Human Brain Stereology From Vertical Sections

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Aims and objectives

The aim of this presentation is to provide an update to the Radiological Community of recent developments in modern design stereology relevant to the measurement of the volume and surface area of the human brain using Magnetic Resonance Imaging (MRI). In particular, we present pertinent practical information from our recent publication in the Journal of Microscopy (Cruz-Orive et al. 2013), which particularly concerns brain surface area estimation, and in which detailed aspects of relevant theory are also to be found.

Measurement of the volume and surface area of the human brain has been an important topic in Neuroscience for over a century. The last few decades have seen the increasing application of stereological methods - notably the Cavalieri method - to estimate brain volume using noninvasive imaging techniques (Mayhew & Olsen, 1991; McNulty et al., 2000; Garcia-Finana et al., 2003). The stereological estimation of pial or subcortical (i.e. grey-white matter boundary) surface area in human brain from Magnetic Resonance (MR) images, however, is less common. Theory (Baddeley et al., 1986; Baddeley & Jensen, 2005) exists to estimate surface area from vertical sections and recent applications to human brain include Roberts et al. (2000), Ronan et al. (2006), Fernandez-Viaderon et al. (2008) and Furlong et al. (2013).

To apply the above mentioned stereological vertical sections method (Baddeley et al., 1986) in combination with MRI requires that a so-called vertical direction is defined (e.g. the inferior-superior axis of the brain) and beginning at a random angle about this axis two or more MR images are reformatted through the 3D image at constant angular intervals. A test system containing a uniform array of cycloids is overlaid on each so-called MR vertical section image with the short axis of the cycloids parallel to the vertical direction and intersections between the test system and the boundary of the surface of interest on the vertical section are recorded. The size of the angular interval between the series of vertical sections, the interval between the sections within each series and the density of the cycloids on the test system will all influence the precision of the estimate. Fortunately, the 3 levels of systematic sampling mean that high levels of precision are obtained for relatively modest workloads. Furthermore, mathematical formulae have been developed for predicting the variance at each of the sampling levels from the corresponding data (see Cruz-Orive et al. 2014).

MRI is increasingly providing images suitable for manual analysis using stereological methods and increases in field strength from 1.5T, to 3T, and even to 7T, facilitate a faster image acquisition. In particular, a 7T system provides sufficiently high-resolution images in just 5-10 min, on which the pial surface can be seen clearly in its entirety. Simultaneously, image analysis techniques are being developed for automatic image analysis. For example, FreeSurfer software (Fischl, 2012) is now in routine use for
extraction of the pial surface of healthy subjects studied at 3T. Further development
is, however, required to segment 'pathological' brain scans at 3T, and at present the
FreeSurfer software is not sufficiently developed for routine application at 7T owing to
altered soft tissue contrast (i.e. cerebral cortex and meninges are isointense) and greater
image inhomogeneity (i.e. bias field) at 7T compared to 3T. Stereology can therefore
be seen as having a niche for enabling convenient analysis of 'pathological' brains at
3T, or of all brains at 7T. #Also, it may be useful to perform stereology on a brain
already segmented using the FreeSurfer software. For example, the segmentation could
be inspected at just the points of intersection between the relevant structures and the
cycloid test systems required for the stereological analysis.

As stated above all of the details of this study are to be found in Cruz-Orive et al. (2014).
Two specific aspects are highlighted here. Firstly, the results of a single application of the
stereological vertical sections method are compared with those obtained using the fully
automated image analysis pipeline available in FreeSurfer software for estimation of the
surface area of the pial surface of the human brain using Magnetic Resonance Imaging
(MRI) at 3T. Secondly, the reliability of formulae developed for predicting the precision
of the stereological surface area estimates (in particular, the so-called 'fakir formula' for
predicting the contribution to the total error variance due to the use of the cycloid test
system) is investigated via simulations performed on the segmented image using a new
software that we have developed called StereoTool.

**Methods and materials**

A 3D MR image of the brain was obtained for a healthy 20 year-old female subject
(body weight: 50 kg, body height: 1.5 m) at the Neurospin Centre of the CEA, Gif-sur-
Yvette near Paris, France. Approval for the study was given by the local Research Ethics
Committee, and the volunteer subject gave fully informed written consent. In particular,
the 3D MR image was acquired with 1 mm isotropic voxels on a 3T TRIO system
(Siemens Medical Systems, Erlangen, Germany) using an Inversion Recovery prepared
T1-weighted FLASH sequence was used with acquisition parameters of TR = 2300 ms,
TE = 2.98 ms, TI=900 ms and flip angle 9 degrees, in 13 min 7 s.

The 3D MR image was transferred to a PC running Linux software and input to
FreeSurfer software (Fischl, 2012) for segmentation using the standard FreeSurfer
pipeline which provided triangulation-based surface representations of the outer (pial)
and the subcortical surfaces of the brain. Subsequently, by using the 3D modelling
software Blender (http://www.blender.org/) 3D Boolean operations were applied to
remove those parts of the closed-surface representation provided by FreeSurfer which
were not part of the surface of interest (e.g. to remove the corpus callosum). To measure
cortical volume automatically the triangulation-based surfaces had to be closed so as to
form a complete bounding surface for the cerebral cortex. This was done separately for each cerebral hemisphere again using Blender software.

