



# Methods for cerebellar imaging: cerebellar subdivision

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Despite its great importance for a variety of behavioral and cognitive domains, the cerebellum has been understudied using human imaging, thereby delaying the development of computational tools that allow us to analyze its anatomy and function. Owing to its homogeneous architecture, finding ways to subdivide the cerebellum into meaningful parcels is one of the major challenges in the field. Although functional parcellations have been developed, the anatomical subdivision of the cerebellum is an active topic of research. This review describes the recent evolution of the computational techniques for automatic subdivision of the human cerebellum. Then, the difference between anatomical and functional parcellations is discussed, highlighting the importance of choosing the right tool for the problem at hand.

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## Introduction

Nestled underneath the tentorium in the posterior cranial fossa, the cerebellum is a remarkable brain structure. It plays an essential role in motor coordination [1] and cognitive function [2]. The cerebellum is divided into two hemispheres and a midline zone called the vermis. Further subdivision is based on hierarchical folds known as lobes (anterior, superior posterior, inferior posterior, and flocculonodular) and lobules (identified by Roman numerals I–X). Brodmann areas have been used to subdivide the cerebral cortex for more than a century [3]. However, unlike the cerebrum, the cerebellum has a rather homogeneous cytoarchitecture, and although some evidence is increasingly

challenging this assumption [4], the cerebellar lobules are still the preferred way to subdivide the structure. Different groups have advanced the application of computational techniques to segment the cerebellum with the objective of developing a fully automatic algorithm. From atlas-based approaches to the newest deep learning models, each novel software alternative becomes more accurate and requires less human intervention. In a data-driven world, such methods are essential for advancing our understanding of the cerebellum and its role in brain function and dysfunction. It is worth noting that different methods have their advantages and limitations, requiring investigators to choose wisely to maximize the possible insights of their research.

## The early days

Twenty years ago, if you were interested in analyzing the human cerebellum using magnetic resonance images, your options were limited to either whole-brain toolboxes or manually delineating the areas of interest for volumetric analysis. In either case, the available options were either inaccurate or time-consuming. The Diedrichsen laboratory pioneered the development of a specialized atlas for the human cerebellum [5]. Spatially Unbiased Infra-tentorial template (SUIT) improved the accuracy of cerebellar imaging analysis, and although it may require some manual intervention, the process was fully automated. The success of SUIT derived from its ability to isolate the cerebellum from the rest of the brain and then normalize the subjects' cerebellum into an unbiased template. The toolbox was upgraded with a probabilistic atlas of the cerebellar lobules and included in a variety of analysis packages [6]. Just a few years after SUIT was made available, other research groups presented alternative options for cerebellar segmentation using a variety of methods. Powell et al. evaluated four different approaches: atlas-based, probabilistic, artificial neural networks, and support vector machines, and showed that the machine learning algorithms outperformed the template and probabilistic-based methods when examining the relative overlap [7].

However, the software developments continued in different directions. Multiple-object geometric-deformable model (MDGM) was proposed by Bogovic et al. [8]. This approach incorporated level sets, a computer vision model that can perform numerical computation on curves and surfaces. Bogovic demonstrated the performance of MDGM in cerebellar segmentation, reporting outperforming similar methods [8]. The Rapid Automatic Segmentation of the human Cerebellum And its Lobules (RASCAL) presented by Weier et al. incorporated a patch-based label fusion after

nonlinear registration [9]. Although good performance was assessed using leave-one-out cross-validation between manual and automatic segmentations, RASCAL was not compared with other algorithms. A different approach presented by Park et al. created high-resolution multitemplates of the cerebellum [10] and adapted MAGeT segmentation [11] to test its performance. This multi-atlas-based approach yielded numerous independent segmentations that are fused using a voxel-by-voxel voting procedure. Further improvement using a multi-atlas approach was published by Price et al. with the Cerebellar Analysis ToolKit (CATK) [12]. CATK uses the Bayesian Active Appearance Modeling framework, which follows a deformable surface paradigm and includes a mechanism for incorporating contextual knowledge. Two more patch-based methods were developed: Plassard et al. used a fusion technique that extends the ideas of the Selective and Iterative Method for Performance Level Estimation [13], and Romero et al. created CERES [14], which took inspiration from previous developments such as multi-atlas, patch-based, and nonlocal label fusion. Initially, CERES used a library of manually segmented cases, however, given its implementation as an online-only resource, all new cases that are processed using a web browser are now included in the template library to increase the accuracy of the system.

Each new method represented an improvement for lobular segmentation; however, it was not until 2018 when Carass et al. evaluated some of the most popular algorithms for automatic lobular segmentation of the human cerebellum in a friendly competition [15]. Although the results were not outstanding, CERES2 showed statistical improvement over the second- and third-place methods in a one metric and comparable performance in the rest of the evaluation. It is important to note that the second and third place were not any of CERES predecessors but new implementations that used machine learning models.

