Curvilinear Feature Extraction From Stacks of Neuron Images

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ABSTRACT

A new approach is proposed for extracting explicit representations of 3-D curvilinear features from stacks of 2-D images. The images, which are of brain tissue, were obtained by confocal microscopy and the features represent the dendritic tree structure surrounding a neuron.

Voxels with a high probability of being on the centre-lines of the dendrites are identified first. Then a combination of a 3-D minimum spanning tree and a 3-D minimum cost path algorithm is used to automatically extract explicit centre-line representations of the curvilinear features. The final objective of the image analysis is to produce, as automatically as possible, generalised cylinder models of the dendritic structures which are then used for studying neuronal morphology and function. In this paper we concentrate on the algorithms used to extract the centre-line representation.

Keywords: curvilinear network extraction, Euclidean distance transform, 3-D thinning algorithm, 3-D minimum spanning tree, 3-D minimum cost path.

1. INTRODUCTION

In this paper we are concerned with the analysis of micrographs of brain tissue taken using a laser scanning confocal microscope. The micrographs show images of the dendritic tree surrounding a neuron at different levels through the tissue. An example of a single image in the stack is shown in Fig. 1(a). The objective is to extract an explicit representation of the tree structure as a generalised cylinder model in order to study both neuronal function and neuronal morphology.[1] Manual digitisation of the paths of the dendrites in each of the 2-D images, followed by a manual correlation between successive layers in order to extract the 3-D structure of the dendritic tree, is particularly labour intensive. Here we are concerned with the development of image analysis techniques which can accelerate the process of 3-D feature extraction and reconstruction.

Research on three dimensional stacks of images has attracted increasing interest in recent years as collection techniques have evolved. Software packages are now available which provide many sophisticated image processing and visualisation capabilities for 3-D image stacks. It is important to emphasise that we are not primarily concerned with visualisation. The aim is to extract a continuous and explicit model which can be used for analysing the electro-chemical properties of the dendritic structure.

There is a substantial body of work on the extraction of explicit representations of curvilinear features from single 2-D images. Techniques for the extraction of explicit representations of 3-D structures from 3-D image stacks are less well developed and it is on this problem that we concentrate.

The complete process involves the extraction of a centre-line representation of the dendritic tree followed by a process to build a generalised cylinder representation around the centre-lines. In this paper we will concentrate on the algorithms for the extraction of the centre-line representation.

The structure of the paper is as follows: Section 2 gives a brief examination of related work. Section 3 contains an overview of our approach to the extraction of 3-D curvilinear features and Section 4 gives details of the algorithms for extracting the centre-line representation. In Section 5 we present some preliminary results and finally, in Section 6, conclusions and a brief look at future work are presented.

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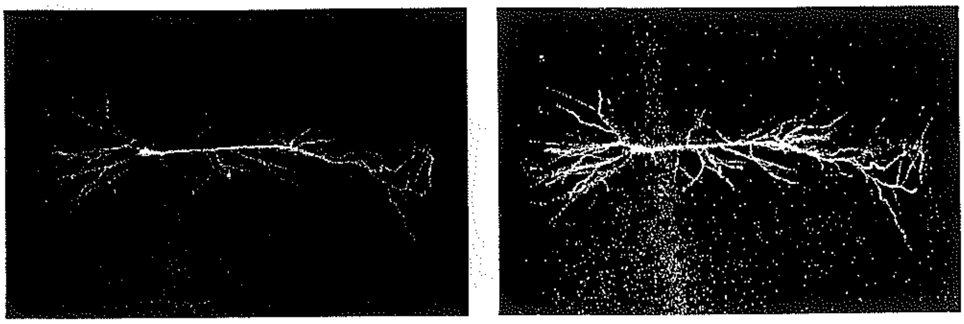

(a) The raw image (b) A contrast stretch of (a)

Figure 1. The seventh image in the stack with size 768x512

2. RELATED WORK

Curvilinear features are one of the most common feature types found in digital images. Whether the image is of a man made or natural scene, many objects appear as curvilinear features. Arteries in medical images, road and river networks in remotely sensed images, characters and other line work in engineering drawings are all examples of curvilinear features.

A range of techniques have been proposed for tackling 2-D curvilinear extraction. Many of the approaches may be classified as either finders or trackers. Finders are essentially operators which give a response at a point in the image which is related to the likelihood that the point is a desired feature point. Such approaches typically require an additional stage to link feature points together to provide a connected representation of the complete feature. Trackers find (or are given) a start point on the feature and then track along the feature using some of its properties to keep it on the correct path. Another approach, and one which is increasingly popular for a wide range of feature recognition tasks, is to use a cost function to represent some property of the feature so that the lower the cost at a point, the more likely it is to be on the feature. Feature extraction then becomes a problem of cost minimisation.

Extraction and reconstruction of 3-D curvilinear structures from image stacks is less well established than for 2-D images and has evolved from totally manual methods involving labour intensive measurement and analysis, through a range of semi-automatic techniques requiring varying amounts of operator activity and software/hardware interaction. Turner et al[1] give an interesting review of approaches to the 3-D reconstruction and analysis of neuron images and Stockley et al[2] describe a semi-automatic approach to 3-D neuron reconstruction. The system involves a manual digitisation stage but the final model can be visualised in 3-D and provides morphological data for passive electrical simulations.

Gerig et al.[3] have proposed a prototype system that extracts 3-D curvilinear structures from volume image data and transforms them into a symbolic description which represents topological and geometrical features of tree-like objects. Their application is the characterisation of the cerebral vascular system. The approach begins with an initial segmentation obtained by 3-D hysteresis thresholding leading to the identification of seed regions which have a high chance of belonging to the structure of interest. Voxels with intensities between a lower and upper threshold are only selected if they are connected to a seed region. A skeletal structure is derived by 3-D binary thinning, approximating the centre-lines while fully preserving the 3-D topology. The thinning algorithm is a pseudo-parallel algorithm proposed by Tsao and Fu.[7] An estimation of object width is calculated by a separate 3-D Euclidean distance transform and a raster-to-vector transformation converts the maximally thinned voxel lists into a vector description.

Based on Gerig et al.'s symbolic description of the 3-D structure of blood vessels, Kawata et al.[8] have proposed another prototype system that provides the anatomical information of blood vessels using cone-beam CT. Their method first extracts a segmentation of blood vessels by simple thresholding of the 3-D image. Then centre-line structures are extracted by using a thinning algorithm for a 3-D binary image. A distance transformation is used to obtain the radius at the location of the centre-line. Finally, a graphic description of the blood vessels is obtained.

Although the systems described above are used to extract 3-D structures from blood vessel images, they can in principle be applied to any vascular component images. However the images used were MR and CT data and visualisation techniques for MR/CT data are well established.[4] CT and MR volumes are easily segmented into regions that correspond to different substances such as air, soft tissue and bone. In live tissue confocal microscopy the structures encountered have similar absorption and reflectance characteristics as most cells are essentially translucent. This makes automatic identification of boundaries and structures much more difficult. Also, the fine scale of the structure detail is smaller than the wavelength of light and hence beyond the resolution limit.

Pudney et al.[5] have proposed a method for 3-D confocal microscope images. They use a local energy technique to detect the surfaces of features in the volume data. The local energy is computed by Wavelets. The paper shows that 3-D local energy is an available means of detecting surfaces in confocal microscope data. More recently, a new method has been proposed for restoration of 3-D quasi-binary images from confocal microscopy by Herzog et al..[6] The method has been applied to the extraction of dendritic trees in neuron images.

In this paper, the method we have developed to obtain the skeleton of neurons from a stack of neuron images has some similarity to the approach of Gerig et al but also uses a cost minimisation technique based on local image properties.

3. OVERVIEW OF THE METHOD

In the previous section, the use of cost minimisation techniques was mentioned. An example of the application of this approach to the extraction of roads and rivers from remotely sensed images was given by Dobie and Lewis.[9] In their paper, a method of using a minimum cost path (MCP) technique to delineate a path between specific points on a required feature was presented. The cost function may be based on image properties and path curvature. The MCP algorithm is based on an extension to D'Esopo's method.[14] Although the method is optimal, it has the disadvantage that it only extracts a single path, specified by its end points.

More recently Dobie et al[10] described an extension to their method which extracts networks of curvilinear features from 2-D images. The approach identifies points in the image with a high probability of being on the network and extracts the broad topology of the feature using a minimum spanning tree(MST) to link the points into a tree. The MST algorithm treats the high probability points as nodes in a graph and finds the graph which minimises the total cost of the arcs whilst simultaneously passing through each of the nodes. The costs used to construct the spanning tree are directly related to the linear distance between points considered. Finally, a minimum cost path algorithm is used between points on the tree to refine the linear sections of the network so that they follow the path of the underlying feature more closely. In this stage the costs are related to image features and may include a path curvature term. The method assumes the correct topology of the tree is obtained by applying the MST alone. The MCP simply refines the path between nodes. To use such costs when constructing the MST, rather than linear distance, would be more accurate but computationally very expensive.

In this paper we describe extensions and modifications to this approach in order to tackle the problem of extracting curvilinear tree-like structures from 3-D image stacks. The method combines the MST and MCP algorithms more closely so that the topology extraction makes some use of the MCP calculations.

The new approach may be summarised as a series of stages. In the first, the aim is to identify voxels in the image stack which have a high probability of being on the centre-lines of the curvilinear features. This is achieved by reducing the image noise and exposing the dendritic structure using a simple threshold applied to the image stack. A 3-D thinning algorithm is then used to produce the set of high probability centre-line voxels.

In the next stage the high probability voxels are linked to form the 3-D skeleton of the dendritic structure using a combination of a 3-D MST algorithm and a 3-D MCP algorithm. The high probability points are linked into the spanning tree, initially on the basis of Euclidean distance, but this is refined within sub-volumes of the stack by calculating the minimum cost path between high probability points. The cost calculation is based on path curvature and image intensity information from the original image stack.

In the third stage of the process the skeleton of the dendritic tree structure is used as the starting point for the estimation of cylinder model parameters. Searching out from the skeleton for the boundaries of the dendrites, the algorithm is able to approximate the dendrites by cylinders with linearly changing radii so that each section of dendrite may be represented by the centre-line coordinates and the radius for each end of the cylinders.

In this paper we will concentrate on the algorithms developed to extract the centre-line representation of the dendritic tree. Details of the final stage will be covered in a future paper.

4. DETAILS OF THE FEATURE EXTRACTION STAGES

4.1. High Probability Voxel Detection

The first stage of the algorithm involves the indentification of voxels which have a high probability of being on or near the central axes of dendrites. An important requirement at this stage is that sufficient points are found over the dendritic structure to be able to capture the full skeleton of the dendritic tree in the second stage of the algorithm.

There are several possible approaches to finding the high probability points. Although the dendrites appear as bright pixels in each image, simple thresholding is inadequate as there is a substantial amount of noise. This can be seen in Fig. 1(b) where a contrast stretch of the image in Fig. 1(a) is shown. One approach would be to use mathematical morphology, opening the stack with a set of 3-D cylindrical structuring elements. However, the use of a simple threshold followed by isolated point removal and the application of a 3-D thinning algorithm, to retain only the significant centre-line points, was found to provide an appropriate set of high probability voxels with which to enter the second stage.

The aim of the 3-D thinning algorithm is to reduce clusters of voxels, identified in the feature, to just those on the centre-line. This is achieved by eroding voxels from the boundary of the feature inwards and demands the following three requirements:

1. Preservation of the feature topology
2. Preservation of the centre-line
3. One voxel width output

In order to achieve these requirements we developed a 3-D thinning algorithm which is a combination of Toriwaki and Yokoi's(T-Y) skeletonisation algorithm[11] and a fast Euclidean distance transform.[12] Since T-Y's algorithm deletes voxels sequentially and breaks requirement 2 above, the output from the Euclidean distance transform is used to support the process of centre-line identification. The value in the distance transform data set represents the Euclidean distance from the feature edge.

The T-Y algorithm is performed by deleting voxels one by one. Voxels in an object are divided into two groups: preserved voxels and unpreserved voxels. The unpreserved voxels may be deleted, but this is not mandatory. The preserved voxels are defined as those voxels which satisfy preservation conditions. If a voxel can be deleted, the topological properties should not be changed. Many papers discuss the topological properties associated with thinning. In fact, T-Y's paper[11] derives necessary and sufficient conditions to delete a 1-voxel.

As the sequential deletion depends on the scan mode, results could be different if the scan mode has been changed. This results in the possibility that the skeleton is not located on the centre-line of the object. Our new algorithm is designed to avoid voxels on the centre-line being deleted. The extra condition is added to preserve those voxels which are possibly on the local centre-lines. This is achieved by applying the fast Euclidean distance transform[12] to the binary data set and using the resulting distance from edge data to restrict deletions.

If all the centre-line points could be identified correctly in this first stage of the algorithm, the second stage would be unnecessary and the task would be greatly simplified. However, image noise and other artifacts of the image capture process result in discontinuities in the features. These are overcome by the employment of the linking process using a minimum spanning tree and minimum cost path algorithm in the second stage of the process.

4.2. Extracting the 3-D Curvilinear Centre-line Structure

In the second stage the aim is to link all the high probability voxels in the stack using a 3-D minimum spanning tree(MST) algorithm. This connects all the high probability points together in a way which minimises the total length of the tree. In the first instance, the criterion for linking is the Euclidean distance, but as each new point is connected, a sub-volume of the image stack is considered around the point on the tree and the point being added. Other unconnected points are considered in the sub-volume and the most appropriate connection is made based on the minimum cost path(MCP) calculation using a 3-D implementation of a modified d'Esopo algorithm.[13,14,9] This links the points into the tree structure by a curvilinear path rather than the straight links of the MST. A pseudo-code representation of the algorithm is shown in Fig. 3.

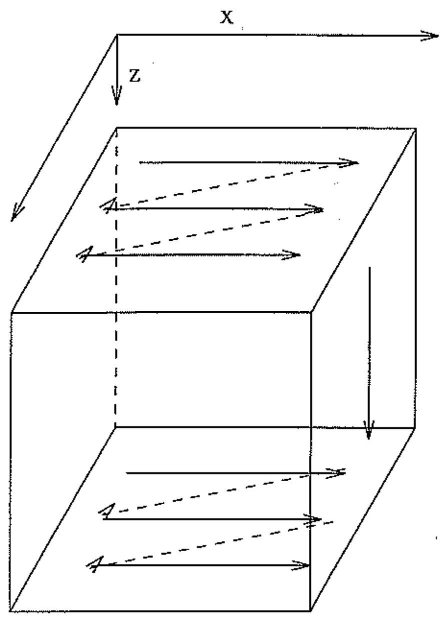

Figure 2. Scanning mode in 3-D

The cost function being minimised includes a term associated with the inverse of the voxel brightness since the dendrites are bright voxels against a dark background. A path curvature term is also included to favour straighter paths over those which are rapidly curving.

The function to be minimised may be expressed as follows:

$$-\sum_{\underline{x}\in S} I_{\underline{x}} + k\sum_{\underline{x}\in S} C_{\underline{x}}$$

where $I_{\underline{x}}$ is the image intensity at voxel position $\underline{x}$, S is the set of voxels along the path, $C_{\underline{x}}$ is an estimation of the curvature of the path at voxel position $\underline{x}$ and k is a constant which determines the relative strength of the term.

In this stage, two stacks of images are needed. One is the stack of images containing only high probability points obtained by the first stage. It is used as a guide for looking for points on the dendritic tree to be linked via the MST/MCP. The other is the stack of raw images which is used for extracting the detailed paths of the curvilinear features using the MCP algorithm.

The MST/MCP algorithm in 3-D

The set of high probability voxels, obtained in stage one, are divided into two groups. Those which have been spanned are called tree points, those not yet spanned are called non-tree points. The aim of the 3-D MST algorithm is to transfer all points from outside the tree to inside the tree.

Although the correct connectivity is often achieved by choosing the next point to add in on the basis of Euclidean distance, this is not always the case. To minimise the likelihood of error, other points outside the tree around the nearest point are also considered and selected on the basis of the minimum cost path. The combined MST/MCP algorithm is summarised by the following steps.

1. The start point for the algorithm is a high probability point which is the one first met by a specific scan mode (see Fig. 2) in the stack of high probability images. The start point is added to the tree.

```
for each node
        node.cost      = infinity
        node.direction = undefine
start_node.cost = 0

initialise(path_info)

append_to_queue(start_node)

while queue not empty
        node = remove_head_from_queue()

for each neighbour of node
        update(path_info,neighbour)
        new_cost =
node.cost+cost_from(neighbour,node,path_info)

if new_cost<neighbour.cost
                neighbour.cost      = new_cost
                neighbour.direction = dir_from(node)

        if neighbour has never been in queue
                append_to_queue(neighbour)

        if neighbour is not currently in queue
                prepen_to_queue(neighbour)
```

Figure 3. The extended d'Esopo algorithm

2. A search is made outwards from the starting point by increasing one voxel each time, until the nearest point outside the tree is found. There may be several possible points which are the same distance from the starting point and also other points which are almost the same distance away as the nearest in terms of Euclidean distance.
3. The candidate point to link in to the network must be chosen from among these points.
4. If L is the distance from the start point to its nearest neighbour all other points within a distance $F * L$ of the start point are considered, where F is some factor greater than 1. In our case we have used $F = 1.2$.
5. We now have one start point (A) and several candidate points. The next step is to extract a sub-volume of the stack of raw images containing these points and then choose the point to be added as that with the minimum cost path from the start point using the MCP algorithm on the raw data set.
6. The start point (A) and the chosen end point (B) of the MCP are then added to the tree.
7. The new end point (B) is taken as the next start point in order to establish if there are more points which may be added to the tree. At this stage the search for further points which may be added to the current branch is limited to a distance around B equal to L, the distance from A to B. This process is repeated until no further points can be added to the current branch within the allowed search distances.
8. The algorithm then searches around all nodes on the tree for the nearest points to the tree on the basis of Euclidean distance. The overall nearest point is chosen for consideration. Once again, a sub-volume is defined in which to apply the MCP process to choose the best point to add in.
9. The new branch is then grown as above and the process repeated until all points have been added into the tree.

Computational complexity

If the number of tree points is n, then at least n comparisons with neighbouring points are required. In addition, for each tree point, the algorithm involves d times searching non-tree points, where d represents the distance in voxels, starting at 1 voxel away. The distance d is different for each tree point, so for each individual network, the whole computation times is $\sum_{i=1}^{n} d_i$. The computational complexity of the MST algorithm depends on both d and n. Our current MST algorithm is made significantly faster by avoiding repeated searching and comparison. It starts at a specific tree point with the minimum searching distance set to the distance at which the search around that point previously stopped, rather than searching from distance 1 to d and performing this for each tree point.

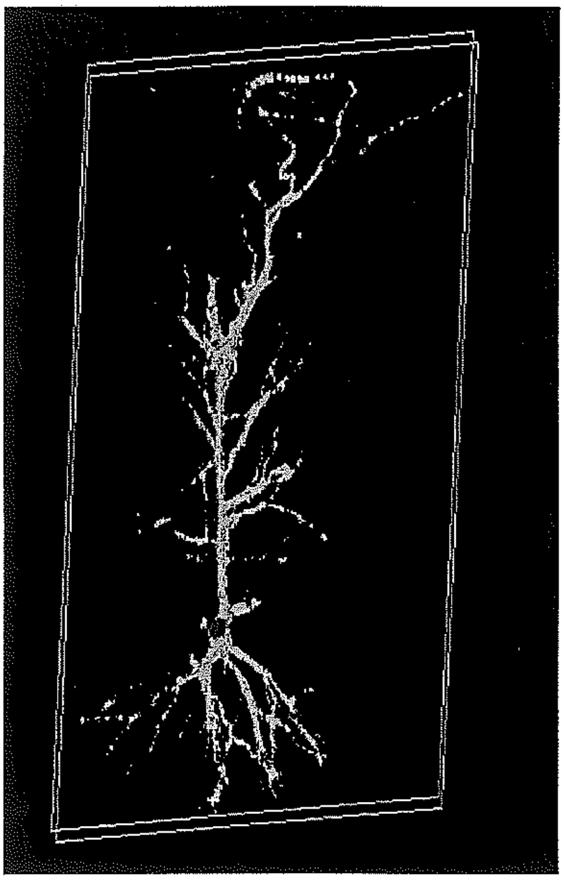

Figure 4. A binary stack with size 655x271x15, threshold value is 57

5. PRELIMINARY RESULTS

The centre-line extraction process has been tested using synthetic image stacks representing various 3-D tree like structures and was found to deliver accurate centre-line representations. The application to a real dendritic tree stack has also been tested. The original stack was 768x512x15 voxels but a sub-stack was used which was large enough to include the required tree. The dimensions of the sub-stack were 677x271x15. A visualisation of the stack using AVS after the initial threshold has been applied is shown in Fig. 4. Evidence of the fragmentation problem can be seen quite clearly.

The result of high probability voxel detection is shown in Fig. 5. The binary stack of Fig. 4 has been thinned and small isolated voxel clusters (typically clusters with fewer than four voxels) have been removed.

