

Two-Step Watershed Segmentation of Epithelial Cells

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Applications in Biology

Heidelberg, September 2nd, 2011

1 Introduction

- Biological background
- Challenges

2 Segmentation

- Proposed method
- Comparison with Voronoi-based segmentation

3 Feature analysis

4 Conclusion

1 Introduction

- Biological background
- Challenges

2 Segmentation

3 Feature analysis

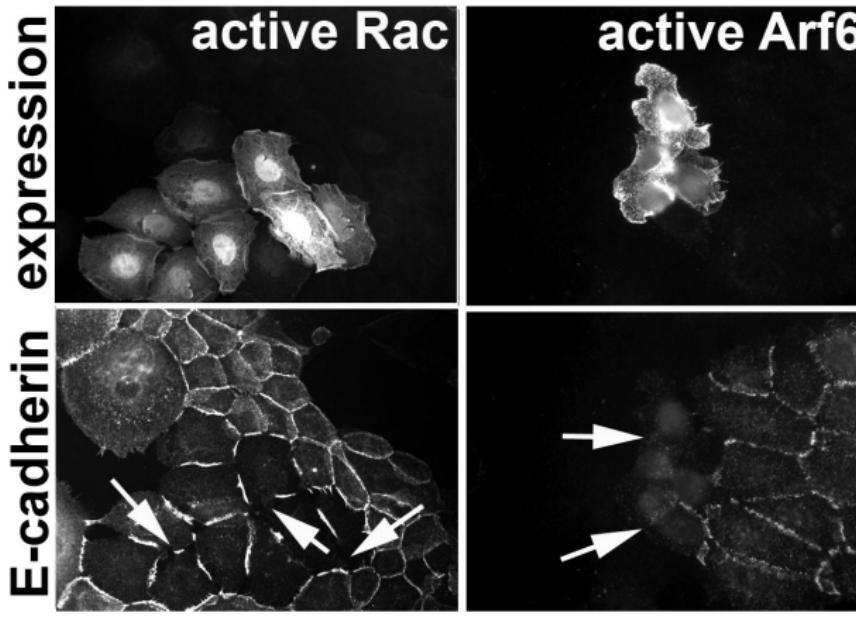
4 Conclusion

Biological background

- Cells junctions **essential for life** of multi-cellular organisms
- E-cadherin:
 - ▶ master cell-cell adhesion receptor
 - ▶ frequently disrupted in different pathologies and cancer

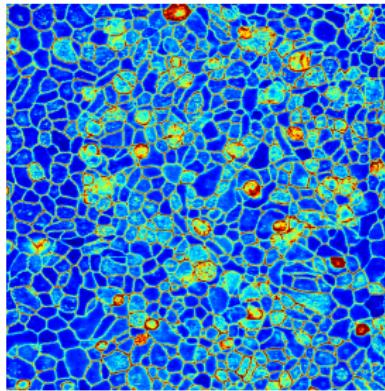
Biological background

During malignancy different oncogenes
disassemble junctions to enable cell invasion

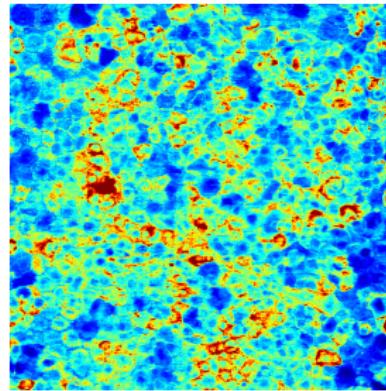


High-Throughput RNAi Screens

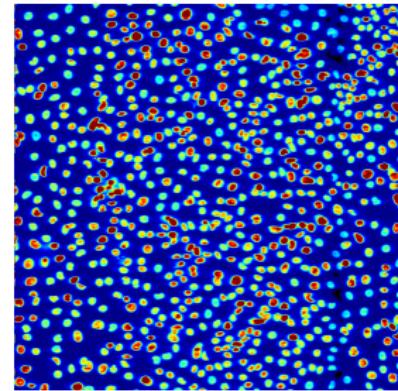
- Studying the **stability of junctions** for *Cancer Research*



E-cadherin

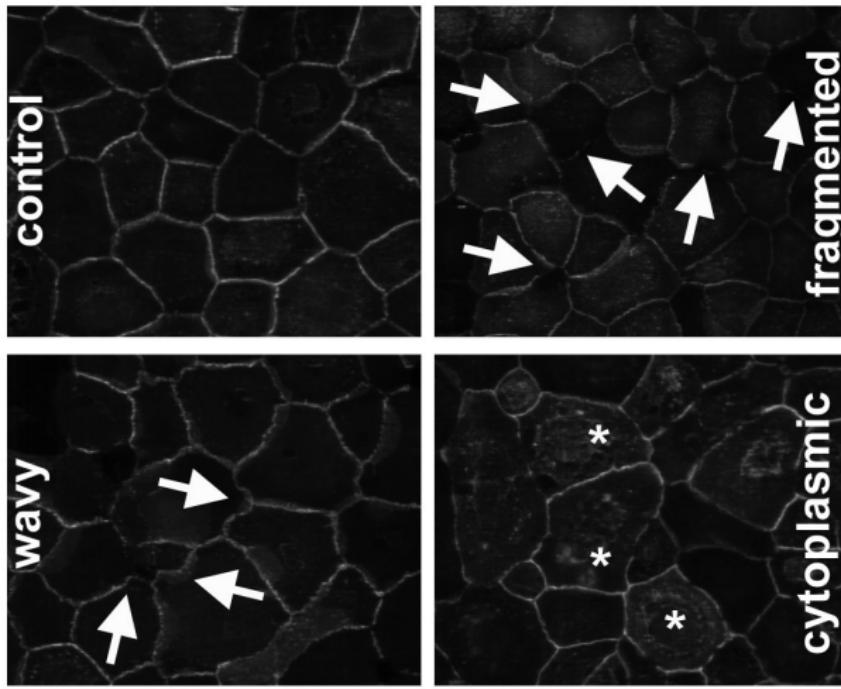


Actin



Nuclei

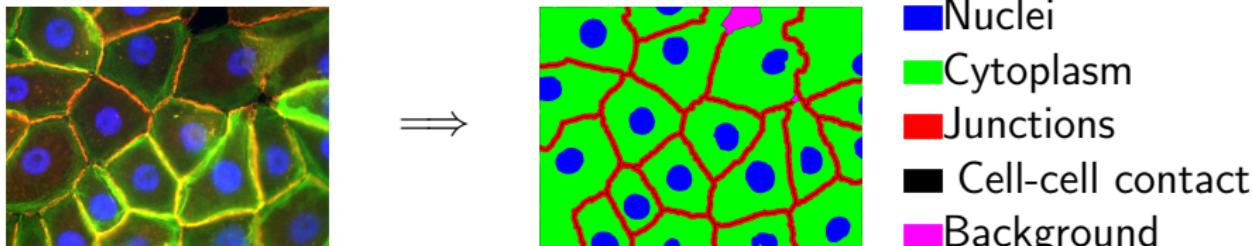
Junctions phenotypes



Challenges

Accurate cell **segmentation** for **per cell** measurements:

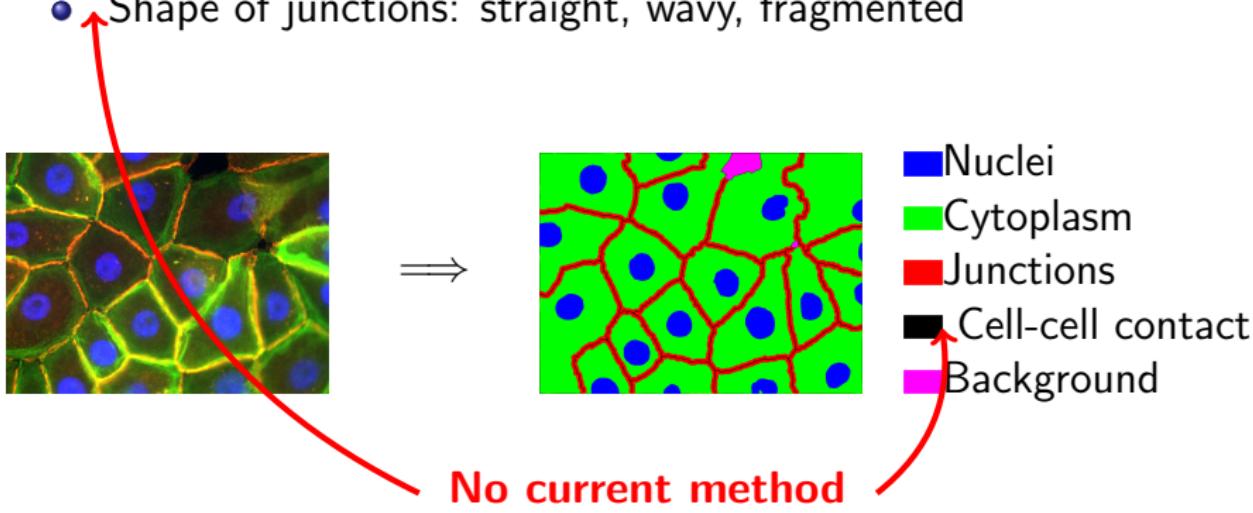
- E-cadherin & Actin localization/quantification
- Shape of junctions: straight, wavy, fragmented



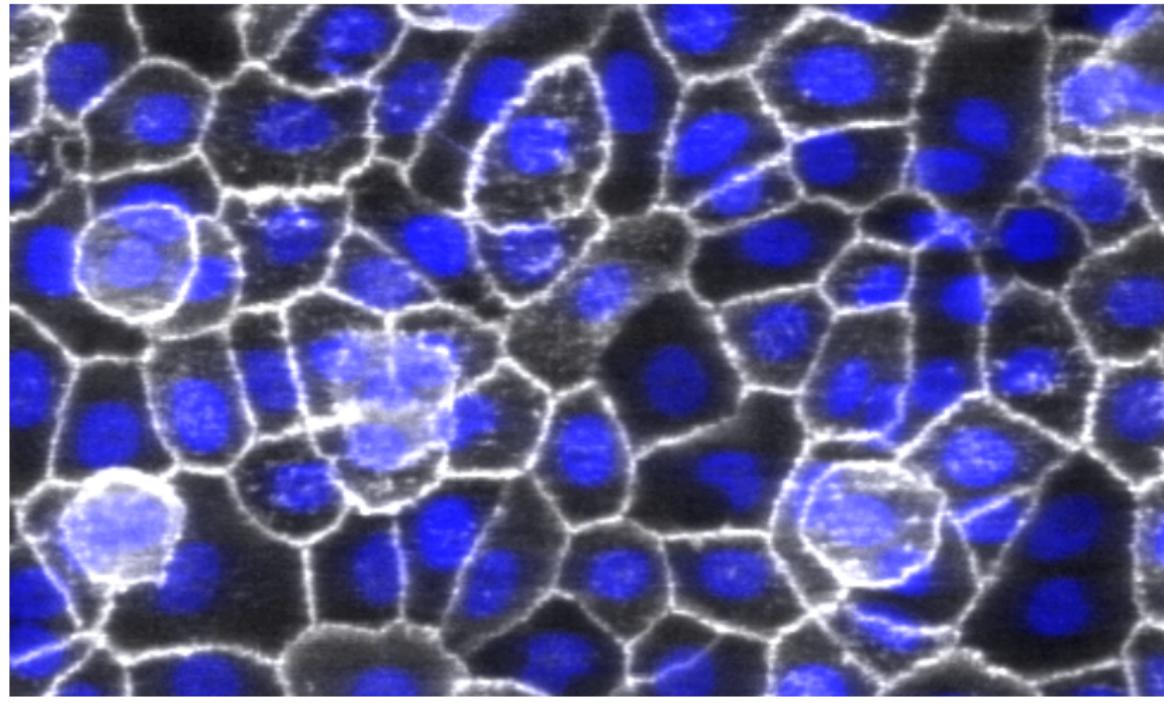
Challenges

Accurate cell **segmentation** for **per cell** measurements:

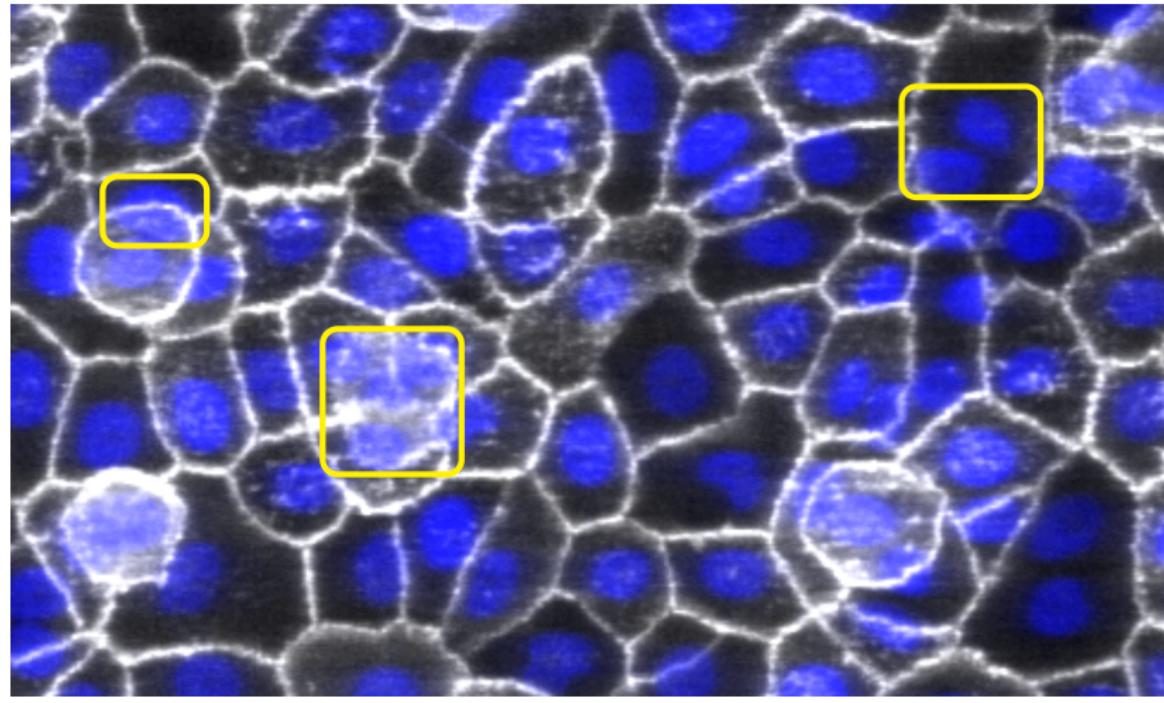
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Challenges



