Cytek®
Aurora
Say Hello to a New Reality
A prodigy incorporating a unique combination of patent-pending innovative technologies that takes flow cytometry to the next level of performance and flexibility.

With up to five lasers, three scattering channels, and 64 fluorescence channels, the Aurora suits every laboratory’s needs, from simple to high-complexity applications. A paradigm shifting optical design provides unprecedented flexibility, enabling the use of a wide array of new fluorochrome combinations without reconfiguring your system for each application. The state-of-the-art optics and low-noise electronics provide excellent sensitivity and resolution. Flat-top laser beam profiles, combined with a uniquely designed fluidics system, translate to outstanding performance at high sample flow rates.

The end result is a system that delivers high quality data where rare and dim populations are easily resolved, regardless of assay complexity.

SpectroFlo® software offers an intuitive workflow from QC to data analysis with technology-enabling tools that simplify running any application.

The Cytek team has reimagined every aspect of cytometry hardware and software to deliver an instrument that fulfills every scientist’s needs.

- **Maximum Channels**
  - 64 fluorescence channels of detection over the full emission spectra.

- **Maximum Colors**
  - 40 colors demonstrated including fluorochromes with emission spectra in close proximity to each other.

- **Maximum Sensitivity**
  - Sensitivity redefined using state-of-the-art optics and low-noise electronics.

- **Maximum Flexibility**
  - No changing optical filters for any fluorochrome.
  - Use any commercially available fluorochrome excited by the onboard lasers.

- **Maximum Accessibility**
  - A powerful, high value system that is accessible to a wide range of users.
Aurora’s Revolutionary Technologies: From Vision to Reality

The Aurora is capable of up to 67 detection channels (64 fluorescence channels, FSC, blue laser SSC, and violet laser SSC) and is empowered by revolutionary technologies, including:

1. Proprietary high sensitivity Coarse Wavelength Division Multiplexing (CWDM) semiconductor detector arrays, enabling more efficient spectrum capture for dyes emitting in the 365-829 nm range.
2. High bandwidth electronics design scalable up to 67 channels.
3. Robust vacuum fluidics system enables ultimate flexibility in sample input formats.
4. Exceptional small particle detection is enabled by violet laser scatter, narrow beam height, and proprietary flat top laser design.
Resolving Challenging Dye Combinations

The detection of some fluorochrome combinations by conventional flow cytometry presents a challenge due to high amounts of spectral overlap (Figure 1, 4). The Aurora addresses this challenge by using differences in full emission spectra signatures across all lasers to clearly resolve these combinations, even if the populations are co-expressed (Figures 2, 3, 5, and 6).

**Example 1: APC and Alexa Fluor 647**

*Figure 1:* Spectrum plots from a conventional spectrum viewer shows heavy overlap between APC and Alexa Fluor 647.

*Figure 2:* Spectrum plots from a four laser Aurora show distinct signatures for APC and Alexa Fluor 647.

*Figure 3:* Whole blood from a healthy donor was stained, lysed, washed, and analyzed on a four laser Aurora system. Subsets of NK and NK T cells that co-express CD56 Alexa Fluor 647 and CD8 APC were easily identified. For comparison, blood from the same donor was stained with CD56 PE and CD8 APC and yielded similar percentages of NK and NK T cells, demonstrating that APC and Alexa Fluor 647 combined did not impact results.

**Example 2: BFP, GFP, and mCherry**

*Figure 4:* Spectrum plots from a conventional spectrum viewer.

*Figure 5:* Spectrum plots from a four laser Aurora show distinct signatures for BFP, GFP and mCherry.

*Figure 6:* AB2.2 mouse embryonic stem cells were genetically modified to stably express BFP, GFP and mCherry under the control of different fate marker promoters. The stable cell line generated was then cultured under differentiation conditions, harvested, and analyzed on a four laser Aurora system to assess the expression of fluorescent proteins. Auto-fluorescence extraction was used to enhance results. Sample courtesy from Luigi Russo, Hannah L. Sladitschek and Pierre Neveu, Cell Biology & Biophysics, Neveu group, EMBL.
See More Clearly with Autofluorescence Extraction

Aurora’s implementation of full spectrum cytometry enables the use of autofluorescence extraction to further improve data clarity. Certain sample types, such as yeast and tumor samples, present the challenge of high autofluorescence. For these challenging applications involving highly autofluorescent particles, let the software’s autofluorescence extraction tool bring new levels of resolution.

