PERCEPTUAL GROUPING OF MEMBRANE SIGNALS IN CELL-BASED ASSAYS

H. Chang[12], *K. L. Andarawewa*[1], *J. Han*[1], *M.H. Barcellos-Hoff*[1] *and B. Parvin*[1]

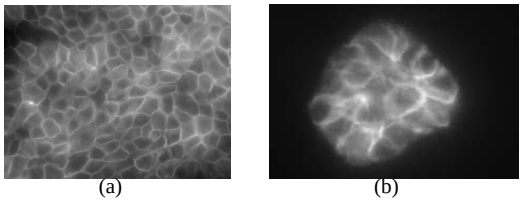
[1]Lawrence Berkeley National Laboratory, Berkeley, CA 94720
[2]Institue of Automation, Chinese Academy of Sciences, Beijng, China

ABSTRACT

Membrane proteins organize themselves in a linear fashion where adjacent cells are attached together along the basal-lateral region. Their intensity distributions are often heterogeneous and may lack specificity. Grouping of these linear structures can aid in segmentation and quantitative representation of protein localization. However, quantitative analysis of these signals is often hindered by noise, variation in scale, and perceptual features. This paper introduces an iterative voting method for inferring the membrane signal as it relates to continuity. A unique aspect of this technique is in the topography of the voting kernel, which is refined and reoriented iteratively. The technique can cluster and group membrane signals along the tangential direction. It has an excellent noise immunity and is tolerant to perturbations in scale. Application of this technique to quantitative analysis of cell-cell adhesion mediated by integral cell membrane proteins is demonstrated.

1. INTRODUCTION

Epithelial cells *in vivo* form sheets and complex hollow tubes or spheres. In cell culture, traditional growth on rigid substrata results in a sheet of cells, i.e. a monolayer, while providing flexible substrata allows epithelial cells to form tissue-specific structures. For example, mammary epithelial cells form hollow spheres (e.g., acini) in substrata that are composed of proteins of the basement membrane, but form hollow tubes when cultured in proteins from the stromal extracellular matrix. Both monolayers and complex structures require cell-cell adhesion mediated by integral cell membrane proteins. One such protein, E-cadherin, is pathoneumonic for normal epithelia and is lost during cancer. Research in the area of quantitative analysis of cell-based assay has spanned learning techniques using texture-based features for characterizing patterns of protein expression [4], geometric techniques using nonlinear filtering and curve evolution [2], and shape regularization for segmentation of subcellular compartments [9]. While segmentation of nuclear regions provides context for localization studies [6], probe features also need to be delineated for certain antibodies. In this paper, a new method for quantifying E-cadherin that is bound to the basal-lateral region of the cell is presented. Loss of E-cadherin can be related to invasion and is a potential precursor for cancer initiation. These signals correspond to locally linear features that delineate cell boundaries as shown in Figure 1. However, the membrane signal may have nonuniform intensity around the cell boundary and may even be perceptual at certain locations along the boundary. It is well known that symmetry, closure, and continuity are preattentive processes in the human vision system that can aid in object-level delineation and recognition [1].



(a) (b)

Fig. 1. Membrane signals from 2D and 3D cell culture models: (a) 2D system, and (b) 3D system.

The proposed method allows inference of saliency from incomplete boundary information through voting and perceptual grouping, and is implemented through the refinement of a set of specifically tuned voting kernels. Spatial voting has been studied for at least four decades. Hough introduced the notion of parametric clustering in terms of well-defined geometry, which was later extended to the generalized Hough transform. In general, voting operates on continuity and proximity, which can occur at multiple scales, e.g., points, lines, or lines of symmetry. The novelty of our approach is in defining a series of kernels that vote iteratively along the radial or tangential direction. Voting along the radial direction leads to the localization of the center of mass [5, 6], while voting along the tangential direction enforces continuity. At each iteration, the kernel orientation is refined until it converges to a focal response. Several variations of these kernels have been designed and tested. For example, for inferring radial symmetries, kernels are cone-shaped and their maximum strength is expressed at the center of the cone [2, 6]. In this context, the voting kernels are initially applied along the gradient directions, then, at each consecutive iteration and at each grid location, kernel orientations are realigned along the maximum responses. In the case of continuous boundary inference, the voting kernels are applied along the normal to the gradient direction. The topography of the kernel is also refined and focused as the iterative process continues. The method is applicable to perceptual shape features, has excellent noise immunity, is tolerant to variations in target shape scale, and is applicable to a large class of application domains.

