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Image Processing Software for 3D Light Microscopy

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Key Words
3D · Microscopy · Software · Image processing · Image analysis

Abstract
Advances in microscopy now enable researchers to easily acquire multi-channel three-dimensional (3D) images and 3D time series (4D). However, processing, analyzing, and displaying this data can often be difficult and time-consuming. We discuss some of the software tools and techniques that are available to accomplish these tasks.

Introduction

Advances in light microscopy now enable researchers to easily acquire multi-channel three-dimensional (3D) images and 3D time series (4D). Conventional light microscopes produce two-dimensional (2D) images that provide a limited amount of information about the organization of cells and tissues in three dimensions. Several computer-controlled fluorescence microscopy systems are available commercially which can collect a stack of closely-spaced 2D images, then use software to display and manipulate the stack as a single 3D image. The availability of a variety of specific fluorescent probes allows these microscopes to be used to study a wide range of physiological parameters in fixed and living cells, tissue sections, and even live animals [1–4]. But it is the availability of 3D image analysis software which runs on low-cost personal computers that makes 3D and 4D image-based research practical. This review examines some of the software tools and techniques that are available to process, analyze, and display these images.

Most 3D imaging software can be divided into five functional modules: image acquisition, processing, rendering, segmentation, and measurement (fig. 1). The software will include one or more of these functional modules. Commercial software often consists of a base program, with additional modules that can be purchased as needed. Some programs can be customized by the user to perform additional tasks.

Image Acquisition

Image acquisition programs, which control microscopes and associated imaging hardware, are used to collect and store stacks of parallel planar (2D) images in files. These 3D files are then passed to other programs to be processed, displayed, and analyzed. Unfortunately, there is no standard file format for 3D images, so some microscope manufacturers have created their own formats, while others use TIFF or variants of TIFF.

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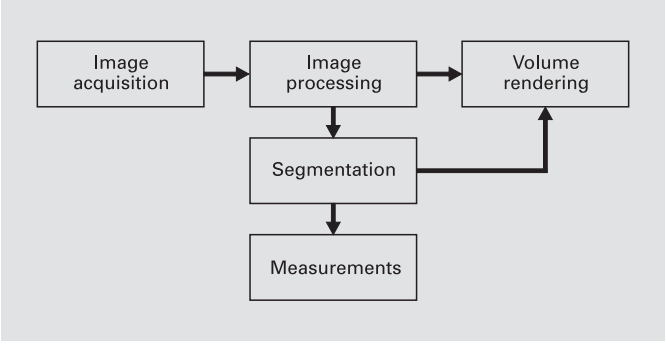

Fig. 1. Data flow diagram of the 3D imaging pipeline.

Manufacturers often provide free image viewer programs for their files that permit you to display and print images. However, additional software is needed to perform sophisticated image processing, analysis, and volume rendering. When evaluating 3D imaging software, make sure that the software that you are considering fully supports the file formats used by your microscopy system(s). This is important because these files contain more than just images. They often include information on microscope configuration and imaging parameters, and some of this information is used by 3D image processing, rendering, and measurement software. One project that is trying to standardize how microscopy data are stored and retrieved is the Open Microscopy Environment (www.openmicroscopy.org).

Image Processing

Various image processing operations may need to be performed on 3D images before they are displayed or analyzed. Some operations attempt to correct for distortion introduced by microscope optics and tissue during image acquisition (e.g. deconvolution, spherical aberration correction), or spectral overlap of fluorophores (e.g. linear unmixing), while ratiometric operators are used when studying ion concentration or pH, and various filters and intensity transformations may be used to improve the visibility of structures. The simpler image processing operations, such as intensity and color transformations, are often performed interactively during volume rendering.

One particularly interesting image processing technique is deconvolution, which can improve the visibility of small structures in some images by increasing resolution and contrast. Deconvolution can also be useful in quantitative studies [5, 6]. However, care should be taken when selecting a deconvolution algorithm, since some algorithms are not suitable for quantitative photometric studies. See the review by Wallace et al. [7] for a more detailed discussion of deconvolution. Most commercial microscopy-oriented imaging programs provide 3D deconvolution modules, including Huygens, Volocity, Imaris, analySIS, Amira, MetaMorph, and Image-Pro Plus. Some of them use the AutoDeblur software developed by AutoQuant. However, there are only a few free 3D deconvolution programs, notably XCOSM (www.omrfcosm.omrf.org/xcosm) and plug-ins for ImageJ [8] by Besson and Dougherty. Figure 2 shows an example of using deconvolution on an image of a fetal mouse kidney.

