

Yanxi Liu
Tianzi Jiang
Changshui Zhang (Eds.)

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Volume Editors

Yanxi Liu

Carnegie Mellon University

School of Computer Science

The Robotics Institute

4109 Newell-Simon Hall, 5000 Forbes Ave., Pittsburgh, PA 15213

E-mail: yanxi@cs.cmu.edu

Tianzi Jiang

Chinese Academy of Sciences

National Laboratory of Pattern Recognition

Institute of Automation

100080, Beijing, China

E-mail: jiangtz@nlpr.ia.ac.cn

Changshui Zhang

Tsinghua University

Department of Automation

Beijing, 100084, China

E-mail: zcs@mail.tsinghua.edu.cn

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Table of Contents

Computational Anatomy and Computational Physiology for Medical Image Analysis <i>Nicholas Ayache</i>	1
Analyzing Anatomical Structures: Leveraging Multiple Sources of Knowledge <i>Eric Grimson, Polina Golland</i>	3
Advances on Medical Imaging and Computing <i>Tianzi Jiang, Xiaobo Li, Gaolong Gong, Meng Liang, Lixia Tian, Fuchun Li, Yong He, Yufeng Zang, Chaozhe Zhu, Shuyu Li, Songyuan Tang</i>	13
Tracking of Migrating and Proliferating Cells in Phase-Contrast Microscopy Imagery for Tissue Engineering <i>Takeo Kanade, Kang Li</i>	24
Cardiology Meets Image Analysis: Just an Application or Can Image Analysis Usefully Impact Cardiology Practice? <i>J. Alison Noble</i>	25
Computer Vision Algorithms for Retinal Image Analysis: Current Results and Future Directions <i>Charles V. Stewart</i>	31
3D Statistical Shape Models to Embed Spatial Relationship Information <i>Jurgen Fripp, Pierrick Bourgeat, Andrea J.U. Mewes, Simon K. Warfield, Stuart Crozier, Sébastien Ourselin</i>	51
A Generalized Level Set Formulation of the Mumford-Shah Functional with Shape Prior for Medical Image Segmentation <i>Lishui Cheng, Xian Fan, Jie Yang, Yun Zhu</i>	61
A Hybrid Eulerian-Lagrangian Approach for Thickness, Correspondence, and Gridding of Annular Tissues <i>Kelvin R. Rocha, Anthony J. Yezzi, Jerry L. Prince</i>	72
A Hybrid Framework for Image Segmentation Using Probabilistic Integration of Heterogeneous Constraints <i>Rui Huang, Vladimir Pavlovic, Dimitris N. Metaxas</i>	82

A Learning Framework for the Automatic and Accurate Segmentation of Cardiac Tagged MRI Images <i>Zhen Qian, Dimitris N. Metaxas, Leon Axel</i>	93
A Local Adaptive Algorithm for Microaneurysms Detection in Digital Fundus Images <i>Ke Huang, Michelle Yan</i>	103
A New Coarse-to-Fine Framework for 3D Brain MR Image Registration <i>Terrence Chen, Thomas S. Huang, Wotao Yin, Xiang Sean Zhou</i>	114
A New Vision Approach for Local Spectrum Features in Cervical Images Via 2D Method of Geometric Restriction in Frequency Domain <i>Viara Van Raad</i>	125
Active Contours Under Topology Control – Genus Preserving Level Sets <i>Florent Ségonne, Jean-Philippe Pons, Eric Grimson, Bruce Fischl</i>	135
A Novel Multifaceted Virtual Craniofacial Surgery Scheme Using Computer Vision <i>A.S. Chowdhury, S.M. Bhandarkar, E.W. Tollner, G. Zhang, J.C. Yu, E. Ritter</i>	146
A Novel Unsupervised Segmentation Method for MR Brain Images Based on Fuzzy Methods <i>Xian Fan, Jie Yang, Yuanjie Zheng, Lishui Cheng, Yun Zhu</i>	160
A Pattern Classification Approach to Aorta Calcium Scoring in Radiographs <i>Marleen de Bruijne</i>	170
A Topologically Faithful, Tissue-Guided, Spatially Varying Meshing Strategy for Computing Patient-Specific Head Models for Endoscopic Pituitary Surgery Simulation <i>M.A. Audette, H. Delingette, A. Fuchs, O. Astley, K. Chinzei</i>	178
Applying Prior Knowledge in the Segmentation of 3D Complex Anatomic Structures <i>Hong Shen, Yonggang Shi, Zhigang Peng</i>	189
Automatic Extraction of Femur Contours from Hip X-Ray Images <i>Ying Chen, Xianhe Ee, Wee Kheng Leow, Tet Sen Howe</i>	200