An intuitive explanation of the differences between variational and iterative voting models of segmentation follows. Both are iterative; however, in variational models [9], geometric constraints are specified and then regularized for continuity. In voting models, geometric constraints are embedded in the shape of the kernel, while smoothness constraints are incorporated in how the topography of the kernel decays smoothly from an ideal response. For example, consider separation of three synthetic overlapping blobs, as shown in Figure 2. Iterative voting [6] collapses each blob to its local cen-

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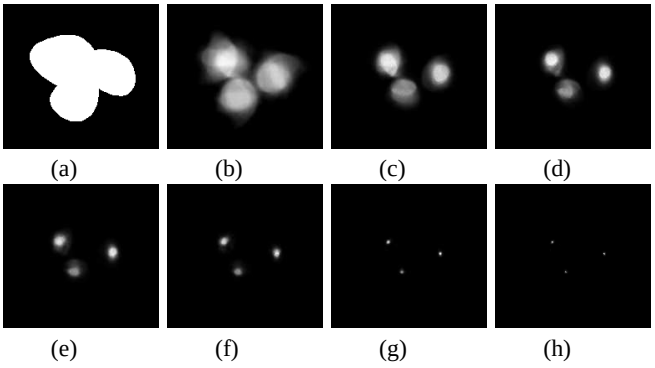


Fig. 2. Detection of radial symmetries for a synthetic image with multiple overlapping objects: (a) original image; (b)-(g) voting landscape at each iteration; and (h) final localization of centers of mass.

troid through applications of kernels, as shown in Figure 3. These kernels project gradient information inward, along the radial direction, to infer an approximation to the center of mass. In this case, the desirable saliency is encoded in the topography of the kernel, which has maximum strength at the center of the cone.

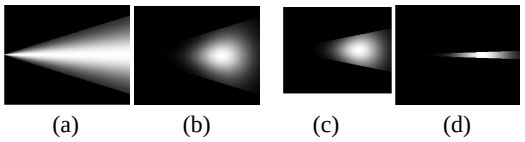


Fig. 3. Kernel topography: (a-e) Evolving kernel for the detection of radial symmetries (shown at a fixed orientation) has a trapezoidal active area with maximum strength at the center for inference of center of mass.

The organization of this paper is as follows. Section 2 provides a brief review of the previous research. Section 3 describes the basic idea and detailed implementation of tangential voting. Section 4 demonstrates the experimental results. Section 5 concludes the paper.

2. REVIEW OF PREVIOUS WORK

The difficulties in the detection of saliency are often due to variations in scale, noise, and topology. Other complexities originate from missing data and perceptual boundaries that lead to diffusion and dispersion of the spatial grouping in the object space. Techniques for grouping local features into globally salient structures have incorporated dynamic programming [7], clustering and graph theoretic methods [8], and tensor voting [3]. While these techniques differ in their concepts, they share a common thread of using continuity and proximity along the minimum energy path to infer global saliency.

The proposed method falls into the category of iterative techniques, which are adaptive to geometric perturbation and typically produce more refined results. This method shares several attributes with tensor-based voting [3]; however, it differs in that it is iterative and is scalar.

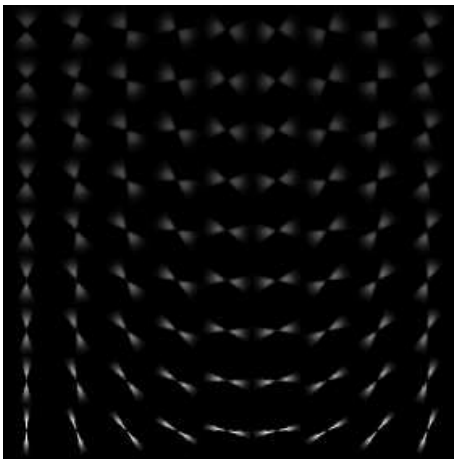


Fig. 4. Kernel topography: Oriented kernels for inference of continuity are bidirectional, and their energy dissipates as a function of distance. Initially, the energy is dispersed (top row), but becomes more focused (bottom row).

