



MACSQuant® Tyto® Instrument short instructions

Photomultiplier tube (PMT) calibration

These short instructions are valid for MACSQuantify™ Tyto Software 3.2 and later.

The reproducibility and stability of the fluorescence signal over time is of vital importance. To ensure a stable measurement that is independent of time, the instrument needs to be calibrated. Calibrate the MACSQuant Tyto Instrument using the MACSQuant Tyto Calibration Beads (# 130-122-730). A daily calibration accounts for potential differences in laser performance or PMT sensitivity by adjusting the voltages accordingly.

The MACSQuant Tyto Calibration Beads contain blank beads, bright beads, and three populations of single-stained fluorescent beads yielding intermediate signals in the target channels. The beads serve as a reference to establish predetermined, lot-specific median fluorescence intensity (MFI) values for each channel. This is achieved by adjusting the voltages on each of the PMTs for the appropriate channel during the automated PMT calibration process.

It is recommended to calibrate the instrument at the beginning of every working day.

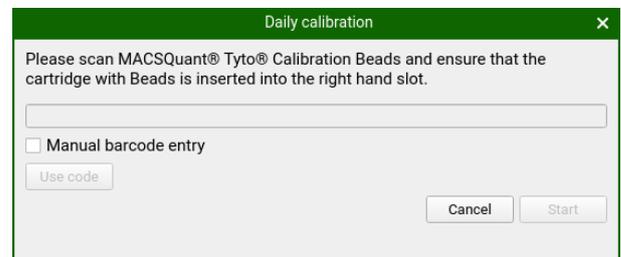
Automated PMT calibration

- 1 Switch on the MACSQuant Tyto Instrument. Wait at least 30 minutes to warm up the optical bench before performing the automated PMT calibration.

The warm-up time is tracked by the MACSQuant Tyto Instrument. If calibration is started before a period of 30 minutes, a pop-up window indicates the warm-up time. It is documented in the **MACSQuant Tyto calibration report** and in the audit trail if the PMT calibration was performed before a warm-up period of 30 minutes.

- 2 Prime a cartridge. Refer to the **MACSQuant Tyto instrument short instructions Cartridge priming and sample loading**.
- 3 Vortex the MACSQuant Tyto Calibration Beads for 10 seconds to break up aggregates.
- 4 Add three drops of MACSQuant Tyto Calibration Beads to 1.5 mL MACSQuant Tyto Running Buffer and vortex. Fill the diluted MACSQuant Tyto Calibration Beads into the cartridge.
- 5 Scan and insert the cartridge.
- 6 Scan the barcode of the MACSQuant Tyto Calibration Beads.

- 7 Optional: If scanning of MACSQuant Tyto Calibration Beads is not possible, click the  **Calibration** button in the toolbar. Select the **Manual barcode entry** checkbox to enter the barcode of the MACSQuant Tyto Calibration Beads manually.



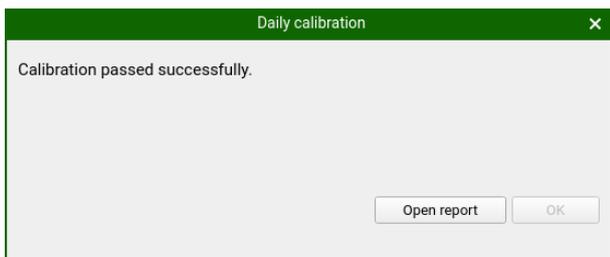
- 8 To start the calibration, click **Start**. The voltage for each channel is automatically adjusted during calibration.
- 9 The calibration results for each channel are presented as dot plots, histograms, and as a tabulated summary. See **Figure 1** as an example of a successful calibration.
- 10 The successful calibration is indicated for each fluorescent channel by the column **Info** giving the status **passed**. Furthermore, the table header of the calibration analysis page shows **Calibration: passed** and the instrument progress bar reports **calibration ok**. See **Table 1** for details about the shown parameter. The calibration table is automatically saved as HTML file.

2024-08-23 Calibration: passed

Procedure Version: 1.1.0

Channel	Gain	V	ΔV (last)	ΔV (init)	Stain Index	CV	Info
BSV	500	500	—	—	—	—	n/a
V1	400	388	-4	-4	271.9	14	passed
V2	400	438	-7	-7	792.0	15	passed
SSC	300	300	—	—	—	—	n/a
BSB	500	500	—	—	—	—	n/a
B1	400	482	-5	-5	710.8	9	passed
B2	400	538	-6	-6	1638.7	9	passed
B3	400	565	-6	-6	357.1	9	passed
B4	400	544	-6	-6	23.6	11	passed
BSR	500	500	—	—	—	—	n/a
R1	400	496	-2	-2	2050.0	15	passed
R2	400	542	-8	-8	215.1	16	passed

11 Click **Open report** to open the **MACSQuant Tyto calibration report**. The calibration report is automatically saved as PDF file.



Gains and threshold of the PMT calibration are loaded as a default instrument setting when a new workspace is generated via **File > New workspace**.

Parameter	Explanation
Gain	Normalized Smart Gain
V	Voltage on PMT
ΔV (last)	Difference in voltage on PMT compared to last successful calibration
ΔV (init)	Difference in voltage on PMT compared to last successful initial calibration
Stain Index	$(MFI_{pos} - MFI_{neg}) / 2 \times SD_{neg}$
CV	Coefficient of variation based on MFI
Info	Info on success of calibration

Table 1: Parameters of the PMT calibration shown in the analysis template, MFI: Median fluorescence intensity

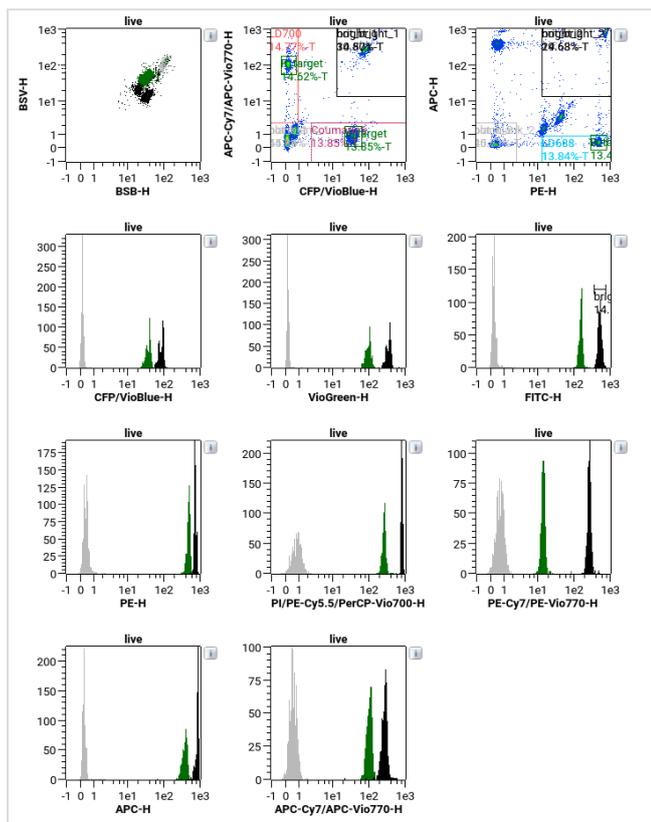


Figure 1: Calibration results of a MACSQuant Tyto Cell Sorter (gray: blank beads; black: bright beads; green: fluorescent beads for the respective target channels)



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