Accelerating cell culture media optimization with an iterative Bayesian approach

Cell culture media optimization is a fundamental but resource-intensive step in both life sciences research and biomanufacturing. Media composition directly influences cell growth and proliferation, the yield of target compounds, and the quality of the manufactured product, yet media formulation typically involves tuning dozens of interacting components such as amino acids, carbon sources, growth factors, and cytokines. These interactions are often highly non-linear, making systematic optimization challenging.

Conventional approaches such as one-factor-at-a-time (OFAT) or statistical design of experiments (DoE) are widely used but scale poorly with increasing complexity. Both approaches become increasingly impractical when more than 15–20 factors are considered, often requiring approximations based on preliminary screening designs. In addition, these methods are not inherently designed to accommodate categorical design factors, such as different carbon or nitrogen sources, which rapidly expand the design space and further increase experimental burden.

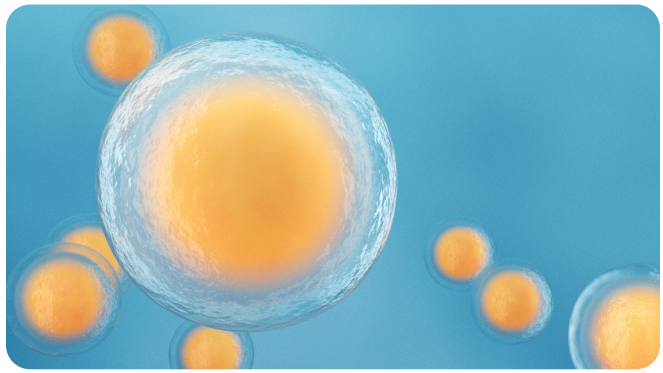
To address these limitations, researchers at the Massachusetts Institute of Technology developed an iterative, Bayesian optimization (BO)-based framework for accelerated cell culture media development, published in *Nature Communications*.[1] The framework begins with a small initial set of experiments used to build a Gaussian Process (GP) surrogate model. The model then informs the selection of subsequent experiments, balancing exploration of unknown regions of the design space with exploitation of promising conditions. With each new dataset, the GP model is updated, and the process continues iteratively until convergence.

The study demonstrated the framework using *ex vivo* peripheral blood mononuclear cell (PBMC) culture as a model system for iterative media optimization. Experimental readouts such as cell viability and counting, which were used to update the BO model at each iteration, were generated using the Cellaca™ MX high-throughput cell counter, providing the feedback required to drive each cycle of the optimization loop.

Publication highlights

- Researchers at MIT used an iterative Bayesian optimization framework to accelerate cell culture media development.
- They identified a blend that improved PBMC viability to 75-80% in just 24 experiments.
- The Cellaca™ MX high-throughput cell counter provided the viability and cell count measurements needed to drive each cycle of the optimization loop.

This content is based on work published by Narayanan et al. in Nature Communications (2025). https://doi.org/10.1038/s41467-025-61113-5



revvity

PBMC viability and homeostasis

Maintaining primary immune cells, such as PBMCs, *ex vivo* is notoriously difficult, with standard commercial media frequently leading to reduced viability and shifts in the distribution of cell types. This presents a significant challenge for applications including drug cytotoxicity studies, co-culture models of the tumor microenvironment, and immunotherapies based on specific lymphocyte subpopulations such as T cells and natural killer (NK) cells.

The researchers first applied the BO framework to optimize the composition of cell culture media and maximize PBMC viability after 72 hours in culture. They hypothesized that combining four commercially available media (DMEM, AR5, XVIVO, and RPMI) in different ratios could yield a composition capable of maintaining high cell viability during extended *ex vivo* culture.

Cell viability and counting were performed before and after incubation in bioreactors. Following the 72-hour culture period, cells from replicate conditions were pooled and treated to prevent aggregation and ensure accurate counting. Viability was assessed using acridine orange/propidium iodide (AOPI) fluorescent staining, which enables simultaneous detection of live and dead cells, with counts performed in three to four replicates on the Cellaca MX high-throughput cell counter. The resulting viability measurements and cell count data informed subsequent rounds of BO-guided media optimization.

