PDA Midwest Chapter 18th Annual
Microbial Contamination & Control
13-14 May '26
Northbrook, IL

AI-Enhanced Signal Interpretation in Rapid Microbial Methods (RMM)

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Agenda

RMM Support Systems
AI Augmenting Analysis
The Extent of AI's Power

See also: PDA Technical Report No. 33 (Revised 2026)
Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods, *Technical report in preparation*

RMM Support Systems

RMM component systems

System	*Description*	*Examples*
Technology platform	Instrumentation or analytical technologies generating data	Luminometer, Flow cytometry, PCR, laser fluorescence, impedance splitter
Detection means	The biological or physical signal used to identify microorganisms	ATP bioluminescence, nucleic acid amplification, optical scattering, $CO_2 \uparrow$
Method validation	Demonstration that the RMM performs as compendial methods	Specificity, inclusivity/exclusivity, LOD, accuracy, precision, false results rate
Regulatory acceptance	Compliance with pharmacopeial and regulatory expectations	Guidance from U.S. Pharmacopeia chapters <1223> and <1225>

RMM detection means

Detection means	*Biological basis*	*Assay description*	*Example technology*
Cell growth	Microbial proliferation or microcolony formation	Detect indirectly by measuring metabolic activity or early growth associated with microbe replication	Culture systems measuring CO_2 increase, O_2 decrease, impedance changes; auto-microcolony detection
Cell viability	Viability markers as ATP, enzymes, membrane integrity	Detect living microbes by rating biochemical indicators without prolonged cell growth	ATP bioluminescence, enzyme substrate assays, fluorescent viability staining, flow cytometry
Cell component	Structural or biochemical components of microbes	Detect microbes by measuring cellular structures / molecules (cell walls, proteins, lipids, pigments).	Laser-induced fluorescence, solid-phase cytometry, Raman Spec., MALDI-TOF mass spectrometry
Nucleic acids	Microbial DNA or RNA sequence presence	Detect and sometimes quantify / identify microbes by amplifying or sequencing nucleic acids	PCR, qPCR, RT-PCR, amplification next-generation sequencing (NGS), DNA hybridization assays
Optical imaging	Direct visualization or optical signatures of microbial cells	Detect microbes through optical signatures / digital imaging of cells captured on filters or surfaces	Flow imaging microscopy, light-scattering particle counters, holographic microscopy

AI Augmenting Analysis

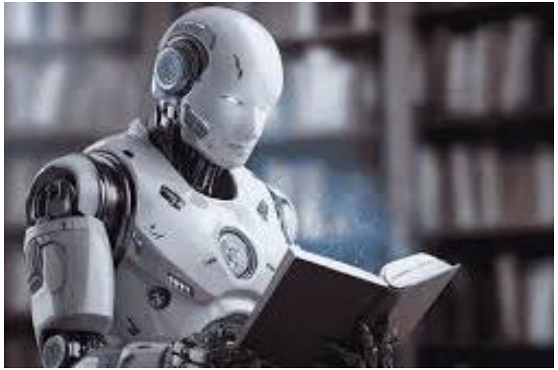
From minor to monumental

Stand-alone, scripted AI applications

- Autocomplete / predictive text
- Face recognition / phone unlocking

Networked, autonomous AI applications

- Self-driving cars / drone swarms
- Stock trading / medical monitoring



AI power beyond classical capabilities

AI does what eg, Nelson rules, cannot

- Employs cross-modal and multivariate representations, not isolated values
  - Models disparate data in latent space; captures nonlinear, multidimensional interactions
- Handles high dimensionality without any pre-specification
  - Uses representation learning to compress thousands of eg, spectra, cytometry channels
- Detects complex, nonlinear patterns and weak signals
  - Unlike PCA/PLS, it can identify subtle signatures of early, slight, stressed contamination
- Integrates context and adapts to non-stationarity
  - Learns a context-dependent variable behavior from environmental and process variables
- Produces probabilistic, decision-ready outputs
  - Delivers calibrated probabilities or uncertainty for contamination class, viability, and load

Stassen, Schmucki, Valero, Manzano, *From Traditional Statistics to Adaptive Multivariate Models…*, PDA J. of Pharm. Sci.and Tech., *In Press*

