



A pharmacokinetic-pharmacodynamic model characterizing antibiotic-induced morphological changes in *Pseudomonas aeruginosa* from time-lapse microscopy images

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Background

- Pseudomonas aeruginosa* (PA) are Gram-negative, rod-shaped bacilli that may cause severe lung, intra-abdominal, and bloodstream infections as well as urinary tract infections.
- Bacteria are known to change morphology when exposed to antibiotics, and such changes may be early predictors of cell death and an excessive endotoxin release.
- Time-lapse microscopy enables real-time imaging of live cells, and may provide longitudinal, morphological insights into the bacteria-drug interactions.

Aims:

- Develop an image analysis method to process time-lapse microscopy images
- Investigate the effect of various concentrations of meropenem (MEM) and colistin (COL) on morphological changes in *P. aeruginosa* over 24 hours by developing a pharmacokinetic-pharmacodynamic (PKPD) model that includes morphological data

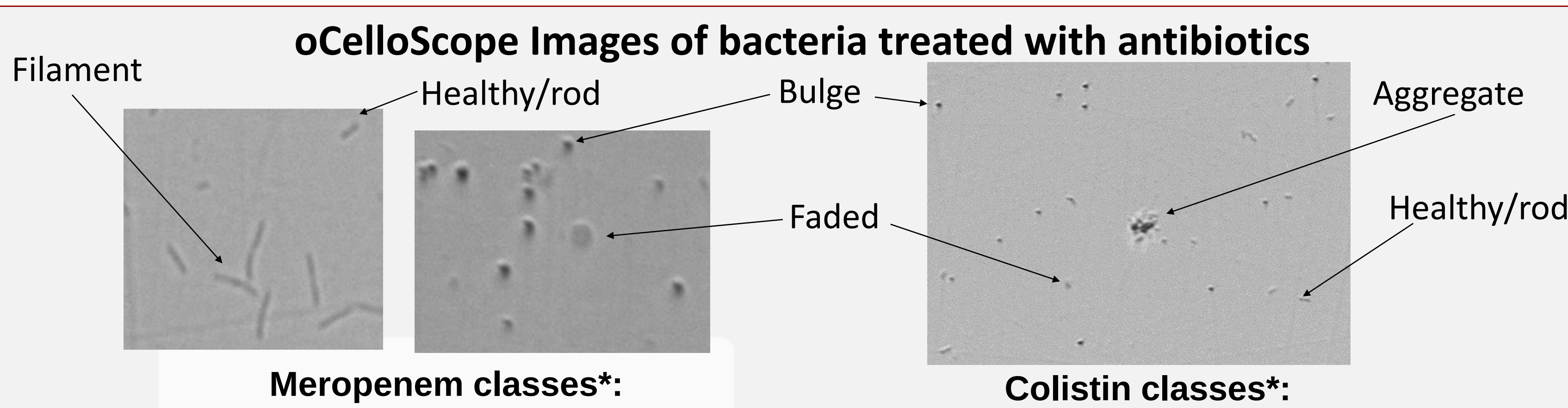
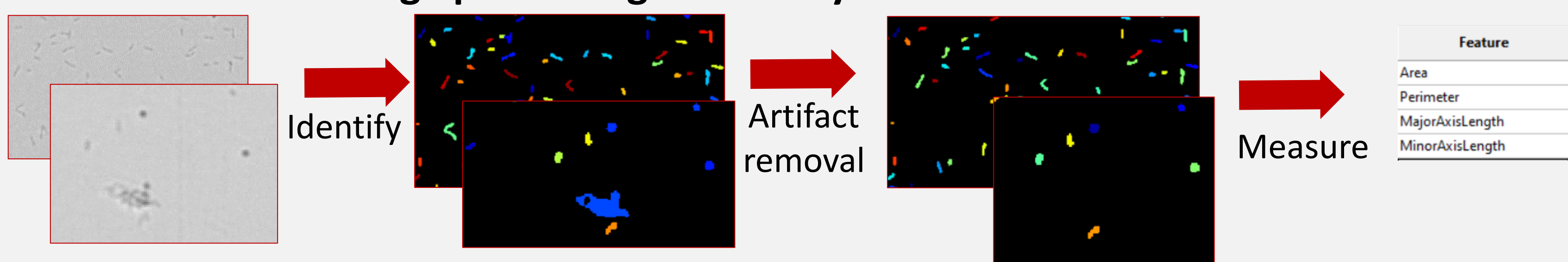
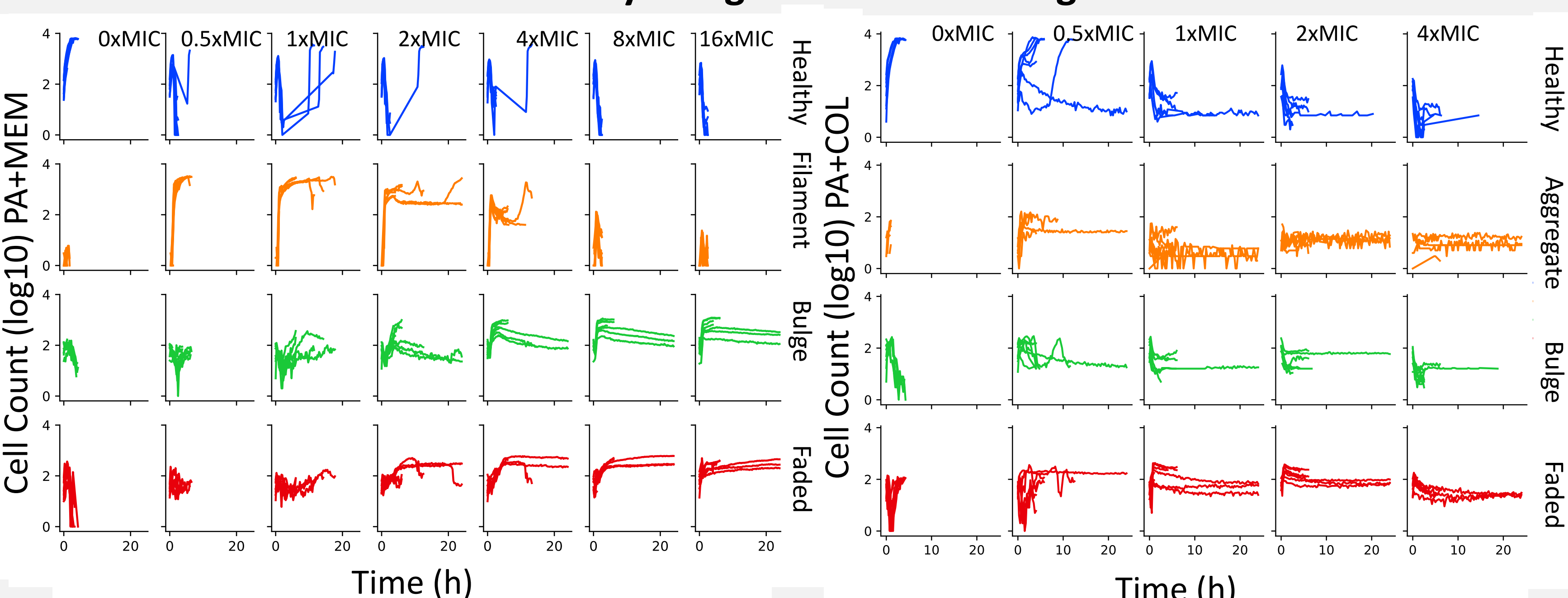


