Visualization of Time Dependent Confocal Microscopy Data

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Abstract

The microscopic analysis of time dependent 3D live cells provides considerable challenges to visualization. Effective visualization can provide insight into the structure and functioning of living cells. This paper presents a case study in which a number of visualization techniques were applied to analyze a specific problem in cell biology: the condensation and de condensation of chromosomes during cell division. The spatial complexity of the data required sophisticated presentation techniques. The interactive virtual reality enabled visualization system, `proteus`, specially equipped for time dependent 3D data sets is described. An important feature of `proteus` is that it is extendible to cope with application specific demands.

CR Categories and Subject Descriptors: I.3.3 [Computer Graphics]: Picture/Image Generation; I.3.6 [Computer Graphics]: Methodology and Techniques

Additional Keywords: biomedical imaging, volume visualization, virtual reality

1 Introduction

In the quest for understanding of biological processes that underlie control of gene expression, there is a strong need for methods to study the structural and functional organization of the cell nucleus. Recent progress in the luminescent labeling of cell components and the use of digital 3D microscopy allow biologists to generate time dependent volume data describing in detail specific processes of the living cell. Due to their complex nature, four dimensional structural analysis is difficult, if not impossible, if traditional analysis techniques are used.

Visualization techniques such as volume rendering [1] and isosurfaces [2] are useful for the analysis of three dimensional volume data. Kaufman et al. describe *BioCube* [3], a system for the visualization of 3D cellular objects. The system specifically focuses on surface discrimination and shading. Sakas et al. [4] used volume rendering for the visualization of confocal data. Virtual reality devices further enhance the perception and understanding of these structures by stereo-scopic vision and head tracking. A first system for the study of three dimensional biological volume data in a virtual environment was Crumbs [5]. Crumbs is a proof-of-concept system for the study of and interaction with volume data in the Cave™.

The visualization of complex 3D time dependent multi channel data demands a combination of features not found in standard visualization systems. Therefore a special visualization system, called `proteus`, was developed. `proteus` distinguishes itself in three ways. First, it has basic support for the visualization of 3D time dependent multi channel data. Second, it has an open ended architecture in which application specific demands can be easily incorporated. Third, it can be used in fully immersive, semi-immersive, and desktop environments.

In this paper a specific case of biological interest is discussed which is typical for many biological volume data analysis problems: the analysis of movement of chromatin during cell division. In the next section the biological background of the case is presented. In section 3 the `proteus` system used for the visualization is described. Section 4 presents the results of the analysis of the time dependent 3D data of the cell nucleus. The last section summarizes our conclusions.

2 Biological background

The regulation of gene expression at the level of DNA and regulatory proteins that bind to it is understood in quite some detail. However, the role of higher order chromatin folding in the interphase nucleus is less well understood. Using state of the art fluorescent DNA/chromatin labeling techniques in combination with confocal microscopy [6], biologists are able to produce time dependent 3D data sets of DNA/chromatin in the cell nucleus in living cells

To this end, cells were used that express green fluorescent protein [7] tagged histone H2B[1]. Histones are key components of chromatin. They constitute a protein octamere, around which the DNA is wrapped almost two full turns. This histone octamere plus the DNA together constitutes the nucleosome, the basic building block of chromatin in all eukaryotic cells. In the experiments discussed in this paper the GFP-labeled H2B allowed chromatin to be visualized in living cells and movement was followed in time and space using 3D confocal microscopy. Time series of 3D images were made of the process of decondensation of the chromatin after cell division (mitosis). During mitosis chromatin is densely packed in chromosomes. After mitosis, part of the chromatin decondensates to form a new nucleus. The aim of these experiments was to analyze the movement of chromatin during formation of the cell nucleus of the newly formed daughter cell.

