Live Neuron Morphology Automatically Reconstructed From Multiphoton and Confocal Imaging Data

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Losavio BE, Liang Y, Santamaría-Pang A, Kakadiaris IA, Colbert CM, Saggau P. Live neuron morphology automatically reconstructed from multiphoton and confocal imaging data. J Neurophysiol 100: 2422–2429, 2008. First published August 13, 2008; doi:10.1152/jn.90627.2008. We have developed a fully automated procedure for extracting dendritic morphology from multiple three-dimensional image stacks produced by laser scanning microscopy. By eliminating human intervention, we ensure that the results are objective, quickly generated, and accurate. The software suite accounts for typical experimental conditions by reducing background noise, removing pipette artifacts, and aligning multiple overlapping image stacks. The output morphology is appropriate for simulation in compartmental simulation environments. In this report, we validate the utility of this procedure by comparing its performance on live neurons and test specimens with other fully and semiautomated reconstruction tools.

INTRODUCTION

Dendritic morphology is a key determinant of neuronal computation (Mainen and Sejnowski 1995; Rall 1977). Long, thin dendrites endow the neuron with two physiologically important properties. First, they maximize surface-to-volume ratio, thereby enabling large numbers of synaptic contacts onto small structures. Second, dendrites isolate synaptic and voltage-gated currents by electrical compartmentalization (Levy 1989; Mel 1993). Since neuronal integration involves complex interactions between large numbers of nonlinear voltage-gated processes, it is not straightforward to infer the relationships between the structure and specific functions of neuronal dendrites. Thus model building and computer simulation are essential to produce viable theories of neuronal computation (Ascoli 2006; Carnevale et al. 1997; Hoffman et al. 1997; Poirazi and Mel 2001). Fortunately, it has become straightforward to perform complex simulations incorporating both neuronal morphologies and ion channel kinetics due to the availability of powerful simulation tools (Brette et al. 2007; Carnevale and Hines 2006). However, for a variety of reasons reflecting both the complexity and individual variability of neurons as well as the relatively weak constraints of the models, one cannot simply present an input and produce an output that necessarily reflects biological reality (Holmes et al. 2006).

To better understand the role of dendrites in neuronal computation, the better understanding of dendrites in neuronal computation requires that can be used for computer simulation is an important goal (Ascoli 2006; Migliore et al. 2005). Available databases have been limited in scope because of the relatively large effort involved in the mainly manual computer-aided reconstruction methods currently in use (e.g., NeuronLucida, MicroBrightField, Williston, VT). To address this need, a number of semiautomated and automated systems are under development (Brown et al. 2005; Evers et al. 2005; Wearne et al. 2005; Wouterlood et al. 2002). Throughput, consistency, and accuracy should improve by reducing the need for the investigator to make individual measurements of dendrite length, diameter, and position (handling dendritic spines is outside the scope of this report). Our own interests in developing a system for automated reconstructions come from the need to acquire electrophysiological data and morphological reconstructions from the same neurons. Advances in functional optical imaging have improved our ability to monitor physiological processes in dendrites (e.g., local Ca²⁺ concentration) from fine structures (Hoogland and Saggau 2004) and from multiple discontinuous sites (Iyer et al. 2006; Reddy et al. 2008). Despite these advances, there is always a limit on the overall acquisition bandwidth. Thus, the acquisition methods allow either high temporal or high spatial resolution, but not both simultaneously. Thus there is need to determine the optimal sites for functional imaging. If the morphology of the neuron is known at the outset of an acute experiment, quantitative criteria can be used to decide where functional imaging has a high likelihood of yielding useful (i.e., constraining) information. Even in cases where such precision is not necessary, producing neuronal libraries of paired morphological and functional data nevertheless remains an important goal.

A typical on-line experiment studying neuronal computation consists of an initial structural imaging phase, where confocal or multiphoton image stacks are acquired. During an intermediate phase, a morphological reconstruction of the neuron is produced and used to choose functional imaging sites. The functional imaging comprises the final phase of the experiment. Such a scenario places a number of requirements on the imaging and computational approaches. Here we describe a procedural pipeline that combines optical imaging and computational reconstruction (the suite of software is called Online Reconstruction and Imaging of Neurons, or “ORION”), which was designed to meet these requirements, a validation of the
component methods, and the resulting morphological reconstructions.

