



# CytoFLEX nano Flow Cytometer

SHIFT PERSPECTIVE, ACHIEVE MORE



ACCELERATING  
*answers*

 **BECKMAN  
COULTER**  
*Life Sciences*

# Breaking the Boundaries of Extracellular Vesicle Detection

Extracellular vesicle (EV) analysis is critical in many labs across the life sciences, yet it remains a challenging task. As EVs vary in their size, composition, and cell origin, discerning their heterogeneity helps researchers understand the role they play in physiological processes and disease. However, until now, there has been no single-particle method that offers the data depth required to reach meaningful conclusions.

Instead, as an EV researcher, you may often turn to using two, three and often more techniques to tell a cohesive story, which typically requires multiple hardware platforms that cannot be reliably standardized. As a result, there is a desire for an instrument that can support multiple orthogonal methods of measurement, creating continuity across datasets for both single-particle and bulk analysis.

Flow cytometry is a well-established technique that offers **high sensitivity and throughput**. Thanks to its ability to analyze individual particles, **flow cytometry is ideal for characterizing heterogeneous EVs**. But, if you're unfamiliar with flow cytometry, you need to understand how to interpret data and must be confident in the results. And crucially, current flow cytometers are not designed to detect and characterize EVs smaller than 100 nm — **until now**.

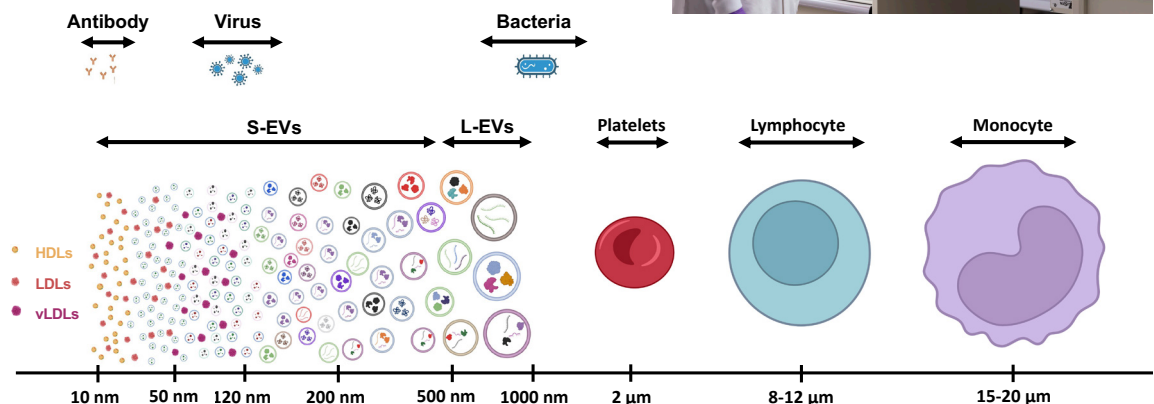
Meet the **CytoFLEX nano Flow Cytometer**. A pivotal development in flow cytometry, the CytoFLEX nano analyzer enables the analysis of **EVs at least as small as 40 nm\* with ease while simultaneously offering up to 6 separate fluorescent channels of detection**, to deliver full characterization.

With the CytoFLEX nano Flow Cytometer, you can expect:

- Increased **size sensitivity** from 1  $\mu\text{m}$  to at least 40 nm\*
- **Flexibility** for your experimental design with **6 fluorescent channels** and **5 side scatter channels**
- **Consistency of results** with **>90%** volumetric counting accuracy and **<1%** carryover between samples



**Figure 1:** The size of different cells and extracellular vesicles. The CytoFLEX nano Flow Cytometer can detect, characterize, and determine the size of EVs down to at least 40 nm\*.



Building on the strengths of the CytoFLEX Flow Cytometer Platform, we've continued to develop innovative fluidic and optic systems to enable you to **apply flow cytometry to the nanoscale with precision and sensitivity**.

\*Characterized using 40 nm polystyrene beads and triggering on violet side scatter.

# Nano Flow Cytometry: Sensitive, Flexible, Consistent

Specifically designed for the analysis of EVs at least as small as 40 nm, the CytoFLEX nano Flow Cytometer empowers you to stop using multiple techniques so helps save you from time-consuming and laborious workflows that have poor repeatability. Now, you can experience a **sensitive, consistent** and **flexible** solution to advance your research.

“The CytoFLEX nano analyzer sets a new standard as it does the work of nanoparticle tracking analysis (NTA), dynamic light scattering (DLS) and a flow cytometer all in one instrument.”

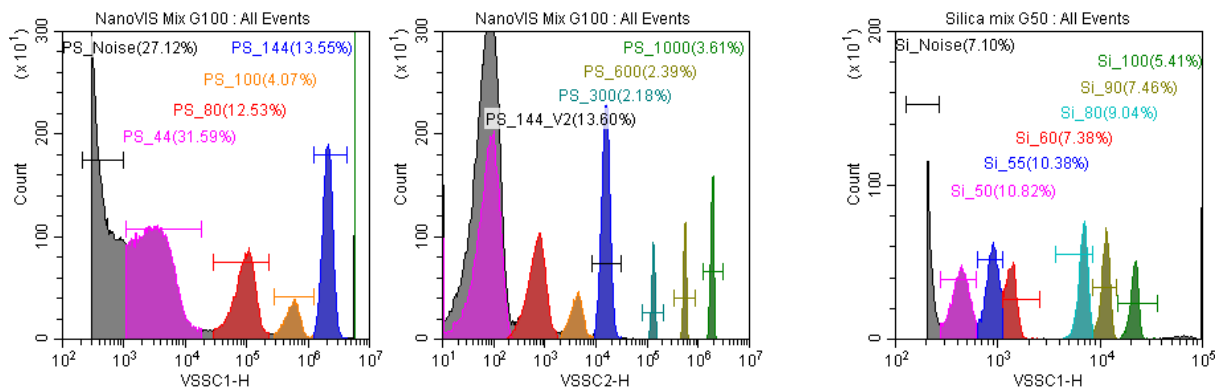
- **John Tigges, Director, Flow Cytometry Core Facility and Center for Extracellular Vesicle Detection, Beth Israel Deaconess Medical Center**

## Sensitive

### EXPLORE WHAT YOU COULDN'T BEFORE

With **unparalleled sensitivity** and **resolution** that enables the **characterization** of smaller EV populations in heterogeneous samples, the CytoFLEX nano Flow Cytometer delivers more insights from your sample. The increased sensitivity of the system opens up the possibilities of what you can study, including small EVs, protein complexes, lipids, and cholesterol.

