BD Influx System

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BD Biosciences
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Outline

Influx overview:
1. Principle of flow cytometry
2. BD Influx 6-way sorter

Sort theory and application:
1. Principle of sorting
2. Accurdrop technology: Decide drop delay
3. Sort Mode: Purity, Recovery and Speed
4. Sorting Strategy and tip
5. Application

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What is Flow Cytometry?

- Flow = Fluid
- Cyto = Cell
- Metry = Measurement

- A variety of measurements are made on cells, cell organelles, and other objects **suspended in a liquid** and flowing at rates of **several thousands per second** through a flow chamber.

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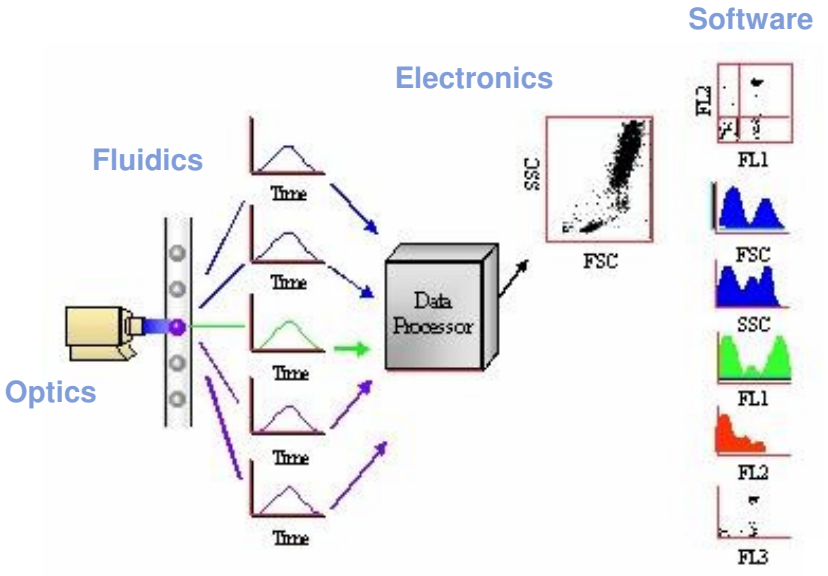
System Overview



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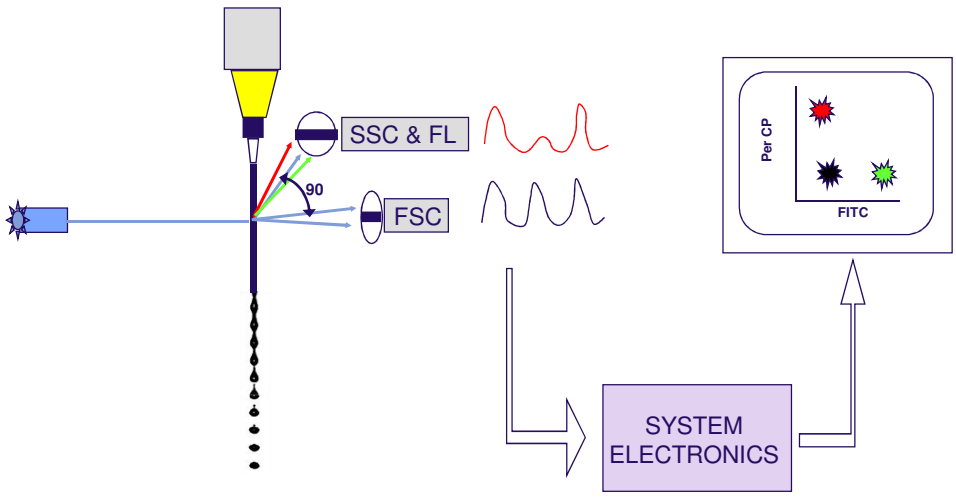
Influx System Overview



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Subsystems

Fluidics

To introduce and focus the cells for interrogation and create a stable breakoff for sorting

Optics

To generate and collect the light signals

Electronics

To convert the optical signals to proportional digital signals, process the signals, and communicate with the computer

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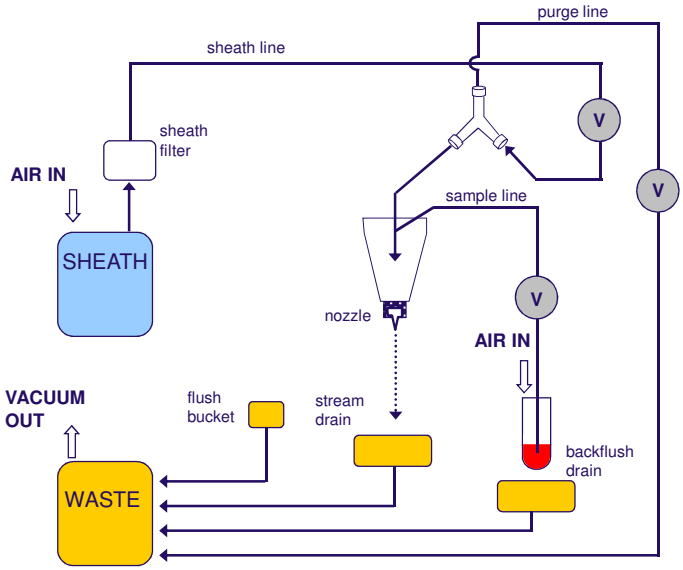
Influx Fluidics



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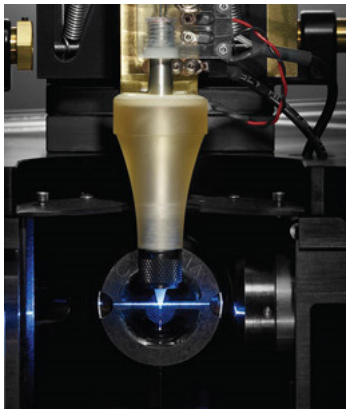
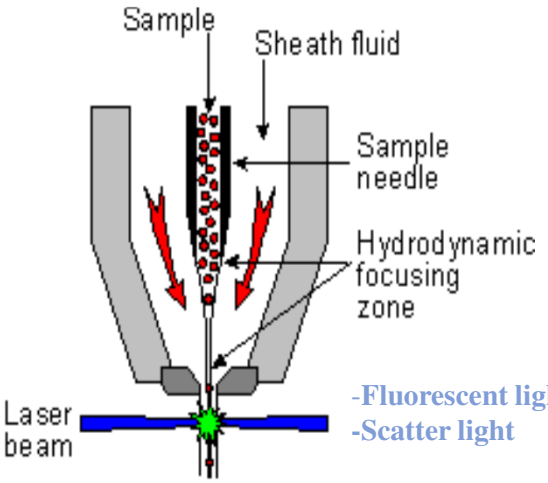
Hydrodynamic Focus





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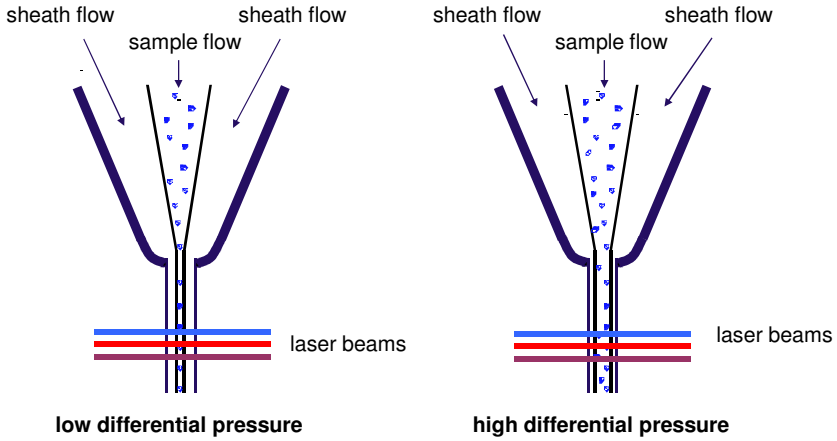
Sample Flow



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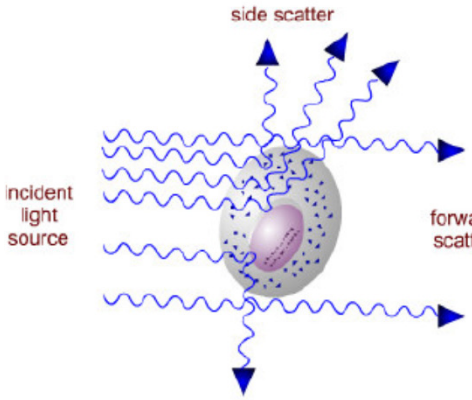
Scatter Light



SSC
Internal Complexity
Granularity

FSC
Size
Shape
Surface
Refractive index

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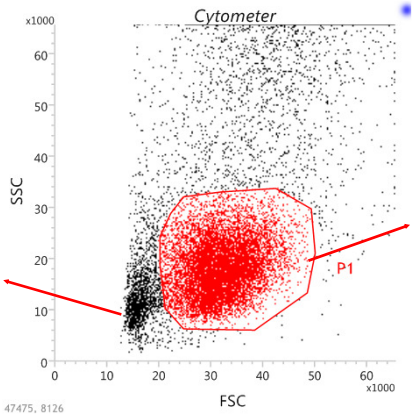
Scatter Light indicating physical properties of cell

