

Optimizing Cell Harvest with Inline Particle Analysis



METTLER TOLEDO

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This application note identifies common harvest challenges resulting from intensification of upstream cell culture processes and shows how inline measurement and characterization can be leveraged to optimize harvest operations.

Case studies drawn from industry partnerships demonstrate how inline particle analysis technology provided by METTLER TOLEDO has been used in this regard, streamlining development and minimizing potential rework downstream.

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The Harvest Bottleneck

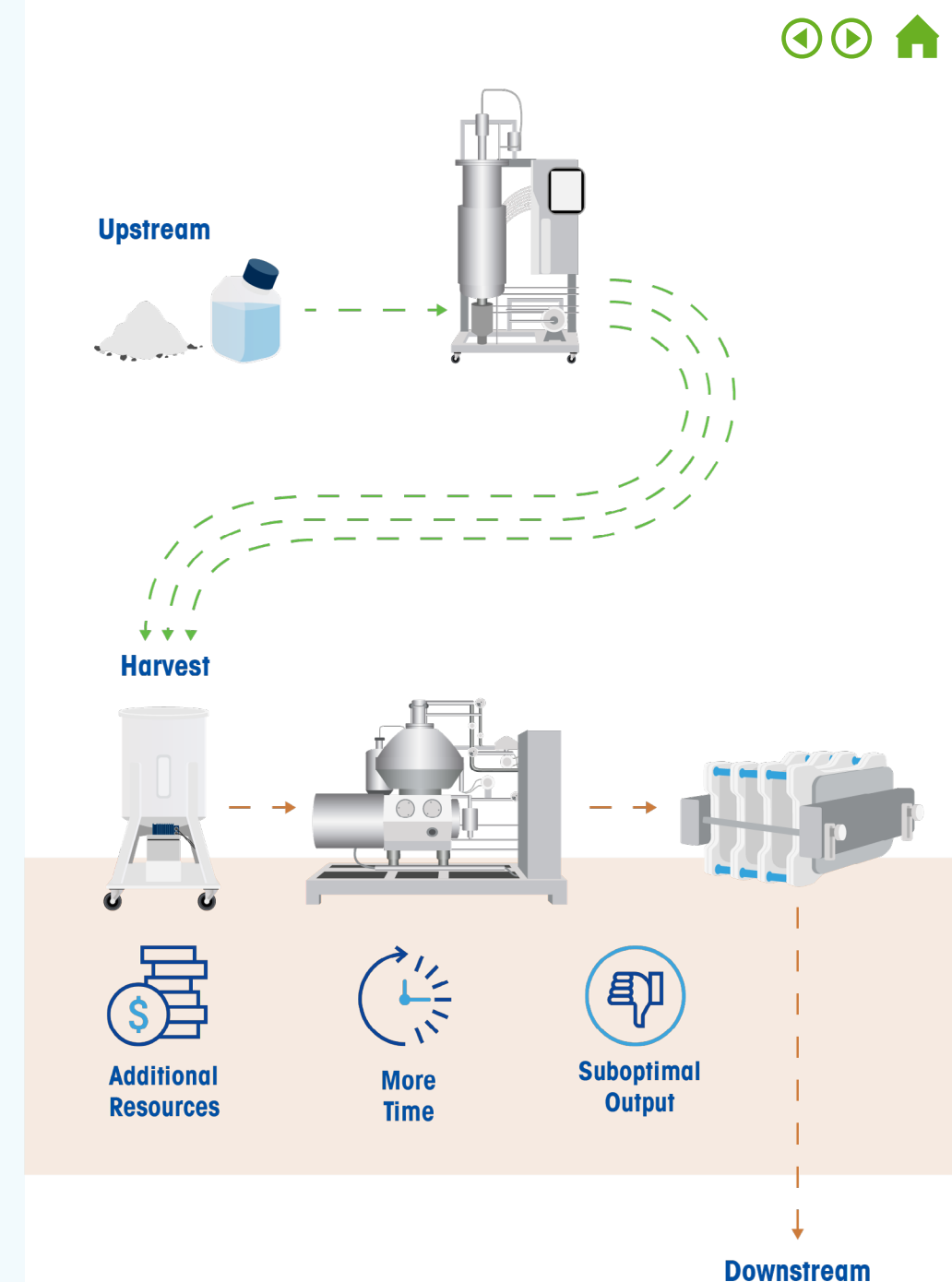
An Industry-Wide Problem

Cell harvest is a crucial unit operation linking upstream and downstream bioprocessing. Harvest operations such as pre-treatment, centrifugation, and filtration mark the first step in separating any biotherapeutic from the medium in which it is grown. The results of this step therefore impact all future development and purification processes.

