White Paper

Bio-IT World 2005 Best Practices Awards
Confocal Microscopy Data Analysis:
A Real-Time Image Analysis and Visualization Solution

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1.0 Abstract

Microscopy scientists currently have to work in an environment where there are multiple standards for image and annotation data. Furthermore, automated algorithms for identifying image features generally perform poorly when asked to interpret the complex structures observed in microscopy samples.

This article presents results from a research project executed by SGI in collaboration with the National Cancer Institute's Advanced Biomedical Computing Center and the National Cancer Institute's Image Analysis Laboratory. Our project goal was to develop new tools that accelerate the workflow for microscopy scientists through novel visualization, analysis, and collaboration capabilities. We present our solution, show how it facilitates efficient analysis of large datasets, and discuss results of several tests on microscopy samples provided by NCI for evaluation.

SGI proposed the development of an interactive segmentation application for microscopy datasets using an optimized process on SGI parallel-processing computers. Scalable graphics provides interactive viewing of the datasets and segmented views in a three-dimensional rendered interface. Direct user interaction with a 3D-volume is possible using the hardware-assisted rendering capability of SGI® OpenGL Volumizer™. With our solution, a biologist can selectively run and review results of segmentation algorithms interactively as they are performed.

The end result of our project is an extendable, open environment for segmenting and interacting with microscopy datasets using graphical widgets inside a realistic three-dimensional interface. As the datasets from instruments continue to grow in size, this solution is ready for processing large datasets because analysis algorithms can be run across shared-memory multiprocessors in the Silicon Graphics Prism™ architecture.

2.0 Background, Vision and Project Goals

The power and capability of confocal microscopes have developed substantially in recent years as multiple vendors each add unique features to their products. Unfortunately, there is still a lack of standard formats and processes available to biologists, particularly those who employ multiple instruments. Each instrument comes with its own software and capabilities vary between vendors. Currently, biologists have to divert their focus away from their core investigations to reformat sample data and utilize multiple software analysis tools from different vendors.

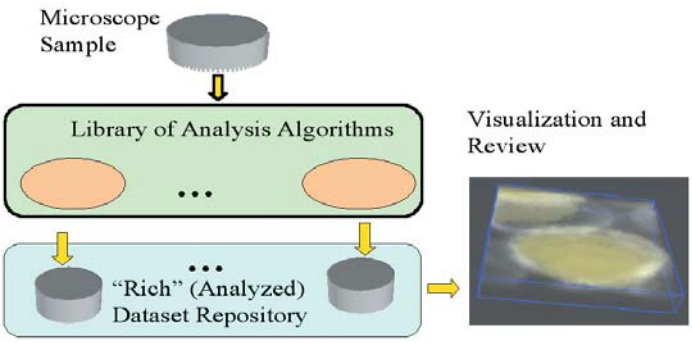

Figure 1 - Multiple Views of Datasets

SGI believes in establishing a computing foundation that "amplifies" a scientist's capability to understand, interpret, and analyze observed microscopy results. Currently, the lack of robust, accelerated segmentation algorithms has been a bottleneck limiting computer-assisted interpretation of microscopy datasets. Our combined analytical and visualization components enable a researcher to create and catalog a variety of different "views" of their observed data, each created by a separate automated analysis. These "derived datasets" (for example, consider a segmentation, then a cluster labeling, and then a per-cell volume calculation) can be viewed independently or combined to represent a fully interpreted dataset. We see this concept illustrated in Figure 1.

As they are developed, additional analysis algorithms can be added to the library of tests available for execution on source datasets. After computer-assisted analysis, the "rich" dataset repository will contain a set of correlated views of the original source. We see this process culminating in an automatic biological analysis system – providing a powerful aid to future cancer research.

In the scope of work described here, we focused on the initial rendering and segmentation. However, the work accomplished laid the foundation for future projects, such as an interface for a rich data repository, to develop this "smart" dataset repository.

3.0 Solution Components

Our solution contains subsystems that handle dataset interaction, volume visualization, segmentation, creation of 3D models corresponding to image features, and storage of analytical results. In the following paragraphs, we further describe some of our system capabilities.

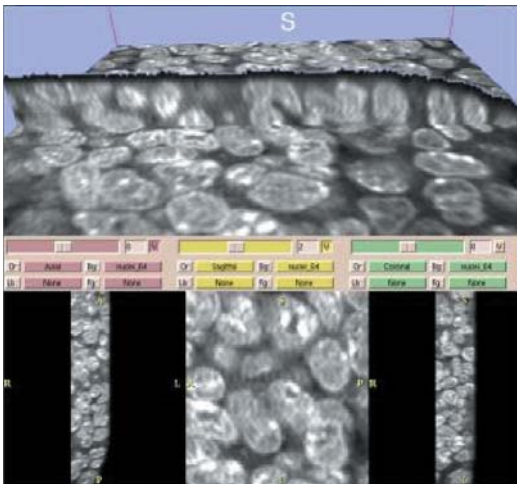

Figure 2 - 3D Slicer User Interface

Interactive User Interface

The problem of loading, viewing, analyzing, annotating, and storing volumetric datasets is a problem common to microscopy scientists and clinical imaging researchers. We decided to leverage a previous investment made by the clinical imaging community and employ the existing 3D Slicer user interface (Slicer2005)[1] . 3D Slicer is an open source project that allows researchers to interactively examine and process medical imaging datasets, such as MRI and CT scans. Slicer handles ingesting volumes from multiple data formats and provides graphical widgets for the user to efficiently examine features in the dataset. An example screenshot of Slicer working on a microscopy sample is shown in Figure 2. Slicer provides other tools for positioning fiducials, measuring distances between arbitrary points in the dataset to measure features, creating histograms of the dataset, and others.

