numera

From Cell-Containing Samples to Analysis-Ready Filtrate

Advanced Filtration in Automated Bioprocess Sampling

EXECUTIVE SUMMARY

Many bioprocess samples cannot be transferred directly from a bioreactor to an analytical instrument. Cell-containing samples often require clarification, dilution, or other preparation steps before analysis. If this sample preparation remains manual, it can become a bottleneck in Process Analytical Technology workflows and can limit the value of automated sampling.

Numera®'s Tape Filtration Unit (TFU) was developed to automate the preparation of cell-containing bioprocess samples. The system was evaluated and optimized through a series of studies using mammalian CHO cells and microbial yeast samples. These studies investigated the filtrate volume, cell damage, cell removal, and dilution in relation to the filter pore size, pressure, pump speed, circulation time, loop volume, and post-cleaning drying parameters, as well as system performance during repeated filtration.

The results show that Numera®'s filtration technology can generate analysis-ready filtrate under defined operating conditions. Under verified CHO test conditions, 99.9% cell removal efficiency was achieved. Repeated system testing included 500 filtration cycles using yeast (50 g/L dry cell weight) and CHO samples at approximately 25 million cells/mL. Filtration parameters were optimized for mammalian applications, and additional work demonstrated parameter optimization for yeast samples at various biomass concentrations.

The evidence supports Numera® as an automated filtration platform for defined mammalian and microbial bioprocess applications, while also showing that filtration performance is application-dependent and should be matched to sample type, biomass, analytical requirements, and operating conditions.

SAMPLE PREPARATION CHALLENGE

Automated sampling is a key enabler of data-rich bioprocessing, but collecting a sample is only the first step. Many analytical instruments require a defined sample condition. Samples may need to be cell-free, diluted, clarified, or transferred without excessive delay. If the preparation step remains manual, the overall workflow remains partially dependent on operator availability and manual consistency.

Academic literature has identified sample preparation and sample transfer as important remaining gaps in automated bioprocess monitoring [1]. This is particularly relevant for PAT workflows, where the goal is to generate timely, reliable data for process understanding and control.

The challenge is especially pronounced in cell-containing samples. Mammalian and microbial cultures may contain high cell densities, cell debris, extracellular products, proteins, metabolites, and matrix components. These can interfere with downstream analyzers, clog fluidic paths, or bias analytical results. For this reason, clarification or filtration is often required before analysis.

Filtration-based sample preparation must balance several competing requirements: sufficient filtrate volume,

effective cell removal, low dilution, low carryover, acceptable sample integrity, and reliable repeated operation. Filter pore size is one important parameter in this balance. Smaller pore sizes, such as 0.22 µm, may reduce debris in the filtrate but can also clog more quickly and reduce volumetric yield. Larger pore sizes, up to 1.0 µm, can still remove cells and may support higher filtrate volume with less clogging, but they allow more subcellular debris or particles to remain in the filtrate. Pressure, pump speed, circulation time, cleaning intensity, and drying settings must therefore be selected based on the sample type, analytical requirements, and the desired balance between filtrate quality and yield.

NUMERA®'S TAPE FILTRATION UNIT

Numera®'s tape filtration unit is designed to automate the filtration of bioprocess samples within the sampling workflow. The system uses filter tape and controlled fluid-handling parameters to separate cells from the liquid phase and generate filtrate for downstream storage or analysis.

Figure 1: Numera® 3 Filtration Module

A key feature of Securecell's Filtration Unit is the tangential flow across the filter membrane. Instead of relying only on direct flow through the membrane, the sample suspension is circulated within the filtration unit by a pump. This tangential movement helps control the interaction between the cell-containing suspension and the filter surface during filtration. Depending on the desired filtrate volume, two configurations are possible: one with a 2-mL loop and one with a 4-mL loop.

In addition to the circulation settings, compressed air can be used to create a pressure difference between the sample side and the filtrate side. This pressure difference supports and accelerates filtration. Both the applied pressure and the circulation speed and duration can be adjusted, allowing the filtration method to be adapted to the sample type and analytical requirements.

Another important design element is the filter membrane. The membrane is mounted on a roll and advances to provide a fresh filter section for each sample. This supports reproducible filtration conditions from sample to sample and reduces the risk that material retained from a previous filtration affects the next sample. Filters with different pore sizes are available, enabling the filtration setup to be selected according to the required balance between cell removal, debris retention, filtrate volume, and clogging risk.

