Attune NxT: Acoustic Focusing Flow Cytometer

Experience the Ultimate Speed without Compromising Performance

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Life Sciences Solutions

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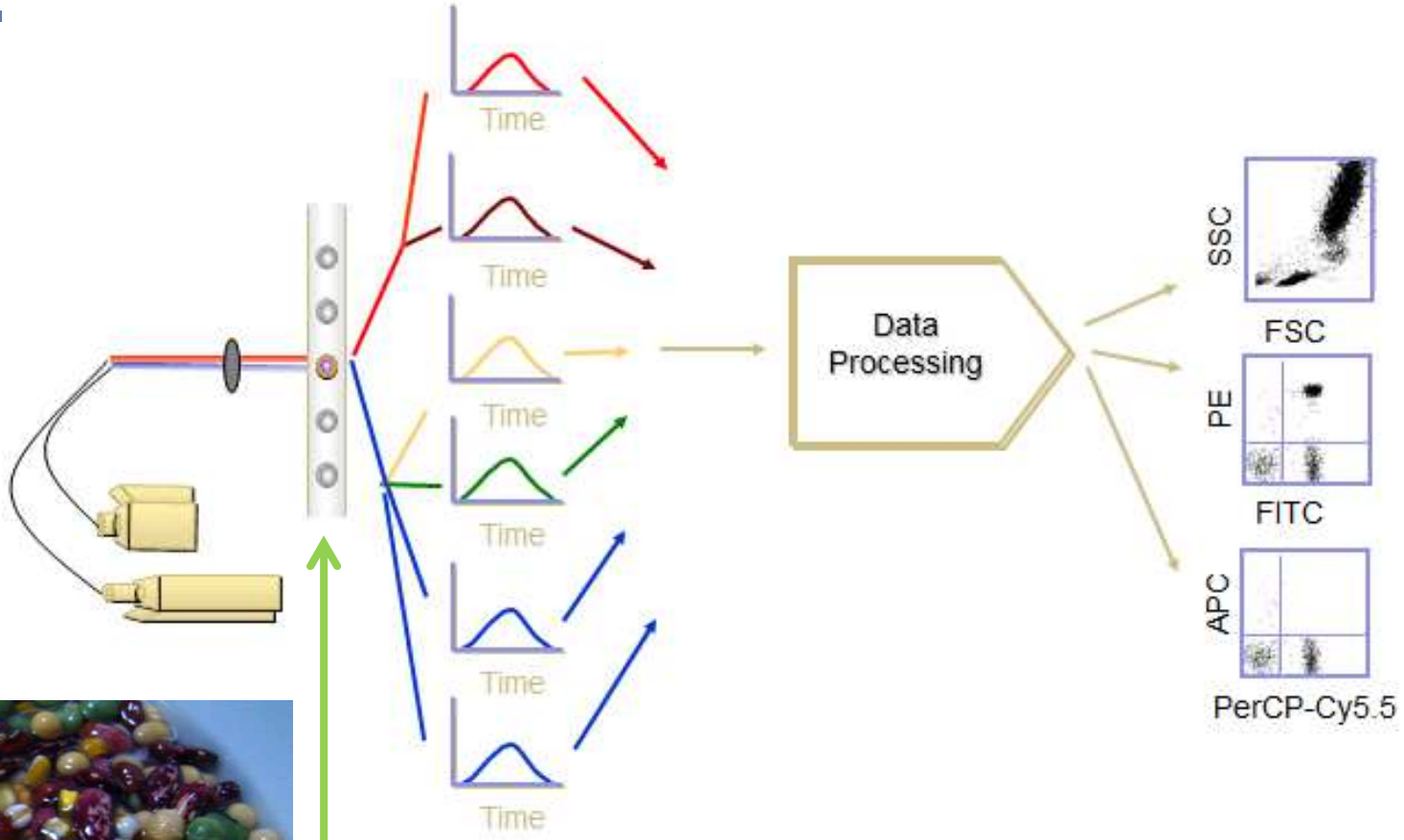
Flow Cytometry: Finding Cells of Interested



- Regulatory T Cells
- NKT cells
- Cancer stem cells
- Circulating tumor cells…etc.

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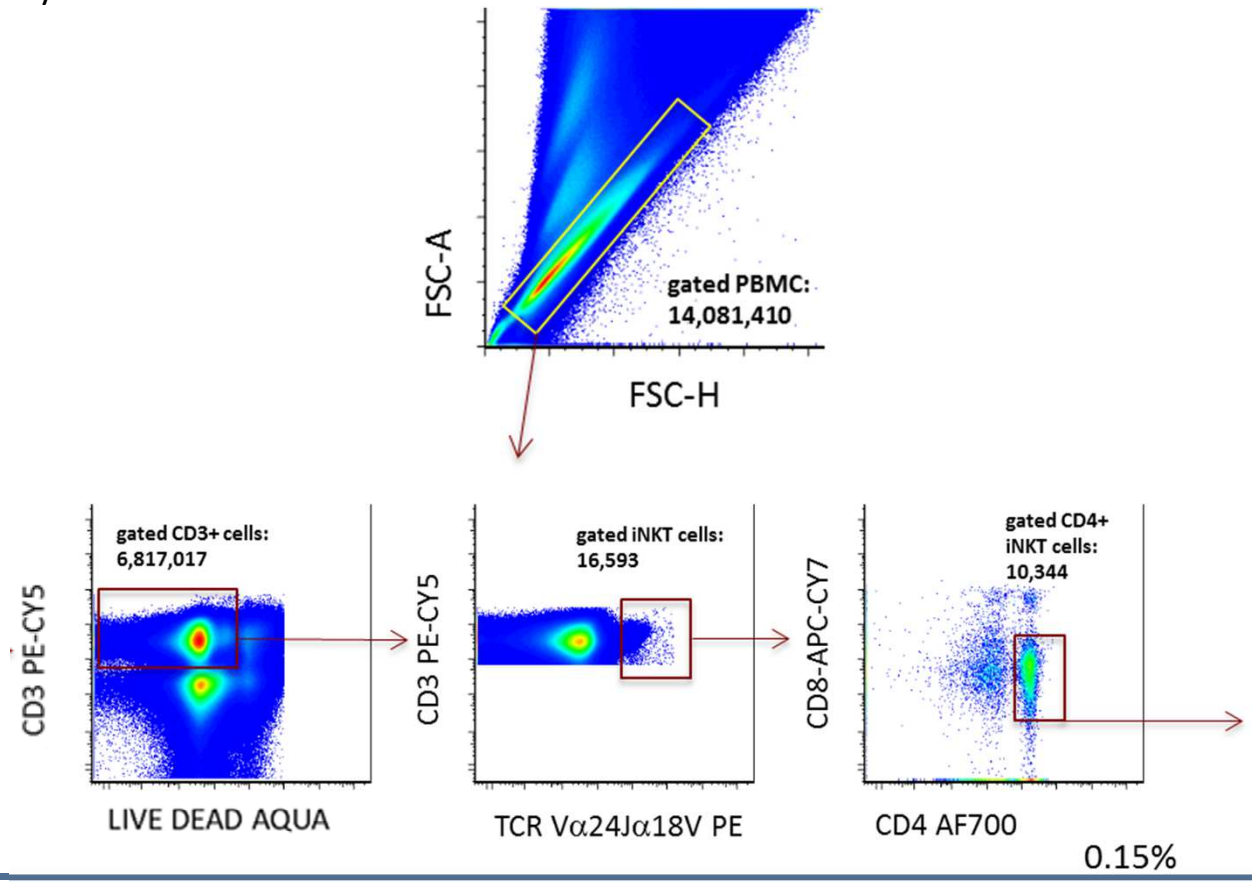
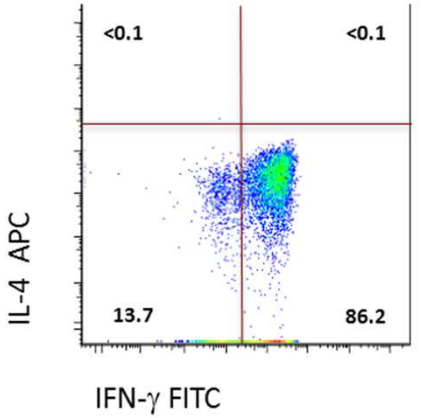
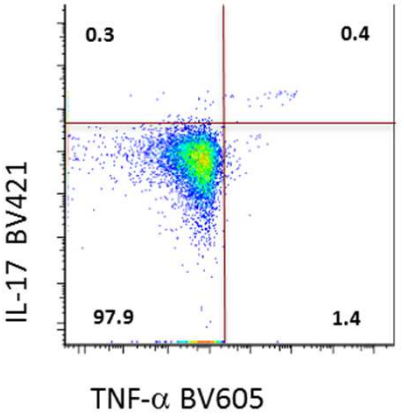
Rare Event Detection

Production of 4 different cytokines (IL-4, IL-17, IFN-g and TNF-a) by human peripheral blood iNKT cells that express CD4. Peripheral blood mononuclear cells (PBMC) were stimulated for 4 hours with 200 ng/mL PMA plus 1 ug/mL ionomycin at 37° C.







Data Courtesy of Andrea Cossarizza's lab (Modena, Italy)

