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Automated Tracing of Horizontal Neuron Processes During Retinal Development

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Abstract

In the developing mammalian retina, horizontal neurons undergo a dramatic reorganization of their processes shortly after they migrate to their appropriate laminar position. This is an important process because it is now understood that the apical processes are important for establishing the regular mosaic of horizontal cells in the retina and proper reorganization during lamination is required for synaptogenesis with photoreceptors and bipolar neurons. However, this process is difficult to study because the analysis of horizontal neuron anatomy is labor intensive and time-consuming. In this paper, we present a computational method for automatically tracing the three-dimensional (3-D) dendritic structure of horizontal retinal neurons in two-photon laser scanning microscope (TPLSM) imagery. Our method is based on 3-D skeletonization and is thus able to preserve the complex structure of the dendritic arbor of these cells. We demonstrate the effectiveness of our approach by comparing our tracing results against two sets of semi-automated traces over a set of 10 horizontal neurons ranging in age from P1 to P5. We observe an average agreement level of 81% between our automated trace and the manual traces. This automated method will serve as an important starting point for further refinement and optimization.

Keywords

horizontal retinal neuron; retinal development; automated tracing; segmentation algorithm

INTRODUCTION

Lineage studies have clearly established that the 7 major classes of retinal cells are generated from multipotent progenitors in an evolutionarily conserved birth order¹. Ganglion cells, cone photoreceptors and horizontal cells are produced first; amacrine cells and rod photoreceptors are next; and bipolar cells and Müller glia are produced last^{1, 2}. It has been proposed that retinal progenitors undergo unidirectional changes in competence during development so that early-stage progenitor cells are competent to give rise only to early-born cell types and late-stage progenitors give rise only to late-born cell types³. Therefore, cell cycle exit must be precisely coordinated with these intrinsic changes in retinal progenitor cell competence during development^{4, 5}.

A major challenge in studying retinal development is discerning effects on cell fate specification from those on proliferation. Genetic perturbations that affect cell fate may have secondary effects on retinal progenitor cell proliferation. Similarly, changes in retinal progenitor cell proliferation affect the generation and thus proportions of different retinal cell types⁵. Only by studying these developmental events concurrently can we begin to identify the primary effect and distinguish it from the secondary effects. Downstream events of retinogenesis, such as differentiation, migration, and synaptogenesis are similarly interconnected. Defects on the proliferation of retinal progenitor cells may lead to subsequent changes in differentiation and synaptogenesis of late-born retinal cell types. For example, postmitotic differentiating amacrine cells born early during development secrete factors that affect the competence of proliferating retinal progenitor cells⁶. Changes in amacrine cell differentiation can therefore have profound secondary effects on retinal development.

Previous studies have demonstrated that Rb and its related family members (p107 and p130) can impact retinal progenitor cell proliferation, cell fate specification, differentiation, and synaptogenesis. Specifically, we observed that in P12 retinae, horizontal cell processes extend apically into the ONL and form ectopic synapses^{7, 8}. It is not known if the apical extension of horizontal neuron processes is a failure of these processes to properly reorganize during maturation from the apical to horizontal orientation or if it reflects post-maturation sprouting. To begin to study this question, we have generated *Gad67-GFP; Chx10-Cre;Rb^{Lox/Lox}* mice expressing GFP in developing horizontal neurons. Using retinae from different stages of development we can visualize the defects in horizontal cell maturation for the first time. However, a major obstacle is analysis of the morphology of individual horizontal neurons during development. Currently, only semi-automated algorithms are available for this analysis that require significant investment of time for each cell.

