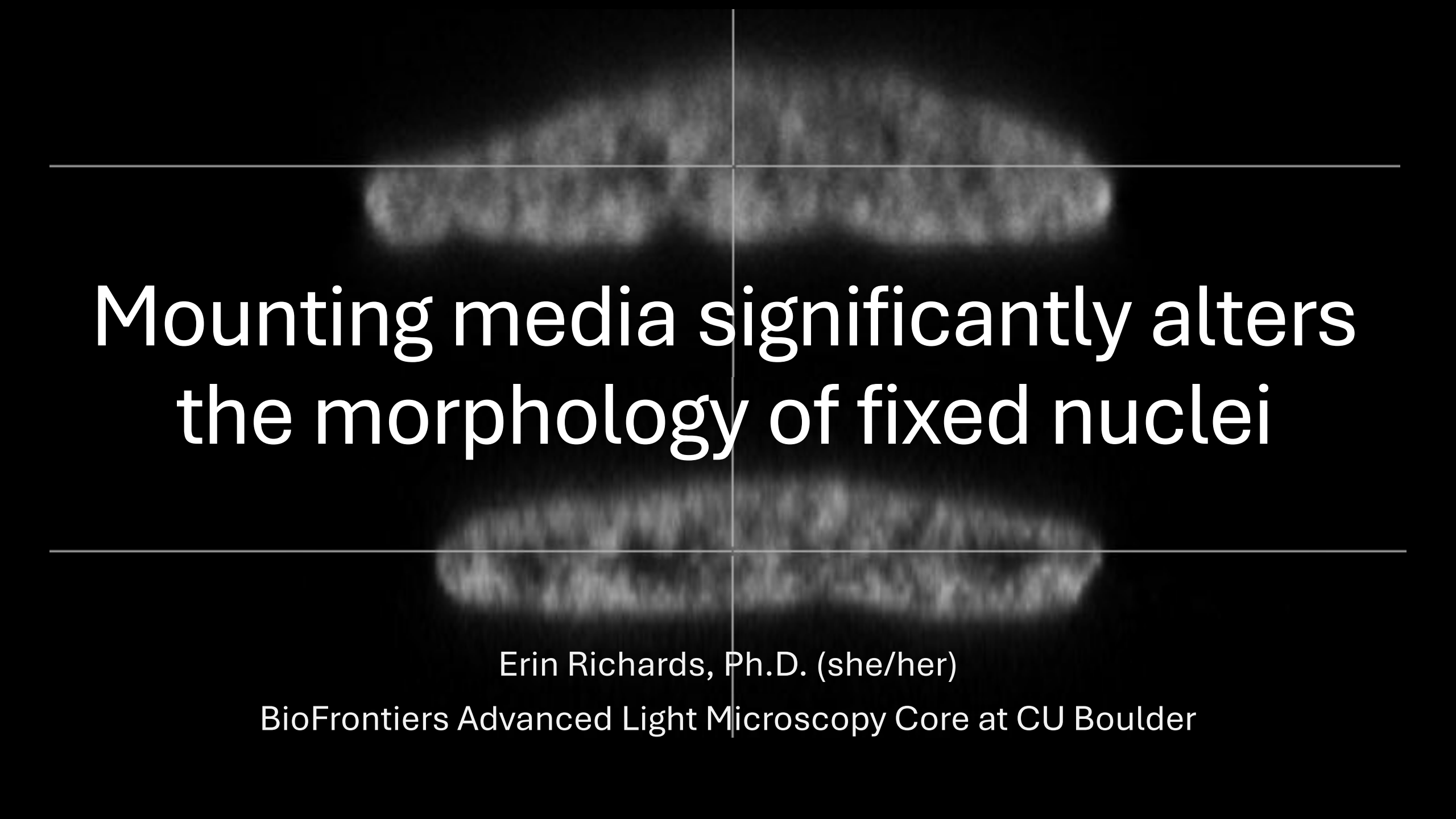


Mounting media significantly alters the morphology of fixed nuclei

Erin Richards, Ph.D. (she/her)

BioFrontiers Advanced Light Microscopy Core at CU Boulder

3D information and loss of signal. Glycerol-based or uncured mountants will preserve 3D information (critical for morphology and colocalization analysis) better than hardening mountants, which cause the sample to flatten. Mountants

