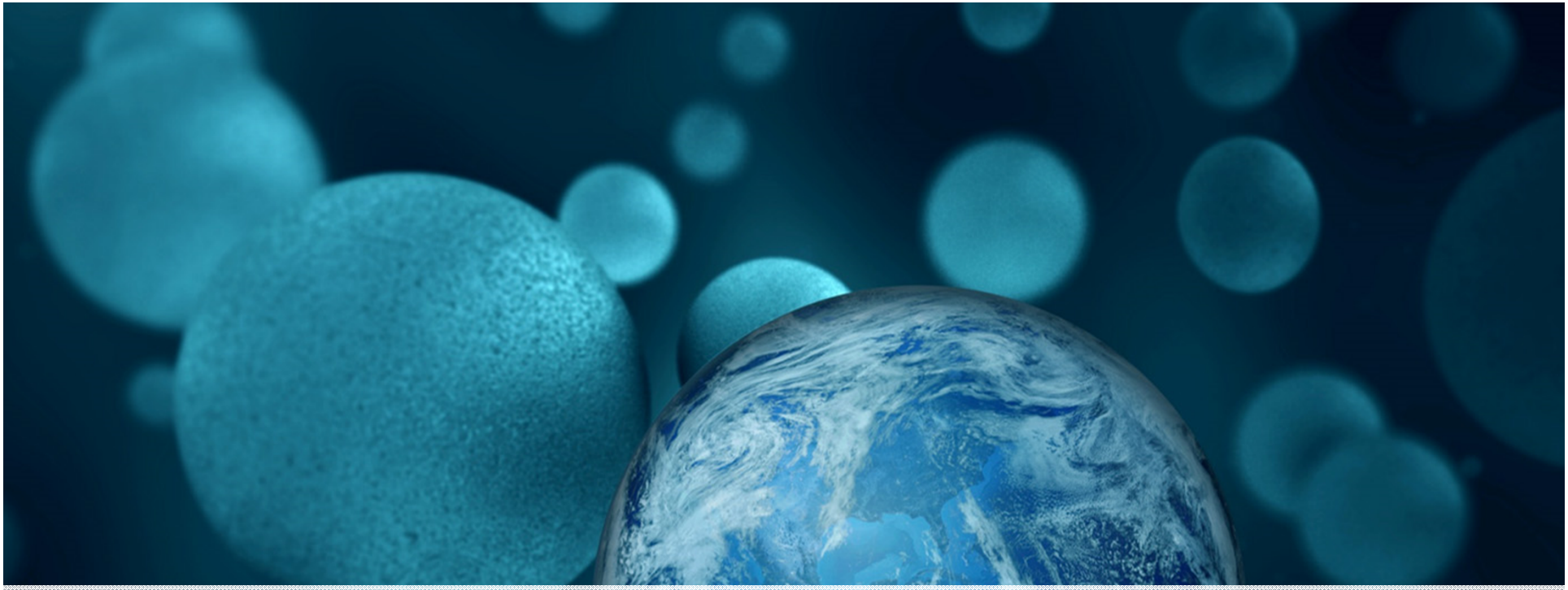

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Better, Stronger, Faster - your BY-onic Cytometer

August 15, 2018
Dolores Ciufo, FAS

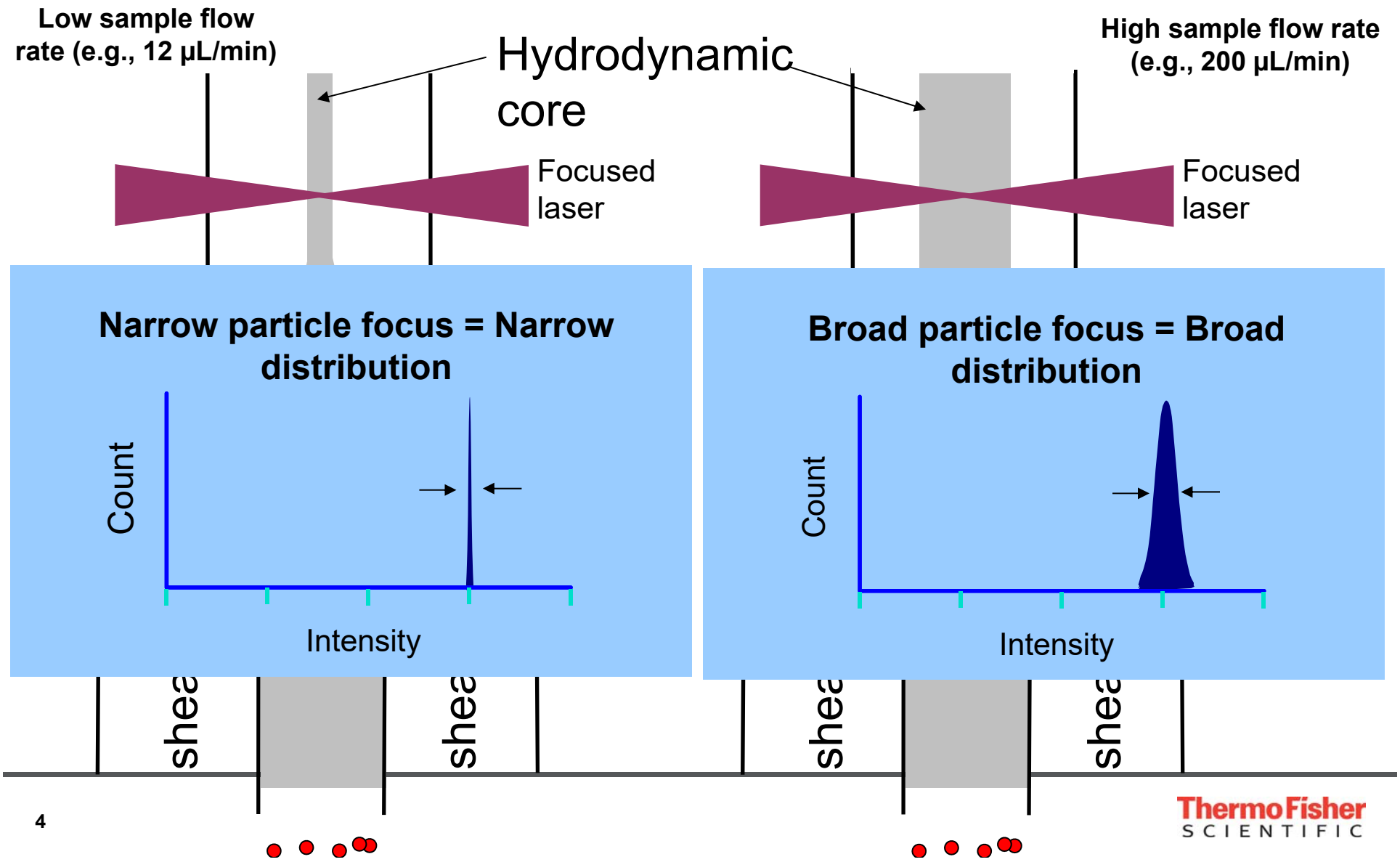
	Instrument	Lasers (excitation beam); Pre-set configuration (nm)						Number of fluorescent detectors (by laser)
		I Laser	II Laser	III Laser	IV Laser	V Laser	VI Laser	
SPECTRAL Analyzer	Cytek Aurora	488	405	640	561	no	no	**48** (14/16/8/10)
ADVANCED Analyzers	BD LSR II-1	488	405	355	640	561	no	**16** (3/4/2/3/4)
	BD LSR II-2	488	405	445	640	561	no	**16** (3/4/2/3/4)
	BD LSR-Fortessa	488	405	355	640	561	no	**18** (3/5/3/3/4)
	ThermoFisher Attune NxT	488	561	no	no	no	no	**7** (3/4)

Agenda

- Attune® NxT™ Systems
  - Instrument
  - Autosampler
- Fluidics and Optics
- Best practices
- Software overview