The dataset consisted of a series of fifty 3D data sets. Each data set was a stack of optical sections of 256×256 voxels each. The number of optical sections in the stacks varied over time due to the flattening of the cell as the cell cycle progresses. The initial number of optical sections was 30, decreasing to 18 sections at later time points. To make processing easier extra optical sections were

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[1]HeLa cells expressing H2B-GFP[8] were kindly provided by H. Kimura of the University of Oxford

added, so that all sets contained an equal number of sections. The voxel size is $90 \times 90 \times 700$ nanometer. Due to physical characteristics of a confocal microscope the optical resolution along the z-axis is at least four times lower than in the x-y plane. The 3D images are corrected for this by scaling in the z-direction during rendering. The time step between subsequent scans varies from 30 seconds in the beginning to 180 seconds at the end of the process. The total amount of storage required for the data is 98 MB.

3 Visualization system

In this section the `proteus` system is described. The philosophy of the system, its capabilities and some implementation details are described.

For the visualization of time dependent volume data in VR the `proteus` system was developed (See Figure 1). The system runs on a wide range of hardware ranging from a Cave™ to a standard desktop computer. The VR setup which was mainly used consists of a large back projected screen, head tracked shutter glasses and a wand for three dimensional input. `proteus` was made using the PVR-system [9]. In a desktop environment the system can be used with the mouse as input device.

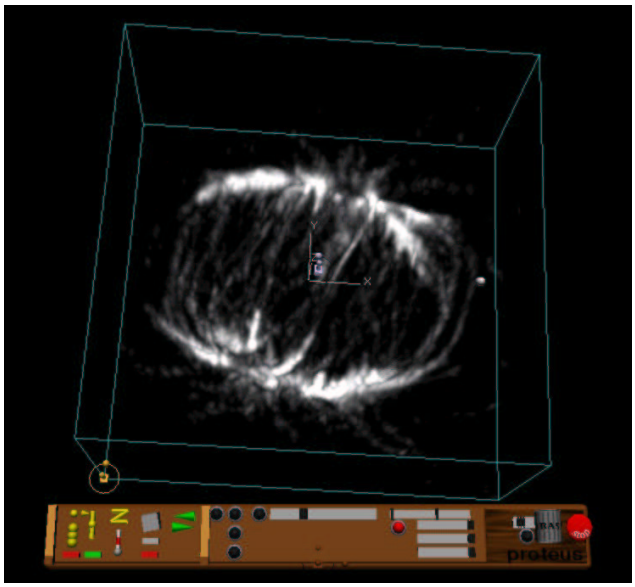

Figure 1: User interface to the `proteus` system

`proteus` offers volume rendering and isosurface [2] algorithms for the visualization of multi channel volume data. Volume rendering at near interactive speed is achieved using Volumizer [10]. Also, the system offers a number of tools for further inspection and analysis of the data. Region selections can be made interactively for the inspection of details of the data. The `proteus` architecture is such that new visualization techniques or interaction primitives can be easily integrated.

A direct manipulation interface based on grab and click is used for interaction. A “virtual hand” connected to the wand is used as a pointing device. The user interface consists of handles, buttons and sliders. Handles are used to move or rotate objects. Buttons and sliders, placed on a three dimensional tool bar, serve the purpose of input of boolean or numeric values. Buttons can be any shape for example the trash can shown at the right in figure 1.

Caching is used at several places in the system to provide the speed needed for interactive work without losing flexibility due to extensive preprocessing. The volume data for different time steps is loaded on demand and kept in a cache as long as the memory is not needed for other data. Also, derived data is cached. For example isosurfaces and bin-trees (used to speed up isosurface calculation) for different time steps are calculated on demand and kept in a cache. By using the cache animation through the time steps runs much faster after a first calculation run.

For the biologists, analysis using `proteus` in a virtual environment was only part of the use of visualization. They also need visualization tools for collaboration and communication. During the analysis process the users not only wanted to see the data themselves but also discuss their findings with colleagues. Furthermore, images are essential in publications to illustrate concepts found during analysis. The system contains tools to capture single images or image sequences while working with the system. In this way images or movies can be generated.

