Cell Segmentation and Tracking in Phase Contrast Images using Graph Cut with Asymmetric Boundary Costs

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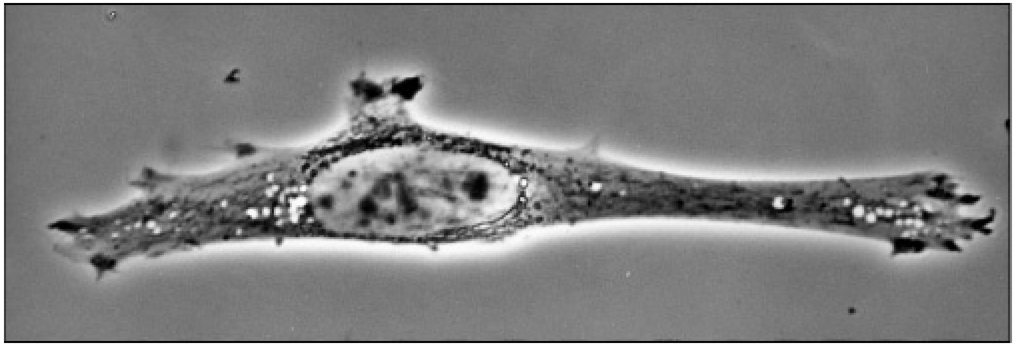
Outline

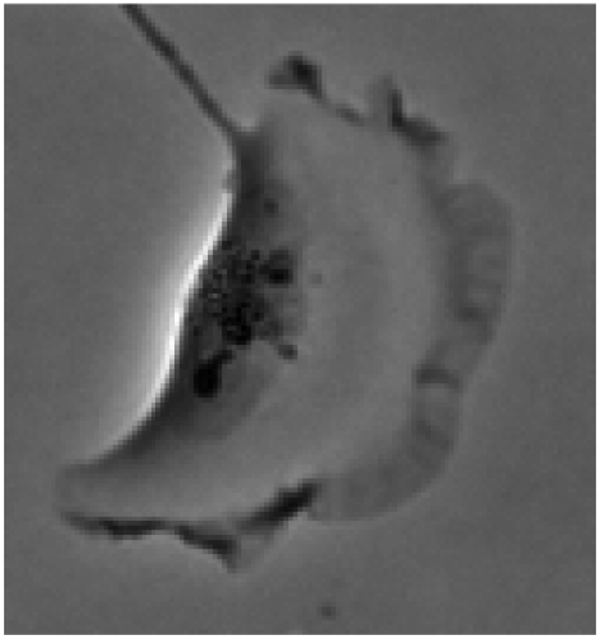
- Introduction
- Method
  - Segmentation
  - Tracking
- Experiments
- Conclusion

Phase contrast microscopy

(A) Bright-field



(B) Phase-contrast 50 µm

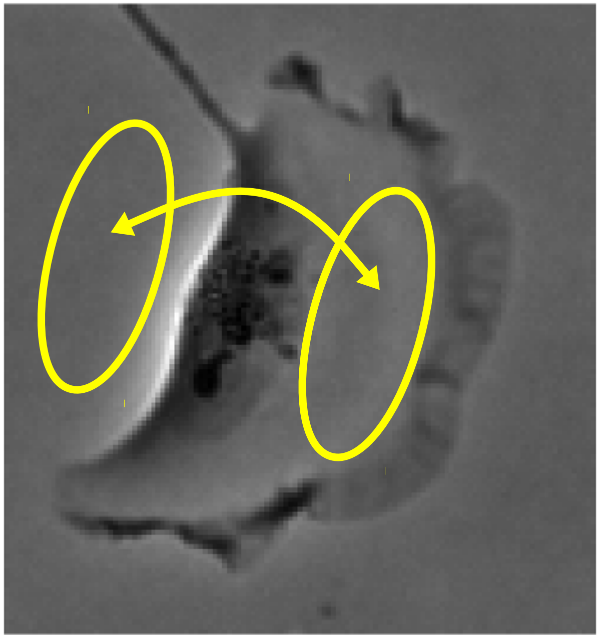


Phase-contrast

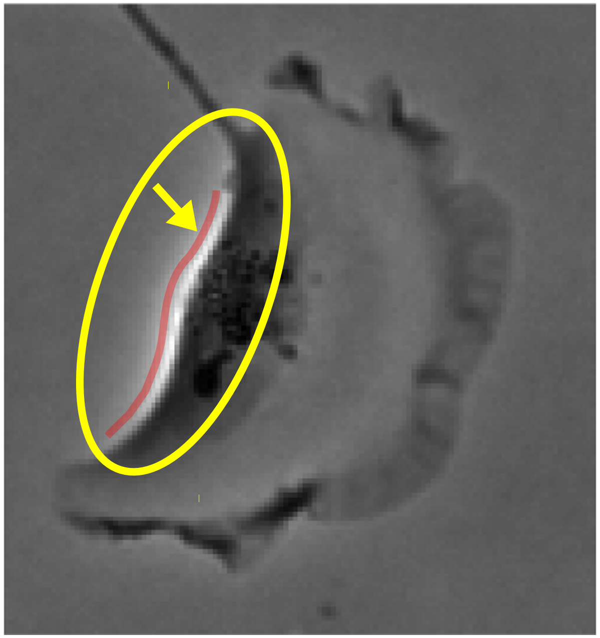
Figure: B. Alberts et al., Molecular Biology of the Cell, 4th Edition, 2002.

