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GEOSTATISTICAL APPROACHES TO MICROBIAL IMAGE QUANTIFICATION  
AND CLASSIFICATION

by

Alexandru-Ionuț Petrișor

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University of South Carolina

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Major Professor and  
Co-Director of Dissertation

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Co-Director of Dissertation

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Committee Member

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Committee Member

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Committee Member

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Committee Member

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Dean of The Graduate School

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## DEDICATION

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## ABSTRACT

The study of biofilm properties has been facilitated by advances in microscopy, such as confocal scanning laser microscopy used in conjunction with analytical imaging, digital analysis, and semi-automated image processing. The outcomes of these approaches are enumeration of bacteria, determination of growth, viability, and changing metabolic conditions, assessment of the microstructure of biofilm, micro-environmental analyses, quantification of biodiversity, and computer control of microscope stage.

The concept of remote sensing involves the acquisition of information about a system without being in direct contact with it. Therefore, microscopy is a particular type of remote sensing. Traditionally, remote sensing has been used in conjunction with Geographic Information Systems to study small-scale phenomena in geology, geography, and other Earth sciences. The present study is based on the hypothesis that remote sensing and digital image processing techniques can be used in conjunction with Geographic Information Systems and spatial statistics to quantify heterogeneity of the microbiological world at several spatial scales (micrometers to cm).

The microbiological system investigated is that of marine stromatolites, the oldest known fossil macrostructures on Earth. Formation of stromatolites continues presently in isolated areas of the Bahamas. The microbially mediated micro-architecture of

stromatolites is critical to understanding the global microbial and biogeochemical processes that influence element cycling on Earth, as well as its prehistoric environmental conditions. Of particular interest are microboring processes, calcification and distributions of sulfate-reducers bacteria, analyzed in relationship with the formation of stromatolites.

In addition to stromatolites, remote sensing and Geographic Information Systems methods were used to reconstruct and estimate biovolumes, and estimate concentrations of homogeneously sized particles. Polymeric microspheres with known diameters were used to create a ground truth for microscopic structures. Several image enhancement techniques (contrast enhancement and filtering) were compared with regard to their impact on image classification.

The results indicated that the approach used in this research represents a viable tool in a field where the development of quantitative imaging is in the early stages. The methodology has a significant potential for automation, and could find more applications in more fundamental microbiological research, and also at different spatial scales of eukaryote cellular biology.

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Graphics* 28, 21-31

## APPENDIX

### PUBLICATIONS AND PRESENTATIONS BASED ON THE RESULTS PRESENTED IN THE DISSERTATION

#### 1. Publications

##### A. Peer-reviewed articles

Petrișor, A. I., and Decho, A. W. (2004). Using Geographical Information Techniques to Quantify the Spatial Structure of Endolithic Boring Processes within Sediment Grains of Marine Stromatolites. *Journal of Microbiological Methods* 56(1), 173-180

Petrișor, A. I., Cuc, A. I., and Decho, A. W. (2004). Reconstruction and Computation of Microscale Biovolumes Using Geographical Information Systems: Potential Difficulties. *Research in Microbiology*, in press

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2004). Quantifying CaCO<sub>3</sub> microprecipitates within developing surface mats of marine stromatolites using GIS and digital image analysis, *Geomicrobiology*, submitted 04/08/2004

Kawaguchi, T., Petrișor, A. I., and Decho, A. W. (2004). Changing in-situ microspatial distributions of sulfate-reducing bacteria and eubacteria within surface mats of marine stromatolites: Quantitation using combined FISH and GIS-image analyses



approaches, manuscript in preparation for submission to *Applied Environmental Microbiology*

B. Conference full papers (published in corresponding volumes of Proceedings)

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2003). An introduction to bacterial geography. In: Țepelea I., Antal C. (Eds.), *Proceedings of the 27th Annual Congress of the American-Romanian Academy of Arts and Sciences 1* (pp. 158-162). Polytechnic International Press

C. Published abstracts

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2002). Using GIS and Remote Sensing Techniques to Assess Spatial Variability Within Bacterial Biofilms", *Abstracts of Graduate Students Presentations- Graduate Student Day 2002*, The Graduate School, University of South Carolina, pp. 45

Petrișor, A. I., and Decho, A. W. (2003). Imaging Fluorescent Polymeric Microspheres Using a Scanning Laser Confocal Microscope to Reconstitute and Estimate the Volume. *Proceedings of the Annual Meeting of the Southeastern Microscopy Society 24*, pp. 12

