

Comparison of volume estimation methods for pancreatic islet cells

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ABSTRACT

In this contribution we study different methods of automatic volume estimation for pancreatic islets which can be used in the quality control step prior to the islet transplantation. The total islet volume is an important criterion in the quality control. Also, the individual islet volume distribution is interesting – it has been indicated that smaller islets can be more effective. A 2D image of a microscopy slice containing the islets is acquired. The input of the volume estimation methods are segmented images of individual islets. The segmentation step is not discussed here. We consider simple methods of volume estimation assuming that the islets have spherical or ellipsoidal shape. We also consider a local stereological method, namely the nucleator. The nucleator does not rely on any shape assumptions and provides unbiased estimates if isotropic sections through the islets are observed. We present a simulation study comparing the performance of the volume estimation methods in different scenarios and an experimental study comparing the methods on a real dataset.

Keywords: Islets of Langerhans, volume estimation, local stereology, nucleator

1. INTRODUCTION

Islets of Langerhans isolated from pancreases of cadaver donors can be transplanted to patients for treatment of specific cases of diabetes. The islets vary in shapes and size, with their diameter ranging approximately from 10 to 700 μm . While the success of the therapy depends on the net volume of the transplanted islets, it has been indicated that small islets might have better engraftment and hence be more effective.¹ In the quality control step, microscopical images of a few samples are usually manually evaluated (for an illustration of such image of an islet sample see Figure 1 below). Attempts have been made to take advantage of image analysis methods and image segmentation in order to construct automatic volume estimation methods.² These are preferred to the manual evaluation, enabling to analyze a significantly larger number of samples and thus reducing the sampling related uncertainty.

In this contribution we focus on methods for estimation of the volume of three-dimensional objects (islets) based on two-dimensional observation (digital image obtained by classical or confocal microscopy, segmented prior to the volume estimation; the segmentation step is not discussed here). The traditional method³ relies on the assumption of a spherical shape of the islet and, based on the diameter of the observed 2D islet profile, uses a conversion table with 50 μm range increments to calculate the estimated islet volume. This procedure introduces unnecessary rounding to the estimation.

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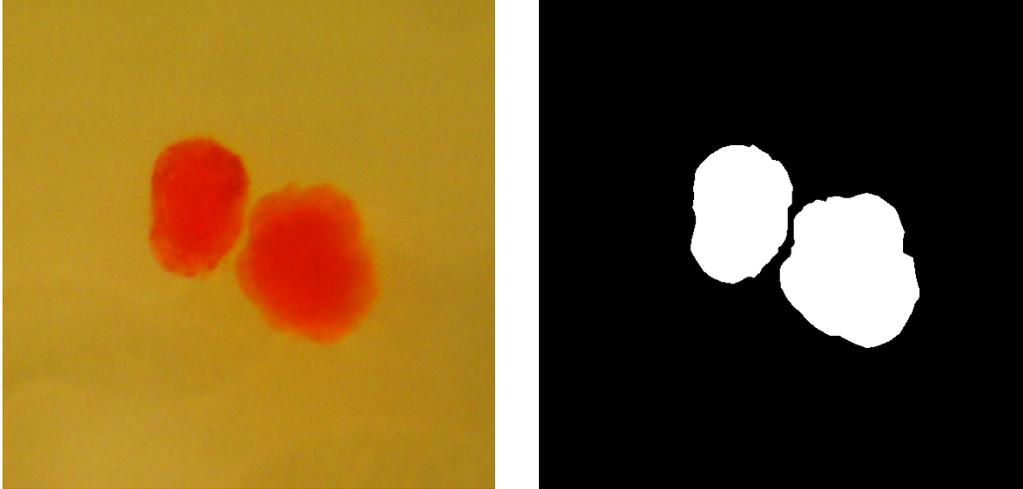


Figure 1. Example of the original image (left, bright orange islets on light brown background – the electronic version contains the figure in color) together with the segmented binary image used for volume estimation (right).