**Example 1: PrimeFlow™ RNA Assay**

Human U937 cells were subjected to the PrimeFlow™ RNA Assay. The cells underwent a series of hybridization steps to label mRNA for HMBS, a low expressed gene (~10 copies/cell), with Alexa Fluor® 488. The sample was run on the Aurora and analyzed using SpectroFlo® software with two different strategies, one with autofluorescence extraction and one without.

Spectrum plots of unstained and Alexa Fluor 488 stained cells acquired on the Aurora. Note that the two spectra heavily overlap.

**Example 2: mCherry Expressing HeLa Cells**

HeLa human cells were transformed with a CRISPR-Cas9 target vector carrying an mCherry reporter. Expression of mCherry is driven by the endogenous promoter of the knocked-in gene. The cells were harvested 32 hour post-infection and analyzed on a four laser Aurora system to assess integration of the fluorescent protein. Autofluorescence extraction was used to enhance the resolution. Sample courtesy of Malte Paulsen, Flow Cytometry and Cell Sorting Facility, EMBL.

Spectrum plots of unstained and positive mCherry cells acquired on the Aurora. Note that the two spectra heavily overlap.
Small Particles in Full View

With its onboard 100 mW 405 nm laser and highly sensitive violet SSC detector, particles nearing 100 nm in size can be analyzed. Aurora opens the door to a wide variety of small particle applications, taking what was once hidden and placing it in full view.

**Example 1: ApogeeMix**

Resolution of ApogeeMix from Apogee Flow Systems (www.apogeeflow.com), a mixture of silica and polystyrene (PS) beads ranging from 110 nm to 1300 nm, when acquired on the Aurora. The smallest particles can be easily identified above background.

Data analyzed using FCS Express 6 by De Novo™ Software.

**Example 2: ViroFlow**

Murine Leukemia Virus (MLV-124 nm ±14 nm) genetically engineered to express superfolder GFP (sfGFP) as a fusion protein with the viral envelope glycoprotein.

The plots on the right show:

A) Buffer only
B) MLV with no sfGFP (MV-M-Zero)
C) MLV with sfGFP-Env (MV-M-sfGFP)

All samples were run on a three laser Aurora using violet SSC as a threshold trigger. Virus reference particles were provided by ViroFlow Technologies (www.viroflowtechnologies.com).

Data analyzed using FCS Express 6 by De Novo™ Software.
More Choice, Greater Flexibility, Easier Setup

The optical design combined with the unmixing capability in SpectroFlo® software allows greater fluorochrome choice, panel flexibility, and easy setup without having to change filters. The three laser configuration provides outstanding multi-parametric data for a wide array of applications. Markers and fluorochromes in a 24-color panel designed for identification of circulating cell subsets in human peripheral blood are summarized in the table below:

<table>
<thead>
<tr>
<th>SPECIFICITY</th>
<th>FLUOROCHROME</th>
<th>SPECIFICITY</th>
<th>FLUOROCHROME</th>
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</table>

24-COLOR DATA

On the next page, this 24-color panel is demonstrated in a normal healthy human whole blood donor using a lyse wash prep.

The 24-Color Panel Includes Many Highly Overlapping Dyes:

- APC/Fire™ and PE/Dazzle™ are the trademarks and property of BioLegend, Inc.
- Brilliant Violet™ is a trademark of Sirigen Group Ltd.
- BD Horizon™ and Brilliant Blue (BB) are trademarks of BD Biosciences.
- Alexa Fluor®, eFluor®, and Super Bright are trademarks of Thermo Fisher Scientific.
- Cy® and CyDye® are registered trademarks of GE Healthcare.
- Allophycocyanin (APC) conjugates: US Patent No. 5,714,386
  - APC-Cy7: US Patent Number 5,714,386.
  - Trademarks are the property of their respective owners.
A New Reality:
3 Lasers, 24 Colors, Unparalleled Resolution

Granulocytes

- CD14 BV510
- CD16 eFluor 450
- HLA DR APC/Fire 750
- CD11c BB515
- CD11b PerCP-Cy5.5
- CD4 BV750
- CD16 eFluor 450

Monocytes

- CD14 BV510
- CD16 eFluor 450
- HLA DR APC/Fire 750
- CD11c BB515
- CD11b PerCP-Cy5.5
- CD4 BV750

Lineage Negative Cells

- CD19 SB 436
- CD56 BV711
- HLA DR APC/Fire 750
- CD16 eFluor 450
- CD11c BB515
- CD123 Alexa Fluor 647

