Optimizing Signal Detection: Exploring the iQue® 5 High-Throughput Screening (HTS) by Cytometry Platform's Gain Adjustment Capabilities

Technical Note

This document offers guidance on using the gain adjustment feature available on the iQue® 5 High-Throughput Screening (HTS) by Cytometry Platform to enhance the detection and measurement of fluorescent signals. The findings emphasize the channel-specific nature of gain adjustment, showcasing its ability to provide precise control over signal amplification, thereby ensuring optimal separation and accurate measurement of fluorescence intensities.

Introduction

Signal adjustment is a frequent challenge in cytometry, especially when striving for improved sensitivity and resolution to analyze cells with low expression levels of target proteins or to bring bright signals within a measurable scale. Flow cytometry is a technique used to analyze cell populations based on physical and fluorescence characteristics. Achieving optimal results requires understanding of fluorochromes, spectral overlap, compensation, and the sensitivity of the instrument. Common challenges include signal loss, fluctuations in fluorescence intensity, high background noise, non-specific cell staining, variability in results from day to day, and incorrect instrument settings. The iQue® High Throughput Screening (HTS) Platform and integrated Forecyt® software offers a wide range of user-friendly experimental design settings, ensuring accurate results. This document provides guidance on the use of gain adjustment, a new feature available on the iQue® 5 Platform.

Gain Adjustment

Flow cytometry empowers the use of multiple dyes within the same experiment while offering a large dynamic range of detection. To achieve the best separation of populations and highest signal to noise ratio, sample preparation and acquisition set-up need optimization. Adjusting reagent concentrations is very effective for staining protocols but not possible for all assay formats. One example is the overexpression of fluorescent proteins that often remains uncontrollable. For those assays, changing the gain as a measure of signal amplification by the detector becomes a powerful tool. It allows the increase of sensitivity to enhance faint signals or decrease the sensitivity to bring bright populations on scale whilst balancing data resolution and noise from the detector. Optimizing the gain enhances signal detection, improves resolution, and boosts the signal-to-noise ratio, especially for low-abundance markers and noisy regions.

This results in more accurate analysis, enabling the identification of rare cell populations and subtle cellular changes. The iQue® 5 Platform is fitted with individual detectors for each channel and therefore supports a greater degree of signal gain control. Each channel is controlled to equivalent or greater levels than observed previously but with a finer degree of control. The gain is adjusted within the iQue Forecyt® software on the protocol tab using a sliding scale between -100% to +100%, with the default being at 0% (Figure 1). This feature provides the user with ultimate flexibility when working with fluorochromes of varying intensities.

Signal adjustment using the iQue® 5 Platform is both straightforward and efficient. To showcase this feature, Jurkat cells were stained with iQue® Cell Proliferation and Encoding Dye V/Blue at various concentrations (0.3-5 mM, 1 in 2 dilution) to provide a range of five distinct intensities. Data was collected across a range of gain settings by adjusting the V445 fluorescence channel using the "Signal Adjustment" box in the iQue Forecyt® software. For this study, gain was adjusted in 20% intervals from -100 to 100% to assess signal sensitivity and is shown as mean fluorescence intensity (MFI, Figure 2).

