Raman single-use technology for bioprocessing

Real-time composition analysis for biopharmaceutical development and manufacturing

Offering at a glance

- A flexible Raman sampling portfolio for reusable or single-use bioprocessing, including:
  - Raman optic system for single use
  - Raman bio multi optic and bio sleeve
  - Raman Rxn-46 probe
- Each probe system uses a patented self-focusing lens with automatic alignment for optimal spectral quality and transferability

Endress+Hauser
People for Process Automation

Single-use technology

The wave of the future in bioprocessing

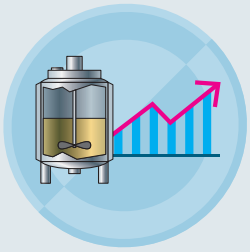
Single-use technology has transformed the world of biopharmaceutical production from process development to commercial drug manufacturing. Single use refers to any process contact equipment designed to be used once, whether for one batch or an entire manufacturing cycle, before being replaced. Components, assemblies, and systems for single use are highly advantageous because they eliminate the need for expensive and labor-intensive end-user cleaning, sterilization, and component validation. Single-use systems also provide cost savings due to reduced use of materials, energy, and water, along with lower cross-contamination risk and faster manufacturing times.

Single-use adoption The adoption of single-use technology for bioprocessing continues to increase, driven by more targeted and personalized medicine, increasing costs, and time-to-market pressures. Recent developments in single-use systems have rendered them more user-friendly and efficient, enabling continuous operation and plug and play modules. With these enhancements, manufacturers can now progressively convert their disposable production facilities into interconnected, fully enclosed unit operations with continuous processing.

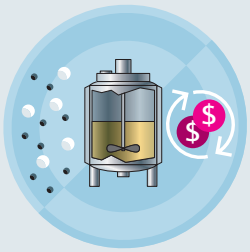
No compromise Nevertheless, the risks of using disposable components remain, primarily the possibility of migration of undesired materials from the plastic materials used in their construction. While material quality standards need to be maintained to ensure product quality, improvements in accuracy, resilience and robustness are required to allow more accurate process control and minimal chance of batch loss.

The need for reliable single-use sensing To satisfy these new requirements, more effective single-use sensing technologies are in demand. Single-use process measurement technology must offer equivalent reliability and accuracy compared to their reusable counterparts. In addition, it must still provide key attributes such as minimized hold-up volume, an ergonomic design, full cGMP compliance, sterility, and cost optimization.

Your trusted partner for Raman single-use bioprocess measurement technology Built upon our long history in cGMP manufacturing with many proven successes, Endress+Hauser has developed an innovative portfolio of Raman spectroscopy single-use bioprocess analysis tools that meet all these requirements. For critical bioprocess monitoring and control, you can rely on our Raman instrumentation and global service experts to fully support your single and multi-use biopharmaceutical manufacturing operations.

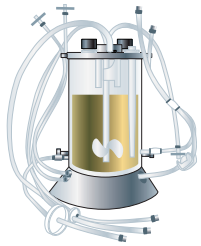


$38.8
billion (USD)
market size for single use bioprocessing globally by 2030

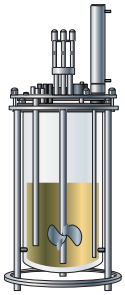


50%
of new biotech projects
use single-use technology

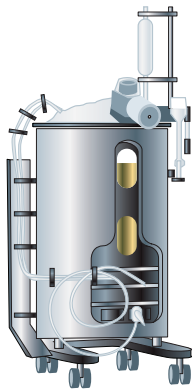
Single-use vs multi-use bioreactor setups



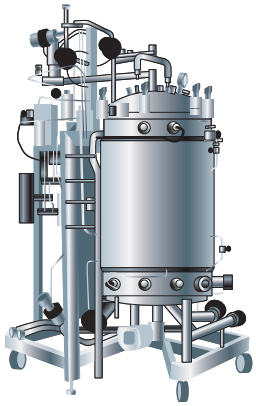
Process development (PD, lab-scale) single-use bioreactor



PD multi-use bioreactor



Production-scale single-use bioreactor



Production-scale multi-use bioreactor

Most biopharmaceutical operations today employ a mix of single-use and multi-use vessels, instrumentation, and sensors. A single-use bioprocessing setup typically contains a benchtop disposable vessel (lab-scale) and/or a disposable bag and multi-use vessel (pilot and production scale). Single-use bioreactors are typically flexible systems that can easily be moved, removed, or expanded depending on production demand. In contrast, multi-use bioreactors are made of glass (lab-scale) or stainless steel (pilot and product scale) and they are more permanent setups typically built to a specified capacity.

