Journal of Neuroscience Methods 178 (2009) 197–204

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Detection of the optimal neuron traces in confocal microscopy images

Zlatko Vasilkoski, Armen Stepanyants*

Department of Physics and Center for Interdisciplinary Research on Complex Systems, Northeastern University, 110 Forsyth St., Boston, MA 02115, USA

ARTICLE INFO

Article history:
Received 1 September 2008
Received in revised form 5 November 2008
Accepted 7 November 2008

Keywords:
Axon
Dendrite
Morphology
Reconstruction
Confocal microscopy
Skeleton
Optimization

ABSTRACT

Quantitative methods of analysis of neural circuits rely on large datasets of neurons reconstructed accurately in three dimensions (3D). Due to the complexity of neuronal arbors, large datasets of reconstructed neurons must be generated with automated algorithms. Here, we attempted to automate the process of neuron tracing from sparsely labeled 3D stacks of confocal microscopy images. Our algorithm involves two steps. In the first step, the segmented image of neurites in the stack is voxel-coded. Centers of intensity of consecutively coded wave fronts are connected into a branched structure, which represents a coarse trace of the neurites. In the second step, this trace is optimized with the modified active contour method, which tends to maximize the intensity along the trace while keeping it under tension. To assess the performance of the algorithm we used manual reconstructions of neurons and converted them into artificial stacks of intensity images. These images were traced using the developed algorithm and quantitatively compared to the corresponding manual traces. The optimal traces were on average 6.0% shorter than the manual traces. This reduction in length resulted from the smoothness of the optimal traces, which, in comparison to the manual ones, were built out of shorter segments, and, as a result, were 3.3% less tortuous. The average distance between the optimal and the manual traces was 0.14 μm, and the average distance between their corresponding branch-points was 2.2 μm, illustrating good agreement between the traces.

1. Introduction

It is virtually impossible to understand how the brain functions without detailed knowledge of neural circuits. Axons and dendrites of neurons can be labeled with a multitude of cell labeling techniques and visualized in three dimensions (3D) with confocal scanning microscopy (Conchello and Lichtman, 2005). Such images contain valuable information about the connectivity in the brain, despite the facts that only small subsets of neurons are labeled in typical experiments to allow for quantitative analysis, and that synaptic connectivity cannot be resolved reliably on the level of light microscopy. The first restriction can be partially alleviated by new circuit labeling techniques such as multicolor Brainbow (Livet et al., 2007; Lichtman et al., 2008), which allows for labeling of a much larger subsets of neurons through the introduction of different colors. Transsynaptic tracing (Wickersham et al., 2007), which identifies the pre-synaptic partners of a given cell, or array tomography (Micheva and Smith, 2007) can alleviate the second restriction on the visualization of neural circuits.

In spite of the above limitations, quantitative methods of analysis of axonal and dendritic arbors of sparsely labeled neurons have already resulted in numerous findings about synaptic connectivity [see e.g. Elston and Rosa, 1997; Kozloski et al., 2001; Duan et al., 2002; Stettler et al., 2002; Lubke et al., 2003; Binzegger et al., 2004; Stepanyants et al., 2004, 2008; Li et al., 2005; Shepherd et al., 2005; Markram, 2006; Jefferis et al., 2007; Lee and Stevens, 2007]. These methods rely on accurate reconstruction of labeled neurons, which currently can only be achieved with manual reconstruction software tools, such as the ones included in Neurolucida (MicroBrightField Inc.), NeuronJ (Meijering et al., 2004), NeuronStudio (CNIC, Mount Sinai School of Medicine), and FilamentTracer (Bitplane Inc.). However, the manual reconstruction is extremely time consuming and depends on the diligence and the subjective judgment of the user. There is an obvious need to automate the neuron reconstruction process.

Automated reconstruction of neurites from confocal microscopy stacks of images typically contains several of the following steps (Russ, 2007): image restoration (denoising and deconvolution), registration of images within individual image stacks, image segmentation, tracing, feature extraction (cell bodies, spines, boutons, synapses, branch-points), and registration of stacks of images. The purpose of this study is to develop and evaluate the performance of an automated algorithm for neurite tracing. To focus on this aim, we assumed that all the remaining reconstruction steps have been already accomplished or will be performed after the tracing is completed. In particular, we assumed that neurites had been successfully segmented from the background, i.e. they contain no loops or broken segments.

* Corresponding author. Tel.: +1 617 373 2944; fax: +1 617 373 2943.
E-mail address: a.stepanyants@neu.edu (A. Stepanyants).

0165-0270/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2008.11.008

There are generally several classes of algorithms, which can be used to obtain the traces of segmented liner branched structures in 3D. One class of algorithms is based on image thinning methods, which are used for extracting skeletons from binary images [see e.g. Lee et al., 1994; Palagyi and Kuba, 1998]. The basic idea behind these methods is an iterative removal of voxels from the surface of the segmented image in a way that preserves the topology of the contained structure. When applied to noisy images of neurites, such algorithms produce voxellated skeletons, which do not follow precisely the intensity in the image and often contain erroneous short branches.

Multi-scale 3D line enhancement filters (Sato et al., 1998; Streekstra and van Pelt, 2002), that are based on the eigenvalues of the Hessian matrix, can facilitate centerline visualization and detection. These filters can extract centerline voxels in non-branched linear structures. Once the centerline is detected, fitting models can be used to determine the most optimal trace of the linear structure. One of the most frequently used models is the active contour model introduced by Kass (Kass et al., 1988; Frangi et al., 1999; Schmitt et al., 2004) that fits the shape of a curve to the skeleton. In most of these models, the user sets the branch-points and they remain fixed during the fitting process.

