# Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures

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# ABSTRACT

Large variations occur in brain anatomical structures in human populations, presenting a critical challenge to the brain mapping process. This study investigates the major impact of these variations on the performance of atlas-based segmentation. It is based on two publicly available datasets, from each of which 17 T1-weighted brain atlases were extracted. Each subject was registered to every other subject using the Morphons, a non-rigid registration algorithm. The automatic segmentations, obtained by warping the segmentation of this template, were compared with the expert segmentations using Dice index and the differences were statistically analyzed using Bonferroni multiple comparisons at significance level 0.05. The results showed that an optimum atlas for accurate segmentation of all structures cannot be found, and that the group of preferred templates, defined as being significantly superior to at least two other templates regarding the segmentation accuracy, varies significantly from structure to structure. Moreover, compared to other templates, a template giving the best accuracy in segmentation of some structures can provide highly inferior segmentation accuracy for other structures. It is concluded that there is no template optimum for automatic segmentation of all anatomical structures in the brain because of high inter-subject variation. Using a single fixed template for brain segmentation does not lead to good overall segmentation accuracy. This proves the need for multiple atlas based solutions in the context of atlas-based segmentation on human brain.

Keywords: inter-subject variation, human brain structures, atlas-based segmentation, segmentation accuracy

# 1. INTRODUCTION

Medical image segmentation plays an increasingly important role in several medical imaging applications. As manual segmentation is tedious, time consuming and subject to considerable inter- and intra operator variability, the development of automated methods is of great interest. Atlas-based methods have been widely used as a powerful approach when a reference or atlas template is available<sup>1,2</sup>. However, human brain structures vary significantly between individuals, seriously hampering the segmentation process<sup>3</sup>. This study aims at investigating how the overall accuracy of all brain structure segmentation using one single template is limited by inter-subject variation.

In the context of this work, we define an atlas as being composed of two 3D volumes: an anatomical image and the labeled structures of the image. In a standard atlas-based segmentation problem, a mapping between the atlas anatomical image and the image to be segmented must be determined. The determined mapping allows the propagation of the labeled structures onto the MR image, resulting in the segmentation of this image. The process of computing the mapping is referred to as registration. The better the registration maps the atlas image to the image with unknown segmentation, the more accurate the results of segmentation are. Normally a combination of rigid and non-rigid methods with numerous degrees of freedom such as the Demons<sup>4,5</sup> and Morphons<sup>6,7</sup> is required. Depending on particular applications, one may use a publicly available atlas such as Talairach, MNI, and Colin27, or construct an atlas from a dataset. Given a representative dataset, selecting a subject that could lead to the best segmentation accuracy when its constructed atlas is adopted in an atlas-based segmentation framework is crucial. Some strategies have been proposed for automatic optimum atlas selection purpose<sup>8,9,10</sup>.

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N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

It is well recognized that individuals differ substantially in brain volumes, distribution of gray and white matter, gyral patterns and cytoarchitecture<sup>11</sup>. Extreme variations in brain structure present fundamental challenges to human brain mapping<sup>3</sup>, and correspondingly limit the segmentation accuracy. Whereas human brain varies tremendously depending on several factors, especially from subject to subject, a lot of atlas-based segmentations still involve one subject as a template. Provided with an appropriate registration approach, whether one could obtain good automatic segmentations for all brain regions by using a single reference is a critical question and has not been fully answered. With this study, we show that because of high inter-subject variation in human brain structure, these solutions will not give satisfying results on all structures. Instead, multiple atlas based techniques<sup>12,13</sup> are required.

#### 2. MATERIALS AND METHODS

Each subject  $S_i$  in a dataset consists of an anatomical image  $I_i$  and a manual segmentation of that image  $A_i$ . Of all N subjects in the dataset, one was selected as an atlas  $S_j$   $I_j$ ,  $A_j$  and was used to automatically segment the other images of the dataset  $I_i$   $1 \le i \le N, i \ne j$  as illustrated in Figure 1. The result was then compared, for every other subject, to the segmentation which was manually traced by expert. This process was carried out for all subjects. Therefore, every subject was selected respectively for being the atlas in atlas-based segmentation.