Raman spectroscopy and single-use As the industry transitions from batch to continuous processing and single to multi-use, manufacturers continue to seek reliable in-line Raman spectroscopy-based measurement methods that meet strict cGMP requirements but are flexible enough to be used in either single-use or multi-use bioprocessing environments.

Requirements of single-use Raman instrumentation

- High reliability
- High accuracy
- Specifications similar to stainless steel
- cGMP compliance
- Traceable calibration
- Digital communication

With regard to the process analysis technology (PAT) initiative of the US Food and Drug Administration (FDA), it is necessary to monitor several process parameters to ensure the quality of biotechnological products. In this context, sensors for online monitoring in disposable bioreactor systems are urgently needed."[1]

[1] Eibl, Regine. Single-Use Technology in Biopharmaceutical Manufacture. Available from: VitalSource Bookshelf, (2nd Edition). Wiley Global Research (STMS), 2019

Endress+Hauser Raman single-use sensors

Accurate and cGMP compliant with a lower cleaning burden

Endress+Hauser has risen to the challenge to develop a suite of versatile single-use sensor technology for the future of biopharmaceutical manufacturing. We now offer scalable single and multi-use Raman sampling systems that provide full bioprocess transparency from development to cGMP, regardless of your bioreactor setup mix.

For the past several years, Endress+Hauser has collaborated with Thermo Fisher Scientific to deliver our trusted Raman probe sampling technology to customers via a disposable Raman fitting integrated into their single-use bioreactors (SUBs) bags. In 2020, we partnered with Sartorius to introduce our Raman analysis into their smallest bioreactor setups (Ambr® 15 and Ambr® 250), as well as Biostat STR® large-scale SUBs for clinical and commercial manufacturing.

Our latest hybrid innovation comprises a specially designed multi-use optic that works in conjunction with a disposable bio sleeve for maximum bioreactor sampling flexibility and efficiency. Whether for single-use or traditional multi-use bioreactors, fed-batch or perfusion, process development or cGMP, Endress+Hauser is continually innovating to provide all our customers with reliable Raman composition measurement. You can rely on Endress+Hauser's Raman and application expertise to help you improve your bioprocesses to gain better process insight, faster development, and improved outcomes.

The Endress+Hauser Raman difference

Not all Raman is created equal

Endress+Hauser is the global leader in Raman instrumentation for laboratory, process, and manufacturing environments in the Life Science industry. Biopharmaceutical manufacturers particularly rely on our Raman analyzers and probes to help drive lab-to-process optimization. Our Raman bioprocessing technology stands apart from other alternatives in the market because we offer:

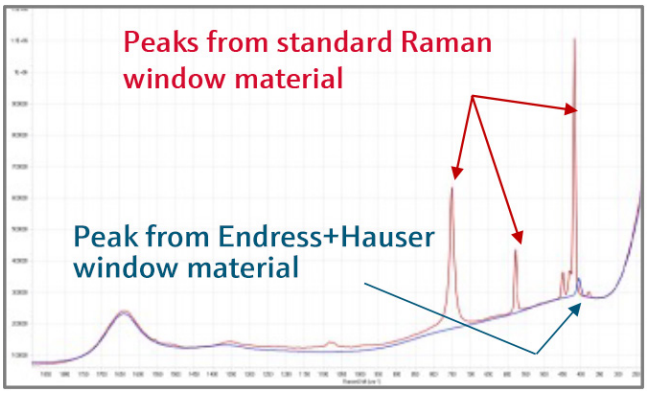
- 30+ years of lab-to-process Raman leadership, expertise, innovation, and reliability
- 20+ years of cGMP expertise (10+ years leading the bioprocess PAT journey), with proven compliance, method transfer, and up time
- Proven successes documented in countless biopharmaceutical customer publications and other industry publications
- ISO 9001:2015 certification and experience hosting many successful audits by leading pharma/biopharma companies and suppliers
- The security of partnering with the Endress+Hauser Group and its network of global and local support process automation experts
- The most trusted, robust, scalable, and reliable Raman analyzer systems on the market
- Superior bioprocessing probes known for having the highest quality of contact materials and the most flexible sampling capabilities
- A demonstrated ability to simplify process equipment complexity and ease method transferability from lab-to-manufacturing
- Experience, training, support, data modeling, and advanced analytics services to allow companies to achieve rapid ROI
- Pharma Manufacturing's 2020 Pharma Innovation Award for the Rxn-46 bioprocessing probe

High-quality probe windows

Optimized for the unique requirements of bioprocessing

Low background window design for single or multi-use
Endress+Hauser's Raman bioprocess sampling probes and optics are designed to meet the specific application and installation needs of bioprocessing. Serving as the "eyes" into your bioprocesses, quality of design is of the utmost importance. Throughout the biopharmaceutical industry, Endress+Hauser Raman probes are known for having the highest quality of contact materials and the most flexible sampling capabilities.

