

Application of Virtual Reality in Volumetric Cellular Visualization

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Abstract

Recent advancement in high-resolution confocal imaging has provided valuable novel insights into structural relationships within cells and tissues *in vitro* and *in vivo*. Development in volume rendering technique enables visualization of 3D dataset in real-time. Here, we present a system in which 3D models are reconstructed from the stacked 2D images obtained with a confocal microscope, and subsequently visualized using volume rendering technique. Using the reconstructed 3D model, we apply virtual reality method to provide an intuitive way to view and manipulate the 3D cellular environment.

Keywords: confocal imaging; volume rendering; reconstruction; visualization; virtual reality

1. Introduction

As living cells and tissues are three-dimensional structures, the spatial relationship between their components is often at issue. However, an analysis of this kind is hampered by an image of a specimen being degraded by out of focus information from planes below and above the current focal plane. This problem has been eliminated or partly eliminated by confocal microscopy. In a confocal microscope the specimen is imaged by a combination of point illumination and point detection, which virtually eliminates the out-of-focus information thus allowing effective serial optical sectioning of a specimen^[3]. By stepping the focal plane through a tissue, one can create its 3D reconstruction.

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Long Paper

The coupling of the confocal microscope with computer technology has greatly enhanced the capabilities of the microscope allowing further video-enhancement and analysis of the image obtained. As a result, analysis of the three dimensional architecture of cells which cannot be accomplished by conventional light microscopy is now possible. Concurrent with the explosive development of confocal microscopy, the development of fluorescently tagged antibodies and probes for localization of proteins, nucleic acids, lipids, ions, etc., that can be visualized in living cells has led to new avenues of research^[14]. Here, we concentrate on proteins that are important in normal cell function and pathophysiology. For example, lipoprotein (a)^[1,2] is an important risk factor implicated in the development of atherosclerosis. A second example is calreticulin, an endoplasmic reticulum calcium-binding chaperone, that is important in cell calcium homeostasis and synthetic activity as well as in heart and brain development^[15,16]. The intracellular scaffold protein, actin, which is responsible for maintenance of the cytoskeleton, is another good example.

In the present report, we describe an approach to 3D reconstruction of high-resolution 2D confocal image datasets. Specific-purpose hardware for volume rendering is utilized to enhance the performance in terms of speed and manipulation. Furthermore, virtual reality technique is developed for construction of a direct and realistic environment to analyze and manipulate the 3D cellular objects.

2. High-resolution Confocal Imaging

High-resolution confocal imaging of cells and their internal structures labelled with fluorescence probes is now routinely performed in most life science research laboratories^[3]. Figure 1 illustrates an example of human cervical cells, HeLa, double labelled with primary antibodies against apolipoprotein(a) (mouse monoclonal anti-human apolipoprotein(a) followed by a TRITC-conjugated anti-mouse secondary antibody) and apolipoprotein E (rabbit polyclonal anti-human apolipoprotein E followed by a FITC-conjugated anti-rabbit secondary antibody).

Confocal microscopy offers improved detection of self-luminous object by virtue of removing out-of-focus background noise due to the use of a pinhole at the front of signal detector^[4,5]. A series of images in *x-y* plane are recorded along the *z* axis of a microscope, so that the entire labeling can be subsequently volume rendered and analyzed quantitatively. These serial images are commonly referred to as the image stack. All of the images in the image stack in most cases are aligned along *x*, *y* and *z*-axes. Typical image sizes are 256×256, 512×512, and 768×512. The selection of the image size is usually dependent on desired size of the final 3D dataset (the series of 2D images collected).

For example, an image stack consisting of 50, 512×512 8-bit images occupies 12.8MB of storage space.

