Simplifying Progress

Comparison Guide

Ambr® 250 High Throughput

Advancements From First to Second Generation for Enhanced Process Characterization Studies

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Abstract

This document provides an overview of the differences between the Ambr® 250 High Throughput Generation 1 and the Ambr® 250 High Throughput Generation 2. We first outline the new features, in particular the improved scale-down performance thanks to continuous gassing, before comparing the performance between the two systems.

These enhancements mark significant progress in the process characterization capabilities of scale-down bioreactors, offering biopharmaceutical companies new avenues to refine their processes and accelerate development timelines. With improved scalability, data integrity, analytics, and user experience, the Ambr® 250 High Throughput Generation 2 sets a new standard for efficiency and precision in bioprocessing.

Introduction

Since its launch in 2013, the Ambr® 250 High Throughput system has been adopted globally across the biopharmaceutical industry. The Ambr® 250 High Throughput supports small volumes and has a compact footprint, significantly enhancing bioprocess development capacity and throughput. This has enabled biopharmaceutical companies to advance quality-by-design (QbD) approaches and accelerate development timelines while significantly reducing the cost and environmental impact of their operations.

Time-saving single-use bioreactors combined with powerful software and highly consistent automation allow upstream scientists to achieve greater precision, reduce manual labor, and streamline their interactions with the system. The robust and flexible user interface enables process developers to design and implement a wide variety of process control schemes.

A key feature of the Ambr® 250 High Throughput is its proven scalability to large-volume bioreactors.[1–4] In many cases, the system has been fully implemented in late-stage process development as a qualified scale-down model of both pilot and production-scale bioreactors,[1–4] highlighting its value as a robust process development tool.

These diverse features have established the Ambr® 250 High Throughput as the industry standard for high throughput bioreactor systems, with installations at most major biopharmaceutical companies and contract development and manufacturing organizations (CDMOs). The system has been deployed across a range of mammalian and microbial cell culture applications, including clone and strain screening, media development, process optimization, process characterization, and commercial manufacturing support.

This has created a uniquely powerful combination of flexible capabilities covering a wide range of process types and operating schemes, along with the benefits of globally standardized technology. This synergy offers significant benefits throughout the upstream process development workflow as projects progress, including technology transfer, in-sourcing and out-sourcing, operator training, and transposable skillsets, both within and between companies, locally and globally.

However, every technology operates within limits, and there is always potential for improvement. The Ambr® product development team at Sartorius constantly strives to evolve and improve our products, further expanding the capabilities of the bioprocess industry and simplifying progress in the development of new biopharmaceutical products supporting unmet patient needs.

Through close collaboration with the diverse community of Ambr® 250 High Throughput users, we have developed the second-generation Ambr® 250 High Throughput system ´Ambr® 250 High Throughput Generation 2´. We identified, developed, and implemented technical improvements in many key areas, with a particular focus on enhancing the system's capability as a qualified scale-down model for process characterization studies. While the Ambr® 250 High Throughput is already well established in many major biopharmaceutical companies, broader adoption for process characterization across the industry will enable users to benefit from improved data quality, more refined processes, faster time-to-clinic and time-to-market, and reduced costs. These advantages are particularly valuable for the large, thorough, and controlled experimental programs required for process characterization.

Key New Features of the Ambr® 250 High Throughput Generation 2

Advanced Gassing Module
Provides continuous gas flow to the vessels, better representing large-scale bioreactor performance and control

Four-Way Gas Supply
Valve enables switching between nitrogen and air for different runs

Key New Features of the Ambr® 250 High Throughput Generation 2

Redesigned and Optimimized Software User Interface
Streamlines user interactions while maintaining core structure and functionality

User Access Control, Authentication, and Data Integrity
Helps to satisfy regulatory requirements

Key New Features of the Ambr® 250 High Throughput Generation 2

New CO_2 Exit Gas Sensor
Increases service life, reduces drift, and automates calibration

Online BioPAT® Viamass Capacitance Sensors
Matches analytical capabilities to larger scale Sartorius bioreactors for continuous growth and cell density monitoring

Six Additional Vessel Variants
Increases workflow flexibility with capacitance and pCO_2 sensors, alongside an improved microsparger design in fed-batch and perfusion vessels

Key New Features of the Ambr® 250 High Throughput Generation 2

Keypad With Lighting and Alarm Controls
Quick access buttons for cabinet lights, muting alarms, and stopping the liquid handler

Embedded Vision System
Enables remote monitoring of the system's status, facilitating troubleshooting and reducing out-of-hours checks

Exterior Analytics Furniture
An interconnectable design supports simple setup, and multiple computers can be operated from a single user interface

New Tip Waste Bin
Reduces frequency of tip bin emptying

Sartorius Tips and Flexible Deck
Increases process flexibility with 1 mL and 10 mL tips, and more operator walk-away time

Scale Down Performance

The supply and control of dissolved gases is a major topic in the scalability of bioreactor processes. Geometric factors and physical principles such as k_La, bubble regime and agitation, are among the most critical aspects. The Ambr® 250 High Throughput Generation 2 includes a major end-to-end improvement of the gas flow schemes (Table 1). We worked intensively with supply partners through many design iterations to replace the pulsed gas supply with a new and improved advanced gas flow control module, which provides a fully continuous and independent gas supply to both sparger and headspace in parallel (Figure 1). This feature delivers an exceptional turndown ratio, providing fine control from low- to high-end for mammalian and microbial cultures, all in a single, flexible module.