Cell Line



Side Scatter

Cell debris

Major Cell population

Forward Scatter

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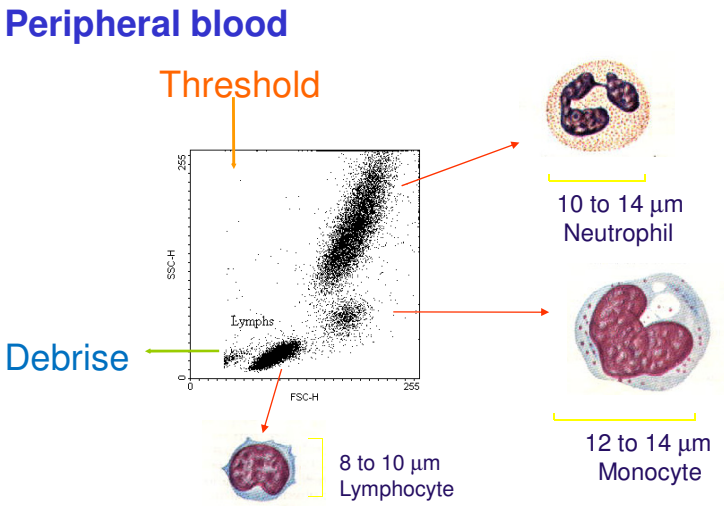
Scatter Light indicating physical properties of cell



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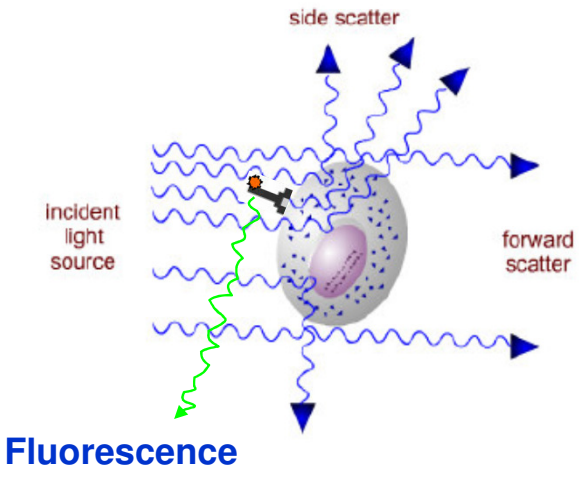
Fluorescent Light



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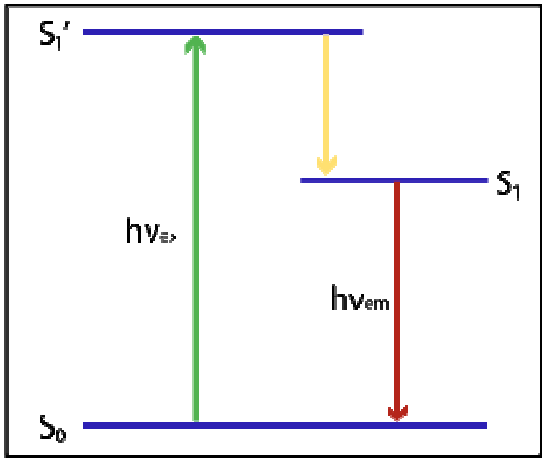
Fluorescent Light



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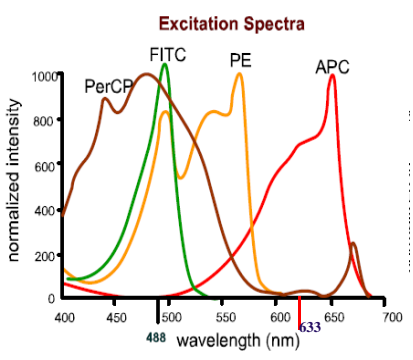
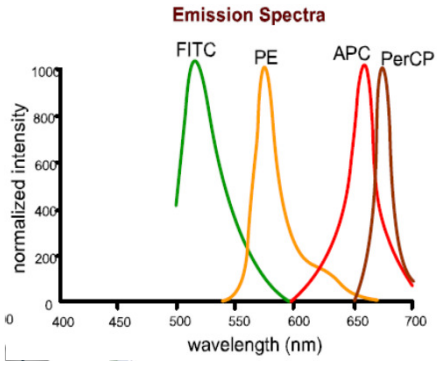
Fluorescent Dye- Excitation and Emission





Excitation: Which laser can generate signals

Emission: Which detector to collect signals

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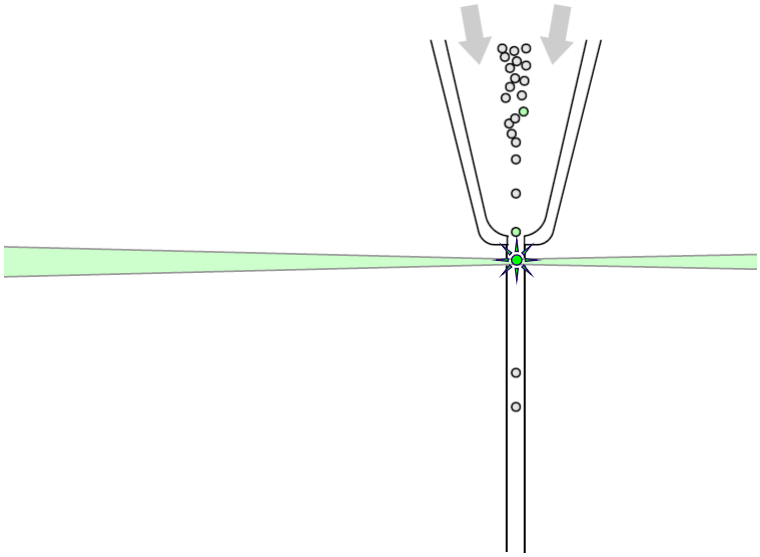
Stream-in-Air Excitation



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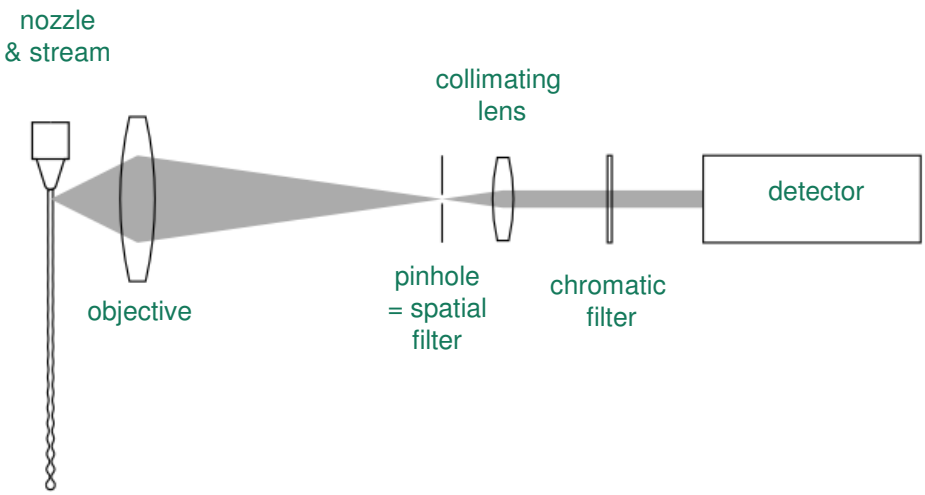
Collection Optics



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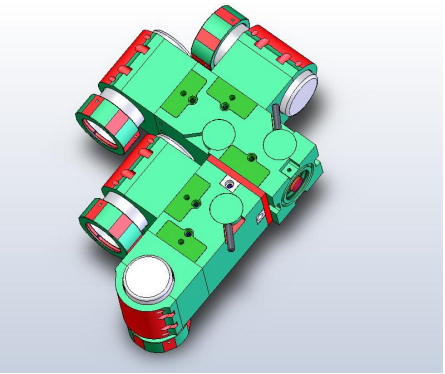
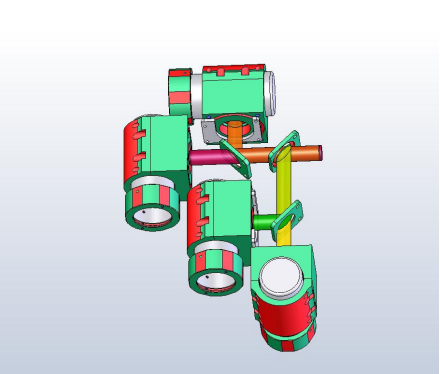
Detector Block

