Fringe projection for the study of bacteriological growth

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Abstract: - We study here the three-dimensional structure found in the limit of the disc agar diffusion test performed over bacteria cultures. In order to achieve this, we have developer a simple optical method, based on the projection of fringes. This method, also allows determining the quality of the sample by measuring the transmission of such fringes through the gel acting as the base for the culture of bacteria.

Key-Words: - Inhibition halos, antibiogram, biological growth, bacterial colonies, self-affine structure, fractal dimension

1 Introduction

One of the clearest examples of the application of fractal geometry [1,2] is related with the diffusion of biological systems, as is the case of bacteria culture and the evolution of its boundaries [3-6]. Different cases of application have been studied, but in them the structure is projected over the pixel array of a CCD camera. This means that the three-dimensional structure of the diffusion has not been considered. This is clear when the agar diffusion test is considered, which consists in the spreading of a sample that presumably contains bacteria, over an agar based culture media, then disks soaked in different antibiotics are placed on the plate with the culture [7-9].

In the present work, we show images of the diffusion halos obtained by a stereoscopic microscope with a 40X magnification, this allows the analysis of the boundary structure in different planes [10,11], representing this way, a more detailed case than the case for 2D images.

2 Fractal structures in biomedicine

It is clear that fractal geometry represents a better approximation to the biological irregular forms [12,13] when compared with Euclidian geometry. We take this fact into account in order to represent the irregular structure formed in the agar diffusion test.

Fractal geometry may prove to be an unifying theme in biology [14], since it permits generalization of the fundamental concepts of dimension and length measurement. Most biological processes and structures are non-Euclidean, displaying discontinuities, jaggedness, and fragmentation. Classical measurement and scaling methods such as Euclidean geometry, calculus and the Fourier transform assume continuity and smoothness. However, it is important to recognize that while Euclidean geometry is not realized in nature, neither is strict mathematical fractal geometry. Specifically, there is a lower limit to self-similarity in most biological systems, and nature adds an element of randomness to its fractal structures. Nonetheless, fractal geometry is far closer to nature than is Euclidean geometry.

3 Fractal dimension

In order to calculate fractal dimension, we can use the box-counting method [15,16]. For a measure M_δ, with a number δ of divisions in the image F, we have the exponential law:

$$M_\delta(F) \sim c\,\delta^{-s} \tag{1}$$

being *c* y *s* constants, and s is the dimension of *F*, which is defined by:

$$s = -\lim_{\delta \to 0} \frac{\log(M_\delta(F))}{\log(\delta)} \tag{2}$$

If the images have irregularities in 3D, which represents a generalization of previous works, instead of bidimensional boxes, then three-dimensional boxes must be considered over the surface [17].

The results for the calculation of fractal dimension, for the case of bacterial growth, shows a three-dimensional variation, which must be taken into account for a more general study of the evolution of the boundaries.

4 Fringe projection and quality of the prepared sample

Now, we show the optical method developed for evaluating the irregular structure and the quality of the sample preparation for the agar diffusion test. This method is based in the projection of fringes [18,19] by the transmission through the halos zone which is formed around the disks soaked with antibiotics. This zone is transparent because bacterial growth is inhibited by the specific antibiotics, while the rest of the agar in the disk is not. Fig. 1, shows a schematic for this method, where a laser is used and the fringes can be seen in the halos by one of the microscopes objectives.