The filtration workflow is therefore not treated as a fixed universal procedure. Instead, relevant parameters can be defined and optimized, including pressure, pump speed, circulation time, pore size, loop volume, and drying settings after cleaning.

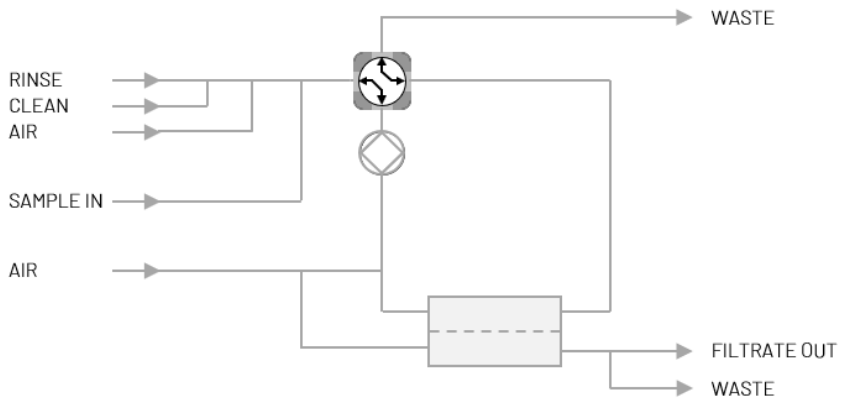


Figure 2: Simplified schematic representation of the Numera® 3 Tape Filtration Unit

The flexibility of the procedures is important because the optimal filtration strategy depends on the application. CHO cells and yeast cells differ in size, mechanical properties, biomass concentration, and process matrix. A filtration strategy that works for one sample type may not automatically transfer to another without adjustment. For this reason, Numera®'s filtration approach is designed to support application-specific parameter optimization rather than a single fixed filtration setting.

MAMMALIAN CELL FILTRATION PROCESS OPTIMIZATION

Studies focused on optimizing the filtration process for mammalian cell applications, particularly CHO cell cultures. The objective was to generate sufficient filtrate while minimizing cell damage. For the tested CHO application, the target was defined as a 50% filtrate yield, corresponding to 1 mL of filtrate from a 2 mL sample, with CHO cells at approximately 25 million cells/mL and a 2 mL loop configuration.

The studies evaluated three central filtration parameters: filtration pressure, circulation speed, and circulation time. Together, these parameters determine how the cell-containing suspension moves across the filter membrane, how strongly filtration is supported by the pressure difference between the suspension and filtrate side, and how long the cells are exposed to circulation through the pump and loop.

Cell integrity (or damage) was measured by lactate dehydrogenase (LDH) with Cobas c111. For each sample, the LDH difference (Delta) was calculated between the processed sample and the reference sample.

$$\Delta\text{LDH} = LDH\ cc_{\ processed\ sample} - LDH\ cc_{\ refernce\ sample}$$

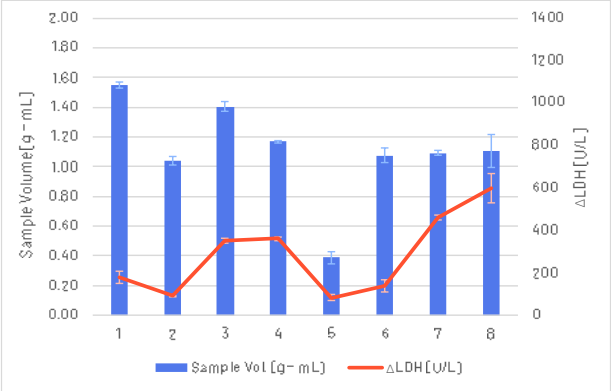
Sample volume was measured gravimetrically (g = mL).

