



# Practical methods for segmentation and calculation of brain volume and intracranial volume: a guide and comparison

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**Background:** Accurate segmentation and calculation of total brain volume (BV) and intracranial volume (ICV) (further—volumetry) may serve various clinical tasks and research studies in neuroscience. Manual segmentation is extremely time consuming. There is a relative lack of published broad recommendations and comparisons of tools for automated volumetry, especially for users without expertise in computer science, for settings with limited resources, and when neuroimaging quality is suboptimal due to clinical circumstances. Our objective is to decrease the barrier to entry for research and clinical groups to perform volumetric cranial imaging analysis using free and reliable software tools.

**Methods:** Automated volumetry from computed tomography (CT)/magnetic resonance imaging (MRI) scans was accomplished using 3D Slicer (v. 4.11.0), FreeSurfer (v. 7.1.1), and volBrain (v. 1.0) in a cohort of 39 patients with ischemic middle cerebral artery territory brain infarcts in the acute stage. Visual inspection for accuracy was also performed. Statistical analysis included coefficient of determination ( $R^2$ ) and Bland-Altman (B-A) plots. A multifaceted comparison between 3D Slicer, FreeSurfer, and volBrain from practical user perspective was performed to compile a list of distinguishing features.

**Results:** BV: FreeSurfer, 3D Slicer, and volBrain provide similar estimations when high quality T1-MRI scans with 1 mm slices (3D scans) are available, whereas 3 mm and thicker slices (2D scans) introduce a dispersion in results. ICV: the most accurate volumetry is provided by 3D Slicer using CT scans. volBrain uses T1-MRIs and also provides good results which agree with 3D Slicer. Both of these methods may be more trustworthy than T1 MRI-derived FreeSurfer calculations.

**Conclusions:** All three studied tools of automated intracranial and brain volumetry—3D Slicer, FreeSurfer, and volBrain—are free, reliable, require no complex programming, but still have certain limitations and significant differences. Based on our investigation findings, the readers should be able to select the right volumetry tool and neuroimaging study, and then follow provided step-by-step instructions to accomplish specific volumetry tasks.

**Keywords:** Brain volume (BV); segmentation; intracranial volume (ICV); magnetic resonance imaging (MRI); computed tomography (CT)

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## Introduction

Accurate segmentation and calculation of total brain volume (BV) and intracranial volume (ICV) (further—volumetry) may serve research studies in neuroscience. Since these volumetric measurements can be used to predict clinical outcomes in space-occupying pathology such as stroke or tumor, such data can be invaluable to clinicians as well. For example, in patients with malignant middle cerebral artery infarcts (a space-occupying pathology), the ability to accommodate swelling in the cranium has prognostic implications. Over time, growing cerebral edema leads to transtentorial herniation and brainstem compression with a high mortality rate (1). Often the survivors are left severely disabled (2). In theory, a patient with a smaller brain will have more room to accommodate the mass effect from the edema than a patient with the same size infarct and intracranial space, but larger brain (3). Therefore, knowing BV and ICV is important in research studies that model spatial compensation reserve as it relates to pathological volumes.

Manual segmentation of BV and ICV is extremely time consuming. There is a relative lack of published broad recommendations for automated volumetry, especially for users without expertise in computer science and for settings with limited resources or when neuroimaging quality is suboptimal due to clinical circumstances. In addition to a relative lack of instructions in medical literature for how to collect these data, there also exists a knowledge gap regarding the accuracy and validation of existing volumetry methods (4). Therefore, we recognize the need to decrease the barrier to entry for research and clinical groups to perform volumetric cranial imaging analysis using free and reliable software tools while providing the comparative guidance and instructions that can be used efficiently without special programming knowledge. Though we also recognize that many automated and manual software exist for volumetric imaging analysis of the brain and skull, we have narrowed our focus to include free non-commercial platforms which are cited in neuroscience literature and are easy to use—FreeSurfer, 3D Slicer, and volBrain.

### *FreeSurfer*

FreeSurfer is a free, open-source software developed in 2012 by scientists at the Laboratory for Computational Engineering at the Athinoula A. Martinos Center for Biomedical Engineering (Charlestown, MA, USA) and is

available at <https://surfer.nmr.mgh.harvard.edu> for Linux and Mac OS platforms (5). FreeSurfer automatically performs all the steps of brain segmentation along with labeling of anatomical brain structures, and statistical volumetry analysis using T1 magnetic resonance imaging (MRI) scans (4–6). The FreeSurfer pipeline performs segmentation automatically from a command line (6). Of existing free tools, we chose FreeSurfer because it is a widely used research tool for volumetric analysis in neurodegenerative diseases (7) and is a common standard to which other clinical software are compared (4,8–10). Though FreeSurfer is easy to use, it requires downloading and installation which can be a complicated step in implementing the software package. It also requires high computational power and is not certified for clinical use (4). It can only utilize T1-MRI scans of the brain.

### *3D Slicer*

3D Slicer (Slicer) is another free, open-source software originally developed between the Massachusetts Institute of Technology Artificial Intelligence Laboratory, the Surgical Navigation and Robotics Laboratory, and Surgical Planning Laboratory at the Brigham and Women's Hospital (Boston, MA, USA) in 1998–1999 (11–18). Slicer is available at <https://www.slicer.org> for Linux, Mac, and Windows. Slicer is used for biomedical image informatics, processing, and visualization and has been under continuous development by The Slicer Community since conception. We chose to include Slicer in this study because it is a relatively versatile, user friendly software with a robust online discussion forum for user help. In contrast to FreeSurfer, Slicer can use a variety of imaging modalities, including MRI and computed tomography (CT) scans of the brain and other organs. It provides skull stripping, thresholding, and manual editing tools through a simple graphical user interface (GUI). These tools often require additional modules or “extensions”, many of which are made by third parties. Slicer does not provide the degree of granular segmentation and structure labeling that FreeSurfer and volBrain do. However, Slicer gives easier control to the user for fine-tuning the output of the software.

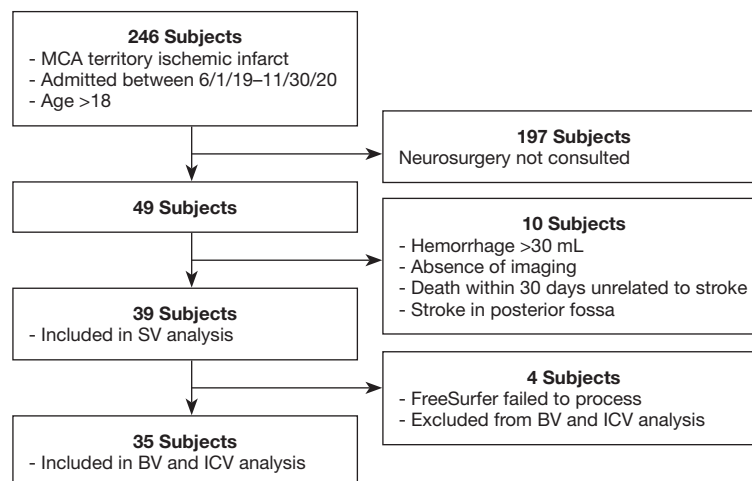
### *volBrain*

volBrain is a free, online platform developed by José V. Manjón (Valencia Polytechnic University, Valencia, Spain) and Pierrick Coupé (University of Bordeaux, Bordeaux,

**Table 1** Demographics of subject population in BV and ICV analysis

Gender	Number of subjects (percentage of total)	Average age (years) $\pm$ standard deviation
Male	16 (45.7%)	60.06 $\pm$ 15.60
Female	19 (54.3%)	58.84 $\pm$ 17.50

BV, brain volume; ICV, intracranial volume.



**Figure 1** Inclusion/exclusion criteria for this cohort of subjects. MCA, middle cerebral artery; SV, stroke volume; BV, brain volume; ICV, intracranial volume.

France) and is available at (<https://volbrain.upv.es>). The volBrain pipeline provides a fully automated method of obtaining BV and ICV from T1 MRI data and does not require any installation, configuration, or training (19). volBrain also conveniently provides easy instructions on its website interface for using the pipeline. To use volBrain, the user must register for access to the system and upload a single anonymized T1 MRI study in neuroimaging informatics technology initiative (NIFTI) format (other compression formats also accepted). The jobs uploaded to the volBrain web server are then distributed across multiple available machines, reducing the computational load. volBrain then generates CSV and PDF reports containing results, which are then emailed to the user. In some brain regions and study populations, volBrain results are comparable to, if not better than, state of the art methods such as FreeSurfer (19).

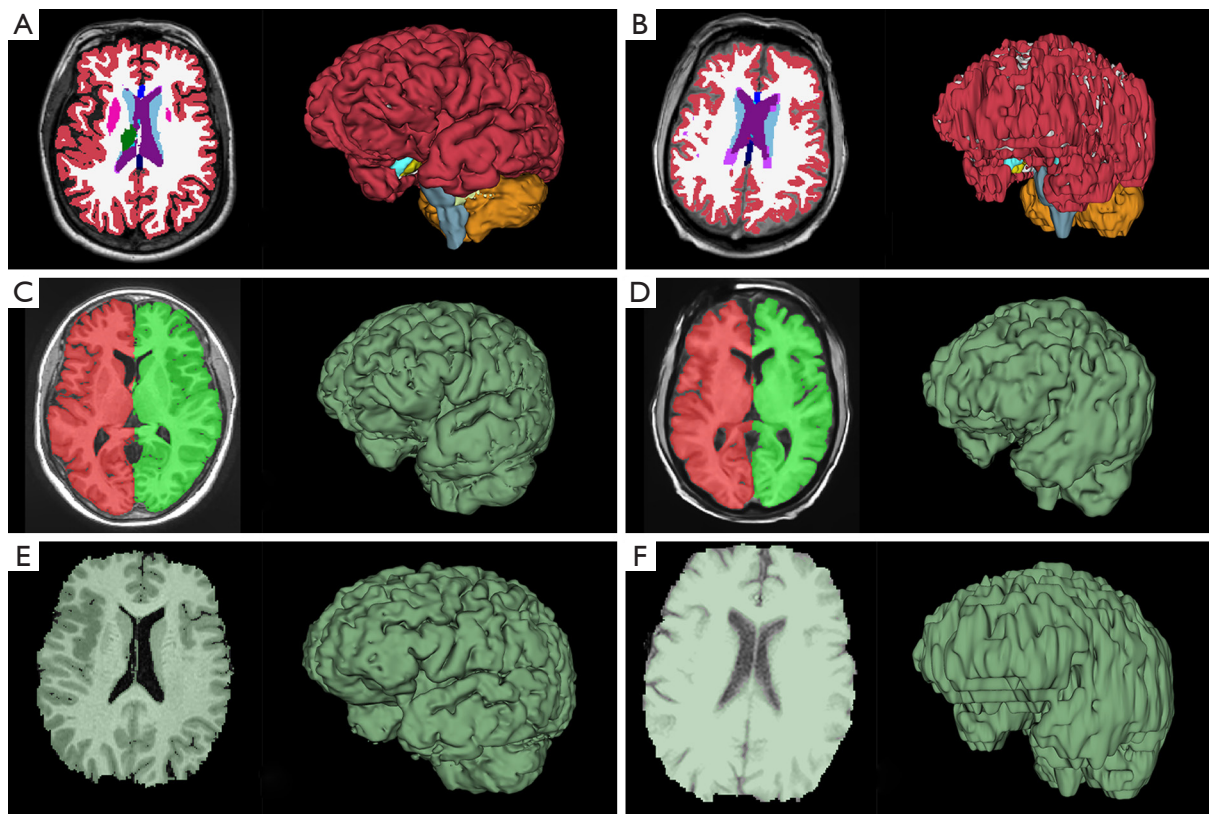
This study was designed with the intention to investigate the inter-method reliability, accuracy, and practicality of using these volumetry tools. We present the following article in accordance with the MDAR checklist (available at [https://qims.amegroups.com/article/view/10.21037/qims-](https://qims.amegroups.com/article/view/10.21037/qims-21-958/rc)

21-958/rc).

