Collaborative Visualization in the Browser for Segmentation, Tracking, and Lineaging with 5-D Biological Microscopy Images

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***Abstract*—The reliable analysis of 5-D fluorescence microscopy image data requires effective visualization tools. Visualization is essential for the development, validation, and dissemination of the analysis algorithms that extract features from the images. The ubiquitous availability of hardware accelerated graphics in the modern web browser will enable a paradigm shift in the analysis of complex biological microscopy data. Here we describe a tool called CloneView 3-D that provides high quality rendering of 5-D image data. The computational analysis results can be overlaid on the image data and explored interactively. The system can be hosted on any HTML server for stand-alone visualization. We have also developed a light weight custom server that enables synchronized viewing between multiple connected clients which enables interaction with image analysis tools in a scripting environment such as MATLAB.**

I. Introduction

High dimensional images captured by confocal fluorescence microscopy enable the study of live proliferating cancer and stem cells in their intact tissue micro-environment. Microscopes with incubators are capable of keeping tissue alive for prolonged periods and produce 5-D image data (x, y, z, λ, t) where λ represents different fluorescence imaging channels. The analysis of this data requires segmentation, tracking and lineaging. Segmentation delineates the individual cells. Tracking establishes temporal correspondences between segmentation results. Lineaging establishes parent-daughter relationships. Visualization is a fundamentally important task in the analysis of 5-D microscopy images. The ability to visualize the image data together with analysis results is essential for developing and evaluating analysis algorithms and for validating the analysis results to ensure that they are error-free. Visualization is also useful in itself as a tool for discovery and for generating new hypotheses.

The analysis of complex biological microscopy image data often involves teams of engineers and biologists working together. To enable geographically separated interdisciplinary teams to collaborate on the analysis of 5-D microscopy data, there is a need for visualization tools that can run on any modern computing device and that do not require download, installation, and maintenance of large software programs.

We have developed an interactive multi-user 5-D image viewer called ***CloneView 3-D***. CloneView 3-D uses WebGL, HTML5, and Javascript and implements custom ray-casting shaders. The client can be used with any generic HTML server or can be used with our custom server that includes multiuser communication and integrates with scripting environments to enable interactive visualization of the image analysis.

The capability of this new tool is illustrated using images collected from explant of an adult stem cell niche present in the mammalian forebrain. This stem cell niche, the subventricular zone, includes a variety of cell types that regulate stem cell behavior. Analysis of 3D images of fixed specimens is valuable to study the relationship of the stem cells to other cells in the niche such as blood vessels. Movies of living explants provide unique insight into the dynamics occurring as stem cells divide and produce daughter cells that differentiate and migrate out of the niche.

II. Related Work

Recently tools have been created to display high dimensional datasets in an interactive manner [1], notably Vaa3D [2], BioImageXD [3], Fiji [4], and Icy [5]. The Lever [6] and Lever 3-D [7] software that we have previously developed is unique in that it allows the segmentation, tracking, and lineaging results to be displayed along with image data. The main drawback to these tools is that they need to be installed and tested on multiple computers, operating systems, and hardware which slows development. To alleviate these problems and expedite development, programmers are turning to web based solutions. Web applications are typically device agnostic and operate across platforms, along with having only one instance of code to maintain. Another benefit to a web application is the ability to share with others without requiring them to install custom software. This can ease collaboration and communication between geographically separated parties.

bioWeb3D [8], Bisque [9], and xtk [10] are programs that run in a web browser and display high dimensional image data with annotations. They do not, however, have integrated image analysis tools nor a way to view tracking or lineaging data. CloneView 3-D allows image processing tool kits, like that of MATLAB, and direct access to the data being displayed. This gives the programmer the ability to develop, execute, and display the results of new algorithms in real-time. Being able to develop new algorithms directly in front of the domain expert is a powerful tool that will allow for rapid prototyping and validation.

