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Bioreactor Intelligence for Real-Time Monitoring and Automation of Stem Cell Cultivation

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Summary:
We present innovative approaches to develop novel sensor-intelligence devices for scaling up and standardizing the production of complex human induced pluripotent stem cells and their derivatives (e.g., neural cells). Preliminary results for the optical at-line sensors show good performance results for classification and segmentation of stem cell aggregates.

Keywords: deep learning, potentiostat, spheroids, embedded vision, process analytical technology

Motivation and Objectives

The quality-assured and reproducible production of complex cell models derived from induced pluripotent stem cells (iPSCs) is a fundamental prerequisite for the successful transfer of these models in e.g., drug development pipelines of pharmaceutical industry as well as cell therapy in clinical applications. Our research is pursuing several highly demanded approaches to develop new sensor-intelligence devices for scaling up and standardizing the production of complex iPSC-based three-dimensional (3D) cell models, known as spheroids or organoids.

The various approaches are to be integrated into a standard commercial suspension bioreactor system “CERO 3D Incubator & Bioreactor” (OMNI Life Science GmbH & Co KG, Bremen, Germany), which is commonly used for the cultivation of iPSC-derived 3D cell aggregates. The system does not yet measure parameters directly in the bioreactor tubes, except pH. This means that no statements can be made about e.g., glucose or lactate concentration, dissolved oxygen or other values relevant to spheroid maturation. Furthermore, quality control of the spheroids is a manual, invasive, time-consuming and user-dependent process. The concept of our sensor-intelligence device considering the following objectives:

- Scalable parallelization and automation of production processes for 3D cell cultures.
- Optimized cultivation and expansion of sensitive cell models, such as human iPSCs, and their differentiation into cardiomyocytes, neurons or lung cells using online sensors.
- AI-supported, non-invasive assessment of cell cultures (e.g. size, growth curve, morphology) during the ongoing production process, possibly with the option of feedback-control.
- Data connectivity and integration into quality assurance systems or laboratory information management systems by implementing standardized interfaces and protocols.

System Architecture

Our system employs optical and electrochemical sensors. Optical sensors capture morphological information of spheroids (e.g. size, shape, homogeneity), while electrochemical sensors measure characteristics of the medium (e.g. pH, dissolved oxygen, glucose / lactate). By combining this heterogeneous data, we plan to further assist biologists in the production of 3D cell cultures and to potentially fully automate the process in the future. Fig. 1 shows the demonstrator of the modified commercial bioreactor with integrated sensor intelligence.

Optical sensors are either integrated directly inside the incubator space (i.e. in-line) to monitor the spheroids inside the rotating tubes [1] or used at-line by attaching a microfluidic system to the bioreactor, where a microscope captures images of the spheroids drawn from the tubes [2]. Extraction of the spheroids is done by a special adapter with sterile metal straws which is inserted through a modified lid into the bioreactor.

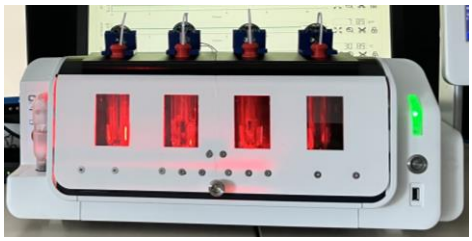

Fig. 1. Modified "CERO 3D Incubator & Bioreactor" with integrated sensor intelligence.

A peristaltic pump gently transfer the spheroids out of the tubes through a transparent microchannel, where images are taken for subsequent classification and analysis, and back to the tubes. Two separate deep learning models based on the YOLO5 (You Only Look Once) and YOLO8 architectures [3, 4] were trained respectively for these two types of optical sensors to detect, classify, and segment the spheroids. The results of the models are used to calculate morphological characteristics.

Electrochemical sensors are integrated inside the bioreactor tubes by the same adapter, which is employed for the spheroid extraction. Highly integrated measurement electronics with wireless data interface and four high-precision potentiostat circuits measures up to five parameters simultaneously (temperature, pH, glucose, lactate, and dissolved oxygen) per rotating tube. Sensor data is transmitted via Bluetooth Low Energy (BLE). Fig. 2 shows the compact measurement electronics with a diameter of 30 mm. Alternatively, the sensors are implemented into the microfluidic system leading to the at-line analysis. However, we focus on results for the optical at-line sensor in this paper.

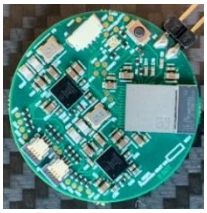

Fig. 2. Electrochemical measurement electronics with four potentiostat circuits and BLE interface.

Validation

The trained machine learning (ML) model for the optical at-line sensor processes each image in roughly 16 ms (± 6 ms), allowing the microscope camera to operate at around 40 FPS. Thus, the system can, in its current setup, process 5 ml sample volume per minute. Mean average precision at an intersection over union threshold of 0.50 (mAP50) of the trained model is 0.68. Throughout the process, the system provides real-time updates of descriptive statistics, offering an overview of the cultivation progress. Fig. 3 shows a visualization of the ML model's results. To assess the potential influence of spheroid transport through the peristaltic pump, we conducted two types of experiments:

1. On the third day, one tube was processed, leaving the final 10 mL untouched. This step was repeated twice on the same day using a tube with more than 10 mL of content. As a result, four tubes were obtained, each containing 10 mL of medium with spheroids.
2. In a separate experiment, 10 mL of medium containing spheroids was pumped through the at-line circuit and analyzed by the system on days 3, 5, and 7.

The spheroids in all 8 tubes were then cultivated under standard conditions for 10 days in total. Fig. 3 displays spheroids from human iPSC-derived neural stem cells (EBiSC cell line UKKi011-A) after 10 days of growth. The results suggest that the physical stress exerted by the system does not impact the spheroids' morphology.

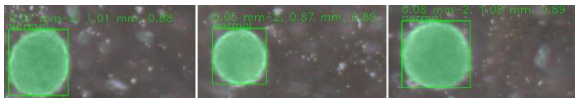

Fig. 3. Example of the ML model's output: classification, segmentation taken on day 10 of cultivation.

Conclusion

The reported outcomes indicate promising results for the optical at-line sensors. By enhancing the integration of our system, we will generate more data to further improve the performance and accuracy of our ML models and use heterogeneous data generated by the optical and electrochemical sensors to achieve our previously stated goals.

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