

Periodic technical report – Summary for publication



iConsensus
Integrated control and sensing platform for
biopharmaceutical cultivation process high throughput
development and production

Project Number: IMI2 Project 777397

Project Acronym: iConsensus

1 What is the problem/issue being addressed?

Biopharmaceuticals are large molecules, most often proteins, used for therapy, e.g. antibodies. The biopharmaceuticals are produced using cells in well-regulated bioprocess in bioreactor.

Health technology is highly profitable but spending the highest percentage of its revenues on R&D. The development of a new drug costs about 1 billion dollars and takes ≥ 10 years. Reducing cost and time for the development of the manufacturing process represent very large savings and is essential to be first to market.

The worldwide community of bioprocessing is in quest for more rational tools to understand, predict and optimize industrial biopharmaceutical manufacturing.

2 Why is it important for society?

iConsensus aims to bring a disruptive new technology platform for mammalian-cell based production of biopharmaceuticals, to reduce the time and costs for the process development and lead to better manufacturing control. This improvement influences the time-to-market and gives competitiveness advantage, higher consistency, efficacy and robustness for the biopharmaceutical industry, generating as well reduced cost, failure and risk for product shortage.

The project clearly supports European Innovation, competitiveness and industrial leadership while giving tools to improve the European well-being and societal challenges.

3 What are the overall objectives?

Cell cultures of mammalian cells, e.g. CHO cells, used for the production of a biopharmaceutical are complex mixtures of ≥ 70 components, of which monitoring provide process knowledge however is a very large and tedious task. All these components can have an effect on the process performance, the purification process or on the product quality, which is the reason why monitoring all these compounds is advantageous.

To address this, iConsensus provides innovative analytical, hardware, software and high-throughput solutions for the development, monitoring and control of cell culture, with an analytical platform including on-line/at-line sensors and methods measuring multiple factors. The analytical platform is aimed to be integrated to bioreactor, enabling feedback control implementation.

4 Technologies

4.1 High-throughput microbioreactors (MBR)

Novel microbioreactors for mammalian cell culture development by m2p/Beckman and RWTH based on microtiter plates designed and tested by creating prototypes. The system has been verified with an industrial CHO cell line with 96 cultures with pH and DO individual regulation.

Novel high-throughput microbioreactors for screening in mammalian cell cultures verified with CHO cells with pH and DO controls (m2p/Beckman, RWTH)

<https://publications.rwth-aachen.de/record/854042>

<https://publications.rwth-aachen.de/record/825312>

<https://publications.rwth-aachen.de/record/843965>

4.2 CE chip platform

New methods based on capillary electrophoresis (CE) to measure different components in the culture, e.g. amino acids, sugars developed and transferred to chip format, by KTH and Kantisto. Transfer of capillary electrophoresis methods to CE chip setups demonstrated for 3 types of metabolites.

New methods of capillary electrophoresis (CE), also in chip format for analysis of culture components, e.g. amino acids, sugars (KTH, Kantisto), and new CE methods for vitamins and antibody

