

Electronics

- Photodetectors
 - Photodiode
 - Avalanche Photodiode
 - Photomultiplier
 - Charged Coupled Device
- Signal Amplifiers
 - Linear
 - Logarithmic
 - Scales: linear vs log
- Analog-Digital Converters
- Pulse
 - Intercept Point
 - Genesis
 - characteristics: A, H, W
- Threshold – Trigger
- Window Gate – Window Extension
- Area Scaling Factor
- Time Delay
- Noise
- Data Representation
 - Creation of a Histogram
 - Types of Plots
- Compensation
- FMO



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Photodetection

- Required in almost all electro-optic systems
- The photodetector receives the collected light energy and converts it into electrical energy and this in turn converted to digital information.
- It gives a measure of the intensity or irradiance of the incident light.
- There are DIFFERENT type of detectors:
 - Photodiodes
 - Photomultipliers
 - CCD



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Photodiode

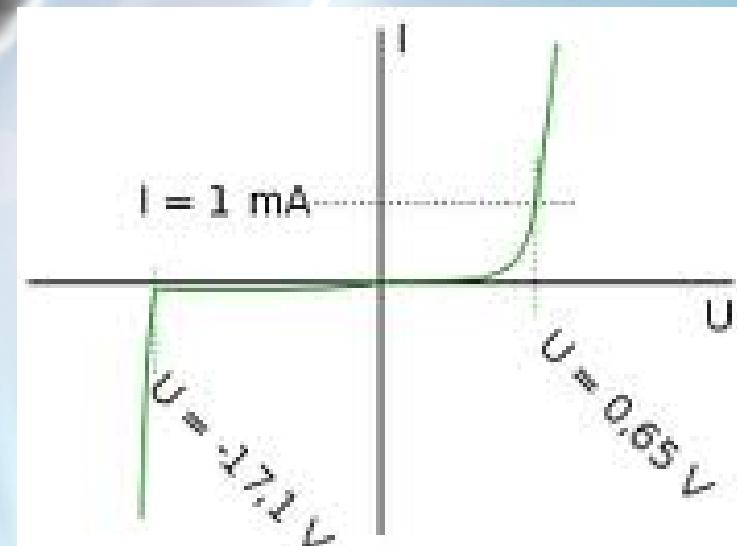
A **PHOTODIODE** is a type of photodetector used for strong signals and when saturation is a problem (FSC detector)



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Avalanche Diode

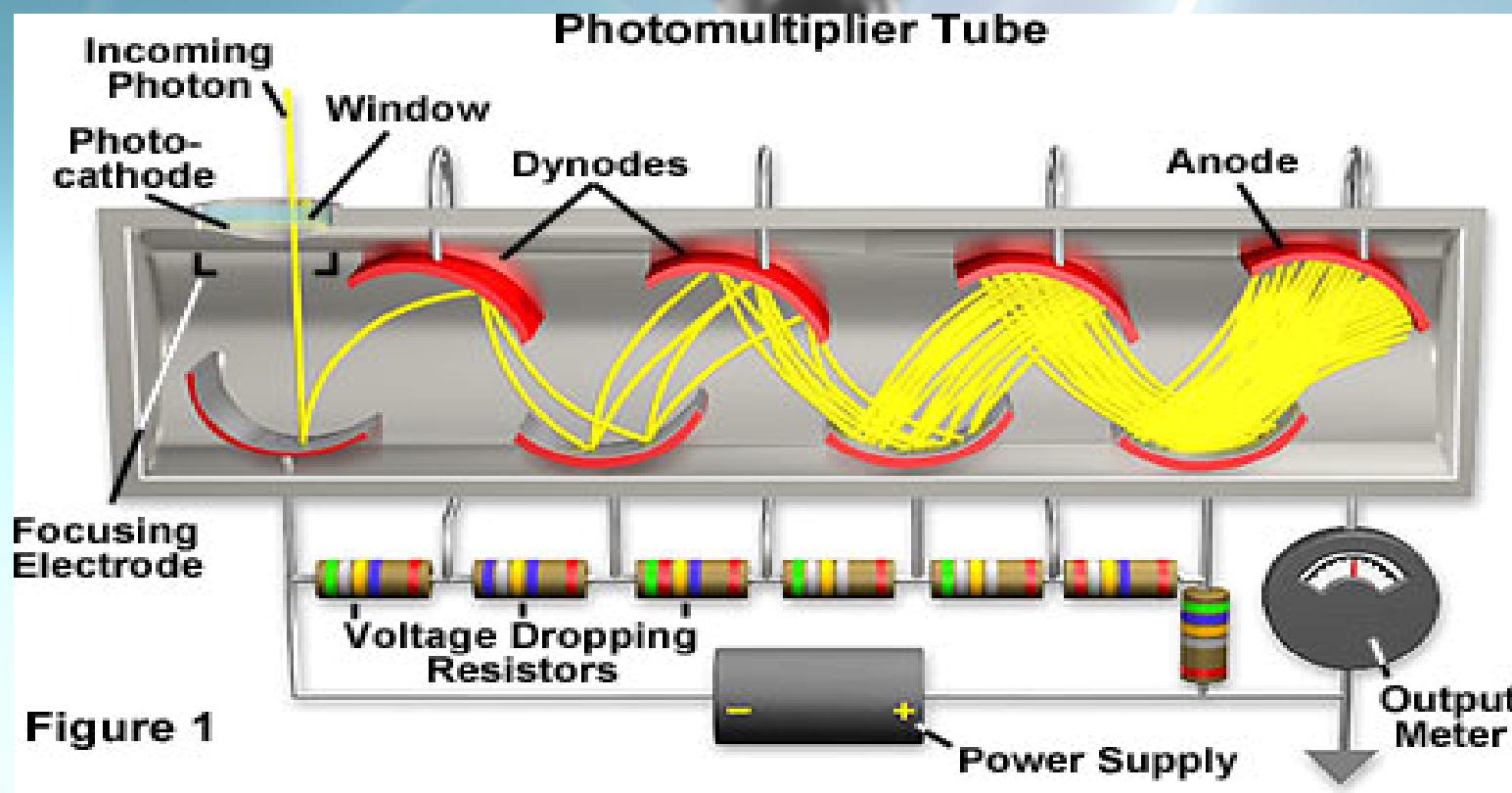
- The Avalanche Diode (APD) detector provides enhanced performance in the long wavelength region.



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Photomultiplier

- A **PHOTOMULTIPLIER**, is more sensitive than a photodiode
- It is useful for light detection of very weak signals as are SSC and fluorescence parameters



Photomultiplier

- Video



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CCD



- A **CHARGE-COUPLED DEVICE (CCD)** is a detector allowing the conversion of incoming photons into electron charges and thus **IMAGE**.
- They are generally less sensitive, but can capture larger image areas. They are greatly improving in sensitivity and noise levels.



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Signal Amplifiers

An **amplifier** is an electronic device that increases the voltage, current, or power of a signal.



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Signal Amplifiers Applications



Signal Amplifiers

An **amplifier** is an electronic device that increases the voltage, current, or power of a signal.

Flow cytometers are equipped with two types of amplifiers:

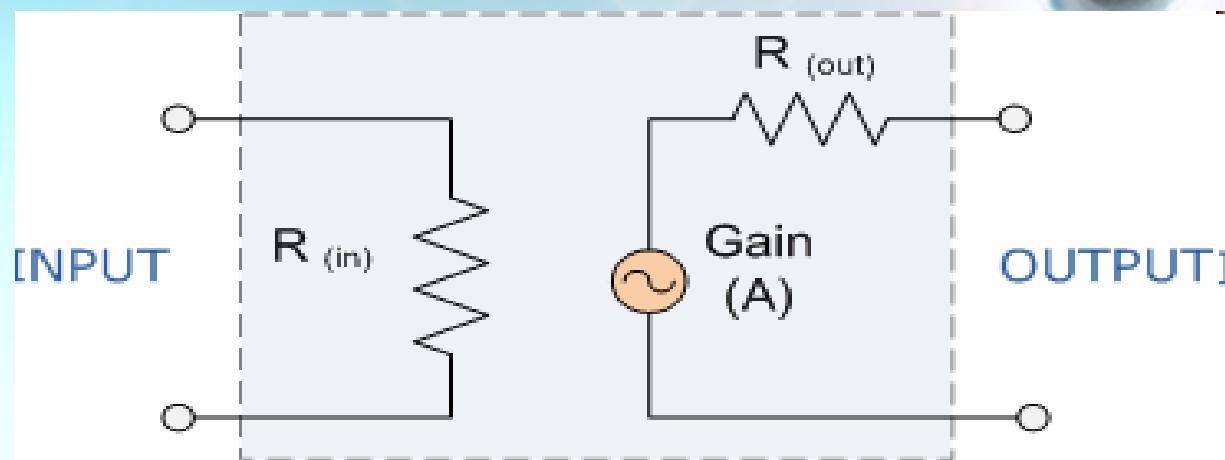
- Linear amplifier
- Logarithmic amplifier



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Signal Amplifiers

- A **LINEAR** amplifier is an electronic circuit whose output is proportional to its input, but capable of delivering more power into a load.
- The gain of the amplifier can be adjusted.
- It is used in cases where there is NOT a broad dynamic range range e.g DNA analysis



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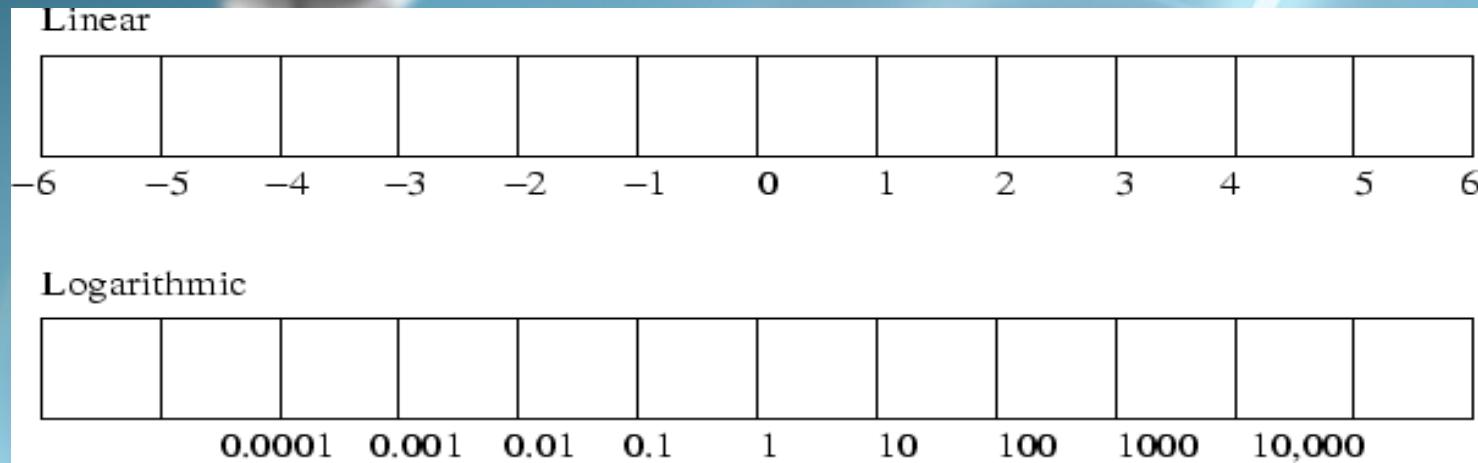
Signal Amplifiers

- A **LOGARITHMIC** amplifier (log amps), produce an output signal proportional to the logarithm of the input signal
- There is no gain setting on a logarithmic amplifier
- They have a wider dynamic range and can expand the scale of weak signals and contracts the scale of strong signals.
- They are widely used for measurements of parameters that vary over a wide dynamic range that result from the biological heterogeneity of many samples subjected to flow cytometry.e.g., cell surface immunofluorescence.



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Scales line - log

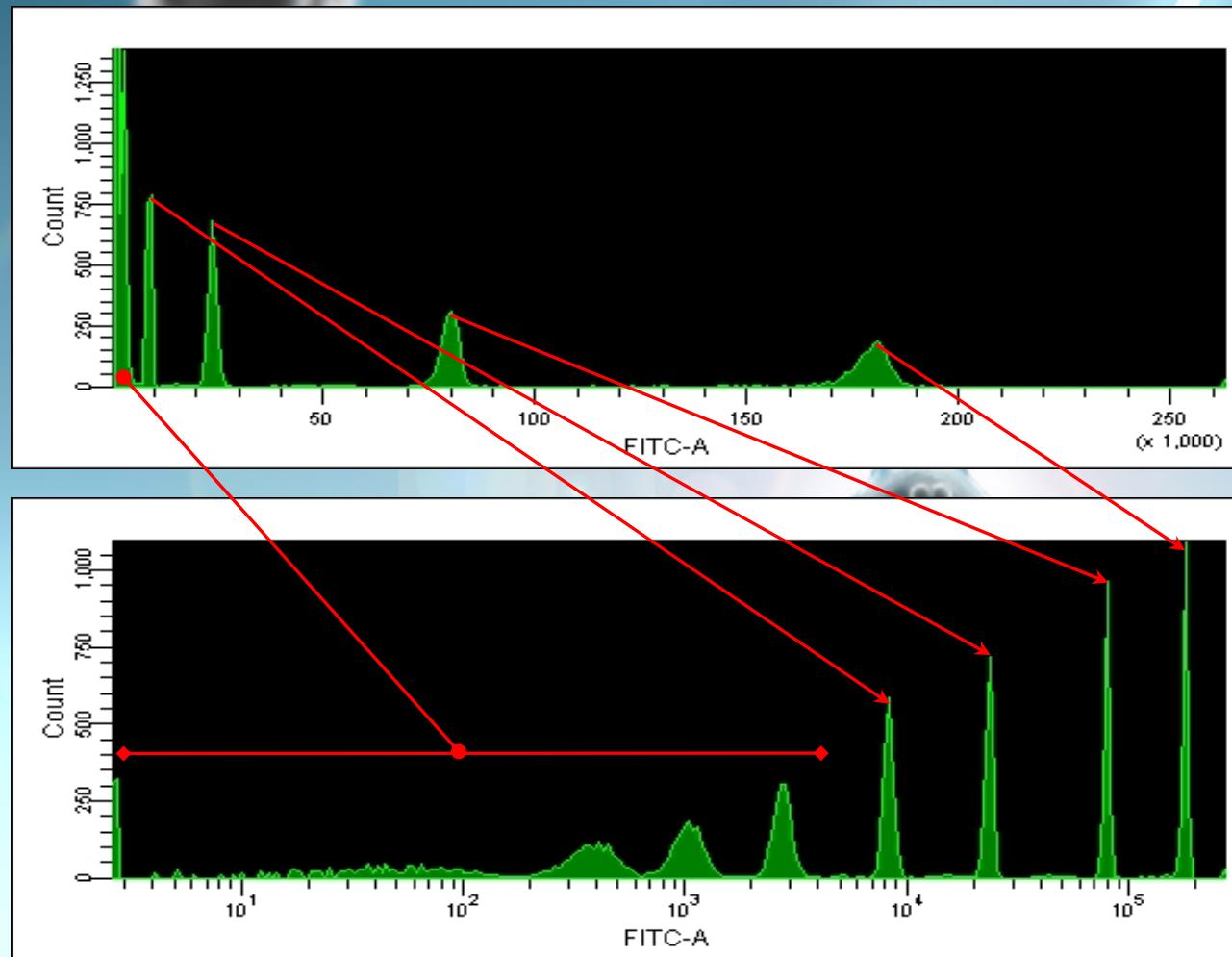


- Moving one unit to the right increment by 1 on the linear scale and multiplies by a factor of 10 on the logarithmic scale.
- **LOGARITHMIC** scales are used when the actual light intensities cover such a large range that it would be difficult to represent them on a **LINEAR** scale.



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Scales line vs log



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Electronics

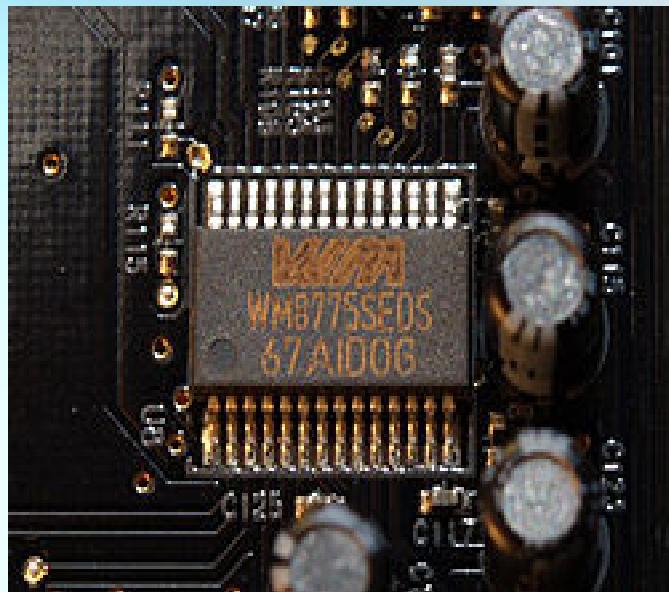
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Analog - Digital Converters

- Once an electrical signal from a photodetector has been amplified it's intensity is analyzed and recorded by a device called **ANALOG-TO-DIGITAL CONVERTER (ADC)**. Each channel represents a specific light intensity range
- Depending on the intensity given from the cell it is recorded on a different channel.

