Lacunarity Analysis of Microvascular Morphology in Human Retina

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Abstract—**Fractal analysis provides means for the quantitative assessment of geometric patterns in one, two, and three dimensions. It is aimed at analysis of graphical shapes that belong to a class of fractal objects that are characterized by the self-similarity over different scales. Various structures in nature are fractals and fractal analysis techniques are widely used for analysis of biomedical images. One such example of application is analyzing blood vessel structure in the human retina that can be extracted from digital images captured by fundus camera. The most commonly used fractal analysis is estimation of fractal dimension using various box-counting methods for mono- and multi-fractals. Although two fractal images can have the same fractal dimension they can have very different appearance and structure. One can appear as a structure that fills most of the available space, while the other can have a lot of empty areas. These differences can be quantified by lacunarity parameter, which has greater value in images with less space-filling properties. This paper focuses on the estimation of the lacunarity parameter implemented in the Python programming language, which is aimed at lacunarity analysis of microvaculae morphology in human retina. The implementation is validated by comparison with the results obtained by ImageJ, a commonly used software for analysis of biomedical images. The value of the lacunarity analysis is demonstrated on a set of actual images of human retina associated with different medical conditions.**

Index Terms—**Box-counting method; fractal analysis; lacunarity; image analysis; Python; retinal microvasculature.**

I. INTRODUCTION

The fractal and lacunarity analysis have been around for some time and their usage is recognized in different areas of science [1,2]. Fractal analysis techniques are becoming widely used in medicine for analysis of biomedical signals and images [3-5]. One such example of application is analyzing blood vessel structure in human retina that can be extracted from digital images captured by fundus camera [6,7].

The microvascular system of the retina is an integral part of the microcirculation of the human body that can be studied directly, *in vivo,* in a simple and non-invasive way by using digital photography. This approach gives us information not only about the health of the eye, but also represents one of the standard diagnostic methods used to detect complications of systemic diseases such as diabetes mellitus and hypertension [8]. Vascular networks have fractal structure, so this fact is often used to differentiate healthy retinal microvasculature from the one with pathological changes [9]. Healthy vascular network is characterized by higher complexity and higher fractal dimension, while various systemic and eye-specific pathological processes in most cases cause vascular pruning and lower vascular complexity. Decreased fractal dimension of the microvascular network is a nonspecific marker of pathological process in the organism, because by itself, it cannot show what pathological process caused it. We previously showed that specificity of fractal analysis results can be increased by adding to the analysis of microvascular geometry the lacunarity dimension that measures gappiness of the image [10]. This approach uses the fact that two fractal images can have the same fractal dimension although they have very different appearance resulting from different space filling properties of each image [11,12]. Therefore, when analyzing geometric patterns in biomedical images, in addition to fractal dimension, it may be useful to estimate the lacunarity of the structure and include it in the analysis [13,14].

This paper shows a scripting tool for quantitative analysis of microvascular network in human retina based on estimation of the lacunarity analysis, that was developed by using Python programming language. The implementation is validated by comparison with the results obtained by ImageJ, a commonly used software for analysis of biomedical images [15]. The value of the lacunarity analysis is demonstrated on a set of actual publicly available images of human retina associated with different medical conditions.

II. MATERIALS AND METHODS

A. Images of Retina

For this research, we used a publicly accessible High - Resolution Fundus (HRF) image database containing 45 raw color images of retina. Images were captured with a Canon CR-digital fundus camera with a 45° field of view and resolution of 3504 × 2336 pixels [16,17]. The database contains 3 groups of images: 15 images of healthy retina, 15 images of retina showing signs of diabetic retinopathy, and 15 images with signs of glaucoma. Each image comes with a

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corresponding gold standard manual vessel segmentation binary image. In the present study, our focus was on these binary images representing retinal microvasculature.

B. Lacunarity Estimation

Similarly to the estimation of box counting fractal dimension, we determine E that represents a set of all scales, i.e. all square sizes by which we will cover the given image [14,18]. The maximum value for $\varepsilon \in E$ may be min (n, m), where n and m are the length and width of the image, and the smallest value for ε is limited by the image resolution. Namely, the smallest value for ε must be greater than or equal to 1px. We can start from the smallest scale, squares with side length of ε, and increase it according to the algorithm in order to form the set E. For images of retina this is illustrated in Fig. 1. As opposed to fractal dimension, where each square is checked whether the square covers fractal objects or not, when determining the lacunarity we count how many pixels in the square actually belong to the object.
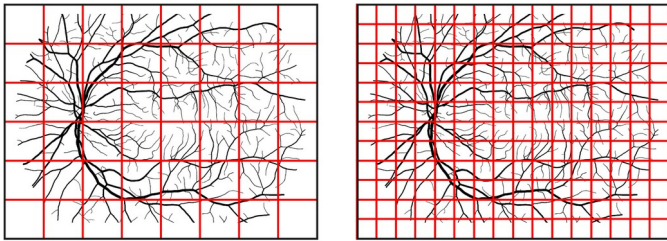

