High Content Analysis of Invadopodia Imaging

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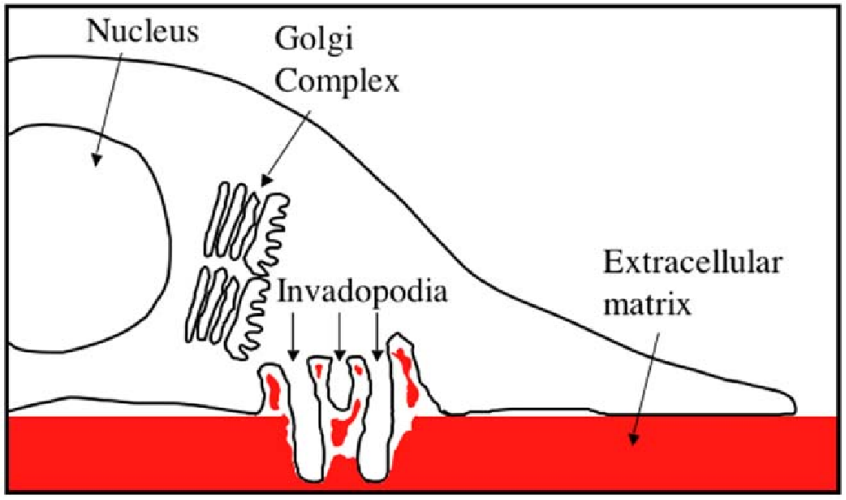
Invadopodia Degrade the Extracellular Matrix

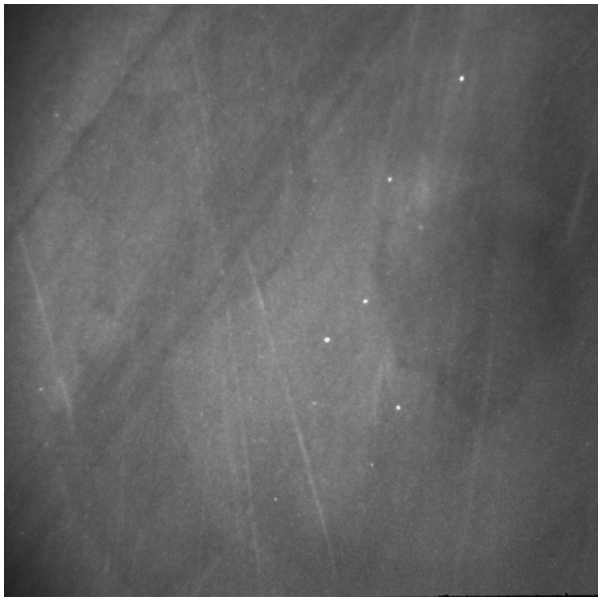
- For metastasis to occur, the extracellular matrix must be degraded
- Dynamic intra-cellular structures that contain many proteins

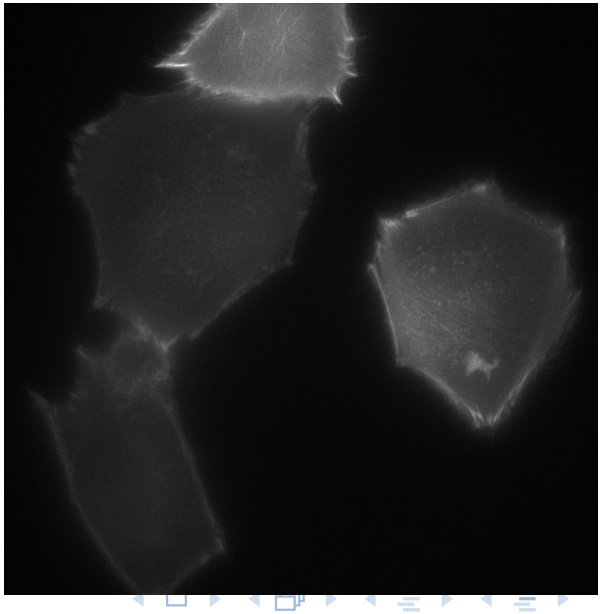


Ayala, et al. Euro J of Cell Bio, 2006

Using Microscopy to Quantify Invadopodia

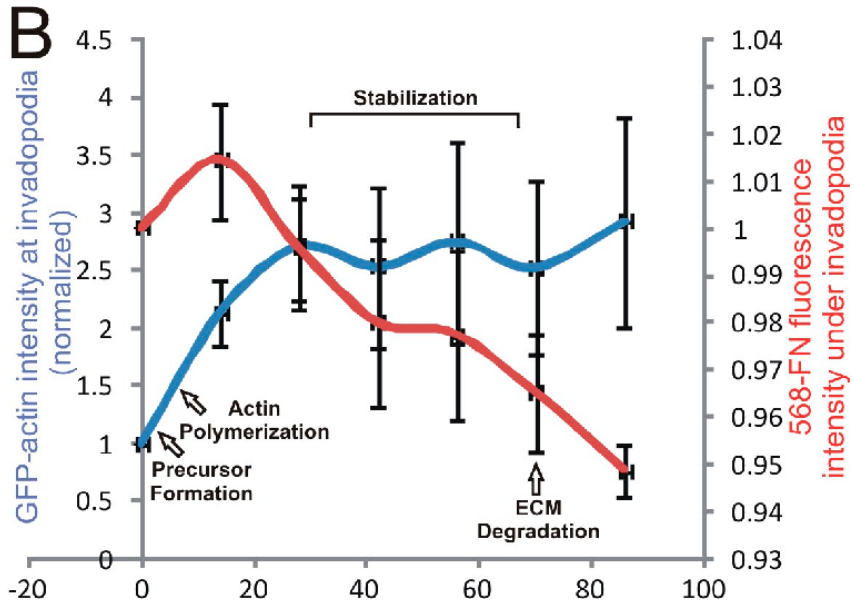
- Primary method to watch invasion uses collagen matrix mixed with fluorescent dye
- As invadopodia form and matrix broken down, dye diffuses away





