Published in final edited form as:

J Neurosci Methods. 2009 May 15; 179(2): 338–344. doi:10.1016/j.jneumeth.2009.02.007.

A Semi-automatic Image Segmentation Method for Extraction of Brain Volume from In Vivo Mouse Head Magnetic Resonance Imaging using Constraint Level Sets

Mariano G. Uberti^{a,b}, Michael D. Boska^{a,b}, and Yutong Liu^{a,b}

^a Center for Neurovirology and Neurodegenerative Disorders, University of Nebraska Medical Center, Omaha, NE 68198-5880

^b Department of Radiology, University of Nebraska Medical Center, Omaha, NE 68198-5880

Abstract

In vivo magnetic resonance imaging (MRI) of mouse brain has been widely used to non-invasively monitor disease progression and/or therapeutic effects in murine models of human neurodegenerative disease. Segmentation of MRI to differentiate brain from non-brain tissue (usually referred to as brain extraction) is required for many MRI data processing and analysis methods, including coregistration, statistical parametric analysis, mapping to brain atlas and histology. This paper presents a semi-automatic brain extraction technique based on a level set method with the incorporation of user-defined constraints. The constraints are derived from the prior knowledge of brain anatomy by defining brain boundary on orthogonal planes of the MRI. Constraints are incorporated in the level set method by spatially varying the weighting factors of the internal and external forces and modifying the image gradient (edge) map. Both two-dimensional multi-slice and three-dimensional versions of the brain extraction technique were developed and applied to MRI data with minimal brain/non-brain contrast T₁-weighted (T₁-wt) FLASH and maximized contrast T₂-weighted (T₂-wt) RARE. Results were evaluated by calculating the overlap measure (OM) between the automatically segmented and manually traced brain volumes. Results demonstrate that this technique accurately extracts the brain volume (mean OM = 94 %) and consistently outperformed the region growing method applied to the T₂-wt RARE MRI (mean OM = 81 %). This method not only successfully extracts the mouse brain in low and high contrast MRI, but can also be used to segment other organs and tissues.

Keywords

brain extraction; image segmentation; level set; mouse brain; MRI

Introduction

Mice have been widely used as models for neurodegenerative disease. Many neuropathological models have been developed using mice, including, but not limited to, HIV-1-induced encephalitis (HIVE) (Persidsky et al., 2005), Parkinson's disease (Przedborski and Vila,

Correspondence should be addressed to: Yutong Liu, Ph.D., Department of Radiology, 985880 Nebraska Medical Center, University of Nebraska Medical Center, Omaha, NE 68198-5880. Phone: (402) 559-8340, Fax: (402) 559-1011, E-mail: E-mail: yutongliu@unmc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

2003; Ebadi et al., 2005), Alzheimer's disease (Emilien et al., 2000), and amyotrophic lateral sclerosis (Julien and Kriz, 2006). Magnetic resonance imaging (MRI) is a powerful tool for neuroscience research providing direct non-invasive three-dimensional (3D) visualization and assessment of the mouse brain morphology and biochemistry in vivo. MRI can be used to acquire data from either selected brain regions or the entire head. The scans typically include non-brain tissues such as the skull and the soft tissue covering the skull. Segmentation of the brain from non-brain tissue in MRI is required for image processing and analysis methods, including brain volume measurements, surface rendering, coregistration with histology, coregistration of MRI acquired at different times, and segmentation of brain neurostructures. The segmentation of brain from non-brain tissue has been referred to as brain extraction and skull stripping. It is called brain extraction throughout this manuscript.

Several automated or semi-automated brain extraction techniques have been developed for human head MRI (Smith, 2002; Lee et al., 2003; Boesen et al., 2004; Rex et al., 2004; Segonne et al., 2004a, 2004b; Fennema-Notestine et al., 2006; Hartley et al., 2006; Zhuang et al., 2006; Chiverton et al., 2007). These techniques can be categorized into region-based and edge-based methods that employ MR image intensity or gradient, respectively, to distinguish the brain from non-brain tissues. Recently, the mean brain shape models have been incorporated with region- and edge-based methods to generate more accurate brain extraction results (Zhuang et al., 2006; Chiverton et al., 2007). In human head the non-brain tissues consisting of bone (skull), skin, and muscle generate different signal intensities in most MRI scans as compared to brain. The gap between the brain and non-brain surrounding tissue is relatively large and the gap combined with intensity differences results in a strong gradient on MR images. These characteristics of the human head make the region- and edge-based techniques suitable methods for the brain extraction. On the other hand, the mouse brain imaged at high-field, especially with T₁-weighted (T₁-wt) MRI, is surrounded by tissues that have similar image intensity to the brain. In addition, the gap between the brain and the non-brain tissue is very narrow, which results in weak edges. At several locations the edge cannot even be observed at an isotropic spatial resolution of 150 μ m. Therefore, human brain extraction techniques are error prone when applied to mouse brain MRI. To the best of our knowledge, no automatic methods exist capable of accurate mouse brain extraction, especially in T₁-wt mouse brain MRI. To solve this problem, we developed a level set method with prior knowledge and evaluated this technique in a variety of MRI sequences against other methods and manual delineation of mouse brain borders.

