# THREE DIMENSIONAL FUNCTIONAL CARTOGRAPHY OF THE HUMAN BASAL GANGLIA BY REGISTRATION OF OPTICAL AND HISTOLOGICAL SERIAL SECTIONS

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## ABSTRACT

In functional neurosurgery, there is a need for accurate localisation of the functional targets. One example is given by Parkinson's disease. The surgical intervention is based on the introduction of electrodes in the subthalamic nucleus. This nucleus is targeted on pre-operative stereotactic MR acquisitions. But MR imaging of the basal ganglia is intrinsically limited, first by image resolution, and second by the relationship between the measured MR signal and the real anatomy, not clearly understood. On the other hand, detailed and accurate cartography of the basal ganglia can be performed on *post mortem* histological serial sections. Indeed, histology overcomes the limitations of MR imaging. Moreover, staining of histological sections allows to recover functional information. But histology is by nature two-dimensional. An histological data set consists in a series of disorganized serial sections, as three dimensional shape information was lost during sectioning. Therefore, the first step toward the integration of histological and MR information is to perform a reliable three dimensional reconstruction of the histological volume. Acquisition of photographs during sectioning, showing the histological sections before sectioning, as well as fiducial landmarks, allows to reconstruct a volume with three dimensional integrity, and is further used to register each histological section with its corresponding optical section.

## 1. INTRODUCTION

Advances in image guided stereotactic neurosurgery and stimulation technology have given rise to a reappearence of the use of functional neurosurgery for the treatment of movement disorders, e.g. Parkinson's disease or dystonia. The intervention is based on the stereotactic introduction of electrodes in disease-specific nuclei of the basal ganglia, e.g. for Parkinson's disease a small, deeply located, nucleus called the subthalamic nucleus (STN) [1]. The nucleus is targeted on pre-operative stereotactic MR acquisitions [2]. In these procedures, the surgical success depends primarily on the accurate localisation of the target.

MR imaging of the basal ganglia, despite technological and clinical progress over the last decade, appears to be intrinsically limited by two factors. First, the resolution of clinical routine MR images is nowadays around $1mm^3$, thus limiting the level of detail of the images to the gross features of the basal ganglia; second, and more fundamentally, the MR signal, being a measure of tissue physical properties, provides us with a representation of the underlying anatomical reality of the organ being imaged. The relationship between the measured signal and the real anatomy is not always completely understood. For example, for Parkinson's disease, the STN is targeted on pre-operative MR acquisitions (T1 and T2-weighted) [2]. On the T1 image (acquired in stereotactic conditions), this nucleus is undistinguishable, and on the T2 image (not in stereotactic conditions, due to geometric distortions), an hyposignal at the STN level is observed, but a clearly defined relationship between this signal and the STN is still debated. This introduces uncertainty on the nucleus localisation. Consequently, electrophysiological study and clinical testing are performed during the intervention to refine the pre-operatively determined target position. This causes the intervention to last on average 10 hours. Therefore, allowing more accurate pre-operative localisation of the functional targets appears to be a key issue.

Detailed and accurate cartography of the basal ganglia can be performed on histological serial sections. Indeed, histology, consisting in the study of postmortem autopsy tissues, overcomes the limitations of MR imaging, allowing higher level of detail and direct observation of anatomical reality [3, 4]. Moreover, staining of histological sections can provide functional information, e.g. Calbindin immunoreactivity which can distinguish the associative and sensorimotor territories of the striatum [5]. Therefore, anatomical and functional cartography of the complete basal ganglia can be performed on histology, and the corresponding features accurately outlined. Nevertheless, before to use such a cartography for target localisation in functional neurosurgery, reliable three dimensional reconstruction from the

