Image Analysis on Bacteria Time-Lapse Movies

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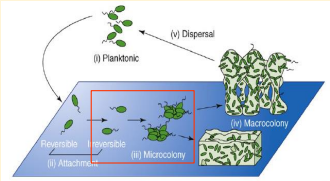
Motivation-Problem Statement

Biofilm formation is a multidisciplinary phenomenon that can be studied from many different perspectives according to:

- The state of development
- The type of biological approach (transcriptomics, proteomics, metabolomics, etc.).



Microscopic analysis, predominantly of Gram-negative proteobacteria, led to a general description of biofilm formation as a temporal process involving transition through distinct stages of multicellular organization. These stages have been identified in [1] as:

i. planktonic
ii. attachment
iii. micro-colony
iv. macro-colony
v. dispersal

Efficient Bacterial Counting! Why is it necessary?

By automating tracking of micro-colonies and single-cells we can quantify their attributes, so as to investigate their response to micro-environment perturbations.

By analyzing time lapse cell "movies", we can quantify:

- ✓ Colony attributes (e.g. area, growth curves)
- ✓ Cell attributes (e.g. area, length, width, orientation, fluorescence, distance from the colony centroid)
- ✓ Division time
- ✓ Rate of Change (RoC) of an attribute (e.g. elongation rate).

What has been done so far...

Solutions already proposed:

The most significant packages are TLM-Tracker [2], CellTracer [3] and MicrobeTracker [4].

But they suffer from several limitations...

Table 1. Software packages overview

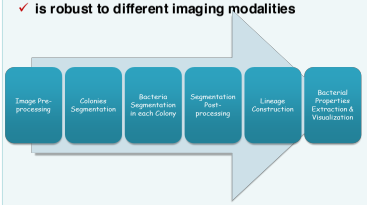
Software	Tracking Multiple colonies	Robustness to dataset quality	Parameterization	Optical/ Confocal images	Phase contrast/ fluorescent images
CellTracer	no	no	complex	optical	both
MicrobeTracker	no	no	complex	both	both
TLM Tracker	no	no	complex	optical	both
Developed Image Analysis Pipeline	yes	yes	simple	both	both

Lack of generality: they are not able to process images acquired by several imaging settings (microscope type, imaging modality etc.)

Our proposal: Six discrete Phases

The proposed pipeline...

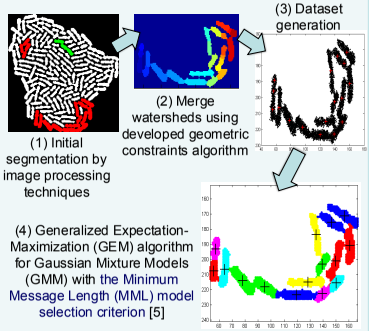
- ✓ offers high throughput analysis
- ✓ needs simple parameterization
- ✓ tracks multiple colonies in time-lapse movie
- ✓ constructs the lineage of initial single-cells
- ✓ provides visualization and quantification of cell attributes
- ✓ is robust to different imaging modalities



Overview of the proposed method. The main steps are bacterial segmentation in each colony and lineage construction.

Methods

Bacterial segmentation in each colony



(1) Initial segmentation by image processing techniques

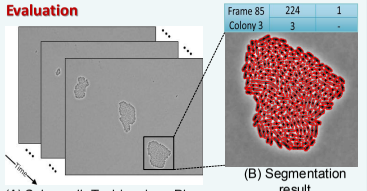
(2) Merge watersheds using developed geometric constraints algorithm

(3) Dataset generation

(4) Generalized Expectation-Maximization (GEM) algorithm for Gaussian Mixture Models (GMM) with the Minimum Message Length (MML) model selection criterion [5]

Results

Evaluation

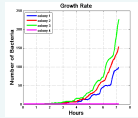


(A) *Salmonella* Typhimurium, Phase Contrast Dataset [6]

(B) Segmentation result

Dataset A	Proposed
Recall	99.43%
Precision	96.84%
F-measure	98.12%
TPs	6856
FPs	224
FNs	39

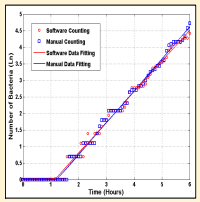
(C) Evaluation Overview



(D) Growth curve of each colony

Results contd.

