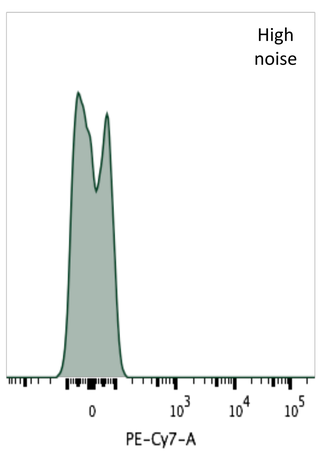
A Simple Method for Characterizing Cytometer Noise

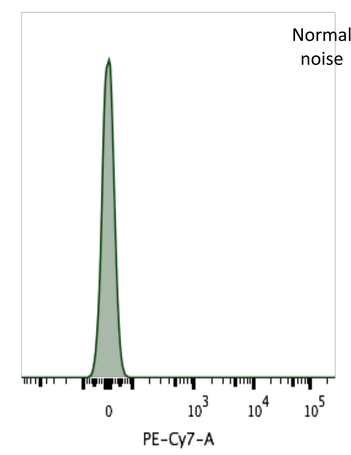
Raymond Lannigan, Eric Chase, Lisa Nichols
Cytek Development Inc., Fremont CA, USA

Background:
Noise level is an important parameter in determining efficacy in cytometer performance. Cytometer noise has both electronic and optical contributions, which together can decrease a cytometer's low end resolution. The authors developed a simple method using common blank/DIM polystyrene beads. Utilizing this four step method, one is able to visualize and identify the noise for further corrective action. This procedure is used in conjunction with other QC methods.

Step 1- Electronic Noise



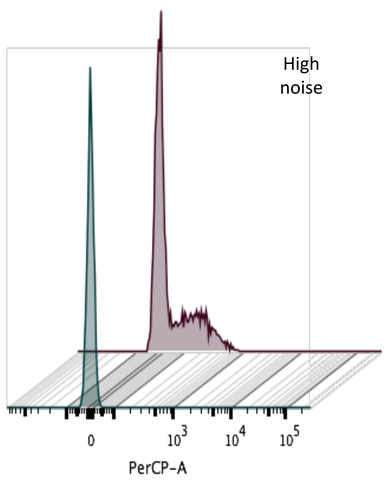
Subset Name	SampleID	Robust SD : APC-Cy7-A
CST Dim	Electronic Noise	56.3



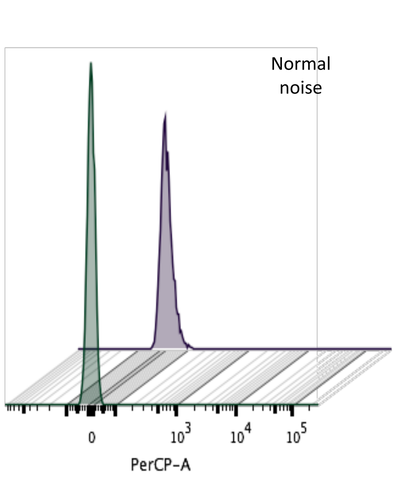
Subset Name	SampleID	Robust SD : APC-Cy7-A
CST Dim	Electronic Noise	25.8

Step 1 legend: CST dim beads acquired on a BD LSRII before and after repairing the electronics.

Step 2- PMT Noise



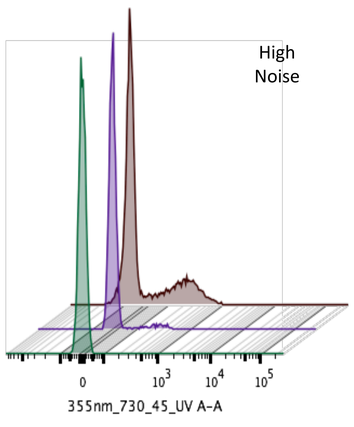
Subset Name	SampleID	Robust SD : PerCP-A
Blanks	Electronic Noise	13.4
Blanks	PMT Noise	99.5



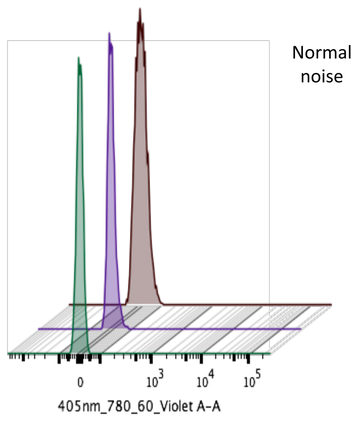
Subset Name	SampleID	Robust SD : PerCP-A
Blanks	Electronic Noise	15.3
Blanks	PMT Noise	23.3

Step 2 legend: Blank beads acquired on a BD LSR Fortessa™ before and after repairing the Per-CP PMT.