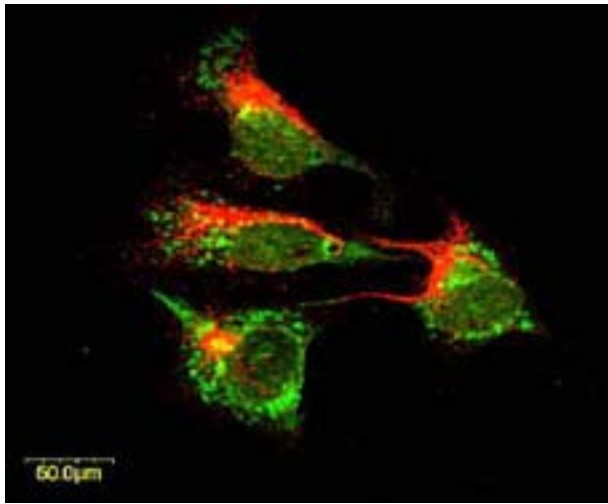


Figure 1: HeLa cells visualized with confocal microscopy after double labelling with antibodies against apolipoprotein(a)(red) and apolipoproteinE (green).



Figure 2: An Olympus Fluoview FV500 confocal microscope

The confocal microscope used in our system is a point-scanning, point-detection confocal laser scanning microscope (Olympus Fluoview™ 500; Figure 2).

Figure 3 shows a series of images of frozen sections of mouse brain in which cell nuclei were labelled with a DNA-specific label(blue) while calreticulin was detected with specific

antibodies(green). The image size is 384×512 and the image stack consists of 30 images.

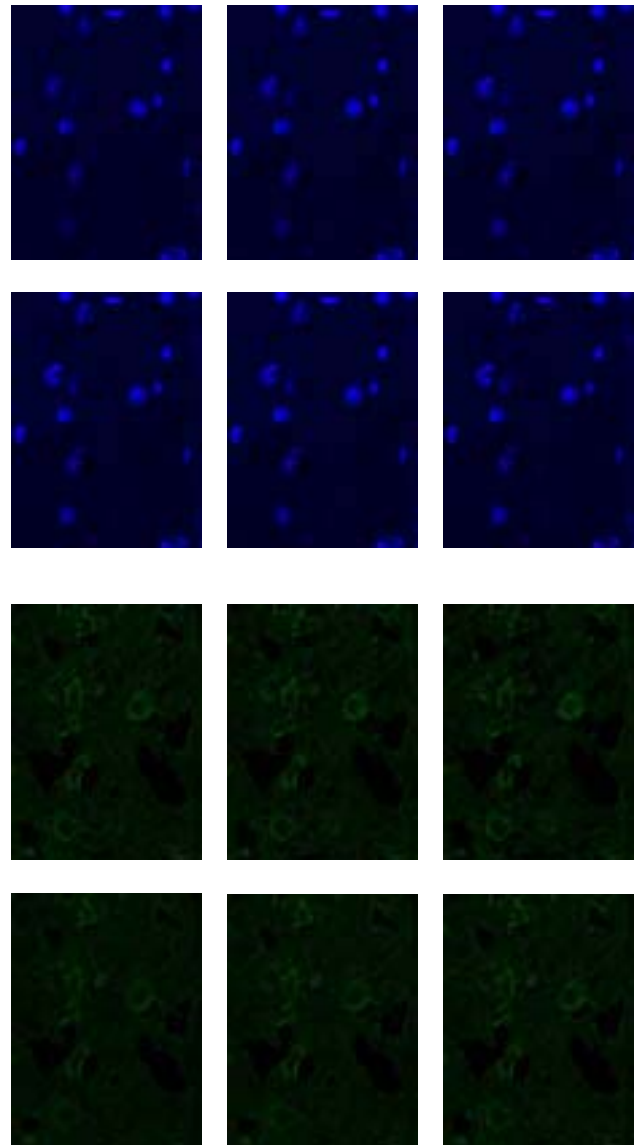


Figure 3: Two-dimensional scanning of cell nuclei labelled with a DNA marker (blue) and calreticulin labeled with antibodies (green). This stack of images taken in x - y plane has been subsequently used for volume rendering.

3. Virtual Reality Based Intracellular Visualization

To interpret intracellular structures, we applied volume rendering and virtual reality to visualize volumetric datasets derived from stacks of 2D confocal sections. In this section, the visualization pipeline and volume reconstruction are explained. The volume rendering technique based upon the ray-casting algorithm is

discussed. And the implementation of virtual reality technique on the reconstructed 3D cellular objects is detailed at the end.

