



# CMEIAS® v4.0: ADVANCED COMPUTATIONAL TOOLS OF BIOIMAGE INFORMATICS 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 upgrade is being developed to analyze microbial (i) morphological diversity related to the database of currently described bacteria; (ii) abundance (e.g., cell density, biovolume, biomass carbon, biosurface area, cumulative length); (iii) ecophysiology (e.g., metabolic activity, autecology and phylogeny using color segmentation of fluorescent molecular probes), and (iv) *in situ* spatial / landscape ecology. CMEIAS includes plugins with many new object analysis/classification features, new tools to help edit images before analysis (Object Separation, Color Segmentation), Excel add-Ins that prepare, 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 plot-based spatial distribution analyses of microbial colonization patterns. CMEIAS is currently being used to analyze microbial colonization patterns and intensity 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 interactions 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 bioimage informatics software is released at our project website <<http://cme.msu.edu/cmeias/>>, adds computational power to many types of quantitative microbial ecology research, and is especially valuable in filling gaps when combined with molecular-based and other methods of polyphasic community analysis.

### CMEIAS® Software Development

Mission Statement:  
Provide scientific community with comprehensive, innovative PC computing tools of bioimage informatics analysis software designed to strengthen quantitative, microscopy-based approaches for understanding microbial ecology, at spatial scales relevant to the individual microbes and their ecological niches.

### 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  $\mu$ m spatial scales
- Understanding the significance of spatial scale is an important challenge that will likely explain some of the diversity found *in situ* in microbial communities.

### CMEIAS-IT® Image Analysis

Version 4.0  
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<http://cme.msu.edu/cmeias/>  
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### CMEIAS® v4.0 Object Analysis

Extracts and reports up to 83 user-selected measurement attributes from each individual object (bacterium)

Measurement Attribute	Unit	Value
Area	$\mu^2$	10.12
Perimeter	$\mu$	11.83
Length	$\mu$	11.83
Width	$\mu$	11.83
Volume	$\mu^3$	11.83
Surface Area	$\mu^2$	11.83
Centroid X	$\mu$	11.83
Centroid Y	$\mu$	11.83
Centroid Z	$\mu$	11.83
Surface Area	$\mu^2$	11.83
Volume	$\mu^3$	11.83
Surface Area	$\mu^2$	11.83
Volume	$\mu^3$	11.83
Surface Area	$\mu^2$	11.83
Volume	$\mu^3$	11.83
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Surface Area	$\mu^2$	11.83
Volume	$\mu^3$	11.83
Surface Area	$\mu^2$	11.83
Volume	$\mu^3$	11.83

### CMEIAS® Biovolume Formula Selection

The accuracy of 17 biovolume formulas was evaluated on images of model populations and communities compared to their true volume measured by water displacement

Overall accuracy of biovolume formulas ranged from 4.97% to 49%. Some popular formulas are grossly inaccurate for certain microbial morphotypes!!

The CMEIAS-1 & 2 formulas had the highest overall accuracy (98%) since they are adaptive to cell shape

9 formulas currently included in CMEIAS v4.0

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

### Major Microbial Morphotypes Classified by CMEIAS

Cocci (C); Spirals (S); Curved Rods (CR); U-Shaped Rods (UR); Regular Rods (RR); Unbranched Filaments (UF); Clubs (CL); Ellipsoids (E); Rudimentary Branched Rods (RBR); Prosthecates (P); Branched Filaments (BF)

### Hierarchical Classifier for Bacterial Morphotypes

Measurement Features: 14-dimensional space

Hierarchical tree classifier for CMEIAS Morphotypes

### CMEIAS® 3MU Classifier & Edit Feature

Results window of output data, copy to Excel

### CMEIAS Size Border Cluster Analysis Tool

Inputs: Object Analysis data array  
Reports: Object Analysis data array  
Using 1,000 Monte Carlo iterations

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

CMEIAS-3MU Classifier, User-Defined Size Border File

### CMEIAS v4.0 Extracts 5 Major Types of Ecologically Relevant Information from Digital Images of Microbial Communities

Morphological Diversity  
Abundance  
In situ Spatial Ecology  
In situ Metabolic Activity  
In situ Autecology / Phylogeny

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

In Situ Spatial Colonization Behavior Biogeography, Spatial Autocorrelation

Eco-Physiology Alometry/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 Object Separation Tool

Provides automatic splitting and manual editing of touching objects

### CMEIAS® Color Segmentation

Version 1.0  
Chandan K. Reddy, Colin A. Gross, and Frank B. Dazzo  
<http://cme.msu.edu/cmeias/>  
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### CMEIAS Spatial Ecology Analysis