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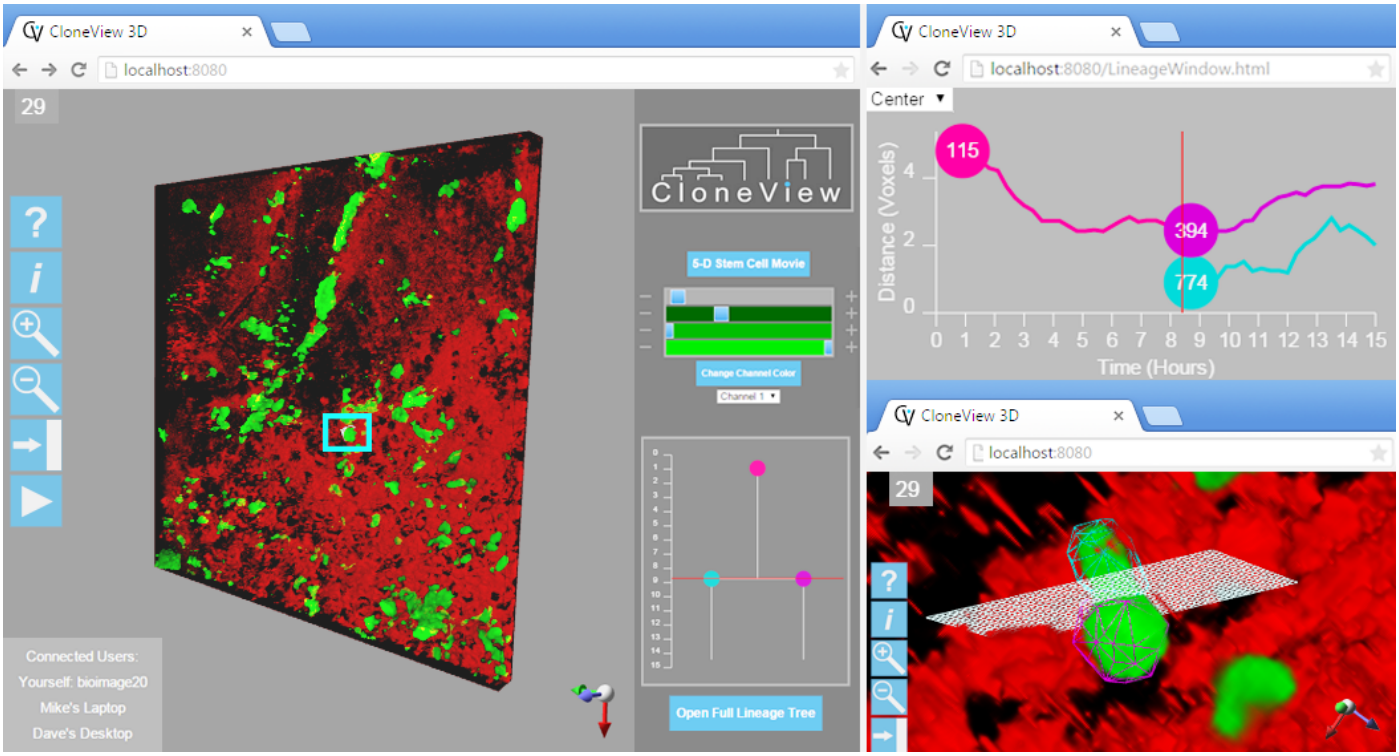

Fig. 1. CloneView 3-D is a WebGL/HTML5 application that enables distributed visualization of 5-D image data with segmentation, tracking, and lineaging overlaid. The main view window (left) allows the viewer to interact with the image data in real-time. The user can rotate the image in 3-D, zoom, explore the time sequence, change the viewing intensity of each channel's voxels independently and toggle the display of segmentation and tracking results. Changes are reflected on all connected browsers. Distances between structures can be encoded on the lineage tree (top right). The bottom right panel shows a zoomed view of a mitosis showing daughter segmentations and cleavage plane.