The new gas flow control scheme more closely aligns with larger-scale bioreactors, enhancing scalability. Changes to the bubble regime led to increased k_La (Figure 2), enabling the system to support higher cell density cultures and | or cell types with higher oxygen demand. The reduced air | O_2 flow rates required to support the same culture oxygen demand may also reduce foaming.

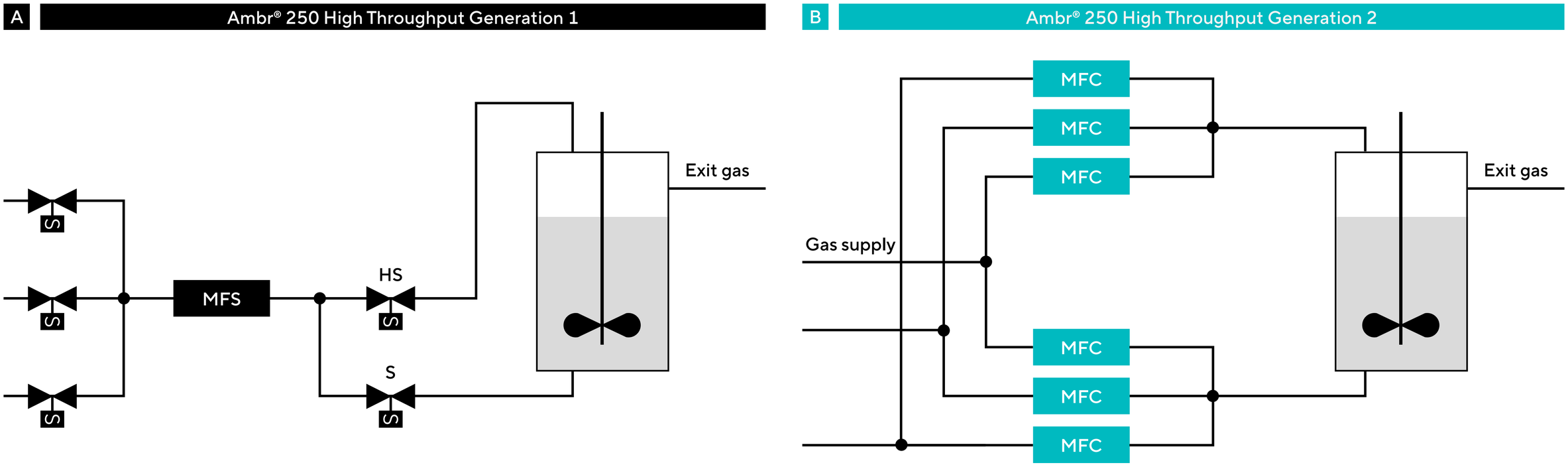
More refined control of dissolved gases in the culture also supports process performance and experimental consistency (Figure 3). Gas supply control is fully independent for the sparger and headspace, offering greater scope for pCO_2 modulation to better match pCO_2 profiles in larger reactors.

Finally, fine control over the mixture of the bioreactor supply gas allows for fully automated recalibration of exit gas sensors (where fitted), as directed by the user through the software interface.

Table 1: *Comparison of Gassing Systems in the Ambr® 250 High Throughput Generation 1 and Generation 2*

Ambr® 250 High Throughput Generation 1	Ambr® 250 High Throughput Generation 2
Three-way gas supply connection (N_2 | air, O_2, and CO_2)	Four-way gas supply connection (N_2, air, O_2, and CO_2)
Three process gases, fixed by supply connection	Three process gases, selected by the user via the software interface
Process gas supply flowrate modulated by the valve on | off time (intermittent gas supply at a fixed flowrate)	Process gas supply flowrate modulated by value open % (continuous gas supply at a variable flowrate)
Three pulsed (on | off) valves for gas metering, one mass flow sensor, plus pulsed valve/s directing gas to sparger and/or headspace	Three mass flow controllers to the sparger, plus three mass flow controllers to the headspace
Generation 1 exit gas CO_2 sensor	Generation 2 exit gas CO_2 sensor with increased sensor life and reduced drift
Generation 1 exit gas CO_2 sensor calibration via service engineer	Generation 2 exit gas CO_2 sensor calibration is fully automated, via the user interface

Figure 1: *Schematic of **(A)** Pulsed Gas Supply in the Ambr® 250 High Throughput Generation 1 vs. **(B)** Continuous Gas Supply in the Ambr® 250 High Throughput Generation 2*



Note. MFS = mass flow sensor, MFC = mass flow controller

Figure 2: *k_La Values for Ambr® 250 High Throughput Generation 2 (•) Compared to Generation 1 (•) at 250 mL Fill Volume*

