

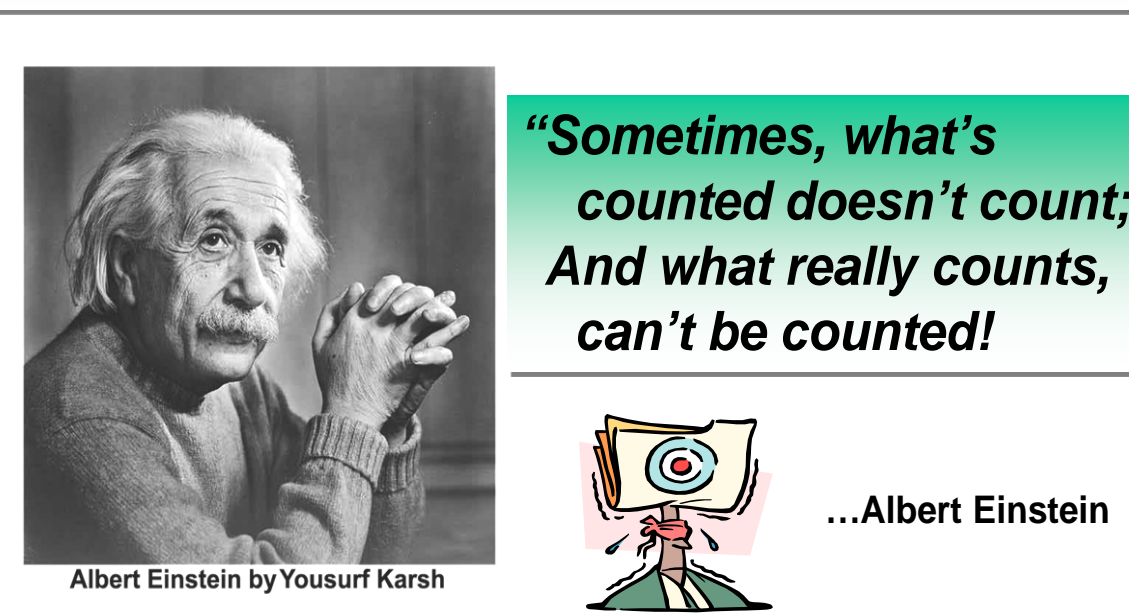
CMEIAS® v3.10: ADVANCED COMPUTATIONAL TOOLS OF IMAGE ANALYSIS SOFTWARE DESIGNED TO STRENGTHEN MICROSCOPY-BASED APPROACHES FOR UNDERSTANDING MICROBIAL ECOLOGY



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A major challenge in microbial ecology is to develop computing tools that can extract ecologically important information from digital images of microbial populations and communities at single cell resolution, and analyze their structure *in situ* without cultivation. Several microbial ecologists, mathematicians and computer scientists are addressing this challenge by developing a software package called CMEIAS (Center for Microbial Ecology Image Analysis System). CMEIAS v1.27 (available at <<http://cme.msu.edu/cmeias/>>) applies pattern recognition algorithms to classify microbial morphotypes with 97% accuracy. A CMEIAS-IT v3.10 upgrade is being developed to analyze microbial (i) richness and abundance of morphological diversity related to the database of currently known bacteria; (ii) abundance (cell density, biovolume, biomass carbon, biosurface area, cumulative length); (iii) metabolic activity, autecology and phylogeny using color segmentation of fluorescent molecular probes, and (iv) *in situ* patterns of spatial distribution during surface colonization. CMEIAS-IT v3.10 includes plugins with many new object analysis / classification features, new tools to help edit images before analysis (Object Separation, Color Segmentation, Photoshop Actions), Excel Add-Ins that compile, tabulate, analyze and plot CMEIAS data, a fractal dimension analyzer, new exploratory cluster analysis tools to optimize the decision boundaries for classification of the operational morphological units with unlimited morphological diversity, and a Quadrat Maker tool for spatial distribution analyses of microbial colonization. CMEIAS is currently being used to analyze spatial patterns of microbial colonization on plant roots, shifts in vaginal microflora in health and BV disease, freshwater biofilms on pebbles and surface polymers on glass, and *in situ* spatial scale of bacterial cell-to-cell communication at single cell resolution.

