Repulsive force based snake model to segment and track neuronal axons in 3D microscopy image stacks

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The branching patterns of axons and dendrites are fundamental structural properties that affect the synaptic connectivity of axons. Although today three-dimensional images of fluorescently labeled processes can be obtained to study axonal branching, there are no robust methods of tracing individual axons. This paper describes a repulsive force based snake model to segment and track axonal profiles in 3D images. This new method segments all the axonal profiles in a 2D image and then uses the results obtained from that image as prior information to help segment the adjacent 2D image. In this way, the segmentation successfully connects axonal profiles over hundreds of images in a 3D image stack. Individual axons can then be extracted based on the segmentation results. The utility and performance of the method are demonstrated using 3D axonal images obtained from transgenic mice that express fluorescent protein.

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Introduction

Three-dimensional reconstruction of images of tubular biological objects, such as blood vessels and neuronal processes, has become an active research topic. Such reconstruction can reveal geometrical features (e.g., length, diameter, and orientation) and topological characteristics (e.g., connectivity and branch order) of the biological circuits, which are crucial in understanding the physiology or development of a biological system. One area where this kind of reconstruction is particularly useful is the arbors of individual axons. For example, a “motor unit” which are the axon and muscle fiber targets innervated by a motor axon has been analyzed by tracing the entire arbor of the axon and identifying all the innervated muscle fibers (Keller-Peck et al., 2001; Kasthuri and Lichtman, 2003). Such reconstruction has been made possible by the development of transgenic mice in which only small subsets of motor axons express fluorescent proteins (Feng et al., 2000; Walsh and Lichtman, 2003). Reconstructing larger numbers of axons has not been possible because of the difficulty of segmenting and extracting individual axons when many are running in a tightly fasciculated bundle. It is thus important to develop computational algorithms that will segment individual axons from both the background and other nearby axons. We are particularly interested in making these approaches fully automatic because of the large size of fully resolved confocal data sets of neuromuscular connections (tens of GB).

The absolute requirement for reconstruction is to correctly segment individual objects contained in the specimen. The segmentation process faces several obstacles: each axonal profile usually has irregular shape, orientation, light intensity, and contrast. In addition, different axons are frequently intertwined into tight bundles with limited space between their boundaries, which tends to make the space between objects often brighter than the background. Moreover, the resolution of the images is limited by physical laws of diffraction, leading to ambiguous borders when axons are closer than 0.25 μm. In order to overcome these problems, several schemes of segmentation have been proposed in the literature, including adaptive thresholding, region growing (grassfire), watershed, active contours (snakes), algorithms based on the variational principles, wavelets, and combination of multiple approaches (Jiang and Mojon, 2003; Mahadevan et al., 2004). However, all these methods suffer from various shortcomings that make them insufficient to segment axons in fasciculated bundles. For instance, watershed algorithm tends to over-segment; region growing, on the other hand, tends to under-segment. Ordinary active contour methods also perform unsatisfactorily when the...
image contains nearby objects whose intensities are added up at their mutual boundary.

Once segmented in a single 2D plane, each individual axon then needs to be traced throughout the image stack, for which several methods have been suggested. For example, rank statistics-based method has been developed to trace neuronal processes and vasculature from two-dimensional confocal microscope images (Al-Kofahi et al., 2003). Al-Kofahi et al. (2002) presented a different 3D tracing method to locate axons in confocal image stacks. Wavelet based segmentation has been proposed to segment axons based on multiscale edges in 3D (Dima et al., 2002). Fuzzy logic based method has also been developed to process airway in 3D (Park et al., 1998).

In this paper, we propose a snake-based algorithm to segment and trace the 3D objects in an image stack. The advantage of this approach is that it retrieves the topological information robustly from the data. Since the classic snake model is prone to give incorrect segmentation when objects of interest are close to each other, a repulsive force based approach is deployed to address this problem and improve the segmentation performance. To improve image quality, we preprocess raw images by morphological operations to enhance contrast, non-linear filtering to remove noise, and anisotropic diffusion to enhance edges.

