Using the BD™ Cytometer Setup and Tracking (CS&T) System for Instrument Characterization and Performance Tracking

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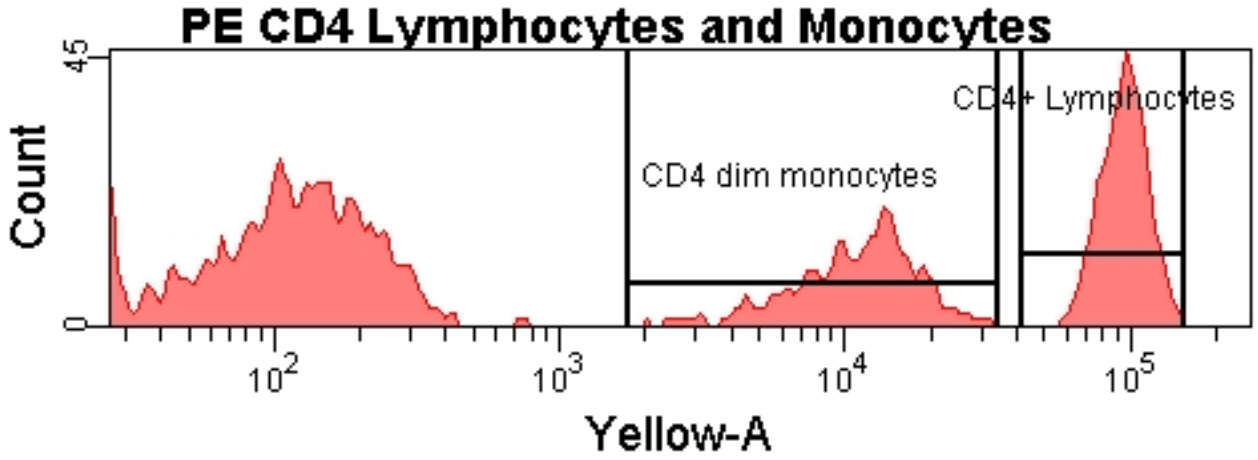
Outline

- **Definitions**
  - Resolution sensitivity
  - Factors affecting resolution sensitivity
    - Qr: Fluorescence detection efficiency
    - Br: Background “noise”
    - Electronic Noise (SDen)

- **Cytometer Setup &Tracking (CS&T)**

- **Choosing gain settings (MFI)**
    - Optimizing PMTVs
    - Linearity
    - Ensuring equivalent fluorescence intensities (MFI) over time: application settings
    - Standardizing setup across instruments

Key Performance Factors in High-Quality Flow Cytometry Data



- Resolution of subpopulations, including dim subpopulations
  - **Sensitivity**
- Relative measured values of fluorescence
  - **Linearity and accuracy**
- Reproducibility of results and cytometer performance
  - **Tracking**
- Comparison of results across time and amongst laboratories
  - **Standardization**

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Instrument Sensitivity: Two Definitions

- **Defining sensitivity**
  1. **Threshold**: Degree to which a flow cytometer can distinguish particles dimly stained from a particle-free background. Usually used to distinguish populations on the basis of Molecules of Soluble Equivalent Fluorochrome (MESF).
  2. **Resolution**: Degree to which a flow cytometer can distinguish unstained from dimly stained populations in a mixture.

- **How to measure instrument-dependent sensitivity?**
  - Resolution sensitivity is a function of three independent instrument factors:
    - Qr
    - Br
    - Electronic noise (SDen)

Defining Instrument Performance and Sensitivity: Qr, Br, and SDen

Characterizing the Cytometer

Cytometry 33:256–259 (1998)

Evaluating Fluorescence Sensitivity on Flow Cytometers: An Overview

James C.S. Wood[1]* and Robert A. Hoffman[2]

[1]Beckman Coulter, Inc., Miami, Florida

[2]Becton Dickinson Immunocytometry Systems, San Jose, California

Received 9 June 1998; Accepted 17 June 1998

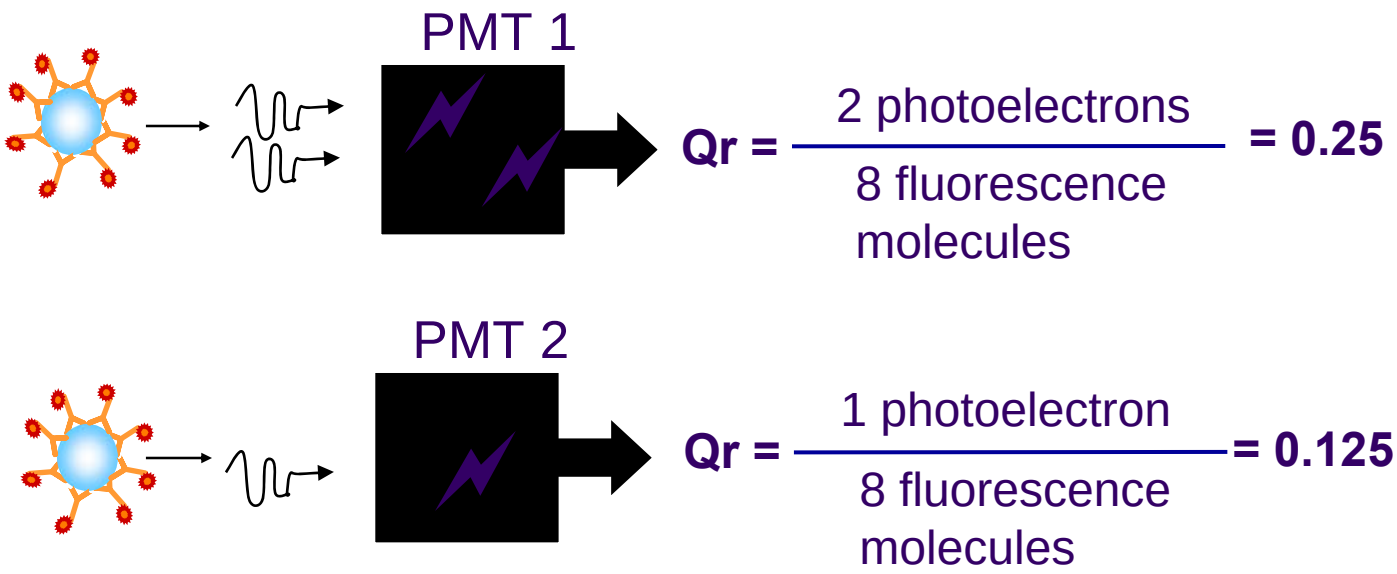
The current paradigms for assessing fluorescence sensitivity on flow cytometers do not provide an adequate assessment of an instrument's ability to detect and measure weak fluorescence on stained particles. The capability to resolve dimly stained populations depends on two factors: the background noise (B), and the efficiency (Q) with which the fluorescence from the fluorochrome molecules are converted to photoelectrons. Any single statistical measure of fluorescence histogram distributions will be unable to uniquely characterize an instrument. Therefore, neither of the routinely used methods (detection threshold and delta channel) measure sensitivity completely and unambiguously. We show the limitations of these methods and propose that instrument sensitivity be characterized in terms of both background noise and detection efficiency in order to determine better the capability to detect and resolve weakly fluorescent particles. Cytometry 33: 256–259, 1998. © 1998 Wiley-Liss, Inc.

Key terms: fluorescence; sensitivity; resolution; flow cytometry

Relative Q: Qr

- **Qr** is *photoelectrons per fluorescence unit* and indicates how bright a reagent will appear on the sample when measured in a specific detector.

$$Qr = \frac{\#\ \text{photoelectrons}}{\#\ \text{fluorescence molecules}}$$



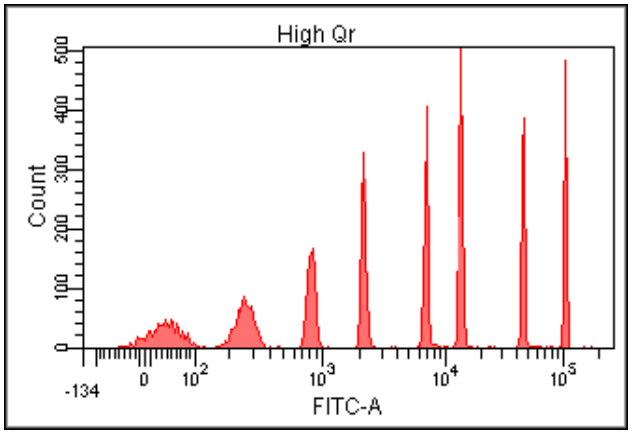
Why is Qr important?

A system with a higher Qr has a better resolution than a system with a lower Qr.

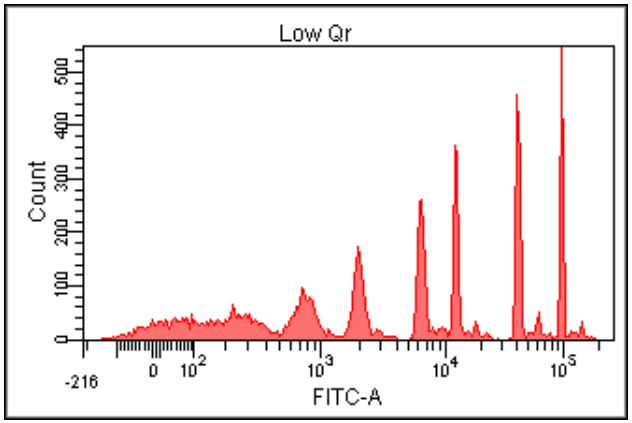
Low Qr value = lower resolution

High Qr value = higher resolution

High Qr



Low Qr



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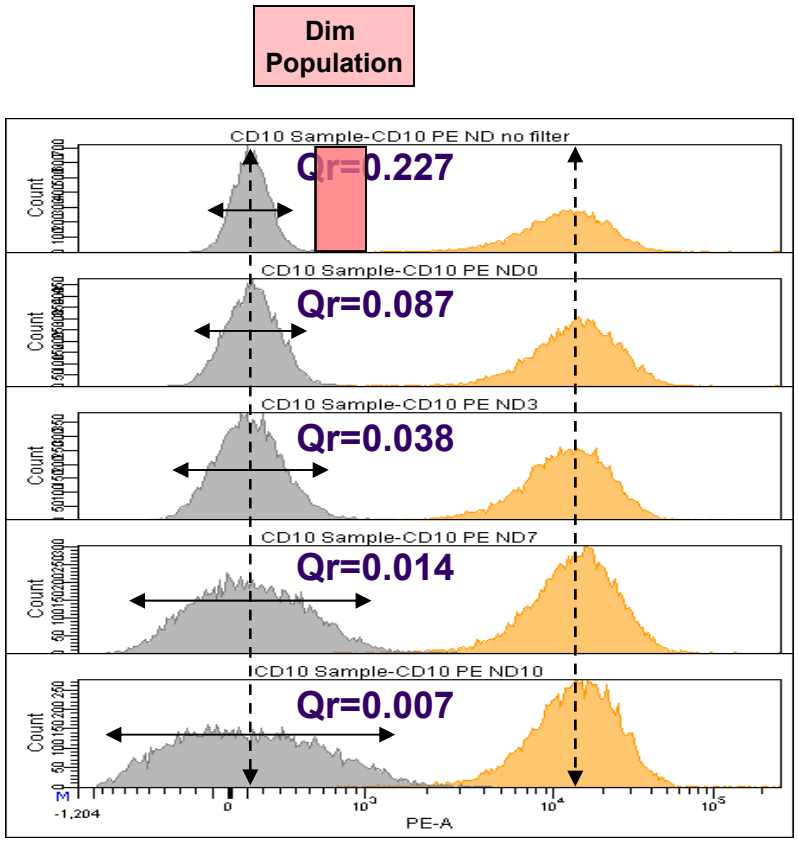
What Factors Affect Qr?

