

Potential Long-distance Signaling by Blastocoel-traversing Thin Filopodia

Movies:

[http://groups.google.com/group/embryophysics/web/filopodia-7-october-2009?
pli=1](http://groups.google.com/group/embryophysics/web/filopodia-7-october-2009?pli=1)

Presented in the Embryo Physics Course <http://www.embryophysics.org>

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By

Michael V. Danilchik

Integrative Biosciences, School of Dentistry

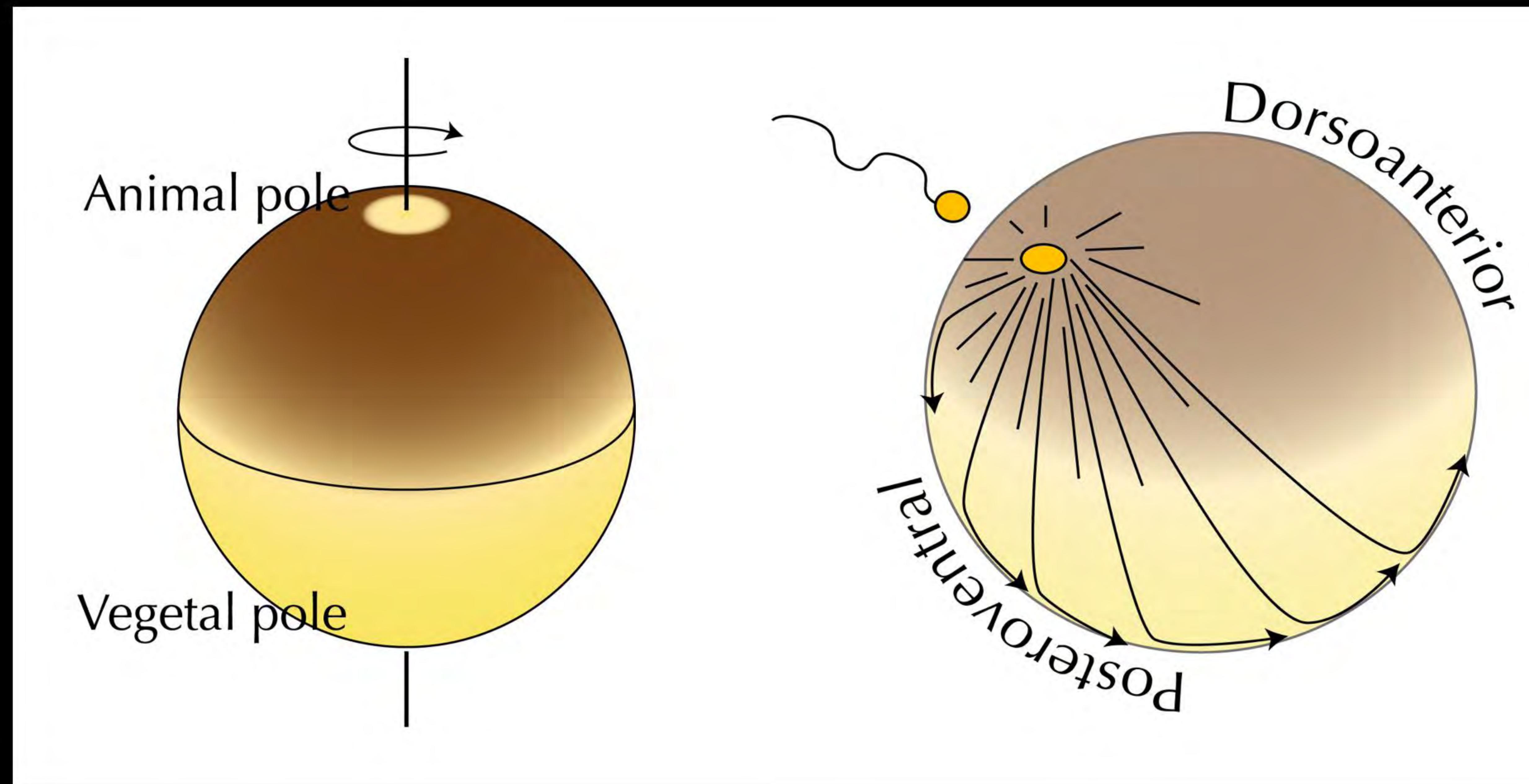
Oregon Health & Science University

danilchi@ohsu.edu



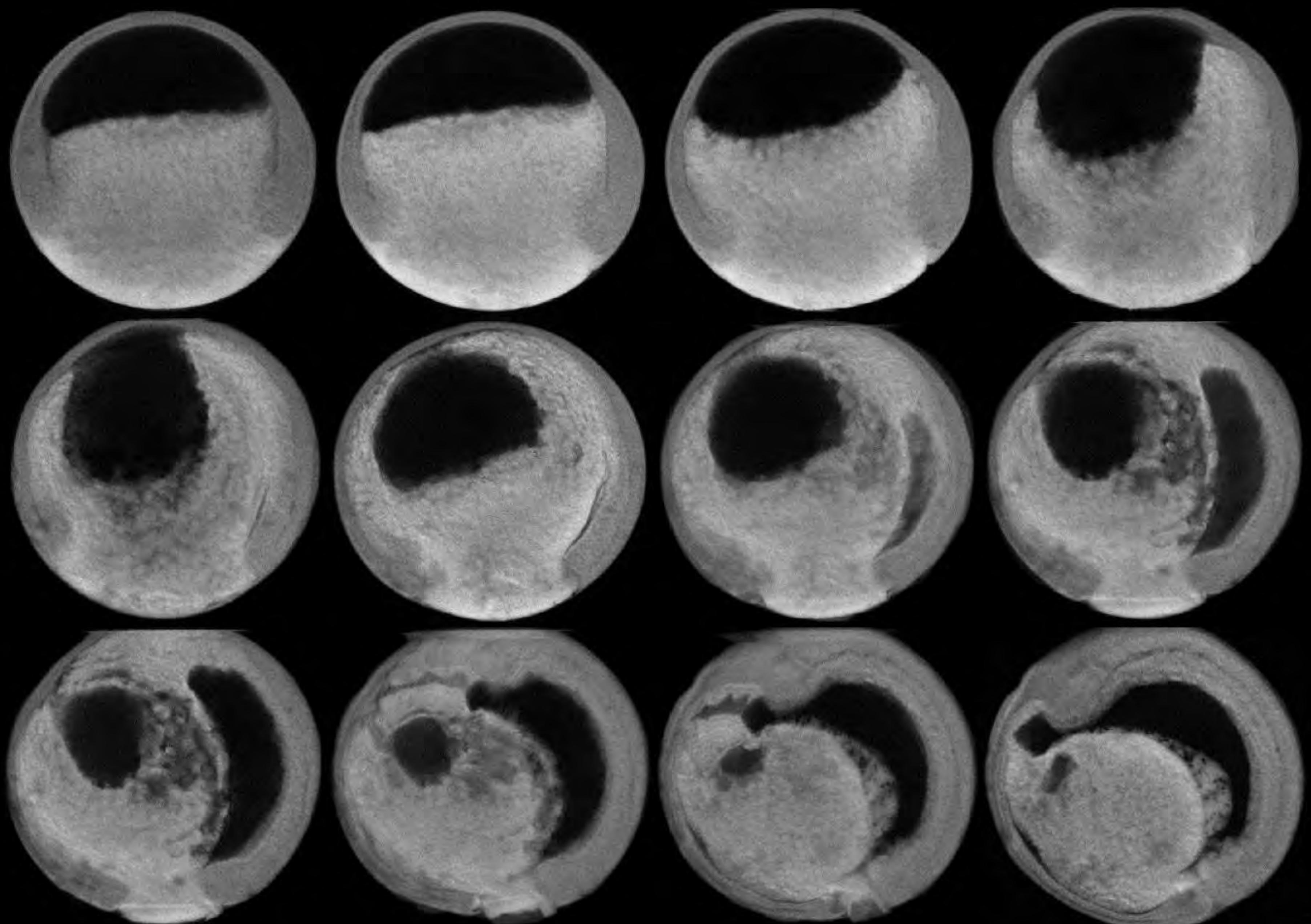
Potential Long-distance Signaling by Blastocoel-traversing Thin Filopodia

Mike Danilchik
OHSU, Portland Oregon
danielchi@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.

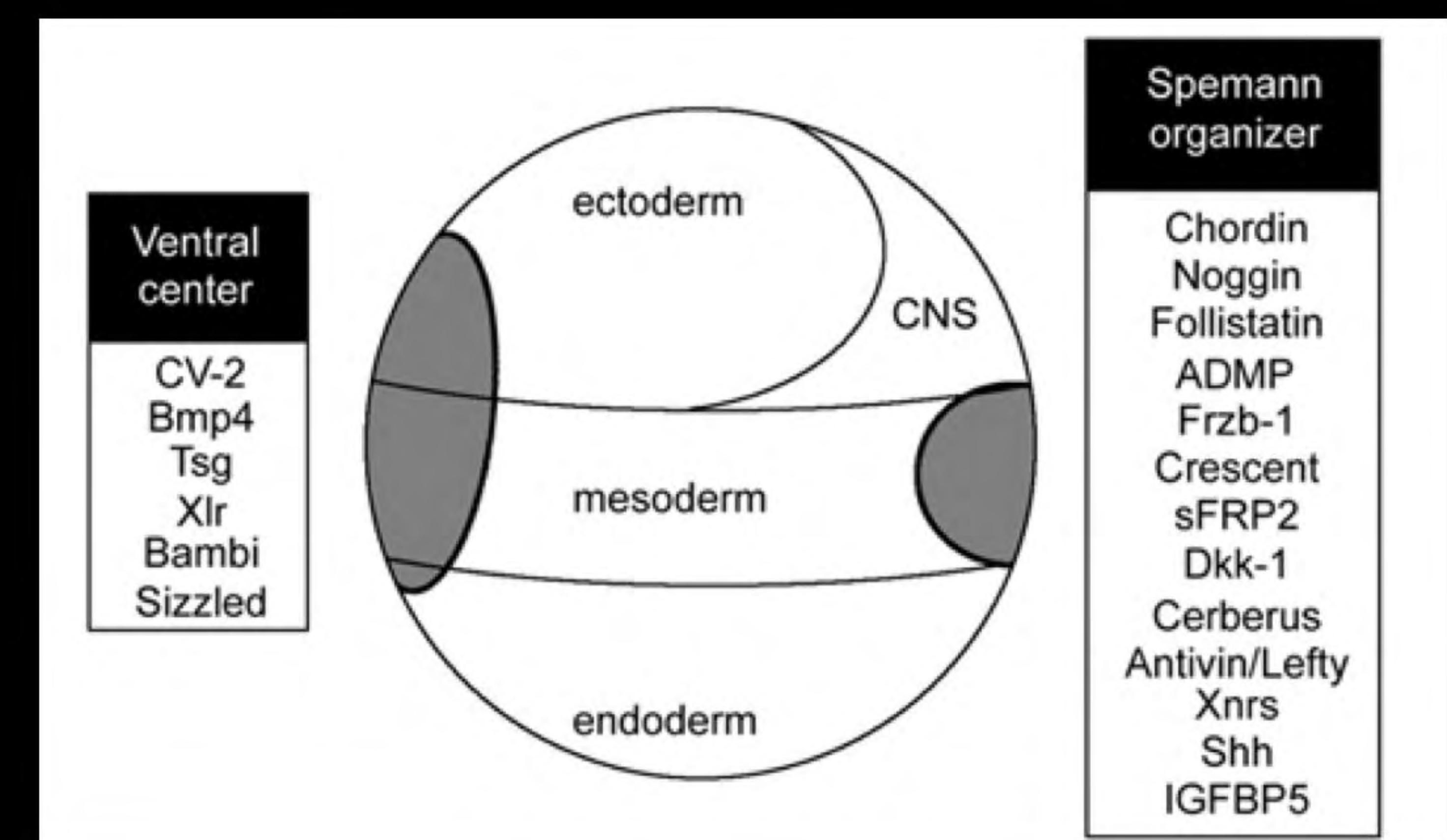
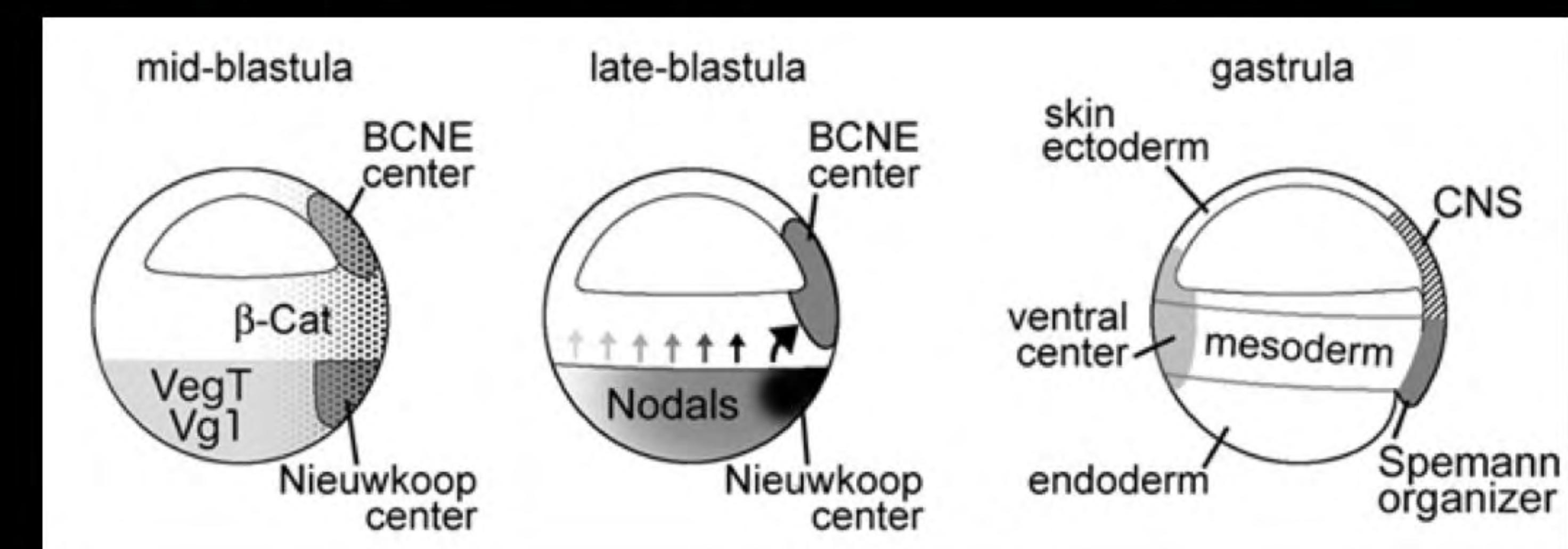
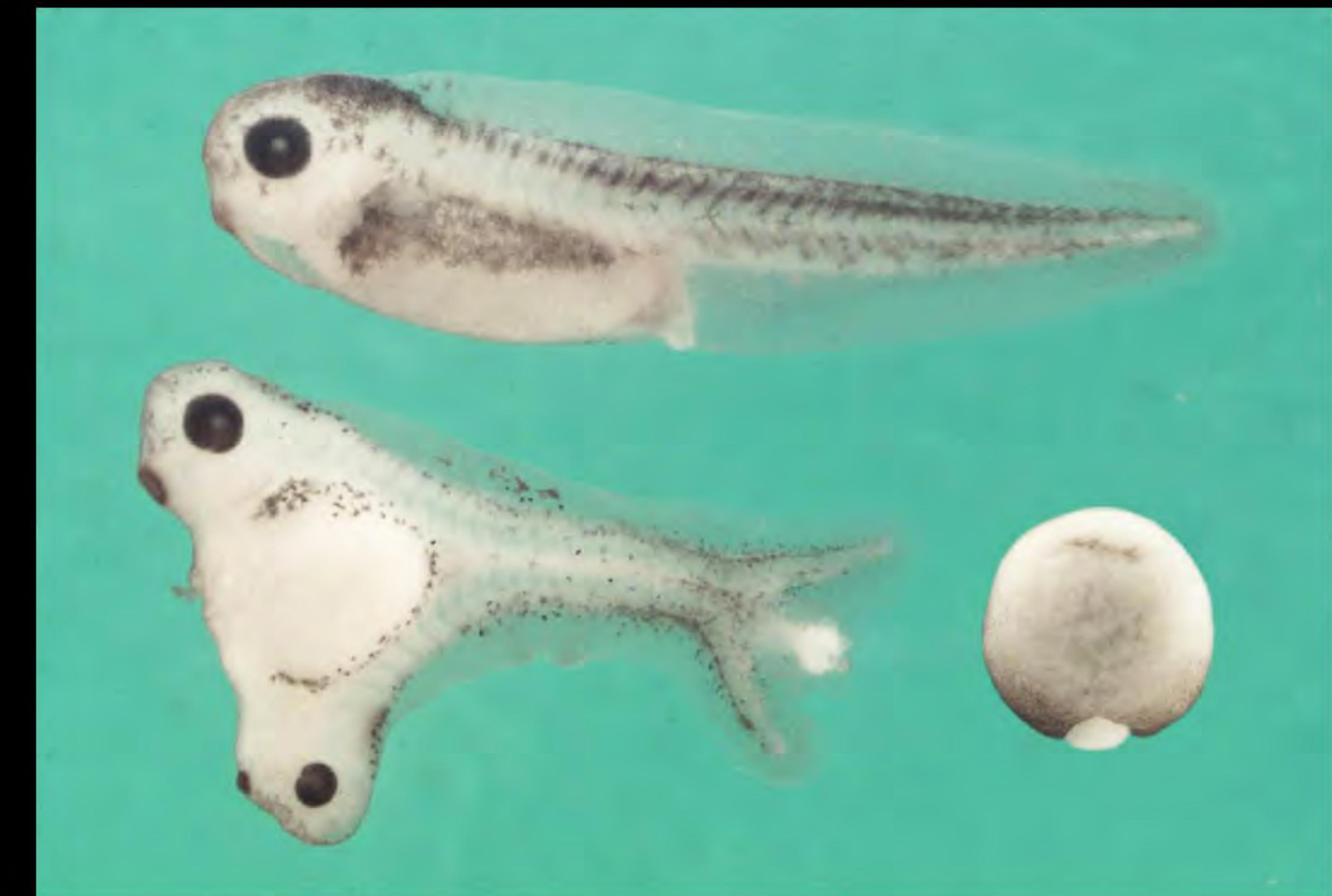
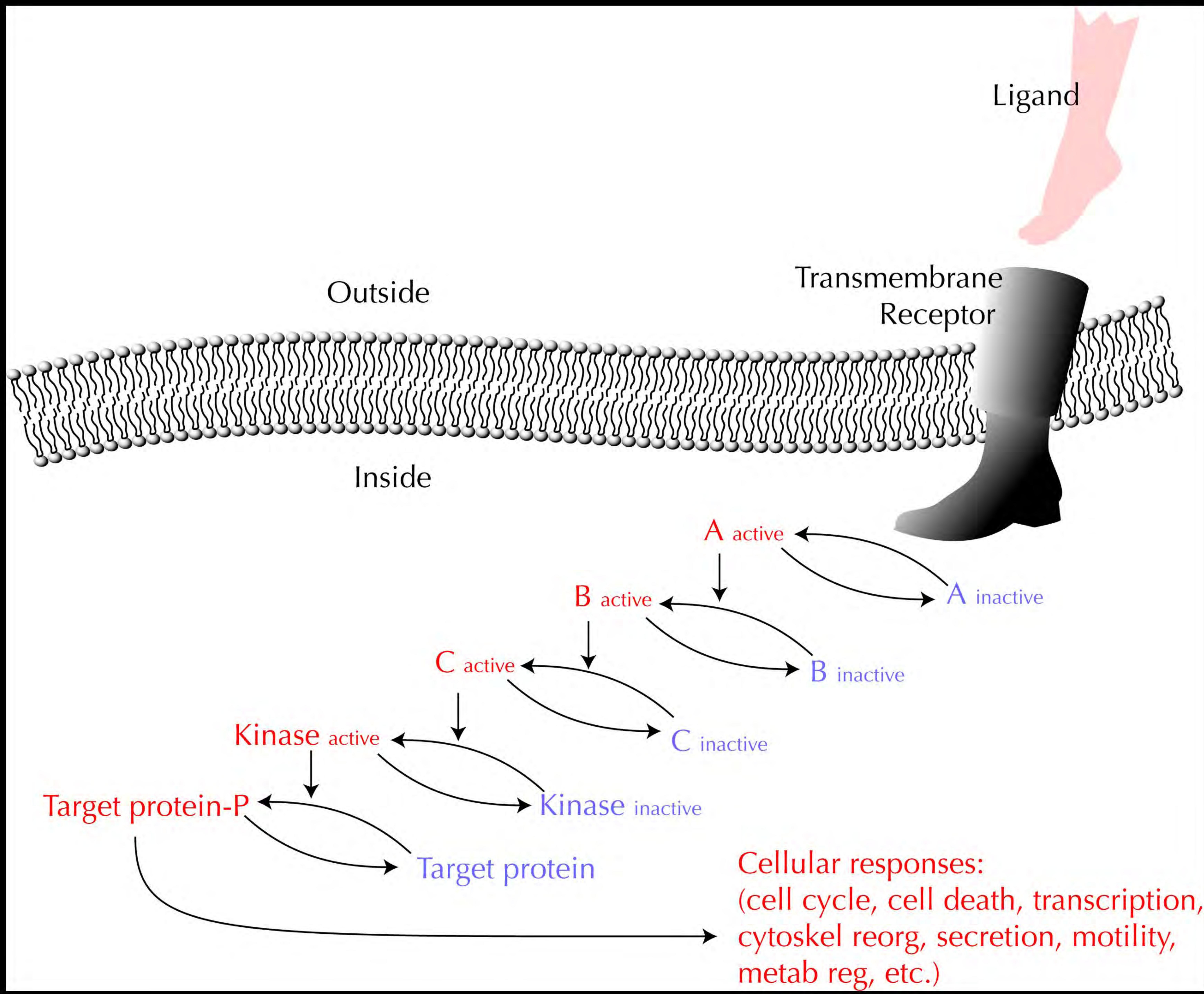
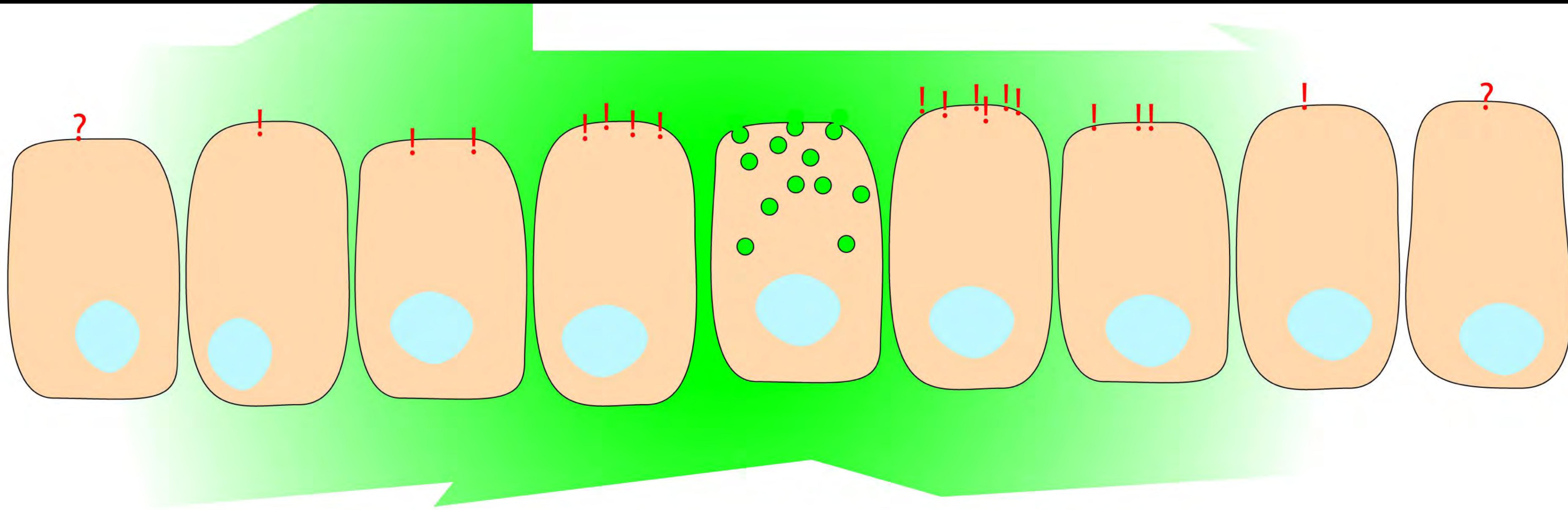


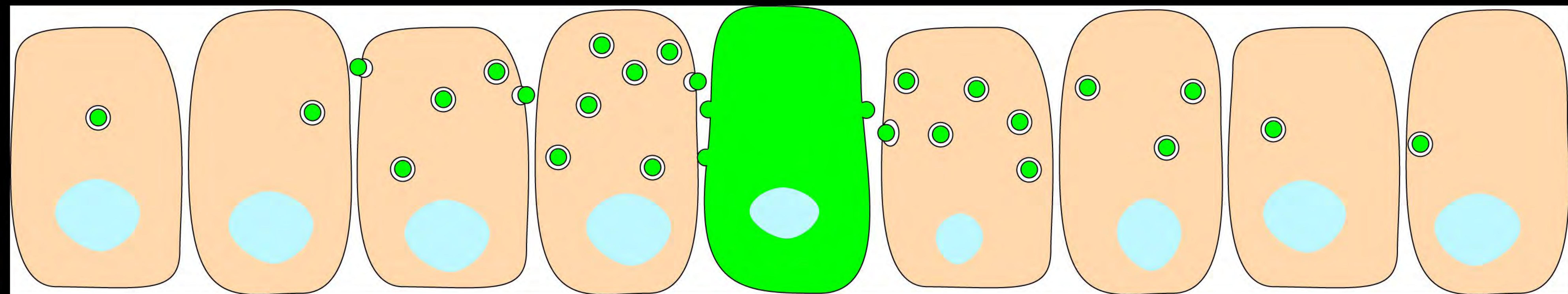
TABLE 1. The 17 intercellular signaling pathways*

- | |
|---|
| Early development and later |
| 1. Wnt pathway |
| 2. Receptor serine/threonine kinase (TGF β) 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 κ 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 |