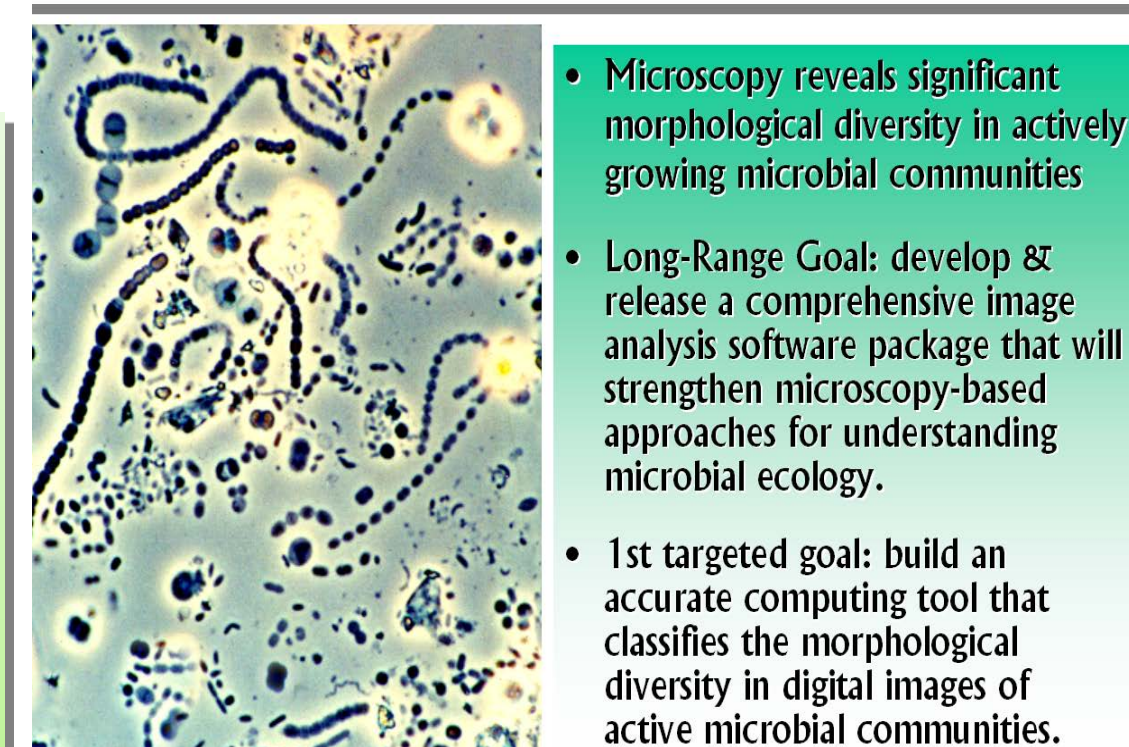
Dilemma in Quantitative Sciences



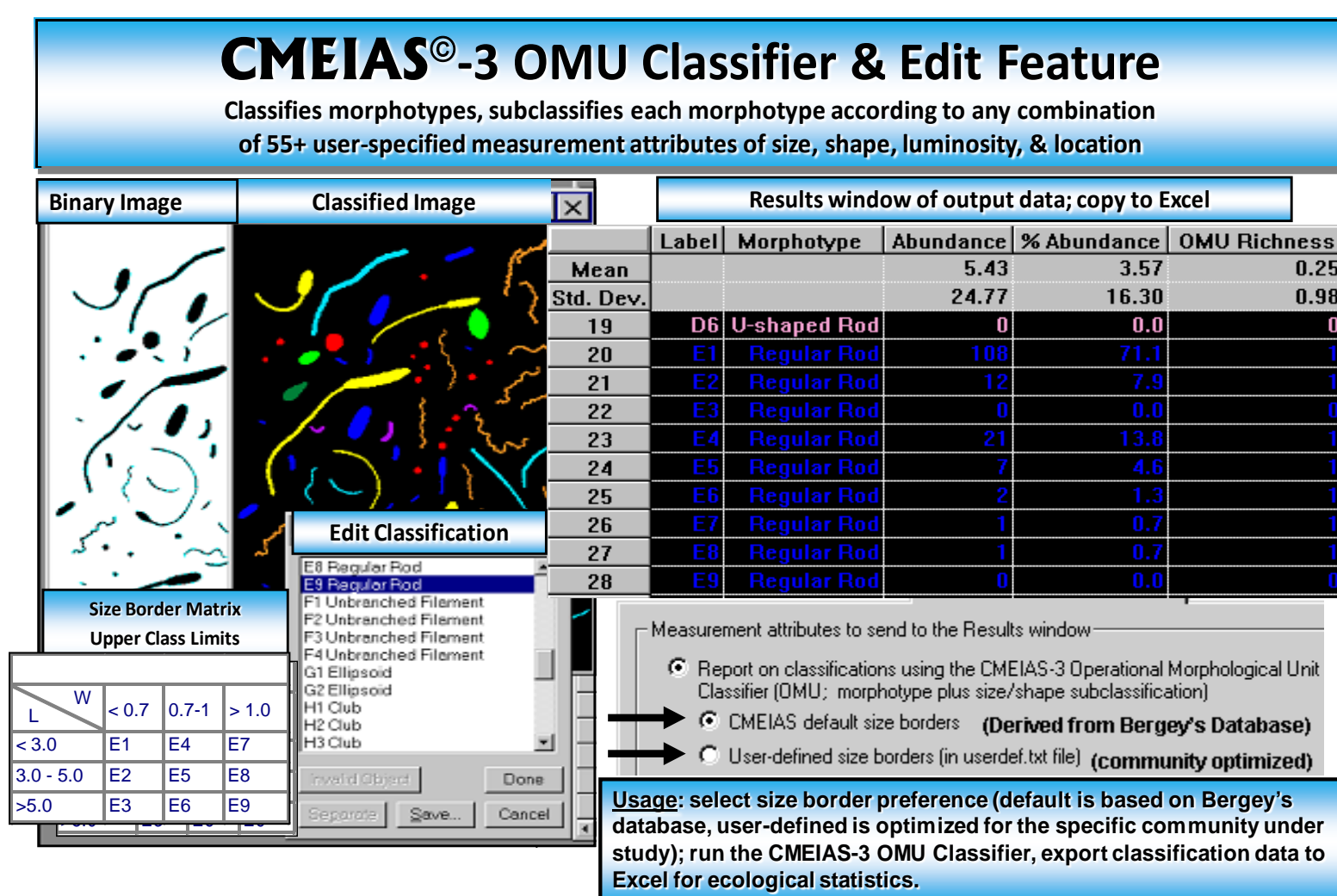
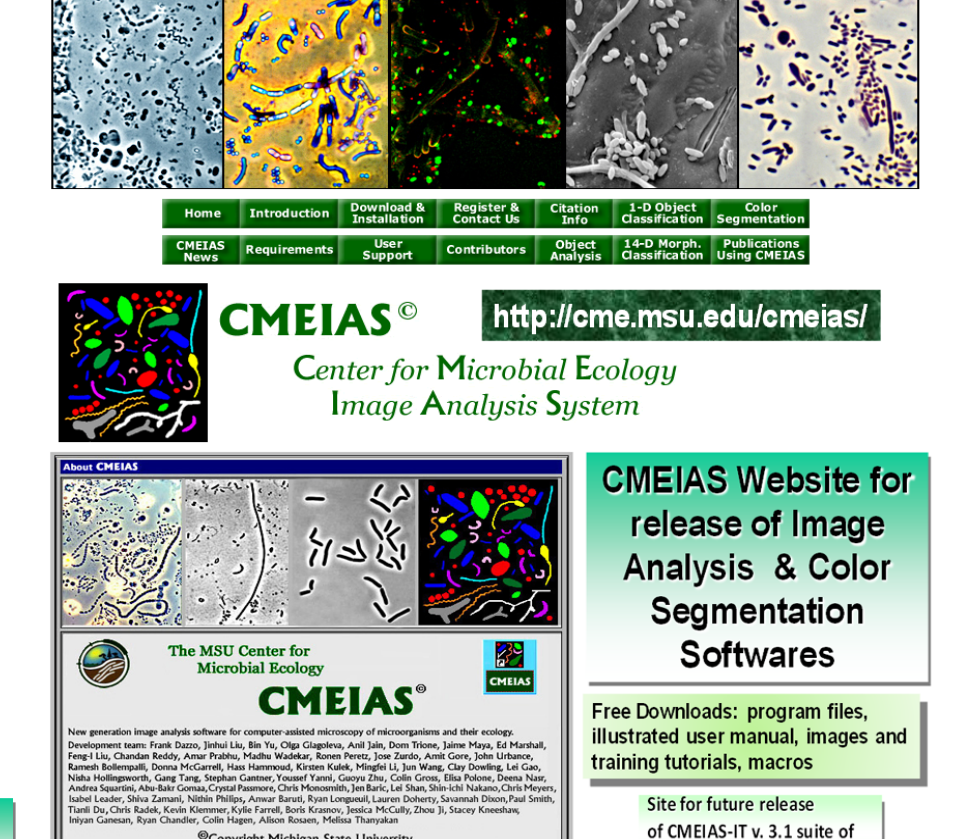
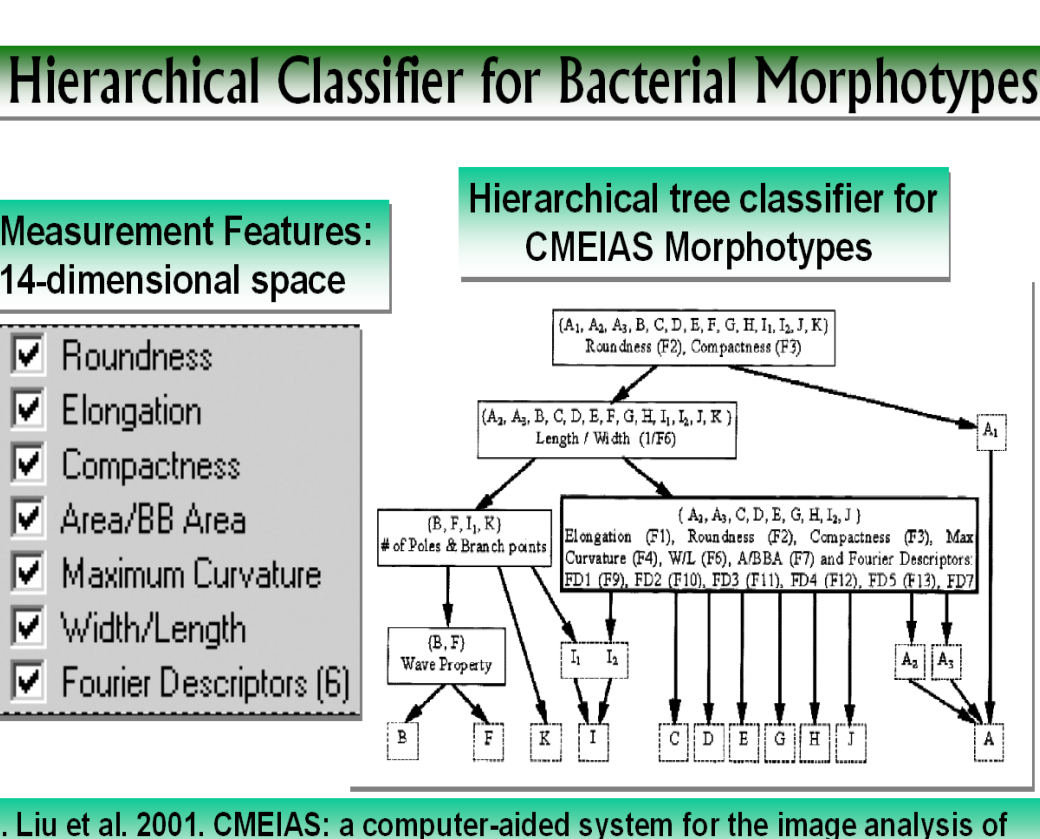