All of our bioprocess probes share identical materials and Raman collection areas—from lab to manufacturing scale, single to multi-use. Our probe window material was specifically selected for low interference to avoid complications caused by sapphire tips that can generate large spectral peaks and obscure certain measurements. In addition, our patented optical design features a self-focusing lens with automatic alignment which delivers high quality, repeatable spectra for every bioprocess measurement. Our Raman probe technology can be used for multiple, real-time upstream bioprocess measurements from lab to cGMP for single or multi-use.

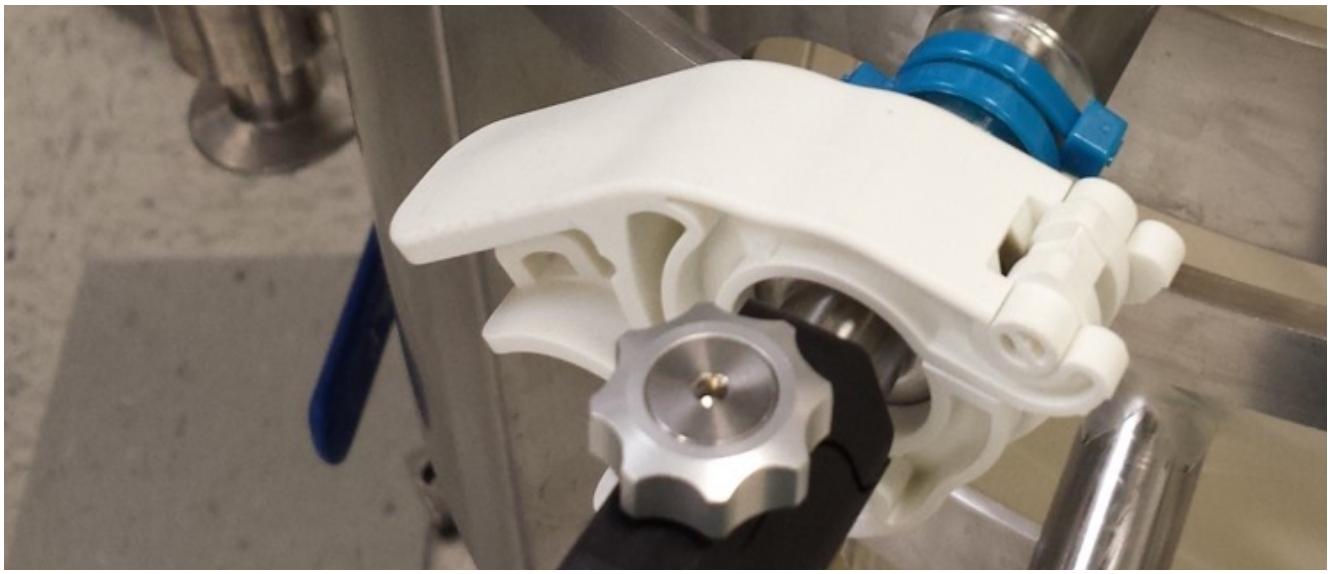


Top-quality bioprocess sampling material and design
- Corrosion-resistant materials
- High-purity, low-background windows
- Bubble shedding characteristics
- High spectral quality and reproducibility
- Fixed focus design

One single-use (or multi-use) probe for many real-time bioprocess measurements

Cell culture
- Glucose
- Glutamine
- Glutamate
- Lactate
- Ammonium
- Osmolatity
- Viability
- VCD
- TCD
- Titer

Fermentation
- Glucose
- Acetate and other metabolites
- Optical density/biomass
- Sugars/sugar alcohols (MeOH, EtOH, glycerol, maltose, fructose, maltotriose, DP4+, etc.)



Raman optic system for single use

Bioprocess probe with sterile, disposable fittings for SUBs

Endress+Hauser introduced single-use Raman sampling technology to single-use bioreactor systems (SUBs) several years ago by working in concert with the leading SUB vendors on the market. Since then, we have been successfully offering our Raman optic system for single use comprised of a Rxn-10 probe, a reusable non-contact optic, and a unique disposable fitting that integrates with SUBs.