**METHODS**

**Tissue preparation**

Brain slices of rat hippocampus were obtained according to the Institutional Animal Care and Use Committee at Baylor College of Medicine. Anesthetized Sprague-Dawley rats were transcardially perfused with ice-cold solution containing (in mM): 110 ChCl, 2.5 KCl, 1.25 Na,PO₄, 25 NaHCO₃, 0.5 CaCl₂, and 7.5 MgCl₂. All chemicals were obtained from Sigma-Aldrich unless otherwise noted. Hippocampal slices (350 μm thick) were transferred to solution containing 125 NaCl, 2.5 KCl, 1.25 Na,PO₄, 25 NaHCO₃, 2 CaCl₂, and 2 MgCl₂, 1.3 ascorbate, and 3 pyruvate for 20 min at 34°C and then at room temperature for 40 min before the imaging experiment.

**Capillary preparation**

Polyimide-coated glass capillaries of 2 ± 1- and 5 ± 2-μm inner diameters were supplied by Polymicro Technologies. Short segments (~40 mm) of capillary were cleaved with a carbide scribe and had about 10 nm of cladding removed by quickly passing the tips through the flame of a Bunsen burner. After being allowed to cool, they were filled with fluorescent dye by capillary action, suspended in a solution containing 100 μM Alexa Fluor 594 (Invitrogen-Molecular Probes) for 30 min. The dye-filled capillaries were glued at an angle of about 8° to the bottom of a glass-bottom petri dish and immersed in water for imaging.

**Imaging**

Visually identified pyramidal neurons from the hippocampal CA1 region were patch-dialyzed with 100–200 μM Alexa Fluor 594 or Alexa Fluor 555 (Invitrogen-Molecular Probes) fluorescent dye solution containing (in mM) 120 K-glucuronate, 20 KCl, 10 HEPES, 2 MgCl₂, 0.2 EGTA, 4 Mg-ATP, 0.3 Tris-ATP, and 7 phosphocreatine for ≥15 min. After the initial dye-filling period, volume data sets were collected using the raster-scanning functionality of the microscope and an objective stepper motor (see following text). In most instances, several overlapping volumes were required to capture all the dye-filled dendrites. Two different optical imaging systems were used in this study. Multiphoton images were collected with a custom Nikon PCM2000 scan system (Iyer et al., 2002), modified to use excitation from a Coherent Chameleon Ti:S laser tuned to 810 nm (~27 mW). This system scans with a lateral resolution of 640 × 480 pixels (corresponding to 192 × 144 μm), whereas the axial resolution was set to 0.5 μm. The emission light was filtered with a Chroma HQ600/200 filter (no excitation filter was used). Each optical section represents the rolling average of three frames at the same plane. Given an acquisition time of about one frame/s, each stack comprised of 50–150 μm required about 10 min. Two to seven volumes were required for the neurons reported here (sometimes requiring structural imaging for ≥1 h).

Confocal images were collected with an Olympus Fluoview 300 Confocal Microscope system. The built-in HeNe laser provided excitation at 543 nm. Single 1.024 × 1.024 optical sections (i.e., no frame averaging) were collected at 0.5-μm axial resolution (~3 s per frame).

**Reconstruction**

There are two major challenges to produce an accurate morphological model. The first challenge relates to the relatively poor quality of raw, unprocessed structural images of the biological specimen. Uneven diffusion of fluorescent dye inside the cell results in a high variation in contrast among the dendrite structures. Furthermore, a low signal-to-noise ratio, resulting from various sources that generally do not follow a Gaussian distribution, makes simple filtering ineffective. This unavoidable effect is mostly due to the low illumination power of the scanning device that must be used to prevent photodamage of cellular function. Finally, important structures of interest are near the theoretical limit of optical imaging resolution (e.g., small spines and dendrites). The second challenge is related to the accurate modeling of the cell as a branched tree using cylinders for shape representation. A realistic morphological model should be expressed as a single branched tree where the starting point is in the center of the soma. Representing the neuron as a connected branched tree involves 1) an accurate estimation of branch lengths and diameters and 2) the correct detection of branch points. An additional challenge is created by the spines studded along the dendrites, making the dendrites “irregular” tubular structures. Proper segmentation of a neuron must include only the regions that belong to the cell, excluding external objects that may be present, the patch pipette being the most prominent. A final, practical consideration is that processing the information of a single cell may require allocating large amounts of memory (typically several gigabytes of RAM).

