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Application note
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PAT solutions for the future of bioprocessing 4.0

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Pharmaceuticals have a prominent role in healthcare and their manufacturing requires innovative tools, scientific and engineering knowledge, and must at the same time comply with strict regulations.

Efficient bioprocess development is therefore a challenging endeavor: timely monitoring and control of Critical Process Parameters (CPPs) and Critical Product Attributes (CPAs) are crucial to design, analyze, and control a bioprocess. The goal is not to have to test the quality of the product, but that it is built-in by design. Implementing Process Analytical Technology (PAT) and advanced data analytics is of paramount importance to comply with demands by regulatory bodies, while at the same time efficiently and quickly develop a robust production process.

PAT relies on concepts that are applicable to all manufacturing:

1. **Process understanding**

The process is understood if all the critical sources of variability are known, managed by the process and the product quality attributes are predicted.

2. **Principles and tools**

CPPs and CPAs must be identified to establish an effective bioprocess. For example, perturbations and modification are challenging to be detected, collecting representative samples through automatic sampling and at-line analyzers help monitoring and controlling. Different classes of tools are included in the PAT system: multivariate tools for design, data acquisition and analysis; process analyzers; process control tools; and continuous improvement and knowledge management tools.

3. **Strategy for implementation**

PAT principles should be applied during the development phase to be able to implement the manufacturing and regulatory system at the same time.

PAT promises higher quality, reduced cost of goods, and faster time to market ensuring different parameters: reduction of variations in CPPs; increased process robustness; fast and predictive up- and downscaling of the process; reduced human intervention, reduced risk of human error, reduced risk of contamination and timely correction of irregularities;

dynamic adjustment to different culture conditions; complexity reduction; increased throughput. Despite those numerous fantastic benefits, the systematic implementation in the industry is lagging behind the ambitious goals set by the PAT initiative in 2004 and delayed by technological limitations that are just being gradually overcome.

Integrated smart bioreactor set-ups will be key to enable the effective orchestration of various hardware classes and the advanced analytics performed in software. Securecell targets this bottleneck with its PAT tools Numera®, an automated sampling and sample preparation system, and Lucullus®, a process information management system. Some example applications have demonstrated their capabilities to close the prevalent integration gap.

Bioprocess control and data management software – Lucullus®

Process control is a central task in bioprocess development and production. Real-time availability of analytical data is the base for process understanding and production empowerment. A variety of data coming from usually disparate data sources need to be merged in a database to get a complete understanding and better insights into process modeling. Both capabilities are the fundament of collecting and later applying process knowledge in control strategies.

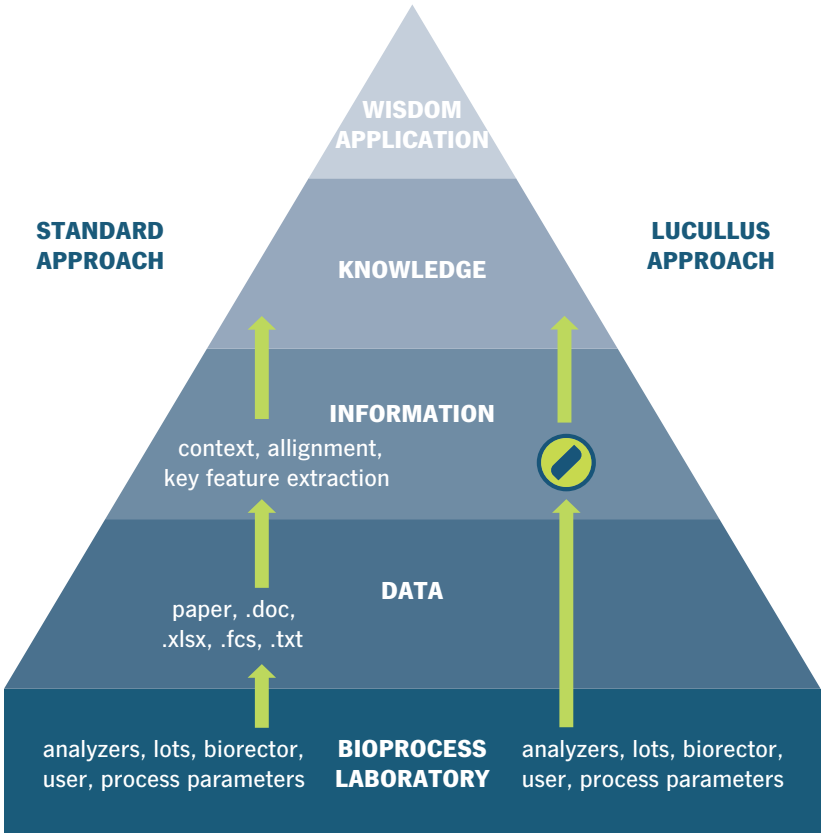

Fig. 1 Practical approach from data to information to knowledge to wisdom or a final application. Illustration of the Lucullus® PIMS approach, which enables a significant speed-up in process development based on extensive data and information management.