- Laser power
- Optical efficiency
- PMT sensitivity (red spectrum)
- Poor PMT performance
- Dirty flow cell
- Dirty or degraded filter

Qr: Anti-CD10 PE Example

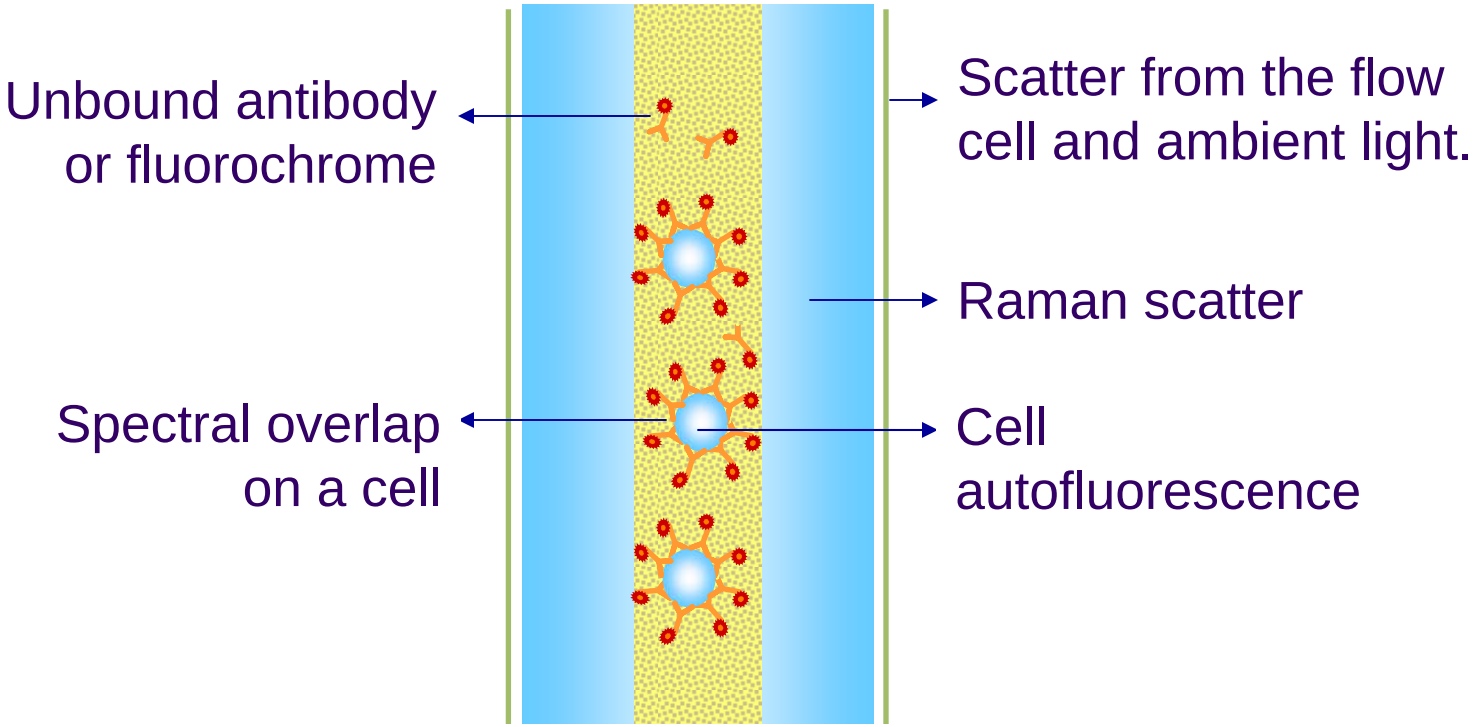
The laser and detectors were attenuated by ND filters over a 30-fold range to illustrate the effects of decreasing detector sensitivity on population resolution.



Trans-mission	SI Corrected
100%	83
35.5%	55
17.8%	35
7.1%	20
3.5%	14

Relative Background: Br

• **Br** is a measure of true optical background in the fluorescence detector, which helps indicate how easily (dim) signals may be resolved from unstained cells in that detector.

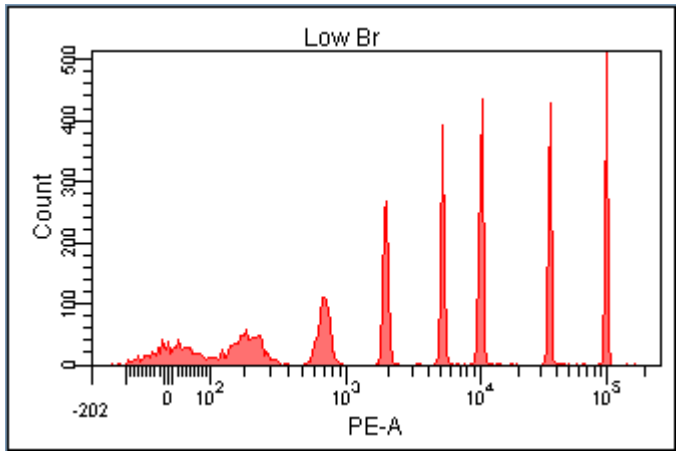


- Factors affecting Br: dirty flow cell, damaged optical component

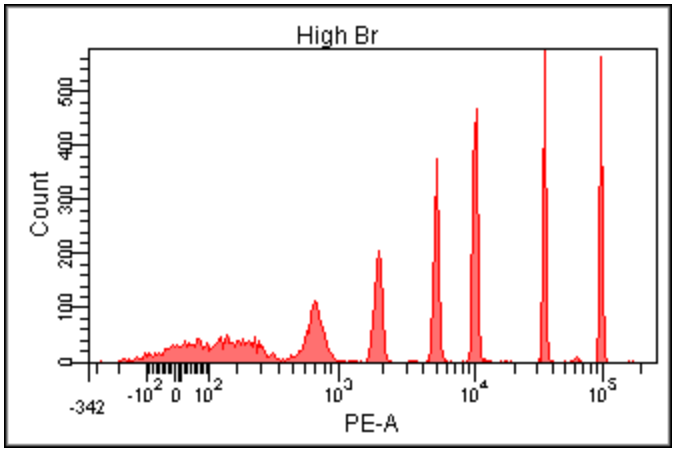
Why is Br important?

- High Br widens negative and dim populations.
- High Qr value = lower resolution
- Low Qr value = higher resolution

Low Br

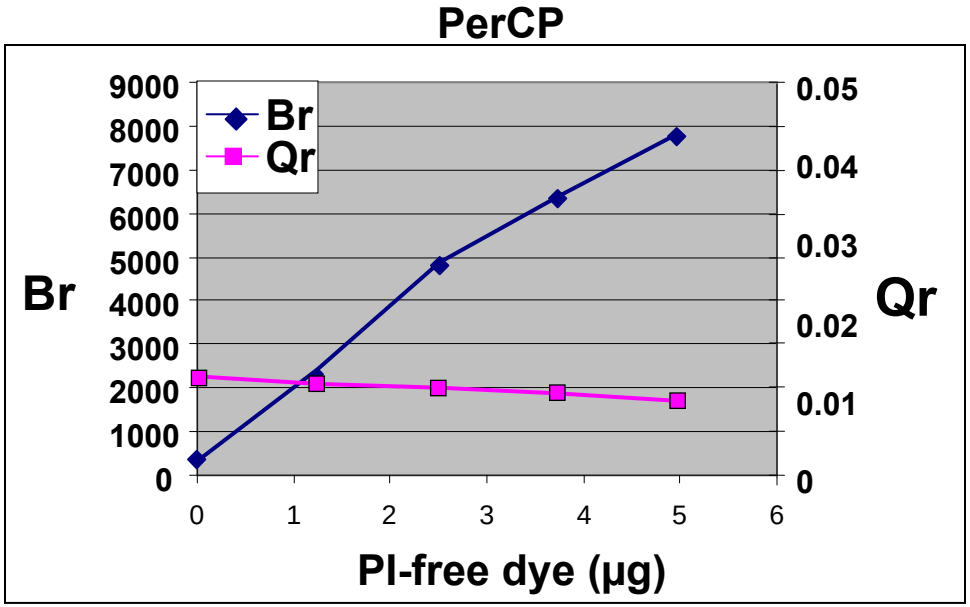


High Br



Br: Optical Background from Propidium Iodide

- **Example: It is common to use propidium iodide (PI) to distinguish live from dead cells. Propidium iodide was added in increasing amounts to the buffer containing beads, and Qr and Br were estimated.**



- **Residual PI in your sample tube will increase Br, which will reduce sensitivity.**

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Electronic Noise (SDen)

- **SDen** is the background signal due to electronics:
  - Contributed by:
    - PMT connections / PMT noise
    - Cables too near power sources
    - Digital error
- Broadens the distribution of unstained or dim particles
  - **Increases in electronic noise results in <u>decreased resolution sensitivity</u>**

  Most important for channels with low cellular autofluorescence
    - APC-Cy™7, PE-Cy™7, PerCP-Cy™5.5

Summary: Instrument Performance and Sensitivity

- Qr and Br are independent variables, but both affect sensitivity.
  - Increases in Br or decreases in Qr can reduce sensitivity and the ability to resolve dim populations.
  - $\text{Sensitivity}_{\text{relative}} \propto \sqrt{\frac{Qr}{Br}}$
  - On digital instruments, BD FACSDiva™ software v6 and CS&T provides the capability to track performance data for all of these metrics, also allowing users to compare performance between instruments.

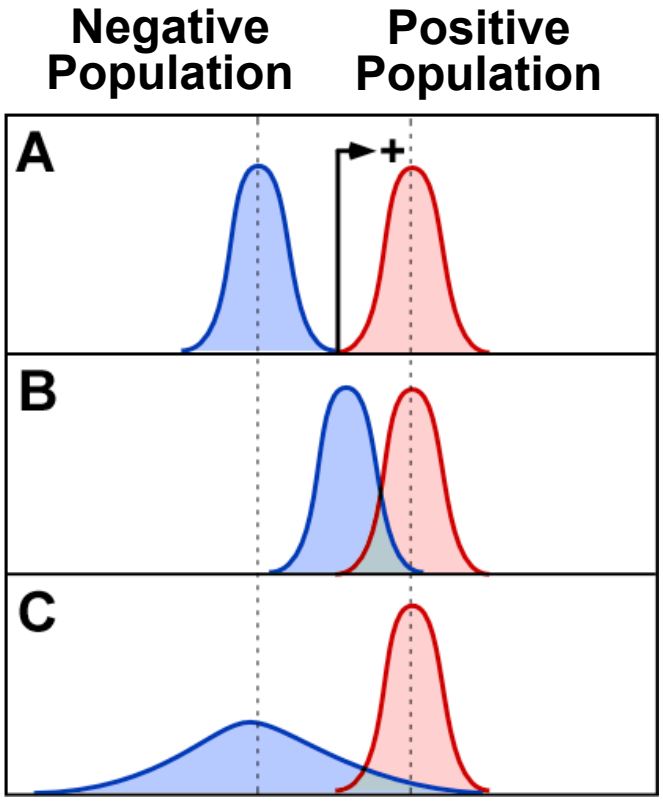
- Instrument performance can have a significant impact on the performance of an assay.

Resolution vs. Background

Negative population has
low background
Populations well resolved

Negative population has
high background
Populations not resolved

Negative population has
low background
high CV (spread)
Populations not resolved

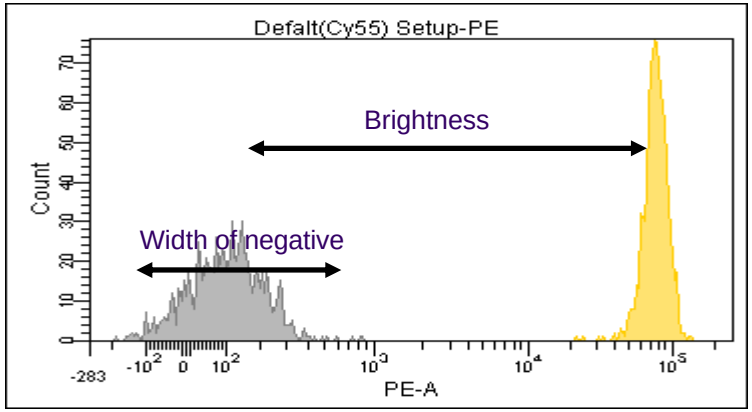


The ability to resolve populations is a function of both background *and* spread of the negative population.

Measuring Sensitivity: The Stain Index

- The **Stain Index** is a measure of reagent performance on a specific cytometer, a normalized signal over background metric.

$$\text{Stain Index} = \frac{\text{Brightness}}{\text{Width of Negative}} = \frac{\text{median}_{\text{positive}} - \text{median}_{\text{negative}}}{2 \times \text{rSD}_{\text{negative}}}$$

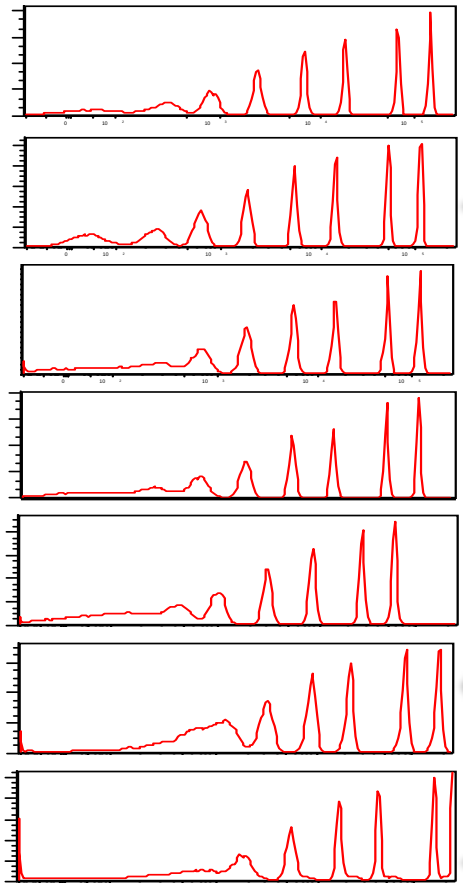


- The brightness is a function of the ***assay*** (antigen density, fluorochrome used).
- The width of the negative is a function of:
  - ***Instrument performance*** (Qr, Br, and SDen) [single-color]
  - ***The assay***
    - (Fluorescence spillover / compensation) [multicolor]
    - The cell population

Example: Stain Index, Qr, and Br Across Laboratories

Example : FITC Channel

Lab	SI	Qr	Br
1	77.4	0.015	233
2	134.5	0.028	216
3	55.6	0.015	976
4	80.5	0.01	298
5	66	0.01	613
6	13.7	0.007	2322
7	16.2	0.018	2768