- **Visualize transparent objects** with high contrast at cell borders

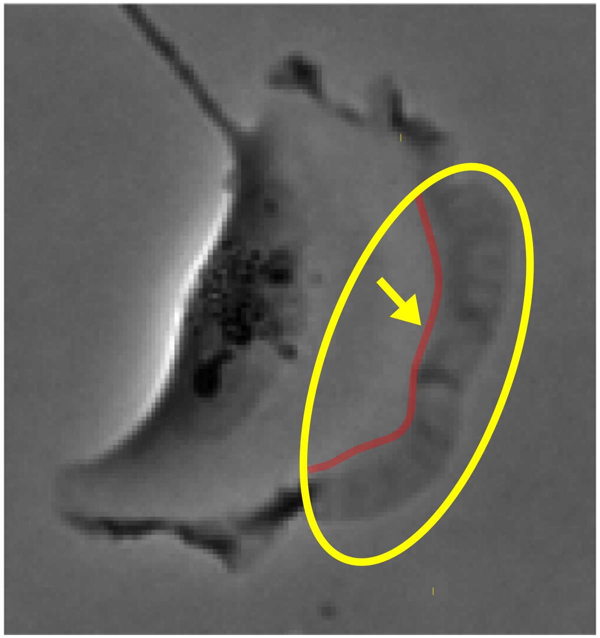
Phase contrast microscopy



Shade-off



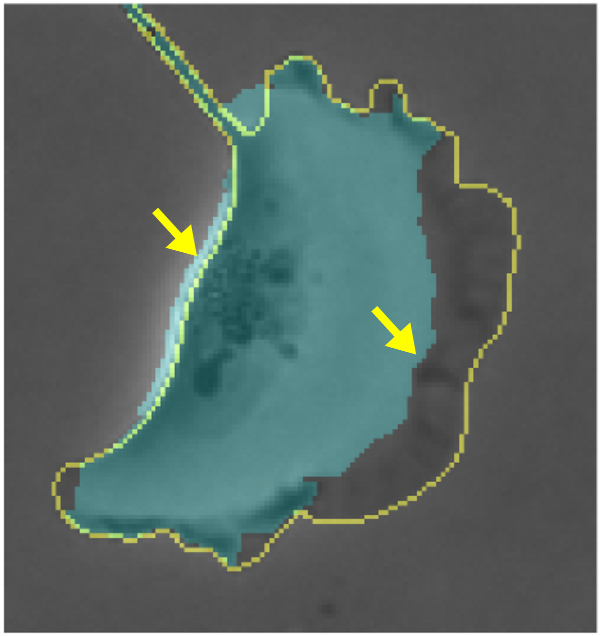
Halo pattern



Strong edges inside and outside the cell

- Drawback: **Various artifacts**

Standard segmentation algorithms

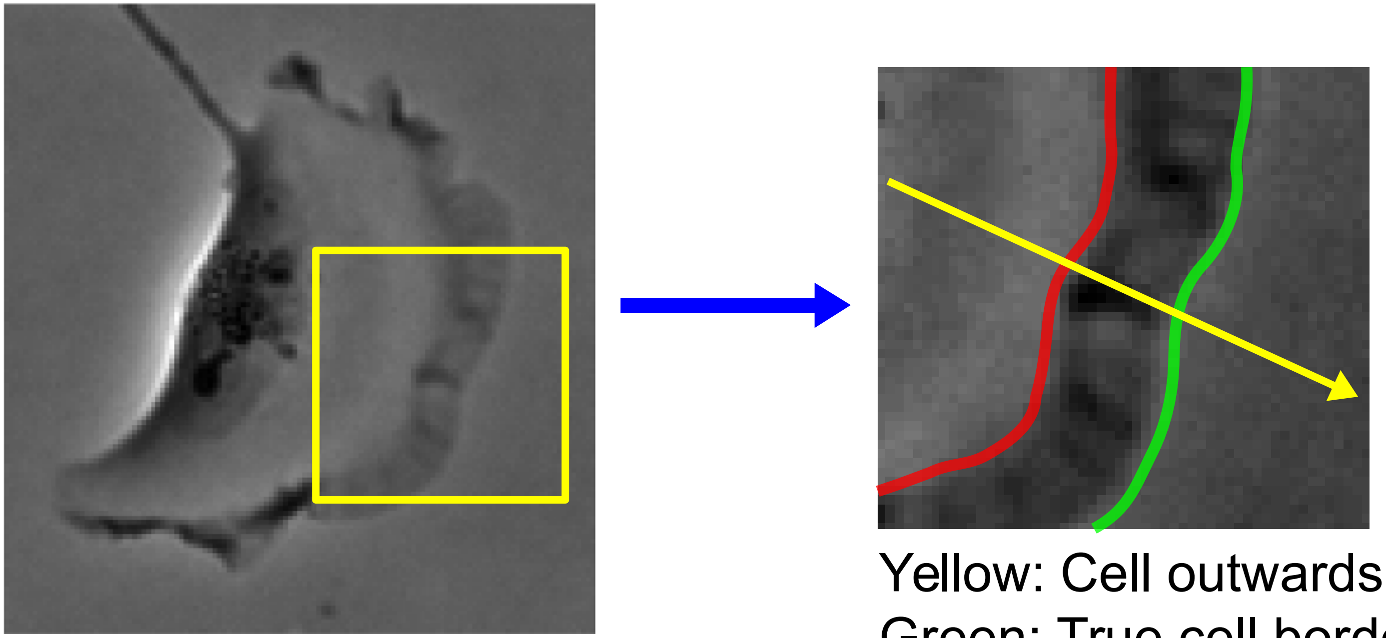


Cyan: Graph cut segmentation result
Yellow: Our manual ground truth

- Standard edge-based segmentation algorithms fail
- Traditional graph cut with **symmetric boundary costs**.

Our approach

- True cell borders appear as **dark-to-bright** transition
  (positive phase contrast microscopy)



Yellow: Cell outwards direction
Green: True cell border
Red: Wrong cell border

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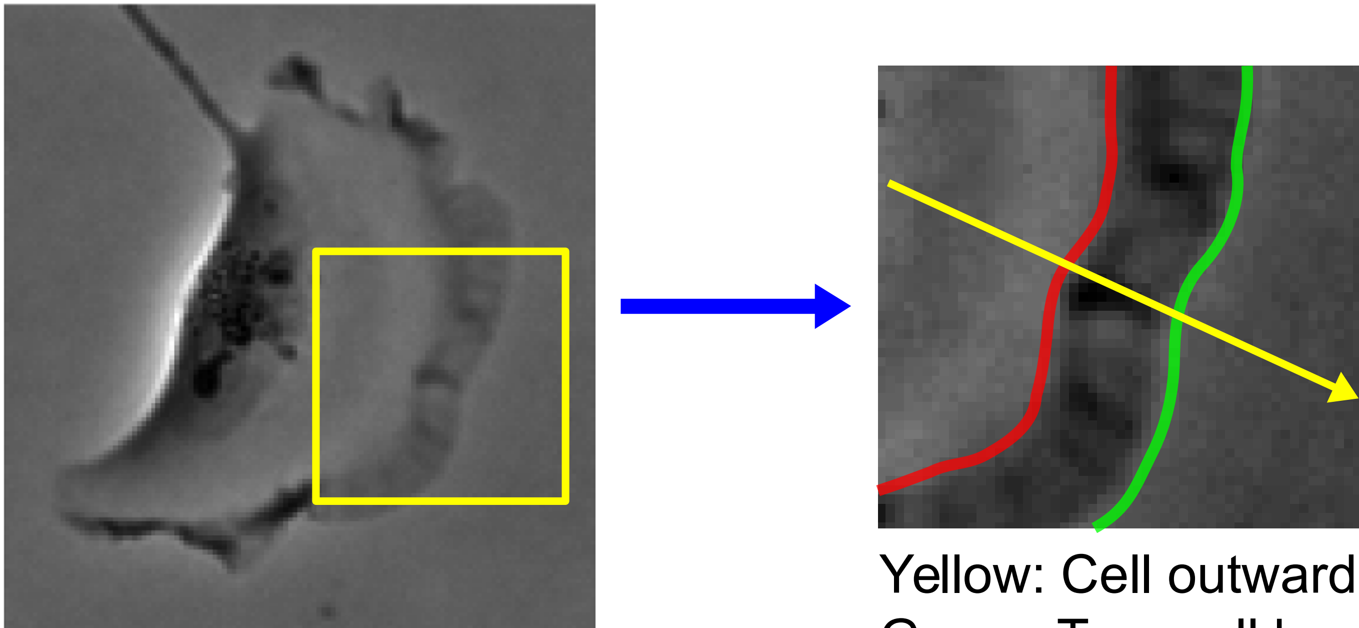