### The machine learning era

Back in 2008, Powell et al. showed evidence that machine learning algorithms were promising tools for neuroimaging, and specifically for cerebellar segmentation [7]. Machine learning techniques use a training set of images to learn a predictive model that assigns class probabilities to each voxel [16]. Usually, the training set provides enough examples to learn the underlying data structure and, at the same time, variability to generalize to new examples. In their report, Powell et al. evaluated two machine learning algorithms: artificial neural networks and support vector machines. At that time, the computational cost of training artificial neural networks was high, especially when working with high-resolution tridimensional images. However, advances in computing power and availability propelled the use and development of artificial neural networks. In their approach, Powell et al. utilized a standard three-layer fully

connected feed-forward network [7]. First, the images underwent a transformation into a set of vectors that were used as input. Each node in one layer is connected to all the nodes in the subsequent layer, defining this network as feed-forward due to the unidirectional flow of information. The weights of each node are adjusted through back-propagation, facilitating the training of the network. In comparison, the networks used by the teams that finished in the second and third place in the 2018 competition [15] are more advanced and specifically designed to work on images. In both cases (LiviaNET and DeepNet), the teams used convolutional neural networks. This type of network performs a series of filtering operations via convolution. This filtering creates new versions of the original image, in which certain features are passed over to the next layer. After a series of filters, the output of a convolution layer identifies features that are highly distinctive and that are used in the following layers to assign the label of a given voxel. In addition of using convolutional layers, DeepNet presented a U-Net architecture. In this type of network architecture, a series of convolutional layers extract features and reduce the resolution of the image for the next layer, once this process is finished, a new number of layers restore the resolution of the images to present the result at full resolution. In other words, this architecture consists of a contracting path to capture context and a symmetric expanding path that enables precise localization [17]. The name DeepNet is related to Deep Neural Networks (also called Deep Learning) and is given by the number of layers and complexity of the model. Currently, deep learning is being used in a number of applications, outperforming other approaches when there is plenty of data (several thousands of annotated examples) to train the model (please see Ref. [16] for a detail description of artificial neural networks). In 2020, Han et al. presented the Automatic Cerebellum Anatomical Parcellation using U-net with Locally Constrained Optimization (ACAPULCO) [18••], which employs a cascade of two three-dimensional convolutional neural networks. One network is dedicated to locating the cerebellum and cropping the image, while the second network segments the structure into lobules. ACAPULCO outperformed CERES2 in several tests and demonstrated similar performance in others. The network complexity of ACAPULCO surpasses its predecessors, necessitating the use of data augmentation to enhance the results. As previously mentioned, deep learning models require a substantial amount of data to learn the underlying data structure and mitigate overfitting, which refers to classifying similar cases but failing to generalize to new instances. Data augmentation is employed when training data examples are limited. The original data undergo transformations through linear or nonlinear operations such as flipping, translation, rotation, scaling, and so on, to generate new examples and improve model performance. In their study, Han et al. compared the results of their network trained with and without data augmentation, highlighting how the network trained without data augmentation failed to accurately label certain parts of the cerebellum [18••].

Additionally, ACAPULCO is the first freely available algorithm for cerebellar segmentation packaged within a singularity container. A singularity container allows researchers to download and use the software package without having to deal with dependencies, which often pose problems due to version upgrades and system incompatibilities. This also provides an advantage over CERES, which limits the number of cases that can be processed on their servers with a free account.

The latest algorithm for cerebellar segmentation is CerebNet [19•]. In their report, Faber et al. describe CerebNet as an adaptation of fastsurfer [20], an advanced deep learning architecture originally designed to segment the entire brain into 95 classes. CerebNet employs a U-Net architecture composed of three two-dimensional convolutional neural networks, each dedicated to a specific axis (axial, sagittal, and coronal). These networks are later combined in an aggregation step that compares the label probability of each position. Faber et al. utilized various data augmentation techniques, including linear, nonlinear, and intensity deformations, to train the network. Their results demonstrate improved performance compared with ACAPULCO and SUIT. The authors claim that no data pre-processing is necessary before using their algorithm, and the run time is faster than that of previous methods. It is important to note that CerebNet is the first algorithm evaluated using data from a clinical population. In this regard, the authors state that CerebNet achieves a high level of detail, particularly noticeable at the intricate boundary between cerebellar white and gray matter.

### The developing cerebellum

Segmentation of the cerebellum in neonates and infants is a critical task in neuroimaging analysis. It aids in the understanding of early brain development, as well as neurodevelopmental disorders. Compared with the adult brain, the segmentation of the neonatal brain poses unique challenges [21]. The neonatal brain undergoes rapid growth and structural changes that limit the usability of software designed for adults. In 2019, an extension of SUIT was fine-tuned to work on neonates by creating a neonate-specific template [22]. SUIT-N uses the same approach than SUIT, providing tools for normalization and volume calculation. However, the low tissue contrast due to low myelination in newborns, limited its ability to accurately segment between gray and white matter [22]. To overcome this problem, Sun et al. used machine learning and a dataset of infants aged between 6 and 24 months from the UNC/UMN Baby Connectome Project [23]. In their approach, the reliable segmentation of the two-year-old cerebella is gradually propagated to the younger subjects using semi-supervised transfer learning [24]. This approach was recently updated from semisupervise to self-supervise learning, meaning that less manual intervention is

required during training, tissue probability maps were included, and their validation was done in multisite data [25•]. It is important to note that cerebellar segmentation on newborns is still work in progress. Currently, the biggest limitation falls on the image quality, to allow us differentiate tissue types, and data availability to train deep learning models.