Recent advancements in upstream processing, derived from cell line development, fed-batch, and perfusion bioreactors, have significantly increased the productivity of fermentation and cell culture processes. While these developments have enabled the production of increasingly complex and specialized biotherapeutics, they have also applied new pressures to harvest and subsequent downstream unit operations. Isolating and purifying the increased volume and variability of bioreactor output often takes more time and resources than expected to ensure that suboptimal material is not passed to downstream operations. The result is an industry-wide bottleneck, beginning at harvest.¹

Modifications to centrifuge design, pre-treatment technologies, and depth filtration format and media have been developed to improve mechanical separation and attempt to overcome the bottleneck.² However, selecting the appropriate tool or method is time-consuming and no single approach can address batch-to-batch variability effectively. Additional insight into the material being processed is needed.

1. Challener, C. (2020). Cell harvesting steps separate the good from the bad. BioPharm International, 33(10), 28–30. <https://www.biopharminternational.com/view/cell-harvesting-steps-separate-the-good-from-the-bad>
2. Dryden, W. A., Larsen, L. M., Britt, D. W., & Smith, M. T. (2021). Technical and Economic Considerations of Cell Culture Harvest and Clarification Technologies. Biochemical Engineering Journal, 167. <https://doi.org/10.1016/j.bej.2020.107892>



Common Harvest Challenges

The Need for Insight

Common harvest challenges resulting from the increased biomass and complexity of modern bioreactor output continue to arise. Often, these challenges compound. For example, variability between batches may result in debris escaping into the centrate for one batch, but not another. If enough escape over time, depth filters may become clogged.

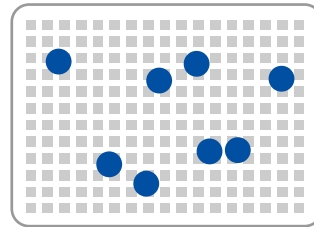
Traditional measurement tools typically do not provide detailed or accurate enough information to overcome these challenges. Techniques like inline turbidity correlate to mass, but offer no insight into particle system composition, size, shape, or count. Other techniques like laser diffraction require sonification and dilution, which can alter the sample. Inline measurement and characterization of particles, as they exist in process, is needed to detect potential issues and address problems before they happen.



Inconsistent Upstream Material

Variation of parameters during cell culture processes creates variability in cell mass, cell viability, behavior, and drug substance concentration

Impact: Difficult to optimize pre-treatments, centrifugation conditions, and filter capacity



Incomplete Recovery

Drug substance left behind in organism debris or on filters

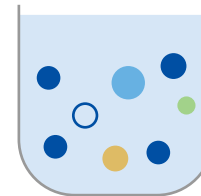
Impact: Reduced harvest and final product yield



Incomplete Separation

Drug substance is not fully separated from other biologic material

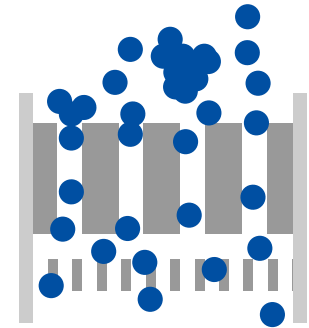
Impact: Lower yield and purity, and often requires additional processing steps



Particle Discharge into Centrate

Impurities or excess debris escape into centrate

Impact: Additional purification or processing to maintain product quality



Clogged Depth Filters

Adding filters for redundant capacity or replacing filters due to clogging

Impact: Increased space requirements, production time, and cost

Solution: Inline Particle Analysis For Real-Time Process Insight

Many common challenges can be solved by integrating existing technologies capable of characterizing complex particle systems as they exist in process. Inline particle analyzers like EasyViewer and ParticleTrack enable automated, real-time data capture and analysis of particle size, shape, and count, dramatically improving the ability to understand, optimize, and control particle systems for efficient and effective harvest.