Yellow: Cell outwards direction
Green: True cell border
Red: Wrong cell border

- Search for segmentation mask that favors dark-to-bright transitions at its boundary
- Graph cut with **asymmetric boundary costs**

Related work

- Kanade et al.: Two-step reconstruction approach
  - Reconstruct abs. phase image & apply basic threshold techniques
  - Fails if sample contains light absorbing structures
- Ambühl et al.: Morphological image processing and level sets
  - Handle halo artifacts by changing image during level set evolution
- Magnusson et al.: Winner ISBI Cell Tracking Challenge 2014
  - Strong tracking approach & Segmentation based on bandpass filtering, thresholding and watershed transform

(1) K. Li and T. Kanade, “Nonnegative mixed-norm preconditioning for microscopy image segmentation,” Proceedings of IPMI, pp. 362–373, 2009.
(2) M.E. Ambül, C. Brepsant, J.-J. Meister, A.B. Verkhovsky, and I.F. Sbalzarini, “High-resolution cell outline segmentation and tracking from phase-contrast microscopy images,” JOM, vol. 245, no.2, pp. 161–170, 2012.
(3) K. Magnusson, J. Jaldén, and H. M. Blau, Cell tracking using bandpass filtering and the viterbi algorithm, Description of the algorithm available at: http://www.codesolorzano.com/celltrackingchallenge/

Related work

- Kanade et al.: Two-step reconstruction approach
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- Boykov et al.: Asymmetric boundary costs in min-cut
  - Propose asymmetric boundary costs for segmentation

  → *Never been applied to phase contrast microscopy*

(1) K. Li and T. Kanade, "Nonnegative mixed-norm preconditioning for microscopy image segmentation," Proceedings of IPMI, pp. 362–373, 2009.
(2) M.E. Ambül, C. Brepsant, J.-J. Meister, A.B. Verkhovsky, and I.F. Sbalzarini, "High-resolution cell outline segmentation and tracking from phase-contrast microscopy images," JOM, vol. 245, no.2, pp. 161–170, 2012.
(3) K. Magnusson, J. Jaldén, and H. M. Blau, Cell tracking using bandpass filtering and the viterbi algorithm, Description of the algorithm available at: http://www.codesolorzano.com/celltrackingchallenge/
(4) Y. Boykov and G. Funka-Lea, "Graph cuts and efficient n-d image segmentation," IJCV, vol. 70, no. 2, pp. 109–131, 2006.

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Segmentation energy functional

- Cost function (Region & boundary term)

$$E(M) = \lambda \cdot R(M) + \boxed{B(M)}$$

Mask $M : \Omega \to \{0,1\}$,
$\Omega \subset \mathbb{R}^2$

- Boundary term

$$B(M) = \int_\Omega C_{\text{edge}}\left(\underbrace{\langle \nabla M(\mathbf{x}), -\nabla I(\mathbf{x}) \rangle}_{\substack{\text{intensity derivative } d \\ \text{(perpendicular to mask boundary)}}}\right) d\mathbf{x}$$

Image I
∇M unit normal vector on mask boundary, and $\mathbf{0}$ elsewhere

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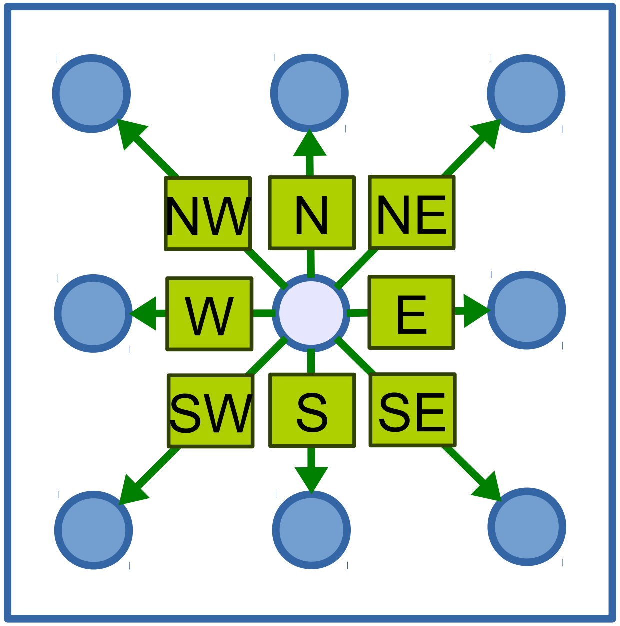
- **Asymmetric** boundary penalties **(dark-to-bright)**

$$C_{\text{edge}}(d) = \begin{cases} \exp\left(-\frac{d^2}{2\sigma^2}\right) & \text{if } \boxed{d > 0} \\ 1 & \text{else.} \end{cases}$$

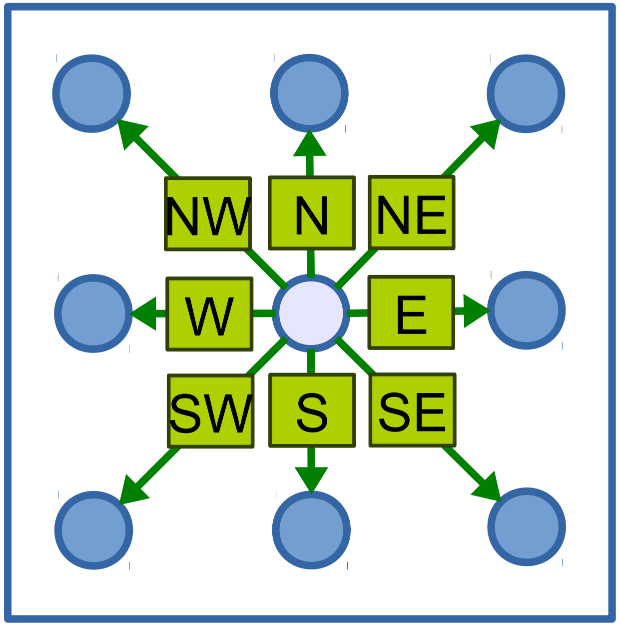
→ **directed graph** with asymmetric edge weights

Symmetric vs. asymmetric penalties



3x3 pixel neighborhood, **Edges and weights** (only outwards edges shown)