Challenges



1 Introduction

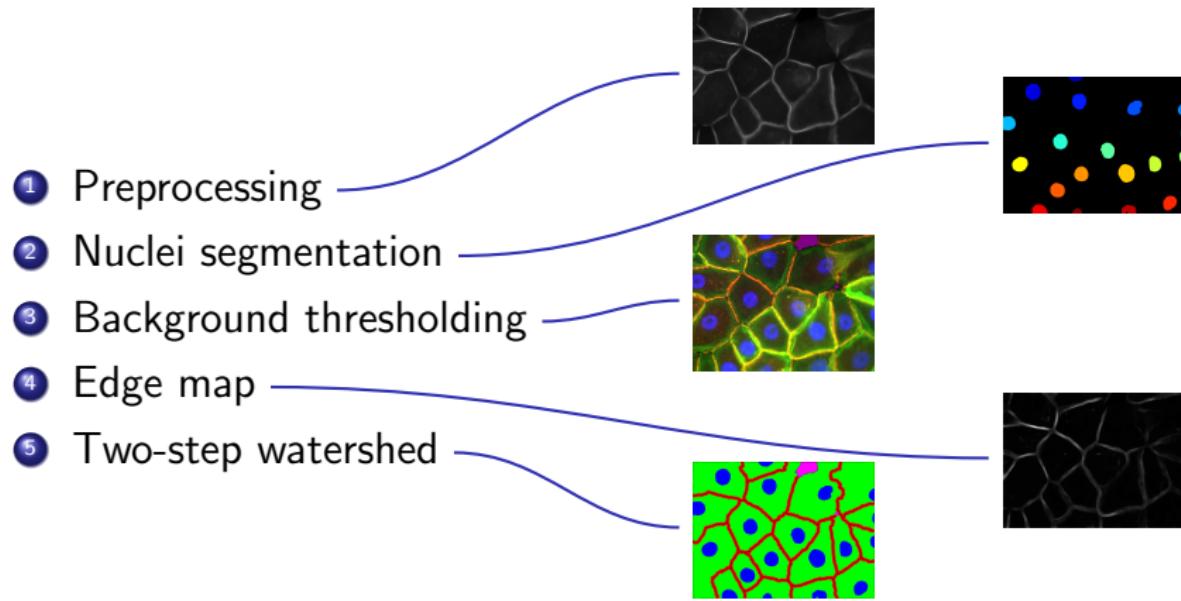
2 Segmentation

- Proposed method
- Comparison with Voronoi-based segmentation

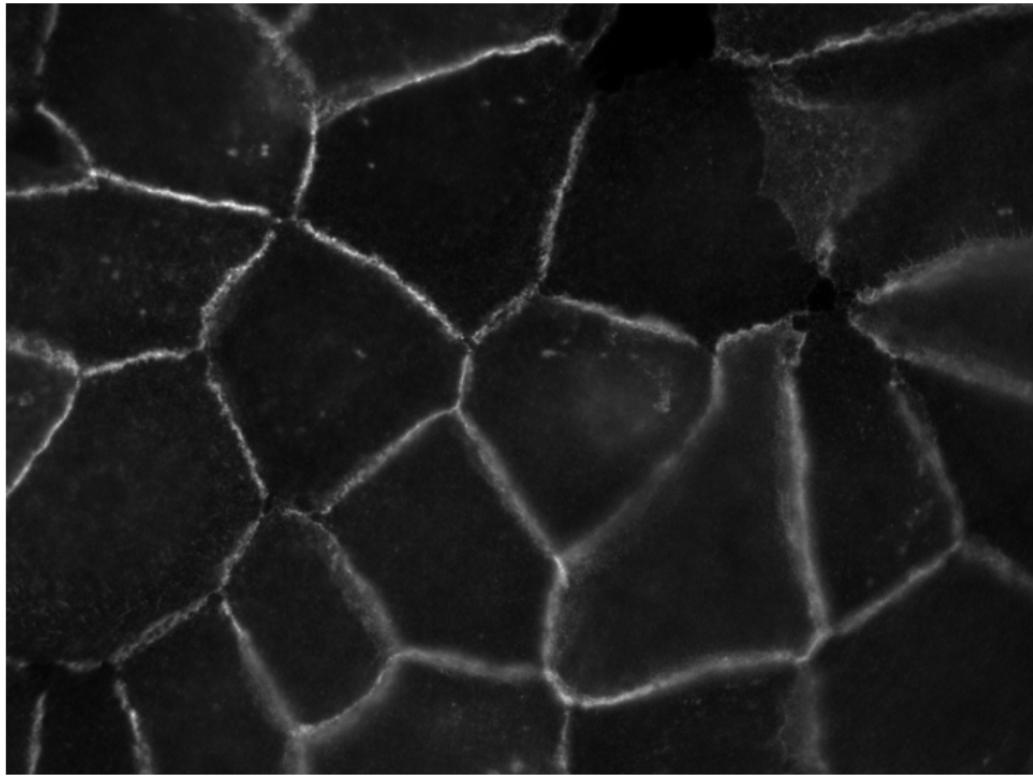
3 Feature analysis

4 Conclusion

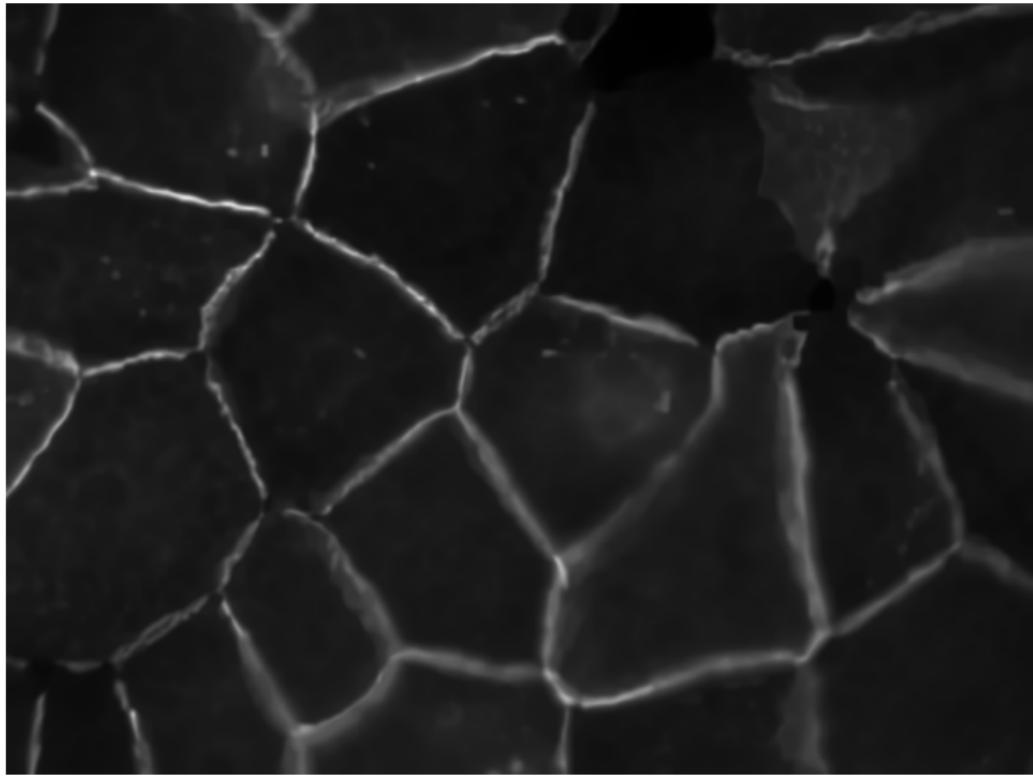
Segmentation pipeline overview



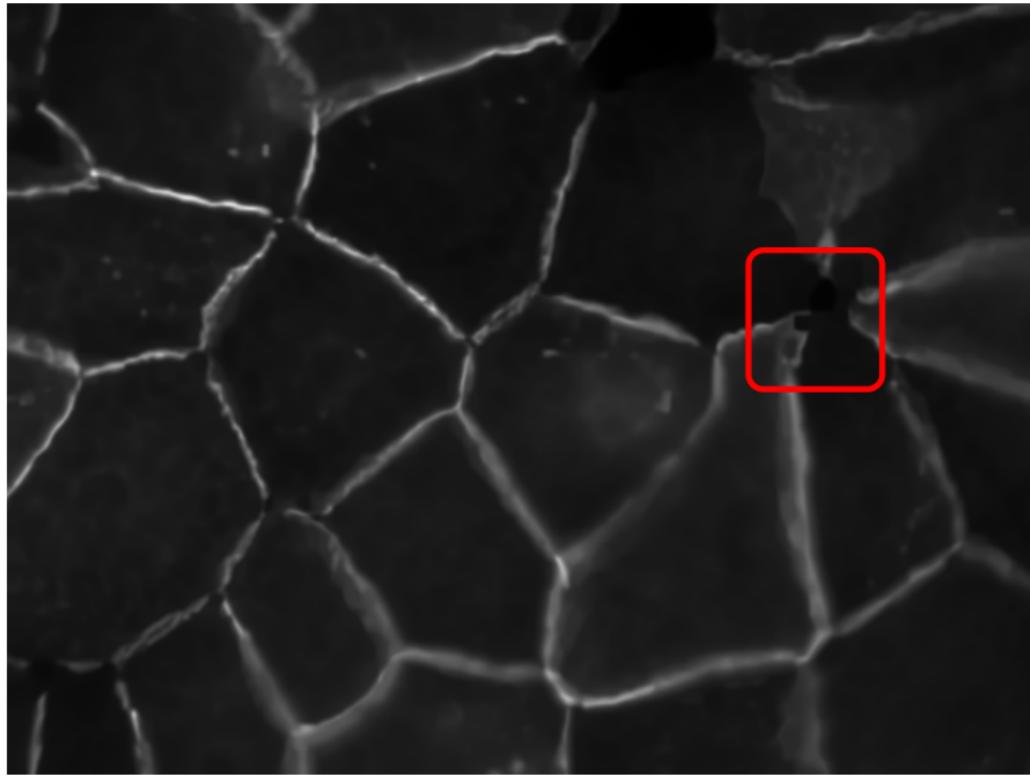
Junctions image



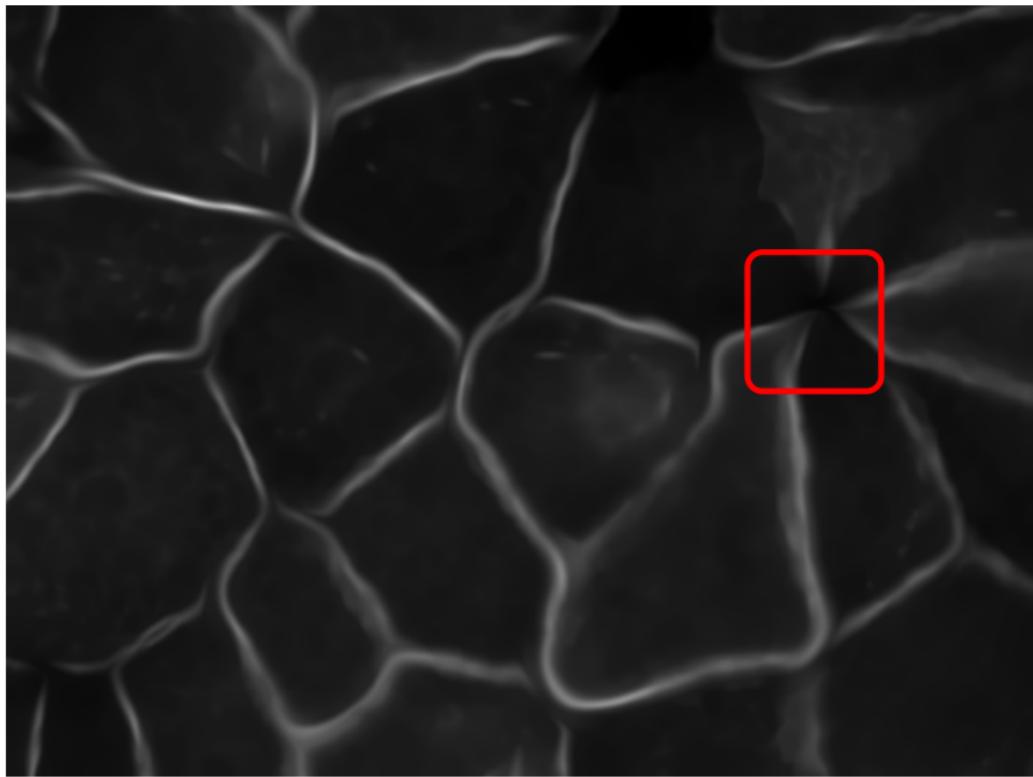
Block-matching filtering (BM3D, Dabov et al., 2007)



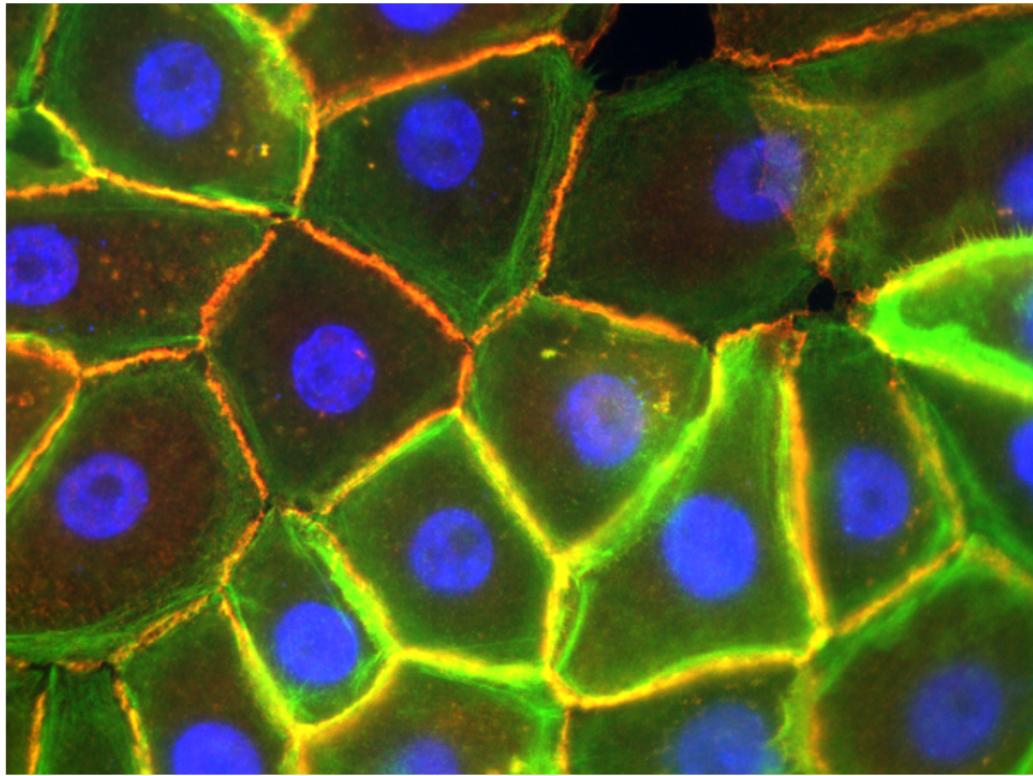
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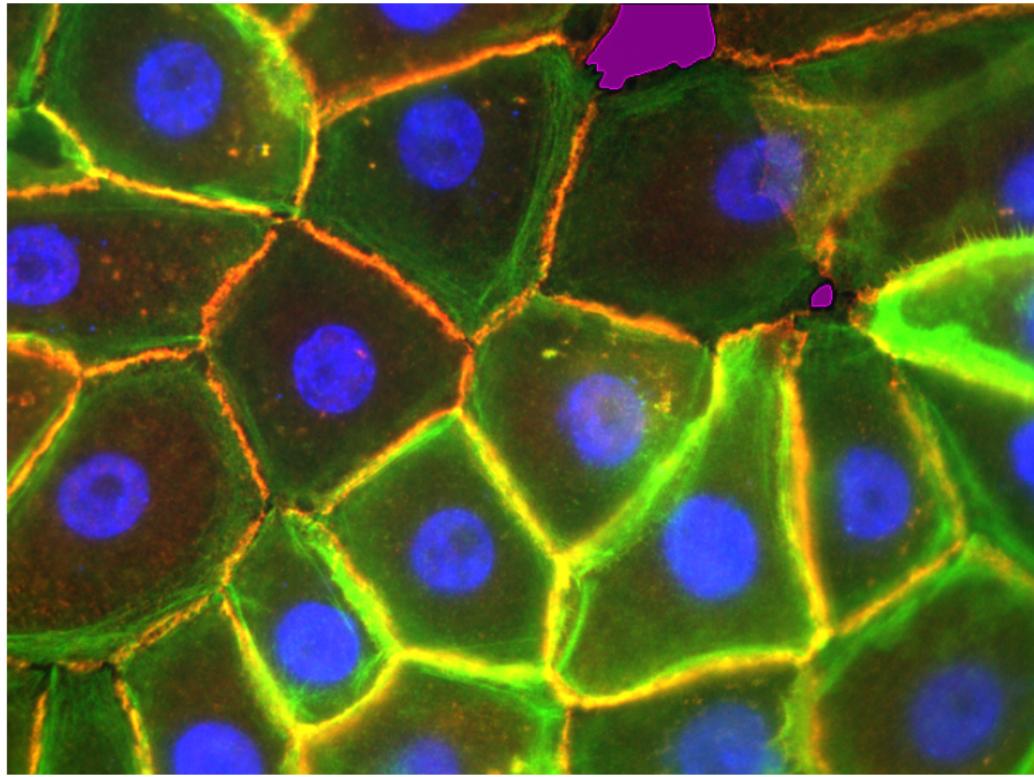
Coherence enhancing diffusion (CED, Weickert, 1999)



