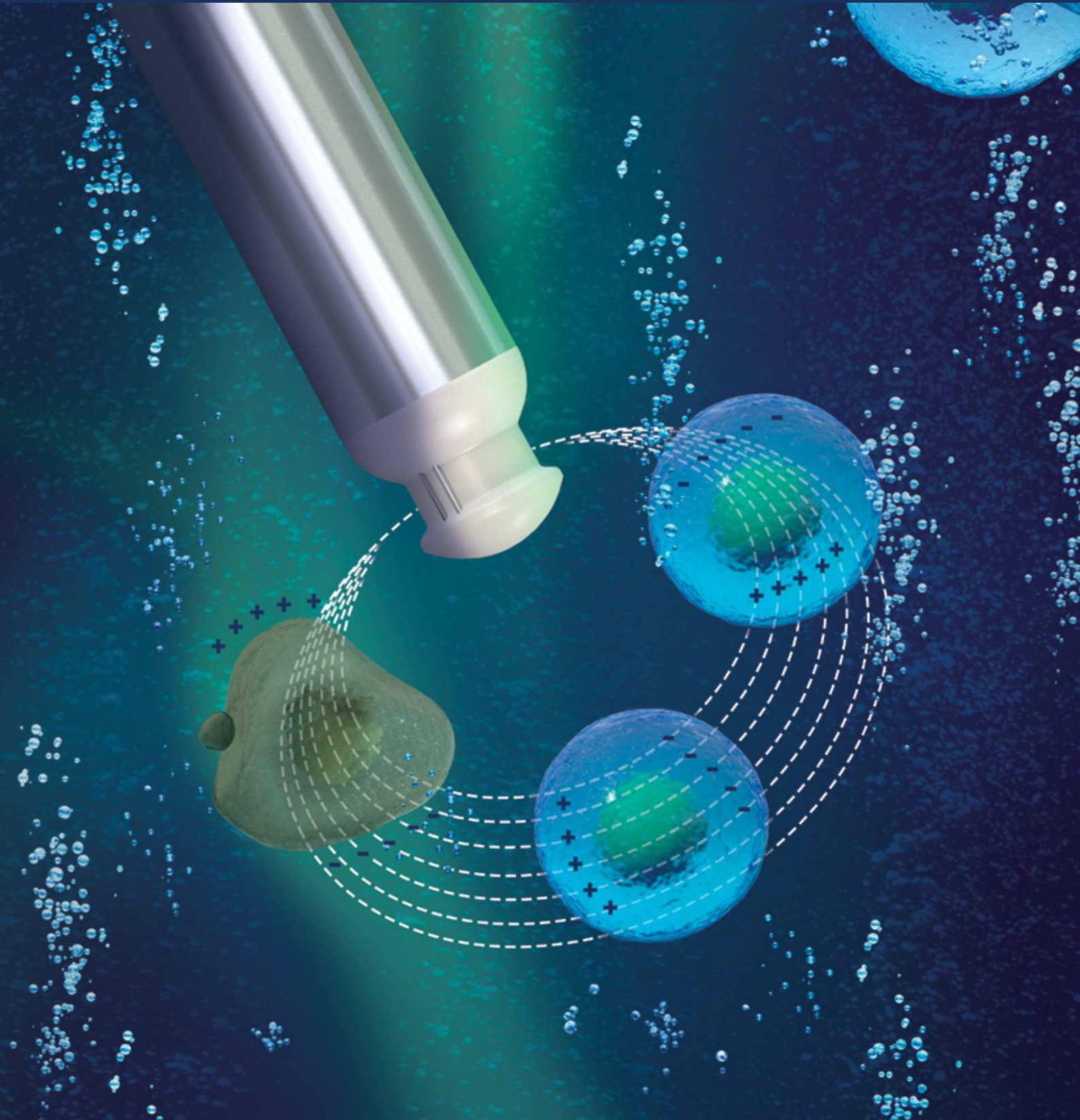


HAMILTON



Cell Density

Applications eBook



Publication Overview



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Introduction Section 1

Explore the advanced capabilities of Hamilton's on-line cell density sensors—widely adopted from applications such as feed control to continuous processing. This eBook presents a comprehensive collection of relevant data and references, highlighting the successful integration of these sensors in diverse scientific and industrial applications.