In response to recent developments in cellular imaging techniques, a significant amount of research in the image processing community has been devoted to automating the analysis of neuronal morphology in laser scanning microscopy images. The majority of this work is focused on automatic tracing of neurites in 2D and 3D images of single neurons^{9, 10, 11}, i.e., locating the centerlines of the axon and dendrites and reconstructing the branching structure. The images used in these studies typically only contain one visible cell, and thus there is no challenge to distinguish between neurites of different cells. Although recent literature claims good performance for state-of-the-art neuronal reconstruction algorithms on these types of images¹², publicly available implementations have not worked well on retinal neuron image stacks in which processes from nearby cells are intertwined with those from the cell of interest; thus, further research is needed to adapt existing techniques to this data. In this study, we describe a first-generation automated algorithm for identifying cells and tracing horizontal neurons in large populations of cells with defects in synaptogenesis. We present

quantitative results that demonstrate the effectiveness of our automated approach compared with manual and semi-automated tracing.

MATERIALS AND METHODS

Cellular Imaging

Retinae from *Chx10-Cre; Rb^{Lox/Lox}; GAD67-GFP* transgenic mice were isolated and flatmounted in glass coverslips. They were placed in a custom-made culture chamber on the stage of a two-photon laser scanning microscope (TPLSM) system. The entire depth of the retinae was scanned in 0.5 μ m z-step increments to achieve maximum resolution resulting in approximately 400 image files per scan. In each image with the 40x objective, 15-20 developing horizontal neurons were captured (e.g., Fig. 1). Sub-volumes roughly centered on individual cells were manually cropped from the original volumes. The large volumes were 512×512×300 voxels in size, and each sub-volume was roughly 150×150×300 voxels in size.

RESULTS

Manual/Semi-Automatic Tracing of Developing Horizontal Neurons

Manual or semi-automatic tracing of individual cropped neurons was performed using the Imaris software (Bitplane Inc). Initially, each cropped cell was visualized in the “Slice Mode” in order to estimate the maximum diameter of the soma and of the processes. These values were then used as “Start point” and “End point” on the filament tracer mode. Next, we ran the software’s automatic filament detection. However, it was frequent that “end-points” were recognized within the soma area or on the borders of the cropped image, leading to fake processes that were easily detected and manually deleted. An additional correction performed by users on the tracings initiated by Imaris software was to manually connect every primary process to a single start point within the soma. These procedures were independently performed by 2 human evaluators.

Development of a Segmentation Algorithm

We assume that the input to our algorithm is a 3-D TPLSM image stack. Representative images showing whole-field and segmented neuron TPLSM volumes acquired from GAD67-EGFP P4 mice are shown in Figs. 1 and 2. In the cropped neuron volumes, we typically expect to find only one soma in each cropped image; however, depending on the density of neurons in the region, portions of other somas may be present in the image along with some of their dendrites, and dendrites from different somas may appear to contact each other.

Our approach to tracing retinal neurons is divided into four stages: (1) soma detection and segmentation, (2) dendrite thresholding, (3) skeletonization, and (4) graph analysis. Segmenting the somas provides boundaries that are used in determining the starting points of the dendrites. The thresholding step yields a binary volume, which is fed to the skeletonization algorithm to reduce every structure to exactly one voxel in thickness. Once we have computed this skeletonized volume, we compute a dendritic graph from which various morphological features can be derived. These stages are explained in more detail below.

Soma Detection and Segmentation—Soma detection was not necessary for the individual cropped neurons due to the fact that each cropped volume was manually centered on the soma of interest; however, no such manual centering was done in the whole-field images. In order to automatically detect the multiple somas in these larger images, we first

performed a grayscale morphological erosion of the intensity volume using a 3-D spherical structuring element roughly equal in size to that of the average soma. We then computed the mean μ and standard deviation σ of the values in this resulting volume, and we applied a threshold equal to $\mu + 6\sigma$. The resulting binary volume was grouped into connected regions, and the centroid of each connected region was taken as a soma center.

Segmenting the somas is necessary for several reasons. First, it serves as a basis for determining which dendritic segments belong to the neuron of interest. Second, it enables us to determine important morphological characteristics of the dendritic tree, including branching order and angle of dendritic segments. In addition, finding the boundary of the soma enables us to pinpoint the beginning of the dendrites and thus more precisely measure their length.

