

Streamlining Drug Discovery with High-Throughput Screening by Cytometry

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Introduction

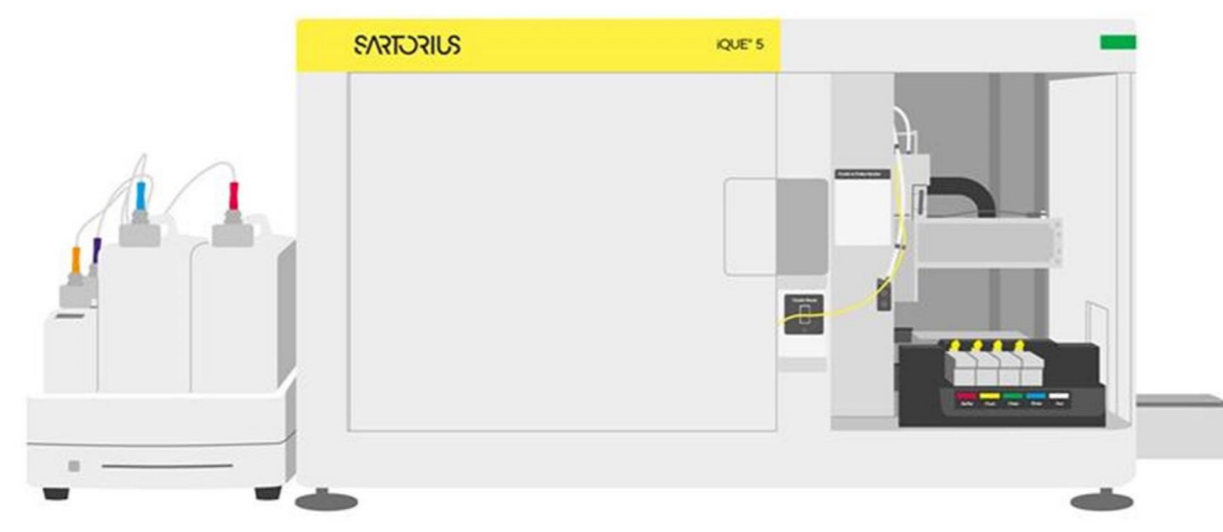
- In Drug Discovery, researchers often face challenges such as large sample sets, complex experiments, and limited resources.
- High-throughput screening using cytometry significantly boosts scientific research by enabling efficient analysis of complex cellular interactions and responses.
- This method supports multiplexed analysis of cell phenotype and function within a single well.
- Drug discovery screens typically involve lengthy experimental

timeframes and are susceptible to disruptions due to fluidics issues. A crucial aspect of this approach is the integration of clog detection, which is essential for minimizing downtime.

- We will explore the features and the operational benefits of the iQue[®] 5 HTS Platform that empower researchers to generate accurate, biologically relevant insights quickly, thereby maximizing productivity and accelerating discovery and innovation in life sciences.

High-Throughput Screening by Cytometry

- Scientists use flow cytometry to investigate complex biological processes as part of routine cell analysis workflows.
- To keep pace with market demands, scientists need a faster way to generate biologically relevant data from high-throughput research workflows.
- The iQue[®] Platform combines the ability to run complex panels using both cells and beads in the same well with the speed and throughput required for HTS workflows.
- The next generation iQue[®] 5 instrument builds on the established features of the existing iQue[®] 3 Platform to enhance the customer experience and support extra flexibility.



- Equipped with adaptable automation capabilities, it facilitates seamless integration with automation solutions, enhancing high-throughput screening and minimizing the need for manual intervention.
- Accelerated market-leading speed with the use of the patented air gap technology to deliver 384-well sampling in <20 min, 96-well <5 min.
- The platform is available in both a 3 laser (Violet, Blue, Red) and 4 laser (Violet, Yellow, Blue, Red) format for improved flexibility and easier panel design to enable greater workflow support.
- VBR format with up to 19 channel capacity.
- VYBR format with up to 27 channel capacity.
- Improved Clog detection and management with air-gap sensor for simplified monitoring.
- Easily set individual channel gains with SIPM.
- Improved usability, fluidics and data analytics including higher volume acquired in less time with reduced carry over between samples.

