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TITLE:

Three-Dimensional Shape Modeling and Analysis of Brain Structures

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SUMMARY:

We introduce a semi-automatic protocol for shape analysis on brain structures, including image segmentation using open software, and further group-wise shape analysis using an automated modeling package. Here, we demonstrate each step of the 3D shape analysis protocol with hippocampal segmentation from brain MR images.

ABSTRACT:

Statistical shape analysis of brain structures has been used to investigate the association between their structural changes and pathological processes. We have developed a software package for accurate and robust shape modeling and group-wise analysis. Here, we introduce an pipeline for the shape analysis, from individual 3D shape modeling to quantitative group shape analysis. We also describe the pre-processing and segmentation steps using open software packages. This practical guide would help researchers save time and effort in 3D shape analysis on brain structures.

INTRODUCTION:

Shape analysis of brain structures has emerged as the preferred tool to investigate their morphological changes under pathological processes, such as neurodegenerative diseases and aging[1]. Various computational methods are required to 1) accurately delineate the boundaries of target structures from medical images, 2) reconstruct the target shape in the form of 3D surface mesh, 3) build inter-subjects correspondence across the individual shape models via shape

parameterization or surface registration, and 4) quantitatively assess the regional shape differences between individuals or groups. Over the past several years, many methods have been introduced in neuroimaging studies for each of these steps. However, despite the remarkable developments in the field, there are not many frameworks immediately applicable to research. In this article, we describe each step of the shape analysis of brain structures using our custom shape modeling tools and publicly available image segmentation tools.

Here, we demonstrate the shape analysis framework for brain structures through the shape analysis of the left and right hippocampi using a dataset of adult controls and Alzheimer's disease patients. Atrophy of the hippocampi is recognized as a critical imaging biomarker in neurodegenerative diseases[2-4]. In our shape analysis framework, we employ the template model of the target structure and the template-to-image deformable registration in the shape modeling process. The template model encodes general shape characteristics of the target structure in a population, and it also provides a baseline for quantifying the shape differences among the individual models via their transitive relation with the template model. In the template-to-image registration, we have developed a Laplacian surface deformation method to fit the template model to the target structure in individual images while minimizing the distortion of the point distribution in the template model[5-7]. The feasibility and robustness of the proposed framework have been validated in recent neuroimaging studies of cognitive aging[8], early detection of mild cognitive impairment[9], and to explore associations between brain structural changes and cortisol levels[10]. This approach would make it easier to use the shape modeling and analysis methods in further neuroimaging studies.

PROTOCOL:

Brain MR images were acquired as per the protocol approved by the local institutional review board and ethics committee.

NOTE: The tools for shape modeling and analysis can be downloaded from the NITRC repository: https://www.nitrc.org/projects/dtmframework/. The GUI software (DTMModeling.exe) can be executed after extraction. The publication list on the shape modeling tools can be found in the project webpage: http://cgv.kaist.ac.kr/brain. See **Figure 1**.

1. Brain MR image segmentation

1.1. Acquire brain MR images of individual subjects and brain segmentation masks.

NOTE: Usually, we acquire T1-weighted MR images for analyses of brain structures. We assume that the MR images are pre-processed for gradient non-linearity correction and intensity inhomogeneity correction using N3[11], improved N3 methods[12], or FSL-FAST[13]. Some freely available tools for automatic segmentation of human brain structures are listed in **Table 1Error! Reference source not found.**

1.2. Correct the segmentation results manually.

NOTE: Open GUI software supporting manual segmentation are listed in **Table 2**. Manual segmentation protocols for the brain structures can be found here[14-16]. A video guide on manual segmentation for hippocampus is here[17]. We describe the protocol for hippocampal segmentation in the next section.

1.2.1. Open the T1-weighted MRI and the automatic segmentation results using the **Open File** menu.

1.2.2. Load the Segmentation plugin by clicking **Window Menu | Show | Segmentation**.

1.2.3 Correct the segmentation mask using the **Add**, **Subtract**, and **Correction** tools in the **Segmentation** plugin.

1.2.4 Save the corrected segmentation mask in Nifti format using the **Save** menu.

2. Manual editing of hippocampal segmentation

NOTE: We introduce a protocol for manually editing of brain segmentation using the GUI modeling software based on the MITK workbench (http://www.mitk.org/). The MITK workbench provides various functions for the manual and automatic segmentation and medical image visualization. We demonstrate the manual editing process for the left and right hippocampi. Steps for manually editing[18] the result of the automatic hippocampal segmentation are as follows.

2.1 Open the T1-weighted MR image and the results of the automatic hippocampal segmentation using the MITK workbench software.

2.2 Load the Segmentation plugin in the MITK workbench by clicking on the menu **Window | Show View | Segmentation**.

2.3 Select the coronal view by clicking the right-hand side icon that appears in the top right-hand side corner of the **Display** window.

2.4 Edit the binary mask of each hippocampus (i.e., left and right) in the coronal view, starting from the hippocampal head to the body as follows.

2.4.1 Scroll throughout the volume until the uncus is found. Include the uncus in the hippocampal mask where it is present.

2.4.2 Edit the mask of the hippocampal body after the uncus has receded using the **Add** and **Subtract** function in the **Segmentation** plugin.

2.4.3 Continue editing the hippocampal mask until the hippocampal tail is found. As the pulvinar nucleus of the thalamus recedes superior to the hippocampus, the fornix emerges.

2.4.4 Finish editing the last coronal slice of the hippocampus in which the entire length of the fornix is visible but not yet continuous with the splenium of the corpus callosum.

NOTE: Cerebrospinal fluid (CSF) spaces can be contained within the hippocampal regions. The CSF spaces can be removed from the hippocampal masks using the **Subtract** tool in the segmentation plugin of the MITK workbench. it may be easier to define the hippocampal regions entirely and then go through all coronal slices from the hippocampal head to tail for the removal of CSF spaces.

2.4.5 Follow the same process for editing the binary masks of both hippocampi.

NOTE: The **Add**, **Subtract**, and **Correction** tools of the **Segmentation** plugin in the MITK workbench can be used for the manual editing. The **Correction** tool is easy to handle small errors in the segmentation mask by performing addition and subtraction according to user input and the segmentation mask without additional tool selection.

2.5 Save the binary masks for left and right hippocampi in Nifti format (nii or nii.gz) using the **Save** menu in the MITK workbench software.

NOTE: The binary masks of left and right hippocampi should be saved separately for the subsequent hippocampal shape model steps.

3. Group template construction

NOTE: After the segmentation and manual editing for all subjects, the individual shape modeling requires the template model of the target structure. We construct the template model from the average binary mask for a population, acquired using "ShapeModeling" plugin in the MITK Workbench. Steps of the template model construction using GUI software are as follows.

3.1 Load the ShapeModeling plugin using the menu function: **Window | Show View | Shape Modeling**.

3.2 Open a directory containing the binary masks of a study population by clicking the **Open Directory** button in the **ShapeModeling** plugin.