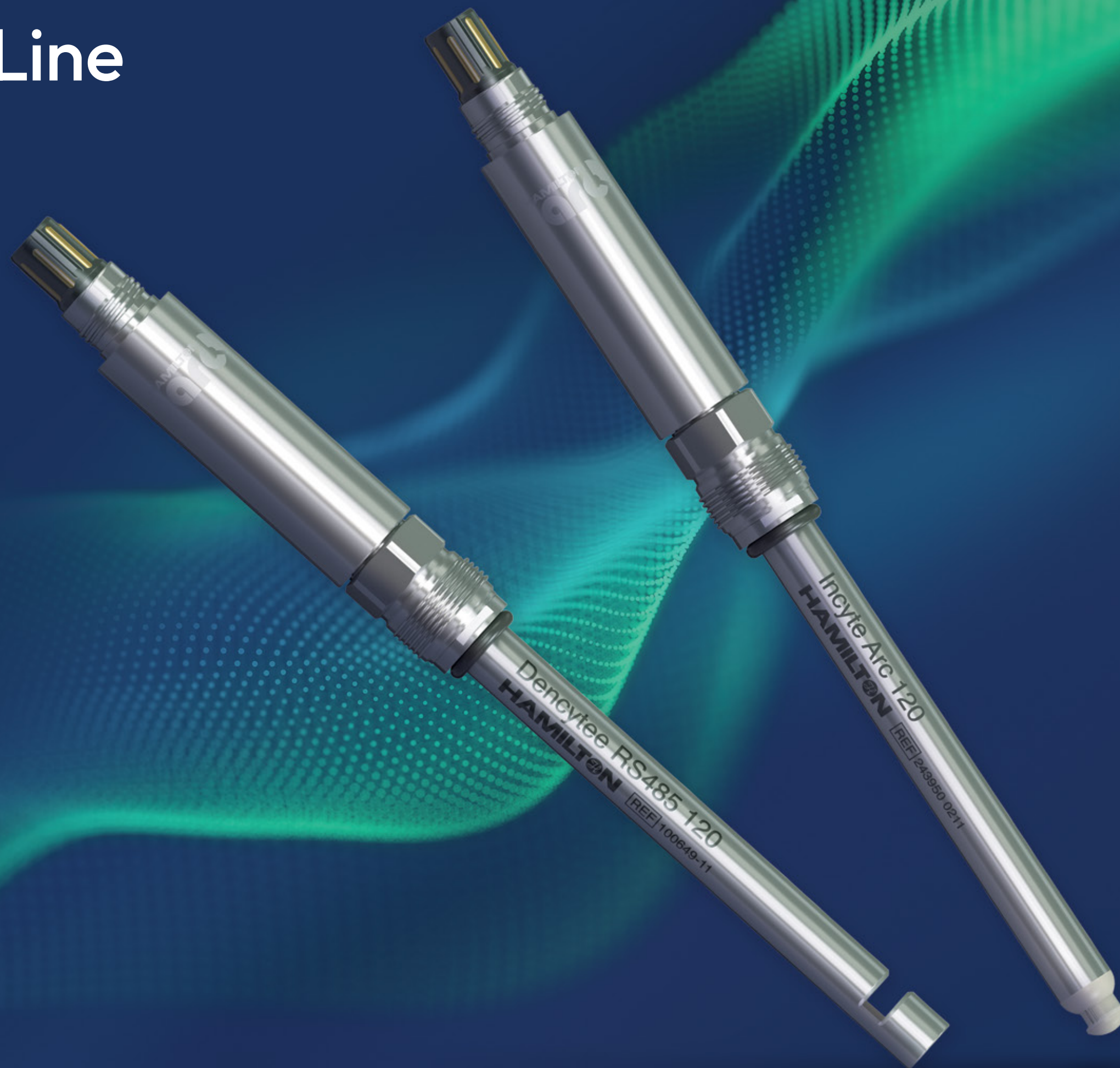
Discover On-Line Cell Density

Innovative Methods that Improve and Streamline Cell Density Monitoring

Traditional cell density measurement and monitoring is time- and labor-intensive. It also lacks real-time, continuous data and makes assumptions based on off-line sampling and manual cell counting.

Hamilton has revolutionized and modernized this process. Rather than counting the cells directly, Hamilton cell density sensors use the principles of permittivity measurement (capacitance per area) and light absorbance which correlate to the cell count in solution at the production line. It's an improvement that saves time and allows for advanced process control.

The following pages detail real applications of these sensors and measurement principles to show the benefits and opportunities offered by this shift in technology.



Your Way for On-Line Measurement



Incyte Arc®

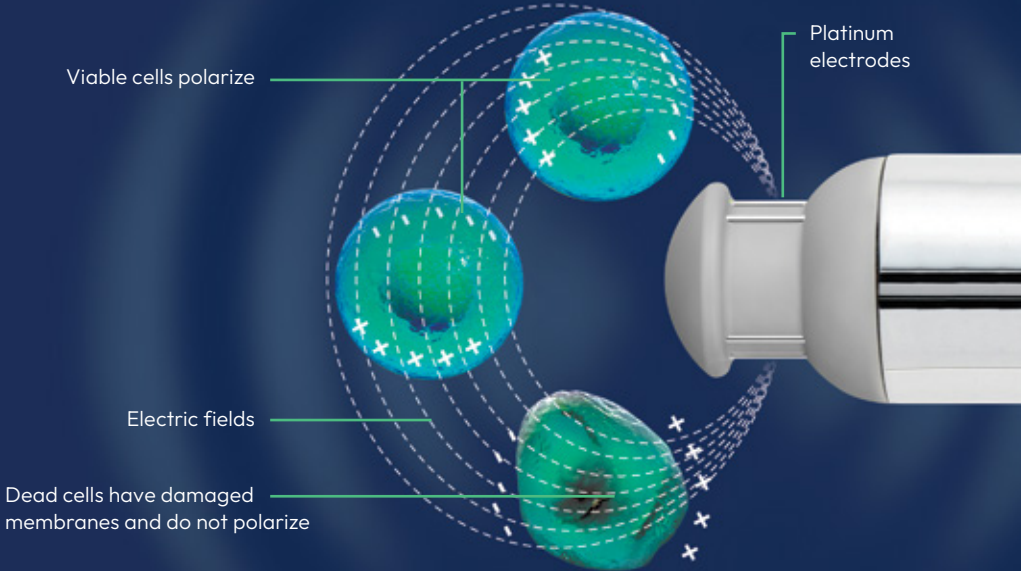
Viable Cell Density (VCD)

The Incyte® sensor enables real-time, on-line measurement of viable cells without the need for sampling. Typical applications include mammalian cell culture, yeast, and high-density bacterial fermentation. The measurement is independent of changes in media, microcarriers, dead cells, and debris. On-line measurement of viable cells makes it possible to detect events and respond in real time without off-line sampling.

- Increase Yield and Lower Production Costs.
- Detect Changes in Cell Physiology with Frequency Scanning.
- Enable Process Control for Improved Process Consistency.
- Early Detection of Process Deviations.

How it Works

The Incyte measurement principle is based on capacitance. In an alternating electrical field, viable cells behave like small capacitors. The charge from these capacitors is measured by the sensor and reported as permittivity (capacitance per area).



Dencytee Arc®

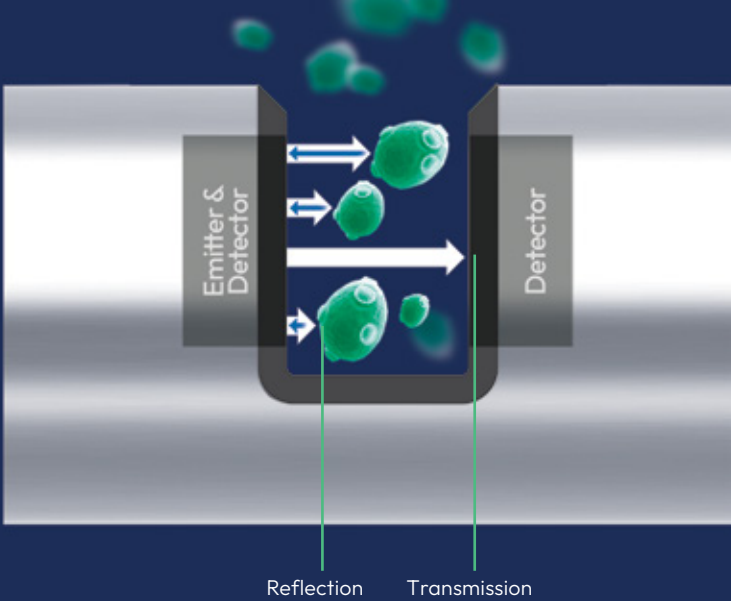
Total Cell Density (TCD)

The Dencytee Arc® sensor performs on-line measurement of Total Cell Density in solution. The sensor is designed to measure the turbidity of the cell suspension. The measurement is made at NIR (near-infrared) wavelengths so it is insensitive to changes in media color. All particles and molecules that scatter light at 860 nm will be detected, including living and dead cells as well as cell debris. This measurement is effective after inoculation when cells are expanding quickly but concentrations are low, making capacitance-based readings less reliable.

