TU Delft

Bioprocess Engineering

In-line Raman spectroscopy for process characterization

Dr.ir. Marieke Klijn
Assistant Professor

NLab Discussion Group
25 – November - 2025

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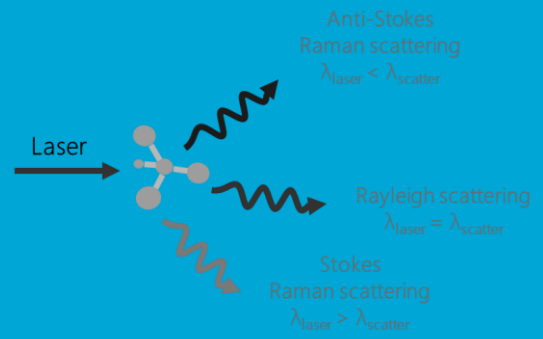
Content

I.

Raman spectroscopy

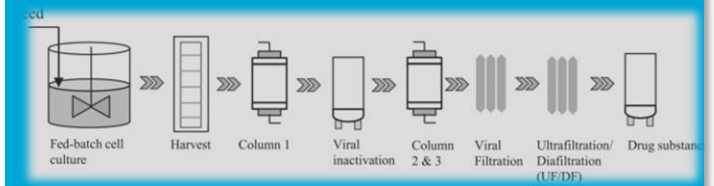
Introduction



II.

Process characterization

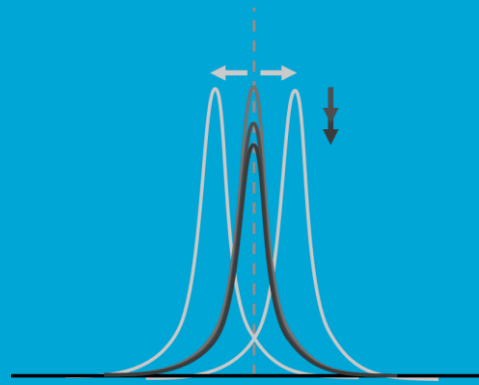
USP & DSP



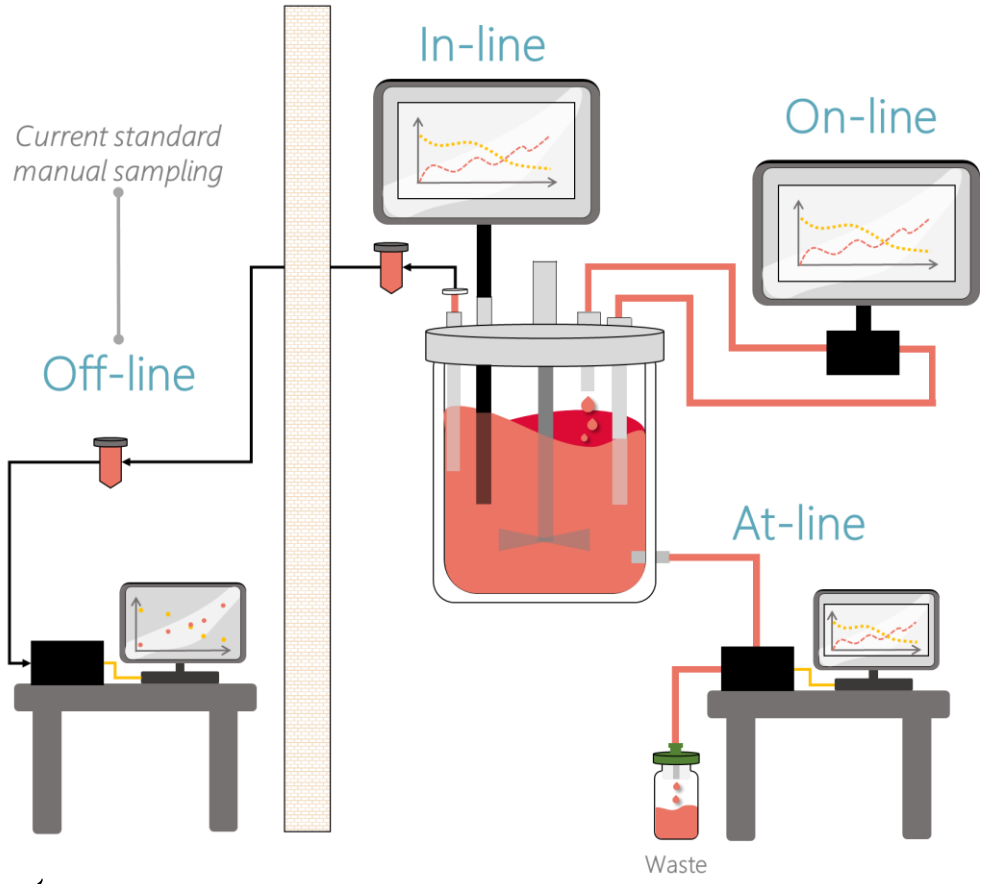
III.

Signal distortion

Impact bioreactor parameters



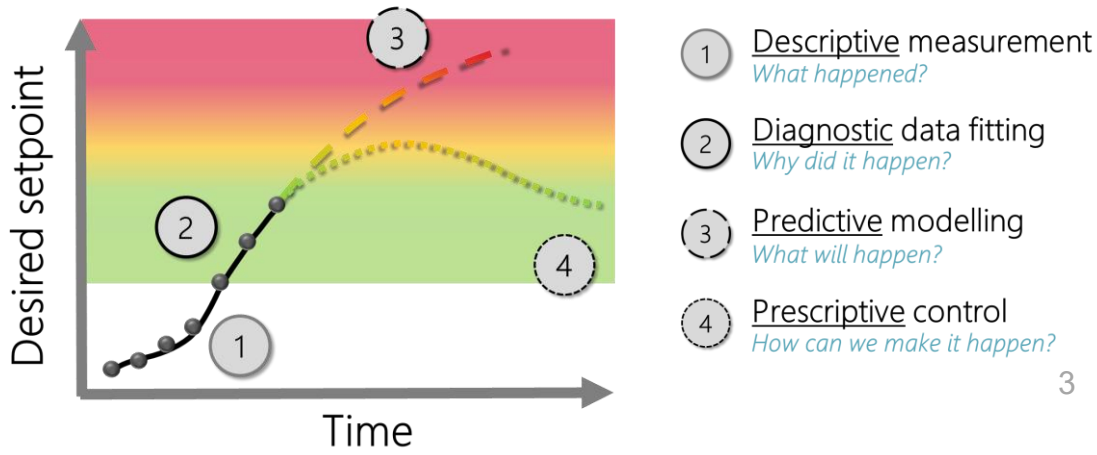
Process Analytical Technology



Capture relevant (inherent biological) variation & critical attributes

Understand impact on process performance & product quality

Establish risk-based thresholds and robust (automated) process decisions



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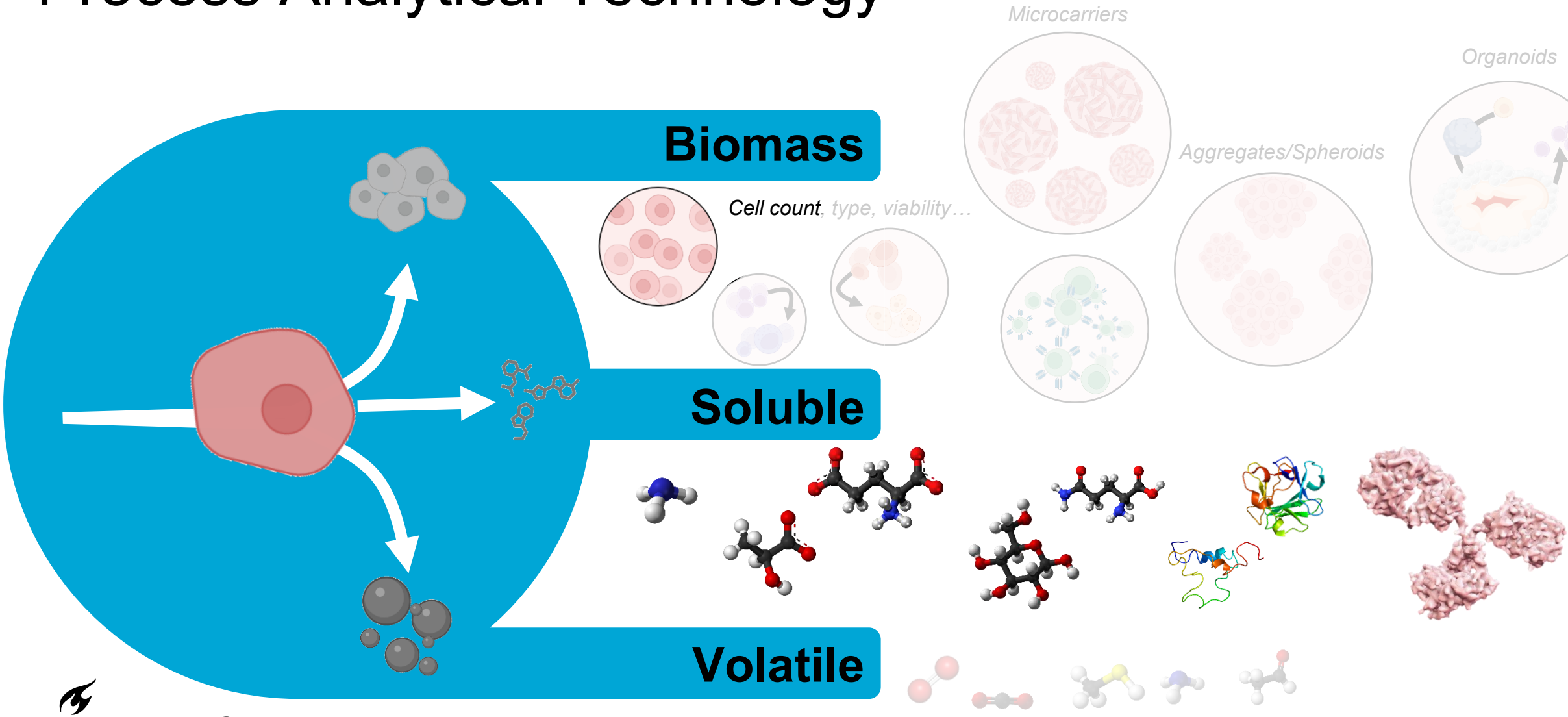
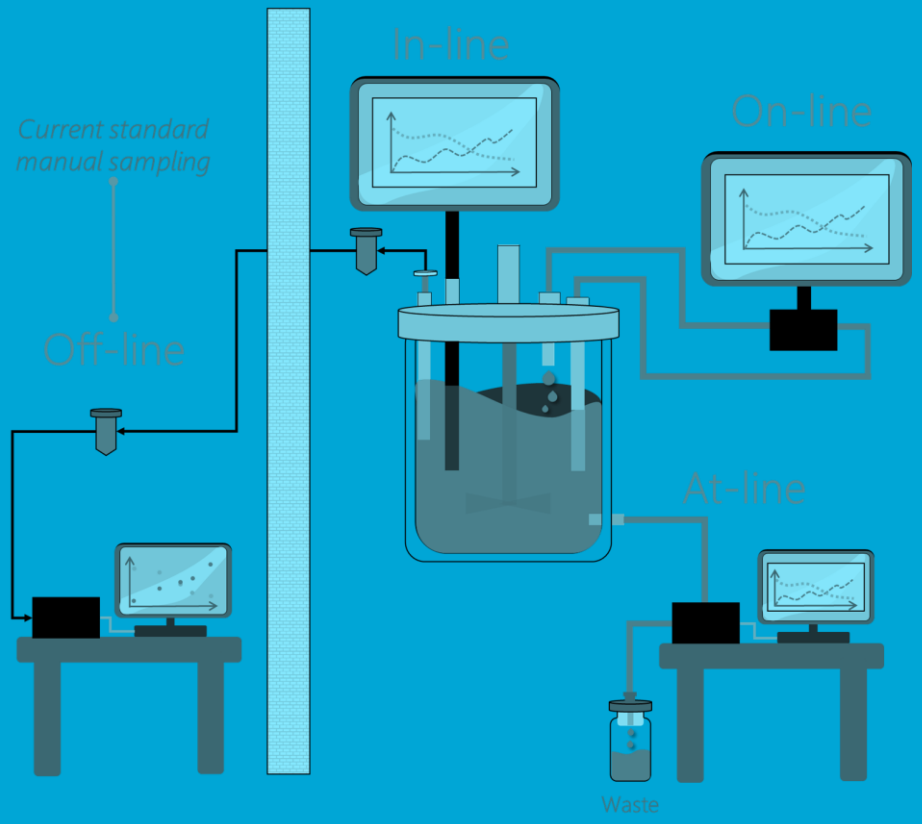
Process Analytical Technology





