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Visualization Software for 3D Video Microscopy: A Design Study

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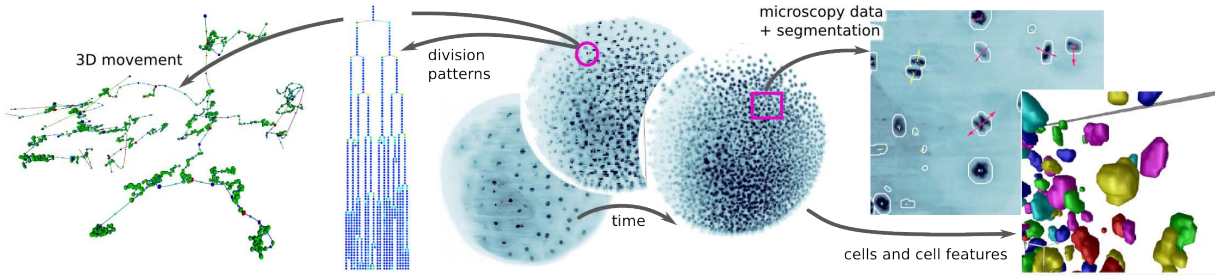

Figure 1: *Visualizations for the analysis of 3D video microscopy data: The rich data requires a combination of SciVis and InfoVis techniques to depict the different aspects of information. Interaction mechanisms, focus and context techniques, as well as linking and brushing connect the different views on the data.*

Abstract

Modern microscopy techniques allow for fascinating new insights into the development of life. They produce, for example, digital 3D+T records of living embryos that reveal how a single cell develops into a complex specimen consisting of thousands of cells. To cope with the huge amount of data, dedicated software is required to help biologists visualize and analyze their data. In close cooperation with experts from biology and image processing, we developed a software for the visual analysis of 3D videos of growing organisms, which is introduced in this paper. We detail the implemented visualizations, the GUI design, and the incorporated interaction methods. We include results of the application of our software in three groups focusing on organismal studies and detail lessons learned of the two-year cooperative software development.

Categories and Subject Descriptors (according to ACM CCS): J.3 [Computer Applications]: Life and Medical Sciences—Biology and genetics

1. Introduction

Recent advances in microscopy research enable biologists to record living organisms as they grow from a single cell into a complex organism consisting of thousands of cells. The resulting videos feature an unprecedented resolution in space and time, from which individual cells can be identified and tracked in 3D over time. Such data permits to address questions about the morphogenetic processes underlying animal and plant development that could so far not be addressed due to lack of suitable imaging and analysis techniques. While so far biologists had to use reductionist approaches, they can now study the development of whole organisms from the earliest stages up to the full-grown viable animal, and observe how cells form tissues and organs. Moreover, they can now analyze the behavior of cell populations, document the variability of behavior across specimens, and compare healthy to pathological situations.

All the information required for this work is already contained in the recorded data, i.e., in the 3D+T videos. Automatic analysis, however, remains challenging due to the lack of appropriate software. In this paper, we describe a software that helps biologists visualize cell data in 3D video mi-

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croscopy. Our contributions are: (i) description of the data processing pipeline in video microscopy, (ii) requirements analysis for this application area, (iii) presentation of our visualization software, (iv) explanation of design choices and lessons learned from the interdisciplinary work.

2. Biological Background – Organismal Studies

The techniques we are going to discuss are designed for the visual analysis of 3D+T data of developing multicellular organisms such as growing embryos or plants.

For translucent organisms such data can be acquired using optical microscopy such as the recently developed light sheet-based fluorescence microscopy (LSFM) [HSD*04, KSWS08, KK11]. The general idea is to label cell nuclei with a fluorescent marker that is stimulated during recording and shows up as high intensity values in the resulting data. A strength of LSFM is the very low invasiveness of the method that allows recording for extended periods of time (hours to days) without any damage to the specimen. The 3D videos feature a very good spatio-temporal resolution and signal-to-noise ratio, which allows for automatic segmentation of small individual structures such as cells and cell nuclei [OLOD*10, LKL*11]. The high temporal resolution permits reliable automatic tracking of these structures over time with low error rates [LKL*11].