Volume Rendering

Volume rendering programs are used to create 2D projection images showing 3D stacks of cross-sectional images from various points of view. A few of these programs can also display sequences of 3D stacks collected over an extended period of time, which can be very useful in developmental studies [9]. Volume rendering programs can also usually perform simple image processing, including adjusting brightness, contrast, color, and opacity. Some volume rendering programs use the 3D graphics processor found on video boards in many personal computers to accelerate the rendering process, permitting the user to more quickly and easily manipulate 3D images. When selecting volume rendering software, it is crucial that your video board be equipped with one of the processors supported by the software, and that your computer has an appropriate amount of memory.

Most of the major commercial imaging programs for microscopy include a volume rendering module. There are also several free volume rendering programs available, but only a few of them can import the 3D image files produced on microscopy systems. Voxx [10] and VisBio [11] are two such programs. Free volume rendering plug-ins are also available for ImageJ. Figure 3 shows an example of volume rendering of the ureteric tree of a fetal mouse kidney using Voxx.

Image Segmentation and Analysis

Segmentation is the process of separating an image into discrete regions. For example, DAPI-labeled nuclei can be segmented from an image of cultured cells. The

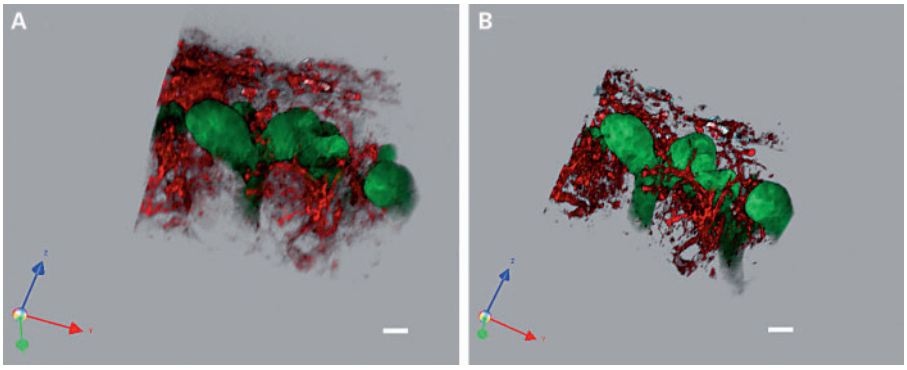

Fig. 2. Confocal images showing increased resolution and contrast after deconvolution (**B**) of ureteric tree ampullae (green) and blood vessels (red) in fetal HoxB7-GFP mouse kidneys (**A**). The image stack was deconvolved using 25 iterations of the maximum entropy-based algorithm in the restoration module, then volume rendered using the visualization module, in Volocity. Scale bar = 20 μm.

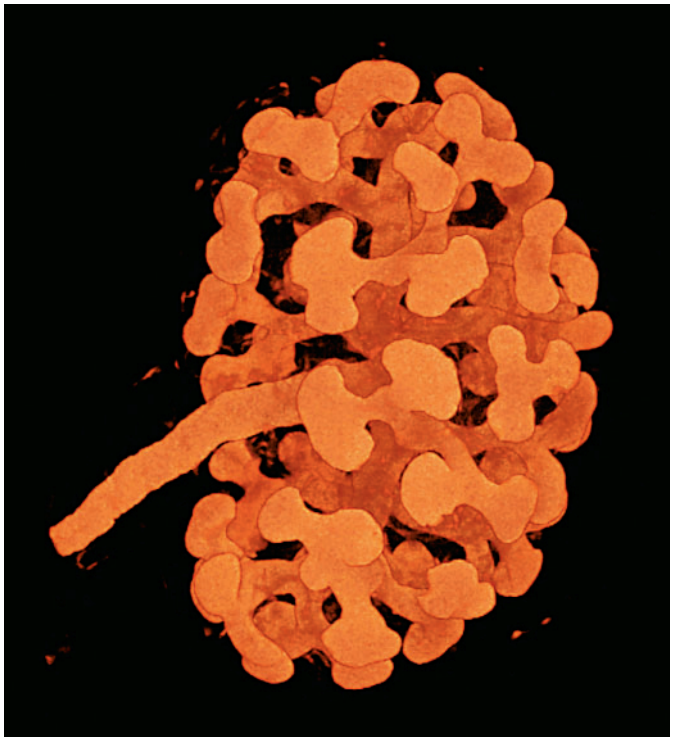

Fig. 3. Volume rendering of the ureteric tree in a fetal HoxB7GFP mouse kidney using Voxx.