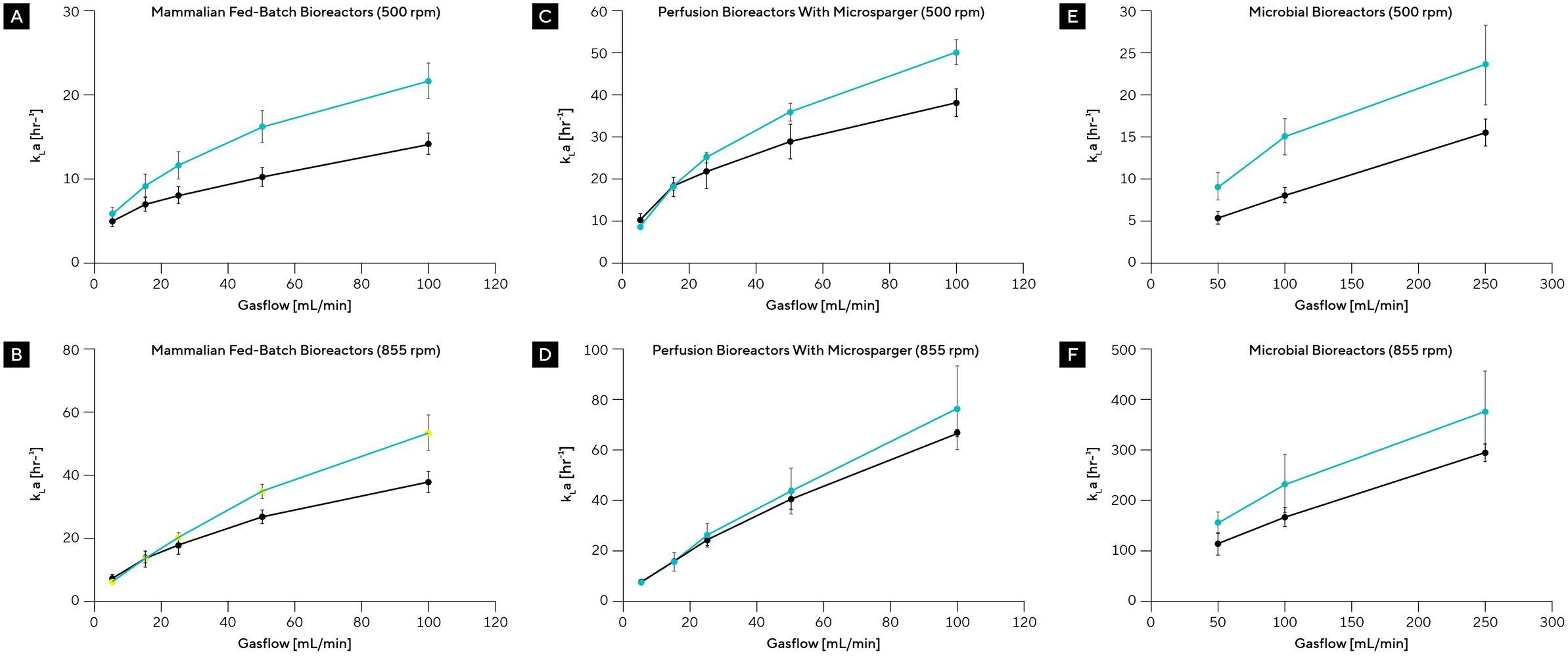
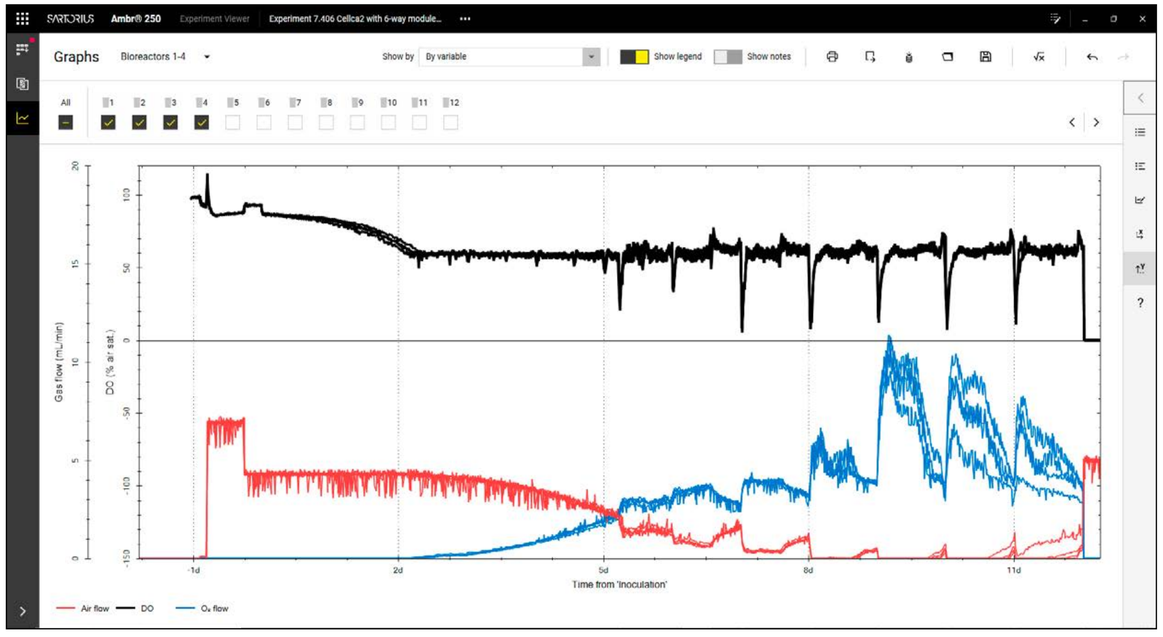