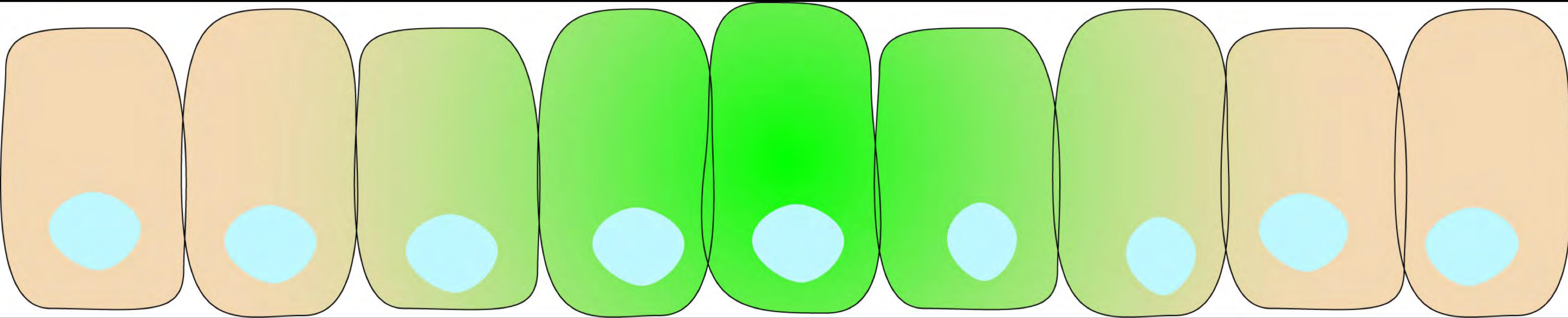




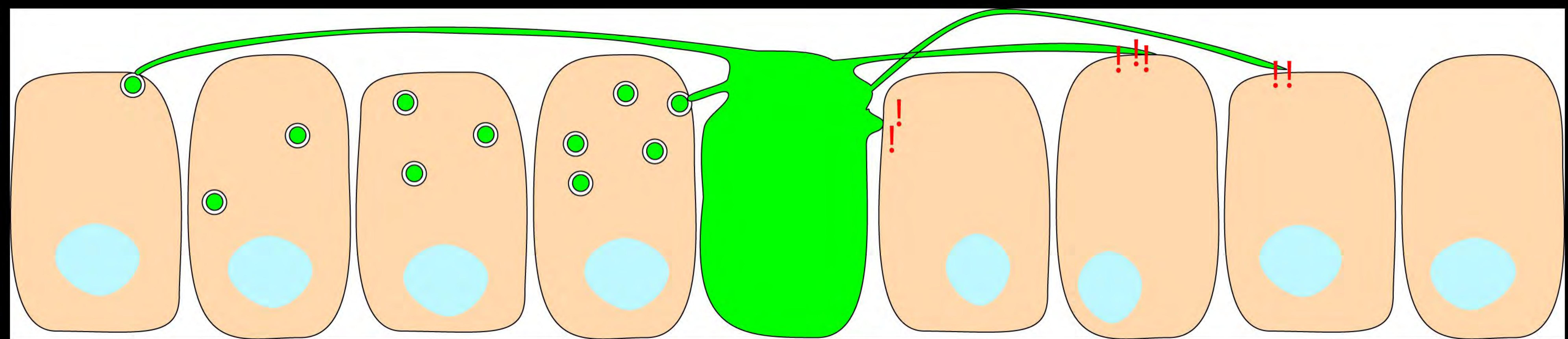
signaling via secreted ligand



signaling via transcytosis



signaling via gap junctional continuity



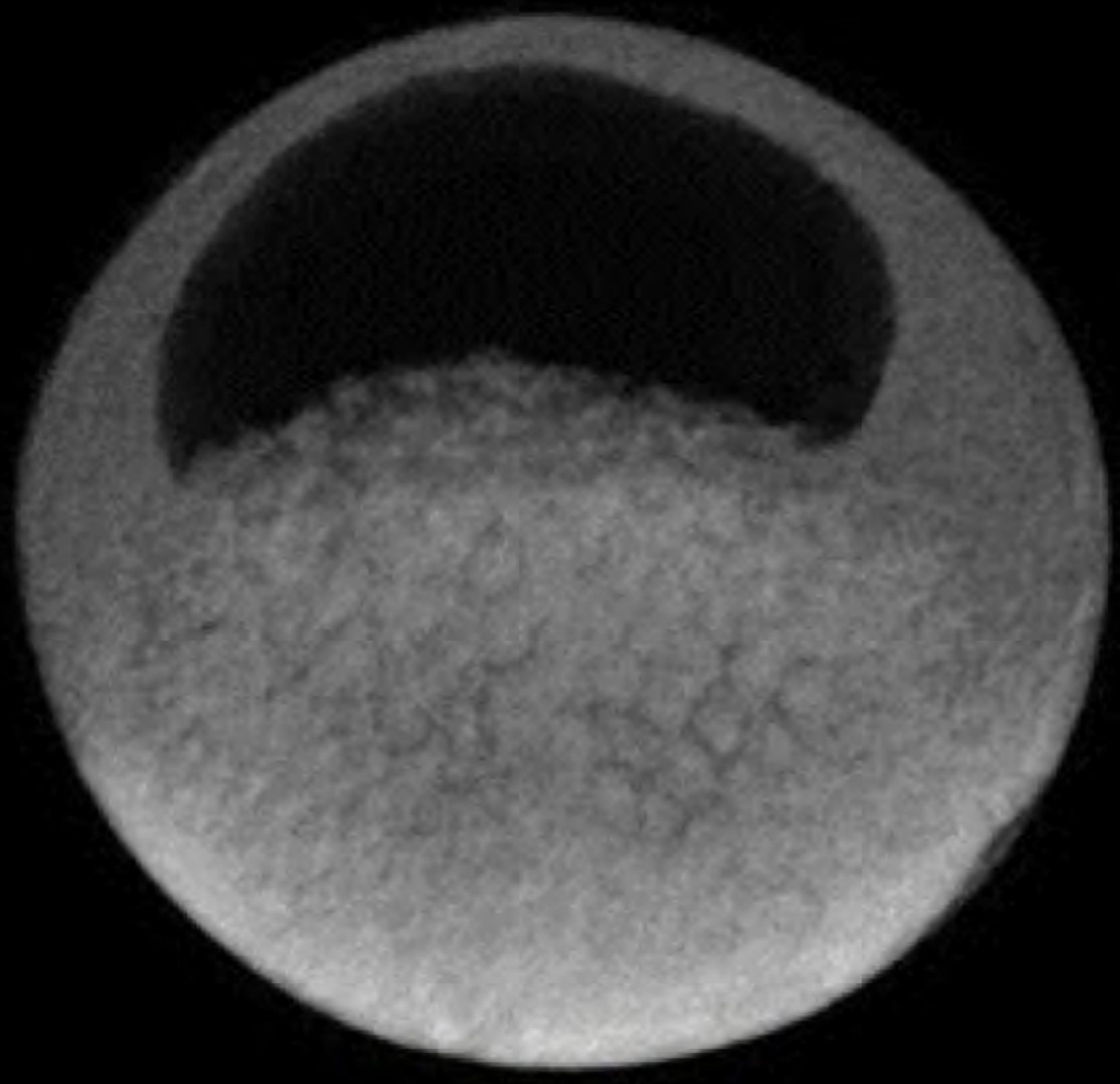
signaling via direct contact

cleavage, a morphogenetic stage...

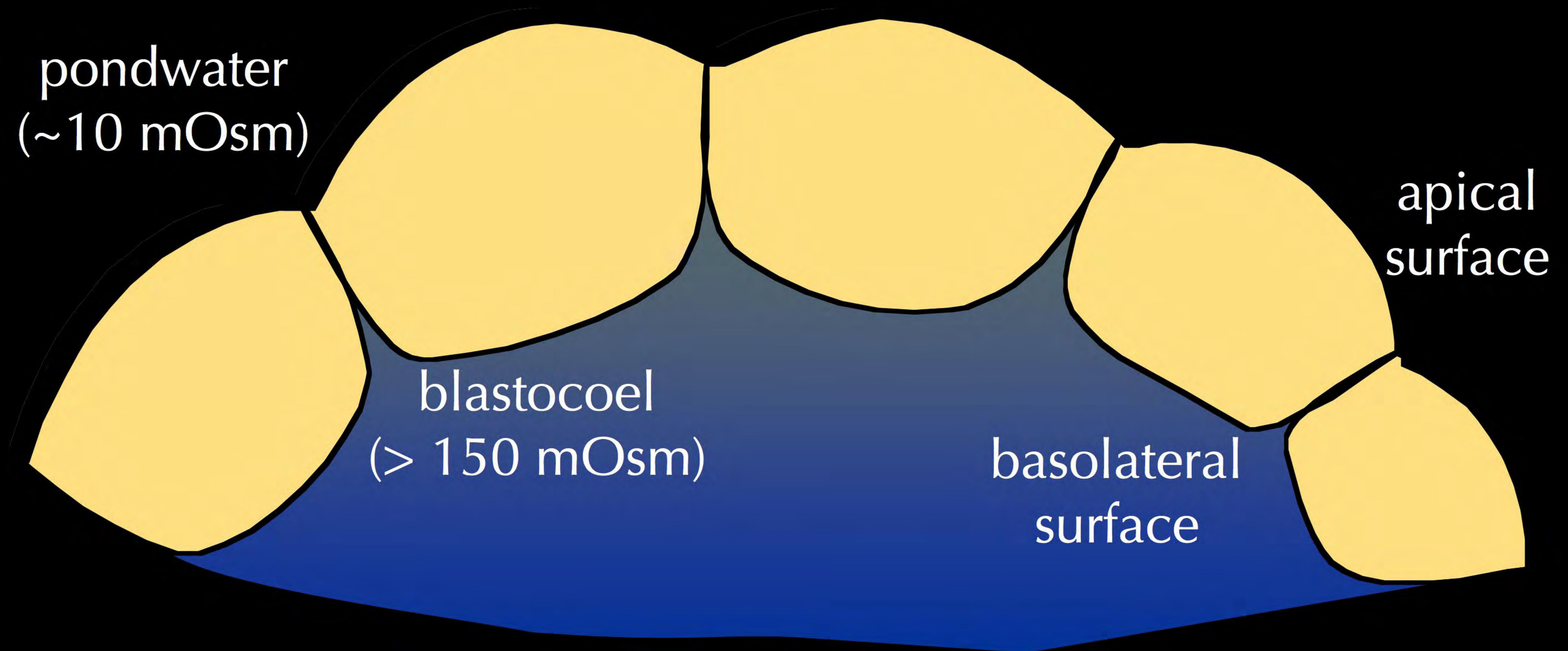
- rapid, ~synchronous cell divisions
- reliance on maternal transcripts and proteins
- cytoplasmic localizations
- membrane specializations (epithelialization)
 - establishment of basolateral domain
 - cell cell adhesion... compaction
- preparations for gastrulation

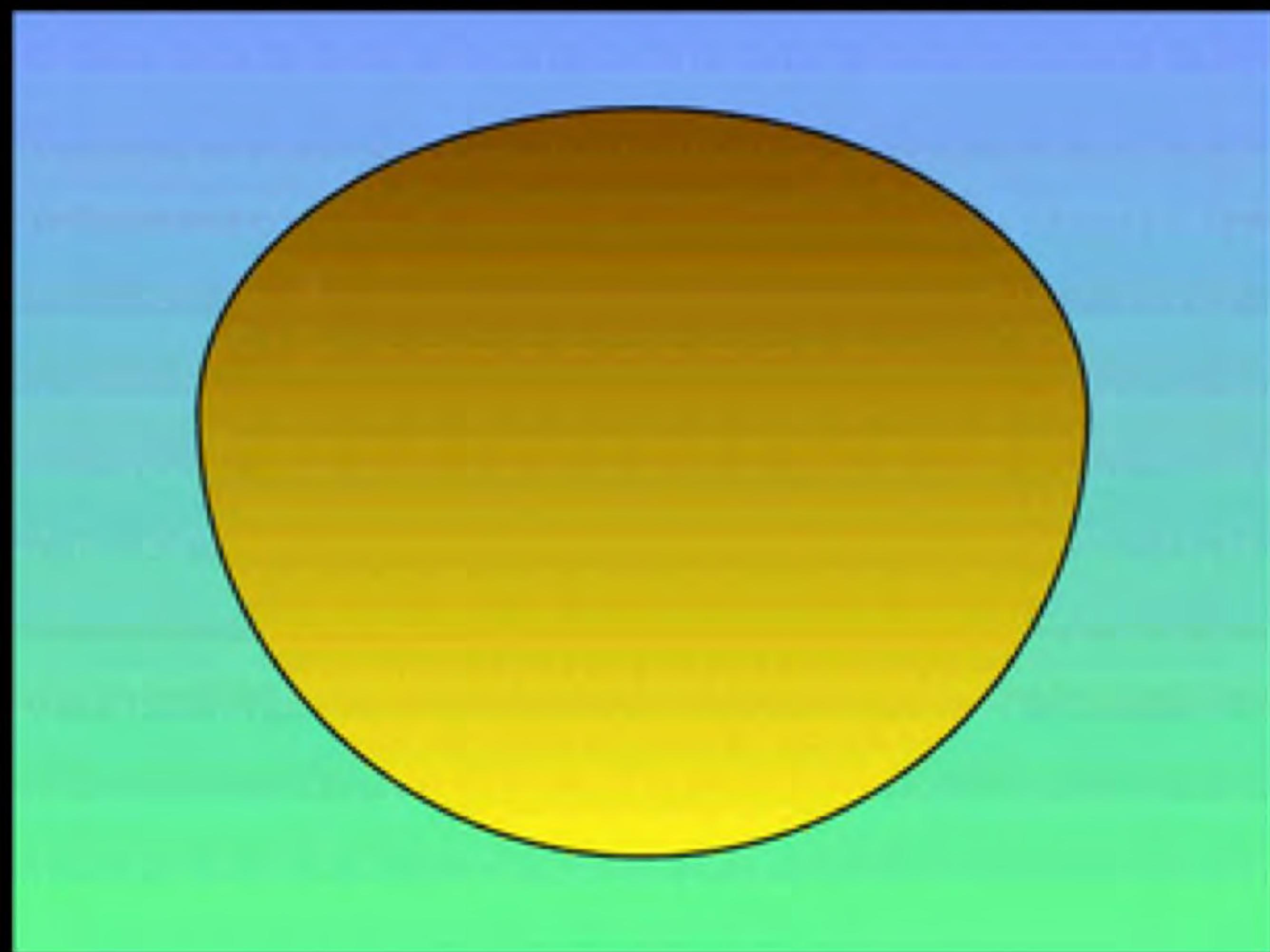


Movie 1



Movie 2

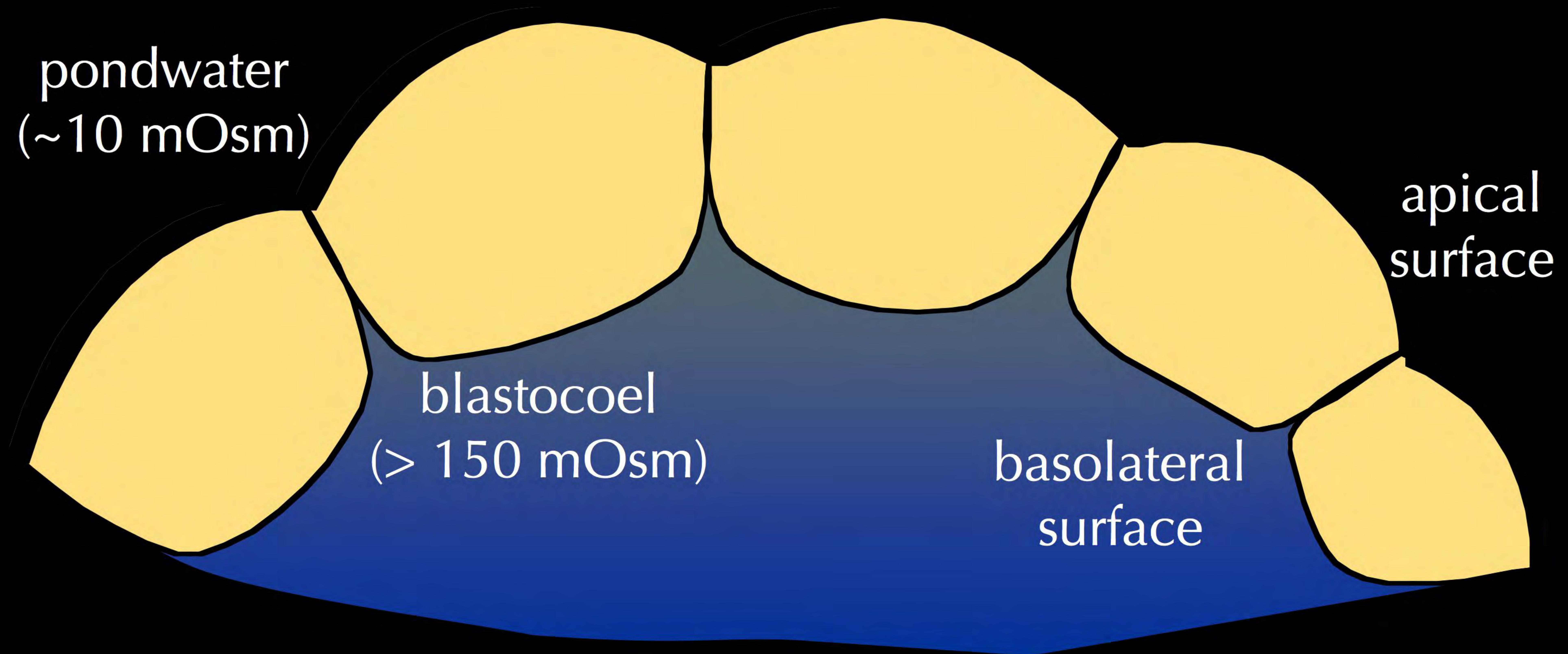


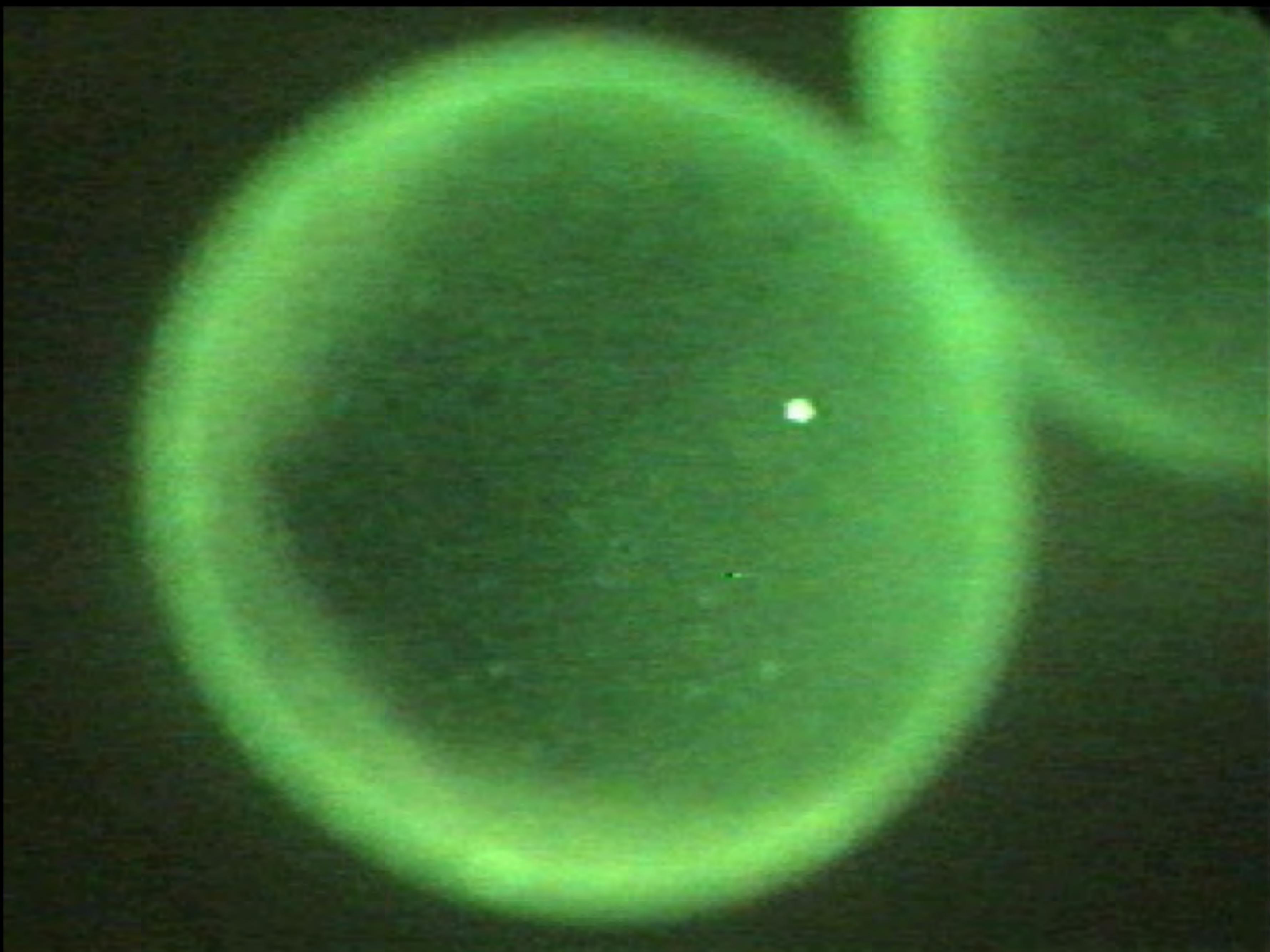


Movie 3



Movie 4





control

Movie 5

Green = fluorescent lectin
(binds surface carbohydrates)

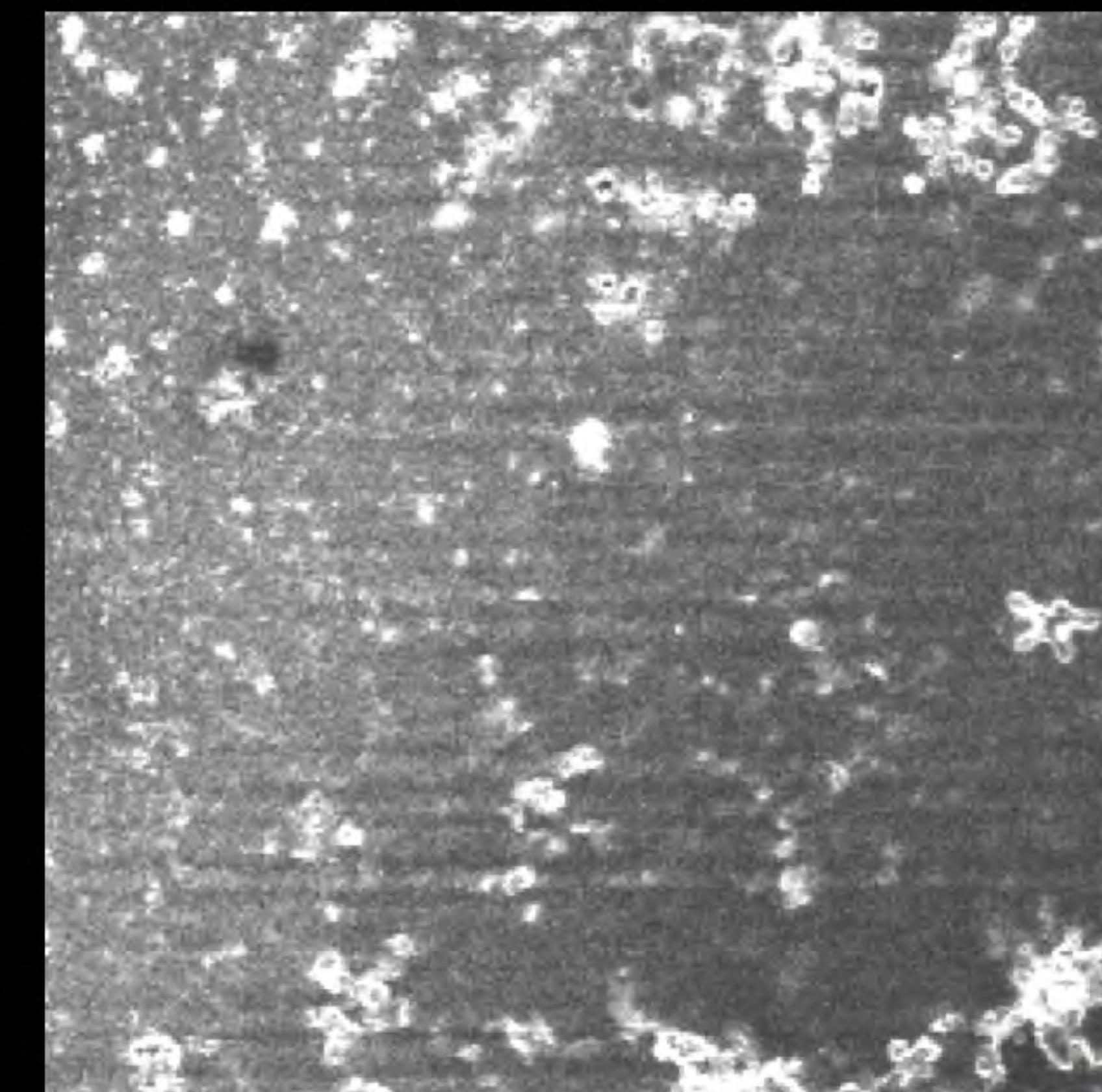
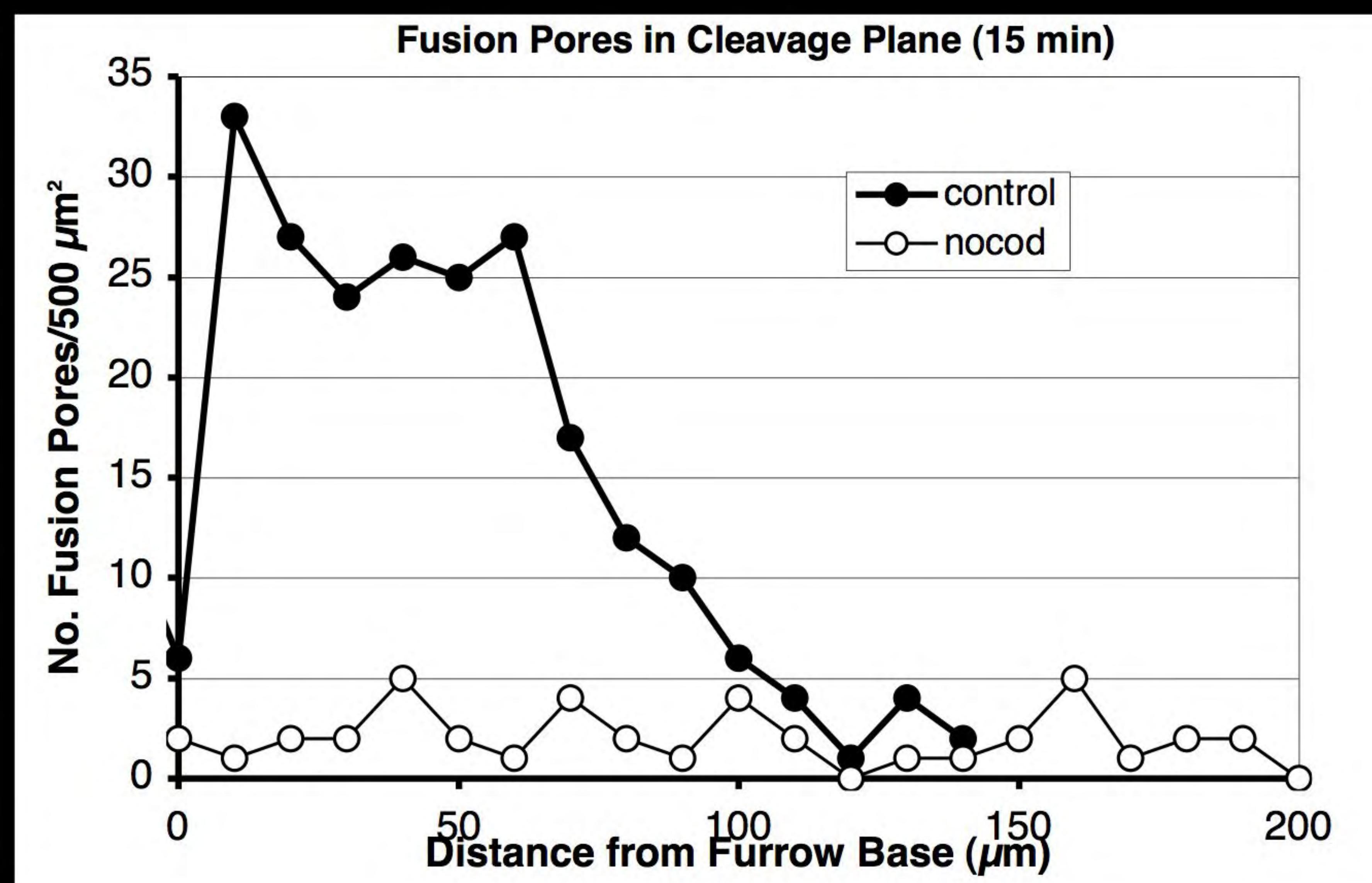
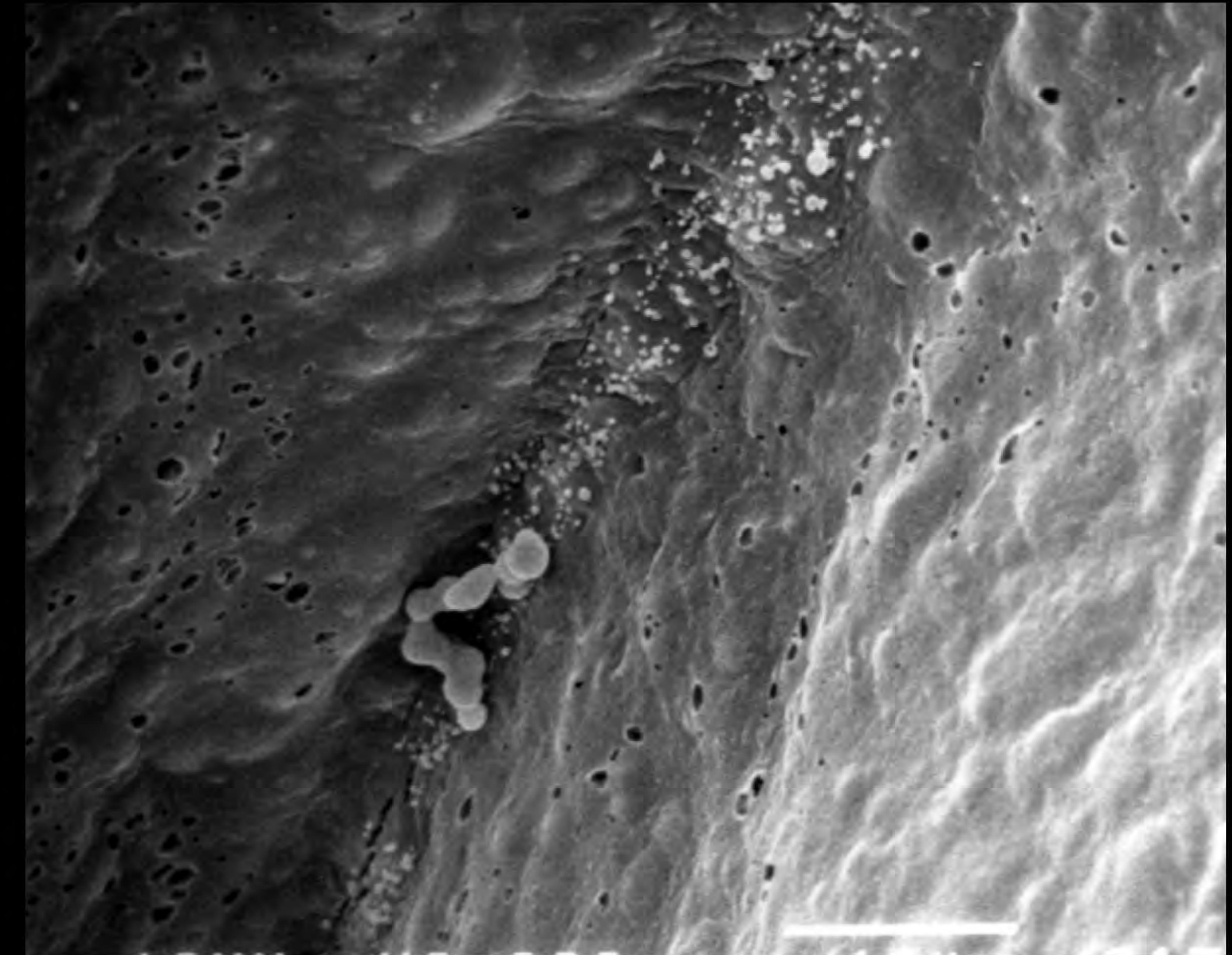
Membrane expansion
& furrow progression
both require
microtubules