### Functional parcellations

A plethora of functional Magnetic Resonance Imaging studies investigating the role of the cerebellum in motor, cognitive, and affective processes have showed cerebellar activity patterns that extend across lobules. Similarly, clinical studies have shown that cerebellar degeneration patterns are not contained within specific anatomical regions. Finally, the development of functional parcellations for the cerebral cortex stimulated the exploration of similar ideas in the cerebellum.

Buckner et al. presented the first cerebellar parcellation based on resting-state fMRI [26]. In their seminal paper, Buckner et al. argue that the cerebellum is connected to the cerebral cortex via polysynaptic circuits and those relationships can be explained with fMRI. To test this idea, the authors compared the signal of each cerebellar voxel with the signal of 17 different networks in the cerebral cortex. The results showed that the cerebellar area dedicated to each network is proportional to the network's area in the cerebrum (with a few exceptions such as the visual cortex). This research group presented an upgraded version of this approach in Xue et al. [27•]. In this paper, they show a completely novel network map revealing a new cerebellar subdivision that does not seem to align with anatomical landmarks. Using a larger dataset and a similar approach, Marek et al. showed that cerebellar networks are reliable, and that at the individual level, cerebellar networks are more variable compared to those in the cerebral cortex [28].

Ren et al. applied a different approach [29], instead of relying on the intrinsic activity of the cerebral cortex, the authors used a clustering algorithm to group voxels with similar activity, following the idea that voxels with similar activity should be part of the same network. Ren et al. used normalized cut spectral clustering [30] to assign labels to each cerebellar voxel, creating a set of parcellations with different number of clusters. Their results show that their approach outperformed previous parcellations when selecting the same number of clusters. A new improvement was presented by King et al. [31••] with a parcellation that was based on task-fMRI data. In their report, King et al. argue that during task execution, the brain activity is richer than during rest, hence using task-related fMRI leads to improved identification of functional regions of the cerebellum. To this end, the authors acquired the Multi Domain Task

Table 1

## List of software tools for cerebellar subdivision.

Name	Description	Year	Reference
SUIT	Template and normalization	2006	[5]
RASCAL	Patch-based label fusion	2014	[9]
MAGeT	Multi-atlas voxel-voting	2013	[11]
CATK	Bayesian Active Appearance	2014	[12]
CERES	Multi-atlas patch-label fusion	2017	[14]
ACAPULCO	Locally constrained 3D U-Net	2020	[18]
CerebNet	Combined 2D U-Nets	2022	[19]
SUIT-N	Neonatal template	2019	[22]
UNC/UMN	Infant self-supervised learning	2023	[24]
Buckner parcellation	Resting-state-derived parcellation	2011	[26]
Ren parcellation	Normalized cut spectral clustering	2019	[30]
King parcellation	Task-based regularized regression	2019	[31]

Battery (MDTB) that consisted of 47 different task conditions [31••]. Then, they used regularized regression, and a winner-takes-all approach to define the label of each cerebellar voxel. Additionally, they developed a metric to evaluate the performance of a functional parcellation based on the fMRI signal characteristics [32] instead of only geometric features. Their results showed that the MDTB parcellation outperformed previous resting-state approaches and confirmed that lobular segmentation is not suitable to explain fMRI data. It is important to note that in all the reviewed methods, some boundaries are weaker than others and those definitions depend on the parameters defined by the researchers. In fact, the probability of a voxel being a member of a specific cluster can be described as a gradient (see Ref. [33] for a review). However, the discussion of functional gradients is out of the scope of this review.

## Conclusion

Algorithms for the automatic segmentation of the human cerebellum have evolved significantly over the years (Table 1). From the first high-resolution atlas, a variety of statistical and machine learning approaches have improved the performance of our analytic tools. Currently, Convolutional Neural Networks and U-Net architectures are leading the competition for the most accurate and fast algorithms for segmentation. While computational power becomes more accessible and our datasets are increasing in the number of examples, one can expect that more complex deep learning models will be developed. Without a doubt, this trend will subsequently be translated into functional parcellations. It is important to remember that many of the algorithms reviewed here, use stochastic processes that can result in differences even when analyzing the same dataset [34]. The assessment of replicability (one subject, one dataset) and reproducibility (one subject, two datasets) of an algorithm should be a common practice before selecting your analysis pipeline. Finally, based on the evidence showing that anatomical landmarks are unrelated to functional profiles and degeneration patterns,

the use of lobular definitions, especially as a processing step (e.g. extracting values or averaging signals) should be reconsidered and justified. We will always be able to go back to lobular definitions for reporting the results in a familiar nomenclature, however, functional definitions in the cerebellum, eventually might be as common as the default mode network is for the cerebral cortex.

## Data Availability

No data were used for the research described in the article.

## Declaration of Competing Interest

I, Carlos Hernandez Castillo declare no conflict of interest related to this submission.

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