Materials and methods

Materials

Fig. 1 shows the maximum intensity projection (MIP) of motor axons in a peripheral muscle nerve connecting to the omohyoid muscle on the $x$–$y$, $x$–$z$, and $y$–$z$ planes. The axons express YFP (Yellow Florescence Protein) in their cytoplasm under the control of the regulatory element of the ubiquitous nervous system gene Thy-1 (Feng et al., 2000). Three-dimensional stacks of images of fluorescently labeled processes are obtained by using confocal microscope.

Preprocessing

As discussed above, methods have been proposed to trace axons in 2D and 3D images; however, the problem we are interested in is different. As shown in Fig. 1, the major task in our problem is to separate one axon from its neighbors, in addition to separate axons from the background. This task could be challenging because neuronal processes may appear touching each other due to low resolution and the orientation of the images. We extend the standard snake model to a repulsive force model to correctly track each axon. Given a 3D image stack $u(i, j, k), i = 1, \ldots, I, j = 1, \ldots, J, k = 1, \ldots, K$, where $i, j, k$ are index of the image in the $x$, $y$, and $z$ direction, the method processes one slice of the image at a time. Without loss of generosity, we assume that the method proceeds along the $z$ direction and processes 2D image in the $x$–$y$ plane, i.e., $u(\cdot; \cdot; k)$, each time. Certain features of the images that can be used to constrain the algorithm and thus are exploited in the processing.

1. The $x$–$y$ dimension of the image stack is much larger than the $z$ dimension of the stack, which means that the 3D objects extend approximately parallel to the $x$–$y$ plane. However, each 3D object may turn at large angles in the $x$–$y$ plane.
2. 3D objects never overlap or intersect with each other. However, they can be very close, and subsequently the contrast is low in the space between them. In this case, the corresponding 2D objects on the cross-section appear to be nearly contiguous to each other.
3. Within each 3D object, the light intensity is not uniform.
4. The boundary of each 3D object is reasonably smooth. Thus, consecutive cross-sections of the same 3D object give 2D objects that are similar in location, shape and area.

Fig. 1. Maximum intensity projection of motor axons on (a) $x$–$y$ plane, (b) $x$–$z$ plane, (c) $y$–$z$ plane. Note the higher resolution of image on the $x$–$y$ plane. The size of the 3D image is 512 by 512 by 156 in the $x$, $y$, and $z$ direction.
As indicated in problems 1 and 2, the contrast between two adjacent axon processes can be very low due to their proximity. The low contrast makes it hard to separate the axons by computational means, and, sometimes, even by human analyst, see Fig. 2. To overcome this problem, we propose an image contrast enhancement method to preprocess the axon image and prepare it for the next computational step, i.e., snake evolution segmentation.

Raw images are preprocessed with morphological top and bottom hat filtering. The top-hat transform is the difference between the original image and its opening, which is defined as the set of foreground parts of an image that fit a particular structuring element. The bottom-hat transform is the difference between the original image and its closing, which is the collection of background parts of an image that fit a particular structuring element. Fig. 3(a) shows a raw image. The top and bottom hat filtered images are subtracted to remove uneven background and enhance contrast, as shown in Fig. 3(b). The contrast was further enhanced by taking the square of the normalized image as shown in Fig. 3(c). However, the squaring amplifies shot noise and creates discontinuity of the gradient of the image. The first problem is solved with median filtering, see Fig. 3(d). The discontinuity in the gradient field is corrected by using anisotropic diffusion (Broser et al., 2004; Acton, 1998), which smooths strong edges more than weak edges. Given an image \( u(x, y) \), its smoothed version is modeled as \( u(x, y, \tau) \) where \( \tau \) parameterizes the amount of smoothing. Smoothing is achieved by solving the following evolution equation (Carmona and Zhong, 1998):