What's more, the CytoFLEX nano analyzer's resolution **lowers the limit of detection** to distinguish both smaller EVs (as shown in Figure 2) and their low-abundance cargo. It allows you to resolve 10 nm variations to properly characterize polydisperse populations\* (shown in Figure 3). With the CytoFLEX nano Flow Cytometer, you can see individual markers for cargo and nucleic acids paired with scatter intensity and membrane associated fluorescence. Individual rare events are able to be differentially identified and characterized as a result.



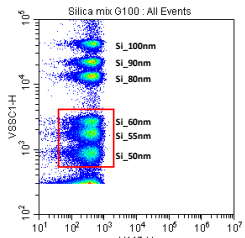
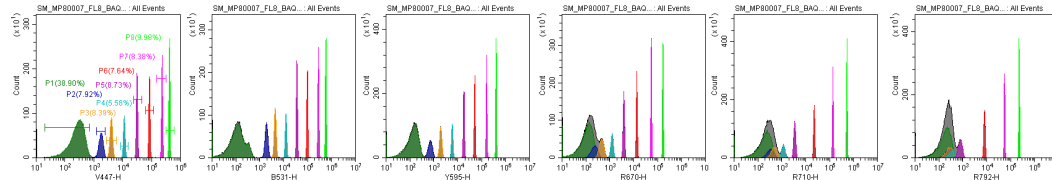
**Figure 2:** The resolution of the CytoFLEX nano Flow Cytometer enables the characterization of smaller EV populations in heterogeneous samples. Mixed sizes of polystyrene beads, including those as small as 40 nm, were detected using the CytoFLEX nano analyzer. These scatters demonstrate the exceptional resolution in sizing available with this flow cytometer.

“On an EV, you might have only 10 copies of an antigen, and with current flow cytometers we do not have the ability to measure those, so the main benefit of the CytoFLEX nano Flow Cytometer is that with its improved scatter and fluorescence sensitivity, you can measure much smaller particles and detect low density antigens.”

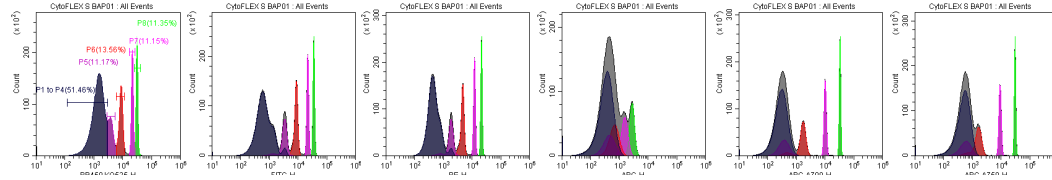
- **Erika Duggan, Research Associate at Cellarcus Biosciences**

\*as determined with silica beads less than 100 nm in diameter.

## CytoFLEX nano



## CytoFLEX S

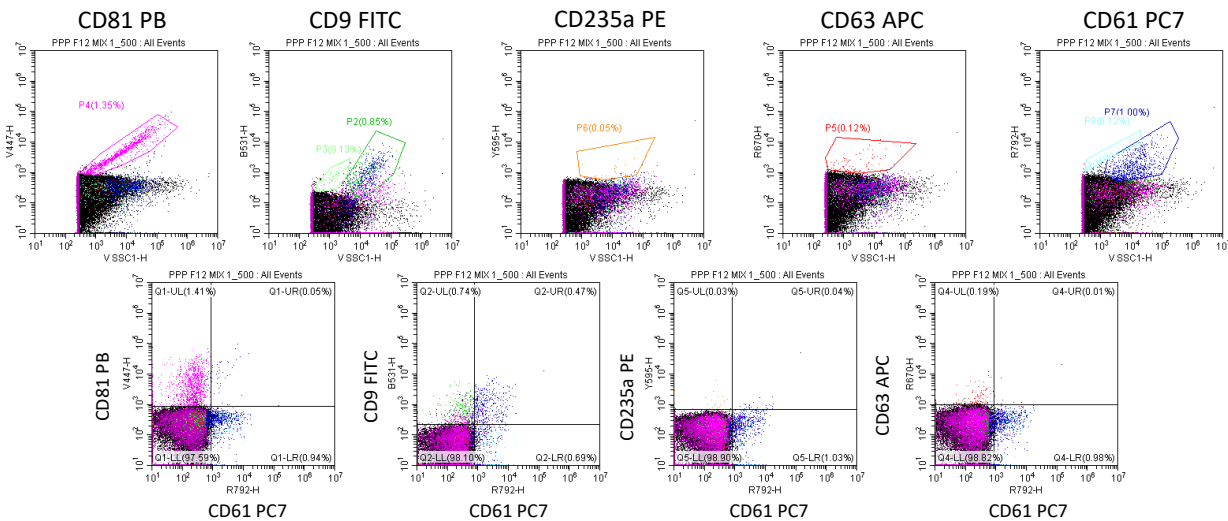


**Figure 3:** The sensitivity of the CytoFLEX nano Flow Cytometer can be used to resolve 8 peak multicolor multi-intensity beads to observe differences in protein and nucleic acid cargo by fluorescence. When compared to the CytoFLEX S Flow Cytometer, the CytoFLEX nano analyzer provides improved resolution at this size level. The scatter shows that it is possible to visualize 10 nm dynamic range separation as determined with silica beads less than 100 nm in diameter.