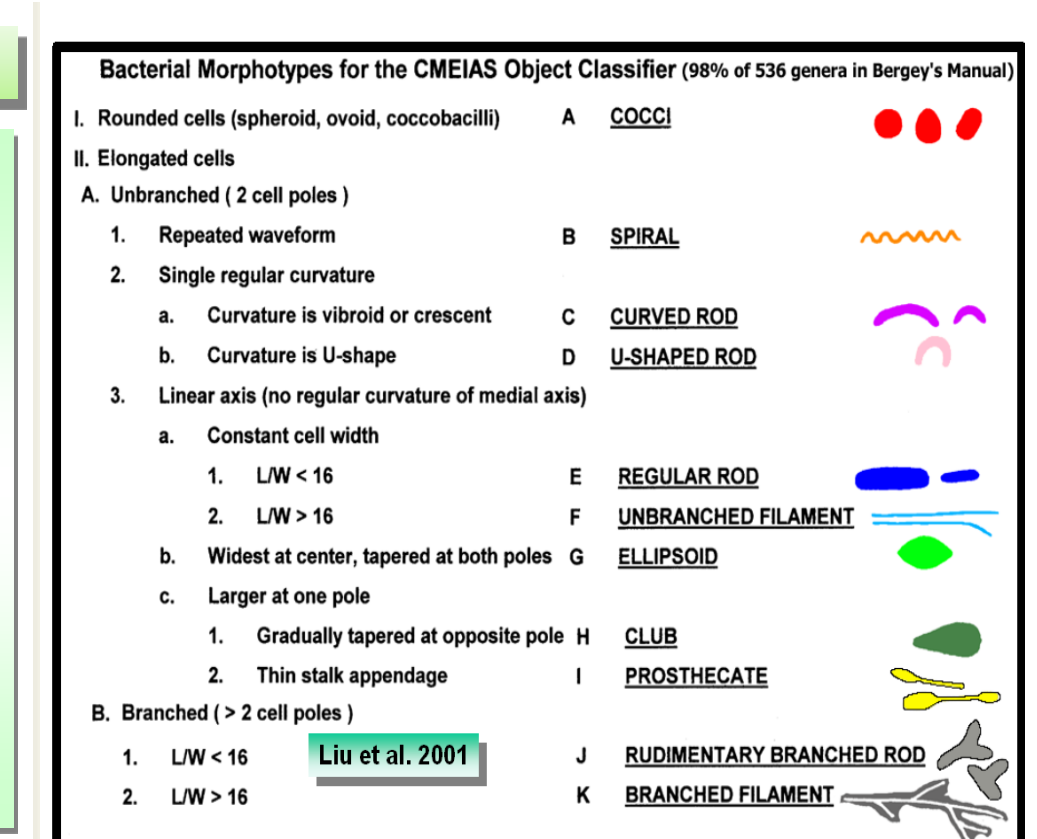
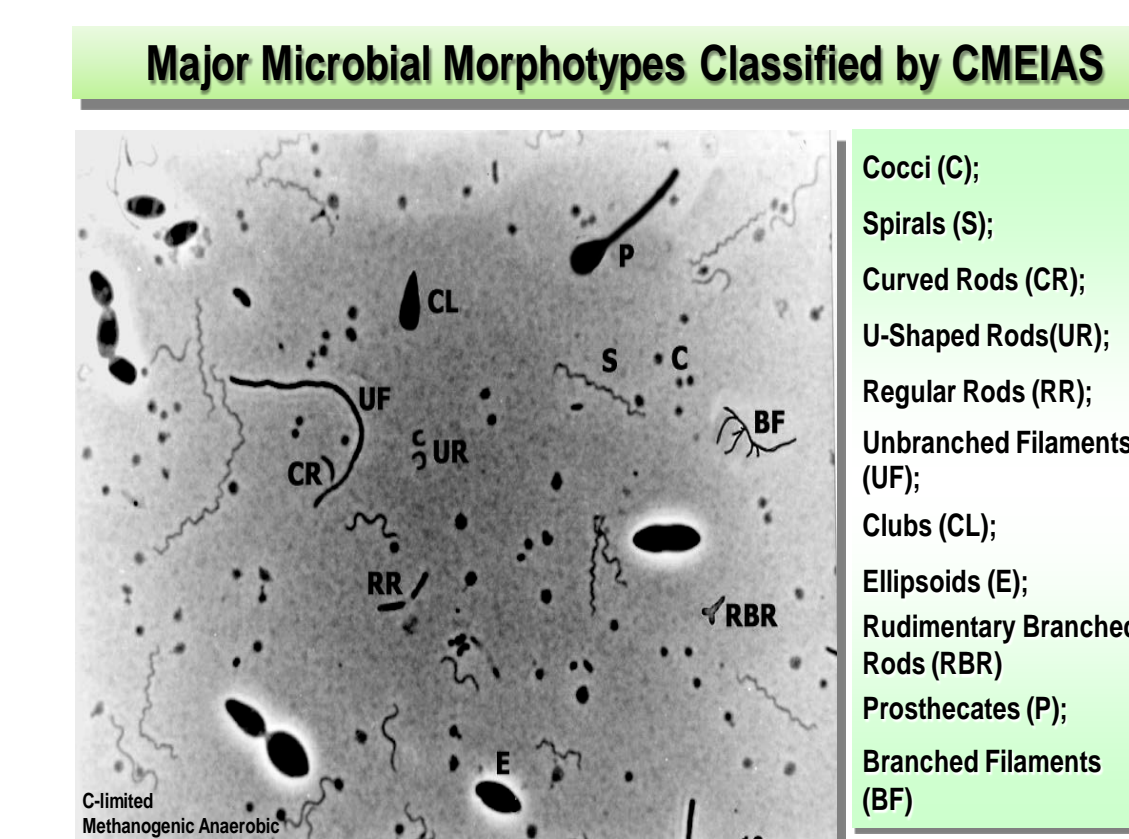
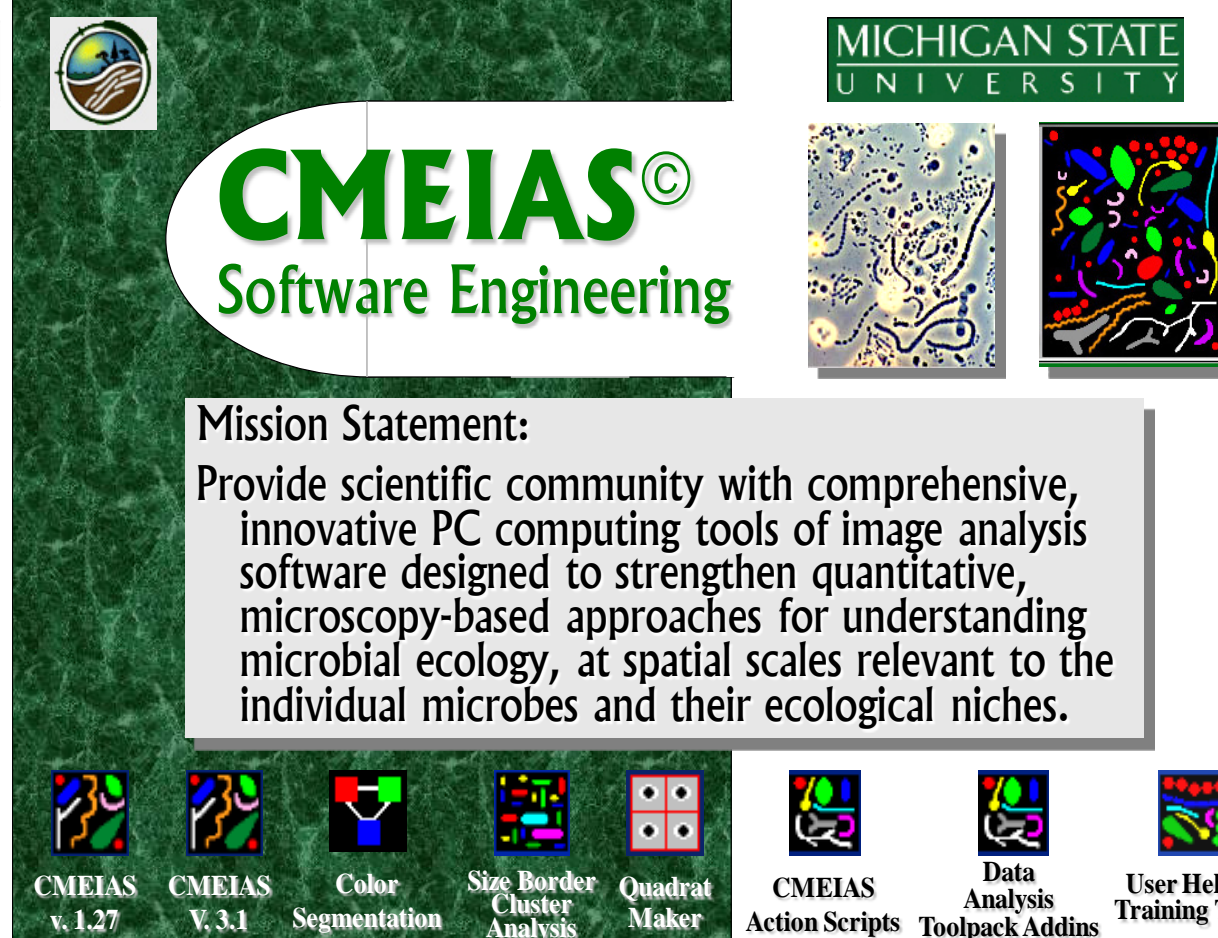
Why is Microscopy So Important in Microbial Ecology?