Automatic Landmarking of 2d Medical Shapes Using the Growing Neural Gas Network <i>Anastassia Angelopoulou, Alexandra Psarrou, José García Rodríguez, Kenneth Revett</i>	210
Biomedical Image Classification with Random Subwindows and Decision Trees <i>Raphaël Marée, Pierre Geurts, Justus Piater, Louis Wehenkel</i>	220
Combining Binary Classifiers for Automatic Cartilage Segmentation in Knee MRI <i>Jenny Folkesson, Ole Fogh Olsen, Paola Pettersen, Erik Dam, Claus Christiansen</i>	230
Computer-Aided Diagnosis (CAD) for Cervical Cancer Screening and Diagnosis: A New System Design in Medical Image Processing <i>Wenjing Li, Viara Van Raad, Jia Gu, Ulf Hansson, Johan Hakansson, Holger Lange, Daron Ferris</i>	240
Constrained Surface Evolutions for Prostate and Bladder Segmentation in CT Images <i>Mikael Rousson, Ali Khamene, Mamadou Diallo, Juan Carlos Celi, Frank Sauer</i>	251
Curvilinear Structure Based Mammographic Registration <i>Lionel C.C. Wai, Michael Brady</i>	261
Deformable Registration for Generating Dissection Image of an Intestine from Annular Image Sequence <i>Suchit Pongnumkul, Ryusuke Sagawa, Tomio Echigo, Yasushi Yagi</i> ...	271
Distance-Intensity for Image Registration <i>Rui Gan, Albert C. S. Chung</i>	281
Efficient Population Registration of 3D Data <i>Lilla Zöllei, Erik Learned-Miller, Eric Grimson, William Wells</i>	291
Efficient Symbolic Signatures for Classifying Craniosynostosis Skull Deformities <i>H. Jill Lin, Salvador Ruiz-Correa, Raymond W. Sze, Michael L. Cunningham, Matthew L. Speltz, Anne V. Hing, Linda G. Shapiro</i>	302
Elastic Interaction Models for Active Contours and Surfaces <i>Albert C.S. Chung, Yang Xiang, Jian Ye, W.K. Law</i>	314

Estimating Diameters of Pulmonary Nodules with Competition-Diffusion and Robust Ellipsoid Fit <i>Toshiro Kubota, Kazunori Okada</i>	324
Fast 3D Brain Segmentation Using Dual-Front Active Contours with Optional User-Interaction <i>Hua Li, Anthony Yezzi, Laurent D. Cohen</i>	335
Improved Motion Correction of fMRI Time-Series Corrupted with Major Head Movement Using Extended Motion-Corrected Independent Component Analysis <i>Rui Liao, Martin McKeown, Jeffrey Krolik</i>	346
Local or Global Minima: Flexible Dual-Front Active Contours <i>Hua Li, Anthony Yezzi</i>	356
Locally Switching Between Cost Functions in Iterative Non-rigid Registration <i>William Mullally, Margrit Betke, Carissa Bellardine, Kenneth Lutchen</i>	367
Multi-modal Image Registration by Quantitative-Qualitative Measure of Mutual Information (Q-MI) <i>Hongxia Luan, Feihu Qi, Dinggang Shen</i>	378
Multi-scale Vessel Boundary Detection <i>Hüseyin Tek, Alper Ayvaci, Dorin Comaniciu</i>	388
Non-rigid Registration for Colorectal Cancer MR Images <i>Sarah L. Bond, J. Michael Brady</i>	399
Quantizing Calcification in the Lumbar Aorta on 2-D Lateral X-Ray Images <i>Lars A. Conrad-Hansen, Marleen de Bruijne, François Lauze, Laszlo B. Tanko, Mads Nielsen</i>	409
Real-Time Simulation of Deformable Soft Tissue Based on Mass-Spring and Medial Representation <i>Shaoting Zhang, Lixu Gu, Pengfei Huang, Jianfeng Xu</i>	419
Registration of 3D Angiographic and X-Ray Images Using Sequential Monte Carlo Sampling <i>Charles Florin, James Williams, Ali Khamene, Nikos Paragios</i>	427

Registration of PET and MR Hand Volumes Using Bayesian Networks <i>Derek Magee, Steven Tanner, Michael Waller, Dennis McGonagle, Alan P. Jeavons</i>	437
Segmentation and Volume Representation Based on Spheres for Non-rigid Registration <i>Jorge Rivera-Rovelo, Eduardo Bayro-Corrochano</i>	449
Segmentation of 3D CT Volume Images Using a Single 2D Atlas <i>Feng Ding, Wee Kheng Leow, Shih-Chang Wang</i>	459
Segmenting Brain Tumors with Conditional Random Fields and Support Vector Machines <i>Chi-Hoon Lee, Mark Schmidt, Albert Murtha, Aalo Bistritz, Jörg Sander, Russell Greiner</i>	469
Segmenting Cardiopulmonary Images Using Manifold Learning with Level Sets <i>Qilong Zhang, Robert Pless</i>	479
Shape Based Segmentation of Anatomical Structures in Magnetic Resonance Images <i>Kilian M. Pohl, John Fisher, Ron Kikinis, W. Eric L. Grimson, William M. Wells</i>	489
Simultaneous Segmentation and Motion Recovery in 3D Cardiac Image Analysis <i>Ling Zhuang, Huafeng Liu, Wei Chen, Hujun Bao, Pengcheng Shi</i> ...	499
Spatial and Temporal Analysis for Optical Imaging Data Using CWT and tICA <i>Yadong Liu, Guohua Zang, Fayi Liu, Lirong Yan, Ming Li, Zongtan Zhou, Dewen Hu</i>	508
Stereo Matching and 3-D Reconstruction for Optic Disk Images <i>Zhang Kai, Xu Xi, Zhang Li, Wang Guoping</i>	517
Total Variation Based Iterative Image Reconstruction <i>Guoqiang Yu, Liang Li, Jianwei Gu, Li Zhang</i>	526
Voronoi-Based Segmentation of Cells on Image Manifolds <i>Thouis R. Jones, Anne Carpenter, Polina Golland</i>	535
Parcellating the Intra-splenium Based on the Traced Fiber from Tractography <i>Gaolang Gong, Tianzi Jiang, Sheng Xie, Fuchun Lin</i>	544