## Flexible

### YOUR EXPERIMENT, YOUR WAY

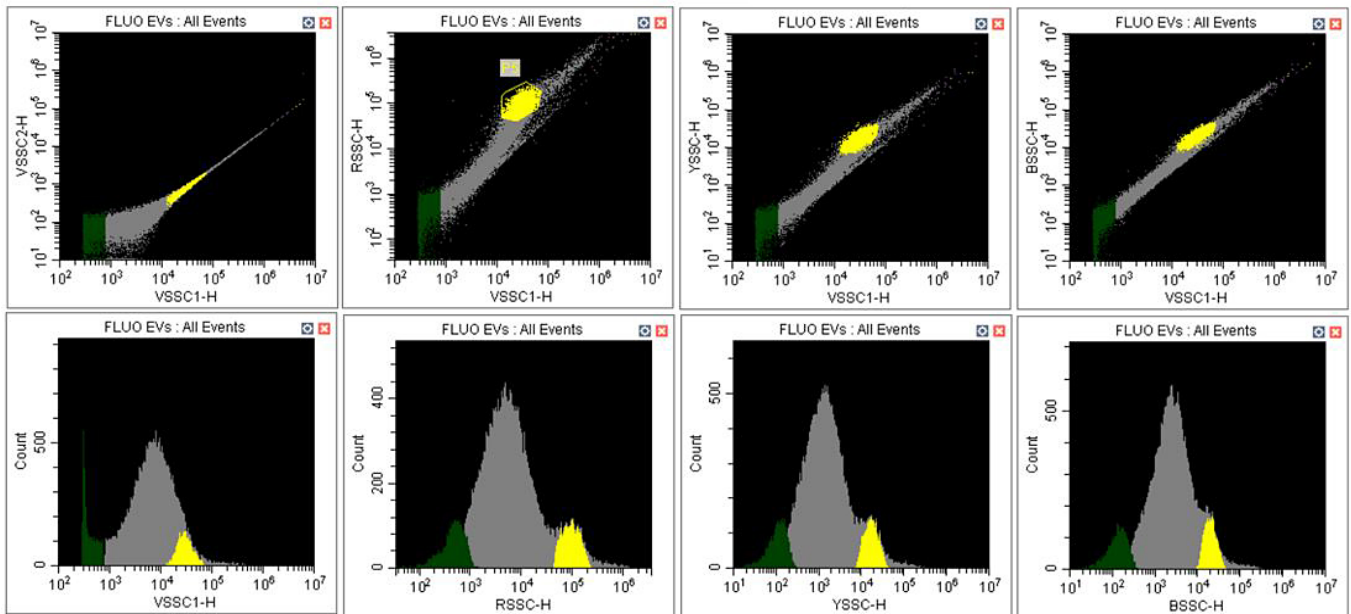
One of the primary advantages of employing the CytoFLEX nano Flow Cytometer for analyzing your extracellular vesicle (EV) populations is its ability to efficiently characterize smaller particle groups. It offers phenotyping capabilities using up to 6 channels, accommodating a large variety of dyes suitable for EVs. This feature enables you to design experiments more flexibly, allowing you to gather the necessary data with fewer constraints.



**Figure 4:** The multicolor phenotyping capacity of the CytoFLEX nano analyzer enables better data. Here, a 5-color panel staining was used, without interference by antibody aggregates. The CytoFLEX nano Flow Cytometer can detect and resolve a minimal platelet contamination (CD235a in PE), and a low percentage of plasma EV positive for CD63 in APC.

## OPENING UP EXPERIMENTAL POTENTIAL

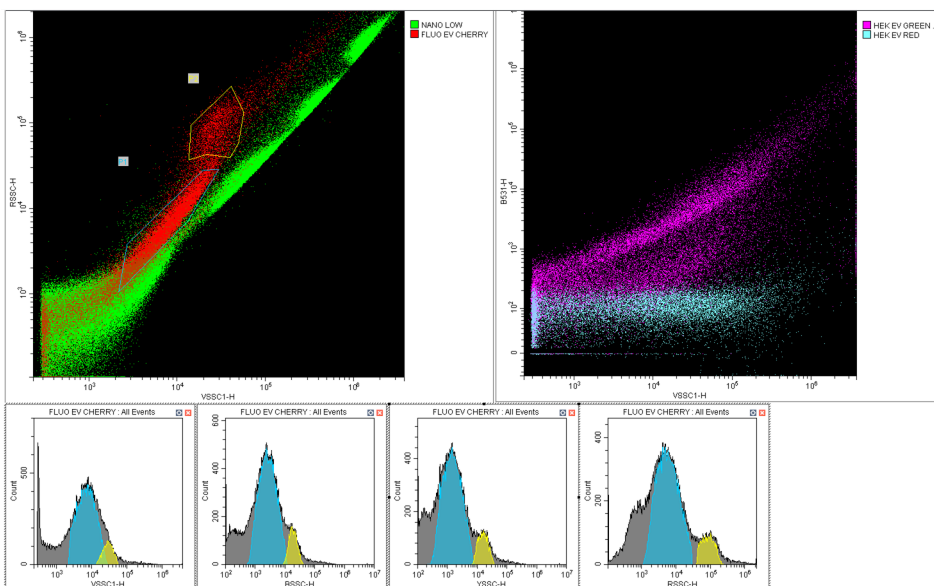
The CytoFLEX nano analyzer expands the possibilities of your research by offering 5 side scatter channels. Investigate new populations by analyzing the ratios of different scatters, without relying on dyes for identification or separation.



**Figure 5:** The CytoFLEX nano Flow Cytometer can separate populations that would not have been identifiable without the use of dyes and the violet side scatter alone, as illustrated by the plots above. Commercially available EVs were analyzed using the 5 different scatter colors to identify the different populations of EVs in the sample.

“Having all these scatters at hand together with the greater sensitivity gives you the power to ask, what could I do now? It opens up new research possibilities.”

– John Tigges, Director, Flow Cytometry Core Facility and Center for Extracellular Vesicle Detection, Beth Israel Deaconess Medical Center



**Figure 6:** With the CytoFLEX nano Flow Cytometer, different sizes of EVs in a heterogeneous sample can be easily identified. Commercially available rEVs (SAE0193, Sigma-Aldrich) were analyzed using two different scatter colors to identify the different sizes of EVs in the sample.

## Consistent

### CONSISTENT PERFORMANCE FOR RELIABLE, REPRODUCIBLE RESULTS

When you are working with particles so small, it is critical to minimize contamination to get reliable results. Instrument cleanup is therefore more important than ever. If you are a flow cytometry user, you know this can be a daunting and time-consuming task that often results in significant portions of downtime between samples.

