Simplifying Progress
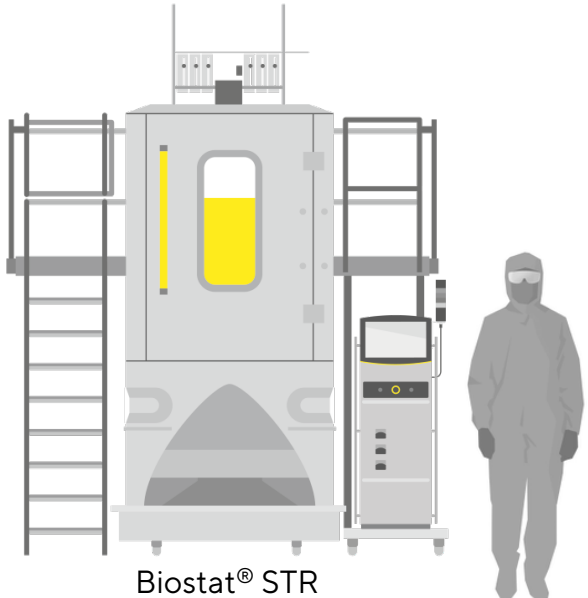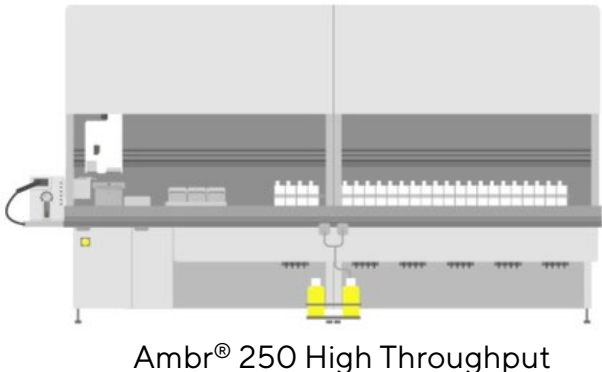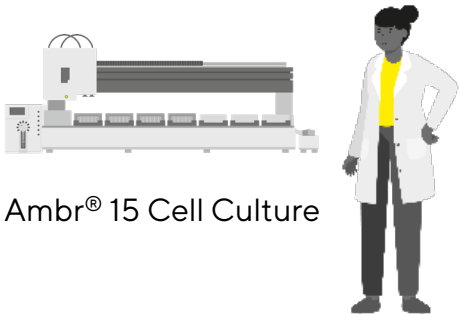
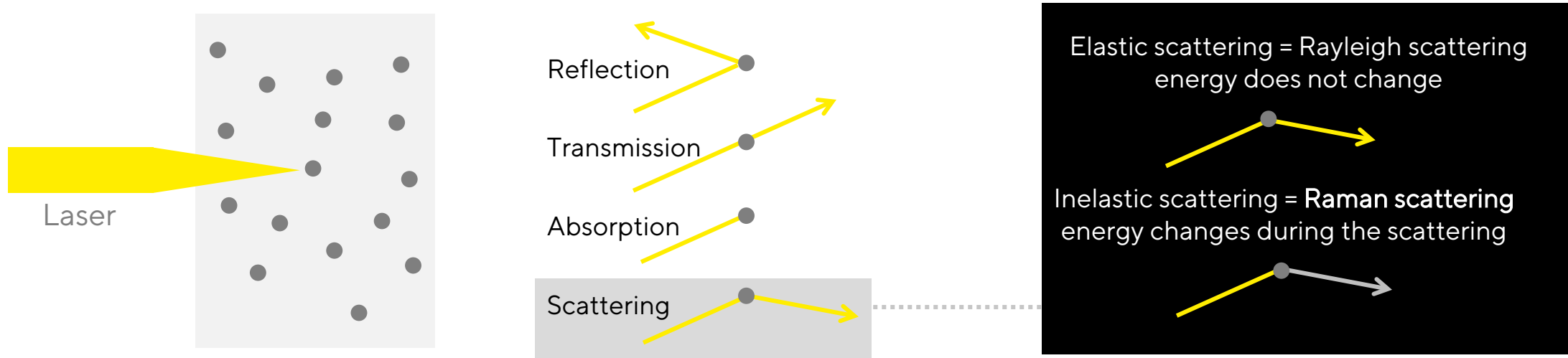# Introducing BioPAT® Spectro: Unlocking the Potential of Raman Spectroscopy

Veronica Brivio - PAT Product Manager, Dan Kopec - PAT Specialist for North America | September 2022

SARTORIUS

BioPAT® Spectro Introduces Raman Spectroscopy Into Ambr® and Biostat STR® to Enable Quality by Design (QbD)

Ambr® 15 Cell Culture

Ambr® 250 High Throughput

Biostat® STR

SARTORIUS

Raman Is a Laser Scattering Technique With High Molecular Specificity

Reflection

Transmission

Absorption

Scattering

Elastic scattering = Rayleigh scattering
energy does not change

Inelastic scattering = **Raman scattering**
energy changes during the scattering

Laser

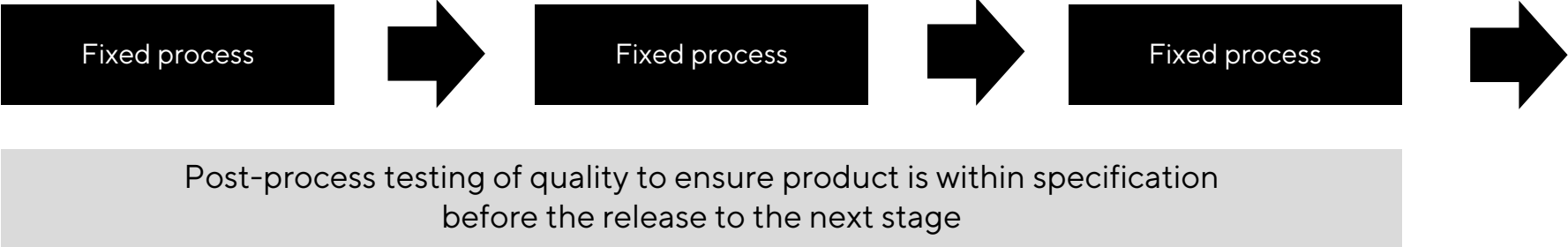A laser is projected into the bioreactor

- Non-invasive
- In-line

The laser interacts with molecules in a
non-destructive fashion

Inelastic scattering is measured as a Raman spectrum

- Gives "molecular fingerprint", enabling qualitative and quantitative analysis of several analytes, such as glucose, lactate, titer etc
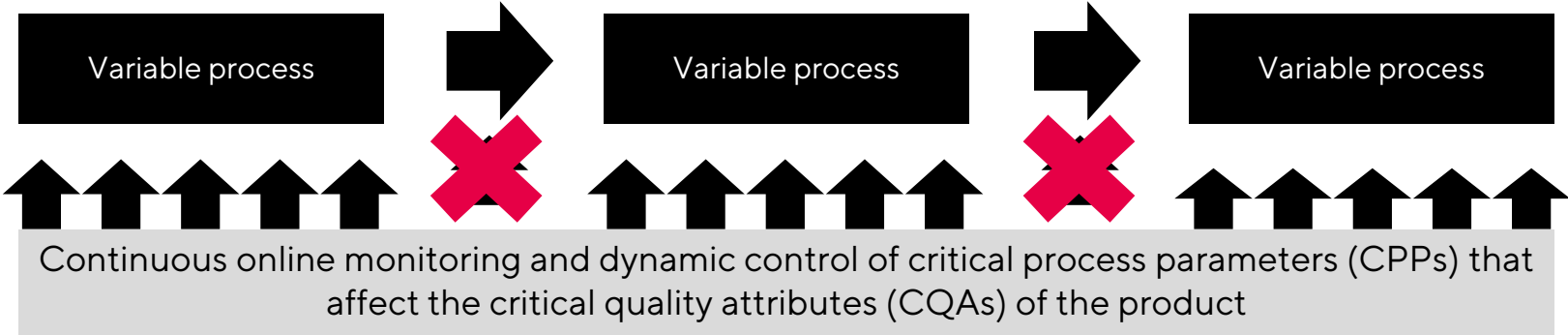- Weak signal requires longer acquisition time

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Quality by Design Enables Consistent and High Product Quality

Traditional process – Post-process quality testing

| Fixed process | | Fixed process | | Fixed process | |

Post-process testing of quality to ensure product is within specification before the release to the next stage

Variability in product quality and quantity

PAT process – Quality by Design (QbD)

| Variable process | | Variable process | | Variable process | |

Continuous online monitoring and dynamic control of critical process parameters (CPPs) that affect the critical quality attributes (CQAs) of the product

Consistency in product quality and quantity

Real-time release

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Raman Spectroscopy Is Complex and Not Well Integrated With Single-Use

Expectation

Reality

Process development

Full DOE and model building is too laborious and expensive

missing automation and high throughput integrations

Data management

Data from different sources require manual alignment

requires expert data scientist and is error prone
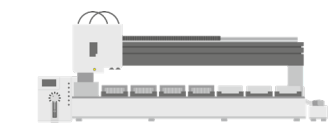
SU manufacturing

No comprehensive single-use integration

immersion probes suffer from stray light in transparent bags

Limited scalability due to low-robustness models and lack of scalable hardware

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BioPAT® Spectro Was Designed to Meet Three Key Requirements

- Enable Raman spectroscopy in high throughput process development
- Facilitate and improve the model building and data management process
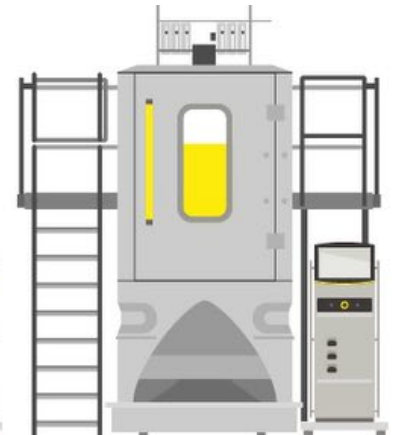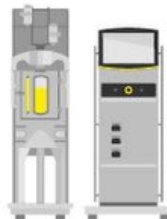- Full single-use integration and scalability for commercial manufacturing

'[BioPAT® Spectro is] the next killer app for Raman as it enables the technology to expand beyond manufacturing and into PD.'

