

AI-Driven Analysis of Bacterial Swarming Physics for Early Biofilm Detection: A Review

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Abstract-- Bacterial swarming is a type of bacterial surface-associated motility known in such bacteria as *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Bacillus subtilis*. It consists in modification of bacterial cellular physiology, flagellation, surface sensing, secretion of extracellular products, and collective migration. Bacterial swarming is important for bacterial adaptation, surface colonization, and interaction with biofilm-associated life styles. Traditional evaluation of swarming is based on traditional observation and measuring of colony or swarm size, which does not cover the whole complexity of bacterial movement.

Artificial Intelligence and computer vision technologies offer new ways for transformation of bacterial swarm images into the quantitative traits like surface coverage, colony morphology, branching patterns, texture, expansion rate, and movement dynamics. This review discusses the biology of bacterial swarming, traditional techniques for swarm evaluation, and the possibility of the use of artificial intelligence and computer vision technologies for quantitative analysis of swarming. Special attention is paid to the possibility of the correlation between evaluated swarming traits.

Keywords-- Bacterial swarming; Artificial intelligence; Computer vision; Swarming dynamics; Biofilm; Image analysis.

I. INTRODUCTION

Bacteria can adopt various types of movement that permit them to react to environmental cues and attach to various surfaces. Swarming is a unique mode of organized movement on a semisolid surface in which a group of bacteria moves as one across it. This type of motility is distinct from swimming, a movement adopted by bacteria individually while suspended in liquid media. Swarming has been widely researched in various species, such as *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [1–3]. Changes in cell shape, flagellar motion, secretion of extracellular polymeric substances, surfactants, intercellular communication, and surface sensing are among the modifications that may accompany the evolution of the swarming behavior phenotype.

For instance, in *P. aeruginosa*, rhamnolipid-mediated decrease in surface tension is one of the factors underlying coordinated surface motility, while quorum sensing systems are involved in the control of multiple physiological functions that characterize collective behavior [2,4].

Swarming phenomenon is particularly intriguing due to the linkages as well as distinct nature of two biological processes – motility and surface colonization/biofilm formation. Biofilms are organized microbial communities that live in the extracellular matrix and adhere to either a biotic or an abiotic substrate [5]. Motility can influence surface colonization, although the relation between motility and biofilm formation varies among various bacteria. Therefore, swarming behavior cannot be considered equal to biofilm formation. The conventional way of assessing the bacterial swarming process is through determining the diameter/area of bacterial colonies during certain time periods. These measurements are straightforward yet give little insight into the morphological details of swarm growth.

There have been recent developments in areas of artificial intelligence (AI), machine learning and computer vision that provide new opportunities for analysis of biological images. Artificial intelligence-enabled image analysis can be used to detect bacteria, segment colonies, measure morphological traits and analyze temporal changes. Such techniques are gaining popularity in the field of microbiology for colony detection, susceptibility testing, microscopy and biofilms quantitation [6-8].

Indeed, the use of AI for studying bacterial swarming is thus one of the emerging fields at the intersection of microbiology, imaging, and computational biology. Instead of using AI for replacing the microbiology experiments, it can also help in extracting quantitative information from images.

Swarming is a group behavior that is dependent on biological as well as environmental parameters. Surface characteristics, nutrients, agar levels, humidity, temperature and bacterial densities may affect the formation of swarmers.

For many bacteria, surface contact causes a change in physiology that allows for collective motility. For *P. mirabilis*, swarming is related to differentiation into long and flagellated swarm cells [1]. For *B. subtilis*, coordinated movement involves the use of flagella, surfactin production, and other extracellular factors [3].

Rhamnolipids in *P. aeruginosa* have been shown to influence surface motility through alteration of surface properties and allowing mobility of bacteria [2]. Many genes involved in bacteria behavior are also regulated by quorum-sensing systems but involvement of quorum sensing in swarming depends on species and environment [4].

Bacterial swarming therefore can be considered as the result of the interaction of several elements:

Surface sensing → physiologic adaptation → motility → group mobility → spatial swarm formation

This process should not be interpreted as a single universal pathway because different bacterial species employ different regulatory mechanisms.

A. Relationship between Swarming and Biofilm Formation

The formation of a biofilm is a multistep process which includes surface attachment, growth, matrix production, maturation and, often, dispersion [5,9,13]. Motility might play a role in the surface exploration and colonization stage, but biofilm formation implies a number of physiological and structural transformations.

