3-D Reconstruction of Macroscopic Optical Brain Slice Images

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Abstract. We present a method for reconstruction of macroscopic optical images of *post-mortem* brain slices to form a 3-D volume. This forms a key part of a series of procedures to allow *post mortem* findings to be accurately registered with MR images, and more generally provides a method for 3-D mapping of the distribution of pathological changes throughout the brain. In this preliminary work, four brains from a study of Creutzfeldt-Jakob disease were examined. After brain fixation, the pathologist cut coronal slices several mm thick through the brain. The anterior and posterior faces of each slice were photographed. We show that the 2-D co-registration of each such pair of images was most effectively obtained if the slice was placed in a jig before photographing. Fiducials on the jig were detected automatically and point-based rigid registration computed. For co-registration between slices, i.e., across a single cut, an intensity-based method for 2-D non-rigid registration is used which provided satisfactory results. By propagating the 2-D registrations through the volume and using the known slice thickness, the 3-D volume was reconstructed. **Keywords:** registration; magnetic resonance image-to-pathology correlation; Creutzfeldt-Jakob disease.

1 Introduction

Study of the pathological correlates of MR signal abnormalities is important in many neurological conditions. Traditionally, the localisation of both MR and pathological abnormalities is based on naming of anatomical regions by the clinician, radiologist, or pathologist. In some circumstances, this is sufficient to ensure the correspondence between regions, but for many applications more accuracy would be advantageous. We are studying the distribution of MR intensity abnormalities in Creutzfeldt-Jakob disease (CJD), in which various regions of grey matter may show hyperintensity in proton density and T2-weighted images. Different types of CJD appear to show

different distributions of change, but more data are needed. Pathology changes revealed on microscopy include varying degrees of neuronal loss, spongiform change, gliosis and prion protein staining. Proper registration between the pathological samples and MR will help to establish the causes of the MR changes. We believe that this will in turn lead to a better understanding of the diagnostic and prognostic implications of *in vivo* imaging and the patterns of spread of disease in the brain, and could help to guide biopsy. In the present paper, we focus on a key process which will aid the co-registration of microscopic data and MR images from the same individual : namely, the reconstruction of whole brain slices into a 3-D volume. This reconstruction also provides a 3-D mapping of the distribution of pathological changes throughout the brain, which is an important goal in its own right.

3-D reconstruction of optical images of large slices can be facilitated if specialised research procedures are used. For example, the optical data in the “Visible Human” were obtained from complete axial sections through the deep frozen cadaver using a macrocryotome [14]. The thin sections themselves were not photographed, the images being acquired from the cut surface of the as-yet-unsectioned cadaver, in a sequence already registered with each other. Such an approach cannot be used for the majority of clinical studies in which conventional pathology procedures must be included. Also, in CJD, any instruments used to cut the brain are likely to become permanently contaminated and can never again be used for any non-CJD studies. Normally, the brain is removed by the pathologist and fixed in preservative. Some weeks later, the brain is cut into coronal slices. From these slices, small rectangular blocks about 1 or 2cm square are cut from selected regions, and from these blocks very thin sections are cut for staining and microscopic examination. We have developed methodology which can be integrated into such pathology routines.

There are several approaches to reconstructing small field-of-view thin sections to form a 3-D volume approximating the block [5,2,7,8,10,13]. Confocal microscopy allows 3-D data to be acquired directly, which is equivalent to 50 or more microscopical sections. However, for human studies, the blocks must be registered with the macroscopic slices, which are at a scale suitable for matching with MR images, and the requirements for reconstructing the macroscopic slices to form a large volume differ from those for reconstructing the small blocks.

