

# VisBio: a Flexible Open-Source Visualization Package for Multidimensional Image Data

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## Introduction

Over the past few years there has been a dramatic improvement in microscopy acquisition techniques, in effective imaging modalities as well as raw hardware performance. As the microscopist's available tools become more sophisticated and diverse—e.g., time-lapse, Z sectioning, multispectra, lifetime,  $n^{\text{th}}$  harmonic, polarization, and many combinations thereof—we face a corresponding increase in complexity in the software for understanding and interpreting the resultant data. With lifetime imaging, for example, it is overwhelming to study the raw numbers; instead, an exponential curve-fitting algorithm must be applied to extract meaningful lifetime values from the mass of photon counts recorded by the instrument.

For the past several years, the Laboratory for Optical and Computational Instrumentation (LOCI, <http://www.loci.wisc.edu>) has been working toward a complete infrastructure for acquisition, storage, visualization and analysis of such multidimensional microscopy data (1). In this article we will describe our efforts with VisBio—an open source, cross-platform application for the visualization and analysis of such data, with an emphasis on flexibility, scalability, ease of use and connectivity with other useful software applications such as ImageJ (2), MATLAB and the Open Microscopy Environment (OME) (3).

VisBio has been developed to combat several cumbersome realities of the current and emerging microscopy landscape:

- **Size:** Datasets are often difficult to work with effectively due to their growing size—sometimes larger than the computer's available memory.
- **Complexity:** Datasets may possess numerous dimensional axes, some spatiotemporal (X, Y, Z, time) and others not (lifetime, spectra, polarization), requiring several forms of analysis before the analyst can perform even basic visualization.
- **Variety:** Datasets are stored in myriad different file formats and numbering schemes—many proprietary—further impeding analysis.

To overcome these issues, we have built VisBio with a powerful multidimensional data engine that targets the goals of scalability, flexibility and connectivity (4). We have also arranged the software to be

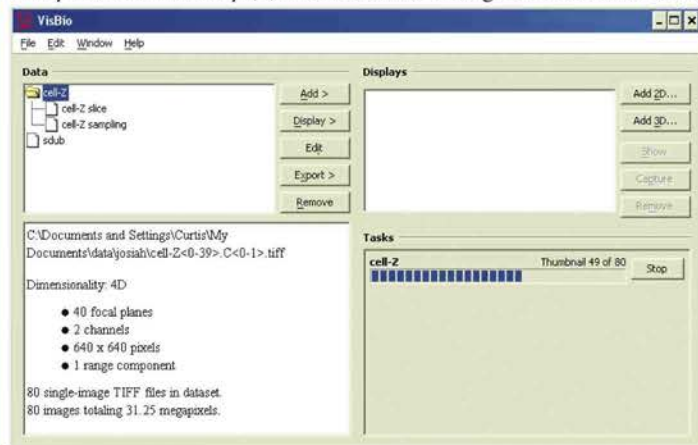


Figure 1: The multidimensional data engine generates low-resolution thumbnails for a dataset. The data panel shows datasets, some derived from others using the arbitrary slice and data sampling transforms.

(we hope) easy to use, and included a built-in help manual describing each feature in detail.

## Multidimensional data engine

VisBio is aware that image planes can be distributed across a variety of dimensional axes, and imposes no limit on the number of such axes within a dataset. When files are imported into VisBio from disk, the software analyzes the file naming structure and image metadata to identify potential dimensions. If it is not 100% certain of the nature of an axis (e.g., Time, Slice, Channel, Lifetime, Spectra), it takes a “best guess” and allows the user to override its selection.

As of v3.22, VisBio supports more than 25 file formats common in microscopy. Recently, we realized that the scope of our file format support was large enough to warrant its own package, LOCI Bio-Formats [<http://www.loci.wisc.edu/ome/formats.html>], which includes additional tools such as an ImageJ plugin for importing supported formats directly into ImageJ.

Though the program gathers information to fully understand the dataset, it does not read in and store all image planes in memory. Instead, when the dataset is first loaded, VisBio spawns a background task that generates a low-resolution thumbnail for each image plane (Figure 1), to allow quick browsing of datasets larger than the computer's available memory. When more detail is required, the program maintains scalability by reading the required image planes from disk on the fly.