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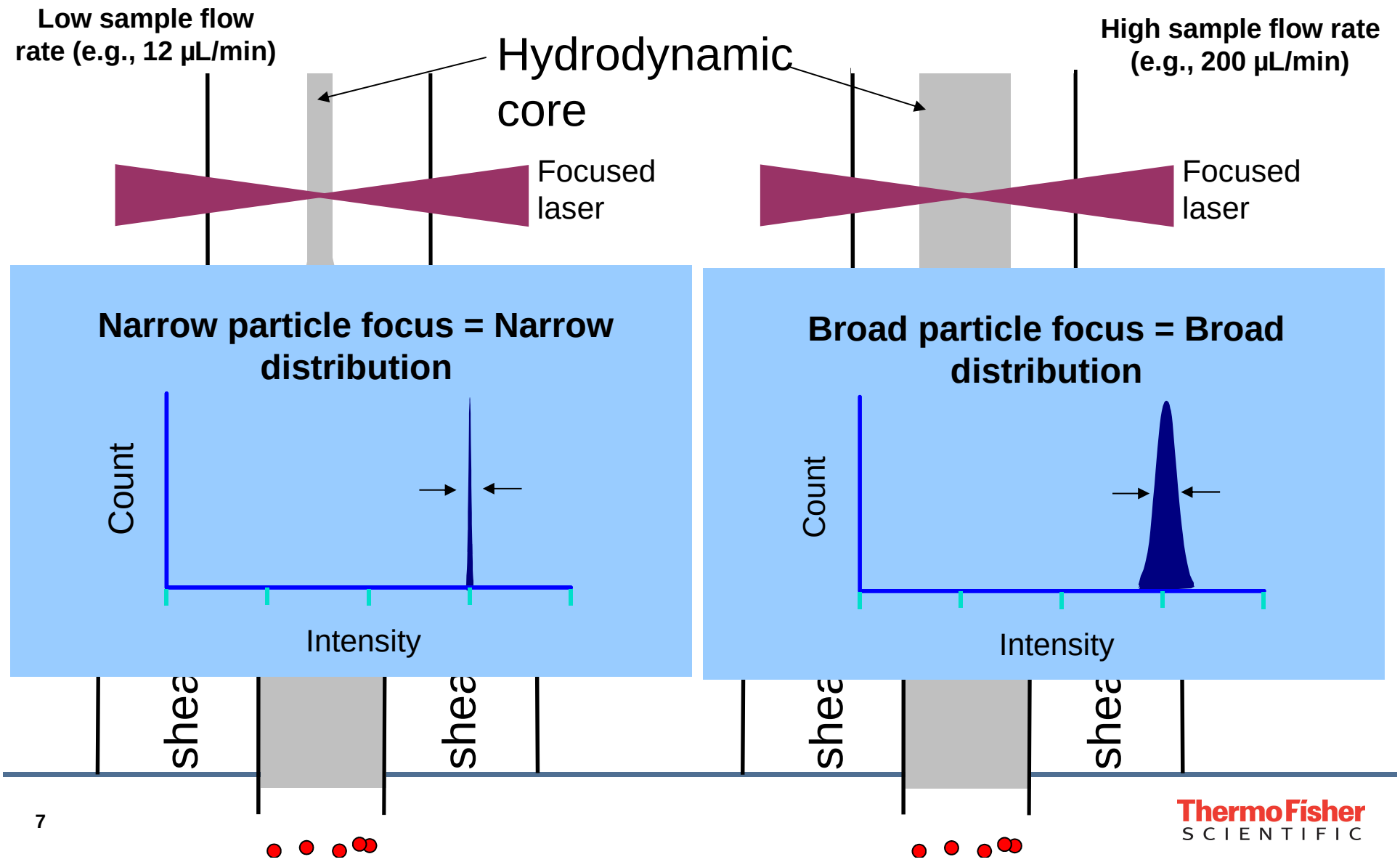
Rare Event Detection: Speed is the Key!!!

http://www.lavastorm.com/blog/2012/05/02/ouch-finding-a-needle-in-a-haystack-data-audits-in-the-big-data-era/

Traditional hydrodynamic focusing

Particle positioning in laser is important



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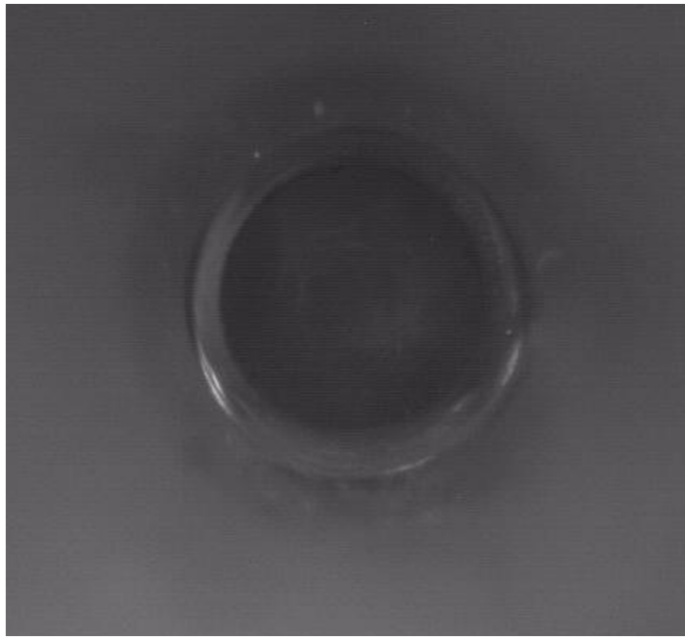
Acoustic-Focusing Technology

Acoustic Focusing
- Acoustic waves focus particles/cells
- Low CV's at any flow rate

Detection Speed:
Up to 35,000 events/sec

Sample input rates:
- 12.5 ul/min – **1 ml/min**
- 10X faster than traditional systems
- Cells tightly focused at the point of laser interrogation regardless of the sample-to-sheath ratio.



End-on view of capillary

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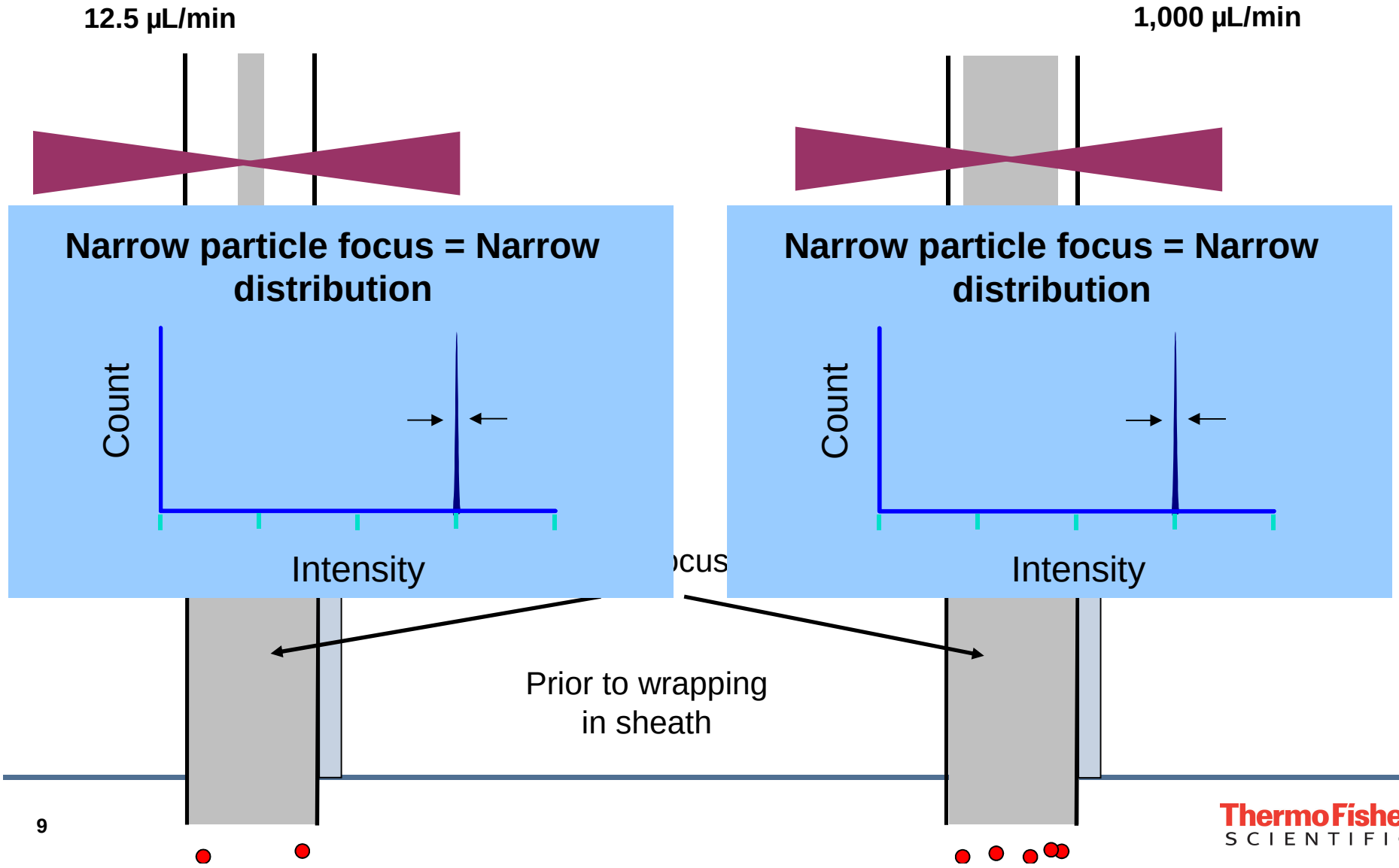
Acoustic focusing

High sample input flow rates allow for more sample flexibility



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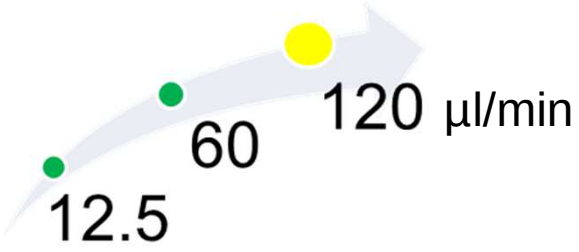
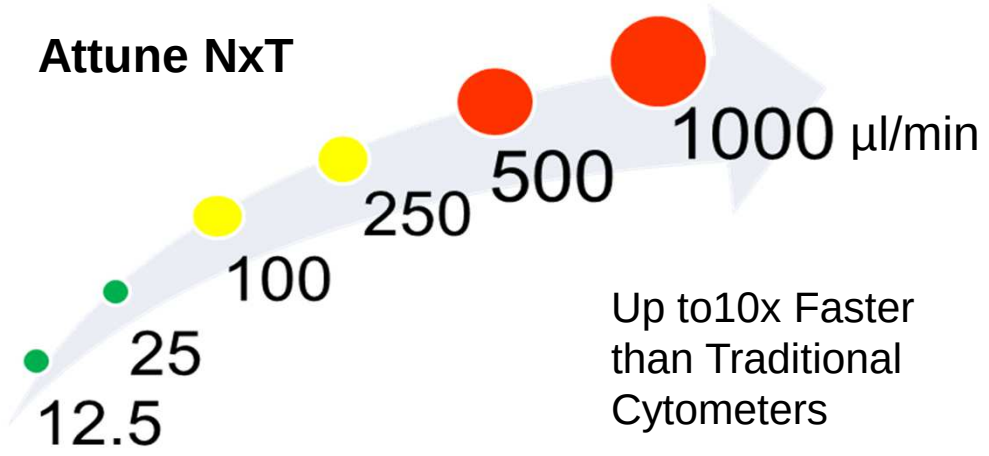
Comparable Results at Fast Detection Speed