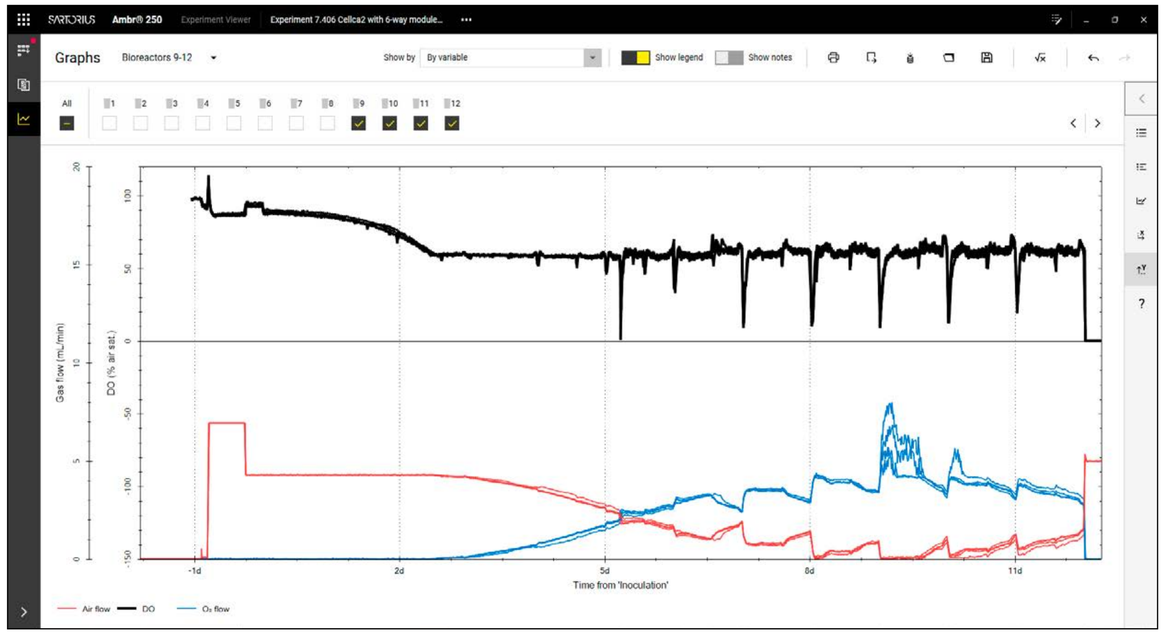