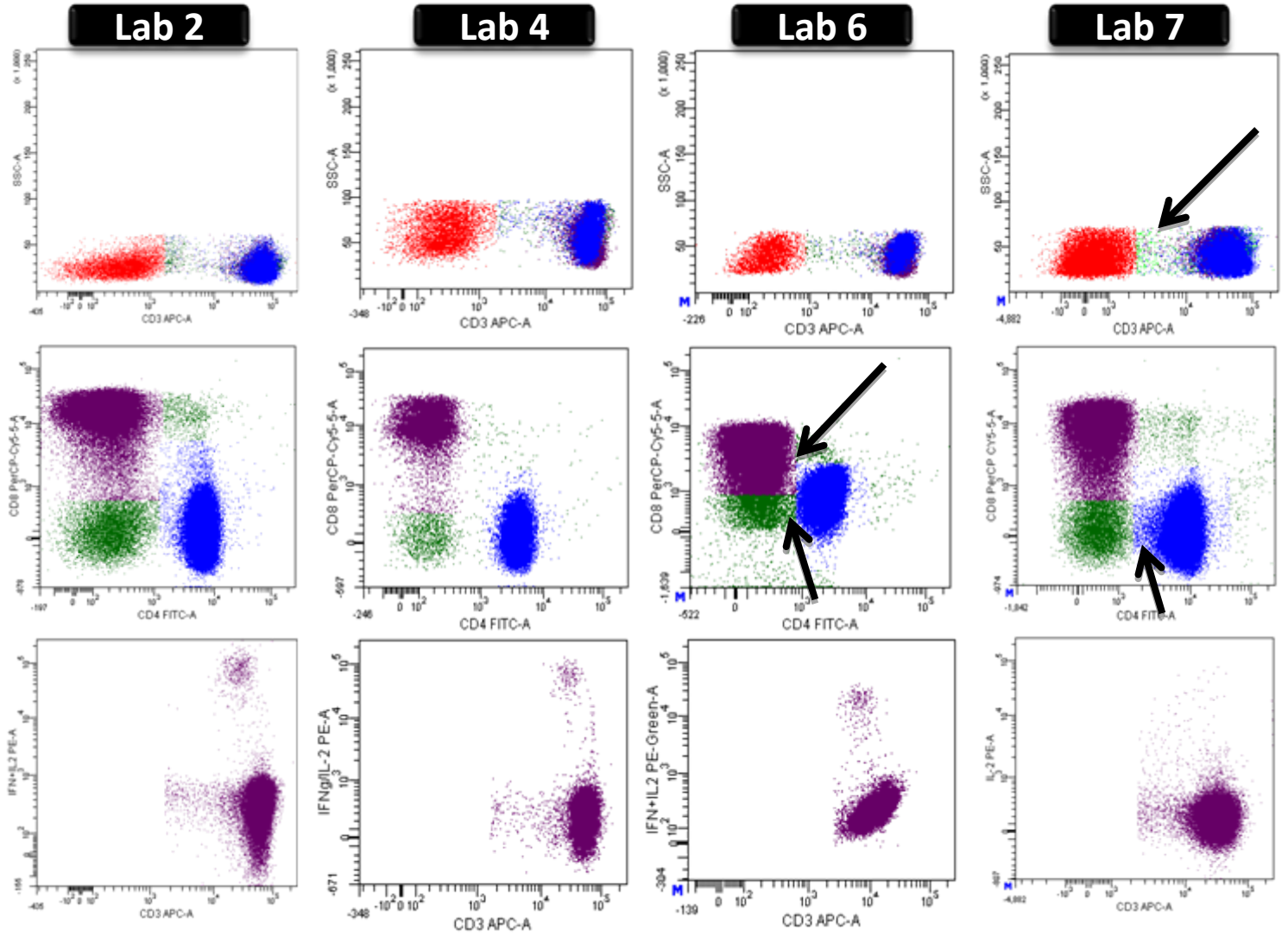
High SI = Good Resolution; correlates with high Qr and low Br

Low SI = Bad Resolution; correlates with low Qr and high Br

Low SI = Bad Resolution; correlates with high Br

Impact of Instrument Performance in Quality Assurance of Multicolor Flow Cytometry Assays.
Jaimes MC,[1] Stall A,[1] Inokuma M,[1] Hanley MB,[1] Maino S,[1] D'Souza MP,[2] and Yan M.[1] **ISAC 2010**
[1]BD Biosciences, San Jose, CA 95131; [2]Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Correlation of SI, Qr, and Br with Assay Performance



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BD Cytometer Setup & Tracking (CS&T) System

CS&T Definition

CS&T is a fully automated software and reagent research system, unique to BD digital cytometers, providing:

- Characterization
- Setup
- Tracking

CS&T: Functions and Benefits

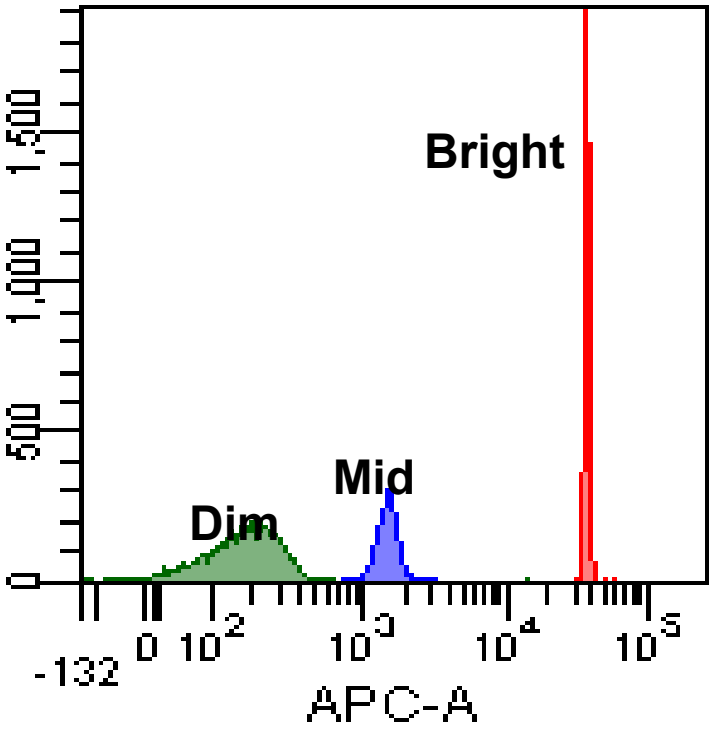
- **Functions of the CS&T system:**
  - Defines and characterizes baseline performance
  - Optimizes and standardizes cytometer setup
  - Tracks cytometer performance

- **Benefits of the CS&T system:**
  - Provides consistent, reproducible data every day
  - Simplifies design of multicolor experiments
  - Yields higher quality data from multicolor experiments
  - Offsets day-to-day instrument variability
  - Enables standardization between instruments and sites
  - Allows early identification of degrading cytometer performance

System Components: Beads (1)

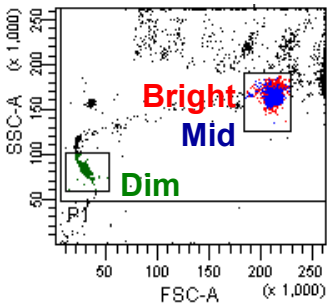
- The CS&T bead set consists of uniform beads of different intensities designed to fully characterize the flow cytometer

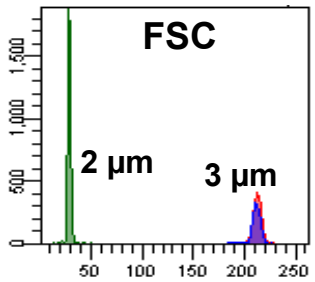
- **Bright Beads—**negligible photoelectron contribution to CV
- **Mid Beads—**large photoelectron contribution to CV
- **Dim Beads—**significant noise/background contribution to CV

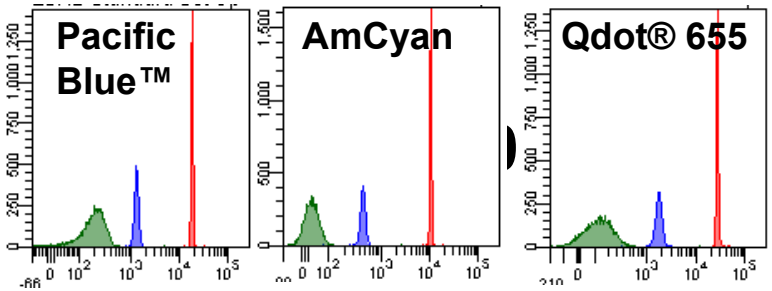


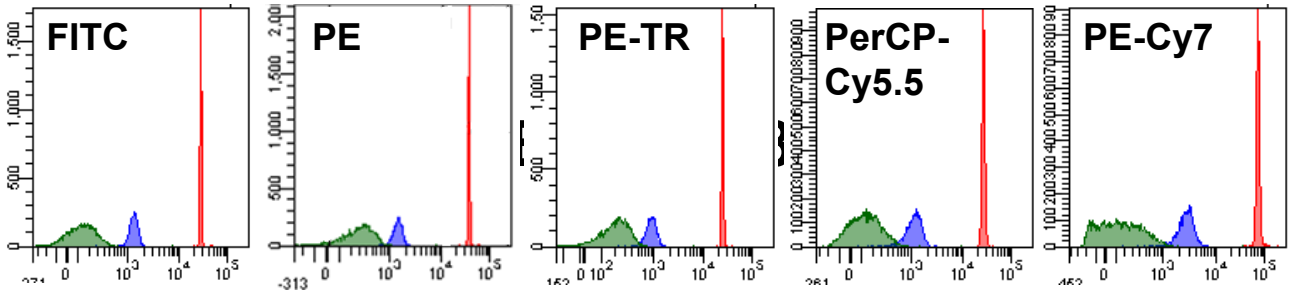
System Components: Beads (2)

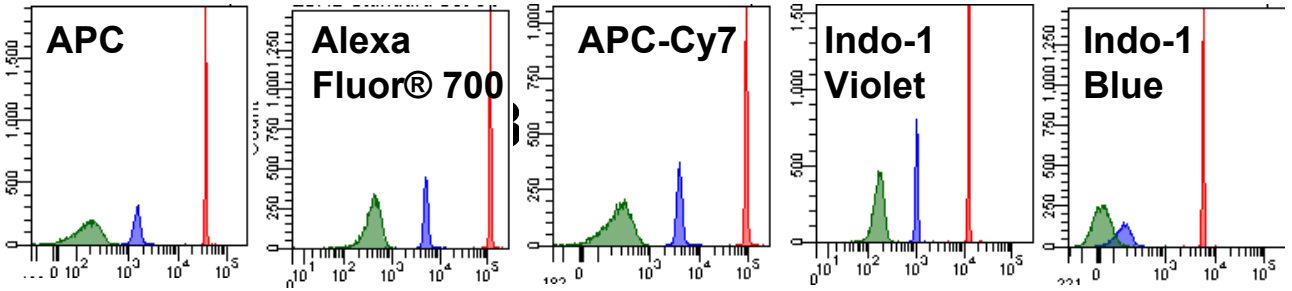
The beads are designed to be excited by all currently supplied lasers and emit in the respective detectors for virtually any fluorescent dye.









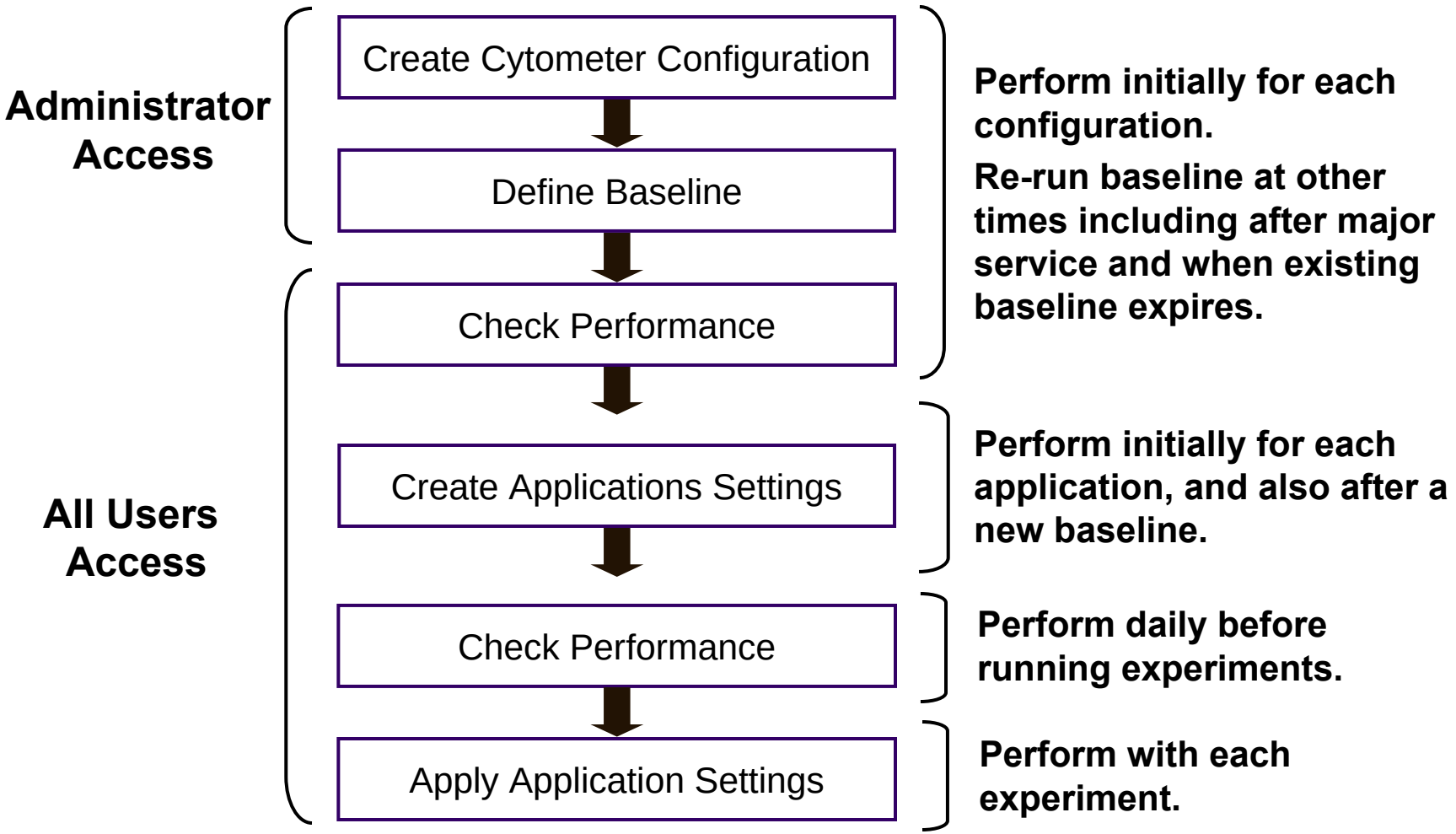


Pacific Blue is a trademark, and Alexa Fluor® and Qdot are registered trademarks of Molecular Probes, Inc.