Movie 6



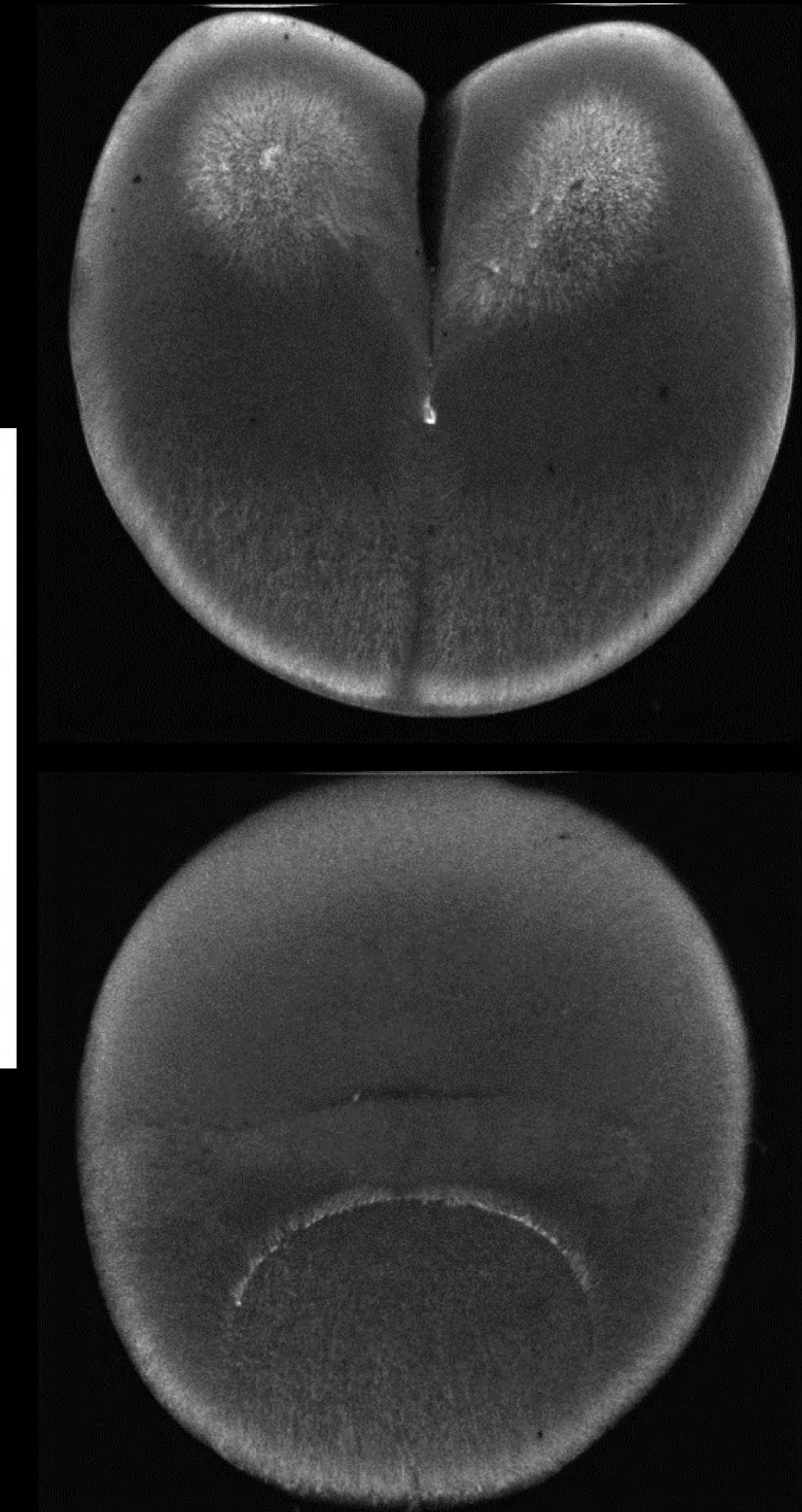
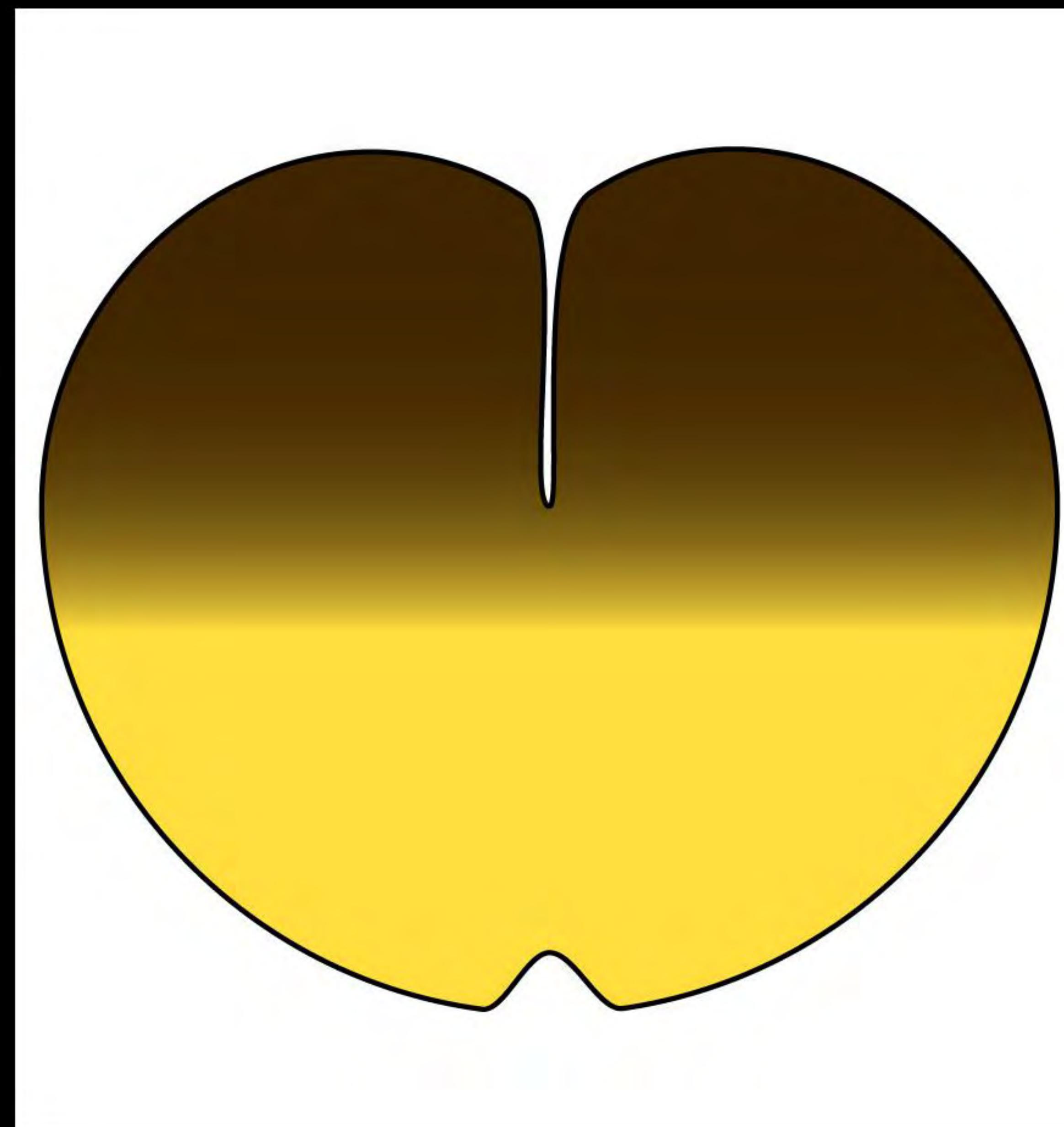
+ nocodazole
(microtubule inhibitor)

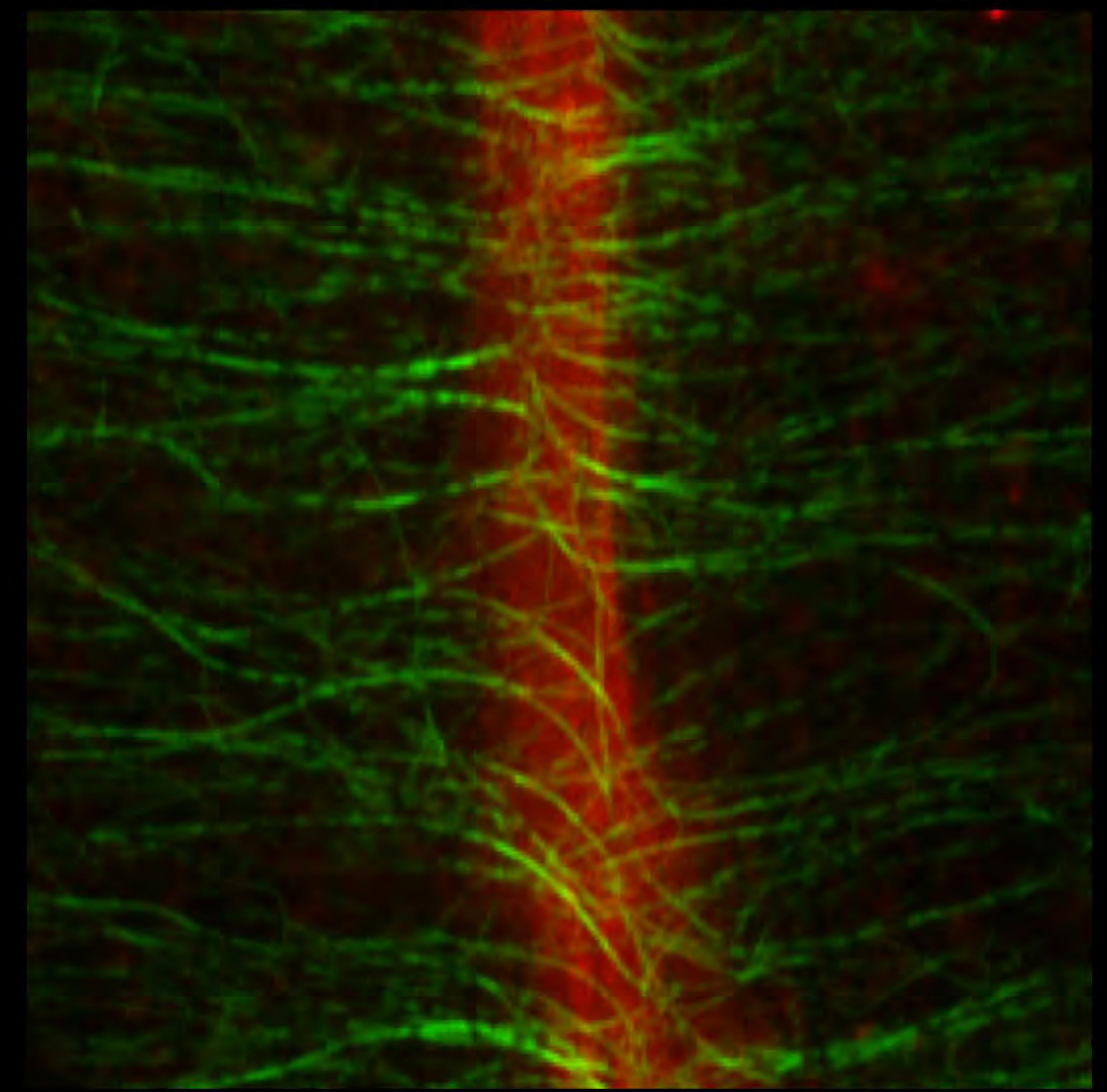
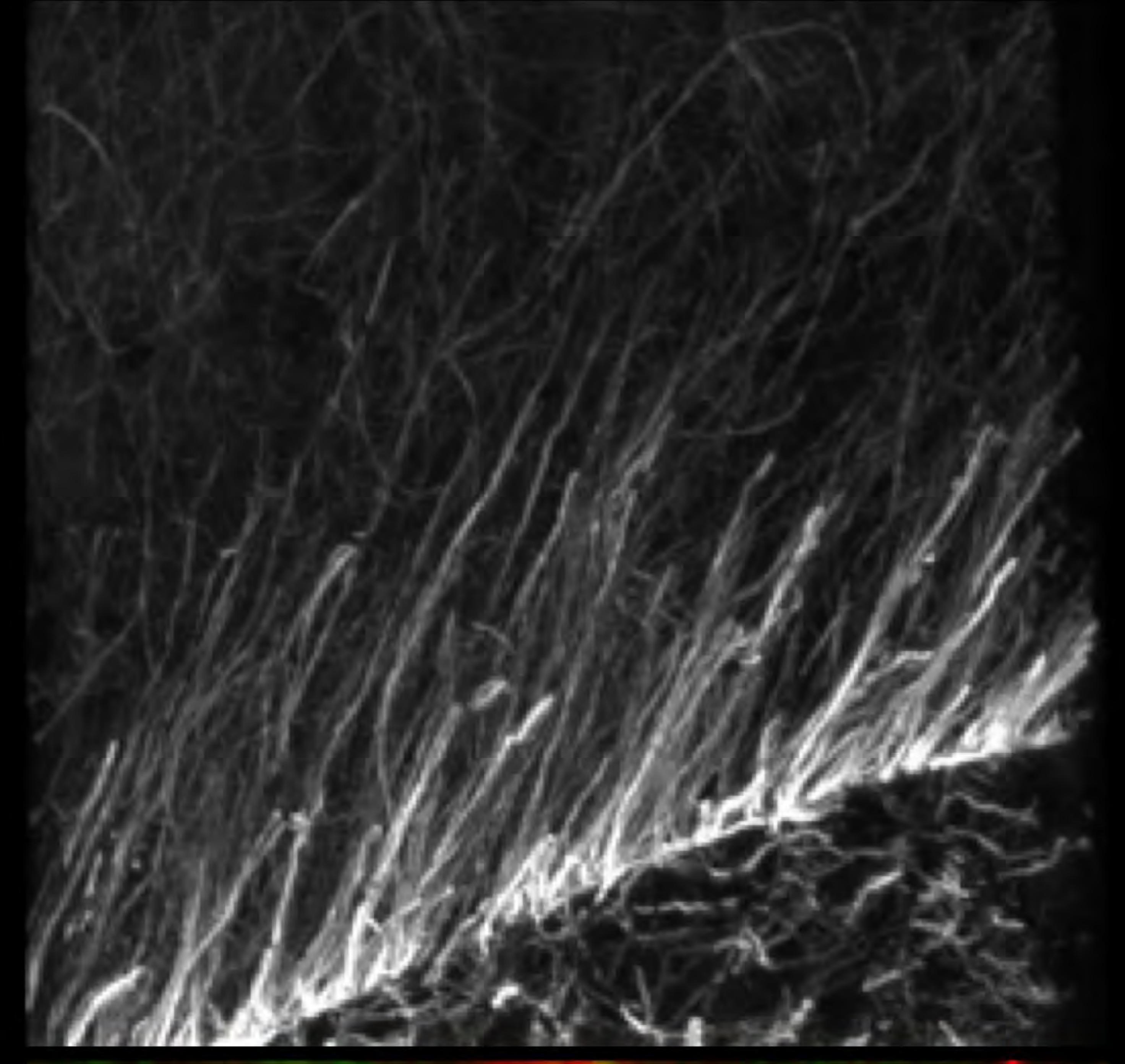
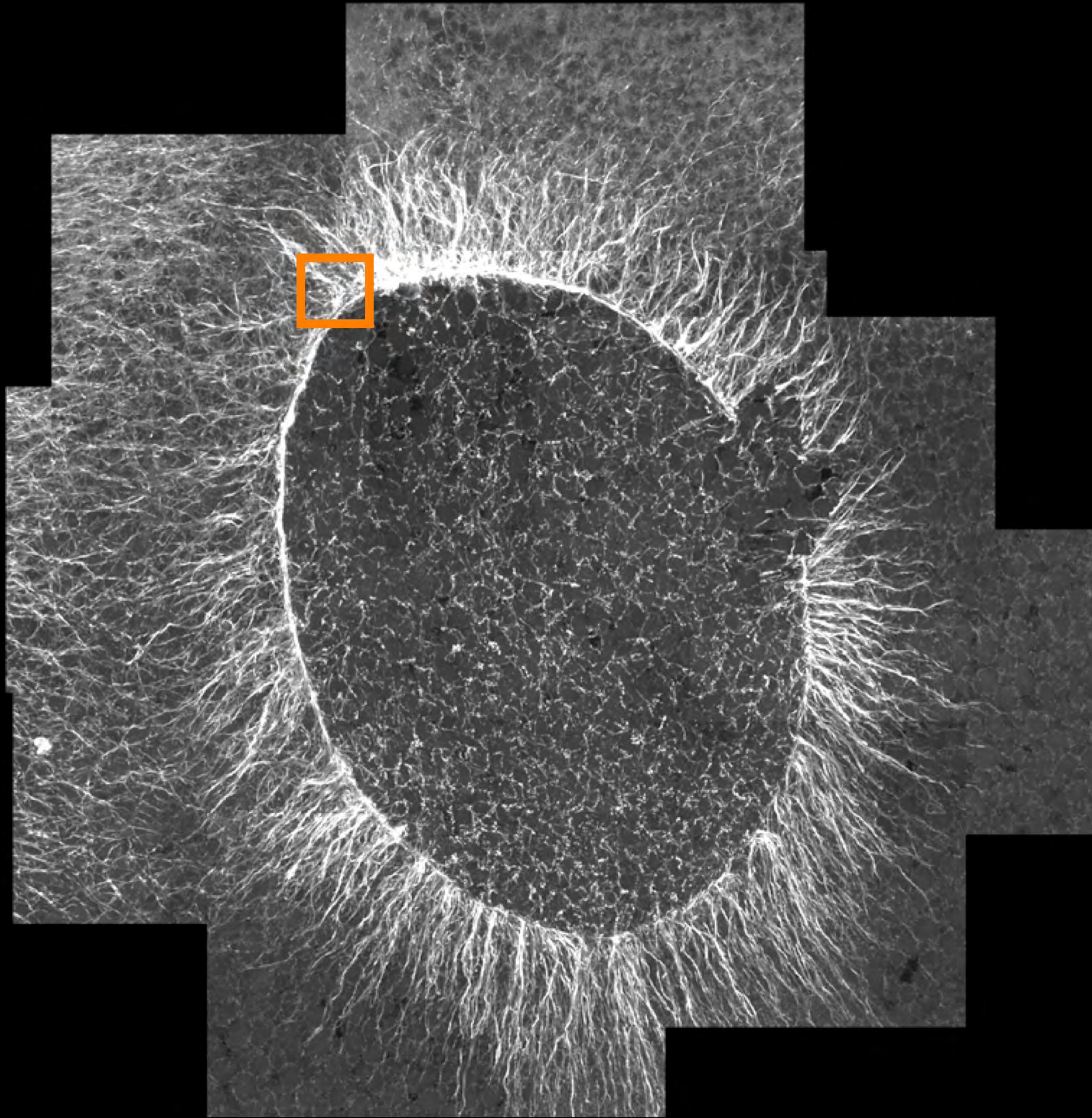
Localization of exocytotic site depends on furrow microtubules