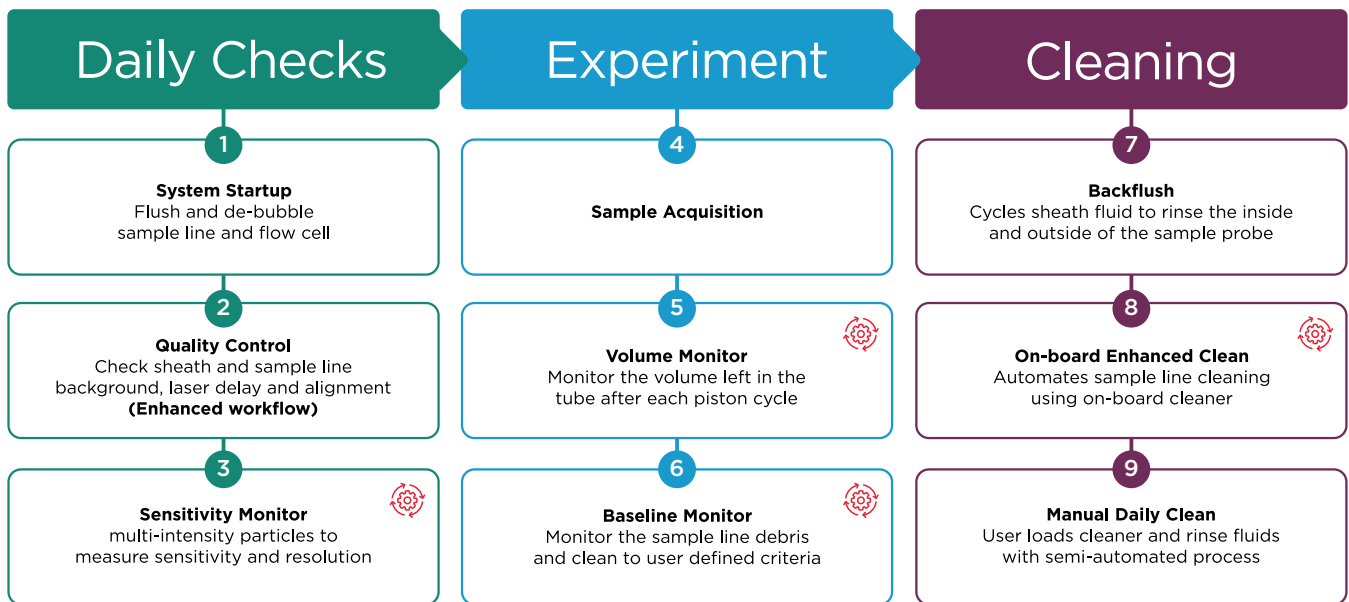
To help alleviate the cleanup burden and ensure reliable results, the CytoFLEX nano Flow Cytometer comes with **built-in, automated tools** that help you **identify and fix any instrument contamination**. These tools make sure you get **less than 1% carryover** from sample to sample, ensuring accurate and consistent counting results so you can trust your data. It also avoids clogging issues, keeping instrument performance consistent and minimizing instrument hands-on time and maintenance. Additionally, the high-precision, low-volume syringe pump in use with the instrument achieves 90% accuracy from sample to sample in determining volumetric counting.

“The instrument itself has in-built mechanisms to check what your background is like, washing and cleaning cycles that ensure that your instrument is clean, and different aspects to help you troubleshoot when there is noise in the system. All of these things provide a confidence level that you are now analyzing single EVs.”

- Vera Tang, Core Facility Manager,  
University of Ottawa

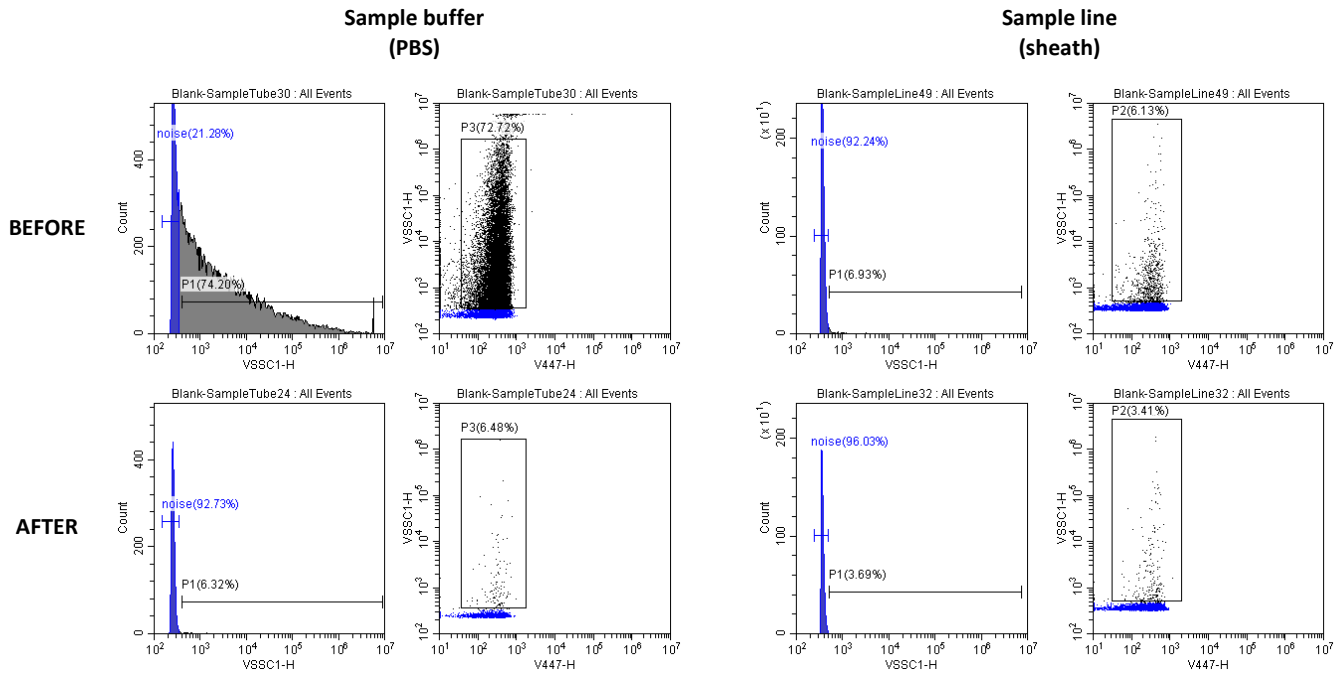
### MINIMAL CONTAMINATION, MAXIMUM CONSISTENCY

The CytoFLEX nano analyzer's built-in quality control (QC) system uses a three-step process: system, cleanliness, and sensitivity. This workflow contains several additional checks to ensure instrument consistency and data reliability. These checks are conveniently automated to save you time in front of the instrument.



 Automated in the CytoFLEX nano flow cytometer

There is access to automatic checks to assess the instrument's cleanliness using the sheath fluid by checking the background and creating a threshold to pass QC. Moreover, the Baseline Monitoring feature instantly checks the cleanliness of both the instrument and buffer while you run your experiment, saving significant time. But that's not all – conveniently, the CytoFLEX nano Flow Cytometer also allows you to quickly check the instrument's performance throughout the day in only a few minutes. Combined, these comprehensive checks make sure you obtain reliable, reproducible data from your precious samples.



