Two-dimensional sample entropy analysis of rat sural nerve aging

Luiz Eduardo Virgilio da Silva[1], Antonio Carlos da Silva Senra Filho[2],
Valéria Paula Sassoli Fazan[3], Joaquim Cezar Felipe[2], Luiz Otavio Murta Junior[2]

***Abstract*— Entropy analysis of images are usually performed using Shannon entropy, which calculates the probability of occurrency of each gray level on the image. However, not only the pixel gray level but also the spatial distribution of pixels might be important for image analysis. On the other hand, sample entropy (SampEn) is an important tool for estimation of irregularity in time series, which calculates the probability of pattern occurrence within the series. Therefore, we propose here an extension of SampEn to a two-dimensional case, namely $\mathbf{SampEn}_{2D}$, as an entropy method for extracting features from images that accounts for the spatial distribution of pixels. $\mathbf{SampEn}_{2D}$ was applied to histological segments of sural nerve obtained from young (30 days) and elderly (720 days) rats. Morphometric indexes, such as the total number of myelinated fibers and the average myelinated fibers area and perimeter were also calculated. Results show that $\mathbf{SampEn}_{2D}$ can extract useful information from histological nerve images, classifying elderly rat image as more regular than young rat. As $\mathbf{SampEn}_{2D}$ is related to irregularity/unpredictability, we can conclude that the proposed method is complementary to morphometric indexes. Further studies are being built to validate $\mathbf{SampEn}_{2D}$.**

I. INTRODUCTION

Entropy analysis is widely used in different fields of science [1]. For time series analysis, approximate entropy (ApEn) arose as an important method for use with short and noisy time series [2]. Later on, sample entropy (SampEn) method was proposed as an improvement of ApEn, which was known to present two important bias [3]. Essentially, both ApEn and SampEn are irregularity measurements. The higher the time series regularity, the lower the entropy value [4]. Regularity is close related to predictability. Regular time series means there are no surprise within its values, their values (or patterns) are highly predictable. On the other hand, irregularity is related to unpredictability.

In image processing field, entropy analysis is usually related to Shannon entropy computation. In this case, entropy is estimated from individual pixels occurrency probabilities, obtained from histogram. However, those probabilities do not take into account the pixels spatial distribution, although they might contain relevant information about the process the image represents.

In this study we propose an extension of SampEn, namely SampEn_{2D}, as an entropy measurement for images that takes into account the spatial information of pixels. Probabilities are calculated following SampEn definitions, adapted for the two-dimensional case. SampEn_{2D} was then applied to histological images of sural nerve obtained from a young and an elderly rat.

[1]Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, SP, Brazil
[2]Department of Computing and Mathematics, Faculty of Philosophy, Sciences and Languages of Ribeiro Preto, University of São Paulo, SP, Brazil
[3]Department of Surgery and Anatomy, School of Medicine of Ribeirão Preto, University of São Paulo, SP, Brazil

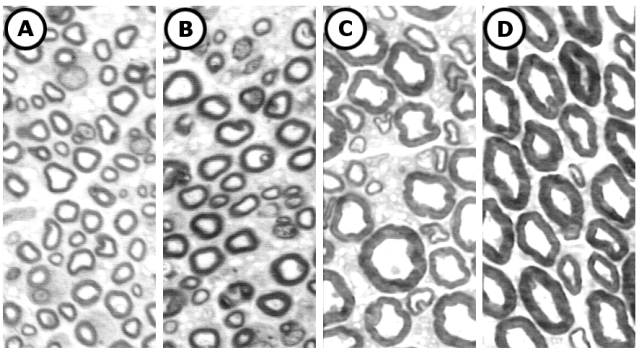

Fig. 1. Examples of rat sural nerve transverse section stained with 1% toluidine blue. Figures (a) and (b) correspond to excerpts of rat aged 30 days and figures (c) and (d) to excerpts of rat aged 720 days.

II. MATERIALS AND METHODS

Int this study we used light microscopy images obtained from two Wistar rats, aged 30 and 720 days. A semithin transverse section of sural nerve was obtained for each rat and stained with 1% toluidine blue, which is used to mark lipids (myelin sheath). Images were optically magnified with oil immersion lens (100 x), optovar (1.6 x) and camera (0.5 x), eventually using a computational magnification (8x) [5], [6]. Fig. 1 shows two examples of the endoneural space of each rat's sural nerve (30 and 720 days).