- 488-nm, 4-c1 m1 1 ck (examp1e)1





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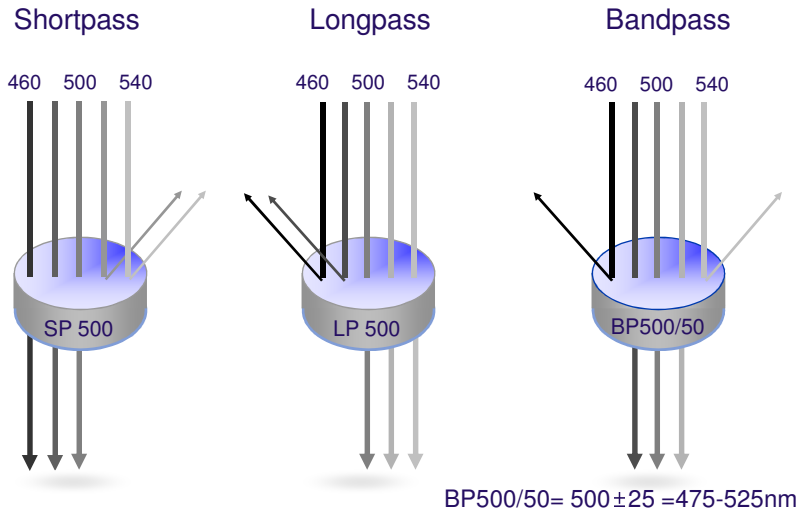
Optical Filters



BP500/50= 500±25 =475-525nm

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Influx Optics Configuration

3 lasers-14 color system (5B-6V-3R)

Excitation1a1	t1ction1Rang1	Examp1 F1uo1och1om1
488 B1u1 a1 (200mW)1	530/401	FITC1
	580/301	PE1
	610/701	PE-TR1
	682/401	Pe1CP-Cy5.51
	750LP1	PE-Cy71
405 Vio1 t a1 (100mW)1	425/261	BV421
	520/351	BV5001
	610/201	BV6051
	660/201	BV6501
	710/501	BV71
	780/601	BV7861
640 R1d La1 (120mW)1	670/301	APC1
	720/401	APC-A exa7001
	750LP1	APC-Cy71

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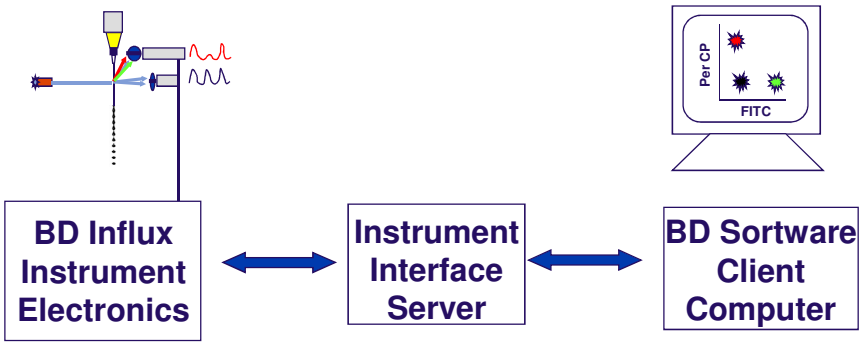
Electronics System Overview



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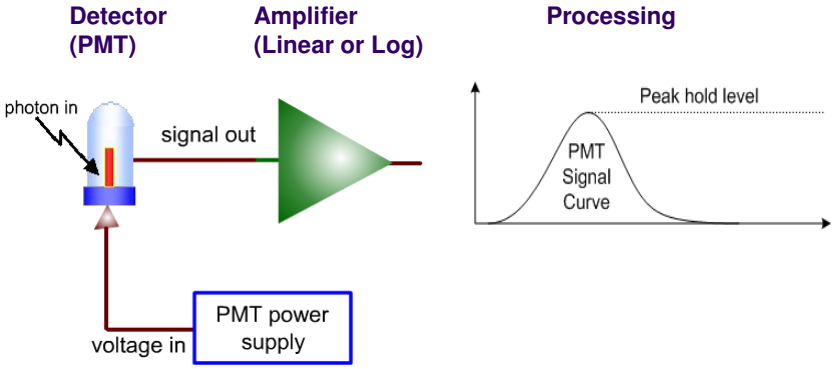
Electronics



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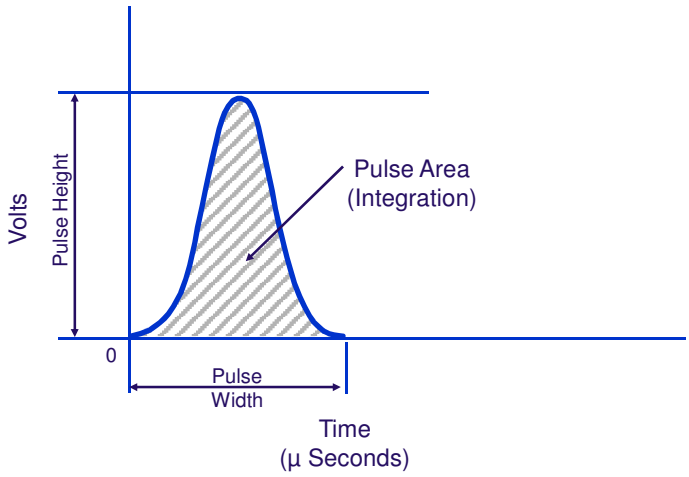
Quantification of a Voltage Pulse



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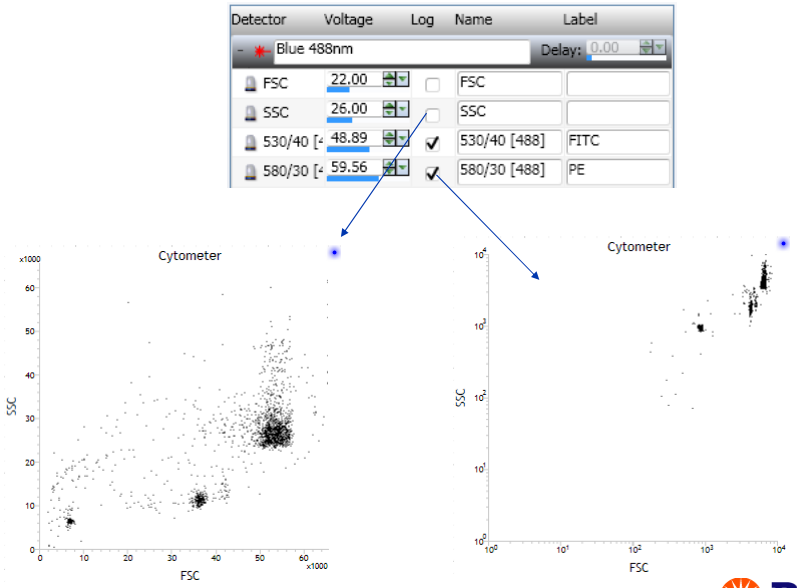
Linear vs Log Amplification



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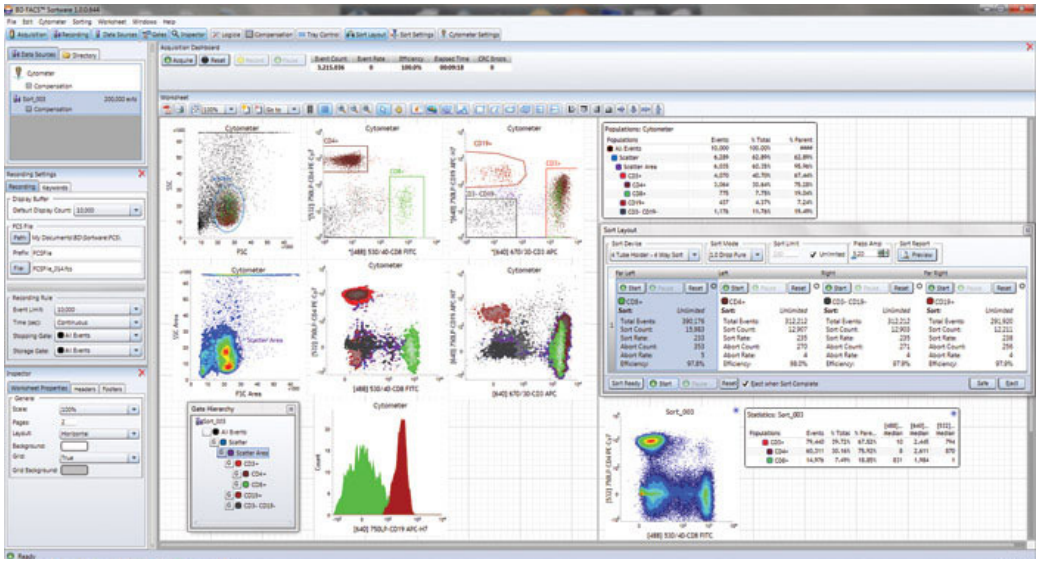
Sortware Software display data



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BD Influx 6-way sorter

Purify target cell or particle population into various device With different choice of nozzle size 70µm, 86µm, 100µm, 140µm, 200µm (optional)

Collection device:
5ml tube, 15ml tube, 50ml tube
96 well, 384 well, slide, customized device

