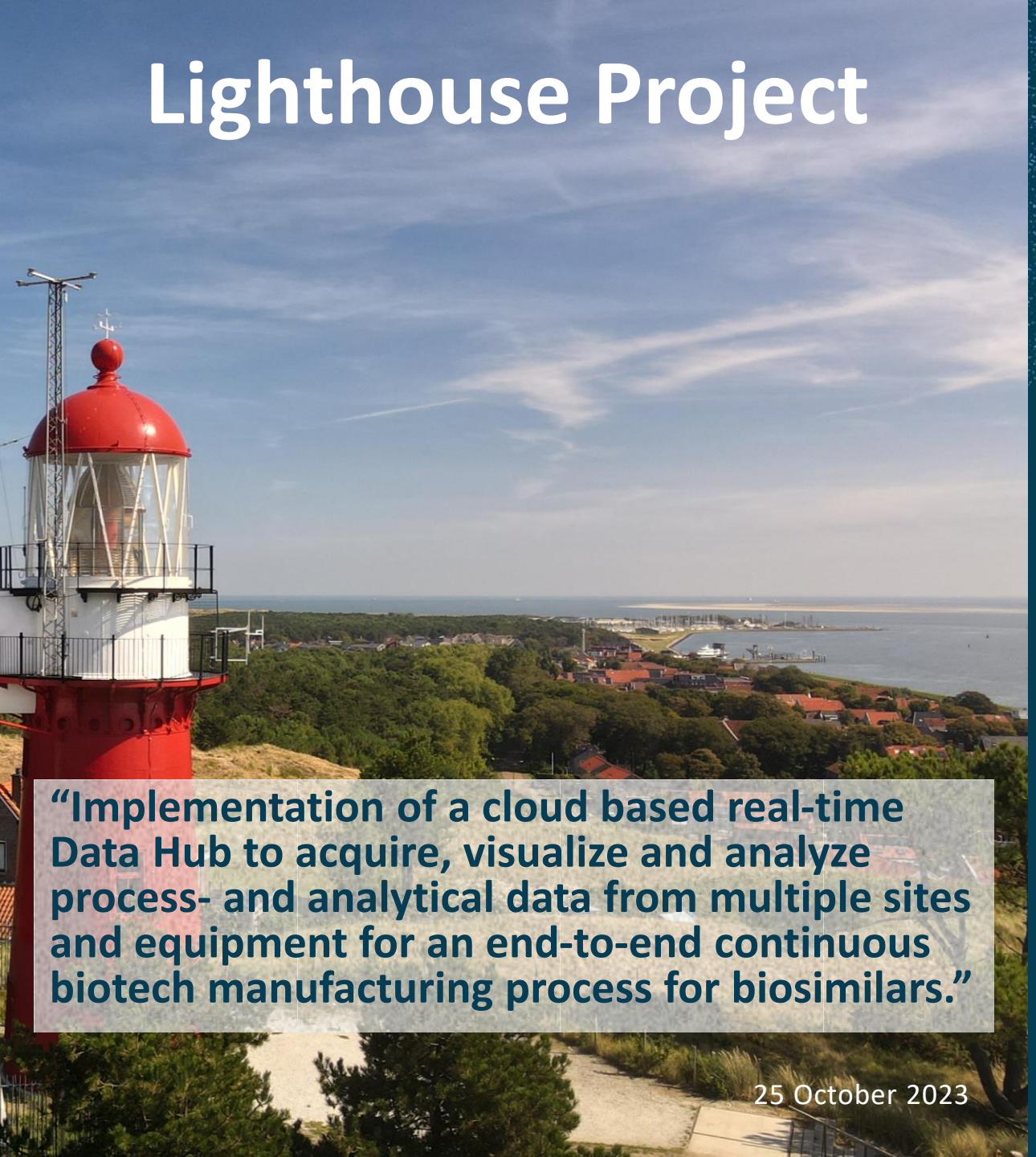
OCTOBER 25, 2023

# Lighthouse Project

BIOSNA PHARMA

Ir J.F. van de Laar, MSc

# Lighthouse Project



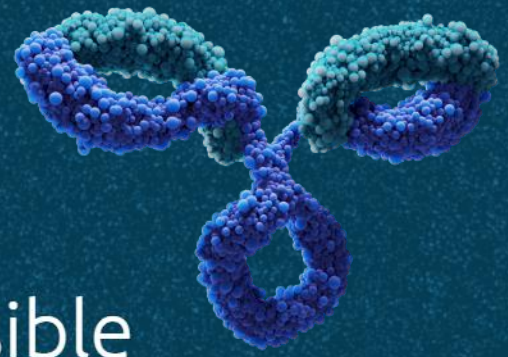
**“Implementation of a cloud based real-time Data Hub to acquire, visualize and analyze process- and analytical data from multiple sites and equipment for an end-to-end continuous biotech manufacturing process for biosimilars.”**

25 October 2023

Biosana PHARMA


SARTORIUS

Ir J.F. van de Laar, MSc



# Making **biologics affordable** and accessible for all patients

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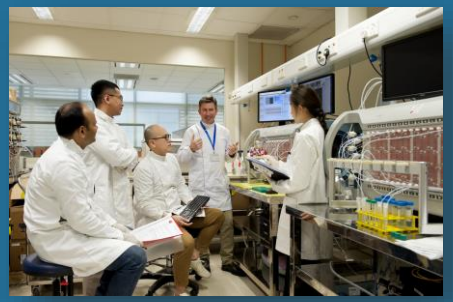
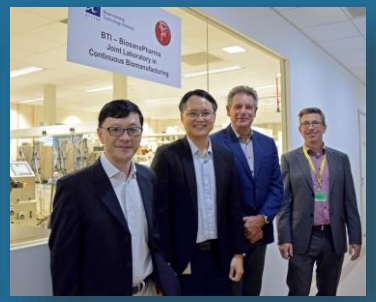
# Biosana Pharma






- Monoclonal antibodies and biosimilars - lead candidate: Omalizumab ➔
- Successful in phase I clinical studies and analytical bio similarity
- First continuous end-to-end manufacturing platform (Mycenax, 2018) ➔



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# Project & 3C process introduction

- Development of a continuous manufacturing platform for our lead mAb product: a biosimilar to Omalizumab Xolair®




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jm BIO ONNE

Skids.AlarmsOverview

192.168.30.208

**PC618**

| State | Group | Type | Name | AlarmComment | TimeLCT | Limit | CurrentValue | Operator | AlarmDuration |
|---|---|---|---|---|---|---|---|---|---|
| No active alarms | | | | | | | | | |