\[
u_s = c\left(au_{\eta\eta} + bu_{\zeta\zeta}\right)
\]

where \( a \) and \( b \) control the smoothing directions and depend on the gradient of image \( u \), \( c \) controls the amount of smoothing, and \( u_{\tau} \) denotes the derivation with respect to \( \tau \). \( \eta \) and \( \zeta \) represent the gradient direction and the direction perpendicular to the gradient, respectively. Carmona and Zhong (1998) employed second order differentiations rather than traditional first order ones to diffuse image and obtain impressive results. In our evaluation, we find that the Hessian method of (Carmona and Zhong, 1998), second-order directional derivation diffusion yields better results than the original Perona–Malik method (Perona and Malik, 1990).

\textbf{Repulsive snake model}

Our algorithm for 2D segmentation is based on the snake (active contour) scheme. Snakes are deformable curves that can move and change their shapes to conform to object boundaries (Kass et al., 1987; Xu and Prince, 1998). The movement and deformation of snakes are controlled by internal forces, which are intrinsic to the geometry of the snake and independent of the image, and external forces, which are derived from the image. These forces are defined in such a way that the evolution of the snake will eventually lead it to conform to some features of

![Fig. 2. Raw images chosen from 4 continuous 2D slices.](image-url)
interest, for instance, the boundaries of objects in the image (Xu and Prince, 1998). Snake models have been applied to various image processing tasks, including motion tracking, segmentation, shape modeling and edge detection.

According to the representation of the curves, snake models can be classified into three major categories: point-based snakes that consist of an ordered collection of points sometimes called snaxels (Kass et al., 1987; Gao et al., 1998), parametric snakes that are represented as continuous functions (Staib and Duncan, 1992; Chakraborthy et al., 1996; Figueiredo and Leitao, 2000; Brigger et al., 2000), and geometric snakes that are based on level sets (Caselles et al., 1993; Malladi et al., 1995; Amadieu et al., 1999). In this work, we developed a parametric snake to track axons in 3D microscopic image.

The 2D parametric snake is a curve \( C(s, t) \) in a given image \( u \) parameterized by \( s \in [0,1] \), with the energy functional:

\[
E(C) = \int_0^1 \left( \frac{1}{2} [x|C'(s)|^2 + \beta|C''(s)|^2] \right) ds - \lambda \int_0^1 \left( |\nabla u(C(s))| \right)^p ds
\]

where \( \alpha, \beta \) and \( \lambda \) are positive coefficients, and \( C' \) and \( C'' \) denote the first and second derivative of \( C \) with respect to \( s \). The first integral is the internal energy which measures the smoothness of the curve, and the second integral is the external energy which attracts the curve toward the object boundary. Minimizing the energy functional \( E(C) \) creates a particular smooth curve \( C \) which maximizes \( |\nabla u(C(s))| \), an edge detector. A general edge detector is defined as:

\[
g(|\nabla u(C(s))|) = \frac{1}{1 + |\nabla G_d u(C(s))|^p}, \quad p \geq 1
\]

where \( G_d u \), a smoother version of \( u \), is the convolution of the image with a Gaussian kernel. Obviously, the function \( g(|\nabla u|) \) is positive in homogeneous regions and zero at the edges. \( E(C) \) is minimized iteratively by considering the curve as a function of an abstract time \( t \), i.e., \( C(s, t) \). Starting from an initial guess \( C(s, t=0) = C_0 \), the curve is deformed according to the following evolution equation until it reaches equilibrium

\[
C_t = \alpha C'' - \beta C'' + \nabla g.
\]

This original snake model has several drawbacks. In the presence of weak edges, it may leak into neighboring objects. It may also be trapped at local maxima, especially in noisy images. The blurring kernel in (2) helps to increase the capture range of the snake, but it is difficult to determine appropriate parameters. In order to increase the capture range, Xu and Prince (1998) presented a new external force for parametric snake models, which is briefly discussed next.