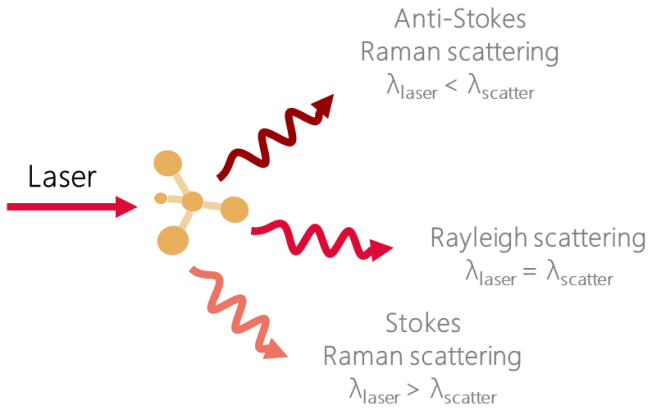
In-line Raman spectroscopy

In-line

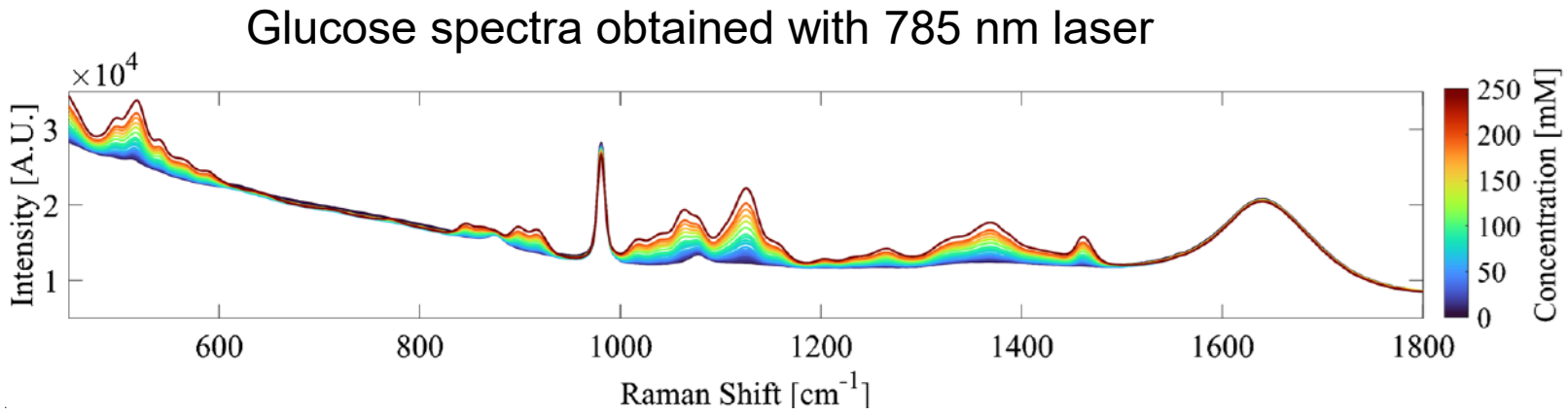
https://www.us.endress.com/en/field-instruments-overview/optical-analysis-product-overview/Raman-bio-multi-optic-sleeve?t.tabId=product-overview

On/At-line

Basics of Raman spectroscopy



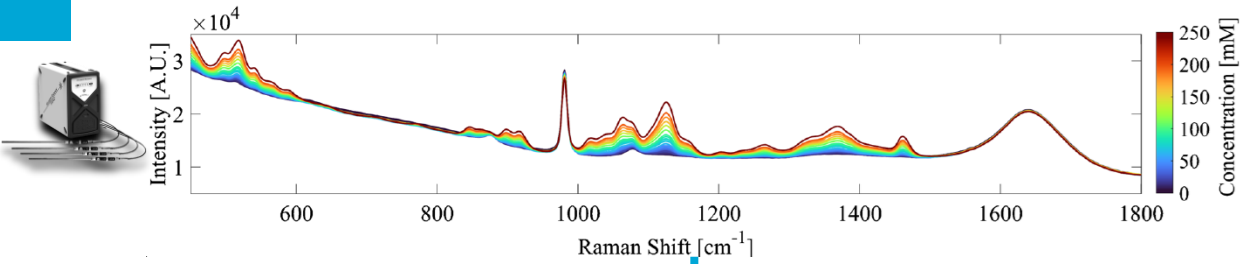
Light-matter interaction



Peak height
Abundance

Peak position
Identity

From spectrum to read-out



Peak position
Identity

Peak height
Abundance

Separate for each target analyte

Continuous in-line measurement

Manual sampling off-line measurement

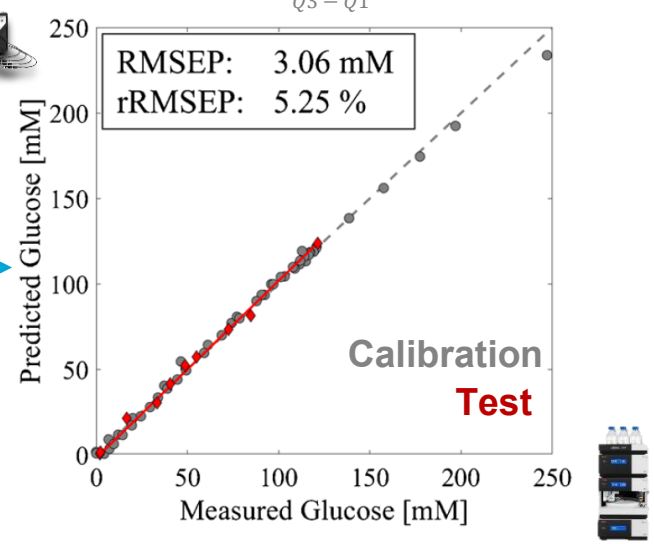
X-data

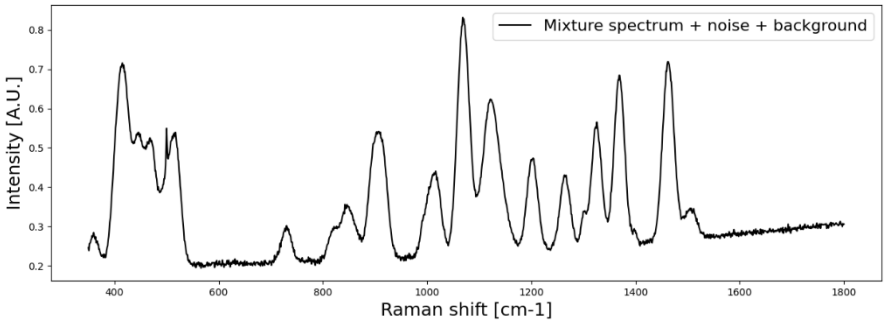
Y-data

Chemometric model

$\hat{y} = x_{pred}\hat{b} + e$

$rRMSEP = \frac{RMSE}{Q3 - Q1} \cdot 100\%$



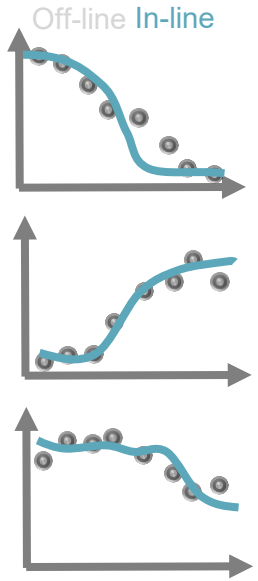


Single spectrum

Model 1

Model 2

Model 3



Multiplexed readout

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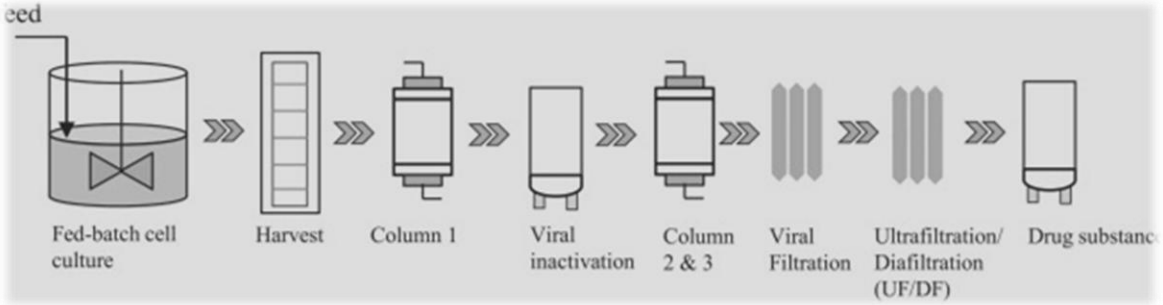
II. Applications

