

# Appendix:

## Laboratory Manual ACE-IBIS Infant Study Image Processing Pipelines

Update: October 2011

- **Image Processing Methods and Pipelines (UNC, Utah, Wash U)**
- **Guidelines for segmentation of anatomical structures (UNC)**
- **Geometric phantom calibration of structural MRI (MNI)**

<http://www.niral.unc.edu/>

<http://ibis-network.org/unc/mri/roiprotocols.htm>

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# Software and Tutorials:







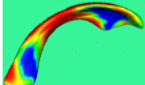


<http://www.niral.unc.edu/download-software>



Free software download

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## Currently Supported Software

	Download	Description
	<a href="#">Go to the ShapeWorks webpage</a>	ShapeWorks software is an open-source distribution (initially developed by the SGI Institute) of a new method for constructing compact statistical point-based models of ensembles of similar shapes that does not rely on any specific surface parameterization: <ul style="list-style-type: none"> <li>▸ Data preprocessing</li> <li>▸ Group-wise particle-based shape correspondence</li> <li>▸ Data visualization</li> </ul>
	<a href="#">Go to the DTIPrep webpage</a>	DTIPrep performs a "Study-specific Protocol" based automatic pipeline for DWI/DTI quality control and preparation: <ul style="list-style-type: none"> <li>▸ Image/diffusion information check</li> <li>▸ Interface-wise and gradient-wise intensity and motion check</li> <li>▸ Head motion and Eddy current artifact correction</li> <li>▸ DTI computation</li> </ul>
	<a href="#">Go to the AutoSeg webpage</a>	AutoSeg is an end-to-end application that performs automatic brain tissue classification and structural segmentation: <ul style="list-style-type: none"> <li>▸ Atlas-based EM tissue segmentation</li> <li>▸ Atlas-based subcortical structure segmentation and lobar parcellation</li> <li>▸ Regional histogram analysis</li> </ul>
	<a href="#">Go to the GAMBIT webpage</a>	GAMBIT is an end-to-end application allowing Group-wise Automatic Mesh-Based analysis of cortical Thickness as well as other surface area measurements: <ul style="list-style-type: none"> <li>▸ Voxel-based cortical thickness</li> <li>▸ White matter cortical surface creation and inflation</li> <li>▸ Group-wise shape correspondence using ShapeWorks</li> </ul>
	<a href="#">Go to the ARCTIC webpage</a>	ARCTIC (Automatic Regional Cortical Thickness) is a cross-platform end-to-end application allowing individual analysis of cortical thickness. It can be run within Slicer3 as an external module or directly as a command line: <ul style="list-style-type: none"> <li>▸ Brain tissue segmentation</li> <li>▸ Lobar cortical thickness analysis</li> <li>▸ White and gray matter mesh creation</li> </ul>
	<a href="#">UNC Cortical Thickness</a>	UNC Cortical Thickness Analysis based on ITK
	<a href="#">NITRC SPHARM PDM page</a> <a href="#">Local SPHARM shape analysis page</a>	The SPHARM shape analysis toolset is used for doing local statistical shape analysis. ITK based.
	<a href="#">Go to the FiberTracking webpage</a>	The FT tool is a cross-platform system to explore diffusion tensor images and to reconstruct fiber tracts: <ul style="list-style-type: none"> <li>▸ Platform independent</li> <li>▸ Fiber tracts between ROI</li> <li>▸ FA and ADC generation</li> </ul>
	<a href="#">Go to the DTIChecker webpage</a>	DTIChecker is a program to check and correct DTI images <ul style="list-style-type: none"> <li>▸ Slice artifacts detection</li> <li>▸ Motion artifacts detection and correction</li> <li>▸ Average creation</li> </ul>

## ABC: Atlas Based Classification of multichannel MRI

Detailed descriptions of ABC are available online at:

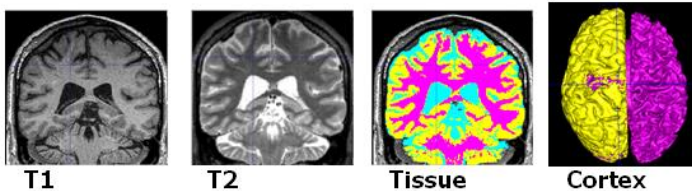
<http://www.na-mic.org/Wiki/index.php/Projects:UtahAtlasSegmentation>

Free download from the NITRC method's repository: <http://www.nitrc.org/projects/abc>

[3DSlicer11] 3DSlicer, A multi-platform, free and open source software package for visualization and medical image computing, download at <http://www.slicer.org/>

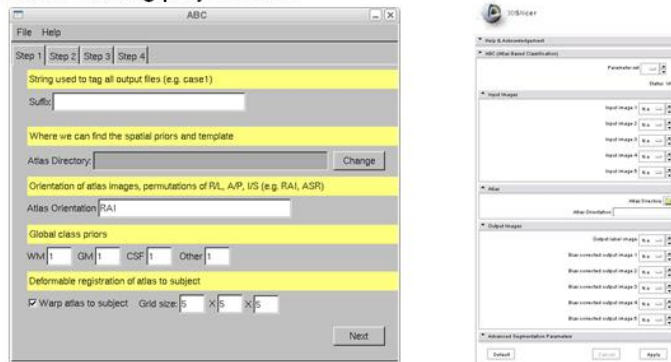
[Prastawa2005] Marcel Prastawa, John H. Gilmore, Weili Lin, Guido Gerig, Automatic Segmentation of MR Images of the Developing Newborn Brain, Medical Image Analysis (MedIA). Vol 9, October 2005, pages 457-466

### ABC: Atlas Based Classification



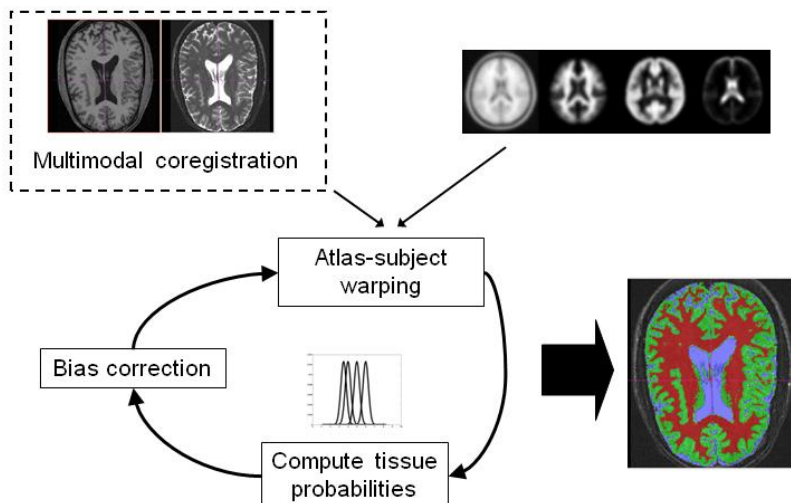
Available as stand-alone binary or Slicer plugin:

<http://www.nitrc.org/projects/abc>



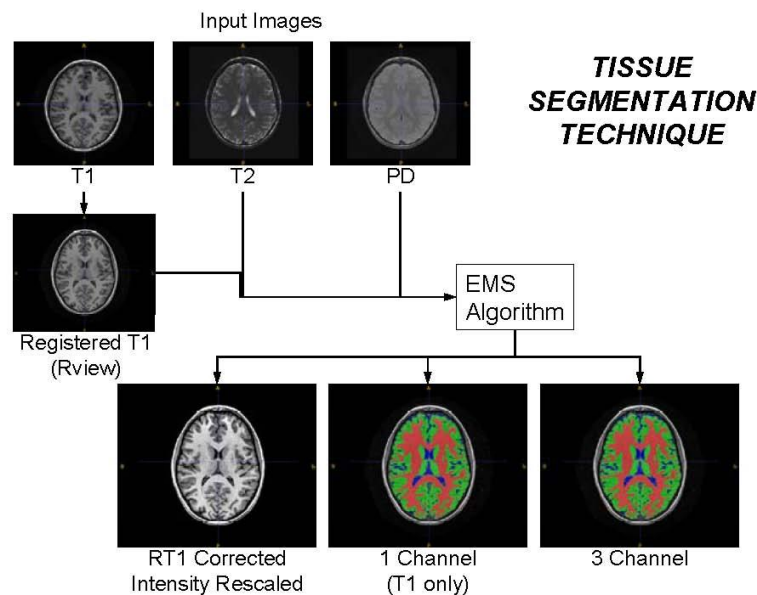
### ABC: Atlas Based Classification

Fully automatic segmentation pipeline



## Full Brain Tissue Segmentation

MRI data are analyzed in a multi-step process designed to facilitate morphometric and volumetric analysis. In a preprocessing step, the T1W data are registered to the ICBM probabilistic brain atlas so that all brains are analyzed and displayed in a common standard coordinate system. The registered T1W images are the basis for a multi-channel segmentation using an automatic, atlas-based brain tissue segmentation program (Expectation Maximization Segmentation or EMS) (Van Leemput, Maes et al. 1999) to separate brain tissue into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF). For the multi-channel segmentation, T2W data (and PD if available) are co-registered to the T1W data within the segmentation algorithm. A separate single channel segmentation using only the registered T1W image is also performed for additional processing purposes. Total brain gray and white matter, and CSF from the multi-channel segmentation are reported and used to calculate Inter-Cranial Volume (ICV = GM+WM+CSF).

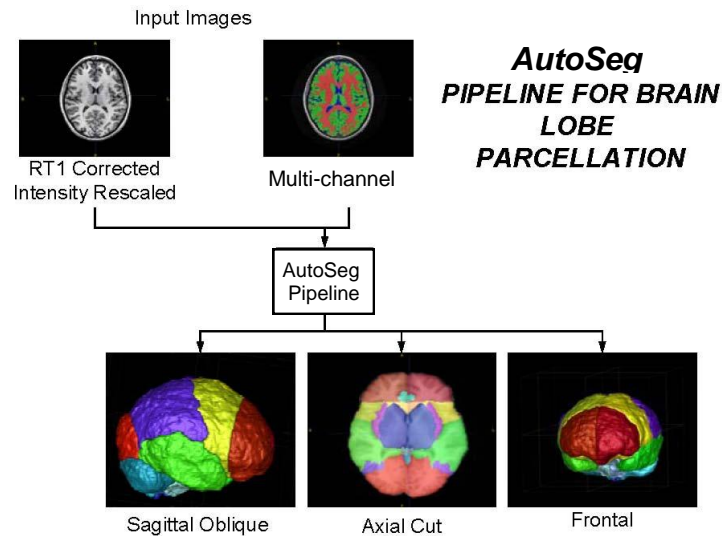


Further analysis seeks to identify and localize changes in brain tissue and sub-cortical structures. These analyses include a lobar parcellation pipeline and a sub-cortical segmentation routine. Both processes are highly automated and require minimal user intervention. Lateral ventricular volume is measured by a semi-automated level-set evolution method (Yushkevich et al., 2006).

The final piece of the analysis is a cortical thickness tool, which leverages the lobar parcellation to provide regional measurement of gray matter thickness. All data are then entered into a spreadsheet, by raters blind to subject identity and risk status.

## Automatic Lobar Parcellation

The registered and intensity-rescaled T1W image and the multi-channel files from the EMS segmentation are used in conjunction with parcellation atlas files as input for the AutoSeg tool's pipeline for high dimensional warping lobar brain parcellation. AutoSeg creates a unique parcellation map for each individual case that determines gray matter, white matter and CSF volumes for the regions defined on the parcellation atlas. Results can be written directly into a spreadsheet. The data reported are right and left volumes for the following regions and lobes: Parietal, Occipital, Temporal, Frontal, Prefrontal, Cerebellum, Corpus Callosum, Sub-cortical, Frontal and Parietal Cingulate, Insula, and Brainstem.

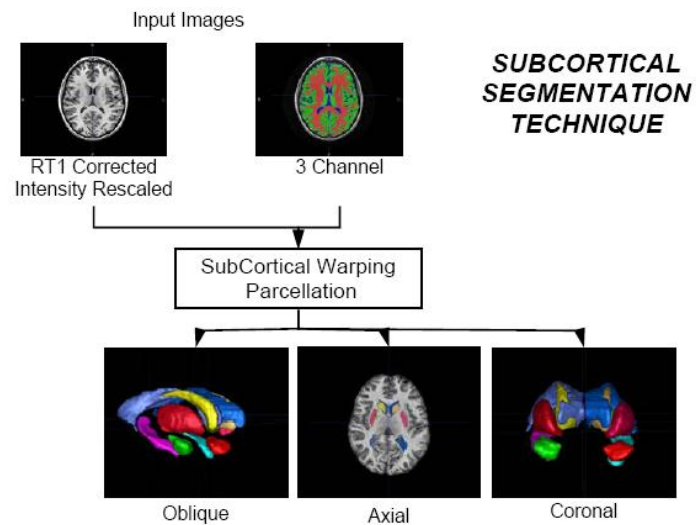


## Automatic Subcortical Segmentation

The automatic sub-cortical segmentation requires the registered and intensity-rescaled T1W image and the multi-channel segmentation files as input files. The segmentation routine works by computing an affine and a fluid transformation which maps an atlas (for which sub-cortical ROI's have been segmented) to an individual case.

The same transformations calculated for the atlas are then applied to the sub-cortical structures. The probabilistic ROI's are thresholded and the binary images collected into a single image file.

The sub-cortical segmentation algorithm automatically segments and determines volumes for right and left amygdala, caudate, hippocampus, lateral ventricle, globus pallidus and putamen.