- Ecological processes change with the spatial scale at which they are observed and measured.
- Thus, knowing how scale influences our observations and measurements is critical
- Many interactions between individual microbes and their physical, chemical and biological environment occur at μm spatial scales
- Understanding the significance of spatial scale is an important challenge that will likely explain much of the diversity found *in situ* in microbial communities.

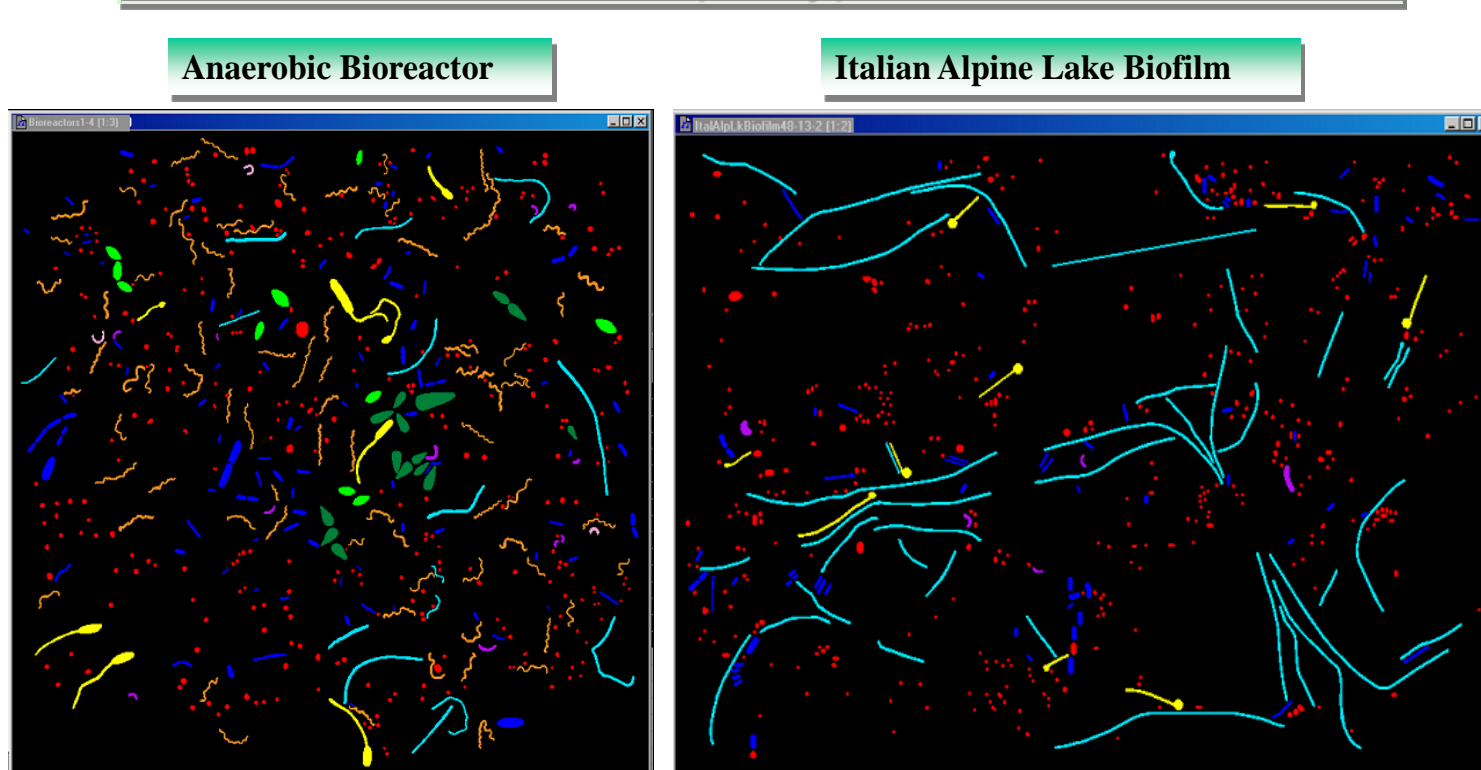
CMEIAS Inspiration from Bergey's Cover



- Microscopy reveals significant morphological diversity in actively growing microbial communities
- Long-Range Goal: develop & release a comprehensive image analysis software package that will strengthen microscopy-based approaches for understanding microbial ecology.
- 1st targeted goal: build an accurate computing tool that classifies the morphological diversity in digital images of active microbial communities.



Microbial Community Analysis Using the CMEIAS Morphotype Classifier



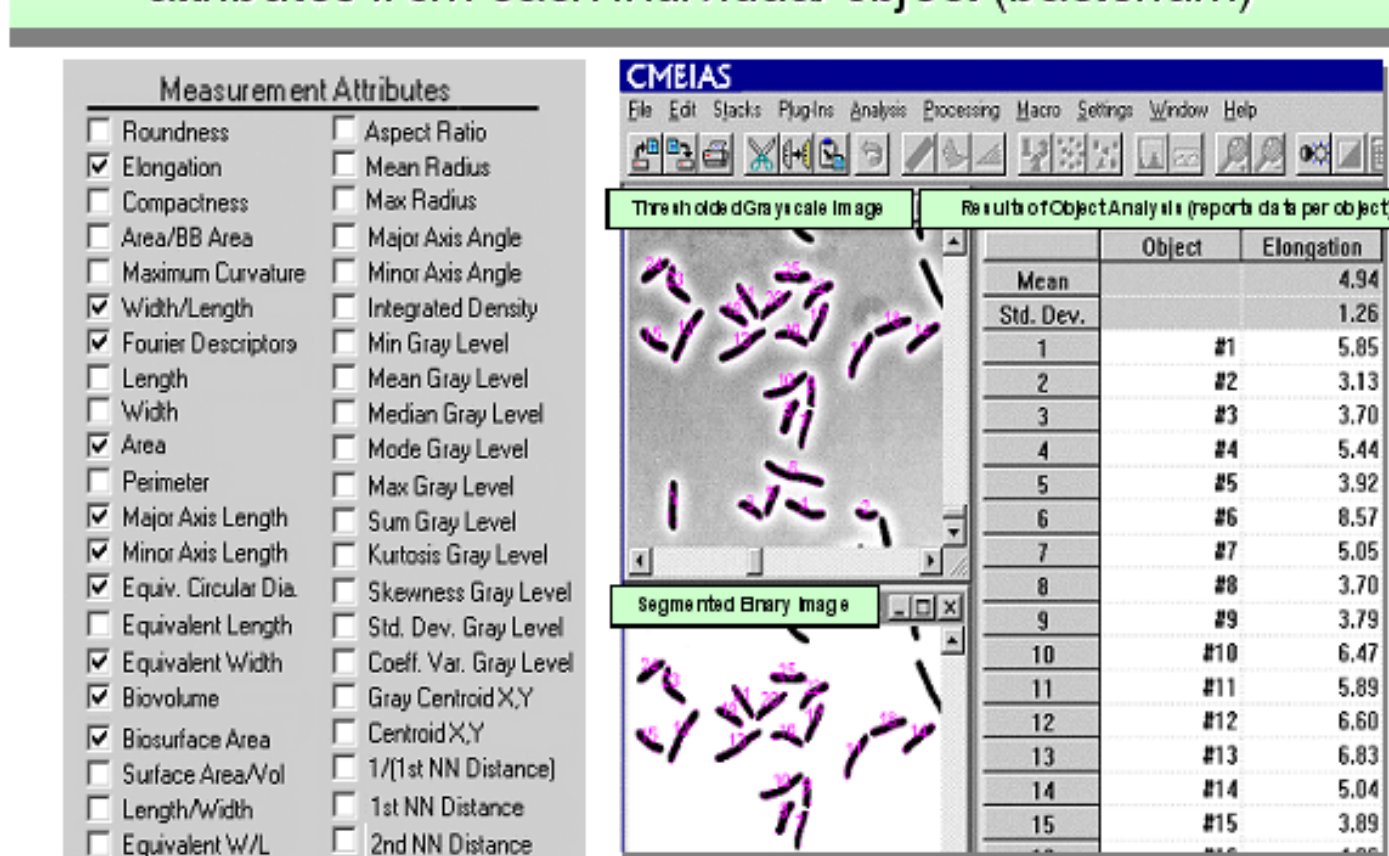
Morphological Diversity/Adaptations Local Community Dynamics Bacteriophage Stress / Refuge Perturbation / Succession / Stability

In Situ Spatial Patterns Colonization Behavior Biogeography Spatial Autocorrelation