Sort Mode:
2 way, 4 way, 6 way, single cell, index sorting

Optional:
BSC, AMO, temperature control

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Sorting Overview

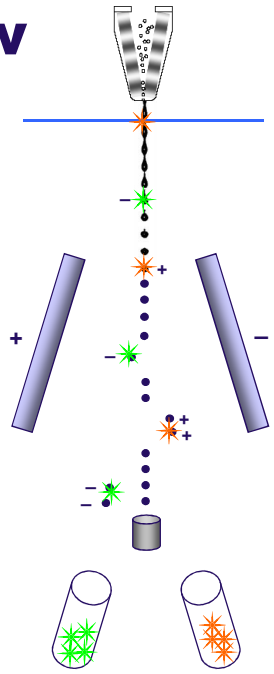
.1 Pass ce1s th1 ugh the ase1 ne at 1 a t1me1

2.1 C1 ect and ana yze s1gna1s f1 m 1 each ce1 t1 dete1m ne wh1ch ce1s 1 t s1 t1

3.1 Cha1ge the st1eam as the d1 p et 1 c nta n ng a ta1get ce1 1 eaks 1ff1

4.1 Def ect the cha1ged d1 p et nt1 1 the app1 p1ate c1 ect1 n tu e 1 1 dev ce1

5.1 A1 w uncha1ged d1 p ets t1 pass 1 t1 waste1



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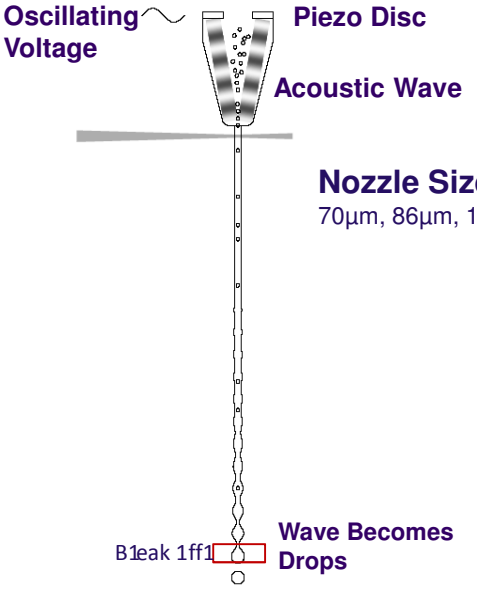
Drop Formation



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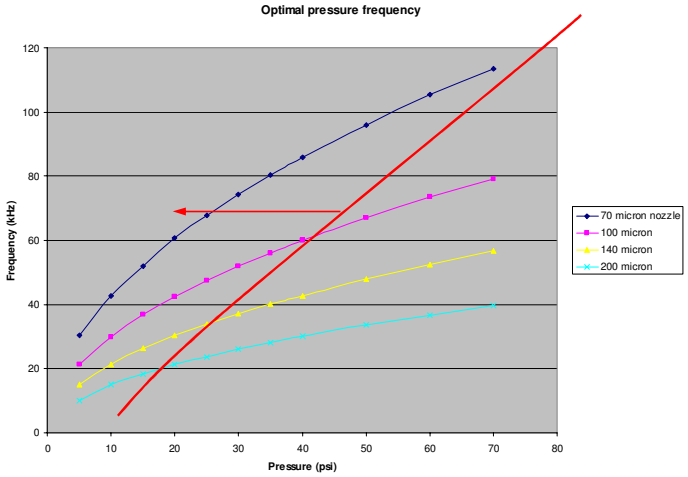
Pressure/Frequency



Recommended
pressure/frequency combinations

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BD Influx Nozzle/Pressure/Frequency setting

Nozz1 1iz1	P1 u1 (p1i)1	F1 qu1ncy (kHz)1
701	**351**	**~75-761**
701	401	~801
701	521	~86.81
701	601	~99.21
701	**651**	**~98.21**
861	**251**	**~501**
861	301	~481
861	331	~581
861	**401**	**~651**
001	**01**	**~361**
001	61	~29.21
001	7-181	~271
001	201	~371
001	201	~391
001	241	~39.001
001	271	39.21
001	271	45.61
001	321	~48.61
401	**51**	**~ 3.91**
2001	**2.81**	**~6.21**

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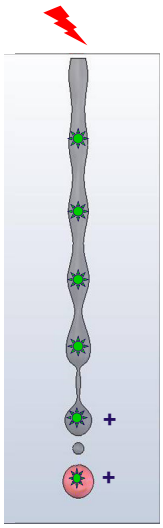
Drop Charging



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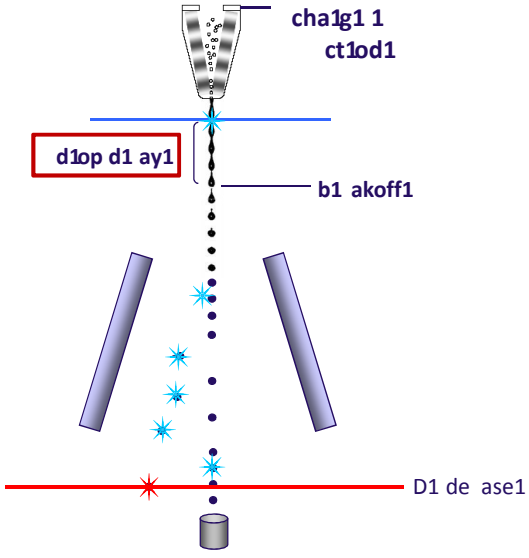
Drop Delay

BD FACS™ Accudrop technology

- Accudrop beads
- Diode laser
- Camera
- Optical filter



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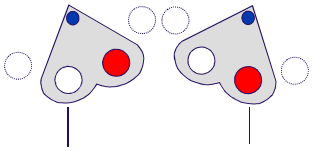
Drop Delay using BD FACS Accudrop

- When drop delay is set correctly, Accudrop beads will be depleted from center stream.
- Use the Accudrop filter so that only the beads show in the stream camera.



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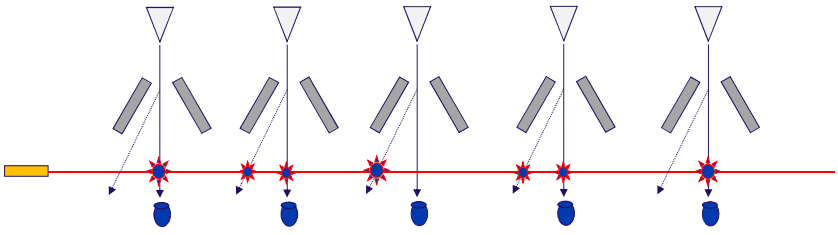
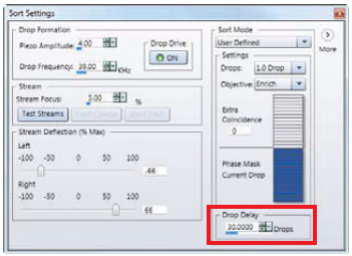
Accudrop

Correct Drop Delay





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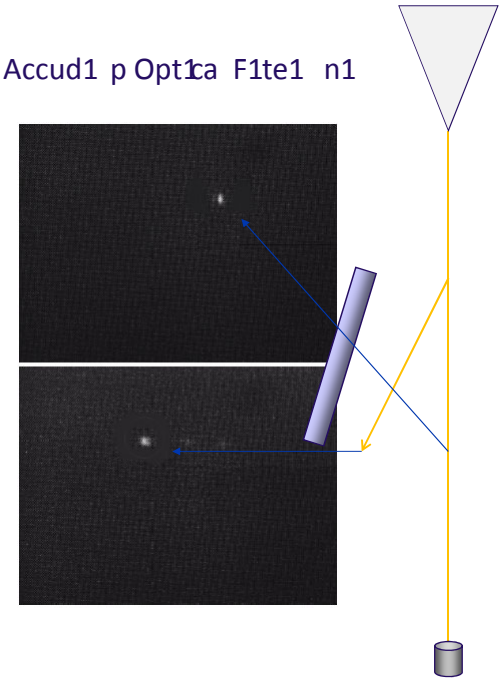
Accud1 p Opt1ca F1te1 n1



Not Correct Drop Delay
Accudrop beads still in the center stream

Correct Drop Delay
Accudrop beads all in deflected side stream

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Sort Mode

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Drops	Sort an additional drop if the cell is on edge of drop
Objective	Enrich (no coincidence) Purify (coincidence with override) Single (coincidence no override)
Extra Coincidence	Adjust how much phase into adjacent drops to abort due to non-target cells
Phase Mask Current Drop	Will only sort if cell is in designated portion of droplet

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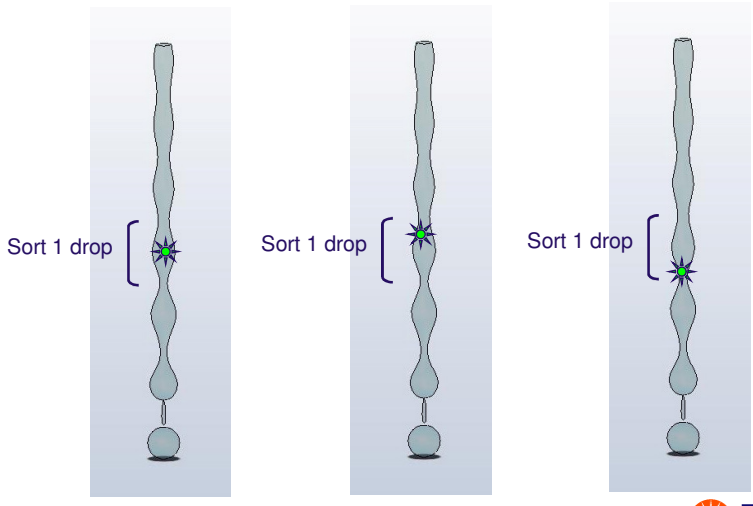
Numb1 of 1 op1

1.0-drop setting

- Sort 1 drop, regardless of the cell's position within the drop.
- There's a chance that cell is actually in other drops.



