Journal of Structural Biology 186 (2014) 49–61

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Robust membrane detection based on tensor voting for electron tomography

Antonio Martinez-Sanchez [a], Inmaculada Garcia [b], Shoh Asano [c], Vladan Lucic [c], Jose-Jesus Fernandez [d,*]

[a] Supercomputing and Algorithms Group, Associated Unit CSIC-UAL, Universidad de Almeria, 04120 Almeria, Spain
[b] Supercomputing and Algorithms Group, Dept. Computer Architecture, Universidad de Malaga, 29080 Malaga, Spain
[c] Max-Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
[d] National Centre for Biotechnology, National Research Council (CNB-CSIC), Campus UAM, Darwin 3, Cantoblanco, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 13 December 2013
Received in revised form 20 February 2014
Accepted 24 February 2014
Available online 10 March 2014

Keywords:
Segmentation
Image processing
Electron tomography
Membrane
Tensor voting
Steerable filters

ABSTRACT

Electron tomography enables three-dimensional (3D) visualization and analysis of the subcellular architecture at a resolution of a few nanometers. Segmentation of structural components present in 3D images (tomograms) is often necessary for their interpretation. However, it is severely hampered by a number of factors that are inherent to electron tomography (e.g. noise, low contrast, distortion). Thus, there is a need for new and improved computational methods to facilitate this challenging task. In this work, we present a new method for membrane segmentation that is based on anisotropic propagation of the local structural information using the tensor voting algorithm. The local structure at each voxel is then refined according to the information received from other voxels. Because voxels belonging to the same membrane have coherent structural information, the underlying global structure is strengthened. In this way, local information is easily integrated at a global scale to yield segmented structures. This method performs well under low signal-to-noise ratio typically found in tomograms of vitrified samples under cryo-tomography conditions and can bridge gaps present on membranes. The performance of the method is demonstrated by applications to tomograms of different biological samples and by quantitative comparison with standard template matching procedure.

1. Introduction

Electron tomography (ET) enables three-dimensional (3D) visualization and analysis of the subcellular architecture and macromolecular organization of cells and tissues in situ at a resolution of a few nanometers (Lucic et al., 2013). This technique involves the acquisition of electron microscopy projection images of a specimen at different orientations. These images are then combined by means of tomographic reconstruction methods to yield the 3D volume (Fernandez, 2012).

Segmentation of the 3D volume into its constitutive structural elements is key for their interpretation. However, it proves to be challenging because of a number of factors such as the crowded cellular environment, the distortion caused by the missing wedge and noise, which is particularly high in ET of fully hydrated and vitrified samples (cryo-ET) (Volkmann, 2010; Fernandez, 2012). Thus, segmentation constitutes a major bottleneck in ET, especially in those studies intended to visualize the subcellular architecture under cryo-conditions. Although several computational segmentation methods have been presented, none has shown general applicability yet. As a consequence, manual segmentation is still a method of choice.

Software packages often used in ET have been gradually including segmentation procedures based on the most known computational techniques (Watershed transform and thresholding (Volkmann, 2002; Cyrklaff et al., 2005)). This makes segmentation a semi-automatic process, thus facilitating its use. In the last several years, there have been significant advances towards computational detection of specific structural features within tomograms, like membranous structures, filaments and microtubules (Lebbink et al., 2007; Sandberg and Brega, 2007; Moussavi et al., 2010; Nurgaliev et al., 2010; Rigort et al., 2012; Weber et al., 2012). Many of these methods rely on some sort of template matching. That is, they search for a template that is suited to the targeted feature, typically by means of cross-correlation techniques. In contrast to these methods, we have recently developed a differential geometry-based segmentation that is particularly suited for membranes

* Corresponding author. Address: National Centre for Biotechnology, National Research Council (CNB-CSIC), Campus UAM, C/ Darwin 3, Cantoblanco, 28049 Madrid, Spain. Fax: +34 91 585 4506.
E-mail address: jj.fernandez@csic.es (J.-J. Fernandez).

http://dx.doi.org/10.1016/j.jsb.2014.02.015
1047-8477/© 2014 Elsevier Inc. All rights reserved.

