Accuracy in Fluorescent Cell Image Segmentation Algorithms

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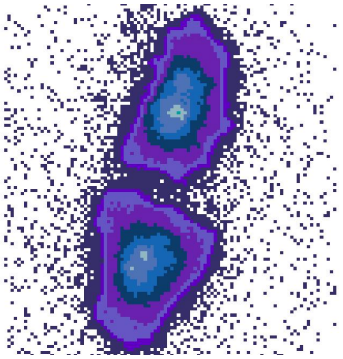
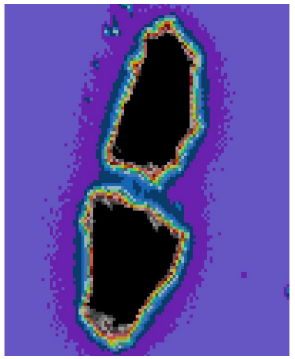
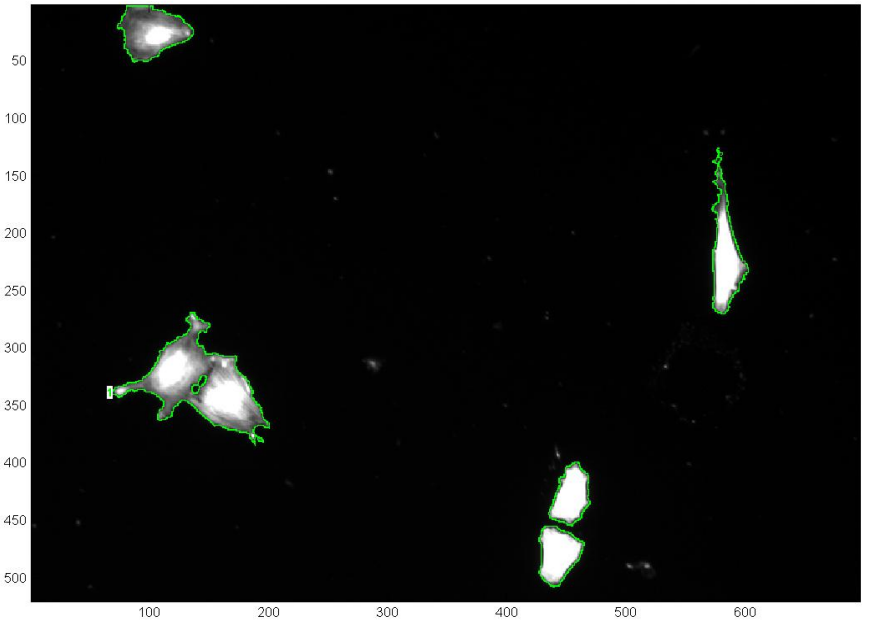
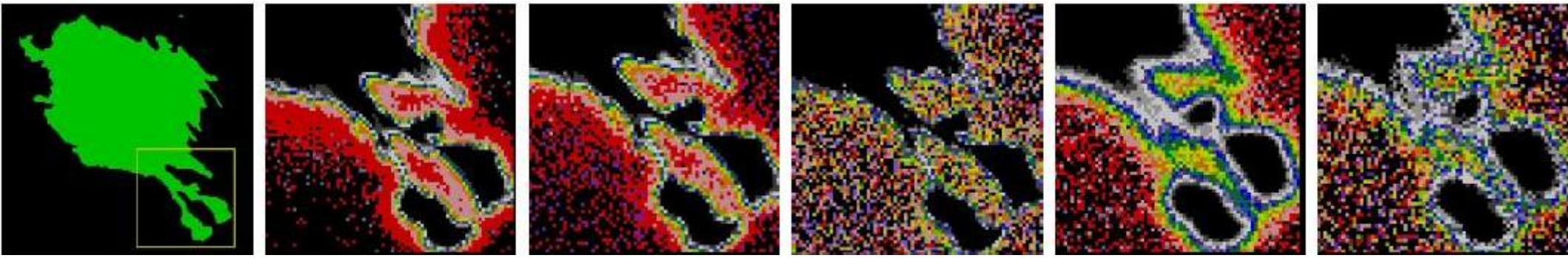






Outline – Segmentation Accuracy

- Image data created for this purpose
- Initial segmentation and visualization studies
- New segmentation method to create reference data for larger scale testing
- Extended Edge Neighborhood: new metric to analyze segmentation accuracy



Data Description

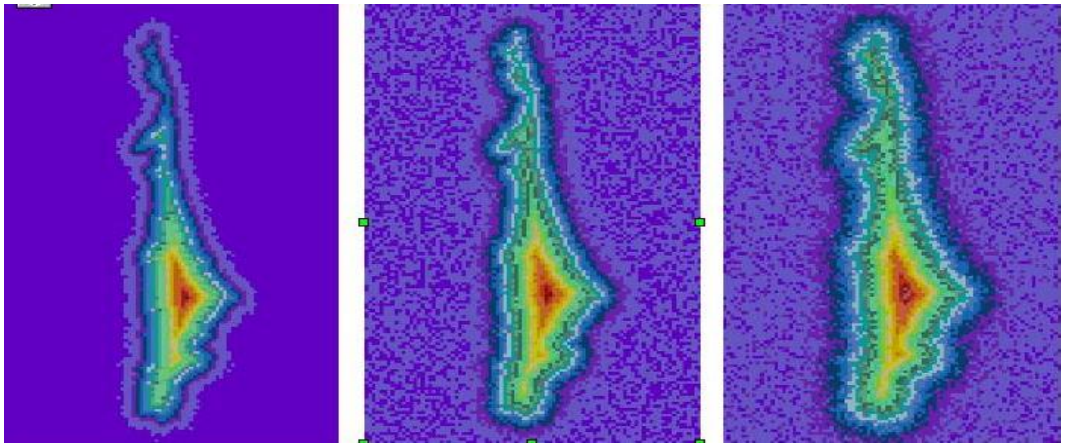
- 2 cell lines:

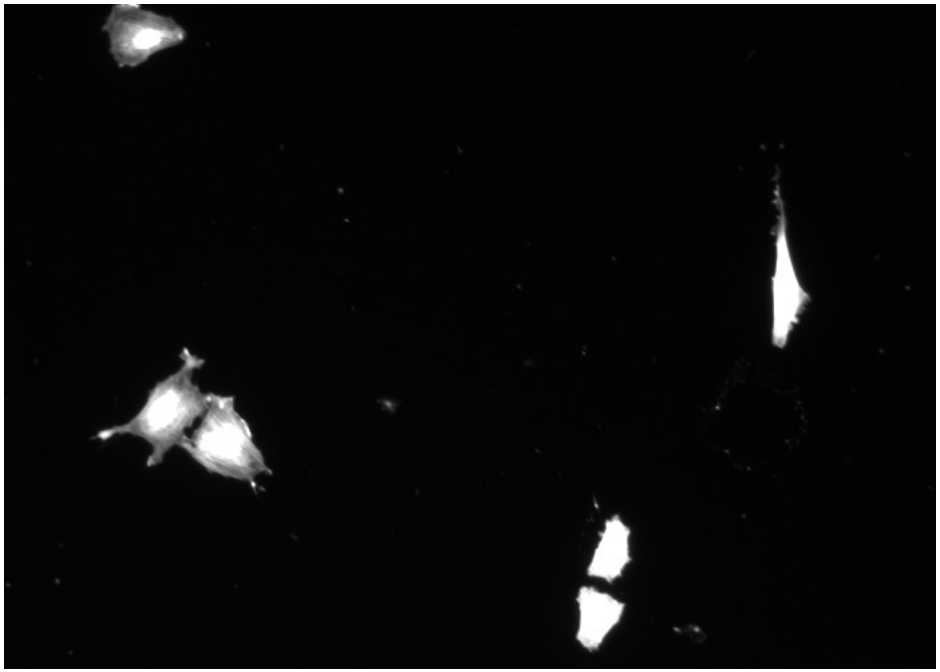
A10 smooth rat cells

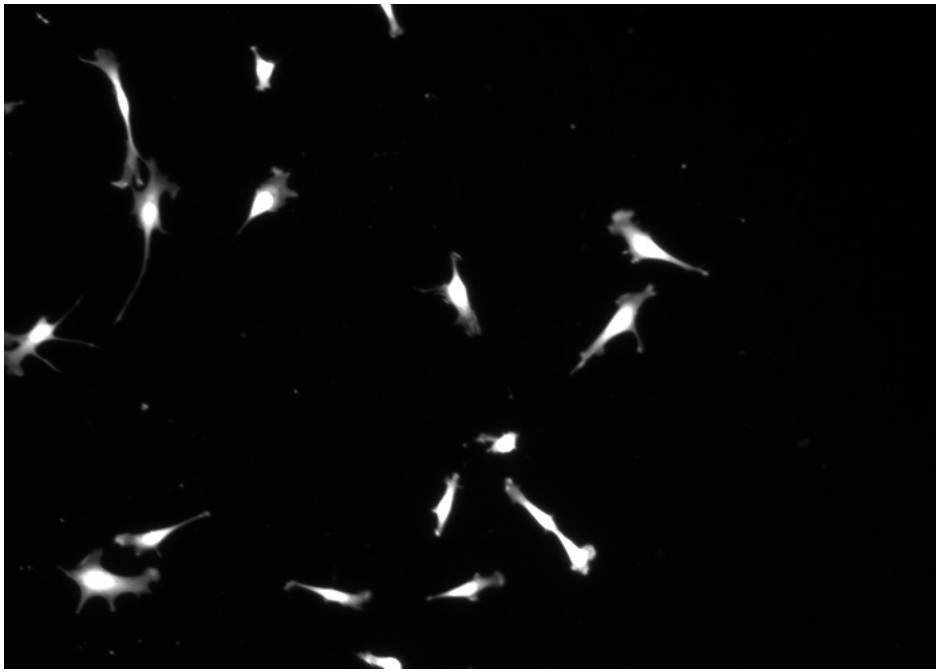
NIH3T3 fibroblasts

- 5 imaging conditions:

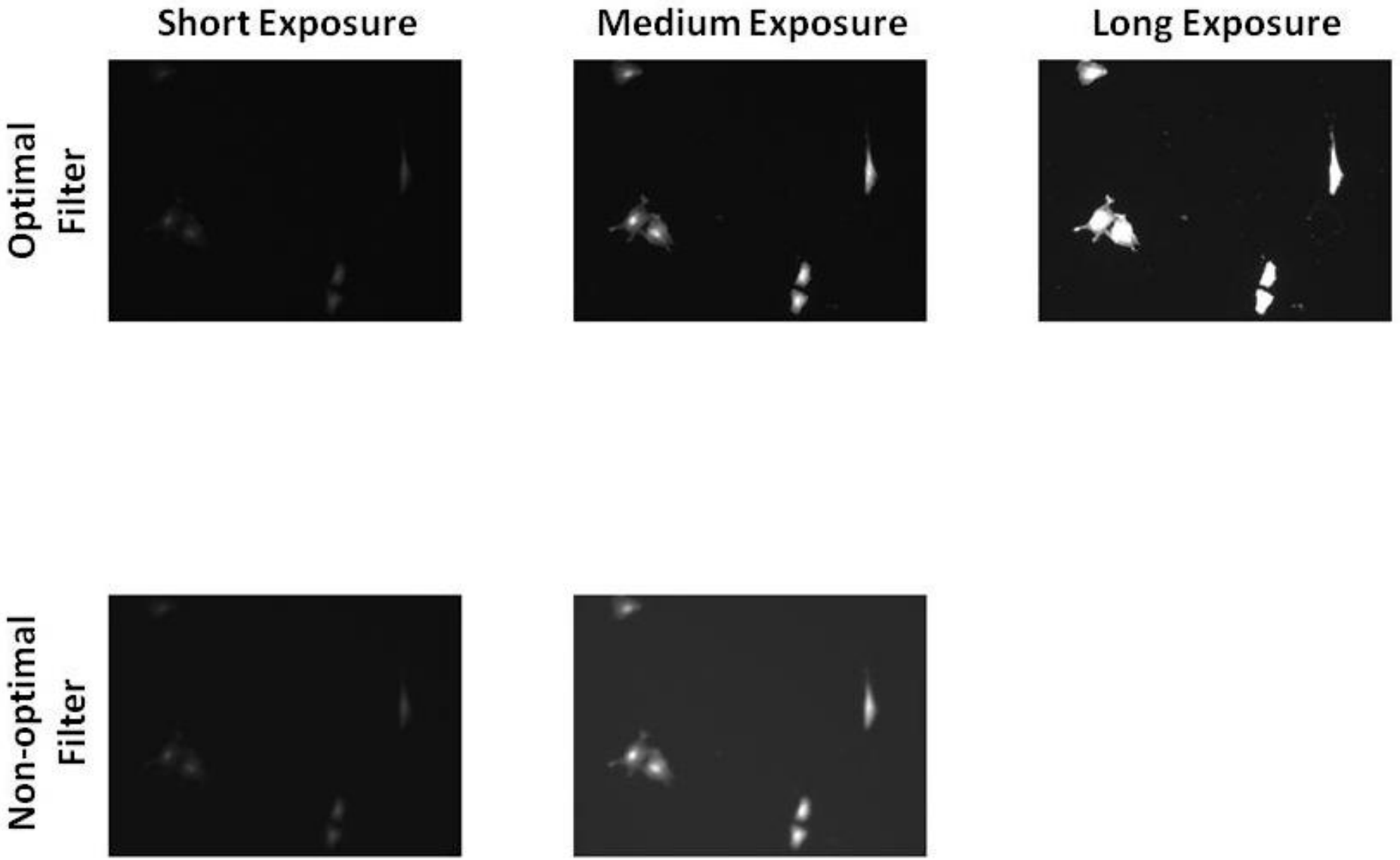
vary illumination; exposure





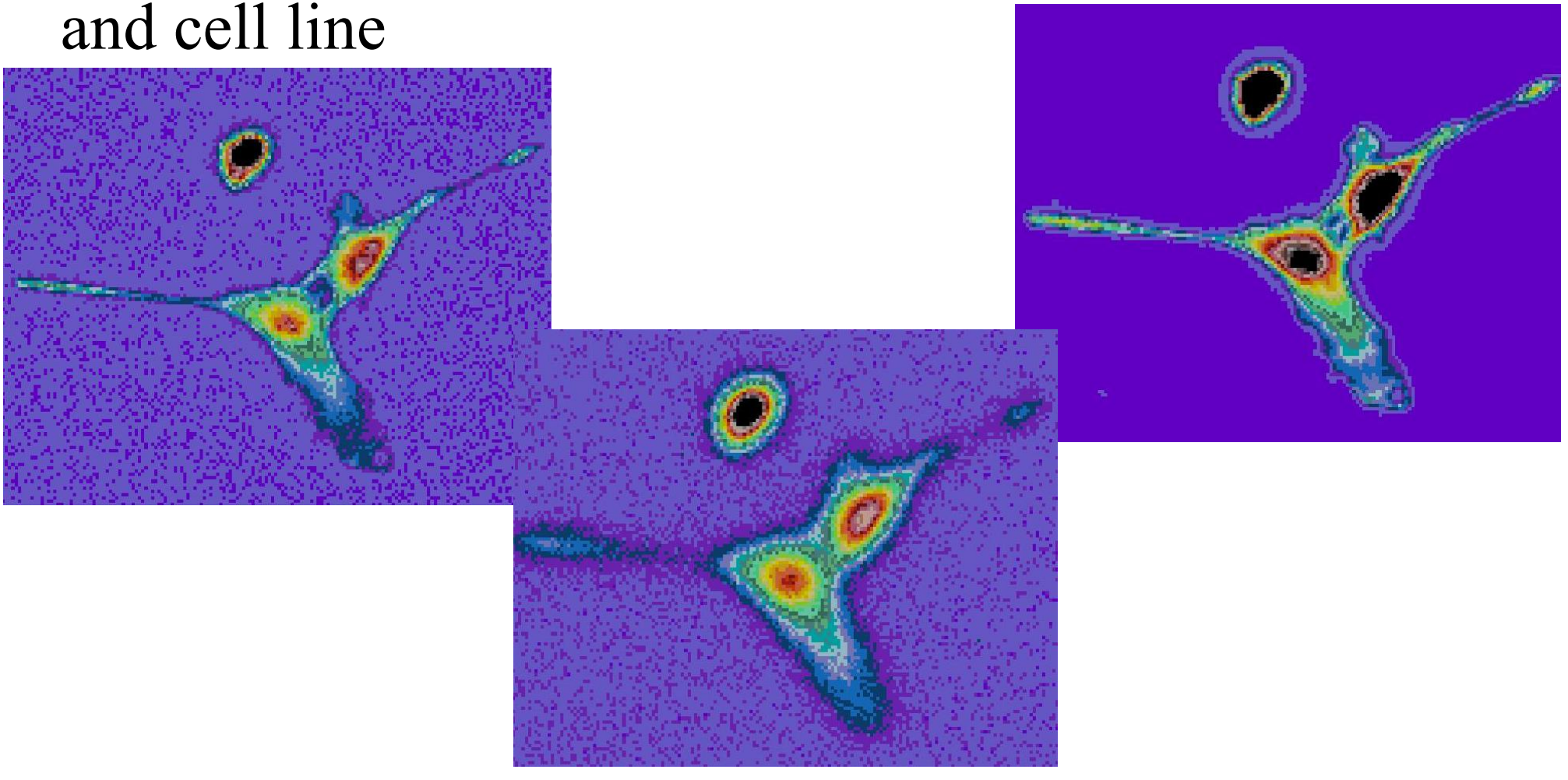


Imaging Conditions



Initial Segmentation Studies

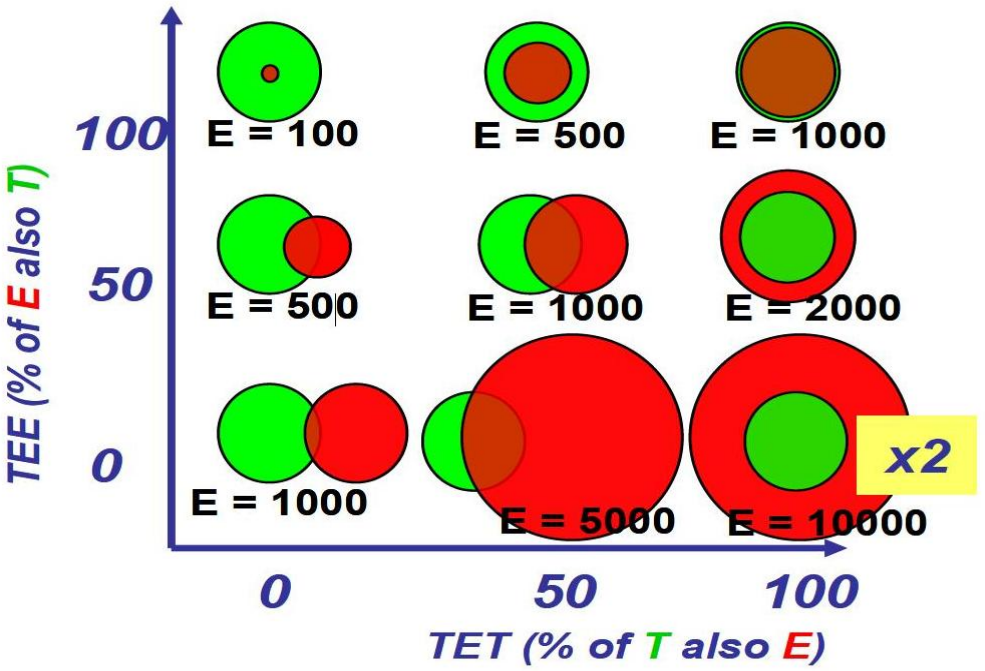
- Compare 9 segmentation algorithms, across imaging condition and cell line, 16 images, 71 cells
- Visualization of image clarity across imaging condition and cell line



Bivariate Indexes

- Many comparisons use Jaccard Similarity Index:
- Reference data set A, and segmentation mask B:

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}.$$



Bivariate Indexes

$$TET = |T \cap E|/|T|, 0.0 \leq TET \leq 1.0$$

$$TEE = |T \cap E|/|E|, 0.0 \leq TEE \leq 1.0$$

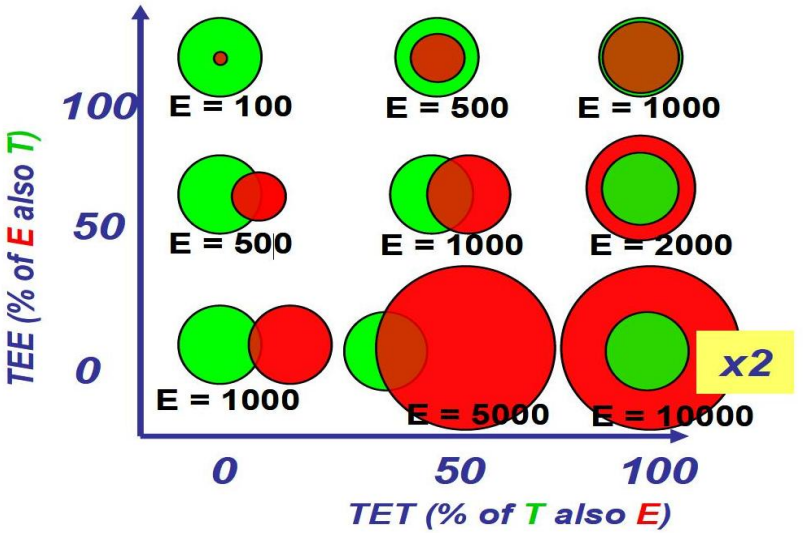
Divides performance into 4 regions:

Dislocation: TET and TEE small

Overestimation: TET large, TEE small

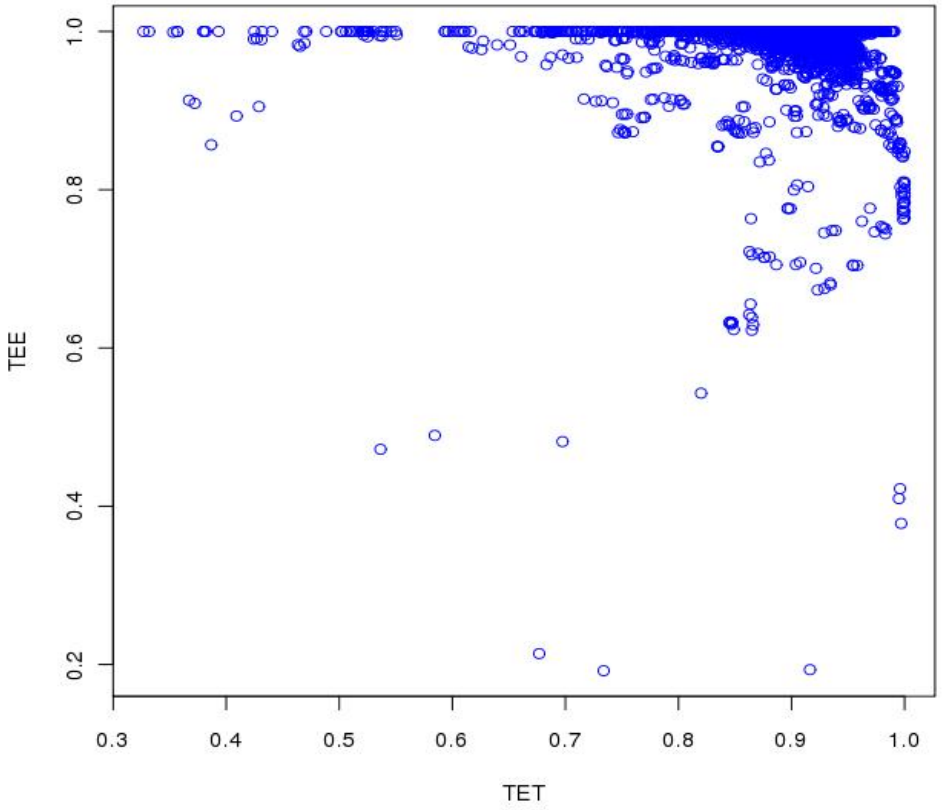
Underestimation: TET small, TEE large

Good: TET, TEE large



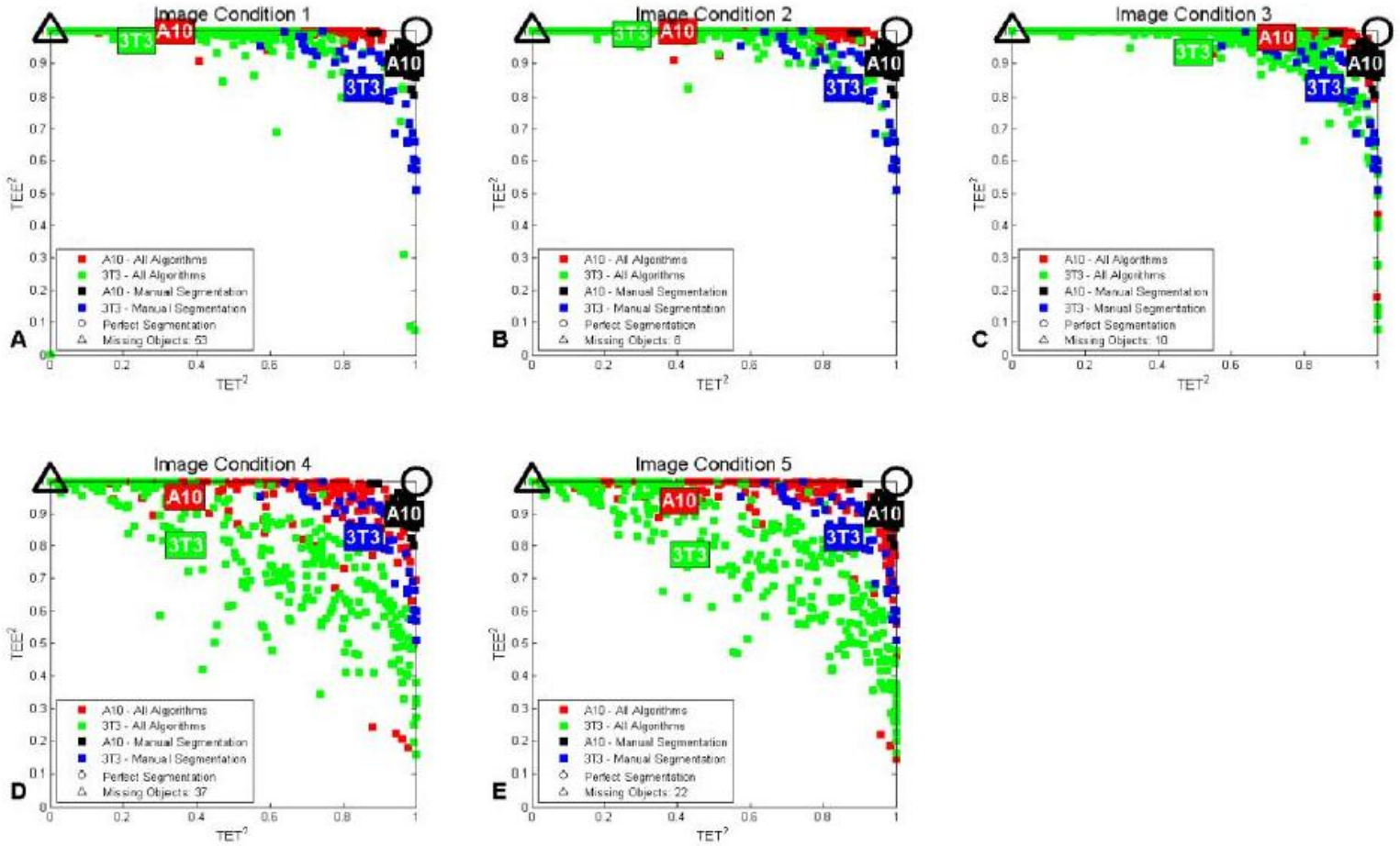
Example of Bivariate Evaluation

- 5-means clustering undersegments, in general
- Define a Segmentation Distance to (1.0,1.0) perfect segmentation