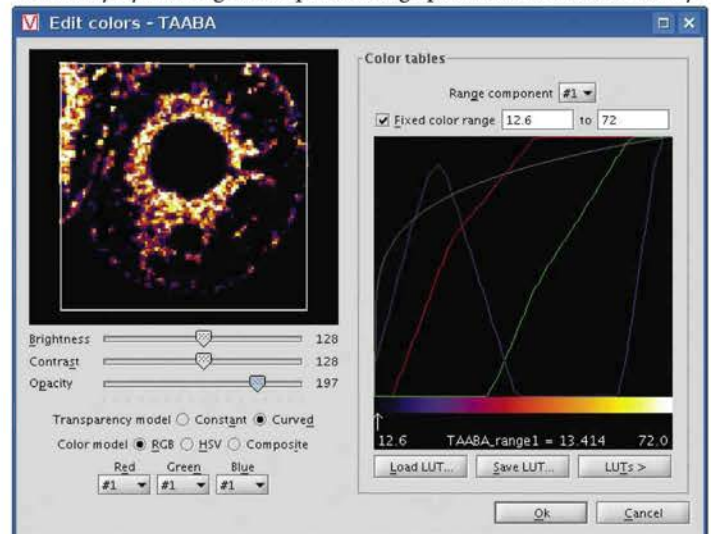


Figure 2: The color settings dialog box shows a colorization of a two-photon dataset of a two-cell hamster embryo labeled with a mitochondria-specific dye, Mitotracker-X-Rosamine. Images were collected as a Z-series of five optical sections, 0.5 microns apart, by Dr. Jayne Squirrell of the University of Wisconsin-Madison.

VisBio's data engine does not merely maintain a list of currently loaded datasets, but rather a tree of currently available data objects (Figure 1). Each dataset represents one branch of the tree, with VisBio providing several “data transforms” to produce derivative data objects from the datasets. For example, VisBio includes a “data sampling” transform for reducing the size of a dataset across any dimension. Simple image scaling can be performed by reducing the image resolution (X and Y axes), and the number of planes distributed across each additional dimensional axis can be decreased as well.

These data transforms can also be performed sequentially, such as in the case of combined spectral lifetime data, where first an exponential curve fit must be applied to each spectral channel, and then the resulting lifetimes must somehow be reduced with a spectral weighting or stripping algorithm to produce the final values for visualization. Functionality within VisBio specific to spectral and lifetime data is still under development, but the flexible infrastructure has been designed to allow for a variety of approaches in processing such data, regardless of



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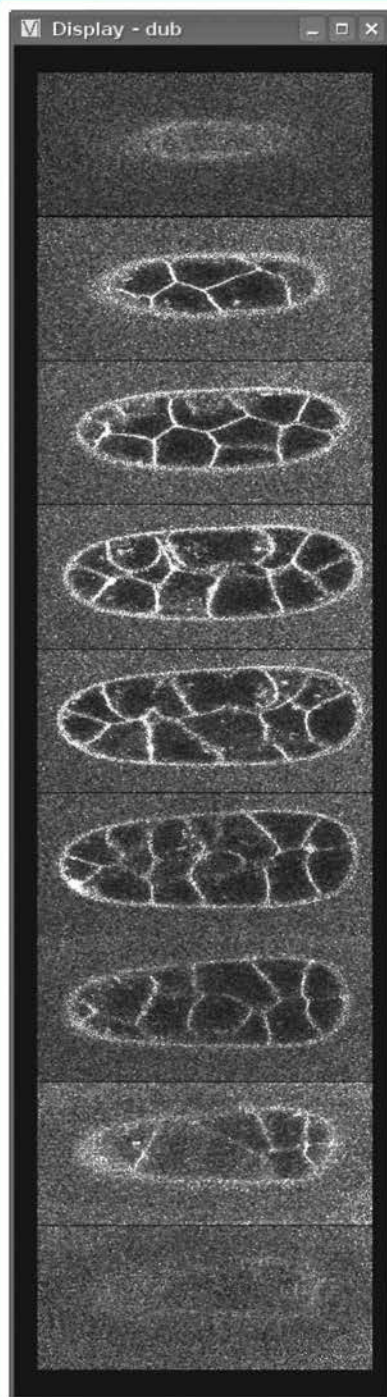


