Release Notes

Bergisch Gladbach, 2025-06-27

MACSQuantify™ Tyto® Software 3.2

These Software Release Notes provide the users with information about software changes, new features, improvements, and bug fixes.

In general, the MACSQuantify Tyto Software version 3.2 is designed for running on the 3-laser, 8-color MACSQuant® Tyto® Cell Sorter, and on the brand new 12-color, 4-laser MACSQuant® Tyto® Lux Cell Sorter. Similar to its predecessor, the MACSQuantify Tyto software 3.2 is also based on a more secure and closed Miltenyi Biotec's proprietary MBCore OS, based on Linux®.

This software introduces a range of exciting new automated features, including "Automated Compensation" to adjust for spillover, "Automated Priming" for direct cartridge priming on the instrument and "Automated Coincidence Aborts" to detect and automatically abort coincidence events. It also introduces "Sort Modes" for the first time on MACSQuant Tyto instruments, enabling users to achieve Ultra Yield and Ultra Purity, or choosing one of the balanced modes in between them. Additionally, the software now supports the new "Large Cell" (LC) cartridge type on the MACSQuant Tyto instruments. Furthermore, this release enhances overall software stability.

For compliance with 21 CFR Part 11, the software offers an optional module that includes an improved user management system (UMS), audit trail, and run reports with e-signatures, available for an additional cost (#160-003-602) and requiring installation by service during a scheduled visit.

The MACSQuant Tyto Lux Cell Sorter, equipped with four lasers, also receives an exclusive "Laser Modes" feature, allowing users to select from three laser modes that enable more efficient use of collinear blue and yellow lasers.

Please note: An upgrade of the "Back Scatter Module", which enables scatter-based sorts and sorting with dim markers on the MACSQuant Tyto instrument might be necessary depending on the hardware status of your instrument. Users who have already upgraded their hardware as part of MACSQuantify Tyto Software version 1.0 or higher installation can upgrade their software directly to version 3.2, which will be performed

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by service engineers during the next planned maintenance visit. For the customers without service contracts, this software update can be requested for additional cost using MACSQuantify™ Tyto® Software Update Service (#160-003-797). This software is compatible on the MACSQuant Tyto Cell sorter with Abeco 3 PCs with CFast card and higher (from S/N :10041 onwards). The newly produced MACSQuant Tyto Cell Sorters, and the new MACSQuant Tyto Lux Cell Sorter have this software preinstalled.

Operating system MBCore 0.45.0

The update to our new OS MBCore equips the MACSQuant Tyto Cell Sorter with a Miltenyi-own OS. MBCore is based on a long-term support (LTS) Linux-kernel (for more information please visit Linux Kernel 5.4.170) and it is intended to replace the currently embedded Windows 7 with Extended Security Updates.

MBCore, manages both hardware and software resources of the MACSQuant Tyto Cell Sorter; it provides a platform for the data acquisition software MACSQuantify Tyto Software 3.2 as well as access to network resources, data exchange, and live support. The software runs in kiosk mode, to reduce unauthorized access to the system. This software version supports the exFAT file system; this is also required on external drives for mounting purposes.

Security and MBCore

MBCore is a Miltenyi-own OS with an up-to-date cybersecurity concept for client protection against outside attacks to safeguard sensitive information and instrument operability relevant in the healthcare and pharmaceutical sectors.

MBCore ensures software stability, sustains long-term performance, and data integrity by collecting, transmitting, and storing data according to ALCOA principles (ALCOA info). MBCore is delivered as an image and forms a container for all other software components. Thus, MBCore is protected from system modification within a running system, as well as any permanent virus and rootkits. Moreover, the auto execution for plug-and-play devices is switched off by default, which minimizes the risk of malware spreading. The data acquisition software MACSQuantify Tyto Software 3.2, included in MBCore image, is, hence, a kiosk-mode software, isolated from the operating system level in a container, and executed with read-rights only. Moreover, MBCore itself lacks a user-facing interface by design to ensure (depending on

the instrument and software package) audit-trailed traceability, as well as enforce data integrity; indeed, only interaction within the designated instrument software that provides necessary functionalities to the user have been made available.

MBCore was created using the widely adopted Yocto Project, commonly used in medical devices [ref. Yoctoproject]. MBCore provides concurrently multiple threads of execution on the MACSQuant Tyto Cell instrument built-in PC CPU and, thus, enables better application performance. Also, the main instrument application is restricted to a maximum of 90% hardware resources, regarding CPU and memory, to prevent the system from freezing. MBCore runs on a “separate-from-customer-data-drive” (industry grade CFast) and has been hardened following Debian hardening guide.