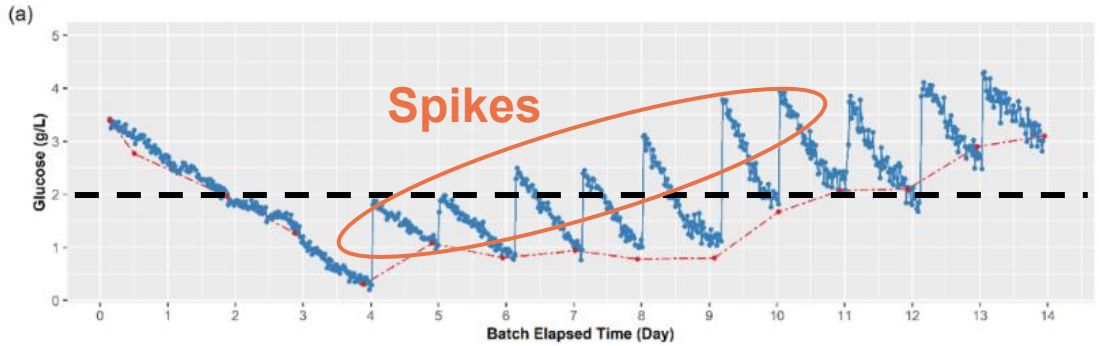


Upstream processing

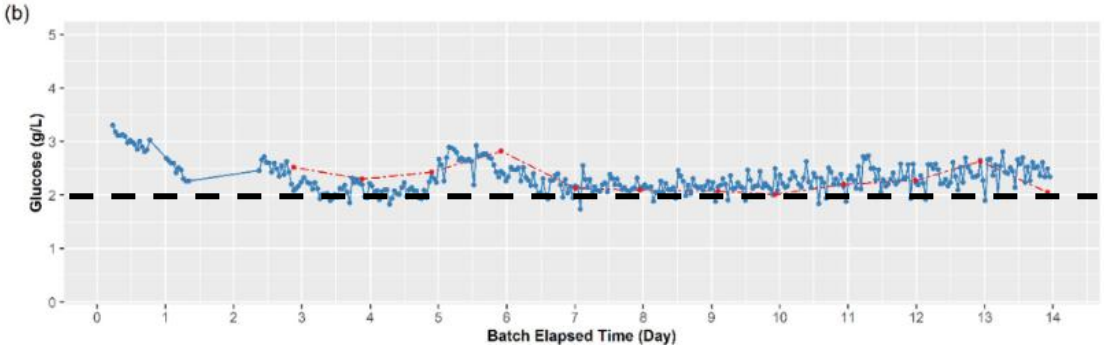
Feeding strategy optimization

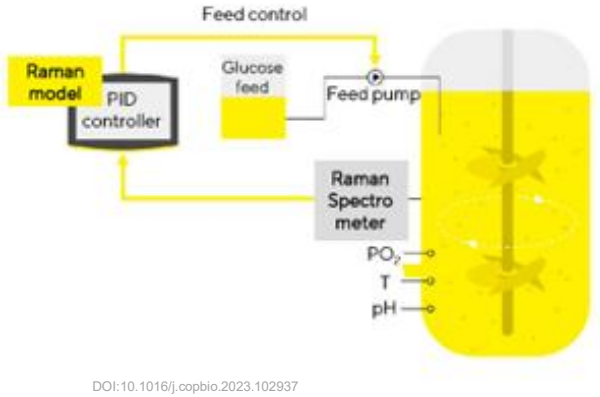
Bolus

Raman
Off-line



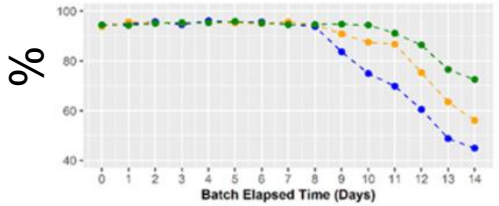
Automated (target: 2 g/L)





DOI:10.1016/j.copbio.2023.102937

Cell viability

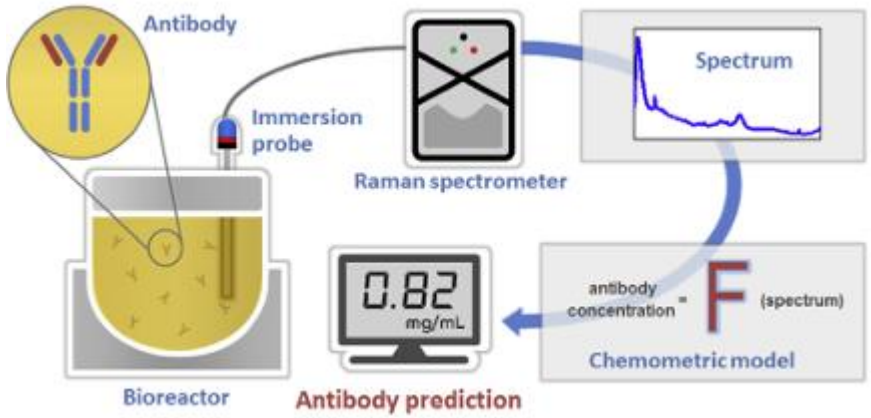


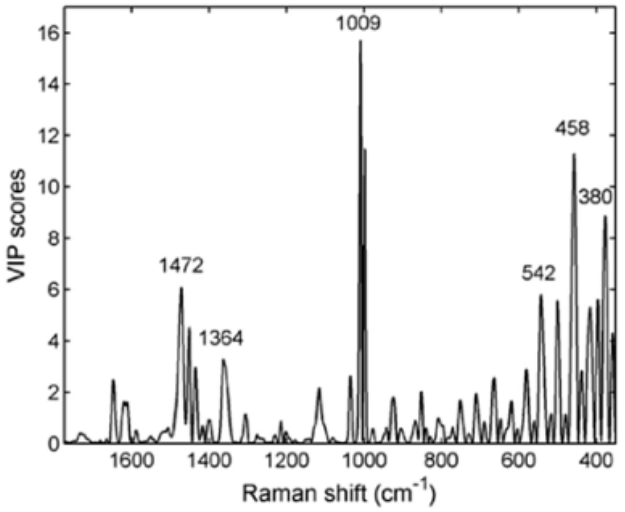
Automated
Automated
Bolus

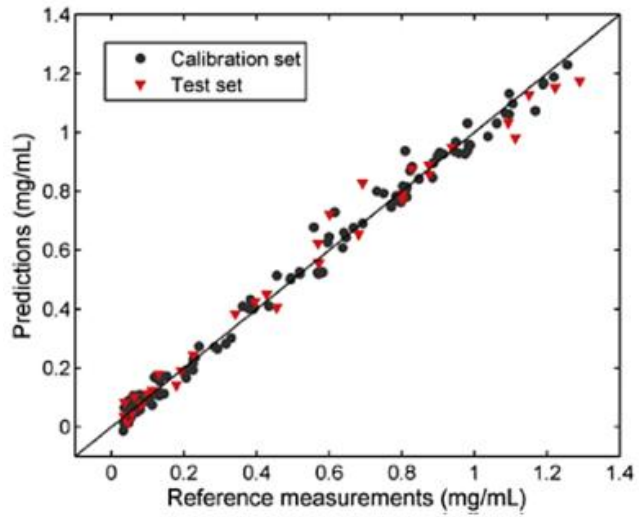
TU Delft

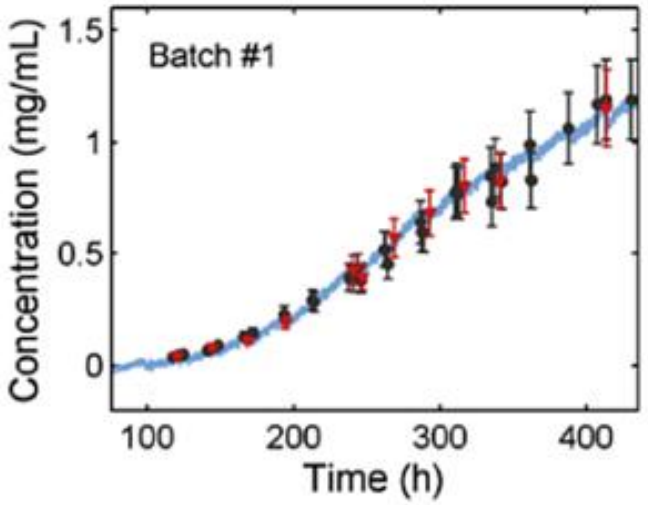
DOI: 10.1002/btpr.3371

Upstream processing









Raman spectroscopy
Calibration
Test

Product monitoring

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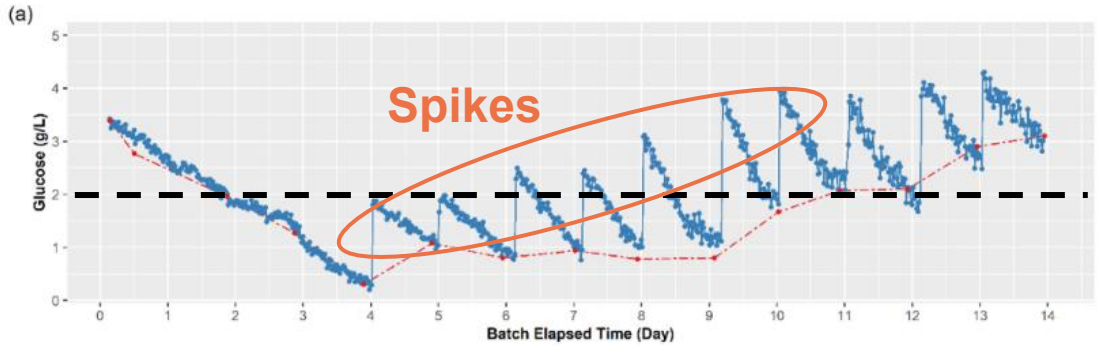
DOI: 10.1016/j.aca.2015.08.050

Upstream processing

Feeding strategy optimization

Bolus

Raman
Off-line



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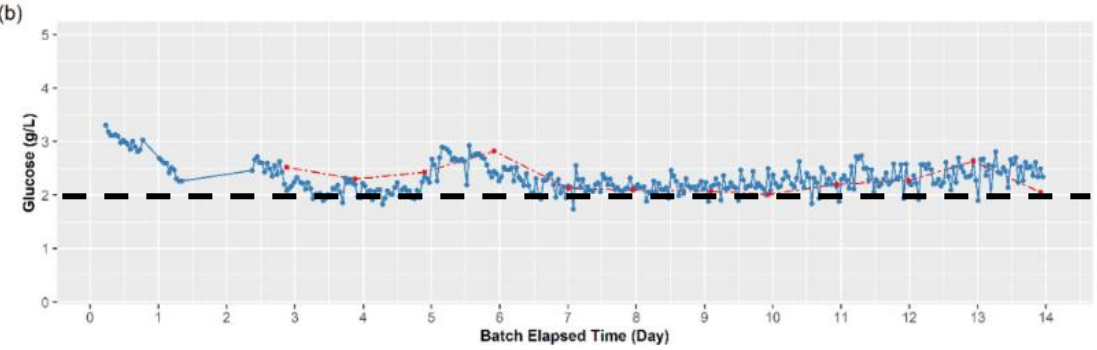

