Integrating Information and Volume Visualization for Analysis of Cell Lineage and Gene Expression during Embryogenesis

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Abstract
Dramatic technological advances in the field of genomics have made it possible to sequence the complete genomes of many different organisms. With this overwhelming amount of data at hand, biologists are now confronted with the challenge of understanding the function of the many different elements of the genome. One of the best places to start gaining insight on the mechanisms by which the genome controls an organism is the study of embryogenesis.

There are multiple and inter-related layers of information that must be established in order to understand how the genome controls the formation of an organism. One is cell lineage which describes how patterns of cell division give rise to different parts of an organism. Another is gene expression which describes when and where different genes are turned on. Both of these data types can now be acquired using fluorescent laser-scanning (confocal or 2-photon) microscopy of embryos tagged with fluorescent proteins to generate 3D movies of developing embryos. However, analyzing the wealth of resulting images requires tools capable of interactively visualizing several different types of information as well as being scalable to terabytes of data.

This paper describes how the combination of existing large data volume visualization and the new Titan information visualization framework of the Visualization Toolkit (VTK) can be applied to the problem of studying the cell lineage of an organism. In particular, by linking the visualization of spatial and temporal gene expression data with novel ways of visualizing cell lineage data, users can study how the genome regulates different aspects of embryonic development.

Keywords: Information Visualization, Volume Visualization, Large Data Visualization, Cell Lineage, Integrating Infovis/Scivis.

1 Introduction
This paper describes an application for analysing the cell lineage and gene expression during the embryogenesis. The approach taken in this application is to combine spatial visualization, such as volume rendering of the confocal microscopy images of embryo together with the information visualization, such as tree visualization of the lineage.

Section 2 is a quick summary of related work in the areas of Cell Lineage, Information Visualization, and Information and Scientific Visualization. Section 3 describes the problem that the application is trying to solve. It also defines the concepts of Cell Lineage and relationship between the Cell Lineage and visualization. Section 4 describes the application and addresses the reproducibility of the results presented in this paper. Finally Section 5 provides information about the future development of the application and the areas where future research can be performed.

2 Related Work
Visualization for biological applications has evolved in many different forms. Currently biologists communicate by using set of de facto established standards that have flourished in their technical literature. One of the commonly used information visualization techniques relates to the display of genealogical trees. This is important in the fields of ecology, evolution and cell biology, in particular for the study of cell lineages in the latter.

Combining genealogical trees information visualization with 3D visualization of confocal microscopy involves the use of a collective set of technologies related to computer graphics, image analysis and visualization. The scalability of these technologies is put to the test with the large amounts of data that biological research can produce.

The following sections describe related work in the areas of cell lineage determination, information visualization and large data processing.

2.1 Cell Lineage
Cell lineage refers to the pattern of cell divisions during embryonic development. It is an important and long studied problem in biology. This is especially true for C. elegans a microscopic roundworm that became a popular model organism for biological research in part due to its invariant cell lineage. Life begins in C. elegans with the fertilized egg cell termed P0. The fertilized egg undergoes a number of rounds of cell division, cell death, and cell differentiation leading to the formation of the adult organism. Importantly, in C. elegans this pattern of division, death, and differentiation is the same in all organisms which allowed the complete lineage from the 1 cell fertilized egg to the 959 cell adult organism to be established through repeated observation of different individuals and manual reconstruction [18, 19]. More recently, confocal microscopy has advanced to the point that 3D movies of much of embryonic development in C. elegans can be captured with high enough resolution to segment and track cell lineages automatically from a single individual [5]. Software tools have also been developed to automatically annotate the segmented cells with their individual identities [6].

In toto imaging in which high performance laser-scanning (confocal or 2-photon) microscopy is used to capture 3D movies of every cell in a developing tissue means it is now possible to extend cell lineage analysis to organisms that do not have an invariant lineage, such as vertebrates which are more medically relevant [11]. For in toto imaging, embryos are labeled to allow all the cells to be segmented such as through the use of a nuclear localized green fluorescent protein and a membrane localized red fluorescent protein. 3D movies are then taken on a laser-scanning microscope and special software such as GoFigure is used to segment and track the cells [11]. Importantly, transgenic organisms can be used to
The challenge of elucidating the cell lineage of an organism is usually attacked in multiple stages. First, the microscopy images are processed for segmenting the individual cells. Second, the cells are associated across different time points in order to establish which cells result from the division of cells in a previous time point. Third, visualization tools must be used for analyzing the cell lineage data along with the spatial information.