The mouse brain extraction technique reported in this manuscript is based on *active models* with the incorporation of prior knowledge. In the past decades, active models have been used extensively for image segmentation. These methods use an initial contour (in two-dimensional (2D) images) or surface (in 3D images) and update the contour/surface iteratively toward the boundaries of the object. Active contour/surface models can be broadly classified as either parametric or geometric models. The parametric active contour/surface (also called snakes) (Kass et al., 1987) are represented explicitly as parameterized curves or surfaces in a Lagrangian framework, whereas the geometric active contour/surface, which is based on level set method (Osher and Sethian, 1988) are represented implicitly as level sets of a function in higher dimensions that evolves in an Eulerian framework (Caselles et al., 1993; Malladi et al., 1995).

Our technique is based on a level set method. Level set methods represent object boundaries as the zero level set of an implicit function defined in a higher dimension, usually referred to as the *level set function*, and evolves the level set function according to a partial differential equation (PDE). The evolving PDE of the level set function is derived from the problem of minimizing defined energies on the level set function. The energy functions are derived from the image properties such as intensity and gradient. Level set methods present several

advantages over the parametric active contours. First, the contours represented by the level set function may break or merge naturally during the evolution allowing automatic adaptation to topological changes. Second, the level set function always remains a function on a fixed grid, which allows efficient numerical schemes such as substituting finite difference approximations for the spatial and temporal derivatives. Third, intrinsic geometric properties of the contour front may be easily determined from the level set function. Fourth, by simply extending the array structures and gradients operators, propagating fronts can be easily followed in three spatial dimensions (Caselles et al., 1993; Malladi et al., 1995).

However, re-initializing schemes of the level set methods have the undesirable side effect of moving the zero level set away from its original position (Gomes and Faugeras, 2000). In this regard, Li et al (Li et al., 2005) introduced a new variational formulation that forces the level set function to be close to a signed distance function, and therefore completely eliminates the need of the costly re-initialization procedure. The variational level set formulation has three main advantages over the traditional level set formulations: 1) significantly larger time step can be used thus speeding up the curve evolution; 2) level set functions can be more efficiently initialized than the signed distance function; 3) the proposed level set evolution can be implemented using a simple finite difference scheme (Li et al., 2005).

The developed mouse brain extraction technique is based on the level set variation introduced by Li et al (Li et al., 2005) and incorporates higher level constraints obtained from prior knowledge and understanding of mouse brain anatomy. We denominated our technique as Constraint Level Sets (CLS). The constraints were manually defined, and their introduction allows the algorithm to overcome the noted problems of minimal gaps and poor to non-existent gradients at the brain boundary. 2D and 3D implementations of the algorithm were evaluated on mouse head MRI acquired using 3D T₂-wt RARE and T₁-wt FLASH sequences, which generate high and low contrast between brain and non-brain tissue, respectively. These results were compared with the results obtained using a standard seed based region growing algorithm and ultimately with the gold standard –manual segmentation.

