

# Automatic Dynamic Range Selection – Simplified Assay Setup with the Thermo Scientific Varioskan LUX Multimode Reader

## Key Words

- **Dynamic range** – The breadth of signal range an instrument can read, from the minimum to the maximum detectable.
- **Sensitivity** – The lowest concentration the instrument can separate reliably from the background.
- **Photomultiplier tube (PMT)** – The light detector used in many microplate readers.

## Goal

This technical note describes how the automatic dynamic range selection feature of the Thermo Scientific™ Varioskan™ LUX multimode reader can ease your workflow and help you achieve optimal measurement results in microplate assays based on measuring fluorescence or luminescence.

## Introduction

Most modern microplate readers use a Xenon flash lamp as their light source for wider wavelength range and improved sensitivity over the traditional halogen lamp. However, Xenon lamps reduce dynamic range, requiring PMT gain voltage adjustments to achieve a wider range.

Adjusting the PMT gain on microplate readers is a commonly performed procedure to achieve the optimal sample signals for fluorescence and luminescence assays. This manual trial and error process is somewhat laborious and can result in loss of sensitivity or over-saturation of signals. Some microplate readers employ an “auto” adjustment where one PMT gain voltage is selected for the entire plate based on a designated high or low signal sample. This also can be an issue as one adjustment is not usually appropriate for the full range of samples on the microplate.

The automatic dynamic range selection feature in the Varioskan LUX multimode reader is designed to ensure the instrument detects samples reliably at a wide signal and concentration range. The instrument automatically adjusts the measurement gain for every sample on the microplate individually. Gone are the days when the scientist needed to perform test runs to optimize measurement gain settings separately for each assay and plate. This innovative feature allows the Varioskan LUX multimode reader to provide high sensitivity and a wide dynamic range for every run without manual adjustments. It allows the user to measure low and high signal samples reliably within the same assay and helps him/her get consistent results day to day.

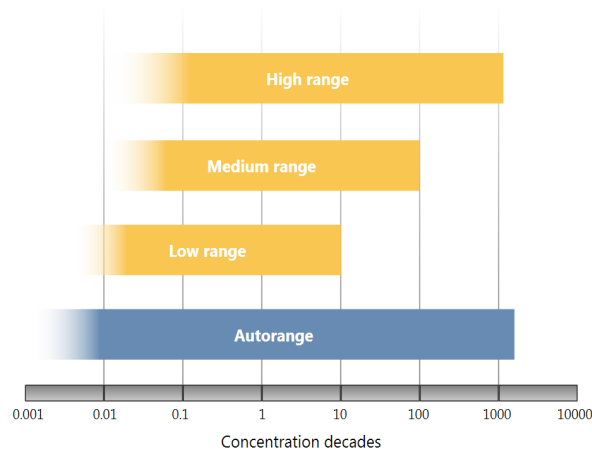


Figure 1. The automatic dynamic range selection helps achieve optimal measurement readings every time, even if the assay includes samples with extremely high and low signals.

## How it works

The Varioskan LUX multimode reader is designed to select the optimal measurement settings automatically during a run. In order to determine the optimal PMT gain voltage for a sample, a flash test measurement is performed. Based on the emission signal level from this trial flash measurement, the instrument selects the optimal gain voltage and performs the actual measurement. The cycle is repeated when the next sample is measured and a new gain is selected for that well. This sequence continues adjusting the gain for each sample until the entire plate is read. The test measurement occurs very quickly, so the overall read time for the plate is still very fast – a 96-well plate can be read in as little as 15 seconds.

Traditionally, when one adjusts the gain, the shift in signal makes it difficult to compare results from one setting to the next. The Varioskan LUX instrument overcomes this obstacle by incorporating a PMT gain calibration, making it possible to use several different PMT gain voltages to measure a set of samples. The system utilizes signals of varying intensity from internal references that cover the whole dynamic range. Based on this calibration data, the instrument forms a conversion table where relative differences between the PMT gain voltages are normalized, and defines light intensity levels where each PMT gain voltage should be used. The effect of this PMT gain calibration is shown in Figures 2 and 3.

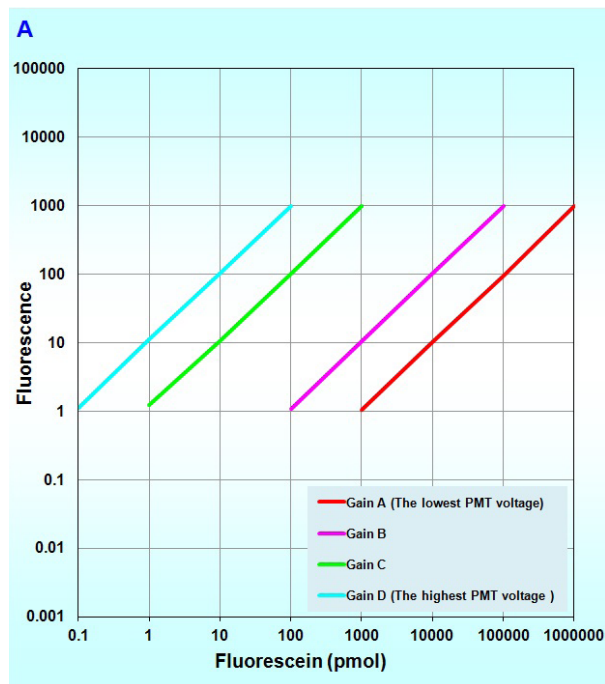


Figure 2. Curves representing data before PMT gain calibration.