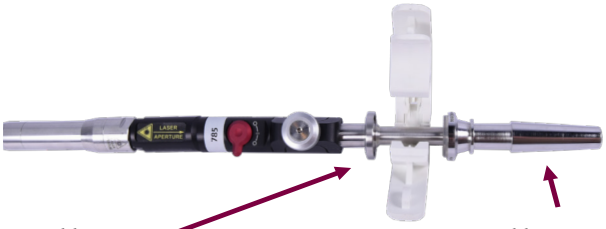
Single-use, disposable bioreactor vessels often use a flexible plastic bag as opposed to a more permanent reusable culture vessel. These bags commonly include one or more sensor ports enabling the contents of the bag to be sampled and monitored. In the past, a tradeoff existed between the expense and complexity of Raman sampling probes needed for accurate *in situ* analysis versus the disposable bag assembly. An additional challenge was how to measure in real time without breaking sterility. Our Raman optic system for single use solves these issues by offering a disposable pathway to a ready-to-use gamma-sterilized window, allowing operators to get Raman data from a SUB without compromising sterility or optical performance.

Developed according to industry standards for single-use sensors, our Raman optic system for single use provides the same accuracy, repeatability, and composition model transferability as our traditional, multi-use Raman probes. Its disposable SUB couplings not only facilitate precise Raman sampling, but the reusable part of the probe features a patented self-focusing lens for automatic alignment. This optical design assures the ultimate spectral quality and reproducibility. Endress+Hauser Raman disposable fittings come in multiple configurations, such as Barb-shaped or PG13.5 port compatible. The Raman optic system for single use has been tested and implemented at multiple biopharmaceutical companies. cGMP qualification is complete with leading SUB vendors, and integration with additional SUB vendors is underway.

Benefits at a glance

- Developed to industry standards for single-use sensors
- Spectral quality to match standard Raman bioprocess probes
- Gamma sterilizable
- E & L testing conducted by an independent agency
- Patented self-focusing lens for automatic alignment
- Tested and supplied by multiple SUB vendors
- Full data compatibility across our bioprocess probe portfolio
- Qualification complete with leading SUB vendors
- Quick and easy calibration and verification*

*Compatible with the multi optic calibration and verification kit



Reusable part
Rxn-10 probe with Raman optic system for single use

Disposable / SU part
A disposable fitting is delivered and discarded with the SUB bag

The Raman optic system for single use works with leading SUBs as follows:

- A unique, disposable Raman fitting is delivered to the SUB vendor who installs, gamma sterilizes, and delivers it to the end user as a component of the SUB
- A reusable, non-contact Raman optic is connected to the disposable fitting/port using a standard clamp
- The Raman optic is connected to the Rxn-10 probe
- The Rxn-10 probe is paired with a Raman Rxn2 or Rxn4 analyzer
- The SUB bag with the disposable Raman fitting is discarded after use

SUB compatibility

Our disposable Raman fittings have been sold with Thermo Fisher Scientific SU Bioreactors and Fermenters since 2018, with thousands of fittings utilized to date.

Disposable fitting as delivered (cap not shown)

Connected to reusable Raman optic system for single use

Customer installation example

Raman bio multi optic and bio sleeve

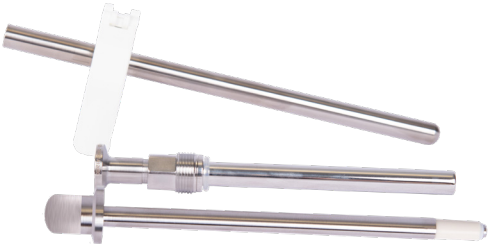
Versatile immersion optic system with disposable sleeve offers the utmost flexibility for reusable or single-use benchtop bioreactor setups

Adapted from our Raman optic system for single use concept, the Raman bio multi optic and bio sleeve offering takes disposable sampling innovation to the next level. Pairing the Rxn-10 probe with a specially designed multi-use optic that fits into a disposable sleeve succeeds in delivering reliable composition measurement with greater ease of use, less maintenance, and lower cross-contamination risk. The use of disposable parts improves the user experience for bioprocessing applications and offers the utmost flexibility for use in either reusable or single-use benchtop bioreactor setups.

The Rxn-10 probe, equipped with a dual-part bio multi optic and bio sleeve, provides accurate, real-time, *in situ* measurements of crucial parameters in your bioprocess. The disposable bio sleeve eliminates the need for either the probe or optic to survive frequent sterilizations and other issues associated with long term re-usability of process contact components. Although suitable for single use, the stability of the bio sleeve has been rated to 50 autoclave cycles when used in conjunction with a bio sleeve desiccator.

Like our single-use optic, the bio multi optic features a patented self-aligning lens for the utmost spectral quality and reproducibility. Bio sleeves are not required for probe calibration and verification steps, so they can be inserted into bioreactors at any time. They can also remain in bioreactors for cleaning and between batches for the ultimate Raman-ready set up. The multi-optic is required for probe calibration and verification, but since it does not contact the process, these steps can be conducted at any time, even mid-batch if necessary. This convenience significantly boosts the overall efficiency of the entire bioprocessing operation.

