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Photodetection in Flow Cytometry

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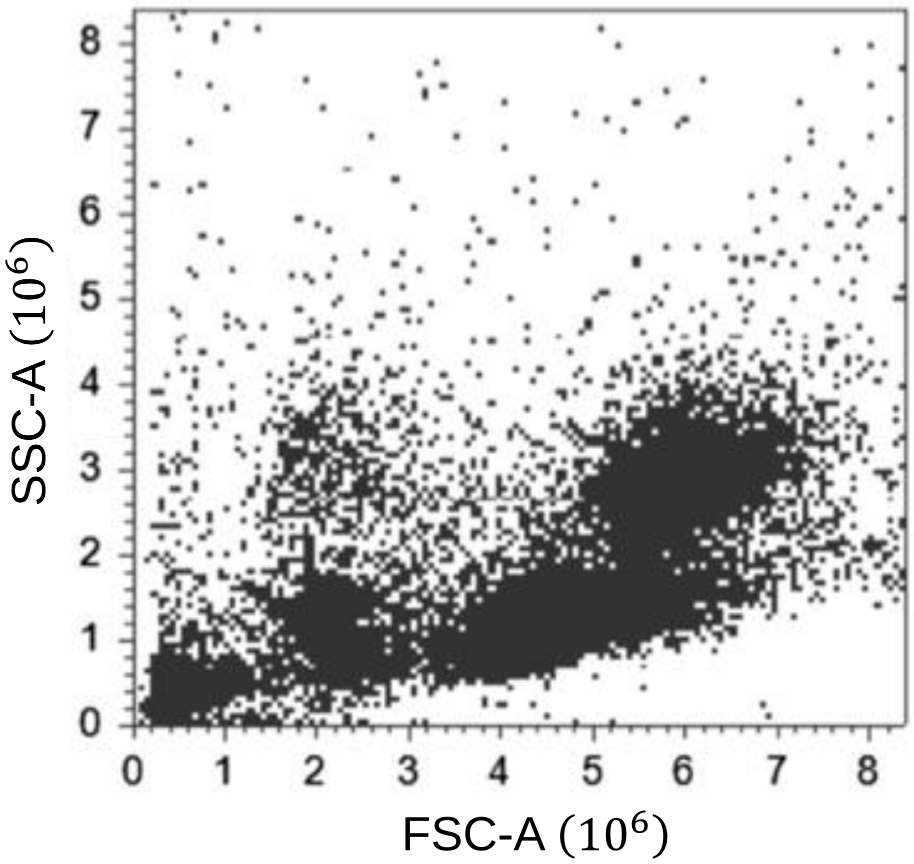
Index

- Introduction to Flow Cytometry
- Photodetection
- How are Scattered Plots Affected?

Motivation



How does photodetection affect the science depicted in the scatter plots?

Introduction to Flow Cytometry

Flow Cytometer Consists of Three Components:

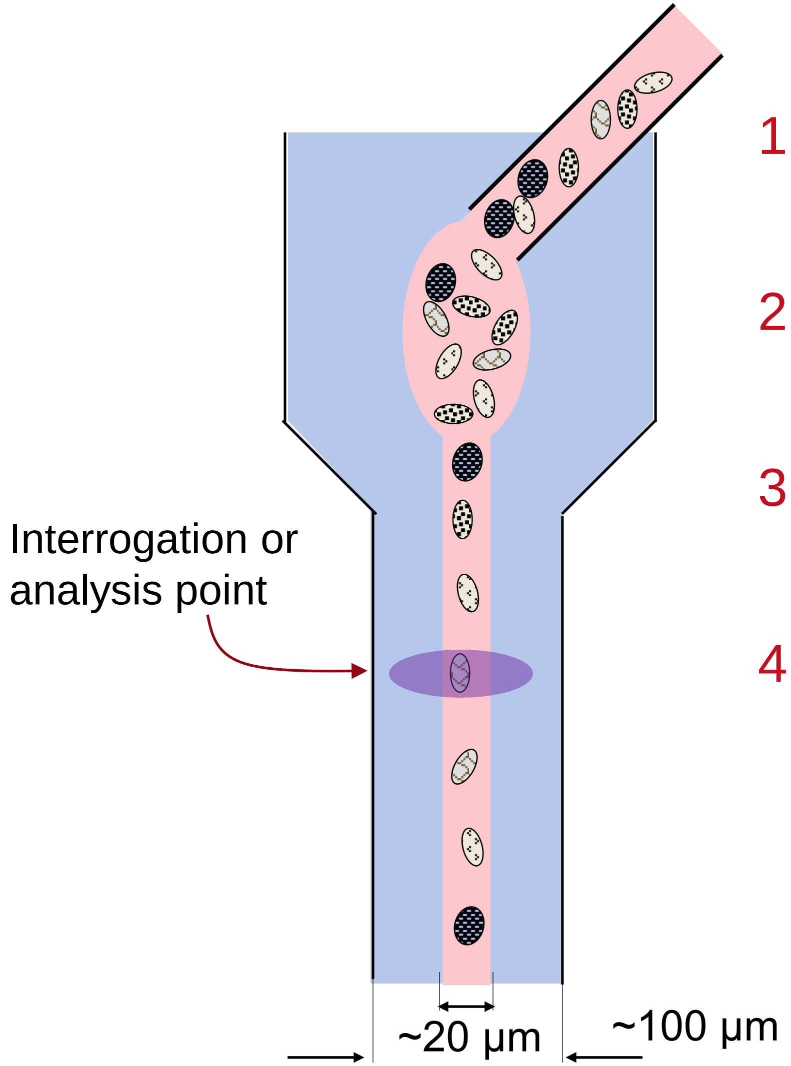
Fluidics

Optics

Electronics

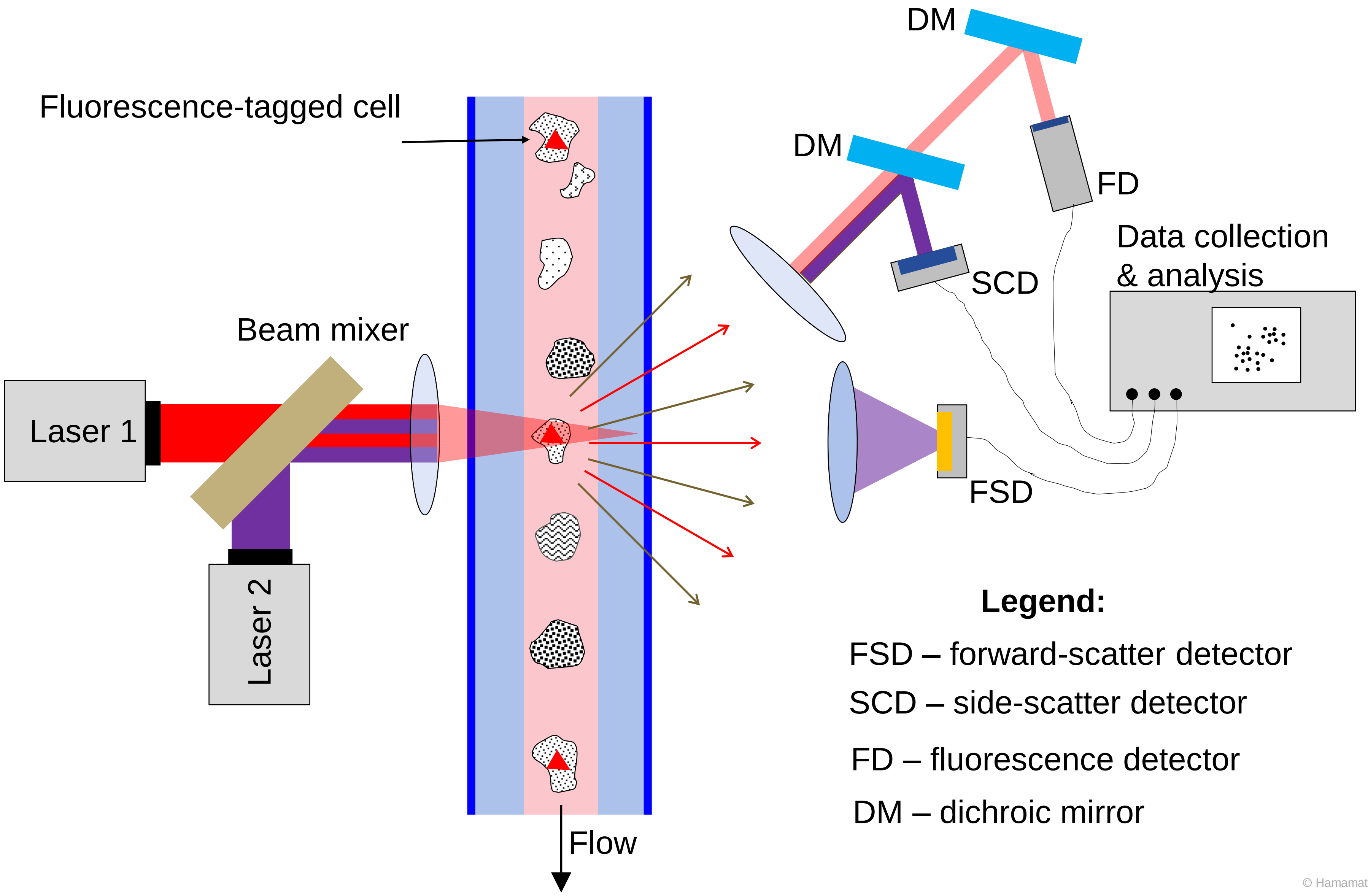
Photodetectors bridge these two components

Fluidics



1. Hydrodynamic focusing creates a narrow flow, known as the core, where the cells arrange in a one-by-one file
2. The diameter of the core depends on the pressure difference between the sheath fluid and the core fluid.
3. Only one cell at the time should be passing through the interrogation point.
4. Sampling rate $\sim 1{,}000 - 100{,}000\ s^{-1}$

Optics



Legend:

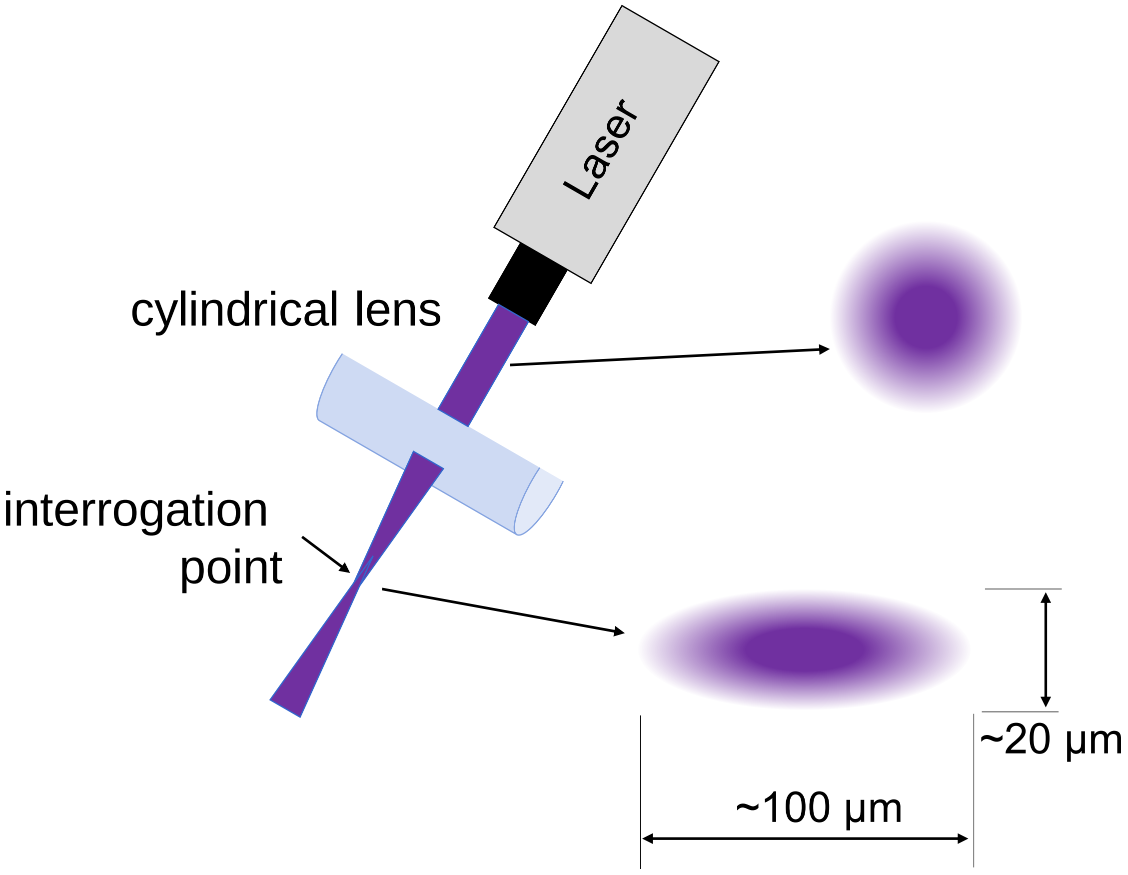
FSD – forward-scatter detector

SCD – side-scatter detector

FD – fluorescence detector

DM – dichroic mirror

Interrogation Point



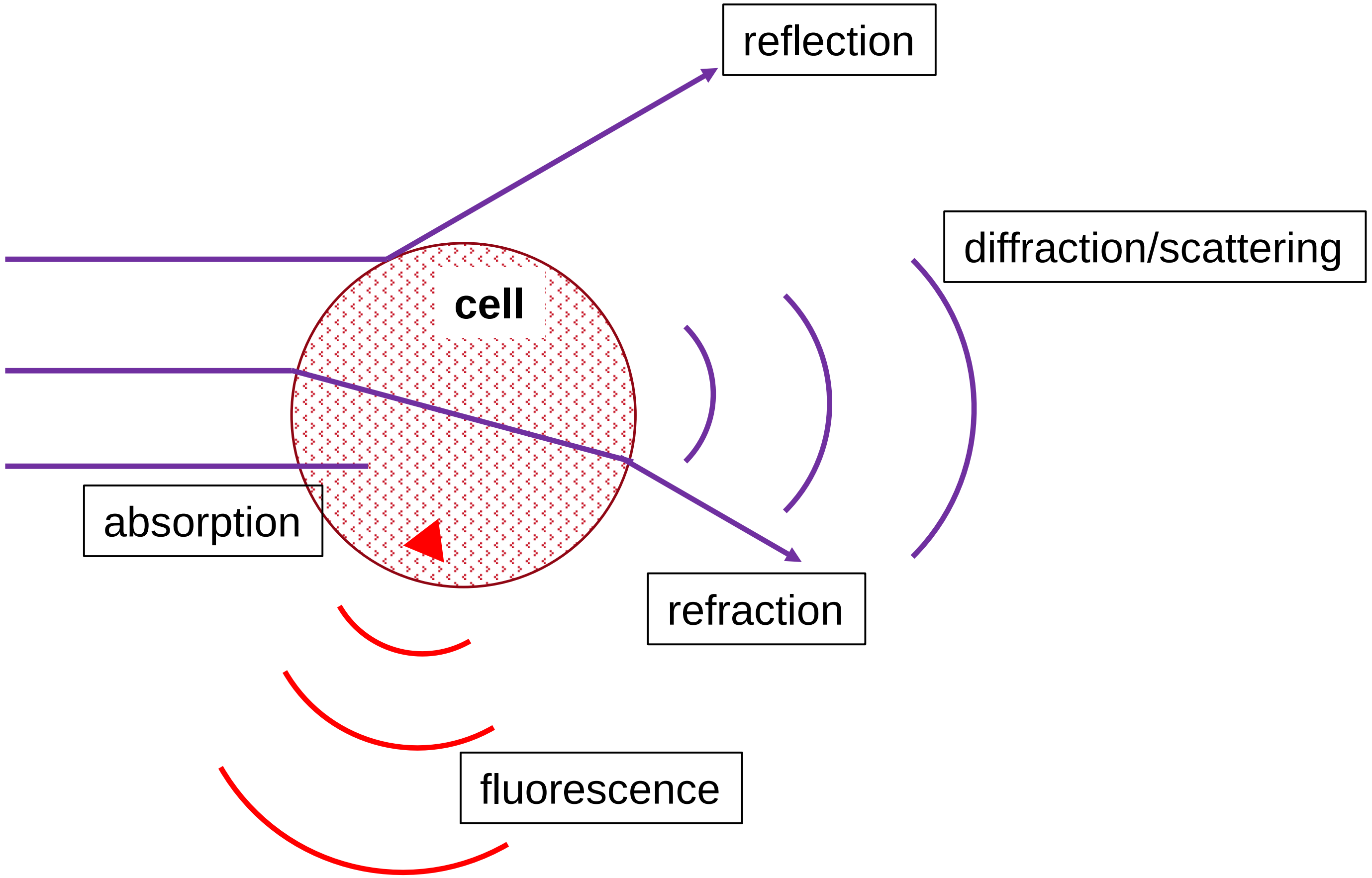
Beam profile (typically Gaussian) before cylindrical lens (**not to scale**)

Beam profile at the interrogation point (**not to scale**). The long axis is perpendicular to the flow.

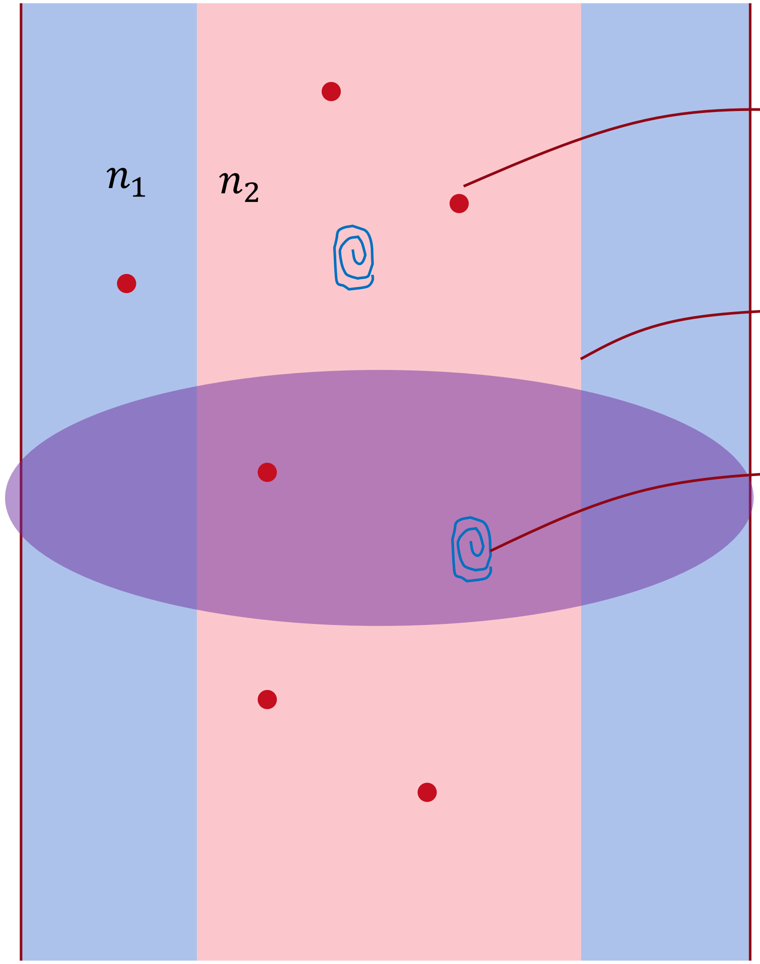
Light Sources

1. Solid-state lasers are the most common illuminators in modern flow cytometers
2. These lasers are compact, light weight, and can provide up to 150 mW of output power
3. Typical wavelengths [in nm]: 488, 505, 514, 532, 552, 561, 594

Interaction of Light with the Cell: Signal Generation



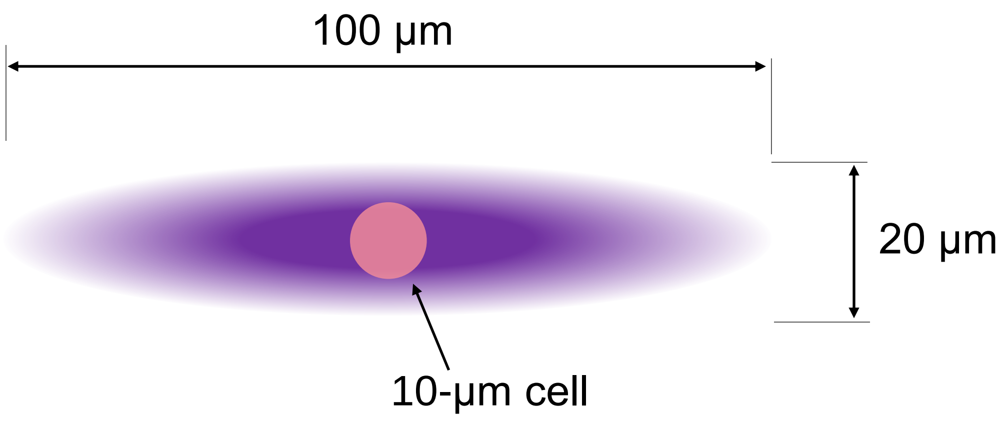
Spurious Light



Spurious light can be due to:

- Impurities
- Index of refraction differences
- Flow turbulence

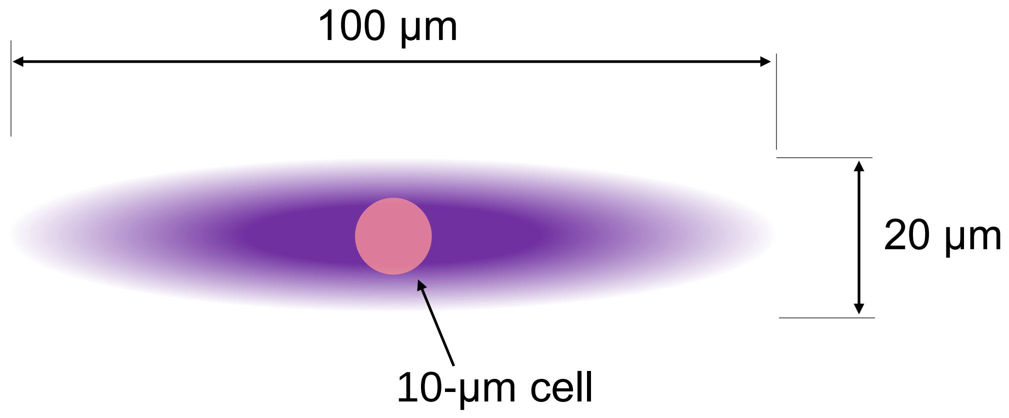
Signal Formation



1. Suppose we have 20-mW laser with $\lambda = 488$ nm
2. About 10% of the power (or 2 mW) will illuminate a 10-µm cell as it passes through the interrogation point. The amount will be proportionally larger/smaller for a cell that is larger/smaller
3. The implied illumination intensity is about 2.55×10^7 W/m^2, which for 488-nm photons gives 6.27×10^{25} photons per m^2 per s