3.1 Visualization Pipeline

The visualization pipeline used here can be represented as a simple flow chart as shown in Figure 4. The inputs to the pipeline

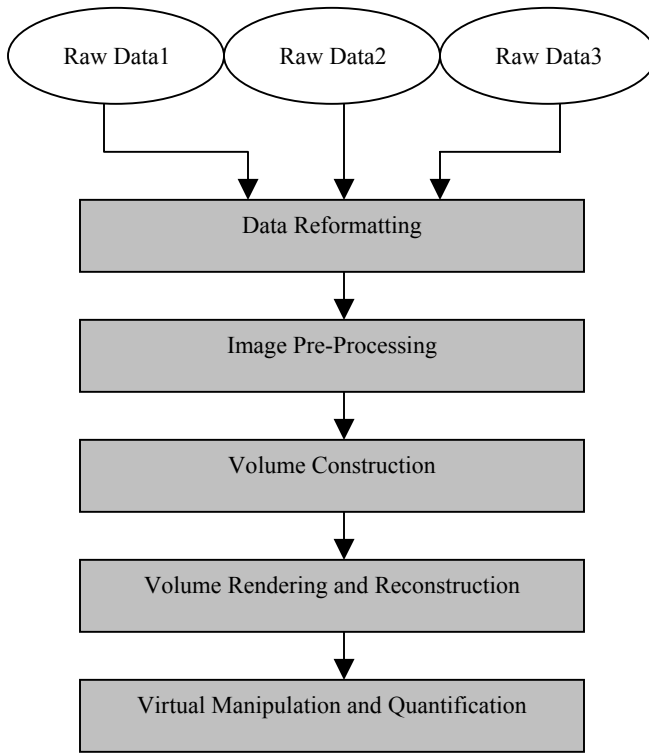


Figure 4: Pipeline of VR Based Visualization

are the raw volumetric datasets, generated from a confocal microscope in three data channels with different colors. The first job with our system is data reformatting. This is to convert the confocal image into a format suitable for volume rendering. Several image processing works are next performed on raw data. These pre-processing jobs include noise removal and smoothing, in order to enhance the contrast and the signal-to-noise ratio of original images. 3D volume can then be constructed and, consequently, interactive volume visualization and manipulation can be performed to reconstruct the 3D cellular structure. With the visualized images and the aid from interactive tools, virtual environment can then be built up to quantify the 3D cellular structure.

3.2 Pre-Processing

The raw confocal images produced by the confocal microscope usually have poor contrast, and poor signal-to-noise ratio. To improve the performance of final images or to meet some specific requirements, it is required to do some kinds of pre-processing on the raw confocal datasets. For example, using threshold technique, some trivial background noises could be eliminated and the image