For example, the work performed on *P. aeruginosa* revealed sophisticated interplay between motility, quorum sensing, secretion of extracellular products and biofilm formation [4,9]. Nevertheless, an increase in swarming ability does not necessarily imply formation of a more intense biofilm. There are a lot of factors which determine the relationship.

Thus, swarming morphology may be regarded as a promising phenotypic characteristic which reflects bacterial surface behavior, rather than a replacement for biofilm assay.

It is an important distinction in constructing AI predictive models. An AI model trained only on swarming pictures should not be referred to as a biofilm predictor unless its predictions have been verified against independent biofilm assays such as measurement of biofilm biomass, viable cell count, extracellular matrix content or existing microplate-based biofilm assays.

B. Standard Analysis of Bacterial Swarming

A simple way to quantify swarming is through measurement of the diameter of the swarm at chosen intervals. This test is cheap and useful for the early stages of research.

But swarming bacteria are not always in the form of a circle. Depending on the strain and experiment parameters, their shape may be dendritic or concentric. Therefore, diameter is not a sufficient parameter to describe a phenotype.

Other parameters may include:

- Swarm area
- Surface coverage
- Perimeter
- Circularity of the colony
- Growth speed
- Irregularity of the edge
- Branching pattern
- Texture
- Radial growth
- Time-dependent morphological changes

These parameters will give a more complete description of swarm formation.

II. APPLICATION OF ARTIFICIAL INTELLIGENCE IN SWARMING ANALYSIS

AI-based image analysis could further the capabilities of traditional methods through analyzing many images.

A generalized framework of AI would be:

Image acquisition → Image pre-processing →
 Segmentation → Feature extraction →
 Classification/quantification → Biological interpretation

A. Image Acquisition

The images could be captured using a phone's camera, digital camera, stereoscope or laboratory imaging device. In the case of quantitative studies, proper lighting, camera distance, plate placement and imaging time must be kept constant.

The use of a cheap smartphone imaging device could be helpful in the initial phases of research.



B. Image Segmentation

Segmentation separates the bacterial swarm from the surrounding agar or background.

Traditional thresholding can be useful for simple images. Machine-learning approaches can provide more flexible segmentation when image background, illumination or swarm morphology varies.

U-Net is an established deep-learning architecture widely used for biomedical image segmentation [10,11,12]. Its encoder-decoder architecture allows extraction of image features followed by reconstruction of a pixel-level segmentation map.

For bacterial swarming, a segmentation model could theoretically generate a mask corresponding to the swarm region.

C. Feature Extraction

Once the swarm region has been segmented, quantitative characteristics can be calculated.

Morphological features

- Area
- Perimeter
- Circularity
- Aspect ratio
- Edge complexity
- Branching characteristics

Spatial features

- Radial expansion
- Surface coverage
- Density distribution

Temporal features

- Change in area over time
- Expansion rate
- Movement of the swarm front

These measurements can provide a more detailed description than visual inspection alone.

D. Computer Vision and Deep Learning Techniques

There exist several types of artificial intelligence techniques which could be applied for analyzing the images of bacteria.

E. Convolutional Neural Networks

A convolutional neural network is able to recognize image features directly from image pixels. The CNN could be trained to classify the images into groups like:

- Not swarming
- Beginnings of swarming
- Well-developed swarming

The technique needs to be validated on the labeled images by microbiology experts.

F. U-Net-Based Segmentation

U-Net is particularly suitable when the objective is to identify the exact boundary of a biological structure [10]. In a swarming experiment, the output could be a pixel-level mask representing the swarm.

The segmented image can then be used to calculate area, perimeter and other morphological parameters.

G. Object Detection

Object-detection algorithms can identify specific structures within images. YOLO-type architectures are widely used for real-time object detection. Their application to bacterial swarming would require an appropriately labelled dataset and careful validation.

Because a swarm is usually a continuous spatial structure rather than a collection of clearly separated objects, segmentation may be more appropriate than object detection for some experimental designs.

H. Quantitative Analysis of Swarm Morphology

Assisted image analysis by AI allows studying features which are not easily quantifiable otherwise.

I. Surface Coverage

It is possible to calculate the share of the plate covered with the swarm as follows:

Surface coverage (%) = Swarm area / Available plate area $\times 100$

J. Swarm Growth

Images captured at various points in time allow estimating the rate of change of the swarm area or the radius as:

Expansion rate = Change in swarm size / Time interval

The exact measurement units are dependent on the specific experimental setup.

K. Edge Structure

Boundary of the swarm can be analysed based on perimetric or shape characteristics. More advanced methods will involve studies of branching structure and fractal characteristics.