Under these considerations, a morphological reconstruction algorithm must be able to 1) trace dendrite branches with high variations of contrast, 2) estimate dendrite lengths and diameters as well as branching points, 3) remove external objects if they are present, and 4) produce a realistic morphology in the presence of noise. Figure 1 presents our automatic reconstruction pipeline, which consists of deconvolution (optional, not included in this figure), denoising, registration, dendrite segmentation, and morphological reconstruction.

**DECONVOLUTION.** An experimental point spread function (PSF) is estimated by acquiring a volume image stack of sparsely scattered latex beads (diameter ~0.2 μm) that are labeled with a fluorescent dye similar to those used in these experiments. These beads simulate ideal point light sources; by averaging many of them (~15), a robust estimate of the microscope’s PSF can be obtained. This PSF is then used to deconvolve the three-dimensional (3D) image using a standard maximum-likelihood method. In our experience, this step is rarely necessary for multiphoton data sets, but could be beneficial for confocal data sets.

**DENOISING.** To robustly remove noise without corrupting the neuron structure, we use the UH-FAST (University of Houston—Frames-based Adaptive hysteresis Thresholding) algorithm. Unlike the classical wavelet-based denoising algorithms where the structure could become corrupted, our approach uses a multidirectional filter that has the advantage of detecting edges along the main axes and diagonals in 3D space. This significantly improves the true positive rate on structures of known and indeterminate size (Santamaria-Pang et al. 2008).

**REGISTRATION.** Many dendrites extend beyond the typical field-of-view (FOV) of laser-scanning microscopes that use objective lenses of high magnification. Multiple-image volumes are therefore necessary to fully capture the entire neuron structure. We are thus required to merge and align the multiple data sets to a single volume. The experimentalist supplies estimated X–Y–Z offsets between each stack (which are obtained when moving the preparation relative to the microscope). To measure similarity during the automated registration process, we use the sum of mean-squared differences for each voxel in the two images. This measure is then minimized using a limited-memory Broyden–Fletcher–Goldfarb–Shanno (BFGS) minimization with simple bounds (Zhu et al. 1997).

**SEGMENTATION.** Our approach to dendrite segmentation is based on constructing a probability volume (VP), derived from a statistical dendrite-shape model. We estimate the probability of a voxel belonging to a dendrite by assigning high probability values to voxels close...
to the dendrite medial axis and low probability values to voxels close to the dendrite boundaries. Such a statistical shape model captures irregular tubular-shape variations estimated from structural shape features. Unlike the classical approaches to detect regular tubular objects, our approach generalizes the detection of both regular and irregular tubular objects to the detection of both regular and irregular tubular objects (Santamaría-Pang et al. 2007a).

MORPHOLOGICAL RECONSTRUCTION. The goal of this reconstruction algorithm is to automatically express the cell morphology in terms of lengths and diameters of a single connected branched tree of approximating cylinders. We use the VP, estimated in the segmentation procedure to perform centerline extraction as follows: First, the soma center point is automatically detected by performing connected component analysis in the probability volume. Second, we automatically estimate the dendrite terminal points. Third, the optimal path that connects each terminal point with the soma center point is computed, defined as the path that travels along the dendrite medial axis and has a minimum length. To find the optimal path that connects each terminal point with the soma center point, we solve the eikonal equation of the form

\[ |D[VP(x)]||T(x)| = 1 \]

where D is the distance transform volume, calculated from the VP computed in the segmentation step. The pipette artifact is automatically removed if present (Santamaría-Pang et al. 2007b).

The ORION software is available at http://cbl.uh.edu/ORION/research/software.