The following case studies highlight recent examples that show how in-depth particle system insight provided by METTLER TOLEDO technologies has been used to improve process understanding, optimize pre-treatment steps, and shorten process development time, resulting in more robust harvest operations.



ParticleTrack™ with FBRM® technology is a probe-based instrument that is inserted directly into the process to track changing particle size and count in real time, at full process concentrations. Particles, particle structures, and droplets are monitored continuously as experimental conditions vary, providing scientists with the evidence required to deliver consistent particles with the required attributes.

EasyViewer™ is a powerful, inline, probe-based imaging tool that captures high-resolution images of cells, debris, agglomerates, bubbles, droplets and other particles as they exist in process. Advanced image analysis enables the monitoring of process changes and quantification of particle size and count in real time.

Case Study One

Optimized Acidification Improves Filtration

Design a scaled-down acid pre-treatment method to precipitate soluble cell by-products and improve filtration, thereby minimizing costs and speeding time to market.

Situation: Centrifugation and filtration failed to fully separate DNA and host cell proteins from an antibody in cell broth.

Problem: Clogged depth filters, reduced chromatography column load capacity, and an overall decrease in process efficiency.

Limitation: Existing tools used to characterize the pre-treatment process required offline analysis, which wasted valuable product, and lacked detailed particle morphology data needed to optimize acidification parameters for efficient filtration at scale.

Potential Implications:

- Wide safety margins during filtration increase membrane cost and processing time
- Filter failure or inefficiency leads to lost time, reduced capacity, and can negatively affect quality attributes
- Reduced chromatography capacity requires additional resin, increasing cost as well as operational and analysis time

Solution and Benefits

Researchers developed a scaled down, real-time, and in-situ method to optimize acidification using particle analysis probes. EasyViewer and ParticleTrack were used to image and measure particle size distribution over the entirety of an acidification reaction carried out at 400 mL scale. pH was also monitored.



