DIVA: Natural Navigation inside 3D Images Using Virtual Reality

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Abstract

As three-dimensional microscopy becomes commonplace in biological research, there is an increasing need for researchers to be able to view experimental image stacks in a natural three-dimensional viewing context. Through stereoscopy and motion tracking, commercial virtual reality headsets provide a solution to this important visualization challenge by allowing researchers to view volumetric objects in an entirely intuitive fashion. With this motivation, we present DIVA, a user-friendly software tool that automatically creates detailed three-dimensional reconstructions of raw experimental image stacks that are integrated in virtual reality. In DIVA's immersive virtual environment, users can view, manipulate and perform volumetric measurements on their microscopy images as they would to real physical objects. In contrast to similar solutions, our software provides high-quality volume rendering with native TIFF file com-

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patibility. We benchmark the software with diverse image types including those generated by confocal, light-sheet and electron microscopy. DIVA is available at `https://diva.pasteur.fr` and will be regularly updated.

Keywords: Virtual Reality, Microscopy, Data Visualisation, Image Processing, Data Treatment, Human-in-the-Loop

2010 MSC: 00-01, 99-00

1. Introduction

Technological advances in the fields of optical and electron microscopy have enhanced our abilities to discern three-dimensional (3D) biological structures via slice-based tomography. Entire organisms can be imaged at sub-cellular resolution and the complex interplay between 3D geometry and biological activity explored [1]. Yet, gaining an intuitive understanding from these complex raw data remains a challenge, as natural modes of 3D visualisation are lacking. Namely, viewing 3D data on a computer monitor while simultaneously using a mouse to interact and extract information is tedious and difficult, *e.g.* clicking inside a 3D object on a 2D screen is a nontrivial task.

Recently, virtual reality (VR) has reemerged as a technology of interest in a host of applications due in large part to new low-cost consumer headsets. Through the seamless integration of stereoscopy, motion tracking and total immersion, VR provides a natural means to visualize 3D structures. Interactions with the aid of VR controllers are intuitive as they are performed as if the data were physically present to the user. Today, numerous initiatives have focused on taking advantage of this technology in the domains of education and scientific research [2, 3, 4, 5, 6]. Recent studies have additionally highlighted the benefits of immersive viewing for handling 3D data which include efficiency and enhanced intuition relative to standard monitor-based visualisation [5, 6]. Various companies and laboratories laboratories have begun to leverage this technology for scientific image visualisation with different volume representation and interaction approaches, a comparison of which is made in Table 1.

Table 1: A comparison of currently existing VR solutions. Recommended requirements are based on publicly available information and configurations used in cited articles. All solutions run on Windows 10 with an Intel Core i7 or greater CPU.

	Arivis InViewR	IstoVisio syGlass	ConfocalVR	VRNT	TeraVR	DIVA
Native TIFF Compatibility	No	No	No	No	No	Yes
Recommended Requirements	16GB RAM, 8GB VRAM	32GB RAM, 8GB VRAM	Unavailable	64GB RAM, 8GB VRAM	64GB RAM, 8GB VRAM	8GB RAM, 6GB VRAM
Desktop Mode	No	No	No	No	No	Yes
Time Series Compatibility	Yes	No	No	No	No	Yes
Multi-User Compatibility	Yes	Yes	No	No	No	No
Applications	Generalized	Generalized	Confocal Microscopy	Neurobiology	Neurobiology	Generalized
License	Commercial	Commercial	Free	Free	Free	Free
Reference	[7]	[8]	[9]	[10]	[11]	

VR is a promising vehicle through which experimentally acquired images can be better understood. It allows instantaneous understanding of complicated 3D morphologies and, notably, through controller-aided interaction it allows entirely natural selection of voxels in 3D space. In our view, image visualisation in VR should be readily available to all research laboratories (as software such as ImageJ and Icy are [12, 13]) and it must provide tools adapted for processing the enormous diversity of microscopy images.

With this motivation, we introduce DIVA, a complete software application that allows easy integration and navigation inside any 3D scientbific image using VR. DIVA uses standard 8- and 16-bit TIFF image stacks and hyperstacks (*i.e.* time series images) as input to instantaneously generate interactive volumetric reconstructions. DIVA does not require any image pretreatment nor does it require conversion to intermediate file formats, as is the case in most 3D image visualization software. Accordingly, it can be used to visualise any type of microscopy image regardless of the imaging modality, ranging from confocal to electron microscopy. The following sections introduce the features that distinguish DIVA as a powerful human-data interaction context.