## Methods

### Subjects

A cohort of patients with space-occupying pathology was chosen to test the performance of volumetry tools in a clinical setting. Our cohort (determined retrospectively) included 39 patients (subjects) treated at a university hospital between June 1st, 2019 and November 30th, 2020 with ischemic middle cerebral artery territory brain infarcts in the acute stage. MRI and CT scans were obtained within 72 h of the last known well time as part of a routine ischemic stroke workup. Subject characteristics can be found in Table 1 and the inclusion/exclusion criteria can be found in Figure 1. All scans were acquired in digital imaging and communications in medicine (DICOM) format. Radiographic scans are completely anonymized and there are no patient identifiers mentioned within the text. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Review Board at the University of



**Figure 2** Examples of BV segmentations. (A) FreeSurfer BV using 3D T1 MRI. (B) FreeSurfer BV using 2D T1 MRI. (C) volBrain BV using 3D T1 MRI. (D) volBrain BV using 2D T1 MRI. (E) 3D Slicer BV using 3D T1 MRI. (F) 3D Slicer BV using 2D T1 MRI. The (A,C,E) segmentations and (B,D,F) segmentations are derived from two different subjects. BV, brain volume; MRI, magnetic resonance imaging.

Arkansas for Medical Sciences and individual consent for this retrospective analysis was waived.

### Volumetry

Volumetry from CT/MRI scans was accomplished using 3D Slicer (v. 4.11.0), FreeSurfer (v. 7.1.1), and volBrain (v. 1.0).

We employed FreeSurfer for T1 MRI analysis using the recon-all function. The graphic representations of segmented volumes were imported into the FreeView visualization module for visual inspection. See *Figure 2A,2B* for examples of FreeSurfer brain segmentations.

We used the volBrain pipeline for T1 MRI analysis. We extracted the Brain (BV) and Intracranial Cavity (ICV) from the PDF data report and visually checked the Intracranial cavity extraction and Macrostructures result images (see *Figure 2C,2D* for examples).

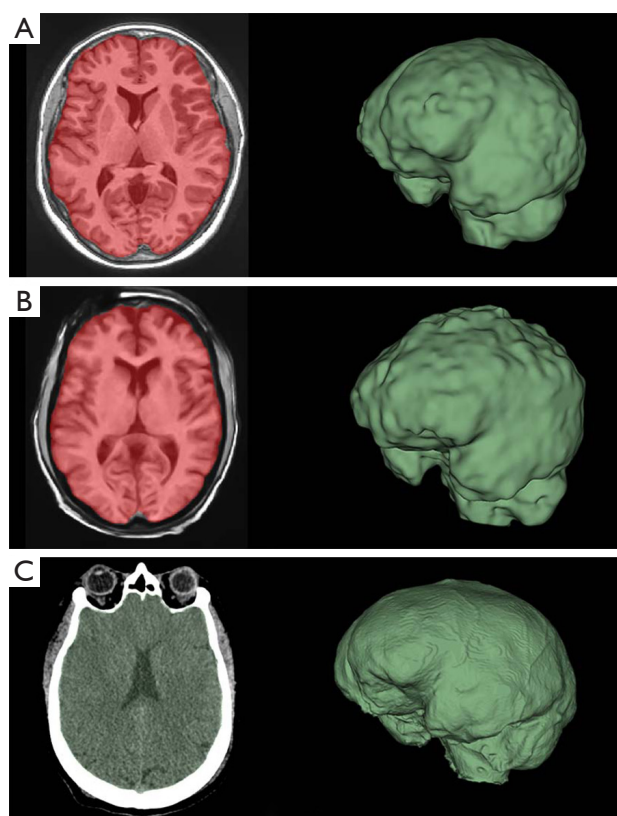
We used 3D Slicer for T1 MRI, non-contrast head CT, and head CT angiogram (CTA) analysis. Slicer

segmentations of BV and ICV were created using a thresholding tool and specialized segmentation extensions such as SwissSkullStripper (20) and Wrap Solidify (21) with minimal use of manual tools such as painting and erasing. In our study, Slicer segmentations of BV and ICV were performed by an investigator (TH) who was blinded to the results from FreeSurfer (performed by different investigator; JH) and volBrain (performed later). Visual inspection for accuracy was performed in the Slicer viewer (see *Figure 2E,2F* for examples).

Each subject in our cohort had specific pathology (ischemic stroke). In each case, we measured the size of the stroke (stroke volumes, SV) on B1000 diffusion-weighted MRI scans (see [Appendix 1](#)). Our data processing resulted in segmentation and calculations of BV, ICV, SV for each subject.

For every volumetry tool used in this study, we exported a sample segmentation into Slicer for 3D visualization and to show large scale differences in accuracy between methods





**Figure 3** Examples of ICV segmentations. (A) volBrain ICV using 3D T1 MRI. (B) volBrain ICV using 2D T1 MRI. (C) 3D Slicer ICV using a non-contrast CT scan. ICV, intracranial volume; MRI, magnetic resonance imaging.

and scan quality (Figures 2,3). The exception was ICV segmentation using FreeSurfer. At the time of this writing, we are aware of no graphic export tools in the current FreeSurfer version for visual verification of intracranial segmentations.

### Data analysis

Results were analyzed in StatPlus in Microsoft Excel. Data analysis included coefficient of determination ( $R^2$ ) with 95% confidence interval (CI) and P value between segmentation methods for BV and ICV. Table 2 provides  $R^2$  values that can be detected with the given sample sizes at 80% and 90% power. Bland-Altman (B-A) plots were generated to evaluate agreement between methods. In the B-A plots (Figures 4,5), the solid horizontal line represents the mean difference between the two methods. The middle-dashed lines represent the 95% CI of  $\pm 1.96$  standard errors of the

**Table 2**  $R^2$  values that can be detected with the given sample sizes at 80% and 90% power

Power	n=39	n=35	n=22	n=13
80%	0.1862	0.2058	0.3120	0.4846
90%	0.2389	0.2627	0.3883	0.5776

mean difference between methods. The outside solid lines represent the limits of normal, which are  $\pm 1.96$  standard deviations from the mean.

### Practical user experience

A multifaceted comparison of volumetry tools from a practical user perspective was performed to compile a list of distinguishing features which has been summarized in Table 3.

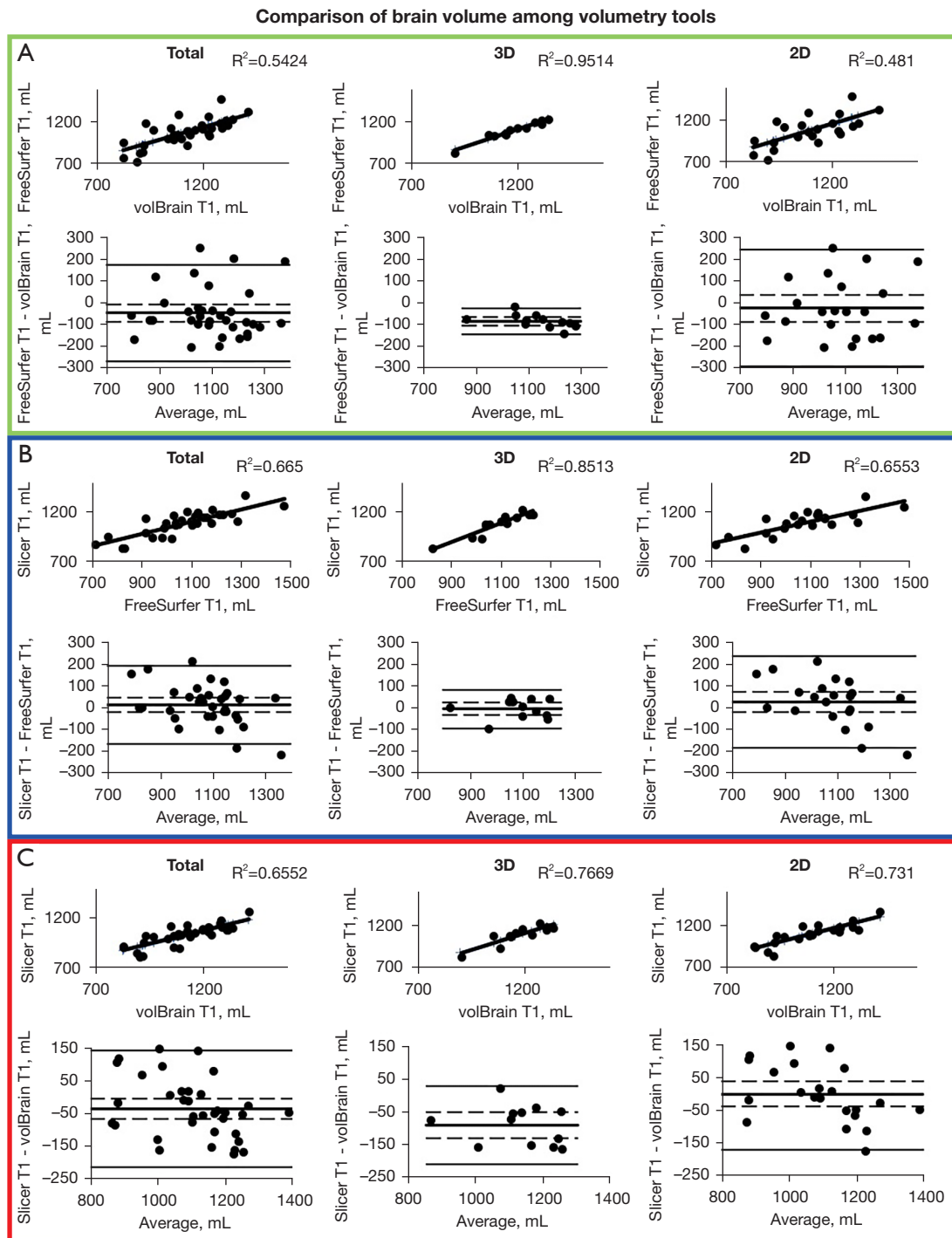
### Instructions

Based on our explorations we were able to consolidate the workflows into detailed instructions for FreeSurfer and Slicer in the Appendix 1. Our aim in providing these instructions are to increase the accessibility for clinical groups regarding volumetric analysis. Highly helpful instructions for using volBrain are included on the volBrain website with video tutorials (<https://volbrain.upv.es/instructions.php>).