1. Lucullus® PIMS for biochemical engineering at TU Wien

Prof. Herwigs at TU Wien performs a wide variety of processes from waste to value to biopharmaceuticals using several organisms, such as extremophilic organisms, microbial, yeasts, fungi and mammalian cells, and numerous reactor systems combined with different equipment. The lab set-up requires extreme flexibility and continuous implementation of process chains and control strategies. Lucullus® enables flexible set-ups of bioreactors systems; intuitive designs and implementation of basic and complex chains; monitoring and control for efficient and robust bioprocesses; centralized, secure data management and basis for knowledge generation; and speed-up of process development and reduction in time to market. link

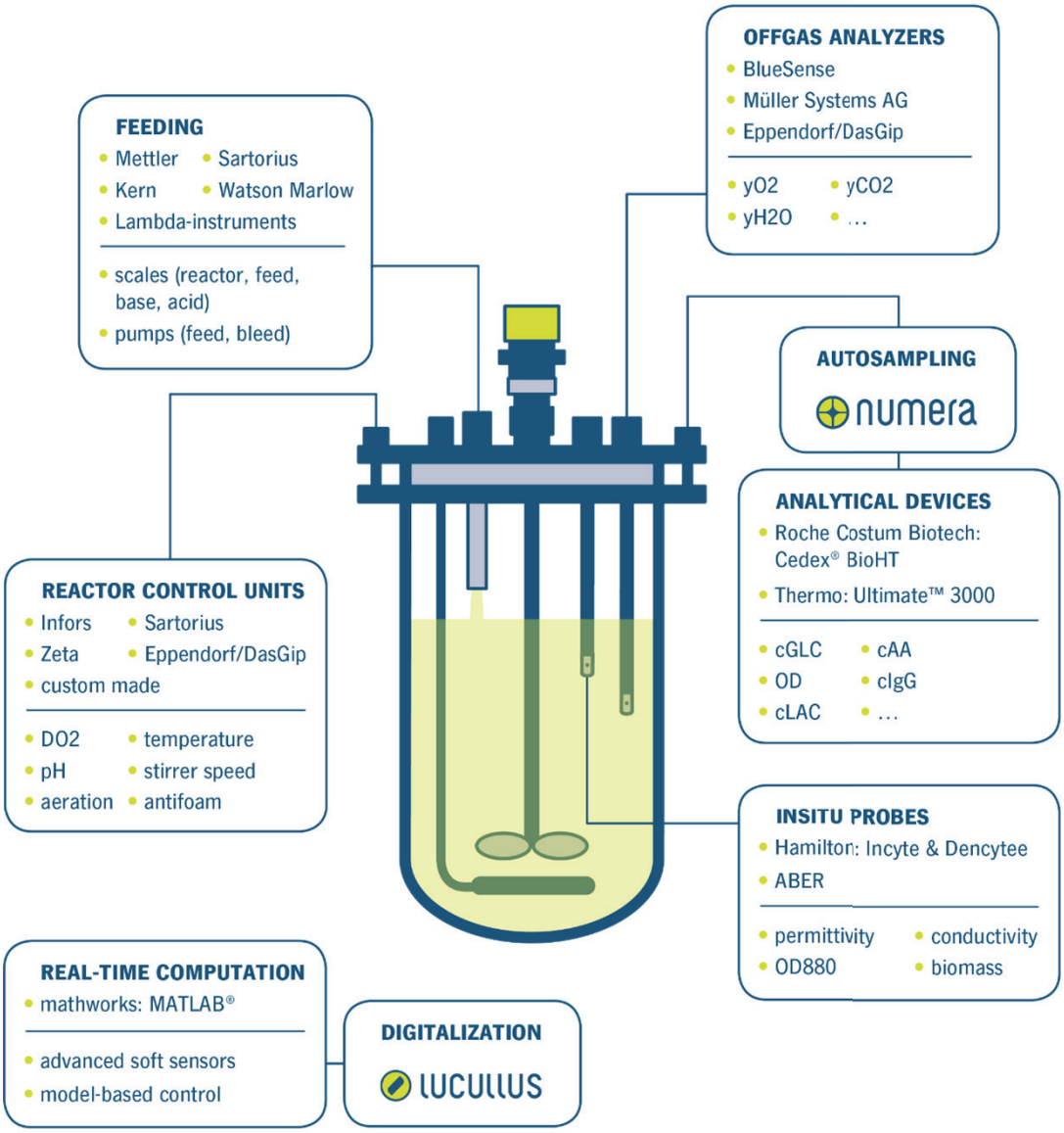

Fig. 2 Summary of devices and interfaces of Lucullus® at TU Wien.

