

Further, we demonstrated the use of ClearVolume for long-term time-lapse imaging with an OpenSPIM microscope⁵. ClearVolume was readily integrated into the μ Manager (www.micro-manager.org/) plug-in to allow live 3D visualization. We used ClearVolume to remotely monitor a developing *Drosophila melanogaster* embryo to check on sample drift, photobleaching and other artifacts (**Supplementary Video 5**). Time-shifting allows inspection of the data at any given point in time during acquisition (**Supplementary Video 6**).

In addition, ClearVolume can be used for aligning and calibrating light-sheet microscopes. The overall 3D point spread function of the optical system and the full 3D structure of the beam (for example, Gaussian or Bessel beam) can be visualized (**Supplementary Videos 7 and 8**). To aid manual alignment of the microscope, ClearVolume computes image sharpness in real time and provides audiovisual feedback (**Supplementary Video 9**). Finally, ClearVolume computes and visualizes sample drift trajectories and makes that information available through an interface for stabilization of the sample (**Supplementary Video 10**).

ClearVolume can also be used on other types of microscopes such as confocal microscopes (**Supplementary Video 11**). Going beyond microscopy, ClearVolume is integrated with the popular Fiji⁶ (<http://fiji.sc>) and KNIME (<http://www.knime.org/>) software packages, bringing real-time 3D+t multichannel volume visualization to a larger community (**Supplementary Videos 12–14**). Furthermore, ClearVolume's modularity allows any user to implement additional CPU- or GPU-based modules for image analysis and visualization.

In summary, ClearVolume makes live imaging truly live by enabling direct real-time inspection of the specimen imaged in light-sheet microscopes. The source code of ClearVolume is available at <http://clearvolume.github.io>.

Note: Any Supplementary Information and Source Data Files are available in the online version of the paper (doi:10.1038/nmeth.3372).

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BigDataViewer: visualization and processing for large image data sets

To the Editor: The increasingly popular light-sheet microscopy techniques generate very large three-dimensional (3D) time-lapse recordings of living biological specimens¹. The need to make large volumetric data sets available for interactive visualization and analysis has been widely recognized². However, existing solutions build upon proprietary file formats requiring that data are copy converted before visualization, or they use dedicated servers to generate virtual slices that are transferred to client applications. In practice, the latter leads to frame rates (under ten frames per second) that are insufficient for a truly interactive experience. An easily accessible and extensible open-source solution for interactive arbitrary virtual reslicing of very large volumes and time series of volumes has been missing (**Supplementary Note 1**).

We fill this gap with BigDataViewer (BDV), a Fiji plug-in³ to interactively navigate and visualize large image sequences from both local and remote data sources. The client software renders an arbitrarily oriented virtual slice through global 3D coordinate space (**Fig. 1a**). Individual image stacks, each representing a view of a multiview selective-plane illumination microscopy (SPIM) data set, can be displayed independently or as a color-coded composite (**Fig. 1a**). Brightness and color can be adjusted for each view separately. The viewer allows free translation, rotation and zoom for image stacks as well as movement between time points. Thus, multiterabyte recordings can be navigated smoothly (**Supplementary Video 1**).

We achieve this performance on very large data by using an efficient client-side renderer and an intelligent loading and caching scheme. To render any virtual slice, only a small fraction of the image data is relevant and gets loaded into memory. The navigation is further accelerated by caching recently visited locations in memory. If images are available at multiple scales, only the most relevant scales for display are requested. This avoids aliasing artifacts at zoomed-out views and facilitates interactive browsing: low-resolution data are loaded rapidly, providing immediate feedback, whereas high-resolution detail is filled in subsequently (**Supplementary Note 2**).

For large time series, we developed a custom open-source data format that is optimized for fast random data access at various scales (**Supplementary Note 3**). Each image is stored as a chunked multidimensional array at successively reduced resolutions. We build on HDF5 as an established portable data format that provides efficient input and output, supports unlimited file sizes and has built-in and extensible compression facilities. Metadata, such as SPIM view registrations, are stored as XML. The proposed format integrates seamlessly with Fiji's plug-ins for SPIM image processing, allowing control and visualization of the intermediate steps of the pipeline (**Fig. 1b–e** and **Supplementary Video 2**). We provide Fiji plug-ins for reading and writing the format so that any image that opens in Fiji can be stored as HDF5 and viewed with the BDV plug-in (**Supplementary Note 4**).