- Simple On-Line Measurement of Cell Growth.
- Reliable Values During the Growth Phase.
- Improved Linearity at High Concentrations.
- Early Detection of Process Deviations.

How it Works

The Dencytee Arc sensor measures over a 5 mm sensing gap. It has a light source and one detector on one side, and a second detector on the other side of the gap. Depending on the cell concentration, the light is absorbed and reflected by the cells. Dencytee Arc combines the transmission at the front detector and reflection at the back detector into a single measurement value of the on-line total cell density.



To measure both low and high cell densities with a high-quality signal, the sensor is also able to measure the reflected light of the cells. This is especially beneficial at high cell densities.

2

Applications Section 2

Integration of a Photobioreactor Into the MaMBA Facility as Part of a Human-Centered Life Support System



Algea Process, Photobioreactor

www.researchgate.net/publication/372495017

Paul Große Maestrup, Ksenia Appelganc, Saurabh Band, Florian Stechmann,
Vera Hagemann, Anna Förster, Cyprien Verseux, Christiane Heinicke : July 2023

The integration of a photobioreactor (PBR) into the Moon and Mars Base Analog (MaMBA) facility as part of a bioregenerative life support system (BLSS) for space habitats was investigated as part of a research program aiming to develop a human-centered life support system (LSS).

Photobioreactor System:

- Cultivates *Anabaena* sp. (a filamentous cyanobacteria) for oxygen production via photosynthesis.
- Equipped with multiple sensors to monitor environmental parameters such as temperature, pH, dissolved oxygen, and optical density (OD).
- Uses sterilized BG110 medium and pressurized air for cultivation.
- Sensors include Hamilton's pH and OD sensors to ensure accurate monitoring of growth conditions.

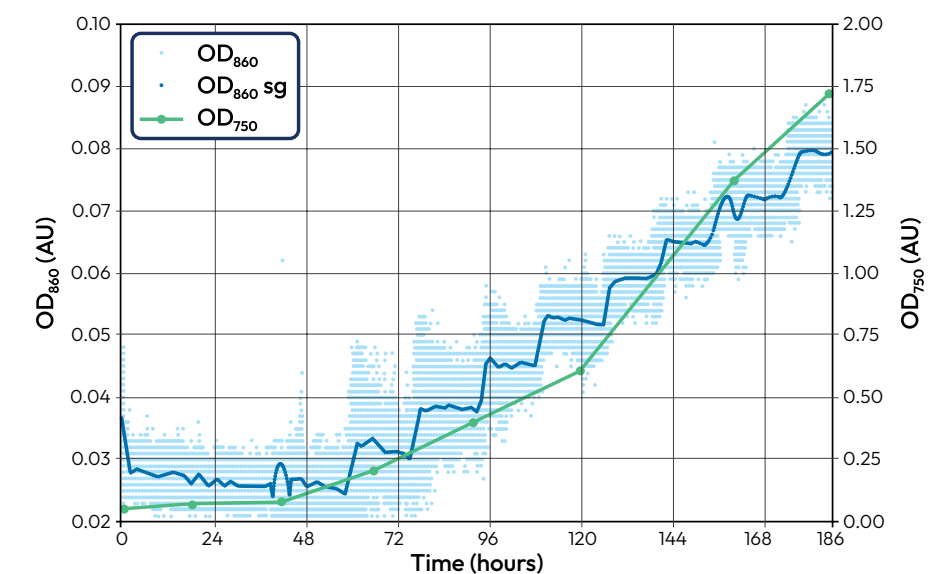


Figure 1: (Left) Configuration of the PBR. (Right) Course of OD₇₅₀ and OD₈₆₀ during the eight-day experiment with the improved configuration. The measurements at OD₇₅₀ are indicating a typical growth curve for cyanobacteria. Although the measurements OD₈₆₀ are less accurate, they have a similar progression to the measurements at OD₇₅₀. The sensor setup was optimized using the Arc Wi Adapter (OD₈₆₀ sg), resulting in minimized noise and a smoother signal.

Conclusion: The integration of Hamilton sensors into the PBR system significantly contributed to the accurate monitoring and control of growth conditions, ensuring the success of the experiments. This study highlights the importance of reliable sensor technology in developing human-centered LSS for space habitats, paving the way for further advancements in bioregenerative life support systems.

Scaling Fed-Batch & Perfusion Antibody Production Processes in Geometrically Dissimilar Stirred Bioreactors



Antibody Production, CHO Cells, Scale-Up, High Cell Density

<https://www.mdpi.com/2227-9717/12/4/806>

Vivian Ott, Jan Ott, Dieter Eibl, and Regine Eibl : April 2024

This study investigates the scaling of fed-batch and perfusion antibody production processes in different geometrically dissimilar stirred bioreactors, focusing on achieving high cell densities and productivity. The primary objective was to transfer processes between cylindrical stirred lab-scale bioreactors and Thermo Scientific's cubical HyPerforma DynaDrive Single-Use Bioreactor.

- Multiple bioreactor systems were used, including 250 mL shake flasks, Ambr 250 modular vessels, 3 L HyPerforma Glass bioreactors, and 50 L HyPerforma DynaDrive bioreactors.
- Experiments included fed-batch and perfusion cultivations using a trastuzumab-producing CHO K1 cell line.
- Different feeding strategies (bolus and continuous) were evaluated.
- Cultivation parameters such as temperature, stirrer configuration, and feed addition were optimized for each bioreactor type.
- Used XCell ATF single-use devices for cell retention.
- The Hamilton Incyte Arc permittivity probe was used to control the bleed pump based on real-time viable cell volume (VCV) measurements.
- Maximum viable cell densities (VCDs) of 49×10^6 cells/mL achieved in the HyPerforma DynaDrive bioreactor.