Reference: “Practical Flow Cytometry” by Shapiro

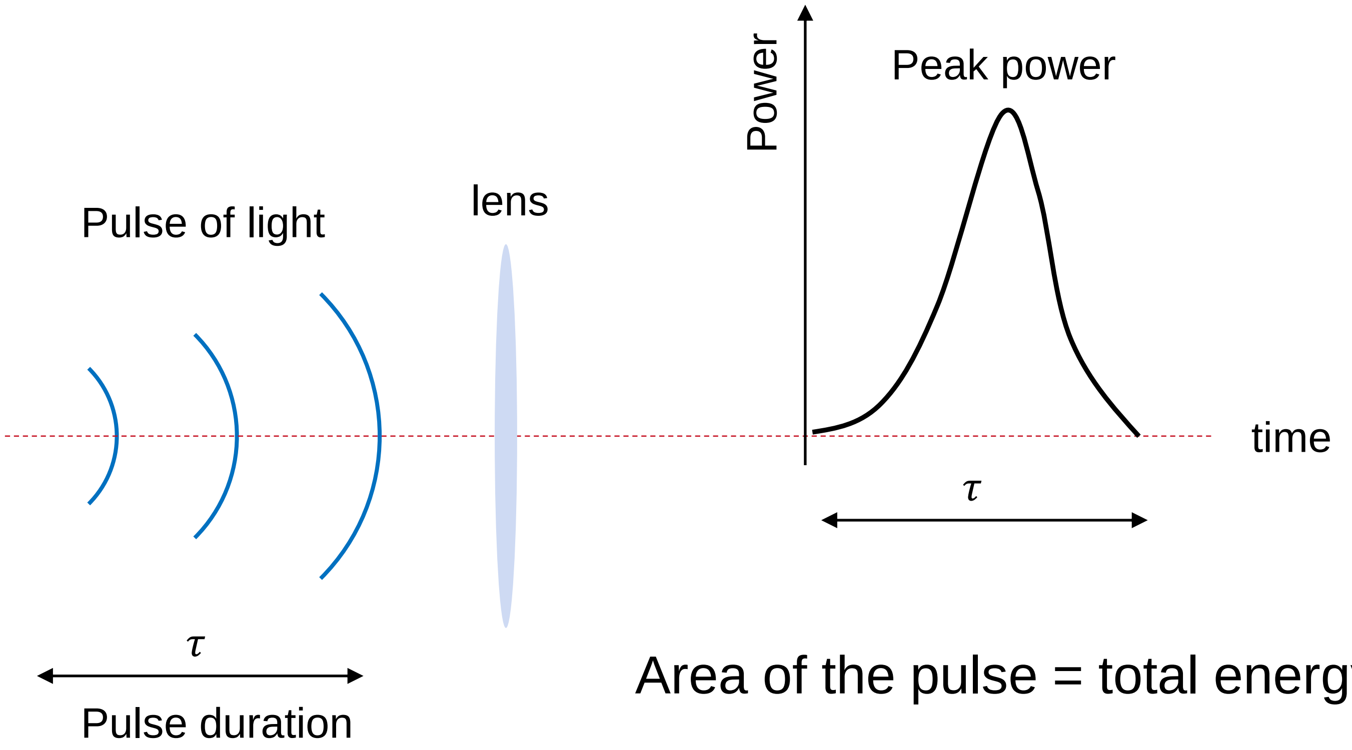
Signal Formation



4. Thus the 10-µm cell is illuminated with 4.9×10^{15} photons per s.
5. If the interrogation rate is 1,000 cells per second, a cell will scatter a total of about 4.9×10^{12} photons

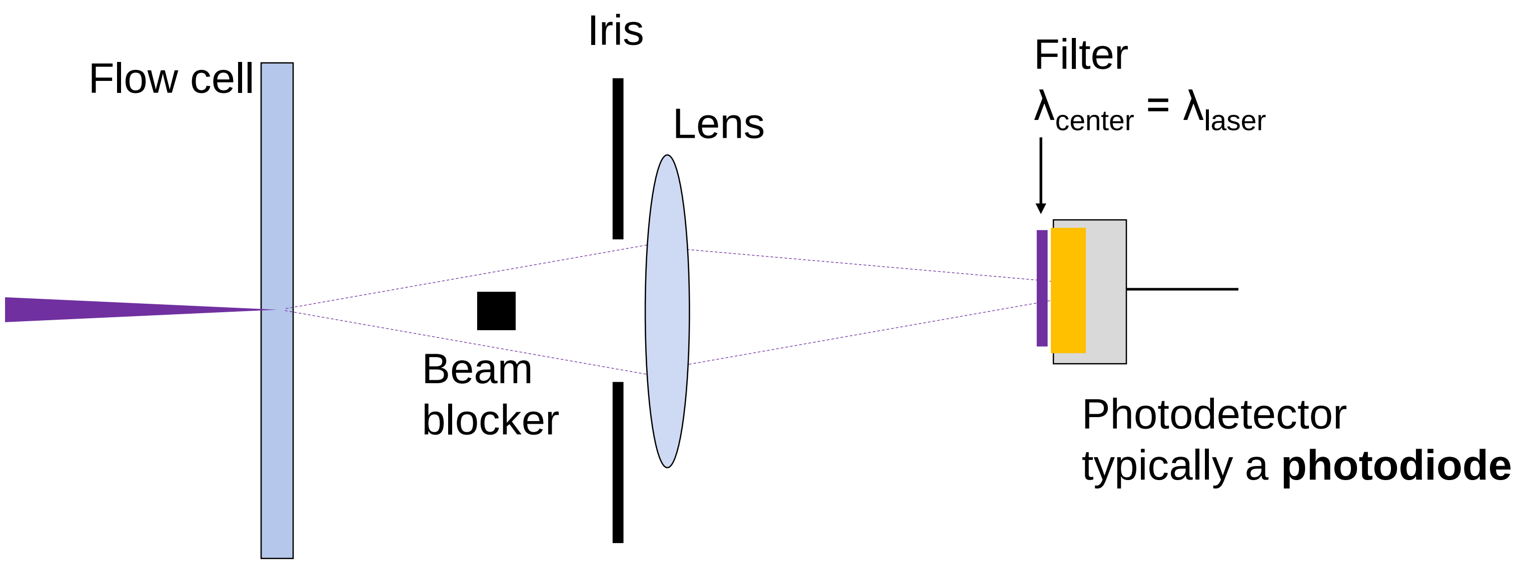
Reference: “Practical Flow Cytometry” by Shapiro

Anatomy of a Pulse



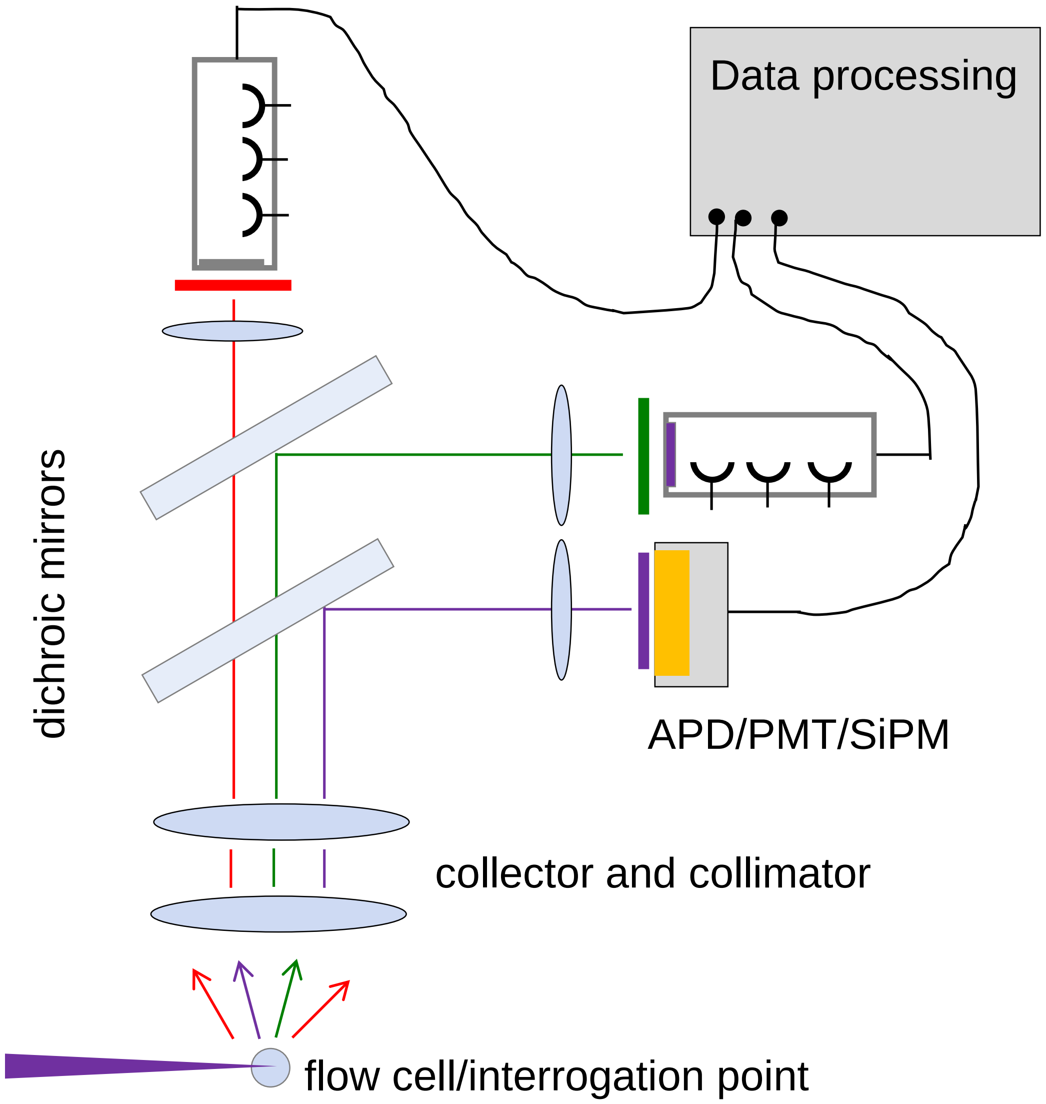
Area of the pulse = total energy in the pulse

Forward–Scatter Detection



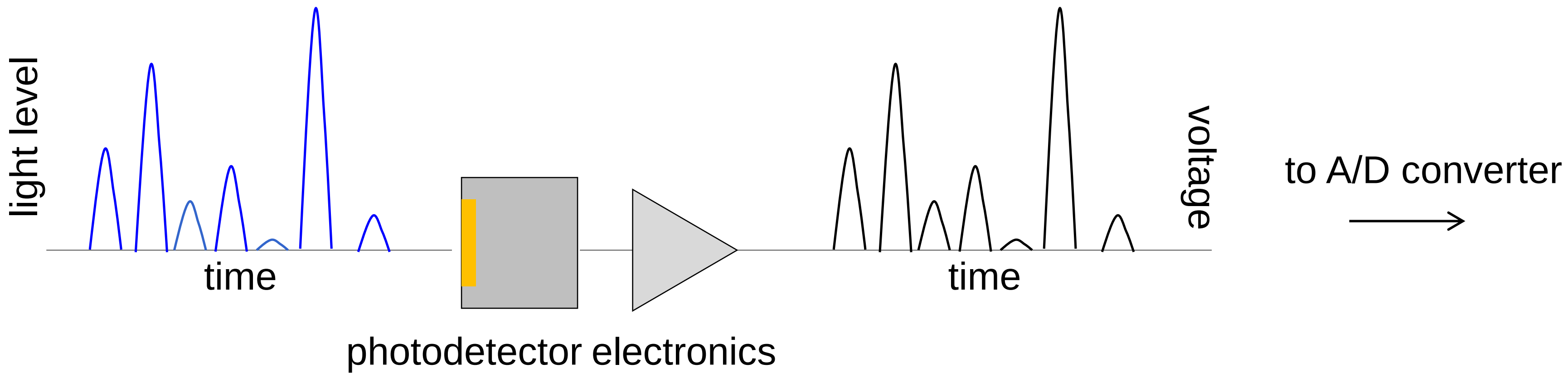
1. The forward scatter signal is primarily determined by the size of the interrogated cell
2. The peak forward scatter power at $\lambda = 488$ nm using 2-μm microspheres and 20-mW laser is about 4 μW

Side-Scatter Optical Setup



1. Light is a mixture of scattered laser light and fluorescence
2. Light level is much lower than in the forward scatter
3. Multiple fluorescence wavelengths can be present
4. Need to use photodetectors with intrinsic gain

The Role of the Photodetector



A photodetector + electronics convert the input light signal (a pulse) into electrical pulse (voltage as a function of time)

