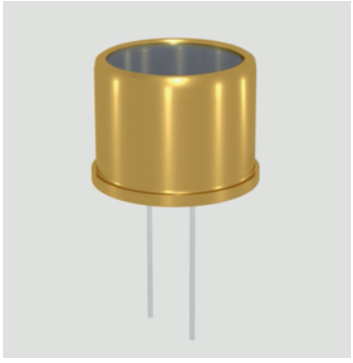
# Gain Independent Compensation

**Two types of photon detectors are used to amplify and then convert light into electronic signals in flow cytometry. The traditional detector is the photomultiplier tube (PMT). Avalanche Photodiode (APD) are another type of photon detector.**

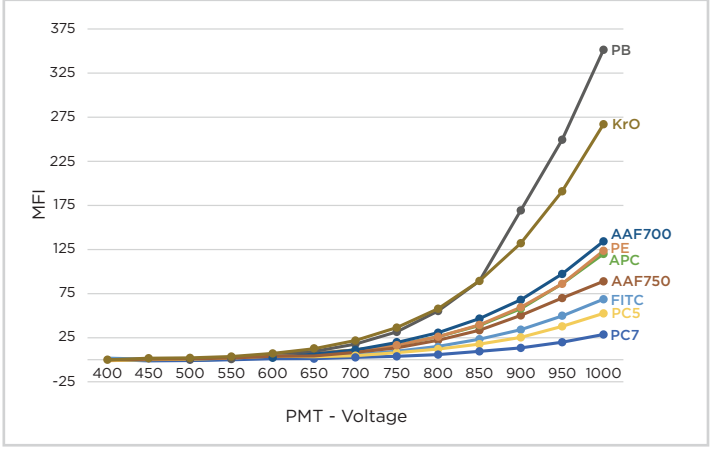
**A voltage is applied that accelerates the electron through the detector to amplify the signal. Increasing the voltage increases the energy of the electrons and thereby amplifies the signal. A measure of the amplification is a unitless quantity, called the Gain.**

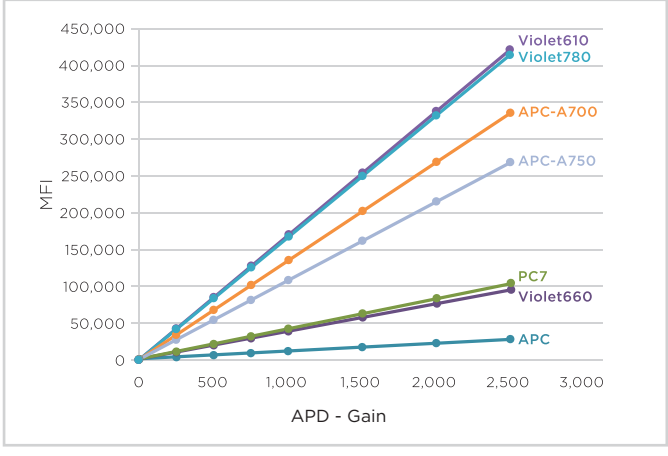
Photomultiplier Tube (PMT)



Avalanche Photodiode (APD)

## Signal Intensity versus Gain: Photomultiplier Tube Versus Avalanche Photodiode

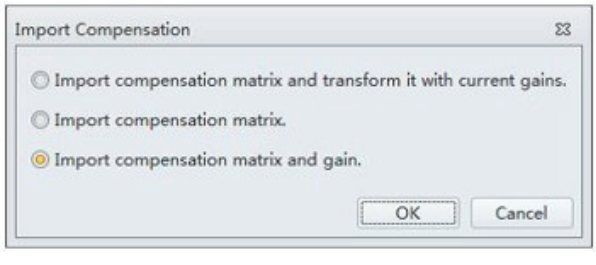



In a PMT, the gain isn't linear. For example, if the voltage is increased by a factor of 2, the resulting signal, or MFI, does not double. Non-linear detection means that measurements taken at different voltages cannot be compared. Consequently, compensation needs to be empirically measured at every setting for each experiment.