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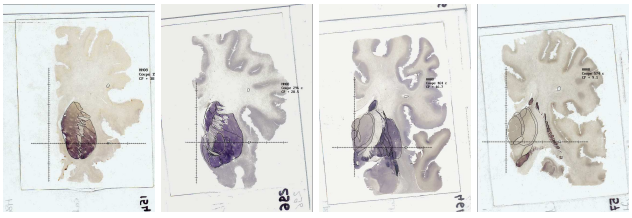
histological sections must be done, as histology being by nature two-dimensional, i.e. histological data consist of a series of discontinuous serial sections. Also, this cartography has to be registrable with the MR acquisitions of the patient, in order to report the outlined features on the patient's anatomy. Resuming, we aim at constructing a three dimensional, anatomical and functional, as well as registrable, cartography of the basal ganglia, based on histology. For doing this, a *post mortem* MR study was conducted on a cadaver's head, 36 hours after death, insuring the MR signal to be very similar to an *in vivo* MR image, and the brain was then extracted and processed for histology. When fusion of MR and histological data will be performed, the *post mortem* MR image will allow to report the cartography on the patient's anatomy, by its registration with the patient's MR image.

In this paper, we focus on the reconstruction problem, i.e. how to get a reliable three dimensional reconstruction from the histological sections, thus providing a three dimensional anatomical and functional cartography of the basal ganglia.

## 2. MATERIAL AND METHODS

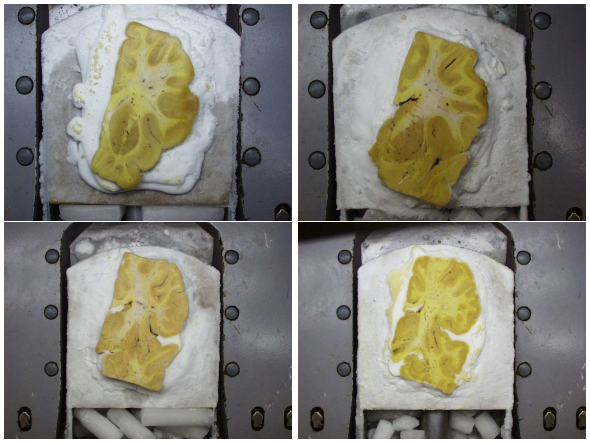
### 2.1. Material

A human brain, obtained 36 hours after death, was stored in 4% paraformaldehyde for 8 days and in phosphate buffer with sucrose for 7 days. One hemisphere was sectioned into 3 blocks (1.5 cm thick) in order to favour a better fixation, and stored frozen at $-40^o$C. The blocks were cut into 70 $\mu$m thick sections which were collected serially. Sectioning was done on a Tetrander Jung freezing microtome. Sections were treated according to different immunohistochemical procedures. One out of ten sections (thus every 700 $\mu$m) was stained for Calbindin immunoreactivity to reveal functional territories. After staining, histological sections were scanned, and structures and territories of the basal ganglia were outlined (Figure (a)). During sectioning, photographs



(a) Calbindin immuno-stained histological sections.

of the unstained surface of the frozen brain, together with part of the cryomicrotome including 6 screws, were taken for one out of ten sections (Figure (b)). There was therefore a corresponding optical section for each histological stained section.



(b) Photographs taken during brain sectioning.

### 2.2. Three dimensional reconstruction

Reconstruction of the histological volume followed two steps. First, we built a three dimensional reference volume from the optical sections (2.2.1) using a feature-based registration method, the screws of the cryomicrotome acting as fiducial markers. Then each histological section was registered with its corresponding optical section (2.2.2) using an intensity-based method.

#### *2.2.1. Feature-based alignment of the optical sections*

In order to reconstruct a reference three dimensional volume from the optical sections, we used a feature-based registration method (rigid ICP - Iterative Closest Point algorithm), where the 6 screws of the cryomicrotome served as fiducial markers. These screws were detected automatically by combination of thresholding and connected component analysis. Alignment of the optical sections using these markers guaranteed the integrity of the resulting three dimensional reconstruction. The resulting volume presented intensity variations from slice to slice, due to the photographs acquisition and scanning process. In order to get an homogeneous volume, these variations were corrected by histogram equalisation.