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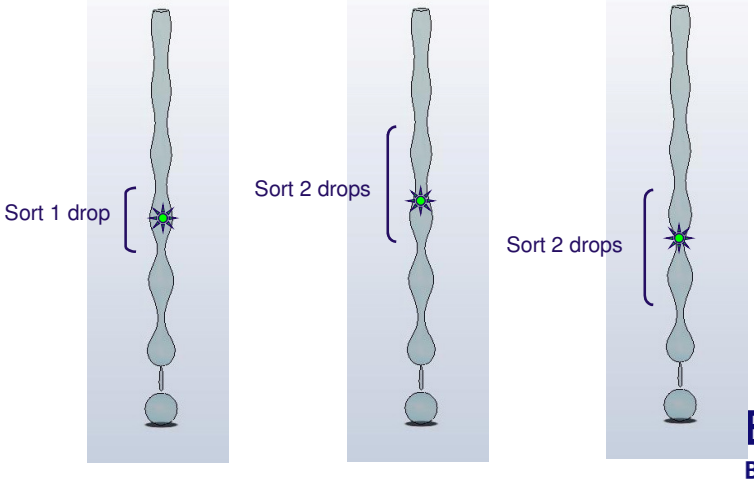
Number of Drops

1.5-drop setting (50%x1 drop+50%x 2 drop=1.5 drop)

- If the cell is in the center of the drop, sort 1 drop.
- If the cell is at the edge of the drop, sort 2 drops.
- You could get more accurate cell count compare to 1.0 drop

1.5 drop is equivalent to Yield mask of 16 on the Aria.



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Objective

The Objective setting influences sort **purity** and sort **efficiency**.

• **Enrich.** Disables all coincidence. Sort as much as you can, for rare cell population

• **Purify.** Enables coincidence and coincidence override
(2 target cells in same drop or within the extra coincidence zone).

• **Single.** Enables coincidence and disables coincidence override to ensure that only one target event can be sorted. (single cell sorting for plate)

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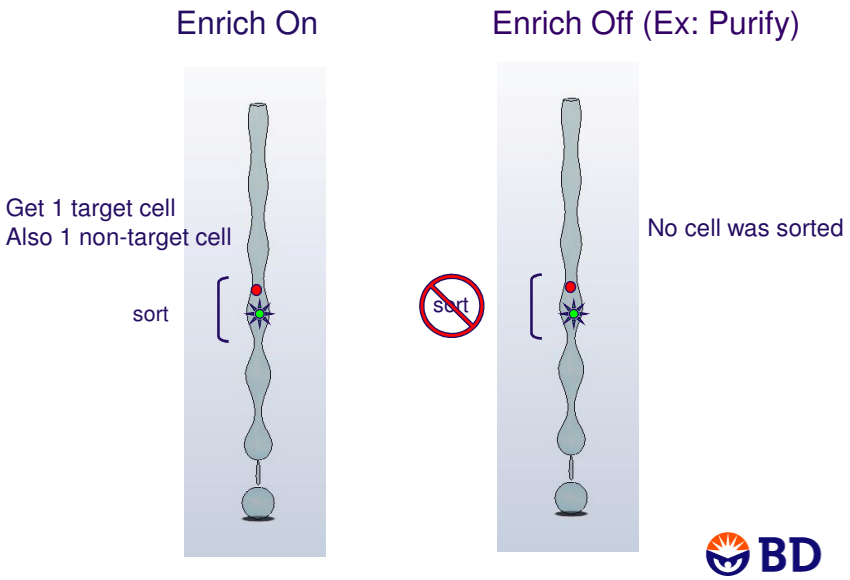
Enrich Setting



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Standard Sort Modes

.0 D1 p Y e1d1	-d1 p s1 t1 .0 D1 p C1 nc1dence1 N1 phase gate1	If y1u need t1 get the h1ghest 1 c1m1 nat1 n 1f pu ty and y1e1d1
.5 D1 p Pu e1	.5-d1 p s1 t1 .5/2.5 D1 p C1 nc1dence1 N1 phase gate1	If y1u need an exact c1unt and 1 h1gh pu ty1
2.0 D1 p En1ch1	2-d1 p s1 t1 N1 C1 nc1dence1 N1 phase gate1	If the 1ec1ve1y 1f ta1get ce1s 1s 1 m1 e 1mp1 tant than pu ty1
.0 D1 p S1ng1e1	-d1 p s1 t1 .5 D1 p C1 nc1dence1 0/16 D1 p Phase Mask1	If y1u need t1 s1 t 1a1ge 1 st1cky 1 ce1s and need an exact c1unt1 If y1u need t1 s1 t s1ng1e ce1s 1nt1 1 p1ate we1s1

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Sorting Tips

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Sort Performance

- Depends on what you want:
  - –Purity
  - –Recovery
  - –Yield
  - –Viability

} Speed !!

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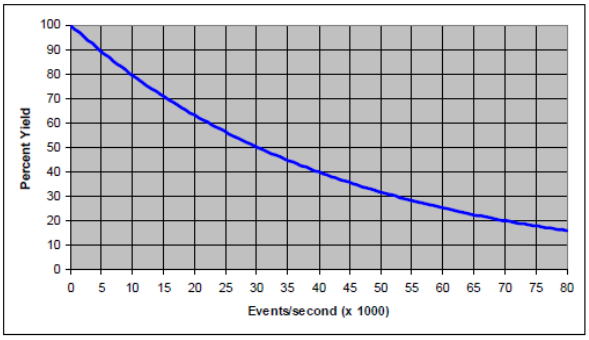
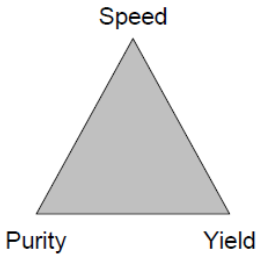
Sort Performance

- Speed vs. Yield/Recovery





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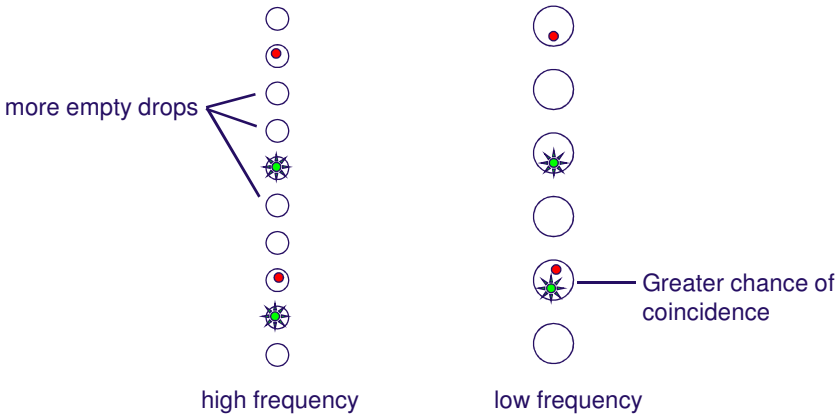
The Importance of Frequency



same event 1ate, h ghe1 f1equency w1 have 1 h ghe1 eff c ency and 1ec1ve1y1

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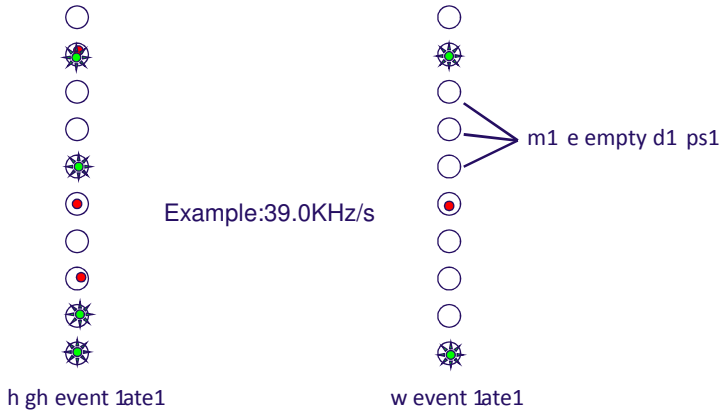
The Importance of Event Rate