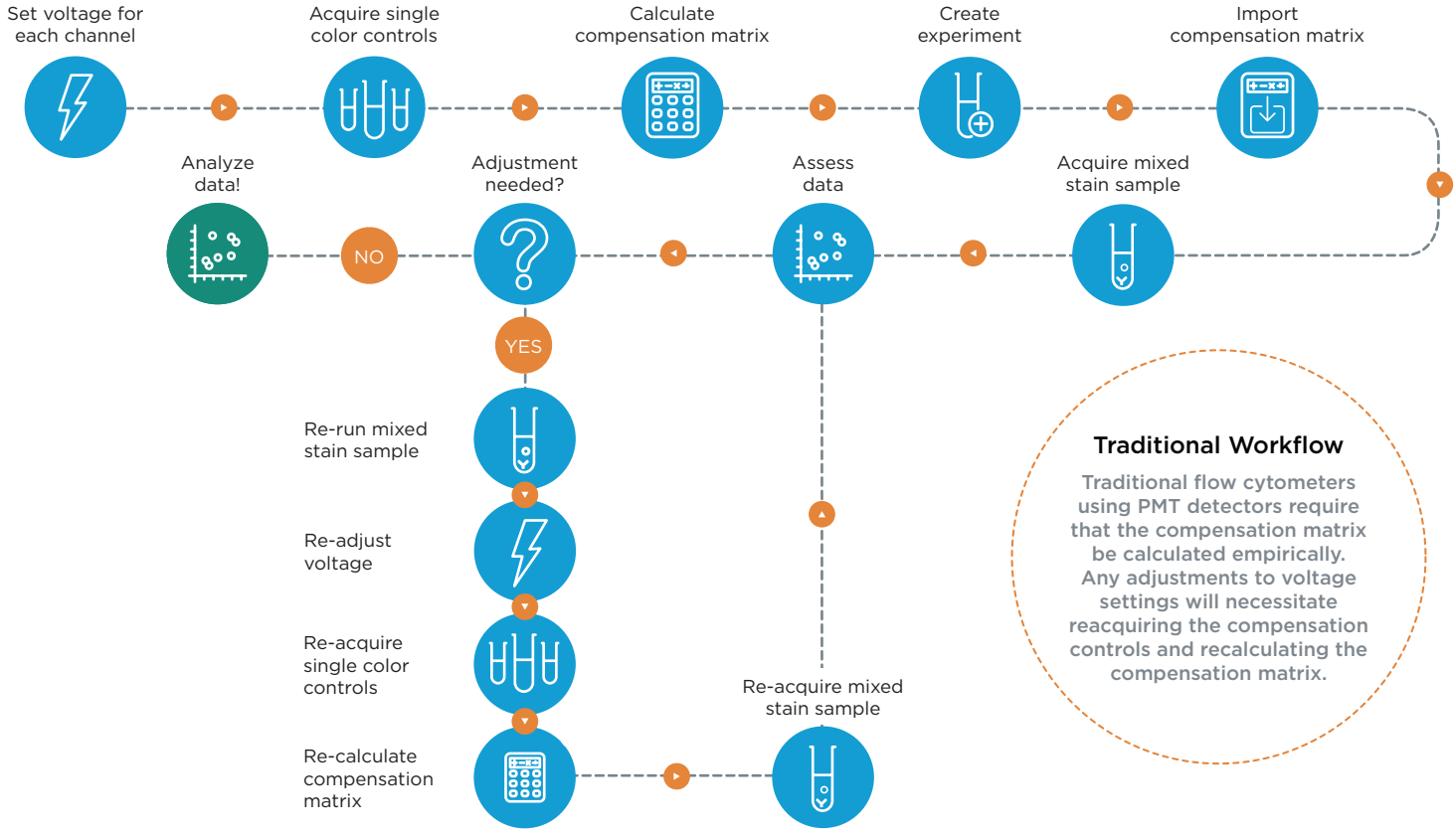
Due to the reproducible semiconductor manufacturing process the gains in an APD can be calibrated for a linear response. Measured intensities are linear to the detector gain setting. This linearity means that a compensation matrix obtained at one gain setting can be used for experiments at different gain settings.