A specially designed multi optic calibration and verification kit is available that includes all the necessary hardware for calibrating and verifying the calibration of the multi optic (and Raman optic system for single use) when connected to an Rxn-10 probe, paired with a Raman Rxn analyzer.



Benefits at a glance

- Patented self-focusing lens with automatic alignment for optimal spectral quality and transferability
- Improved ease of use and calibration
- Reduced probe service and maintenance
- Simplified workflow for greater process efficiency
  - Greater flexibility in calibration and verification schedules
  - Interchangeable probes in process without downtime
  - Redundancy made easy
- PG13.5 threaded connector for headplate entry
- Autoclavable, gamma sterilizable, and compatible with standard cleaning protocols
- Expanded compatibility with additional SU bioprocessing containers
- Eliminates cross-contamination risk for single-use set ups
- Compatible with Endress+Hauser flow assembly CYA680

Bio multi optic

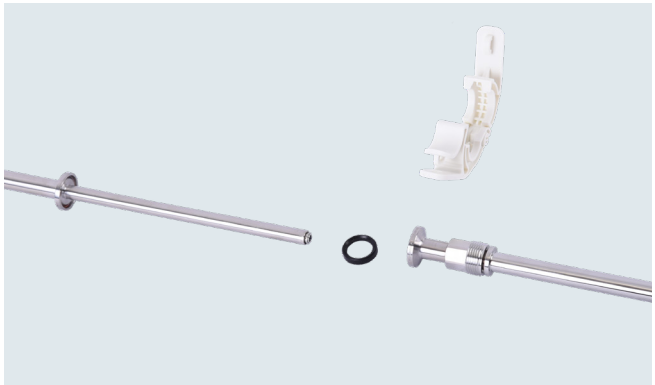
- A reusable optic that does not contact the process/ sample
- Directly connects to the Rxn-10 probe
- No maintenance required beyond regular calibration and verification

Bio sleeve

- Disposable sleeve that contacts the process/sample
- Flexible design
  - Multi use - can be sterilized and cleaned by the user. Replace after 50 autoclave cycles
  - Single use - can either be sterilized by the user and discarded after one use, or integrated into a SUB, sterilized by the SUB vendor, and discarded with the SUB. For the latter, SUB vendor testing and qualification is required

How to use Endress+Hauser resuable bio multi optics with disposable bio sleeves in benchtop bioreactors:

- A reusable multi optic is attached to a Rxn-10 sampling probe using a simple snap-in-place design.
- The bio sleeve is inserted into a benchtop bioreactor's PG13.5 port and autoclaved as necessary. For autoclaving, a sleeve desiccator cap is used.
- The non-contact multi optic is inserted into the bio sleeve and secured with a standard clamp.
- The Raman Rxn2/4 analyzer initiates data collection using the analyzer's embedded Raman RunTime control software.
- Real-time composition measurements are taken *in situ* via the attached Rxn-10 probe connected to the reusable bio multi optic inside the disposable bio sleeve.



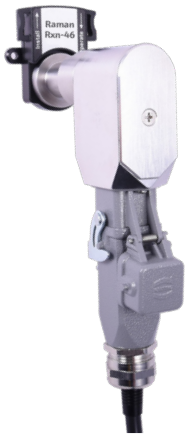
Raman Rxn-46 probe

Bioprocess probe compatible with BioPAT® Spectro by Sartorius for Ambr® 15, Ambr® 250, and Biostat STR®

The Raman Rxn-46 probe is a highly unique offering in our robust bioprocessing probe series, optimized specifically to fit the BioPAT Spectro platform by Sartorius. This collaboration represents the first instance of Raman being brought down to the Ambr-scale and Biostat STR single-use product lines. It offers the market an ideal interface to high throughput development through single-use commercial manufacturing. By providing fast, reliable, and accurate measurement of key process variables from lab-to-process, the Rxn-46 probe empowers bioprocessing companies to more easily scale-up and scale-out from development to cGMP while complying with strict quality standards.

Benefits at a glance

- Enables faster, easier, and more robust model building via integration with Ambr 15 and Ambr 250
- Allows high throughput process development which supports QbD
- Provides efficient transfer to Biostat STR for single-use manufacturing
- Offers a scale-independent interface from 15 ml in the laboratory to 2000 L in the production suite
- Requires no probe cleaning, sterilization, or frequent maintenance due to non-contact sampling



2020 Pharma Innovation Award winner by Pharma Manufacturing Magazine

How the Rxn-46 probe works with BioPAT Spectro by Sartorius for Biostat STR for single use

Raman Rxn2 and Rxn4 analyzers (single or four channel) with 785 nm wavelength are compatible with the Rxn-46 probe for single-use manufacturing. The probe and analyzer work together with the Sartorius system as follows:

- Raman probes attach to the BioPAT Spectro single-use port.
- Ports come ready to use and fully qualified.
- Probe connection to the port is fast and simple.
- Raman collection is isolated from bubbles and light (no additional light blocking is required).
- Raman RunTime software initiates data collection from Biostat STR 50L – 2000L single-use bioreactors.
- Raman results can be sent to DCS or SCADA systems for automated bioreactor monitoring and control.

