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A Stereological Assessment Method for Estimating the Surface Area of Cycloids

Yüzey Alanının Hesaplanmasında Cycloid Metoduna Dayandırılan Stereolojik Bir Değerlendirme

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Abstract

Objective: In this study, we sought to determine differences in estimations of surface area made by classical vertical uniform random (VUR) section series and vertical section series obtained perpendicular to a fixed horizontal plane.

Materials and Methods: One volunteer subject (male, 25 years of age) with no neurological deficit was chosen at random from a bank of controls in the magnetic resonance (MR) image data library of the Department of Radiology. First, a soccer ball with known geometrical features (radius: 9.75 cm) was imaged using a T1-weighted MR scanner at 5-mm thickness (total 40 sections) to test the validity and reliability of surface area and volume measurements obtained via stereological methods. Second, T1-weighted MR section profiles were obtained from a volunteer individual. Surface area and volume estimation procedures were carried out using the Stereo Investigator 6, MicroBrightField, Inc., USA.

Conclusions: We determined that there are no differences in either surface area or volume estimations made using VUR sections and direct vertical sections. We have performed an exhaustive series analysis with a variety of objects.

Key Words: Calculating surface area, Cycloids, Stereology, Volume estimation

Özet

Amaç: Bu çalışma, klasik dikey düzenli rastgele (DDR) seri kesitler ile yatay düzleme dik olan dikey seri sabit kesitlerin ölçümleri arasında fark olup olmadığını anlamak için yapıldı.

Gereç ve Yöntem: Magnetik rezonans (MR) resimlerinin bulunduğu Radyoloji bolumu arşivi kontrol bankasından rasgele alınan ve hicbir norolojik hasarı bulunmayan gonullu bir kişi (erkek, 25 yaşında) secildi. İlk olarak geometrik özellikleri bilinen (yarıçap: 9.75 cm) futbol topunu T1-weigthed MR cihazı kullanılarak 5 mm kalınlığında MR görüntüleri (toplam: 40 kesit) alınarak yüzey alanı ve hacim hesaplama sonuçlarının stereolojik metot ile doğruluğu ve güvenirliliği test edildi. İkinci olarak T-1 weigthed MR tarayıcı cihazı kesit profilleri gönüllü bireyden elde edildi. Yüzey alanı ve hacim hesaplama prosedürleri Stereo Investigator 6. MicroBrightField. Inc. USA. Kullanılarak yapıldı.

Sonuç: Çalışmamız sonucunda, ilgilenilen geometrik şekillerin enine ve boyuna kesitlerinde DDR kesitler ile direk dikey kesitler arasında hem yüzey alanı hesaplanması açısından hem de hacim hesaplanması açısından hiçbir farlılık bulunamamıştır.

Anahtar Kelimeler: Yüzey alanı hesaplanması, Cycloid, Stereoloji, Hacim hesaplanması

Introduction

Quantitative examination of human cerebral cortex using a variety of methodological approaches has provided useful information in clinical studies and improved our understanding of the structure-function relationship. These techniques are based on in vivo magnetic resonance (MR) images. One such method is stereology, which is a sampling technique used to generate mathematically unbiased estimates of the geometric properties of three-dimensional structures based on two-dimensional slices of the object [1]. One of the most important quantitative parameters is the surface area, which is an important value in many areas of biology, such as the interface between capillaries and tissues, microvilli and the intestinal lumen, inspired air and dissolved gases in the alveoli, as well as connections between neurons, glial cells and synapses [2]. Useful techniques for estimating the surface area of complex objects have been produced using isotropic uniform random (IUR) sectioning [3] and vertical uniform random (VUR) sectioning approaches [4]. These methods have been successfully applied to a variety of biological tissues and images [5-7]. Although it is theoretically possible to use both IUR and VUR sectioning procedures to estimate surface area efficiently and without bias, this has proved difficult in practice, particularly when considering in vitro MR sectioning. For this reason, few studies have been published on this subject [8-9].

In this study, we sought to determine any differences between estimations of surface area made using classical VUR section series and those made using vertical section series that were obtained perpendicular to a fixed horizontal plane.

