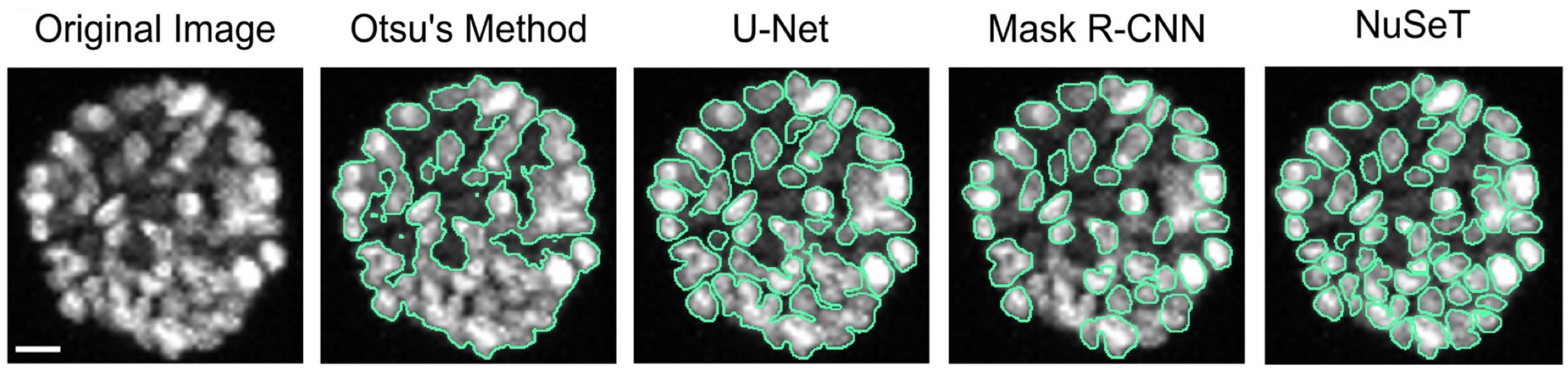
Unveiling Sporulation: Image Segmentation and Tracking for Bacterial Cell Analysis

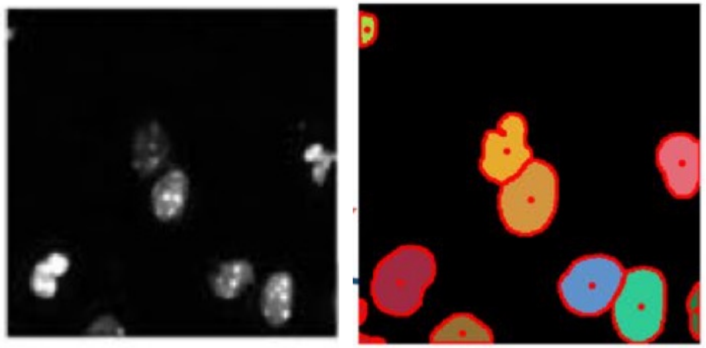
Mahsa Ghasemi, October 2024

Deep Learning

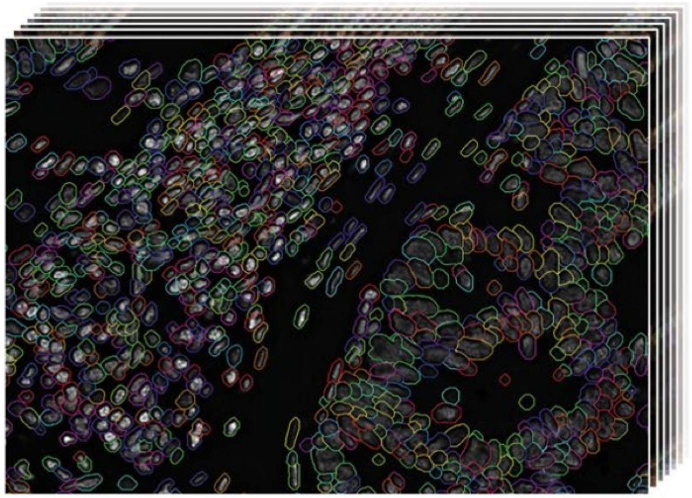
U-Net and Region Proposal Networks, 2020



