Using TIRF (Total Internal Reflection Fluorescence) Microscopy to Measure Actin Filaments

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Introduction/ Background

Actin:

- Maintains shape of cells and responsible for cell motility
- Monomers can polymerize into bundles or filaments
- Associated with cancer metastasis

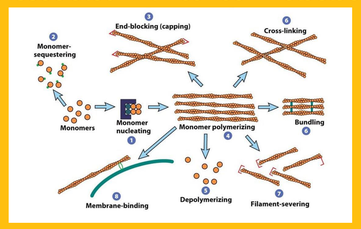


Figure 1: Actin can form many different structures through interaction with actin binding proteins.

(John Wiley & Sons, 2005)

Total Internal Reflection Fluorescence (TIRF) Microscopy:

- Evanescent wave only excites fluorescent molecules near surface of coverslip, eliminating high background of epifluorescence.
- Can use to watch actin filaments grow in real time

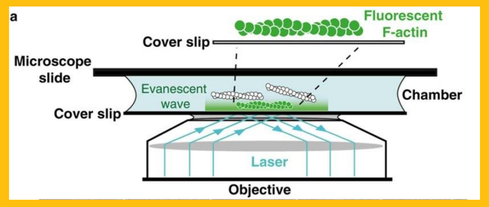


Figure 2: TIRF microscopy allow us to visualize individual actin filaments as they polymerize over time.

Zimmermann, D. *et al.* (2016)

Actin polymerization has three stages:

1) Nucleation or lag phase
2) Elongation, polymerization or growing phase
3) Treadmilling or steady growth phase.

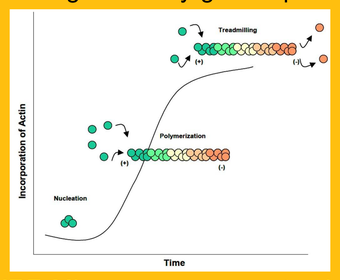


Figure 3: Shows the three stages of actin polymerization over time.

(Maisa Garcia Arguinzonie, Wurzburg Univ. Diss. 2003)

Using TIRF Microscopy we can track different filaments and catch what phase our filament lengths are in, over time we watch them grow and complete each stage.

Hypothesis

Frozen actin will polymerize at the same rate as fresh actin.

Materials and Methods

Using TIRF Microscopy to measure our actin filaments. We can make a movie of the growth of our filaments by taking snapshots every 5 seconds over a span of 20 minutes. We can then use our movie to measure the filaments to find the filament length. Using the length, slice, and time we can then make a graph and find the slope of our polymerization of that filament. We want to compare fresh versus frozen because fresh can go bad after a certain amount of time, and by freezing the actin, it allows us to use when needed.

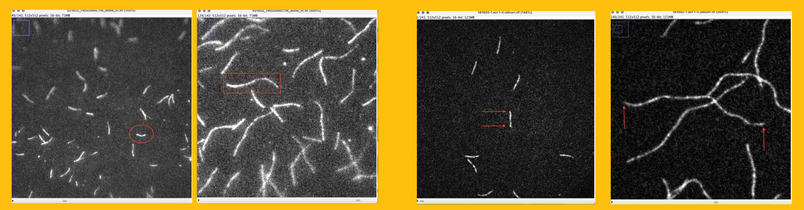


Figure 4: Still image frames from TIRFm timelapse recordings show progression of actin polymerization for frozen (left two panels) and fresh actin (right two panels) at two different time points (0 and 350sec). Red marks indicate filament measured in each.

Results

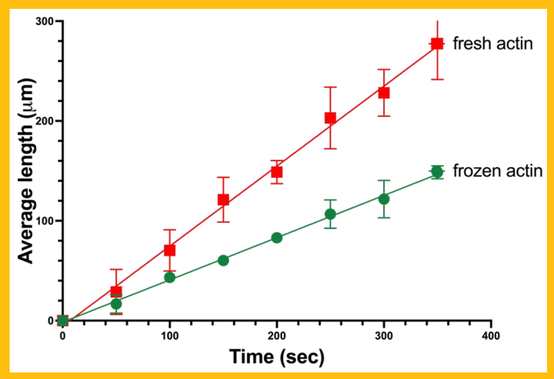


Figure 5: Shows the average length (µm) for fresh and frozen actin vs. time (sec). This is after normalizing the data so it could be read at the same starting time and length.

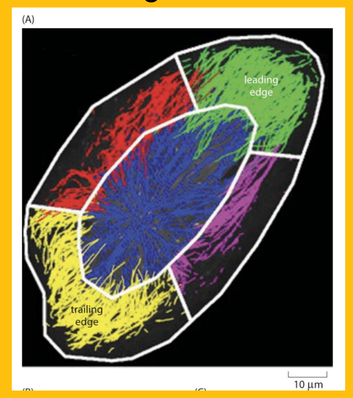
- Frozen Actin polymerization rate = 25.38 µm/min
- Fresh Actin polymerization rate = 48.06 µm/min

Conclusions

- We can get consistent actin polymerization rates using hand-quantitation of TIRFm images.
- Our initial data indicates that frozen actin polymerizes half as fast as fresh actin.

Future Directions

The next steps are adding a protein that we know enhances polymerization called Palladin Ig3 to our frozen actin and then quantitating the polymerization rates, then comparing our measurements to our fresh actin with added Palladin Ig3.



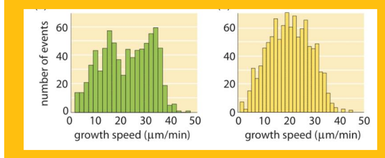


Figure 6: Growth rates of actin filaments in vivo. (A) Growth rates in different regions of a human endothelial cell. (B) Growth speeds at the leading edge of the cell. (C) Growth speeds at the trailing edge of the cell. (Adapted from KT Applegate, Journal of Structural Biology 176 (2011) 168–184)

References

- Figure 1: Cell and Molecular Biology, 4/e, 2005 John Wiley & Sons
- Figure 2: Zimmermann, D. *et al.* (2016) Methods Mol Biol. 1369:151-79
- Figure 3: Maisa Garcia Arguinzonie, Wurzburg Univ. Diss. 2003
- Figure 6: KT Applegate, Journal of Structural Biology 176 (2011) 168-184

Acknowledgements

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