This procedure has been applied to simple organisms such as the Caenorhabditis elegans, which produces only 1090 cells during development (the adult contains only 959 cells because 131 cells under programmed death during development). However, when addressing more complex organisms such as the Zebrafish the data that needs to be processed gets scaled by two or three orders of magnitude.

This paper describes the type of visualization functionalities that are needed by the community of biologist studying embryogenesis, and how many of them are being implemented in the Visualization Toolkit (VTK). Scalability is one essential requirement for software tools to satisfy the needs of data processing for larger organisms. VTK’s existing parallel infrastructure has already proved to be scalable to terabytes of data and therefore provides a platform for seamlessly developing information visualization applications suitable for the study of embryogenesis.
3.1 Following Cell Lineage

The process of embryogenesis starts with the zygote cell dividing into two cells of approximately half the size of the zygote. These two cells divide each one into two daughter cells, which continue to divide. The division process is not totally symmetric. At every stage, the daughter cells have slightly different concentrations of proteins, and slightly different geometric relationships with their neighbors. These subtle composition differences, along with further exchanges of information via molecular signals between the cells, drive the process of differentiation by which the cells become specialized into particular tissues and organs. The daughter cells also arrange themselves in particular spatial patterns. For example, the first two divisions of the *C. elegans* zygote define the basic body-plan of the organism; each cell being associated to one of the anterior, posterior, ventral and dorsal axes of the worm.

The history of cellular divisions that sequentially tracks the parents of a particular cell up to its original ancestor, the zygote cell, is called cell lineage. Simple organisms such as the *C. elegans* worm tend to have a deterministic cell lineage, meaning that for any given embryo, the cells divide following exactly the same pattern. More complex organisms tend to have a deterministic lineage only in the early stages of embryogenesis and then, as large numbers of cells of similar type are required, they tend to generate these cells by following non-deterministic, although likely stereotypic, patterns.

![Figure 1: Complete cell lineage of *C. elegans.*](image)

Studying the cell lineage of an organism is important because it helps in the understanding of how a particular cell defines its fate in the organism. It also helps to understand how mutations and environmental factors may perturb the embryogenesis process and result in malformed organisms. In the context of studying gene functionality, the cell lineage also provides a baseline layer upon which the behavior of genes can be laid out across space and time as the organism develops. For example, if a biologist is testing the hypothesis that a particular gene is required for the formation of the eyes, tracking the expression of this gene across the cell lineage of the embryo will permit to verify if different levels of expression of this gene may make a difference in how the eyes get formed.

Cell lineages are also extremely important in tissue-engineering applications where undifferentiated stem cells are cultivated in order to produce tissue suitable for use as grafts for particular organs. This is an important treatment for patients suffering from severe burns and muscular degeneration, for example. In this context, understanding the lineage of the cells that are to be produced helps to select the appropriate ancestor cells and culture conditions that could be used as starting point for cultivating the tissue.

Studying the cell lineage of an organism, however, is a very challenging task and to date has only been done for a few simple organisms. It typically requires a scientist to carefully observe the development of the embryo under a microscope and to track each one of its cellular divisions. The task grows more and more challenging as the development of the embryo progresses and more and more cells occupy the field of view. The recent combined use of fluorescence confocal microscopes and automated imaging technologies has provided better tools for confronting the challenge of tracking thousands of cells over time. A recent and very promising approach to the study of embryogenesis is to acquire 4D datasets by grabbing 3D confocal microscopy datasets repeatedly over time as the embryo develops. These datasets are then processed using image segmentation algorithms in order to identify the location of the cells at every time [11, 17, 5].

Once the cells have been segmented from the images, the sequence of cellular divisions that led to the creation of each one of the cells has to be traced over time in order to reconstruct the links between each daughter cell and its parent. Current methodologies for tracing cell lineages involve a combination of automated tracing followed by supervised post-processing. The automatic tracing is based mostly on interpreting the spatial location of the cells with respect to the body-plan axes, and identifying the spatio-temporal continuity between the parent cells and its daughter cells. The supervised post-processing stage is performed by an expert interactively editing a graphical representation of the lineage structure [5, 6].

3.2 Information and Spatial Visualization

While many software systems provide tools in either spatial-temporal or information visualization, they have not been integrated in a meaningful way. Here we describe the differences between spatial and information visualization, and how they should be used together to make an effective system.

In spatial visualization, the data to be visualized has some inherent spatial information associated to each data point (i.e., a space reference frame that users easily relate to). The challenge of spatial visualization is to transform the data in such a way as to effectively show important features in the data.