4 Results

This section describes the analysis process of the data described in section 2 using the `proteus` system described in the previous section. The analysis of the chromatin decondensation data progressed in a number of steps. For each step the role and requirements for visualization were different. The focus in this section will be on the visualization techniques used and the conclusions drawn based on the visualizations.

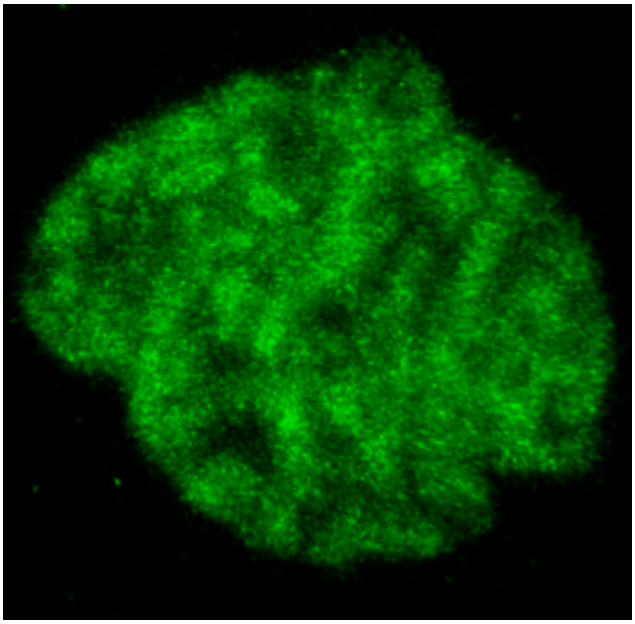

Figure 2: Volume visualization of the data during the decondensation process.

In the first step a need to get an overview of the data was considered most important. Volume rendering was used because this presents the data in a natural and intuitive way (see figure 2). Also, volume rendering was the representation which comes closest to the slice images the researchers were familiar with. Volume rendering, although the speed was not high enough for effective interactive visualization, gave insight into the global structure of the data. Based on the visualization, two factors were identified which complicated the visible tracking of the structures in the nucleus: First, the overall movement of the cell nucleus due to movement of the cell and changing of the scanning origin of the microscope. Second, the data was noisy which would interfere with automatic feature extraction.

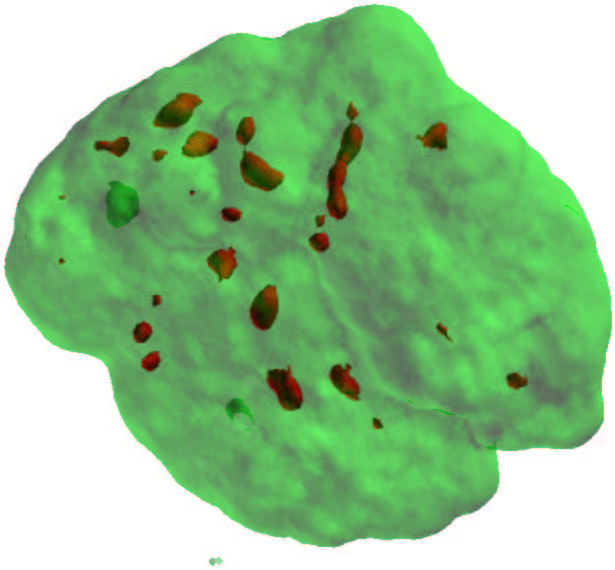

Figure 3: Iso surfaces in the filtered data. A low threshold value was used to show the boundary of the nucleus (green transparent isosurface). The dense parts of the chromatin are shown in red.

