# Automated Registration of Histology sections with ex-vivo MRM volumes

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**Introduction:** Magnetic Resonance Microscopy (MRM) is a technique that is well suited for the development of new biomarkers[1,2], although validating these biomarkers requires comparison of the MRM volume with histology sections. Finding the MRM slice corresponding to the histology section is however a time consuming and tedious task, due to the unknown orientation of the cut plane of the histology section and the local deformations that occur during the slicing. For the automation of this process, a 2D-3D registration is desired. Only a few articles have been published on the registration of histology with MRM[3] and even less address the problem of the registration of a single histology section to an ex-vivo MRM volume[4]. We present an automated registration method that registers a single 2D histology section with the MRM volume of the mouse brain.

**Methods:** For the experiment we used 3 mouse brains, which were removed from the skull and fixed. The MRM images were acquired with a Bruker 9.4 Tesla scanner with a T1 imaging protocol, resulting in a 256x256x256 volume, with an isotropic resolution of 0.078125 mm per voxel. After imaging with the MRM scanner, 4 HE-stained histology sections were created from different locations. The histology images were acquired with a Leica light microscope with an image size of 2600 x 2060 pixel, resulting in an isotropic resolution of 0.00323 mm per pixel. We had to exclude 4 sections, which were cut into pieces during the slicing of the sections.

To guarantee a registration algorithm that is robust for noise and imaging artifacts, we used a preprocessing step on both modalities. To enhance the contrast of the histology section (figure 1A), we use the characteristics of the HE-staining. The red channel of the color image shows uniform intensity values, the green channel shows low values on spots of the nucleii and structures with much cytoplasm. After subtracting the red channel from the green channel, the structures of interest are enhanced as shown in figure 1B. Next, the background is removed and the image is inverted for a better registration result (figure 1C). The MRM-volume was segmented in 21 different structures selected by their visibility in the histology sections. An example of the segmentation is shown in figure 1D. Our group is currently working on an automated atlas-based segmentation of ex-vivo mouse brain MRM volumes. Since the automatic segmentation is not finished yet, we did the segmentation manually for now.

The registration algorithm is implemented using ITK[5] and concerns regular registration algorithms[6,7]. To find the global location of the section in the MRM volume, we created binary images from the segmented volume and the histology sections using a threshold. These binary images were registered with a 2D-3D registration method using the mean squared distance as similarity metric. Since, the registration is meant to be a quick search through the entire mouse brain, we set the optimizer to a fast convergation and allowed only translation in the x, y and z direction. The final slice was found using the MRM segmentation and the preprocessed histology section. As a similarity measure we used mattes mutual information and set the optimizer to a slow convergation. Since the orientation of the cut plane of the histology section is unknown we also allowed rotation around the x, y and z axis. As starting position we used the result of the global registration.

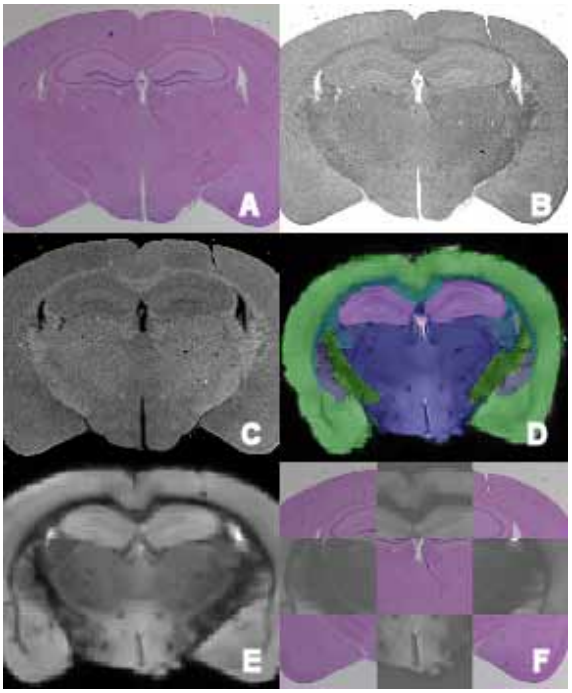


**Figure 1.** The preprocessing of the data and the registration: **A.** The original histology coupe that should be fitted. **B.** The resulting image by subtracting the red channel from the green channel. **C.** The inverted image used for registration. **D.** The segmented MRM volume used for registration. **E.** The automatically found MRM slice. **F.** The MRM slice compared to the original histology.

**Results:** The results of the algorithm were compared visually: First, by manual comparison of the automatically found MRM slice with the histology section and second, by stepping through the MRM volume to find a better matching slice. An example of the found slice and the visual comparison is shown in respectively figure 1E and 1F. The algorithm returned in 7 of the 8 histology sections the best matching MRM slice. The 1 failure was the only section of the cerebellum. In the segmentation of the MRM volume the grey and white matter were both assigned to 'cerebellum', although the histology has a clearly visible distinction between both structures. This difference in appearance may have caused the failure.

**Conclusion:** This paper presents the first very promising results of the registration of a single histology section to a MRM volume, but it can be improved in several ways: First, the manual segmentation of the MRM will be replaced by automated segmentation since it takes to much time for biologists to segment the volume manually and it will be more objective, too. Next, we will investigate the possibilities for global and local deformations to compensate for the deformations in the histology section and to use the MRM segmentation for the annotation of the histology section. Further, we will investigate the possibilities for partial matching. We tested the algorithm only on undamaged histology sections, however most histology sections in daily routine are damaged caused by the slicing procedure.

**Acknowledgement and References:** [1] C.T.Farrar et al., "A dual Histology stain/MRI contrast agent for molecular imaging of the brain," Proc. ISMRM, 2005, p. 313. [2] Y.Z. Wadghiri et al., "In vivo detection of Alzhemer's plaques in transgenic mice: Requirement for targeted contrast agents," Proc. ISMRM, 2005, p. 358. [3] P.A. Yushkevich et al., "Using MRI to build a 3D reference atlas of the mouse brain from histology images," Proc. ISMRM, 2005, p. 2809. [4] G. Li et al., "Registration of in-vivo MR images to triphenyltetrazolium chloride stained sections in small animal models," Proc. ISMRM, 2005, p. 1029. [5] T.S. Yoo et al., "Engineering and algorithm design for an image processing API: A technical report on ITK - The Insight Toolkit," Proc. Med. Meets Virtual Reality, 2002, pp. 586–592. [6] J.B.A. Maintz and M.A. Viergever, "A survey of medical image registration,," Med. Image. Analysis., vol. 2, no. 1, pp. 1–16, 1998. [7] D.L.G. Hill et al., "Medical image registration," Phys. in Med. and Biol., vol. 46, no. 3, pp. R1–R45, 2001.