To evaluate the reconstruction results produced by our fully automated software pipeline, we compared the results from the same input data set to Neurolucida (MicroBrightField), a commercially available morphology tracing software suite. Three human tracers with various levels of familiarity with neuron structure and tracing methodologies used the standard manual tracing features on a denoised, prealigned supervolume in “stack import” mode. We also used the AutoNeuron module of Neurolucida (MicroBrightField) to compare our results with those of a commercially available automated neuron reconstruction system. We realized optimal reconstruction results with AutoNeuron by setting the “Sensitivity” parameter to a value of 65.

Simulation

Impedance calculations and synaptic input simulations were carried out in the NEURON simulation environment (Carnevale and Hines 2006) using the “Adaptive Time Step” feature at 37°C. For all the models, axial resistivity was set to 200 Ω·cm, membrane capacitance to 1 µF/cm², and membrane conductance to 2 × 10⁻¹⁰ S/cm², with a reversal potential of -70 mV. Input and transfer impedances were computed at 10⁻⁶, 1, 10, 100, and 1,000 Hz. Synaptic input was simulated via double-exponential conductance changes (\( \tau_{\text{rise}} = 0.5 \) ms, \( \tau_{\text{decay}} = 1.0 \) ms, \( \text{amp} = 2 \times 10^{-3} \) μS) triggered by phantom presynaptic events via NEURON’s built-in NetCon and NetStim classes (Carnevale and Hines 2006).

RESULTS

Fully automated pipeline

Transferring multiple, unregistered image volumes of hippocampal CA1 pyramidal neurons to our software suite results in the fully automated (i.e., without user intervention) reconstruction of accurate morphology, ready for simulation, in several minutes (see Fig. 1). An optional preprocessing step in which the raw input volumes were deconvolved to account for the distributed nature of laser excitation through real microscope objective lenses was found to be qualitatively and quantitatively unnecessary for the imaging systems used here. We used a frames-based adaptive hysteresis thresholding (UH-FAST; Santamaria-Pang et al., unpublished observations) algorithm that is optimized for the quality of data produced by our imaging systems and the conditions of our typical optical neurophysiology experiment. The UH-FAST algorithm reduces noise while preserving structural details (such as fine dendrites and spines) and structural integrity (see Fig. 1, “Denoising”). Our denoising procedure reduces the pixel intensities of background objects (i.e., non-dye-filled neuronal structures), thus increasing the effective signal-to-noise ratios (Santamaria-Pang et al., unpublished observations). Because
the confocal microscope data tended to be noisier, we observed the most improvement in denoising for data sets produced with that instrument. In contrast, it was possible for multiphoton data sets to be reconstructed without deconvolution and without denoising. Since dendrites of CA1 neurons are typically larger than the FOVs of either imaging system, the next processing step automatically aligned and merged multiple 3D data sets (see Fig. 1, “Registration”). The resulting single volume is then segmented, the pipette image is removed, and the morphology is reconstructed. This results in a single, connected tree with precise dendrite locations, lengths, and diameters (Fig. 1, “Reconstruction”). The data of this cell morphology are fed into a simulation engine (i.e., NEURON) for computational studies. Figure 2 illustrates that our software suite has not been optimized for one particular data set and that ORION performs well for neurons of the CA1 class and is robust for inputs with differing amounts of noise (especially those observed in data from our confocal system; see bottom row).

Qualitative comparison of ORION to other automatic and manual tracing methods

To assess the results produced by ORION, we selected a well-filled neuron that had been imaged with our multiphoton (MP) imaging system (i.e., seven partially overlapping volumes, 2,546 × 912 × 121 voxels). We compared (Fig. 3) the morphological reconstruction from our algorithm (ORION, designated “OR”) to another automated tracing program (AutoNeuron module of Neurolucida, designated “AN”) and to manual tracings performed by three human tracers (using the base version of Neurolucida, designated as “H1,” “H2,” and “H3,” respectively). Because AutoNeuron does not automatically register multiple volumes or remove the pipette artifact, a single, registered volume with the pipette removed was used as the input data set. Visual comparison of the entire neuron (Fig. 3, middle column) and selected subtrees of the same neuron (Fig. 3, red, green, and blue boxes) illustrate that all tracers (humans and computers) concur on the general shape, placement, and orientation of the dendrite. When compared with a maximum intensity projection of the raw data (i.e., one perspective of the input data), our software detected nearly all the same branches as did the other tracers, with minimal gaps, missing branches (filled arrowheads), or spurious segments (open arrowheads).