Ambr® 15 Cell Culture
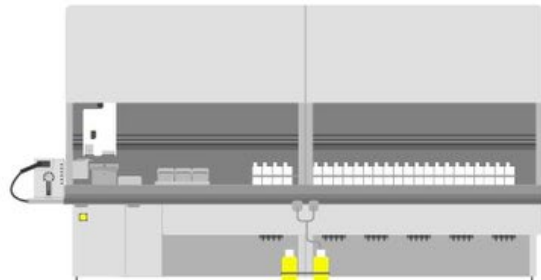
Ambr® 250 High Throughput
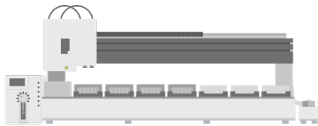
Biostat® STR

Aspen Alert, March 2020

SARTORIUS

Raman Spectroscopy Use Cases

Model building	Process development	Single-use manufacturing

Ambr® 15 Cell Culture

Ambr® 250 High Throughput

Biostat® STR

SARTORIUS
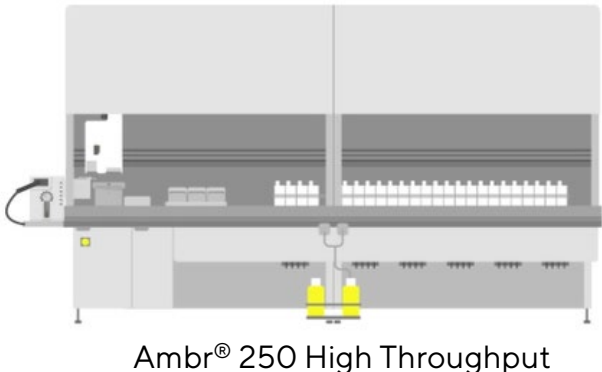
Raman Spectroscopy Use Cases

Model building	Process development	Single-use manufacturing

Ambr® 15 Cell Culture

Ambr® 250 High Throughput

SARTORIUS
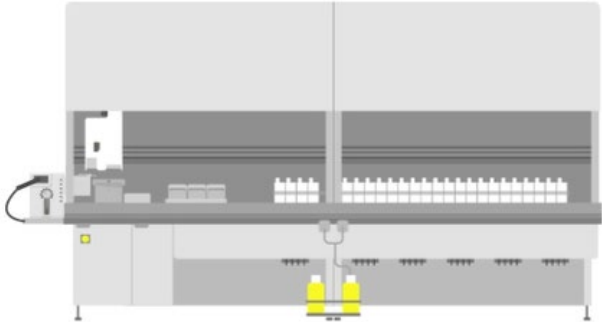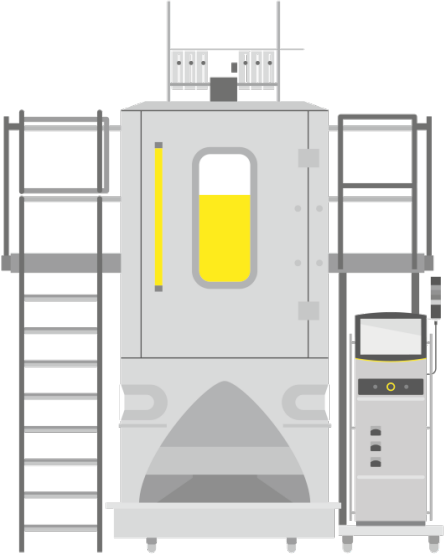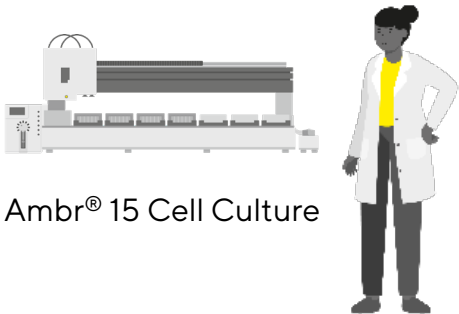
Quantitative Raman Spectroscopy Requires Model Building

Laser

Raman spectrum

	Concentration
Analyte X	3 g/L
Analyte Y	5 g/L

Offline data

Multivariate data analysis software (SIMCA®)

Model file

Laser is projected into the bioreactor

Raman scattering is collected as spectral data

A multi-variate data analysis software builds a statistical model that predicts the analyte concentration from the spectral data

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Conventional Model Building Is Laborious, Expensive and Time-Consuming

Spectrometer

SIMCA

1 Data acquisition
Run at least 2x4 benchtop bioreactors

2 Data consolidation & contextualization
Manually consolidate and align spectral and offline data

3 Model building
based on 6 runs

At least 2 x 12 days

At least 1 day

1 day

At least 26 days

- Time consuming
- Operator involvement
- High media consumption

- Requires data scientist
- Very time consuming
- Error-prone manual task

- Models built on only a few runs
- Limited DOE design space

Low robustness models with limited transferability to manufacturing

SARTORIUS

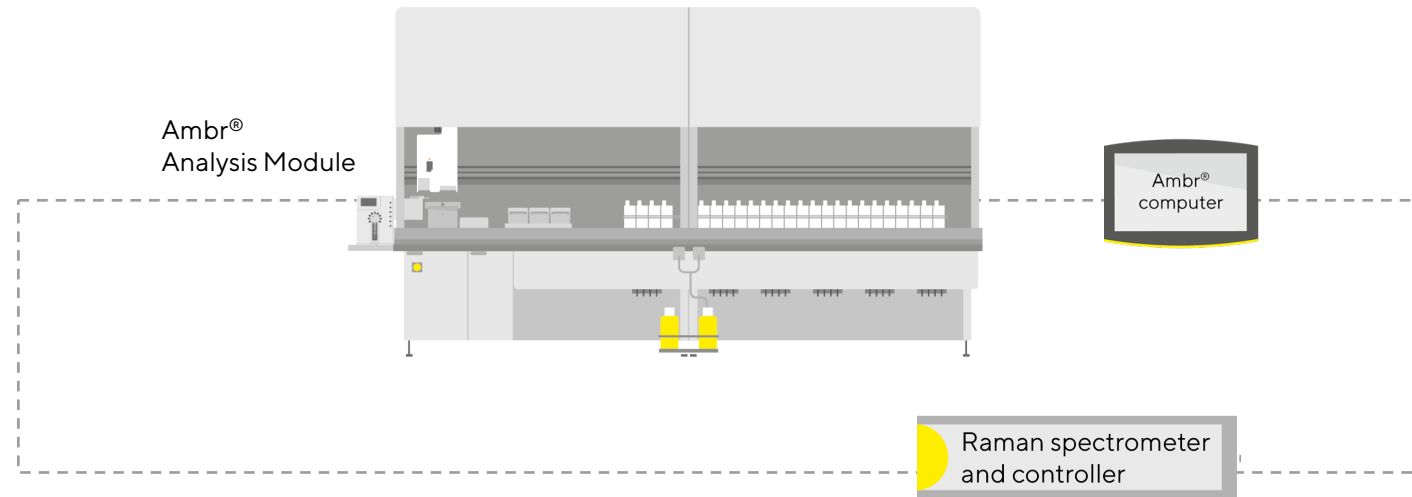# BioPAT® Spectro is integrated in Ambr® 15 and Ambr® 250 High Throughput

Connection of a Endress+Hauser Raman spectrometer probe

Connection of a Tornado Raman spectrometer probe

Data Acquisition and Consolidation Is Fully Automated in Ambr®

Ambr®
Analysis Module

Ambr®
computer

Raman spectrometer
and controller

3rd party Raman spectrometers (Kaiser Optical Systems and Tornado Spectral Systems) are fully integrated into the Ambr® and can be controlled via the Ambr® software. Spectral data is collected in the Ambr® software.
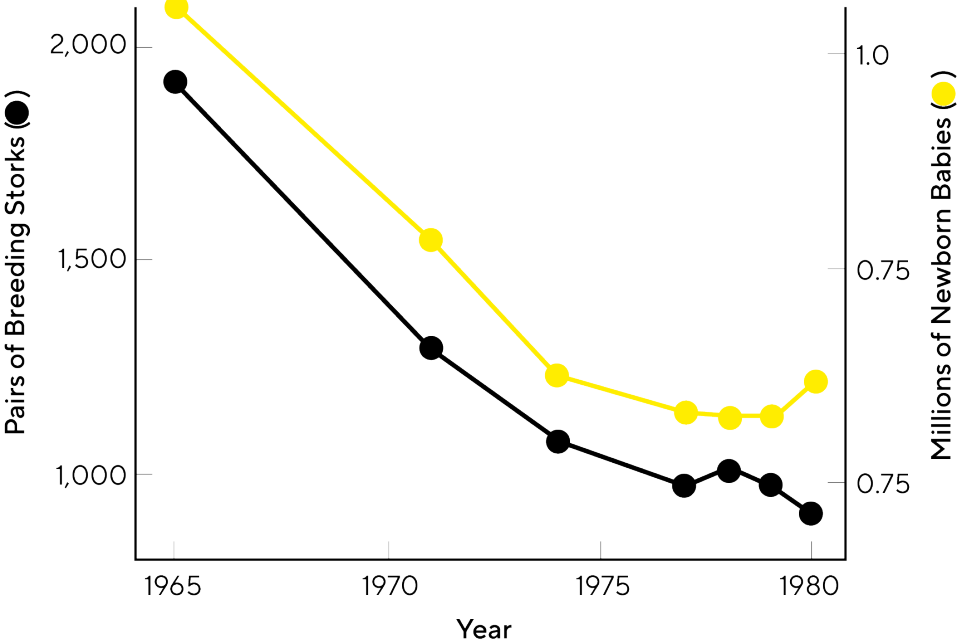
Data Acquisition and Consolidation Is Fully Automated in Ambr®
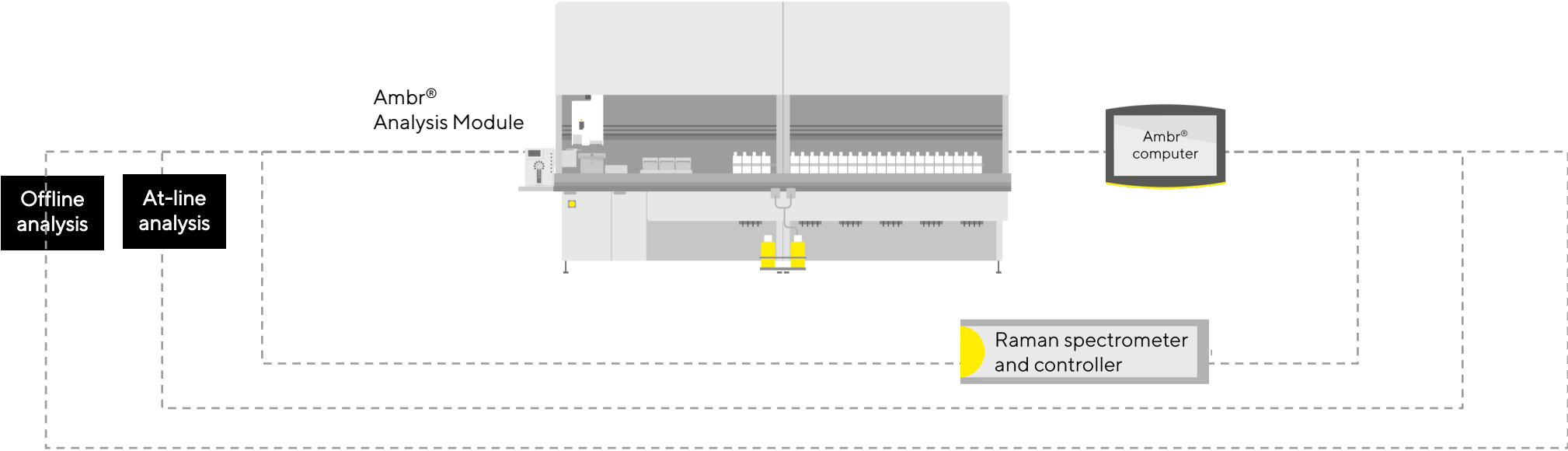
The data from integrated analyzers is collected and automatically aligned with the spectral data.
Offline data can be added manually during the run.

