anitoa

Maverick qPCR

Real-Time Fluorescence Quantitative PCR Instrument Instructions for Use Manual

Version 1.85

The instruction manual must be properly placed in the product box during shipment.

The user is required to keep this manual in a safe place so that it can be consulted when needed.

Imported by: Anitoa Systems, LLC

Manufactured by: Anitoa Biotechnology (Hangzhou) Co., Ltd.

Please read this instruction manual carefully before use

Warranty

Anitoa Systems, LLC (hereinafter referred to as Anitoa) warrants that the Maverick Quantitative PCR System you are using has been fully tested and performs as described in the manual. The instructions and safety warnings given in this instruction manual must be followed to use the instrument, otherwise the warranty does not apply.

Software description

The software is a necessary tool for the operation of the instrument. For improving its performance and reliability, Anitoa has the right to modify its functions or design, etc., in advance or subsequently without informing the clients, and Anitoa has all the intellectual property rights of the modified version.

Responsibility statement

Anitoa is not responsible for direct or indirect incidental damages arising from non-compliance with the operating instructions or incorrect use of the Maverick Real-Time Fluorescence Quantitative PCR System. Only Anitoa 's technicians or its authorized agents may inspect or provide parts for the instrument in question, and we are not responsible for direct or indirect collateral damage resulting from the user's disassembly and replacement of parts. The responsibility of Anitoa is limited to the repair of the machine and the replacement of the parts, but not for the results of the experiments.

Intellectual property statement

Contents

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1. Important notes

Intended use

The Anitoa Maverick Real time fluorescence quantitative PCR instrument is an in vitro diagnostic device intended to perform fluorescence-based PCR to provide detection of nucleic acid sequences in various specimens derived from human, animals, or environmental samples.

The Anitoa Maverick Real time fluorescence quantitative PCR instrument is intended to be used by trained professionals in combination with in vitro diagnostic assays.

The following safety measures must be observed during all phases of operation, maintenance, and servicing of this instrument. Failure to observe these measures or the warnings and precautions indicated in this manual will likely undermine the safety standards for which the instrument was designed and manufactured and the intended scope of use of the instrument.

Instrument grounding

To ensure the personal safety of the operator, please use the power adapter provided by the manufacturer, which has a 10A three-prong grounding plug at the input end. When using the adapter, please use a grounding socket that matches the plug to ensure that the input power line of the instrument is reliably grounded.

1) Use of power supply

Before the instrument adapter is connected to the power cord, it must be ensured that the AC power supply voltage (100 to 240 VAC) and frequency (50/60Hz) are consistent with those required by the instrument adapter. When making the power cord connection, make sure that the instrument power switch is off. Do not touch the power switch and power cord with wet hands. It is prohibited to disconnect the power cord when the instrument is not powered off. It is forbidden to touch the power cord to the hot surface of the instrument. Do not clean the instrument when it is not disconnected. Please turn off the power when the instrument is no longer in use.

2) Power cord

The instrument should normally use the power cord supplied with it. If the power cord is broken, it must be replaced without repair. When replacing the power cord, it must be

replaced with the same type of power cord of the same specifications. When this instrument is in use, do not place anything on the power cord, and do not place the power cord in a place where people move around.

3) Power cord plugging and unplugging

Power cord plugging and unplugging must be the correct handheld plug operating parts, plug insertion should ensure that the plug is completely, tightly inserted into the socket, do not pull hard when pulling out the plug, or yank the power cord.

Placement of the Instrument

1) The instrument should not be placed in a location where it is difficult to disconnect the power supply.
2) The instrument uses semiconductor cooling and fan-assisted heat dissipation, so when placing the instrument, ensure that there is no obstacle within 15cm around the instrument, and when multiple instruments are used at the same time, the distance between each instrument should be not less than 30 cm.
3) The instrument should be placed in a place with low humidity, less dust, and far from water sources (such as pools, water pipes, etc.), with good ventilation, no corrosive gas or strong magnetic field interference, and avoiding direct sunlight and strong light sources. The table where the instrument is placed should be horizontal and stable.
4) High ambient temperature will affect the testing performance of the instrument or cause malfunction. Do not use this instrument in the place of direct sunlight and strong light source to avoid affecting the fluorescence detection of the instrument, and should be far away from heating, stove, and all other heat sources.
5) Turn off the power when stop working. When the instrument is not used for a long time, cut off the power, unplug it, and cover it with soft cloth or plastic film to prevent dust and foreign objects from entering.

Precautions

1) During the test operation, avoid liquid dripping on the instrument.
2) The consumables and reagents used in the test should be disposed of according to the relevant standards and should not be discarded or dumped at will.
3) If there are hazardous substances in the test, related training must be conducted before using them.
4) After use, the hazardous substances should be handled and stored properly in strict accordance with the relevant regulations.
5) The test personnel who operate the instrument need to be trained and have relevant qualifications.
6) When handling toxic, corrosive, or infectious substances, safety goggles and gloves must be worn.
7) It is strictly forbidden to touch the metal module when the instrument is running and for a period of time just after the operation to avoid burns.

8) It is strictly forbidden to open the instrument during the operation of the instrument, otherwise it will cause abnormal experimental results.

After-sales service

1) After receiving the instrument, please confirm the relevant content on the after-sales warranty card and contact the shipping unit if you have any questions.
2) After unpacking the instrument, please keep the packing box and packing materials properly for use when returning to the factory for after-sales service.
3) Before sending the instrument to the maintenance department, the instrument must be disinfected.
4) After the instrument is delivered to the maintenance department and unpacked, the maintenance personnel must disinfect the instrument immediately.

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Packaging, storage, and transportation marking

Table 1-1 Package storage and transportation identification

Symbol	Title	Description	Position
	Place carefully and gently	This symbol is used to indicate that the product is a precision instrument and should be handled carefully and gently.	On the packing carton
	Upward	This symbol is used to indicate that the instrument must be kept upward during handling, storage and use, and must not be placed sideways or upside down to avoid damage to the instrument.	On the packing carton
	Afraid of getting wet	This symbol is used to indicate that the instrument must not be stored in a humid environment or in a place where it can be splashed with liquid.	On the packing carton
	Stacking 5 layers	This symbol is used to indicate the maximum number of layers of vertical stacking overlap allowed for a box.	On the packing carton
	Anti-bumping	This symbol is used to indicate that the instrument should be handled, stored, and used with care to avoid any impact on the performance of the instrument.	On the packing carton

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Instrument Identification Information

Table 1-2 Instrument identification information

Symbol	Description	The location on the instrument where the symbol will appear
	Watch out for high temperatures	On the equipment
	Production date	On the equipment nameplate
	CE mark	On the equipment nameplate
	Pay attention to safety	On the equipment nameplate
	In vitro diagnostic medical instrument	On the equipment nameplate
	E-waste, pay attention to the classification	On the equipment nameplate
	Product number	On the equipment nameplate
	Serial number	On the equipment nameplate
	Instruction manual	On the equipment nameplate
	Biological hazards	On the equipment nameplate
	FCC mark	On the equipment nameplate

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2. Product overview

Anitoa Maverick is a portable, high performance real time quantitative PCR system with an optional large 10-inch touch screen for integrated operation. Maverick is equipped with a multi-channel fluorescence optical system, powered by Anitoa's ultra-low-light CMOS bio-imaging sensor. Maverick is optimally suited for applications where portability, minimal space, fast time-to-result is required. Applications of Maverick are point-of-care molecular diagnostics test (POCT), food safety and environment testing, agriculture, or research lab use where bench space is limited. The product can be widely used in universities and research institutes, CDC, Entry-exit Inspection and Quarantine Bureau, Public Security Criminal Evidence Identification Center, veterinary stations, food companies and pharmaceutical companies.

Figure 1.1 Maverick compact qPCR system

Features

- Compact and rugged design. No internal moving parts, and no need for calibration.
- Multi-wavelengths up to 4-channels fluorescence sensing capability[1].
- Cross platform software for Windows® and Android® OS, with cloud-ready connectivity.
- Low power requirement. External supply with ~90W active peak power for both 4 well and 8 well devices. External supply with ~144W active peak power for 16 well devices.
- Battery-backup option for outdoor use and power loss protection.
- Efficient and fast: with ultra-fast temperature rise and fall system and unique fluorescence collection chip technology, fast detection can be achieved without specific consumables.
- Touch operation: available 10-inch LCD touch screen, integrated touch operation, no external computer, simple and convenient.
- Lightweight and portable: compact and portable body (247 x 188 x 145 mm for 16 well devices), light weight (3.6 kg for 16 well devices), easy to move, strong environmental adaptability.
- Stable and reliable: the whole machine has no moving parts, and the structure is sturdy and durable without regular calibration even after a long time of use.
- Intelligent management: optional 4G module allows remote management or cloud management of experimental data as needed.
- Multiple options: Support 2 or 4 fluorescence channels (more channels can be customized), suitable for most of current dyes, no cross interference between channels, no need for regular calibration maintenance.
- Stable light source: independent LED light source for each fluorescence channel, stable and non-decaying LED light source, no need for regular replacement.
- High sensitivity chip: the unique "Ultra-Low-Light CMOS Image Sensor (CIS) Chips", millisecond-level extremely fast shooting, stable and reliable data.

[1] Support Intercalating dyes, hydroxyls probes and FRET probes

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Key parameters

Capacity (# of wells)	4, 8, 16
Channels (# of Fluorophores per well)	4 (1. FAM/SYBR Green; 2. JOE/HEX/VIC/TET; 3. ROX/Texas Red; 4. CY5/LIZ/Cy5.5).
Multiplex capability	Up to 4 targets per well
Minimum detection threshold	4 copies
Dynamic range	>1.0E9
Signal Interface	USB 2.0,
Excitation source	High endurance LED, with built-in collimating lens
Detector	Ultra-low-light CMOS Bio-imaging chip
Thermal system	Solid-state, Peltier-based
Tube/plate formats	0.2mL, 4 or 8-tube strip
Reaction volume	10uL – 60uL
Excitation Range:	460nm – 670nm
Emission Range	510nm – 720nm
DNA probes supported:	DNA binding dyes (e.g. SYBR Green), hydrolysis probes (e.g. TaqMan probe) and hybridization probes (e.g. FRET probes).
Temperature Uniformity	<= ±0.25°C
Temperature control resolution	<= ±0.1 °C
Temperature Ramp Rate	6°C/s heating; 4°C/s cooling
Detection accuracy	Ct value CV <= 3%
Size and weight	MQx4: 198 mm (L) x 134 mm (W) x 98 mm (H) MQx8: 147 mm (L) x 182 mm (W) x 99 mm (H) MQx16: 247mm (L) x 188mm (W) x 145mm (H)
Weight	MQx4: 1.2 kg MQx8: 1.45 kg MQx16: 3.6Kg

Power supply	MQx4 and MQx8: DC 10.5 V, 90 W MQx16: DC 15V, 9.6A, 144W (Adapter provided that accepts 110V/240V AC)

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Applications

- Point-of-care molecular diagnostics[2]
- Food safety test
- Environmental microbial-threat monitoring
- Agriculture DNA testing
- Forensic testing
- Research and educational lab use
- Drug quality assurance testing

[2] Clinical clearance required.

Instrument external dimension

Figure 1.2 shows the external dimension of the Maverick qPCR instrument.

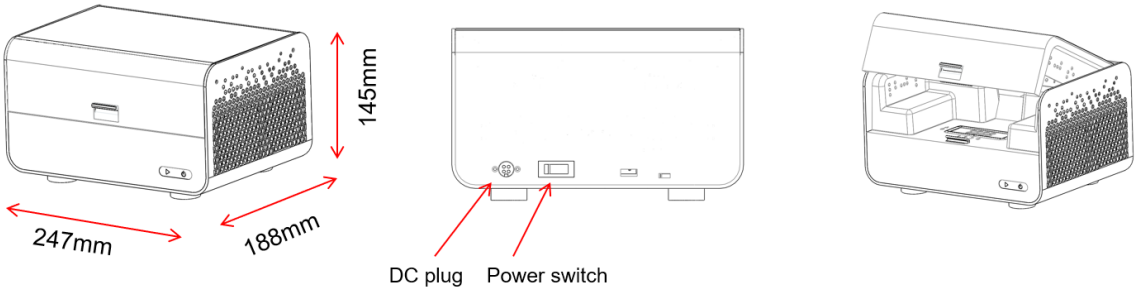
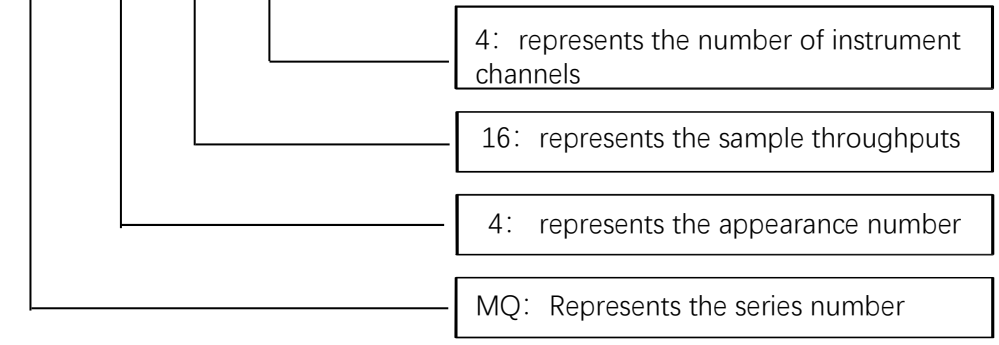


Figure 1.2 Maverick compact qPCR system MQx16 product dimension

Specification model description

Example: MQ 4 16 4



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Instrument models (4,5-series)

Table 2-1 Instrument models

Model	Channel	Sample throughput
MQ4162	2 channels	16 wells, touchscreen
MQ4164	4 channels	16 wells, touchscreen
MQ4162	2 channels	16 wells
MQ4164	4 channels	16 wells

Instrument models (1-series)

Table 2-3 Instrument models

Model	Channel	Sample throughput
MQ1044	4 channels	4 wells
MQ1042	2 channels	4 wells
MQ1084	4 channels	8 wells
MQ1082	2 channels	8 wells

3. Hardware set-up guide

This chapter describes the use and storage conditions of the MQ4 ultra-portable quantitative fluorescence PCR instrument, its structural components, removal of the fixture, installation/uninstallation of the software, and preparation for power-up.

Environmental conditions

1) Transportation and storage conditions of the instrument
   a. Environmental temperature： - 5℃ ~ 40℃ ；
   b. Relative humidity：≤75%
2) Working conditions requirements
   a. Environmental temperature：15℃~35℃
   b. Environmental humidity：35%~75%
   c. Input voltage： DC 15V 9.6A

Unpacking

1) The outer packaging of the product is a cardboard box, filled with shock-absorbing foam inside, after unpacking, first check whether the items you receive are missing or damaged.
2) If the outer packaging of the product is obviously damaged during transportation, please do not use it and contact the manufacturer and authorized distributor in time.
3) Check the completeness of the provided accessories against the packing list (Table 3-3-1).
4) If the instrument or accessories have been damaged or lost in transit, please inform the shipping company personnel and our customer service personnel.

Check the packing list

After opening the box, please check the contents of the box according to the packing list, if the items are found to be damaged or missing, please contact the manufacturer and the authorized distributor immediately.

Table 3-3-1 Packing list

Accessory	Quantity
Fluorescence	1

Quantitative PCR System	
Power Cord	1
Power Adapter	1
USB Cable	1
Instruction Manual	1
Factory Inspection Report	1
Warranty Card	1
Certificate of Conformity	1

Power up the instrument

The instrument is powered through an external AC/DC power adapter. The power adapter accepts AC input ranging 110V to 240V and produces DC output of 15V.

The DC power input connector port is located at the back of the instrument. Once the power is provided, the green power indicator light will turn on.

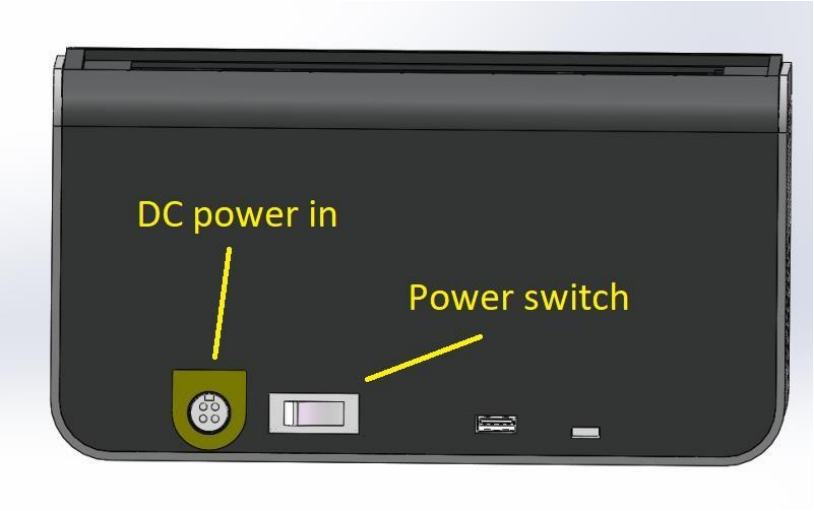

Figure 2.1 Maverick qPCR USB and power connections

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Connect to a PC via USB

The instrument is equipped with an USB port at the back, we can connect the instrument to a Windows® based PC via the supplied USB cable.

For instruments that are equipped with a touch screen, there is no need to connect the instrument to a host PC or Tablet.

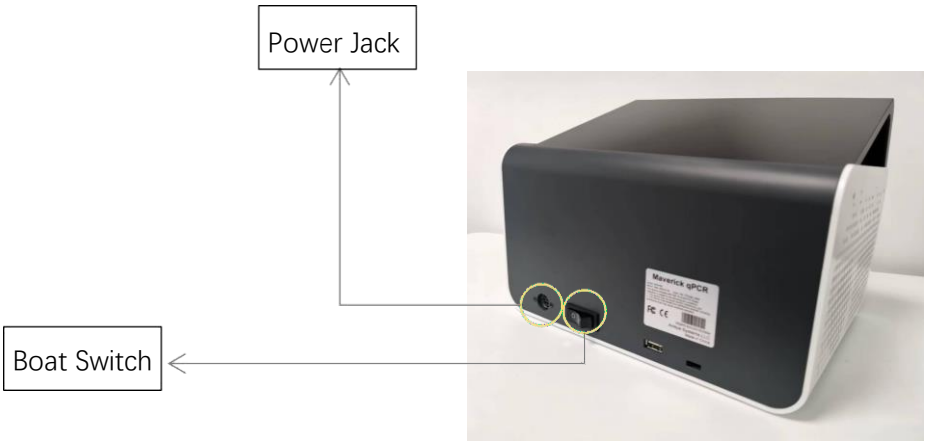
Light indicator states

There are two LED indicator lights. The green light is used to indicate power supply. As long as the instrument is powered, the green LED indicator will stay on.