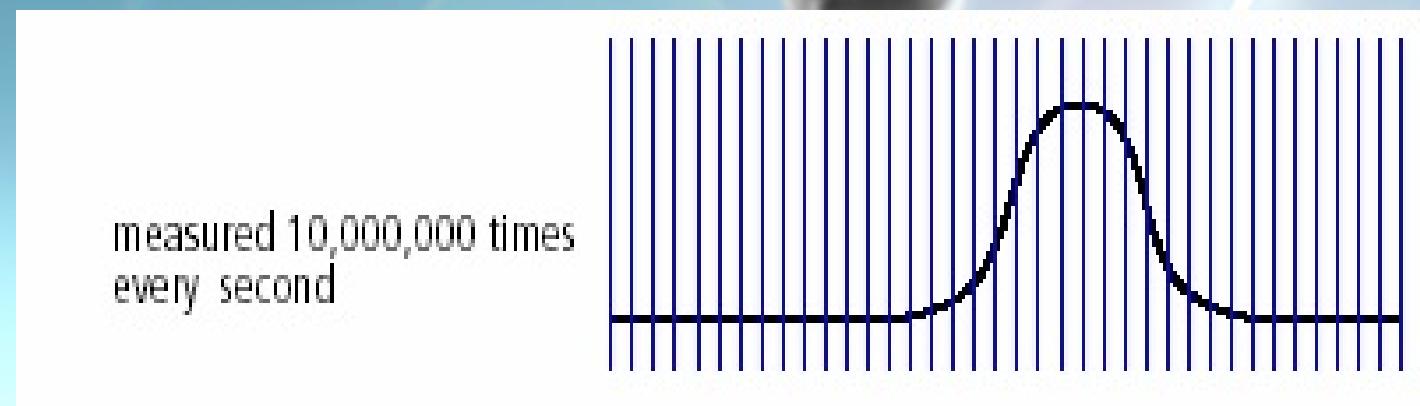


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Analog – Digital

The voltage pulse is given a digital value by the Analogue-to-Digital Converter (ADC).

The resolution of the data will depend on the ADC and is described in two terms:



SAMPLING RATE in megahertz

Each analog signal coming from the preamp board is digitized continuously at 10MHz in the channel DAQ board.

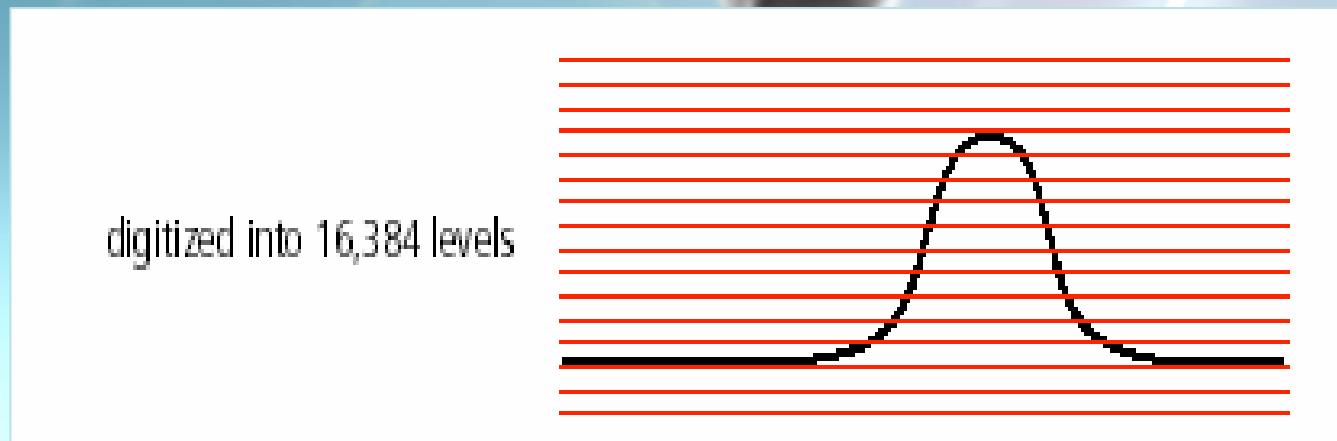


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Analog – Digital

The voltage pulse is given a digital value by the Analogue-to-Digital Converter (ADC).

The resolution of the data will depend on the ADC and is described in two terms:



RESOLUTION in bit

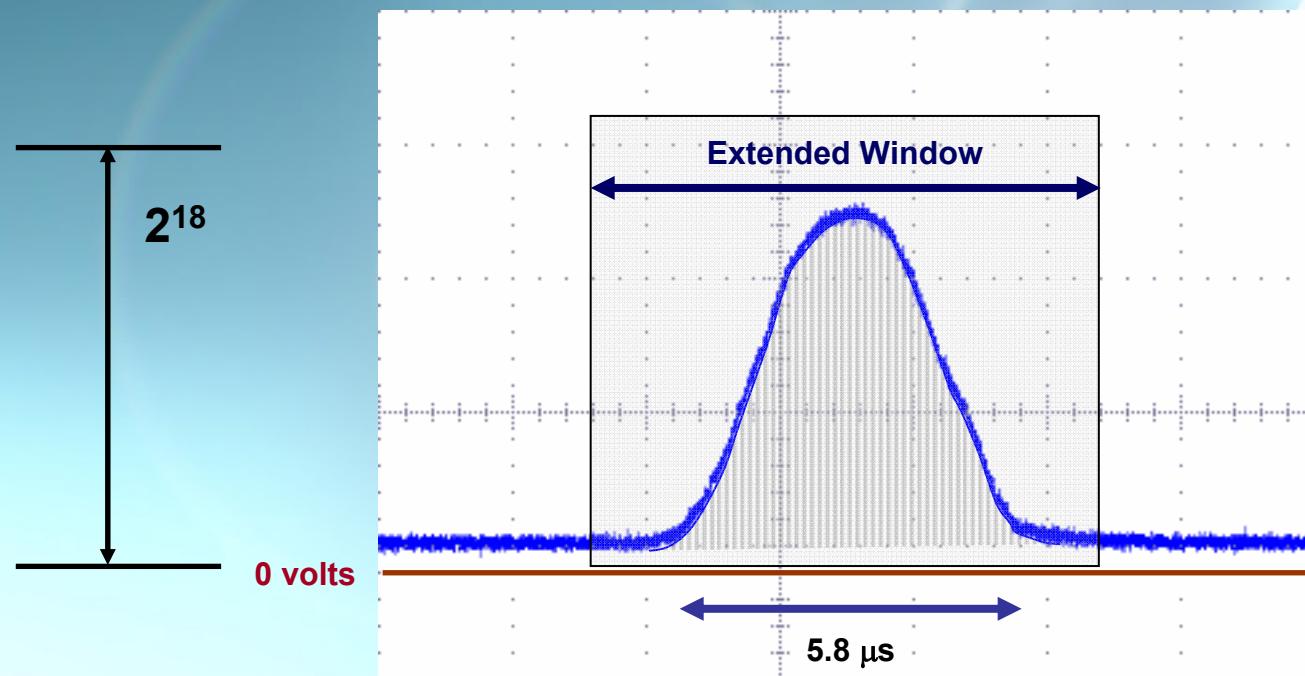
For example, an 8-bit converter will yield 256 channels, and a 10-bit converter gives 1024 channels resolution.



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A Continuously Digitizing Cytometer

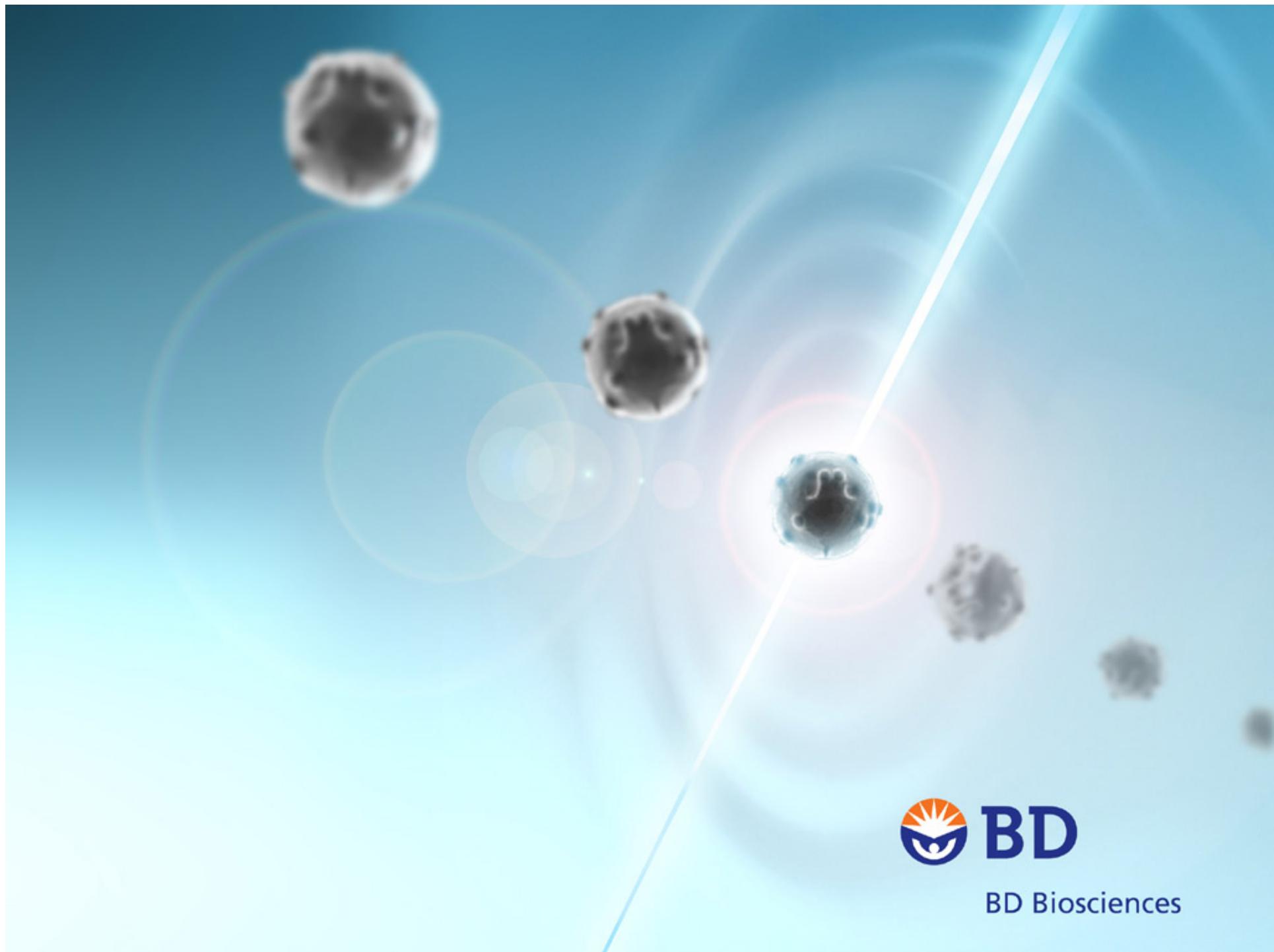
10 MHz ADC: 5.8 μ sec pulse has ~ 58 observations



Pulse area is a measurement of fluorescence (18 bit resolution)



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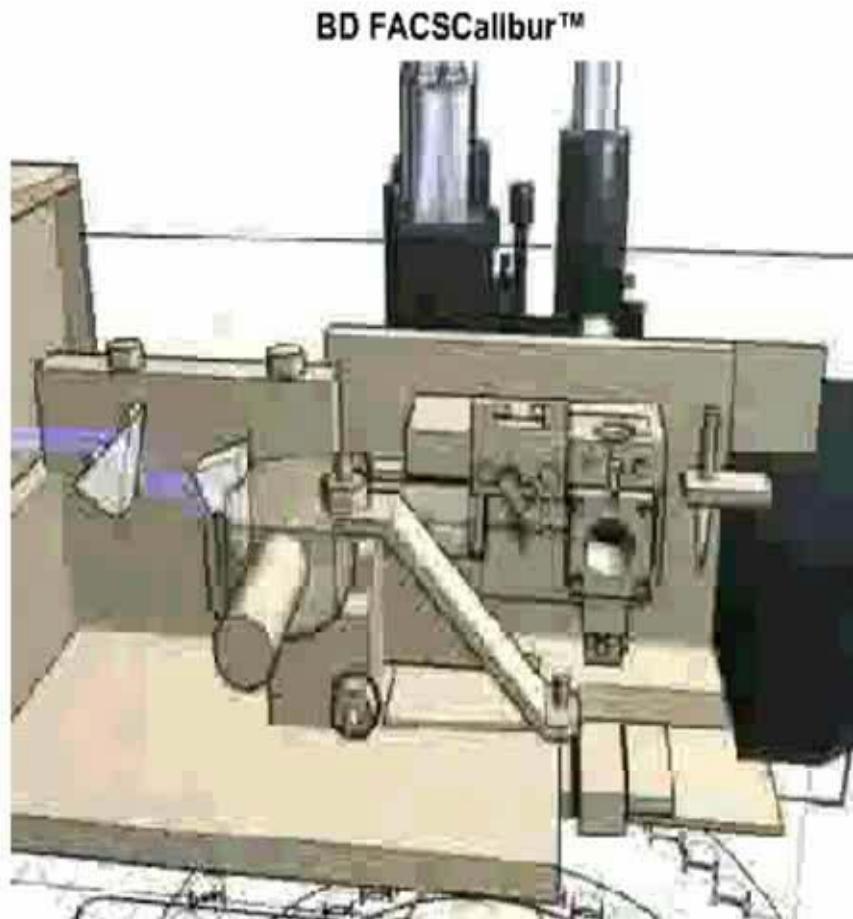
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Intercept Point



The laser is the excitation light source for BD cytometers.

Laser is an acronym for light amplification by stimulated emission of radiation.

Lasers generate intense beams of coherent light.

Event Genesis

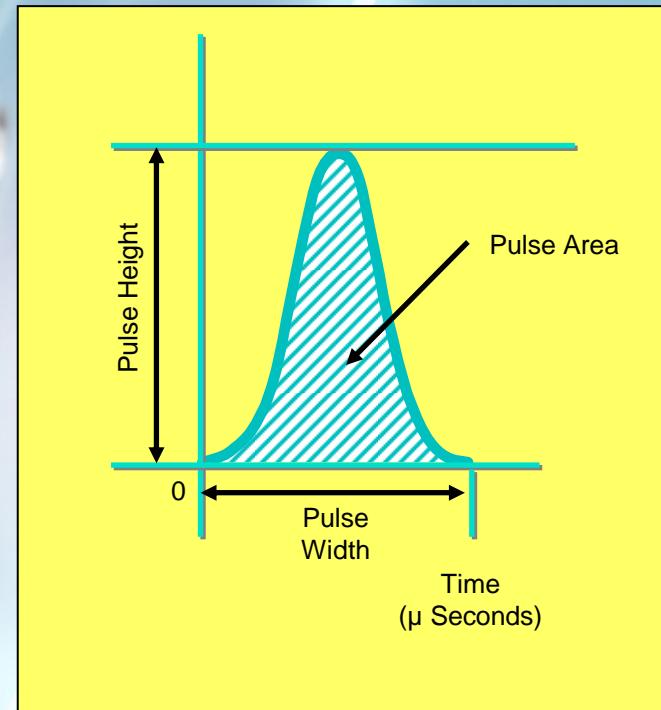
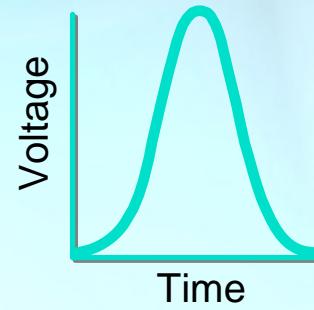
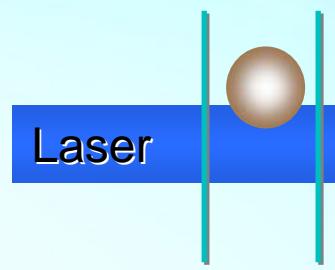
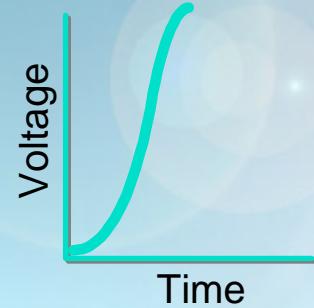
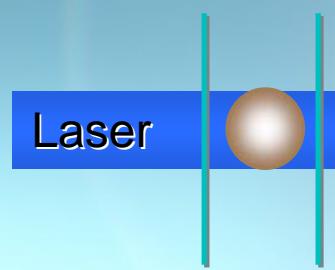
Signal Processor

The signal processor creates measured values of pulses as digital numbers. Measured values include pulse peak, pulse area, and pulse width. Measurements that combine results from two or more detectors include fluorescence compensation and ratios. The measured values may be linear or logarithmic.