Data Acquisition and Consolidation Is Fully Automated in Ambr®

SIMCA®

SIMCA®-ready data file
- spectral data
- offline data
- process data

Ambr®
computer

Ambr®
Analysis Module

Offline analysis

At-line analysis

Raman spectrometer and controller

After the run, a consolidated and contextualized data file can be exported from the Ambr® software, ready for model building in SIMCA®.

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BioPAT® Spectro in Ambr® Is Designed for Building Causal Models
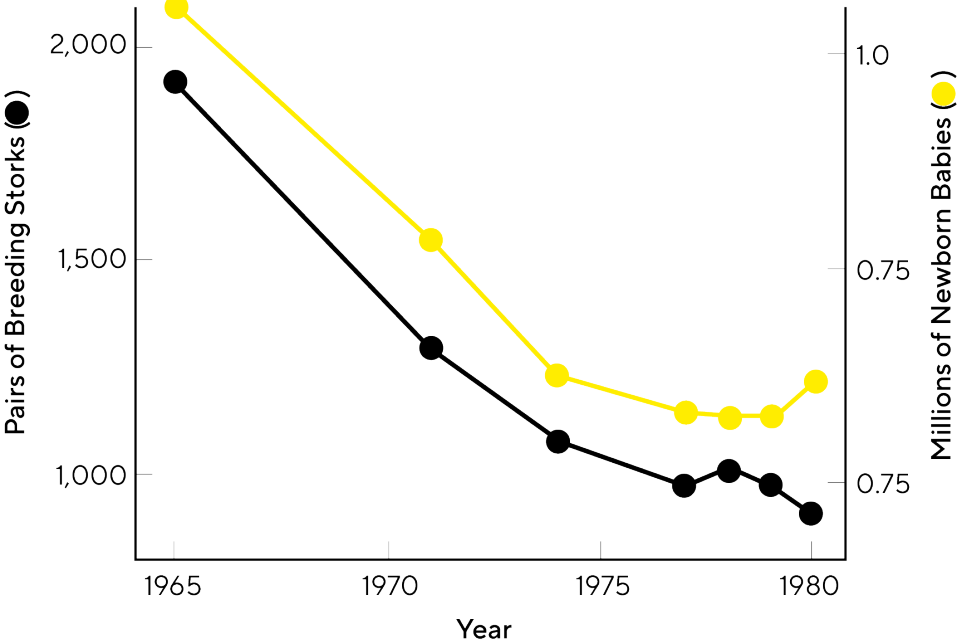

SIES, H.A new parameter for sex education. Nature 223. 495 (1988)

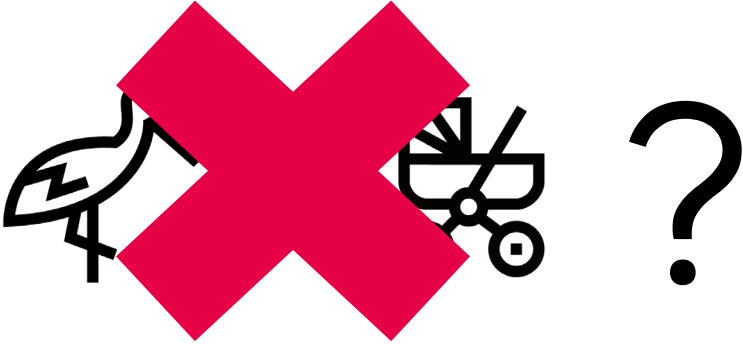Image: © John Lund/Getty Images

SARTORIUS

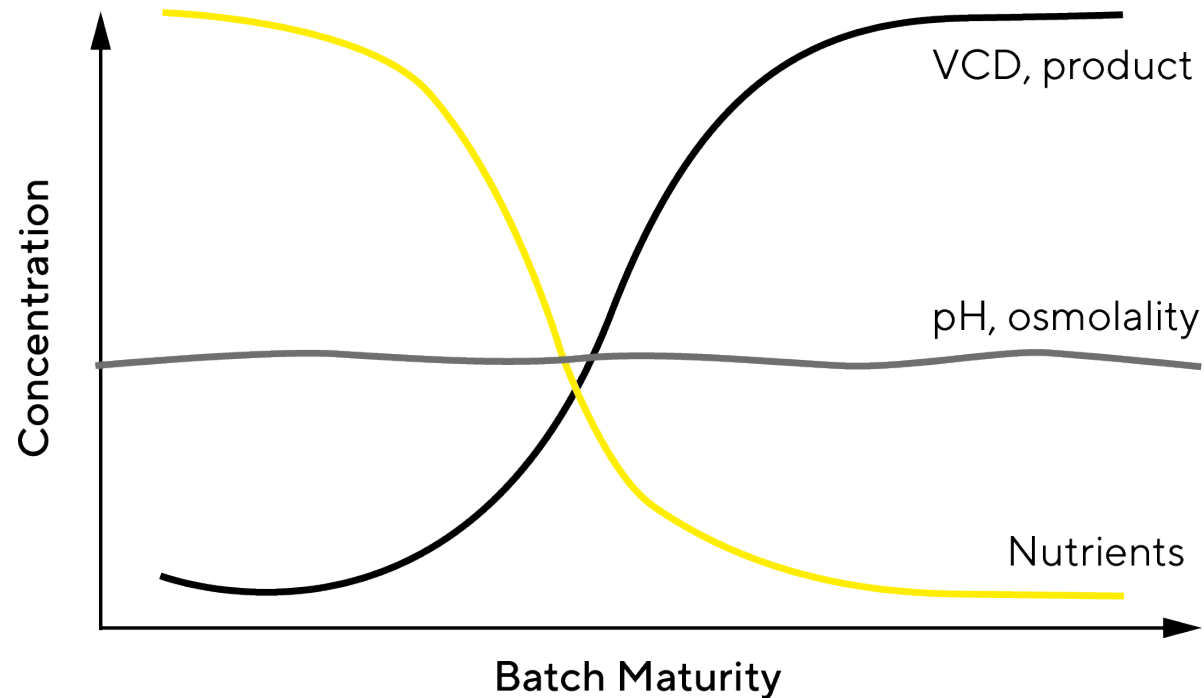# BioPAT® Spectro in Ambr® Is Designed for Building Causal Models

SIES, H.A new parameter for sex education. Nature 223. 495 (1988)

Correlation does not imply causation!

SARTORIUS

BioPAT® Spectro in Ambr® Is Designed for Building Causal Models

Random correlations are a risk in model building
- Analyte concentration changes follow similar patterns
- statistical models cannot distinguish causal from non-causal correlations
- Risk mitigation: Use techniques to break correlations

SARTORIUS

BioPAT® Spectro in Ambr® Is Designed for Building Causal Models

Experimental plan

24 vessels in Ambr® 250 or Ambr® 15

18 vessel DOE breaks correlations and increases model robustness	6 golden batches

Inoculation density	Glucose set point / profile	pH	Dissolved oxygen

Automated spiking

Measure (unspiked) sample → Robot takes a new sample and adds stock solution from a well plate and mixes → Measure spiked sample
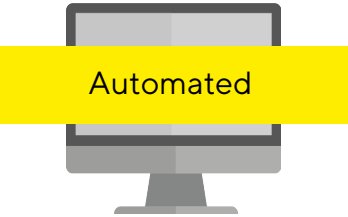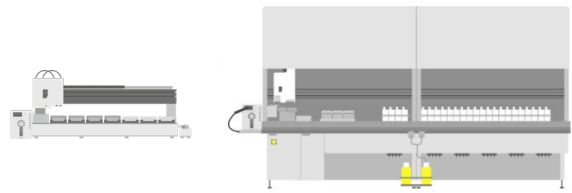
Design of Experiment (DOE)
→ Different process trajectories & analyte trends
→ Trains model for predicting even during process deviations
→ Breaks correlations & increases model robustness

Analyte spiking
→ Introduces a step change in concentration of an analyte
→ Breaks correlations with non-spiked analytes
→ Increases the concentration range of the model

SARTORIUS

BioPAT® Spectro Is the Perfect Tool for Spectroscopy Model Building

Automated

SIMCA

1

Data acquisition
Automated data acquisition & consolidation Run 12, 24 or 48 bioreactors in parallel
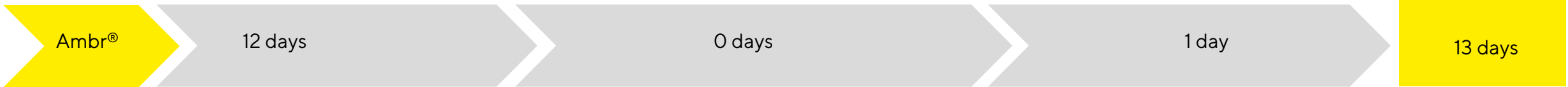
2

Data consolidation & contextualization
Manually consolidate and align spectral and offline data