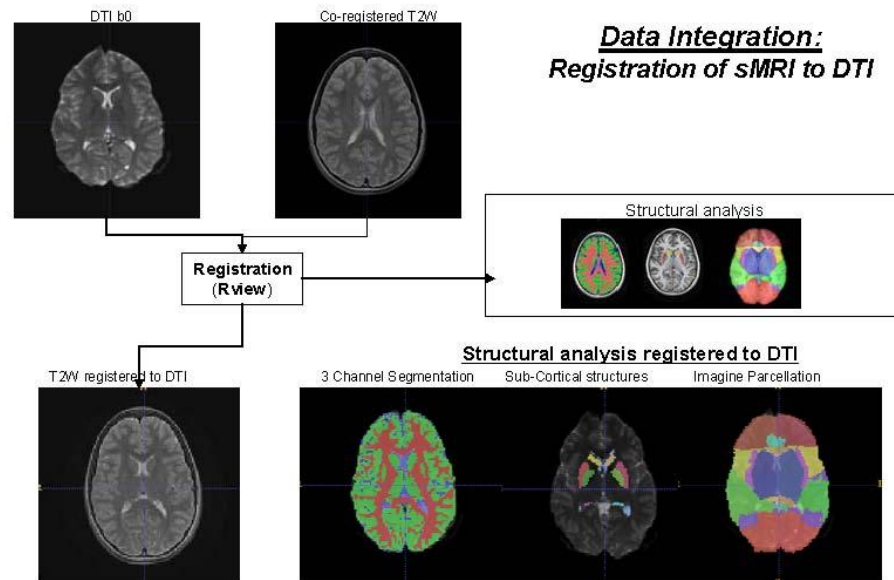


### Results: Volumes for Left and Right

Amygdala	Lateral Ventricle
Caudate	Globus Pallidus
Hippocampus	Putamen

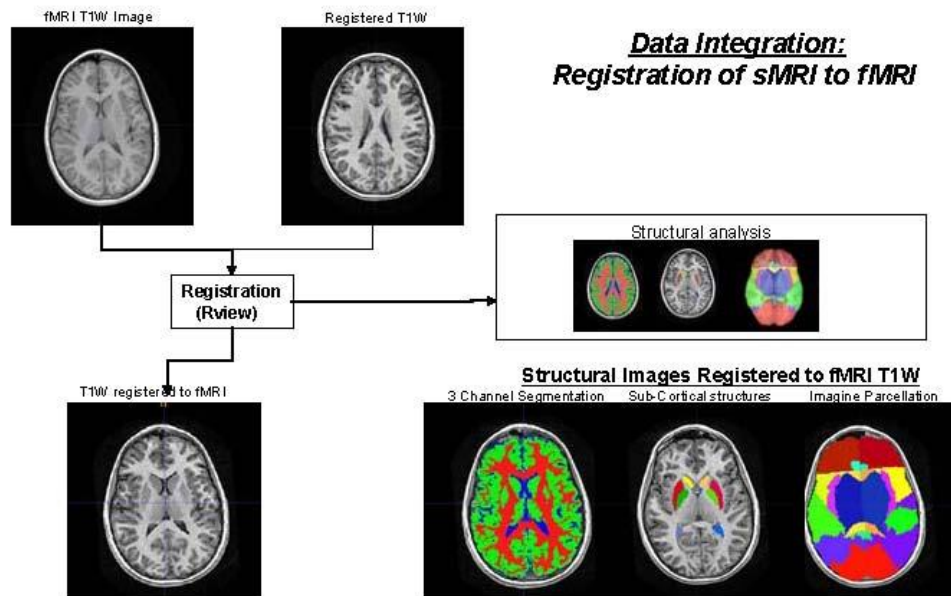
## Registration of sMRI Analysis to DTI

Registration of the sMRI gray level and segmentations to the Diffusion Tensor Image (DTI) coordinate space was accomplished by calculating a transformation that maps the registered T2W image to the DTI b0 image. This same transformation was then applied to the sub-cortical ROI's and multi-channel EMS segmentation. For the lobar parcellation, the registered stripped-brain T1W was registered to the DTI b0 image and that transformation used on the Imagine lobar parcellation.



## Registration of sMRI Analysis to the fMRI

Registration of the sMRI gray level and segmentations to the functional scan data (fMRI) coordinate space was accomplished by calculating a transformation that maps the structural registered T1W image to the fMRI T1W image. This functional T1 is a coarser resolution image than the structural but is acquired in the same coordinate space as the fMRI scans. The transformation was then applied to the structurally registered sub-cortical ROI's and multi-channel EMS segmentation. For the lobar parcellation, the registered stripped-brain T1W was registered to the fMRI T1 image and that transformation used on the lobar parcellation.



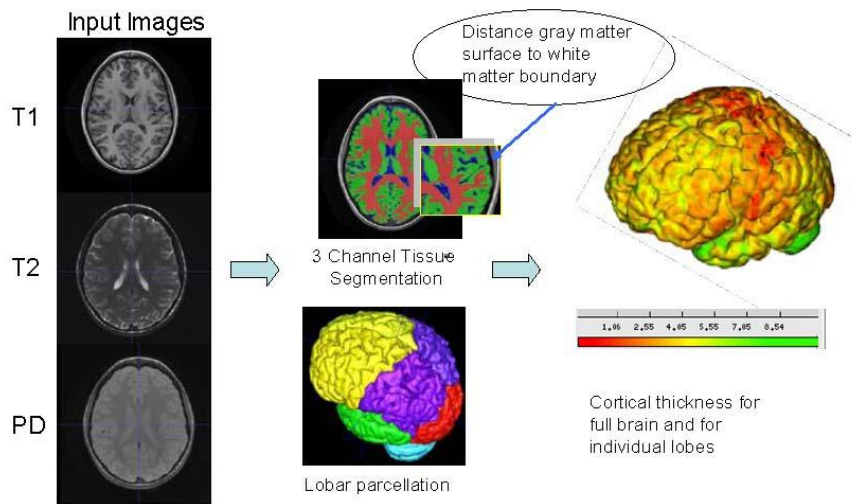


## Cortical Thickness Measurement

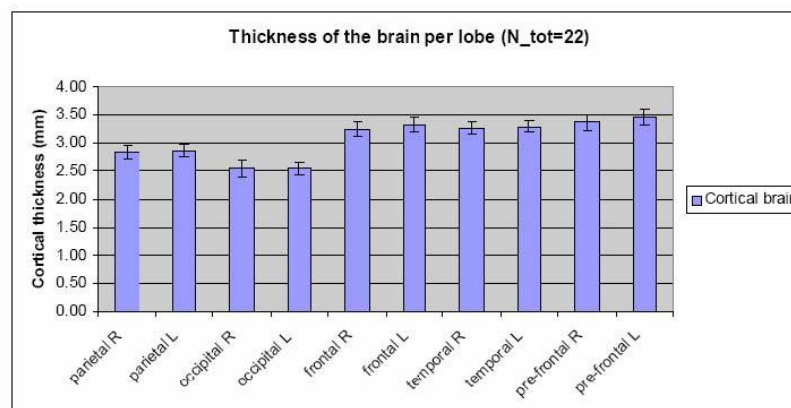
The cortical thickness tool is used for measuring the thickness of the brain gray matter. It uses the segmentation file and lobar parcellation produced by the AutoSeg tool to determine the average distance between the white matter and gray matter. The tool then reports the average cortical thickness in millimeters for each label of the parcellation file. These results can then be compared as displayed in the following chart.

A comprehensive tutorial with details about methodology and tool are found at:  
<http://www.ia.unc.edu/dev/tutorials/CorticalThickness/index.htm>

## Cortical Thickness Procedure



## Cortical Thickness per Lobe



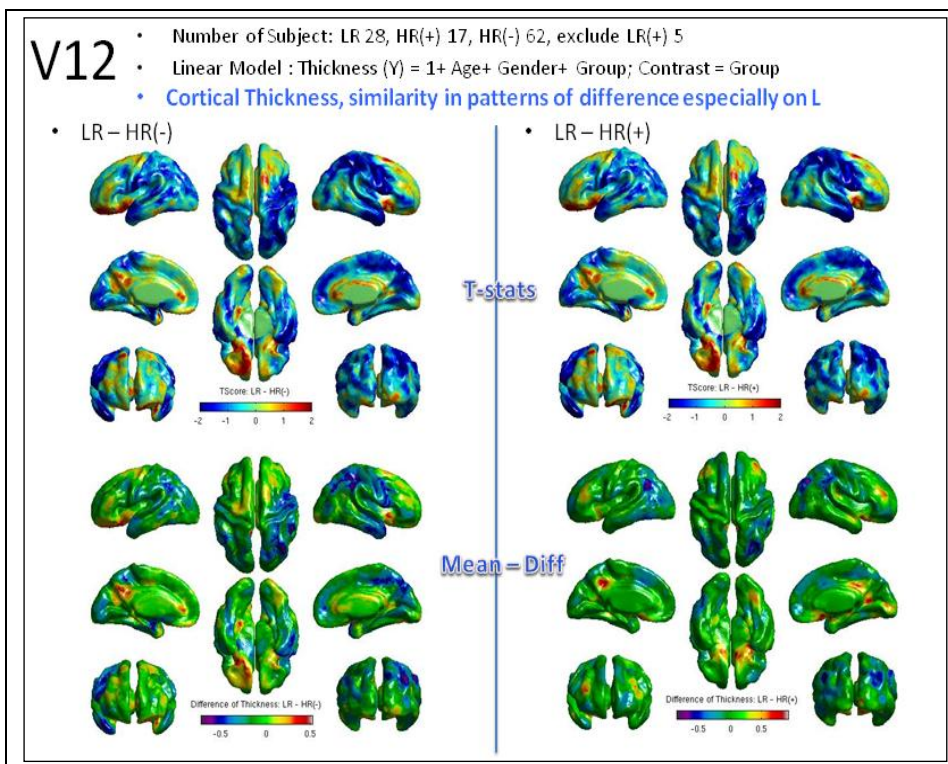
Cortical thickness across all age groups per lobe shows the pattern  
prefrontal  $\approx$  frontal  $\approx$  temporal  $>$  parietal  $>$  occipital

## Cortical Thickness Analysis with CIVIT UNC/MNI Collaboration

CIVIT workflow [Ducharme11, Shaw08, Shaw06] for cortical thickness and surface area analysis specifically adapted for this young age. In CIVIT, white matter surfaces are extracted via a shrink-wrap deformable surface evolution into the prior white matter tissue segmentation. This is then deformably extended to follow local laplacian distance and intensity fields to the gray matter surface to form the pial surface. These steps also determine measurements of local cortical thickness and surface area. The generated surfaces are overlaid on the gray-scale images to ensure anatomical correctness. The cortical surfaces are then mapped to a spherical domain for registration with a prior atlas using the cortical, sulcal features. This registration enables the extraction of corresponding cortical thickness and surface area measurements, which are analyzed using the SurfStat procedure developed by the Statistical Analysis Group.

- [Ducharme11] Ducharme S, Hudziak JJ, Botteron KN, Ganjavi H, Lepage C, Collins DL, Albaugh MD, Evans AC, Karama S, Brain Development Cooperative Group. Right Anterior Cingulate Cortical Thickness and Bilateral Striatal Volume Correlate with Child Behavior Checklist Aggressive Behavior Scores in Healthy Children. *Biol. Psychiatry* 2011 Apr.
- [Shaw06] Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, Gogtay N, Evans A, Rapoport J, Giedd J. Intellectual ability and cortical development in children and adolescents. *Nature* 2006 Mar.;440(7084):676–679.
- [Shaw08] Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP. Neurodevelopmental trajectories of the human cerebral cortex. *J Neurosci* 2008 Apr.;28(14):3586–3594.

The figure below shows application of the CIVIT pipeline to ACE-IBIS data of 12mo infants.





## What is ITK-SNAP?

Download: <http://www.itksnap.org/>

SNAP is a software application used to segment structures in 3D medical images. It provides semi-automatic segmentation using active contour methods, as well as manual delineation and image navigation. In addition to these core functions, SNAP provides a number of supporting utilities. Some of the core advantages of SNAP include

- Linked cursor for seamless 3D navigation
- Manual segmentation in three orthogonal planes at once
- Simplified user interface for selecting parameters for the active contours method
- Support for many different 3D image formats
- 3D cut-plane tool for fast post-processing of segmentation results
- Extensive tutorial

**Tutorial: Getting Started with ITK-SNAP** <http://www.itksnap.org/docs/viewtutorial.php>



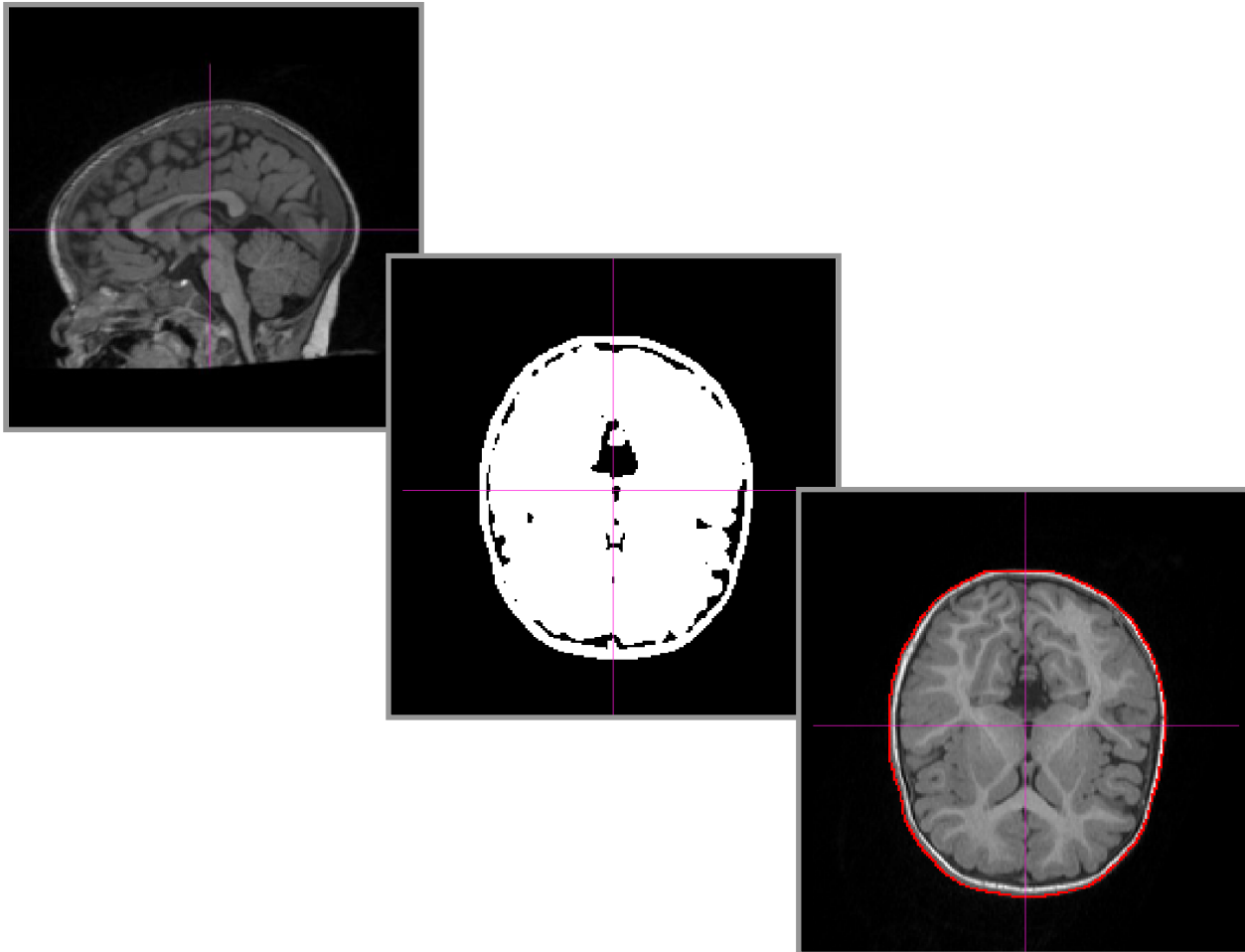
# Head Circumference



The Head Circumference program allows you to calculate the head circumference based on MRI data. Head Circumference uses fourier harmonics to parametrize the contour of the head and gives the length of this contour.