<https://doi.org/10.1002/elps.202100122>

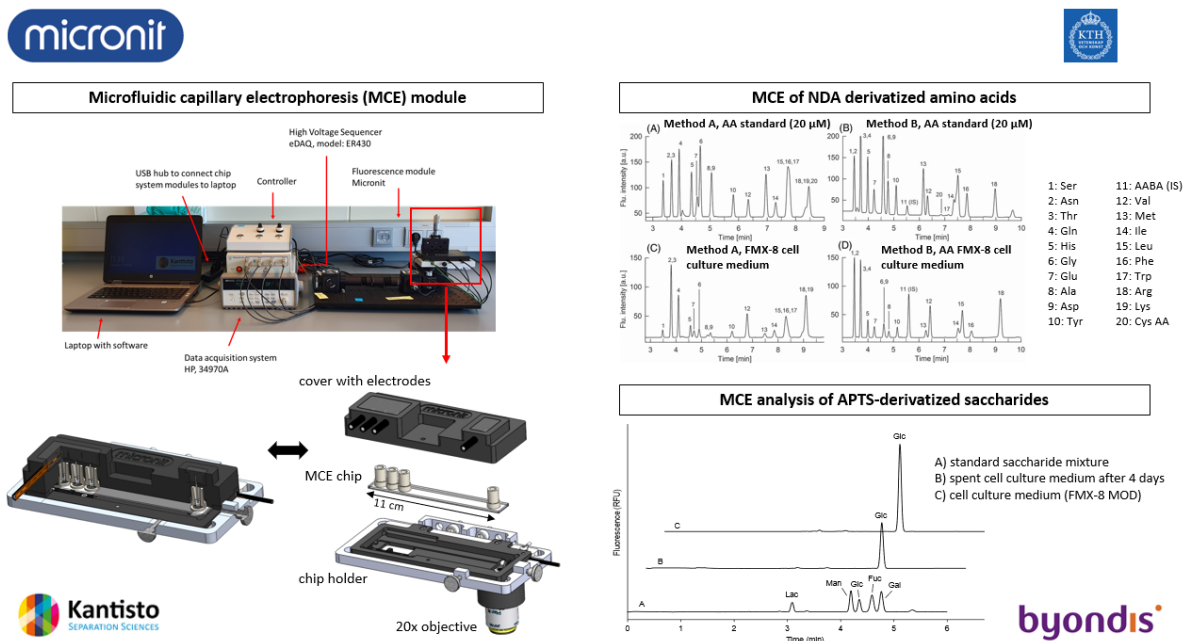
<https://doi.org/10.1002/biot.202100325>

<https://doi.org/10.1002/elps.202100213>

<https://doi.org/10.1155/2022/2819855>

<https://doi.org/10.1002/elps.202200144>

<https://doi.org/10.1016/j.trac.2023.116975>



Overview of the CE-chip technology

4.3 Optical on-line sensors

New sensors based on optical properties created by Presens and proof-of-concept's demonstrated. New formats for pH, O₂ and CO₂ sensors, including autoclavable sensors. Sensors

successfully tested by industrial partners in cell culture processes. Optical sensors integrated in microfluidic module, part of ALIAS.

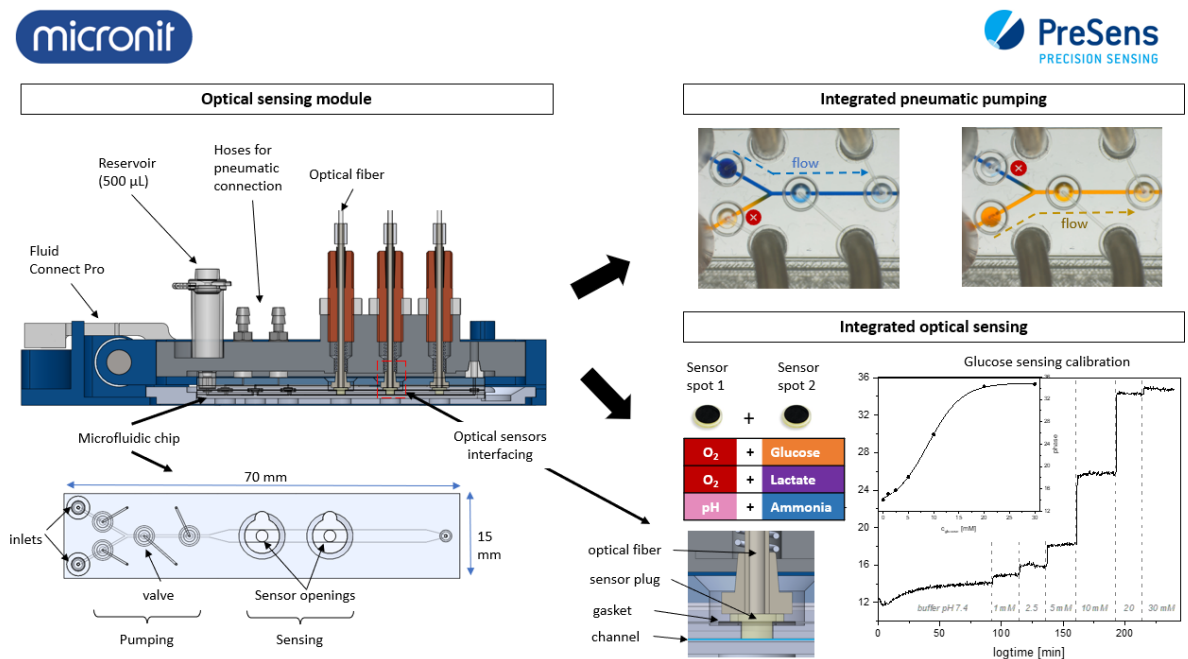
New optical on-line sensors (Presens) with successful tests by industry