Other volume estimation methods also assume specific shape and orientation of the islets.² We aim to investigate the performance of these methods and compare it to the performance of a simple local stereological method, the nucleator.^{4,5} Our motivation is that the nucleator does not depend on any shape assumptions and it is an unbiased volume estimation method if a randomly oriented section through the object is observed.

We have performed a simulation study in which we assess the performance of the volume estimators in question for ellipsoids with random semiaxes lengths. We consider two types of observation: randomly oriented sections through the ellipsoid center (this corresponds to optical sections obtained by confocal microscopy) and orthogonal projections to a randomly oriented plane (this is an approximation to classical microscopy imaging). To the best of our knowledge, a suitable stereological method for volume estimation from a single observed planar projection of the object is not available in the literature, hence we investigate the performance of the nucleator also in this case.

Finally we apply the volume estimation methods to samples of rat islets observed by classical microscopy. As an independent measure of the islet volume we have also experimentally determined the total DNA content per sample. This is technically more simple to do for rat islets than for human islets which are often wrapped in the so-called exocrine tissue.

2. METHODS

The traditional way to express the volume of islets in a sample is to give the number of so-called islet equivalents (IEQ). The number of IEQ in a sample is obtained by dividing the actual volume of the islets by the volume of a (hypothetical) spherical islet with diameter of $150 \mu\text{m}$, see Ref. 3.

Suppose that we have recorded a digital image of the islet profile (section or projection) and the image has been segmented in order to separate the islet from the background. This means that our data consist of a binary image of the islet. See Figure 1 for an illustration.

2.1 Sphere volume with Ricordi table

The classical method for islet volume estimation was proposed in Ref. 3. It consists of manual determination of the islet diameter for (nearly) circular islets or estimation of a “representative diameter” for non-circular islets. The islets are then categorized according to their estimated diameter into classes of $50 \mu\text{m}$ increments. Finally, the number of islets in each category is multiplied by the corresponding factor (given in the so-called Ricordi table) to obtain the number of IEQ in the category. Summing over the categories provides the total number of IEQ in the sample.

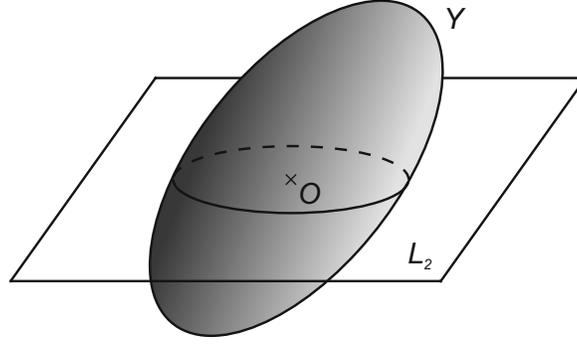


Figure 2. Illustration of the principle of local stereology: a three-dimensional particle Y is sectioned by an isotropically oriented plane L_2 through the reference point O . The planar section $Y \cap L_2$ forms the observation and is used to estimate the volume of Y .

In the present study we use an automatic version of this procedure – the manual measurements of the diameter are replaced by the automatic procedure described in Ref. 2. For each islet the area A of its observed (segmented) profile is automatically determined and the diameter d of a circle with the same area is calculated using the formula $d = \sqrt{4A/\pi}$. The islet is then categorized based on this diameter of approximating circle, using the Ricordi table.

The original Ricordi table classifies every islet with diameter $> 350 \mu\text{m}$ into the same category. This means that the volume of very large islets can be severely underestimated by this procedure. We use an extended version of the Ricordi table which adds extra bins for larger islets, again with $50 \mu\text{m}$ increments. The corresponding multiplication factors were determined in the same way as in the original Ricordi table.³ Islet volume estimated by this method will be denoted \widehat{V}_S^R .

2.2 Sphere volume

This volume estimation method is a version of the previous one: once the diameter d of the segmented islet profile is determined (using the formula $d = \sqrt{4A/\pi}$ where A denotes the area of the segmented profile) the islet volume is estimated as the volume of a sphere with diameter d , see Ref. 2. In this way the unnecessary rounding introduced by using the Ricordi table is avoided. Islet volume estimated by this method will be denoted \widehat{V}_S .