Traditional hydrodynamic focusing

Particle positioning in laser is important



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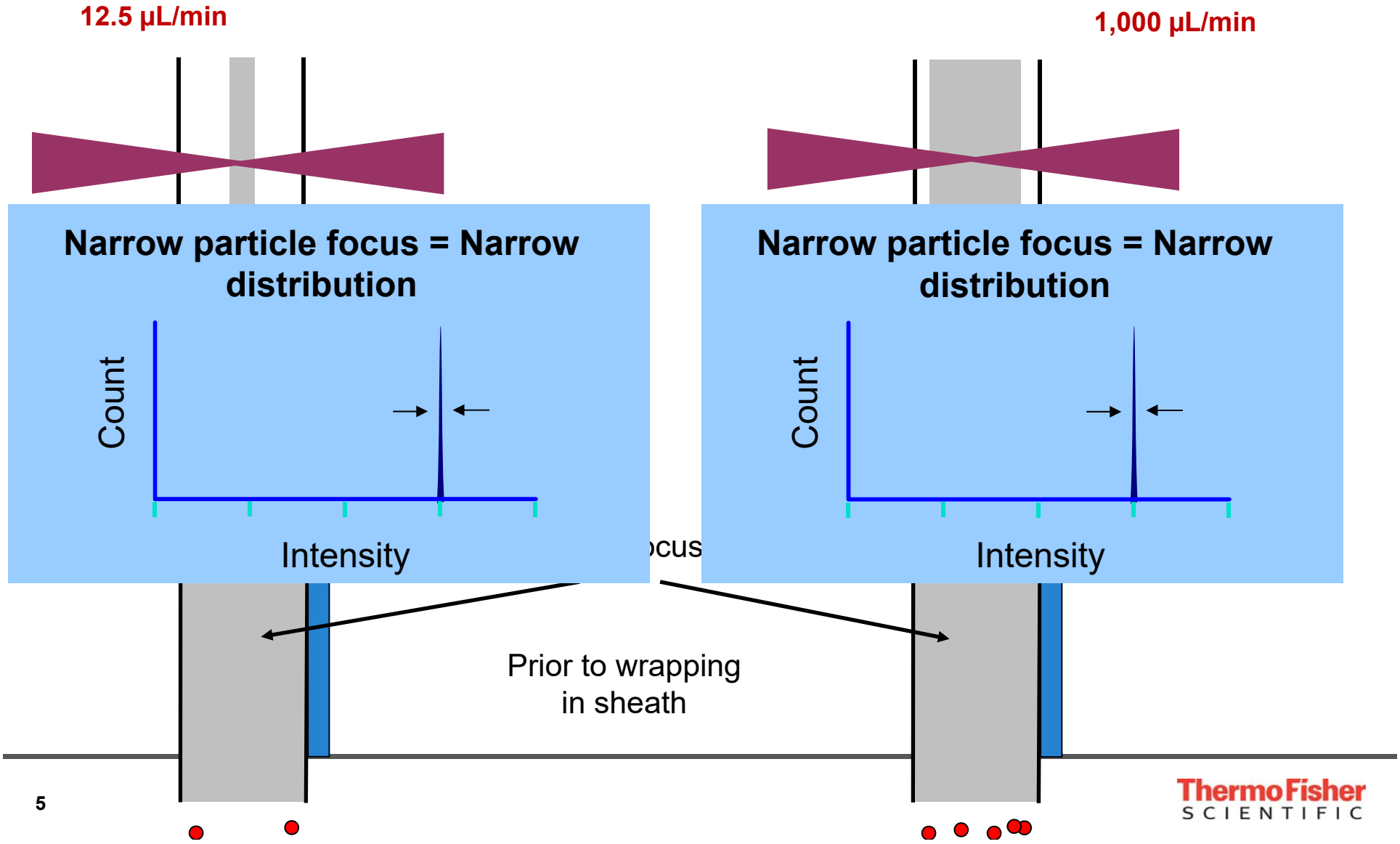
Acoustic assisted hydrodynamic focusing

High sample input flow rates allow for more sample flexibility



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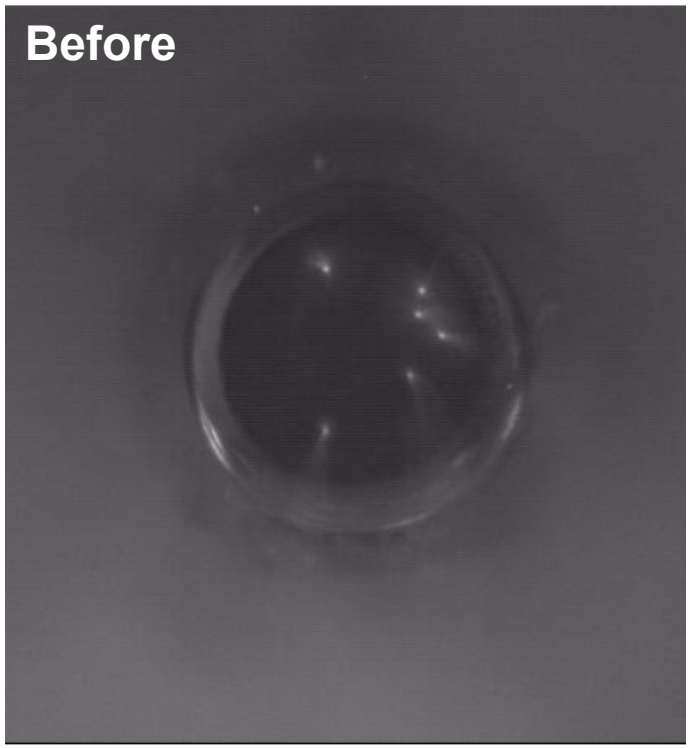
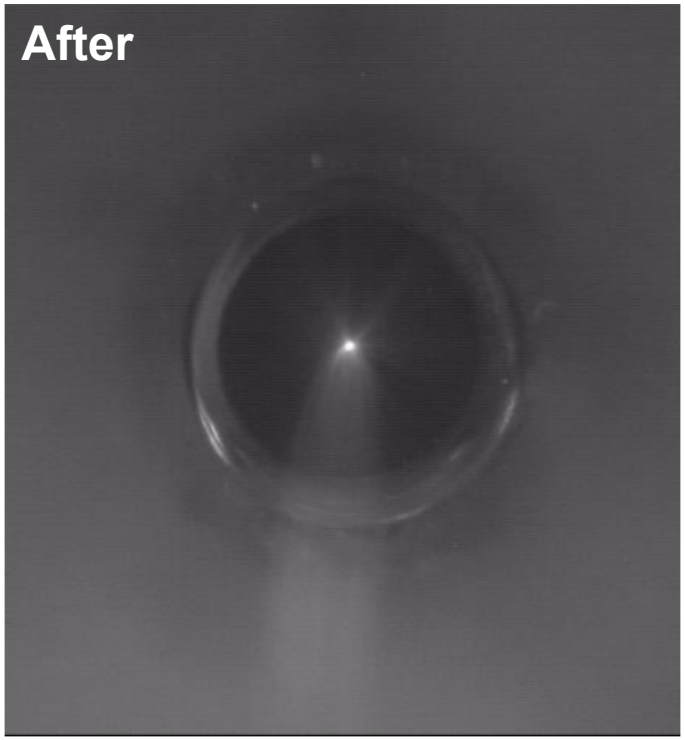
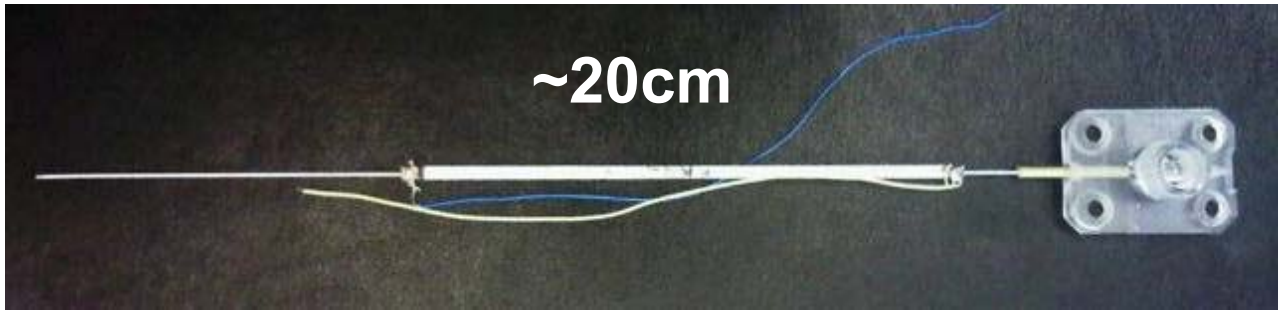
Acoustic focusing