Materials and Methods

1. Magnetic Resonance Imaging (MRI)

Adult male C.B.-17 severe combined immunodeficient (SCID) mice (n = 5) were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center. Animals were allowed to acclimate to the animal center for one week before the experiment. Mice were anesthetized by inhalation of 1.5% isoflurane in a 70% nitrous oxide/30% oxygen mixture prior to MRI acquisition. Anesthetized animals were mounted in a custom-made stereotactic holder with a breathing monitor and placed into the MRI system for imaging. Anesthesia level was varied during the acquisition from 0.5% to 1.5% isoflurane to maintain the breathing rate between 40–80 breaths per minute. Breathing rate was monitored using an MRI compatible physiological monitoring system (Model 1025, SA Instruments, Stony Brook, NY). 3D high resolution T₁-weighted (T₁-wt) MRI scan was acquired from each animal using the Fast Low Angle Shot (FLASH) sequence followed by a 3D high resolution T₂-weighted (T₂-wt) Rapid Acquisition with Relaxation Enhancement (RARE) sequence in a Bruker (Karlshere, Germany) 7 T MRI system operating Paravision 4.0. Data were acquired from mouse brains using a 25 mm birdcage coil. Acquisition parameters for the T₁-wt FLASH sequence were TR/TE = 50/5 ms, flip angle = 35°, averages = 2, total acquisition time = 27 min. For the 3D T₂-wt RARE sequence, acquisition parameters were optimized based on proton density (PD), T₁ and T₂ measurements to maximize the contrast between the brain and surrounding tissues in a relatively short scan time as described in the following section. The acquisition parameters used were TR = 800 ms, effective TE = 30 ms, RARE factor = 16,

averages = 1 for a total acquisition time of 8.5 min. In both sequences, the field of view (FOV) was 20×20×20 mm with a matrix of 256×128×128 (voxel size = 78×150×150 μm). The reconstruction matrix size was 256 isotropic (voxel size = 78 μm isotropic) for both scans.

2. Optimization of T₂-wt MRI using RARE sequence

PD, T₁ and T₂ values were measured in one mouse to optimize the 3D T₂-wt RARE sequence using the Bruker 7 T scanner and birdcage coil (Table 1). RARE with variable TR was used for T₁ measurement and multislice, multiecho CPMG phase cycled MRI for PD and T₂ measurements. T₁-, PD- and T₂-wt images were also acquired. PD and relaxation times vary with location; the values shown in Table 1 were measured at the region of interests (ROIs) close to the brain/non-brain boundary. It can be seen that PD and T₁ in brain tissue is almost the same as in non-brain tissue, and T₂ in brain is 1.5–2 times the T₂ of non-brain tissue. The T₂ contrast is proportional to $|e^{-TE/T_2^b} - e^{-TE/T_2^{nb}}|$, where the superscripts “b” and “nb” are the abbreviates of “brain” and “non-brain”, respectively. Its change with TE is plotted in Figure 1A. The T₂ contrast is maximized using RARE when TE = 26 ms. In this study, due to the physical limitations of scanner gradients, TE was set at 30 ms. The 3D T₂-wt image of the mouse using the optimized RARE sequence and the T₁-wt image using the FLASH sequence described in the preceding section are shown in Figure 1B and C. The signal-to-noise ratio (SNR) was measured for both T₁- and T₂-wt images. The SNR of the T₂-wt RARE images was about 12 compared to 20 in the T₁-wt FLASH at this resolution and scan time.

3. Brain Extraction

A. 2D and 3D Level Set Method with the Incorporation of Constraints

Level Set Method: The brain extraction technique was developed based on a level set method (Li et al., 2005). Level set methods represent a 3D surface C as the cross section of the graph of a four dimensional (4D) function ($m = \varphi(x, y, z)$ or $\varphi(x, y, z, m)$ in implicit format) taken at $m = 0$, where φ is the function of x, y, z and m . This can be formulated by: $C = \{(x, y, z) | \varphi(x, y, z) = 0\}$. The function φ is usually called the level set function; the surface C is called the zero level surface (Osher and Sethian, 1988). Level set methods start with an initial zero level surface and update the surface iteratively toward the object boundaries. The movement of the zero level surface is represented by $C(t) = \{(x, y, z) | \varphi(t, x, y, z) = 0\}$ resulting from the evolution of the level set function φ . The evolution of φ can be written in the following general form (Osher and Sethian, 1988):

$$\frac{\partial \varphi}{\partial t} + F|\nabla \varphi| = 0, \quad (1)$$

which is called the level set equation. The function F is the speed function. For this study, the function F depends on the image gradient and the level set function φ . Employing a recently introduced variation of the level set methods (Li et al., 2005), Equation (1) can be written as:

$$\frac{\partial \varphi}{\partial t} = \mu \left[\Delta \varphi - \text{div} \left(\frac{\nabla \varphi}{|\nabla \varphi|} \right) \right] + \lambda \delta(\varphi) \text{div} \left(g \frac{\nabla \varphi}{|\nabla \varphi|} \right) + \nu g \delta(\varphi), \quad (2)$$

where Δ is the Laplacian operator, δ is the Dirac function, and g is the gradient map. The first term at the right hand side corresponds to the internal force, penalizing the deviation of the level set function from the previous iteration. The second and third terms correspond to the external force that drives the zero level surface toward the locations with high gradient (edges). μ , λ and ν are the weighting factors of the forces. By choosing the appropriate values for the

weighting factors, the internal and external forces together drive the surface toward and converge at the brain boundary.