2.3 Prolate volume

An approximating ellipse with semiaxes lengths a and b , $a < b$, is fitted to the segmented islet profile by the algorithm described in Ref. 6. The islet volume is then estimated as the volume of a prolate ellipsoid with semiaxes lengths equal to a, a and b , respectively.² Islet volume estimated by this method will be denoted \widehat{V}_P , thus the corresponding formula is $\widehat{V}_P = (4/3) \cdot \pi a^2 b$.

2.4 Nucleator

The nucleator is a local stereological method for volume estimation of a three-dimensional object based on physical or optical sections through the object.⁴ Its main advantage is that no assumptions about the object shape are needed.

Let Y be the three-dimensional object with a uniquely determined reference point O in its interior, e.g. the center of mass, or the nucleus or nucleolus in case of biological cells. Furthermore, let L_2 be a randomly (isotropically) oriented plane passing through the reference point, see Figure 2. The section $Y \cap L_2$ is observed and used for volume estimation.

The classical nucleator proposed in Ref. 4 used only a few measurements in the section $Y \cap L_2$ to estimate the volume of Y . In this paper we use the so-called integrated version of nucleator.⁵ This version uses information in the whole section $Y \cap L_2$, which results in lower variability of the estimates compared to the classical nucleator.

The volume estimate using the integrated nucleator is obtained by the formula

$$\widehat{V}_N(Y) = \int_{Y \cap L_2} \text{dist}(x, O) \, dx,$$

where $\text{dist}(x, O)$ is the Euclidean distance between the point x and the reference point O . The integral can be discretized in a natural way and used for volume estimation if the section $Y \cap L_2$ is recorded as a binary image.

Both the classical and the integrated nucleator are unbiased volume estimators, with respect to random orientation of the section plane L_2 , see Ref. 4,5. Thus, the mean value (more precisely, the expected value) of the estimate is the true volume of Y .

We stress here that the nucleator can be used to estimate the volume of both convex and non-convex objects. This plays an important role in the context of the islet volume estimation as the clinical experience indicates that the islets can sometimes be non-convex. This is a favourable feature of the nucleator since the sphere or prolate volume estimation methods are hard to justify for non-convex objects.

Throughout this paper we use the center of mass of an observed (segmented) islet profile $Y \cap L_2$ as the reference point O . We use this to approximate the position of the true (but unknown) center of mass of the 3D islet Y . Islet volume estimated by this method will be denoted \widehat{V}_N .

3. EXPERIMENTS AND RESULTS

3.1 Simulation study

In order to assess the performance of the volume estimation methods described above we have performed a simulation study with random ellipsoids. Let a , b and c denote the semiaxes lengths of an ellipsoid. We have fixed $a = 1$ and let b and c be independent, identically distributed random variables with uniform distribution on the interval $[1; x]$, where we take $x = 2, 4$ or 8 , respectively.

In the clinical practice it is usual to analyse samples containing a large number of islets in order to determine the total islet volume in the sample. To mimic this situation we have generated, for each choice of x , 1000 random samples, each containing 100 non-overlapping ellipsoids with random semiaxes lengths (as described in the previous paragraph). Each of the ellipsoids was randomly isotropically oriented.

For each ellipsoid we have determined its section parallel to the (x, y) -plane and passing through the ellipsoid's center. This corresponds to an optical section obtained by a confocal microscope. We have also determined for each ellipsoid an orthogonal projection to the (x, y) -plane. This is a rough approximation to an image obtained by classical optical microscopy. We stress here that the sections and projections are calculated analytically from the ellipsoid's dimensions and orientation. Thus, there is no need for image segmentation here.

We have calculated volume estimates based on each elliptical section or projection by the three estimation methods described in Sections 2.2-2.4 and summed up to obtain the estimate \widehat{V} of the total volume in each sample. In the simulation study we have disregarded the Ricordi method from Section 2.1 because there is no natural analogue of the original Ricordi table.