Materials and Methods

Subjects

One volunteer subject (male, age 25) with no neurological defi-

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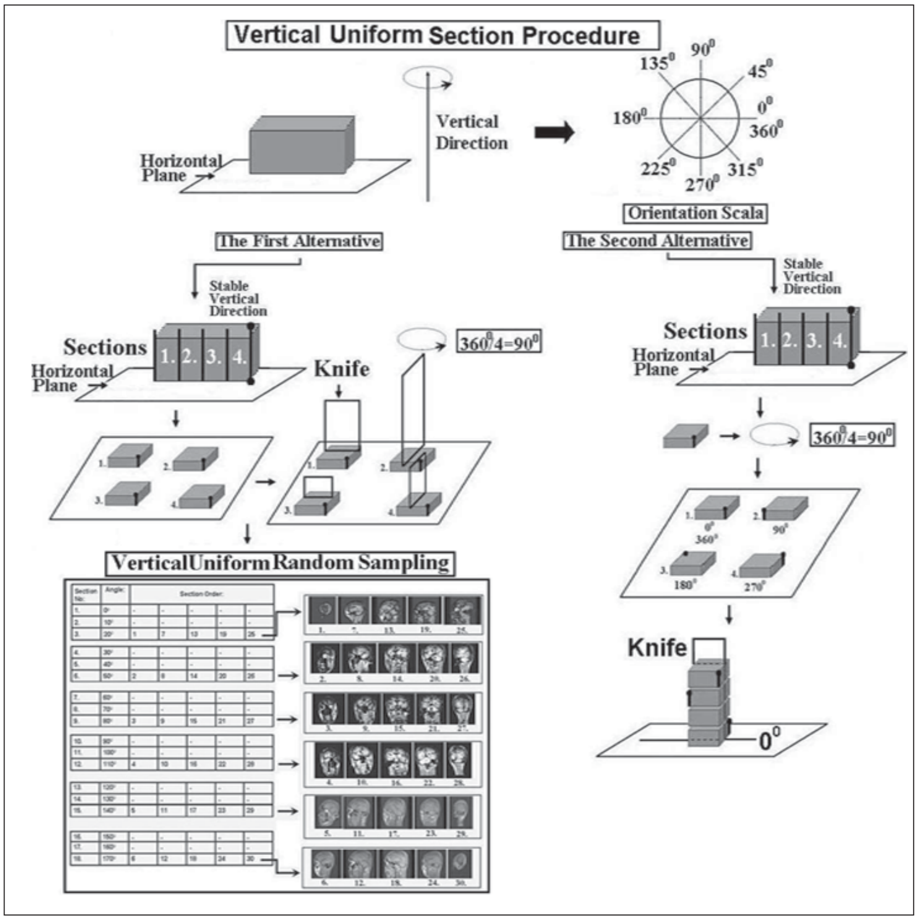

Figure 1. *The principle of the vertical uniform section (VUR) procedure is shown. Vertical uniform random sampling (VURS), used in the present study, is illustrated (boxed image-see Figure 2). The object of interest is laid parallel to the horizontal plane and the object is cut perpendicularly in a random manner. The resulting vertical sections are placed on a transverse plane that is parallel to the horizontal plane. It is important that the object be placed in the same direction (0°) as the previously detected internal marker. VUR sections are obtained by uniformly, randomly and systematically rotating a knife. Alternatively, vertical sections may be obtained by cutting the object perpendicular to the horizontal plane. These sections are uniformly and randomly rotated and have a systematic uniform random position. In the next step, these sections are placed over the other sections, and finally they are cut with a knife at a stable position. Because the second method cannot be applied to in vivo studies, it was not used in the current study.*

cit was chosen at random from a bank of controls in the MR image data library in the Department of Radiology, Medical Faculty, Ataturk University, Erzurum. The ethics committee at the Medical Faculty, Ataturk University approved this study.

Collection of Magnetic Resonance Images

First, a soccer ball with known geometrical features (radius: 9.75 cm) was imaged using a T1-weighted MR scanner at 5-mm thickness (total 40 sections) to test the validity and reliability of the surface area and volume measurements obtained using stereological methods.