CMEIAS Community Analysis

Spatial Abundance 2-D & 3-D Architecture

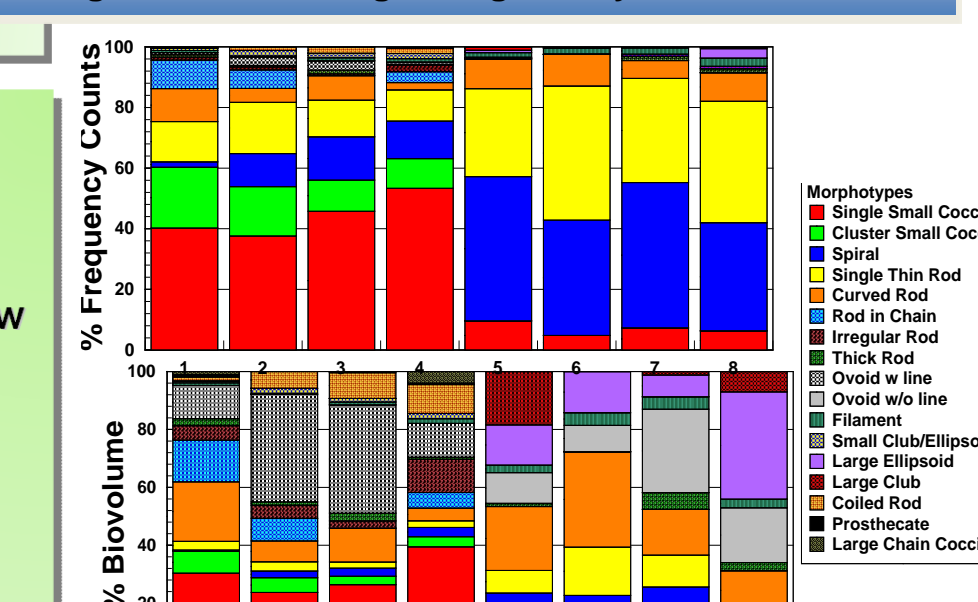
CMEIAS v3.1 Object Analysis



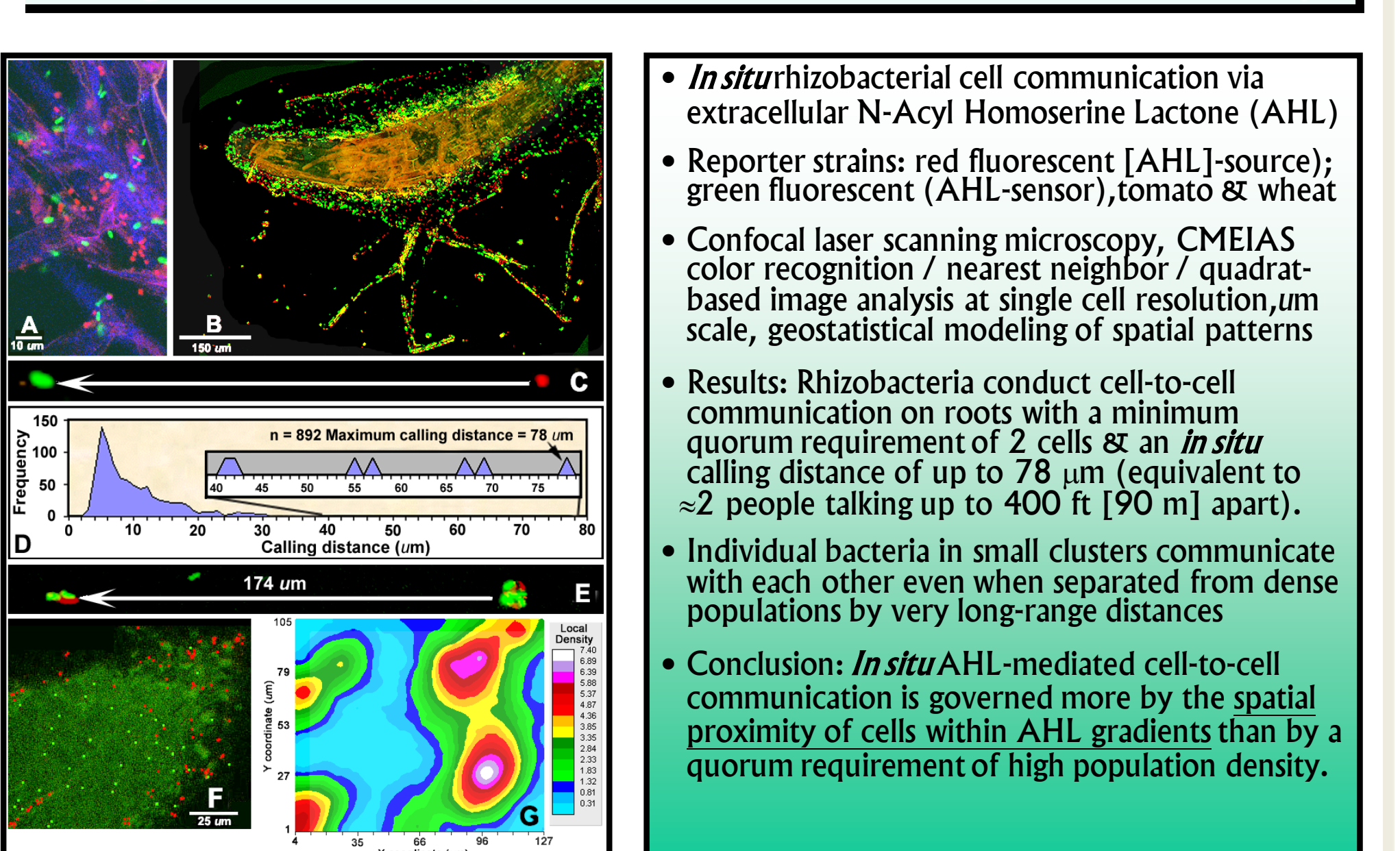
Examples of Ecophysiology Traits of Microbial Communities Revealed by In Situ CMEIAS Image Analysis at Single-Cell Resolution

- Cell biovolume relates to allometric scaling of metabolic rates, allocation of utilizable nutrient resources & overall status of growth physiology
- Cell surface area/biovolume relationships relate to accessibility and uptake of nutrients, especially at low limiting concentrations
- Cell elongation adaptations to predatory stress
- Spatial clustering of attached cells reflects their colonization behavior & impacts on cell-cell interactions (cross-feeding, communication, amensalism)

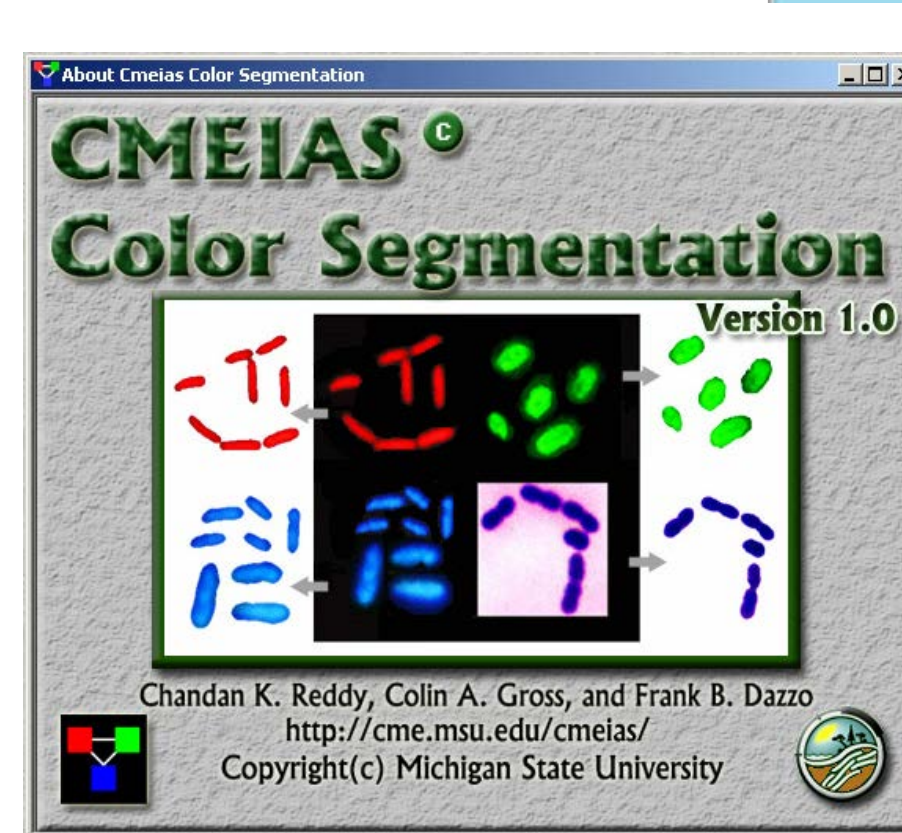
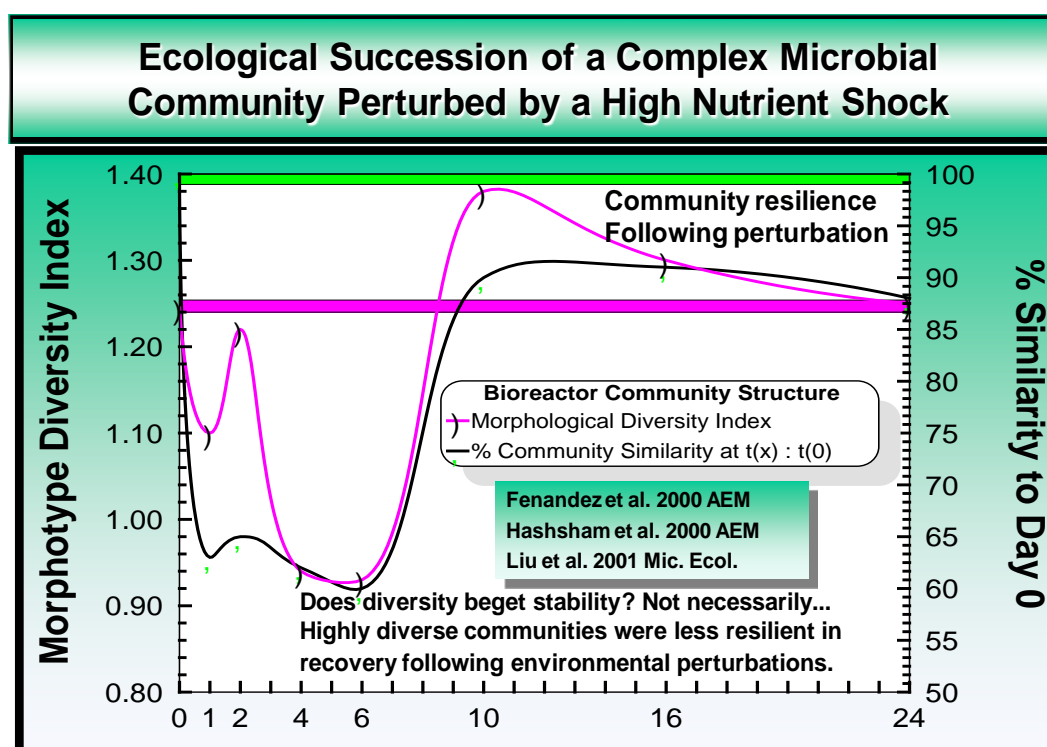
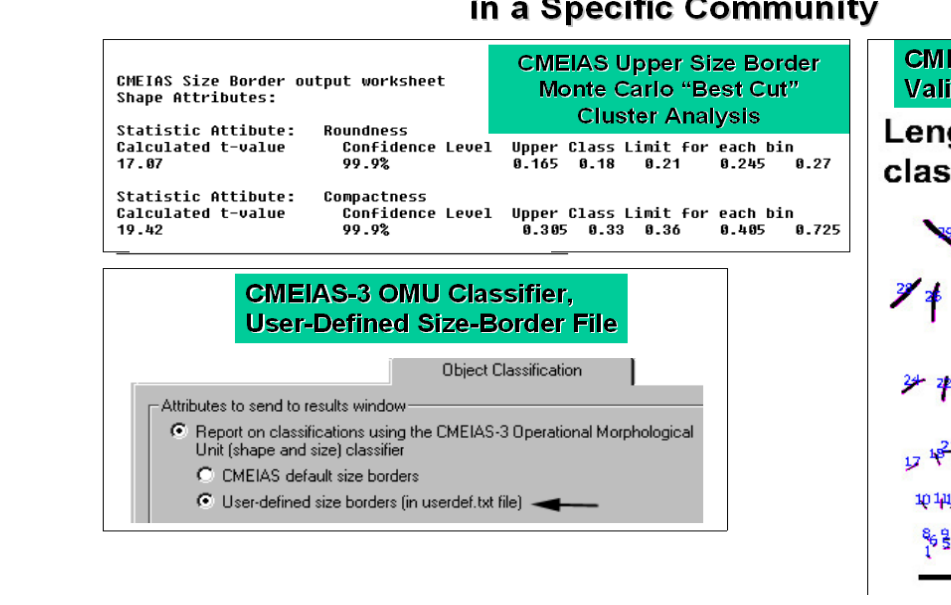
Abundance measured by cell biovolume significantly impacts on computed community diversity, dominance and evenness (CMEIAS data, anaerobic methanogenic Bioreactor Chemostat Communities, J-evenness = 0.65 using cell, 0.81 using # weighted by biovolume).