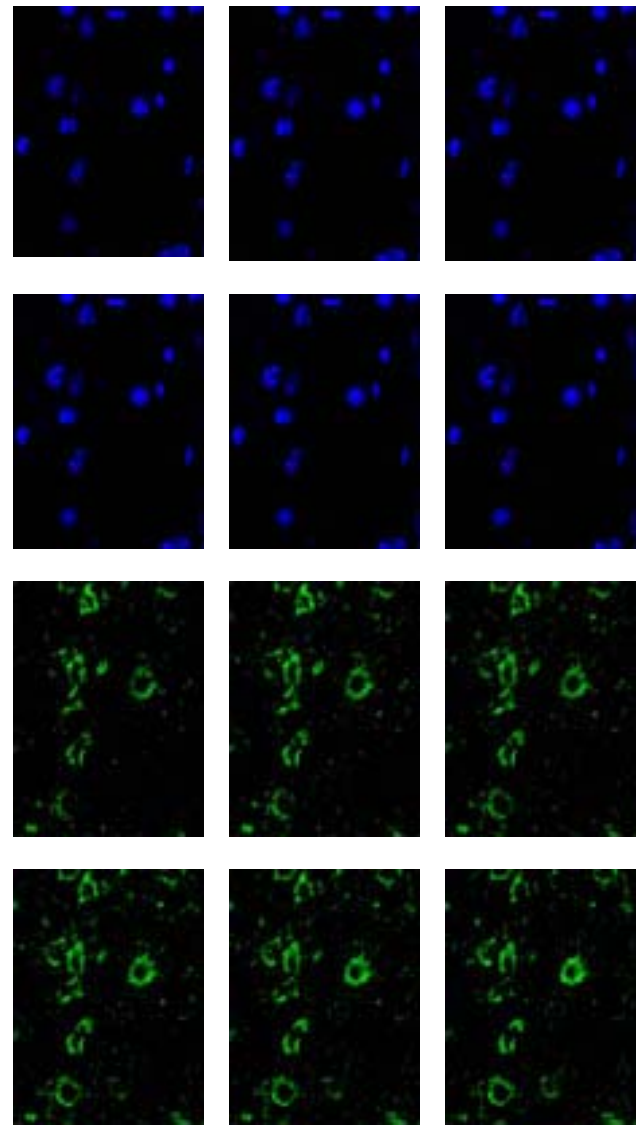


Figure 5: The processed confocal images of cell nuclei labelled with a DNA marker (blue) and calreticulin labeled with antibodies (green) shown in Figure 3.

contrast could be enhanced easily. Figure 5 illustrates the resultant images of the confocal images shown in Figure 3 after pre-processing.

3.3 Volume Reconstruction

A volume dataset is a three-dimensional array of data values associated with points in a three-dimensional space. Each element in the three-dimensional array is called a volume element, or voxel (in analogy with pixel, which denotes a picture element of a two dimensional image). A set of three-dimensional integer coordinate (x, y, z) denotes the position of a voxel within the three-dimensional array.

In our case, the cellular volume is made up of the series of confocal images. Each voxel is mapped to one pixel on the two-dimensional confocal image. The x and y -axis positions of the voxel in the volume are the same to the position of the pixel in the two-dimensional confocal image. And the z -axis position of the voxel is the order of the two-dimensional confocal image in the image stack.

Sometimes, the confocal image stack may have unequal resolutions in spatial and lateral directions. For example, in some datasets, two adjacent pixels in an image are $0.3 \mu\text{m}$ apart while two consecutive images are $0.4 \mu\text{m}$ apart. In this case, the x , y and z coordinates of a voxel must be multiplied by separate factors, (p , q , and r for example) to obtain its positions in physical space ($p \times x$, $q \times y$, $r \times z$).

3.4 Volume Rendering

Volume rendering, or direct volume rendering, is a technique of displaying volumetric data sets as a two-dimensional image. It does not involve the intermediate representation of volume data to geometric primitives; rather the volume is directly visualized by projecting the data onto an image screen. Over the past two decades, volume rendering has become an important visualization method for a wide variety of applications^[6-10]. With its various applications, volume rendering was proved to be effective, simple and fast to display the surfaces from sampled volumetric data.