Figure 3: The 3D view shows an image stack using an orthogonal projection for comparison between image planes. Dataset is a *C. elegans* embryo undergoing cell fusion and imaged by multiphoton microscopy, provided by Dr. William Mohler of the University of Connecticut-Farmington.

order (the order the objects appear on the display list) to determine which image planes are beneath which others, and how translucent they are. Each RGB lookup table is fully manipulable by dragging the mouse, and color tables can be saved to disk in ImageJ's LUT (lookup table) format. Several preset tables are provided, as well as common tools for color adjustment such as brightness and contrast sliders. For quick adjustment of transparency, an opacity slider is provided that

the dimensional complexity.

### Visualization and analysis

VisBio provides the ability to visualize image data in two and three dimensions with the VisAD visualization toolkit (5), a Java library for discrete numerical data processing and visualization. The display logic has been kept completely separate from the data engine, so that data import, transforms and export to disk or to other software can all be performed without the overhead of creating a potentially expensive visualization.

That said, at its core, VisBio was designed as a tool for visualization and analysis, providing 2D and 3D display capabilities, with interactive pan, zoom and rotation. Any number of data objects may be overlaid within a single display, and each data object may be added to multiple displays to present different visualizations. When the application is aware of the dataset's physical dimensions, the aspect ratio is automatically adjusted to match; in any case, the aspect ratio is fully configurable. The dataset may be quickly browsed using the dimensional sliders or arrow keys to step through dimensional positions, with the low-resolution thumbnails used first, and full resolution images read from disk and displayed after a user-defined delay has elapsed.

Complete control is provided over color mappings including transparency (Figure 2). Each color channel is assigned its own RGB lookup table with alpha, with the final color value at each pixel being generated from the sum of the table values for each channel. In the case of multiple data objects within a single display, the transparency values are used together with the rendering

functions in one of two ways: constant, which sets the alpha value across the entire table to a single value; and curved, which sets the value according to an exponential function such that the leftmost color value is totally transparent, the rightmost color value is totally opaque, and the slider controls the nature of the curve from min to max—the minimum slider value bends the curve downward such that the entire table is nearly transparent, the maximum slider value bends the curve upward such that the entire table is nearly opaque, and the central slider value is a straight line.

Displays in 3D show stacks of image slices along a designated “stack axis” (typically the Z axis if one exists). Slices can be toggled individually or *en masse* to provide the most effective picture of the data. The projection may be toggled between perspective (realistic) and parallel (orthogonal) display modes, with the latter being useful for generating tall stacks of images with identical orientations (Figure 3). A variety of other settings are provided, including scale bars, bounding box and slice highlighting.

Semi-transparent volume rendering can be employed to produce a volume visualization with interactive rotation (Figure 4). The algorithm functions by interpolating the data to an  $N \times N \times N$  cube, with user-defined  $N$  between 64 and 192 (to conserve memory), and generating planes at six orientations, one per cube face. When the display is rotated, the orientation most parallel to the plane of view is used, as though the user were looking down through the planes. To experience the illusion of a volume, the transparency controls described above should be employed to cull unwanted elements by assigning small alpha values within their color ranges, while emphasizing desired structures by assigning large alpha values within their color ranges. Needless to say, this type of volume rendering works best when wanted and unwanted areas do not have a lot of color overlap. Often, the curved transparency model with transparency slider set between 70 and 130 will provide a reasonable volume rendering, since structures are often colored more brightly than the background is.

