

APPLICATION OF MODIFIED SUBJECTIVE SURFACE METHOD TO 3D CELL MEMBRANE IMAGE SEGMENTATION

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Abstract. In this paper, we study 3D cell membrane image segmentation where the segmented surface is reconstructed by the use of 3D digital cell membrane image information and information that is obtained from thresholded 3D image in a local domain. The segmentation method is based on evolution of surface that is governed by a nonlinear PDE, the modified subjective surface equation. A semi-implicit finite volume scheme was used for the numerical discretization of the proposed model. The method was applied to real data representing 3D microscopy images of cell membrane within the zebrafish pectoral fin.

Key words. Image segmentation, subjective surface method, level set method, finite volume method, semi-implicit scheme, cell microscopy images, zebrafish.

AMS subject classifications. 65M08, 35K61, 68U10.

1. Introduction. Image segmentation is the process of subdividing the image domain into constituent parts. It is known to be one of the fundamental, difficult and most studied problems in image processing. There are many approaches to image segmentation (see e.g., [13, 6, 4, 2]) but in this paper, we focused on the subjective surface segmentation method. In image processing, this segmentation approach was introduced in [10, 11], studied and applied in [10, 11, 1, 8, 12, 14, 7]. Subjective surface segmentation method is based on the idea of segmentation function evolution that is governed by a geometrical diffusion model (see e.g., [12] and references contained therein for more details).

In applications, it is usually not easy to obtain optimal result using the subjective surface segmentation approach if the object that is intended to be segmented has internal structures or edges. The reason, may be attributed to the fact that this approach works with edge information throughout the process of segmentation. Hence, spurious edges or edges within the internal structures in an object of interest are also not disrespected during segmentation. For segmentation of membrane images which is the focus of this paper, the classical subjective surface segmentation approach seems not to give optimal result. This is, probably, as a result of the nature of membrane images. To improve the quality of the final segmentation result, we use the idea of local thresholding of image intensity values within a ball of appropriate radius around the approximate center of a membrane image. So, the idea is to presmooth the membrane image (to be segmented); apply the local thresholding to the presmoothed

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image; combine the information obtained from the thresholding with the information from the original membrane image intensities to get a segmentation result.

2. Main results.

2.1. Mathematical model. Let $I^0 : \Omega \rightarrow \mathbb{R}$, $\Omega \subset \mathbb{R}^3$ be the intensity function of a 3D membrane image. Let $\alpha = \min_{x \in B(c,r)} G_\sigma * I^0(x)$, $\beta = \max_{x \in B(c,r)} G_\sigma * I^0(x)$, where G_σ is a smoothing kernel, $B(c, r)$ is a ball with radius r centered at c , a given approximate central point inside the membrane image to be segmented. Then the threshold value which is used during the process of local thresholding may be chosen as $t_r = \eta \alpha + (1 - \eta) \beta$, with $\eta \in [0, 1]$ and the ball radius may be chosen with respect to the approximate size of the membrane image to be segmented. So, the concept of local thresholding is to set all intensity values in the local neighborhood of center c to β if they are above t_r and α otherwise.

Our method is based on solution of the following modification of subjective surface equation (see also [12])

$$(2.1) \quad u_t = |\nabla u| \nabla \cdot \left(G^0 \frac{\nabla u}{|\nabla u|} \right)$$

where $G^0 = g(\delta |\nabla G_{\sigma_1} * I^0| + \theta |\nabla G_{\sigma_2} * I^{t_r}|)$; u is the unknown segmentation function; g is the Perona-Malik function typically defined as $g(s) = \frac{1}{1+Ks^2}$; $K > 0$ is a parameter chosen empirically [9]; I^{t_r} is 3D image intensity of thresholded image within a ball of radius $r > 0$; $\delta, \theta \in [0, 1]$ determine the influence of information obtained from thresholding and membrane image intensities in the segmentation process; G_{σ_1} and G_{σ_2} are the smoothing kernels. Equation (2.1) go along with the following Dirichlet boundary conditions

$$(2.2) \quad u(t, x) = u^D \in [0, T] \times \partial\Omega,$$

and the initial condition

$$(2.3) \quad u(0, x) = u^0(x) \in \Omega.$$

u^D is assumed without loss of generality to be 0. We note here that, if $\delta = 1$ and $\theta = 0$ then (2.1) reduces to the subjective surface segmentation model [10].