In order to locate the boundaries of the somas of interest, we use each soma center as the seed point for a level-set segmentation algorithm based on the Chan-Vese energy¹³. This procedure results in a 3-D closed surface that represents the boundary of the soma. A representative soma segmentation result is shown in Fig. 3. The resulting segmentation is used as the boundary of each soma.

Thresholding the Dendritic Tree—After the somas have been located and segmented, we proceed to segment the dendritic tree. Since we use a skeletonization approach, we must first find an appropriate threshold in order to produce a binary image. Ideally, we want to find a threshold that perfectly captures the structure of the dendritic tree; however, this becomes a challenge due to several factors: low signal to noise ratio, dendritic structures that are small relative to the image resolution, gaps in fluorescence along dendrites, and the presence of clutter in the image. Too high a threshold will tend to split dendrites into disconnected segments, whereas too low a threshold might capture too much non-cellular material and cause separate dendrites to falsely connect.

The search for an optimal threshold was performed by employing two assumptions: (1) a threshold separates cellular material from background, and (2) the background intensity is relatively uniform apart from clutter objects and has a lower average intensity than that of cellular material. Therefore, if starting with a high threshold and gradually stepping down, a point at which the threshold value transitions from cell to background will be reached, and the object of interest will suddenly increase in size as large amounts of background are included with the object. This approach attempts to detect this volumetric explosion as the threshold is decreased. Then, by backing off slightly from the threshold at which the big increase in size occurred the final threshold is determined. This procedure is illustrated in Fig. 4.

To describe this procedure in more detail, we start with a threshold equal to the maximum intensity value within the soma of interest. We then decrease this threshold by 10% of its current value, apply the threshold to the image, identify the connected binary component that coincides with the soma of interest, and record the number of voxels in this component. We then compute the change in this number of segmented voxels compared to the previous threshold, and we calculate whether this change exceeds a predefined amount δ . If not, we decrease the threshold and repeat the process; if so, then we halt the process and go back a predefined number of threshold values N . In our experiments, we used $\delta=20000$ and $N=9$, which were determined empirically to work well on our datasets. Once we have found the appropriate threshold, we apply this threshold to produce a binary image.

3-D Skeletonization—Many 3-D binary skeletonization algorithms have been proposed in the literature¹⁴⁻¹⁶, each with its own strengths and weaknesses. Some approaches

guarantee structures of unit width, which typically comes at the expense of higher computational cost, whereas other approaches employ approximations and shortcuts to reduce computation time and produce a result that is “mostly” of unit width. The guarantee of unit width greatly simplifies subsequent analysis of the dendritic tree, since it is straightforward in such cases to identify branch points by examining connectivity. For this reason, we chose to use the iterative approach proposed by He et al.¹⁷. In each iteration of this approach, we first identify deletable points by determining whether each object point is a “simple point” (see definition in He et al.¹⁷), a non-tail point, and a member of the outermost layer of the object. The deletable points are then ordered by number of neighbors, and points with fewer neighbors are deleted first. The algorithm iterates until no deletable points remain. An example of the 3-D skeletonization process is shown in Fig. 5.

Ideally, we would like the final skeleton to coincide with the brightest part of the dendrite so as to follow the approximate centerline; however, because the skeletonization algorithm operates on a binary image, intensity information is ignored during the skeletonization process, and no such brightness properties can be guaranteed. For this reason, we modify the algorithm such that deletable points are ordered by the intensity value of the corresponding voxel in the grayscale image. Points are deleted in order from dimmest to brightest by means of a linked list, and each time a point is deleted, any of its neighbors that become deletable are properly inserted into the list. In this way, the final skeleton tends to more closely follow the brightest portion of the dendrites, resulting in a more accurate centerline.