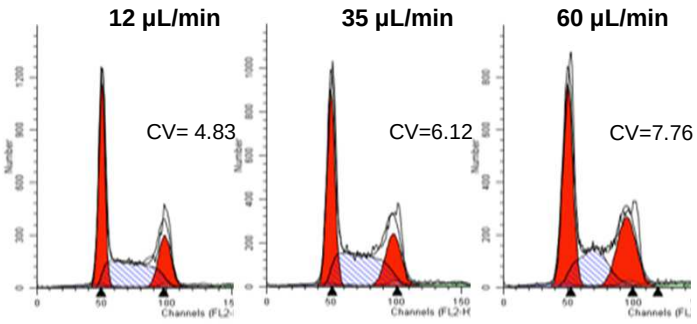
Traditional Cytometers



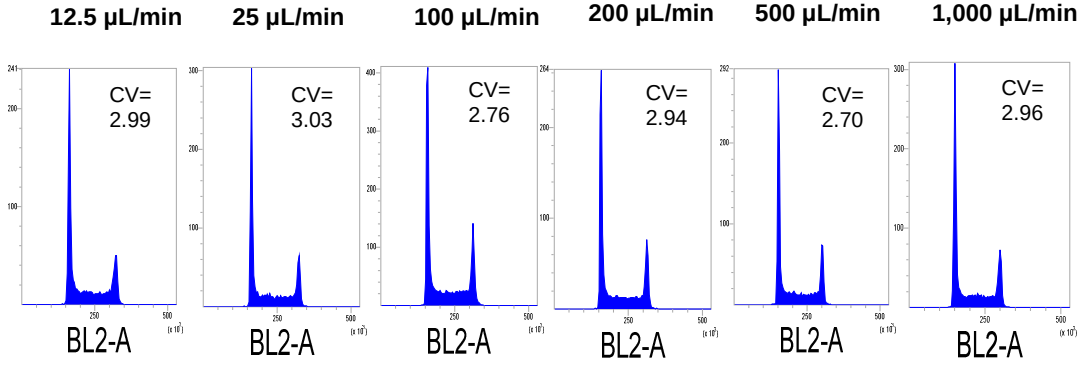
Attune NxT



Up to10x Faster than Traditional Cytometers



Hydrodynamic Focusing Only



Acoustically Enhanced Hydrodynamic Focusing

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Achieving Maximum Event Rates

- Event rate of a sample is universal and does not depend on the properties of the flow analyzer platform
- Event rate of a sample is determined by two values:
  - Sample input flow rate
  - Sample concentration

Sample Flow Rate (ul/min)

Sample Concentration (cells/mL)	12	25	60	100
1.0E+04	2	4	10	17
5.0E+04	10	21	50	83
1.0E+05	20	42	100	167
5.0E+05	100	208	500	833
1.0E+06	200	417	1,000	1,667
2.0E+06	400	833	2,000	3,333
4.0E+06	800	1,667	4,000	6,667
6.0E+06	1,200	2,500	6,000	10,000
8.0E+06	1,600	3,333	8,000	13,333
1.0E+07	2,000	4,167	10,000	16,667
2.0E+07	4,000	8,333	20,000	33,333

Most Flow Cytometry is Done at a Few Hundred Events/s

Dilute samples

Moderate samples

Only sample to achieve Maximum Event Rate

Achieving Maximum Event Rates

- Addition of Acoustic Assisted Hydrodynamic Focusing yield high event rates (even for dilute samples)
- Extended Sample Input Flow Rates allow for higher analysis rates (10x) at lower concentrations
- Maximum Event rate of instrument is not restricted to a corner case
- Centrifugation is now optional

Acoustic Assisted Hydrodynamic Focusing

Sample Input Flow Rate (ml/min)

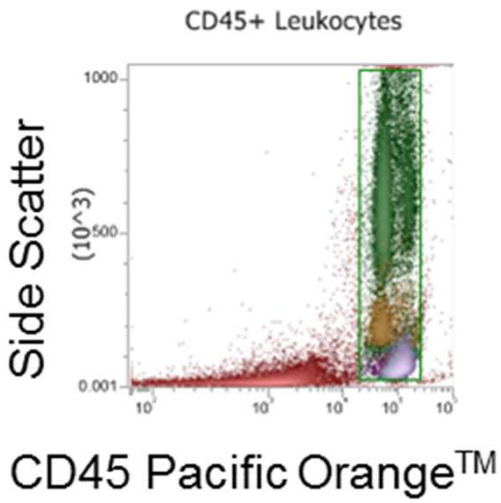
Sample Concentration (cells/mL)	12	25	60	100	200	500	1000
1.0E+04	2	4	10	17	33	83	167
5.0E+04	10	21	50	83	167	417	833
1.0E+05	20	42	100	167	333	833	1,667
5.0E+05	100	208	500	833	1,667	4,167	8,333
1.0E+06	200	417	1,000	1,667	3,333	8,333	16,667
2.0E+06	400	833	2,000	3,333	6,667	16,667	33,333
4.0E+06	800	1,667	4,000	6,667	13,333	33,333	
6.0E+06	1,200	2,500	6,000	10,000	20,000		
8.0E+06	1,600	3,333	8,000	13,333	26,667		
1.0E+07	2,000	4,167	10,000	16,667	33,333		
2.0E+07	4,000	8,333	20,000	33,333			

Improved Collection Times

- Start with 1 million cells (PBMCs) per ml
- Collect CD45 Positive Gated Cells

		Competitor A		Competitor B		Competitor C		Attune NxT	
	Cells	14 ul/min	66 ul/min	12 ul/min	120 ul/min	12 ul/min	60 ul/min	500 ul/min	1000 ul/min
Seconds	10,000	42.8	9	50	5	50	10	1.2	0.6
Minutes	100,000	7.1	1.5	8.3	0.8	8.3	1.7	0.2	0.1
Minutes	1,000,000	71.3	15.0	83.3	8.3	83.3	16.7	2.0	1.0
Hours	10,000,000	11.9	2.5	13.9	1.4	13.9	2.8	0.3	0.2

12 minutes



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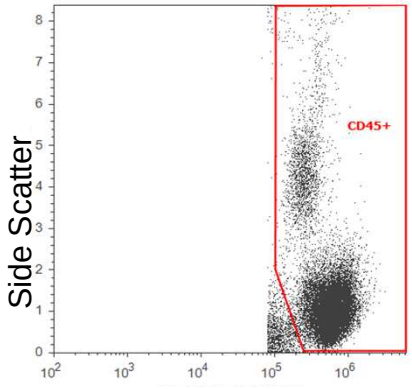
No-Lyse No-Wash Applications



Dilute your samples, not your data quality

Immunophenotyping mouse whole blood presents a challenge due to the limited sample volume available (≤100 µL/day/animal) particularly in longitudinal studies.

Small volumes limit the ability to perform multicolor phenotyping experiments with the required compensation and fluorescence-minus-one (FMO) controls

The sample dilution required in no-lyse, no-wash methods (to achieve low coincidence with red blood cells and platelets) generally dilutes the cell sample to such an extent that the time required to acquire sufficient events at the flow rates available in traditional instruments is extremely long.

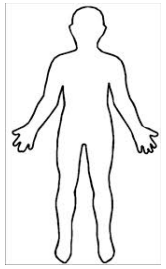
No-Lyse No-Wash Applications

Simplified sample preparation workflows

Generic sample preparation workflow

Collect Sample






Total sample prep time ~1.5-2.5hrs

Process to Single cell Suspension 5-10 min

Wash 5-10 min

Lyse red blood cells or enrich for white blood cells 30-60min

Wash 5-10 min

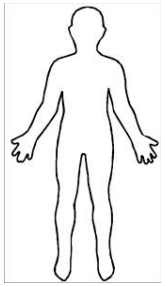
Stain 15-30 min

Wash 5-10 min

Acquire Samples

Add 1-2 hours for intracellular markers

No Lyse/No Wash sample preparation workflow




Collect Sample

Lyse red blood cells or enrich for white blood cells 30-60min

Wash 5-10 min

Stain 15-30 min

dilute

Wash 5-10 min

Acquire Samples

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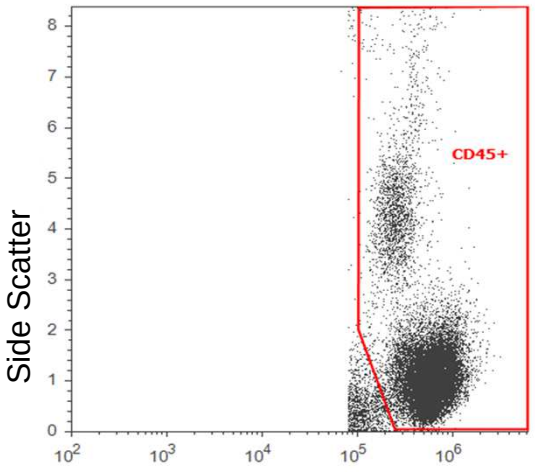
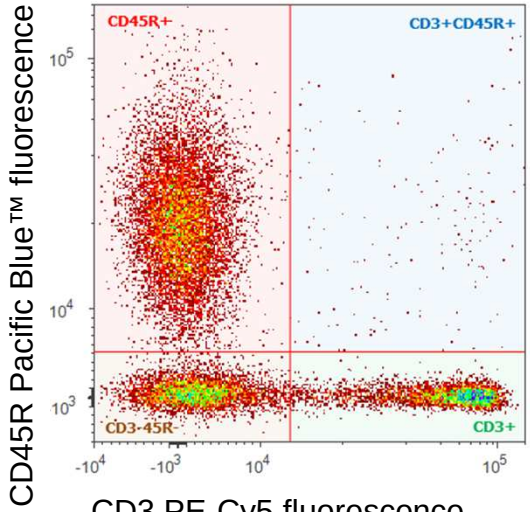
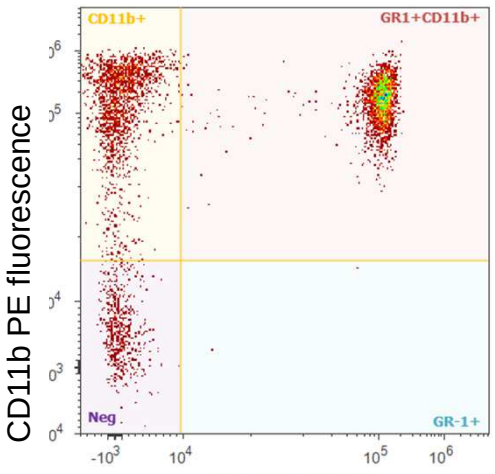
Mouse: No-lyse No-wash 5-color Immunophenotyping







- Use 5µl whole blood with 45µl antibody labeling solution
- Incubate 30 min RT
- Add 2mL PBS
- Use CD45 fluorescence for threshold to eliminate RBCs
- Run on Attune® at 500 µl/min
- Use AbC™ Total beads for compensation
- No cell prep necessary