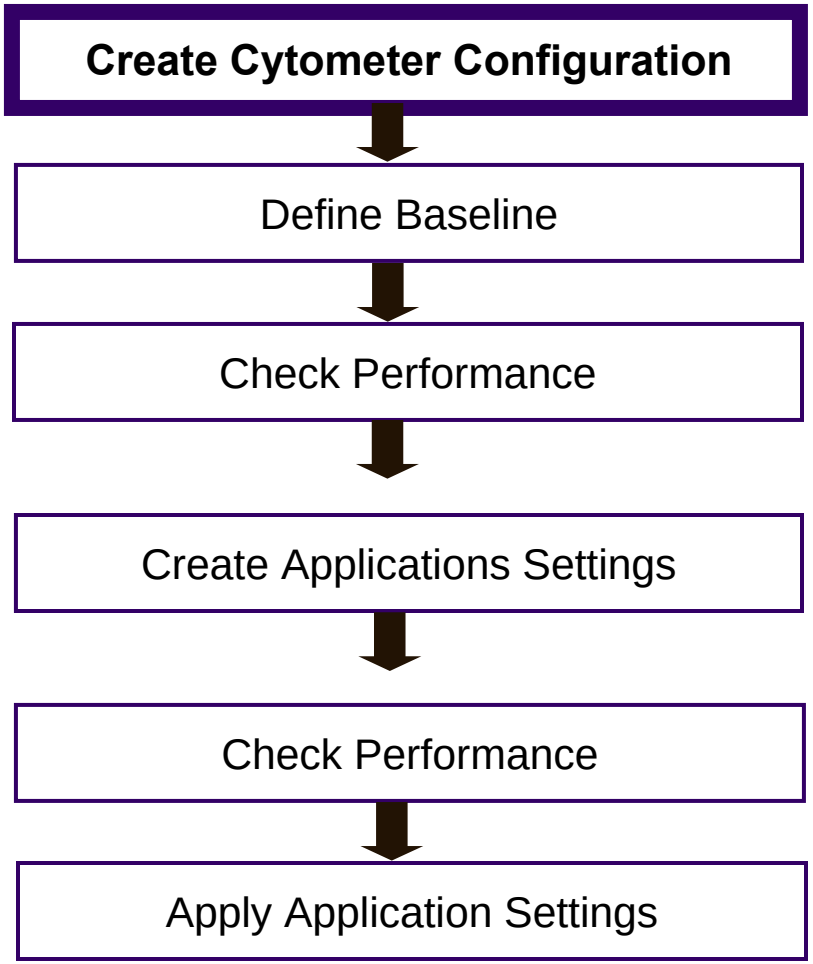
System Components: Software

- Very flexible system using user-defined:
  - Cytometer configurations to support any number of lasers, parameters, and nozzles
  - Fluorochrome parameters, filters, and mirrors including Q-dots and new fluorochromes
  - Alarm boundaries for tracking performance
  - Application settings

CS&T Workflow

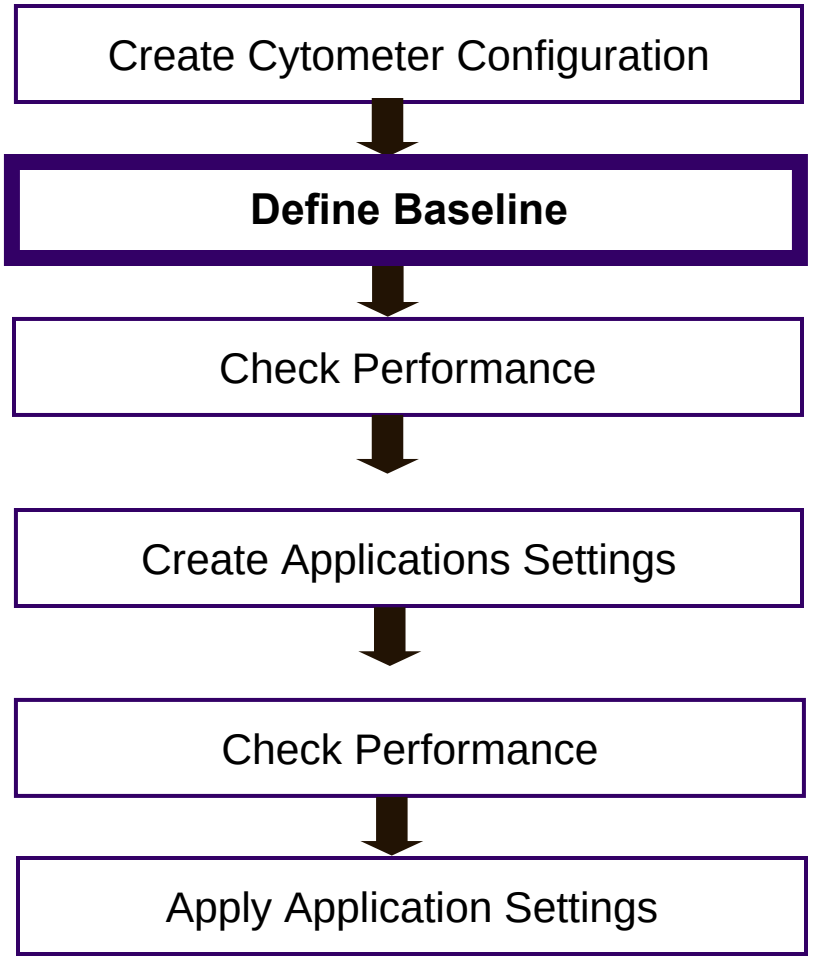


CS&T Workflow



Defines the optical layout of the cytometer. Includes filters, mirrors, and parameter labels.

CS&T Workflow

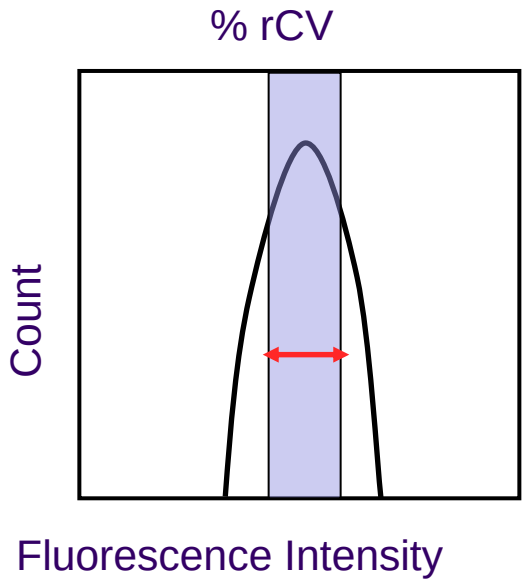


Determines the baseline performance of the cytometer by measuring key factors such as linearity, detector efficiency, optical background, and electronic noise.

Determines a target MFI for each parameter. Determines a linear range for each parameter.

Laser Alignment: Robust Coefficient of Variation – %rCV

- % rCV = 100 x 0.7413 (75th percentile – 25th percentile)/Median
- Used as a measurement of resolution because it is less sensitive to outliers.



- Major factors contributing to the CV of measurements:
  - Laser beam shape and alignment
  - Laser power

Baseline Laser Settings

- Characteristics of the lasers are measured and reported.
- The laser delay, area scaling factors, and the window extension are set.
  - For the BD FACSAria™ family of cytometers, the laser delay is set for a specific sheath pressure.
  - Area scaling may need to be optimized for different cell types.

Laser Settings

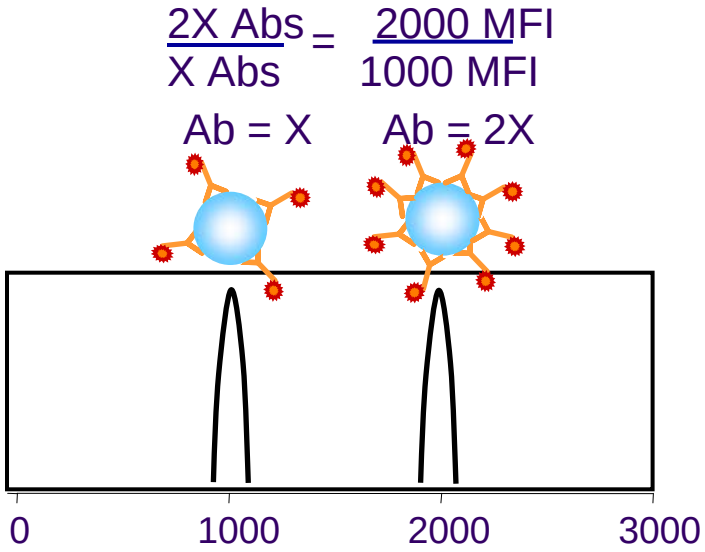
Laser	Power (mW)	Power Spec. (mW)	Current (mA)	Current Spec. (mA)	Delay (Trigger on FSC)	Delay (Trigger on Fluorescence)	Area Scaling Factor
Violet	57.85	44.93 - 67.39	N/A	N/A	-31.30	-31.30	1.26
Blue	20.35	16.27 - 24.41	1.43	1.16 - 1.74	0.00	0.00	1.30
Red	19.79	16.40 - 24.60	N/A	N/A	31.96	31.96	1.18

Window Extension: 7
FSC Area Scaling Factor: 0.91

30

Linearity

- **Defined as proportionality of output (MFI) to input (Fluorescence/ # of photons)**



- **Important for fluorescence compensation**
  - Compensation of data in the last decade involves subtraction of large numbers
  - Small errors (non-linearity) in one or both large numbers can cause a large absolute error in the result

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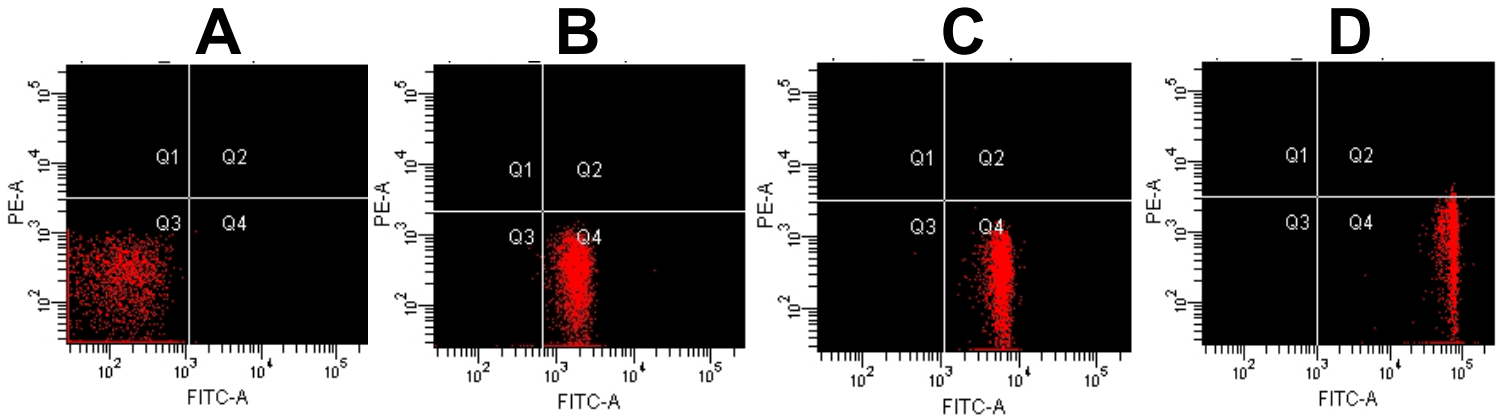
Effect of Non-Linearity on Compensation

- Compensation of data in the last decade involves subtraction of large numbers
- Errors (non-linearity) in one or both large numbers can cause a large absolute error in the result