To obtain an automatic segmentation, the Morphons registration was applied to find the correspondence between the anatomical image of the atlas being used and the image to be segmented. The resulting deformation field  $T_{j,i}$  allows the propagation or warping of the atlas segmentation  $A_j$  to the image, resulting in the segmentation of this image  $A_{j,i}$ . The anatomical agreement between the automatic and the manual segmentations was measured using the Dice index. These numerical results were then subjected to multiple comparisons to statistically compare all atlas pairs with respect to segmentation accuracy. The significant tests, with significance level 0.05 and Bonferroni correction, were carried out to analyze the difference in means of all possible pairs considering all brain structures at a time and particular structures.



Figure 1. Segmentation of all subject i  $(i \neq j)$  using subject j as the atlas.

## 2.1 Data

The experiments were carried out on two different datasets, which were extracted from two publicly available resources. Examples of both datasets are shown in Figure 2.

Dataset 1: MR brain data of 17 subjects were acquired from the database available through the Internet Brain Segmentation Repository (IBSR)<sup>14</sup>. The T1-weighted images were already "positionally normalized" into the Talairach alignment. The manual delineations were done by experts at the Center for Morphometric Analysis of Massachusetts General Hospital, Harvard Medical School. The images were processed by the CMA 'autoseg' bias field correction routines and manually labeled with the NVM, an open-source software tool for making precise quantitative neuro-anatomical measurements in volumetric image data. This study considered 32 basic structures in each subject of the dataset as all segmented structures.

Dataset 2: 17 subjects were randomly extracted from the database LPBA40<sup>15</sup>, which consists of 40 T1-weighted brain images and the corresponding labels. This database is available at the Laboratory of Neuro Imaging (LONI), University of California, Los Angeles (UCLA). The images were already preprocessed using the existing LONI protocols to produce skull-stripped volumes and aligned to the MNI305 atlas. 56 structures were manually labeled in each of the subjects according to the LONI custom protocol based on BrainSuite software.

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

In the experiments, we refer to a subject in a dataset by its identification number [1,...,17].



Figure 2. Examples of two datasets.

# Preprocessing

As the presence of non-brain tissue makes it problematic for the registration, skull-stripping needs to be done for images in dataset 1 as the very first step of the framework. To perform skull-stripping, we constructed a binary mask based on the expert segmentation. Since the brain structures were not fully labeled in some cases, mathematical morphology operators such as closure were necessarily applied to obtain a solid mask. The structuring element used was a square whose size is [2,2].

To reduce the computational cost, the volumetric images were cropped to remove unnecessary background: from 256x256x128 voxels to 175x175x128 voxels (dataset 1) and from 217x181x181 voxels to 185x150x150 voxels (dataset 2).

# 2.2 Image registration

In the context of this study, atlas-based approach treats the segmentation problem as a registration problem. An appropriate registration algorithm, therefore, is indispensible to obtain satisfying segmentation results. Registration was performed in two steps: a rigid registration providing global alignment of the data followed by a non-rigid deformation.

# Rigid registration

Despite the images in dataset 1 being normalized to the Talairach system, and those in dataset 2 being aligned to the MNI305 atlas, rigid registration was needed to improve the global alignment. We applied a rigid translation with 3 parameters using the Sum of Squared Difference (SSD) as the similarity metric. For each dataset, a subject was randomly selected as the reference. All remaining subjects were then translated to this reference.

# Deformable registration: the Morphons

Morphons registration uses an iterative process in a multi-resolution framework to find the correspondence from an image to a target image. At each resolution scale, the following steps are executed iteratively: displacement estimation, deformation field accumulation and deformation. Local indication on how to deform the moving (reference) image to make it more similar to the fixed (target) image is found in the displacement estimation step. One of the salient points of this method is that it estimates the incremental displacement field based on the local phase computed for every voxel. The local phase information is found by applying a set of quadrature filters on the images. Each filter is corresponding to a particular direction. The displacement field, computed from the local phase difference, is then used to update the deformation field. Normalized convolution<sup>16</sup> is employed instead of a regular smoothing method for the regularization of the updated field. In the deformation step, the moving image is morphed toward the fixed image based on the resulting deformation field.

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

It is important to notice that a certainty measure is adopted in the accumulation and regularization steps. As the quadrature filters are applied to measure the displacement of local structure, using the response amplitude as a certainty measure yields large certainties at edges, lines and surfaces. This quadrature phase difference measure is beneficial because it is invariant to image intensity and weak gradients. Besides, using quadrature phase for measuring the similarity between the images makes the method less sensitive to the variations in the intensity of the data.

