Image processing and interactive visualization of confocal microscopy images

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ABSTRACT

The essence and methodology is proposed for image processing and interactive visualization of confocal microscopy images. Firstly, an intensity compensation algorithm is applied to reduce noises resulted from light absorption and scattering by objects and particles in the volume through which light passed. Secondly, a deconvolution algorithm based on Maximum A Posteriori is applied to improve the image stacks’ quality. Lastly, the ray-casting algorithm is used to reconstruct the fine details in the volume. Furthermore, interactive control panel is integrated to provide users an intuitive interface to view the cells and its structures through translation, rotation and zoom in/out operations. Experimental results demonstrate that the speed and versatility of the program allows for convenient, fast, interactive examination of unknown cell structures even on commodity PC without any other special hardware. The program is also able to visualize dynamically changing temporary structures conveniently in addition to static images.

Key words: Image processing; volume visualization; virtual reality; laser scanning confocal microscopy.

1. INTRODUCTION

After several years of research and development in hardware and software, the techniques of understanding complex topologies in the macroscopic scale are now considered well established. However, the structures in the microscopic scale show even much more complex than those of organs. For example, one can list structures of nerve cells, tissues, muscles, blood vessels etc., showing
beautiful, complex, and still unexplored patterns. In order to understand the spatial relationship and internal structures of such microscopic probes, tomographic series of slices are required. Laser scanning confocal microscopy (LSCM) is a relatively novel method allowing for a true tomographic inspection of microscopic probes. In fact, LSCM is generally believed to be one of the most significant advances in optical microscopy in the 20th century and it has become a widely accepted tool for optical imaging in biological and material sciences.

LSCM is relatively new in obtaining tomographic structures in the microscopic scale [1–7], probably due to the relative ease to acquire high quality images from specimens and the growing number of applications in cell biology relying on imaging fixed and living cells or tissues. However, how to process confocal data sets still remains a puzzle. Our objective is to carry out an applicable method about computer-aided image processing and interactive visualization of confocal microscopy data sets, including pre-processing steps to improve the image stacks' quality and interactive visualization method to reconstruct the fine details, with several valuable examples demonstrated.

2. PRINCIPLES OF LASER SCANNING CONFOCAL MICROSCOPY

The principle of LSCM is shown in Fig. 1 [1–2]. Visible or ultra violet laser emission is focused on the first confocal pinhole and then onto the specimen as a diffraction limited light spot. The primary incident light is then reflected from particular voxel elements or emitted from fluorescent molecules excited within it. Emissions from the object return along the primary laser light pathway and depart from it by lateral reflection from (or passage through, depending on the instrument) a dichroic mirror onto the second confocal pinhole. This aperture is confocal with the in-focus voxel elements in the specimen. The virtual elimination by defocusing of all distal and proximal flanking emissions at this physical point assures that the light passing onto the detector, a sensitive photodetector is specifically derived from in-focus object voxels with a resolution. In LSCM, only in-focus light is imaged, whereas out-of-focus light is rejected by the confocal pinhole [1, 2]. The light spot can be scanned in successive $z$ sections to image the entire object by means of a precise stage motor.

LSCM offers several advantages compared to the conventional wide-field optical microscopy. However, the image data sets acquired by LSCM show several characteristics requiring special processing techniques to make the method applicable [1, 3, 4]:
1. Typical datasets are so large that they require efficient processing methods and adequate computer memory.

2. Fluorescently labeled samples characteristically have low signal levels so that microscopy images are typically much noisier than CT and MRI images.

3. Since excitation of fluorescence also destroys fluorophores through photobleaching, the signal to noise ratio decreases with the collection of each focal plane of an image volume. Consequently, microscopy image volumes are usually sensitive to small changes in rendering parameters, which leads ordinary volume visualization algorithms to fail to capture the delicate structures.

4. The image data sets usually have unequal resolutions in the plane and the depth directions. A visualization method therefore has to be able to perform with "blocks" or unequal size lengths instead of cubic voxels.