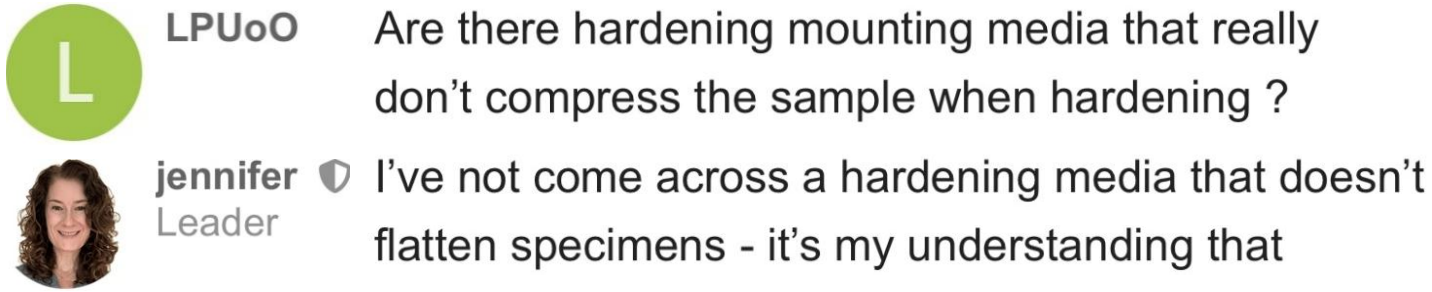
Jonkman *et al.* 2020 Nature Protocols

all dyes typically used for 3D-SIM (34). In contrast, hardening mounting media, such as Prolong Gold (Life technology) despite having good antifading properties, flatten the cells substantially and compromise nuclear morphology.

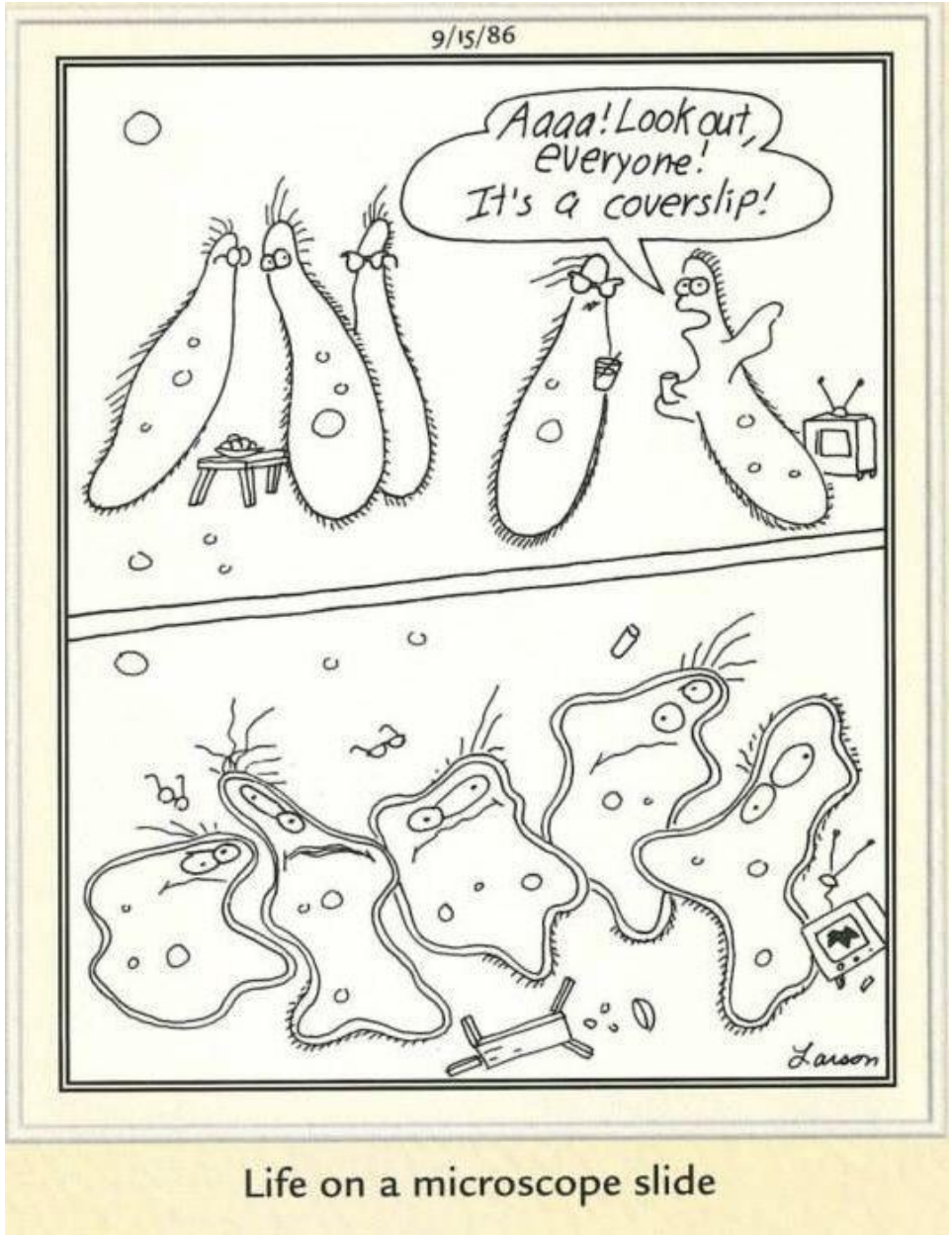
Markaki 2013 Methods in Molecular Biology

to immersion oil (~1.518). Some groups report shrinkage of the samples upon hardening.