✓ Cross Validation



Cross Validation with the primary model of *Baranyi and Roberts* [7]

Table 2. Parameters induced by manual counting

Col.	μ_{max} (h^{-1})	Lag (h)	R^2
1	0.808	1.373	0.991
2	1.038	1.846	0.995
3	0.965	1.225	0.994

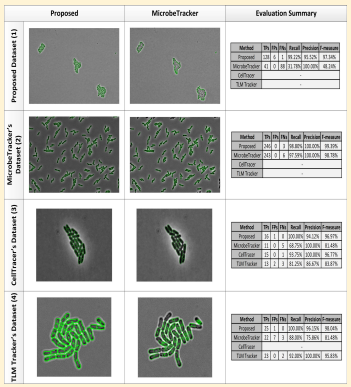
Table 3. Parameters induced by software counting

Col.	μ_{max} (h^{-1})	Lag (h)	R^2
1	0.813	1.377	0.992
2	1.031	1.865	0.995
3	0.928	1.143	0.994

μ_{max}: Maximum growth rate, Lag: Lag phase

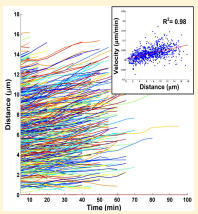
Models induced by manual and software counting are almost identical.

✓ Comparison Evaluation

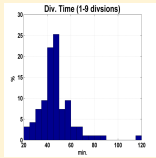


Column 1 and 2. Segmentation/cell detection results of the developed and MicrobeTracker. No segmentation results are provided for CellTracer and TLM Tracker since they failed to analyze all time-lapse movies. Column 3. Evaluation summary of each method. Our method exhibits F-measure ≥96% for all cases.

Visualization and Quantification of Cell Attributes



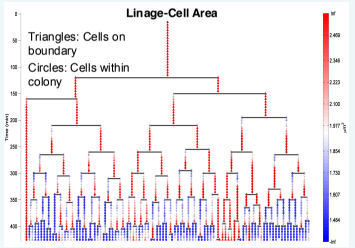
(A) Cell's distance from the colony's centroid as function of time (time-series). Max. distance from colony centroid and cell velocity are correlated (scatterplot).



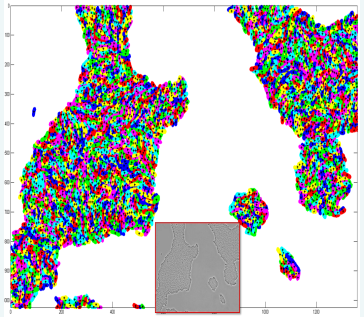
(B) Distribution of division times of 9 generations

Results contd.

✓ Tracking Cells



✓ Going on very large scale (3236 bacteria)



Conclusions

We present a fully automated tool, which enables high throughput analysis of time lapse movies, and we proved its robustness and universality by thorough evaluation. Our method:

- **introduces universality to microscopy and imaging modalities**
- **preserves bacterial shape accurately**
- **extracts and visualizes single cell attributes**

References

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Acknowledgments

This work was supported by the action THALIS: "Biological Investigation Of the Forces that Influence the Life of pathogens having as Mission to Survive in various Lifestyles; BIOFILMS", which has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF)-Research Funding Program: THALES. Investing in knowledge society through the ESF.

ABSTRACT

Time-lapse microscopy now enables detailed imaging data generation and monitoring of dynamic cellular processes at the single cell level. Recent studies have highlighted the usage and importance of this technology for investigating biological noise in gene regulation, cell growth and proliferation etc. Mathematical and statistical model development is of growing interest in capturing and testing hypothesis regarding the dynamical behavior of biological systems. Modeling bacterial communities forming biofilms relies on the efficient and accurate extraction of information from time-lapse microscopy data (image frame sequences) of growing bacterial colonies. However, the analysis of such "cell movies" data is currently very time consuming and error prone since it is essentially performed by human-experts. In this thesis we address this important limitation in a multi-resolution image analysis framework.

We have developed a methodology for identifying accurately the boundaries of individual bacterial cells and tracking them from frame to frame so as to construct the cells' genealogy (bacterial cell segmentation and lineage tree construction) even in large-size microbial communities where there is great difficulty in identifying the individual cell boundaries. The automated and novel pipeline of algorithms we have developed combines methods from image processing and machine learning to segment and track bacteria precisely.

The pipeline has been tested and evaluated with two different cell movies datasets and several images produced by different labs. The developed methodology has been shown to achieve high F-measure score (above 95%) in each evaluation case. It can be applied to different image modalities, such as phase contrast, bright field, and fluorescent, produced by optical and confocal microscopy. Using extensive experimentation we demonstrate the robustness and reliability of the proposed pipeline regardless of the image modality used. Our image processing pipeline is fully automated, computationally efficient and suitable for high throughput analysis of bacterial cell movies without any human intervention on its calibration.

SUBJECT AREA: Image Analysis and Machine Learning

KEYWORDS: bacterial segmentation, cell counting, cell lineage construction, cell feature extraction and visualization, expectation-maximization.