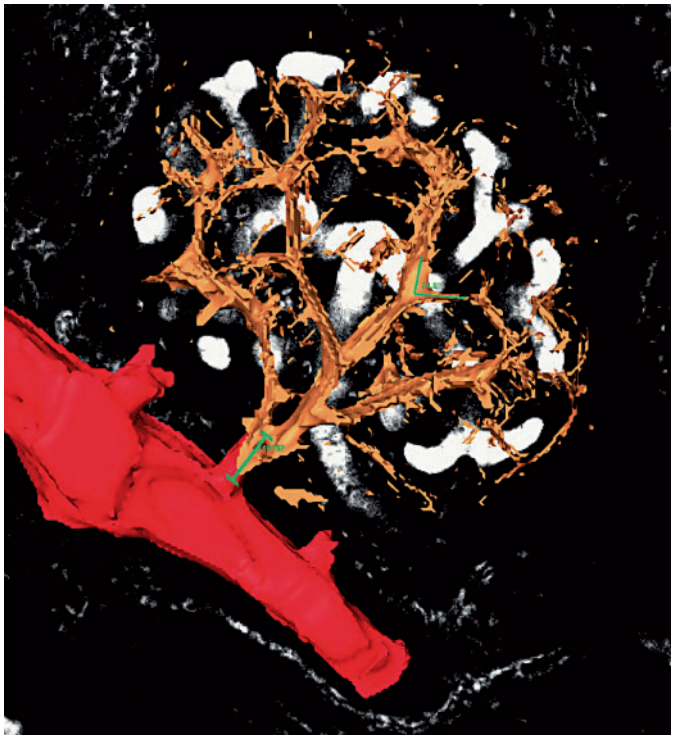

Fig. 4. Image segmentation of a fetal HoxB7-GFP kidney using Amira. The dorsal aorta (red) and renal vasculature (orange) were isolated using a combination of active contours and manual segmentation.

segmented nuclei can then be counted or measured. Segmentation software can use several properties to group pixels or voxels, including intensity, intensity gradients, color, shape, orientation, and connectivity. Automatic segmentation is the ideal, but tracing the boundaries of regions by hand is still commonly done, due to the complexity of many biological structures. Once structures have been segmented, morphometric and photometric measurements can be made in the segmented regions. For example, measuring colocalization of different fluorophores is such a task. Figure 4 shows an example of segmentation of a fetal mouse kidney using Amira [for more information on segmentation, see 12–14].

Table 1. Microscopy-oriented programs to process 2D and 3D images

Program	Website
Free	
ImageJ	http://rsb.info.nih.gov/ij
VisBio	www.loci.wisc.edu/visbio
Voxx	www.nephrology.iupui.edu/imaging/voxx
Commercial	
Amira	www.amiravis.com
analySIS	www.soft-imaging.com
AutoQuant X	www.aqi.com
Huygens	www.svi.nl
Image-Pro Plus	www.mediacy.com
Imaris	www.bitplane.com
MetaMorph	www.moleculardevices.com
Volocity	www.improvision.com

Voxx and VisBio are volume rendering programs, while the other programs can be used to segment and measure images.

Software

There are many programs available for 3D image processing, but only a few of them can handle the multi-channel 3D and 4D images produced by confocal and two-photon microscopes. A list of several microscopy-oriented programs is included in table 1, along with website addresses where information on each program can be found. Commercial software developers usually provide free trial versions of programs that can be downloaded from their websites. These either provide limited-time access to all of the features in the program, or access to a subset of the features with no time limit. Most of the commercial imaging programs have a similar set of features, which makes it more difficult to decide which software to purchase. You should always process some of your images using the trial version of the software, to make sure that it really can do the job. You should also consider ease of use. And finally, look at the total cost of ownership: the price of the base program, additional modules, multi-user licenses, and yearly software updates.

Most commercial 3D imaging software is expensive, so limited funds may force individual researchers to use whatever program(s) their institution has already purchased. Consequently, many researchers use a combination of commercial software and free non-commercial software. ImageJ [8] is probably the most widely used of the noncommercial imaging programs, augmented by many user-developed plug-ins. An online manual describing ImageJ and some of the plug-ins used in microscopy can be found at www.uhnresearch.ca/facilities/wcif/imagej.

Conclusions

There are many 3D imaging programs available, but only a few of them have been designed for 3D light microscopy. Before selecting such software, you should ask the following: (1) Will this software be able to import files from all of the microscopes that you use? (2) Does the software include all of the features that you need? For commercial packages, how many additional modules will you have to purchase to meet your analysis needs? (3) Will you need to use more than one program to analyze your images? If so, can image and data files created by each program be read by the other programs? (4) Do you have the required hardware (e.g. enough memory, suitable video board) to use the imaging software?

Given the expense of commercial software, and the long learning curve associated with most imaging software, one should thoroughly examine the capabilities of the programs and applicability to both present and future projects before making a purchase.

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