Automatic Characterization of Focal Adhesions in TIRF Microscopy Images

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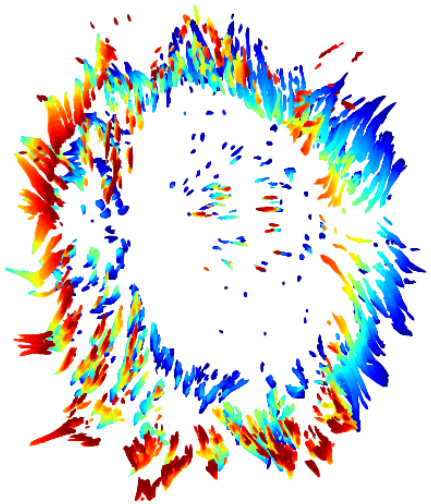
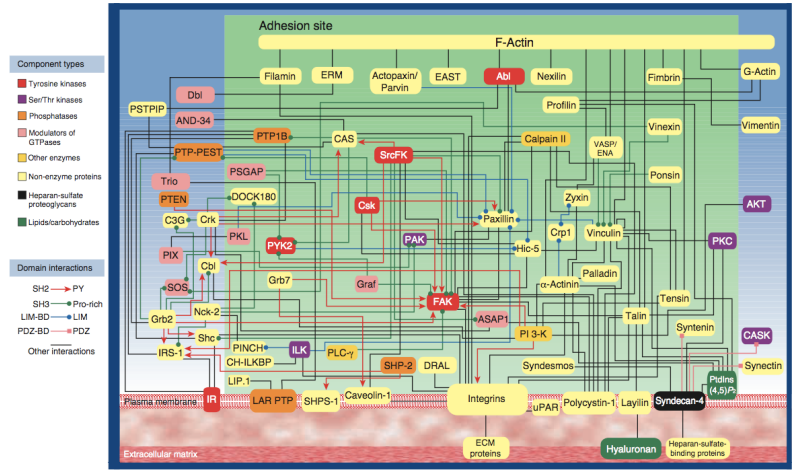


Focal What?

- Points of contact between the substrate and cells
- Consist of dozens of dynamically recruited proteins
- Important in understanding how cells navigate and sample the environment



E. Zamir and B. Geiger. JCS, 114(20):3583-3590, 2001.

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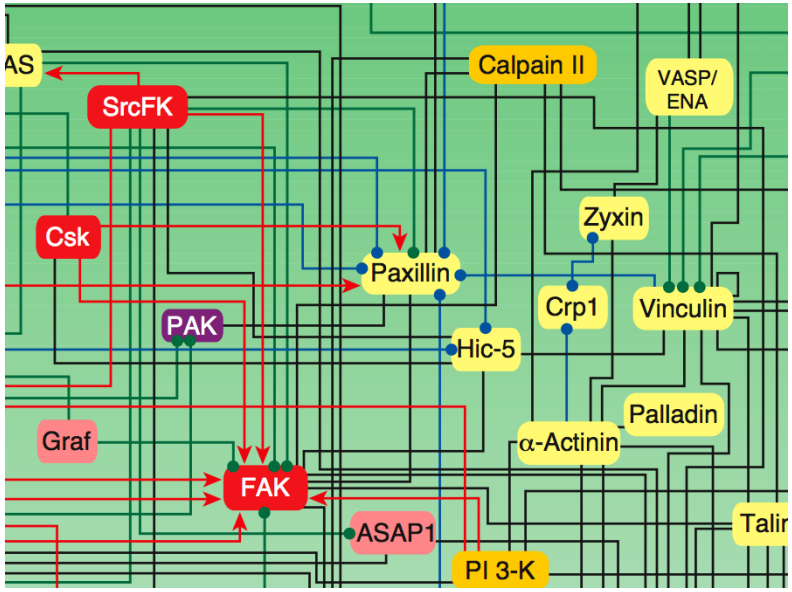

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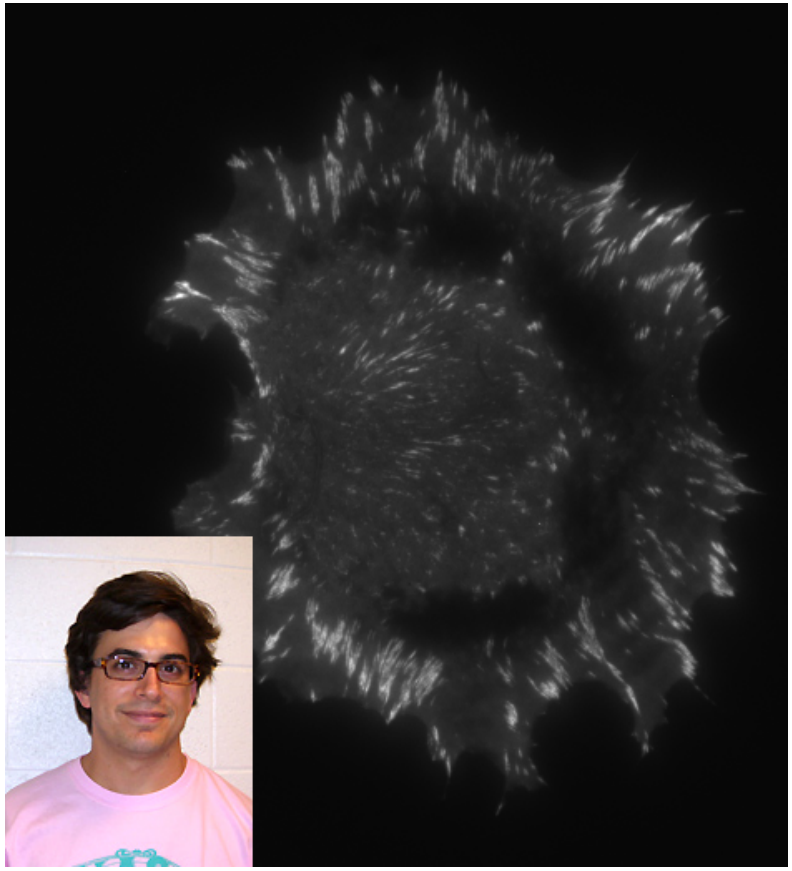
Paxillin Info/Example Movie

- Paxillin - adhesion scaffolding protein
- Recruited to adhesions early, remains associated
- Data consists of time lapse images of GFP-labeled Paxillin in 3T3 fibroblasts