Fig. 1. Covering the fractal objects with different box scales

The lacunarty at each scale ε is estimated using the following formula:

$$\Lambda(\varepsilon) = \left(\frac{\sigma}{\mu}\right)^2 \tag{1}$$

where σ is the standard deviation, and μ is the mean of the number of pixels counted for each square with sides of length ε. The following statistical formulas are used:

$$\mu = \frac{1}{n}\sum_{i=1}^{n} x_i \tag{2}$$

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \mu)^2} \tag{3}$$

where x_i is the total number of pixels in each square and n is the total number of squares at the given scale ε.

As a final step, for a given geometric structure, the lacunarity Λ for a given image is estimated as an average value of all $\Lambda(\varepsilon)$ previously calculated for all scales ε [13].

C. Three Variants of the Estimation Algorithm

We implemented three variants of the method for lacunarity estimation algorithm based on the approach how the squares at different scales ε were placed over the geometric object we are analyzing, as illustrated in Fig. 2. The first variant of the algorithm uses standard non-overlapping placement of squares

over the object until the whole image is not covered. This process is repeated at each scale ε and the total number of squares needed to cover the image depends on the size of the image and selected scale ε.

The second approach uses gliding overlapping boxes to cover the image at each scale ε [19]. Here we have a much larger number of total squares needed to cover the image and the number depends on how much do we move/glide each square between the iterations.

Finally, we implemented an approach to lacunarity estimation with random placement of squares over the image at each scale as it can be done for calculations of fractal dimension [20]. The total number of squares at each scale is arbitrary for each step, but it should be at least equal to the total number of squares with sides ε needed to cover the whole image.

As for the settings, the following values were used for for the scales ε: {3, 5, 7, …,19}, both in our script and in the ImageJ program. As for the gliding box approach, when "gliding" the box, each next box was moved 50% to the right, and 50% down when moving to the next row. Finally, for the random placement method, we used the number of boxes for each scale ε to be the same as the the total number of squares of size ε needed to cover the whole image.
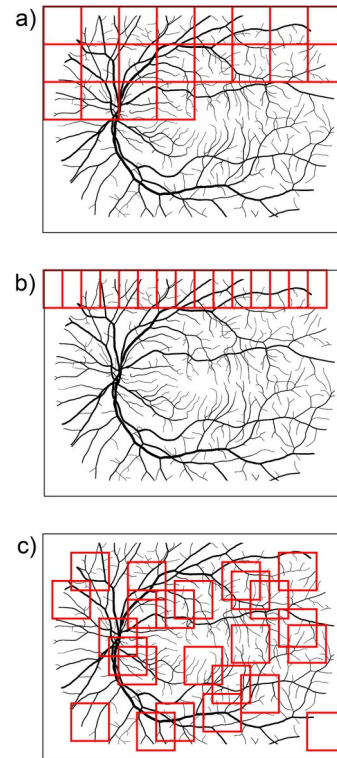

Fig. 2. Three variants of the algorithm: a) Standard non-overlapping boxes, b) Gliding box approach, c) Random box placement

D. Programming and Statistical Analysis Tools

The algorithm for lacunarity analysis is developed using the Python programming language and the Jupyter Notebook scientific computing software [21]. The Jupyter Notebook provides a web-based collaborative environment for experimenting with the code and the intermediate results can

be accessed using a standard web browser. Statistical analysis of the results was performed and visualized using R programming language.

E. Validation Using ImageJ Software

Validation of the lacunarity analysis results obtained using our Python script is done by the comparison with the results obtained by ImageJ software and FracLac plugin [15,22]. ImageJ is a public domain Java program that is commonly used for image analysis in biomedicine. All 45 binary images from the HRF image database that represent blood vessels microvascular network were processed in order to compare lacunarity parameter estimates calculated by ImageJ and all three variants of the presented new software tool were implemented in Python.

