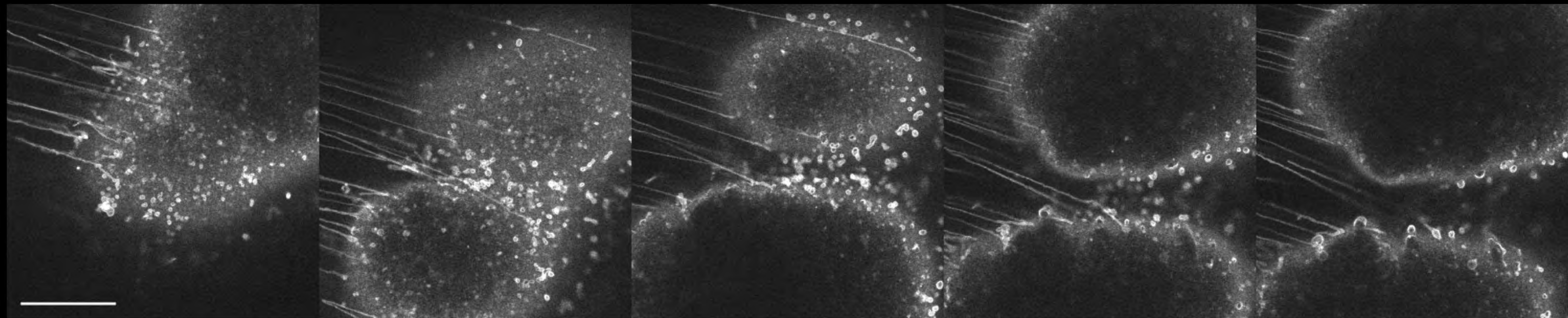
eGFP-mem  
Blastocoel buffer  