**Original GVF model**

The Gradient Vector Flow (GVF) model is an extension of the original snake idea. In the GVF model, the external force term in (1) is replaced by a vector field \( v \), which is a diffusion of the gradient.
vectors of a grey level or binary edge map derived from the original image (Xu and Prince, 1998). The reaction diffusion equations:

\[ \begin{align*}
  v_t &= \mu \nabla^2 v - (v - \nabla f)^2, \\
  v(t = 0) &= \nabla f
\end{align*} \]

leave the external force field approximately unchanged from its original value when it is near object boundaries, or \(|\nabla f|\) is larger. Elsewhere in the image, this diffusion process “interpolates” field v from \(\nabla f\) between object boundaries.

Because the information from strong object boundaries is propagated throughout the image by the diffusion process, the GVF model has a much larger “capture range” than the original snake model and is considerably less sensitive to the choice of initialization (Jacob et al., 2004; Ray et al., 2002; Ray and Acton, 2004; Zimmer et al., 2002).

Although the GVF snake model has proven to outperform standard snake model in detecting concave boundaries (Xu and Prince, 1998), it does not solve the problem of locating weak boundaries. As pointed out in Zimmer et al. (2002), the diffusion process (4) progressively leads to strong boundaries and overwhelsms the influence of weak boundaries. This defect makes snake to deform to incorrect segmentations even when the initialization is positioned closely to the correct object boundary. Another problem of GVF snake model is that it cannot correctly segment objects that are adjacent to each other (Xie and Mirmehdi, 2004). When two objects are nearby, the GVF force between them produces an “equilibrium line,” illustrated in Figs. 4(a) and (b). If the initialization contour is placed outside the equilibrium line, the snake will deform to the wrong direction, or rest in the equilibrium line if the initial contour lies within the equilibrium line. These two limitations greatly affect the application of snake in tracking problems, especially in our cases that axons may be close to each other. When two axons are close to each other, the corresponding snake of one axon is likely to be attracted to the other axon, while remaining attached to its legitimate object. In some cases, one snake even evolves and moves into the boundary of the neighboring axon due to the weak boundaries between the two axons. To overcome this problem, we develop a GVF snake model based on repulsive force between two or multiple objects.

**Repulsive GVF model**

To correctly separate axons close to each other, we exploit the prior information that axons are separated and employ a repulsive force to push the snakes toward their legitimate objects. This repulsive force can be obtained by reversing the gradient direction of neighboring objects.

Assume that an image \(u(x, y)\) consists of two objects, \(O_1\) and \(O_2\) that are close to each other and the rough areas of the two objects are known beforehand, see Fig. 5. We want to determine an evolution that will continuously attract a robustly placed initial curve \(C_1\) toward the boundary of object \(O_1\) and \(C_2\) toward that of object \(O_2\). From the edge map of \(u(x, y)\) and the discussion above, we know that if the initial contour \(C_1\) is placed beyond

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Fig. 4. Test the snake deforming in two close circular objects. (a) Two circular objects, (b) result obtained from GVF snake model, (c) result of our repulsive GVF model. In both panels b and c dotted lines represent initialized circles and deformation process, and solid lines are the resulting evolution curve. Line AB represent the “equilibrium line” produced by the two opposite edge forces from the two circles.