**Username:** HALGROUP\jvdlaar

Change User

Log out 0

AVEVA SYSTEM PLATFORM

**General Settings**

**Auto Logout Time:** 600 s

**Tia Portal Version:** PC618_Siemens_V1.0.0

**Stored Checksum**

MF F0 74 C6 69 99 0A A7 66

CD 9D 2E AE D6 57 F9 59 FE

VI 80 F6 6D 00 7F B2 DD 84

Harvest BC 95 63 FE C9 3B E8 BB

Filtratrion 6F AA 37 09 ED A5 AE FB

Skids

Critical 33

High 42

Low 2

Mode: Enable

Active 59

UnAcked 62

Running

**SMS Settings**

Alarm Messages: 24

Warning Messages: 36

interval: 00:01:00

Send

Area Alarms — Set PlantState

MF — 6 7 — Running — Mode Enable

Harvest — 7 8 — Running — Mode Enable

PCC1 — 1 — Running — Mode Enable

VI — 6 7 — Running — Mode Enable

PCC2 — 1 — Running — Mode Enable

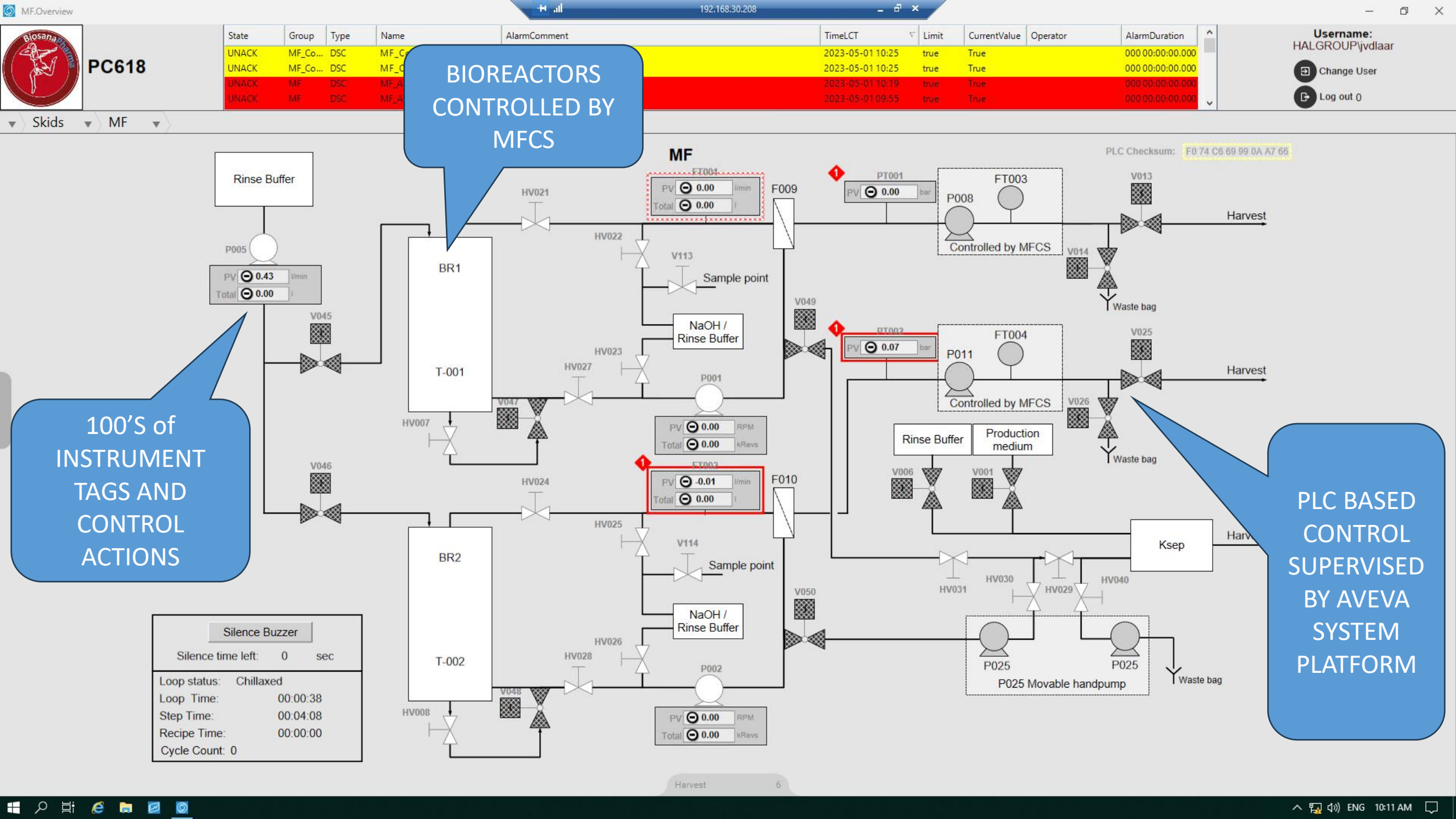
AIXNF — 9 10 — Running — Mode Enable

CD — 4 6 — Running — Mode Enable

7 SKIDS CONNECTED:

- MF
- HC
- PCC1
- VI
- PCC2
- AIX-NF
- CD

ENG 10:11 AM


MF.Overview
192.168.30.208
PC618
State
Group
Type
Name
AlarmComment
TimeLCT
Limit
CurrentValue
Operator
AlarmDuration
UNACK
2023-05-01 10:25
2023-05-01 10:19
2023-05-01 09:55
000 00:00:00.000
Username:
HALGROUP\jvdlaar
Change User
Log out 0
Skids
MF
BIOREACTORS CONTROLLED BY MFCS
100'S of INSTRUMENT TAGS AND CONTROL ACTIONS
PLC BASED CONTROL SUPERVISED BY AVEVA SYSTEM PLATFORM
MF
PLC Checksum:
F0 74 C6 69 99 0A A7 66
Rinse Buffer
P005
PV 0.43 l/min
Total 0.00 l
BR1
T-001
BR2
T-002
HV021
HV022
HV023
HV024
HV025
HV026
HV027
HV028
HV007
HV008
V045
V046
V047
V048
V113
V114
Sample point
NaOH / Rinse Buffer
FT001
PV 0.00 l/min
Total 0.00 l
FT002
PV -0.01 l/min
Total 0.00 l
P001
P002
PV 0.00 RPM
Total 0.00 kRevs
F009
F010
PT001
PV 0.00 bar
PT002
PV 0.07 bar
P008
FT003
Controlled by MFCS
P011
FT004
Controlled by MFCS
V013
V014
V025
V026
V049
V050
Harvest
Waste bag
Rinse Buffer
Production medium
V006
V001
Ksep
HV031
HV030
HV029
HV040
P025
P025 Movable handpump
Silence Buzzer
Silence time left: 0 sec
Loop status: Chillaxed
Loop Time: 00:00:38
Step Time: 00:04:08
Recipe Time: 00:00:00
Cycle Count: 0
Harvest 6
ENG 10:11 AM

PC618

| State | Group | Type | Name | AlarmComment | TimeLCT | Limit | CurrentValue | Operator | AlarmDuration |
|---|---|---|---|---|---|---|---|---|---|
| ACK | Harvest | Lo | WE005.PV.Lo | Std ACK - not in use | 2023-05-30 10:12 | 0.0 | 0.17000000... | jvdlaar | 000 00:00:00.000 |
| ACK | Harvest | LoLo | WE005.PV.LoLo | Std ACK - not in use | 2023-05-30 10:12 | 12.0 | 0.17000000... | jvdlaar | 000 00:00:00.000 |
| ACK | Harvest | DSC | PT016.OutOfRangeWarning | Std ACK - not in use | 2023-05-30 10:12 | Fault | True | jvdlaar | 000 00:00:00.000 |
| ACK | Harvest | Process | V053.OpAlarm | Std ACK - not in use | 2023-05-30 10:12 | true | True | jvdlaar | 000 00:00:00.000 |

Username:
HALGROUP\jvdlaar
Change User
Log out 0

Skids ▾ Harvest ▾

MF 1

# Harvest

PLC Checksum: BC 95 63 FE C9 3B E8 BB

Loop status: No recipe running
Loop Time: 00:00:00
Step Time: 00:00:00
Recipe Time: 00:00:00
Cycle Count: 10

Silence Buzzer
Silence time left: 0 sec

SB
T011

HV041
From KSep
Sample point

P012
PV 0.00 ml/min
Total 0.00 l

Depth Filter
F001

PT015
PV 0.24 bar

PT016
PV 1.38 bar

V053
SF
F002
HV042

V054
SF
F003
HV043

SF filter swap

Harvest
T014
ST001
PV 0.0 RPM

WE005
PV 0.17 kg

V029
Sample point
To PCC1

PCC1 interaction

PT001
PV 0.00 bar
P008
FT003
Controlled by MFCS
V013
From MF1
V014
Waste bag

PT002
PV 0.07 bar
P008
FT004
Controlled by MFCS
V025
From MF2
V026
Waste bag

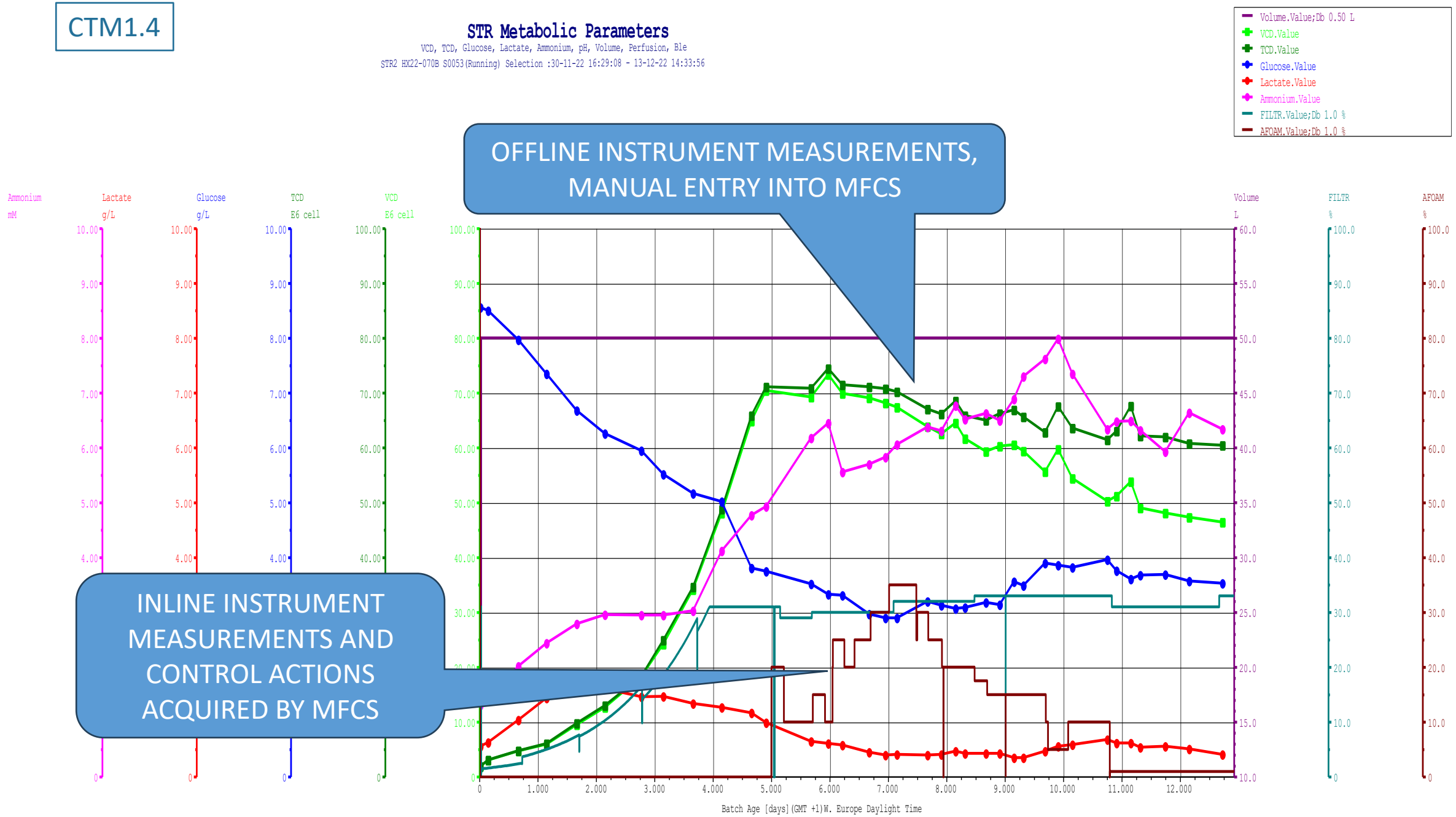
HYBRID CONTROL BY AVEVA SYSTEM PLATFORM, MFCS AND UNICORN