2.2. Numerical discretization.

2.2.1. Time discretization. Semi-implicit approach which is known to guarantee unconditional stability is used for time discretization of (2.1). Assume that equation (2.1) is solved in time interval $I = [0, T]$ and N equal number of time steps. If the time step is denoted $\tau = \frac{T}{N}$, then the time discretization of (2.1) is given by

$$(2.4) \quad \frac{1}{\sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}} \frac{u^n - u^{n-1}}{\tau} = \nabla \cdot \left(G^0 \frac{\nabla u^n}{\sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}} \right),$$

where ε is a regularization parameter (Evans - Spruck [3]), u^0 is given initial segmentation function, and u^n , $n = 1, \dots, N$ is the solution of the model in time step n .

2.2.2. Space discretization. For space discretization (see also, [12]), we used similar notations as those used in [8]. Although the step by step details of the discretization process is given in [12], for purpose of completeness we include these steps here also.

Let the finite volume mesh containing the voxels of 3D image be denoted \mathcal{T}_h , while V_{ijk} , $i = 1, \dots, N_1$, $j = 1, \dots, N_2$, $k = 1, \dots, N_3$ denote each finite volume. For each $V_{ijk} \in \mathcal{T}_h$, let h_1, h_2, h_3 be the size of the volumes in x_1, x_2, x_3 direction. Let the volume of V_{ijk} and its barycenter be denoted by $m(V_{ijk})$ and c_{ijk} respectively. Let the approximate value of u^n in c_{ijk} be denoted by u_{ijk}^n . For every $V_{ijk} \in \mathcal{T}_h$, we denote the set of all (p, q, r) such that $p, q, r \in \{-1, 0, 1\}$, $|p| + |q| + |r| = 1$ by N_{ijk} , the set of all (p, q, r) , $p, q, r \in \{-1, 1\}$ by M_{ijk} , and the set of all (p, q, r) such that $p, q, r \in \{-1, 0, 1\}$, $|p| + |q| + |r| = 2$ by P_{ijk} . For each $(p, q, r) \in N_{ijk}$, denote the line connecting the center of V_{ijk} and the center of its neighbor $V_{i+p, j+q, k+r}$ by σ_{ijk}^{pqr} and its length $m(\sigma_{ijk}^{pqr})$. We denote the planar sides, area and normal of finite volume V_{ijk} by e_{ijk}^{pqr} , $m(e_{ijk}^{pqr})$ and ν_{ijk}^{pqr} respectively. Let x_{ijk}^{pqr} be the point of intersection of the line σ_{ijk}^{pqr} and planar side e_{ijk}^{pqr} . Furthermore, for each $(p, q, r) \in M_{ijk}$, let s_{ijk}^{pqr} denote the vertices of the finite volume V_{ijk} , and for each $(p, q, r) \in P_{ijk}$, let the midpoints of the voxel edges be denoted y_{ijk}^{pqr} . The approximate value of u^{n-1} in x_{ijk}^{pqr} , y_{ijk}^{pqr} and s_{ijk}^{pqr} , with (p, q, r) belonging to the appropriate index set, is denoted by u_{ijk}^{pqr} ; the time index is omitted, as only the values from the time level $n - 1$ will be needed at these points.

With these given notations, if we integrate (2.4) over finite volume V_{ijk} , we have that

$$(2.5) \quad \int_{V_{ijk}} \frac{1}{\sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}} \frac{u^n - u^{n-1}}{\tau} dx = \int_{V_{ijk}} \nabla \cdot \left(G^0 \frac{\nabla u^n}{\sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}} \right) dx.$$