Download: <http://www.ia.unc.edu/dev/download/index.htm>

The figure below shows application of the Head Circumference Tool to ACE-IBIS data of a 12mo subject.



## CCSeg: Corpus Callosum Segmentation Tool

CCSeg allows a semi-automatic segmentation of the corpus callosum and provides volume information. The corpus callosum can be segmented in a large dataset via command line or scripted use of the tool. All results are visually reviewed, and can be manually corrected as necessary in the tool's graphical user interface.

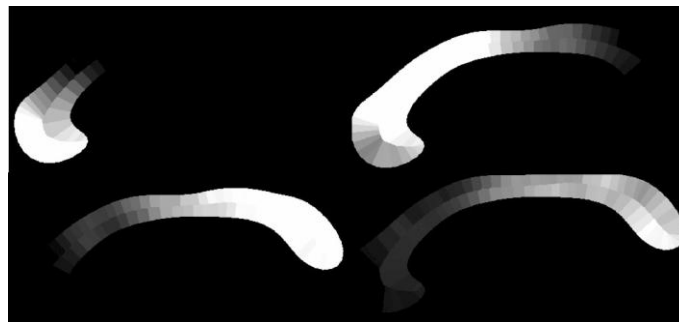
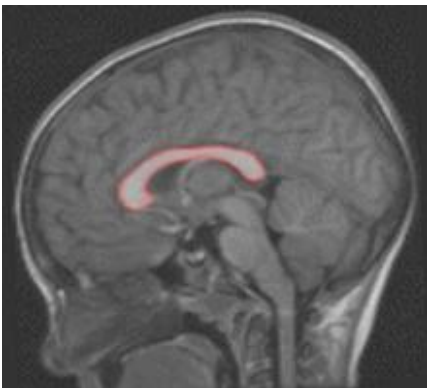
- Vachet, C., Yvernault, B., Bhatt, K., Smith, R.G., Gerig, G. Hazlett, H.C., Styner, M., Automatic corpus callosum segmentation using a deformable active Fourier contour model, to appear in Proc. SPIE 8317, 2012, in print

**Input:** CCSeg requires as input a corpus callosum atlas, 3D T1w image, and accompanying tissue segmentation label image.

**Output:** The primary output results from CCSeg include the final full corpus callosum segmentation, probability maps of the CC subregions, and area measurements of the full CC and its subregions. Other output include images and parameters used during computation.

**Software & Atlas download:** [http://www.nitrc.org/frs/?group\\_id=466](http://www.nitrc.org/frs/?group_id=466)

**Tutorial download:** [http://www.nitrc.org/docman/?group\\_id=466](http://www.nitrc.org/docman/?group_id=466)



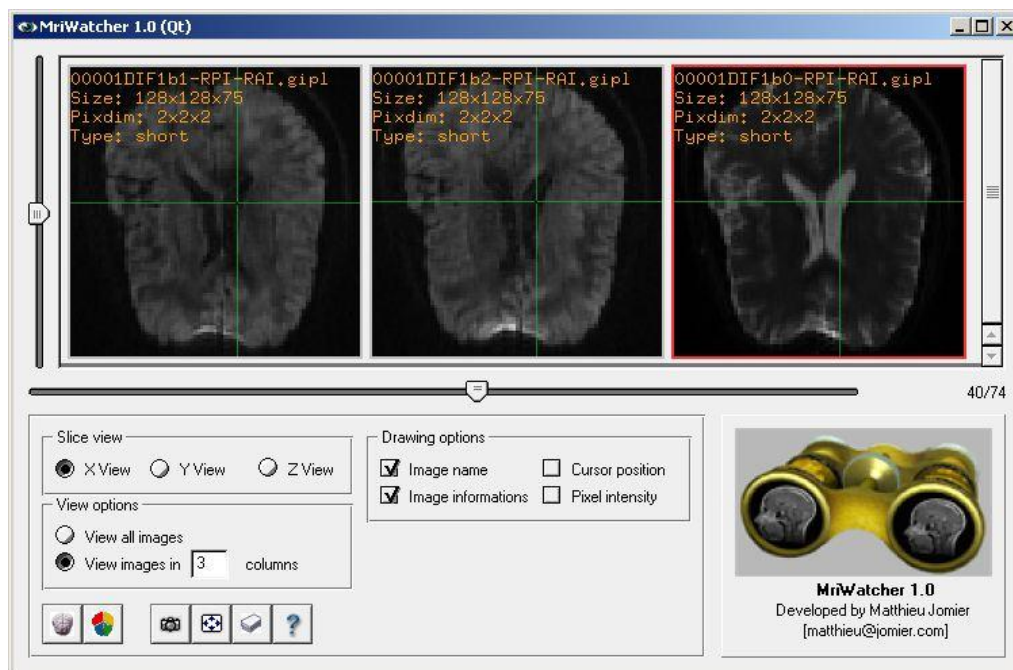


# MriWatcher



MriWatcher is a visualization tool for MRI images. It uses coupled cursors to show the differences between images. You can load an overlay images and save screenshots.

Download: <http://www.ia.unc.edu/dev/download/index.htm>

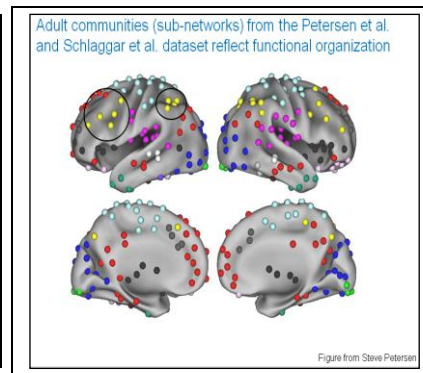
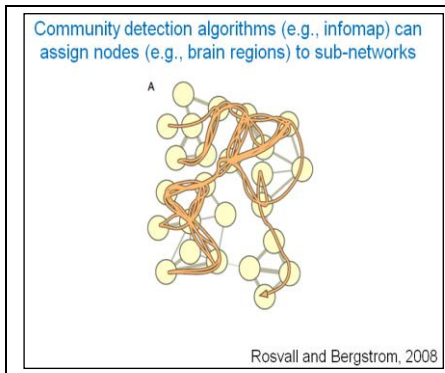


## Processing Pipeline fcMRI BOLD (Wash U, John Pruett)

A comprehensive processing pipeline to explore network behavior, community (sub-network) composition, and functional connectivity strengths applicable to infants has been installed at Wash U. the procedure includes the following processing steps:

- **Registration:** Registration of IBIS high-risk age-specific structural atlases to our fcMRI analysis space, allowing direct translation of fcMRI analyses to spatial coordinates for the IBIS Network's sMRI & DTI analyses. One of several current BOLD processing atlas registration sequences involves high-risk infant BOLD → high-risk infant T2 → ACE/IBIS high risk age-specific T2 target → collaborator Jeff Neil et al.'s term infant T2 target → our "711-2B" space.
- **BOLD qc measure:** Review of atlas fits by site and measures of BOLD signal standard deviation vs pre-processed BOLD run root-mean-square movement by site.
- **Preprocessing:**
  - Steps for basic fcMRI pre-processing include:
  - band-pass filtering ( $0.009 \text{ Hz} < f < 0.08 \text{ Hz}$ ), spatial smoothing, and regressing: 6 movement parameters, the whole brain signal, and ventricular and white matter signals (and their 1st derivatives).
  - rigorous BOLD motion "scrubbing" routine,
  - correlation of BOLD time course from each (meta-analytically defined) region with that for every other region (Pearson's  $r$ ), creating a square correlation matrix ( $229 \times 229$ ) for every scan (e.g., see [Dosenbach07]), resulting in 26,106 correlations between region pairs for each scan,
  - application of Fisher's z-transform to correlation values to ensure normality → these functional connections (Fisher's z-transformed  $r$ -values) are the matrix elements used for many of the following analyses.
- **Graph theory-based network analyses (Matlab tool):** Tool for graph theory-based metrics (small world metrics [Watts98]: clustering coefficients and path lengths; and assortativity [Newman02]) to test for potential differences in functional network efficiency and resiliency. Infomap [Roseval07, Roseval08] and Modularity Optimization [Newman06] will provide converging solutions to community assignments in these infants. To explore relationships between the fcMRI data and behavioral features and symptoms (e.g. ASD), we can correlate changes in functional connectivity (for each matrix cell) with continuous rating scale scores (e.g., from Autism Diagnostic Observation Schedule sub-scales, Mullen Scales of early Learning). This approach will associate patterns of functional connectivity within and between certain brain networks with graded measures of functioning across the normal range, the range of sub-threshold autistic symptoms, and through mild to severe levels of autistic dysfunction.
- **Predictions of ASD and dimensional measures of behavior from fcMRI patterns:** Complementing above approaches by Support Vector Machine (SVM)-based approaches for visualization of regions and connections that provide robust multivariate classifications of the groups [Ben-Hur08, Ecker10, Jaekel09]. SVM-related computations are carried out using the Spider Matlab Machine Learning Toolbox (<http://www.kyb.tuebingen.mpg.de/de/bs/people/spider>) implemented in MATLAB 7.8.0 (R2009a; The Mathworks, Natick, MA), as well as functions available as part of the MATLAB Bioinformatics, Curve-fitting, and Statistics Toolboxes and in-house MATLAB code.
- **SVM classifications is using**
  - "soft-margin" SVMs, as suggested [Hsu03], and linear, or nonlinear (which provide slightly greater accuracy in certain circumstances [Ben-Hur08]: namely radial basis kernels) decision functions.
  - Initial reduction of the number of features (in this case, the Fisher's z-transformed  $r$ -values) speeds up computation and improves performance. Therefore, we conduct an initial univariate feature-filtering, t-tests (two-sided, not assuming equal variance) compare every feature across the classes (e.g., ASD or no diagnosis), separately on the training set of each "leave one out cross-validation step where the test samples are excluded prior to t-testing, and the features are then separately ranked by their  $p$ -values in ascending order.

- The highest ranked features are retained and the rest are eliminated. For unequal sample numbers we compute area under the ROC (receiver operating characteristic) curve (AUC) instead of the standard accuracy.
- Statistical significance of results is assessed using the binomial probability distribution function in Matlab 7.8.0. (R2009a; The Mathworks, Natick, MA).



## Citations:

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- Newman, M.E.J., Assortative mixing in networks. Physical Review Letters, 2002. 89(20): p. 208701 %U <http://www.ncbi.nlm.nih.gov.beckerproxy.wustl.edu/pubmed/12443515>.
- Rosvall, M. and C.T. Bergstrom, An information-theoretic framework for resolving community structure in complex networks. Proceedings of the National Academy of Sciences of the United States of America, 2007. 104(18): p. 7327-7331 %U <http://www.ncbi.nlm.nih.gov.beckerproxy.wustl.edu/pubmed/17452639>.
- Rosvall, M. and C.T. Bergstrom, Maps of random walks on complex networks reveal community structure. Proceedings of the National Academy of Sciences of the United States of America, 2008. 105(4): p. 1118-1123 %U <http://www.ncbi.nlm.nih.gov.beckerproxy.wustl.edu/pubmed/18216267>.
- Watts, D.J. and S.H. Strogatz, Collective dynamics of 'small-world' networks. Nature, 1998. 393(6684): p. 440-2.

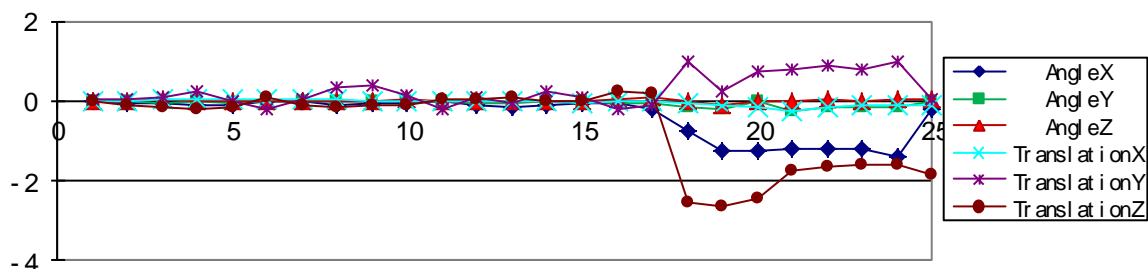


## DTIPrep: Tool for automatic quality control for DWI

Zhexing Liu, Yi Wang, Guido Gerig, Sylvain Gouttard, Ran Tao, Thomas Fletcher, Martin A. Styner, Quality control of diffusion weighted images, SPIE Medical Imaging, February 2010

DTIPrep performs the following functions on input DWI sequences:

- 1) **Image information checking:** In this step, common image information, such as dimension, sizes, origin, spacing, space and space directions is checked against the values in the DWI acquisition protocol.
- 2) **DWI cropping and/or padding:** Group analysis of DTI usually requires same image sizes. If image sizes are found to be mismatched, the user can choose to crop and/or pad the DWIs to the same size as defined in the protocol.
- 3) **Diffusion information checking:** Includes checking the b-value, diffusion sensitive directions and measurement frame. Due to the poor compliant situation of diffusion information storage in Dicom files, close attention should be paid to the diffusion related information.
- 4) **Slice-wise intensity related artifacts checking:** Computes the correlations between successive slices in each gradient and examines them at each slice position across all the diffusion gradients volumes. Large deviation from the mean of all the gradients means a dramatic intensity change or an intensity artifact. If the DWI is multiple b-valued, the correlations are fitted against the b-values using a quadratic model.
- 5) **Interlace-wise Venetian blind artifact checking:** Detects Venetian blind like artifacts via the correlations and motion parameters between the interleaved parts for each gradient volume. The correlations are examined across all the diffusion gradient volumes and are also fitted for multiple b-valued DWIs with a quadratic model. Motion parameters are found by rigid registration between the interleaved parts. User-selected translation thresholds are usually set relative to the corresponding voxel size and rotation thresholds to 0.5 degree.
- 6) **Baseline averaging:** State of the art DTI protocols usually use more than 6 diffusion sensitive directions. A non-diffusion weighted baseline image is advised to get acquired for every 6-8 gradients. Thus the resulting DWI scans consist of more than 1 baseline image. These baseline images are averaged in order to be used as a registration template during the eddy-current and head motion correction procedure. If there is motion between the baseline scans, the average is acquired in an iterative way by registering them to an optimized position.
- 7) **Eddy-current and head motion artifacts correction:** We integrate a tool developed by Ran Tao in University of Utah (<https://gforge.sci.utah.edu/gf/project/dwi-processing>). In this tool, all diffusion gradients are registered to the baseline using affine transformation. After the image transformation and re-sampling, the corresponding diffusion sensitive direction vector is updated with the transformation.
- 8) **Gradient-wise checking:** This is designed to detect the residual motion artifacts after eddy-current and head motion corrections.
- 9) **DTI estimation and computation:** DTI is estimated from the QC'd output of the previous steps and DTI indices are computed. The user can choose estimation via standard least squares, weighted least squares, non-linear least squares or maximum likelihood.