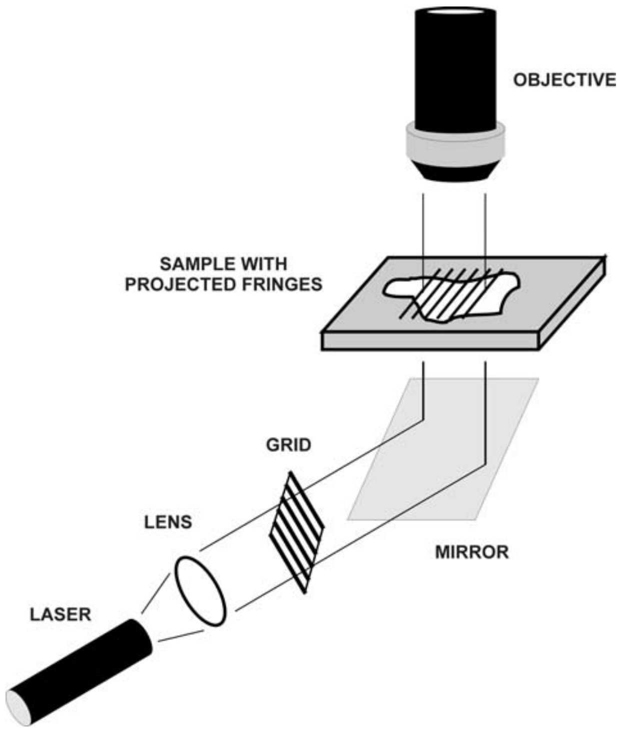


Figure 1- Experimental setup with fringe projection (transmission).

Figure 2- Image of the fringes projected on the halos zones.

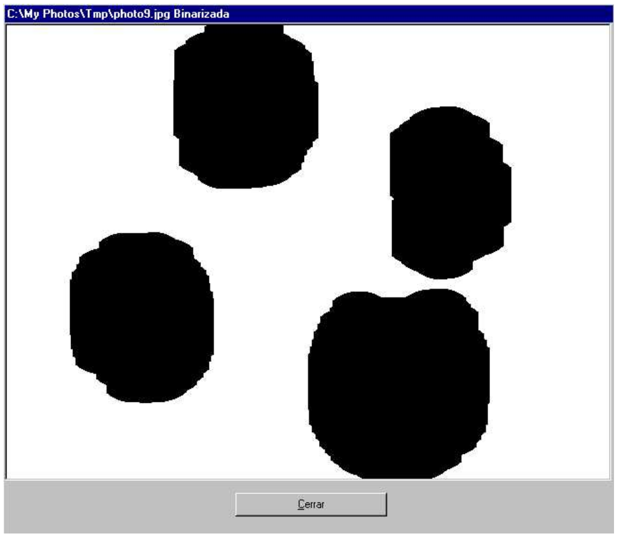
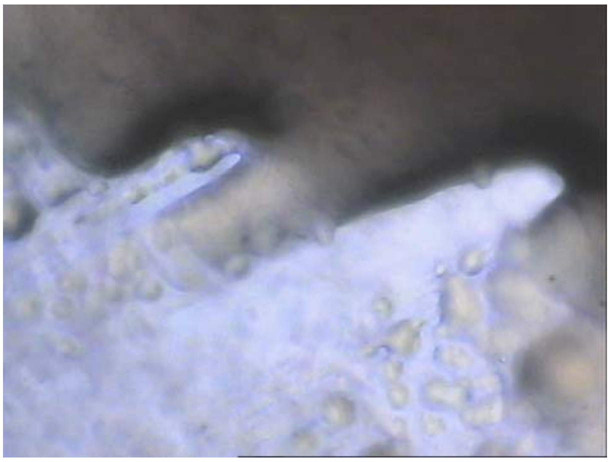


Figure 3- Contour of the halos zones obtained with digital image processing.

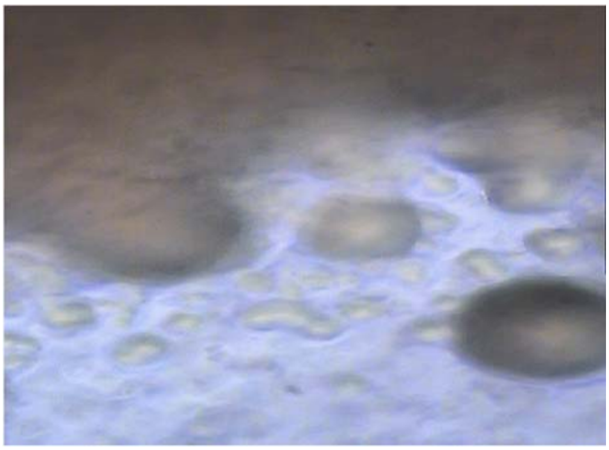
The obtained fringes are shown in Fig. 2. Some sections with fringes can be also observed which show the areas where positioning of the disks is not adequate since the sample spreaded is not thick enough. By processing of the image, we can obtain the contours shown in Fig. 3, which allow estimating the fractal dimension of the sample and the study of its boundary evolution [20].