3

Model building
Based on at least 10 runs

| Ambr® | 12 days | 0 days | 1 day | 13 days |

- Save 80% of operator time
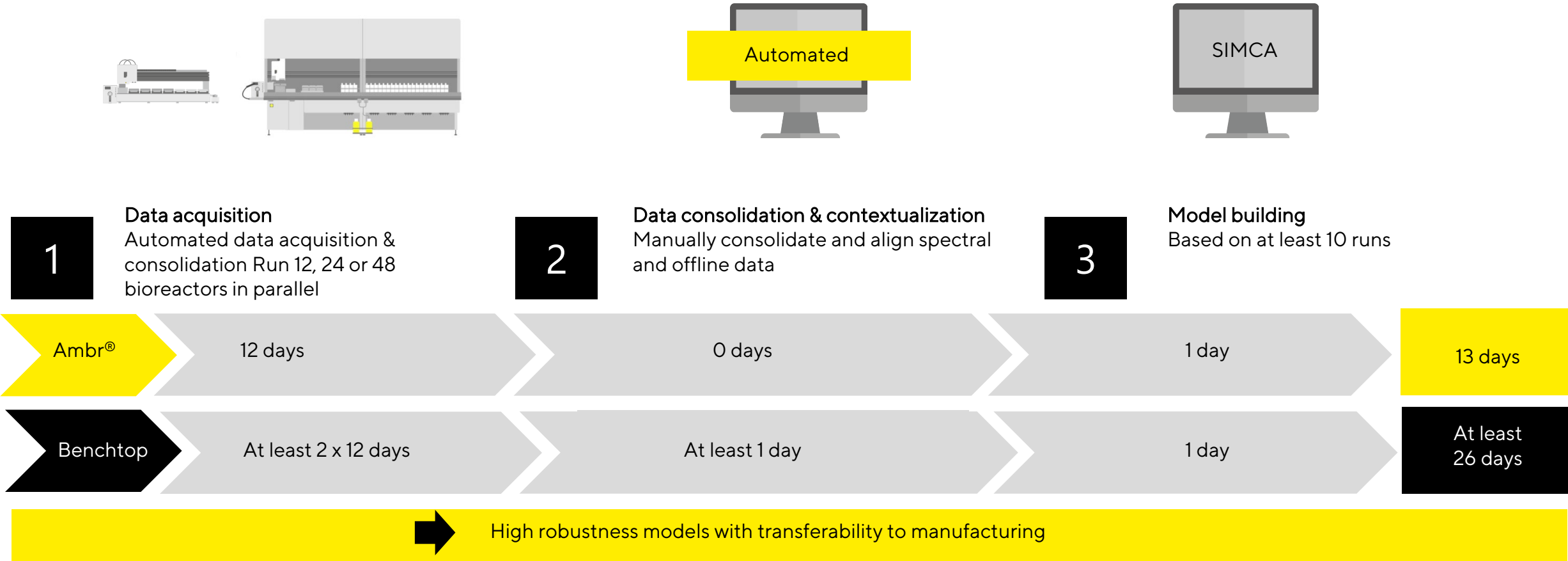- Save 95% of media costs
- Automated spiking breaks correlations

- Fully automated alignment of process, spectral and offline data
- Ready for important into SIMCA®

- Models built on many runs
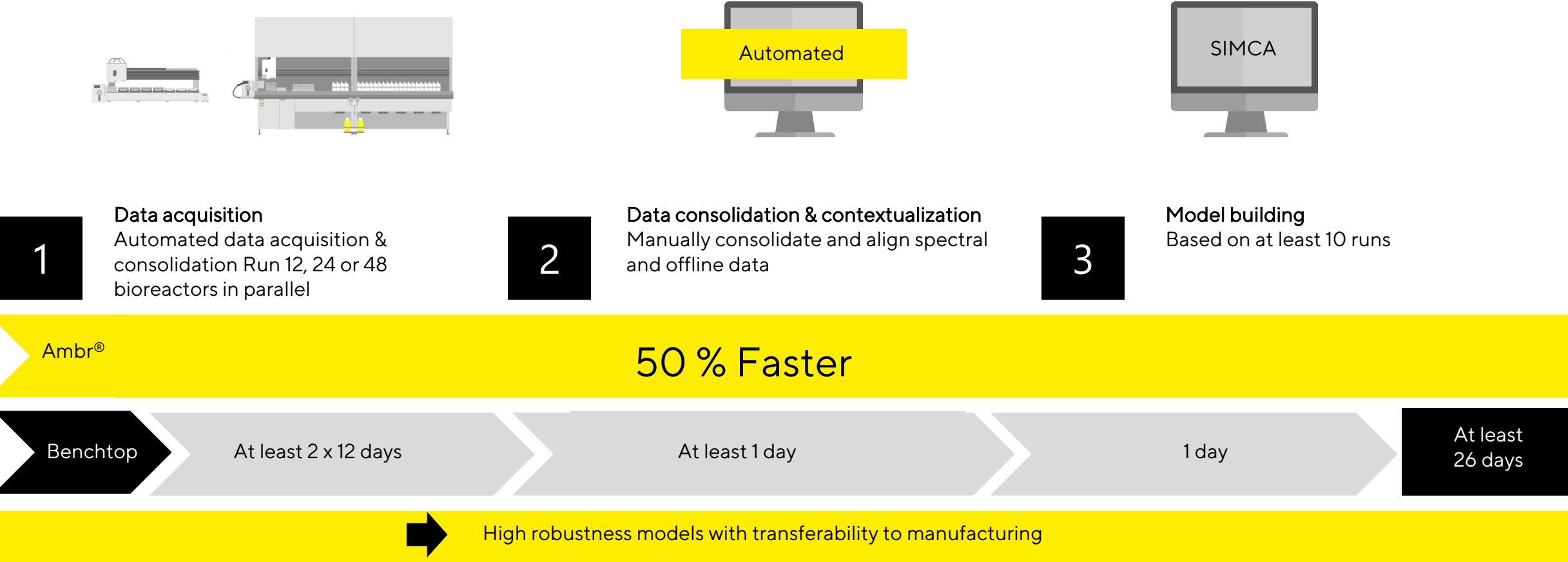- Large DOE design space
- All process data used

➤ High robustness models with transferability to manufacturing

SARTORIUS

BioPAT® Spectro Is the Perfect Tool for Spectroscopy Model Building

Automated

SIMCA

1 **Data acquisition**
Automated data acquisition & consolidation Run 12, 24 or 48 bioreactors in parallel

2 **Data consolidation & contextualization**
Manually consolidate and align spectral and offline data

3 **Model building**
Based on at least 10 runs

Ambr®	12 days	0 days	1 day	13 days
Benchtop	At least 2 x 12 days	At least 1 day	1 day	At least 26 days

High robustness models with transferability to manufacturing

SARTORIUS

BioPAT® Spectro Is the Perfect Tool for Spectroscopy Model Building

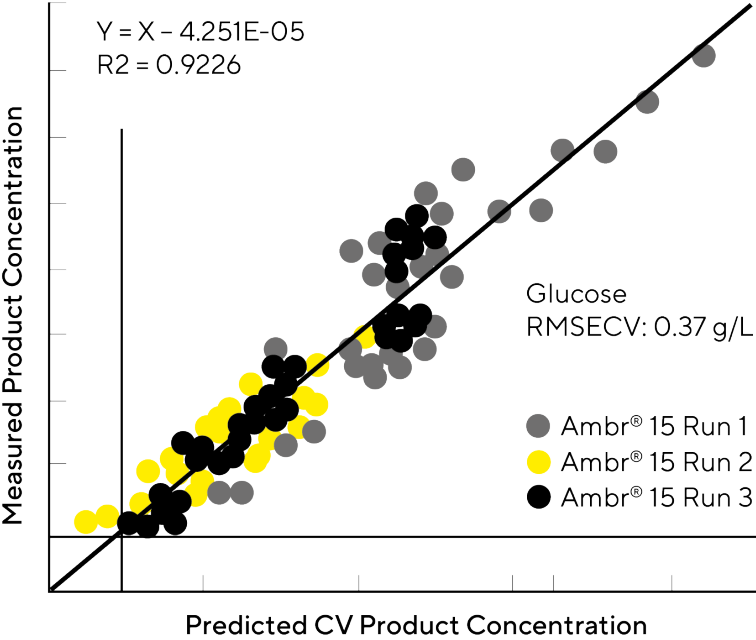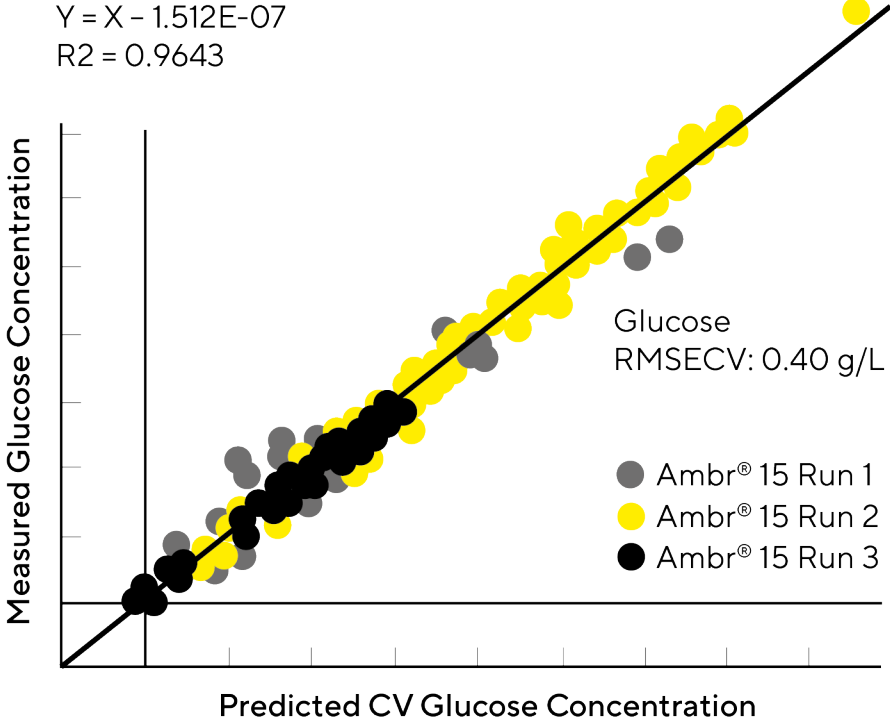Automated

SIMCA

1 Data acquisition
Automated data acquisition & consolidation Run 12, 24 or 48 bioreactors in parallel

2 Data consolidation & contextualization
Manually consolidate and align spectral and offline data

3 Model building
Based on at least 10 runs

Ambr®   50 % Faster

| Benchtop | At least 2 x 12 days | At least 1 day | 1 day | At least 26 days |

High robustness models with transferability to manufacturing

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Model Building In Ambr® 15 Cell Culture

Predictive Models

$Y = X - 1.512E\text{-}07$
$R2 = 0.9643$

Measured Glucose Concentration (y-axis)
Predicted CV Glucose Concentration (x-axis)

Glucose
RMSECV: 0.40 g/L

- Ambr® 15 Run 1
- Ambr® 15 Run 2
- Ambr® 15 Run 3

$Y = X - 4.251E\text{-}05$
$R2 = 0.9226$

Measured Product Concentration (y-axis)
Predicted CV Product Concentration (x-axis)

Glucose
RMSECV: 0.37 g/L

- Ambr® 15 Run 1
- Ambr® 15 Run 2
- Ambr® 15 Run 3

Reproduced with kind permission from Ruth Rowland-Jones, GSK Stevenage. Performed with a prototype spectroscopy integration and a Tornado Raman spectrometer.