Prior Quantification Methods are Low Throughput

- Reliant on by hand counting
- number of cells with invadopodia
- number of invadopodia per cell
- tracking degradation over time



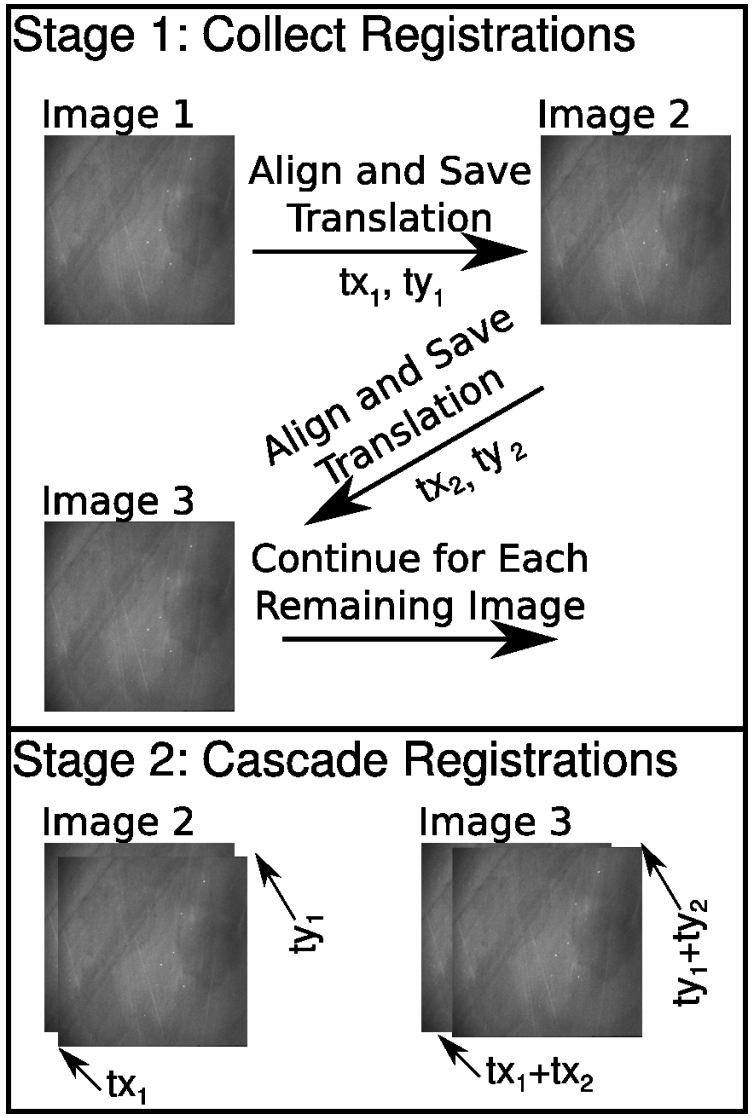
Oser et al., J. Cell Biol., 2009

Image Processing Overview

1. register images
2. find the bright actin puncta
3. track the puncta
4. classify puncta as invadopodia or not
5. analyze the data associated with the invadopodia

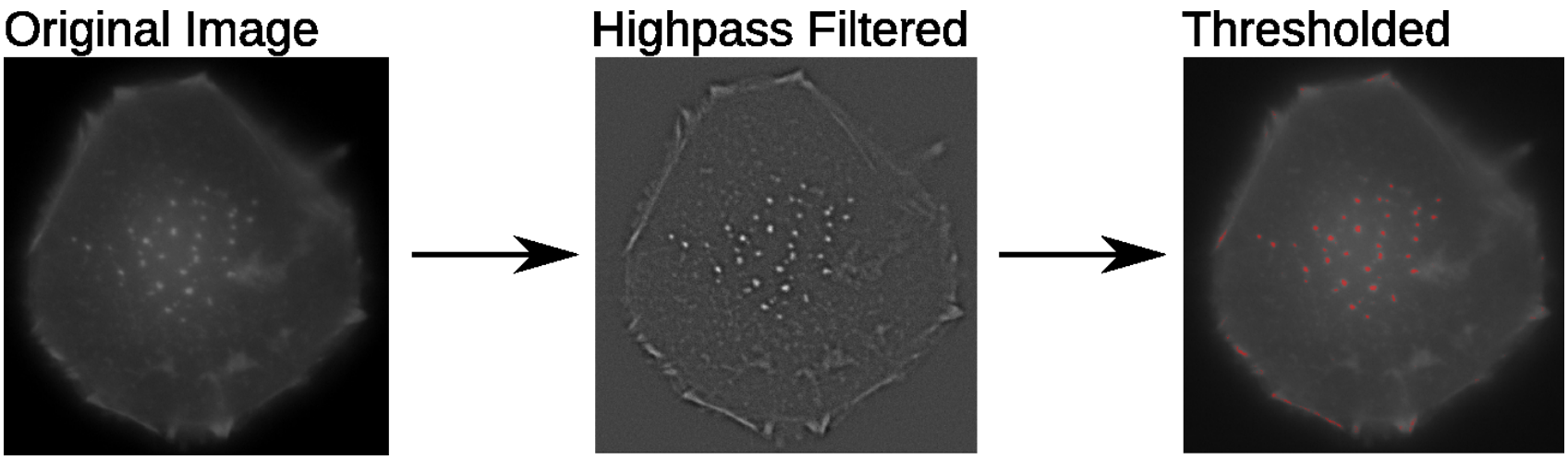
Dealing with Drastic Diagonal Drift in Demented Daguerreotypes

- major problem for the tracking algorithm
- registration and appropriate methods to deal with shifted images needed