CD45-FITC
CD45R-Pacific Blue™
CD11b-PE
CD3-PE-Cy5
GR-1-Pacific Orange™

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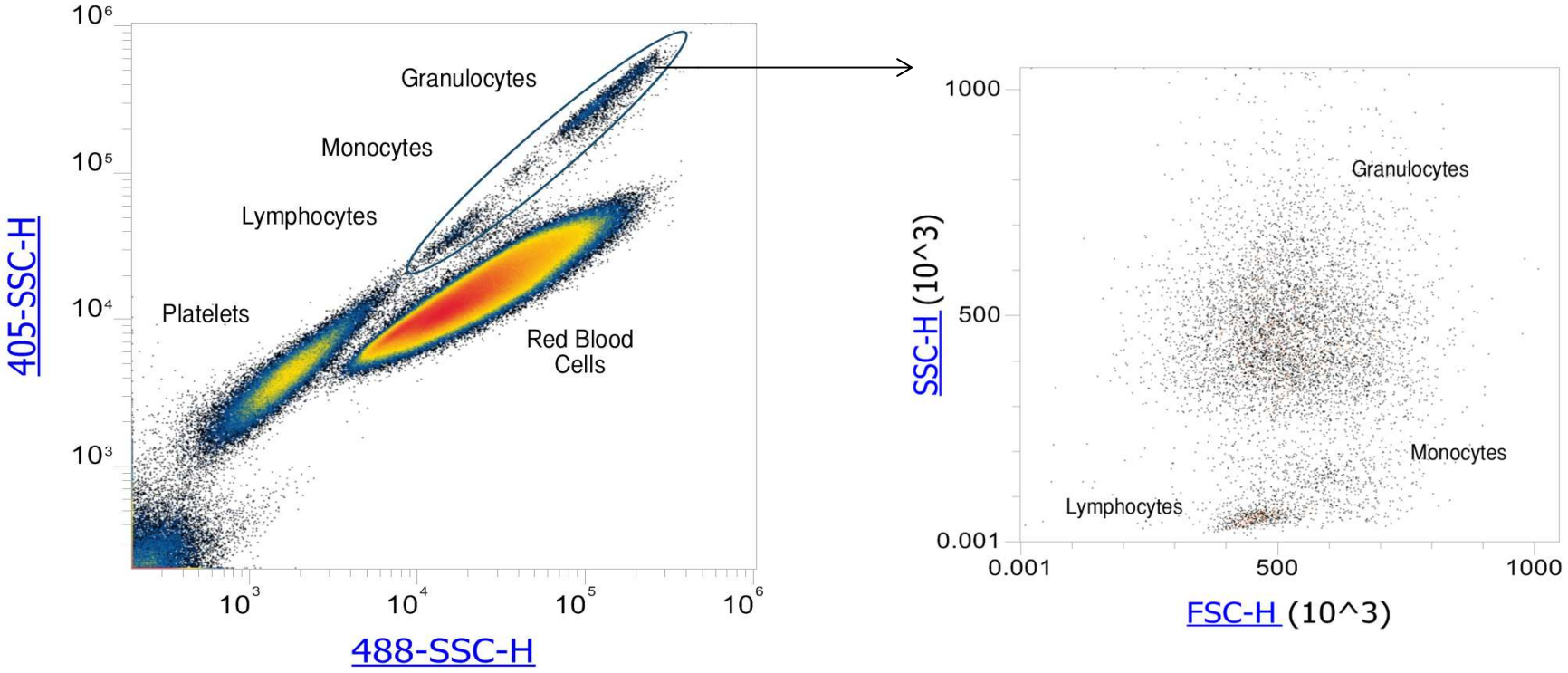
Benefits for NLNW Assay

- Saves sample preparation time
- Avoids cell loss and damage due to lysis and centrifugation
- Reveals true biology

NLNW using 405 and 488 nm SSC

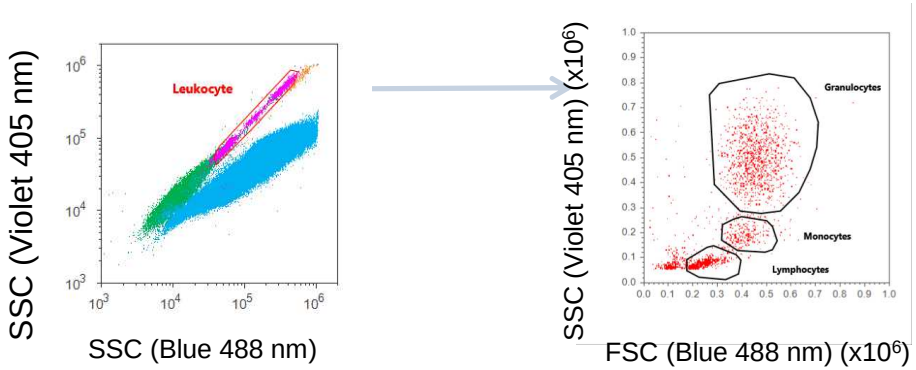


Data courtesy Jordi Petriz, Josep Carreras Leukemia Research Institute, Barcelona, Spain

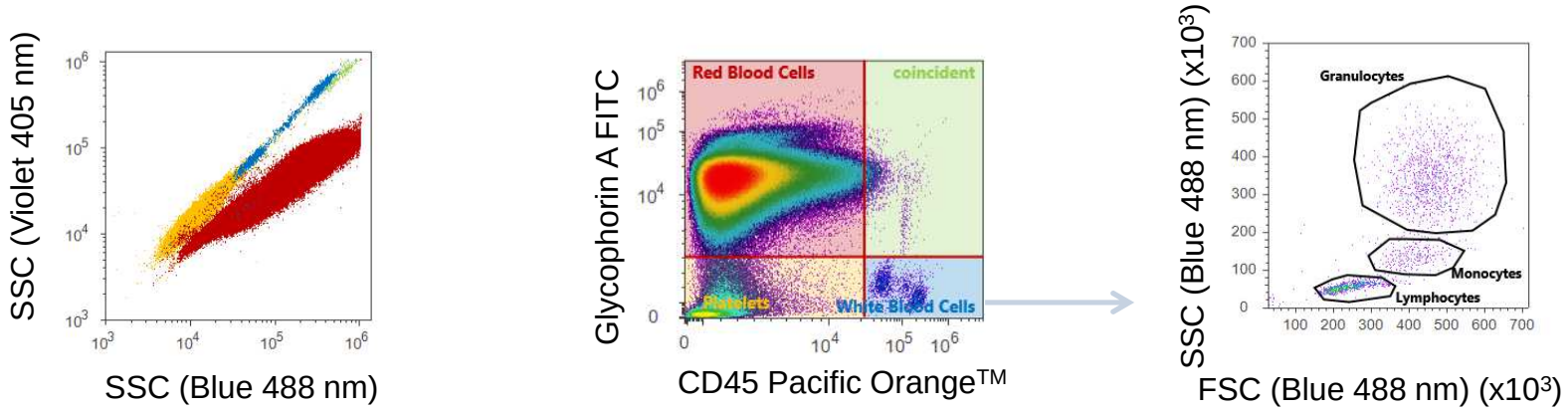
No Lyse/No Wash assays on the Attune® NxT

1. Dual SSC – Blue Laser SSC/Violet Laser SSC

- Utilization of Violet Scatter to differentiate white from red blood cells



2. **Fluorochrome-conjugated CD45 and Glycophorin-A Abs** can be used to differentiate white and red blood cells



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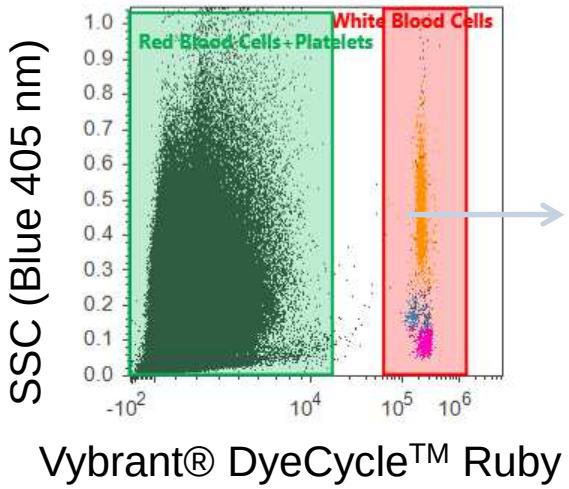
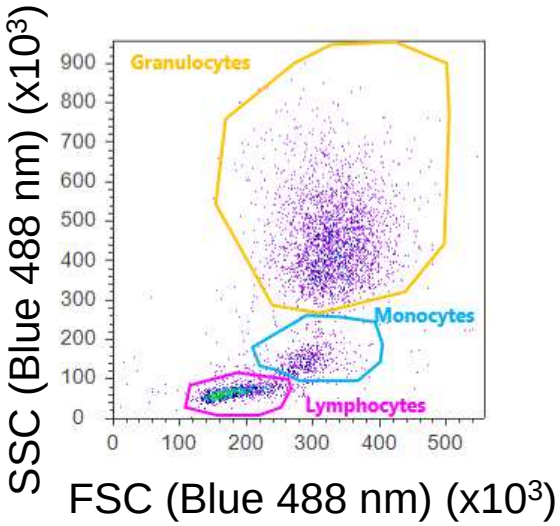
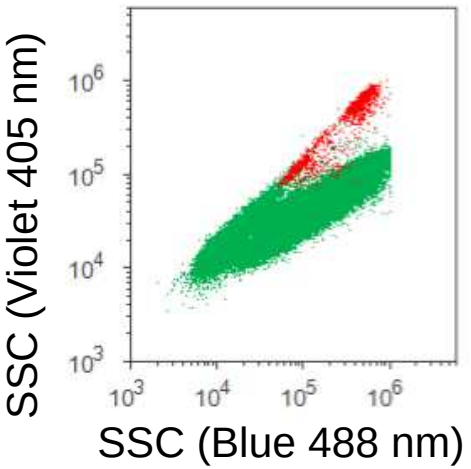
Live Cell DNA binding dyes

3. **DNA binding dyes**

Fluorescent probes that bind DNA in live cells can differentiate nucleated white blood cells from anucleate red blood cells







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Small in size, BIG in performance

- **Footprint (H x W x D):**
  - 16 in × 23 in × 17 in
  - 40 cm × 58 cm × 43 cm
- **Weight:**
  - 29 kg (64 lb)
- **Electrical requirements:**
  - 100–240 VAC, 50/60 Hz, <150 W

Smaller footprint in valued lab space

Smallest footprint on the market for a 4 laser system with individual laser pinhole collection and dedicated PMT's

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Attune NxT: Acoustic Focusing Flow Cytometer

Optics

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Flexible and Upgradeable Optical Configuration

Modular Design

- **Lasers:**
  - Choose from 1-4 Lasers
- **Detection Channels:**
  - FSC, SSC
  - Up to 16 Detection Channels