Movie 7

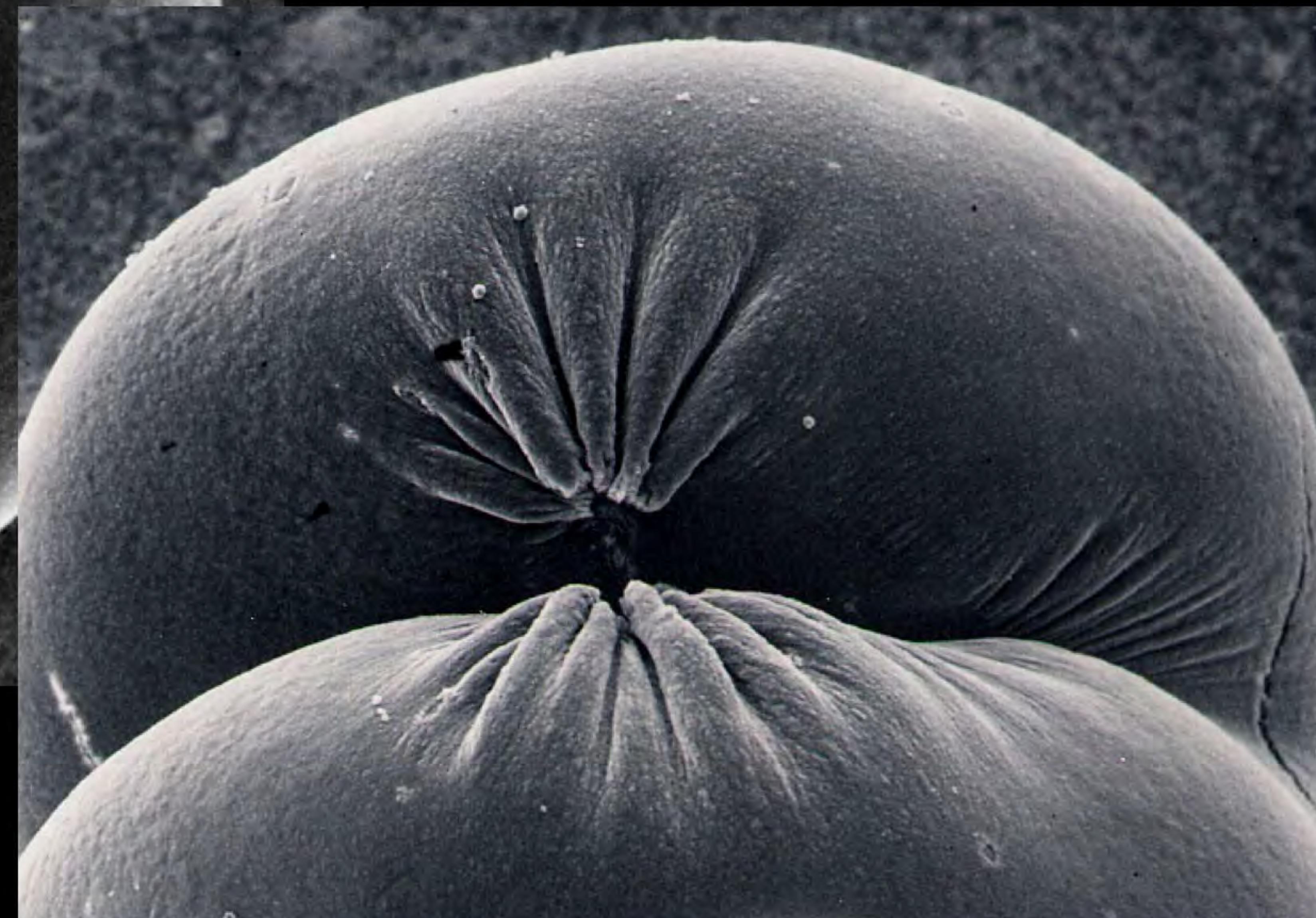
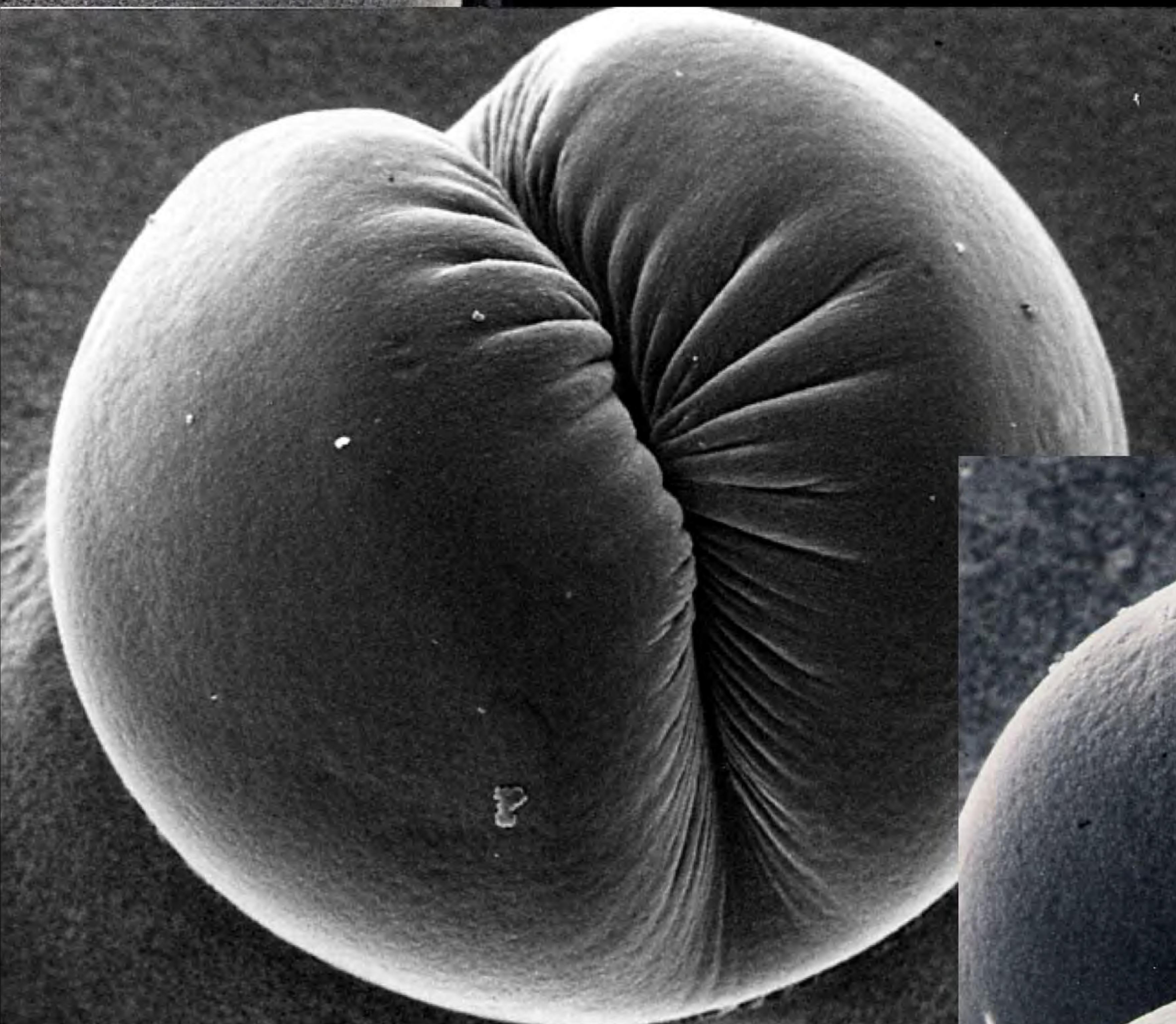
FM 1-43 (fluorescent lipid dye)



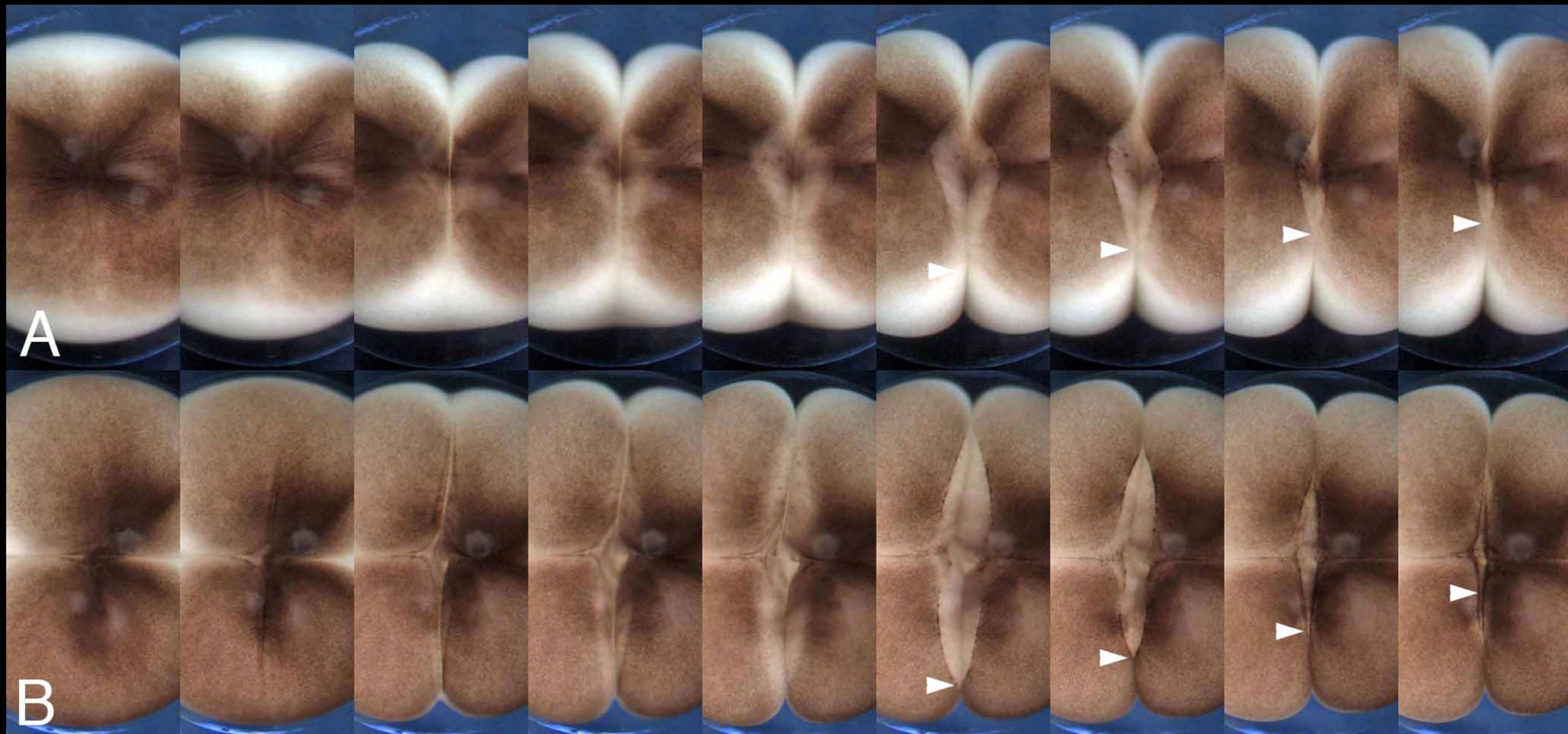


microtubules microfilaments

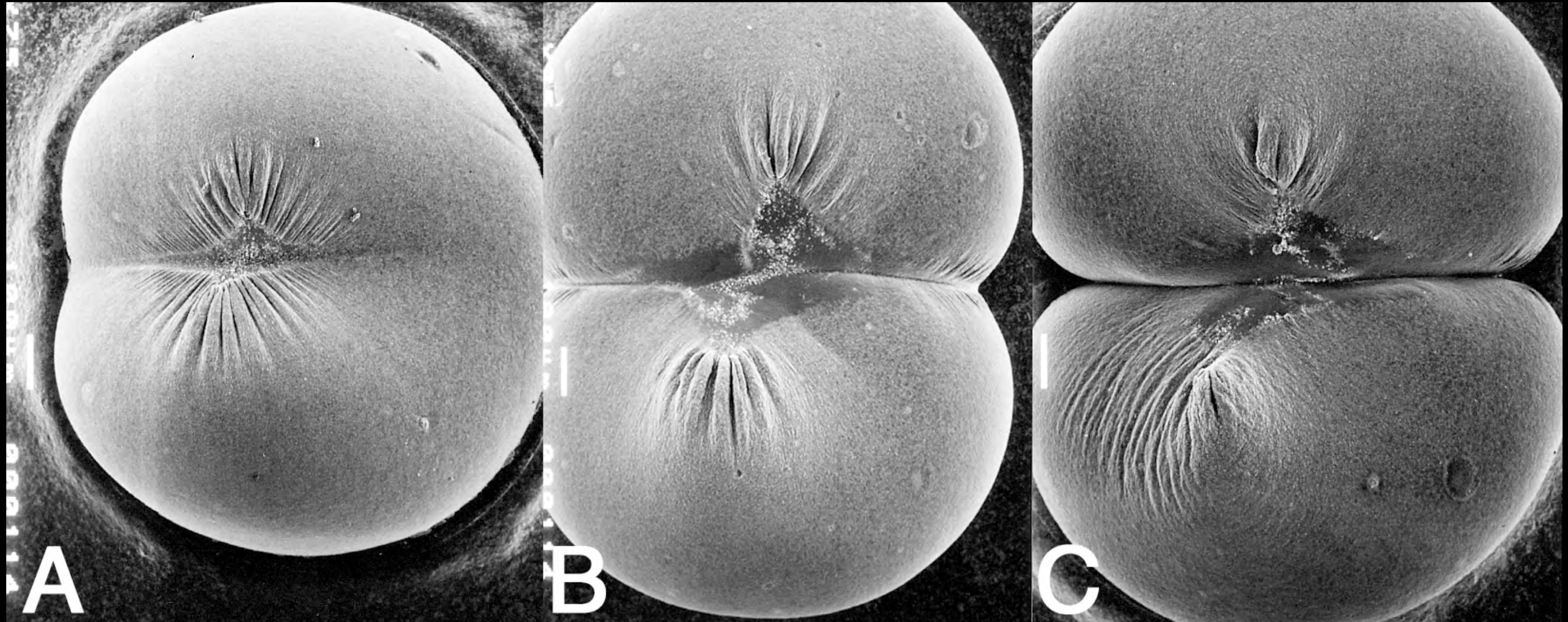
Cleavage furrow closure: stress folds marginal protrusive zone basolateral protrusive zone



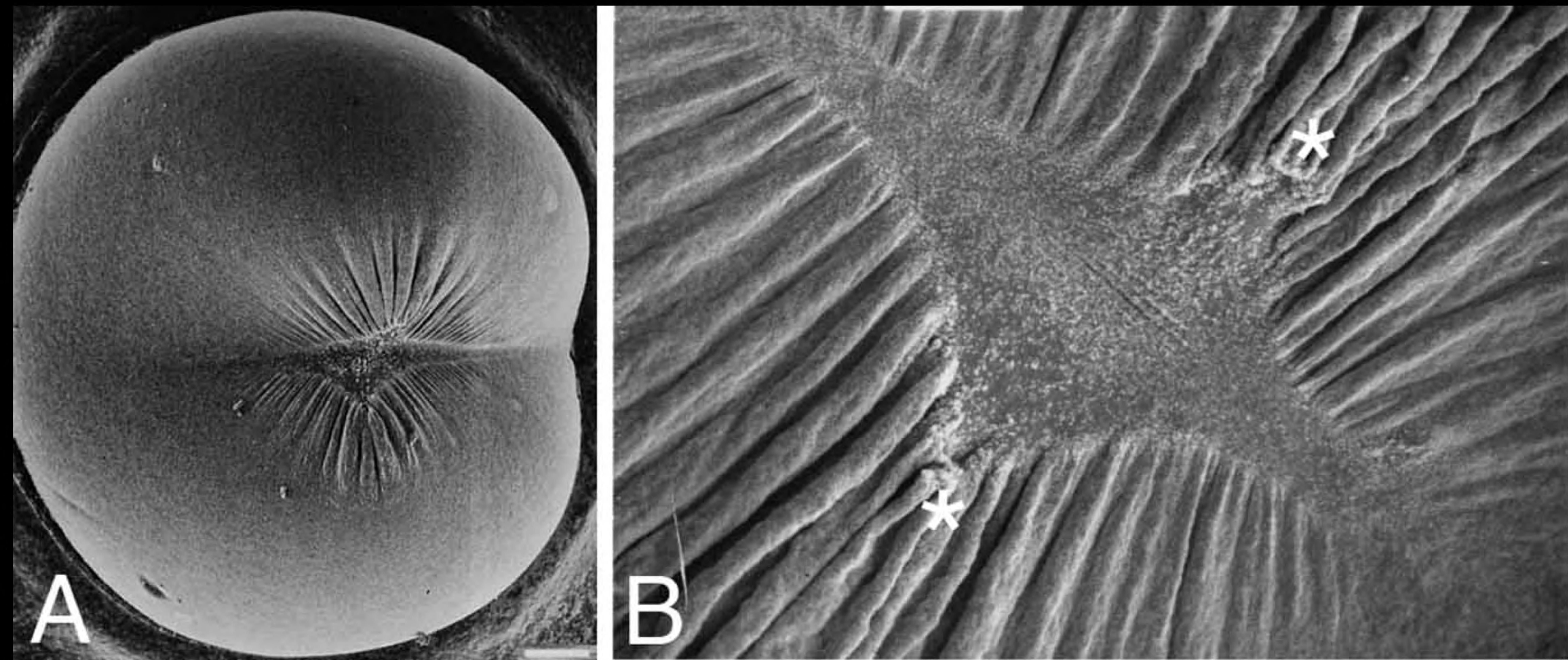
Cleavage furrow closure via apical zippering & basolateral adhesion

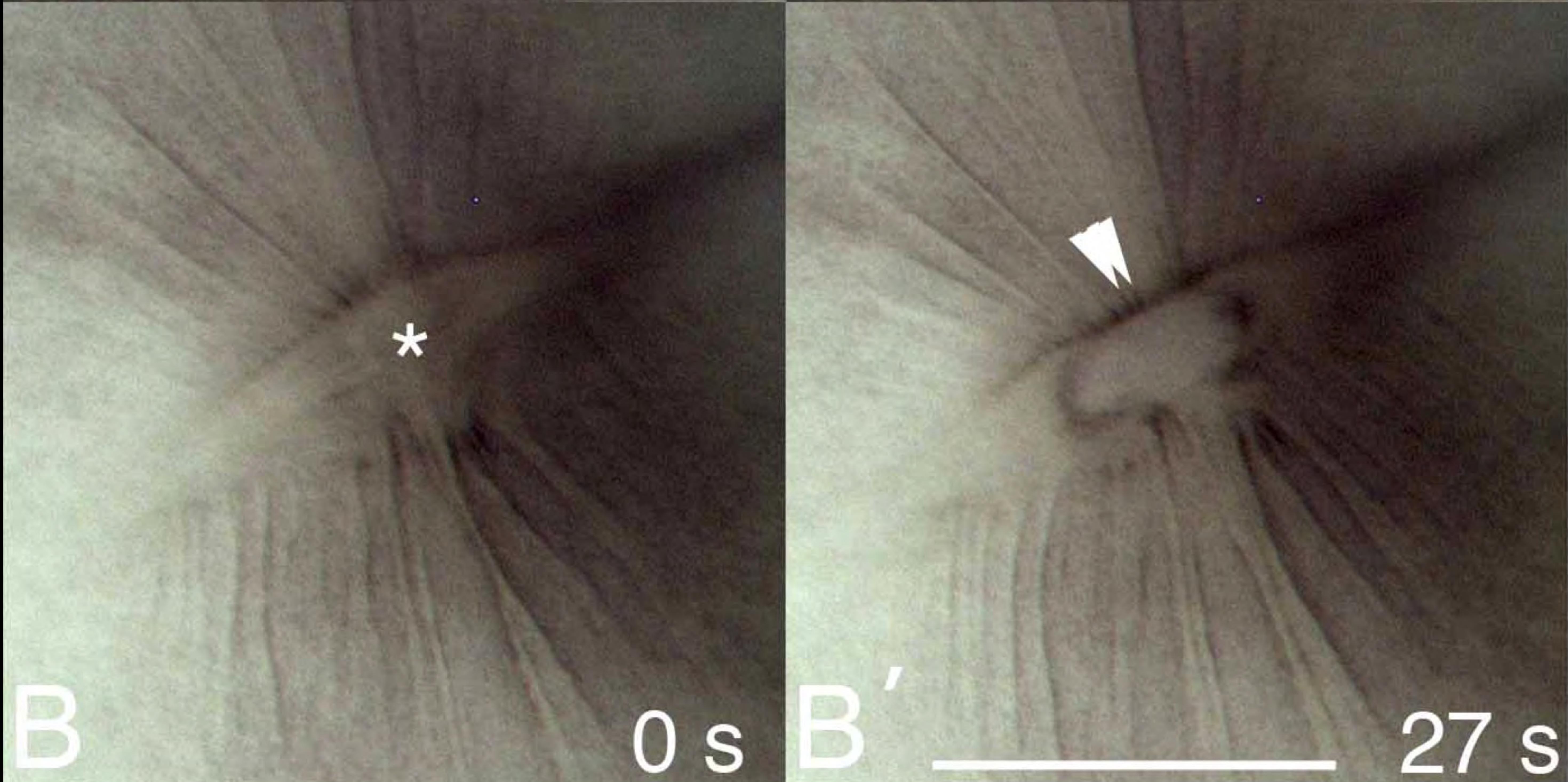
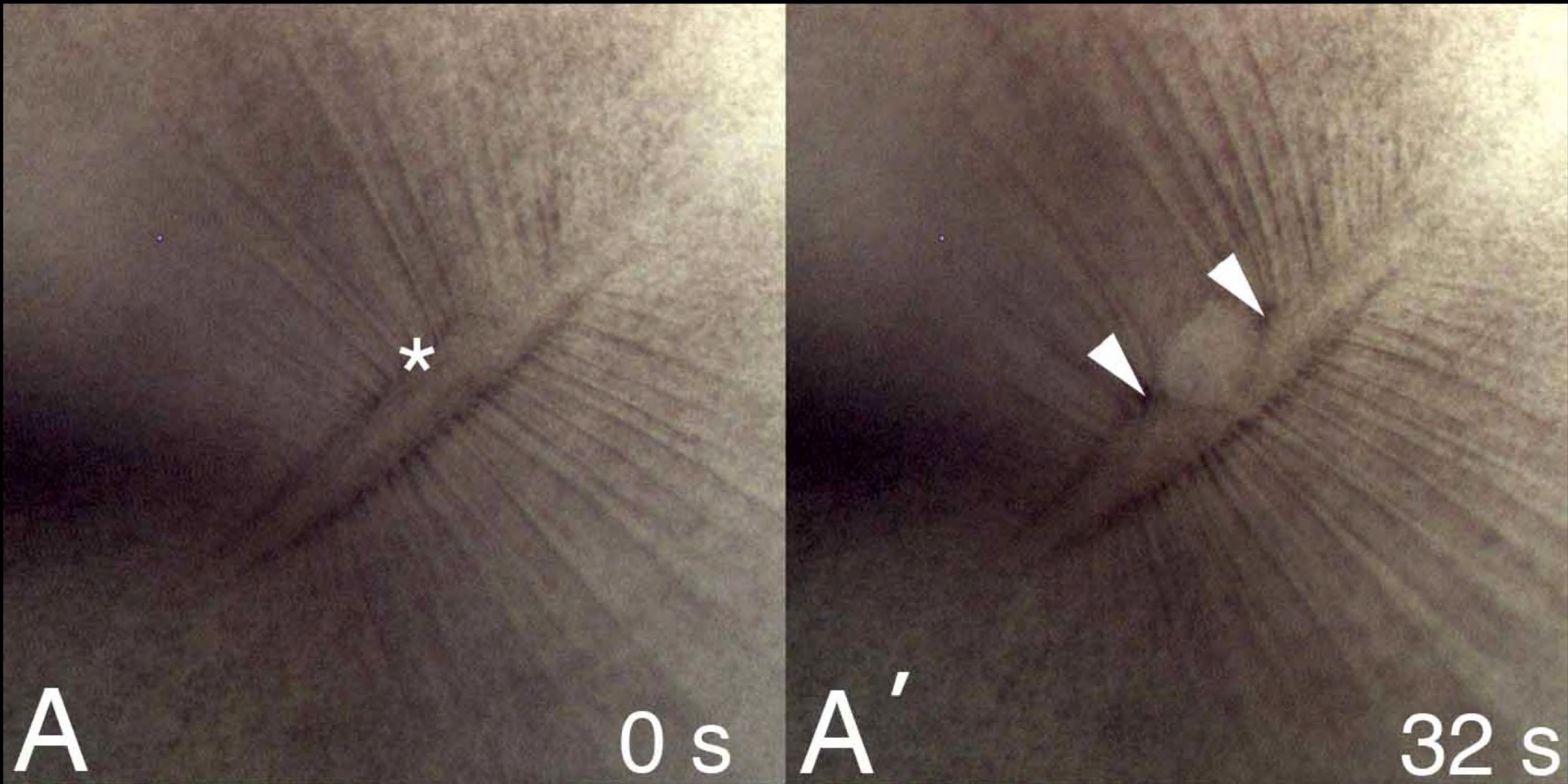


Stress folds ("tension lines")