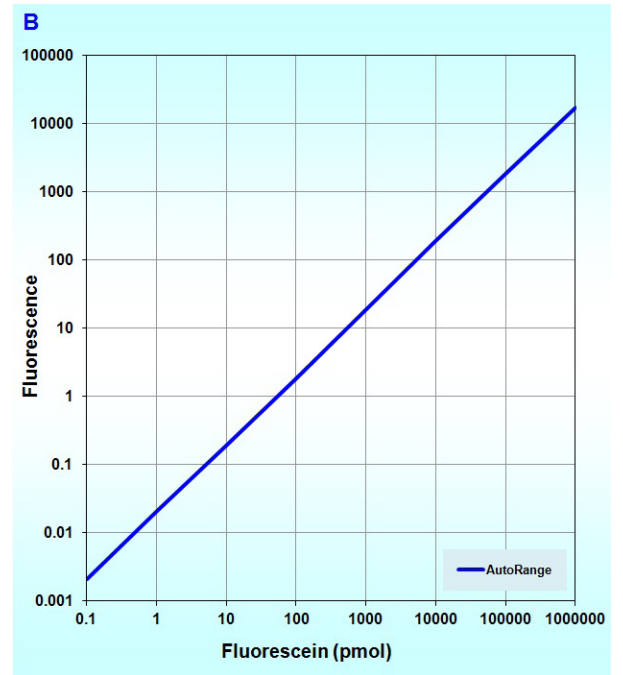


Figure 3. When several PMT gains are calibrated against each other a continuous linear dynamic range over seven orders of magnitude can be obtained. Each gain is responsible for measuring a certain area of the dynamic range.

Figure 4 illustrates the benefits of automatic dynamic range selection compared to a corresponding manual selection, which was performed on a microplate reader from another manufacturer. With a microplate reader employing a manual measurement gain setting, the result is always a compromise between high sensitivity and wide dynamic range. If the measurement gain is manually optimized for high sensitivity for detecting low concentrations, the dynamic range becomes narrow, thus losing the ability to detect samples with high concentrations (yellow curve). If the measurement gain is manually optimized for detecting samples at a wider concentration range, the sensitivity suffers, making it impossible to detect low concentration samples (red curve). The Varioskan LUX automatic dynamic range selection does not compromise. The instrument is able to detect signals reliably at a wide concentration range, offering both high sensitivity and wide dynamic range automatically without manual optimization (blue curve).

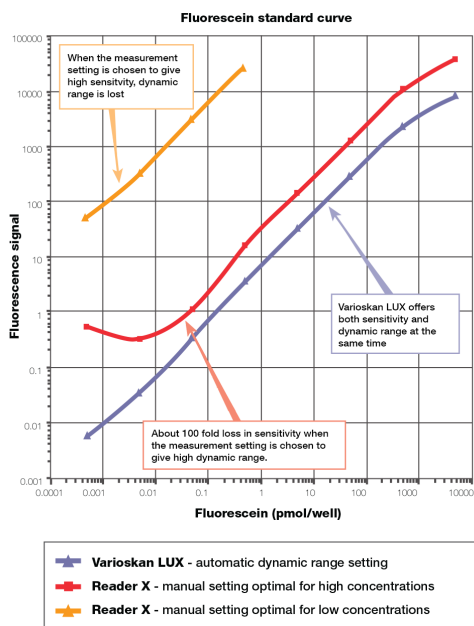


Figure 4. Example of dynamic range and sensitivity achieved in a fluorometric measurement of a fluorescein concentration series performed by Varioskan LUX reader (blue curve) and a microplate reader from another manufacturer (yellow and red curves).

Without the automatic dynamic range selection, the measurement setup of a fluorometric or luminometric microplate assay is often complicated and time-consuming, as the PMT gain has to be optimized separately for each plate. In addition, without the automatic PMT gain calibration, the results from assay to assay and from day to day are not necessarily comparable.

## Conclusions

The automatic dynamic range selection feature of Varioskan LUX multimode reader provides a range of benefits, including:

- Eases measurement setup
- Eliminates need for repeat test runs for gain optimization
- Helps save precious samples (not wasted on test runs)
- Helps ensure both high sensitivity and wide dynamic range
- Enables detection of both high and low signal samples within an assay
- Helps users get comparable results from assay to assay

The Varioskan LUX microplate reader's automatic dynamic range selection feature simplifies measurement setup in fluorescence and luminescence assays. The sophisticated internal calibration system allows the instrument to automatically adjust its settings for optimal sensitivity while maintaining a wide dynamic range, helping users obtain optimal measurement readings every time.

[www.thermoscientific.com/varioskanlux](http://www.thermoscientific.com/varioskanlux)

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