Power cord connection

1) Adapter connection: the adapter supplied with the instrument should be used to connect the adapter to the instrument.
2) Power cord connection: use the power cord provided with the instrument. When connecting, the instrument power switch should be in the "off" state, and then turn on the instrument switch after connecting.

How to use the instrument



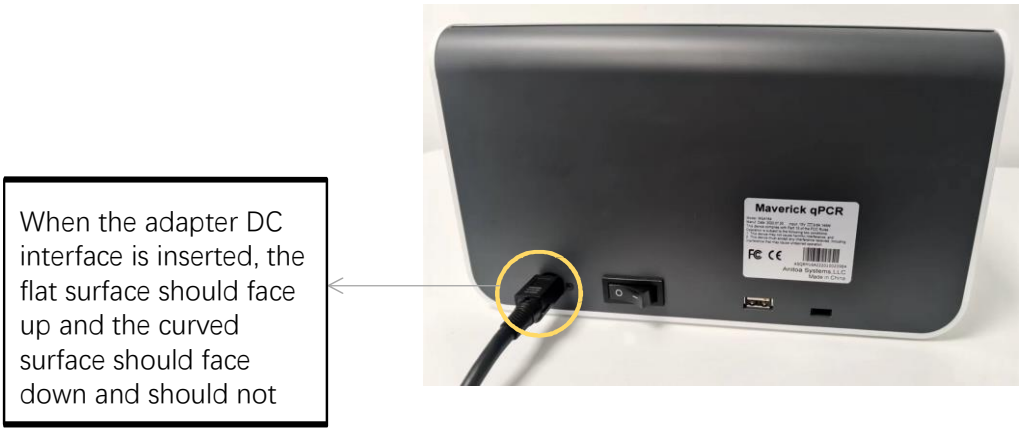


Figure 3-5-1 Back of the instrument

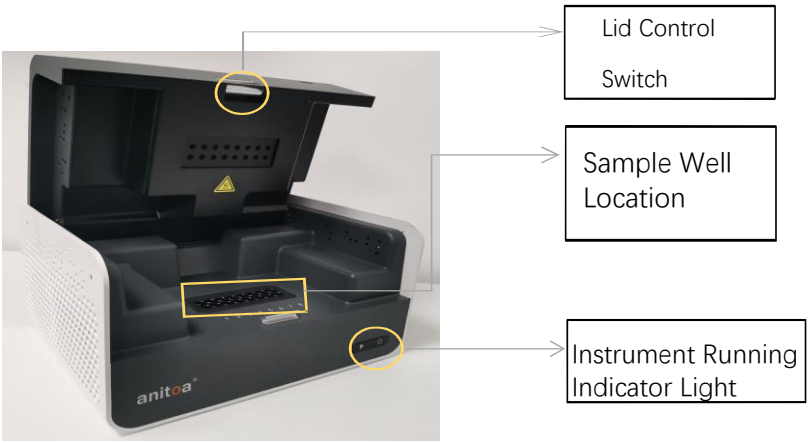


Figure 3-5-2 Front of the instrument

1) Insert the DC connector into the power jack of the instrument and hear the "click" sound to indicate that it is inserted in place, as shown in Figure 3-5-1.
2) Turn on the instrument power switch (boat switch) and the instrument power indicator lights up (green).
3) Open the flap of the instrument and put the collected sample tubes into the sample table position shown in the figure.

Close the flap, run the test, the instrument starts normally and then the operation indicator lights on (blue).

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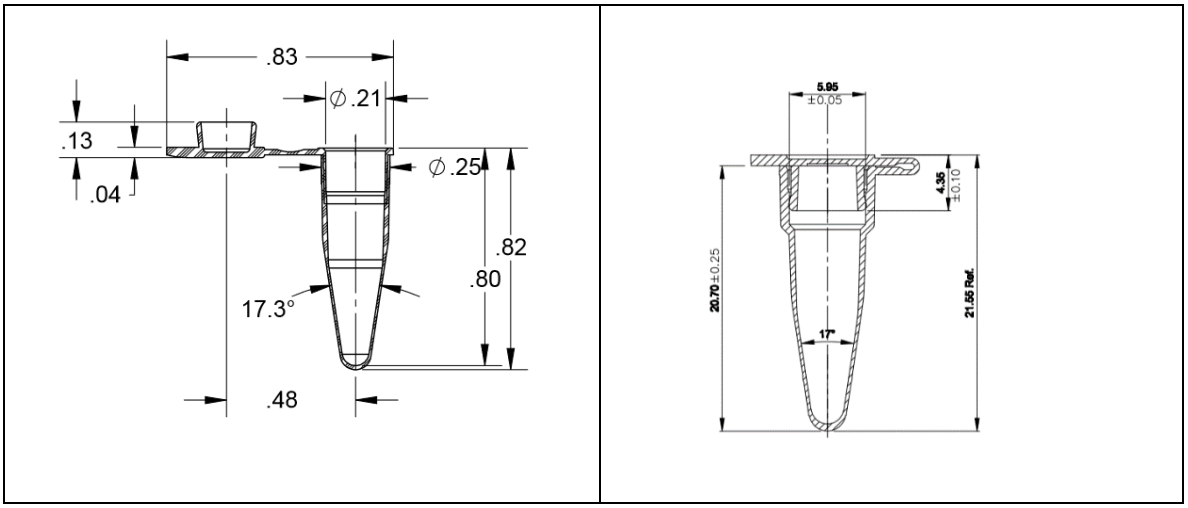
qPCR tube/plasticware requirement

Maverick qPCR requires the use of full transparent qPCR tubes with flat caps. See figure below for examples.

Below are drawings and dimension of the tubes we recommend both in English and Metric units



Some examples of the tubes that are proven to work in Maverick instruments:

UltraFlux® i PCR Tubes with Caps - 3247-00 from SSIBio
(https://www.ssibio.com/pcr/snapstrip-ultraflux-i-ultraflux-z-pcr-tubes/ultraflux-i-pcr-tubes-with-caps/3247-00-detail)

EasyStrip™ Plus Tube Strip with Attached Ultra Clear Caps AB2005 from Thermo Fisher
(https://www.thermofisher.com/order/catalog/product/AB2005)

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Axygen® 0.2 mL Polypropylene PCR Tube Strips and attached Flat Cap Strips, 8 Tubes/Strip, 8 Flat Caps/Strip, Clear, Product Number PCR-0208-AF-C

(https://ecatalog.corning.com/life-sciences/b2b/US/en/Genomics-%26-Molecular-Biology/PCR-Consumables/PCR-Tubes-and-Strip-Tubes/Axygen%C2%AE-PCR-Strip-Tubes/p/PCR-0208-AF-C)

4. Software User Guide (Windows-based Software)

System Requirement

The Maverick software Windows version required Microsoft Windows 10 or later (Including Windows 11). The desired hardware spec. for the laptop (or desktop) system is listed below:

PC Laptops

	Minimum Specifications	Recommended Specifications
OS	Windows 10	Windows 10
Processor	Intel Core i3 or equivalent	Intel Core i5 or better*
Memory	4 GB	8 GB or more
Wireless Network Adapter	802.11g/n	802.11n/ac/ax
Hard Drive	80 GB hard drive space	120 GB hard drive space or larger

- *If use AMD CPU systems, The AMD Ryzen 5 or better is recommended.*

Install the software

The software is delivered as pair of installation files: setup.exe, and Installer.msi. Run setup.exe and follow instructions to install the software.

If the file is one compressed file, please decompress it first. If you do not have a compress/decompress utility installed on your computer, go get one at: http://www.7-zip.org . It is free.

The installed application file will be in C:/Anitoa/Maverick by default.

To launch the Maverick software, click the Windows® Start button on the left bottom corner of the desktop and find Maverick software. See below:

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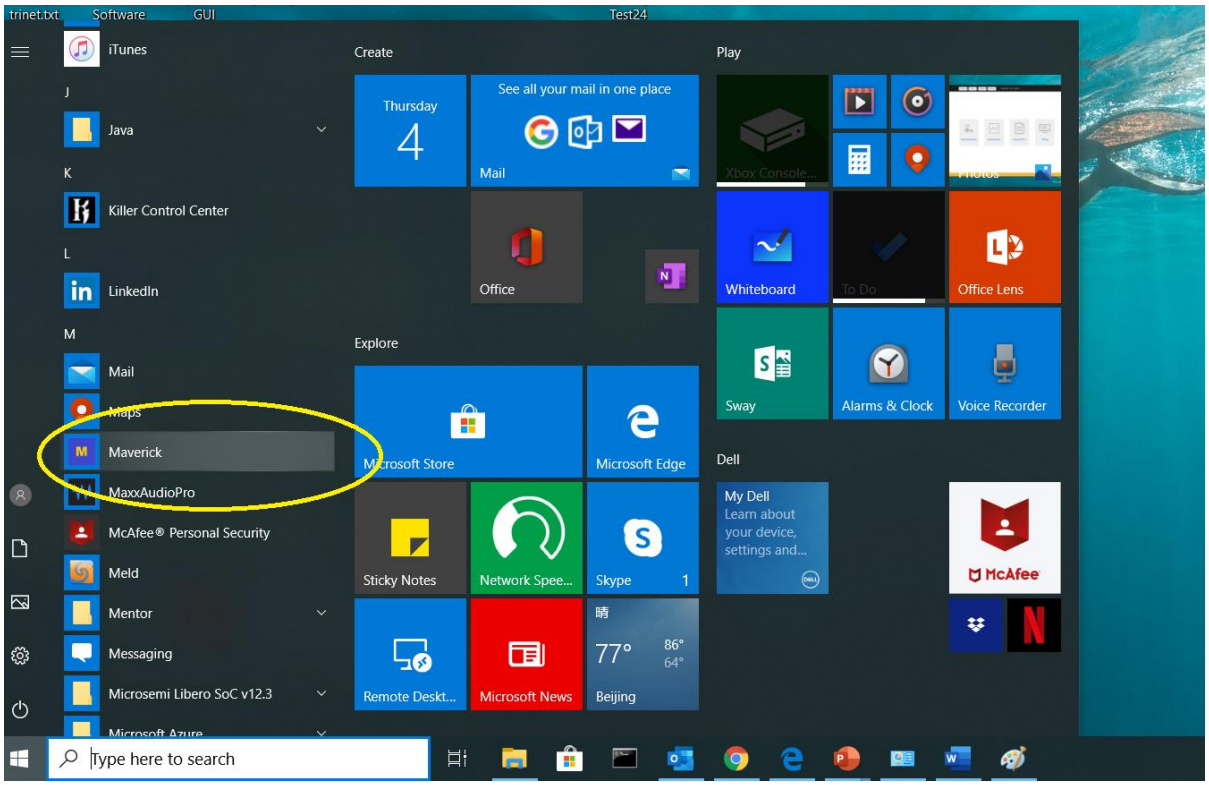

An icon for Maverick software will also appear on the desktop. We could launch the software by clicking on this icon.

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Launching the software

When the Maverick PC software is launched, after a brief delay for device initialization, the front page will display as shown in Figure 4-1.

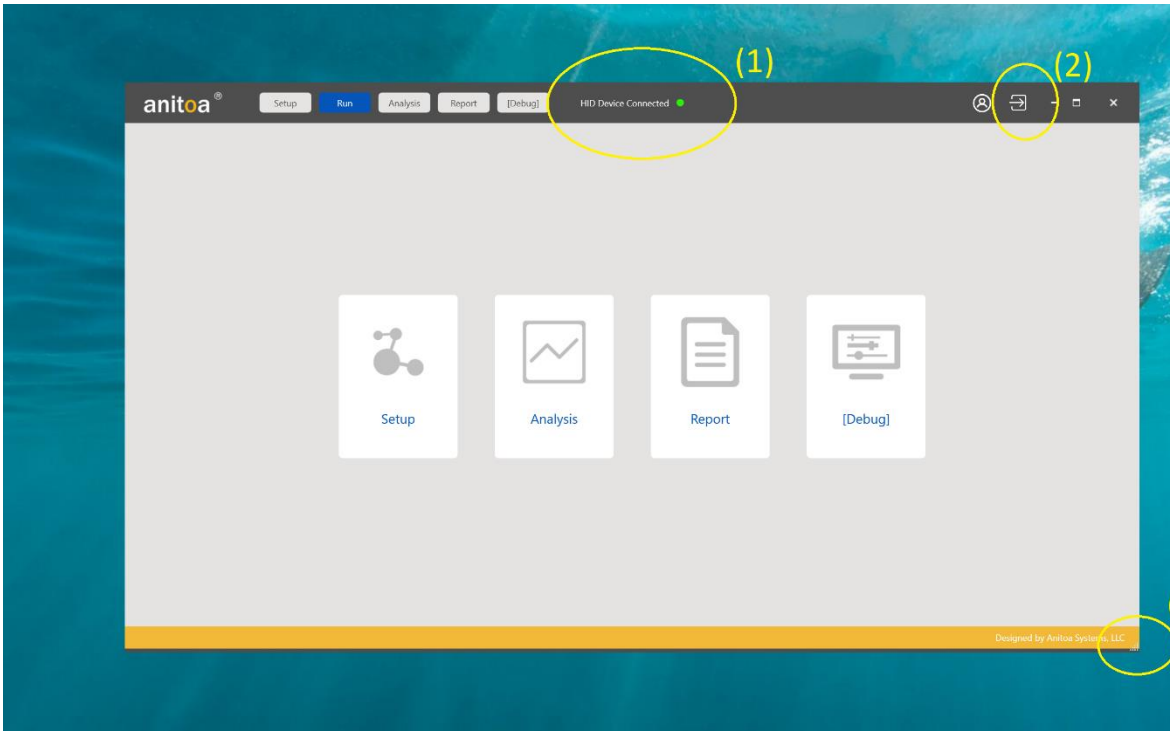

Figure 4-1 Maverick software front page

Several notable features are marked in Figure 4-1. Marker (1) is an indicator of whether the Maverick USB device is found. If found, a message “HID Device Connected” is shown along with a green dot. We call it HID because the Maverick instrument uses USB HID device class.

The home button

Marker (2) is the home button. At any time of running this software, click this button to return to this front page.

Resizing the window

Marker (3) is the place to drag to resize the Maverick software windows.

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The setup page – Name setup

Click on the “Setup” button will take us to the setup page (Figure 4-2). Here we can set up the experiment. Here we can name the experiment and all the samples in the wells. We can also name and select the fluorescence channels for qPCR fluorescence reading.

A default date-coded experiment name is given on this page when we first enter. Feel free to change it to something you choose.

Samples and fluorescence channels set up

The software automatically detects the number of reaction wells the connected instrument can support. Each reaction well can be associated with a sample. In this page, the user can enter a name for each sample. It a sample well name field is left empty (white spaces), it is considered that the sample does not exist for that well.

It is important to select the fluorescence channels (Marker (2)) we need to use for the experiment. The selection of the channels depends on the type of fluorescence dyes used in the assay design. We can also give a name for each selected channel

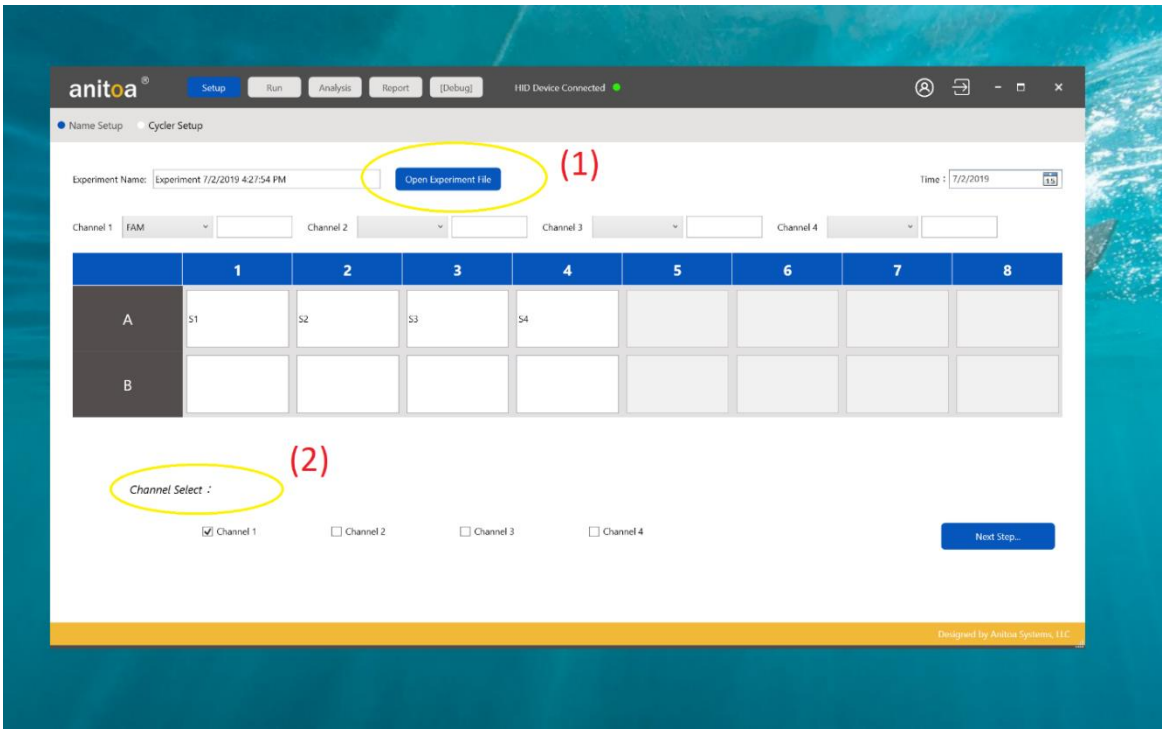

Figure 4-2 The setup page – sample setup

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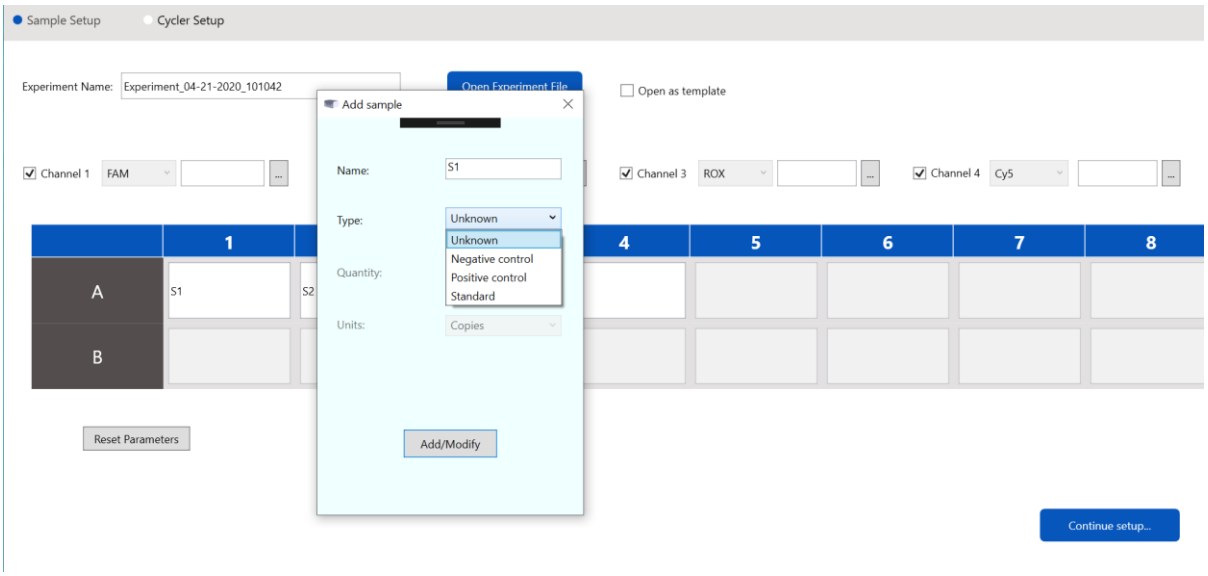
Open existing experiment for template file

We can also load an existing experiment file or template file by clicking on the “Open experiment file” button (Marker (1)).