<https://www.presens.de/knowledge/publications/application-note/cell-culture-monitoring-in-stirred-tank-bioreactor-with-optical-ph-sensors-1717>

<https://www.presens.de/knowledge/publications/application-note/evaluation-of-an-optical-co2-probe-for-long-term-monitoring-in-stirred-tank-bioreactors-1715>

<https://www.presens.de/knowledge/publications/application-note/evaluation-of-an-optical-o2-probe-and-sensor-spots-for-long-term-measurements-in-stirred-tank-bioreactors-1713>

<https://www.presens.de/knowledge/publications/application-note/online-co2-monitoring-in-cho-cell-culture-1759>



Overview of the optical sensing stand-alone module

4.4 Spectrometry and chemometry support

Generic chemometric PLS prediction model by Hohenheim Univ. created to predict glucose, glutamate and lactate concentration with errors < 5 % using Raman spectra from different CHO cultures and different Raman spectrometers.

Generic chemometric partial least square (PLS) prediction model (Hohenheim Univ.) to predict glucose, glutamate and lactate levels using Raman spectra from various CHO cultures and spectrometers

doi.org/10.3390/s22155581

Prediction of amino acids and glycosylation in perfusion process (KTH)

<https://doi.org/10.1016/j.bej.2022.108426>

2D fluorescence for monitoring in microtiter plates

<https://www.mdpi.com/2306-5354/9/9/438>

<https://doi.org/10.1186/s13036-023-00332-0>

<https://doi.org/10.3390/fermentation9020095>

4.5 Holographic methods

New concept for the quantification of cell density based on holographic image. Successful evaluation at GSK with very close correlation (<10% error) with reference. The method detects as well early viability drop and early apoptotic cells. System integrated to high-throughput bioreactors.

New quantification of cell density by holographic image (Iprasense), with successful test at GSK and integration to high-throughput bioreactors.

<https://www.iprasense.com/wp-content/uploads/2023/02/NORMA-4S-APPLICATION-NOTE-A-fully-Automatic-Cell-Counter-for-High-Throughput.pdf>

<https://www.iprasense.com/applications/viable-cell-density-monitoring-in-bioreactor/#>