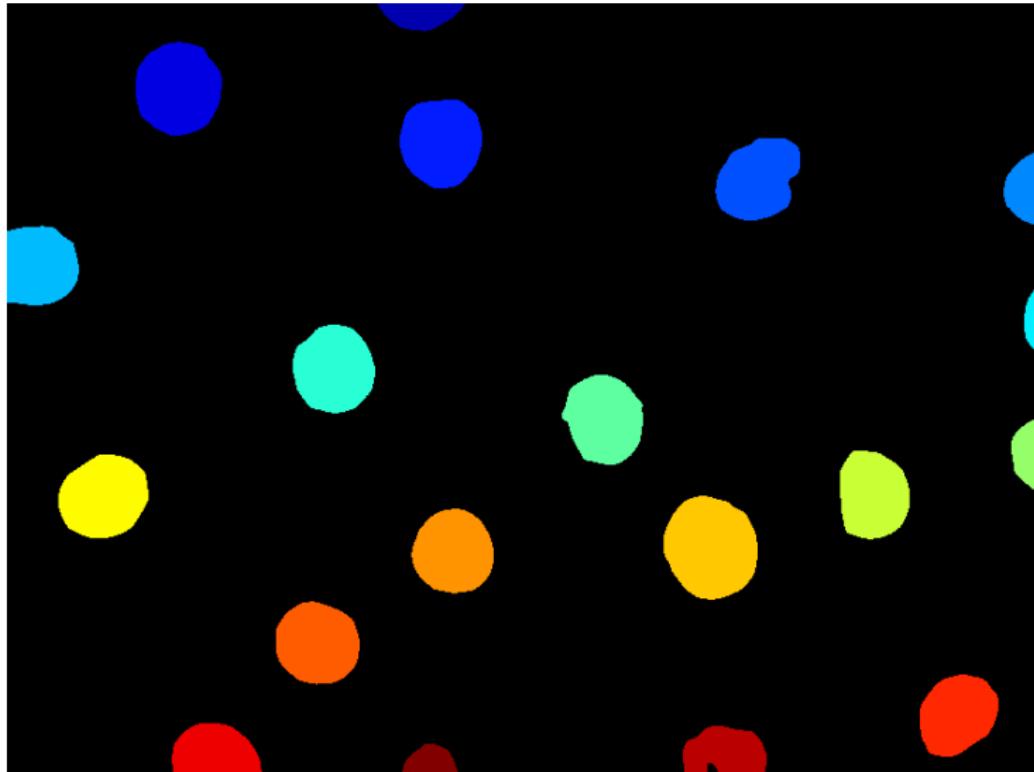
Background thresholding on Actin



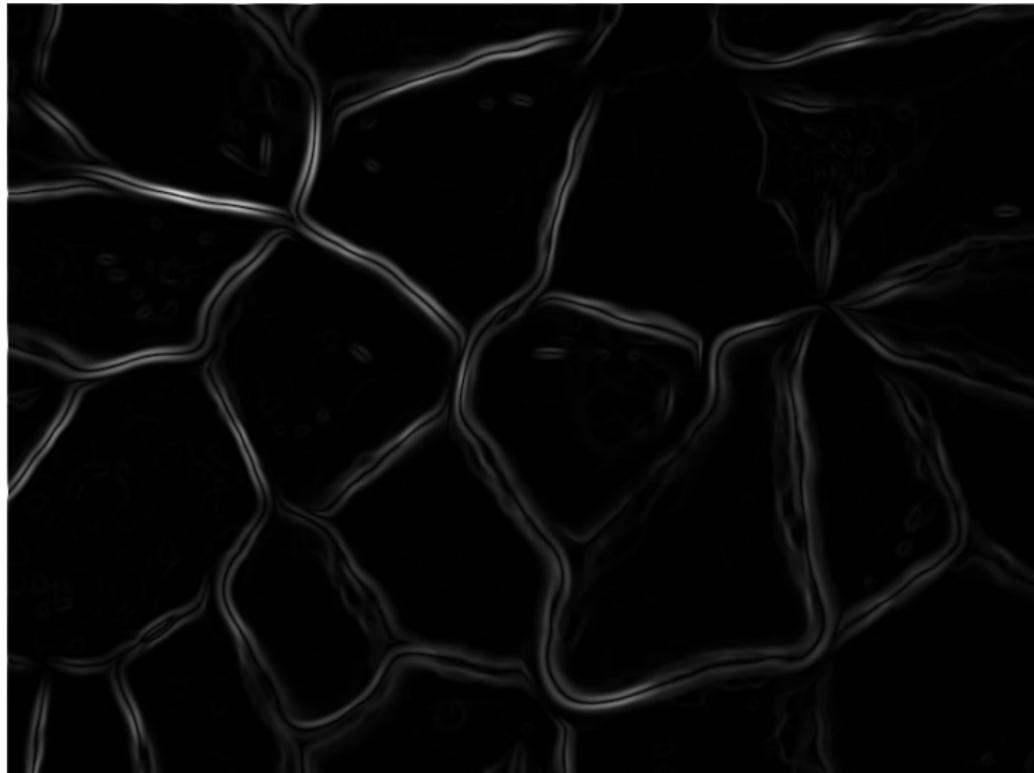
Background thresholding on Actin



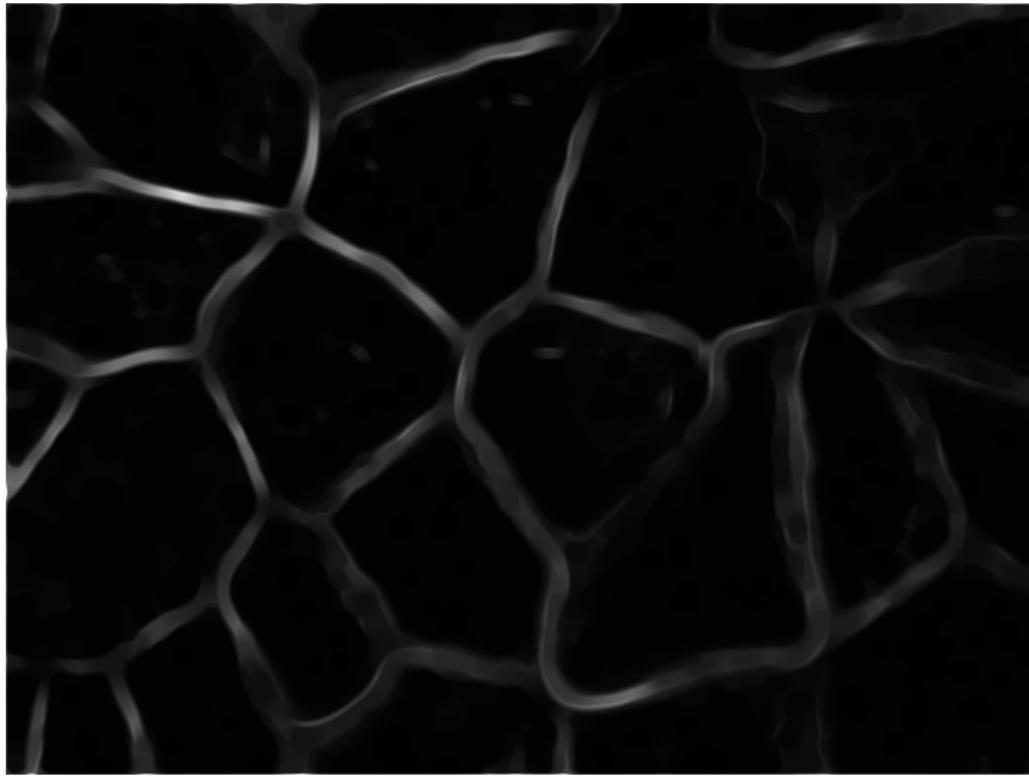
Adaptive thresholding + filling holes on Nuclei



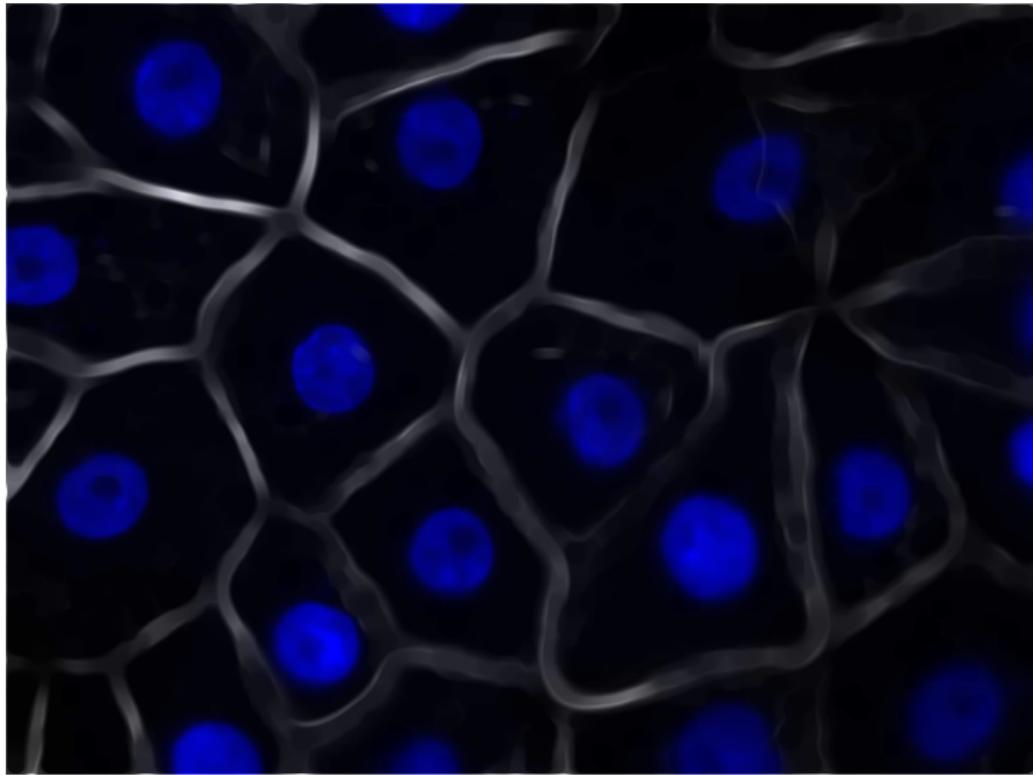
Sobel filter + morphological closing on *Junctions*