When this step is done, we can click on the “Next step” button to continue setting the thermal cycling program.

Additional sample property setup

By double clicking the sample grid in the Sample setup window, the user can set up additional information about the sample by entering values in a pop-up window shown below. Additional property includes type of sample as well as standard quantities.



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The setup page – Cycler setup

Here we can setup the thermal cycler program. We could choose the type and number of thermal periods and steps, assign time period and temperatures. We could have up to two Pre-Denature periods. The main thermal cycling period can have 2, 3, or 4 steps. We can also define at which step the fluorescence capture is done. Lastly, we have a hold period whose temperature and time period are configurable.

A cycler program can have several periods; and each period can have one or more steps. For example, we could have pre-denature period, cycle period and hold period. In the cycle period, we can have many repeated cycles consists of denature, annealing, and extension steps.

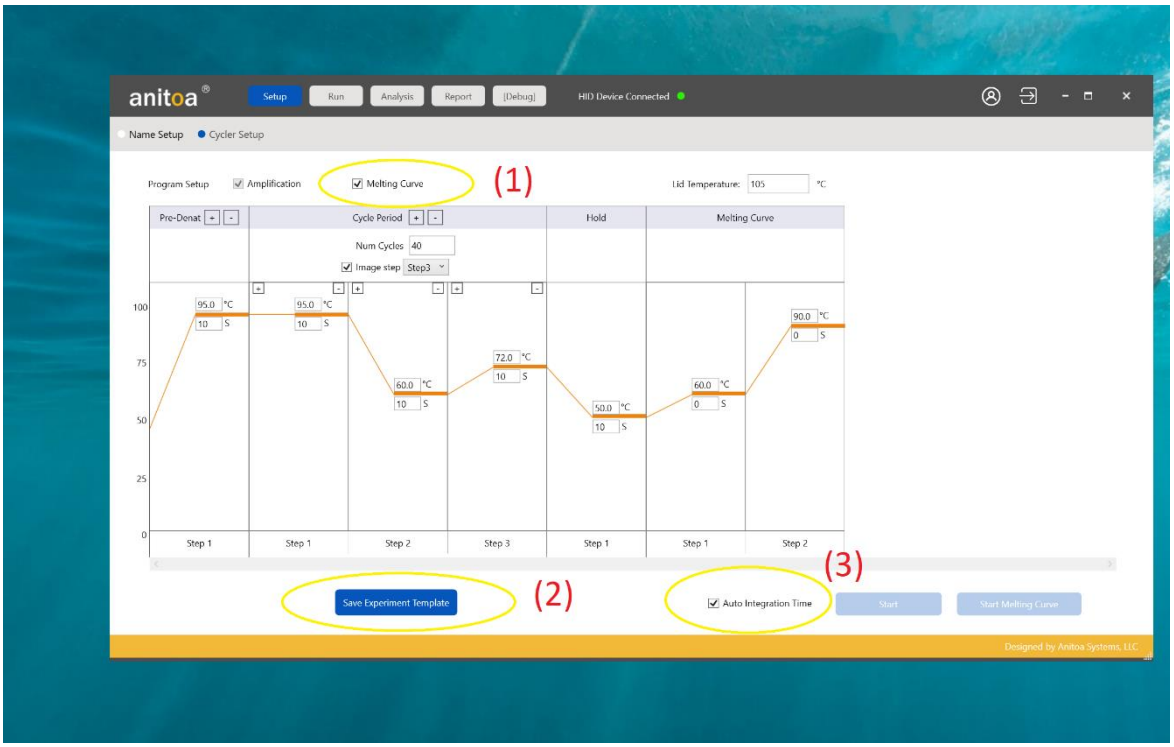

Figure 4-3 Cycler setup

qPCR amplification thermal cycling program starts when the user clicks on the “Start” button.

Melting curve analysis period

We can also add a Melt Curve (Marker (1)) analysis period at the end of the main thermal cycling program. The Melt curve analysis can be triggered either manually by clicking the start melt analysis button, or automatically by checking “” check box (not done yet).

Hot lid temperature and reaction volume

In this page, we can also set the desired hot lid temperature and reaction volume. The latter (reaction volume setting) is used for data analysis only. It will not affect how the experiment is run.

Saving template

At this step, we can also save the settings to a template file (Marker (2)). So next time when we perform a similar experiment, we can just load the template file instead of manually entering all the information again.

Auto Integration Time

This is an option to let the instrument automatically choose the optical sensor integration time (i.e. Exposure time of the fluorescence camera) according to the fluorescence strength of the assay (Marker (3)). The goal is to adjust the sensitivity of the fluorescence camera so that the signals from the chemistry fall into the appropriate dynamic range of the sensors and yield the best signal to noise ratio (signal quality). We recommend leaving this check box checked.

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Running the experiment and monitor status

In the setup page – cycler setup, when we are satisfied with all the settings and loaded the samples, we can start running the experiment by click the “Start…” button.

Before the instrument starts to execute thermal cycling program, it will automatically check to ensure the lid is closed and the main power is applied. If either of these conditions is not met, the software will show popup windows with warnings. We could correct these programs and start again by simply press the “Start…” button again.

When the qPCR cycler starts to run, the software will automatically switch to Run pages. The first Run page will allow us to monitor hot lid and reaction well temperature. We can easily see if the temperature is going through the cycling programs or not.

Running amplification program

When running normal qPCR amplification program, the amplification window will real time monitor the fluorescence signal from the samples. In addition, the Run-Amplification Curve page will also show the current run status and estimated remaining run time.

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Running melt analysis

When running Melt analysis, the “Run-Melt curve” will show the fluorescence change rate as function of temperature.

Figure 4-4 Melt analysis

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Analysis

There are two analysis pages: Amplification analysis and Standard curve analysis. The latter is used for determining the quantity of the DNA targets using standard curve analysis method.

Amplification analysis

This will show the amplification curves for all samples. We can select the samples and study, set several parameters to this analysis.

Ct Threshold

The threshold for determining Ct is expressed as the percentage of the saturating level of the amplification levels after normalization. The default Ct threshold is 10%. The bigger this percentage, the more delayed the Ct values are.

Ct Low limit

The minimum value of Ct is set through this parameter. This setting is helpful in determining the base fluorescence value for amplification curve analysis. In most qPCR experiments, the concentration of the analytes prior to amplification is low, such that the Ct value cannot be less than 13 or so.

Normalize

When displaying amplification curve, we usually normalize the fluorescence values. This will allow us to visualize Ct threshold value. This choice will not affect the result of the analysis.

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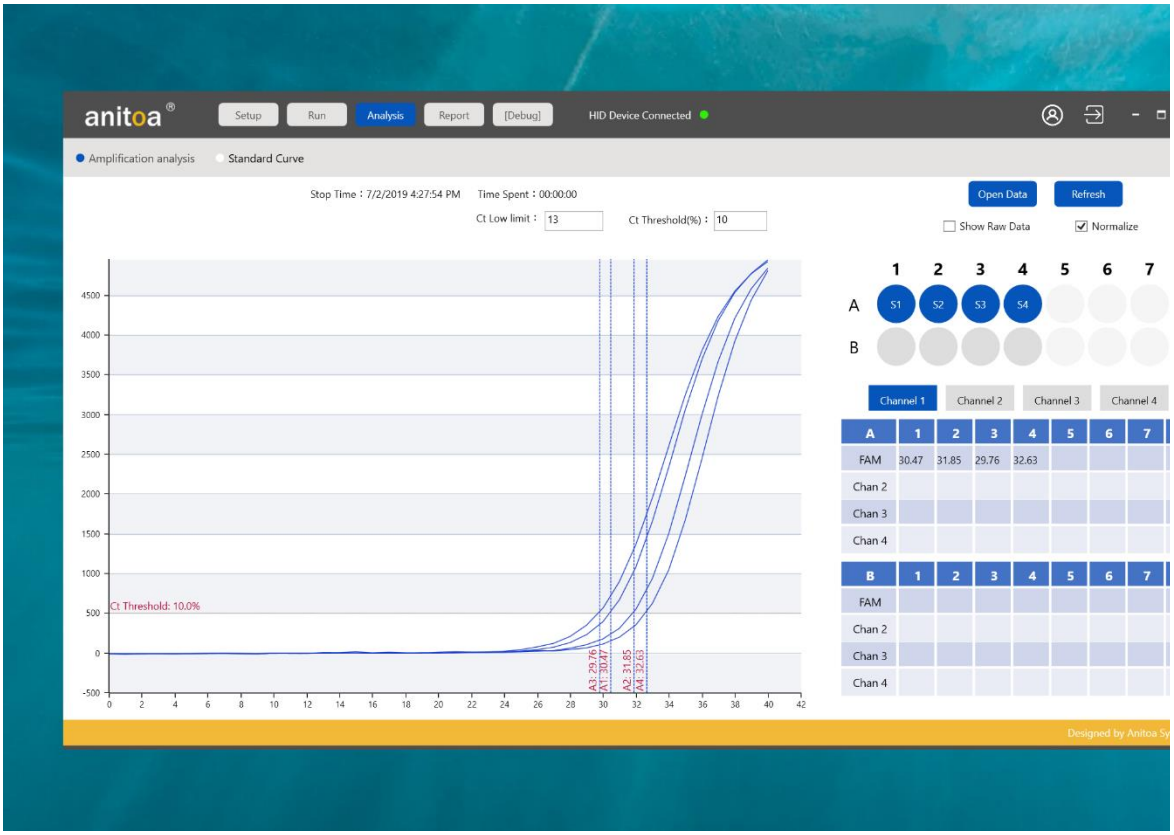


Figure 4-5 Amplification analysis

Positive vs Negative results

For each sample's DNA target, we get a positive vs negative result. Generally, the negative result will be shown as "Neg" and the positive result will be shown with a Ct value between Ct low limit and maximum cycle number. If we mouse over the Ct value in the data table, we will see some additional parameters that justifies the positive/negative test. These parameters include the saturation fluorescence level, amplification efficiency and fluorescence reading signal to noise ratio.

False positive results

Sometimes a negative result is the result of a "false positive" result. If we mouse over the result, we see that the "Confidence" level of positive is below a threshold, for example, 10%. The confidence threshold is adjusted through advanced setting window.

Further discussion of how to judge positive vs. negative results is given in Appendix B.

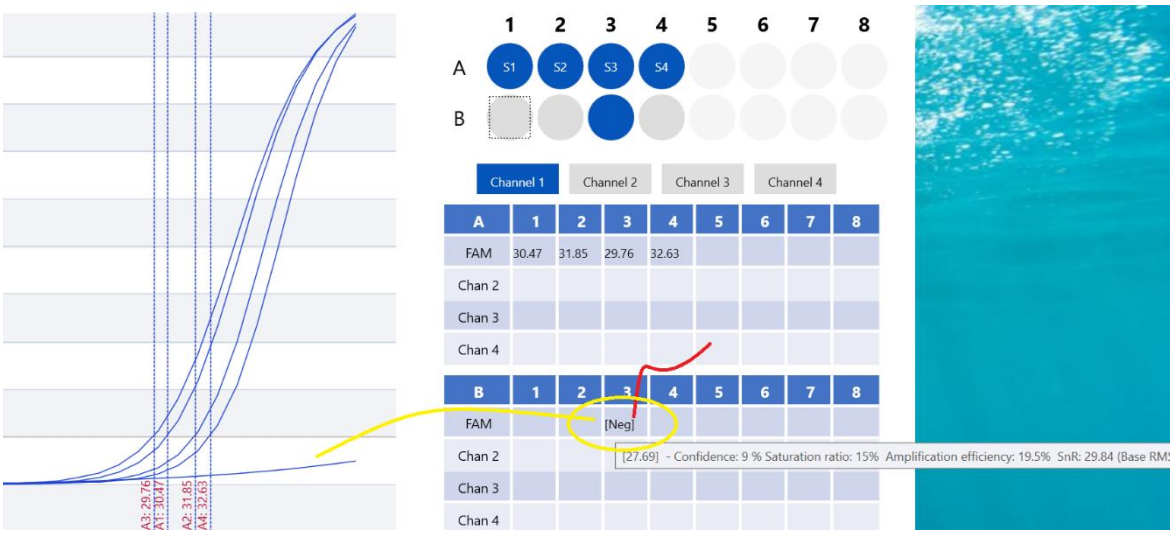


Figure 4-5 Positive vs Negative analysis

.

Standard Curve Analysis

Standard curve analysis is used to determine the absolute concentration of a sample by comparing to other samples with known amount of analyte concentration. We called the samples with known concentration “standards” and the ones with concentration that needs to be determined “unknowns”.

For such analysis, the “standards” and “unknowns” should be in the same fluorescence channel to ensure they are comparable. Thus, standard curve analysis is always performed on one specific channel.[3] We have to first the select the specific channel and perform such analysis.

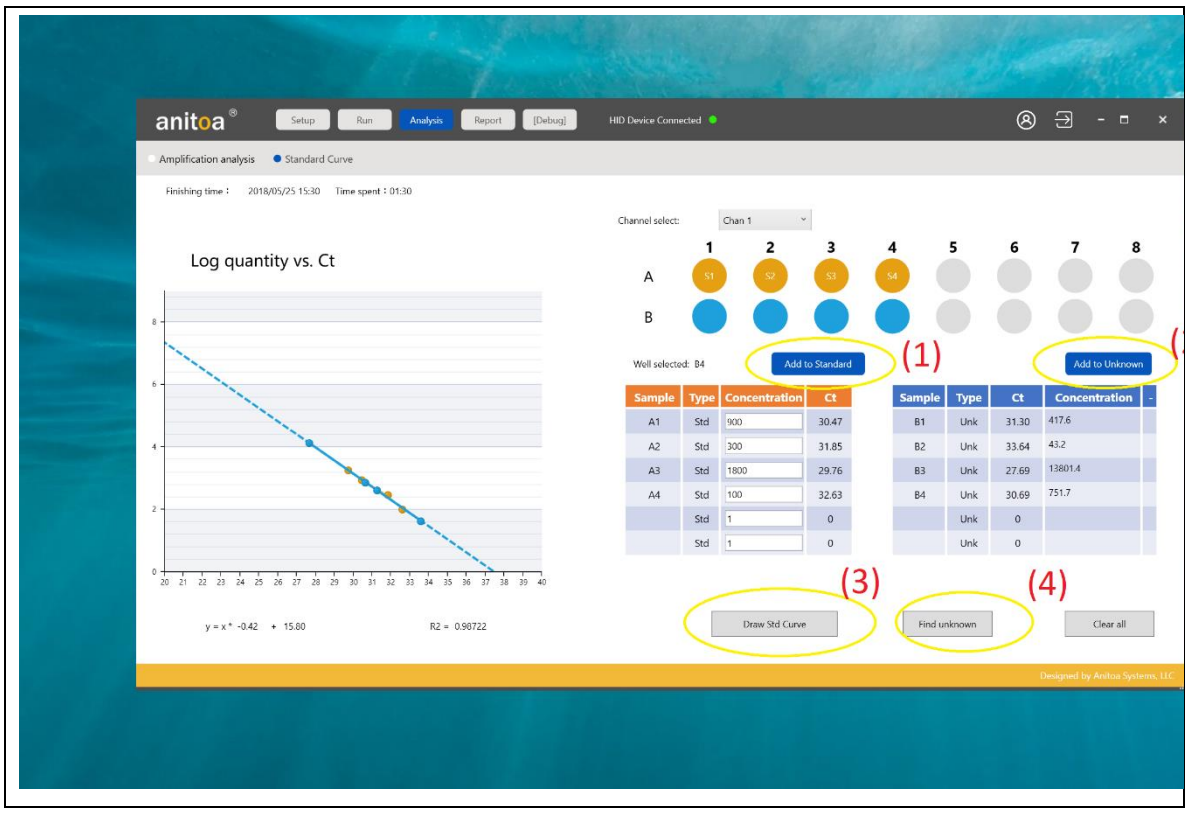


Figure 4-7 Standard curve analysis page

[3] We usually also make sure no other competing DNA targets and primer/probe sets are present in standard curve analysis samples, unless in very low concentration.