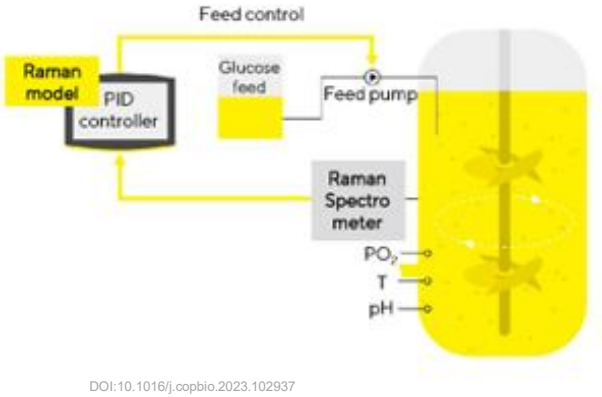

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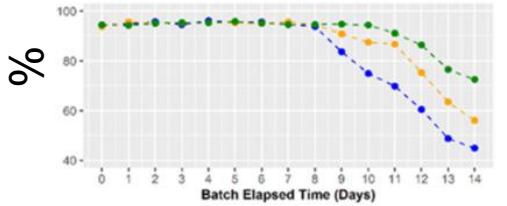
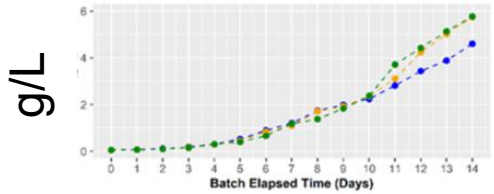
Cell viability



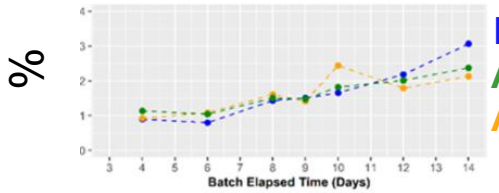
Automated
Automated
Bolus

Titer (mAb)



Automated
Automated
Bolus

Glycosylation



Bolus
Automated
Automated

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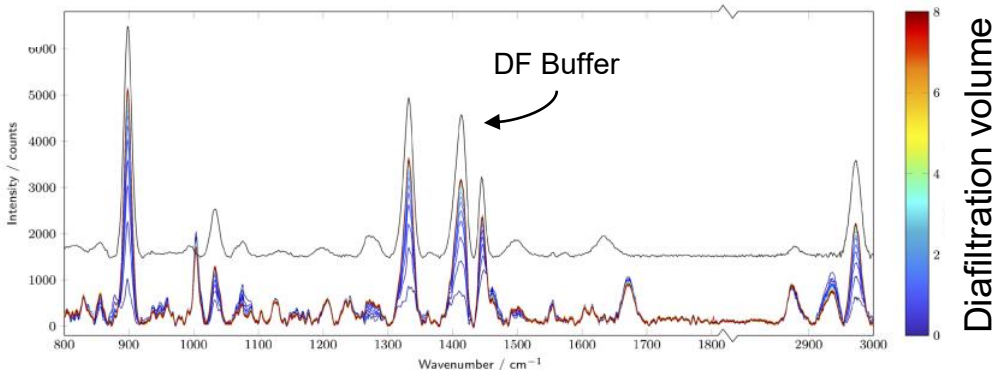
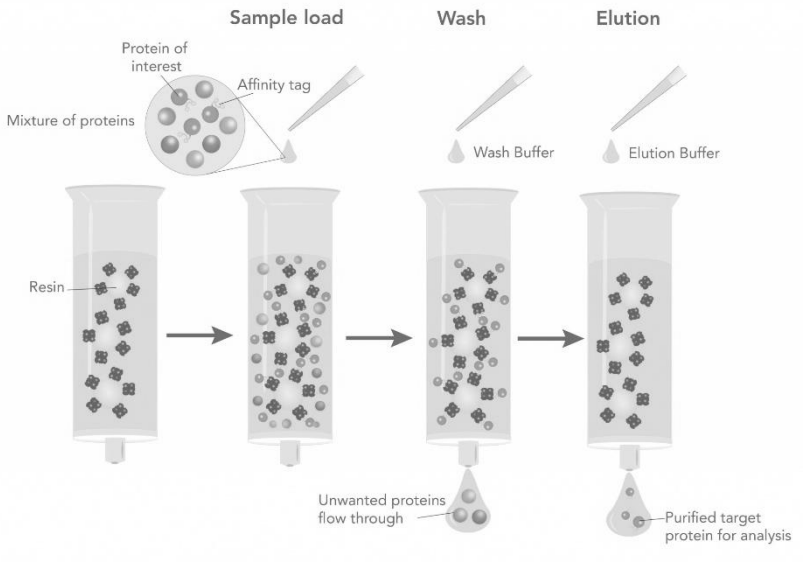
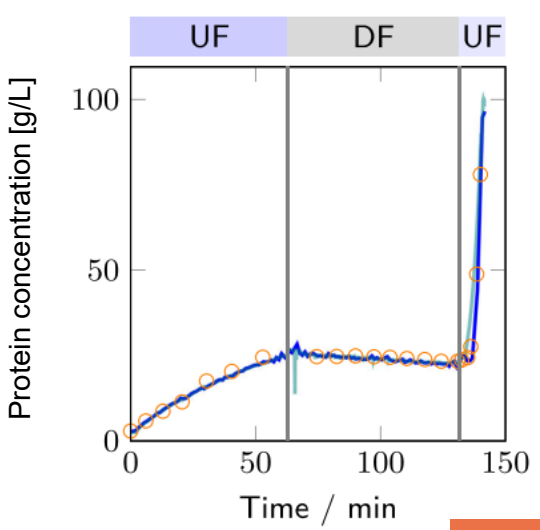
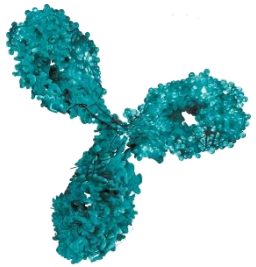
DOI: 10.1002/btpr.3371

