Supplementary materials for the paper:

V3D: A Real-Time 3D Visualization System for the Quantitative Analysis of Large Bioimages and Its Applications to Neuroscience

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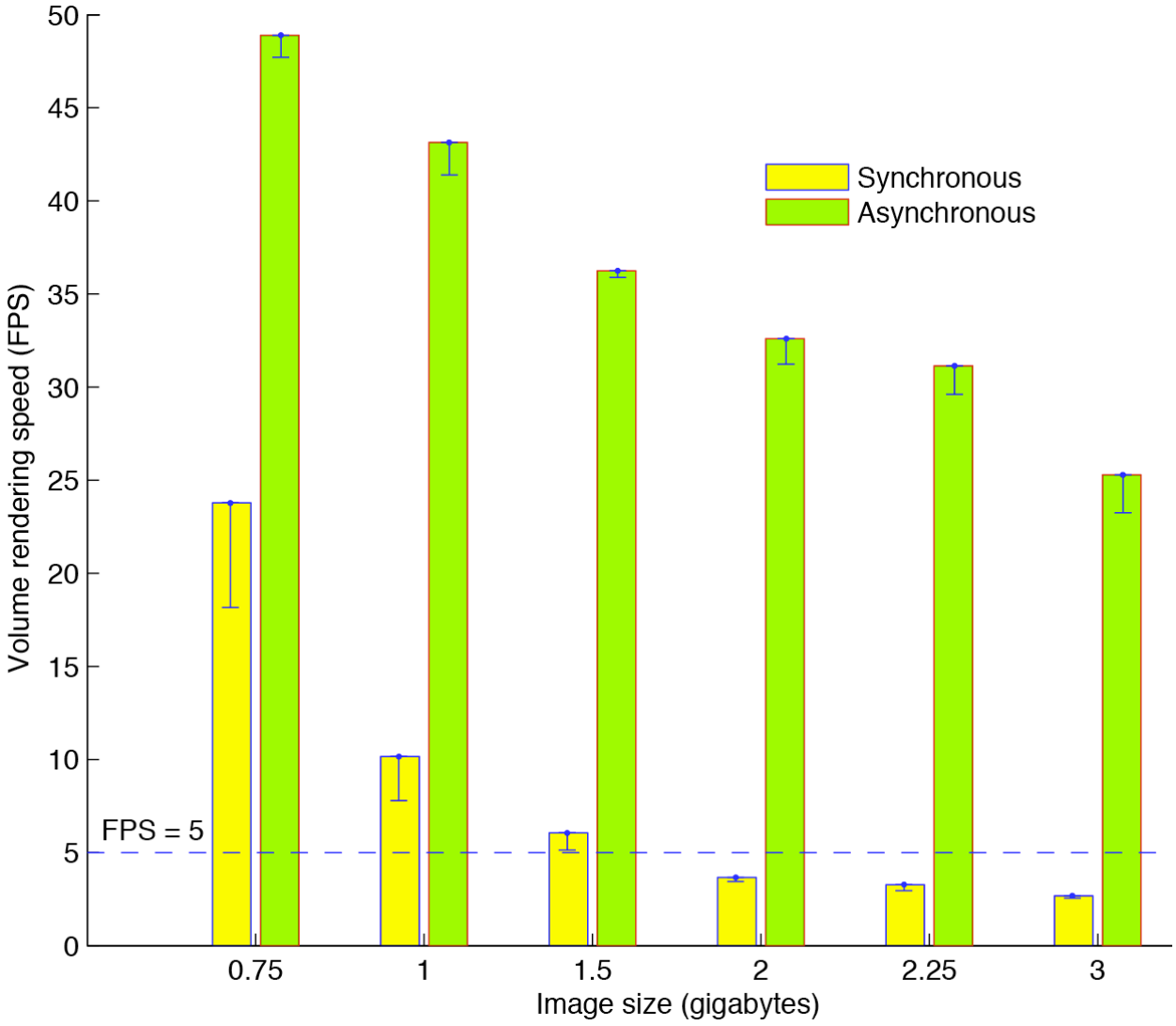
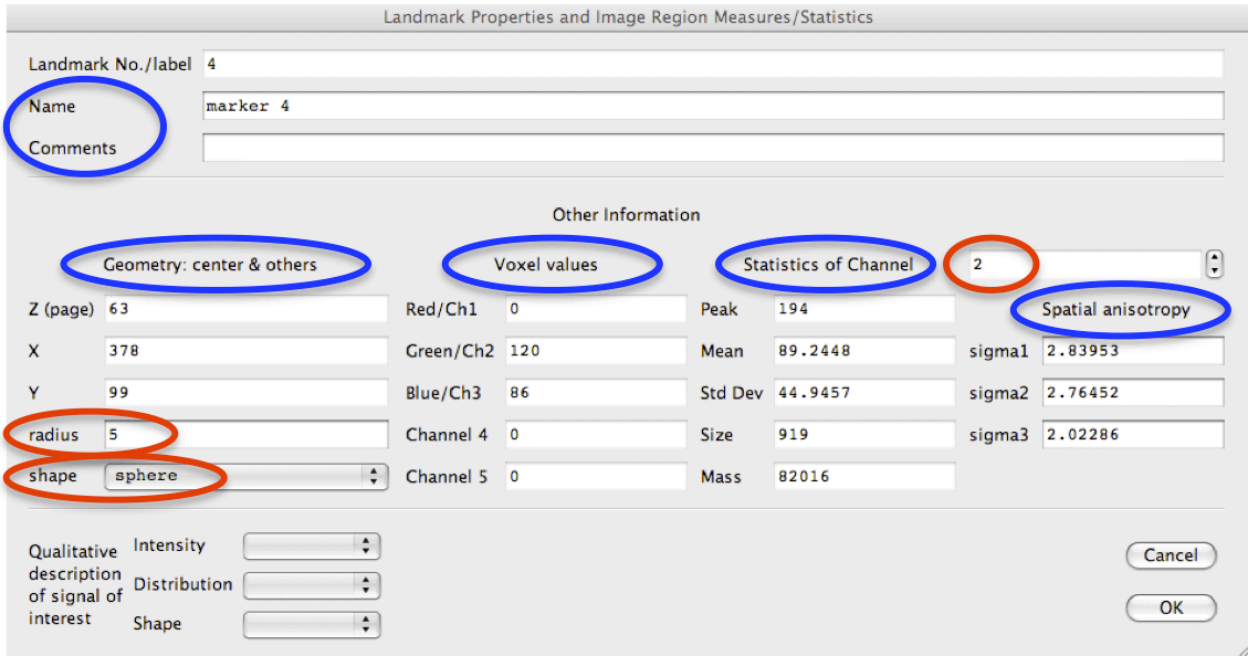
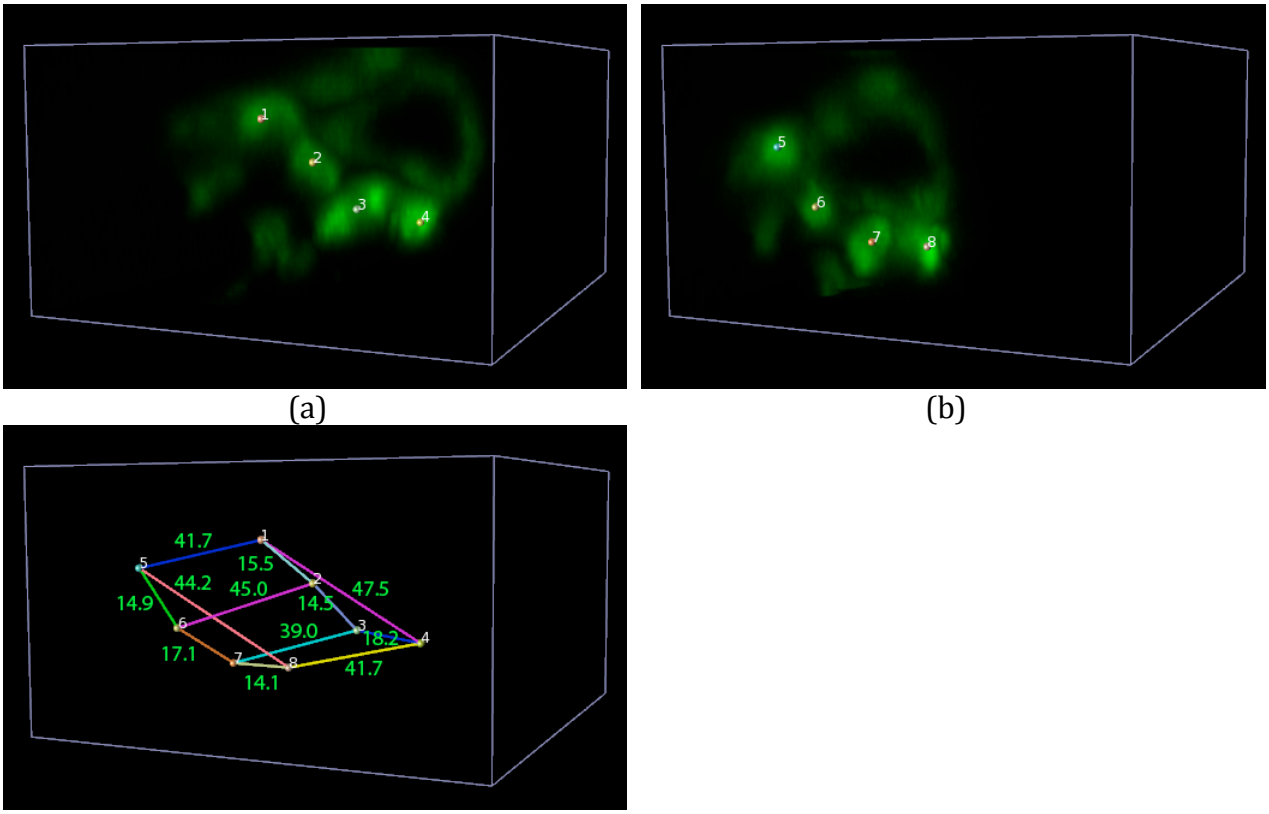
Supplementary Figures.