256-cell

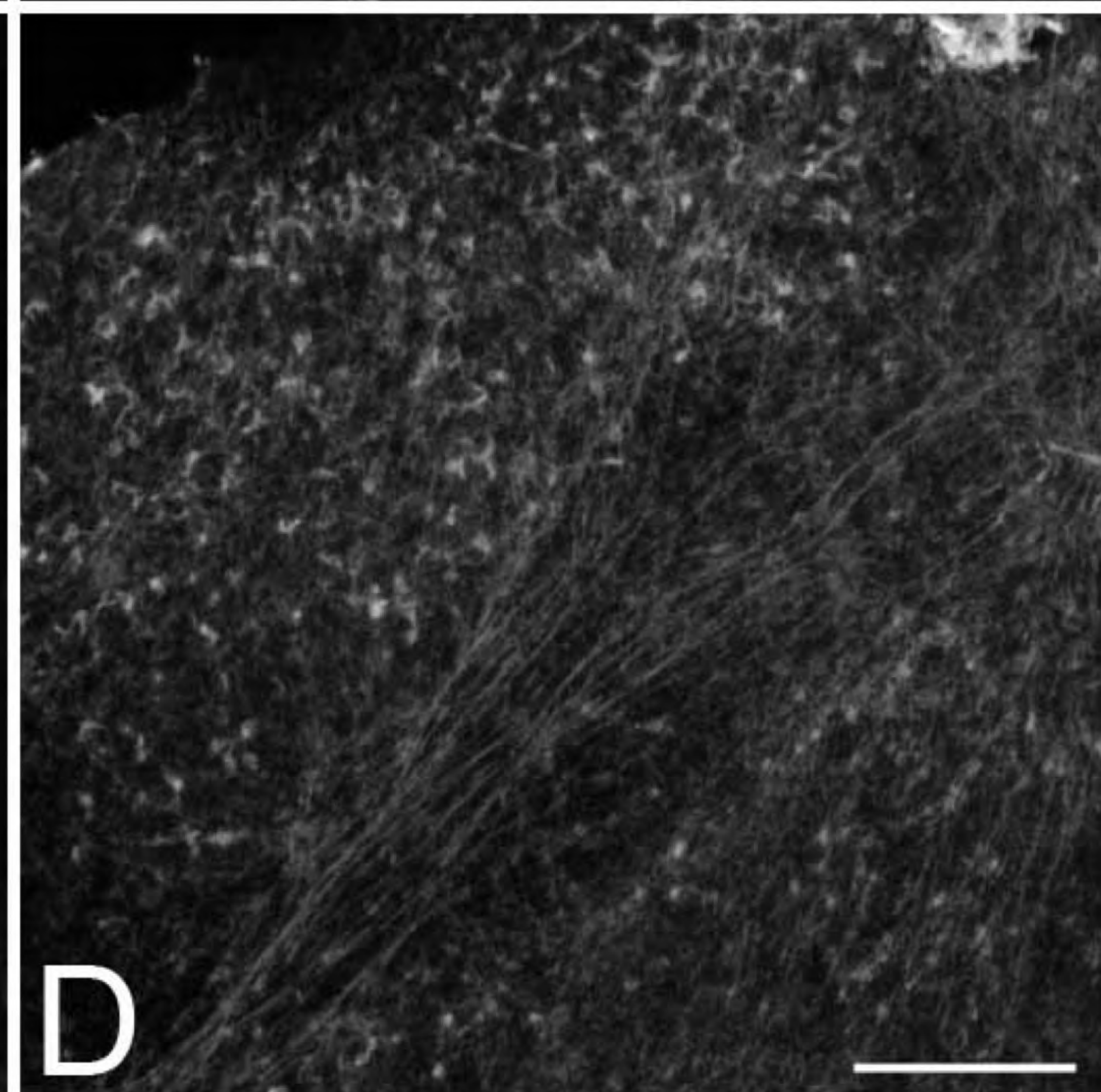