Current to Voltage Conversion

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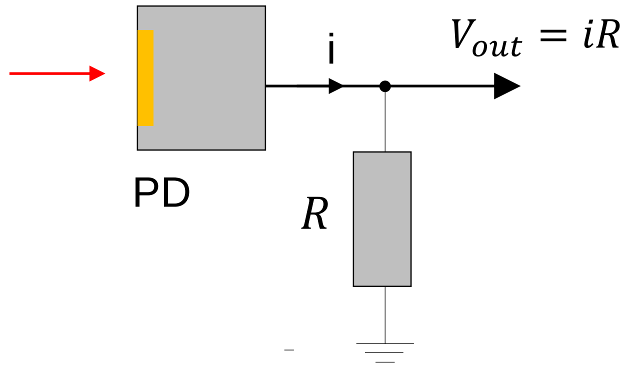
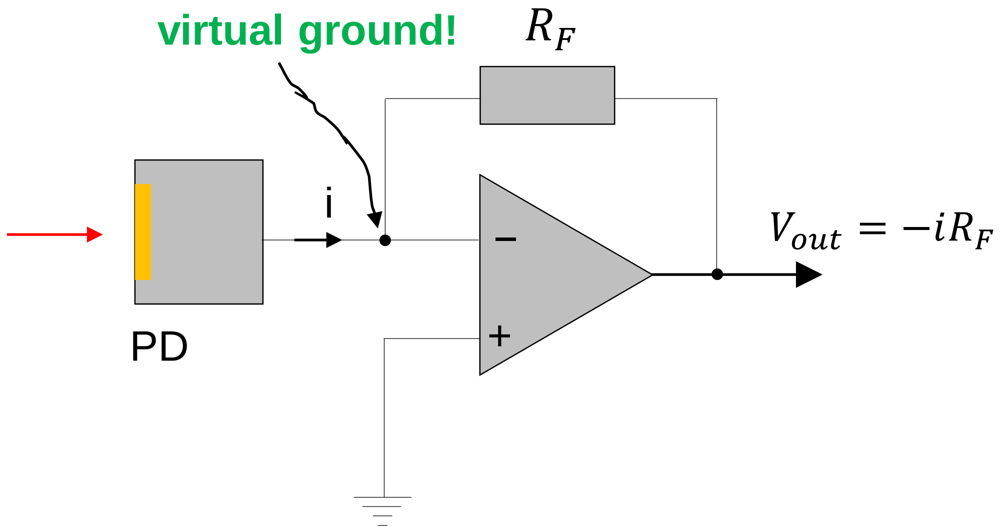




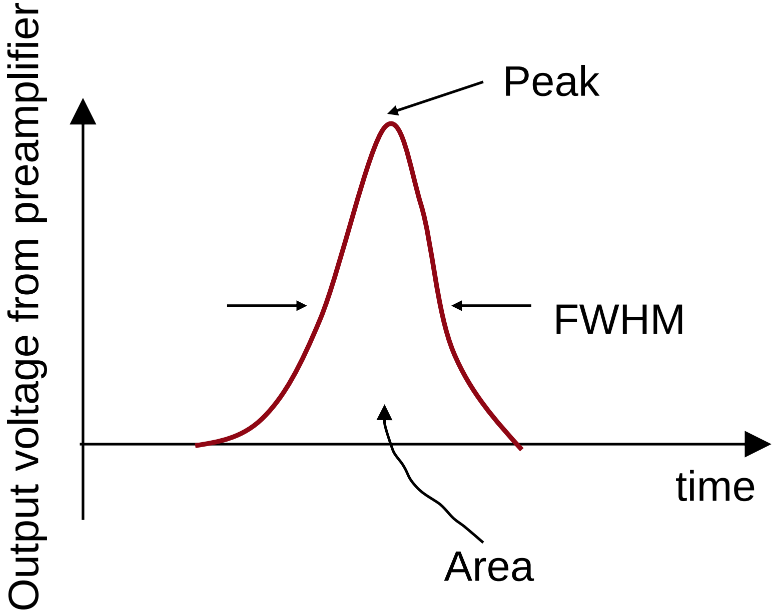
Resistive termination

- + Simple circuit and inexpensive
- + Well-behaved frequency response
- + Well-understood noise (Johnson)
- – Loads the PD, can lead to non-linearity

Transimpedance amplifier (pre-amplifier)

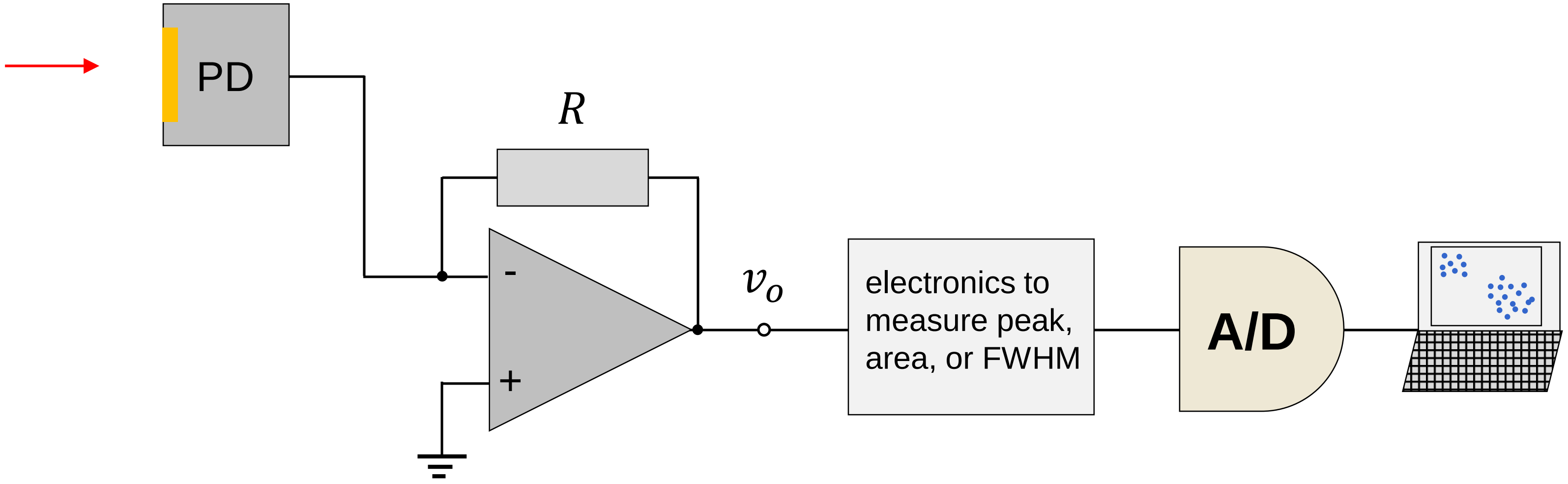
- + Virtual ground, no loading of the PD
- – Complex noise and frequency response
- – Needs biasing

What is Measured?



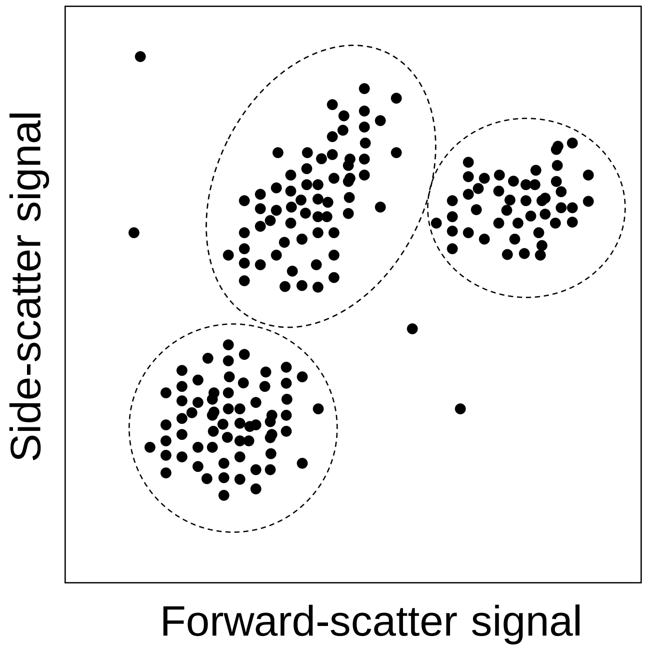
The front-end electronics can be set up to measure the peak value of the pulse, its FWHM, and/or area under the curve. These different measurements provide specific information about the cell.