XIV Table of Contents

Motion Compensation and Plane Tracking for Kinematic MR-Imaging <i>Daniel Bystrov, Vladimir Pekar, Kirsten Meetz, Heinrich Schulz,</i> <i>Thomas Netsch</i>	551
Author Index	561

Voronoi-Based Segmentation of Cells on Image Manifolds

Thouis R. Jones¹, Anne Carpenter², and Polina Golland

¹ MIT CSAIL, Cambridge, MA, USA

² Whitehead Institute for Biomedical Research, Cambridge, MA, USA

Abstract. We present a method for finding the boundaries between adjacent regions in an image, where “seed” areas have already been identified in the individual regions to be segmented. This method was motivated by the problem of finding the borders of cells in microscopy images, given a labelling of the nuclei in the images. The method finds the Voronoi region of each seed on a manifold with a metric controlled by local image properties. We discuss similarities to other methods based on image-controlled metrics, such as Geodesic Active Contours, and give a fast algorithm for computing the Voronoi regions. We validate our method against hand-traced boundaries for cell images.

1 Introduction

Image cytometry, the measurement of cell properties from microscopy images, has become an important tool for biological research. In particular, high-throughput experiments rely on automatic processing of images to deal with the large amount of data they produce [3]. A fundamental operation in cell-image analysis is identifying individual cell boundaries. This is often difficult because there are many different staining protocols, leading to dramatically different appearances for cells. Moreover, the difference between cell interior and cell border may not be very pronounced (see Figures 1 and 2). Identification of individual cells allows much more powerful analysis of the resulting data than methods that provide only mean measurements for cell populations. For example, expression data from a protein interaction chip cannot differentiate a bimodal population and a unimodal population if they have the same mean expression levels. Measurements of individual cells prevents conflation of subpopulations.

It is almost always the case that the nuclei of cells are more easily identifiable, because they have a more uniform appearance and shape, are brighter relative to the background when stained, and do not abut one another, as cells do. They are also usually interior to the cells. This leads us to phrase the problem of segmenting cells as one of identifying boundaries between regions given “seeds” in individual regions from which to start the segmentation. Current methods for identifying cells for image cytometry sometimes use a fixed offset around the nuclei. However, this fixed offset requires tuning to different cell types, and does not provide information about phenotypes that cause changes in cell size or shape [2].

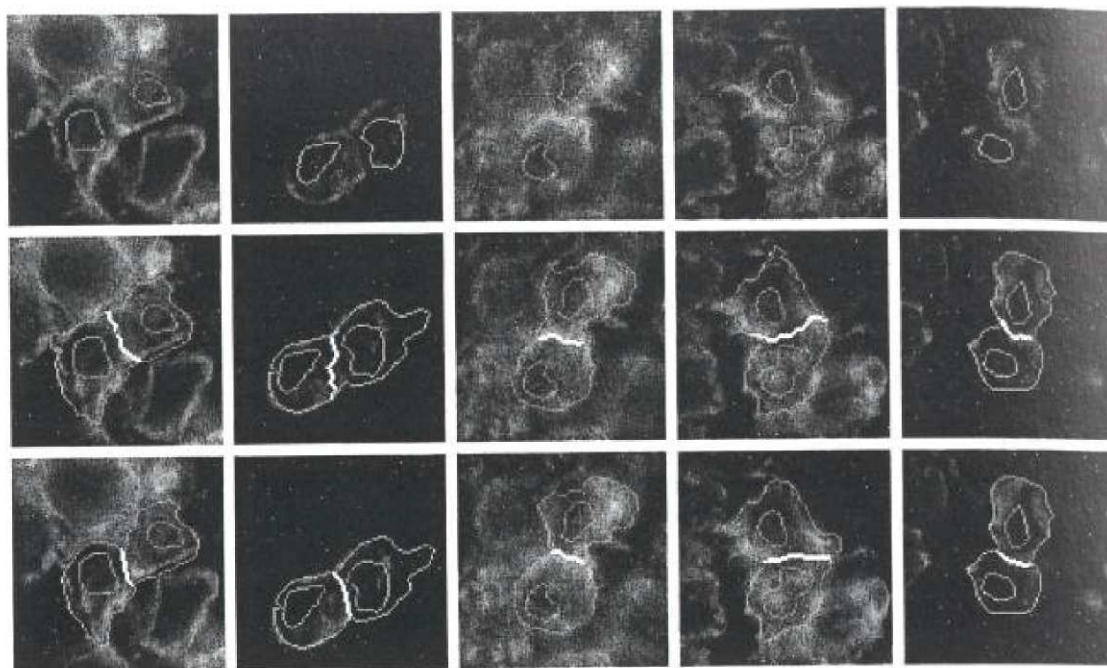


Fig. 1. Typical results from our algorithm. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