End-on view of capillary

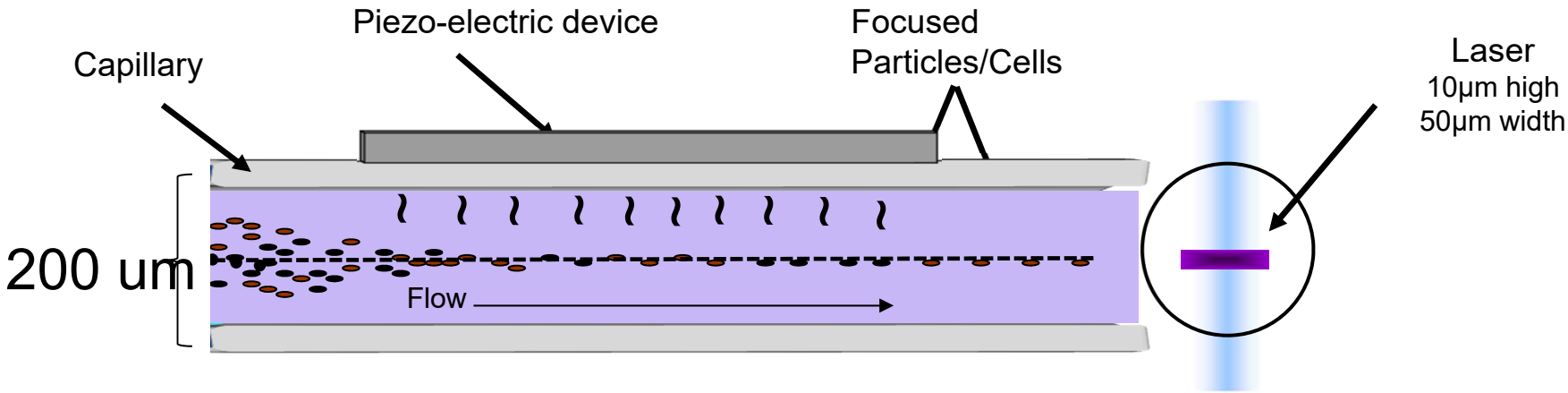




Acoustic Focusing Capillary



Acoustic Waves – similar to ultrasound used to visualize a fetus *in utero*



- Flow rate can be increased while maintaining resolution

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Clog resistant

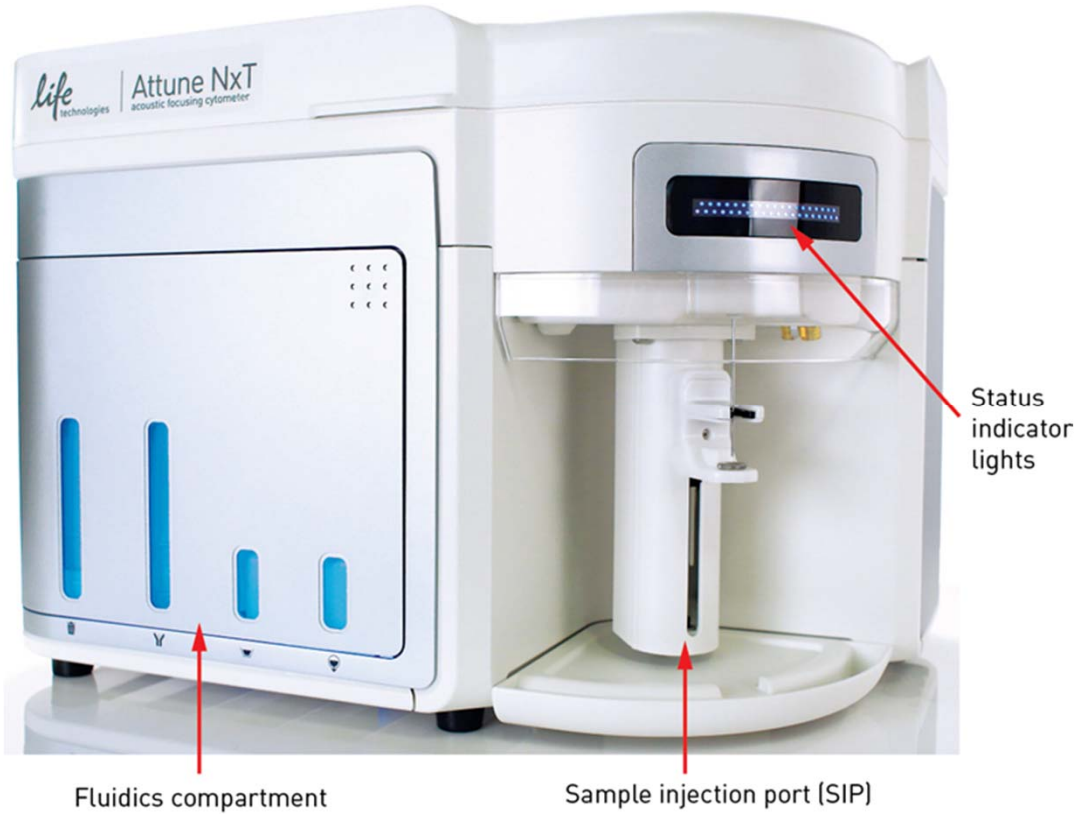
Acoustic focusing

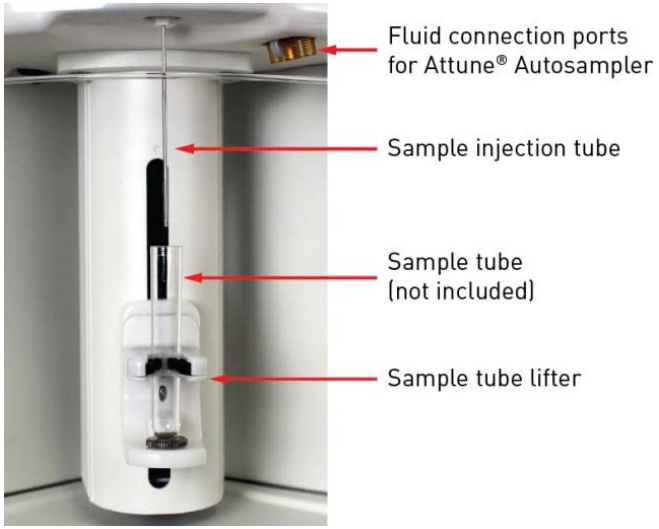
200 um diameter flow cell / ≥ 200 um fluid lines