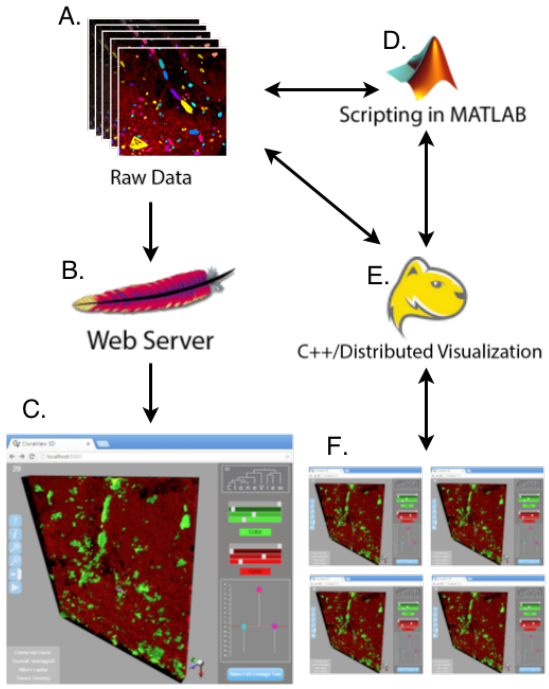

Fig. 2. CloneView 3-D can be used for either stand alone or collaborative visualization. In stand alone mode (A, B, C) the image data, analysis results and Javascript client code can be hosted on any web server. For collaborative visualization a custom server based on the Mongoose embedded web server (E) manages communications among clients and optionally between clients and a connected image analysis / scripting environment such as MATLAB.

III. Methods

We have developed an interactive 5-D image viewer that runs in any modern web browser called ***CloneView 3-D***. With a WebGL enabled browser, users are able to view 5-D (x, y, z, λ, t) images sequences along with segmentation, tracking, and lineaging results. CloneView 3-D has three major components: WebGL, HTML5, and Javascript for rendering and user interface; a light weight custom server for distributed viewing capabilities; and the ability to integrate into scripting development environments like MATLAB. The first component uses A, B, and C in Fig. 2 and can act as a stand-alone web visualization tool installed on any computer running a web server. When using our custom server derived from the Mongoose code base [11] (E in Fig. 2), each browser window is synchronized so that any user can manipulate the current view (F in Fig. 2). This server is written to allow MATLAB direct access to the raw data and analysis results (Fig. 4), enabling manipulation and viewing in real-time across all clients. By exploiting the convenience of a scripting language, this tool has the ability to greatly reduce the time it takes to develop new image analysis algorithms.

A. WebGL

Full rendering capabilities in the browser allows utilization from any location and avoids the need to install and update local software. However, not all browsers have the same support for WebGL. Chrome and the new Microsoft Edge have the best support with Firefox close behind, but Internet

