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Structure enhancement diffusion and contour extraction for electron tomography of mitochondria

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ABSTRACT

The interpretation and measurement of the architectural organization of mitochondria depend heavily upon the availability of good software tools for filtering, segmenting, extracting, measuring, and classifying the features of interest. Images of mitochondria contain many flow-like patterns and they are usually corrupted by large amounts of noise. Thus, it is necessary to enhance them by denoising and closing interrupted structures. We introduce a new approach based on anisotropic nonlinear diffusion and bilateral filtering for electron tomography of mitochondria. It allows noise removal and structure closure at certain scales, while preserving both the orientation and magnitude of discontinuities without the need for threshold switches. This technique facilitates image enhancement for subsequent segmentation, contour extraction, and improved visualization of the complex and intricate mitochondrial morphology. We perform the extraction of the structure-defining contours by employing a variational level set formulation. The propagating front for this approach is an approximate signed distance function which does not require expensive re-initialization. The behavior of the combined approach is tested for visualizing the structure of a HeLa cell mitochondrion and the results we obtain are very promising.

1. Introduction

To date, it is firmly established that mitochondrial function plays an important role in the regulation of apoptosis (Green and Reed, 1998; Obeid et al., 2007). For instance, following a variety of cell death signals, mitochondria exhibit early alterations in function and morphologic changes, such as the opening of the permeability transition pore or mitochondrial megachannel (Frank et al., 2001; Zamzami et al., 2007). There is also strong evidence that defects in function may be related to many of the most common diseases of aging, such as Alzheimer dementia, Parkinson's disease, type II diabetes mellitus, stroke, atherosclerotic heart disease, and cancer. This is founded on the observation that mitochondrial function undergoes measurable disturbance accompanied by drastic morphologic alterations in the presence of these multisystem diseases (Frey et al., 2006; Munnich and Rustin, 2001; Tandler et al., 2002).

Concurrent with the aforementioned conceptual advances there has been a significant increase in the types of tools available to study the correlation between mitochondrial structure and function. Along with the now classic methods for isolating mitochondria and assaying their biochemical properties, there are new and powerful methods for visualizing, monitoring, and perturbing mitochondrial function while assessing their genetic consequences (Marco et al., 2004; Pon and Schon, 2007). Electron tomography (ET) has allowed important progress in the understanding of mitochondrial structure. This imaging technique currently provides the highest three-dimensional (3D) resolution of the internal arrangement of mitochondria in thick Section (Perkins and Frey, 1999; Mannella et al., 1994). Nevertheless, the interpretation and measurement of the structural architecture of mitochondria depend heavily on the availability of good software tools for filtering, segmenting, extracting, measuring, and classifying the features of interest (Frey et al., 2002; Perkins et al., 1997).

This paper is organized as follows: Section 2 presents an overview of anisotropic nonlinear diffusion models in image processing in general, and in electron microscopy in particular. The level set method is also presented briefly as it is applied to the extraction of contours in images. In Section 3 we propose a new image smoothing and edge detection technique for electron tomography as an extension to the model proposed by Bazán and Blomgren (2007). This approach employs a combination of anisotropic nonlinear diffusion and bilateral filtering. In Section 4 we exhibit the

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performance of the combined approach for visualizing the structure of a HeLa cell mitochondrion with very promising results. We end this paper with a summary and discussion in Section 5.

2. Related work

In this section we present an overview of anisotropic nonlinear diffusion models in image processing and electron microscopy. The level set method is also presented briefly as it is applied to the extraction of contours in images. We only review here the works that serve as background to the model we propose in Section 3. For an excellent and comprehensive survey of diffusion methods in image processing we refer the interested reader to the book by Weickert (1998) and the references therein. Two very good references for the level set method are the books by Osher and Fedkiw (2003) and by Sethian (1999).

2.1. Nonlinear diffusion in image processing

Nonlinear diffusion is a very powerful image processing technique used for the reduction of noise and enhancement of structural features. It was first introduced to the image processing community by Perona and Malik (1990) as an attempt to overcome the shortcomings of linear diffusion processes, namely the blurring of edges and other localization problems. Their model accomplishes this by applying a process that reduces the diffusivity in areas of the image with higher likelihood of belonging to edges. This likelihood is measured by a function of the local gradient $|\nabla u|$. The model can be written as

$$u_t - \nabla \cdot \left(g\left(|\nabla u|^2\right) \cdot \nabla u\right) = 0, \tag{1}$$

for $t > 0$, on a closed domain Ω, with the observed image as initial condition $u(\mathbf{x}, 0) = u_0(\mathbf{x})$, and homogeneous Neumann boundary conditions $\langle g \cdot \nabla u, \mathbf{n} \rangle = 0$, on the boundary $\partial\Omega$. Here, $\mathbf{n}$ denotes the outward normal to the domain's boundary $\partial\Omega$, and $\langle g \cdot \nabla u, \mathbf{n} \rangle$ indicates the inner product $\int_{\partial\Omega}(g \cdot \nabla u) \cdot \mathbf{n}\, ds$. In this model the diffusivity has to be such that $g(|\nabla u|^2) \to 0$ when $|\nabla u| \to \infty$ and $g(|\nabla u|^2) \to 1$ when $|\nabla u| \to 0$.