GeneSegNet, 2023



CellSeg (Mask R-CNN), 2022

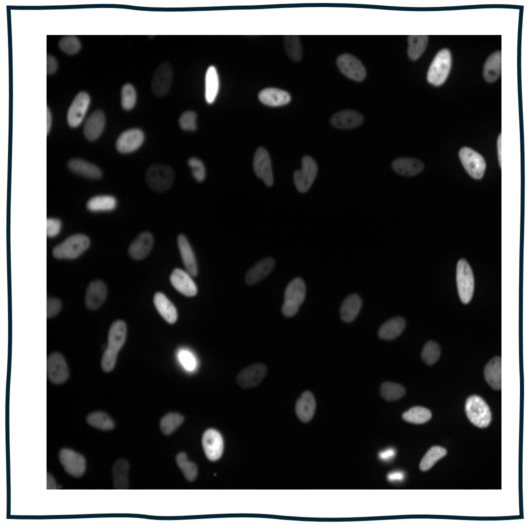


Cell segmentation and tracking

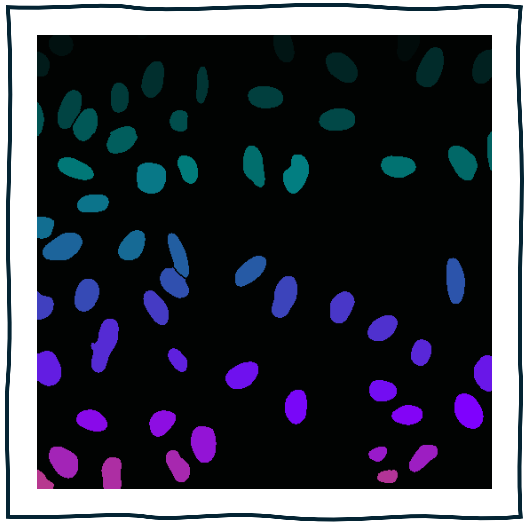
Deep Learning Library: A Python-based library for single-cell analysis using TensorFlow

- Cell segmentation in 2D and 3D
- Cell tracking.

Raw Image



Tracked Image



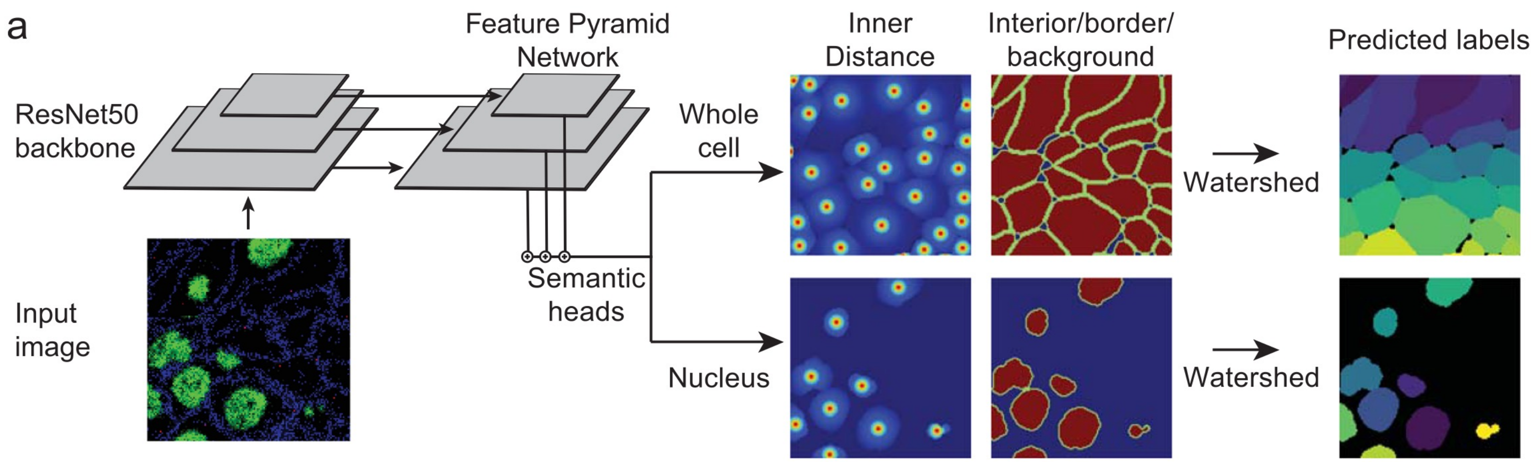
* Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning, Noah F. Greenwald et al, 2022