UNICORN INTERFACE INTEGRATED INTO AVEVA SYSTEM PLATFORM

PCC1 5

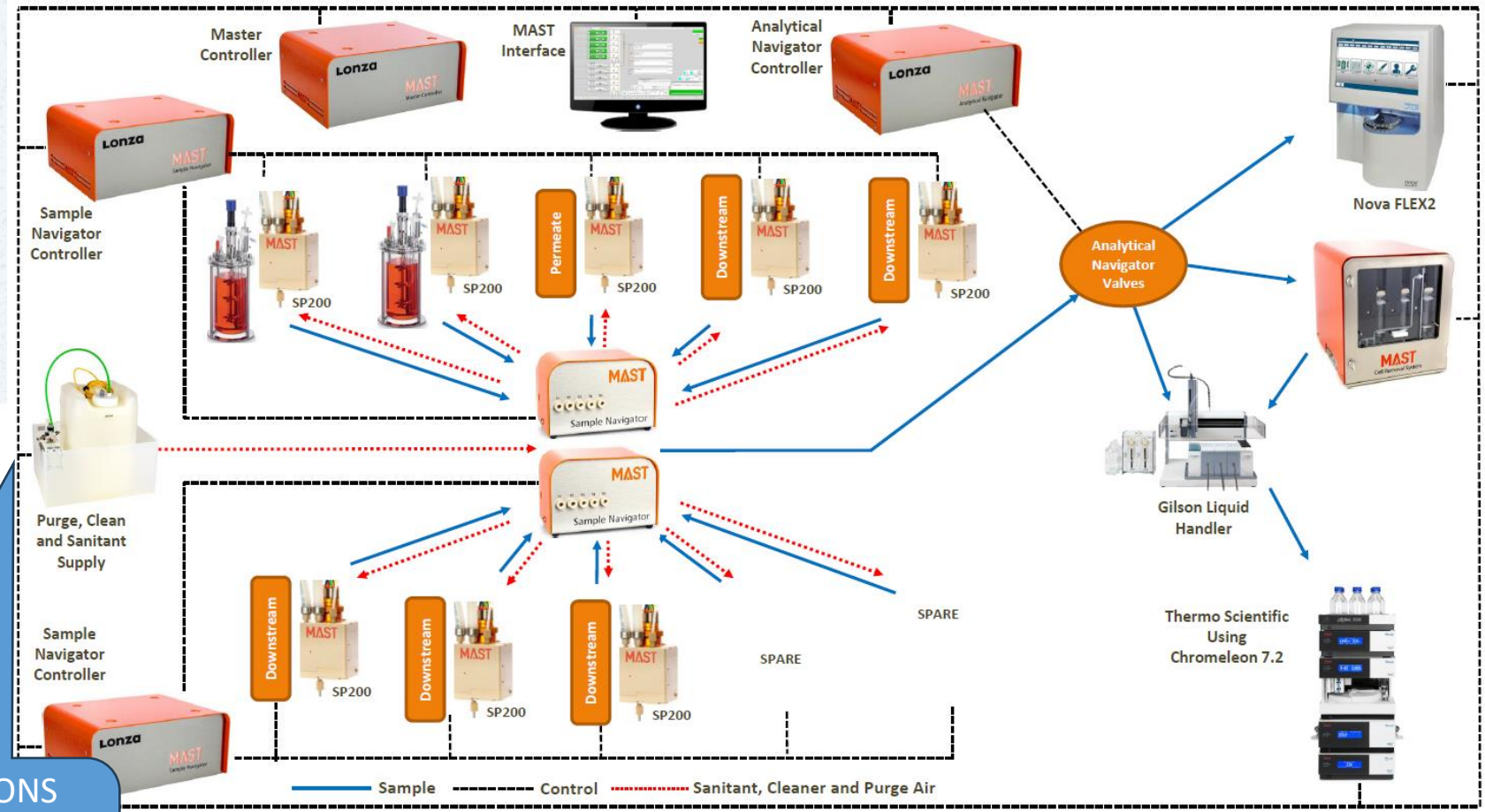
ENG 10:12 AM

CTM1.4


STR Metabolic Parameters
VCD, TCD, Glucose, Lactate, Ammonium, pH, Volume, Perfusion, Ble
STR2 HX22-070B S0053(Running) Selection :30-11-22 16:29:08 - 13-12-22 14:33:56
Volume.Value;Db 0.50 L
VCD.Value
TCD.Value
Glucose.Value
Lactate.Value
Ammonium.Value
FILTR.Value;Db 1.0 %
AFOAM.Value;Db 1.0 %
OFFLINE INSTRUMENT MEASUREMENTS, MANUAL ENTRY INTO MFCS
INLINE INSTRUMENT MEASUREMENTS AND CONTROL ACTIONS ACQUIRED BY MFCS
Ammonium mM
Lactate g/L
Glucose g/L
TCD E6 cell
VCD E6 cell
Volume L
FILTR %
AFOAM %
Batch Age [days](GMT +1)W. Europe Daylight Time

# In-process sampling and at-line testing

| SP/MN | Sample point |
|---|---|
| STR1 | Bioreactor 1 |
| STR2 | Bioreactor 2 |
| SP1 | Sample point for STR1 |
| SP2 | Sample point for STR2 |
| SP3 | MF Permeate |
| SP4 | Clarified harvest |
| SP6 | Acidification bag( for ProtA elute) |
| MN1 | kSep |
| MN2 | ProtA flow through |
| SP7 | Post-VI surge bag |
| SP8 | CEX elute |
| MN5 | CEX flow through |
| SP9 | CD Bag |
| DS | D |




AUTOMATED SAMPLE LOCATIONS THROUGHOUT PROCESS TO CELL CULTURE ANALYZER AND PROT A, SEC AND CEX CHROMATOGRAPHY



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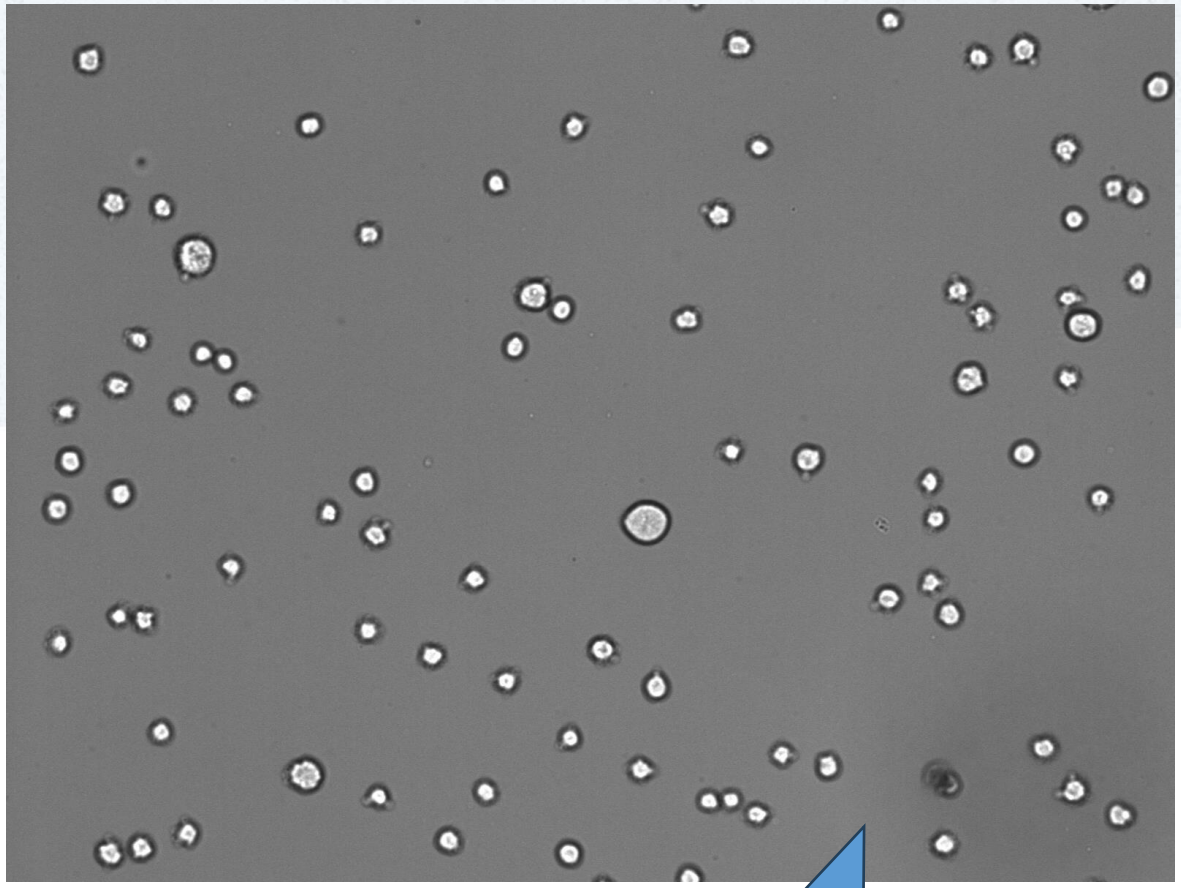
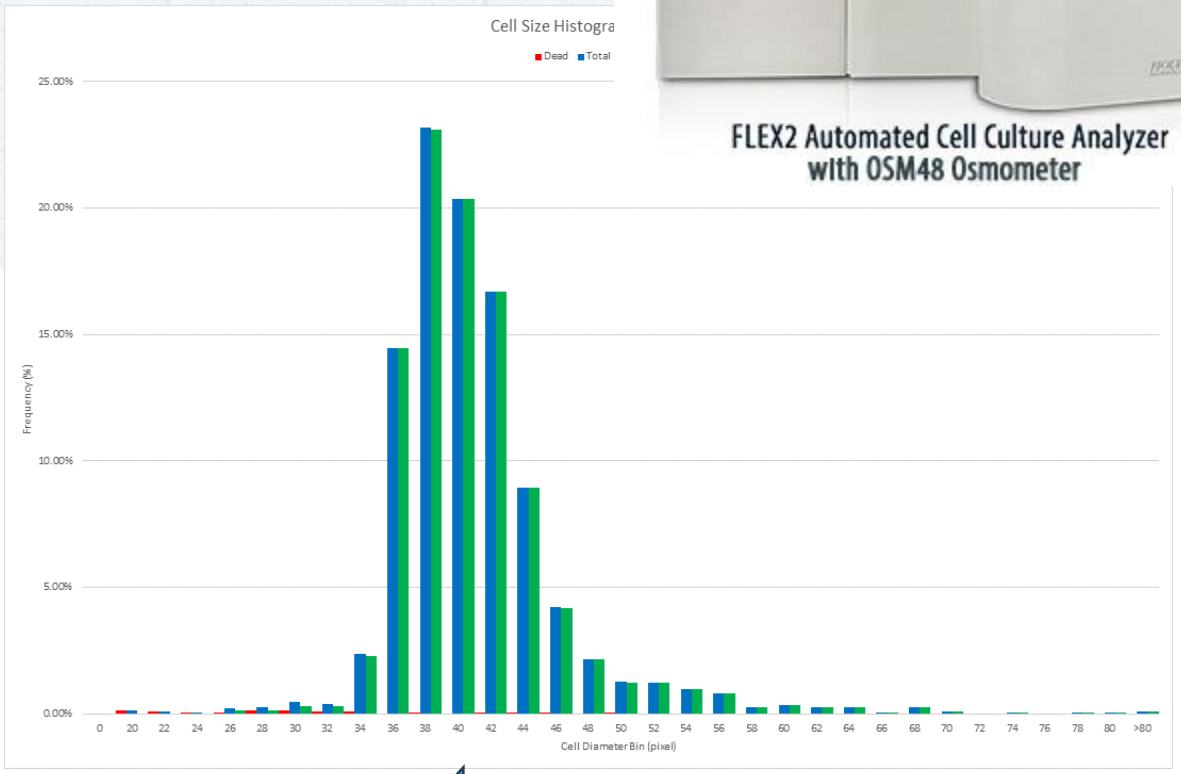
# NovaMedical BioProfile® FLEX2: cell size distributions (growth phase)