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Model Building In Ambr® 15 Cell Culture

Predictive Models

Y = X – 0.0001769
R2 = 0.9753

Measured Product Concentration

Predicted CV Lactate Concentration

Lactate
RMSECV: 0.29 g/L

● Ambr® 15 Run 1
● Ambr® 15 Run 2
● Ambr® 15 Run 3

Y = X + 1.208E-05
R2 = 0.8187

Measured Glutamate Concentration

Predicted CV Glutamate Concentration

Glutamate
RMSECV: 0.15 g/L

● Ambr® 15 Run 1
● Ambr® 15 Run 2
● Ambr® 15 Run 3

Reproduced with kind permission from Ruth Rowland-Jones, GSK Stevenage. Performed with a prototype spectroscopy integration and a Tornado Raman spectrometer.

Model Building In Ambr® 15 Cell Culture

Results – Single Bioreactor Time Course Comparison

Glucose Concentration
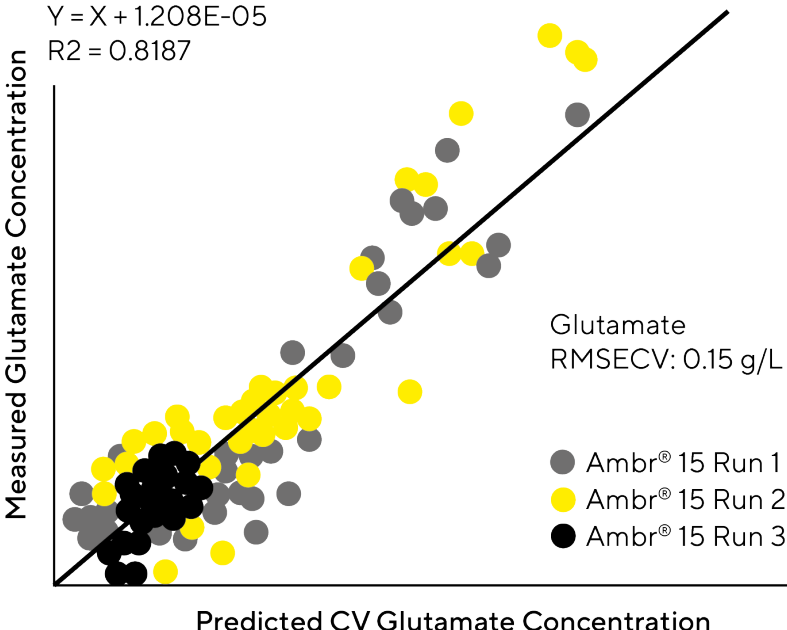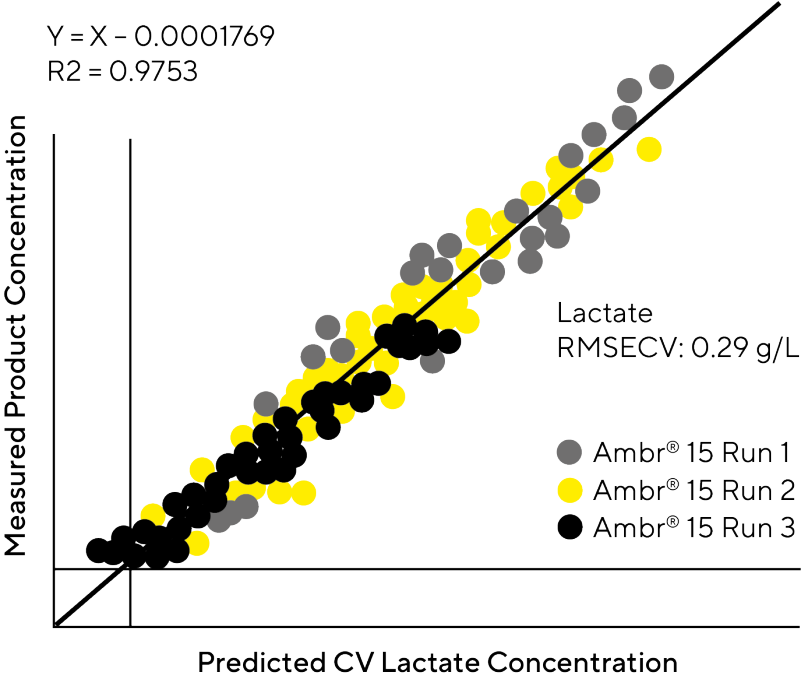
Lactate Concentration
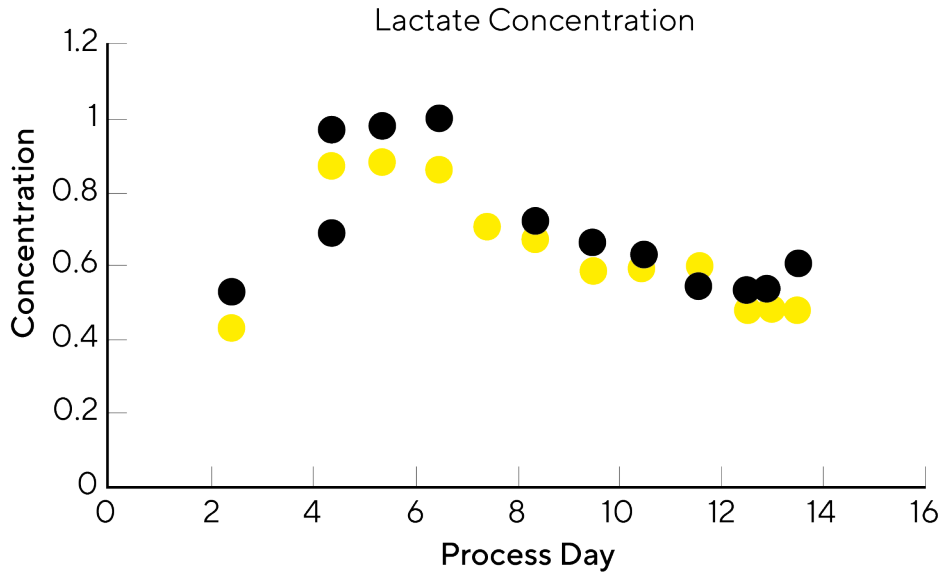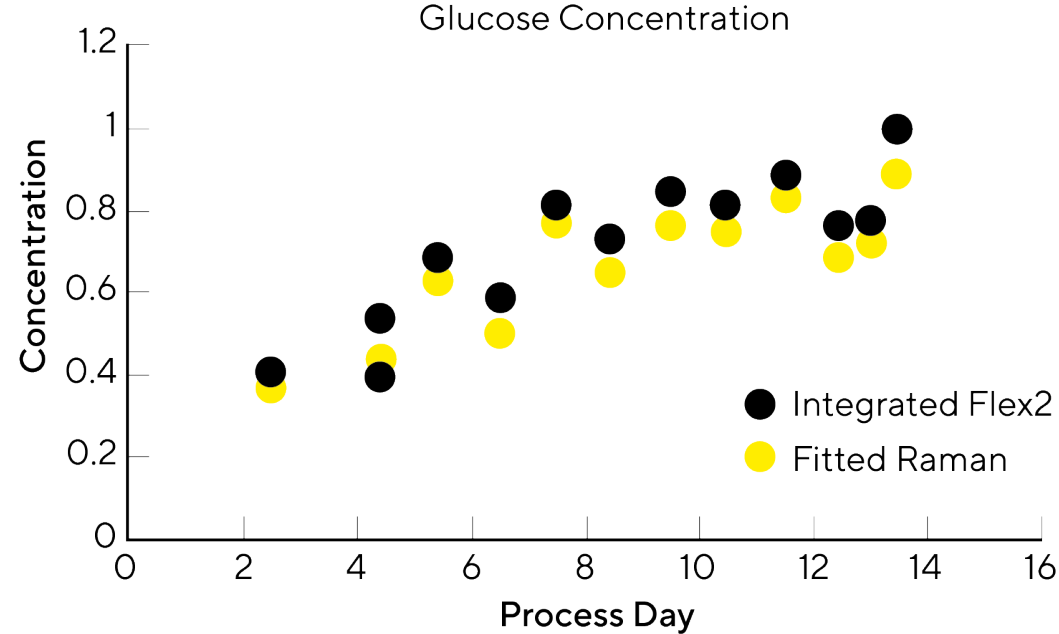
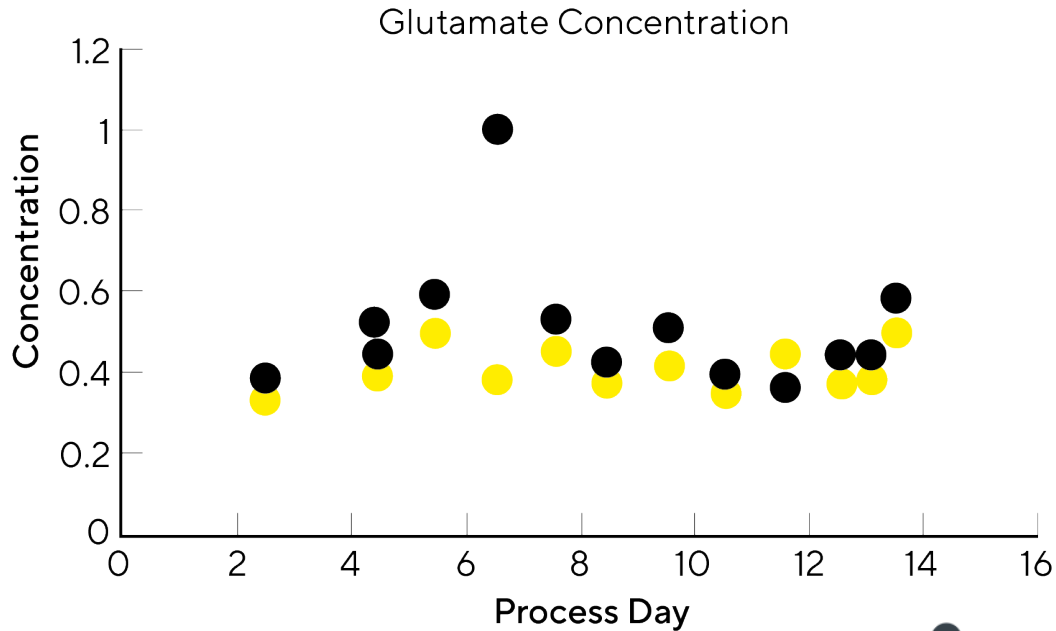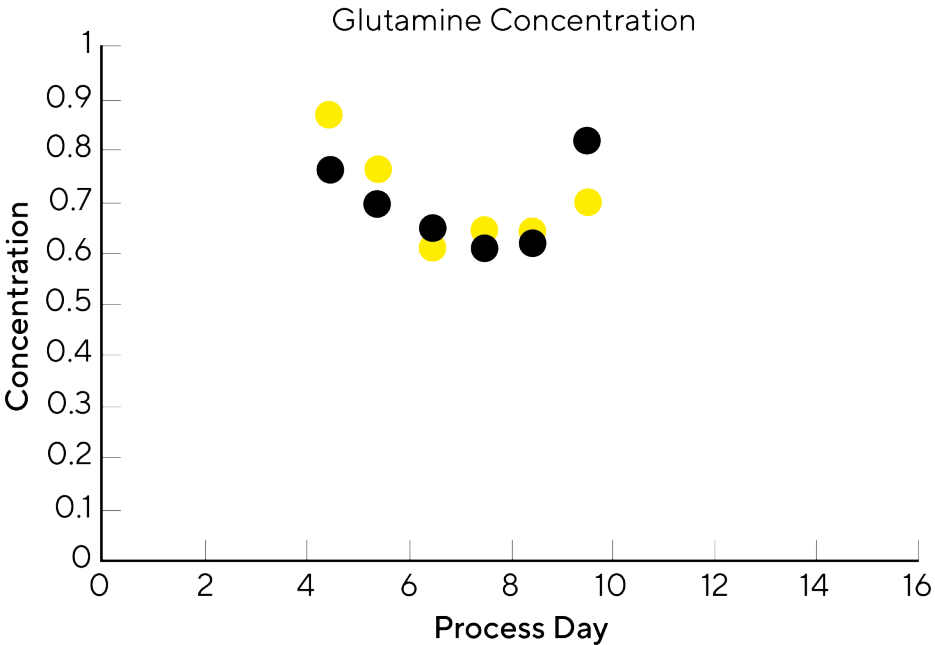All concentrations normalized to highest Flex2 Value