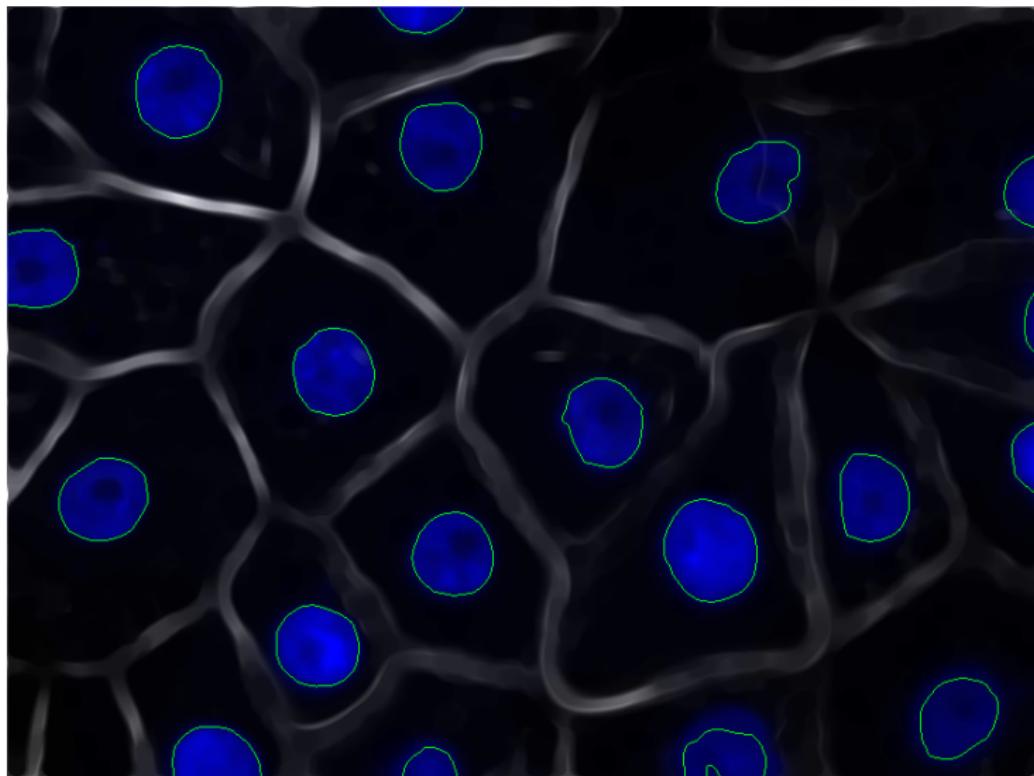
Sobel filter + morphological closing on *Junctions*



