Article

Estimation of the Volume and Volume Fraction of Brain and Brain Structures on Radiological Images

Bünyamin Şahin* and Amani Elfaki

ABSTRACT

Size changes of the brain is monitorized for clinical or research purposes. Stereological methods provide some techniques to obtain quantitative information about the size of the brain or size relation of its components within the whole. The Cavalieri principle is the main technique to estimate the volume of brain and its components. Using this technique, the volume of any object could be estimated from a set of slices through the object, provided that they are parallel, separated by a known distance. The cut surface areas of the sections are assessed and the multiplication of the total cut surface area with the mean of the section thickness provides an estimation of the volume of the examined object. The point-counting and planimetry are two methods for the assessment of sectional cut surface areas in the Cavalieri principle. Sometimes, the volume of brain could not provide comparative information among the groups. Scientists have documented several factors that contribute to the size of brain. Factors related to brain growth, such as gender and physical size, are thought to influence the maximal size of an individual's brain. Comparing solely the brain volumes or its components between two groups will not provide reliable data. At this point, the volume fraction method of stereological approaches proposes the solution. The volume fraction is simply expressed as the fraction of component within the reference volume. Both methods could be used digitally or they can be applied on the printed films. There are many studies describing the techniques and its applications. However, mostly they contain advanced information that is not suitable to be digested by newcomers. In the present paper we gave simple information on the application of both techniques. We also discussed the factors affecting the volume estimations on radiological images. Examples of applications and their solutions are also provided.

Key Words: brain, volume, volume fraction, stereology, the Cavalieri principle, imaging, magnetic resonance, computed tomography

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Introduction

Stereological methods provide quantitative data about three-dimensional structures using two-dimensional images. Volume and volume fraction approaches of the stereological methods are very commonly used for clinical and research purposes. With the combination of sectional radiological imaging tools they provide quantitative data about the total size or size relations of the organs or structures in living subjects.

There are numerous studies and papers describing those two mentioned principles. However, most of them contain very intricate information about the techniques. In the present paper, we aimed to give enough information to the readers so that they can easily apply the methods presented here.

1. Estimation of Volume

Recent studies revealed that the morphological changes of the brain are resulted in functional changes. For example, the volume of cortical structures devoted to a function often influences the quality of a person's ability to perform that function. For this reason, many of studies focused on evaluating the volume of total brain or its components to search on functions (Till *et al.*, 2011; Hashimoto *et al.*, 2011).

Brain volume can be estimated using the Cavalieri principle of volume estimation by means of consecutive serial sections (Mayhew *et al.*, 1996). A simple approach to obtain such information was demonstrated over 300 years ago by the Italian mathematician Bonaventura Cavalieri. He pointed out that the volume of

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any object could be estimated from a set of slices through the object, provided that they are parallel, separated by a known distance. In the Cavalieri principle, the cut surface areas of the sections are assessed and the multiplication of the total cut surface area with the mean of the section thickness provides an estimation of the volume of the examined object (Gundersen *et al.*, 1981; Roberts *et al.*, 1992; Roberts *et al.*, 2000).

To avoid bias, the first section must be placed at a uniform and random position in a constant interval of length (*t*) i.e. to start the scanning always at, for example, one cm from the right tip of the object will introduce an unknown amount of bias in general. Moreover, the series of sections must encompass the object entirely. The direction of cutting does not affect the unbiasedness property, but will affect the estimation precision in general. Thus an unbiased estimate of volume can be obtained by multiplying the total area of the section cut surfaces through the structure on all the sections as it shown below formula:

$$V = t \times \sum A \, \mathrm{cm}^3 \qquad (1)$$

Where $\sum A$ denote the sum of section areas in cm² and *t* is the sectioning interval in cm for the consecutive sections.

Sectional imaging modalities have provided an opportunity for volumetric quantification of the brain and its components (Mayhew and Olsen, 1991). Both computed tomography (CT) and magnetic resonance (MR) imaging may produce reliable measurements of intracranial cavity and its content. Magnetic resonance imaging offers optimal soft tissue contrast resolution and multiplanar capability without the use of ionizing radiation. However, CT imaging is still a powerful modality for central nervous system imaging and for subsequent routine intracranial volume measurements because of the reduced scanning duration, the availability and the detailed depiction of bony structures (Laughlin and Montanera, 1998; Sugouros *et al.*, 1999; Mazonakis *et al.*, 2004).

As it seen in the first formula the section thickness and the sectional surface area of the consecutive slabs are required for the estimation of volume. In CT and MR machines, the section thickness and the interval between sections are generally fixed distances. Therefore, the sum of section thickness and the interval must be recorded as the distance between sections.

In the second step, the sectional cut surface areas are required to be estimated. Point-counting and planimetry are two methods for the assessment of sectional cut surface areas in the Cavalieri principle.

1.1 Estimation of Volume Using Point-counting Method

The point-counting method is generally suitable for the estimation of structures printed on films or photographs and the structures showing very intricate surface area in sections. The point-counting method consists of overlying each selected section with a regular grid of test points, which is randomly positioned. A test point is a cross shaped lines (+) and it is said to hit the object if a previously assigned corner of the intersection of the cross lines **(Figure 1)**. After each superimposition, the number of test points hitting the structure of interest on the sections is counted **(Figure 2)**. Finally, the volume of the structure is estimated by multiplying section thickness, total number of points and the area represented per point in the grid (Sahin and Ergur, 2006). The unbiased estimator is written as follows:

$$V = t \frac{a}{p} \sum p \qquad (2)$$

Where *t* is the section thickness, $\sum P$ denotes the total point counts and (a/p) represents the area associated with each test point, corrected for any change of scale in the images as it is printed on the hardcopy films (Sahin *et al.*, 2003).