Table 1: Parameter combinations for the tests based on preliminary tests and an experimental design plan

Test #	Pressure [bar]	Circulation Time [sec]	Pump Speed [rpm]
1	0.1	112	30
2	0.1	107	20
3	0.3	120	20
4	1.2	30	152
5	0.1	30	106
6	0.1	96	20
7	0.85	30	174
8	0.1	120	70

Table 2: Results for cell damage (ΔLDH) and yield (sample volume)

Test #	ΔLDH [U/L]	Error	Sample Vol [g = mL]	Error
1	178.2	28.7	1.55	0.02
2	90.8	3.1	1.04	0.03
3	352.5	10.8	1.40	0.03
4	361.3	7.2	1.17	0.01
5	83.4	14.2	0.39	0.04
6	136.3	28.1	1.08	0.05
7	458.9	12.6	1.09	0.02
8	597.0	70.1	1.10	0.11



For CHO cultures at approximately 25 million cells/mL, the final tests identified suitable settings for filtration pressure, circulation speed, and circulation time. These settings enabled the system to reach the defined filtrate yield target under the tested conditions while limiting cell damage.

The studies also showed that cell density has a direct influence on filtration behavior and sample integrity. In particular, circulation time must be adapted to cell density. If the sample is circulated for too long, cells remain exposed to mechanical stress while passing repeatedly through the pump and loop. This can increase cell damage and lead to the release of intracellular components such as LDH. The report therefore indicated that a dynamic circulation strategy should be implemented to minimize cell damage across varying cell densities.

These results demonstrate that Numera®'s TFU can be systematically optimized for mammalian cell filtration. Rather than applying one fixed filtration method to all samples, the process can be adjusted to the specific cell density and application requirements. For CHO cultures under the tested conditions, the optimized parameter set provided the basis for generating analysis-ready filtrate from cell-containing samples while maintaining a controlled balance between filtrate yield and sample integrity.

YEAST FILTRATION APPLICATION TESTING

Microbial and yeast processes introduce additional filtration challenges because biomass concentrations can be high and sample matrices can differ substantially from mammalian cell culture. Numera®'s TFU was therefore also evaluated for yeast applications to determine whether the filtration concept could be adapted to high-biomass microbial samples.

The objective of the yeast study was to maximize filtrate yield while minimizing process time. The defined performance target was to recover 75% of the input volume using 0.45 µm filter tape for both 2 mL and 4 mL loop configurations. The test covered yeast cultures with approximately 60 g/L DCW for the 2 mL loop, and approximately 33 g/L DCW for the 4 mL loop.

Optimization focused on filtration pressure, circulation pump speed, circulation time, and loop volume. The results showed that circulation speed influenced filtration performance differently depending on the loop configuration. For the 4 mL loop, lower circulation pump speeds produced more filtrate than higher pump speeds. For the 2 mL loop, circulation speed had less impact on filtrate yield. Based on these findings, a circulation speed of 20% was recommended, consistent with the strategy used for mammalian cell cultures.

With circulation speed fixed, suitable circulation times were then determined to reach the 75% yield target. The results showed a clear trade-off between filtrate yield, biomass concentration, and process time, particularly for the 4 mL loop. Higher biomass concentrations generally required longer circulation times, while lower concentrations allowed the defined yield to be reached faster.

Under ideal test conditions, using fresh culture with minimal debris, the practical limits for reaching 75% yield were approximately 60 g/L DCW for the 2 mL loop and approximately 30 g/L DCW for the 4 mL loop. At lower biomass concentrations, shorter circulation times could achieve comparable or higher yields, similar to the behavior observed in mammalian cell filtration.

Table 3: Filtrate volume vs. circulation time for the 2 mL loop setup

Circulation Time [s]	Filtrate Volume [mL]	Error
30	1.10	0.04
50	1.25	0.04
75	1.40	0.06
100	1.50	0.07
120	1.53	0.06