laser



A Continuously Digitizing Cytometer



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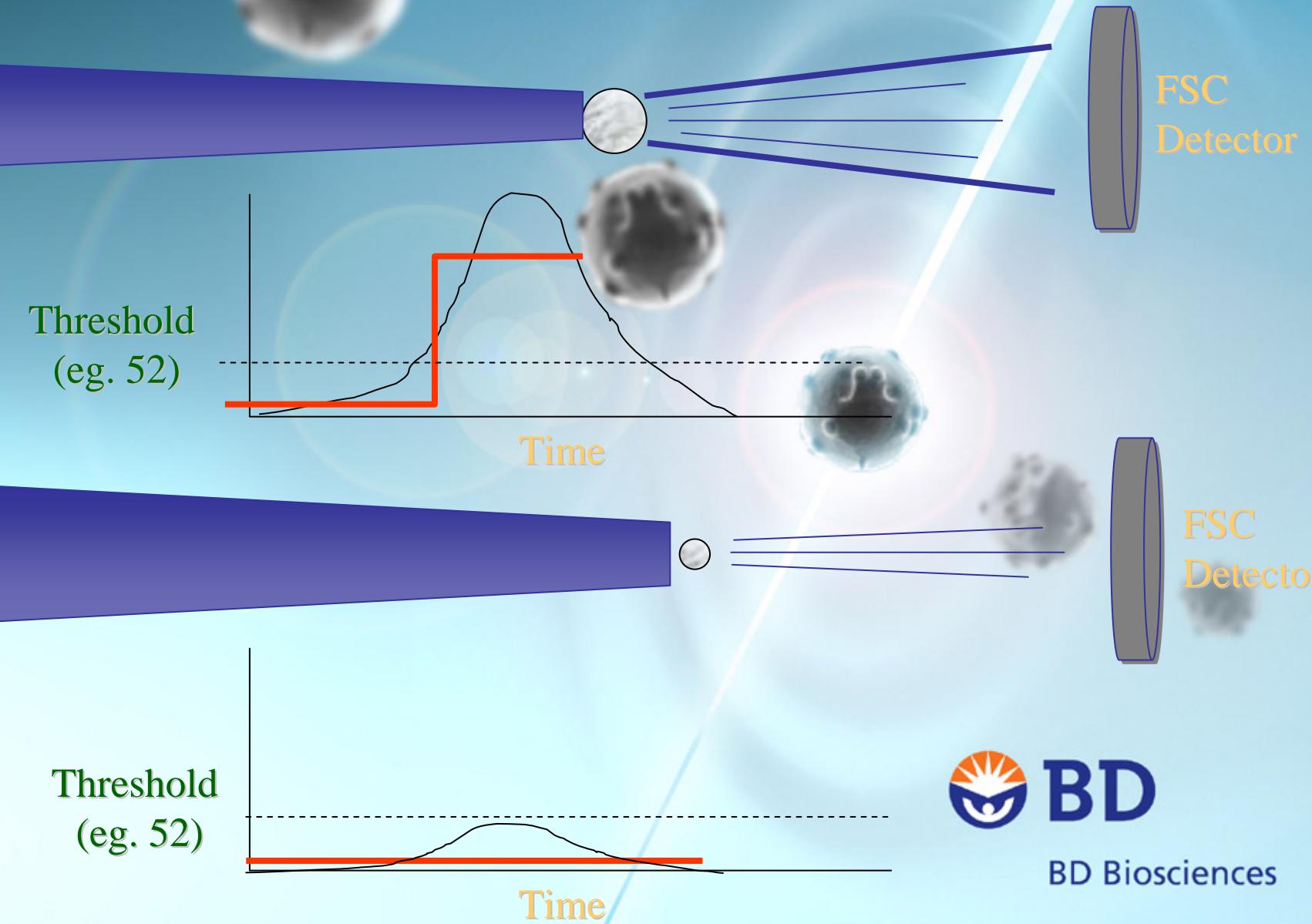
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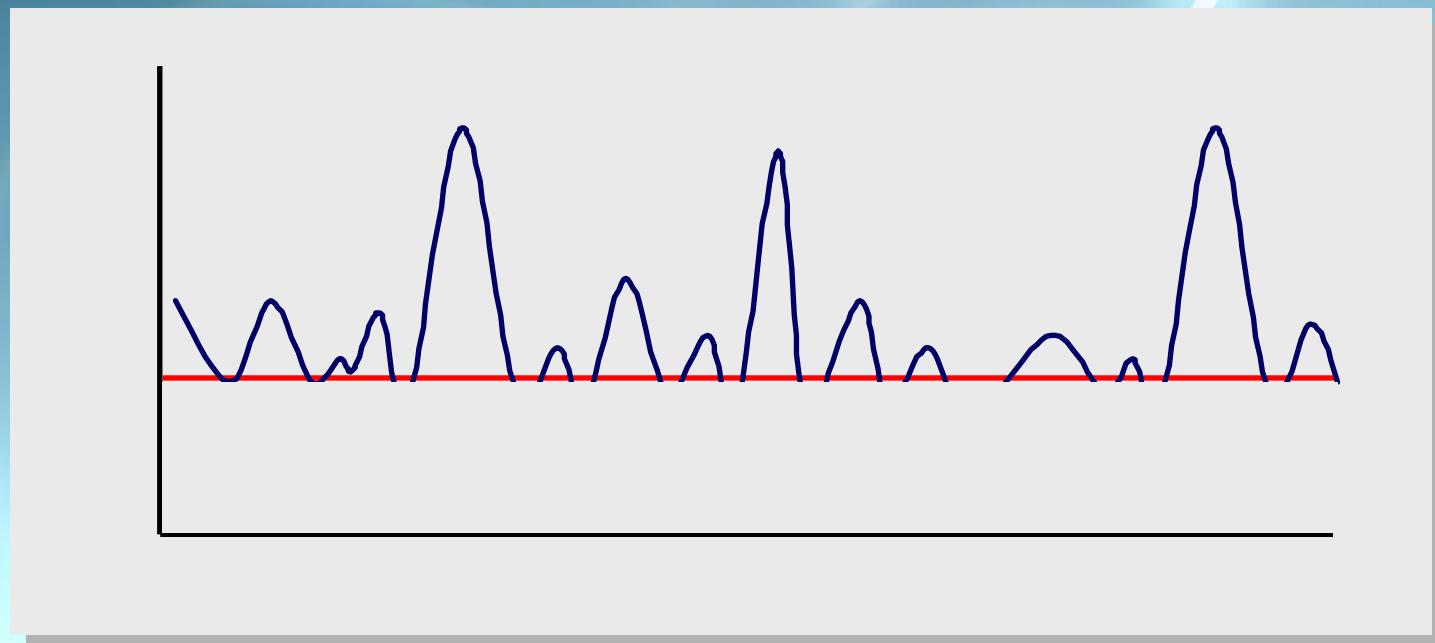


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Threshold

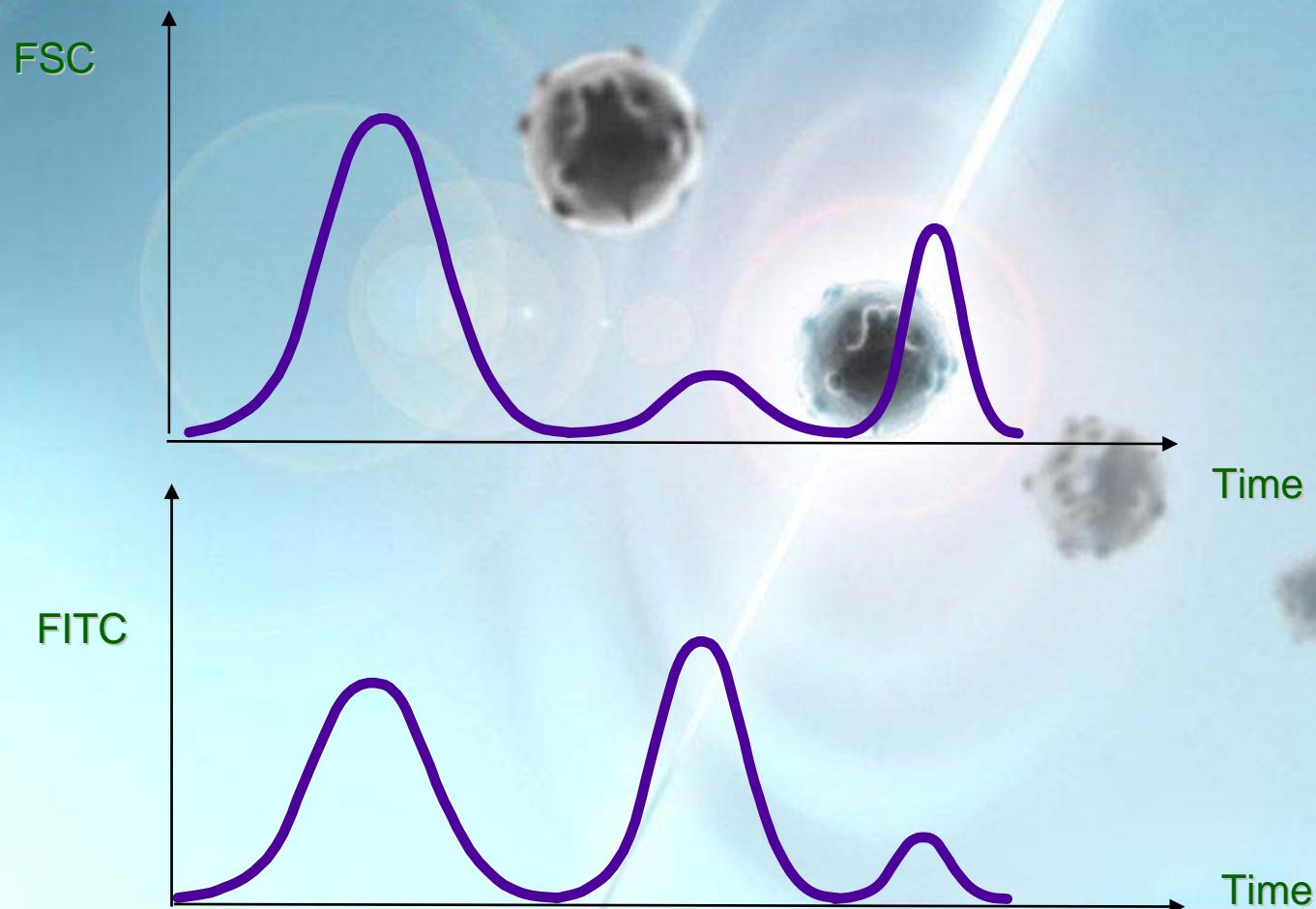


Threshold

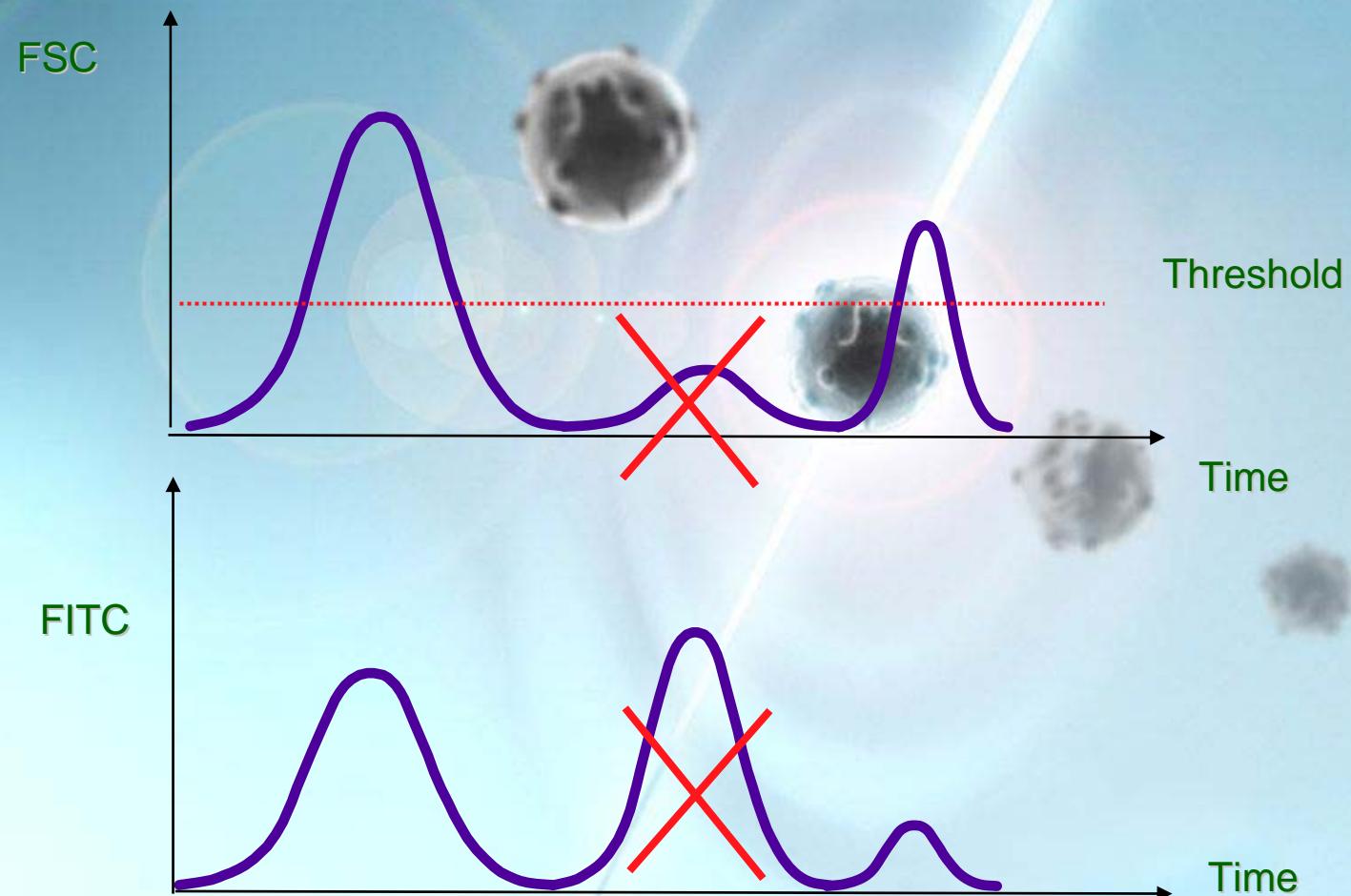


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Threshold Trigger



Threshold - Trigger



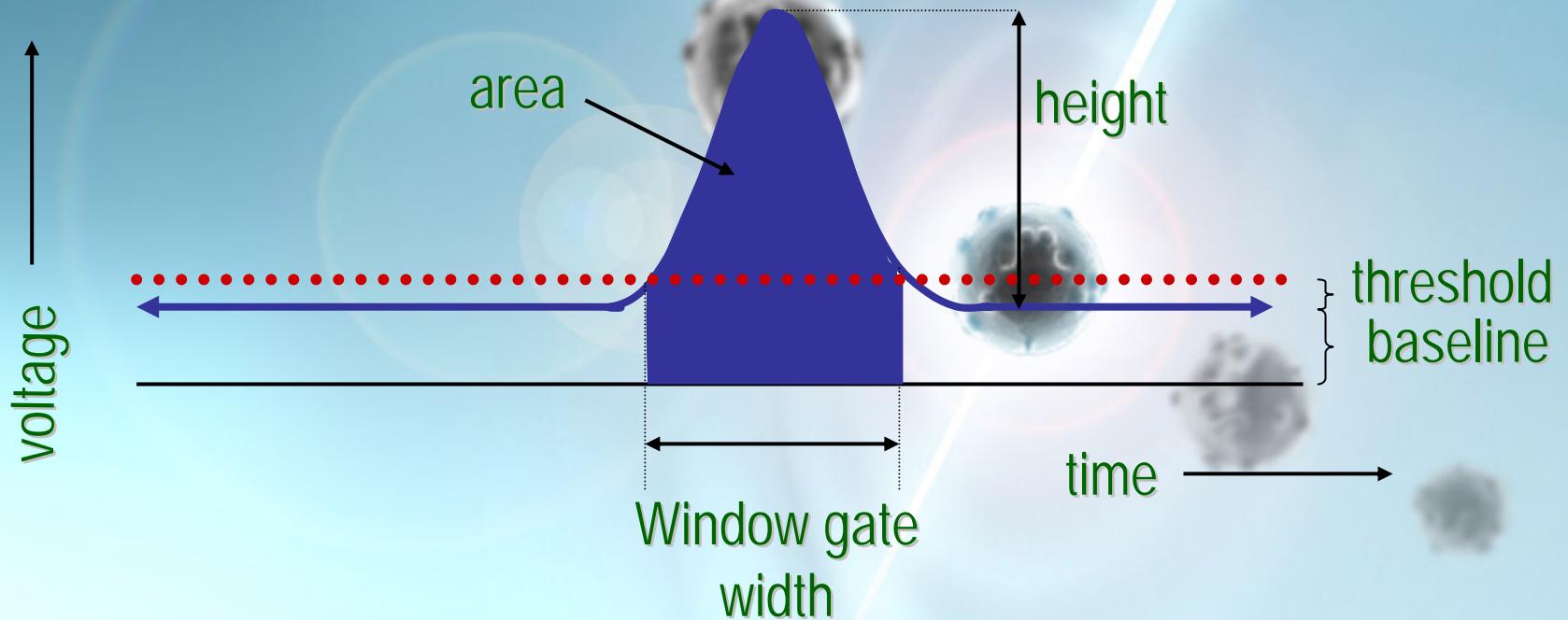
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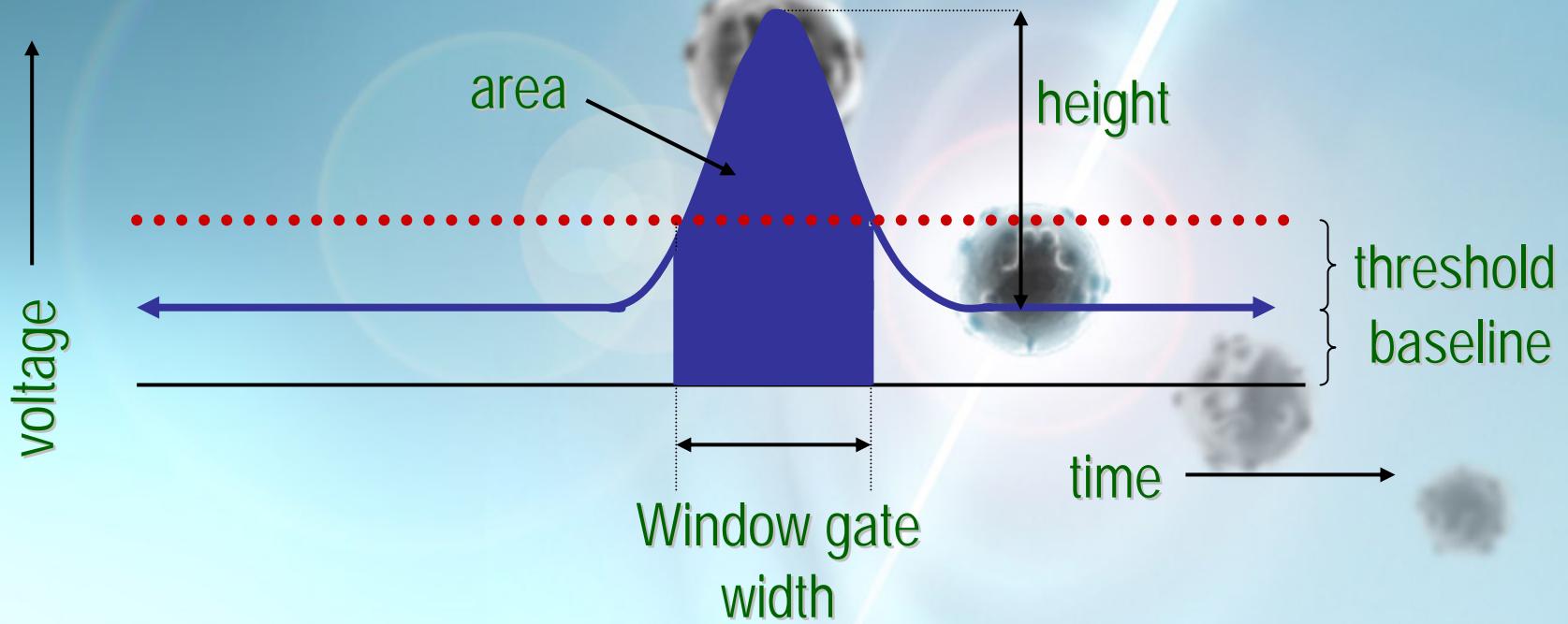
BD Biosciences