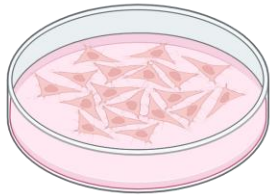
Wurm *et al.* 2010 Methods in Molecular Biology



Conversation on the forum *image.sc microlist* (2019)

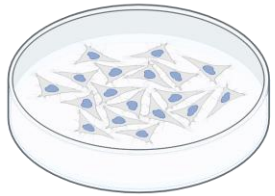


Gary Larson 1986 The Far Side



Culture cells.

Fix & stain.



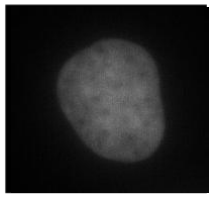
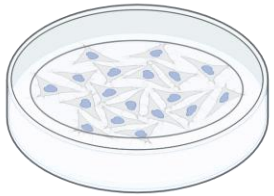


Image fixed nuclei in buffer.

Mount & cure.



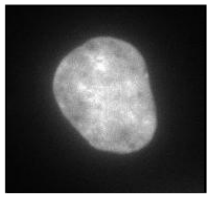
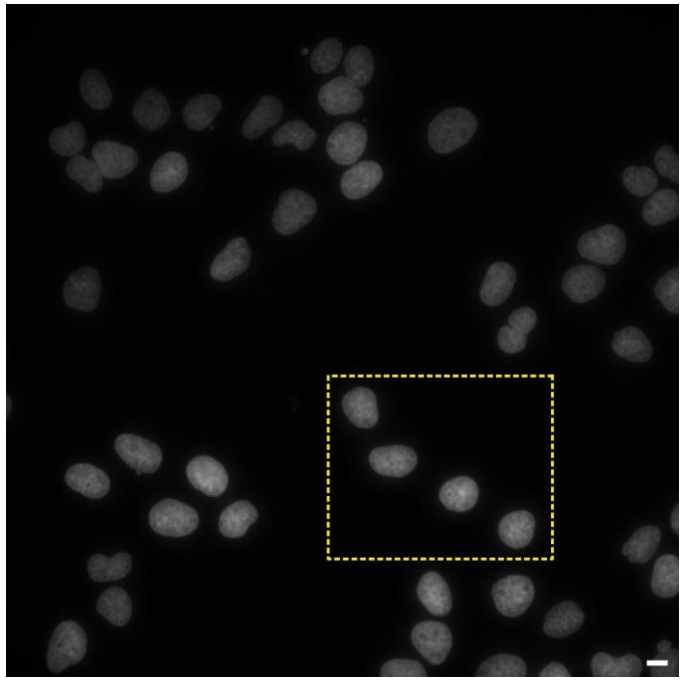
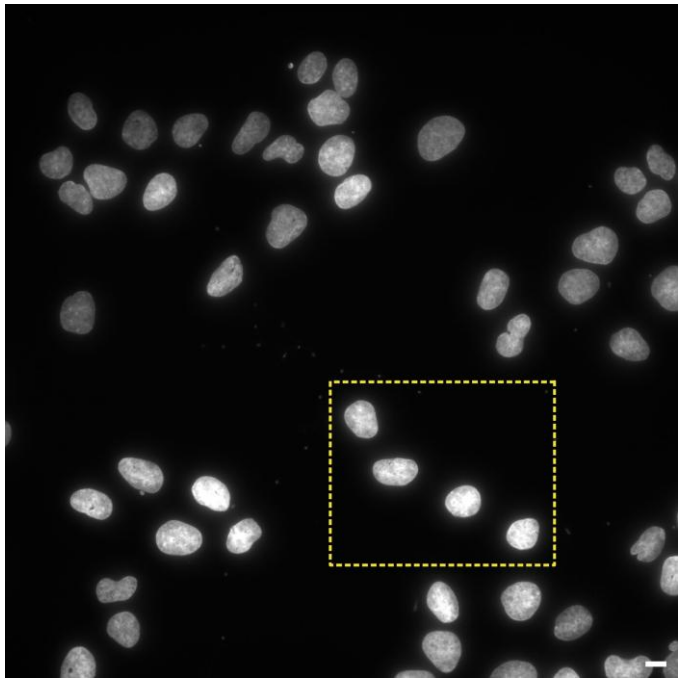