B Cells

- CD19 SB 436
- CD1c BV605
- CD27 APC
- IgD PE/Dazzle 594
- CD38 PerCP-eF710

NK Cells

- CD56 BV711
- CD16 eFluor 450
- CD57 PE-Cy7
- CD8 BV570

NK T Cells

- CD127 APC R700
- CD27 APC
- CD56 BV711
- CD127 APC R700

Double Negative Cells

- CCR7 BV421
- CD45RA Alexa Fluor 488
- CD28 BV785
- CD38 PerCP-eF710
- HLA DR APC/Fire 750
- PD-1 BV650
- CD95 PE-Cy5

Treg Cells

- CD25 PE
- CD127 APC R700
- CD38 PerCP-eF710
- HLA DR APC/Fire 750
- PD-1 BV650
See More with the UV Laser

With the addition of the UV laser and a total of 64 fluorescence detectors, Aurora now has the power to take highly-multiplexed assays beyond 30 colors. Incorporation of the UV laser takes the Aurora platform to the next level.

35-Color Panel

Markers and fluorochromes in a 35-color panel are summarized in the table below. Human peripheral blood mononuclear cells were stained, washed, and acquired on a five laser Aurora.

<table>
<thead>
<tr>
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<th>Violet</th>
<th>Blue</th>
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<td>Specificity</td>
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</table>

LIVE/DEAD™ is a trademark Thermo Fisher Scientific.
cFluor™ is a trademark of Cytek Biosciences.
Spark Blue™ is a trademark of BioLegend.

**t-SNE and FlowSOM Analysis**

(t-SNE analysis of 35 colors immunophenotyping panel using OMIQ software (www.omiq.ai). FCS files including only CD45+, singlets, and live cells were analyzed in OMIQ software. Scaling was optimized and t-SNE analysis was done using GPU t-SNE algorithm for all donors (top row). One cell subset was present only in donor one (see arrow in top row). Colored-continuous scatterplots for donor one showing marker expression in this unique subset are shown in the second and third rows. Clustering analysis by FlowSOM visualized by GPU-tSNE, shows metacluster two expressing CD3+/CD4+/CD57+/PD-1+/CD45RO+/CD95+/CD56+/CD45RA-/CCR7-/CD27-...
2D Dot Plots

CD4+ T Cells

CD8+ T Cells

Double Negatives T Cells

Monocytes

NKT Cells

TCRγδ Cells

B Cells

Regulatory T Cells

Dendritic Cells

Cytek® Aurora
Say Hello to a New Reality

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SpectroFlo® Software Guided Workflows

The new SpectroFlo software offers an intuitive workflow from QC to data analysis with technology-enabling tools that simplify running any application.

**QC and Setup:**
Run Daily QC to monitor instrument performance and add reference controls.

**Extra Tools:**
Unmix data using controls from different experiments or apply virtual filters to your data.

**Users:**
For administrative controls.

**Preferences:**
Customize the software appearance. Set default plot sizes, text sizes and fonts, gate colors, print layout, statistics table options, and more.

**Experiment Workflow:**
From the Acquisition menu, you can start a new experiment and get to your data in three simple guided steps.

**Step 1: Create Your Experiment**
Create your experiment, choose fluorochromes, and add labels, tubes, worksheets, and stopping criteria in this guided workflow.

**Step 2: Acquire Your Tubes**
Load and acquire your samples.

**Step 3: Unmix Your Data**
Visualize your reference controls spectra using our unique unmixing wizard.
Get to Know Our Automatic Micro-Sampling System (AMS)

Meet the AMS

The AMS offers preset and user adjustable settings that allows the loader to be fine tuned to your experimental requirements. The AMS is specifically designed to streamline experimental workflow and seamlessly integrates into the Aurora. The AMS also offers ease of use and low carry over.

- **Reliable and easy**
  - **Reliable** 96 well plate acquisition maximizes productivity.
  - **Easily change** between plates and tubes in a matter of seconds.

- **Three throughput modes**
  - **Optimized** acquisition speeds, from low carry over to high throughput.

- **User customizable modes**
  - **Fully customizable** with a suite of user modes to fit a variety of applications and workflows.
Cytek Careers

Cytek Biosciences is dedicated to enhancing our customers' user experience. The Aurora system is backed by our world-class service and support team that can provide phone or field based assistance. Various levels of maintenance options are available to meet your needs now, and in the future.

Technical Support

We have a worldwide team of field service engineers and technical application specialists at your service. To maintain your instrument and keep it running well, you can choose the right service contract for your needs. Our technical applications specialists are here to support you with application related questions such as troubleshooting experiments, understanding or troubleshooting software behaviors, and more.

For help choosing the right service contract, contact your sales representative at sales@cytekbio.com.

For service and application support, contact us at support@cytekbio.com.