Finer comparison by closer inspection of the selected subtrees emphasized the good agreement of the different tracers. Each region contains generally the same segments in similar locations. Furthermore, each tracing well overlays the raw data maximum intensity projection (white background pixels) and each tracer was able to properly distinguish the closely spaced and crossing branches in the blue subregion. Tracer AN appears to report generally larger diameters (quantitatively demonstrated in the following text), whereas tracer H1 updated X–Y–Z diameter points less frequently, resulting in a blocky appearance of the dendrite. Our software suite, OR, reported the smallest diameters with tapered cylinders (Fig. 3, top row, green zoom-in), resulting in smooth segments that follow the tortuous dendrite. Furthermore, ORION and the human tracers were able to properly disregard spines for accurate diameter estimation (green zoom-ins). ORION’s results are therefore comparable in quality to other automated and manual methods.

Quantitative comparison of ORION to other automatic and manual tracing methods

To quantitatively compare the completeness and accuracy of our reconstruction method, we collected several metrics that included total dendritic length, surface area, and diameter. Figure 4 illustrates dendritic diameter measurements, for the subtree in the red box of Fig. 3, for each tracer versus the diameters determined by our software suite. Each data point represents a comparison of the diameter of a single segment reported by a tracer to the diameter of the segment reported by ORION which was geometrically closest in 3D space. The other computer tracer (AutoNeuron) consistently estimated...
diameters to be larger than those reported by ORION, as represented by the data points clustering above the slope = 1 dotted line (e.g., diameters reported by ORION as about 2 μm were reported as 3 μm by AutoNeuron). The human tracers did not systematically over- or underestimate diameters relative to ORION.

A quantitative comparison of the reconstruction results is presented in Table 1. ORION reported the second largest total...
dendritic length of 6,747 μm, only slightly shorter than the longest found by a human tracer at 7,150 μm, suggesting that ORION is more successful at detecting the full dendritic tree. On the other hand, whereas the other automated reconstruction method (AN) reported the shortest total dendritic length (5,698 μm), it yielded the largest total surface area (47,195 μm). This could be the result of a consistent overestimation of dendrite diameter. Indeed, the average dendrite diameter as reported by AN was the largest in the range of the tracers at 2.8 μm. AN also generated the largest minimum overall diameter (1.6 μm) and the largest maximum diameter (15 μm). ORION indicated the smallest average diameter (1.0 μm), with the minimum and maximum values falling in the middle of the tracers’ distribution. Highlighting the accuracy problems inherent in manual tracing by humans, two of the human tracers reported minimum diameters of 0.1 or 0.2 μm, below the theoretical optical resolution of the imaging system. Thus our software suite produces results that quantitatively fall inside the extremes of the results produced by other computer and human tracers.

To determine the accuracy of the different tracers, we analyzed the reconstructions of simple structures of known size: fluorescent dye-filled glass capillaries of 2- and 5-μm inner diameter (Table 2). We also investigated the effects of deconvolution on the accuracy of the reconstruction results. In general, the tracers reported more accurate results when using data sets that had not been deconvolved: the average error among the five tracers was 0.26 μm for the nondeconvolved 2-μm capillary data set—a size similar to that of a typical hippocampal CA1 thin dendrite—whereas it was 0.52 μm (underestimated) for the same data set that had been deconvolved. There was much smaller observable difference in the aggregate accuracy of the tracers when measuring the diameter of the 5-μm capillary deconvolved and nondeconvolved data sets (0.47 μm, likely because the relative error imposed by the PSF was smaller. Our automated reconstruction procedure reported diameter errors of 4.5 and 8.4% for the nondeconvolved 2- and 5-μm capillaries, respectively. These errors were among the smallest for all the tracers, human and computer, testifying to the accuracy of our algorithm.