Incorporation of Prior Knowledge into the Level Set Method: The prior knowledge of the brain anatomy needs to be represented in a way that is fast and easy to define by the user and in a format where the prior knowledge can be automatically employed in the level set method. In the presented method the prior knowledge is represented by planar contours of brain boundaries defined on sagittal and axial planes. These contours are referred to as orthogonal contours to distinguish them from the active contour of the level set method. The number, positions, and shapes of these orthogonal contours depend on the user and must accurately and effectively represent the brain boundary. In this study, three sagittal and two axial brain contours were defined (Figure 2B, and G). The orthogonal contour planes need to be carefully selected and the orthogonal contour shapes carefully defined to include or exclude extrema of the brain depending on the user's segmentation goal. In this study, paraflocculi and olfactory tubercle were excluded from the orthogonal contours because they would not appear in histological analysis. Registration with histology was a secondary goal not included in this work.

To incorporate the prior knowledge represented by the orthogonal contours, constraint points were extracted from the orthogonal contours on coronal slices. The constraint points served the level set method in two ways: they were used to define the initial zero level contours and to constraint the contours from evolving beyond the constraint points (Figure 2, H and I). In the multislice 2D CLS, initial contours on coronal slices were generated by connecting the constraint points using line segments. These initial contours were stacked to form the closed initial zero level surface for the 3D level set method. Contrary to the edges, which attract the active contour, the constraints trap the contour and block its propagation. To incorporate the constraints in the level set method, the weighting factors in Equation (2) were spatially varied. Instead of being constants, they are varied at the constraint points by increasing μ (deviation penalty), decreasing λ , and changing the sign of ν (both λ and ν are for external force). In this study, we set the weighting factors for 2D CLS to $\mu = 0.04$ and $\lambda = 4.0$, $\nu = -2$ for T₁-wt FLASH and modified to $\lambda = 3.0$, $\nu = -1$ for T₂-wt RARE. The constraint points were set to $\mu = 0.05$, $\lambda = 0.0$, and $\nu = 2$ in 2D CLS. The weighting factors were modified for 3D CLS in both acquisition methods to $\lambda = 5.0$, $\nu = -3$ and the constraint points were modified to $\nu = 1$. Table 2 shows the pseudocode of the algorithm implemented in this study.

Programming Environment: The CLS methods were programmed in Matlab® (The MathWorks Inc, Natick, MA), version 7.5 (R2007b) on a Dell Precision workstation with an Intel 2.2GHz Core2 Duo CPU and 4GB RAM. The operating system (Windows XP) and Matlab were both in 64bit version.

B. Seed based region growing algorithm (SBRG)—The SBRG algorithm in Analyze® (AnalyzeDirect, Lenexa, KS) was used for brain extraction on the optimized T₂-wt RARE MRI. A seed was located on the brain tissue of a middle coronal slice. Lower and upper thresholding limits were adjusted to exclude as much non-brain tissue as possible while preserving all of the brain tissue.

C. Manual Segmentation—Manual segmentation was performed on the optimized T₂-wt RARE MRI in a slice by slice fashion, by three experienced investigators using the ROI function in Analyze® software. Brain contours were manually traced on coronal slices using freehand ROI. To be in accordance with the constraints used in the level sets, paraflocculi and olfactory tubercle were also excluded during manual segmentation.

4. Comparison of Results

Overlap measure (OM) defined by:

$$OM = \frac{V_{\cap}}{(V_1 + V_2)/2}, \quad (3)$$

was calculated to measure the overlap $V_{\cap}$ of two volumes V_1 and V_2 . OM ranged between 0 and 1 with 0 indicating no overlap and 1 indicating an exact match between the two volumes. OM was first used to evaluate the variance in manual results obtained by three experienced investigators. For each animal, we calculated the OM of the manually traced brain volumes by different investigators in pair-wise fashion. In addition, we compared the inter-investigator OMs for all animals using the Student's t-test with the Bonferoni correction for pair-wise calculations (for 3 pair-wise comparisons $\alpha = 0.05/3 = 0.0167$).