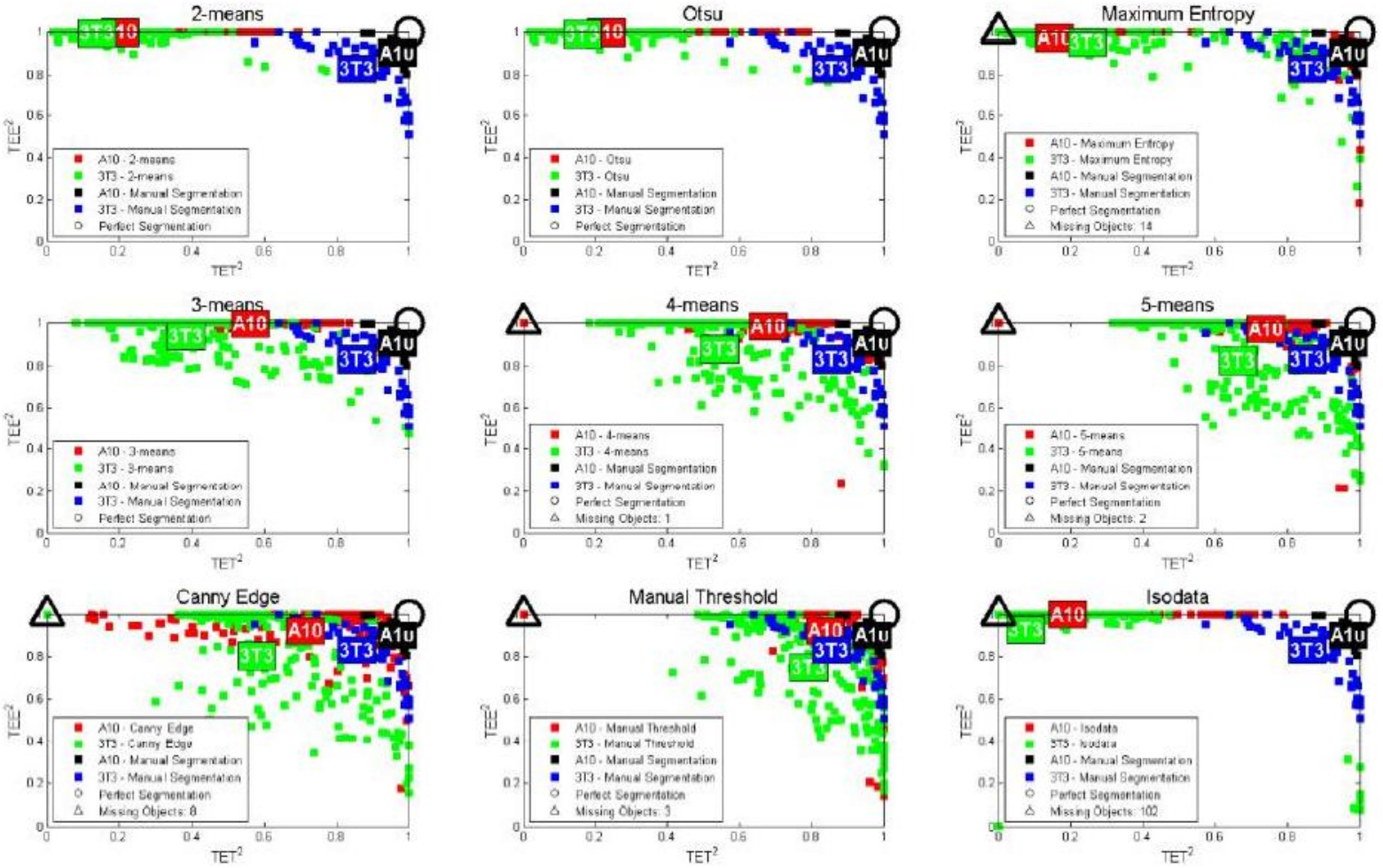


Initial Results – 71 Cells

- Segmentation accuracy: related to both imaging condition and cell geometry

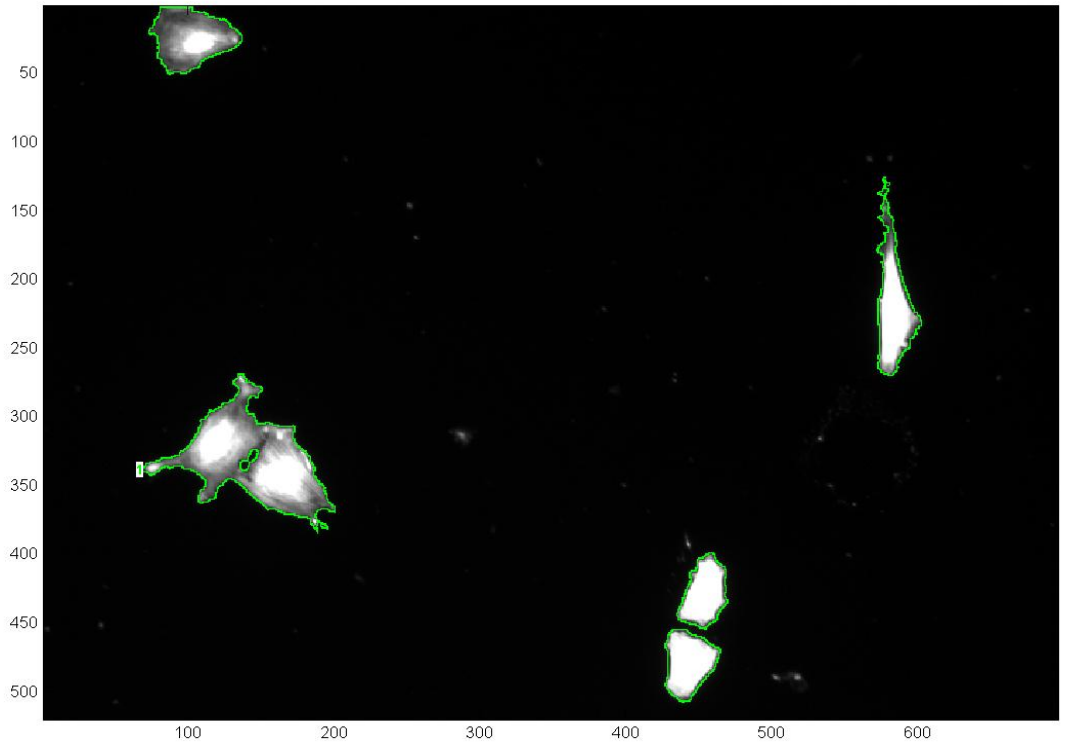


Initial Results: 9 Segmentation Methods



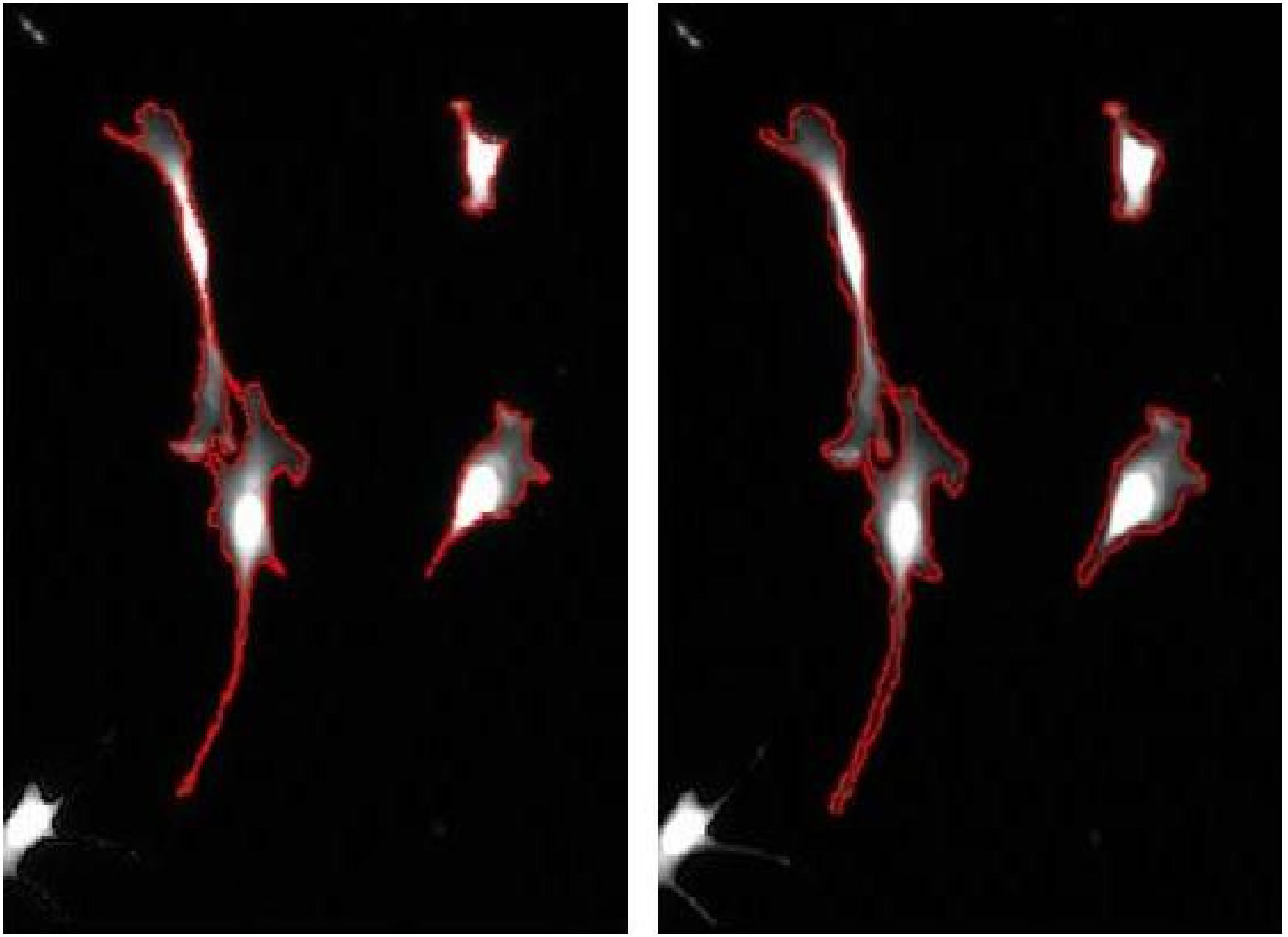
Scaling up to 40000 cells

- Reference data: currently collected manually
- New segmentation technique allows us to collect reference data automatically, based on human vision: how the eye determines a boundary