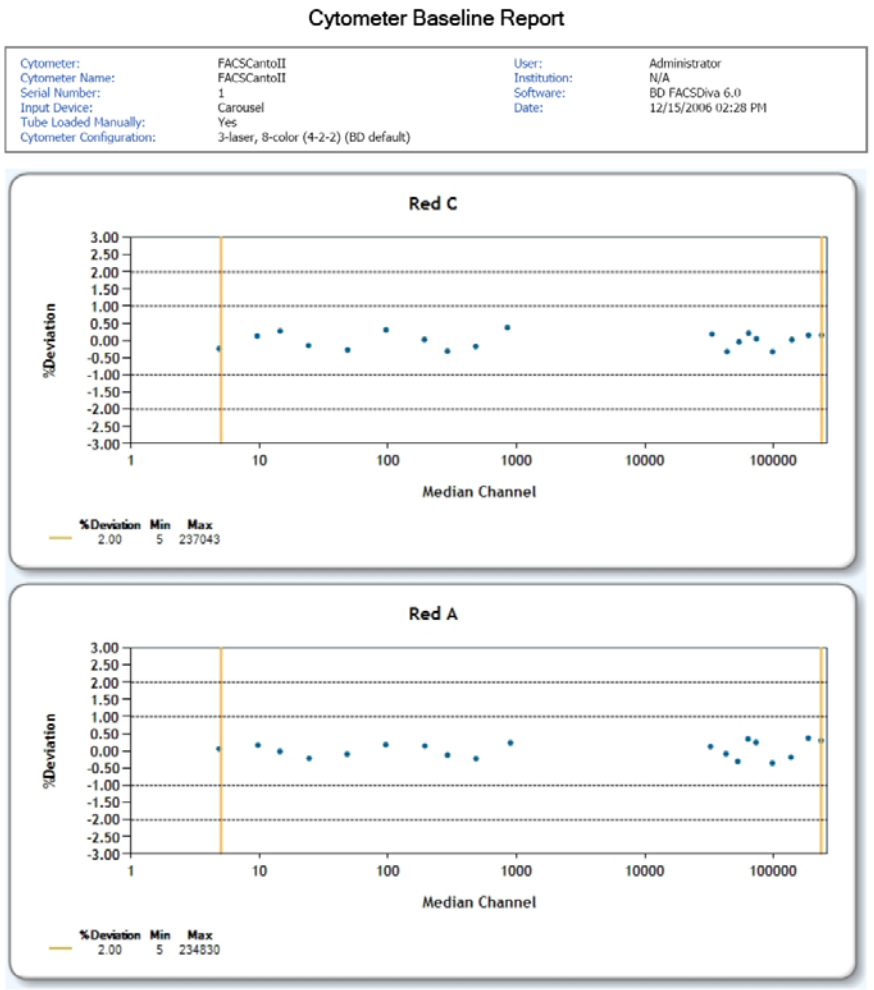


Channel	Median Fluorescence Intensity			
FITC	68	1,796	5,921	73,000
PE	80	75	79	365

BD CompBead™ compensation particles stained with varying levels of FITC-Ab.
Compensation was set using samples A & C.
This instrument had 2% deviation from linearity above 50,000.

CS&T Baseline Report—Linearity

- CS&T reports the linearity range for every fluorescence detector (±2.0% deviation) in the cytometer baseline report
- Users can print out data plots for any detector



Cytometer Configuration

Cytometer Baseline Report

Cytometer:	LSRII	User:	Administrator
Cytometer Name:	LSRII	Institution:	N/A
Serial Number:	1	Software:	BD FACSDiva 6.1.2
Input Device:	HTS	Date:	06/10/2009 11:17 AM
Tube Loaded Manually:	Yes		
Cytometer Configuration:	4-Blue 2-Violet 2-355UV 2-Red (BD default)		

Setup Beads

Bead Product: CST Setup Beads, Part #: 910723
Lot ID: 16295, Expiration Date: 05/31/2010
Bead Lot Information: Available

Detector Settings

Laser	Detector	Parameter	PMTV	New Target Value	Old Target Value	Bright Bead %Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV	Dim Bead Median Channel	Dim Bead % Robust CV
Blue	FSC	FSC	705	125000	125000	6.14	113653	6.42	19530	28.66
Blue	E	SSC	327	125000	125000	7.04	120708	7.02	58556	6.51
Blue	D	FITC	585	24583	27157	4.59	1203	13.44	124	58.47
Blue	C	PE	499	28355	27438	4.45	1090	18.43	216	64.49
Blue	B	PerCP-Cy5-5	680	50516	50677	7.26	1957	30.98	201	112.65
Blue	A	PE-Cy7	725	36849	42011	8.19	968	37.57	58	208.01
Red	B	APC	580	62375	59540	4.44	3487	12.09	223	49.61
Red	A	APC-Cy7	509	62017	59991	5.63	2591	11.94	184	51.78
Violet	B	Pacific Blue	457	28601	27118	3.75	1775	10.87	279	40.60
Violet	A	AmCyan	590	118431	111103	3.55	5421	9.18	370	57.17
355 UV	B	Indo-1 (Violet)	504	41452	50009	4.30	1221	9.36	150	39.97
355 UV	A	Indo-1 (Blue)	561	73730	79680	5.43	1162	46.64	90	464.12

Detector Settings (Continued)

Laser	Detector	Parameter	Linearity Min Channel	Linearity Max Channel	Slope	Intercept	Electronic Noise Robust SD	Qr	Br
Blue	FSC	FSC	N/A	N/A	0.0039	2.3	N/A	N/A	N/A
Blue	E	SSC	N/A	N/A	7.6606	-14.2	N/A	N/A	N/A
Blue	D	FITC	150	235857	7.4916	-16.3	22.1	0.0117	844
Blue	C	PE	39	238086	7.6178	-16.1	22.2	0.0522	576
Blue	B	PerCP-Cy5-5	70	231619	7.7379	-17.2	25.3	0.0022	232
Blue	A	PE-Cy7	49	225549	7.5107	-16.9	23.5	0.0075	60
Red	B	APC	115	241344	7.5553	-16.1	22.2	0.0130	112
Red	A	APC-Cy7	192	247690	7.5549	-15.7	28.1	0.0033	1853
Violet	B	Pacific Blue	22	235649	7.5324	-15.6	26.7	0.0190	2284
Violet	A	AmCyan	33	235285	7.3995	-15.4	30.1	0.0058	4624
355 UV	B	Indo-1 (Violet)	88	237003	7.3375	-15.2	24.3	0.0937	517
355 UV	A	Indo-1 (Blue)	76	237479	7.6409	-16.1	25.0	0.0087	1616

PMTV: optimized PMT voltages

New Target Value: MFI target for bright bead

Bright Bead % Robust CV

Linearity Min Channel and Linearity Max Channel: linear range

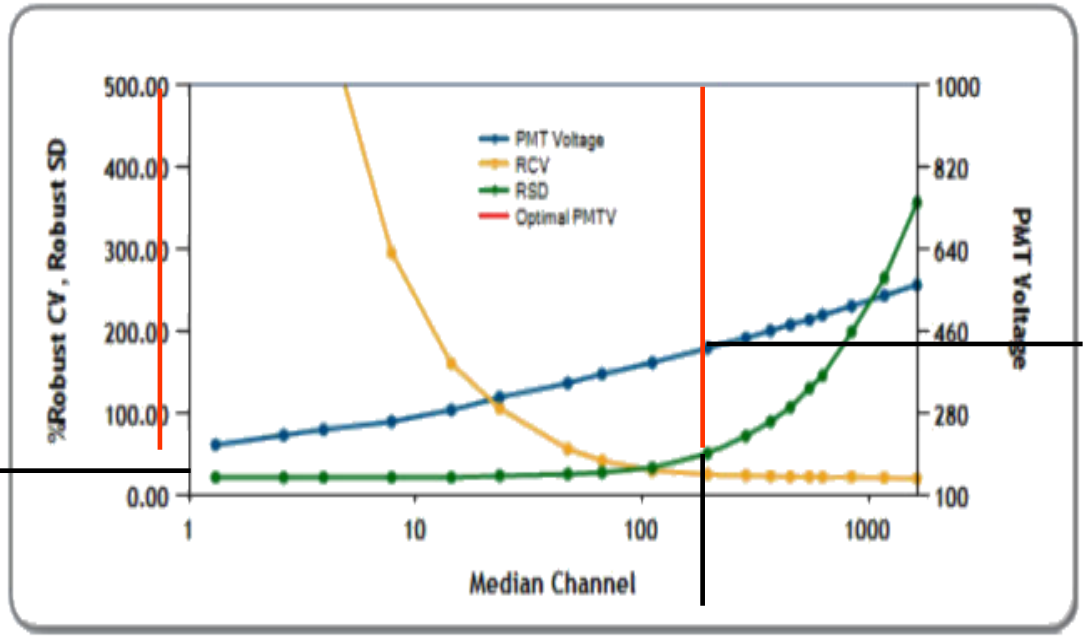
Electronic Noise (EN) Robust SD

Qr: detector efficiency

Br: background

Baseline PMTVs and MFI Target Values

- For each parameter, CS&T determines a baseline PMTV that places the dim bead at an MFI equal to 10 x SD_{EN}.



SD_{EN} = 18.11

PMTV = 451

MFI = 10 x SD_{EN} = 181.1

What are Target Values?

The MFI target value is the MFI of the bright bead at the baseline PMTV.

Detector Settings

Laser	Detector	Parameter	Target Value
Blue	FSC	FSC	125000
Blue	E	SSC	125000
Blue	D	FITC	24583
Blue	C	PE	28355
Blue	B	PerCP-Cy5-5	50516
Blue	A	PE-Cy7	36849
Red	B	APC	62375
Red	A	APC-Cy7	62017
Violet	B	Pacific Blue	28601
Violet	A	AmCyan	118431

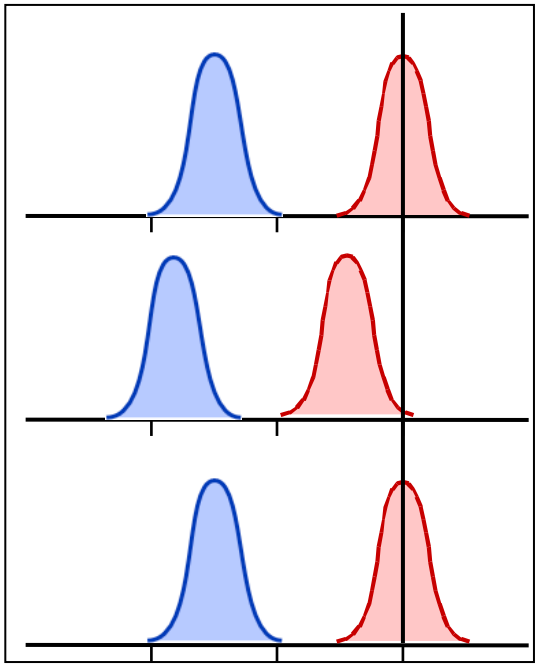
Why are Target Values Important?

By determining the voltage needed to place the bright beads at the target value, CS&T supports tracking and reproducibility of results over time.

550 V Day 1

550 V Day 2

560 V Day 2



On Day 2, the CS&T performance check determines that the PMT voltage must be raised from 550 to 560 to remain at the target value.

Using Application Settings for BD™ CBA Flex Set Assays

- **Application settings enable reproducible MFI values, experiment to experiment.**
  - **Accurate, direct comparison of data over time, across sites**

Day 0:

Application settings for a 30-plex BD Cytometric Bead Array (CBA) created using control CBA beads and the SOV for the red channels determined

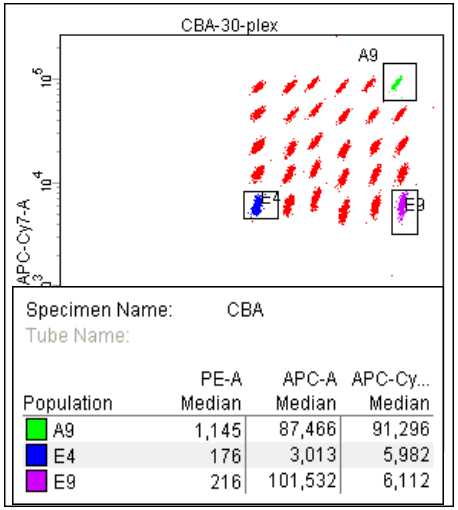
Subsequent days:

Setup was done using standard CS&T setup. No other controls.

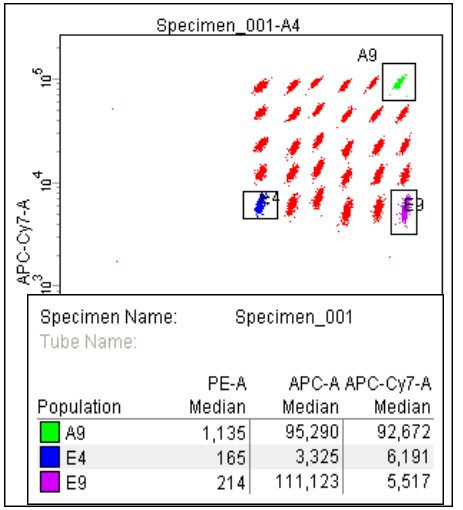
Application settings applied to the experiment, and SOVs from Day 0 experiment copied.

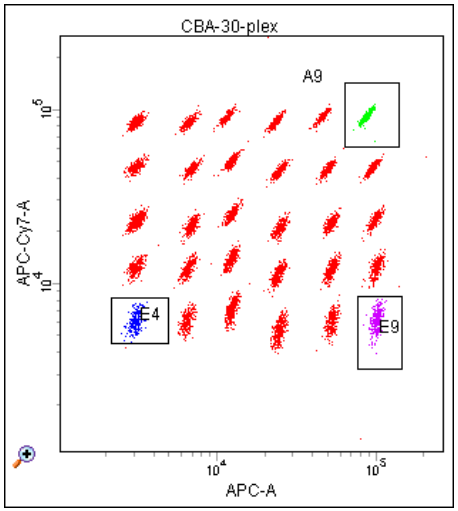
This is data post-compensation.