Slicer also provides a plug-in architecture to include extensions, such as new rendering and analysis algorithms. Our team utilized plug-ins to extend Slicer to implement hardware assisted visualization using the SGI OpenGL Volumizer API.

Visualization

To facilitate efficient visualization of tissue and microscopy volumes, SGI used OpenGL Volumizer which efficiently handles large volumes by pre-loading the voxels into high-speed texture memory in graphics pipelines and allowing interactive speed rendering. As the view is interactively moved, OpenGL Volumizer regenerates appropriate geometry to render with high image quality, yet maximized rendering speed.

OpenGL Volumizer handles extremely large volume datasets through its use of volume roaming, multiresolution rendering (SGI2002)[2] , and scalable graphics hardware (SGI2004). These capabilities allow rendering operations to move freely through a volumetric dataset and achieve an interactive-speed application frame rate that would otherwise be impossible.

As a test case, SGI received a microscopy dataset from NCI ABCC and used OpenGL Volumizer to visually isolate nuclei and cell walls without the need for off-line image processing or costly segmentation algorithms. This “visual segmentation” and visual review was performed interactively by the user through interactive volume transfer functions (a feature available with SGI OpenGL Volumizer.) In Figure 3, a dataset stained for cell wall structure is shown in the Slicer interface with OpenGL Volumizer-accelerated rendering.

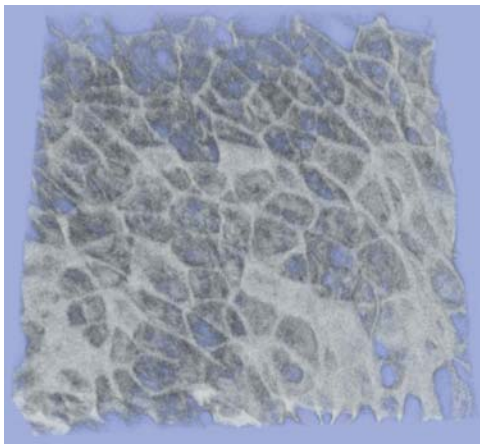

Figure 3 - SGI Volumizer

Segmentation

Since one of our goals was to facilitate the development of new microscopy-specific image interpretation algorithms for microscopy, we wanted a software library for segmentation that would ease new algorithm creation. Segmentation is a computationally intense application and is often too slow for use in interactive applications unless the implementation runs multithreaded on a parallel processing computer. Since multithreaded algorithm development is tedious and time-consuming, we looked for an environment that hides as much of the parallel processing details as possible while still providing scalable performance on shared memory computers, such as the Silicon Graphics Prism visualization system or the SGI® Altix® scalable computing system.

The National Library of Medicine Insight Segmentation and Registration Toolkit (ITK)[3] is an open-source software system designed originally to support the Visible Human Project. Currently under active development, ITK employs leading-edge segmentation and registration algorithms in multiple dimensions and provides a software system that can be extended for microscopy-specific analysis. Using ITK allows both the clinical imaging and microscopy communities to employ a common analysis framework and share effort. For a history of its conception and more information on ITK, please refer to (Yoo2005)[4] or (http://www.itk.org).

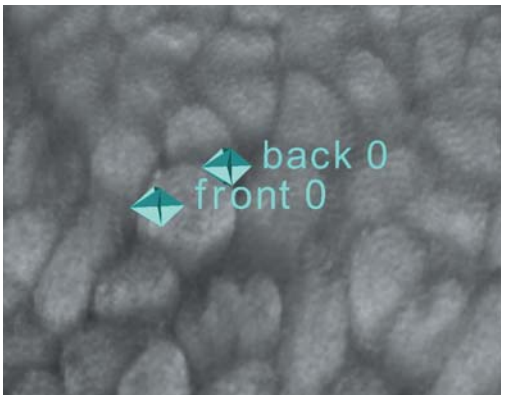

Figure 4 - Interactive Measurement

4.0 Results

To evaluate our system, we used a dataset taken by a Zeiss LSM 410 confocal microscope stained to display cellular nuclei. In the following paragraphs, we describe several processes performed during the analysis and interpretation of the volume captured from the microscope.

Marking and Measuring:
The scientist is able to place and query 3D cursors throughout the dataset under analysis. Employing user-controllable 3D markers (see Figure 4) we selected a nucleus and measured it interactively by placing two locatable markers at observable nucleus boundaries. The markers can be used in multiple ways, according to the user's purposes – including visual feature highlighting, interactive measurement, or as seed points for analytical algorithms.