Second, T1-weighted MR section profiles were obtained from a volunteer individual. The right sagittal axis of the volunteer's brain was assigned a starting point called "zero degrees" (0°), and then the whole brain was scanned with MR perpendicular to the axial plane in 5-mm thick sections (slice gap: 0 mm). The same process was applied using all other interesting angles at a systematic interval of 10^0 in the same direction (perpendicular to the axial plane). Approximately 540 images were obtained from the subsequent series (30 images from 0°, 30 images from 10°, etc.), and the collection consisted of images obtained at angels of 20°, 30°, 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 130°, 140°, 150°, 160° and 170°.

Stereological software

Surface area and volume estimation procedures were applied using the Stereo Investigator 6, MicroBrightField Inc., USA.

Image data

MR image data were transferred to a workstation and converted from DICOM in order to estimate the surface area and volume of various structures using stereological methods.

Sampling Procedures

I. Sampling for a soccer ball

Forty section images (section thickness, 5 mm) obtained by scanning throughout a soccer ball without applying any sampling procedure were used for both stereological analyses and calculation according to the formulas for volume and surface area of a sphere.

II. Vertical Uniform Random Sampling (VURS) Protocol for MR Images

After all of the MR images were transferred to a workstation and converted from DICOM, a sampling procedure was applied according to stereological principles described in the VURS protocol (Figure 1, 2).

In this study, a systematic range of 30° was accepted. In the first sampling procedure (VURS-1), randomness began at 20° (Table 1, Figure 2). The first image was taken from first section at 20°, the second image was taken from second section at 50°, the third image was taken from second section at 80°, and so on. All sampling procedures at this stage are summarized in Table 1 (Figure 2).

Table 1. The first vertical uniform random sampling procedure (VURS-1); randomness was 20°

Section No:	Angle:	Section Order:				
1	0°	-	-	-	-	-
2	10°	-	-	-	-	-
3	20°	1	7	13	19	25
4	30°	-	-	-	-	-
5	40°	-	-	-	-	-
6	50°	2	8	14	20	26
7	60°	-	-	-	-	-
8	70°	-	-	-	-	-
9	80°	3	9	15	21	27
10	90°	-	-	-	-	-
11	100°	-	-	-	-	-
12	110°	4	10	16	22	28
13	120°	-	-	-	-	-
14	130°	-	-	-	-	-
15	140°	5	11	17	23	29
16	150°	-	-	-	-	-
17	160°	-	-	-	-	-
18	170°	6	12	18	24	30

In the second sampling stage (VURS-2), the systematic range was the same (30° was considered acceptable), but randomness began at 10°. All sampling procedures during the second stage are summarized in Table 2.

In the third sampling stage (VURS-3), the systematic range was the same (30° was considered acceptable), but randomness began at 0°. All sampling procedures during the third stage are summarized in Table 3.

The volume and surface area formulas were applied separately to section images obtained as described above according to stereological principles (Figure 3).

II. Use of the Modified Vertical Section Procedure on MR Section Profiles

After all MR images were transferred to a workstation and converted from DICOM, each image was separately assessed to estimate surface areas and volumes. All section profiles obtained were perpendicular to a fixed horizontal plane in each angel (0°, 10, 20°, 30°, 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 130°, 140°, 150°, 160° and 170°).

The volume and surface area formulas were applied according to stereological principles to the section images as described above (Figure 4).

Stereological Estimations

I. Surface Area and Volume Calculation for a Soccer Ball

The ball used in our calculations was sphere-shaped with a radius of 9.75 cm. Its volume and surface area can be calculated by the volume and surface area formulas for a sphere ($V=4/3 \cdot \pi \cdot r^3$, $SA= 4 \cdot \pi \cdot r^2$).