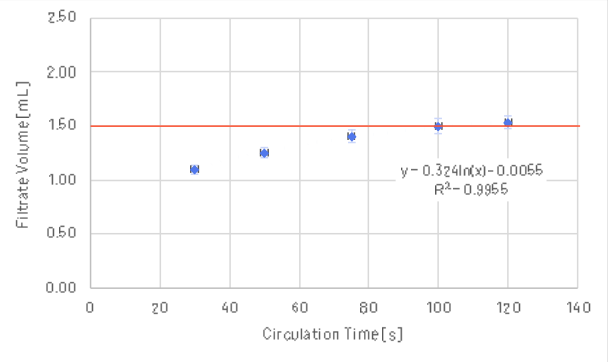
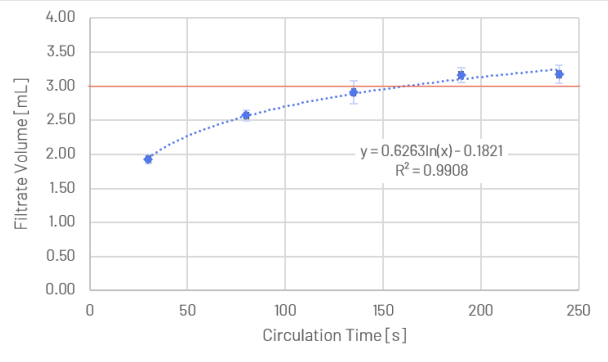


Table 4: Filtrate volume vs. circulation time for the 4 mL loop setup

Circulation Time [s]	Filtrate Volume [mL]	Error
30	1.93	0.06
80	2.57	0.08
135	2.91	0.17
190	3.16	0.10
240	3.18	0.13



These findings broaden the relevance of Numera®'s filtration technology beyond mammalian cell culture. They show that the TFU can be adapted to microbial samples through parameter optimization, while also confirming that filtration performance depends strongly on biomass concentration, loop configuration, and process time. For yeast applications, the optimized parameter strategy provides a practical basis for generating filtrate from high-biomass cultures while balancing yield and processing speed.

DILUTION CONTROL AND DRYING OPTIMIZATION

Filtration workflows include cleaning and rinsing steps, which may leave residual liquid in the system. If it is not properly managed, this residual liquid can dilute subsequent samples and affect analytical accuracy.

The TFU drying optimization study addressed this issue by optimizing drying parameters after cleaning. The objective was to reduce sample dilution effects due to residual rinsing solution remaining in the flow paths after cleaning to a threshold of a maximum of 2.5%. The optimized settings reduced dilution bias in both 2 mL and 4 mL loop configurations. Final recommended drying settings were defined based on pressure and drying time parameters.

The tests were performed using a glucose solution with a concentration of approximately 3 g/L. Glucose concentrations were measured using a Cobas c111 analyzer.

Bias calculation: $\bar{x}\ \text{bias} = \frac{Glc_{processed\ sample} - Glc_{ref}}{Glc_{ref}} * 100\,[\,\%\,]$

Table 5: Average bias among 15 samples with optimized drying parameter settings for the 2 mL and 4 mL configurations.

		2 mL Loop		4 mL Loop	
Reference sample	$c(Glc_{ref})$	3.070 g/L	± 0.01 g/L	3.062 g/L	± 0.01 g/L
Av. Value Processed Sample	$c(Glc_{procees\ sample})$	3.002 g/L	± 0.01 g/L	3.018 g/L	± 0.01 g/L
Bias		**-2.26%**	**± 0.32%**	**- 1.37%**	**± 0.47%**

This result is relevant because dilution directly affects calculated analyte concentrations. A filtration system that removes cells but introduces uncontrolled dilution would not be suitable for quantitative analytical workflows. Numera®'s optimization works therefore addressed not only cell removal, but also the analytical quality of the prepared sample.

REPEATED FILTRATION AND CELL REMOVAL

The main Numera® 3 system verification provides the strongest system-level evidence for filtration performance. Under defined CHO test conditions, the system achieved 99.9% cell removal efficiency, demonstrating its ability to generate clarified filtrate from cell-containing mammalian culture samples.

Repeated filtration performance was also verified under demanding test conditions. The system completed 500 filtration cycles using both high-biomass yeast samples at 50 g/L DCW and CHO samples at approximately 25 million cells/mL, with a 2 mL sample loop volume and 0.45 µm filter pore size. These tests demonstrate that filtration performance was evaluated not only as a single operation, but as a repeated automated workflow under biologically relevant sample conditions.

Filter tape capacity was also assessed. The system requirement of 250 filtrations with one filter tape coil was reached, and the results indicate that up to 375 filtrations can be achieved depending on the application and operating conditions.

These results are significant because they evaluate filtration as a repeated automated system function rather than as a single isolated event. For real-world use, repeated operation is essential. A useful automated filtration system must not only work once; it must perform reliably over many cycles.