Four Laser Optical Configuration

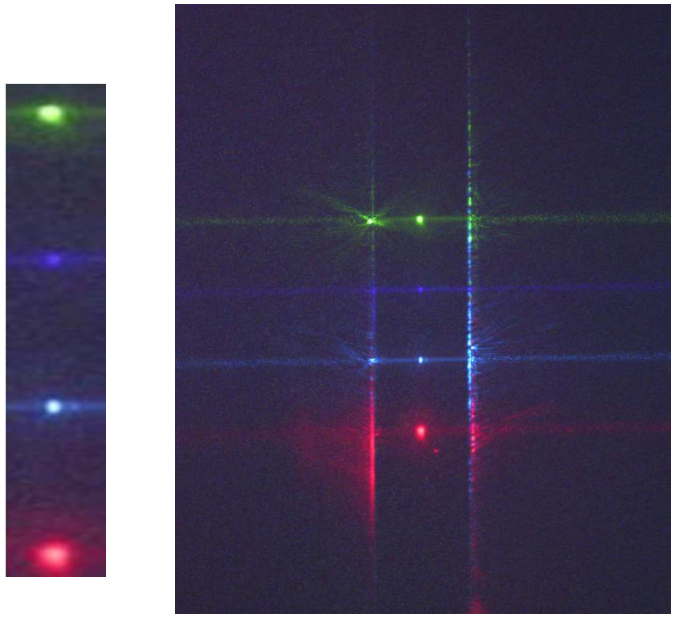
16 Detection Channels

- Violet 405 nm
  - 4 Colors
  - Optional SSC
- Blue 488 nm
  - 3 Colors
  - FSC/SSC
- Yellow 561 nm
  - 4 Colors
- Red 637 nm
  - 3 Colors

Excitation Laser	Emission Filter (nm)	Channel	Recommended Dyes
Violet - 405 nm	440/50	VL1	"Alexa Fluor® 405 Pacific Blue™"
	512/25	VL2	Pacific Green™
	603/48	VL3	Pacific Orange™ Qdot® 605
	710/50	VL4	Qdot® 705
Blue - 488 nm	530/30	BL1	Alexa Fluor® 488 FITC
	590/40	BL2	PE-Alexa Fluor® 610 PE-Texas Red® PE
	695/40	BL3	PE-Alexa Fluor® 700 Tri-Color® PE-Cy®5.5 PerCP PerCP-Cy®5.5 Qdot® 705
Yellow - 561 nm	585/16	YL1	PE
	620/15	YL2	PE- Alexa Fluor® 610 PE-Texas Red®
	695/40	YL3	PE-Alexa Fluor® 700 PE-Cy®5.5 Qdot® 705 Tri-Color®
	780/60	YL4	PE-Cy®7 Qdot® 800
Red - 637 nm	670/14	RL1	APC Alexa Fluor® 647 Qdot® 655
	720/30	RL2	Alexa Fluor® 680 Alexa Fluor® 700 APC-Alexa Fluor® 700 Qdot® 705
	780/60	RL3	APC-Alexa Fluor® 750 APC-Cy® 7 Qdot® 800

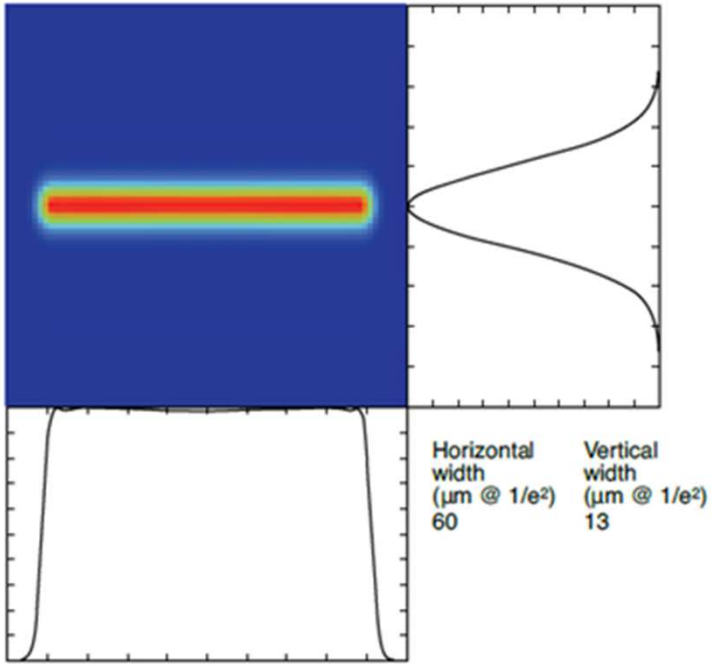
Spatially Separated Lasers

- **Spatially Separated Lasers**
  - All lasers spatially separated
    - Co-linear lasers limit the combination of colors with similar emission
  - Improved compensation for multi-color panels
    - More choices for colors
    - 6 color experiments with no compensation with 4 laser instrument
  - Minimal compensation for popular dyes
    - Example: FITC vs. PE



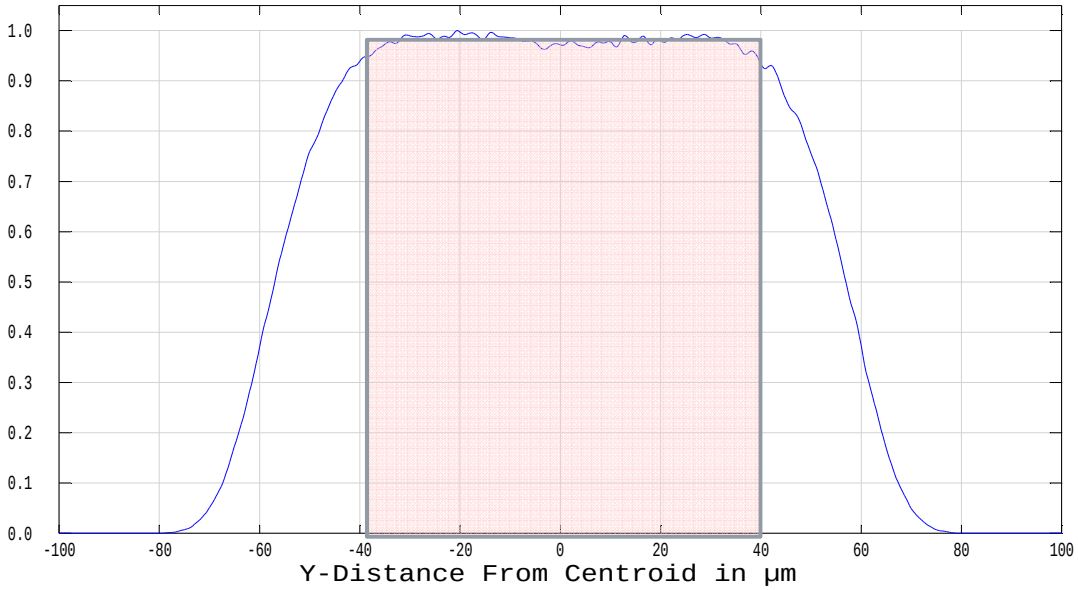
Beam Profile: Flat-Top Lasers

The Attune™ NxT Flow Cytometer uses flat-top lasers, with emission profiles which have an intensity profile that allows a much wider window of alignment.



Flat-Top Beam Shaping (>60% at 3-sigma)

Uses aspheric optics to "re-shape" the beam



Things to LOVE about the Flat-Top Lasers:

- ~2x reduction in scattered light
- CV guaranteed AT the flow cell
- Lateral shift? No problem

Alignment

With these lasers, slight shifts in the alignment do not affect sensitivity and CVs, because they have a higher tolerance for misalignment, allowing them to maintain high sensitivity and low CVs

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Lasers: Superior signal efficiency

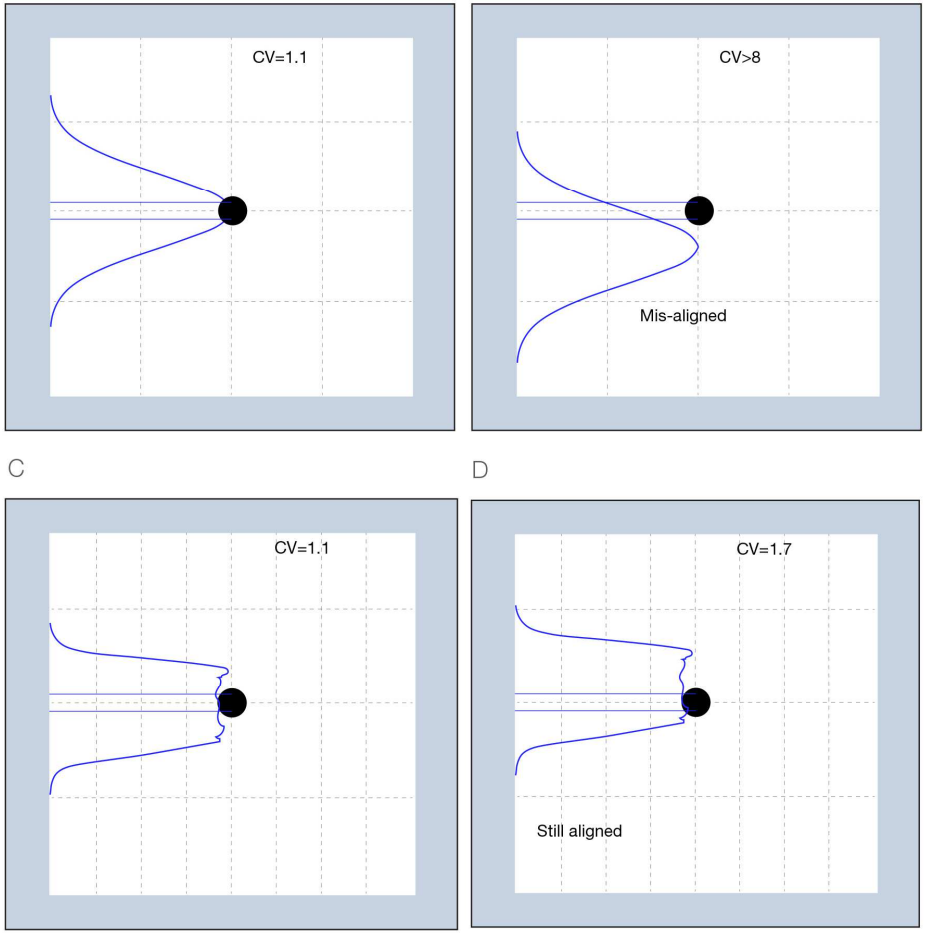
Emission profile:

Gaussian: Intensity increases until it hits a maximum, small window for alignment, prone to alignment shifts leading to loss of sensitivity and high CVs.