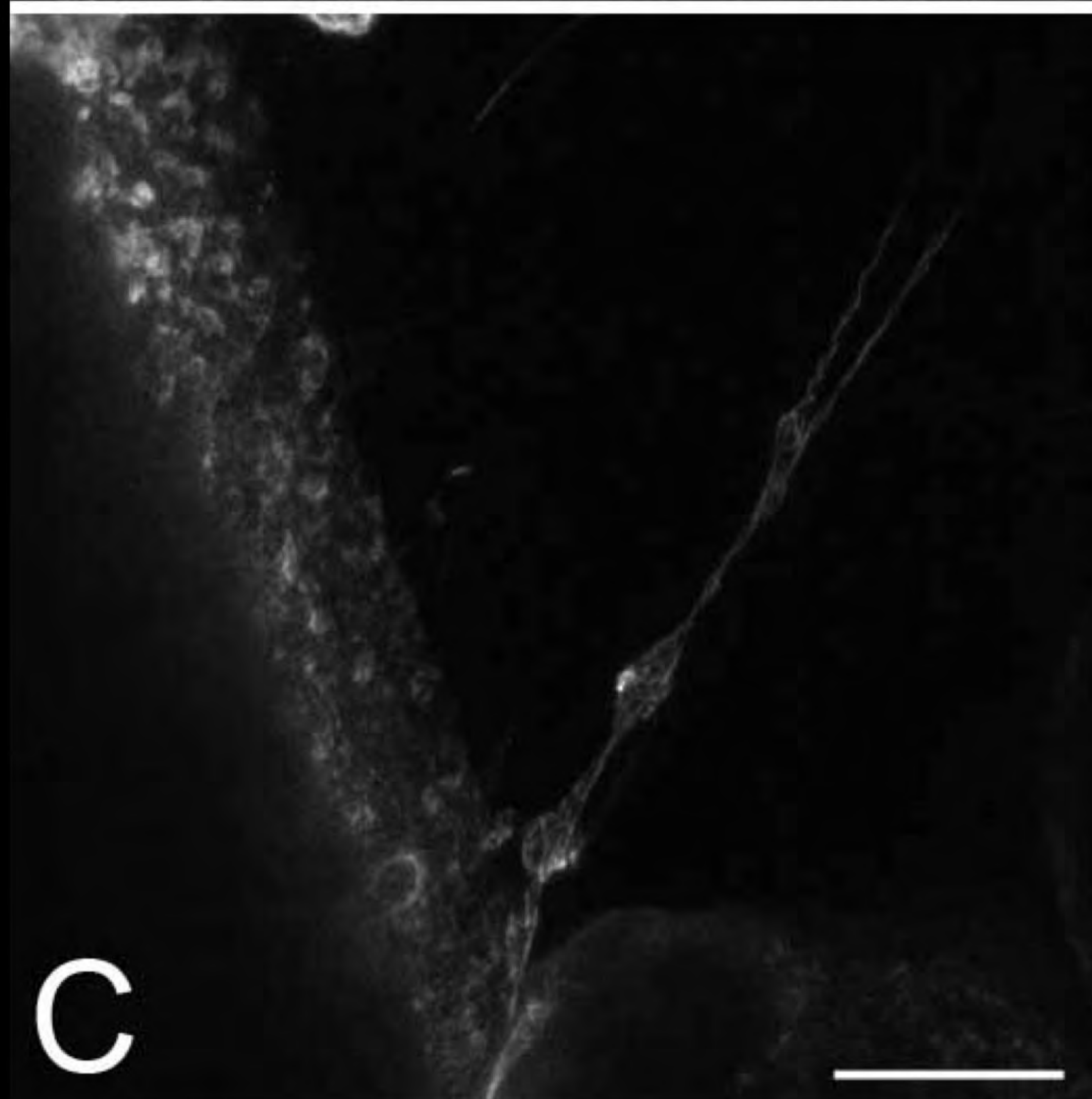
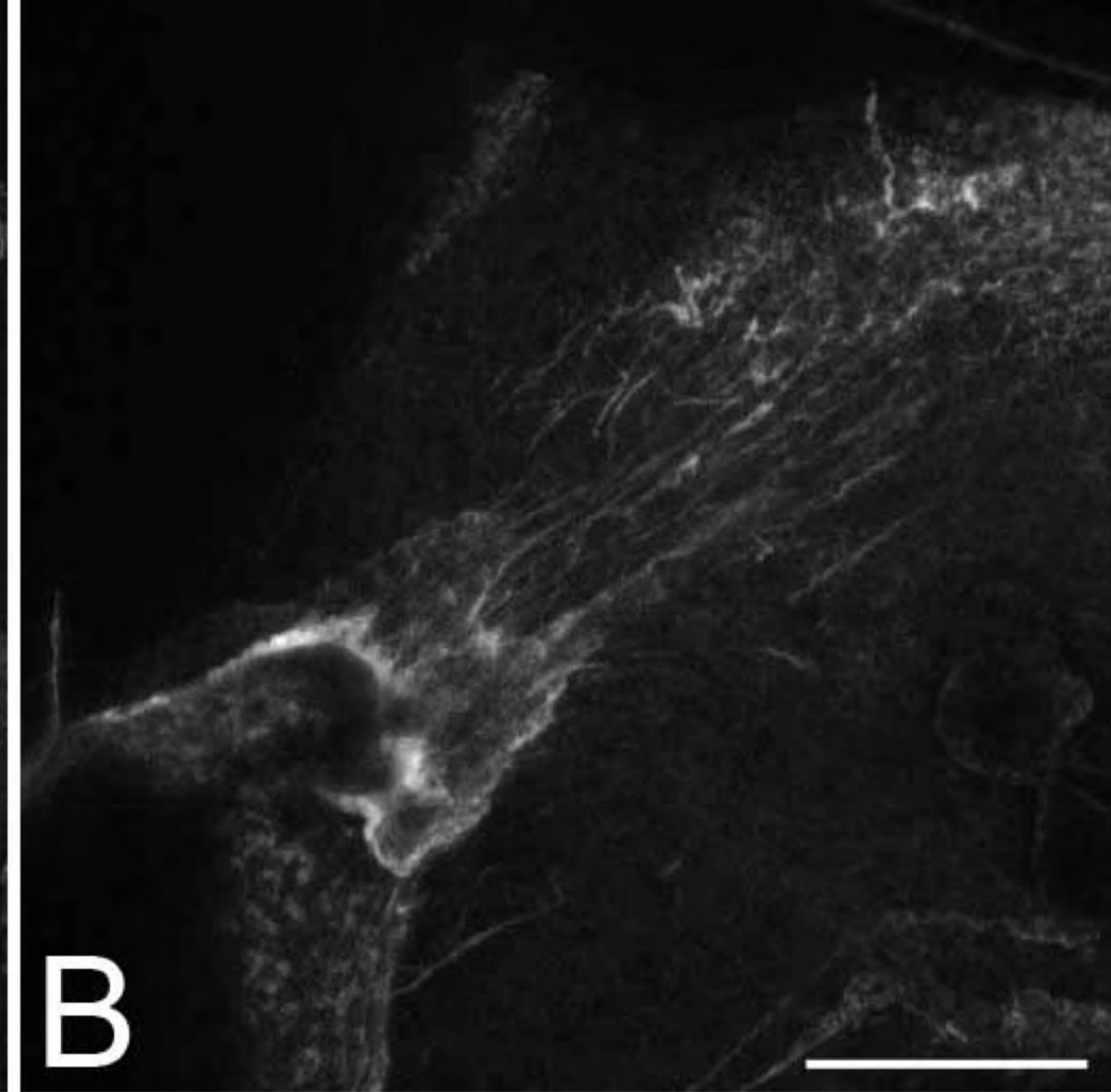