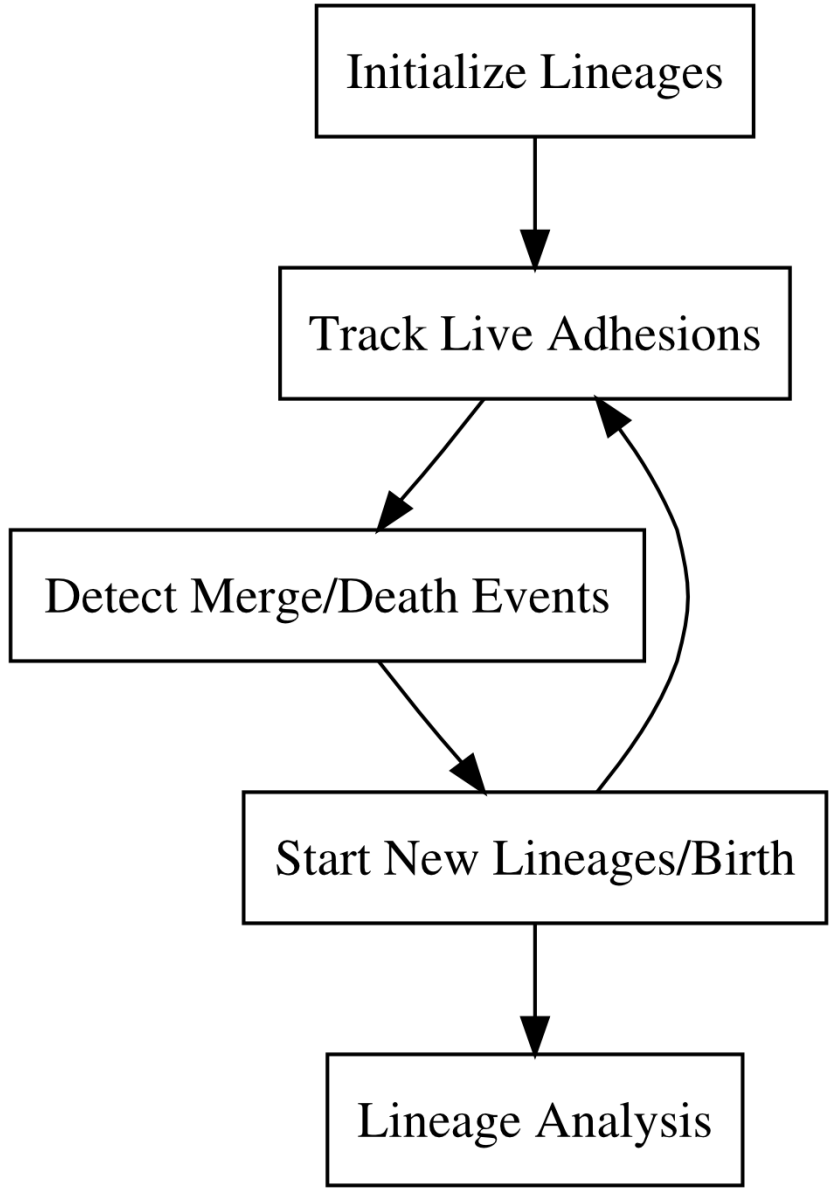
Finding the Actin Puncta

- based on a method developed to identify focal adhesions
- uses a high-pass filter to identify puncta in differing background fluorescence levels



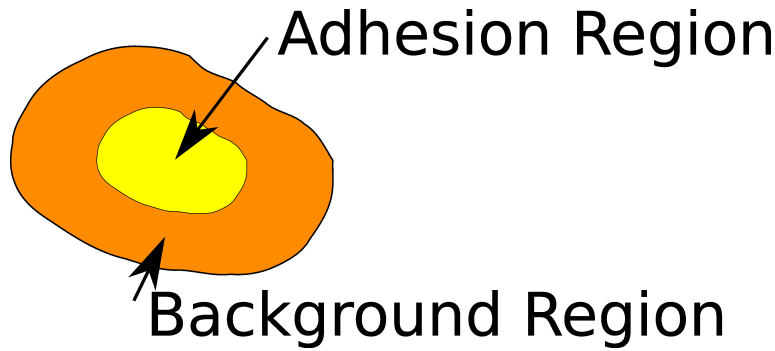
Tracking the Puncta

- system relies on overlap in puncta from frame to frame
- if overlap fails, uses distance between puncta centroids