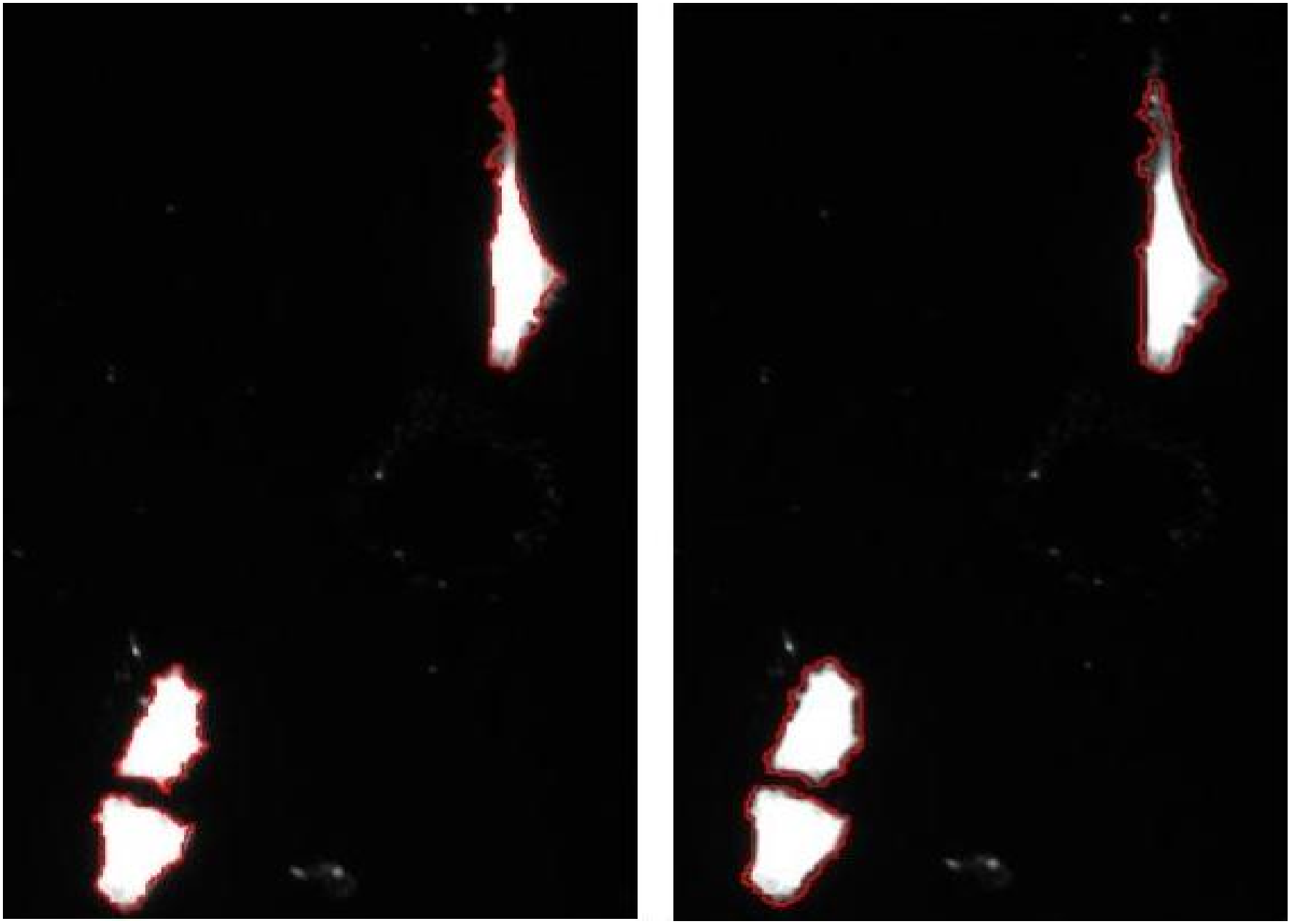


Consistency in Manual Segmentation

- Analyze reference data sets created manually

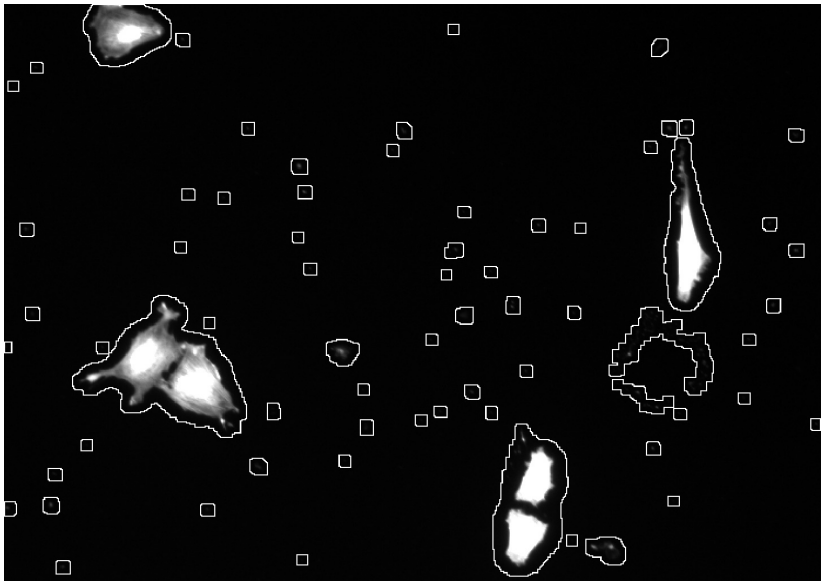


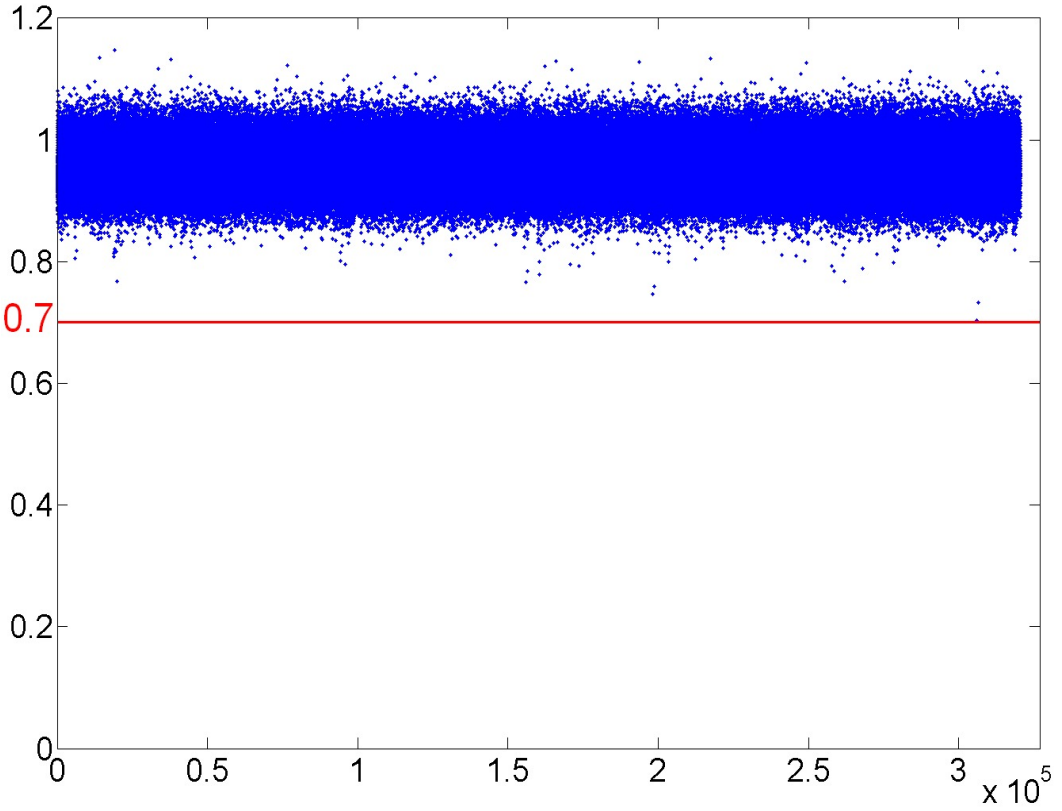
Consistency in Manual Segmentation



Defining How Humans Look at Cell Edges

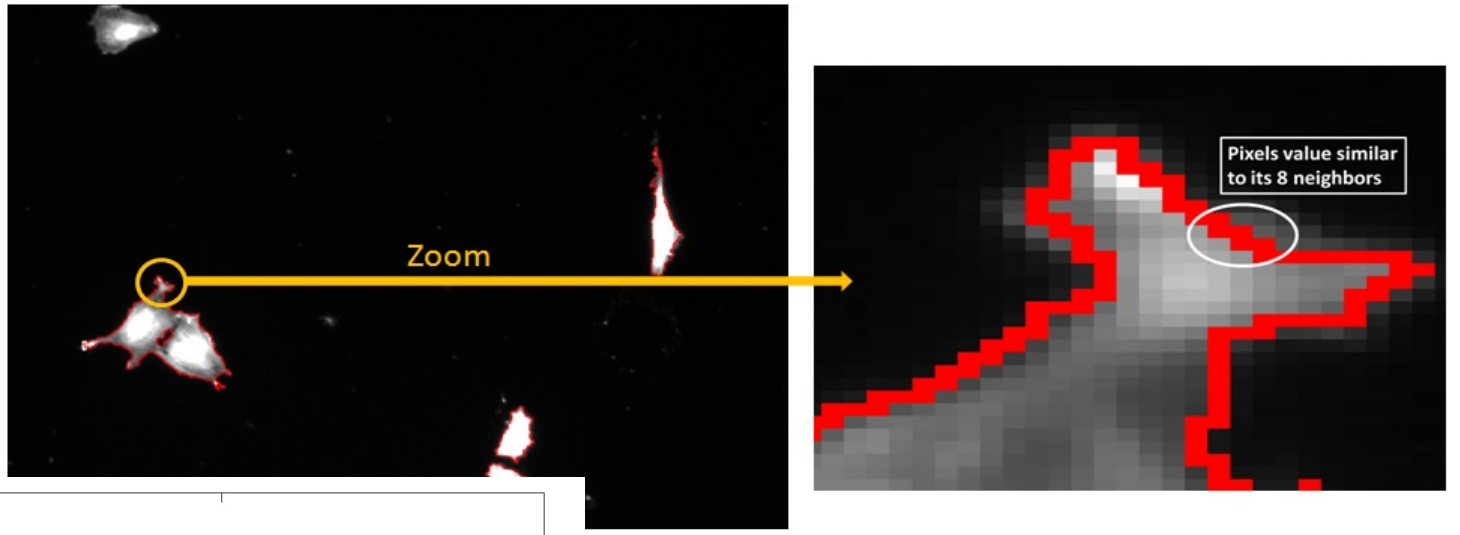
- Background pixel intensities should be close to one another