(Martinez-Sanchez et al., 2011, 2013). We first proposed a method that uses a local membrane detector based on the Hessian tensor (Martinez-Sanchez et al., 2011). Later, we improved this detector and extended the abilities of the framework to characterize and classify the detected membranous structures (Martinez-Sanchez et al., 2013). Nevertheless, this local membrane detector still presented several limitations. Namely, gaps that may appear on membranes due to experimental imaging conditions were not properly filled. Also, membrane-attached structures were not discarded and were instead segmented as part of the membranes. A non-trivial postprocessing stage was required in these cases to actually extract the membrane voxels. Finally, the detector used was suitable for membranes with ridge-like (i.e. local maximum) profile, and was therefore unable to identify edge-like structures (e.g. membrane of vesicles having dense interior).

In this work, we propose a more robust local membrane detector. The method is based on broadcasting differential information through the 3D space using the tensor voting algorithm (Tong et al., 2004). In this way, nearby voxels that belong to the same membranous structure enhance each other's structural information. As a result, the new local detector can fill the gaps present on membranes, disregards structures apposed to the membranes, and it is more robust against low signal-to-noise ratio, thus simplifying the postprocessing stage. In this work we also show that membranes having ridge-like and edge-like profiles can be detected using the same procedure but different tensor that provides the differential information.

2. Background on local membrane detection

2.1. Hessian tensor-based detection

It is assumed that, at a local scale, a membrane can be modeled as a plane-like structure with membrane density profile (in the direction perpendicular to the membrane) following a Gaussian function. That is, the membrane profile is ridge-like, its density decreases as a function of the distance from the center of the membrane.

Membrane detection starts with the application of a scale-space operation on a grayscale volume, typically implemented as Gaussian low-pass filtering. This step is used to isolate the information at a given scale σ, thus filtering out noise and all features smaller than the scale. An additional benefit of this step is that scale-space can smooth membranes making their profile closer to Gaussian. Typically, σ is set to the thickness (expressed in voxels) of the targeted membrane (Martinez-Sanchez et al., 2011).

Previously, our local detector for ridge-like membranes was based on the Hessian tensor (Martinez-Sanchez et al., 2011). This tensor provides information about the second order density variation, as it is defined as:

$$\mathbf{H} = \begin{bmatrix} \frac{\partial^2 L}{\partial x^2} & \frac{\partial^2 L}{\partial x \partial y} & \frac{\partial^2 L}{\partial x \partial z} \\ \frac{\partial^2 L}{\partial x \partial y} & \frac{\partial^2 L}{\partial y^2} & \frac{\partial^2 L}{\partial y \partial z} \\ \frac{\partial^2 L}{\partial x \partial z} & \frac{\partial^2 L}{\partial y \partial z} & \frac{\partial^2 L}{\partial z^2} \end{bmatrix} \quad (1)$$

where L denotes the volume after the scale-space operation and $\frac{\partial^2 L}{\partial i \partial j}$ $\forall i,\ j \in (x, y, z)$ are its second order partial derivatives. As a result of the eigen-analysis of the Hessian tensor, three orthogonal eigenvectors $\vec{v}_i$ and their corresponding eigenvalues λ_i (representing second order derivatives along $\vec{v}_i$) are obtained, which characterize the local structure around any voxel of the volume. If we assume $|\lambda_1| > |\lambda_2| > |\lambda_3|$, then the first eigenvector $\vec{v}_1$, i.e. the one whose eigenvalue exhibits the largest absolute value, points to the direction of the maximum curvature. If the local structure is a plane, $\vec{v}_1$ points to the direction perpendicular to the plane and the following relationship holds $|\lambda_1| \gg |\lambda_2| \approx |\lambda_3|$. This led us earlier to propose a local detector (so-called membrane strength, M) defined as follows (Martinez-Sanchez et al., 2011):

$$M = \begin{cases} \frac{\left(|\lambda_1| - \sqrt{\lambda_2 \lambda_3}\right)^2}{|\nabla L|^2} & \lambda_1 < 0 \\ 0 & \text{otherwise} \end{cases} \quad (2)$$

where $|\nabla L|$ denotes the gradient of the volume L resulting from the scale-space operation.