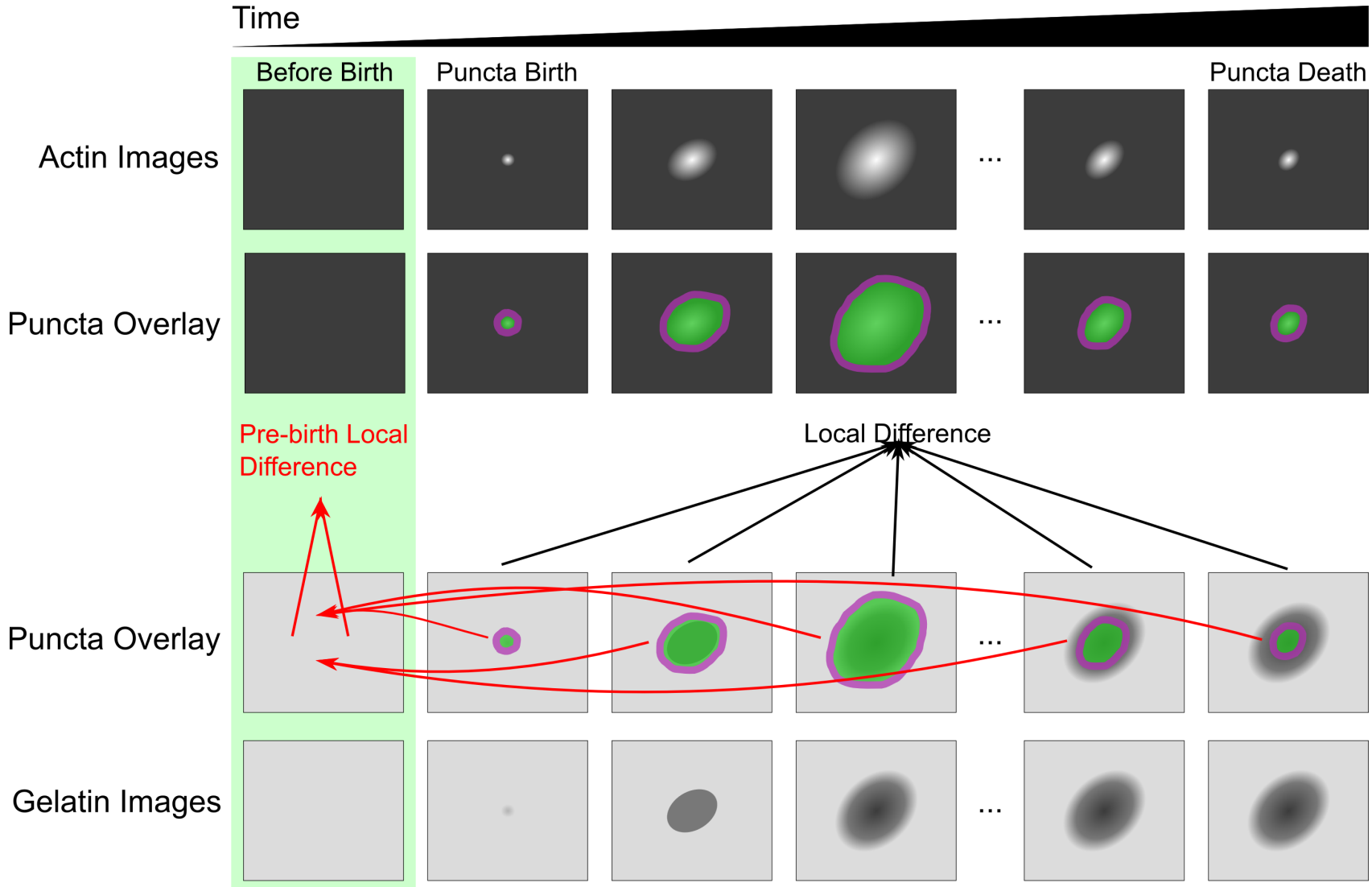


Filtering to Find Invadopodia

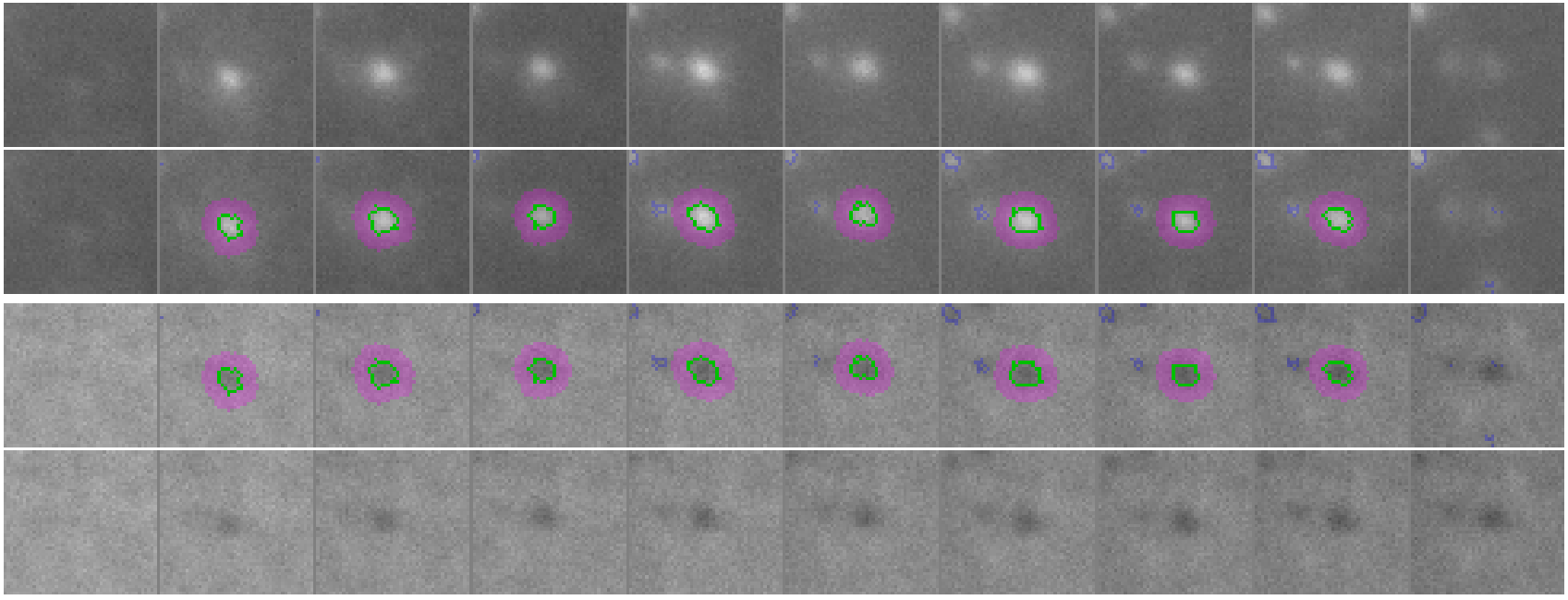
- longevity
- doesn't merge with another puncta
- didn't split off from another puncta
- decrease in gelatin underneath puncta
  - local background difference