Moreover, MBCore OS updates are encrypted, and the system will only apply image updates if signatures are verified, and updates decrypted. Updates of the MBCore OS will be provided through MACSQuantify Tyto Software updates and they will be delivered by Miltenyi Biotec upon request. As a security feature, services run under the unprivileged user, and access to Miltenyi-Biotec service functionalities is restricted via the cryptographic network protocol SSH.

By default, any incoming traffic from outside is blocked. However, the MACSQuant Tyto instrument can be safely connected to a network to allow a centralized data management as well as enable users to access and store files on remote computers and servers. Remote access is made possible via a temporary communication upon user request: MBCore supports NFS and SMB data transfer protocols to push data from instrument to network shares and servers. Pulling data onto the instrument, as well as domain controller integration functionalities are blocked. User rights and permissions are locally managed as part of MACSQuantify Tyto Software in order to allow different user actions. In addition, improved and secure Live Support is available on the MACSQuant Tyto instrument. Ports for remote support by Miltenyi Biotec are filtered (port 443 or 5938), so that a connection can be established. Remote access is made possible via temporary communication and upon user request.

New Features

1. MACSQuantify Tyto Software version 3.2 employs **“Automated Compensation”** to compensate the spectral overlap of individual fluorochromes detected in more than one fluorescence channel. The automated compensation works either with single-stained cells, or single-stained MACS® Comp Bead Kits (# 130-104-693 for anti-REA).
2. MACSQuantify Tyto Software version 3.2 now includes **“Automated Priming”** to automatically pre-wet the channel to both positive and negative collection chambers on the MACSQuant Tyto instrument directly. Automated priming can be done using the sample itself with no additional pipetting steps.
3. **Sort Modes:** MACSQuantify Tyto Software Version 3.2 introduces four automated sort modes—Ultra Yield, Ultra Purity, and two balanced modes in between—that allow seamless optimization between purity and yield based on experimental requirements.. Coincidence aborts, valve timing, and sort aborts are key parameters that are fine-tuned in each sort mode to achieve the desired outcomes.
4. The Sort Modes include **“Automated Coincidence Detection”**, which helps in automatic detection of two or more cells sticking to each other which can be misidentified as single cells. This is integrated as a check box called “Enable coincidence aborts”. This checkbox can be selected individually for each sort mode. Per default, it is enabled for all sort modes, except for the Ultra Yield mode.
5. MACSQuantify Tyto software version 3.2 also powers MACSQuant Tyto Lux. It has an exclusive feature called **“Laser Mode”**, which gives the flexibility to select the mode to control the Yellow laser.
6. MACSQuantify Tyto Software version 3.2 also offers features to support compliance enabling according to 21 CFR Part 11 (#160-003-602, to order please visit **www.miltenyibiotec.com**), i.e. lightweight directory access protocol (LDAP) connection, user management system (UMS) with configurable user permissions, signature of run reports with a signature reason, tracked activities in the audit trail. The audit trail report can be viewed and exported for documentation in a human readable format.

7. MACSQuantify Tyto Software version 3.2 now supports a new cartridge type called **"MACSQuant Tyto Cartridge LC (Large Cell)"**.
8. MACSQuantify Tyto Software version 3.2 also now includes **SMB** and **NFS** network share protocols for data backup to external server locations.

Improvements

MACSQuantify Tyto Software version 3.2

- Automated Initial calibration can now be also performed by the admin users in addition to the service users. We recommend the admin users to perform initial calibration **once every 6 months**.

- New layout of automated PMT calibration results
    1. Color grading of histograms is changed to be colorblind-friendly, and consistent with our MACSQuant analyzers.
    2. Calibration results table reorganized
    3. SI and CV gets ranking (A and E) for pass/fail criteria.

- Real time sort statistics are improved to show the new parameter "Coincidence aborted", which is shown as the percent of all the triggered events.

- Lock screen, which was a 21 CFR Part 11 exclusive feature, was moved to be a default feature for both RUO and 21 CFR Part 11 users for ensuring the security and preventing unauthorized access to the instrument

- The sort controls & cartridge eject buttons under Misc Controls are now moved to the small orange touchscreen monitor.