Emission Filter	Laser			
	405 nm	488 nm	561 nm *	640 nm
445/45 nm	✓ Pacific Blue			
525/45 nm	✓ BV510	✓ FITC		
586/20 nm	✓ BV605	✓ EYFP	✓ PE	
615/20 nm	✓ Qdot 605	✓ PI	✓ PE-Dazzel594	
667/30 nm	✓ BV650	✓ PerCP	✓ PE-Cy5	✓ APC
695/40 nm	✓ BV711	✓ PerCP-Cy5.5	✓ PE-Cy5.5	✓ AF680
725/40 nm	✓ BV750	✓ PerCP-eFluor7	✓ PE-AF700	✓ AF700
780/60 nm	✓ BV786	✓ PE-Cy7	✓ PE-Cy7	✓ APC-Cy7

Table 1: Laser and channel details for iQue[®] 5 Platform * 561 nm not on 3 laser (VBR) instrument

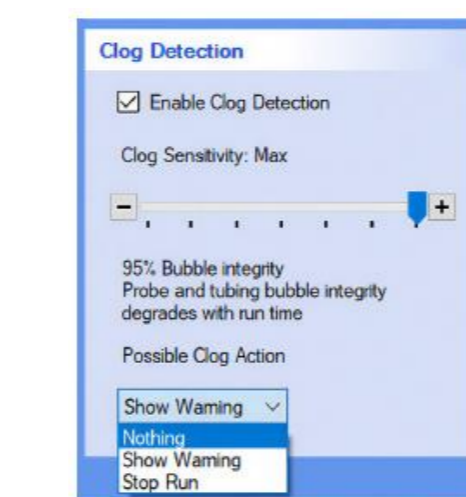
Automatic Clog Detection and Management

- Clogging is a known issue in flow cytometry, to address this the iQue[®] 5 Platform has innovative design features to help minimize clogs and aid in the management to reduce sample loss.
- The fluidics assembly has been redesigned, with the use of tubing material which has an increased rigidity and resistance to pump pressures to prevent fluid path inconsistencies and reduce frequency of clogs.



Bubble Sensor

- The platform has a bubble sensor which provides a visual indicator of the air gap as it passes the sensor.
- Under normal operation, this appears as a rhythmic flashing green light, the frequency dependent on the length of sip time. It provides a clear indication of even air gaps.
- If a solid light or no light is visible, this indicates a fault, possibly due to a blockage in the probe and tubing assembly or fluid link tubing.



Clog Management

- Within the iQue Forecvt[®] Software users choose to enable the clog detection feature which uses features of the bubble frequency and timing as an indicator for disrupted flow.
- Disruption is measured as a combination of changes to the frequency of the bubbles passing the sensor, fragmented bubbles within the fluid phase or deviations from the number of bubbles expected.
- The sensitivity of detection can be set at varying levels. As a guide, default sensitivity will flag an issue when <75% bubble integrity is detected, or when 5-7 wells are missing.
- If issues are detected various actions can be pre-set – “Nothing”, “Show Warning” or “Stop Run”.