Same F1equency, 1 we1events 1ate w1 g1ve y1u h1ghe11
ec1ve1y 1ut w1 c1nsume m1 e t1me1

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Sample Preparation Considerations

- Enrich rare cell population if possible
- Avoid cell clumps
  - Always filter your cells before sort!
  - Use Accutase instead of Trypsin
  - Treat cells with DNAse
- Use appropriate sample buffer
  - PBS, HBSS or phenol-red free culture media w/ 25mM HEPES, 5mM EDTA and 1~2% FBS or 0.1~0.2% BSA to maintain cell viability
- Use viability dye to confirm cell viability before sort

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Summary: Sorting Considerations

- Collection tube:
  Pre-coated with 1% BSA or 10% FBS overnight and filled with appropriate collection buffer
  - 5ml Falcon tube: 2ml
  - 15ml centrifuge tube: 7ml
- Change collection tubes periodically
- Temperature control
- Event (Threshold) rate:
  1/10~1/4 drop drive frequency for better yield

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Compensation Theory

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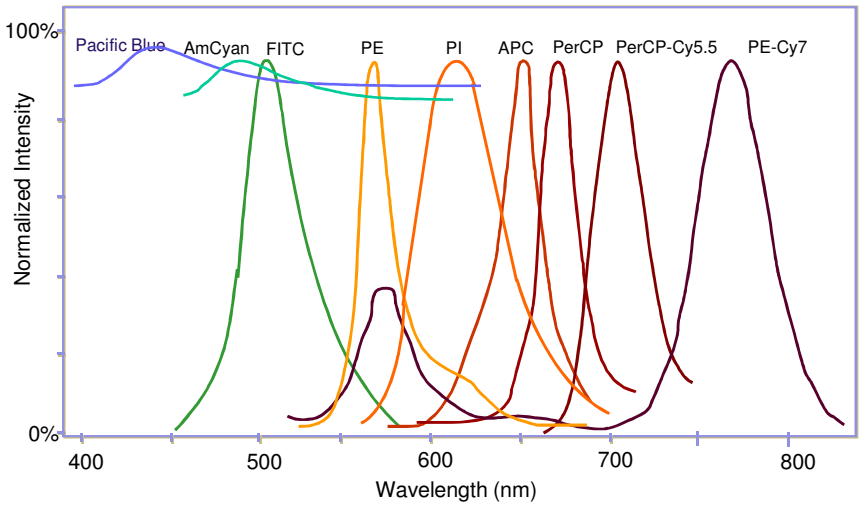
Emission Spectra: Spectral Overlap



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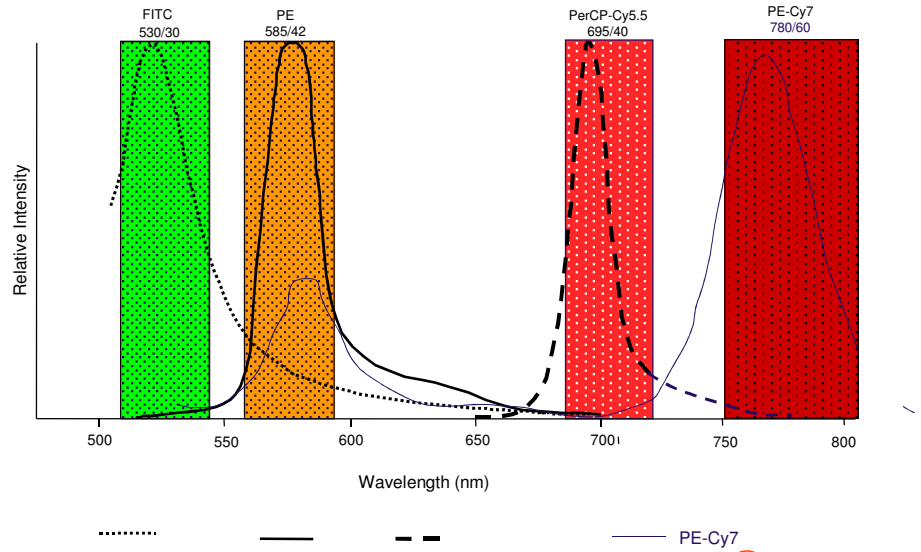
Spillover



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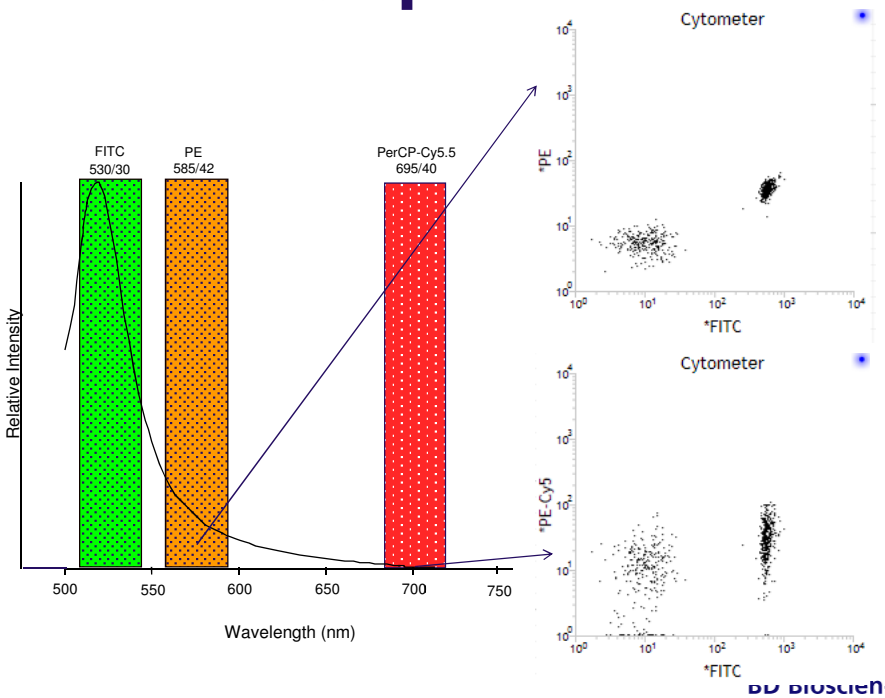
FITC Spillover



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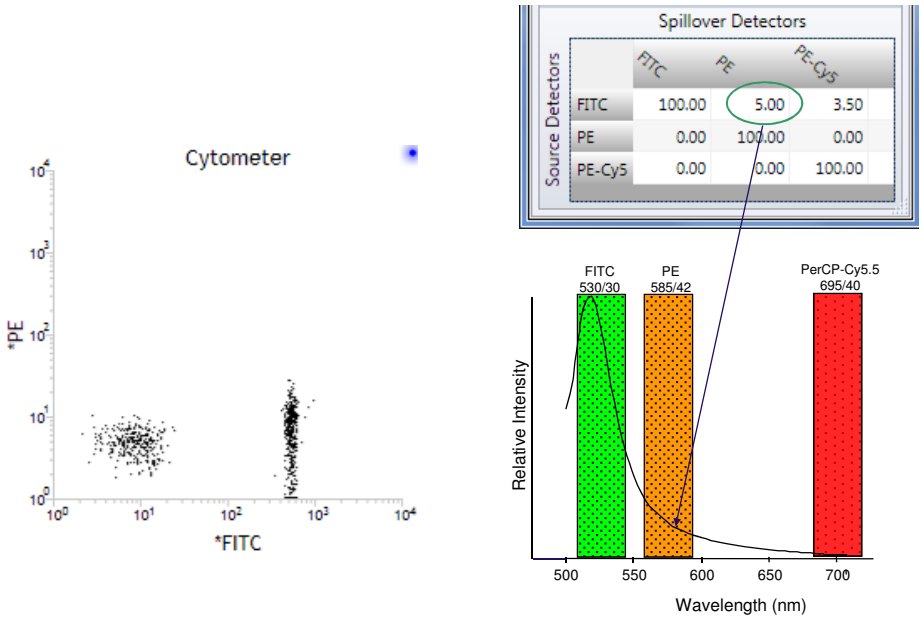
FITC Compensation