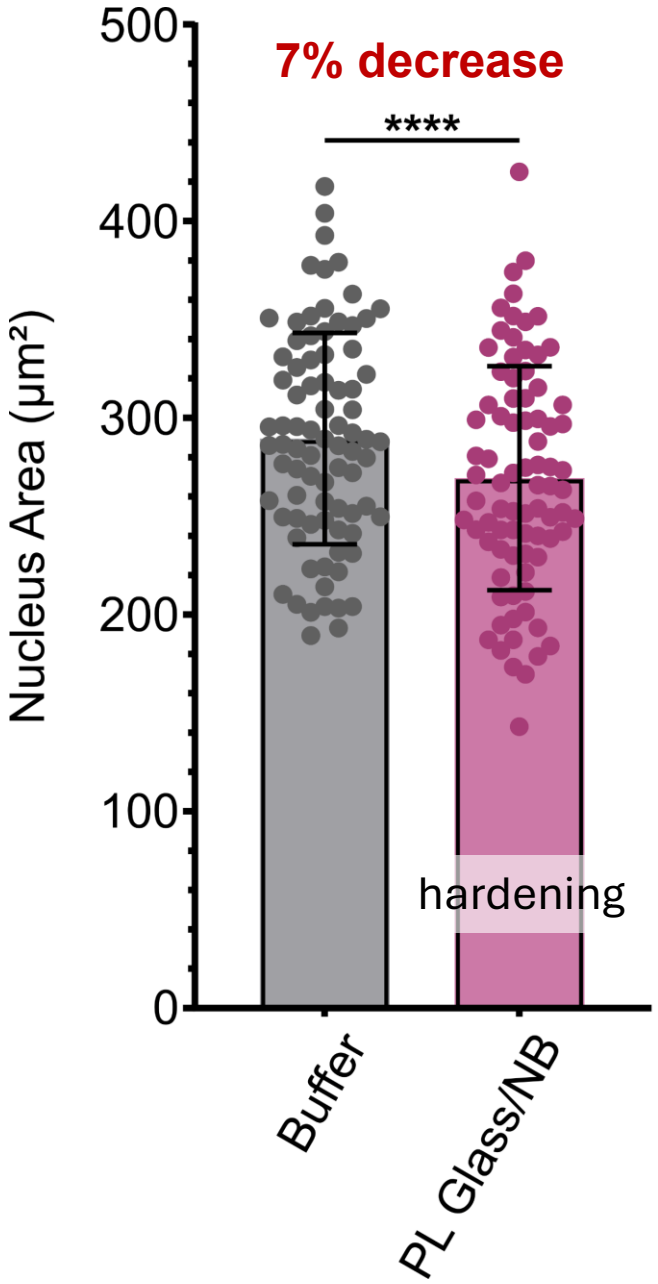
Image fixed and mounted nuclei in 2D.



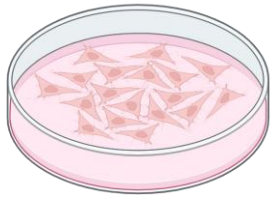
Buffer



PL Glass/NB (hardening)

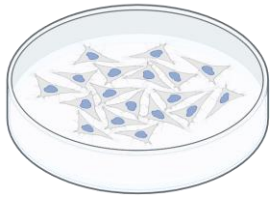


Images and image analysis courtesy of Megan Broe. Images acquired on a Nikon Ti-E Widefield with a 40X air NA 0.95 objective.
**** = $p < 0.0001$.



Culture cells.

Fix & stain.



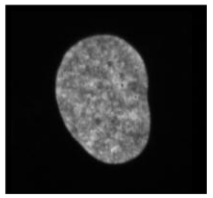
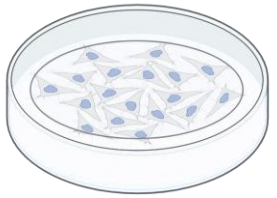


Image fixed nuclei in buffer.

Mount & cure.