* *Deep Learning Automates the Quantitative Analysis of Individual Cells in Live-Cell Imaging Experiments*, *David A. Van Valen et al, 2016*

https://github.com/vanvalenlab/deepcell-tf?tab=readme-ov-file

Mesmer algorithm

- A deep-learning-based method
- Whole-cell and nuclear segmentation
- ResNet50 backbone and Feature Pyramid Network

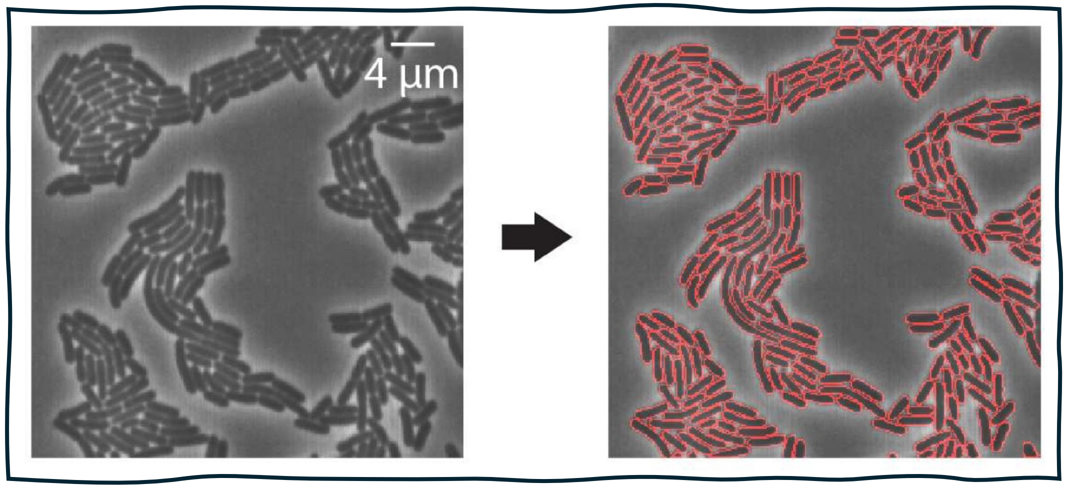


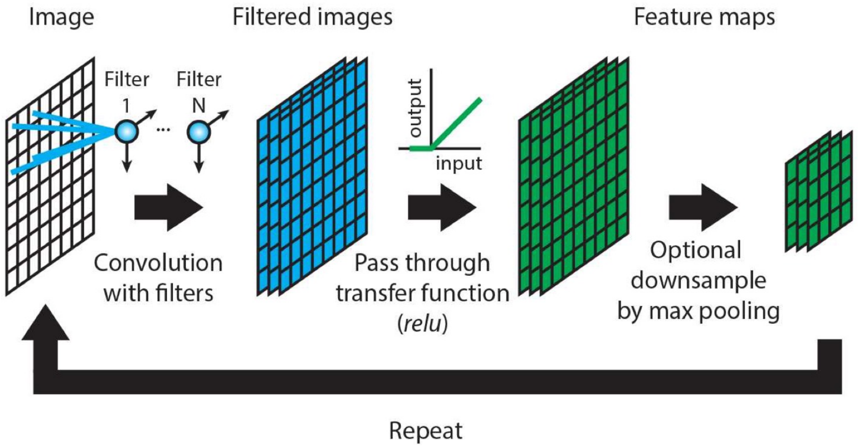
* Whole-cell segmentation of tissue images with human-level performance using large-scale data annotation and deep learning, Noah F. Greenwald et al, 2022