Flat-top lasers: 2x reduction in scattered light, CV guaranteed at the flow cell, and slight shifts in the alignment do not affect sensitivity

Flat-top vs. Gaussian Lasers

Flat top lasers reduce need for alignment adjustments



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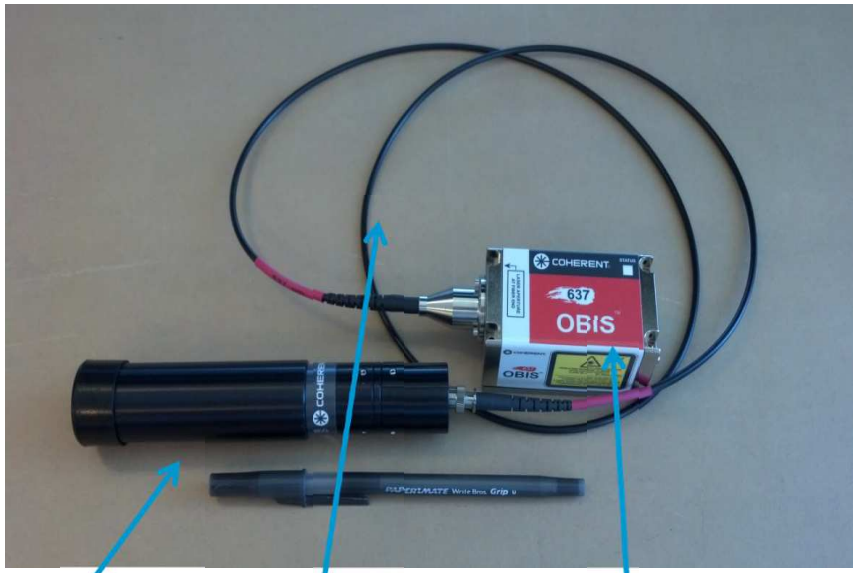
Maximized Laser Lifetime

Lasers Only on During Acquisition

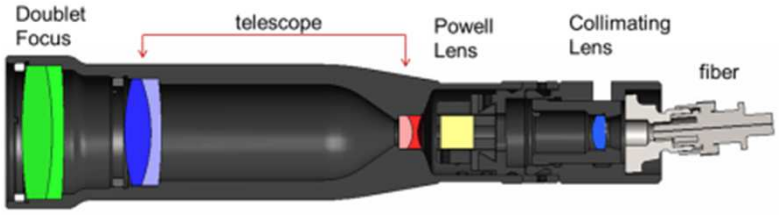
- 10X Increase in Lifetime
- Minimizes Down Time

- 50 mW 405nm
- 50 mW 488nm
- 50 mW 561nm
- 100 mW 638nm



- Pre aligned and welded fiber to laser interface

- Pre aligned fiber to BSO interface



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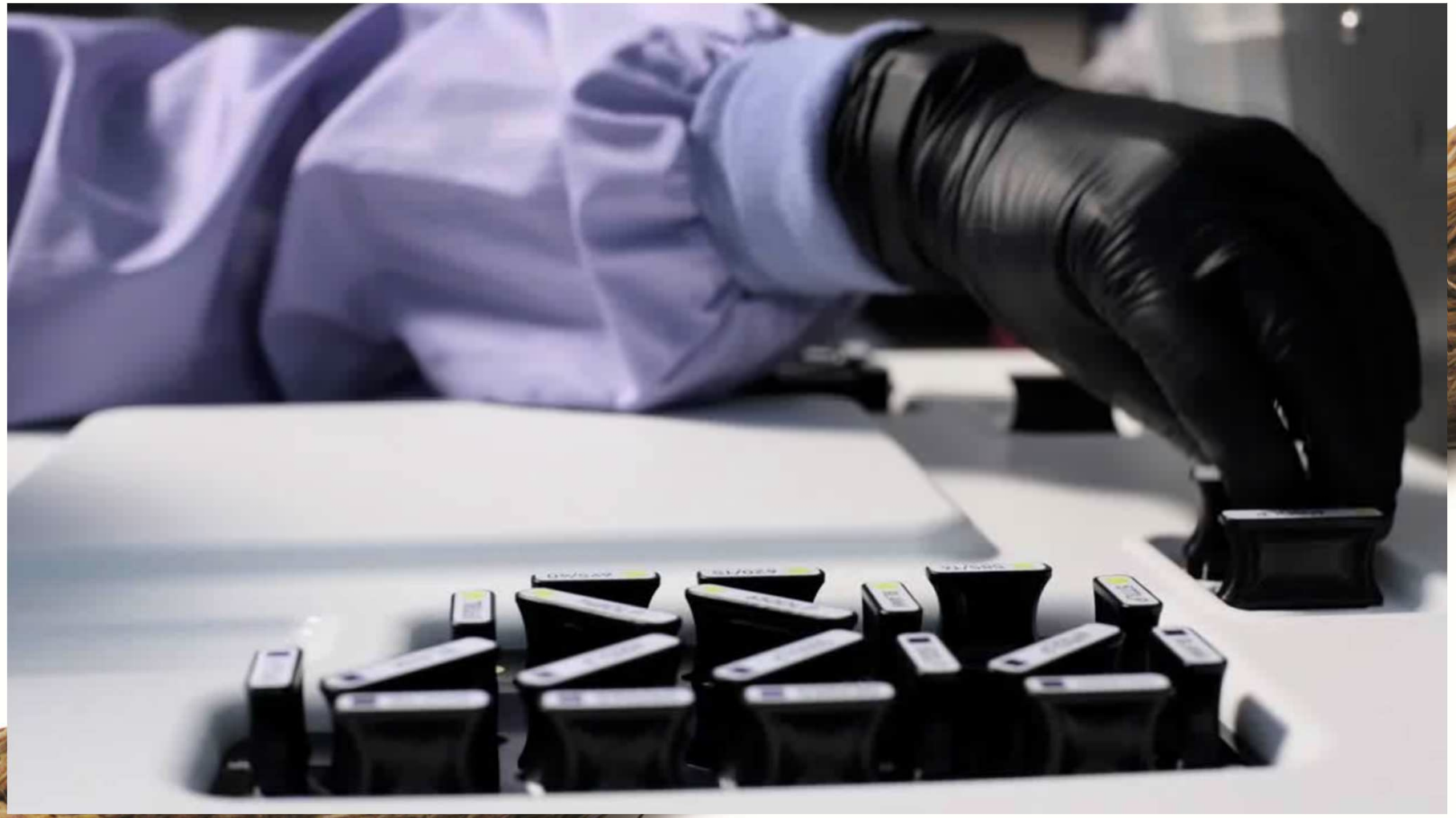
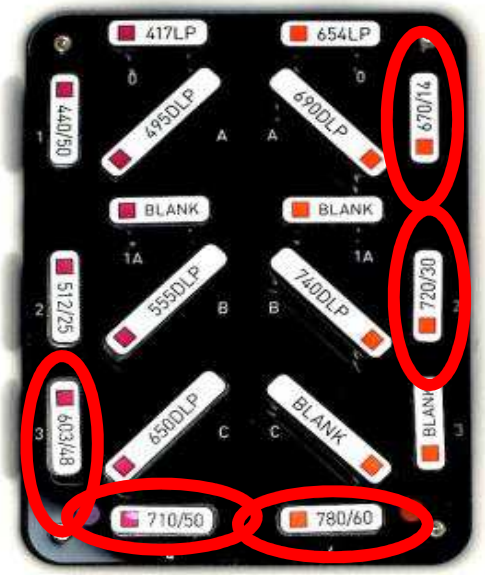
Exchangeable Optical Filters

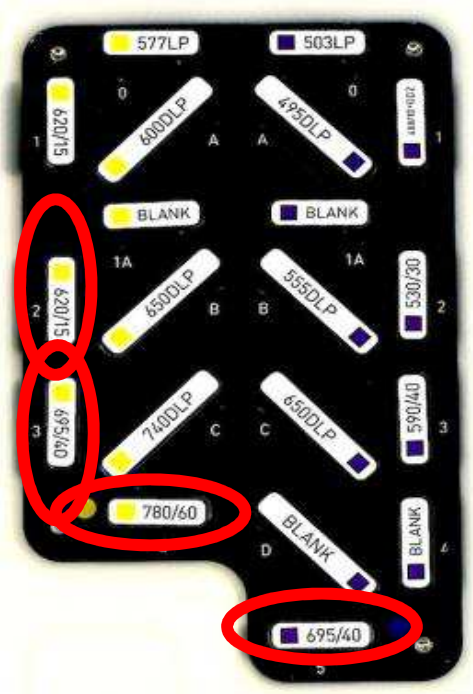


Detectors Optimized to Individual Channel Spectrum

- Hamamatsu PMT's customized for reduced noise
- Detectors optimized to the spectrum of each individual collection channel

Fiber-Coupled Collection Blocks





- Enhanced Multialkali Red Sensi

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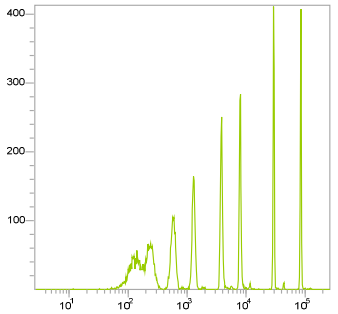
Superior sensitivity and precision

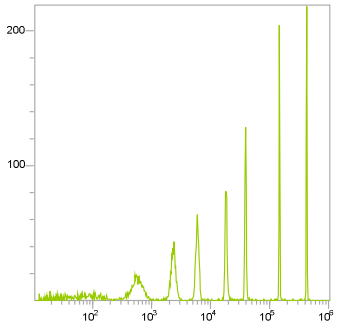
Attune® NxT cytometer demonstrates precision across all flow rates

Fluorescence sensitivity with 8 Peak Rainbow Beads:

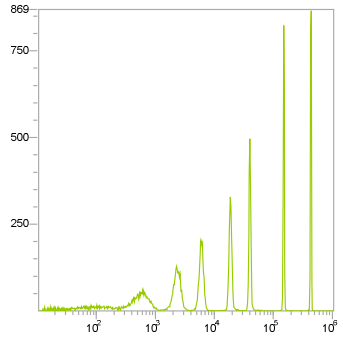
- ≤80 MESF FITC
- ≤30 MESF PE
- ≤70 MESF APC



Hydrodynamic instrument at 12.5 µL/min



Attune® NxT 12.5 µL/min



Attune® NxT 500 µL/min

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Attune NxT: Acoustic Focusing Flow Cytometer

Fluidics

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On Board Fluidics

- **Fluid storage:**
  - All fluids stored within instrument with active fluid level sensing
- **Standard fluidic tanks:**
  - 1.8 L focusing fluid tank
  - 1.8 L waste tank
  - 175 mL shutdown solution tank
  - 175 mL wash solution tank
- **Nominal fluid consumption:**
  - 1.8 L/day

Easy access to tanks for refilling

Smooth and Accurate Sample Delivery

- **Sample delivery:**
  - Sample delivered by positive displacement syringe pump for volumetric analysis
- **Volumetric cell counts:**
  - Live/dead analysis
- **Sample tube format:**
  - Any tubes from 17x100mm to 8.5x45mm
    - 5mL conical tubes
    - Eppendorf tubes
- **Sample analysis volume:**
  - 40 µL–4 mL
- **Sample rates:**
  - 12.5–1,000 µL/min
- **Unused sample return option:**
  - Sample returned into plate or tubes

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Clog Resistant

- Attune® NxT uses a positive displacement syringe pump for the sample
  - ✓ Samples are pushed through the system with a direct displacement (and volumetric) pump
  - ✓ Pump pushes potential clogs through with pressure that builds steadily if there is a restriction