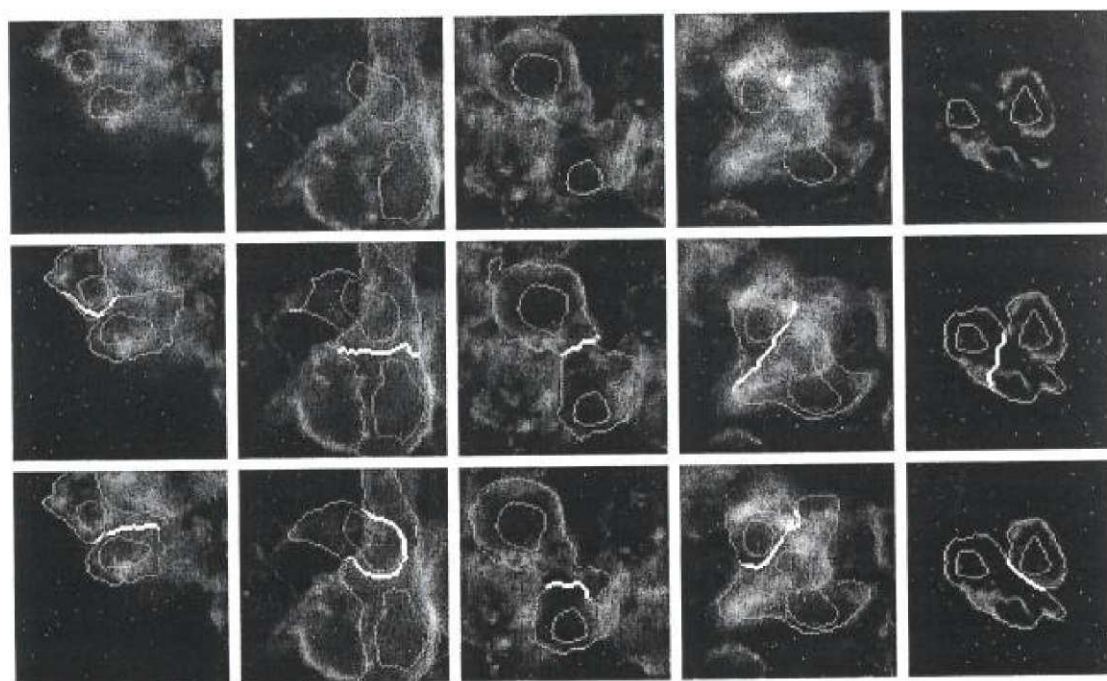


Fig. 2. Five worst outliers from evaluation set, as measured by distance between manual and automatic segmentation boundaries. Internal shapes are seed regions, taken from manual segmentation of nuclei, and are the same in both rows. Bold lines show cell/cell boundaries that are compared. Top row: Cell images, with nuclei outlined. Middle row: Automatic segmentation with our method. Bottom row: Manual segmentation.

Another common approach is to use watershed segmentation to identify cell boundaries [1,8], however, this is often fragile. Watershed segmentation treats the image as a height field, and segments pixels according to which minimum a drop of water would flow to if placed on that pixel in the height field. Morphological operations are used to impose a limited set of minima, equivalent to our seed regions. Watershed is quite unstable, because a single noisy pixel can allow large groups of pixels to change segmentation, by creating a gap leading to a different minimum. We avoid this fragility in two ways: first, by comparing neighborhoods of pixels rather than individual pixels, and second, by including a regularization factor to provide reasonable behavior when the image data does not contain a strong enough edge between two seed regions. In the limit, our regularization approaches a 2D Voronoi segmentation, i.e., pixels are assigned to the nearest seed region measured in the image plane, without reference to image features.

Our approach is to define a metric in the image plane and to calculate distances from seed regions according to this metric. Pixels are then assigned to cells according to their distance from the corresponding nucleus under that metric. The metric uses information about image edges, both their strength and their orientation, as well as a regularization term corresponding to inter-pixel distance within the image.

2 Method

Our method operates by computing a discretized approximation of the Voronoi regions of each seed on a manifold with a metric controlled by local image features. The metric defines the incremental distance in a particular direction in the image/manifold. Its behavior is such that adjacent pixels with similar surrounds are close to one another, while pixels whose surrounds differ are treated as farther apart.