Let the average value of $A_\varepsilon = \sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}$ in finite volume V_{ijk} be denoted by $\bar{A}_{\varepsilon,ijk}^{n-1}$. Considering the fact that u^n and u^{n-1} are assumed to be piecewise constant over a finite volume mesh and using the divergence theorem we obtain that

$$(2.6) \quad m(V_{ijk}) \frac{u_{ijk}^n - u_{ijk}^{n-1}}{\tau} = \bar{A}_{\varepsilon,ijk}^{n-1} \sum_{N_{ijk}} \int_{e_{ijk}^{pqr}} G^0 \frac{\nabla u^n}{\sqrt{\varepsilon^2 + |\nabla u^{n-1}|^2}} \cdot \nu_{ijk}^{pqr} dS.$$

If the normal derivative $\nabla u^n \cdot \nu_{ijk}^{pqr}$ is approximated by $(u_{i+p, j+q, k+r}^n - u_{ijk}^n) / m(\sigma_{ijk}^{pqr})$ and $A_{\varepsilon,ijk}^{pqr;n-1}$ and G_{ijk}^{pqr} are defined to be the averages of A_ε and G^0 respectively on e_{ijk}^{pqr} then (2.6) reduces to

$$(2.7) \quad m(V_{ijk}) \frac{u_{ijk}^n - u_{ijk}^{n-1}}{\tau} = \bar{A}_{\varepsilon,ijk}^{n-1} \sum_{N_{ijk}} m(e_{ijk}^{pqr}) G_{ijk}^{pqr} \frac{u_{i+p, j+q, k+r}^n - u_{ijk}^n}{A_{\varepsilon,ijk}^{pqr;n-1} m(\sigma_{ijk}^{pqr})}.$$

Equation (2.7) simplifies to

$$(2.8) \quad u_{ijk}^n = u_{ijk}^{n-1} + \frac{\tau}{m(V_{ijk})} \bar{A}_{\varepsilon,ijk}^{n-1} \sum_{N_{ijk}} m(e_{ijk}^{pqr}) G_{ijk}^{pqr} \frac{u_{i+p, j+q, k+r}^n - u_{ijk}^n}{A_{\varepsilon,ijk}^{pqr;n-1} m(\sigma_{ijk}^{pqr})};$$

which further simplifies to

$$(2.9) \quad \left(1 + \frac{\tau}{m(V_{ijk})} \bar{A}_{\varepsilon,ijk}^{n-1} \sum_{N_{ijk}} G_{ijk}^{pqr} \frac{m(e_{ijk}^{pqr})}{A_{\varepsilon,ijk}^{pqr;n-1} m(\sigma_{ijk}^{pqr})} \right) u_{ijk}^n - \frac{\tau}{m(V_{ijk})} \bar{A}_{\varepsilon,ijk}^{n-1} \sum_{N_{ijk}} G_{ijk}^{pqr} \frac{m(e_{ijk}^{pqr})}{A_{\varepsilon,ijk}^{pqr;n-1} m(\sigma_{ijk}^{pqr})} u_{i+p,j+q,k+r}^n = u_{ijk}^{n-1}$$

DEFINITION 2.1 (Global rescaling (see also, [12])). *Let $\vartheta = \min_{\Omega} u_{ijk}^n$ and $\zeta = \max_{\Omega} u_{ijk}^n$ for each time step n . Then the rescaled version of u_{ijk}^n given by (2.9) is obtained by the following relation $u_{ijk}^n = \frac{1}{\zeta - \vartheta} (u_{ijk}^n - \vartheta)$. As a result, we have that for each time step n , the rescaled version of u_{ijk}^n is in $[0, 1]$ and it is used in the next time step.*

Equation (2.9) together with zero Dirichlet boundary condition, represents a system of linear equations which can be solved efficiently by the Successive Overrelaxation (SOR) method. Finally, we note how to determine the average values G_{ijk}^{pqr} , $A_{\varepsilon,ijk}^{pqr;n-1}$ and $\bar{A}_{\varepsilon,ijk}^{n-1}$ either in voxels or on voxel sides. To determine these quantities, we used the reduced diamond cell strategy (see [8]). The idea of the reduced diamond cell approach is to drop the values in the eight corner points denoted by $c_{i,j+q,k+r}$, $c_{i+1,j+q,k+r}$, with $q, r \in \{-1, 1\}$ and as a result reducing the stencil from 18 to 10 points. Hence, from the computational standpoint, this approach reduces the computational cost.