Figure 3: *CHO Fed Batch Cell Culture Gas Flow Traces from Ambr® 250 High Throughput* ***(A)*** *Generation 1 (N=4) and* ***(B)*** *Generation 2 (N=4) systems, Visualized in the Generation 2 Software*

A Ambr® 250 High Throughput Generation 1



B Ambr® 250 High Throughput Generation 2

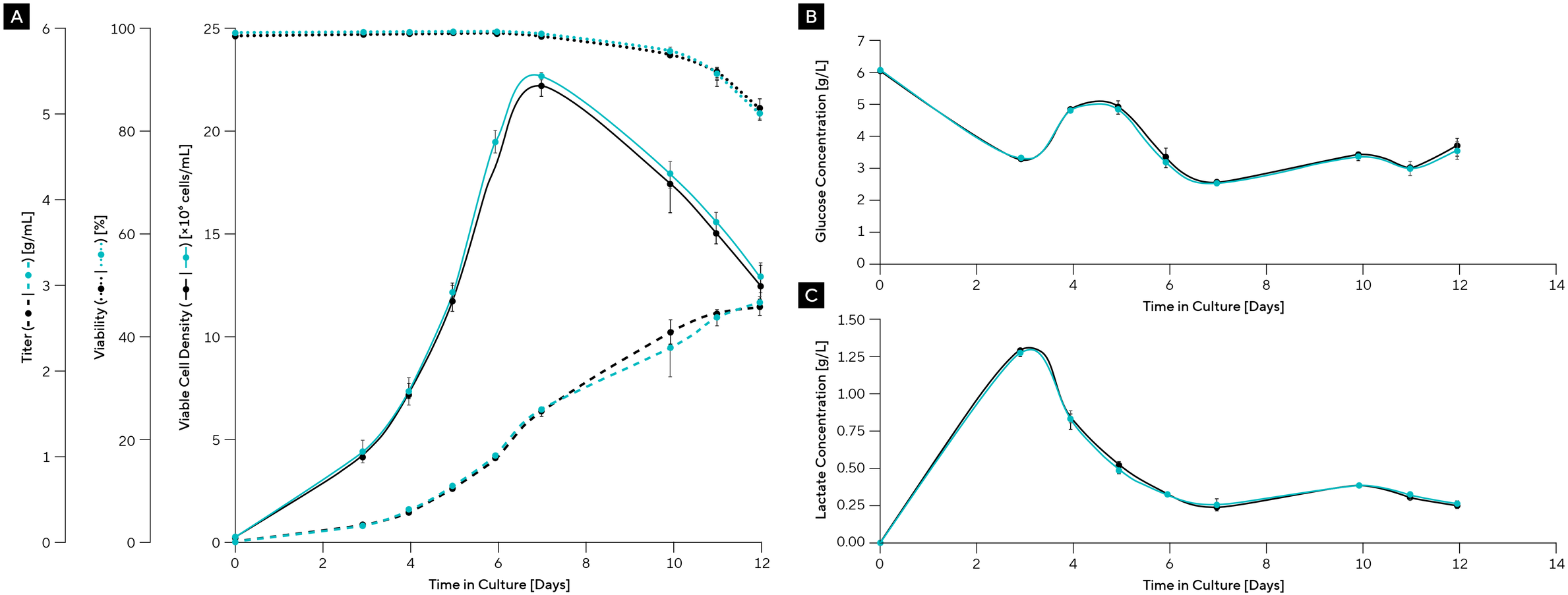


Cell Culture Performance

The continuous gas supply provided by the new advanced gassing module provides more refined control of dissolved gases in culture, supporting process control (dissolved oxygen and pH) and experimental consistency (Figure 3). Due to the higher k_La with continuous gas supply, the O_2 flows required for the same culture are also noticeably reduced (Figure 3, blue lines). Importantly, our non-optimized fed-batch CHO cell culture test shows similar performance and consistency for the Ambr® 250 High Throughput Generation 1 and Generation 2 in terms of viable cell density (VCD), viability (%), titer, and metabolites (Figure 4).

These results show that, when identical process conditions are used, the Ambr® 250 High Throughput Generation 1 and Ambr® 250 High Throughput Generation 2 can be operated in parallel or at different stages of the workflow with very similar cell culture performance results. We anticipate that further work with the enhanced technical capabilities of the Ambr® 250 High Throughput Generation 2 will allow end users to further optimize small-scale process conditions and scale-down models, improving match to large-scale process performance. This will further support the implementation of the Ambr® 250 High Throughput Generation 2 as a qualified scale-down model for late-stage characterization studies.

Figure 4: *Comparison of **(A)** Viable Cell Density, Viability, and Titer; **(B)** Glucose; and **(C)** Lactose in Fed-Batch CHO Cell Cultures in the Ambr® 250 High Throughput Generation 1 (N=4) (●) and Ambr® 250 High Throughput Generation 2 (N=4) (●)*



Software

The Ambr® 250 High Throughput Generation 2 software user interface streamlines operator workflows, reducing clutter and cognitive load, and allowing for faster user interactions.

For new users implementing the Ambr® 250 High Throughput Generation 2 to replace manually operated benchtop bioreactor systems, some training of bioreactor operators will be required. In organizations with large benchtop installations, this may mean retraining many manual bioreactor operators and scientists to operate the Ambr® 250 software.
To facilitate this transition, the new Ambr® 250 High Throughput Generation 2 user interface has been redesigned and optimized according to industry standard guidelines (ISO 9241-210:2019 "Human-centred design for interactive systems“). These improvements make the software easier to navigate for new users, including more visual cues and supporting information presented to the user at the point of interaction.

For existing users of the Ambr® 250 High Throughput Generation 1, while the user interface has a new look and feel, the core functionality and underlying structure have been fully preserved. Enhanced user guidance embedded directly in the software design and the new Help Center supports the quick transition to the new user interface with only modest familiarization time. This Help Center contains the software manual as a series of separate PDF documents, divided by system function or topic, a search function, and a direct link to the Sartorius Learning Centre, which hosts the current Ambr® 250 High Throughput training videos.

The transition of experimental protocols (process definitions) and data files to Ambr® 250 High Throughput Generation 2 is straightforward, as the new software is fully backward compatible with existing processes and output data files created using previous software versions (Figure 3).
To facilitate software platform version management for both Ambr® 250 High Throughput users and Sartorius, Generation 2 software will be made available to existing users of the Ambr® 250 High Throughput Generation 1 systems with an eligible service contract through annual preventative maintenance visits from 2026 onwards.

Data Integrity

User requests for improvements around data integrity have been prioritized and implemented. This facilitates the deployment of the Ambr® 250 High Throughput system where the data package will form part of a regulatory submission. Please note that Ambr® 250 High Throughput Software was not developed for GMP use and is not 21 CFR 11 compliant.

Added software features supporting data integrity:

- **User login** — the software is now password protected so that the user actions and process changes can be tracked in the audit.
- **Audit process changes** — any changes made to the process (recipe) are tracked and highlighted for each user.
- **Tamper-evident data files** — enables users to demonstrate that underlying files of an experiment have not been tampered with (manually edited).
- **Sample ID configuration** — addressing the requirement for unique sample identifiers, sample IDs can now be automatically created in a user-defined format, seamlessly interfacing with existing data workflows and improving data tracking.
- **Master recipes** — users can now save a process as a 'master process' that remains unchanged, providing a reference point. While the file can be edited to run a process, any changes create a new process file, preserving the master version.
- **Configuration and process comparison** — users can compare processes and system configurations to better track changes made during process characterization campaigns.