Sample syringe delivery

Attune® NxT System

Status indicator lights
Green, Amber, Blue, Multicolor





Any tube that fits on the tube lifter will work

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On board fluids

1ml Sample Syringe

Volumetric delivery:
Accurate counts
Clog resistance

Color coded Fluid Lines

Black Sensor Connections

Notifies when filling/emptying is needed

Color coded Fluid Lines

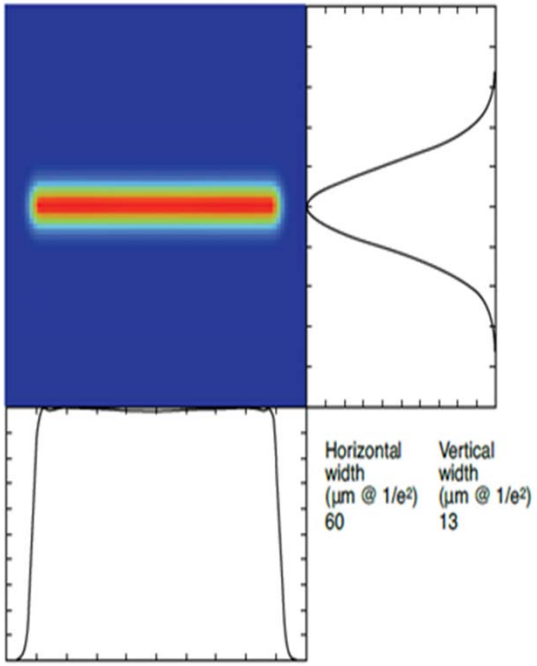
Waste

Focusing Fluid

Wash Fluid

Shutdown Fluid

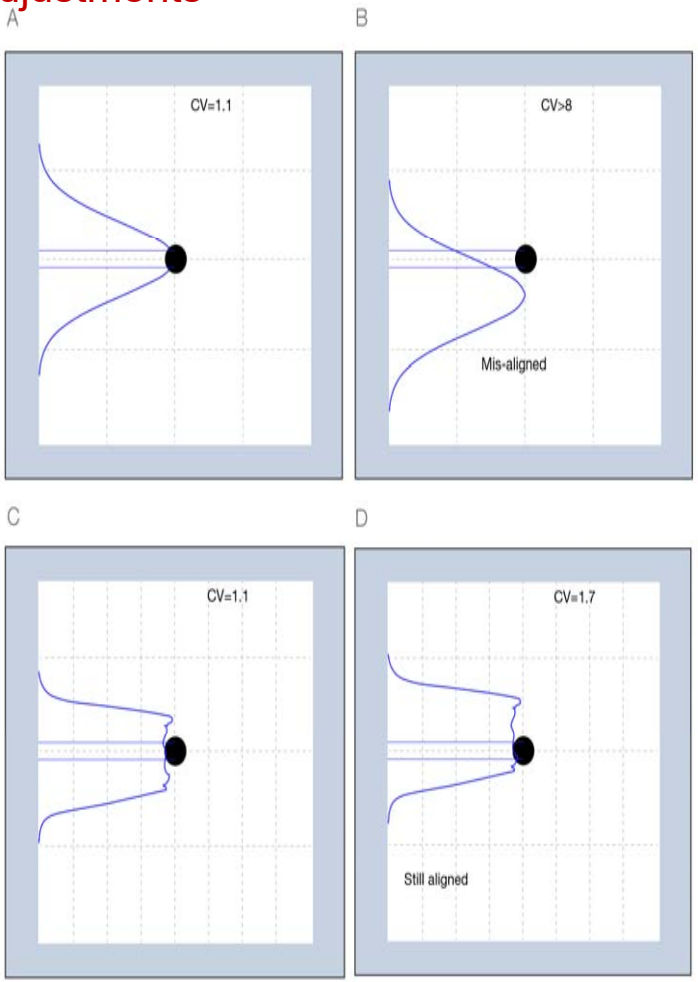
Beam Profile: Flat-Top Lasers



- consistent laser energy excitation
- ensures resulting data is due to biological, not instrument variation

Flat-top vs. Gaussian Lasers

Flat top lasers reduce need for alignment adjustments



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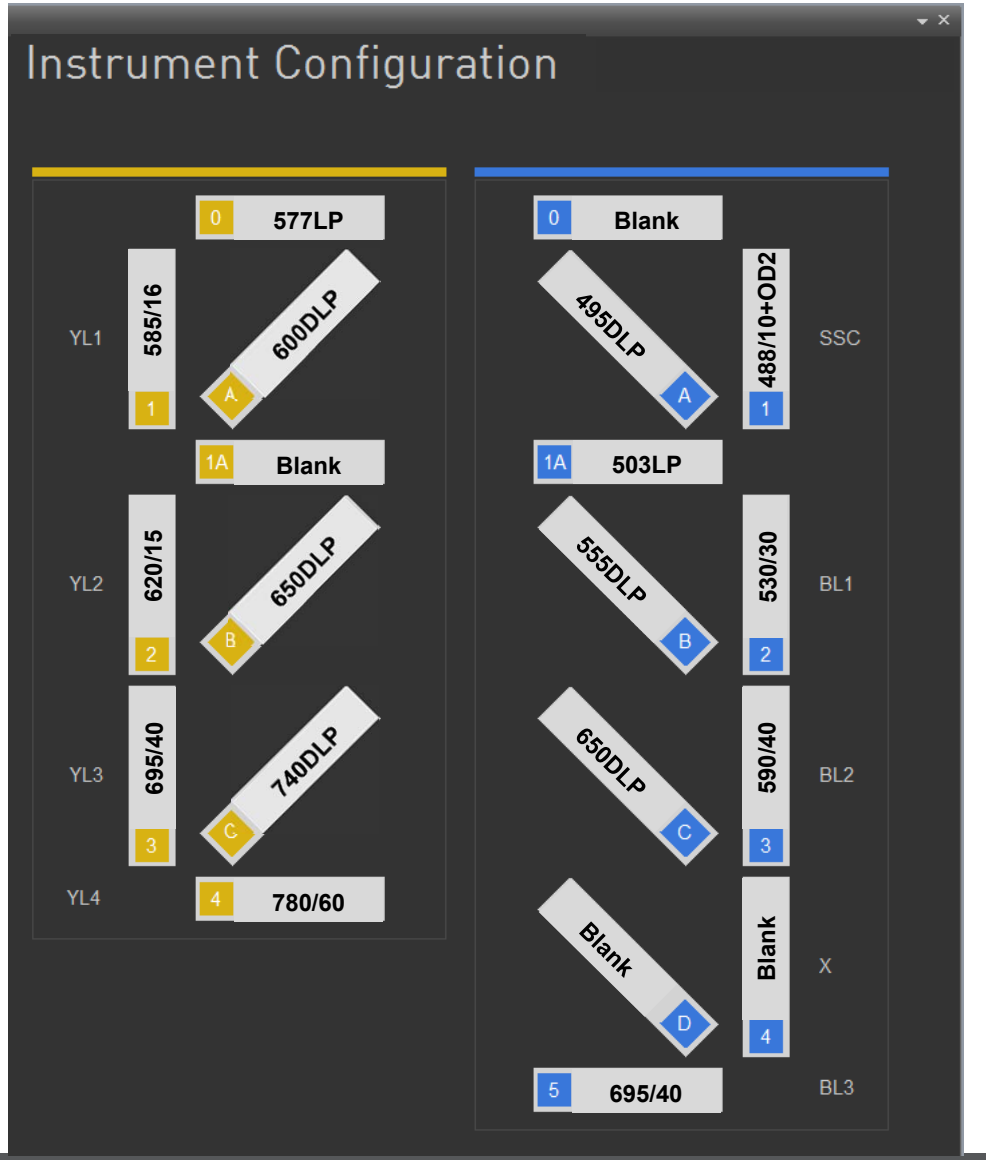
Blue Yellow - Configuration

Blue	488 nm laser	50 mW
Yellow	561 nm laser	50 nm

9 parameters (7 fluorescent detectors)