Fig. 5. Modified gradient direction and its intermediaries. (a) Simulated image, assuming left circular in subject to be \(O_1\) and right one to be \(O_2\), (b) the associated gradient flow and the “equilibrium line” denotes the critical area of boundary influence. If we place the initial curve \(C_1\) beyond this line, the force will attract the curve to wrong direction, (c) reversed gradient direction. The initial curve will deform to the right direction even if initial curve is placed close to the neighborhood object, (d) GVF force vector, and (e) repulsive GVF force vector.
“equilibrium line” \( AB \), then part of curve \( C_1 \) will progressively evolve into the boundary of \( O_2 \) under the influence of its external force, see Figs. 5(b) and (d). This limits the application of snake evolution in axon tracking since initialization needs to be carefully chosen. If \( O_1 \) and \( O_2 \) are close to each other, it is almost impossible to choose a suitable initialization, which is independent of the attraction of nearby object. However, by reversing the direction of the force of the neighboring objects, as shown in Figs. 5(c) and (e) and examples in the next section, we obtain better performance in terms of achieving a robust initialization and ensuring the snakes evolve in the right direction. This reversing force acts like the role of the membrane of axons that keep two axons separated.

Mathematically, we use \( f_e \) to denote the edge forces of the image including objects \( O_1 \) and \( O_2 \). Such forces can be derived as the force \( \nabla g \) in (3). We use the term influence parameters \( I_{p_1} \) and \( I_{p_2} \) to denote the range parameters of \( O_1 \) and \( O_2 \), respectively. Such parameters can be chosen as the curve which is parallel to the objects of interest and encloses them within a distance of \( \delta \), see Fig. 6. We assume that the rough area and shape of the objects \( O_1 \) and \( O_2 \) are known. The assumption is valid since the axon does not change its position and shape abruptly. Then the repulsive force for object \( O_1 \) can be calculated as:

\[
f_{r1} = \left\{ \begin{array}{cl} -\nabla g(I(x)) & x \in I_{p1} \\ \nabla g & x \in I_{p2} \end{array} \right. \tag{6}
\]

where \( \nabla g \) is the edge force. Repulsive force for object \( O_2 \) can be similarly defined. In order to increase the capture range of snake we adopt the diffusion method (4) of (Xu and Prince, 1998). This approach encourages snake to deform robustly in the right direction, even when the initial contour is close to other objects. In fact this approach moves the equilibrium line to a much larger area, as indicated in Fig. 4(c). Similarly the repulsive force can be applied to another neighboring object, for example, the force can be applied to object \( O_2 \). With the same step, we can have the repulsive force from \( O_1 \), and this repulsive force plus object boundary information will keep the initialization curve \( C_2 \) to its legitimate object \( O_2 \).

**Repulsive snake to segment axons**

Our method consists of the following steps: for each image, we apply our preprocessing techniques to obtain high contrast images. The initialization of the snakes can easily be done manually on the first frame of the sequences of images and allows the curve to evolve to correct boundaries. As axons do not change their positions and shapes much between consecutive frames, it is reasonable to use the final contour of frame \( n \) as the initialization contour of frame \( n+1 \), thus allowing a straightforward automatic initialization that link axons positions across frames. To compensate for the difference in positions of an axon on two consecutive frames, we need a correction procedure to ensure that the initialization on frame \( n+1 \) is in proper position and snakes deform to the right boundaries. We use the radii and areas of axons as a criterion of evaluating proper initialization and adaptively adjust the repulsive force to control the snake deformation.

**Update radius and area of a snake**

The smoothness of the 3D axon guarantees that the center of gravity (CoG) of an axon on frame \( n \) and \( n+1 \) does not shift abruptly. We exploit this fact by using the following equation to validate snake evolution result. Without loss of generality, we assume there is only one axon in the image, then we update its CoG on frame \( n+1 \) by

\[
c_{g}(n+1) = \frac{1}{n+1} \sum_{p=1}^{n} \theta^{(n-p)} c_{g}(p) + \tilde{c}_{g}(n+1)
\]

where \( \theta \) is a weighting factor that assigns more weight to the most recent frames and less weight to the frame far away from frame \( n+1 \). In this work, we set \( \theta \) to 0.95.

**Necessary constraints on repulsive snake deformation**

As described above, axons do not change their shapes abruptly, which implies that area of each axon will vary slightly between consecutive frames. Therefore, it is reasonable to assume that the absolute difference in areas of a axon on two consecutive frames is lower than some threshold. We can incorporate this information in the validation process if the area of an axon changes above the threshold between frames.