**Figure:** Motion plot for gradient-wise checking shows large motion occurred after gradient-17.



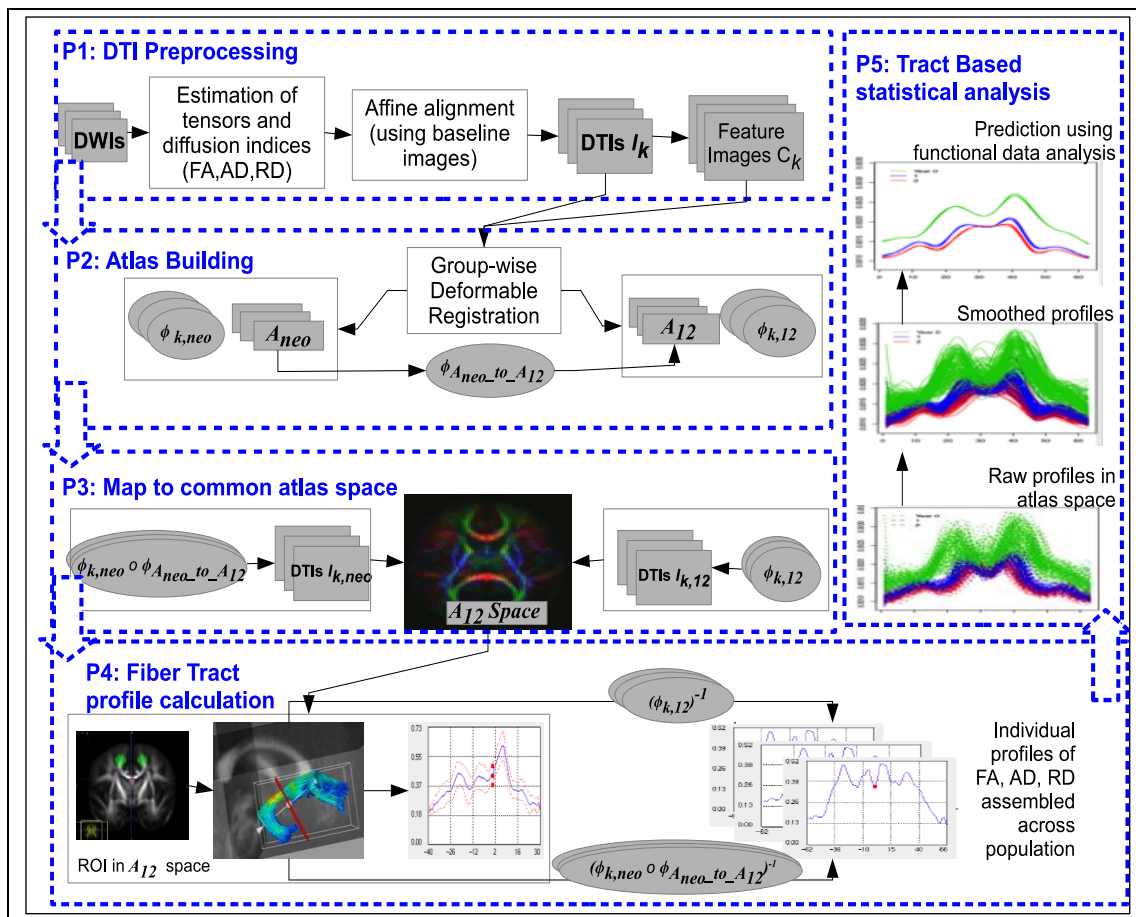
**Figure:** Example intensity artifacts detected with DTIPrep

## DTI: Processing Pipeline: Modules P1 to P5

Detailed description of methodologies for fiber tract parameterization and atlas-based group analysis:

Isabelle Corouge, P.Thomas Fletcher, Sarang Joshi, Sylvain Gouttard, Guido Gerig, Fiber Tract-Oriented Statistics for Quantitative Diffusion Tensor MRI Analysis, Medical Image Analysis 10 (2006), 786 - 798

Casey B. Goodlett, P. Thomas Fletcher, John H. Gilmore, Guido Gerig. Group Analysis of DTI Fiber Tract Statistics with Application to Neurodevelopment. NeuroImage 45 (1) Supp. 1, 2009. p. S133-S142



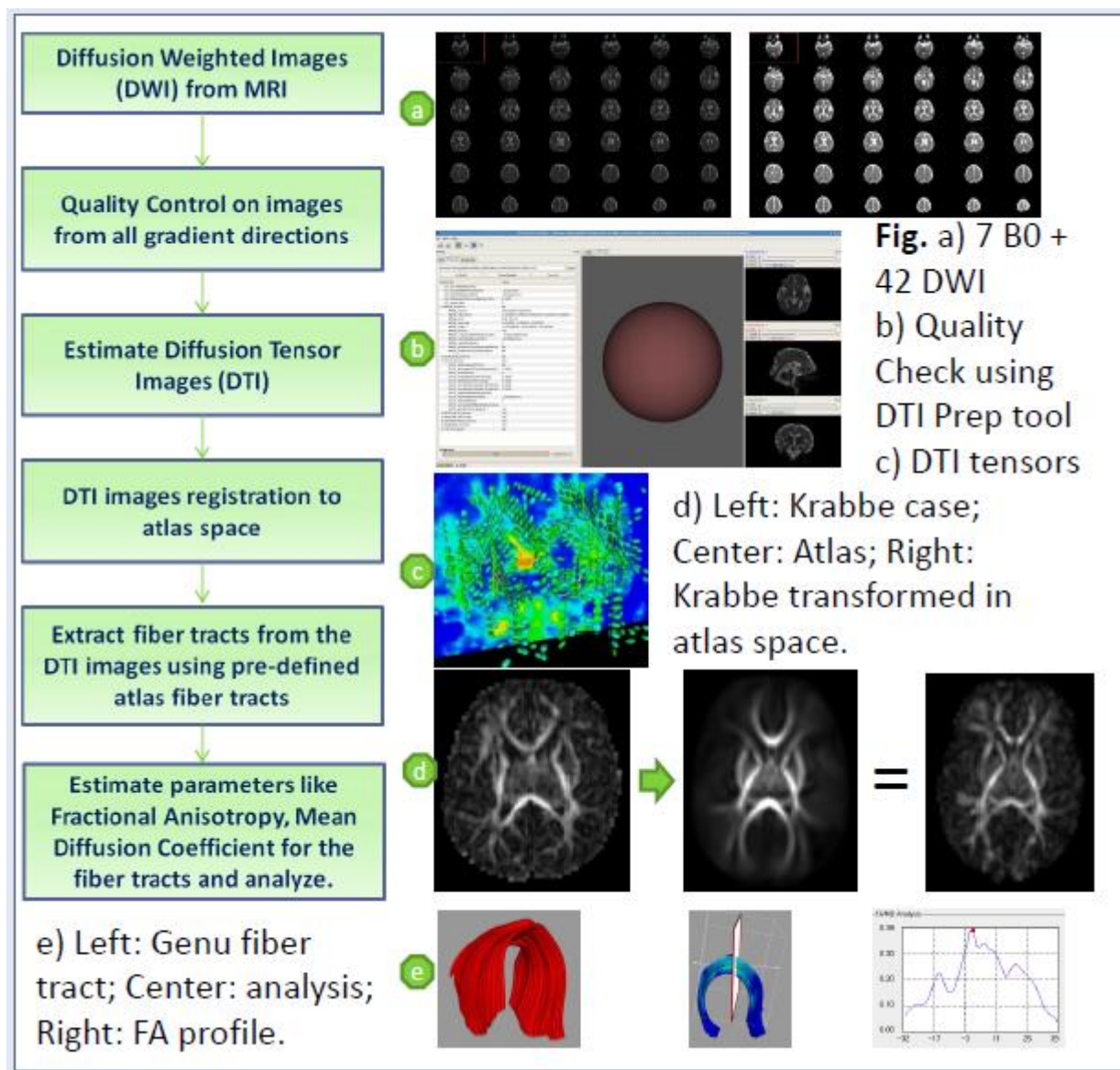
Flow chart of the DTI tract-based analysis. The first part is DTI preprocessing including DWI corrections, computation of DTI indices of FA, RD and AD. The second part covers atlas construction with an illustration graph of the steps including 1) construction of 1&2 year atlas, 2) construction of neonate atlas, and 3) transforming the neonate atlas to 1&2 year atlas for a final atlas. The third part shows procedures of tract profile calculation. The fourth part tells the tract-based analysis with figures showing raw tract profiles in atlas space and smoothed profiles

## DTI: Processing Pipeline

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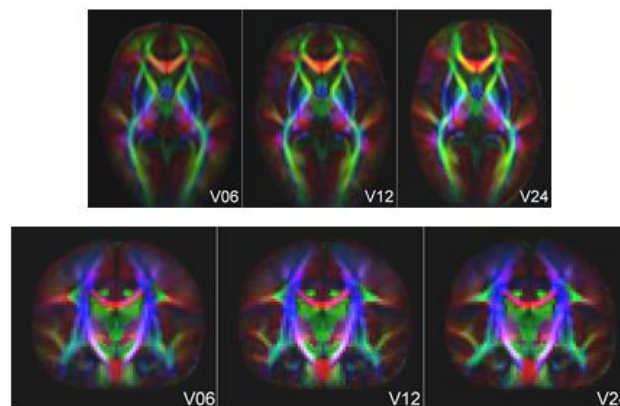
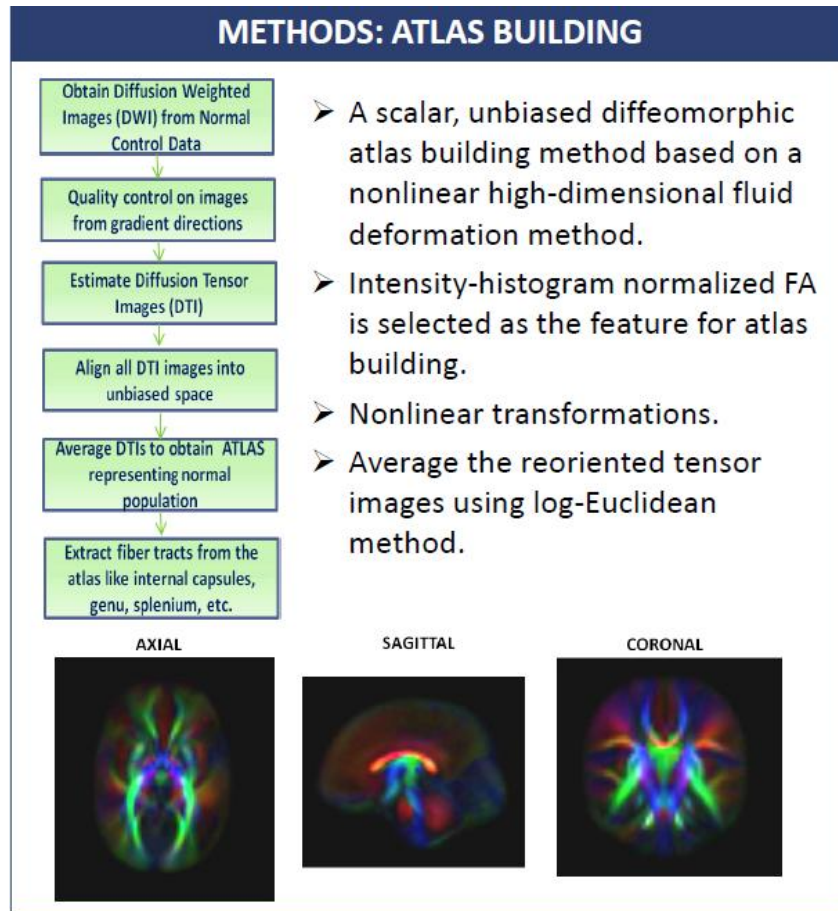
Casey B. Goodlett, P. Thomas Fletcher, John H. Gilmore, Guido Gerig. Group Analysis of DTI Fiber Tract Statistics with Application to Neurodevelopment. NeuroImage 45 (1) Supp. 1, 2009. p. S133-S142



## DTI: Building of unbiased atlas template (module 2)

Detailed description of methodologies atlas building:

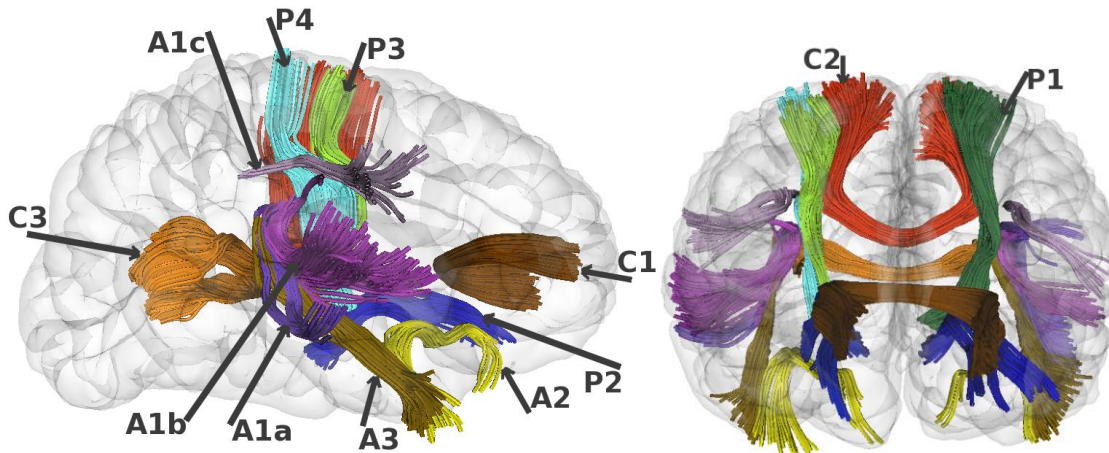
[Joshi2004] Joshi S, Davis B, Jomier M, Gerig G. Unbiased diffeomorphic atlas construction for computational anatomy. In: Thompson PM, Ratnanather T, Poldrack RA, Nichols TE, eds. *Supplement issue on mathematics in brain imaging*. Elsevier; 2004:S151-S160. Casey B. Goodlett, P. Thomas Fletcher, John H. Gilmore, Guido Gerig. Group Analysis of DTI Fiber Tract Statistics with Application to Neurodevelopment. *NeuroImage* 45 (1) Supp. 1, 2009. p. S133-S142