Excitation Laser	Emission Filter (nm)	Channel	Recommended Dyes	Fluorescent Proteins
Blue - 488 nm	530/30	BL1	Alexa Fluor® 488 FITC	eGFP Emerald eYFP
	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	mOrange RFP dTomato
	620/15	YL2	PE-Alexa Fluor® 610 PE-Texas Red®	mCherry DsRed mKate mStrawberry
	695/40	YL3	PE-Alexa Fluor® 700 PE-Cy®5.5 Qdot® 705 Tri-Color®	
	780/60	YL4	PE-Cy®7 Qdot® 800	



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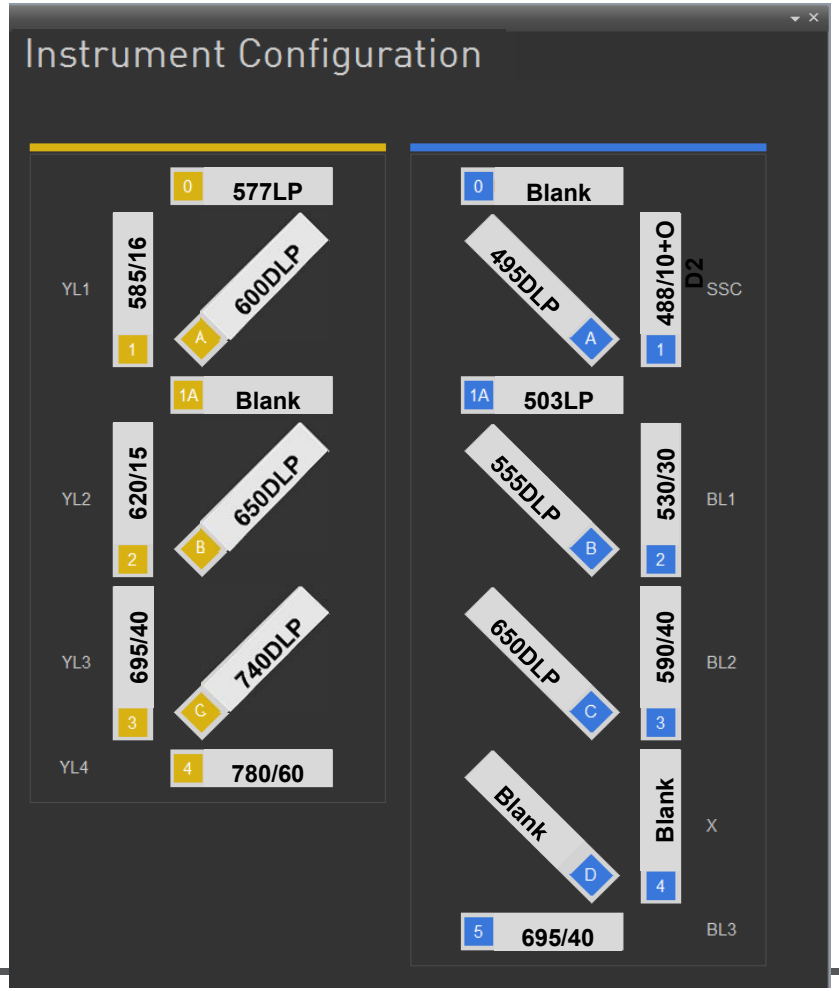
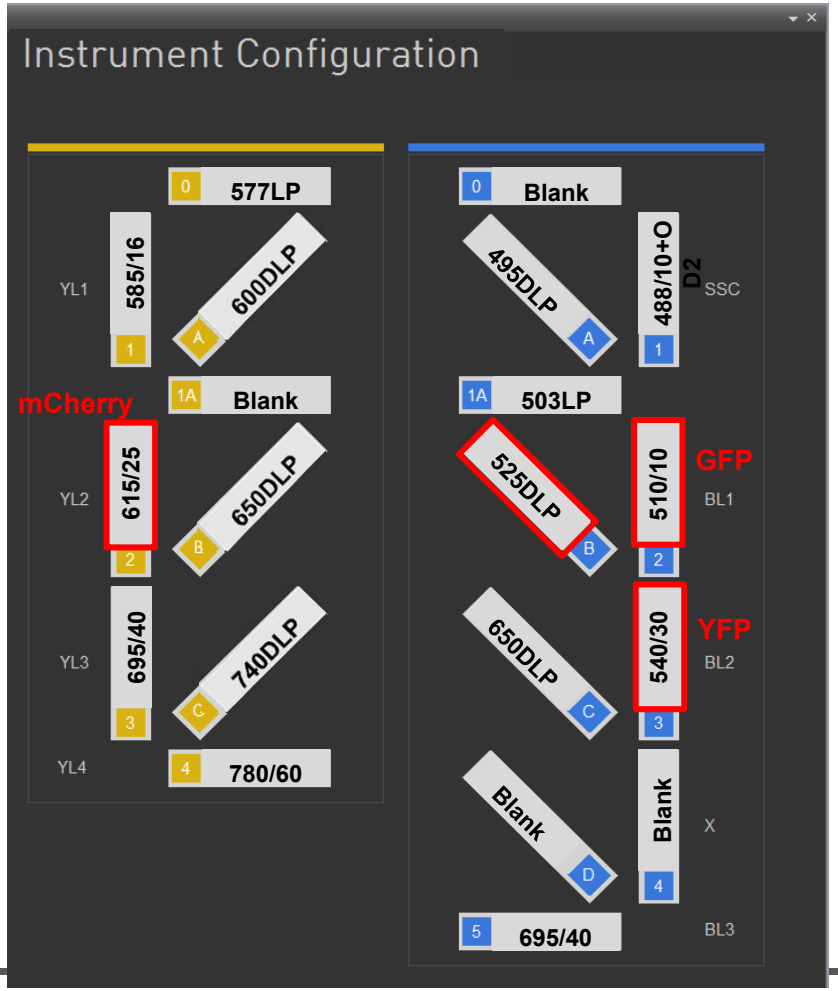
Fluorescent protein optimization filters

Default filters

mCherry	615/25 BP filter
GFP	510/10 BP filter
YFP	540/30 BP filter
	525 dichroic LP





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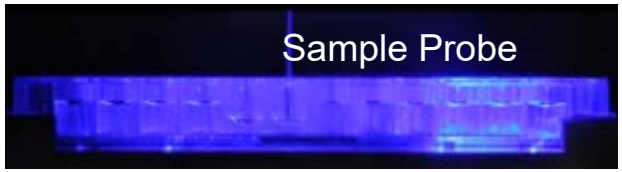
Fluorescent proteins

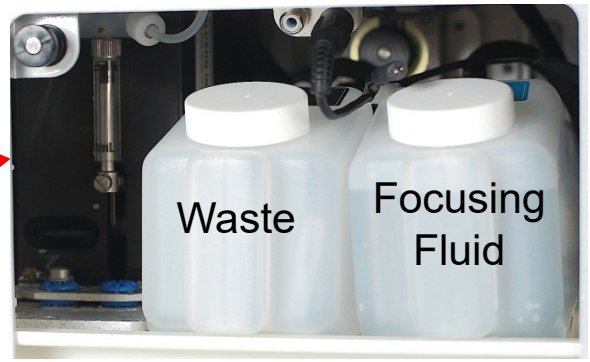
Table 1. Spectral characteristics of fluorescent proteins commonly used in flow cytometry.