Table 2. The second vertical uniform random sampling procedure (VURS-2); randomness was 10°

Section No:	Angle:	Section Order:				
1	0°	-	-	-	-	-
2	10°	1	7	13	19	25
3	20°	-	-	-	-	-
4	30°	-	-	-	-	-
5	40°	2	8	14	20	26
6	50°	-	-	-	-	-
7	60°	-	-	-	-	-
8	70°	3	9	15	21	27
9	80°	-	-	-	-	-
10	90°	-	-	-	-	-
11	100°	4	10	16	22	28
12	110°	-	-	-	-	-
13	120°	-	-	-	-	-
14	130°	5	11	17	23	29
15	140°	-	-	-	-	-
16	150°	-	-	-	-	-
17	160°	6	12	18	24	-
18	170°	-	-	-	-	-

Table 3. The third vertical uniform random sampling (VURS-3); procedure (Systematic range: 30°, randomness: 0°)

Section No:	Angle:	Section Order:				
1	0°	1	7	13	19	25
2	10°	-	-	-	-	-
3	20°	-	-	-	-	-
4	30°	2	8	14	20	26
5	40°	-	-	-	-	-
6	50°	-	-	-	-	-
7	60°	3	9	15	21	27
8	70°	-	-	-	-	-
9	80°	-	-	-	-	-
10	90°	4	10	16	22	28
11	100°	-	-	-	-	-
12	110°	-	-	-	-	-
13	120°	5	11	17	23	29
14	130°	-	-	-	-	-
15	140°	-	-	-	-	-
16	150°	6	12	18	24	-
17	160°	-	-	-	-	-
18	170°	-	-	-	-	-

Section No:	Angle:	Section Order:				
1.	0°	-	-	-	-	-
2.	10°	-	-	-	-	-
3.	20°	1	7	13	19	25
4.	30°	-	-	-	-	-
5.	40°	-	-	-	-	-
6.	50°	2	8	14	20	26
7.	60°	-	-	-	-	-
8.	70°	-	-	-	-	-
9.	80°	3	9	15	21	27
10.	90°	-	-	-	-	-
11.	100°	-	-	-	-	-
12.	110°	4	10	16	22	28
13.	120°	-	-	-	-	-
14.	130°	-	-	-	-	-
15.	140°	5	11	17	23	29
16.	150°	-	-	-	-	-
17.	160°	-	-	-	-	-
18.	170°	6	12	18	24	30

