Journal of Neuroscience Methods 170 (2008) 332–344

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

An assessment of methods for aligning two-dimensional microscope sections to create image volumes

R. Beare [a,*], K. Richards [b], S. Murphy [b], S. Petrou [b], D. Reutens [a]

[a] Faculty of Medicine, Nursing and Health Sciences, Monash University, 246 Clayton Road, Clayton 3168, Australia

[b] Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville 3010, Victoria, Australia

Received 4 April 2007; received in revised form 9 January 2008; accepted 9 January 2008

Abstract

This study assessed five different methods for aligning microscope images of Nissl-stained sections of mouse brain to form three-dimensional image volumes. Methods exploiting both image content and information from un-sectioned tissue were investigated. The accuracy of reconstruction was estimated using fiducials with known physical properties, demonstrating that methods exploiting tissue content produced distorted image volumes while a method using artificial fiducials produced the most accurate and unbiased alignment. Methodological issues relating to methods of volume reconstruction are discussed and it is recommended that methods using information from un-sectioned tissue be used wherever possible.

Keywords: Volume reconstruction; Tissue section alignment; Comparison of methods

1. Introduction

Reconstruction of volume information from 2D tissue sections is important in applications requiring characterization of anatomical structure and 3D properties of tissue and organs. The process of reconstruction involves correction of misalignment between consecutive slices introduced by sectioning, mounting and image acquisition. Several techniques have previously been employed to automatically align sections. These include fiducial markers based on needle or drill holes, tubes and tissue array cores (Bussolati et al., 2005; Dogdas et al., 2007; Goldszal et al., 1995; Simonetti et al., 2006; Streicher et al., 1997) as well as approaches that exploit tissue content information from adjacent slices (Cohen et al., 1998; Ju et al., 2006; Ourselin et al., 2001b) or different image sources (Dauguet et al., 2007; LONI, 2006).

All reconstruction methods involve estimation of a transformation function that registers a section with its neighbor(s). Registration methods are differentiated by the form of this transformation and the information used to estimate it. A rigid body (3 parameter) transform describing a translation and rotation is the simplest transform suitable for section alignment and is appropriate when local distortions are not significant. More complex non-linear transformations are often used when local distortions are significant. However, procedures estimating non-linear transformations are usually initialized using a rigid body transformation, and are therefore partially dependent on the quality of the simpler transformation.

The quality of registration is very difficult to determine because, in most cases, no information about the un-sectioned tissue is available. A standard approach involves inspection of a slice through the reconstructed volume orthogonal to the original sections—if the contours of anatomical structures appear smooth the registration is considered acceptable. This is a subjective estimate of quality that is insensitive to smoothly varying distortions of the volume, which retain good local structure.

Some registration methods have been validated using images, which are generated by a procedure that maintains alignment, such as cryo-planing (Ourselin et al., 2001b). This is a valuable exercise, but cannot be considered to validate the method when applied to specimens with different characteristics, such as different stains or tissue types. Artificial markers for cross-validation (Streicher et al., 1997) and information from the un-sectioned tissue, like block face images (Kim et al., 1997; Ourselin et al., 2001a) have also been used as a quality metric, but information of this sort is not available for all tissue types.

* Corresponding author at: Faculty of Medicine, Nursing and Health Sciences, Monash University, Level 5, Block E, 246 Clayton Road, Clayton 3168, Australia. Tel.: +61 3 9594 5508.

E-mail address: Richard.Beare@med.monash.edu.au (R. Beare).

0165-0270/$ – see front matter
doi:10.1016/j.jneumeth.2008.01.012

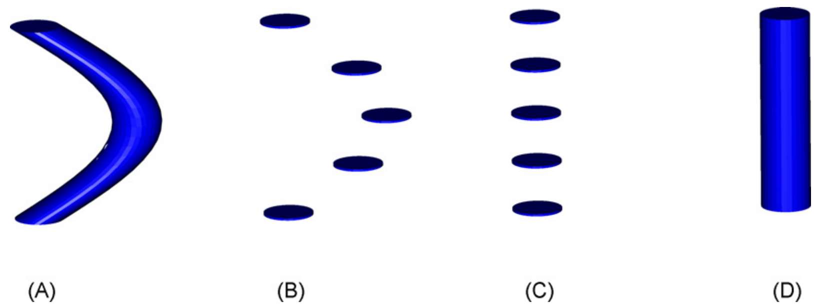

Fig. 1. (A) Original object—a bent rod with uniform cross-section. (B) Sections cut from object. (C) Sections aligned by maximizing overlap resulting in a straight rod. (D) Reconstructed object with incorrect shape—"reconstructing a cucumber from a banana" (Streicher et al., 1997).

