Flow Cytometry

Attune Xenith Flow Cytometer

Frequently asked questions

1. What is the Attune Xenith Flow Cytometer?

The Attune Xenith Flow Cytometer is an advanced spectral-enabled flow cytometry instrument designed by Thermo Fisher Scientific. It features acoustic focusing technology and advanced spectral capabilities, making it ideal for high-throughput, precise cellular analysis. This innovative instrument leverages acoustic-assisted hydrodynamic focusing to deliver high sensitivity and fast evaluation of multiple parameters, enhancing the quality and efficiency of cellular research. For more detailed information, you can visit thermofisher.com/attunexenith.

2. How does acoustic focusing technology work in the Attune Xenith Flow Cytometer?

Acoustic focusing technology uses sound waves to align cells within the flow cell rather than relying on hydrodynamic forces. This allows for more accurate positioning of cells, leading to faster processing speeds and higher data quality. In the Attune Xenith acoustic assisted hydrodynamic focusing is used where acoustic focusing works in tandem with traditional hydrodynamic focusing to ensure accuracy at flow rates over 100 µL/min.

3. What are the speed advantages of the Attune Xenith Flow Cytometer?

The Attune Xenith offers significant speed advantages, processing samples up to 5 times faster than most spectral flow cytometers on the market. This is made possible by its acoustic focusing technology, which ensures efficient and precise sample alignment. As a result, the Attune Xenith significantly reduces time-to-results and increases throughput, making it an excellent choice for high-demand, high-throughput laboratories.

4. How does the Attune Xenith Flow Cytometer minimize clogging issues?

The Attune Xenith uses positive displacement fluidics that maintain a specified volumetric flow through the system. If a clog is detected the system will work to maintain the specified volume by exerting increased pressure. This “over pressure” can result in displacing clogs and enables the instrument to continue running. This is combined with a wide-bore flow cell reduces the risk of clogs occurring.

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5. What types of samples can the Attune Xenith Flow Cytometer handle?

The Attune Xenith Flow Cytometer can handle a wide variety of sample types, including tumor and tissue samples, blood, and other complex biological materials. Its robust design and advanced fluidics ensures reliable performance even with challenging samples.

6. What is spectral unmixing, and how does the Attune Xenith Flow Cytometer utilize it?

Spectral unmixing is a process that differentiates multiple fluorophores based on their unique spectral signatures. The Attune Xenith Flow Cytometer uses the Ordinary Least Squares algorithm for unmixing to accurately identify and analyze multiple fluorophores within a single sample, enhancing the detail and precision of multicolor analysis.

7. How many lasers and detectors does the Attune Xenith Flow Cytometer have?

The Attune Xenith Flow Cytometer is equipped with 6 lasers, 51 fluorescent detectors, and 6 scatter channels. The system supports both traditional compensation and spectral unmixing, offering flexibility for various research applications.

8. Can the Attune Xenith Flow Cytometer be used for high-throughput screening?

Yes, the Attune Xenith Flow Cytometer is well-suited for high-throughput screening. It is compatible with Invitrogen CytKick Max autosampler, which allow for the efficient processing of both standard and deep well 96- and 384-well plates, streamlining workflows and increasing productivity.

9. What maintenance features does the Attune Xenith Flow Cytometer offer?

The Attune Xenith Flow Cytometer has automated maintenance procedures that have been developed to minimize the hands-on time from the user. These include Startup, Shutdown, Rinse, SIP Sanitize, Debubble, Unclog, Deep Clean, Flow Cell Clean, System Decontamination and a Long term shut down (for use when the system will be out of use for extended periods). When running plates the system can be set up to automatically shut down at the end of the plate without the need for user interaction. The Attune Xenith Fluid Cart holds most of the fluids needed to run the maintenance functions including wash solution, shut down solution and bleach and water for onboard dilution for cleaning the sample lines.

10. What are the benefits of using the Attune Xenith Flow Cytometer for rare event detection?

The Attune Xenith's speed and reduced time to results make it ideal for detecting statistically significant rare event populations, such as stem cells or circulating tumor cells. Acquiring the large number of cells needed to get to a minimum of 500 cells in the population of interest for rare events is often achieved by highly concentrating cells which risks clogging instruments and loss of cells due to coincidence through the laser. With the acoustic focusing technology in the Attune Xenith Flow Cytometer cells can be run without concentrating, in a faster time which reduces the risk of clogs, reduces the loss of events through coincidence and reduces the time to results.