Reproduced with kind permission of Merck MSD, Mike Nelson presented this at AICHE 2019, performed with a prototype spectroscopy integration and a Tornado Raman spectrometer
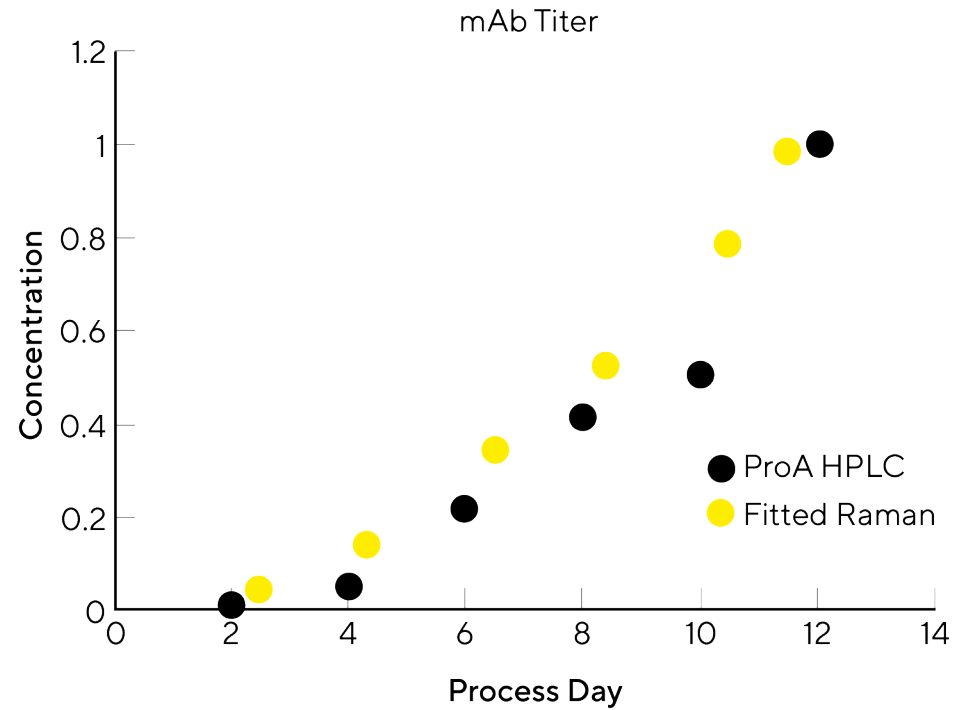
Model Building In Ambr® 15 Cell Culture

Results – Single Bioreactor Time Course Comparison

Reproduced with kind permission of Merck MSD, Mike Nelson presented this at AICHE 2019, performed with a prototype spectroscopy integration and a Tornado Raman spectrometer

Model Building In Ambr® 15 Cell Culture

- Picked one specific bioreactor to show time course data

- Raman model tracks well with reference data

- Could conceivably control process using only fitted Raman model

mAb Titer

● ProA HPLC
● Fitted Raman

Reproduced with kind permission of Merck MSD, Mike Nelson presented this at AICHE 2019, performed with a prototype spectroscopy integration and a Tornado Raman spectrometer
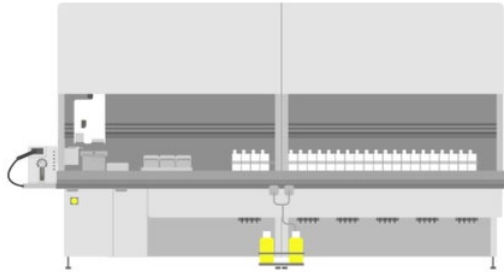
Raman Spectroscopy Use Cases

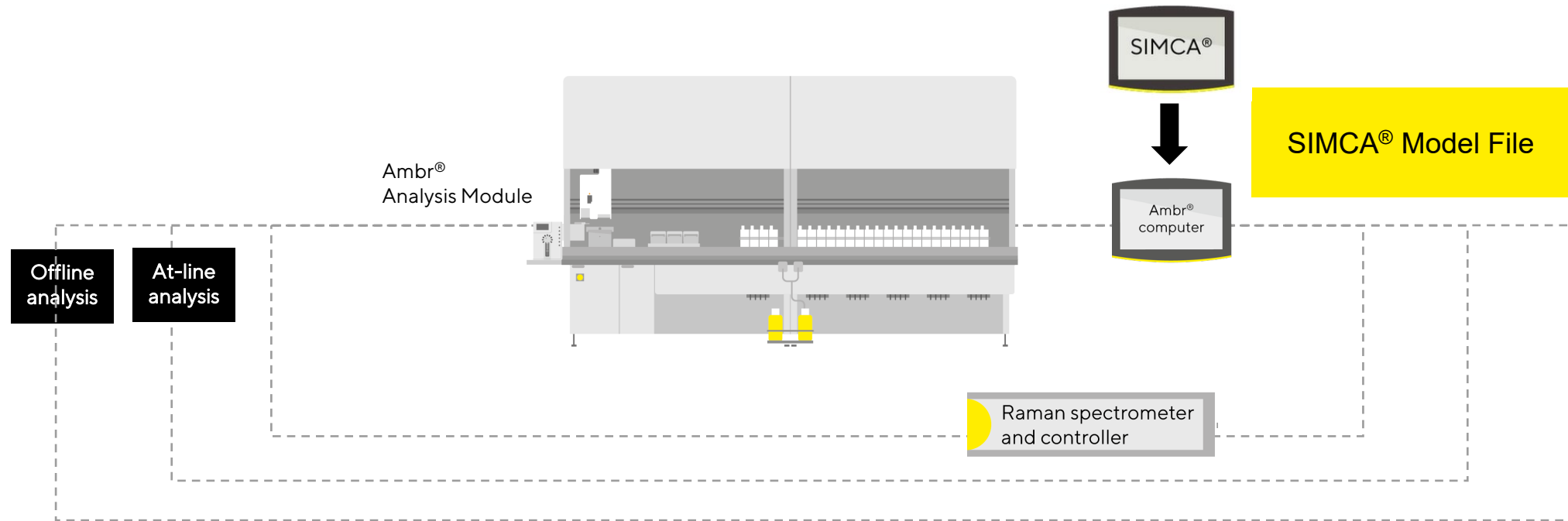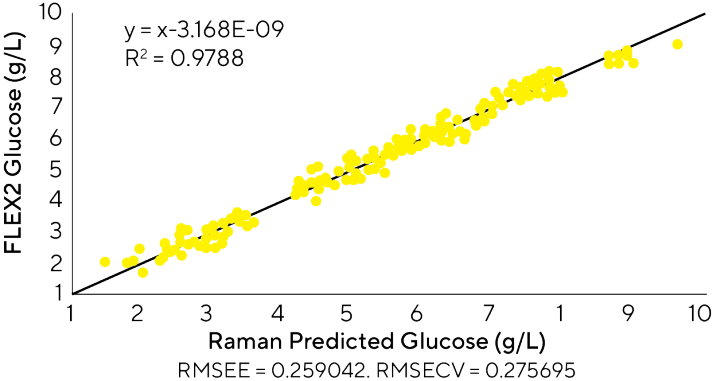| Model building	Process development	Single-use manufacturing

Ambr® 15 Cell Culture

Ambr® 250 High Throughput

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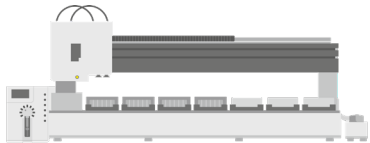
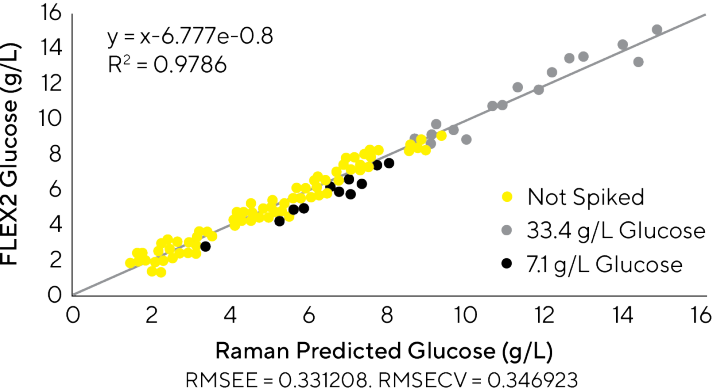# Raman Spectroscopy in High-Throughput Process Development