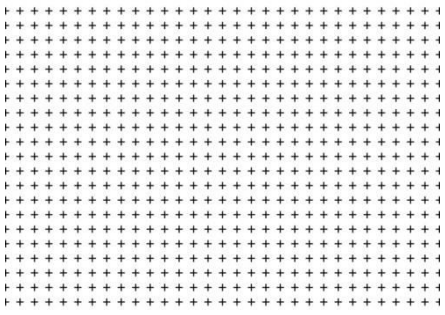

Figure 1. A point counting grid.

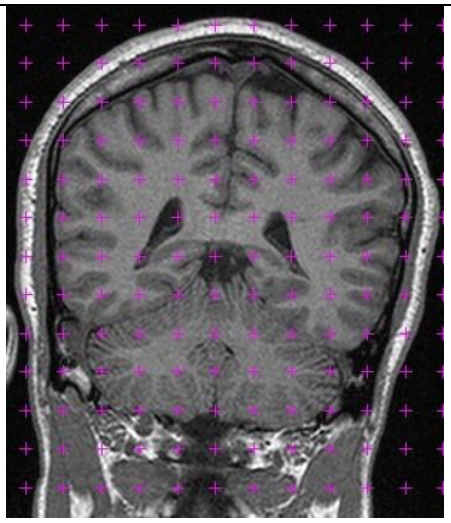

Figure 2. A coronal section of brain and a point- counting grid superimposed on it.

In the sectional radiological studies, the examined structures are reduced in size during printing. Therefore, it is crucial to know the actual representative area for the points. The magnification or reduction correction may sometimes be tedious. Using the following formula for the scale correction of radiological images will solve the problem:

$$V = t \times \left(\frac{SU \times d}{SL} \right)^2 \times \sum P \qquad \textbf{(3)}$$

where t is the section thickness of consecutive sections, SU is the scale unit of the printed film, d is the distance between the test points of the grid, SL is the measured length of the scale printed on the film and $\sum p$ is the total number of points hitting the sectioned cut surface areas (Sahin and Ergur, 2006).

1.3. Estimation of Volume Using Planimetry Method

The planimetry is a method suitable for digitalized images. It consists of manual and semi-automated techniques. The manual planimetry involves manually tracing the boundaries of objects of interest on images of sections is the most commonly used technique. In semi-automated planimetry either threshold of images or tolerance adjustment of selection tools are used. In the threshold method the gray scale colour range of the images was determined using upper and lower limits of the scale. Therefore, the region within the range of certain gray colour is labelled. Finally, the areas of labelled regions are estimated (**Figure 3**). For the tolerance adjustment it is similar to the threshold method. The selection tool is adjusted to delineate the selection area within the certain tolerance limits. After adjustment of tolerance percentage the selection tool is touched over the interested region and the boundaries of the areas are selected and the surface is estimated (**Figure 4**). The sum of the measured areas of sections obtained by planimetry is multiplied by the section thickness and the volume of the structure is estimated using the first formula (Sahin and Ergur, 2006).

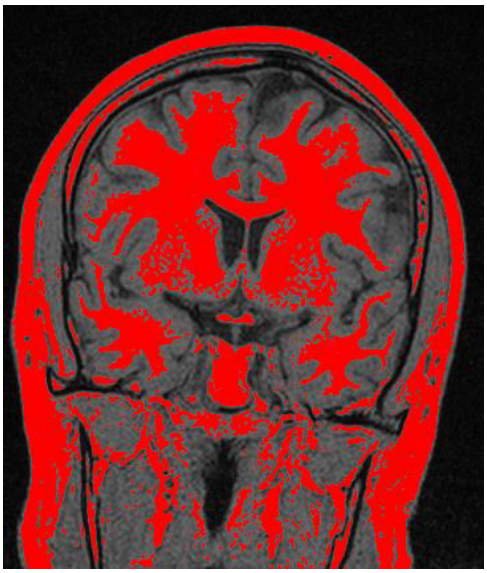

Figure 3. A coronal MR image of the brain. Application of the threshold for white mater.

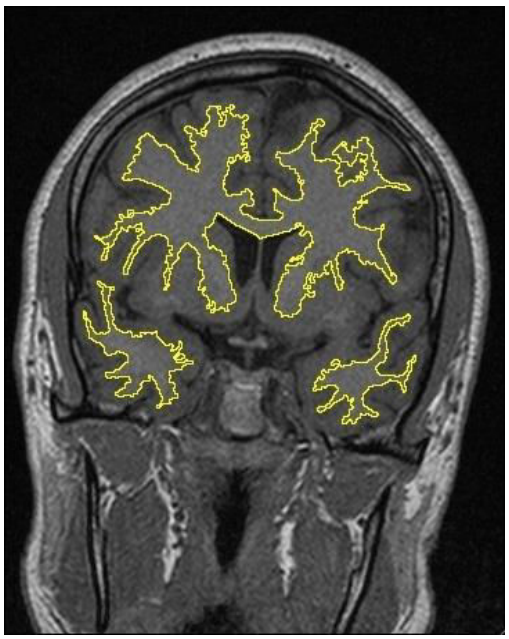

Figure 4. A coronal MR image of the brain. Application of the selection tool for white mater.