Fluorescent protein	Excitation max (nm)	Emission max (nm)	Channel on Attune NxT Flow Cytometer
Azurite, TagBFP, mTagBFP, mTagBFP2, Cerulean, ECFP, TagCFP, AmCyan	383, 400, 400, 400, 433, 439, 458, 458	450, 456, 456, 456, 475, 476, 480, 489	VL1 (440/50), VL2 (512/25)
T-Sapphire	399	511	VL3 (603/48)
LSS-mKate1, LSS-mKate2	463, 460	624, 605	BL1 (530/30 or 510/10*)
TurboGFP, EGFP, TagGFP, emerald GFP (emGFP)	482, 483, 484, 487	502, 506, 507, 509	BL1 (530/30 or 510/10*)
TagYFP, TurboYFP, EYFP, Topaz, Venus, Citrine	508, 508, 514, 514, 515, 517	524, 524, 527, 527, 528, 529	BL2 (574/26 or 590/40)† or (540/30)*
mKOm, mOrange, mOrange2, Kusabira Green Orange, E2 Orange	548, 548, 549, 548, 540	559, 562, 565, 561, 561	YL1 (585/16)
DsRed, DsRed2, DsRed-Express, tdTomato, TagRFP, mStrawberry, mCherry, mKate, mKate2, TurboFP635 (Katushka)	553, 553, 553, 554, 555, 574, 587, 588, 588, 588	583, 583, 584, 581, 584, 596, 610, 635, 633, 635	YL2 (620/15 or 615/25*)
mPlum, HcRed, mRaspberry, mNeptune, E2Crimson	590, 592, 598, 599, 611	649, 645, 625, 649, 646	YL3 (695/40)

*Bandpass emission filter used with the Invitrogen™ Attune™ NxT Fluorescent Protein Filter Kit (optional, Cat. No. 100022775). †The 574/26 filter set is standard on all Attune NxT instruments that are not configured with a yellow laser. The 590/40 filter set is included on Attune NxT instruments configured with the yellow laser.

Autosampler

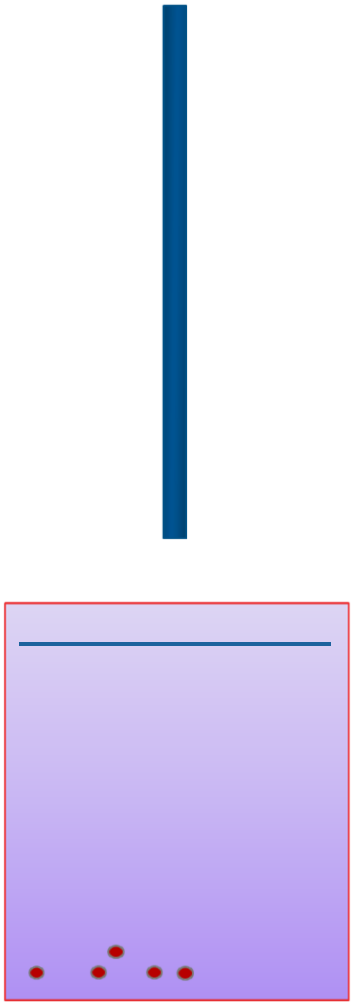




- Plate formats: 96 or 384 well plates, standard or deep well ***Round/U***, V-bottom and flat

- Plate experiments can include tubes
- Runs plate/records wells horizontally or vertically
- Mixes by pipetting up/down (user sets # of mixes)
- Probe is rinsed between wells (user sets # rinses)

Autosampler Mixing procedure



The user sets:
- The plate type
- The total sample volume
- The number of mixes
  (over mixing may cause bubbles)

The system defines:
- The liquid level in well
- The probe position
- The mixing method

Mixing sample by aspiration instead of shaking ensures homogeneity of the sample and maintains cell viability