In a first step, biologists are currently working towards the reliable detection of individual cells and their tracking over time. This requires the further improvement in image quality, as well as the continuous improvement of the robustness of the segmentation and tracking algorithms. This becomes particularly critical in later time steps, when the organism consists of thousands and up to tens of thousands of densely packed cells. At this stage, prior to the actual data analysis, visualization becomes a crucial part when it comes to verification of the automated post-processing steps, as errors in the segmentation and tracking accumulate in later analysis.

During our joint work with three research groups imaging different biological specimens (flies, fish and plants), we identified the following requirements:

- Visualization of (i) large volumetric time-dependent data with many small details, (ii) multiple data channels, e.g., microscopy and segmented data, (iii) evolving cells, (iv) multivariate features derived from the input data, e.g., cell size, shape, and texture parameters.
- Data mining and statistical tools to analyze the data.
- Video functionality to animate evolving processes.
- Graphical user interface for easy interactive use.
- Interaction methods that link the different visualizations.

3. Related Work

The need for better analysis software has already been expressed in recent years and O'Donoghue et al. [OGG*10] define as central requirements: good usability, incorporation of visual analytics algorithms, and multi-scale representations of the data. CellBioVis-software already available are Simi Biocell [SHMS97] and Angler [MBD97], which render 3D image series and cell lineages, while concentrating on the application to *C. elegans*. AceTree [BBM*06, MBBW06] is a lineage viewer and editor. In this context, there also exists Virtual Worm-base [BKM05], which is an interactive 4D *C. elegans* database also including tools for visualization purposes. CellProfiler Analyst [JKW*08] is an open-source software to explore and analyze image-based data. Weber et al. [WLK*05] describe a framework for the validation of three-dimensional segmentation that combines different rendering modes for volumetric and segmented data. Wylie et al. [WB09] and Cedilnik et al. [CBI*07] visualize and validate 3D lineage data and therefore, propose the combination of scientific visualization and information illustration in a single visualization framework, called Titan project, which is part of the Visualization Toolkit (VTK). Mosaliganti et al. [MMHL08] develop visualizations of microscopic biological structures that provide a better rendering of relevant geometric structures.

4. Software Design

As several research groups working on different biological questions expressed urgent need of a software tool to investigate their data and none of the existing programs provided sufficient support for the visualization of cell data in 3D microscopy videos, we developed a novel system. In the following sections we will first explain the central visualization algorithms, along with their special adaptations to the given data and a brief discussion of why we chose each technique. Afterwards, we will discuss the user interface, the interaction mechanisms, and the implementation of our program.

4.1. Supported Visualizations

Volume Rendering A central requirement for the visualization software are algorithms to render the raw data. We support direct volume rendering [HKRs*06] and maximum intensity projection (MIP) (Fig. 1 center). MIPs are already well accepted in the bioimaging community [OGG*10] as they are an easy to implement and widely used technique to render image stacks, the most common image modality in this community. Moreover, the fixed alignment with a given axis eases the spatial orientation.

We provide default nonlinear transfer functions that make the resulting renderings resemble images from light microscopy. The resulting images have a familiar look and feel and emphasize the relevant structures, which makes them easier to analyze. The MIPs are additionally annotated with labels, time step information, scales, bounding box and color bars to provide all relevant information. With the mouse wheel the time step can be interactively manipulated, which allows for rapid analysis of time-dependent data.

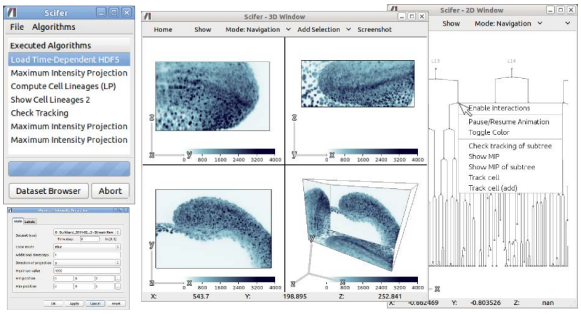

Figure 2: *Software design: The GUI in Scifer consists of multiple windows (left to right: main window (top), algorithm dialog (bottom), 3D window, 2D window).*