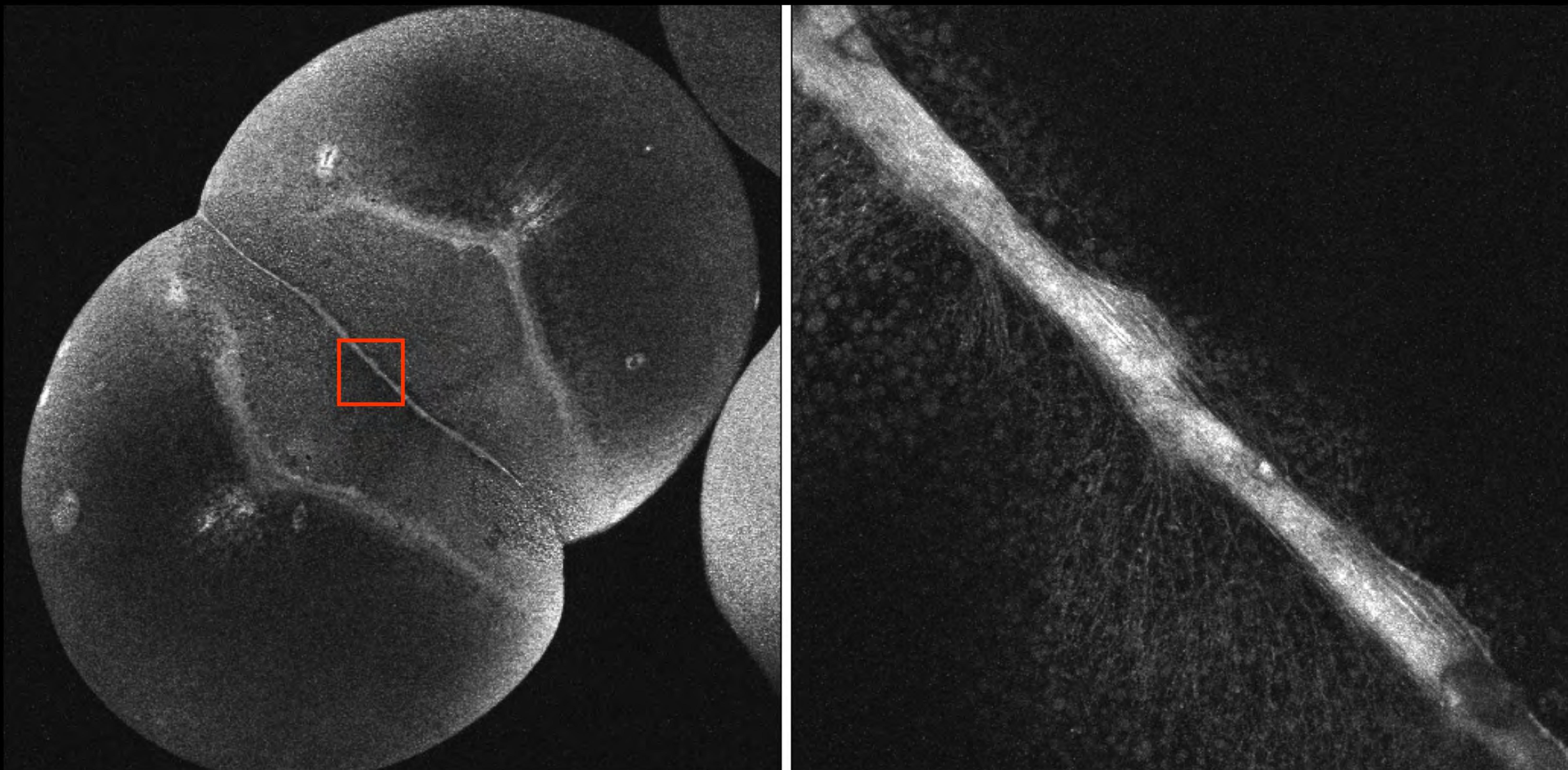


Marginal contractility isn't a function
of contractile ring



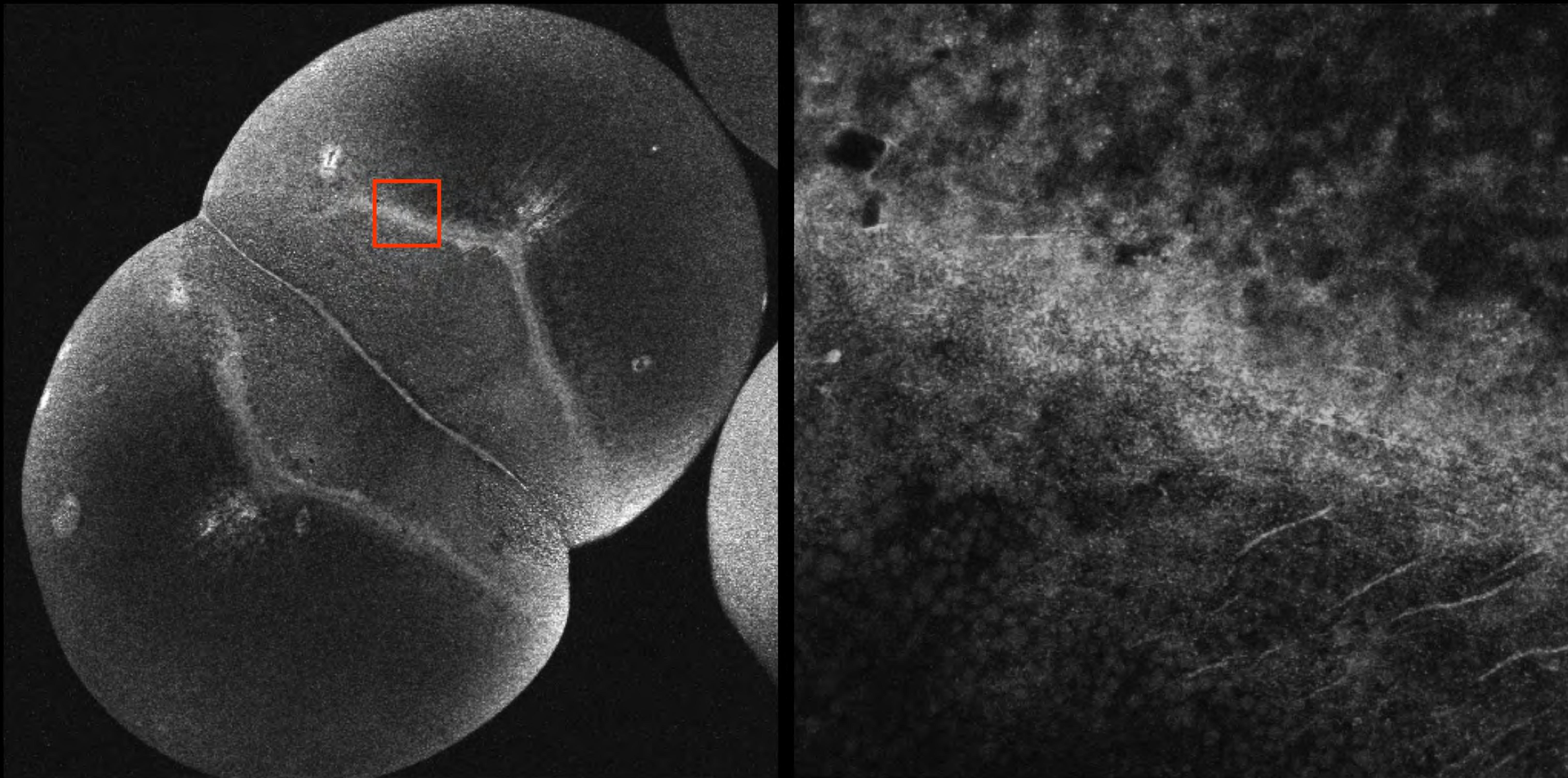


Parallel bundles of contractile f-actin



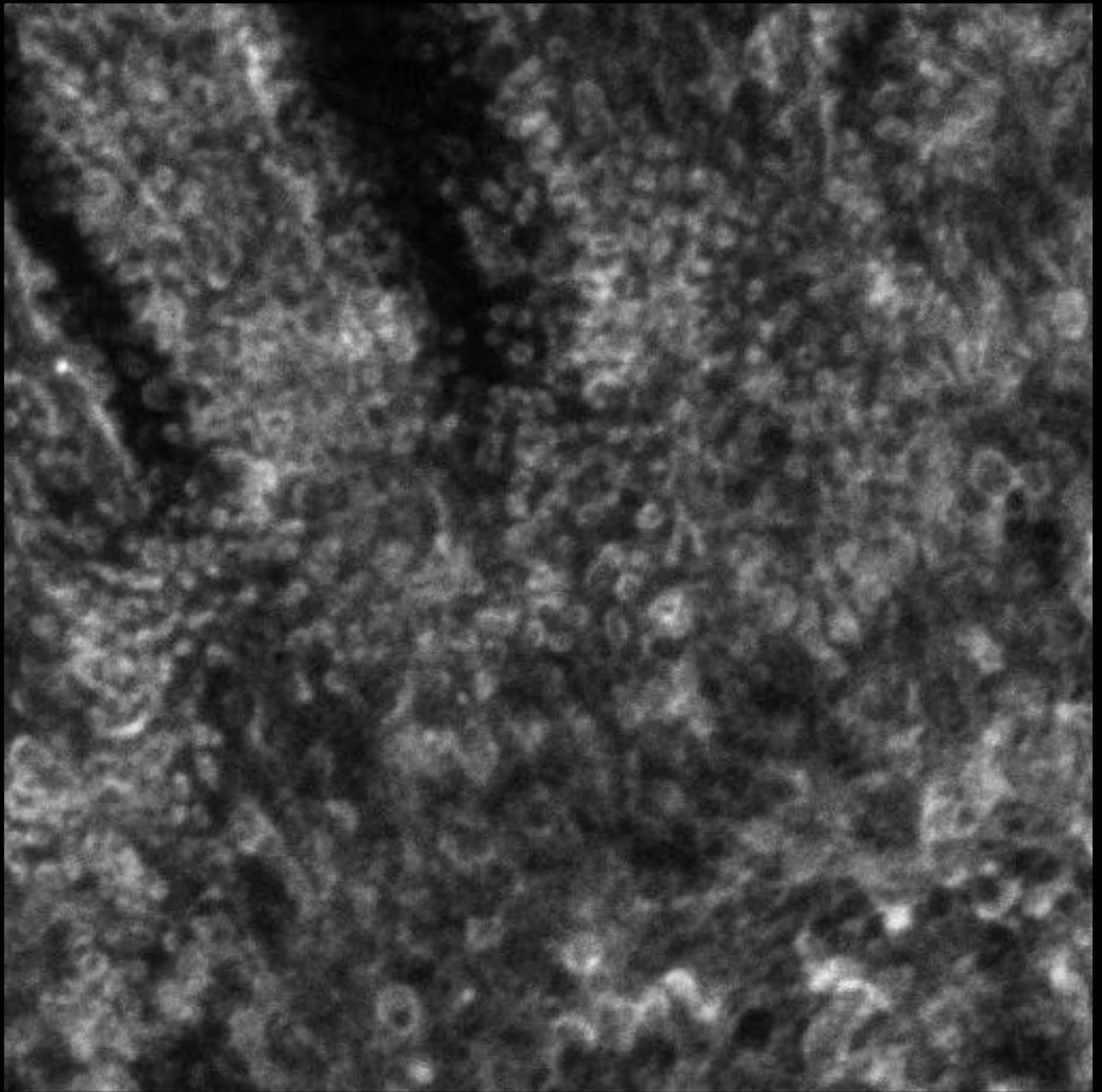
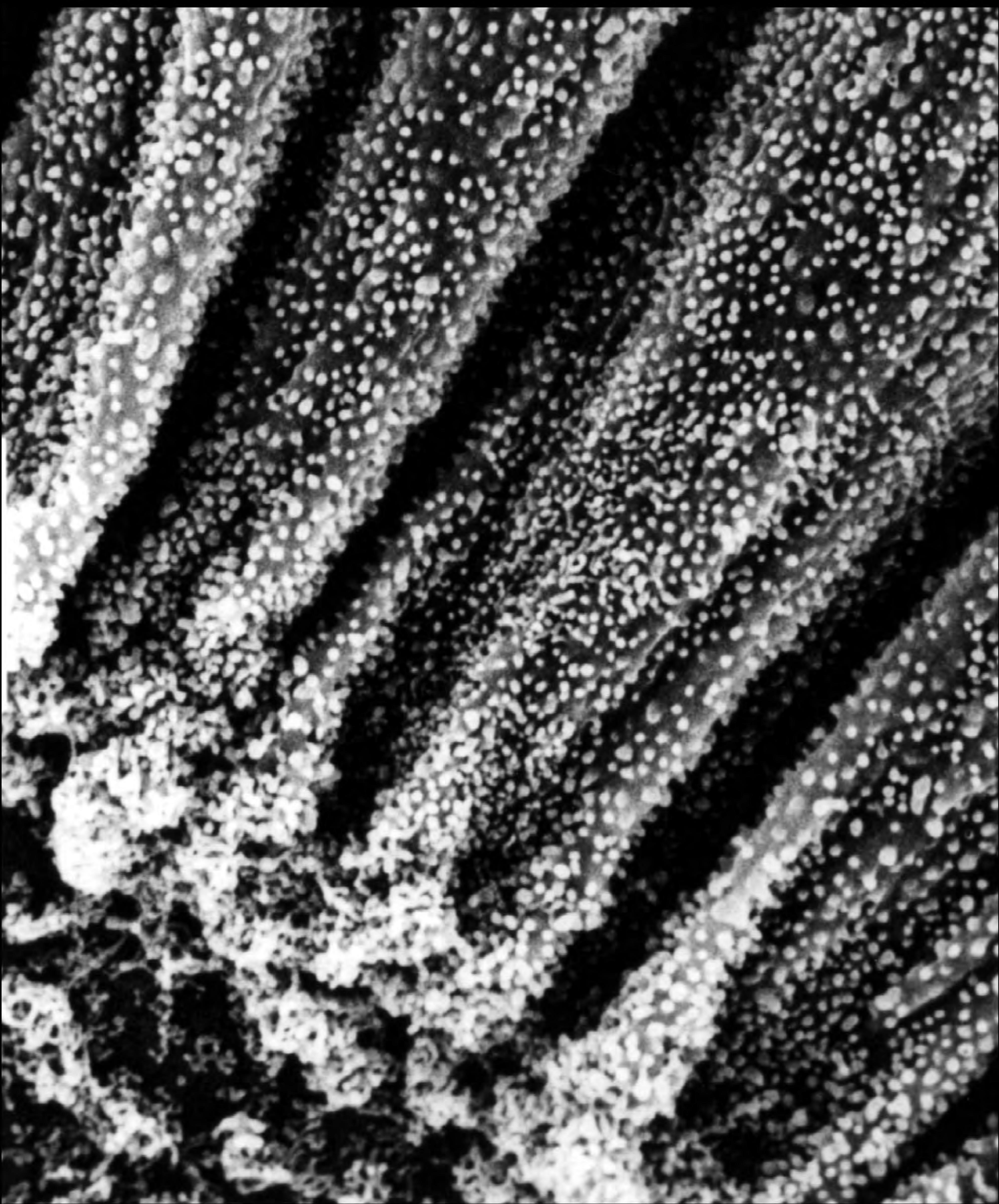
Rh-phalloidin

Amorphous band of contractile f-actin



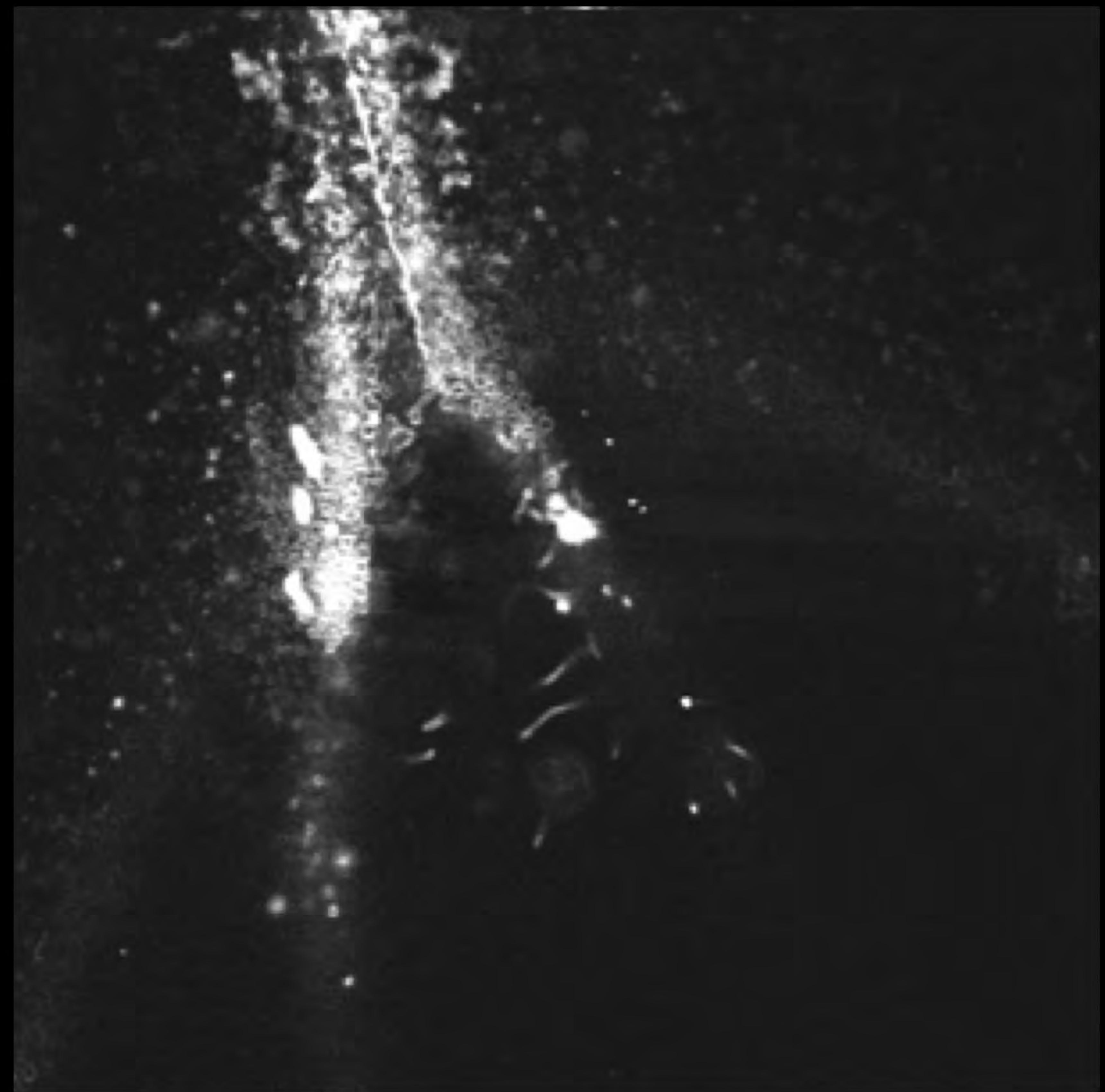
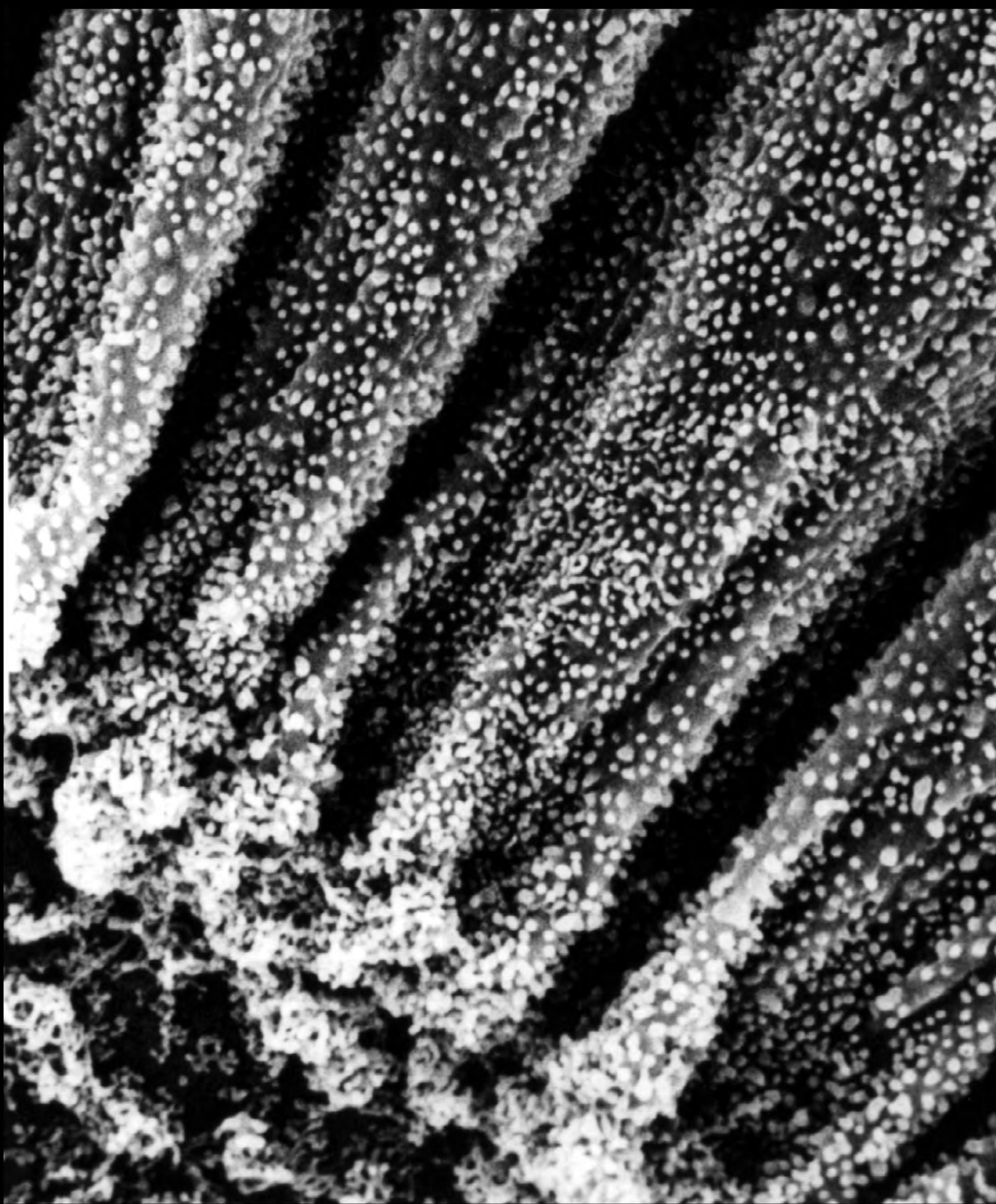
Rh-phalloidin

