Detection and morphological quantification of dendritic spines from in vivo two-photon images

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INTRODUCTION

Despite significant advances in the development of automated image analysis algorithms devoted to the study of neuronal structures, current software tools offer only rudimentary capabilities for the analysis of dendritic spines. For example, they cannot reliably estimate spine volume and shape and typically require time-consuming and labor-intensive expert intervention. The problem is especially challenging in in-vivo imaging, where the difficulty of extracting morphometric properties of spines is compounded by lower image resolution, higher noise levels due to numerous labeled processes, and tissue motion due to blood circulation and respiration.

To address this challenge, we introduce a new computational framework for the automated extraction and quantitative analysis of dendritic spines from in vivo two-photon imaging. This framework includes: (i) a new algorithm for 3D image segmentation tailored to robustly extract dendritic tree features even under very low signal-to-noise ratio; (ii) a centerline algorithm that computes the central axis of dendritic branches; (iii) an algorithm that detects spine location with respect to the centerline trace and extracts the complete 3D structure of dendritic spines.

METHODS

Two-photon imaging: We implanted cranial windows in ketamine/xylazine-anesthetized adult GFP-M transgenic mice (age at surgery, 80–100 days), which express enhanced GFP under the flo1 promoter. The skull overlying the right visual cortex was removed and replaced with a coverglass window, leaving the dura intact. Animals recovered from surgery for at least 30 days before imaging. Tissue was fixed at the time of recording in 4% paraformaldehyde in PBS. Imaging was carried out at high resolution (1024 × 1024 pixels, 0.5 μm per pixel, 0.5 μm z step size). Image analysis: Specific Matlab routines have been developed to perform image segmentation [1], centerline tracing [2] and detection points located on all protrusions as well as the terminal points of the neurites.

RESULTS

To manually validate our algorithm for spine detection, we select non-overlapping boxes of fixed size containing a relatively small number of spines to ensure accuracy of the manual count. We first compute the 2D projection of the centerline along the z-direction and then randomly select points on the centerline and draw boxes of size 70 by 70 μm for these points to count the spines. We then sequentially discard overlapping boxes until no more of such boxes exist. By so doing, we obtain a total of 12 non-overlapping boxes.

Figure 4 shows the 2D projected view of the centerline and random selection of non-overlapping boxes with their centers on the centerlines of one of the segmented volumes.

Table 1 Statistical Analysis

<table>
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<tr>
<th>Data Set</th>
<th>No. of Sub-volumes</th>
<th>True Positive</th>
<th>False Positive</th>
<th>False Negative</th>
<th>Precision(%)</th>
<th>Recall(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>13</td>
<td>13</td>
<td>14</td>
<td>91.43</td>
<td>90.85</td>
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<tr>
<td>2</td>
<td>20</td>
<td>97</td>
<td>15</td>
<td>12</td>
<td>86.60</td>
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<td>3</td>
<td>11</td>
<td>94</td>
<td>5</td>
<td>6</td>
<td>95.35</td>
<td>91.18</td>
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</table>

Precision is defined as the ratio of the number of relevant records retrieved to the total number of irrelevant and relevant records retrieved. Recall is the ratio of the number of relevant records retrieved to the total number of relevant records in the ground truth database. The table shows that the performance of our method is very competitive since precision and recall percentages over the combined data are 91.76 and 91.55 respectively.

CONCLUSION

We have developed a multi-step automated procedure for locating points on all protrusions as well as the terminal points of neurites. This computational pipeline includes dedicated modules for automated image segmentation, automated tracing of the centerlines of dendritic and axonal branches, a routine which computes the points which lie on the spines, and finally an algorithm extracting the complete 3D structure of dendritic spines. This framework enables the computation of a wide range of in-situ features such as spine length, spatial distribution and spine volume in a high-throughput fashion. We illustrate our approach for the automated extraction of dendritic spine features in time-series multi-photon images of layer 5 cortico-excitatory neurons from the mouse visual cortex. Our computational framework facilitates the development of a scalable software platform that can rapidly and objectively process large-scale fluorescent images of complex neuronal networks.


Fig. 1 Volume segmentation

Fig. 2 Centerline tracing and endpoint detection

Fig. 3 Spine detection

Fig. 4 Sub-volume selection

Fig. 5 Spine volume extraction