To evaluate the performance of an automated segmentation method (CLS or SBRG), the OM of the automatically extracted brain volume and the manually traced volume of each investigator was first calculated for each animal. The mean and standard deviation of OMs were then calculated across the three investigators. To compare the automatic results (i.e. 2D versus 3D CLS on both T₁-wt and T₂-wt MRI, 2D CLS on T₁-wt versus on T₂-wt, 3D CLS on T₁-wt versus on T₂-wt, 2D CLS on both T₁-wt and T₂-wt versus SBRG on T₂-wt, 3D CLS on both T₁-wt and T₂-wt versus SBRG on T₂-wt), Student's t-tests with one tail paired distribution and a critical value $\alpha = 0.05$ were performed on the OMs of all animals. All statistical analysis was performed using Microsoft Excel.

Results

Figure 2 shows an example of the brain extraction on the T₁-wt image of one mouse using the 3D CLS method. Image renderings were obtained using the image visualization software Amira (Visage Imaging, Chelmsford, MA). Figure 2A shows a maximum intensity projection of the mouse whole head MRI before extraction of the brain. It can be seen that the brain parenchyma had intensities similar to the peripheral tissues, which resulted in poor brain versus non-brain contrast. Figure 2B and G show the orthogonal brain contours drawn on specific sagittal and axial slices (five contours per mouse were drawn in this study). The constraint points, automatically extracted from the contours, are shown on Figure 2C and H. Figure 2D and I show the initial zero level surface constructed from the constraint points. Figures 2E and J show the final zero level surface after reaching the brain boundary. The segmented brain volume is shown in Figure 2F. With the incorporation of the constraints, the surface correctly identified the brain boundary and the CLS method accurately segmented the brain tissue.

Comparison of Manual Results

First of all, we compared the brain extraction manual results obtained by three experienced investigators. Results showed a total mean for the manual results of OM = 0.964 with SD = 0.007. No significant difference between manual results obtained from different investigators was found ($p > 0.0167$ for all groups).

Comparison of 3D versus 2D CLS Implementations

Results in Figure 3 show that the 2D CLS performed with slightly greater accuracy than the 3D CLS ($p = 0.050$) for the T₁-wt FLASH MRI (Figure 3A). On the other hand, there was no statistically significant difference between 2D CLS and 3D CLS on the optimized T₂-wt RARE MRI ($p = 0.077$) (Figure 3B). Generally speaking, the performance difference between 2D and 3D CLS was minor and significant only in cases of negligible brain/non-brain contrast.

Comparison of CLS Techniques on T₁-wt FLASH MRI versus T₂-wt RARE MRI

Both the 2D and 3D versions of CLS show improved performance ($p = 0.005$ and $p < 0.001$, respectively) when applied on the optimized T₂-wt RARE MRI versus the T₁-wt FLASH MRI as shown in Figure 4A and 4B, respectively. As expected, improving contrast between brain and non-brain tissues improves the performance of the extraction method.

Comparison of CLS Techniques to SBRG on T₂-wt RARE

The 2D CLS consistently provided better brain extraction on both low contrast (T₁-wt FLASH, $p = 0.037$) and high contrast (T₂-wt RARE, $p = 0.037$) as compared to the SBRG algorithm used only on the high contrast T₂-wt RARE MRI (Figure 5A). Likewise, the 3D CLS consistently provided better brain extraction from both low contrast (T₁-wt FLASH, $p = 0.047$) and high contrast (T₂-wt RARE, $p = 0.028$) compared to the standard SBRG algorithm on the optimized T₂-wt RARE (Figure 5B). Clearly, the 2D and 3D versions of the CLS implemented in this study outperformed the traditional SBRG algorithm.

CPU Time versus Resolution

Head MRI resolution was varied to investigate the performance (CPU time) of the brain extraction. At $256 \times 256 \times 256$, the 3D CLS required more than 8 hours to perform the extraction compared to 1.7 hours for 2D CLS. The CPU time decreased in both implementations with decreasing resolution. The CPU time decrease with data size was more dramatic in the 3D than 2D CLS. At $128 \times 128 \times 128$ and $96 \times 96 \times 96$, 2D and 3D CLS required almost the same time (24 and 7 minutes, respectively).

In summary, the variability of the manual brain extraction introduced by three different experienced investigators resulted in a mean OM for the manual results of 0.964. CLS accurately extracted the mouse brain, with an OM = 0.939 and a standard deviation of 0.012 for a total of $N = 60$ overlap comparisons. The confidence interval at the 0.05 alpha level was $Rn_{CI0.05} = [0.936, 0.941]$ with the minimum observed in a T₁-wt FLASH MRI using 3D CLS ($OM_{Min} = 0.899$), and the maximum on the T₂-wt RARE MRI using a 2D CLS ($OM_{Max} = 0.960$). Therefore, an OM between 0.936 and 0.941 will be achieved with 95% confidence applying CLS to mouse brain MRI with similar SNR and resolution as used in this study.