The cross-sectional surface area of brain structures can be measured by means of the planimetry method using some kinds of free software. The Osirix, DicomWorks, ImageJ and etc. could be found freely on the Internet. Especially, the last one is distributed by the National Institutes of Health, the United States of America. The final release of the ImageJ has good tools for the estimation of sectional surface area of the interested structures.

If the radiological images are in Dicom format it is not required to adjust the image size with the software since they contain information about size of scaling of images. However, the images except Dicom formatted require adjustment of software with the size of images. Before the estimation, the scale of the image should be set in the software. Following the setting of the image size, the sectional surface areas are estimated using planimetry on each section. If the interested region is distinguished clearly from the surrounding structures, threshold processing of the program can be used. In this case, clicking on the interested surface is enough to delineate the boundaries of the interested surface. Then, the software automatically calculates the number of pixels enclosed by the traced contours on each section and provides the cross-sectional area of the interested structure on a slice-by-slice basis.

1.3. Planimetry Versus Point-counting

Efficiency is an important topic in the stereology and regarding the efficiency the user may choose either point-counting or planimetry techniques. Imaging technique or the composition of organ may produce sectional images with clear boundaries from the surrounding structures. In these cases, the usage of planimetry may be very practical. Researcher could separate the region of interest using threshold or tolerance adjustment of selection tool. Sometimes, organs or structures may not show clear boundaries from the neighbouring organs. The two techniques of semi-automated planimetry do not work in this condition. Sometimes, the region of interest may intermingle with the other regions. Therefore, manual planimetry may be inevitable.

The printed films especially in retrospective studies obligate the researcher to use point-counting method. Moreover, some of the organs or the interested structure may consist of collection of isles. The delineation of individual isles may be very difficult. Additionally, some of the structures within an organ should be excluded from the organ. Therefore, the application of planimetry may be impossible. Most of the studies adopting the point-counting technique for organ volume estimations have mentioned that this volumetric approach is superior to the technique of planimetry (Roberts *et al.*, 1992; Mazonakis *et al.*, 2002). There are limited studies performing measurements to compare the two volumetric techniques. Gong *et al.* (1999) reported that the planimetry technique should be preferred approach for the measuring tumour volume. However, most of the stereological studies report that the point-counting method is the most efficient way for the estimation of organ volumes (Mazonakis *et al.*, 2002, Sahin an Ergur, 2006; Sahin *et al.*, 2007).

1.4. The Section Thickness and Windowing

Previous studies showed that the estimated volume is strictly affected by the section thickness of the radiological sectional images (Emirzeoglu *et al.*, 2005; Sahin and Ergur, 2006; Sahin *et al.* 2008). The volume of internal organs increases when the section thickness is increased. Controversially, higher section thicknesses resulted in lower intracranial cavity volume. These two conditions could be described as over/under-estimation of volume due to the over/under-projection of the images.

The main reason for the under-estimation may be the partial voluming artefact of sectioning in radiological imaging. Partial voluming artefact is the result of sectioning and the thickness of sections in MR imaging and CT scans. It is well known that partial voluming arises when a voxel contains different types of tissues. As a result, the reconstructed intensity ascribed to this voxel in the displayed image represents an intermediate value that does not correspond to any real tissue. The partial volume effect is more pronounced in thick sections and can considerably influence the accuracy of the obtained volume measurements (Nawaratne *et al.*, 1997). The intensity of signal cannot be separated within each voxel when it arises from different tissue compartments and this

produces what is referred to as partial voluming artefact. To fix the contribution of effect of section thickness standard section thickness should be used for the research studies (Sahin and Ergur, 2006). Additionally, gold standard studies may provide some correction formulas for the different section thicknesses (Emirzeoglu *et al.*, 2005; Sahin and Ergur, 2006; Sahin *et al.* 2008).

Windowing is an adjustment procedure to see the contrast differences among the structure in radiological imaging. During the windowing of frames, different levels of settings to obtain best view are chosen. These adjustments are related to the nature of scanned structure and the imaging technique. Diederichs *et al.* (1996) showed that a proper windowing must be chosen to obtain maximum intensity projections. In the living subjects, windowing process may produce over/under-projection of the structures. Hence, the obtained results may be resulted in over/under-estimation. Using standard windowing level may decrease bias in quantitative studies on radiological images.

1.5. Inter- and Intra-observer Variation

The reproducibility of the techniques is important for the assessment of their reliability. There are some studies in the literature evaluating the inter-rater variance using the Cavalieri principle on sectional radiological images. Their results showed little inter-rater variation (Sahin *et al.*, 2003; Odaci *et al.*, 2003; Akbas *et al.*, 2004).

There are little studies evaluating intra-rater variation of the estimation of brain volume using the MR images. Ronan *et al.* (2006) reported that the limits of estimates for the volume of brain were felt within ±2 SD's of the population and there was a good agreement between repeated measures for the total brain volume data. They used point counting method for the assessment of brain volume in the mentioned study. Bermel *et al.* (2003) developed a semi-automated measure of brain parenchymal fraction using commercially available edge-finding and thresholding software. Their intra-rater variability was very low and they concluded their software could be used to quantify the brain structures within 30 minutes.