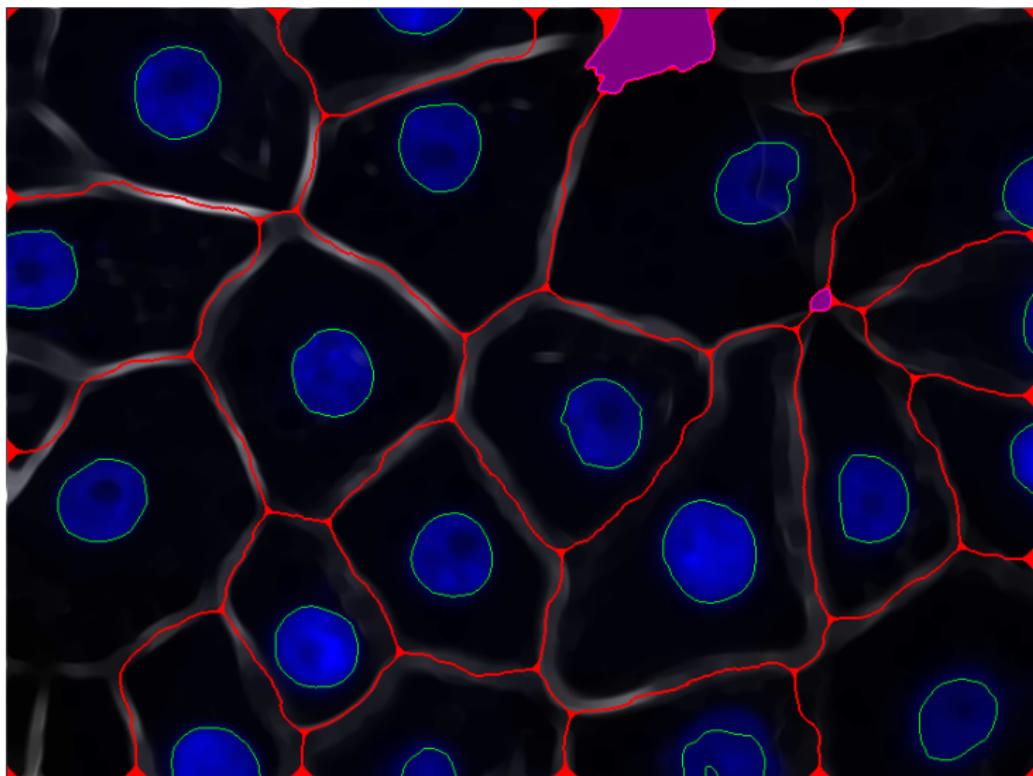
1st watershed step on Edge map



1st watershed step on Edge map



1st watershed step on Edge map

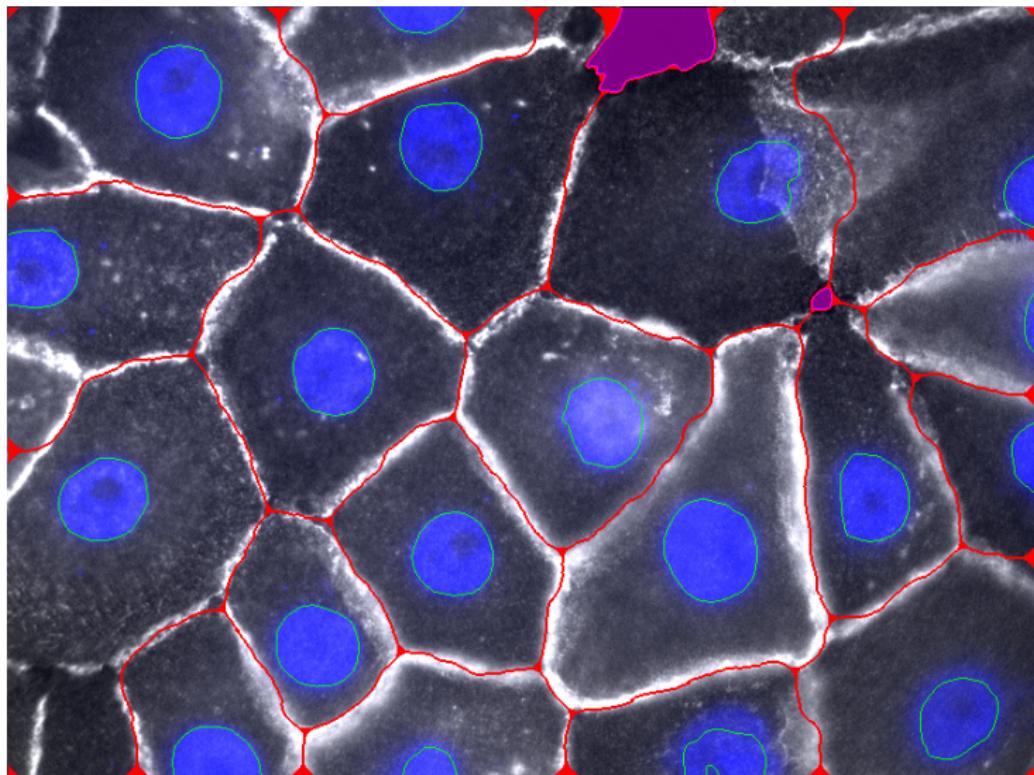


Two-Step Watershed Segmentation

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Eroding cells at junctions

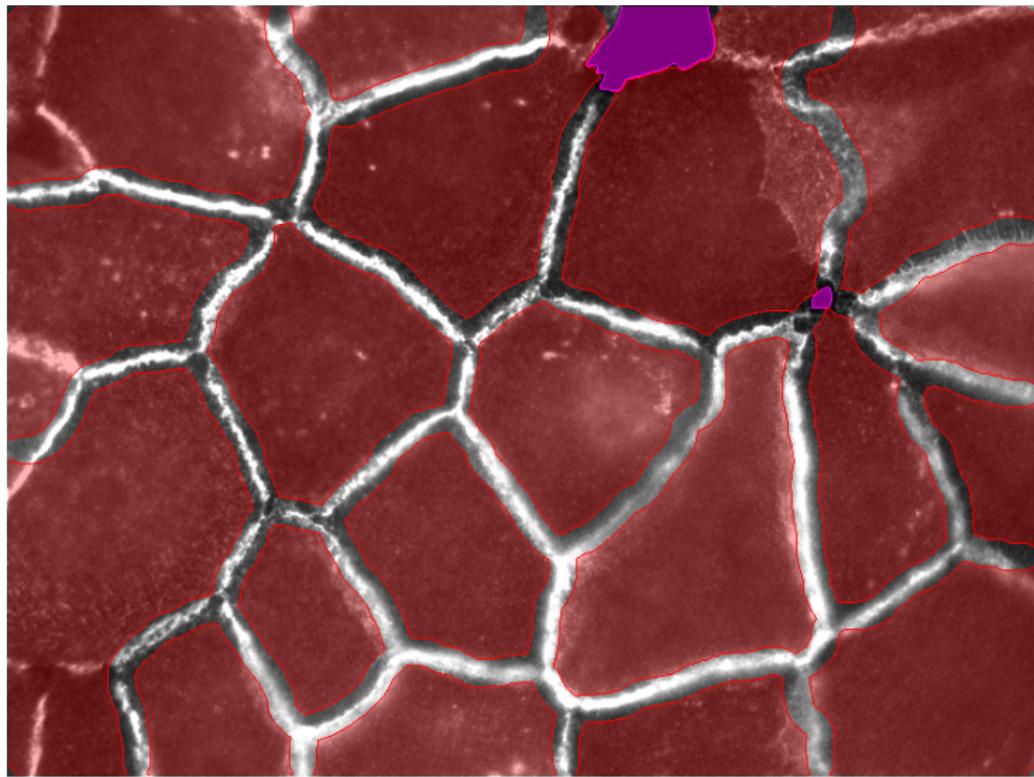


Two-Step Watershed Segmentation

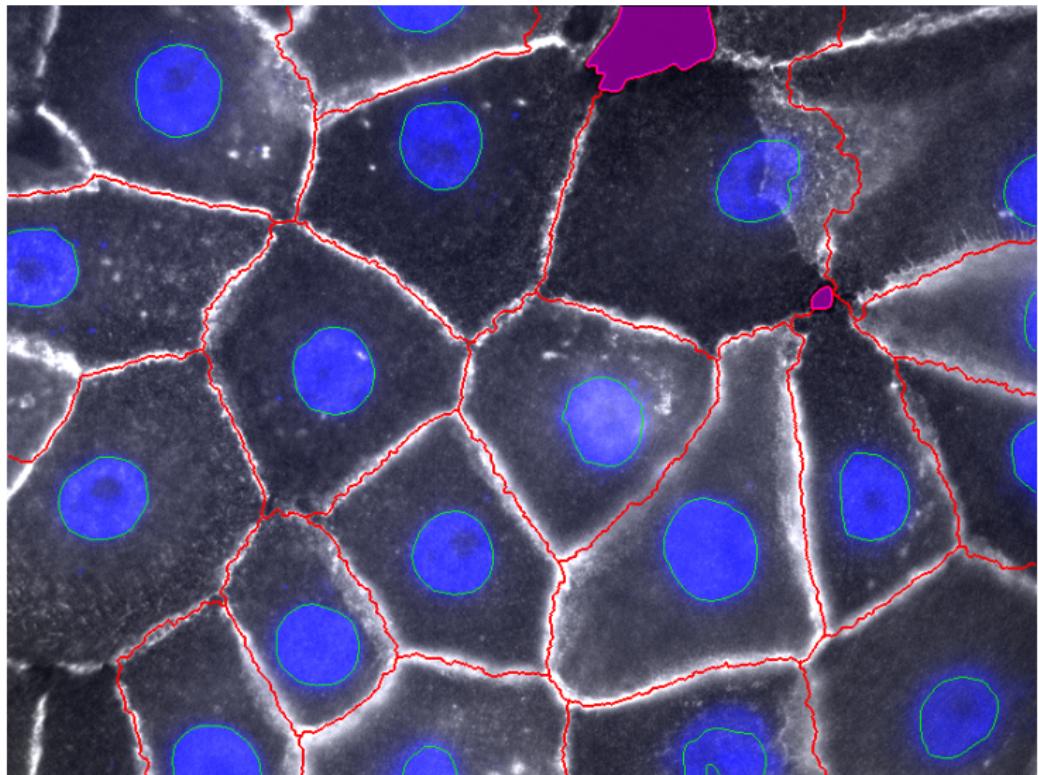
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Eroding cells at junctions



2nd watershed on raw Junctions



Two-Step Watershed Segmentation

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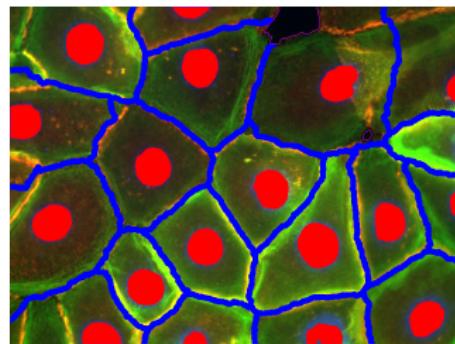
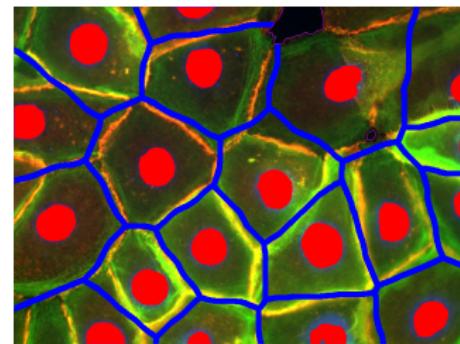
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Comparison with Voronoi-based segmentation

Comparison with Voronoi-based segmentation

- Voronoi-Based Segmentation (*T. Jones et al., 2005*)
 - ▶ implemented in *CellProfiler* and *Bioconductor* (*propagate*)

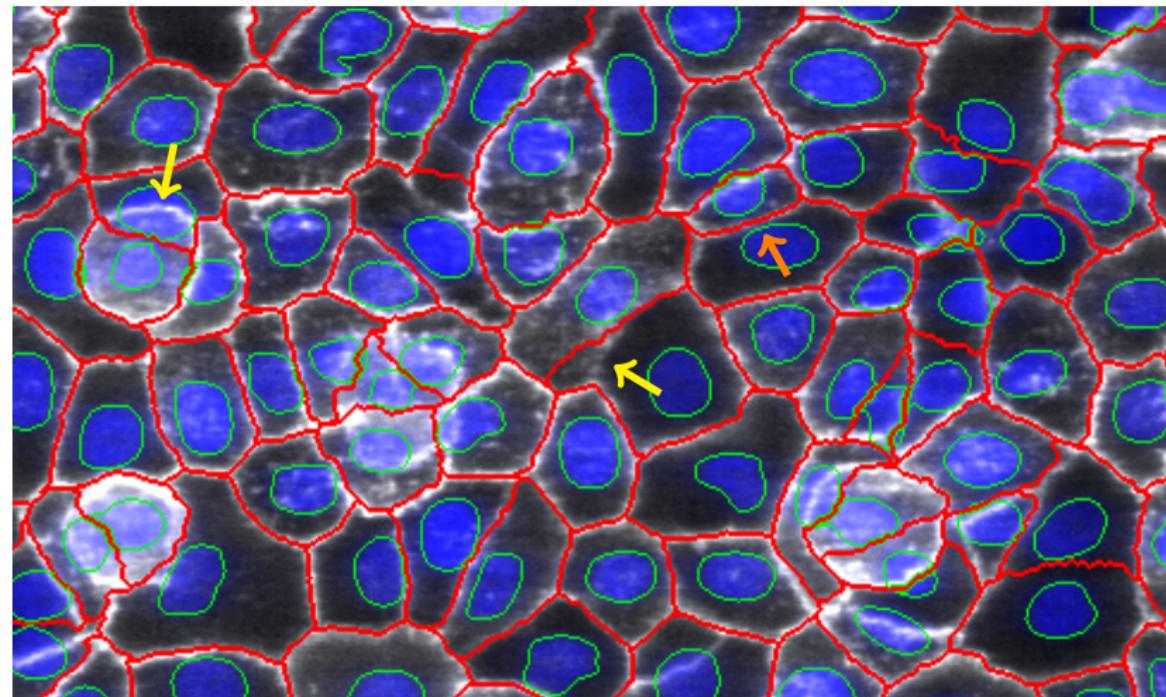
$$\text{Pixel distance} = \frac{\text{Gradient} + \lambda \times \text{Euclidean distance}}{1 + \lambda}$$

