Extended Dynamic Range for flash light sources

Introduction

Certain instruments, such as Cytation 5 and Neo2, when paired with Gen5 v2.07 or higher, now have an extended dynamic range in fluorescence mode. This allows automatic adjustment of the detector's gain to match sample signal intensity. The result is a mode that does not require manual gain adjustment and provides results over a very broad range of signal intensity, on a scale of 0 to 10,000,000 RFU. In each read mode, the final sample result corresponds to the result that would have been obtained at the highest PMT gain.

Another version of extended dynamic range for continuous light sources has long been available, e.g. tungsten and luminescence, that is on a scale of 0 to 5,800,000 units. Note that extended dynamic range is not available for spectral scans.

Reader Factory Calibration

To support the extended range mode, each reader is calibrated at the factory. The calibration procedure uses ultra-stable light sources to calibrate three PMT gains (low, medium, high). The high PMT gain is fixed for all readers, and the medium and low PMT gains are automatically calculated based on measurement of the stable light sources.

Multiplication factors are then applied to correct the data measured at the two lower gains, giving an equivalent high-gain result. Because different optical systems and detection modes do not have equivalent light throughput, there are three sets of calibrated numbers:

- one for the monochromator optics (all read modes)
- one for the filter optics in fluorescence intensity (FI) and fluorescence polarization (FP) modes
- one for the filter optics in time resolved fluorescence mode (TRF)

The calibration data is saved onboard the instrument. **Table 1 shows the high PMT data (highlighted in gray / italic font) is the same for all compatible units.** The medium and low PMT data (highlighted in blue) is calibrated for each instrument at the factory. The numbers shown are examples from an actual instrument.

	PMT Low Gain	PMT Medium Gain	*PMT High Gain*
	Instrument specific Calibrated at BioTek	Instrument specific Calibrated at BioTek	*Same for all units*
Monochromator	Gain = 47 Factor = 251.1	Gain = 68 Factor = 16.2	*Gain = 100* *Factor = 1*
Filter – FI and FP	Gain = 42 Factor =254.4	Gain = 61 Factor =16.0	*Gain = 90* *Factor =1*
Filter - TRF	Gain = 68 Factor = 289.0	Gain = 100 Factor =17.2	*Gain = 145* *Factor =1*

Table 1: *Example of instrument calibration data.*

Example of Read Process

In this example, we consider a reading procedure using the monochromator in fluorescence intensity mode. The PMT gain numbers and multiplication factor used in this example correspond to this read mode in Table 1. The same process applies to the filter system; the only differences are the gain and multiplication factors used.

- The three tables below show how low, medium and high samples are processed. Numbers in **bold underlined** in tables 2, 3 and 4 come from, or are derived from the calibration table (Table 1)
- Table 2: two high wells are read at a low PMT setting. A well is considered high if the initial reading at the medium PMT gain is above 10,000 RFU
- Table 3: two low wells are read at a high PMT gains setting. A well is considered low if the initial reading at the medium PMT gain setting is below 10,000 RFU / Medium PMT factor (**16.2** in this example); 10,000/16.2 = **617** RFU.
- Table 4: one medium well read at a medium PMT setting. This well is considered medium because the initial reading at the medium PMT setting falls between the two above criteria.
- In each case, the final well result is the equivalent data you would get at the highest PMT gain. In this specific example, the data is equivalent to the entire microplate being read at a PMT gain of 100.

Step#	Description	Purpose	A1 signal (very high)	A2 signal (high)
1	1 flash – PMT gain = **68**	Evaluate sample signal	Over-range	25,261 RFU
2	Compare data to criteria	Decide sample category	RFU >10,000	RFU >10,000
3	Set PMT gain	Read sample with proper gain	**47** (low)	**47** (low)
4	Read	Collect sample data	12,421 RFU	1,638 RFU
5	Apply factor	Correct sample data	x **251.1**	x **251.1**
6	Final data	Report data	3,118,913 RFU	411,301 RFU

Table 2: *Example high signal well processing when the monochromator optical system is used to measure fluorescence intensity.*

Step#	Description	Purpose	A4 (low)	A5 (very low)
1	1 flash – PMT gain = **68**	Evaluate sample signal	502 RFU	0 RFU
2	Compare data to criteria	Decide sample category	RFU<**617**	RFU<**617**
3	Set PMT gain	Read sample with proper gain	**100** (high)	**100** (high)
4	Read	Collect sample data	8,122 RFU	453 RFU
5	Apply factor	Correct sample data	**1**	**1**
6	Final data	Report data	8,122 RFU	453 RFU

Table 3: *Example of low signal well processing when the monochromator optical system is used to measure fluorescence intensity.*

Step#	Description	Purpose	A3 (medium)
1	1 flash – PMT gain = **68**	Evaluate sample signal	4,965 RFU
2	Compare data to criteria	Decide sample category	**617**<RFU <10,000
3	Set PMT gain	Read sample with proper gain	**68** (medium)
4	Read	Collect sample data	4,967 RU
5	Apply factor	Correct sample data	**16.2**
6	Final data	Report data	80,465 RFU

Table 4: *Example of medium signal well processing when the monochromator optical system is used to measure fluorescence intensity.*

Final Microplate Data Reported to the User

In each case, the final well result is the equivalent data that would have been obtained at the highest PMT gain. In this specific example, the data is equivalent to the entire microplate being read at a PMT gain of 100.

	1	2	3	4	5
A	3,118,913	411,301	80,465	8,122	453