In the processing step that followed, the movement of the cell was filtered out using a center of mass estimation, and the noise was suppressed using a low pass filter. At this stage more specific aspects of the data became of interest: the analysis of movements of dense parts in the chromatin data. Iso surfaces were useful for analysis of the motions of these dense parts over time. This is due to the fact that the dense parts can be accurately characterized by a specific data value. Because the position of dense parts relative to the boundary of the nucleus was important, transparent isosurfaces were introduced to show the boundary of the nucleus using a low iso value (see figure 3). By analyzing the motions of the dense parts, it became clear that the apparent direction of these dense parts was mainly outward without much reorganization inside the nucleus. To test this hypothesis the dense spots in the data were detected using Largest Contour Segmentation [11]. In this method the locations of maximum intensities are detected in the data and a region around these maxima in which the data is within a certain range close to the maximum is determined. Then the center of gravity of the found regions is calculated. This procedure gave about 40 objects per time step. These objects were tracked from using a mutual nearest neighbor algorithm.

The noise and inter time step movement proved too difficult for robust application of object detection and tracking algorithms. Therefore, automatic detection was combined with manual editing. The extensibility of the `proteus` system allowed for integration of the output feature detection and tracking algorithms into the visualization of the raw data. The mass-centers found by feature detection were shown as spheres. The number of time steps for which the spheres are shown can be controlled by the user. The tracks found are shown as cylinders between spheres. Color coding was used to show the different tracks or the time step in which the mass center was found. The tracks can be manually edited using direct manipulation in places where the algorithm was not capable of calculating the tracks. By visual inspection of the movement of objects using isosurfaces and volume rendering it was in many cases possible to improve the found tracks. By using the tracking algorithm in combination with the track editing tool the main tracks of dense chromatin spots were found (see figure 4).

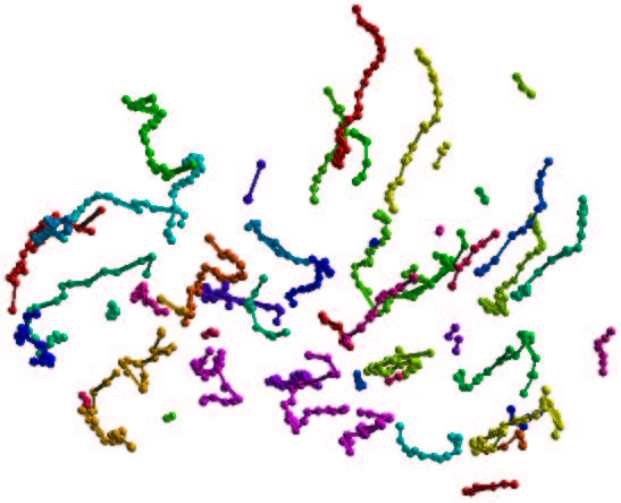

Figure 4: Tracks of dense parts in the data for 22 time steps.

5 Conclusions

In this paper a case study of the visualization of time dependent confocal data was presented. The `proteus` system is used for the analysis of chromatin condensation and decondensation during mitosis. `proteus` is an open ended system for which many case specific techniques have been developed.

The study demonstrated that visualization can serve a useful purpose in the time dependent confocal microscopy data. The main lessons learned from this case study are:

- Visualization is only one part of the analysis process. The visualization system used must be flexible enough to cooperate with the other parts of the process. The consequence of this is that the visualization system must be designed as an open system in which the output of other analysis tools can be incorporated.
- There is no one “silver bullet” visualization technique. Each visualization technique has its strength and weakness. Therefore a whole repertoire of techniques should be offered for use.
- In the analysis process, visualization is also used for collaboration and communication. In order to be effective, results found in a highly sophisticated environment should be recordable, such that the results can be reproduced for discussion or publication.

We believe that in other areas where time dependent volume data is studied, the described strategies and techniques can be applied as well. In the future we hope to extend `proteus` by integrating additional analysis tools. In this way we hope to achieve a tight integration of automatic feature detection and interactive visual analysis of the data.

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