Additionally, in the reduced diamond cell approach, the values of u^{n-1} are obtained from the midpoints y_{ijk}^{pqr} of the voxel edges. These values are given approximately for each $(p, q, r) \in P_{ijk}$ by

$$\begin{aligned} u_{ijk}^{pq0} &= \frac{1}{4} \left(u_{ijk}^{n-1} + u_{i+p,j,k}^{n-1} + u_{i,j+q,k}^{n-1} + u_{i+p,j+q,k}^{n-1} \right), \\ u_{ijk}^{p0r} &= \frac{1}{4} \left(u_{ijk}^{n-1} + u_{i+p,j,k}^{n-1} + u_{i,j,k+r}^{n-1} + u_{i+p,j,k+r}^{n-1} \right), \\ u_{ijk}^{0qr} &= \frac{1}{4} \left(u_{ijk}^{n-1} + u_{i,j+q,k}^{n-1} + u_{i,j,k+r}^{n-1} + u_{i,j+q,k+r}^{n-1} \right). \end{aligned}$$

The components of the averaged gradient on e_{ijk}^{pqr} are approximated by 2D diamond cell approach in orthogonal planes which use the values u_{ijk}^{pqr} given above (see also [8]). This means that

$$\begin{aligned} \nabla^{p00} u_{ijk}^{n-1} &= \frac{1}{m(e_{ijk}^{p00})} \int_{e_{ijk}^{p00}} \nabla u^{n-1} dx \\ &\approx \left(p(u_{i+p,j,k}^{n-1} - u_{ijk}^{n-1})/h_1, (u_{ijk}^{p,1,0} - u_{ijk}^{p,-1,0})/h_2, (u_{ijk}^{p,0,1} - u_{ijk}^{p,0,-1})/h_3 \right), \end{aligned}$$

$$\nabla^{0q0} u_{ijk}^{n-1} = \frac{1}{m(e_{ijk}^{0q0})} \int_{e_{ijk}^{0q0}} \nabla u^{n-1} dx$$

$$\approx \left((u_{ijk}^{1,q,0} - u_{ijk}^{-1,q,0})/h_1, q(u_{i,j,q,k}^{n-1} - u_{ijk}^{n-1})/h_2, (u_{ijk}^{0,q,1} - u_{ijk}^{0,q,-1})/h_3 \right),$$

$$\nabla^{00r} u_{ijk}^{n-1} = \frac{1}{m(e_{ijk}^{00r})} \int_{e_{ijk}^{00r}} \nabla u^{n-1} dx$$

$$\approx \left((u_{ijk}^{1,0,r} - u_{ijk}^{-1,0,r})/h_1, (u_{ijk}^{0,1,r} - u_{ijk}^{0,-1,r})/h_2, r(u_{i,j,k+r}^{n-1} - u_{ijk}^{n-1})/h_3 \right).$$

Hence, we obtain that the approximations $\nabla^{pqr} u_{ijk}^{n-1}$ for the gradient at the points x_{ijk}^{pqr} , with $\nabla^{pqr} u_{ijk}^{n-1}$ denoting the approximation of the gradient in the barycenters x_{ijk}^{pqr} of voxel side e_{ijk}^{pqr} . Using the same approach for the computation of gradients of image intensities, the following approximations are obtained as well:

$$(2.10) \quad G_{ijk}^{pqr} = g \left(\delta |\nabla^{pqr} I_{\sigma_1;ijk}| + \theta |\nabla^{pqr} I_{\sigma_2;ijk}^{thr}| \right),$$

$$A_{\varepsilon,ijk}^{pqr;n-1} = \sqrt{\varepsilon^2 + |\nabla^{pqr} u_{ijk}^{n-1}|^2}, \quad \bar{A}_{\varepsilon,ijk}^{n-1} = \sqrt{\varepsilon^2 + \frac{1}{6} \sum_{N_{ijk}} |\nabla^{pqr} u_{ijk}^{n-1}|^2}.$$