Outline

Introduction

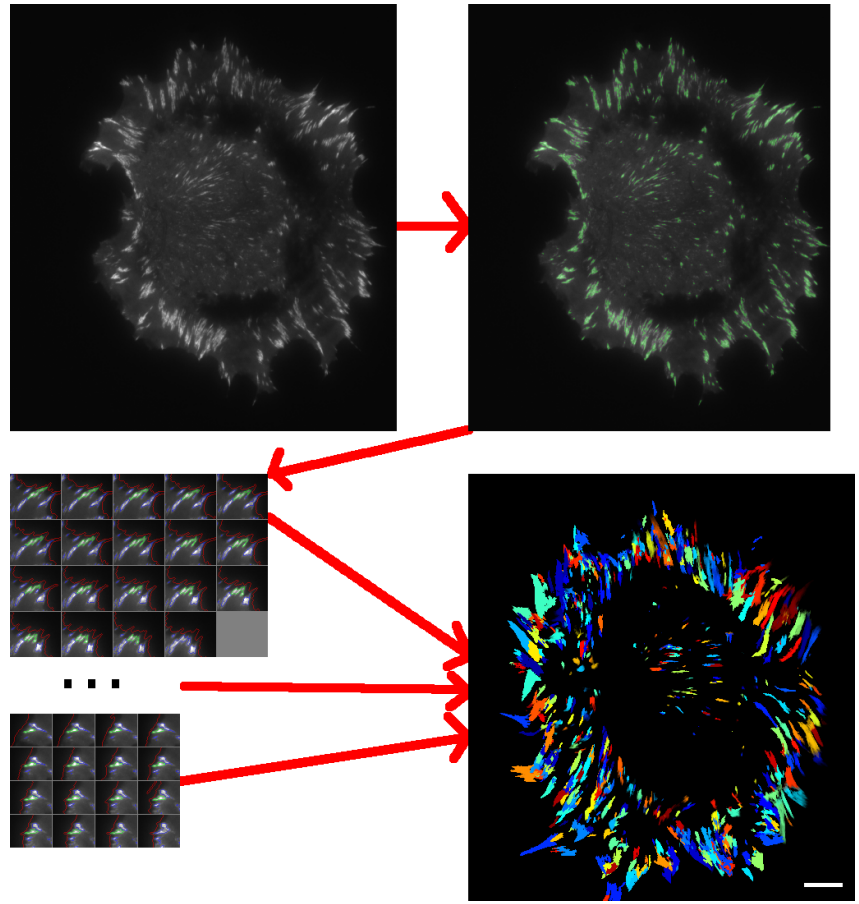
Finding/Tracking Adhesions
- Identifying Focal Adhesions
- Visualizing the Results

Adhesion Properties

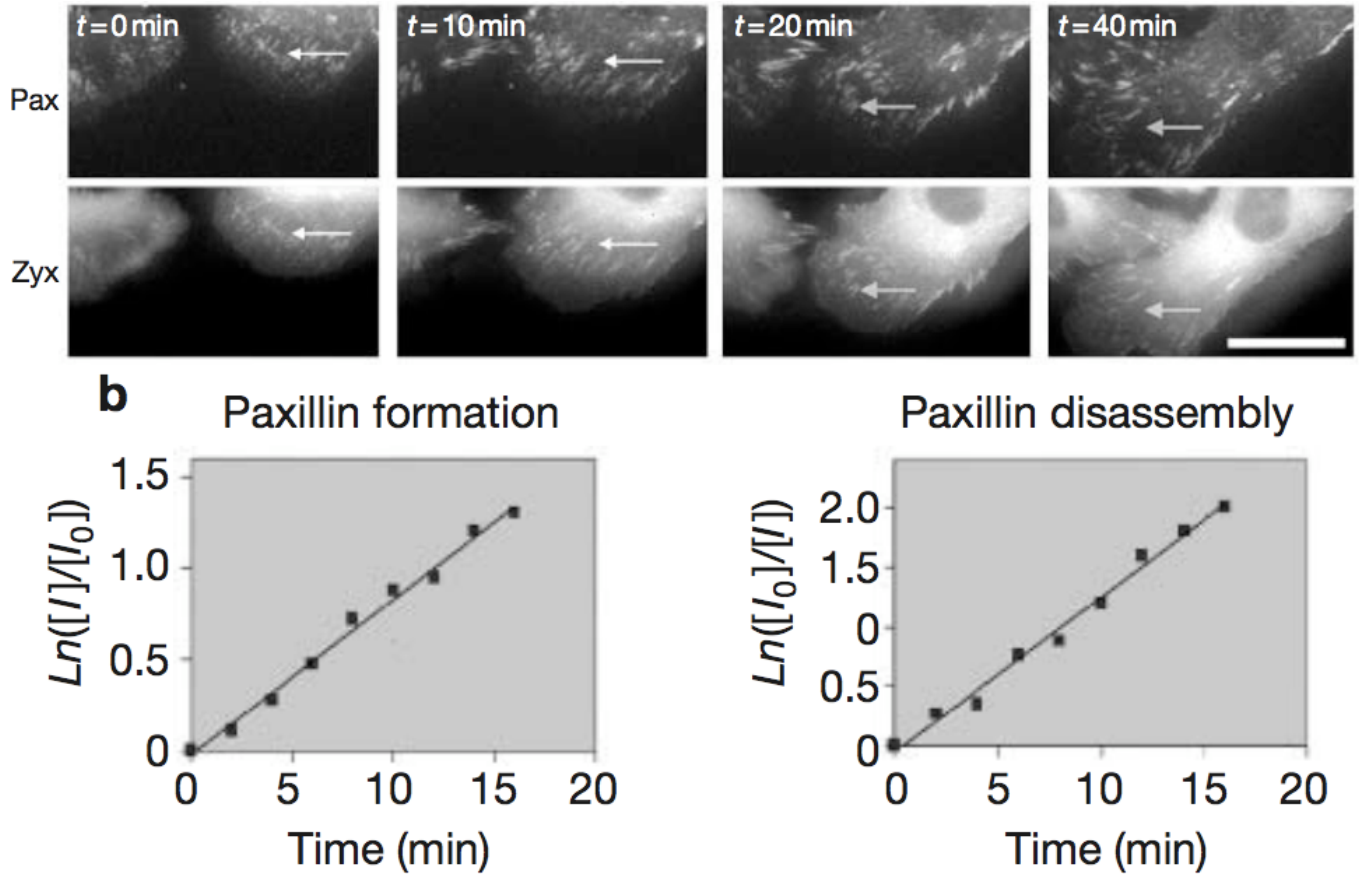
Conclusions

Identification Overview

- Prior Methods
- Multistage process
  - Finding Adhesions
  - Tracking Adhesions
  - Visualization



