TUNABLE TENSOR VOTING FOR REGULARIZING PUNCTATE PATTERNS OF MEMBRANE-BOUND PROTEIN SIGNALS

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ABSTRACT

Membrane-bound protein, expressed in the basal-lateral region, is heterogeneous and an important endpoint for understanding biological processes. At the optical resolution, membrane-bound protein can be visualized as being diffused (e.g., E-cadherin), punctate (e.g., connexin), or simultaneously diffused and punctate as a result of sample preparation or conditioning. Furthermore, there is a significant amount of heterogeneity as a result of technical and biological variations. This paper aims at enhancing membrane-bound proteins that are expressed between epithelial cells so that quantitative analysis can be enabled on a cell-by-cell basis. We propose a method to detect and enhance membrane-bound protein signal from noisy images. More precisely, we build upon the tensor voting framework in order to produce an efficient method to detect and refine perceptually interesting linear structures in images. The novelty of the proposed method is in its iterative tuning of the tensor voting fields, which allows the concentration of the votes only over areas of interest. The method is shown to produce high quality enhancements of membrane-bound protein signals with combined punctate and diffused characteristics. Experimental results demonstrate the benefits of using tunable tensor voting for enhancing and differentiating cell-cell adhesion mediated by integral cell membrane protein.

Index Terms— Perceptual grouping, membrane-bound protein, segmentation

1. INTRODUCTION

Epithelial cells compose monolayers in culture by forming cell-cell adhesion mediated by integral cell membrane proteins. One such protein, E-cadherin, is pathoneumonic for normal epithelia and its down regulation is associated with motility, epithelial-mesenchymal transition (EMT) and cancer initiation. 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 [1], geometric techniques using nonlinear filtering and curve evolution [2], and shape regularization for segmentation of subcellular compartments [3]. While segmentation of nuclear regions provides context for localization studies, 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. At optical resolution, E-cadherin is visualized as locally linear features that delineate cell boundaries as shown in Fig. 1. However, the membrane signal may have nonuniform intensity around the cell boundary, be punctate (e.g., connexin) or diffused (e.g., E-cadherin), and may even be perceptual at certain locations along the boundary.

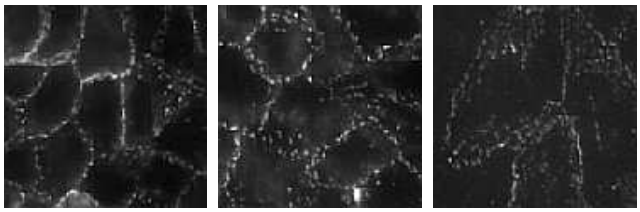


Fig. 1. Membrane-bound protein has a complex pattern of localization along the cell membrane. Signal has an additional punctate pattern on top of an existing diffused signal.

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 [4]. The proposed method allows inference of linear structures from noisy, often incomplete boundary information. It involves perceptual grouping of pixels through voting. Grouping through voting has been studied for at least five decades. As examples, Hough introduced the notion of parametric clustering in terms of well-defined geometry, which was later extended to the generalized Hough transform. [5] developed an iterative tangential voting system that employs tunable kernels to refine paths of low curvature in images. [6] proposed a general purpose voting framework that uses deformable tensors to reveal perceptual structures. In general, voting operates on continuity and proximity, which can occur at multiple scales, e.g., points, lines, or lines of symmetry. We build upon the tensor voting framework [6] and the iterative scalar framework [5] in order to produce an efficient method to detect and refine perceptually interesting linear structures in images. The novelty of our method is the extension of the tensor voting framework to precisely detect and refine linear structures at different scales, by iteratively tuning the tensor fields as pixel orientations are better defined. Although similar itera-

tive tuning is proposed in [5], to the best of our knowledge, no analogy has been made within tensor voting.