Spatial visualization algorithms may operate on scalar data (e.g. color mapping, contouring), vector data (e.g. oriented glyphs, warping, streamlines), tensor data (e.g. tensor ellipsoid glyphs), or may perform other advanced geometric or topological operations (e.g. cut planes).

With information visualization, the problem is abstracted one level further. Most of the time, there is no inherent spatial-temporal information given to information (no intuitive perceptual framework). Instead, the raw information consists of an arbitrary number of fields assigned to a set of entities, and a perceptual framework must be constructed. The data for information visualization may come from a wide variety of sources, such as financial or marketing data. In certain cases there may also be a predefined set of linkages between objects, for example in communication graphs or corporate hierarchies. These linkages may be explicit, or could be constructed implicitly (e.g. via a similarity metric between objects). As with spatial data, the information must then be filtered and transformed in order to extract the most useful information from the data.

The additional task of information visualization involves assigning a physical location to each entity. There are a wide variety of ways to perform this operation, including projections, graph layout, tree maps, and parallel coordinates. The resulting data containing physical locations may then be filtered in a variety of ways using spatial visualization filters.

One case where spatial and information visualization may be used together is when visualizing metadata. For simple experiments with a small number of datasets, the user may be able to keep track of the meaning of datasets by their filename or location on the file system. However, as the number of datasets becomes large as in a scientific experiment, the user must then rely on metadata in order to (1) find relevant dataset(s) and (2) view information about loaded datasets. This metadata, taken collectively from a number
of datasets, may be considered a data source itself. If the meta-
data from numerous datasets are stored in a single location, such
databases, along with a pointer to the full datasets (e.g. via a
filename), the system should be able to perform some additional
advanced operations. First, the metadata could be read in and vi-
sualized using information visualization techniques. For example,
the metadata for a series of experiments could be plotted using a
parallel coordinates plot in order to view relationships between ex-
periment runs along with interesting outliers. This metadata should
be linked to the full datasets so that the user may open the rele-
vant datasets. These datasets may then be visualized with spatial
visualization algorithms.

4 METHODOLOGY

The application was implemented using VTK, Titan, and Qt. Each
toolkit aided towards the ease of implementation. VTK and Titan
provide several algorithms for spatial and non-spatial data process-
ing, as well as visualizing. The application utilizes the flexibility of
the toolkits to perform tasks that are usually hard in the traditional
Information Visualization toolkits. For example, to define the time
front, the iso-contour line was applied to the tree visualization.

All the toolkits used in this application are cross platform toolk-
its. As such they not only cross platform boundaries, but also
domains-of-use boundaries. By leveraging the large user communities
of these two toolkits, the application benefits from out-of-the-
box ideas borrowed from the related fields.

This section describes the implementation and usage of the appli-
cation, as well as the scalability of technology and reproducibility
of this project.

4.1 Application

The basic pipeline of the application can be seen on the Figure 2.
There are two distinct branches of the visualization pipeline. On
one hand, the actual cell lineage is visualized using Titan module.
The confocal microscopy volumetric images, on the other hand, are
visualized using the more traditional volume rendering capability
of VTK. The selection of the transfer function used for volume ren-
dering as well as the selection of the time frame of the 3D data to be
rendered, out of the 4D collection of confocal microscopy images,
is determined by the selection of nodes within the InfoVis part of
the pipeline. In this way, it is possible to navigate the cell lineage
tree and to obtain 3D visualizations of the embryo at a stage that is
consistent with the selections made on the cell lineage tree display.

4.1.1 Information Visualization Pipeline

The information visualization components of the application are
provided by the Titan Informatics Toolkit[20]. Titan is designed,
developed, and supported by Sandia National Laboratories.

Titan provides the ability to extract meaningful knowledge from
large information sources through various visualization and interac-
tion techniques. Titan has been added onto the existing capabilities
of the VTK, which allows rich integration between information vi-
sualization and scientific visualization.

There are a number of data object types which may store various
geometric objects such as image data, volume data, or polygonal
data. Titan defines additional types for tables, trees, and graphs.
For the cell lineage viewer, we use the tree structure. A tree holds
a hierarchy of entities. In the case of a cell lineage, the cells are
the entities and the hierarchy is naturally defined by the parent and
child cells, which in effect creates a binary tree.