Modeling nonlinear relationships I

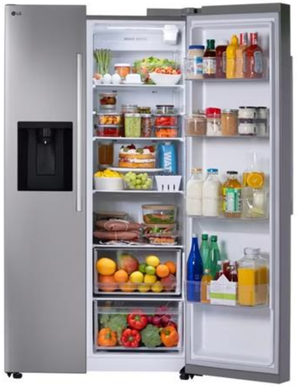
AI model unique capability

Modeling nonlinear relationships w/o prespecifying functional form

Example common application

A household refrigerator

- non-stationary (door openings)
- latent variables (frost thickness)
- coupled relationship (stored items)



Modeling nonlinear relationships II

Example in RMM application

Reaction of marker luminescence is generally equal to ATP concentration

But the measured relative light unit (RLU) to viable cell count is actually

- nonlinear
- non-stationary
- context-dependent
- signal sat. / compression
- state dependent: ATP per cell
- bounded by substrate/ enzyme effect
- non-linear chemistry to detector transduction

What the AI model learns

RLU values not proportional to viable cells

- media components can inhibit photogenic enzymes
- ATP expression levels / kinetics is cell-type specific
- incubation times can alter ATP production/ accumulation kinetic
- at high RLU the count no longer scales proportionally
- at low RLU, signal is dominated by noise/background

Wu, C.-C., et al (2025). Predictive fermentation control of Lactiplantibacillus plantarum using deep learning for real-time batch outcome forecasting [Preprint]. arXiv / PMC repository.

Integrating heterogeneous data

AI model unique capability

Integrates heterogeneous data types within a unified modeling framework

Example in RMM application

AI- based screening can integrate ATP luminescence + flow cytometry + environmental and process data

This unified framework reveals

- sample state understanding:
  eg, low RLU from low cell count *vs* low metabolism
- environmental data: conditions predictions
- time-series shape: growth vs noise

Manzano, T. & Whitford, W., AI-Enabled Digital Twins in Biopharmaceutical Manufacturing, BioProcess International, 21(7–8) July–August, 2023

Characterization of uncertainty

AI model unique capability

Explicitly characterizes of uncertainty and variability (e.g., probabilistic prediction and confidence estimation)

Example in RMM application

Considering low-level contamination near the limit of ATP luminescence detection

AI model, eg, Bayesian neural network, trained on

- process covariates (eg, temp., media lot)
- ATP RLU time series or endpoint
- orthogonal RMM signals (eg, flow cytometry)

Instead of a point estimate, the model produces

- posterior distribution: P (CFU | RLU, covariate)
- prediction interval (eg, 95% probability of failure)
- probability of exceeding spec.: P(CFU>limit)

Wu, C.-C., et al (2025). Predictive fermentation control of Lactiplantibacillus plantarum using deep learning for real-time batch outcome forecasting [Preprint]. arXiv / PMC repository.

High-dimensional spaces

AI model unique capability

Operates in high-dimensional spaces where classical regression is underdetermined

Example in RMM application

Detection of weak, distributed multivariate signals and higher-order interactions

- high-dimensional operation: hundreds of features
- captures multiple nonlinear interactions
- performs implicit feature selection
- integrates heterogeneous modalities, such as
  - spectral data (Raman)
  - scalar signals (ATP based intensity)
  - distributional data (cytometry populations)

Manzano T, Whitford W. Artificial Intelligence Empowering Process Analytical Technology and Continued Process Verification in Biotechnology. GEN Biotechnology. 2025 Feb 1;4(1):23-8.

Temporal & longitudinal data

AI model unique capability

Handels of temporal & longitudinal data, including non-stationary process behavior

Example in RMM application

Enables trajectory-level inference of microbe activity

Employs process dynamics both between and within batches, eg

- longer lag phases
- altered growth rates
- distribution of lag phases

Revealing, eg

- media degradation
- environmental drifting
- organism population changes

Sever, E. A., et al, A novel rapid bioluminescence-based antimicrobial susceptibility testing method using ATP consumption kinetics for Enterobacterales. Frontiers in Microbiology, 2024 15, 1357680.