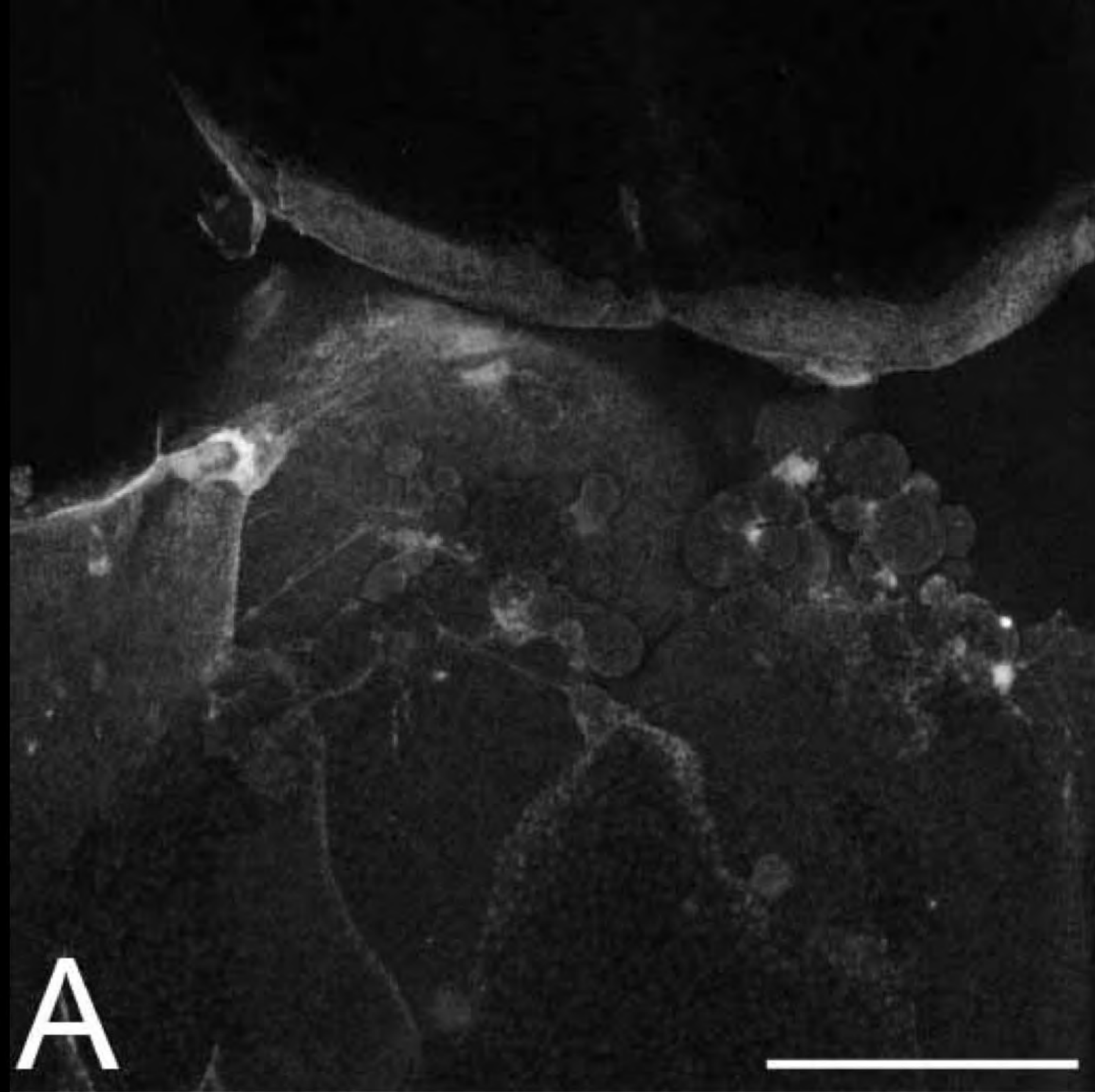
# Traversing filopodia



