Analysis, Reconstruction and Visualization of Malignant Brain Tumors: A Case Study in Data Synthesis

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ABSTRACT

There has been a lot of interest in modeling the development of malignant brain tumors and in analyzing their shape and growth. Here we explore how a multidisciplinary approach, combining modern visualization and image processing techniques with experimental studies can augment the understanding of tumor development. In this case study, we analyze histological slices of a microscopic brain tumor and reconstruct these slices into a 3D representation. We process these slices to: 1) identify tumor boundaries, 2) isolate proliferating cells, and 3) segment the tumor into regions based on the density of proliferating cells. We then reconstruct the 3D shape of the tumor using a constrained deformable surface approach. This work provides a synthesis of image processing and scientific visualization techniques. It allows the analyst to 1) see properties of histological slices in the 3-D environment, with animation, 2) switch 2-D "views" dynamically, 3) see relationships between the 3-D structure and the structure on a plane, and 4) integrate custom image processing filters specially designed to reveal physiological properties. Using this method we were able to provide a very interesting result to the neuro-oncology team, and shed light on the limitations of a widely held assumption about the shape of the growing microscopic tumor.


Keywords: visualization, image processing, biomedical imaging, volume visualization, data synthesis

I INTRODUCTION

The outcome of patients with primary malignant brain tumors such as glioblastoma multiforme (GBM) remains very poor, in spite of all current treatment efforts. This is related to the rapid growth of these tumors and their extensive tissue invasion. One novel promising approach to understand such multicellular behavior better is to combine experimental and computational modeling and simulate these tumors as self-organizing complex dynamic biosystems. Innovative computational models and effective visualization may therefore be able to provide exciting insights into the structure of these tumors and provide a better understanding of their growth and behavior.

The widely taken approach in analyzing the behavior of the GBM focuses on describing and following the tumor growth as a function of time. This virtual approach takes into account that the growth presumably starts from a single mutated cell, passes through a so called multicellular tumor spheroid (MTS) stage, approaching the macroscopic stage with detectable lesion, diagnosis and death [2]. Hence, according to several models, at each step of its growth a macroscopic GBM tumor can be seen as an idealized multicellular sphere [1]. This is shown in Figure 1, representing a cross-section of an idealized tumor. In studying the growth of these solid tumors it is particularly important to understand the proliferative activity of the tumor core and the behavior of the proliferating cells. Three cross-sections of an actual microscopic GBM tumor are shown in Figure 2. The pictures are obtained with an established method in immunohistochemistry, MIB-1 labeling, which stains the proliferating tumor cells brown. In our case, everything not stained can be alive or dead, but is considered non-proliferating. The topmost image in Figure 2a shows the central slice of the tumor, with the diameter of the main sphere 464µm. The picture in the middle shows the cross-section of the tumor 35µm from the center, with the diameter 456µm. Finally, the bottom slice corresponds to the cross-section of the tumor 77µm from the center, with 321µm in the diameter.

II OUR APPROACH

The objective of this study is to develop a method for the automatic analysis of these histological slices and effective visualization of the 3D tumor boundary. We use a multidisciplinary approach, which combines morphological image processing (to segment the slices, isolate the proliferating cells, count them, analyze their spatial distribution within each slice, and segment the slices into areas of different proliferative activity) with scientific visualization (to reconstruct the tumor boundaries). We have carefully designed these image-processing algorithms to highlight physiological processes and constraints and have selected scientific visualization methods to preserve this structure in 3D reconstruction. Once all these issues are resolved, the proposed approach can be used to simulate the

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growth dynamics of the tumor with respect to different parameters. This could contribute to a better understanding of the malignant brain tumors.

III THE METHOD

Our method consists of the following steps: 1) image segmentation into the tumor/nontumor regions and extraction of the tumor boundaries, 2) detection and isolation of the proliferating cells, 3) segmentation into regions based on proliferation activity, and 4) reconstruction of the tumor shape. In the following sections we describe major processing operations within each step of the analysis. The brief overview of the mathematical morphology is given in the Appendix.

III.1 Image Normalization

Since the histological slices were prepared independently of each other, some variations in the luminance values were obtained. A normalization process was undertaken to make sure that the luminance values in all three slices occupy the same range of values and that the illumination of the extracellular region was the same in all slices.