2 Methodology

Four fixed *post-mortem* brains were examined for this preliminary study. Using a guide, the pathologist cut coronal slices of constant thickness through the specimen. For brains 3 & 4, only one hemisphere was available, and the slices were 12mm thick. For brains 1 & 2, 7mm slices were cut through the whole cerebrum. The next step was carried out in preparation for co-registration of images from the two faces of the same slice (within-slice registration). For the first brain, 6 to 8 needle tracks were made through each slice with a 1mm trocar which had been dipped into Indian ink. A small guide was used to assist in making the track perpendicular to the cut faces of the slice. After photographing one face, the slice was turned over by hand to reveal the other face. For brains 2 - 4, a perspex (Plexiglass) jig was constructed (Fig. 1) to hold

each slice during photography. Accurate circular fiducial marks were machined at each corner. Initially, the brain slice was laid on one plate of the jig, and the second plate then screwed down firmly by hand on top of the slice. Photographs were then obtained of both faces. For brains 1 and 2, these were colour transparencies which were subsequently digitised at high resolution (3887 x 2590 pixels, 24-bit colour scale). For brains 3 and 4, a digital camera with a resolution of 1600x1200 pixels was used.

Fig. 1. Jig to hold each slice during photography of anterior and posterior faces.

To reconstruct the original 3-D relationships, two different types of registration must be computed. The first is to register the anterior (A) and posterior (P) sides of each slice, i.e. within-slice registration. The second is to register the images of surfaces on either side of the cut which separates two adjacent slices, i.e. between-slice registration. Slices were numbered from front to back. Thus “P6” refers to the posterior face of slice 6.

2.1 Within-Slice Co-registration Using Artificial Landmarks

The cut surfaces on each side of a slice are separated in the direction perpendicular to the faces by several millimetres (the thickness of the slice). We therefore cannot rely on sufficient similarity between image features to match them correctly. Instead, we explored methods using artificial landmarks, evaluating two options.

Method 1 For the first brain, the needle track landmarks were identified on the images by visual inspection and the centre of the landmarks marked by the pathologist with a cursor. Corresponding marks on the A and P faces were labelled and a standard 2-D rigid co-registration between the sets was computed by minimising the sum of the squared Euclidean distances between the centres.

Method 2 For the second brain, the jig fiducials were visible as circles on a contrasting background at the corners of the image. A program based on a Hough transform was implemented, to detect the circles automatically, to localise their centres to sub-pixel accuracy, and to estimate their radius. 2-D rigid registration between the A and P faces using the circle centres was then calculated. To determine the accuracy and reproducibility of co-registration using the jig, a phantom slice 7mm thick was constructed. This had black rubber faces and 16 circular white discs on each face, constructed so that these circles were exactly opposite each other on the two faces (Fig. 1).

2.2 Between-Slice Intensity-Based Registration

The surfaces on either side of a single cut were derived from the cleavage of a single tissue plane and natural features on the two cut surfaces should correspond exactly to each other. However, distortions occur during cutting and during later movement of a slice, so a non-rigid registration method is essential. A general purpose non-rigid registration framework was used, which computed an initial rigid registration followed by a second affine stage and finally a free-form stage. The results after each stage were examined separately. For each stage, a multi-resolution block matching step was followed by regularisation, as described below.

Block Matching [8] Block matching algorithms have been used for medical image registration by several groups [e.g 3,4] as well as our own [8]. For the first (coarse scale) the image was divided into large square blocks. For each block centre χ_k in the reference image a corresponding block centre γ_k in the floating image was found by moving the floating block until a similarity measure was optimised. Large blocks were used in the first iteration (to provide a rough estimate of the necessary large displacements). This was followed by iterations using progressively smaller blocks (down to a minimum of 4x4 pixels) to generate smaller but more accurate displacements. The complexity could thus be kept constant for each iteration. The similarity measure used was the grey level correlation ratio which is an appropriate choice for this application where the true relationship between blocks will tend locally to be at least affine [9]. The output of the block matching was a relatively sparse field of displacement vectors.