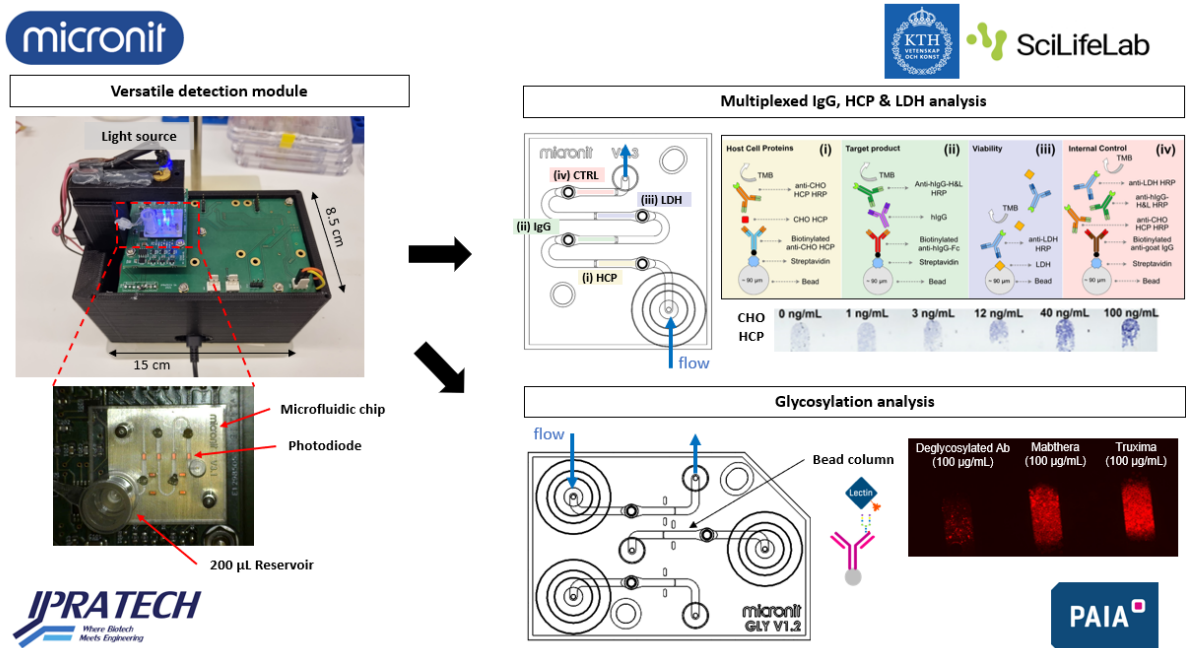
4.6 Affinity-based microfluidics

Development and validation of the microfluidic bead-based immunoassays completed using samples from a Rituximab-producing CHO cell bioreactor by KTH. Micronit's COC devices used for integration of colorimetric assay for CHO HCP and IgG detection in stand-alone module (Ipratech). DNA quantification addressed using (i) bead-based detection strategy with a DNA intercalator, and (ii) novel enzyme-free method based on competitive hybridization assay for detection of specific nucleic sequences with sensitivity in the pM-range in less than 30 minutes.

Novel microfluidic bead-based immunoassays validated with antibody producing CHO cell bioreactor culture (KTH), with Micronit's COC devices, for host cell proteins and antibody detections in stand-alone module (Ipratech), and fast DNA quantification by bead-based detection

10.1021/acssensors.0c01884

10.1016/j.copbio.2021.06.018



Overview of the affinity-chip technology

4.7 Analysis of glycosylation

PAIA has continued the validation of its lectin based detection technology with samples from Bayer on different antibody glycoforms. Simplified glycan assay workflow created to facilitate the transfer into microfluidics. First results from samples of EFPIA partners show that this approach is feasible.

Technology successfully transferred to microfluidics and measurement of mannosylation in cell culture samples achieved with detection limits < 1%.

4.8 Total integration, ALIAS, and data management, MID

'At-Line Integrated Analytical System', 'ALIAS', created. Architecture and functionalities of ALIAS defined and put in place, with the monitoring information database (MID). ALIAS is a liquid handler, integrating the sample preparation, the stand-alone modules of affinity-based microfluidics, optical sensing and CE-chip, enabling ≥ 10 analyses to monitor bioreactor cell cultures, with < 1 mL volume, achieved 1-3 times/day.

4.9 Modelling of cell cultures

Development of a fed-batch process (KTH) with data used for modelling. Mechanistic kinetic model by column generation and statistical approach for the kinetics (KTH), and data-driven models (UMons) generated. Model able to predict the process behaviour. Software sensors predicting unmeasurable variables. Robust model predictive control formulation (UMons) with several external disturbances validated in simulations.

Mechanistic kinetic model of a fed-batch process by column generation and statistical approach for the kinetics (KTH), and data-driven models (Univ. Mons), able to predict the process behaviour

<https://doi.org/10.1016/j.compchemeng.2023.108164>

Software sensors predicting unmeasurable variables and robust model predictive control with several external disturbances validated in simulations.

5 Exploitation and dissemination

The achievements of iConsensus have been numerous and bearing high promises, thanks to the collaboration of the experts of academia with the SME's and interaction of the EFPIA partners. The new analytical stand-alone modules and ALIAS have good commercial potential, which will be pushed forward, by iConsensus partners. The sustainability of iConsensus achievements is also guaranteed by the publications in 17 peer-reviewed articles, 5 application notes and a patent application. The dissemination has also taken place at conferences as 20 lectures and 8 posters.

6 Progress beyond the state of the art

iConsensus aims at developing a new platform for real time analysis of the components.

Nowadays, the development of cultivation processes uses empirical methods supported by statistics, and high-throughput, where only very few parameters are easily quantified, leading to suboptimal outcome. When more efforts are put on the analytical technology, this requires enormous resources and time. iConsensus brings solutions by far pioneer, with no equivalent product today, with real-time solutions and advanced MBR. Besides the importance of innovative tools in R&D it is also crucial to have reliable, fast and qualified analytical tools for manufacturing processes. Data and knowledge gained during development should be usable in a straight line in the final manufacturing process. To use the same analytical tools throughout the development and life cycle management of a process/product can be highly beneficial for the control and robustness of the final manufacturing process.