Confirm impurity precipitation in real time



Identify ideal point for transfer to filtration, without sampling



Increased operational capacity and consistency of filtration and chromatography



Save valuable time and resources



Maximize recovery and increase overall process efficiency

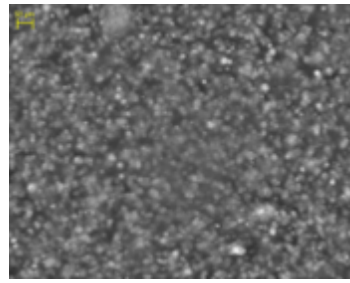
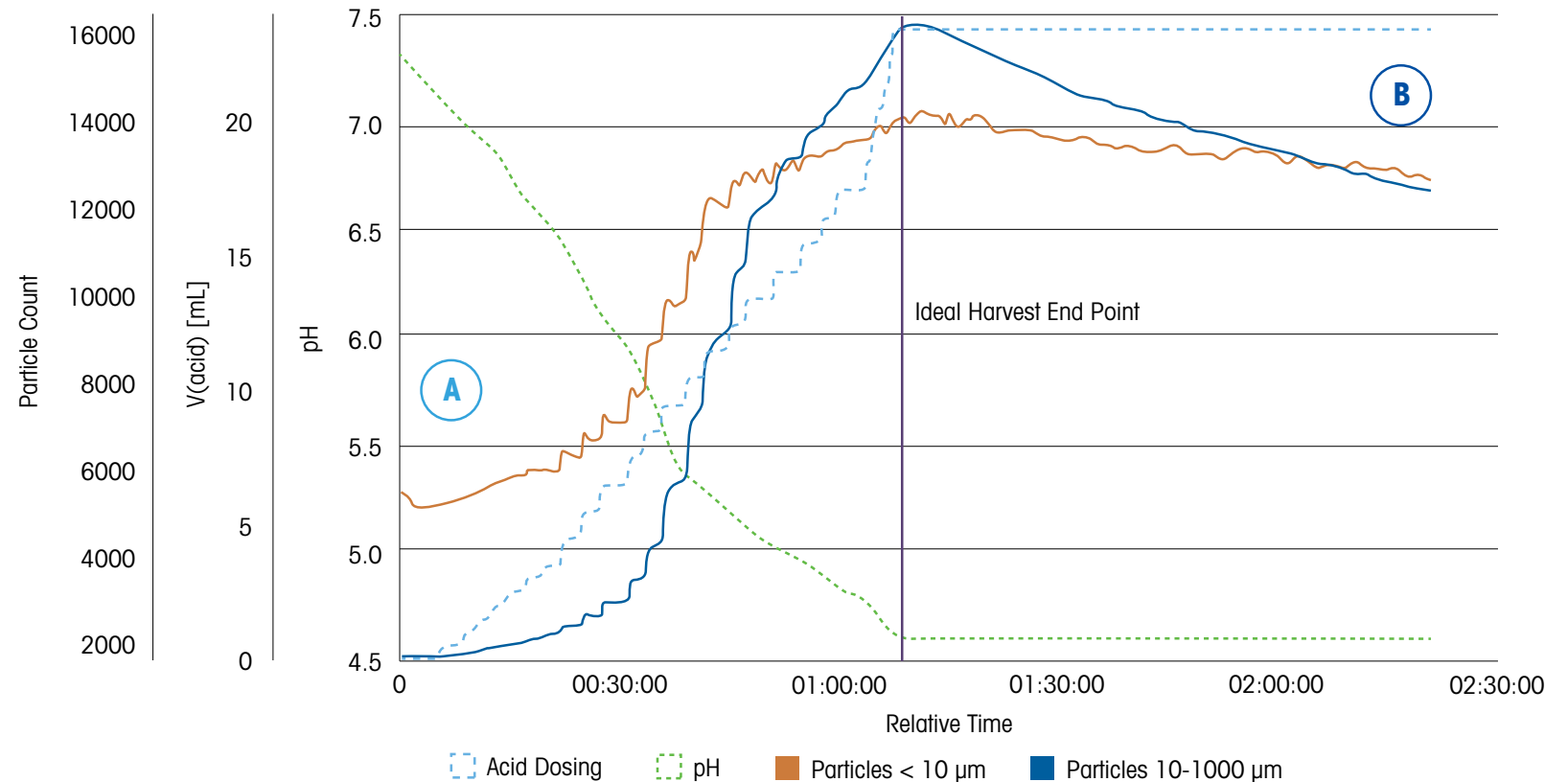
Case Study One

Key Findings & Observations

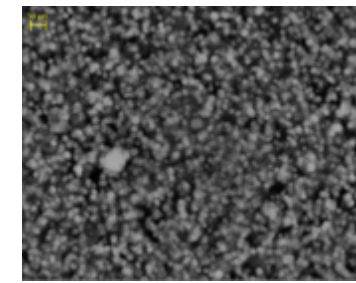
Acidification Optimization

Determine ideal acidification conditions for effective impurity filtration

- **Visual comparison:** Clear morphological differences in impurity precipitation at different reaction points (Image A vs. B)
- **Process understanding:** Image B correlated with reduced impurities in recovered product
- **Identify operating range:** Major precipitation occurs between pH 6.5 – 5.2
- **Detect harvest endpoint:** No major precipitation below pH 4.8; missed by offline techniques
- **Identify “degradation”:** Particles begin to degrade over time after dosing and continued mixing; indicating process is sensitive to hold time



A Raw Cell-broth



B Final Acidified Product

Case Study Two

Optimized RNA Recovery

Develop a method to optimize RNA precipitation for recovery from filters through measurement of particle morphology as a function of processing parameters, minimizing product and resource loss.

Situation: RNA isolation and purification are difficult due to RNA's tendency to degrade, form complex structures, and adhere to surfaces. Chromatography is expensive and time consuming. Therefore, an alternative method using RNA precipitation and capture on filters followed by dislodging and re-solubilization was studied.

Problem: Particle attributes and interactions with filters during RNA precipitation were believed to cause repeated process failures.

Limitation: Existing particle characterization tools did not provide insight necessary to identify cause or predict failure.

Potential Implications:

- Batches are frequently lost due to precipitated RNA lost on infrastructure
- Particle mischaracterization using existing methods slows development and increases time to market
- Reverting to chromatography costs up to 12 times more than filtration due to resin cost

Solution and Benefits

Researchers used EasyViewer and ParticleTrack to image and characterize RNA precipitants and flocs inline during a series of capture and re-solubilization experiments. Several parameters were varied and correlated with other properties such as sedimentation, filter capacity, and efficiency of re-solubilization. Data collected confirmed failure due to increasing fines and diffuse particle morphology. Insights gained enabled prediction of poor recovery and provided critical information for optimizing scale-up of efficient RNA recovery.