Regularisation The hierarchical nature of the block matching algorithm contributed to regularisation of the vector field, but a substantial fraction (about 20%) of vectors were generated from blocks with few features, or with features in the two images that did not represent anatomically corresponding structures. These usually appeared as outliers in the vector field. We therefore adopted robust methods for further regularisation, which was performed following block matching in each of the rigid, affine and free-form stages. For regularisation in the rigid registration stage, a least trimmed squares (LTS) strategy was used to estimate the two-by-two rotation matrix R and a translation vector $t = (t_1, t_2)$ which best superimposed the points $\{\chi_k\}$ and their counterparts $\{\gamma_k\}$. The LTS method is far more robust to outliers than the classical least squares method [12] and can cope with up to 50% of outliers. Following block matching at the beginning of the affine stage, the LTS method was

also used for regularisation, but without the rigidity constraints. For the free-form stage, a weighted Gaussian median filter [1] which performs well with outliers was used to convert the sparse deformation field into a dense, regularised field.

2.3 3-D Reconstruction by Propagation of 2-D Registrations through the Volume

The known slice thickness was used to calculate the antero-posterior coordinate (“z” value) of each image. The alternating 2-D within-slice rigid- and between-slice non-rigid- registrations were propagated through the volume to allow each image to be transformed in “x” and “y”. After 3-D reconstruction, images of the two faces of the same cut have the same “z” value; we have not at this stage fused, or chosen between, these two coincident images systematically, but we arbitrarily selected one of the pair for display purposes as in Fig. 4.

For propagation using affine non-rigid registrations between slices, the transforms between each image pair were all parametric and propagation was a relatively straightforward analytic process (left half of Fig. 2). It is assumed that all P images have been flipped to bring all images into the same orientation. Let $\mathbf{I}(A_i)$ be the A (anterior) image coordinates of the i th slice and the $\mathbf{I}(P_i)$ its P (posterior) image. Let $\mathbf{T}_R(P_i, A_i)$ be the rigid transformation between these two images and $\mathbf{T}_A(P(i+1), A_i)$ be the affine transformation between the P face of slice i and the A face of the next slice $i+1$. The geometric transformations can be propagated from the A face of slice 1 to the P face of slice k so that $\mathbf{I}(P_k)$ is transformed into the coordinate frame of $\mathbf{I}(A_1)$:

$$\mathbf{I}(P_k, A_1) = \mathbf{T}_R(P_1, A_1) \times \mathbf{T}_A(P_2, A_1) \times \mathbf{T}_R(P_2, A_2) \dots \mathbf{T}_A(P(k-1), A(k)) \times \mathbf{T}_R(P_k, A_k) \times \mathbf{I}(P_k)$$

However, propagation of free-form registrations required interpolation and image resampling for each new slice (right half of Fig. 2). To reduce the need to extrapolate the free-form vector fields outside the boundary of an image, the propagation direction is normally chosen to run from larger to smaller slices. Free-form propagation involves the following steps (Fig. 2):

- (a) Within-slice rigid registration between A_i and P_i
- (b) Resampling of P_i to form P_i'
- (c) Between-slice non-rigid registration between P_i' and $A(i+1)$, generating a regularised free-form vector field (note that an affine stage is embedded in this).
- (d) Within-slice rigid registration between $A(i+1)$ and $P(i+1)$
- (e) Transformation of the vector field from (b) using $\mathbf{T}_R(P(i+1), A(i+1))$ from step (d)
- (f) Warping of $P(i+1)$ to form $P(i+1)'$ which is then in the same 2-D coordinate frame as A_i .

Steps (c) to (f) are repeated, up to the last slice in the propagation sequence.

Fig. 2. Flow chart showing methods for propagating 2-D non-rigid registrations through a sequence of images. As an example, propagation from A5 to P7 is shown. Left: affine propagation (analytical). Right: free-form

3 Results

3.1 Within-Slice Rigid Registration

Method 1 (needle track landmarks) After registration the root mean square and maximum errors (Euclidean distances) between corresponding needle track landmarks were examined as intrinsic measures of the consistency of the results. There was a wide range of errors. For the best slices, the errors were 1.1 mm RMS and 1.6mm maximum. However, for the worst slices, the errors were 13.1 mm RMS and 21.6mm maximum.