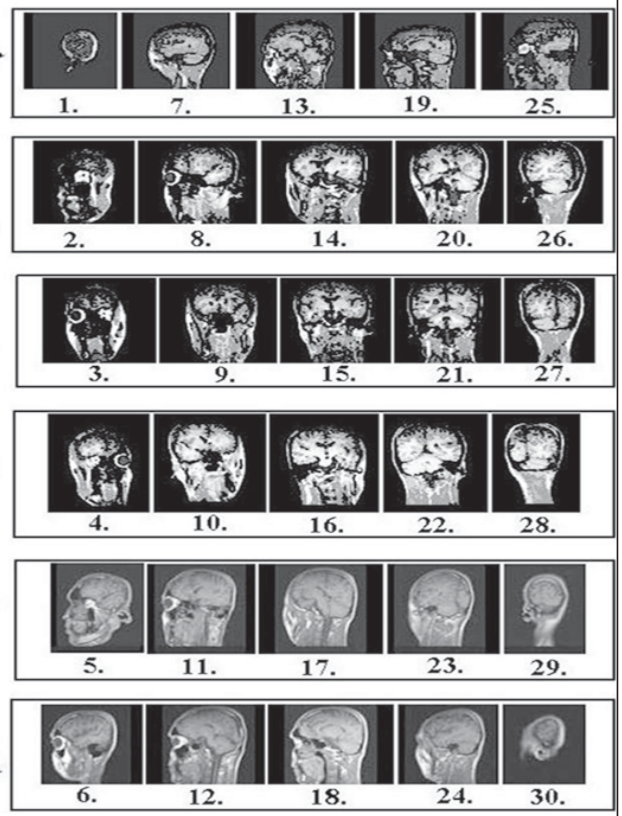

Figure 2. *The vertical uniform random strategy used in this study. First, a whole brain was scanned to produce 5 mm T1-weighted consecutive MRI sections at an angle of 0° (Sagittal: perpendicular to horizontal axis). Then the same procedure was applied to all angles separately (From angle of 10° to 170°). This resulted in approximately 540 section profiles from one brain (31 sections at a sagittal angle of 0°, 30 sections at an angle of 10°, etc.). Second, a systematic random sampling procedure was carried out in order to obtain sections. A total of 18 series (angles of 0°-170°) was divided into six groups. Thus every group contained three series, for example the 1st, 2nd and 3rd series consisted of angles of 0°, 10° and 20°. Finally, the systematic interval at 30° was determined and random sampling was performed at angles of 0° (Table 3), -10° (Table 2) and -20° (Table 1) in the first, second and third VURS respectively. For example, we chose the 1st, 7th, 13th 19th and 25th section profiles from the 20° series, the 2nd, 8th, 14th 20th and 26th section profiles from the 50° series, the 3rd, 9th, 15th 21st and 27th section profiles from the 80° series, and so on. Procedures for estimating both surface area and volume were applied to 30 section profiles according to stereological principles.*

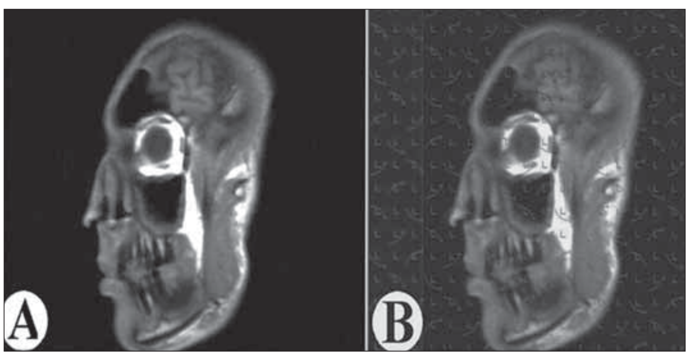

Figure 3. A) *a sagittal MR image used in the study. This image was transmitted to a PC with a stereo-investigator.* ***B)*** *the same image was superimposed on a point counting grid (for volume estimation) and intersections (for surface area estimation) together.*

II. Surface Area and Volume Estimation for a Scroll Ball and MR sections

Volume estimation

A volume estimation of any structure of interest can be obtained efficiently according to the principle of Cavalieri [10] regardless of shape or size. The volumes for each section were estimated by the following formula:

$$\hat{V} = t \times \frac{a}{p} \times \sum_{i=1}^{m} P_i$$

where V is the volume of the object of interest (tumor) in one section plane, t is the section thickness, a/p is the interpoint area, and ΣP is the number of points touching the tumor in that section. After this formula is applied as described in other sections, the estimate of total volume can be obtained from:

$$\hat{V}_{\underline{total}} = V_1 + V_2 + \ldots V_n$$

Surface area estimation

The surface area any structure may be estimated according to the principle of surface area from vertical sections [4] regardless of its shape or size.

Table 4. An example of total volume and CE of a brain section in our study (angels of 60)

Section Number	Number of Point (P)	A PXP	B PXP_i	C PXP_{i+1}
1	4	16	88	108
2	22	484	594	748
3	27	729	918	1134
4	34	1156	1428	1496
5	42	1764	1848	2142
6	44	1936	2244	1980
7	51	2601	2295	2448
8	45	2025	2160	2205
9	48	2304	2352	2400
10	49	2401	2450	2597
11	50	2500	2650	2450
12	53	2809	2597	2703
13	49	2401	2499	2254
14	51	2601	2346	2346
15	46	2116	2116	2438
16	46	2116	2438	2392
17	53	2809	2756	2650
18	52	2704	2600	2600
19	50	2500	2500	2100
20	50	2500	2100	2250
21	42	1764	1890	2100
22	45	2025	2250	2205
23	50	2500	2450	2100
24	49	2401	2058	1764
25	42	1764	1512	1344
26	36	1296	1152	756
27	32	1024	672	576
28	21	441	378	231
29	18	324	198	162
30	11	121	99	44
31	9	81	36	0
32	4	16	0	0
Total	1225	54229	53674	52723

$$\hat{S} = 2.T.(a/l).\sum_{i=1}^{j} Li$$

The surface area was estimated using the following formula: where *T* is the slice separation, *a/l* is the area per length of a cycloid, *Li* is the number of intersections generated on the i^{th} slice and *j* is the total number of slices.