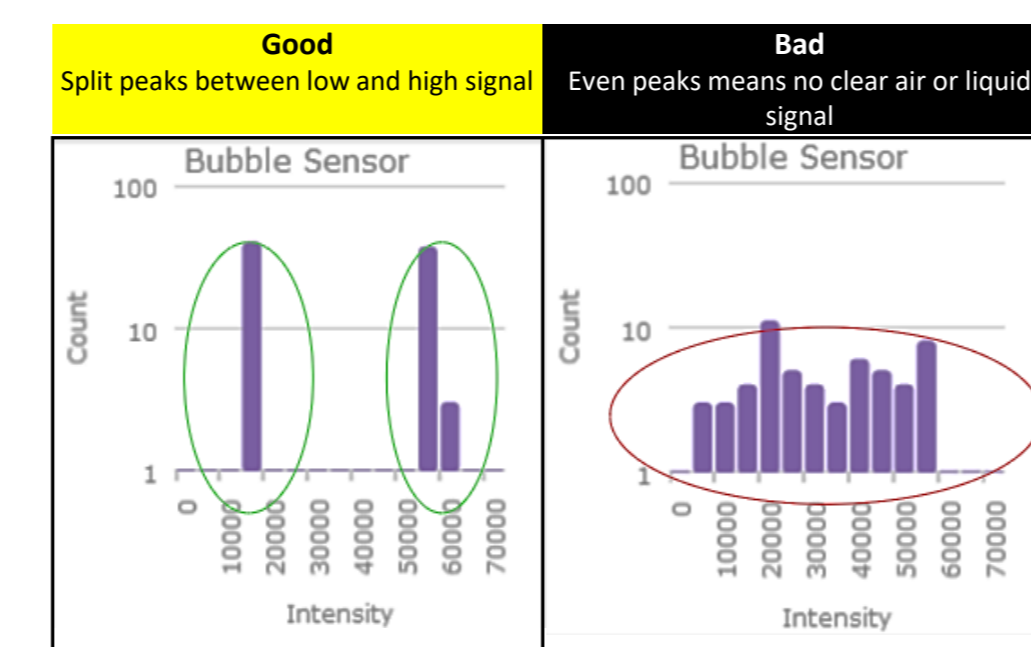


Figure 1: Bubble Sensor Data

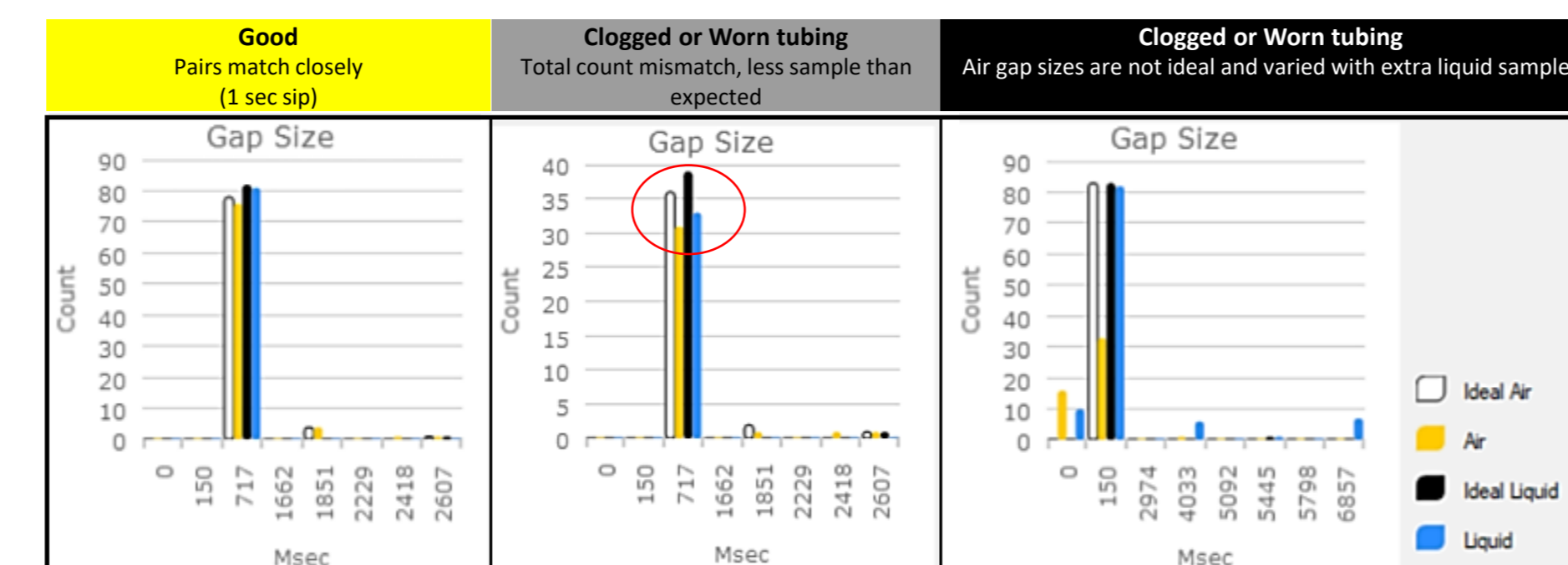


Figure 2: Gap Size Data

Detection of Clog

- When an issue is detected, the software will present the data as two graphs.
- Bubble Sensor data (Figure 1) – Displayed as two clear peaks split between high and low intensity values representing the air and liquid slugs (left).
 - If disrupted or “shredded” bubbles or unexpected bubble sizes are detected there will be no clear split in populations (right).
- Gap Size data (Figure 2) - Describes the gap sizes for the air gap and sample slugs as a timing measure.
 - It is the difference between the ideal gap data (clear/black bars) and the measured gap data (yellow/blue bars) that is important.
 - Potential clogs can appear as a discrepancy in ideal and measured values (middle, ring) or random air and liquid data (right).

Clog Log

- Forecvt will store all details of completed runs.
- Summary of run settings, any warnings detected and details on the warning trigger.
- The log is stored and can be exported as a CSV file if required for further processing.

Gain Adjustment

- Adjusting reagent concentrations is effective for optimizing staining protocols but not possible for all assays e.g. the overexpression of fluorescent proteins. For those assays, changing the gain as a measure of signal amplification becomes a powerful tool, allowing the increase of sensitivity to enhance faint signals or decrease to bring bright populations on scale.