Step 3: Optical Background Noise



Subset Name	SAMPLE ID	Robust SD : 355nm_730_45_UV A-A
Blanks	Electronic Noise	16.0
Blanks	PMT Noise	16.5
Ungated	Optical Background	256



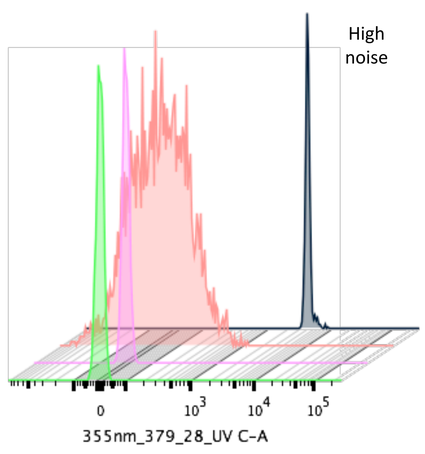
Subset Name	SampleID	Robust SD : 405nm_780_60_Violet A-A
Blanks	Electronic Noise	15.4
Blanks	PMT Noise	16.5
Ungated	Optical Background	28.6

Step 3 legend: Blank beads acquired on a BD LSR Fortessa™. Demonstrates high optical background in a UV fluorescent channel and low optical background in the violet fluorescent channel. Both channels have a long stokes shift.

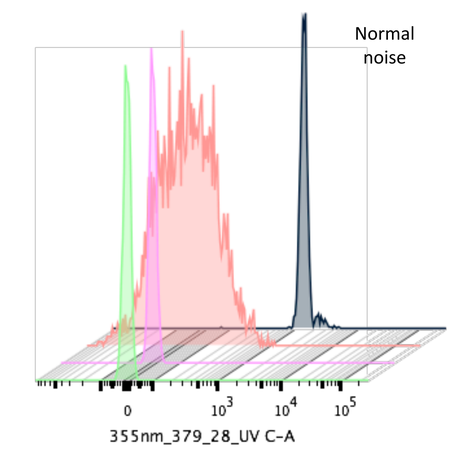
Methods:
To characterize noise level we used a 4-step method with either blank and DIM (CST) beads and used their Robust Standard Deviation (rSD) and/or mean as a measure of the noise contribution to the signal.

- **Step 1**: Trigger the cytometer on Forward Scatter and turn all PMTs to zero volts (ie. Electronic noise)
- **Step 2**: Trigger the cytometer on Forward Scatter, turn the PMTs to operating voltage and block the light path (ie. Electronic + PMT noise)
- **Step 3**: Trigger the cytometer on Forward Scatter noise, keep all PMTs at operating voltage and unblock light paths. (ie. Electronic + PMT noise + optical background noise)
- **Step 4**: Run blank beads with PMT at operating voltage, light paths unblocked. (ie. Electronic + PMT noise + optical background noise + bead scatter). Step 4a troubleshoots laser scatter.

Step 4- Optical Scatter



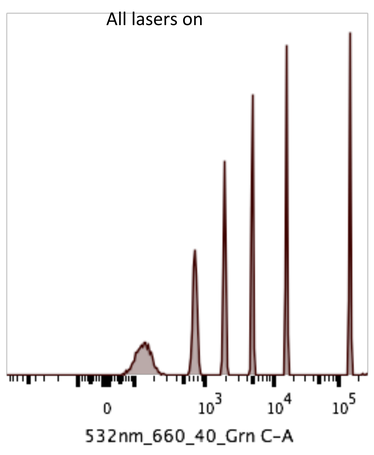
Subset Name	SampleID	Robust SD : 355nm_379_28_UV C-A
Blanks	Electronic Noise	14.1
Blanks	PMT Noise	13.5
Ungated	Optical Background	130
Blanks	Bead Scatter	323



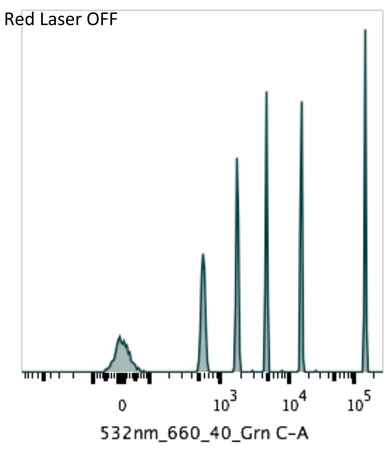
Subset Name	SampleID	Robust SD : 355nm_379_28_UV C-A
Blanks	Electronic Noise	14.1
Blanks	PMT Noise	13.5
Ungated	Optical Background	130
Blanks	Bead Scatter	181

Step 4 legend: Blank beads acquired on a BD LSR Fortessa™ with violet laser on and off.

Step 4a- Optical Scatter(QbSure® beads)



Blanks: **Geometric Mean** : *532nm_660_40_Grn C-A*: 150
Blanks: **Robust SD** : *532nm_660_40_Grn C-A*: 43.0



Blanks: **Geometric Mean** : *532nm_660_40_Grn C-A*: 2.28
Blanks: **Robust SD** : *532nm_660_40_Grn C-A*: 25.7

Step 4a legend: QbSure® beads acquired on a BD LSR Fortessa™ with red laser on and off

Conclusions:
We have developed this as a common troubleshooting practice for diagnosing noise problems in the cytometers we service. If step 1 SD is >30, we will troubleshoot in the electronics area of the cytometer. If Step 1 SD is <30 and step 2 SD is >50, then we troubleshoot in the PMT area. If Step 1 and Step 2 are normal, and step 3 SD is >150 then we troubleshoot the light path, and flow cell area. It has been our experience that step 4 noise is unlikely to be high without one of the previous steps being high. Step 4 is the closest condition to running cells and can be useful in determining laser scatter noise. This scatter noise raises the resolution limit and decreases the dynamic range.

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