The Ambr® software is able to read SIMCA® model files and can predict analyte concentrations from the spectral data for process monitoring and control.
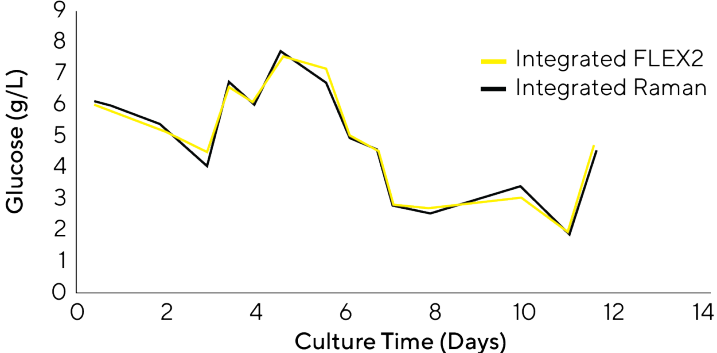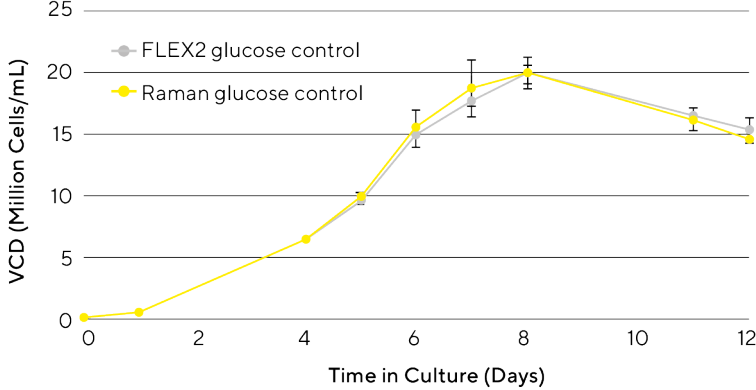
Automated Feed Control in Ambr® Using Raman Spectroscopy

y = x-3.168E-09
R² = 0.9788

FLEX2 Glucose (g/L) vs Raman Predicted Glucose (g/L)

RMSEE = 0.259042. RMSECV = 0.275695

y = x-6.777e-0.8
R² = 0.9786

Not Spiked
33.4 g/L Glucose
7.1 g/L Glucose

FLEX2 Glucose (g/L) vs Raman Predicted Glucose (g/L)

RMSEE = 0.331208. RMSECV = 0.346923

Glucose model built without spiking range: 2 – 9 g/L

Glucose model built with spiking range: 2 – 15 g/L

Integrated FLEX2
Integrated Raman

Glucose (g/L) vs Culture Time (Days)

FLEX2 glucose control
Raman glucose control

VCD (Million Cells/mL) vs Time in Culture (Days)

Raman predictions and NovaFlex 2 measurements give comparable results

Cell culture profiles are very similar for glucose control based on Raman or Nova FLEX2 assays
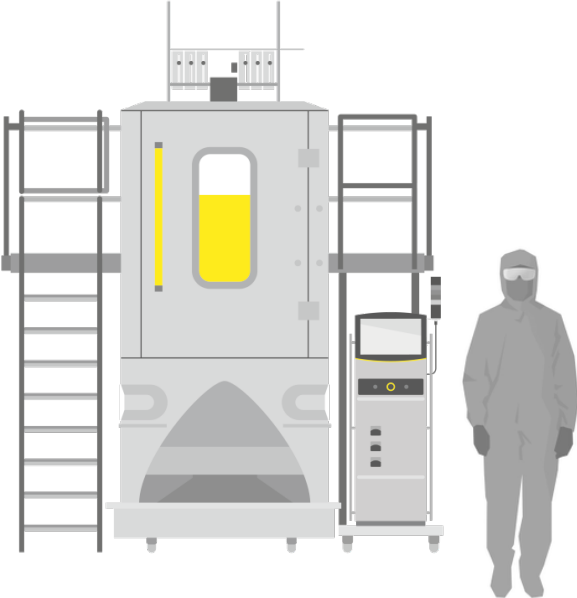
Data source: Ambr® 250 High Throughput, with Kaiser Raman spectrometer and probe; Marek Hoehse and Mike Sibley, Sartorius; Glucose set point: 5 g/L using bolus additions
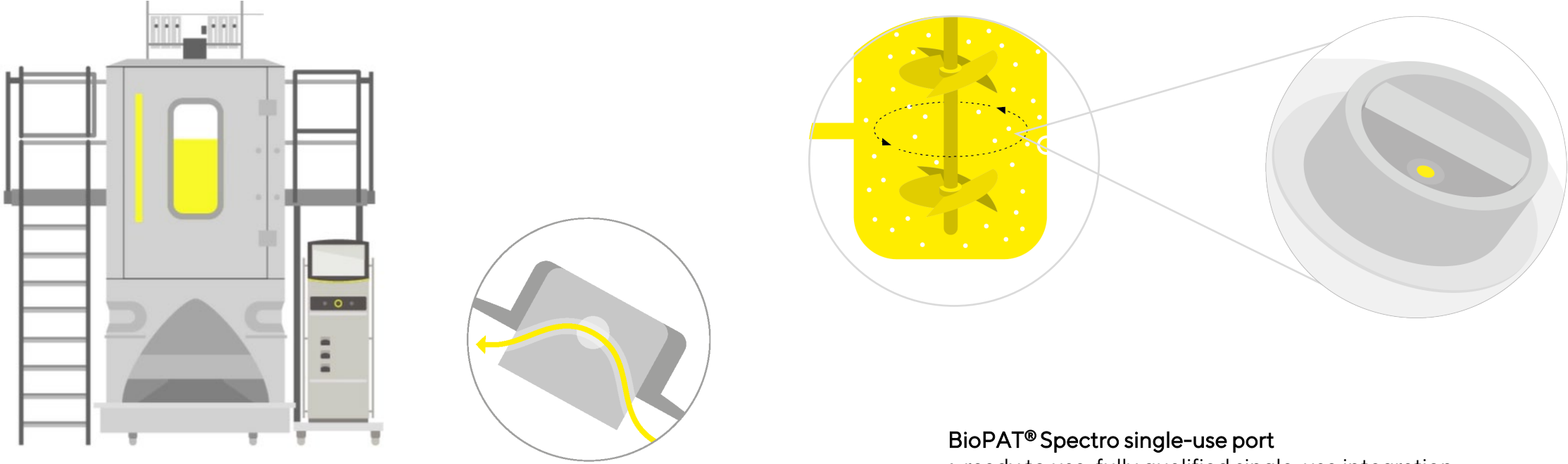
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Raman Spectroscopy Use Cases

Model building	Process development	Single-use manufacturing

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BioPAT® Spectro Single-Use, Inline Integration in Flexsafe STR®

BioPAT® Spectro single-use port
- ready to use, fully qualified single-use integration
- isolation of sample from light and bubbles
- faster set-up time

SARTORIUS

BioPAT® Spectro Single-Use, Inline Integration in Flexsafe STR®

BioPAT® Spectro single-use port in Flexsafe STR® bag

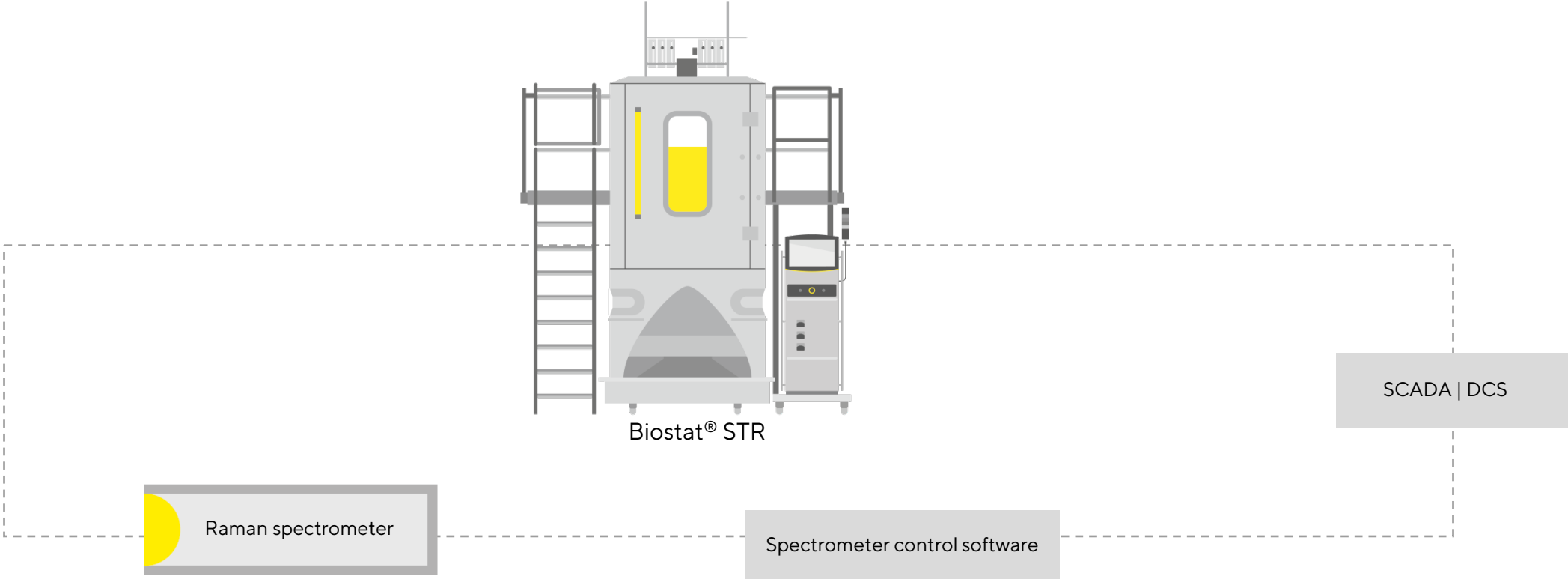Connection of a Endress+Hauser Raman spectrometer probe

Connection of a Tornado Raman spectrometer probe)