Analytical Capabilities

The Ambr® 250 High Throughput Generation 2 now has an option for embedded capacitance measurement, providing online monitoring of BioPAT® Viamass (capacitance) in bioreactor vessels equipped with BioPAT® Viamass sensors. This feature has been developed and tested for cell culture applications (it has not been tested with microbial cultures).

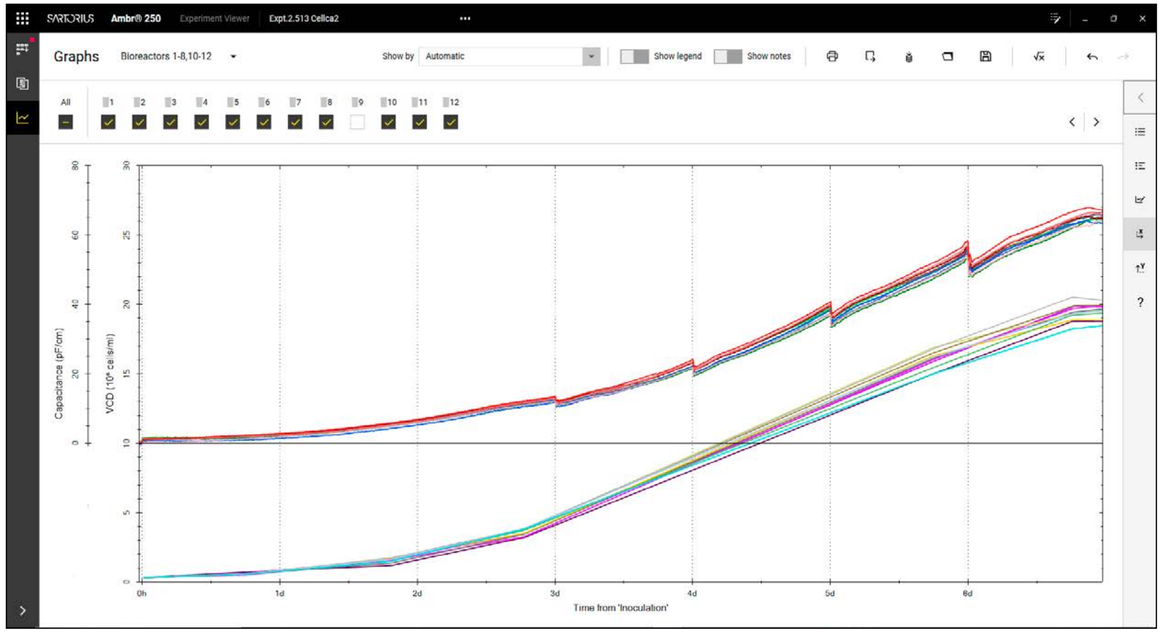
BioPAT® Viamass provides continuous, consistent, and sensitive readings, with process events such as cell culture dilution by daily feed additions clearly visible (Figure 5A). BioPAT® Viamass capacitance correlates well with VCD counts (Figure 5B), and the software includes a calibration function to allow automated calculation of 'continuous VCD value' based on a combination of online capacitance and offline cell count.

Feedback process control operations based on viable cell density (e.g., inoculation transfer, parameter shift, feed addition, transfection, harvest) can be better optimized when based on an online value, both with respect to time resolution and reducing variability associated with sampling and optical cell counting. The capacitance technology used is aligned with BioPAT® Viamass throughout the Sartorius bioreactor range, supporting the scalability of both bioreactor processes and associated analytics.

Electronics for capacitance signal measurement are held within capacitance clamp plates, included as part of the Ambr® 250 High Throughput BioPAT® Viamass Option. This analytical option is suitable for a range of mammalian cell culture applications, including new perfusion vessel variants and a small number of fed-batch vessel variants, fitted with both single-use pCO_2 sensor spots and single-use capacitance sensors.

Figure 5: ***(A)*** *Continuous BioPAT® Viamass Capacitance Trends Compared to Parallel Daily VCD Counts, for a 7-Day Fed-Batch CHO Cell Culture (N = 11),* ***(B)*** *Scatter Plot of BioPAT® Viamass Capacitance and VCD Counts*