BDV has a modular architecture that separates data access, caching and visualization (**Supplementary Note 5**). We rely on the generic image processing library ImgLib2 (ref. 4) to provide abstract interfaces between the modules. This enables the data back end to hide implementation details such as chunking and caching. The modular architecture makes it easy to add alternative data back ends for data sources other than the custom HDF5 format.

Any image that can be opened in Fiji through LOCI (Laboratory for Optical and Computational Instrumentation) Bio-Formats⁵ can be viewed using BDV. We also support access to other hierarchical data formats similar to our SPIM-optimized HDF5, such as the Imaris file format. Furthermore, BDV can directly access the online image services CATMAID⁶ and OpenConnectome⁷, making massive serial-section electron microscopy data sets available in previously inaccessible orientations to complement fixed-orientation web browser-based visualization (Fig. 1f–i and Supplementary Video 3). In addition, we developed a web service (Supplementary Note 6) that allows users to access our custom HDF5 format remotely, enabling web browser–like access to image data stored online from within Fiji. In all cases, the data back end provides cached virtualized access to image data, resulting in the illusion that all of the data are present in memory.

The virtualized access is a powerful way to present remote and/or extremely large data sets for computation as well as visualization. In Fiji, we use our framework to make raw images of the data sets available as virtual stacks and then run standard image processing tools on arbitrarily large images. For example, a SPIM time-lapse image series may be registered, fused and deconvolved without being locally stored on the processing computer. Moreover, it is straightforward to programmatically access the pixel data using standard ImgLib2 interfaces, which means that existing code for filtering and segmentation will work without modification (Fig. 1j). Similarly, the visu-

alization front end can be programmatically extended to display additional data, such as image processing results or annotations (Fig. 1k). Thus the BDV is a tool for visualization and processing of multiview data from light-sheet microscopy as well as a general solution for dealing with large, terabyte-sized data sets from any imaging modality. BDV is included in the default Fiji installation. Documentation is available at <http://fiji.sc/BigDataViewer>.

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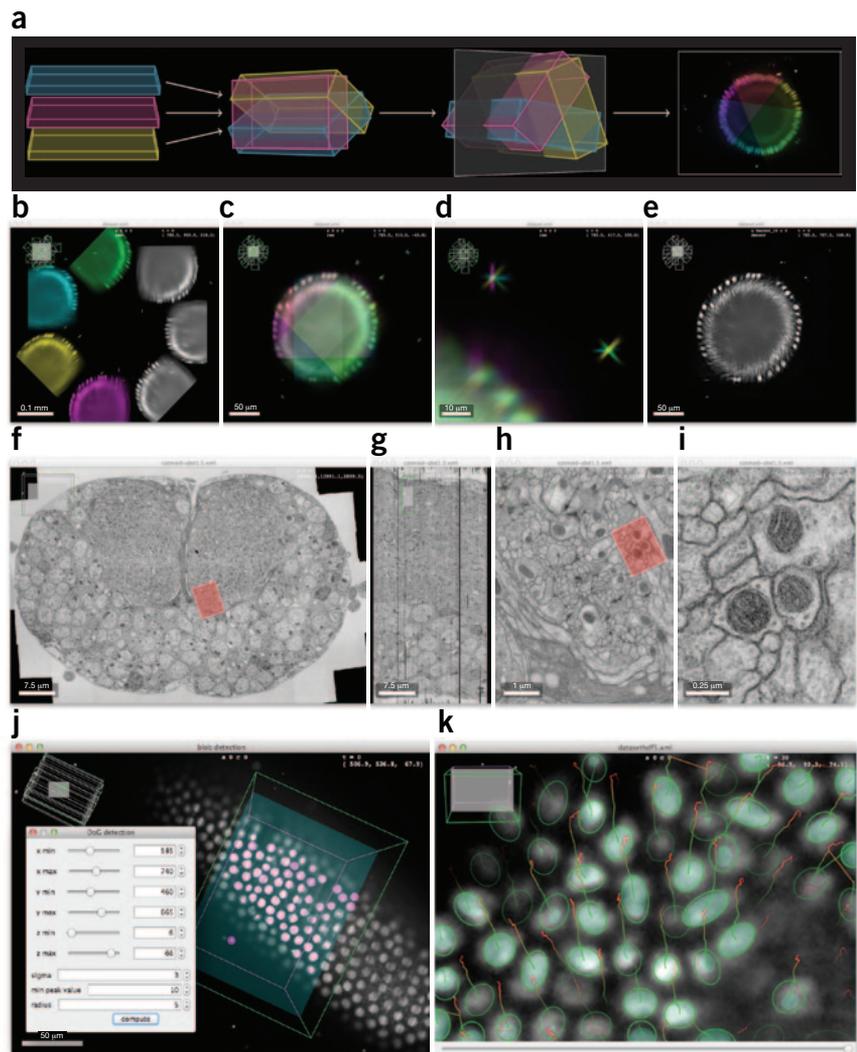
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AUTHOR CONTRIBUTIONS