2. Lucullus® PIMS for event-based E. coli process control

E. coli are cultured in a pET expression system and auto-induction medium. Typically, the start of induction coincides with a temperature shift. In the first example, the shift is performed based on an in-line optical density (OD) signal; in the second one, Numera® in combination with an HPLC monitors the consumption of glucose and lactose, and the trigger of the shift is based on substrate consumption and the behavior of the culture. In both processes, Lucullus® controls and automatically initiates the temperature shift. The triggering was efficiently performed by Lucullus® based on real-time data. The combination of Numera® and Lucullus® allows a control strategy based on direct measurement of the analyte of interest and holds a huge potential for optimizing the process in a science-based manner. Process variability can be decreased, and productivity stabilized resulting in a robust process.

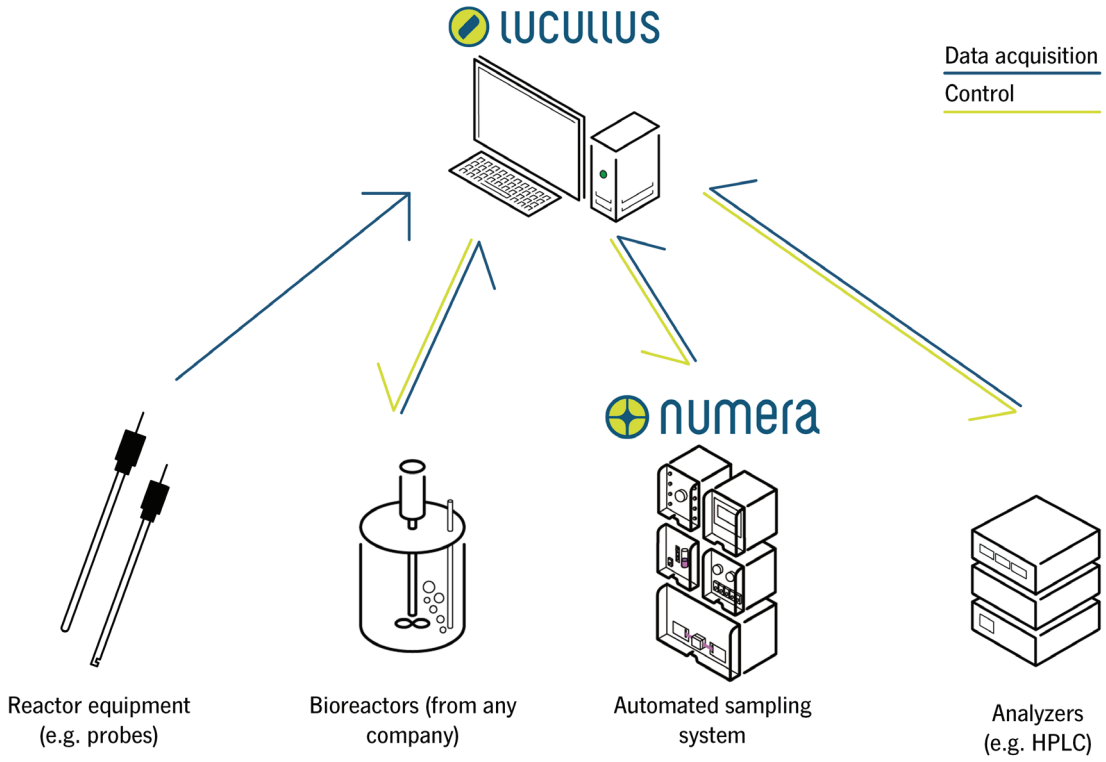

Fig. 3 Summary of devices` interactions monitored and controlled by Lucullus®

Automated sampling and analytics – Numera®

Sample-based monitoring has various advantages: direct measurement of specific analytes; usage of highly automated off-line analyzers; reliability through application of reference methods; extensive parallelization; flexibility and storage of reference samples for later analysis. Numera® allows maintaining those benefits, while still automating those typically manual workflows connected to it. Numera is an accurate (error of 0.4%, can be increased by sample dilutions), highly precise, long-term sterile system that can perform high-frequency low volume sampling.

1. On-line HPLC and data integration

A bioreactor containing a CHO culture was equipped with a Numera® connected to an HPLC, while Lucullus® acted as the overarching solution to control all the parameters and control inputs. Due to the systems's high precision, the HPLC calibration (0.03-0.01% error) can be performed with Numera® and the dilution factor directly calculated into Lucullus®. Reference analytics can be brought directly to the process and metabolites (e.g. B-vitamins and IgG) be monitored for process characterization. Link

Fig. 4 Numera® for automated sampling and on-line HPLC analysis including a multiplexer, dilution and filtration module as well as an autosampler which is connected to the UltiMate 3000 HPLC system.