3.3 Click the **Template Construction** button in the **ShapeModeling** plugin.

3.4 Check the mean shape mesh and save it in stereolithography (STL) format using the **Save** menu.

4. Individual shape reconstruction

NOTE: At this step, we perform the shape modeling for individual subjects using **Start Shape**

Modeling button in the "ShapeModeling" plugin. We list the software parameters of this plugin in **Table 3**. Detailed explanation on each parameter can be found here[5]. Steps of the individual shape reconstruction using GUI software are as follows.

4.1 Load T1-weighted MR image and its segmentation mask using the **Open File** menu.

NOTE: We use the T1-weighted MR image for visual validation.

4.2 Check the modeling parameters in ShapeModeling plugin and modify if necessary.

NOTE: If the template model is not deformed or the distance between the template model and the image boundary is large, it is recommended to increase the boundary search range. If some geometric distortions are found, increasing maxAlpha and minAlpha with step 0.5 would help to resolve the issue. It is important to check the voxel intensity for the target object in the segmentation mask. If the value is not 1, intensity parameter should be changed accordingly.

4.3 Click the **Shape Modeling** button to run the shape modeling process and check the result in the 3D view of MITK workbench.

4.4 Repeat steps 4.2 and 4.3, when the template model is not fitted to the image boundary closely.

NOTE: The template model is visualized with the segmentation mask in the sagittal, coronal, axial, and 3D view of the MITK workbench. The template surface is not deformed when the distance between the template model and the image boundary is less than a threshold which is one tenth of the smallest voxel size.

4.5 Save the modeling result in a stereolithography (STL) format using the **Save** menu in MITK framework.

5. Group-wise shape normalization and shape difference measurement

NOTE: At this step, we align the individual shape models to the template model and compute the point-wise shape deformity between the corresponding vertices between the template model and the individual shape model. Steps for the shape deformity measurement are as follows.

5.1 Select the shape model of a subject in the **Data Manager** of the MITK workbench.

NOTE: Users can select multiple models for the deformity measurement.

5.2 Perform the deformity measurement by clicking the **Measurement** button in the **ShapeModeling** plugin.

REPRESENTATIVE RESULTS:

The shape modeling process described here has been employed for various neuroimaging studies

on aging[6,8,10] and Alzheimer's disease[5,9]. Especially, this shape modeling method showed its accuracy and sensitivity in the shape analysis on the hippocampus for an aging population of 654 subjects[8]. A quantitative analysis of the software and the publicly-available software, ShapeWork, LDDMM-TI, and SPHARM-PDM, can be found here[5]. We describe many open tools from MR image preprocessing to brain segmentation in **Table 1**, **Table 2**, and **Table 4**.

Figure 2 is a diagram of the shape modeling framework using the template models of target structures. The template models represent general shape characteristics of the brain structures in a population. **Figure 3** presents the deformation of the hippocampal template model for individual shape reconstruction. The method induces a large-to-small scale deformation of the template model to minimize the distortion of its point distribution while restoring individual shape characteristics. **Figure 4** shows the reconstructed shape models of two subjects with their segmentation masks. **Figure 5** shows the aligned individual shape models, their average model, and the shape difference vectors with an individual shape model. **Figure 6** presents the average shape deformity maps, projected onto the average model, for two groups with small and large brain tissue volume (BTV). We selected subjects whose BTV is greater or less than a standard deviation from the mean of a healthy aging population of 51 subjects[5]. The shape deformity maps of two groups present opposite patterns of hippocampal shape difference at corresponding regions.

FIGURE LEGENDS

Figure 1. GUI software for the shape modeling and analysis

Figure 2. Steps of the shape modeling using the template models for brain structures

Figure 3. Deformation of the template model (orange) for individual shape reconstruction. Color map: vertex-wise deformation magnitude (mm).

Figure 4. Examples of individual shape modeling of the hippocampus

Figure 5. Aligned individual shape models, their average model, and the shape difference vectors with an individual shape model. Left: Aligned individual shape models (white) and their average model (blue). Right: Point-wise shape difference vectors between the average model and an individual model.

Figure 6. Average shape deformity of two groups with small and large brain tissue volume (less or greater than one standard deviation from the population mean) in a healthy aging population

Table 1. List of open software widely used for automatic segmentation of brain structures

Table 2. List of open software for manual segmentation and visualization

Table 3. Parameters for the individual shape reconstruction

Table 4. List of open software widely used for brain MR preprocessing and skull stripping

DISCUSSION:

In summary, we have described the software pipeline for the shape analysis on brain structures including (1) MR image segmentation using open tools (2) individual shape reconstruction using a deformable template model, and (3) quantitative shape difference measurement via transitive shape correspondence with the template model. Statistical analysis under the false discovery rate (FDR) correction is performed with the shape deformity to investigate the significance of morphological changes of brain structures, associated with neuropathological processes.

Our modeling pipeline internally use in-house tools to construct a template model from subject images. The steps for the template construction are as follows: (i) Compute the group average mask via iterative alignment of subject images to an average image which evolves at each iteration. (ii) Generate a 3D surface mesh from the average mask using marching cubes method[20]. (iii) Resample the surface mesh using a mesh resampling using the ACVD tool (https://www.creatis.insa-lyon.fr/site/en/acvd.html). The number of the template model can be set in the ShapeModeling plugin.

The individual shape reconstruction is based on a progressive template deformation method. This method allows a large-to-small scale deformation to minimize the geometric distortions of the template model while restoring the individual shape details by propagating the template model to image boundaries. The deformation method is limited to the structures with spherical topology. Against this limitation, we have introduced structure-specific constraints in the shape modeling of brain third ventricle, which has a hole by interthalamic adhesion[6]. However, the structure-specific constraints are not supported by the current version of our software.

The individualized shape models are aligned in common space using the generalized Procrustes algorithm[19]. Here, we use the similarity transformation (isotropic scale, translation, and rotation) for the shape model normalization. The local shape differences are determined by the displacement vector between the corresponding vertices of the individual surface models and their mean shape model. The shape deformity at each vertex is computed as the signed Euclidean norm of the displacement vectors which are projected onto the vertex normal of the mean model. The detailed steps of the statistical shape analysis can be found here[5].

For the accuracy evaluation of the shape modeling, we use 3 metrics: Dice coefficient, mean distance, and Hausdorff distance. The Dice coefficient represents volume overlap between the reconstructed model and the target segmentation mask. The mean distance is the average distance between them, and the Hausdorff distance is the maximal distance between them. Lower distances and higher Dice coefficient indicate better accuracy. For the hippocampus study[5], the Dice coefficient was 0.85-0.9, the mean distance was around 0.3 mm, and the Hausdorff distance was 2 mm. However, these results depend on the volumes and shape details of target structure. Volume difference and surface roughness can be used as indicators for the accuracy

and shape quality[5].