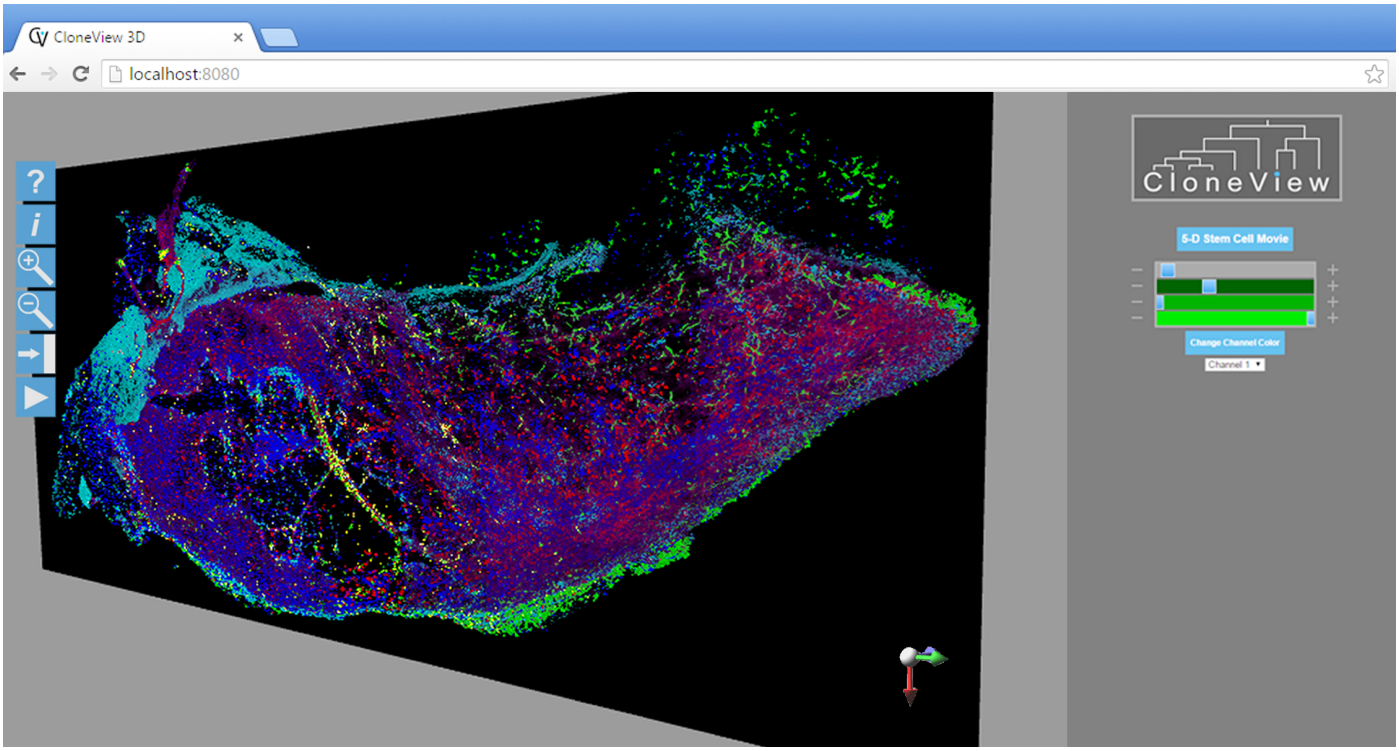

Fig. 3. Interactive visualization of a complex 3-D image of an intact, microdissected mouse adult brain neural stem cell region, the sub-ventricular zone. The image is a montage of 39 overlapping image stacks. The channels label blood vessels and ependymal cells (red); oligodendrocytes precursors (yellow); migrating neuroblasts (cyan); GFAP positive stem cells, and mature astrocytes (green); transit amplifying cells (magenta); and DAPI stained cell nuclei (blue). The image has 10,098 $\times$ 4,491 $\times$ 60 (x, y, z) voxels spanning approximately 4.5mm $\times$ 2.5mm $\times$ 60μm.

Explorer has only recently added WebGL support and has limited abilities to run complex shaders. We have created custom shaders for each browser to maximize compatibility. WebGL can take advantage of hardware acceleration provided by graphic processing units (GPU) and performs best on computers with dedicated GPUs that have enough memory to fit the entire image sequence. CloneView 3-D can still be viewed on computers with integrated GPUs that use system memory, however frame rates will be lower. Most modern computers and even laptops are shipping with high quality dedicated GPUs and these limitations are more typical for smaller low powered devices such as tablets and phones.

To view a 3-D texture in the browser, a complex volumetric ray-casting [12] shader was created using WebGL's shader language. WebGL (1.0) allows for 16 2-D texture units with maximum dimensions varying between browser versions. The 5-D data shown in Fig. 1 has 50 2-channel 3-D images, each with a dimensions of 512 $\times$ 512 $\times$ 25, which is 2,500 image slices. Each image stack for a given frame needed to be placed in a 2-D mosaic with max dimensions of 8,192 $\times$ 8,192, chosen based on the data presented by WebGLStats [13]. WebGL Stats shows that over 75% of currently installed browsers have the capability to load textures of that size. Loading time of image and segmentation data can vary depending on Internet connection speed. Images are compressed and saved as `JPG` files and have a typical size of 1.5 MB per frame per channel. If there is not enough memory to store all of the time sequence on the GPU, WebGL will use system memory to cache data until needed. A 64-bit browser is necessary for uncompressed image sequences that are greater than 4 GB in size. The theoretical limit is up to 16 channels to be blended for each frame and as many frames as can fit into system ram.

CloneView 3-D uses Javascript to load segmentation from the associated `JSON` files into the shaders. Segmentation, tracking, and lineage data stored in `JSON` format has a typical size of 4 MB per frame and is dependent on how many objects appear in each frame. The shaders overlay this segmentation data onto the 5-D image data which can be selected and toggled interactively. Tracking and lineage data is also loaded and is displayed as a simple lineage tree in the main viewing window (left panel in Fig. 1) or a more detailed lineage tree that has distance between objects encoded on the Y axis (top right panel in Fig. 1). Mitotic events are also highlighted by displaying a cleavage plane between the separating cells to help to visualize the polarity of the event (bottom right panel in Fig. 1).