- Attune® NxT flow cell is 200 um in diameter. This is relatively large in the industry

- Attune® NxT flow path is back-flushed after every sample
  - ✓ Back-flush is into an orifice that is larger than the sample path
  - ✓ Any accumulation in the forward path is cleared back into a larger orifice after every sample

Attune® Autosampler

Efficiency with high sample throughput

- Designed for minimal clogging
- Compatible with standard and deep well 96/384-well plates
- Simultaneous control of tubes or plates
- Easily attached and detached from instrument
- Automated cleaning for easy maintenance

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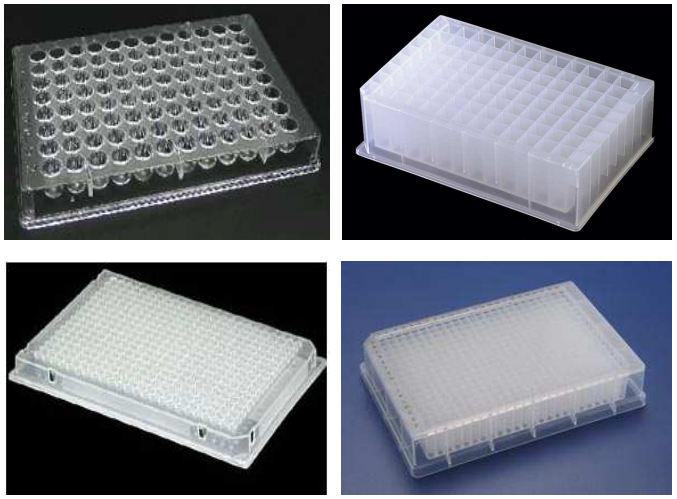
Attune® Autosampler

Compatible plate types

- Flat, round, and V-bottom
- 96 deep well
- 96 well, standard depth
- 384 deep well
- 384 well, standard depth



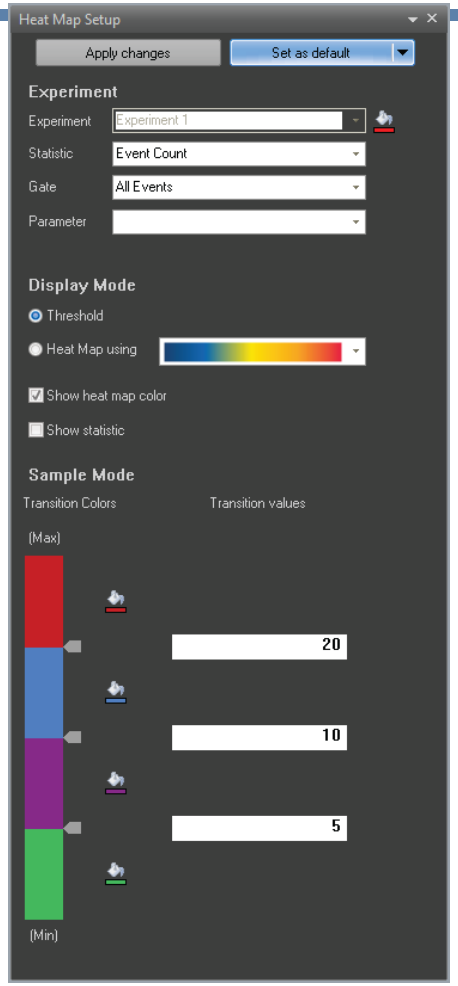
Mixes sample by aspiration

- Enable homogeneity of sample
- Maintain cell viability

Built-in flexibility

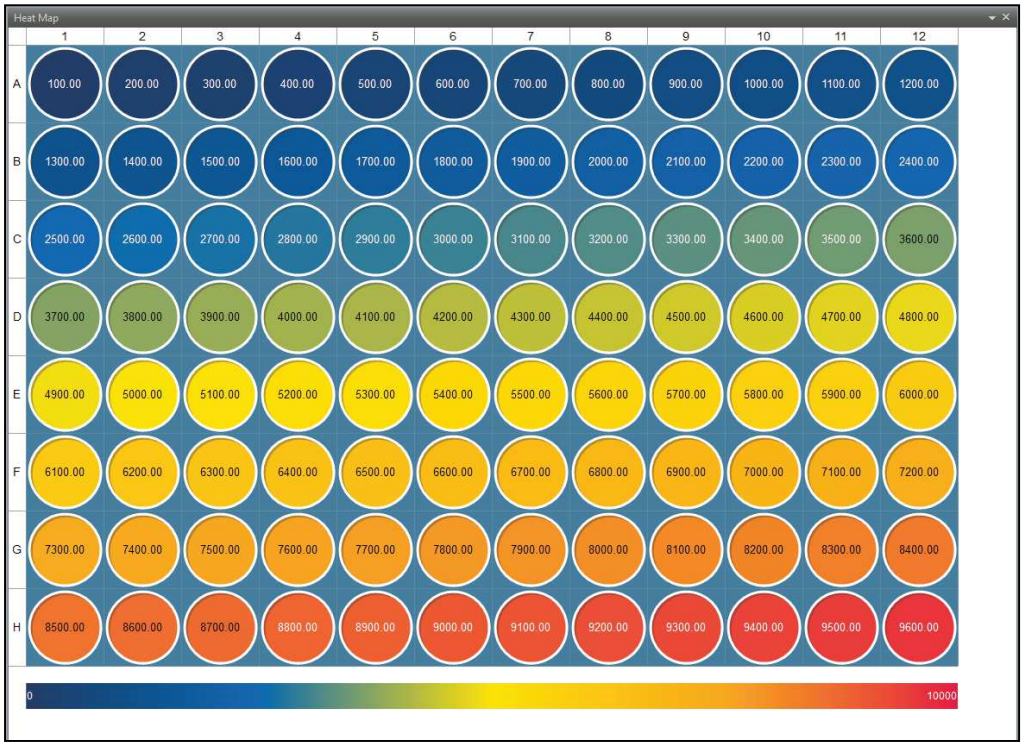
- Choose number of washes
- Choose amount of mixing
- Choose number events or volume to be analyzed

Heat Mapping



The values overlaid on each well in the heat map are the measured percentages of dead cells in the individual wells. Minimal variation is observed in propidium iodide fluorescence across the entire plate, with a CV of 1.44% for the entire data set (96 wells).

The Attune™ NxT Autosampler heat map function identifies variation within a parameter across a 96-wellplate. Live and heat-killed THP-1 cells were stained with 2 µg/mL propidium iodide, dispensed into a 96-well V-bottom plate, and run at a Standard collection rate of 500 µL/min with 2 mix cycles per well and 2 rinse cycles between wells. Propidium iodide was excited using a 488 nm laser (640 LP).



On the **heat map**, a color gradient graphically represents the percentage of propidium iodide–positive cells (dead cells).

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Attune NxT: Acoustic Focusing Flow Cytometer

Attune NxT Software

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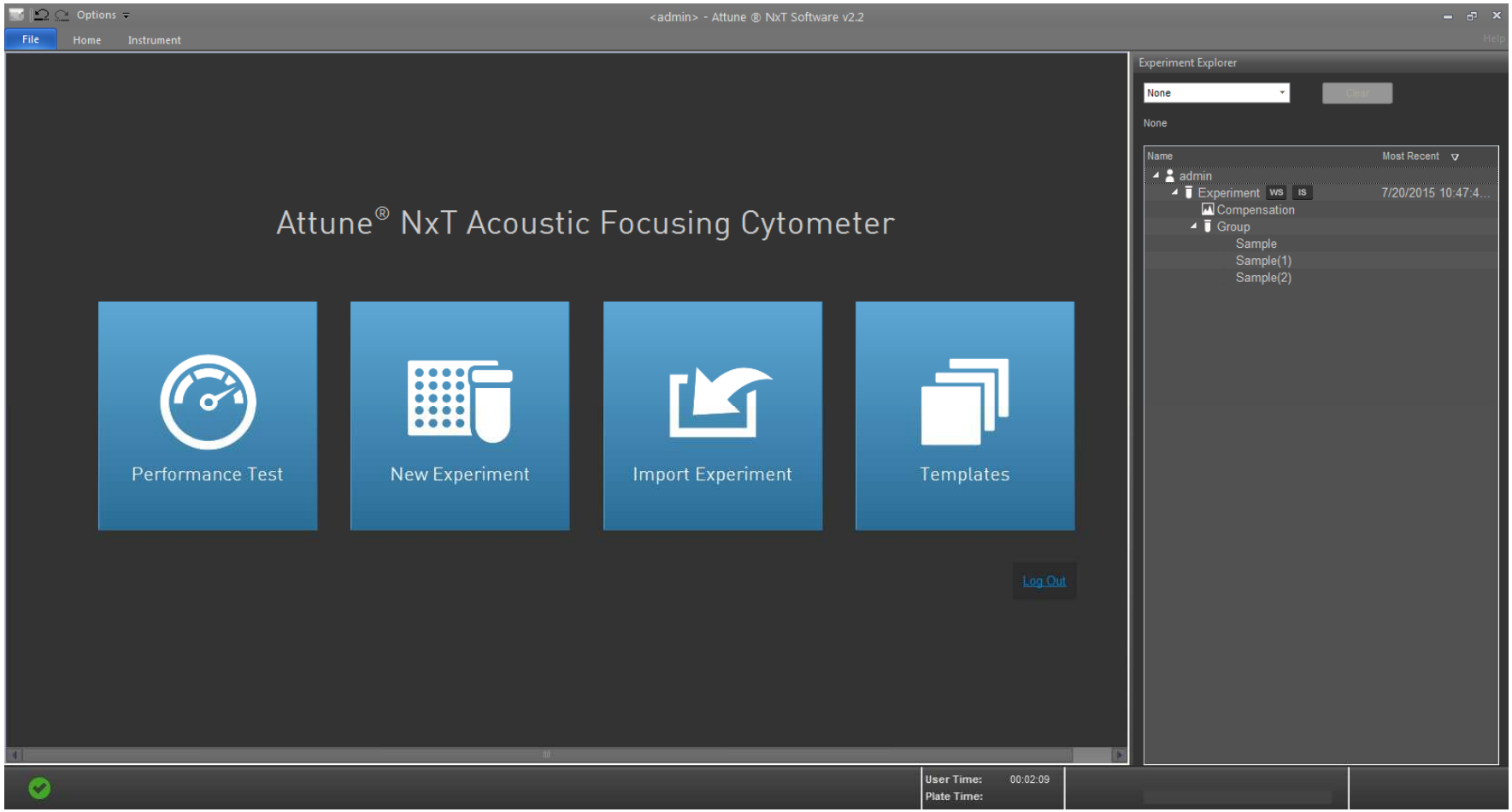
Home Page



