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Free-D: an integrated environment for three-dimensional reconstruction from serial sections

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Abstract

Three-dimensional (3D) reconstruction is a powerful tool to investigate complex neuroanatomical organizations. 3D models are often generated by piling up registered segmentations carried out on serial sections labeled by histological means. However, these models suffer limitations (incompleteness and lack of statistical representativity), which can be overcome by model averaging and fusion. These operations require an appropriate reconstruction environment allowing the simultaneous processing of several data sets. This paper describes the first release of *Free-D*, a software designed for the reconstruction of 3D models generated from stacks of serial sections, in the perspective of model averaging and fusion. A unique graphical user interface integrates the 3D reconstruction tools. Several large stacks (tens of gigabytes) including hundreds of images having heterogeneous characteristics (size, resolution, depth, etc.) can be simultaneously processed, thus complying to most encountered experimental situations. This first version of *Free-D* constitutes the required environment for the future integration of the averaging and fusion algorithms currently developed in our group and illustrated here with preliminary results.

Keywords: Three-dimensional reconstruction; Serial sections; Neuroanatomy; Surface modeling; Neuroinformatics

1. Introduction

Anatomical and functional neuroimagery of the laboratory small animal has seen the recent advent of a number of three-dimensional (3D) techniques such as confocal microscopy (Fine et al., 1988; Dailey, 1996), microscopic magnetic resonance imaging (Narasimhan and Jacobs, 1996; Benveniste et al., 2000), microscopic X-ray tomography and micro-positron emission tomography (Phelps, 2000). However, 3D reconstruction from serial sections remains the tool of choice to study the distribution of specific tracers over wide spatial extents at tissular resolution. In this methodology (Toga, 1990), the specimen is serially cut into slices whereupon structures of interest are revealed using specific histological processing. The digitized slice images are then segmented by delineating the identified structures. Using rigid geometric transformations (translation and rotation), the images are registered. The piling-up of the registered segmentations allows the reconstitution of the studied structures as graphical 3D models. Despite its indisputable value as an aid to elucidate complex spatial neuroanatomical organizations, 3D reconstruction suffers from two main limitations. First, each reconstructed model may be incomplete because of the practical impossibility to label in a single experiment as many distinct structures as desired. Second, each model is not statistically representative since it is generated from data collected on a unique animal. Generating models that are both complete and representative necessitates that data gathered from distinct experiments on as many animals be integrated into unique, generic representations (Banrezes et al., 2003). Due to inter-individual and experimental variabilities, this can only be achieved by model fusion, a process involving the spatial normalization of the individual models. The availability of a user-friendly 3D reconstruction software, designed in the perspective of 3D model averaging and fusion, is thus essential. Such a software should take into account the need to simultaneously process several image stacks and multiple 3D models.

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Apart from commercial softwares (e.g., Amira (TGS), Imaris (BitPlane), Metamorph (Universal Imaging Corp.), Neurolucida (MicroBrightField)), a number of non-commercial 3D reconstruction tools are available (Huijsmans et al., 1986). Most of them are designed as suites of independent programs (Smith, 1987; Hessler et al., 1992; Kremer et al., 1996) or as function libraries (Chen et al., 1996), each component of which is dedicated to a particular step of the reconstruction process. Reconstructing a model encompasses several segmentation/registration/visualization cycles, because errors generally remain undetected until the 3D model is displayed. Thus, using a collection of non-communicating programs makes the reconstruction process inefficient and error-prone since data files have to be repeatedly fed through a pipeline of applications without any immediate 3D visual feedback on registration and segmentation actions. Furthermore, most of the existing reconstruction softwares were developed under the Unix operating system on Silicon Graphics machines because 3D rendering requires high memory and graphical performances. This hampers software diffusion and accessibility. Lastly, none of the existing reconstruction software was designed to allow the simultaneous processing of distinct data stacks in the perspective of model fusion.

A software integrating into a unique graphical user interface all the required functionalities for 3D reconstruction had been developed by our group (Roesch et al., 1996). However, this software, running under the Microsoft Windows operating system, presented only a limited number of 3D rendering functionalities. Moreover, since it used its own graphical library, it could not benefit from the low-cost accelerated graphics hardware now available for PCs. Furthermore, it was not designed for model fusion.

We have thus undertaken the development of *Free-D*, a 3D reconstruction software designed in the perspective of fusion and quantitative analysis of 3D models. Special care has been taken to avoid dependency upon platform and operating system, to minimize constraints regarding input data characteristics and formats and to maximize flexibility and interactivity, while ensuring software evolvability. We describe here the first release of *Free-D* that offers a collection of 3D reconstruction tools illustrated on rat neuroanatomical data. It will constitute the software environment for the integration of the fusion algorithms currently developed in our laboratory.

2. Material and methods

2.1. Software development

Free-D is developed under the Linux operating system and is written in the C++ programming language. The Qt library (Dalheimer, 1999) was used to program the graphical user interface. This library is freely available for Unix/Linux platforms (http://www.trolltech.com). Qt editions for Windows and MacOs X also exist. The different Qt versions are mutually source compatible. Using this library thus makes the software easily portable to platforms other than Linux. Portability is further facilitated by relying on the standard OpenGL library (Woo et al., 1999) for 3D rendering, which is also available on most common platforms (http://www.opengl.org). Besides, low-cost PC graphics cards are now available, which offer high performance for 3D rendering, thanks to dedicated hardware acceleration. It is thus possible to simultaneously reach high graphic quality and fluid user interactivity for dynamic model visualization and exploration on any laboratory PC.

2.2. Experimental data

The use of *Free-D* for neuroanatomical studies is illustrated in this paper with data from a study carried out in our group concerning the spatial organization of sacral parasympathetic nucleus (SPN) neurons in the male rat lumbo-sacral spinal cord (Banrezes et al., 2002). Tracing protocols are detailed elsewhere (Banrezes et al., 2002). Briefly, spinal neurons were retrogradely labeled following pseudo-rabies virus (PRV) injections into pelvic organs (corpus cavernosum of the penis and dome of the bladder) or by applying WGA-HRP at the central end of the sectioned pelvic nerve. Animals were subsequently anesthetized and perfused. Spinal cord segments L5-S1 were dissected out and serially cut with a cryostat into coronal sections 20 μm (WGA-HRP) or 30 μm (PRV) thick. Following histological processing for revealing the tracers, every other section was retained for 3D reconstruction. Fig. 2B displays the image of one such section obtained after PRV injection into the corpus cavernosum of the penis. Fig. 2C shows a magnified portion of this image containing PRV-labeled neurons.

3. Results

Free-D is routinely used in our laboratory on standard PC machines running the Linux operating system. It has also been compiled and run on Silicon Graphics machines running Irix. The required hardware configuration depends on the volume of image data to be manipulated. The minimum required memory amount is about 256 Mb. Hardware-accelerated graphic cards are required for a fluid interactive 3D rendering.

3.1. Image and stack files

3.1.1. Slice images

The input image format in *Free-D* is the Tagged Image File format (TIFF). TIFF is a widespread platform independent format to which most other image formats can be converted. Using this format thus guarantees compatibility with almost all existing image acquisition systems and softwares. Moreover, a large spectrum of image types,