III. RESULTS AND DISCUSSION

First, we performed the sanity check using arbitrary images of known fractals. We compared the numbers obtained with new Python script and ImageJ and observed matching results in up to two decimal places (data not shown). Next, we used the 45 images coming from the open access HRF image database containing retina images from three groups of subjects: 15 images from patients with diabetic retinopathy (DR), 15 images from patients with glaucoma (G), and 15 images of healthy retina (H). We observed a very good alignment of the results coming from ImageJ and all three approaches implemented in our script as depicted in Fig. 3. The best alignment with the ImageJ is achieved with the gliding box approach, but the computing time was slightly longer than the time that was needed for the standard non-overlapping box approach. As for the method with random placement of boxes, it yielded very good results, even with the number of boxes in each step that is equal to the total number of squares of size ε needed to cover the whole image, let us call it M. This method becomes even more accurate when we use a number of boxes that is much larger than the number M, i.e. 5x, 10x, 50xM, but in those cases the calculation would take much more time. Therefore, the approach with random placement of boxes can be very accurate, but the computing time for this method would be the longest out of the three.
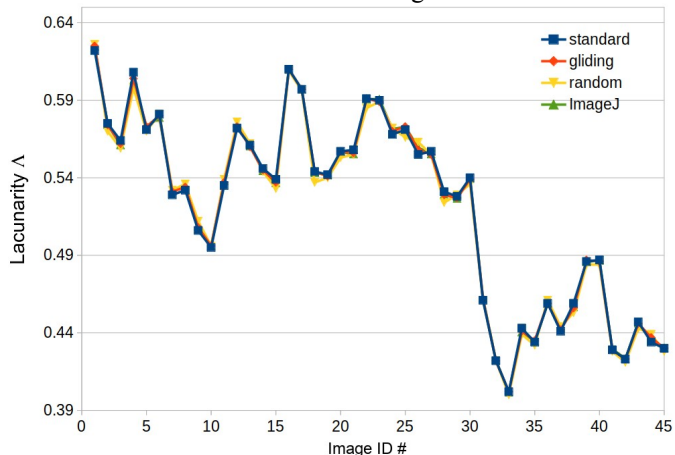

Fig. 3. Results for lacunarity estimation vs. image ID# : a) ID# 1-15 DR, b) ID# 16-30 G; c) ID# 31-45 H

The main evaluation criteria for our research was to perform a comparison of our implementation in Python to ImageJ for the type of experiments we plan to do in the near future. For that purpose we performed statistical analysis in order to compare the three groups of images coming from the HRF image database. As shown in Table 1, there is a very good match of the mean values and standard deviations for the results coming from all three variants of the Python method and ImageJ program. The mean lacunarity distributions for each group of images are also depicted by using box plots in the Fig. 4. It can be seen that the data coming from all four methods, all three variants and ImageJ, display almost identical distributions.

TABLE I
COMPARISON WITH IMAGEJ SOFTWARE: HRF IMAGE DATABASE

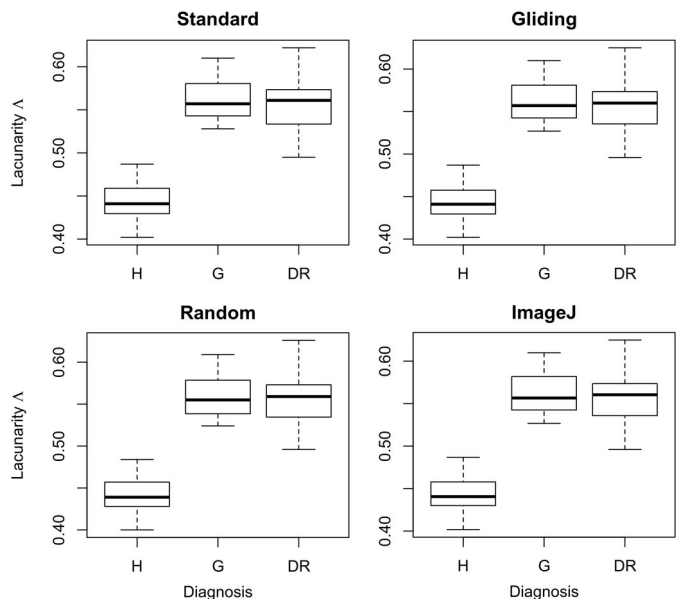| Method vs. Diagnosis	Healthy (H)	Glaucoma (G)	Diabetic Retinopathy (DR)
Standard	0.4438±0.0234	0.5626±0.0249	0.5557±0.0348
Gliding	0.4438±0.0232	0.5624±0.0254	0.5559±0.0343
Random	0.4424±0.0231	0.5606±0.0256	0.5556±0.0334
Image J	0.4439±0.0232	0.5627±0.0255	0.5559±0.0339

Note: showing values mean ± SD

Fig. 4 Statistical visualization of the mean lacunarity distribution for each group of retinal images (H- healthy, G-glaucoma, DR- diabetic retinopathy) Distributions of data points were obtained with our script for three variants for lacunarity calculation in Python vs. results obtained with ImageJ program

In addition, a one-way ANOVA with Tukey post-hoc test was used to analyze if the mean lacunarity dimension is different among the different diagnoses. More importantly, in each case, the p value obtained with the statistical analysis led to the same conclusion regardless which method for the estimation of lacunarity parameter was used (Fig. 5). These results validated the data obtained with three variants of lacunarity estimation from our script versus the results obtained by ImageJ program and FracLac plugin.