In situ CMEIAS Analysis of Cell-to-Cell Communication by Rhizobacteria during Root Colonization (Gantner et al. 2006 FEMS Mic. Ecol. 56: 188-194)

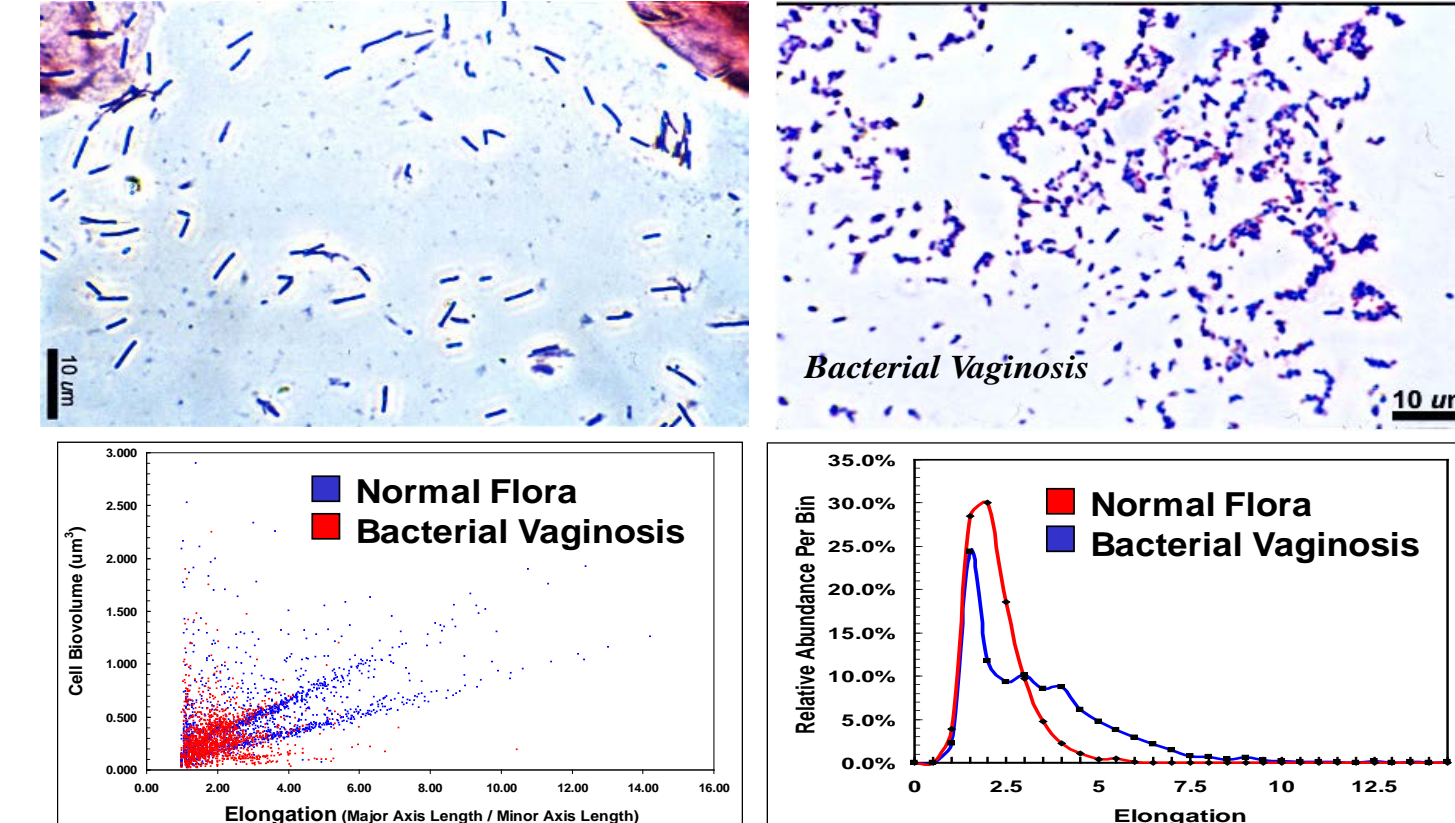


- In situ* rhizobacterial cell communication via extracellular N-Acyl Homoserine Lactone (AHL)
- Reporter strains: red fluorescent [AHL]-source; green fluorescent (AHL-sensor), tomato & wheat
- Confocal laser scanning microscopy, CMEIAS color recognition / nearest neighbor / quadrat-based image analysis at single cell resolution, μm scale, geostatistical modeling of spatial patterns
- Results: Rhizobacteria conduct cell-to-cell communication on roots with a minimum quorum requirement of 2 cells & an *in situ* calling distance of up to 78 μm (equivalent to ≈ 2 people talking up to 400 ft [90 m] apart).
- Individual bacteria in small clusters communicate with each other even when separated from dense populations by very long-range distances
- Conclusion: *In situ* AHL-mediated cell-to-cell communication is governed more by the spatial proximity of cells within AHL gradients than by a quorum requirement of high population density.

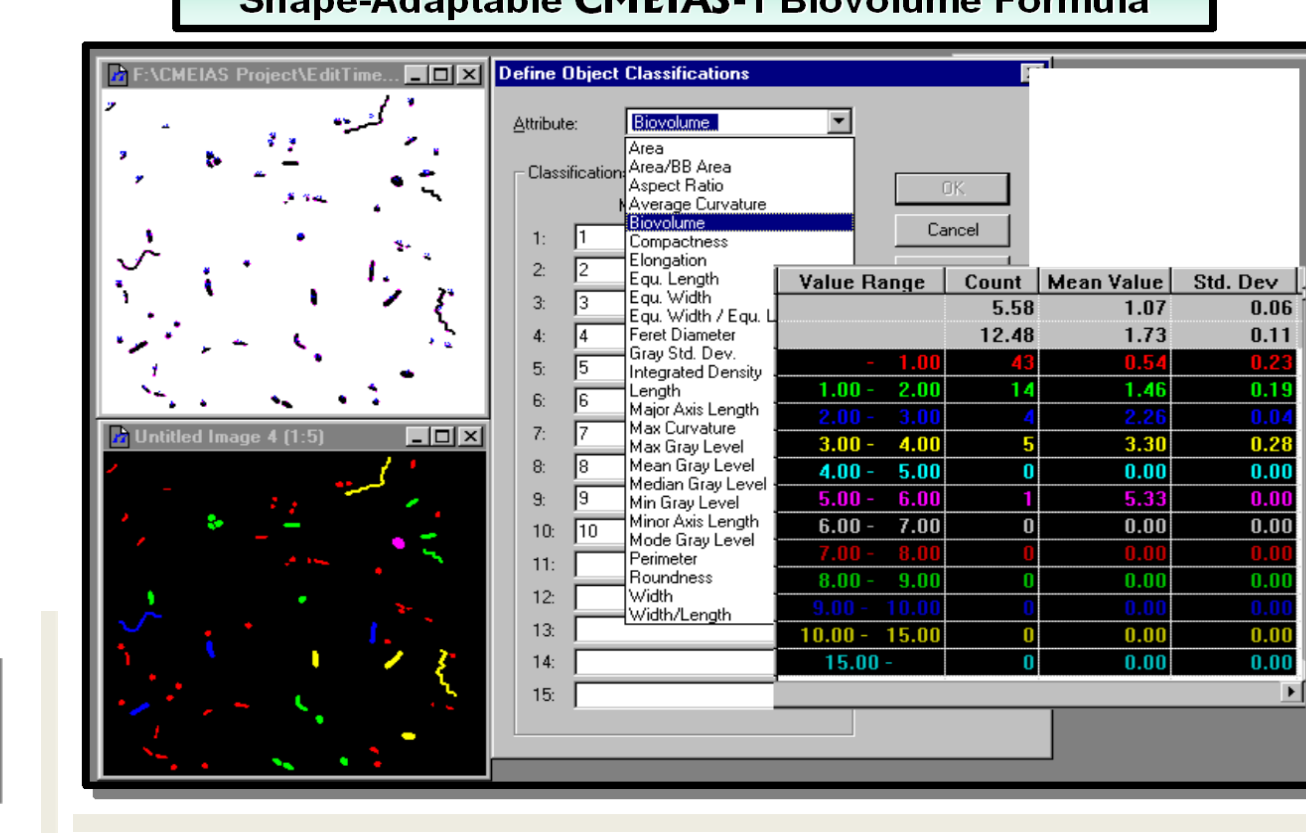


Eco-Physiology Allometry/Metabolism Resource Ecology Growth / Productivity Local Cell-Cell Interactions