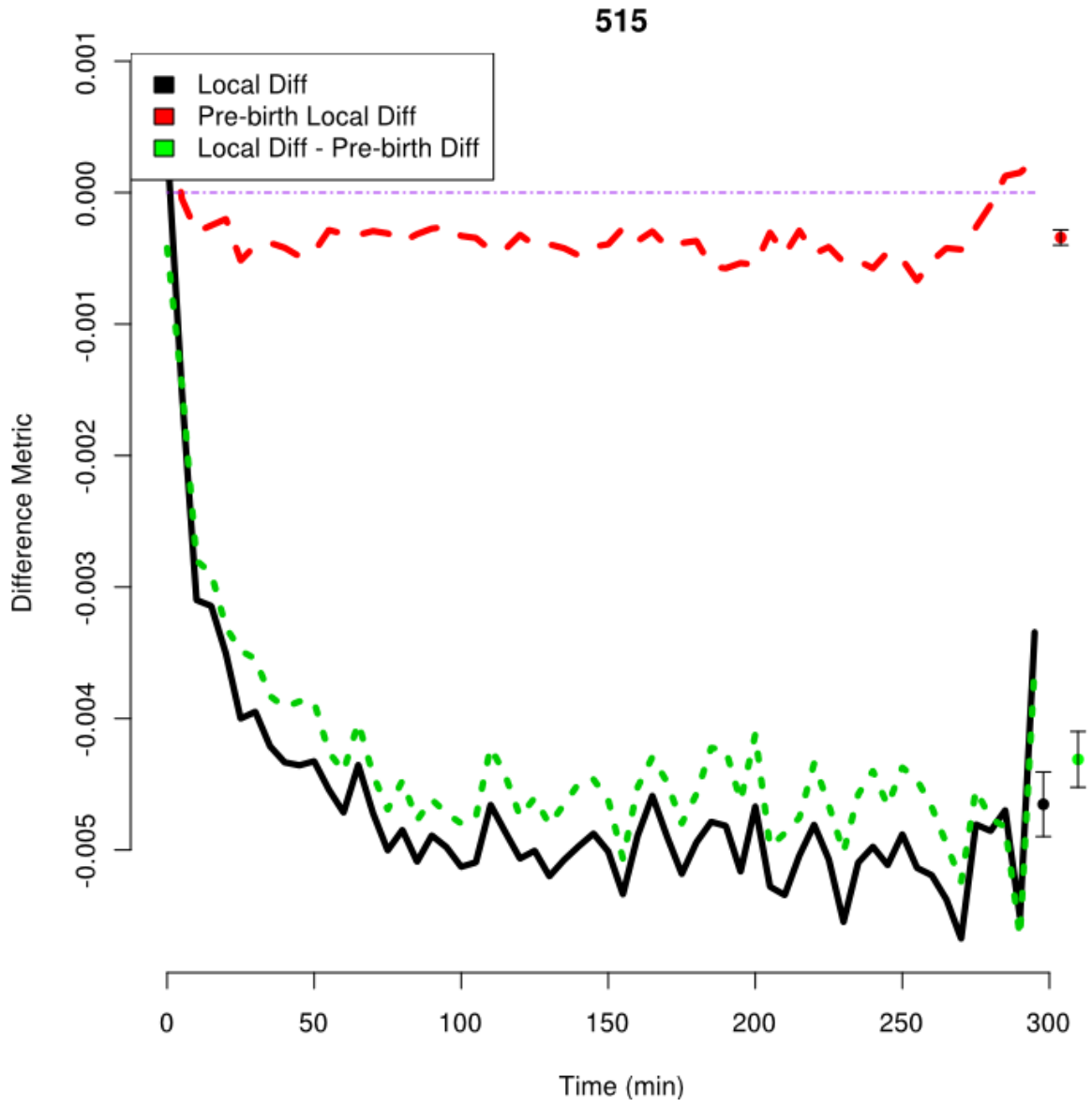
Overall Local Difference Filtering Cartoon