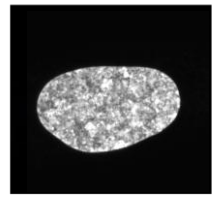
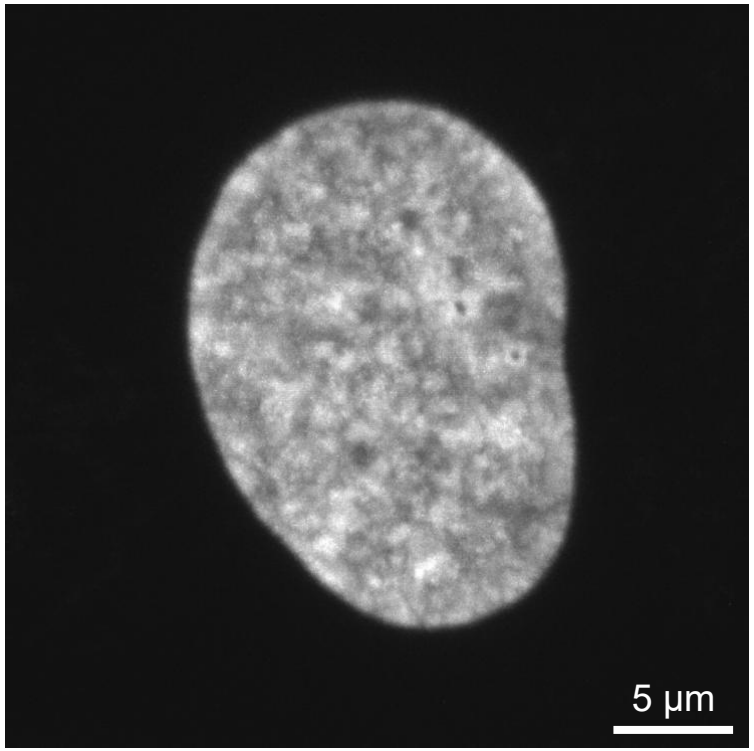