Training

We offer two days of in-depth, interactive, hands-on training with each new instrument installation. If you later have a need for additional training, we offer a shorter one day refresher option. To learn more, speak to your Cytek sales representative or email us at sales@cytekbio.com.

Online Resources

As Cytek grows so will the tools on our website to enhance your experience with your full spectrum cytometer. For example, you can interact with other Aurora and Northern Lights users and our technical applications support staff in the Aurora User Community (contact your sales representative to learn how to activate your account). Need to learn more about dyes used on the Aurora? Click on the reagents tab to find panel design examples and other optimization tools.

Visit www.cytekbio.com regularly as we introduce more exciting tools throughout the year.
Specifications

Optics

EXCITATION OPTICS

OPTICAL PLATFORM
Aurora contains a fixed optical assembly with the capacity to be configured with up to five spatially separated laser beams. Laser delays are automatically adjusted during instrument QC.

LASERS
Base model three-laser configuration: 405 nm: 100 mW, 488 nm: 50 mW, 640 nm: 80 mW
Available laser upgrades: 355 nm: 20 mW, 561 nm: 50 mW

BEAM GEOMETRY
Flat-Top laser beam profile with narrow vertical beam height optimized for small particle detection.

EMISSION OPTICS

EMISSION COLLECTION
Fused silica cuvette coupled to high NA lens for optimum collection efficiency to optical fibers.

FORWARD AND SIDE SCATTER DETECTION
FSC: high-performance semiconductor detector with 488nm bandpass filter
SSC: two high-performance semiconductor detectors with 405nm and 488nm bandpass filters

FLUORESCENCE DETECTORS
Proprietary high sensitivity Coarse Wavelength Division Multiplexing (CWDM) semiconductor array per laser enabling more efficient spectrum capture in the 365-829 nm range. No filter changes required for any fluorochrome excited by the 355 nm, 405 nm, 488 nm, 561 nm, 640 nm lasers.

STANDARD OPTICAL CONFIGURATION
Violet detector module: 16 channels unevenly spaced bandwidth from 420-829 nm.
Blue detector module: 14 channels unevenly spaced bandwidth from 498-829 nm.
Red detector module: 8 channels unevenly spaced bandwidth from 652-829 nm.

4 and 5 Laser Options:
Yellow-Green detector module: 16 channels unevenly spaced bandwidth from 365-829 nm.

Fluidics

SAMPLE FLOW RATES
Low: 15 µL/min, Medium: 30 µL/min, High: 60 µL/min, Plate high-throughput mode: 100 µL/min

FLUIDIC MODES
Long clean, SIT flush, Purge filter, Clean flow cell

MANUAL SAMPLE INPUT FORMATS
12x75 mm polystyrene and polypropylene tubes

Workstation

Workstation specifications may vary between laser configuration

OPERATING SYSTEM
Windows® 10 Pro 64-bit

PROCESSOR
Intel® Core™ i7 processor

RAM
64 GB

HARD DRIVE
500GB SSD and 1 TB SATA

VIDEO PROCESSOR
NVIDIA® GeForce

MONITOR
32” UHD 4K Monitor

Installation Requirements

Dimensions (W x D X H)

INSTRUMENT DIMENSIONS
Without loader: 54 x 52 x 52 cm
With loader: 58 x 62 x 52 cm

INSTRUMENT WEIGHT
Instrument weight (5 lasers): 71 kg
Loader weight: 13 kg

RECOMMENDED WORKSPACE
165 x 76 x 132 cm

Room Requirements

POWER
100-140 VAC, 15A or 200-250 VAC, 10A

HEAT DISSIPATION
500 W with all solid-state lasers

TEMPERATURE
15–28°C

HUMIDITY
20%-85% relative non-condensing

AIR FILTERING
No excessive dust or smoke

LIGHTING
No special requirements

Regulatory Status

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Software

SPECTROFLO® SOFTWARE
Live unmixing during acquisition
Developed specifically to streamline assay setup, data acquisition, and file export
Automated QC module
Autofluorescence extraction
Raw and Unmixed FCS 3.1 files

Electronics

SIGNAL PROCESSING
Digital signal processing with automatic window gate adjustment.
22-bit 6.5 log decades.
Threshold using any single parameter or combination of parameters.

PULSE SHAPE PARAMETERS
Pulse Area and Height for every parameter. Width for scatter parameters and one fluorescence parameter for each laser.
For more information, email us at: sales@cytekbio.com or call 1-877-922-9835

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+31 (0) 20 765 3440

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2-9-1 Ikenohata Taito-ku
Tokyo 110-0008 Japan

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