These measurements need to be taken carefully as morphology depends on the concentration of agar, nutrients, humidity and other factors of the experiment.

L. Image Texture

Image texture analysis might potentially reveal the differences in spatial distribution and texture structure. Such measurements as gray-level co-occurrence matrix have been used in image analysis.

However, roughness of the swarm's boundaries does not necessarily mean that it is more virulent. It should be biologically validated.

M. Potential Applications in Pharmaceutical and Microbiological Research

AI-assisted swarming analysis may have several research applications.

III. ANTI-SWARMING SCREENING

Compounds can be tested for their effects on bacterial surface motility. AI-assisted image analysis may allow quantitative comparison of treated and untreated cultures.

A. Anti-Biofilm Research

Because motility and biofilm-associated behaviours can interact, swarm analysis may be used as one component of anti-biofilm research.

Importantly, inhibition of swarming should not by itself be interpreted as proof of biofilm inhibition. A separate biofilm assay is required.

B. Comparative Analysis of Clinical or Environmental Isolates

Different bacterial isolates can be compared using standardized imaging and quantitative morphological measurements.

C. Natural Product Screening

Plant-derived compounds and other chemical candidates can be evaluated for effects on bacterial motility. AI may help process large numbers of images and identify differences in swarm morphology.

D. Educational and Undergraduate Research

Low-cost imaging combined with open-source image-analysis software may provide students with an accessible introduction to quantitative microbiology and AI-assisted research.

IV. PROPOSED LOW-COST WORKFLOW

A practical laboratory workflow could be:

Standardized swarm plate → Smartphone imaging → Image preprocessing → AI/image segmentation → Area and morphology measurement → Time-series analysis → Comparison with experimental controls

For a research-grade AI model, a dataset should contain sufficient variation in bacterial strains, growth conditions, imaging conditions and swarm phenotypes.

The dataset should ideally be divided into:

Training set → Validation set → Independent test set

This prevents the model from being evaluated only on images that are very similar to those used during training.

V. LIMITATIONS AND CHALLENGES

AI-assisted swarming analysis has several limitations.

First, bacterial swarm morphology is strongly affected by experimental conditions. Differences in agar concentration, nutrient composition, incubation temperature, humidity and imaging conditions can influence the observed phenotype.

Second, an AI model trained on a limited dataset may not generalize to other bacterial species or laboratory conditions.

Third, image-based measurements do not directly establish molecular mechanisms. A change in swarm morphology may result from altered growth, motility, surfactant production or other physiological effects.

Fourth, AI predictions require biological validation. High classification accuracy on a dataset does not necessarily mean that the model has biological predictive value.

Finally, standardized datasets and reproducible imaging protocols are necessary before AI-based swarm analysis can be widely adopted.

VI. FUTURE PERSPECTIVES

Future studies may integrate time-lapse imaging with machine-learning algorithms to investigate how swarm morphology changes over time. Combining image-derived features with independent measurements of biofilm biomass, motility, gene expression and extracellular matrix production could allow the development of biologically validated predictive models.

Future systems could combine:

Swarm velocity + surface coverage + morphology + texture
 + bacterial growth parameters → experimentally validated
 biofilm outcome

This integrated approach could potentially support screening of anti-motility and anti-biofilm compounds.

Smartphone imaging may further reduce the cost of image acquisition and make quantitative microbiology more accessible. However, smartphone-based approaches require calibration and standardized lighting before quantitative measurements can be compared reliably between experiments.

VII. CONCLUSION

Bacterial swarming is a complex collective behaviour influenced by motility, surface sensing, extracellular products, cell signalling and environmental conditions. Conventional swarm analysis based primarily on diameter provides useful information but does not capture the full spatial and temporal complexity of swarm development.

Artificial intelligence and computer vision provide promising approaches for converting bacterial swarm images into quantitative data. Segmentation, morphological analysis and time-series image analysis may provide measurements of swarm area, surface coverage, boundary characteristics and expansion dynamics.

At present, AI-assisted swarming analysis should be considered a research and analytical tool rather than a validated standalone predictor of biofilm formation or bacterial virulence. Future studies combining standardized imaging, machine learning and independent microbiological validation may establish whether swarm-derived features can reliably contribute to prediction of biofilm-associated phenotypes.

The combination of low-cost imaging, quantitative image analysis and microbiological experimentation represents a promising direction for pharmaceutical microbiology, anti-virulence research and undergraduate research.

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