For stereological analysis of the pial and subcortical surfaces, the software StereoTool developed in-house was used to perform the following tasks. The intersection between the 3D triangulated surface model and test planes in a vertical sections sampling design (two series of vertical sections with an angle of 90 between them (Fig 1)), was computed as a finite union of planar polygonal curves whose links are the straight line segments determined by the test plane in the hit triangles. In Fig 2 (prepared using 3D Slicer software), the polygonal approximations corresponding to each of the 2D trace sections obtained from the 3D pial and subcortical surfaces are represented in red and blue colours, respectively. On the section plane containing the polygonal trace curves, a grid of cycloid test curves was superimposed with an established mechanism of randomness and the software automatically counted all the intersections between the polygonal traces and the curves of the grid.

**Images for this section:**

![Fig. 1: Relative positions of the sectioning planes for the two series of vertical sections obtained at sampling angles of 37 and 127 degrees (i.e. separated by 90 degrees) about the vertical direction are shown above the corresponding images on which are displayed traces of the pial (red) and subcortical (blue) surfaces.](image-url)
Fig. 2: (A) Vertical section number 5 from the first vertical section series in Fig 1 with the pial surface shown in red and which is displayed separately in (B). In (C) and (D) the cycloid test system is shown overlain on (A) and (B), respectively. The points of intersection between the cycloids and the pial surface are marked by filled and open circles in (C) and (D), respectively.
Results

The total surface area of the pial and subcortical surfaces and the total volume of the cerebral cortex in each cerebral hemisphere were computed using 3D Slicer software (http://www.slicer.org). For the pial surface, the total number of triangles for the left and right hemispheres were 232941 and 234485, corresponding to areas of 926.29 and 936.20 cm², respectively, and an overall total of 1863 cm². This compares with a value of 1924 cm² which was obtained from the stereological analysis presented in Figs 1 and 2 (for further details see Cruz-Orive et al., 2013).

The availability of FreeSurfer and the development of our in-house software StereoTool enabled us to perform for the first time the simulation of the vertical sections method and this revealed the following interesting aspects.

The surface area estimator from vertical Cavalieri sections at a single orientation is relatively insensitive to that orientation. This is not warranted in general, and it suggests that the pial surface is fairly isotropic - at least around the chosen vertical direction. The surface area estimator obtained from two mutually perpendicular Cavalieri series (called the two-series estimator) is the average of the corresponding two one-series estimators, and as expected from the preceding paragraph, the observed correlation between these two one-series estimators was negligible.

Contrary to the surface area estimators, which are relatively insensitive to section orientation, the model predictors of their error variances are rather sensitive to orientation. In particular, the instability of these predictors increases as a series of Cavalieri sections becomes nearly sagittal, because the brain is the union of two hemispheres delimited by the very deep mid sagittal sulcus. This instability is transmitted directly into the variance predictors. Nonetheless, most of the instability could be removed by replacing the estimate of a relevant coefficient (called the smoothness constant) with a fixed value which, in principle, may depend upon the type of surface under study.

The prediction of the third-stage variance component due to intersection counting with cycloid test curves remains unavailable in its generality. The so-called 'fakir predictor' used in the present study is only tentative. The main reason that it is proposed and applied is because, thanks to FreeSurfer and the in-house StereoTool software packages, the predictor could be tested against the empirical 'true' variance obtained from Monte Carlo simulations on the real images. The results are encouraging (see Fig 3) suggesting that it is a reasonable predictor for practical purposes (for further details see Cruz-Orive et al., 2013).
**Fig. 3:** Model predictors of the coefficient of error due to intersection counting on the pial traces with the cycloid test system plotted against the corresponding empirical values obtained by Monte Carlo replications. The checks were performed with grids of three different sizes on each of the six sections from each of the two series of vertical sections shown in Fig 1. The distance $2d$ in mm is the height of the fundamental tile of the cycloid test grid (see Fig 2).
Conclusion

FreeSurfer software and stereological methods provide highly complementary resources for investigations of the morphology of the human brain. We can surely expect that FreeSurfer and other software packages will continue to develop so that in general it will become possible to establish combined MR image acquisition and analysis protocols for reliable automatic measurement of the volume and surface area of the human brain. Stereological methods are, however, also important. They provide a mathematically rigorous and efficient means for checking measurements obtained using, for example, FreeSurfer, for potentially analysing datasets that may not have been acquired using established protocols and for studying compartments and structures of interest for which an automatic solution is not yet available. Furthermore, as demonstrated here the advances in computing and information processing which have led to the development of FreeSurfer are also being harnessed to support advances in stereology. In particular, whereas stereology has previously generally been a completely manual approach using, for example, microtomes, photography and acetate sheets bearing the relevant test systems, etc., software is increasingly becoming available for the convenient computer-based application of stereological methods. For instance, with StereoTool we have been able to perform the computer-based simulations for the first time in stereology, in order to test the reliability of formulae developed to predict the precision of the stereological vertical sections method. Such an approach is likely to motivate further developments in stereology.

Whether FreeSurfer or stereology are used to measure the surface area of the brain a fundamental consideration remains, namely the studied brain is likely to be only one subject from a cohort for which the population mean is the parameter of interest. In this case, a decisive component emerges, namely the biological variance among subjects. This component, together with the desired difference one wants to detect among population means, and the desired power of the test, determine the optimal choice of the number of subjects (and of the sections to be sampled and intersections to be counted per subject when stereology is used). The problem, and the details concerning its solution, are considered in detail for instance in Cruz-Orive et al. (2004).

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References


