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AN INTRODUCTION TO BACTERIAL GEOGRAPHY

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Bacterial colonies secrete a matrix of extra-cellular polymeric substances to form biofilms under most environmental conditions. Formation of biofilms has relevance to public health, medicine and environmental sciences, and also impacts agriculture, industry, and other economic aspects. Scanning confocal laser microscopy was used in conjunction with fluorescent lectin probes to obtain digital images of biofilms. Digital image analysis and GIS were used to assess and quantify the structure and spatial variability within biofilms. Classification algorithms were developed using remote sensing techniques to identify the elements contained in each image. The final outputs were maps of the sections through the biofilm. Various statistical approaches were used extensively to analyze the spatial structure and diversity of the biofilms. We are hoping that this new technique will facilitate the understanding of the biofilm structure, of various microbial processes, and lends itself to applications in other fields.

1. Introduction

Biofilms are formed by bacterial colonies encapsulated in an extra-cellular polymeric substances matrix. They rapidly form under most environmental conditions [1]. The study of biofilms has been facilitated by advances in microscopy, such as the scanning confocal laser microscopy (SCLM) used in conjunction with analytical imaging [2], digital analysis [4], and semi-automated image processing [1]. Various approaches based on SCLM have been developed to assess the structure [1] and spatial variability within biofilms [2].

The purpose of our study was to create a method to utilize digital images of biofilms collected from SCLM used in conjunction with fluorescent lectin probes in a combined approach using remote sensing, digital image processing and, ultimately, GIS tools. The final output should be a map of the image that may be analyzed using various techniques from spatial statistics. This tool will ideally quantitatively analyze changes that occur in bacterial biofilms due to the presence of contaminants (in natural biofilms) or antibiotics (in colonies of pathogen bacteria), and detect changes in biofilm architecture over different spatial scales. Applications may include the study of biofilm formation and growth, bacterial colonization, and the determination of enzymatic activities [6].

2. Materials and Methods

The images we used were series of images of sections through biofilms like the following ones. Figure 1 displays a section through a biofilm formed by a single bacterial species [6].

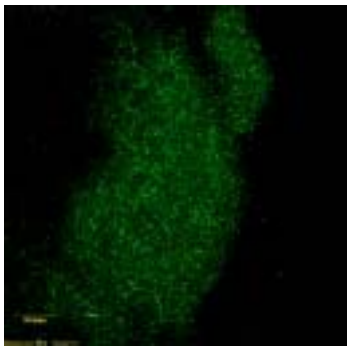


Figure 1. Section through a biofilm formed by a single bacterial species

Figure 2 displays a section through a bacterial biofilm from a Bahamas stromatolite [8].

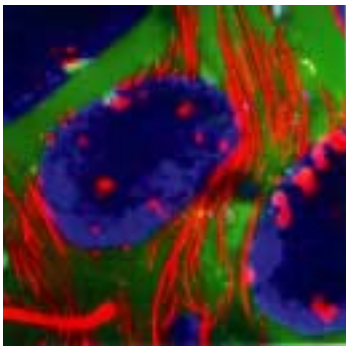


Figure 2. Image of a section though a bacterial biofilm from a Bahamas stromatolite

Confocal microscopy is an example of remote sensing, defined as “the acquiring of data about an object without touching it” [9]. This statement is illustrated below by a comparison between aerial photography (Figure 3) and a diagram of the principle of confocal microscopy [10] displayed in Figure 4. Similarities with respect to the definition in [9] can be easily noticed [6].

Figure 3. Remote sensing: aerial photography

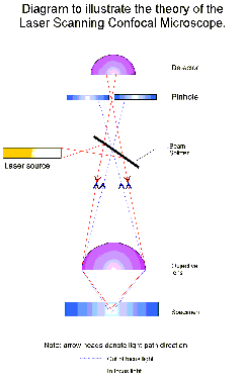


Figure 4. Remote sensing: confocal microscopy

Simplistically, and ignoring the special path of light in aerial photography or confocal microscopy, it may be argued that the major difference between remote sensing of biofilms using confocal microscopy and remote sensing of the Earth using aerial photography or satellite imagery reduces to scale as indicated in Figure 5 [7].

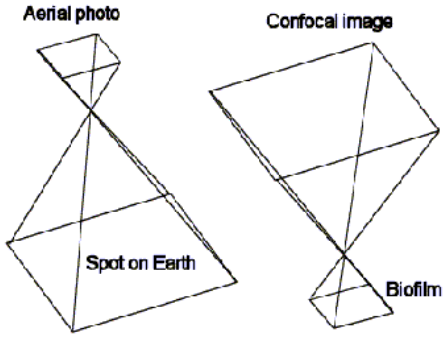


Figure 5. If the details related to the path of light in aerial photography and confocal microscopy are ignored, the difference between the two examples of remote sensing reduces to scale

The approach used in this study does not differ substantially from aerial photography. In both cases, the images are processed using digital images processing techniques.