Dendritic Network Analysis—Formation of a one-voxel-thin skeleton facilitates the computation of a graph representation of the dendritic structure. Our goal is to represent the neuron as a graph consisting of nodes and edges, where the edges correspond to dendritic segments and the nodes correspond to either the soma or the branching or end points of the dendrites. Finding such a representation allows us to easily compute topological features such as number of primary branches (i.e., those connected directly to the soma), branch length, and branch angle. The positions of these branches, given by the underlying skeleton, also enable the computation of morphological features such as branch thickness and taper rate.

We compute the dendritic graph as follows. First, we identify all “node points” in the binary skeleton, which are defined as skeleton voxels that have either more than two neighbors (branch points) or exactly one neighbor (end points). Node points are grouped into “nodes”, which are defined as connected groups of node points that include both the soma and the dendritic branch points. We then identify “segment points”, which are defined as skeleton voxels that have exactly two neighbors. Segment points are grouped into “segments”, which are defined as connected groups of segment points. By these definitions, a skeleton can be reduced to a system of segments and nodes, where segments are analogous to graph edges. Each segment will be connected to two nodes, one on either end. Note that it is possible to have a skeleton in which a pair of nodes is connected by more than one “edge”, and it is also possible that a segment could loop and connect to the same node at both ends; however, since we do not apply any formal graph analysis techniques here, such anomalies are not problematic.

In order to facilitate analysis of the dendritic graph, we assign a branch ordinal to each dendritic segment, e.g., primary, secondary, tertiary, etc. Primary segments are defined as segments that are connected to the soma node, secondary segments are defined as segments that are connected to primary segments, and so on. An example of a dendritic graph color-coded by branch order is shown in Fig. 6.

Extension to Whole-Field Image Analysis

In volumes containing multiple neurons, we may observe instances of two separate dendrites from the same soma or from two different somas coming into close proximity or contact with each other. Due to the limited resolution of image acquisition system, the skeletonization algorithm will often connect these dendrites; thus, it is necessary to determine a break point to separate these connected dendrites into two separate entities. Individual dendrites typically do not change direction sharply; however, when two separate dendrites are connected in the skeleton volume, there is often a sharp transition from dendrite to dendrite. In order to find the point of connection, we compute the instantaneous angle between neighboring segments of five voxels along the dendrite, starting at one soma and continuing to the other soma. The transition point appears where the highest change in the instantaneous angle occurs and exceeds a predefined threshold. We then break this combined dendrite into two separate dendrites at the closest node point to the transition point. In our algorithm, we used a threshold of 60 degrees.

Quantitative Results on Cropped Neurons

In order to evaluate the accuracy of our automated tracing approach, we assembled a test set consisting of ten cropped neuron volumes. These ten neurons were chosen from a larger set of cropped neuron volumes for their relatively high contrast, measured by comparing the average brightness of the soma to the mean and variance of the background values. The dendritic structure of each of the ten neurons was manually traced by two different neurobiologists using the Imaris software suite. Soma measurements were not compared in this study.

For each neuron, we compared our automated tracing result to each of the manual tracings separately (A-1 and A-2). We also performed an integrated comparison (A-3) in which the automated trace was evaluated against only those dendrites that were agreed upon by both neurobiologists. Finally, we compared the manual traces against each other (1-2) in order to gain a sense of the accuracy of the ground truth itself. Because artifacts from the manual tracing included trace lines that extended inside the soma, we only compared lines outside our detected soma boundary. For the individual comparisons, we performed a two-way evaluation: (1) *correctness*, i.e., the percentage of dendrites in the automated trace that were within a distance d of some dendrite in the manual trace; and (2) *completeness*, i.e., the percentage of dendrites in the manual trace that were within a distance d of some dendrite in the automated trace. For a given manual trace, we averaged the correctness and completeness values for each manual to yield a single agreement metric for each neuron.

