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Application News

Culture Optimization Software CellTune

Efficient Optimization of Culture Conditions Using the CellTune Culture Optimization Support Software

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User Benefits

- CellTune can investigate culture conditions more efficiently with fewer experiments, reducing the time and developmental costs required to optimize culture conditions.
- Even users with minimal knowledge of statistical analysis can easily extract important parameters from large volumes of measured data and propose optimized culture conditions.
- CellTune facilitates the simultaneous optimization of multiple components across four or more dimensions beyond the human abilities.

■ Introduction

Optimizing culture conditions is a critical step in developing and producing pharmaceuticals by cell culture. However, there are countless combinations of culture media components and culture parameters, so determining optimum culture conditions is a very time-consuming and costly process of trial and error that is largely reliant on the experience and intuition of skilled workers. This Application News presents a case study in which the AI-based culture optimization software CellTune streamlined the optimization process using fewer culture experiments to improve the antibody productivity.

CellTune was developed through joint research between Shimadzu and Epistra Inc.

■ Feed Culture Conditions

Twelve feed culture media were prepared with commercial feed culture media (Table 1), where 1× is the originally designated concentration of the product) and used to culture Chinese hamster ovary (CHO) cells. Culture samples were collected every 24 hours between days 3 and 7 of each culture and used to measure the antibody concentration and culture environment conditions (cell count, pH, dissolved gas, osmotic pressure, etc.) with BioProfile FLEX2 (Nova Biomedical). In addition, the samples were analyzed for 144 metabolites in the culture supernatant with LC/MS/MS Method Package for Cell Culture Profiling Ver.3. More information about Cell Culture Profiling Ver. 3 can be found in Application News No. 01-00498A-EN.

Table 1 Feed Media Conditions

Flask	Commercial Feed Medium	Glucose	Glutamine
1	1 ×	10 g/L	Added to commercial feed medium for a final concentration of 6 mM
2	1 ×	10 g/L	
3	1 ×	17.5 g/L	
4	1 ×	17.5 g/L	
5	1 ×	25 g/L	
6	1 ×	25 g/L	
7	2 ×	10 g/L	
8	2 ×	10 g/L	
9	2 ×	17.5 g/L	
10	2 ×	17.5 g/L	
11	2 ×	25 g/L	
12	2 ×	25 g/L	

■ Identifying Important Components with the Feature Extraction Module

CellTune has two functional modules: a feature extraction module and an AI optimization module. The feature extraction module identifies parameters for optimization in four steps: (1) dataset coupling, (2) data cleansing, (3) selection of data for analysis, and (4) statistical analysis by partial least squares (PLS) regression and correlation analysis (Fig. 1). In this case study, (1) datasets were coupled by connecting each culture environment measured data with each LC/MS data, based on culture flask ID and culture sampling times. Next, (2) data cleansing was performed to remove noise and impute missing values; then (3) data obtained on day 5 and day 6 of the culture were selected for analysis; and (4) data from each day were subjected to naïve PLS regression. Variable importance for prediction (VIP) scores, which indicate the importance of a particular variable, were calculated, and components of the culture media that correlated with the antibody productivity were extracted. Components with a VIP score larger than 0.8 on both day 5 and day 6 of culture were deemed to be important (Table 2). The individual computational processes used to perform steps (1) through (4) are presented in the CellTune software as pre-linked analysis recipes (Fig. 2), allowing users to obtain results simply by selecting an analysis recipe and setting parameter thresholds.

Table 2 List of Important Components Extracted by CellTune

Substance name	VIP: day 5	VIP: day 6
Histidine	1.36354	1.328939
Cystine	1.358055	1.327764
Tryptophan	1.357963	1.316705
Phenylalanine	1.351867	1.341002
Tyrosine	1.351663	1.331608
Methionine	1.350939	1.33506
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Thymine	0.801899	1.011638