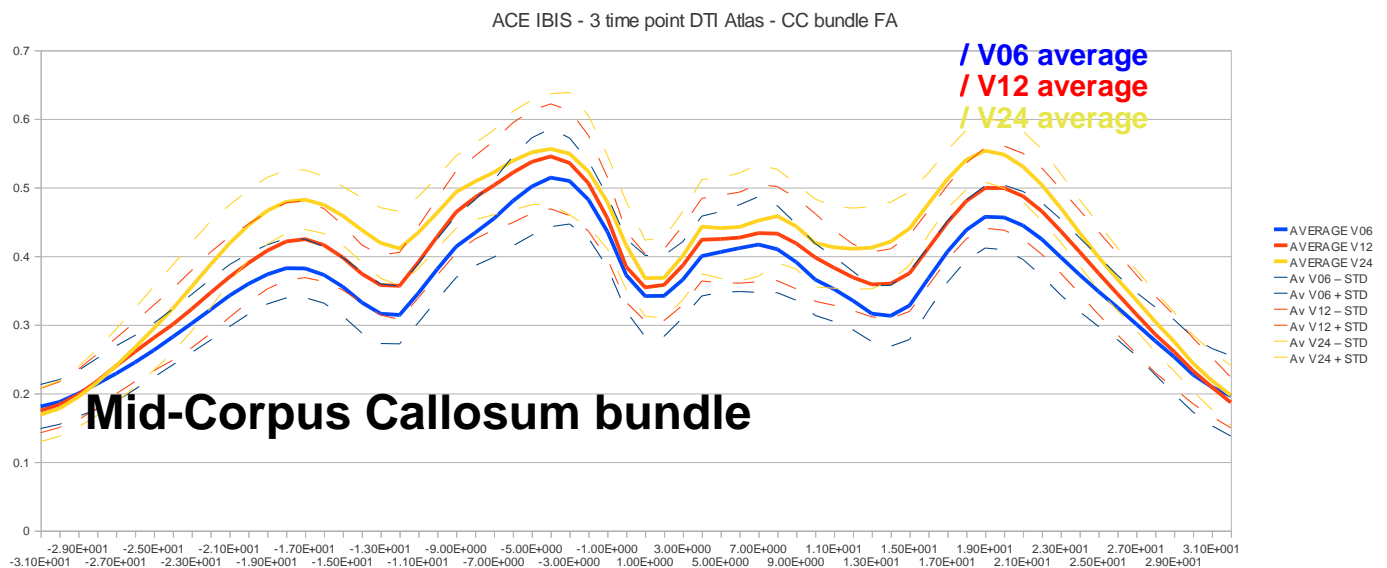
Color FA - Average of the 3 time points mapped in the atlas space



## DTI: Tractography and Analysis (modules 4,5)



3D visualization of 21 fiber tracts in sagittal and coronal views. C1, genu corpus callosum (CC); C2, body CC; C3, splenium CC. A1a, arcuate-inferior-temporal tract; A1b, arcuate-superior-temporal tract; A1c, arcuate-superior tract; A2, uncinate fasciculus; A3, inferior longitudinal fasciculus (ILF); P1, posterior limb internal capsule (PLIC); P2, anterior limb internal capsule (ALIC); P3, motor tract; P4 sensory tract.



Along tract plot of FA along mid-cc bundle, illustrating age change of tract diffusivity.

## UNC/UTAH Neuro Image Research and Analysis Lab MRI Region of Interest Definitions

The following pages contain excerpts of the manual tracing protocols from the UNC Neuro Image Research and Analysis Lab (NIRAL). . Traces guided by these manual tracing protocols were used in building many of the atlases which are now used in NIRAL studies. Automated segmentation of structures is performed using high-dimensional warping, results are then visually inspected for accuracy, and can be edited if necessary.

The NIRAL employs a variety of measurement protocols in our studies of the brain and its subregions. Measurement protocols are either adapted from existing protocols in use by other research groups, or are developed by our own group. Protocols developed by our own group are guided by methods described in the literature, available methods for similar measurements, classic brain atlases, and expert pediatric neuroradiologists. When adapting or developing protocols for measurements in our longitudinal MRI studies of brain development, our group is confronted with the challenges of pediatric brain images. Protocols from other research groups often require at least some adaptation to accommodate pediatric images; the level of adaptation required depends upon the complexity of the brain region of interest and the timeline of brain development.

Each Region of Interest protocol excerpt indicates major boundaries of the region, tool used for defining the region, approximate time required for completion of the protocol, and established rater reliability.

The complete protocols are available on the following website:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

### Processing Tools

Several tools are employed in the measurement of MRI data in the NIRAL, ranging from fully automated (e.g., ABC for tissue segmentation) to semi-automated (e.g., ITK-SNAP for lateral ventricle segmentation) to fully manual tools (e.g., ITK-SNAP for amygdala segmentation).

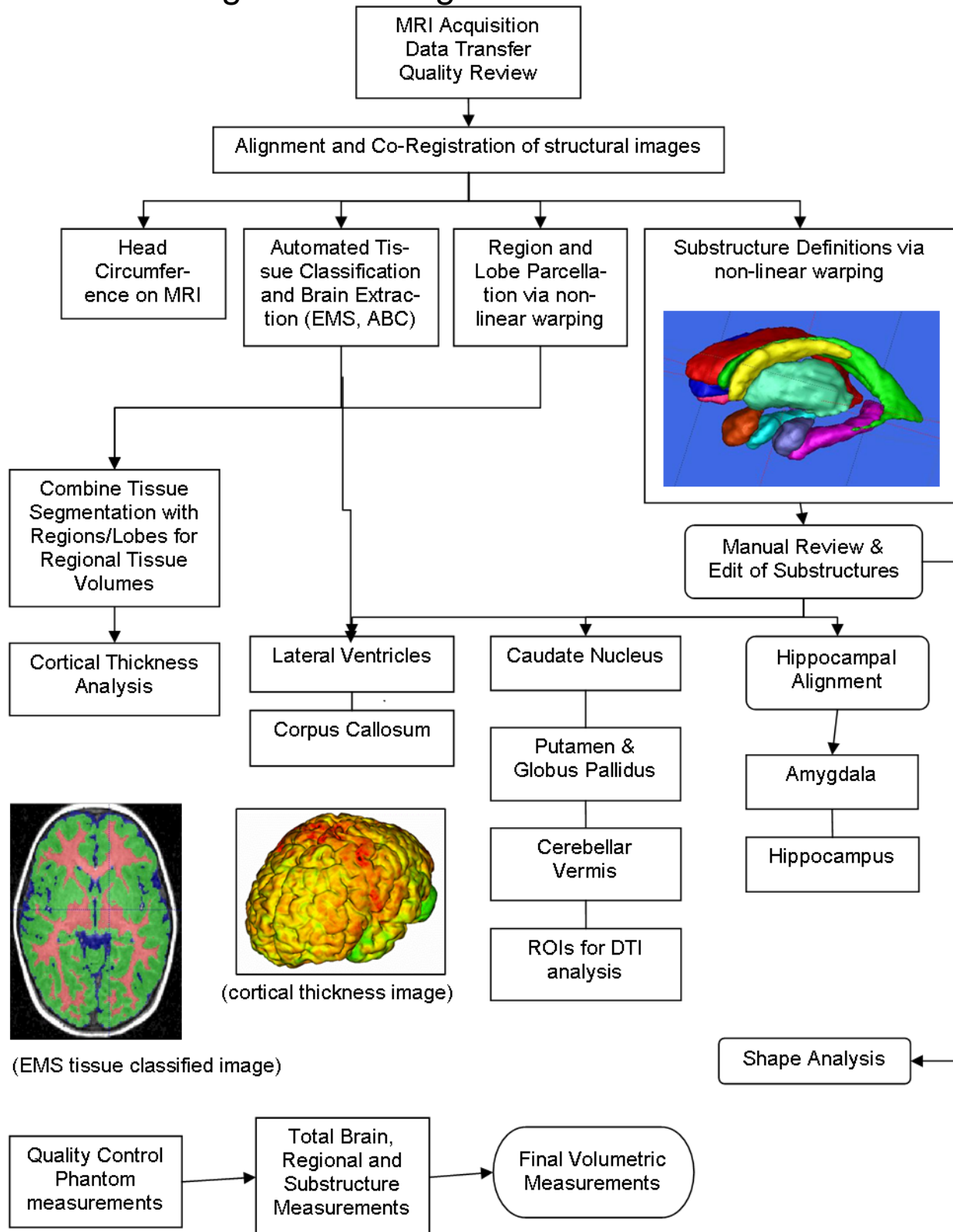
A core tool for user-supervised segmentation is ITK-SNAP, which allows manual tracing in all three planes with 2-D overlay and 3D visualization, as well as semi-automated 3D snake segmentation via 3D geodesic level sets. ITK-SNAP (or a previous version of SNAP) is the tool used for the majority of the methods employed in our current research.

Several image processing tools and accompanying tutorials are available on the following website:  
<http://www.ia.unc.edu/dev/download/index.htm>

### Reliability Procedures

The NIRAL takes great care in developing valid, reliable, and reproducible volumetric measurement protocols for various brain regions. Our standard procedure for establishing reliability is to have at least two raters perform the measurement protocol on a set of 15 images, which includes 5 images repeated three times in random order. Intra-rater reliability of greater than 0.90 is typically required for a rater to be deemed reliable. Requirements for inter-rater reliability vary based on difficulty and size of structure, but are typically greater than 0.90 for large structures or when two raters will be sharing the workload, or greater than 0.80 for smaller structures, or when only a single rater will be tracing the full data set. Our group always establishes rater reliability on pediatric brain images from own dataset.

# Image Processing Flow: Structural MRI



# Regional and Lobe Parcellation

Aim: Parcellate the pediatric brain into lobar regions in an efficient, reliable and anatomically meaningful manner.

Method Development: The lobar regions were defined, following anatomical and geometrical rules, on the same gray level average template used for tissue segmentation. Tracing of the lobar regions on the template image was performed in ITK-SNAP in a systematic order. The order of tracing allowed the boundaries of more easily defined regions to guide the more difficult regions.

Method Implementation: The parcellated template is applied to each subject image via non-linear high-dimensional fluid deformation, thus automatically labeling the lobar regions of the new images. The resulting warped parcellations are reviewed for accuracy. The parcellation for each subject is then combined with that subject's tissue classification image (from EMS, ABC or IGM) to obtain white matter, gray matter and cerebral-spinal fluid volumes for each lobe and region. Volumes for regional measurements, such as right and left cerebrum, are obtained by adding together multiple individual lobes.

The major anatomical boundaries are described below. The complete manual lobe parcellation protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

## 1) Cingulate Gyrus

The cingulate gyrus is bounded at its anterior and superior extents by the cingulate sulcus, at its inferior extent by the callosal sulcus and corpus callosum, and at its medial extent by the interhemispheric fissure. The lateral boundary is a straight line across the WM, connecting the depths of the cingulate and callosal sulci. The posterior boundary is the cingulate sulcus, where visible; and a line to corpus callosum or extent of the brain, where the cingulate sulcus is not visible.

## 2) Insular Cortex

The insular cortex consists of the gray matter in the depth of the Sylvian Fissure. The superior, inferior, anterior, and posterior boundaries are traced at the center of the insular sulcus. The lateral boundary is CSF within the Sylvian Fissure. The medial boundary is the lateral edge of the extreme or external capsule.

## 3) Corpus Callosum

The corpus callosum is bounded at its superior, anterior and posterior boundaries by the callosal sulcus and the cingulate gyrus trace. The lateral ventricle forms most of the inferior boundary. The lateral boundary is traced as a straight line between the callosal sulcus and the lateral-superior corner of the caudate nucleus or lateral ventricle.

## 4) Cerebellum

Surrounding CSF forms the posterior, inferior, and lateral boundaries of the cerebellum. The superior boundary is the inferior extent of the cerebrum. The boundary between the cerebellum and brainstem is the point where the cerebellar peduncles join with the brainstem. The boundary with the middle cerebellar peduncles is traced in the axial view by drawing a straight line between cranial nerve V and the notch of the fourth ventricle. Cerebellar peduncles are included with the cerebellum.

## 5) Subcortical Area

This region is traced for the purpose of exclusion from other regions. It is not intended to measure individual subcortical structures. The following areas are traced as part of the subcortical exclusion area: caudate, thalamus, putamen, globus pallidus, internal, external and extreme capsules, and ventricles within this region. In general, the trace follows the "external" boundaries the caudate, external or extreme capsule, and the thalamus. The superior and posterior boundaries are the callosal sulcus, and the anterior boundary is the extent of the caudate nucleus. The inferior boundary is the inferior extent of the thalamus and a horizontal cut plane separate the mid-brain from the superior extent of the pons. Where cutting across white matter at the



superior extent of the internal capsule, a straight line is drawn from the corner of the corpus callosum trace, to the corner of the putamen, and then across the external capsule to the insula trace.

6) Brainstem

The brainstem is separated from the subcortical area by a horizontal cut plane at the superior extent of the pons. The boundary of the brainstem with the cerebellum has already been determined by the cerebellum trace. The inferior extent of the brain is determined as one slice inferior to the inferior extent of the cerebellum.

7) Frontal Lobe

The frontal lobe is bounded at its superior, anterior and lateral extents by CSF at the surface of the brain. The medial boundary is the interhemispheric fissure, and existing traces of interior structures. The posterior boundary is the central sulcus. The inferior boundary is CSF in the anterior portions, then the Sylvian Fissure, and in the more posterior slices, a line drawn (in the coronal plane) across the central white matter between the central sulcus and the inferior extent of the corpus callosum. The complete frontal lobe can be separated into “frontal” and “prefrontal” (see below).

8) Prefrontal Lobe

The prefrontal lobe is separated from the frontal lobe by a vertical cut plane at the anterior extent of the corpus callosum. The prefrontal lobe consists of the frontal lobe portion that is anterior to the corpus callosum, while the “frontal” lobe consists of the portion between this cut plane and the central sulcus.