T.P. and S.S. wrote the software. S.S. and T.P. devised all algorithms. T.P. and S.P. designed the file format and integrated it with SPIM registration. T.P., S.S. and P.T. conceived of the project and wrote the paper.

Figure 1 | Overview of the BDV rendering principle and functionality. (a) Rendering algorithm. Source image volumes are transformed into a common global space. Then the global space is transformed into the current viewer frame, which is aligned with the screen plane $z = 0$. Pixels on this plane are rendered to the viewer canvas to produce a final image. (b–e) Integration with Fiji's SPIM image processing. Individual angles of a light-sheet microscopy data set can be interactively viewed before registration (b) and after registration (c). (d) Zoomed-in view of fluorescent beads after registration. (e) The results of a multiview deconvolution and other processing steps can be incorporated into the data set and viewed in a common global space. (f–i) Online data sources. An electron microscopy data set of 1.5 segments of the ventral nerve cord of a first instar *Drosophila* larva (458 sections, each section consisting of ~70 overlapping image tiles, imaged at 4 nm/pixel with 50-nm section thickness) is available online through the web browser-based CATMAID viewer. BDV improves interactive visualization of the data set with arbitrary 3D navigation using the same intuitive interface as for local data. (f) One z section of the data set. (g) Resliced x section. (h,i) Zoomed-in views of the areas shaded red in f and h, respectively. (j,k) Extensibility and processing. Source volumes are accessible and processing results can be visualized via standard interfaces. As an example, a blob-detection algorithm is run on a subvolume selected by the user, and results are visualized in BDV (j). (k) Results of an automatic cell-tracking algorithm⁸ are overlaid on the source data for visual inspection.



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MAGI: visualization and collaborative annotation of genomic aberrations

To the Editor: We introduce Mutation Annotation and Genome Interpretation (MAGI), an open-source web application (<http://magi.cs.brown.edu/> and **Supplementary Software**) for exploration, annotation and integration of public and private cancer genomics data (**Fig. 1**, **Supplementary Fig. 1** and **Supplementary Methods**). MAGI is, to our knowledge, the first tool to support interactive, bidirectional user interactions between public and private cancer genomics data sets¹ (**Supplementary Table 1**). This bidirectional interaction enables researchers to leverage large public data sets in the analysis of their own cancer samples while also facilitating the expert-sourcing of public data sets through collaborative annotation. The software (**Supplementary Fig. 2** and **Supplementary Table 2**) can also be installed locally. MAGI includes several key features, detailed as follows.