Downstream processing

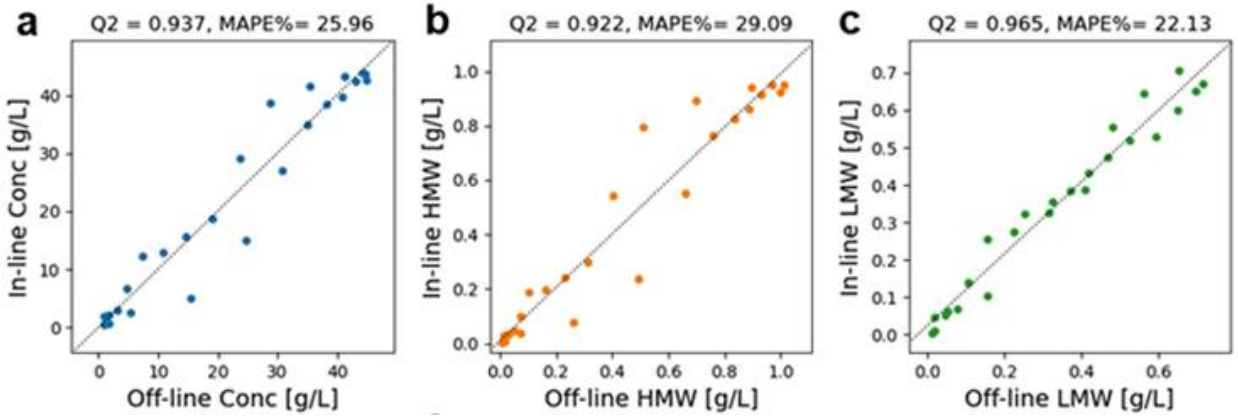








Off-line SEC
In-line UV/Vis
In-line Raman



Dynamic filtration

In-line CQA determination

10.1007/s00216-022-04477-7

10.1016/j.chroma.2024.464721

Opportunities & challenges

Insight

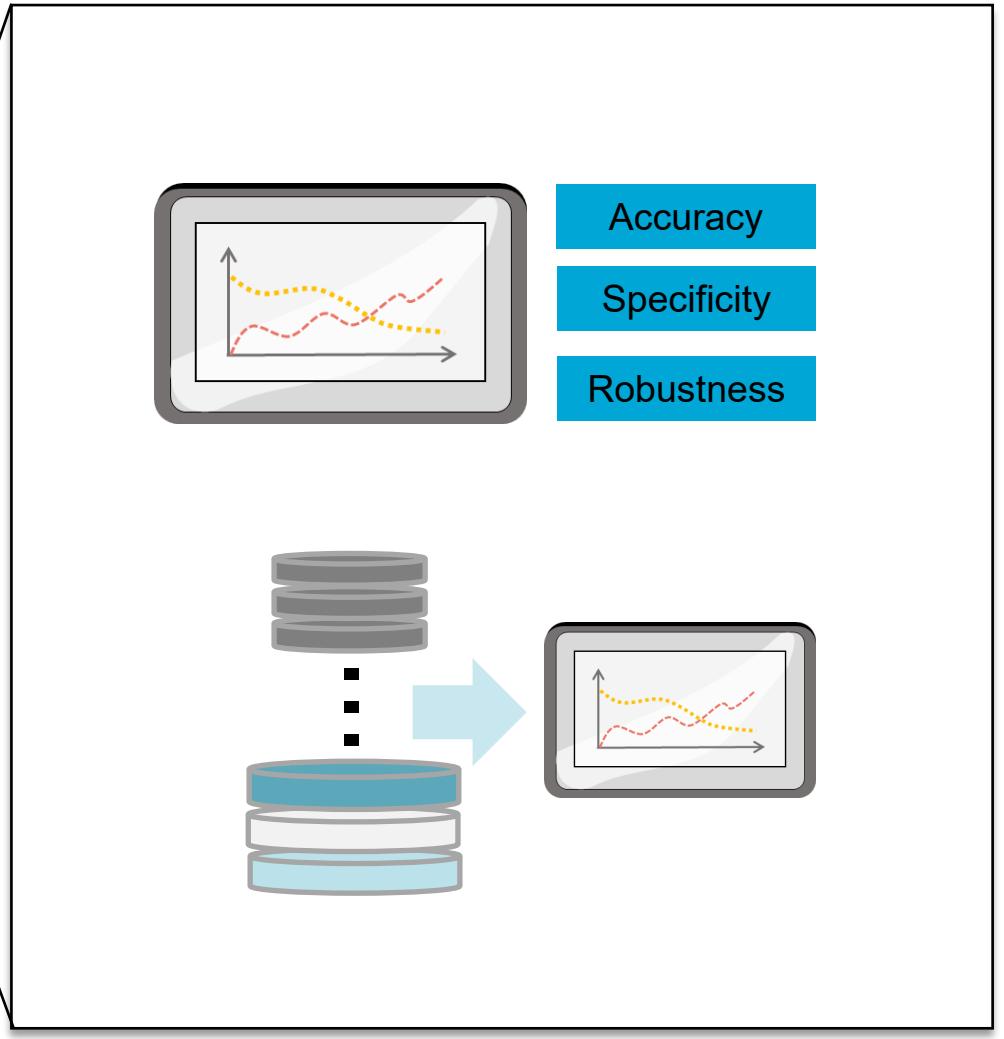
Development

Automation

Evaluation

Transferability

Early-stage use



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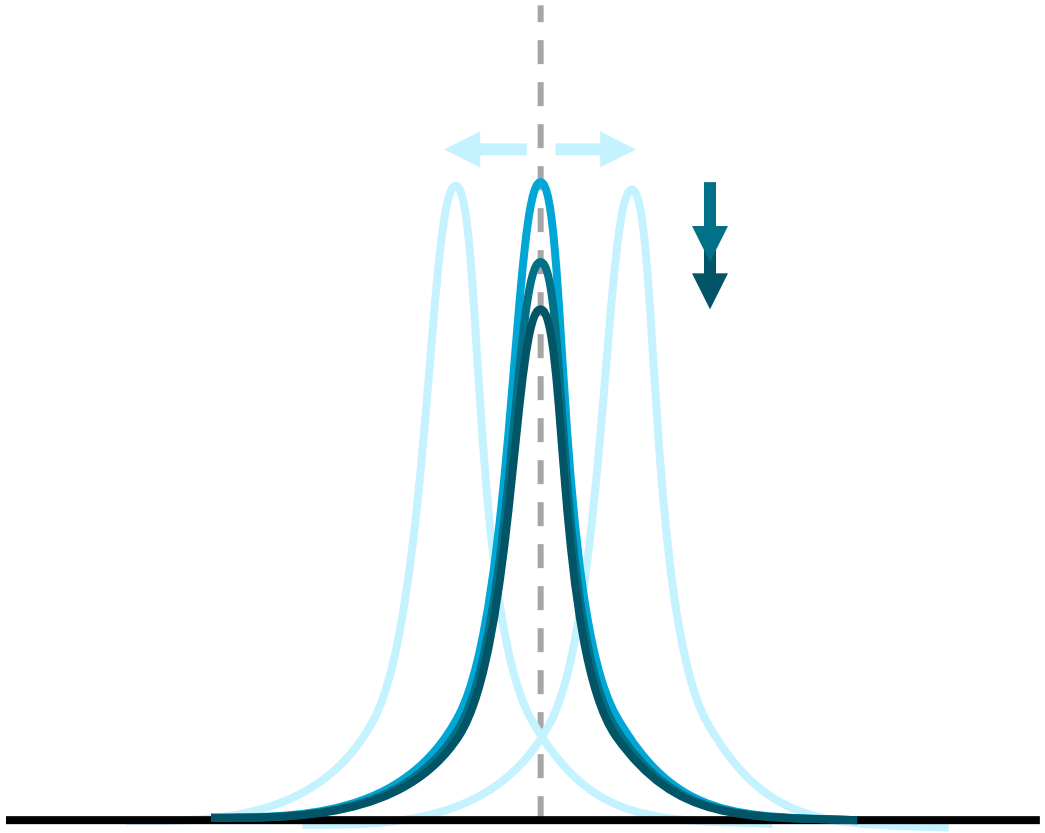
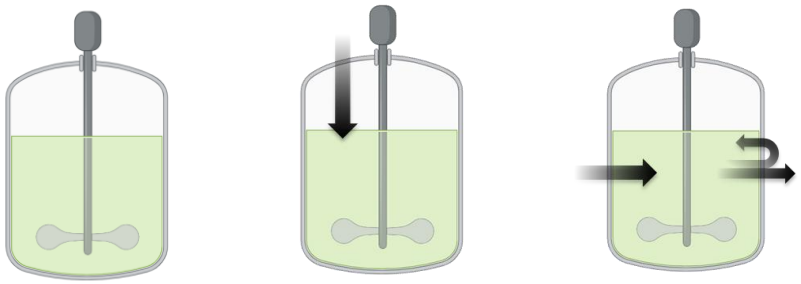
III.
Signal distortion

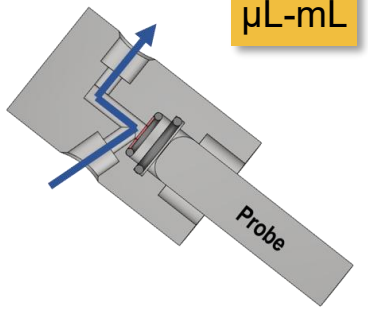


Signal understanding



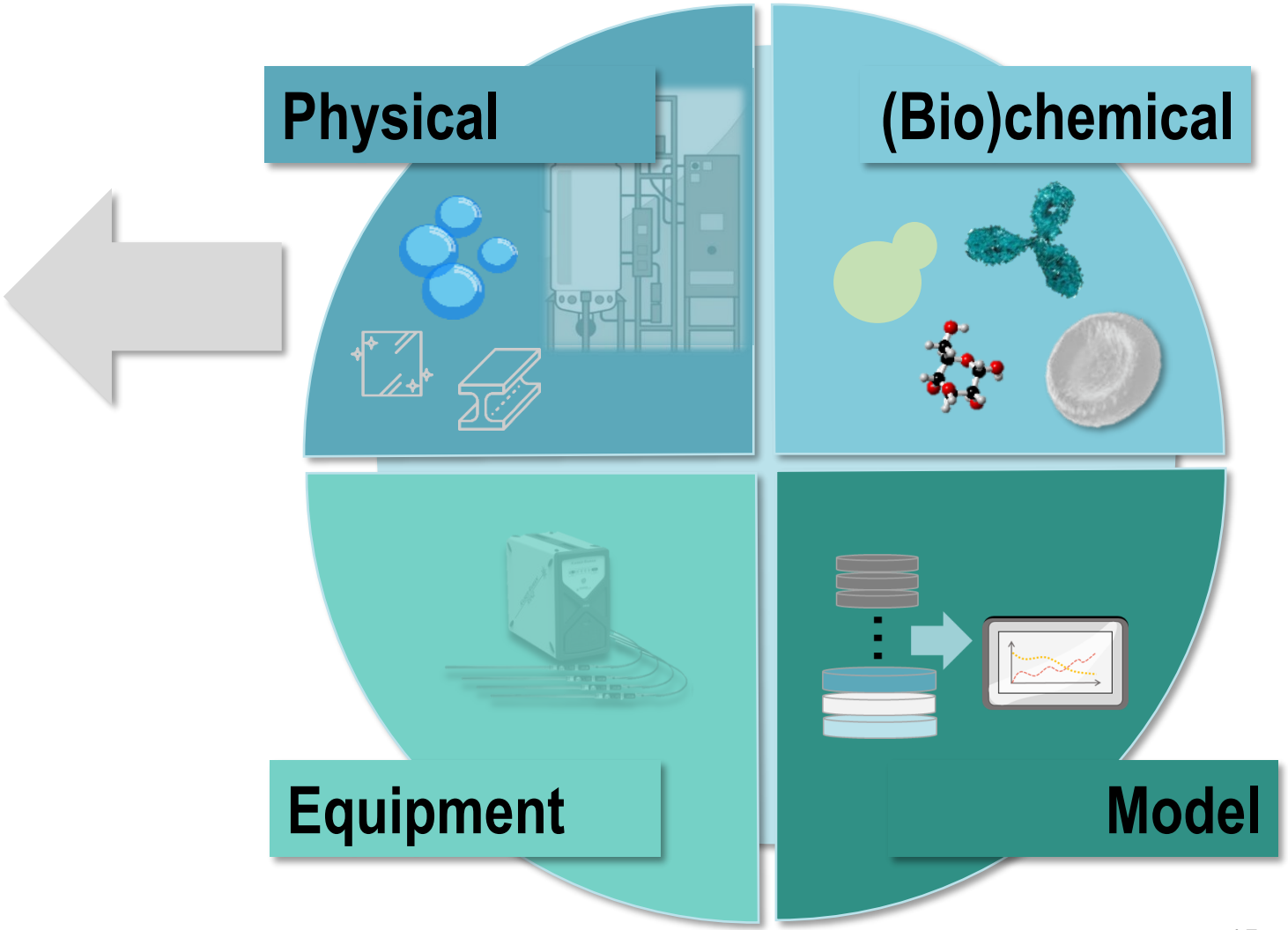
Sharing data between processes

mL-L



µL-mL

Miniaturization of measurements



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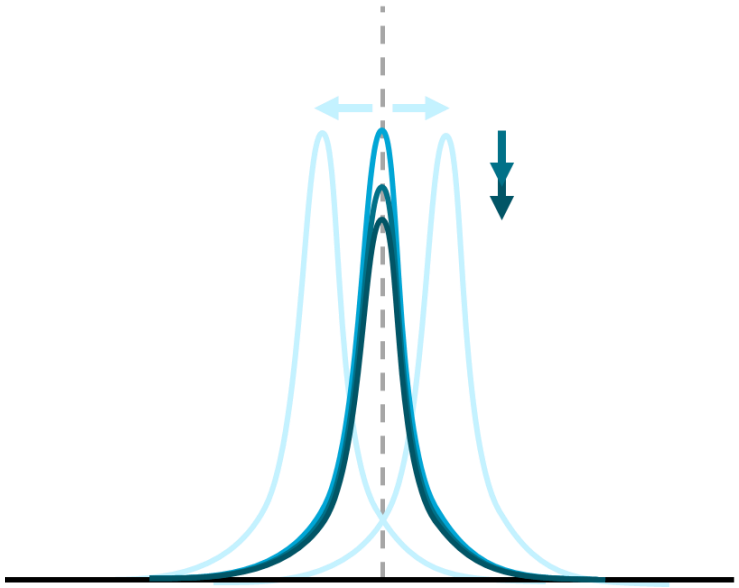
Signal understanding | Set-up

Work performed by
Maarten Klaverdijk



Quantify peak and intensity shift

Four matrices
Demi water
Synthetic media
Synthetic media (25 g/L glucose)
Synthetic media (50 g/L glucose)

Four peaks
Immersion probe window (406 cm^{-1})
Water HOH-bending (1642 cm^{-1})
When present
Media sulphate (981 cm^{-1})
Glucose COH-bending (1125 cm^{-1})