Symmetric boundary penalties



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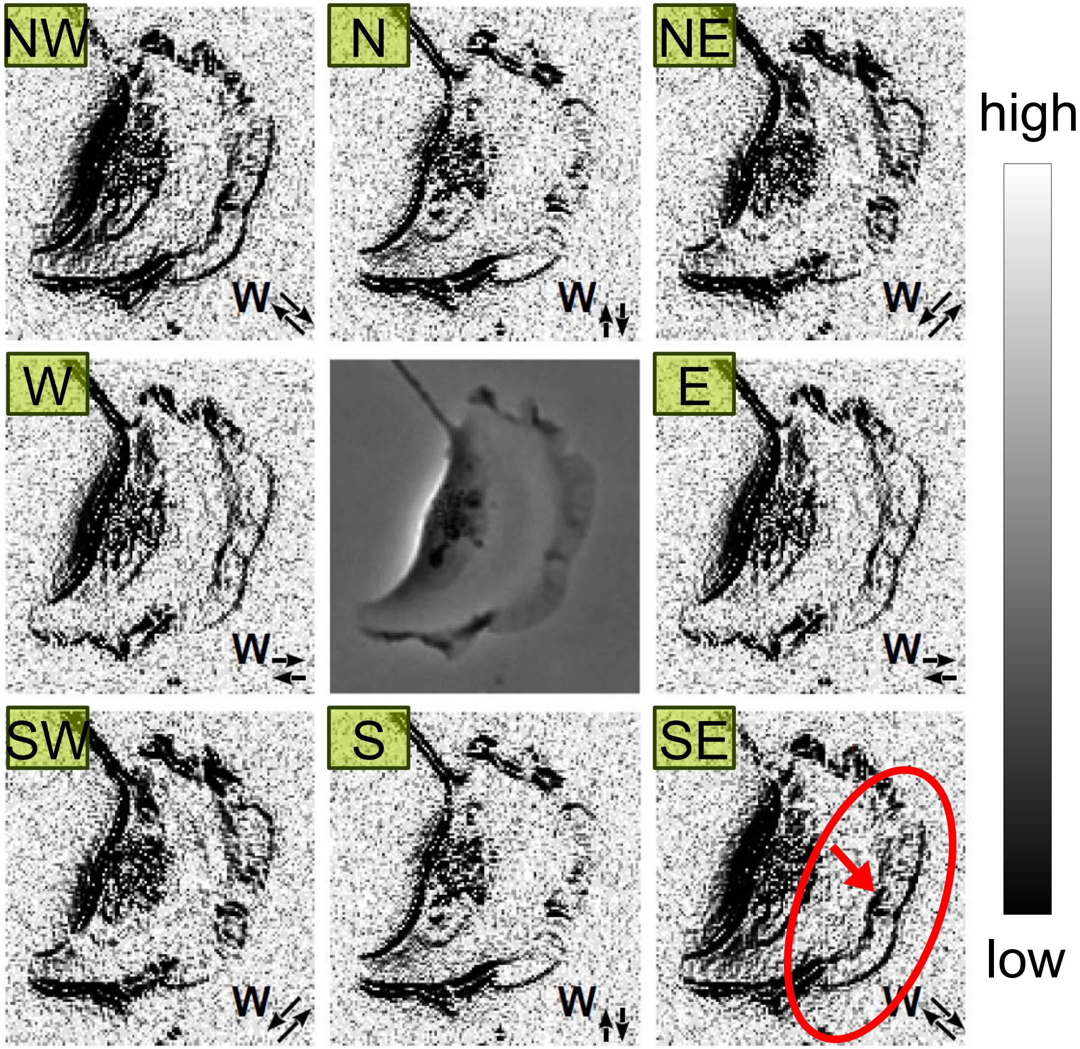
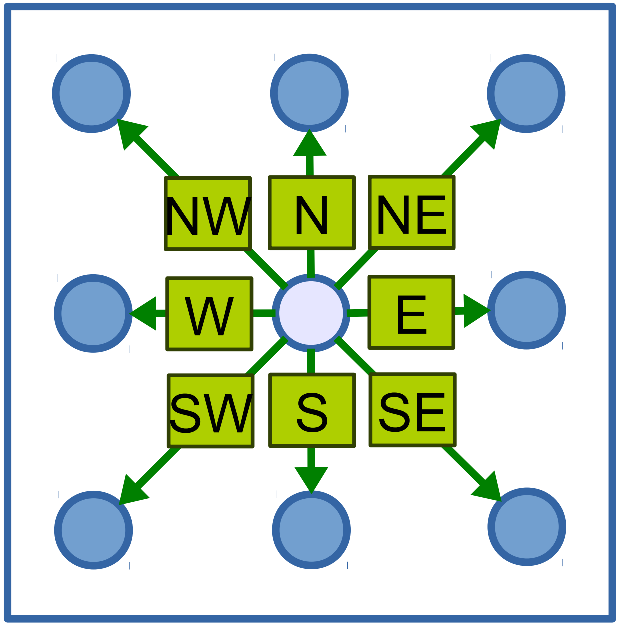


- Low costs at **wrong cell borders** (bright-to-dark transitions)