SARTORIUS

Integrating Endress+Hauser Raman spectroscopy into Ambr enables Quality by Design (QbD) methods that are scalable to all sizes of Biostat STR single-use bioreactors

Quick and easy calibration and verification
A specially designed Rxn-46 calibration and verification kit is available that includes all the necessary hardware for calibrating and verifying the calibration of the Rxn-46 probe when paired with a Raman Rxn analyzer.

Please see the “Raman bioprocessing solution compatibility with BioPAT Spectro by Sartorius” brochure for more information about the integration of our Rxn-46 probe and Biostat STR single-use manufacturing product line from Sartorius.

The scalability advantage

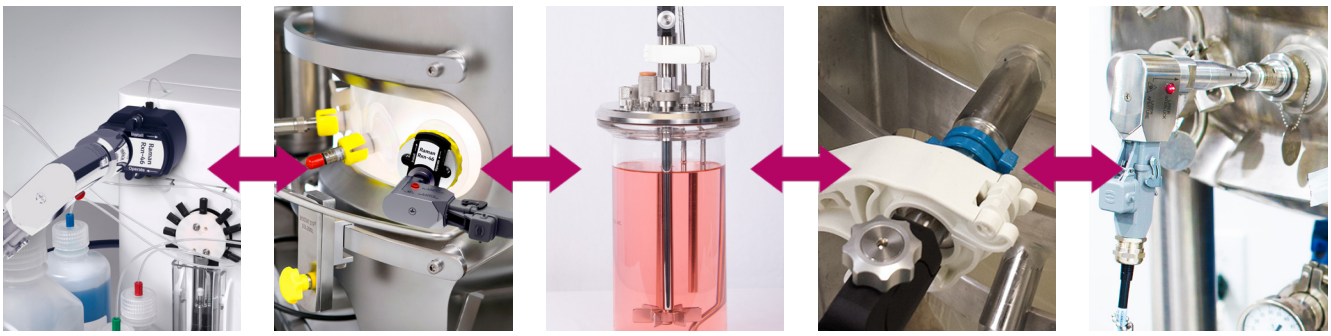
Purposely designed Raman bioprocessing probes for easy scalability from lab to cGMP for multi or single-use

Raman transferability across all scales

All Endress+Hauser Raman bioprocessing probes offer the ultimate in scalability for both single and multi-use facilities from process development to commercial production. Transfer of data models from lab-to-pilot-to-manufacturing across our Raman probes – including our Rxn-10 probe with bIO-Optic and Rxn-45 probe – is well demonstrated in literature.[2]

This easy scalability is due to several design features, such as:

- Identical materials and Raman collection areas for all probes
- Window material optimized specifically for low interference
- Rugged instrumentation with simple analyzer and probe calibration, standardization, and performance verification

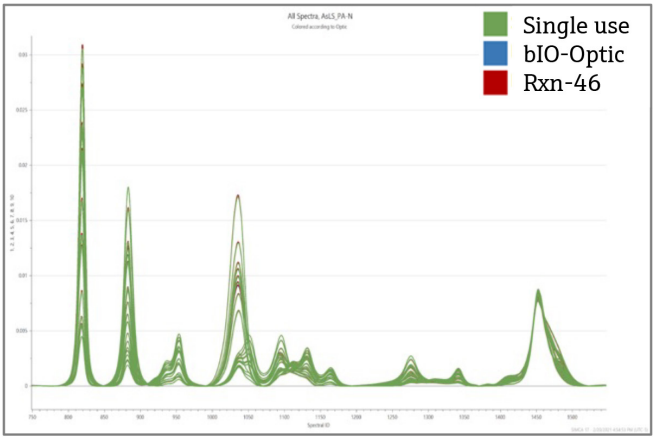


Example of cross-scale Raman analysis using analyzers and bioprocessing probes designed to facilitate scalability from micro and mini-bioreactor systems through to single-use and multi-use bioreactors of all sizes

Transferability across our Raman single-use probes

For biopharmaceutical manufacturers employing a mix of multi and single use setups, Endress+Hauser Raman analyzer systems produce model data that is compatible with them all. Using 24 different samples, Raman models created with spectra from our Rxn-10 probe with bIO-Optic and our Raman optic for single use were shown to transfer well to samples collected with our Rxn-46 probe and vice versa.