Elfaki *et al.* (2011) used both the combination of manual delineation and semi-automated planimetry. The intra-rater repeatability was similar to the findings of both studies. Their results showed that the reproducibility of the obtained data is good. When we checked the result of point-counting, manual planimetry and semi-automated planimetry there is good agreement for the intra-rater comparisons. Although previous studies used very different strategies for measuring brain volumes in neurodegenerative diseases, they all achieved relatively low intra-rater variability, with mean coefficient of variations ranging from 0.2 to 2.9% (Rovaris 2000).

1.6. The Coefficient of Error

Stereological methods provide coefficient of error (CE) to the researcher making appropriate changes on the sampling or estimating procedures. The first CE formula is proposed by Matheron in 1965 (Gundersen *et al.*, 1999). Following the release of that formula, many of formulas have been proposed for appropriate estimates (García-Fiñana and Cruz-Orive, 2000). In 2003 García-Fiñana *et al.* built on this work to further develop the error prediction formulae for the volume estimator.

Since the huge number of available CE predictors, researcher may confuse to decide on which one is the most suitable. Still, it must be considered that the CE predictors only give an idea to the researcher about the sufficiency of the number of sections and points hitting the interested structure. By this way, a researcher can see the potential variability in any given volume measurement. When the CE of these measurements is high, it can generate obvious problems in accuracy and hence interpretation. These problems may arise if too few slices or too few points are taken for volume estimation. The observer is eligible to change the spacing of points in the grid or the number of slices available in any sectional study to obtain a reasonable CE value. It is also important to note that an appropriate grid size and the number of slices required for volume estimation of an object is crucial at the beginning, there is no need to calculate the CE value for repeated sessions. This reduces the calculations by using just the third equation for point counting. A CE value lower than 5 % is within acceptable range (Gundersen and Jensen, 1987). Empirically, we may say that choosing a point density in the grid so that 300-400 points were counted for each section

series to yield coefficients of error at 5% or less.

In the point-counting method the CE of estimates can be estimated by means of the following formula:

$$CE=\left(\sum_{i=1}^{m}P_i\right)^{-1}\times\left[\left(0.0724\times(b/\sqrt{a})\times\sqrt{n\times\sum P}\right)+\left(\frac{1}{12}\times\left(\left(3\times\sum_{i=1}^{m}P_i^2-Noise\right)-\left(4\times\sum_{i=1}^{m-1}P_i\times P_{i+1}\right)+\left(\sum_{i=1}^{m-2}P_i\times P_{i+2}\right)\right)\right)\right]^{1/2} \quad (4)$$

Where, i=1, 2, ..., m is the number of sections, P is the number of point hitting the sectioned surface areas, *Noise* is the value obtained above and the others are constants (Gundersen and Jensen, 1987). Noise gives information on the complexity of the examined cut surface area of the specimen. Calculation of the CE using these equations requires knowledge of the value of a dimensionless shape of coefficient $(b/\sqrt{a})$, which is equivalent to the mean boundary length of the profiles divided by the square root of their mean area, is a measure of the average shape of the profiles through the structure of interest on the sections. It can be determined using the diagram proposed by Gundersen and Jensen (1987). A simple diagram is also shown in Figure 5. *0.0724* is a constant. n is the total number sections and $\sum p$ is the total number of points hitting the sectioned cut surface areas of the interested structure.

In the planimetry method the CE of estimates may be obtained using the following formula:

$$CE=\left(\sum_{i=1}^{n}A_i\right)^{-1}\times\left[\frac{1}{12}\left(3\sum_{i=1}^{n}A_i^2-4\sum_{i=1}^{n-1}A_iA_{i+1}+\sum_{i=1}^{n-2}A_iA_{i+2}\right)\right]^{1/2} \quad (5)$$

Where, i=1, 2, ..., m is the number of sections. A is the measured area of the sections using planimetry and the others are constants. This formula allows the researcher to evaluate the area changes and the measured cut surface areas in the consecutive section series (Mazonakis *et al.*, 2002).

1.7. Example of Volume Estimation

Aim

Estimation of volume of right hemisphere in human using point-counting method on serial MR images.

Imaging acquisition procedure

Structural MR imaging was done using a Siemens 1.5 Tesla Magnetom Avanto Vision System. T1-weighted images were obtained using three-dimensional acquisition by Magnetization Prepared Rapid Acquisition. Slice distance is 1.0mm, the field of view is 250 read, 192mm phase, TR=1657ms, TE=2.95ms, bandwidth 180Hz/pixel, flip angle 15°, ECHO spacing=7.5ms, phase resolution=100%, slice resolution=50%, and acquisition time=5 minutes and 18 seconds. The images are in coronal section without contrast media.

Morphometric measurements were conducted using the ImageJ. The software is in the public domain and was downloaded from the Internet (available at the site: http://rsb.info.nih.gov/ij/). It runs on any computer systems. Measurements from images can be stored separately.

The Dicom image of patient was transferred to the software and converted into stack. Systematic random sampling was done since the number of slices in coronal plane about 192 sections. The sampling fraction was 1/10 for the brain. This mean, for example take the 5th section as first than go on 15, 25, 35 for the brain. Therefore, the section interval for the brain was 1 cm. Finally, 15 to 17 sections containing brain images were obtained.