We extracted image excerpts of 212 x 474 pixels from each microscopy image, resulting in 12 segments for rat aged 30 days and 15 segments for rat aged 720 days.

In addition, several morphometric indexes were also calculated for nerve images, namely the total number of myelinated fibers, the average ratio between axonal diameter (discarding myelin) and total fibre diameter (g ratio), the average area and perimeter of myelinated fibers and the percentage of occupancy of the myelinated fibers (percentage of the total cross-sectional area of the endoneural space occupied by the myelinated fibers) [5], [6].

A. Sample Entropy 2D ($SampEn_{2D}$)

The extension of SampEn for a two-dimensional method was developed so as to maintain the original purpose of SampEn, i.e. an irregularity measure. In short, SampEn

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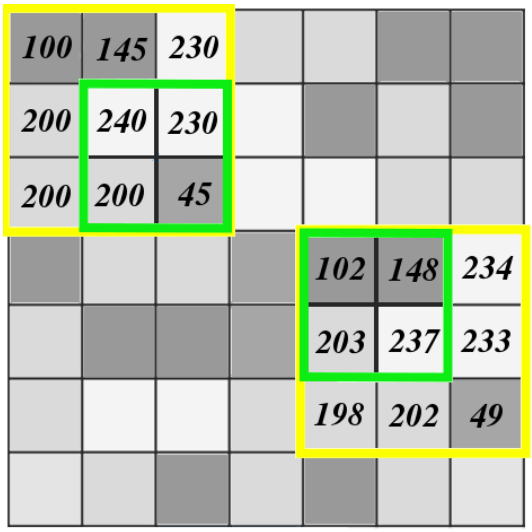

Fig. 2. Example of SampEn$_{2D}$ pattern comparison scheme. Considering the first squared window of size 2 ($m = 2$), the algorithm search for similar spatial patterns, where each corresponding pixels variation are limited to $r = 5$ gray levels. The illustrated pattern at right represents a match for both $m = 2$ (solid line) and $m = 3$ (dashed line). With images that are represented by a more regular pattern, SampEn$_{2D}$ tend to show a lower value compared to images with more irregular patterns.

quantifies the probability that m-length similar patterns will still be similar for $m+1$. Two patterns are considered similar if each corresponding point within the patterns are distant at most r from each other [3], [4]. This probability can be achieved by computing the total number of m and $(m+1)$-length patterns matches. The ratio between those values gives the conditional probability of finding $(m+1)$-length similar patterns, given they are similar for m.

It is easy to note that periodic or very regular time series tend to present the same number of similar patterns for both m and $m+1$. The opposite occurs for unpredictable or very irregular time series, where m-length similar patterns might not remain similar for the next point. For more details, see for example [7].

The two-dimensional SampEn method proposed here, namely SampEn$_{2D}$, defines two-dimensional m-length patterns (squared windows) in place of one-dimensional patterns used in SampEn. Likewise SampEn, each m-length pattern is compared to all other m-length patterns within the image. Pattern match is considered if every pixel within one pattern differs no more than r from the corresponding pixel at the comparing pattern. Fig. 2 shows one example of pattern comparison step of SampEn$_{2D}$.

Average occurrence probability is calculated for all m and $(m+1)$-length patterns (namely $U^m(r)$ and $U^{m+}(r)$, respectively). Finally, SampEn$_{2D}$ is calculated as the logarithmic ratio of $U^m(r)$ and $U^{m+}(r)$. The ratio between the total number of m and $(m+1)$-pattern matches would give the same results. However, this is not suitable here as the number of matches found for images generally overflows the computational memory. A formal definition of SampEn$_{2D}$ is given below.

First, consider an arbitrary image $u(i,j)$ with n_i width and n_j height. Let $x_m(i,j)$ be the set of pixels of u ranging columns j to $j+m-1$ and lines i to $i+m-1$, i.e., $x_m(i,j) = [u(i,j), u(i,j+1), \ldots, u(i,j+m-1), u(i+1,j), u(i+1,j+1), \ldots, u(i+m-1,j+m-1)]$. In a few words, $x_m(i,j)$ is the m-length squared subset of pixels with origin placed at (i,j). The total number of pixels in the image is $N = n_i * n_j$.