Figure 1. Principle of Laser Scanning Confocal Microscopy.
5. The structures of the objects to be examined are often partially or entirely unknown, which leads to the critical need for interactive navigation and searching capabilities.

3. IMAGE PROCESSING AND INTERACTIVE VISUALIZATION METHODOLOGY

3.1. Intensity Compensation Algorithm

Firstly, an intensity compensation algorithm is applied to reduce noises resulted from light absorption and scattering by objects and particles in the volume through which light passes, shown in Fig. 2. Consider light propagation from point \( r_0 \) to \( r \) in a transparent medium. According to the Lambert-Beer Law, the effect of light absorption and scattering can be generally described by

\[
I(\lambda, r) = I_0(\lambda, r_0) \exp(-\alpha |r_0 - r|)
\]

where \( I_0 \) is the light intensity and \( \lambda \) is the wavelength. Here \( |r_0 - r| \) is the path length and \( \alpha \) represents the sum effect of the volume absorption and scattering power per unit length of the medium. Many elements affect the value of \( \alpha \), e.g. the attributes and characteristics of glass slide, microscopic glass, mounting medium and so on. Without the loss of generality, it is assumed that \( \alpha \) is only associated with the voxel intensity at \( r \) measured in the confocal images in order to simplify it. The essence of intensity compensation algorithm is that the intensity value of every pixel in image stacks above the focal plane is multiplied by factor \( \alpha \) and then accumulated to compensate the light attenuation in the focal plane.

Figure 2. Interaction of Organism and Light.
3.2. Deconvolution Algorithm Based on MAP

The time in acquiring one confocal image is about 1s in many popular LSCM, which is much longer than the time of physiological process. Therefore, the size of the pinhole is usually increased to decrease the image acquire time, and then a deconvolution algorithm is used to account for image degeneration. Maximum A Posteriori method, a natural candidate for solution by Expectation-Maximization algorithm, is used based on Bayes formula.

The EM algorithm is often used in estimation problems that would be easy, if the values of some discrete “hidden” variables were known. The hidden variables are the cell details of the voxels in our experiment. The EM algorithm is iterative, and alternates between two steps: the “E step” where the hidden variables are estimated based on the most recent estimate of the “ordinary” variables and the “M step” where the ordinary variables are estimated based on the most recent estimate of the “hidden” variables [8]. EM algorithm always converges in 7–15 iterations in practice, meaning that the location and value of the maximum bias is within a predefined tolerance of values in the previous iteration.

The algorithm used in experiment is started on the E step, with the bias field initialized to zero at all voxels. The steps are described as following:

Step 1: input original data

\[ \phi^0 = \phi = \sum_{\xi, \xi} \varepsilon(\xi, \xi) \] and \[ \phi = \sum_{\xi, \xi} \phi(\xi, \xi) \]

Step 2: compute power spectrum

\[ \phi^0 = \phi = \sum_{\xi, \xi} \varepsilon(\xi, \xi) \] and \[ \phi = \sum_{\xi, \xi} \phi(\xi, \xi) \]

Step 3: compute

\[ H^{(k)}(\xi, \xi) = \frac{H^{(k)}(\xi, \xi) G(\xi, \xi)}{G^{(k)}(\xi, \xi) \phi(\xi, \xi)} \]

Step 4: iterate according to those formulas:

\[ \phi^{(k+1)}(\xi, \xi) = \phi^{(k)}(\xi, \xi) + H^{(k)}(\xi, \xi) \]

\[ H^{(k+1)} = \frac{G(\xi, \xi) \phi^{(k+1)}(\xi, \xi)}{N \phi^{(k+1)}(\xi, \xi)} \]

\[ k = k - 1 \]

Step 5: if \( k \neq 0 \) then go to STEP3; otherwise operate inverse Fourier transform on \( \rho^{(k+1)}(\xi, \xi) \) and output the result.
3.3. Ray-Casting Algorithm

In volume rendering, the most popular method is ray-casting algorithm, shown in Fig. 3. This algorithm 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 [5–6]. The details of ray-casting algorithm are described by Levoy [9].