Marginal protrusions
are actin-filled



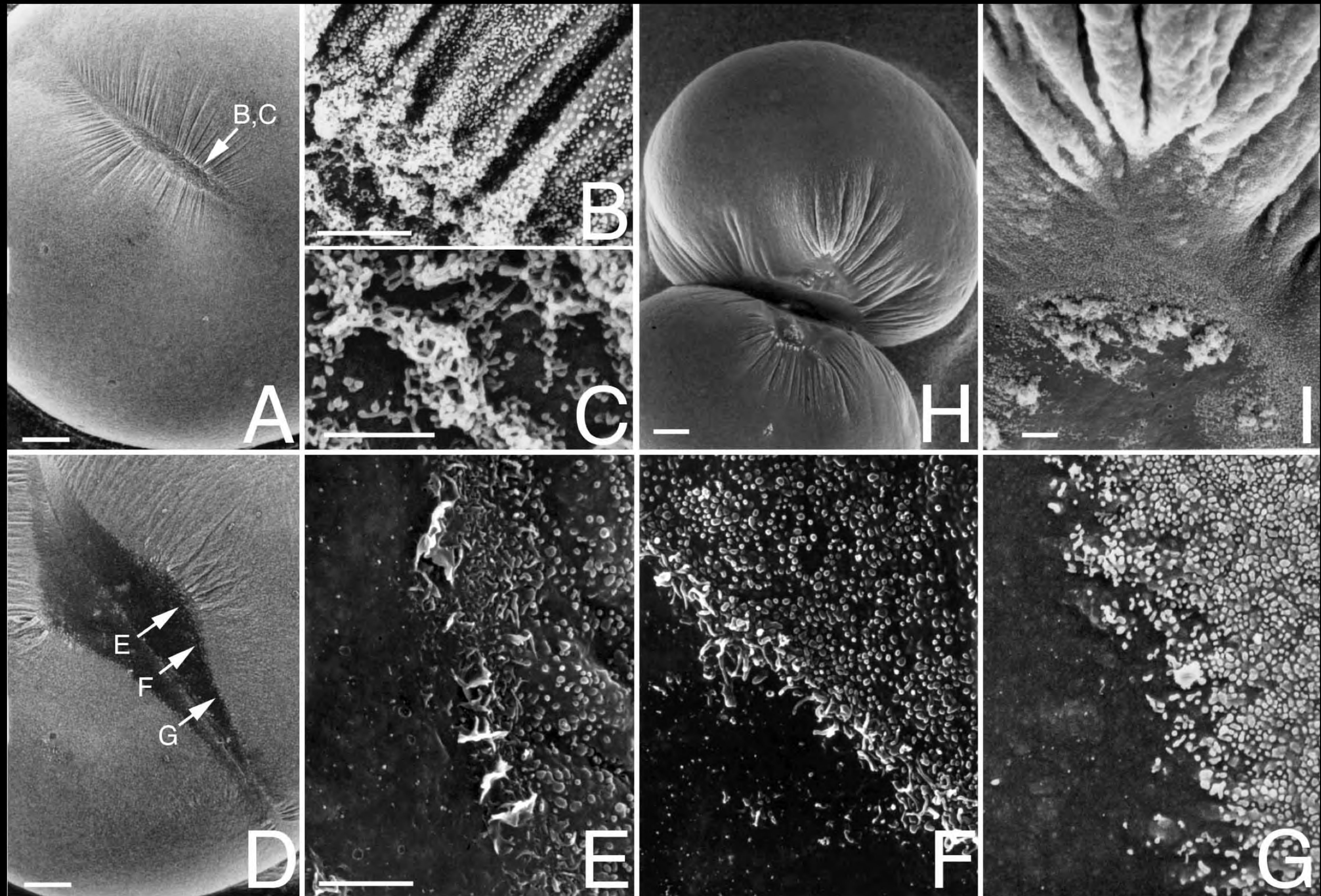
Rh-phalloidin, 2-cell

... and motile

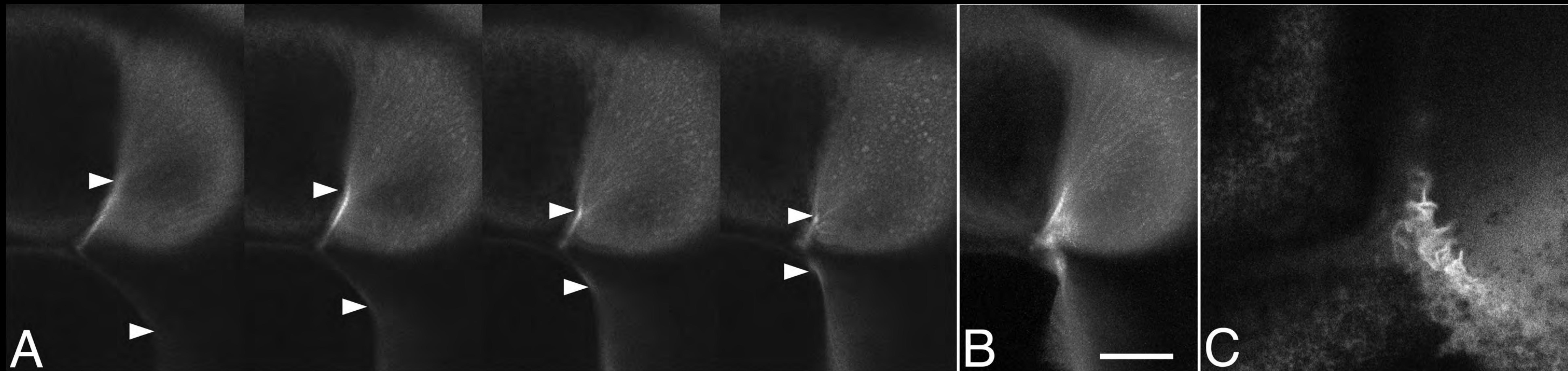


FM1-43
Movie 8

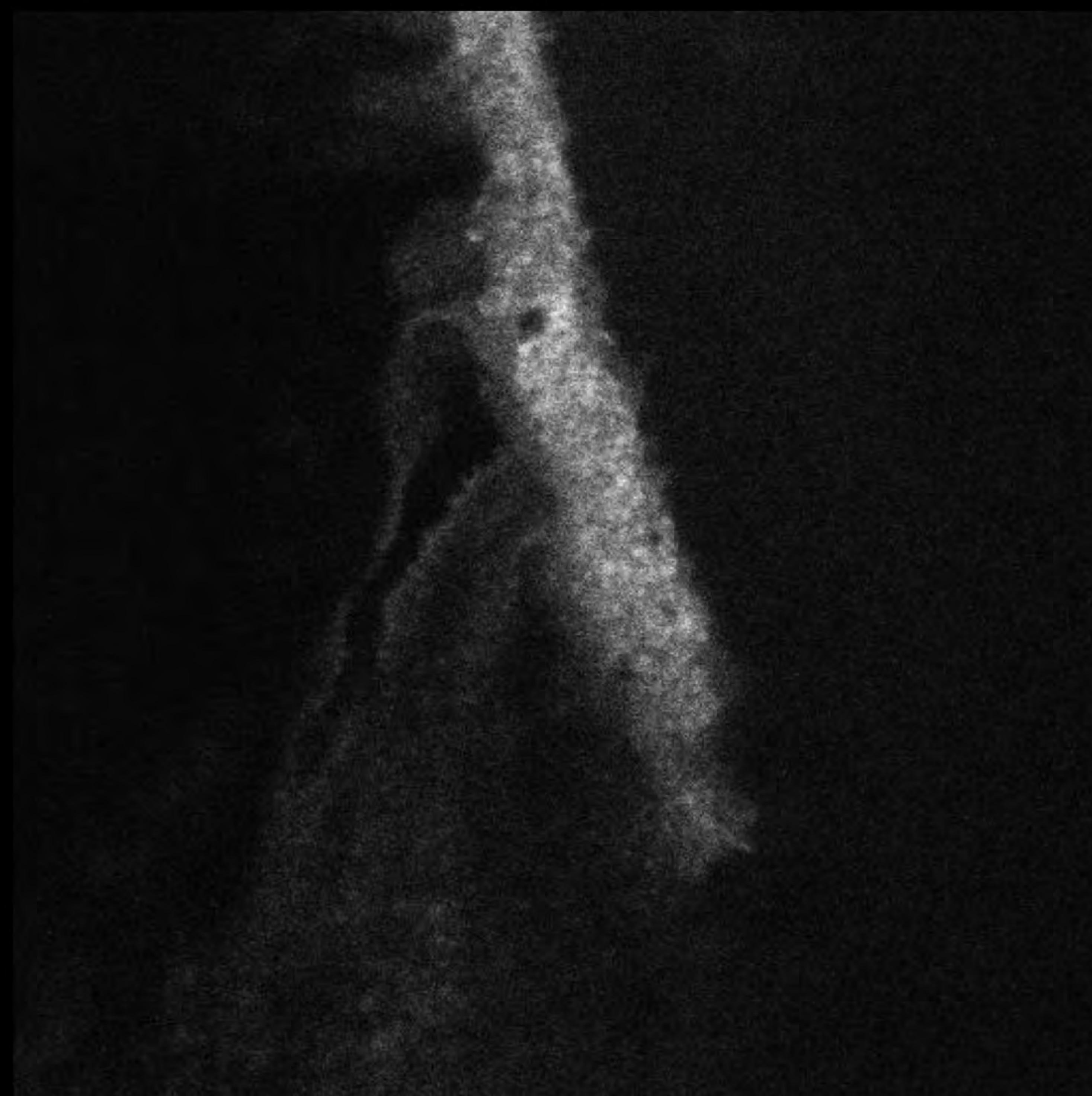
Marginal Protrusive Zone



Protrusions generate tension along marginal zone

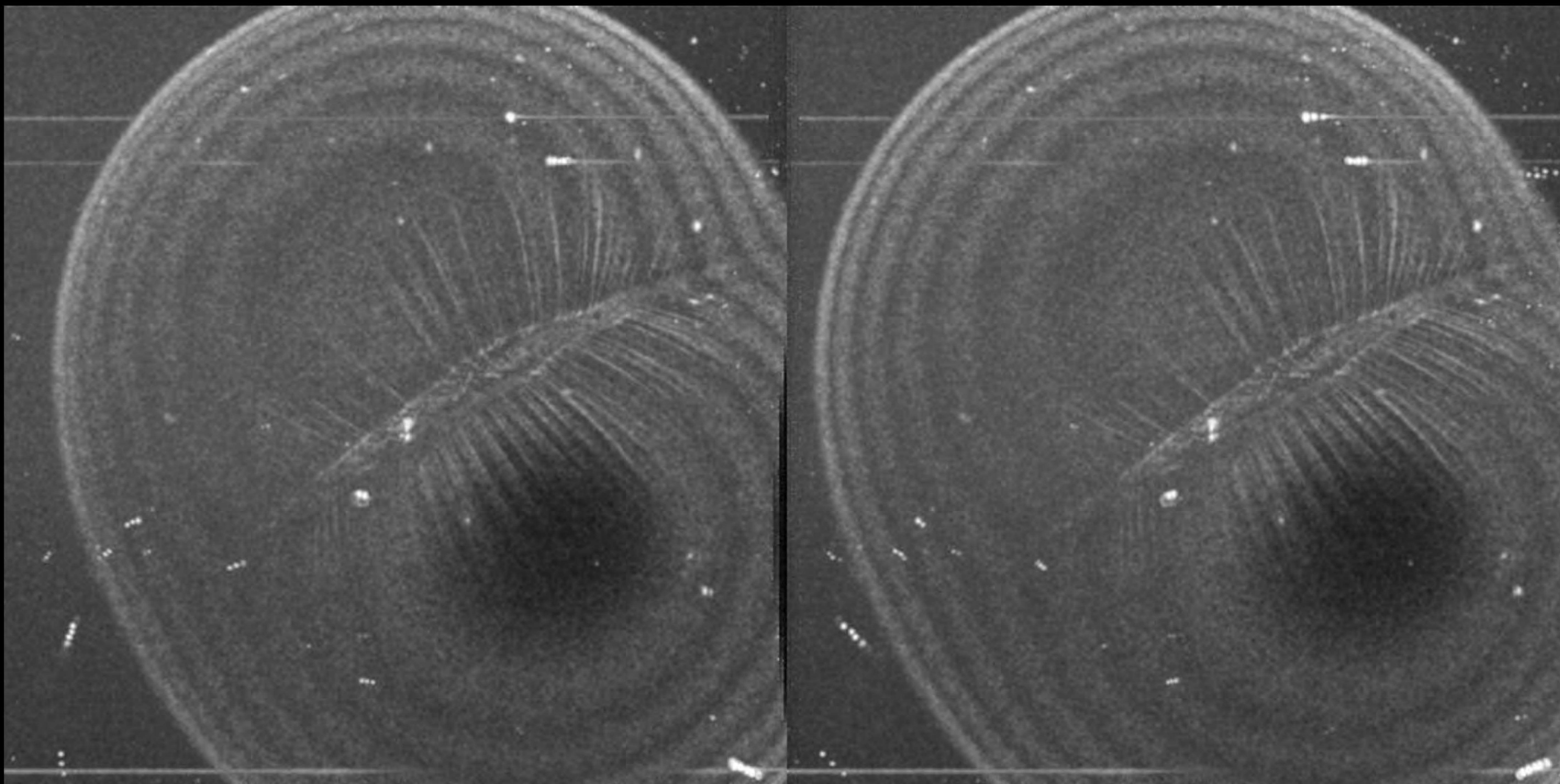


eGFP-rGBD



Movie 9

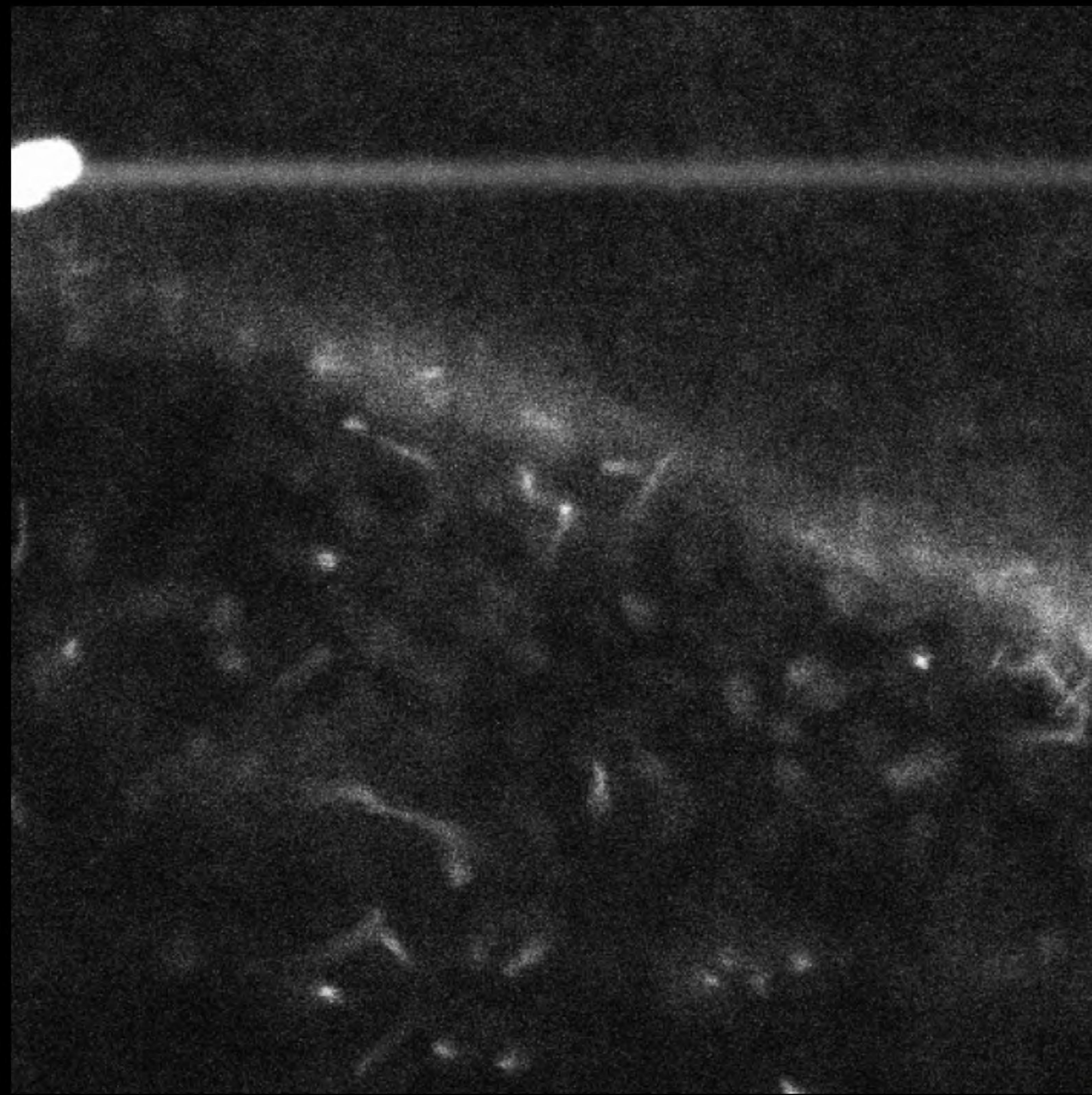
Basolateral Protrusive Zone



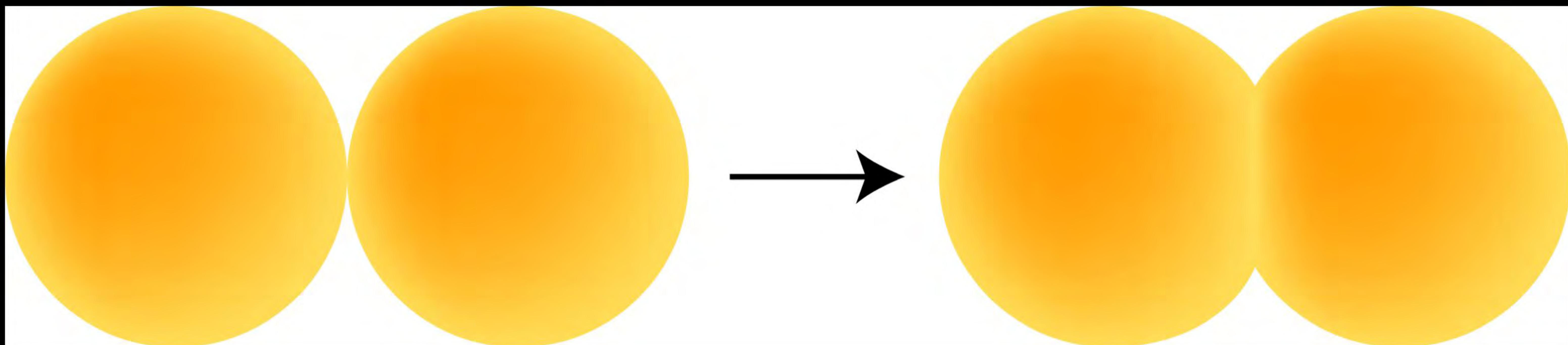
Movie 10

FM1-43

Basolateral Protrusive Zone

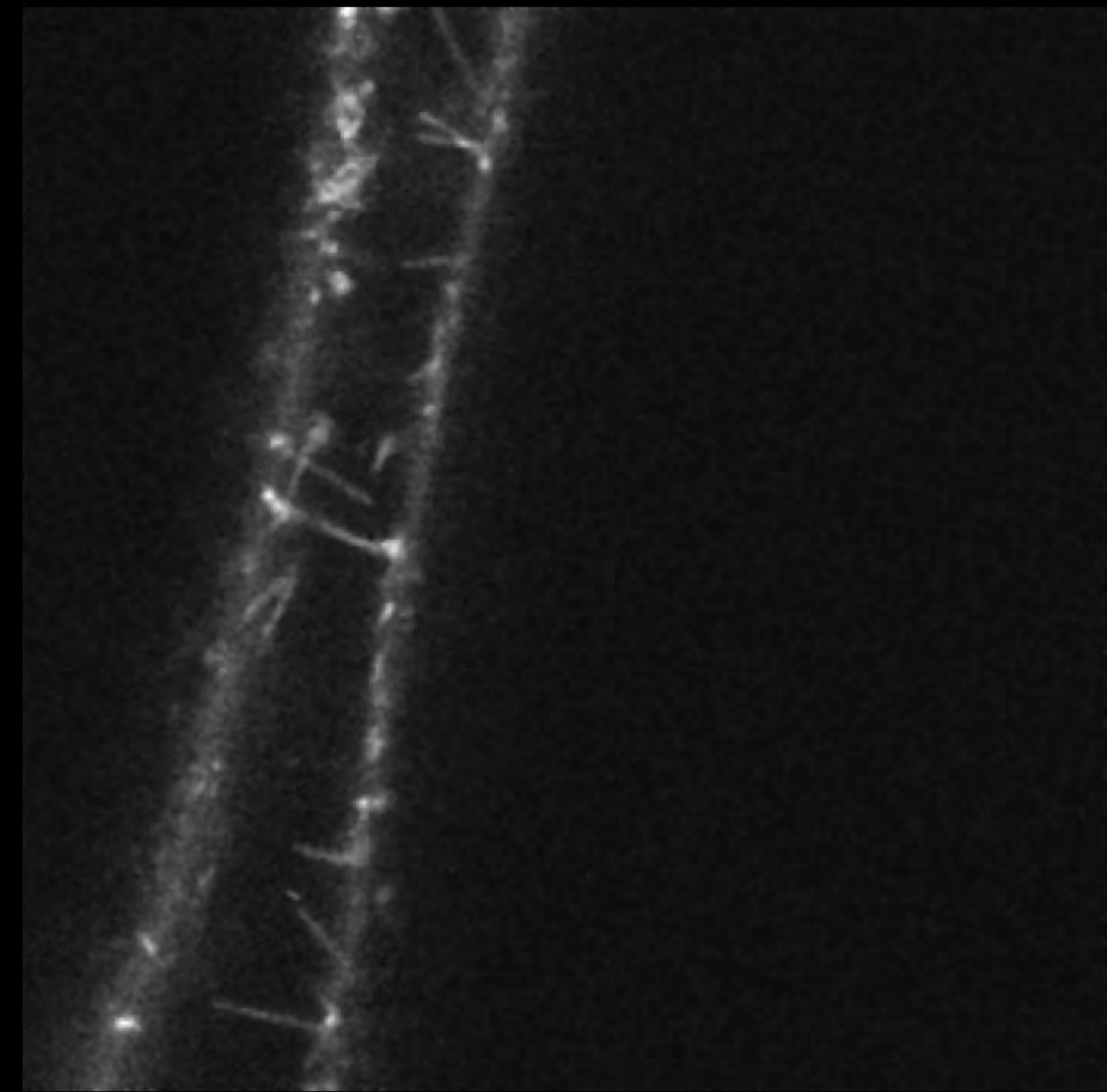
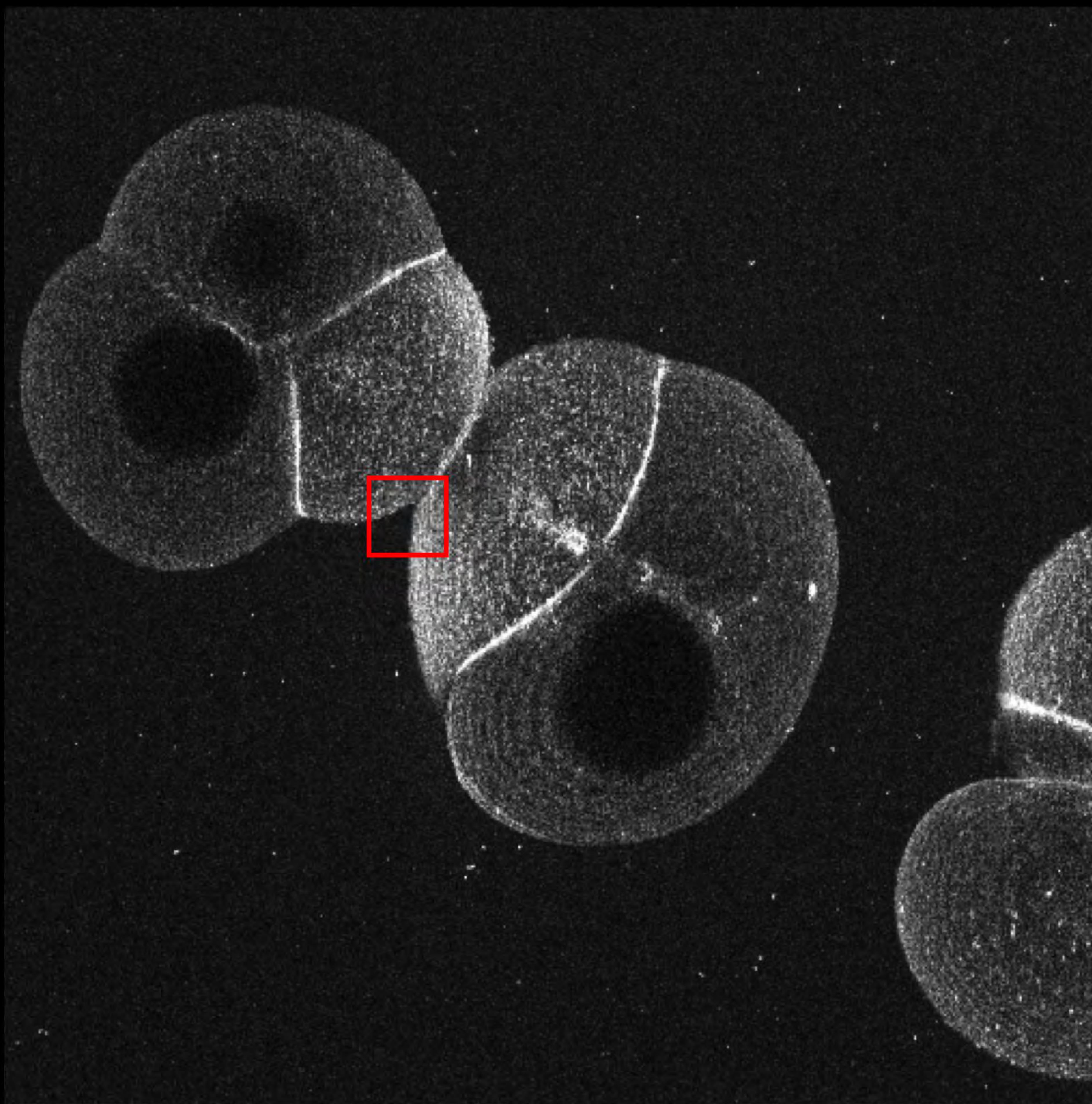


Movie 11



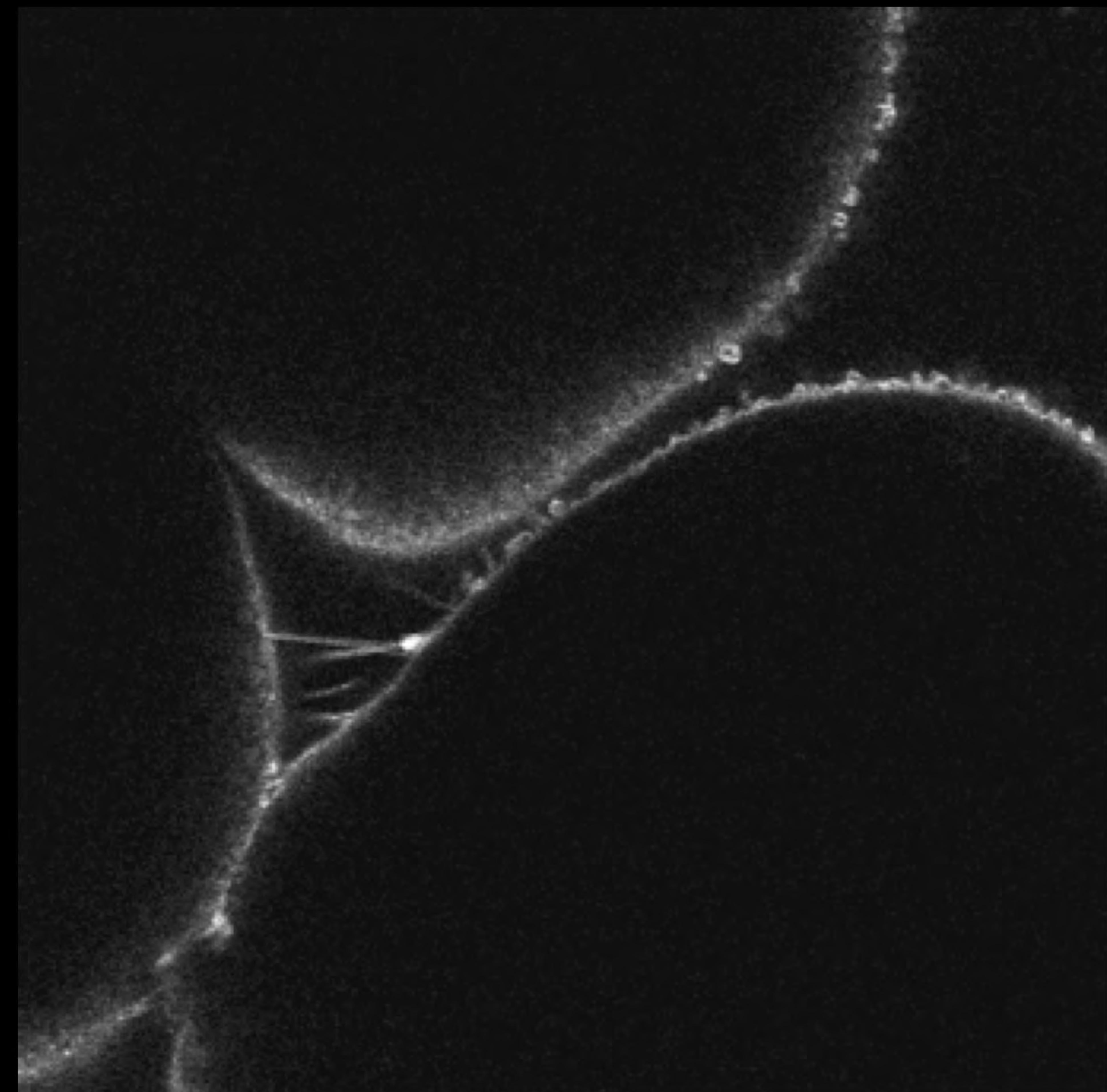
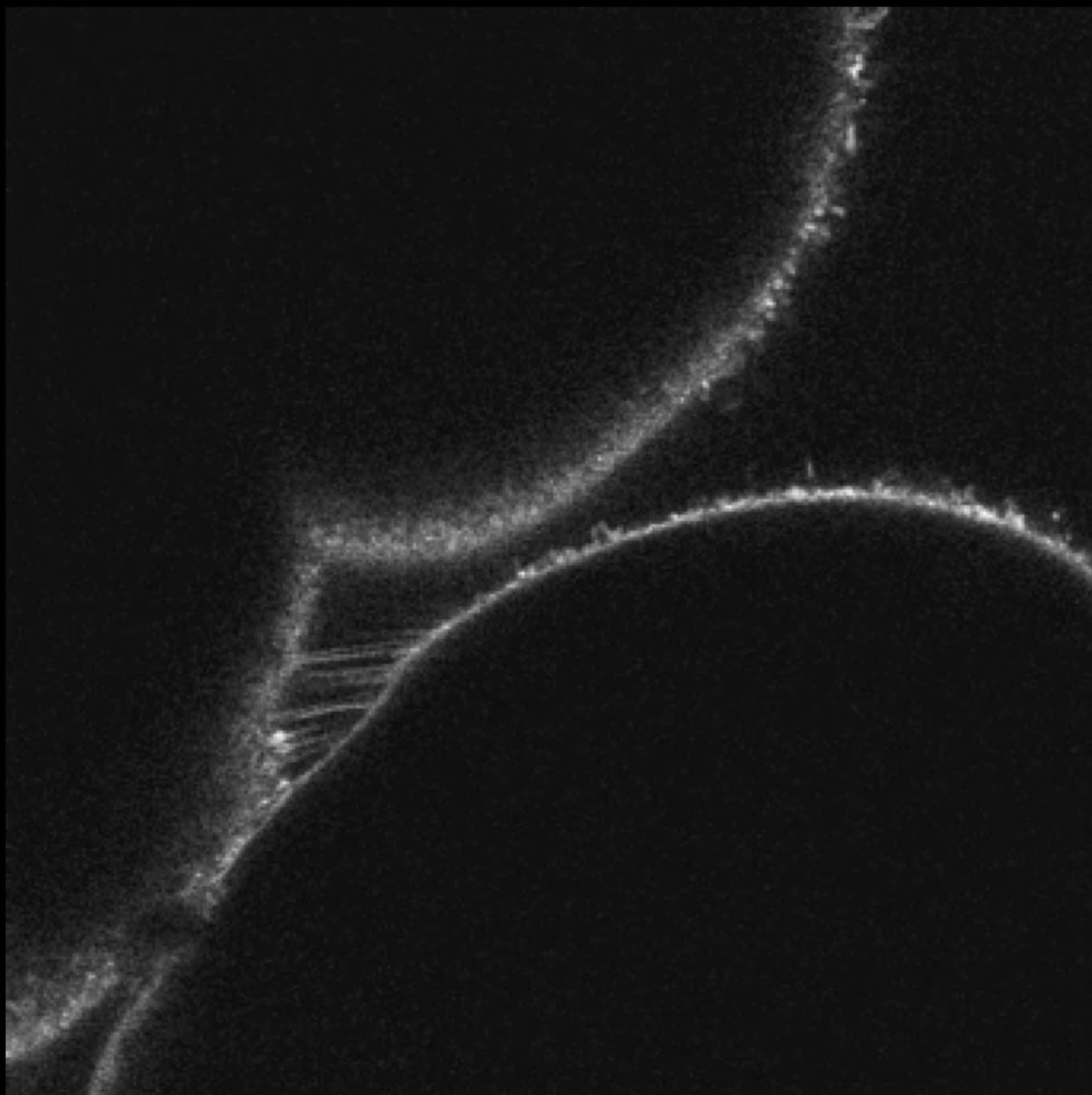