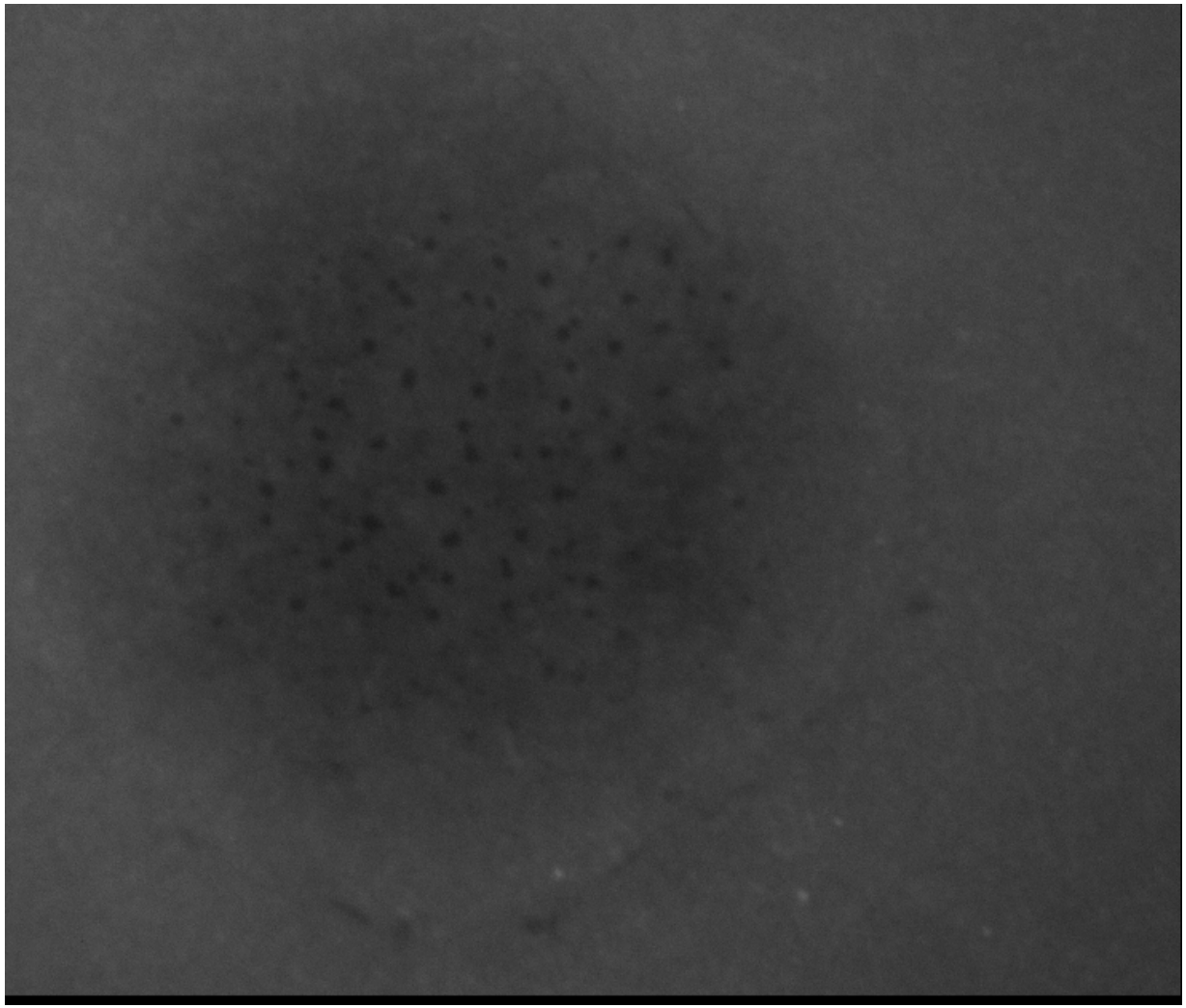
Real Puncta Time-lapse



Quantifying the Local Difference Measurement



Visualizing the Results - Highlighting Invadopodia