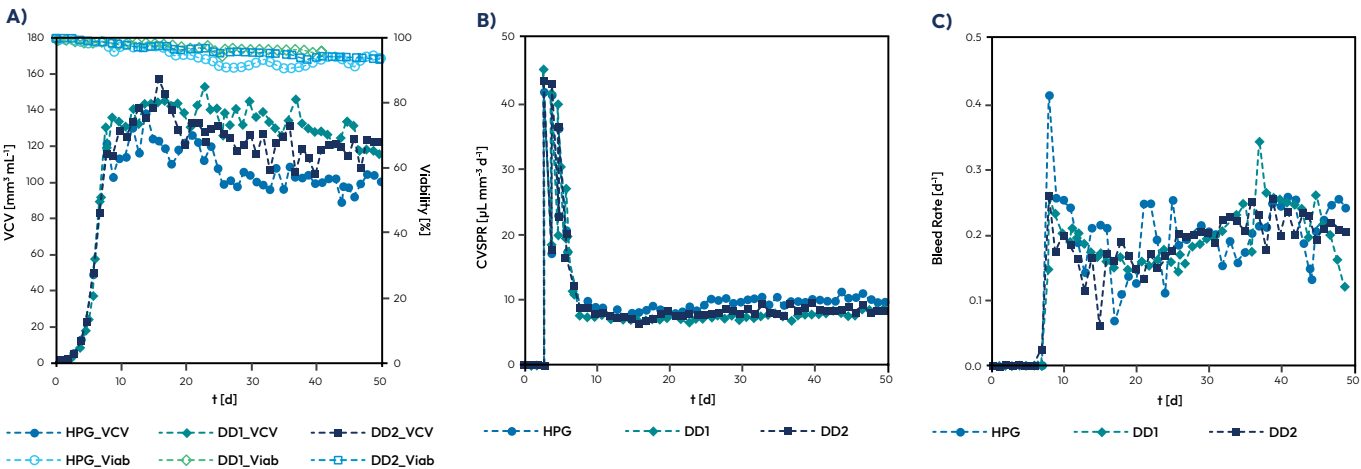


Figure 2: Course of (A) VCV and viability, (B) viable cell volume specific perfusion rate (CVSPR), and (C) bleed rate during the three perfusion experiments performed with continuous feeding (nomenclature: HPG = HyPerforma Glass bioreactor, DD = HyPerforma DynaDrive bioreactor, Viab = viability).

Conclusion: The study showed the successful scaling of antibody production processes in geometrically dissimilar bioreactors. The Hamilton Incyte Arc permittivity sensor played a crucial role in monitoring and controlling cell density in perfusion processes, contributing to high productivity and consistent product quality. This research highlights the importance of advanced sensor technology in optimizing biopharmaceutical production at different scales.

Process Intensification by Inoculating Antibody Production Bioreactors Directly from Cryovials



<https://onlinelibrary.wiley.com/doi/full/10.1002/cite.202300189>

Jan Ott, Vivian Ott, Peter Neubauer, Dieter Eibl, and Regine Eibl : February 2024

By directly inoculating bioreactors from ultra-high cell density working cell bank (UHCD-WCB) cryovials, this study demonstrates a simplified and more efficient approach to antibody production, reducing labor and streamlining workflows.

- The study utilized different bioreactor systems including 250 mL shake flasks, Ambr 250 modular vessels, and a 50 L HyPerforma DynaDrive Single-Use Bioreactor.
- Direct inoculation from cryovials was compared to standard inoculum production in shake flasks and Ambr bioreactors.
- The Hamilton Incyte Arc probe controlled the bleed pump based on real-time viable cell volume (VCV) measurements.
- Perfusion rate adjustments were made to maintain a cell-specific perfusion rate.
- Successful inoculation of the DynaDrive bioreactor with one cryovial, expanding directly in the production bioreactor.

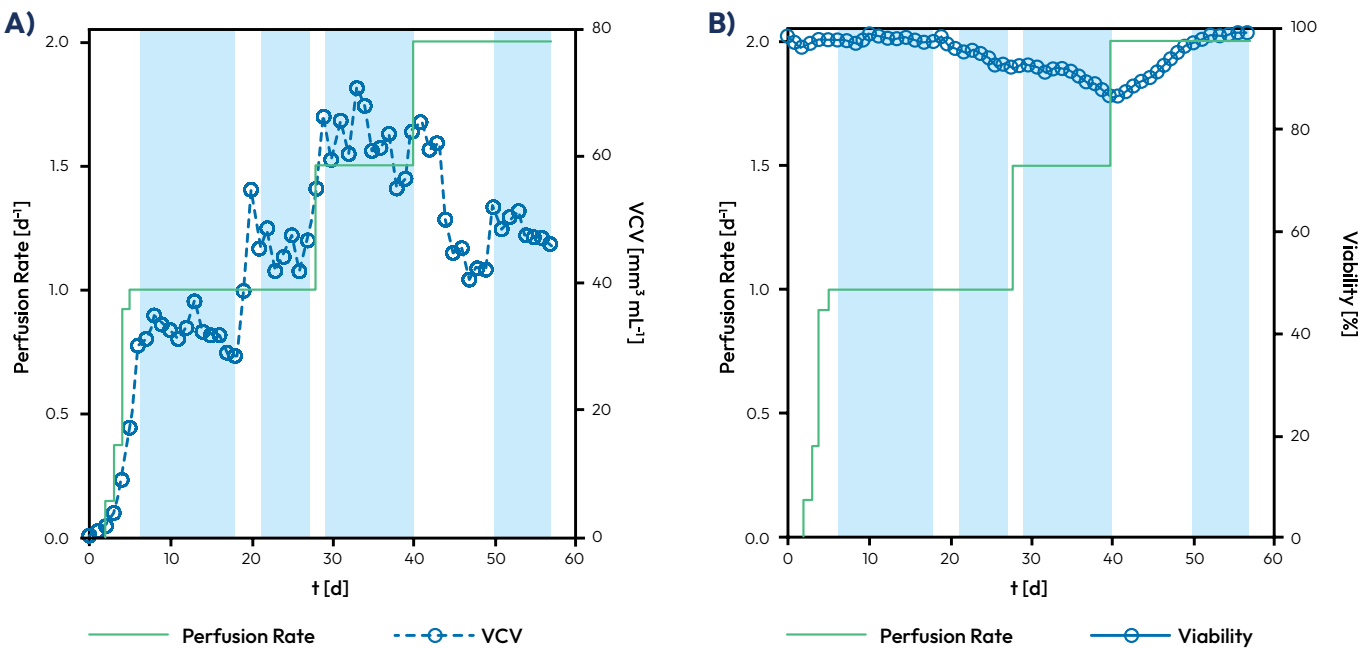


Figure 3: Time course of (A) perfusion rate and VCD, and (B) perfusion rate and viability during the 2L perfusion cultivation (N = 1).