Four process parameters
Temperature
Bubbles
Biomass
Viscosity

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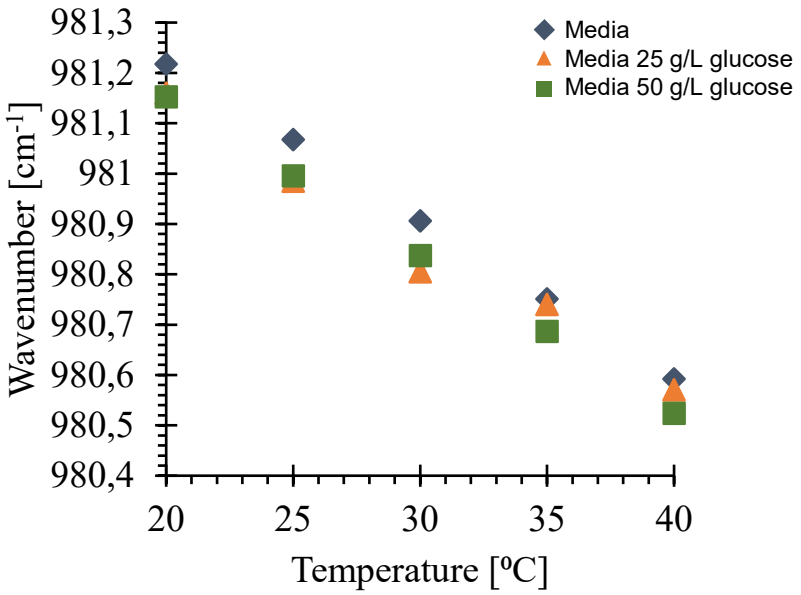
Klaverdijk et al. (2025)

Signal understanding | Sulphate peak example

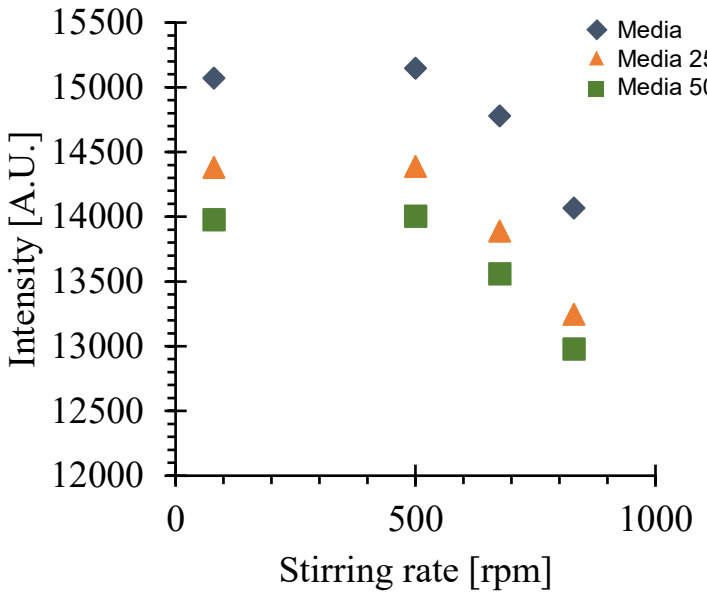
Work performed by
Maarten Klaverdijk

2-6% decrease due to peak shift



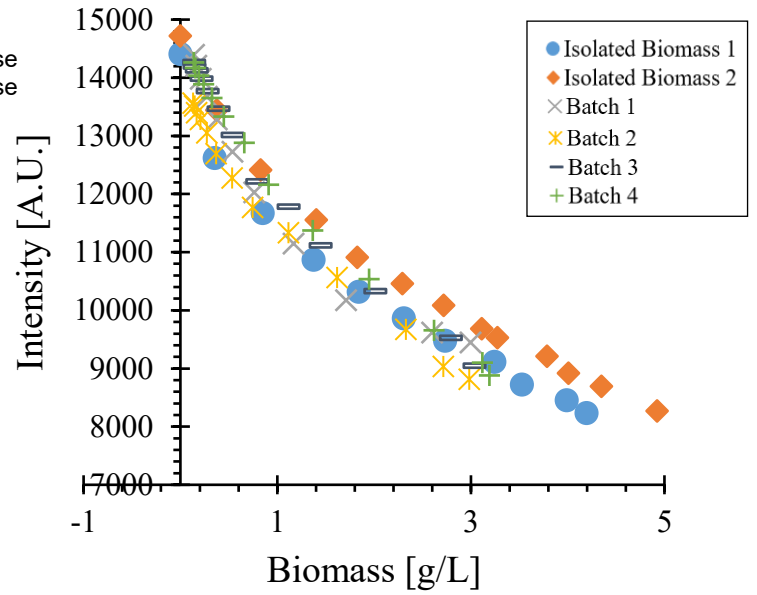
Linear correlation

7-8% intensity decrease



Measurement conditions

~44% intensity decrease



Data pre-processing

Understanding the impact on data from different systems

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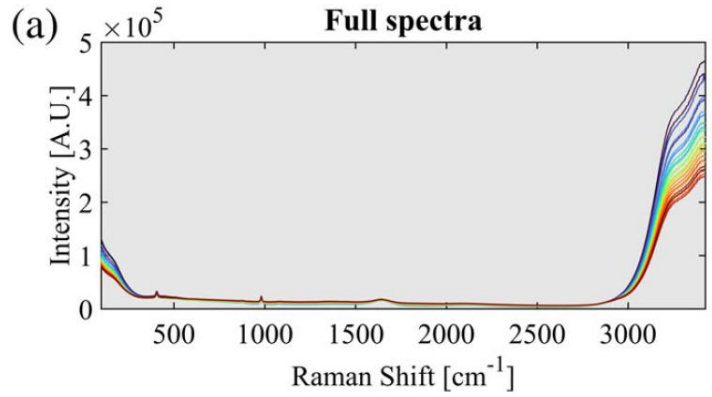
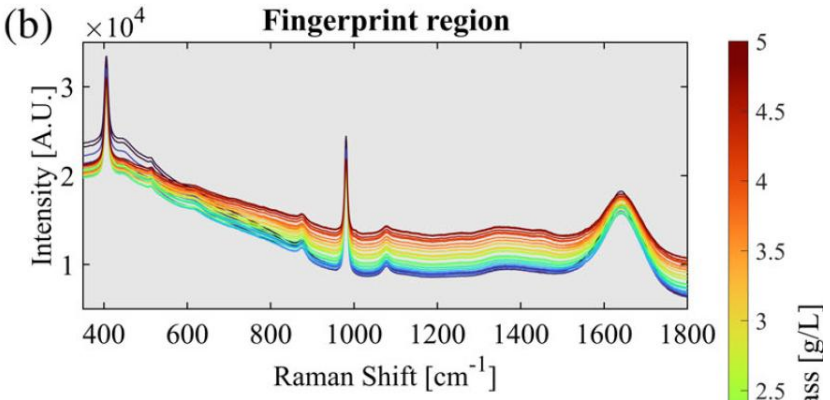
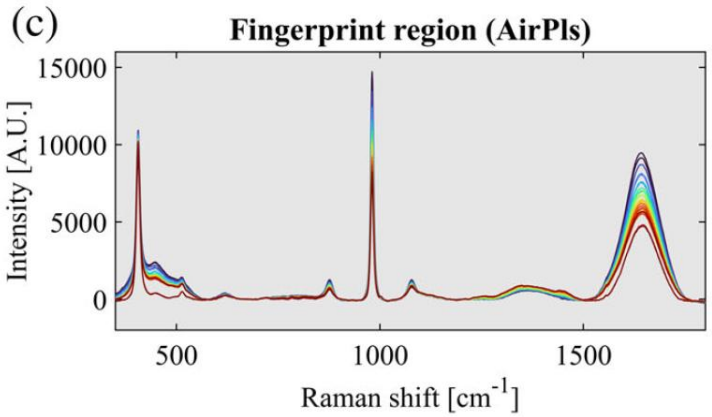
Examples given for sulphate peak (981 cm^{-1})

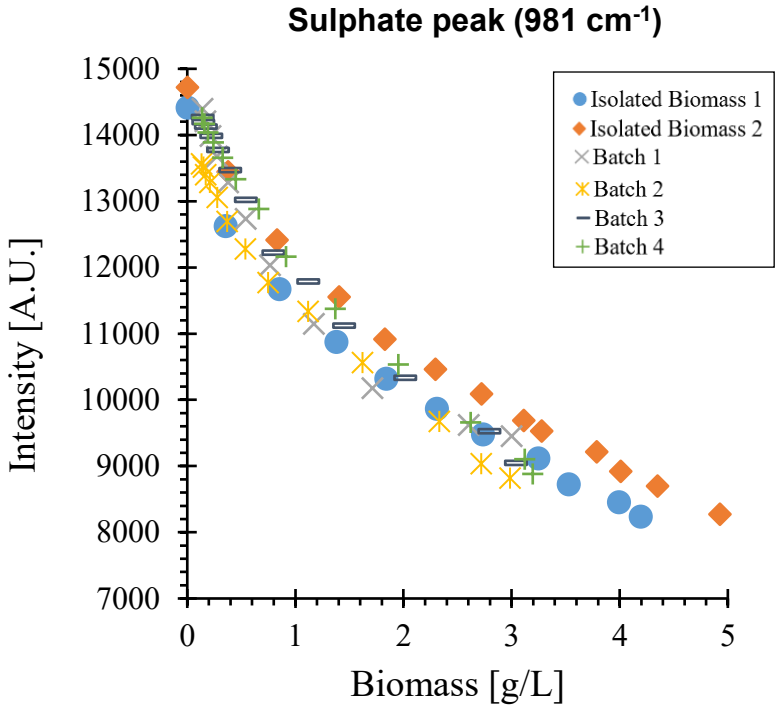
Signal understanding | Biomass

Work performed by
Maarten Klaverdijk









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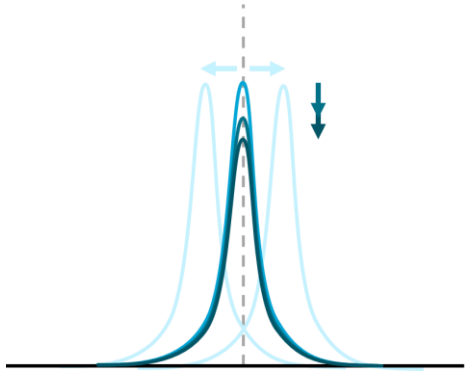
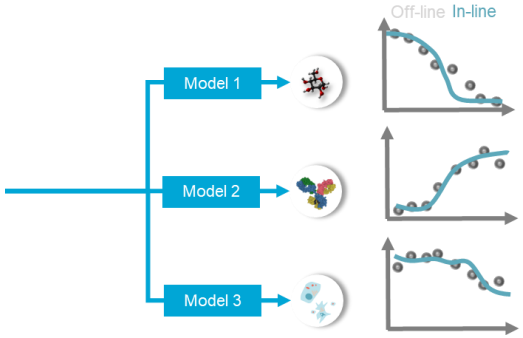
Summary