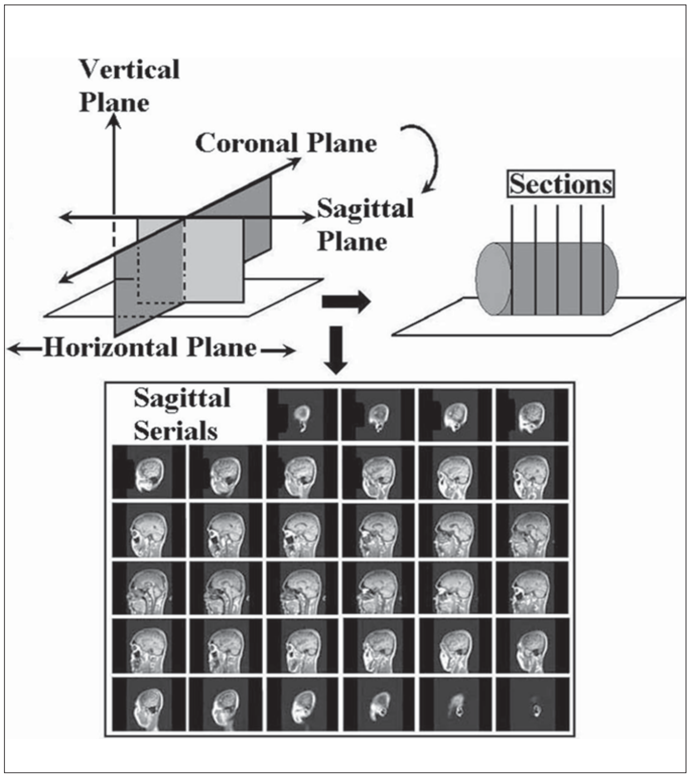

Figure 4. *In the first image, all planes, related to this study are illustrated. Serial sections (MR images in the study) at the fixed plane (angle of 0°, sagittal: Perpendicular to horizontal axis) were handled. All serial images (31 section profiles) in which the brain was viewed are shown. Estimates of volume and surface area were made according to stereological principles.*

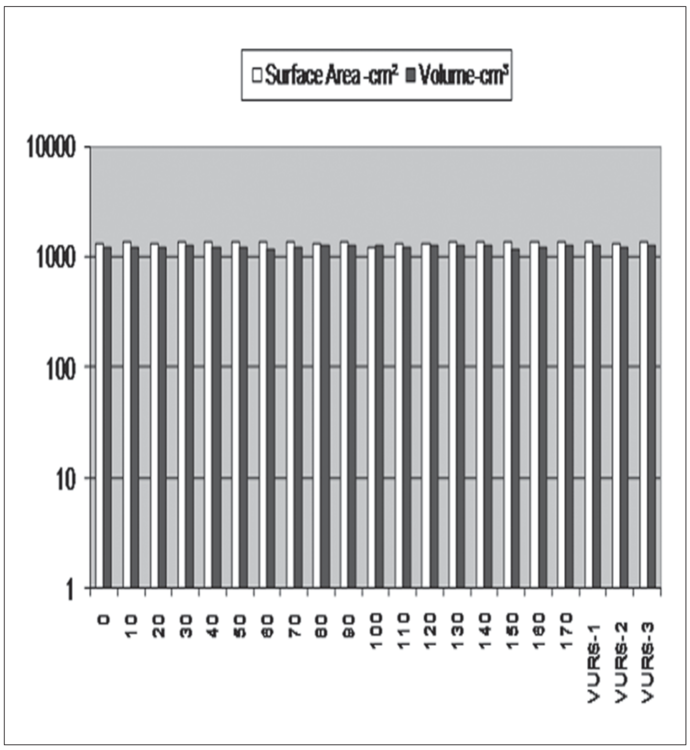

Figure 5. *Surface area and volume values estimated in the study.*

Table 5. An example of total surface area and CE of a brain section in our study (angels of 60)