For ease of use, we also distribute a Matlab script together for generating the list files and running the command line tools for each step. Currently, we have tested the tools in Linux, MacOS, and Windows. The significance of the in-house software is that it is fully automated for template-based shape modeling and measurement. We have validated its robustness and accuracy with various data sets of aging and Alzheimer's disease populations[5]. Furthermore, there are many approaches using the shape modeling method on different human organs.

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DISCLOSURES:

The authors declare that there is no conflict of interest.

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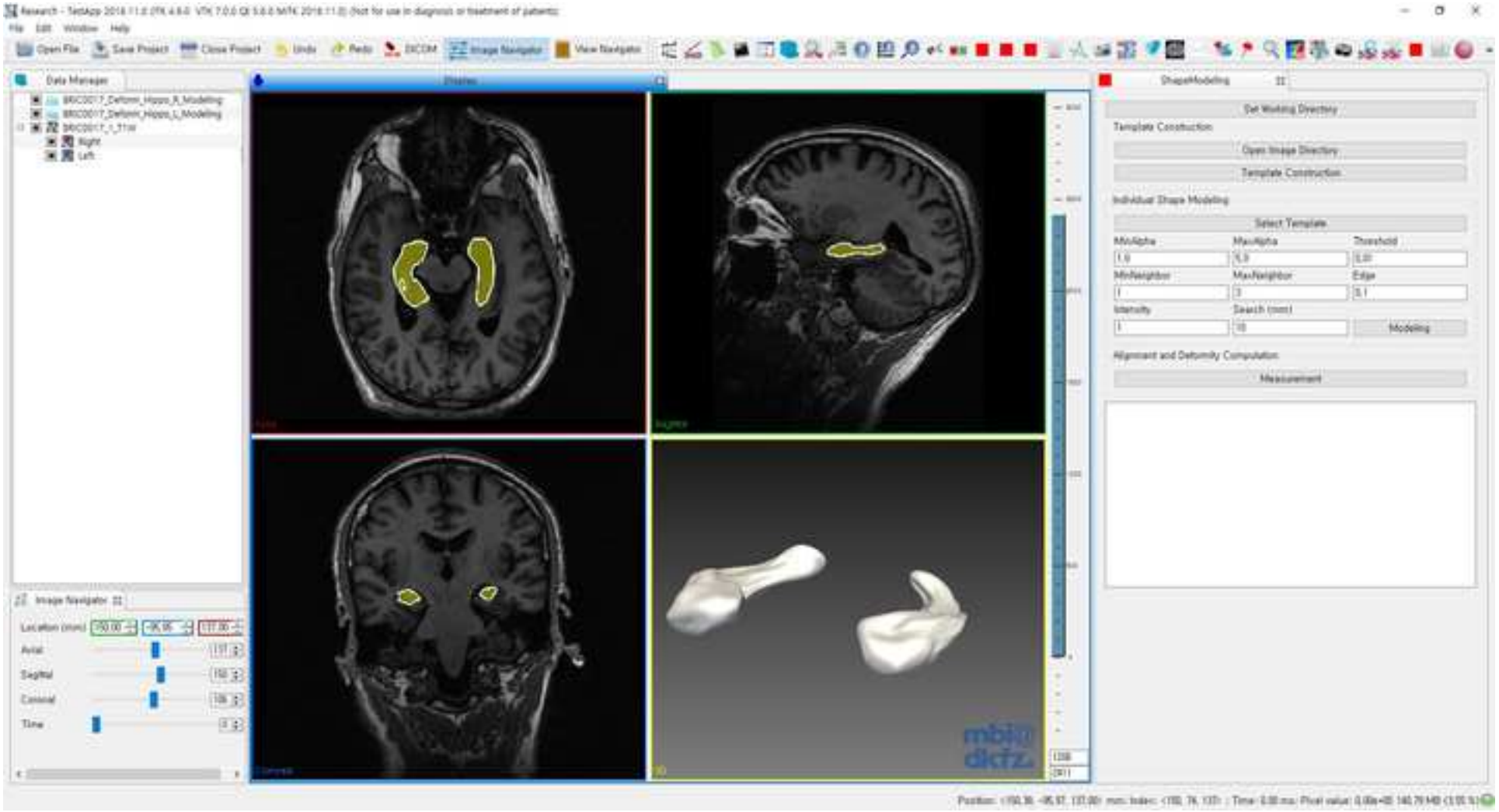