Conclusion: Direct inoculation of production bioreactors from UHCD-WCB cryovials is shown to be feasible and beneficial in this study. The Hamilton Incyte Arc probe was crucial for real-time monitoring and control of cell density, contributing to the successful implementation of both fed-batch and perfusion processes. This method can significantly simplify production, reduce labor and space requirements, and enhance productivity in antibody manufacturing.

Influence of Cell-Specific Parameters in a Dielectric Spectroscopy Conversion Model Used to Monitor Viable Cell Density in Bioreactors



<https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/biot.202300028>

Adèle Schini, Bartolomeo De Canditiis, Célia Sanchez, Michaël Pierrelee, Karine-Elise Voltz, Laurent Jourdainne : September 2023

For accurate viable cell density (VCD) monitoring in bioreactors using dielectric spectroscopy, this study demonstrates the critical need to account for cell-specific parameters such as cell size, membrane capacitance, and cytoplasm conductivity when developing and applying predictive models.

- Experiments conducted in various bioreactor setups with different cell lines, including CHO cells and yeast.
- Off-line measurement of cell size, membrane capacitance, and cytoplasm conductivity.
- Development of conversion models to correlate real-time capacitance data with VCD.
- Identified key cell-specific parameters that significantly influence the accuracy of dielectric spectroscopy models.
- Real-time monitoring with Hamilton Incyte Arc sensors provided reliable data for model development.
- Highlighted the impact of variations in cell size and membrane capacitance on model performance.

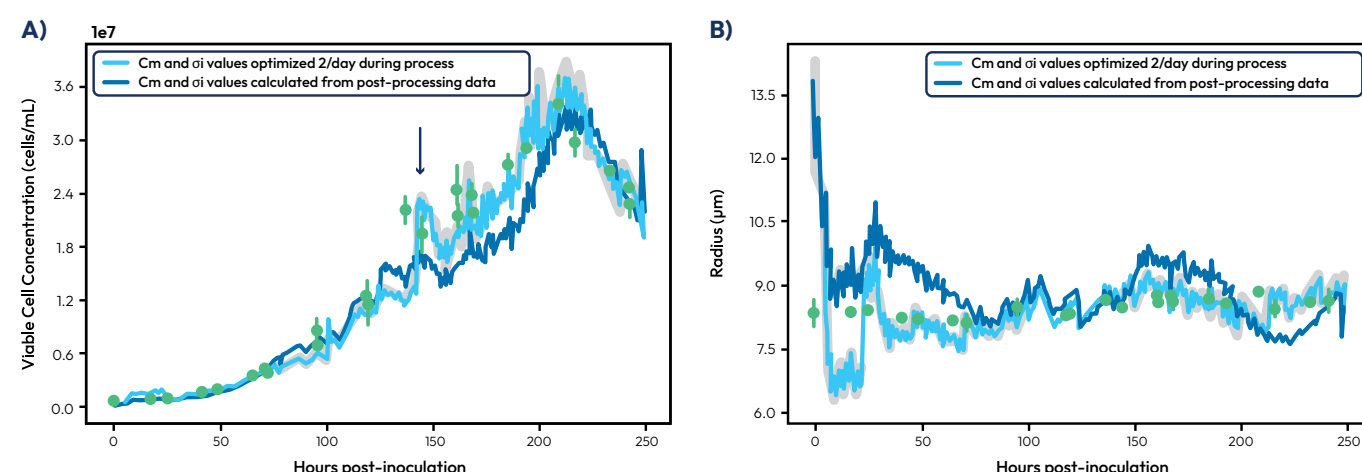


Figure 4: Comparison of the “adjusted” model with C_m and a_i values calculated twice per day during process (light blue curve) with the “fixed” model with constant C_m and a_i values calculated for the studied cell line (dark blue curve). (A) Viable cell concentration (VCC) estimate. (B) Radius estimate. Green dots: reference values obtained by sampling and off-line analysis. Error bars: off-line 95.0%-confidence interval. Gray highlighting: on-line 95.0%-confidence interval.

Conclusion: The study underscores the importance of considering cell-specific parameters in dielectric spectroscopy models used for monitoring VCD in bioreactors. The Hamilton Incyte Arc capacitance sensor was instrumental in collecting real-time data, which, when combined with off-line measurements, led to more accurate and reliable VCD predictions. This research contributes to the optimization of bioprocess monitoring and control, supporting more efficient and scalable biomanufacturing processes.

Advancing Raman Model Calibration for Perfusion Bioprocesses Using Spiked Harvest Libraries



<https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/biot.202200184>

Patrick Romann, Jakub Kolar, Daniela Tobler, Christoph Herwig, Jean-Marc Bielser, Thomas K. Villiger : November 2022

To enable real-time monitoring of multiple analytes in complex bioreactor matrices, this study develops a systematic Raman calibration workflow using spiked harvest libraries for perfusion bioprocesses.

- Perfusion bioreactors (Labfors 5 Cell, Infors HT) inoculated with a CHO-K1 cell line.
- Bioreactor conditions: 36.5°C, 50% DO (maintained by Hamilton's VisiFerm DO Arc), and pH 7.07 (maintained by Hamilton's EasyFerm Plus Arc).
- Capacitance measurements with Incyte Arc sensor to control VCV at 12%.
- Perfusion rate set at 1.3 reactor volumes per day (RV/day).
- Off-line calibration using the flow cell setup and subsequent model verification during perfusion runs.