Discussion

Accurate brain extraction is required for many in vivo neuroimaging studies. However, as mentioned above, to the best of our knowledge no automatic brain extraction method exists specifically for murine MRI. The method developed in this work has advanced the ability of preclinical neuroscience research using mouse models of neurodegenerative disease by automating and improving the accuracy of the delineation of the mouse brain. Low contrast scans, such as high resolution T₁-wt FLASH is used for many studies, including structural imaging, cell tracking with super paramagnetic iron oxide (SPIO) particles, and tumor imaging with gadolinium contrast agents. However, the low contrast between brain and non-brain tissues in these scans makes the brain extraction difficult. In this study, we used the T₁-wt FLASH MRI to evaluate our technique on low contrast images. On the other hand, T₂-wt RARE MRI was used to compare brain extraction performance on high contrast images.

Despite increasing applications in medical image segmentation, incorporation of constraints has rarely been applied (Mitchell et al. 2002). Several properties are desirable for the constraints, including 1) the ability to effectively represent the prior knowledge of the object to be segmented; 2) ease of definition by the user, 3) seamless incorporation into level set methods and 4) automation of the initial contour using the constraints. The method presented in this study meets these desired properties. With minimum knowledge of the mouse brain

anatomy and by quickly browsing through the head MRI, an investigator or technician can locate the areas with weak edges, define brain contours, and place constraints at these locations. The contours were defined on orthogonal (sagittal and axial) 2D slices to avoid complex 3D visualization and manipulation. Seamless incorporation of the constraints was achieved in this study by changing the weighting factors of the internal and external forces at the constraint points. Additionally, the constraint points were used to automatically define the initial surface by using them as the vertices of the surface, eliminating the need for the user defined initial surface (Figure 4D). This also assured the initial surface to be close to the brain boundary, which reduced the time required for the surface to converge to the brain boundary.

It took less than one minute for an experienced experimenter to manually draw each orthogonal contour and a total less than five minutes to draw five contours on a mouse. The constraint points derived from the contours were automatically obtained by the algorithm and therefore did not require any additional user's time. By comparison, it requires about 3 hours to manually trace the coronal slices through the brain with the same data (256 imaging slices in coronal direction with 180 slices containing brain tissue).

At the image resolution of $256 \times 256 \times 256$ used in this study, the results did not show a marked tendency of the CLS for better accuracy in the 2D implementation (2D CLS) as compared to the 3D implementation (3D CLS). However, the 2D CLS was around 5.5 times faster than the 3D CLS version, which makes the former the more efficient option for brain extraction at this resolution. It is opportune to mention that the objective of this study pointed toward the ability of the CLS to accurately extract the mouse brain rather than the computational efficiency of the technique. Therefore, for fast implementation and flexibility, the algorithm described in this work was prototyped using a high level scripting program (Matlab) and run on a standard Windows platform. Improvements in speed can easily be achieved by translation of the code to a more efficient programming language, use of faster computers with more efficient operating systems (Unix based) and ultimately by the use of parallel processing with multislice 2D CLS.

As expected, the CLS performed better on the T_2 -wt RARE MRI (mean OM = 95%) than on the T_1 -wt FLASH MRI (mean OM = 93%). The reduced accuracy of the CLS results on T_1 -wt FLASH is a result of several factors. As described above, on the T_1 -wt FLASH the brain/non-brain border cannot be discerned at certain locations. Drawing orthogonal contours at these locations generates variable results based on the experience of the user. Moreover, the increased brain/non-brain contrast on T_2 -wt RARE MRI (Figure 2C) allowed the CLS algorithm to more accurately converge on the boundaries of the brain volume.

The accuracy and potential utility of the CLS algorithm was compared to a standard SBRG algorithm on the contrast optimized T_2 -wt RARE images to assess whether improved contrast would be sufficient to match the performance of CLS with standard algorithms. Both 2D and 3D CLS versions were more accurate than a standard SBRG algorithm on the optimized T_2 -wt RARE (mean OM of 94 % vs 81 %, respectively). We found in our study that the SBRG algorithm can extract the brain in most cases, however the final results contained extra tissues attached to brain and lack of brain tissue in some regions that made the results less robust than the CLS method. There are two main reasons why the SBRG algorithm is not accurate: 1) even contrast is increased on T_2 -wt RARE, still there are regions where the intensity difference is minimal and therefore the delimiting boundary goes beyond the brain parenchyma, and 2) the intensity range that defines the brain parenchyma is not absolute, slightly varying as we moved from rostral part of brain toward the caudal part, resulting in inaccurate brain tissue definition in some slices. It is reasonable to believe that these problems will be more severe as the contrast-to-noise ratio of the image decreases making the CLS a more suitable candidate for the brain extraction over a broad range of image types.