**Figure 7:** The Baseline Monitor assesses the sample line and sheath line for residues, allowing constant awareness of potential contributors to sample readings. If the results of the Baseline Monitor do not meet expectations, it automatically triggers the selected cleaning workflow to return to baseline. The system re-assesses between each cleaning cycle and stops when acceptance criteria are met.

## QC Report

Scatterspheres Lot No.: 2692901	QC Date: 2024-01-16 09:44
Scatterspheres Expires: 2024-10-02	Cytometer SN: MP80006
Fluorospheres Lot No.: BAQ03	Cytometer Name: CytoFLEX nano
Fluorospheres Expires: 2024-10-02	Sample Flow Rate: 1µL/min
Detector Configuration: Default-Configuration	

### Laser

Laser	Delay(µs)	Default Delay(µs)	Difference Delay(µs)	Power(mW)	Target Power(mW)	Result
Violet	0.00	0.00	0.00	116	108-132	✔
Blue	0.00	0.00	0.00	49	40-60	✔
Yellow	0.00	0.00	0.00	30	25-45	✔
Red	-0.48	0.00	-0.48	108	90-110	✔

### Sheath Flow Rate/Background

Item	Value	Target	Unit	Result
Sheath Flow Rate	1801	1801	1600-2000 µL/min	✔
Threshold (VSSC1-H)	398	398	≤600	✔
Sheath Noise	6	6	≤80 Events/Sec	✔
Sample Noise	5	5	≤150 Events/Sec	✔

### Side Scatter Assessment

#### Event Rate

Value	Target	Unit	Result
197	197	≥ 50 Events/Sec	✔

#### Side Scatter Assessment Result

Channel	Gain	Target Gain	%Diff Target Gain	Median	Target Median	%Diff Target Median	FD	Target FD	Result
VSSC1	86	89	-3.54	1429937.0	1420000.0	0.70	8.50	1.00	✔
VSSC2	181	181	0.00	41014.0	40000.0	2.53	8.20	1.00	✔
BSSC	68	68	0.00	465571.3	460000.0	1.21	7.19	1.00	✔
YSSC	108	108	0.00	265961.9	260000.0	2.29	6.08	1.00	✔
RSSC	170	170	0.00	865046.6	860000.0	0.59	4.59	1.00	✔

### Fluorescence Assessment

#### Event Rate

Value	Target	Unit	Result
187	187	≥ 50 Events/Sec	✔

#### Fluorescence Assessment Result (Single Laser)

Channel	Gain	Target Gain	%Diff Target Gain	Median	Target Median	%Diff Target Median	rCV(%)	Target rCV(%)	Result
VFSC	1241	1241	0.00	1011360.0	1000000.0	1.14	-	-	✔
V447	839	839	0.00	426396.9	410000.0	4.00	3.40	10.00	✔
B531	1123	1052	6.75	91220.6	90000.0	1.36	4.46	10.00	✔
Y595	1279	1201	6.49	167632.5	170000.0	-1.39	4.52	10.00	✔

Figure 8: Instrument QC report.

The built-in monitoring features of the CytoFLEX nano analyzer are integral to the instrument's workflow, and ensuring the accuracy, reliability and repeatability of your experimental data.

### Sensitivity Monitoring

The optional Sensitivity Monitor feature uses specifically designed multi-fluorescent multi-intensity beads to confirm the system meets the target values for all fluorescent channels, so you can have confidence in the reliability of your experimental data.

### Volume Monitoring

Allows you to supervise the volume before acquisition. This feature enables the software to track the remaining sample in the tube and alert users when there is insufficient sample to prevent costly errors.

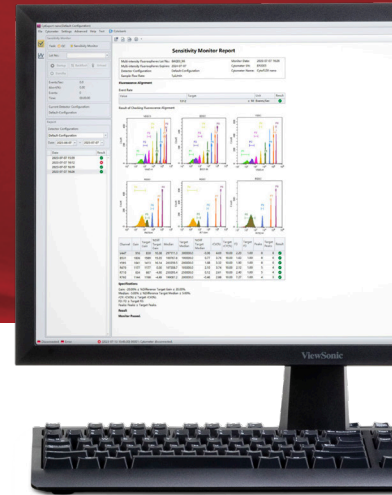
### Baseline Monitoring

Enables you to set acceptance criteria and select the type of cleaning the instrument will undertake to reach them. The Baseline Monitor can be started at any time during the day to automatically check the sample line and automatically clean it if it does not meet the defined acceptance criteria.

# More Than an Instrument: Advanced Software. Expert Support.

## CytoFLEX nano Flow Cytometer

More than an instrument:  
an appropriate method to get  
publishable data.



While the flow cytometer itself is a critical component of your workflow, proper use of the tool is essential for obtaining accurate and reliable results. The CytoFLEX nano Flow Cytometer software is compatible with the MIFlowCyt-EV framework for standardized reporting of flow cytometry data. When paired with our recommended calibration method, this enables you to visualize your data in absolute units, and provides the recommended data for reporting and method-to-method comparison.

But that's not all. When you buy a CytoFLEX nano Flow Cytometer, you don't just get an instrument and its software — you receive expert support. As part of the CytoFLEX nano analyzer purchase, our applications team will come on-site to teach you and your users how to operate the instrument and our recommended method for data calibration, allowing you to analyze and report in absolute units.



# Enhanced Efficiency with More Powerful Optics and Fluidics

The marriage between fluidics and optics is critical when enabling the detection of smaller biological nanoparticles. Based on our CytoFLEX principles, we have fully redesigned optics and fluidics to develop the unique capabilities of the CytoFLEX nano Flow Cytometer:

- Increased laser power and purpose-designed optical excitation path for maximized examination of each particle.
- Sample and sheath flow rates are reduced\* to increase exposure times, helping to collect more light and detect the EVs in your sample with more efficiency.
- Sample delivery via piston pump provides more stability and accuracy of volumetric counting.
- Sheath fluid paired with a user-exchangeable 5 nm filter and baseline monitoring options minimizes noise from debris.
- Daily QC check-ups eliminate nano and micro bubble elimination and control debris.