Optimize precipitation enabling filtration as a viable alternative to chromatography



Increase efficiency and retain yield



Speed development and time to market



Reduce potential cost by up to 12x compared to chromatography method

Case Study Two

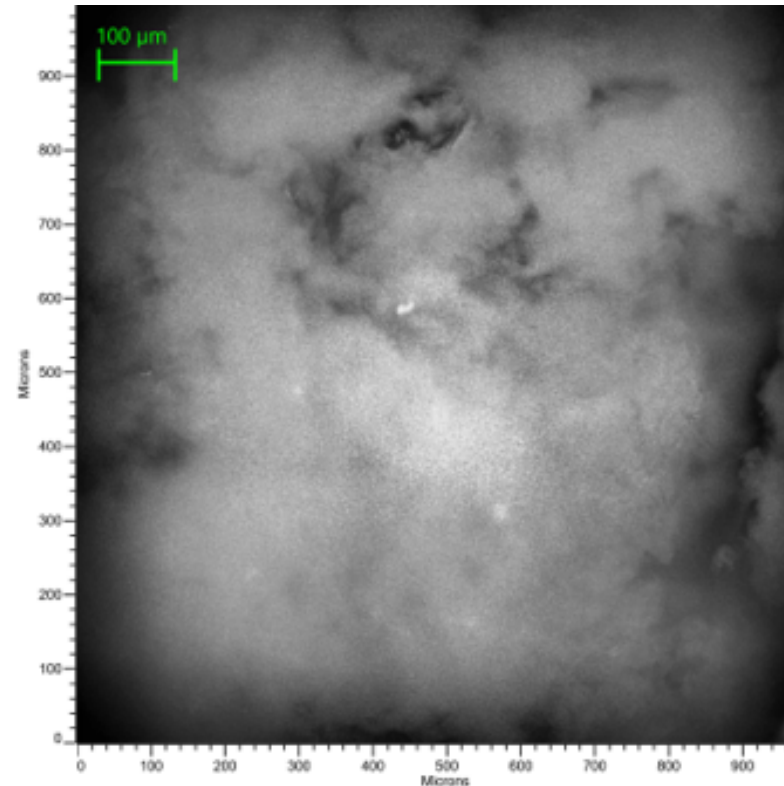
Key Findings & Observations

RNA Alcohol-Precipitation

Gain insight into particle morphology to maximize recovery

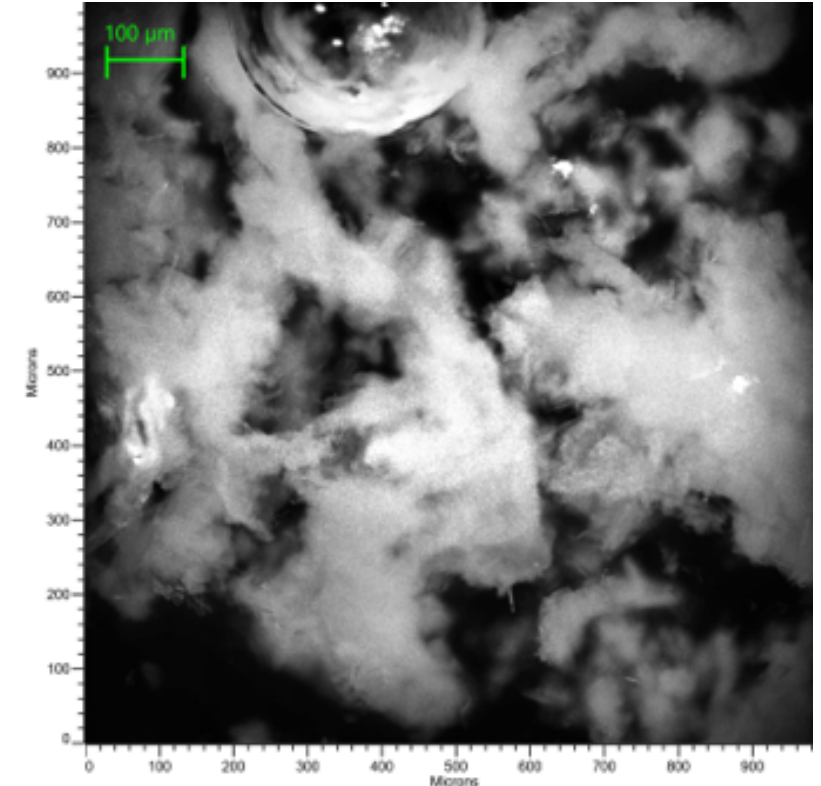
- **Visual comparison:** Varied conditions resulted in starkly different particle system appearance (Image A vs. B). Pixel intensity corresponds to size and density of particulate matter. Pure solution appears black while dense flocs, which reflect more light, appear white. Large areas of gray correspond to dispersed clouds of small particles with little separation between solution and aggregates.
- **Correlate to process outcome:** Poor recovery correlated to particle size, density, morphology, and count. Dense, larger flocs (Image B) released more easily from filters.