- Now adds the functionality to recognize the GMP cartridge types (MACS® GMP Tyto® Cartridge HS and MACS® GMP Tyto® Cartridge)

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Bug fixes

Bug description	Status
1. New workspace overwrites already opened workspace if the save dialog is cancelled	fixed
2. Incorrect pop-up messages while copying LDAP certificates from USB flash drive	fixed
3. Trigger threshold channels are not updated under the graphs as per selection	fixed
4. Reboot title and message after changing the options needs to be more descriptive	fixed
5. Compensation table is transposed when exporting as FCS file	fixed
6. No user folder is preselected when user has private and public permissions	fixed
7. Flow controls UI flickers when user checks and unchecks the Auto Pressure button continuously	fixed
8. In audit trail report, the Auto Pressure valve shows as “false” when sort starts	fixed
9. Saving channel annotation field blank causes issues with login	fixed
10. Annotations are not updated in compensation matrix when changed on experiments tab	fixed
11. First double-click to maximize the plot causes it to overlap	fixed
12. Audit trail stops working after instrument remains on lock screen for over 24hrs	fixed
13. USB flash drive not recognized in Copy dialog if it is inserted before startup	fixed
14. Y-axis label of “Trigger rate vs Elapsed time” is truncated	fixed
15. Audit trail system (ATS) report: discrepancies in login and logout messages	fixed
16. Error message headlines were truncated	fixed
17. Space availability of USB flash drive not changed after copying the file	fixed
18. Pop-up dialogs in UMS when a user was set inactive, password expired, are very generic	fixed
19. Progress bar is not shown when the lock screen is activated	fixed

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Known Issues

Bug description	Workaround
1. SW crashes when users save workspaces containing .mqd files and opens another workspace	Recommend not saving .mqd files as part of workspaces
2. Unchecking DHCP without manually setting a nameserver always results in using a fallback server	Don't uncheck the DHCP if there is no DNS available
3. If the default is set to use "Automated Priming", the older workspaces from previous SW versions will show "Automated Priming" enabled	Make sure to check this and accordingly uncheck it if automated priming is not needed
4. Few USB flash drives show very slow copying speeds	Use a different USB flash drives from different manufacturer. We are investigating which models are very slow
5. Improper unmounting of USB flash drive causes errors	Eject the USB flash drive every time to ensure proper unmounting
6. Auto coincidence detection in Sort Modes doesn't work optimally with MACSQuant Tyto Cartridge LC or debris-rich samples	Do not use auto coincidence aborts when MACSQuant Tyto Cartridge LC or debris-rich samples are used. Instead, use manual singlet gating on all the lasers
7. Incorrectly formatted file names leads to SW crash	File naming should always end with this ".%Num%.mqd". Do not remove this
8. Sample ID carries into the file name of PMT calibration	Open a new workspace to avoid this issue
9. Audit trail report cannot be signed with special characters	Do not use these (") and (\) characters while signing the audit trail reports
10. .mqd/.fcs files do not contain instrument settings	Instrument settings can be retrieved from saved workspaces, copied from the Run Reports, or from the audit trail entries.
11. In SMB server configuration after multiple failed login attempts, no longer prompts the user for a password	Use the copy dialog to enter the trigger the password dialog

12. Software crashes when user clicks “Print” when no printer is added	Will be fixed in next patch release. Not a usual workflow
13. Coincidence abort rates on reopening run reports display inconsistencies	Will be fixed in next patch release. Use the original PDF exported at the end of run
14. Admin users cannot directly delete the files saved in public location to free up the hard disk space after a backup	Admin users need to have “delete” right option assigned for Tyto public files to clear these files

Recommendations

Consideration	Recommendation
1. Changing from “balanced” or “yellow off” laser modes to “yellow only” on MACSQuant Tyto Lux causes events to disappear under noise threshold	To avoid that events are cut off by the noise threshold, increase the Back Scatter Blue voltage, or decrease the noise threshold when using the “yellow only” mode.
2. PE-Vio 615 (B+Y3) has high spillover into the B+Y4 channel. Detection of PerCP-Vio 700 (B+Y4) might not work well in combination with PE-Vio 615 on the MACSQuant Tyto Lux Cell Sorter	Use PE-Vio 670 instead of PerCP-Vio 700 in the B+Y4 channel
3. PE-Vio 670 (B+Y4) has high spillover into the R1 channel. Detection of APC (R1) might not work well in combination with PE-Vio 615 on the MACSQuant Tyto Lux Cell Sorter	Use Vio R667 instead of APC in the R1 channel
4. The compensation is dependent on the laser mode. Acquisition and sorting must be performed with the same laser mode as the compensation measurement	Perform the compensation for each laser mode individually. When switching laser modes, a pop-up message is shown to reset the spillover matrix
5. The compensation is dependent on the cartridge type. Acquisition and sorting must be performed with the same cartridge type	Use same cartridge type for compensation and to sort

6. Cross-staining of beads during the automated compensation process can lead to a poor compensation calculation	Refer to the MACSQuant Tyto Instrument short instructions „Compensation“ for optimal MACS Comp Beads preparation and compensation. Immediately proceed with the autocompensation after mixing the single stains. Do not store the mixed compensation control sample
7. Using "Trigger (fluorescent) threshold" leads to inconsistencies in purity percentages while sort modes are used	Do not use trigger threshold during sorting where higher purity is desired as it hides the events from sort logic and coincidence handling