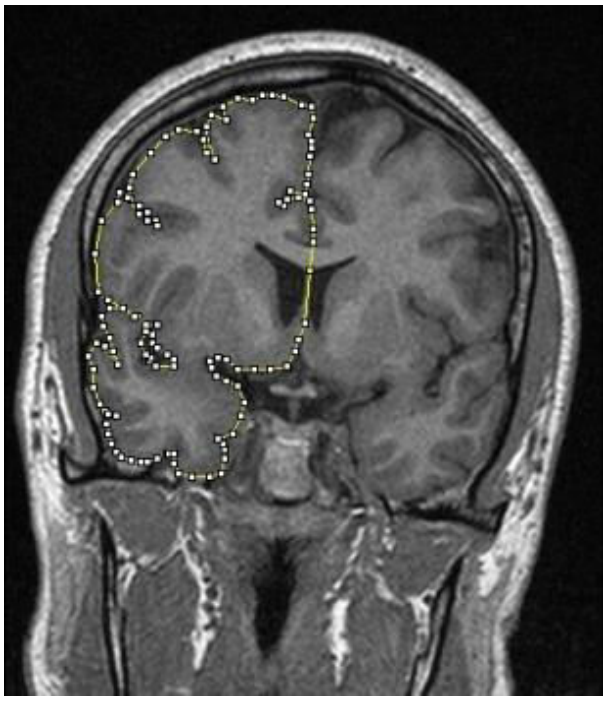

Figure 5. Application of manual planimetry for the delineation of right hemisphere in ImageJ.

Procedure

The outer boundaries of the hemisphere were manually delineated **(Figure 5)**. The volume of cerebral hemisphere was estimated by the multiplication of total sectional surface area with the section interval (i.e. 1cm) at its shown in the fourth formula. The CE of estimate was also predicted using the 1st formula. All calculations were done using the Microsoft Excel spreadsheet. The surface area data were transferred from ImageJ to the Excel. Finally, all the calculations were done automatically **(Table 1)**.

Table 1. Sectional cut surface area of the right hemisphere and estimation of volume. Section thickness is 10mm and the areas are in mm^2.

A, Section No	Bi Surface Area
1	886
2	1519
3	2365
4	2950
5	2950
6	3145
7	4310
8	4658
9	4911
10	4825
11	4662
12	4802
13	4704
14	4275
15	4032
16	3465
17	2720
18	2035
19	628
20	0
Total	63842

$V = t \times \sum A =$ 0×63842=638420mm²=638.42 cm³ CE=0.72%.

2. Estimation of Volume Fraction

The volume of brain and its components can be estimated by the Cavalieri principle as described in the previous chapter. The human brain does, however, vary widely in size (Knutson *et al.*, 2001). To date, scientists have documented several factors that contribute to this variation. Factors related to brain growth, such as gender and physical size, are thought to influence the maximal size of an individual's brain (Raz, *et al.* 1998, Sgouros *et al.*, 1999). Comparing solely the brain volumes or the volumes of other intracranial structures between two groups (i.e. control and experimental groups) will not provide reliable data (Sgouros *et al.*, 1999).

The volume fraction of a component within a reference volume is a simple parameter used in biomedical science (Howard and Reed, 1998; Mattfeldt *et al.*, 2003; Mattfeldt *et al.*, 2004). It is used to express the proportion of a phase or component within the whole structure. The volume fraction of an *X* phase within a *Y* reference volume is simply expressed as follows:

$$V_V(X,Y) = \frac{\text{Volume of } X \text{ phase in } Y \text{ reference space}}{\text{Volume of } Y \text{ reference space}} \quad (6)$$

Where the $V_V(X,Y)$ indicates volume fraction of *X* phase within the *Y* reference volume. Using this approach, V_V (*hippocampus, brain*), V_V (*cortex, total brain volume*) and V_V *(tumour, hemisphere*) can be estimated. Volume fraction ranges from 0 to 1 and is often expressed as a percentage (Howard and Reed, 1998).

As it described before, the section thickness and the representing area per point should be known for the estimation of volume. In case of knowing all the requirement of volume estimations is available; the volume fraction of any particular structure could be estimated by the formula given above. Sometimes, the images may not contain information mentioned above. In this case, throwing a point-counting grid over the images and counting the number of images hitting the reference and phase could be used

for the estimation of volume fraction. For these cases, the volume fraction of a phase can be estimated using the formula:

$$V_V(X,Y) = \frac{\sum P_X}{\sum P_Y} \quad (7)$$

Where, $\sum p_x$ indicates the number of points hitting the X phase and $\sum p_Y$ the number of points hitting the reference space i.e. Y.

Usually, the phase within the reference space is smaller in size. In this case, the use of a simple point counting grid can provide sufficient sampling opportunity for the section cut surface area of the reference space, but not for the phase. The combined point counting grids (CPCG) could be used to give equal sampling opportunity to both of them. A combined point counting grid is composed of two sets of points of different densities on the same grid. **Figure 6** illustrates a CPCG that has four fine points (crosses and encircled crosses) per coarse point (i.e. encircled crosses only). We can describe this grid as a CPCG with 1/9 area fraction. The area per point associated with each coarse point is thus four times larger than that of each fine point; one should consider that encircled points are used as both fine and coarse points (Kalkan *et al.*, 2007).