\*Compared to CytoFLEX flow cytometer



PERFORMANCE		
<b>Violet Side scatter sensitivity</b>	VSSC1: 40 nm relative to polystyrene nanoparticles VSSC2: 80 nm relative to polystyrene nanoparticles	
<b>Scatter detection dynamic range</b>	VSSC1 for small range 40-150 nm polystyrene nanoparticles VSSC2 for large range 80-1000 nm polystyrene nanoparticles	
<b>Violet forward scatter sensitivity</b>	300 nm relative to polystyrene nanoparticles	
<b>Fluorescence sensitivity and resolution</b>	Simultaneous detection of fluorescence on six fluorescence detectors, using 500 nm CytoFLEX nano Multi-intensity Fluorospheres V447: 8 peaks    8531: 8 peaks    Y595: 8 peaks R670: 6 peaks    R710: 5 peaks    R792: 4 peaks	
<b>Fluorescence rCV</b>	rCV ≤ 10% (using QC Fluorospheres at 1 μL/min)	
<b>Carryover between Samples</b>	≤ 1% <sup>a</sup>	
<b>Acquisition speed</b>	Maximum electronic acquisition speed	16000 events/second with ≥ 95% yield
	Recommended maximum sample acquisition speed	5000 events/second to avoid possible swarming or coincidence situation
<b>Volumetric counting accuracy</b>	> 95% <sup>b</sup>	

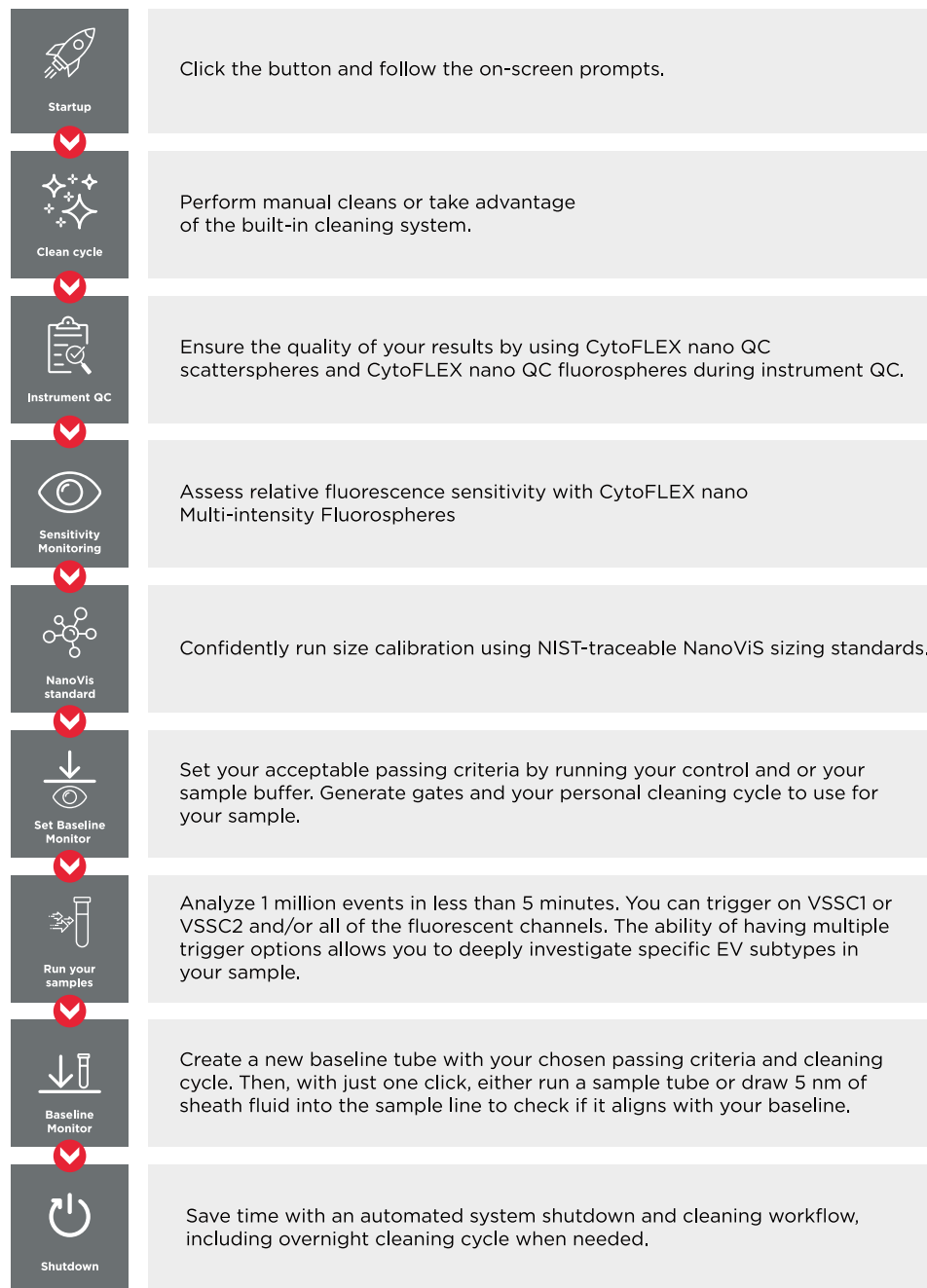
a. Tested with polystyrene nanoparticles.

b. Tested with 144 nm QC Scatterspheres on 3 units under the conditions: record 3 minutes at the sample flow rates of 1μL/min, 2μL/min, 3μL/min, 4μL/min, 5μL/min, 6μL/min respectively, repeat 5 times, then calculated the average total events for each speed, compared the calculated the volumetric counting accuracy with the theoretical total events (the known concentration 1.3\*10<sup>8</sup> x volume).

..... *Accelerating Answers* .....

# How to Use the CytoFLEX nano Flow Cytometer

The CytoFLEX nano Flow Cytometer is designed with ease of use and workflow optimization in mind. The intuitive and user-friendly CytExpert software used for acquisition and data analysis is the same as that used for the CytoFLEX platform, ensuring a seamless transition for users with minimal training time required. Enhancements to the CytExpert workflow are specifically tailored to address the challenges of EV analysis, such as inter-sample management and return-to-baseline. With the CytoFLEX nano analyzer, you can trust that your workflow will be streamlined and efficient, allowing you to focus on generating reliable and reproducible results.



“Compared to other single-particle methods, the CytoFLEX nano analyzer greatly reduces the burden of manual sample management while offering advanced tools to ensure instruments are operating from a common baseline.”