For detailed descriptions about this registration method, we refer the readers to articles describing this method thoroughly<sup>6,7,16</sup>. In this study, we implemented the algorithm in a 8 scale multi-resolution framework, using 6 quadrature filters and Gaussian Kernel function with variance 1.5 for the normalized convolution. The automatic segmentation was found by warping the atlas segmentation using the deformation field computed in the registration to the fixed image.

#### 2.3 Anatomical agreement measurement

The anatomical agreement is quantified using the Dice index, also referred to as Dice Metric (DM), a realization of volume overlap measurement. This metric measures the volumetric overlap of two structures by taking the intersection and the union.

$$DM = \frac{2 V_{\text{fixed}}^{i} \cap V_{\text{fixed}}^{i}}{V_{\text{moving}}^{i} + V_{\text{fixed}}^{i}}$$
(1)

 $V_{moving}^{i}$  and  $V_{fixed}^{i}$  denote the volume of structure i in the moving image and the fixed image, respectively. The volume is computed as the total number of voxels which form that structure.

Perfect overlap of the manual and automatic segmentations leads DM to its maximum value 1. A complete mismatch between the volumes results in DM = 0. The more the volumes are overlapping, the larger the value of DM is ( $0 \le DM \le 1$ )

#### 2.4 Statistical analysis

After the automatic segmentations were obtained, one way ANOVA testing was carried out to statistically analyze the effect of inter-subject variation on the segmentation accuracy. For every possible subject pair, we applied one way ANOVA to check the significant difference of segmentation accuracy using two different atlases. The median of the alignment measurements of all structure pairs quantitatively represents the segmentation accuracy. Besides, the analysis was also done at structure level. We performed the same pairwise comparisons but considered one particular structure at a time. In this case, the Dice metric of the overlap measurement is used directly to indicate the segmentation accuracy. General Linear Model (GLM) procedure was applied for multiple comparisons at significance level 0.05 in conjunction with Bonferroni adjustment.

#### 3. RESULTS AND DISCUSSION

In this section, we present the segmentation results and the analysis on the underlying effect of inter-subject variation.

Experimental results showed good performance of the Morphons. To assess the registration results, we compared the anatomical alignment using every subject as an atlas after rigid and non-rigid registration with the anatomical agreement after rigid registration only. Figure 3 shows obvious improvement in image positioning applying the Morphons.

These registration results are comparable to those in Arno Klein's comprehensive study<sup>17</sup> about 14 non-rigid registration methods on the same databases. The average, taken across all subjects, of the median Dice measurements is 0.78 on dataset 1 and 0.72 on dataset 2; whereas in that study<sup>18</sup>, it lies between 0.34 and 0.52 on dataset 1, and ranges from 0.6 to 0.81 on the whole database LPBA40 depending on the registration method used. Besides, these results show that there is a limit in the agreement between the expert and automatic delineations made by registering an atlas with current registration techniques. The Morphons registration has the same results as other registration techniques for this sake. An illustrative example of the segmentation results can be found in Figure 4.

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

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Figure 3. Registration result. The alignment measurement of every subject pair results in a vector whose components are corresponding to the measurements for all structures (32 for dataset 1 and 56 for dataset 2). The median of these components was used as the representative for the vector. We had 16 median values resulting from 16 measurements between a subject and the remaining ones. The average of these values is plotted as a single metric associated to the measurement for this subject.



Figure 4. An example of segmentation results: from left to right: intensity image, manual segmentation and automatic segmentation; from top to bottom: slice with different views (axial, sagittal, and coronal).

To choose the best subject of the database, it is important to analyze the significant difference in terms of segmentation accuracy when different atlases are used. The inter-subject comparisons revealed that no subject in either of the databases was a good template for all structures. No subject was found to be superior to more than 4 subjects among the other 16 subjects regarding segmentation accuracy. All cases in which there is significant difference are: in dataset 1, subjects 5, 7, 14, 16 are superior to subject 10; in dataset 2, subject 13 is superior to subjects 3, 4, 11, 16, and subjects 8, 10, 12, 17 are superior to subject 4, 16.

The overlap measurement distribution is presented in Figure 5. Each boxplot, associated to an atlas being used for segmenting the other 16 subjects, displays the distribution of 16 values that represent the overlap measurements for those subjects. Each value is the median of the vector consisting of the agreement measurements between automatic and expert segmentations for all structures of a subject to be segmented. The boxplot corresponding to MUL represents the distribution of 14 measurements when three atlases were used in a combining way for segmenting the other 14 subjects.