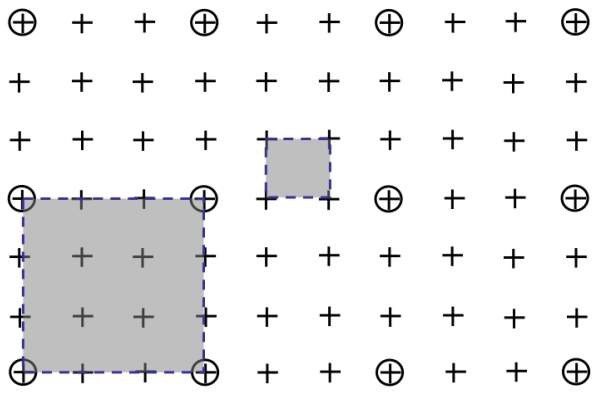

Figure 6. An example of combined point counting grid with 1/9 area fraction. While an encircled cross represents a larger area, each crosses without regarding the encircle represent 1/9 fraction of the large areas.

The volume fraction of a component within the organ can be estimated by placing the CPCG over the section series, counting the number of coarse points that hit the reference space including the phase, and counting the number of all points hitting only the phase. As the ratio of fine to coarse points is 1/9, a slightly modified version of the 7th equation can be used to estimate the volume fraction of a component within the subject.

$$V_V(X,Y) = \frac{\sum P_X}{9x\sum P_Y} \quad (8)$$

In the new formula, none of the parameters in the volume estimation equation is required except the number of points hitting the phase and the reference space. This new approach is not affected by the reduction/magnification ratio of the images for each group (Kalkan *et al.*, 2007).

2.1. Example of Volume Fraction Estimation

Aim

Estimation of volume fraction of the right lateral ventricle within the right hemisphere on serial MR images.

Background

The same images with same sections sampling fraction (1/10) were used for the estimation of volume fraction of the lateral ventricle. Therefore, the section thickness of images was 1 mm and interval is 9 mm, total section thickness is 10 mm.

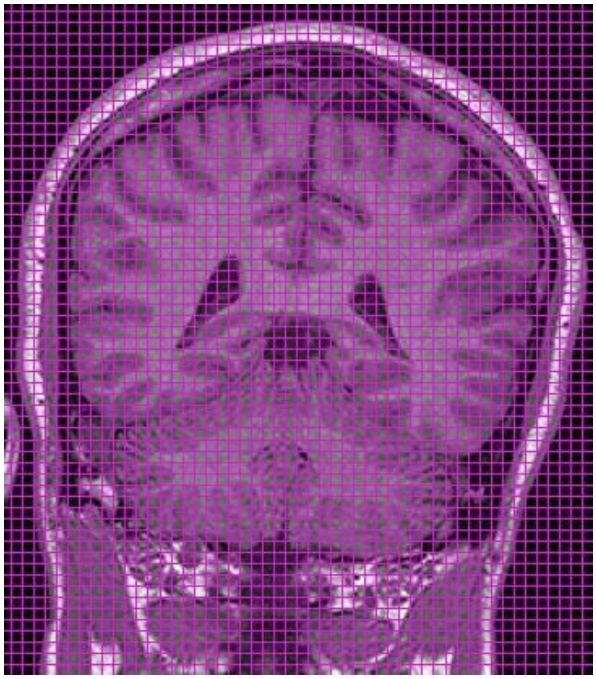

Figure 7. An MR image of brain section in coronal plane. A point-counting grid with 10mm^2 representative area was superimposed on the image.

Procedure

Images were transferred to the ImageJ and slice number was reduces in 1/10 fraction. We obtained 20 images totally. Grids with 10mm² and 200mm² representative areas were randomly superimposed over the images for the right lateral ventricle and right hemisphere, respectively **(Figure 7)**. Therefore, the area fraction sample of the grids was 1/20. The number of points hitting the ventricle and hemisphere were counted as it shown Table 2. The volumes of each structure were estimated using the second formula. Volume fraction of the lateral ventricle is estimated.

Table 2. Recorded numbers of points hitting the lateral ventricle and hemisphere.

Number of Points (ΣP)		
Section No:	Ventricle	Hemisphere
1	0	0
2	0	0
3	0	8
4	0	14
5	0	16
6	10	20
7	9	23
8	15	25
9	12	25
10	6	26
11	5	24
12	9	25
13	16	24
14	5	20
15	0	20
16	0	19
17	0	14
18	0	11
19	0	4
20	0	0
	$\Sigma P_{(ventricle)}$=87	$\Sigma P_{(hemisphere)}$=318

Estimation of volumes using 2nd formula:

$\boldsymbol{V_{ventricle} = t \times a/p \times \Sigma A} = 1 \times 87 \times 0.1 = 8.7\ cm^3$

$\boldsymbol{V_{hemisphere} = t \times a/p \times \Sigma A} = 1 \times 318 \times 2 = 636\ cm^3$

Estimation of volume fraction of lateral ventricle using point counts:

$$\hat{V}v(ventricle,hemisphere) = \frac{\sum P_{Ventricle}}{20\sum P_{hemisphere}} = \frac{87}{20x318} = \frac{87}{6360} = 1.37\%$$

Summary and Outlines

In the presented paper we described the volume and volume fraction estimation of stereological methods. For the application of the Cavalieri principle, the consecutive images of brain encompassing the entire structure should be used. At least 10 sections should be used for the brain volume. The interested structure must have clear boundaries from the surrounding structures. Since there are clear biological boundaries among hemispheres, cerebellum and brain stem it is possible to estimate the volume of those mentioned structures. Moreover, the volume of cortex, white matter, ventricles and gray matter collections within the brain can be estimated. Fail to distinguish the boundaries of interested structures may contribute certain amount of bias.

