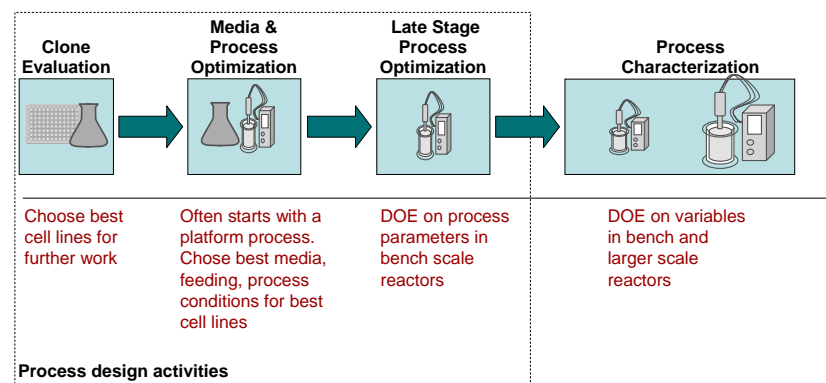


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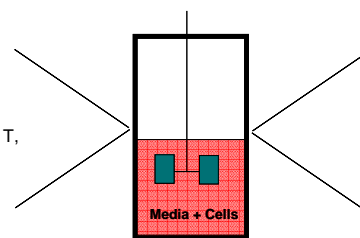
Use of scale-down models in process development



The need for multi-factorial DOE has never been greater

Parameter Choices

- Cell line
- Media components
- Feed strategy
- Control settings for pH, T, O₂, CO₂, others



Process Outcomes

- Growth
- Titer
- Critical Quality Attributes (CQAs)
- Other attributes

Disturbances

The potential design space is vast, and first principles models do not exist to link inputs and outputs.

The potential design space is complicated by presence of categorical factors, and curvature of responses.

Experiments to understand links between process robustness to process design are likely broader than present-day optimization studies. The number of trials needed grows quickly with number of factors considered.

The SimCell System



A new model is needed to enable multi-factorial experimentation with high throughput and high data quality.

Micro-Bioreactor Array (MBA) is designed as a bioreactor model.
Working volume: ~700 microliters
Optical measurement of DO, pH, total cell density

Robotics system for high-throughput.

Automated experiment execution and control
Capacity for 210 MBAs = 1,260 cell culture reactors

Software and data solutions support multi-factorial experimentation.

Experiment design, execution and analysis
Import and integrate offline data sets

Design of Experiments

Factor	Levels		
pH	-		+
DO	-		+
Feed 1	-	0	+
Feed 2	-	0	+

Four factor, mixed level full factorial design yields 36 unique experimental combinations.

180 individual micro-bioreactor cultures conducted in a single two week experiment.

CHO cell line producing monoclonal antibody
13 day fed-batch experiment with pH, DO and glucose control

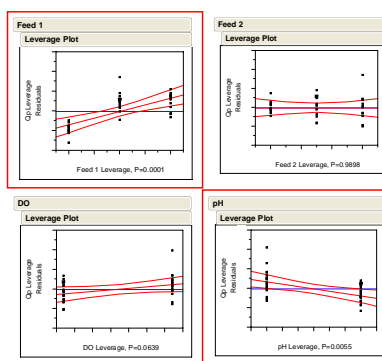
On-line measurements:

pH, DO, total cell density

Off-line measurements:

- viability – Guava EasyCyte
- glucose – Tecan SpectraFluor Plus w/ Biovision Assay Kit
- titer – ForteBio Octet
- intact IgG – Caliper LabChip 90

Linking process parameters and outcomes



Leverage plots for Qp show its sensitivity to manipulations of process parameters.

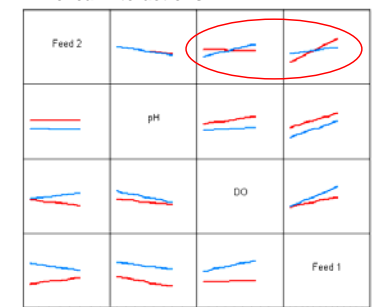
In this case, pH and Feed 1 were found to have significant affects on specific productivity.

While Feed 2 did not have a significant effect, it does interact with other process factors.

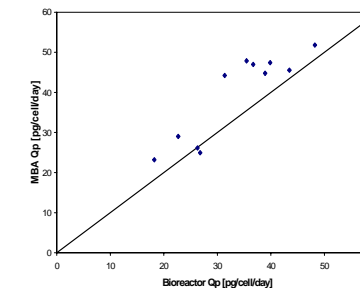
Simplified optimization schemes might miss such interactions.

Similar analyses can be applied to IVCC, titer, purity or other attributes to rank input parameters by importance and reveal the critical factors for optimization and control of variability.

A more detailed analysis can reveal interactions...