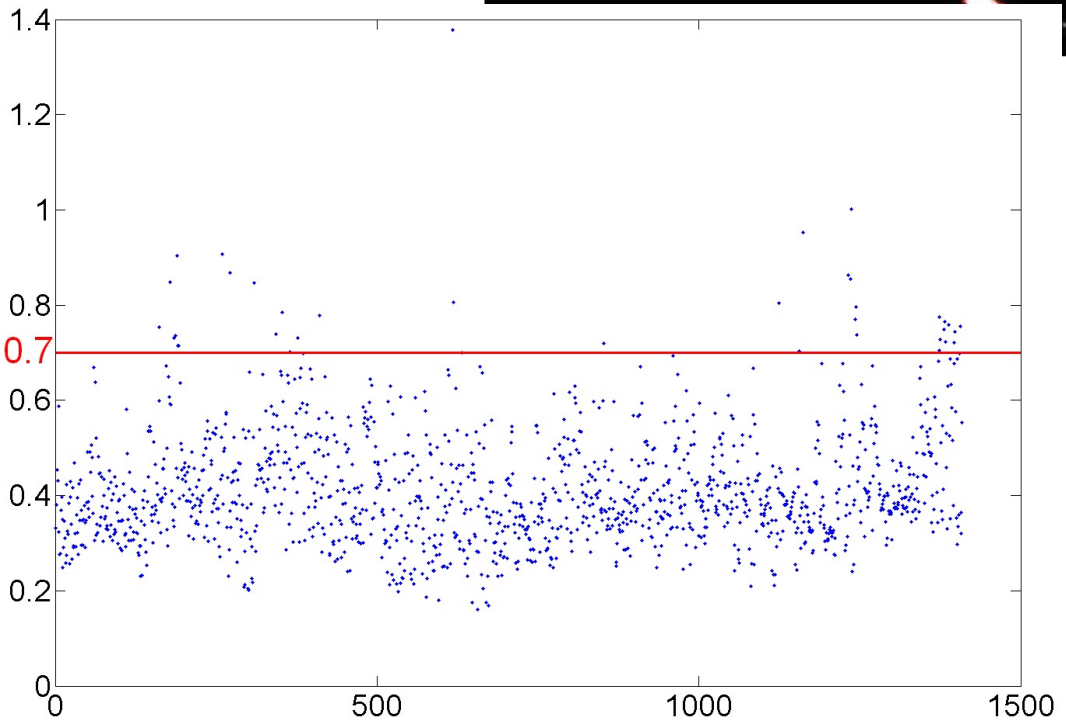




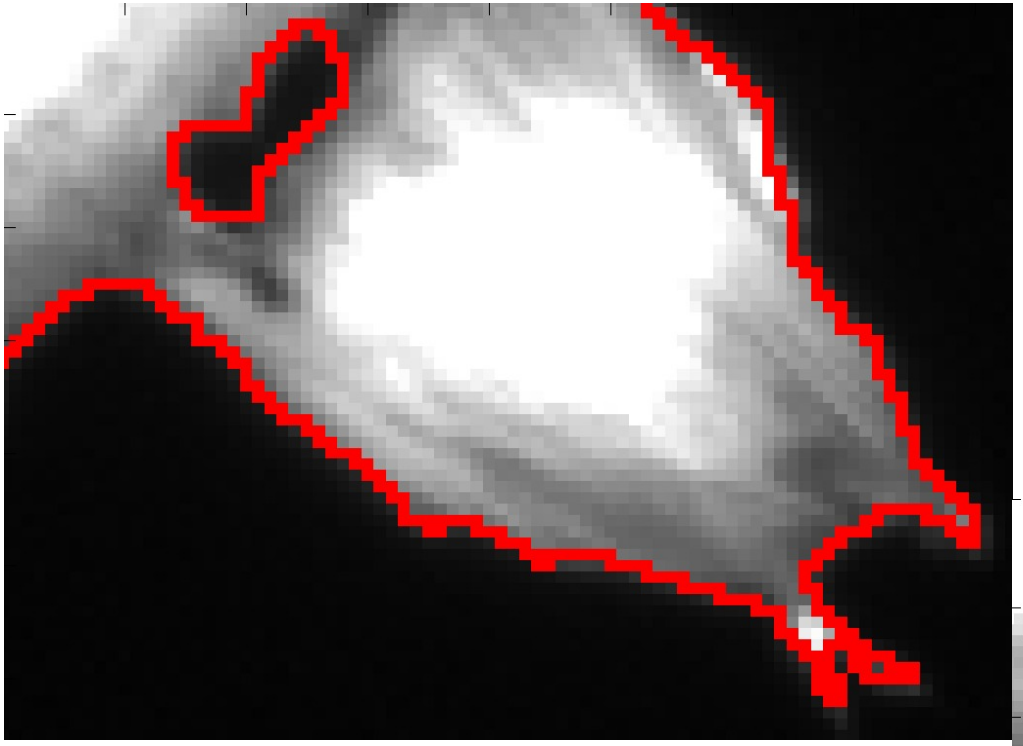
Defining How Humans Look at Cell Edges

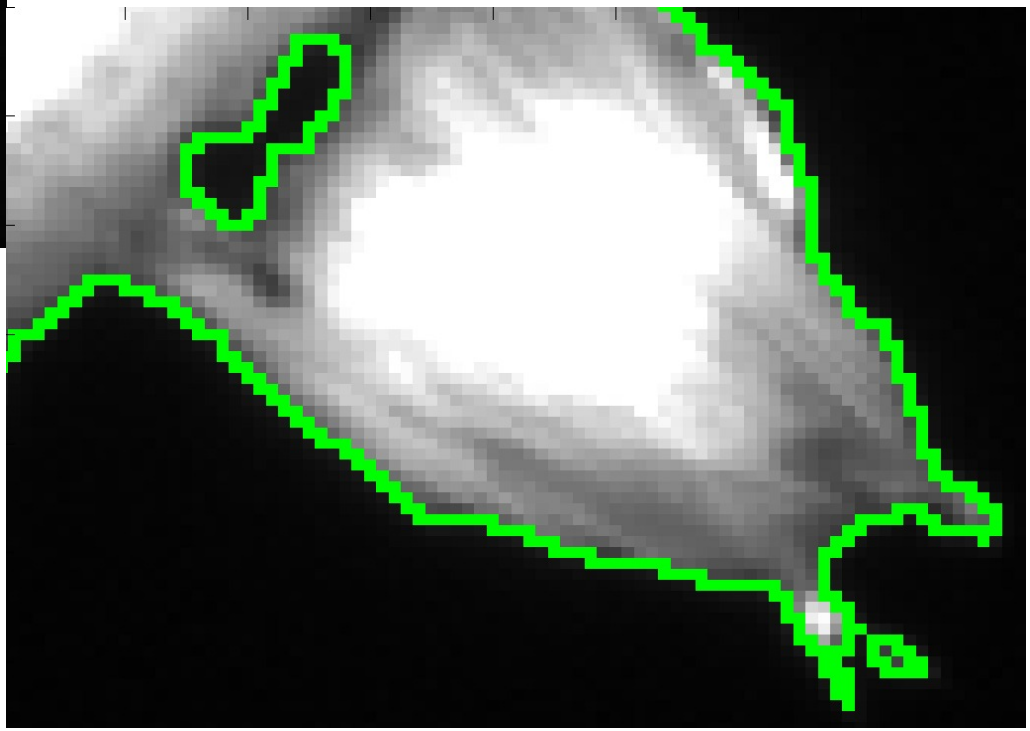
- Edge pixel intensities should vary by a lower ratio





Compare with Manual Segmentation





Bivariate Analysis Manual Segmentation

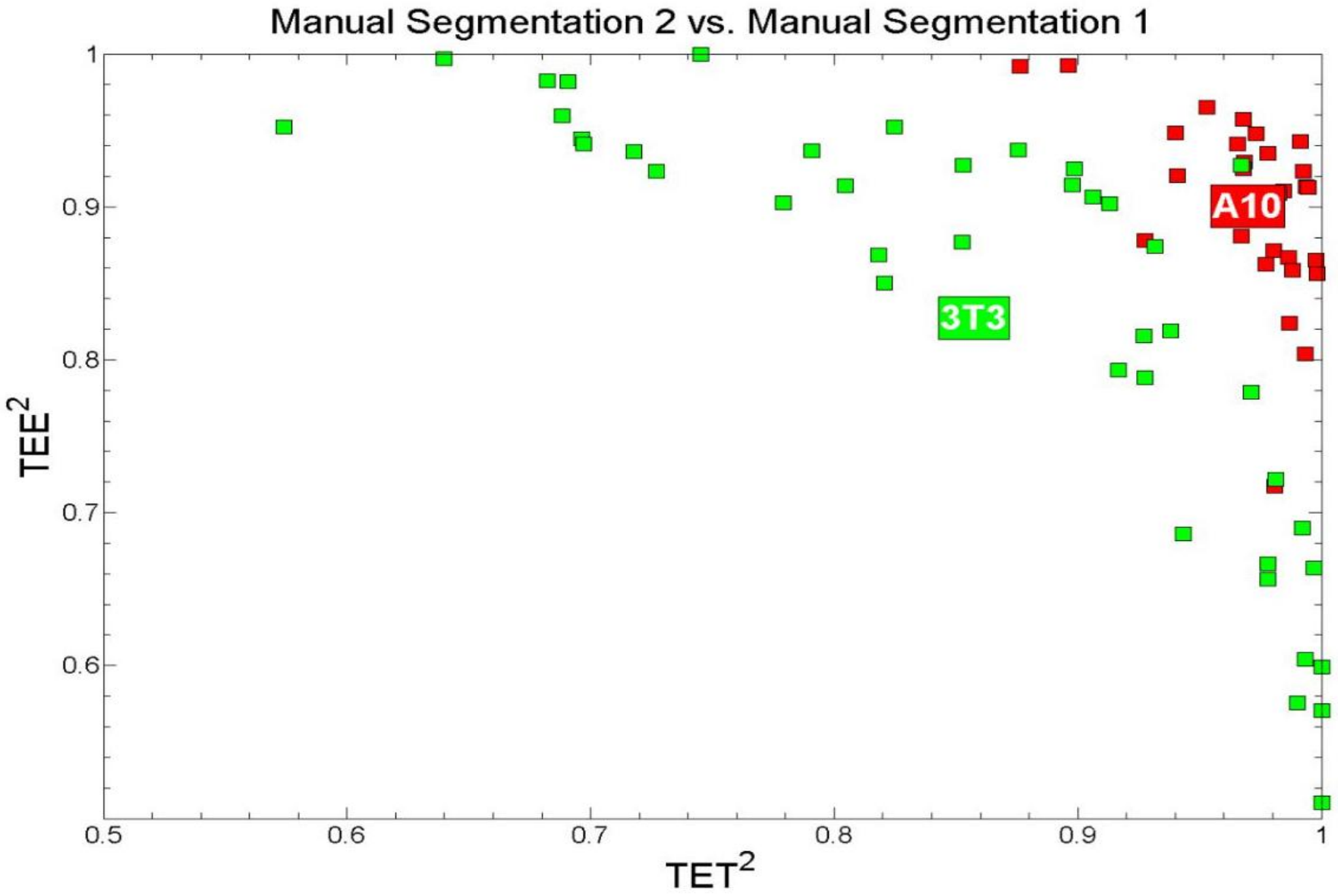
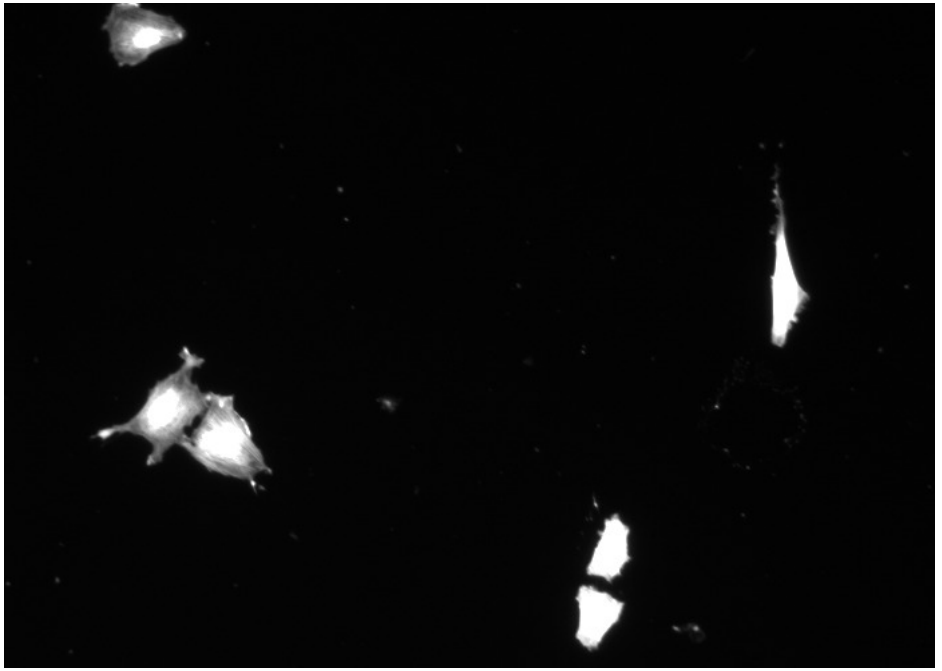
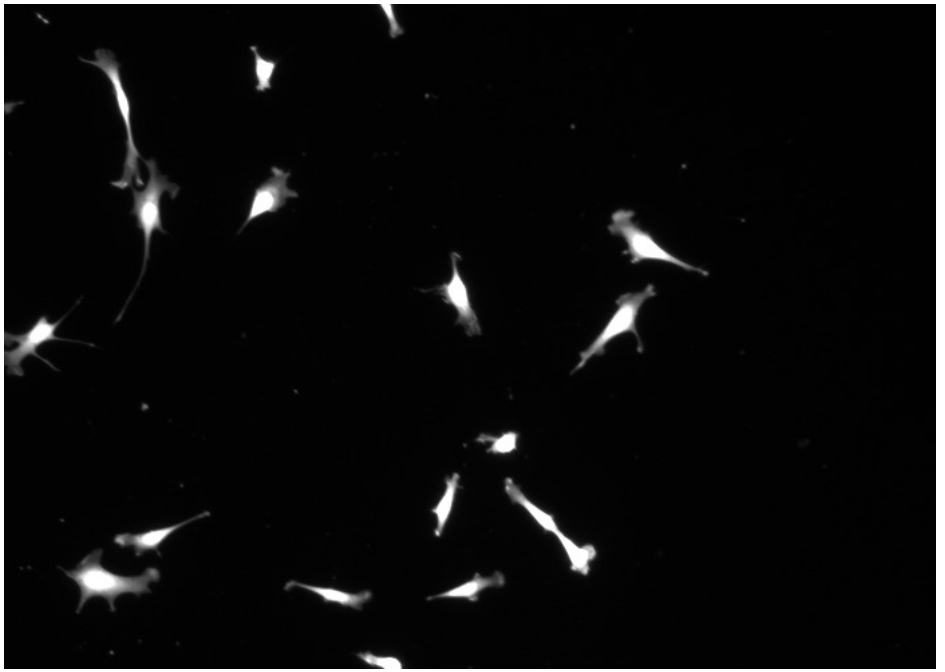