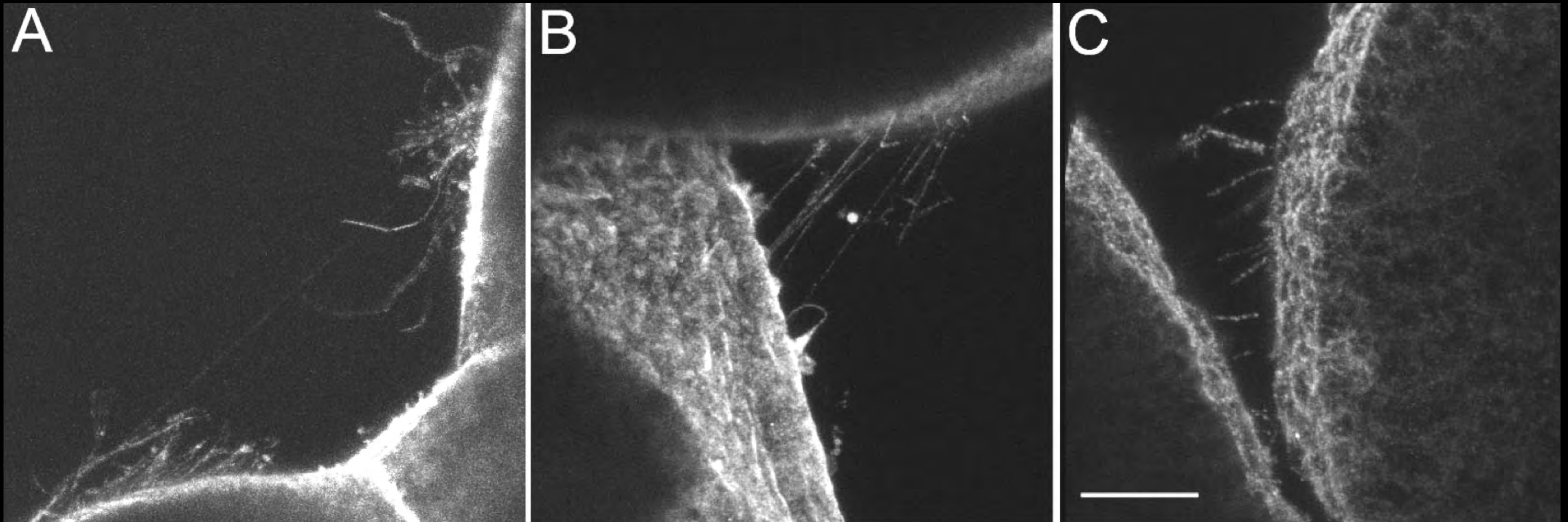
eGFP-mem  
Blastocoel buffer  
256-cell

Long filopodia remain tautly connected  
to nonadjacent cells during cleavage