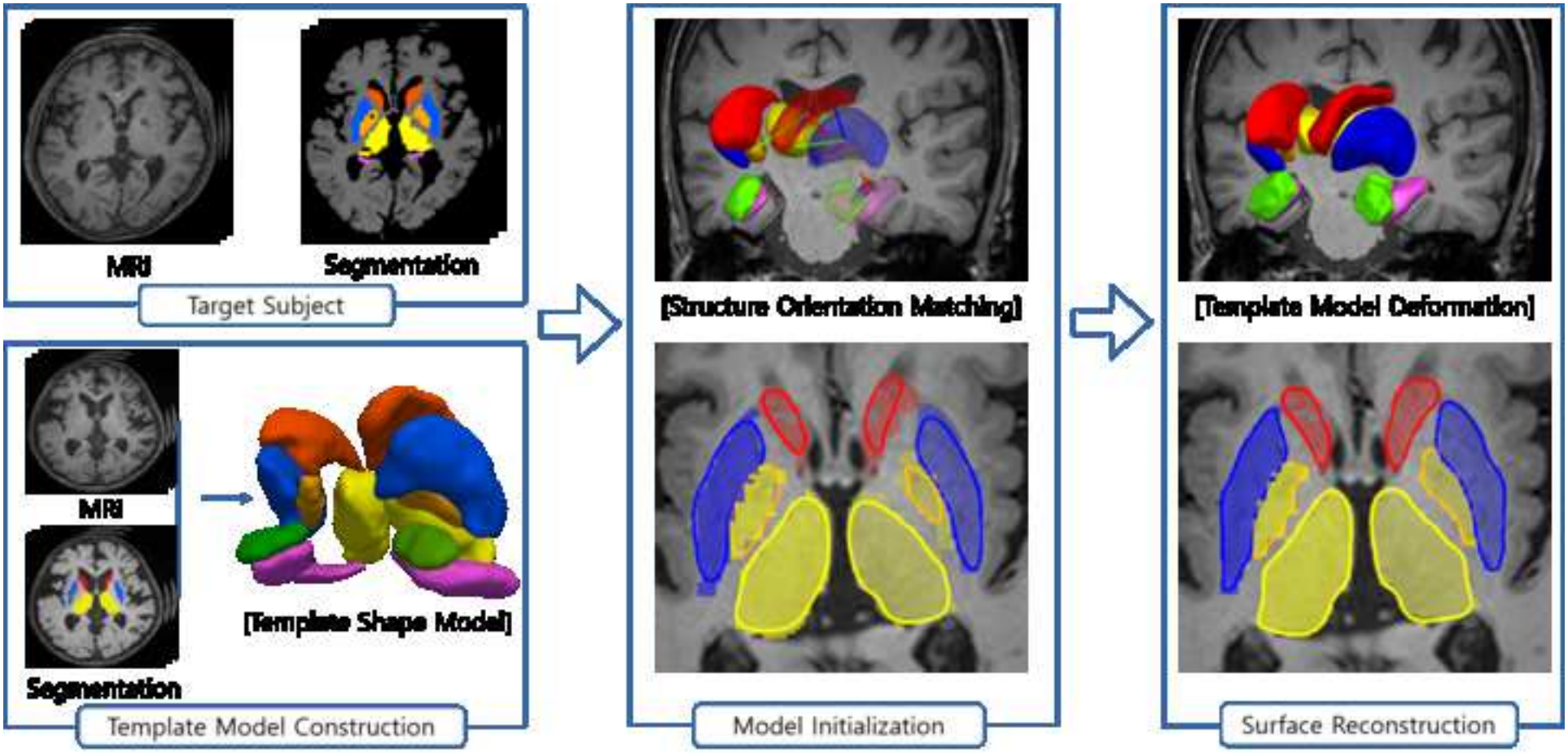

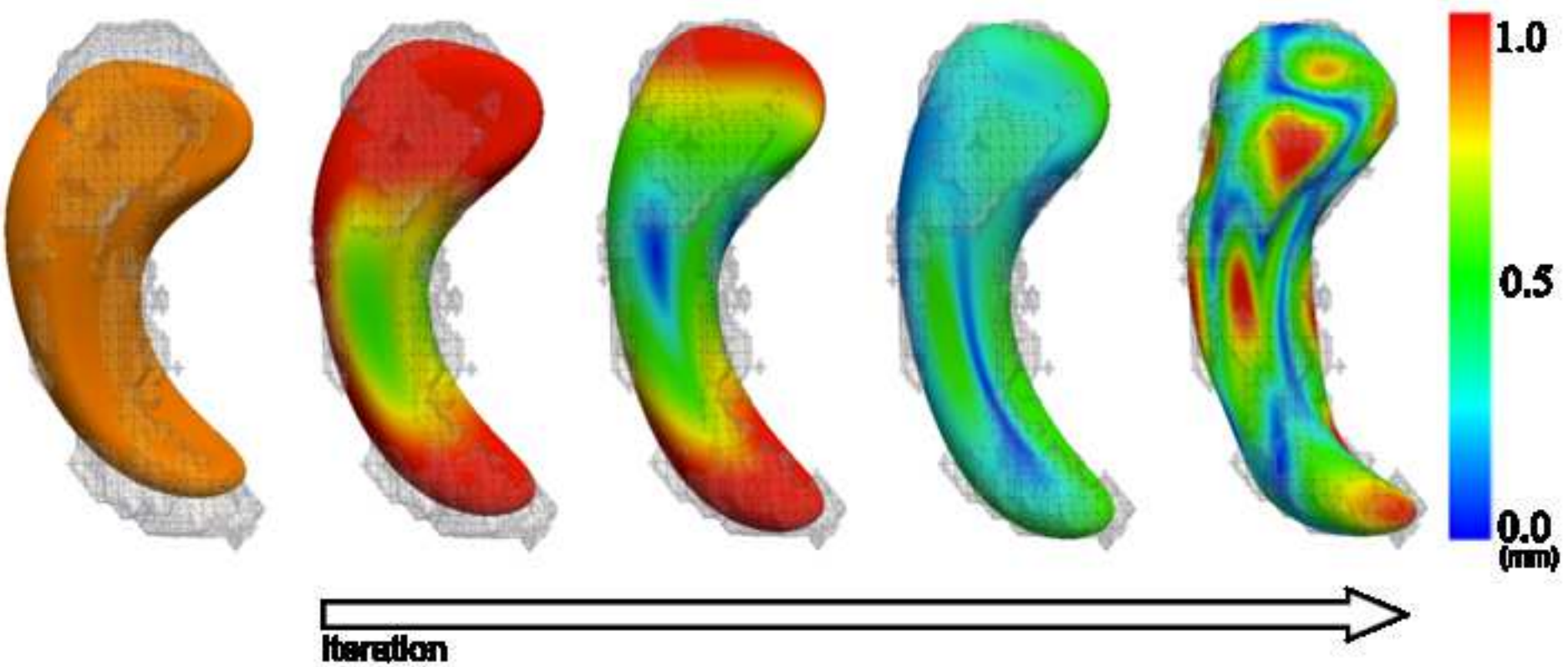

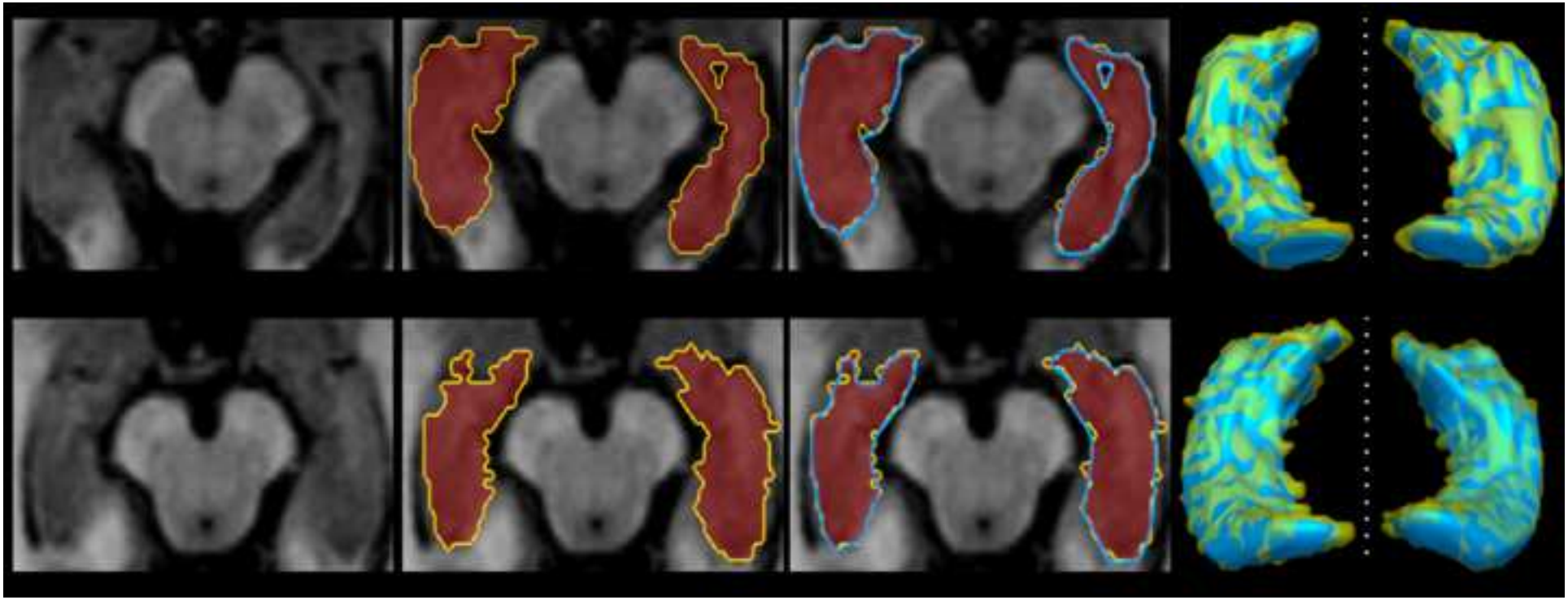

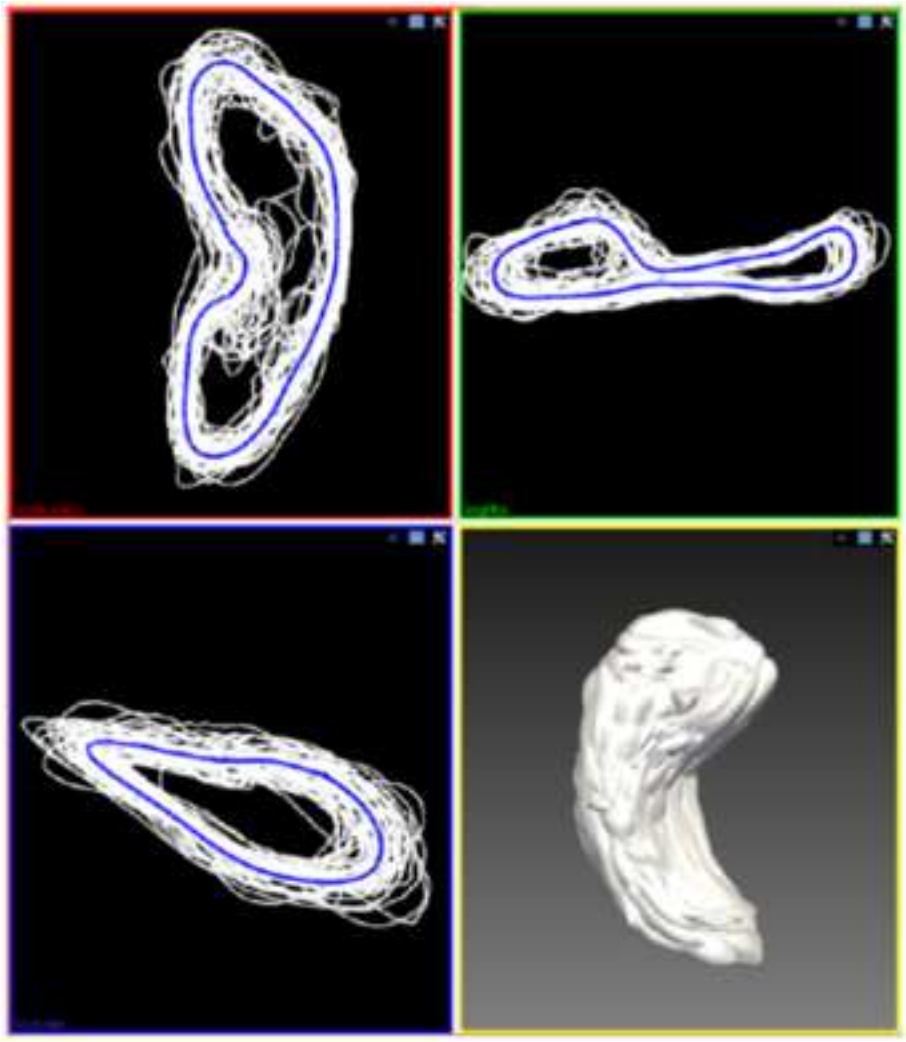

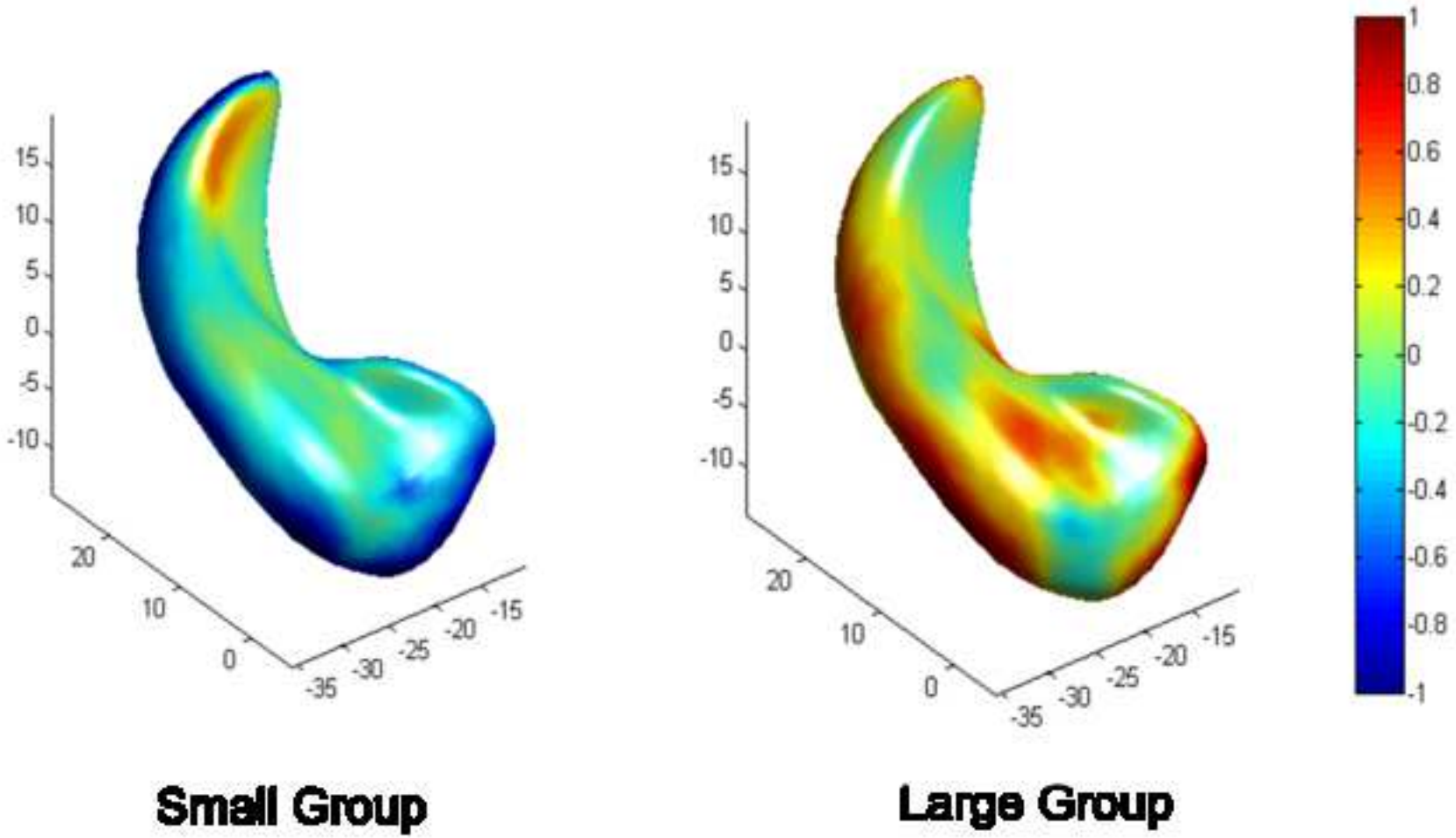

Table

Name	Description	System
ALVIN	Lateral ventricle segmentation	Linux
FIRST	Subcortical structure segmentation in FSL	Linux, Mac
FAST	Tissue classification tool with the correction for spatial intensity variations	Linux, Mac
FreeSurfer	Voxel-wise full brain segmentation	Linux, Mac
TOADS-CRUISE	Automatic brain segmentation tool	Linux, Mac
NiftySeg	Automatic brain tissue classification tool	Linux, Mac
BrainSuite PVC tool	Brain tissue classification tool in BrainSuite package	Windows, Linux, Mac

Organization	Link
King's College London	https://www.nitrc.org/projects/alvin_lv/
University of Oxford	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST
University of Oxford	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FAST
Athinoula A. Martinos Center for Biomedical Imaging, MGH	https://surfer.nmr.mgh.harvard.edu/
Johns Hopkins University	https://www.nitrc.org/projects/toads-cruise
King's College London	https://github.com/KCL-BMEIS/NiftySeg
University of Southern California	http://brainsuite.org/

Name	Description	System
MITK	GUI software providing plugins for semi-automatic (e.g. region growing and watershed thresholding) and manual image segmentation	Windows, Linux, Mac
3D Slicer	GUI software for medical image processing and 3D visualization. Segment Editor in 3D Slicer is a module for manual segmentation	Windows, Linux, Mac
ITK-Snap	GUI software for semi-automatic (active contour method) and manual segmentation	Windows, Linux, Mac
GIMIAS	GUI software for biomedical image computing. Manual segmentation plugin is supported.	Windows, Linux
MRICron	GUI software for NIFIT format image viewer. It also supports volume rendering, ROI region drawing, and statistical tools	Windows, Linux, Mac
Mango	Multi-platform image viewer supporting surface visualization, ROI editing, and image analysis	Windows, Linux, Mac

Organization	Link
German Cancer Research Center	http://mitk.org/wiki/MITK
Brigham and Women's Hospital, Inc.	https://www.slicer.org/
University of Pennsylvania and University of Utah	http://www.itksnap.org/pmwiki/pmwiki.php
University of Sheffield	http://www.gimias.org/
University of South Carolina	http://people.cas.sc.edu/rorden/mricron/index.html
University of Texas Health	http://ric.uthscsa.edu/mango/index.html