Image Features for Consistent Manual Segmentation

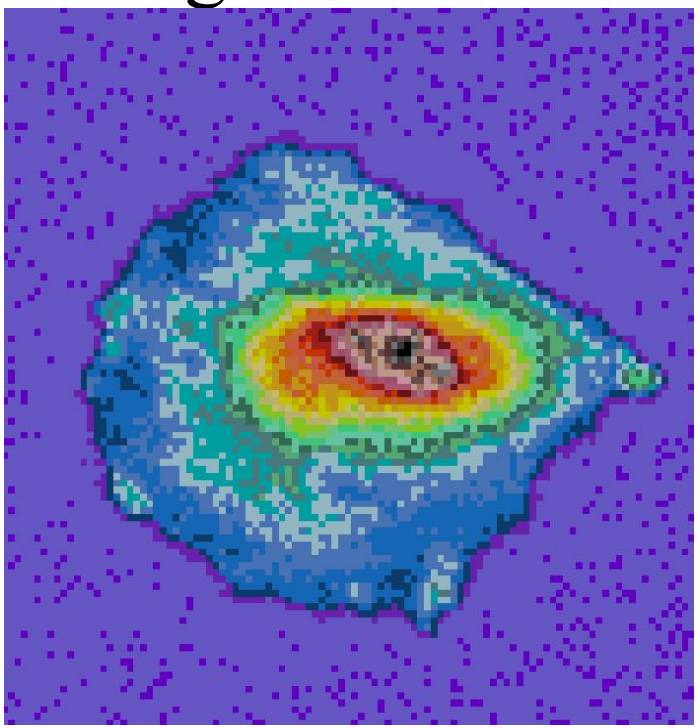
- Cell Size
- Roundness: Perimeter to Area Ratio

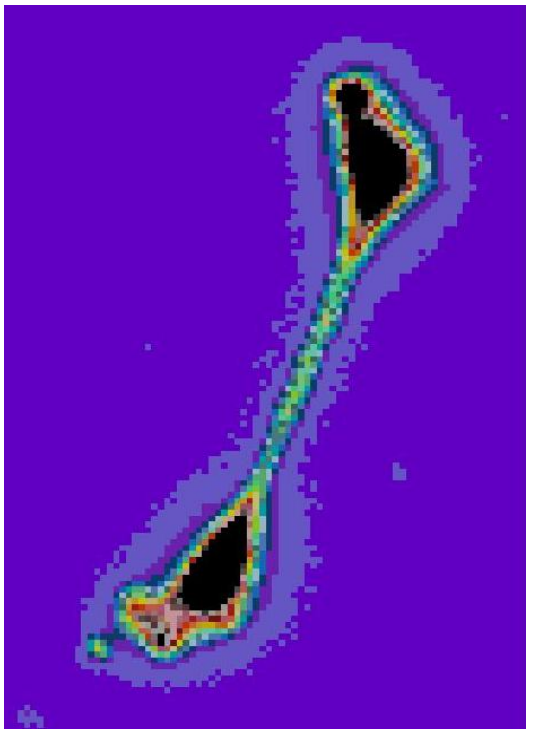


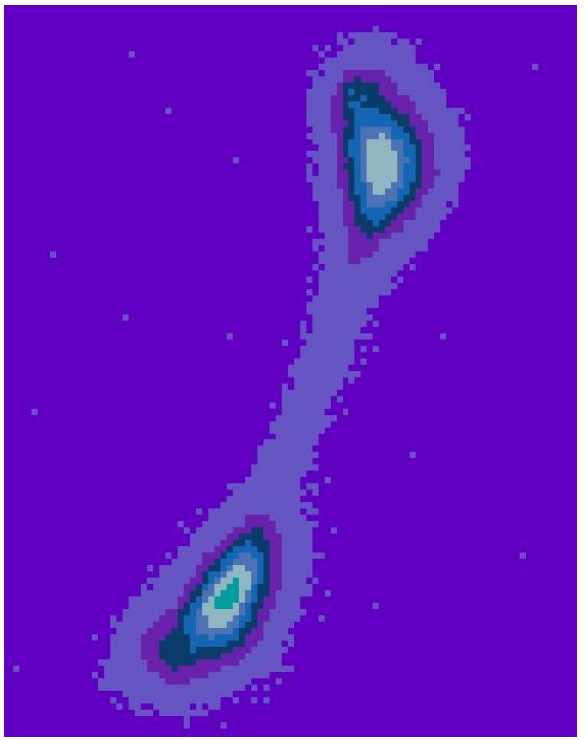


Extended Edge Neighborhood

- Fraction of pixels near cell edge, at risk for segmentation error
- Function of edge quality, defined by gradient
- Function of cell geometry: are most pixels at the edge?

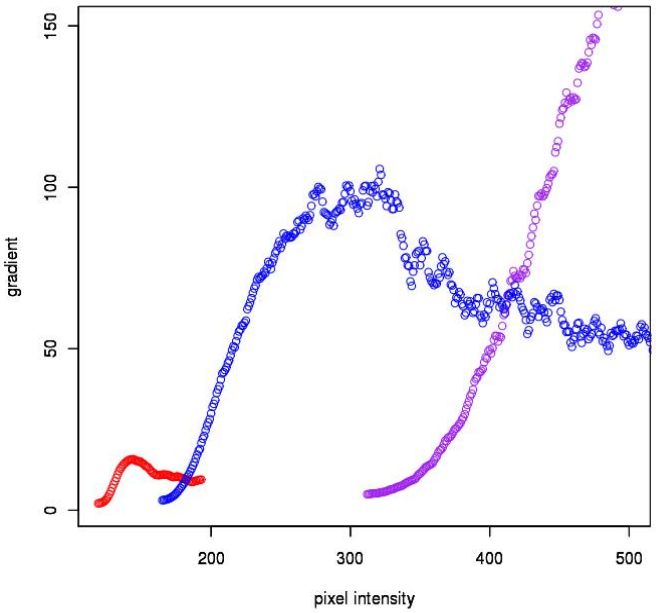


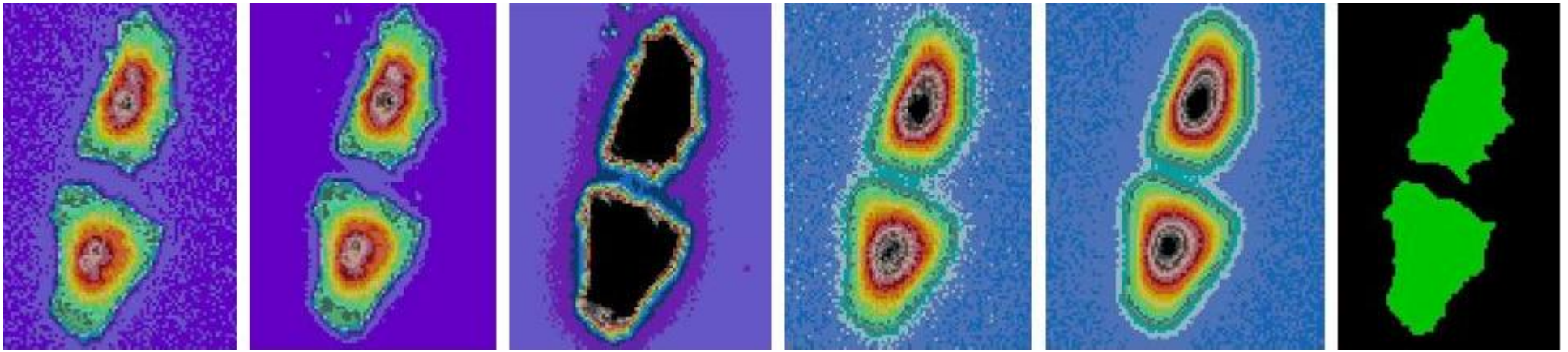




Edge Quality Calculation

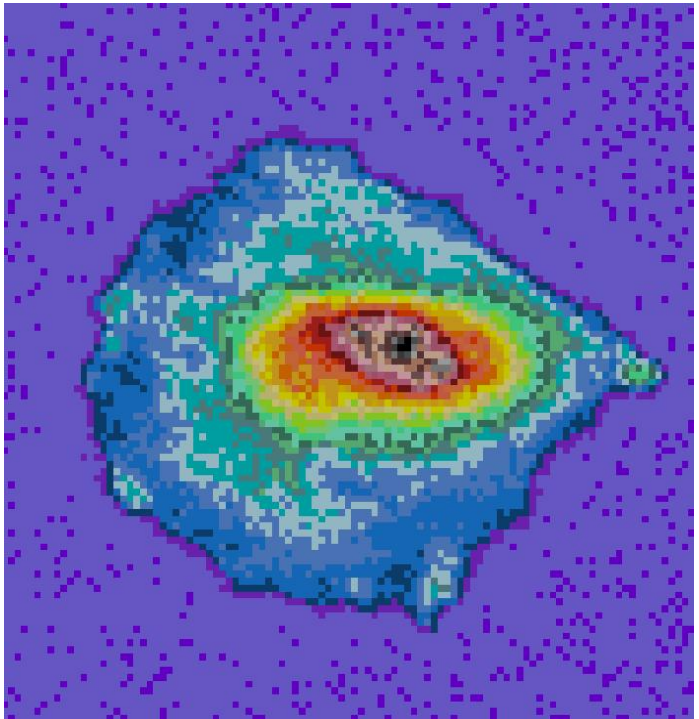
- Based on the pixel intensity gradient at the cell edge
- Used to measure thickness of cell edge

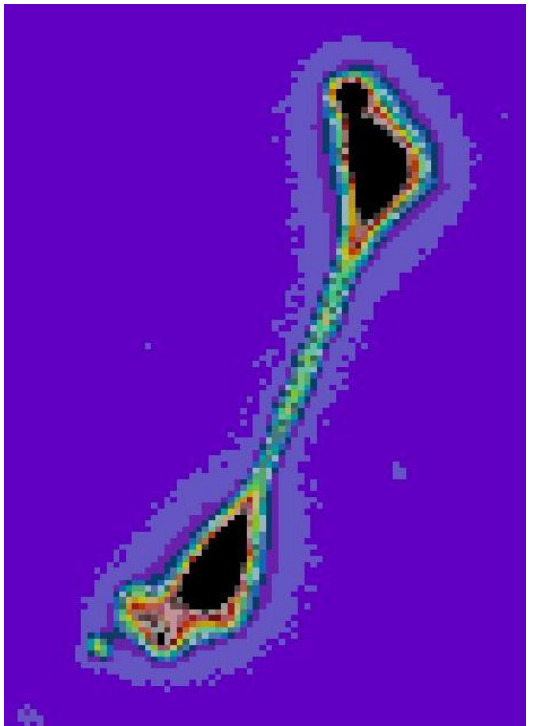


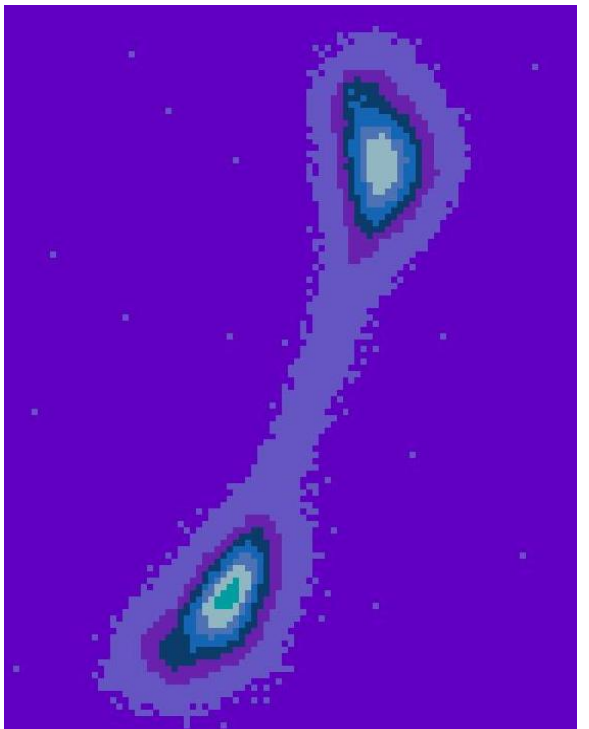


Extended Edge Neighborhood (EEN)