A: Recovers Poorly



System characterized by a large cloud of small particles adhered to filters, resulting in a lost batch

B: Recovers Well



System characterized by dense, large flocs with clear separation released well from filters

Case Study Three

Optimized Centrifugation for Filtration

Dynamic control of centrifugation parameters for improved depth-filtration, speeding processing time and enabling early detection of conditions leading to filter blockage.

Situation: Variable bioreactor output made optimization of centrifugation parameters difficult.

Problem: Excessive particle escape overburdened depth filters, leading to eventual blockage and/or reduced capacity downstream.

Limitation: Turbidity and offline analysis methods did not provide the type or speed of information needed to characterize the effect of centrifugation parameters on centrate output.

Potential Implications:

- Additional screening runs; increased cost and processing time due to oversized filters
- Filter failure or inefficiency leads to lost time, reduced capacity, and can negatively affect quality attributes
- Reduced chromatography capacity requires additional resin, increasing cost as well as operational and analysis time
- Incorrect process controls increase process failures and waste development time

Solution and Benefits

Bioprocess engineers used EasyViewer and ParticleTrack inline at the centrate outlet to image and characterize particle size and count as a function of flow rate and bowl speed. Five conditions were evaluated for total processing time, ability to remove excess particles, and effect of filter capacity on performance.



Enable early detection of potential issues and dynamic control of centrifuge output



Increase operational capacity and consistency of filtration and chromatography



Reduce filter and resin costs



Inline particle size distribution and counts allow researchers to run fewer small-scale filter studies, reducing project time and resources