Table

Parameter
--minAlpha
--maxAlpha
--thresholdAlpha
--minRing
--maxRing
--edge
--intensity
--range
--init

Description
Minimum weight for internal force preserving Laplacian coordinates of template model (default: 1.0)
Maximum weight for internal force preserving Laplacian coordinates of template model (default: 5.0)
Threshold parameter to reduce the alpha weight gradually during the template deformation (default: 0.01)
Minimum level of neighborhood (default: 1)
Maximum level of neighborhood (default: 3)
Weight parameter for external force (default: 0.1)
Voxel value for target structure in segmentation mask
Boundary search range (default: 5.0)
Template model initialization using iterative closest algorithm (default: 1 (true))

Table

Name	Description	System
MINC N3	Non-parametric non-uniformity normalization (N3) method	Linux, Mac
ANTs N4BiasCorrection	N4ITK: Improved N3 method in Advanced Normalization Tools (ANTs) software package	Windows, Linux, Mac
SkullStrippingToolkit	Skull stripping tool using a level-set based fusion method	Matlab
ROBEX	Skull stripping tool using a brain surface fitting method	Linux, Mac
FSL BET	Skull stripping tool in FSL pacakge	Linux, Mac, Windows
BrainSuite bse tool	Skull stripping tool in BrainSuite pacakge	Windows, Linux, Mac

Organization	Link
McGill University	https://www.nitrc.org/projects/nu_correct
University of Pennsylvania	https://sourceforge.net/projects/advants/
University of North Carolina	https://www.nitrc.org/projects/skulltoolkit
University of California, Los Angeles	https://www.nitrc.org/projects/robex/
University of Oxford	https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET
University of Southern California	http://brainsuite.org/processing/surfaceextraction/bse/

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Date:	July 24, 2019

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Dear Dr. DSouza,

We greatly appreciate your patience and the reviewers' constructive comments on our manuscript. We have revised the manuscript accordingly. Please find attached a point-by-point response (written in blue color) to each comment/suggestion.

Sincerely,

Jinah Park, Ph.D.
Associate Professor
School of Computing, Korea Advanced Institute of Science and Technology

Editorial Comments:

• Please print and sign the appropriate Author License Agreement, then scan and upload it with your manuscript files. UK-funded article are usually need to be published under open access only. I have attached a blank copy.

