



Why Is An Important Feature of a Fluorescence Microplate Reader?

What is dynamic range?

Dynamic range of a fluorescence instrument indicates the range of fluorescence intensity that is detectable by the detector been used. It is determined by the minimum and maximum detection limits of an instrument. For example, if a fluorescence instrument can detect signal between 100~100,000 counts (means it can not detect signal weaker than 100 counts, or higher than 100,000 counts), then the dynamic range of this fluorescence instrument is $100,000 / 100 = 4$ orders of magnitude (4 decades).

What determines the dynamic range?

Dynamic range of a fluorescence plate reader is determined by the choice of the detector and its operating conditions. The majority of fluorescence microplate readers use photomultiplier tube (PMT) as the detector, operating in the photon counting mode. Most of them use analog detection with 12 or 16 bit A/D converters and thus have very narrow dynamic range, typically 4 to 5 orders of magnitude. The high level of dark count, which is measured without excitation light, further lowers the usable dynamic range of these microplate readers to below 3 to 4 orders of magnitude.

What is the dynamic range of FluoDIA T70?

The FluoDIA T70 uses a photomultiplier tube as its detector, operating in the photon counting mode. Designed with the highest sensitivity and widest dynamic range in mind, FluoDIA T70 can count up to 16,000,000 photons/counts per second. It is the only plate reader currently available that can detect signals of 7 orders of magnitude. With a background count of less than 10 counts per second, FluoDIA T70 has a usable dynamic range of over 6 orders of magnitude.

Why didn't dynamic range gain as much attention as sensitivity?

Since a fluorescence measurement usually requires detection of a very low signal, sensitivity has been given the most priority in determining whether an instrument is superior to the others. Also, since traditional fluorescence detection measures only one or two samples at a time, it is relatively easy to adjust the volume of a sample so that its signal will fall into the required detection range of a particular instrument.

Why is dynamic range very important for a plater reader?

Fluorescence plate readers are designed for measuring tens to thousands of samples at one measurement. Among these many samples, some may have very weak signals; others may have very strong signals. The researchers normally do not know which signal is strong and which one is weak before the measurement. A wide dynamic range is the only way to ensure all samples will be properly measured under the same condition, at the same time, without further unwanted intervention.

Why does the superior dynamic range of the FluoDia T70 benefit the customer?

The FluoDia T70 allows researchers to screen hundreds to thousands of samples in a more accurate, cost-effective and labor-effective way. For example, a FRETbased assay, with very high positive control samples and very low negative control samples, as shown below, can be measured easily by FluoDia T70.

	1	2	3	4	5	6	7	8	9	10	11	12
A	1307	1353	1351	1415	207633	205665	213669	213813	321816	342309	340780	346943
B	39854	43068	42679	43561	376868	383949	388818	391907	83806	85701	84037	84168
C	423784	416485	429870	433983	123534	123417	126323	124026	475405	486168	478255	514543
D	152831	153205	149728	159637	523862	521169	521430	521268	235831	238398	236649	227584
E	567108	572461	571902	585111	328056	323006	337417	351189	555397	536083	567301	587206
F	277643	275838	286024	285020	601830	585967	616517	622041	402356	432157	414008	433686
G	578987	611468	621673	627637	483504	456800	479885	498480	609028	610949	588437	616158
H	483907	507926	513158	517847	9560754	9568734	9754994	9721788	8635459	8763264	850	864

In this 96-well microplate, blank control samples (wells H11-12, in gray color) have readings below 103 counts, negative control samples (wells A1-4, in blue color) have readings below 104 counts, positive control samples (Wells H5-10, in red color) have readings above 107 counts, and the testing samples (in pink and green) has readings between 104(green) to 106 (pink) counts. *With no further adjustment of samples, only FluoDia T70 plate reader can detect all these samples.* If a researcher uses a plate reader with a dynamic range of 5 decade, he can only read those wells that have less than 105 counts. If a researcher uses a plate reader with a dynamic range of 6 decades, of which there are very few, he can also collect data from wells that are between 105 ~106 counts, but not from positive control wells (> 107 counts). In order to accurately measure all the samples at the same time, they have to repeat the measurement after specific cells that have exceeded the dynamic range are adjusted to fit within the dynamic range of the platereader. This adjustment is both cost and labor intensive and it further introduces artifacts into the measurement.

Conclusion: *Dynamic range is a very important specification of any fluorescence microplate reader. With a dynamic range of 7 orders of magnitude, FluoDia T70 is the only way to guarantee accurate, cost-effective and labor effective measurement of tens to thousands of fluorescence samples.*

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