9) Occipital Lobe

The occipital lobe is bounded at its posterior and posterior-lateral extents by CSF at the surface of the brain. The cerebellum forms the inferior boundary. The medial-anterior boundary is formed by the parietal-occipital sulcus. The lateral-anterior/superior boundary is determined by a straight line drawn (in the coronal plane) across the white matter to the most lateral occipital gyrus. The medial occipital-temporal gyrus is included with the occipital lobe.

10) Temporal Lobe

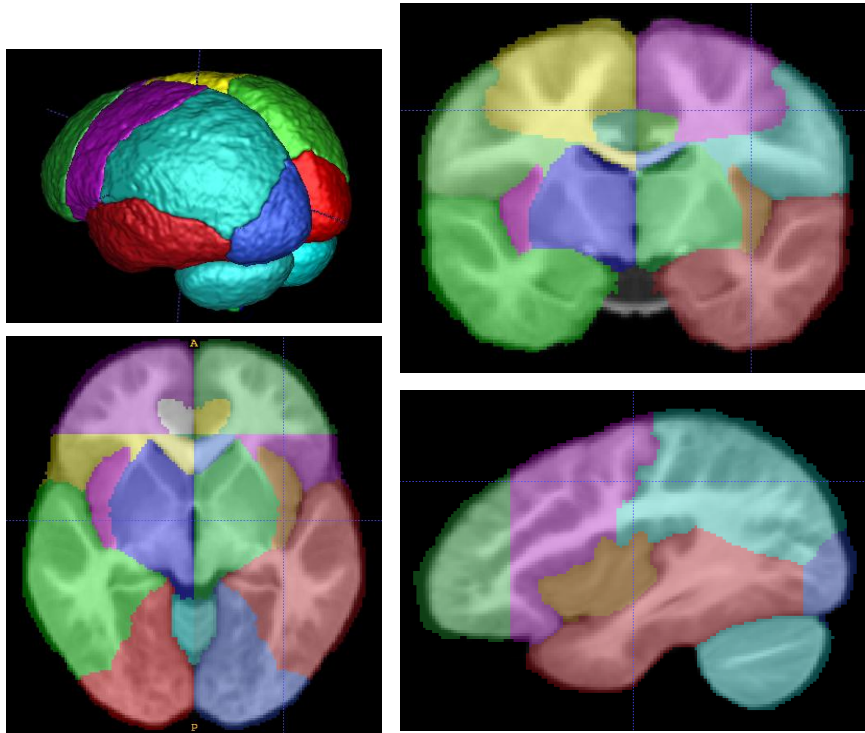
The temporal lobe is bounded at its anterior, lateral, and inferior extents by CSF at the surface of the brain. The Sylvian Fissure is the superior boundary. The medial boundary is formed by CSF, and the existing internal traces. The posterior-superior boundary is determined in the sagittal plane by drawing a line from the upper extent of the Sylvian Fissure, through the parallel sulcus (horizontal posterior segment) to the occipital lobe trace or surface of brain (in lateral slices). The lateral occipital-temporal and fusiform gyri are included with the temporal lobe.

11) Parietal Lobe

The parietal lobe consists of all remaining cortex and central white matter not already included with other lobes. The superior and lateral boundaries are CSF at the surface the brain. The anterior boundary is the central sulcus. The posterior boundary is the occipital lobe. The inferior boundary is determined by the existing traces of interior structures. The isthmus is included with the parietal lobe.

## References

Fukui et al., 2000 (cerebellum, subcortical, cortical lobes)  
IPL, University of Iowa (cortical lobes)  
Joshi et al., 1997; 2000 (fluid deformation)  
Kates et al., 1999 (cortical lobes)  
Rademacher, et al. 1992 (cingulate, insular cortex)  
SPNL, Stanford University (cingulate)  
Türe, et al. 1999 (insular cortex)  
Whalley et al., 1999 (temporal)



# Caudate Nucleus

Tool and Method: ITK-SNAP is a semi-automated 3D segmentation tool controlled by a user-defined threshold window, initialization, and region-growing parameters. The threshold and region-growing steps automatically find tissue boundaries and label the caudate (as identified by initialization). The output segmentation label can then be manually edited as necessary. Although the caudate does require some manual editing to exclude the nucleus accumbens, the ITK-SNAP method is much more reliable and efficient than a fully manual protocol.

Images and Required Time: The caudate nucleus is traced on high resolution T1 images in standard ACPC alignment. Time required to complete one case is approximately 30 minutes.

Established Reliability:

Average intra-rater = 0.97

Average inter-rater = 0.96

The key boundaries and methods are described below. The complete caudate nucleus tracing protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

Preparation & Sampling Steps

- 1) Trace sample regions in the caudate. These will be used to measure image statistics, which will guide the threshold values. They will also be used as initialization regions for the 3D segmentation snake.
  - a) Sample the caudate on five sagittal slices, with separate samples for each hemisphere.
  - b) Save the sample voxel counts and record the mean intensity for each hemisphere.

3D Snake Segmentation: Pre-processing & Initialization

- 2) Preprocess the image to apply a threshold window to the image, isolating the GM from surrounding tissues.
  - a) Select the In/Out snake and use the sample mean intensity to set the upper and lower bounds of the threshold window. The intensity range and smoothness value are standardized for all caudate segmentations within a study.
- 3) Initialize the snake with the sample you have already traced.

3D Snake Segmentation: Running the Snake

- 4) The Snake Control parameters are standardized for caudate segmentation all images.
- 5) Run the 3D snake segmentation. The 3D region-growing snake will automatically find the boundaries of the caudate on the threshold image.
  - a) Allow the snake to advance for a set number of iterations. Scroll through the image to see if the segmentation has sufficiently filled the caudate.
  - b) The caudate is considered sufficiently filled when the medial, superior, and lateral edges of the pre-processed threshold image are reached. The segmentation should also reach to at least two slices inferior to the most inferior axial slice on which the anterior commissure crosses the midline.
- 6) Repeat the above steps for the caudate in the opposing hemisphere.

Manually Editing the 3D Snake Segmentation

The values chosen for the snake parameters optimize segmentation of the caudate: the values maximize caudate inclusion, while minimizing leakage into connected gray matter (GM) structures. The 3D snake segmentation easily finds boundaries between GM and surrounding white matter (WM) and cerebral-spinal fluid (CSF), but there is no way for it to distinguish between connected GM structures, such as the caudate and nucleus accumbens. Therefore, certain errors are unavoidable, and these errors are manually edited.

To make the most of the increased reproducibility among raters and across time of the 3D region-growing segmentation (versus manual segmentation), minimal manual editing is done. Manual editing follows specific guidelines and is done only where the segmentation has gone excessively beyond the caudate. Key edits are described below.

#### Edits in the Axial Plane

- 1) Minimal or no editing may be required in the following regions. Review each region and edit only if the label deviates excessively from the boundary.
  - a) The medial-anterior edge of the caudate label. Due to partial voluming, the shared boundary of the corpus callosum (WM) and ventricle (CSF) appears to be about the same intensity as the caudate gray matter and is therefore sometimes incorrectly included in the caudate label.
  - b) The tail of caudate may require additional labeling.
  - c) The boundary between the thalamostriate vein and the WM just anterior to the thalamus. In T1 images, blood vessels are often very similar in intensity to GM. Due to this similarity in intensity, and the spatial proximity to the caudate, the thalamostriate vein is sometimes included in the caudate label.
  - d) The lateral edge of the caudate, where the label may have leaked across the internal capsule into the putamen. Remove the portion of the label extending past the center of the internal capsule.
- 2) The inferior extent of the internal capsule is a landmark used in the coronal view, in conjunction with the inferior extent of the lateral ventricle, to exclude the nucleus accumbens. This boundary is marked in the axial view by the following steps:
  - a) Go to the axial slice two slices inferior to the most inferior slice on which the AC connects across the midline.
  - b) Without changing the axial slice level, go to the coronal view, and beginning anterior to the caudate, page posteriorly until the putamen appears. Stop on the most anterior slice in which the putamen appears.
  - c) Without moving the crosshairs, return to the axial view. Delete all portions of the caudate label including and posterior to the horizontal crosshair (which marks your current coronal slice level).
  - d) Page inferiorly, deleting this posterior portion of the label, until you have moved all the way through the caudate label.

#### Edits in the Coronal Plane

- 3) In the first two or three coronal slices containing caudate label, remove isolated groups of label pixels on the medial side of the ventricle (only if they are obvious outliers).
- 4) In slices anterior to the putamen, significant regions of the caudate not labeled at its very inferior extent should be added.
- 5) When the putamen appears and CSF of the ventricle is visible, begin using a straight line cut to exclude the nucleus accumbens. On this slice, the inferior portion of the caudate label will be abruptly truncated from your the axial view edits. Draw a straight line connecting the lateral inferior point of the caudate label and the inferior point of the ventricle. All parts of the caudate label below this line should be erased.
  - a) Continue using the straight line cut to exclude the nucleus accumbens until the inferior extent of the caudate narrows significantly and the caudate and nucleus accumbens can be structurally distinguished.
  - b) From this point and posterior, use the structural boundaries of the caudate. The inferior boundary is the inferior extent of the narrow portion of the caudate.
- 6) Review the other boundaries of the caudate label. Do not edit anything on the lateral boundary (caudate boundary with internal capsule).

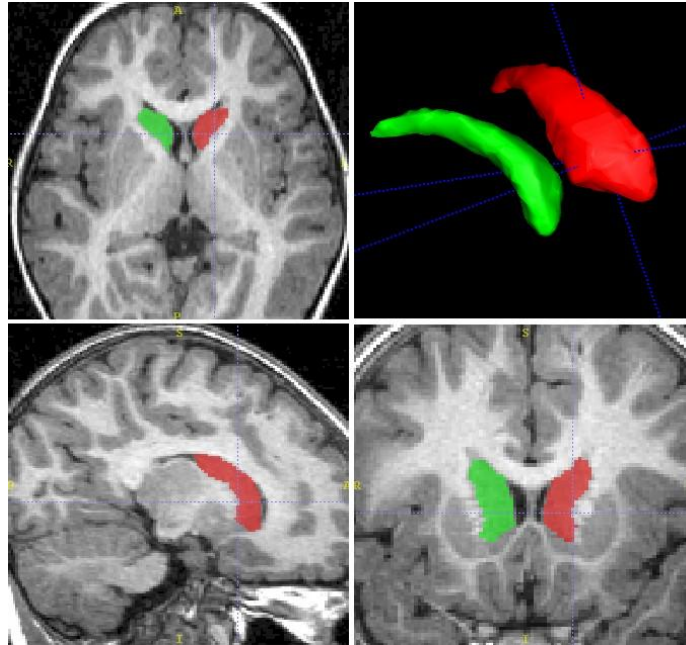
#### Edits in the Sagittal Plane

- 7) In the sagittal view, there will be an indented right angle in the label at the inferior extent of the caudate head, which is a result of tracing rules used to exclude the nucleus accumbens. To make the trace more anatomically appropriate, fill in this area by drawing a straight line connecting the two inferior points of the label where it forms the right angle.

Review the caudate label in all three views.

Reference

IPL, University of Iowa



# Putamen and Globus Pallidus

Tool and Method: The manual tracing features of ITK-SNAP are used to initially label the putamen and globus pallidus as a combined structure and then use designated landmarks to separate the two structures. This method takes advantage of the more reliable borders of the combined structure, while only needing to define the shared border once for both structures. Defining the border between the putamen and globus pallidus uses the “paint over” feature of ITK-SNAP, which allows the user to re-label an existing segmentation label.

Images and Required Time: The putamen and globus pallidus are traced on high resolution T1 images in standard ACPC alignment. Time required to complete one case, for both structures, is approximately two hours.

Established Reliability:

Average intra-rater: combined = 0.97, putamen = 0.97, globus pallidus = 0.93

Average inter-rater: combined = 0.97, putamen = 0.97, globus pallidus = 0.83

The key boundaries and methods are described below. The complete putamen and globus pallidus tracing protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

Tracing Methods

The major boundaries of the combined putamen and globus pallidus (GP) are the internal and external capsules. These boundaries are usually best seen the axial view. The more inferior boundaries are traced in the coronal view. When tracing around the level of the anterior commissure (AC), the user must follow distinct rules to maintain reliability. The separation of the putamen and GP is predominately accomplished in the coronal view.

Tracing the combined putamen and globus pallidus

- 1) Begin tracing in the axial view. In superior axial slices, the putamen is bounded medially, anteriorly, posteriorly by the internal capsule, and laterally by the external capsule.
- 2) When the GP appears and forms part of the putamen’s medial boundary, trace the combined putamen and GP structure.
  - a) The internal capsule forms the medial, anterior, and posterior boundaries, and the external capsule forms the lateral boundary.
  - b) If the portion of the external capsule along the putamen’s lateral border is not visible, trace a straight line between the visible anterior and posterior points of external capsule along the putamen’s lateral border.
- 3) The last axial slice traced should be one superior to the slice in which the AC extends laterally, partially separating the putamen and GP.
- 4) Switch to the coronal view. The superior portion of the structure is already labeled from the traces done in the axial view. The inferior portion will be labeled in the coronal view. The putamen and GP are bounded laterally by the external capsule, and medially and superiorly by the internal capsule.
- 5) Separate the nucleus accumbens from the putamen by tracing a vertical line from the most lateral, inferior point of the internal capsule to the most inferior point of the gray matter structure bounded by the external capsule. All gray matter lateral to this line is defined as putamen.
- 6) When the GP appears medial to the putamen, trace the combined structure. The inferior border of the GP marks the top level of the vertical line traced as the inferior medial border of the putamen.

- a) As tracing continues posteriorly, the internal capsule may not completely form the putamen and GP medial border. In this case, stop tracing at the most lateral edge of the internal capsule and draw a vertical line to the inferior boundary of the putamen and GP (do not trace GM directly inferior to the internal capsule).
- 7) When the AC appears, it defines the inferior boundary of the GP and the inferior medial boundary of the putamen. Use the following rules to exclude non putamen/GP gray matter structures inferior to the AC:
  - a) All gray matter directly inferior to the AC is excluded. Trace a vertical line adjacent to the lateral edge of the AC, stopping at the inferior boundary of the putamen/GP, if visible. If not visible, stop level with the inferior boundary of the AC.
  - b) If the AC is not visible, trace a straight line between the most medial point of the external capsule or temporal stem and the most lateral point of the internal capsule.
  - c) Moving posteriorly, the AC concentrates in size and slides under the putamen and GP, medial to lateral. Trace a vertical line medial to the AC, stopping level with the inferior boundary of the AC (thus “cutting out” the AC).
  - d) When distinct intensity differences are not visible, the anterior perforated substance is excluded by tracing a straight line from the inferior medial corner of the AC to the lateral inferior extent of the internal capsule. To exclude the amygdala and other non putamen/GP gray matter, continue tracing this straight line, but no lower than horizontal.
- 8) As the AC disappears, the temporal stem defines the inferior lateral boundary of the putamen and globus pallidus. Trace the straight line (no lower than horizontal) forming the inferior medial boundary from the internal capsule to the superior medial corner of the temporal stem. More posteriorly, the inferior boundary can be structurally identified, and this line is no longer needed.
- 9) Be sure to trace all of the putamen as it extends inferiorly toward the temporal lobe.
- 10) Review the segmentation label in all views.