Asymmetric boundary penalties



3x3 pixel neighborhood,
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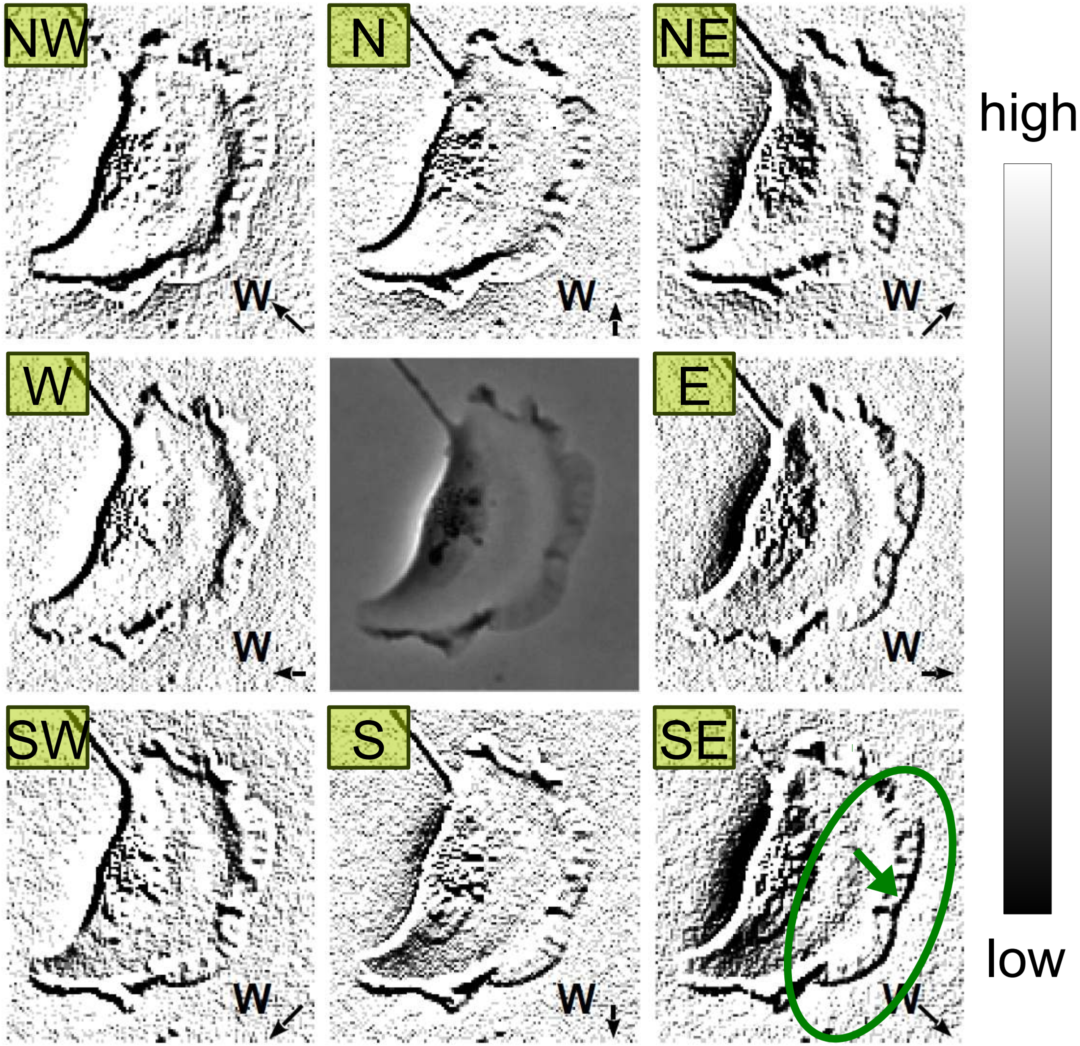
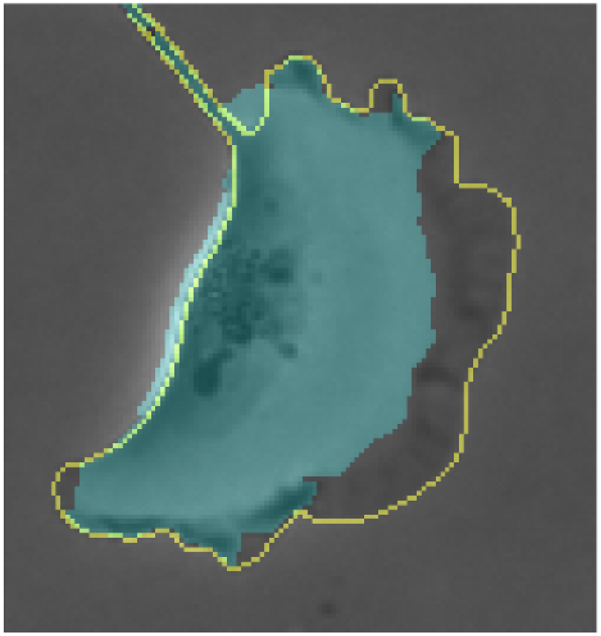
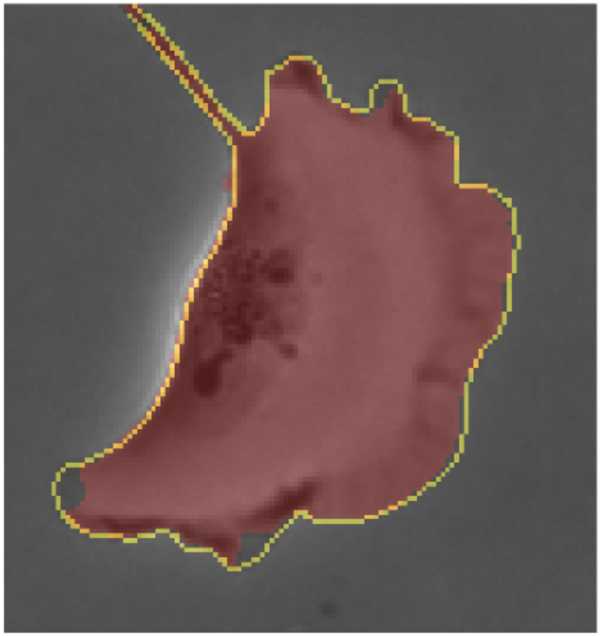


- Low costs at **correct cell borders** (dark-to-bright transitions)

Asymmetric boundary penalties



Cyan mask: Segmentation result of graph cut with **symmetric costs**
Yellow: Our manual ground truth



Red mask: Segmentation result of **proposed method**
Yellow: Our manual ground truth

Regional penalties

- Standard graph cut **(negative log-likelihood)**

$$R(A) = \sum_{p \in \mathcal{P}} R_p(A_p) \quad \text{(regional term)}$$

$$R_p(\text{"obj"}) = -\ln \Pr(I_p | \text{"obj"}) \quad \text{(object penalty)}$$

$$R_p(\text{"bkg"}) = -\ln \Pr(I_p | \text{"bkg"}) \quad \text{(background penalty)}$$

→ **hard constraint**

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$\rightarrow$ **hard constraint**

- In our approach

$$R(M) = \int_{\Omega} M(\mathbf{x}) \cdot C_{\text{obj}}(I(\mathbf{x})) d\mathbf{x} \quad \text{(regional term)}$$

$$C_{\text{obj}}(v) = \frac{P(v|\mathcal{B}) - P(v|\mathcal{O})}{P(v|\mathcal{O}) + P(v|\mathcal{B})} \quad \text{(data costs)}$$

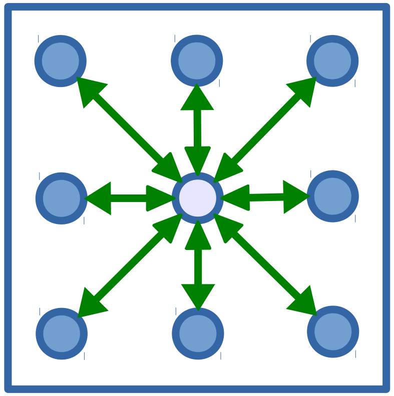
Intensity v
$P(v|\mathcal{O})$ and $P(v|\mathcal{B})$
from fore-/background
intensity histograms

$\rightarrow$ **soft constraint**

Optimization

$$E(M) = \lambda \int_\Omega M(\mathbf{x}) \cdot C_{\text{obj}}(I(\mathbf{x}))d\mathbf{x} + \int_\Omega C_{\text{edge}}\left(\langle \nabla M(\mathbf{x}), -\nabla I(\mathbf{x})\rangle\right) d\mathbf{x}$$

- Enery minimization problem
- Discretize edge term into 8 directions
  → combinatorial optimization problem
- Solve efficiently by a **min-cut approach**