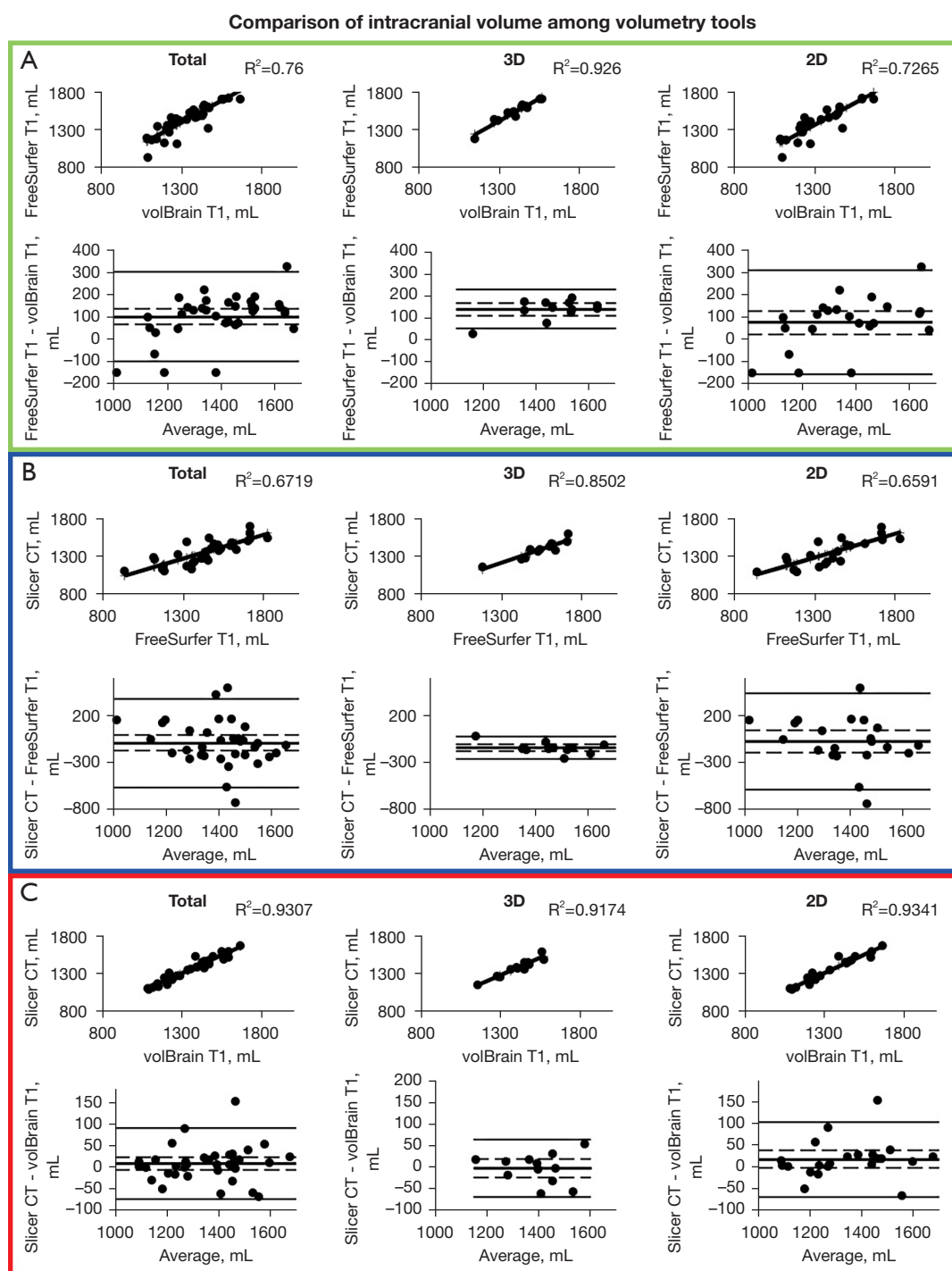
## Results

### Processed scans

In this study, we analyze the inter-method reliability of FreeSurfer, Slicer, and volBrain using real-world scans obtained in a clinical setting where subjects could have been critically ill and/or uncooperative, requiring faster diagnostic scans with MRI slice intervals sometimes greater than 1.1 mm. Therefore, there were two major categories of MRI in this study: 14 subjects had high quality sagittal MRIs with 1.1 mm slice intervals (3D scans), 25 subjects had lower quality axial MRIs with 3–6 mm slice intervals (2D scans); 1 subject (7.1%) in the 3D group and 3 subjects (12.0%) in 2D group failed to complete the recon-all function on FreeSurfer for unknown reasons and were excluded from analysis. Three of these 4 subjects also failed to complete the volBrain pipeline. To keep the sample size consistent, the remaining subject was also removed from all other analysis. This left 13 3D subjects and 22 2D subjects



**Figure 4** BV analysis. FreeSurfer-volBrain (A), Slicer-FreeSurfer (B), and Slicer-volBrain (C) separated by Total, 3D, and 2D scans. Analysis includes coefficient of determination ( $R^2$ ) (above) and B-A plots (below). Total of 35 subjects. Slicer, 3D Slicer; BV, brain volume; B-A, Bland-Altman.



**Figure 5** ICV analysis. FreeSurfer-volBrain (A), Slicer-FreeSurfer (B), and Slicer-volBrain (C) separated by Total, 3D, and 2D scans. Analysis includes coefficient of determination ( $R^2$ ) (above) and B-A plots (below). Total of 35 subjects. Slicer was used to process CT scans for our ICV analysis (in contrast to our BV analysis, in which Slicer was used to process T1 MRI data). Slicer, 3D Slicer; CT, computed tomography; ICV, intracranial volume; B-A, Bland-Altman; BV, brain volume; T1 MRI, T1-weighted magnetic resonance imaging.

**Table 3** Summary of key features of FreeSurfer, 3D Slicer, and volBrain

Software	FreeSurfer	3D Slicer	volBrain
Authors and developers	Laboratory for Computational Engineering at the Athinoula A. Martinos Center for Biomedical Engineering (Charlestown, MA, USA)	Massachusetts Institute of Technology Artificial Intelligence Laboratory, the Surgical Navigation and Robotics Laboratory, and Surgical Planning Laboratory at the Brigham and Women's Hospital (Boston, MA, USA)	José V. Manjón (Valencia Polytechnic University, Valencia, Spain) and Pierrick Coupé (University of Bordeaux, Bordeaux, France)
Internet address	<a href="https://surfer.nmr.mgh.harvard.edu">https://surfer.nmr.mgh.harvard.edu</a>	<a href="https://www.slicer.org">https://www.slicer.org</a>	<a href="https://volbrain.upv.es">https://volbrain.upv.es</a>
Requires download and installation	Yes	Yes	No (i.e., the tool is online)
OS platform	Linux, Mac OS	Linux, Mac OS, Microsoft Windows	Not applicable
Input image modalities	T1-MRI	T1-MRI, DWI-MRI, CT, CTA	T1-MRI
Input file type	DICOM	DICOM	NIFTI
Need for interaction with human user	Minimal	Significant	Minimal
Need for software add-ons	No	Yes	No
Processing speed	Slower	Fast	Fast
Brain anatomical structure labeling	Automatic	Not available	Automatic
Volumetry of distinct pathological imaging findings	Not available	Semi-automated	Not available

MRI, magnetic resonance imaging; DWI, diffusion weighted imaging; CT, computed tomography; CTA, computed tomography angiography; DICOM, digital imaging and communications in medicine; NIFTI, neuroimaging informatics technology initiative.

for analysis.

For every subject, segmentation of the intracranial space on Slicer was performed using high resolution CT scans (both non-contrast and CTAs, with slice thickness 1 mm or less). This method was 100% successful and resulted in a visually accurate segmentation of ICV for every subject.

### Volumetry

Results of the BV correlations between each method are displayed in *Figure 4*. ICV correlation results are displayed in *Figure 5*. Coefficient of determination, 95% CI, and P value for each correlation are shown in *Table 4*. *Table 2* shows  $R^2$  values that can be detected with the given sample sizes at 80% and 90% power. For almost every comparison between methods, our group of subjects with 3D scans showed higher BV and ICV correlations compared to our group of subjects with 2D scans. All correlations were statistically significant ( $P \leq 0.05$ ).

### BV analysis

FreeSurfer and volBrain with 3D scans show the best correlation ( $R^2=0.9514$ ) and agreement for all BV methods we compared (*Figure 4A*). In *Figure 2* we display the visual difference in BV segmentation between two subjects (left-3D MRI; right-2D MRI). It is clear that using 3D MRIs on all three of our reported platforms produces more accurate BV segmentations. This is most evident in the differences in quality of the three-dimensional segmentations between *Figure 2A,2C,2E* (3D scans) and *Figure 2B,2D,2F* (2D scans).

Interestingly, there is a relatively wide discrepancy in correlation between 3D and 2D scans in our FreeSurfer-volBrain comparison (*Figure 4A*). FreeSurfer-Slicer and volBrain-Slicer comparisons showed a smaller difference between 3D and 2D scans in both cases (*Figure 4B,4C* respectively). We suggest that good inter-method reliability for BV can only be shown between FreeSurfer and volBrain using 3D T1 MRIs. However, compared to FreeSurfer and Slicer, volBrain might provide a better



**Table 4** R<sup>2</sup> values and corresponding 95% CI assessing the association between volume estimates obtained using different imaging methods

Quality	Comparison	R <sup>2</sup>	95% CI	P value
ICV				
Total	Slicer_CT vs. FreeSurfer	0.6719	0.4543 to 0.7743	<0.0001
	FreeSurfer vs. volBrain	0.7600	0.5867 to 0.8355	<0.0001
	Slicer_CT vs. volBrain	0.9307	0.8739 to 0.9522	<0.0001
3D	Slicer_CT vs. FreeSurfer	0.8502	0.5224 to 0.9041	<0.0001
	FreeSurfer vs. volBrain	0.926	0.7231 to 0.9484	<0.0001
	Slicer_CT vs. volBrain	0.9174	0.7980 to 0.9632	<0.0001
2D	Slicer_CT vs. FreeSurfer	0.6591	0.3462 to 0.7818	<0.0001
	FreeSurfer vs. volBrain	0.7265	0.4532 to 0.8262	<0.0001
	Slicer_CT vs. volBrain	0.9341	0.8525 to 0.9578	<0.0001
BV				
Total	Slicer_T1 vs. FreeSurfer	0.6650	0.4351 to 0.7648	<0.0001
	FreeSurfer vs. volBrain	0.5424	0.2766 to 0.6770	<0.0001
	Slicer_T1 vs. volBrain	0.6552	0.4316 to 0.7631	<0.0001
3D	Slicer_T1 vs. FreeSurfer	0.8513	0.5640 to 0.9140	<0.0001
	FreeSurfer vs. volBrain	0.9514	0.8410 to 0.9713	<0.0001
	Slicer_T1 vs. volBrain	0.7669	0.3980 to 0.8712	<0.0001
2D	Slicer_T1 vs. FreeSurfer	0.6553	0.3325 to 0.7756	<0.0001
	FreeSurfer vs. volBrain	0.481	0.1337 to 0.6610	<0.0004
	Slicer_T1 vs. volBrain	0.7310	0.4605 to 0.8291	<0.0001

The methods being compared are described in the Comparison column of the table. P values are from tests comparing the observed R<sup>2</sup> value to 0 (zero; no association). CI, confidence intervals; ICV, intracranial volume; Slicer, 3D Slicer; CT, computed tomography; BV, brain volume; T1, T1-weighted magnetic resonance imaging.

segmentation when 2D scans are used. By comparing *Figure 2D* (volBrain) with *Figure 2B* (FreeSurfer) and *Figure 2F* (Slicer), volBrain provides better large-scale accuracy when using 2D scans.