.

Steps to perform standard curve analysis:

Step 1:

Select the fluorescence channel for such analysis

Step 2:

Add standard samples – we can select the samples that are standard and click “Add the standard” button (Marker (1)) to add to standard table. We can then assign the concentration numerical values to these standard samples by typing into the table.

Step 3:

Draw the curve by clicking “Draw Std Curve”. (Marker (2))

Step 4:

Add unknown samples to unknown table (Marker (3))

Step 5:

Calculate concentration for unknown samples (Marker (4)).

.

Save experiment and Print Report

Open report page to print report and save experiment.

The experiment file will contain all the settings and experiment results.

Two report formats will be used for report generation. The excel file report is hand editable. The PDF report is easier to store and use on different platforms.

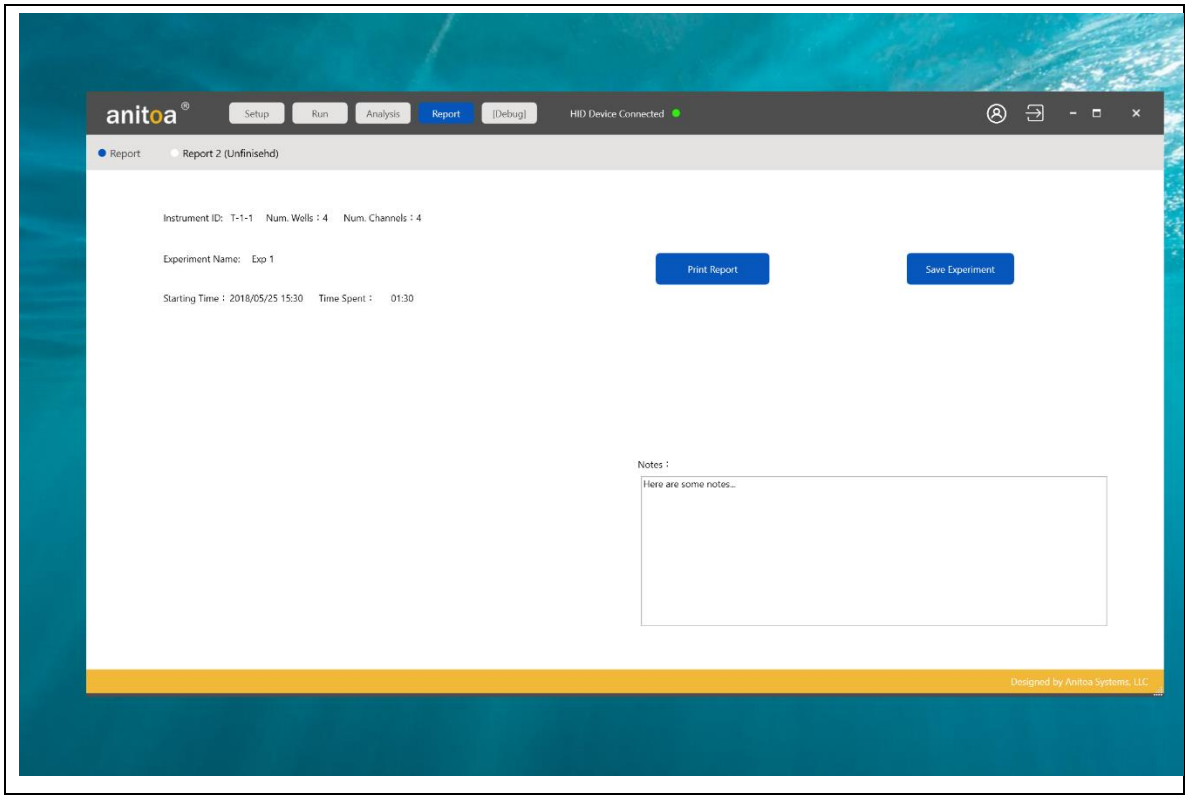


Figure 4-8 Save and export page

In this page, we can also enter the operator's name and some comments. These will be saved with the experiment file.

5. Software Operation Guide (Android Software)

Anitoa qPCR software can be used to set up experiments, run experiments, and collect, analyze and manage experimental data. The software contains three main functional modules, namely "Test", "Data " and "Setup".

1) "Test" module: It mainly includes creating new experiments, setting experimental parameters, importing experimental templates and running experiments.
2) "Data" module: mainly contains experimental data analysis, upload, import data, export data, export PDF, standard curve.
3) "Setup" module: mainly contains upload configuration, user management, user switching, WLAN, Bluetooth, language, virtual keyboard, date and time, display, application information, version update, software version check, instrument number.

Launch software

1) After the instrument is turned on, it automatically enters the main interface of the software - Test.

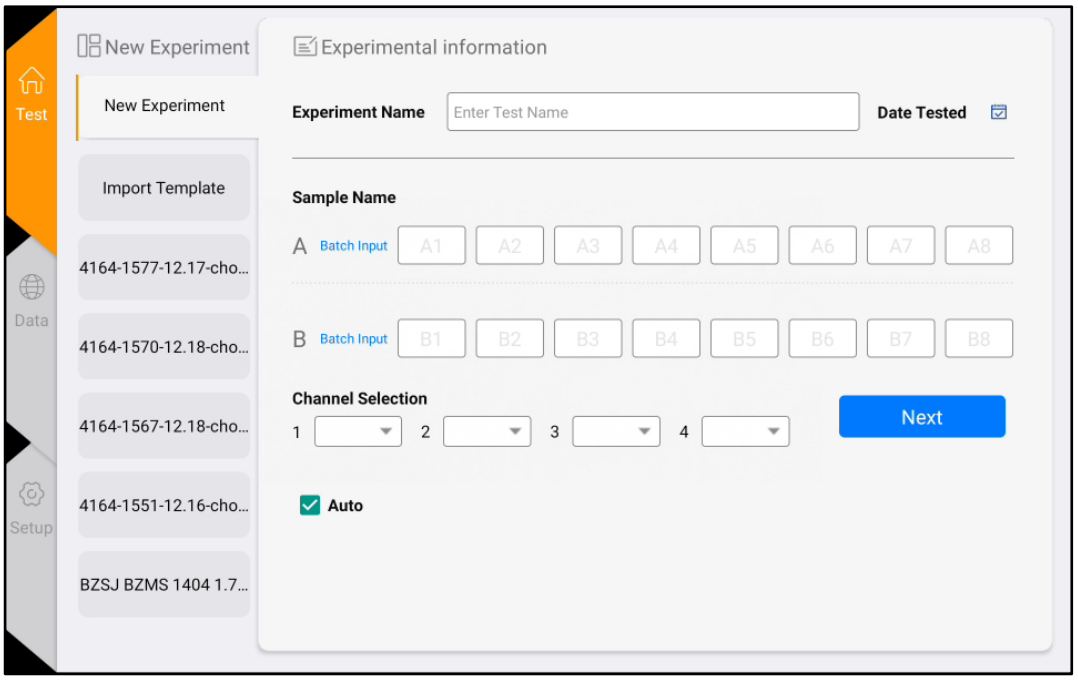


Figure 5-1-1 Main interface of the software

Experimental settings

1) In the Test interface, click <New Experiment>(Figure 5-2-1) the right panel is the Experiment Setting 1, in the Experiment Setting 1 (Figure 5-2-2), enter the experiment name, select the corresponding channel, select the well position, and enter the sample information, etc., and click "Next" to enter the Experiment Setting 2.

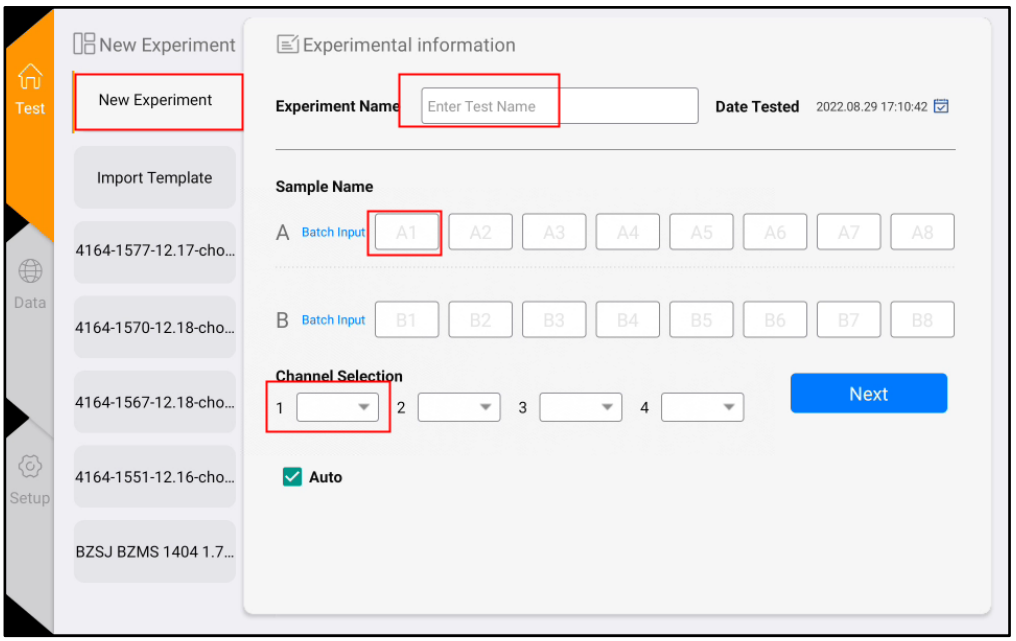
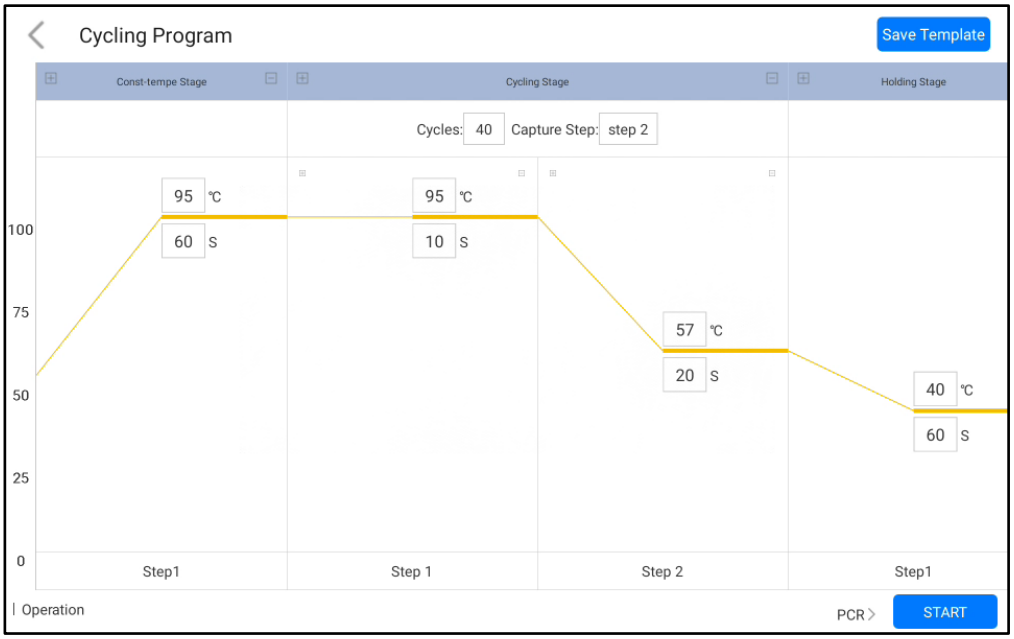


Figure 5-2-1 New experiment interface

2) In Experiment Setting 2 (Figure 5-2-3), set the reaction program (parameters such as reaction

temperature, reaction time, number of cycles, photo stage, etc.).

Figure 5-2-2 Experimental Setting 2



Save Template

1) Click the "Save Template" icon in the upper right corner to save the current template.

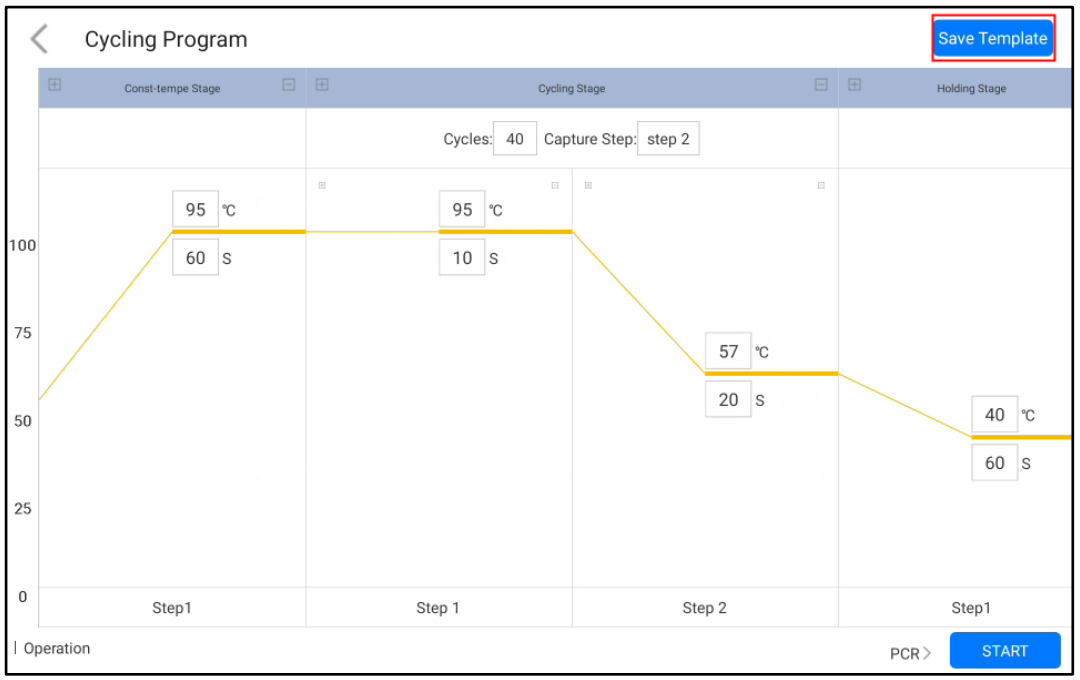


Figure 5-3-1 Program setting interface

2) The saved templates will appear in the "Test" interface for selection. Click the second option "Import Template" to import other experiment templates.

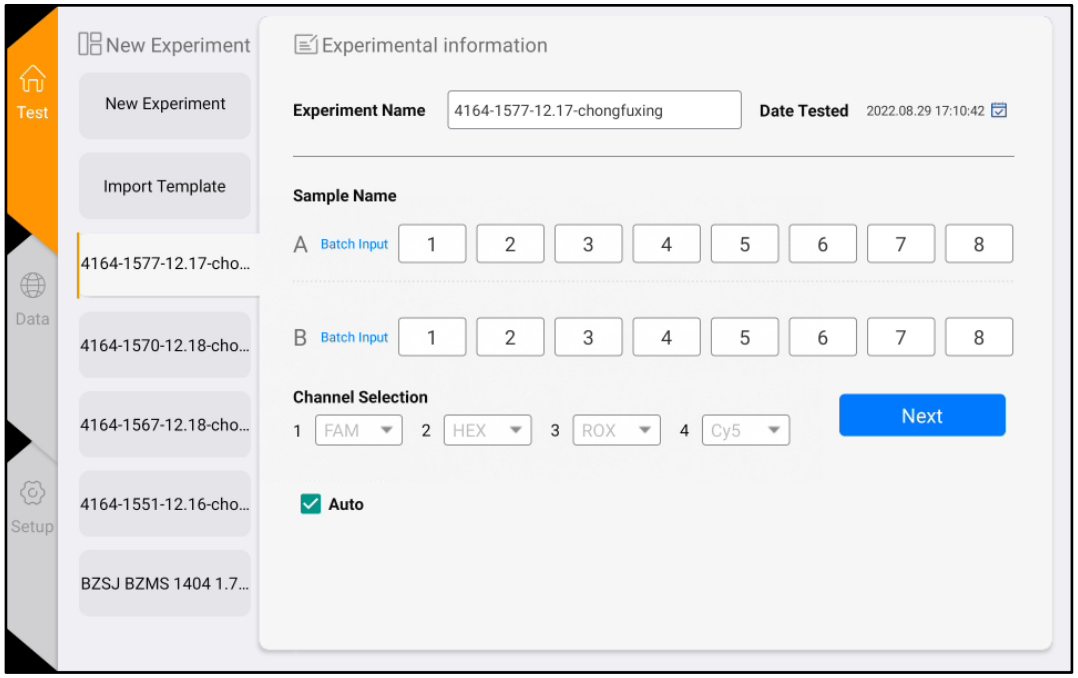


Figure 5-3-2 View the template page

Run the experiment

After the experiment settings are completed, click the "Start" button to start the experiment and enter the "Running" interface. Click "Force to stop" at the top right corner to stop the experiment.

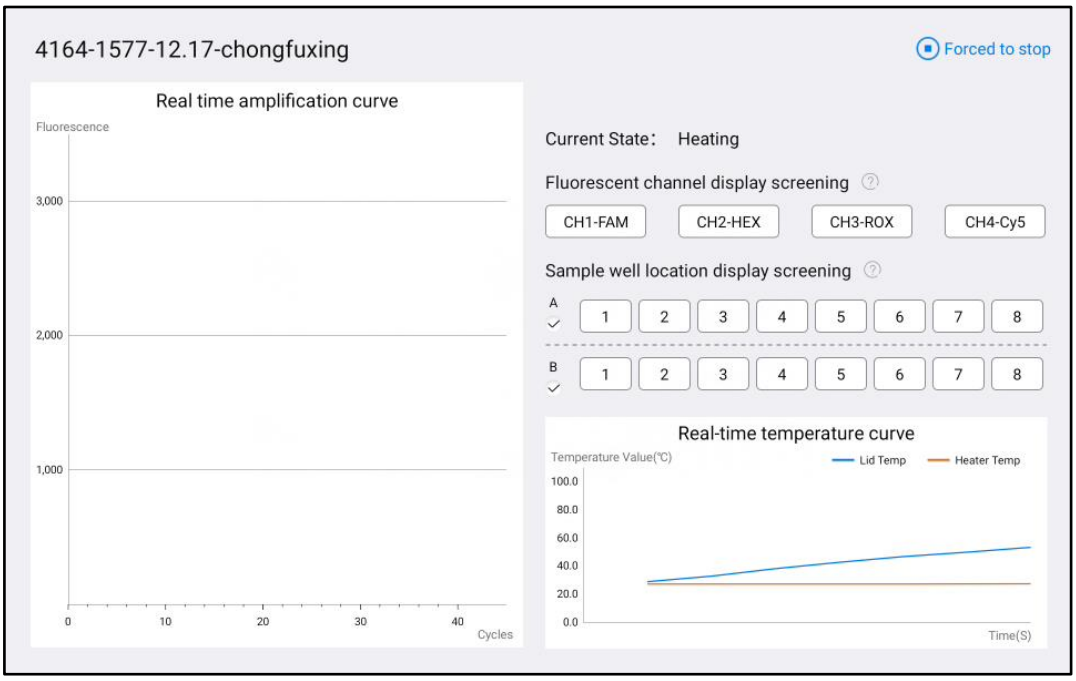


Figure 5-4-1 Running interface

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In the experiment running interface click the channel buttons and sample well buttons on the right side to filter the image information, and the sample wells have an all-select button to filter the whole row.