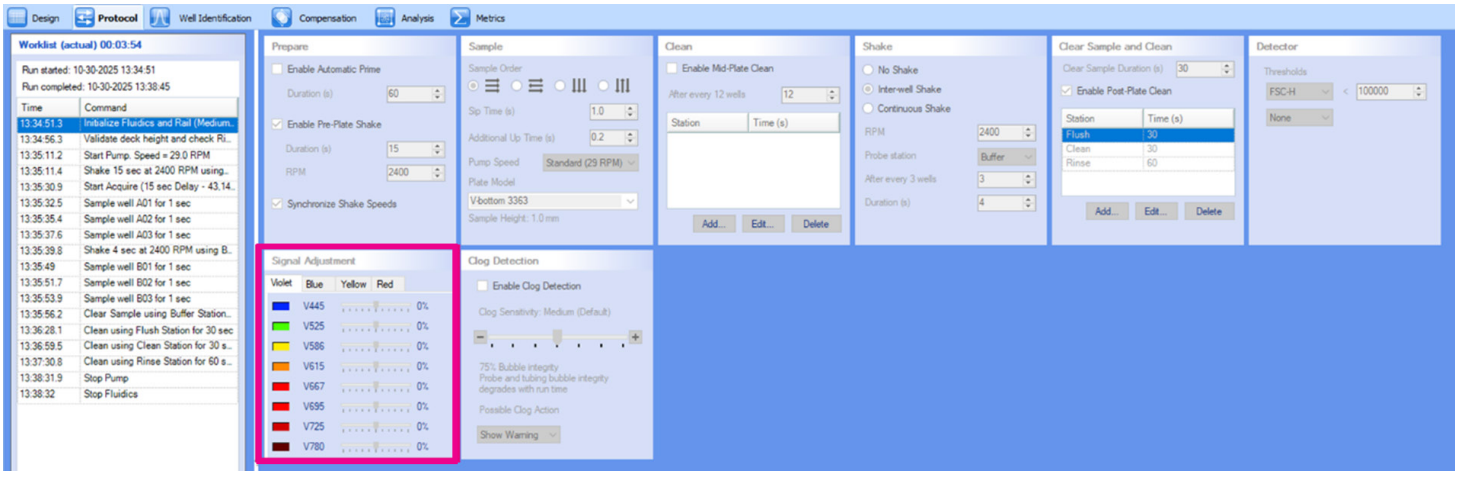

Figure 1: Experiment setup showing the location of the Signal Adjustment window (outlined in magenta box) in the iQue Forecyt® software.

- Figure 2 C and D: – demonstrates data collected at the default setting (0%) where all five intensities can be visualized, although the brightest (Teal) is close to the upper detection limit.
- Figure 2 A and B: -100% signal adjustment has shifted all peaks to the left but there is some merging or lose of definition of dimmer signals.
- Figure 2 E and F: - +100% signal adjustment has sifted peaks to the right resulting in the top concentration fluorescent signal being too high and off the scale for effective measurement.
- Figure 2 G: - illustrates the effect on MFI across the full range of signal adjustment and dye concentrations. The data demonstrates there is more control to decrease a signal (~3 log) than increase (~0.5 log) within the software.
  - Clear to see when signals merge either at the top or bottom end of the scale.
  - Optimal separation was observed with default (0%) setting.
- Figure 2 H: - Overlay of bright population over signal reductions to show left shift in population.