Parallel, Interactive Segmentation:
Through the user interface, a level set segmentation algorithm from ITK was invoked on the sample to identify individual nuclei with minimal user interaction required. The interface provides the biologist an opportunity to guide the segmentation, which is required for microscopy even using today's best segmentation algorithms. Our environment provides segmentation algorithms from the state-of-the-art clinical image analysis system (ITK) embedded in an interactive, immersive, accelerated interface. The biologist invokes and reviews automated algorithms all through a 3D view of her or his dataset (see Figure 5). We achieved speedups of 40% over a single processor when we used dual CPUs, and further speedups as we added additional processors. On our test dataset, the biologist received a visual update of algorithm progress every 10-15 seconds.

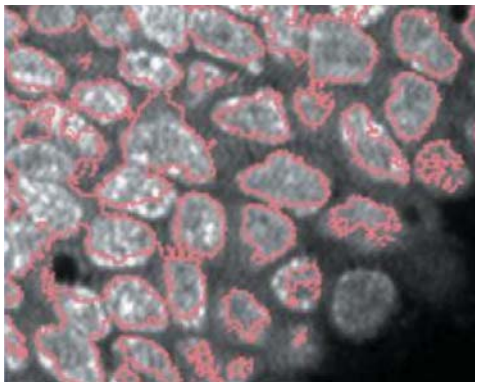

Figure 5 - Interactive Segmentation

Visual Insight:
In addition to automated analysis, the user is embedded in a three-dimensional view of their volumetric dataset. He or she can draw conclusions on the observed volume through direct manipulation of the mapping function between source voxels and colors displayed on the screen. Using this technique, the biologist can review the processing accuracy of algorithms by comparing algorithmically-created segmentation boundaries with source voxels. Here in Figure 6, we show how a user detected a previously undetectable, invisible, circular feature because the red voxels behind the invisible feature show through to the user. This "punch through" was visible only after the user manipulated the color lookup table, and hadn't been detected looking only at the image histogram or segmentation results.

5.0 Future Work

To increase the efficiency of a team of scientists working together to analyze a set of laboratory observations, we plan to further extend our microscopy research system through integrating with a scientific image and metadata database system, such as the Scientific Image Database (SIDB) (SIDB2005)[5] or the Open Microscopy Environment (OME) (OME2005)[6]. Once the database integration is completed,

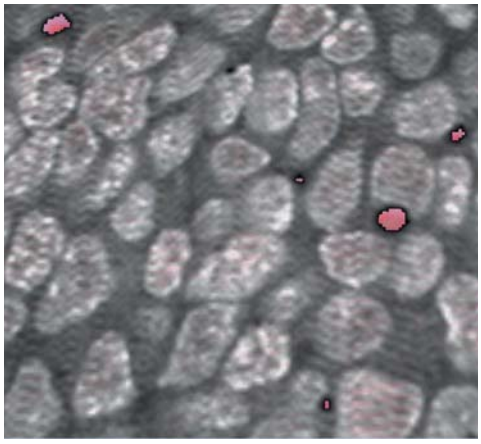

Figure 6 - Features Discovered

our microscopy environment will be a powerful analysis client to a database rich enough to store annotations, notes, and observations from a team of scientists, either co-located or geographically dispersed.

Improving the speed and scalability of volume rendering using SGI's OpenGL Volumizer and scalable graphics hardware is a second area of future work to extend our system. Images of ever increasing size are being captured by today's confocal microscopes. These images are, even now, becoming harder to visualize and analyze using today's available software packages. With the current microscopes and their ability to capture multi-dimensional datasets, scalability will be a primary requirement for microscopy analysis software.

6.0 Conclusion

Our microscopy solution employs parallel processing resources on the Silicon Graphics Prism for high speed segmentation and volume interaction. Our project utilizes the mature 3D Slicer GUI and an expandable and multithreaded segmentation library (ITK). Hardware-accelerated dataset rendering is provided by OpenGL Volumizer. This solution is available on, Linux®64, Linux32, IRIX®, and Windows® – making this application deployable on many different platforms and operating systems. Our collaborators at NCI are just now beginning to use this environment, and are expecting noticeable efficiency improvements in their efforts to study the small-scale biological processes that can spread the onset of cancer.

7.0 References

(SGI2003) OpenGL Mulipipe SDK Users Guide, Version 3.0.1, *SGI Technical Publication 007-4239-004,* 2003.

[1] Slicer2005 www.slicer.org

[2] SGI2002 SGI OpenGL Volumizer 2 Programmer's Guide, *Technical Publication 007-4389-005,* 2002.

[3] ITKApp2005 NLM Insight Registration and Segmentation Toolkit; http://www.itk.org/HTML/Applications.htm

[4] Yoo 2005 Yoo, T. S., and Ackerman, M. J., Open source software for medical image processing and visualization, *Communications of the ACM,* Vol 48, Number 2, February 2005, pp. 55-59.

[5] SIDB2005 The Scientific Image Database homepage; http://sidb.sourceforge.net/

[6] OME2005 The Open Microscopy Environment homepage; http://openmicroscopy.org/

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