COMPLEX DEVICES

PICTURES




VECTOR DATA



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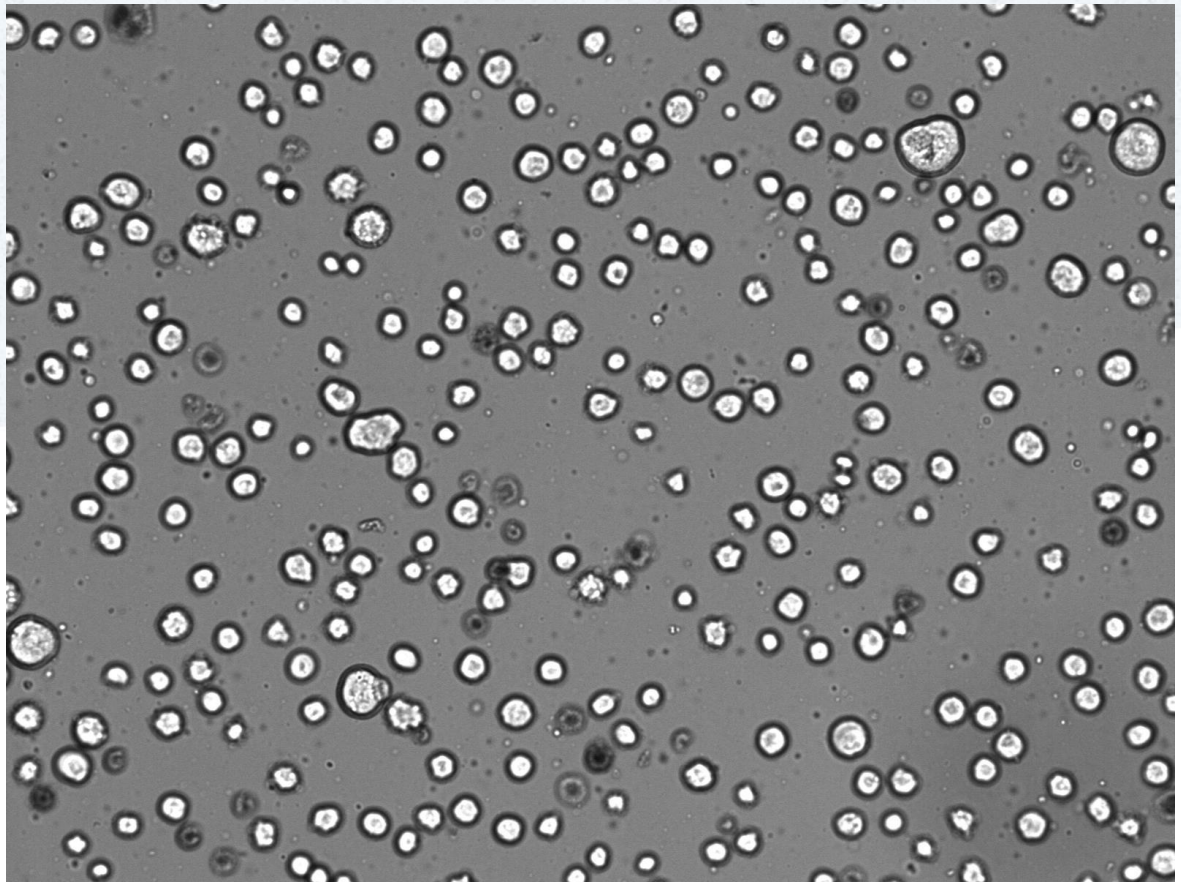
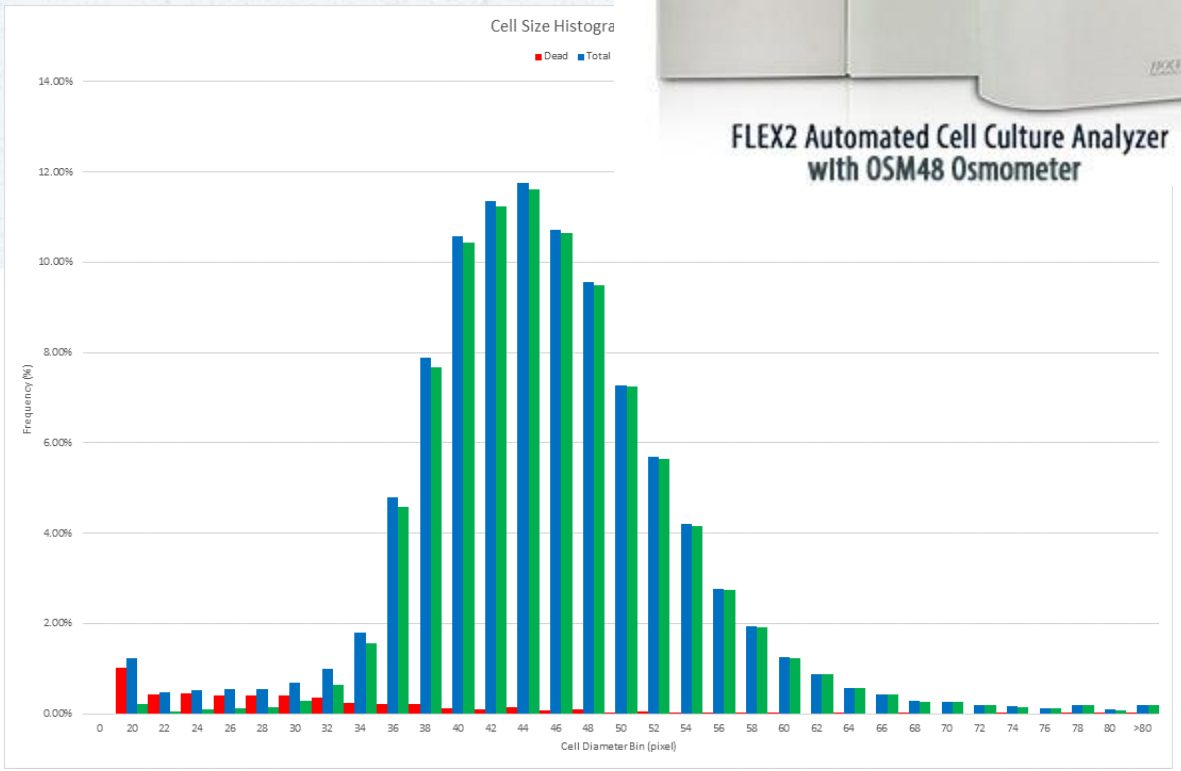
# NovaMedical BioProfile® FLEX2: cell size distributions (production phase)







FLEX2 Automated Cell Culture Analyzer with OSM48 Osmometer



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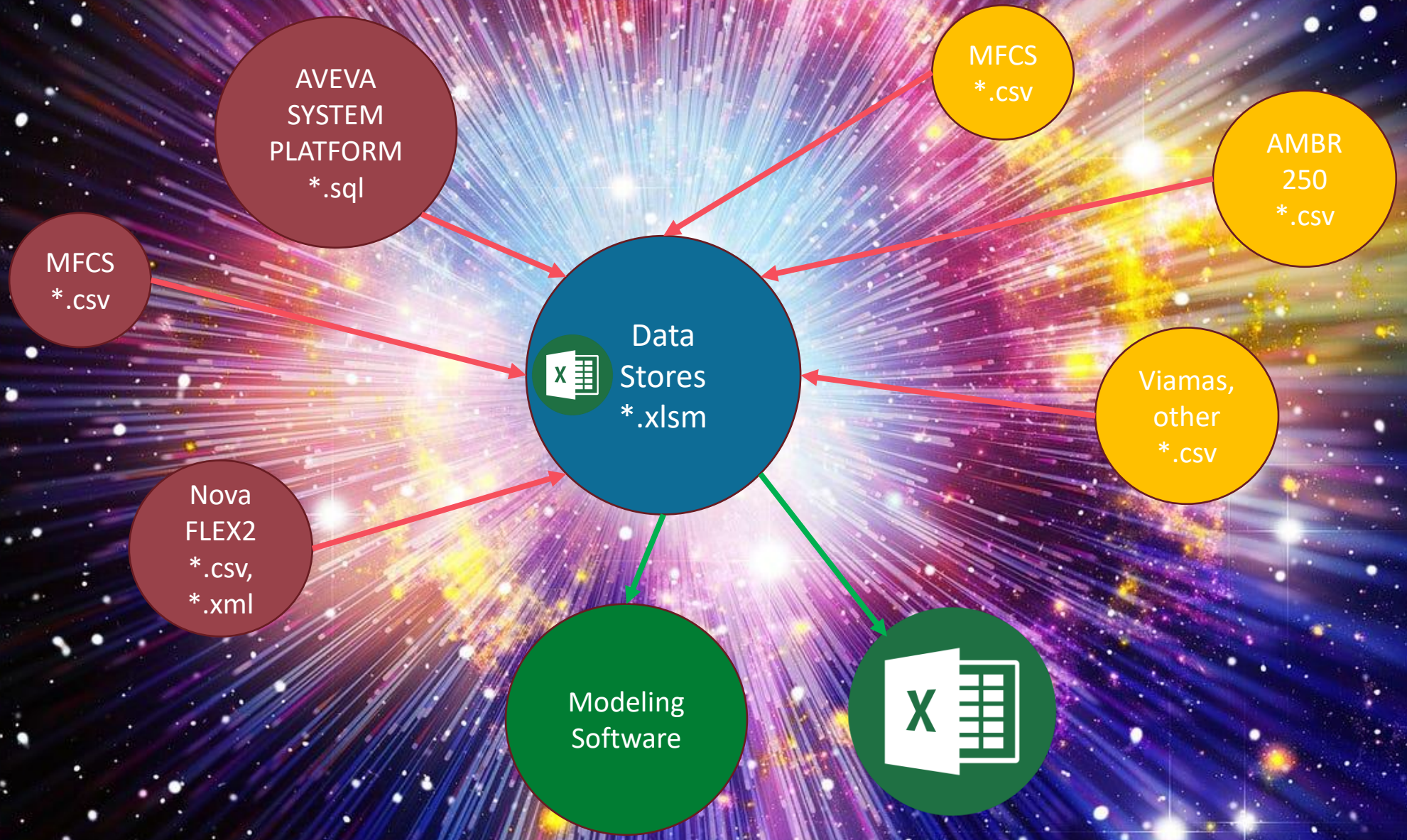
# In the beginning… there was chaos



Pharma & Life Sciences | Singapore & The Netherlands

# BiosanaPharma requires a reliable cloud-based data platform for continuous manufacturing

## Challenge

- Massive amount of process parameters and attributes at different scales and sites.
- Data stored across several different databases and devices, e.g., MFCS, NovaFlex, AVEVA, Excel, etc, as well as in paper files.
- Real time scale-up performance monitoring, failure mode and excursion monitoring needed.
- Connection needed to commercially available modelling packages.

## Solution

Using AVEVA Data Hub as cloud data platform to collect data from various sources at the development laboratory and the manufacturing site is promising to ensure comparability, data integrity and contextualization.

