Registration of anatomical MRI and histological sections for rat brain

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Introduction

To fully exploit the complementary information from MRI and histology, both modalities need to be co-registered. Two separate issues have to be addressed: obtain a 3D histological block from 2D sections, register the 3D histological volume to the 3D MRI volume. We propose an original approach in which we use external markers to obtain a 3D histological volume from a set of 2D histological sections. Then, we compare two different rigid registration methods to spatially match MRI and histological data.

Material and Methods

A healthy rat underwent MRI at 2.35T (SMIS console). T_1-weighted images were obtained using a MDEFT approach (spatial resolution 333x333x333µm, 4 averages). The animal was sacrificed and the brain frozen in isopentane and stored at -80°C. All subsequent operations were performed at -25°C, inside a cryotome. The brain was placed in a mold and surrounded by four needles (Fig. 1). Neg-50® at 4°C was quickly poured into the container. The mold was then rapidly removed from the support and cooled from the bottom in liquid nitrogen. After embedding, the temperature of the cryotome was raised to -8°C, temperature at which the four needles can be withdrawn. The four needle tracks were filled with an acrylamide solution containing indian ink. After polymerization, the block was stored at -80°C.

To sample the whole brain, 254 slices (20µm thick) were obtained with a 60µm gap. Pertex was applied on each marker to protect the markers prior to staining. A classic hematoxylin-eosin approach, which generates a T_1-like contrast, was then performed after which sections were scanned (spatial resolution 21x21x20µm).

Section registration was achieved based on the markers' centroids which were determined using a convolution mask on each corner of each section. The 3D histological volume was reconstructed with a rigid registration approach (2 translations, 1 rotation) using the markers as landmarks (1). MRI skull striping was performed using snakes with manual initialization via ITK-Snap software.

MRI brain and histological scans were then registered in two ways. A rigid global registration (SPM2) was performed using grey level intensities and a similarity index based on square differences (2). A rigid local registration was computed based on a block matching approach (BMA) and using a similarity index based on correlation coefficient (3). To evaluate the importance of sampling the whole brain, the histological dataset was decimated (24 slices out of the 254 were kept) and the two registration methods (BMA and SPM2) were performed.

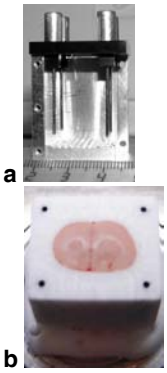

Fig. 1. (**a**) Mold and four needles. (**b**) Embedded brain during cutting. Markers appear as black dots.

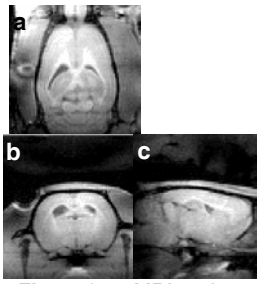

Fig. 2. MRI data acquired in vivo in axial (**a**), coronal (**b**), and sagittal (**c**) orientations.

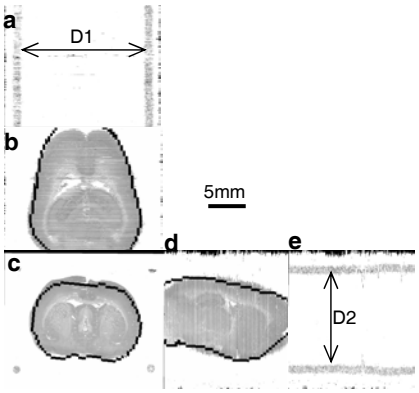

Fig. 3. Reconstructed histological volume. Images (**a**) and (**e**) are taken through the markers. The black contours on images (**b**), (**c**), and (**d**) represent the contours of the corresponding MRI sections displayed on Fig. 2 after registration using SPM2.

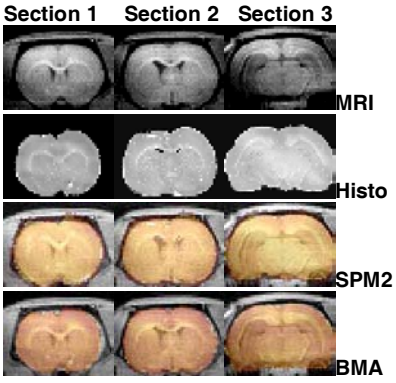

Fig. 4. Three sections from the rat brain: MRI data, histological data, overlay of SPM2-registered histological data over MRI, and overlay of BMA-registered histological data over MRI.

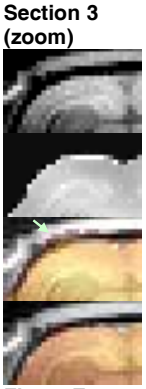

Fig. 5. Zoom on a part of section 3 from Fig. 4. Arrow indicates pixels outside MRI contour.

Results

Fig 2 and Fig 3 show MRI and histological data respectively. The quality of the histological registration can be evaluated from the markers visible on images (3a) and (3e). After registration, the mean inter-marker distances are D1=16.98±1.49mm and D2=12.67±1.03mm, which correspond to the mold (D1mold=17.15mm, D2mold=12.65mm). The MRI contours (black lines on 3b-d) match approximately the contours of the histological sections registered to the MRI data using SPM2. Fig. 4 shows overlays of SPM2 and BMA registered histological data over MRI data. Visual inspection indicates that BMA performs a better registration than SPM2, as it can be seen on Fig. 5: SPM2 places histological pixels outside the MRI brain contours. Both SPM and BMA registered the decimated histological dataset as well as original histological dataset (data not shown).

Conclusion

The proposed approach allows a coarse registration of MRI and histological modalities. BMA (local rigid transformation) appears to perform better than SPM (global rigid transformation). Results also indicate that sampling the entire brain (254 slices) does not seem to provide additional registration quality over a coarse sampling (24 slices). Further analysis is needed to quantify the quality of the approach.

References

(1) Simonetti et al. J. Neuroscience Methods, 2006; 158:242. (2) Friston et al. Human Brain Mapping, 1995; 2:165. (3) Ourselin et al. Image and Vision Computing, 2001; 19: 25.