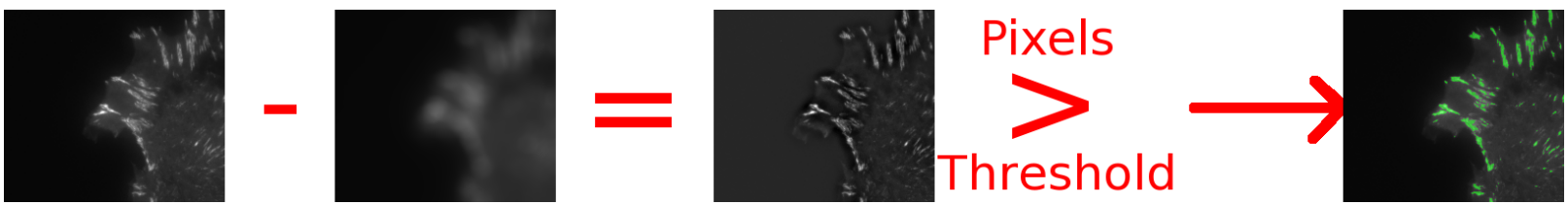
Prior Methods



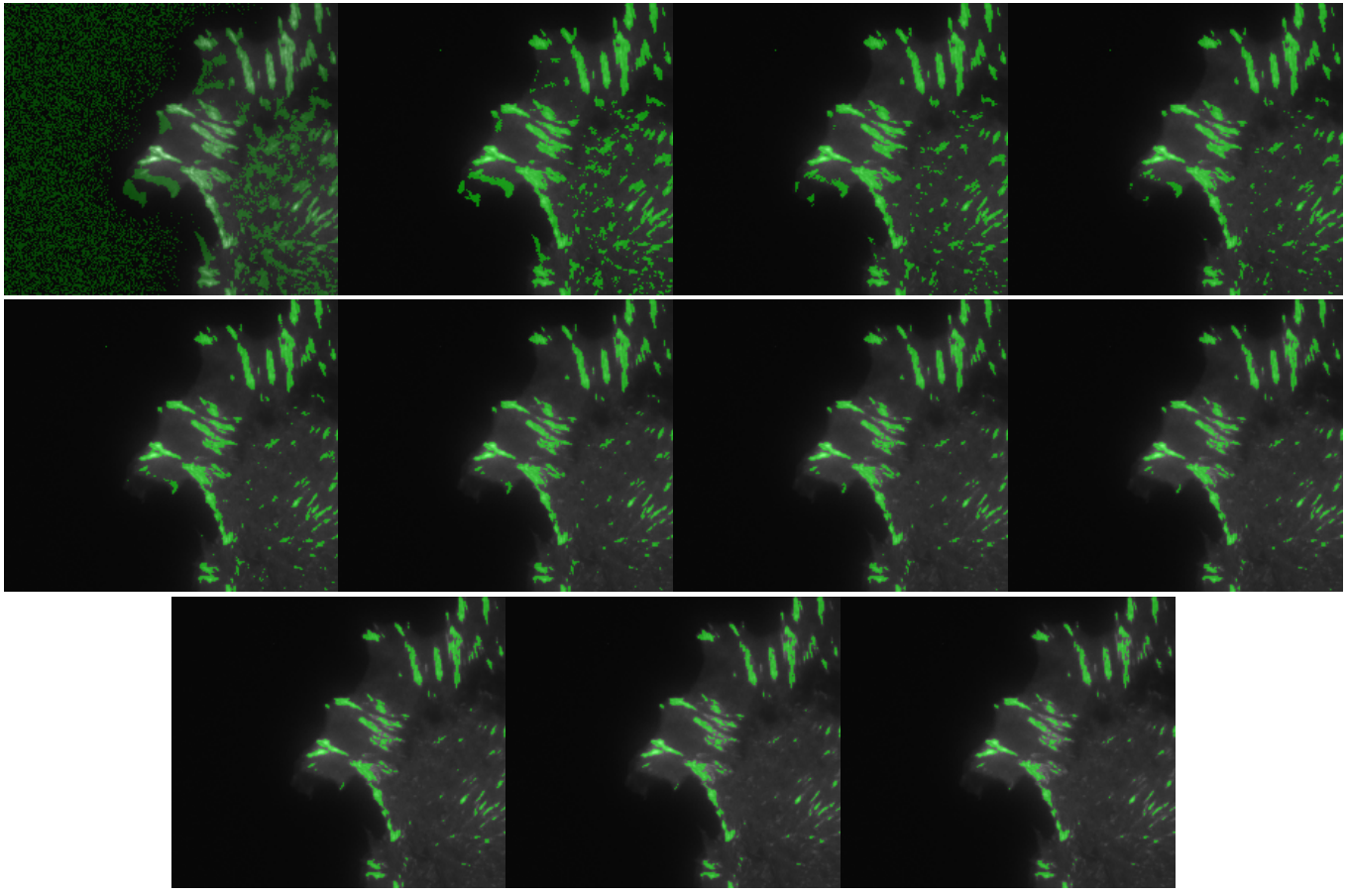
D. Webb, et al. NCB, 6(2):154-161, 2004.

Determining Adhesion Pixels

- Technique adapted from E. Zamir, et al. JCS, 112(11):1655-1669, 1999.
- High-pass image filter applied
- Threshold used to select pixels