### ICV analysis

FreeSurfer ICV segmentations correlate well with Slicer and volBrain when using 3D scans (R<sup>2</sup>>0.850 in both cases) (*Figure 5A,5B*). However, though these are strong correlations, our B-A plots indicate that FreeSurfer consistently overestimated ICV compared to Slicer and volBrain (*Figure 5A,5B*). Using 3D scans, FreeSurfer overestimates ICV by an average of 143.43 mL (10.40%) compared to volBrain (T1) (P<0.0001) (95% CI: 115.72–171.13) and 148.09 mL (10.78%) compared to Slicer (CT)

(P<0.0001) (95% CI: 111.14–185.03). *Figure 3* does not include a three-dimensional segmentation for ICV using FreeSurfer, because FreeSurfer does not provide an ICV segmentation.

The Slicer method of segmenting the intracranial space is not split into 3D and 2D scans because CT scans were used; however, we compared this method with FreeSurfer and volBrain for each respective subject (*Figure 5B,5C*). ICV segmentations from CT scans using Slicer were visually accurate (*Figure 3C*). We also show that volBrain creates convincing segmentations of ICV (*Figure 3A,3B*), which correlate and agree very well with this Slicer method (R<sup>2</sup>=0.9307) regardless of scan quality (*Figure 5C*). Due to high correlation, agreement, and visual accuracy, we report excellent inter-method reliability between volBrain (T1) and Slicer (CT) segmentations of ICV.

## Discussion

Though there is no study that confirms the accuracy of these methods compared to “ground truth”, there are some important considerations in acquiring an accurate segmentation. A method’s accuracy can be visually assessed by its ability to differentiate a region of interest from other parts of the scan (small-scale) and its ability to create an acceptable segmentation (large-scale), which is inherently limited by scan quality and slice interval (for examples, see the segmentations overlaid on T1 axial MRI sections and the differences in segmentation quality between 2D and 3D scans in *Figures 2,3*).

### Visualization of segmentations

Though FreeSurfer provides a visualization GUI, Freeview, which allows for checking of segmentations in orthogonal planes, it does not provide a user-friendly method of assessing the large-scale accuracy of the total segmentation in three-dimensional view.

An advantage of using Slicer in this study is its ability for quality checking of segmentations in three dimensions. Not only were we able to check our Slicer-generated segmentations using the Slicer viewer, but we also report methods of importing FreeSurfer and volBrain results into this viewer. In this study, these methods were employed to generate *Figures 2,3*. Such simple methods of visualization can be invaluable to a research group with limited expertise in computer science (see [Appendix 1](#) for more information).

### FreeSurfer

Though some studies have raised questions about the accuracy of FreeSurfer volume calculations (22,23), FreeSurfer is often used as a standard against which other automated volumetry software are often compared (4,8-10). Using the described methods, the results of BV calculations in our study are similar between FreeSurfer, Slicer, and volBrain when good quality 3D T1-MRI scans are used. However, the inter-method reliability between these methods is lesser when using 2D T1-MRIs with higher slice thickness. It is important to note, that the FreeSurfer website does recommend using MRI data with thickness no larger than 1.5 mm (24). This means that any research group interested in reliable volumetric analysis of the brain must ensure that high-quality 3D T1 MRIs are acquired. This may be a limiting factor for volumetric imaging

analysis in certain clinical settings.

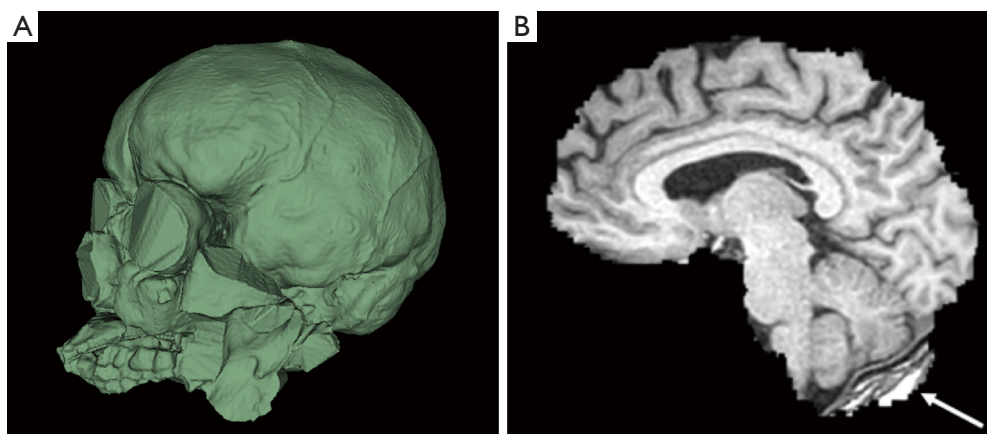
It has been suggested by the developers of FreeSurfer that it would be best to calculate ICV by directly counting voxels; however, the difficulty of distinguishing between cortical bone and CSF on T1 images due to similar MR intensity prevents them from doing so (25). Instead, FreeSurfer utilizes a relationship between the ICV and the BV following the approach of Buckner *et al.* (26) and scales the BV to correspond to that of a known atlas image, and then uses the corresponding scaling factor to estimate ICV (22,23). According to a study by Klasson *et al.* (22), this FreeSurfer method of estimating ICV is biased by total BV. Another study published in 2015 showed that FreeSurfer calculates an ICV which correlates to manual segmentation with an  $R^2=0.801$  (23). In this study, though FreeSurfer ICV showed high correlation with other methods, it appears to overestimate the ICV by a significant margin compared to other methods which show strong agreement. These overestimations and the inability to check the FreeSurfer ICV segmentation cast significant doubt upon the accuracy of FreeSurfer ICV calculations.

FreeSurfer offers an advantage by providing a segmentation and volume for every anatomical brain structure (*Figure 2A,2B*), which can be useful for further analysis if needed.

### 3D Slicer

Slicer consistently provides a visually convincing segmentation of the total intracranial space when high resolution CT scans are used—in contrast to FreeSurfer which only provides an estimated ICV without a visual segmentation, thus precluding visual inspection for accuracy. If high resolution non-contrast CT is not available, a modern CTA can be used for the above-described method of ICV segmentation but may require more manual editing (see instructions in [Appendix 1](#) for further information). Notably, we identified a commonly encountered ICV segmentation error using CT scans (*Figure 6A*), but this was overcome using the procedure we provide in the [Appendix 1](#).

ICV from CT is decidedly accurate compared to MRI because of the contrast between tissue and bone and the minimal geometric distortions inherent to CT technology as opposed to MRI (27). We suggest comparing this method to other commercial volumetry software in future studies and potentially using Slicer and modern volumetric CT scans as a method of choice for accurate ICV calculations.



**Figure 6** 3D Slicer segmentation errors commonly encountered in this study. (A) Slicer ICV error involving the segmentation escaping from the intracranial space into the soft tissue. (B) SwissSkullStripper failure to remove part of the skull on a T1 MRI. Arrow indicates section of skull included in segmentation. ICV, intracranial volume; T1 MRI, T1-weighted magnetic resonance imaging.

BV segmentation using Slicer with T1 MRIs requires skull stripping and thresholding of the brain. In our experience, this is a less accurate and more tedious segmentation method compared to FreeSurfer and volBrain which are fully automated. At times, Slicer removes part of the brain when stripping the skull. Other times, Slicer leaves part of the skull “un-stripped” (*Figure 6B*). These errors can be fixed using manual tools, but the process can be tedious in many cases (see [Appendix 1](#)).

### volBrain

In this study, we found volBrain to be the most efficient and user-friendly method we explored. The instructions found online are easy and self-explanatory, which overcomes some of the most difficult aspects of implementing volumetric imaging analysis. To convert the subject DICOM to NIFTI, the user must simply install dcm2nii and “click and drag” the DICOM file into the GUI. volBrain results include screenshots in axial, coronal, and sagittal planes (examples of axial screenshots are included in *Figure 2C,2D*). Additionally, volBrain provides processed NIFTI files on its website which can be loaded as data into Slicer (see [Appendix 1](#)) or MRICron (volBrain recommended) for 3D viewing. volBrain results also include an analysis of the asymmetry of the brain, the percentage of the intracranial cavity occupied by brain tissue (see Intracranial reserve analysis in the [Appendix 1](#)), and subcortical structure labeling.

In *Figure 2C,2D*, we show that volBrain provides

sufficiently accurate visual segmentations of the brain even when 2D scans are used. Similarly, *Figure 3A,3B* show that volBrain produces good intracranial cavity segmentations regardless of scan quality. However, these segmentations are not as accurate as those derived from Slicer using CT scans (*Figure 3C*). Nevertheless, of the methods we tested with T1 MRI data, volBrain provides the most reliable segmentations of BV and ICV if T1 MRI quality is limited. In contrast to FreeSurfer, there is no explicit recommendation regarding T1 MRI slice thickness on the volBrain website.

### Supra- and infratentorial volume

Another advantage to FreeSurfer and volBrain are their ability to automatically separate the supratentorial and infratentorial brain compartments. There are clinical situations where interplay between supratentorial and infratentorial volumes becomes a critical factor in determining spatial and temporal dynamics of brain shift (which are upward and downward transtentorial brain herniations). A few common clinical examples are posterior fossa tumors, malignant middle cerebral artery strokes, supratentorial intra- and extra-axial hematomas, and obstructive hydrocephalus. Therefore, it may be important for investigators studying intracranial space-occupying lesions to know accurate supra- and infratentorial compartment volumes.

Since the tentorium cerebelli is dome-shaped, segmenting these volumes using Slicer would require

tedious manual correction in three dimensions. If investigators require an estimation of BV above or below the tentorium cerebelli, FreeSurfer consistently provides visually accurate segmentations using high quality scans. volBrain can also provide an estimation of the supratentorial volume if the user subtracts the cerebellum and brainstem (both provided in result file) from the total BV. Therefore, it may be practical to use FreeSurfer or volBrain to calculate these volumes in a study involving space-occupying pathology.

### ***Workflow efficiency***

It is important to consider the efficiency of a software in clinical practice or research setting. FreeSurfer data processing is time consuming and requires substantial computing power (FreeSurfer processing took roughly 6 h on our PC workstation running Ubuntu 20.04.1 with an AMD Ryzen 5 1600 processor and 32 GB of DDR3 memory), which may not suit well to time-sensitive clinical practice today. Its practicality will likely improve following Moore's law, which observes that transistor density on computer chips roughly doubles each year.

Slicer was significantly faster than FreeSurfer in our experience and could provide ICV and BV segmentations of one subject in  $\approx 30$  minutes on our workstation. Slicer, however, requires more user interaction through a GUI—in contrast to FreeSurfer which performs roughly 6 h of unsupervised calculations after typing a few simple commands.

volBrain was the most efficient and easy to use. Due to online nature of volBrain, it avoids the need for a dedicated workstation but, at the same time, requires imaging study anonymization in order to comply with patient confidentiality. In addition, volBrain cannot directly process DICOM format images and requires a simple file conversion using a free program (dcm2nii) followed by an upload to the volBrain website. Unlike FreeSurfer, volBrain provides quicker processing time and allows for multiple subject processing at once (up to 10 scans daily with the current version) (19).