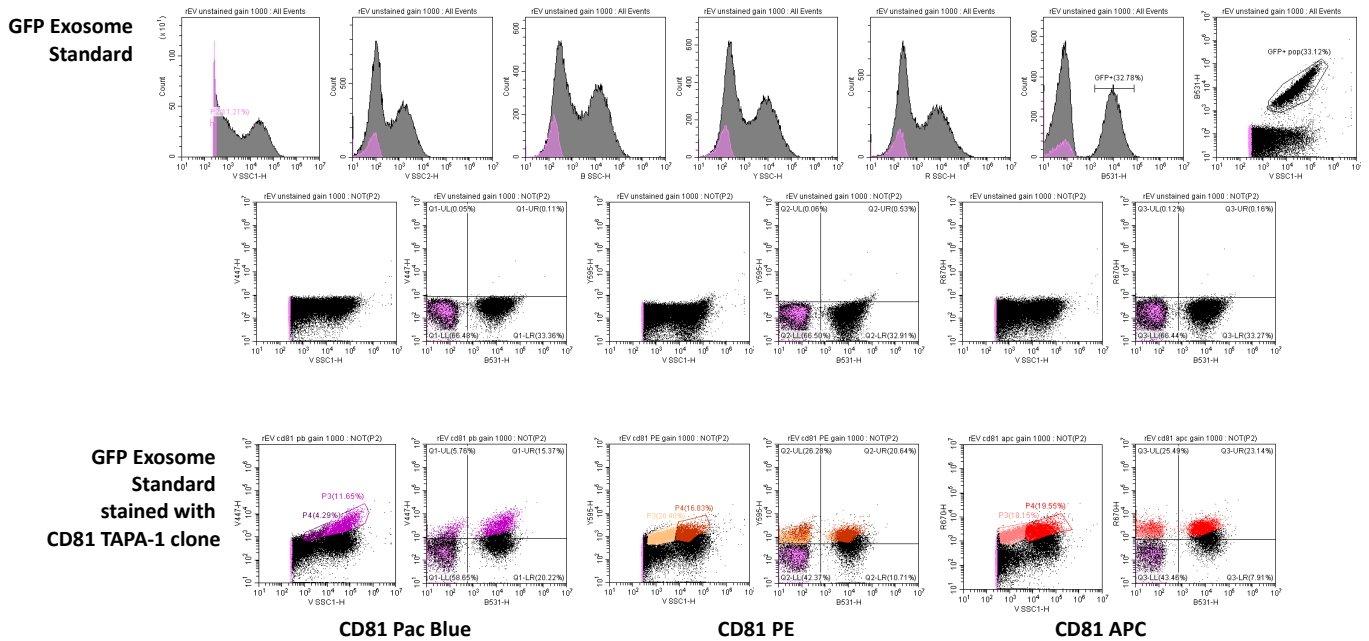
– Olesia Gololobova,  
Co-Founder and  
Manager of the  
Extracellular particle  
Characterization  
and Enrichment Lab,  
The Johns Hopkins  
University School of  
Medicine

# Deeper Insights Into Extracellular Vesicles

EVs are carriers of biologically active molecules that can travel to different targets and execute specific biological functions. These particles come in a range of subtypes, structures, and sizes.

EVs provide crucial insights into the health and disease profiles of the cells and organs in which they originate. By detecting, counting, and characterizing EVs, researchers can more easily **investigate disease states**, and **improve novel methods for therapeutics associated with cell and gene therapy**.

Their small size, however, makes EVs challenging to characterize by traditional flow cytometry. Now, the CytoFLEX nano Flow Cytometer provides the only accurate method to **quantify, measure, and identify EVs at least as small as 40 nm\***. (Figure 6).



**Figure 10:** For the first time, the CytoFLEX nano Flow Cytometer allows the study exosomes of all sizes. One antibody staining was completed on commercially available GFP Exosome Standard. With traditional flow cytometry, characterization is incomplete, as only exosomes down to 100 nm can be detected. The CytoFLEX nano analyzer can fully characterize all exosomes.

# Service and Support Packages

With the CytoFLEX nano Flow Cytometer, Beckman Coulter Life Sciences offers more than just an instrument, we **provide a partnership**. You invest in our flow cytometers and cell sorters for the long term — and we want to be with you every step of the way.

A service plan can help **minimize instrument downtime** and workflow disruptions in your research, year after year. **We offer multiple service contract options based on your lab's needs and budget.**

- Contact your local service representative for response times.
- Reliability updates include hardware, software, and instrument modifications to recommended levels.
- Basic operator training performed at your facility by a Beckman Coulter field service engineer.
- Accidental damage to the instrument(s) that affects its functionality.
- Contact your Beckman Coulter sales or service representative for more information on features and eligible instruments.

Plan Benefits	Beckman Coulter Life Sciences Service Plans				
	WARRANTY PERIOD		POST-WARRANTY PERIOD		
	Warranty	Start-Up Care	Prevention Plus	Protective	Comprehensive
Certified Parts & Labor	•	•	20% off	•	•
Travel Expenses <sup>1</sup>	•	•	20% off	•	•
Annual Preventive Maintenance		•	•	•	•
Annual Health Check <sup>2</sup>		•		•	•
Priority Onsite Response Time <sup>3</sup>		•		•	•
Remote Technical Support	•	•	•	•	•
Software & Engineering Updates <sup>4</sup>		•	20% off	•	•
Annual Basic Operator Training <sup>5</sup>		•	20% off	•	•
Accidental Damage Occurrence <sup>6</sup>		50% off			50% off
BeckmanConnect Remote Connectivity <sup>7</sup>	•	•	•	•	•
Relocation Support <sup>8</sup>		•			•



1. Travel expenses are covered within 100 miles of a Beckman Coulter Service hub and are only for Beckman Coulter service technicians.
2. Proactive service check performed by a Beckman Coulter representative.
3. Contact your local service representative for response times.
4. Reliability updates include hardware, software and instrument modifications to recommended levels.
5. Training performed at your facility by a Beckman Coulter field service engineer.
6. Accidental damage to the instrument(s) that affects its functionality. Repair services for accidental damage are reduced by 50% including 50% on product replacement if deemed necessary
7. Contact your Beckman Coulter sales or service representative for more information on features and eligible instruments.
8. Includes one-time de-installation and re-installation of the instrument(s). All costs associated with the shipping, transportation and logistics of the instrument(s) are the sole responsibility of the laboratory.

# Accessories and Consumables

To keep your system operating at the highest standard over time, we offer a range of spare parts, reagents, and plastic consumables.