3. APPROACH

The membrane signals correspond to the negative curvature maxima at a given scale within the image space. But curvature features are noisy and may suffer from undesirable artifacts. The process is initiated by voting with a Gaussian kernel at each image feature point. Let $F(x_o, y_o)$ be the curvature feature at location (x_o, y_o) in the image. Let (x_n, y_n) be a point in the neighborhood of (x_o, y_o) that can be influenced with a kernel applied at position (x_o, y_o). The initial voted image is then represented as

$$V(x_n, y_n) = \sum_{(x_n, y_n) \in Neighbor(x_o, y_o)} \{F(x_o, y_o) * G_{(x_o, y_o)}(\sigma)\} \tag{1}$$

The refinement of the voted image is iterative, involving application of a more focused kernel at the next iteration along the α direction.

$$\alpha = \arctan \frac{V_{yy} - K_{max}}{V_{xy}} \tag{2}$$

Where V_{yy} and V_{xy} are the local derivatives of the voted image, and K_{max} is the maximum curvature computed from the Hessian of the voted image. The shape of the kernels, shown in Figure 4, indicates whether the energy distribution of the kernel is focused or dispersed. Initially, the energy is dispersed; however, at each consecutive iteration, the energy becomes more focused and at the same time the kernel orientation is redirected along the direction of maximum response, as shown in Figure 5a, and the entire process is shown in Figure 5b. These voting kernels are precomputed and indexed for rapid retrieval.

$$V(x_n, y_n) = \sum_{(x_n, y_n) \in Neighbor(x_o, y_o)} \{F(x_o, y_o) * Kernel(\sigma, \theta, \alpha)\} \tag{3}$$

Iterative Voting

1. *Initialize the parameters:* Initialize $r_{\max}, \Delta_{\max}$, and a sequence $\Delta_{\max} = \Delta_N < \Delta_{N-1} < \cdots < \Delta_0 = 0$. Set $n := N$, where N is the number of iterations, and let $\Delta_n = \Delta_{\max}$.

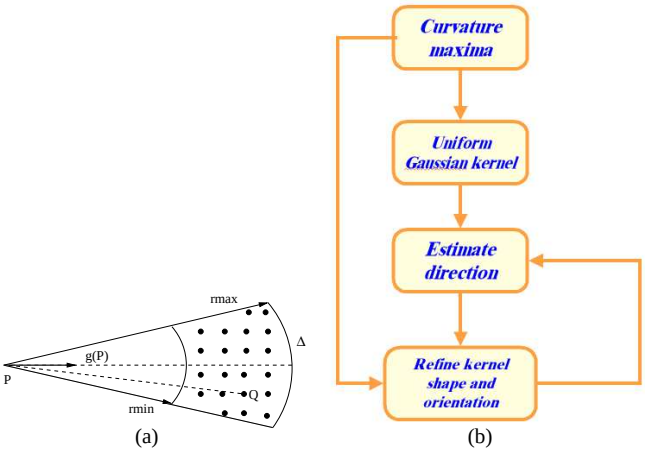


Fig. 5. (a) Redirection of the kernel in the next iteration; and (b) general flow of the algorithm.

$r_{\max}$ refers to the extent of the voting influence along a given orientation. $r_{\max}$ decays as a function of the distance to the voting pixel.

2. *Initialize the saliency feature image:* Define the feature image $F(x, y)$ to be the local external force at each pixel of the original image. The external force is set to the maximum (negative) curvature, which corresponds to the membrane signal.
3. *Initialize the voting magnitude:* Apply the isotropic voting of Equation 1.
4. *Update the voting direction:* Compute the Hessian of the voted landscape and construct an orientation map based on Equation 2.
5. *Refine the angular range:* Let $n := n - 1$, apply Equation 3, and repeat steps 3-5 until $n = 0$.