For each sample we know the true total volume V of the ellipsoids in the sample. We can calculate the relative bias and the mean squared relative error (MSE) of an estimate \widehat{V} as the mean value of $(\widehat{V} - V)/V$ and the mean value of its square, respectively. These characteristics are given in Table 1 for estimates based on the sections through the ellipsoid center and in Table 2 for estimates based on the orthogonal projections.

As seen from Table 1—and confirmed by its theoretical properties—the nucleator is an unbiased volume estimator if it is applied to images of optical sections. Both the sphere and prolate volume estimators have negative bias in this case, i.e. they underestimate the actual volume. This underestimation is more severe if we allow for more variable shapes of the ellipsoids (longer interval for the semiaxes lengths b and c). Also, the MSE of the nucleator is the lowest among the three methods in all the situations due to its unbiasedness.

Table 2 shows that all the estimators suffer from relatively large bias if the projections are observed. This in turn results in large MSEs, the larger the longer is the interval for semiaxes lengths b and c . The lowest relative bias and MSE are exhibited by the prolate volume estimator.

Table 1. Simulation study: volume estimates based on sections through the ellipsoids' center. Relative biases (upper rows) and mean squared relative errors (MSE, lower rows) for the estimated total volume in a sample are given for the three situations: the semiaxes lengths b and c have uniform distribution on the interval given at the top of the column.

	[1; 2]	[1; 4]	[1; 8]
\widehat{V}_S - rel. bias	-0.025	-0.105	-0.235
\widehat{V}_S - MSE	0.001	0.014	0.061
\widehat{V}_P - rel. bias	-0.170	-0.368	-0.559
\widehat{V}_P - MSE	0.030	0.138	0.317
\widehat{V}_N - rel. bias	-0.000	0.002	0.000
\widehat{V}_N - MSE	0.001	0.003	0.007

Table 2. Simulation study: volume estimates based on the orthogonal projections of the ellipsoids. Relative biases (upper rows) and mean squared relative errors (MSE, lower rows) for the estimated total volume in a sample are given for the three situations: the semiaxes lengths b and c have uniform distribution on the interval given at the top of the column.

	[1; 2]	[1; 4]	[1; 8]
\widehat{V}_S - rel. bias	0.078	0.422	1.312
\widehat{V}_S - MSE	0.007	0.185	1.764
\widehat{V}_P - rel. bias	-0.072	0.095	0.722
\widehat{V}_P - MSE	0.006	0.016	0.560
\widehat{V}_N - rel. bias	0.103	0.532	1.580
\widehat{V}_N - MSE	0.011	0.290	2.536

3.2 Experiments on real dataset

We have analyzed 10 samples containing different amount of rat pancreatic islets, stained prior to imaging and observed by classical microscopy. Digital image of each sample was recorded with resolution $2.4 \mu\text{m}$ per pixel, i.e. one image corresponds to one sample, possibly containing many individual islets. The islets were then manually segmented. Three independent manual segmentations of each image were combined using the majority vote method to provide a single segmented image for each sample, see Figure 1 for an example.

The segmented binary images were then used for volume estimation, using the package FIJI.⁷ First we estimated the volume of each islet in the image and then summed these volumes to obtain the total islet volume in the particular image. Also, the total DNA content (in ng) was experimentally determined for each sample. This is an independent measure of the total islet volume in each sample.

The islets in our study had rather round, regular shape. In such situations it can be expected that the sections through a centrally positioned reference point do not differ much from the profiles observed by classical microscopy. Hence we also used the nucleator to approximately estimate the islet volume.

When comparing the different methods of volume estimation we first studied the Pearson correlation coefficients of the estimated volume with the amount of DNA in each sample. All the correlations were higher than 0.993, with only negligible differences between the methods. We also calculated the correlation between each pair of volume estimates corresponding to different methods. All the correlations were higher than 0.997 – this indicates that the volume estimation methods are almost perfectly linearly dependent.