Another powerful feature VisBio possesses is the ability to slice the image stack at arbitrary angles to provide cross-sectional views at various points. Arbitrary slicing is implemented as a data transform, with each slice being defined as a new data object beneath the object

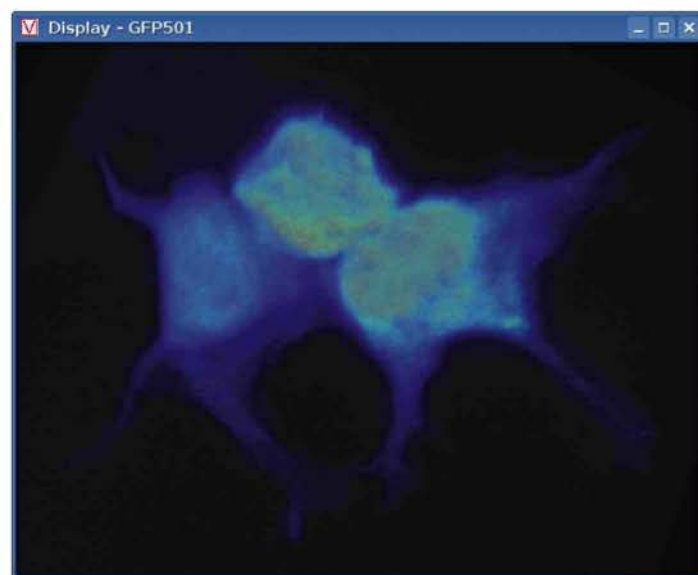


Figure 4: Three-dimensional reconstruction of human embryonic kidney cells (HEK293) that were transfected with a plasmid construct which expresses GFP. Cells were fixed with 3% paraformaldehyde and observed under a laser scanning confocal microscope. The GFP signal is observed throughout the cell. Dataset provided by Dr. Carrie Graveel, Mr. Lance Rodenkirch and Dr. Peggy Farnham of the University of Wisconsin-Madison.





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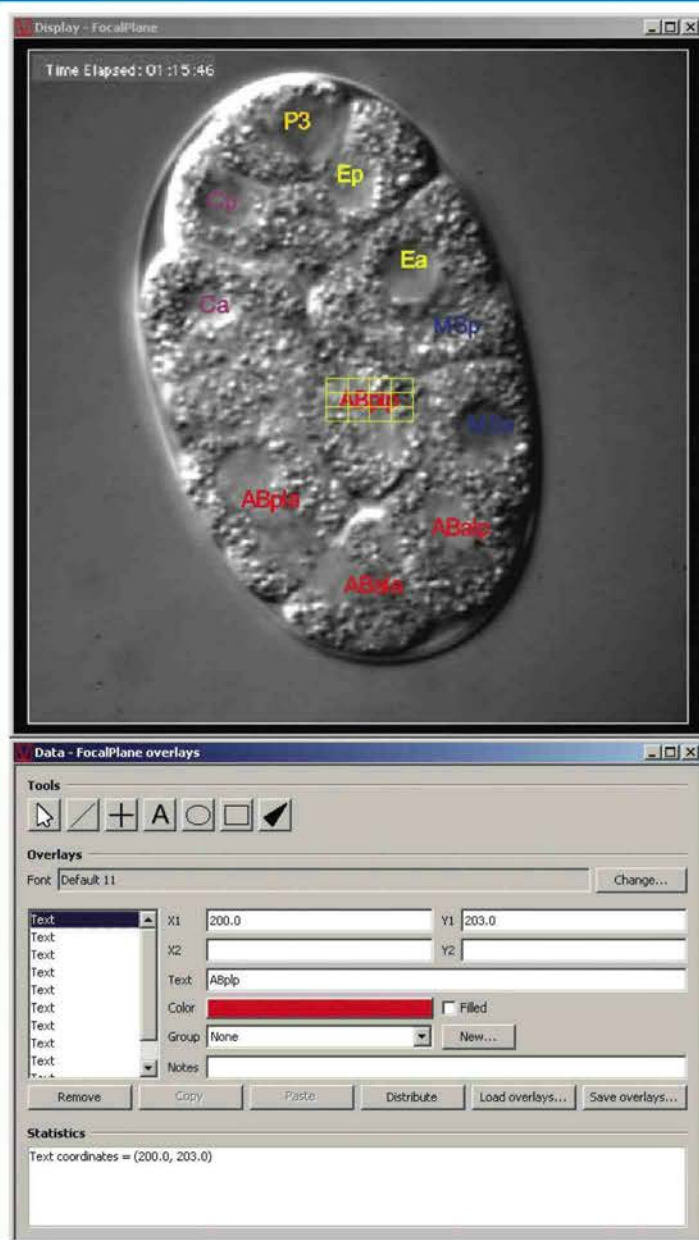