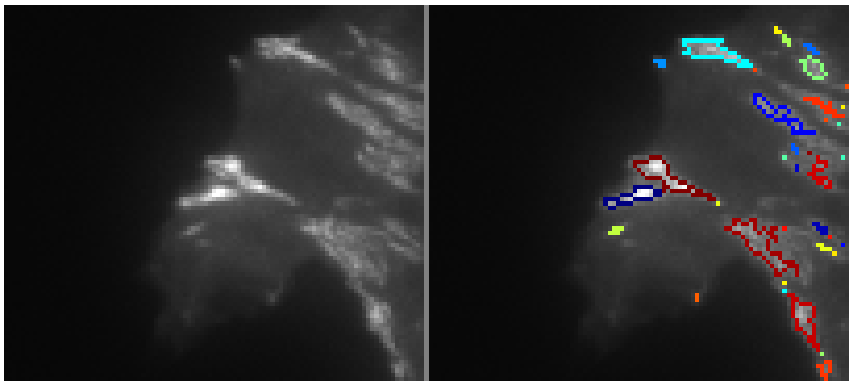


Threshold Variation

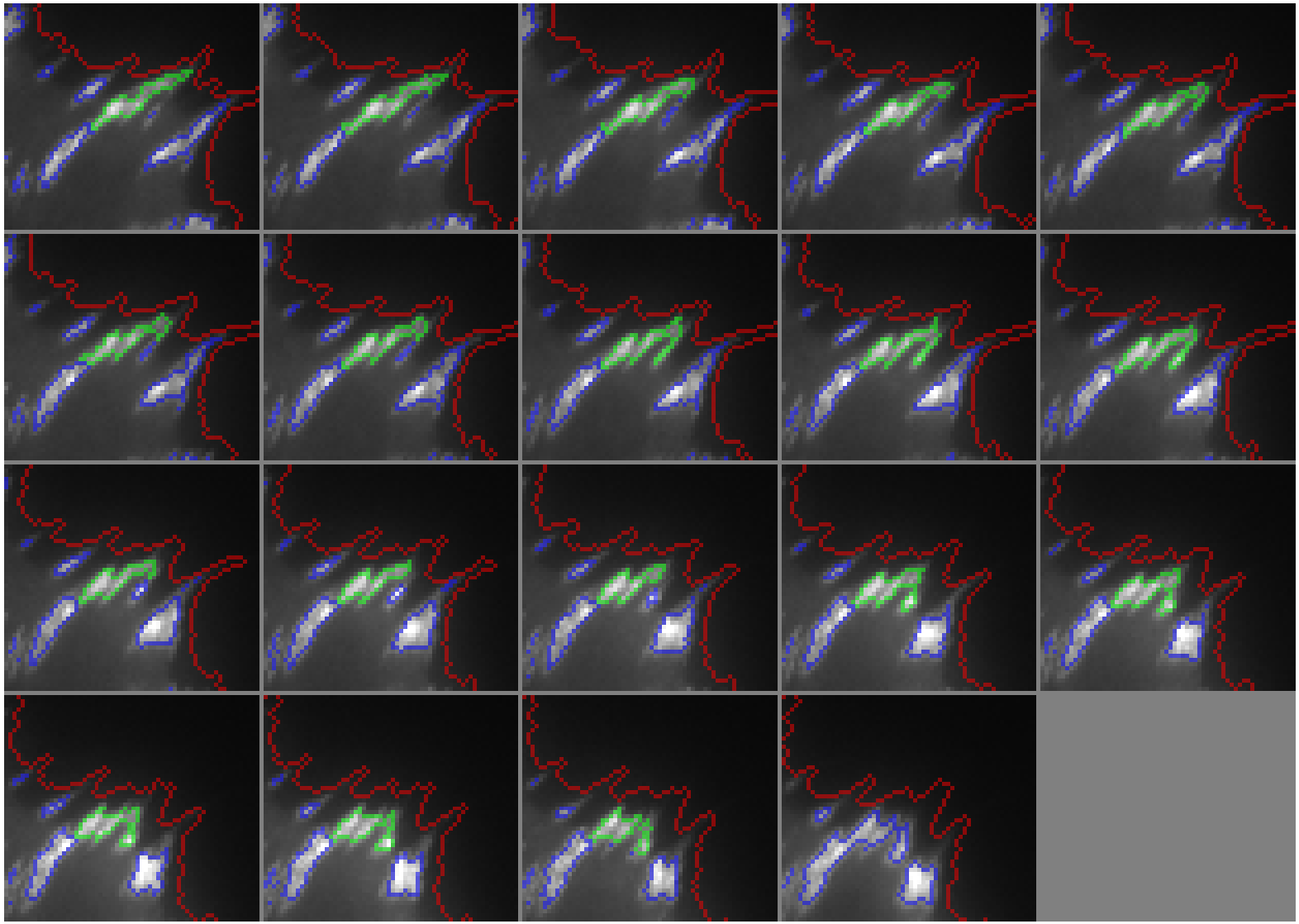


Assigning Pixels to Adhesions

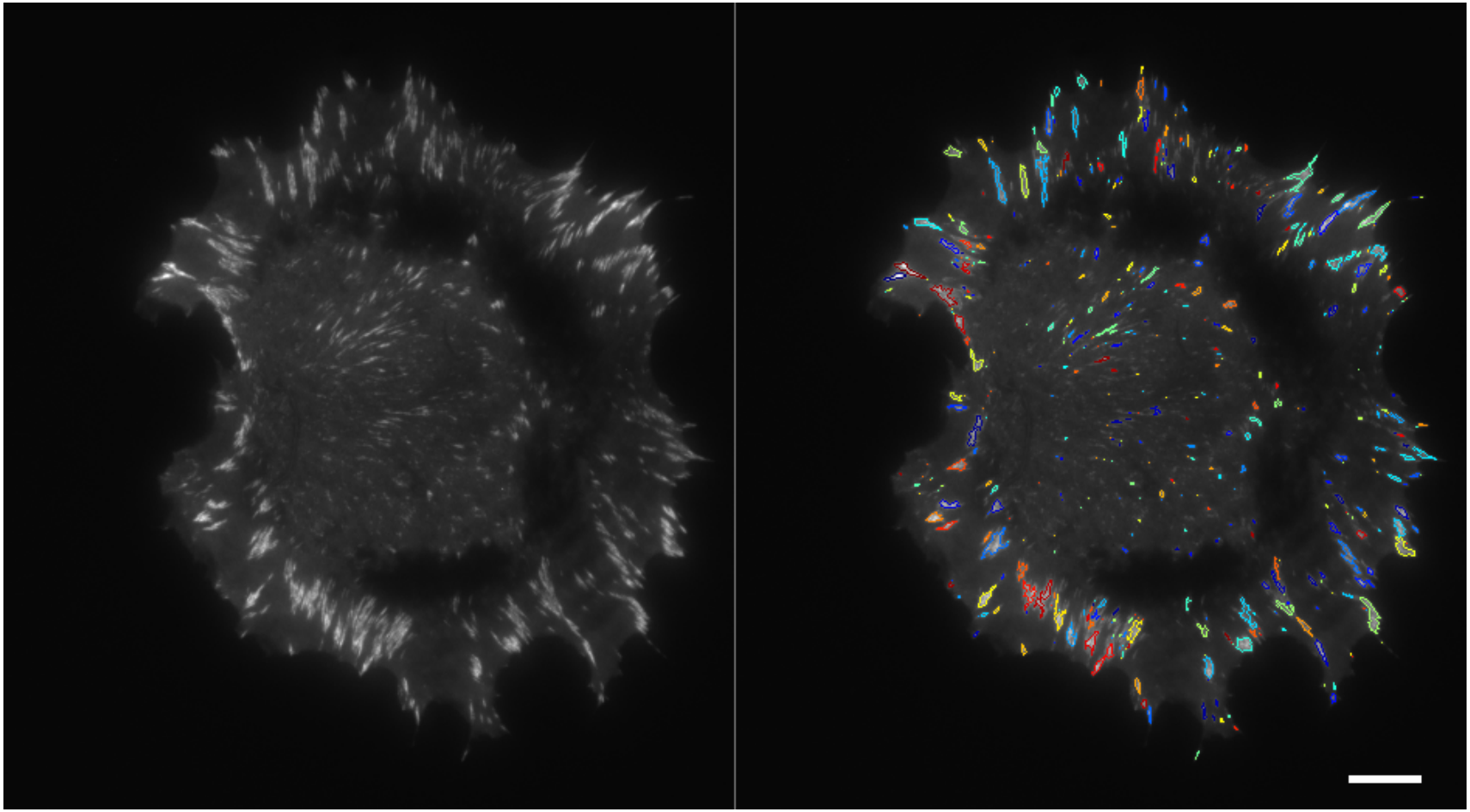
- Technique adapted from E. Zamir, et al. JCS, 112(11):1655-1669, 1999.
- High-passed pixel values are sorted, then added singly
- Each added pixel, joins a current adhesion or starts a new adhesion
- Merging limited to small adhesions