However, it is possible to form only $N_p = (n_i - m) * (n_j - m)$ different squared patterns because the last m columns and lines cannot be used as a pattern origin for both m and $(m+1)$-length patterns, following the same unidimensional algorithm properties [3]. Two-dimensional SampEn is defined by

$$SampEn_{2D}(m, r, N_p) = -\ln \frac{U^{m+1}(r)}{U^m(r)} \tag{1}$$

where

$$U^m(r) = \frac{1}{N_p} \sum_{i=1}^{N_p} U_i^m \tag{2}$$

$$U_i^m = \frac{[\#\text{ of } x_m(i,j) \mid d[x_m(i,j), x_m(i_1,j_1)] \leq r]}{N_p - 1} \tag{3}$$

and

$$U^{m+1}(r) = \frac{1}{N_p} \sum_{i=1}^{N_p} U_i^{m+1} \tag{4}$$

$$U_i^{m+1} = \frac{[\#\text{ of } x_{m+1}(i,j) | d[x_{m+1}(i,j), x_{m+1}(i_1,j_1)] \leq r]}{N_p - 1} \tag{5}$$

The distance function d is defined by

$$d[x_m(i,j), x_m(i_1,j_1)] = \max_{k,l}(|u(\alpha,\beta) - u(\alpha_1,\beta_1)|). \tag{6}$$

where $k = 1, \ldots, m$, $l = 1, \ldots m$, $\alpha = i + k - 1$, $\alpha_1 = i_1 + k - 1$, $\beta = j + l - 1$ and $\beta_1 = j_1 + l - 1$. The distance function is ideally the same of SampEn, here extended for the two-dimensional case. In Equations (3) and (5), the origin points (i,j) and (i_1,j_1) must be different to exclude self-matches.

It is worth noting that, as the comparisons between patterns in SampEn$_{2D}$ are made by each single corresponding pixels and only for the original rotation, rotating variant patterns will not match in SampEn2D. According to the definition proposed here, SampEn2D assumes that such patterns within images are not similar structures.

One-dimensional SampEn definition of m and r parameters is a difficult task [8], [9]. For example, in analysis of heart rate variability series, parameters common choices are $m = 2$ and $r = 0.15$ or $r = 0.20$ percent of signals standard deviation [2], [10], [3], [7]. For the new two-dimensional approach, we expected those parameters to play similar roles. However, changing m or r by the same amount in one and two-dimensional methods might have different aftereffect, once for time series patterns we have m comparison while for images there are m^2 comparisons.

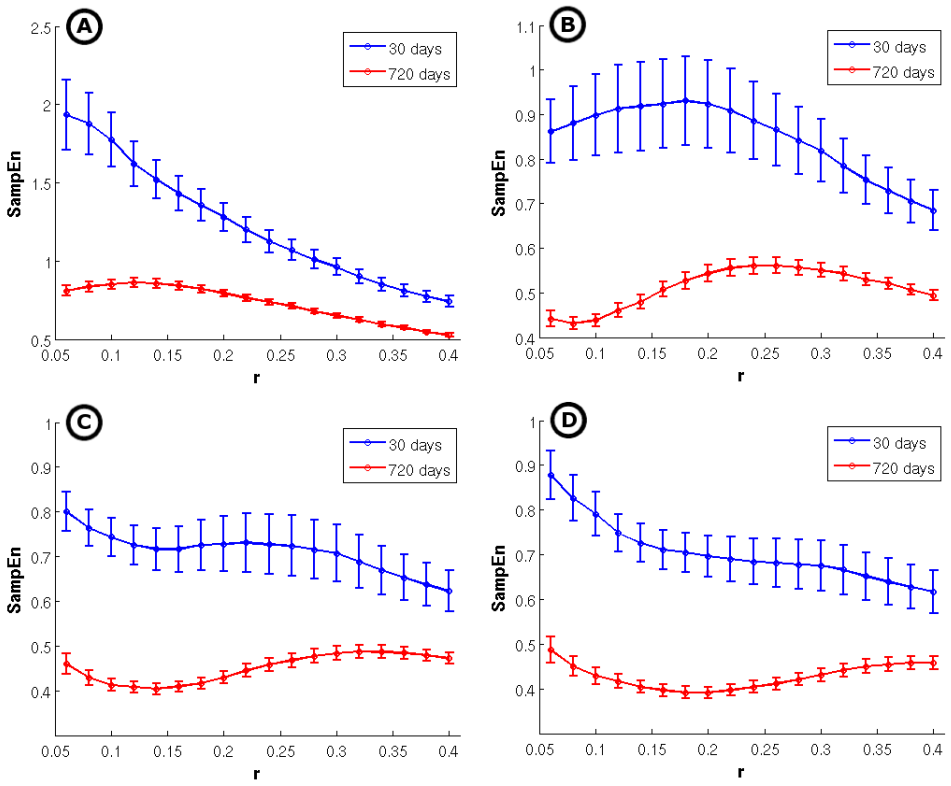

Fig. 3. SampEn$_{2D}$ values for the two sural nerve images (rats aged 30 and 720 days). For each plot, horizontal axis represents r parameter ranging from 0.06 to 0.40; (a) $m = 1$, (b) $m = 2$, (c) $m = 3$ and (d) $m = 4$. Error bars are standard errors within each segment, extracted from the same nerve image.