Window Gate



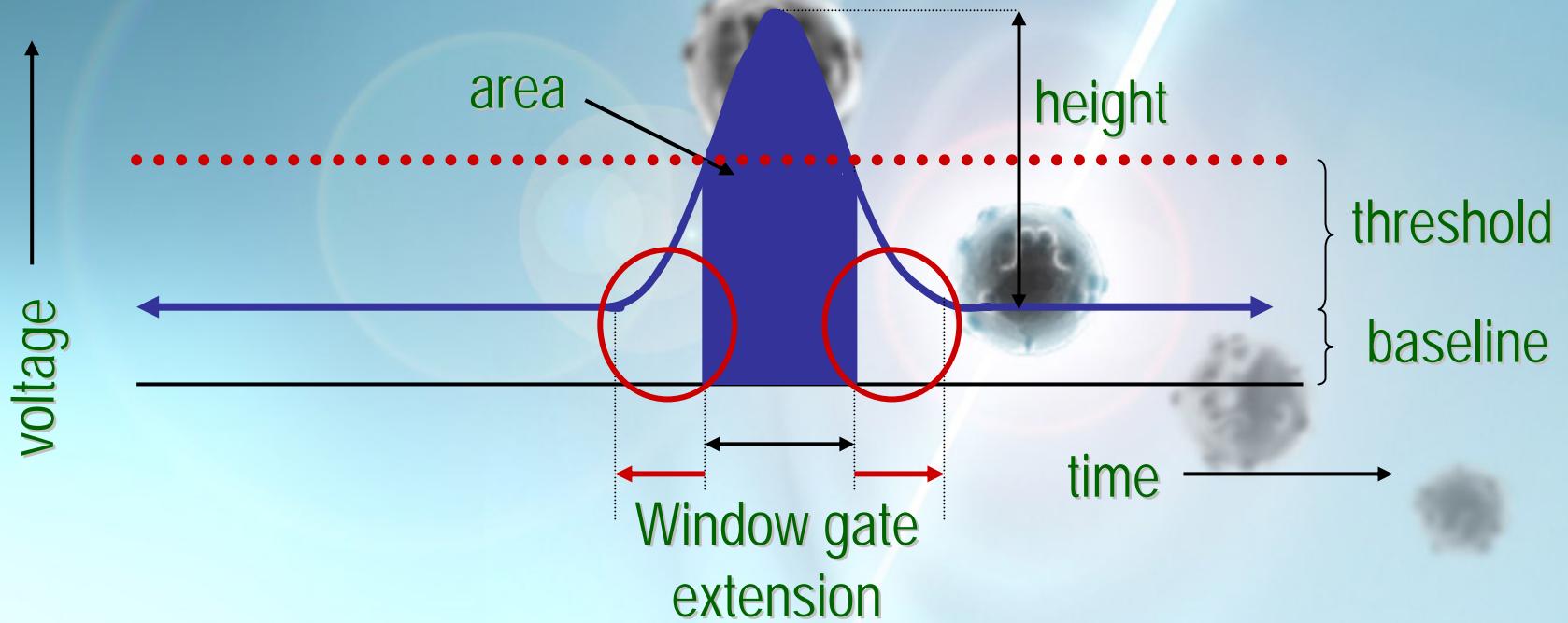
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Window Gate



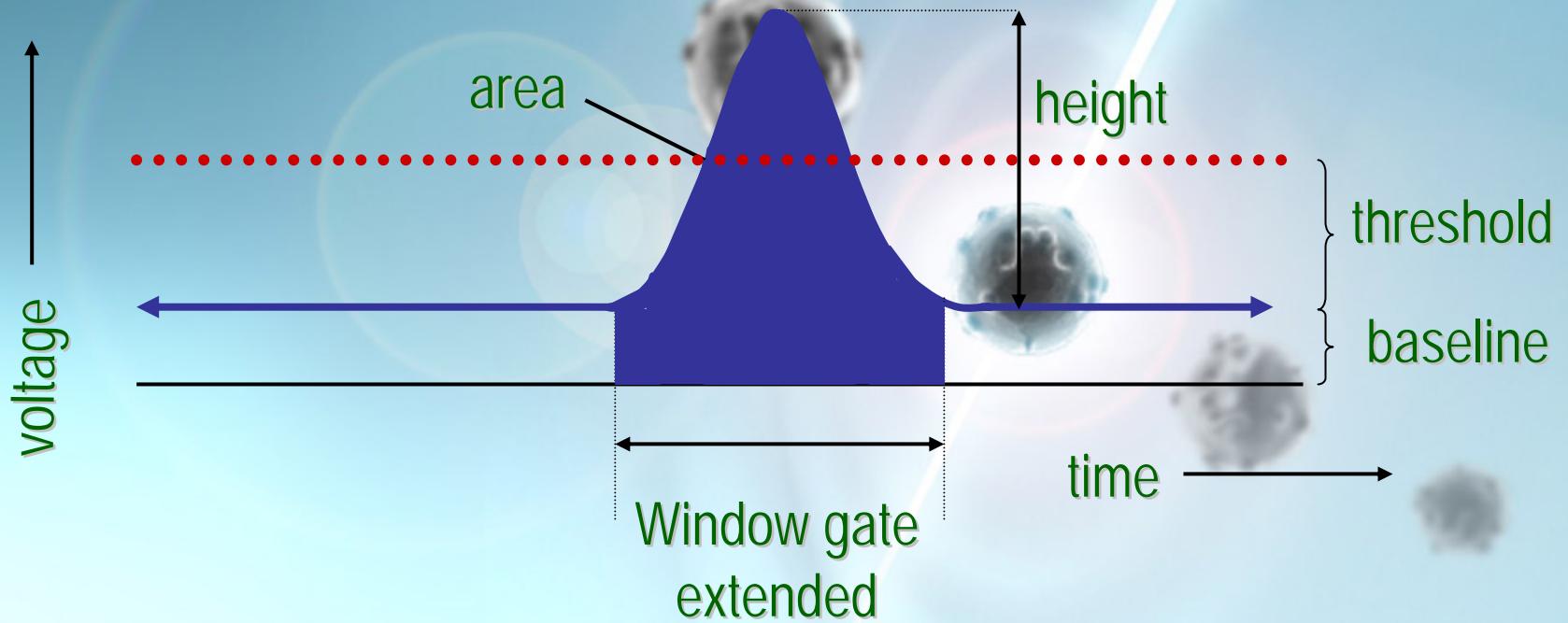
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Window Gate Extension



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Window Gate Extension



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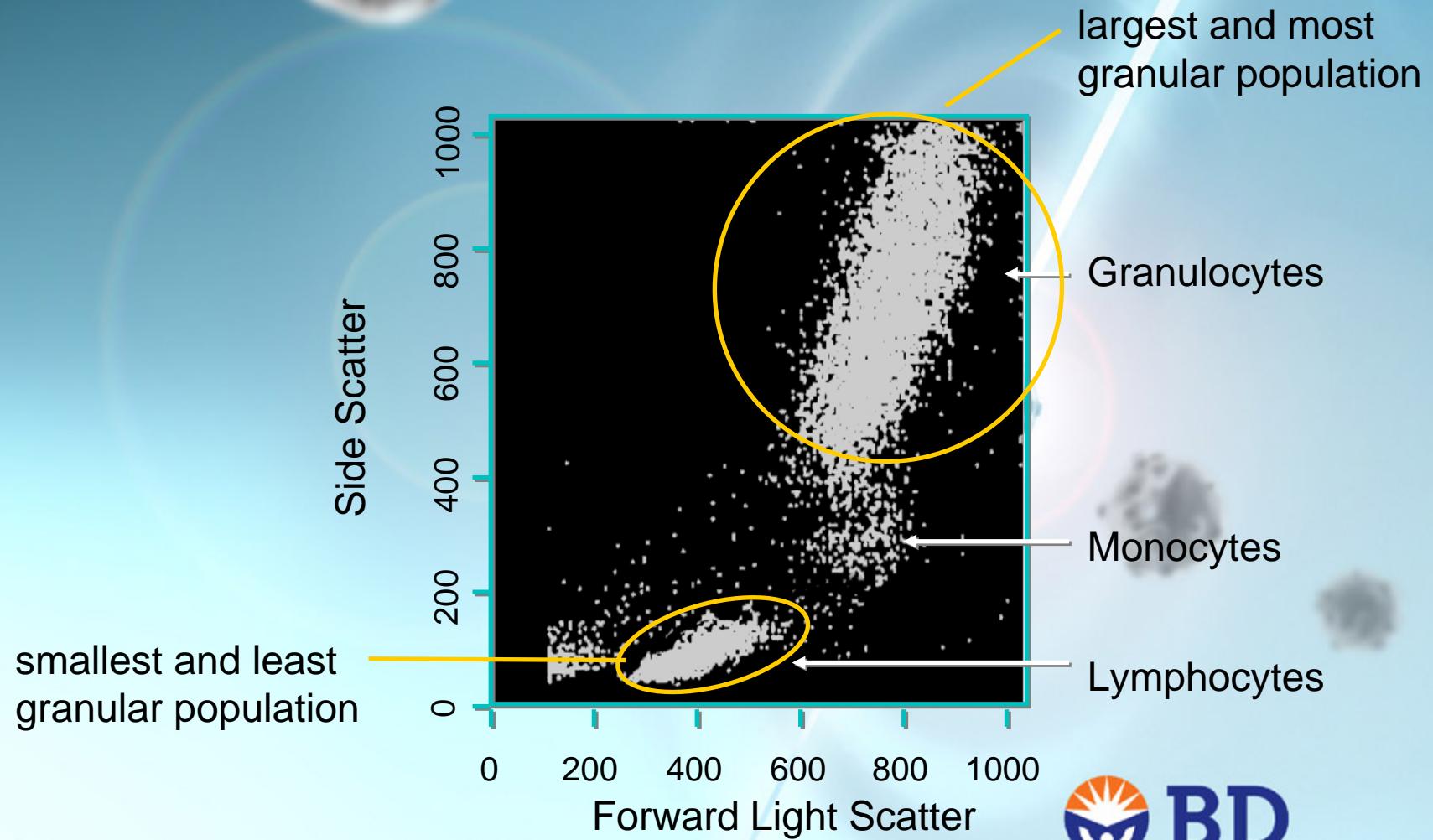
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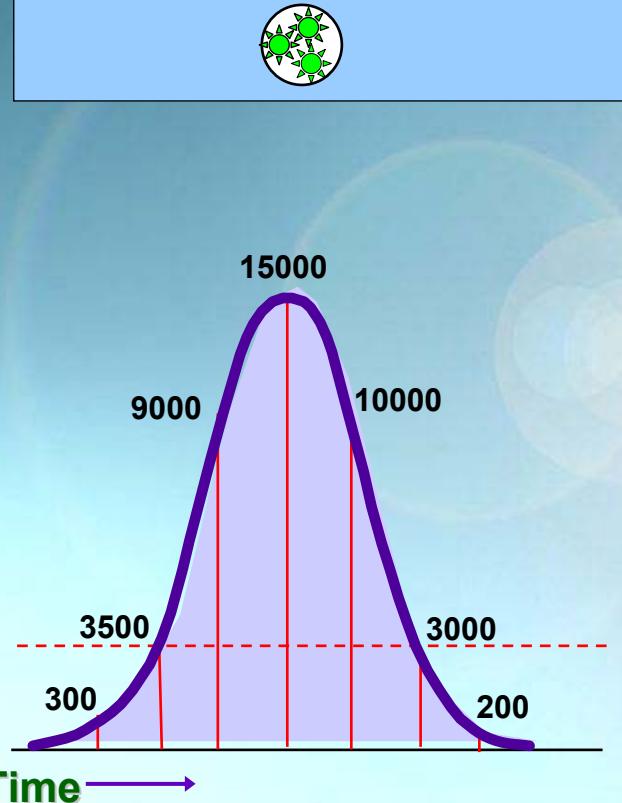
Area Scaling Factor