## Results

**Simple, secure data sharing allows the customer:**

a) **A single point-of-truth, well contextualized, well searchable real-time and historical data.**
b) **Efficient real-time visualization and off-line analysis.**
c) **Process parameters and quality attributes and excursions using SIMCA On-line MVDA models.**

**The AVEVA Data Hub solution is a powerful tool for process development as well as manufacturing and can become the hub for the future of biopharma**

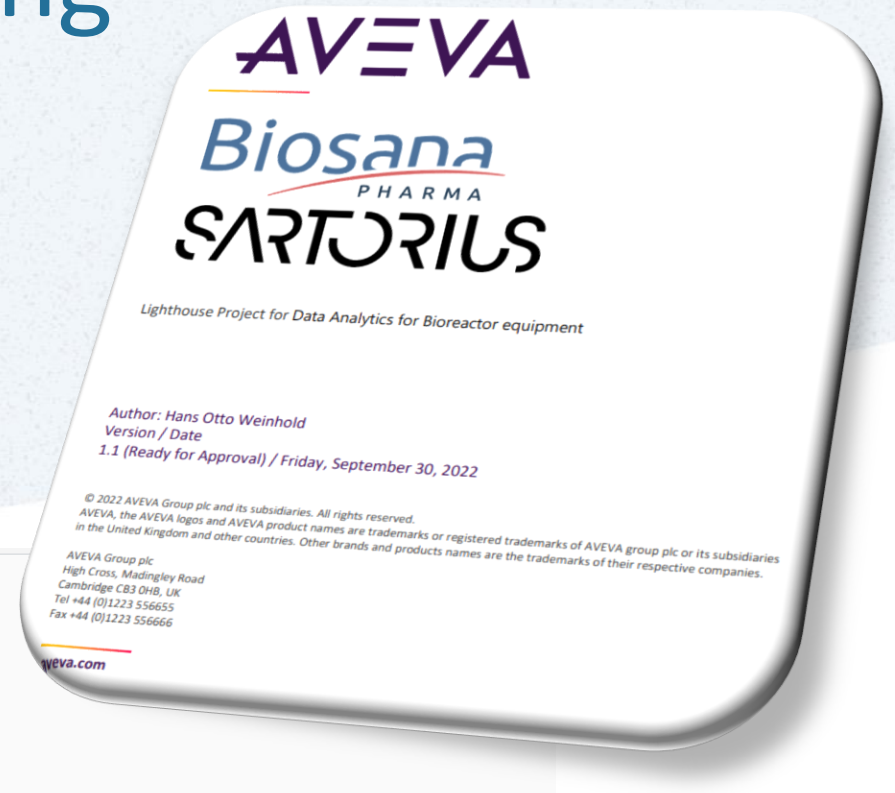
*"The future of biopharma is "lights out" continuous end-to-end processing.*
*The cost of goods of antibodies will decrease a factor 10 in the next decade by innovation, driven by pressure on originators and choice of health authorities for biosimilars.*

*Maarten Pennings, CTO, BiosanaPharma*

Learn more

AVEVA

# Lighthouse Project: Acquisitioning and Sharing Data through ADH




**Biosana-Satorius**

Biosana-Sartorius

| | |
|---|---|
| Tenants | 2 |
| Sharing Status | All Tenants Active |

**Biosana-Satorius**

8bc8cc64-625e-4c93-939d-4082090e5bb5

Biosana-Sartorius

All Tenants Active

*Created 20.02.2023*

Tenants | My Members | My Groups | My Clients

Filter tenants...

**Administrative Tenant**

| Name | Status | Users (11) | Clients (1) | Streams (6816) | |
|---|---|---|---|---|---|
| BiosanaPharma | Active | 4 | 0 | 6816 | |
| Sartorius Stedim Biotech GmbH | Active | 7 | 1 | 0 | |

# Data Sources – Singapore Laboratory

| Source System | Location | Protocol | In scope for Lighthouse project |
|---|---|---|---|
| **Ambr 250 – OPC UA** | **Singapore** | **PI Adapter for OPC UA** | **Yes** |
| Spectroscopy Module – OPC UA/File | Singapore | Python Script to process files | No |
| **MFCS 3.1 – OPC DA/UA** | **Singapore** | **PI Adapter for OPC UA** | **Yes** |
| KML100 – Serial (tbd) | Singapore | | No |
| Levitronics, tbd | Singapore | | No |
| **ABER Viamass – Modbus Serial – Modbus TCP bridge** | **Singapore** | **PI Adapter for Modubus TCP** | **Yes** |
| BioSMB – OPC DA/UA (Beckhoff PLC) | Singapore | PI Adapter for OPC UA<br>(Dianomic Foglamp) | No |
| **Novaflex 2 – CSV Files** | **Singapore** | **PI Adapter for Structured Files**<br>**Image processing through custom tool** | **Yes** |
| ThermoFisher HPLC – tbd<br>HPLC Data Analytics<br>Chromatogram data | Singapore | | No |
| Offline data (Lab) – CSV/Manual data | Singapore | PI Adapter for Structured Files/Simple Web interface | No |
| Unicorn, SQL Database | Singapore | PI Adapter for RDBMS | No |



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# Data Sources, Netherlands Manufacturing Site

| Source System | Location | Protocol | In scope for Lighthouse project |
|---|---|---|---|
| **AVEVA System Platform Historian 2020R2 (Backup)** | **Leiden** | **Backup SPH Server – PI Connector for Wonderware – PI Server – PI to ADH** | **Yes** |
| Unicorn (Backup), SQL Database | Leiden | PI Adapter for RDBMS | No |
| **MFCS 3.1 (Backup) – CSV Files** | **Leiden** | **PI Adapter for Structured Data Files** | **Yes** |
| **Novaflex 2 – CSV Files** | **Leiden** | **PI Adapter for Structured Data Files** | **Yes** |
| ThermoFisher HPLC – tbd<br>- HPLC Data Analytics<br>- Chromatogram data | Leiden | | No |
| MAST Sampling System, tbd | Leiden | | No |
| Offline data (Lab) – CSV/Manual data | Leiden | PI Adapter for Structured Data Files/Simple Web interface | No |



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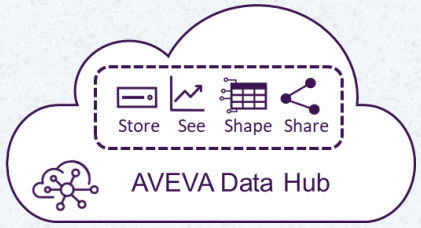
# Data Flow – Halix Manufacturing Site




CMO domain controller

Control room / home
CMO Laptops, through VPN connection

MAST/ Analytical

MFCS SCADA

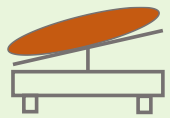
AVEVA System Platform

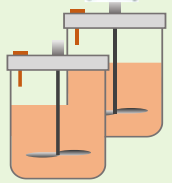
Unicorn SCADA

Bioreactors

DCU (PLC)

DCU (PLC)





Perfusion and harvest skids

DSP skids

Chromatography



* Data was imported into AVEVA Data Hub from back-ups due to GMP restrictions

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# AVEVA Data Hub – Bringing in data from everywhere

| | | |
|---|---|---|
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.CER - unnormalized, integrated | AMBR_SP_2.ambr 250.Bioreactor 12.CER - unnormalized, integr… |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.CER - unnormalized | AMBR_SP_2.ambr 250.Bioreactor 12.CER - unnormalized |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.CER - integrated | AMBR_SP_2.ambr 250.Bioreactor 12.CER - integrated |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.CER | AMBR_SP_2.ambr 250.Bioreactor 12.CER |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Cell viability | AMBR_SP_2.ambr 250.Bioreactor 12.Cell viability |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Cell density | AMBR_SP_2.ambr 250.Bioreactor 12.Cell density |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Cap off | AMBR_SP_2.ambr 250.Bioreactor 12.Cap off |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Calcium | AMBR_SP_2.ambr 250.Bioreactor 12.Calcium |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed volume removed from biore… | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed volume removed fro… |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed total volume_SP | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed total volume_SP |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed total volume | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed total volume |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed to level total volume_SP | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed to level total volume… |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed to level total volume | AMBR_SP_2.ambr 250.Bioreactor 12.Bleed to level total volume |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Bicarbonate | AMBR_SP_2.ambr 250.Bioreactor 12.Bicarbonate |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Batch name | AMBR_SP_2.ambr 250.Bioreactor 12.Batch name |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Base volume pumped | AMBR_SP_2.ambr 250.Bioreactor 12.Base volume pumped |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Base pulse flow on time_SP | AMBR_SP_2.ambr 250.Bioreactor 12.Base pulse flow on time_SP |
| ☐ | AMBR_SP_2.ambr 250.Bioreactor 12.Base pulse flow off time_SP | AMBR_SP_2.ambr 250.Bioreactor 12.Base pulse flow off time_SP |