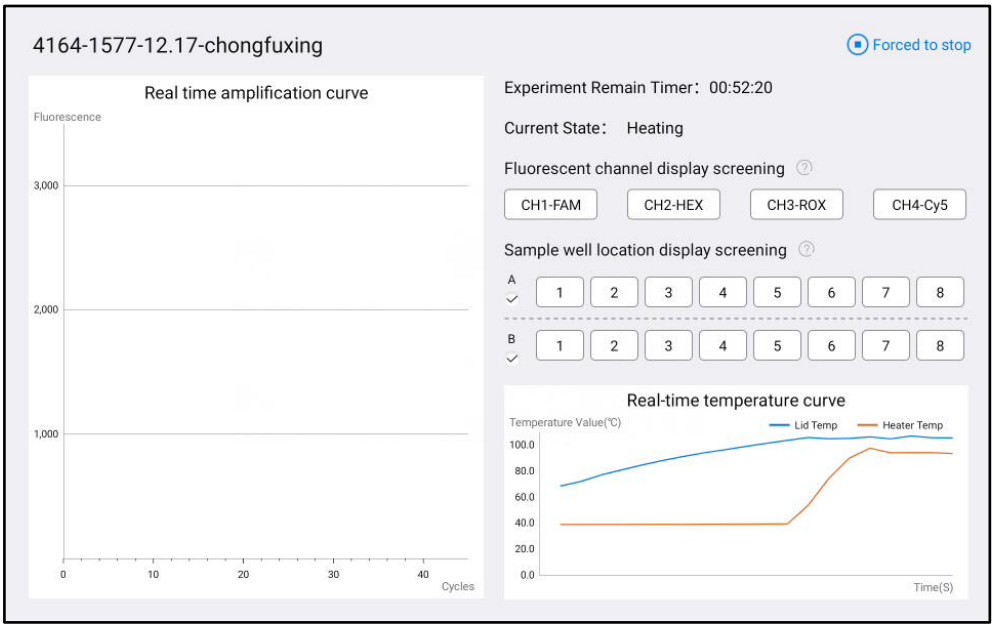


Figure 5-4-2 Running interface

Experimental analysis

1) Click the "Date" button on the main page to enter the "Result Analysis" page, as shown in Figure 5-5-1.

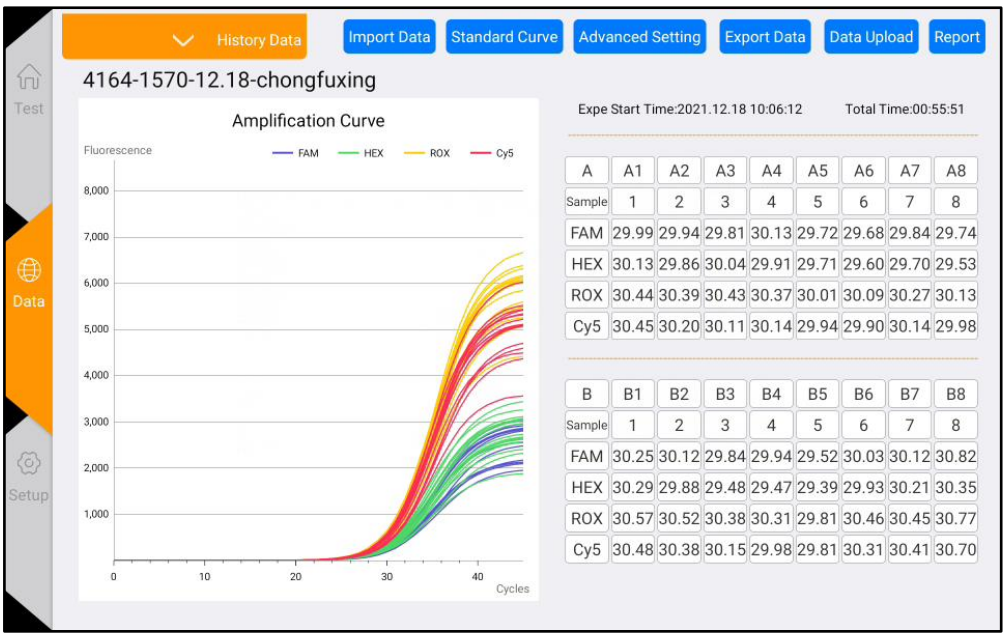


Figure 5-5-1 Result analysis interface

2) Click the History Data button above, you can pop up the historical experimental data results, select any one of the experimental results, you can view the data, such as Figure 5-5-2.

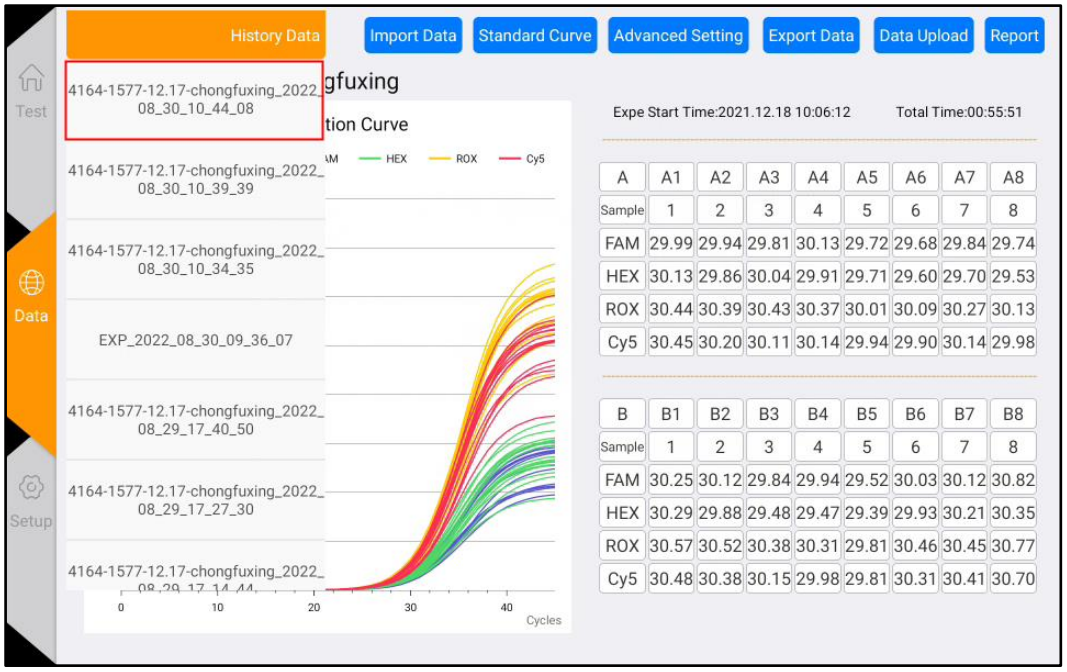


Figure 5-5-2 Date list

3) Experimental analysis (click the Advanced Setting button)

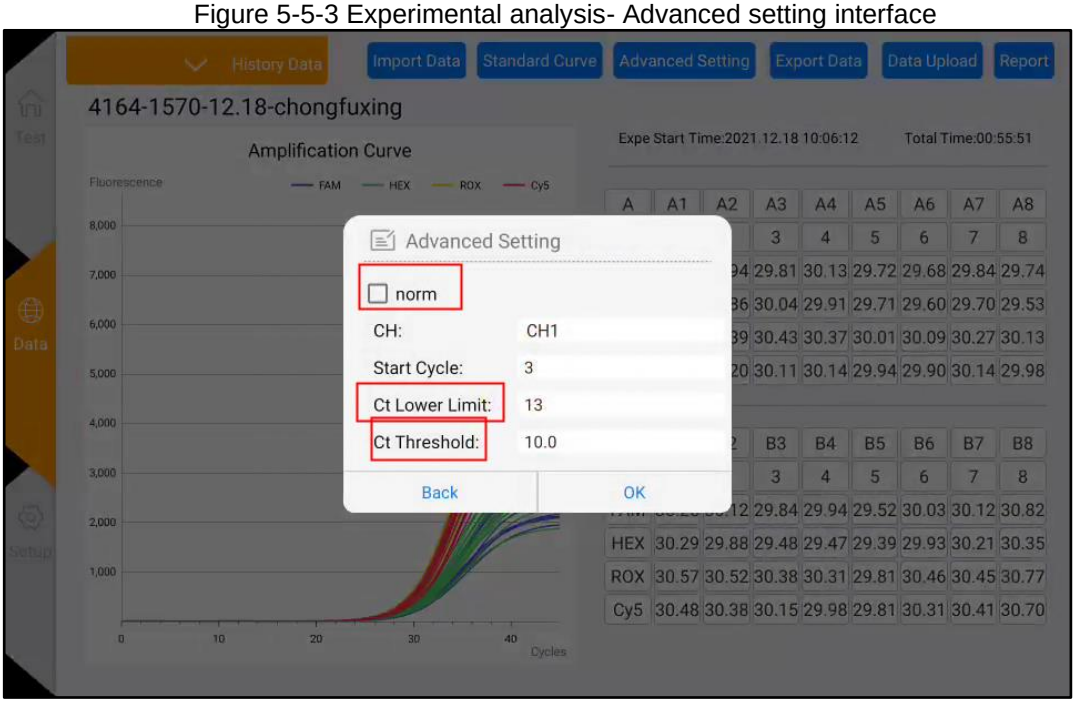
a. Adjust the baseline Ct lower limit.

b. Adjust Ct threshold percentage: default is 10%.

c. Normalize: normalize the amplification curve without affecting the results.

.

Figure 5-5-3 Experimental analysis- Advanced setting interface



d. Click on the channel and well buttons in the data table on the right to select the wells and channels for data analysis. (Gray button means not selected)

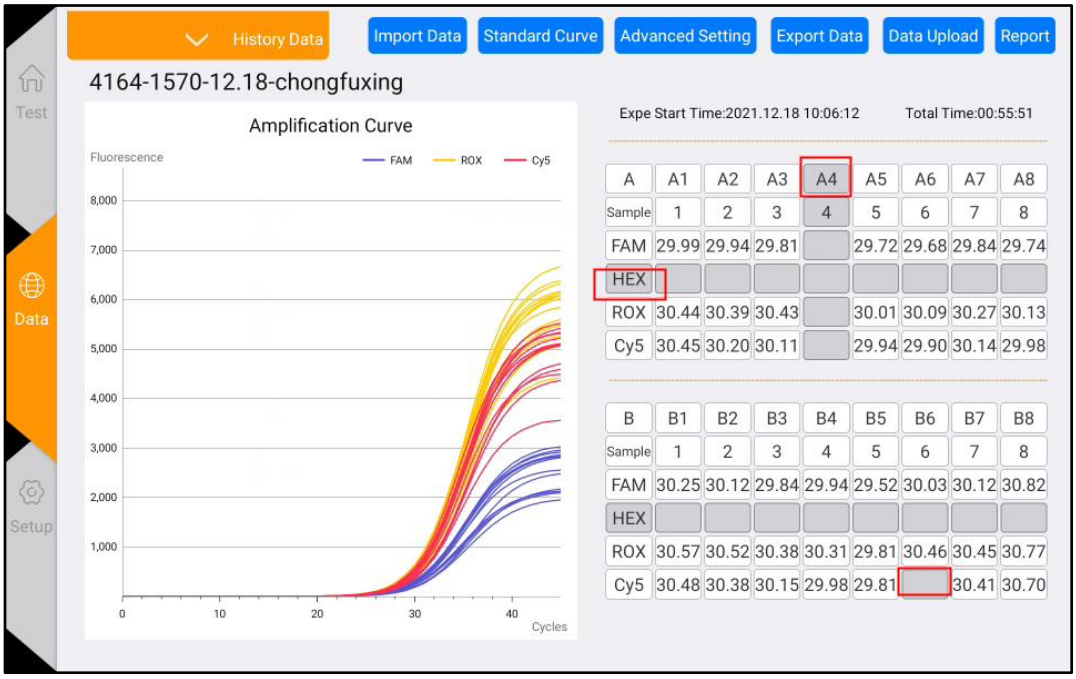


Figure 5-4-5 Data filtering interface

4) Calculate the standard curve

a. Click the Standard Curve button in the data interface to enter the standard curve interface (as

shown in Figure 5-5-4).

b. Manually input the standard concentration, calculate the standard curve, and save it.

c. Then select Unknown Points, click Find Unknown, and select Use Saved Standard Curve to calculate the concentration of the unknown sample based on the Ct value.

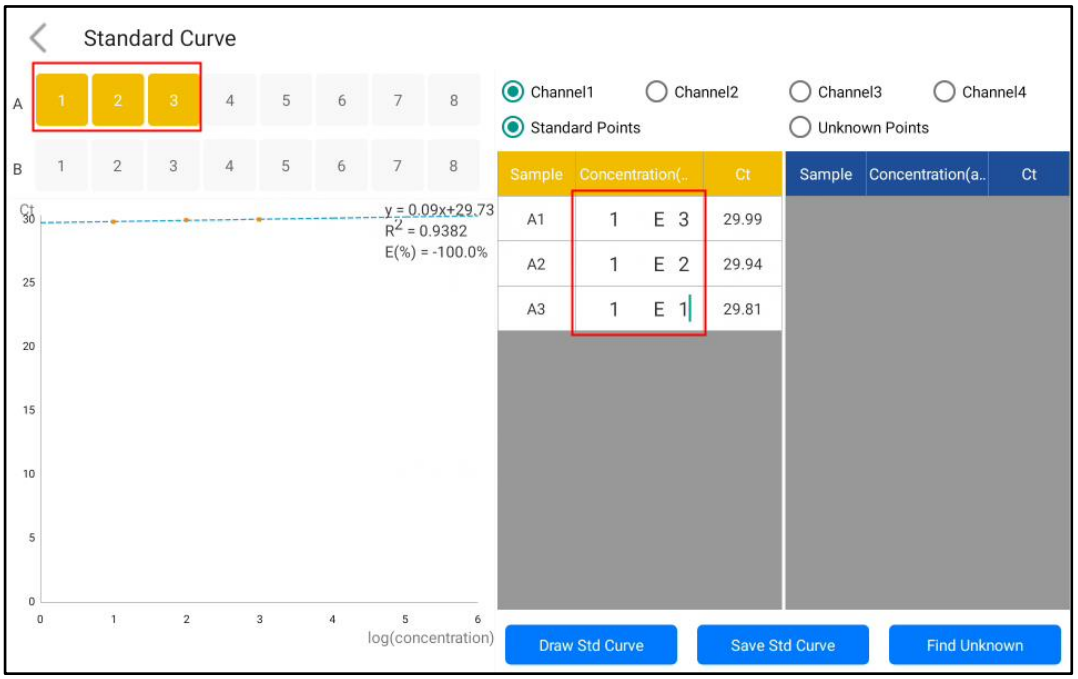


Figure 5-5-4 Standard curve interface

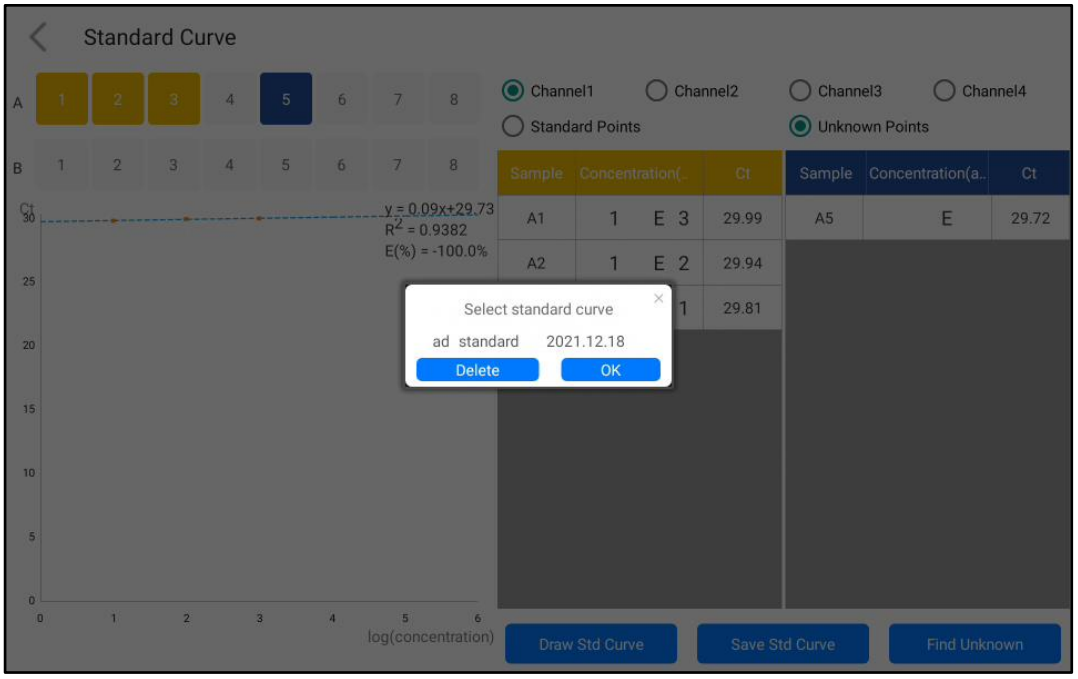


Figure 5-5-5 Selecting the standard curve used for the calculation

.