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Area Scaling

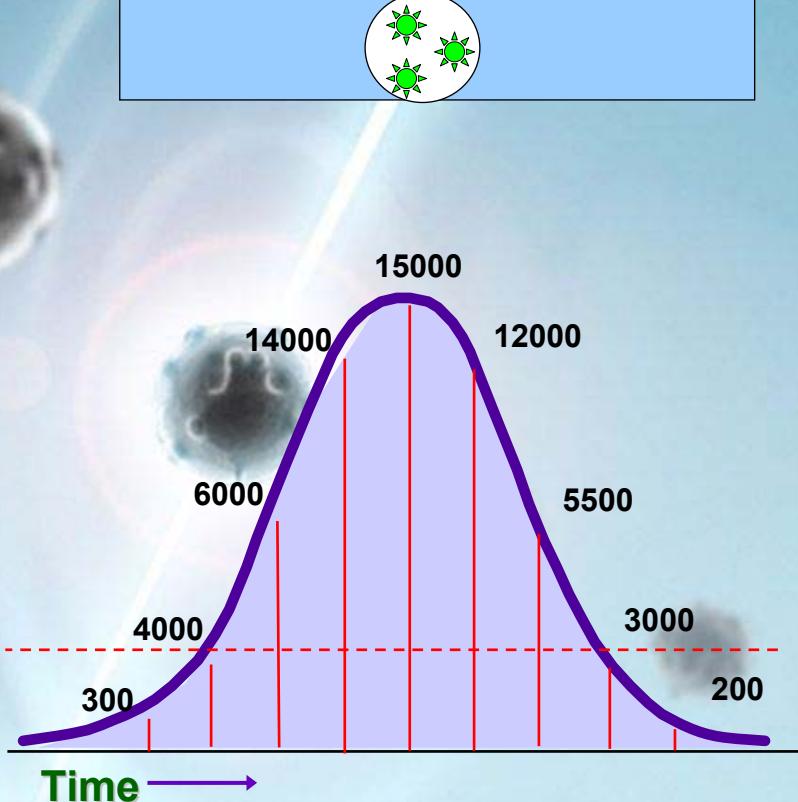
Small particle



Height = 15K

Area = 40K

Large particle

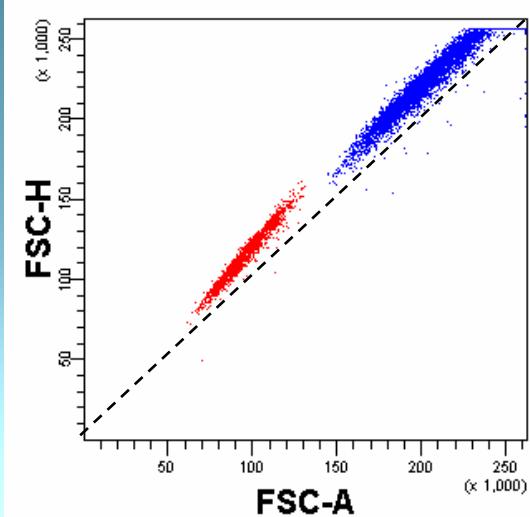


Height = 15K

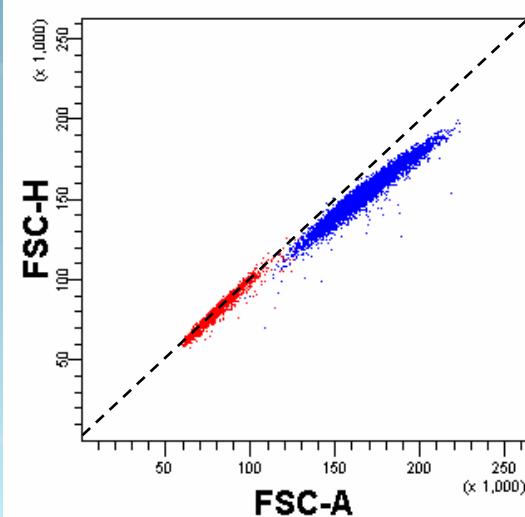
Area = 60K

Area Scaling

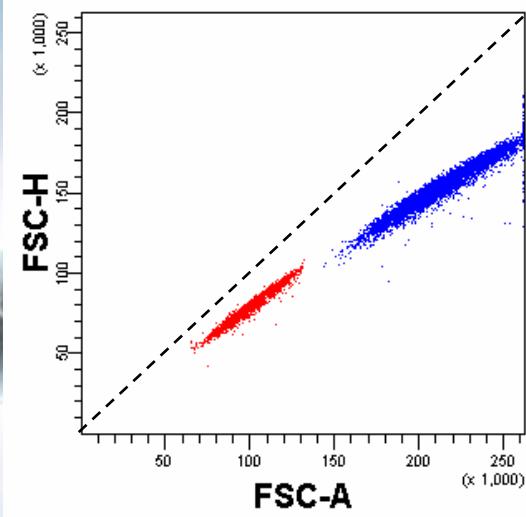
Incorrect



Correct



Incorrect



Height signal is saturated

Area scaling is adjusted for smaller population in mixture

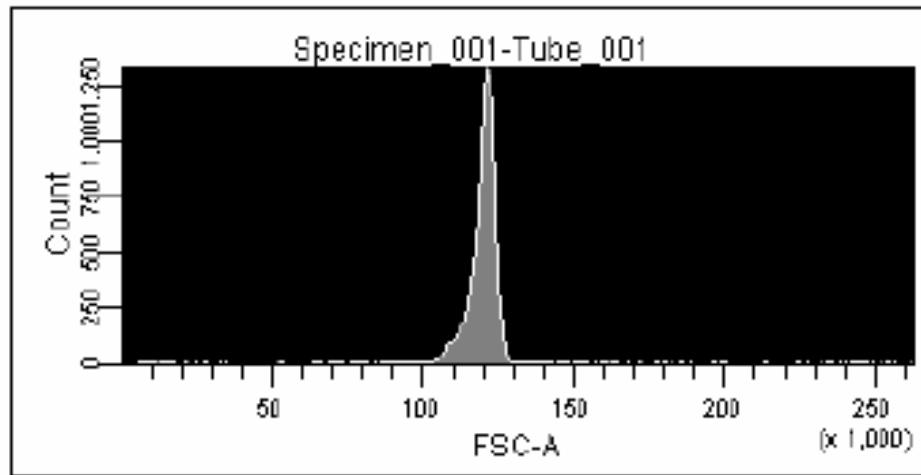
Area signal is not linear



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Area Scaling Factor

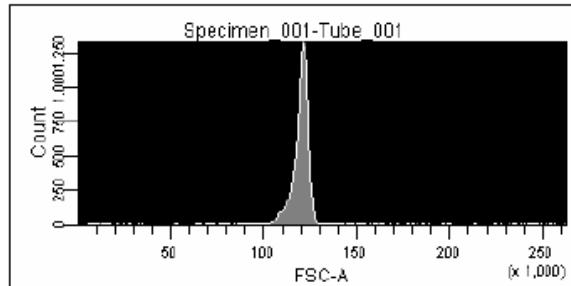
0.75



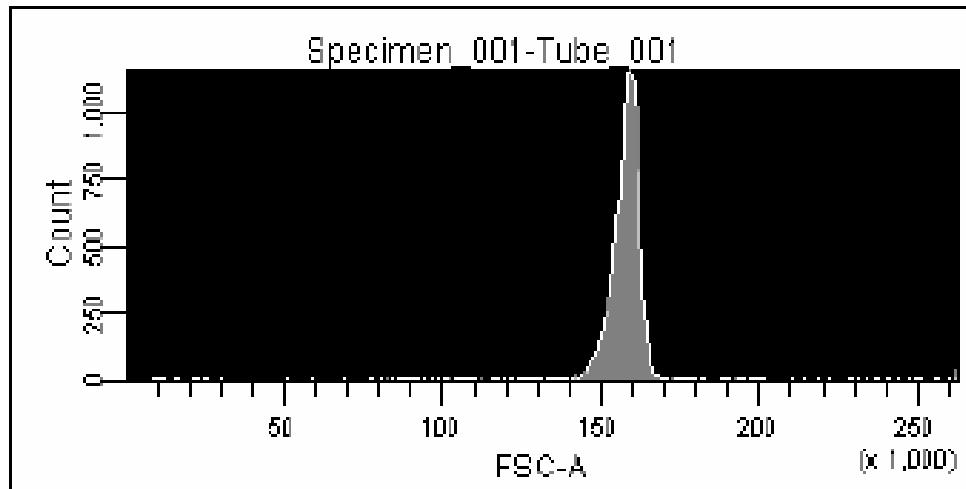
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Area Scaling Factor

0.75



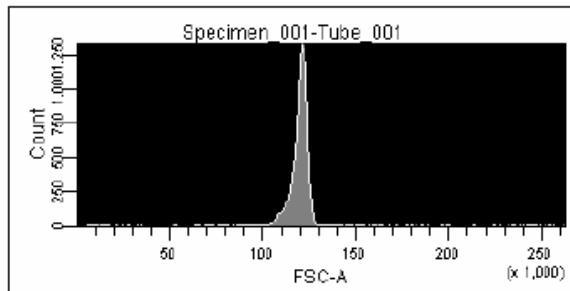
1.00



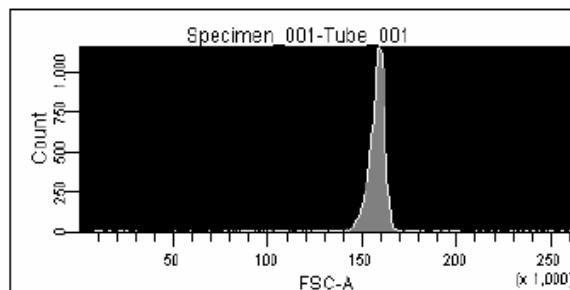
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Area Scaling Factor

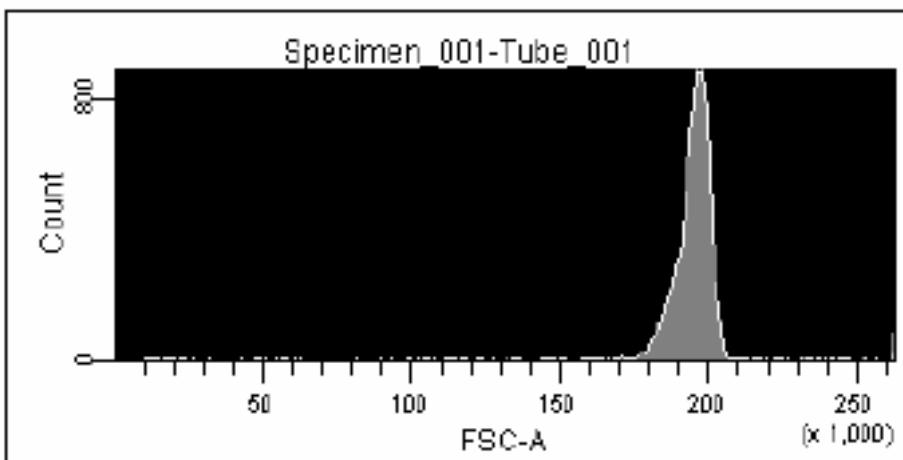
0.75



1.00



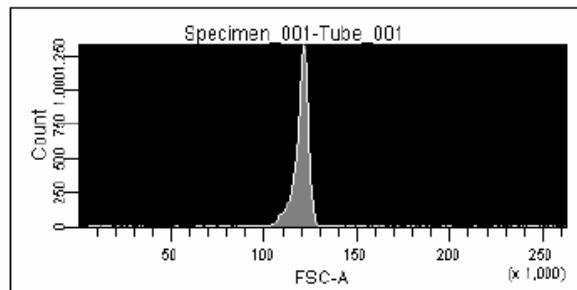
1.25



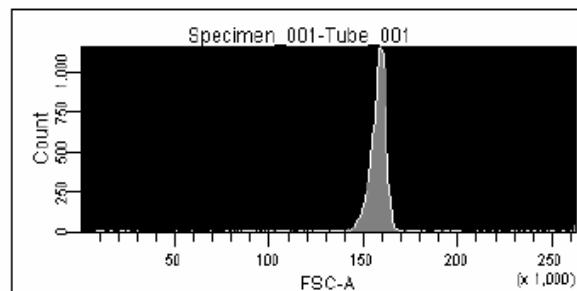
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Biosciences

Area Scaling Factor

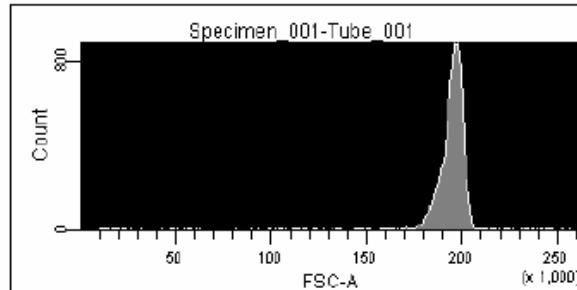
0.75



1.00

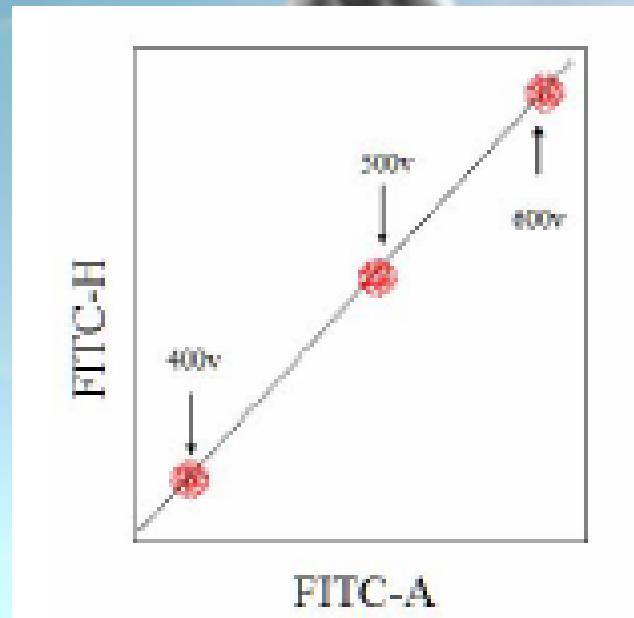


1.25



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Area Scaling Factor



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Electronics

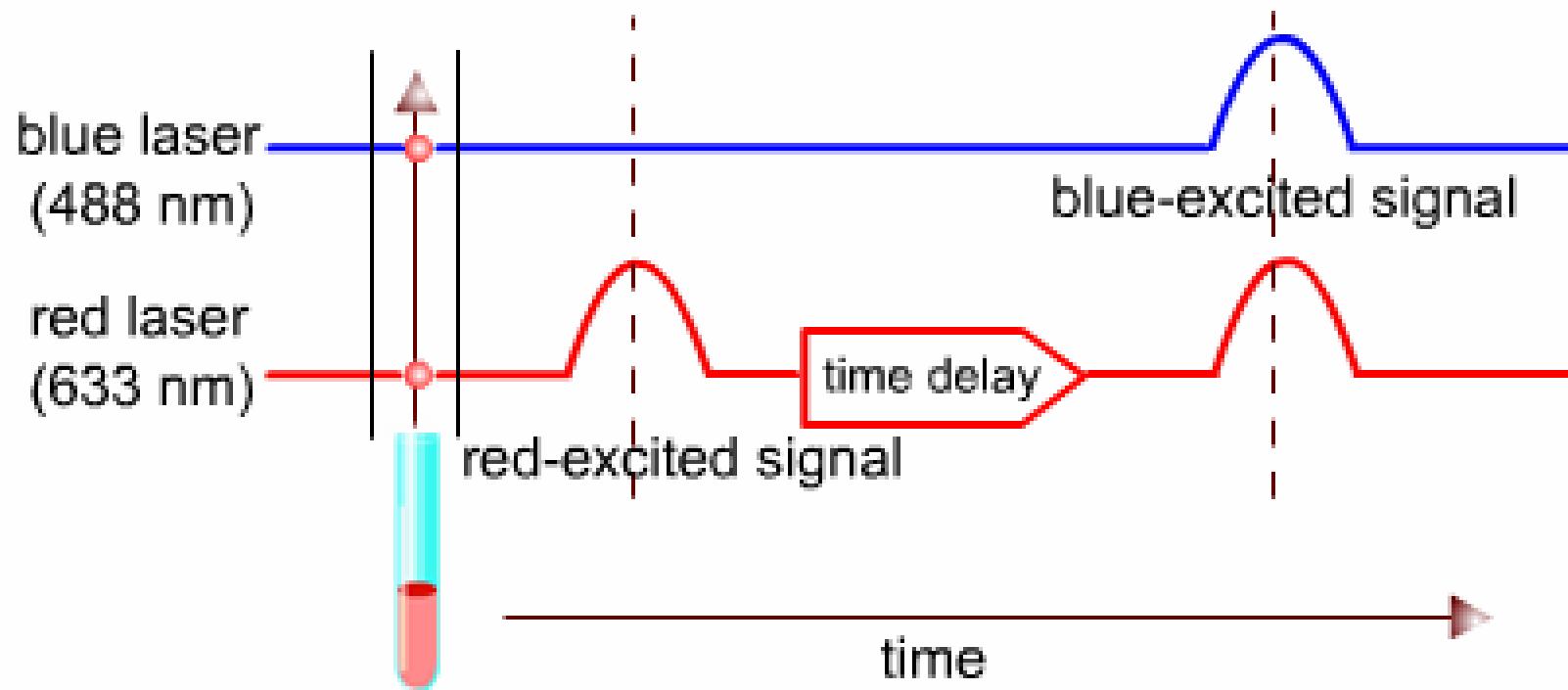
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Time Delay

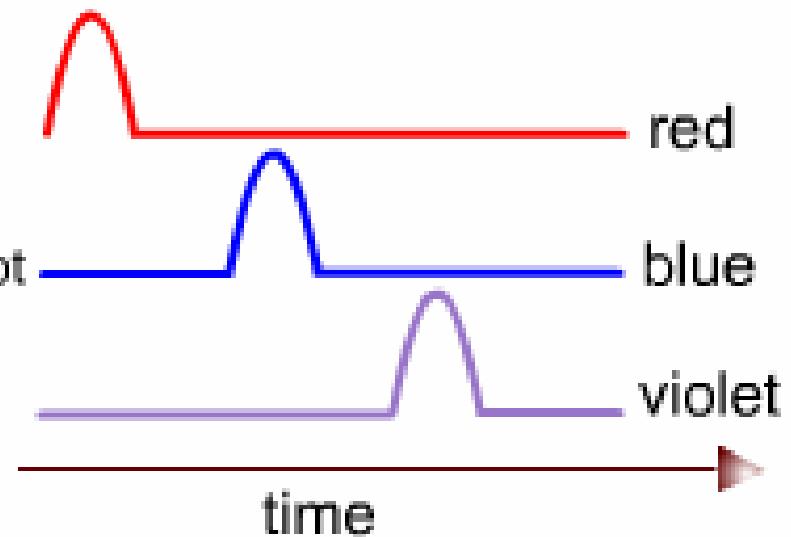
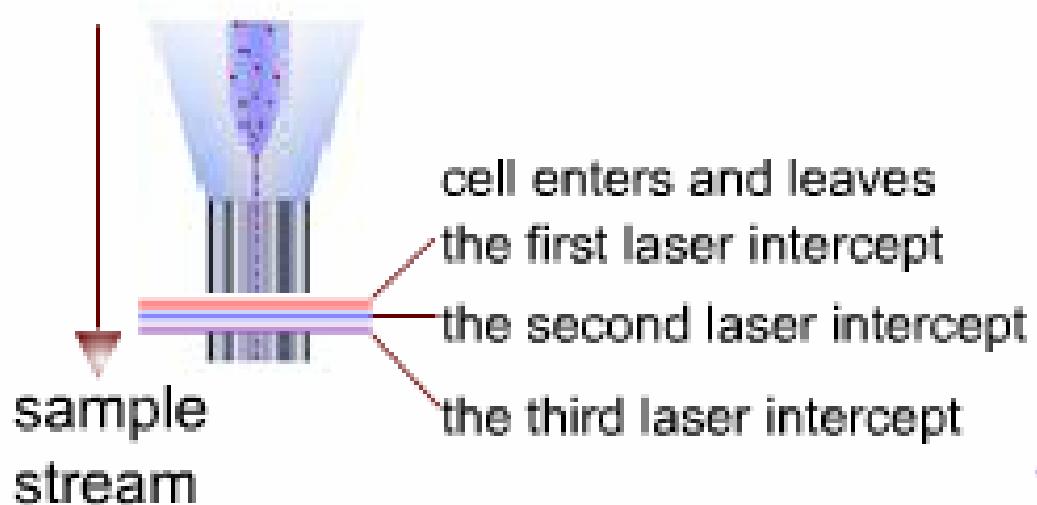
BD FACSCalibur™