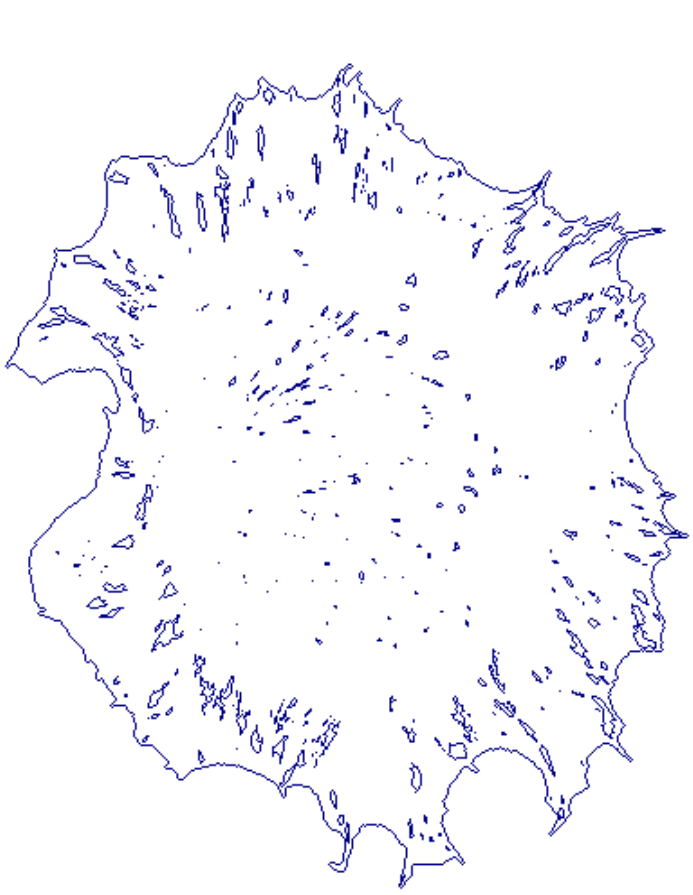
Visualizing Single Adhesions

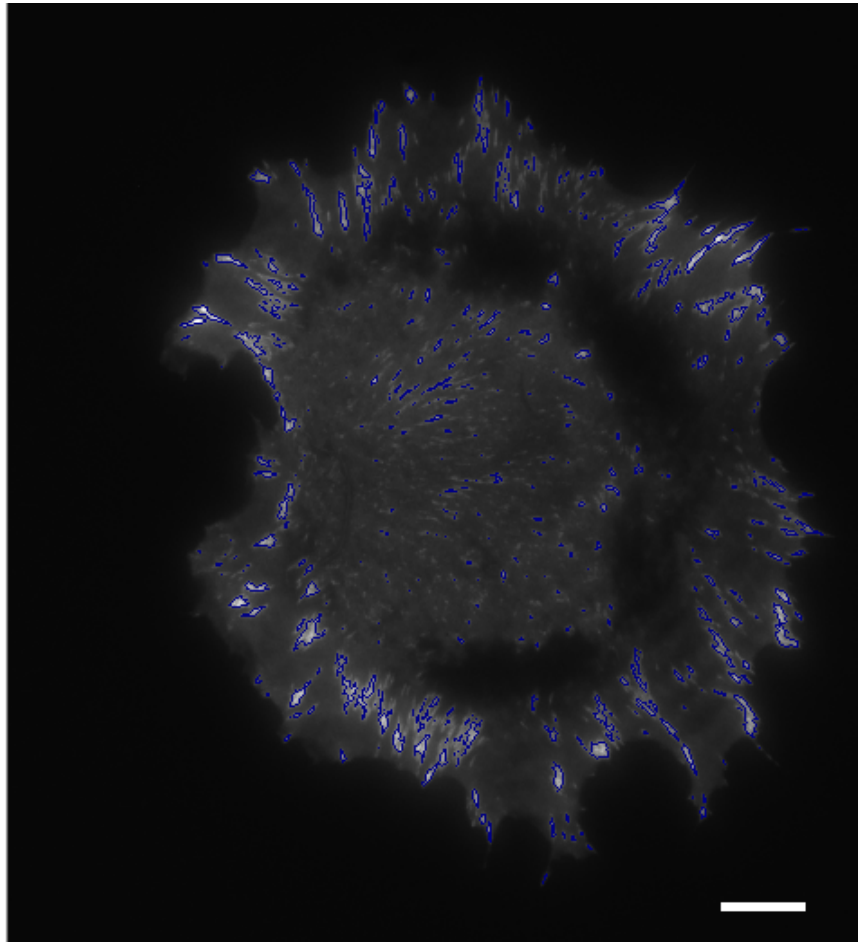


Tracking Example Movie - Unique Colors



Tracking Example Movie - Birth Time Colors

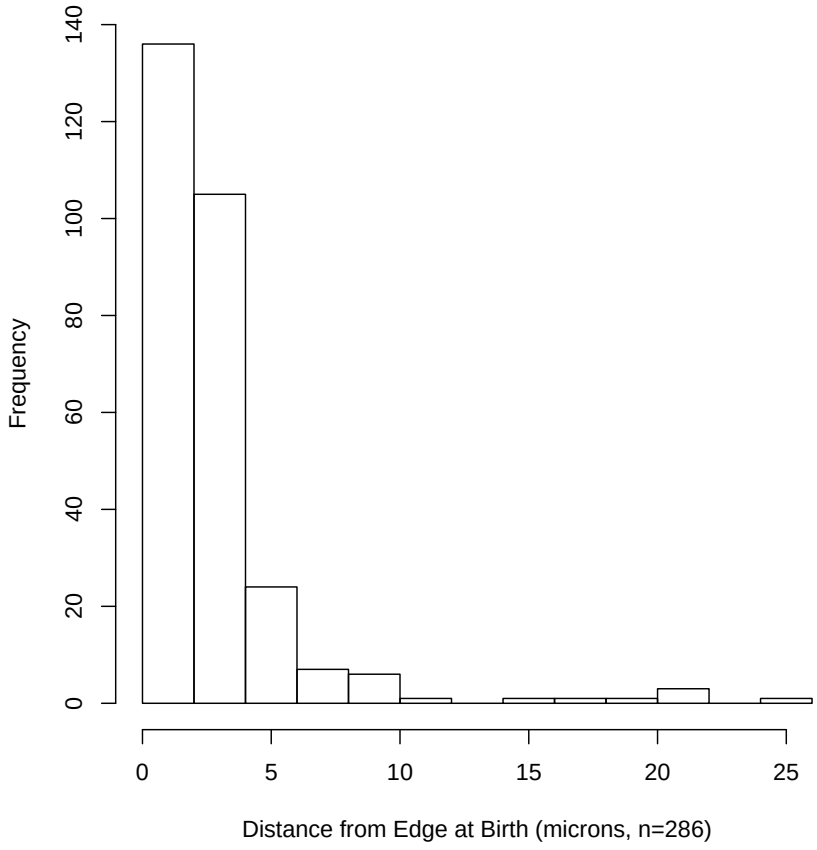


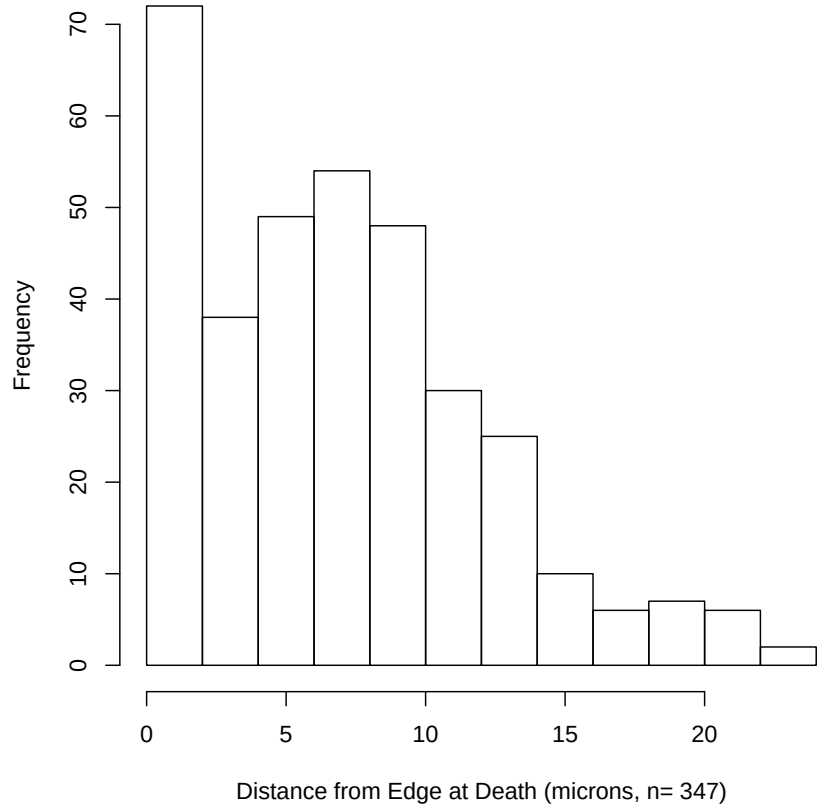


Properties Overview

- General Adhesion Lifecycle properties:
  - Adhesion birth and death locations
  - Adhesion lifetimes
- Assembly and Disassembly Rates