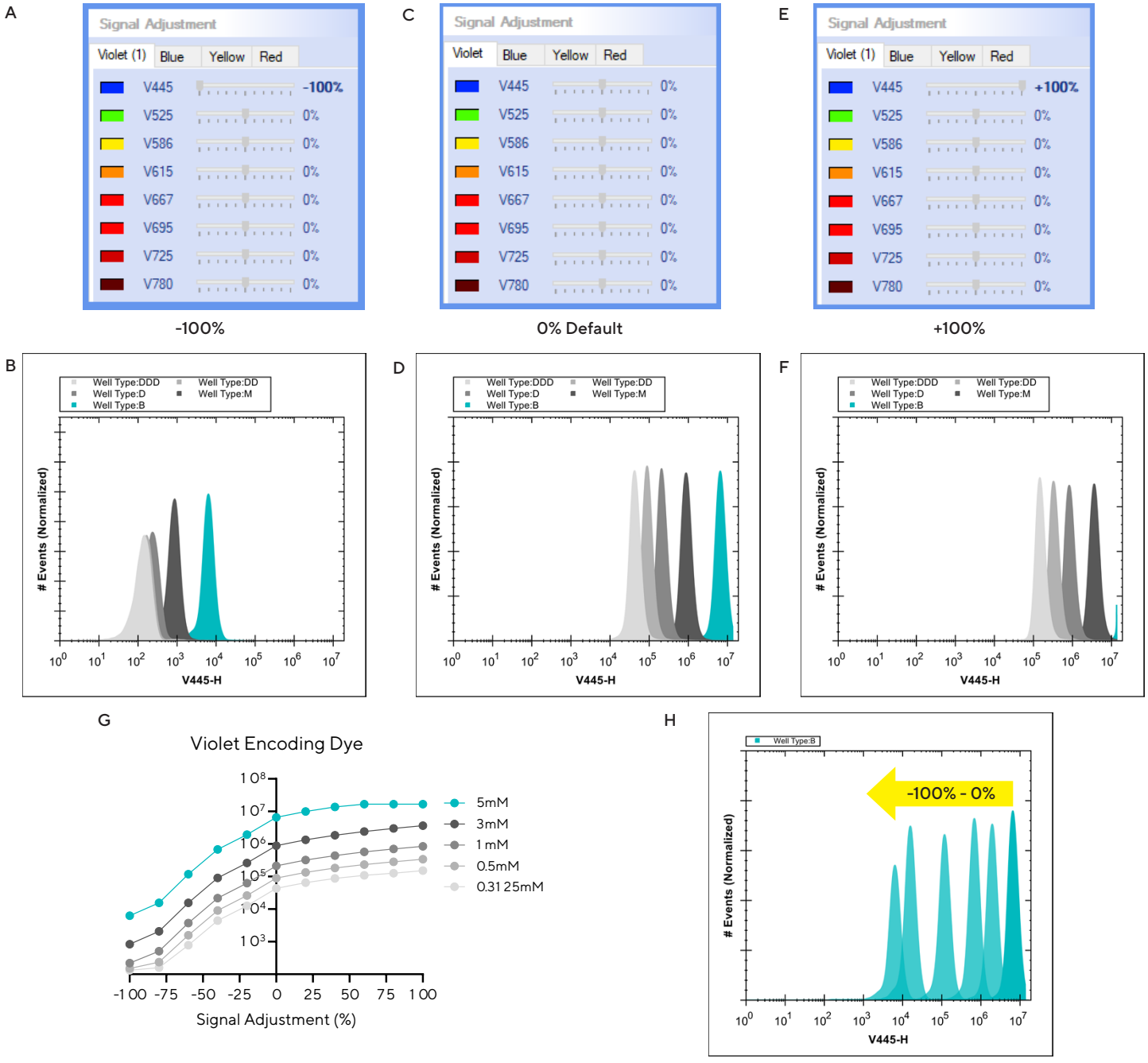

Figure 2: Data to demonstrate effect of signal adjustment. The signal was adjusted to -100% for the violet fluorescence channel (V445), resulting in a leftward shift in the histogram (A, B). The signal was set to the default of 0% (C, D). The signal was adjusted to its upper limit of +100%, resulting in a rightward shift (E, F). G) The Mean Fluorescence Intensity (MFI) graph illustrates the dilution of Violet Encoding Dye fluorescence intensity across various signal adjustment setups. H) overlay of the bright (5 mM) population across gain adjustments.

Further testing to showcase the gain adjustment feature

To demonstrate that gain adjustment is channel independent, the same set up was used to test the effect of adjusting an alternative neighbouring channel (V525) to look at the effect on signal (V445).

- Figure 3A illustrates that adjusting the gain in V445 channel (closed symbols) only affected signal measured in that channel and not any signal collected in the V525 channel (open symbols).
- Figure 3B demonstrates that when the V525 channel was adjusted to -100%, the signal detected in that specific channel V525 is changing as intended (pink bars) while there was no effect on the signal of neighbouring channel V445.
- Figure 3C shows the same data but as a dot plot of the V445 or V525 population movement when the gain is reduced by 100% in either channel.