### **Conclusions**

This study provides results of volumetric imaging analysis of the brain and intracranial space in a clinically representative cohort of subjects. All three tools—3D Slicer, FreeSurfer, and volBrain—are free, reliable, require no

complex programming, but still have certain limitations and significant differences.

Using high-resolution non-contrast CT scans and CTAs, Slicer provides a simple and visually accurate method of segmenting the intracranial cavity. volBrain also provides a fully automated, visually accurate, and easy method, which agrees with Slicer. Both of these methods may be more trustworthy than T1 MRI-derived FreeSurfer ICV calculations, which may overestimate the true value even when using high-quality 3D T1 scans. For BV, all three of our methods showed good correlation and agreement, with visually accurate segmentations when using 3D scans. volBrain may provide a better segmentation of the brain when T1 MRI quality is limited.

Slicer allows for processing of multiple types of scans, while FreeSurfer and volBrain only use T1 MRI scans. Slicer also allows for easy manual editing of the output and 3D visualization of the results, while FreeSurfer and volBrain require complex procedures to edit and visualize their segmentations in three dimensions. However, unlike Slicer, both FreeSurfer and volBrain allow for fully automated BV and ICV calculations.

Our study highlights the importance of acquiring high quality volumetric (3D) T1 MRI scans if these methods of segmentation are to be implemented for research or integrated into clinical workflow. volBrain provides the easiest method for segmentation, whereas Slicer provides the most user involved segmentation experience. We provide a practical guide to decrease the barrier to entry for research and clinical groups to perform volumetric imaging analysis with these methods. The readers should be able to select the right volumetry tool based on our study findings and then follow our step-by-step instructions to accomplish specific volumetry tasks.

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## Footnote

**Reporting Checklist:** The authors have completed the MDAR checklist. Available at <https://qims.amegroups.com/article/view/10.21037/qims-21-958/rc>

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <https://qims.amegroups.com/article/view/10.21037/qims-21-958/coif>). VP reports that he is a consultant for Medtronic. Consulting fees are paid directly to University of Arkansas for Medical Sciences. VP did not receive any funds. Medtronic Navigation provided equipment (laptop computer) and software (StealthViz) for the study. VP owns Medtronic Plc (MDT) stocks. The other authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Radiographic scans are completely anonymized and there are no patient identifiers mentioned within the text. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional board of the University of Arkansas for Medical Sciences and individual consent for this retrospective analysis was waived.

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Appendix 1

Further considerations and limitations

3D Slicer volumetry results

Slicer provides two different types of volumetry results: labelmap volumes and closed surface volumes. The developers of the application have reported minimal differences between the two methods of volume calculation, as long as the method is used consistently (28). They likely reflect different internal algorithms of Slicer. In our study, we chose labelmap volumes, as they were the default output. We generated a closed surface 3D representation of BV, ICV, and SV for visual inspection to detect segmentation errors. We also reviewed segmentations in orthogonal planes but did not use Closed Surface statistics for any of our calculations.

Intracranial volume with 3D Slicer

If high resolution non-contrast CT is not available, a modern CTA can be used for the described method of ICV segmentation but may require more manual editing due to the hyperdense cerebral vessels that can be “mistaken” as a bone by Slicer due to almost similar Hounsfield unit value or CT density (discussed in Instructions for segmentation using 3D Slicer). Of note, the module Wrap Solidify takes more time to process CTAs (minutes) than non-contrast CT scans (seconds).

Importantly, if the CT scan was done with a gantry tilt, it will provide inaccurate ICV segmentations, due to

distortion of the sagittal plane image. Thus, 0-degree gantry tilt CT scans or CTA must be used for this method on the current Slicer versions.

FreeSurfer limitations

FreeSurfer was unable to process T1 MRI scans of 4 subjects (10.2%) and these were excluded from the study. Of note, one BV segmentation on FreeSurfer was not anatomically accurate in one patient as the clivus was segmented as brainstem. Table S1 shows FreeSurfer processing errors.

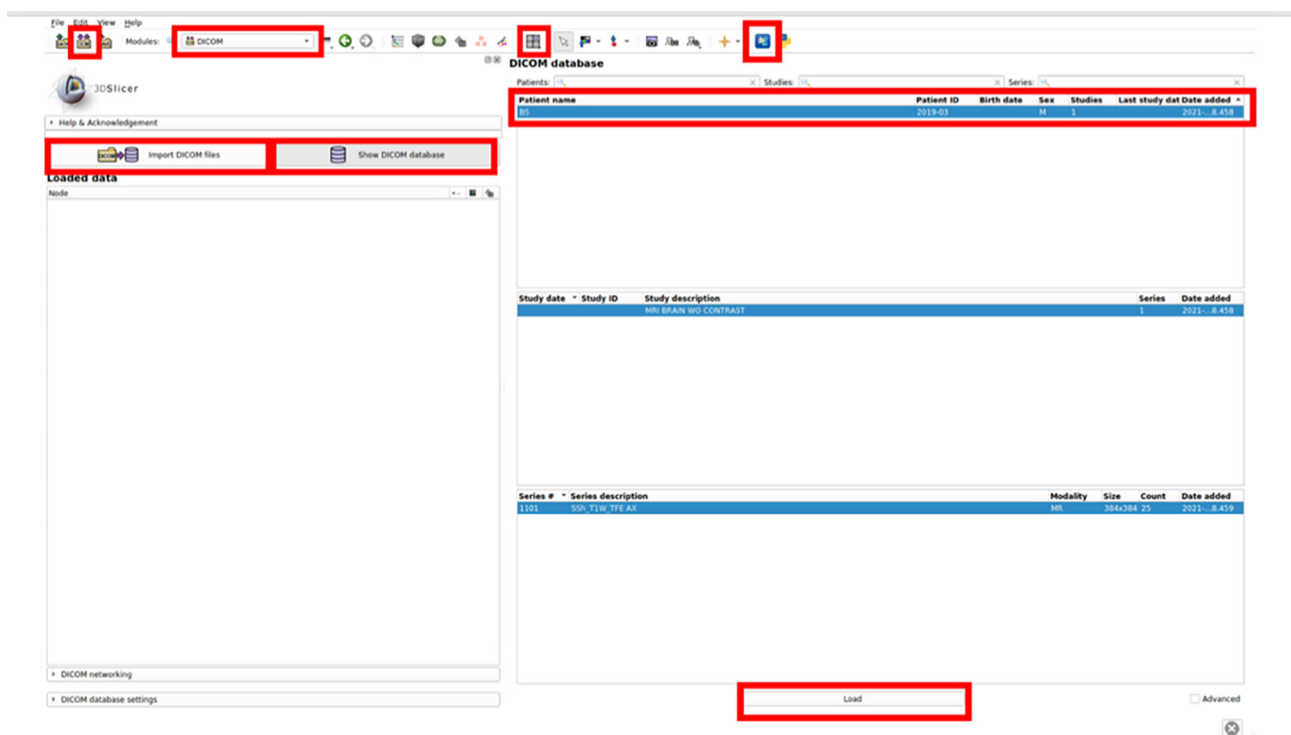
For FreeSurfer version 5.2 and later, the brainstem is not included in the Morphometry Stats and Global Measure of Volume, because the volume of brainstem changes depends upon the field of view of the volume acquisition (29). In this study, we added the brainstem volume in the aseg.stats output file from FreeSurfer, in efforts to counteract a false negative skew of the data.

According to Gronenschild *et al.*, it is important to use the same version of FreeSurfer and the same OS and workstation (30). Thus, all segmentations in this study were created using the same workstation and the same version of FreeSurfer and Slicer. Though the scans in our study were obtained on different MRI scanners, it has been shown that FreeSurfer total brain volume measurements are consistent across MRI field strengths (31), but it is important to note that there was variation among T1 quality in our study. FreeSurfer does recommend good quality T1-weighted MRI scans with slice intervals no larger than 1.5 mm (24).

Table S1 FreeSurfer processing errors

Error case	Scan quality	Problem with T1 MRI	FreeSurfer error log	Acceptable Slicer T1 segmentation? (+/-/?)
1	Axial 2D 3 mm	Defect in T1 MRI (missing part of left temporal lobe)	“Topological defect” <sup>†</sup>	-/?  Defect apparently interfered with skull stripping and output a white brain mask
2	Sagittal 3D 1.1 mm	Left hemisphere defect (possible massive infarct invading ventricle)	“Topological defect” <sup>†</sup>	-/?  Skull stripping removed part of occipital lobe
3	Axial 2D 3 mm	Likely movement artifact, and right temporal lobe displaced in coronal plane	“Talairach failed!”	+
4	Axial 2D 6 mm	Unable to import MRI correctly into FreeSurfer	“Talairach failed”	+/?  Segmentation appeared blocky: likely attributable to slice thickness
5	Axial 2D 3 mm	Clivus segmented as cerebellum	N/A	+

<sup>†</sup>, often happens when cerebellum or dura are not removed from wm.mgz.



**Figure S1** 3D Slicer GUI of DICOM database. Relevant icons are highlighted in red. GUI, graphical user interface; DICOM, digital imaging and communications in medicine.

The current FreeSurfer version unable to export segmentations to be further used on modern surgical navigation systems; at the time of this writing, the authors were unable to find an additional postprocessing solution for this. However, FreeSurfer and volBrain segmentations can be uploaded into Slicer for visual inspection in 3-dimensions (see below), with the exception of FreeSurfer ICV segmentations.