GIS is defined as a "decision support system involving the integration of spatially referenced in a problem solving environment" [11]. An example of how GIS works is presented in Figure 6 [12].

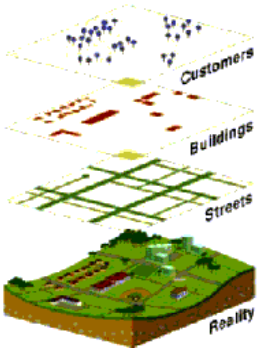
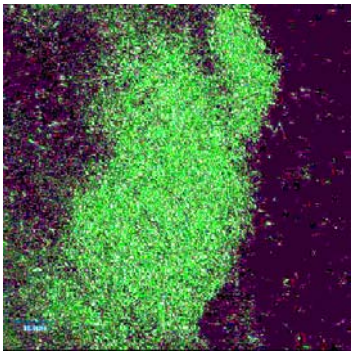


Figure 6. How does GIS work: GIS stores information about the world as a collection of thematic layers that can be linked together by geography

Change detection, available in Erdas Imagine, may be used to analyze confocal images. Similar results may be obtained using Geographical Information Systems (GIS) to detect changes within biofilms as a result of various environmental conditions or stressors. This method is not new as indicated in a 1968 study where industrial sites in Franklin County, SC, were mapped in two years, 1954 and 1958, and the changes were tracked by overlaying the two images [13]. The choice of this example is not random, given the fact that bacteria may appear to the naked eye as dots dispersed over a continuous surface. If the bacterial images are obtained correctly, then a similar approach can be used to determine how are the enzymatic activities within a biofilm influenced by various environmental conditions or stressors [6].

3. Results and Discussion

A first step in this study was an enhancement of the picture through contrast stretching [14], in order to eliminate the noise and facilitate the separation of bacteria from the background. This method makes the range of the lookup table vary linearly from the minimum statistics value to the maximum statistics value in the input (X) direction and from 0 to the maximum brightness value in the output (Y) direction [15]. Figure 7 displays a comparison between the initial image and the enhanced image. The min-max contrast stretching yielded the best results. The enhanced image was used in the following steps as reference.



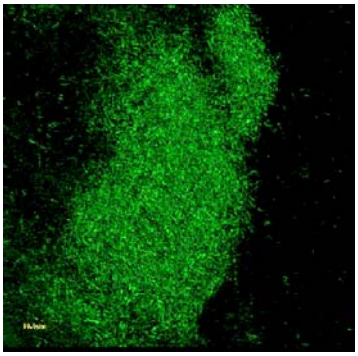
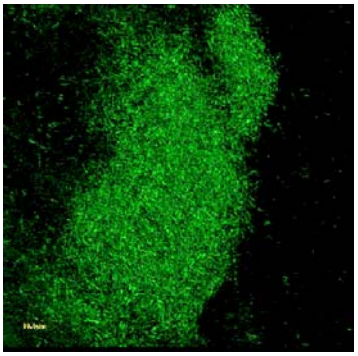


Figure 7. Comparison between the original image (first) and the same image enhanced using min-max contrast stretching (second)

In the next step, a 3 x 3 high pass filter was applied to the enhanced image. The role of high-pass filters is to underline the edges and the size of the filter was chosen to minimize the blurring effects [14]. Figure 8 displays the differences between the reference image and the filtered image. The first classification method used to separate the bacteria from the background was density slicing [14]. Figure 9 presents the results of applying this method to the reference image and to the filtered one as well. Colors were chosen according to the original image.



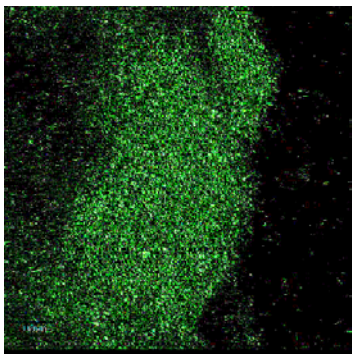
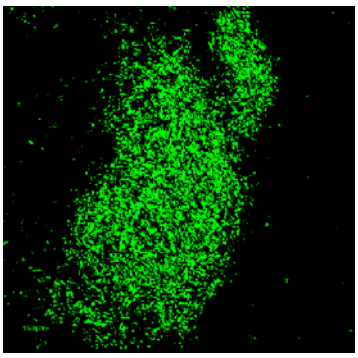


Figure 8. Comparison between the reference image (first) and the same image filtered using a 3 x 3 high-pass filter (second)