Response: We submitted the Author License Agreement for the standard access. Although one of the authors is UK-based, the software development and further development study included for this video publication are mostly supported by Korean research grants. The author from the UK also confirmed that there was no obligation with Open Access in this case.

• The manuscript will benefit from thorough language revision as there are a number of grammatical errors throughout. Please thoroughly review the manuscript and edit any errors.

Response: We regret that there were some problems with English. The manuscript was carefully revised to improve English grammar and readability.

• **Abstracts:**
1) Reduce the summary to 50 words.

Response: We reduced the summary as the suggestion.

• **Protocol Language:** Please ensure that ALL text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.
1) Examples NOT in imperative voice: lines 79, 81,82, 86-94, etc
2) Please use complete sentences in all your steps.

Response: We re-wrote the protocol section in the imperative tense. We also removed unnecessary descriptions (line 86-94) about the command-line tools and open software in the protocol section for filming.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. **Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc) to your protocol steps.** There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:
1) Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the guidelines of your institutions human research ethics committee.
2) 1.2.A: Unclear what is done, please describe or cite a reference (only if you are not indicating this for filming).
3) 1.3: Unclear what is done, please describe or cite a reference (only if you are not indicating this for filming).
4) 1.1-1.4: These are not real steps, only discussion of choices to make. Please rewrite as complete steps with sufficient details.
5) 1.5.B.iii: unclear what is done here.

Response: We added the ethics statement before the numbered protocol: “Brain MR images were acquired as per the protocol approved by the local institutional review board and ethics committee.” We also rewrote the protocol section 1 to describe clear steps for filming in the section.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

Response: We adjusted the numbering following the instruction.