Mitigate stability issues such as sheer; improve product quality

Case Study Three

Key Findings & Observations

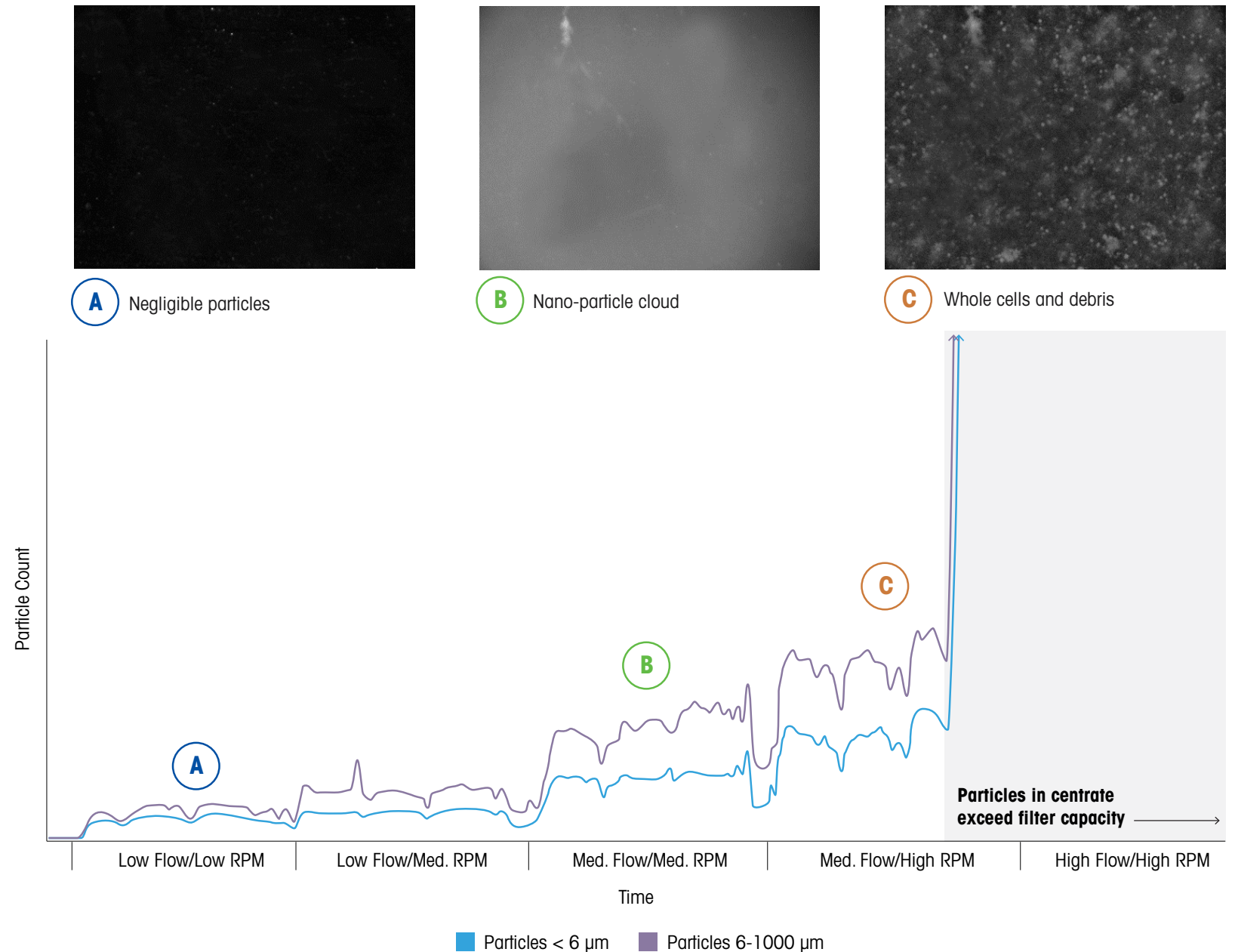
Centrifugation and Depth Filtration DoE

Characterize centrate particle system as a function of centrifugation conditions

Feed flow = 0.25 – 0.4 L/min;

Speed = 8200 – 10500 RPM

- **Process understanding:** Particle count increases across all sizes with increased flow rate and RPM.
- **Granular insight:** Particle size and count continue to increase within condition after Med. Flow/Med. RPM.
- **Predict issue:** Med Flow/High RPM eventually exceeded bowl capacity, leading to exponential increase in particle count (C) that would likely block filters. Continued particle breakthrough at higher conditions determined to exceed limits of unit operation.



Additional Resources

Webinars and Guides for Bioprocess Optimization

Harvest Applications

Webinar: Examples of Inline Harvest Characterization Through Particle Analysis

Acidic precipitation of nucleic acids in cell culture broth is evaluated to improve filtration and pre-chromatography impurity clearance.

► [Click to Learn More](#)

Webinar: Centrifugation and Depth Filtration Process Development

Learn how GSK developed and optimized centrifugation and depth filtration processes for mAb purification.

► [Click to Learn More](#)

Webinar: Demonstration of Inline Particle Analysis PAT for Bioprocess Harvesting

How to expedite bioprocess development of cell-culture harvest, flocculation, and depth-filter sizing.

► [Click to Learn More](#)

Other Applications

Guide: Innovative Techniques for Downstream Bioprocessing

Learn how process analytical technology is empowering your peers to transform day-to-day workflows and make significant downstream process improvements.

► [Click to Learn More](#)

Guide: Biopharmaceutical Manufacturing

Discover how the right tools to monitor and understand your biological process, from process development to full-scale manufacturing, help optimize productivity while minimizing time-to-market.

► [Click to Learn More](#)

Webinar: Automated Metabolite Control for Bioprocess in Vaccine Manufacturing

Manual control of metabolites during fed-batch bioprocesses in stirred tanks is time consuming and often leads to poor bioprocess control and reduced yield.

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