The graph at the right depicts the Raman spectra of all samples collected across three different probes overlaid. It demonstrates that the spectral response across each probe is identical (using standard spectral pretreatments).



24 samples x 2 x 3 probe types = 144 overlaid spectra

[2] https://link.springer.com/article/10.1007/s00216-021-03727-4

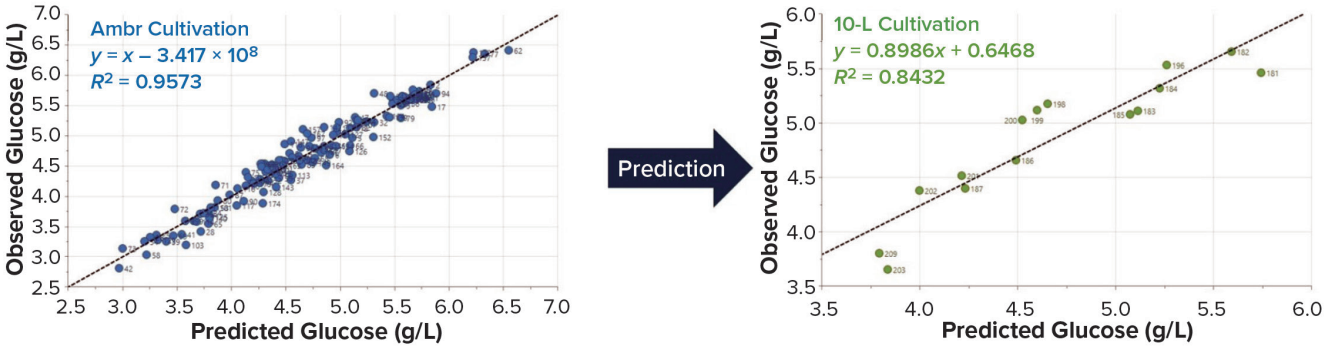
Application success

Successful Raman model transfer from Ambr-scale to benchtop bioreactors

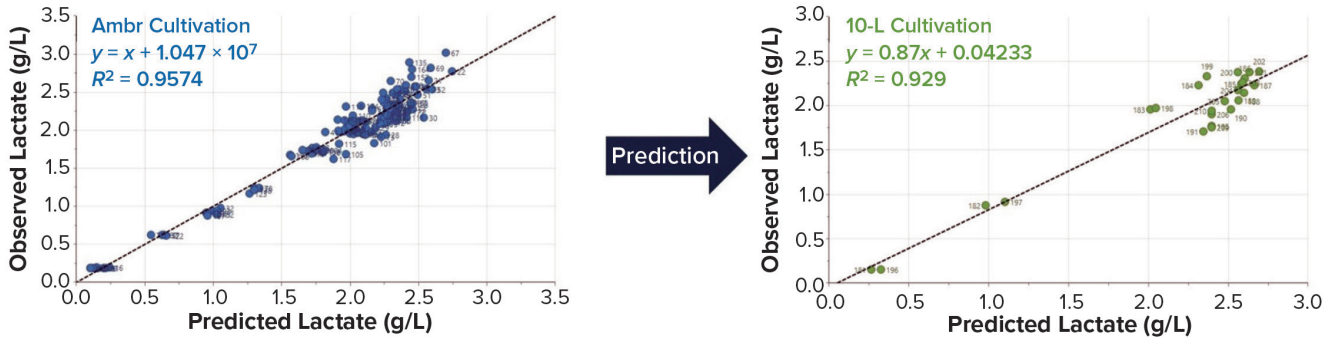
A critical capability for analytical technology is interoperability between hardware formats and scales, as well as transferability of analyte calibrations. Data gathering can be significantly more efficient when done during development cycles, and the value of that efficiency is often realized when the resulting models are utilized in the more costly manufacturing environment. Recognizing these facts, Bayer recently published data from a series of experiments designed to test the functionality of Endress+Hauser Raman when applied to significantly different formats and scales of bioreactors.[1]

The scale-up/scale-down test analyzed data bidirectionally, collected in Sartorius Ambr 250 and 10L benchtop bioreactors. The Ambr set up consisted of a BioPAT Spectro flow cell coupled to a Rxn-46 probe, while the 10L set up utilized a Rxn-10 probe with a 220 mm bIO-Optic. The authors built PLS regressions for glucose and lactate at each scale and then used the scale-specific models to predict the analytes at the alternate scale. The precision of the measurements remained within functional specifications for both analytes in scale-up and scale-down tests. In the case of regressions built with the high throughput capability of the Ambr system transferred to the 10L scale, the root mean squared error of prediction (RMSEP) for glucose was 0.29 g/L and 0.38 g/L for lactate.