Section Number	Number of Intersection (G)	A GXG	B GXG_i	C GXG_{I+1}
1	13	169	299	325
2	23	529	575	690
3	25	625	750	725
4	30	900	870	1080
5	29	841	1044	1102
6	36	1296	1368	1656
7	38	1444	1748	1482
8	46	2116	1794	1656
9	39	1521	1404	1755
10	36	1296	1620	1584
11	45	2025	1980	1260
12	44	1936	1232	1408
13	28	784	896	952
14	32	1024	1088	1344
15	34	1156	1428	1496
16	42	1764	1848	1932
17	44	1936	2024	1540
18	46	2116	1610	1702
19	35	1225	1295	1120
20	37	1369	1184	1184
21	32	1024	1024	1312
22	32	1024	1312	1120
23	41	1681	1435	1394
24	35	1225	1190	1085
25	34	1156	1054	1156
26	31	961	1054	620
27	34	1156	680	748
28	20	400	440	420
29	22	484	462	308
30	21	441	294	126
31	14	196	84	0
32	6	36	0	0
		A	B	C
Total	1024	35856	35086	34282

Estimation of the surface area per unit volume

This method is used to estimate the surface area per unit volume. The surface area per unit volume was estimated using the following formula [4]:

$$\hat{S}_V = \frac{2 \cdot \sum_{i=1}^{n} I_i}{\frac{l}{p} \cdot \sum_{i=1}^{n} P_i}$$

where I is the number of intersections counted and L is the total length of all cycloids in all slices. Pi is the point count per sample slice.

Error Prediction Estimations

I. Error Predictions for the Cavalieri Estimation:

The point density of the point counting grid was designed to obtain an appropriate coefficient of error (CE) for images of the serial sections (Table 4). CE and coefficient of variation (CV) were estimated according to Gundersen and Jensen's formula [10].

$$\text{Noise} = 0.0724 \times (b/\sqrt{a}) \times \sqrt{n \times \sum P} = 0.0724 \times 5 \times \sqrt{32 \times 1225} = 71,67$$

Noise is a value representing the complexity of the examined cut surface area of the specimen, $b/\sqrt{a}$ is equivalent to the mean boundary length of the profiles divided by the square root of their mean area, n is the section number that is examined, and ΣP the number of points touching each whole section.

$$Var_{SRS}(\sum_{i=1}^{n} a) = (3 \cdot (A\text{-Noise}) - 4 \cdot B + C)/12 = (3.(54229-71,67)-4X53674+52723/12=41,58$$

Where $Var_{SRS}(\sum_{i=1}^{n} a)$ indicates variance of total area in the systematic random sampling (SRS). These data indicate the sufficient number of sections required to obtain an appropriate variation for section samples. A, B and C are the total numerical values for the data in the related column of Table 1.

$$Total_{var} = \text{Noise} + Var_{SRS} = 71,67 + 41,58 = 113,25$$

$$CE(\sum P) = \frac{\sqrt{Total_{Var}}}{\sum P} = \frac{\sqrt{113,25}}{1225} = 0,0086$$

CE is the last calculated value. The highest limit of CE that is generally accepted is 5% [10].

II. Error Predictions for the Surface Area Estimation

$$VAR_{SRS} = \frac{3A - 4B + C}{12} = \frac{3x35856 - 4x35086 + 34282}{12} = 125,5$$

$$CE(S) = \frac{\sqrt{VAR_{SRS}}}{\sum_{i=1}^{n} I_i} = \frac{\sqrt{125,5}}{1024} = 0,012$$

Table 6. Results of estimated brain surface area and volume on MR images both obtained according to vertical uniform random sampling procedure and directly vertical section that was perpendicular to a fixed horizontal plane at the angle of 0°-180° separately