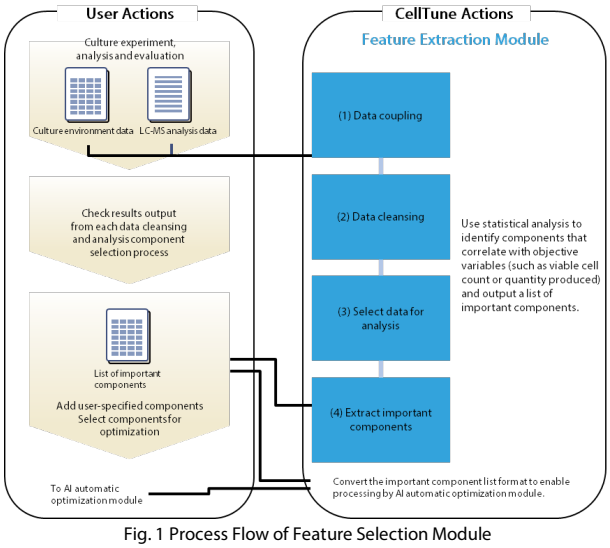

Fig. 1 Process Flow of Feature Selection Module

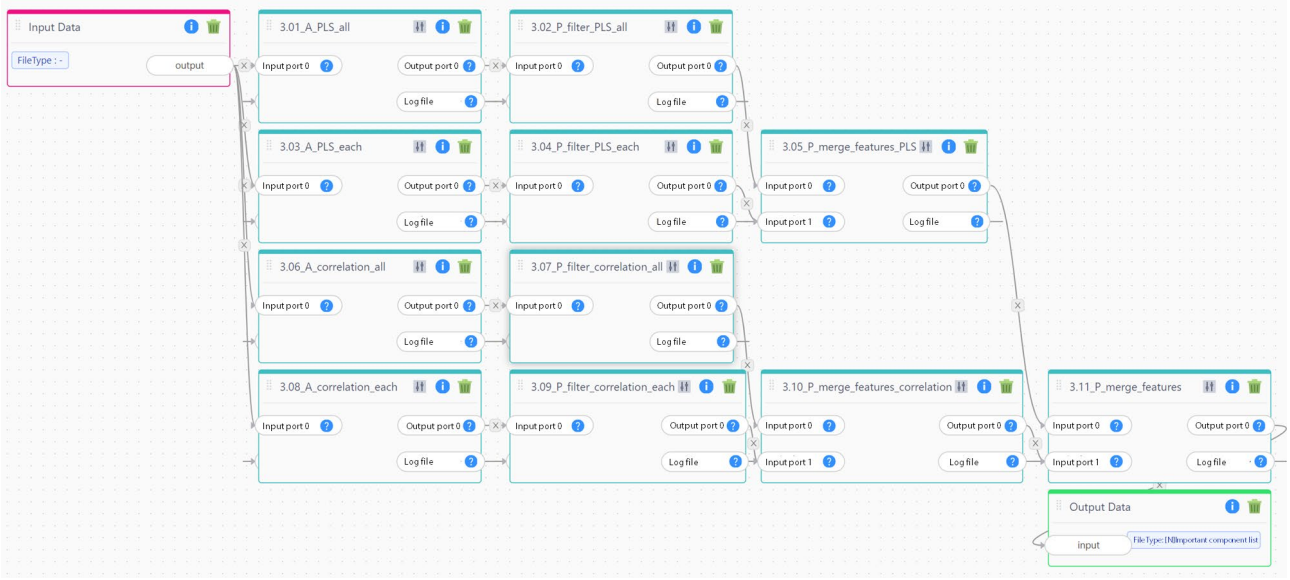

Fig. 2 Example Analysis Recipe

■ Optimizing the Feed Culture Media Composition with the AI Optimization Module

The AI optimization module generates an experimental design that investigates optimal conditions of variables for optimization. The user sets an objective variable, selects which variables to optimize from the important components identified by the feature extraction module (and adds arbitrary variables if needed), sets upper and lower limits of conditions for the variables, and enters the control conditions in a prescribed format. CellTune then generates an experimental design consisting of a user-specified number of experiments. This allows the user to simultaneously optimize multiple variables beyond human abilities, and values are provided in up to three decimal places, enabling fine-tuning.

In this case study, glucose and four other user-specified components were added to the top eight important components extracted by the feature extraction module, giving 13 total variables for optimization. The antibody concentration multiplied by the viable cell count was set as the objective variable. The goal of optimization was to maximize this objective variable. CellTune generated an experimental design with 12 experimental conditions, including one control (Table 3). Following this experimental design, the prepared feed culture media were added on day 3 of the culture, and the antibody concentration and viable cell counts were assessed on day 6. The experimental design and the objective variable results (performance) were then re-entered into CellTune to generate a new experimental design. This process of generating an experimental design, performing culture experiments, and determining results is termed a single round. Three rounds were performed in this case study. Experimental design and score data entered into CellTune generate a prediction model of the performance based on the experimental design and the performance data, and the next experimental design is generated focusing on the high-performance areas, enabling efficient search for optimal conditions.

CellTune can also provide a visualization of optimization results. The history plot feature plots the results for each flask for each round of experiments and creates a line graph showing the maximum values trend with each round. This enables the user to understand at a glance the performance of all the culture experiments. In this case study, the antibody concentration increased with each round of optimization, improving by approximately 40 % compared to the control conditions (Fig. 3). The summary plot feature displayed the results of each round of experiments (Fig. 4). It showed that each component targeted for optimization (explanatory variables and green bars) varied less between culture conditions after each round of experiments, and the culture performance (objective variable and orange bars) increased overall with each round of experiments, demonstrating that optimization was achieved. In this case study, the summary plot showed that optimization became saturated, and the feed medium composition was optimized in three rounds of experiments, demonstrating how it provides a visual guide to the degree of optimization being achieved.