Another approach is vectorial tracking (Can et al., 1999; Al-Kofahi et al., 2002; Srinivasan et al., 2007; Wang et al., 2007). This class of algorithms exploits local image properties to recursively trace linear structures. The algorithm starts from a seed point, which is typically provided by the user, and tracks the structure of interest by exploring the nearby voxels. The algorithm can be combined with a dynamic real-time updating of the trace direction by the user (Meijering et al., 2004). Vectorial tracking is suitable for semi-automated image analysis of high contrast images. However, this method is not appropriate for precise fully automated tracing of branched structures because the branch- and end-points of the structure have to be specified by the user.

Currently, there are no fully automated tools, which would reliably trace complex neuronal morphology, e.g. axonal arbors of cortical pyramidal neurons. Most methods require from the user to perform pattern recognition tasks, such as identification of branch- and end-points. In this study, we described an automated algorithm for determining the optimal traces of already segmented neurite images. This computerized method of neuron tracing requires no user involvement. It consists of two steps, voxel-coding and optimal tracing, which are described in the first part of this paper. In the second part, we compared the results obtained with the automated tracing procedure to the manual neuron reconstructions.

2. Materials and methods

2.1. Manual neuron traces

In this study we used dendritic arbors of 20 pyramidal cells reconstructed from layer 3 of monkey superior temporal cortex and prefrontal area 46 (Duan et al., 2002, 2003). These neurons were retrogradely labeled with Lucifer Yellow and reconstructed in 3D with custom-designed morphometry software NeuroZoom (Nimchinsky et al., 1996, 2000). Neuron reconstructions were obtained from http://NeuroMorpho.Org (Ascoli, 2006) in the SWC format (Cannon et al., 1998).

2.2. Converting neuron traces into image stacks

For every manually reconstructed cell, we generated a 3D stack of intensity images by using the following procedure. We assumed that the underlying neurite fluorescence, F_N, is distributed uniformly throughout the arbor of the labeled neuron. Since in the present study we are only interested in finding the optimal traces of already segmented images, background level of fluorescence, F_B, was assumed to be zero. To simulate the light scattering in the tissue and in the microscope we linearly blurred the fluorescence of the labeled tissue by convolving it with a point spread function (*PSF*). For convenience, the *PSF* was chosen to be Gaussian with equal standard deviations in all three dimensions, $\sigma_{x,y,z} = 1\ \mu m$. As a result of this procedure, we obtained the expected fluxes of photons arriving at the detector from individual locations in the tissue. These fluxes, integrated at the detector over time, result in the expected counts of photons arriving from individual voxels in the image stack, $\mu(x, y, z)$. For simplicity, voxel size was chosen to be $s = 1\ \mu m$ in all three dimensions. The actual photon counts, $n(x, y, z)$, were randomly chosen from the Poisson distributions, $P(n|x, y, z)$, with the corresponding expected values, $\mu(x, y, z)$. This procedure can be summarized as:

$$
\begin{aligned}
PSF(x,y,z) &= \frac{1}{(2\pi)^{3/2}\sigma_x\sigma_y\sigma_z}\exp\left[-\frac{x^2}{2\sigma_x^2}-\frac{y^2}{2\sigma_y^2}-\frac{z^2}{2\sigma_z^2}\right]\\
\mu(x,y,z) &= s^3\int F(x',y',z')PSF(x-x',y-y',z-z')\,dx'\,dy'\,dz' \qquad (1)\\
P(n|x,y,z) &= \frac{\exp[-\mu(x,y,z)]\mu(x,y,z)^n}{n!}.
\end{aligned}
$$

In this expression, $F(x, y, z)$ is the underlying fluorescence of the tissue. It assumes the values of F_N for points belonging to the interior of the neurites and F_B for the exterior points.

To create an artificial image stack from a manually traced neuron we first voxellated the trace by assigning values of $F_N = 20\ \mu m^{-3}$ to all the voxels overlapping with the trace and $F_B = 0\ \mu m^{-3}$ to the rest of the voxels. This procedure yielded the function $F(x, y, z)$, which was next convolved (second expression in Eq. (1)) with the *PSF* to produce the expected photon counts in the image stack, $\mu(x, y, z)$. Next, the actual photon counts, $n(x, y, z)$, were randomly generated for each voxel from the corresponding Poisson distributions (last expression in Eq. (1)). In order to avoid loops, voxels with zero actual photon counts in the interior of neurites were assigned the photon counts of one. Finally, the intensity $I(x, y, z)$ in the image stack was obtained by scaling down the actual photon counts to the [0, 1] interval. The value of $F_N = 20\ \mu m^{-3}$ was chosen because it produced image stacks, where the intensity along the neurites was similar to or more variable than that in typical confocal microscopy images of neurites (Fig. 1). Our method is not sensitive to particular values of this parameter.

2.3. The optimal trace

We begin the description of the method for finding the optimal traces of linear branched structures in confocal microscopy images by first looking at a simplified example. Consider a 3D continuous intensity image, $I(\vec{r})$, of a non-branched line with known start- and end-points, $\vec{r}_i$ and $\vec{r}_f$. One can employ active contour models (Kass et al., 1988) to obtain the optimal trace of this image. To this end, a fitness function has to be constructed which favors smooth traces connecting points $\vec{r}_i$ and $\vec{r}_f$ in a way that maximizes the intensity along the trace.

We used a parametric representation of the trace line, $\vec{r}(t)$, $t \in [0, 1]$, which originates at the point $\vec{r}(0) = \vec{r}_i$ and terminates at the point $\vec{r}(1) = \vec{r}_f$. On the one hand, the optimal trace has to pass through the high intensity places in the image and on the other hand, the trace has to be kept under tension to prevent it from zigzagging from one high-intensity voxel to another. The trade-off between these two requirements can be captured by the maximization of the fitness functions containing some of the following terms (Kass et al., 1988;