Angels	Number of Section	Test Point Area Per Cycloids Length -cm^2	Number of Intersection	Surface Area -cm^2	Volume Associated With Test Point -cm^3	Number of Test Point	Volume-cm^3
0	31	1,32	1001	1321,32	0,98	1260	1234,8
10	31	1,32	1031	1360,92	0,98	1272	1246,56
20	29	1,32	999	1318,68	0,98	1230	1205,4
30	30	1,32	1034	1364,88	0,98	1281	1255,38
40	30	1,32	1040	1372,8	0,98	1229	1204,42
50	31	1,32	1026	1354,32	0,98	1272	1246,56
60	32	1,32	1024	1351,68	0,98	1225	1200,5
70	31	1,32	1033	1363,56	0,98	1235	1210,3
80	32	1,32	1007	1329,24	0,98	1278	1252,44
90	32	1,32	1035	1366,2	0,98	1308	1281,84
100	32	1,32	933	1231,56	0,98	1291	1265,18
110	33	1,32	1003	1323,96	0,98	1231	1206,38
120	32	1,32	1020	1346,4	0,98	1284	1258,32
130	32	1,32	1027	1355,64	0,98	1293	1267,14
140	30	1,32	1036	1367,52	0,98	1303	1276,94
150	29	1,32	1043	1376,76	0,98	1221	1196,58
160	29	1,32	1047	1382,04	0,98	1237	1212,26
170	30	1,32	1029	1358,28	0,98	1286	1260,28
VURS-1	29	1,32	1028	1356,96	0,98	1279	1253,42
VURS-2	29	1,32	1010	1333,2	0,98	1269	1243,62
VURS-3	30	1,32	1029	1358,28	0,98	1279	1253,42

Results

We obtained approximately 540 images from the subsequent series (30 images from 0°, 30 images from 10°, etc.) consisting of angels of 20°, 30°, 40°, 50°, 60°, 70°, 80°, 90°, 100°, 110°, 120°, 130°, 140°, 150°, 160° and 170°. All stereological sectioning procedures are summarized in Table 1, Table 2, Table 3, Figure 1 and 2. Our stereological estimations of total volume and surface area were significantly similar ($P < 0.05$) according to directly vertical sections and VURS sections (Table 6).

Discussion

Stereology is an unbiased sampling technique whereby the object of interest is sectioned into a series of two-dimensional slices. This method can generate important data about the three-dimensional structure the object [8].

Human beings have long been interested in the regular and macroscopic metric parameters of the three-dimensional universe, as well as microscopic and irregular parameters such as volume, number, length and surface area. For structures such as cones or cylinders, volume and surface area can be easily calculated by applying known formulas. However, if a structure has irregular geometrical features, then complex processes are inevitability required. Currently, stereological methods are among the techniques suggested to estimate the metric properties of irregular sutures. It is easy, efficient and practical to apply some of these methods, such as volume estimation using the Cavalieri method, both in vivo and in vitro. However, determination of the surface area of complex and irregularly-shaped objects, especially objects in living organisms, is very difficult and not practical in terms of sampling procedure. Stereological principles for estimating surface area consist of either isotropic uniform random sampling (IURS) or VURS procedures [4].

The VURS procedure described by others and in our study is both difficult to understand and not practical to apply, particularly in *in vivo* situations, despite the fact that it is an efficient and useful approach to estimate the surface area of interesting objects. The most important disadvantage is the increased workload in the processing of the tissue due to the embedment and re-embedment [12]. This burden may be tolerated in macroscopic or perhaps microscopic studies that are conducted in vitro, but in radiological studies, to obtain and analyze VUR is not practical for a typical radiology unit. In our study, we found that approximately 540 sections, 5-mm thick, were required for the VURS procedure.

We estimated brain surface area from MR images that were obtained according to the VURS procedure as described in Materials and Methods. Directly vertical sections were obtained perpendicular to a fixed horizontal plane at angles of 0°-180°. We determined that there is no difference in the estimation of surface area or volume between VURS sections and directly vertical sections using an exhaustive series of a test object. We found no difference between surface area estimates using VURS sections or directly vertical sections in our empirical study. This result is consistent with a previous study that was carried out by Robert N, et al. (2000) [9]. In this study, cerebral hemispheres were sampled with 12 exhaustive series of vertical sections at 15° intervals about a fixed vertical axis [9] and surface area estimations were done with cycloid test lines [4]. Our study is similar to the study of Robert N, et al. (2000) with respect to the use of directly vertical sections at different angels to estimate surface area, but we also tried to obtain MR sections for estimating the surface area according to a vertical uniform random sampling procedure in order to compare with two approaches.

Conflict of interest statement: The authors declare that they have no conflict of interest to the publication of this article.

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