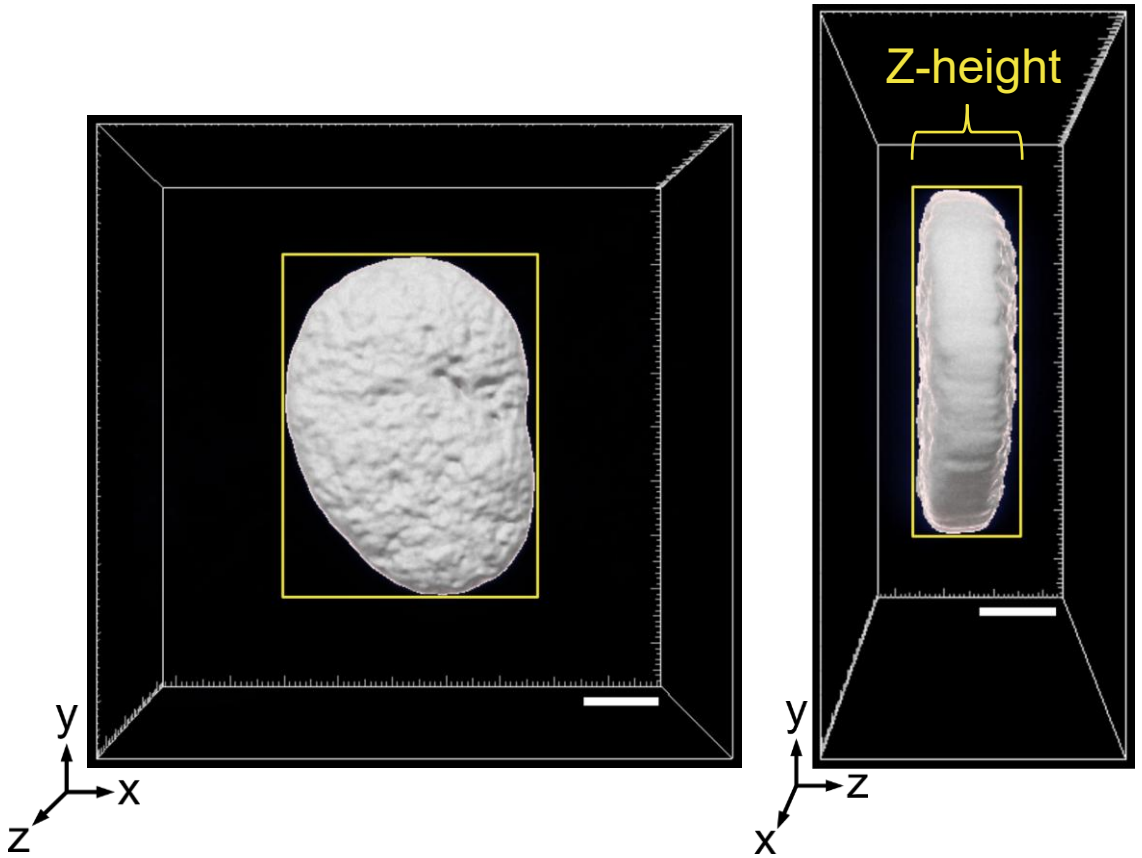
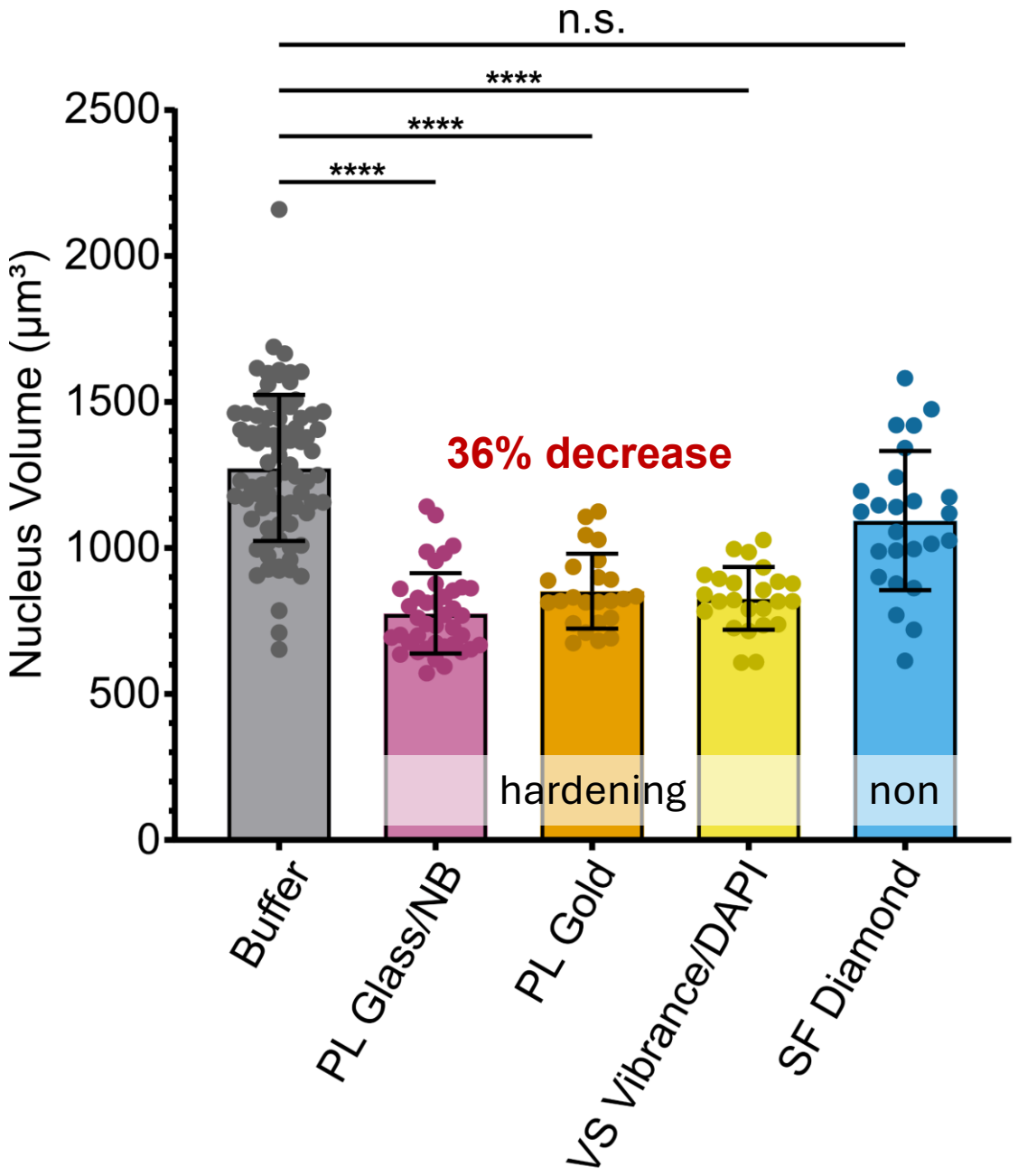