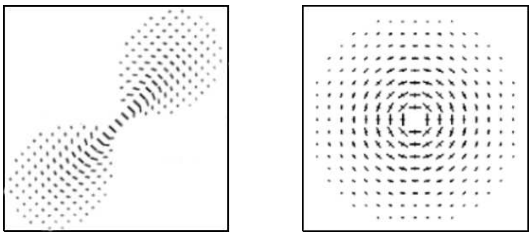
In a nutshell, our method starts by encoding each pixel in an image as an unoriented tensor, whose size is proportional to pixel intensities. A first tensor voting pass is executed using a ball field, i.e. votes are propagated radially, as no initial orientation is known. This allows tensors to start their characteristic structural deformation that consequently reveals, although still inaccurately, the presence or not of perceptual lines in the image. Although the classical tensor voting would stop at this stage, we proceed with consecutive tensor voting passes aiming at refining previous results. These consequent iterations are performed with stick fields, i.e. votes are concentrated along pixel's tangent, most probable continuation for a line passing through this pixel. The concentration of the votes through stick fields is possible because the first voting pass naturally produces an estimation of the orientation at each pixel. Note that our method independs on initial measures of gradient or curvature as initial guesses to the location of lines, differing significantly from [5]. One interesting observation is that the stick fields are gradually tuned, i.e. the field aperture is reduced as the voting iterations proceed and the orientation estimations become more and more accurate. The method is applicable to detection of linear features, has excellent noise immunity, is tolerant to changes in target scale, and applicable to a large class of application domains.

The rest of this paper is organized as follows: Section 2 describes the tensor voting framework and its application to perceptual grouping of linear structures. Section 3 introduces our method, extending the concepts of the tensor voting framework. Experimental results are shown in Section 4 and some conclusion are presented in Section 5.

2. THE TENSOR VOTING FRAMEWORK

In the framework proposed by [6], perceptual grouping is achieved by vote casting between elements of an image. Such elements are represented as tensors, mathematical entities whose capability of encoding magnitude and orientation make tensor voting particularly efficient for detection of perceptually organized structures, such as edges, lines and regions. In 2D, tensors can be represented geometrically as ellipses or analytically as 2 by 2 matrices. Initialized with an arbitrary size and shape (given respectively by the eigenvalues, λ_1, λ_2, and eigenvectors, e_1, e_2, of its analytical representation), input tensors are gradually deformed due to the accumulation of votes cast by other neighboring tensors. Votes are also tensors composed of certain magnitude and orientation, which encode the Gestalt principles of proximity, smoothness and good continuation. Depending on the nature of the input elements, a priori information about their orientation can be available or not. Therefore, tensor voting offers two possible vote casting configurations: one that concentrates the votes according to the input orientation (stick field - Fig. 2(a)) and another one that casts votes radially (ball field - Fig. 2(b)). The voting fields are the composition of all votes that can be cast from a tensor located in the center of the field to its neighboring tensors. Given two tensors positioned in the image, the angle θ, arc length s and curvature κ between them is used to produce the vote V from one another, as shown by Equation 2, where N is the vector normal to the smoothest path between the two tensors and is given by $[-sin(\theta) \;\; cos(\theta)]^T$. Note that the stick field exists only at $\theta \leq 45°$.

$$V = e^{-\frac{s^2 + c\kappa^2}{\sigma^2}} NN^T \tag{1}$$



(a) (b)

Fig. 2. Tensor voting fields. (a) Stick field - when an estimate of the initial orientation is known, and (b) Ball field - when no orientation information is known.

(a) (b) (c)

Fig. 3. Example of perceptual grouping through tensor voting. A set of (a) input elements are (b) encoded as tensors, whose (c) resulting deformations reveal a curve.

The tensor deformation imposed by accumulating the strength and orientation of the votes eventually reveals behavioral coherence among image elements. In other words, elements that lie on the same salient feature (e.g. a curve or a region) strongly support each other and deform the tensor at those sites according to the underlying structure orientation. Therefore, each kind of structure is expected to produce tensors of a particular shape: very elongated tensors (high $\lambda_1 - \lambda_2$) for lines, and more rounded ones (low $\lambda_1 - \lambda_2$) for regions. Fig. 3 exemplifies how a set of (a) input elements are (b) encoded as tensors, whose (c) deformations resulting from accumulated votes reveal an underlying salient linear structure. The method is robust to considerable amounts of outlier noise and does not depend on critical thresholds. The only free parameter is the scale factor σ, which denes the range of the voting neighborhood. Some more detailed information can be found in [6].