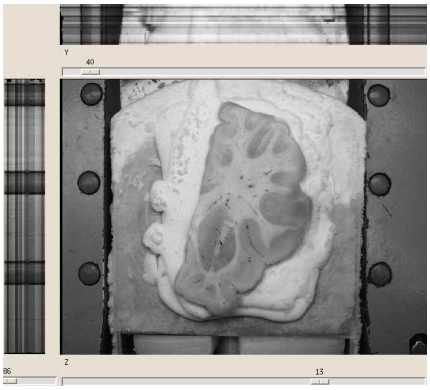
#### *2.2.2. Reconstruction of the histological volume*

Using the optical reconstructed volume as a geometrical reference, each histological section was registered with its corresponding optical section. Registration was performed with the block matching algorithm [6], an intensity-based two-steps method consisting in selective local-based correspondance computation, followed by robust transformation estimation, these two steps being embedded in an iterative multi-scale scheme. Correlation coefficient was used as the similarity measure for computation of the correspondances, and affine transformations were estimated.

Selective local-based correspondance computation and robust estimation of the transformation made this algorithm particularly adapted to the problem, capable to cope with distortions due to the processing of the histological sections. Indeed, most of the correspondances were found in highly contrasted regions, i.e. around the basal ganglia, and irrelevant correspondances were considered as outliers during robust estimation.

## 3. RESULTS

First, feature-based alignment of the optical sections was performed. Figure (c) shows three orthogonal views of the reconstructed optical volume from optical sections of the first brain block. Notice the resulting alignment of the microtome screws on lateral views, as well as intensity variations from slice to slice. Alignment of the complete optical sections followed by correction of intensity variations can be seen in Figure (e) - left.

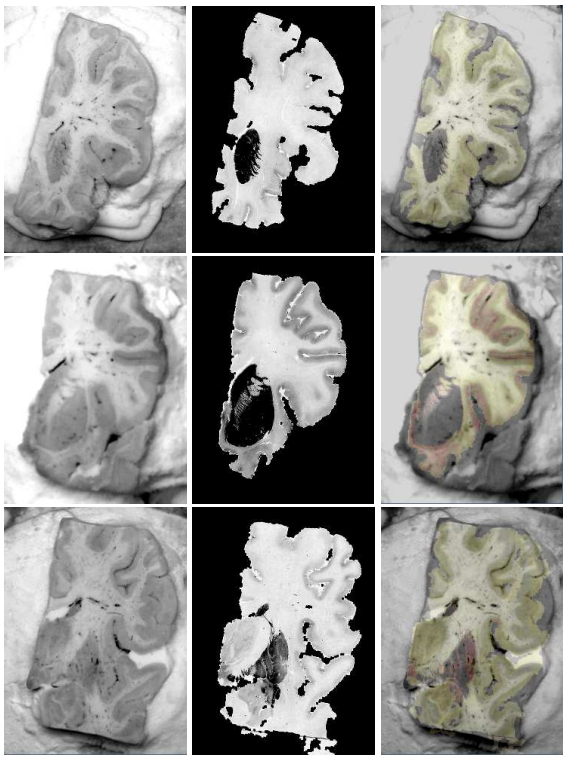


(c) Rigid alignment of the optical sections.

Then, histological sections were registered with the corresponding optical sections. On Figure (e), some results are presented: optical sections (left column), registered corresponding histological sections (middle column), and both sections fused (right column), in order to visually assess the quality of the registrations. Stacking-up the registered histological sections lead to a reliable three dimensional histological volume (Figure (e) - right). Finally, the structures and territories outlined on the histological sections were deformed following the transformations found by registration of the histological and optical sections, leading to a three dimensional cartography of the basal ganglia (see Figure (f)).

## 4. DISCUSSION

To improve pre-operative target localisation in functional neurosurgery, we aim at building a three dimensional functional cartography of the human basal ganglia, based on histological data. In this paper, we focused on the reliable three dimensional reconstruction from histological sections. Accurate description of anatomical structures and functional

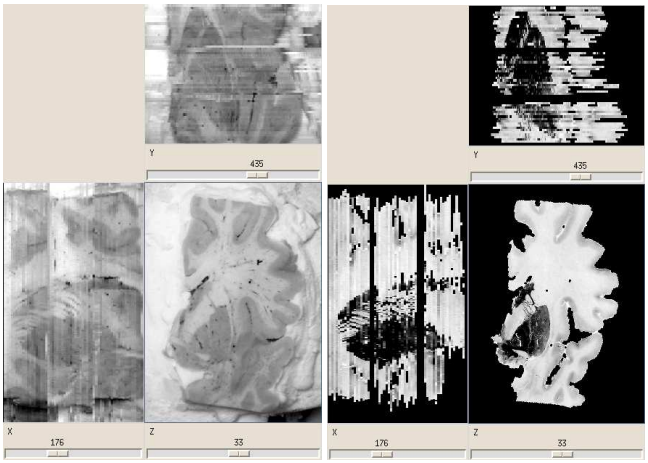


(d) Registration of histological and optical sections.