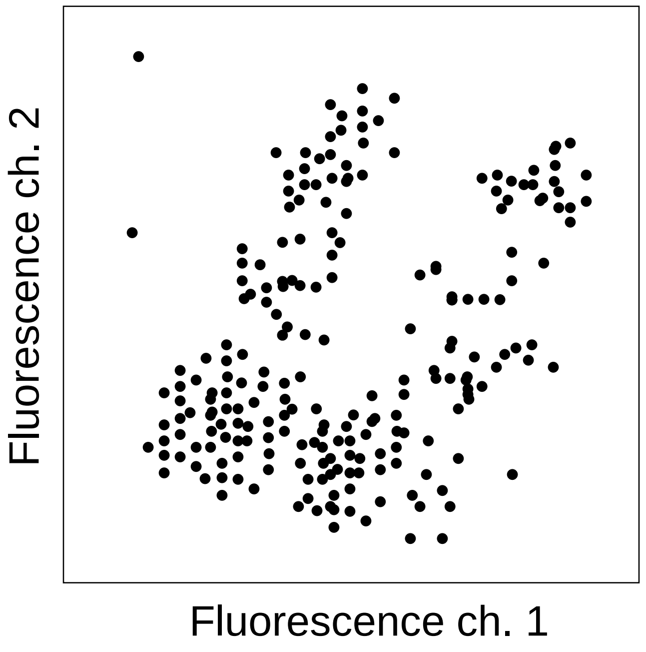
Detection and Data Processing Chain



Pre-amplifier

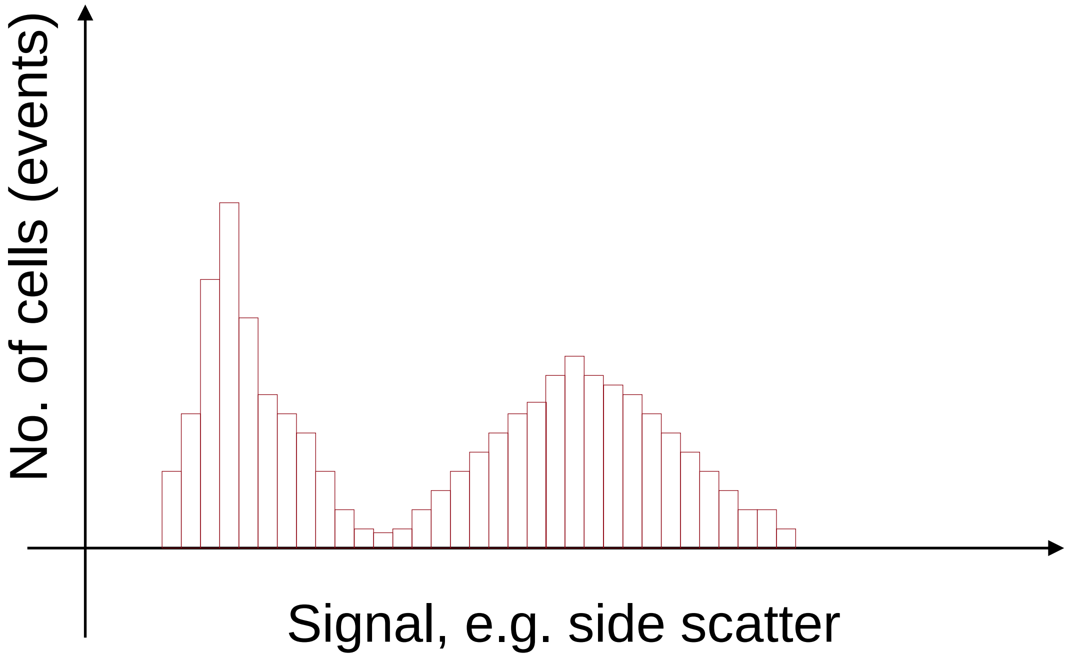
Scatter Plots





Scatter plots are ubiquitous to flow cytometry

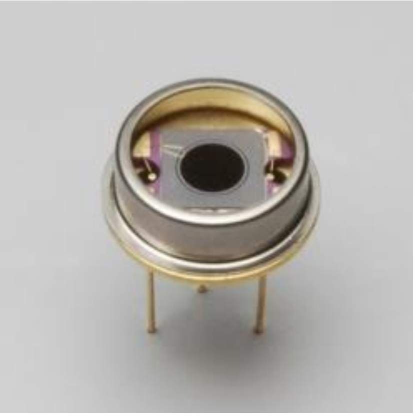
Histograms

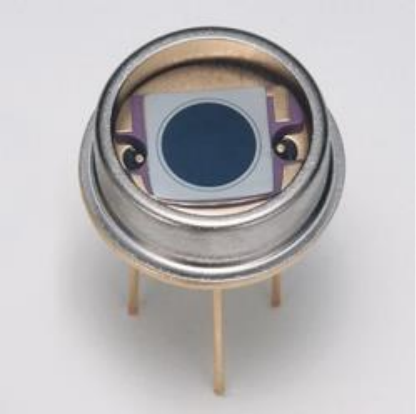


Histograms of a given measured quantity are also common

Photodetection

Point Photodetectors used in Flow Cytometry





PMT PD APD SiPM

PMT – photomultiplier tube

PD – photodiode

APD – avalanche photodiode

SiPM – silicon photomultiplier

Photomultiplier Tube

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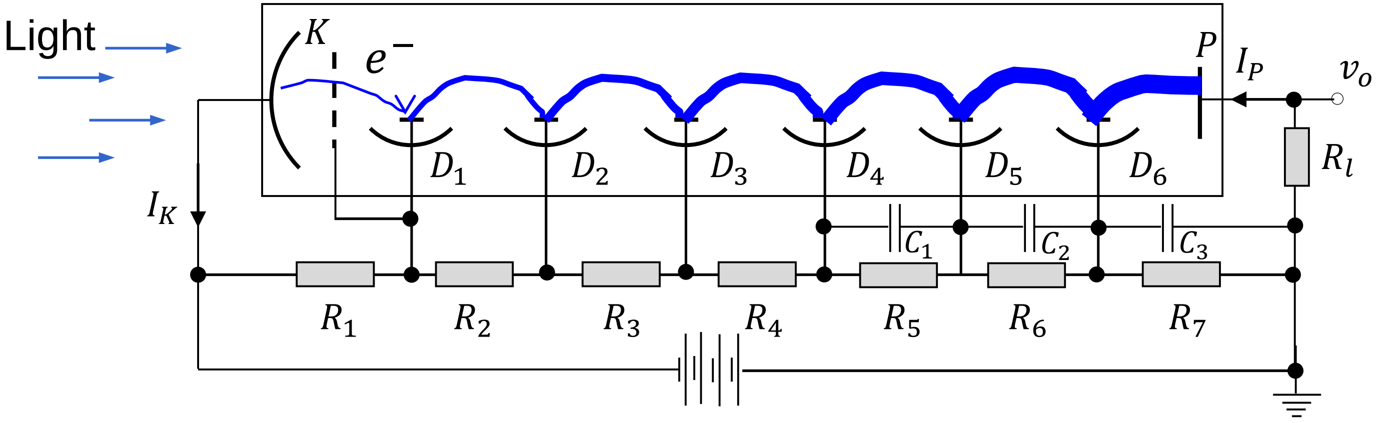
K – cathode $D_1, \ldots D_6$ – Dynodes P – anode



$V \sim 1000$ V

Gain: $10^4 - 10^8$

There are two essential phenomena involved in the operation of a PMT: *extrinsic photoelectric effect* and *electron secondary emission*.

Solid-State Photodetectors

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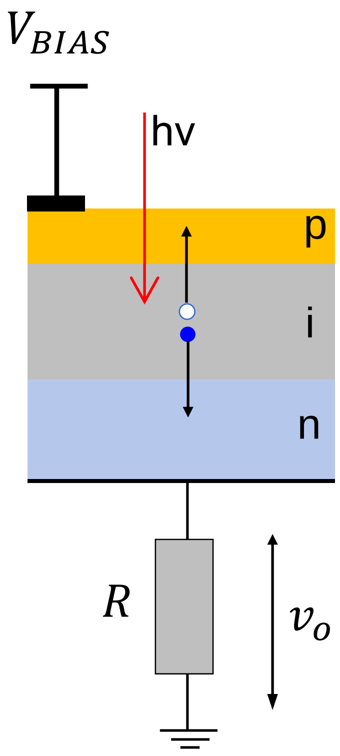
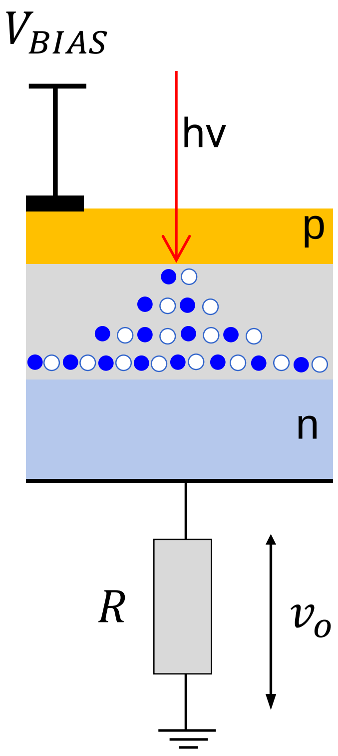
Si PIN photodiode



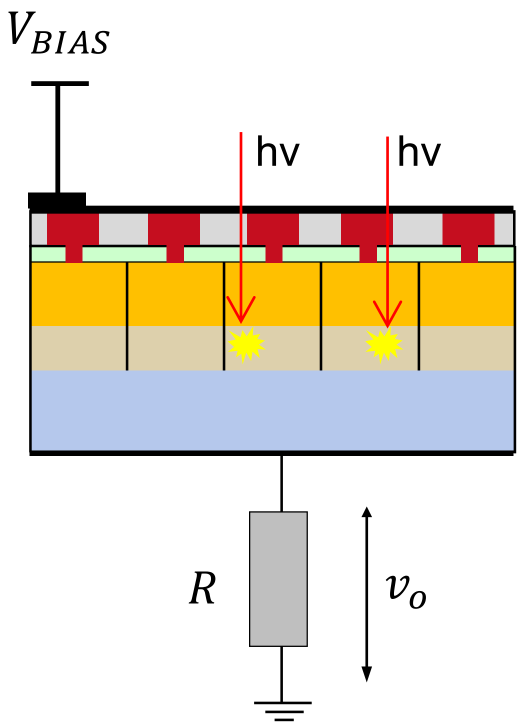
Gain =1

Si APD



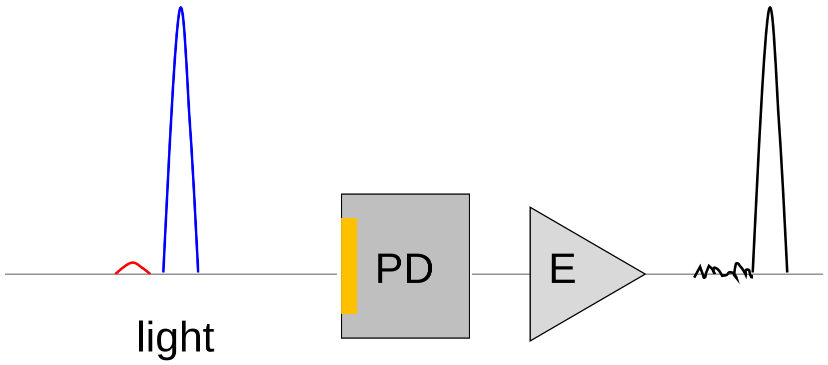
Gain up to ~100

SiPM



Gain ~10^6

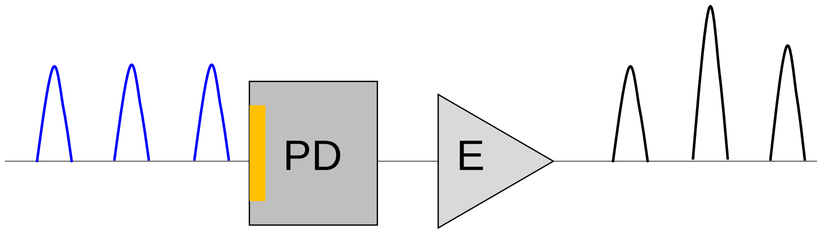
Detection Characteristics: Photosensitivity



Can we detect the “red” pulse?

A photodetector’s *effective* photosensitivity depends on its quantum efficiency (function of wavelength) and **intrinsic gain**.

Detection Characteristics: Gain Variations



1. Random gain variation of a photodetector increases noise, which translates into a larger scatter of the measured quantity
2. All photodetectors with intrinsic gain exhibit gain variation. The contribution to noise is expressed with *excess noise factor* F

Gain and Excess Noise

	μ	F	
PMT	$10^5 - 10^7$	~ 1.2	$F \approx \frac{\delta}{\delta - 1}$
APD	$1–100$	$\sim 3 - 4$	$F \approx \mu^{0.3}$
SiPM	$10^5 - 10^6$	~ 1.1	$F \approx 1 + P_{ct}$

Legend

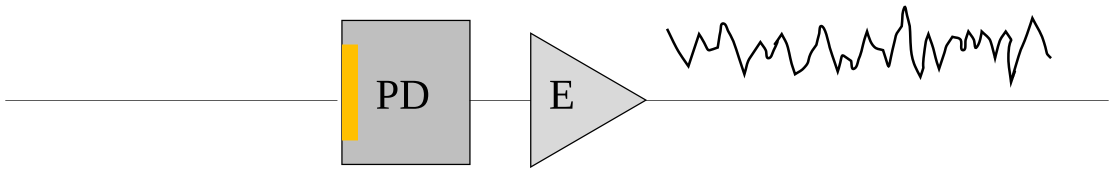
μ – Intrinsic gain of a photodetector

F – Excess noise factor

δ – Gain of the first dynode in a PMT (typically ~ 4)

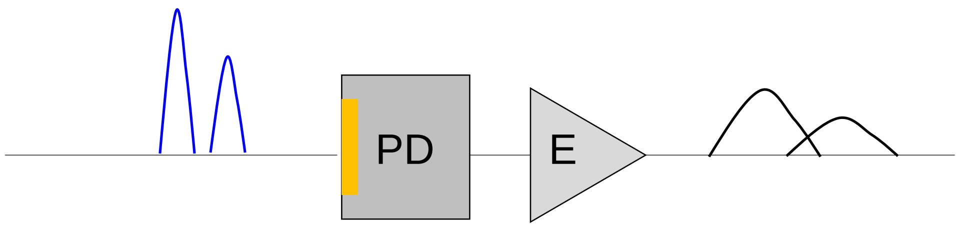
P_{ct} – Probability of crosstalk in a SiPM (typically less than 10%)

Detection Characteristics: Dark Current



1. Photodetector’s dark current results in the output signal offset from zero
2. The variation around the mean is noise
3. The magnitude of dark current depends on the photodetector’s bias voltage and temperature

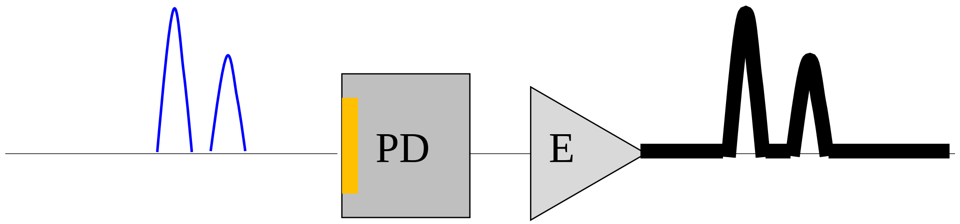
Detection Characteristics: Bandwidth



Is there an output signal pileup?