3. TUNABLE TENSOR VOTING

We build upon the tensor voting and iterative scalar framework in order to produce an efficient method to detect and refine perceptually interesting linear structures in images. Our method starts by encoding each pixel in an image as an unoriented tensor, whose size is proportional to pixel intensities ($\lambda_1 = \lambda_2 = I_{ij}$). A first tensor voting pass is executed using the ball field (Fig. 2(b)), as no initial pixel orientation is known. This allows tensors to start their characteristic structural deformation that consequently reveals, although still inaccurately, the presence or not of perceptual lines in the image. Although the classical tensor voting would stop at this stage, we proceed with consecutive tensor voting passes aiming at refining previous results. These consequent iterations are performed with stick fields (Fig. 2(a)), as the first voting naturally produces an estimation of the orientation at each pixel. One interesting observation is that the stick fields are gradually tuned (i.e. the field aperture is reduced) as the voting iterations proceed and the orientation estimations become more and more accurate (Fig. 4). At each iteration, tensors that do not deform as lines (low $\lambda_1 - \lambda_2$) are eliminated so their influence is not accounted into the following iterations. This tuning process eventually concentrates the votes only over real lines, producing better, enhanced results. The iterations stop when the aperture of the voting field is small enough to produce the same field as in a previous iteration or at $\theta = 1°$. Fig. 5 depicts the process.

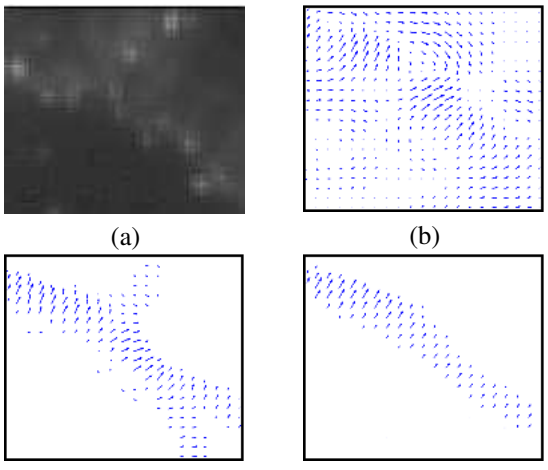


Fig. 4. The signal along the curvelinear path is gradually refined by using tunable filters: (a) Original image. (b) After first iteration (ball voting). (c) After four iterations (at $\theta = 30°$). (d) Final result (at $\theta = 5°$).

4. EXPERIMENTAL RESULTS

In order to demonstrate the benefits of our tunable tensor voting, we apply it to membrane-bound protein signals (e.g. E-cadherin). The tunable tensor voting is employed in quantifying E-cadherin that is bound to the basal-lateral region of the cell. At optical resolution, E-cadherin is visualized as locally linear features that delineate cell boundaries as shown in Fig. 1. However, the membrane signal may have nonuniform intensity around the cell boundary, be punctate and diffused, and may even be perceptual at certain locations along the boundary. Our dataset consists of 270+ 1344x1024 images with membrane signals presenting these characteristics.

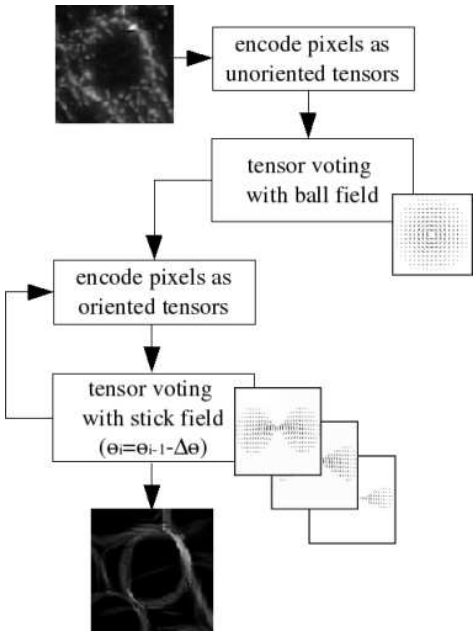


Fig. 5. Regularization of membrane signal by tunable tensor voting. The primary theme is the feedback loop for iterative change of the voting aperture for continuous refinements.

Fig. 6 shows a membrane image (a), the results of initial, ball voting (b), intermediate and final results (c-e) of the tuning process, evidencing the (f) membrane signal enhancement achieved. The enhancement 6(f) is clear if compared with the original membrane signal 6(a).

Fig. 7 shows into more details (a) the punctate and diffused membrane-bound protein signal, and a comparison between the results of detecting lines with (b) classical tensor voting, and (c) our tunable tensor voting. Note that by iterating over the result obtained first by a regular application of tensor voting, pixels are determined with more precision to belong to the membrane.

Fig. 8 shows some enhancements produced by our method. Images on the left side are cuts of the original images, while those on the right are their respective enhanced versions. In general, membrane signals are highly dispersed along the cell membrane. This is mainly due to the wide field microscopy and the influence of out-of-focus light. Note that even in the presence of noisy and dim signals, our method is able to infer lines interpolating punctate, diffused patterns.