General Dynamics Properties





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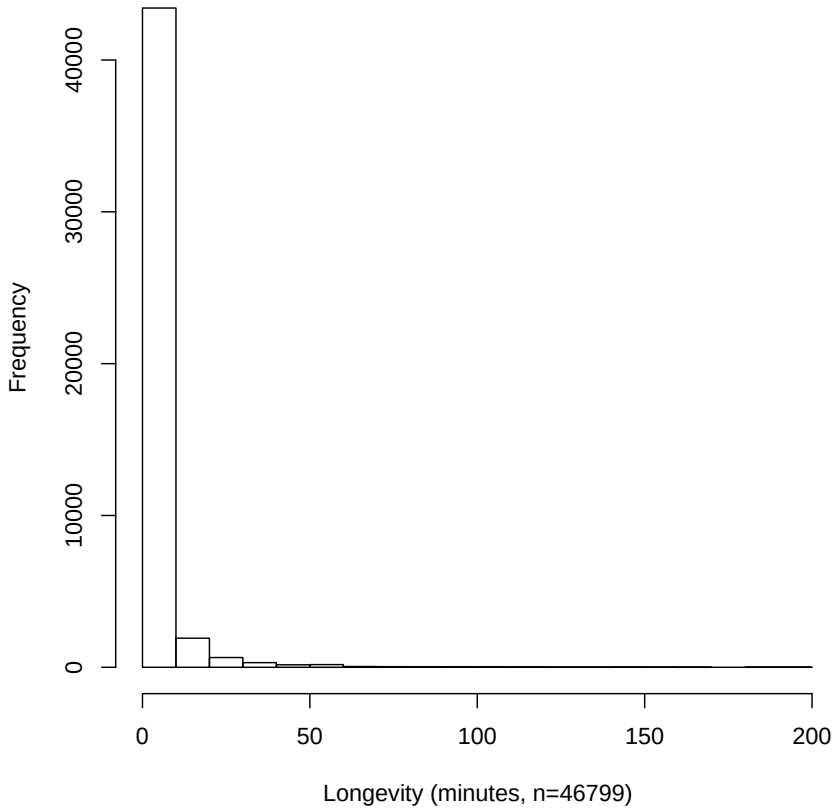
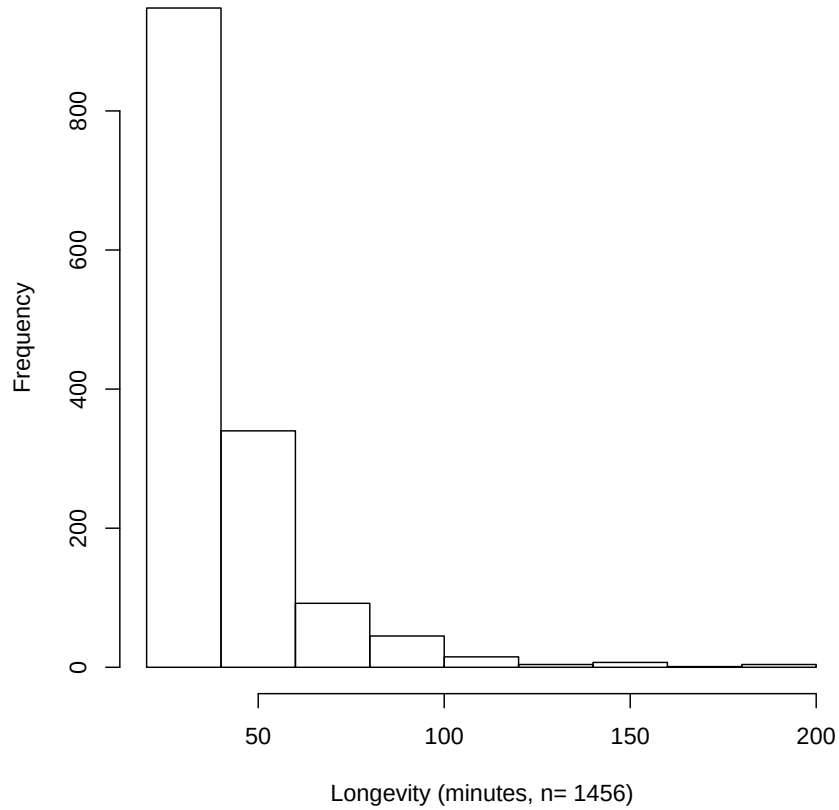
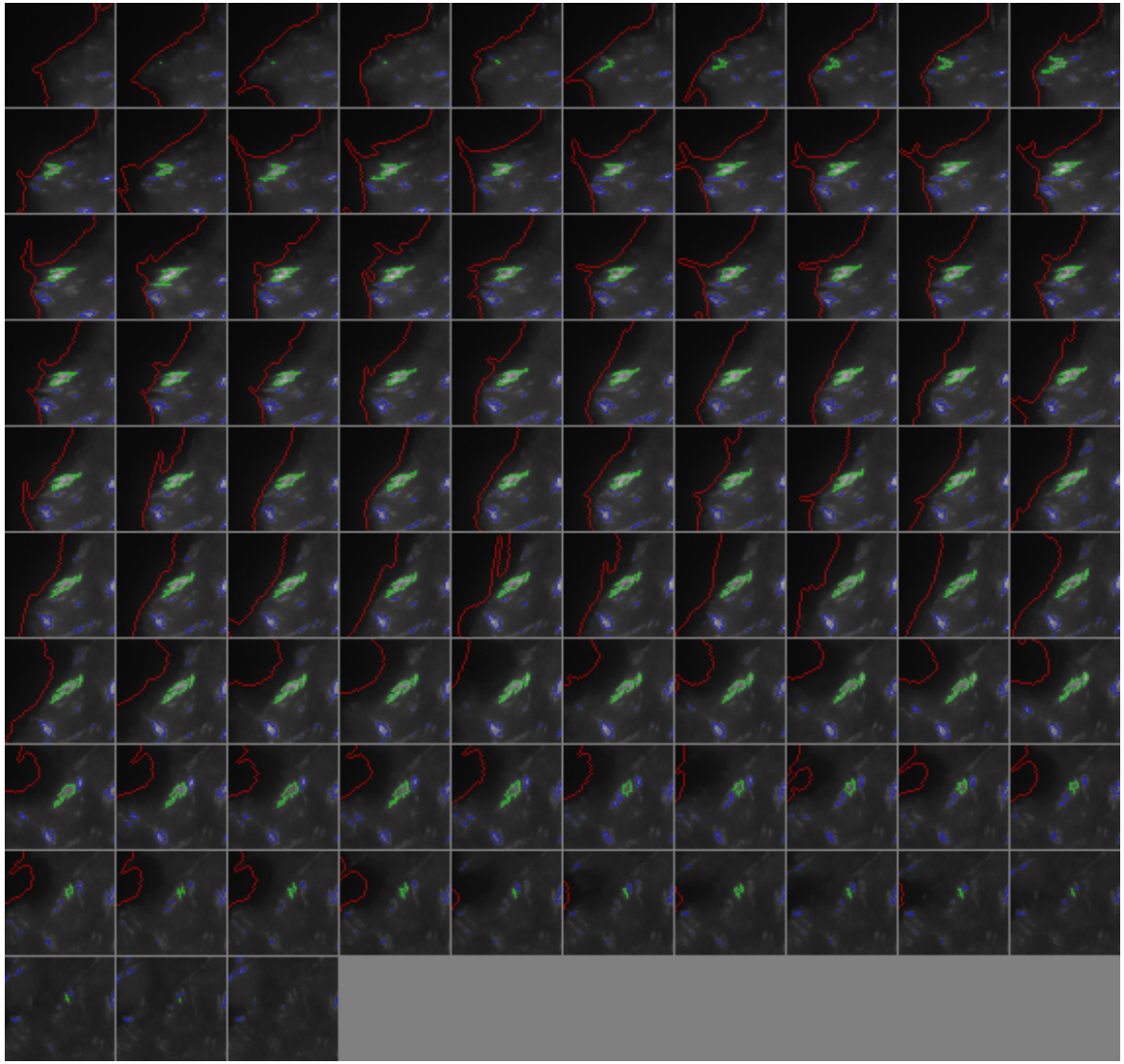