Notwithstanding the practical success of the Perona–Malik model, it presents some serious theoretical problems such as (i) ill-posedness (Nitzberg and Shiota, 1992; Weickert and Schnörr, 2000); (ii) non-uniqueness and instability (Catté et al., 1992; Kichenassamy, 1997); (iii) excessive dependence on numerical regularization (Benhamouda, 1994; Fröhlich and Weickert, 1994). The last observation motivated an enormous amount of research towards the incorporation of the regularization directly into the partial differential equation (PDE), to avoid too much implicit reliance on the numerical schemes. A variety of spatial, spatio-temporal, and temporal regularization procedures have been proposed over the years (Alvarez et al., 1992; Catté et al., 1992; Cottet and Germain, 1993; Weickert, 1994b, 1996b, 2001; Whitaker et al., 1993). In Section 2.2 we describe one of the variants to the Perona–Malik model that has been successfully used in electron microscopy, and in Section 3 we propose a new model based on a combination of anisotropic nonlinear diffusion and bilateral filtering.

2.2. Anisotropic nonlinear diffusion in electron tomography

One way of introducing regularization to the Perona–Malik model is through anisotropic diffusion. Förstner and Gülch (1987) and Bigün and Granlund (1987) concurrently introduced the matrix field of the structure tensor for image processing, and it is the basis for today's anisotropic diffusion models. The main idea behind these models is to construct the orthogonal system of eigenvectors $\mathbf{v}_1, \mathbf{v}_2$, of the diffusion tensor $\mathbf{D}_\sigma$ in such way that they will reveal the presence of edges, i.e., $\mathbf{v}_1 \| \nabla u_\sigma$ (parallel) and $\mathbf{v}_2 \perp \nabla u_\sigma$ (perpendicular). Then one chooses appropriate (corresponding) eigenvalues that will allow smoothing parallel to the edges and avoid doing so across them. The main advantage of anisotropic diffusion models over their isotropic counterparts is that they not only account for the modulus of the edge detector, but also its directional information.

Cottet and Germain (1993) and Weickert (1994a, 1996a) were among the first authors to propose anisotropic nonlinear diffusion models for image processing. They devise a diffusivity matrix of the form

$$\mathbf{D}_\sigma = [\mathbf{v}_1 \quad \mathbf{v}_2 \quad \mathbf{v}_3] \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \begin{bmatrix} \mathbf{v}_1^{\mathrm{T}} \\ \mathbf{v}_2^{\mathrm{T}} \\ \mathbf{v}_3^{\mathrm{T}} \end{bmatrix}, \tag{2}$$

where the vectors $\mathbf{v}_i$ are the eigenvectors of the image's structure tensor $\mathbf{J}_\sigma = \nabla u_\sigma \cdot \nabla u_\sigma^{\mathrm{T}}$ or its convolved version $\mathbf{J}_\rho = G_\rho \times \mathbf{J}_\sigma$, where $u_\sigma = G_\sigma \times u$ and G_σ, G_ρ are Gaussian kernels of width σ, ρ, respectively. The parameters λ_i are functions of the eigenvalues, $\mu_1 \geqslant \mu_2 \geqslant \mu_3$, of the structure tensor $\mathbf{J}_\sigma$ (or $\mathbf{J}_\rho$). Together, the eigenvalues μ_i and the eigenvectors $\mathbf{v}_i$, characterize the local structural features of the image u, within a neighborhood of size $O(\rho)$. Each eigenvalue μ_i reflects the variance of the gray level in the direction of the corresponding eigenvector $\mathbf{v}_i$, while each parameter λ_i controls the diffusion flux in the direction of $\mathbf{v}_i$ and has to be chosen carefully.

Anisotropic nonlinear diffusion in electron microscopy was introduced by Frangakis et al. (1999, 2001). Based on the works of Weickert(1998, 1999a,b), they chose the parameters λ_i to create a hybrid model that combines both edge enhancing diffusion (EED) and coherence enhancing diffusion (CED). EED is based on the directional information of the eigenvectors of the structure tensor $\mathbf{J}_\sigma$, and its aim is to preserve and enhance edges. CED is based on the directional information of the eigenvectors of the convolved structure tensor $\mathbf{J}_\rho$, and is intended for improving flow-like structures and curvilinear continuities. To combine the advantages of EED and CED, the Frangakis–Hegerl model uses a switch based on comparing an ad hoc threshold parameter to the local relation between structure and noise $(\mu_1 - \mu_3)$. The threshold parameter is based on the mean value of $(\mu_1 - \mu_3)$ in a subvolume of the image containing only noise. EED is used when the difference $(\mu_1 - \mu_3)$ is smaller than the threshold parameter. When it is larger, the model switches to CED for the enhancement of line-like structure patterns. In a separate publication, Frangakis et al. (2001) applied the hybrid model to two-dimensional (2D) and 3D electron tomography data and compared it with conventional methods as well as with wavelet transform filtering. They concluded that the model exhibits excellent performance at lower frequencies, achieving considerable improvement in the signal-to-noise-ratio (SNR) that greatly facilitated the posterior segmentation and visualization.

Fernández and Li (2003, 2005) proposed a variant to the model by Frangakis et al.(1999, 2001) for ET filtering by anisotropic nonlinear diffusion, capable of reducing noise while preserving both planar and curvilinear structures. They provided their model with a background filtering mechanism that highlights the interesting biological structural features and a new criterion for stopping the iterative process. The Frangakis–Hegerl model diffuses unidirectionally along the direction of minimum change, $\mathbf{v}_3$, and efficiently enhances line-like structures (where $\mu_1 \approx \mu_2 \gg \mu_3$). It was argued by Fernández and Li (2003) that a significant number of structural features from biological specimens resemble plane-like structures at local scale. Therefore, they defined a set of metrics to discern whether the features are plane-like, line-like, or isotropic. The metrics they defined are: