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# Local tracing of curvilinear structures in volumetric color images: Application to the Brainbow analysis

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In this study, we compare two vectorial tracing methods for 3D color images: (i) a conventional piecewise linear generalized cylinder algorithm that uses color and edge information and (ii) a principal curve tracing algorithm that uses the gradient and Hessian of a given density estimate. We tested the algorithms on synthetic and Brainbow dataset to show the effectiveness of the proposed algorithms. Results indicate that the proposed methods can successfully trace multiple axons in dense neighborhoods.



## 1. Introduction

It is impossible to throughly understand the function of a complex system without understanding its basic elements. For that reason morphology (geometry and topology) of neurons is of broad interest, in order to comprehend the function and connectivity of neurons as well as to detect miswiring as may occur in Alzheimer's and Parkinson's diseases [25,19]. Various imaging techniques have been utilized to uncover morphology. Resolution limitations in optical imaging, problems related to sample preparation and most dominantly, the diversity and the complexity of neuronal arbors are some of the major challenges in neuroinformatics. With recent improvements in 3D imagery techniques, high volumes of data are available as image stacks, but obtained images are not always discriminative and conclusive. Hence, they require tedious and, most of the time, impractical manual processing. Therefore, reliable and efficient techniques are needed to process these data with minimum initialization/parametrization and easy user intervention.

Significant research effort has been dedicated to the segmentation and analysis of curvilinear objects that arise in various contexts (e.g. vascular networks, bronchia) [44,6,38,15,55]. In general, segmentation is the first step to outline an object. Further analysis is needed to extract the topology information from the segmented image stack, which ranges from unsupervised skeletonization techniques to model based approaches. A recent literature review for the analysis of linear branched structures can be found easily [34]. However, it is safe to claim that all tree extraction methods in the literature seek the underlying ridge (trace) of the curvilinear structure.

Definition of a ridge has been studied in various contexts. In statistical signal processing, ridges of functions or data clouds have been studied under the concept of principal curves [24,26,40]. In images conditions for pixels being on the ridges are also discussed [18,37]. Although these previous works defined local conditions for ridges or samples from the principal curves, they do not answer the connectivity of such samples in space [18,37,40].

Earlier attempts to uncover the connectivity of samples from ridges used the topological skeletonization of data. The skeleton of an object is obtained by removing the exterior pixels/voxels to obtain the underlying geometry (centerline) of the structure [13,29,41]. These morphological methods applied to binary data where segmented images are already provided beforehand, are very sensitive to noise level, resulting in disconnections in the skeleton.

Another set of algorithms employ active contour models or level-sets. These techniques optimize some suitable energy function that combines appearance and geometry terms through scalarization (e.g. linear combination). Unlike unsupervised clustering based approaches, prior shape models can also be incorporated into the

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optimization, which can be solved by efficient graph path search algorithms, such as Dijkstra's [17,45,50,12]. Shape priors are incorporated to the optimized energy function [39,36,38,45,10,52,55] to highlight the object boundaries. These approaches are shown to work well in a variety of scenarios for segmentation with careful tuning. However, tuning operation requires a detailed level of topological and structural understanding of the dataset in order to define energy functions that will succeed.

In conjunction with the contour models, multi-scale enhancement methods have also been employed to highlight certain curvilinear structures [44,21,17,47,35,53,56,43,22]. Eigenanalysis of the Hessian matrix of the image intensity is a popular approach to identify objects (e.g. vessels), where eigenvalue ratios can be related to local curvature.

In general, a major challenge in graph construction is parameter optimization. It is possible to end up with global solutions that are infeasible due to the selection of parameters or initializations, and tuning of these parameters get harder as the dimension of data increases. For that reason, most of the proposed 3D methods seek 3D associations between 2D image segmentation results [52,45,10,11,54]. However, such approaches increase the complexity by creating problems not present in 3D (e.g., occlusions), and try to address them with arbitrary heuristics.

In another track of algorithms, local 3D approaches construct a local graph representation for the data to simplify the search space without introducing complexity. For large datasets, where search space increases exponentially with the data dimension, these methods provide fast and efficient locally optimum solutions for the tracing problem [2,10,52,11,54]. Algorithms in this category generally start from a given seed point with an initial direction vector. Optimality measures are similar to the ones that are defined for the global solutions, but they process only the proximity of the points in the feature space. Therefore they are also called "exploratory algorithms" which provide fast solutions for the optimum trace/minimum path problem. Generalized shape models can be classified in this category, where tubular objects are modeled as piecewise-linear cylinders with varying radius [27,21,2,1,15,7]. These methods locally fit the shape model to the wall of the tubular objects. Usually the shape is assumed to be a cylinder, but it can be more complex (superellipsoid) depending on the available computational power [49] or simpler (sphere) depending on the application [30].

Brainbow images, as shown in Fig. 1, are obtained by a recently developed technique for acquiring 3D colored confocal microscopy imagery that depict neuronal networks in the central nervous system [32,23]. They present a great opportunity for neuroscientists to study brain structure and function. By staining individual neurons using a combination of fluorescent proteins, each neuron can potentially be labeled with a distinct color (a maximum of approximately 90). However, given the complexity and the density of arboring structures, as well as due to the randomness in the staining process and protein expression, selectivity on the color

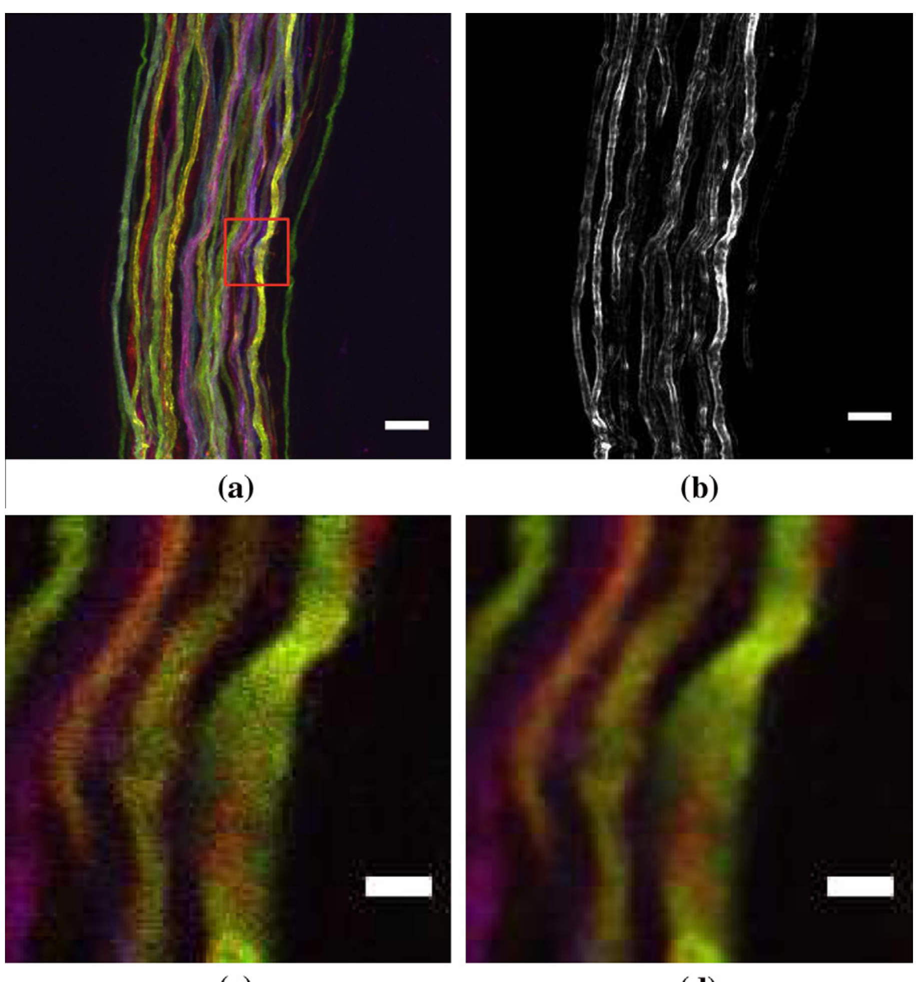


**Fig. 1.** (a) MIP of Brainbow image stack showing a bundle of axons of motor neurons. (b) MIP of estimated edge map of the image stack. (c) Zoomed single slice view of the region depicted with red box in (a). (d) Same region after 3D bilateral denoising. Scale bars are 100 pixels in (a and b) and 20 pixels in (c and d) respectively.