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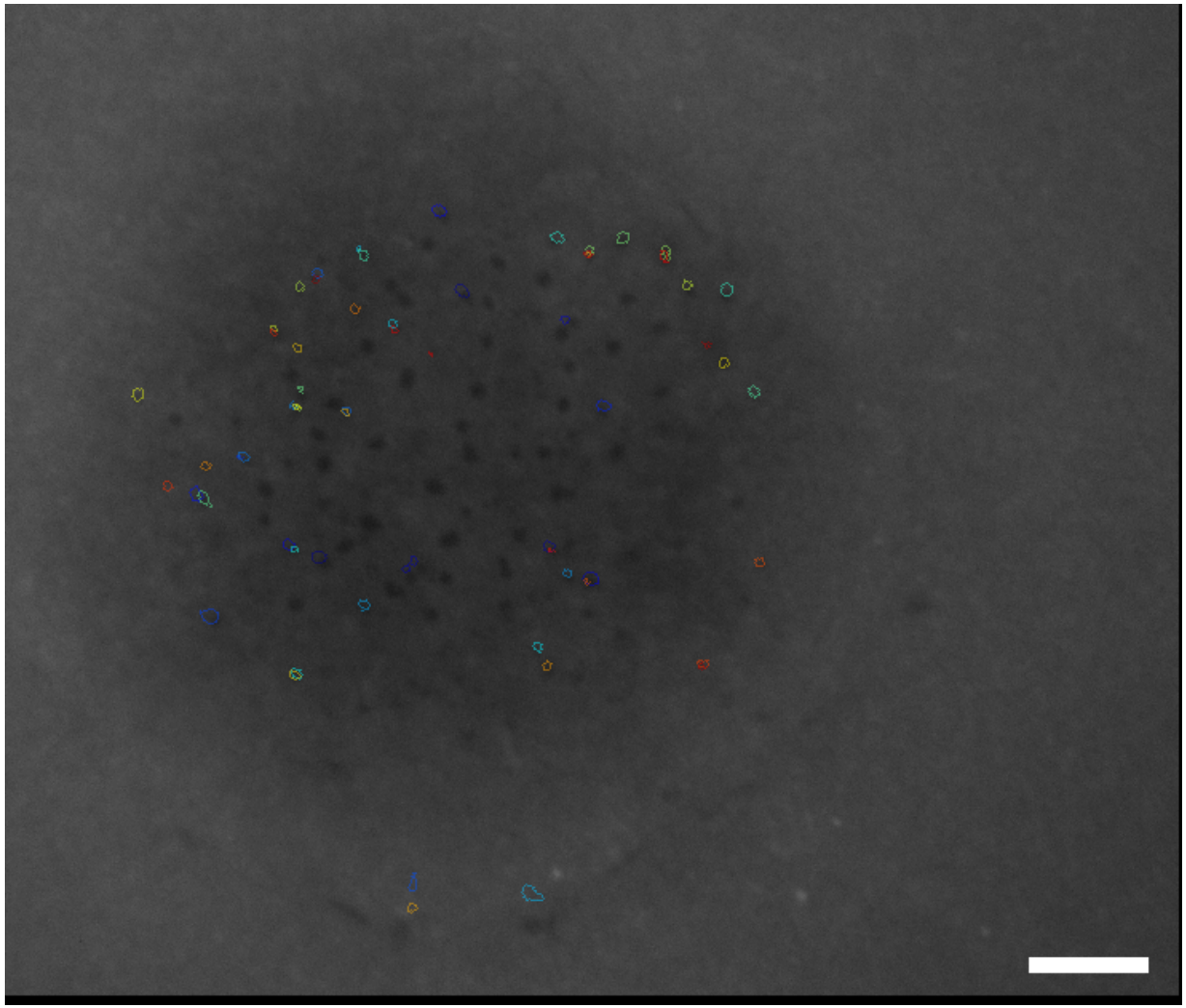
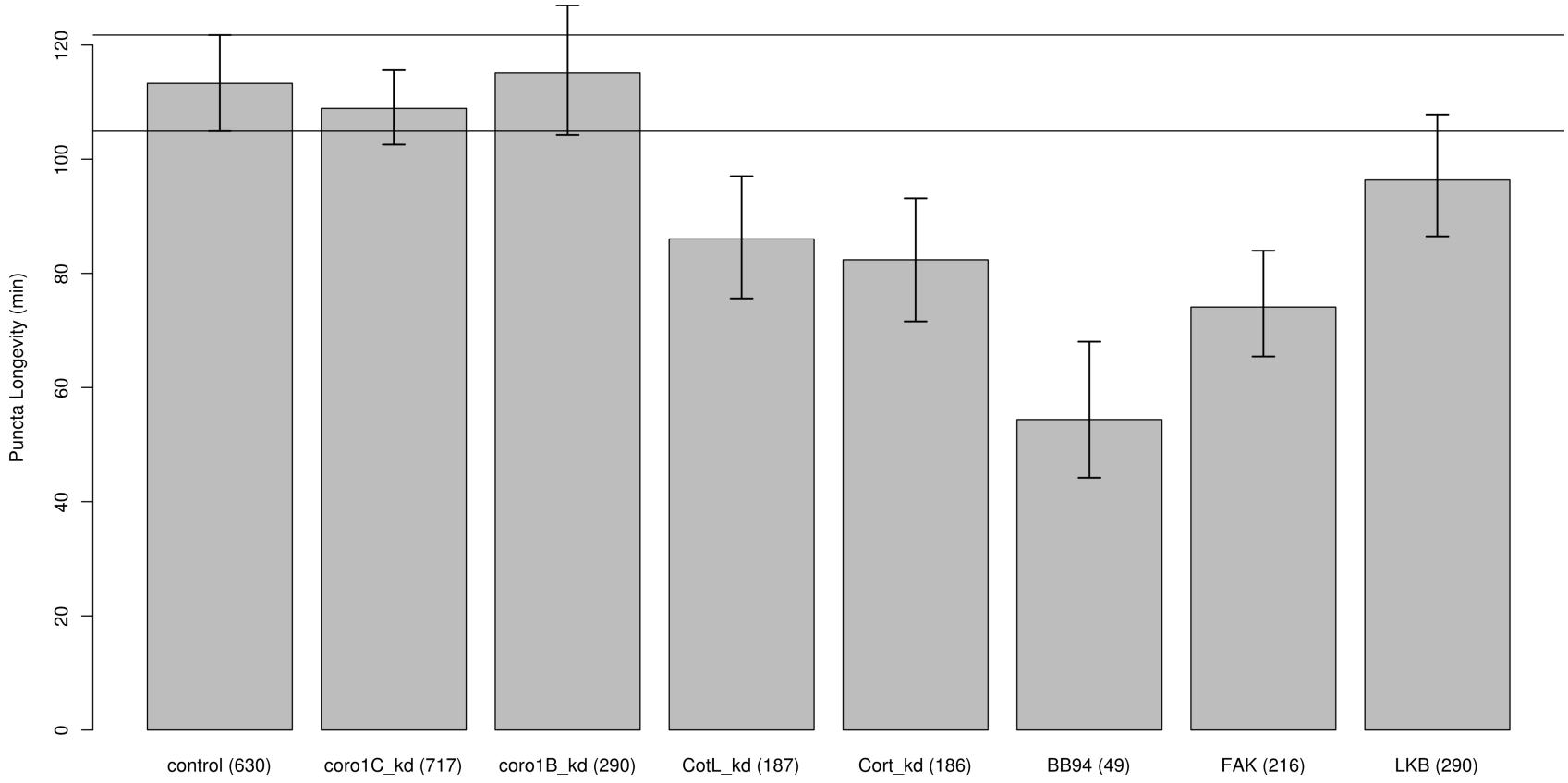