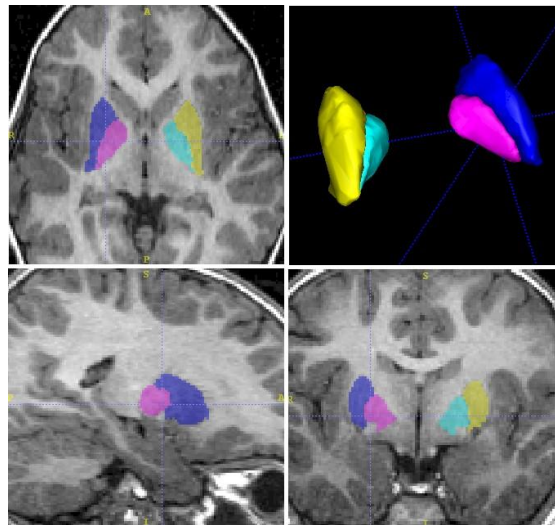
#### Tracing the border between the putamen and globus pallidus

- 1) Perform all traces separating the putamen and globus pallidus in the coronal view. No changes will be made to the external boundaries of the combined structure, which have already been traced.
  - a) Set ITK-SNAP to “paint over” the existing label with a new GP label. The boundary between the putamen and GP should be traced precisely, but the rest of the trace simply needs to encompass the existing label.
- 2) Begin anterior to the putamen and globus pallidus. When the GP appears, there will be an abrupt shape change in the medial border of the combined structure label, and a notch in the internal capsule at the superior end of the putamen/GP border on the T1 image. This notch will be used in most slices as the landmark for the superior end of the putamen/GP border.
  - a) In these anterior slices, the landmark for the inferior end of the putamen/GP border is the where the most inferior point of the internal capsule meets the putamen/GP label medial border.
  - b) Trace the border between the putamen and GP, following the intensity difference between the two structures, and approximating a circular arc between the superior and inferior landmarks.
- 3) When the AC appears, the combined structure label will form a right angle with the GP superior to the AC, and the putamen lateral to the AC. The landmark for the inferior end of putamen/GP border is the superior lateral corner of the AC. As the AC concentrates in size and slides under the label, continue tracing all regions lateral to the AC as putamen, and medial to the AC is GP.
- 4) In posterior slices, as the AC merges into the temporal stem, the putamen envelops the posterior of the GP. The putamen/GP border is defined as follows:
  - a) Use the intensity difference between the putamen and GP, if visible.

- b) When there is not a visible intensity difference, the inferior landmark for the putamen/GP border is the right angle juncture of the combined label and the most medial superior edge of the AC.
  - c) The arc shape of the putamen/GP border soon becomes a straight line.
  - d) Check the border between the putamen and GP in the axial view; all regions anterior the AC are putamen, and all regions posterior to the AC are GP.
- 5) When the AC disappears, the inferior landmark for the putamen/GP border is use the most medial point of gray matter that extends between the two branches of white matter into the temporal lobe.
  - 6) The last slice on which to trace in the coronal view is the most posterior slice on which the label extends into the temporal stem.
  - 7) In the sagittal view, trace as GP any remaining combined label region directly posterior to the region already labeled as GP.
  - 8) Review the putamen and globus pallidus labels in all views.

#### Reference

IPL, University of Iowa





# Amygdala

Tool and Method: Images are first aligned along the long axis of the hippocampus. The manual tracing features of ITK-SNAP are used to label the amygdala. This tracing protocol was originally developed by the Center for Neuroscience and the M.I.N.D. at University of California at Davis.

Images and Required Time: The amygdala is traced on high resolution T1 images aligned along the hippocampus. Time required to complete one case is approximately one and one half to two hours.

Established Reliability:

Average intra-rater = 0.90

Average inter-rater = 0.78

Average inter-site vs. U.C. Davis (adult images) = 0.92

The key boundaries and methods are described below. The complete amygdala tracing protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

Tracing Overview:

Trace the amygdala in the coronal plane. Use the axial plane to review traces and exclude the putamen. Use the sagittal plane to review traces and define the rostral extent of the amygdala.

Tracing in the Coronal Plane

- 1) The caudal section of the amygdala appears dorsal to the inferior horn of the lateral ventricle and hippocampus, and lateral to the optic tract. Begin tracing the amygdala from the dorso-lateral extent of the optic tract, and continue tracing the ventral surface of the amygdala along the ventricle, then amygdala-hippocampal transition area, medial surface of the brain, to the starting point at the dorso-lateral extent of the optic tract.
  - a) In caudal slices, the putamen forms the lateral border of the amygdala. If this border is seen, trace a line from the dorsolateral extent of the optic tract directly lateral to the amygdala/putamen border. If this border is not seen, extend the horizontal line laterally to the white matter.
    - i. Follow either the putamen/amygdala border, along the white matter, or (if necessary) a line directly ventral until the lateral ventricle is reached.
  - b) In caudal slices, the ventral border of the amygdala is formed by the lateral ventricle, then more rostrally by the hippocampus.
    - ii. The ventro-medial portion of the amygdala extends just ventral to the optic tract.
    - iii. More ventral, this border is formed by the amygdala-hippocampal transition area (excluded from the amygdala). If this border is ambiguous, define the boundary by tracing a line perpendicular to the optic tract.
    - iv. More dorsal, the border is formed by the medial surface of the brain.
- 2) When the medial surface of the brain extends further lateral than the optic tract, use the dorso-lateral extent of the medial surface as the dorsal border of the amygdala. Draw a horizontal line laterally from this point to the white matter. The putamen is no longer present and the lateral border is formed by white matter. Follow the curve of the white matter to the lateral ventricle.
- 3) Farther rostral, the hippocampus forms the ventral border of the amygdala, divided by a thin section of white matter (alveus). The amygdala-hippocampal transition area will no longer be present, and the ventral border of the amygdala will be fairly horizontal (from the lateral border to the medial surface of the brain at the semiannular sulcus).
- 4) Farther rostral, the hippocampus recedes medially and the entorhinal cortex begins to form the dorso-medial border of the amygdala. The most dorsomedial point of the amygdala is at the semiannular sulcus on the medial surface of the brain.

- a) White matter separates the amygdala from entorhinal cortex medially. If this is difficult to see, trace a straight line dorso-medially from the most medial point of the white matter (ventral to the amygdala) to the semiannular sulcus.
- 5) As the hippocampus and lateral ventricle disappear, white matter forms the dorsal, lateral, and ventral borders. The medial border is formed by the entorhinal cortex and the dorsomedial border is formed by the medial surface of the brain.
- 6) Farther rostral, the medial surface of the brain extends farther lateral, separating the temporal lobe from the rest of the brain. At this point, the dorsal border of the amygdala is defined by the surface of the brain.
- 7) Continue tracing the amygdala until it is indistinguishable.

#### Editing in the Horizontal (transverse) Plane

- 8) In dorsal slices, the putamen is an elongated tail extended caudally from the caudal portion of the amygdala. To exclude the putamen, follow the white matter along the lateral border of the amygdala as it extends caudally. Trace a straight line (medial-caudal diagonal) through the putamen, to the white matter on the medial side of the putamen just lateral to the thalamus. Farther ventral, trace to the medial surface of the brain.

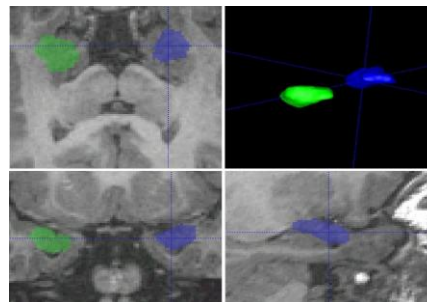
#### Editing in the Sagittal Plane

- 9) The rostral extent of the amygdala is defined by following the natural curvature of the ventral surface along the white matter as it extends rostrally. If it is not possible to follow the white matter to the surface of the brain, follow the natural curvature from the most rostral tip of the white matter to the medial surface.
- 10) Check the amygdala-hippocampal border. The border appears as a diagonal line from the dorso-caudal tip of the amygdala to the ventro-rostral tip of the hippocampus.

Review amygdala label again in the coronal plane.

#### References

Schumann et al., 2004  
UC Davis Center for Neuroscience



# Hippocampus

Tool and Method: The semi-automated MOJO tool is used for landmarking and segmenting the hippocampus via high-dimensional deformation of a template. The MOJO tool guides the user through the process of landmarking, calculating the deformed template, and calculating the volume of the hippocampus.

Images and Required Time: The T1 gray level image, unaligned and in Analyze format, is the input to the MOJO tool. Approximate time to complete one case is 45 minutes to one hour.

Established Reliability:

Average intra-rater = 0.95

Average inter-rater = 0.81

The key boundaries and methods are described below. The complete hippocampus segmentation protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

- 1) After loading the T1 image into MOJO, standardize the image intensity using the LevelWidthAdj command.
  - a) Calculate a white matter intensity average by clicking in the anterior, middle, and posterior of the corpus callosum and averaging the three values.
  - b) Calculate a gray matter intensity average by clicking in the anterior, middle, and posterior of the caudate and averaging the three values.
  - c) Use the command line tool LevelWidthAdj, with the gray matter and white matter averages as input, to calculate values for “Level” and “Width.” Enter the output values into the user interface scroll bars to adjust the intensity of the image, and then save the intensity-adjusted image.
- 2) Set the reference points for the anterior commissure (AC) and posterior commissure (PC), and set the boundary box limits at the periphery of the brain. Save the aligned image.
- 3) The right and left hippocampus are segmented one at a time, using the same rules for setting the landmarks. Landmarks will first be set at the head and tail of the hippocampus. Landmarks for superior, inferior, lateral and medial borders will then be defined on each of five cross sections of the hippocampus, between the head and tail landmarks.
  - a) Roughly set the head and tail landmarks, and then select the H-T axial button. The oblique view shows the image aligned along the long axis of the hippocampus.
  - b) Refine the head and tail landmarks, and then proceed with the landmarks on the five slices.
- 4) Setting the Tail landmarks:
  - a) In the sagittal view, click on the farthest posterior point of the hippocampus tail. The landmark should be on the most posterior slice of the hippocampus. Scroll right and left to verify location.
  - b) Set the Tail landmark in the oblique view.
- 5) Setting the Head landmarks:
  - a. In the coronal view, scroll anteriorly until the amygdala grows and the hippocampus head is a small gray matter circle bordered by a thin white matter line. Verify anterior location in the sagittal view.
  - b. In the oblique view, check that all of the hippocampus is behind the selected slice level. Set the Head landmark.
- 6) Set Slice 1 landmarks:

Superior at the most superior portion of gray matter, slightly above the alveus in CSF. Inferior at the most inferior portion of gray matter. Lateral at the most lateral portion of the gray matter. Medial at the most medial portion of the gray matter, above the uncus notch.
- 7) Set Slice 2 landmarks:

Superior at the most superior portion of gray matter, above the alveus. Inferior at the most inferior portion of gray matter. Lateral at the most lateral portion of the gray matter. Medial at the most medial portion of the gray matter of the hippocampus above the most lateral end of the ambient cistern.

8) Set Slice 3 landmarks:

Superior at the most superior portion of gray matter of the hippocampus on the white matter between the hippocampus and the tail of the caudate. Inferior at the most inferior portion of gray matter. Lateral at the gray matter boundary of the hippocampus and lateral ventricle. Medial at the gray matter boundary of the subiculum and the ambient cistern.

9) Set Slice 4 landmarks:

Superior at the most superior portion of the gray matter. Inferior at the most inferior portion of gray matter. Lateral at the gray matter boundary of the hippocampus and lateral ventricle. Medial at the gray matter boundary of the hippocampus and ambient cistern. Place the landmark half in gray matter and half in white matter.

10) Set Slice 5 landmarks:

Superior at the most superior portion of gray matter. Inferior at the most inferior portion of gray matter. Lateral at the gray matter boundary of the hippocampus and lateral ventricle. Medial at the gray matter boundary of the hippocampus and ambient cistern.

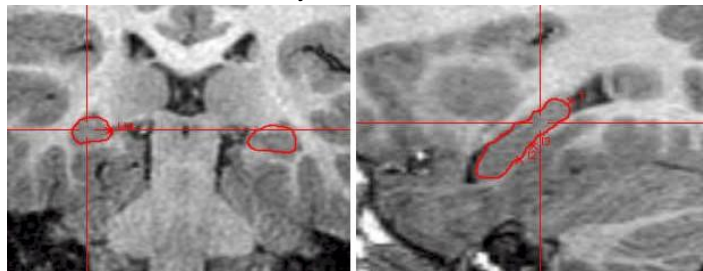
11) Check the accuracy of all landmarks in the oblique view along the axis of the hippocampus using the H-T axial and H-T coronal buttons, and then save the landmark definitions file.

12) Start the deformation script (MojoDeform), using the image and landmark files as input. After about 20 minutes, the surface files will be complete.

13) Load the surfaces and page through the hippocampus to review accuracy of the definition. If a substantial portion of the hippocampal border has been missed, adjust the landmarks and re-run the script.

References

Csernansky et al., 1998



# Lateral Ventricles

Tool and Method: ITK-SNAP is a semi-automated 3D segmentation tool controlled by a user-defined initialization and region-growing parameters. The region-growing features of ITK-SNAP are used in conjunction with the CSF soft segmentation output from EMS to label the lateral ventricles. The output label can then be manually edited as necessary. The 3D display and cut plane tool of ITK-SNAP are employed to separate the right and left lateral ventricles.

Images and Required Time: The lateral ventricles are labeled on the CSF soft segmentation output images from a dual-channel EM segmentation, with a high resolution images (T1w and T2w) as the input. The T1w and T2w images are used for final review and editing of the label. If a project's only high-resolution image is a T1w, then a T1-only EM segmentation is performed. Time required to complete one case is approximately 15 minutes.