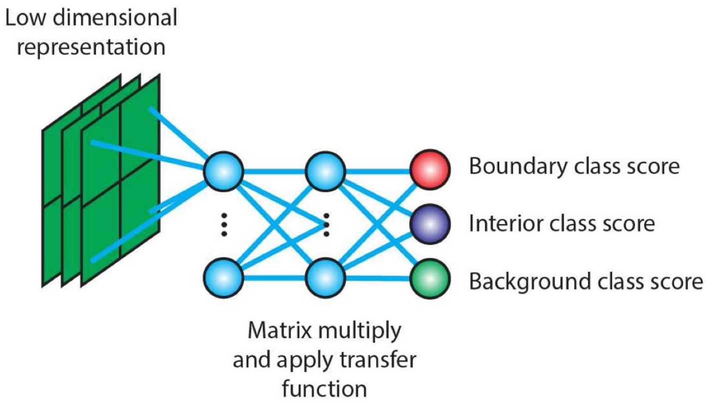
Conv-nets

- Deep convolutional neural networks
- Handle both fluorescent and phase microscopy images
- Dimensionality Reduction

Result







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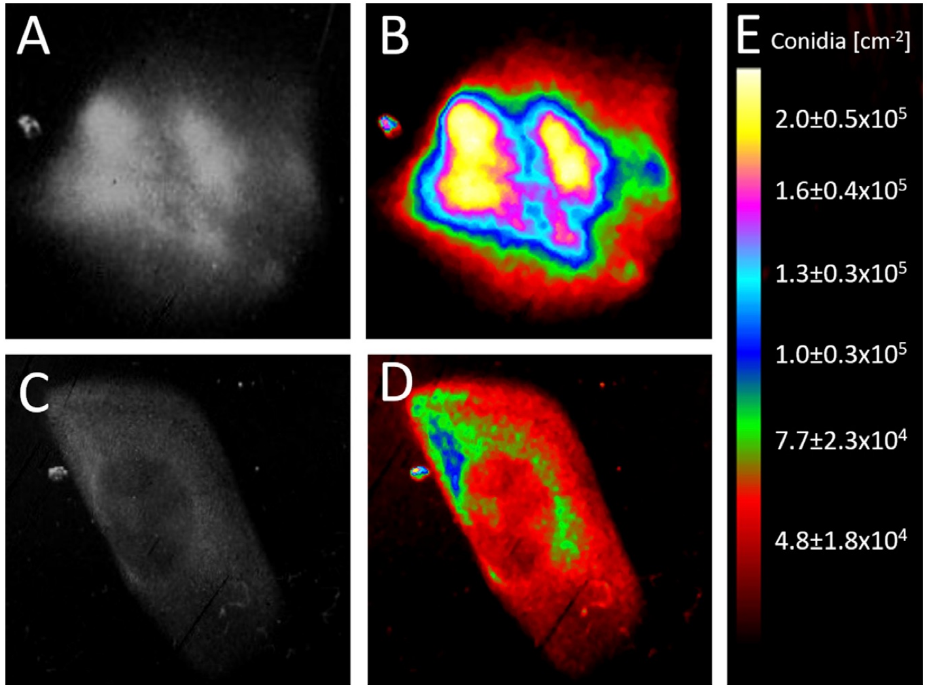
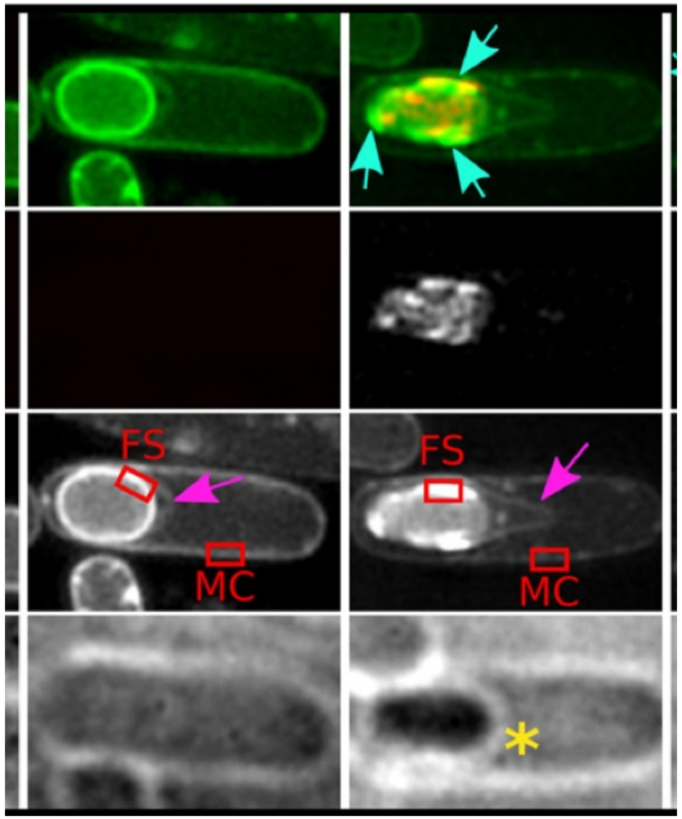
Sporulation Zones