In summary, this work demonstrates an improved semi-automated mouse brain extraction technique based on a level set method with the incorporation of prior knowledge. This technique has been validated by manual delineation of the mouse brain borders and application to both low and high contrast MRI scans. This technique will be broadly applicable to both high and low contrast segmentation of brain as well as other organs and tissues.

Acknowledgments

The authors thank Jennifer Bradley, Erin McIntyre, Melissa Mellon, Lindsay Rice, and Jennifer Sullivan for technical support. The work was partially supported by the Nebraska Research Initiative (3132050655) and by NIH (5P01 NS043985-04, 5P01 NS043985-05, and 2P01 NS043985-06A1).

References

- Boesen K, Rehm K, Schaper K, Stoltzner S, Woods R, Luders E, Rottenberg D. Quantitative comparison of four brain extraction algorithms. *NeuroImage* 2004;22:1255–1261. [PubMed: 15219597]
- Caselles V, Catta F, Coll T, Dibos F. A geometric model for active contours in image processing. *Numer Math* 1993;66:1–31.
- Chiverton J, Wells K, Lewis E, Chen C, Podda B, Johnson D. Statistical morphological skull stripping of adult and infant MRI data. *Computers in biology and medicine* 2007;37:342–357. [PubMed: 16796998]
- Ebadi M, Brown-Borg H, El Refaey H, Singh BB, Garrett S, Shavali S, Sharma SK. Metallothionein-mediated neuroprotection in genetically engineered mouse models of Parkinson's disease. *Brain Res Mol Brain Res* 2005;134:67–75. [PubMed: 15790531]
- Emilien G, Maloteaux JM, Beyreuther K, Masters CL. Alzheimer disease: mouse models pave the way for therapeutic opportunities. *Arch Neurol* 2000;57:176–181. [PubMed: 10681074]
- Fennema-Notestine C, Ozyurt IB, Clark CP, Morris S, Bischoff-Grethe A, Bondi MW, Jernigan TL, Fischl B, Segonne F, Shattuck DW, Leahy RM, Rex DE, Toga AW, Zou KH, Brown GG. Quantitative evaluation of automated skull-stripping methods applied to contemporary and legacy images: effects of diagnosis, bias correction, and slice location. *Human brain mapping* 2006;27:99–113. [PubMed: 15986433]
- Gomes J, Faugeras O. Reconciling distance functions and Level Sets. *J Visual Communic and Imag Representation* 2000;11:209–223.
- Hartley SW, Scher AI, Korf ES, White LR, Launer LJ. Analysis and validation of automated skull stripping tools: a validation study based on 296 MR images from the Honolulu Asia aging study. *NeuroImage* 2006;30:1179–1186. [PubMed: 16376107]
- Julien JP, Kriz J. Transgenic mouse models of amyotrophic lateral sclerosis. *Biochim Biophys Acta* 2006;1762:1013–1024. [PubMed: 16675207]
- Kass M, Witkin A, D T. Snakes: Active contour models. *International Journal of Computer Vision* 1987;1:321–331.
- Lee JM, Yoon U, Nam SH, Kim JH, Kim IY, Kim SI. Evaluation of automated and semi-automated skull-stripping algorithms using similarity index and segmentation error. *Computers in biology and medicine* 2003;33:495–507. [PubMed: 12878233]
- Li, C.; Xu, C.; Gui, CFMD. Level Set Evolution Without Re-initialization: A New Variational Formulation. *IEEE Computer Society Conference on Computer Vision and Pattern Recognition*; 2005.
- Malladi R, Sethian JA, Vemuri BC. Shape modeling with front propagation: a level set approach. *IEEE Trans Patt Anal Mach Intell* 1995;17:158–175.
- Osher S, Sethian JA. Fronts propagating with curvaturedependent speed: algorithms based on Hamilton-Jacobi formulations. *J Comp Phys* 1988;79:12–49.
- Persidsky Y, Potula R, Haorah J. Rodent model systems for studies of HIV-1 associated dementia. *Neurotox Res* 2005;8:91–106. [PubMed: 16260388]
- Przedborski S, Vila M. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model: a tool to explore the pathogenesis of Parkinson's disease. *Ann N Y Acad Sci* 2003;991:189–198. [PubMed: 12846987]