We introduce a Riemannian metric defined in terms of the image $\mathcal{I}$ and a regularization parameter λ , as

$$\mathbf{G} = \frac{\nabla \mathbf{g}(\mathcal{I}) \nabla \mathbf{g}^T(\mathcal{I}) + \lambda \mathbf{I}}{1 + \lambda}, \quad (1)$$

where $\mathbf{I}$ is the 2×2 identity matrix. The function $\mathbf{g}$ maps images to images, and in our application is generally a small-radius blur. The effect of this blur is to combine a weighted neighborhood in the gradient computation, to avoid relying too much on single (noisy) pixel values. Infinitesimal distances under $\mathbf{G}$ are measured by

$$\|d\mathbf{x}\|_{\mathbf{G}}^2 \equiv d\mathbf{x}^T \mathbf{G} d\mathbf{x} = \frac{(d\mathbf{x}^T \nabla \mathbf{g}(\mathcal{I}))^2 + \lambda (d\mathbf{x}^T d\mathbf{x})^2}{\lambda + 1}. \quad (2)$$

The first term in the numerator of (2), $\|d\mathbf{x}^T \nabla \mathbf{g}(\mathcal{I})\|^2$, increases distances measured parallel to large gradients in $\mathbf{g}(\mathcal{I})$. The regularization effect of λ can be seen by

$$\lim_{\lambda \rightarrow \infty} \|d\mathbf{x}\|_{\mathbf{G}}^2 = d\mathbf{x}^T d\mathbf{x} = \|d\mathbf{x}\|_2^2, \quad (3)$$

i.e., $\mathbf{G}$ becomes more Euclidean as λ increases.

Given (2), we can compute the distance between any two points in an image as the shortest path between those points. We use a discretized approximation, i.e., a chamfer

distance, applied to an 8-connected neighborhood. This approximation to the distance is generally no worse than 10% of the correct value [7], which for our application is generally not more than a single pixel.

Pixels overlapping seed regions are initialized to be distance 0 from their seed, and the distances of the remaining pixels are computed by Dijkstra's algorithm [6]. Each pixel is labelled with the seed it is closest to, i.e., the Voronoi region of that seed in the manifold defined by (2). Computing the seed-to-pixel distances in this manner also makes it trivial to limit the segmentation to a predetermined foreground region (for example, the result from a global thresholding step).

The computation of Voronoi regions places the boundary between two adjacent regions at pixels that are equidistant from each seed, as measured on the manifold from (2). The inter-pixel distance is larger where the image is changing more according to $\nabla g(\mathcal{I})$, so boundaries tend to align with image differences. The regularizing parameter λ allows the user to make the manifold more "flat" according to prior knowledge about region shapes, and the choice of g (e.g., the radius of smoothing) controls how sensitive distances are to small features. In this work, we use a narrow (3- or 5-pixel radius) Gaussian blur for g .

2.1 Connection to Geodesic Active Contours

Our algorithm is related to Geodesic Active Contours [5]. The full details of that work are not given here, but we discuss the connection briefly.

Active contours can be seen as finding a shortest path in a Riemannian space, where distances between pixels are defined by an edge stopping function $g : \mathbb{R}^+ \rightarrow \mathbb{R}^+$.¹ Examining equation (8) from Caselles *et al.* [5] helps establish the similarity:

$$\text{Min} \int_0^1 g(|\nabla \mathcal{I}(\mathcal{C}(q))|) |\mathcal{C}'(q)| dq \quad (4)$$

where $\mathcal{I}$ is the image, $\mathcal{C}(q)$ is the curve on image that we are minimizing over, and q is the parameter along the curve. The edge stopping function g is strictly decreasing and positive, with $g(\infty) = 0$. The effect of g 's interaction with $\nabla \mathcal{I}$ is such that the minimum curve follows larger gradients in the image.

The minimization can also be written as (equation (12) of [5])

$$\text{Min} \int_0^{\mathcal{L}(\mathcal{C})} g(|\nabla \mathcal{I}(\mathcal{C}(s))|) ds \quad (5)$$

where s is the arclength parameter for $\mathcal{C}$, and $\mathcal{L}(\mathcal{C})$ is the length of $\mathcal{C}$. Therefore, active contours can be seen as seeking a minimum length curve where the length depends on image characteristics [5].

Our goal is different from active contours, since we hope to find boundaries between regions corresponding to different seeds. However, we do seek shortest paths with a distance metric controlled by image characteristics. We do not wish to follow boundaries, as in active contours, but rather to avoid them (equivalent to making boundaries "far")

¹ In this subsection, g is an edge stopping function, not the same as g in the previous subsection.

from the seed regions in our formulation). However, just as Voronoi regions can be defined in terms of shortest paths, our segmentation algorithm can be defined in terms of shortest paths defined by equation (1) and image properties. The metrics in the two approaches differ, specifically in their treatment of edges in the image: edges in the active contour setting have a metric that makes points along the edge closer, while our metric makes the points across the edge more separated. Moreover, active contours use a directionally uniform metric, while ours is not, since it is larger across edges rather than along them. However, the goal is the same: allow the computation of inter-pixel distances that simplify the problem at hand and map it to a simpler framework. In both cases, the problem reduces to that of finding shortest paths (though this is simply a jumping-off point for active contours to more powerful and efficient methods such as level-sets).