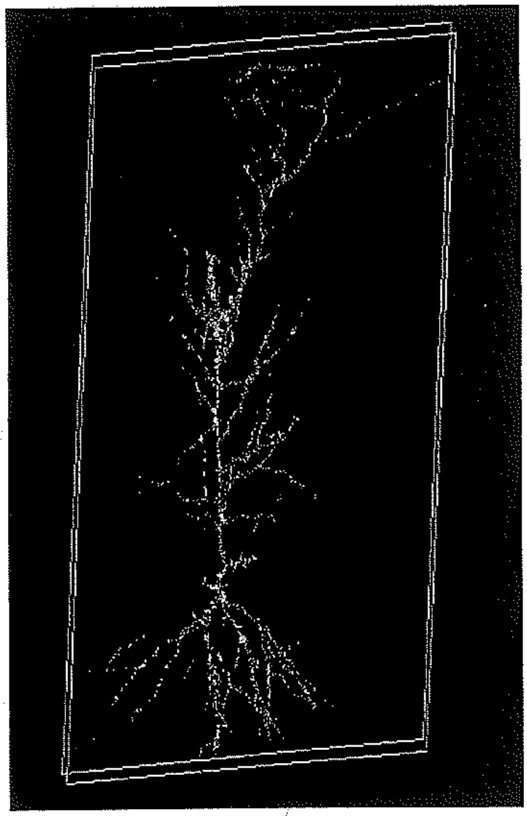

Figure 5. High probability voxels obtained voxel detection

Finally, the centre-line tree structure has been obtained by applying the combined MST/MCP algorithm using the high probability voxels and the original image stack as the starting point. The 3-D tree structure representing the centre-lines of the dendrites has been displayed using the geometry viewer in AVS (see Fig. 6). Visual inspection and comparison with the individual images in the raw image stack reveals that a useful representation has been obtained without any manual intervention. Although the representation is not perfect in every detail, the broad structure and complexity of the dendritic tree is well modeled and provides a valuable starting point either for an interactive edit to refine the model or for the extraction of the generalised cylinder representation in the final stage of the image analysis process.

For the stack illustrated above, the whole procedure to the end of the centre-line extraction stage takes about 60 minutes on an SGI O_2 workstation with a 174 MHz processor and 128 Mbytes of main memory.

6. CONCLUSION AND FUTURE WORK

An automatic method has been implemented for extracting curvilinear features from stacks of neuron images and representing them as an explicit 3-D centre-line model. As the boundaries of some of the image features have almost the same intensity as noise caused by the brain tissue, it is difficult to obtain continuous curvilinear centre-line representations of one voxel width by using a simple threshold and 3-D thinning algorithm. Therefore, further image processing techniques are needed to extract the underlying features using both the raw image data and the result of the 3-D thinning algorithm. This method overcomes the discontinuity problem due to noise and the unreliability of 3-D thinning. It provides a useful tool for feature extraction from large image data sets in 3-D. Although it has

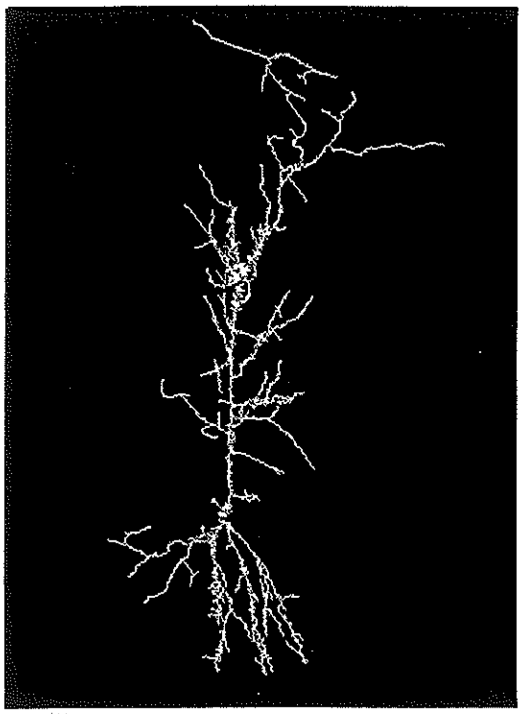

Figure 6. Curvilinear features extracted from the stack of Raw Images

been applied to confocal microscope image data, it could also be applied to other 3-D curvilinear feature extraction problems.

One of the main advantages of this method over earlier approaches is that it avoids fragmentation of the dendritic tree due to noise through the combined use of the minimum spanning tree and minimum cost path algorithms. A continuous and explicit model of the 3-D curvilinear features is obtained.

To build a 3-D generalised cylinder model of the neuron, the centre-line representation is used as a starting point for the final stage. Work on this procedure is still in progress and will be described in a later paper.

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