

Applied Biosystems Attune® Acoustic Focusing Cytometer Differentiating Features



Instrument Model Number: Attune® Acoustic Focusing Cytometer

Instrument Description: The Attune® Acoustic Focusing Cytometer combines Acoustic Focusing Technology with flow cytometry.

Manufacturer: Life Technologies

Life Technologies is the manufacturer and sole vendor of the Attune® Acoustic Focusing Cytometer (Part Numbers 4445315 (Blue/Violet) and 4469120 (Blue/Red)) in the United States or Canada.

Technology overview

Acoustic Overview

Acoustic focusing technology is a patented technology (Kaduchak et.al. U.S. Patent 7,304,957) that replaces or partially replaces hydrodynamic focusing of cells with focusing derived from acoustic radiation pressure forces. Until this ground breaking technology was introduced to a flow cytometer in 2010, traditional cytometers relied upon hydrodynamic focusing, which uses high volumetric sheath flow to limit the injected sample into a small core stream that is accelerated through the optical interrogation spot of a tightly focused laser beam to focus and transport cells. In comparison, acoustic focusing exploits the physical differences between cells and its carrier medium using ultrasound waves (over 2 MHz) to position cells into a single focused line along the central axis of a flow channel. (Ward, M, et al, 2009). The ability to focus cells into a precise line with or without sheath fluid can offer many advantages over the traditional flow cytometers.

Acoustic Focusing Cytometry Applications

The Applied Biosystems Attune® Acoustic Focusing Cytometer is labeled “For Research Use Only, Not for use in diagnostics procedures”. It is not intended for any animal or human therapeutic or diagnostic use.

Attune® Acoustic Focusing Cytometer is very flexible and, subject to the applicable label license, can be use for but not limited to, the following research applications:

- Immunology-immunophenotyping
- Apoptosis

- Cell proliferation Assays
- Cell Cycle Analysis
- Microbiology
- Fluorescent Protein Detection
- Phosphorylation Studies/Signal Transduction Studies
- Oceanographic Studies
- Absolute Cell Counting
- Stem Cell Analysis
- MHC Tetramers
- Rare Event Detection
- Phagocytosis
- FRET Studies
- FISH
- Viability Assays

Acoustic Focusing Advantages

The advantages of acoustic focusing over other methods include:

- The ability to precisely measure cells without loss of sensitivity at high sample rates.
- The ability to adjust sample rate ranging from 25 $\mu\text{L}/\text{min}$ - 1000 $\mu\text{L}/\text{min}$
- The ability to adjust the time that a cell spends in laser beam by increasing or decreasing flow rates.
- The ability to analyze a wide range of sample concentrations including very small initial sample sizes
- The ability to reduce the background noise by reduction in free label concentration using dilution of sample

Over the past several years, multicolor flow cytometry has become a leading tool for the detection of single cells and their characteristics some of which include: size, granularity, antigen expression, membrane integrity. Using flow cytometry rare cell populations can be identified in a larger diverse cellular population. Because of the high quality data, flow cytometry is commonly used to provide quantitative data for microscopic analyses.

The Attune® Acoustic Focusing Cytometer contains the following differentiating features and benefits:

Instrument

1. The instrument can process samples at sample flow rates between 25 and 1000 μL per minute providing both high sensitivity and high flow rates. This capability also provides a high degree of flexibility in the types of analysis that can be performed.
2. The instrument part number 4445315 uses 405 nm and 488 nm solid state diode lasers, permitting the use of violet and blue laser dye reagents, enabling low compensation experiments while instrument part number 4469120 uses a 488nm and 638 nm solid state diode laser, permitting the use of blue and red laser dyes enabling the use of existing optimized reagent panels
3. The instrument can acquire eight parameters on a single sample: six color fluorescence data plus forward and side scatter. The multi-parameter capability provides the flexibility to obtain exceptionally high levels of information from a single sample.
4. The instrument permits the adjustment of the photomultiplier gain allowing user control over collection of experimental data.
5. The instrument includes a complete compensation matrix that will allow end user to compensate the signal from every channel out of every other channel.
6. The instrument permits the adjustment of the optical configuration by the end user without the need for a service engineer by using plug and play dichroic mirrors and band-pass filters, providing greater flexibility in reagent selection.
7. The instrument optical alignment has been designed to be highly stable so that under normal operation and without moving the instrument the customer is not required to align optics.
8. The instrument provides at least 6 decades of resolution enabling high data accuracy And can capture up to 20,000 events/second allowing high data collection rates
9. The instrument can be used with 100V-240V without modification or special plug requirements.
10. The instrument uses small amounts of fluids (averaging less than 1L per day total fluid consumption) helping to minimize fluid expense and waste disposal costs.
11. The instrument has calibrated sample volume delivery, which enables ability to perform absolute cell counts without use of bead product.
12. The instrument has been engineered to lessen the potential for clogging by the use of 200 micron flow cell and tubing.

Instrument On-Board Computer

13. Instrument's 23 inch monitor allows visualization of data acquisition and data analysis modules without the need for additional monitor.