Figure 5: Overlays displayed over a developing *C. elegans* embryo illustrate the organism's lineage from single cell to 100-cell. Dataset provided by Lisa Peterson and Fong-Mei Lu of University of Wisconsin-Madison.

to be sliced; thus, multiple simultaneous slices are possible. The slice angle is defined by the rotational parameters yaw and pitch, with location controlling where the slice appears along the slicing line. The slice resolution is also independently configurable; at modest resolutions, the arbitrary slice can be animated in real time, while at higher resolutions the slice appears crisper with more detail. The software also allows the user to select whether the slice is recomputed while the sliders are being adjusted, or only after the mouse button is released.

VisBio also comes with an overlays feature allowing the construction of glyphs in various shapes atop image planes in 2D (Figure 5). The overlays logic is implemented as a data transform, with each set of overlays being defined as a new data object beneath the marked up object. Possible overlay types include markers, lines, ovals, boxes, arrows and text. Overlays can be individually colored and divided into groups. Results can be saved or exported to a tab-delineated text file for use with a spreadsheet application.

#### Export features and connectivity

VisBio provides several ways to export data. The data engine allows direct export of a data object to TIFF files on disk, sent straight

into ImageJ (which is automatically launched), or uploaded to an OME server online. Alternately, a "snapshot" can be taken of an active display and saved to disk or sent to ImageJ for further processing. Animations of display transitions (rotation, pan and zoom motion) can also be recorded and saved as movies or image sequences on disk.

For data import, VisBio is capable of downloading images from an OME database. Such datasets function much as though they had come from files on disk, except that VisBio accesses the database to obtain needed image planes on demand.

VisBio also includes the capability to connect to a MATLAB installation, or to an external program interface (using command line parameters with standard input and output). Thus, it is straightforward for programmers to define a VisBio data transform driven by a MATLAB script or specially formulated external application. However, these interfaces are quite early. They currently work by passing each image plane into the function and retrieving a resulting image plane—it is not yet possible to provide non-image results or perform calculations across a range of image planes.

#### Future directions

We are working to improve VisBio's database connectivity and support for image metadata. Our OME-TIFF file format [<http://www.loci.wisc.edu/ome/ome-tiff.html>] and metadata support within our Bio-Formats package are a good start toward metadata standardization, but more work is still needed in this area to fully reap the benefits made possible by metadata-rich acquisition. We eventually hope to store not only metadata recorded at acquisition time, but also information derived within VisBio either automatically or manually. For example, we are working toward a system where overlays could be performed within the software then stored into a database under the name of the person who created them. Later, another investigator could update the overlays, and his changes would be recorded under his name, with a revision system for storing multiple versions of the information.

We will continue to enhance support for spectral and lifetime datasets, and intend to provide robust tools for handling such image types. The MATLAB and command line interface capabilities provide steps toward this end, but we currently do not package any lifetime- or spectra-specific algorithms with VisBio. We also plan to expand on the interface to allow the passing of more complex parameters and the ability to define more complex user interfaces, so that VisBio can provide (for example) a full-featured lifetime curve fitting user interface driven by a pluggable curve fitting algorithm written in MATLAB, C++ or other language.

We also plan to expand the overlays feature to allow the grouping of multiple glyphs across a dimensional axis to define higher-dimensional "region of interest" (ROI) structures. For example, circling a nucleus on every slice of a Z series identifies it in 3D. Such ROIs would be very useful for computing statistics such as volume, or for 3D visualization with rendering techniques such as coring.

Our current efforts involve improvement of VisBio's data handling and display efficiency, with a major new release of the application slated for early summer 2006. ■

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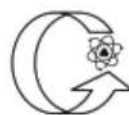
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