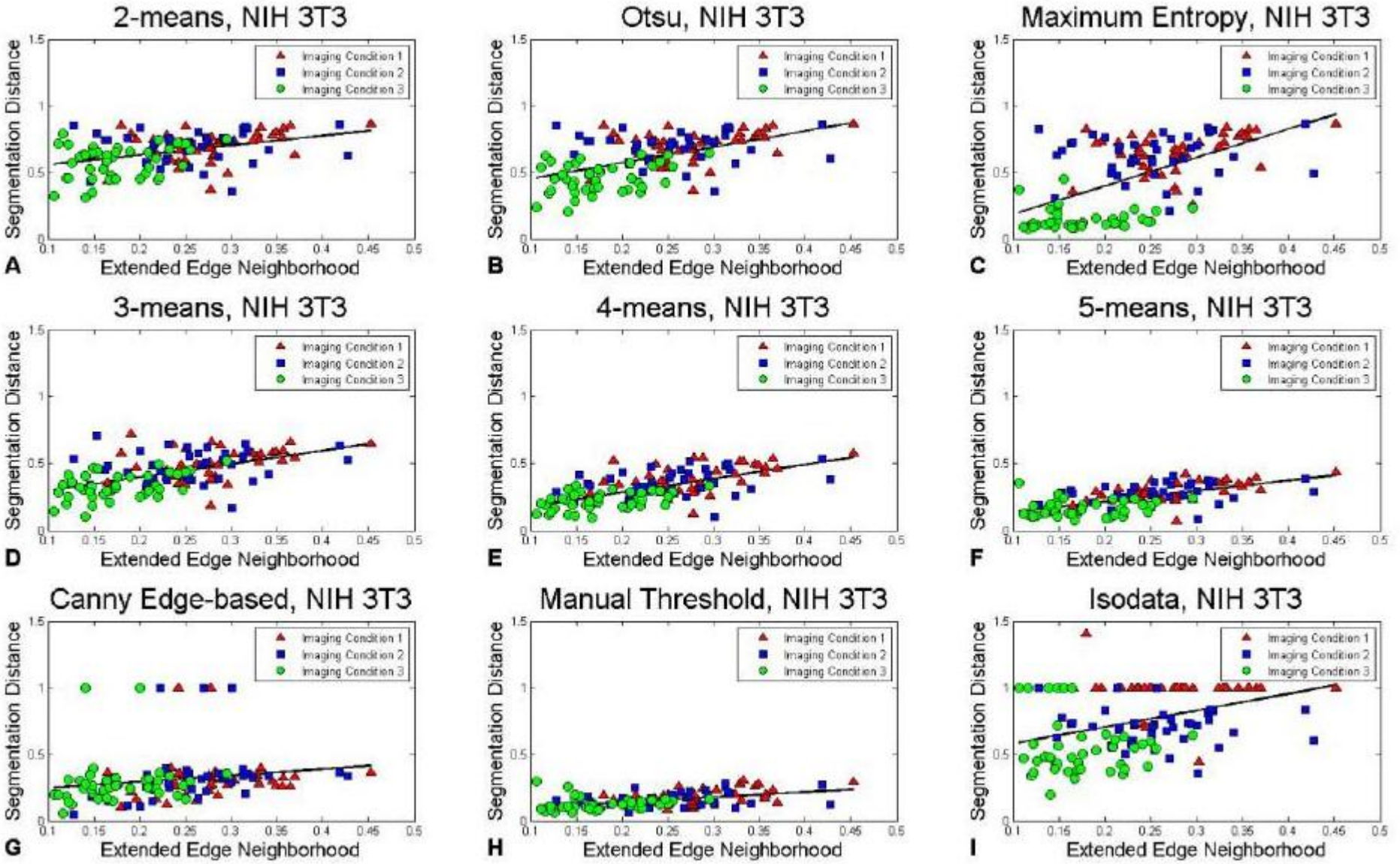
- Edge pixels = thickness of edge * perimeter
- Ratio of Edge pixels/Total pixels
- EEN: 0.1 (high edge quality, large, round)
  EEN: 0.4 (high edge quality, small, spindly)
  EEN: 1.0 (low edge quality, small, spindly)





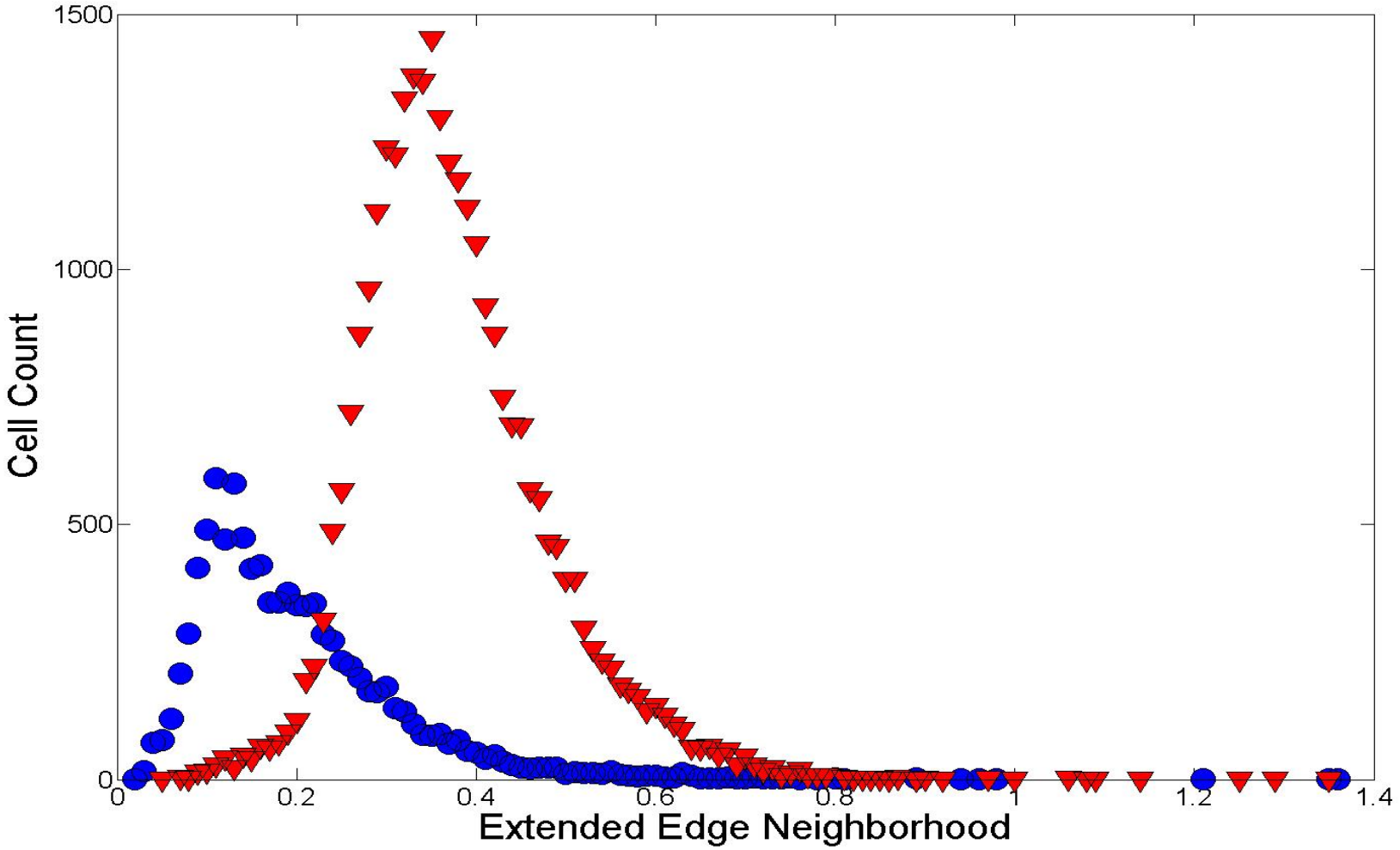


Previous Studies: EEN

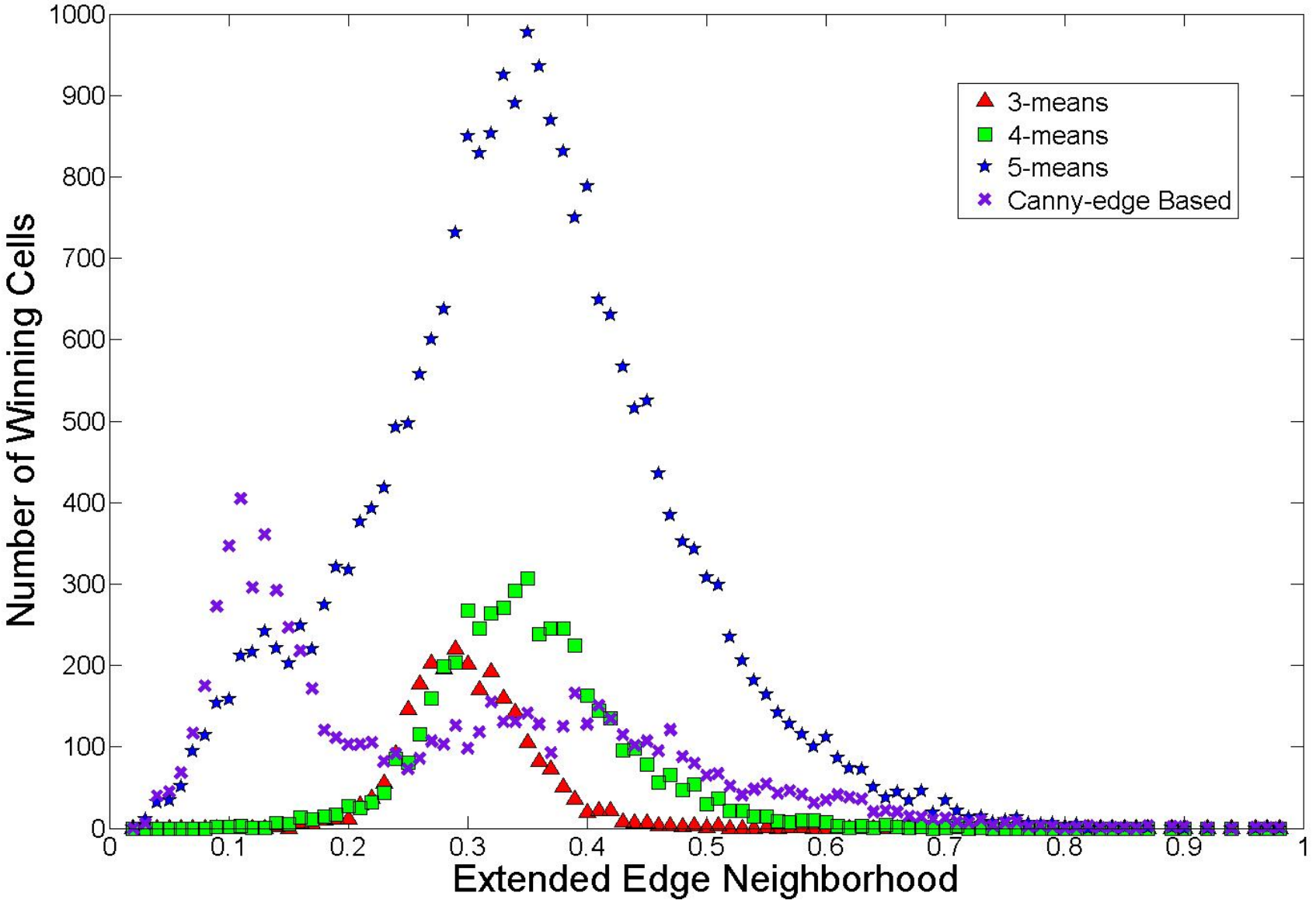


Best Algorithms From Previous Study: 40 000 Cells

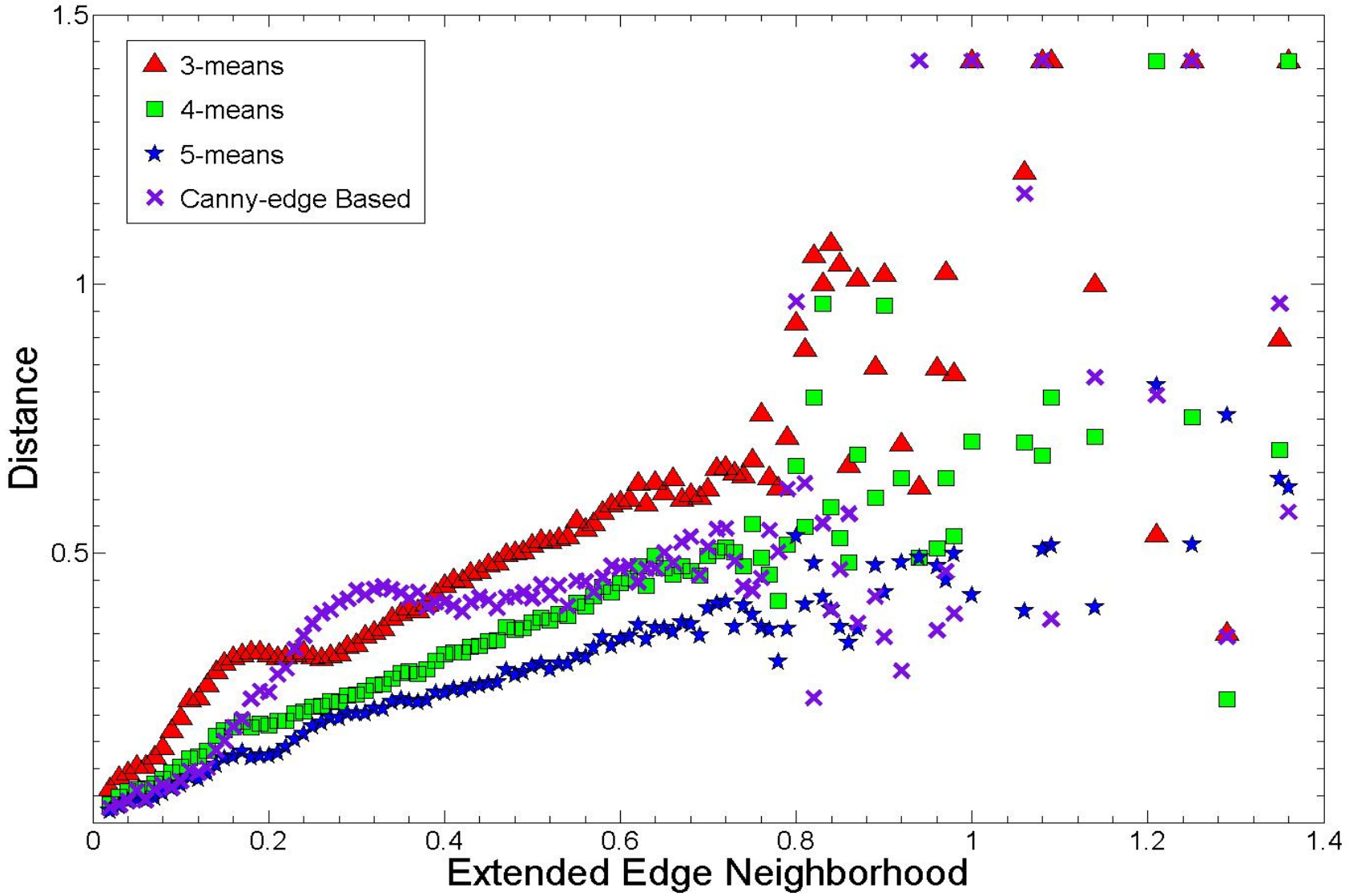
- First look at cell distributions as a f(EEN)