III.2 Extraction of Tumor Boundaries

The first step is to segment the tumor region, consisting of the dark proliferating spots and light nonproliferating areas, from the uniformly light background (see Figure 2a). To do so we use grayscale morphological processing (as introduced in the Appendix). First, we process each image to remove all the light regions within the tumor. This is achieved with grayscale opening (grayscale dilation followed by erosion, using a 19×19 pixel structural element, corresponding to 1/50 of the image). This produces a bimodal image and simple intensity thresholding can be applied to identify the tumor region. After the tumor region is isolated, we eliminate the background noise and refine the region boundary by performing binary closing with a 7×7 disk-shaped structural element. The result of these operations is illustrated in Figure 2b.

III.3 Detection of Proliferating Cells

The next step is to identify the proliferating cells within the tumor region. With the MIB-1 monoclonal antibody, only the proliferating cells are stained dark brown, therefore we first identify the proliferating candidates by extracting the pixels with low luminance values. We defined the next step in the cell extraction process in order to effectively capture the physiological constraints. Since we assumed that at the microscopic level, each proliferating cell is a sphere of roughly 3µm diameter, we want to keep only those regions that satisfy this criterion. Moreover, cells without sufficient growing room do not proliferate. Therefore, we perform binary closing with a 3×3 pixel structural element which eliminates candidates smaller than 3µm, separates connected cells and in addition eliminates segmentation noise. Figure 2c shows the detected cells for each histological slice.

III.4 Computing the Density of Proliferating Cells

To detect areas of different proliferation within each tumor slice, we start with the corresponding cell map (see Figure 2c). For each slice we compute the local variance of the luminance values and divide the range into four regions representing four levels of cell density. The local variance is computed using neighborhood large enough to capture the spatial activity but small enough to avoid averaging out the variance estimate. Consequently, for the adequately chosen neighborhood (20×20 pixels), the areas of different cell activity will map into different variance values. Figure 2d represents the variance image segmented into four regions, corresponding to the areas of high, medium, low and no proliferation.

III.5 3D-Shape Reconstruction

Starting from the spherical model of tumor growth, a constrained deformable surface is used to reconstruct the tumor from the histological data. In more details, a geodesic active surface [5] is evolved toward the minimal surface which intersects the given data slices at the segmented boundaries (shown in Figure 2b) and passes through the assumed sphere/ellipsoid wherever the histological data is missing. From the practical point of view, a level set method [6] is used to implement this active contour (more specifically, the convergence rate has been improved significantly thanks to the narrow band optimization [8]). Once the implicit model of the tumor has been obtained, the visualization tool "IBM Open Visualization Data Explorer" [7] is used to extract an explicit model from the implicit one and to display the obtained surface transparently. Other meaningful objects, such as texture-mapped planes...
IV RESULTS: USING THE METHOD TO ANALYZE TUMOR GROWTH

We begin our discussion with the analysis of the central histological slice of the tumor (the topmost slice in the Figure 2a), corresponding cell density map (Fig. 2d) and map of the proliferating cells (Figure 2c). The first point to be noticed is the substantially higher density of proliferating cells in the bulging area (84% MIB-1 staining, whereas the total slice only shows a 30% MIB-1 positivity). Moreover, it appears that single invasive tumor cells are only shed from the bulging area (indicated through yellow dots in Figure 2a) supporting not just a topographic but also a quantitative linkage between tumor cell proliferation and invasion. The comparison between this picture and the corresponding cell density map (Figure 2d) shows that the areas with the highest cell density (red and green regions) are close to onset of the bulging area. Interestingly, from the map of proliferating cells (Figure 2c), we observe that the bulging area also shows central regions of lower cell count. This may be related to the high metabolic demand of the rapidly dividing cell population, which eventually leads to nutrition deprived cell sections (black regions with low cell density).

However, the most interesting (and the most surprising) result was the actual reconstruction of the tumor volume, since we realized that it deviates significantly from a perfect sphere. There are several possible explanations for this asymmetry. The microscopic brain tumors used in this pilot in vitro experiment would presumably continue to grow spherically if they were kept floating within the original tissue culture medium. However, when such “spheroids” are placed into a heterogeneous, structured extracellular matrix gel [9], an idealized spherical model may represent the tissue culture medium. However, when such “spheroids” are placed into a heterogeneous, structured extracellular matrix gel, an idealized spherical model may represent the in vitro experiment would presumably continue to grow spherically if they were kept floating within the original tissue culture medium.