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Time Delay

BD FACSaria™

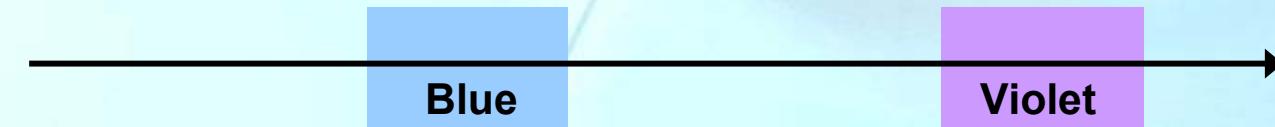
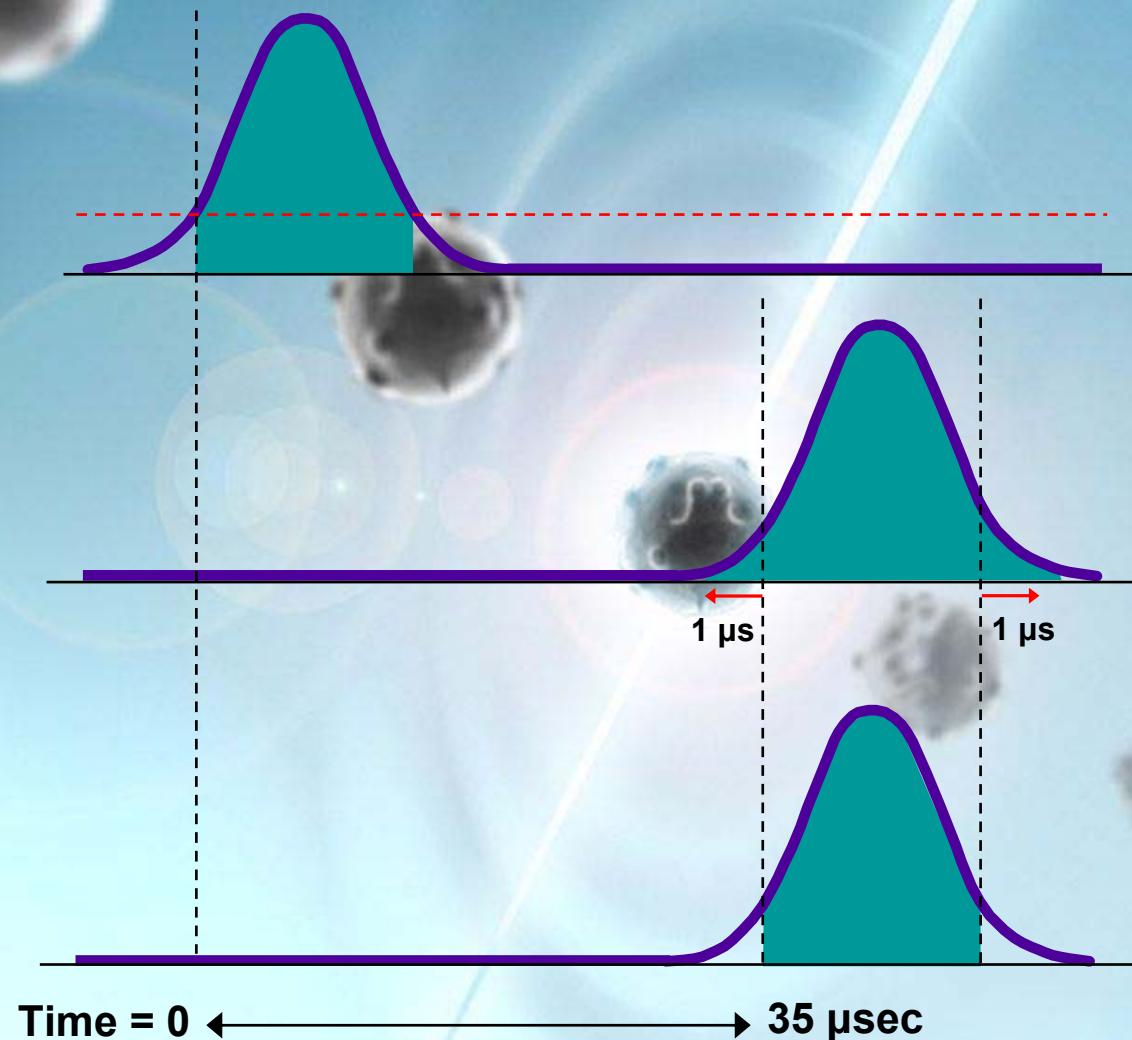


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Correct time delay

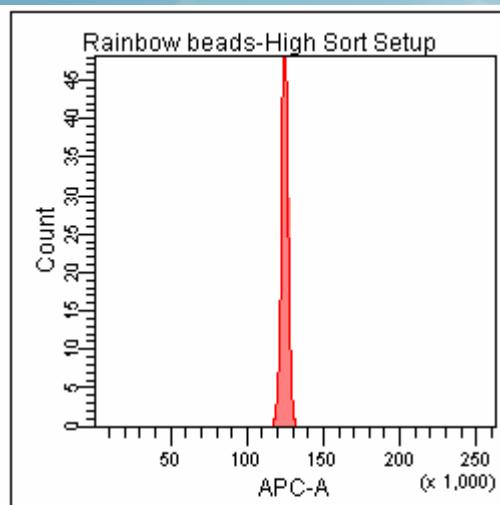
Window
Extension = 2

Window
Extension = 0



Correct time delay

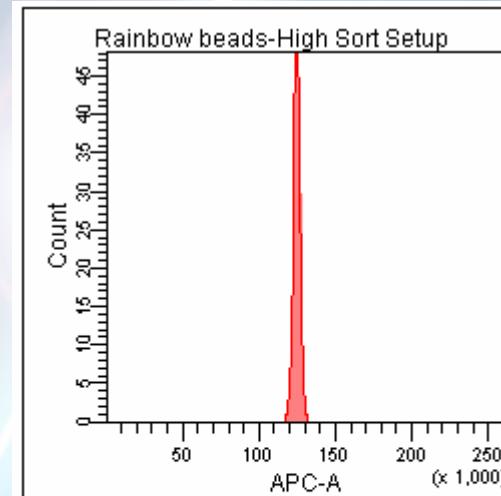
Window Extension = 2



Tube Name: High Sort Setup
Record Date: Mar 28, 2006 12:57:26 PM
\$OP: Administrator

Population	APC-A Mean	APC-H Mean
P1	124,572	127,868

Window Extension = 0



Tube Name: High Sort Setup
Record Date: Mar 28, 2006 12:57:26 PM
\$OP: Administrator

Population	APC-A Mean	APC-H Mean
P1	124,572	127,868

BD Biosciences

Incorrect time delay

Window
Extension = 2

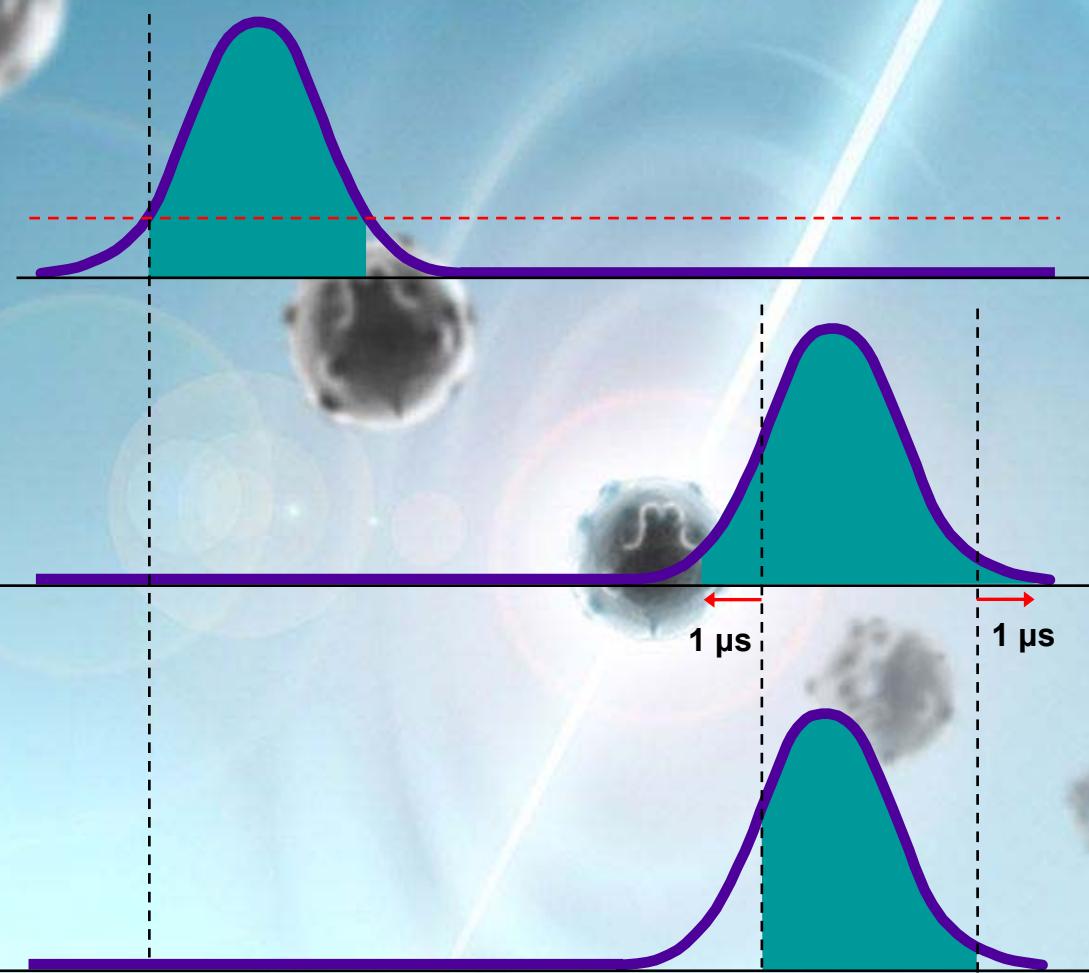
Window
Extension = 0

Time = 0

37 μ sec

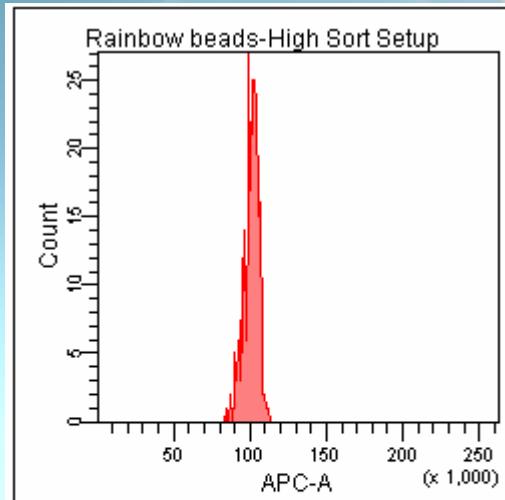
Blue

Violet



Incorrect time delay

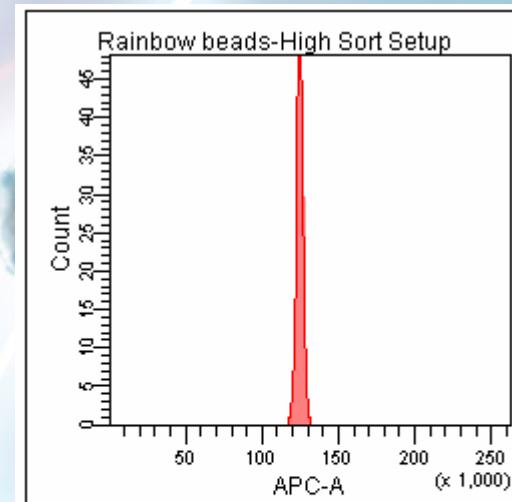
Window Extension =
0



Tube Name: High Sort Setup
Record Date: Mar 28, 2006 12:57:26 PM
\$OP: Administrator

Population	APC-A Mean	APC-H Mean
P1	101,398	127,188

Window Extension
= 2

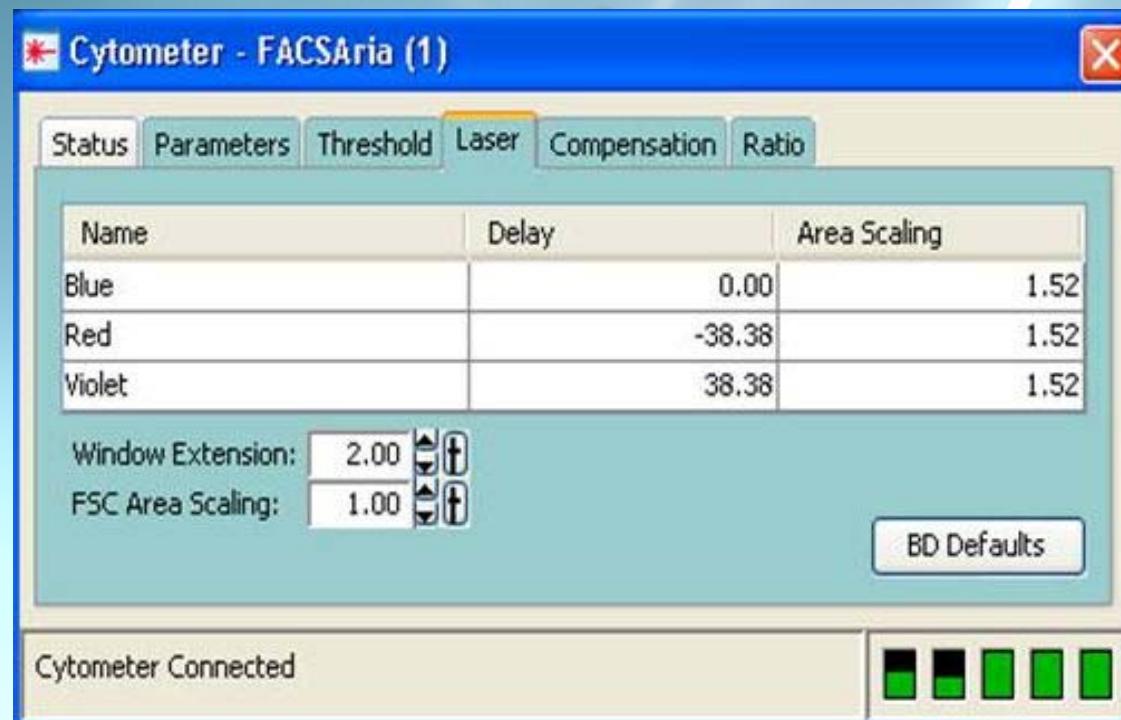


Tube Name: High Sort Setup
Record Date: Mar 28, 2006 12:57:26 PM
\$OP: Administrator

Population	APC-A Mean	APC-H Mean
P1	124,572	127,868

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Parameters – Laser Settings



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Time Delay

Picture from microscope



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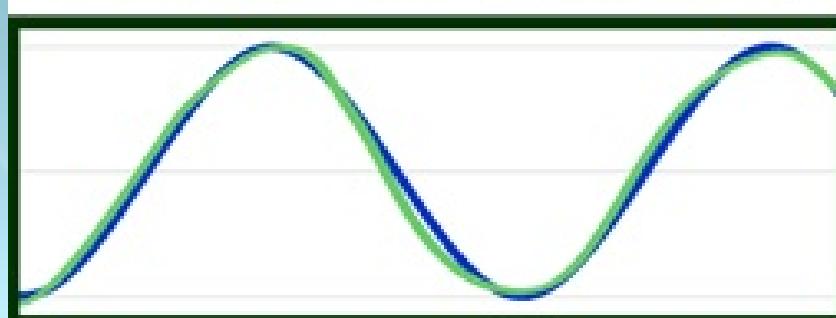
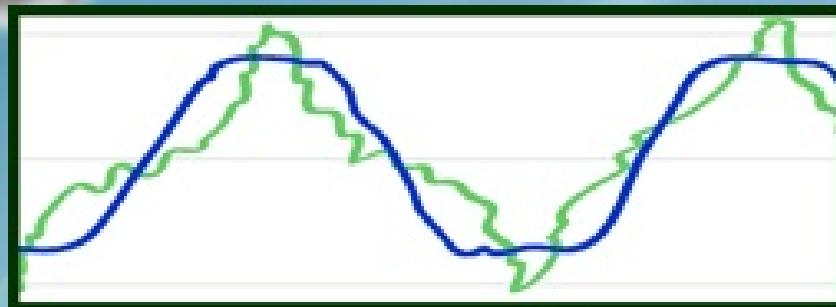
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NOISE