To date there has been no study comparing methods using a consistent quality measure.

This paper investigates the registration quality of a number of approaches applied to celloidin-embedded, Nissl-stained, sections of mouse brain using a rigid body transformation. Biases introduced by reconstruction processes are the focus of the study.

The registration methods investigated in this paper include two content-based schemes, one shape-based scheme, one method using artificial fiducials in the embedding material and one using additional-embedded material. The mechanism for measuring quality was based on artificial markers with known characteristics in the brain. The approach for validation was aimed at detecting biases introduced by the registration methods rather than estimating local smoothness.

2. Hypothesis

The hypothesis being tested in this paper is whether anatomical structures can lead to distorted volumes when volume reconstruction of tissue section images is based on image content. This issue is best explained using a simplified model. Consider a three-dimensional shape constructed from a bent rod of uniform but unknown cross-section, as illustrated in Fig. 1. This object is then cut into thin sections using a series of equally spaced, parallel cuts, which are too thin to provide useful information about the angle of the vertical edge. This process is analogous to standard histology sectioning and imaging.

The task is then to assemble the original object from the sections without prior knowledge of shape. If an approach akin to image registration is used then each section will be aligned with its neighbors so that overlap is maximized, leading to a straight, vertical rod.

This paper tests whether analogous problems caused by dominant structure in tissue sections leads to measurable distortion of the reconstructed volume.

It is well known that the distortions introduced by sectioning and mounting can be quite complex and non-linear and therefore should be corrected with non-linear transforms. However, non-linear transforms are usually generated by estimating an initial simple linear transform followed by corrections of local error using localized, non-linear warping. The first step in testing whether there is a problem with this approach is verifying whether estimates of linear transforms using image content-based schemes can be susceptible to biases of this sort. If bias is present then it will be necessary to test whether the non-linear transformation estimation step corrects it. We anticipate that initialization of a non-linear warping with a biased linear transform will produce a biased, but smooth, volume.

The distortion of celloidin sections by the cutting process has been shown to be dependent on the blade angle, with a combination of compression and shear of the sections being observed (Dempster, 1942). The characteristics of distortion due to the cutting process remain consistent throughout the volume provided the blade angle is kept constant. Minimal additional distortion is caused by the process of mounting sections on slides due to the mechanical strength of celloidin. This is very different to other sectioning methods, such as frozen sections, which experience much more complex and section dependent distortion during the mounting process. If the correct rigid body transforms are used to reconstruct a volume from a set of celloidin sections the result will be a distorted version of the original volume, with the distortion being dependent on the sectioning blade angle and therefore consistent throughout the volume. Such a volume can therefore be used to test for biases introduced by the registration process. The deformations experienced by frozen sections are much more complex and depend on the exact conditions of the mounting step. The manual nature of the mounting step means that the distortion varies widely between sections and the lack of mechanical strength of the frozen embedding material means the distortions are severe. Rigid body transforms are not complex enough to correct such distortions.

This study therefore selected celloidin embedding because it provides a good platform for testing whether biases are introduced during the reconstruction process.

2.1. Background to registration methods

The most important image analysis technique used in this study is image registration, which is widely applicable in medical and biomedical imaging. This section provides an overview of image registration techniques so that the approaches used in this study can be understood.

Image registration is the process of estimating a transformation that makes one image similar to another. The form of the transform and the approach to estimating it differentiate image registration approaches. Transformations are typically classified as linear or non-linear. Examples of linear transformations include rigid body (shift and rotation) and affine while non-linear