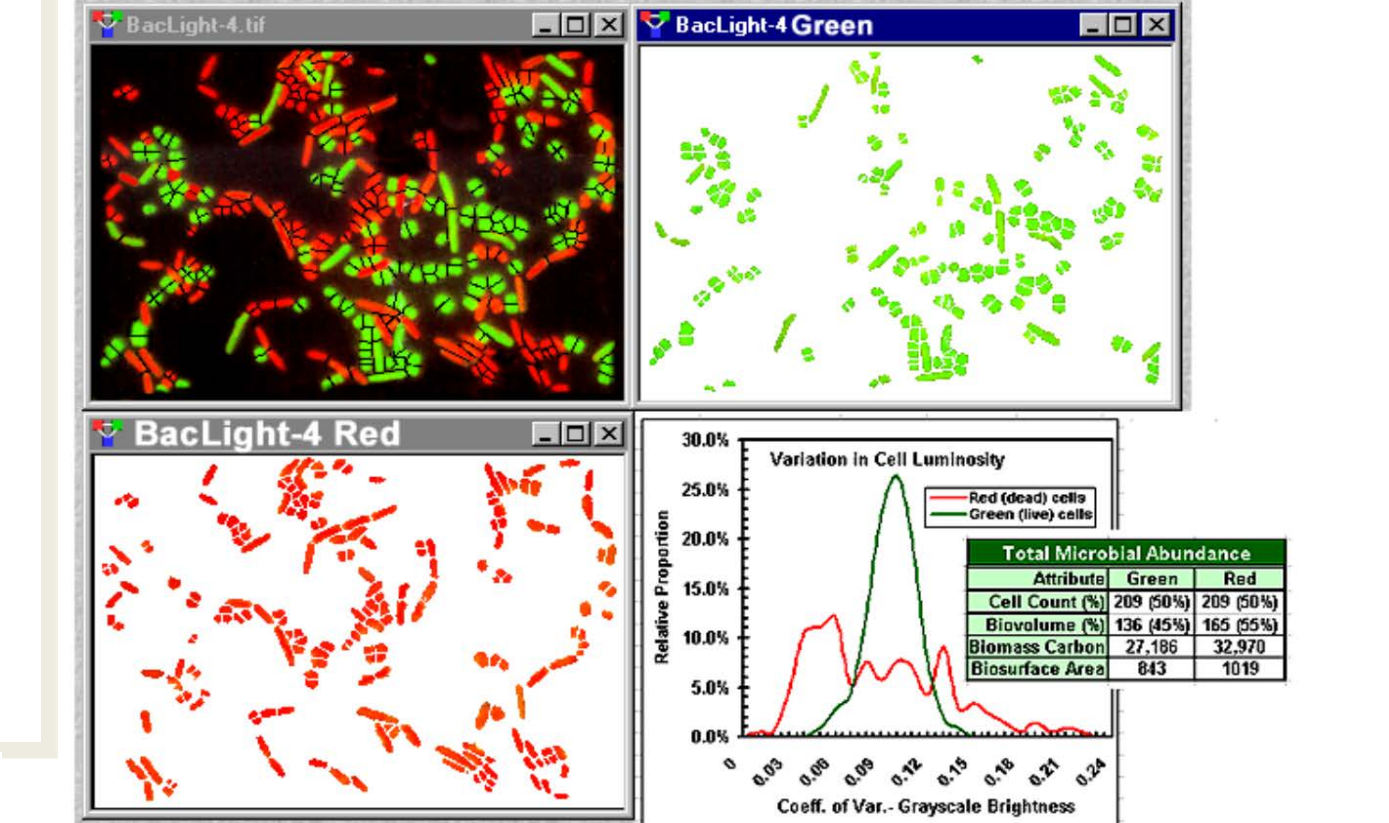
Human Vaginal Microflora: "Normal" vs. Bacterial Vaginosis



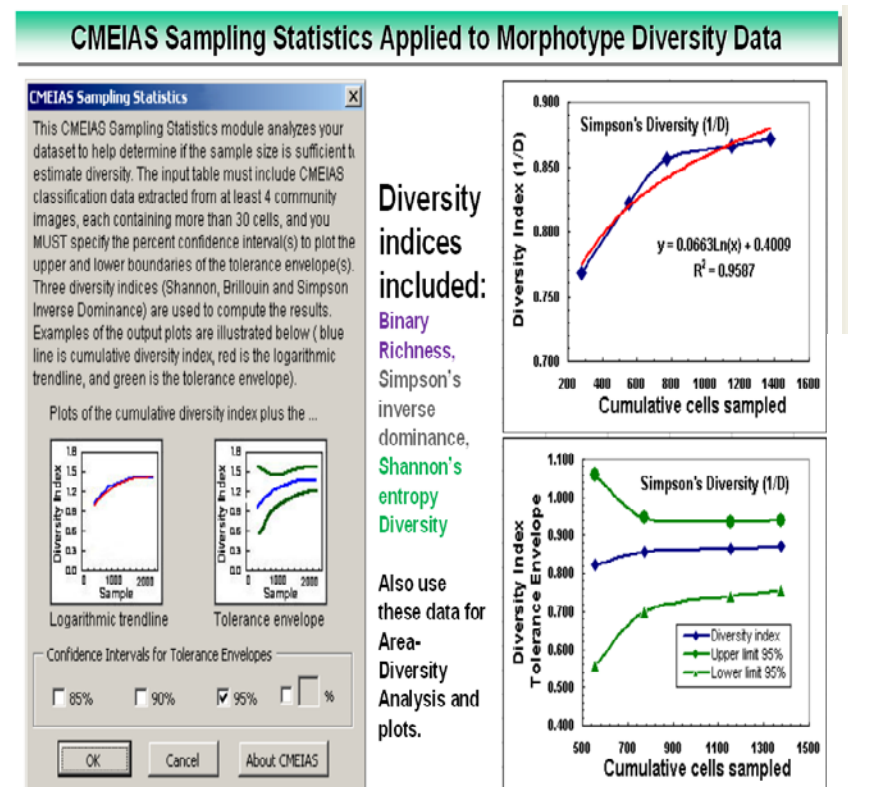
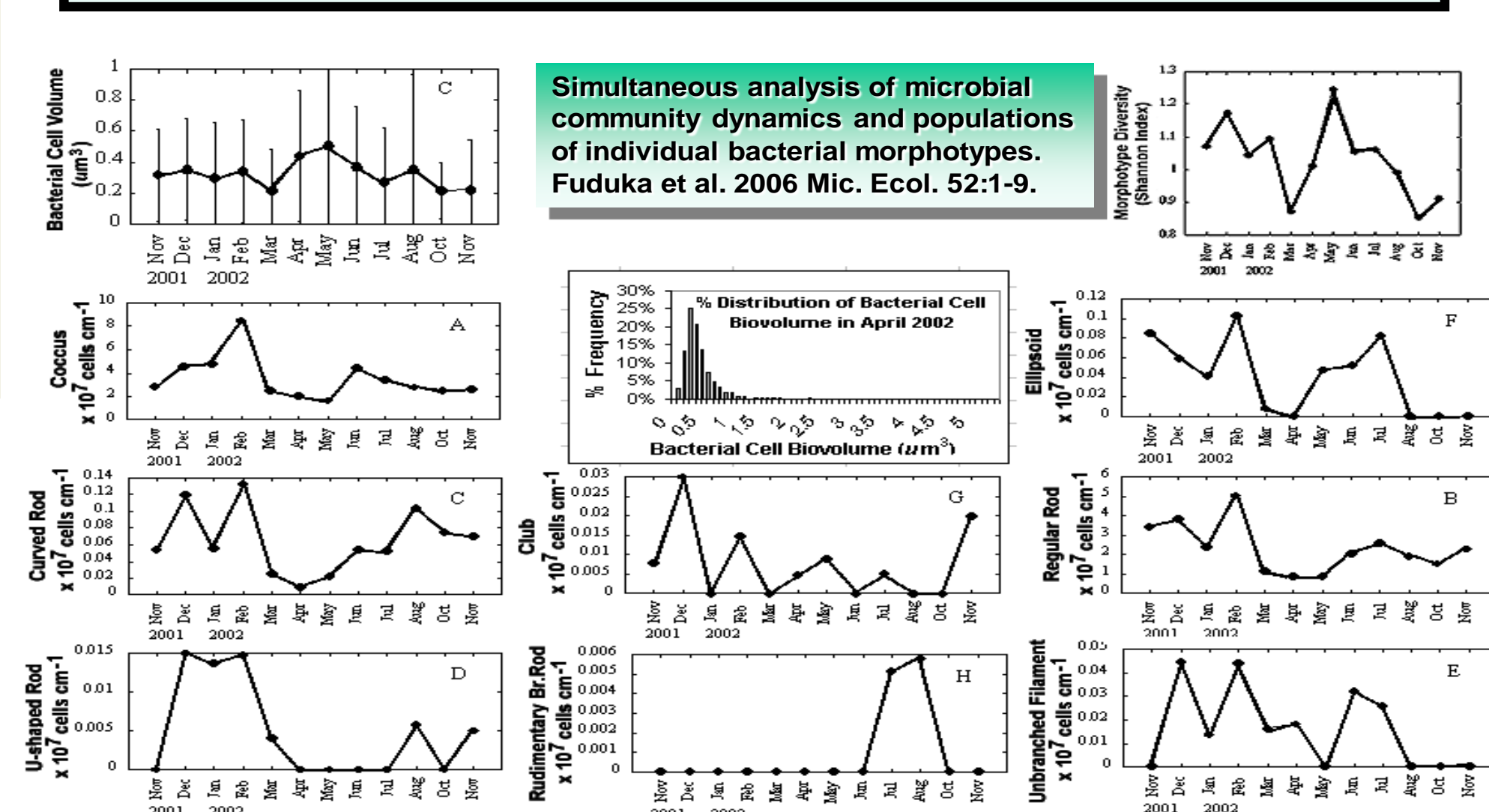
Distribution of Microbial Abundance using the Shape-Adaptable CMEIAS-1 Biovolume Formula