### Instructions for segmentation using 3D Slicer

#### Getting started and loading a DICOM image

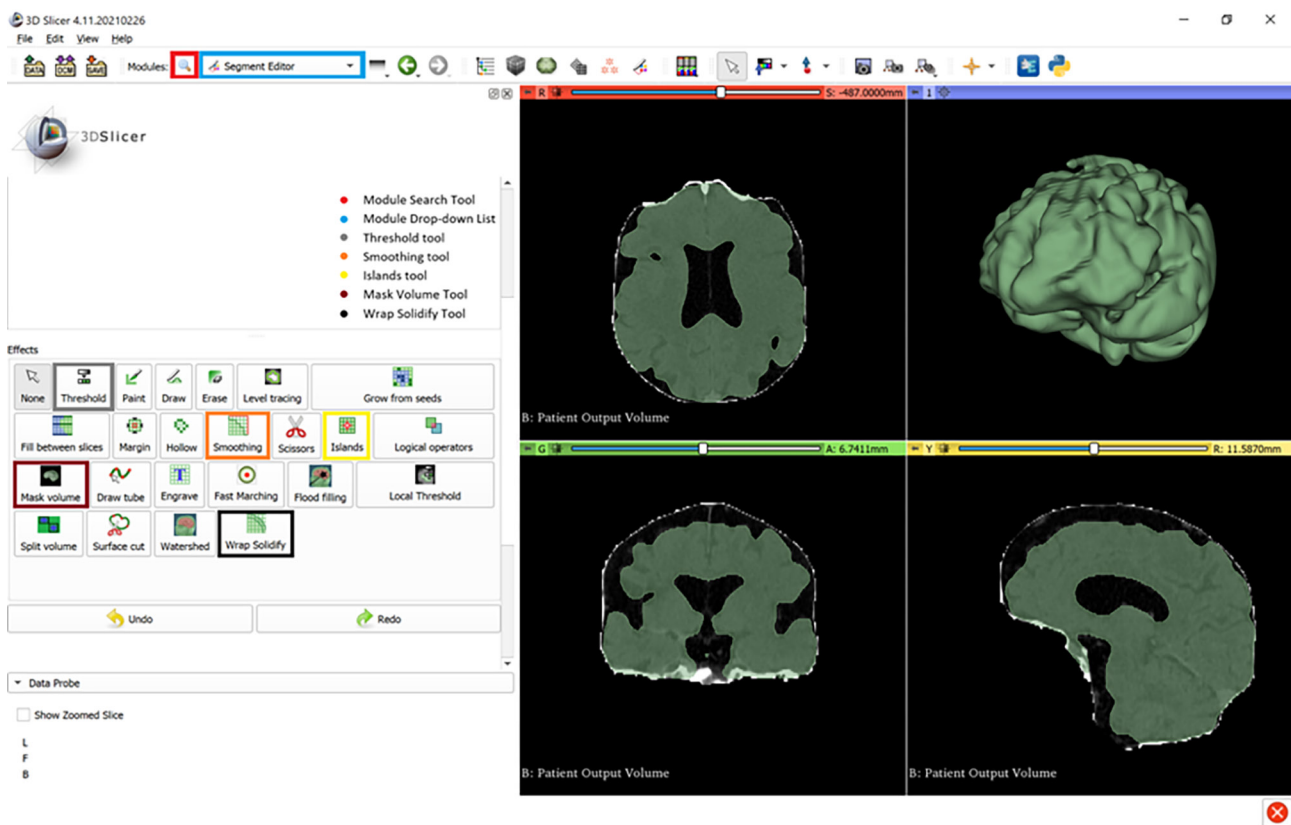
1. Using the Extensions Manager (*Figure S1*), Install 3D Slicer extensions: *SegmentEditorExtraEffects*, *SurfaceWrapSolidify*, and *SwissSkullStripper* (20).
2. Click the DCM icon in the top left of the GUI (*Figure S1*).
3. Click *Import DICOM files* (*Figure S1*).
4. Find the DCM files of interest in the local file system.
5. Click *Import*.
6. Click *Show DICOM database* (*Figure S1*). The DICOM database should be visible instead of the Viewer on the

right side of the screen.



7. In the DICOM database, click the correct patient under *Patient Name* (*Figure S1*) and click *Load* (bottom of *Figure S1*).
8. Most of the modules for these instructions will be in the *Modules* drop-down menu in the application menu at the top of *Figure S1*.
9. The view can be adjusted in the *Layout Toolbar* in the application menu.
  - a. It is recommended to use the *Four-Up* view in this study.
10. More information can be found on navigating the GUI on the 3D Slicer documentation page (32).

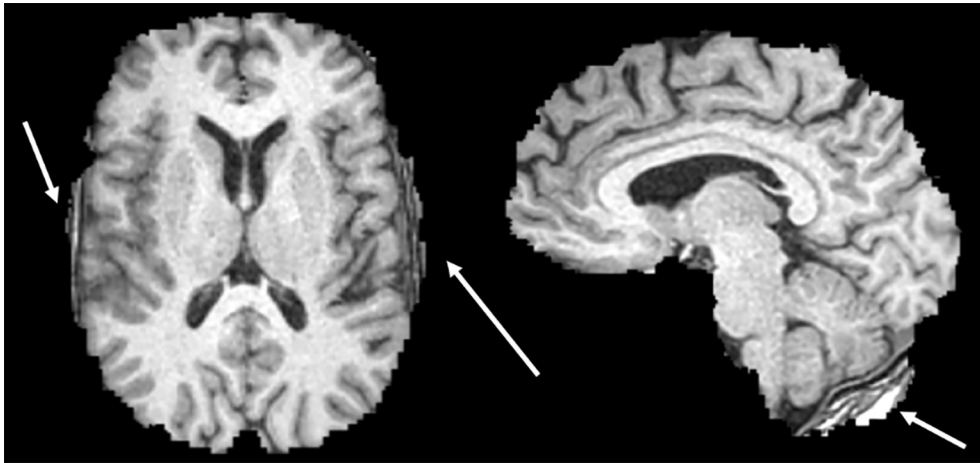
#### Brain volume segmentation using T1 MRI

- The skull must first be removed via skull stripping.
- 3D Slicer version 4.11.0 needs additional installation of *Swiss Skull Stripper* (for instructions on how to install, see above).
- Load T1 MRI DICOM file.
- Select *Swiss Skull Stripper* module within the *Segmentation* tab under the *Module Drop Down List*



**Figure S2** 3D Slicer graphical user interface of Segment Editor module. Relevant icons are highlighted in color.

- menu (Figure S2).
- The *Input Patient Volume* should be the T1 MRI DICOM.
- Click the *Patient Output Volume* drop-down menu.
  - Choose “Create New Labelmap Volume”.
  - The *Patient Mask Label* should be listed as the same name as the one created above (i.e. “Patient Output Volume”).
- Click *Apply*. This will create a multicolored label map of the brain.
  - Note: sometimes skull stripping contains errors (Figure S3).
- Click the *Volumes* module.
- Change *Active Volume* to the volume named above (i.e., “Patient Output Volume”).
- Choose *Gray* from the *Lookup Table* under the *Display* tab. This will create an output volume with intensity contrast between the brain and ventricles.
- Change module to *Segment Editor*. Under *Master volume*, select *Patient Output Volume*.
- Click *Add* .
- Click the box  next to the *Segmentation* drop-down menu to align slice views to the segmentation.
- Under *Effects*, select *Threshold*, and tweak the threshold intensity range to include all white and gray matter of brain, but not so broad that it includes any part of the ventricles. (Note: skull stripping is often imperfect (Figure S3), and areas of skull, dura, optic nerve, or posterior eye may appear outside the brain. Imperfect skull stripping can be manually removed using the scissor tool, the eraser tool (select sphere brush, in 3D plane), and smoothing effect (select *opening remove extrusions*) in the *Segment Editor* module. There are, occasionally, some hyperintensities noted in the ventricles which can also be manually excluded (see Figure S2 for relevant segmentation effects).
- Select the *Segment Statistics* module under *Quantification*. Select the segmentation that was previously created and click *Apply*.
- The brain volume will be found under *Volume* (calculated by the *Labelmap Statistics* plugin) (28).



**Figure S3** Skull strip errors in 3D Slicer.

### Intracranial volume segmentation using CT/CTA

- These instructions are heavily derived from the methods outlined by Lassoan on GitHub at <https://lassoan.github.io/SlicerSegmentationRecipes/CTSkullStripping> (33).
- Load non-contrast CT or CT angiogram DICOM file. Of note, current Slicer versions require CT scans acquired with 0-degree gantry tilt.
- Choose “Segment Editor” module → click “Add” button to create a new segment. (be sure the *Master Volume* is the CT scan).
- Select “Threshold” effect and set intensity range of bone (from approximately 300 to maximum value) → click *Apply* button.
- Remove small spots of segmentation left in the brain by using the *Islands* effect (choose *Keep largest island* option) → click *Apply* button.
  1. Note: CT angiogram may have hyperdense vessels with contrast in the intracranial space. In some instances, these vessels are not removed by the *Islands* effect. These can be manually removed using the *Eraser* tool (also choose *sphere brush* and *erase in 3D*). Be careful not to erase the segmented skull.
- Select *Wrap Solidify* effect
- Set *Region* to *Largest cavity*; enable *Split cavities* and set *Cavity Size* slider to 8 mm → click *Apply* button. This may take several minutes.
- The resulting segmentation should resemble *Figure S4A*. However, sometimes the segmentation has errors resembling *Figure S4B*.

- If this happens, undo the *Wrap Solidify* step by clicking *undo*.
- There are 2 options to fix this segmentation error.
  1. If the thresholding step did not include all of the skull margins, this is your problem. Adjust the thresholding intensity range to include all of the skull.
  2. If option 1 does not work, use the *paint* tool to paint the sinuses (click *Sphere brush* and *Edit in 3D* views) (*Figure S4C*). Note: This works best in the sagittal view. Then re-do the *Wrap Solidify* step.
- Select *Scissor* effect and delete segmentation below foramen magnum plane (this works best in the sagittal view).
- Calculate volume using *Segment Statistics* module (28).

### Stroke volume segmentation using DWI B1000 MRI

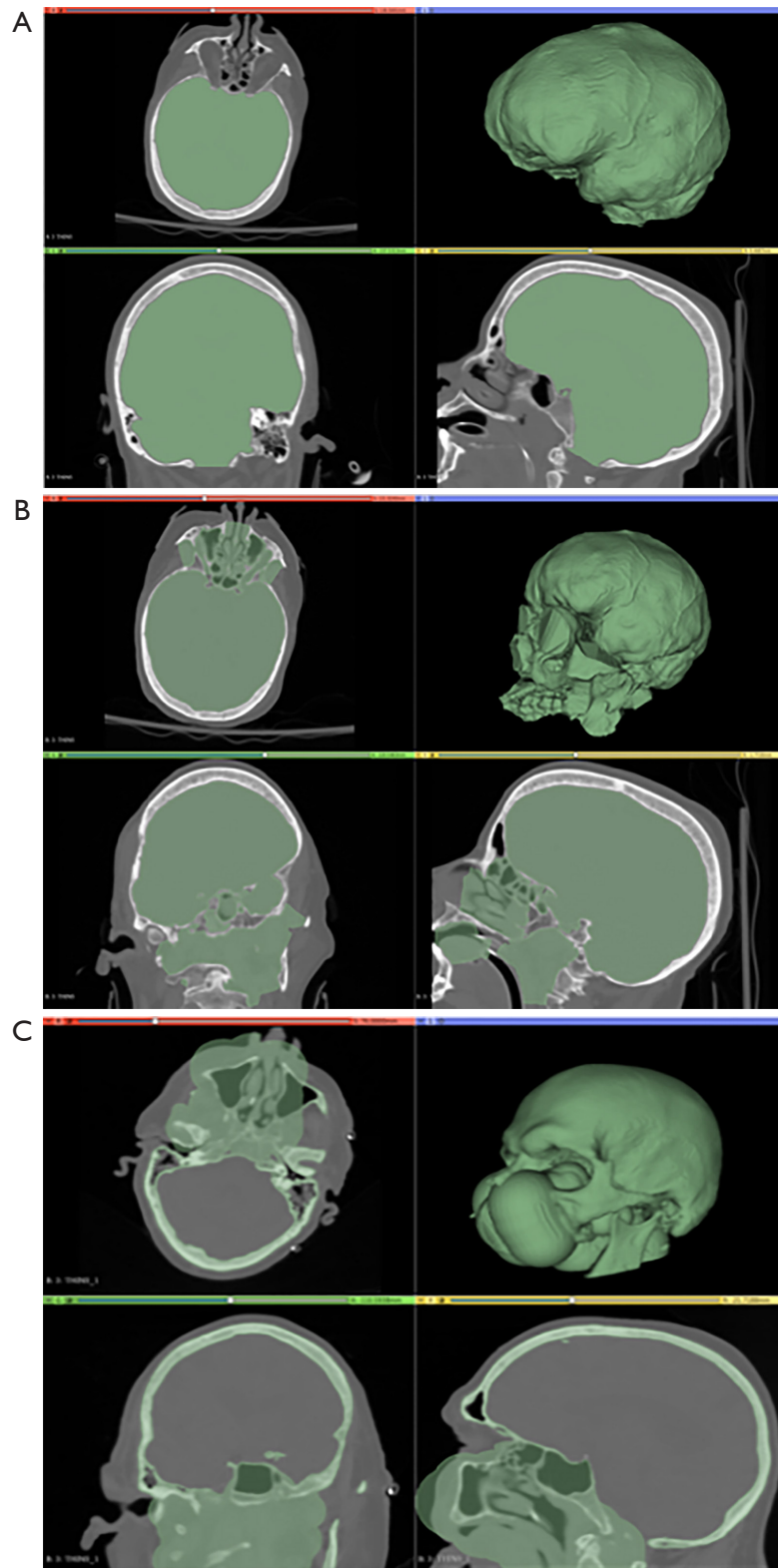
- Load B1000 DWI MRI.
- Choose *Segment Editor* module and create new segmentation.
- Tweak the threshold intensity range to include the entire area of hyperintensity that indicates infarcted tissue.
- Click *Apply*.
- Calculate volume using *Segment Statistics* module.
- Results are seen in *Figure S5*.