Supplementary Figure 1. The volumetric image rendering speed of V3D visualization engine tested on a Mac Pro machine. For each image size, both the peak speed (green and yellow bars) and the respective standard deviation (black line-ranges) of at least 10 speed-test trials are shown. The tests were done on a 64bit Mac Pro machine with an ATI Radeon HD 4870 graphics card, which has only 512M video memory.



Supplementary Figure 2. The V3D tool for quantitative measuring and annotating any 3D spatial location in an image. Blue circles: image content attributes that can be measured or annotated; red circles: the user-changeable parameters for quantitative measuring.



(a) (b)

(c)

Supplementary Figure 3. Quantification of the displacement of relative locations of neurons in a live *C. elegans* 5D imaging experiment. Shown is a portion of the nerve ring neurons imaged using selective plane illumination microscopy (SPIM). Green: gcy-5:GFP-tagged neurons. The data (image series) contains 126 time points, each is a 4D image stack.
(a) Direct pinpointing the centers of four neurons at time 1.
(b) Direct pinpointing the centers of the same neurons at time 2. Because of the 5D display of the entire image series, the identification of the corresponding neurons at different time points is straightforward.
(c) 3D measuring of the global displacement of neurons and their relative location displacement during the imaging period.

Supplementary Note. Comparison of V3D and other tools.