The next stage in our work would be to couple this representation with the data from the volumetric growth of the tumors over time, and simulate this growth on the 3D representation, i.e. to simulate the 3D-shape reconstruction at different time points. Furthermore, we intend to match our reconstruction to other methods for visualizing tumor bodies, such as 3D confocal microscopy, and compare the slices from both representation. Finally, an important step towards building a more complex tumor model is devising a method for reconstructing both the tumor shape and tumor histology. One long-term goal is to find correlations in the tumor morphology among the histological slices, use them to build a reliable 3D representations of these functional volumetric segments, and ultimately, model and follow their growth dynamics, i.e. simulate dynamical 3D histology. Such temporo-spatial models, which incorporate increasingly detailed biological data are likely to bring exciting new insights to biomedical research and to provide a better understanding of brain tumor biology and growth dynamics.

APPENDIX

The goal of this section is to describe briefly the morphological tools used for processing of the tumor slices. More details can be found in [3]. Mathematical morphology relies on a structure known as the complete lattice. A complete lattice is a set of elements \( \{x_i\} \) with a relation of order, such as greater than or less than. For a discrete gray level functions \( f \) and \( g \), the simplest lattice is the lattice of function where the order is:
\[ f \leq g \text{ if for each spatial position } x, \ f(x) \leq g(x). \]

Moreover, each set \( \{ x_i \} \) possesses a supremum and an infimum defined as maximum lower bound of \( \{ x_i \} \), \( \wedge \{ x_i \} \), and minimum lower bound of \( \{ x_i \} \), \( \vee \{ x_i \} \). In case of discrete signals, infimum and supremum are simply minimum and maximum of the set elements:

\[
\wedge \{ f(x) \} = \min \{ f(x) \}, \ \vee \{ f(x) \} = \max \{ f(x) \}.
\]

Consequently, transformations to be defined on this lattice have to: 1) preserve the structure, i.e. the order, and 2) commute with supremum (infimum) operators. This leads to the definition of basic morphological operations: (1) increasing operators, (2) dilations, and (3) erosions as

1. \( f \leq g \implies \psi(f) \leq \psi(g) \), preserve the structure.
2. \( \psi(\vee \{ f, g \}) = \vee \{ \psi(f), \psi(g) \} \), commute with max.
3. \( \psi(\wedge \{ f, g \}) = \wedge \{ \psi(f), \psi(g) \} \), commute with min.

These equations define a large class of erosion and dilation operators. In our method we used the following two examples: If \( f(x) \) denotes an \( N \)-dimensional signal and \( Mn \) is a window (flat structuring element) of size \( n \), the erosion and dilation by \( Mn \) are given by:

**EROSION:** \[ \varepsilon_n(f(x)) = \min \{ f(x + k), k \in Mn \} \]

**DILATION:** \[ \delta_n(f(x)) = \max \{ f(x - k), k \in Mn \} \]

The most often used morphological operations are based on the composition of erosion and dilation with structuring element. They are:

**MORPHOLOGICAL OPENING:** \( \gamma_n = \delta_n \varepsilon_n \)

**MORPHOLOGICAL CLOSING:** \( \varphi_n = \varepsilon_n \delta_n \)

The opening (respectively closing) simplifies the signal by removing the bright (respectively dark) components that do not fit within the structuring element. If the simplification has to deal with both bright and dark elements, an open-close (\( \gamma_n \varphi_n \)) or a close-open (\( \varphi_n \gamma_n \)) has to be used. Note, that none of these filters is dual, but in practice they approximately remove the same kind of information.

The special (and the simplest) case of mathematical morphology deals with binary sets \( \{ x_i \mid x_i = 0,1 \} \). Consequently, for processing binary signals morphological operators can be defined either as the set or logic operators. For example, the binary erosion and dilation can be expressed as:

**EROSION:** \[ \varepsilon_n(f(x)) = \begin{cases} x, & \text{if } f(x + k) = x, \forall k \in Mn \\ \overline{x}, & \text{if } \exists k \in Mn, f(x + k) \neq x \end{cases} \]

**DILATION:** \[ \delta_n(f(x)) = \begin{cases} x, & \text{if } \exists k \in Mn, f(x + k) = x \\ \overline{x}, & \text{if } f(x + k) \neq x, \forall k \in Mn \end{cases} \]

where \( Mn \) is a binary structuring element of size \( n \).

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**LITERATURE**

Figure 2: Four views of three histological slices of malignant brain tumor: a) photographs, b) extracted tumor contours, c) extracted proliferating cells, and d) segmentation based on the density of proliferating cells.

Figure 3: a) 3D boundary contour, b) 3D boundary with in situ slice displaying histology, c) 3D boundary contour with in situ slice displaying segments of different proliferation activity, and d) 3D boundary contour with three in situ proliferating cell maps (the tumor has been stretched vertically to show all three slices simultaneously).