Gray value image and calculated conidia densities of sporulation zones, 2021



Conidia counting: Estimation of spore density on a surface, indicated by color gradients in the image
Gray value correlation: The relationship between pixel intensity (gray levels) and conidia density

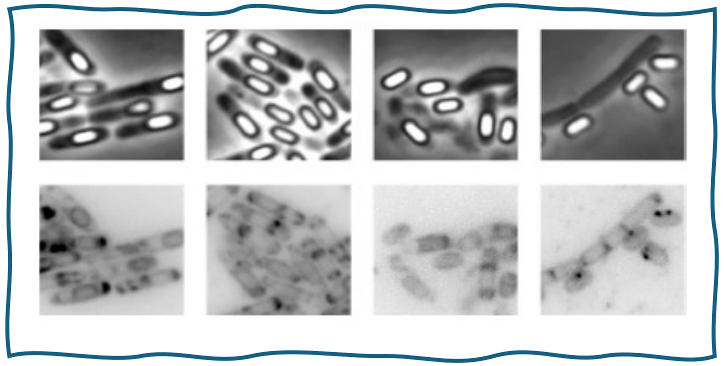
Visualization of coat formation during sporulation in *Bacillus cereus, 2023*



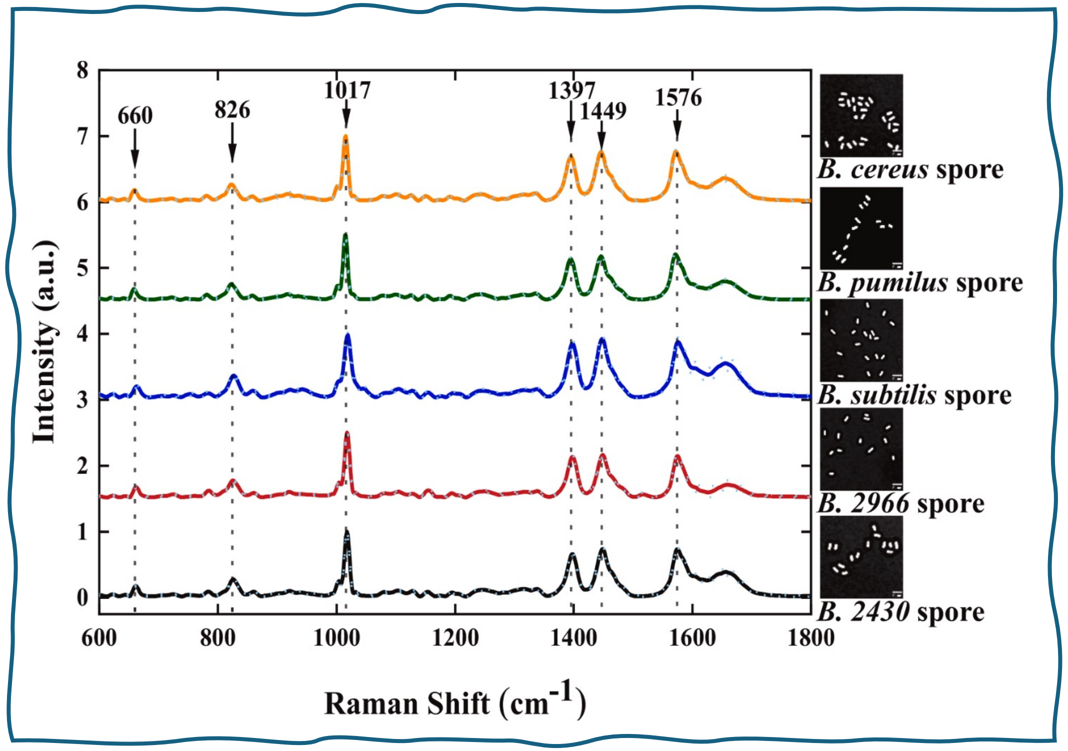
A fluorescence-based method for live visualization of coat formation during *Bacillus cereusn* sporulation, using dyes that bind to spore surface proteins.

Other papers

- *High Resolution Analysis of Proteome Dynamics during Bacillus subtilis Sporulation, Zhiwei Tu et al, 2021*