11. What software tools are available with the Attune Xenith Flow Cytometer?

The Attune Xenith Flow Cytometer is equipped with intuitive software that facilitates efficient data acquisition, analysis, and visualization. The automated voltage setup procedures and user-friendly interface make it accessible to users with varying levels of flow cytometry experience.

12. What makes the Attune Xenith Flow Cytometer stand out from other flow cytometers on the market?

The Attune Xenith Flow Cytometer stands out due to its unique combination of speed, reliability, and optical capabilities. Its acoustic focusing technology ensures precise cell alignment, which enhances data accuracy and reduces the risk of clogging. The instrument offers both spectral unmixing and conventional compensation options, providing flexibility for various experimental needs. Additionally, the Attune Xenith Flow Cytometer is designed to be compatible with high-throughput workflows, making it an excellent choice for laboratories that require efficient and scalable solutions.

13. Is training available for new users of the Attune Xenith Flow Cytometer?

Yes, Thermo Fisher Scientific provides comprehensive training and support for new users of the Attune Xenith Flow Cytometer. This includes hands-on training sessions, detailed user manuals, and ongoing access to experienced technical support staff. A standout feature is the continued complimentary support from a dedicated Field Application Scientist (FAS) team post-purchase, which is unparalleled in the industry. This exceptional level of support ensures that users can fully utilize the capabilities of the instrument and underscores the commitment to customer success.

14. What are the benefits of the NIR (781 nm) laser?

The NIR 781 nm laser offers significant advantages in flow cytometry, including an expanded fluorophore palette, minimized autofluorescence, enhanced multiplexing capabilities, compatibility with existing NIR dyes, and improved signal detection. These benefits collectively enhance the flexibility, sensitivity, and accuracy of flow cytometric analyses, making it a valuable addition to advanced flow cytometry instruments like the Attune Xenith Flow Cytometer. We are finding utility in moving viability dyes to the NIR channels (Live/Dead NIR 876) freeing up the more commonly used channels for other dyes. We are also finding that dyes (e.g. BUV805 and APC Fire 810) can be better spectrally separated by their emission spectra off the NIR laser.

15. What is the fluidics design of the system? Is it pressure based, or syringe driven?

The Attune Xenith features a syringe-driven fluidics design that utilizes positive displacement fluidics. This design offers the advantage of performing absolute counts with an accuracy of +/- 10% without the need for count beads. It also helps resist clogs that can occur with challenging samples, such as tissue or tumor digests. The system is equipped with multiple pressure sensors that monitor for overpressure conditions to protect the instrument from damage, alerting the user to perform an unclog if high pressure is detected. Additionally, a flow sensor is implemented to detect issues, provide long-term monitoring, and confirm the instrument's optimal running condition.

16. What are the advantages of acoustic focusing for spectral cytometry?

Acoustic focusing technology offers several distinct advantages for spectral cytometry. It enhances sample alignment, leading to improved data resolution and accuracy. This technology also reduces sample preparation requirements, allowing for more straightforward and quicker sample processing. Additionally, acoustic focusing increases throughput, enabling the analysis of more samples in less time. It also enhances the signal-to-noise ratio, resulting in clearer and more reliable data. These benefits make acoustic focusing an ideal choice for researchers aiming to achieve high-parameter, high-quality spectral data with greater efficiency and reliability