This Python script is developed as a part of the project with a main goal to study the use of new methods for stratification of patients with Alzheimer's dementia according to the risk for progression of this disease [23]. One of such methods is use of portable digital fundus camera to evaluate microvascular geometry of the retina in elderly people. Despite the fact that the resolution of images captured with a portable non-mydriatic digital fundus camera is relatively small, its use has many advantages. This type of camera is affordable, and also quick and easy to use because does not require prior dilatation of the pupil. It could be used for screening in primary care clinics in a large number of patients. It could be also used in the field outside the outpatient clinic for patients for whom specialized health care is difficult to access. Digital photographs can be subsequently easily transmitted to a diagnostic center by an ophthalmologist for expert analysis [24,25].

The main advantage of the use of this new script is possibility to perform fully automated scripting and control over our *in-silico* experiments that will be performed on segmented digital retinal fundus images of patients with Alzheimers dementia and the subjects with normal cognitive function. An additional control group of subjects will be made of the patients with colon cancer because it has been shown that in people with some cancers, the incidence of Alzheimers dementia is very low, and vice versa – people with Alzheimers dementia rarely have decreased incidence of cancer [26].

The next steps in development of this analysis tool will include integration with automated fractal dimension and multifractal parameters estimation in order to create a single Python library.
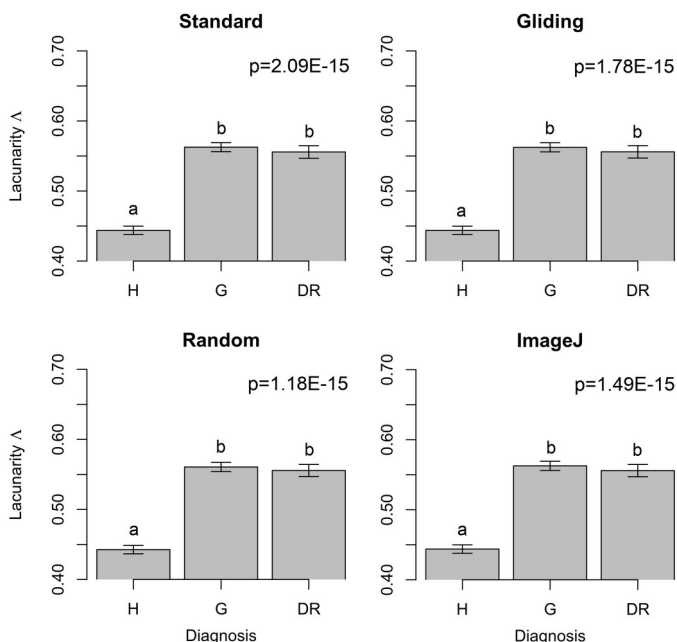
IV. Conclusion

This research focuses on the implementation of a Python library to enable performing automated lacunarity analysis of biomedical images. The paper describes three variants of the implementation aimed for the use when analyzing fractal objects commonly found in biomedical images. The implementation was validated using an open access set of images of human retina. The results obtained by three variants of the method implemented in Python were compared with the results of ImageJ program. It should be pointed out that the method using non-overlapping box placement was the fastest, but not as accurate as the method using gliding box approach. The method with random placement of boxes needs a larger number of boxes than the non-overlapping method in order to achieve desired accuracy and it was the slowest one out of the three. However, the method with random placement of boxes could be very useful if we wanted to estimate the lacunarity of a part of an image that is cut out using an irregular shape. Furthermore, the results for all three methods were statistically analyzed and in each case the statistical analysis led to the same conclusion, regardless which method for the estimation of lacunarity parameter was used. This new tool allows for easy automation of experiments with biomedical images.

Further steps in the research will include improvements of various argument options and additional settings in order to enable advanced and fully automated fractal analysis of fundus images of human retina. Additional image sets will be used including fundus images obtained by a hand-held fundus camera that can be operated by family physicians. Usage of such cameras, combined with the use of fractal analysis and quantification, could potentially allow for development of non-invasive disease detection in early stage.

Fig. 5. One-way ANOVA test using lacunarity from different methods

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