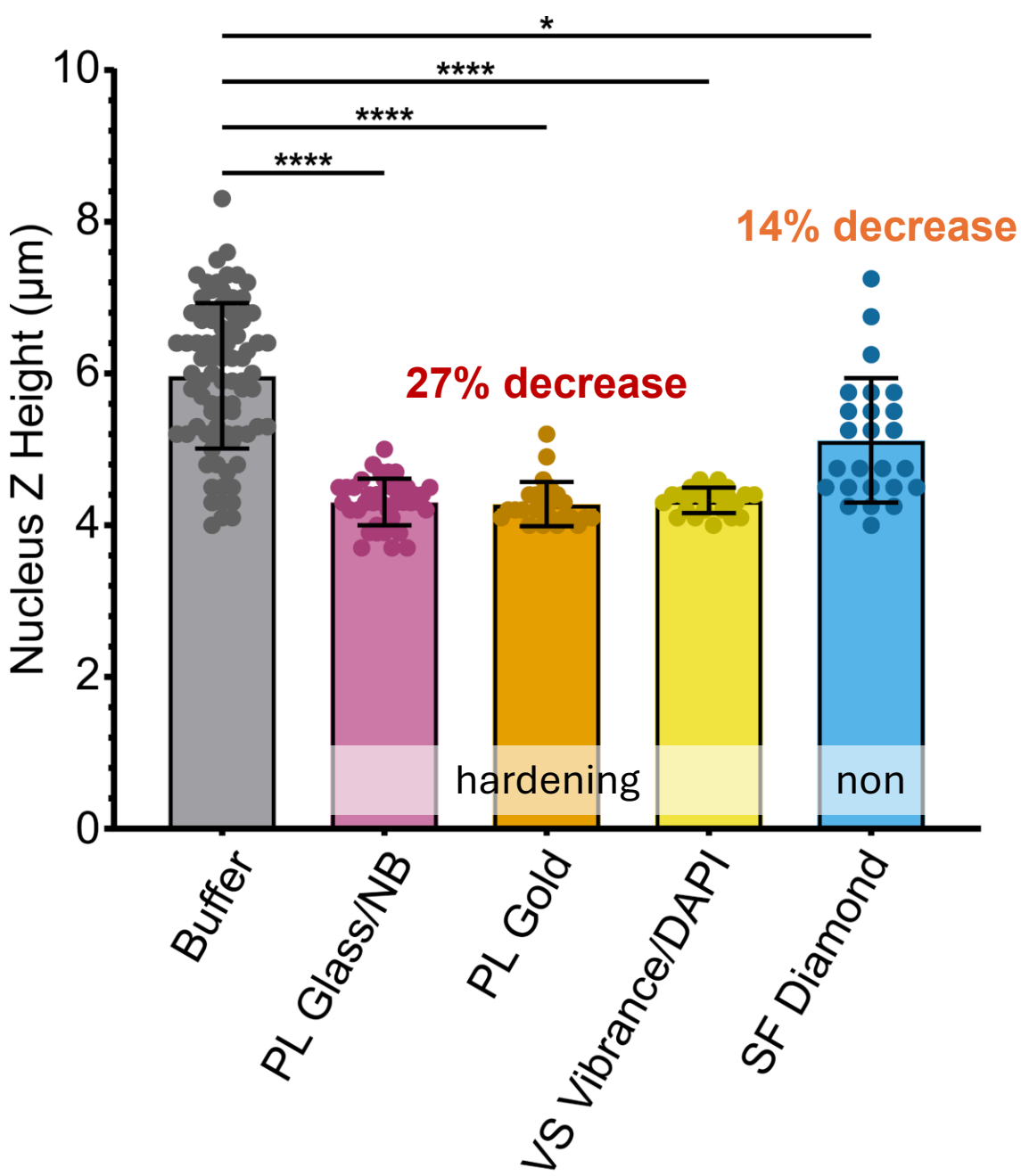
Image fixed and mounted nuclei in 3D.