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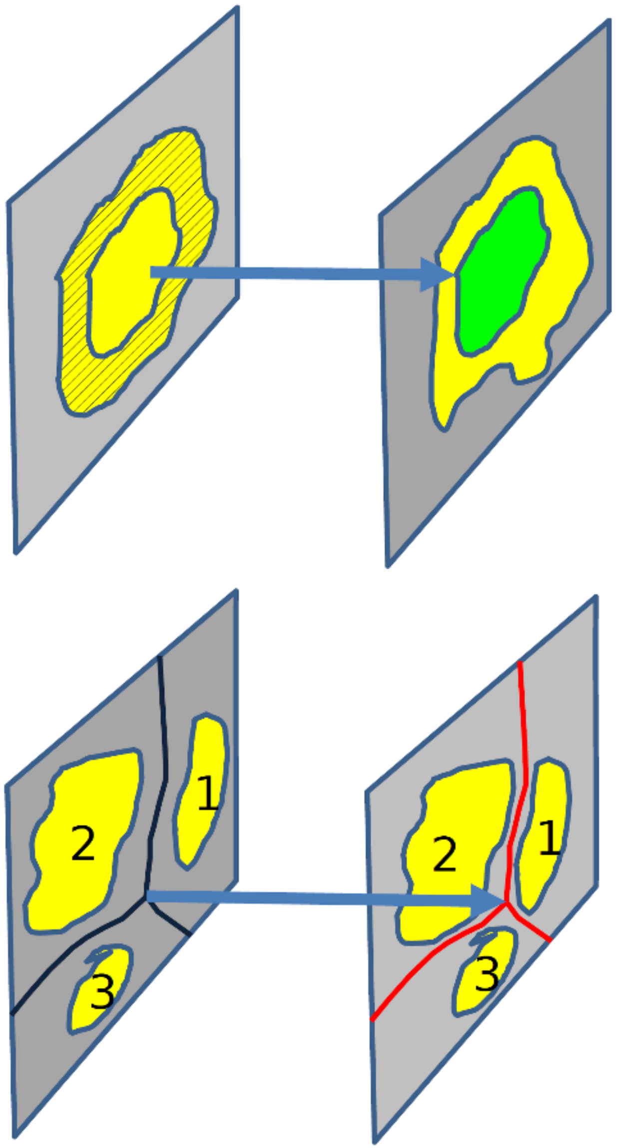
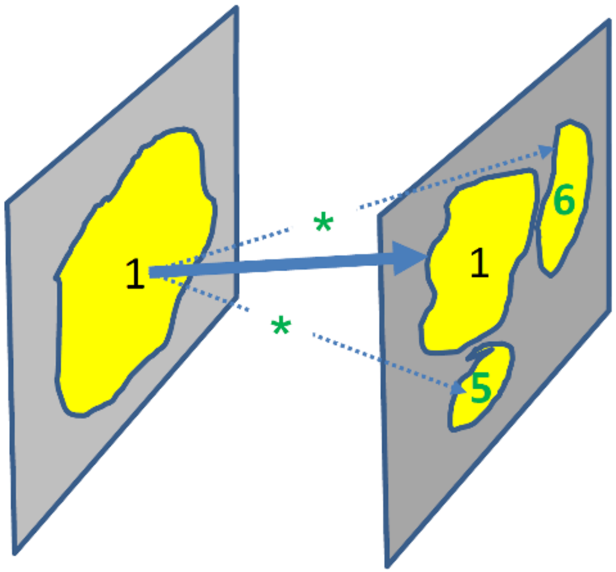


1) Propagate Segmentation Information

a) Foreground information
using eroded mask
→ hard foreground constraint

b) Partitioning information
using borders of „support regions“
→ hard background constraint

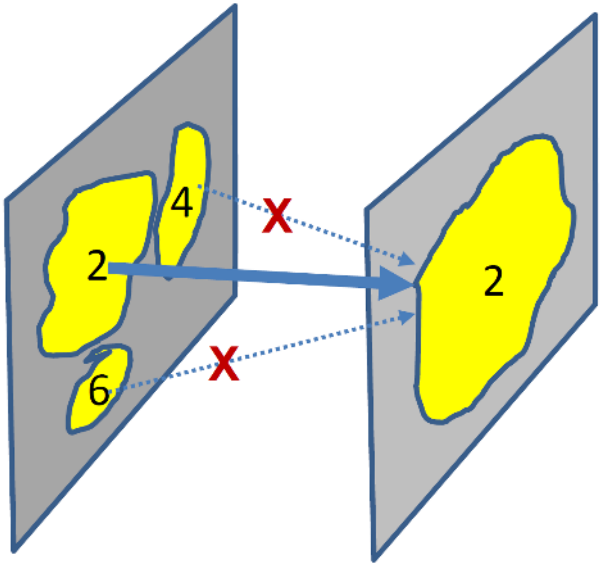
Tracking: Label propagation



2) Propagate Labels to over-lapping Segments using max. IoU

a) Resolve **one-to-many** correspondences

- start new tracks (with new label)



b) Resolve **many-to-one** correspondences

- stop other tracks

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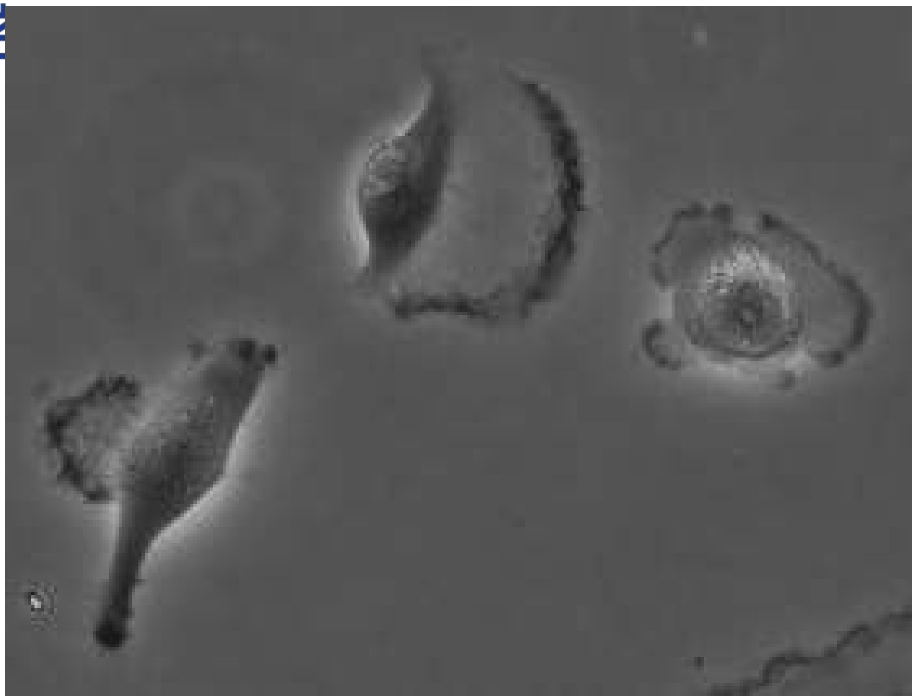
Datasets: ISBI cell tracking challenge[1,2]



Glioblastoma-astrocytoma U373 cells on a polyacrylimide substrate*
Phase contrast microscopy

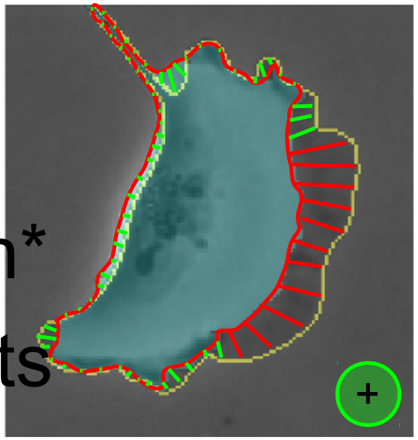
- Strong **shape** variations
- Weak outer **borders**, strong irrelevant inner borders
- **Cytoplasm** has same structure as background

(1) ISBI Cell Tracking Challenge, Available at: http://www.codesolorzano.com/celltrackingchallenge.
(2) M. Maška, V. Ulman, D. Svoboda, P. Matula, and P. Matula, et al., “A benchmark for comparison of cell tracking algorithms,” Bioinformatics, vol. 30, no. 11, pp. 1609–1617, 2014.
*Data provided by Dr. Sanjay Kumar, Department of Bioengineering University of California at Berkeley, Berkeley CA (USA).