### Instructions for segmentation using FreeSurfer

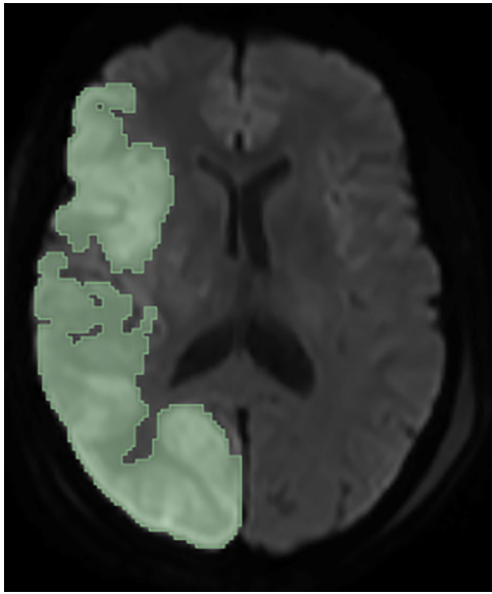
#### Getting started

- Brain volumes and intracranial volumes are calculated using T1 MRI scans on *FreeSurfer* version 7.1.1.





**Figure S4** Intracranial volume considerations. (A) Intracranial volume segmentation using Slicer. (B) Segmentation error due to Slicer difficulty recognizing margins of the intracranial space (best seen on sagittal view). (C) Correction of segmentation error (B) using the paint tool.



**Figure S5** SV segmentation using DWI B1000 MRI. SV, stroke volume; DWI, diffusion weighted imaging; MRI, magnetic resonance imaging.

- Of note: FreeSurfer only processes T1 MRI scans of the brain.
- Open a terminal or command line and set up an environment using the following commands (can copy & paste).
  - o `export FREESURFER_HOME=$HOME/FreeSurfer`
  - o `export SUBJECTS_DIR=$FREESURFER_HOME/subjects`
  - o `source $FREESURFER_HOME/SetUpFreeSurfer.sh`
- Additional Information on installation: <https://surfer.nmr.mgh.harvard.edu/fswiki/Installation>
- Additional Information on setup: <https://surfer.nmr.mgh.harvard.edu/fswiki/SetupConfiguration>
- After *FreeSurfer* (FreeSurfer) is downloaded and installed on a workstation, move all subject DICOM folders to the *freesurfer/subjects* directory.
  - o These instructions assume that DICOM files are stored in a separate folder specifically named for each patient (*patientname*)

#### Brain volume and intracranial volume segmentation using T1 MRI scans

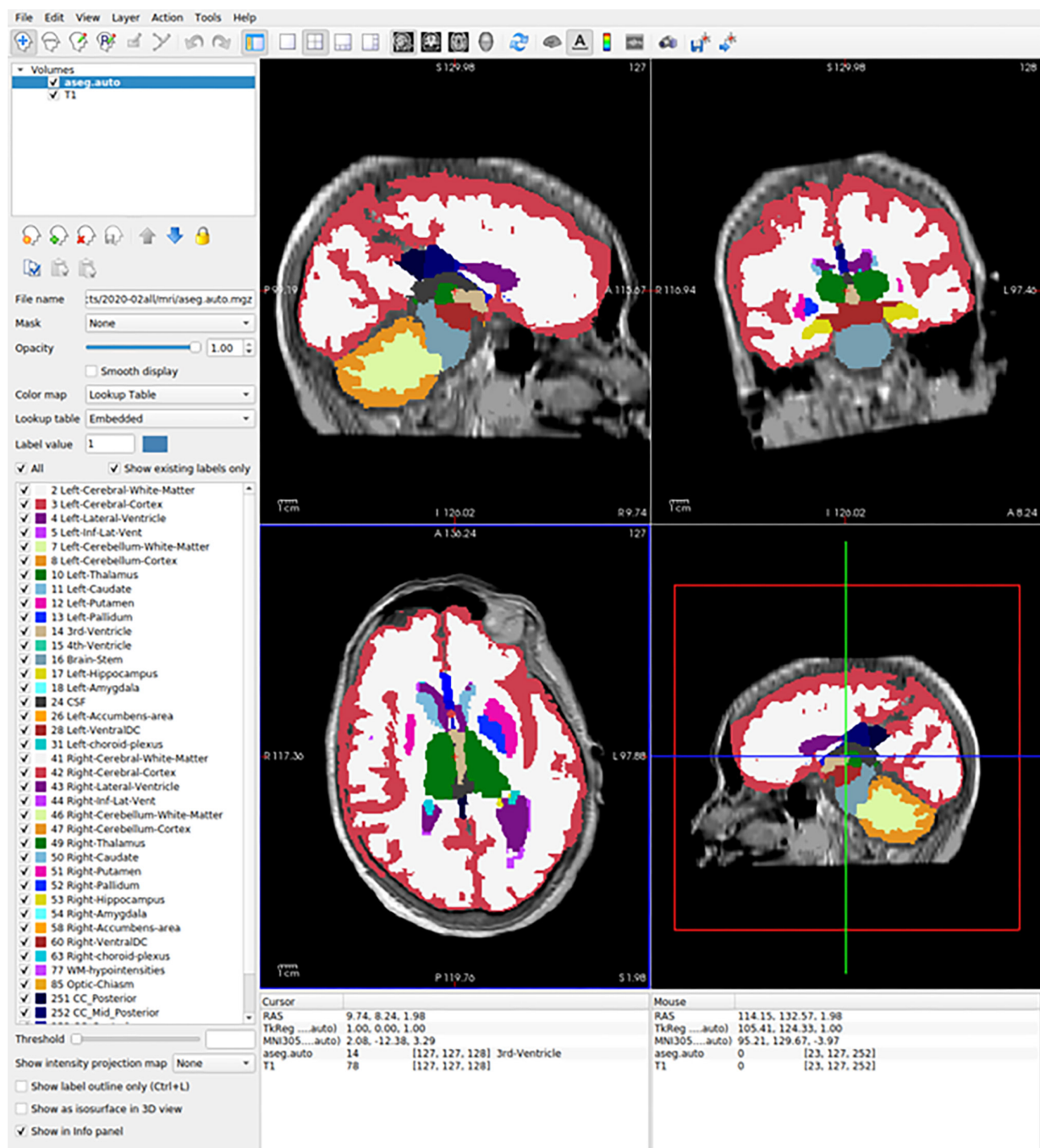
- Within a command line, navigate to the *freesurfer/subjects* directory and locate the directory with the T1

DICOM files of interest (i.e., *patientname*).

- o Ensure that your terminal is *not* in the *patientname* directory, but the *subjects* directory.
- Type:
  - o `dcmunpack -src patientname`
  - o *FreeSurfer* will then interrogate the directory to find the anatomical DICOM file (*anatomical.dcm*)
- Copy the *anatomical.dcm* file.
- Within the command line, navigate into the *patientname* directory.
- Type the following command:
  - o `recon-all -i anatomical.dcm -subject newdirectory -all`
  - o *FreeSurfer* will create a new directory in *freesurfer/subjects* in which all of the output data will be stored.
  - o *newdirectory* can be named anything of your choosing.
- *FreeSurfer* will run recon-all. This may take several hours.
- Volume data can be found in the *aseg.stats* output file under the *stats* directory in the *newdirectory*. The volume is reported in mm<sup>3</sup>, which can be converted to cm<sup>3</sup> or mL.
- The *BrainSegVolNotVent*, provides the brain segmentation volume without the ventricles (BV). Note: the *aseg.stats* file also provides a *SupratentorialVolNotVent* value for the brain tissue above the tentorium cerebelli, excluding the ventricles. The *EstimatedTotalIntraCranialVol* provides the volume of the entire intracranial space.
- The FreeSurfer wiki states that for version 5.2 and later, the brainstem is not included in the global measures of volume because the amount of brainstem changes depending upon the field of view of the volume acquisition (29).
- You can measure the volume of the brainstem and add it to the *BrainSegVolNotVent*.
- Type:
  - o `mri_segstats --ctab-default --seg aseg.mgz --id 16 --sum brainstem.dat`
- This command will provide the volume of the brainstem.

#### Viewing results of recon-all in FreeView

- Navigate to */subjects/patientname/newdirectory/mri*
  - o “*patientname*” in the above line being the earlier directory name chosen for patient name.



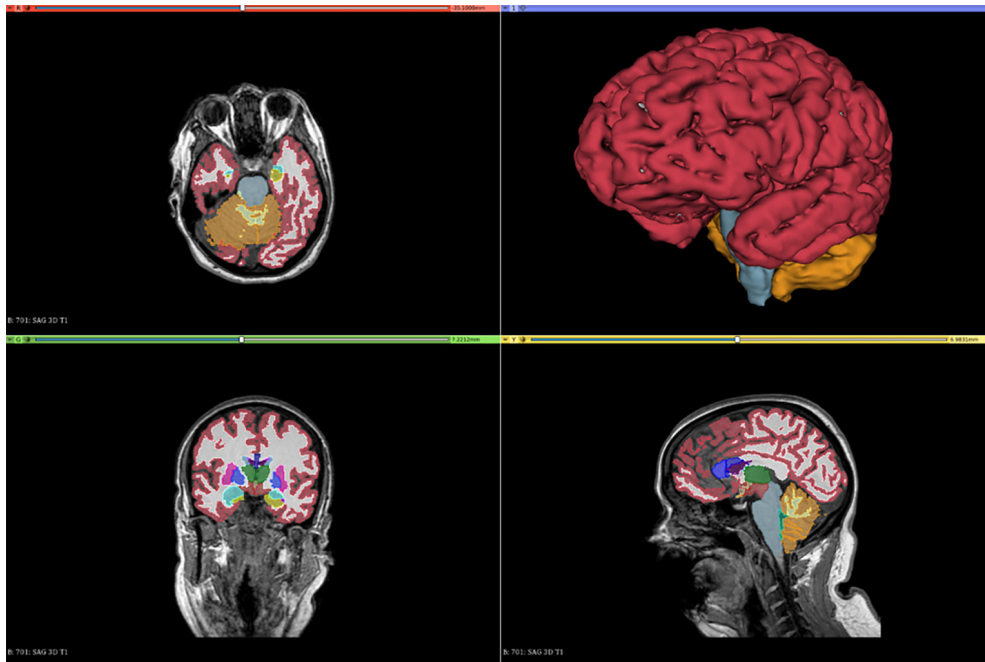
**Figure S6** GUI of FreeView. GUI, graphical user interface.