Using 24 experiments across four iterations (six experiments per batch), the BO framework identified an optimized blend that achieved a statistically significant improvement in viability of 75-80%, compared to approximately 60% for individual media alone (Figure 1).

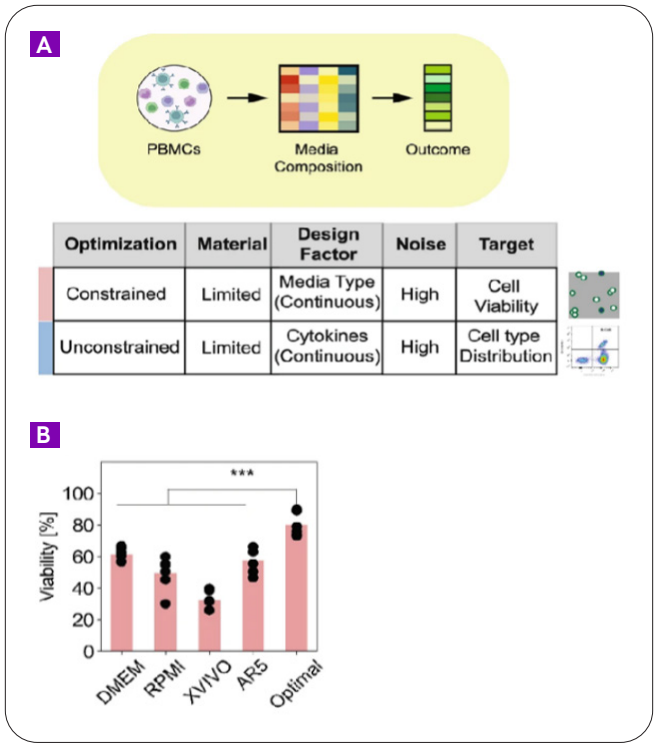


Figure 1: Media blend optimization of PBMC cultures. A) Workflow and study parameters to optimize media composition to maximize viability and maintain homeostasis of PBMCs in culture. B) PBMC cell viability as a function of the optimized media blend compared to individual standard media (DMEM, RPMI, XVIVO, and AR5) represented using data from six biological replicate experiments in each case. Individual double-sided t-test was performed between the standard media and optimal media formulation. *** denotes a p-value < 0.01. Image adapted from Narayanan et al.[1]

The iterative progression of the optimization is illustrated in Figure 2, which shows PBMC cell viability measurements across all experimental iterations. In the early iterations, the algorithm explored a broad range of media compositions, resulting in a wide distribution of viability measurements across experimental conditions (5–75%). As the optimization progressed, the algorithm increasingly focused on more favorable regions of the design space, narrowing the search toward higher viability conditions.