Latent representations

AI model unique capability

Learns of latent representations that capture underlying biological or process structure

Example in RMM application

Reveals potential of *Injured but Viable* microbes

AI model (eg, autoencoder + classifier) trained on:

- multiple RMM assays, such as ATP (RLU)
- environmental data (e.g., disinfectant exposure)
- historical outcomes (eg, eventual vs no growth)

Learns underlying structure, not just correlations, eg:

- new media lot pleiotropy
- new stress condition pleiotropy
- different organism class pleiotropy

Zhu, L., et al, Open-set deep learning-enabled single-cell Raman spectroscopy for rapid identification of airborne pathogens in real-world environments. Science Advances, 11(2), eadp7991.

Support of decision-making

AI model unique capability

Supports of decision-making through predictive simulation and scenario analysis

Example in RMM application

Prediction from low, slightly upward signal trend

Classical approach for “no-growth yet”:

- continue incubation following predefined rules
- not using trajectory shape or contextual data

AI-enabled analysis:

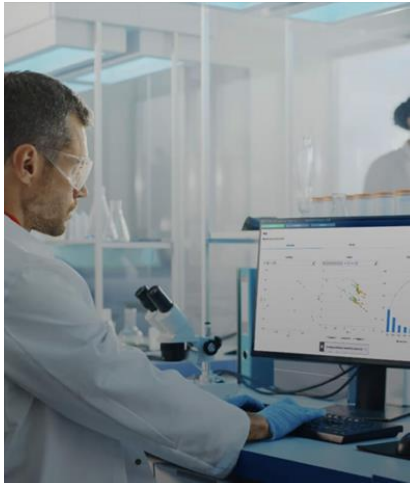
- predicts future distribution over time
- yields probability signal will cross a threshold
- estimates contamination risk given observed data

Miller, M. J. A Novel AI-Native Approach for the Detection, Enumeration, and Identification of Microorganisms Using Transformer Multimodal Spectral Imaging (TMSI), 2026. American Pharmaceutical Review.

The Extent of AI’s power

Exceptions to AI in data analysis

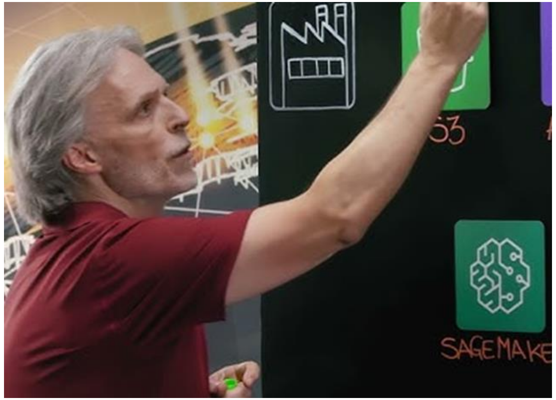
1. Human in the loop
   - human oversight is a scientific / regulatory requirement
2. Not indicated in all analysis
   - for some goals / projects classical maths are sufficient
3. Doesn’t provide mechanistic understanding
   - does not explain why or how specific relationship exist



AI potentials not addressed today

RMM lifecycle (operational deployment) steps

- Technology Assessment / Selection
- Feasibility / Method Development
- Data curation / governance
- Validation (IQ / OQ / PQ)
- Management Functions
- Regulatory Submission
- Implementation & Training
- Risk Identification / Prioritization
- Ongoing Lifecycle Management



Summary

AI provides

- Cross-modal, high-dimension, representational learnings of sample and microbial state
- Sensitive detection of weak, nonlinear, and early-stage contamination signatures
- Context-aware, probabilistic decision support under non-stationary conditions

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Thank You for Your Time

Disambiguation

Covariates are input or explanatory variables included in a model because they may influence the response variable. Covariates may participate in coupled system dynamics or act independently.

Covariance is a statistical value that quantifies the joint variability of two variables (the extent to which they vary together in absolute terms).

Correlation is the normalized form of covariance, expressing the strength and direction of a linear relationship between two variables on a bounded scale (−1 to 1).

Correlated measurements are observations that are statistically dependent; variation in one measurement is associated with variation in another.

Coupled relationships are physical, chemical, or biological interdependencies in which variables influence one another through system dynamics (often bidirectionally or via shared constraints).

Latent variables are unobserved constructs inferred from data (eg, via PCA/PLS) that capture underlying structure by representing shared variance among multiple measured variables.