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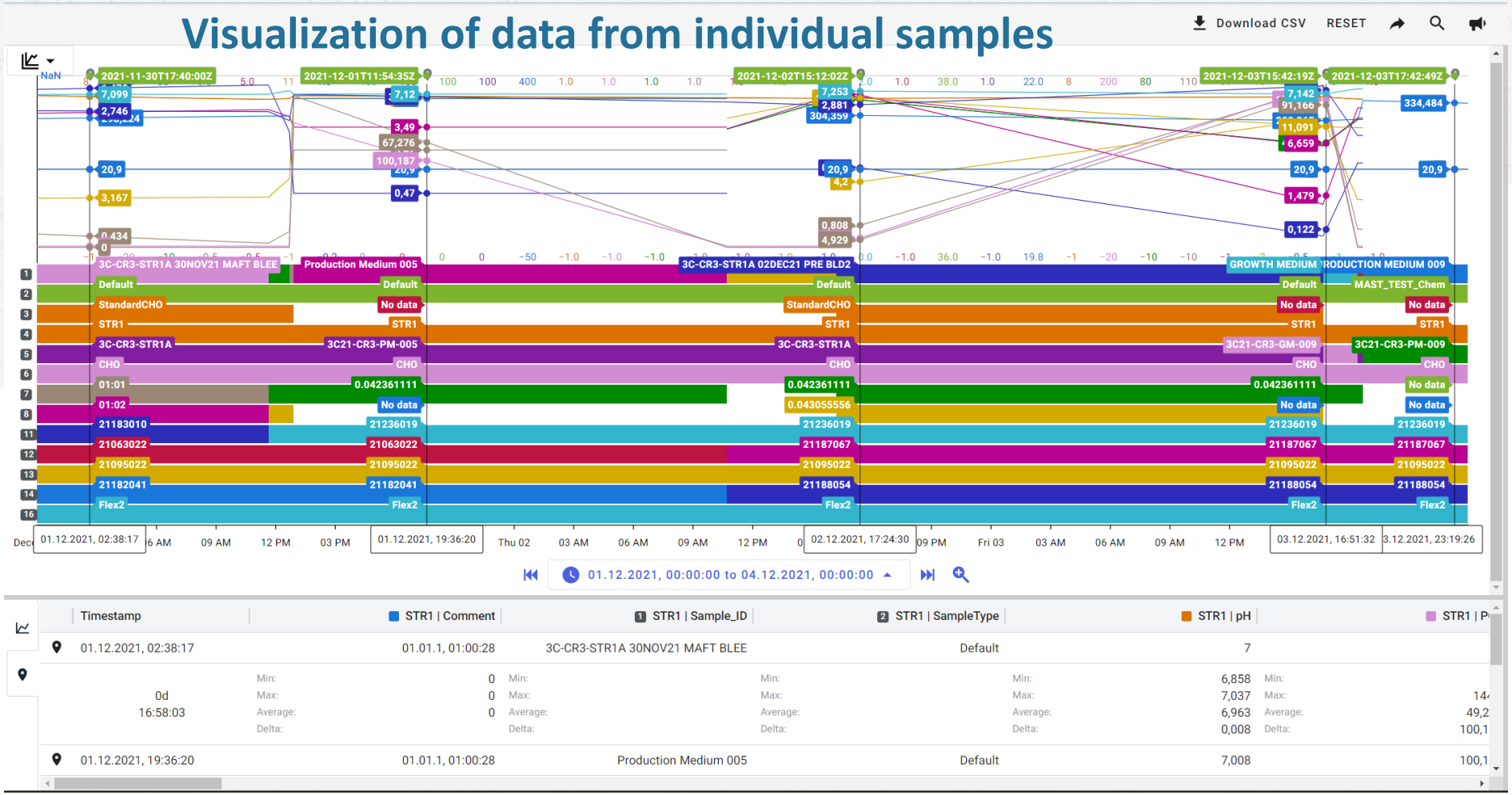
# Asset Contextualization and data visualization

Visualization of Asset Data from AMBR250 Bioreactor

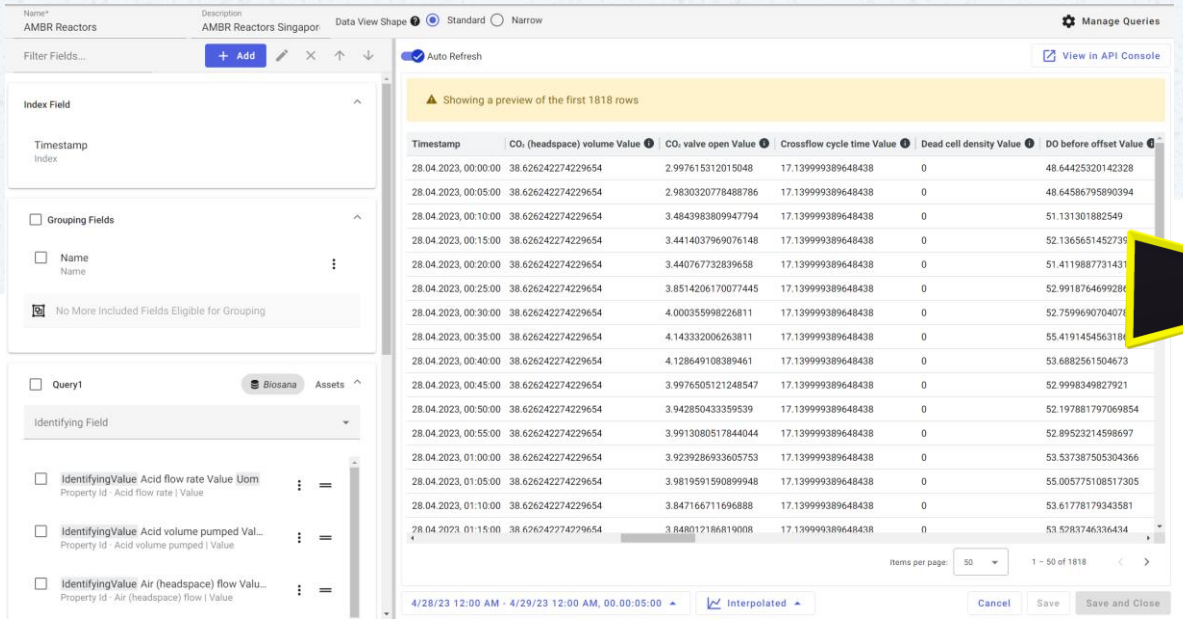
# Data from NOVAFLEX Analyzer

## Visualization of data from individual samples

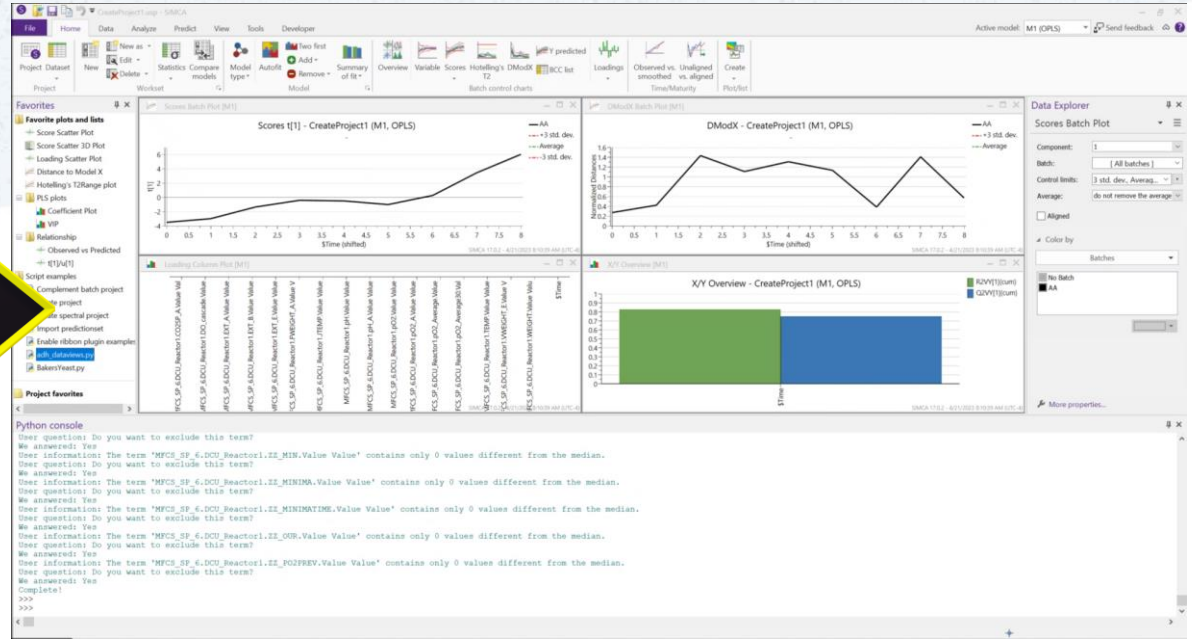


# Use Data Views to expose data to any external modeling applications, e.g. SIMCA

Create a Data View which contextualizes data
From different sources




Consume data through REST API of ADH
using Python module in SIMCA



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OCTOBER 23, 2023

# Insilico tools for optimization of a continuous bioprocess

## Determining optimal feeding strategies

Chris McCready

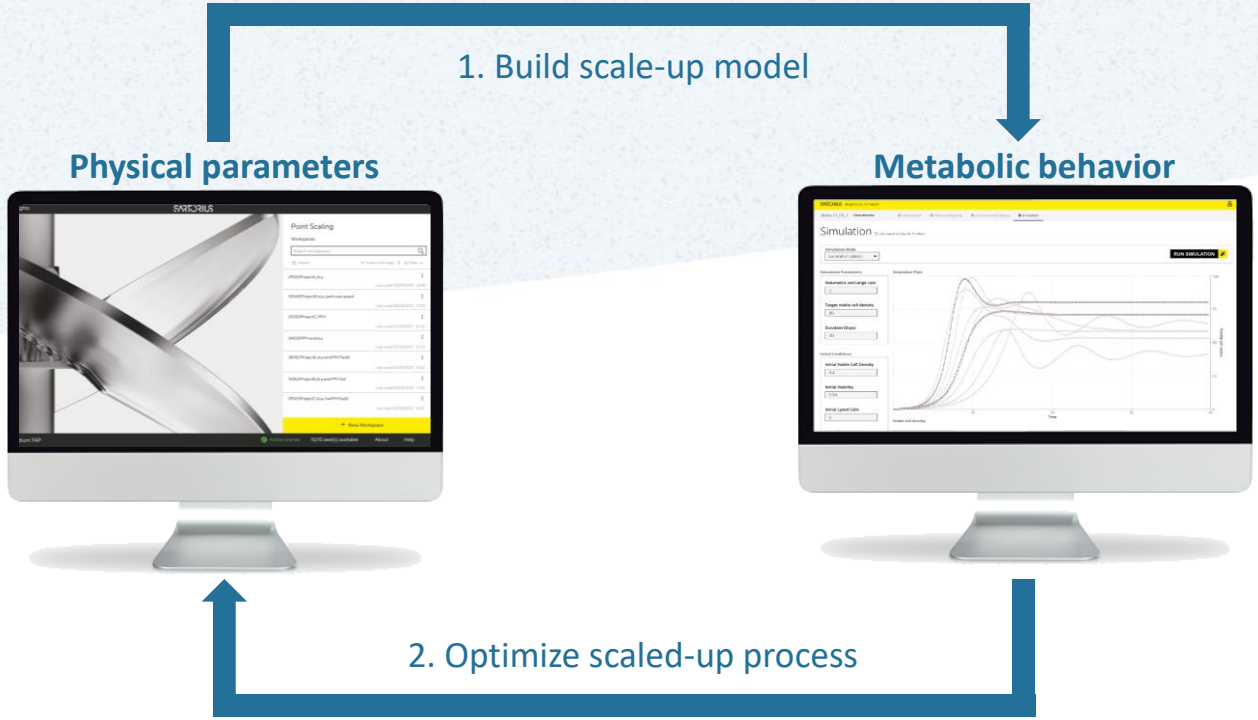
Sartorius Corporate Research | Advanced Data Analytics

# Bioreactor Scaling – The Challenge

# Physical Parameters Versus Metabolic Behaviour

## Bioreactor Specific

- Transfer recipe between scales
- Verify recipe for scale
- Reproducibility and stability of scale and ranges
- Scaling error assessment




## Hardware Independent

- Adjusting process parameters and feeds to optimize process
- Consider during simulation how clones behave in bigger/ smaller scales
- Selecting best cell-lines for intensification and robust scaling