A



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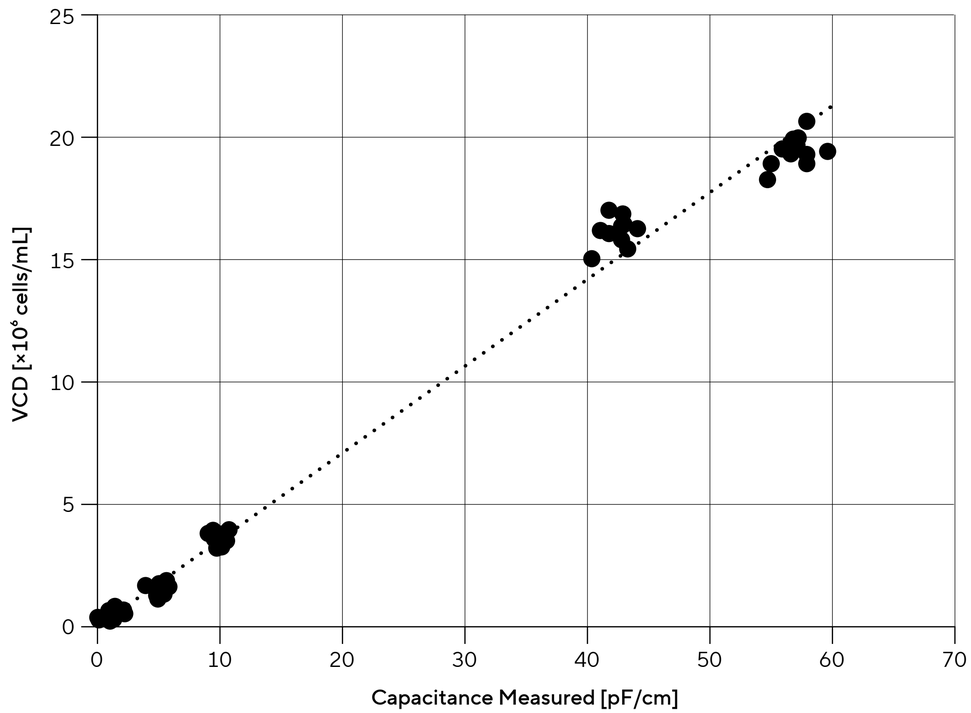


Note. VCD = Viable cell density.

Workflows and Robustness

Vision System

The Ambr® 250 High Throughput Generation 2 features real-time cameras to enhance monitoring capabilities. Each 12-vessel system includes one bed-view camera, while each 24-vessel system is equipped with two cameras. Additionally, a camera is installed on the liquid handler, allowing users to view pipetting and gripper operations, as well as bioreactor stations.

System Cameras
System vision cameras enable users to visually monitor the inside of the workstation cabinet from the system control computer. The camera views are real-time, with no recording or playback available, ensuring the system control computer processing capacity is reserved for process support and control.

Depending on the IT policy and setup, remote desktop capabilities may allow users to monitor and troubleshoot the system status from remote locations. This reduces the need for out-of-hours site visits, supports faster intervention, and improves overall system robustness.

Liquid Handler Camera
The liquid handler camera can be directed to individual bioreactor locations, allowing users to inspect the culture in each bioreactor, view the foam layer, and make decisions about antifoam addition. A design change to the bioreactor cooling jacket, specifically the removal of the bridge, significantly improves visibility into the bioreactor from the front, particularly around the level of the culture surface. The camera also provides a real-time view of pipetting and lid gripper operations, supporting remote intervention and helping to diagnose any alarms, boosting overall robustness.

Integrated Analyzer Furniture

The Ambr® 250 High Throughput Generation 2 has an added option for integrated analytics furniture. This is a set of three laboratory benches for the control PC, monitor, and integrated third-party analyzers, designed for flexible setup according to the analyzer and laboratory configuration. This modular design concept was optimized to simplify the setup of optional integrated analyzers and associated control computers, such as a cell counter, BioProfile® FLEX2, and spectrometer.

The furniture includes an adjustable monitor stand and keyboard, video, mouse (KVM) switch, allowing the user to conveniently switch between and manage the Ambr® 250 High Throughput and integrated analyzer control computers from a single user station.

Transitioning From the Ambr® 250 High Throughput Generation 1 to the Ambr® 250 High Throughput Generation 2

To support user flexibility and extend the life cycle of existing Ambr® 250 High Throughput installations, two major new elements of the Ambr® 250 High Throughput Generation 2 will be made available for field upgrade to most existing platforms (those with serial number 132+), in addition to the Generation 2 software as described above. Field upgrades for both the BioPAT® Viamass monitoring system and the continuous gassing system will be made available after the series production of Ambr® 250 High Throughput Generation 2 systems has been fully established.

The new capabilities of the Ambr® 250 High Throughput Generation 2 will support users of earlier systems (SN 1-131) to move forward with life cycle replacements of these earlier installations where required.

Figure 6: *1 mL and 10 mL Sartorius Ambr® Automation Filtered Pipette Tips*

Keypad With Lighting, Alarm, and Liquid Handler Controls

The new quick-access keypad features buttons for activating cabinet lights, muting alarms, and pausing the liquid handler, and it offers customization options for button press actions.

Pipette Tips and Flexible Deck

The Ambr® 250 High Throughput Generation 2 features an updated flexible bed design compatible with 1 mL and 10 mL Sartorius Ambr® automation pipette tips (Figure 6). The boxes fit into any tip box location on the new flexible bed, and there is no change to the total number of pipette tip box locations. Since 1 mL pipette tips can be used to sample from bioreactor vessels, the new flexible bed provides both increased process flexibility and more walk-away time for operators, because a significantly increased number of pipette tips capable of sampling operations can be placed on the system.