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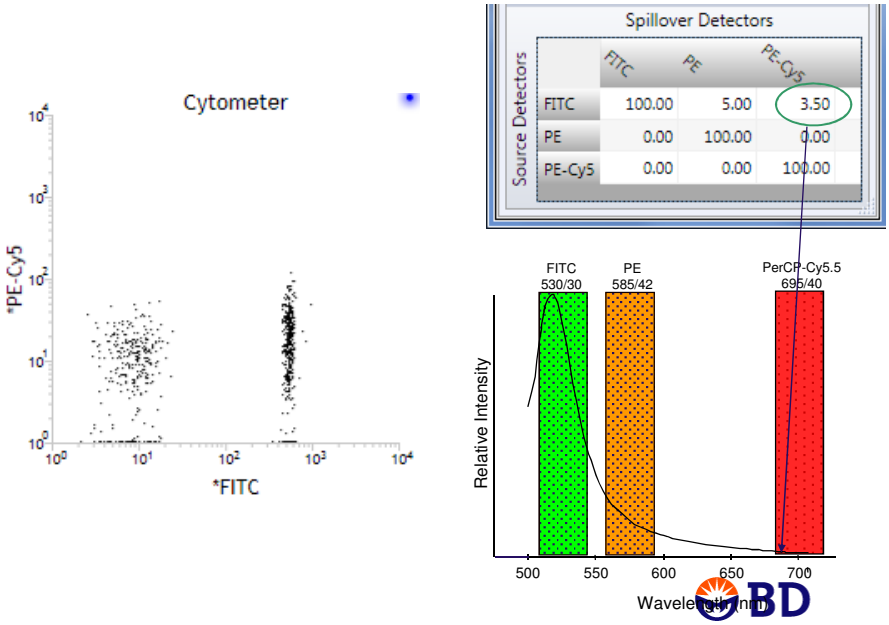
FITC Compensation



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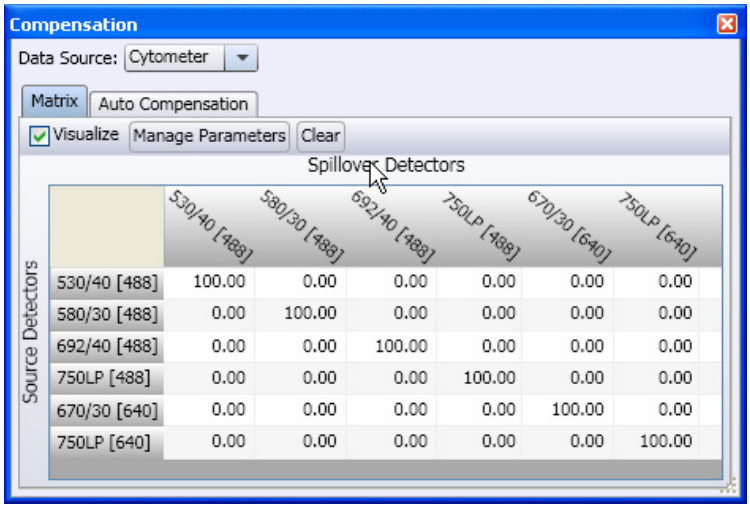
Comp1n1ation Mat1ix1



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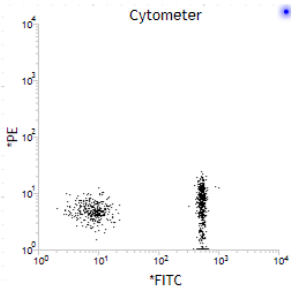
Compensation Examples

Inc1 ect C1mpensat1 n1

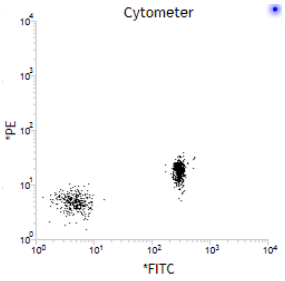
C1 ect C1mpensat1 n1



Statistics: Cytometer

Populations	FITC Median	PE Median
FITC+	530	6
negative	8	5

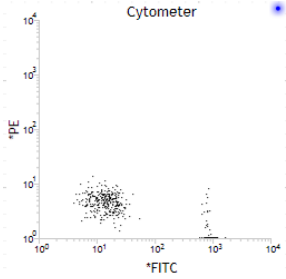
Unde1c1mpensat1 n1



Statistics: Cytometer

Populations	FITC Median	PE Median
FITC+	294	18
negative	5	5

Ove1c1mpensat1 n1



Statistics: Cytometer

Populations	FITC Median	PE Median
FITC+	872	1
negative	14	5

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Application

- Sort different target cell
- Life or death
- Morphology
- Surface antigens
- Gene expression.
- Cell functions.
- DNA content

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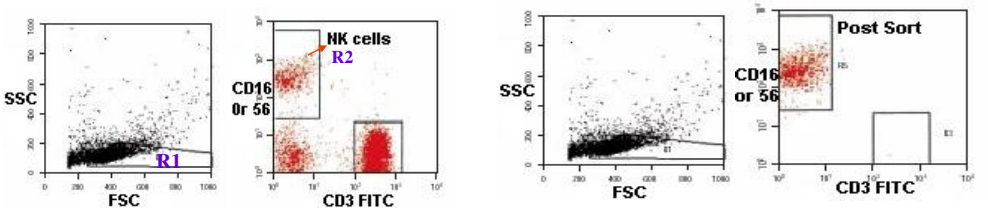
Sorting Cells By Surface Markers

- **So1ting NK C1**
  - – CD3 FITC t1 exc1ude T ce1s1
  - – CD56+CD16 PE t1 nc1ude a1 NK Ce1s.1



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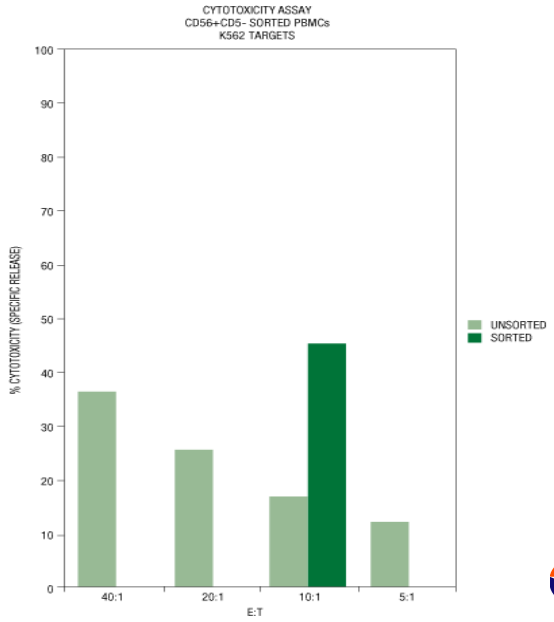
Sorting NK Cells for Cytotoxicitity Studies



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Regulatory T Cell Sorting

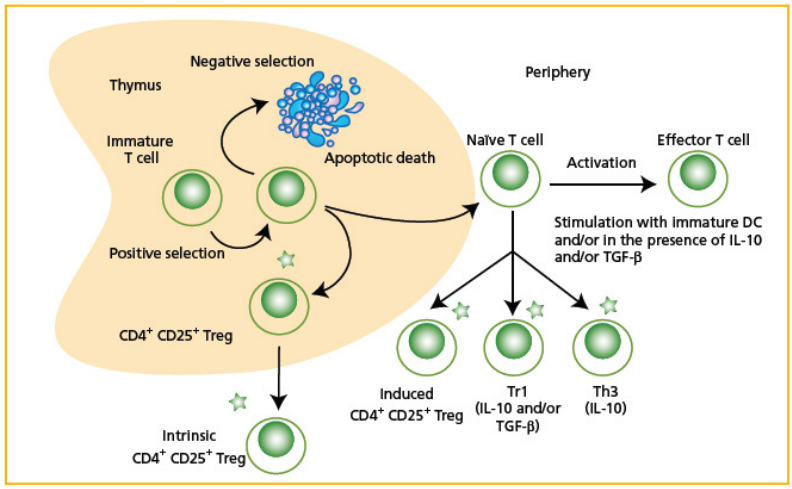

Figure 1. Schematic representation of the role of regulatory T cells in immune function

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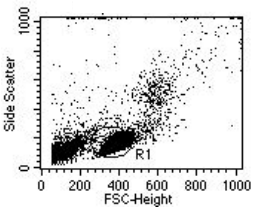
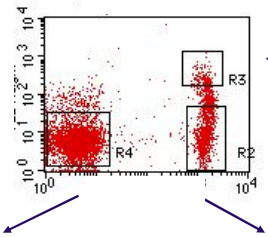
CD4/CD25 Treg sorting



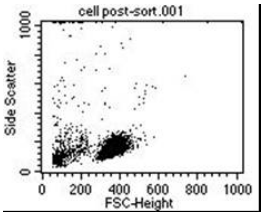


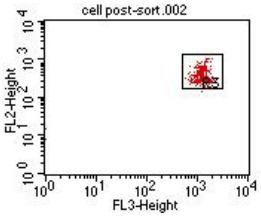
1.35 %

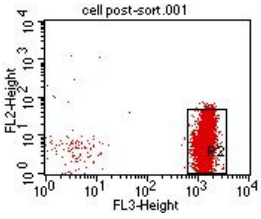
8.94 %

Sort on R1 and R2

sort on R1 and R3







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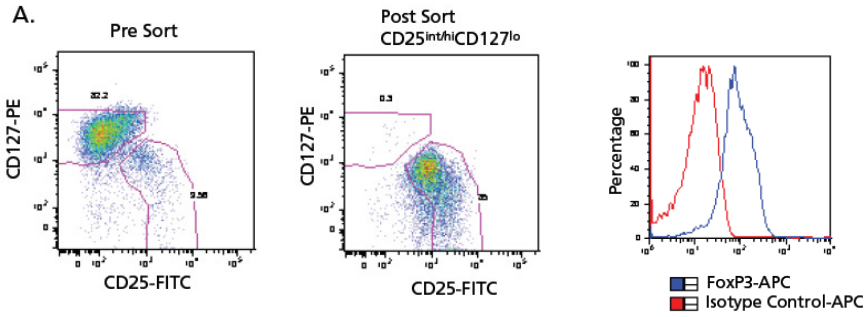
CD4/CD25 High/CD127 Lo