4. EXPERIMENTAL RESULTS

An experiment has been designed to quantify membrane signals (e.g., E-cadherin) and a number of structural features in the model system. This experiment consists of both 2D and 3D cell culture models under control and treated conditions. In this case, the treatment is radiation and TGFβ. The primary rationale for extending the cell culture models to 3D is that they provide a more faithful replication of cell behavior *in vivo* than is possible using the 2D substrata. While the information these cultures can provide is undoubtedly more valuable, the experiments are much harder to set up, and require more advanced quantitative tools for phenotypic characterization [2]. Since the total E-cadherin signal is averaged with respect to the number of cells per image, cells are first counted using a method presented in our earlier papers [2], which also uses iterative voting, but the voting is applied in the radial direction. All samples are stained with nuclear stain, and radial voting provides the basis for cell counting and a more refined nuclear segmentation [2], as shown in Figure 6. Figures 7 and 8 show an example of E-cadherin localization for 2D and 3D cell culture models, respectively. In this experiment, a total of 118 images were collected for the 4 experimental factors (e.g., control for 2D model system, 2D treatment, control for 3D model system, 3D treatment). Voting along the tangential direction enhances the E-cadherin signal while diffusing the background fluorescence signal. We have quantified structural (e.g., organization) and functional (e.g., E-cadherin) features of each model system. Structural features are quantified through the average bending energy of the boundary that represent each colony. Functional features are computed in two ways with similar results. In the first method, voted results are thresholded with a single threshold for the entire data set, then overlaid on the probe channel, where fluorescence signal is accumulated. In the second method, voted energy of each pixel is weighted on the probe channel, and the weighted fluorescence signal is subsequently aggregated. Results of structural and functional analysis are shown in Figure 9. Figure 9a indicates loss of colony organization as a result of double treatments, where organization is quantified by the average curvature along the boundary of the colonies; and Figure 9b indicates that E-cadherin is better preserved in the 3D model systems under identical treatment conditions. An interesting question is the extent that structural (e.g., organization) and functional (e.g., E-cadherin per cell) features are surrogates for a specific treatment. In this context, linear (e.g., linear discriminant analysis) and nonlinear classifiers (e.g., support vector machine) are trained to evaluate the recognition performance. The classification error probabilities are shown in Table 1.

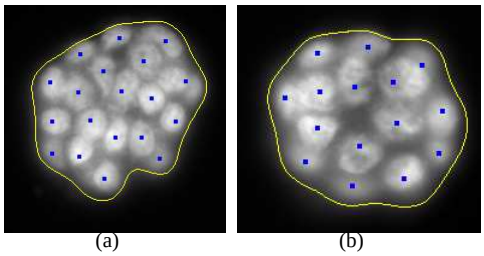


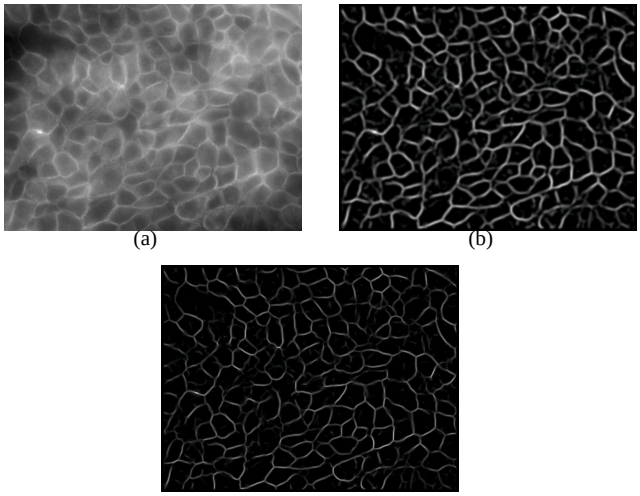
Fig. 6. Two examples of voting results for the nuclear stained 3D cell culture model indicate the detection and counting of nuclear regions.

	Organization	E-cadherin	Organization and E-cadherin
LDA	38%	9%	9%
SVM	49%	11%	13%

Table 1. Estimated probability of classification error using leave-one-out method indicates that E-cadherin is can be used as a proxy for the treatment.