Movie 12
Recovery after low $[Ca^2]$ dissoc⁺



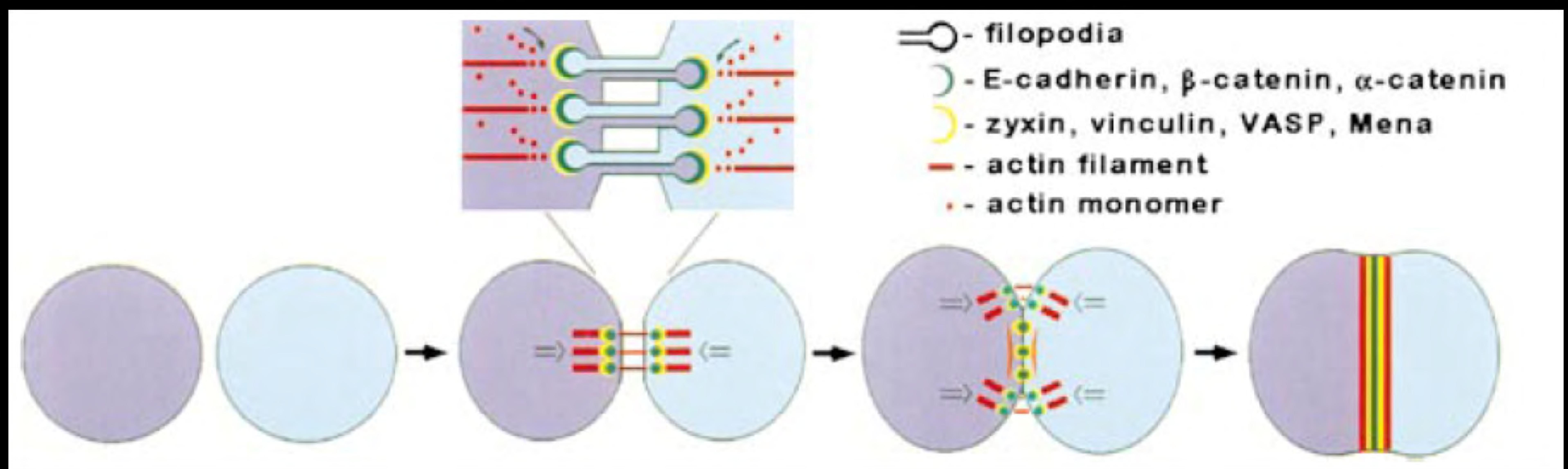
Movie 13

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

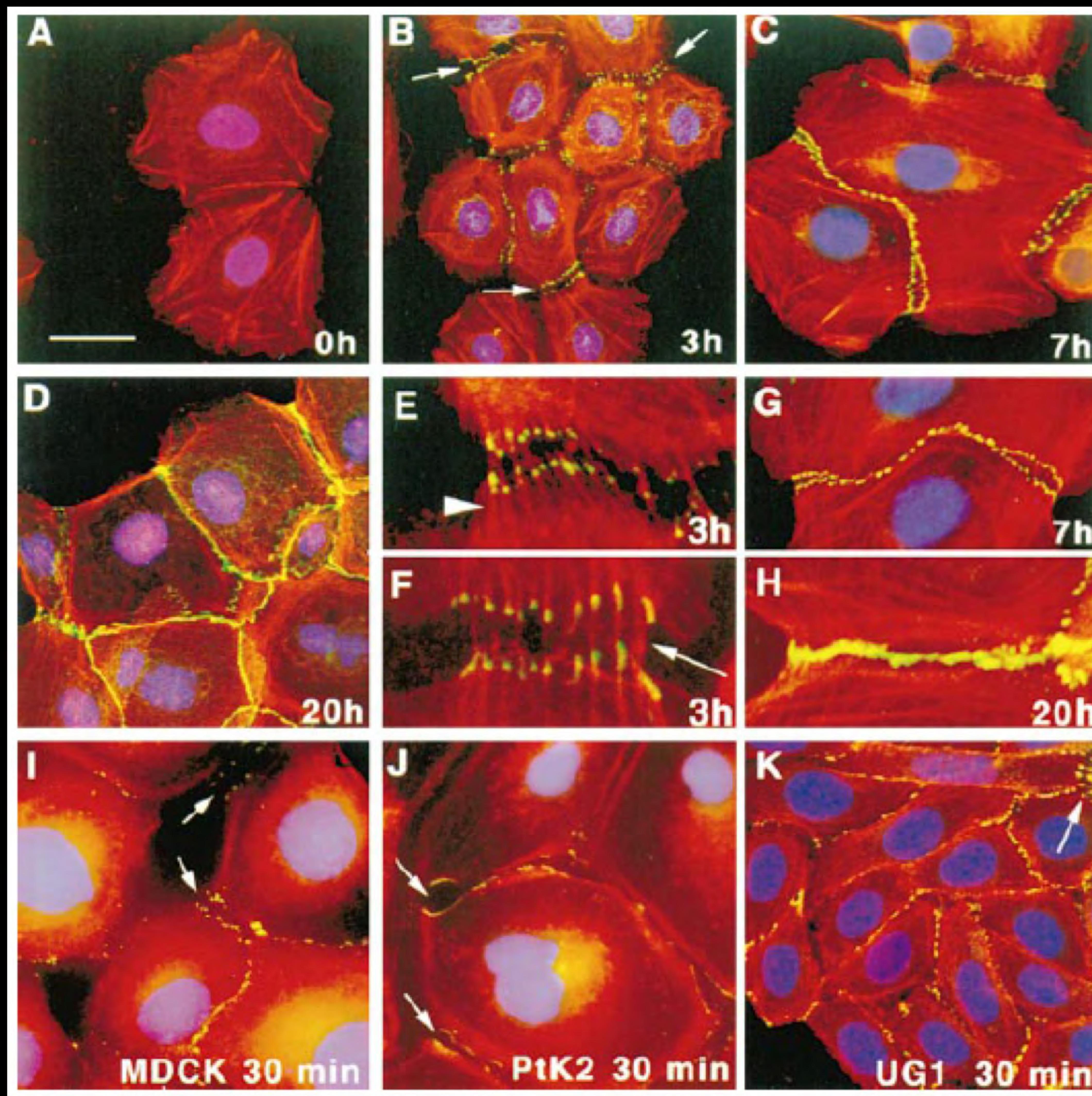


Movie 14

Recovery after low $[Ca^2]$ dissoci⁺



Vasioukhin et al., 2000



Marginal Protrusive Zone

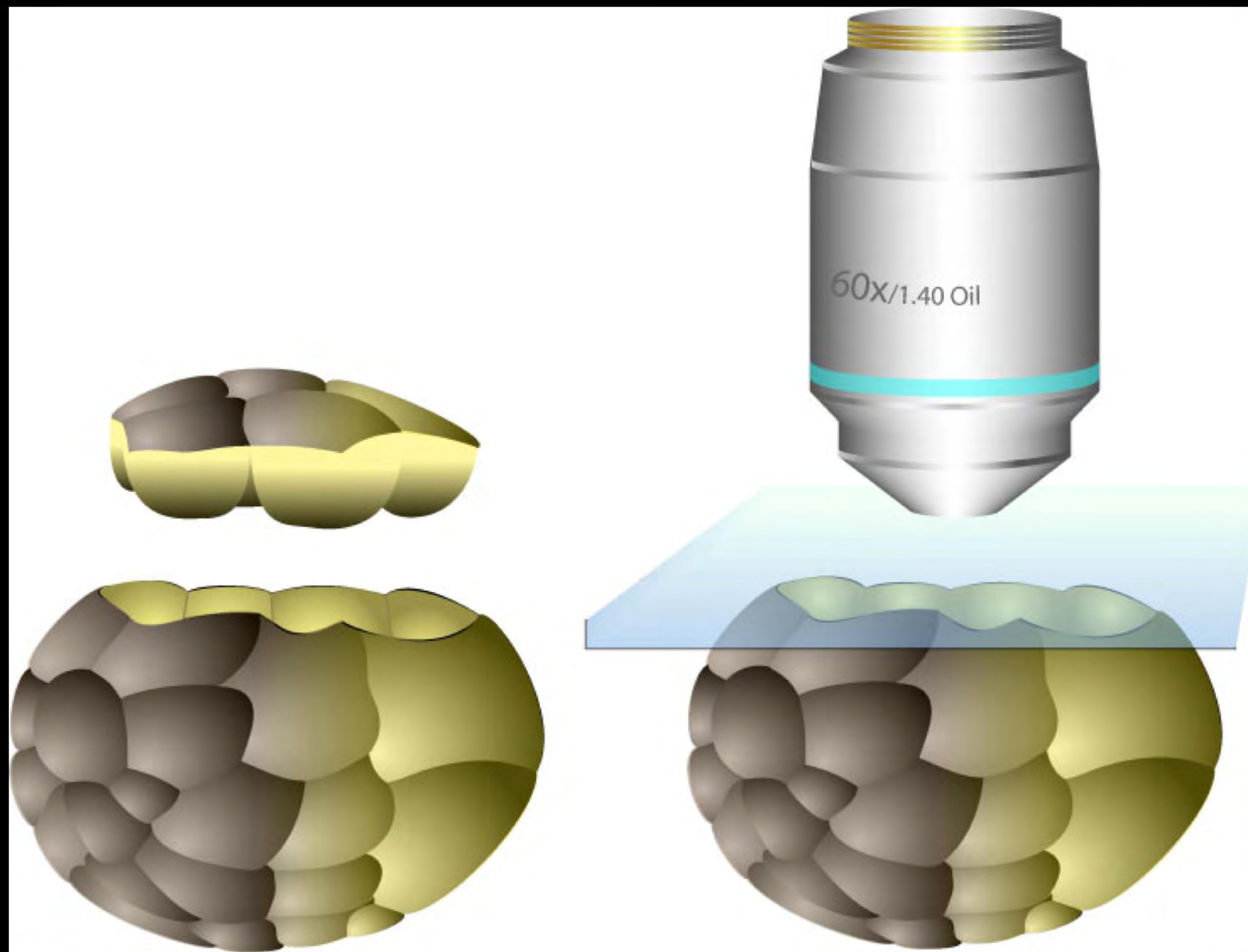
dynamic!

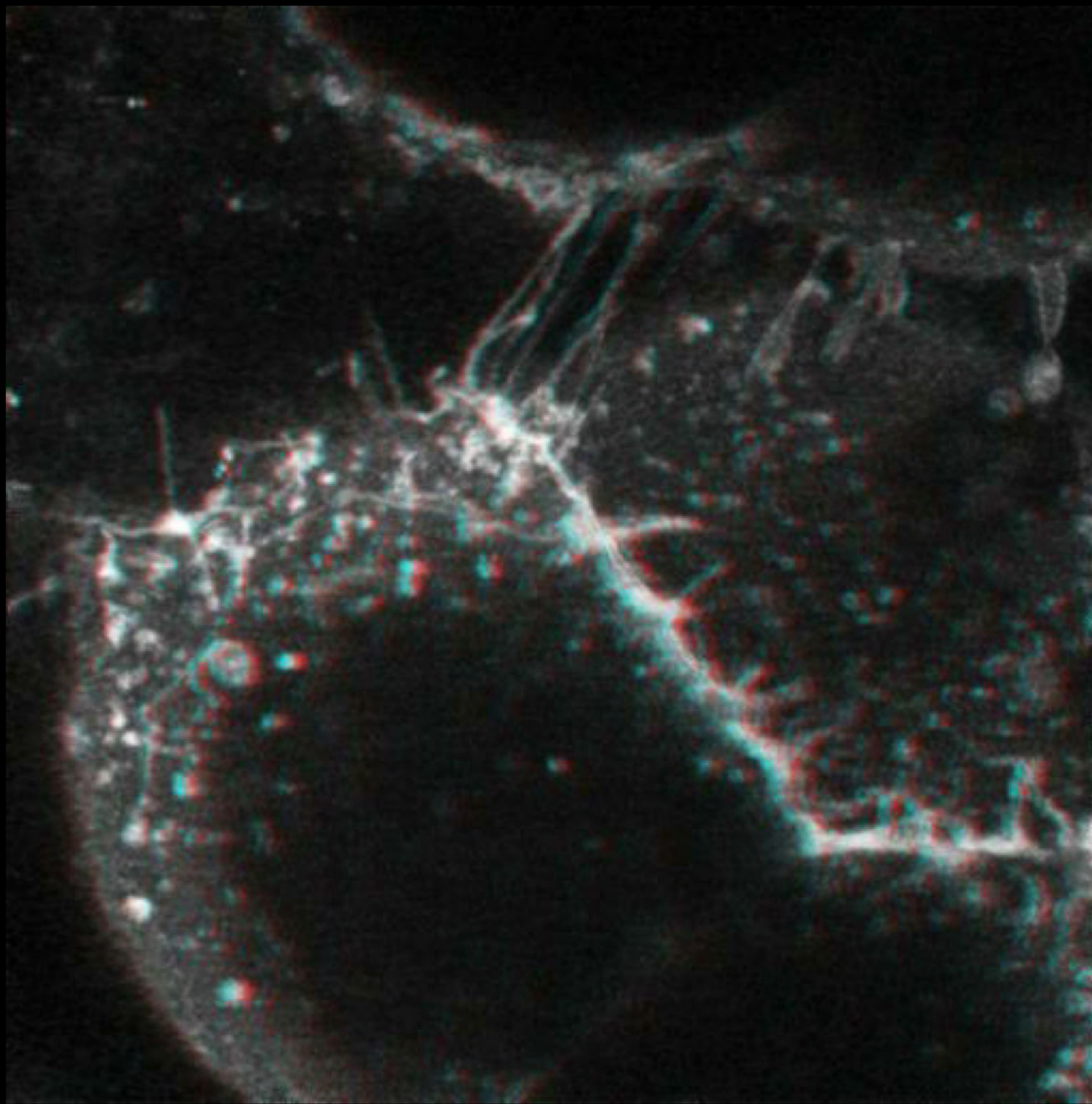
localized actin-based protrusive activity:
tension at apical/basolateral boundary
& at stress folds

Basolateral Protrusive Zone

dynamic!

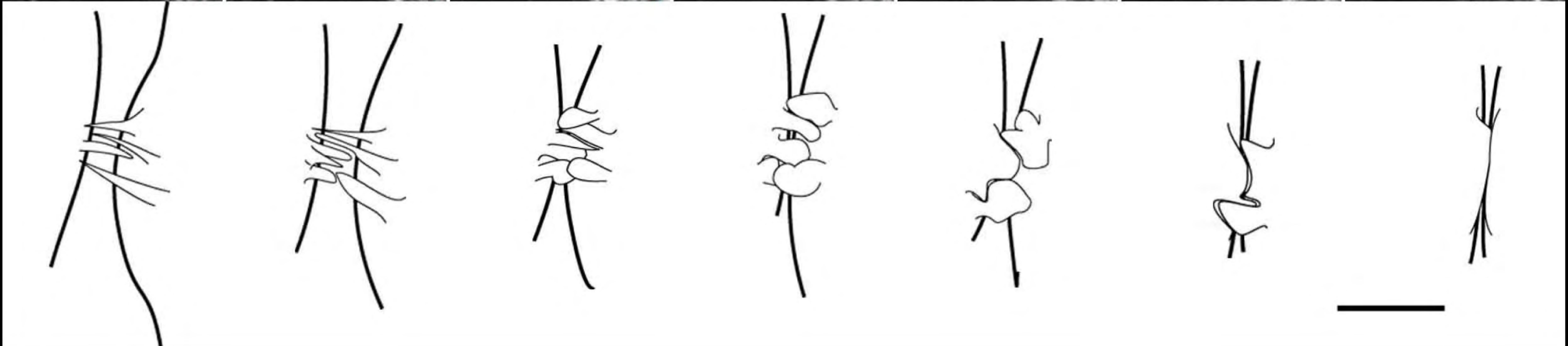
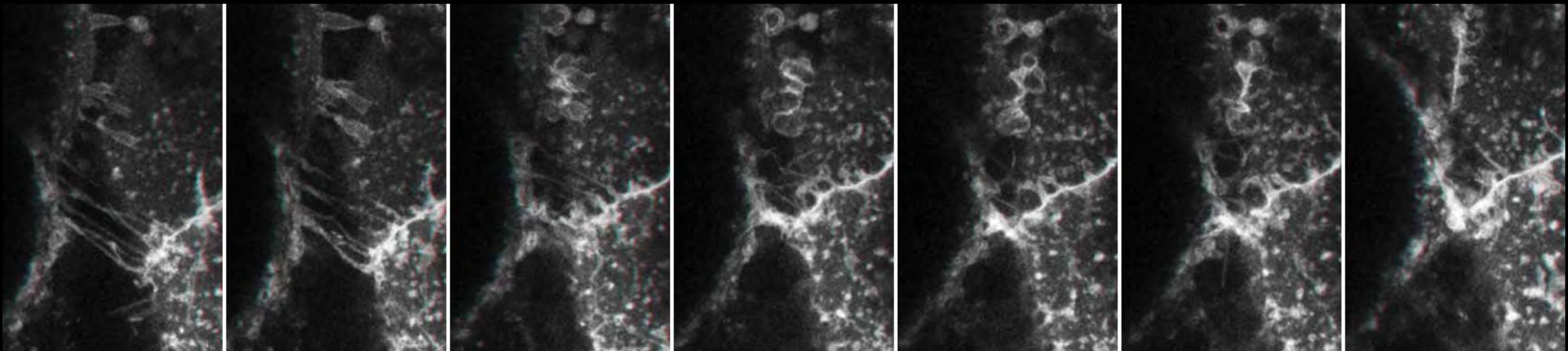
ad hoc reactive to local contact
highly adhesive



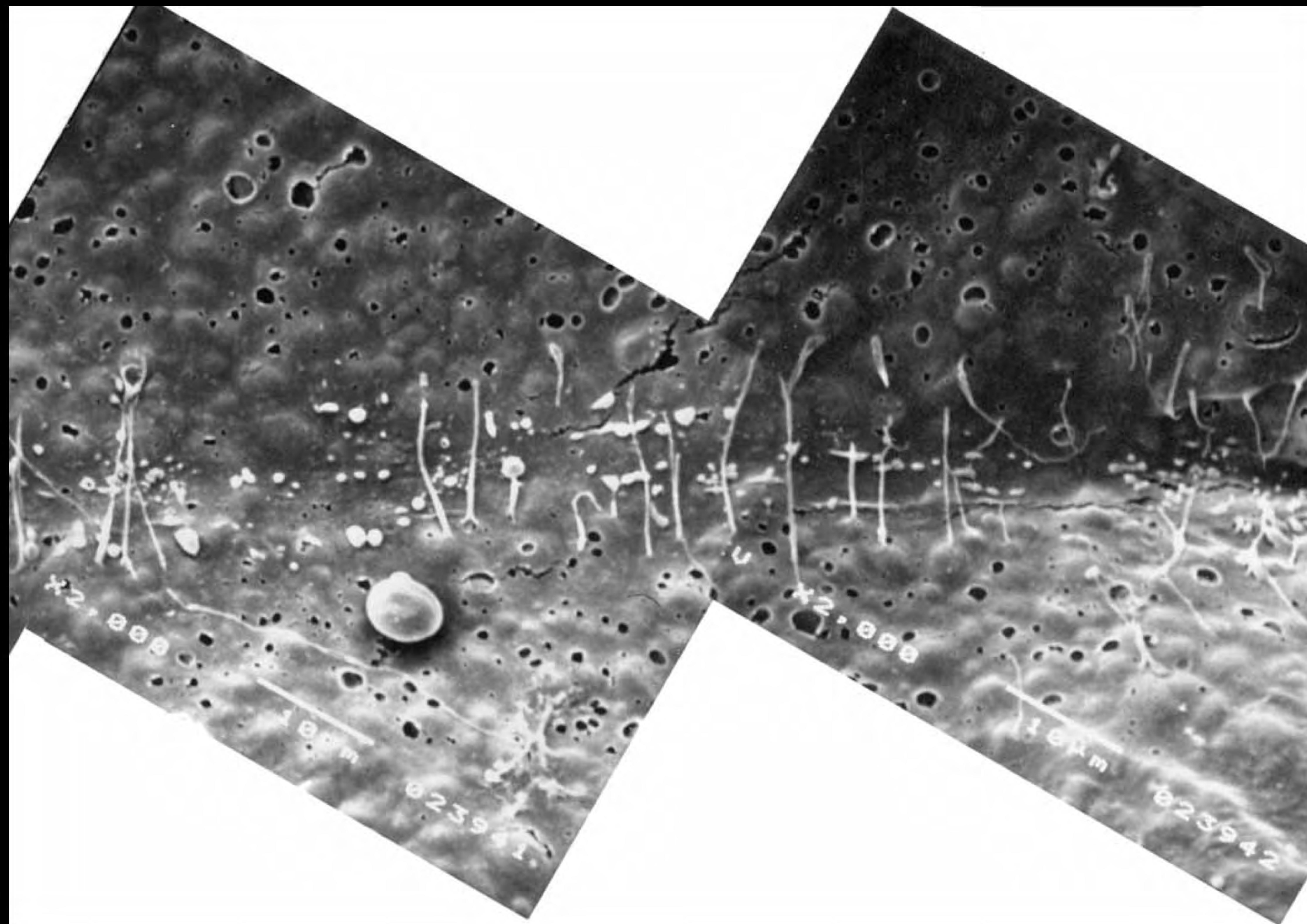


Movie 15

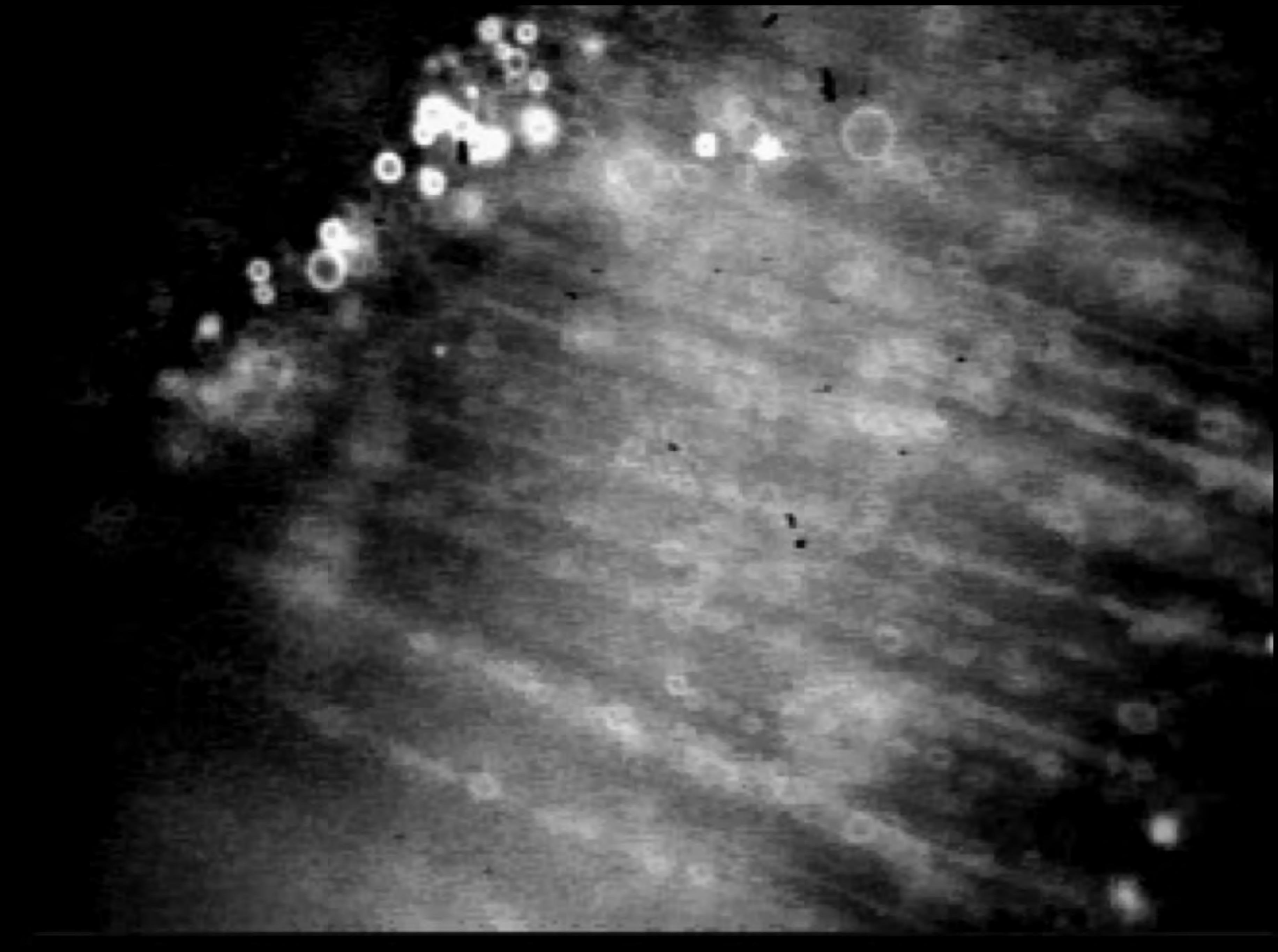
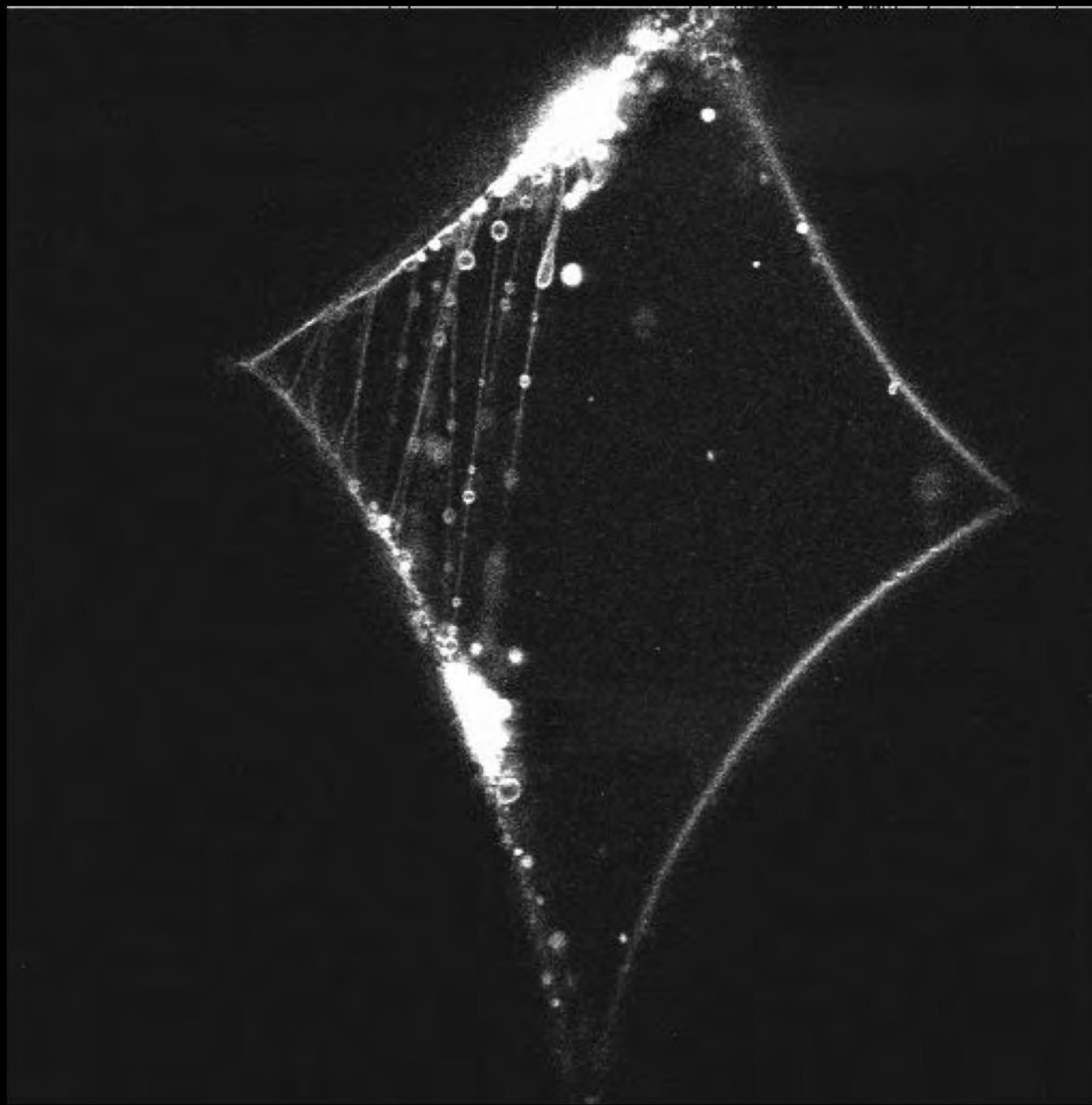
eGFP-mem
Blastocoel buffer
256-cell