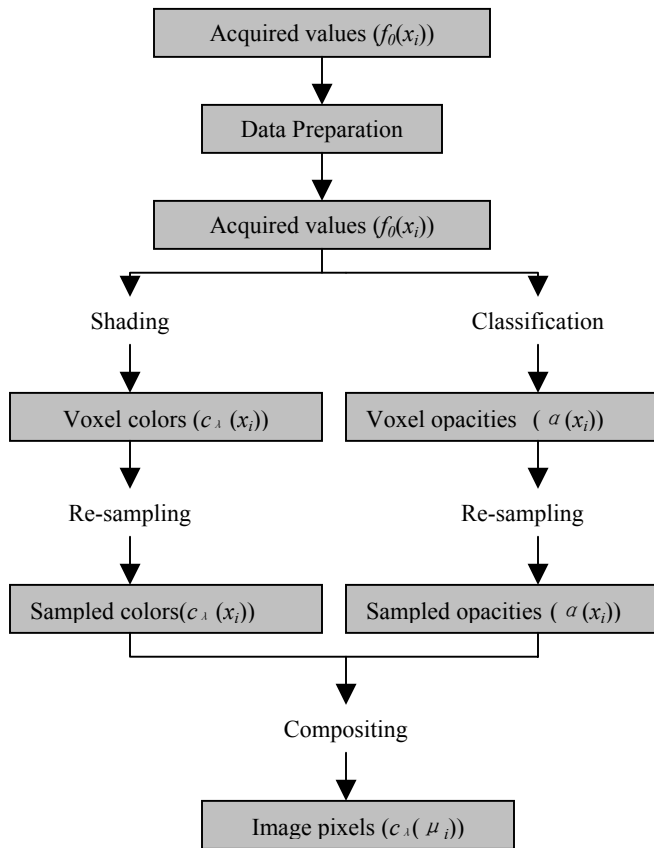


Figure 6: Pipeline of ray-casting algorithm

In volume rendering, the most popularly used method is ray-casting algorithm^[11] (Figure 6), which casts parallel rays from each pixel on the image into the semi-transparent three-dimensional volume. The color intensity of each pixel is obtained by composing the color contributions of each sample point on the ray cast from the pixel point. The details of ray-casting algorithm are described by Levoy^[11].

Direct volume rendering is structure independent, which is different from surface rendering^[17]. But because of the intensive-computing work, the speed of direct volume rendering is generally slow.

3.5 Hardware-accelerated Volume Rendering

Recent advancement in volume rendering enables rendering of full volumes at interactive speeds on an ordinary desktop computer. Earlier efforts towards real-time volume rendering were based mainly on optimized software techniques, such as the Shear-warp rendering algorithm^[12]. Today, volume rendering hardware allows real-time volume visualization on desktop PCs. VolumePro^[13] is the first single-chip real-time volume rendering system that can be implemented on consumer PCs. It can render rectangular volumetric datasets up to 512^3 8-bits voxels in real time using ray-casting algorithm.

With VolumePro's support, the volume model of cellular structure can be reconstructed using the successive 2D scan slices. Figures 7(a)-(c) illustrate the reconstructed and visualized volume model of the image stack of confocal x - y sections shown in Figure 5. With volume rendering, users are able to interact with, and manipulate, the rendered objects and hence attain clearer and more detailed interpretation. Common manipulations include cutting, rotating, and zooming. In our system, these operations can be achieved in real time (see Figures 7(d)-(f)).

Figure 8 shows sequential sections throughout a cell labelled with a fluorescent actin probe. Figure 9 illustrates the rendered volume and some manipulations on it, like rotating, adding a cutting plane through the volume and zooming.

3.6 Intracellular Virtual Reality

Virtual Reality (VR) is a form of simulation in which computer graphics is used to create a realistic world. A user is able to interact with the synthesized world. Interactivity and a captivating power of virtual reality contribute to the feeling of "immersion", of being a part of the action happening on the screen, that users experience.

In some way, VR can help deepen users' perception of intracellular structure and the distribution of proteins in cells. In the virtual environment, users can have the depth feeling of the cell samples and thus will view the cell samples as realistic 3D objects. In this way, the stereoscopic vision enables users to assess the size of an object.