Visual inspection of the quality of the registration was carried out, particularly with reference to the silhouette of the slice image, including slice features in the background which protruded beyond the cut surfaces as these should contribute to the silhouette when viewed from the A or the P side. This revealed the large deformations between different parts of the brain slice, which frequently occurred when a slice was composed of separated fragments that could move independently of each other.

Method 2 (jig landmarks) Experiments with the phantom slice confirmed good performance of the jig (Fig. 1 and Table 1). Images of both faces of the phantom slice were obtained: (a) with the jig moved and replaced at various orientations relative to

the camera (to test the image acquisition, digitisation and image processing steps); (b) after repeatedly dismantling and reassembling the jig (to evaluate mechanical aspects of the jig); and (c) with spacers about 1mm thick at one side of the phantom slice (to simulate the effect of imaging slices having non-parallel faces).

When the jig landmarks were used for registration (the normal situation with brain slices) the error in the phantom slice landmarks (about 0.75mm RMS, Table 1, right hand column) was a measure of the accuracy of the jig. However, the phantom slice itself could not be perfectly accurate, and the intrinsic error of the phantom landmarks (residual error when these same landmarks were used for registration) was about 0.55mm. The intrinsic error of the jig landmarks, calculated in the same way, was about 0.7mm (Table 1).

The intrinsic errors between jig landmarks were also examined when the jig was used for registration of images from brain 2, 3 and 4 (Table 2). These errors were in fact superior to those obtained when the phantom slice was photographed. These results confirmed the reliability of the jig landmark registration when used with real brain slices and also suggested that the image acquisition technique, involving factors such as avoidance of shadows and reflections and use of a digital camera, was improving.

Table 1. Errors in jig landmark positions for phantom slice images averaged across 4 trials of each condition.

		Intrinsic errors ¹ (mm)		Accuracy of jig registration ² (mm)
		Jig landmarks	Phantom landmarks	
Varying jig orientation				
and position with respect to the	RMS	0.80	0.63	0.73
cameras	<i>max</i>	<i>1.13</i>	<i>1.04</i>	<i>1.31</i>
Effect of dismantling and	RMS	0.68	0.50	0.61
reassembling	<i>max</i>	<i>1.07</i>	<i>1.14</i>	<i>1.37</i>
Effect of non parallel jig faces	RMS	0.67	0.55	0.72
	<i>max</i>	<i>0.95</i>	<i>1.20</i>	<i>1.40</i>

¹ residual errors between landmark positions after registration using the same landmarks

² phantom landmark errors after registration using jig landmarks

Table 2. Residual intrinsic errors in jig landmark positions for brain slice images averaged across slices.

		Brain 2	Brain 3	Brain 4
Type of camera		film	digital	digital
Number of slices		3	12	14
Slice thickness, mm		7	12	12
Residual error, mm	(RMS)	0.55	0.19	0.11
	(max)	0.76	0.24	0.16

3.2 Between-Slice Non-rigid Registration

Between-slice registration was computed in stages (rigid, affine, and free-form) which were evaluated by visual inspection of subtraction images and of stacked image sets. Figure 3 shows the successive stages for slices 10 and 11, i.e images P10 and A11, of brain 1. For this image pair, the subtraction images show progressive improvement (i.e. increasing uniformity) at each stage. The residual subtraction features (i.e. areas of non-uniformity) generally were *not* derived from the cut brain surface but from oblique surfaces or from artefacts. This is a good result because such non-corresponding features should ideally not influence the registration of those features which really do correspond.

Fig 3: Processing steps for between-slice registration. Example from brain 1, slices 10 and 11. Middle row: subtraction images to show the residual differences between image P10 (untransformed) and the three stages of transforming flipped slice A11 (rigid, affine and free form stages).