V3D and V3D-Neuron have been tested extensively on a variety of machines (Mac, Linux and Windows). We also compared many other tools with V3D/V3D-Neuron. The interested users and developers should refer to the V3D online documentations for the latest details.

We summarize below the comparisons of our tools with Neurolucida and Image Pro.

- Neither Neurolucida nor Image Pro is able to render multi-gigabyte volumetric data. Image Pro can do some 3D volume rendering, but its visualization engine is slow even for a small data set (compared to V3D's speed using the same computer). More importantly, Image Pro's rendering entails significant loss of signal especially in the dark image area. Image Pro does not support a 3D pinpointing type of interaction (and as a natural result, it does not support directly 3D quantitative measuring/profiling functions as V3D), thus as a *platform software* neither Neurolucida and Image Pro is comparable to V3D with regard to the two critical capabilities, i.e. volume rendering speed and 3D interactability of image content.

- Neurolucida neuron tracing is restricted to 2D plane. It is largely manual. We were unsuccessful in using its automatic module to produce a meaningful 3D reconstruction. Image Pro has some simple functions to do image thinning in 3D, or do region growing in 2D to find the "skeleton" of a structure (limited in 2D plane only). It also supports manual drawing in 2D of the skeleton, but the function was weaker than Neurolucida and thus it would be extremely laborious to use it to produce a meaningful 3D reconstruction. More importantly, Image Pro does not produce a full 3D reconstruction with a ***defined topology***. Thus strictly it cannot be called a neuron reconstruction tool. In contrast, V3D-Neuron is able to produce a 3D neuron reconstruction in a much more efficient way than both tools.

- Both Neurolucida and Image Pro only run on Windows. On the contrary, V3D is cross-platform, - running on Mac, Linux, and Windows.

Supplementary Video Legends.

Supplementary Video 1. 3D visualization of a digital model of a fruit fly brain. Magenta voxels: the 3D volumetric image of a fruit fly brain; green voxels: a 3D GAL4 neurite pattern; colored surface objects of irregular shapes: digital models of various brain compartments; colored tree-like surface objects: two 3D reconstructed neurons.

Supplementary Video 2. Hierarchical visualization of a fruit fly brain.
(a) The global 3D viewer.
(b) Local 3D viewer for region A of Fig. 1c.
(c) The local 3D viewer for region B in Fig. 1c is used for tracing neurite and proofreading the reconstruction in 3D.

Supplementary Video 3. 3D pinpointing methods in V3D.
(a) Pinpointing using 2-clicks.
(b) Pinpointing using 1-click.

Supplementary Video 4. 3D counting of neurons in the arcuate nucleus of the hypothalamus of a mouse brain. For better visibility, only a small trunk of data is displayed. Red: AgrP neurons infected with FLEX-AAV-ChR2-td-tomato virus; blue: DAPI staining indicating the cell bodies of neurons; green spheres: markers indicating the locations of neurons.

Supplementary Video 5. 5D volumetric image visualization and quantitative measuring for *C. elegans* neurons. A series of SPIM images (Supplementary Figure 3) were used. The neuron centers 1∼8 were directly pinpointed. 3D line segments were defined between them for profiling both voxel intensity and distance between the moving neurons.

Supplementary Video 6. The use of V3D-Neuron in visualization, reconstruction, and proofreading of the 3D morphology of a fruit fly neuron.

Supplementary Video 7. A 3D atlas of 111 stereotyped neurite tracts in a fruit fly brain. The width of a tract indicates the spatial variation of its location.

Supplementary Video 8. V3D-Neuron can display a neuron in multiple ways (see Methods).

Supplementary Video 9. Editing a neuron using V3D-Neuron (see Methods).

Supplementary Video 10. Display of multiple neurons in V3D-Neuron. The first half shows how to display the atlas of fruit fly neurite tracts in Figure 6. The second half shows how to display multiple mouse brain neurons.