### Quantitative comparison of simulation results

We assessed the functional consequences of variations in morphology reconstruction by comparing four different simulation results (i.e., input impedance, transfer impedance, current injection, and conductance change) among all tracers (Fig. 5). For the model generated by each tracer, the somatic input impedance was measured at 10^−9, 1, 10, 100, and 1,000 Hz (Fig. 5A). As expected, all five models exhibited increasing attenuation with increasing input frequencies. The model output by our reconstruction suite displayed the highest input resistance (at each frequency) of all the models, with approximately 35 MΩ at low frequencies. This is expected because this model had the smallest surface area and the longest total dendritic length, giving it a relatively long electrotonic length constant. The other computer tracer, AutoNeuron, generated a model that exhibited the lowest input resistance of the cohort, about 15 MΩ at low frequencies. The models generated by the human tracers were distributed between these extremes.

We also simulated measuring the dendritic transfer impedance from a distal apical branch tip to the soma in all five models (Fig. 5B). Again, all five models exhibited increasing attenuation with increasing input frequencies. The model obtained using our software suite displayed the second highest transfer impedance profile (>350 MΩ at low frequencies), with only one of the human tracers higher. Highlighting the high probability for errors when humans manually trace complex dendrites, this tracer recorded several branch narrowings with 0.2-μm diameters, leading to abnormally high transfer impedance for path from this branch to the soma. Thus subjective errors in tracing can severely influence the functional behavior of the resulting model.

### TABLE 1. Quantitative analysis for the morphological reconstruction

<table>
<thead>
<tr>
<th></th>
<th>Total Length, μm</th>
<th>Surface Area, μm²</th>
<th>Diameter, μm</th>
<th>Minimum</th>
<th>Maximum</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td>OR</td>
<td>6,747</td>
<td>20,199</td>
<td>1.0 ± 0.6</td>
<td>0.4</td>
<td>6.47</td>
</tr>
<tr>
<td>AN</td>
<td>5,698</td>
<td>47,195</td>
<td>2.8 ± 1.4</td>
<td>1.6</td>
<td>15.00</td>
</tr>
<tr>
<td>H1</td>
<td>6,327</td>
<td>39,956</td>
<td>2.2 ± 1.2</td>
<td>0.2</td>
<td>11.00</td>
</tr>
<tr>
<td>H2</td>
<td>6,806</td>
<td>42,371</td>
<td>2.3 ± 1.4</td>
<td>0.9</td>
<td>14.40</td>
</tr>
<tr>
<td>H3</td>
<td>7,150</td>
<td>31,461</td>
<td>1.1 ± 1.1</td>
<td>0.1</td>
<td>10.60</td>
</tr>
</tbody>
</table>

Diameter values are means ± SE. Length, surface area, and diameter statistics for the data set used in Figs. 1 and 3 from all tracers.
Further, we simulated a simple dendritic current injection step (500-pA amplitude, 100-ms duration) into the same distal branch tip and recorded the somatic voltage waveform as a function of time (Fig. 5C). This is similar to what electrophysiologists might do if they could establish a recording on a thin branch. The resistor–capacitor (RC) filtering of the intervening dendrite is apparent as the square waveform in response to this input varied from 0.05 to 0.15 mV, with concomitant increased RC filtering. Our model demonstrated a depolarization of about 0.1 mV, a median value.

**DISCUSSION**

Our software suite reconstructs neuronal morphologies from typical confocal or multiphoton image stacks in several minutes, with no human intervention. The entire computational pipeline includes optional (but typically unnecessary) deconvolution, denoising, segmentation, reconstruction, and conversion to file formats suitable for simulation. The generated results are qualitatively equivalent to currently available commercial, semiautomated reconstruction and computer-aided manual reconstruction in that the same overall branching structure is produced. Furthermore, quantitative metrics indicate that our algorithm detects more thin branches than the alternative tracers. It should be noted that our goal was to accurately measure dendrite lengths and diameters; augmenting our algorithms to explicitly account for spines could conceivably improve diameter accuracy. In addition, controls with objects of known size of the scale of thin dendrites suggest that our algorithm is among the more accurate at reporting diameters on the micron scale. Example simulations with models generated from the different tracers show the importance of objective, accurate dendrite representation.

