

ClearVolume: open-source live 3D visualization for light-sheet microscopy

To the Editor: Current state-of-the-art light-sheet microscopes rely on sophisticated control software to perform the acquisition of gigabytes of image data per second over the course of hours or even days. Typically the microscopes acquire data in a first step, and in a second step these data are processed and visualized offline. The delay between data acquisition and data assessment wastes time and storage space. Technology that enables viewing and assessing data during imaging would offer significant advantages. However, even the most advanced microscopes display only the latest image plane acquired or projection while the raw volumetric data are saved to disk^{1–4}.

Ideally, one would be able to instantly visualize and analyze time-resolved multichannel three-dimensional (3D+t) data during acquisition, virtually rotate and zoom into the sample, go back in time to replay dynamic processes of interest, and even access the live data feed remotely. From a biological perspective this would shorten the time needed to tune imaging parameters and assess a sample's viability or relevance. Experimentally, the ability to remotely visualize a 3D+t data stream would facilitate the monitoring of long-term time-lapse imaging. From an optics point of view, live visualization would also be helpful when performing system design and alignment. Indeed, irrespective of the experimentalist's goal, the earlier one can visualize and assess the acquired data in their spatiotemporal entirety, the more effective live light-sheet microscopy becomes in the discovery process.

In this work, we present ClearVolume, an open-source package for live graphical processing unit (GPU)-accelerated 3D+t multichannel visualization. It is designed to easily augment any custom-built light-sheet microscope with real-time visualization capabilities. For that purpose ClearVolume has a simple interface for all major programming languages and frameworks used for controlling light-sheet microscopes: Java, C/C++, Python, LabVIEW and OpenSPIM (μ Manager) (Fig. 1a, Supplementary Videos 1–3 and Supplementary Note 2). Key features especially relevant to light-sheet microscopy are the capability to create instant multiview and multicolor renderings (Fig. 1d) as well as instant rewind and replay of time-lapse recordings (Fig. 1c). Furthermore, ClearVolume provides real-time GPU-based image processing capabilities, such as image sharpness estimation and sample drift tracking (Fig. 1c). Finally, ClearVolume enables live streaming of 3D data in real time over the Internet (Fig. 1b). This effectively decouples the physical location of the microscope from the experimenter's location. For example, in the case of long-term developmental imaging, it is possible to remotely inspect the health of the embryo and the quality of the recorded volumes. Overall, ClearVolume enhances the microscope's ergonomics and permits the immediate 3D perception of the imaged processes.

The value of integrating ClearVolume into a high-speed light-sheet microscope optimized for cellular imaging is demonstrated with a volumetric acquisition of a 12-cell-stage *Caenorhabditis elegans* embryo undergoing mitosis (Supplementary Video 4 and Supplementary Note 1). The embryo could be virtually rotated, and regions of interest, such as cell divisions, could be inspected while 3D data were acquired at six stacks per second.

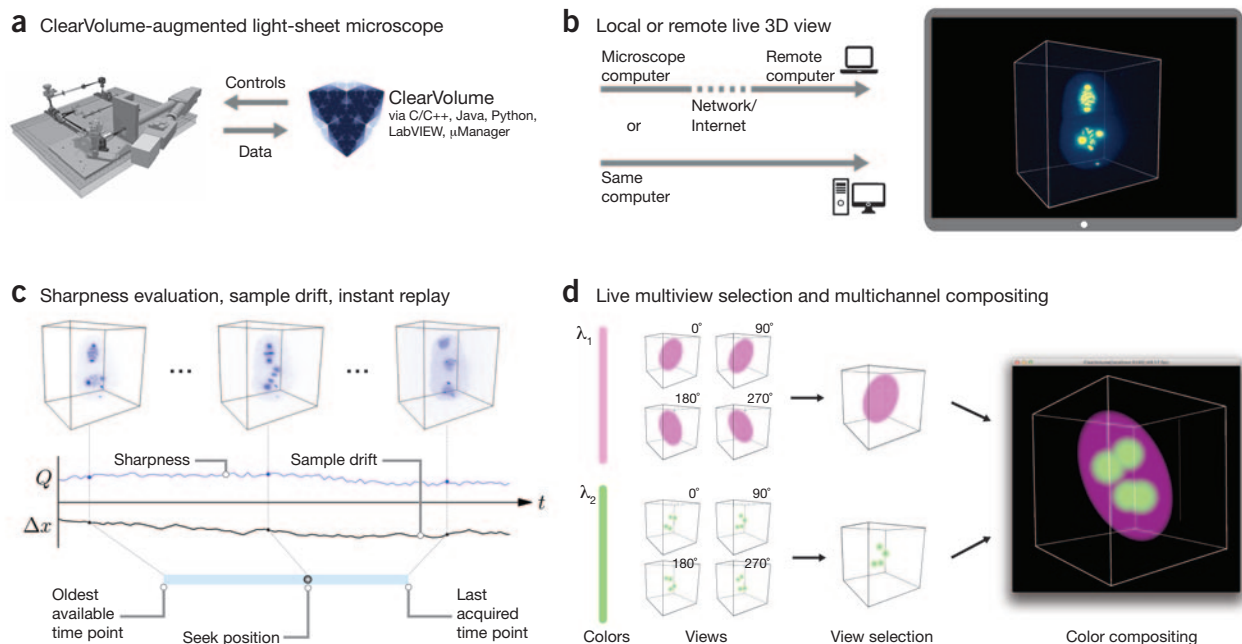


Figure 1 | ClearVolume: an open-source real-time multichannel 3D visualization toolkit for microscopy. (a) ClearVolume can interface with a number of software systems, such as LabVIEW or μ Manager. It also features language bindings for C/C++, Java and Python. (b) The volumetric data acquired by a connected microscope can either be rendered locally or sent to a different computer for remote inspection. (c) In addition to live volumetric rendering, ClearVolume features real-time sharpness and sample drift evaluation, instant replay and arbitrary 2D and 3D overlays. (d) ClearVolume can selectively visualize multiview, multichannel data sets.

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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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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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.