The success of inter-scale and inter-format transferability demonstrated by these results provides valuable evidence that Endress+Hauser Raman technology can provide value throughout the entire development to commercial manufacturing workflow, while being agnostic to cultivation hardware. This capability is particularly valuable in circumstances requiring a great deal of time spent working in small scale development, including the development and production of monoclonal antibodies (mAbs) and cell and gene therapies (CGTs). Pressure to reduce the time needed for iterative development cycles and the overall research-to-production timeline for CGTs could be greatly alleviated by implementing Raman for rapid scale-up and effective scale-down.[1]



Comparing observed and predicted plots for the partial least squares (PLS) model developed to predict glucose concentration using spectral data from the Ambr 250 cultivation (left); glucose prediction for two 10-L cultivations (right)



Observed versus predicted plot for the PLS model developed to predict lactate concentration using spectral data from the Ambr® 250 cultivation (left) and lactate prediction for the two 10 L cultivations (right)

1 Classen, Jens; Langer, Matthäus; Jockwer, Alexander; Traenkle, Jens. "Successful Transfer of Raman Models from Ambr® 250 high-throughput systems to larger scale stirred tank bioreactors"

Technical specifications

Technical specifications

	Raman optic system for single use	Raman bio multi optic and bio sleeve	Raman Rxn-46 probe for Biostat STR®
Analyzer compatibility	Raman Rxn2 or Raman Rxn4 embedded analyzer	Raman Rxn2 or Raman Rxn4 embedded analyzer	Raman Rxn2 or Raman Rxn4 embedded analyzer*
Sampling compatibility	Rxn-10 probe	Rxn-10 probe	Probe is compatible with BioPAT Spectro for Ambr and Biostat STR by Sartorius
Probe configuration	Single or four channel	Single or four channel	Single channel for Ambr Single or four channel for Biostat STR
Laser wavelength	785 nm, 1000 nm	785 nm	785 nm
Spectral coverage	Limited by the coverage of the analyzer being used	Limited by the coverage of the analyzer being used	Limited by the coverage of the analyzer being used
Maximum laser power into probe head	< 499 mW	< 499 mW	< 499 mW
Sample interface	**Temperature:** 0 to 100 °C (32 to 212 °F)	**Bio sleeve, temperature:** -30 to 150 °C (-22 to 302 °F) **Bio sleeve, maximum pressure:** 13.8 barg (200 psig)	**Temperature:** probe is non-contact; operating temp: 10 to 50 °C (50 to 122 °F)
Wetted materials	**Wetted materials (SUB fitting):** 316L stainless steel **Window:** proprietary material, optimized for bioprocesses **Process connection:** varies according to single use bioreactor vendor port and fitting type **Surface finish:** Ra 0.38 µm (Ra 15 µin) with electropolish **Adhesive:** USP Class VI and ISO 10993 compatible	**Wetted materials (bio sleeve) - Body:** 316L stainless steel **Window:** proprietary material, optimized for bioprocesses **Process connection:** PG13.5 **Surface finish:** Ra 0.38 µm (Ra 15 µin) with electropolish **Adhesive:** USP Class VI and ISO 10993 compatible	**Process connection:** Sartorius BioPAT Spectro
Immersible length (mm)	Dimensions vary according to single use bioreactor vendor port and fitting type	**Bio sleeve:** 120 and 220 mm (4.73 and 8.67 in)	n/a
Immersible diameter (mm)	Dimensions vary according to single use bioreactor vendor port and fitting type	**Bio sleeve:** 12 mm (0.48 in)	n/a
Sterilization method	Gamma sterilized	**Bio sleeve:** autoclave, with the use of the sleeve desiccator rated for 50 autoclave cycles Compatible with gamma sterilization	n/a
Calibration method	**785 nm:** multi optic calibration accessory* (recommended) or HCA-785 with single-use calibration adapter **1000 nm:** HCA-1000 with single use calibration adapter	Multi optic calibration accessory* (recommended) or HCA-785 with bio sleeve attached to bio multi optic	Rxn-46 probe calibration cell*
Verification method	**785:** multi optic verification accessory with 70% IPA (recommended) or bIO Sample Chamber with 70% IPA and single use calibration adapter **1000 nm:** bIO Sample Chamber with 70% IPA and single-use calibration adapter	Multi optic verification accessory with 70% IPA (recommended) or bIO sample chamber with 70% IPA and bio sleeve attached to bio multi optic	Rxn-46 probe verification cell

*Analyzers with Raman RunTime v6.2.2+ software

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