Augmented MIPs A second requirement was the provision of algorithms for the validation of the segmentation results and the cell tracking, which is realized using augmented MIPs. The user can choose an arbitrary combination of the following options (Fig. 1 right):

- *MIP + labels*: Renders the MIP and on top the IDs of segmented cell nuclei at the center of mass of the segmented structure.
- *MIP + isolines*: Add outlines of the segmented structures which show the exact boundaries of the segmentation and help identify under- and oversegmentation.
- *MIP + arrows*: Adds to each segmented cell arrows that point in the direction of motion from the current to the next time step. Color coding is used to distinguish between cell movements and cell divisions.

We also tried visualizations in 3D-space, but found them too cluttered and difficult to analyze. Hence, we settled on the maximum projections.

Cell Rendering In several application scenarios, the rendering of individual or few cells is required, e.g., highlighting of cells with specified properties (Fig. 1 right/bottom). Therefore, we include geometric renderings of the cell nuclei. These are either directly generated from the segmented data using the marching cubes algorithm or, if memory and rendering time is crucial, are depicted as ellipsoids that are scaled according to segmented cell size.

Lineage Tree Visualization A cell lineage is the pattern of cell division during development [Chr00] and its analysis is commonly accomplished based on the depiction of the lineage tree, which corresponds to a binary tree. For the tree layout we use a standard algorithm [TBET98] (Fig. 1 left). Dividing cells are represented with circles and moves with lines. Color coding is used to include additional information such as cell velocity or cell size. On demand labels for each node in the tree can be displayed.

Cell Movement in 3D To aid in the analysis of cell movement and division processes, we included two types of visualization. Either evolving cells are rendered in video mode or the cell lineage is rendered in 3D (Fig. 1 left). While the video mode is less cluttered and gives a better sense of concurrent events, the 3D lineages aid in the investigation of 3D motion patterns of cell clusters.

InfoVis for Cell Features A frequent request is the depiction of additional derived features such as cell or lineage properties. For this purpose, we provide a number of information visualization techniques and statistical graphics such as histograms, boxplots, and scatterplot matrices. All these visualizations are interactive and can be linked back to the input data, as will be detailed in section 4.3.

4.2. The GUI

For a convenient depiction and analysis of the different visualization types, the GUI consists of three major windows (Fig. 2). The main window is used to (re-)start algorithms and provides information on the progress of the current algorithm. The 3D window renders the 3D+T data in three orthographic and one perspective view, each of which can be interactively maximized. The 2D window is used for information visualization, such as lineage depictions or statistical information. All windows provide interactions with the mouse when a displayed item is selected. In the menu, the user can change between navigation and interaction mode. In navigation mode, the visualization can be translated, zoomed or rotated. In interaction mode, the navigation is disabled if the user selects an item that provides additional interactions (see Fig. 2(right)).

4.3. Interaction Techniques

A crucial feature during data analysis are the interaction mechanisms. According to the information visualization mantra, *"overview, zoom and filter, details on demand"* [Shn96], we need to provide views on the data at different levels of detail and a set of interaction techniques that link the levels and provide easy to understand navigation between them. While the visualization algorithms detailed above can be found in many visualization systems, interactive navigation between the techniques combined with level of detail approaches is challenging and remains a rare feature that we tried to integrate as widely as possible in our software.

Fig. 3 depicts the different visualization techniques and the navigation between them. On the coarsest level, we depict the entire 3D video microscopy data using volume visualization techniques. By clicking on one of the rendered cells, the user can start the rendering of the corresponding cell lineage and dig deeper into the data. The lineage presents a time-dependent illustration of the cell division patterns. To link this information back to microscopy data, the user can invoke local MIPs from each of the nodes in the lineage. The bounding box of the MIP is either chosen to incorporate the selected cell and a small neighborhood, or can