Petrișor, A. I., and Decho, A. W. (2003). Simulating the Marine Stromatolite Microenvironment Using Fluorescent Microbeads Incorporated in Gel Capsules, *Bulletin of South Carolina Academy of Science 55*, pp. 108

Petrișor, A. I., and Decho, A. W. (2004). Reconstruction and computation of biovolumes:  
potential problems, *Bulletin of South Carolina Academy of Science* 56, pp. 126-

127

#### D. Posters

Petrișor, A. I., Ellis, S., and Decho, A. W. (2001). A Quantitative Approach to Digital

Images Analysis with Applications in the Study of Biofilms Using Confocal Microscopy. Presented in the conferences:

- Graduate Student Day Scholarly Work Poster Session, Russell House, University of South Carolina, Columbia, SC;
- Dispelling the Fear and Discovering the Truth: Linking the Environment and Health, South Carolina Sustainable Universities Initiative, Columbia, SC;
- South Carolina Public Health Association Annual Meeting, Myrtle Beach, SC;
- Science Posters Meeting, organized by Dr. Michael Wargovich and Dr. Joan Cunningham, Columbia, SC.

Petrișor, A. I., Cheval, S., and Decho, A. W. (2004). GIS for the quantification and classification of microscopic images of marine stromatolites. National Conference of GIS Users, Institute of Geography at the Romanian Academy, Bucharest, Romania

#### E. Internet publications

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2003). Quantitative Analyses of Microbial Images Using Feature Analyst, submission for the "Best of Feature Analyst for 2003" contest, November 10, 2003, published on-line at [http://www.featureanalyst.com/solutions/USC\\_project.pdf](http://www.featureanalyst.com/solutions/USC_project.pdf) (retrieved February 10, 2004)

## 2. Oral presentations

Petrișor, A. I., Ellis, S., and Decho, A. W. (2001). Quantitative Analysis of Confocal Microscopy Images of Bacterial Biofilms Using Digital Images Processing Techniques. Biology Spring Symposium, Georgetown, SC

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2002). Using GIS and Remote Sensing Techniques to Assess Spatial Variability Within Bacterial Biofilms, Graduate Student Day Physical and Life Sciences Oral Presentation Session, Russell House, University of South Carolina, Columbia, SC

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2002). Bacteria, Geography, and Statistics: How Do They Work Together?, Biology Spring Symposium, Georgetown, SC

Petrișor, A. I. (2002). An Application of Kriging in the Study of Spatial Variability of Bacterial Biofilms Using Confocal Microscopy, Digital Image Processing, and Geographical Information Systems, 32nd Annual Meeting of the South Carolina Chapter of the American Statistical Association, Russell House, Columbia, SC

Petrișor, A. I., Kawaguchi, T., and Decho, A. W. (2002). An Introduction to Bacterial Geography, 27th Annual Congress of the American-Romanian Academy of Arts and Sciences, Oradea, Romania

Petrișor, A. I., and Decho, A. W. (2003). Reconstruction and Computation of Biovolumes, Graduate Student Day Physical and Life Sciences Oral Presentation Session, Russell House, University of South Carolina, Columbia, SC

Petrișor, A. I., and Decho, A. W. (2003). Assessing and Quantifying the Microstructure of Bahamian Stromatolites Using Image Processing Techniques and GIS, Biology

Spring Symposium, Georgetown, SC (paper received a Honorable Mention for the best presentation)

Petrișor, A. I., and Decho, A. W. (2003). Imaging Fluorescent Polymeric Microspheres Using a Scanning Laser Confocal Microscope to Reconstitute and Estimate the Volume, Annual Meeting of the Southeastern Microscopy Society, Columbia, SC

Petrișor, A. I. (2003). Simulating the Marine Stromatolite Microenvironment Using Fluorescent Microbeads Incorporated in Gel Capsules, South Carolina Academy of Science, Clemson, SC

Petrișor, A. I., and Decho, A. W. (2004). GIS Applications in Microbiology, Biology Spring Symposium, Georgetown, SC

Petrișor, A. I., and Decho, A. W. (2004). A Novel Approach to the Classification and Quantification of Microbial Images: Applications on Bahamian Stromatolites, Graduate Student Day, Russell House, University of South Carolina, Columbia, SC

Petrișor, A. I., and Decho, A. W. (2004). Reconstruction and computation of biovolumes: potential problems, South Carolina Academy of Science, Charleston, SC