2. Implementation

The software architecture on which DIVA is based, entitled *Lean Mapper*, consists in arranging distinct subsystems to interact with each other via a top-level supervisor. Specifically, a data subsystem, which holds loaded imaging data is decoupled from a user-facing interface subsystem. A unique data identifier is used as a reference by the supervisor to trigger events. For example, when the user adjusts a slider to increase the ambient lighting in the interface subsystem, the corresponding event is emitted to the data subsystem which alters the rendered value in real-time. Among the advantages of this data-oriented approach includes low-latency user interactions with data and fluid navigation, especially in VR modes.

An image stack loaded into DIVA is rendered as a 3D volume through bilinear texture interpolation. The user defines the aspect ratio of the volume by specifying the pixel size and stack spacing in a user interface panel.

Developed using the Unity game engine (`https://www.unity.com/`), DIVA includes a dual interface: a desktop mode for viewing on a standard computer monitor and a VR mode where the user enters an entirely artificial immersive environment by means of a VR headset. This design decision stems from the realization that, despite the massive improvement in VR technology, spending large amounts of time in VR space is not a comfortable experience. In DIVA, the VR interface is tailored to efficient scientific analysis of imaging data, while the desktop interface focuses on parameter settings and initial visual screening of the data. Hence, features of the software are implemented in each respective mode separately.

The desktop interface allows the user to modify visualisation parameters such as voxel size and lighting. It also includes a user-friendly transfer function interface (*i.e.* a 3D look-up table), which allows users to specify pixel intensity-dependent opacity and colour by means of absorption and emission curves, respectively, in real-time. In this interface data appears as a 3D volumetric representation on a 2D screen. The volume can be translated, rotated

and scaled using the mouse.

In the VR interface, DIVA renders image stacks as virtual "physical objects" in a virtual room environment. The user can grasp the image stack and navigate inside it via physical manipulation using the VR controller (bundled with all commercial VR headsets). A key VR function included in DIVA to address the complexity within biological image volumes is a handheld clipping plane that allows interactive removal of portions of the rendered volume in real-time (see video demonstration in supplementary material). This feature is essential when extracting information from dense images. A simple-to-use counting and distance measurement tool is additionally included to rapidly and easily perform 3D quantification of imaging data. In tests we have performed, point-to-point measurements with the VR measurement tool are performed roughly an order of magnitude quicker than using a standard 2D image stack viewer such as ImageJ.

The perception of electron microscopy (EM) images benefits greatly from DIVA, in particular. The flexible transfer function interface allows a smooth modulation of pixel-dependent transparencies and colours to reveal structures of interest while suppressing undesired pixel information such as the significant background noise characteristic of EM images. Finally, the VR context enhances the perception and verification of 3D EM structures notably via stereoscopy with fluid interactive rendering.

Examples of uses of DIVA for optical and electron microscopy images are shown in Figure 1 and corresponding videos in the supplementary material.

Critical to any VR experience is maintaining a sufficiently high frame rate to avoid delays upon rapid head movement by the user wearing the headset. In practice, this is a significant design constraint, as volume rendering techniques are computationally costly and must be performed twice during each frame in VR (*i.e.* once for each eye). To address this challenge, DIVA renders volumes through a ray-casting approach that can have its sampling resolution easily adjusted in real-time to ensure a sufficient frame rate for the user [15]. Additionally, the resolution of the rendered image is dependent on how far the camera (observer) is; the image is better resolved in regions in which the user is