- The iQue[®] 5 Platform is fitted with individual detectors for each channel and therefore supports a high level of signal gain control. Gain is adjusted within the software using a sliding scale between -100% to +100% (default at 0%, Figure 3).
- To showcase this, Jurkat cells were stained with iQue[®] Cell Proliferation and Encoding Dye V/Blue at various concentrations (0.31-5 mM) to provide five distinct intensities. Data was collected across a range of gain settings by adjusting the V445 fluorescence channel in 20% intervals from -100 to 100% and is shown as mean fluorescence intensity (MFI, Figure 3).
 - Default setting (0%) all five intensities can be visualized, although the brightest (Teal) is close to the upper detection limit.
 - 100% signal adjustment has shifted all peaks to the left but there is some merging or loss of definition of dimmer signals.
 - +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.
 - Overlay of bright population over signal reductions to show left shift in population.

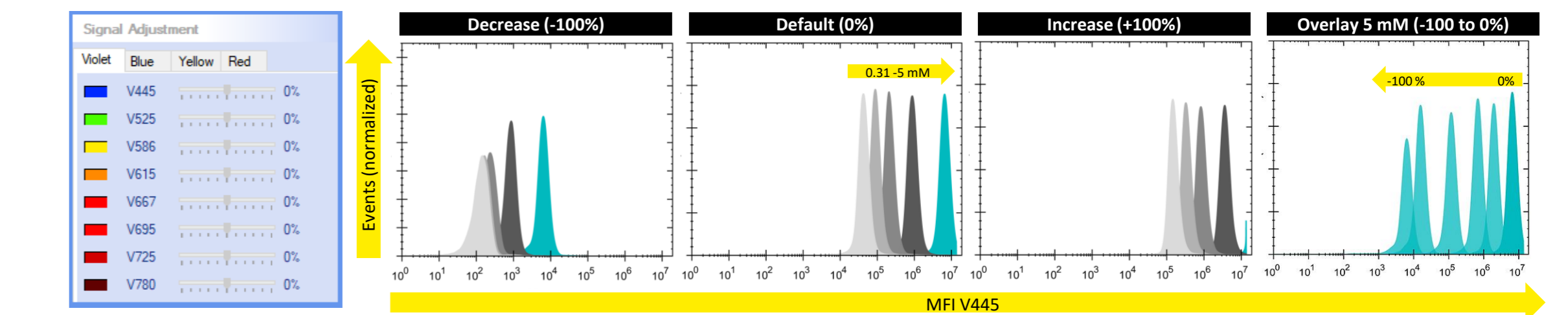


Figure 3: Example of effect of signal adjustment on MFI signal from iQue[®] Cell Proliferation and Encoding Dye.