3 Experiments and Evaluation

In order to provide insight into the behavior of the metric defined in (1), we experiment with synthetic data and adjust λ , trading off the effect of image features on the region segmentation versus the Euclidean distance from the seed. As can be seen in Figures 3 and 4, the segmentation approaches the Voronoi diagram of the seed regions (i.e., ignoring image features) as λ increases. Also, note that in the noisy example less regularization (i.e., a lower value for λ) is needed to achieve similar segmentations because the noise makes the edges in the image less pronounced than in the noise-free example. In effect, increasing noise provides a form of regularization.

Our algorithm is currently part of an automatic image cytometry program [4], and has been used in a variety of experiments with several cell types of varying morphologies. To evaluate our algorithm on real world data, we compare it to the manual segmentation of cell images. Sixteen images taken from *Drosophila* cells stained for DNA (to label nuclei) and Actin (a cytoskeletal protein, to show the cell body) were outlined. First, nuclei were outlined by hand. The nuclear outlines were overlaid on the cell images, and one cell per nucleus was outlined. Our algorithm was then applied to the same cell images and nuclear-outlines data, and the results compared by computing the signed distance between boundary pixels. It effectively computes the distance for each pixel in the automatic segmentation boundary to the corresponding manual segmentation boundary. Hand-outlined nuclei were used, rather than automatically segmented nuclei, since our algorithm does not address nuclei segmentation, and we want the comparison to be as meaningful as possible. In general, automatic segmentation of nuclei is fairly simple (See section 1.)

Our algorithm does not compute a foreground/background separation, but instead relies on a such a label for each pixel to be given as input. For comparing our algorithm to the manual segmentation, we compute the foreground pixels as the union of cells identified in the manual segmentation. Methods exist for automatically choosing a foreground/background labelling, but distinguishing foreground from background is not part of our algorithm.

For the purposes of this comparison, we also use seed regions from hand-outlined nuclei. In general, nuclei are more compact, separated, and brightly stained than cells,

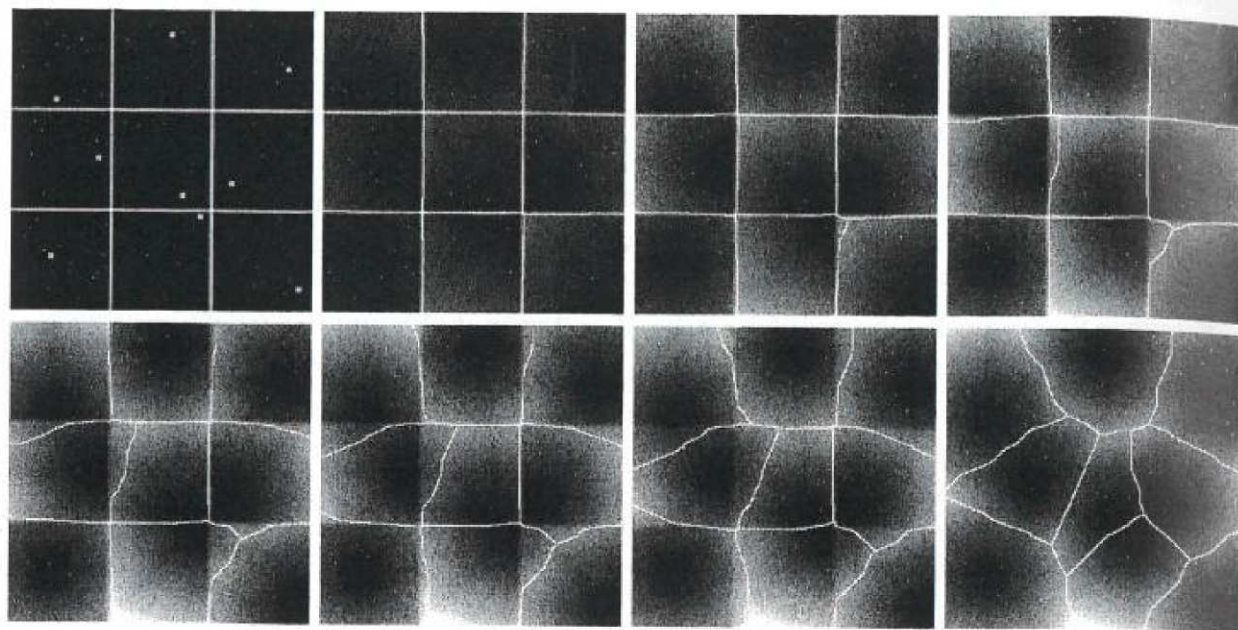


Fig. 3. Synthetic example. The input image is in the upper left, with seed sites marked with dots. From left to right across the two rows, the resulting distances calculated with our metric are shown, with the resulting segmentation overlaid white lines, for λ equal to 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, and 3.0. The segmentation lines follow the ridges in the distances function. As can be seen, as λ increases, the segmentation approaches the Voronoi diagram of the seed regions.