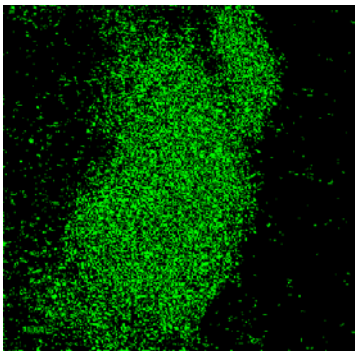


Figure 9. Comparison between the classification through density slicing applied to the reference image (first) and to the filtered image (second)

The final step of this project was to separate the bacteria from the background using supervised classification. This method was preferred as the identification of training pixels is relatively easy and there are other collateral data that may assist the process [14]. The results of this classification are displayed in Figure 10. Colors were chosen according to the original image.

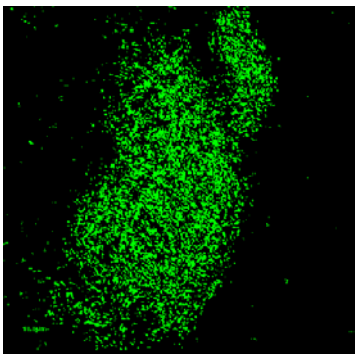


Figure 10. Separation of the bacterial cells from the background using supervised classification

The processes described above may introduce errors at various steps and result into lack of information for some regions. Kriging was used in an attempt to fill in the gaps and produce smooth, continuous maps of the sections. In our attempt to kriging based on the classified image, we used the semivariogram displayed in Figure 11.

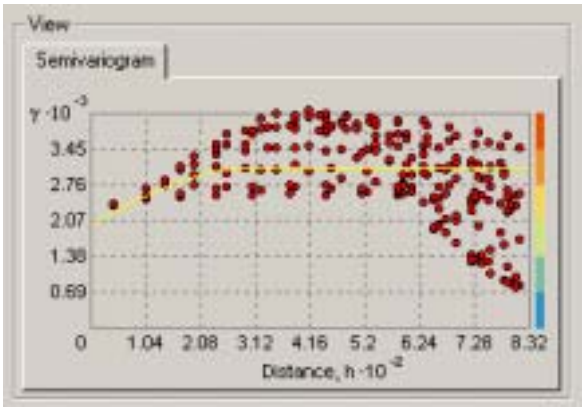


Figure 11. Semivariogram corresponding to the classified map of the section through biofilm

It couldn't be argued whether the maps obtained using kriging provide a better description of the reality. The results were inconclusive toward the final goal of assessing spatial variability within biofilms.

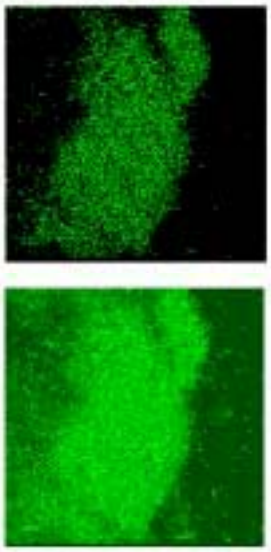


Figure 12. Comparison between the classified map (first) and the prediction map obtained via kriging (second)

The Bahamas stomatolite image was treated in a similar fashion. Figure 13 displays the final result after converting the final Erdas image into a GIS grid representation. Colors were chosen according to the original image.

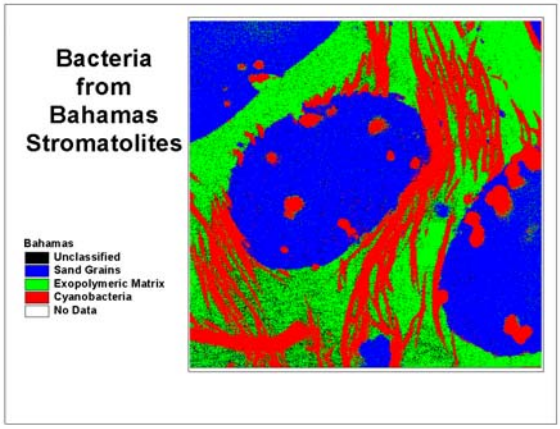


Figure 13. Contrast-enhanced, filtered, and classified image of a section through a bacterial biofilm from Bahamas stromatolites presented as a map using ArcView GIS

Preliminary results indicate that our methodology could lead to a rigorous quantification of the structural variability within biofilms and also of the phenomena related to biofilm formation, growth, and death. The remaining questions are how does our approach compare to similar ones, and whether extrapolation to the volume is possible. Also, an automation of the whole process may represent a tremendous help, but is limited by the use of several pieces of software. The use of spatial statistical procedures in conjunction with our methodology may improve the analytical abilities of this approach.

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