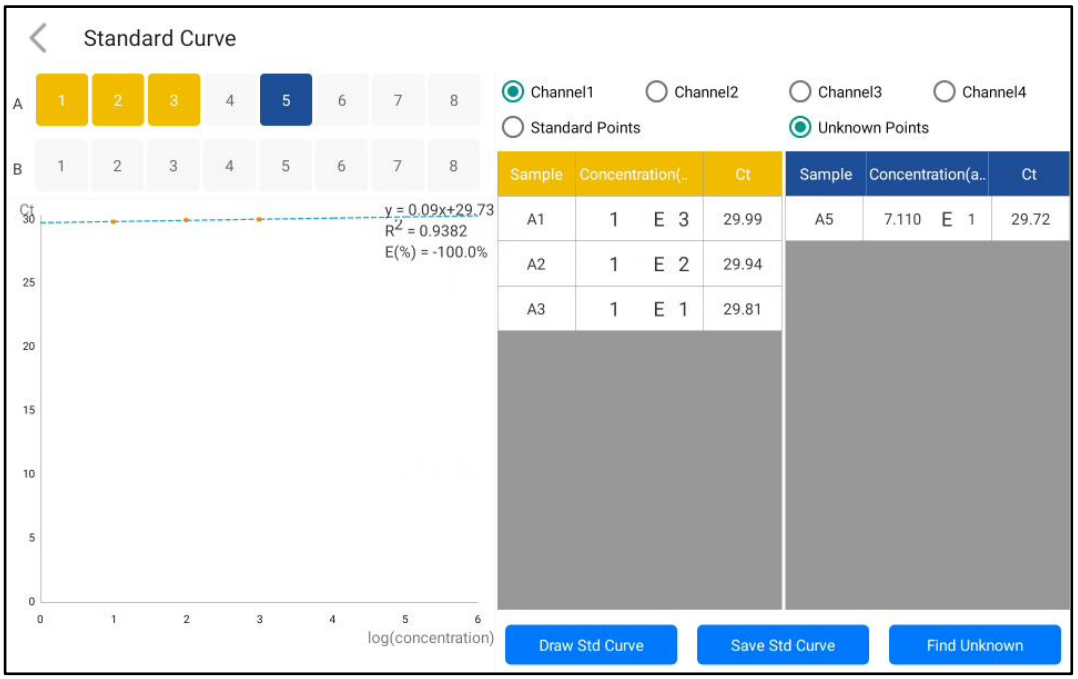
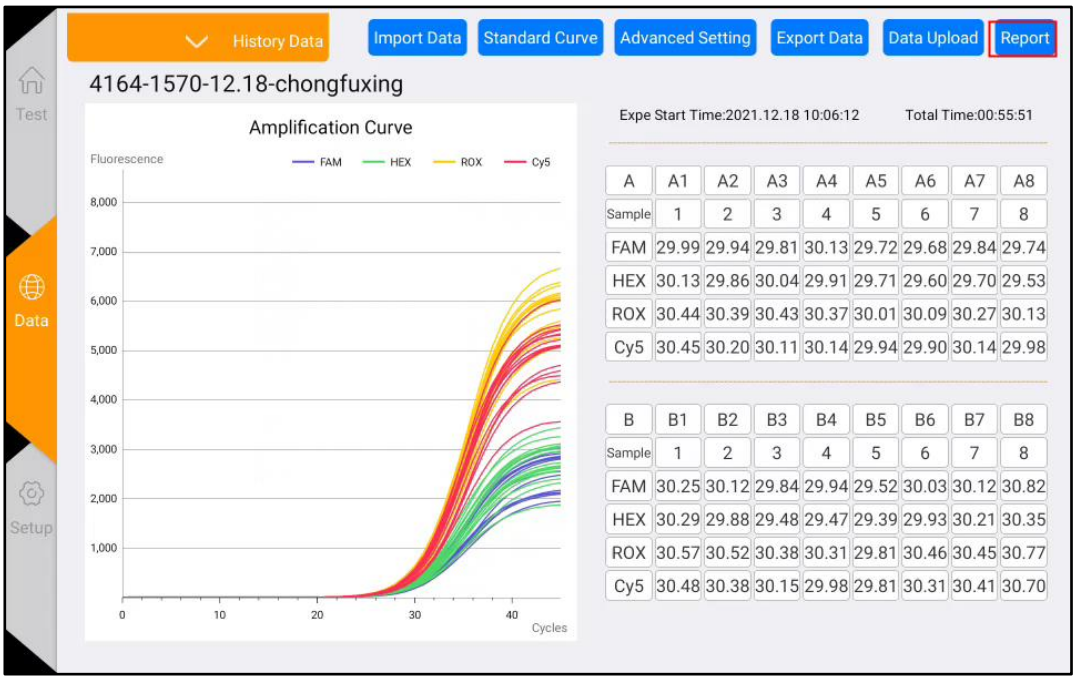


Figure 5-5-5 Calculating unknown points

Data export

1) Click the "Report" button in the experiment analysis interface to enter the PDF preview

.

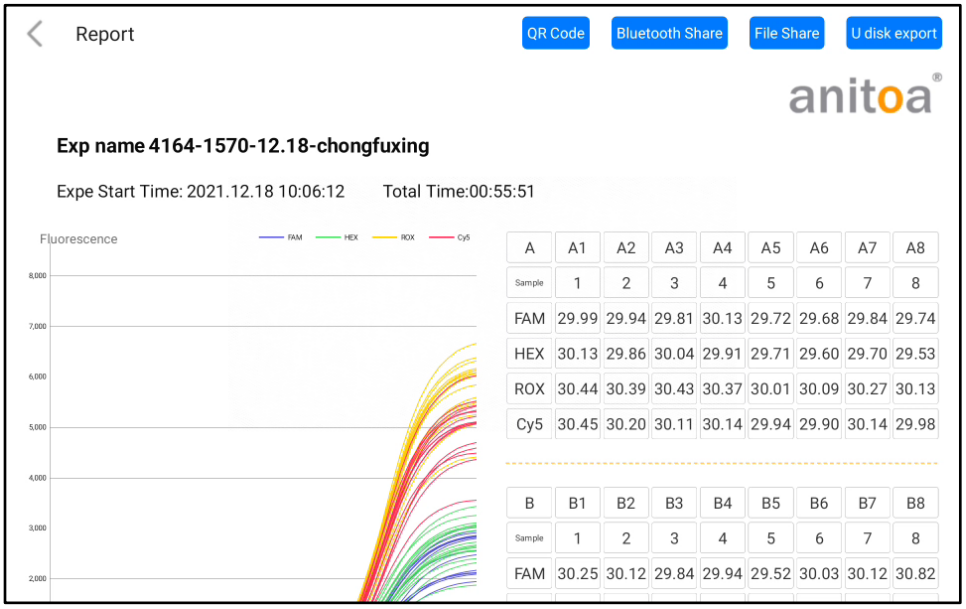


A	A1	A2	A3	A4	A5	A6	A7	A8
Sample	1	2	3	4	5	6	7	8
FAM	29.99	29.94	29.81	30.13	29.72	29.68	29.84	29.74
HEX	30.13	29.86	30.04	29.91	29.71	29.60	29.70	29.53
ROX	30.44	30.39	30.43	30.37	30.01	30.09	30.27	30.13
Cy5	30.45	30.20	30.11	30.14	29.94	29.90	30.14	29.98

B	B1	B2	B3	B4	B5	B6	B7	B8
Sample	1	2	3	4	5	6	7	8
FAM	30.25	30.12	29.84	29.94	29.52	30.03	30.12	30.82
HEX	30.29	29.88	29.48	29.47	29.39	29.93	30.21	30.35
ROX	30.57	30.52	30.38	30.31	29.81	30.46	30.45	30.77
Cy5	30.48	30.38	30.15	29.98	29.81	30.31	30.41	30.70

interface of the experiment report.

Figure 5-6-1 Data export

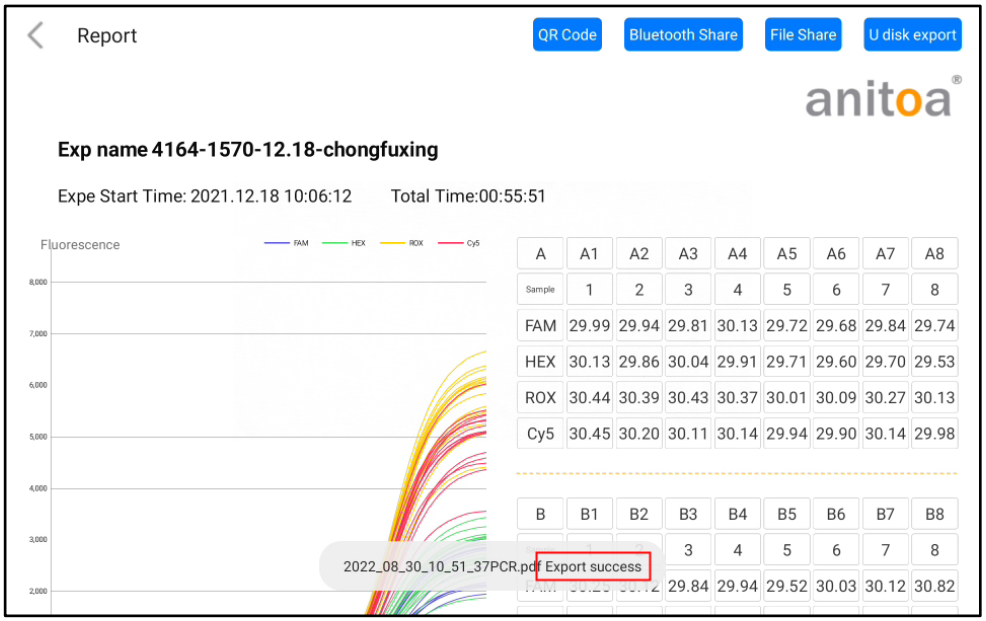


A	A1	A2	A3	A4	A5	A6	A7	A8
Sample	1	2	3	4	5	6	7	8
FAM	29.99	29.94	29.81	30.13	29.72	29.68	29.84	29.74
HEX	30.13	29.86	30.04	29.91	29.71	29.60	29.70	29.53
ROX	30.44	30.39	30.43	30.37	30.01	30.09	30.27	30.13
Cy5	30.45	30.20	30.11	30.14	29.94	29.90	30.14	29.98

B	B1	B2	B3	B4	B5	B6	B7	B8
Sample	1	2	3	4	5	6	7	8
FAM	30.25	30.12	29.84	29.94	29.52	30.03	30.12	30.82

Figure 5-6-2 PDF preview page

2) At present, four sharing options are supported, which are "QR code", "Bluetooth share", "File share" and "U disk export". After inserting the USB flash drive, click the USB export, which means it is saved successfully, as shown in Figure 5-6-3. Note that the USB flash drive needs to be formatted in FAT32 format before use.



A	A1	A2	A3	A4	A5	A6	A7	A8
Sample	1	2	3	4	5	6	7	8
FAM	29.99	29.94	29.81	30.13	29.72	29.68	29.84	29.74
HEX	30.13	29.86	30.04	29.91	29.71	29.60	29.70	29.53
ROX	30.44	30.39	30.43	30.37	30.01	30.09	30.27	30.13
Cy5	30.45	30.20	30.11	30.14	29.94	29.90	30.14	29.98

B	B1	B2	B3	B4	B5	B6	B7	B8
			3	4	5	6	7	8
			29.84	29.94	29.52	30.03	30.12	30.82

Figure 5-6-3 Export success page

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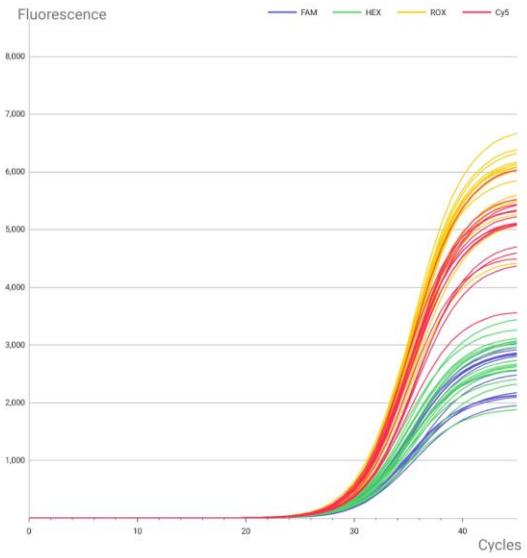
Exp name 4164-1570-12.18-chongfuxing

Expe Start Time: 2021.12.18 10:06:12 Total Time:00:55:51



A	A1	A2	A3	A4	A5	A6	A7	A8
Sample	1	2	3	4	5	6	7	8
FAM	29.99	29.94	29.81	30.13	29.72	29.68	29.84	29.74
HEX	30.13	29.86	30.04	29.91	29.71	29.60	29.70	29.53
ROX	30.44	30.39	30.43	30.37	30.01	30.09	30.27	30.13
Cy5	30.45	30.20	30.11	30.14	29.94	29.90	30.14	29.98

B	B1	B2	B3	B4	B5	B6	B7	B8
Sample	1	2	3	4	5	6	7	8
FAM	30.25	30.12	29.84	29.94	29.52	30.03	30.12	30.82
HEX	30.29	29.88	29.48	29.47	29.39	29.93	30.21	30.35
ROX	30.57	30.52	30.38	30.31	29.81	30.46	30.45	30.77
Cy5	30.48	30.38	30.15	29.98	29.81	30.31	30.41	30.70

Conclusion

Unit: User: Sampling time:

Auditor: Date: 2022-08-30 10:50:32

Figure 5-6-4 Preview of the complete PDF export interface

6. Instrument Maintenance

Instrument cleaning

Regular cleaning

For everyday cleaning requirements, we can use a soft, clean and damp cloth to wipe the external surface of your instrument. After this step, dry your instrument with another soft cloth. Avoid abrasive cloths, towels, paper towels, and similar items that might cause damage. Before cleaning your instrument unplug all external power sources, devices, and cables. Don't get moisture into any openings.

Well cleaning for more contamination removal

If the experiments were not carried out carefully, substances such as spill-over DNA that may cause fluorescence background, cross talk etc. can get into the reaction wells. We could clean the reaction wells with lint-free wipes or swabs. Moreover, to remove potential contaminants, we recommend use 10% bleach solution to clean suspected contamination areas. We could let the cleaning solution on the surface for 10-15 minutes before another wide down with de-ionized water.

Additional recommendations about cleaning

(1) Instrument surface cleaning: the surface of the instrument should be scrubbed regularly with a soft cloth with 75% alcohol, and the instrument should be wiped dry after cleaning.

(2) Reaction wells cleaning：

a. Dust or impurities in the reaction wells can affect PCR amplification and fluorescence detection, and regular cleaning is recommended.

b. To prevent dust from entering the reaction wells, the flip-up cover must be closed when the instrument is not in use.

c. If any reagent enters the sample well, it should be wiped clean with a dust-free soft cloth with anhydrous ethanol.

d. ⚠The power must be turned off and the power cord unplugged before cleaning the instrument.

e. ⚠Do not pour liquids into the reaction module or inside the instrument.

f. ⚠Do not use corrosive solvents or organic solvents to scrub the instrument.

Instrument protection

(1) Do not switch the instrument on and off frequently.

(2) Please use the adapter provided by the original manufacturer.

(3) ⚠Boiling water bath or low temperature holding on the instrument is prohibited.

(4) ⚠It is forbidden to disassemble the instrument by non-original maintenance personnel.

Waste disposal

(1) After each experiment, there are a large number of amplification products in the test tube, which should be disposed of as soon as possible according to relevant regulations to avoid contaminating the laboratory and instruments.

(2) ☣Do not open the cover of the test tube after it is removed from the instrument, otherwise it will easily cause laboratory contamination.

Overheat protection

(1) When the temperature value of the instrument temperature control module exceeds the set threshold (120°C), the device will automatically stop heating up and force all ongoing actions to stop.

(2) After the above-mentioned failure of the heating system, the user should stop using the instrument and promptly contact the manufacturer or distributor for maintenance.

Operation requirements

(1) During use of the instrument, the operator may come into contact with harmful substances or infectious substances, the operator needs to have relevant training and relevant qualifications to handle such tasks.

(2) The operator should operate the instrument in strict accordance with the relevant national regulations.

Preventive maintenance requirement

(1) After every one year of the use the instrument, the user is required to perform actions to check the condition of the instrument as outlined in the steps below:

a. A thorough cleaning following the steps outlined above
b. Careful visual inspection of the instrument to ensure that no visible mechanical damage has occurred. This check should include checking for loose fittings, checking for loose or missing screws. There should be no rattling sound when the instrument is picked up and gently shaked by hands. The plastic surface should not have developed any visible cracks or deep cuts.
c. Whenever possible, the user should run a standard assay to check instrument performance. The assay should have positive and negative controls and the result should match expected outcome of the test.

d. If the instrument is used for quantitative nucleic acid analysis, the user should try to test the performance of the instrument running standard curve analysis. The linearity of the test should match what is expected of the assay used.

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7. Troubleshooting guide

Issue with connecting the power

Please note that the power connector at the back of the instrument has an orientation. Make sure that the connector is properly aligned with this orientation. Do not force the connector in if the resistance is too great. Instead make sure the connector and receptacle are properly aligned.

Also make sure the AC cable is connected to a power source. There is a blue indicator light on the AC/DC adapter. It will lit when the AC cord is receiving power from the AC source.

For some instrument, there is also a power switch at the back. Make sure this switch is at “on” position when we use the instrument.

The test tube would not fit, or the lid would not pressure on test tube when closed

Please make sure to follow direction from section “qPCR tube/plasticware requirement” to select the proper test tube. If not sure, just purchase the brands Anitoa recommended or contact Anitoa for samples of the properly sized qPCR tubes.

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USB connection between PC and instrument cannot be established

If the PC USB port is functional properly (this can be checked by using it for keyboard, mouse or USB disk), and the instrument is properly and securely connected to the PC, the instrument should always be recognized by the PC.

This can be checked by:

Go to settings menu of your Windows®-based PC

Open Device Manager

Look for “Human Interface Devices

You may find the qPCR device being identified as “HID-compliant bar code badge reader”. Unplug and plug in again the instrument will cause this item to disappear and reappear. If this is not happening, please contact Anitoa technical support.