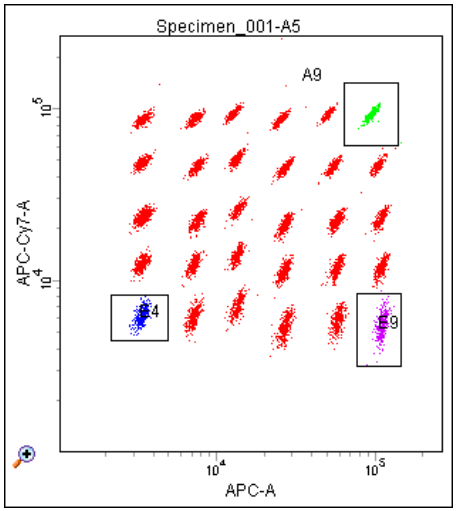
Day 0



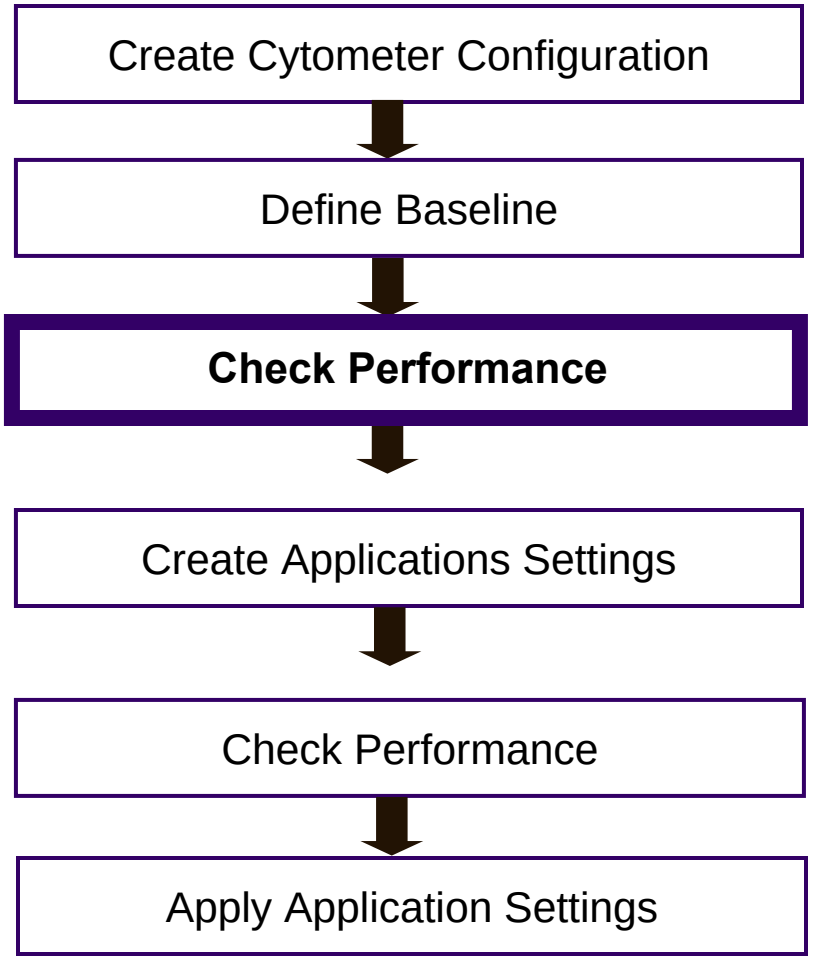
Day 70





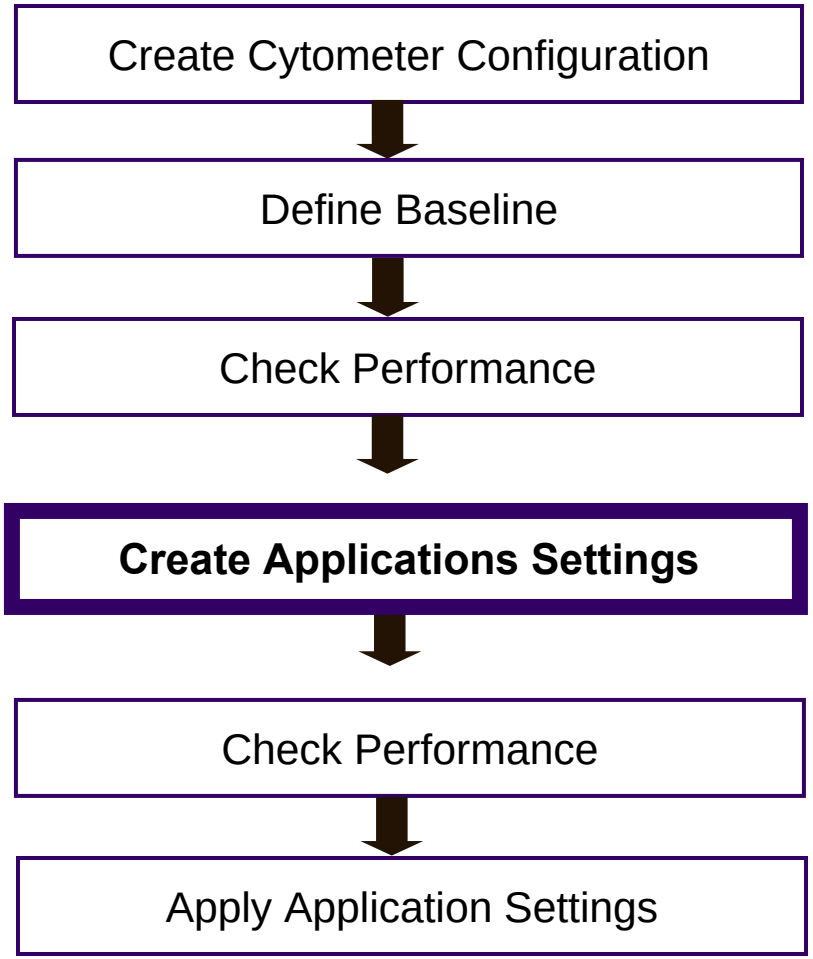


CS&T Workflow



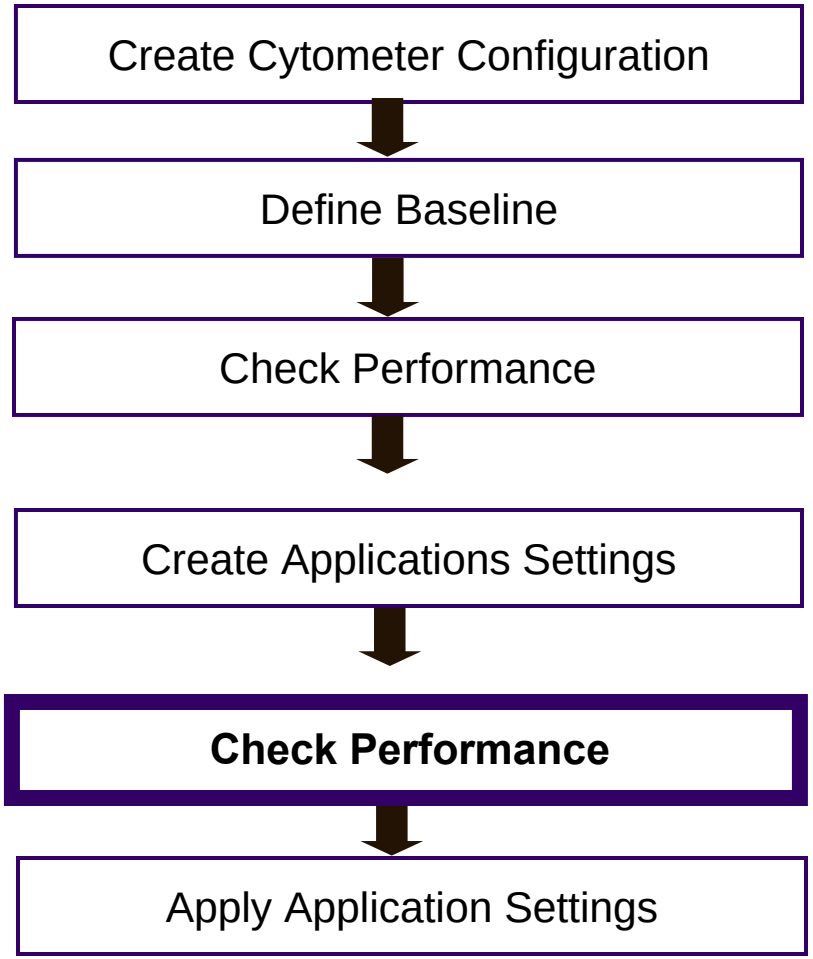
Must be run right after defining the baseline.

CS&T Workflow



Define application-specific or sample-specific settings.

CS&T Workflow



Measures the variation from the baseline and records data for tracking day-to-day cytometer performance.

Adjusts the PMT voltages for each parameter to place the bright bead population at the target value.

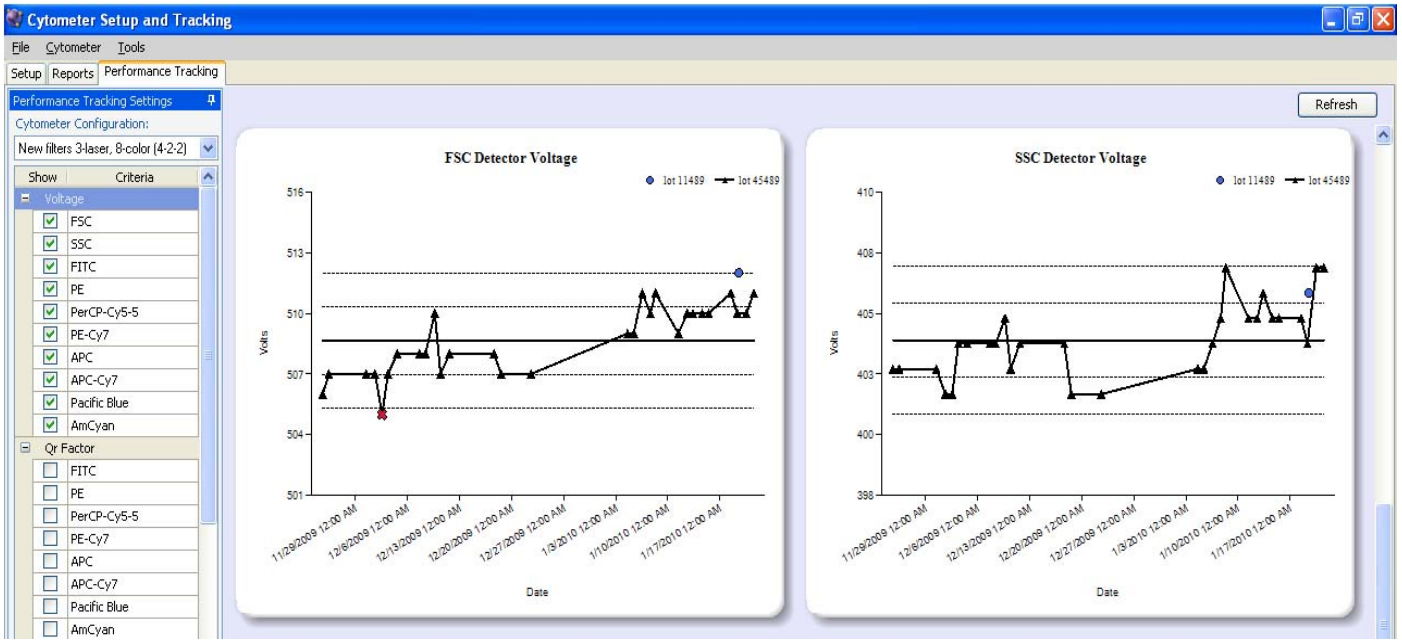
Daily Performance Checks

- Perform at least once a day or before each experiment as required.

- For each fluorescence detector the CS&T module will automatically:
  - Check and adjust laser delays
  - Check and adjust area scaling factors
  - Adjust detector PMT voltages to place bright beads at their target values in each detector
  - Measure performance parameters
    - Detector efficiency (Qr), background (Br), rCVs, and PMT voltages
  - Record and track performance parameters with Levey-Jennings graphs

Performance Tracking

- CS&T automatically tracks all cytometer performance parameters.
- The data is presented in Levey-Jennings plots.