Long filopodia are actin-filled, coated with integrin, & carry vesicles

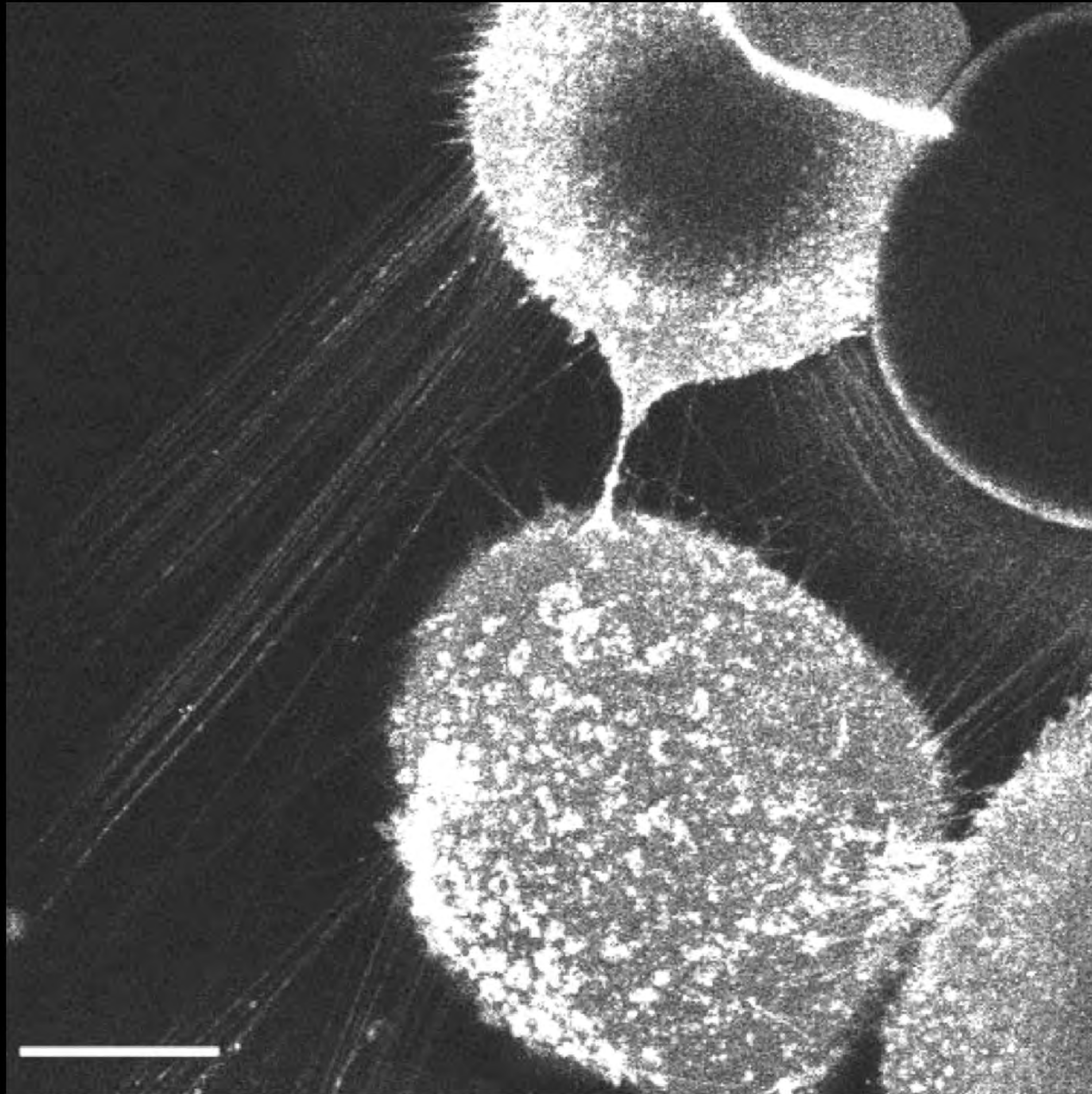


phalloidin

anti- $\beta$  integrin