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

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 $MUL \cong$  multiple atlases

Figure 5. Data distribution. Each boxplot includes a box and whisker plot. The box has lines at the lower quartile, median (center red horizontal line), and upper quartile values. The whiskers extend from each end of the box to the most extreme values within 1.5 times the interquartile range from the ends of the box. Outliers are presented by the red plus signs.

The mean overlap measurement was close to inter-observer agreement<sup>17</sup> proving that the registration method was performing correctly on the whole images. Nevertheless, the minimum Dice values were low as shown in Figure 6, leading to the remark that the automatic segmentations were not satisfactory for all structures.



Figure 6. Scatter plot of the overlap measurements between manual and automatic segmentations of a subject in each dataset.

Figure 7 and Figure 8 present a detailed inter-subject analysis at structure level. In these figures, a colored block on a column reveals that the corresponding subject S has significant difference with k other subjects. The difference is positive (S is superior) or negative (S is inferior) depending on the group to which this subject belongs. Any subject which is superior (inferior) to at least two other subjects is categorized in the group of the most (least) preferred subjects by assigning green (red) color on the corresponding block. Cases in which a subject has significant difference with respect to only one other subject are not reflected. At a certain point on the color code bar, sign "+" or "-", followed by the value of k, indicates positive or negative significance. In the results, the maximum number of subject to which a

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

subject has negative (or positive) significant difference is 16 (or 8). This explains why the color code bar appears to cover the range [-16:8]. The same color lookup table is used for both cases.

The results showed that the two groups vary significantly from structure to structure. Moreover, a template providing superior or comparable segmentation accuracy for some structures can have very low performance compared to other templates at some other structures. For examples, in dataset 1, subject 15 is superior for structures 16, 28, 51 and 60 but inferior for structures 7 and 11. In dataset 2, subject 10 is preferred for segmentation of structures such as 27, 34, 44, 65, 66, and 86 but highly inferior at structure 24, 49, 67, and 81.



Figure 7. [Dataset 1] Multiple comparisons on particular structures. Color codes represent the number of subjects to which each subject is significantly better or worse at segmenting a specific structure in other subjects.

N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010



Figure 8. [Dataset 2] Multiple comparisons on particular structures. Color codes represent the number of subjects to which each subject is significantly better or worse at segmenting a specific structure in other subjects.

In summary, we studied from the experiments that given a good registration method, with regard to all brain structure segmentation accuracy, an atlas that is superior to many other atlases could not be found. This resulted in a suspicion that all atlases in these particular datasets are capable of producing comparable segmentation results. However, by the

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statistical analysis at particular structures, we demonstrated that what mainly explains the above phenomenon is the significant difference in optimum templates used for segmenting different structures. In other words, being the best option for segmenting a structure does not mean that an atlas can yield good segmentation for other structures.

To handle the tremendous inter-subject variations of human brain structure, sophisticated approaches such as multiple-atlas based solutions are needed. Figure 5 shows a comparison on the segmentation accuracy resulting by using one single atlas and using three atlases. In this experiment, three atlases were selected as the most optimum templates of a dataset based on Normalized Mutual Information (NMI) after non-rigid registration<sup>8</sup>. Each atlas resulted in a segmentation. All segmentations of an image were merged together to obtain the final segmentation by assigning to each voxel the label that receives the majority "votes" from the atlases. The median of the Dice index using three atlases (0.82 for dataset 1, and 0.77 for dataset 2) is greater than that resulting by using individual atlas ( [0.77 - 0.79] for dataset 1, and [0.7 - 0.74] for dataset 2).

# 4. CONCLUSION

In this study, we proved the critical effect of inter-subject variations on single atlas based segmentation. There is no template optimum for automatic segmentation of all anatomical structures in the brain because of high inter-subject variation. Although atlas-based segmentation applied for particular human brain structures is a valid approach, using a single fixed template does not lead to good segmentation accuracy for all structures. For such needs, a multiple-atlas based solution should be used instead.

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N.T. Doan, J. Orban de Xivry and B. Macq, Effect of inter-subject variation on the accuracy of atlas-based segmentation applied to human brain structures in Proceedings of SPIE Medical Imaging 2010: Image Processing, B. M. Dawant and D. R. Haynor, 7623, 63, 2010

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