Cytometer Configuration

Cytometer Performance Report

Cytometer:	LSRII	User:	Administrator
Cytometer Name:	LSRII	Institution:	N/A
Serial Number:	1	Software:	BD FACSDiva 6.1.2
Input Device:	HTS	Date:	06/10/2009 11:21 AM
Tube Loaded Manually:	Yes	Cytometer Baseline:	06/10/2009 11:17 AM
Cytometer Configuration:	4-Blue 2-Violet 2-355UV 2-Red (BD default)	P/F:	Pass

Setup Beads

Bead Product: CST Setup Beads, Part #: 910723
Lot ID: 16295, Expiration Date: 05/31/2010
Bead Lot Information: Available

Bright Bead % Robust CV resolving populations

Detector Settings

Laser	Detector	Parameter	Target Value	Actual Target Value	% Difference Target Value	Bright Bead % Robust CV	Mid Bead Median Channel	Mid Bead % Robust CV
Blue	FSC	FSC	125000	122137	-3	4.92	122262	4.88
Blue	E	SSC	125000	123357	-2	6.76	124184	6.91
Blue	D	FITC	24583	23921	-3	4.48	1167	13.07
Blue	C	PE	28355	27848	-2	4.32	1062	17.59
Blue	B	PerCP-Cy5-5	50516	49842	-2	6.97	1919	31.05
Blue	A	PE-Cy7	36849	37212	0	8.20	980	37.17
Red	B	APC	62375	61689	-2	4.29	3442	12.05
Red	A	APC-Cy7	62017	60412	-3	5.47	2521	11.54
Violet	B	Pacific Blue	28601	27987	-3	3.35	1735	10.24
Violet	A	AmCyan	118431	119010	0	3.14	5439	8.83
355 UV	B	Indo-1 (Violet)	41452	40239	-3	4.25	1182	8.89
355 UV	A	Indo-1 (Blue)	73730	72675	-2	5.24	1147	46.48

Detector Settings (Continued)

Laser	Detector	Parameter	Dim Bead Median Channel	Dim Bead % Robust CV	PMTV	Δ PMTV	Qr	Br	P/F
Blue	FSC	FSC	20388	19.92	715	10	N/A	N/A	Pass
Blue	E	SSC	60449	6.43	328	1	N/A	N/A	Pass
Blue	D	FITC	120	57.78	582	-3	0.0126	884	Pass
Blue	C	PE	212	63.27	497	-2	0.0628	691	Pass
Blue	B	PerCP-Cy5-5	200	113.49	678	-2	0.0022	254	Pass
Blue	A	PE-Cy7	57	209.84	725	0	0.0077	58	Pass
Red	B	APC	220	49.03	579	-1	0.0130	96	Pass
Red	A	APC-Cy7	181	50.38	507	-2	0.0037	2039	Pass
Violet	B	Pacific Blue	274	40.49	456	-1	0.0230	2964	Pass
Violet	A	AmCyan	376	55.31	591	1	0.0061	4696	Pass
355 UV	B	Indo-1 (Violet)	145	40.07	502	-2	0.1154	688	Pass
355 UV	A	Indo-1 (Blue)	86	461.63	560	-1	0.0081	1401	Pass

PMTV: optimized PMT voltages

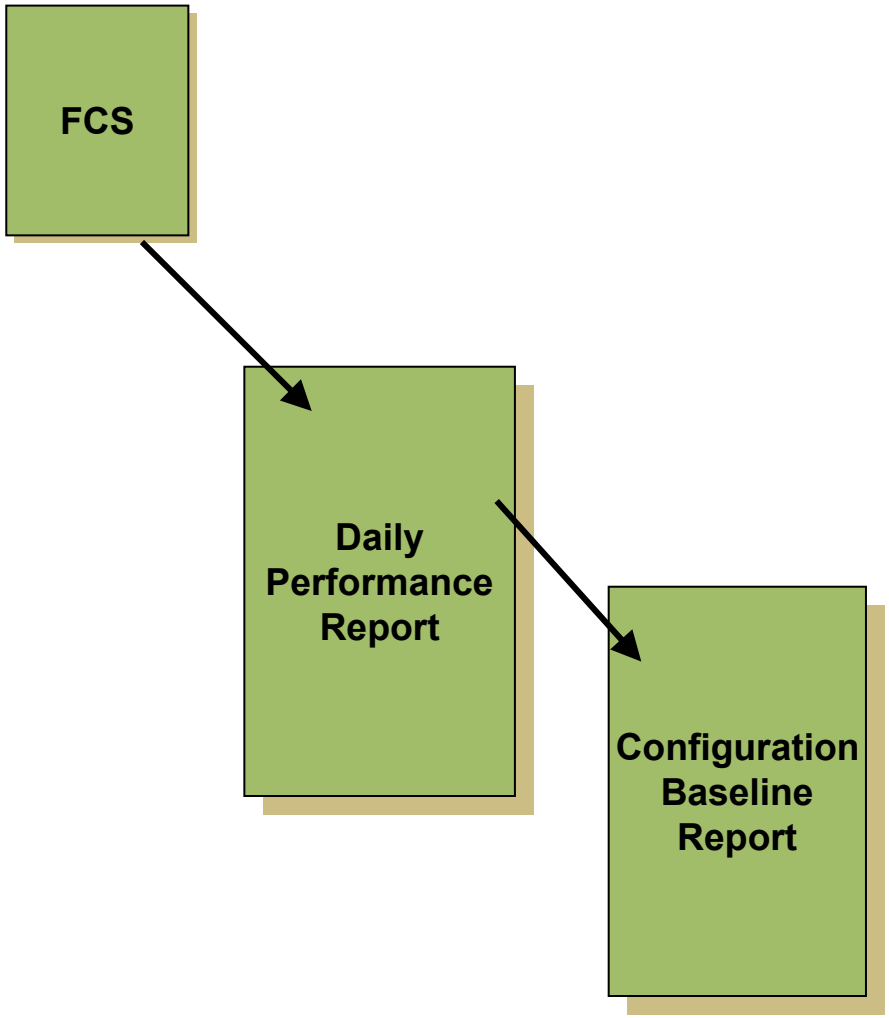
ΔPMTV: change in PMT voltages

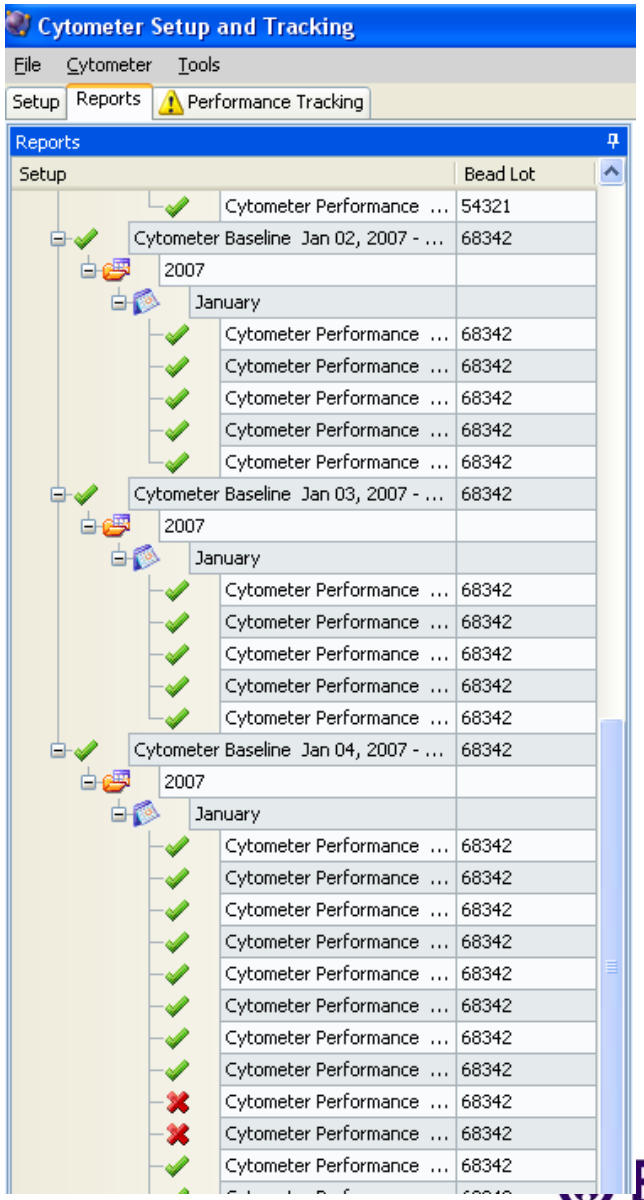
Qr: detector efficiency

Br: background

Performance Data: Reports

All data is directly traceable back to the original baseline report

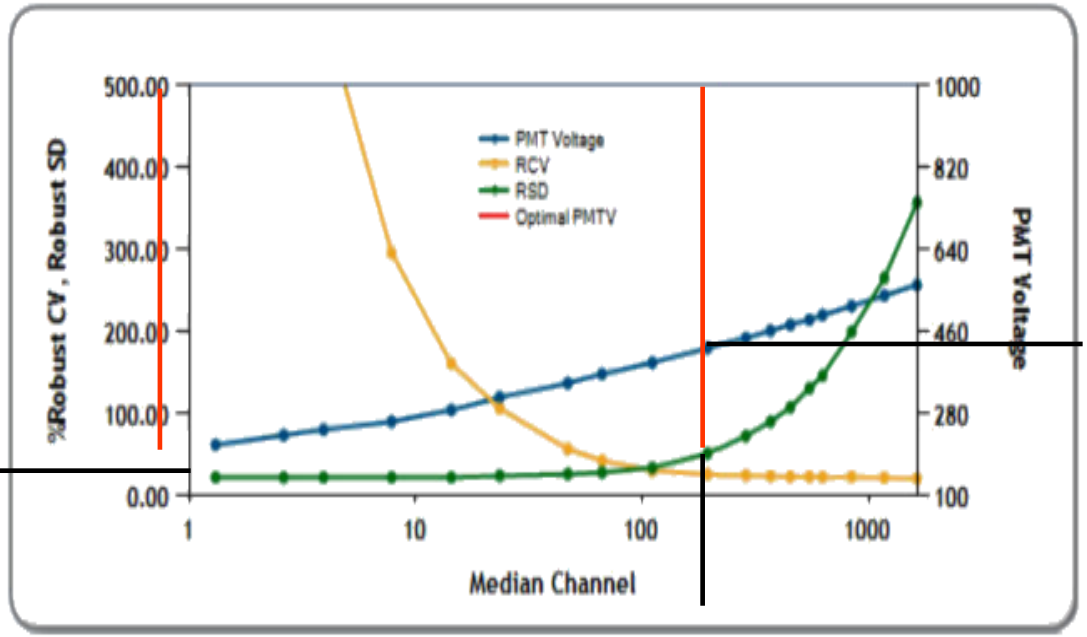




Optimizing Gain Settings (MFI)

Baseline PMTVs and MFI Target Values

- For each parameter, CS&T determines a baseline PMTV that places the dim bead at an MFI equal to 10 x SD_{EN}.



SD_{EN} = 18.11

PMTV = 451

MFI = 10 x SD_{EN} = 181.1

CS&T Settings vs. Application Settings

- One of the criteria for setting gain is to ensure that electronic noise does not impact low-end sensitivity
  - The goal is have the dimmest cells (unstained) where electronic noise is no more than 10% to 20% of the total variance**.**
- An alternative approach—for a given assay, measure the rSD of the negative cells at different gain (PMTV) settings.
  - A good rule—set the gain so that the rSD of the negative cell is greater than 2.5 times the SD of the electronic noise.

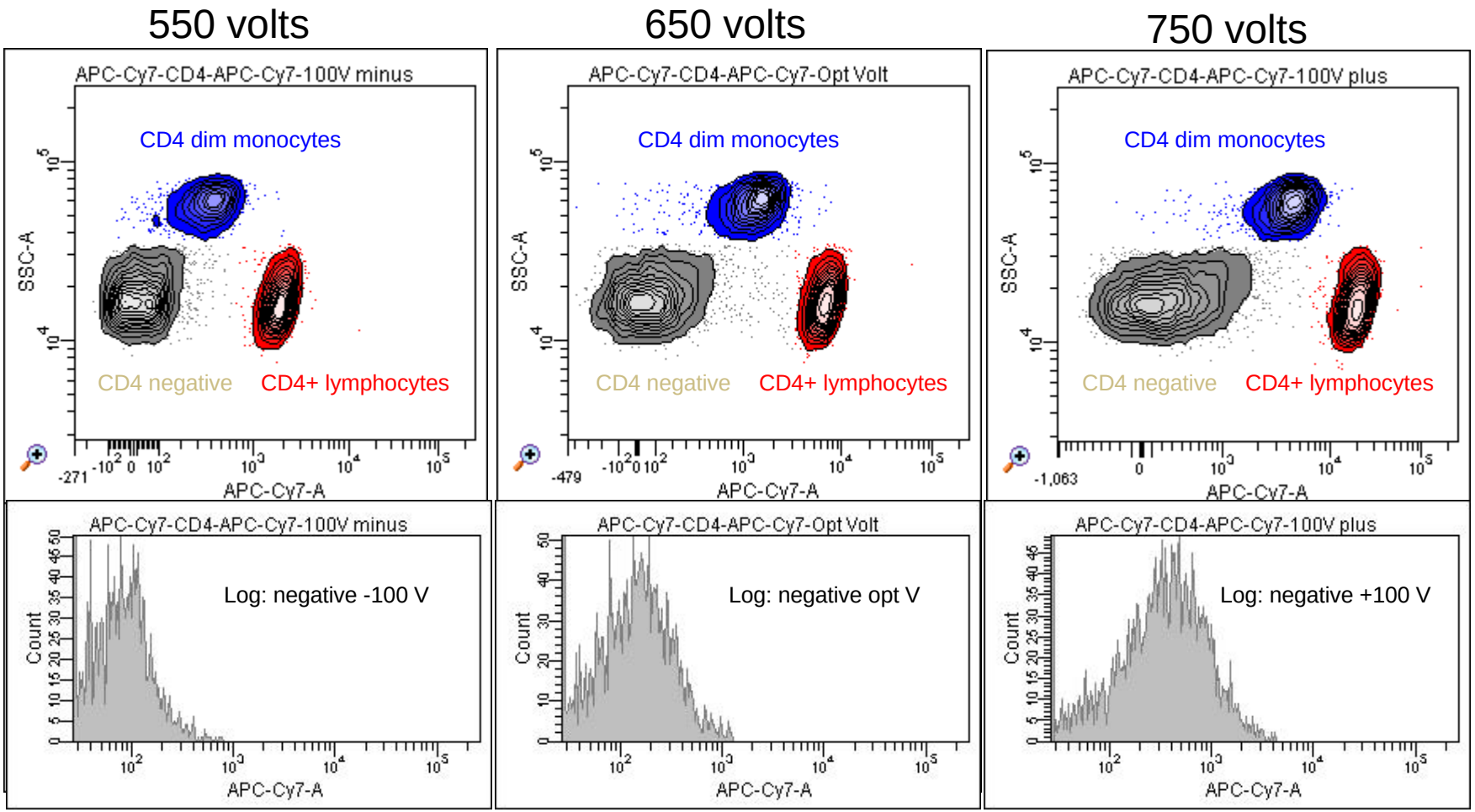
$$\mathrm{rSD}_{\mathrm{Neg\,Cells}} > 2.5 \times SD_{\mathrm{EN}}$$