17. Why does the Attune Xenith Flow Cytometer have PMT's rather than APD's?

The Attune Xenith Flow Cytometer uses Photomultiplier Tubes (PMTs) instead of Avalanche Photodiodes (APDs) due to several critical advantages. PMTs offer high sensitivity, allowing them to detect very low levels of light, which is essential for precise and accurate measurements in flow cytometry. They also provide a wide dynamic range, capable of measuring a broad range of signal intensities from very dim to very bright, thus enabling detailed quantification across various sample conditions. Additionally, PMTs have a fast response time, quickly reacting to changes in light intensity, which is crucial for the rapid data acquisition required in flow cytometry. Their spectral flexibility allows the use of various optical filters to detect different wavelengths of light, enhancing their versatility in multi-parameter analysis. Moreover, PMTs are highly reliable and compatible with advanced optical systems, ensuring consistent and high-quality data collection. In contrast, while some APDs may offer higher quantum efficiency, they suffer from a higher noise floor and limited linearity for bright signals, which negatively impacts the flexibility and accuracy needed to run both compensation and spectral panels on the same instrument. These attributes make PMTs the ideal choice for achieving precise, accurate, and high-quality data in both spectral and conventional flow cytometry applications.

18. How does the Attune Xenith Flow Cytometer perform spectral unmixing?

The Attune Xenith Flow Cytometer performs spectral unmixing by capturing the emission spectrum of each fluorophore across multiple detectors. It uses advanced spectral unmixing algorithms to accurately deconvolute overlapping spectra, ensuring precise identification and quantification of each fluorophore. Specifically, the Attune Xenith employs the Ordinary Least Squares (OLS) algorithm for spectral unmixing, which is a widely used method in spectral systems. This approach allows for accurate separation of signals, even when spectra overlap significantly, ensuring high-quality data for complex multi-parameter analyses.

19. Does Thermo Fisher Scintific recommend beads or cells as controls in spectral unmixing?

Why would one be chosen over the other? Both beads and cells can be used as controls in spectral unmixing, and the choice between them depends on the specific requirements and goals of the experiment. Beads offer consistency, reproducibility, and ease of use, making them ideal for calibration and routine quality control. Cells, on the other hand, provide biological relevance and allow for the characterization of complex spectra and autofluorescence, which can be crucial for certain applications. Therefore, the decision to use beads or cells should be based on the specific needs of the spectral unmixing process and the experimental context. Cells are a good choice if you have plenty of sample, the antibody in use will stain a large and distinct positive population and there will be an internal negative population. Beads are a good choice if your sample is limited, the antibody would only stain a very small subset of cells or the population of cells that would stain would not be distinct. Beads are also only a good choice if the spectral characteristics of the fluorophore are maintained on beads. We recommend that when establishing a panel, a full set of single stain controls of both beads and cells be prepared. After recording the controls user should check the signature of the beas cells and decide on the best choice for each fluorophore. For example, if the spectral signatures are different on beads, then cells will be needs and if cells are brighter than beads then the cells would need to be used for the controls. It is likely a mixture of cells and beads for controls will be the optimal choice.

20. How do users choose between whether they need to run spectral unmixing vs conventional compensation?

Choosing between spectral and conventional flow cytometry depends on your experimental needs and the complexity of your panels. Spectral flow cytometry offers significant advantages for high-parameter detection, improved data resolution, flexibility, simplified panel design, and the ability to handle complex samples. It is also beneficial for sample types with multiple auto fluorescent populations to be explored and for panels where a user may not be familiar with the best peak channel to choose. Conventional flow cytometry remains a robust and effective option for simpler applications and lower-parameter analyses. Conventional compensation can be beneficial for panels of smaller size and/or limited single channel overlap. It can also be beneficial for panels that include very bright dyes such as functional dyes which often require individual detector adjustments (adjusting the full spectral signature to keep these dyes on scale in bright samples may lead to confusing spectral signatures). By considering these factors, you can select the flow cytometry method that best aligns with your research goals and requirements.

21. Can the Attune Xenith Flow Cytometer run no-lyse/ no wash assays in the same way as the Attune NxT Flow Cytometer?

Yes, the Attune Xenith Flow Cytometer has built in FSC and SSC channels off the violet laser so that no-lyse/no wash assays, which exploit the difference in violet light-scattering properties between red blood cells and leukocytes, can be easily run without the need for any changes in filters or need for purchase of additional accessory kits. This integrated capability means that no changes in filters or additional accessory kits are required, making the process straightforward and efficient.

The Attune Xenith Flow Cytometer complements the Attune family of flow cytometers, including the Attune NxT and Attune CytPix.

Learn more at **thermofisher.com/attunexenith**