The available studies reveal that the inter-rater variation is low for the volume estimations. However, the analysis of images for research purposes should be done by the same observer thought the study. Standard section thickness and using the same windowing parameter for the analysis of sectional images are also crucial for the reliability of the studies.

Appendix

The estimation of CE using 4th formula as a spreadsheet. Excel spreadsheet for CE estimation can be as following (B1; section thickness, B2; interval, B3; representation area per point, B4; B5; title, $b/\sqrt{a}$, B6-B25; number of points in sections, B26; sum of points):

=(SQRT(((0,0724*B4)*(SQRT((B26)*(COUNTIF(B6:B25;">0")))))+(((3*(((B6*B6)+(B7*B7)+(B8*B8)+(B9*B9)+(B10*B10)+(B11*B11)+(B12*B12)+(B13*B13)+(B14*B14)+(B15*B15)+(B16*B16)+(B17*B17)+(B18*B18)+(B19*B19)+(B20*B20)+(B21*B21)+(B22*B22)+(B23*B23)+(B24*B24)+(B25*B25))-((0,0724*B4)*(SQRT((B26)*(COUNTIF(B6:B25;">0"))))))-(4*((B6*B7)+(B7*B8)+(B8*B9)+(B9*B10)+(B10*B11)+(B11*B12)+(B12*B13)+(B13*B14)+(B14*B15)+(B15*B16)+(B16*B17)+(B17*B18)+(B18*B19)+(B19*B20)+(B20*B21)+(B21*B22)+(B22*B23)+(B23*B24)+(B24*B25)))+((B6*B8)+(B7*B9)+(B8*B10)+(B9*B11)+(B10*B12)+(B11*B13)+(B12*B14)+(B13*B15)+(B14*B16)+(B

15*B17)+(B16*B18)+(B17*B19)+(B18*B20)+(B19*B21)+(B20*B22)+(B21*B23)+(B22*B24)+(B23*B25)))))/12)))/B26

The estimation of CE using 5th formula as a spreadsheet. Excel spreadsheet for CE estimation can be as following (B1; section thickness, B2; interval, B3; title, B4-B23; section cut surface areas, B26; sum of areas):

=((1/B24*(SQRT(((3*(((B4*B4)+(B5*B5)+(B6*B6)+(B7*B7)+(B8*B8)+(B9*B9)+(B10*B10)+(B11*B11)+(B12*B12)+(B13*B13)+(B14*B14)+(B15*B15)+(B16*B16)+(B17*B17)+(B18*B18)+(B19*B19)+(B20*B20)+(B21*B21)+(B22*B22)+(B23*B23)))-(4*((B4*B5)+(B5*B6)+(B6*B7)+(B7*B8)+(B8*B9)+(B9*B10)+(B10*B11)+(B11*B12)+(B12*B13)+(B13*B14)+(B14*B15)+(B15*B16)+(B16*B17)+(B17*B18)+(B18*B19)+(B19*B20)+(B20*B21)+(B21*B22)+(B22*B23)))+((B4*B6)+(B5*B7)+(B6*B8)+(B7*B9)+(B8*B10)+(B9*B11)+(B10*B12)+(B11*B13)+(B12*B14)+(B13*B15)+(B14*B16)+(B15*B17)+(B16*B18)+(B17*B19)+(B18*B20)+(B19*B21)+(B20*B22)+(B21*B23)))/12))))*100

References

Akbas H, Sahin B, Eroglu L, Odaci E, Bilgic S, Kaplan S, *et al.* Estimation of the breast prosthesis volume by the Cavalieri principle using magnetic resonance images. Aesthetic Plast Surg 2004; 28: 275-280.

Bermel RA, Sharma J, Tjoa CW, Puli SR, Bakshi R. A semiautomated measure of whole-brain atrophy in multiple sclerosis. J Neurol Sci 2003; 208: 57-65.

Diederichs CG, Keating DP, Glatting G, Oestmann JW. Blurring of vessels in spiral CT angiography: effects of collimation width, pitch, viewing plane, and windowing in maximum intensity projection. J Comput Assist Tomogr 1996; 20: 965-974.

Elfaki A, Osman T, Elsheikh A, Hamdoun A, Sahin B. Evaluation of the intra-rater Variation for the Estimation of Volume of Cerebral Structures Using the Cavalieri Principle on Magnetic Resonance Images. Journal of Experimental and Clinical Medicine 2011; 28: 22-25.

Emirzeoglu M, Sahin B, Selcuk MB, Kaplan S. The effects of section thickness on the estimation of live volume by the Cavalieri principle using computed tomography images. Eur J Radiol 2005; 56: 391-397.

García-Fiñana M, Cruz-Orive LM, Mackay CE, Pakkenberg B, Roberts N. Comparison of MR imaging against physical sectioning to estimate the volume of human cerebral compartments. Neuroimage 2003;18: 505-516.

García-Fiñana M, Cruz-Orive LM. New approximations for the variance in Cavalieri sampling. J Microsc 2000; 199 :224-538.

Gong QY, Tan LT, Romaniuk CS, Jones B, Brunt JN, Roberts N. Determination of tumour regression rates during radiotherapy for cervical carcinoma by serial MRI: comparison of two measurement techniques and examination of intraobserver and interobserver variability. Br J Radiol 1999; 72: 62-72.