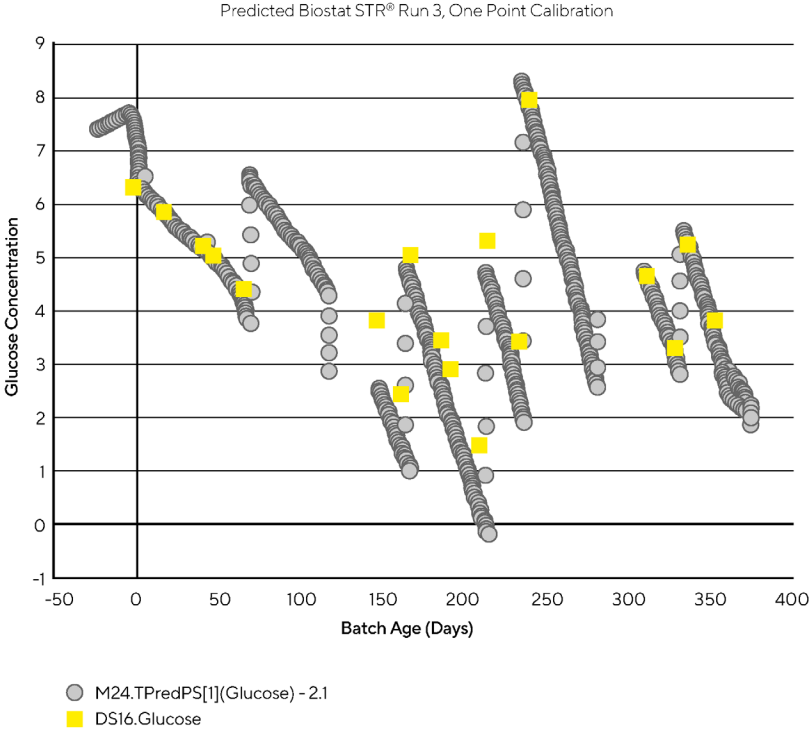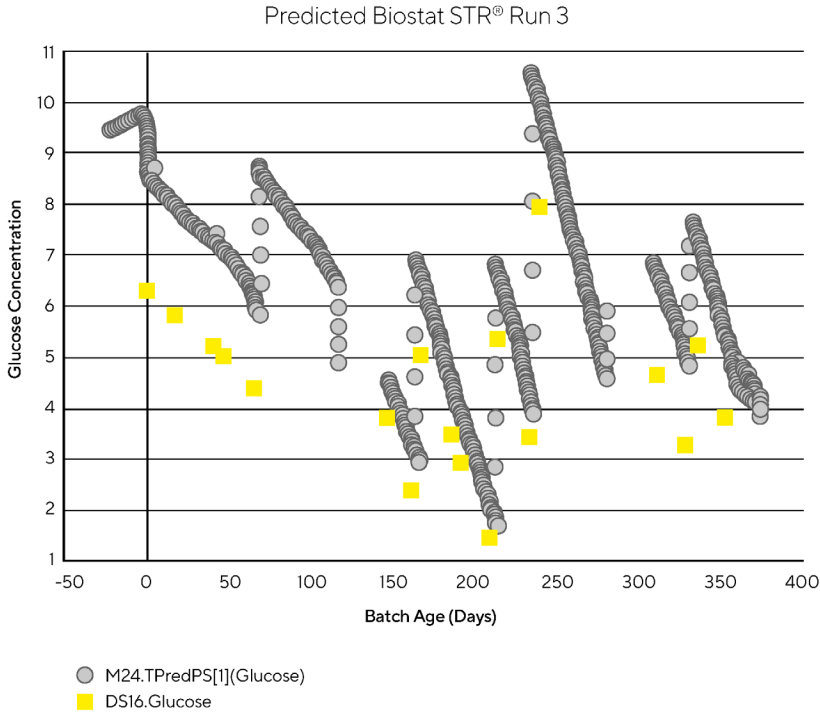**Standardized optical design: same probe for Ambr® and Biostat STR®**

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BioPAT® Spectro integration in Biostat STR®

Biostat® STR

SCADA | DCS

Raman spectrometer

Spectrometer control software

SARTORIUS

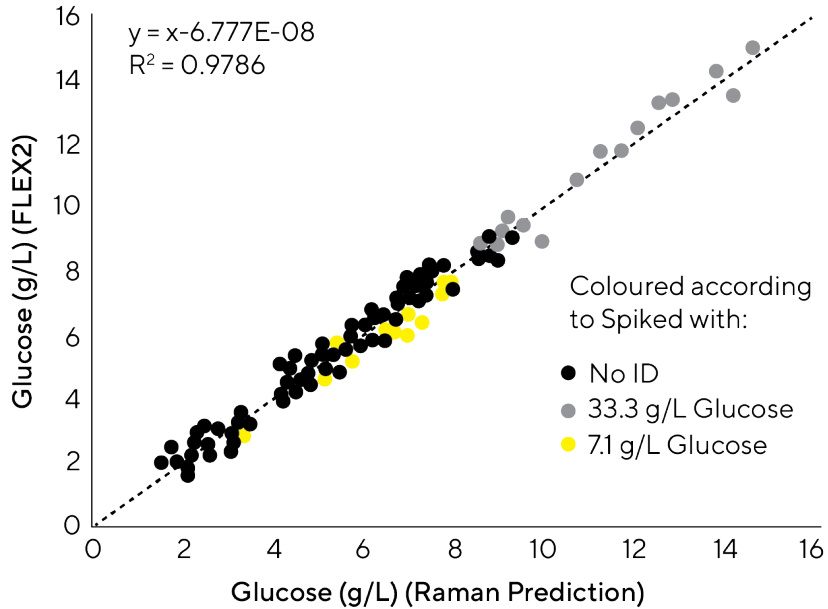# Scalable Models Due to Platform Approach - Model Transfer to 50 L Single-Use Bioreactor

Model developed using Ambr® 15 runs + SUB batch 1
Used to predict SUB batch 3

Potential for Model Transfer

Predicted Biostat STR® Run 3

○ M24.TPredPS[1](Glucose)
■ DS16.Glucose

Predicted Biostat STR® Run 3, One Point Calibration

○ M24.TPredPS[1](Glucose) – 2.1
■ DS16.Glucose

Reproduced with kind permission from Ruth Rowland-Jones, GSK Stevenage. Performed with a prototype spectroscopy integration and a Tornado Raman spectrometer.
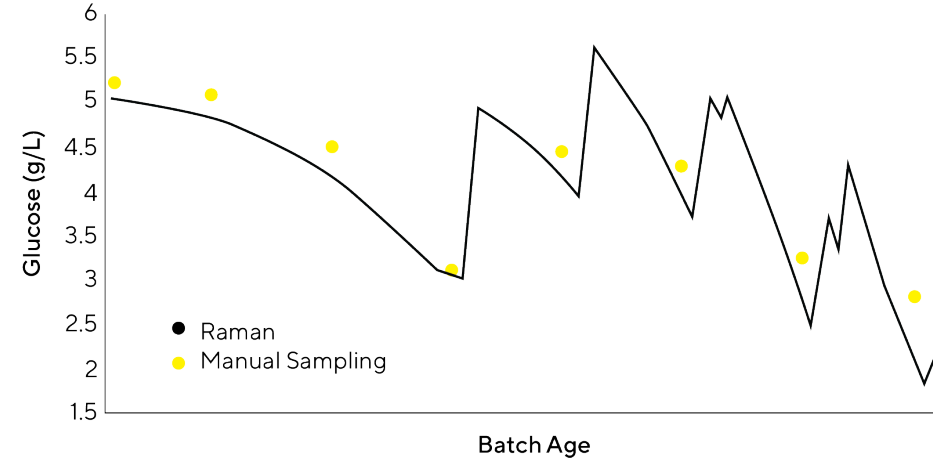
The Platform Approach Enables Model Transfer Across Scales

$y = x - 6.777E-08$
$R^2 = 0.9786$

Coloured according to Spiked with:

- No ID
- 33.3 g/L Glucose
- 7.1 g/L Glucose

Glucose (g/L) (FLEX2) vs Glucose (g/L) (Raman Prediction)

Model building in Ambr® 250

- Glucose model built using two Ambr® runs of eight vessels each
- Spiking two glucose stock solutions
- RMSEcv = 0.34 g/L

- Raman
- Manual Sampling

Glucose (g/L) vs Batch Age

Direct model transfer from Ambr® to Flexsafe STR® 200L

- STR and Ambr® runs happened at different sites
- Different seed train, media lot and reference method were used
- The raw Ambr® model was used without any additional data from an STR
- Offset correction was performed based on first reference measurement

Data source: Ambr® 250 High Throughput & Flexsafe STR® 200, with Kaiser Raman spectrometer and prototype probe; Marek Hoehse and Mike Sibley, Sartorius

SARTORIUS

Enable Rapid and Robust Scale-Up With Scalable Bioreactor Hardware

- From Ambr® 250 to Biostat STR® 2000 Sartorius vessels maintain geometric similarity

- Ensures that mixing and oxygen transfer rates remain consistent over scaling

- Simplifies technology transfer between cultivation scales

Ratio	Ambr® 250	Biostat STR® 50	Biostat STR® 200	Biostat STR® 500	Biostat STR® 1000	Biostat STR® 2000
Vessel Height/Diameter	2	1.8	1.8	1.8	1.8	1.8
Impeller Diameter/Vessel Diameter	0.42	0.38	0.38	0.38	0.38	0.38

SARTORIUS

BioPAT® Spectro: Scalable Raman Spectroscopy in Single-Use

- Robust model building in Ambr® is faster, easier, and more cost effective than possible before

- Quality by design is enabled by introducing Raman spectroscopy to high-throughput process development

- Efficient transfer to single-use manufacturing due to the scalable platform approach
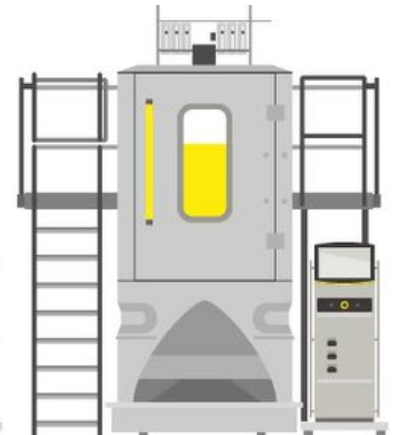

Ambr® 15 Cell Culture

Ambr® 250 High Throughput

Biostat® STR

SARTORIUS
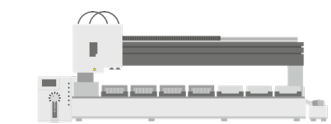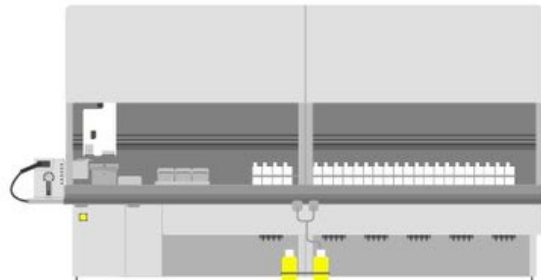
How to Get BioPAT® Spectro?

- All Ambr® 250 High Throughput and Ambr® 15 Cell Culture instruments can be retrofitted
- Quotes can be generated right now, orders will ship starting from 08/2020
- Flexsafe STR® bags with the BioPAT® Spectro single-use ports are available from 12/2020 onwards
- Please contact your local PAT expert for inquiries

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Thank you.

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