In the flow chart of ray-casting algorithm, after the volume data is acquired or imported, the whole volume scene is transformed and clipped according to the viewing direction. Pre-classification and transfer function are applied before the ray traversal. Uniform sampling and interpolation are then carried out along each ray. The color and transparency of each sampling point are accumulated, followed by gradient-based shading. Finally, the output image is altered and displayed.

3.4. Interactive Control Panel

An interactive control panel similar with the Virtual Reality Toolbox Viewer of MATLAB [10], shown in Fig. 4, is designed to view virtual cells and tissues. The center circular button of the viewpoint control resets the camera to the current viewpoint. The right and left arrows around it browse through predefined viewpoints. The headlight toggle controls whether the camera
emits light or not. The center navigation wheel can move the scene in all
directions. There are three navigation methods (walk, examine and fly),
whose behavior associated with the movement modes and navigation zones
are shown in Table 1 [10].

### Table 1. Navigation Methods and Description.

| Movement Mode | Mouse Navigation Description |
|---------------|-------------------------------|
| Walk          | Outer—Click and drag the mouse up, down, left, or right to move the camera in any of these directions in a single plane. Inner—Click and drag the mouse up and down to move forward and backward. Drag the mouse left and right to turn left or right. |
| Examine       | Outer—Click and drag the mouse up and down to move forward and backward. Drag the mouse left and right to pan left and right. Inner—Click and drag the mouse to rotate the viewpoint about the original of the scene. |
| Fly           | Outer—Click and drag the mouse to tilt the view either left or right. Inner—Click and drag the mouse to move the camera up, down, left or right within the scene. Center—Click and drag the mouse up and down to move forward and backward. Move the mouse left or right to turn in either of these directions. |
4. EXPERIMENTAL RESULTS

Fig. 5 shows the reconstruction result and its pseudo image of the heart cell confocal stack acquired by Leica LSCM. The original image format is $512 \times 512$ pixels with 30 focal planes.

Fig. 6 shows the reconstruction results of the same confocal stack acquired by Zeiss LSCM under the same conditions. The original image format is $400 \times 300$ pixels with 21 focal planes. The difference between these two images is that the right one is rendered after image pre-processing, including intensity compensation algorithm and deconvolution based on MAP algorithm. Results demonstrate that the image pre-processing techniques can effectively remove the noises due to light absorption and scattering and result in a much more clear and plain reconstruction image.

Fig. 7 shows the reconstruction results of confocal stack of C. elegans embryo undergoing cell fusion acquired by Bio-Rad LSCM. The original image format is $192 \times 128$ pixels with 9 focal planes and 12 time points. Visualization of time varying volume data is a very complex task, due to their dynamic nature. Static images often suffer from cluttering when many time steps are visualized; on the other hand, animations often don’t allow an in-depth analysis of certain data characteristics. The fanning in time allows to convey similarities.
and differences in the progress of time. Furthermore, a topological relationship between different time steps is observed in much more details.

Fig. 8 shows the reconstruction results of confocal stack of embryonic cortex neurons in SD pregnant mouse, green light indicating Ca²⁺ increase and red light indicating Ca²⁺ decrease.

5. DISCUSSION AND CONCLUSIONS
An applicable method is proposed for image processing and interactive visualization of confocal microscopy images by integrating several algorithms, with several valuable examples demonstrated. Firstly, an intensity compensation algorithm is applied to reduce noises resulted from light absorption and scattering by objects and particles in the volume through which light passes. Secondly, a deconvolution algorithm based on Maximum A
Posteriori is applied to improve the image stacks’ quality. Lastly, the ray-casting algorithm is used to reconstruct the fine details in the volume. Furthermore, virtual reality technique is integrated with volume rendering to provide users an intuitive interface to view the cells and its structures through translation, rotation and zoom in/out operations. Experimental results demonstrate that the speed and versatility of the program allows for convenient, fast, interactive examination of unknown cell structures even on common configuration PC. By testing different datasets the program has been able to significantly improve the task of understanding the internal structure of laser scanning confocal microscopy image stacks. The program is also able to visualize dynamically changing temporary structures conveniently in addition to the static images.

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