Experiments: Boundary detection results

Boundary costs	Seq. 1			Seq. 2		
	F-meas.	Recall	Prec.	F-meas.	Recall	Prec.
Symm.	0.863	0.838	0.889	0.768	0.732	0.808
Asymm. (Equ. 2)	**0.896**	**0.894**	**0.897**	**0.835**	**0.822**	**0.847**

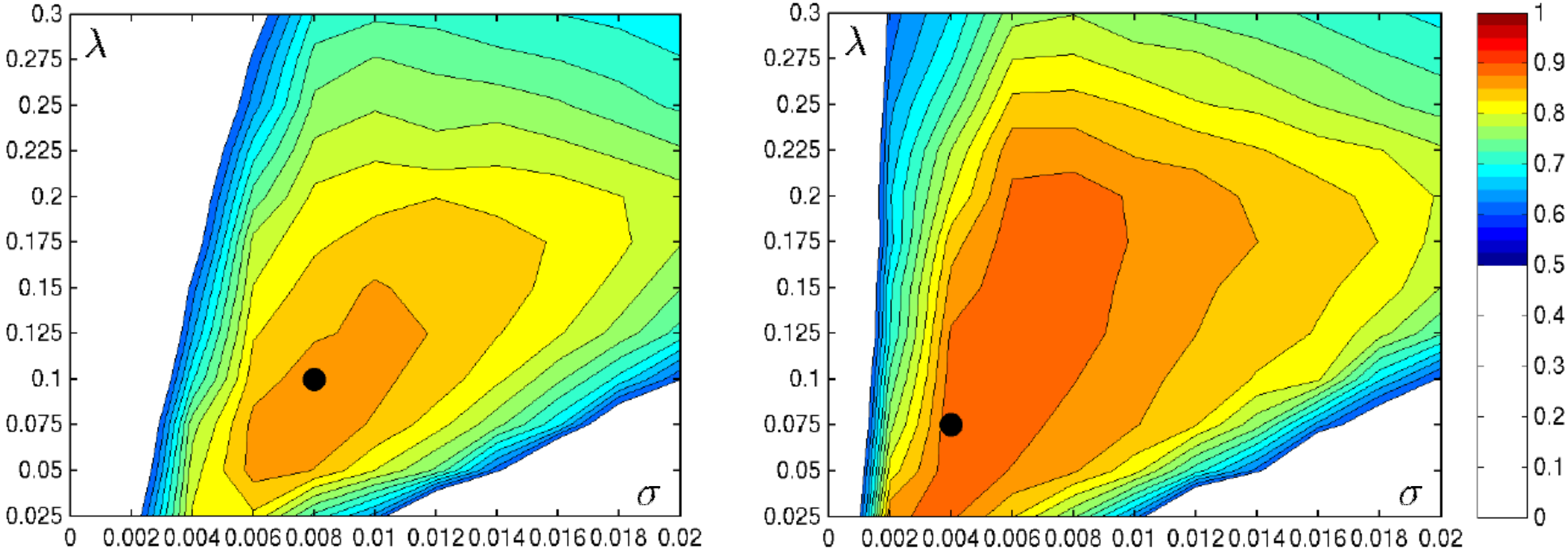
Boundary detection F-measure, recall and precision (4 pixels tolerance)



- **Boundary detection** recall and precision*
- Symmetric vs. asymmetric boundary costs

*Computed using code from „The Berkeley Segmentation Dataset and Benchmark", Available at: http://www.eecs.berkeley.edu/Research/Projects/CS/vision/bsds/.

Stability of results

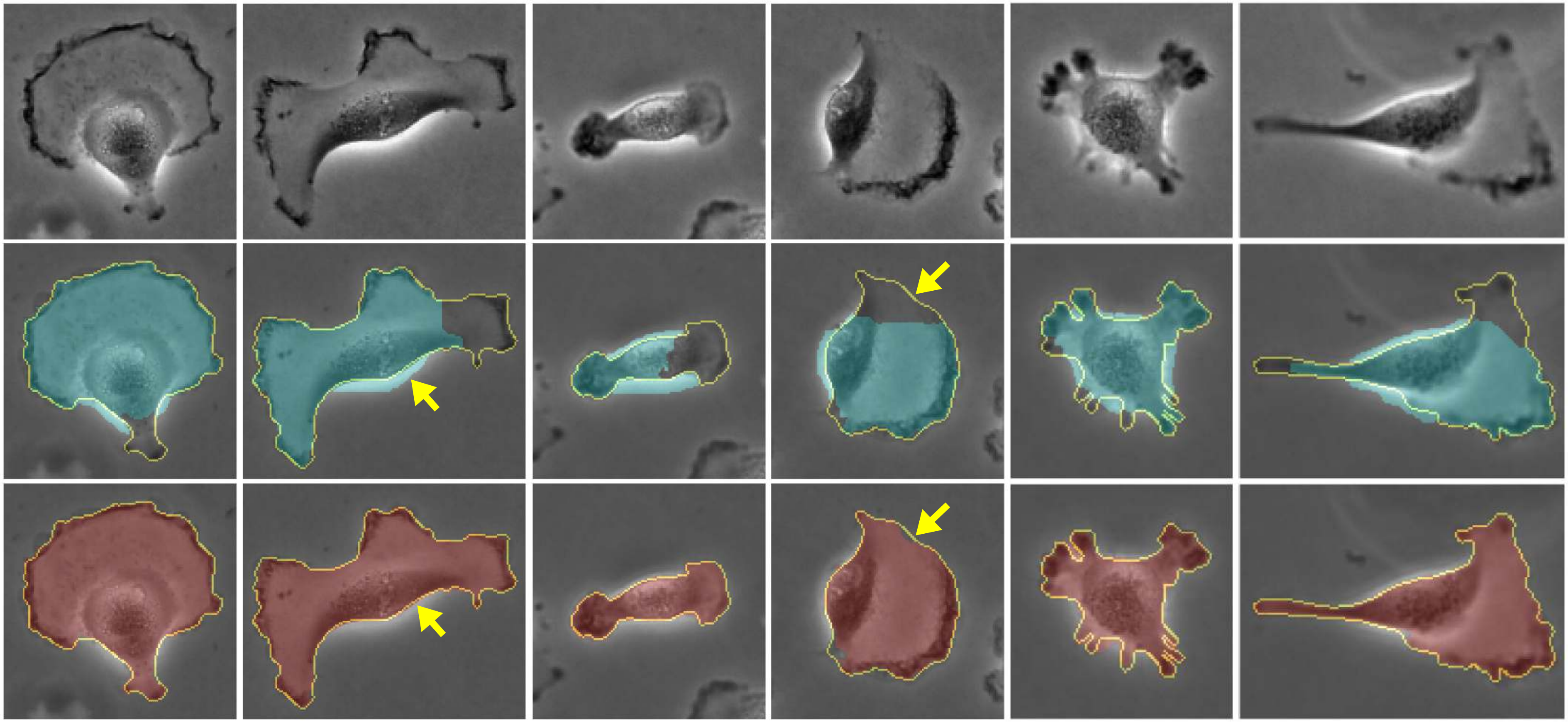


Symmetric boundary costs, F-measure isolines

Asymmetric boundary costs, F-measure isolines

- Boundary detection results across **varying min-cut parameters** lambda and sigma.