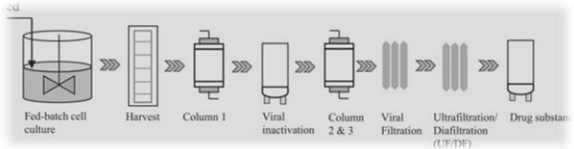


$f(x) = y$

Peak height and position



Real-time & Multiplexed read-out



Upstream & downstream applications

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Bioprocess Engineering

In-line Raman spectroscopy for process characterization

Dr.ir. Marieke Klijn
Assistant Professor

NLab Discussion Group
25 – November - 2025

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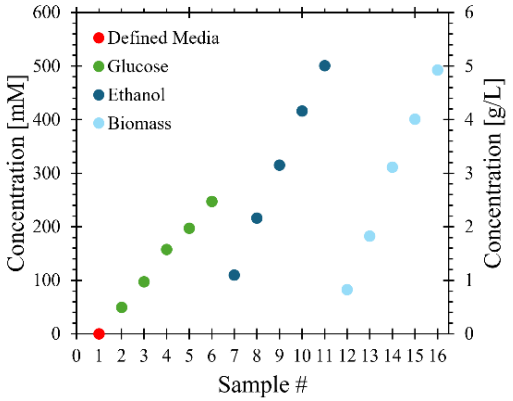
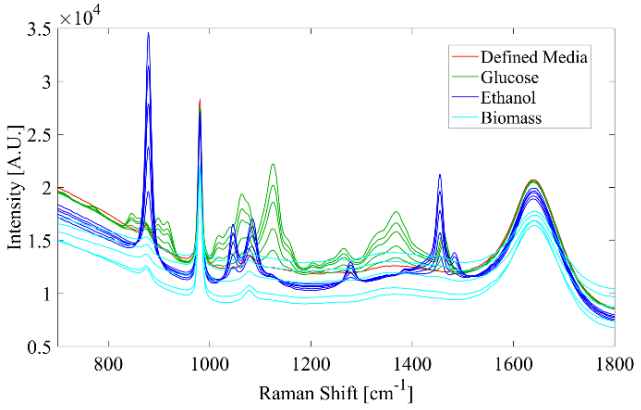
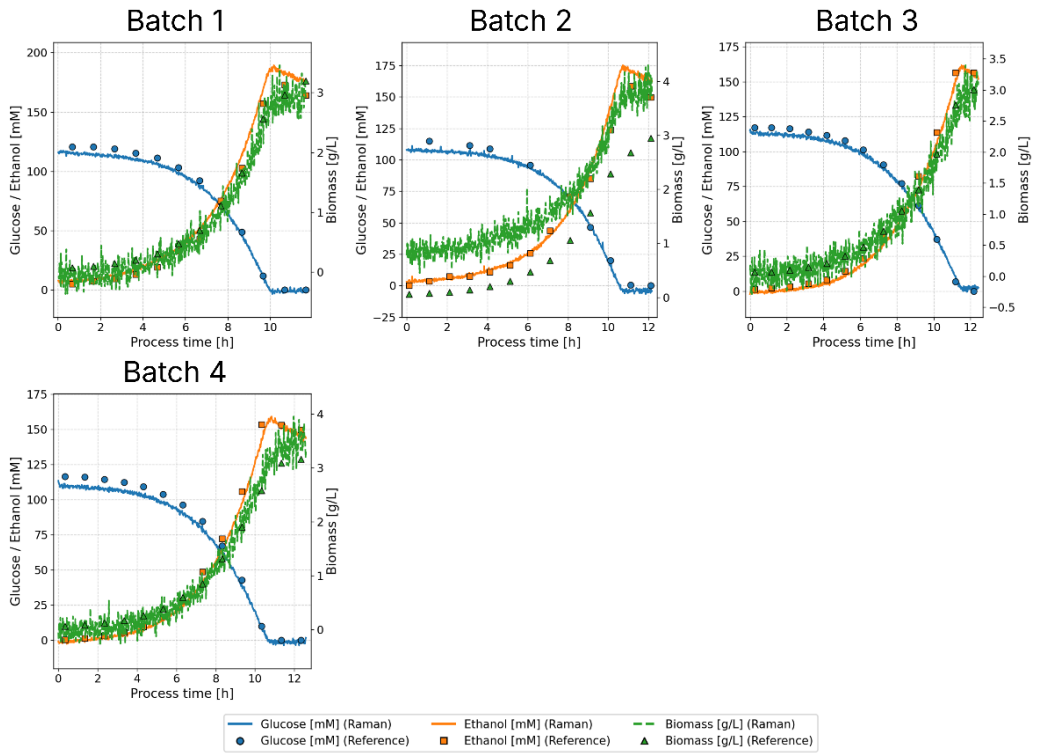
Model calibration | Single compound data

Calibration data





Validation data



Model calibration with only single compound data

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Klaverdijk et al. (2025) under review

Model calibration | Comparison of approaches

Work performed by
Maarten Klaverdijk

Approach	Model	Calibration data		Exp. time	Comp. time	Flexibility	rRMSEP (batch + fed-batch)		
		Total	Source				Glucose	Ethanol	Biomass
Standard	PLS	~36	Process				2.1%*	3.6%*	5.4%*
Single compound	PLS	16	Exp.				4.8%	11.6%	16.2%
	IHM	16	Exp.				4.2%	6.3%	10.0%
HM-synthetic	PLS	125	Synthetic				3.2%	14.5%	256%
HM-augmented	PLS	12+48	Process + Augmented				12.6%	7.8%	-

*Only batch data validation
Fed-batch performance: 30%, 62%, 88%

Isolated and/or synthetic data can help speed up implementation

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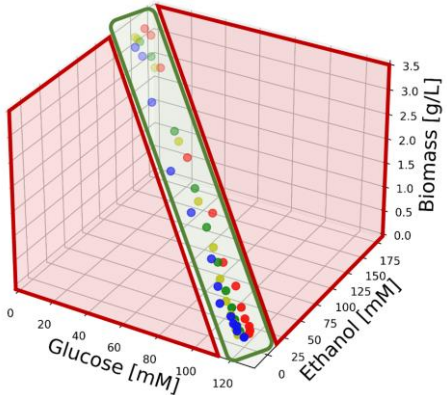
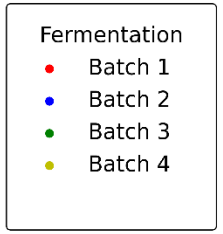
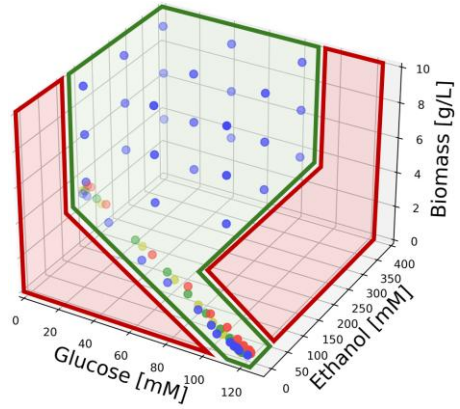
Klaverdijk et al. (2025) under review

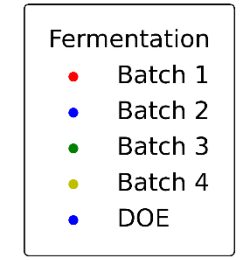
Model transferability | Batch to fed-batch

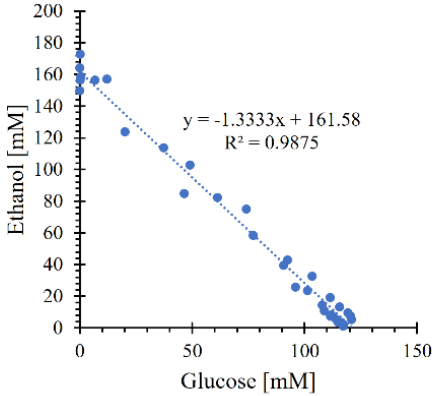
Work performed by
Maarten Klaverdijk

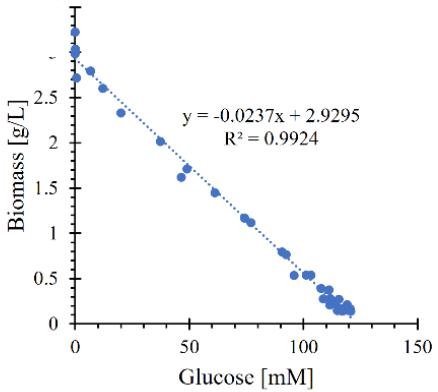


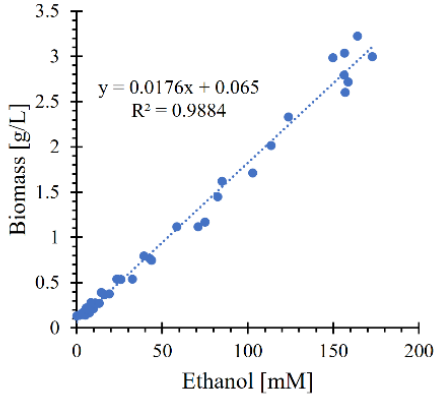












Single compound data supplementation

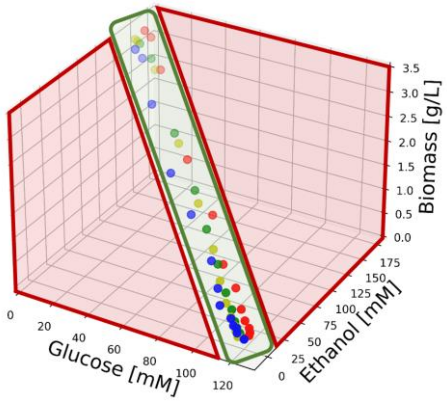
- Calibration
- Predicted

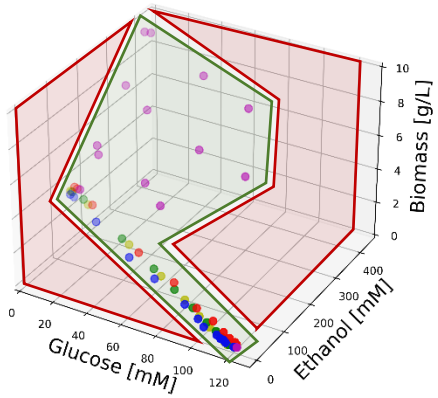
Cross-correlations

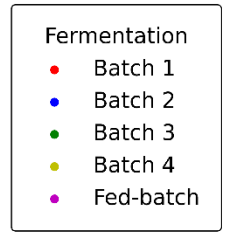
Klaverdijk et al. (2025)