III. RESULTS AND DISCUSSION

Fig. 3 shows the results of SampEn$_{2D}$ for the two sural nerve images. SampEn$_{2D}$ was calculated for r ranging from 0.06 to 0.40 (step 0.02) and m ranging from 1 to 4. It is worth noting that r value is a percentage of image standard deviation. Error bars are standard errors calculated from each segment, extracted from the same nerve image.

SampEn$_{2D}$ curves show that elderly nerve images seems to have a general pattern more regular or more predictable compared to young one, reflected by its greater entropy values. This is true for all sets of parameters calculated (m,r). In general, greater SampEn$_{2D}$ differences between 30 and 720 days images are obtained for low values of r (0.06 to 0.20).

As SampEn and SampEn$_{2D}$ similarity tolerance increases (r), more similar patterns are expected to be found. Usually, it should lead to a decreasing of entropy value. However, this tendency is not observed for the entire r range in Fig. 3. As SampEn and SampEn$_{2D}$ can also be calculated as the logarithmic ratio of m-length and $(m + 1)$-length pattern matches, increasing r could lead to increasing matches of m-length patterns but not of $m+1$. The ratio will increase in this case, as so entropy. Moreover, this situation is more likely to occur in SampEn$_{2D}$ than in SampEn, as the increment on m in the former requests $m+1$ more pixels to be similar to account for a match.

Table I show the values of morphometric indexes. The total number of myelinated fibers (MF tot), the average ratio between axonal diameter (discarding myelin) and total fibre diameter (g ratio), the average area (MF area) and perimeter (MF perim) of myelinated fibers and the percentage of occupancy of the myelinated fibers (MF occup) are show for sural nerve images from rats aged 30 and 720 days.

TABLE I

MORPHOMETRIC INDEXES CALCULATED FOR 30 AND 720 DAYS NERVE SECTION IMAGES. MF AREA AND MF PERIMETER UNITS ARE μm^2 AND μm, RESPECTIVELLY. MF OCCUP IS A PERCENTAGE OF TOTAL NERVE AREA.

Age	MF tot	MF area	MF perim	g ratio	MF occup
30 days	913	13.61	13.98	0.48	31.78
720 days	654	33.98	21.76	0.45	25.49

One can note that virtually all morphometric indexes seems to be different between 30 and 720 days images. Aging usually causes axonal atrophy, which is reflected by a decreasing in g index. Furthermore, the number of myelinated fibers and the percentage occupancy of myelinated fibers are also decreased in rat aged 720 days.

On the other hand, the average area and perimeter of elderly rat is increased compared to young rat. Previous study showed that there is a postnatal growth spurt between 30 and 90 days in rats [5]. Therefore, myelinated fiber in rat aged 30 days is not as developed as in adulthood.

Table I reveals the morphometric changes observed in rat sural nerve with aging. Although SampEn$_{2D}$ does not directly takes into account those metrics, the present study

revels that regularity/predictability characteristics of those images are also altered towards an irregularity loss with aging. It is in accordance to physiological complexity theory, which points to complexity loss with diseases and aging [11].

SampEn_{2D} algorithm was implemented using Java version 7. In a desktop computer with Intel Xeon CPU E5405 2 Ghz Quad Core and 3.9 Gb of RAM, SampEn_{2D} computation time for a sample image with size 300x300 was 95 seconds.

IV. CONCLUSIONS

In this study we propose an extesion of SampEn to a two-dimensional analysis, namely SampEn_{2D}, which extracts information related to the repeatability of patterns within images. Results show that SampEn_{2D} might be useful for histological image analysis. In addition to morphometric indexes, which are generally used to assess histological images of nerves in presence of pathologies and aging, irregularity analysis produced by SampEn_{2D} can also detect different properties of those images, which may not be directly related to morphometry.

Further studies have to be conducted to statistically validate SampEn_{2D} as well as to study its dependence on noise and image size.

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