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 applies pattern recognition algorithms to classify microbial morphotypes with 97% accuracy. A CMEIAS-IT v3.10 upgrade is being developed to analyze microbial (i) morphological diversity related to the database of currently described 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 of biofilm landscapes. CMEIAS includes plugins with many new object analysis/classification features, new tools to help edit images before analysis (Object Separation, Color Segmentation), Excel Com Add-Ins that compile & analyze CMEIAS ecological 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, freshwater biofilm communities on surface polymers on glass, shifts in vaginal microflora in health and BV disease, and the *in situ* spatial scale of bacterial cell-to-cell communication at single cell resolution. These CMEIAS-based applications can facilitate research designed to enhance our understanding of microbial ecology by providing well-documented, accurate, robust and user-friendly computing tools that extract important, quantitative information from digital images of microbes at multiple spatial scales relevant to their *in situ* ecological behavior in various habitats. CMEIAS computer-assisted microscopy adds an exciting new dimension of awesome computational power to many types of quantitative microbial ecology research, and is especially valuable when combined with molecular-based and other methods of polyphasic community analysis.

CMEIAS® Software Development

Why is Microscopy So Important in Microbial Ecology?

CMEIAS-IT® Image Analysis

Version 3.10

CMEIAS® v3.1 Object Analysis

CMEIAS® Biovolume Formula Selection

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

Major Microbial Morphotypes Classified by CMEIAS

Hierarchical Classifier for Bacterial Morphotypes

CMEIAS® SBCAT

CMEIAS Size Border Cluster Analysis Tool

CMEIAS Tools to Build a Custom Size Border File Optimized to Classify the Full Diversity of Operational Morphological Units in a Specific Community

CMEIAS® Color Segmentation

CMEIAS Spatial Ecology Analysis

CMEIAS® Quadrat Maker

CMEIAS v.3.10 Extracts *5 Major Types* of Ecologically Relevant Information from Digital Images of Microbial Communities

Morphological Diversity

In situ Spatial Ecology

Abundance

CMEIAS Computer-Assisted *In situ* Microscopy of Microbial Communities

In situ Metabolic Activity

In situ Autecology / Phylogeny

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

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

CMEIAS Community Diversity and Similarity Analyses Based on Frequency Distribution Data for Optimized Size-Range Clusters

CMEIAS JFrad Fractal Dimension Analysis

Human Vaginal Microflora: "Normal" vs. Bacterial Vaginosis

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 relationship relates 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, xenobiosis)

Regular Rods

3d Surface Plots of RCR Biofilms – 5 different substrata

CMEIAS Image Analysis of Measurement Attributes that Discriminate Biofilm Architecture

Highly-Ranked Attributes:

- % Substratum Coverage
- Areal Porosity
- Foreground Object Area
- Mean Diffusion Distance
- Max Diffusion Distance
- Fiber Length
- Radiality
- Integrated Gray Density
- Clumpiness
- Gray Asymmetry
- Sum Densitometry
- Fractal Dimension
- Georeferenced Cluster Index
- Cmeias-2 Morphotype Class
- Cmeias-3 OMU Class with User-Defined Size Border

Discrimination of Biofilm Architecture by CMEIAS Landscape Ecology Metrics at 1x and 10x Spatial Scales

1x Biofilm 10x Biofilm

Microbial Community Analysis Using the CMEIAS v3.10 upgrade