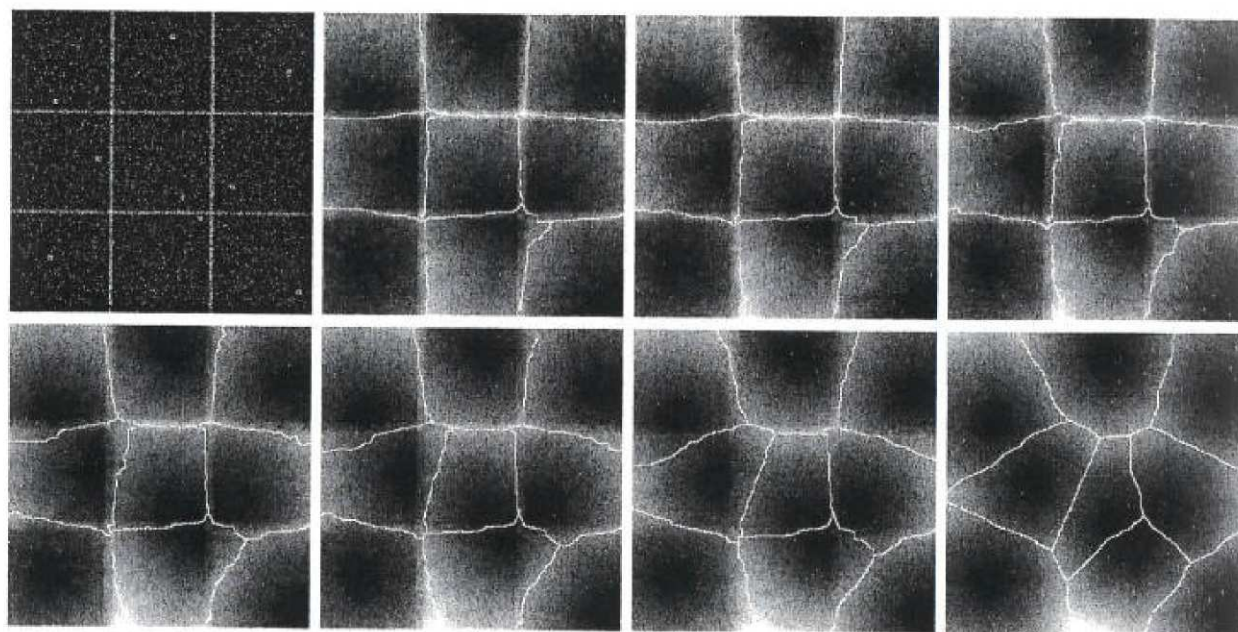


Fig. 4. Synthetic example with noise. The same input as in Figure 3 is used, with zero-mean Gaussian noise with standard deviation 0.5 added to each pixel. Edges were 1.0 and background was 0.0 before noise was added. The layout is the same as in Figure 3, but with λ equal to 0.025, 0.05, 0.075, 0.1, 0.125, 0.2, and 0.75.

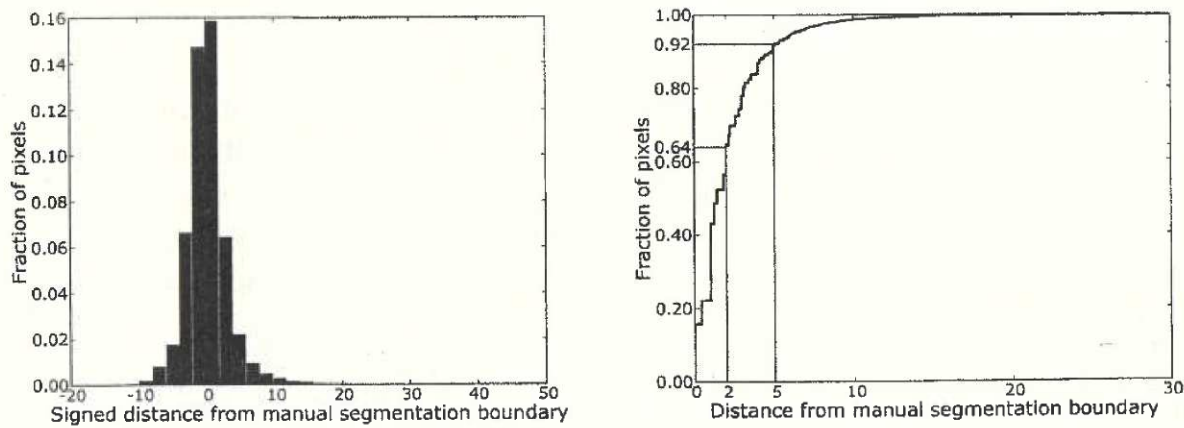


Fig. 5. Combined histogram for the signed distances and cumulative distribution of absolute distances from automatic segmentation to manual segmentation for the sixteen images in our test set

and so are usually easily segmented by simple thresholding. We chose to use the nuclei from manual segmentation in order to keep the conditions for manual segmentation and our algorithm as similar as possible.