.

How do I know if the instrument is functioning properly when everything is plugged in?

In section “Launching the software”, if it shows “My HID Connected”, it confirms that the software can communicate with the hardware correctly.

At this point, we should be able to start an experiment and monitor the reaction well and hot lid temperature change at least. If temperature change does not occur, please contact Anitoa for support of diagnose potential hardware faults.

Touchscreen blank on power up

Some of the models of Maverick qPCR are equipped with a touchscreen interface. If the touchscreen panel is blank on power up, there are several steps to diagnose this event:

1. Make sure the instrument is indeed powered on. See section “Issue with connecting the power” to debug power problem.
2. The screen may have been inadvertently locked up through user settings input. Completely power down the system – unplug power input, switch off the instrument, and then power on the system again.
3. If the steps above are not effective, contact Anitoa support to report possible hardware issue.

Temperature Profile Abnormal

During experiment run, the user can visually judge if the temperatures of the lid and reaction wells rise as expected. If for any reason the lid or the well temperatures do not rise and comedown as expected:

1. Diagnose power problem. Check secure connections are made with the power adapter.
2. If the instrument is able to communicate with the laptop or touchscreen and the power supply connection is good, but the temperatures do not rise as expected, contact Anitoa support to report possible hardware failure.

.

Instrument is running as expected but no data were collected

Make sure the laptop is connected to the instrument via USB cable and make sure that the laptop PC did not go into sleep mode during the experiment.

Many laptops are set to go to sleep mode very quickly when it is on battery power. Disable sleep mode or make sure that the laptop is powered.

When loading an experiment file, unless the user is trying to load it as a template, make sure to uncheck the load as template button. Otherwise only the settings will be loaded, the test data will not be loaded.

.

Additional troubleshooting guide

No.	Failure Phenomenon	Cause Analysis	Processing
1	. The screen shows a black screen	Screen motherboard damage	Must replace the screen motherboard, please contact with the supplier or manufacturer
		If the screen lock function is incorrectly operated, the screen enters the black screen standby mode	Close the lock screen, and enter the test interface directly after the startup
		Screen damage	Need to replace the screen, please contact the supplier or manufacturer
	Software prompts "Abnormal auxiliary heating	Auxiliary temperature selftest abnormal	Please restart the instrument first to confirm, if still cannot solve please contact the supplier or manufacturer
	Abnormal heat-up curve of hot cover	Power supply problems	Verify that the power is plugged in properly
		Hot cover assembly problems	Please restart the instrument first to confirm, if still cannot solve please contact the supplier or manufacturer
2	Unable to turn on	Power supply problems	Verify that the power is plugged in properly
		Switch or power cable damage	Please restart the instrument first to confirm, if still cannot solve please contact the supplier or manufacturer
3	USB flash drive export failed	The USB disk is not in good contact	Reinsert the USB flash drive for confirmation
			Can try to use WIFI, Bluetooth export function
			If the problem persists, contact the supplier or manufacturer
4	HID connection exception	Screen communication exception	Please restart the instrument first to confirm, if still cannot solve please contact the supplier or manufacturer
5	No experimental data after instrument operation	Incorrect setting of experimental parameters	Verify that the thermal cycling parameters and sample parameters are set correctly, and set the fluorescent markers
6	Report upload and print function exception	WIFI setting error	Please check if the instrument WIFI setting is correct, please connect to the network correctly
7	Test time and report time are not synchronized	Time synchronization without network connection	The default time is used when the device is not connected to the network. Please

.

			connect to the network for immediate time update
8	Software prompts "Please close the hot cover"	Instrument flip cover is not closed in place	Re-close the flap to ensure it is closed in place.

.

8. Appendix: PCR data processing – Positive/negative determination and Ct calculation.

For each reaction well and each channel, a series of cycle-by-cycle fluorescence read out data is obtained. The fluorescence read out from the reaction is processed to determine whether the result is positive or negative. This process involves several steps:

1. Alignment of the initial phase of the data. The algorithm estimates the average signal magnitude of the data at the initial cycles and estimate the mean. For subsequent processing, the algorithm will subtract the mean value to align the initial phase of the data along the x axis of the coordinate systems.

2. Estimate the standard deviation of the data in the initial phase of the data points.

3. Determine and refine Ct value

   The algorithm will then compare the magnitude of the data to the Ct threshold value. The Ct threshold is determined with a multiple of the base line standard deviation. If the magnitude of the data consistently exceeds the threshold, the result is considered positive, the cycle number location of Ct threshold crossing is the Ct value of the data.

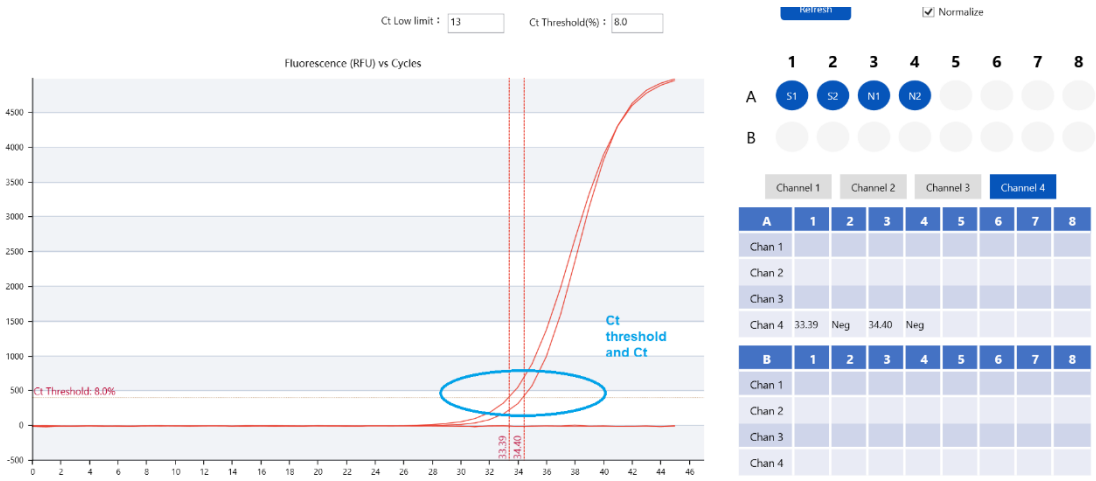

Figure A1 Ct determination

After a reaction data is judged as positive, a sigmoid curve fitting is carried out. The algorithm will use the sigmoid fitting parameters to estimate the saturation magnitude of the data as well as amplification efficiency. Amplification efficiency estimate is provided by looking at the fluorescence strength multiplication during the initial exponential growth phase of the positive reaction data.

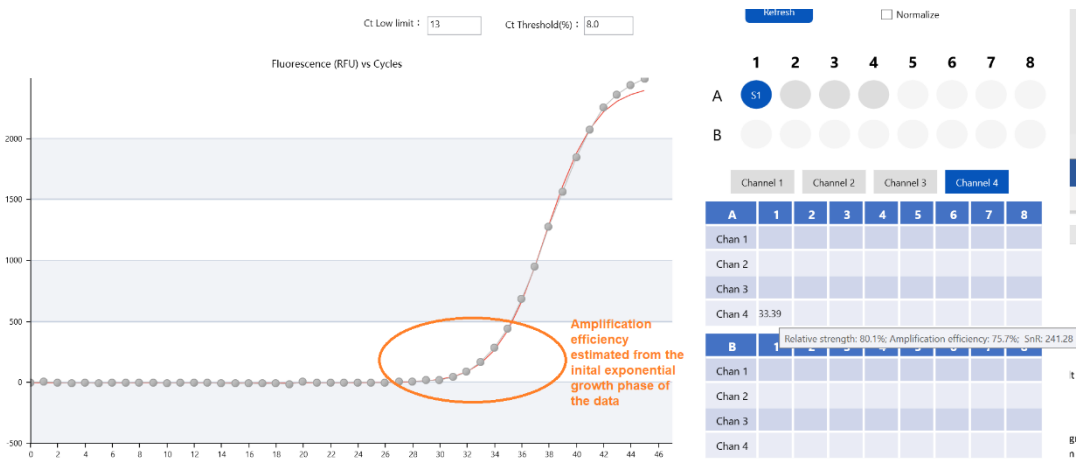

Figure A2 Amplification estimation

Additional data processing method – base slope correction

Sometimes, when we look at the raw fluorescence data, we see a general negative slope during the initial phase of the data.

Without correction, it is difficult to get correct Ct value. Because the standard deviation of the base line signal will be exaggerated. The standard deviation of the initial phase of the curve is used to determine Ct threshold.

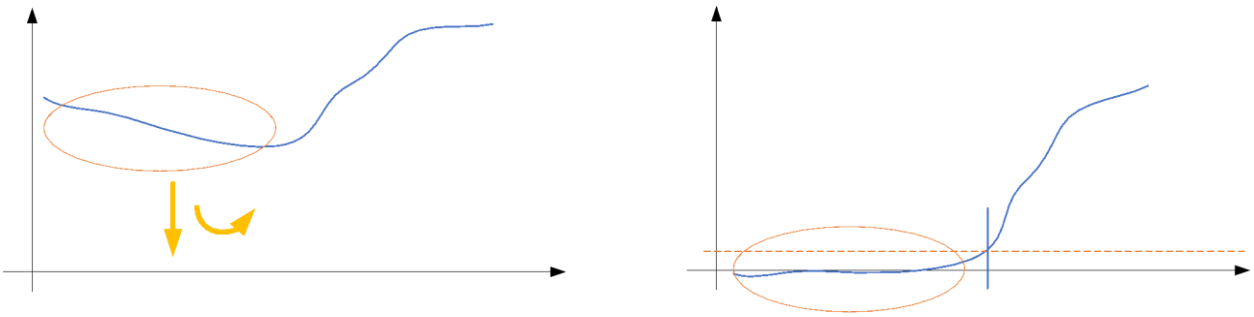

Figure A3 Base Slope Correction

When we perform curve fitting and calculate the Ct, we would translate and rotate the curve to make the initial phase of the curve aligned to the x-axis of the graph.

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Disable data processing to view raw data for debugging purposes

It is possible to disable data processing for debug purposes. Once all data processing methods are disabled, we can view the raw, unprocessed fluorescence data in the analysis window. As shown below, in “Settings”->”Imaging settings” tab the user can selectively disable data processing methods to see the raw data. This is useful for debugging purposes.

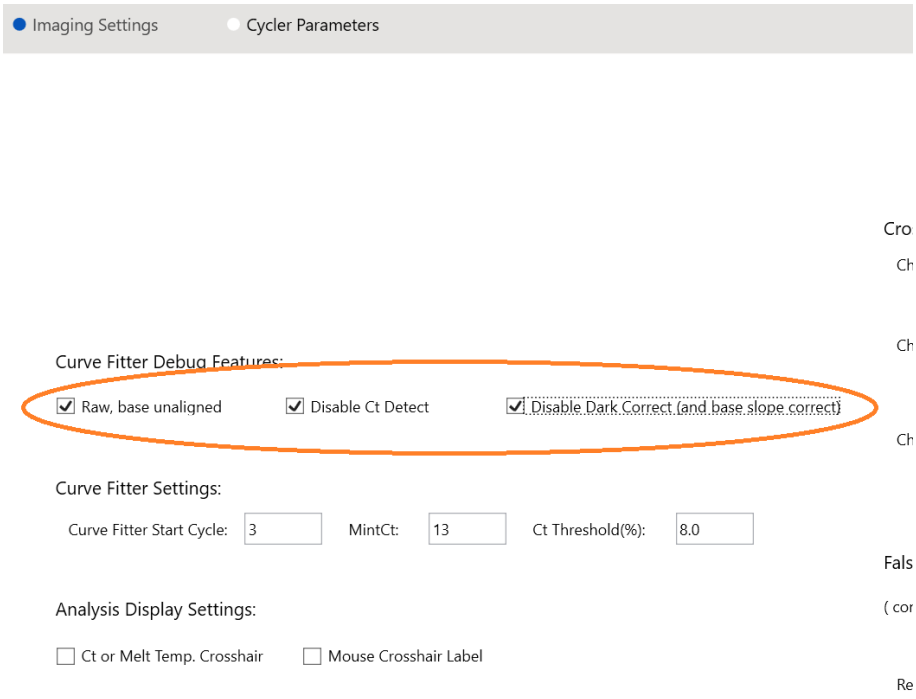

Figure A4, Disable data processing for debugging ppurposes

False positive detection

The Maverick software uses a set of algorithms to analyze positive reaction data and determines whether positive result determination could be false. When a reaction data is judged false positive, it will show as negative, but with special bracket to indicate that it was due to false positive test.

The determination of false positive performed by evaluating several parameters: “relative strength”, “(estimated) amplification efficiency”, “signal to noise ratio (SnR)”. If any of these parameters fall below a certain threshold, the data is judged as false positive. The threshold of these parameters is adjustable by the user. Please note this analysis is only performed on reaction data that is initially judged as positive.

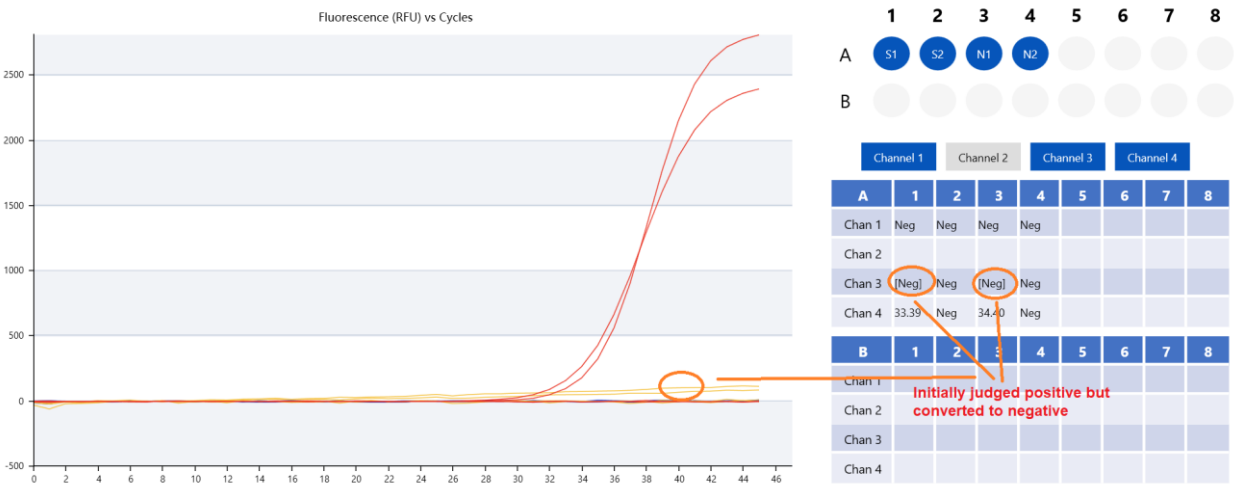

Figure A5, False Positive Determination

Below is a description of these parameters and their effect on false positive detection.

Relative strength (value 0 to 100%):

The algorithm examines the signal strength of a positive reaction data and compare it with all the other positive data from the same channel. The signal strength is defined here as the saturation value (or projected saturation value) of the data after Sigmoid data fitting. If a positive data is particularly weak compare to the other positive reaction data, it may be deemed false positive. The algorithm takes into the account of Ct value in that as Ct value increase, the signal strength at saturation is expected to be weaker. The threshold to trigger a false positive detection based on signal strength is adjustable.

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(Estimated) Amplification efficiency (value 0 to 100%):

The algorithm can estimate the amplification efficiency of the reaction data by calculate the slope of the data when performing sigmoid curve fitting. The optimal amplification efficiency of PCR is 100%, which corresponds to amplicon concentration double every cycle. It is normal for the amplification efficiency to be below 100%. However, if the estimated amplification efficiency of the data is too low, relative to the amplification efficiency of other positive data in the same channel, the reaction data is likely positive. The threshold to trigger a false positive detection based on amplification efficiency is adjustable.

Signal to noise ratio

The algorithm compares the signal strength to the root mean square noise (RMS) of the data. If the value is too low, that means that the signal strength is too low compare to the noise level in the data. This is another indicator of false positive

The default threshold values are:

- Relative strength threshold: 20%
- Amplification efficiency threshold: 20%
- Signal to noise threshold 10.

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The user can adjust the threshold values and save them with the experiment file or template file. As shown below, in “Settings”->”Imaging settings” tab the user can selectively adjust the threshold values.

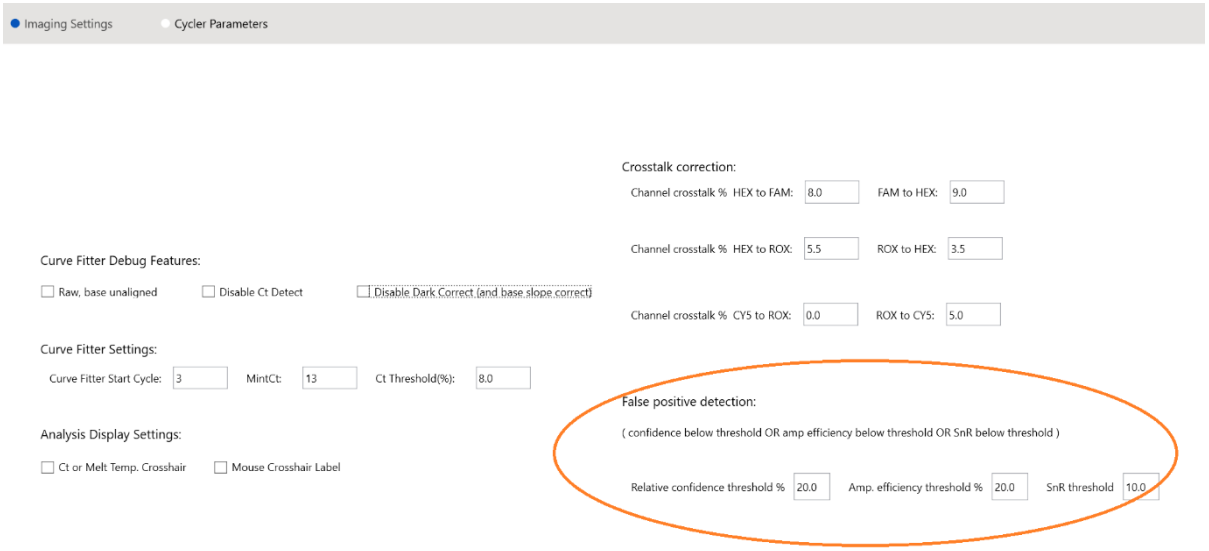

Figure A6 Threshold values for false positive determination

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How to find the Relative strength, Amplification efficiency, and Signal to noise ratio of the reaction data

To adjust the thresholds for false positive detection (assuming the user is not satisfied with the default values), it maybe helpful to find out the Relative strength, Amplification efficiency, and Signal to noise ratio of a given reaction data. This can be done by floating the mouse curser above the Ct value of that reaction data, in the analysis window. See Figure xxx.