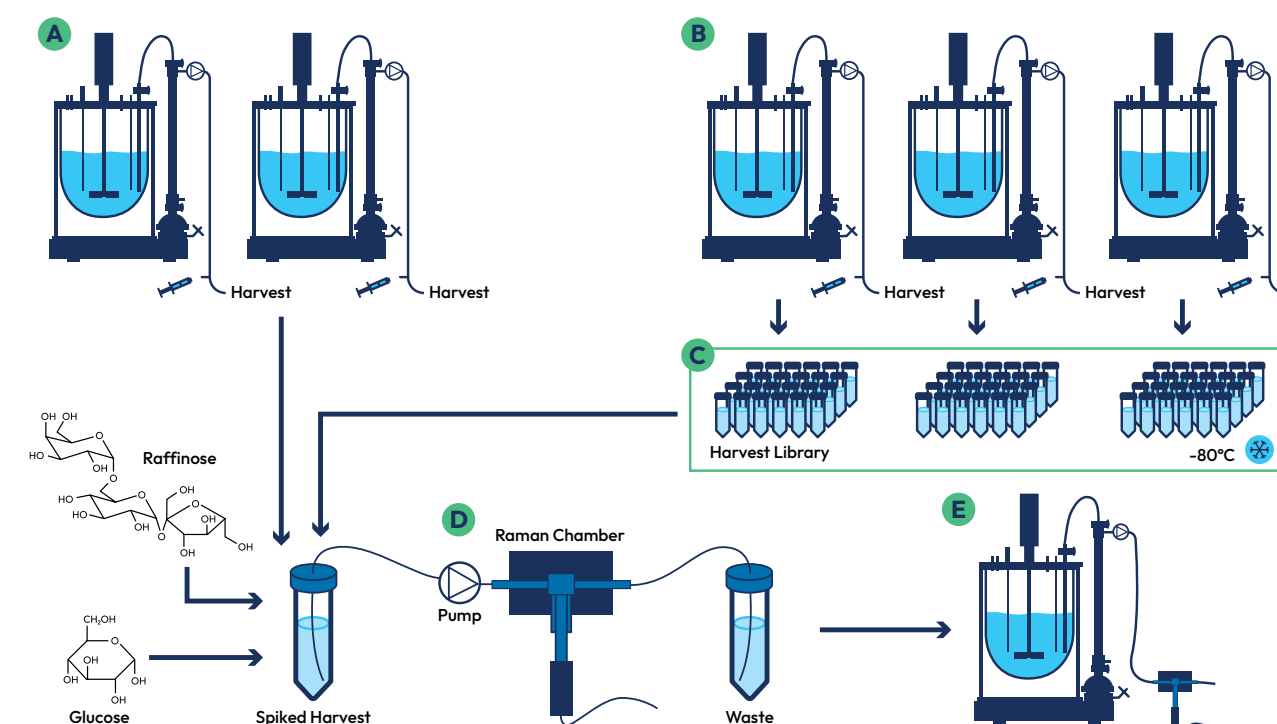


Figure 5: Schematic of the harvest library spiking approach. Fresh harvest samples of two reactors (A) and harvest samples of three reactors (B) which were frozen and stored in a harvest library (C) were used for model calibration. Harvests were spiked with glucose and/or raffinose and pumped through a Raman flow cell (D) to generate Raman calibration data. The flow cell was then connected directly to the bioreactor run (E) for model verification.

Conclusion: The study highlights the successful development and application of a spiking-based Raman calibration workflow for perfusion bioprocesses. The use of Hamilton sensors (VisiFerm DO Arc, EasyFerm Plus Arc, and Incyte Arc) was critical for maintaining optimal bioreactor conditions and providing reliable real-time data. The novel approach reduced calibration time significantly and improved model robustness, facilitating more efficient and scalable bioprocess monitoring.

Integrating Dielectric Spectroscopy for High-Titer Oncolytic Measles Virus Production



<https://www.hamiltoncompany.com/process-analytics/case-studies/app-note-high-titer-oncolytic-measles-virus-production>

Dustin Eckhardt and Prof. Dr.-Ing. habil Denise Salzig : November 2024

Oncolytic measles virus (MeV) is a promising cancer therapy which comes with challenges during biomanufacture for therapeutic applications. Determining the optimal time of harvest (OTH) to maximize infectious viral particle yield is particularly important, but traditional methods for assessing viral infectivity, such as plaque assays and TCID50 assays, are time-consuming and not suitable for real-time analysis during virus production.

To address these challenges, dielectric spectroscopy (DS) of Hamilton's Incyte Arc sensor was used as a non-invasive, in-line measurement of viable cell density that provides insights into cell viability, growth, and morphological changes during the infection process.

- Measles virus (MeV) is produced in Vero cells using Cytodex 1 microcarriers in a 1 L stirred tank reactor (STR) with a 0.5 L working volume.
- Viable cell concentration is monitored in-line using dielectric spectroscopy (DS) by measuring permittivity at 1 MHz.
- Total cell concentration is determined off-line by dissolving cells bound to microcarriers using crystal violet solution.