The tree is read in from a file and loaded into two separate views.
One view is a tree widget which shows the cell hierarchy as an
expandable list of cells. The other view is a tree layout view where
the tree is laid out using either a standard view or a radial view. The
tree is then converted to polygonal data and rendered in the same
way as other data types. Using Titan we are also able to link the
selection in these views.

4.1.2 Volume Visualization Pipeline

To visualize the confocal microscopy images of the embryo, vol-
ume visualization is used. The toolkits provide necessary data
structure and algorithms to perform the volume visualization. The
basic data structure is vtkImageData, which is a three dimensional
block of data (voxels) with origin, spacing, and dimensions associ-
ated with it. There are several volume mappers. The most com-
monly used are fixed-point software ray-casting and GPU-based
ray-casting. Depending on the hardware on which the application
is running, the appropriate mapper is chosen. Figure 8 shows a
volume display from a confocal microscopy image of the embryo
development.

In order to dynamically link the volume visualization to the nav-
igation of the cell lineage tree, the application took advantage of
volume rendering multi-component datasets. A two-component
dataset was produced by using as first components the intensity val-
ues of the original microscopy image and as second components the
cell identifiers produced by AceTree for that specific stage of em-
byro development.
In order to produce the second component, individual cells were segmented from the confocal microscopy images and then labeled using the cell identifiers provided by the results of the cell lineage tracing of AceTree [5, 6]. Since the cell identifier labels are co-registered to the original microscopy images it is then possible to modulate the transfer functions of the volume rendering based on the values of the cell identifiers. Through this mechanism, specific cells can be highlighted or removed from the volume visualization.

Although AceTree already performs the segmentation of the datasets and reconstructs the cell lineage, the intermediate images resulting from the cell labeling process were not available as output of the processing. Therefore, we reproduced the cell segmentations by using image analysis components from the Insight Toolkit (ITK). The image analysis pipeline used is described in Figure 11.

First the confocal microscopy datasets were smoothed using five iterations of a median filter that took into account the strong anisotropy of the pixel dimensions, a factor of almost 1:10. The resulting smoothed images have most of the noise removed and present a consolidated region of almost uniform intensity inside the cells. These smoothed images were then thresholded to be used as the speed image for a Fast Marching level set filter [15, 8]. The fast marching filter was run once for each one of the cells identified by AceTree for the current stage of embryo development. At each one of these iterations, the cell coordinates were used as seed point for the fast marching filter. The output of the fast marching filter was then labeled using the cell identifier produced by AceTree. A single image with the consolidation of all the labels corresponding to the cells of the current developmental stage was generated. The original microscopy image was merged with the image of labels in order to produce a two-components datasets. By creating independent transfer functions for each cells, it was possible to highlight individual cells according to selections made in the cell lineage tree. The volume mapper performs a ray-casting through the volume of data and for each sample point applies the appropriate transfer function. An example of individual cells highlighting is presented in Figure 12.

4.1.3 Interaction

The application provides several interaction scenarios that facilitate the exploration of cell lineage, spatial correspondence of the cells, and gene expression. The simplest and most obvious interaction is zoom and pan in the tree layout and zoom, pan, and 3D rotation in the volume display. When zooming in the tree layout, the system exposes more cell annotation. Similarly, when zooming out the tree layout, the cell annotations will hide to improve visibility.

In the tree widget, the user can expand the subtrees to expose the cells in the subtrees. In addition, similar collapsing of subtrees can be performed in the tree layout. By selecting the cell, the subtree rooted in that cell will be hidden, while the cell will still be visible. The cell with the hidden subtree will be marked as such. An example of collapsing subtree in the tree layout view can be seen in the Figure 13.
Finally, there is an elaborate selection system that links all views. A slider is provided to the user to select the time of embryonic development. This will load appropriate volume data corresponding to that time and draw a line on the tree layout at that stage of development. This can be seen in Figures 6, 14, and 7, where the highlighted lines show the current development time. Figure 10 shows the whole application with the selected volume time loaded.

Another selection mode allows user to select the individual cells, groups of cells, or subtrees of cells. Once the user selects a set of cells in the tree widget, or the tree layout, the same set is selected in the other tree view, as well as highlighted in the volume display. In addition, the genes in the gene expression display are highlighted corresponding to the selected cells. The user can also select the gene set in the gene expression display and cells corresponding to these genes will be selected in other views. Figure 9 shows example where several cells and subtrees of cell lineage are selected in the tree widget and corresponding cells are selected in the tree layout.