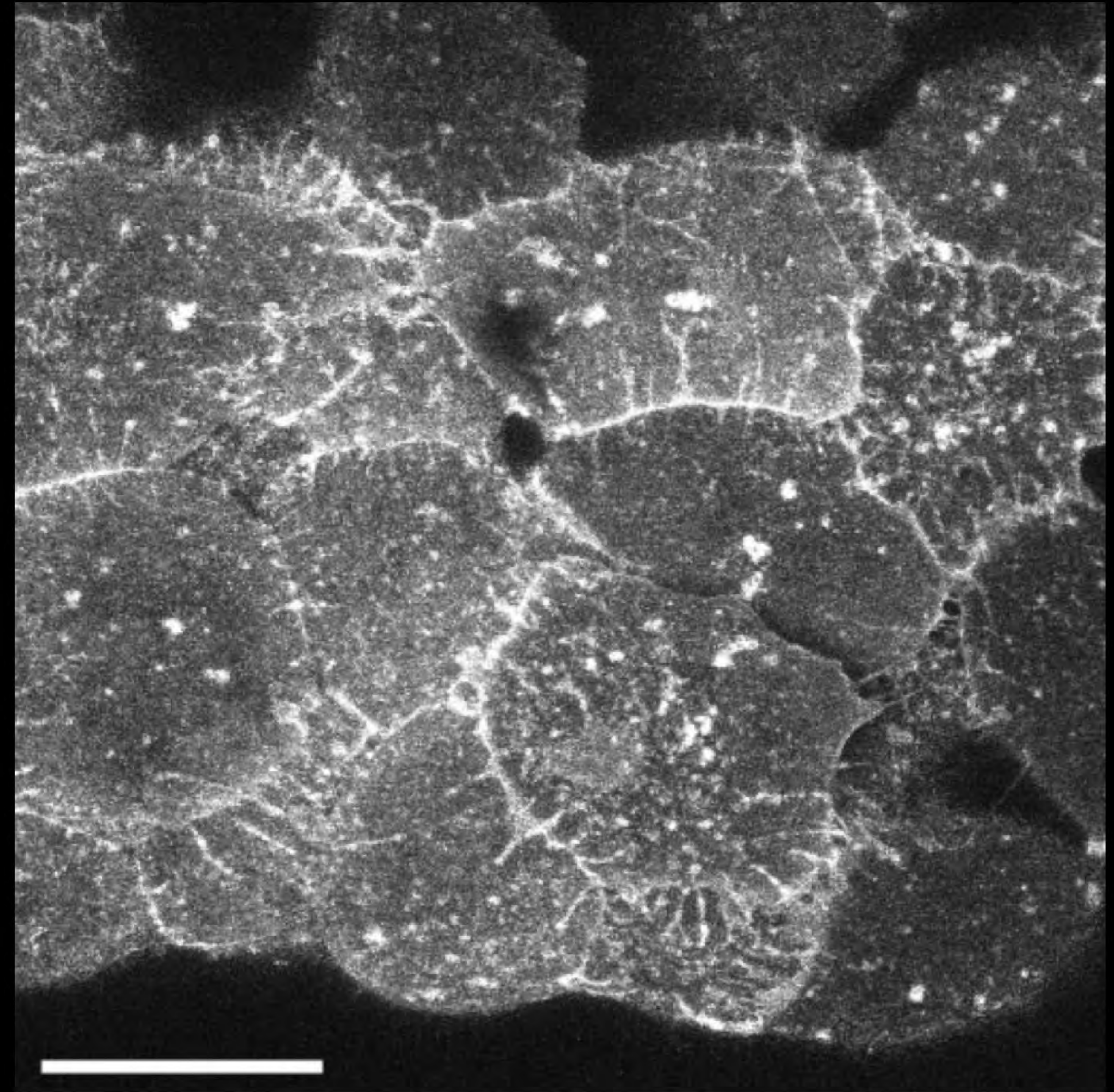
anti-serotonin

# Traversing filopodia disappear after MBT



Bar: 100  $\mu\text{m}$ .

pre-MBT (64-cell)