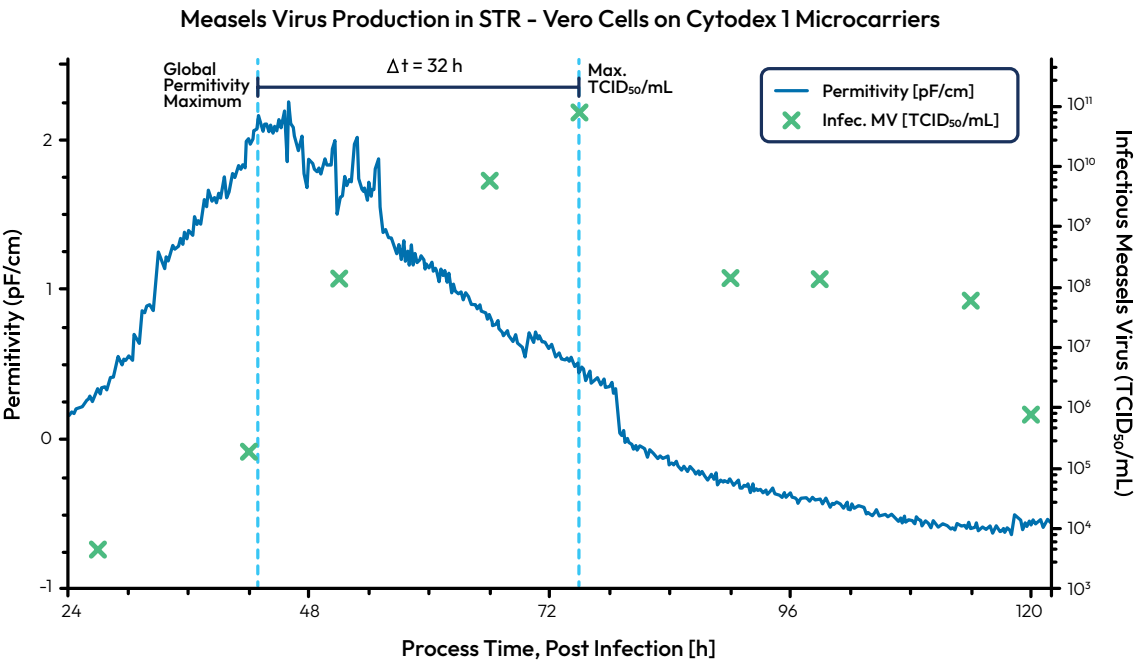


Figure 6: Relationship between global permittivity measurement using Hamilton's Incyte Arc sensor and infectious virus titer.

Conclusion: While DS cannot measure cell concentration in infected cells, it can predict syncytia formation and cell lysis timing, aiding in TOH prediction. The integration of DS is proposed to enhance the efficiency of oncolytic virus production, ultimately benefiting therapeutic applications.

Real-Time Cell Detachment Monitoring in Microcarrier Culture by Incyte Sensors



<https://www.hamiltoncompany.com/knowledge-base/application-note/app-note-real-time-cell-detachment-monitoring-in-microcarrier-culture>

Atefeh Ebrahimian, Harald Kühnel : January 2025

Microcarrier-based platforms have revolutionized scalable cell culture for diverse applications, from vaccine production to cell therapy and cultivated food. These cultures progress through attachment, growth, and detachment phases, and monitoring of the progression of each stage is important for diverse applications of microcarrier-based cell culture, especially for performing timely bioreactor transfer during scale-up and scale-down studies. Traditional off-line sampling and microscopy methods disrupt workflows, reduce precision, and increase contamination risk, especially at larger scales. Therefore suitable alternatives are required. In-line capacitance monitoring was used due to its ability to provide real-time insights into detachment status.

- MA 104 cells were cultured on Cytodex 1 microcarriers a 1-liter DASGIP bioreactor system (Eppendorf, DASGIP® Bioblock parallel system) with a 700 mL working volume.
- Capacitance measurements were performed at a frequency of 1 MHz.
- Permittivity signals were recorded during the cell detachment process (maximum duration of 1 hour).
- Off-line sampling and microscopy assessing cell detachment was used as a comparison.
- A predictive model was developed based on permittivity signals recorded during the detachment process to forecast final detachment outcomes.

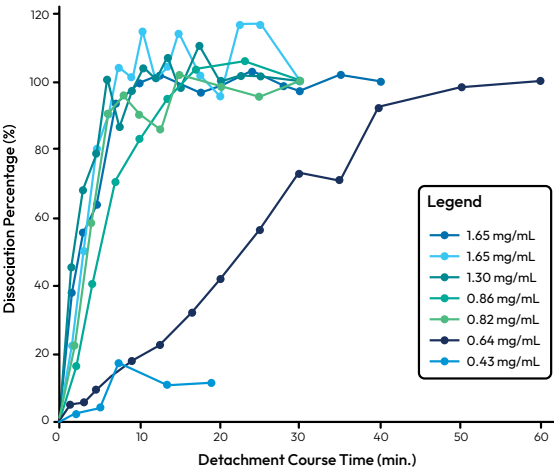


Figure 7: Cell dissociation monitoring by off-line sampling.

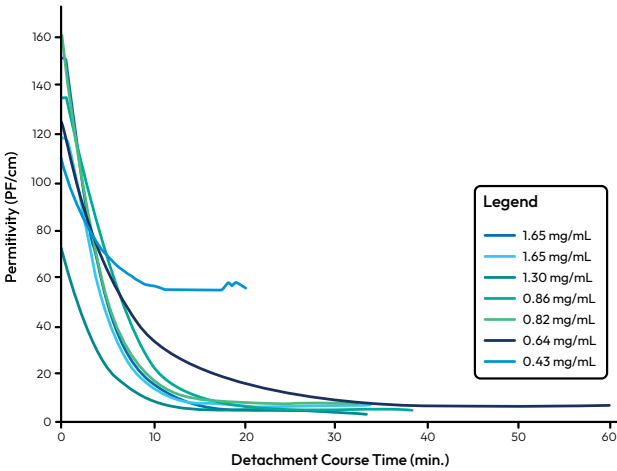


Figure 8: Permittivity signals from two Incyte sensors for two distinct enzyme concentrations: 0.86 g/L and 0.43 g/L.

Conclusion: The result of this study highlight the effectiveness of Hamilton's Incyte sensor for real-time monitoring of cell detachment in microcarrier cultures. This approach enhances detachment efficiency assessment and enables timely decision-making, optimizing bioprocessing workflows. The predictive modeling demonstrates potential for forecasting detachment outcomes, reducing reliance on off-line sampling, and minimizing contamination risks. Overall, the findings underscore the sensor's value in advancing scalable cell culture practices, particularly in applications like stem cell detachment.

In-Line Prediction of Viability and Viable Cell Density Through Machine Learning-Based Soft Sensor Modeling and an Integrated Systems Approach



An Industrially Relevant PAT Case Study

<https://aiche.onlinelibrary.wiley.com/doi/abs/10.1002/btpr.3520>

Sneha Suman, Michaela Murr, Jacob Crowe, Spencer Holt, Jakob Morris, Andrew Yongky, Kyle McElearney, Glen Bolton : November 2024

As the biopharmaceutical industry transitions to digital analytics for enhanced cellular understanding and high-quality product production, the limitations of traditional, labor-intensive off-line measurements become increasingly apparent as these methods often result in time delays, inconsistencies, and potential compromises to product quality. This study proposes using soft sensor models to predict viability and viable cell density (VCD) in CHO cell culture processes using in-line optical density and permittivity sensors. The authors present a simplified empirical model and demonstrate its adoption as part of an integrated systems approach for in-line viability prediction.