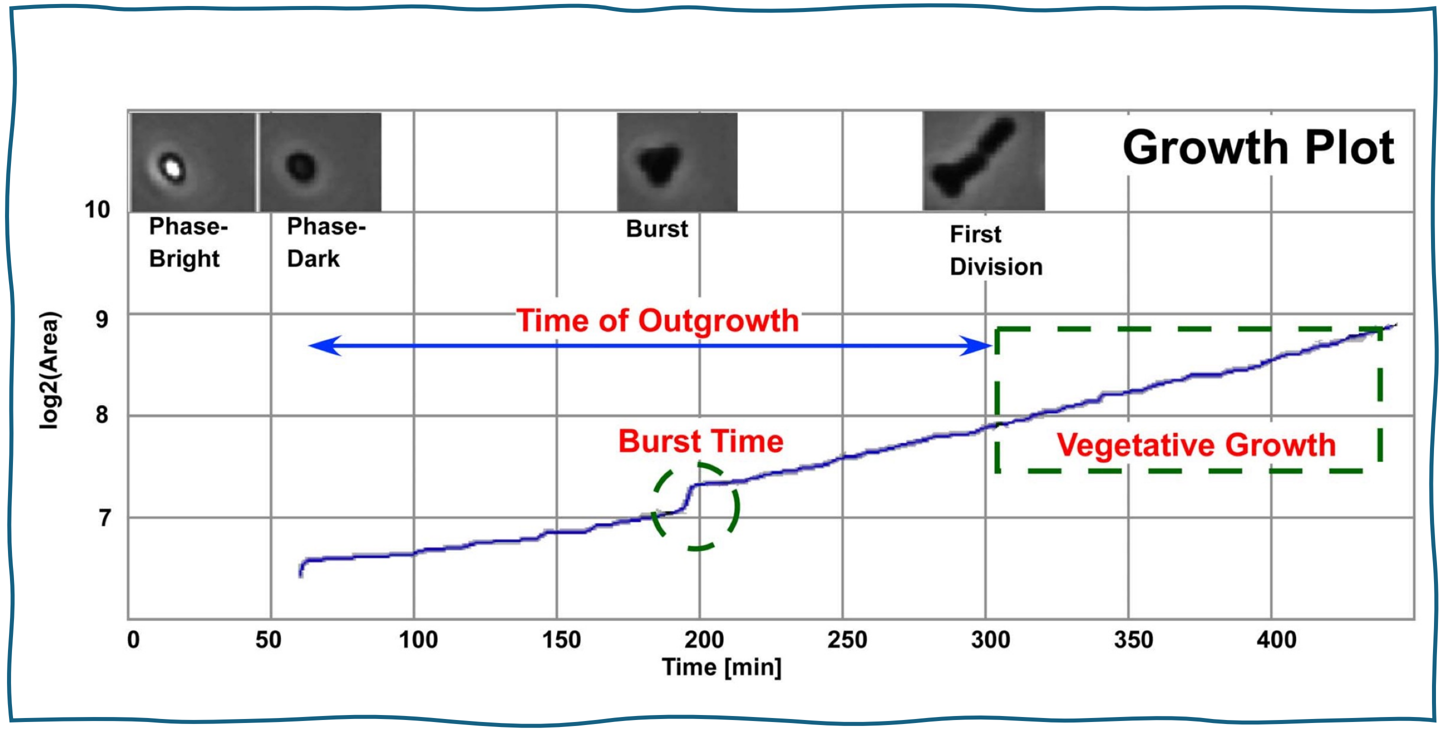


- *Accurate identification of living Bacillus spores using laser tweezers Raman spectroscopy and deep learning, 2022*