For the integrated comparisons, we modified the above definitions slightly to reflect the level of agreement between manual traces. Thus, correctness becomes the percentage of dendrites in the automated trace that were within a distance d of some dendrite in *either* of the two manual traces, and completeness now uses the percentage of *agreed-upon* dendrites in the manual traces, i.e., those dendrites in the first manual trace that were within a distance d of some dendrite in the other manual trace (order is not important). We average these modified scores in the integrated comparisons to yield a single agreement metric for each neuron.

We present comparison data from individual neuron traces in Table 1. Four different agreement metrics are included in the table: (1) automated vs. manual trace #1 (A-1); (2) automated vs. manual trace #2 (A-2); (3) integrated comparison of automated vs. manual traces (A-3); and (4) manual #1 vs. manual trace #2. We present the averages completeness and correctness metrics over the dataset as well as the overall averages of these metrics in

Table 2. Computation time averaged roughly 30 seconds per neuron using unoptimized Matlab code running on a 2.8 GHz Intel Core2 processor.

In order to better understand the source of errors in our automated approach, we quantified the number of erroneous voxels with respect to dendritic branch order, i.e., primary, secondary, tertiary, and quaternary. A pie chart illustrating the distribution of false-detection errors over each type of dendrite is shown in Fig. 7(a). These errors are further broken down by individual cell in Fig. 7(b).

Overall, we evaluated our tracing algorithm on ten cropped horizontal neuron volumes of varying developmental age and compared our results to ground truth traces created by two different experts on the same volumes. The ten cells were selected based on having high measured contrast with respect to the background, which should allow for higher tracing accuracy. The agreement between the two sets of ground truth traces on these cells was only 85.9%, which suggests that even human visual recognition of dendrites in this imagery is a difficult and subjective task. Our automated traces achieved an agreement level reasonably close to this benchmark (79.5%).

Qualitative Results on Whole-Field Images

We demonstrate the applicability of our tracing algorithm to whole-field images by showing tracing results on two sets of images. The first set consists of two whole-field volumes of P6 horizontal cells collected as described in the Imaging Methods section above. No ground truth data was available for these images. Tracing results for these images are shown in Fig. 8.

The second set consists of two images of P2 horizontal neurons from the G42 transgenic mouse line collected and analyzed by Huckfeldt *et al.* in developmental tiling experiments¹⁸. Each volume was at least of size 1024×1024×72 voxels; however, in order to speed up computation time, we downsampled the volume in *x* and *y* by a factor of 4 using bicubic interpolation and anti-aliasing. Limited ground truth data for these images was made available in the form of manually colorized 2-D projection images. Each cell in a selected group of neurons was assigned a different color and colorized to mark the approximate extent of the dendrites belonging to that cell. Tracing results are shown in Fig. 9. These images serve not only to illustrate the applicability of our method to a different set of imaging conditions, but also to further validate the tracing algorithm.

DISCUSSION

Precisely coordinating the proliferation, cell fate specification, migration, differentiation, and synaptogenesis of neurons during retinogenesis is essential for formation of a functional visual system. However, after the retina forms, considerable cellular and synaptic reorganization can occur in response to neuronal stress or injury. One example is Müller glial cell reactive gliosis, which has secondary effects on retinal vasculature, neuronal survival, and synaptic connections^{5, 19}. Müller cells respond to neuronal stress, cell death, and neurotransmitter imbalances. Of particular relevance to OPL synaptogenesis is corrupt rewiring via the formation of complex neurite fascicles and the generation of new synaptic connections throughout the retina; this rewiring can result from retinal degeneration or other stress²⁰⁻²². These data suggest that retinal synapses are not static once formed but can rewire in response to a variety of stimuli. Therefore, it is important to determine if a synaptic defect in knockout mice is caused by the loss of the gene of interest during synaptogenesis or by a secondary effect of rewiring as a consequence of developmental defects or degeneration.