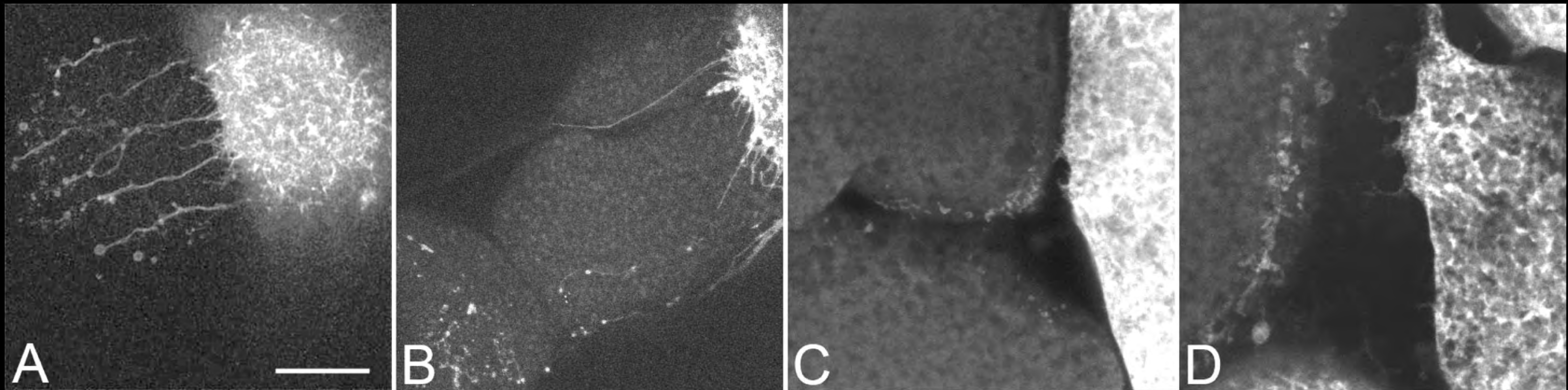


Bar: 70  $\mu\text{m}$ .

post-MBT (st. 7+)



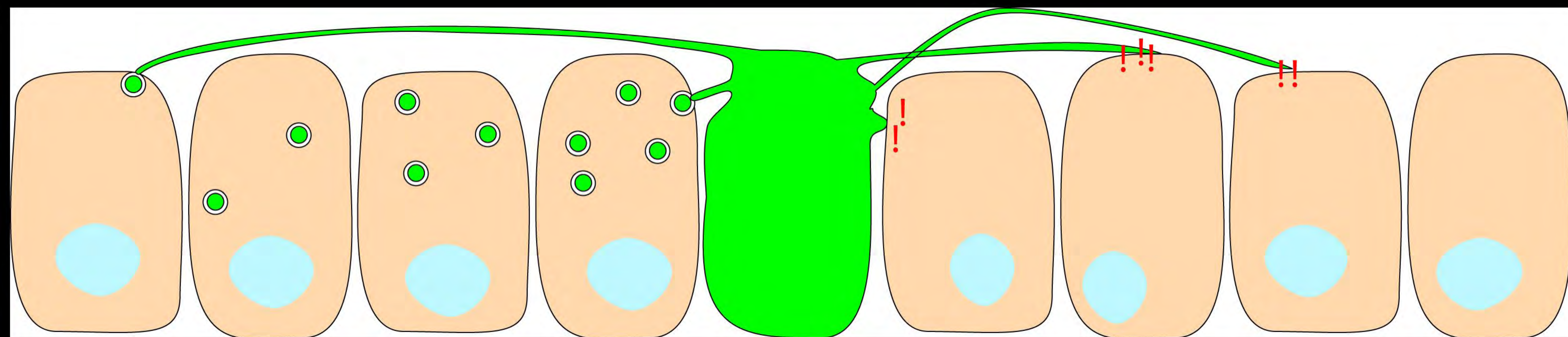
# Long filopodia undergo vesiculation @ MBT



Bar: 24, 47, 35, and 17  $\mu\text{m}$  in A-D, respectively

eGFP, live

Lucifer yellow injected, fixed



signaling via direct contact

# Summary

1. Localized exocytosis along cleavage plane requires MTs
2. New membrane expansion tightly regulated to accommodate added surface area @ cytokinesis
3. Stress folds @ furrow margins generated by local cortical tension, not contractile ring activity
4. Furrow margins = sites of protrusive activity
5. Bared basolateral surfaces = ad hoc protrusive activity
6. Blastocoel contains long filopodia that span the blastocoel prior to midblastula transition (MBT)
7. Role of traversing filopodia unknown. Distribution and role presently under investigation.

# Acknowledgements

Betsy Brown (OHSU)  
Steven Bedrick (OHSU)  
Liz Overton-Harris (Merlo Station High School)  
Chris Cunningham (Univ Oregon)  
  
George von Dassow (Friday Harbor Labs, UW)  
Bill Bement (U Wisconsin)