territories of the human basal ganglia was performed on Calbindin immuno-stained histological sections. To conduct successfully such an immunohistochemical staining, brain fixation had to be done accordingly, i.e. fixation could not be as long as for other histochemical stainings (e.g. Nissl staining revealing cytoarchitecture). Therefore, the brain suffered important distortions during sectioning and histological process, resulting in sections with independant, eventually large, geometric distortions. Alignment of these sections had to be done very carefully, in order to get a reliable three dimensional reconstruction.

Alignment of histological serial sections into a three dimensional volume can be done following a straightforward method. It consists in the registration of consecutive sections two by two and further three dimensional reconstruction by composition of the resulting transformations [7, 8, 9]. Nevertheless, this method is not adequate when the sections have suffered independant two dimensional geometric distortions. Indeed, rigid registration of contiguous sections would not compensate for the distortions, and non rigid registration, which could partially cope with distortions, followed by transformation composition, would not allow to get a reliable three dimensional histological volume in the absence of a three dimensional reference.

In order to build such a reference, following [10, 11], photographs of the unstained surface of the frozen brain were taken. Feature-based alignment of these photographs yield a reference volume, and each histological section was



(e) Three orthogonal slices through the optical (left) and histological (right) reconstructed volumes.

then aligned with its corresponding photograph. This guaranted the three dimensional integrity of the reconstructed histological volume.

## 5. CONCLUSION

In this paper, we have presented a reliable alignment of histological Calbindin immuno-stained sections of the human brain, leading to an accurate anatomical and functional cartography of the basal ganglia. The integrity of the reconstructed histological volume was guaranteed by the previous construction of a three dimensional reference volume from photographs taken during brain sectioning. This work is part of a study which aims at constructing a three dimensional, anatomical and functional, as well as registrable, cartography of the human basal ganglia for accurate localisation of targets in functional neurosurgery. Remaining steps include the fusion of the histological volume with the *post mortem* MR image of the head acquired before histology processing, and registration of this MR image with the patient's MR image, that will allow to report the cartography on patient's anatomy.

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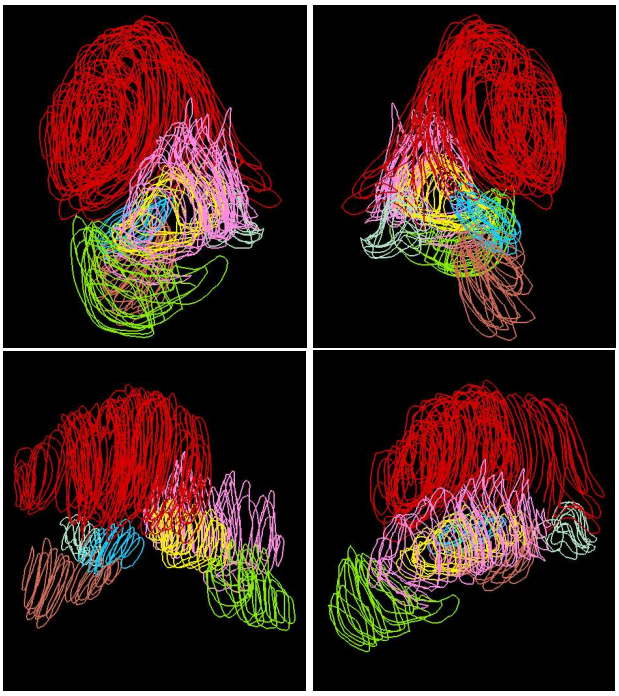

(f) Three dimensional cartography of the human basal ganglia. Four viewpoints: antero-posterior (top left); postero-anterior (top right); antero-medial (bottom left); postero-lateral (bottom right). Only 10 structures and territories are shown here (thalamus nuclei, pallidum, accumbens, substancia nigra, subthalamic nucleus).


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