# The Data Set

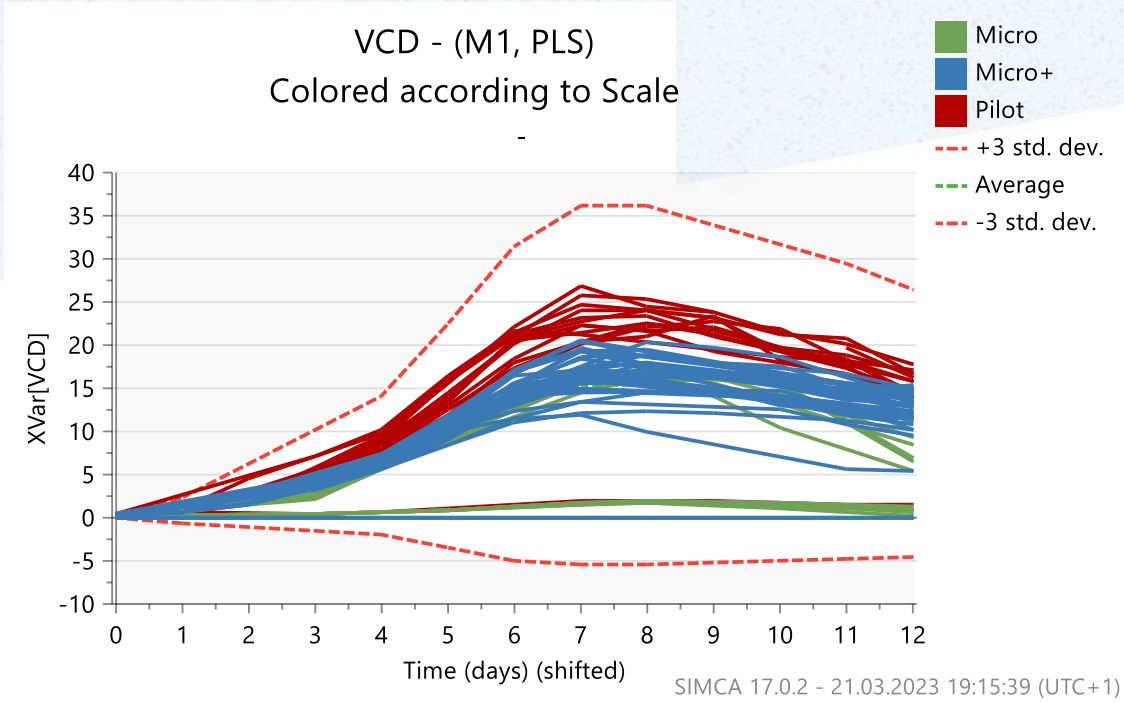
**Data scale: compromises three different scales**

- Micro: Ambr15
- Micro+: Ambr250
- Pilot: 2-2000L

**Data scale: compromises 10 variables**

- From cell counter
- From metabolite analyzer

Step 1: Investigate raw data for obvious errors or strange behavior



# The Difference Identification Plot

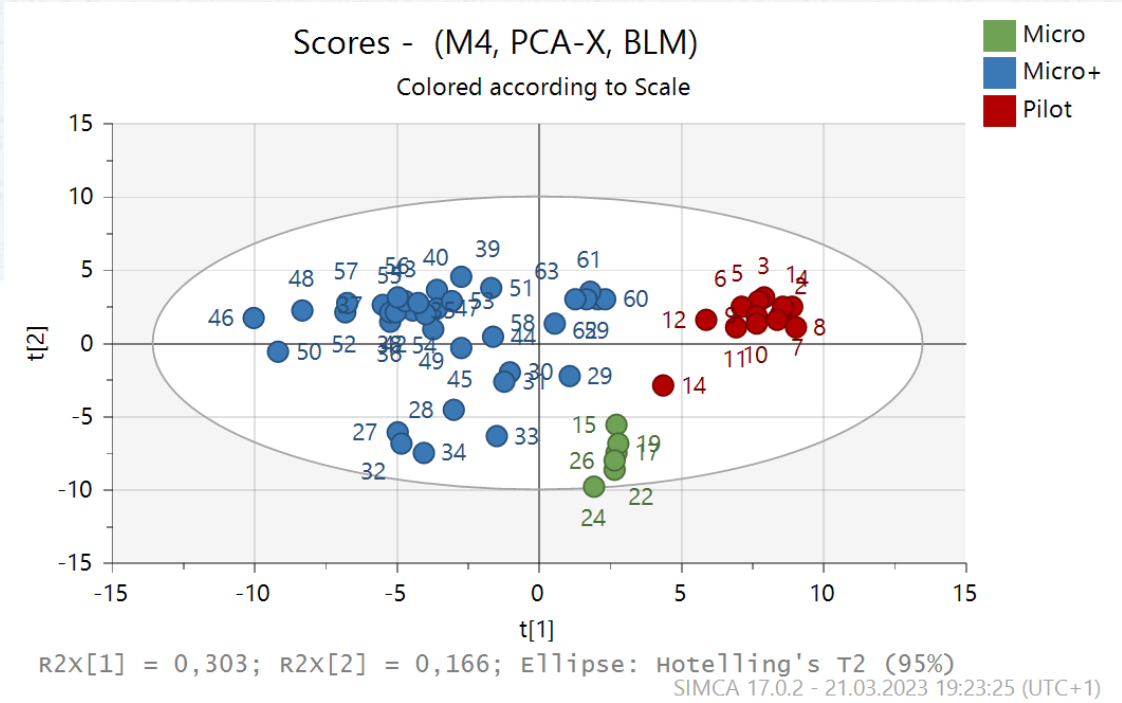
## Condense data with PCA

- Each process represents one batch
- Each dot represents all data for that batch
- The closer the dots together the more similar they are

## Key observation

- Separation according to scale
- Micro+ more scattered compared to pilot
- None of the Micro+ runs is overlapping with pilot group

Step 2: Assess relative distance between runs



# The Problem Understanding Plot

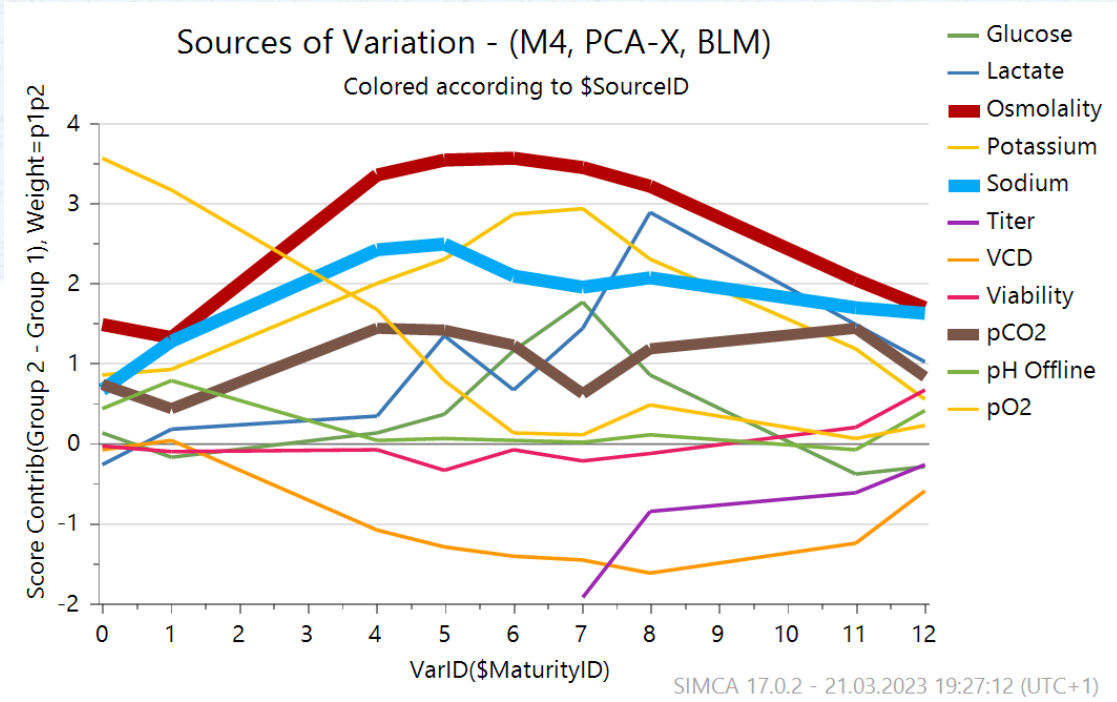
## Use of contribution plot

- Each line represents a parameter
- The further away from the X-Axis the stronger is that parameter contributing to the before observed difference

## Key observation

- Sodium and pCO2 are higher in the Micro+ scale.
- Both are used for pH control and can point towards problems with pH controller in small scale

Step 3: Identify the difference



# Hybrid Cell Culture Model

## Combining mechanistic and data driven modeling

### Purpose

- Calibrate a model to predict process operation
- Determine feeding strategy to optimize product

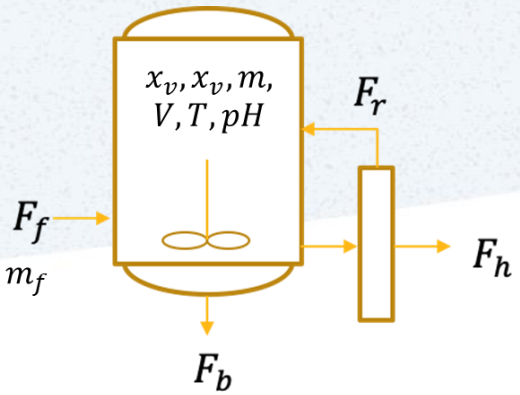
### Opportunity

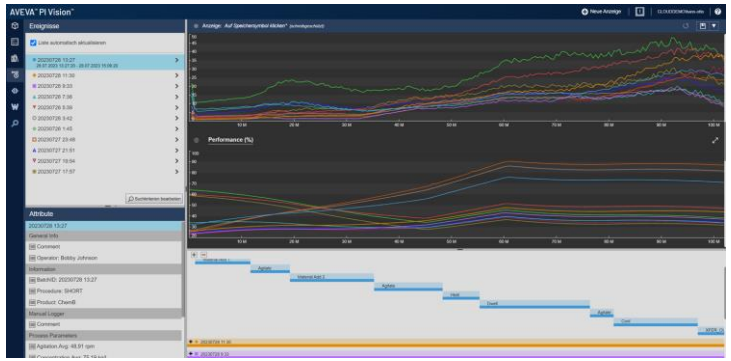
- Wet-lab experiments are time consuming
- Digital simulations to enhance lab results