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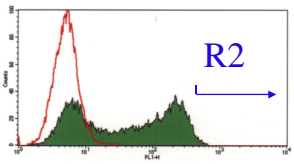
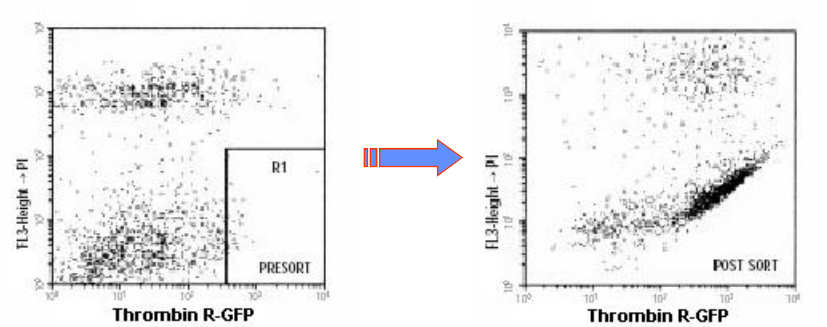
Sorting by Gene Expression

Add viability check.





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Sorting by Gene Expression

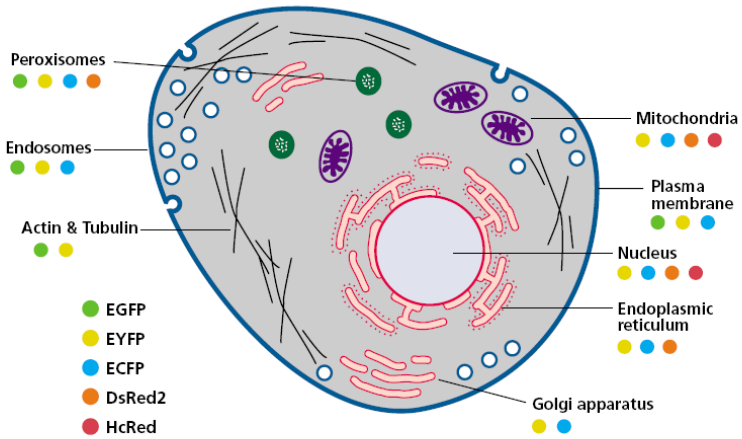

Figure 12. Organelles targeted by BD Living Colors™ Subcellular Localization Vectors.

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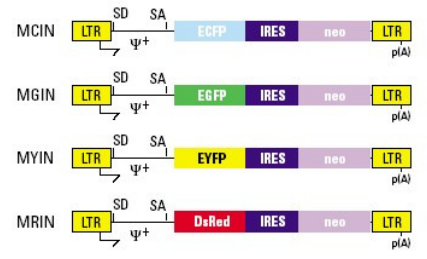
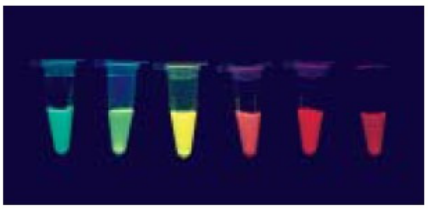
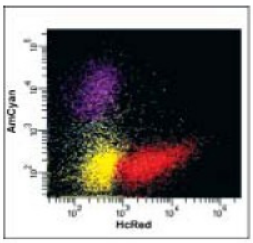
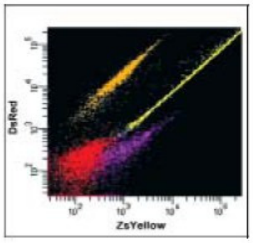
Sorting by Gene Expression









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Rare Cell Sorting tip

- Ra1e ce1 s1 t ng usua1y 1equ es p1e-en1chment steps:1
  - B1ng the sta1t ng pu ty t1 > 5 %1
  - F c1
  - Immune Pann ng1
  - Magnet c Beads (P1s t ve/Negat ve)1

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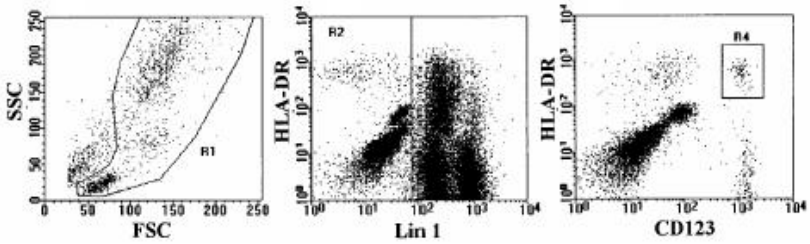
Parameter	CD123+ DCs	CD11c+ DCs	Basophils
Lin 1	-	-/+	-
Anti-HLA-DR	++	+++	-
CD123	+++	+	+++
CD11c	-	+++	+

Immunophenotype of CD11c, CD123 DCs and baso
(fluorescence intensities)

- 4-Color Staining
  - Lineage Cocktail FITC
    - CD3, 14, 16, 19, 20, 56
  - CD123 (IL-3Rα) PE
  - HLA-DR PerCP
  - CD11c APC
  - Controls
  - FACLysing Solution



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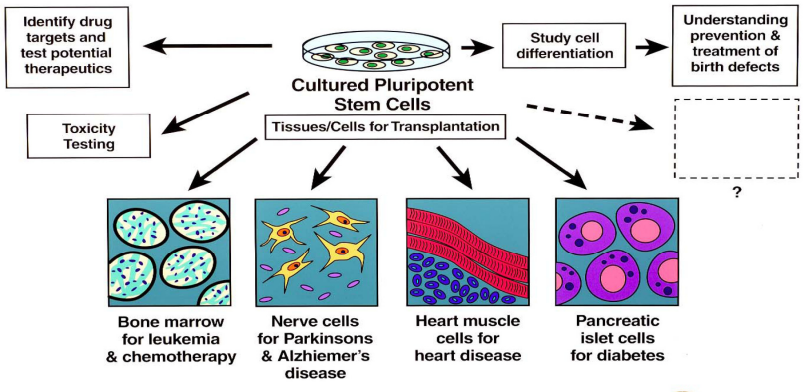
Stem cell sorting



Schematic adapted from http://stemcells.nih.gov/index.asp

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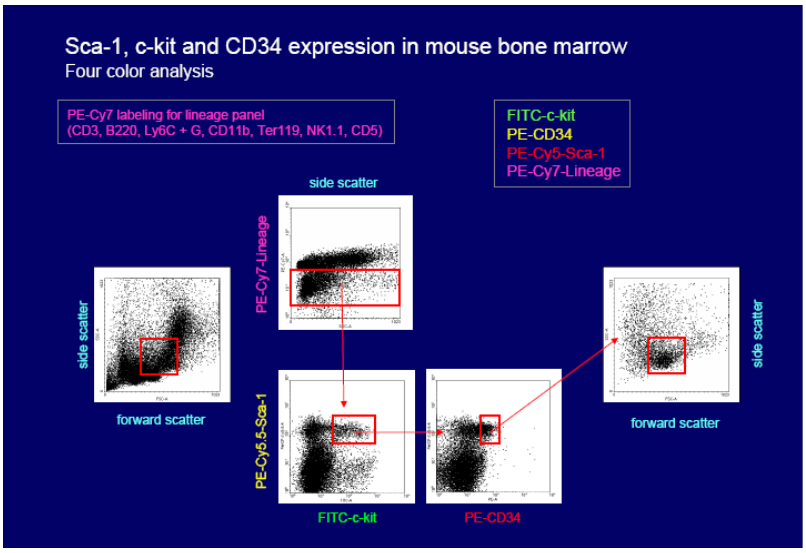
Hematopoietic Stem Cells



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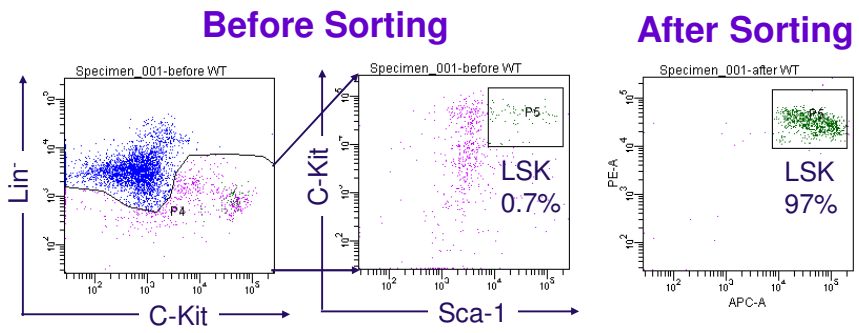
Bone Marrow Stem Cell



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Thank You

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