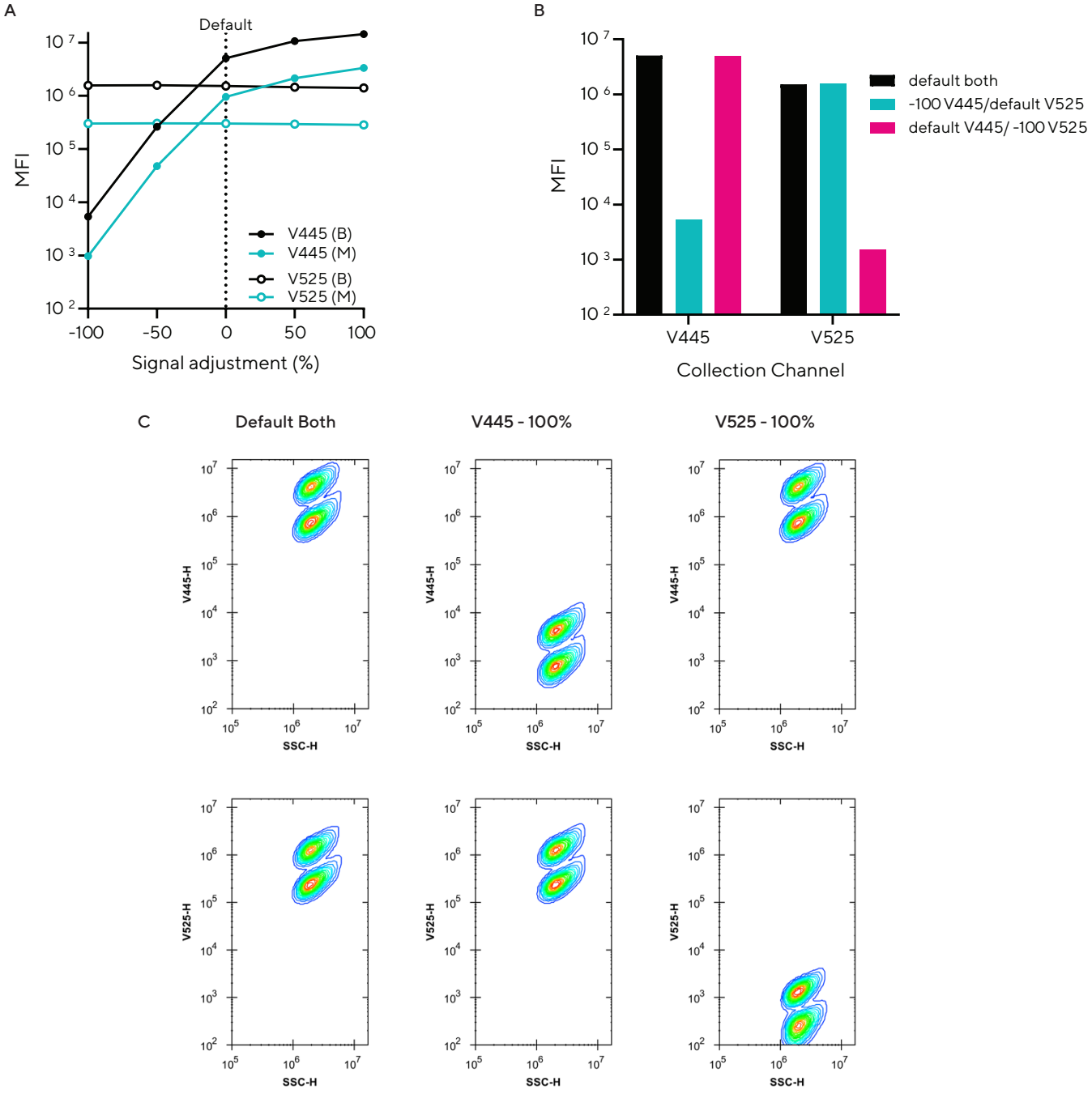

Figure 3: Gain adjustment is channel Specific. A) Signal in the neighbouring channel V525 was tracked for two dye intensities Bright (B) and Medium (M) across a range of V445 adjustments, closed symbols effect on V445 signal, open symbols effect on V525 signal. B) Bar graph to demonstrate change V525 gain (-100%) only effects signal detected in the V525 channel and not V445 (Pink bars). Data for default (Black) and -100% V445 (Teal) shown for bright dye. C) Dot plots of the two populations for both channels showing movement only in respective channels after gain adjustment.

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Status: 01 | 2026 iQue-Optimizing-Signal-Detection-Technical-Note-en-Sartorius