Image processing to identify bacteria in CellProfiler



Classify using Machine Learning



• 78% MEM test accuracy
($\geq 0.73\%$ per class)

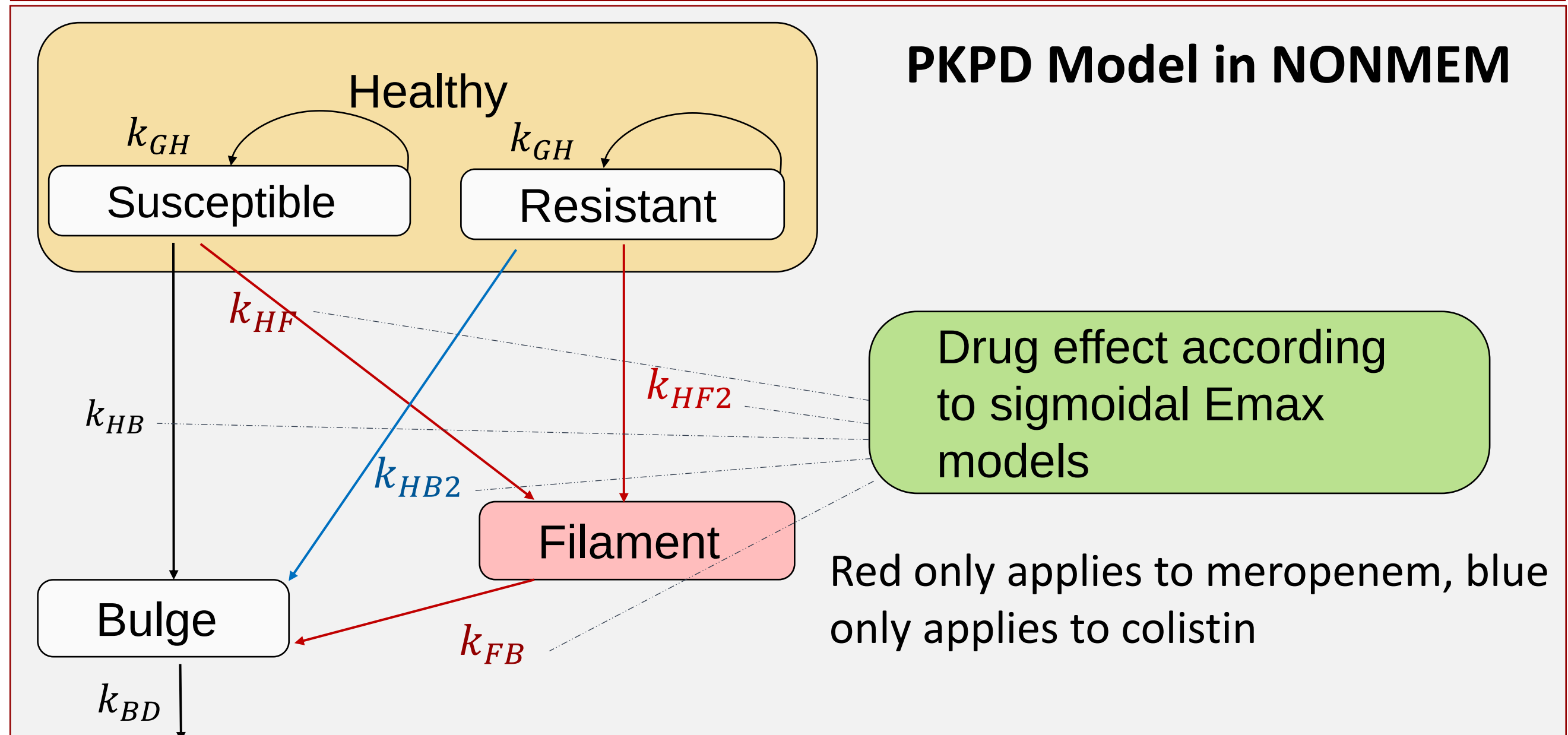
• 77% COL test accuracy
($\geq 0.69\%$ per class)

Machine Learning Count Data Results

- Bacteria reached an upper limit of quantification (ULOQ) of 6300 bacteria/image
- The observed lower limit of quantification (LLOQ) was 1 bacterium/image
- The compiled bacterial morphology data set indicated that at low concentrations ($<0.5xMIC$ MEM, $<1xMIC$ COL), bacteria predominantly remained as healthy bacilli.
- For meropenem, at medium concentrations ($0.5-2xMIC$), healthy cells became filaments, but at higher concentrations ($\geq 4xMIC$) healthy bacilli and any filaments formed bulges.
- For colistin, some bacteria at $1-2xMIC$ formed aggregates, but at $1-4xMIC$ most cells become bulged or faded.