4 Methods: Best for individual cells

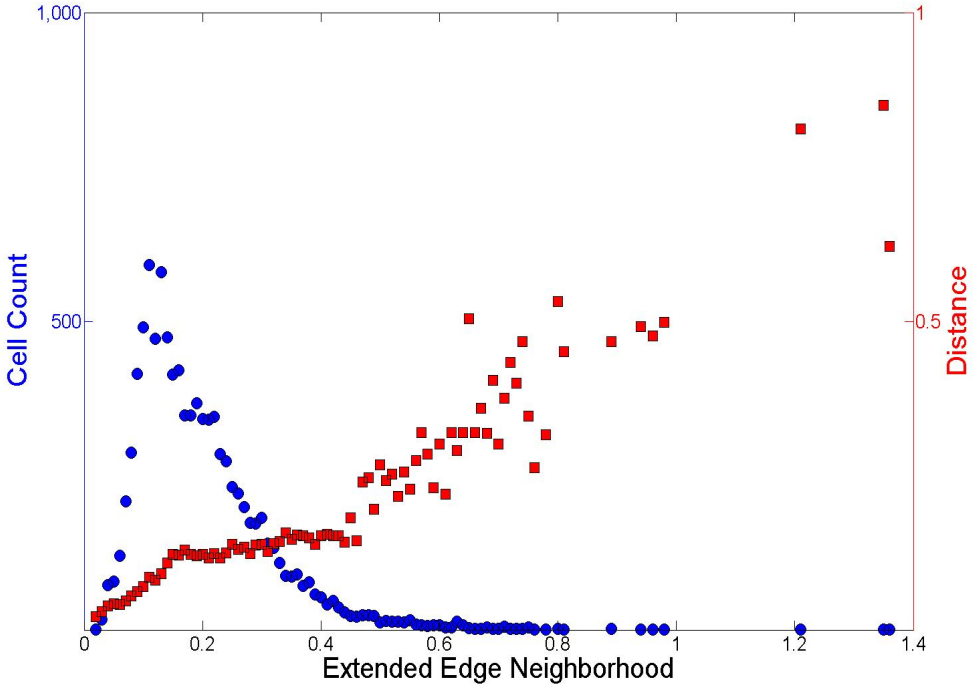


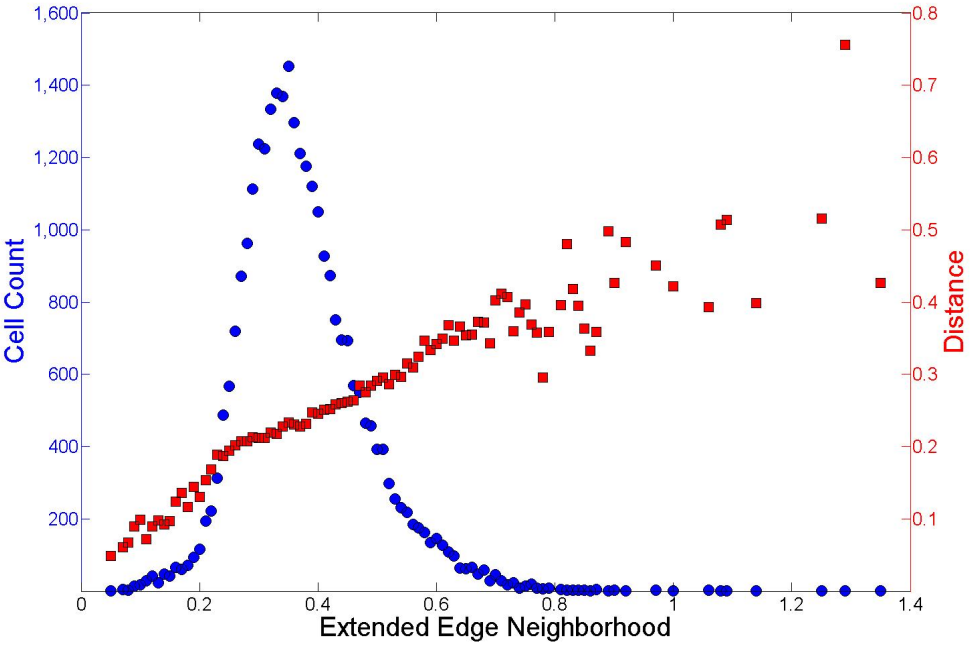
Segmentation Distance vs. EEN



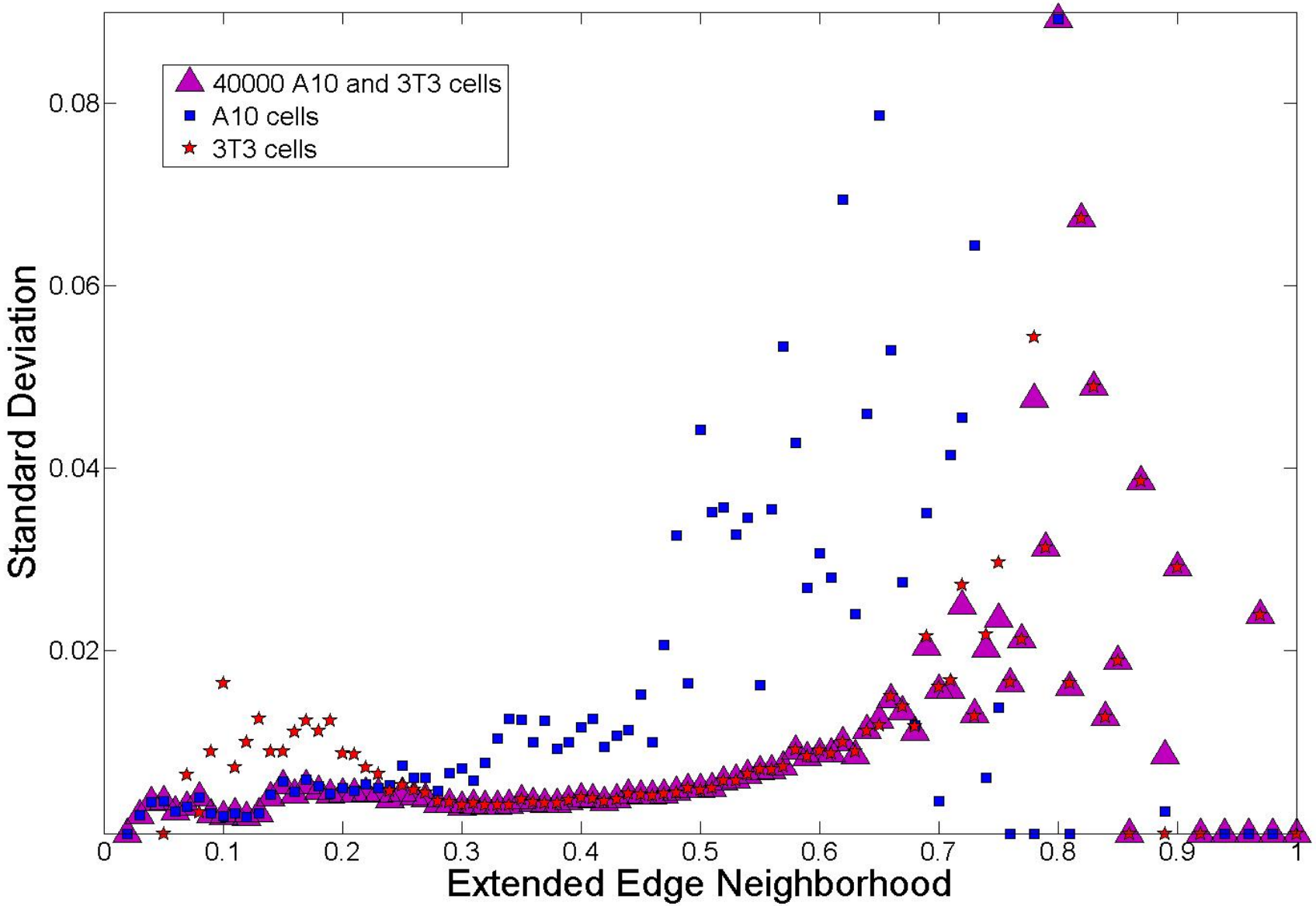
Each Individual Cell Line

- A10 cells: EEN approx. 0.1-0.25
  3T3 cells: EEN approx. 0.2-0.5



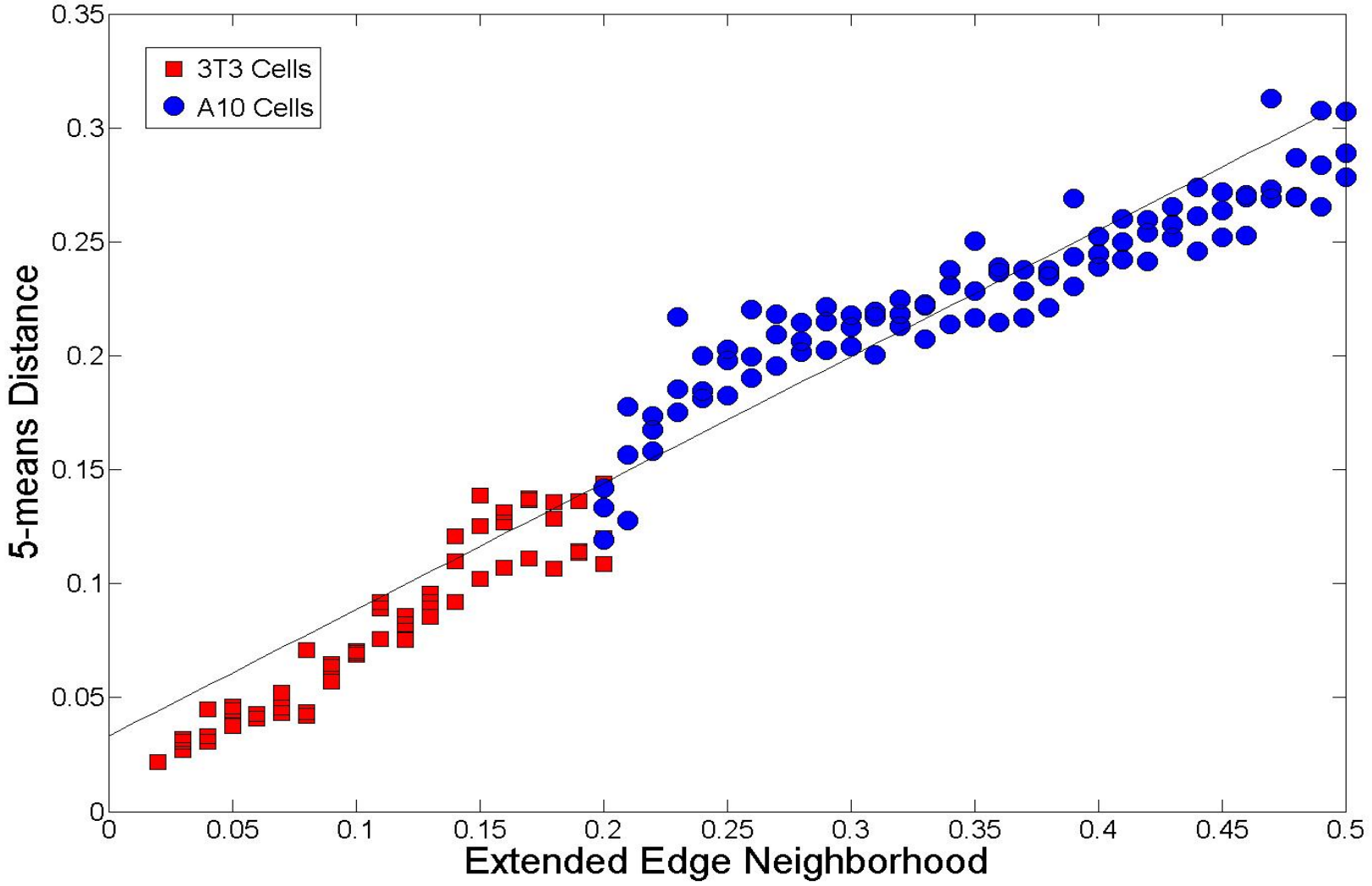


Standard Deviations for Clusters



Combine Data from Each Cell Line

- Distance = 0.15 + EEN * 0.477
  correlation coefficient = 0.9815



Conclusions

- 40000 cells: see relationship between segmentation accuracy and extended edge neighborhood
- In general, cells with higher area-to-perimeter ratios will segment with higher accuracy (larger, rounder).
- Edge quality & segmentation accuracy directly related