 $\lambda = 0$  $\lambda = 1000$

Comparison with Voronoi-based segmentation

Comparison with Voronoi-based segmentation

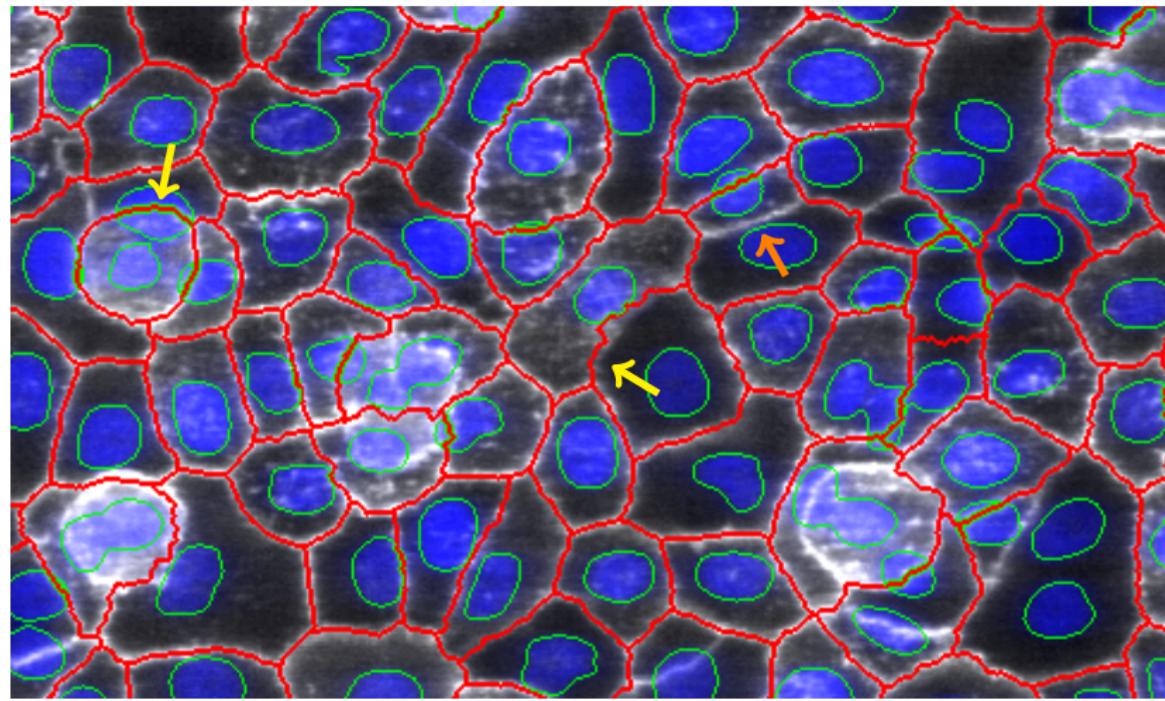
propagate $\lambda = 10^{-5}$



Comparison with Voronoi-based segmentation

Comparison with Voronoi-based segmentation

Proposed segmentation pipeline



1 Introduction

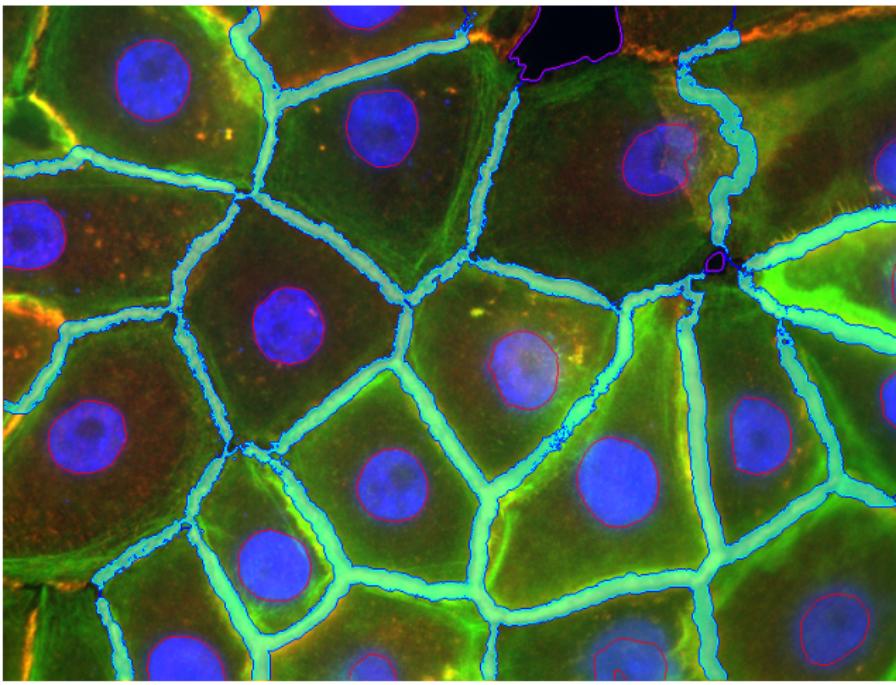
2 Segmentation

3 Feature analysis

4 Conclusion

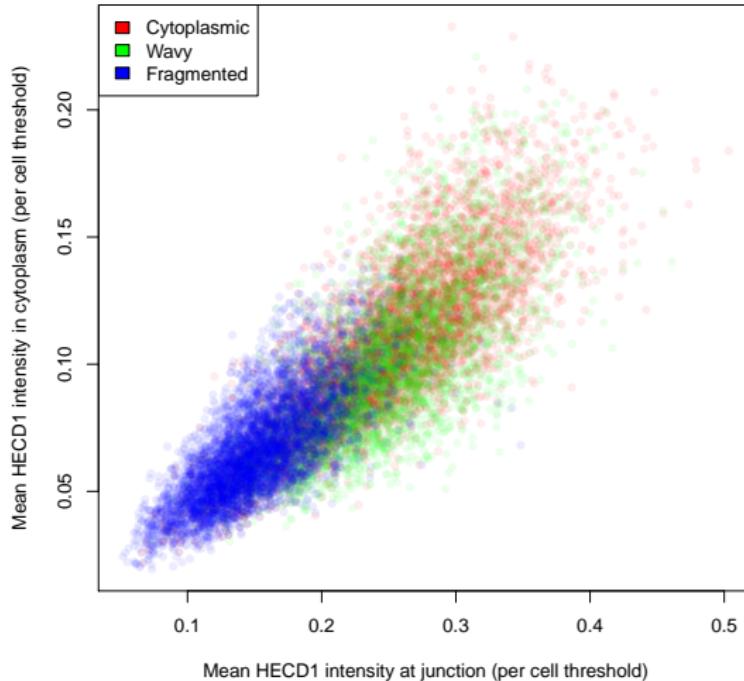
Feature extraction

- Junctions region with **per cell** threshold



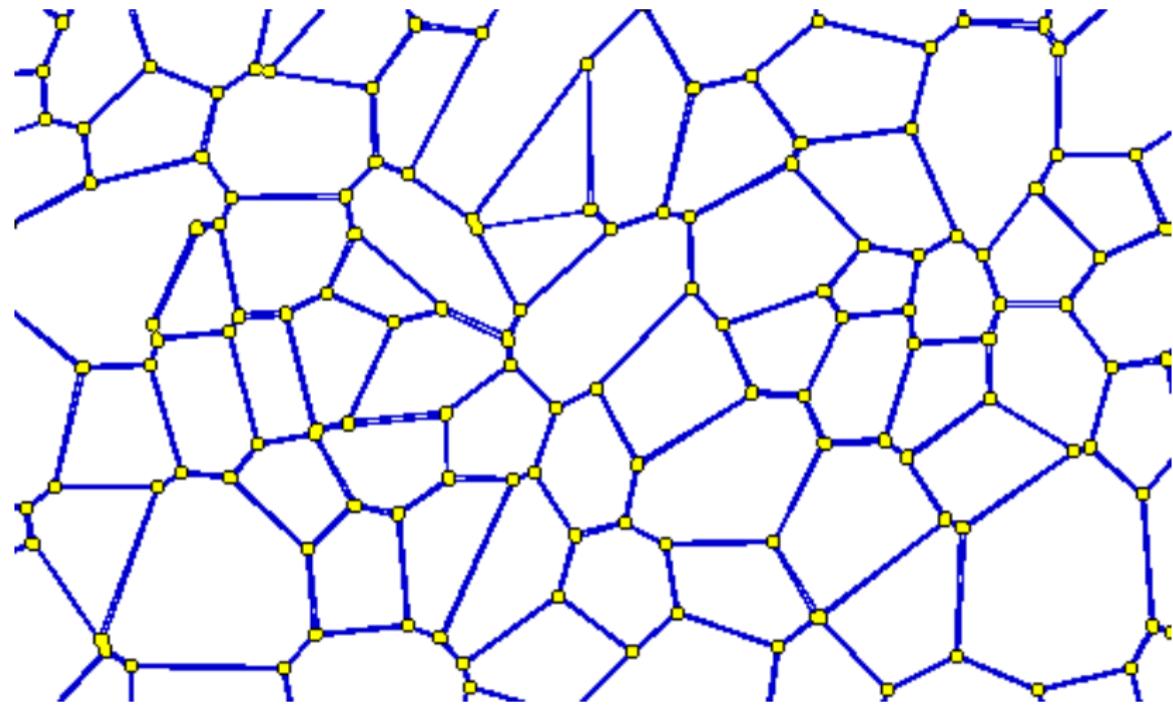
Feature analysis

Mean HECD1 intensity at junctions Vs non-junction



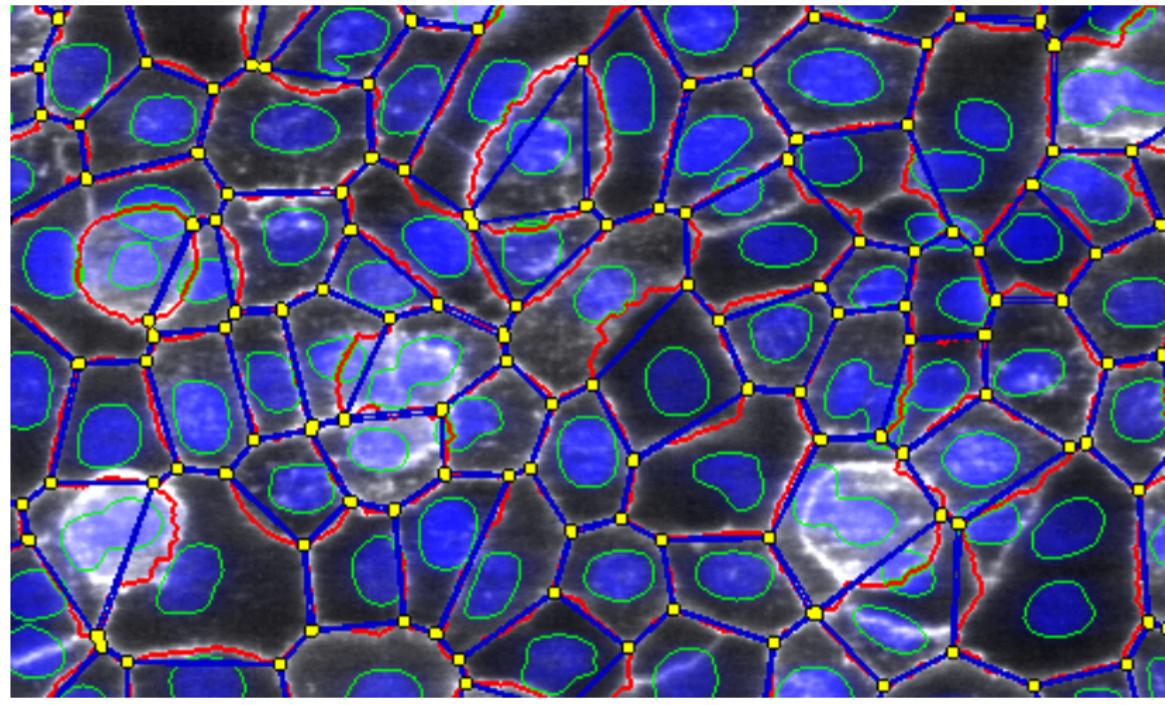
Feature extraction

Corner-to-corner contours



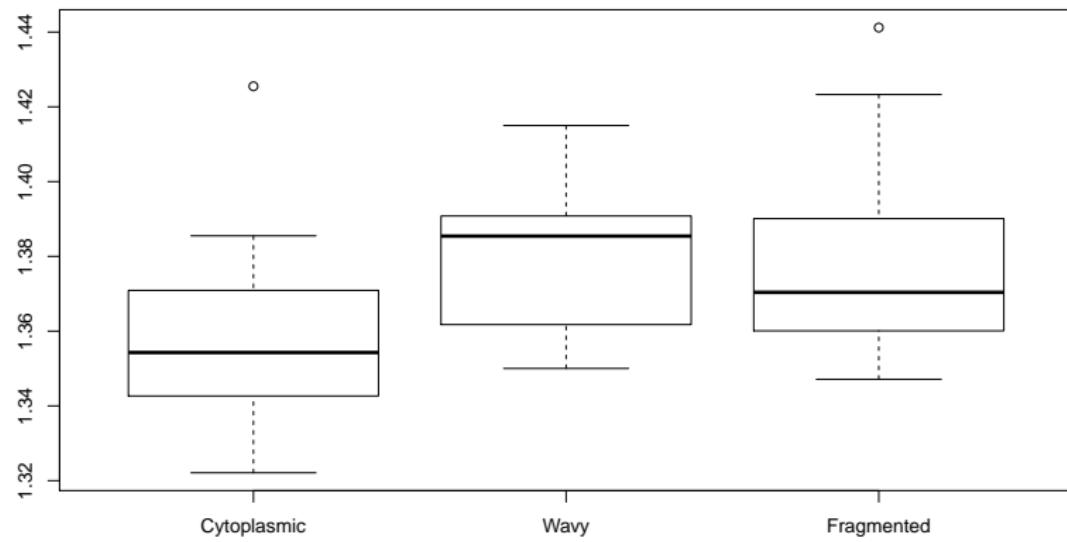
Feature extraction

Corner-to-corner contours



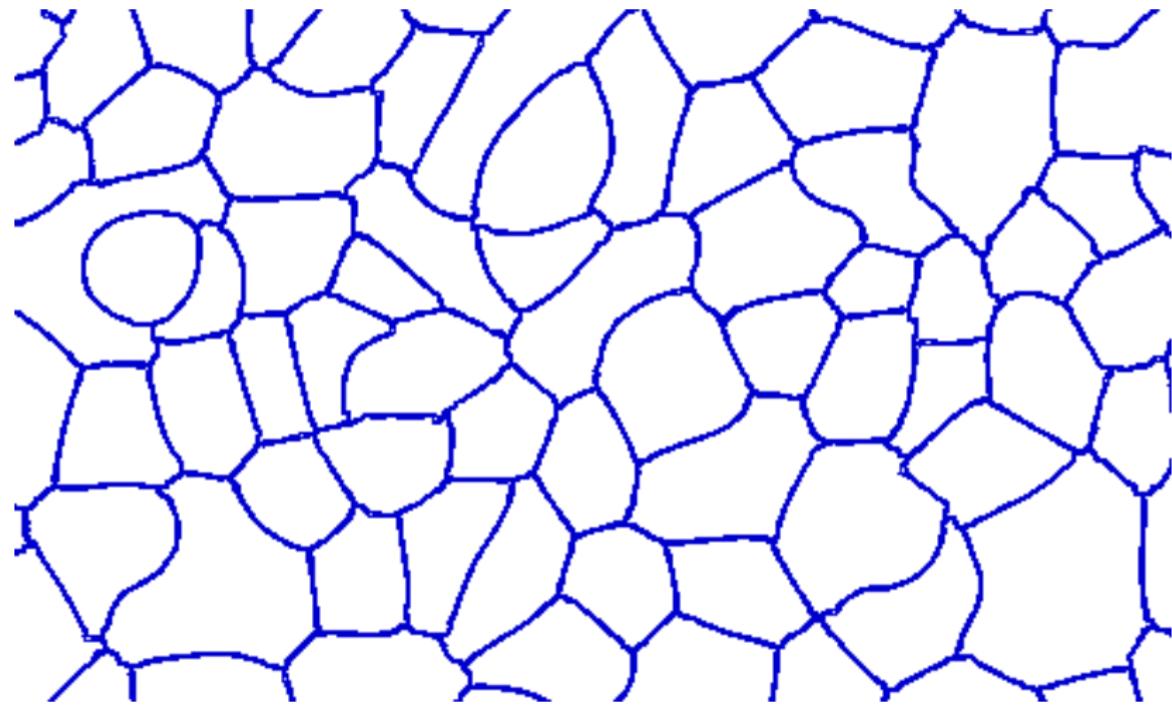
Feature extraction

Cell perimeter / Corner-to-corner perimeter (mean)



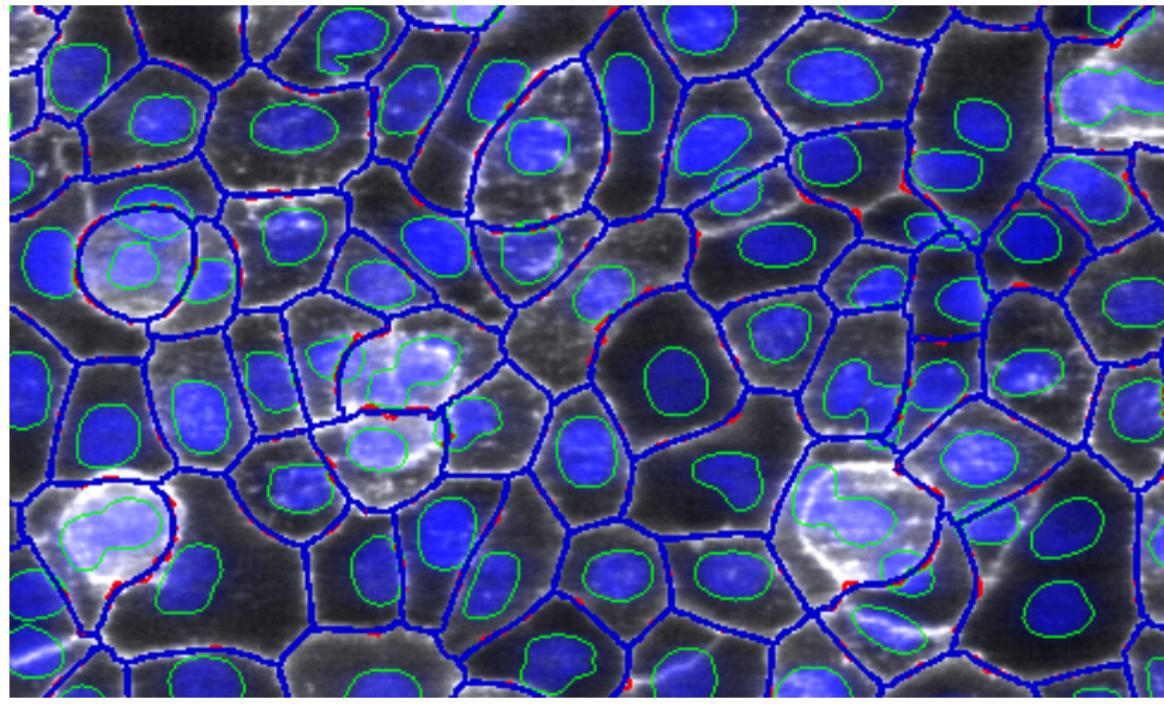
Feature extraction

Smoothed contours



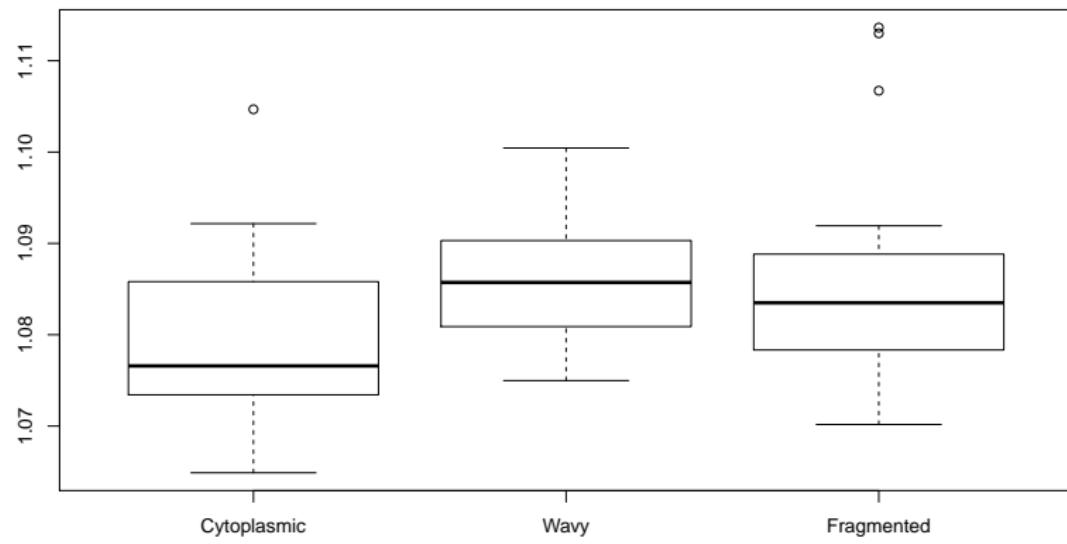
Feature extraction

Smoothed contours



Feature extraction

Cell perimeter / Smoothed perimeter (mean)