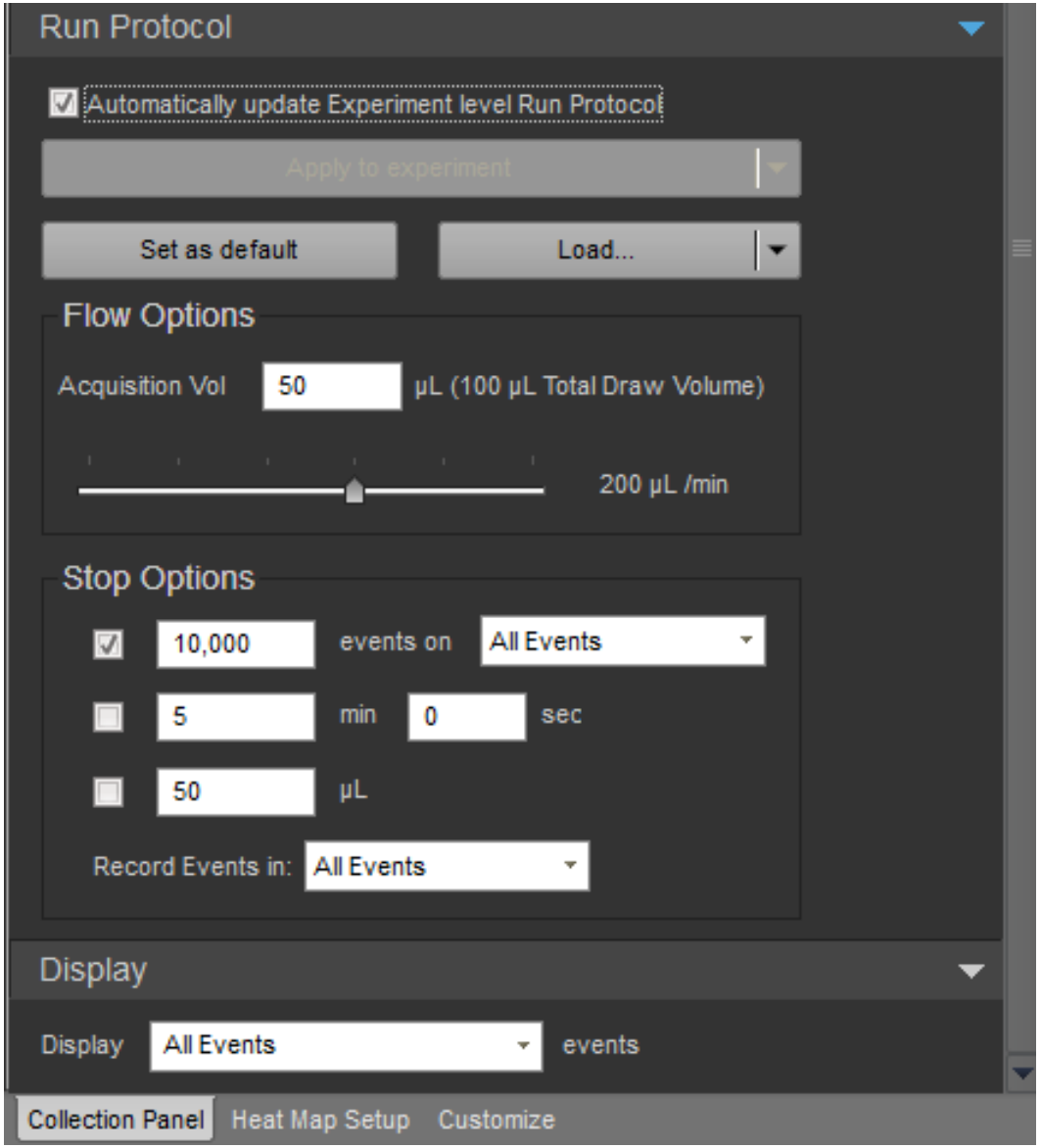
Sample syringe / volumetric delivery

Paradigm shift

Sample is <u>not</u> automatically drawn from tube

Use the ***Collection Panel Run Protocol*** to control data acquisition

Sample is pulled from the tube when ***Run*** is clicked



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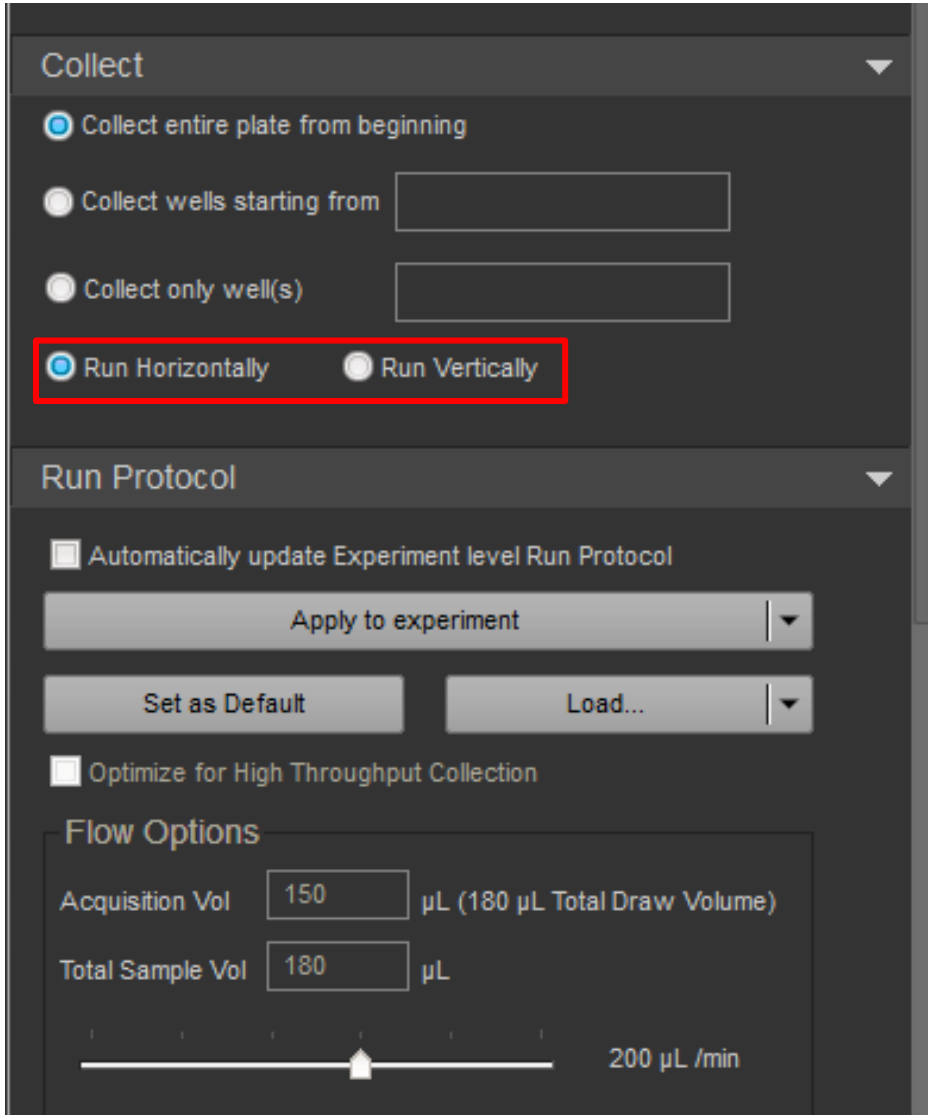
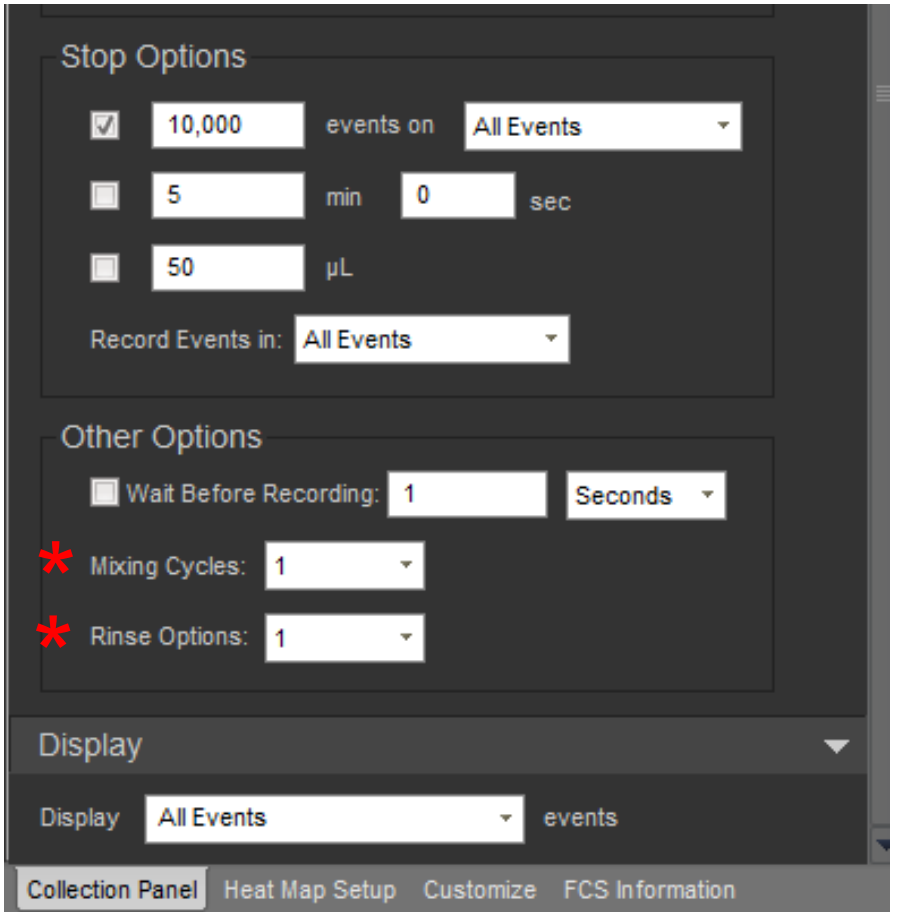
Collection panel for plate experiment





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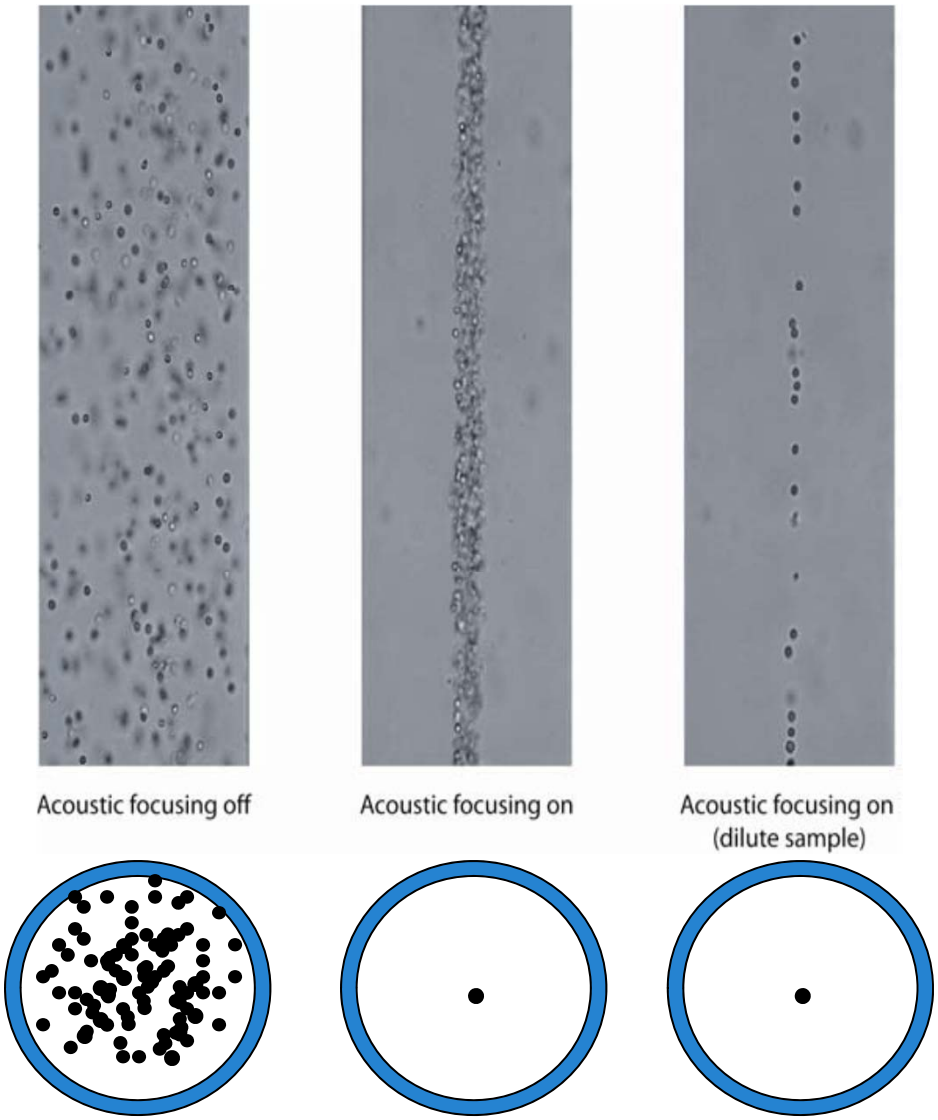
Best Practice – dilute samples



Dilution is the solution!