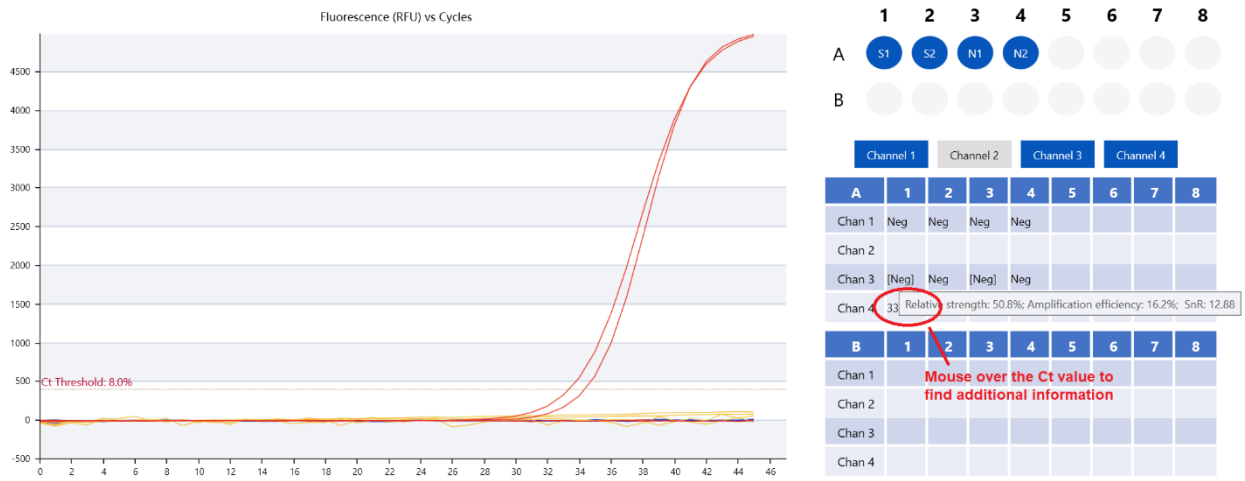

Figure A7 Additional attributes of the data

9. Appendix: Channel Crosstalk Suppression Techniques

The emission spectra of fluorophore materials used in multiplex qPCR assay overlap to some degree. In Anitoa's Maverick qPCR system, we took several measures to suppress such cross talk both in hardware and software. In this section, we explain the software measures to suppress channel cross talk, using experiment data as illustration.

In the Maverick software, cross talk suppression parameters are available, and their default value are chosen according to empirical data from most common multiplex qPCR reagent products used in factory test. These values can be adjusted by user for any specific assay used.

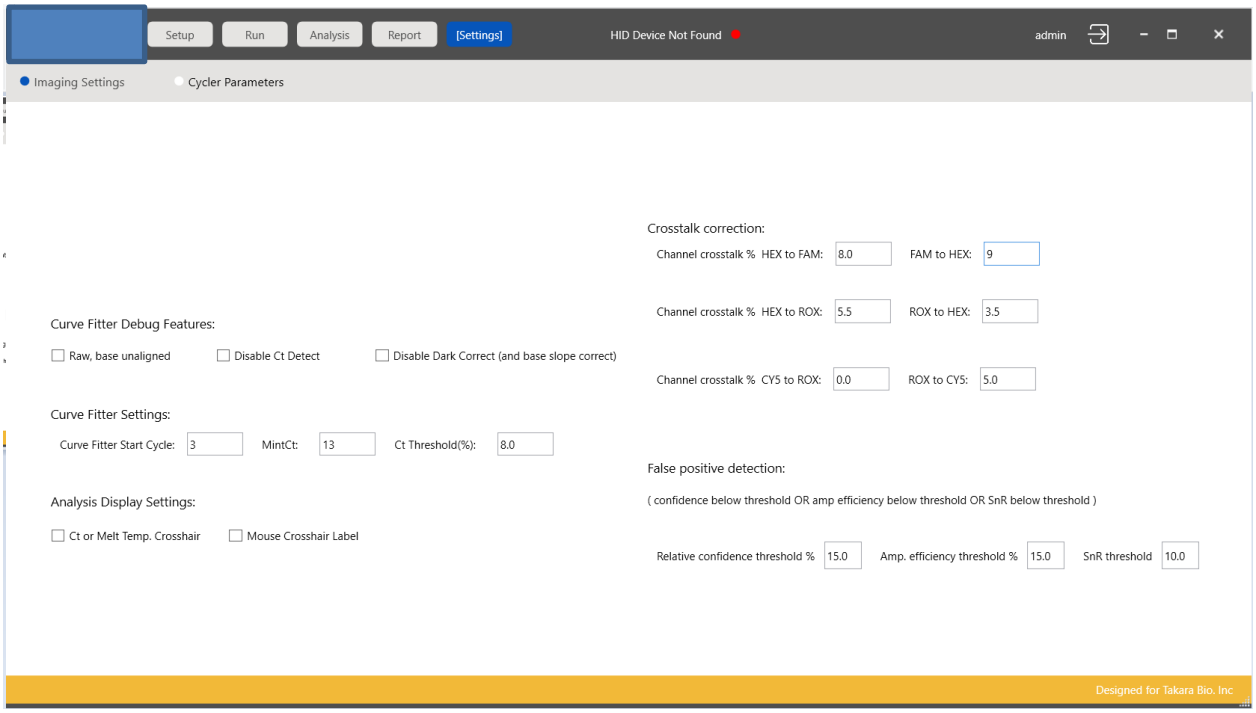

Figure B1. Crosstalk suppression parameters

In Figure 2 below, we show the result of an experiment. In this example, only channel 2 (HEX) and channel 3 (ROX) is shown.

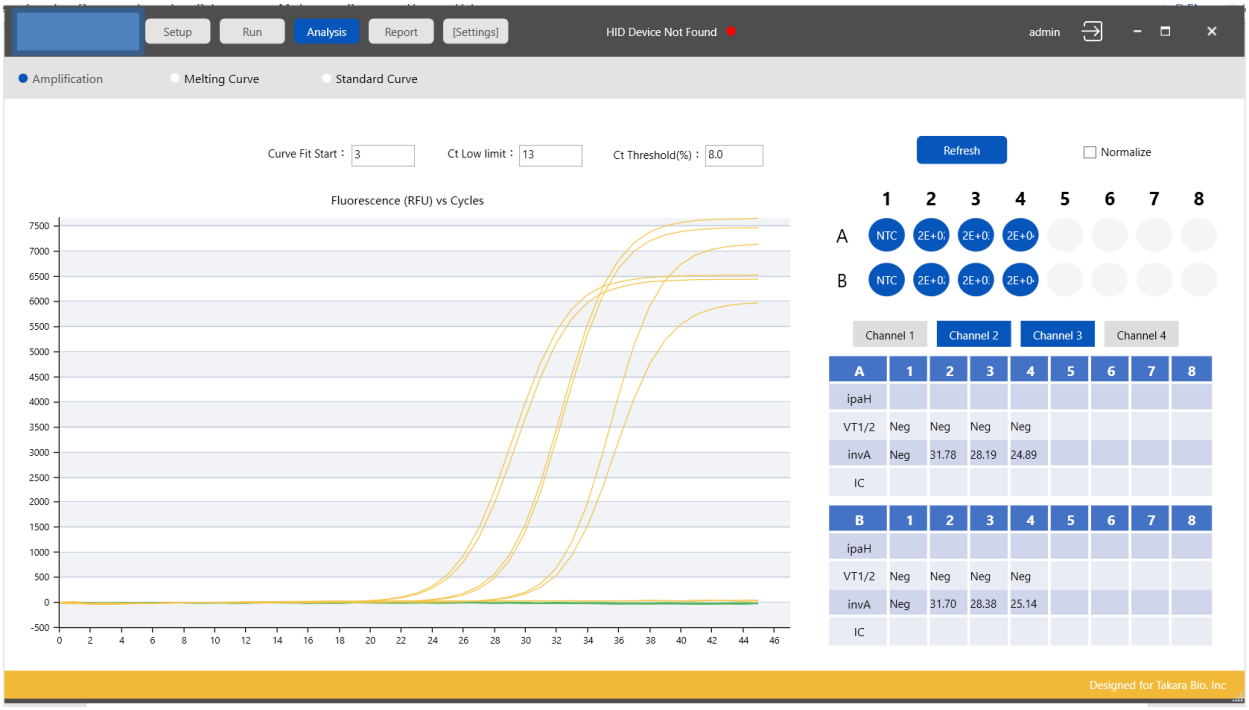

Figure B2. Experiment Data

In this experiment, the ROX channel shows strong amplification of concentration ladder products, while the HEX channel gives all negative results. This agrees with the expectation of the design of the experiment. The fluorescence curve of the HEX channel shows a very clean flat line, this is made possible by the crosstalk suppression method mentioned above.

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Method to adjust cross talk parameters

To illustrate the idea of how cross talk suppression works, let us first go ahead and turn off the crosstalk suppression feature and see what happens. This is done by going to the setting page – step (1), make all crosstalk suppression parameters 0 percent, step (2) let us disable the curve fitting functions to view raw data.

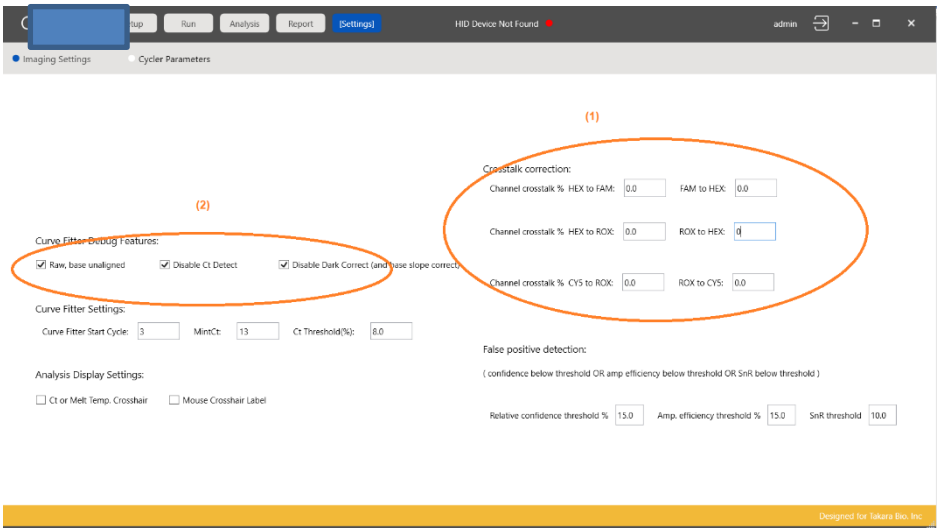

Figure B3 Zeroing all crosstalk suppression ratio parameters, and turn off curve fitting features to view raw data

After these changes, let us go back to the analysis page. Here we now see effect of cross talk from ROX to HEX channel.

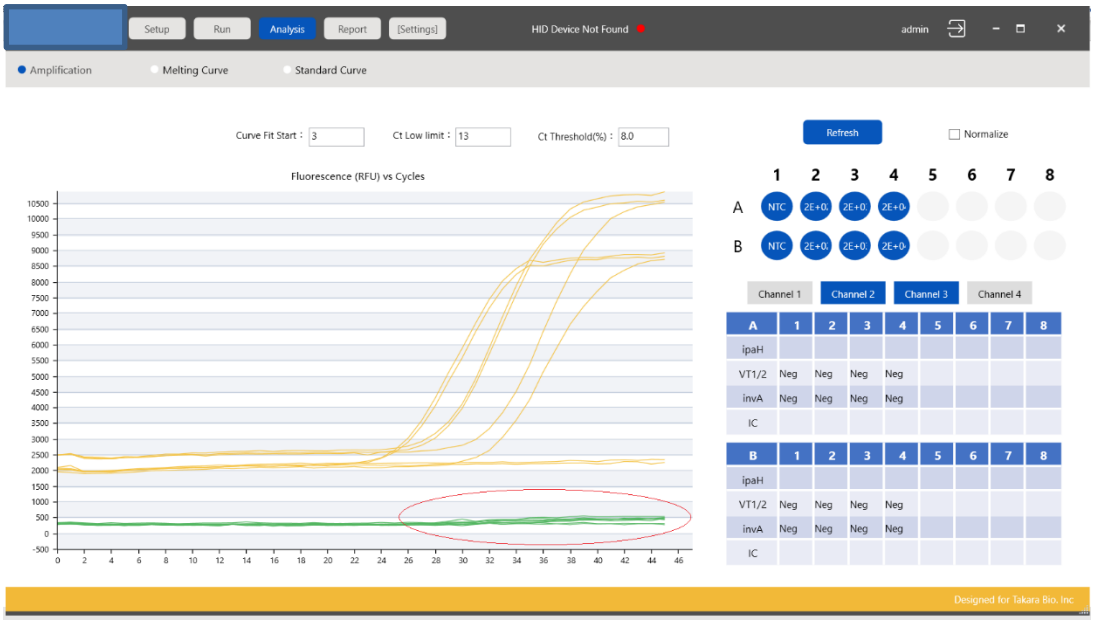

Figure B4, Cross talk is shown graphically with raw data

If we focus on just the hex channel, the effect is clearer:

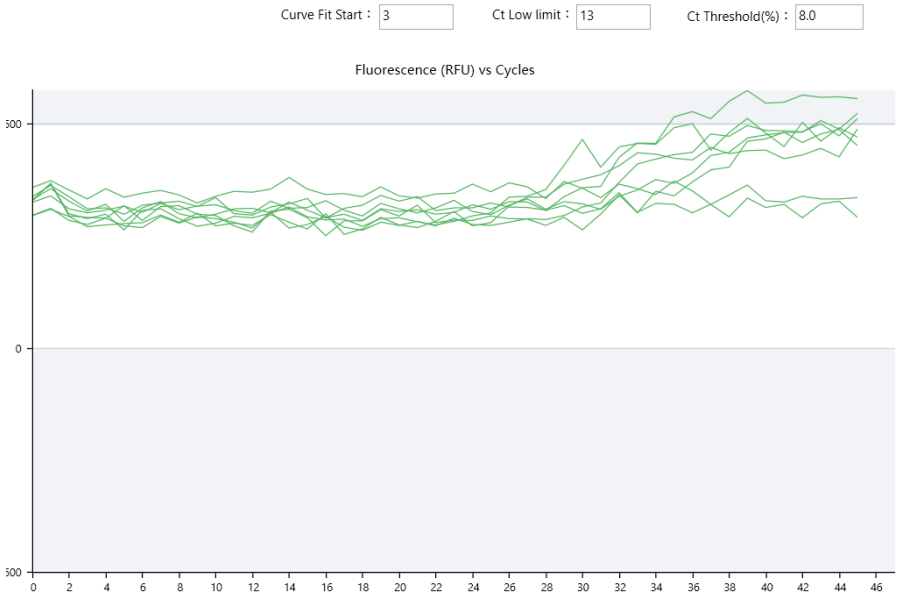

Figure B5, Signal in HEX channel due to crosstalk.

As a result, the hex channel signals maybe judged as positive.

If we then go back to the setting page to add some crosstalk suppression percentage to 3.0, we will find that the crosstalk effect on HEX signal is cleared.

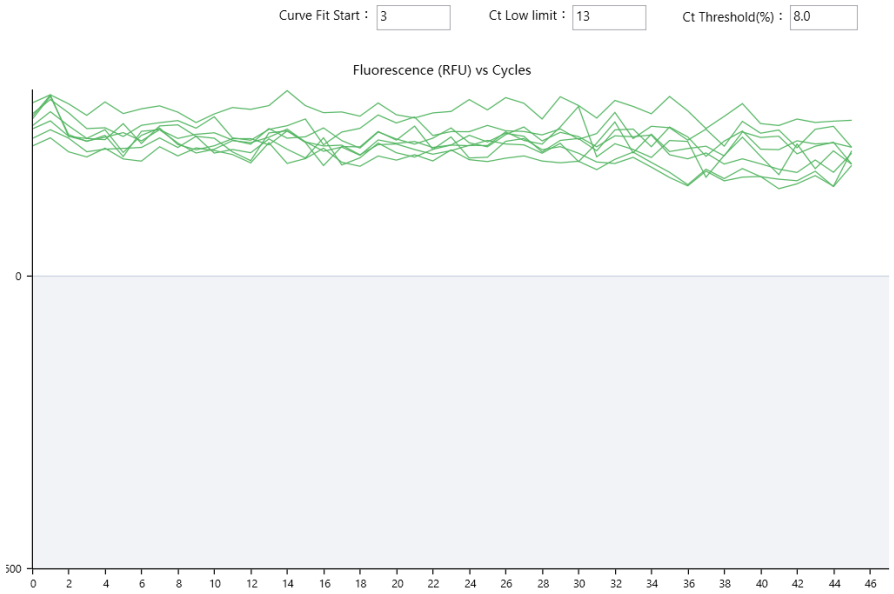

Figure B6 Cross talk effect in HEX channel is suppressed.

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So, we can keep 3 percent as the crosstalk suppression ratio for ROX to HEX crosstalk. Now if we turn back on all the curve fitting functions by unchecking the “Curve Fitting Debug Feature”, we will see the curves in Figure 2.

To summarize, crosstalk suppression parameters can be adjusted if we have assay that we know have strong signal in one channel and supposedly negative result on at neighboring channels.

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10. Change History

Date	*Author*	*Content*
5/20/2022	Yong Hui	Initial draft
8/11/2022	Yan Di	Further refinement
11/13/2022	Zhimin Ding	Adding intentional use clause
1/18/2022	Yong Hui / Zhimin Ding	Adding section for Preventive maintenance requirement.
3/15/2024	Yong Hui, Cai Xu	Update to include information about the newer S models