- o “new directory” is the name of the output directory chosen previously.
- Type the following command: *freeview T1.mgz aseq.auto.mgz*
  - o This will load the original T1 MRI and the brain segmentation in FreeView (Figure S6) for visual

inspection.

### Visualization of volBrain and FreeSurfer segmentation results in 3D Slicer

- volBrain allows the user to download the.nii.gz file. This can be uploaded as “Data” into Slicer. One can



**Figure S7** 3D representation of FreeSurfer segmentation in 3D Slicer viewer.

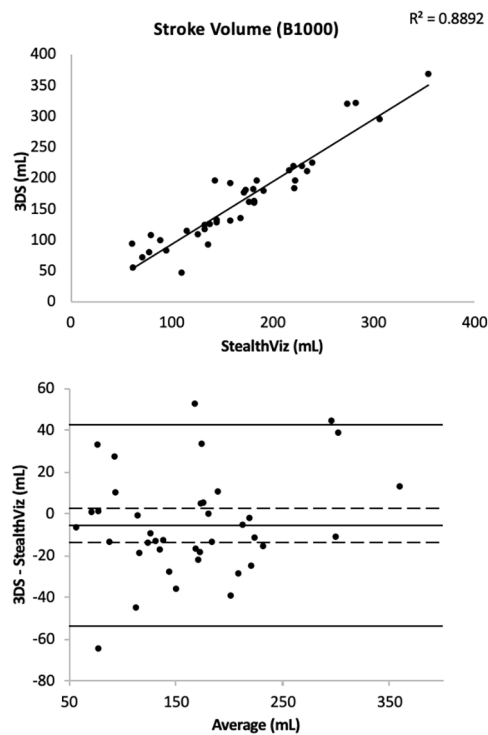
- then visualize the original T1 MRI and segmentation.
- By clicking the “eye” icon, one can view “crisp\_mmni” volume. Click the eye icon to view the volume, then right click the eye icon and click “Show in 3D views as volume rendering”. This will show a volume of the brain with CSF on the outside in the 3D viewer (this will represent the intracranial space).
- Additionally, one can threshold the “crisp\_mmni” volume in Segment Editor to see the brain without the CSF (use segmentation editor).
- One can also load FreeSurfer results into Slicer by loading the “mri” result folder as “data”. Right click the eye icon next to the “brain” volume. Then right click the eye icon and click “Show in 3D views as volume rendering” to see the segmentation in 3D.
- We also utilized the tools fs2dicom, dcmqi, and docker to export FreeSurfer segmentation to a DICOM file and upload into Slicer for 3D visualization (*Figure S7*) (*Figure 2A,2B* in main text).
  - o This would be a difficult step for the user, and we recommend simply loading the mri result folder as data as mentioned above. More information on exporting FreeSurfer segmentation to Slicer can be found at the following links:
  - o <https://github.com/QIICR/dcmqi/>

- o <https://github.com/corticometrics/fs2dicom>
- Exporting FreeSurfer segmentation as a DICOM file for manual editing of errors proved untenable as the DICOM file was unable to be utilized with other segmentation software.

### *Stroke volume analysis*

Here we compare SV calculations between Slicer and manual StealthViz segmentations using B1000 scans. SV was manually segmented on Medtronic StealthViz software v. 1.3.0.26 by senior author (VP). Instructions for using StealthViz is beyond the scope of this study as it is not a free, open-source software. Our SV calculations were made using a simple thresholding tool in Slicer. Manual segmentations via StealthViz were our gold standards for SV calculations, because in addition to a thresholding tool, a manual slice-by-slice correction was also used. StealthViz is our gold standard segmentation method against which we are comparing Slicer. SV correlation between Slicer and StealthViz showed good agreement and a strong correlation with  $R^2 = 0.8892$  (see *Figure S8* and *Table S2*). All 39 subjects were included for SV analysis (demographics included in *Table S3*).

FreeSurfer is unable to process B1000 scans. Thus, if a



**Figure S8** SV correlation between StealthViz and 3D Slicer ( $R^2 = 0.8892$ ). SV, stroke volume.

**Table S2** Demographics of subject population in SV analysis

Gender	Number of subjects (percentage of total)	Average age $\pm$ standard deviation (years)
Male	17 (43.5%)	61.41 $\pm$ 16.06
Female	22 (56.4%)	61.18 $\pm$ 17.55

SV, stroke volume.

**Table S3** SV analysis

Comparison	$R^2$	95% CI	P value
SV			
Slicer vs. StealthViz	0.8892	0.8086 to 0.9228	<0.0001

SV, stroke volume; CI, confidence interval; Slicer, 3D Slicer.

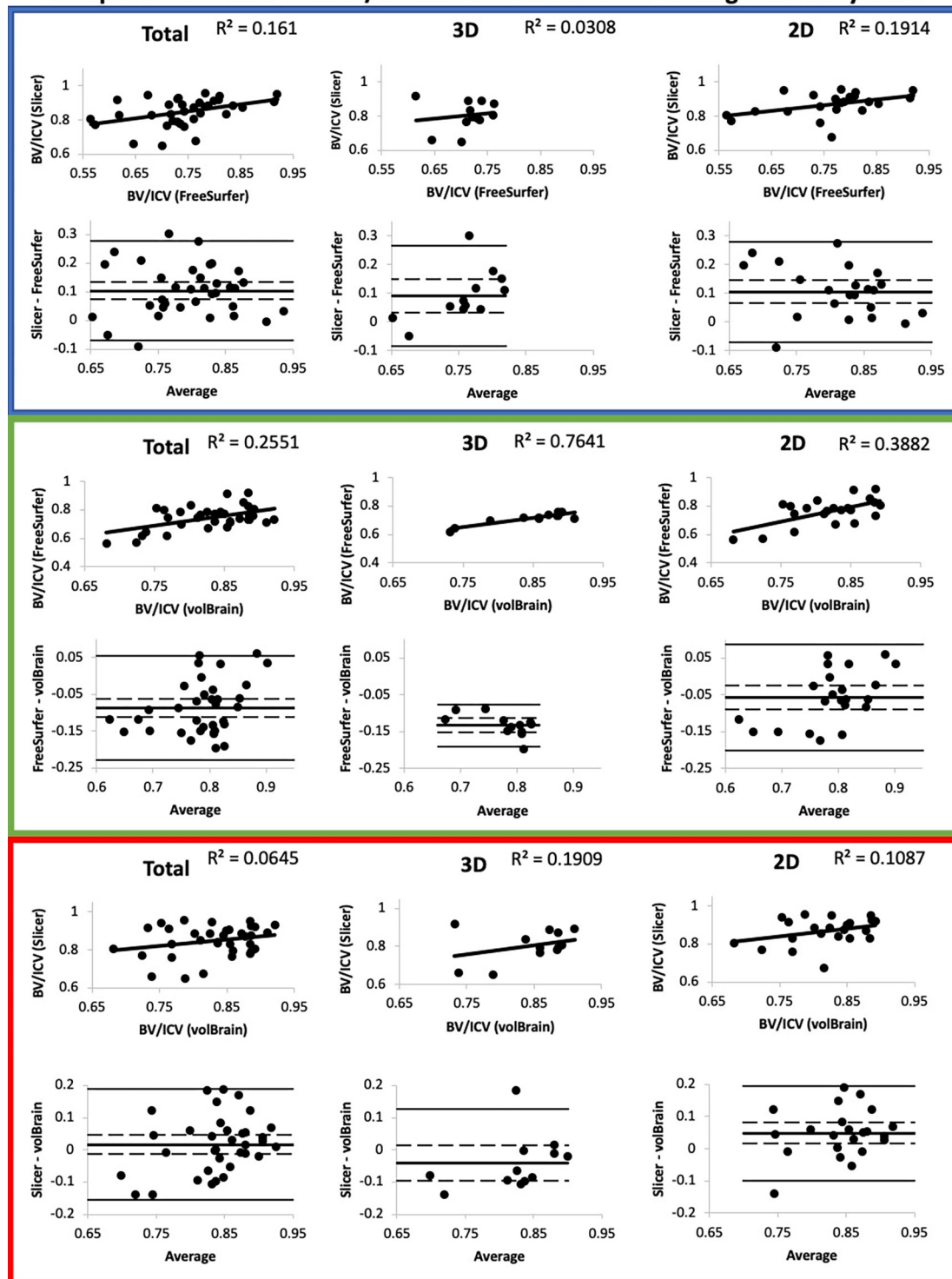
free software is to be used, we recommend using Slicer for SV calculation (see above for instructions).

### *Intracranial reserve analysis*

Here we define “intracranial reserve” as the brain volume to intracranial volume ratio. Intracranial reserve can be relevant in a study involving space occupying pathology such as ischemic stroke. It in theory has prognostic value due to the ability of the skull to accommodate swelling from an ischemic stroke. Refer to *Figure S9* for the inter-method reliability for intracranial reserve. Note that the only acceptable correlation is between FreeSurfer and volBrain using 3D scans. *Table S4* shows the Pearson correlation coefficients, 95% CI, and P value for our intracranial reserve analysis.



## Comparison of Brain Volume/Intracranial Volume Ratio among Volumetry Tools



**Figure S9** Intracranial reserve analysis. For each Slicer ratio, T1 was used for BV and CT was used for ICV. BV, brain volume; ICV, intracranial volume; Slicer, 3D Slicer; CT, computed tomography.

**Table S4** R2 values and corresponding 95% CI assessing the association between volume estimates obtained using different imaging methods

Quality	Comparison	R <sup>2</sup>	95% CI	P value
Ratio of BV to ICV				
All	Slicer vs. FreeSurfer	0.1610	0.0044 to 0.3717	0.0169
	Slicer vs. volBrain	0.0645	0.0000 to 0.2583	0.1409
	FreeSurfer vs. volBrain	0.2551	0.0408 to 0.4600	0.0020
2D	Slicer vs. FreeSurfer	0.1914	0.0000 to 0.4477	0.0417
	Slicer vs. volBrain	0.1087	0.0000 to 0.3658	0.1340
	FreeSurfer vs. volBrain	0.3882	0.0695 to 0.6025	0.0020
3D	Slicer vs. FreeSurfer	0.0308	0.0000 to 0.3310	0.5663
	Slicer vs. volBrain	0.1909	0.0000 to 0.5059	0.1355
	FreeSurfer vs. volBrain	0.7641	0.3714 to 0.8634	<0.0001

The methods being compared are described in the Comparison column of the table. P values are from tests comparing the observed R2 value to 0 (zero; no association). CI, confidence intervals; BV, brain volume; ICV, intracranial volume; Slicer, 3D Slicer.

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