## CytExpert for CytoFLEX Acquisition and Analysis Software Compensation Import




1. Use Suggested Gain settings to create compensation. Use beads to acquire the single color stains. Save to the Compensation Library or export to a Comp file.
2. Import the compensation and gains into the New Experiment.
3. Then adjust gains on the sample if needed, compensation will automatically adjust.

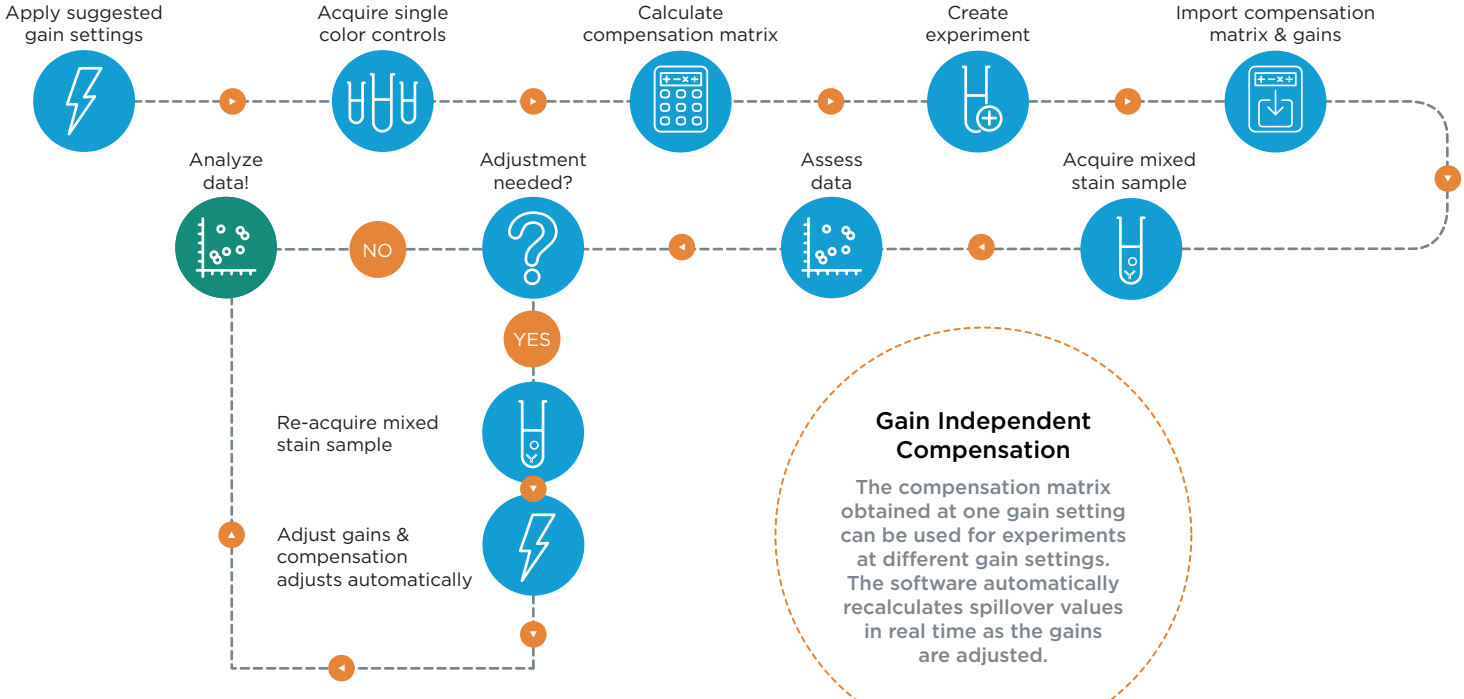
# Experimental Workflow




### Traditional Workflow

Traditional flow cytometers using PMT detectors require that the compensation matrix be calculated empirically. Any adjustments to voltage settings will necessitate reacquiring the compensation controls and recalculating the compensation matrix.

# CytoFLEX Workflow




### Gain Independent Compensation

The compensation matrix obtained at one gain setting can be used for experiments at different gain settings. The software automatically recalculates spillover values in real time as the gains are adjusted.

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