We note that repulsive GVF snake initialization relies on the prior knowledge about the rough area of objects or influence parameters projected from previous frame. However, the projection may, though rare, lead to incorrect initialization when previous segmentation result on frame \( n \) is not accurate enough. Such case may arise where there are two axons close to each other. Then the initialization will lead to suboptimal segmentation on frame \( n+1 \) and such mistakes will be propagated to the following frames. To address the problem, we modify our algorithm to adaptively adjust the repulsive force.

Assume we have well-segmented contours \( C_1 \) and \( C_2 \) for two axons close to each other on frame \( n \), we project the two contours to next preprocessed frame \( n+1 \). We also know the areas and CoG of contours \( C_1 \) and \( C_2 \). Based on these prior knowledge we assign certain statistical “degree,” denoted...
as \( d(i) \), \( i = 1, 2 \) as a convex function, which represents the degree of the reliability of the segmentation contours \( C_i \)

\[
d(i) = 1 - 0.5 \left| \frac{A(C_i) - \bar{A}(C_i)}{A(C_i)} \right| + 0.5 \left| \frac{\bar{c}_{g,i} - \bar{c}_{g,i}}{c_{g,i}} \right|^2
\]

(8)

where \( \bar{A}(C) \) represents the weighted mean area of the object in all slices (similar to (7)), and \( A(C_i) \) denotes the area of object enclosed by snake \( C_i \). Similarly, we use \( c_{g,i} \) to denote the area of object CoG of the axon up to the current frame and \( c_{g,i} \) as the CoG of axon on the current frame. The square operation applying in the second term comes from the isoperimetric inequality which states in some sense that square of length of curve \( C_i \) is “comparable” with the area \( A(C_i) \) and we know \( O_j = \int C_j(t)dt \). We use this parameter to redefine the repulsive force (6) as:

\[
f_{r,i} = \left\{ - \frac{\nabla g_i(I(x))d(i)}{\nabla g_i} \quad x \in \Omega_{g,i} \right\}
\]

(9)

which can adjust the strength of repulsive force adaptively to attain both robustness and adequacy. In fact, in our data, it is typical that

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Fig. 7. Axon tracking and segmentation on an image with six axons. (a–f) Evolution of GVF without repulsive force.
between two objects close to each, one tends to have strong edge force and another has weak edge force in their conjunction part. If we reverse the strong edge force, the energy force of (3) will grow so high that it will push the snake apart from the legitimate edge. Such a problem is caused by strong edge force overwhelming the weak force after the diffusion process. In such cases, $d(i)$ becomes much higher, and we evolve the snake again in the current slice with the revised force. We found that the adaptive revised force based snakes obtain improved results.

Results

In this section, we present the results by applying our method to segment and track axons. Our raw data consist of an image stack obtained from a confocal microscope and resampled along the $x$ axis, which gave 200 image slices. There are six axons in the data set. We use different examples to illustrate the effectiveness of our repulsive snake in segmenting and tracking axons. The first example shows that our method can adequately correct error made...
by the GVF snake in the intermediate steps and ensure the correct tracking. In order to illustrate the performance improvement of repulsive force based snake model, we first used standard GVF to process axon images, see Fig. 7. Fig. 7(a) shows the initialization, and Figs. 7(b)–(e) show the intermediate results of snake evolution. The final result is shown on Fig. 7(f). It is seen that the snakes cannot correctly separate two adjacent axons to the top. Fig. 8 shows the result of GVF snake based on the repulsive force. It is noted that without the repulsive force, axon 1 and 2 are deformed to incorrect segmentation contour, see Fig. 8(a). In order to correct this error we apply our repulsive snake and derive the corrected segmentation results, see Fig. 8(b). This adjustment keeps the snakes evolving into correct boundary, as shown in Figs. 8(c) and (d). Fig. 8(e) shows a case that the snakes make mistakes. However, based on the knowledge from the previous frame and using our repulsive snake again, we can obtain correct segmentation results which place the snake in the boundary of axon 1 and 2 as indicated in Fig. 8(f).