Tech Note: March 2012

Standardizing Application Setup Across Multiple Flow Cytometers Using BD FACSDiva™ Version 6 Software

Ellen Meinelt, Mervi Reunanen, Mark Edinger, Maria Jaimes, Alan Stall, Dennis Sasaki, Joe Trotter

Correctly Setting PMT Voltage Gain Improves Resolution



Factors to Consider for an Optimal Gain Setup

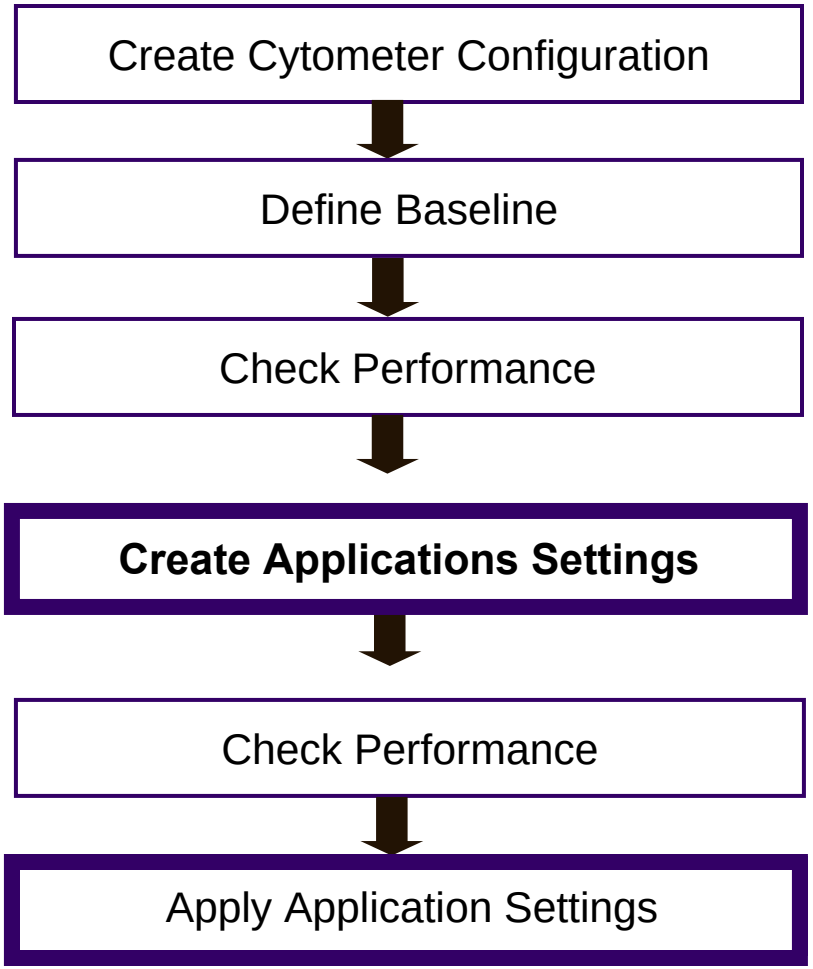
- **Things to consider in *optimizing* the cytometer setup for your specific application:**

  1. ***Electronic Noise* can affect resolution sensitivity**
     - ✓ **A good *minimal* application PMT voltage would place the dimmest cells (unstained) where electronic noise is no more than 10% to 20% of the total variance.**

  2. **Dynamic range assessment for each fluorescence parameter**
     - a) **Are the brightest populations within the *linear range* of the detector?**
       - **Leave room for ~ 2-fold increase in expression levels and ensure the cells are in the linear range of the detector.**
     - b) **Are the compensation controls within the linear range of the detector?**
       - **If positive cells are outside of the linear range, compensation may be inaccurate.**
     - c) **Are the negatives (in a stained sample) too high?**
       - **This is a matter of taste.**

  3. **An optimal cytometer gain setting is one for which both conditions are met.**

CS&T—Application Settings Workflow



Define application-specific or sample-specific settings.

Update application settings to account for day-to-day changes in the cytometer.

Apply updated application settings to your experiment.

Application Settings Scope

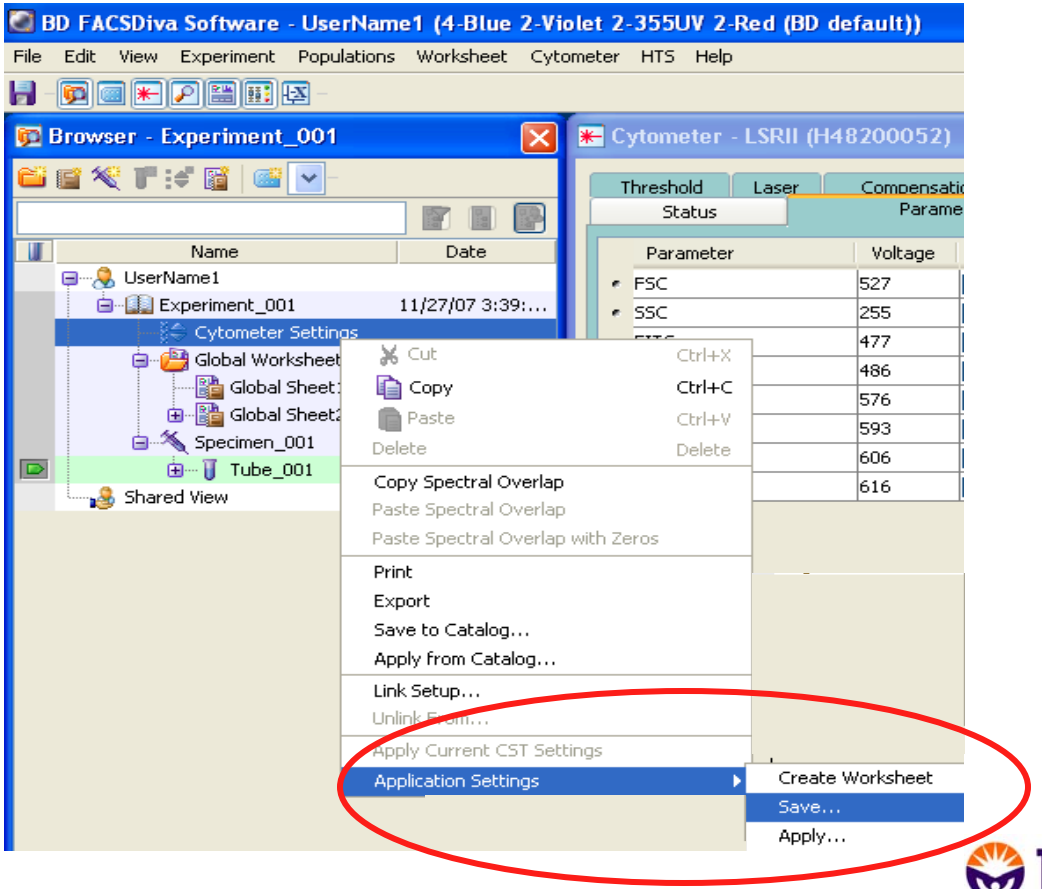
- The GOAL is to optimize settings for the **CELLS** of interest.
- These settings reliably adjust:
  - The negative population(s) to be above the noise on the low end of the measurement.
  - The positive population(s) to be below the top of the linear range at the high end of the measurement.

Creating Application Settings

1. Create an experiment and apply CS&T settings.
2. Delete parameters not used.
3. Adjust area scaling if needed for very small or large cells.
4. Starting with current CS&T settings, run cells and compensation controls, adjusting PMT voltages:
   - Verify that the rSD of the negative population for each fluorescence parameter is 2.5 times the rSD of electronic noise.
   - Verify that the MFI of the positive population for each fluorescence parameter is in the linear region of the scale, and is not greater than 120,000 (for the BD™ LSR II and BD FACSCanto™ II, since this is around half of the linearity max).
5. Right-click the Cytometer Settings icon and select Application Settings > Save.
6. Rename the settings.

CS&T Saves Your Assay-Specific MFI Targets

- **Run a CS&T performance check to standardize the instrument.**
- **Adjust the PMT voltages so that you have the fluorescence intensities (MFIs) that are appropriate for your assay.**
  - **Select "Application Settings-Save" (right-click Cytometer Settings)**
    - **BD FACSDiva 6 / CS&T software remembers the target MFI values.**
  - **These settings can then be applied to future experiments.**
  - **Gives reproducible data**
    - **Experiment to experiment or**
    - **Instrument to instrument**



Summary: Setting Up Your Instrument

- **Only two things need to be done to set up a single instrument for a given type of assay.**

  1. **Set the gain [PMT voltage], to achieve reproducible fluorescence intensity (MFI): this is achieved by creating and using application settings in parallel to use of CS&T.**

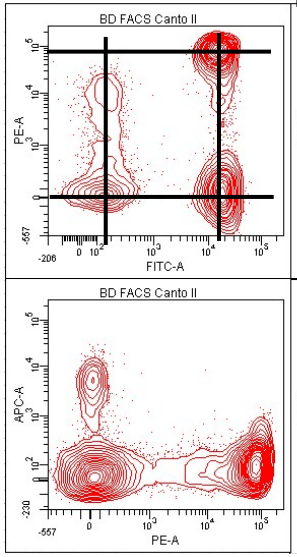
  2. **Correct for background from fluorescence spillover [compensation], which is instrument-dependent.**

- **For comparing results of assays to be run on multiple instruments, it is important that the MFIs are consistent and reproducible between the instruments.**

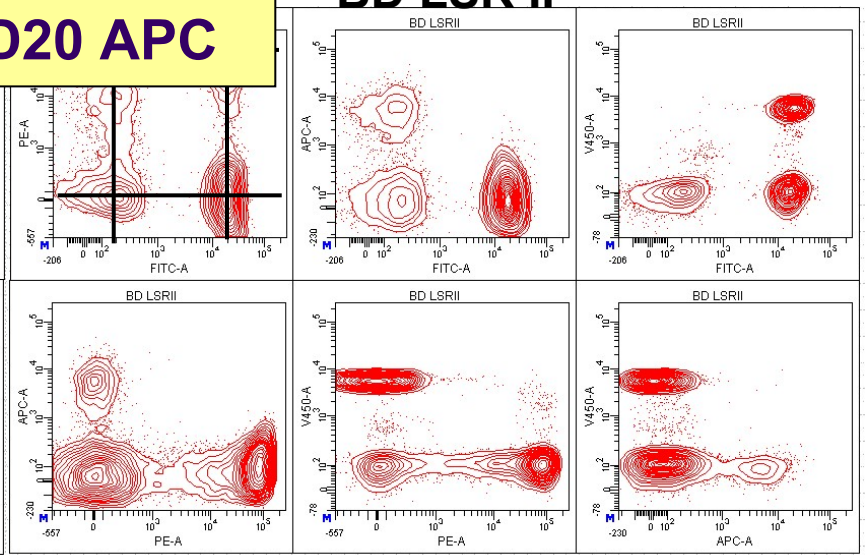
1 Assay – 4 Platforms

- CD4 V450
- CD8 PE
- CD3 FITC
- CD20 APC

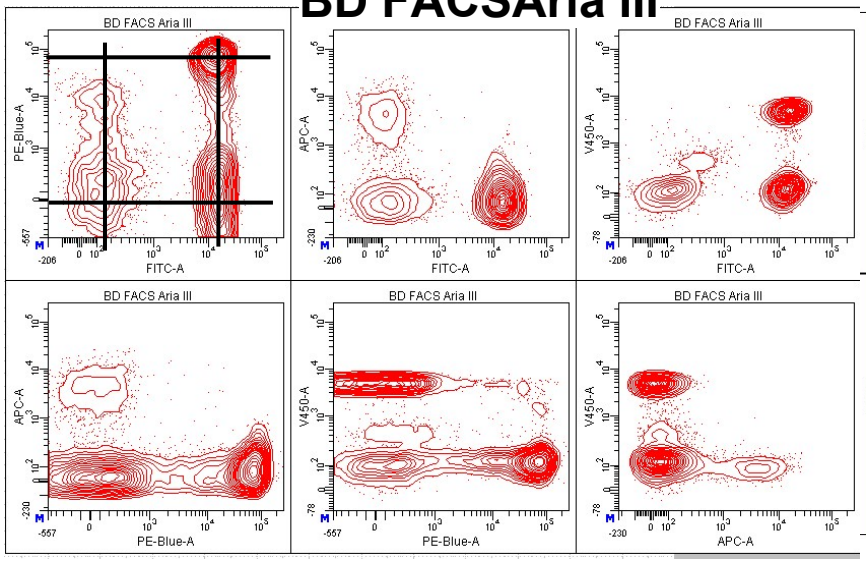
BD FACSCanto II



BD LSR II



BD FACSAria III



BD LSRFortessa