2.3. Numerical experiments. In this section, numerical experiments were performed on biological image of a developing pectoral fin in zebrafish embryo in order to demonstrate the performance of our mathematical model (2.1) on membrane images. In all the experiments performed, isosurface 0.5 is displayed. 3D microscopy images of cell membrane were given to us to process by the research group of Nadine Peyri ras, (CNRS BioEmergences, France, in the framework of ImageInLife EC funded project). In zebrafish, pectoral fin serves as a vertebrate model for limb development. A sheet of cells converges at the prospective fin field location, subsequently proliferates and remodels into a 3D distinct structure. As the bud continues to grow and extends from the body trunk, cells undergo morphological changes to give rise to the fin 3D shape. Ectodermal cells are stretched and potentially play a part in shaping the fin while mesodermal cells decrease in volume in order to accommodate their neighbors given the increased compaction inside the fin. Here, we aim to measure dimensions of different cell types (EVL, ectoderm, lateral plate mesoderm, and somitic mesoderm) and monitor and quantify their shape changes influenced by external and internal forces during the 2D to 3D transition. To label cell nuclei and membrane globally, wild type zebrafish embryos were injected with mRNA encoding H2B-mCherry and eGFP-F at one-cell stage. Embryos were anesthetized and immobilized starting from 18 hours post fertilization and mounted on the upright confocal microscope Zeiss LSM 780. Datasets were acquired at the xy resolution of 0.6386660µm/pixel and 0.8467909µm/pixel z-direction. Nuclei centers were obtained using Difference of Gaussians algorithms and further validated using the software Mov-IT implemented in the Bioemergences Workflow[5]. The nuclei centers were used for construction of initial segmentation function in the presented membrane segmentation method.

We note that in all Figures 2.2–2.5, the first column shows the 3D volume rendering of the original 3D membrane image intensity and the second column shows, in black colour, their corresponding results after segmentation. Also, in Figure 2.1, first column of first row shows the 2D slice (along z axis) of 3D cell membrane image which

is intended to be segmented, first column of second row shows the 2D slice (along z axis) of 3D binarized image which is obtained by local thresholding, second column of first row shows the result of segmentation without local thresholding while second column of second row shows the result of segmentation with local thresholding. The partial reconstruction of the membrane image shown in second column of first row may be attributed to the fact that some internal structures or edges in the image did not allow the segmentation function to get to the actual boundary of the cell. However, with the local thresholding, the segmentation function is able to grow to the boundary of the cell giving rise to the result which is shown in second column of second row.

In the first numerical experiment, $\delta = 1.0$ and $\theta = 0.0$ were used. This choice of parameters reduced equation (2.1) to the classical subjective surface model [10]. Results obtained using these parameters are shown in Figures 2.2 and 2.4. Furthermore, for other numerical experiments, $\delta = 0.8$ and $\theta = 0.2$, $\delta = 0.7$ and $\theta = 0.3$, $\delta = 0.6$ and $\theta = 0.4$ were used. The results obtained using these parameters are shown in Figures 2.3 and 2.5. With these nonzero choices of θ , it can be seen by visual comparison that the results are correct.

Finally, we note that in these numerical experiments, computations were done on a grid with $50 \times 50 \times 40$ voxels, and computational method parameters were set to $h = 0.01$, $\tau = 0.01$, $\eta = 0.95$, $r = 26$, $K = 0.8$ and $N = 100$.

In conclusion, it is not difficult to see that mathematical model (2.1) is a useful and successful generalization of the classical subjective surface model for 3D cell membrane image segmentation.

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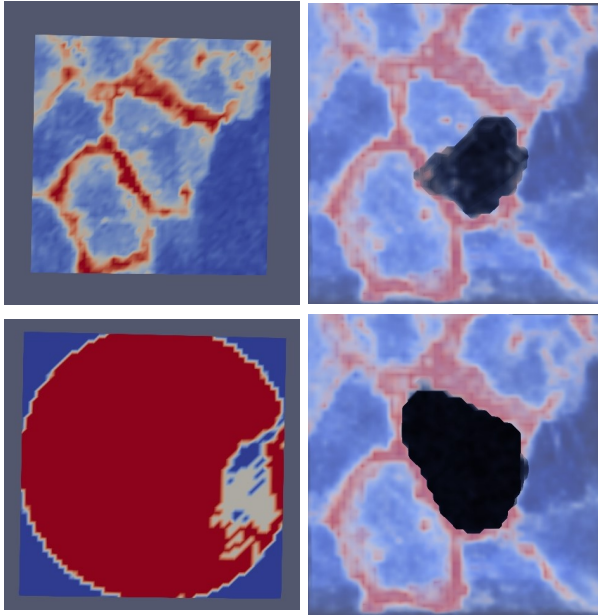