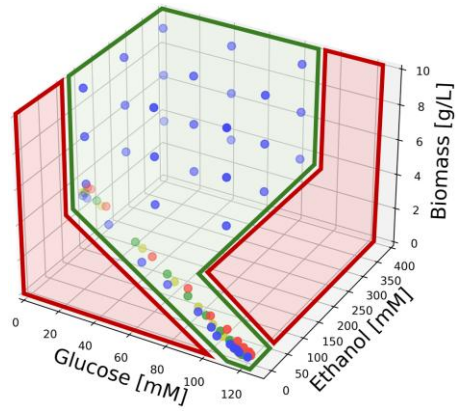
Model transferability | Batch to fed-batch

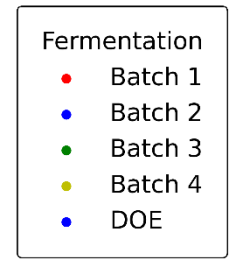
Work performed by
Maarten Klaverdijk



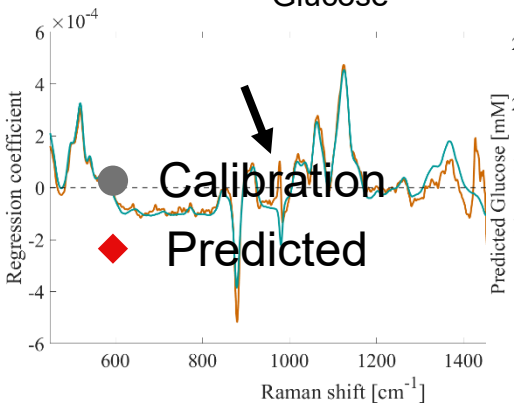


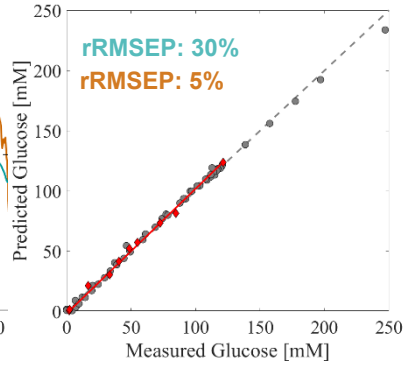




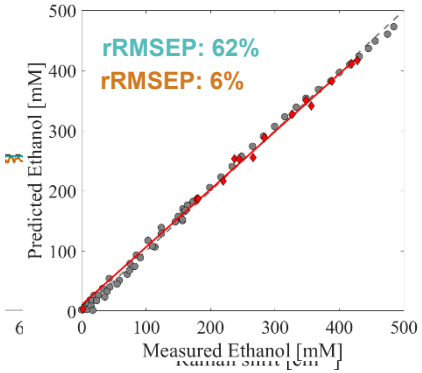


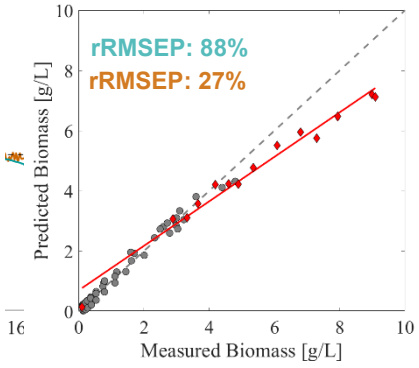
Glucose



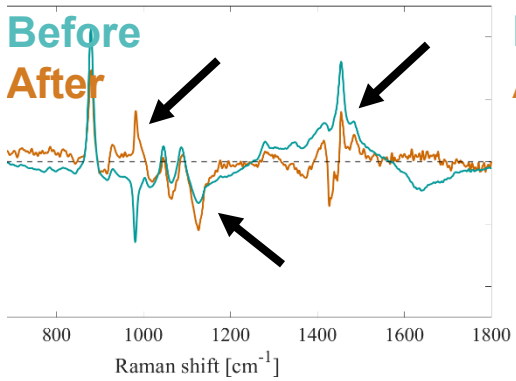


Ethanol





Biomass



Before
After

Expanding model application without additional runs

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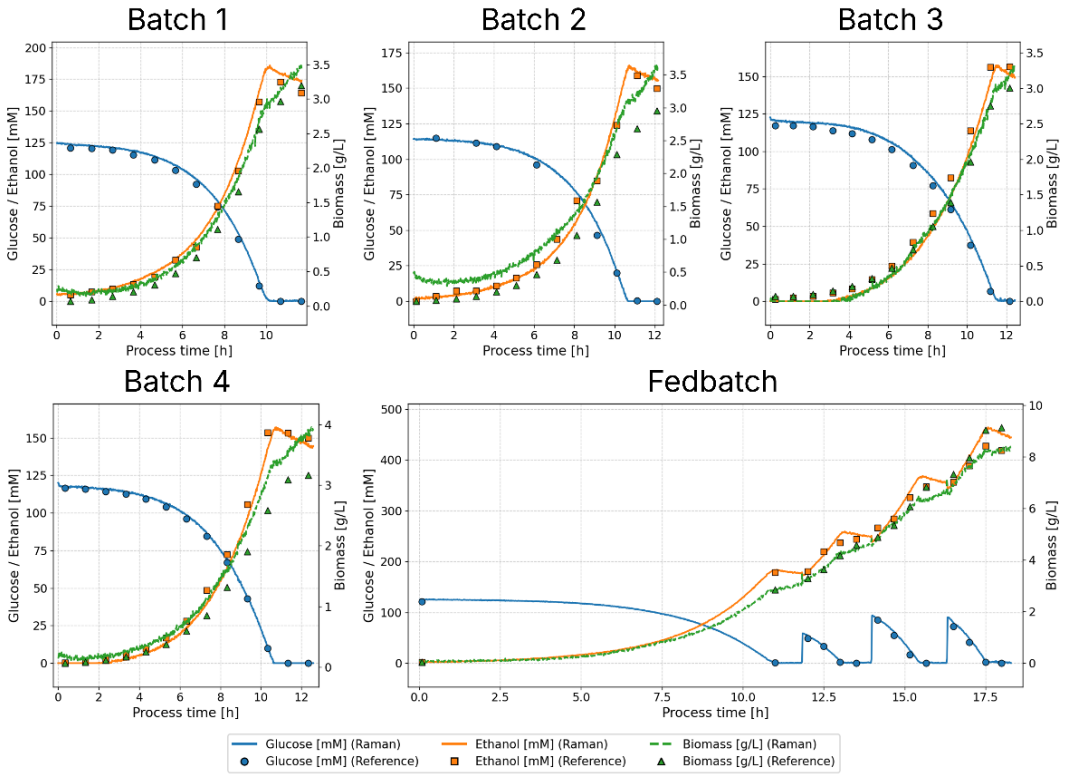
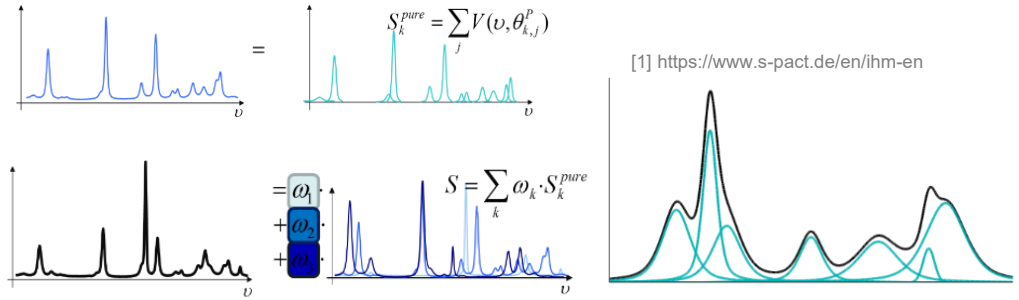
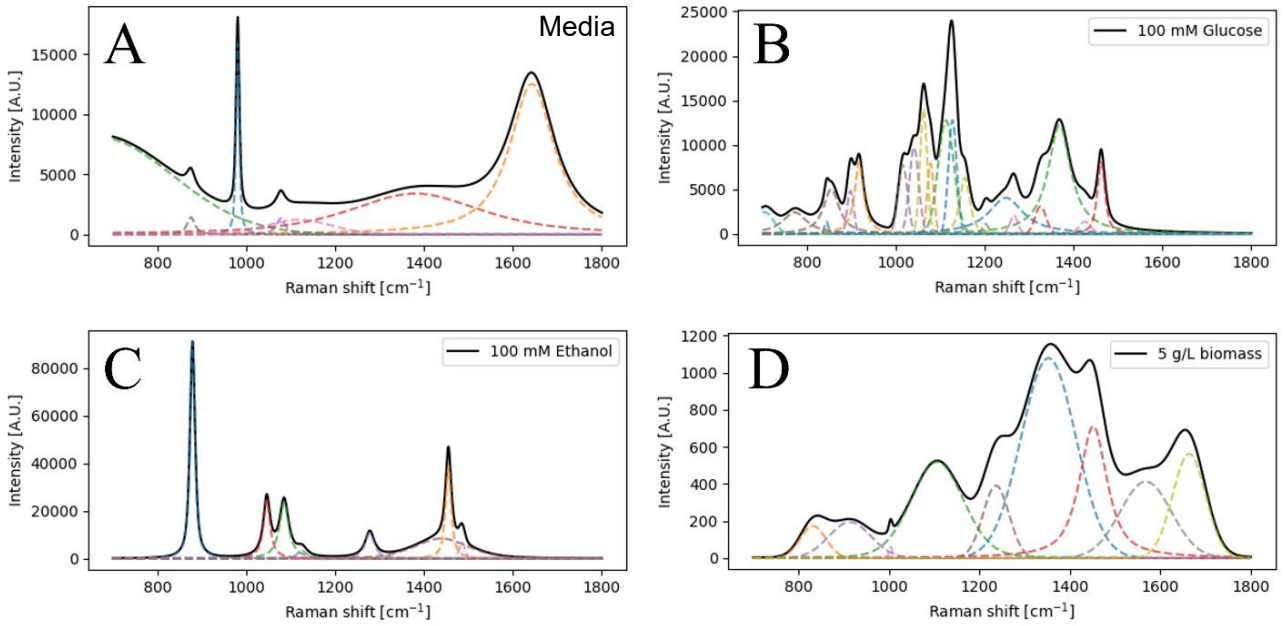
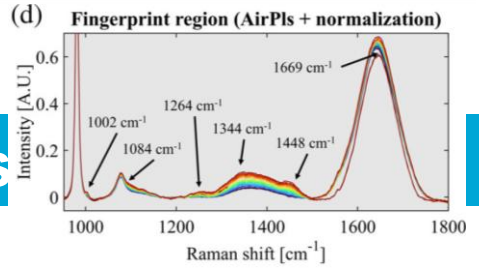
Model calibration | Hard models









Individual indirect hard models fitted to predict process

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Klaverdijk et al. (2025) under review