Conclusions

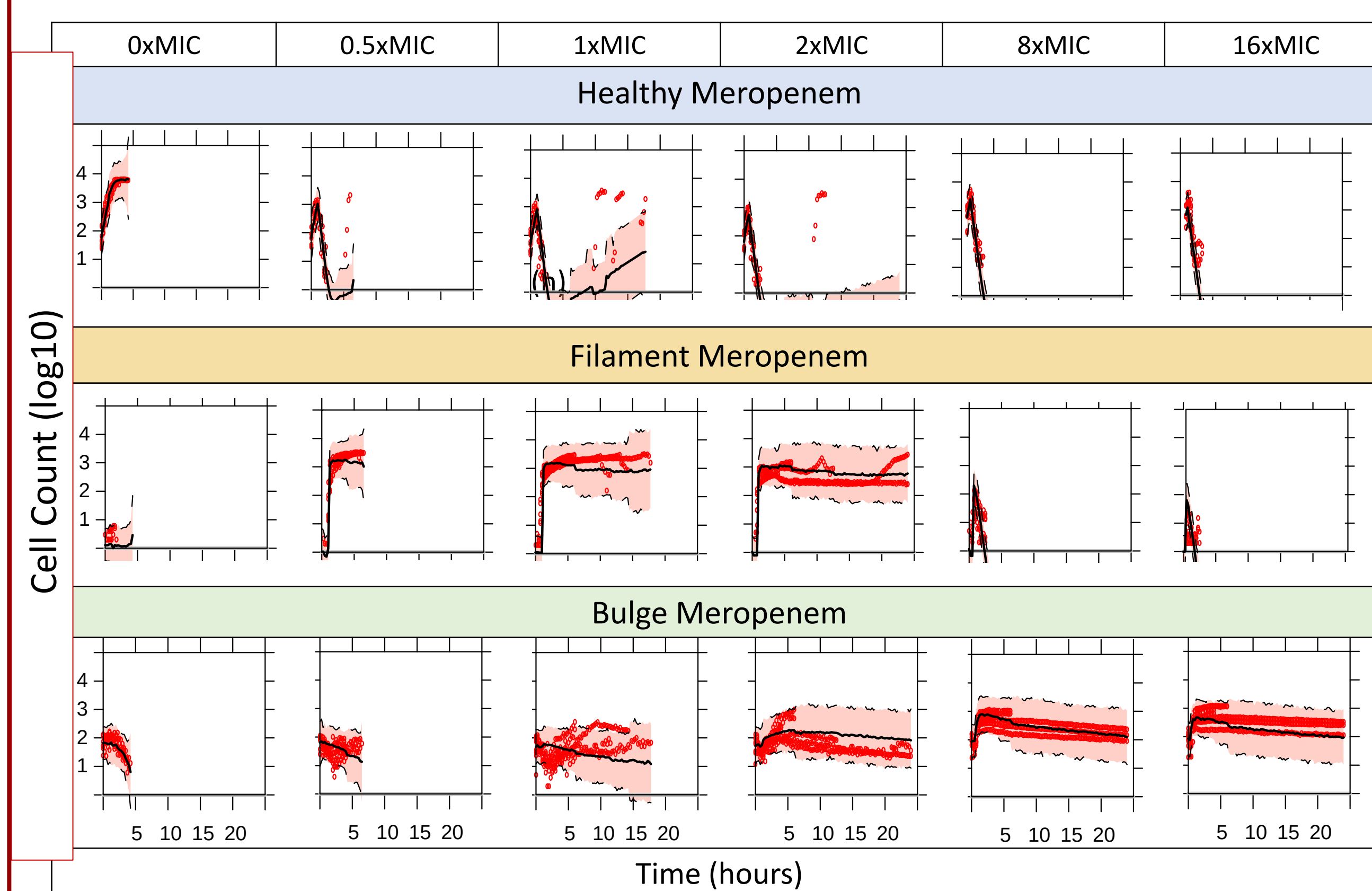
- A CellProfiler pipeline was built and was able to identify, count, and measure bacteria.
- ML models were developed and able to classify bacterial shapes with moderate accuracy (77-78%).
- A PKPD model was used to relate the time course of bacteria morphology to the drug concentration.
- This method may after further refinement help to deepen the understanding of the antibiotic-bacterial interactions.



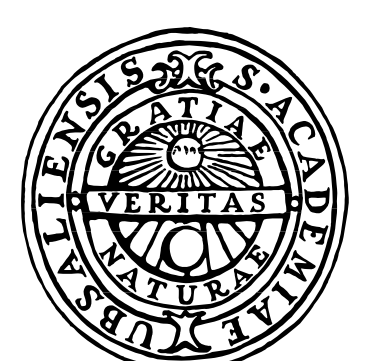
- A PKPD model was developed for healthy bacteria, bulges and filaments (MEM only).
- Faded and aggregate classes were not included in PKPD models.
- A pre-existing resistant subpopulation was used to account for regrowth.
- Drug degradation/adsorption was accounted for according to previous models. (1)
- The maximum number of bacteria (Bmax) was fixed to the ULOQ.
- The M3 method was used to account for LLOQ observations.

Example Visual Predictive Checks

Meropenem VPCs selected to display a range of compartments and concentrations (4xMIC was excluded but resembles nearby xMIC)



Red circles are the observed bacterial counts. Red area (surrounded by black dash lines) corresponds to the 95% CI for the simulated median (shown as solid black lines) from 1000 simulated datasets.



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