Images courtesy of Megan Broe and Emi Bauer. Images acquired on a Nikon A1R laser scanning confocal with a 60X oil NA 1.40 objective.



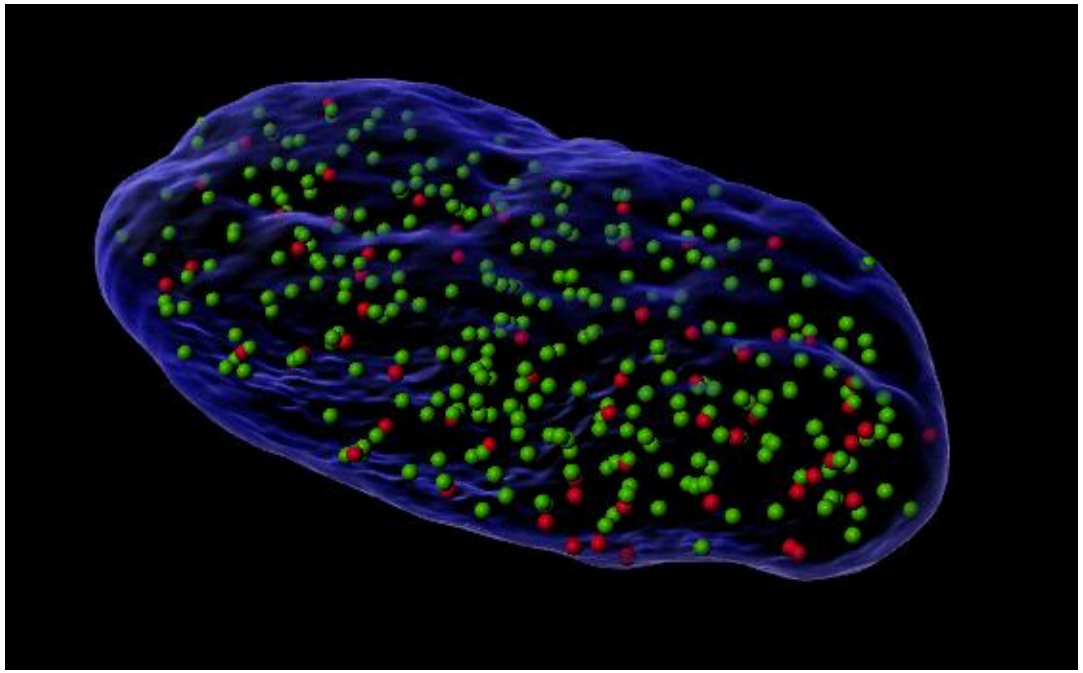


BioF

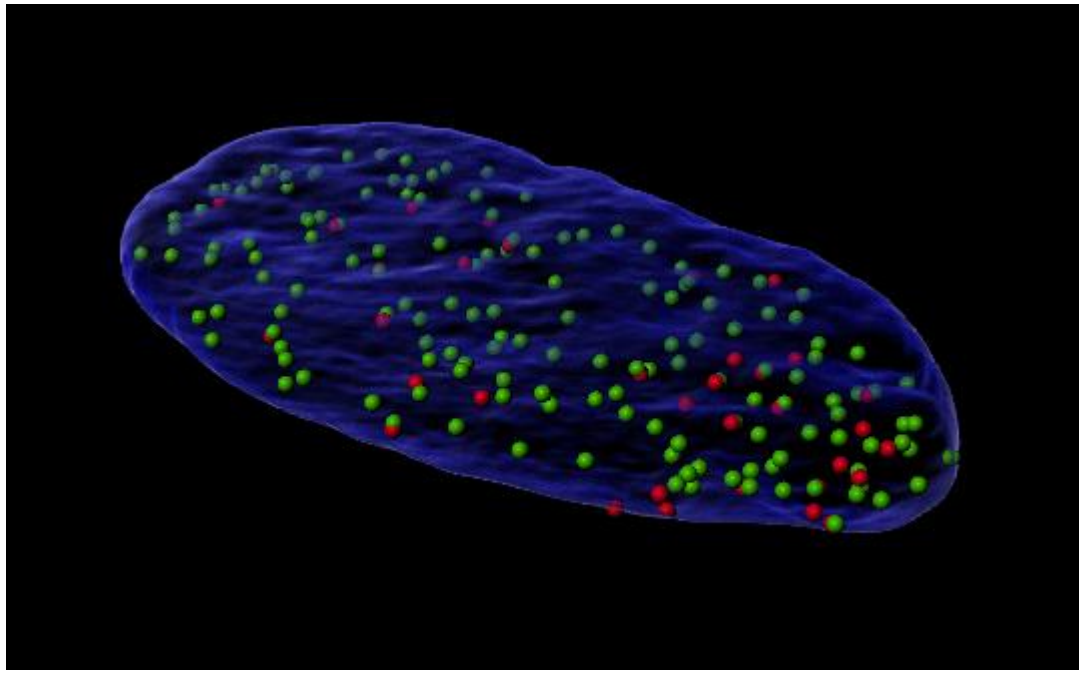
Image analysis courtesy of Megan Broe and Emi Bauer. Images acquired on a Nikon A1R laser scanning confocal with a 60X oil NA 1.40 objective.
n.s. = $p \geq 0.05$, * = $0.01 \leq p < 0.05$, **** = $p < 0.0001$.

Future Directions

Does the nuclear shrinkage from hard-setting mounting medium cause *erroneous* increases in colocalization between nuclear targets?



Buffer



PL Glass/NB
(hardening)

Images and image analysis courtesy of Isabel Ramirez-Aguilar. Images acquired on a Nikon AXR laser scanning confocal with a 100X oil NA 1.45 objective.

Acknowledgements

*Worked on this project directly

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Funding

BioFrontiers Institute at CU Boulder

NIH grants R01CA107098S1 (WF)

1S10RR026680-01A1 (Imaris)

NIST-CU cooperative agreement 70NANB15H226 (A1R)