Established Reliability:

Average intra-rater = 0.99

Average inter-rater = 0.99

The key boundaries and methods are described below. The complete lateral ventricle tracing protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

Preparation Steps

- 1) Run a dual-channel EMS tissue segmentation. The CSF soft segmentation image, "seg3," will be the input image for labeling the lateral ventricles in ITK-SNAP.
- 2) Load the seg3 image in ITK-SNAP. Set the bounding box, making sure all portions of the lateral ventricles are within the box.

3D Snake Segmentation: Pre-processing & Initialization

- 3) Select the right ventricle label and enter the ITK-SNAP "Segment 3D" window.
- 4) Preprocess the image by selecting the In/Out snake and using the default parameters.
- 5) Place bubbles within the lateral ventricles to initialize the snake.
  - a) Place bubbles in both the right and left ventricles (they will be separated later), beginning in superior axial slices and moving inferiorly.
  - b) In the superior slices, place one bubble in each ventricle. As you move inferiorly and the ventricles separate into anterior and posterior portions, place bubble in each portion.
  - c) Also place additional bubbles in portions of the ventricles that appear separated from the rest of the ventricles. This is common in the pediatric images, particularly in the anterior portions, where the ventricles tend to be very narrow, and in the occipital horn.

3D Snake Segmentation: Running the Snake

- 6) Accept the initialization and set ITK-SNAP to update the 3D viewer continuously, which will allow you to see the ventricles grow as the bubbles expand.
- 7) The Snake Control parameters are standardized for ventricle segmentation in all images.
- 8) Run the 3D snake segmentation. The 3D region-growing snake will automatically find the boundaries of the ventricles on the threshold image.
  - a) Allow the snake to advance until the label fills the ventricles.
  - b) Review the ventricle label to be sure that all regions are labeled.
  - c) If additional bubbles are needed, restart initialization and continue from that point.

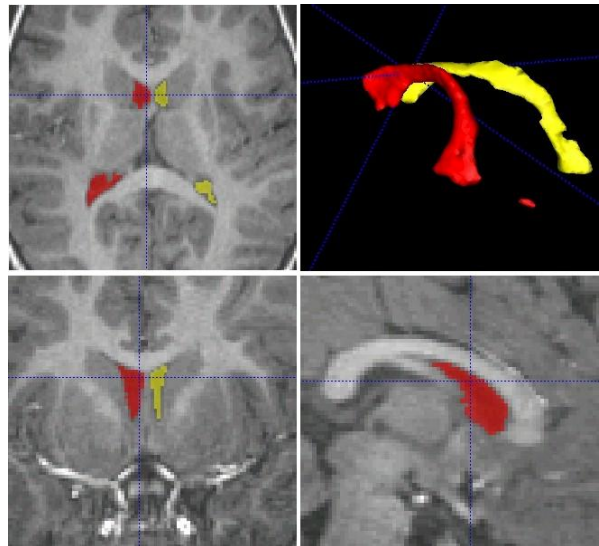
### Editing the Snake Segmentation:

- 9) The snake will often bleed into areas of CSF that are not part of the lateral ventricles, and the excess must be removed. The most common regions that require editing are the third ventricle and interhemispheric space.
  - a) Trace in sagittal slices, removing portions of the label that do not belong to the lateral ventricles.
  - b) Scroll through all views to review segmentation.

### Separating Right and Left Lateral Ventricles

- 10) Display the ventricles in the 3D viewer, and rotate so that the ventricles are displayed directly from the inferior side.
- 11) Set the left ventricle label to paint over the existing right ventricle label. Select the 3D cut plane tool.
  - a) Define the cut plane by clicking directly above the center of the ventricles, and then directly below.
  - b) Rotate the 3D image to view the cut plane. If necessary, reset view and repeat the definition of the cut plane.
  - c) Accept the cut plane. The lateral ventricles will be separated into right and left.
- 12) Review the right/left separation on the 2D slices, and save the segmentation file.

Review work by loading the ventricle segmentation file on top of the gray level image, which will provide a better view of the anatomy than the seg3 image.



# Cerebellar Vermis

Tool and Methods: The manual tracing features of ITK-SNAP are used to label the cerebellar vermis. To provide stability and consistency, the cerebellar vermis lobules are labeled on the two most mid-sagittal slices, and the measurements are averaged to obtain a mid-sagittal area of the cerebellar vermis. The vermis is segmented into three portions: lobules I-V, VI-VII, and VIII-X.

Images and Required Time: The mid-sagittal cerebellar vermis is traced on high resolution T1 images in standard ACPC alignment. Time required to complete one case is approximately 20 minutes.

Established Reliability:

Intra-rater: lobules I-V = 0.99, VI-VII = 0.96, VIII-X = 0.92, total midsag crbl vermis = 0.99

Inter-rater reliability is in progress.

The key boundaries and methods are described below. The complete cerebellar vermis tracing protocol is available at:

<http://ibis-network.org/unc/mri/roiprotocols.htm>

Finding the mid-sagittal slices

- 1) Find the two most mid-sagittal slices of the cerebellum. The midline of the cerebrum is defined by a clearly delineated cerebral aqueduct, fourth ventricle, corpus callosum. The midline of the cerebellum may be slightly different than the midline of the cerebrum. In addition to the above criteria, look for visible primary and prepyramidal fissures of the cerebellum, and an absent or fuzzy-appearing cerebellar tonsil.
  - a) Use the coronal and axial views to verify each slice as midline.

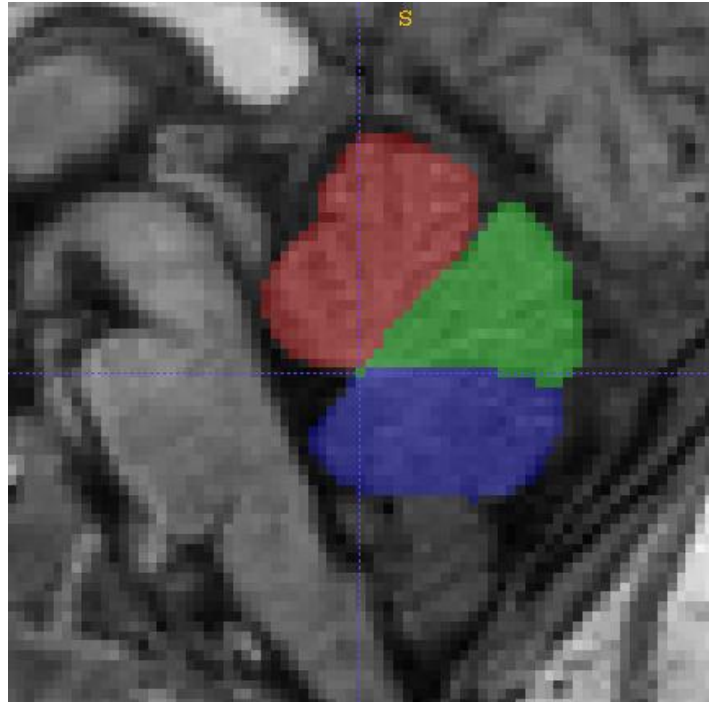
Tracing the mid-sagittal cerebellar vermis

- ❖ Note: Use the ITK-SNAP feature of “draw over clear” when tracing the cerebellar vermis. This will allow the new trace to abut the existing label without requiring a precise trace along the adjoining border.
- 2) Manually trace cerebellar vermis lobules I-V as a unit.
    - a) Begin at the apex of the fourth ventricle.
    - b) Trace the border of the vermis. Follow large sulcal indentations, but do not trace into smaller sulci and thin fissures.
    - c) Continue to the anterior limit of the primary fissure.
    - d) Complete the trace with a straight line from the anterior limit of the primary fissure to the apex of the fourth ventricle.
  - 3) Manually trace cerebellar vermis lobules VI-VII as a unit.
    - a) Begin at the posterior limit of the primary fissure.
    - b) Trace the border of the vermis, continuing to the most anterior limit of the prepyramidal fissure.
    - c) Draw a straight line from the anterior limit of the prepyramidal fissure to the apex of the fourth ventricle.
    - d) Complete the trace by connecting to the primary fissure, allowing the current trace to abut the existing segmentation of lobules I-V.
  - 4) Manually trace cerebellar lobules VIII-X as a unit.
    - a) Begin at the posterior limit of the prepyramidal fissure.
    - b) Trace the border of the vermis, continuing to the apex of the fourth ventricle.
    - c) Exclude the cerebellar tonsil.
    - d) Complete the trace by connecting to the prepyramidal fissure, allowing the current trace to abut the existing segmentation of lobules VI-VII.
  - 5) Repeat the tracing steps for the second mid-sagittal slice.

- 6) For analysis, the average mid-sagittal cerebellar vermis is used. This can be calculated using the voxel count value in the saved Volumes & Statistics file.
- a) Divide the voxel counts by two (2) to obtain the average.
  - b) Multiply the average voxel counts by the pixel dimension scale factor. The pixel dimension scale factor is the product of the pixel dimensions in the superior-inferior direction (Y in a coronal image) and the anterior-posterior direction (Z in a coronal image).

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Courchesne, et al., 1988





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# Geometric Phantom Measurements

## Phantom and Procedures from DCC at MNI

[Fonov 2010] Fonov, V., Janke, A. L., Caramanos, Z., Arnold, D. L., Narayanan, S., Pike, G. B., Collins, D. L., Improved precision in the measurement of longitudinal global and regional volumetric changes via a novel mri gradient distortion characterization and correction technique. H. Liao et al. (Eds.), In: Lecture Notes in Computer Science LNCS, Springer Verlag, Vol. 6326, pp. 324–333, 2010

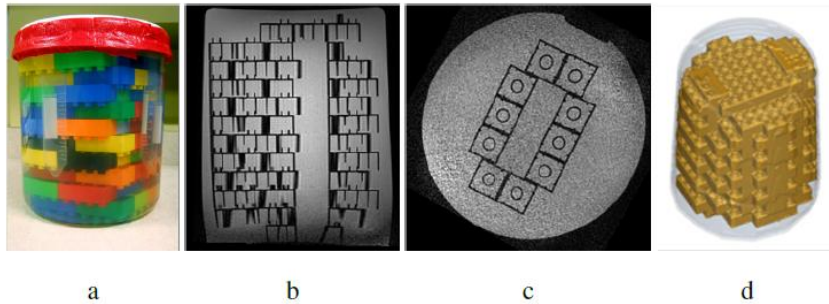
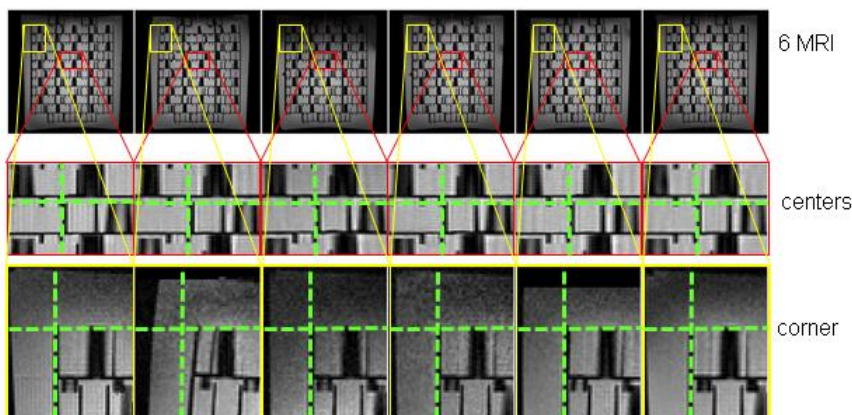


Figure 2. a) Volumetric geometry MRI phantom constructed from Lego DUPLO bricks, b) and c) vertical and axial slices of 3D MRI, d) 3D reconstruction of MRI image of phantom.

**Aim:** Measurement of 3D geometric phantom, calculation of 3D spatial deformations via automatic detection of deformation field to known structures, application of deformations to structural MRI (T1w and T2w).

**Description:** Approach based on imaging a 3-D geometric phantom with easily identifiable, known structures which densely cover the whole volume. The differences between imaged positions of these structures, and their known positions enables an estimation of the deformation field. We constructed a phantom from 125 Lego DUPLO bricks made of ABS plastic (Billund, Denmark) assembled inside a polycarbonate Nalgene 8L container and filled with a water solution of 0.15mM/L  $MnCl_2$  and 2.8g/L NaCl. The MR image of the Lego phantom is linearly aligned and then non-linearly registered to an ideal phantom image created numerically using the knowledge of the location of the Lego DUPLO bricks and their geometric properties. Registration is completed using ANIMAL [Collins97] and results in a dense field of vectors, where non-null vectors indicate the difference in position for different points within the FOV. The distortion correction field is expressed using a spherical harmonic expansion based on a mathematical model of the magnetic field gradients. The distortion fields represent the actual geometric deformation and are applied to calibrate the MR images at each site. A detailed description of the phantom and correction procedures is found in [Fonov2010].

The figure below shows geometric distortion of 6 MRI scans using the same protocol and phantom position in the coils.



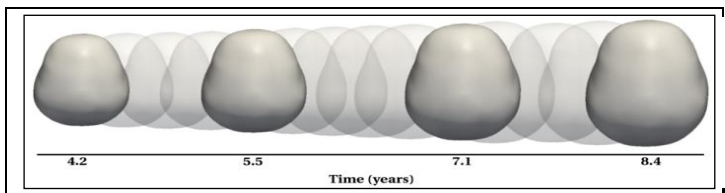
# Longitudinal Shape Analysis

A main emphasis of new image analysis research is put on spatiotemporal analysis of structure and function, i.e. 4D image processing applied to longitudinal data with repeated scans over the age range of interest.

4D shape analysis is developed as part of the NA-MIC project ([www.na-mic.org](http://www.na-mic.org)), which develops open-source software for medical image analysis researchers.

Detailed description of procedure: <http://www.na-mic.org/Wiki/index.php/Projects:Utah2ShapeRegression>

- [Fishbaugh2011] Fishbaugh, J., Durrleman, S., Gerig, G., Estimation of Smooth Growth Trajectories with Controlled Acceleration from Time Series Shape Data, to appear MICCAI 2011, Springer Verlag LNCS, September 2011, in print
- [Fishbaugh2012] James Fishbaugh, Stanley Durrleman, Guido Gerig, A framework for longitudinal data analysis via shape regression, to appear SPIE Feb. 2012, in print
- [Durrleman2011] Durrleman S, Prastawa M, Gerig G, Joshi S., Optimal data-driven sparse parameterization of diffeomorphisms for population analysis, Inf Process Med Imaging. 2011;22:123-34, PMID: 21761651



The figure below shows application of 4D shape analysis to ACE-IBIS data, with color coding illustrating velocity of local growth.