Automation Integration and iQue[®] Qmax Workstation

- The iQue[®] Platform is an automation agnostic system by design and pairs well with a variety of established liquid-handling robotics to provide microplate loading for unattended analysis of samples (96- or 384-well).
- Connection uses the Robotic Integration Automation Programming Interface (API) License via a Serial | TCP connection which includes all the tools necessary including Screen Development Kit (SDK) fully compatible with 21 CFR Part 11 compliance.
- The system can be integrated into larger robotic platforms alongside multiple systems and detectors to amplify data analysis capabilities.
- Combined with automated cleaning and shut-down the system can support an average run of 45 plates/experiment.
- To fully support the use of iQue in HTS workflows the Qmax Workstation can be used to provide an extended run time (up to 48 hours) to support higher plate throughput.
- Alongside the iQue[®] 5, Qmax has been redesigned to incorporate a trolley for improved transportation of fluid tanks, a longer connection hose and fitted with easy fill and empty capability.

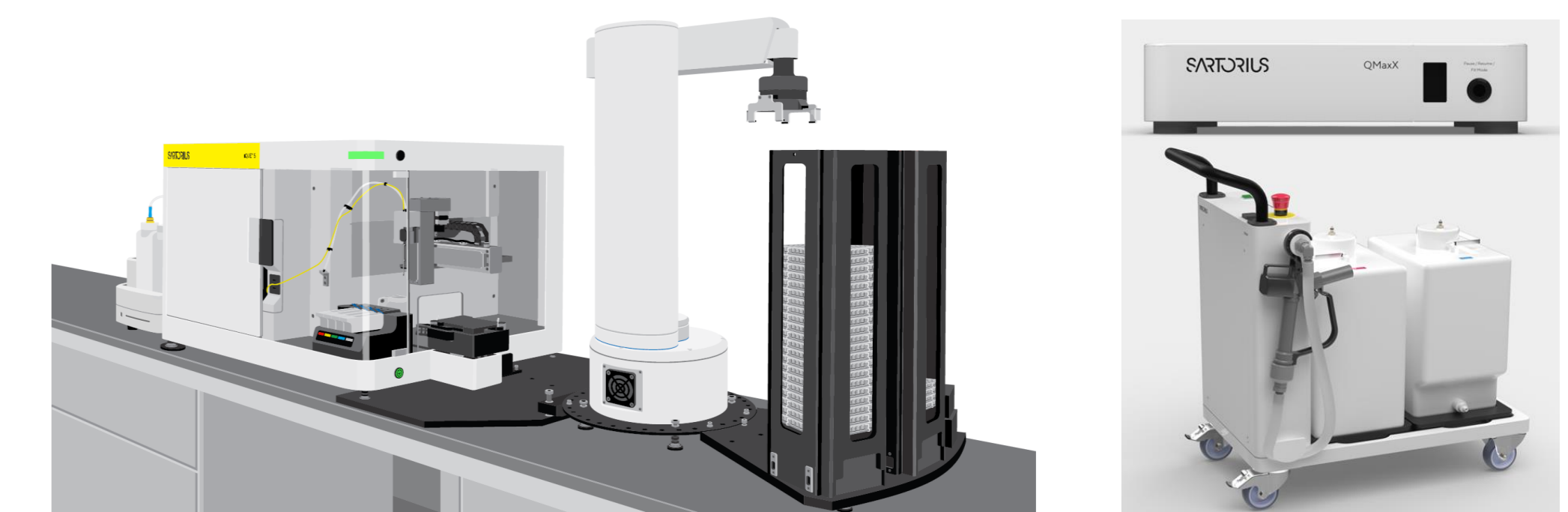


Figure 4: Visualization of iQue[®] integration with plate handling automation and iQue[®] Qmax Workstation