To distinguish between developmental defects and reorganization, one must study multiple stages of development. Although this approach is useful, the possibility remains that between developmental time points examined, changes might be overlooked. An easier way to distinguish between developmental defects and reorganization is to continually monitor the cells as they develop over several days. This is now feasible using 2-photon live imaging and transgenic mouse lines that express GFP in subsets of retinal neurons. In this study, we developed and tested a new algorithm to automatically trace and analyze large numbers of horizontal neurons.

It is of interest to note the integrated agreement metrics, which were 71.5% correctness and 87.0% completeness. In theory, we should aim for 100% agreement in both of these metrics, since they represent more lenient comparisons with respect to the ground truth; i.e., we would like to be able to detect those dendrites that are agreed upon by *all* human evaluators (completeness), and any dendrite we detect should have also been labeled by *at least one* human (correctness). While the completeness score is high, we note that correctness is somewhat lower than desired, indicating that our method, with the parameters used, tended to err on the side of false detections. We observed that many of these false detections were actually dendrites belonging to other nearby neurons. In addition, the vast majority of false detection errors occurred in tertiary or higher-order processes, which can be attributed to a combination of decreased fluorescence (due to smaller process diameter) and the detection of “foreign” dendrites.

In an attempt to improve our detection results, we implemented a strategy for pruning false dendrites from the automated trace. This strategy involved the analysis of four-way intersections in the skeleton in terms of branch angles. Wherever a four-way intersection occurred, we assumed that two dendrites had crossed; thus, we attempted to pair each of the four branches with its counterpart based on trajectory similarity and separate the two dendrites in the resulting network. We observed that while this strategy improved the results for some cells, it actually decreased the matching performance for other cells by several percentage points, resulting in a lower overall performance score (data not shown). Nonetheless, we believe that a more carefully planned and rigorous analysis approach involving both three-way and four-way intersections could result in improved performance in future work.

Given the large amount of variability between the two sets of human evaluations in this study, we expect that some dendritic segments present in the data may have been missed by both evaluators; if this is the case, then not all of the false detections made by the algorithm are necessarily false. Also, certain parameters of the algorithm, in particular the value of N (number of steps to regress after explosion detection), can be tuned to favor more or fewer detections if desired. Nonetheless, it is clear that more manual traces performed by independent evaluators on the same cells would help to more accurately quantify the ground truth and better assess the performance of the algorithm.

Many of the problems arising from false detections are diminished when our algorithm is applied to larger images containing multiple neurons. In order to illustrate this, we applied our algorithm to two sets of whole-field images of mouse retinae. In such scenarios, we would be able to compute aggregate statistical measurements such as dendrite angle and vertical extent over the entire field, where correct associations between dendrites and somas become less important. Nevertheless, comparison of our tracing results to colorized ground truth images of whole-field images shows that we can reasonably distinguish and separate neighboring cells in close proximity to each other.

Having observed that the accuracy of the automated tracing is comparable to manual tracing methods, our automated approach can be applied to larger datasets of horizontal neurons. In particular, computation of various features of the dendritic structure and the soma can be automated, including branch length, branch angle, dendrite classification (e.g., apical/basal/horizontal), planarity of the dendritic field, etc, leading to statistical comparisons between genotypes with respect to retinal development.

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Figure 1.

Representative figures of a whole-field image showing apical-basal (top-down) (A) or cross-sections (side) (B) views of P4 horizontal neurons containing both horizontal and apical-basal processes. Retina from GAD67-EGFP P4 mouse was dissected, flat-mounted, cultured and live imaged on a two-photon laser scanning microscope (TPLSM) system. Each field was scanned every 0.5 μm in the z direction. Image reconstructions were cropped to remove signal from amacrine cells. The voxel pitch is 0.38 μm in the x and y directions.

Figure 2. Representative figures of single horizontal neurons. Retina from GAD67-EGFP P4 mouse was dissected, flat-mounted, cultured and live imaged on a two-photon laser scanning microscope (TPLSM) system. (**A, B**) Two independent horizontal neurons were cropped from larger whole-field image shown in Figure 1. Dendrites not belonging to the cell of interest are visible in (**B**) (arrow).