2. On-line analysis with Cedex® Bio HT – Glucose, lactate, ammonium, and IgG

A bioreactor containing a CHO culture was equipped with a Numera® connected to an HPLC and a Cedex® Bio HT, while Lucullus® acted as overarching solution to control all the parameters and control inputs. Glucose, lactate, ammonium, and IgG were analyzed. The complete measurement of analytes took 31 minutes in total (19 minutes for the Bio HT analysis). The connection between Numera®, Cedex® Bio HT, bioreactor, and Lucullus® enables the efficient monitoring of substrate, metabolite, and product. Link

3. On-line analysis with HPLC and Cedex® Bio HT – Amino acids

A bioreactor containing a CHO culture was equipped with a Numera® connected to an HPLC and a Cedex® Bio HT, while Lucullus® acted as overarching solution to control all the parameters and control inputs. The sample processing required dilutions could be performed by Numera®. A total of 19 amino acids were analyzed on-line with the HPLC system in 34 minutes, the analytical data were available in Lucullus® 11 minutes later. The same analysis took 19 minutes with the Bio HT system and the data were available in Lucullus® 11 minutes later. The monitoring of various analytes was performed without significant errors or system failures. link The higher measurement frequency and significantly reduced random errors resulted in a larger information content per experiment. (A reliable automated sampling

system for on-line and real-time monitoring of CHO cultures. A. Hofer, et al.)

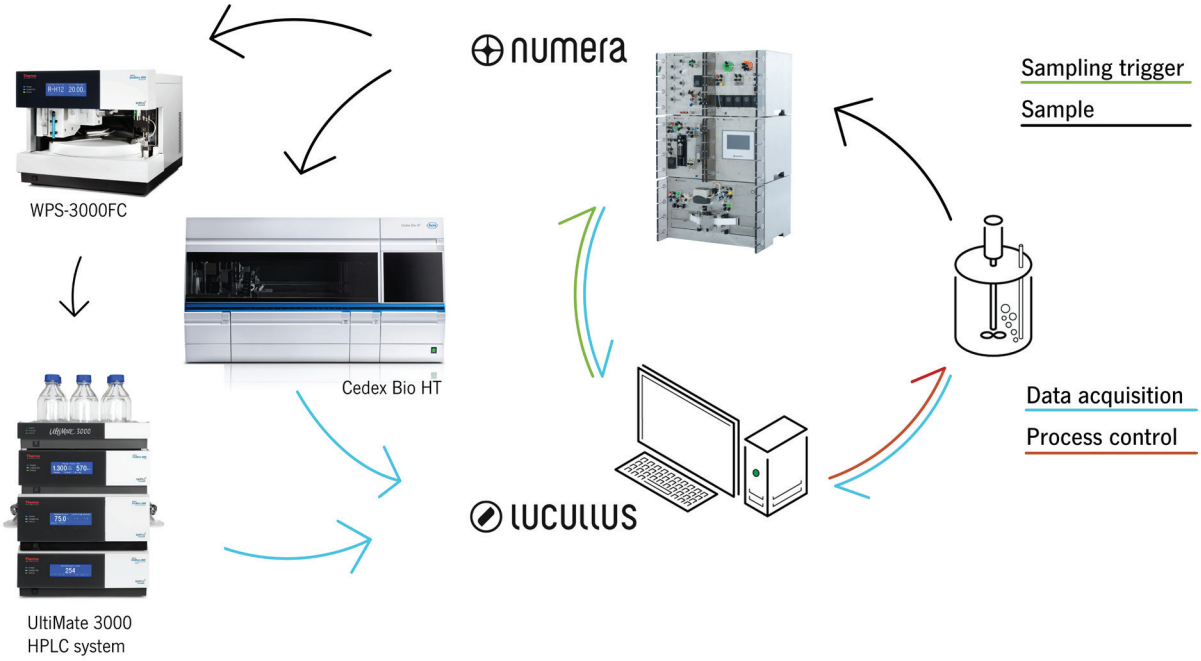

Fig. 5 Lucullus PIMS is the overall software, uniting data acquistion and control of the bioreactor system, Numera and the two analyzers, namely UltiMate 3000 HPLC system and Cedex® Bio HT Analyzer. To implement automated analysis with both devices, Numera is equipped with a routing module, which allows multiple sample distributions, e.g. to the Bio HT. Amino acid analysis by HPLC is realized by transferring the sample from Numera to an analytical autosampler with fraction collector (Thermo Fisher Scientific), where the sample is derivatized and injected.

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