Gundersen HJG, Boysen M, Reith A. Comparison of semiautomatic digitizer-tablet and simple point counting performance in morphometry. Virchows Arch B Cell Pathol Incl Mol Pathol 1981; 37: 317-325.

Gundersen HJG, Jensen EB, Kiêu K, Nielsen J. The efficiency of systematic sampling in stereology-reconsidered. J Microsc 1999; 193: 199-211.

Gundersen HJG, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987; 147: 229-263.

Hashimoto R, Javan AK, Tassone F, Hagerman RJ, Rivera SM. A voxel-based morphometry study of grey matter loss in fragile X-associated tremor/ataxia syndrome. Brain. 2011; 34: 863-878.

Howard CV, Reed MG. Unbiased Stereology: Three-dimensional measurement in microscopy. Oxford: Bios 1998: 39-68.

Kalkan E, Cander B, Gul M, Girisgin S, Karabagli H, Sahin B. Prediction of prognosis in patients with epidural hematoma by a new stereological method. Tohoku J Exp Med 2007; 211:235-242.

Laughlin S, Montanera W. Central nervous system imaging. When is CT more appropriate than MRI? Postgrad Med. 1998;104: 73-76, 81-84, 87-88.

Mattfeldt T, Gottfried HW, Wolter H, Schmidt V, Kestler HA, Mayer J. Classification of prostatic carcinoma with artificial neural networks using comparative genomic hybridization and quantitative stereological data. Pathol Res Pract 2003; 199: 773-784.

Mattfeldt T, Trijic D, Gotffried HW. Incidental carcinoma of the prostate: clinicopathological, stereological and immunohistochemical findings studied with logistic regression and self-organizing feature maps. BJU Int 2004; 93: 284-290.

Mayhew TM, Mwamengele GL, Dantzer V. Stereological and allometric studies on mammalian cerebral cortex with implications for medical brain imaging. J Anat 1996; 189:177-184.

Mayhew TM, Olsen DR. Magnetic resonance imaging (MRI) and model free estimates of brain volumes determined using the Cavalieri principle. J Anat 1991; 178: 133-144.

Mazonakis M, Damilakis J, Maris T, Prassopoulos P, Gourtsoyiannis N. Comparison of two volumetric techniques for estimating liver volume using magnetic resonance imaging. J Magn Reson Imaging 2002; 15: 557-563.

Mazonakis M, Karampekios S, Damilakis J, Voloudaki A, Gourtsoyiannis N. Stereological estimation of total intracranial volume on CT images. Eur Radiol 2004; 14:1285-1290.

Nawaratne S, Fabiny R, Brien JE, Zalcberg J, Cosolo W, Whan A, *et al.* Accuracy of volume measurements using helical CT. J Comp Assist Tomogr 1997; 21: 481-486.

Odaci E, Sahin B, Sonmez OF, Kaplan S, Bas O, Bilgic S, *et al.* Rapid estimation of the vertebral body volume: a combination of the Cavalieri principle and computed tomography images. Eur J Radiol 2003; 43: 316-326.

Raz N, Dupuis JH, Briggs SD. Differential effects of age and sex on the cerebellar hemispheres and the

vermis: a prospective MR study. AJNR Am J Neuroradiol 1998; 19: 65-71

Roberts N, Cruz-Orive LM, Reid NMK, Brodie DA, Bourne M, Edwards RHT. Unbiased estimation of human body composition by the Cavalieri method using magnetic resonance imaging. J Microsc 1992; 171: 239-253.

Roberts N, Puddephat MJ, McNulty V. The benefit of stereology for quantitative radiology. Br J Radiol 2000; 73: 679-697.

Ronan L, Doherty CP, Delanty N, Thornton J, Fitzsimons M. Quantitative MRI, a reliable protocol for measurement of cerebral gyrification using stereology. Magn Reson Imaging 2006; 24: 265-272.

Rovaris, M. Sensitivity and reproducibility of volume change measurements of different brain portions on MR scans from patients with multiple sclerosis. J Neurol 2000; 24 7: 960-965.

Sahin B, Acer N, Sonmez OF, Emirzeoglu M, Basaloglu H, Uzun A, Bilgic S. Comparison of four methods for the estimation of intracranial volume: a gold standard study. Clin Anat 2007; 20: 766-773.

Sahin B, Emirzeoglu M, Uzun A, Incesu L, Bek Y, Bilgic S, *et al*. Unbiased estimation of the liver volume by the Cavalieri principle using magnetic resonance images. Eur J Radiol 2003; 47: 164-170.

Sahin B, Ergur H. Assessment of the optimum section thickness for the estimation of liver volume using magnetic resonance images, a stereological gold standard study. Eur J Radiol 2006; 57: 96-101.

Sahin B, Mazonakis M, Akan H, Kaplan S, Bek Y. Dependence of computed tomography volume measurements upon section thickness: an application to human dry skulls. Clin Anat. 2008; 21: 479-485.

Sgouros S, Goldin JH, Hockley AD. Intracranial volume change in childhood. J Neurosurg 1999; 91: 610-616.

Till C, Ghassemi R, Aubert-Broche B, Kerbrat A,Collins DL, Narayanan S, *et al*. MRI correlates of cognitive impairment in childhood-onset multiple sclerosis. Neuropsychology 2011; 25: 319-332.