The main viewing window allows the user to rotate the image in any direction, zoom, play through the time sequence, change the viewing intensity of each channel independently, and toggle segmentation and tracking results. When using CloneView 3-D through the custom web server (E in Fig. 2), all of these viewing parameter changes are reflected across each browser (F in Fig. 2).

B. Distributed Data Viewing

We have developed a custom web server derived from the Mongoose embedded web server code base [11]. Mongoose is a simple server written in C/C++ that can be run on any computer and is easily modified. Our custom version includes a message passing framework using websockets (E in Fig. 2)

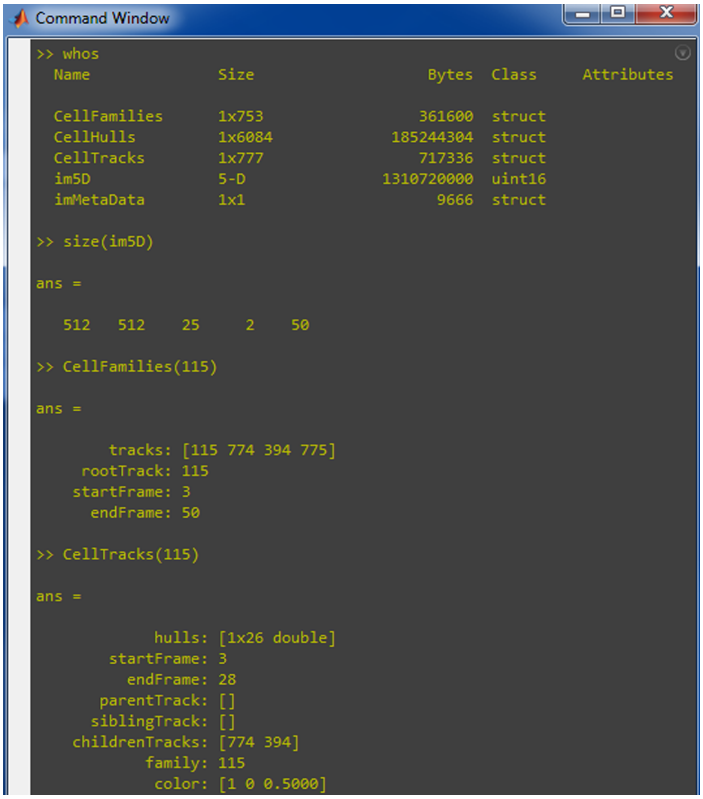

Fig. 4. MATLAB interface to the CloneView 3-D server, showing lineage and track information for the clone displayed in Fig. 2. For each movie, the CellFamilies structure contains the lineage information, the CellTracks contains tracking information and the CellHulls contains segmentation information. All of the time sequence analysis is available for processing and export. im5D is the 5 channel image data where each channel can be processed independently using the image processing tools. Following processing, the display of image data and analysis results can be updated on all attached clients.

ensuring that each attached browser is viewing the same state. Because each browser has a full copy of the data on initial load, these messages are small and updates can be sent in real-time.

This lightweight server can be started directly from a scripting environment like that of MATLAB. By doing so, MATLAB has the ability to manipulate the data (Fig. 4) and update all the viewers at once. MATLAB also receives messages back from the server with the current state and is aware for instance when users select a particular cell. The limits of how many clients can be connected concurrently has yet to be tested, but it is sufficiently responsive with a dozen or so clients all connected to a fast internet connection. By allowing MATLAB complete control over the data, geographically separated teams will now have the ability to develop new segmentation, tracking, and lineaging without the need to send complex programs that need to be installed. Having a domain expert evaluate results immediately after prototyping will increase the pace of innovation and at the same time optimize accuracy.

IV. Conclusion

We have developed CloneView 3-D as a distributed 5-D image viewer that integrates segmentation, tracking, and lineaging results. This viewer can be run on a stand-alone server or as part of a distributed visualization tool that is run from a scripting environment. The ability to run and visualize new image analysis algorithms directly with complex image datasets synchronized between multiple browsers will increase the pace of development. This is only possible now with the advent of WebGL, HTML5, and Javascript along with the availability of hardware accelerated graphics. Geographically separated collaborators will no longer have to install and maintain complex software tools during the development of image analysis tools. This will expedite the development and validation of analysis tools for high dimensional complex data sets such as the study of live proliferating cancer and stem cells in their intact tissue micro-environment.

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