### Process drivers

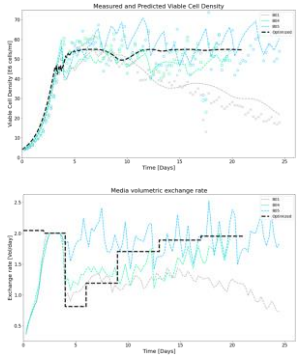
- Feeding (perfusion) rate drives cell behaviour
- Removal of accumulated toxins and inhibitors

### Perfusion bio-process




Simulation model



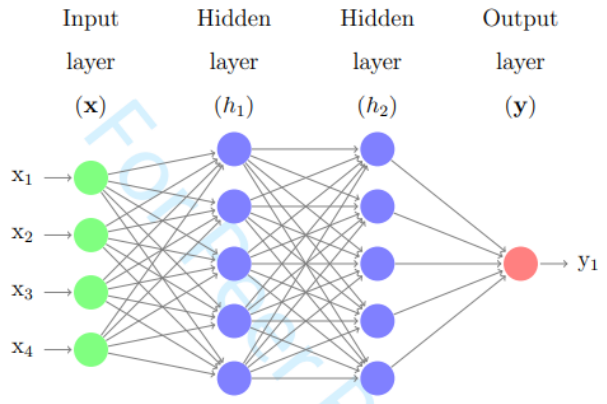

### Mechanistic model of cell growth

Kinetic model containing known phenomena

| | |
|---|---|
| Umax | 0.447214 |
| kd | 0.0349892 |
| kt | 0.00629552 |
| kl | 0.842443 |
| inh | 33.4254 |

- $\frac{d\,x_{vcd}}{dt} = \left(\mu_{eff} - u_{death} - \frac{F_{bleed}}{V}\right) x_{vcd}$
- $\frac{d\,x_{dead}}{dt} = u_{death}\, x_{vcd} - \left(k_{lyse} + \frac{F_{bleed}}{V}\right) x_{dead}$
- $\frac{d\,x_{lyse}}{dt} = \boldsymbol{k_{lyse}}\, x_{dead} - \frac{(F_{harvest} + F_{bleed})}{V} x_{lyse}$ → Tracking of lysed cells
- $\frac{d\varphi_{bio}}{dt} = k_{inh}\, x_{vcd} - \frac{(F_{harvest} + F_{bleed})}{V} \varphi_{bio}$ → Tracking of inhibitory biomaterials
  - $\mu_{eff} = \boldsymbol{\mu_{max}}\, \theta_{sub}\, \theta_{inh}\, f(T, pH, \dots)$ → Growth modifications
  - $\mu_{death} = \boldsymbol{k_{death}} + \boldsymbol{k_{toxic}}\, x_{lyse}$ → Accumulation of toxins
    - $\theta_{inh} = \frac{1}{\left(\frac{\varphi_{bio}}{2\,k_{inh}}\right)^2 + 1}$
- $\frac{d\,\Phi}{dt} = \boldsymbol{k_{\Phi}} x_{vcd} - \frac{(F_{harvest} + F_{bleed})}{V} \Phi$ → Product material balance
  - $\boldsymbol{k_{\Phi}} = f_{ML}(F, T, pH, glc, lac, \dots, \varphi_{bio}, x_{lyse}, \dots)$ → ML model of productivity
- Six coefficients: $\boldsymbol{\mu_{max}}, \boldsymbol{k_{death}}, \boldsymbol{k_{lyse}}, \boldsymbol{k_{toxic}}, \boldsymbol{k_{inh}}, \boldsymbol{k_{\Phi}}$

### ML model of productivity (yield)



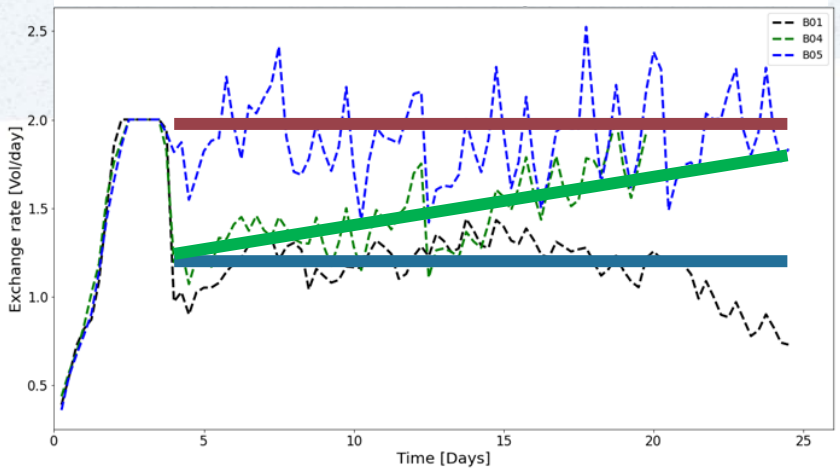
# Model Identification

Calibrating a model to predict cell growth and product production from process operation

## Process drivers

- Feeding strategy
- Process conditions (temperature)

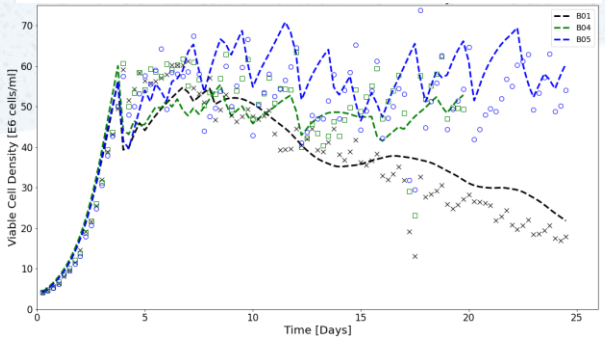
**Feeding experiments**



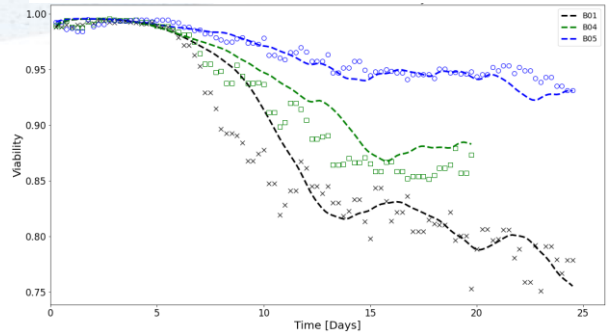

## Process performance

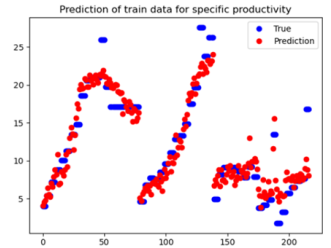
Growth, health and productivity

**Cell growth**

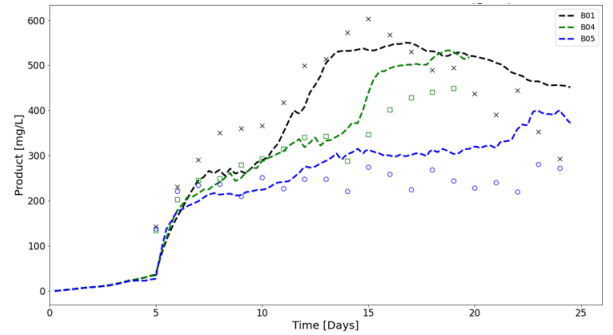



**Cell health**




**Product concentration**





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# Process Optimization

Determine feeding strategy to maximize product yield

## Optimization details

- An optimizer was used to determine feeding at select times throughout the batch

## Objective

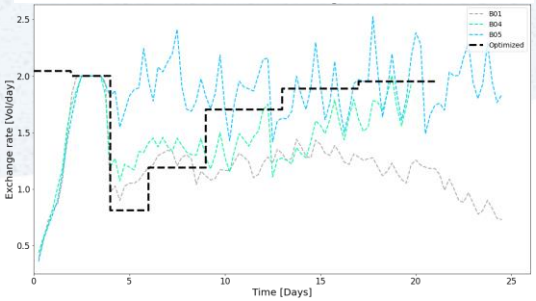
- Maximize product collected

## Take-away findings (process understanding)

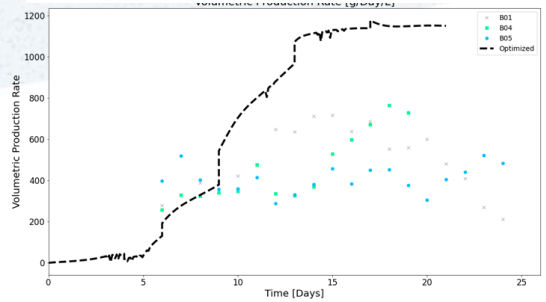
- Accumulated toxins are related to cell diameter
- Cell diameter (stress) correlated with productivity
- Optimal result when balancing “cell stress” with cell health

## Results

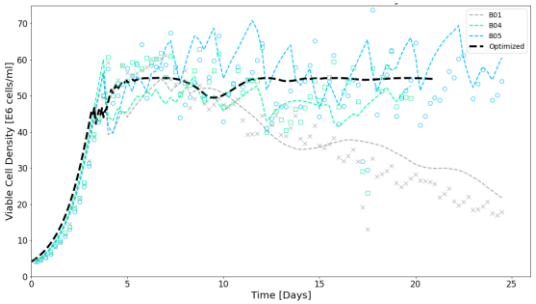
**Optimal feeding strategy**



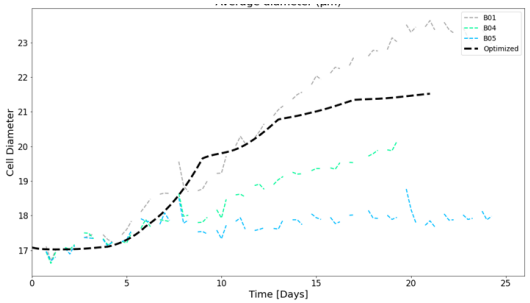

**Product production rate**




**Cell growth**




**Cell health | stress**



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# Thank you.

## Acknowledgements:


**BioSana:**
Maarten Pennings - CTO
Ard Tijsterman - CEO
Bart van Schaik - Data Scientist
Jake Chng - USP Scientist
David George - USP Scientist
Haizat Abdul Haman - USP Scientist

**Sartorius:**
Gerben Zijlstra - Process Technology Manager
Chris McCready - Lead Data Scientist
Gopi Palamadai - SME Data Architecture
Henry Weichert - Technology Consultant PAT
Timo Schmidberger - Principal Data Scientist
Johan Hultman - Manager IoT and Data Analysis

**AVEVA:**
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Fabio Dani - Software Developer Engineer


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