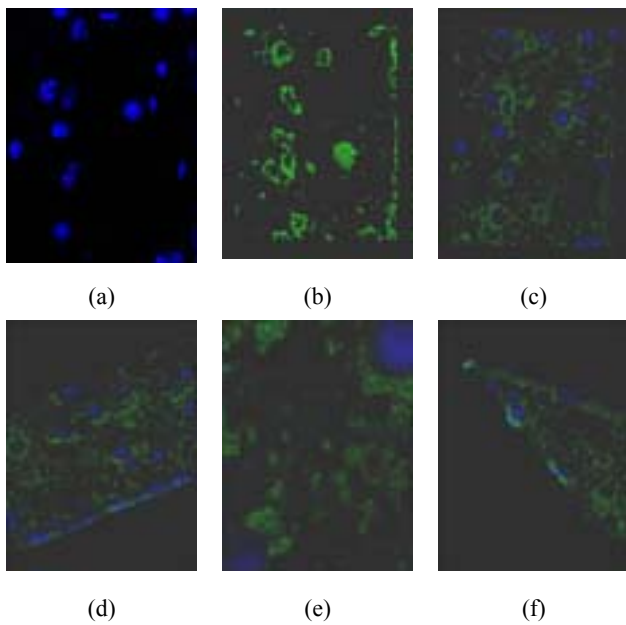


Figure 7: (a) Rendered images of cell nuclei; (b) rendered image of calreticulin; (c) rendered image of combined dataset of cell nuclei and calreticulin; (d) rendered image after rotation; (e) rendered image after zooming in; (f) rendered image after adding a cutting plane through the volume

At first let's have a quick look at how human beings get to know the depth information of 3D objects. 3D objects provide people with perceptions of width, height and depth, but common 2D images could only convey the width and height information. Most people associate depth perception with stereopsis in which both eyes register an image and the brain uses the horizontal shift in image position registered by the two eyes to measure depth.

Hence, based on the human eye stereo viewing principle, to produce the stereo image, we need to generate two images of the local volume for the left and right eyes respectively and display them on the stereo-ready monitor screen. In our system, at first we utilize volume rendering to generate two images of the reconstructed three-dimensional cellular model for the left and right eyes respectively (Figure 10). Subsequently the two images are displayed on the stereo-ready screen. Users wearing a pair of stereo glasses would view the volume as a three-dimensional model with position values in x , y and z directions. Hence, in this virtual environment, three-dimensional manipulation or operation can be efficiently developed with the aids of various interactive tools, such as the data glove and motion capturing devices (see Figure 11).

4. Conclusions

We have built up a comprehensive system for cell biology research. With high-resolution confocal imaging, internal cell structure and distribution of specific proteins can be imaged. After pre-processing, the confocal image stacks can be utilized to reconstruct three-dimensional volumes which subsequently will be visualized using volume rendering technique and displayed on the screen. Furthermore, virtual reality technique is integrated

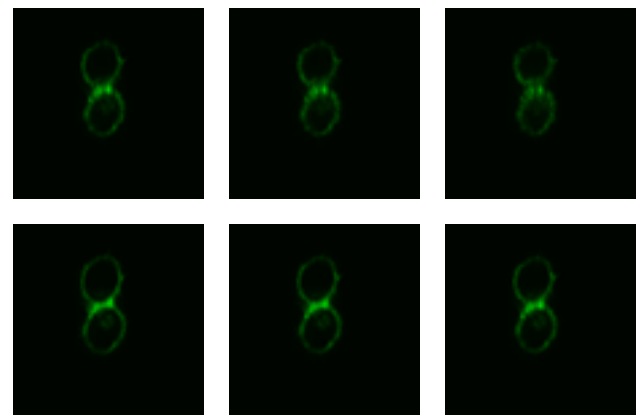


Figure 8: A series of 2D confocal X-Y sections showing a distribution of cytoskeletal protein, actin, throughout a cell.