Our motivation for developing this software suite—aiding the interpretation and guiding the execution of complex optical neurophysiology experiments—places unique constraints on the input data, the processing requirements, and the output format. The advanced optical instrumentation developed in our labs (Iyer et al. 2006; Reddy et al. 2008) permits sophisticated neurophysiological investigations that are not possible by any other current technique. Our rapid laser-scanning technology is able to position a laser beam on many user-defined sites with an aggregate bandwidth of 50 kHz. This enhanced imaging capability is accompanied by an increase in complexity of experimental design, execution, and interpretation. Therefore it becomes eminently useful to have accompanying theoretical data on the actual neurons (rather than generic models) that have been studied and—for the most complex experiments—that are being studied.

The automated reconstruction technique presented here offers several crucial advantages. ORION has been optimized specifically for dendrites, with no human intervention. The entire computational pipeline includes optional (but typically unnecessary) deconvolution, denoising, segmentation, reconstruction, and conversion to file formats suitable for simulation. The generated results are qualitatively equivalent to currently available commercial, semiautomated reconstruction and computer-aided manual reconstruction in that the same overall branching structure is produced. Furthermore, quantitative metrics indicate that our algorithm detects more thin branches than the alternative tracers. It should be noted that our goal was to accurately measure dendrite lengths and diameters; augmenting our algorithms to explicitly account for spines could conceivably improve diameter accuracy. In addition, controls with objects of known size of the scale of thin dendrites suggest that our algorithm is among the more accurate at reporting diameters on the micron scale. Example simulations with models generated from the different tracers show the importance of objective, accurate dendrite representation.

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Our motivation for developing this software suite—aiding the interpretation and guiding the execution of complex optical neurophysiology experiments—places unique constraints on the input data, the processing requirements, and the output format. The advanced optical instrumentation developed in our labs (Iyer et al. 2006; Reddy et al. 2008) permits sophisticated neurophysiological investigations that are not possible by any other current technique. Our rapid laser-scanning technology is able to position a laser beam on many user-defined sites with an aggregate bandwidth of 50 kHz. This enhanced imaging capability is accompanied by an increase in complexity of experimental design, execution, and interpretation. Therefore it becomes eminently useful to have accompanying theoretical data on the actual neurons (rather than generic models) that have been studied and—for the most complex experiments—that are being studied.
Automatically for the data as acquired in our typical optical physiology experiment without loss of generality (i.e., different neuron morphologies are possible), allowing us to quickly generate accurate model morphologies for any pyramidal neuron. The software accepts images of low axial resolution and possible weak and/or incomplete dye filling. The first feature is necessary to minimize full frame imaging for duration and irradiation purposes. The second aspect accommodates the limited dye diffusion times in deference to physiological procedures. It has to be taken into account that filling live neurons with fluorescent dyes normally results in inferior contrast compared with biocytin fills that require histological approaches (e.g., Stuart et al. 1999), leading to generally smaller values of total dendritic length for neurons. Although it is possible that given sufficient time for the fluorescent dye to diffuse and manually adjusting the detector gain to image the thinnest, distal dendrites, the total dendritic length would conform better to previously published values, which is beyond the scope of a typical electrophysiology experiment. A final advantage of ORION is that it is modular and thus easily extensible and adaptable. This makes it feasible to optimize the software suite to generate model morphologies for nonpyramidal neurons (although no modification was necessary for test cases of medium spiny neurons of the striatum). Furthermore, as advanced optical instruments are under continual development, the modifiable nature of ORION can adapt to expanded imaging capabilities (e.g., Gliko et al. 2006; Reddy et al. 2008).

In summary, ORION accomplishes a complete reconstruction on dye-filled portion of dendrites in several minutes, which is well within the typical time frame of an acute experiment. Since the CA1 pyramidal neuron arbor is larger than the field-of-view of microscopes equipped with high magnification objective lenses, our software has been augmented to automatically align and merge multiple image stacks. Furthermore, the software suite automatically detects and removes the imaging artifact of the patch pipette necessary to establish electrical recordings and introduce fluorescent dye. It should be noted that in this study, AutoNeuron and the human tracers were supplied with input data sets that were prealigned and merged into a single volume with the pipette removed. In a typical scenario not using our software, these otherwise time-consuming and demanding tasks would have to be done manually. Finally, the software suite is fully automated, requiring no user intervention. This automation provides maximum accuracy, consistency, and speed while accepting a wide range of input data, freeing the experimentalist to concentrate on the technical aspects of the experiment and the higher-level concepts and scientific principles being investigated.

References