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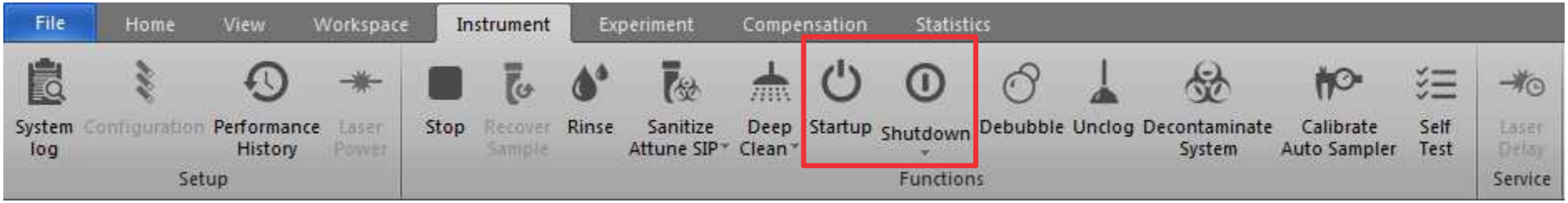
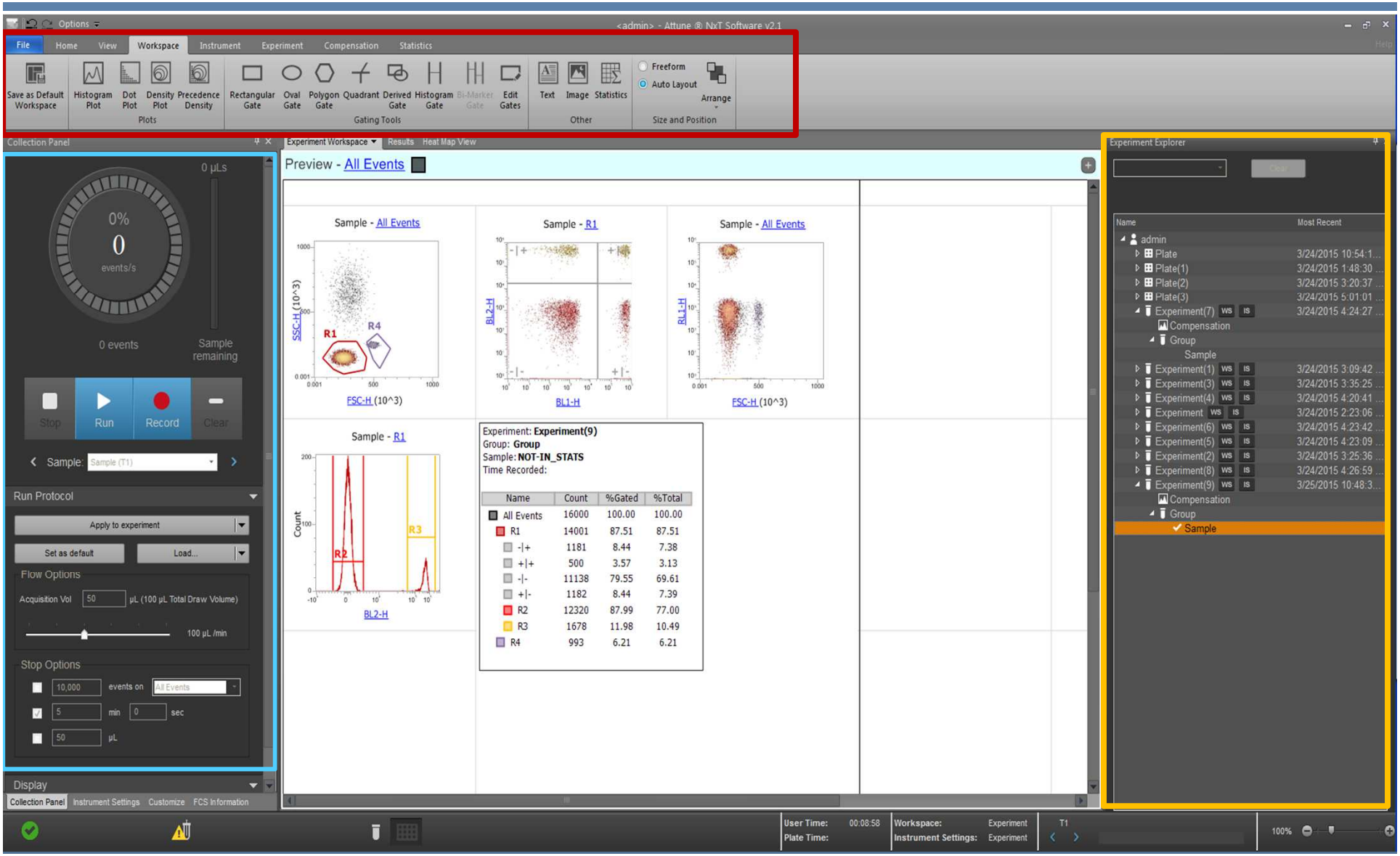
Instrument Control



- Software-controlled fluidics function:
  - Startup: One click, <5 min and ready to go
  - Shutdown: One click, load 3mL of 10% bleach and just leave

Workspace: Decreased Learning Curve



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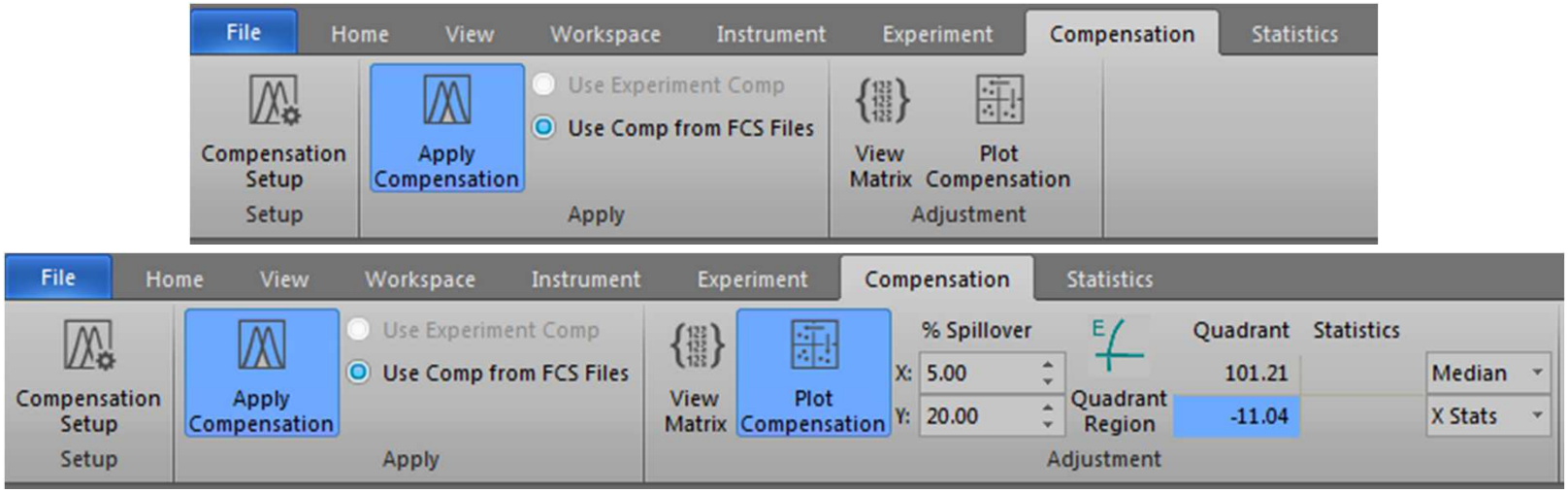
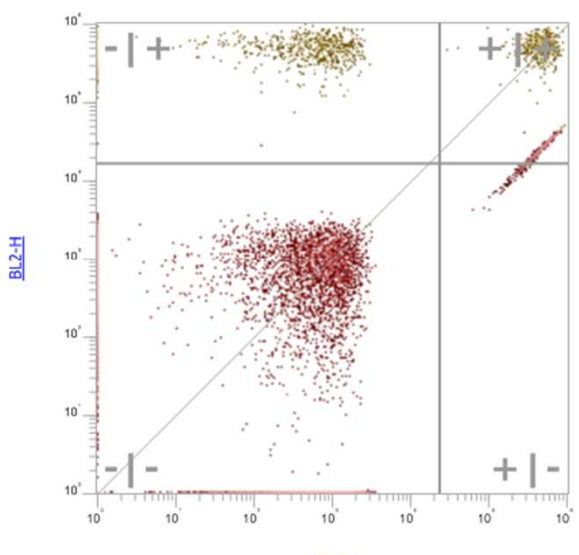
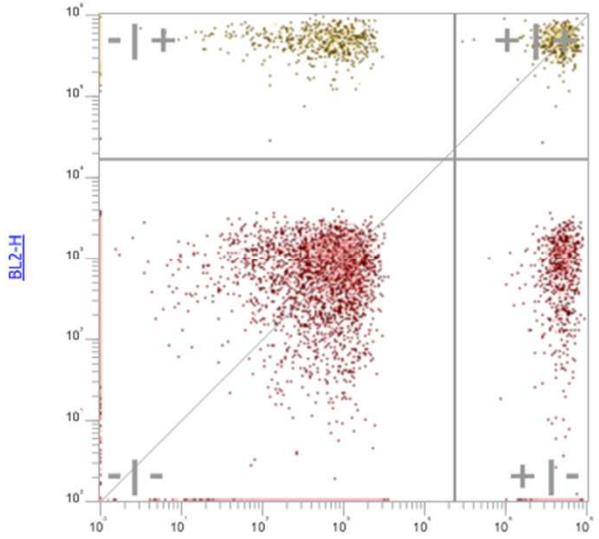
On-Plot Compensation Adjustment



Simple Interface for Compensation



On Plot Compensation - Pre



On Plot Compensation - Post

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Customer’s Vioce

http://www.selectscience.net/SelectScience-TV/Videos/enhanced-identification-and-isolation-of-cancer-cells-using-acoustic-technology/?videoID=3348

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Attune® NxT Acoustic Focusing Cytometer

Summary:

- **Modular Design:**
  - 1 – 4 Lasers
  - 6 – 16 Detection channels
- **Acoustic Focusing:**
  - 10X faster than traditional cytometers
  - Minimal loss in data quality when going fast
- **Automation**
  - Flexibility for customers
  - Consistency across plate/between plates
- **Robust:**
  - Flat-top lasers
  - Clog detection
- **Easy to learn and use:**
  - User guides
  - Intuitive interface

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Questions?

- Mobile Apps
  www.thermofisher.com/apps

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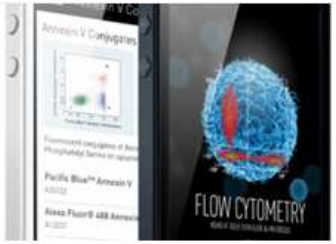

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