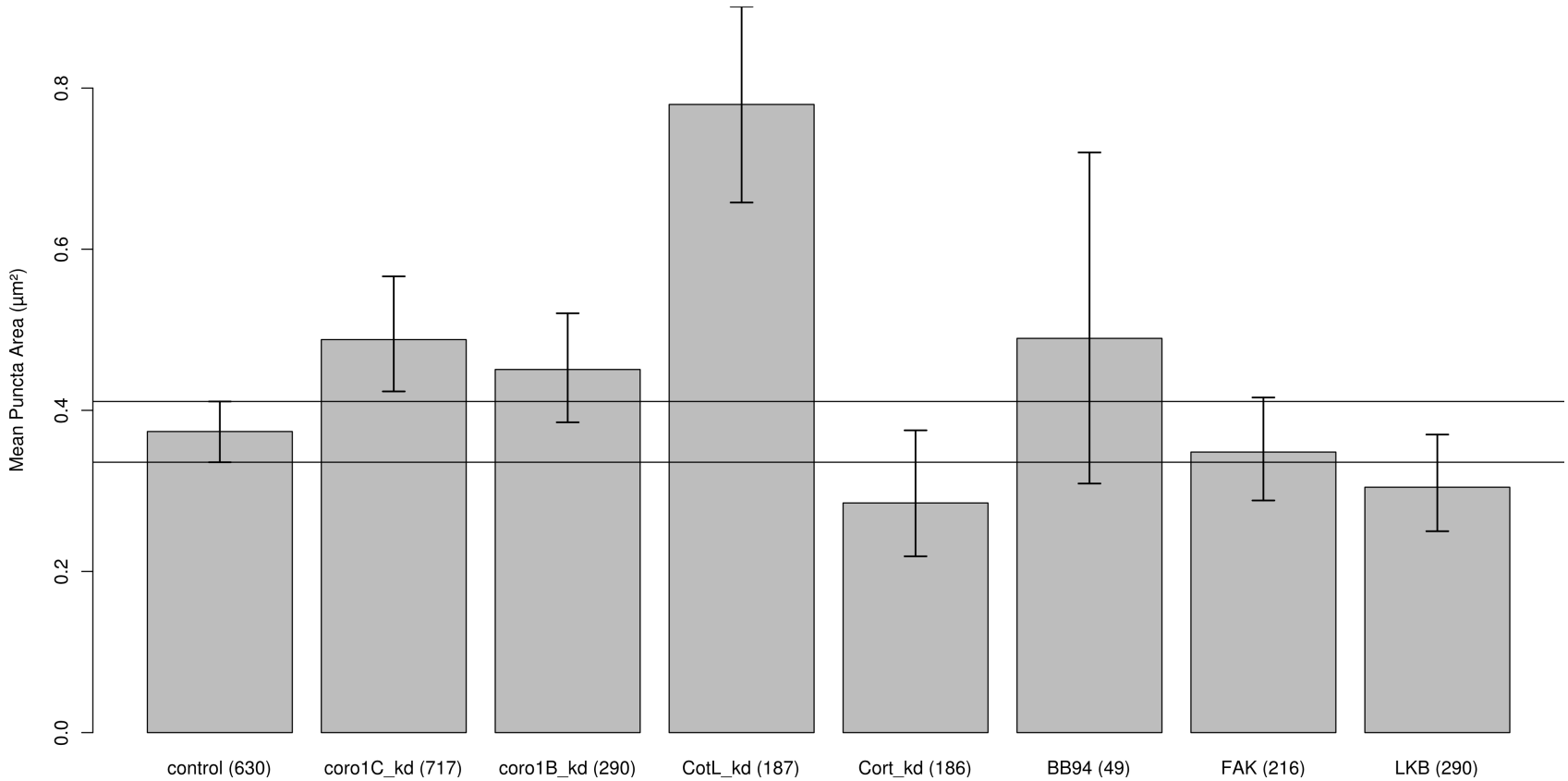
Data Overview

- with the puncta tracked and classified, we can start looking at invadopodia properties
- working with a set of knockdowns

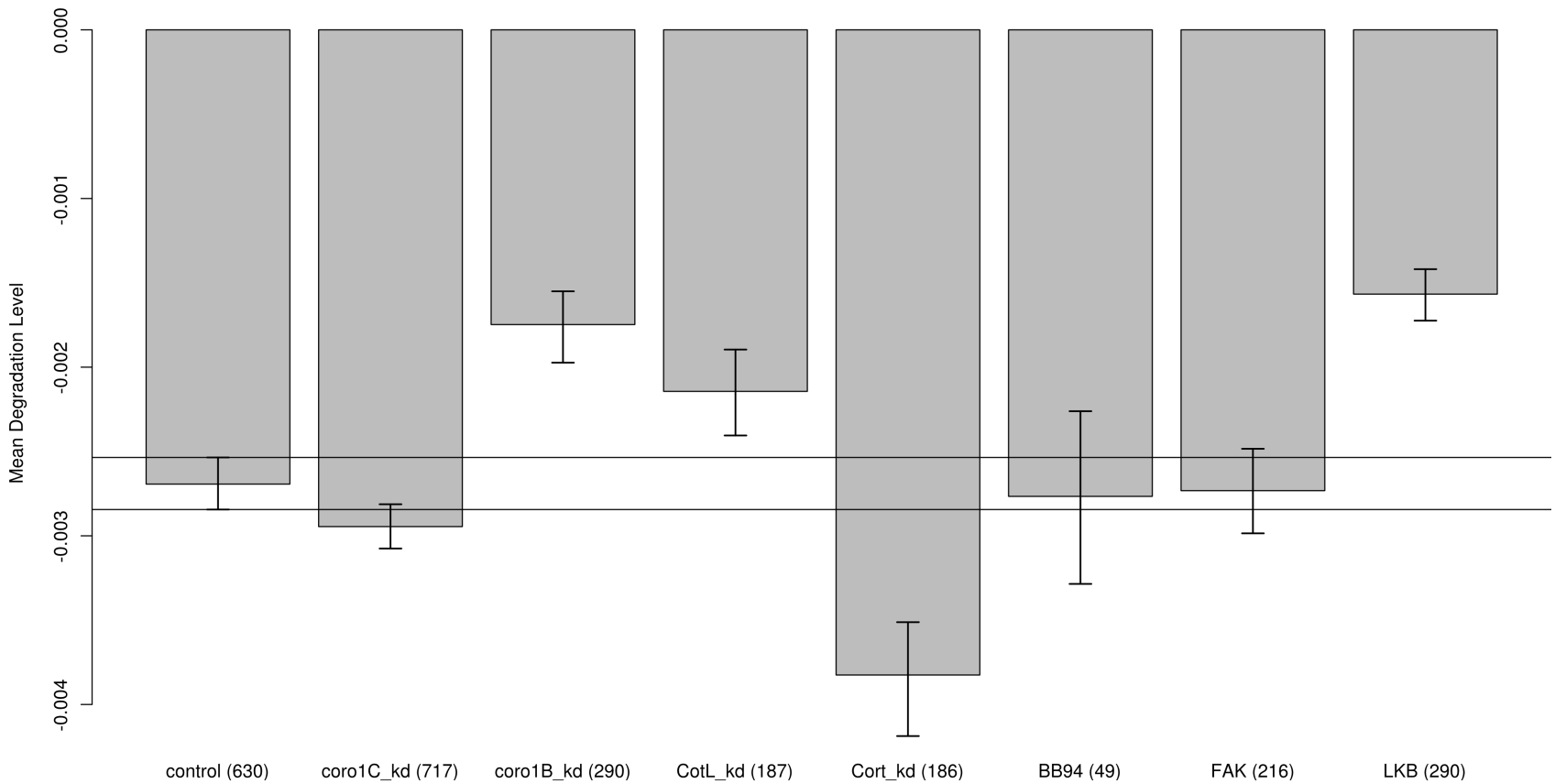
Longevity Comparisons



Mean Puncta Area Comparisons



Mean Local Difference Comparisons



Future Plans

- work on last few knockdowns
- track down false positives in BB94 cells
- add more invadopodia property comparisons (e.g. - distance from edge)
- implement invadopodia producing cells counting software