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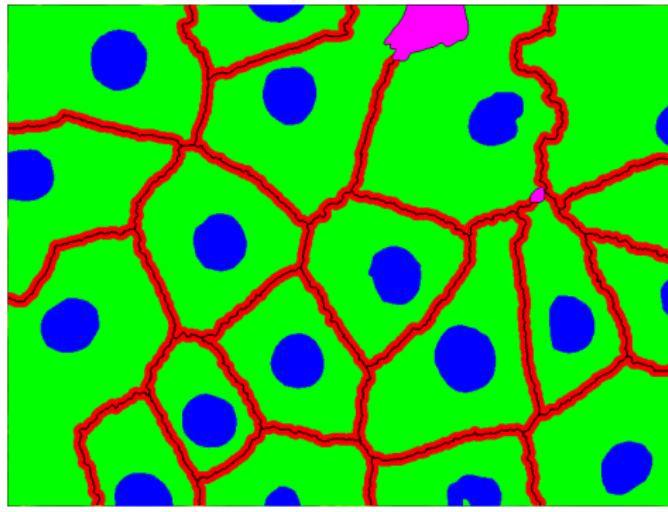
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Main contribution

Novel segmentation method for RNAi depleted cells

- Accurate localization of cell structures
- Allows precise quantification of E-cadherin & Actin



- Nuclei
- Cytoplasm
- Junctions
- Cell-cell contact
- Background

Limitations of our method

- Tailored for specific images: **cell-cell junctions**
- Suffers from watershed *leaks* or *local maxima*
- Unable to recognise “*no-nucleus*” cells

Future work

- Segmentation quality assessment
- Cell under-segmentation
- More feature extraction
- Share code with EBImage authors

Thanks!

watergrow function

- Watershed with seed regions
- Region growing with shape prior

Example with a 5x5 box neighbourhood, 6 votes required:

1	1	1	1	1
3	3	2	3	3
3	3	2	3	3
3	1	1	3	3
3	1	1	3	3

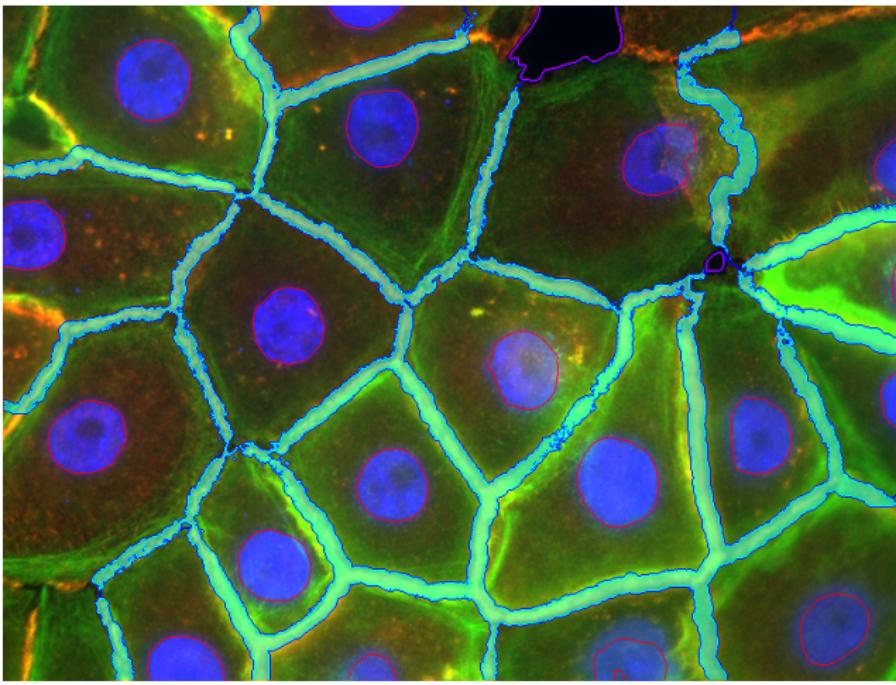
2 will not be included

1	1	1	1	1
3	2	2	2	3
3	2	2	2	3
3	1	1	1	3
1	1	1	1	1

2 will be included

Feature extraction

- Junctions region with **per cell** threshold



Feature extraction

- Junctions region with **constant width**