1. Insufficient bandwidth degrades signal fidelity. At high counting rates this may lead to signal pileup
2. Detection bandwidth is determined by the photodetector and front-end electronics

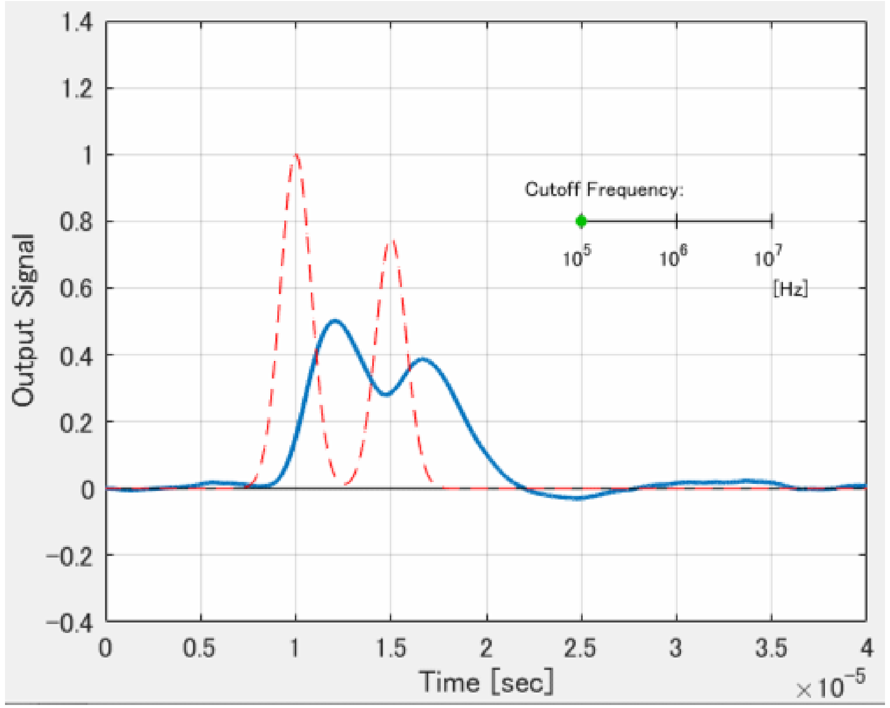
Bandwidth and Noise



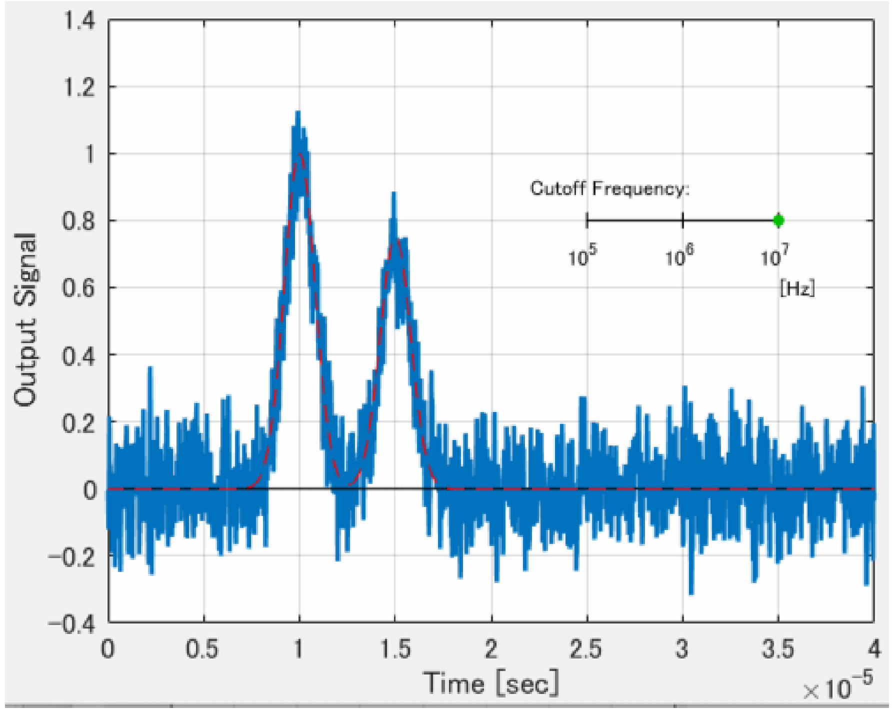
Too much bandwidth adds noise (indicated by the thicker line) but does not improve fidelity

Bandwidth and Noise

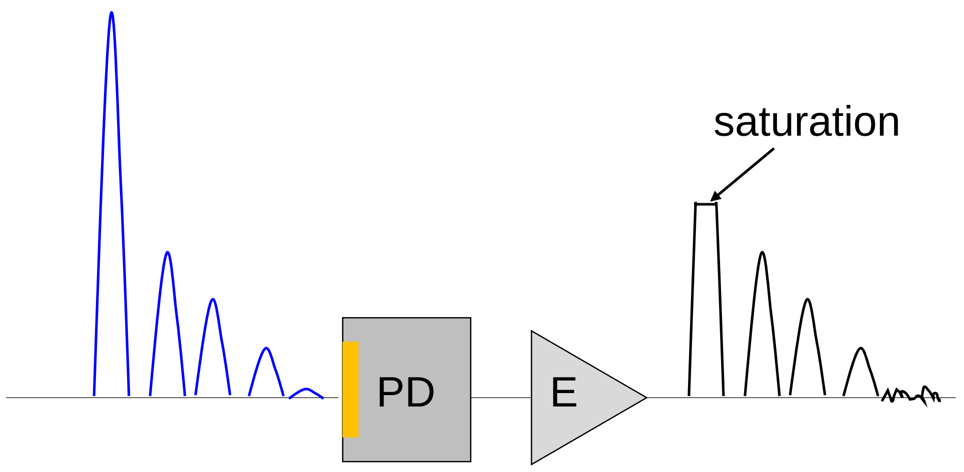
100 kHz bandwidth



10 MHz bandwidth



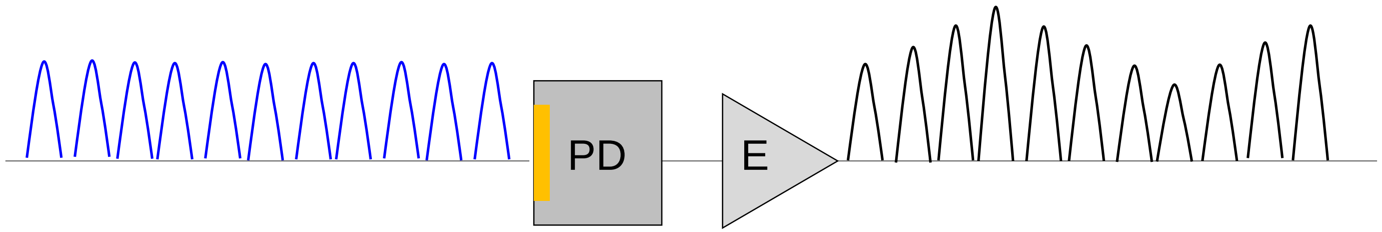
Detection Characteristics: Linearity and Dynamic Range



Is the output proportional to the input?

Linearity and dynamic range depend on the properties of the photodetector and detection electronics

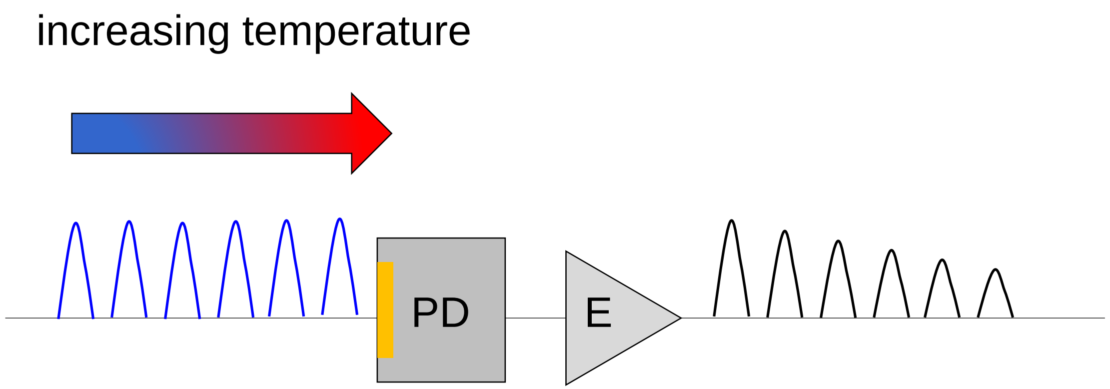
Detection Characteristics: Stability



Does the output vary for a constant input?

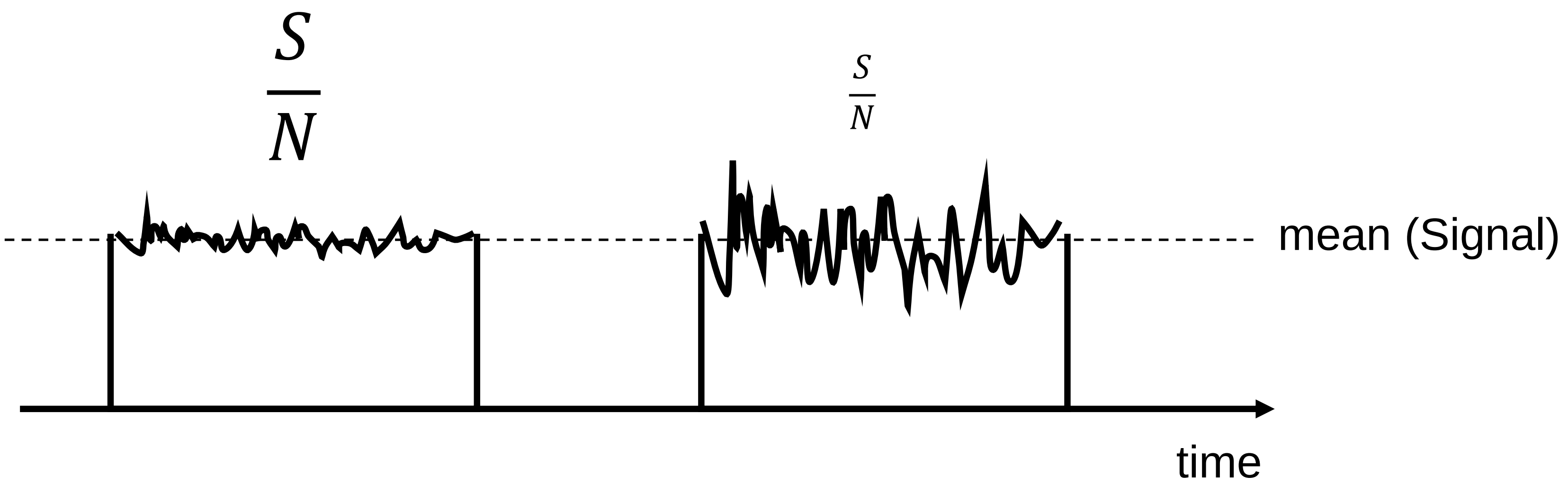
There are numerous factors that can affect stability (at different time scales):
gain variation, temperature drift, 1/f noise,…

Detection Characteristics: Temperature Drift



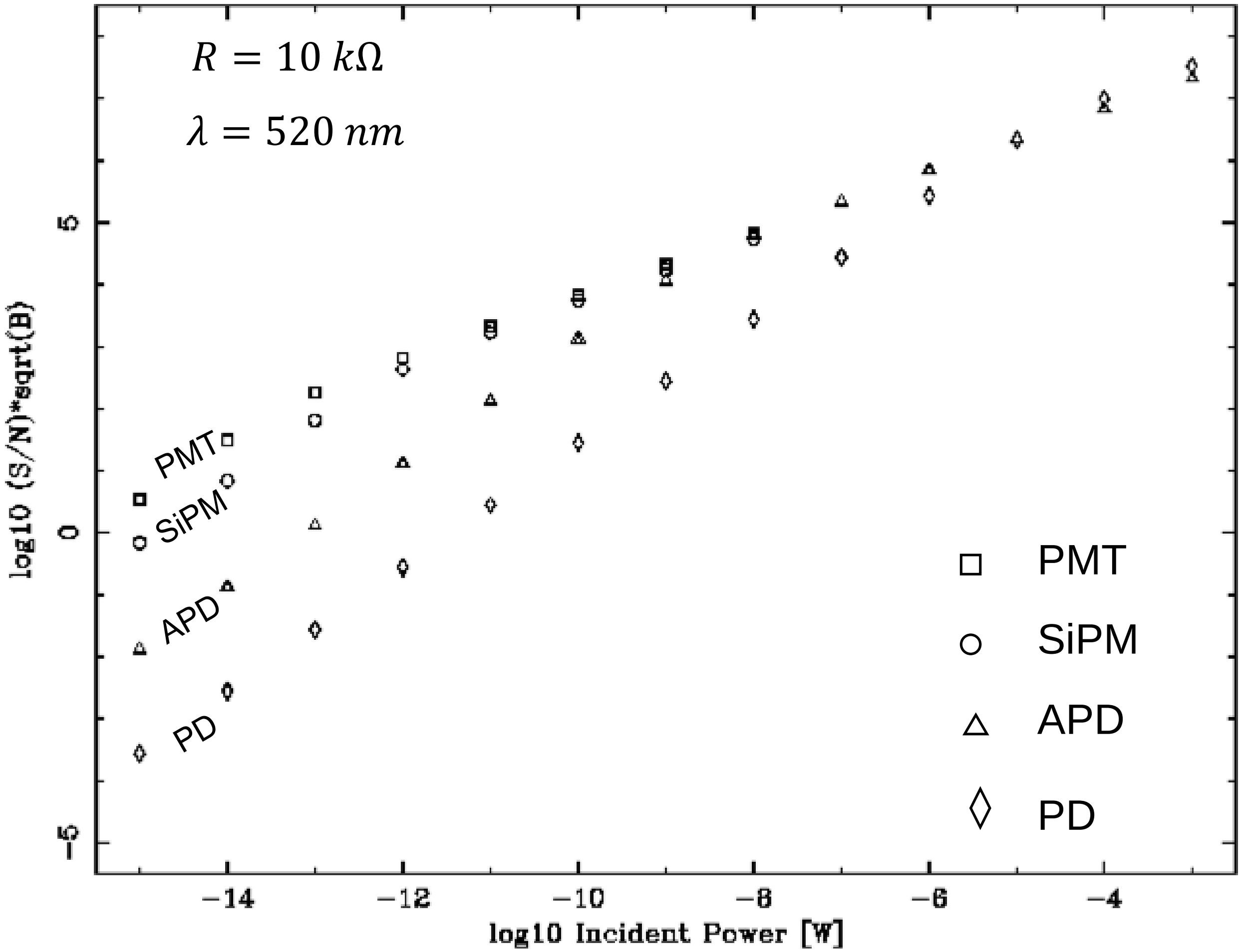
1. If uncompensated, temperature drift will cause gain change of the photodetector. This effect is very strong in APDs and SiPMs
2. Temperature drift also affects noise characteristics

Signal-to-noise ratio



1. $S/_N$ must be greater than 1 for detection to contain useful information
2. $S/_N$ depends on many factors such as incident light power, photodetector’s sensitivity, detection bandwidth, type of frontend electronics, and more…

Signal-to-Noise Ratio



How are Scattered Plots Affected

How are Scatter Plots Affected?