Experiments: Symmetric vs. asymmetric costs



Cyan masks: Graph cut with symmetric costs, Red masks: Our approach with asymmetric costs, Yellow borders: Our manual ground truth

- Improved detection of very **weak boundaries**
- **Halo boundaries** are handled well

Preliminary results: ISBI cell tracking challenge

Group	Av. SEG	Av. TRA
KTH-SE	**0.7953**	**0.9818**
HOUS-US	0.5323	0.9206
IMCB-SG	0.2669	0.9595

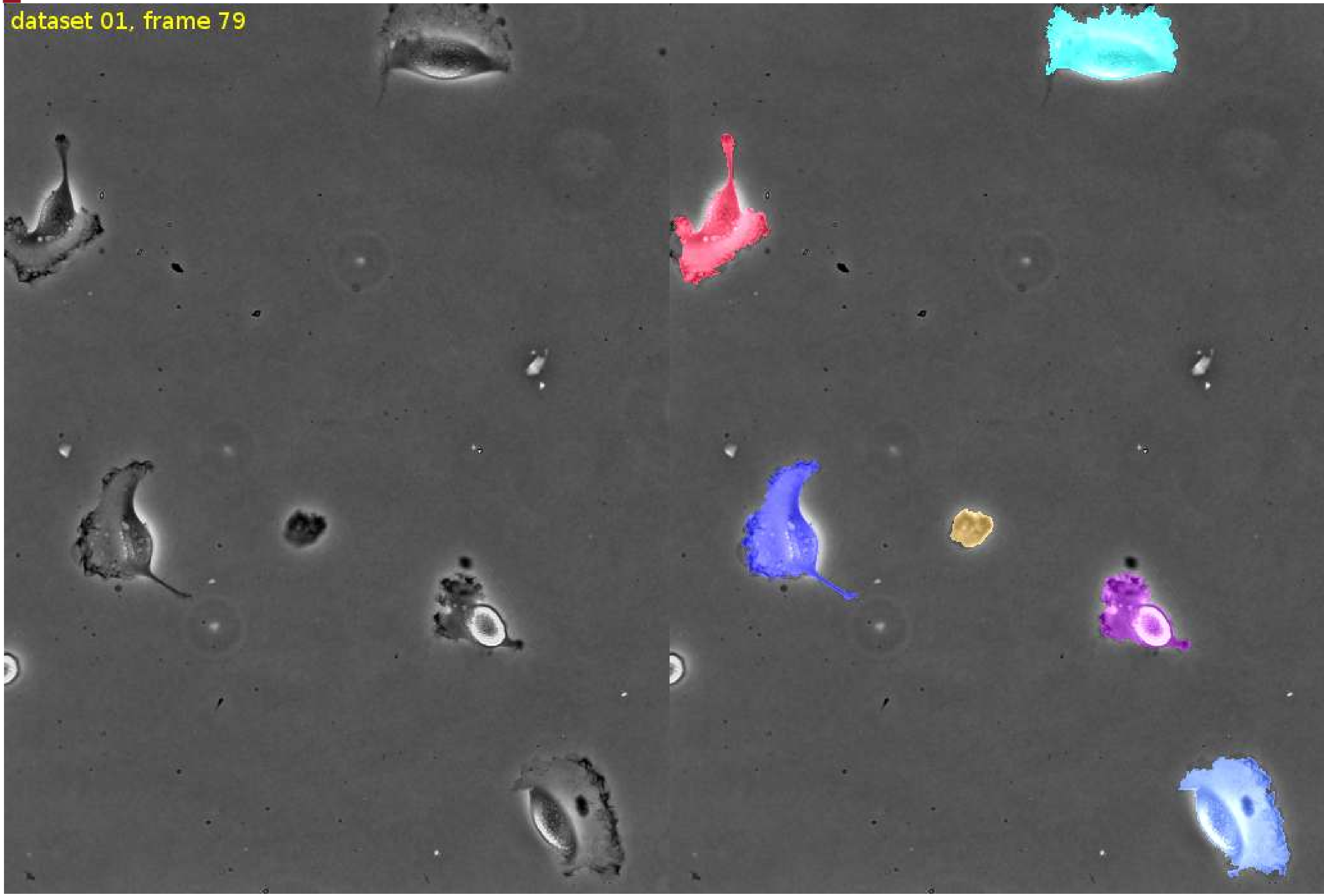
Reported results on the „challenge dataset“

Sequence	Av. SEG	Av. TRA
Seq. 1	0.8648	0.9830
Seq. 2	0.7563	0.9150
Seq. 1+2	0.8105	0.9490

Our preliminary results on the „training dataset“

- Comparison against top ranked methods from last years **ISBI cell tracking challenge**
- Phase contrast dataset: PhC-C2DH-U373

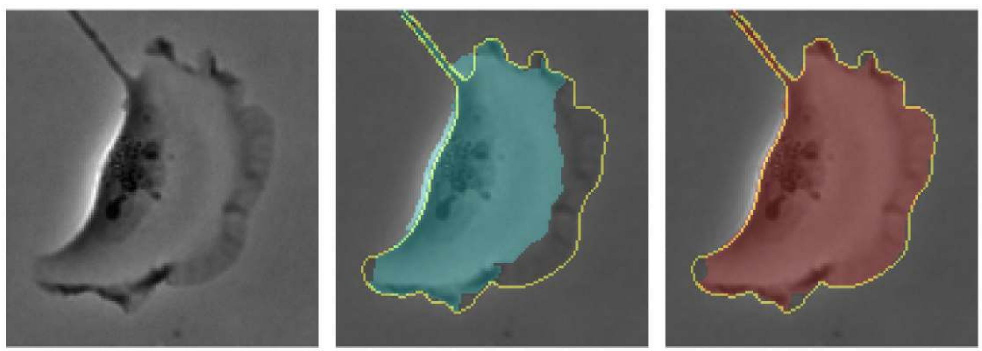
Submitted results: PhC-C2DH-U373



Submitted results: PhC-C2DH-U373

Conclusion

- Direction dependent boundary costs improve segmentation in phase contrast microscopy
- Our approach outperforms standard min-cut segmentation with symmetric costs
- Preliminary results suggest competitive performance with top-ranked methods in the ISBI CTC



→ *Profit for cell segmentation in other modalities*

→ *Open-source MATLAB code (and ImageJ plugin):*
http://lmb.informatik.uni-freiburg.de/resources/opensource/CellTracking/

Thank you!

This study was supported by the Excellence Initiative of the German Federal and State Governments (EXC 294).