Single Adhesion Assembly and Disassembly Rates



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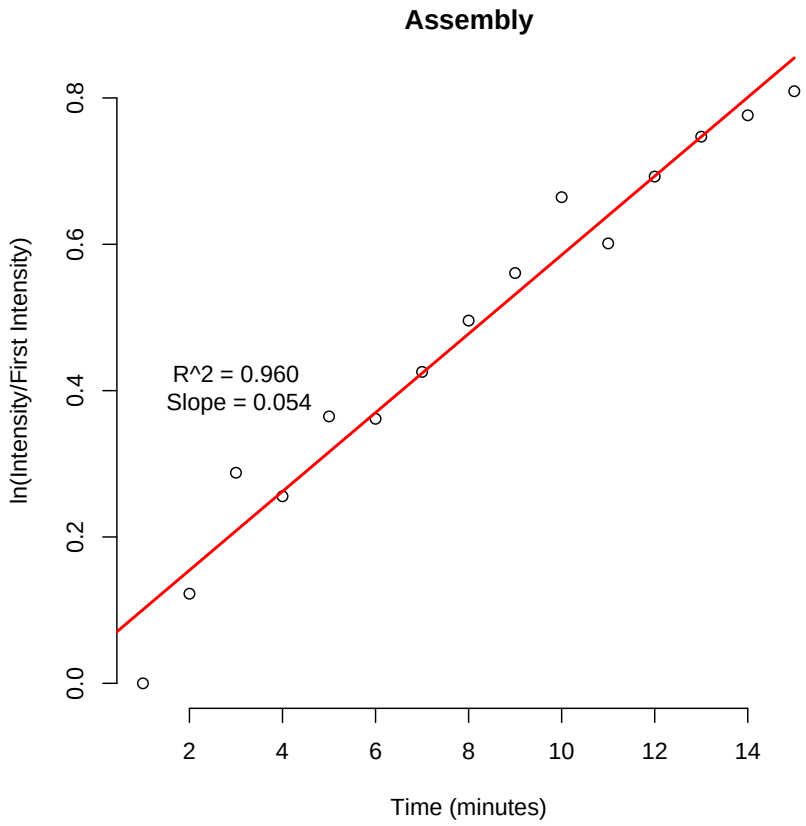
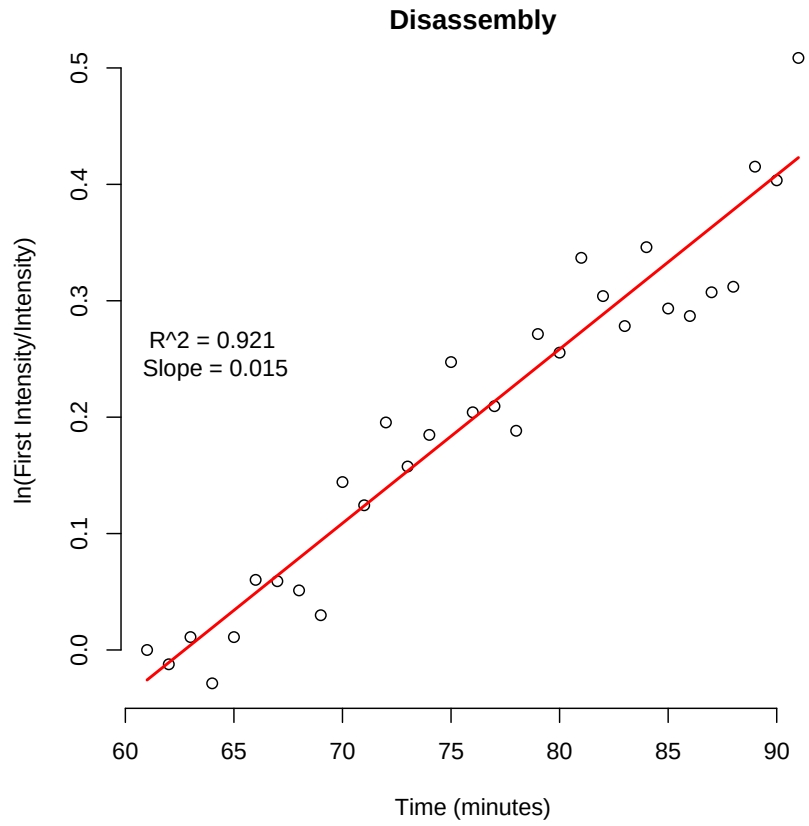
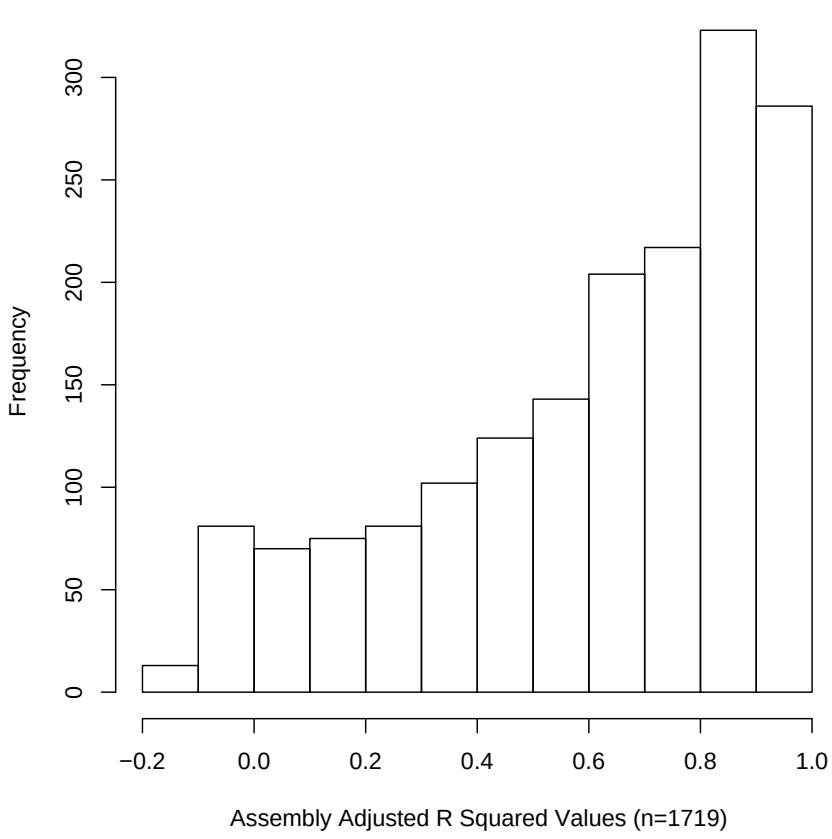
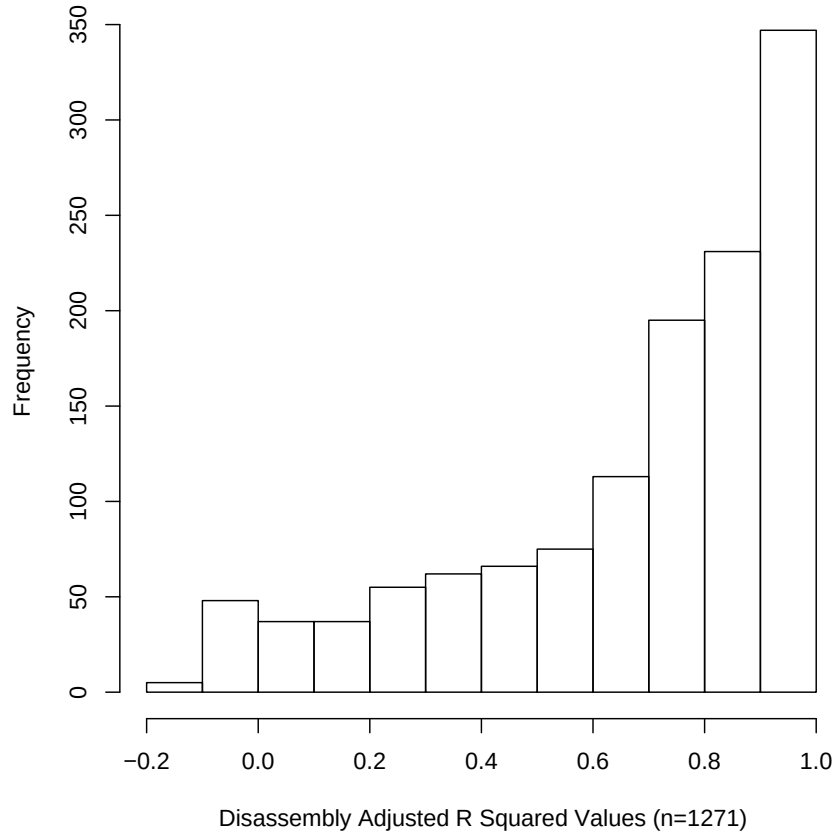