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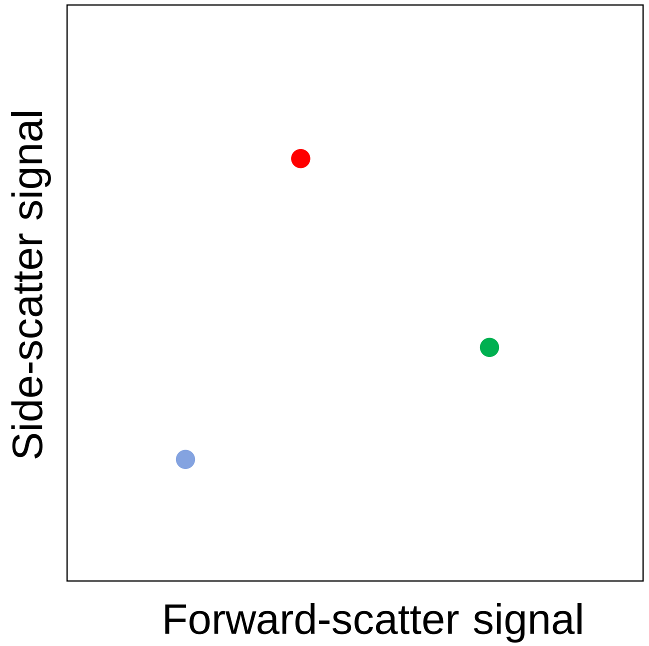
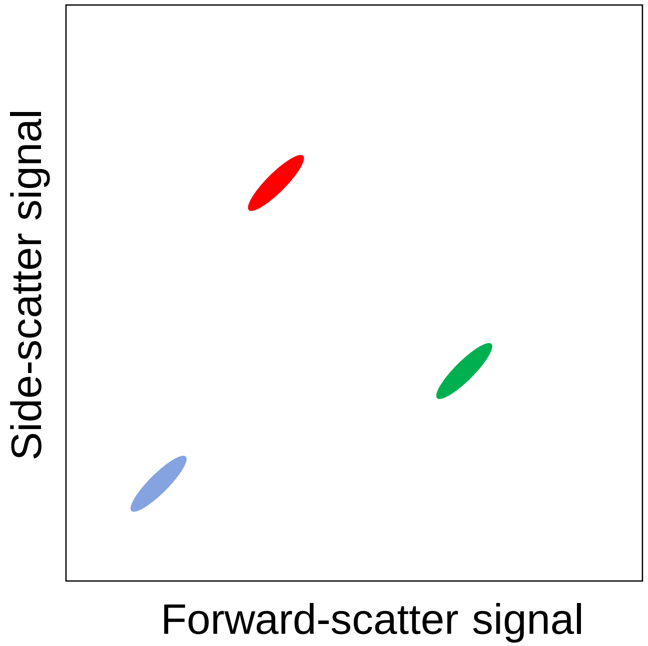
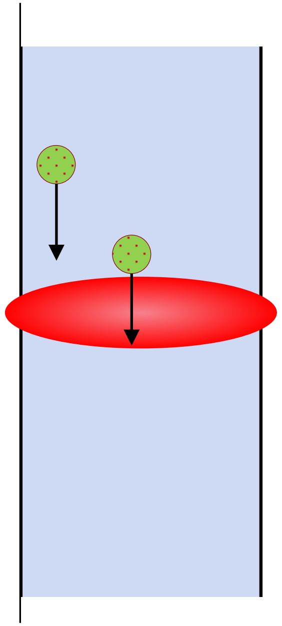
Reference (Ideal)



Power variation due to flow





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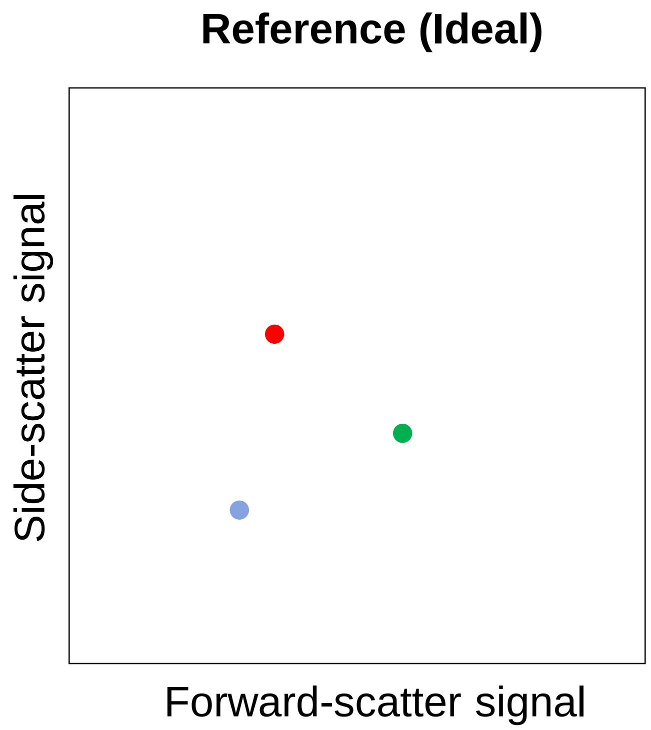

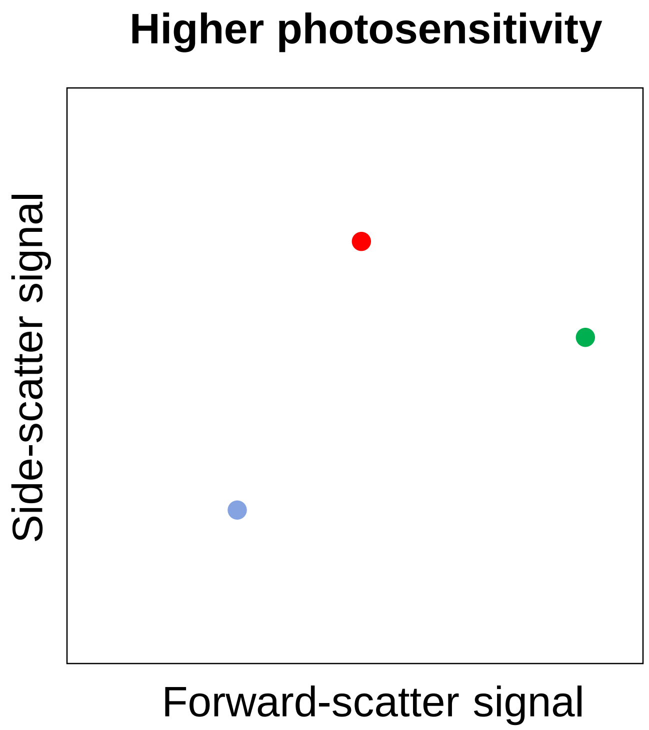

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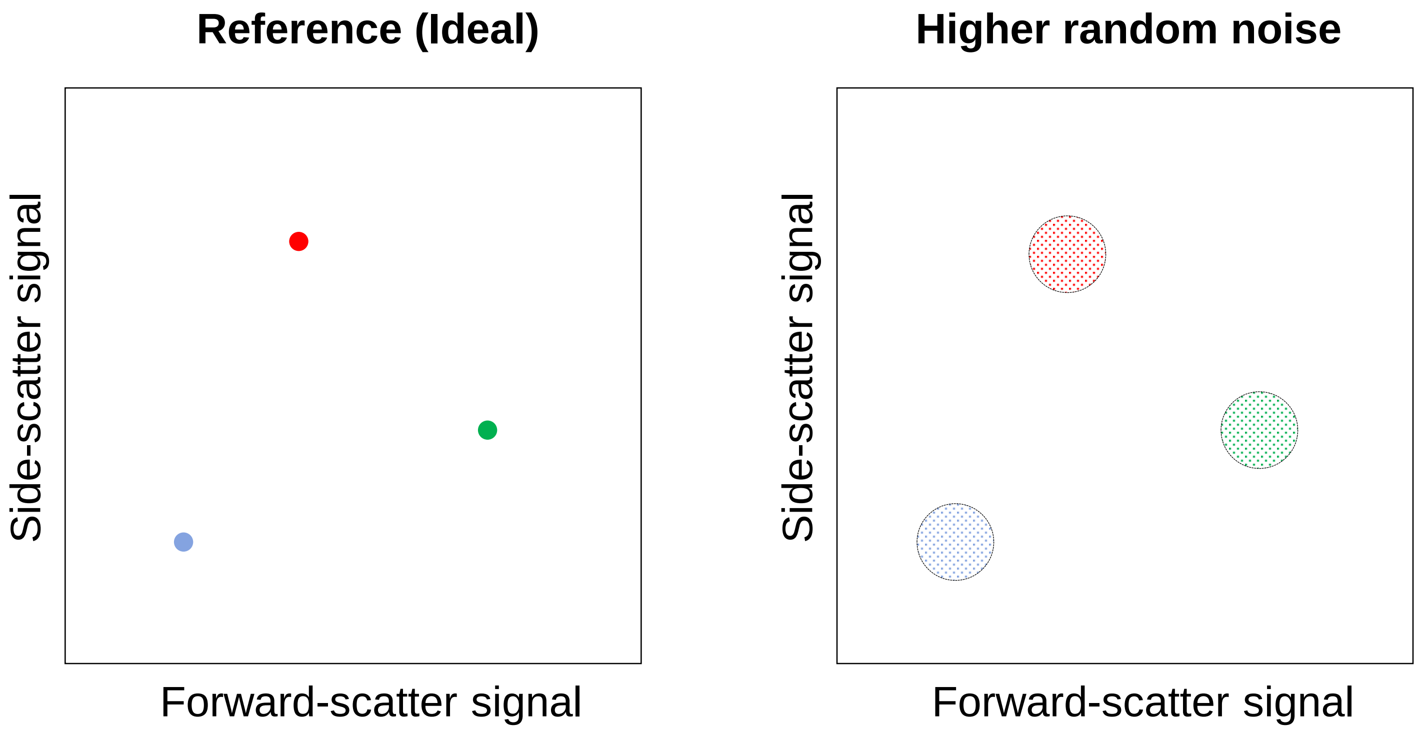