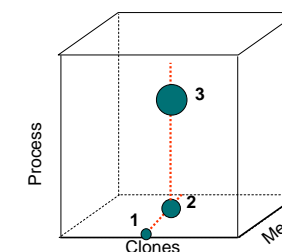
Comparison across several conditions



Excellent correlation (R=0.90) between platforms for specific productivity.

Results from MBA DOE reliably predict performance at bench-scales, more than 1500-fold scale-up.

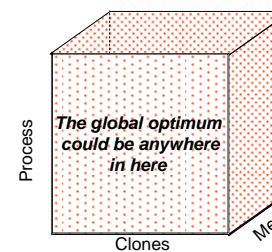
A better way to do process development...



Today's cost and throughput constraints dictate serial optimization:

First clone selection, then media development, then process parameters

This process is not only lengthy; but, can miss important interactions between variables and thus the true optimum.



SimCell's high throughput allows a more complete exploration of the design space in significantly less time.

Multi-objective optimization is also possible:

- Reduced process variability
- Easy purification
- Improved product quality

Verification of select conditions in bench-scale bioreactors



After completion of DOE, select conditions were chosen for verification at the bench-scale.

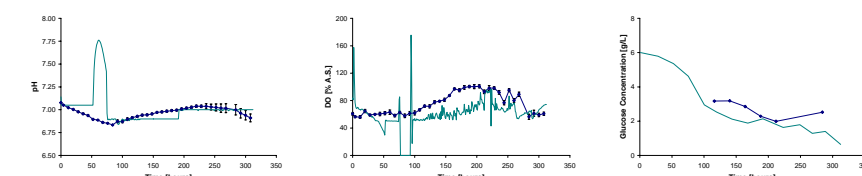
3L Applikon glass vessel with 1L working volume

- Identical process parameters as used in the MBA experiments
- pH, DO and glucose control
- Agitation at 150 RPM for similar shear and mixing profiles
- Daily sampling for cell count, viability and glucose measurement

At the conclusion of the experiment, data from MBAs and bench-scale compared

- Measurement and control of pH, DO and glucose
- Cell growth, viability and IVCC profiles
- Terminal titer, intact IgG, product purity and specific productivity

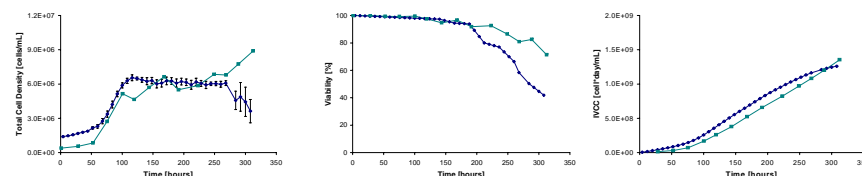
Measurement and control



MBA data represented in blue
Bioreactor data in green

Measurement and control are very similar between the two platforms.

Growth and viability



Differences in late stage viability decline are observed in this particular condition.

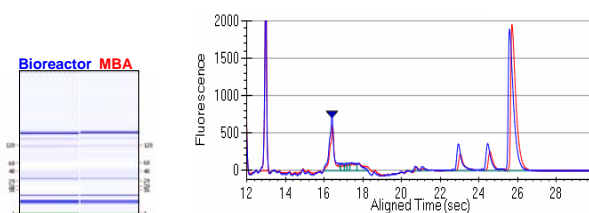
Growth and viability profiles are similar between the two platforms.

Productivity and product quality

	Bioreactor	MBA
Titer [mg/L]	2445	2390
Intact IgG [mg/L]	2378	2621
Purity [%]	60	69
Qp [pg/cell/day]	43.47	45.54

Comparison of terminal product titer and quality across the two systems.

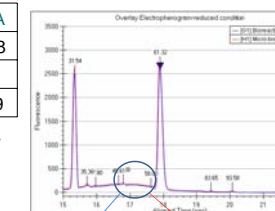
Titer and product quality (Microchip-SDS) are virtually identical.



Non-fully glycosylated forms

	Bioreactor	MBA
Heavy Chain [mg/L]	2435	2498
HC purity [%]	52	51
NFGF [%]	3.95	3.99

Heavy chain content and quality is virtually identical between the two platforms.



Summary and conclusions

High throughput cell culture and analytical systems are needed more than ever, as QbD will require many more cell culture experiments than are done today.

The SimCell System can be used to execute multi-factorial DOE in high throughput.

The resulting cultures can be analyzed with other platforms, such as the ForteBio Octet and Caliper LC90 (or LCGX), to obtain titer and product quality information in very high throughput.

High data content allows statistical analysis across a variety of metrics, such as IVCC, titer and purity with the ability to determine interactions between parameters.

Scalability to conventional bench-scale bioreactors has been demonstrated across multiple conditions indicating that SimCell results can be used with confidence.

High throughput experimentation can be used to conduct clone, media and process optimization in parallel resulting in accelerated process development timelines with savings of up to 24 weeks possible.

Acknowledgements

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Additional Bioprocessors contributors:

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- Brett Schreyer