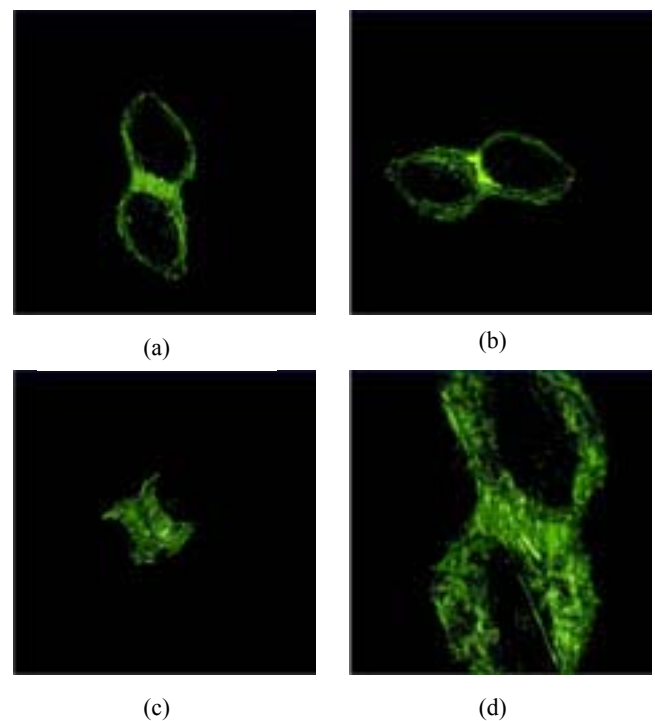


Figure 9: (a) Rendered image of actin; (b) rendered image after rotation; (c) rendered image after adding a cutting plane through the volume; (d) rendered image after zooming in

with volume rendering in order to provide users a direct and intuitive interface to view and interact with the stereo models.

The present work shows feasibility of using stacked 2D images obtained with a confocal microscope for 3D reconstruction and subsequent volume rendering. The major limitation that we face presently is the limited number of sections in confocal stacks. This is due to excessive photobleaching during collection of images. In future, attempts will be made to remedy this problem by use of multi-photon excitation for confocal imaging and image collection.

Some basic manipulations currently can be achieved on the visualized volume. And more quantitative analysis and information extraction of subcellular components are going to be completed in the future.

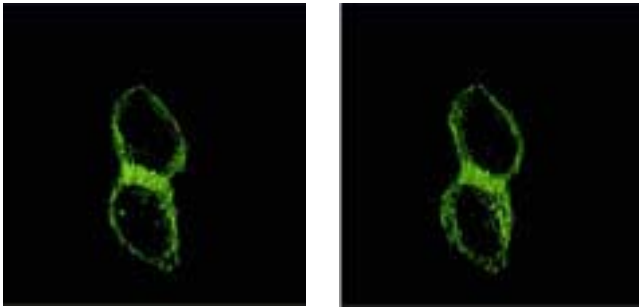


Figure 10: (a) Rendered image of actin for left eye; (b) rendered image of actin for right eye

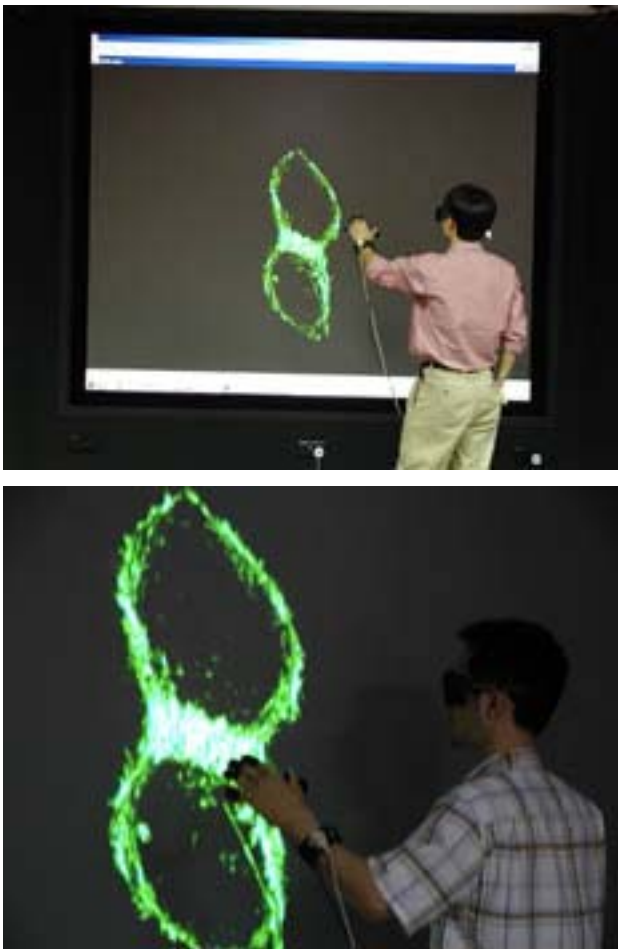


Figure 11: Virtual and interactive environment for volumetric cellular research

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