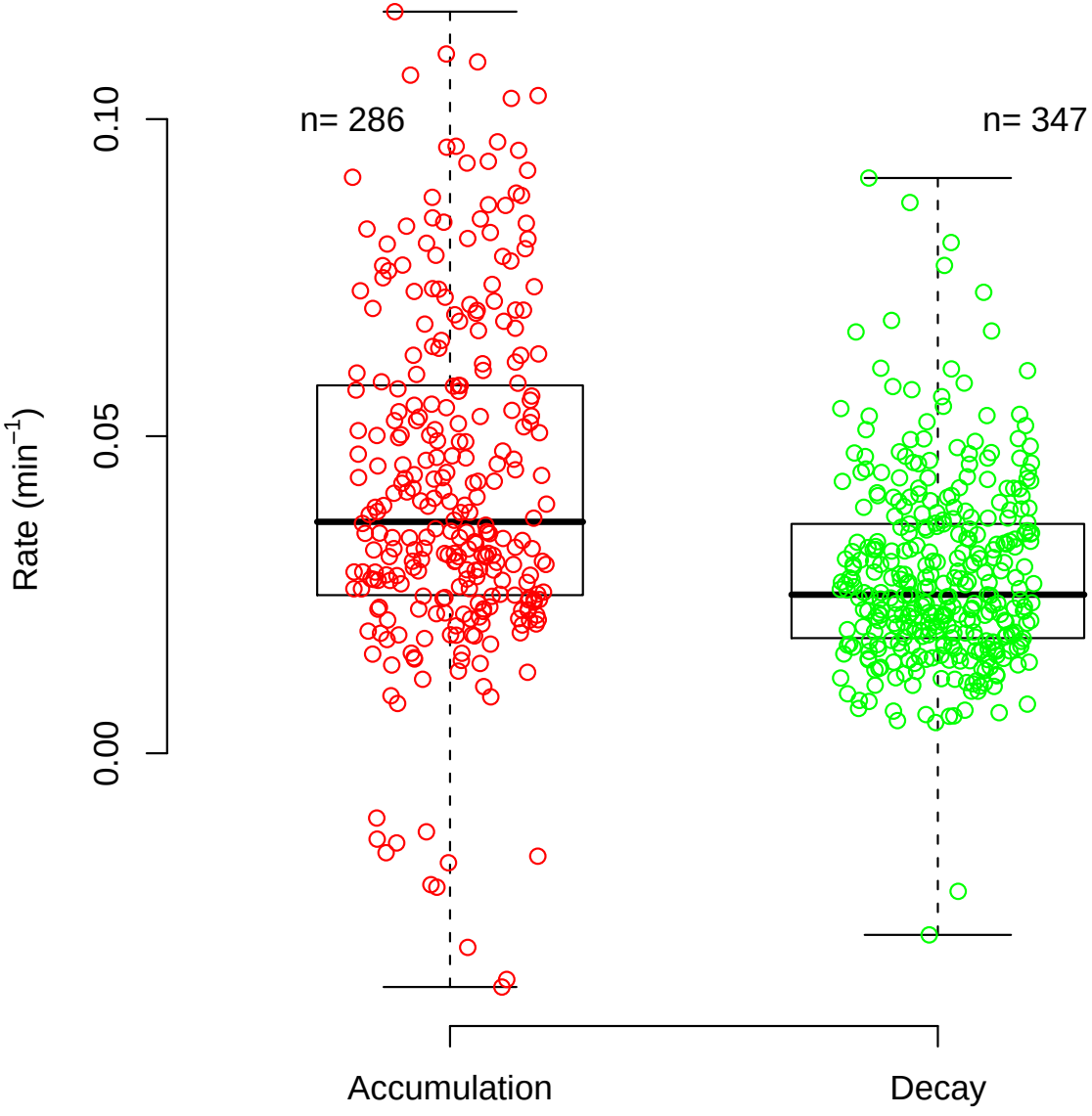


Rate Fit Qualities



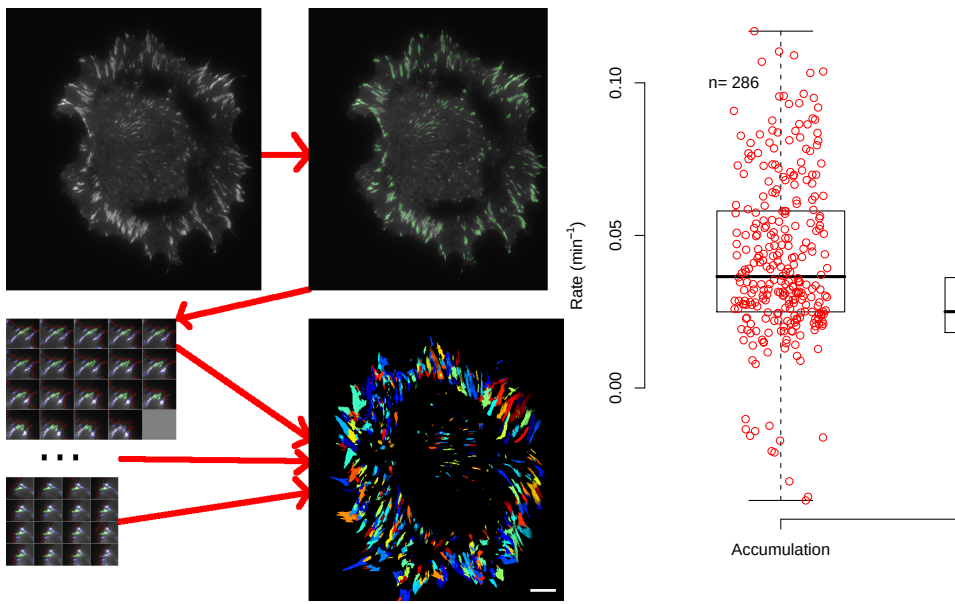


Overall Rate Summary



Conclusions/Future Work

- System for extracting properties for labeled adhesions:
  - Finding
  - Tracking
  - Visualization
  - Properties
- Future Work:
  - integration of biosensor data



Questions?