5 Fringe projection and 3D fractal structure

When we analyzed different sectors of the growth boundary, we can see the great quantity of irregularities on a surface in the three dimensional space. Such irregularities have different levels as shown in Fig. 4 for a sector focused at different planes, in the vertical position, by the stereoscopic microscope.



(a)



(b)

Figure 4- Boundary of the halos at different positions along the vertical axis.

For the case of the considered structures, we must take into account that they are self-affine forms contained in a tridimensional space. This way, and according to studies performed by other authors for the two-dimensional case of bacterial growth and rough surfaces, the mean value of the roughness exponent (H) is related with the fractal dimension by $s=3-H$.

The experimental setting is shown in Fig. 5, where now, the fringes projection is by reflection. This means, fringes are focalized in different levels in order to reconstruct the structures showed in Fig. 4, and that way reaching to a representation of the contour in levels of gray (in a similar way as in Fig. 3). Finally, the fractal dimension obtained with cubic boxes is shown in Fig. 6, where ε is the error obtained in the regression method for the graphic.

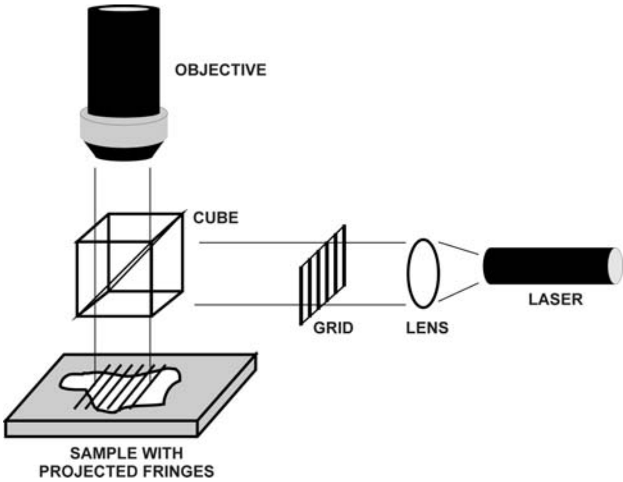


Figure 5- Fringes projection for studying the irregular structure of the boundary.

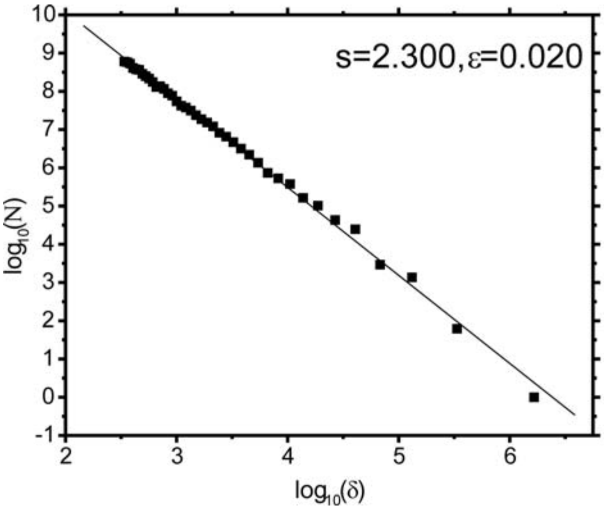


Figure 6- Fractal dimension for the irregular surfaces on the boundary.

6 Conclusions

We presented a method based on fringe projection for its application in the diffusion of bacteria cultures over agar used as a culture media. This method allows an accurate and simple way for studying the fractal characteristics for this diffusion. We also showed that the irregular structure is contained in space, and obtained its fractal dimension by image processing. Also, fringe projection can be used for positioning the disks soaked with antibiotic in areas within the petri dish, where the sample has been spread correctly.

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