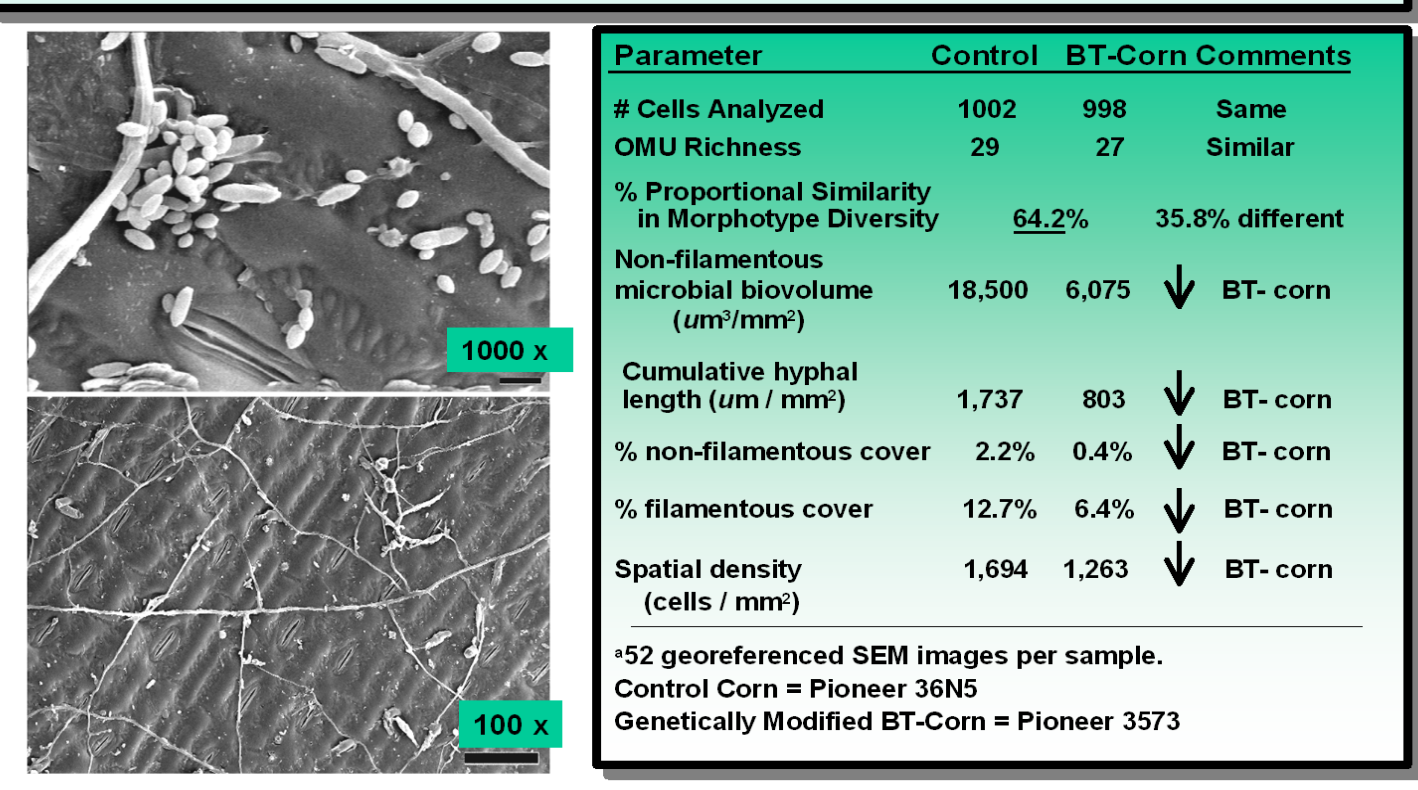
IAS Color Analysis of Bacterial Viability (BacLight Activity Stain)



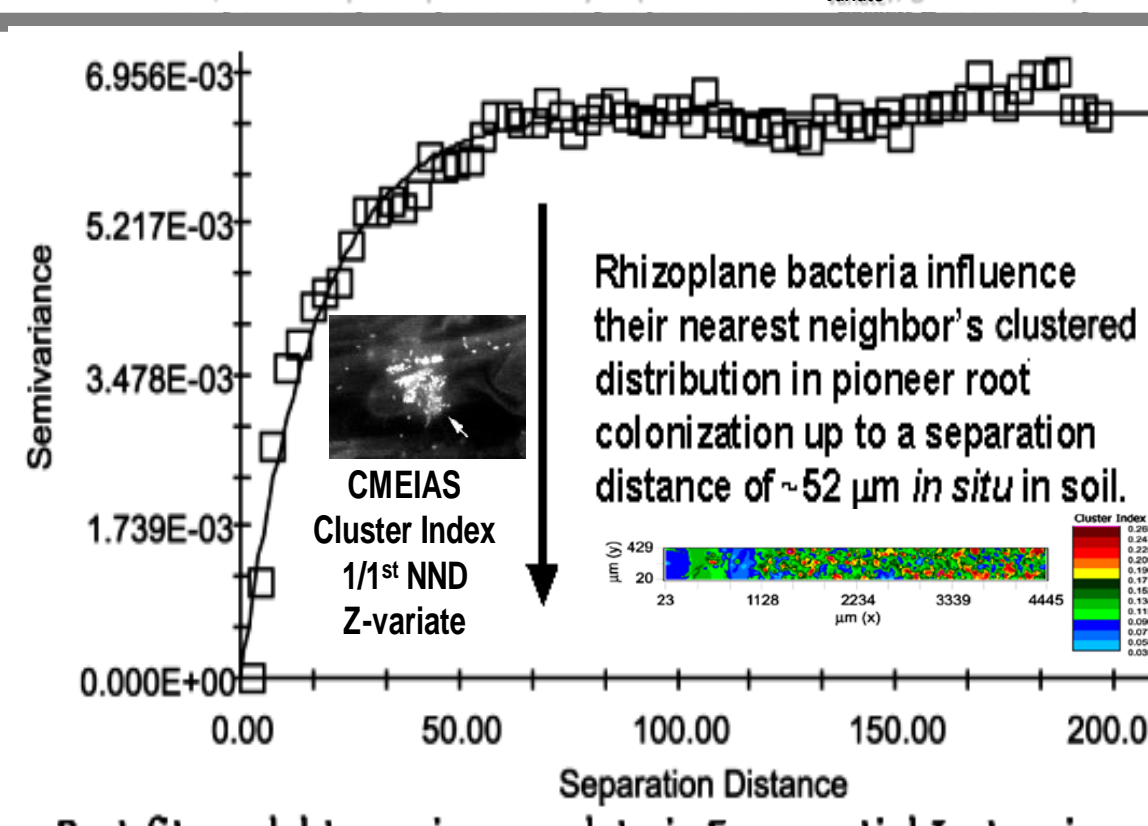
CMEIAS Analysis of Dynamic Seasonal Changes in Microbial Communities in Steambed Epilithic Biofilms



In Situ CMEIAS Analysis of Phylloplane Microbial Communities on Field-Grown Leaves of Control vs. Genetically Modified BT-Corn®



Geostatistics of Pioneer Rhizobacterial Colonization



Best fit model to variogram data is Exponential Isotropic, residual sum of squares = 3.86×10^{-6}

F Dazzo 2004 In A. Varma et al. (ed.) Plant Surface Microbiology, pp. 503-550, Springer-Verlag

3d Surface Plots of RCR Biofilms – 5 different substrata