FIG. 2.1. In this figure, first column of first row shows the 2D slice (along z axis) of 3D cell membrane image which is intended to be segmented, first column of second row shows the 2D slice (along z axis) of 3D binarized image which is obtained by local thresholding, second column of first row shows the result of segmentation without local thresholding while second column of second row shows the result of segmentation with local thresholding.

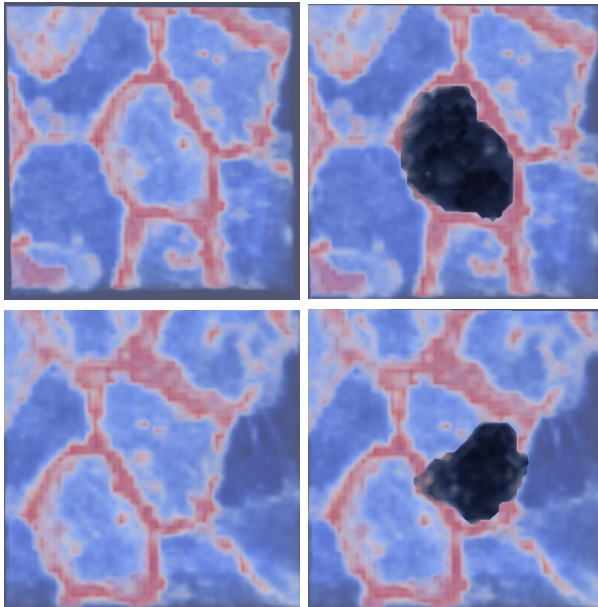


FIG. 2.2. First column of this figure shows the 3D volume rendering of the 3D cell membrane image which is intended to be segmented, while the second column shows the result after application of (2.1) with $\delta = 1.0$ and $\theta = 0.0$.

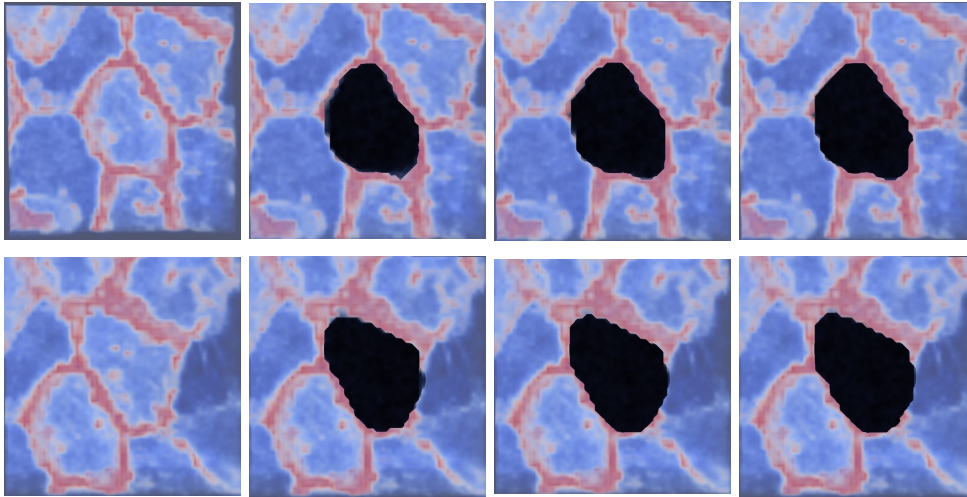


FIG. 2.3. First column of this figure shows the 3D volume rendering of the 3D cell membrane image which is intended to be segmented, while the second column shows the result after application of (2.1) with $\delta = 0.8$ and $\theta = 0.2$, $\delta = 0.7$ and $\theta = 0.3$, and $\delta = 0.6$ and $\theta = 0.4$ respectively.