Under these conditions, our algorithm is only responsible for computing cell-cell boundaries, rather than cell-background boundaries, which are fixed. We therefore only consider the distance between boundary pixels in the two images that have a common cell-label on either border. When evaluating the algorithm, we use the one-sided signed distance (negative inside cells) from the automatic segmentation to the manual segmentation. We set λ in (1) to 0.05 times the distance between the average foreground and background pixel intensities on a per-image basis. This value for λ was found to be close to optimal in our experiments, with fairly stable behavior for a reasonably large range (within a factor of two). Our test set includes a wide variety of cell types, with different sizes and morphologies. In general, most applications would have more homogeneous data, to which λ should be tuned.

Sixteen images made up the test set. Each image was roughly 512x512 pixels on a side, with cells roughly 25 pixels in diameter, and 80 cells per image on average. Across the entire set, there were 21.6k pixels on a cell-cell boundary in the automatic segmentation. A histogram of their signed distances with respect to the manual segmentation is shown in Figure 5. Sixty-four percent (14.0k) of the boundary pixels in the automatic segmentation are within 2 pixels in distance from the corresponding manual boundary. Ninety-two percent (19.8k) of the pixels are within 5 pixels. The accuracy of the hand-labelling is around 3 pixels, based on the width of the marker used to outline the cells.

Typical results on the test set are shown in Figure 1. We show the top 5 worst outliers from the data set, based on maximum boundary distance, in Figure 2. In some cases, the automatic method has “chosen” a different edge in the image to use as the cell-cell boundary. In others, the close proximity of the nucleus to a cell boundary has caused the automatic segmentation to move past the boundary chosen in the manual segmentation, causing large deviations between the two.

4 Discussion

In some segmentation tasks seed regions for segmentation are easily identified. Nuclei act as such seed regions in our application, the segmentation of cells in microscopy images. The more difficult task is finding the boundaries between cells that share a common border. Cell appearance in the images is not uniform, varying depending on the type of cells and the protocol used to stain them, and simplistic approaches are not sufficiently accurate or robust.

We have demonstrated an algorithm for segmentation of image regions based on seed areas that respects image boundaries as defined by a difference operator. The algorithm computes an approximation of the Voronoi region of a seed on a manifold, implicitly defined by a difference operator that operates on image neighborhoods. The only assumption that the algorithm relies on is that images change more near the borders of regions. This is similar to the behavior of Geodesic Active Contours, with an inversion in the behavior of the metrics near edges. We approximate distances using a chamfer-like difference operator, and use Dijkstra's algorithm for computing individual regions quickly.

Our algorithm is currently implemented in an automatic image cytometry package [4], and has been used successfully in several experiments with a variety of cell types and morphologies. We have compared our algorithm to manual segmentation by an expert of microscopy cell images. Our algorithm performs well. There are a few failure cases, perhaps due to the fairly simple prior on cell shape and size that our metric implies. A more complex prior could incorporate measures of, e.g., cell roundness or cell area. Although our method is more accurate for segmenting cells than others, such as using fixed offsets from nuclei or the watershed transform, the true test of its usefulness is in whether it produces more accurate measurements of cellular phenotypes. In order to understand how segmentation accuracy affects measurement accuracy, we plan to validate against data from flow cytometry or another method not based on image processing.

In the future, we would like to explore other choices for g in (1). It might also be possible to use the distance defined by our metric to compute foreground/background labellings and to detect poorly-segmented cells.

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References

1. S. Beucher "The Watershed Transformation Applied to Image Segmentation," *Scanning Microscopy International*, Vol. 6, pp.299–314, 1992.
2. Boutros M., A. Kiger, S. Armknecht, K. Kerr, M. Hild, B. Koch, S. Haas, Heidelberg Fly Array Consortium, R. Paro and N. Perrimon "Genome-wide RNAi Analysis of Cell Growth and Viability in *Drosophila*," *Science*, Vol. 303, pp. 832–835, 2004.

3. A.E. Carpenter and D.M. Sabatini "Systematic Genome-wide Screens of Gene Function," *Nature Reviews Genetics*, Vol. 5, pp. 11–22, 2004.
4. "CellProfiler: versatile software for high throughput image analysis," A.E. Carpenter, T.R. Jones, D.B. Wheeler, D.A. Guertin, I.H. Kang, P. Golland, and D.M. Sabatini, *in preparation*.
5. V. Caselles, R. Kimmel, and G. Sapiro "Geodesic Active Contours," *Int. J. Comput. Vision*, Vol. 22, pp. 61–79, 1997.
6. E. W. Dijkstra "A Note on Two Problems in Connection with Graphs," *Numerische Math.*, Vol. 1, pp. 269–271, 1959.
7. U. Montanari "A Method for Obtaining Skeletons Using a Quasi-Euclidean Distance," *Journal of the ACM*, Vol. 15, pp. 600–624, 1968.
8. C. Wahlby "Algorithms for Applied Digital Image Cytometry," PhD Thesis, 2003

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