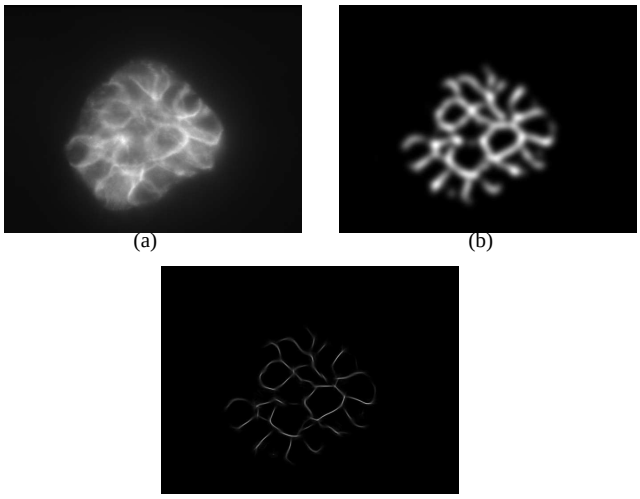
5. CONCLUSION AND FUTURE WORK

Iterative voting for detecting saliency in cell-based assays is introduced and applied to membrane-bound proteins that are responsible for cell-cell contact. The main novelties are (1) re-estimation of voting direction and (2) updating the voting fields by focusing their



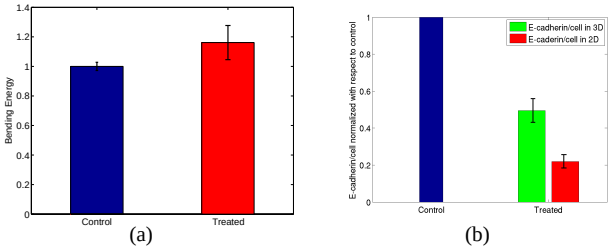
(a) (b) (c)

Fig. 7. Localization of membrane bound protein for 2D cell culture model: (a) original image; (b) initial voting landscape; and (c) the final voting results corresponding to the enhanced boundaries.



(a) (b) (c)

Fig. 8. Localization of membrane bound protein for 3D cell culture model: (a) a slice of the original image of the mammosphere (3D cell culture model); (b) initial voting landscape; and (c) voted results corresponding to the membrane proteins along the points of maximum negative curvature.



(a) (b)

Fig. 9. Phenotypes of organization and E-cadherin stability: (a) Organization of mammosphere measured as the average bending energy of a front enclosing the colony; and (b) E-cadherin is better preserved in 3D than in 2D under identical set of treatment.

energy at each consecutive iteration. We suggest that the iterative voting strategy overcomes the drawbacks of traditional static voting and shares positive attributes of geometric regularization. The voting algorithm provides a general framework for inferring a variety of types of low-level saliency: by simply modifying the kernel shapes and external forces measured from the image (gradient, curvature, etc.), the algorithm may be adapted to an array of cell-based assays. The performance of the method has been demonstrated on real data under multiple experimental conditions.

6. REFERENCES

[1] I. Biederman. *Human image understanding: recent research and theory*. Academic Press, Rosenfeld (editted), 1985.

[2] H. Chang and B. Parvin. Segmentation of three dimensional cell culture models from a single focal plane. In *Proc. Int. Symposium on Visual Computing*, 2006.

[3] G. Medioni, M.S. Lee, and C.K. Tang. *A Computational Framework for Segmentation and Grouping*. Elsevier, 2000.

[4] R. Murphy. Automated interpretation of subcellular locatoin patterns. In *IEEE Int. Symp. on Biomedical Imaging*, pages 53–56, April 2004.

[5] B. Parvin, Q. Yang, J. Han, H. Chang, B. Rydberg, and Barcellos-Hoff. Iterative voting for inference of structural saliency and characterization of subcellular events. *IEEE Transactions on Image Processing*, 16(3):615–623, March 2007.

[6] S. Raman, C. Maxwell, M. Barcellos-Hoff, and B. Parvin. Geometric approach segmentation and protein localization in cell cultured assays. *Journal of Microscopy*, page in press, 2007.

[7] A. Shashua and S. Ullman. Structural saliency: the detection of globally salient structures using a locally connected network. In *Proceedings of the IEEE International Conference on Computer Vision*, pages 321–327, Tampa, FL, 1988.

[8] Z. Wu and R. Leahy. An optimal graph theoretic approach to data clustering: Theory and its application to image segmentation. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 15(11):1101–1113, 1993.

[9] Q. Yang and B. Parvin. Harmonic cut and regularized centroid transform for localization of subcelular structures. *IEEE Transaction on Biomedical Engineering*, 50(4):469–476, 2003.