- Rex DE, Shattuck DW, Woods RP, Narr KL, Luders E, Rehm K, Stoltzner SE, Rottenberg DA, Toga AW. A meta-algorithm for brain extraction in MRI. *NeuroImage* 2004;23:625–637. [PubMed: 15488412]
- Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *NeuroImage* 2004a;22:1060–1075. [PubMed: 15219578]
- Segonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *Neuroimage* 2004b;22:1060–1075. [PubMed: 15219578]
- Smith SM. Fast robust automated brain extraction. *Human brain mapping* 2002;17:143–155. [PubMed: 12391568]
- Zhuang AH, Valentino DJ, Toga AW. Skull-stripping magnetic resonance brain images using a model-based level set. *NeuroImage* 2006;32:79–92. [PubMed: 16697666]

Fig. 1. Optimization of the 3D RARE sequence for maximum brain/non-brain tissue contrast. (A) Based on the proton density (PD), T₁, and T₂ measurements in one mouse, the T₂ contrast was maximized using the RARE sequence when TE = 26 ms. (B) A coronal slice of a mouse brain acquired using the T₁-wt FLASH sequence where it can be seen the very low contrast between brain and non-brain tissues. (C) The same coronal slice acquired using the optimized T₂-wt RARE sequence. Note the improvement in contrast using the optimized T₂-wt RARE sequence.

Fig. 2.

Results of the brain extraction using 3D Constraint Level Set (CLS) on the T_1 -wt image of a mouse. (A) The mouse head MRI. (B) Brain contours were drawn on sagittal and axial slices. (C) Constraint points were extracted from the contours. (D) The initial zero level surface was constructed using the constraint points as vertices. (E) The final surface after 60 iterations. (F) The segmented brain using the CLS method. (G) The contour drawn on the sagittal slice. (H) Constraint points extracted from the contours on a coronal slice. (I) The initial surface contour on the coronal slice. (J) The final surface contour on the coronal slice.

Fig 3. Comparison between 2D and 3D Implementations of the Constraint Level Sets (CLS). (A) CLS on T_1 -wt FLASH MRI. The 2D CLS was slightly better than the 3D CLS but there was not strong statistical difference ($p = 0.050$). (B) CLS on T_2 -wt RARE MRI. The 3D CLS tended to be more accurately than the 2D CLS, however they were not statistically different ($p = 0.077$).

Fig. 4.

Performance of the Constraint Level Set (CLS) on T_1 -wt FLASH MRI versus T_2 -wt RARE MRI. (A) 2D Implementation. The 2D CLS gives significantly better results when applied on the T_2 -wt RARE MRI than on the T_1 -wt FLASH MRI ($p = 0.005$) (B) 3D Implementation. Likewise the 2D CLS, the 3D CLS performed statistically better on the T_2 -wt RARE MRI than on the T_1 -wt FLASH MRI ($p < 0.001$).

Fig. 5.

Comparison of Constraint Level Set (CLS) Technique versus Seed Based Region Growing Algorithm (SBRG) on optimized T₂-wt RARE images. (A) 2D CLS versus SBRG on high-contrast images. The 2D CLS shows statistically significant improvement in brain segmentation using either high (T₂-wt RARE ($p = 0.037$)) or low (T₁-wt FLASH ($p = 0.028$)) contrast images compared to SBRG used with high contrast (T₂-wt RARE) images only. (B) 3D CLS versus SBRG on high-contrast images. As with the 2D CLS, the 3D CLS was also statistically better on both T₂-wt RARE ($p = 0.028$) and T₁-wt FLASH ($p = 0.047$) images compared to the results obtained using SBRG algorithm on high-contrast images (RARE).

Table 1Proton Density (PD), T₁ and T₂ Measurements on Brain Tissue and Non-brain Tissue

	PD (ratio)	T ₁ (s)	T ₂ (ms)
Brain Tissue	1	1.5–2.0	30–40
Non-brain Tissue	1	1.2–2.0	17–20

Table 2
Brain Extraction Procedure Using the Constraint Level Set (CLS) Algorithm

1	Draw brain contours on different sagittal and axial slices
2	Extract constraint points from these contours
3	Set the weighting factors of internal penalty and external image gradient forces with appropriate values
4	Set the weighting factors of internal penalty and external image gradient forces at the constraint points
5	Construct the initial surface from the constraint points
6	Update level set function and zero level surface iteratively