Voltage

Current

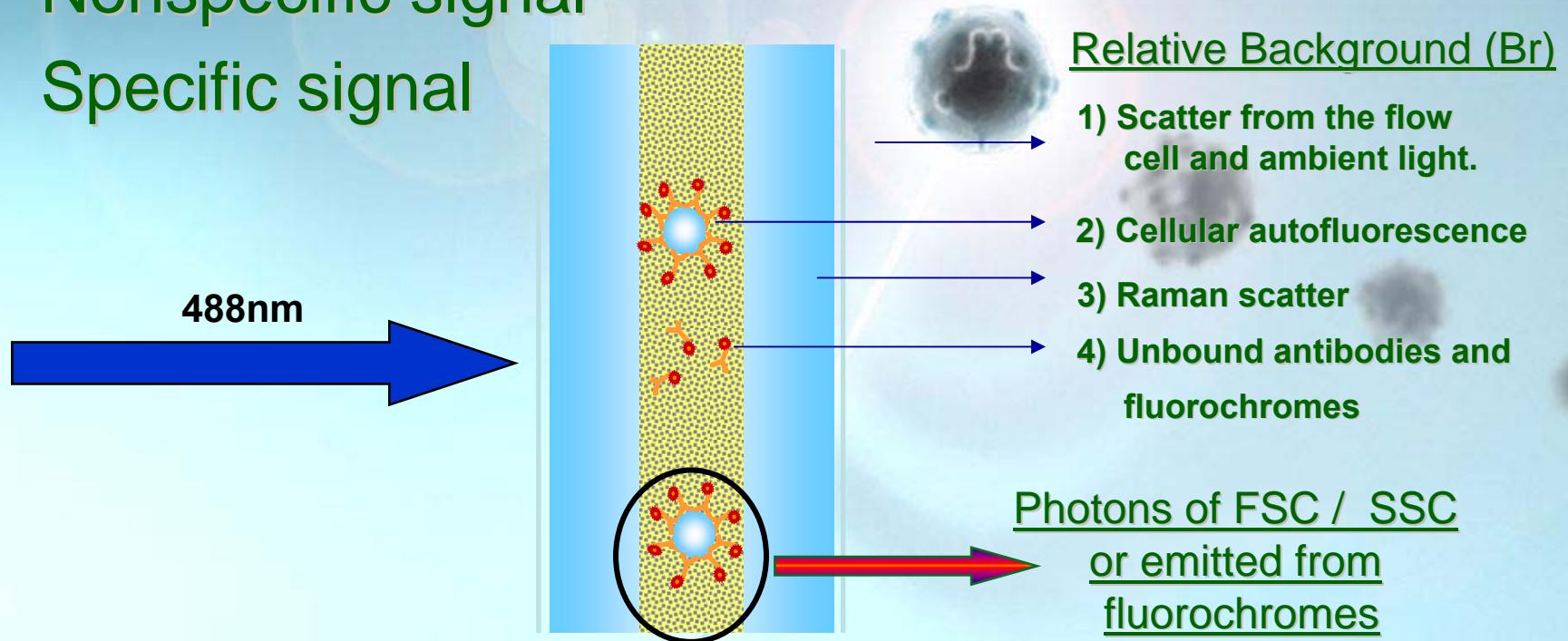


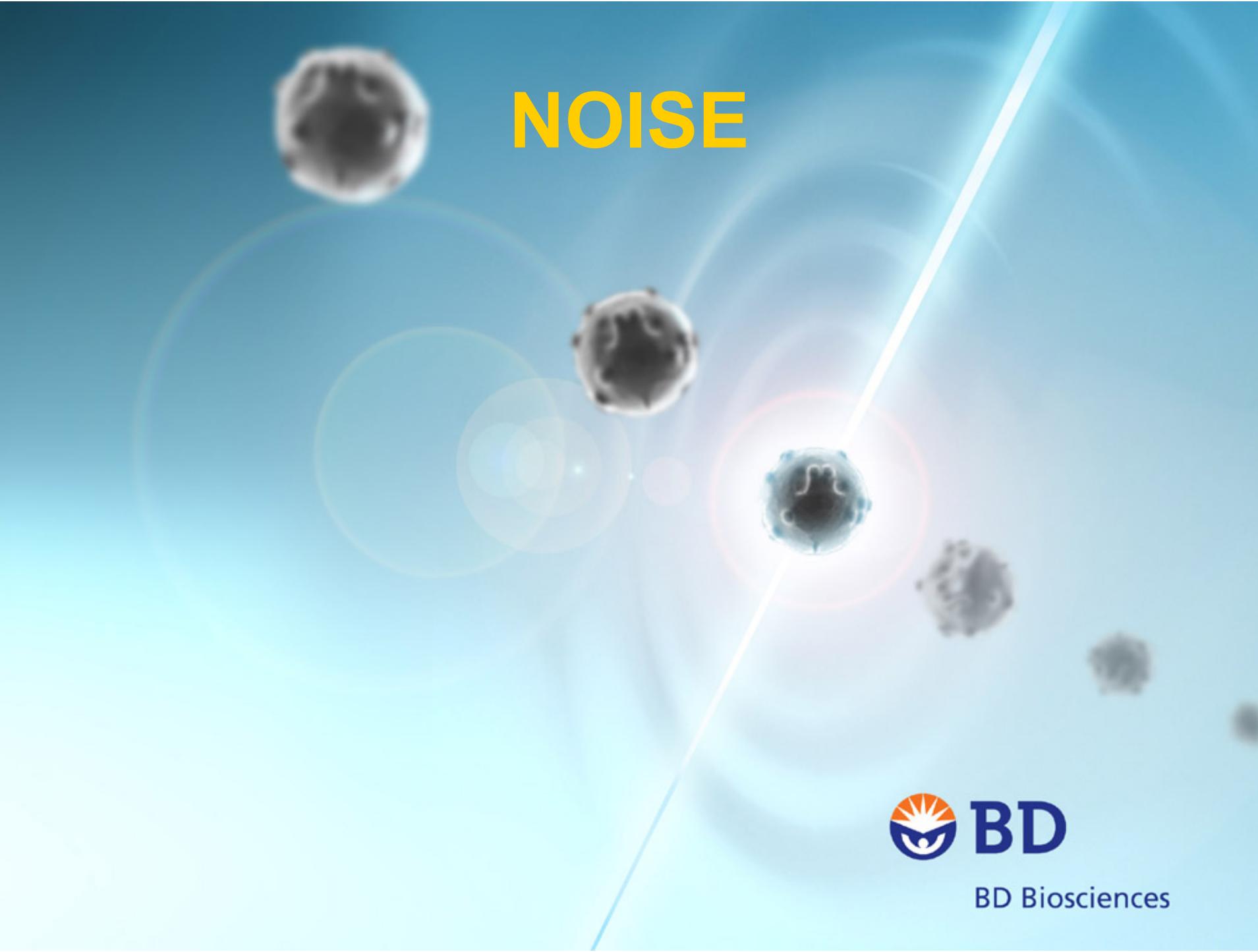
BD Biosciences

Detectors:

Specific signals vs background

- Not all electronic signals represent valid data !
Photons reaching the PMT create two types of signals:
 - Nonspecific signal
 - Specific signal





NOISE



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Electronics

- Photodetectors
 - Photodiode
 - Avalanche Photodiode
 - Photomultiplier
 - Charged Coupled Device
- Signal Amplifiers
 - Linear
 - Logarithmic
 - Scales: linear vs log
- Analog-Digital Converters
- Pulse
 - Intercept Point
 - Genesis
 - characteristics: A, H, W
- Threshold – Trigger
- Window Gate – Window Extension
- Area Scaling Factor
- Time Delay
- Noise
- Data Representation
 - Creation of a Histogram
 - Types of Plots
- Compensation
- FMO

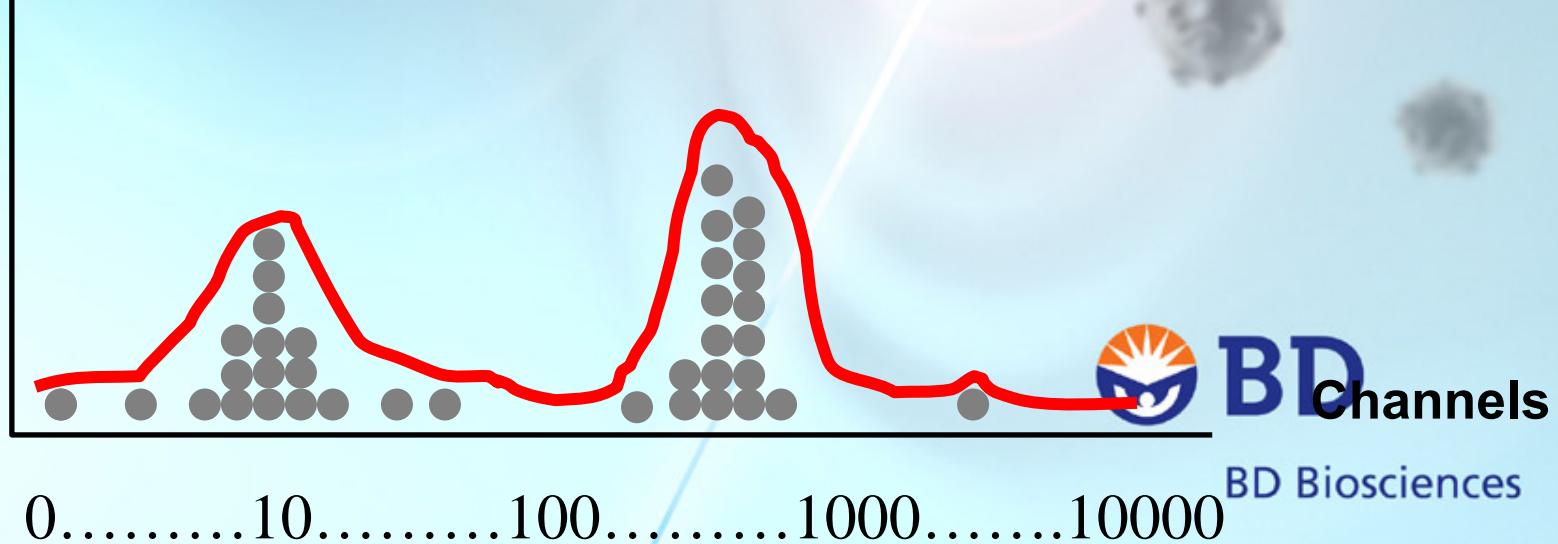


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Creation of a Histogram

Event #	Param 1 FSC	Param2 SSC	Param 3 FITC	Param 4 PE	Param 5 APC
1	100	500	10	650	4
2	110	505	700	700	6
3	90	480	720	670	10
4	95	490	15	720	15

Event Count



 BD Channels

BD Biosciences

Types of Plots

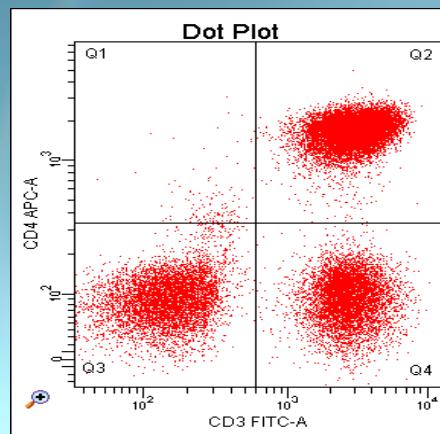
- Single Color Histogram
 - Fluorescence intensity (FI) versus count
- Two Color Dot Plot
 - FI of parameter 1 versus FI of Parameter 2
- Two Color Contour Plot
 - FI of P1 versus FI of P2. Concentric rings form around populations. The more dense the population, the closer the rings are to each other
- Two Color Density Plot
 - FI of P1 versus FI of P2. Areas of higher density will have a different color than other areas



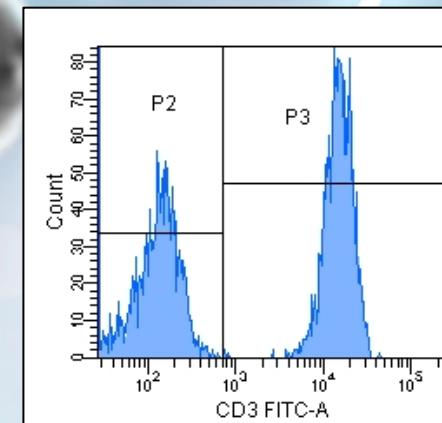
BD Biosciences

Electronics: The Results

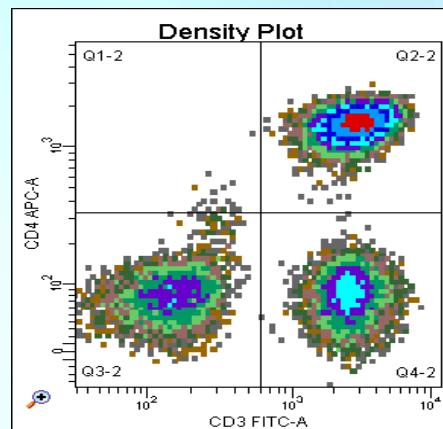
- Different forms for Data Display – Choose the most appropriate



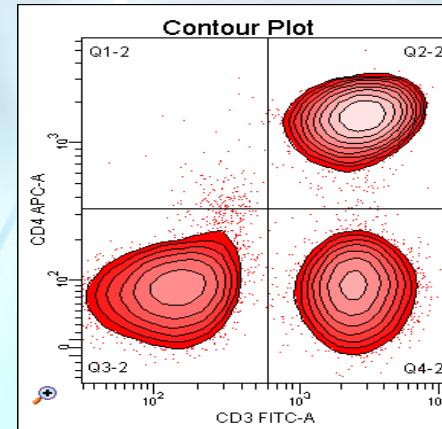
Dot Plot -
Each cell is
one Dot



Histogram Plot –
Each cell a dot in a
channel



Density Plot
Each cell a dot.
Cell-densities
are represented
by colours.

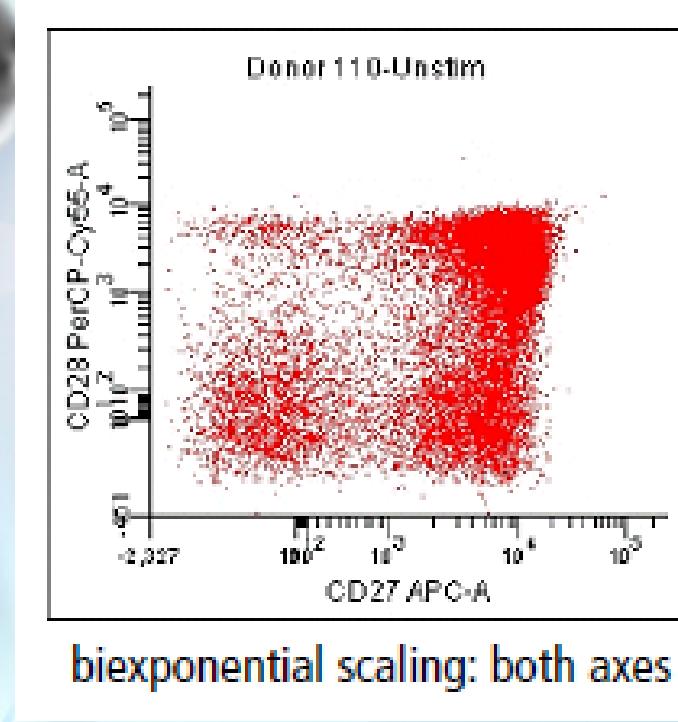
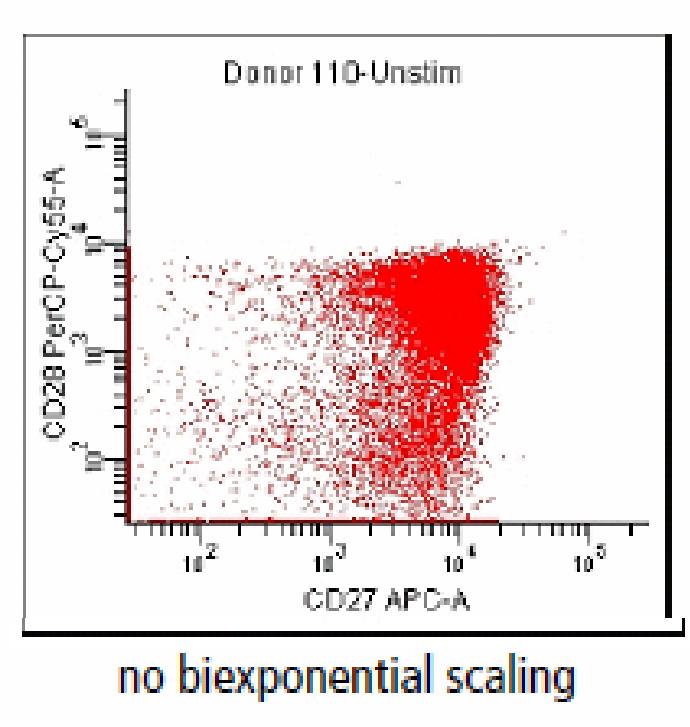


Contour Plot
Each cell a dot.
Cell-densities
are represented
by density-lines.



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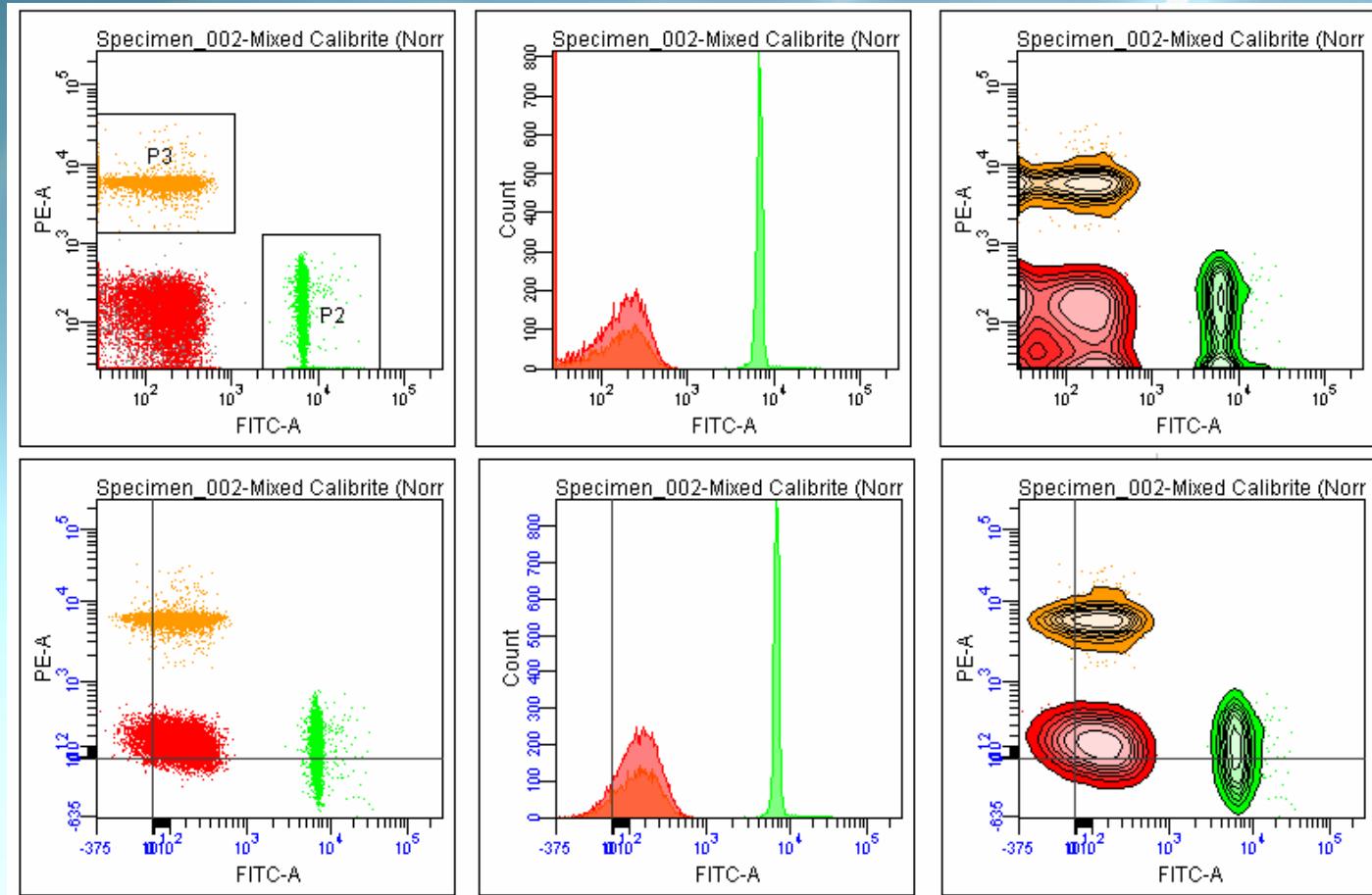
Optimized BiExponential