CMEIAS computing tools:  
Object Analysis (spatial attribute per individual, e.g., nearest-neighbor traits)  
Cumulative Object Analysis (spatial attribute/defined area, e.g., spatial density)  
Quadrat Maker (optimize quadrat sampling to maximize spatial resolution)

Extracts 3 types of data for spatial ecology analysis:  
Point pattern (tests for spatial randomness, predicts colonization behavior)  
Grid-based quadrat lattice (measures heterogeneity in spatial density)  
Georeferenced geostatistics (mathematically model georeferenced data for spatial autocorrelation of user-selected Z-variables, e.g., clustering index, and produce statistically defensible 2-D and 3-D kriging interpolation maps of the Z-variables over the entire spatial domain)

### In situ Metabolic Activity

Segmentation / analysis of colored pixels  
Classification of cell's physiological status & activity based on color discrimination, e.g., Live/Dead, GFP-reporter gene expression, substrate-induced respiration, autofluorescence, FISH-MAR metabolic activity, Gram's reaction  
Allometric scaling implications

### CMEIAS JFrad Fractal Dimension Analysis

Version 1.0  
Development team: Zhou Ji & Frank Dazzo  
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<http://cme.msu.edu/cmeias>

### Human Vaginal Microflora: "Normal" vs. Bacterial Vaginosis

Object Analysis: The ideal model system for exploring medical microbiology applications of CMEIAS computer-assisted microscopy software.

### 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)

### CMEIAS® Quadrat Maker

Version 1.0  
Colin Gross & Frank Dazzo  
<http://cme.msu.edu/cmeias/>  
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### Geostatistics of Pioneer Rhizobacterial Colonization

(white clover seedlings germinated 2-d in soil, optimized wash, acetone orange, CLSM, CMEIAS spatial pattern analysis (cluster index, geostatistics))

### CMEIAS In situ Spatial Distribution Analysis: Immunofluorescence of Rhizobium leguminosarum bv. trifolii 0403 on a white clover rhizoplane

Parameter Measured	Value Obtained
Image Area Analyzed	10,434 $\mu^2$
Cell Count	196
% Substratum Area Covered by Microbes	2.70%
Total Microbial Biomass Carbon	259.0 $\mu$ g
Total Microbial Biomass Nitrogen	51.8 $\mu$ g
Total Microbial Biomass Phosphorus	1,126.6 $\mu$ g
Spatial Density	19.024 cells/ $\mu^2$
Mean 1st Nearest Neighbor Distance	3.9 ± 2.4 $\mu$ m
Mean 2nd Nearest Neighbor Distance	5.7 ± 2.9 $\mu$ m
Spatial Randomness Index	0.68 (clustered)
Variance: Mean Ratio	1.796 (clustered)
Negative Dispersion Index	1.996 (clustered)
Monte Carlo Randomness Index	0.550 (clustered)
Lloyd's Mean Crowding Index	1.595 (clustered)
Lloyd's Patchiness Index	1.995 (clustered)
Holligata's A Index	0.536 (clustered)

### In situ CMEIAS Analysis of Cell-to-Cell Communication by Rhizobacteria during Root Colonization

(Carter et al., 2006 FEMS Mic. Ecol. 56:188-194)

### CMEIAS JFrad - Biofilm Demo

### Regular Rods

Length > 2.0  $\mu$ m

Width < 0.5  $\mu$ m

Width > 0.5 - 0.8  $\mu$ m

Width > 0.8 - 0.8  $\mu$ m

Width > 0.8  $\mu$ m

Width > 0.8  $\mu$ m

### 3d Surface Plots of RCR Biofilms - 5 different substrata

### CMEIAS Image Analysis of Measurement Attributes that Discriminate Biofilm Architecture

Highly-Ranked Attributes:  
Substratum Coverage  
Area Porosity  
Foreground Object Area  
Mean Diffusion Distance  
Fiber Length  
Reliability  
Integrated Gray Density  
Clumpiness  
Grey Asymmetry  
Sum Denisometry  
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

### Ecological Niches for Rhizobium: Includes POPR Endophytes of Cereals

### Vertical stacked quadrat density maps of indigenous cells of Rhizobium leguminosarum bv. trifolii biofertilizer strain E11 colonized on rice roots grown in soil from 4 sites in the Kafr El-Sheikh (KF) and 4 from Algharab (GH) governorates in the Nile delta of Egypt. Note that the pseudocolored scales cover a wider range in Kafr El-Sheikh, with higher overall natural colonization densities.

### Microbial Community Analysis Using CMEIAS-IT v4.0

American Biovector, Italian Alpine Lake Biofilm

### Hierarchical Size subclassification of Lactobacilli

Length 2.0  $\mu$ m splits L. iners from others. Width borders of 0.5, 0.6, & 0.8  $\mu$ m classifies L. gasseri, L. jensenii, L. crispatus & L. reuteri, respectively.