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Best practice: cell concentration and sample flow rate

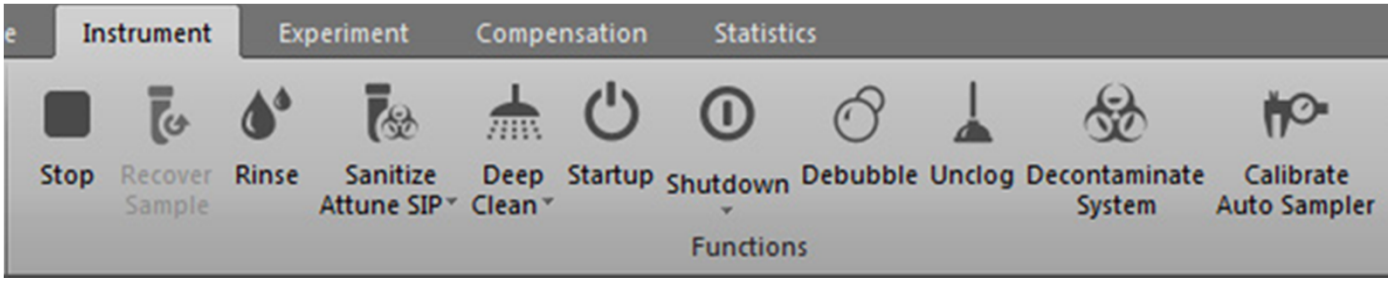
The event rate will approach maximums stated in the column header when samples of stated concentrations are run at the flow rates below.

When acquiring large event files (i.e files with > 10^6 events), plot parameters should not be changed while recording. Maximum file size: 20 Million events

Sample flow rate	***Maximum* sample concentration (35,000 ev/sec)**	***Maximum* sample concentration (8000 ev/sec) – *for accurate counts***	**Flow rate**
1000 µL/ minute	2.1 x 10^6 cells/mL	0.48 x 10^6 cells/mL	- Particles > 4 µm - Predominantly acoustic focusing
500 µL/ minute	4.2 x 10^6 cells/mL	0.96 x 10^6 cells/mL	- Particles > 2 µm - Predominantly acoustic focusing
200 µL/ minute	6.7 x 10^6 cells/mL	1.5 x 10^6 cells/mL	
100 µL/ minute	1.3 x 10^7 cells/mL	3 x 10^6 cells/mL	
25 µL/ minute	5.4 x 10^7 cells/mL	1.2 x 10^7 cells/mL	- Small particles < 2 µm - Best resolution from background for dimly positives assays - Smallest sample core - Predominantly hydrodynamic focusing
12.5 µL/ minute	1.0 x 10^8 cells/mL	2.4 x 10^7 cells/mL	

Let your biology and data quality be your guide. *If good data is obtained while running at 2-8,000 ev/sec, adjust the sample concentration and flow rate to maintain that.*

Best practices – Use routines on the Instrument tab –

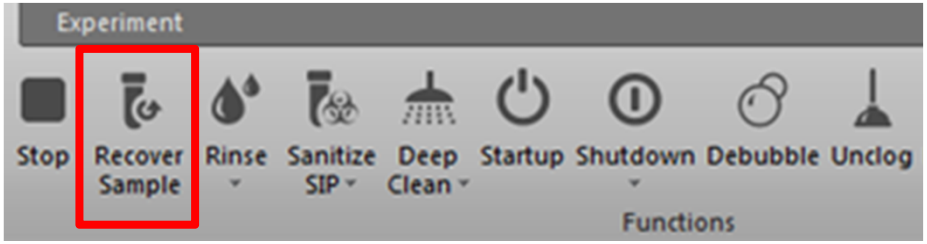


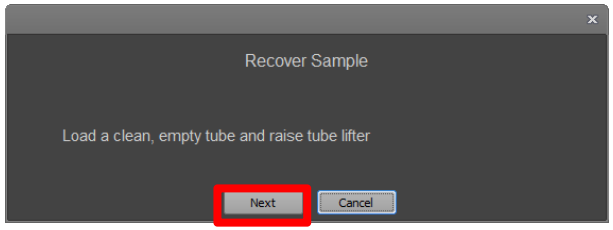
Follow on screen instructions

- **Recover Sample** - returns unused sample volume back to the well or the tube.
- **Rinse -** flushes system between samples. Runs automatically every time the SIP is lowered, but also can be user-initiated.
- **Sanitize Attune SIP** - sanitizes the SIP and sample lines between samples or experiments
- **Deep Clean** - thoroughly washes the system sample lines and flow cell between experiments
- **Debubble** - clears bubbles from the fluidics lines of the cytometer
- **Unclog** - back flush operation to remove clogs from the sample line.

All part of fluidics maintenance

Sample Recovery





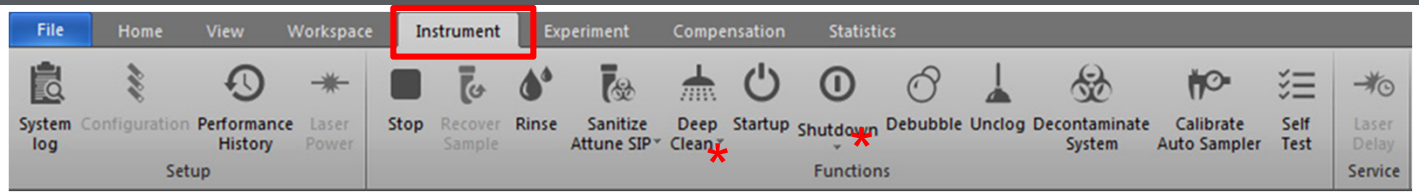
Follow on-screen instructions

Anytime sample remains in the sample loop

Stop option has been reached
Operator clicks stop

NOTE: Must select **recover sample** before lowering the tube lifter

Daily instrument cleaning guide



Between samples	• **Rinse** – automatically initiated when SIP is lowered (for tubes), or set in *run protocol* for plates • **Sanitize SIP** between sticky samples or cell counts
Between users / experiments 2 min	• **Quick Deep Clean** - 30 minute cleaning routine (click on the arrow below the Deep Clean icon to select ***Quick***) • **2x Sanitize SIP / Sanitize Autosampler SIP** (plate experiments) – 1st with 10% bleach per instructions 2nd with MilliQ water.
End of day	• ***Thorough*** **Shutdown** (click on the arrow below the Shutdown icon to select *Thorough*)

Note: Always wipe the outside of the SIP after doing a SIP Sanitize

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Summary: Better – Stronger - Faster

Better – acoustic focusing
volumetric delivery
automated cleaning routines
intuitive software

Stronger – clog resistant engineering
challenging samples
more stable laser alignment

Faster - 6 sample flow rates from 12.5 to 1000 µl/min
dilute samples
rare event analysis