- MAGI generates interactive visualizations of cancer genomics data in a query set of genes with real-time zooming, panning and data filtering (**Fig. 1**). Visualizations include single-nucleotide variants and small insertions and deletions (**Supplementary Figs. 3–5**), copy-number aberrations (**Supplementary Figs. 4–7**), gene expression and protein-protein interactions. These views are linked, highlighting different types of data in the sample (**Supplementary Fig. 8**). The MAGI web application is loaded with genomic data from The Cancer Genome Atlas (TCGA) Pan-Cancer study² and protein-protein and protein domain annotations from various sources. MAGI also shows categorical and continuous attributes for each sample and is initialized with survival time, gender and tumor-purity estimates from the Pan-Cancer data set² (**Fig. 1**).
- Users may upload genomics data—including mutations, gene expression, methylation or sample attributes—directly into MAGI (**Supplementary Table 3**). These private data are available for query, visualization and annotation in conjunction with public

data sets. Data upload uses a simple web form with no local software installation required. MAGI automatically generates summaries of user data sets with interactive graphs, sortable tables and pathway analysis (**Supplementary Figs. 9 and 10**).

- Annotation capabilities facilitate collaborative annotation of mutations and protein-protein interactions in both public and private cancer genomics data sets. Annotations include literature citations, text comments and votes, and these are seamlessly integrated with the interactive visualizations (**Supplementary Figs. 11 and 12**). The MAGI website is initialized with ~40,000 protein sequence change annotations from the Database of Curated Mutations (<http://docm.genome.wustl.edu/>) and from PubMed Central searches.
- A sample view shows all sample features and annotations of aberrations in a given (public or private) tumor sample (**Supplementary Fig. 13**). An annotation score prioritizes the display of aberrations with annotations in the MAGI database. This view leverages information across many samples for single-sample (“ $n = 1$ ”) analyses. The sample view also reveals samples

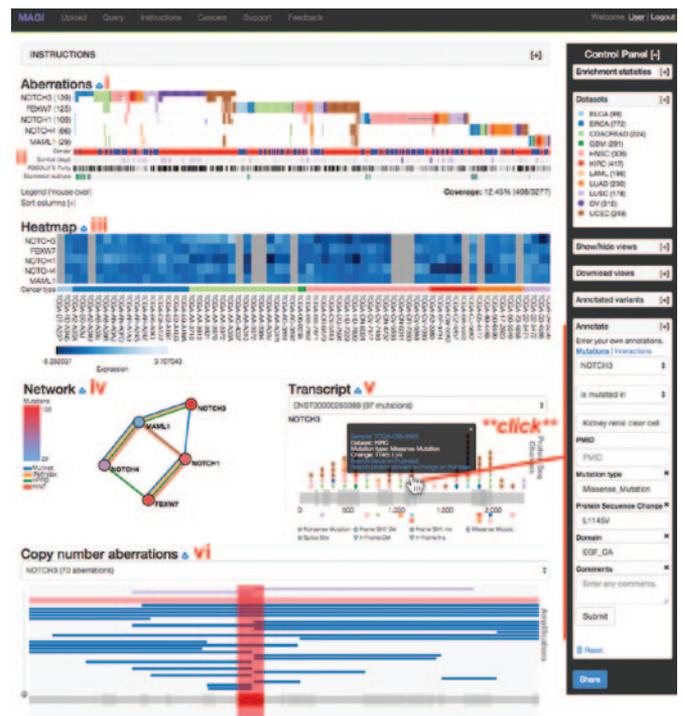


Figure 1 | Screenshot of the MAGI web application displaying mutations in the Notch signaling pathway from TCGA Pan-Cancer data set. (i) The aberrations view shows the pattern of mutations in the gene set across tumor samples. (ii) Additional rows in the aberration and heat-map views show attributes for each sample; displayed here are survival time, gender and estimated tumor purity. (iii) Users can upload and display continuous-valued data (for example, expression or methylation data) in a heat map. Shown here are RNA-seq differential expression values for the TCGA Pan-Cancer data set. (iv) The network view shows the interactions among genes from multiple interaction networks. (v) The transcript view shows the locations and types of the mutations in a transcript of a given gene. (vi) The copy number-aberrations view shows the amplified or deleted segments across tumor samples in a given gene. Annotations are added using a simple web form that records mutation or interaction type, a PubMed ID (PMID) and/or free-text comments. Clicking on an element in any of the views populates this form with the corresponding mutation or interaction, simplifying data entry.