Live Cell Imaging and Outgrowth of Bacillus spores

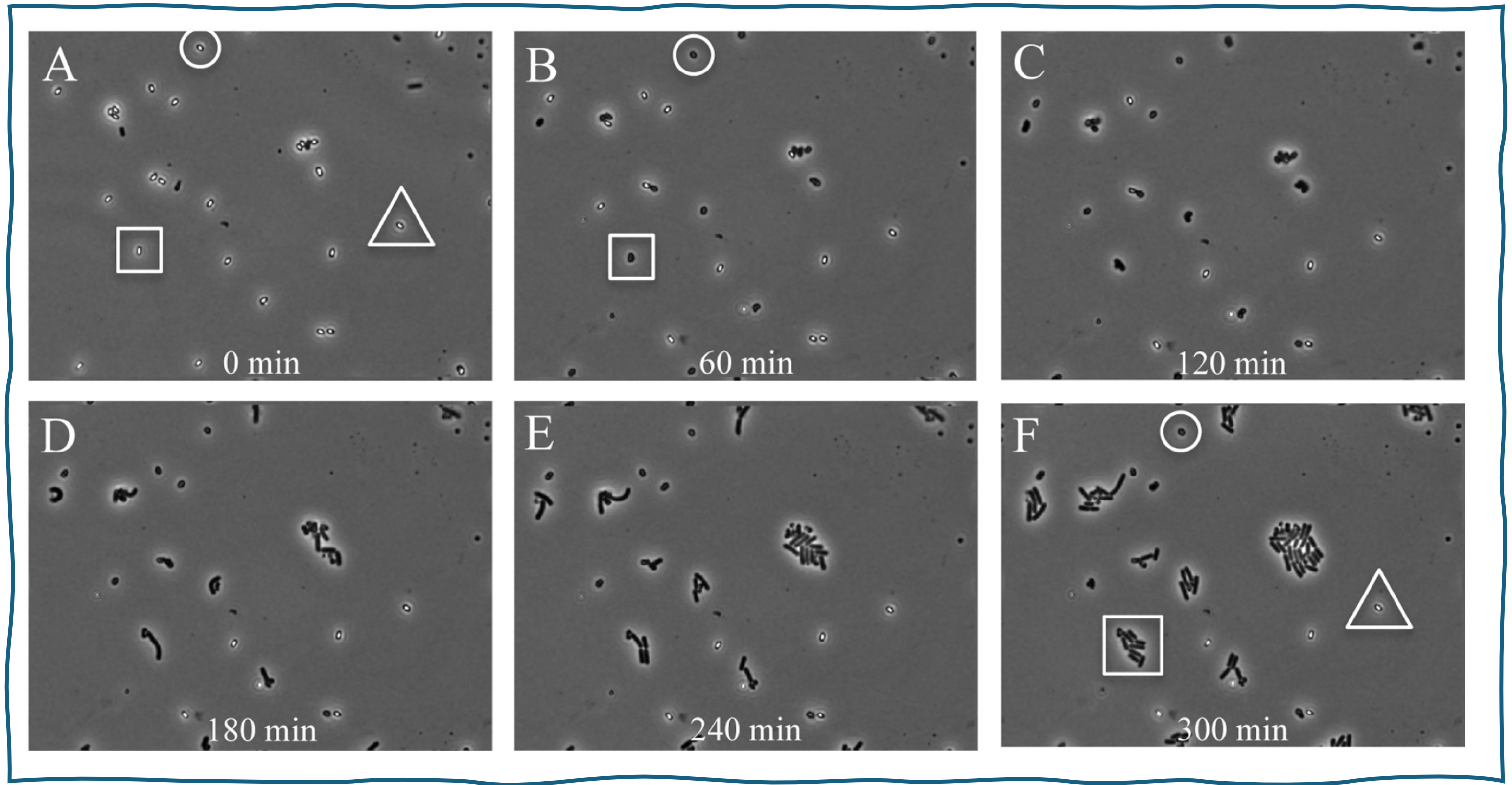
- Creation of a tool called **SporeTracker** for automated analysis of spore germination and outgrowth.
- Development of a **closed air-containing chamber** for live microscopy of aerobic bacterial spores.



Live Cell Imaging and Outgrowth of Bacillus spores

- Heat stress (85°C for 10 min) delayed spore germination and decreased the proportion of spores that could grow

- SporeTracker allowed detailed tracking of phases from spore germination to vegetative growth, showing heterogeneity in the population.



Time-resolved images showing heterogeneous germination and outgrowth of B. subtilis 1A700 spores on minimal medium

** Live Cell Imaging of Germination and Outgrowth of Individual Bacillus subtilis Spores; the Effect of Heat Stress Quantitatively Analyzed with SporeTracker, Rachna Pandey et al, 2013*

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Original article

Hyperspectral imaging and deep learning for detection and quantification of germination in *Bacillus cereus* spores

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Summary Germination of *Bacillus cereus* spores followed by growth and replication of the vegetative cells in food can result in food poisoning and therefore significant economic and health impacts. This study explores a novel approach to detect and differentiate spores and germinated *B. cereus* cells using hyperspectral imaging (HSI) in combination with machine learning using three different germination triggers. HSI could successfully differentiate between dormant spores, germinated cells and structural controls (non-spores). The spectral data in the visible-near-infrared range are sensitive to unique structural and chemical characteristics specific to spores, setting them apart from their vegetative counterparts and non-biological controls. This non-destructive and robust approach shows significant potential for detection and assessment of the physiological state (dormant or germinated). Therefore, HSI is a potential method for the detection of germination in *B. cereus* spores and merits further research and validation.

Keywords *Bacillus*, DL/ML, food, imaging, pathogens, spectroscopy, spores.

Thank you