• **Protocol Highlight:** After you have made all of the recommended changes to your protocol (listed above), please re-evaluate the length of your protocol section. There is a 10-page limit for the protocol text, and a 3- page limit for filmable content. If your protocol is longer than 3 pages, please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.
1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.
2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.
3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.
4) Notes cannot be filmed and should be excluded from highlighting.
5) Please bear in mind that software steps without a graphical user

interface/calculations/ command line scripting without detailed commands and entries cannot be filmed.

Response: We highlighted the essential steps for filming in the protocol section. The filmable content is now less than 3 pages.

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Response: We added two paragraphs to cover the details and limitations of our proposed method.

• **Figures:**
1) Please remove the embedded figures from the manuscript. Figure legends, however, should remain within the manuscript text, directly below the Representative Results text.
2) All figures should be numbered and have figure legends and in-text references.
3) Please expand the legends to adequately describe the figures/tables. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description.
4) Please provide each figure (if multiple panels are present per figure, keep them within 1 file) as an individual SVG, EPS, AI, TIFF, or PNG file.

Response: We revised the figure and table legends in the manuscript and removed the embedded figures which are not necessary in the protocol section.

• **Tables:** Please remove the embedded Tables from the manuscript. All tables should be uploaded to the Editorial Manager site in the form of Excel files. A description of the table should be included with the Figure legends.

Response: We removed the embedded Tables in the manuscript. We upload the corresponding excel file, including all the tables.

• **References:**
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2) Please move the in-text http weblinks (LINES 72, 73, 111) into the reference list, and use superscripted citations.

Response: We revised the references following JoVE instructions.

• **Table of Materials:** Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all

relevant materials/software in separate columns in an xls/xlsx file. Please include items such as software used.

Response: We added more information, such as the maintainers (institution) of the open software, in the tables.

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Comments from Peer-Reviewers:

Reviewer #1:

Manuscript Summary:
This paper represents an end-to-end shape analysis framework on hippocampi. The proposed process combines existing tools and the implementation of the authors' prior work. Overall, the steps are well described and easy to follow, but there are a couple of steps not quite clear. Please see my comments below.

Response: We appreciate very much the reviewer's thoughtful comments and suggestions. Our response to each comment is given below.

Major Concerns:

- The title of this paper is little misleading since it focuses on not all subcortical structures but just hippocampi. Each subcortical structure may require specialized handling, so it's not clear if this framework extends to a general subcortical structure analysis.

Response: We understand the reviewer's comment that our manuscript is mainly on hippocampi rather than dealing the subcortical structures as a whole. Although the main example was a brain hippocampus model, however, the intention of this paper was to describe the steps for shape modeling and analysis applied to subcortical structures. The modeling method has also been verified for brain third ventricle and lateral ventricles as well.

- Is Step 2 mandatory in this framework? This may be necessary if the automated tools fail. What is difference between Step 1-5 and this one?

Response: In this paper, we aimed to introduce the sequential steps from the manual segmentation editing to the shape analysis using our software. The manual segmentation editing is not mandatory for the shape analysis, when using automated segmentation tools. However, it is often required to acquire accurate segmentation masks suitable for clinical research. Step 2 introduces more details of the manual editing with an example of brain hippocampus for filming.

- The Procrustes alignment requires a known correspondence. How does this work on volumetric masks (3-1-A-i)?

Response: We build the template image of brain structure via iterative process to compute the Frechet mean image of subject images. They are aligned non-linearly to an average image that evolves iteratively.

- The template shape is obtained by marching cube, which is later used for the individual surface reconstruction. Does the template model guarantee genus-zero? Any topological correction steps in volume or on surface?

Response: Thank you for your comments. We realize that our description of the template construction has not been enough to explain the steps of the template construction. After the template image construction, we apply morphological operations to remove holes to the template image. Then, we build 3D surface model from the template image using marching cube and surface resampling algorithm. We rewrote the protocol section to describe these details more clearly.

- How is the distance computed between the surface models and their masks? Are the surfaces voxelized back or are the volumes reconstructed as surfaces in some way (perhaps marching cube)? The same question for the Hausdorff distance.

Response: We measure the mean and Hausdorff distance after converting the surface models to volumes.

- The individual surfaces are reconstructed from the initial template that is being updated in the group-wise analysis step. Since the template changes, the shape correspondence might need to be updated. How sensitive is this framework to the initial template? Does this require any step to deform the updated template to generate new individual surfaces?

Response: We first build the template model of the subject images using the template construction process, previously described. Then, the template model is propagated to restore the individual shape characteristics in the subject images. This step is based on a progressive template deformation method allowing a large-to-small scale deformation to minimize the geometric distortions of the template model. This method can maintain the anatomical point-to-point correspondence across subjects while restoring the individual shape details.

Minor Concerns:
- What's the typical number of vertices for the surface reconstruction? How can the users decide the number of vertices?

Response: For hippocampus, the number of vertices was 2463. As the number of vertices increases, the computation time linearly increases. We modified our GUI software to allow users to set the desired number of vertices.

- Figure 2 is little confusing. Does the color indicate the amount of the displacements between successive iterations? For example, why the red color on top turns into the greenish (3rd and 4th iterations)?

Response: We appreciate your comment. Yes. The color indicates the vertex displacements during the individual shape modeling. Red color indicates a larger deformation of the template model. Blue color shows smaller differences in the model deformation. As the template model closes to the image boundaries, the amount of the vertex displacements decreases. We will prepare filming materials for better understanding.

Reviewer #2:

Manuscript Summary:
A protocol is presented for manually segmenting and conducting shape analysis of the hippocampus from MR images.

Major Concerns:
Copy editing is required to correct typographical and grammatical errors.
A complete worked out processing example for an available dataset, including segmentation steps and subsequent review and interpretation of shape analysis results, would be helpful.

Response: We thank the reviewer for the careful review and suggestions. We have revised the manuscript for typos and grammatical errors.

In the protocol section, we have added the detailed steps for the shape modeling and analysis on hippocampus. We have also modified the discussion section to resolve the reviewer's comment.

Minor Concerns:
The presented protocol by itself would be difficult to implement without an accompanying video; however, this is only a minor concern given that a video is available.

Response: We will carefully prepare filming materials for better understanding.

Reviewer #3:

Manuscript Summary:
The manuscript describes a step by step protocol for the analysis of brain structures from MRI scanner images using freely available tools.

Major Concerns:
NA

Minor Concerns:

There are many figures with no Figure identification or figure legends. If these pictures will be included in the manuscript (as they are useful to follow the protocol) they should be identified with a figure number and figure legend or title.

Response: We appreciate the reviewer for reviewing the manuscript carefully. We realized that some figures in the protocol section are not necessary, because most of them will be presented in our video. The figures and tables in the result section are identified with their legends.

Line 102: Please, succinctly describe MITK or refer to table 3.

Response: We describe the MITK workbench briefly in the protocol section 2. We developed a plugin for the MITK workbench to provide the functions for the shape modeling and analysis.

Line 111: The provided link is not working, please provide the correct one.

Response: We thank the reviewer for careful review. We have corrected the link.