4.2 Scalability

The initial prototype was developed using a serial pipeline. This means the largest datasets the application can handle is in the order of thousands of cells and the image size in the order of 10 million voxels. For larger datasets, the combination of data parallelism and parallel rendering has to be employed. VTK natively supports data streaming. That said with the technologies in ParaView, the application can render large volumes in parallel.

The approach taken for the large data is to distribute data spatially and based on the cell lineage subtree the cells appear in. Currently ParaView can already process and render the cell lineage in parallel except of the layout of the actual tree. There are several ways to layout the tree in parallel but for the purpose of this application the actual parallel layout was not developed. It will be however, explored in future work.

4.3 Reproducibility

Reproducibility is a fundamental aspect of scientific work. More fundamental even than the peer-review process. After all, peer-review is just a method for verifying the reproducibility of published technical work. Unfortunately, the practical possibility of reproducing every experiment that is reported in the literature is hampered by the amount of technical resources that are needed for running such experiment. In the fields of image analysis and visualization, software itself account for the most expensive resource required to reproduce technical work. The second most costly resource is the access to the original data used by the authors.

The current availability of open source toolkits, such as VTK and ITK, reduces the amount of effort that readers and reviewers must invest in order to reproduce published work. Further improvements can be made when authors share their source code and data along with the text of their papers. In the preparation of this paper we benefited largely from the fully reproducible publications that distributed not only the original microscopy data of C. elegans embryos but also the executables of the AceTree and StarryNight applications used for generating cell lineages [5, 6, 17]. Thanks to the openness of these authors, we were able to reproduce their results with minimal effort, and then we were able to focus on applying the novel resources of the VTK information visualization framework.

In order to keep up to the standards that made this paper possible, we are also providing the source code used to produce re-
Figure 12: Volume visualization of the embryo developmental stage at 90 minutes, with cells \textit{Eav}, \textit{MSapp} and \textit{ABaraapa} highlighted in red, and cells \textit{Capp} and \textit{ABppapd} highlighted in green. This diagram illustrates how the volume visualization can be linked to selections made on the cell lineage tree display.

Figure 13: Tree Layout view with the collapsed subtree rooted in \textit{ABp}.

Figure 14: Tree Layout view and Volume view with the same time selected.

Figure 15: Lineage Mutation

5 Future Work

There are several aspects of this application that should be addressed in the future. They include improved handling of even larger datasets, adding the capability of comparing lineage trees, and adding an option to search for motives in the branching pattern of lineage trees.

Currently the volume rendering is parallelized, but the tree algorithms are not. When exploring higher organisms, such as zebrafish, the number of cells can be a few orders of magnitude higher. The \textit{C. elegans} lineage has around one thousand cells while analysis of the embryonic development of zebrafish will require analysis of up to a million cells. These much larger trees would benefit from parallelized rendering and traversal.

With higher organisms, the cell lineage is typically stereotypic but not completely deterministic, so the option of comparing trees would be extremely useful. This ability will be essential to define exactly how the lineage normally varies from organism to organism and what patterns are conserved. Furthermore, searching for motives in the branching would provide researchers with a powerful tool to detect mutations and find patterns. Figure 15 shows example mutations from \textit{C. elegans} which result in stereotypic alterations in the pattern of the lineage tree. The normal lineage tree is shown on the left. Certain mutations will cause a level of the lineage tree to be skipped while other mutations will cause a level of the tree to be repeated over and over. As can be seen in the figure the nature of these motives can be fairly complex. Lineage motifs also exists in higher organisms in the context of stem cells. Some stem cells follow a motif where one daughter cell remains a stem cell while the other daughter cell differentiates either immediately or after a few rounds of cell division which serve to expand the number of differentiated cells. Using tree comparison or motif searching will allow normal patterns in lineage trees to be identified as well as allow changes in these patterns caused by genetic mutations to be detected.

6 Conclusion

This paper describes an application for analysing the embryogenesis using the combination of information and traditional visualization. The application uses the new Titan informatics toolkit of the Visualization toolkit to display cell lineage trees, and volume visualization of VTK to display the confocal microscopy images. By linking the selection from the tree views, volume view, and the gene expression view, it provides the research several tools required for effective analysis. The algorithms used to perform the visualization are scalable to much larger models, so it is feasible to envision
analysing much more complex embryos. Most examples in this paper were performed on the dataset of C. Ellegans embryos. However, the application will be extended to perform the same operations on a much larger datasets of zebra fish. In addition, because the application is based on open source, general purpose toolkits, it can be readily extended to perform other analyses, such as searching for motives, gene expression visualization, and cell lineage comparison.

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