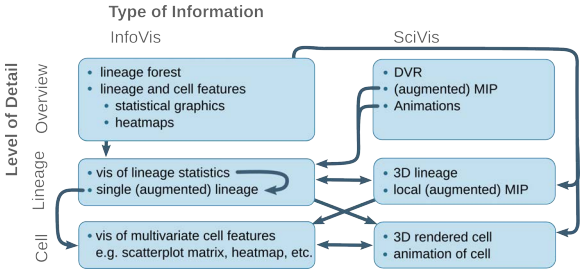

Figure 3: *Visualization techniques and their interactions.*

be automatically extended to incorporate all cells in the subtree of the lineage. Local MIPs can be further enhanced to incorporate information about the segmentation and tracking. An additional option is the color-coding of lineage nodes to help the user find unusual structures in lineages. On the finest level of detail, the user can investigate individual cells and their properties. Cell properties can be studied using an integrated scatterplot matrix. Upon brushing all selected cells are rendered in 3D. The rendering of 3D cells can also be invoked from the lineages and the local MIPs.

4.4. Implementation Details

All presented techniques are part of *scifer* (`http://www.scifer.info`) the visualization software developed in the *Computer Graphics and Visualization* group at Heidelberg University. Scifer is implemented in C++ and algorithms are loaded as individual modules which eases the integration of novel techniques. Scifer supports the following operating systems: Linux (ubuntu) and Mac OS X. Currently (summer 2012) scifer is only available to cooperating groups. If interested in the software, please contact Heike Leitte.

5. Validation and Lessons Learned

The visualization software is currently used within three cooperating groups at Heidelberg university. Data analysis and visualization has so far been accomplished using open-source software (Fiji – `http://fiji.sc/`), commercial tools (amira – `www.amira.com`), and self-coded visualizations in Python. Major bottlenecks were the efficient processing of long time-series (from several hundred up to several thousand time steps) of large volumetric data (2048^3 voxels), and the analysis of data with several thousand cells, where simple renderings easily suffer from occlusion.

Our software is currently used for direct data visualization (MIPs and lineage trees), cell tracking, and the validation of pre-processing steps (segmentation and tracking). The direct visualization is faster than previous techniques and provides more flexibility with respect to visualization settings and the combination of multiple visualization techniques. In case of the validation, we could decrease the manual workload from two weeks (20 time steps, $<$100 cells per time step) to few hours (100 time steps, 1000 cells per time step) for the verification of the segmentation of cell nuclei.

Lessons we learned during the development of the software (3 years) are as follows:

Flexibility of the software: Meeting the needs of application scientists is work in progress and the software design has to be very flexible. *Data readers and writers*: File formats already changed a lot during our joint work. We provide data readers and writers for the formats commonly used in collaborating groups (vtk, netcdf, HDF5) and convert the data after reading into an internal format. Data export (screenshots, videos, SVGs) is also important to make the results usable for the application researchers.

Algorithm parameters: Parameters of the algorithms allow for large flexibility, but are also hard to grasp if there are too many. We try to keep the number of parameters low, build hierarchies in the dialog windows using multiple tabs, and provide good default settings where possible.

Talk to the scientists: Most important are frequent communication and meetings with the application scientists to really understand what they need and how the software can be further improved to achieve their goal. At the current state, the entire team (biology, image processing, and visualization) meets once a month. Additional meetings between individual members of the team are scheduled on demand to discuss, for example, new features of the software (sometimes multiple meetings per week). What we found also very helpful is to visit the other labs and understand their work. Therefore, we organized a "wet lab day" during which the computer scientists were introduced to fish breeding and microscopy. Additional introductory courses to the software programs *ilastik* [SSKH11] (`http://www.ilastik.org`) by the *Multi-dimensional Image Processing* group of Fred Hamprecht (Heidelberg University) and *scifer* helped the entire team get started with the programs.

6. Conclusions

We presented a visualization software for the analysis of organismal cell data recorded using 3D video microscopy. We described the implemented algorithms, GUI, and interaction mechanisms and explained the choices made. The new software helped to decrease human workload significantly and permitted for analysis that was not yet possible. Besides a variety of visualization algorithms from scientific and information visualization, our software provides comprehensive interaction techniques that enable the user to change between multiple levels of detail and different views on the data. While we concentrated in the first step on a versatile software that helps with many every day problems, future work will concentrate on improved analysis techniques that target specific application questions, such as the analysis of multivariate cell features and the analysis of cell lineages, more closely.

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