Additionally, a rigid paper waste container, including a liquid-resistant surface coating, liquid absorption pad, and sealing mechanism is now available for the Ambr® 250 High Throughput pipette tip eject station. This simplifies the collection and disposal of ejected tips and reduces the frequency of tip bin emptying.

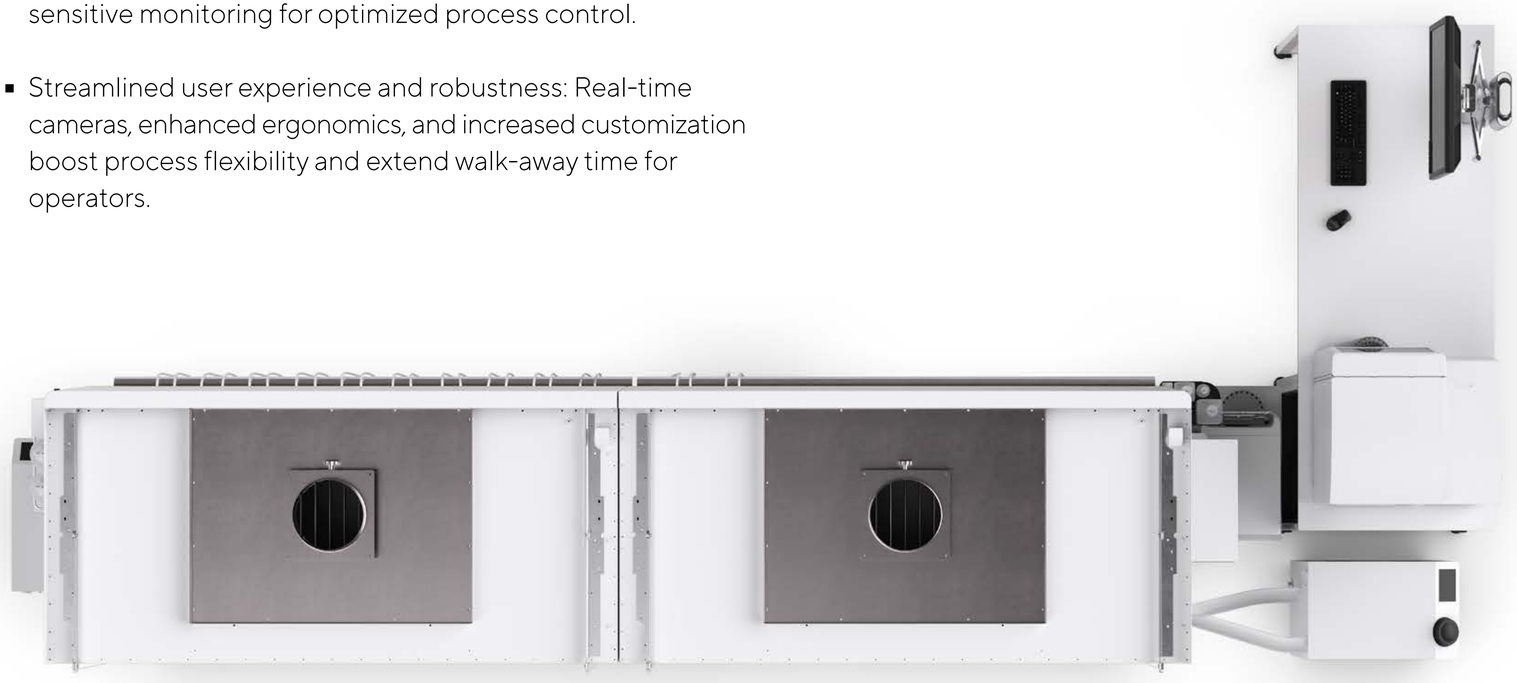
Conclusion

The Sartorius mission to empower scientists and engineers to simplify and accelerate progress in life science and bioprocessing, enabling the development of new and better therapies and more affordable medicines, means we are committed to continuous innovation. Transitioning to the Ambr® 250 High Throughput Generation 2 offers substantial benefits for process characterization in the bioprocess industry.

Key technical advances include:

- Improved scale-down performance: Enhanced continuous gassing provides precise control over dissolved gases, improving scalability and supporting higher cell density cultures.
- Updated software: The new user interface facilitates faster interactions and maintains backward compatibility for a seamless transition.
- Strengthened data integrity: Features like audit tracking and tamper-evident files bolster data integrity, supporting regulatory submissions.
- Enhanced analytical capabilities: Integration of BioPAT® Viamass capacitance measurement offers continuous, sensitive monitoring for optimized process control.
- Streamlined user experience and robustness: Real-time cameras, enhanced ergonomics, and increased customization boost process flexibility and extend walk-away time for operators.

These advancements mean the Ambr® 250 High Throughput Generation 2 offers significantly improved process characterization capabilities, enabling biopharmaceutical companies to refine their processes, accelerate time-to-market, and reduce operational costs.



For further information, visit
sartorius.com/en/products/fermentation-bioreactors/ambr-multi-parallel-bioreactors/ambr-250-high-throughput

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