- Created soft sensor models for predicting cell viability and viable cell density (VCD) using in-line optical density and permittivity sensors integrated into bioreactors.
- Conducted experiments across three scales (3 L, 7 L, 200 L) to collect real-time data on cell culture processes, using run-specific coefficients for accuracy.
- Employed Leave-One-Out Cross-Validation (LOOCV) to validate model performance against off-line measurements, ensuring predictions maintained a $\pm 5\%$ error margin.
- Evaluated over a hundred modeling techniques, selecting the Python Gaussian Process Regressor (GPR) with Matern Kernel as the best-performing model.
- Utilized the integrated models for continuous monitoring of viability and VCD, supporting enhanced process control and data-driven decision-making.

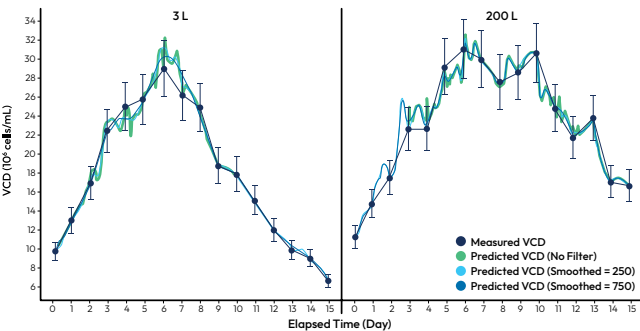


Figure 9: Representative Control Runs at 3 and 200 L-scales comparing off-line measured viable cell density (VCD) and in-line predicted VCD.

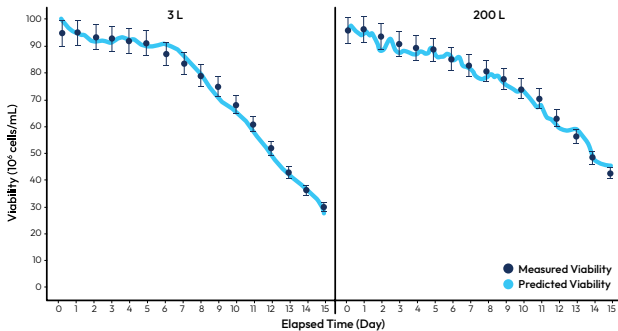


Figure 10: Integrated Systems Approach for Computing Continuous Viability at 3 and 200 L-scales.

Conclusion: The model demonstrated robust real-time in-line prediction of viability and VCD across various scales and process conditions, outperforming conventional models. This study highlights the potential to significantly reduce reliance on labor-intensive off-line measurements. By aligning with regulatory guidelines, these innovative machine-learning-based technologies pave the way for advancements in biomanufacturing, promising improved process control, efficiency, and adherence to quality standards. The findings underscore the critical role of advanced data-processing techniques in minimizing manual measurements, driving innovation within the biopharmaceutical industry.

Application of an Exhausted Fermentation Broth Obtained from Biohydrogen Production in an Apple Orchard: Assessment of Fruit Quality



Fermentation, Recycling of Byproduct

<https://www.mdpi.com/2311-5637/9/7/628>

Michele Galiano, Giulio Demetrio Perulli, Brunella Morandi, Diana Di Gioia : July 2023

This study investigated the use of biohydrogen production byproduct (BEFB) from *Clostridium beijerinckii* DSM34075 as a foliar biostimulant in an apple orchard, focusing on its impact on fruit quality and its potential for sustainable agriculture.

- A 4 L bioreactor (Solaris Biotechnology, Porto Mantovano, Italy) was used for the fermentation process.
- The bioreactor was equipped with pH, electrical conductivity, and dissolved oxygen sensors.
- The Hamilton Dencytee Total Cell Density sensor was used to monitor the cell density.
- Fermentation conditions: 37°C, 500 rpm, pH maintained at 6.0 using NaOH 1 M.
- The fermentation was conducted in fed-batch conditions with periodic collection of the exhausted broth.

Conclusion: BEFB, obtained from biohydrogen production, can be effectively used as a biostimulant in apple orchards. The Hamilton Dencytee sensor was critical in monitoring cell density during fermentation, ensuring optimal production of the biostimulant broth. The application of BEFB resulted in improved fruit weight and dry matter content, making it a promising sustainable agricultural input. Further research is needed to optimize application doses and strategies.

Salmon Cell Suspension Cultures Grown with a Perfusion System Yields Viable Cell Densities >100 Million cells/mL



<https://helda.helsinki.fi/server/api/core/bitstreams/b8187c72-5737-4246-ae0b-59ee959a5659/content>

Daisy Ogawa, Jian Zhang, Michael Kagan, Alex Oreshkov, Grace Zhou, Aaron Elfenbein : October 2023

To advance the commercialization of cultivated meat and seafood, this study investigates a perfusion system for cultivating salmon cell suspensions, achieving viable cell densities exceeding 100 million cells/mL and significantly enhancing bioprocess productivity.

- High perfusion and low perfusion conditions tested.
- High perfusion: 42 vessel volumes (vv), 50L media over 26 days.
- Low perfusion: 36 vv, 43 L media over 27 days.
- Viable cell densities reached >100 million cells/mL with >93% end viability.
- Vi-Cell (Beckman Coulter) for VCD and viability.
- Flex2 (Nova) for pH, dissolved oxygen, CO₂, and metabolite analysis.
- Hamilton Incyte sensor for permittivity measurements as a proxy for viable cell mass.
- Media yield for perfusion bioreactors was lower compared to fed-batch processes (3.7 g/L media for perfusion vs. 10-12 g/L media for fed-batch).
- Identification of critical cell-specific perfusion rate (CSPR) necessary to optimize media consumption and reduce costs.



Conclusion: The study demonstrates that a perfusion system can significantly enhance cell density and bioreactor productivity for cultivated salmon cells. The Hamilton Incyte sensor was crucial for real-time monitoring and control of cell growth, facilitating the successful implementation of the perfusion process. Further optimization is needed to reduce media consumption and production costs, making the process more economically viable for large-scale applications. This innovative approach shows promise for improving the efficiency and scalability of cultured meat and seafood production, supporting the transition from research and development to commercial-scale manufacturing.

Unlock the Secrets of Bioprocessing Excellence



Cell Density Brochure



Biopharma Brochure



Cultivated Food Brochure



PAT in Biopharma White Paper



In-Line Sensors for Process Analytics



Process Analytics Catalog



Cultivated Meat White Paper



PAT in Fermentation White Paper



Process Analytics Cell Density Sensors



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