BD Biosciences

Optimized BiExponential

Algorithm self-adjusts and is automatically scaled



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Compensation

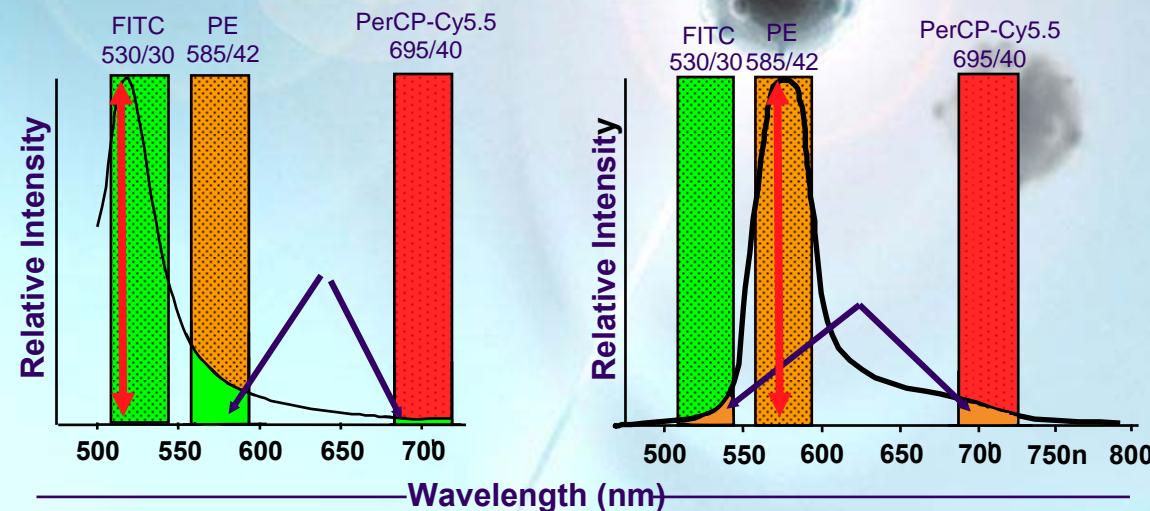
- Basic assumption of flow cytometry:
Exciting a FITC / PE labeled cell with a 488nm laser
only photons emitted by PE reach PE-detector (585 /
42 BP and 556LP)
only photons emitted by FITC reach FITC detector
(530 / 30 BP and 502LP)
and create electronic signals
- Not true for unprocessed data !



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Compensation

- Photons reaching a specific detector are coming from
 - optical background (especially cellular auto-fluorescence)
 - photon spill over from all fluorochromes present in experiment
 - the fluorochrome specific for this detector



Compensation

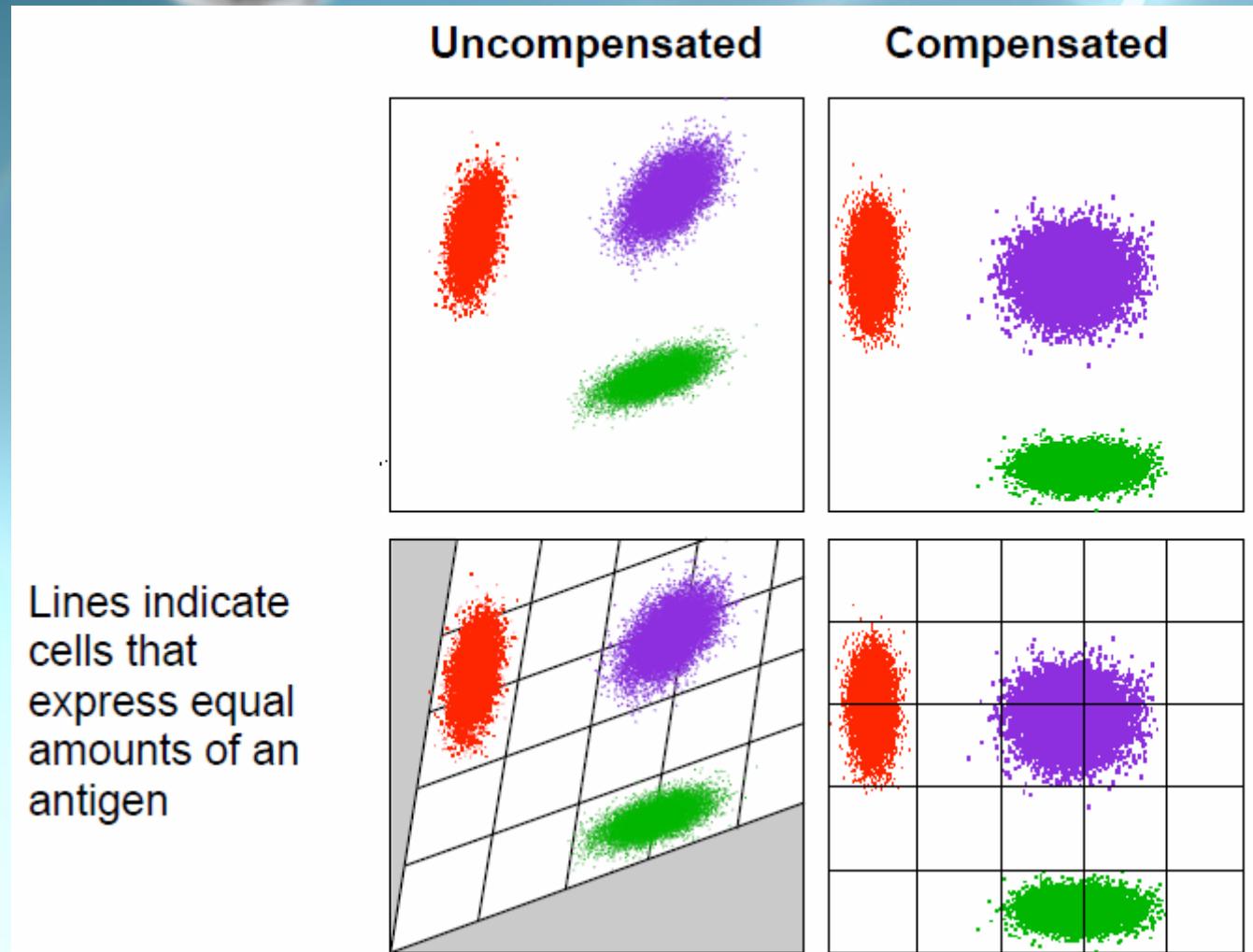
- Compensation is a procedure to
 - determine for each detector electronic background signal due to
 - a) relative background = Br (especially cellular auto-fluorescence)
 - b) photon spill over from all fluorochromes present in experiment
 - subtract from
 - the specific electronic signal induced by the photons of a fluorochrome
 - the nonspecific electronic signal from relative background and spill over



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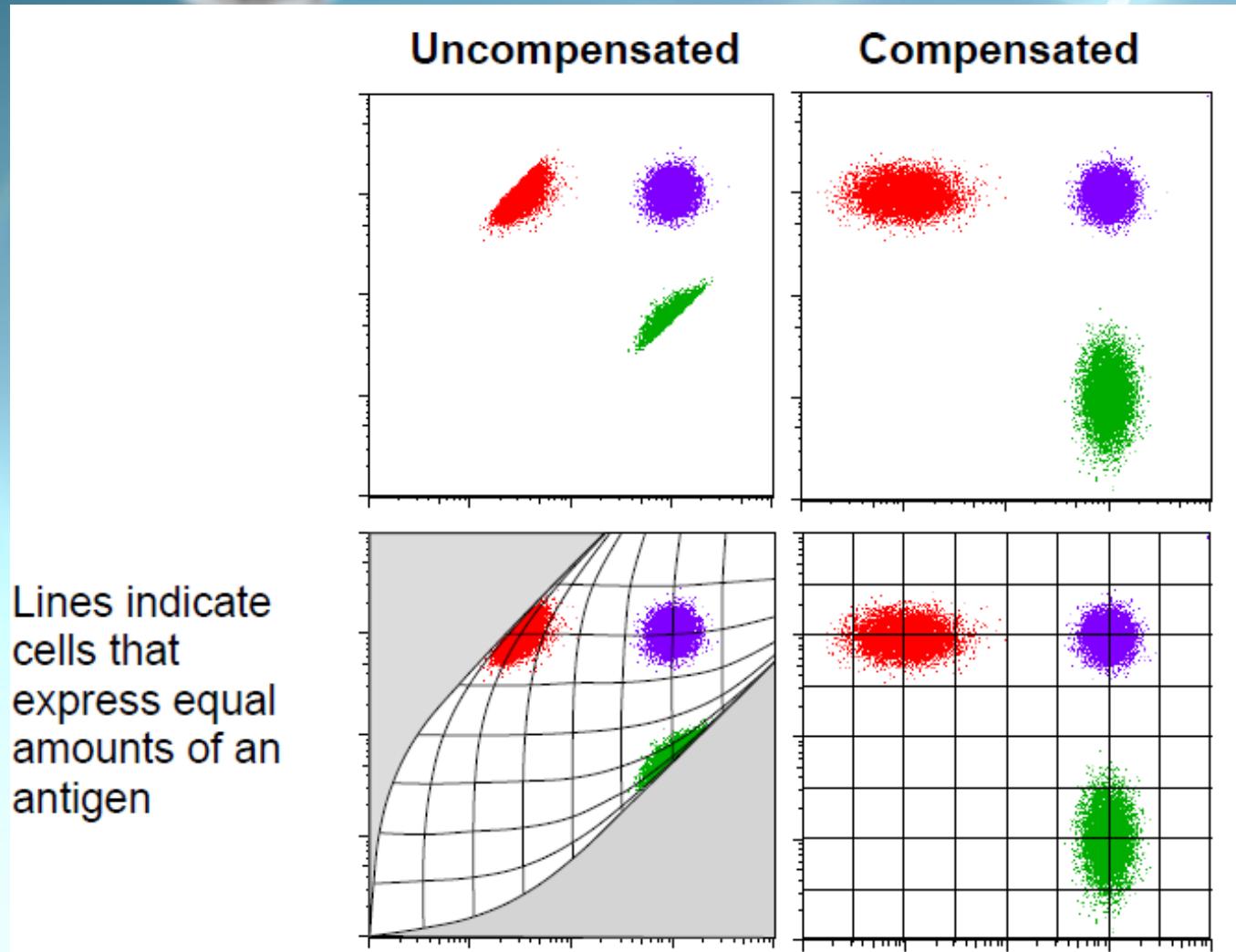
Compensation

...in linear domain



Compensation

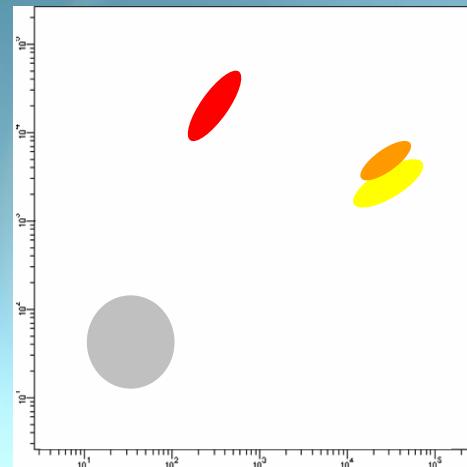
...in log domain



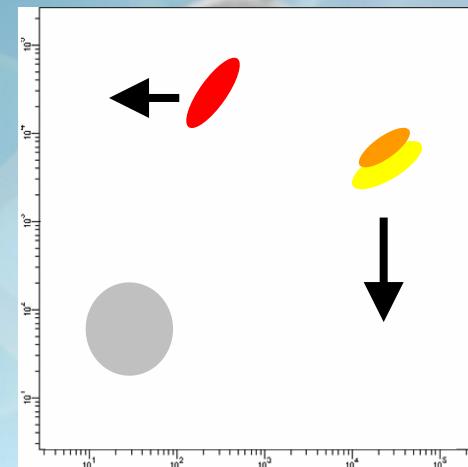
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Compensation

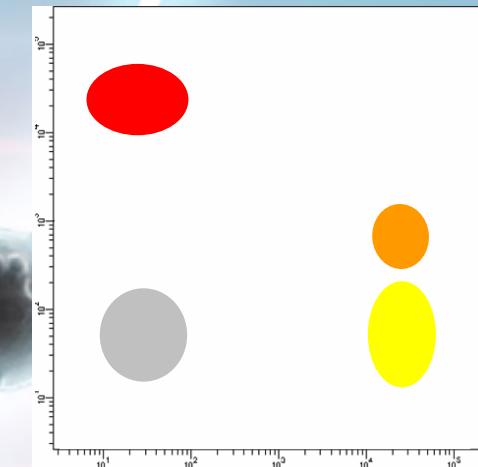
What you see



What you want



What you get



by compensation



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FMO

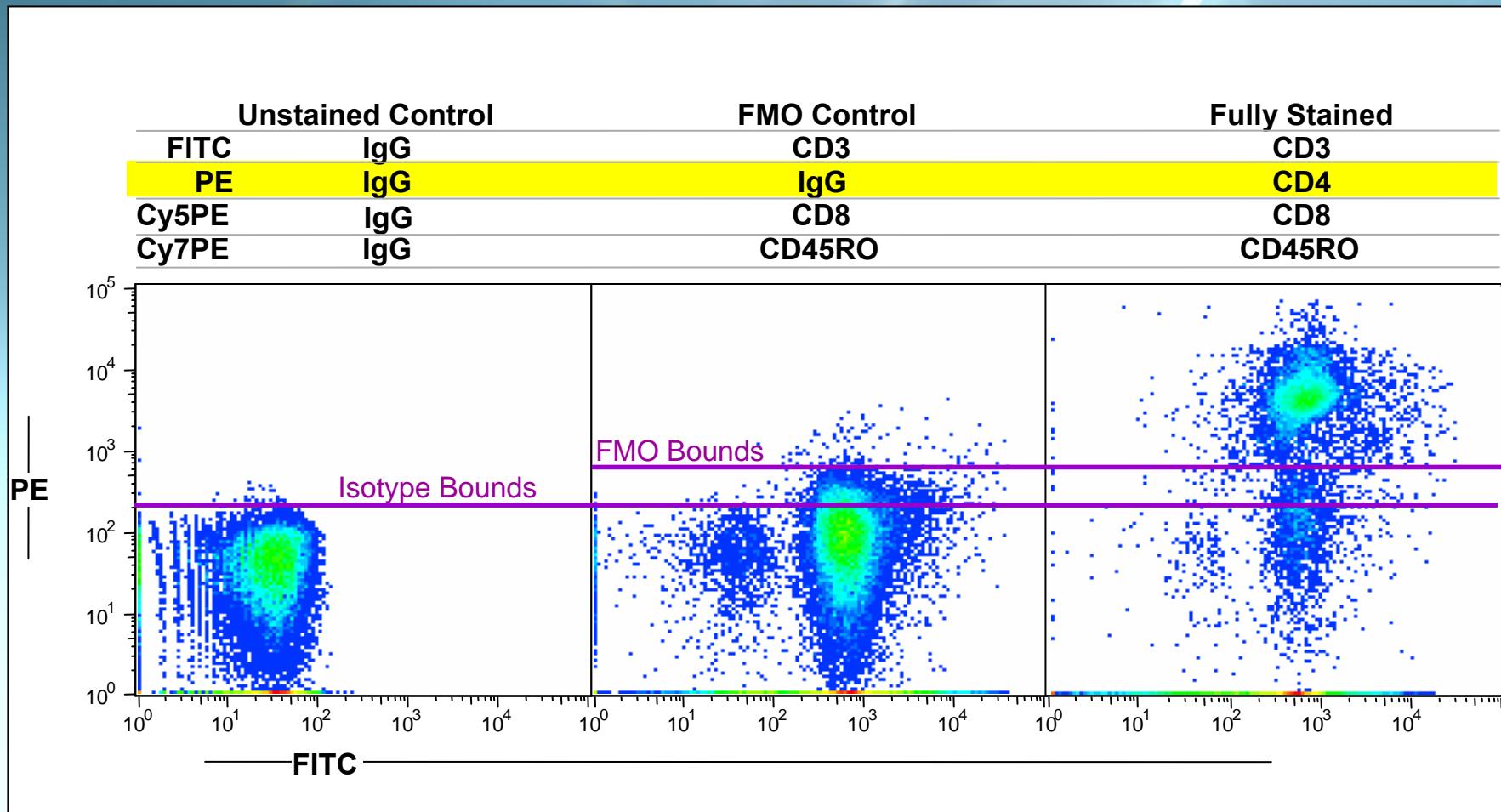
- FMO controls are a much better way to identify positive vs. negative cells
- FMO controls can also help identify problems in compensation that are not immediately visible
- FMO controls should be used whenever accurate discrimination is essential or when antigen expression is relatively low



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FMO

Compensation was properly set for all spillovers



Courtesy Mario Roederer

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