We used two additional steps to detect peaks of M corresponding to membranes. A threshold t_M over M was imposed to select membrane-like voxels. This was then coupled with the non-maximum suppression (NMS) criterion which selects only the ridge points (i.e. the local maxima in the direction perpendicular to the membrane). The use of NMS results in detected membranes being represented as one-voxel thick surfaces in the 3D space. In summary, the local detector for ridge-like membranes is given by the following equation (Martinez-Sanchez et al., 2013), where the first condition represents NMS, δ is a small number and $\mathbf{x} \in \mathbb{R}^3$ denotes a voxel of the volume:

$$\begin{cases} (L(\mathbf{x}) > L(\mathbf{x} - \delta \vec{v}_1)) \text{ and } (L(\mathbf{x}) > L(\mathbf{x} + \delta \vec{v}_1)) \\ M(\mathbf{x}) \geqslant t_M \end{cases} \quad (3)$$

2.2. Structure tensor to detect edge-like membranes

The detector defined in the previous section can detect ridge-like membranes, but it is not suitable for the detection of membranes with edge-like profile where density on one side of the membrane is similar to that of the membrane (e.g. those presented by a densely filled vesicle). Consequently, a new detector for this type of membranes is required. To this end, we focus our attention to the Structure tensor (Weickert, 1998), also known as the second moment tensor, which is given by:

$$\mathbf{J} = \begin{bmatrix} \left(\frac{\partial L}{\partial x}\right)^2 & \frac{\partial L}{\partial x}\frac{\partial L}{\partial y} & \frac{\partial L}{\partial x}\frac{\partial L}{\partial z} \\ \frac{\partial L}{\partial x}\frac{\partial L}{\partial y} & \left(\frac{\partial L}{\partial y}\right)^2 & \frac{\partial L}{\partial y}\frac{\partial L}{\partial z} \\ \frac{\partial L}{\partial x}\frac{\partial L}{\partial z} & \frac{\partial L}{\partial y}\frac{\partial L}{\partial z} & \left(\frac{\partial L}{\partial z}\right)^2 \end{bmatrix} \quad (4)$$

where $\frac{\partial L}{\partial i}$ $\forall i \in (x, y, z)$ are the first order derivatives of the scale-spaced volume L. The eigen-analysis of this tensor proceeds as in the previous case (Fernandez and Li, 2003, 2005). The first eigenvector $\vec{v}_1$, i.e. the one whose eigenvalue exhibits the largest value, points to the direction of the maximum variation. Also, a local plane satisfies $|\lambda_1| \gg |\lambda_2| \approx |\lambda_3|$. However, the Hessian and Structure tensors differ regarding the exact position of the detected surface. For a ridge profile L, the local maximum of the largest eigenvalue ($|\lambda_1|$) of the Hessian tensor corresponds to the maximum of L (Fig. 1(Left)). For an edge profile L, the local maximum of the largest eigenvalue ($|\lambda_1|$) of the Structure tensor corresponds to the inflection point of L (Fig. 1(Right)). Consequently, the Structure tensor is well suited to detect edge-like local structures. Moreover, the output of this edge detector is equivalent to the output of the Hessian-based detector (red curves in Fig. 1). This means that the eigen-analysis of the Structure tensor allows detection of edges and their conversion into ridges. This, in turn, enables application of all our ridge-based methodology to detect membranes with edge profile. Therefore, the ridge-like profiles detected by the Hessian-based detector and the edge-like profiles detected by the Structure tensor-based detector can be further processed in the same way.

In this work, first and second order derivatives required for the components of the tensors have been implemented based on central differences (Frangakis and Hegerl, 2001).