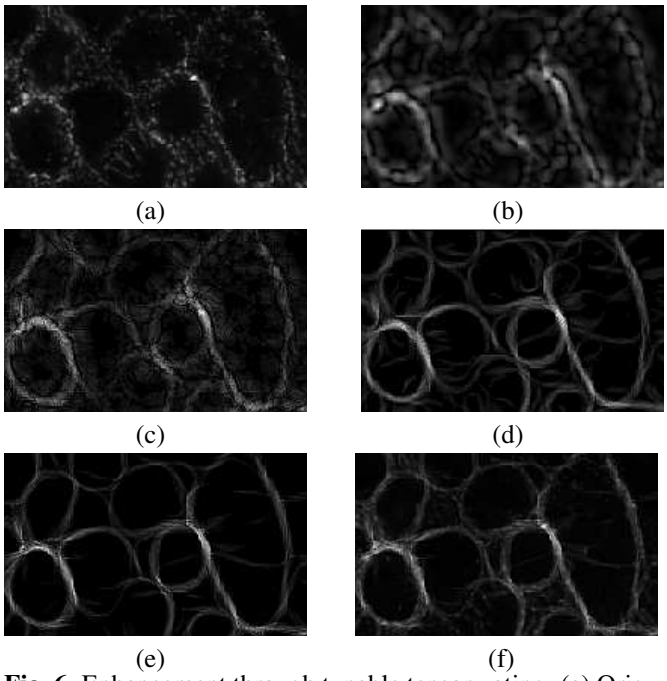


Fig. 6. Enhancement through tunable tensor voting. (a) Original membrane signal. (b) Ball tensor voting result (Also classical tensor voting result). (c) and (d) Results of intermediate tuning iterations. (e) Resulting detected linear structures. (f) Enhanced promoted by tunable tensor voting - (e)+(a).

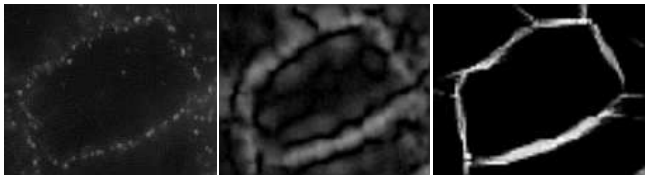


Fig. 7. Comparison between tensor voting and tunable tensor voting. (a) Original membrane-bound protein signal. (b) Processed by classical tensor voting. (c) Processed by tunable tensor voting.

5. CONCLUSIONS AND FUTURE WORK

In this paper, we introduced a tunable tensor voting method, able to detect and refine punctate, diffused membrane-bound protein signals. The method coupled tensor and iterative voting frameworks to leverage advantages of both methods. As a result, complex patterns along a curvelinear path can be regularized and enhanced. Such an enhancement enables membrane-bound protein to be quantified on a cell-by-cell basis. As future work, we plan to extend our method to detect membrane signals in 3D cell cultures. One important remark is that membrane lines in 2D become surfaces in 3D. As well as the tensor voting framework, our method can be naturally scaled to deal with 3D signals.

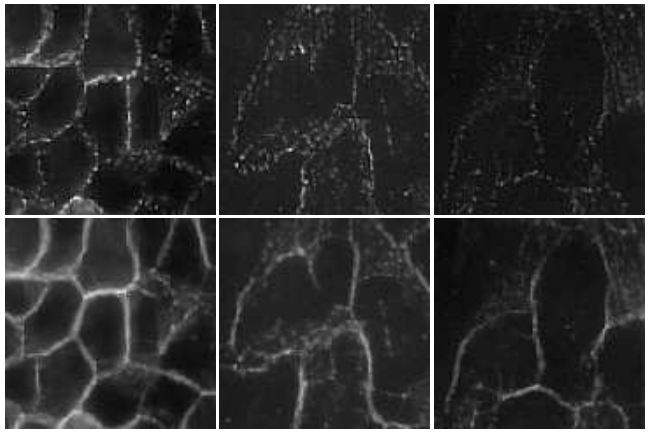


Fig. 8. Results of tunable tensor voting membrane enhancement. Top row - original membrane signals. Bottom row - enhanced images. Even in the presence of noisy and dim signals, our method is able to infer lines interpolating punctate, diffused patterns.

6. ACKNOWLEDGEMENTS

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