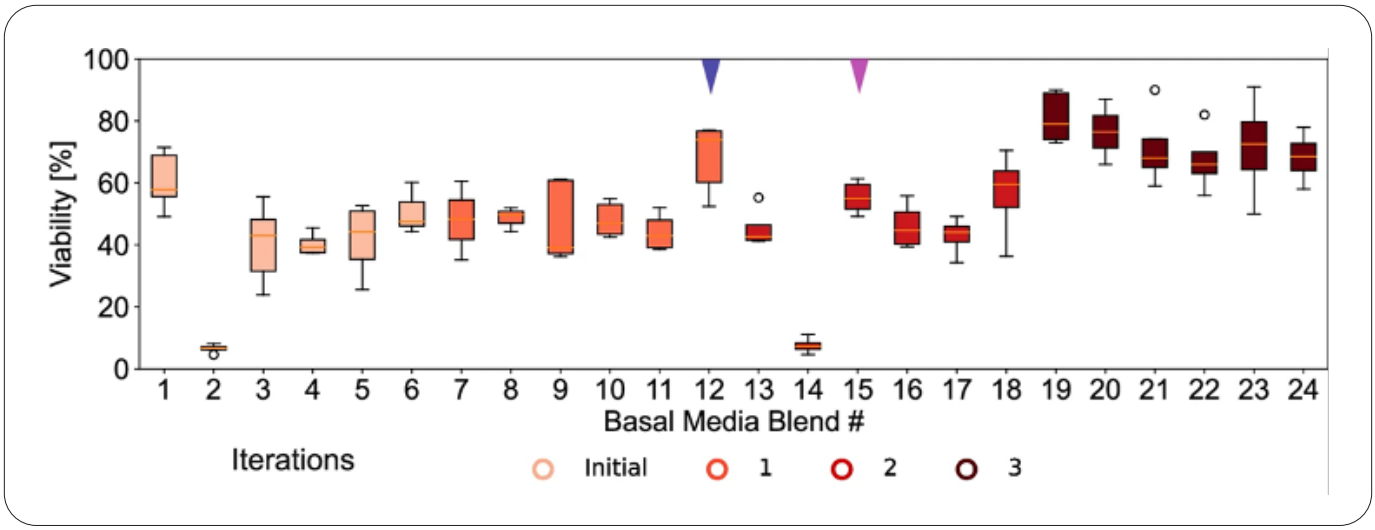


Figure 2: PBMC cell viability across iterative rounds of Bayesian optimization. Evolution of cell viability of the experiments planned in the different iterations indicated as boxplots of the biological triplicate data (with some experiments having up to five replicates). The box spans between first and third quartile encompassing a line for the median. The whiskers extend to the farthest data point that falls within 1.5 times the interquartile range. Points outside this range are indicated as fliers. The progressively increasing shades of red correspond to the increasing iteration number. Image adapted from Narayanan et al.[1]

Notably, the viability-optimized blend did not uniformly maintain the diverse subpopulations of lymphocytes, favoring T cells over B cells and NK cells. To address this, a second sequential optimization was performed using eight design factors (IL-2, IL-3, IL-4, IL-7, IL-12, IL-15, IL-21, and BAFF). In just 12 experiments across two iterations, a combination was identified that preserved both total cell density and the distribution of cell types compared to the *ex vivo* distribution.

Together, these sequential optimization workflows demonstrate the flexibility of the BO framework for addressing multiple objectives in complex primary cell culture systems. Viability and cell counting were performed using the Cellaca MX cell counter with AOPI fluorescent staining at each iteration, providing the experimental feedback required for subsequent cycles of media optimization within the BO model. This iterative workflow highlights the role of viability and cell count measurements in supporting data-driven optimization strategies for complex cell culture systems.

Experimental significance

This work demonstrates that BO-guided iterative experimental design can make complex media optimization more resource-efficient and broadly applicable, particularly for applications where biological material is limited, such as needle biopsies or rare/pediatric cancers. The study also highlights the growing role of iterative, data-driven workflows in cell culture development, where experimental measurements are iteratively incorporated into model-guided optimization strategies.

The Cellaca MX high-throughput cell counter

Optimizing cell culture media typically includes hundreds of different combinations of nutrients at different concentrations, making it time-consuming and requiring frequent cell counts across many samples with replicates. Even with an optimized experimental design, as demonstrated in this publication, a high number of cell counts is needed. The Cellaca MX cell counter with AOPI fluorescent staining is well-suited for high-throughput, precise cell counting with replicates, supporting scientists in maintaining an efficient workflow. To find out more or to discuss your application, contact our specialist team.

Reference

1. Narayanan, H., Hinckley, J. A., Barry, R., Dang, B., Wolffe, L. A., Atari, A., Tseng, Y., & Love, J. C. (2025). Accelerating cell culture media development using Bayesian optimization-based iterative experimental design. *Nature Communications*, 16(1), 6055. https://doi.org/10.1038/s41467-025-61113-5