Traversing filopodia

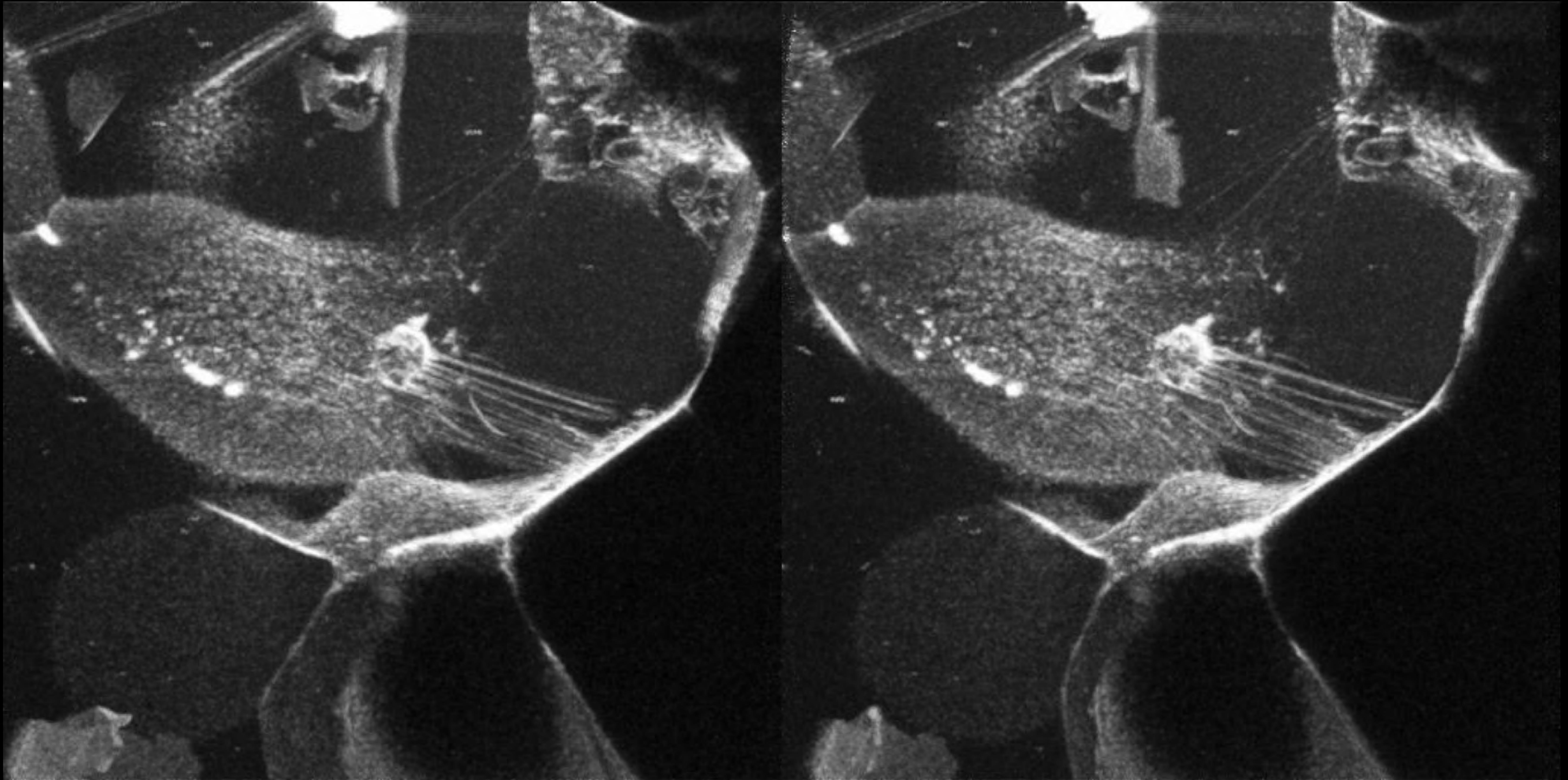


Traversing filopodia



Movie 16

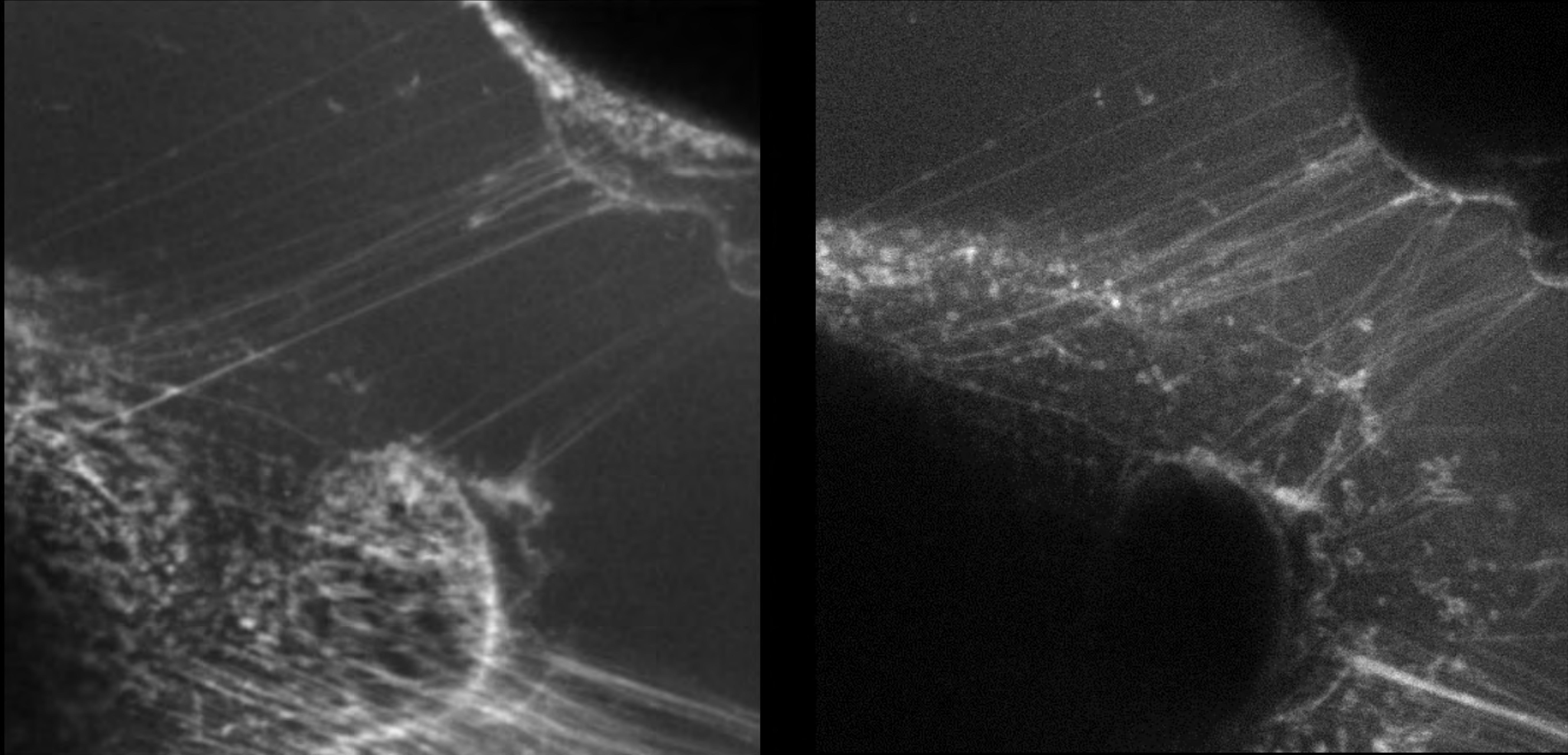
Traversing filopodia



Movie 17

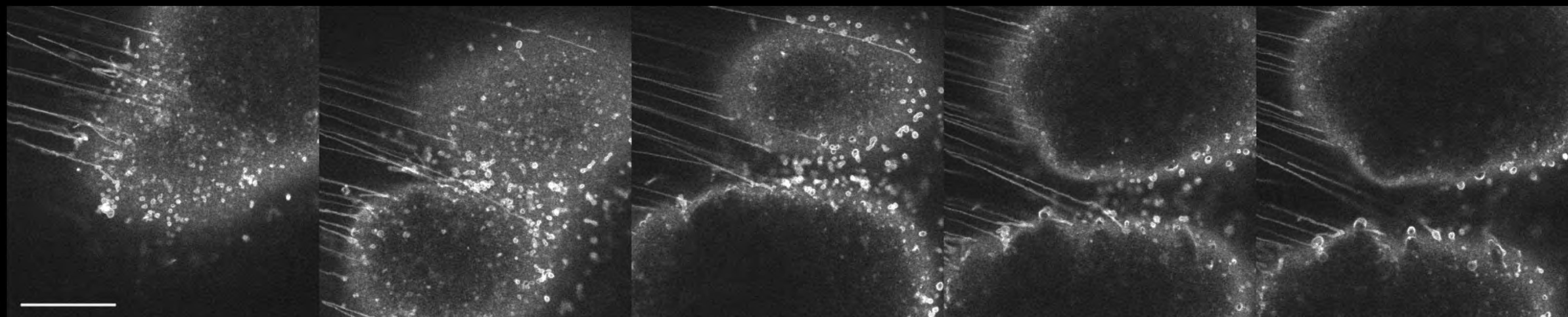
eGFP-mem
Blastocoel buffer
256-cell

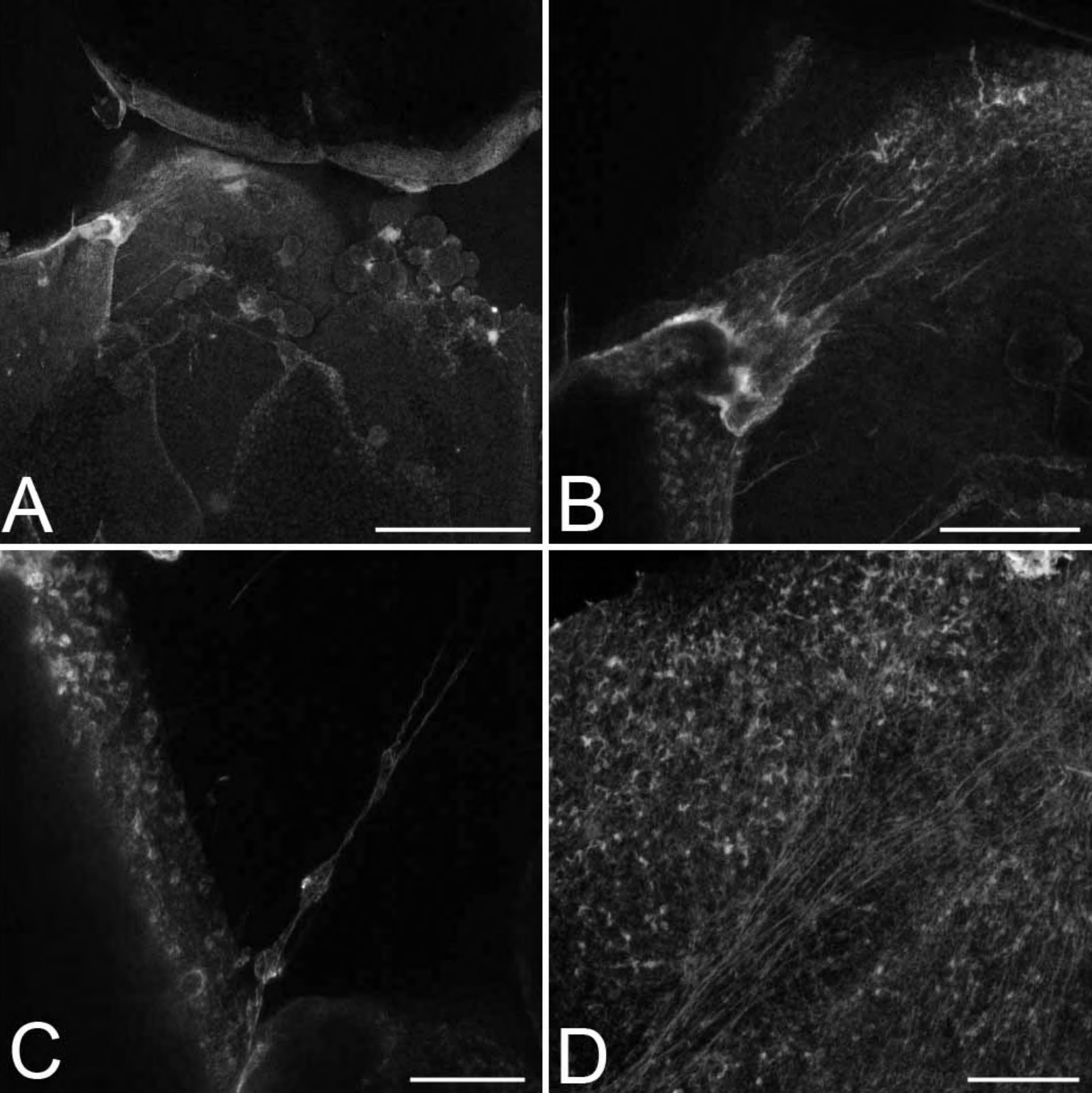
Traversing filopodia



eGFP-mem
Blastocoel buffer
256-cell

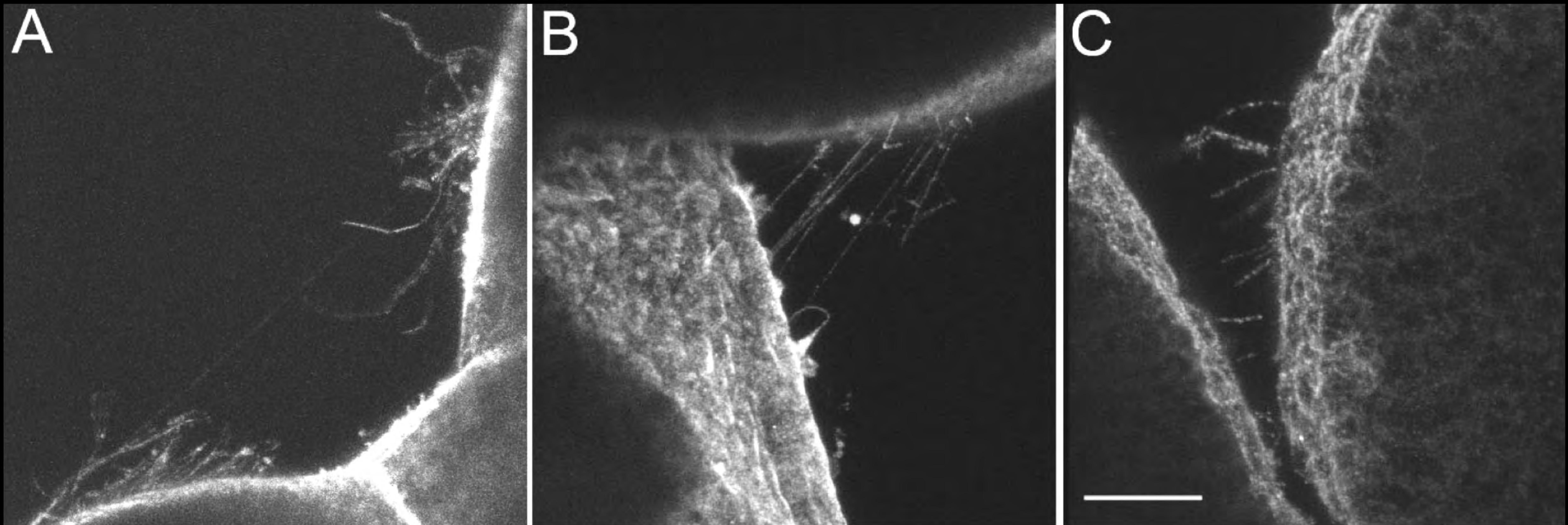
Long filopodia remain tautly connected
to nonadjacent cells during cleavage





Rh-phalloidin, 16-cell

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

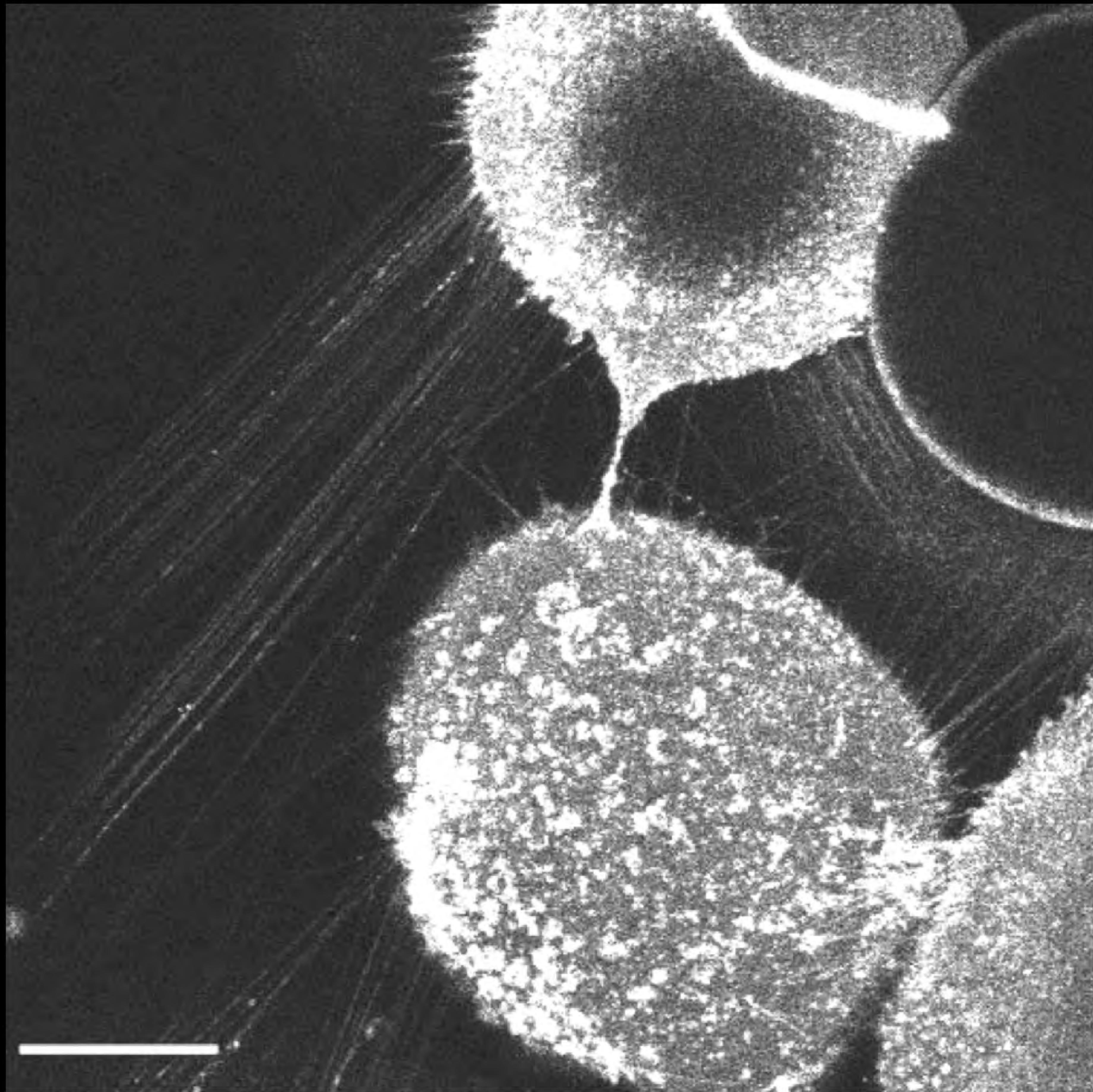


phalloidin

anti- β integrin

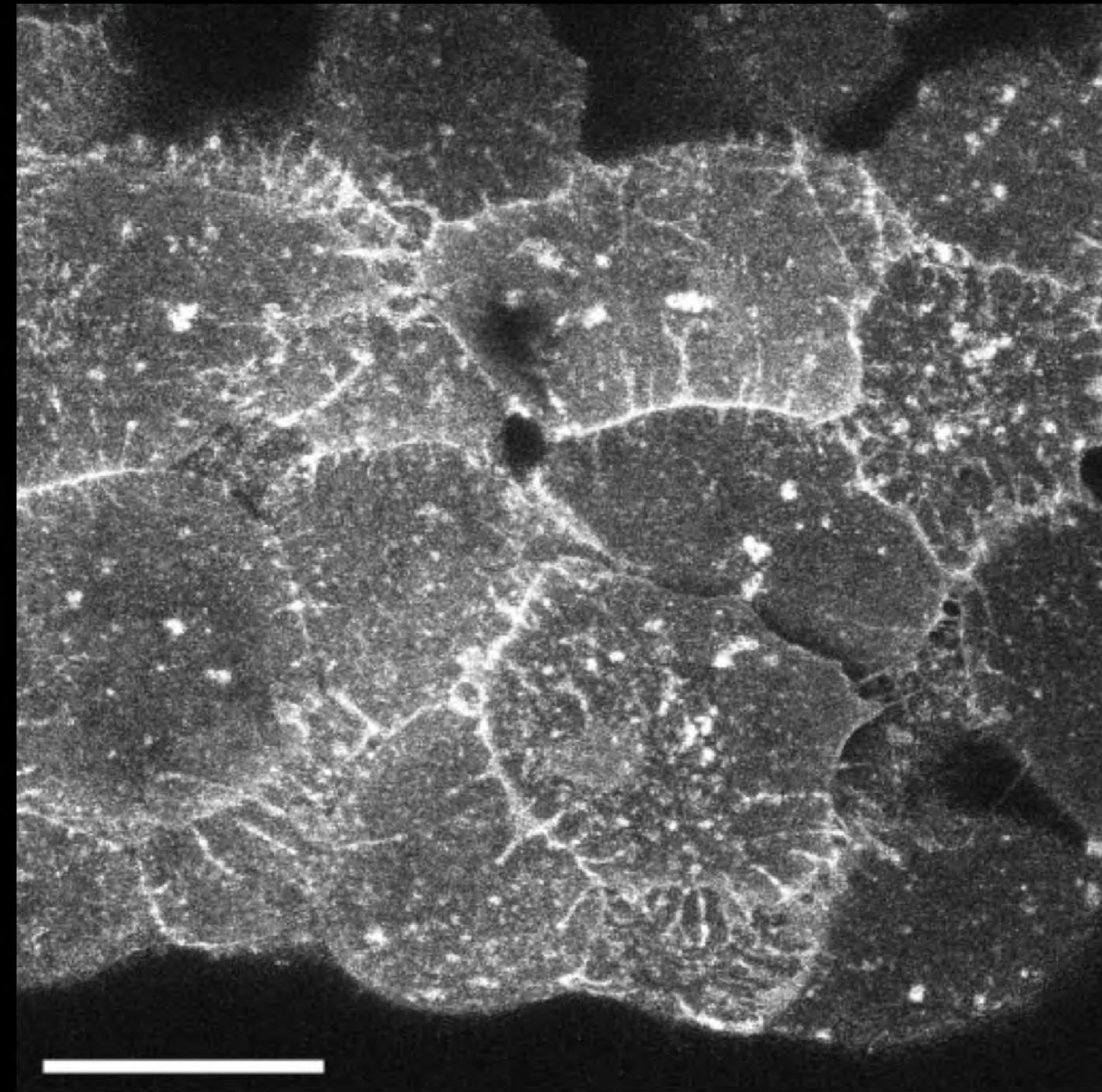
anti-serotonin

Traversing filopodia disappear after MBT



Bar: 100 µm.

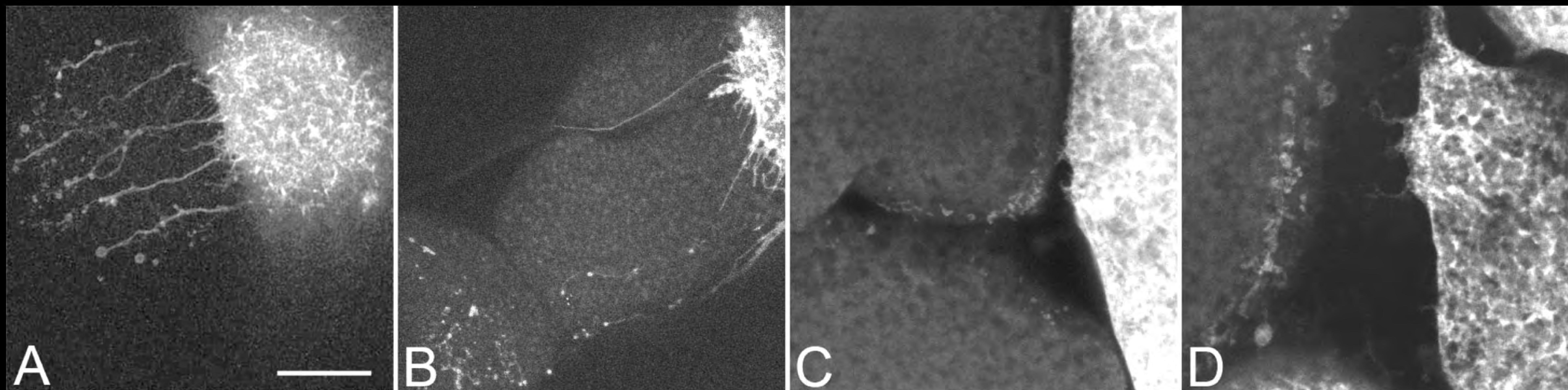
pre-MBT (64-cell)



Bar: 70 µm.

post-MBT (st. 7+)

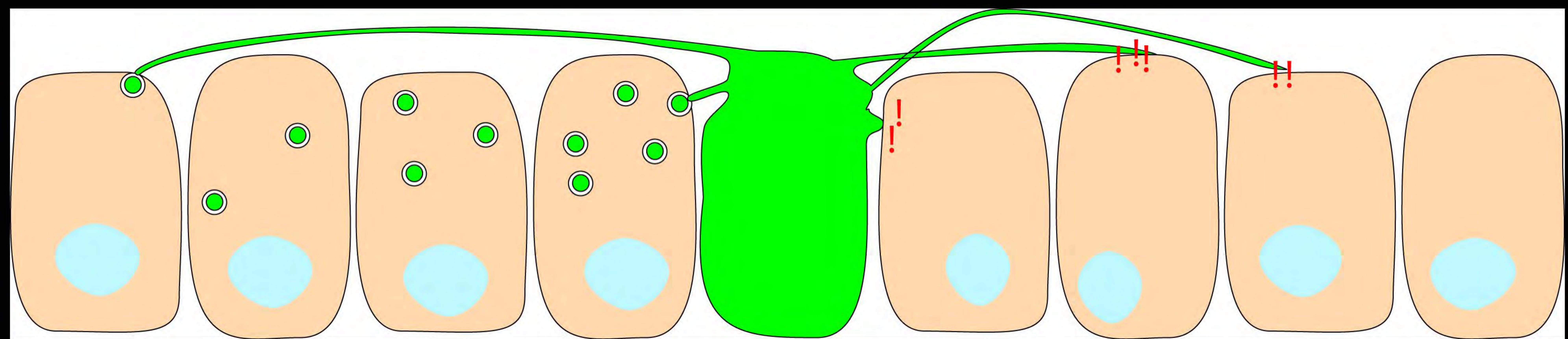
Long filopodia undergo vesiculation @ MBT



Bar: 24, 47, 35, and 17 μm 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)

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Bill Bement (U Wisconsin)