In axon tracking, there are cases that axons are close to each other, see Fig. 8. Starting from the initialized contour, our model can ensure the snakes evolve in the right directions under the effect of the adaptive repulsive force, see Figs. 8(b) and (f). Fig. 9 shows a more difficult case in which axons are much closer to each other. Starting from the automatic initialization from the previous frame,

![Fig. 7.](image)

![Fig. 8.](image)

![Fig. 9.](image)
the intermediate segmentation results are displayed from Figs. 9(a) to (e). By adaptively adjusting the repulsive force, we obtain the final segmentation of Fig. 9(f). It is obvious that axons are well segmented and correctly marked. This self-correcting capability is beneficial to axon tracking as well as to other applications such as cell tracking problems. Furthermore, we also note that the weight factor $0 < \theta < 1$ in Eq. (7) plays an important role in estimating the CoG. If we set $\theta$ to be 0.7 rather than 0.95, as we did in Fig. 8, and rerun the segmentation and tracking process to test the robust reliability value of $C_i$. We found the results are almost the same, see Fig. 10; because the re-evaluation of Eq. (8) minimizes the influence from CoG.

In order to further demonstrate the performance of our algorithm, we use another dataset to test the robustness of the proposed method. There are four axons in the dataset, see Fig. 11, and both axons 2 and 3 have “no boundary.” Our results show that the proposed method can achieve accurate segmentation results. In summary, the method proposed in this paper can segment and track multiple adjacent objects, as shown in Figs. 8 and 10. In both figures, it is noted that there are multiple axons close to each other.

![Fig. 10. Axon tracking and segmentation on the same image of Fig. 8 with repulsive force but with a different CoG value ($\theta = 0.7$), which adjusts the strength of repulsive force.](image-url)
yet our method can successfully segment them and obtain the proper boundaries.

**Discussions and conclusions**

In this paper, we introduced a snake method based on repulsive force to detect the boundaries of the cross-sections of axons in 2D image and connect the boundaries in 3D to track the axons. The evolution of the snakes is controlled by the two parameters, namely, $\alpha$ and $\beta$ of (1) where a large $\alpha$ creates a snake of large contraction while a large $\beta$ favors a final contour of high rigidity and a small $\beta$ allows the snake to deform toward a corner. The adjustment of the two parameters needs to be judiciously determined based on the obtained images and the requirements. The branching patterns of axons and dendrites are of fundamental importance to understand developmental neurobiology and neurodegenerative conditions. Three-dimensional images of axons

![Fig. 11. Segmentation of axons from another data set which has lower contrast between boundaries and some axons are merged into one whole. (a) Axons 1, 2 and 3 are adjacent to each other. (b–f) Segmentation results show that the axons are correctly separated by our repulsive model even there are 'no boundary' between them.](image-url)
elucidate the process of neural innervation and help to establish neuronal circuits involving both the formation of new connections and the elimination of existing connections. Though three-dimensional images of fluorescently labeled processes can be obtained, their branch pattern is difficult to derive due to lack of robust methods of tracing individual axons. We developed a repulsive snake model to segment and track axonal profiles in 3D images. The method processes 2D images in sequence and uses the segmentation result of the previous image as the initialization contours in the next image. To overcome the problem that one snake may merge with another one incorrectly, we introduced repulsive force to keep the two snakes separated. Results obtained from real 3D microscopy images show that the method can obtain robust and accurate results in segmenting neuronal axons. The method can be used in applications like blood vessel and airway segmentation. Future work will focus on developing algorithms to model branches, i.e., split and merge, in axons.

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References