Pages 3-4: I suggest to identify the different key anatomical regions mentioned in the MRI images (uncus, fornix, etc.).

Response: We removed figures in the protocol section, because they will be shown during filming. We will prepare filming materials according to the reviewer's comment.

Line 145: I suggest to indicate the implications or the relevance of "operating on an existing segmentation" or "in a new object".

Response: There was misleading about the correction tool. We have revised the sentences as follows:

"The Add, Subtract, and Correction tools of the Segmentation plugin in the MITK workbench can be used for the manual editing. The Correction tool is easy to handle small errors in the segmentation mask by performing addition and subtraction according to user input and the segmentation mask without additional tool selection."

Line 146: there is a mistake and I guess "the" should be removed.

Response: We removed the word.

Line 157: It is indicated that the command line tool "MeanImageBuilder" is not included in the MITK software library. Please, indicate how to obtain and install it.

Response: The tool is provided with our software package based on the MITK workbench. In the GUI software, the MeanImageBuilder tool is executed in the template construction steps. We have revised the first not in the protocol section to describe how to execute our GUI software.

Lines-199-200: Please, identify the table. I also recommend to include some suggestions and orientation about how to select/optimize the parameters.

Response: We replaced the table with table number and legends in the results section.

Line 205: Please, indicate which criteria should be used to change the parameters.

Response: We added a note in the protocol section to explain how to change the parameters to resolve some issues in the individual shape reconstruction.

Line 210: Please, specify what you mean by enough (a clear criterium should be desirable).

Response: We revised the sentence to describe the algorithm more clearly: “Repeat step 2 and 3, when the template model is not fitted to the image boundary closely. The template model is visualized with the segmentation mask in the sagittal, coronal, axial, and 3D view of the MITK workbench. The template surface is not deformed where the distance between the template model and the image boundary is less than a threshold which is one tenth of the smallest voxel size”

Line 214: Please, indicate the range of values expected for each of the three metrics proposed to consider that the individual shape reconstruction is accurate enough.

Response: It is difficult to define the proper range of the metrics to determine the accuracy of the shape modeling. Lower distances and higher Dice coefficient indicate that the individual models represents the shape details of brain structures more accurately. Volume difference and surface roughness can be another indicators of the model accuracy. We have added a paragraph about the metrics in the discussion section.

It seems to me that in section 5 (line 222) "Group-wise Shape Normalization and Shape Difference Measurement" some information is missing, as it does not follow the same structure compare to previous sections in which you provided (subsections C) brief instructions about how to use the software. Please, use the same structure than in previous sections, and add step by step indications about how to align the individual shape models to a mean shape model, average the aligned individual models, calculate the difference vector, and compute a point-wise shape deformity.

Response: Because the processes for the group-wise normalization and the deformity measurement are automated, we just described how to perform them in

our GUI software. We have added addition paragraph in the discussion section to describe the algorithm briefly:

“The individualized shape models are aligned in common space using the generalized Procrustes algorithm[19]. Here, we use the similarity transformation (isotropic scale, translation, and rotation) for the shape model normalization. The local shape differences are determined by the displacement vector between the corresponding vertices of the individual surface models and their mean shape model. The shape deformity at each vertex is computed as the signed Euclidean norm of the displacement vectors which are projected onto the vertex normal of the mean model. The detailed steps of the statistical shape analysis can be found in [5].”

I would like to thank the authors for their altruistic effort in publishing this protocol.

Response: We really appreciate the reviewer for the helpful and constructive comments and suggestions.

Reviewer #4:

Manuscript Summary:
The manuscript describes a semi-automatic brain structure analysis software. It uses MRI image segmentation and 3D shape analysis to create a validated method and tool.

Major Concerns:
No major concern.

Minor Concerns:

1. Manual segmentation protocols for the brain structures is used for pre-processing. The tools described in the references lists on line 99 only address brain shapes that are not altered. For instance, if the brain shape is altered due to stroke, the segmentation tools seem to cause error in the classification. Does your software work for mainly-intact brain?

Response: We validated our shape modeling software for Alzheimer’s disease, stroke, and normal aging. As the reviewer mentioned, automatic tools can fail to segment the boundaries of brain structures with neurological diseases. In such cases, the manual editing is required. Our software also supports manual editing functions, implanted in the MITK workbench.

Ref:

Kim, Jaeil, Maria del C. Valdes-Hernandez, Natalie A. Royle, and Jinah Park. "Hippocampal shape modeling based on a progressive template surface deformation and its verification." IEEE transactions on medical imaging 34, no. 6 (2014): 1242-1261.

Hernández, Maria del C. Valdés, Jaeil Kim, Ian Whiteford, Xinyi Qiu, Joanna M. Wardlaw, and Jinah Park. "Automatic Hippocampal Multimodal Assessment for Studies of Stroke and Small Vessel Disease." In MIUA, pp. 33-38. 2014.

Hernández, Maria del Carmen Valdés, Simon R. Cox, Jaeil Kim, Natalie A. Royle, Susana Muñoz Maniega, Alan J. Gow, Devasuda Anblagan et al. "Hippocampal morphology and cognitive functions in community-dwelling older people: the Lothian Birth Cohort 1936." Neurobiology of aging 52 (2017): 1-11.

2. There is quite a bit of manual editing involved. Is it possible to use some sort of machine learning to improve the segmentation process? How subjective is the manual editing? Have you done test-retest to measure variability?

Response: We strongly agree with the reviewer's comment. Machine learning techniques can improve the manual editing process and the automatic segmentation. ML-based annotation techniques, such as NVidia's Fast AI Assisted Annotation and Transfer Learning, could be solution for the issue.

Our manual editing steps for hippocampus are based on the manual segmentation procedure, introduced in [18]. In the study, the interrater error was about 4% for left and right hippocampi in volume.

3. Has this software been tested with MRI with different slice thickness? What is the minimum recommended? Please include the MRI properties needed for this software to work the best.

Response: We tested our algorithm with MR images of various slice thickness. We think the isotropic and small voxel sizes are better for representing the shape details of target structure. Note that the computation time of the shape modeling process is independent from the image resolution.

4. In step 5, when you compute the mean shape, does it matter what is included in the group mean? Should there be a lower and upper boundaries to prevent skewing of the shapes?

Response: Currently, our software does not consider outliners in the group mean computation. We implemented a progressive template deformation using Laplacian coordinates to minimize the distortion of the local geometry in the individual shape reconstruction. This method can prevent skewing in the shape models.

5. Does the shape analysis take into account the volume change?

Response: In the shape analysis, it is recommended to normalize the individual shape models with intracranial volume. In our approach, the individual models are normalized in common space using the similarity transformation (isotropic scale, translation, and rotation). We will add more options in our software to select different normalization approaches.

6. I find the discussion short and did not address the limitations based on the representative results.

Response: We added paragraphs on the limitations and more details of our method in the discussion section.

We really appreciate the reviewers for their valuable and constructive comments and suggestions.