Performance

14. The instrument is designed to collect Height, Area, and width measurements on all scatter and fluorescent channels.
15. The instrument part number 4445315 blue/violet configuration combines 2 excitation (405, 488 nm) and 3 emission distinct emission filter sets from each laser to enable collection of up to 6 unique combinations of wavelengths during a single run for multiplexing. The instrument part number 4469120 blue/red configuration combines 2 excitation (488nm with 4 distinct emission filter sets and 638 nm with 2 distinct emission filter sets) to enable the collection of up to 6 unique combinations of wavelengths during a single run for multiplexing.
16. The instrument is capable of analyzing particles in the size range of 1 μm to 45 μm , which matches well with the sizes of commonly cultured cells and cells obtained from other sources.
17. The instrument exhibits lower coefficient of variations (%CV) as compared to hydrodynamic instruments especially at higher sample rates due to the acoustic focusing.

Software

18. The data from the Attune® Acoustic Focusing Cytometer is stored in the industry standard FCS 3.0 file format, allowing the use of other third party software programs to analyze data.
19. The instrument includes a license to software for one user at no cost that is designed to collect and analyze the flow cytometry data.
20. The software has a simple user interface based on the windows platform so that it is easy to navigate and requires minimal training.
21. The software supports data acquisition, data analysis, and functional control of the instrument making the overall operation of the instrument simple and convenient.
22. The software has been designed so that data analysis can be performed real time during data acquisition or post acquisition.
23. The software includes multiple plot types including dot plots, density plots, and histogram plots that include linear, logarithmic, and linear-log axis scales.
24. The software provides automated start up and shut down capability as well as some automated user prompted maintenance functions.
25. The software has simplified automated and manual compensation programs
26. The software allows easy access to up to 10 of the most commonly used experiment templates on the main menu screen.
27. The instrument software provides import/export capabilities of experiment files, FCS files, instrument settings, and compensation settings.
28. The instrument software allows users to start a sample in 3 mouse clicks or less.

29. Instrument software includes statistical analysis tools which can be exported to a CSV file. (Statistics included- Mean, Median, CV, Standard Deviation, % parent, % total, and Concentration)
30. Instrument software allows back gating, overlay plots, derived parameters, and Boolean gating.
31. The instrument software allows for administrative and user log in capabilities.
32. The instrument software allows user easily copy and paste data plots to power point and word documents.
33. The instrument software allows thresholding of up to two parameters simultaneously.

Design

34. The instrument's footprint (H x W x D): 16 in/40 cm x 23 in/58 cm x 17 in/43 cm) is small enough to allow the unit to be placed either on a counter or in a laminar flow hood.
35. No special electrical or environmental requirements (i.e. heating, cooling) are necessary beyond those normally found in a standard laboratory environment.
36. The instrument tube lifter was designed to accommodate 17 mm x 100 mm to 8.8 x 45 mm tubes.
37. The instrument is designed to house all instrument fluid containers on board the cytometer without the use of separate fluidics cart to reduce space consumption and simplify operations.
38. The instrument is designed for user to have easy access to sample lifter for both right hand and left handed individuals.
39. The instrument is designed with minimal weight requirements such that a single individual could move the instrument if necessary.
40. The instrument is designed for convenient access to additional filters and filter holders under the instrument lid.
41. The instrument is designed to operate at temperatures ranging from 15–30°C
42. The instrument is CE marked, UL Listed, and meets CDRH requirements for Laser safety
43. The instrument is developed and manufactured in accordance with quality system requirements that comply with ISO 9001 standards.

Vendor Service and Support

44. The instrument is provided with a limited warranty for a period of one year beginning from the earlier of the date of installation or 90 days from date of delivery. A team of factory trained service engineer is available to perform warranty repairs. Optional post-warranty service contracts including next-business-day on-site repairs are available for purchase.
45. The instrument is provided with a two-day basic user training session by a certified technical scientist (Field Application Specialist) at customer site or a three-day training session at the application support center in Foster City, California or Frederick, Maryland.
46. Vendor is able to supply the necessary consumables to perform instrument performance tracking, daily operation, and maintenance.
47. Vendor offers Telephone Technical Support and Field Applications/Sales/Service Support to answer technical questions, help review data, make recommendations on how to troubleshoot results encountered with flow cytometry experiments.

Green Features

48. The Attune® Acoustic Focusing Cytometer technology utilizes less energy than a standard 60W light bulb.
49. The instrument design uses very low sheath fluid volumes, thereby generating minimal biohazardous waste.

References:

Kaduchak, G., Goddard, G., Salzman, G., Sinha, D., Martin, J.C., Kwiatkowski, C.S., and Graves, S.W. 2008. Ultrasonic Particle Concentration and Application in Flow Cytometry. U.S. Patent 7,340, 957

Ward, M., Turner, P., DeJohn, M., and Kaduchak, G. Fundamentals of Acoustic Cytometry. *Current Protocols in Cytometry*, 1.22.1-1.22.12, July, 2009