Figure 3. Representative figures of soma segmentation by level-set approach: **(A)** Central region of original intensity volume; **(B)** Intensity volume with soma surface (white) overlaid.

Figure 4.

Determination of the dendritic tree threshold. **(A)** Illustration of thresholding approach for the neuron shown in Figure 2B. Progressive increments were used for the threshold adjustment. In each window, the primary connected component of the binary volume resulting from the threshold is shown in red. **(B)** Total thresholded area as a function of the iteration number. **(C)** Change in area δ_A between subsequent iterations. A threshold of $\delta_A=20,000$ voxels, shown as a red dotted line, was used in this segmentation.

Figure 5. Representative figure of 3-D skeletonization approach. (**A, B**) Binary volume before (**A**) and after (**B**) skeletonization procedure of the neuron shown in Figure 2B.

Figure 6. Dendrite ranking system. **(A)** Illustration of the neuron shown in Figure 2B with its original dendritic skeleton and segmented soma overlaid. **(B)** To better illustrate the detection of branching and the branch order, the same skeleton was color-coded according to branch order. Primary processes are shown in pink, secondary processes in yellow, tertiary in blue and quaternary and greater in purple.

Figure 7. Distribution of false detection errors as a function of branch order. **(A)** Pie chart of overall distribution of errors over the entire test set. **(B)** Histogram of errors broken down by individual cell.

Figure 8.

Representative examples of the application of the automated tracing algorithm to two whole-field image stacks. **(A-D)** A representative field of a P6 control retina ($Rb^{Lox/Lox}$) shows that, at this developmental stage, the processes of horizontal neurons are organized in a horizontal orientation as observed in an adult retina. **(E-H)** Horizontal neurons from *Rb-deficient* retina ($Chx10-Cre; Rb^{Lox/Lox}$) have processes that project in both apical and basal orientations. Max projections of *en face* and orthogonal views are shown in **A, C, E, G**; corresponding views of the resulting traces are shown in **B, D, F, H**. The trace of each cell is assigned a random color to show the separation of individual cells as determined by the algorithm.

Figure 9.

Application of the automated tracing algorithm to two P2 horizontal cell images (one per row) from Huckfeldt *et al.*²⁸ (A,D) Colorized max projection of each volume where colors correspond to individual cells and their dendrites. (B,E) 2-D projection of automated tracing results overlaid on each grayscale max projection with matching color scheme. (C,F) Alternate view of tracing results shows the presence of many apical and basal processes in these neurons not discernible in the 2-D overlays.

Table 1

Comparison of tracing results for ten individual horizontal neurons. The age of the cell, four different agreement metrics, and a visual comparison between traces are shown for each cell. In the visual comparison, the first three columns are individual traces (automated, manual 1, and manual 2); the fourth column is an overlay of the two manual traces illustrating where they overlap (white); and the fifth column is a “consensus trace” showing where any two of the three traces overlapped.

Cell #	Age	Agreement (%)				Top-down visual comparison (yellow = automated; cyan = manual trace 1; magenta = manual trace 2)
		A-1	A-2	A-3	I-2	
1	P4	83	71	84	85
2	P1	77	71	81	85	
3	P3	62	70	72	83	
4	P3	70	68	72	88	
5	P5	90	87	91	94	
6	P2	85	81	86	93	
7	P4	96	94	97	93	
8	P5	66	63	70	84	
9	P4	62	71	78	74	
10	P4	59	57	64	80	

Table 2

Agreement metrics averaged over ten neurons.

Comparison metric	A-1	A-2	A-3	1-2
Correctness (%)	64.5	67.8	71.5	N/A
Completeness (%)	85.5	78.6	87.0	N/A
Average (%)	75.0	73.2	79.5	85.9