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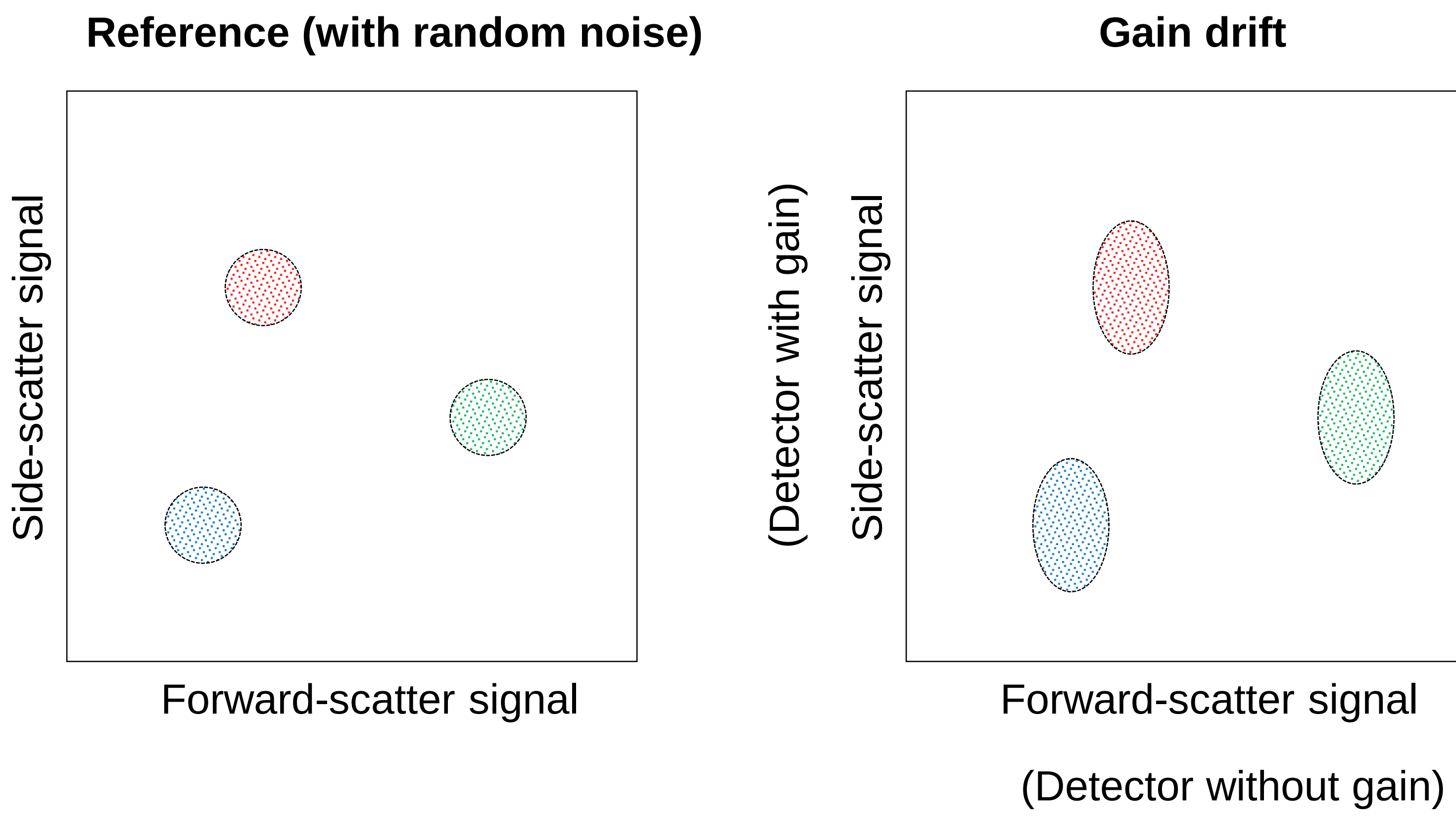

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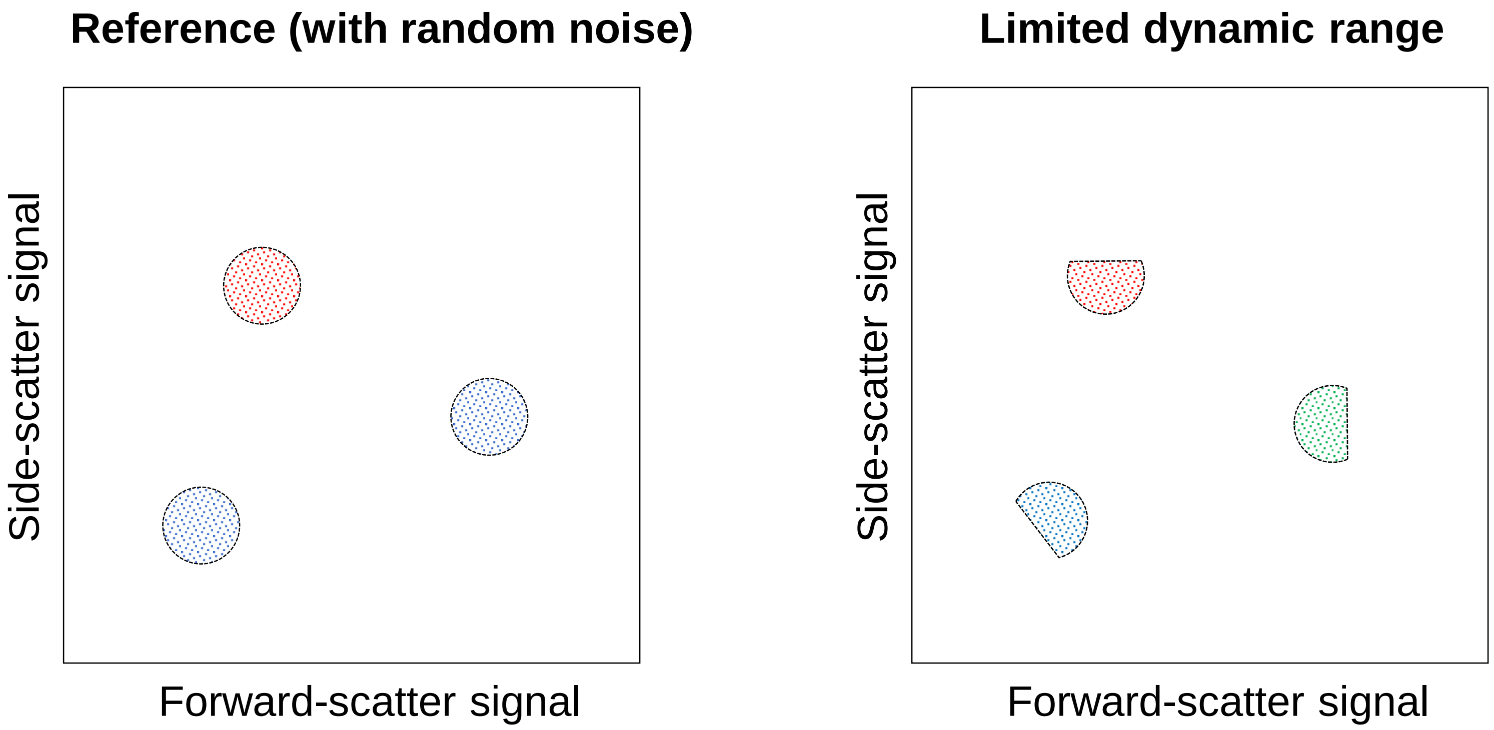

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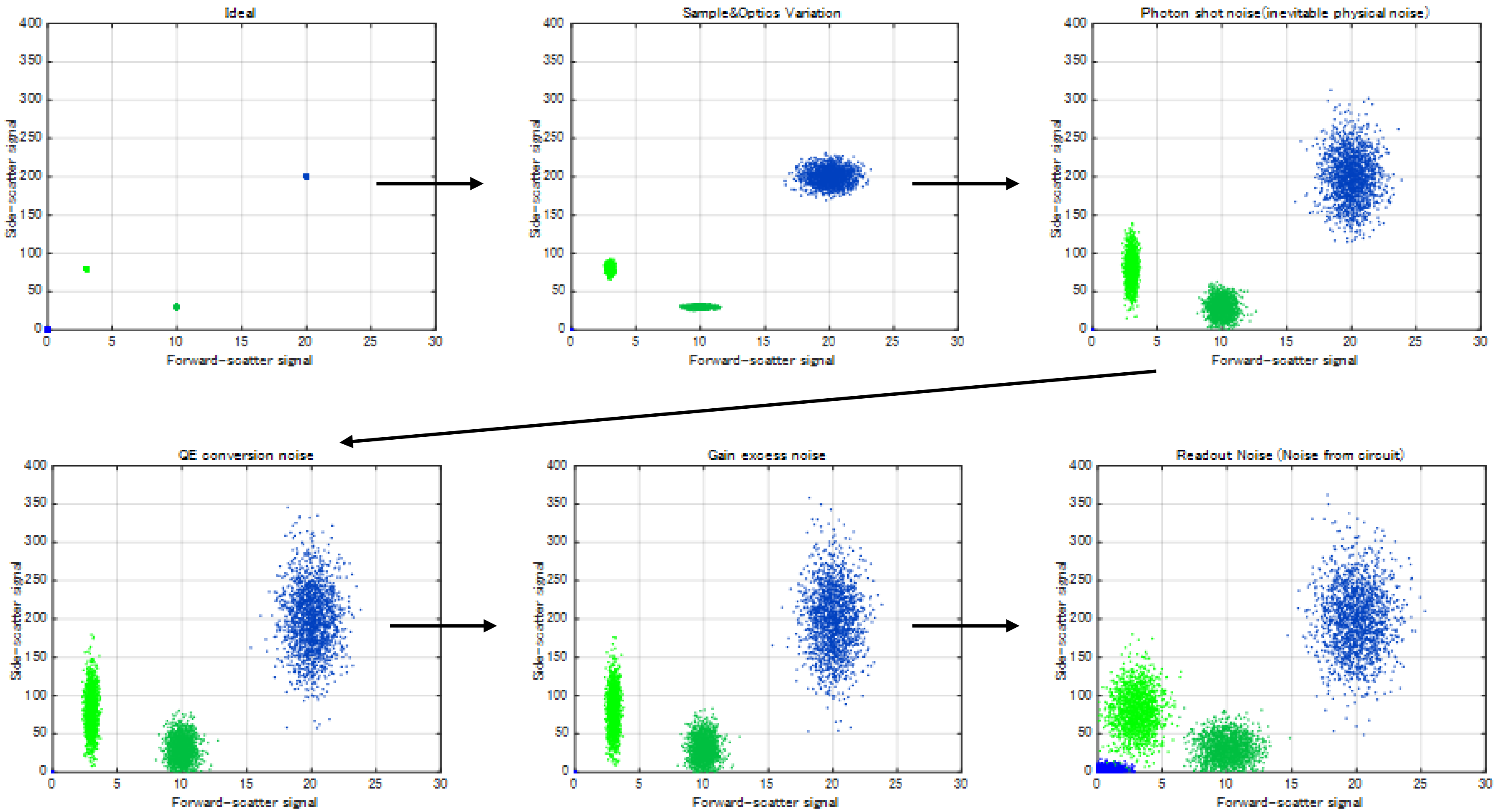

Hamamatsu in flow cytometry

- ✓ Manufactures all types of photodetectors used in flow cytometry
- ✓ Designs and manufactures customized detection circuits and ASICs
- ✓ Manufactures a variety of optical components for flow cytometry
- ✓ Conducts R&D to quickly respond to the changing needs in flow cytometry technology

Hamamatsu in flow cytometry

- ✓ Manufactures all types of photodetectors (APD, SiPM, SPAD, Cameras and PMT) used in flow cytometry
- ✓ Custom integrated optical assemblies from front-end electronics to complete ASICs
- ✓ Manufactures a variety of optical components for flow cytometry
- ✓ Work with customers on custom solutions to quickly respond to the changing needs in flow cytometry technology

Because of our wide offering of optical components, Hamamatsu is unbiased when recommending the correct detector depending on the specific customer's requirements.

Closing Remarks

1. Flow cytometry is a versatile technique to study cells and microparticles
2. Photodetector is an indispensable component of every flow cytometer
3. The choice of the photodetector should be based on the best $\frac{S}{N}$ performance of the detection system
4. Limitations of the detection system will affect scatter plots and histograms masking or distorting science

Thank you

Thank you for listening

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piatek@njit.edu

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2	Emerging Applications - LiDAR & Flow Cytometry	2	2-Jun-20	4-Jun-20
3	Understanding Spectrometer	2	9-Jun-20	11-Jun-20
1 Weeks Break				
4	Specialty Products – Introduction to Light Sources & X-Ray	2	23-Jun-20	25-Jun-20
5	Introduction to Image Sensors	2	30-Jun-20	02-Jul-20
1 Weeks Break				
6	Specialty Products – Laser Driven Light Sources	2	14-Jul-20	16-Jul-20
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8	Mid-Infrared (MIR) Technologies & Applications	2	28-Jul-20	30-Jul-20
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