Next we focused on the ratios of the DNA content per IEQ obtained for the different volume estimation methods. We denote these ratios by \widehat{p}_S^R , \widehat{p}_P and \widehat{p}_N , respectively (the subscripts identify the corresponding volume estimation method).

Selected characteristics of the ratios are given in Table 3. We observe that \widehat{p}_S^R has the largest standard deviation. This can be explained by the additional rounding (compared to the other volume estimation methods) introduced by using the Ricordi table. Relative standard deviations of the other ratios are virtually the same.

Table 3. Numerical characteristics of the ratios DNA per IEQ for different methods of volume estimation. The standard deviations are normalized by the corresponding mean to provide fair comparison of the methods.

	\widehat{p}_S^R	\widehat{p}_S	\widehat{p}_P	\widehat{p}_N
mean [ng/IEQ]	8.3	8.2	9.5	7.9
standard deviation / mean	0.24	0.18	0.17	0.19

Prominent differences are observed in the values of mean for the ratios of the DNA content per IEQ, see Table 3. This implies that the different volume estimation methods, although highly correlated, are not equivalent. Statistically significant differences are observed between the mean of \widehat{p}_P and the mean of \widehat{p}_N (p -value 0.030). For the other pairs the difference of means is not statistically significant (due to the low number of samples).

Ref. 8 reports the average DNA content per IEQ (using the Ricordi table) in rat islets to be 8.19 ng/IEQ for small islets (diameter $< 100 \mu\text{m}$) and 3.62 ng/IEQ for large islets (diameter $> 200 \mu\text{m}$). In our study the average DNA content per IEQ (using the Ricordi table) was 8.3 ng/IEQ for the whole islet population, i.e. our result does not contradict the previous study.⁸

4. CONCLUSIONS

Based on the results of the simulation study, if the sections of the islets are observed, e.g. by confocal microscopy, the nucleator is the method of choice for volume estimation due to its unbiasedness and the lowest MSE among the three considered methods.

If orthogonal projections of the islets are observed (this is a rough approximation to the classical microscopy) the prolate volume estimator performs the best. It has a only a small bias and reasonable values of the MSE if the islets are not much elongated. The sphere volume and the nucleator perform much worse in this scenario. For the nucleator this is caused by the violation of its assumptions.

In the experimental study the sphere volume using the Ricordi table was the most variable in terms of the DNA content per IEQ. We recommend not to use the Ricordi table because of the unnecessary rounding introduced to the estimation procedure.

Among the other methods the prolate volume is the most reliable method in the experimental study – its variability is comparable to the other methods and we may assume that it has the smallest bias (as indicated by the results of the simulation study).

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REFERENCES

- [1] Lehmann, R. et al., “Superiority of small islets in human islet transplantation,” *Diabetes* **56**, 594–603 (2007).
- [2] Nicolauss, N. et al., “Computer-assisted digital image analysis to quantify the mass and purity of isolated human islets before transplantation,” *Transplantation* **86**, 1603–1609 (2008).
- [3] Ricordi, C. et al., “Islet isolation assessment in man and large animals,” *Acta Diabetol. Lat.* **27**, 185–195 (1990).
- [4] Gundersen, H. J. G., “The nucleator,” *J. Microsc.* **151**, 3–21 (1988).
- [5] Hansen, L. V. et al., “The semi-automatic nucleator,” *J. Microsc.* **242**, 206–215 (2011).
- [6] Mulchrone, K. F. and Choudhury, K. R., “Fitting an ellipse to an arbitrary shape: implications for strain analysis,” *J. Struct. Geol.* **26**, 143–153 (2004).
- [7] Schindelin, J. et al., “Fiji: an open-source platform for biological-image analysis,” *Nat. Methods* **9**, 676–682 (2012).
- [8] Huang, H.-H., Ramachandran, K., and Stehno-Bittel, L., “A replacement for islet equivalents with improved reliability and validity,” *Acta Diabetol.* **50**, 687–696 (2013).