Table 3 Example Experimental Design Generated by CellTune

ID	Glutamic acid	Aspartic acid	Glutamine	•••	Cystine	Control?	Notes
Category				•••			
Lower	0.5	12.8	32	•••	9		
Upper	1.35	24.96	54.4	•••	14.37		
Control	0.5	12.8	32	•••	9	TRUE	
Flask 2	1.15	24.32	54.4	•••	9.895		
Flask 3	1	22.4	38.4	•••	14.37		
•••	•••	•••	•••	•••	•••		
Flask 12	0.9	23.68	44.8	•••	9		

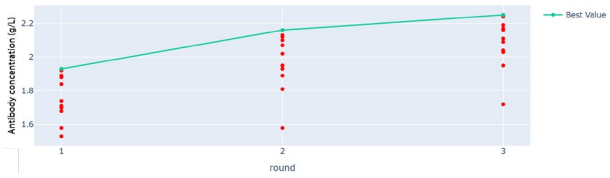

Fig. 3 History Plot

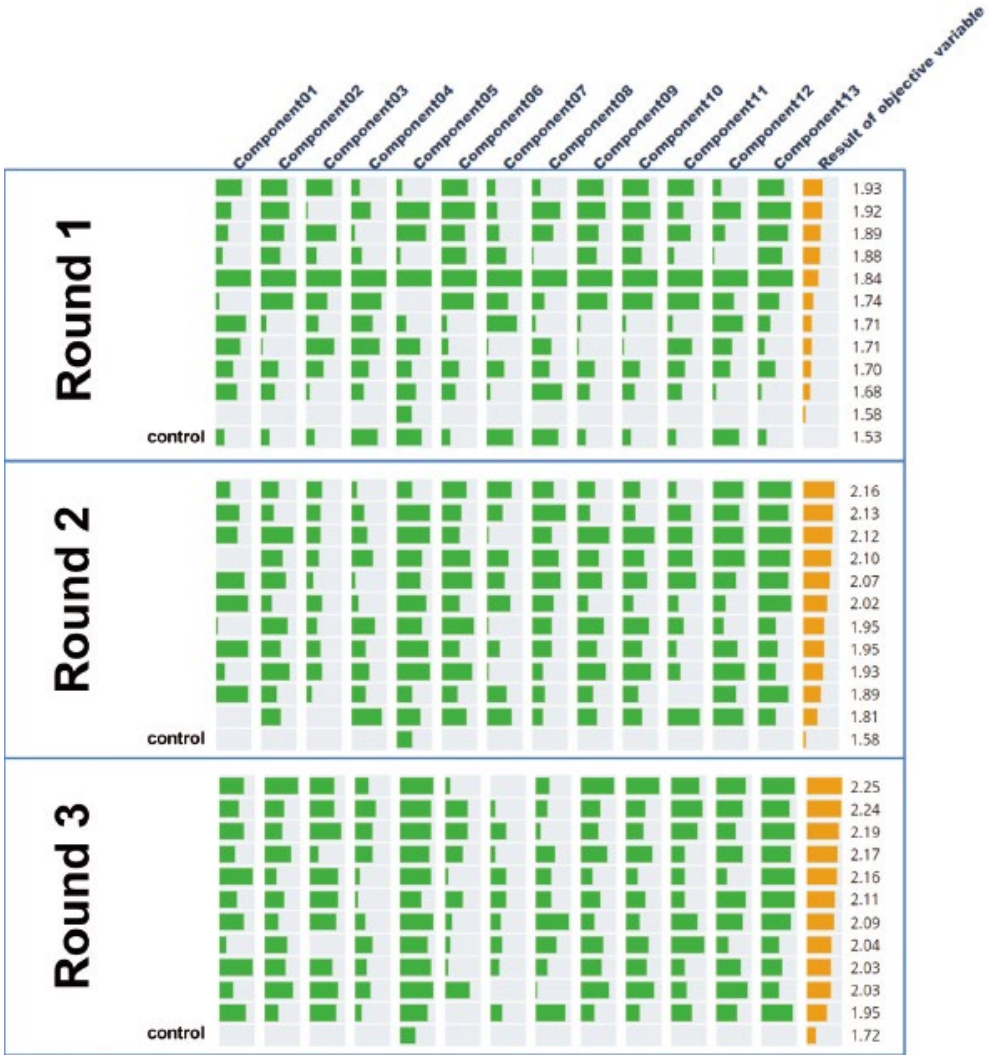

Fig. 4 Summary Plot

■ Conclusion

From a vast amount of measured data, the feature extraction module in CellTune was able to provide a simple method for identifying 13 components for optimization that could improve the antibody productivity. The AI optimization module was used to generate three rounds of optimization experiments, where each round consisted of 12 experimental conditions. This enabled a 40 % improvement in the antibody productivity over the control conditions.

CellTune can be used to streamline the investigation of culture conditions by reducing the number of culture experiments required for optimization, which lowers the time and labor costs involved in optimizing culture conditions.

01-00804-EN First Edition: Dec. 2024

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