3.3 3-D Reconstruction by Propagation of 2-D Registrations through the Volume

For brain 3, the alternating within-slice- and between-slice- registrations were propagated from slice 7 forwards and backwards through the volume. The results

after using the two alternative non-rigid between-slice methods (affine only and free-form following affine) were compared by inspecting the continuity of the outline and of internal image features in the stacks of images. The best results were obtained using the affine method (Fig. 4).

Fig. 4. Completed reconstruction of macroscopic optical brain slice images from brain 3 (only left hemisphere available): surface rendered views. Left image: postero-lateral view; right image: postero-medial view. The methods used were: jig-based (rigid) within-slice registration; affine (non-rigid) between-slice registration; analytical propagation.

4. Discussion

Implications of obtaining surface images from thick slices The thickness of macroscopic brain slices imposes a wide separation between the images of the anterior and posterior cut surfaces. However, it is wrong to imagine the voxels of the 3-D optical reconstruction as having a large size in the “z” direction. Unlike typical tomographic images, the majority of features visible in our optical images lie on or extremely close to the surface of the slice, i.e. there is very little partial volume effect for such features. The optical data should be visualised as very thin slices with a large interslice gap. Nevertheless, this rule is not true for visible features which do not lie on the cut surface, as inspection of the residual details which did not subtract out after between-slice registration revealed. The difference images after non-rigid registration shown in Fig. 3 showed that the large majority of the residues were derived from such non-corresponding features, confirming the good performance of our method.

Needle track vs. jig methods for within-slice rigid registration The needle track method avoided the need for gadgetry in the photographing area, an advantage considering the special precautions needed when handling CJD brains. However, making the needle tracks was at least as time consuming as placing slices in the jig. Furthermore, our result showed that it was impossible to avoid substantial deformations when a slice was turned over to photograph its opposite face. In principle, this might have been corrected by using a non-rigid within-slice registration

method. However, such a method could not be based on natural image features, because the thick slices do not allow the assumption of correspondence of image features. Also, it would have required a large number of artificial needle track landmarks for accurate estimation and this would have caused an unacceptable number of artefacts in the pathological material. A final disadvantage of the needle track method was the difficulty in localising the needle track landmarks, which tended to have a ragged boundary. In contrast, the jig held the slice stably during photography and provided accurate fiducials, thereby allowing reliable rigid registration.

Affine vs free-form methods for between-slice non-rigid registration The most consistent results in the present study were obtained with affine rather than free-form between-slice registration and this may prove sufficient to initialise a reliable registration procedure with 3-D MR data. The affine transformation was applied globally to the 2-D optical image. This had the advantage of allowing propagation between the complete set of slices to be solved analytically, avoiding the compounding of errors arising in image resampling and regularisation of discrete vector fields. However, a global affine transformation applied to a 2-D image does not allow us to represent independent movement of separated fragments. More detailed evaluation of local matching performance is planned.

Implications of expected physical deformations on choice of between-slice registration algorithms Several factors determine the actual deformations which occur during the *post-mortem* studies. The brain tends to shrink during fixation, and if stood on a surface will flatten towards this. Further deformations occur as a result of compression during cutting. It is likely that such deformations can be represented by an affine transformation, which allows differential stretching in “x” and “y” and skew as well as translation and rotation. However, more complex deformations also occur which are mainly determined by the degree of connectedness between parts. When one part is separated from another by a narrow neck of tissue, for example where the two cerebral hemispheres are joined only by the corpus callosum, bending occurs easily along the join. The narrower the neck of tissue along the join, the more readily bending occurs when the slice is cut or (later) when it is moved. With such connections, stretching will usually be limited. However, when there is complete disconnection, e.g. when a fragment such as the anterior pole of the temporal lobe is left completely separated after cutting, movement relative to other parts is only limited by collision and by the care of the pathologist and photographer in maintaining the original relations. The ideal between-slice matching algorithm thus needs to allow unconstrained deformation between fragments, bending but little stretching along narrow necks of tissue, and more constrained (probably affine) deformations locally within intact blocks of tissue.

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