Spare Parts	Part Number	Description
	D02032	Sheath Filter
	Various	Spare Optical Filters

Reagents	Part Number	Description
	C85323	CytoFLEX nano Daily QC Scatterspheres: 144 nm polystyrene scatterspheres
	C85324	CytoFLEX nano Daily QC Fluorospheres: 500 nm polystyrene beads
	C92889	CytoFLEX nano Multi-fluorescent Fluorospheres: 500 nm polystyrene beads
	B51503	CytoFLEX Sheath Fluid
	C86349	CytoFLEX Cleaner - EU REACH-compliant, enzymatic azide-free cleaning solution



## NanoVis Sizing Standards

When using flow cytometry as a characterization tool for EVs, you often need to determine the size of the nanoparticles in the sample. This requires data calibration of the scatter signal intensity. NanoVis are the only ready to use, NIST-traceable nanoscale sizing standards in the market, providing you a new level of confidence in the performance of your instrument and the resulting data. NanoVis nanoscale sizing standards can be used with CytoFLEX and CytoFLEX nano flow cytometers to create calibration curves and check the accuracy of your sizing data.



## Part of the CytoFLEX Platform

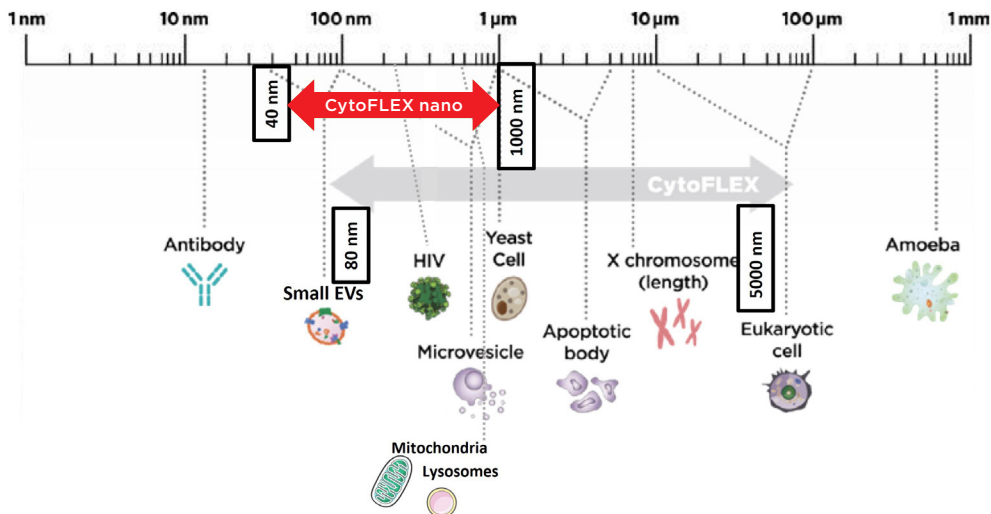
### Familiarity

The CytoFLEX nano Flow Cytometer is a great addition to the CytoFLEX platform. Thanks to its familiar interface, the time needed to train team members to start using the instrument is greatly reduced. And the small CytoFLEX platform footprint remains, making it easy to install in your lab.

“If you come to my lab, any of the regular CytoFLEX users will transfer to the CytoFLEX nano in no time.”

- Alfonso Blanco, Director flow cytometry, University College Dublin

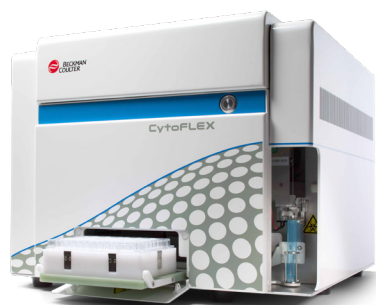
If you are a CytoFLEX user, you can use both platforms synergistically to study the interactions of most cells and EVs. Alternatively, you can use the CytoFLEX Flow Cytometer when your experiments call for higher throughput or to optimize your sample concentration before running them in the CytoFLEX nano Flow Cytometer.



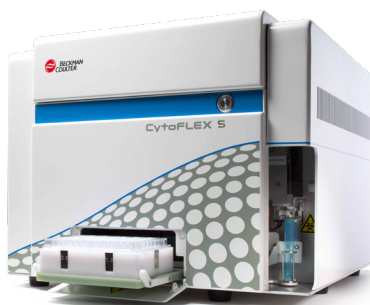
## CytoFLEX Flow Cytometer

The CytoFLEX Flow Cytometer features the capability to detect nanoparticles down to 80 nm\* thanks to its capability to measure side scatter off the violet as well as the blue laser. This allows you to characterize bigger EVs and study them within their cellular context.

\*Characterized using 40 nm polystyrene beads and triggering on violet side scatter.



CytoFLEX Flow Cytometer



CytoFLEX S Flow Cytometer

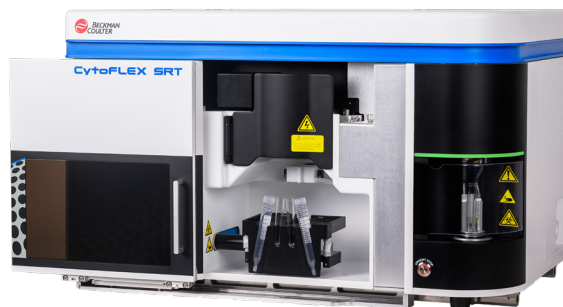


CytoFLEX LX Flow Cytometer

## CytoFLEX SRT Cell Sorter

Responding to the increasing demand for nanoparticle isolation for detailed analysis, the CytoFLEX SRT cell sorter excels in sorting nanoparticles as small as 100 nm\*\*. Drawing on the design principles of the CytoFLEX platform of flow cytometers, this sorter efficiently handles particles smaller than 1 micron. This capability allows you to precisely sort bacteria, virus-like particles, and extracellular vesicles (EVs) down to the 100 nm size range.

\*\*Characterized with 100 nm YG beads and VLP particles



CytoFLEX SRT Benchtop Sorter

# Superior Performance for More Insightful Nanoparticle Analysis

## Choose Beckman Coulter Life Sciences for Benchmark Expertise and Innovation

Our legacy of innovation dates back to 1935. We remain committed to shaping flow cytometry technology to fit seamlessly into your lab's workflow and to provide an optimal user experience. When you choose one of our instruments, you can expect to receive the highest level of expertise, innovation, and quality assurance.

Contact your local Beckman Coulter Life Sciences sales representative.

[beckman.com](https://beckman.com)



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