APPLICATION SCOPE AND PARAMETER OPTIMIZATION

The filtration results presented in this white paper apply to defined sample types, cell densities, system configurations, filter pore sizes, loop volumes, pressure settings, pump speeds, and circulation times. Performance may differ with other cell types, organisms, media compositions, biomass concentrations, analytes, tubing configurations, and analytical requirements.

An important learning from the validation work was that filtration parameters optimized on an isolated TFU test stand did not transfer directly to the final Numera® 3 system without adjustment. Additional subsystem testing, followed by final integration optimization and testing, was required to define suitable parameters for the complete automated sampling workflow. This is important because sample quality depends on the entire fluidic path, including sample withdrawal, circulation, filtration, transfer, and push-back steps.

Filtration performance also involves an application-specific balance between filtrate yield, process time, and sample integrity. Higher filtrate yields can often be achieved, but typically at the cost of longer processing time. For this reason, the optimal filtration strategy should be defined according to the actual analytical goal. In some workflows, maximum filtrate volume may be the priority. In others, shorter cycle time, lower dilution, reduced cell damage, or faster availability of analytical data may be more important.

Cell concentration is another important parameter. The studies showed that filtration settings, especially circulation time, need to be adapted according to cell density. If cells are circulated for too long through the pump and loop, mechanical stress may increase and lead to higher cell damage. At present, this adjustment is not fully automatic. The user needs to define or update the relevant filtration parameters either directly in

Numera® or via Lucullus®, depending on the system configuration and process requirements.

For new applications, Securecell recommends an application-specific assessment of filtration parameters. This ensures that filtrate yield, cell removal, dilution, cycle time, and sample integrity are aligned with the intended analytical workflow.

CONCLUSION

Numera®'s filtration technology addresses one of the central challenges in automated bioprocess sampling: transforming cell-containing process samples into analysis-ready filtrate. Across mammalian and microbial studies, the TFU was characterized, optimized, and verified under defined operating conditions.

The verification and validation results demonstrate effective cell removal under CHO test conditions, repeated filtration over hundreds of cycles, optimized filtration parameters for mammalian and yeast applications, and controlled dilution after cleaning-related drying optimization. The studies also showed that filtration performance depends on the biological sample, biomass concentration, loop configuration, pore size, pressure, circulation speed, and circulation time.

These findings support the use of Numera® as an automated filtration platform for defined bioprocess sampling applications. At the same time, they highlight an important principle for reliable implementation: filtration parameters should be selected according to the intended analytical goal and adjusted to the specific process conditions. By integrating filtration into the automated sampling workflow, Numera® helps reduce manual sample preparation and supports more consistent, data-rich bioprocess monitoring.

CONTACT

The results presented in this white paper are part of Securecell's broader verification and validation activities for the Numera® automated bioprocess sampling system. Numera® is designed to support reliable, automated sampling, sample preparation, and integration with analytical workflows in modern bioprocess development.

To learn more about Numera® and its role in automated bioprocess sampling, visit: www.securecell.ch/numera

For application-specific questions, technical discussions, or to evaluate how Numera could support your bioprocess workflow, please contact the Securecell sales team: www.securecell.ch/contact

Our team will be happy to discuss your process requirements, sampling strategy, analytical setup, and suitable Numera configuration.

Securecell AG, In der Luberzen 29, 8902 Urdorf, Switzerland

References

[1] Hofer et al., A Reliable Automated Sampling System for On-Line and Real-Time Monitoring of CHO Cultures,

RELATED PRODUCTS AND ADDITIONAL INFORMATION

Order Information

FILTER COILS FOR NUMERA® 3	PART NO.
Filter tape 0.22 µm PES Membrane* on coil	006688
Filter tape 0.45 µm PES Membrane* on coil	006689
Filter tape 1.20 µm PES Membrane* on coil	006690
Filter coil without filter tape	006691

Filter Tape is available on coils containing 30 m (~100 ft).

*Note: PES (Polyethersulfone) is a hydrophobic membrane, designed to remove particulates during filtration, and its low protein and drug binding characteristics make it ideally suited for life science applications.

Technical Specification

A technical datasheet covering available filter materials and their technical specifications, such as pore size, flow rate, and chemical resistance, is available to download in our Knowledge Base at help.securecell.ch