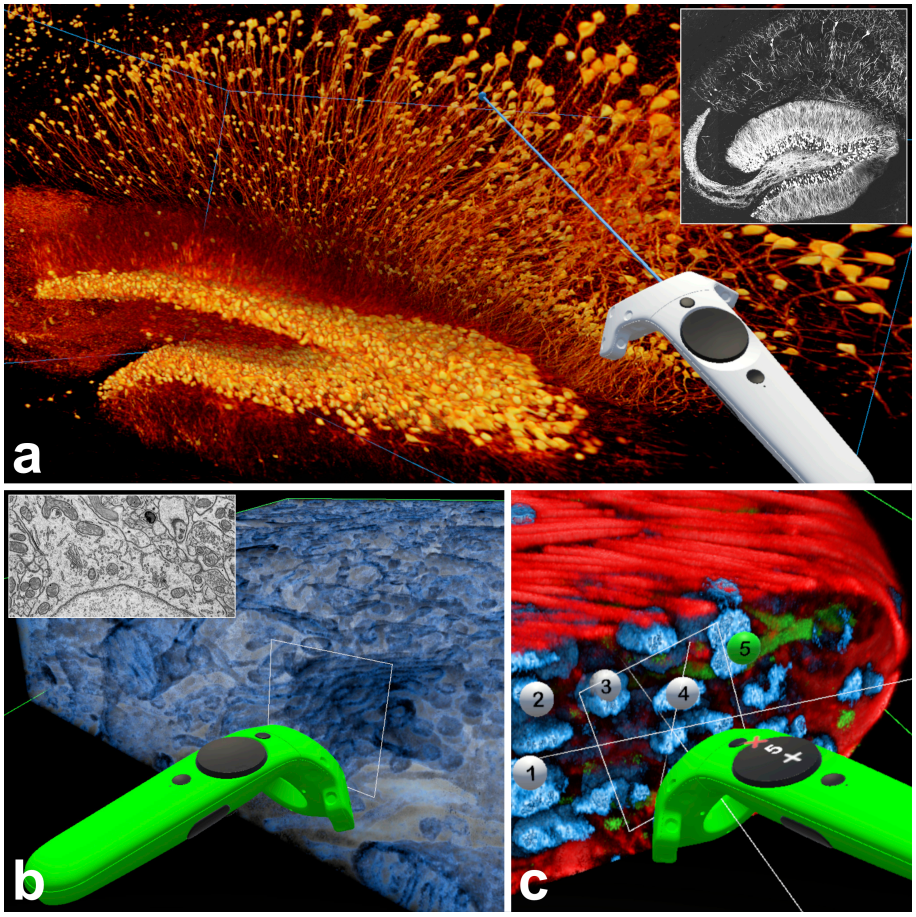

Figure 1: DIVA Usage Examples. Virtual reality visualisation of raw microscopy TIFF image stacks using DIVA. (a) Mouse hippocampus imaged by two-photon confocal microscopy (Thy-1-GFP mouse) with a slice from the raw image shown in inset [14]. (b) Focused ion beam scanning electron microscopy of components of an adult mouse neuron; seen are the Golgi apparatus and mitochondria (raw image is shown in inset). (c) Multichannel confocal acquisition of drosophila testis (red corresponds to actin, blue to nuclei and green to fusome). The positions of nuclei are labeled using a counting tool included with DIVA. In all of the panels, the colour of the VR controller is associated to the action being performed.

interacting or observing. This has the result of maintaining a high frame rate even on modest computer configurations. Accordingly, DIVA runs comfortably on mid-range laptop computers.

Numerous microscopy modalities generate multi-channel or multi-colour image types. Accordingly, DIVA supports visualisation of up to four different channels, whose colour and absorption (transfer function) characteristics can be individually customized. Figure 1(c) shows an example of a multi-colour confocal image rendered in DIVA's VR context. Additionally, time-series images can be moved where dynamic cellular processes can be observed from every angle using VR. The software has integrated sample images for the user to test upon downloading.

3. Applications

To date, DIVA is being used in a number of biological studies. As a pure visualisation tool, it has been used to navigate inside a gamut of microscopy types, not limited to confocal, light-sheet and electron microscopy types. Screen and movie captures are easily made, firmly justifying DIVA's use as a scientific communication vehicle. Notably, enhanced visualisation with DIVA permits easy quantification and measurements to be performed. For example, filamentous structures such as neurons and microtubules can be readily traced (see supplementary video) and counting of structures in dense images can be done rapidly with the aid of the aforementioned volume clipping plane function. All measurements performed in DIVA can be exported in a CSV format. Table 2 below describes a few of the systems and applications DIVA has been used for to date.

An extensive user manual describing different DIVA use cases is included in the supplementary material. Associated to this are video captures demonstrating the main features of the software.

Table 2: Biological systems that DIVA has been used for to date.

System	Microscopy Type	Application
Mouse hippocampus	Confocal	Quantification of structural effects of neural generation in mice
Zebrafish	Confocal	3D Quantification of leukocyte migration
Giant unilamellar vesicles	Confocal	Quantification of curvature of artificial vesicle membranes
Eukaryotic sells	Confocal	Transport of material in tunneling nanotubes
Arabidopsis thaliana	Light-sheet	3D navigation inside plant root
Drosophila larva	Light-sheet	Visualization of entire embryos during development
Phospholipid bilayer	Cryogenic electron microscopy	Identification of local protein densities
Eukaryotic cells	Focused ion beam electron microscopy	Optimized visualization of internal cellular contents

4. Conclusion

We have introduced DIVA, a standalone software that allows instantaneous exploration of complex volumetric microscopy images. DIVA leverages virtual reality as a means to explore and "dive in" experimental data in a natural 3D environment. Freely available for academic use, DIVA is a user-friendly software application compatible with all major VR headset brands (e.g. HTC Vive, Oculus Rift) that use the Windows-based SteamVR standard. Updated versions of the software will be made regularly available on `https://diva.pasteur.fr`.

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