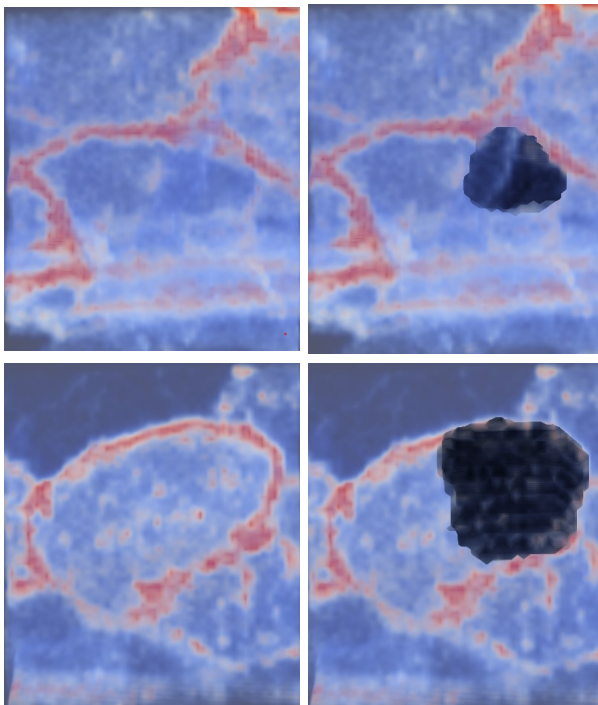


FIG. 2.4. First column of this figure shows the 3D volume rendering of the 3D cell membrane image which is intended to be segmented, while the second column shows the result after application of (2.1) with $\delta = 1.0$ and $\theta = 0.0$.

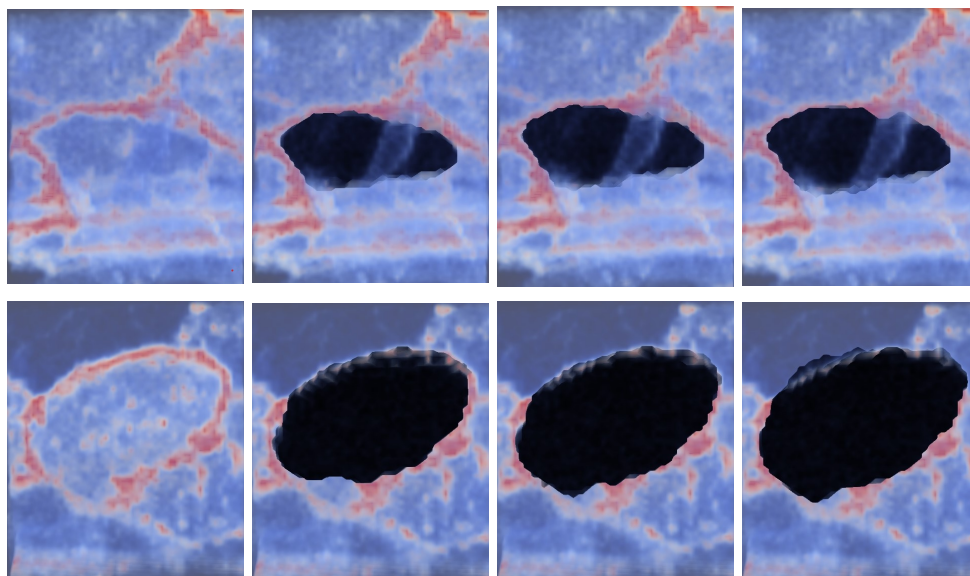


FIG. 2.5. First column of this figure shows the 3D volume rendering of the 3D cell membrane image which is intended to be segmented, while the second column shows the result after application of (2.1) with $\delta = 0.8$ and $\theta = 0.2$, $\delta = 0.7$ and $\theta = 0.3$, and $\delta = 0.6$ and $\theta = 0.4$ respectively.

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