MKT-00171_UM_Nio_Digital_PCR_RevG

Nio® Digital PCR

User manual

Technical support

For technical support inquiries:

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- **Denmark:** techsupport.nordic@bio-rad.com | 00 800 00 24 67 23
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Details available at: www.stillatechnologies.com/patents.

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1 Introduction to Crystal Digital PCR®

1.1 Purpose of the document

This document provides general information for the use of the Nio® Digital PCR instrument for Crystal Digital PCR®. The workflow and required hardware components are described in detail. It is essential to read the User Manual carefully and pay attention to the safety information provided. The instructions and safety information in the User Manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

All documents referenced in this User Manual can be accessed here: https://www.bio-rad.com/fr-fr/product/nio-system?ID=9d6dac6d-4584-a142-2cce-f1b84b48e731

1.2 Overview of the Crystal Digital PCR® Workflow

Crystal Digital PCR® is Stilla Technologies' next-generation technology for the absolute quantification of nucleic acids.

Using cutting-edge microfluidic innovations, this technology integrates the digital PCR process in a single consumable (Figure 1). The sample is first flowed through a network of microchannels and partitioned into a large 2D array of droplets, also called a Droplet Crystal. PCR is then performed within the chip consumables and following the Droplet Crystal is imaged to reveal the droplets that contain amplified targets. The last step consists of an automated data analysis including the detection of all generated droplets and the differentiation of positive droplets to precisely extract the absolute quantity of nucleic acids.

With Crystal Digital PCR®, the combination of powerful image analysis and intuitive visual inspection offers an unmatched level of confidence in the digital PCR measurement, yielding trustful data.

Sample loading	Droplet generation, PCR, reading and analysis on Nio® Digital PCR

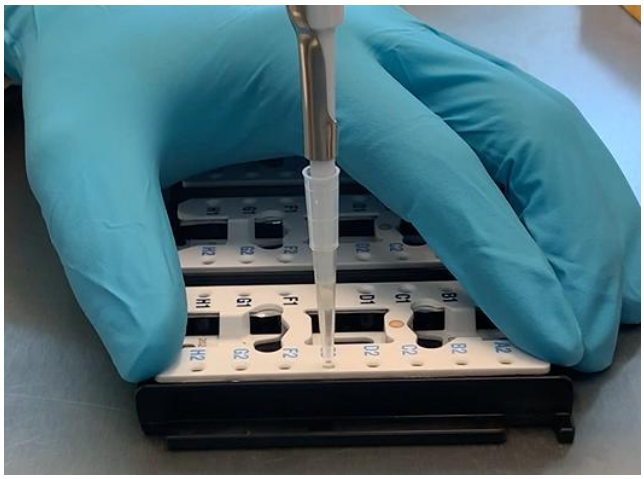


Figure 1: Pipetting Ruby Chip

Prepare the sample for the reaction mix. To ensure accurate quantitative results only use validated dPCR MIX reagents. Load the reaction mix into the wells of the selected chip consumable provided in the chip plate for transportation and instrument operation. Both the chip consumable as well as the chip plate are compatible for sample loading utilizing automated liquid handling stations.

Place the prepared chip plate into the Nio® Digital PCR. Launch the Digital PCR protocol combining partition, amplification and read-out parameters for data acquisition using up to 7 fluorescent channels (Blue, Teal, Green, Yellow, Red, Infra-Red and Purple). Image analysis and data extraction are performed using the Nio® Analyzer software.

2 Intended Use of the Nio® Digital PCR

Crystal Digital PCR® performing droplet generation/amplification and imaging of the Droplet Crystal in up to 7 detection channels in a fully automated single instrument is available with the Nio® Digital PCR.

The Nio® Digital PCR is an all-in-one platform for Crystal Digital PCR® compatible with the microfluidic chip consumables Ruby Chip, commercialized today by Bio-Rad under the product name of RDG16 Cartridges. The Nio® Digital PCR ensures partitioning, thermocycling and droplet crystal imaging of the chips without any intermediate user action.

The Nio® Digital PCR is available in three configurations offering different throughput capacities to match the needs of all digital PCR users. The Nio® E configuration enables running up to 48 samples per run.; The Nio® configurations allow processing of up to 192 samples per run. Lastly, the Nio®+ configuration fits for higher throughput needs and allows running up to 384 samples per run.

The Nio® Digital PCR is a laboratory instrument intended to be used by professional users trained in molecular biological technics. Before using the Nio® Digital PCR, the user must be trained by a Stilla Technologies representative.

In the following, the instrument is referred to as “Nio® Digital PCR” whatever its configuration (Nio® E, Nio® or Nio®+), unless otherwise stated specifically.

The Nio® Digital PCR is intended to be used with the Nio® software suite: Nio® Reader software and Nio® Analyzer software.

The Nio® Reader software functions as the user interface to set up all experiment parameters (amplification, image acquisition) on the Nio® Digital PCR for a single automated Crystal Digital PCR® workflow. Nio® Reader software is available in two modes: Standards or Regulatory. The latter enables Nio® Digital PCR users (customers) to meet the Food and Drug Administration’s regulations on good laboratory practices (GLP) as well as good manufacturing practices (GMP) in pharmaceutical as well as biotechnology industries. Nio® Reader software Regulatory provides the necessary features to permit the Nio® Digital PCR to operate, in compliance with Title 21 of the U.S. Code of Federal Regulations Part 11 (21 CFR Part 11), within a closed system. A closed system is defined as “an environment in which system access is controlled by the persons who are responsible for the content of electronic records that are on the system”. The customers choose between Standard or Regulatory mode when installing or updating Nio® Reader software.

The Nio® Reader software:

- Allows defining the analytical context of the experiments. Experiments can be set up on-demand or dedicated experimental templates can be created for recurring experimental setups.
- Controls the Nio® Digital PCR instrument for the acquisition of the fluorescence images.
- Applies pre-analysis treatments to the acquired images and provides a first quality control in preparation for the detailed downstream experiment analysis performed by the Nio® Analyzer software.
- In Regulatory mode, Nio® Reader software operates in conjunction with naica® Data Service, the latter functions as the Nio® Digital PCR user account manager and ensures the compliance of all Nio® Digital PCR operations with respect to regulation 21 CFR Part 11. In Regulatory mode, locked standard experiments can be generated for routine use.

  Note: The security controls built into Nio® Reader software must be properly configured and administered by the Nio® Digital PCR administrator(s) in the customer’s organization to be secure and in compliance with 21 CFR Part 11. Stilla® Technologies makes no claim that Nio® Reader software is 21 CFR Part 11 compliant in and of itself, nor does the company guarantee compliance for the user. The Nio® Digital PCR user organization must establish policies and standard operating procedures (SOPs) that work in conjunction with the tools provided by Stilla® Technologies to ensure compliance with 21 CFR Part 11.

The Nio® Analyzer software is then used to extract data from the images acquired using the Nio® Digital PCR and, to calculate the absolute concentrations of the targeted nucleic acids.

Both Nio® Reader software and Nio® Analyzer software are pre-installed on the Nio® Digital PCR instrument. Their installers are also available for download on the Stilla Technologies website. Please read the Nio® Analyzer software user manual before use.

Nio® Reader software, Nio® Analyzer software and naica® Data Service are intended for use by laboratory personnel trained in the techniques of Crystal Digital PCR®.

In general, Crystal Digital PCR® can be performed on the Nio® Digital PCR with any type of nucleic acid sample. However, individual sample-type compatibility for digital PCR applications may require a dedicated assay validation by the end-user. Please note also that both sample purity and the extraction method used can influence sample compatibility for digital PCR applications.

The Nio® Digital PCR instruments are intended for Research Use Only. Not for use in diagnostic procedures.

Note:

For DNA analysis, only validated dPCR mix reagents, such as naica® PCR MIX and naica® multiplex PCR MIX reagents are recommended for use to achieve optimal Crystal Digital PCR® performance on Nio® Digital PCR. For RNA analysis, only the validated qScript™ XLT One-Step RT-qPCR ToughMix® (QuantaBio) are recommended for use to achieve optimal Crystal Digital PCR® performance on Nio® Digital PCR.

For detailed instructions for validated chip consumables (Ruby Chips) as well as validated dPCR reagents (naica® PCR MIX, naica® multiplex PCR MIX), refer to the respective Instruction for Use (IFU) documents.

Citing the Nio® Digital PCR instruments in scientific publications, presentations, seminars, etc.

To cite the use of the Nio® E, Nio® or Nio®+ use:
Crystal Digital PCR® (Stilla Technologies, France)
naica® Ruby Chip
Nio® Reader software
Nio® Analyzer software
naica® PCR MIX reagents
naica® PCR MIX
naica® multiplex PCR MIX

3 Materials and equipment

In this section, the instrument is referred to as Nio® Digital PCR whatever its configuration (Nio® E, Nio® or Nio®+) unless specified otherwise.

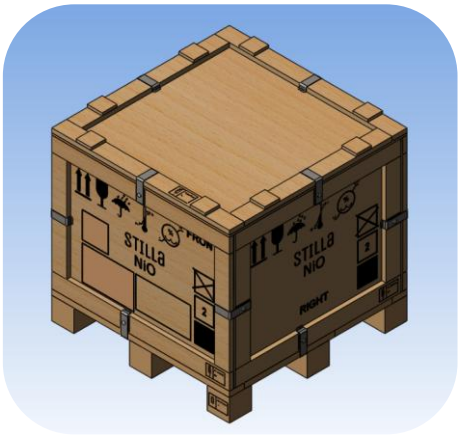


Figure 2: Nio® Digital PCR Packaging

3.1 Nio® Digital PCR packaging

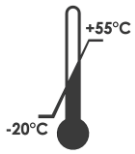
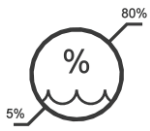
Nio® Digital PCR packaging contains:

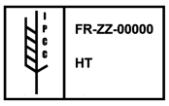
- The main instrument.
- Certificate of conformity.
- Power cable.

The Nio® Digital PCR packaging shall only be handled with a pallet truck and lifted by at least 3 persons.

3.2 Warning labels on the Nio® Digital PCR packaging

The following warning labels are located on the Nio® Digital PCR packaging:

Symbols	
	**Storage limit temperatures** The Nio® Digital PCR shall be stored in its packaging within the limit temperatures indicated on the box: -20°C to +55°C
	**Storage limit humidity** The Nio® Digital PCR shall be stored in its packaging within the limit humidity conditions indicated on the box: 5% to 80% non-condensing
	**Storage direction** The Nio® Digital PCR shall be stored with the arrows pointing up
	**Stacking information** No more than two Nio® Digital PCR should be stacked on each other
	**Caution- Fragile** Handle the Nio® Digital PCR packaging with care
	**Caution - Keep dry** The packaging is to be kept dry at all times

	**Phytosanitary information** Phytosanitary information regarding the wood packaging

3.3 Nio® Digital PCR safety information

- Follow all safety precautions provided in the user manual or on the instrument.
- Observe all general safety precautions which apply to electrical instruments.
- Do not open the housing of the Nio® Digital PCR. Only authorized service personnel should perform service or repairs.
- Do not attempt any repairs or alterations except as expressly described in the User Manual or as instructed by a Stilla Technologies representative.
- Stilla Technologies cannot be held responsible for any damages or injuries arising from the improper utilization of Nio® Digital PCR.
- Always disconnect the instrument from its power source before cleaning or moving Nio® Digital PCR.

3.3.1 Warning labels

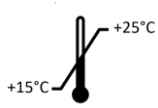
The following warning labels are located on the Nio® Digital PCR instrument (applies for Nio® E, Nio® and Nio®+ configurations):

	**Caution- liquids** **Ensure that no liquids can enter the device. Sample must be loaded outside of the Nio® Digital PCR.** **Caution- environment** **The ventilation of the device must not be covered.** **Caution- shocks** **The instrument housing must never be opened, unless instructed by a Stilla technical representative. The door must be opened only by a user trained by a Stilla technical representative. The instrument housing or door must never be opened, while the instrument is still in operation. Always shut down the instrument and remove the mains plug from the instrument first, prior to opening the instrument housing. If the instrument's door is opened without following the safety rules (shutting down, powering off and unplugging), robotic movements inside the instrument can cause physical contacts or shocks to the operator. When the instrument housing or door is opened at no time any analysis should be initiated, as this initiates the risk for the operator to experience shocks coming from robotic movements.** **Caution- photobiologic** **If the instrument's door is opened without following the safety rules (shutting down, powering off and unplugging), the optical module LEDs can display a risk to cause damages to the operators eyes. Always shut**

	down the instrument and remove the main plug from the instrument first, prior to opening the instrument housing. **Caution- use** **If the device is used in a manner not described in this manual, safety may be compromised.**
	**Caution- hot surface** The instrument housing or door must never be opened, unless instructed by a Stilla technical representative. The instrument housing or door must never be opened, while the instrument is still in operation. Always shut down the instrument and remove the main plug from the instrument first, prior to opening the instrument housing. If the instrument's door is opened without following the safety rules (shutting down, powering off and unplugging), there are risks of burning or scalding due to the thermoblocks that can reach temperatures above 50°C. Only use materials (chips and chip plates) provided by Stilla Technologies, which are heat resistant at temperatures up to 95°C.
	**Caution- the risk of pinching** The instrument housing or door must never be opened, unless instructed by a Stilla technical representative. The instrument housing or door must never be opened, while the instrument is still in operation. Always shut down the instrument and remove the main plug from the instrument first, prior to opening the instrument housing. If the instrument's door is opened without following the safety rules (shutting down, powering off and unplugging), risks of pinching can occur with a manual or automatic opening/closing of the thermoblocks lids.
	**Protective conductor terminal**
	**Caution - Biohazard** The Nio® system is a laboratory equipment instrument that does not display any biohazard risk in itself. However, if biohazardous samples are used within the workflow, the user must respect any local guidelines specific for the sample type as well as to local guidelines and regulations. Always use gloves, masks, glasses and laboratory coat to use the Nio® Digital PCR if biohazard material is involved.

3.3.2 Nio® Digital PCR labeling

Labeling symbols

Symbols	
	Legal manufacturer
	Product reference (part number)
	Product serial number
	Country of Manufacturing: France Manufacturing date. Date Format: YYYY-MM
	Operating temperature range
	Read carefully the User Manual before using the product
	Caution: User manual documentation must be consulted in all cases where this symbol is marked. Using the product without applying the instructions explained in the User Manual and Instructions for Use may result in personal injury or damage the equipment and facilities or may impact the analytical results.
	Alternating current
	Restriction of Hazardous Substances (Directive 2002/95/EC on the restriction of the use of certain hazardous substances in electrical and electronic equipment).
	Waste Electrical and Electronic Equipment (WEEE) mark for Europe

CE	The product conforms to current applicable EC directives.

4 Nio® Digital PCR declaration of conformity

The declaration of conformity can be downloaded at https://www.bio-rad.com/fr-fr/product/nio-system?ID=9d6dac6d-4584-a142-2cce-f1b84b48e731 .

5 Nio® Digital PCR consumables required but not included

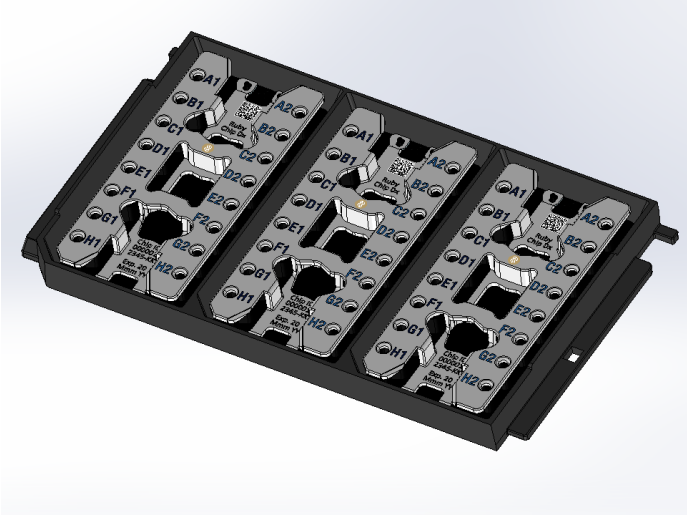


Figure 3: 3 x Ruby Chips in a Chip Plate

The following consumables are required to complete the Nio® Digital PCR workflow for Crystal Digital PCR® and must be ordered separately:

- Chip consumables – Ruby Chip and Chip Plate (C16011)
- naica® IQ/OQ kit (R30001)
- naica® PCR MIX 5X (R10056)
- naica® PCR MIX 10X (R10106)
- naica® multiplex PCR MIX 5X (R10055)
- naica® multiplex PCR MIX 10X (R10105)
- Antistatic wetted wipes (ACL Staticide®, Reference: SW12). The specific product can be ordered from Stilla Technologies as a spare part (PN H10000.472) or directly from the supplier Digi-Key using the reference ST1059-ND. SW12 ACL Staticide Inc | Static Control, ESD, Clean Room Products | DigiKey.
- Precision Wipes must be purchased using the reference - Precision Wipes (Kimtech™ Science, 7552, 1 ply, 213x114 mm) from global standard laboratory suppliers.
- Reagents or consumables for nucleic acid purification.
- Standard consumables and equipment for PCR mix preparation.
- Assay-specific digital PCR reagents, primers and probes.

6 Nio® Digital PCR instruments technical specifications

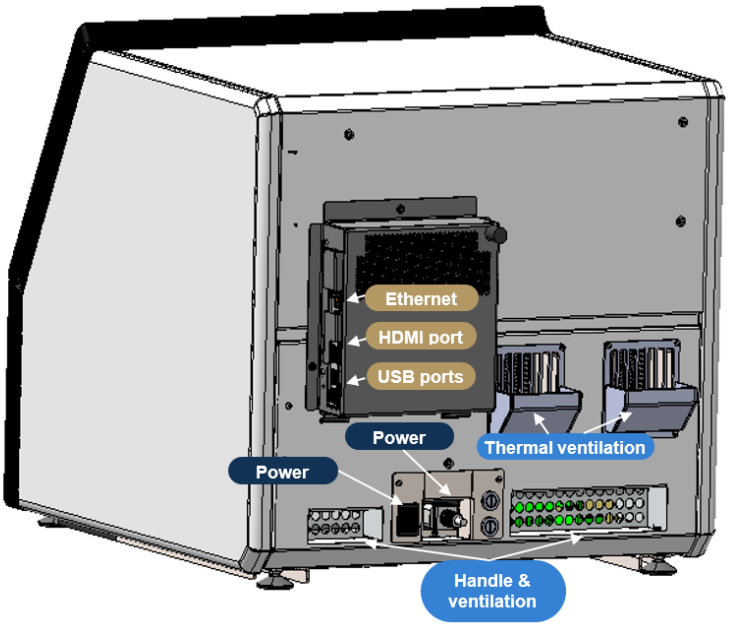


Figure 4: Nio® Digital PCR rear interfaces

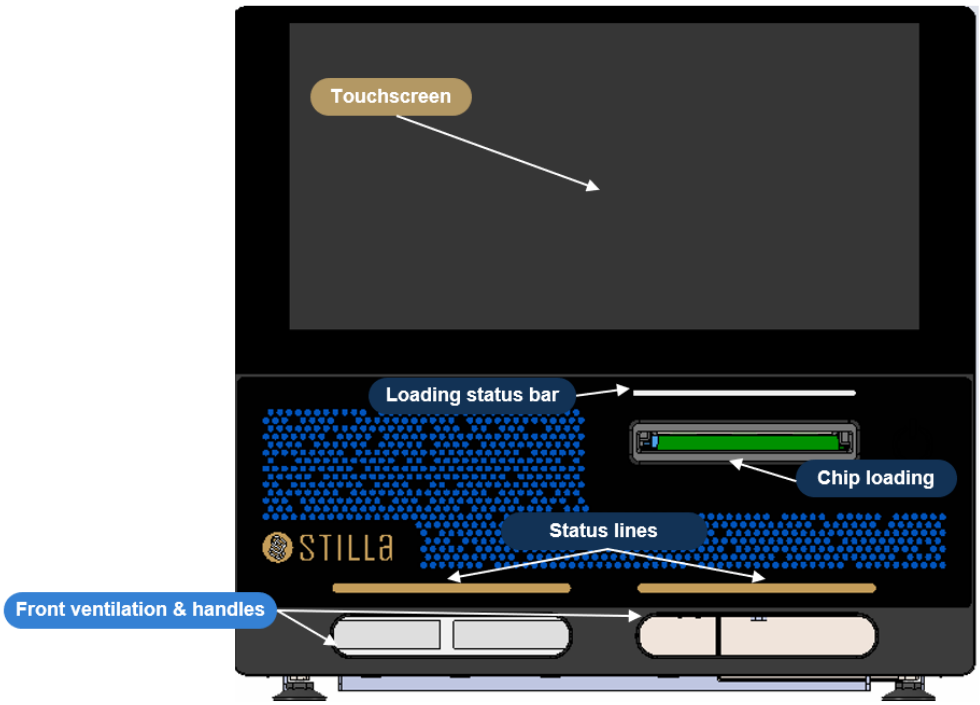


Figure 5: Nio® Digital PCR front interfaces, example for the Nio+ configuration. The color of the droplets on the front differs depending on the configuration of the instrument: blue for Nio+, silver for Nio® and gold for Nio® E.

Technical specifications*	
Instrument analysis capacity	Maximal capacity in a single run: • Nio®+ configuration: 24 Ruby Chips / 8 chip plates • Nio® configuration: 12 Ruby Chips / 4 chip plates • Nio® E configuration: 3 Ruby Chips / 1 chip plate Min capacity in a single run: 1 chip / 1 chip plate (all Nio® E, Nio® and Nio®+ configurations) Each chip plate can be set up for individual experimental parameters. Once the chips from an individual chip plate are completely processed, the chip plate can be unloaded, and the available slot can be used to load a chip plate to sequence an additional experiment.
Thermoblock temperature range**	+25°C to +100°C
Thermoblock control accuracy	± 0.75 °C
Thermoblock uniformity (at 72°C)	± 0.75 °C
Thermoblock adjustable ramping	From 1°C/s to 2°C/s
Thermoblock pressuring gas	Air
Screen	General Touch Display module BBL228 21.5''
Functional interfaces	1 front USB3 4 rear USB3 1 rear HDMI 1 rear Ethernet
Barcode Reader	OEM Barcode scanner RT208 – 1D&2D barcode
Dimensions (Width x Depth x Height)	56 x 65 x 52 cm
Weight	61kg ± 0.5kg
Power supply	100-240V~ (±10%), 50-60Hz
Overvoltage category	CAT II

Technical specifications*	
Power consumption max (W)* **	1750W
Fuse	6,3x32 T 16A. H / 250V~
Noise	61dBA max

* Technical specifications apply whatever the instrument's configuration (Nio® E, Nio® or Nio®+), unless otherwise specifically stated.
**The thermal plate inside the Nio® Digital PCR can be set to operate at temperatures ranging from 25 to 100°C.

The thermal plate temperature should never be set below the operational temperature (room temperature), as this will expose the Peltier elements that regulate the temperature to significant damage and could reduce the operating life of the instrument.

*** The facilities must be in adequation with the maximum power consumption indicated e.g.:

- The facility must be wired with a minimum 10-15 Amp grounded line with 220-240 Volts AC. Dedicated circuit is advised.
- The facility must be wired with a minimum 20 Amp grounded line with 110 Volts AC. Dedicated circuit is advised.

An electrical ground is required. The instrument must be ordered with the power cable in accordance with the standards in the country of use. The instrument is shipped with the dedicated power cable. During installation, always make sure that the power cable is in accordance with the standards in the country of use. If the voltage fluctuates more than 10%, a power line regulator is required.

Do not use electrical adapters. Refer to the list of power supply cables compatible (MKT-00191_PowerSupplyCords_Nio) to know more about regional specificities.

For optimal behavior, Stilla Technologies recommends switching off the instrument at least once a week.

If the voltage fluctuates more than 10%, **a power line regulator (Uninterruptible Power Supply Unit) is advised**.

EMC requirements (applies for all Nio® E, Nio® and Nio®+ configurations)	
Emission class	Class A
Compliant with IEC 61326-1	
*** This instrument is not intended to be used in residential environments and cannot ensure the adequate protection to the radio electrical reception in this kind of environment**	

Warning: Emissions exceeding the levels described above might occur if the instrument is connected to another equipment like external screen, USB drive…

Imaging system (applies for all Nio® E, Nio® and Nio®+ configurations)		
LED	Excitation wavelengths [nm]	Emission wavelengths [nm]
Blue	450-490	505-535
Teal	510-530	540-560
Green	533-557	574-596
Yellow	564-586	600-640
Red	610-640	655-685
Infra-Red	645-695	707-752
Purple	500-540	655-685
Recommended fluorophores	FAM, Yakima Yellow®, Atto®550, ROX, Cy®5, Atto®700/Cy®5.5, DY-521-XL	
***Additional fluorophores within the specified wavelengths can be used, fluorophore selection requires validation depending on the individual Crystal Digital PCR® assay design.**		
Data format	.niodata, .nioexperiment, .nioprotocol, .nioassay	

Embedded instrument PC (applies for all Nio® E, Nio® and Nio®+ configurations)	
Motherboard	ASROCK INDUSTRIAL IMB-1233-WV
Motherboard battery	BATT-LI CR2032 3V/220mAh/55mm BATT-LI CR2032 3V/240mAh/55mm
CPU	I5-12400
RAM	32 GB
System drive (SSD)	2 TB
Operating system	Microsoft Windows 10 IoT Enterprise 2019 LTSC
Interfaces	1 Front USB3 port + 4 Rear USB3 ports (available for mouse, keyboard or USB key/drive connection – a USB3.X external hard drive is recommended for data transfer)

	1 Rear Ethernet port 1 Rear HDMI port
Recommended PC configuration to run Nio® Reader Client application	
Processor	Intel Core i5 or higher, at least 2 cores of 2 GHz or higher
Graphical Card	recommended (equivalent to NVIDIA GeForce GT 1030 or higher)
Screen	at least 1920 x 1080; aspect ratio 16:9
RAM	At least 16 GB
Operating system	Microsoft Windows 10 64bits

Instrument performances (applies for all Nio® E, Nio® and Nio®+ configurations)	
dPCR Quantification uncertainty	+/-10% on 100-25000 cp/ µL

Ambient condition (applies for all Nio® E, Nio® and Nio®+ configurations)	
Pollution degree	2

OPERATION CONDITIONS (applies for all Nio® E, Nio® and Nio®+ configurations)	
Environmental conditions	Clean indoor laboratory
Temperature	+15°C to +25°C
Relative humidity	40% to 80% non-condensing
Altitude	Operating at max. 2000m above sea level  **Warning: To operate beyond 500m, contact support**

STORAGE CONDITIONS (applies for all Nio® E, Nio® and Nio®+ configurations)	
Temperature	+5°C to +40°C

Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa

SHIPPING CONDITIONS (applies for all Nio® E, Nio® and Nio®+ configurations)	
Temperature	-40°C to +60°C
Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa

7 Nio® Digital PCR installation instructions

In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

7.1 Operating requirements

Proper infrastructure requirements for Nio® Digital PCR installation:

- A clean laboratory environment
- A sturdy surface, for supporting up to 65 kg and with a horizontality level defect inferior to 0.3°
- A minimum distance of 20 cm to neighboring objects around the Nio® Digital PCR instrument
- Room temperature between +15°C and +25°C

Warning: The Nio®+ may consumes up to 1750W, please refer to the technical specifications above for more details about power supply requirements.

Warning: the instrument is designed to be used in a PROFESSIONAL ENVIRONMENT FACILITY. It is likely possible that it will not work properly in a DOMESTIC ENVIRONMENT. In case of a performance supposedly affected by electromagnetic interferences, a proper functioning can be restored by increasing the distance between the instrument and the interferences source.

It is advised to perform an electromagnetic environment evaluation before the installation of the instrument.

Warning: Do not use this instrument close to high electromagnetic radiation sources (for instance "RF" sources intentionally non protected) which can disturb its right functioning.

7.2 Instrument installation

The instrument is purchased either in its Nio® E, Nio® or Nio®+ configuration. The definitive configuration of the instrument is performed at initial installation by a Stilla Technologies trained

specialist or Stilla Technologies' distributors staff member as it requires both hardware and software manipulations. The initial installation must always be executed by a trained Stilla Technologies representative. The Nio® E/Nio®/Nio®+ will be installed according to Stilla Technologies specifications. The installation will be documented with an installation notice. The installation notice is required to release the Nio® Digital PCR for customer operation.

The unpacking of the Nio® Digital PCR should only be performed by a Stilla Technologies representative or by a customer only if instructed by Stilla Technologies, then follow the "How to unpack the instrument" procedure.

Three people are required to move the Nio® Digital PCR inside a facility.

The instrument shall always be gripped by the specific handles that are shown on the picture below:

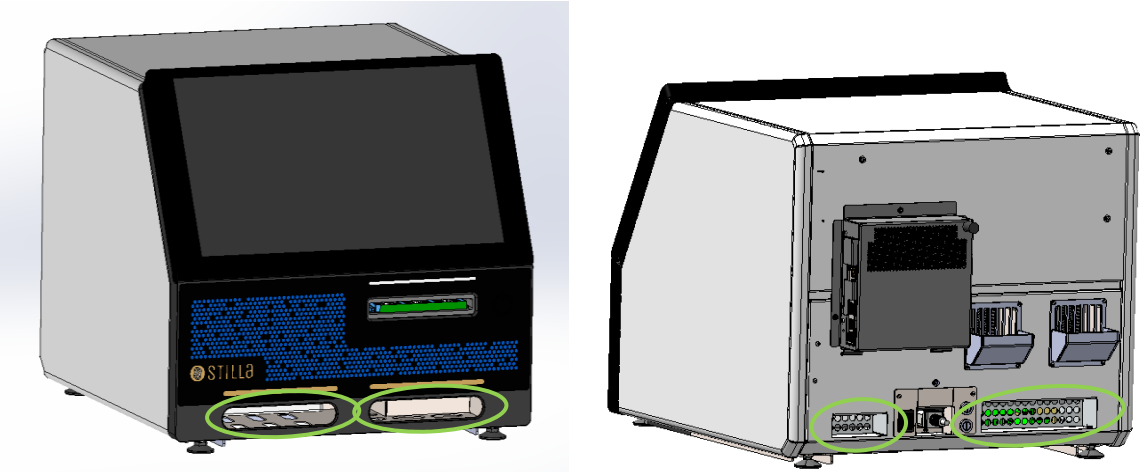


Figure 6: Nio® E /Nio® /Nio® + handles

Only the handles shown in the picture above shall be used to lift the instrument.

The Nio® Digital PCR instrument must be positioned on a sturdy surface able to support 65kg. A minimum distance of 20cm to neighboring objects must be respected to let the right access to all interfaces, the sectioning device (power cord) and to allow good air circulation.

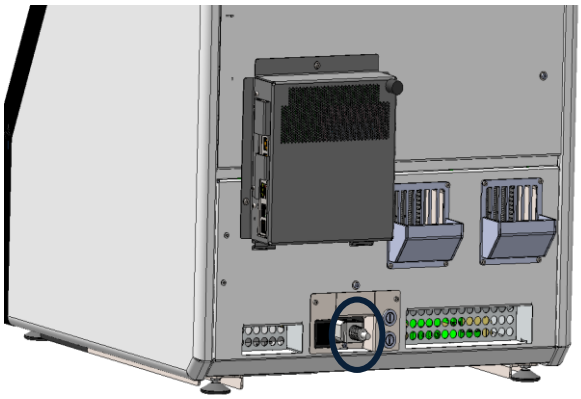


Figure 7: Nio® E /Nio® /Nio® + power plug location

Warning: The back of the instrument, in particular the open parts (ventilation parts – see Figure 4), must be protected from light (sun/window or artificial light) as much as possible (while guaranteeing the 20 cm space) to avoid altering the imaging quality.

Warning: The Nio® Digital PCR instrument must not be placed on a surface where vibrations or shocks can occur.

Plug the Nio® Digital PCR using the power cord supplied by Stilla Technologies at the rear of the instrument. The proper security grounding of the instrument will be ensured by the power cord plugging on a grounded building main plug.

Warning: Only the specific power cord supplied by Stilla Technologies can be used to guarantee the correct functioning and safety of the Nio® Digital PCR and the regulatory compliance.

After installation of the Nio® Digital PCR instrument, its leveling shall be controlled to ensure the expected performances, following the “How to control and adjust the instrument leveling” procedure. While performing the leveling of the instrument, take off the tapes maintaining the thermocycler covers closed, and the screen cover against the door.

Warning: In case of the need for a relocation of the instrument in the facility, contact a Stilla Technologies representative.

7.3 Installing / Updating the Nio® software suite

Nio® Reader software and Nio® Analyzer software’s Standard modes are pre-installed on every shipped Nio® Digital PCR. If applicable, Stilla Technologies trained specialists or Stilla’s distributors specialists update software to their latest versions at installation. If the Regulatory mode is required, Stilla Technologies recommends that the switch from Standard to Regulatory mode is performed during the installation of the instrument by a Stilla Technologies or Stilla’s distributors specialist. The customer must ensure that an IT department administrator is available to assist the 21 CFR Part 11 Nio® Reader and Nio® Analyzer software installation. For the detailed instructions on how to install and configure naica® data Service, please refer to sections 10.1 and 10.2.

The Nio® software suite can also be installed on a personal computer and Nio® Reader software can connect remotely to the instrument.

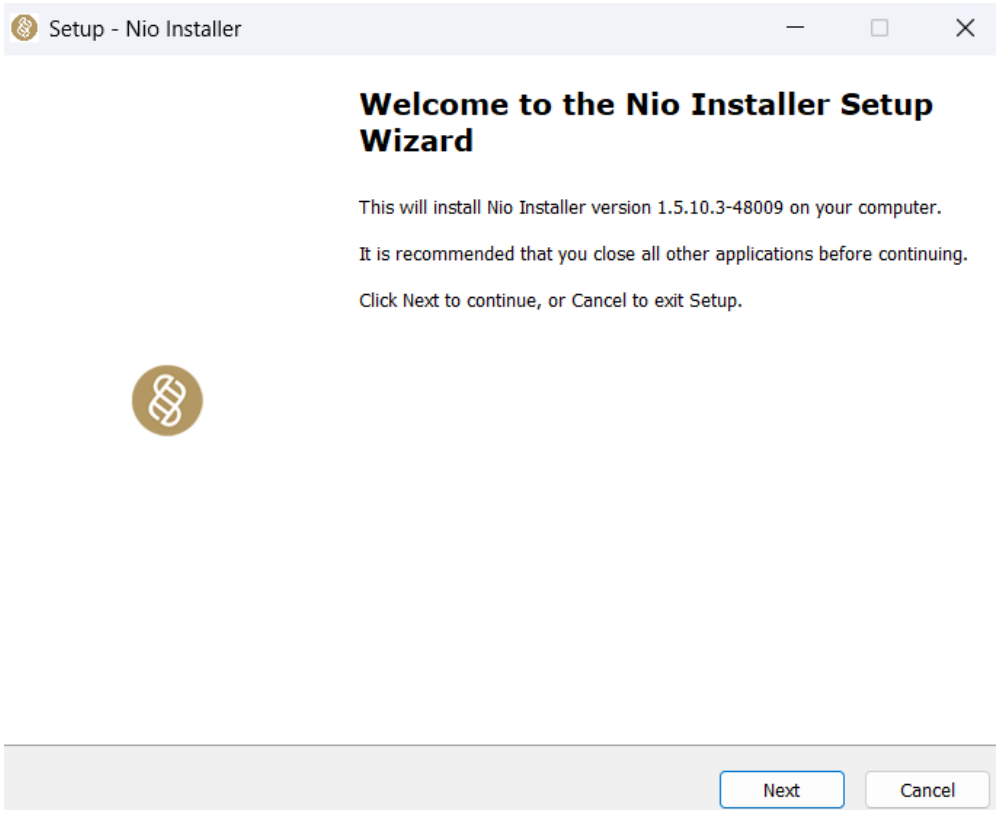
Warning: For optimal performance, Stilla Technologies recommends to perform the analysis of .niodata files with Nio® Analyzer software on a personal computer and not on the Nio® Digital PCR instrument itself, especially when it is running.

7.3.1 Installing / Updating the Nio® software suite

Installers of the most recent versions of the Nio® software are available for download, free of charge, at the following link: https://www.bio-rad.com/fr-fr/product/nio-system?ID=9d6dac6d-4584-a142-2cce-f1b84b48e731

Warning: Please ensure that there are no more Chip Plate in the instrument prior software update.

Nio® Reader software installer guides the user across the installation of either the Standard mode or the Regulatory mode. When launching the installer, the setup wizard pops-up:

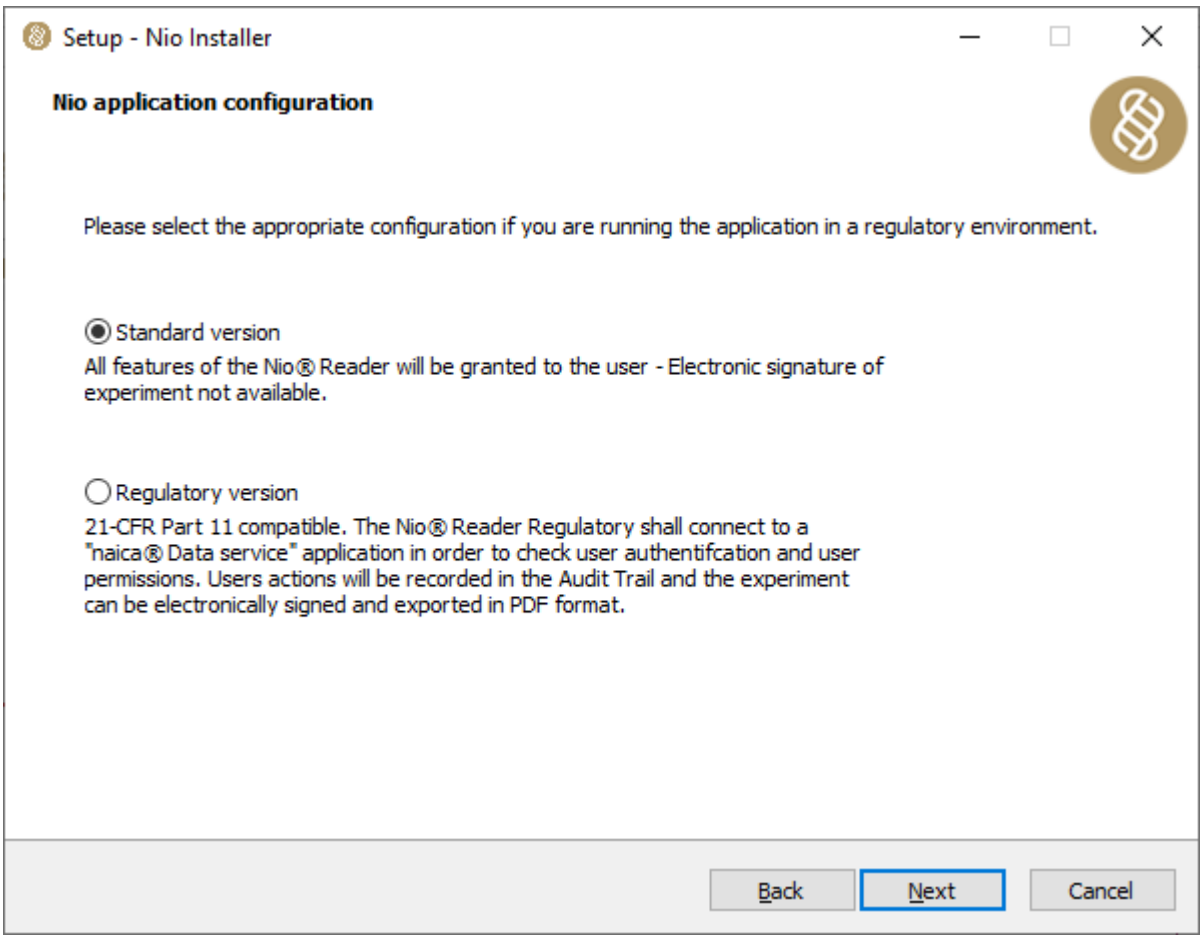


To install Nio® Reader software, click “Next”.

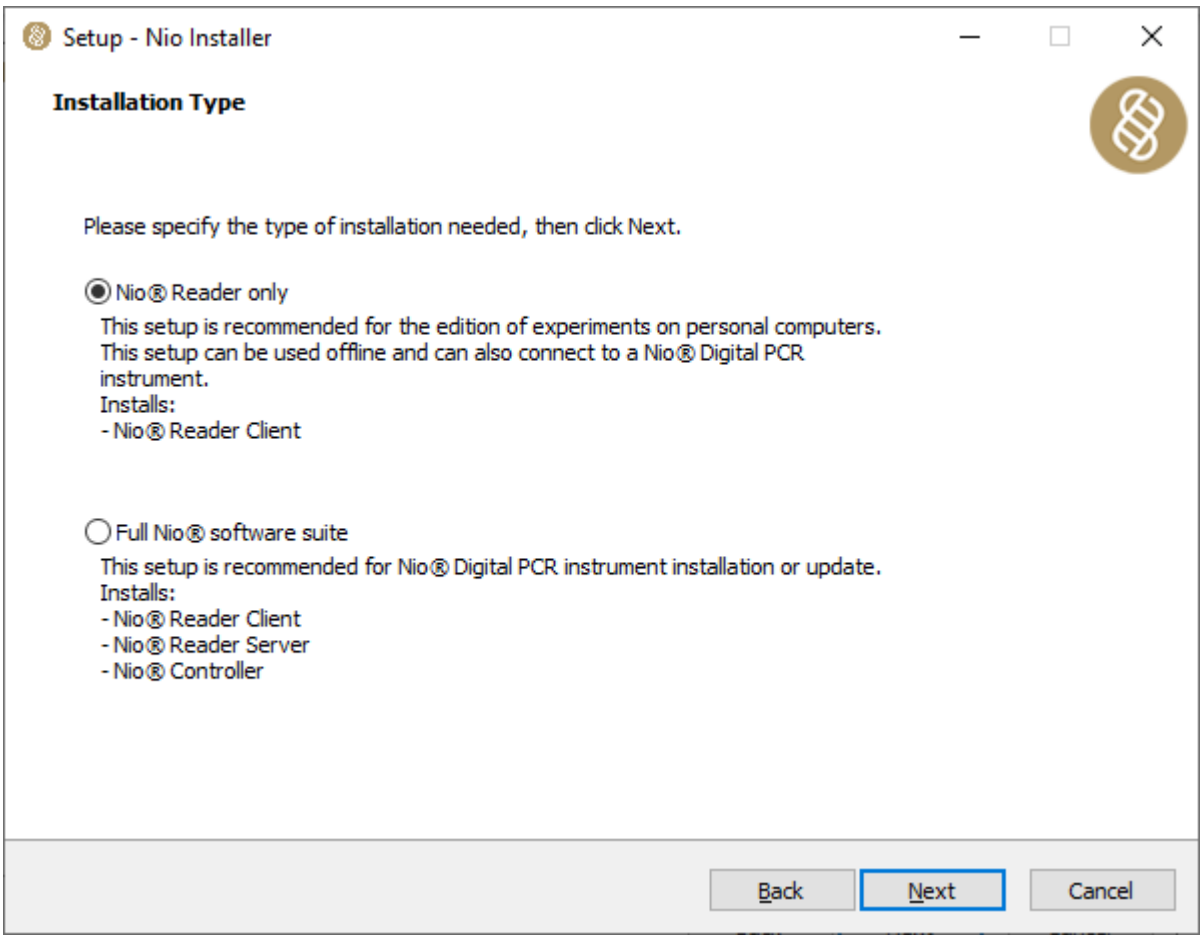
At this stage, the user must select to install either the Standard mode or the Regulatory mode:

- Standard: For users who do not need to operate in compliance with Title 21 of the U.S. Code of Federal Regulations Part 11 (21 CFR Part 11).
- Regulatory: For users needing to operate in compliance with 21 CFR Part 11.

Note: If installing the Regulatory mode, Nio® Reader software functions in conjunction with naica® Data Service. Please refer to sections 10.1 and 10.2 for the detailed instructions on how to install and configure naica® data Service.


Setup - Nio Installer
Nio application configuration
Please select the appropriate configuration if you are running the application in a regulatory environment.
Standard version
All features of the Nio® Reader will be granted to the user - Electronic signature of experiment not available.
Regulatory version
21-CFR Part 11 compatible. The Nio® Reader Regulatory shall connect to a "naica® Data service" application in order to check user authentifcation and user permissions. Users actions will be recorded in the Audit Trail and the experiment can be electronically signed and exported in PDF format.
Back
Next
Cancel

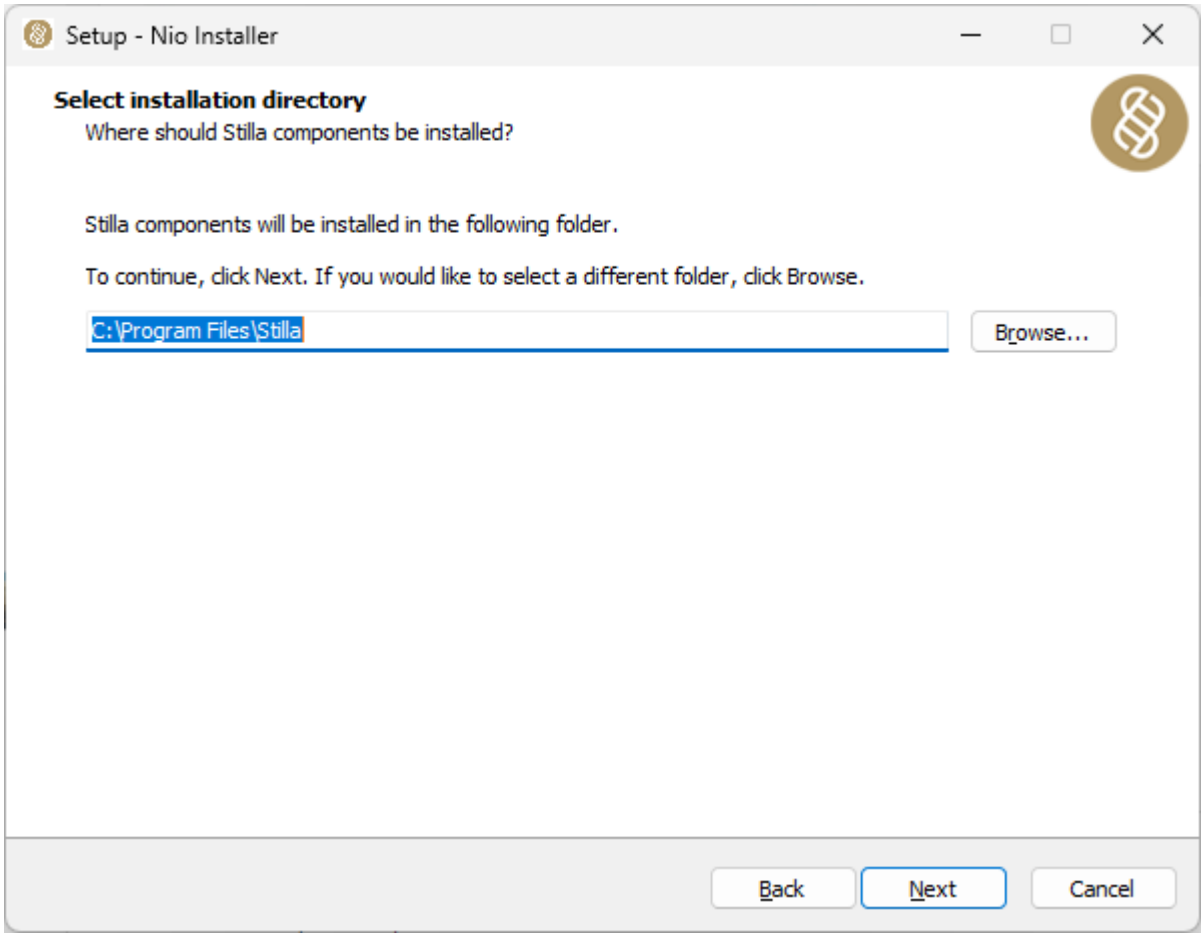
Click “Next”.



Whatever the mode selected at the previous step (Standard or Regulatory), select the type of installation between “Nio® Reader only” or the “Full Nio® software suite”:

- Select the “Nio® Reader only” option when installing Nio® Reader software on a distant personal computer. The necessary application to design experiments (Assays, Protocols and Experiment files) is then installed. It is possible to connect remotely to the Nio® Digital PCR instrument from a distant personal computer. Remote connection to the Nio® Digital PCR instrument allows monitoring runs on the instrument live and from any connected computers. To do so, the network configuration must be properly set prior to software installation. Please refer to section 7.3.2 for instructions details to configure the Nio® Digital PCR instrument to allow remote connection.
- Select “Full Nio® software suite” when updating or installing the software on the Nio® Digital PCR instrument. All the software components are installed, allowing both setting up experiments and piloting the instrument.

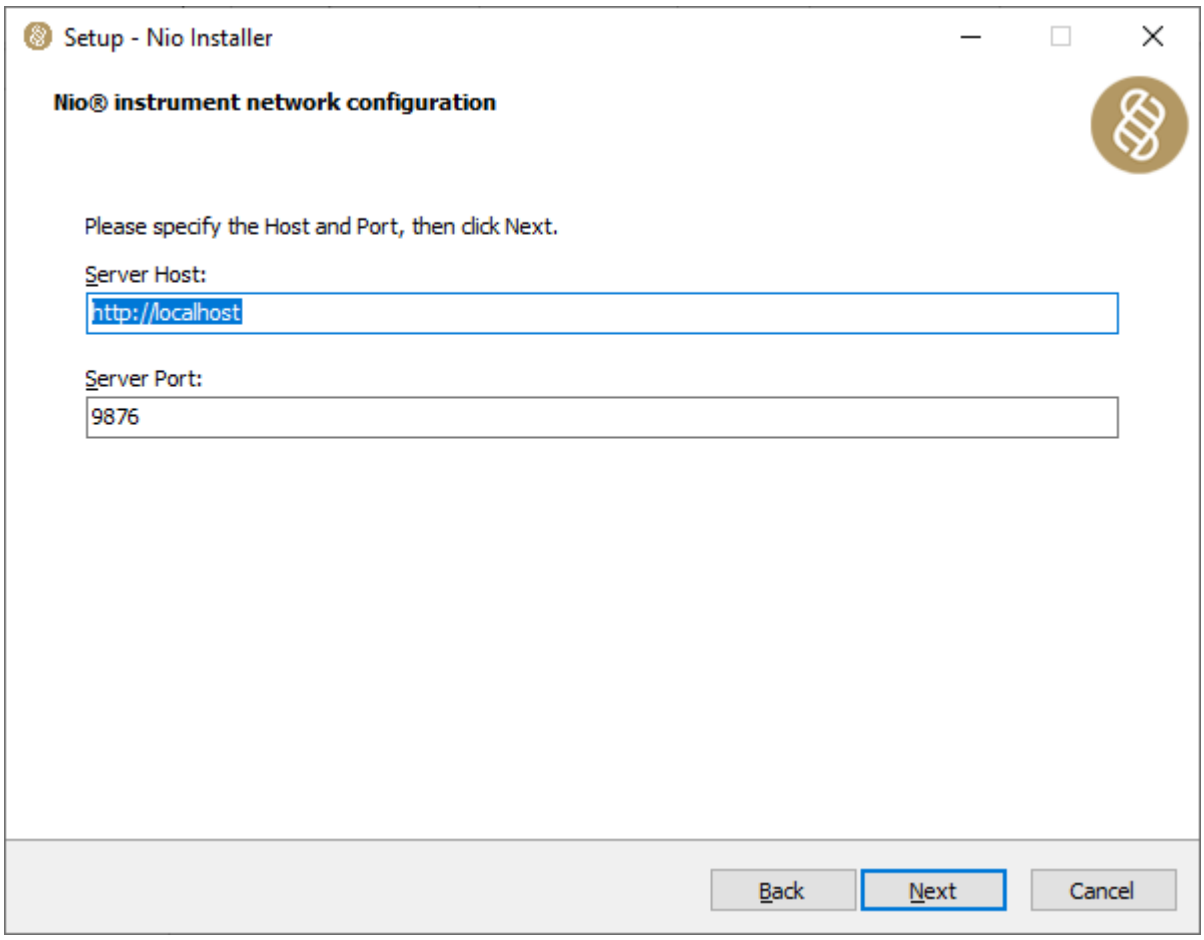
Click Next.



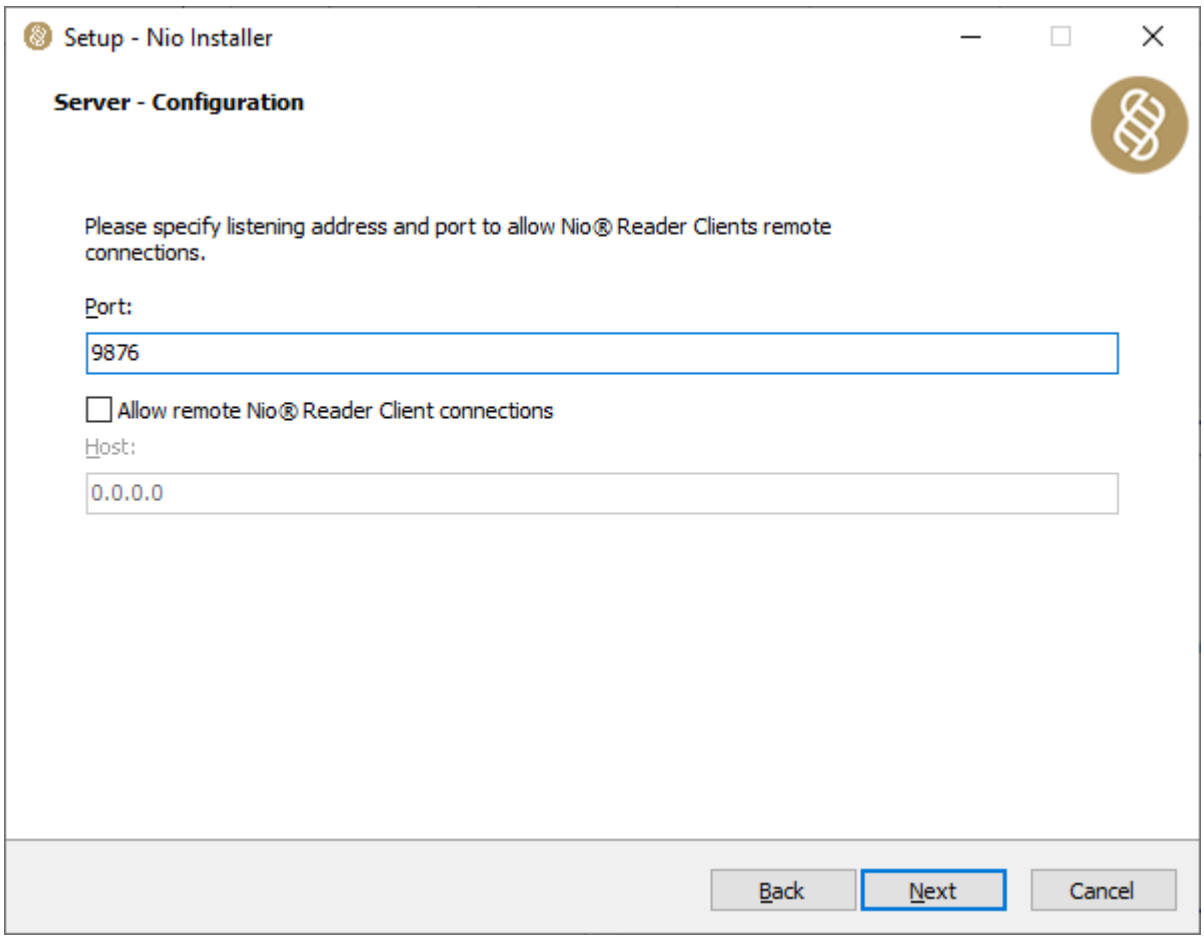
Configure the installation directory and click “Next”. The next step differs depending on the type of installation selected (“Nio® Reader only” or “Full Nio® software suite”):

- When installing “Nio® Reader only”, the pop-up below is displayed to set the network configuration for remote access to the Nio® digital PCR instrument from a distant personal computer.

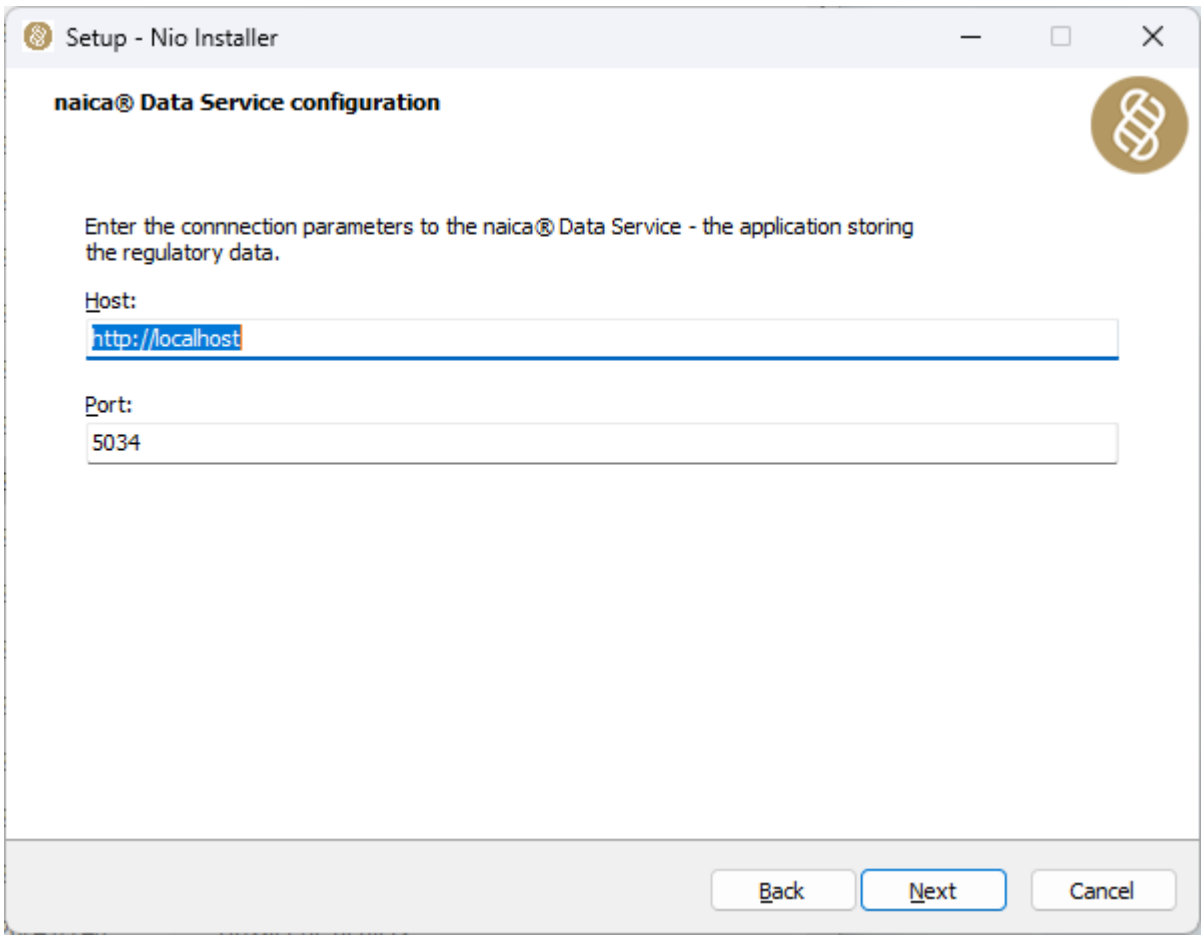
  If the Nio® Reader application is not meant to connect remotely to a Nio® Digital PCR instrument, leave the default settings for Server Host and Port settings displayed on the screenshot below.



- When installing the “Full Nio® Software Suite”, the pop-up below is displayed, to set the Server connection. For the latter, the default value allows any input connection if Windows Defender is properly set (see section 7.3.2 for more details). Please contact your IT department to properly configure these parameters.



If the software install mode was “Regulatory”, the following page will be displayed, asking the parameters to connect to the naica® Data Service for Nio® application. Refer to section 10.2.1.2 for Host and Port entries.



Finish the installation.

When installing Nio® Reader software on a distant personal computer meant to connect remotely to the Nio® Digital PCR instrument, start the Nio® Reader software and view the instrument status and the server connection enabled to verify that the installation proceeded well.

Note: it is possible to change the Nio® Digital PCR instrument's server address and port directly in the Nio® Reader software in the Settings page, if necessary.

Please refer to Nio® Analyzer manual to install or update Nio® Analyzer software either on the Nio® Digital PCR instrument or on a distant personal computer.

7.3.2 How to update Windows Defender to allow remote connection

Note: This is an IT procedure that should be done once on the Nio® Digital PCR instrument to allow remote connection from a distant personal computer.

On the Nio® Digital PCR instrument, create an Inbound port rule.

Open "Windows Security", click on "Firewall & network protection", and then select "Advanced settings" which opens the "Windows Defender Firewall with Advanced Security" window.

Add a new Inbound rule:

- Rule type: port
- Apply the rule to TCP connections and specify the port number of the Nio® Reader Server
- Select "Allow the connection"
- Select the network location types to which this rule applies
- Name the rule and click on finished

Important: this is the procedure to follow if the firewall on the Nio® Digital PCR Instrument is Windows Defender. Should any other firewall be installed, the procedure for allowing external connection in the communication port between the Nio® Reader Client and Nio® Reader Server on the chosen port should be applied on the firewall.

8 User interactions

In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

8.1 Starting up the Nio® Digital PCR

When the instrument is powered off, it's entire front side is turned off, with no lightning visible:

Figure 8: Powered-off Nio® E /Nio® /Nio® +

To power on the Nio® Digital PCR, turn on the power switch at the rear of the instrument:

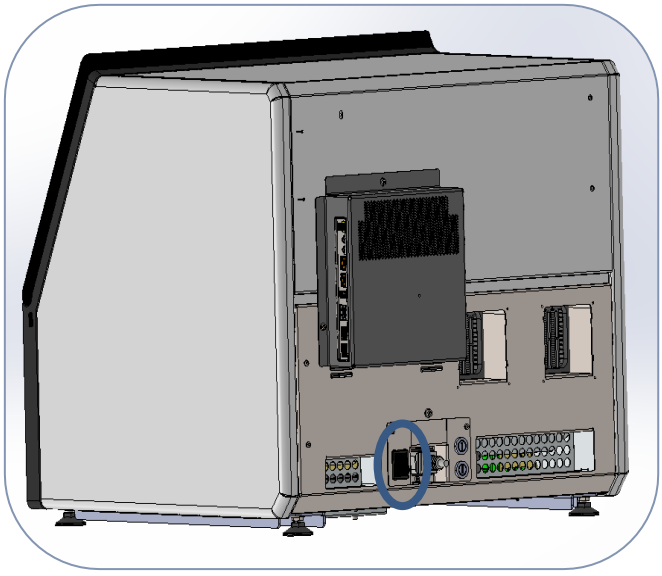


Figure 9: Nio® E /Nio® /Nio® + power switch location

When powered on, the start-up button will appear on the front of the Nio® Digital PCR:

Figure 10: Powered-on and ready to start Nio® E /Nio® /Nio® +

To start up the Nio® Digital PCR, click on the start-up button. The start-up button will start blinking, the droplet panel and the two status lines on the bottom of the front door. They will keep blinking throughout the entire initialization procedure.

Once the Nio® Digital PCR instrument is fully initialized and ready for operation:

- The start-up button disappears.
- The two-status lines are illuminated in gold.
- The droplet pattern and the Stilla logo are illuminated.
- The screen is turned on and presents the Nio® Graphic User Interface (GUI).

Figure 11: Started Nio® E /Nio® /Nio® +

8.2 Screen adjustment

The front upper panel orientation can be adjusted to improve the screen positioning and the ergonomic of use. The panel must be manipulated on its two sides to avoid contact with the tactile part of the screen that will activate the GUI area. The two nontactile areas on the sides of the screen are approximately 4cm wide and are shown in the picture below.

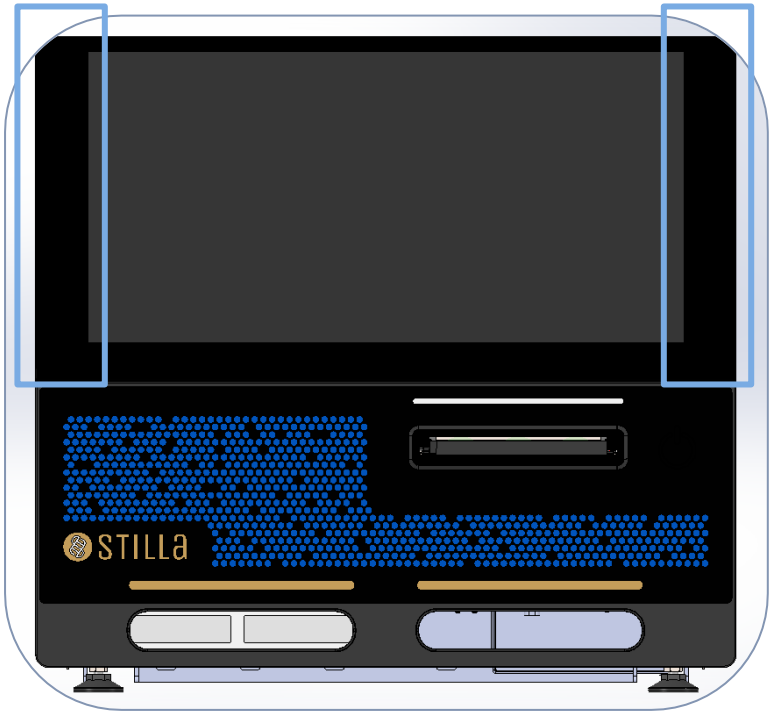


Figure 12: Non-tactile areas on Nio® E /Nio® /Nio® + screen

The orientation of the upper panels allows to position it between two maximal positions:

- Against the front cover of the Nio® Digital PCR at 30° from the vertical.
- A vertical position.

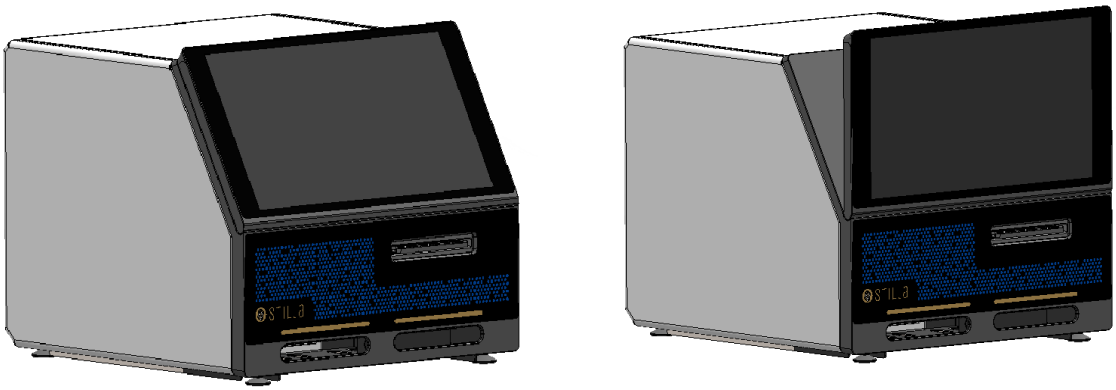


Figure 13: Nio® E /Nio® /Nio® + screen orientation adjustment. Left: 30° tilted from the vertical. Right: vertical.

A mechanical stop prevents to rotate the screen above the vertical position.

Warning: Do not force any movement beyond the specified positions (30° vertical and vertical position) as this may damage the instrument and displays a risk to disconnect / rip off the screen.

Warning: Do not try to manipulate the screen panel while anyone's hand is behind it to prevent any pinching risks.

8.3 Status display

The front panel of the Nio® Digital PCR allows to display information about the status of the instrument:

- The two bottom status lines lightning indicates 4 different instrument status:
  - Bottom status lines turned off: Nio® Digital PCR instrument turned off.
  - Lightning on permanently in Gold: Nio® Digital PCR instrument ready for operation.
  - Lightning turned on blinking: Nio® Digital PCR instrument starting up and initializing.
  - Lightning on permanently in Red: Nio® Digital PCR instrument alert mode.
- The loading status line above the chip plate loading area can display 3 statuses:
  - Loading status light turned off: no chip plate loading/unloading action planned.
  - Loading status light turned on permanently: Nio® Digital PCR awaiting user action to load / unload a chip plate.
  - Loading status light turned on and blinking: Nio® chip plate loading / unloading in progress. Do not block Nio® chip plate loading area.

Figure 14: Nio®+ front view, showing status display zones. The droplets on the front panel of Nio® E (resp. Nio®) shines in gold (resp. silver).

8.4 Switching off the Nio® Digital PCR

The Nio® Digital PCR must be switched off properly, as follow:

- Click on the red shutdown button on the top right corner of the window
- Select 'Shutdown'
- Click on 'Confirm shutdown'
- Wait until the Nio® Instrument Status switches from 'Stopping' to 'Stopped' on the Nio® Reader software window (Instrument Information section of the Homepage) and the status lines are switched off (see section 8.3)
- Once the instrument is switched off, close the Nio® Reader software window
- Switch-off the embedded PC from the Windows menu
- Turn the power switch at the back of the instrument off

9 Nio® Digital PCR Operations in Standard mode

In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

9.1 Chip plate preparation warnings

Only operate validated chip consumables with the Nio® Digital PCR.

Warning: Operating any other than specified chip consumables in chip plates risks damaging the instrument.

The validated chip consumables for Nio® Digital PCR operation are Ruby chips.

The chips are delivered in chip plates. These chip plates must be used to load chip consumables into the Nio® Digital PCR instrument. Operating any other than specified chip plates to insert chip consumables risks damaging the instrument.

Each chip plate can accommodate up to 3 chip consumables. The Nio® Digital PCR can operate any chip number starting from 1 chip consumable.

The Nio®+ configuration of the instrument can be loaded with up to 8 chip plates of 3 chips each.

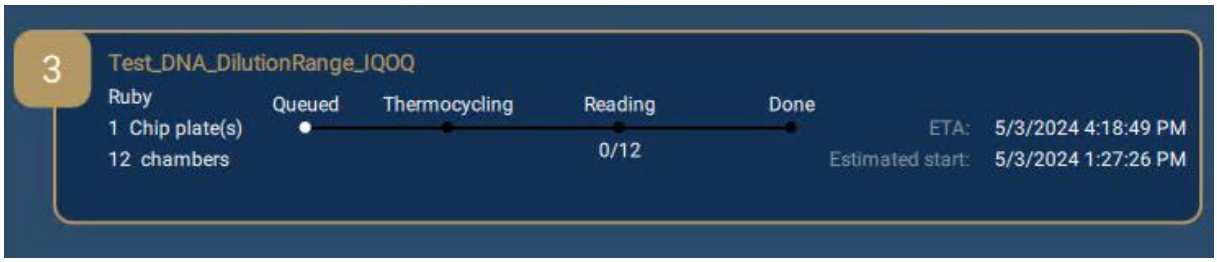
The Nio® configuration of the instrument can be loaded with up to 4 chip plates of 3 chips each.

The Nio® E configuration of the instrument can be loaded with one chip plate of 3 chips each at a time.

For high throughput use (over 4 chip plates loaded in the Nio®+instrument), Stilla recommends that the user:

- Evaluates the stability of the assay over time once loaded in the chip. Contact local Stilla representative for additional information about how to evaluate the stability of assay. For experiments where a high number of chip plates are processed at once, normalization methods can be proposed.
- Ensure that the ambient temperature is within operating temperature range. It is preferable to have a low ambient temperature to ensure the stability of the assay inside the chips in the Nio® Digital PCR.
- When possible, it is recommended to minimize the queuing time of the chip plate in the Nio®+ (i.e. the time between the chip plate loading into the Nio®+ to the start of the run of the chip plate). When possible, users should aim to reduce the queuing time to less than 4hrs, and plan preparation of the chips accordingly. Nio® Reader software displays the

estimated start time of the run of a chip plate in the Run Status page. Note that if a temperature warning is displayed, it is not recommended to launch experiments if a run slot is not immediately available.



Stilla Technologies recommends not reusing an individual chip plate for multiple experiments, as this may display a risk for cross contamination.

For the detailed description of chip preparation including the details for correct chip orientation in the chip plate, please refer to the individual chip consumable Instruction for Use.

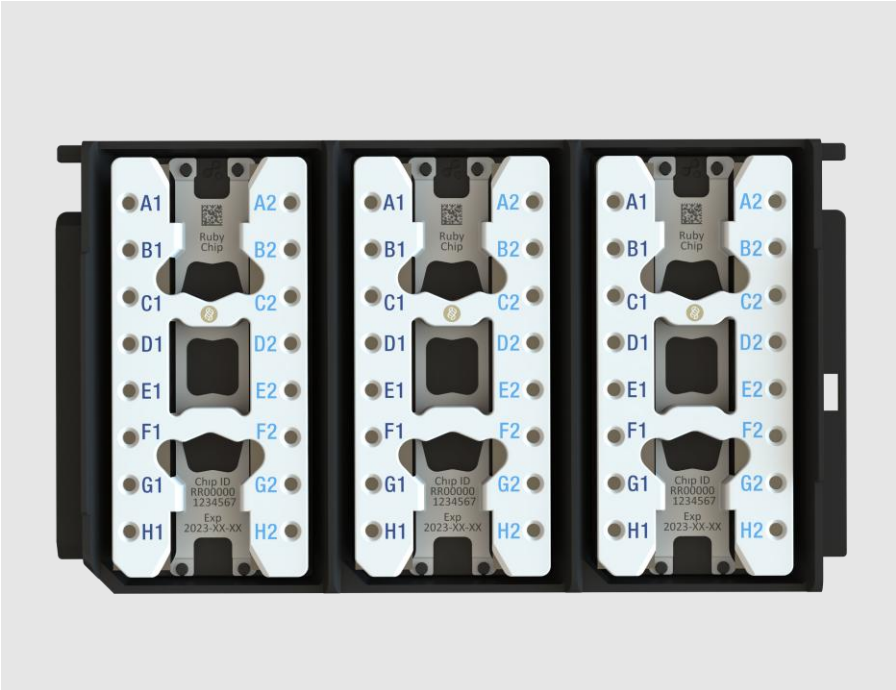


Figure 15: Top view of 3 x Ruby chips in a Chip Plate

9.2 Windows desktop

Once the instrument is started, it is possible to log in with two Windows sessions: User or Admin.

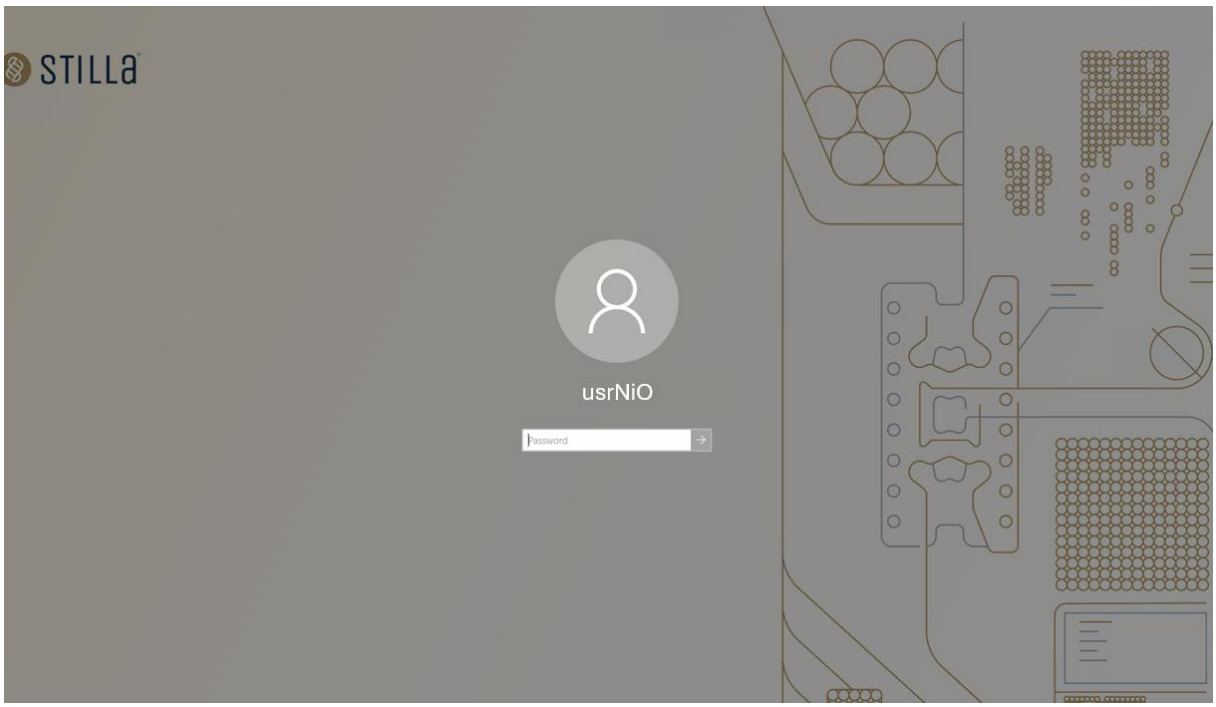


Figure 16: Windows session login page

It is highly recommended for the daily work to log under Windows session using the Non-Admin user, for cyber-security purposes.

Local time considerations: the ETA displayed in the Nio® Reader Client and the timestamps recorded in the. Nio® data file are consistent with the Nio® Digital PCR local time. If the instrument time is modified some inconsistencies may appear between inconsistent time-zoned computers.

Please refer to local IT procedure for session password updates and cybersecurity policy.

Use the Nio® Reader icon to start working with the Nio® Digital PCR.

It is not recommended to launch several Nio® Reader applications on the Nio® Digital PCR instrument, in a single Windows session or across several sessions for multiple users. The Nio® instrument will struggle to provide feedback to the several instances of the Nio® Reader software. Please close the application before switching to another User or Windows session.

At start-up, the warning below may pop-up in two cases:

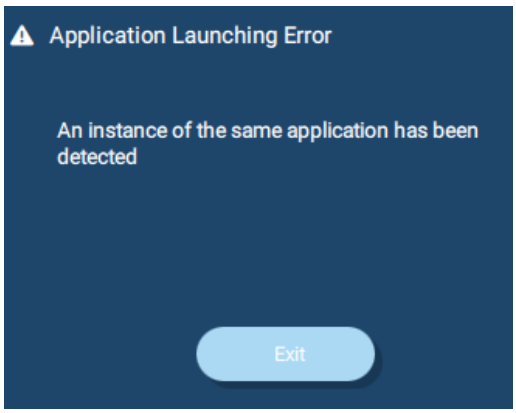


Figure 17: Warning pop-up in case of multi-instance.

- Several instances of Nio® Reader software are opened at the same time under the current user session. Only keep one instance open.
- Or, another instance of Nio® Reader software is opened under another user session on the Nio® Digital PCR instrument. Either close the current session and work on the other one or close the Nio® Reader software instance in the other user session.

9.3 Nio® Digital PCR experiment

The Nio® Reader graphical user interface (GUI) allows to set-up, launch and follow the experiments run on the Nio® Digital PCR.

Warning: It is possible to import or export information to set-up an experiment using an USB stick, always control that the USB stick is not infected by malicious software.

9.3.1 Home Page

The home page is displayed when entering in Nio® Reader software.

The Home page:

- Contains a quick user guide on how to use the instrument,
- Allows to shortcut to the Experiment edition page of recent runs,
- Allows to unload chip plates already present in the instrument.
- Displays the instrument status

Figure 18: Nio® Reader software Home Page

9.3.2 Top Ribbon

The top ribbon is always present on the top of the interface.

It allows to:

- Reach the Home Page,

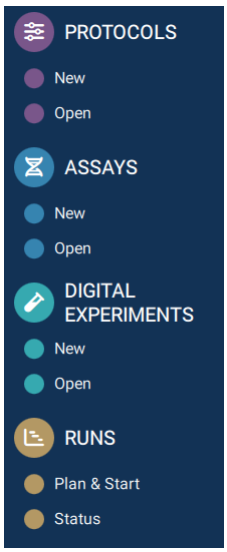
- Reach the Settings and Legal information Page

- Shutdown, Stop all or Restart

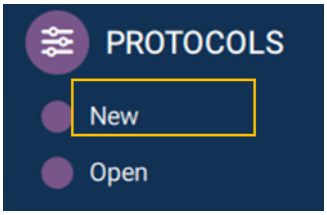
9.3.3 Left Ribbon

The left ribbon allows the navigation between the user interface pages needed to create and run an experiment.

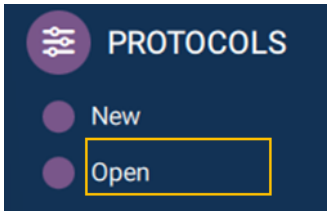
Click on the button under each workflow step name to reach the desired page.



For example, for “protocol” workflow step, create a new protocol by clicking on the “New” button:



Or open an existing protocol by clicking the “Open” button:



9.3.4 Protocol creation

The Protocols page allows to define a new protocol or open an existing one. The protocol specifies the instructions given to the instrument to be executed: mix type, PCR program and scanning conditions (activated channels and their corresponding exposure times).

The following page then allows to set-up or edit a protocol:

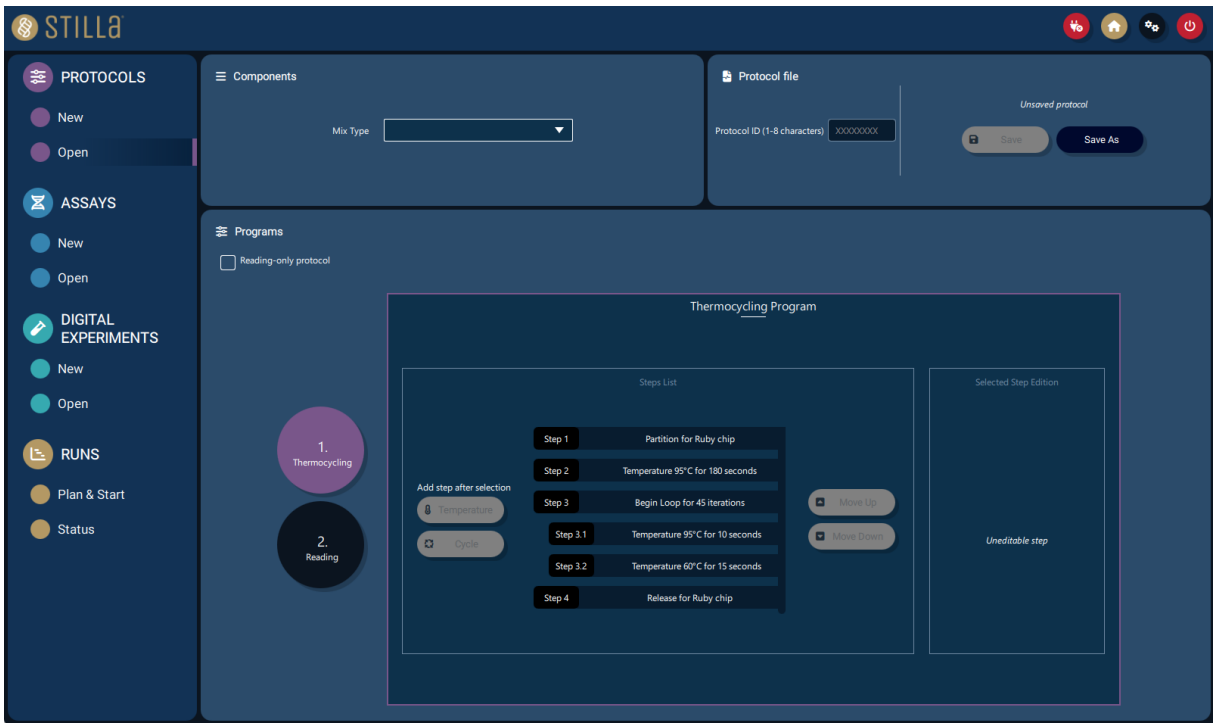
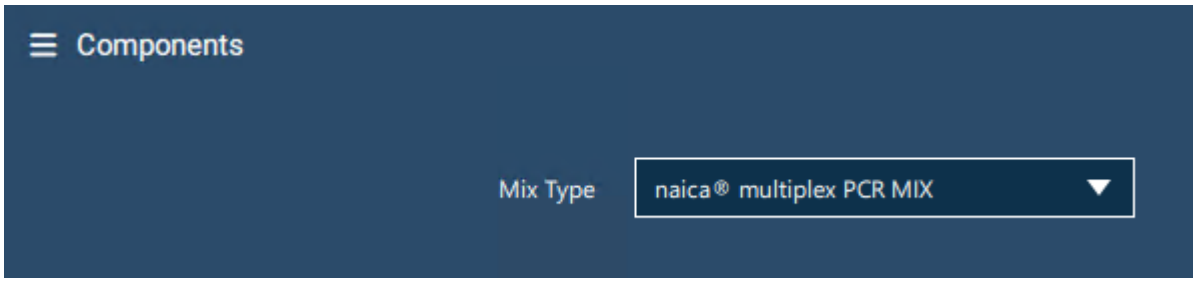


Figure 19: Nio® Reader software Protocol Page

By default, all experiments are composed of 2 steps: Thermocycling and Reading. For a Read-only protocol, it is possible to remove the thermocycling step by clicking the check box “Reading-only protocol)

This page allows to:

- Save the Protocol file and define its name
- Set a protocol ID (from 1 to 8 characters): affects tag to a protocol. This tag is used to easily identify the protocol among others in the experiment edition page, or in split results (see Composite Experiments)
  Note: if protocols created with former versions of Nio® Reader software (v1.0 or v1.1) are used, a default protocol ID will be automatically assigned. This default ID will be “PRT+I” where “I” increments.
- Select the PCR mix used,



- **Warning:** The mix type defines the recommended set of thermocycling and reading parameters used by Nio® Analyzer software for the calculation of the concentration, so it needs to be properly set to match the PCR mix actually used for the experiment. By default, no mix type is selected. The PCR mix is selected among the following list:
  - naica® multiplex PCR MIX for experiments with DNA target(s) and fluorescent probes.

- naica® PCR MIX for experiments with DNA target(s) and Evagreen reporter; Select the “Blue reference” (“Infra-Red reference”) to use the blue (infra-red) channel as reference channel for droplets detection.
- QX700™ One-Step RT-ddPCR Kit for Probes, for experiments with RNA target(s) and fluorescent probes
- QX700™ ddPCR™ EvaGreen® Supermix for experiments with DNA target(s) and Evagreen reporter
-
- qScript® XLT One-Step RT-qPCR ToughMix® is no longer compatible with the new software version 1.5.10.3, it is still compatible with the previous software version 1.3.8.3..

- Define the PCR program:
  - By adding or deleting steps and setting their duration (in sec), temperature (in °C), and ramp rate (in °C/sec).
  - By creating an editable number of PCR cycles.

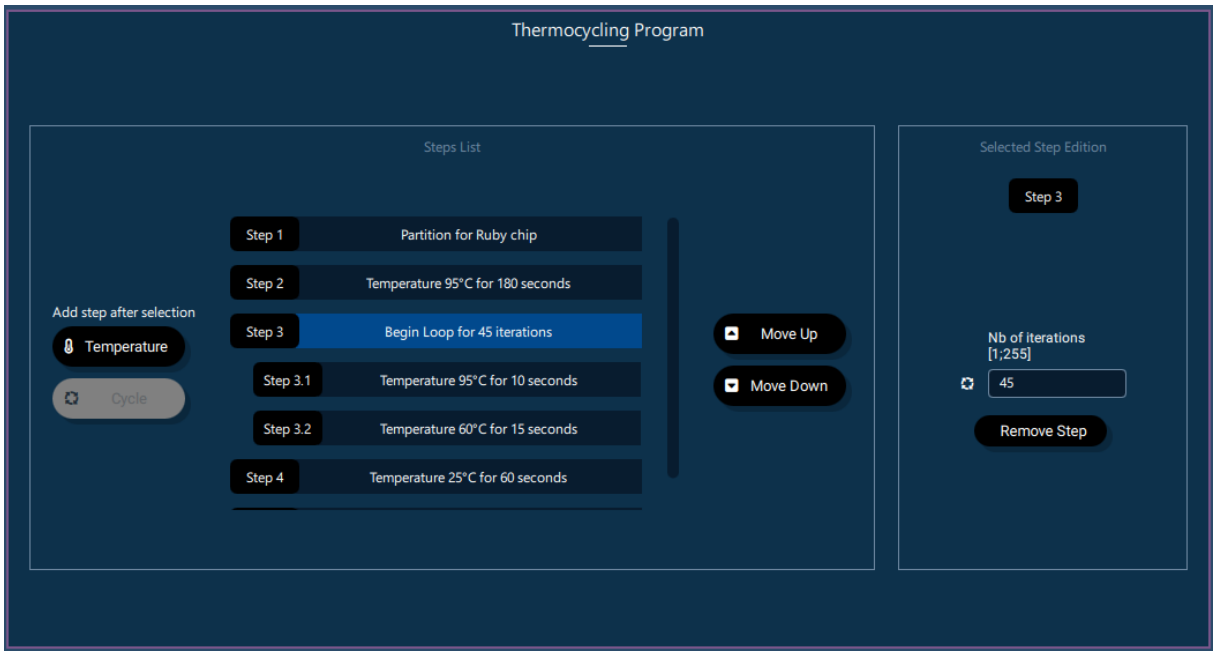


Figure 20: Thermocycling program definition

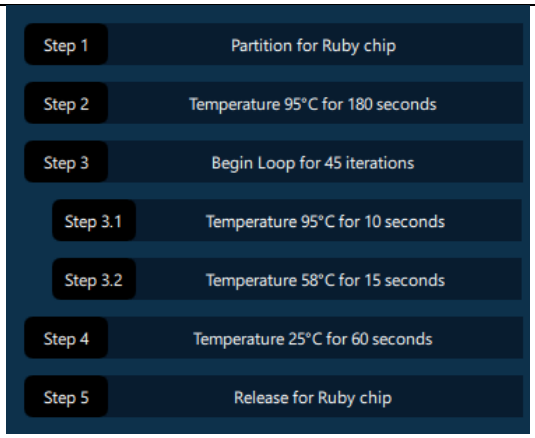
- Define the reading program by:
  - Activating the desired channels.
  - Defining the exposure times for each channel (in ms).

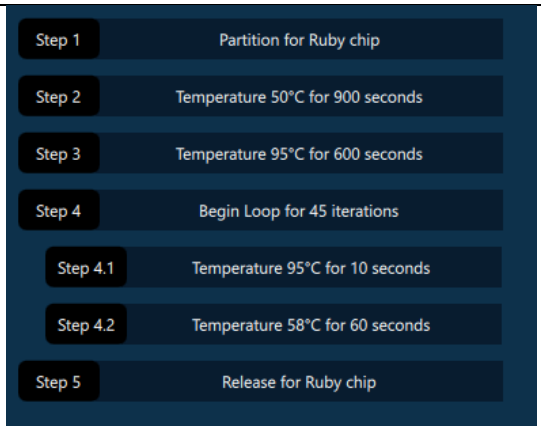
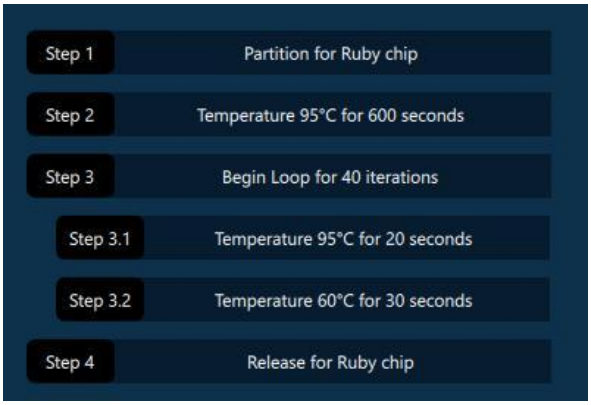
Figure 21: Reading Program definition

When creating a new protocol, the software offers to start either from scratch or from an existing protocol. Five protocol templates are pre-loaded and can be used as starting point

- "Template_PCR-45-cycles_7channels_naica-multiplex-PCR-MIX_RubyChip_v1.1" for experiments targeting DNA with TaqMan® probes.
- "Template_PCR-45-cycles_Evagreen-BlueDetection_naica-PCR-MIX_RubyChip_v1.1" for experiments targeting DNA with Evagreen and using the blue channel as reference channel for droplets recognition.
- "Template_PCR-45-cycles_Evagreen-InfraRedDetection_naica-PCR-MIX_RubyChip_v1.1" for experiments targeting DNA with Evagreen and using the infrared channel as reference channel for droplets recognition.
- "Template_QX700_One-Step RT-ddPCR Probes_45-cycles_7channels_RDG16_v1.1" for experiments with RNA target(s) and fluorescent probes.
- "Template_QX700_ddPCR EvaGreen_40-cycles_RDG16_v1.1" for experiments with DNA target(s) and Evagreen reporter.

The thermocycling programs of each template protocol are described in the table below:

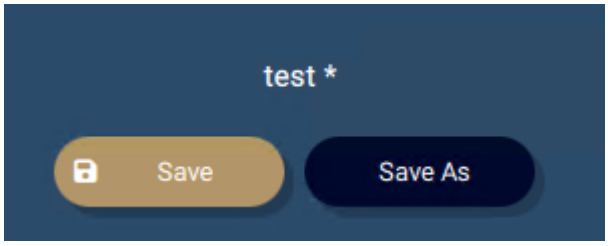
• **Template_PCR-45-cycles_7channels_naica-multiplex-PCR-MIX_RubyChip_v1.1 /** • **Template_PCR-45-cycles_Evagreen-BlueDetection_naica-PCR-MIX_RubyChip_v1.1 /**	

• **Template_PCR-45-cycles_Evagreen-InfraRedDetection_naica-PCR-MIX_RubyChip_v1.1**	
• **Template_QX700_One-Step RT-ddPCR Probes_45-cycles_7channels_RDG16_v1.1**	
• **Template_QX700_ddPCR EvaGreen_40-cycles_RDG16_v1.1**	

Once the protocol is entirely defined, press the “Save” or “Save as” button to save it as a nioprotocol file readily usable in the Experiments panel.

While the protocol is not saved, the mention “unsaved protocol” is displayed on top of both “Save” and “Save as” buttons.

When a protocol is being edited, an asterisk is displayed next to the protocol name warning that there are unsaved changes.



The protocol definition can be done with Nio® Reader software on personal computer, it does not require the use of the Nio® Digital PCR instrument.

9.3.5 Assays creation

The Assays page allows to define a new assay or open an existing one. It regroups information with regards to the targets and fluorophores as well as the way to analyze and display the results in Nio® Analyzer software.

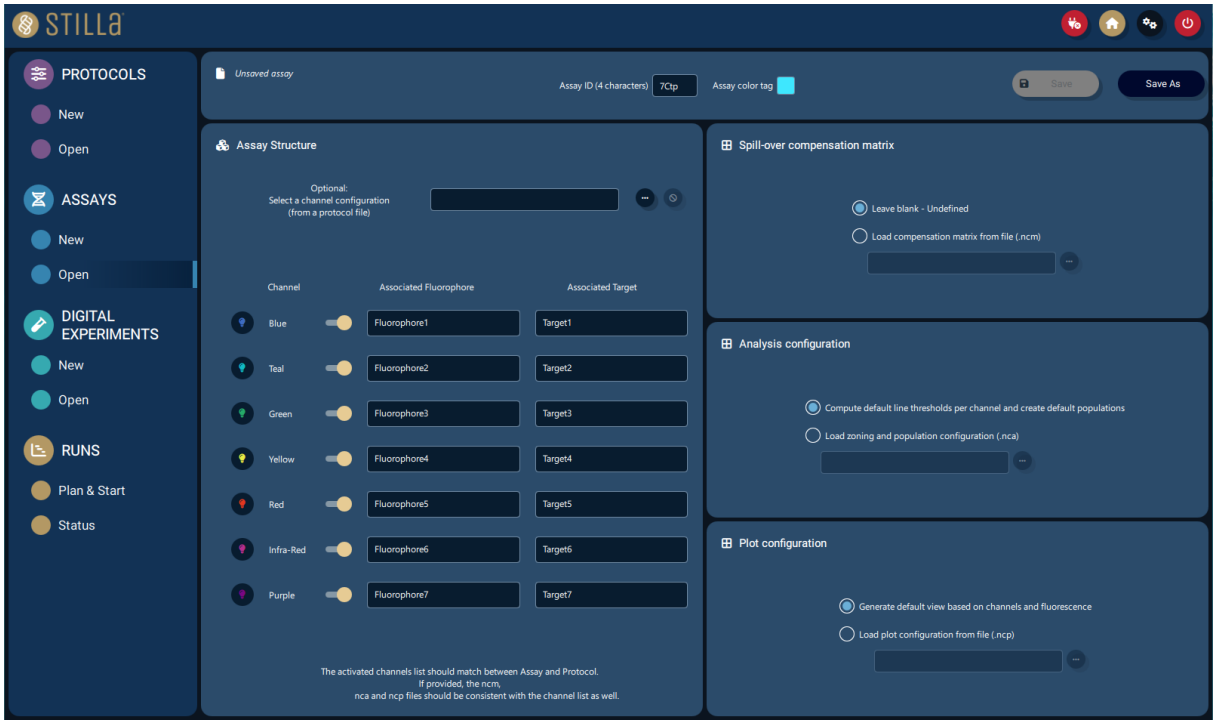
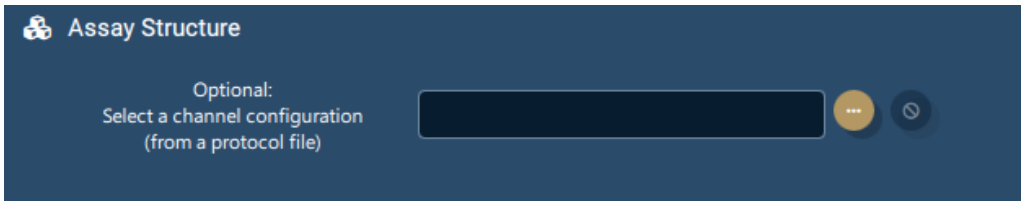


Figure 22: Nio® Reader software Assays page

This page allows:

- Assigning a 4 digits ID and color tag to the assay
- Specifying the structure of the assay, namely:
    - The fluorophores associated to each channel. It is possible to import the list of activated channels from the protocol by loading a .nioprotocol file clicking the three dots button.



    - The target associated to each channel.
- Defining how to analyze and display the results in Nio® Analyzer software by:
    - Loading a spill-over compensation matrix (ncm file)
    - Loading an analysis configuration file (nca file)
    - Loading a plot configuration file (ncp file)

These configuration files are useful in Nio® Analyzer software where they are used (and can be designed), please refer to the Nio® Analyzer software user manual to have more details about them.

When creating a new assay, the software offers to start either from scratch or from an existing assay. Two assay templates are pre-loaded and can be used as starting point:

- “Template_multiplex_7channels_RubyChip_v0.1” with all the seventh fluorescent channels activated,
- “Template_naica-PCR-MIX_Evagreen_RubyChip_v0.1” with the blue, green and infra-red channels activated for Evagreen, None and the AlexaFluor647 fluorescent reporter respectively.

Once the assay is entirely defined, press the “Save” or “Save as” button to save if as a .nioassay file, readily usable in the Experiments panel.

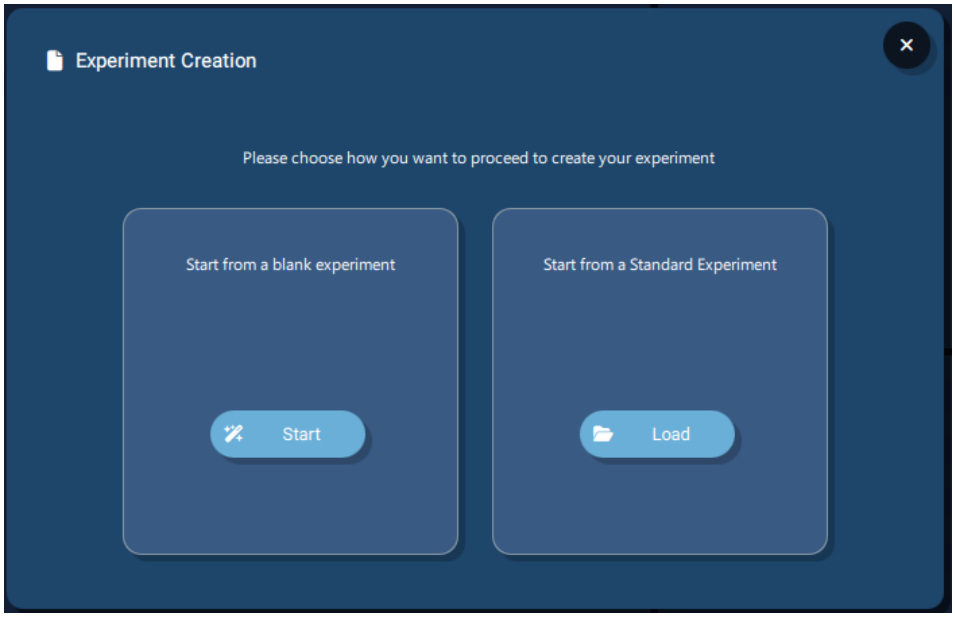
While the assay is not saved, the mention “unsaved assay” is displayed on top of both “Save” and “Save as” buttons.

When an assay is being edited, an asterisk is displayed next to the assay name warning that there are unsaved changes.

The assay definition can be done with Nio® Reader software on personal computers, it does not require the use of the Nio® Digital PCR instrument.

9.3.6 Experiments creation

The Experiments page allows to create a new experiment (from blank or by using a Standard Experiment (.niostdexperiment), and load its parameters as template) or edit an existing one.



When creating an experiment from an existing Standard Experiment, the following parameters are imported in the new experiment:

- The protocols
- The assay
- The pooling (if any)
- The sample data (if any)

The experiment edition step allows to associate the protocol file(s) and the assay file(s) to a set of chips or chambers.

Figure 23: Nio® Reader software Experiment page

In this page, the Chip Plate layout panel allows the visualization of the structure of the experiment at a glance and is used to:

- Name the experiment.
- Specify the layout of the chip plate:
    - At this stage, chips' barcodes can be filled in manually (the barcode must be entered in capital letters) already or left blank until the barcode of each chip is scanned by the Nio® Digital PCR instrument,
    - It is possible to enable or disable a custom set of chambers and/or chips with the "Enable/disable elements" button.

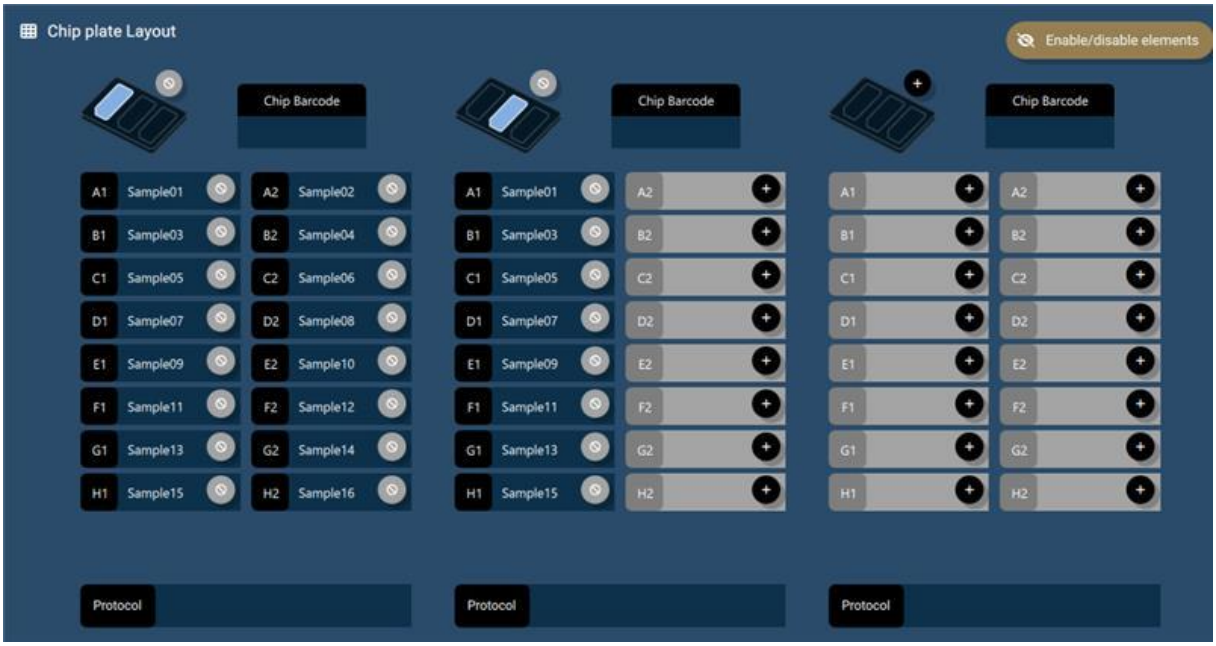


Figure 24: Screenshot of the layout of the chip plate in the Experiment page, when clicking on the "Enable/disable elements" button. In this example, the right column of the middle chip and the right chip are disabled.

- Assign a protocol (resp. an assay) to each enabled chip (resp. chamber) of the chip plate; To do so, the .nioprotocol (resp. nioassay) files must be prior loaded in the experiment.

Load a protocol (resp. an assay) by clicking on “Add” in the Protocol (resp. Assay) section of "Files” menu

Note: it is possible to import several protocol (resp. assays) files at the same time using multiple selection in the file browser popup window.

To assign a protocol to a chip, drag and drop the protocol files from the protocol library to the “Protocol” box under the chip:

Figure 25: Assigning a protocol to a chip

If a protocol has a protocol ID (feature from Nio® Reader v1.2 and above), it is displayed. If a protocol file has been created with Nio® Reader software v1.1 or v1.0, the tag will be missing:

When selecting a protocol and clicking on the “View” button, a pop-up summarizing the protocol content - thermocycling (if any) and reading programs - is displayed:

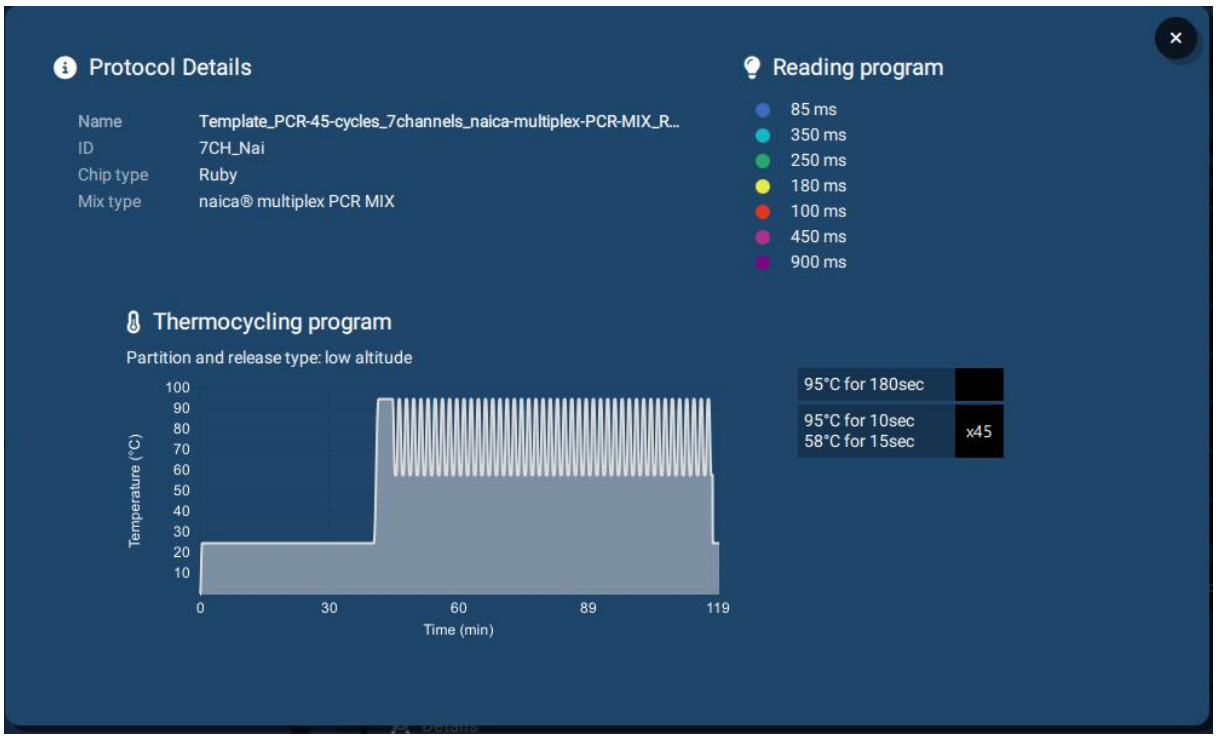


Figure 26: Details of the protocol selected as displayed when clicking on the "View" button

Note: Protocols can differ between the chips of a given chip plate. In this case, the experiment will be considered as a composite experiment and, at the end of the run, the results may be split into several experiment result files (.niodata). Refer to the composite experiments section for additional details.

To assign an assay to a given chamber or set of chambers, drag and drop the assay file from the assay library to the desired chamber or selection of chambers:

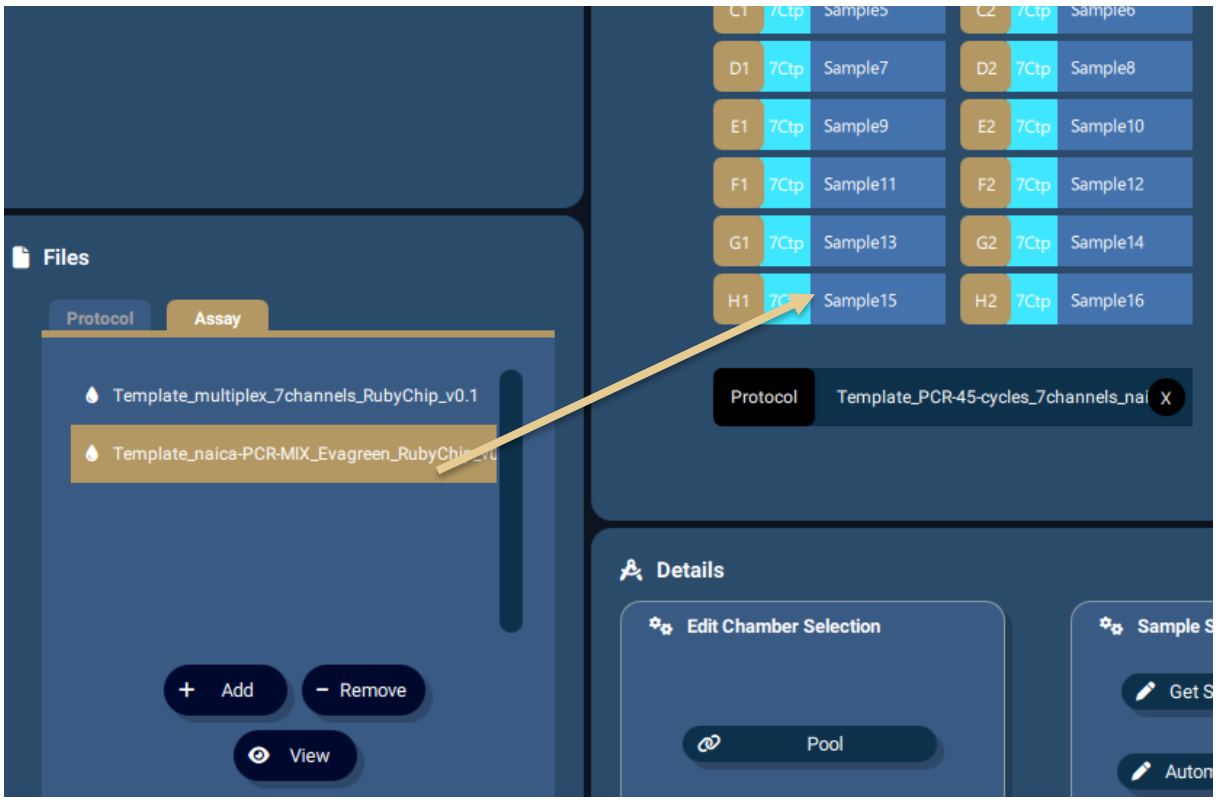


Figure 27: Applying an assay to a chip

Note: the drag and dropped assay will be set on the chamber where it is dropped. If this chamber is selected together with other chambers, all the chambers in the selection will have this assay assigned.

Tips: It is possible to select all the chambers of a chip at once by clicking on the Chip icon next to the Chip barcode field.

Whenever selecting an assay and clicking on the “View” button, a pop-up is displayed summarizing the Assay content:

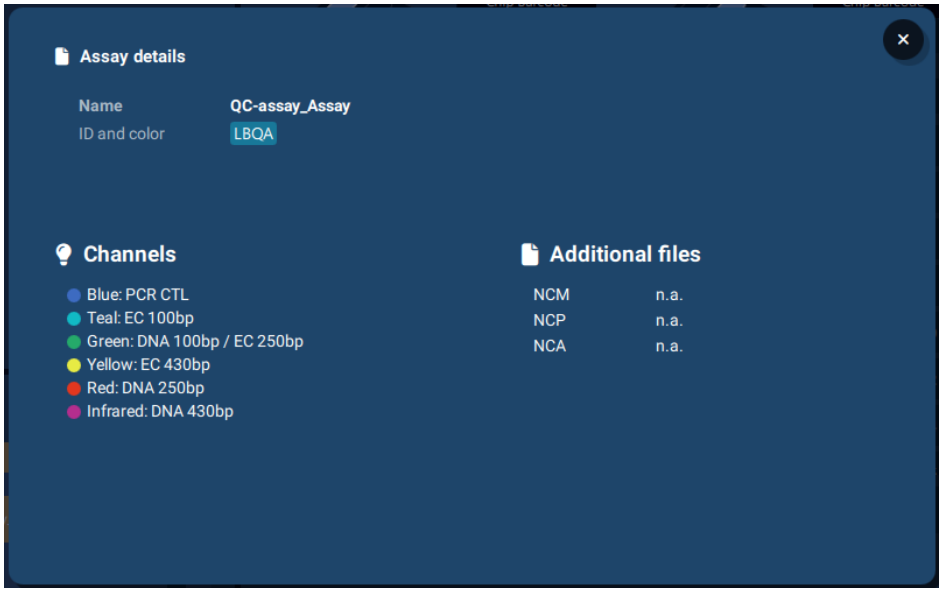


Figure 28: Details of the assay selected as displayed when clicking on the "View" button

Note: It is possible to assign different Assay files to different chambers of the chip plates. In this case, the experiment will be considered as a composite experiment and, at the end of the run, the results may be split into several experiment result files (.niodata). Refer to the composite experiments section for additional details.

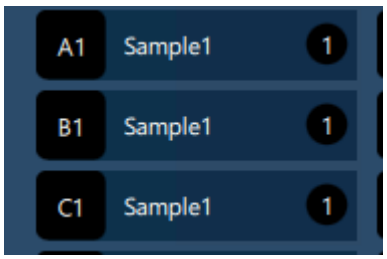
Note: Use the “Enable/disable elements” button to suppress the assignment of an assay to a given set of chambers.

The Details panel of the Digital Experiments page is used to enter further information with regards to the chambers.

In the “Edit Chamber Selection” menu, chambers containing the same reaction mix (i.e. the same sample) can be pooled together and be analyzed as a single chamber. To do this, select the chambers and then click on “Pool”.

Figure 29: Pooling chamber

Once pooled, all samples for those chambers will automatically be assigned the same name and an index.



To unpool those samples, select the pooled samples and click on “Unpool” in “Edit Chamber Selection”.

Note: pooled samples must share the same experiment context: same protocol and same assay.

The sample Settings menu can be used to load sample information from a CSV file by clicking on “get sample from CSV file”. Sample information corresponds to the position of the chamber, the sample name, the sample type – Positive Control, Negative Control, Known or Unknown –, the dilution factor and further contextual information.

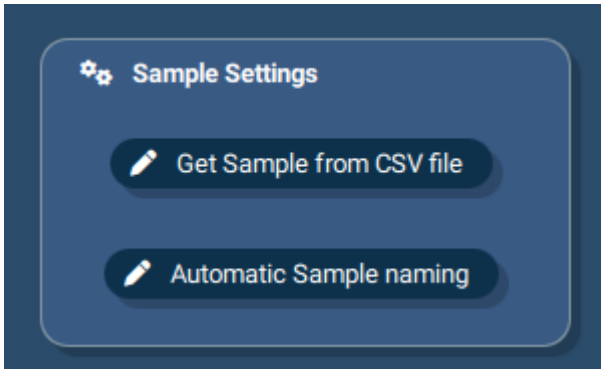


Figure 30: Get sample from CSV file

Template files for the .CSV input are available at C:\Users\Public\Stilla\NioReaderClient.

Two formats are available with different row/chamber order:

- Template_NCT_Input_sortByColumn.csv
- Template_NCT_Input_sortByRow.csv

Chip Position	Chamber	Chip ID	Sample Name	Sample Type	Context	Dilution
Left	A1	B-Chip1	Chip1-A1	Unknown	context1-chipA1	1
Left	A2	B-Chip1	Chip1-A2	PositiveControl	context1-chipA2	2.0
Left	B1	B-Chip1	Chip1-B1	NegativeControl		3.0
Left	B2	B-Chip1	Chip1-B2	Known		4.42
Left	C1	B-Chip1	Chip1-C1	Unknown		
Left	C2	B-Chip1	Chip1-C2	Unknown		
Left	D1	B-Chip1	Chip1-D1	Unknown		
Left	D2	B-Chip1	Chip1-D2	Unknown		
Left	E1	B-Chip1	Chip1-E1	Unknown		
Left	E2	B-Chip1	Chip1-E2	Unknown		
Left	F1	B-Chip1	Chip1-F1	Unknown		
Left	F2	B-Chip1	Chip1-F2	Unknown		
Left	G1	B-Chip1	Chip1-G1	Unknown		
Left	G2	B-Chip1	Chip1-G2	Unknown		
Left	H1	B-Chip1	Chip1-H1	Unknown		
Left	H2	B-Chip1	Chip1-H2	Unknown		
Middle	A1	B-Chip2	Chip2-A1	Unknown		1
Middle	A2	B-Chip2	Chip2-A2	PositiveControl		2.0
Middle	B1	B-Chip2	Chip2-B1	NegativeControl	context2-chipB1	3.0
Middle	B2	B-Chip2	Chip2-B2	Known	context2-chipB2	4.42
Middle	C1	B-Chip2	Chip2-C1	Unknown		
Middle	C2	B-Chip2	Chip2-C2	Unknown		
Middle	D1	B-Chip2	Chip2-D1	Unknown		
Middle	D2	B-Chip2	Chip2-D2	Unknown		
Middle	E1	B-Chip2	Chip2-E1	Unknown		
Middle	E2	B-Chip2	Chip2-E2	Unknown		
Middle	F1	B-Chip2	Chip2-F1	Unknown		
Middle	F2	B-Chip2	Chip2-F2	Unknown		

Figure 31: Example of a filled .csv input file from the Template_NCT_Input_sortByRow.csv template

Alternatively, sample information can be manually edited in the chamber editor. The latter opens when double-clicking on the chamber ID label close to the sample name field of each chamber in the Chip plate Layout.

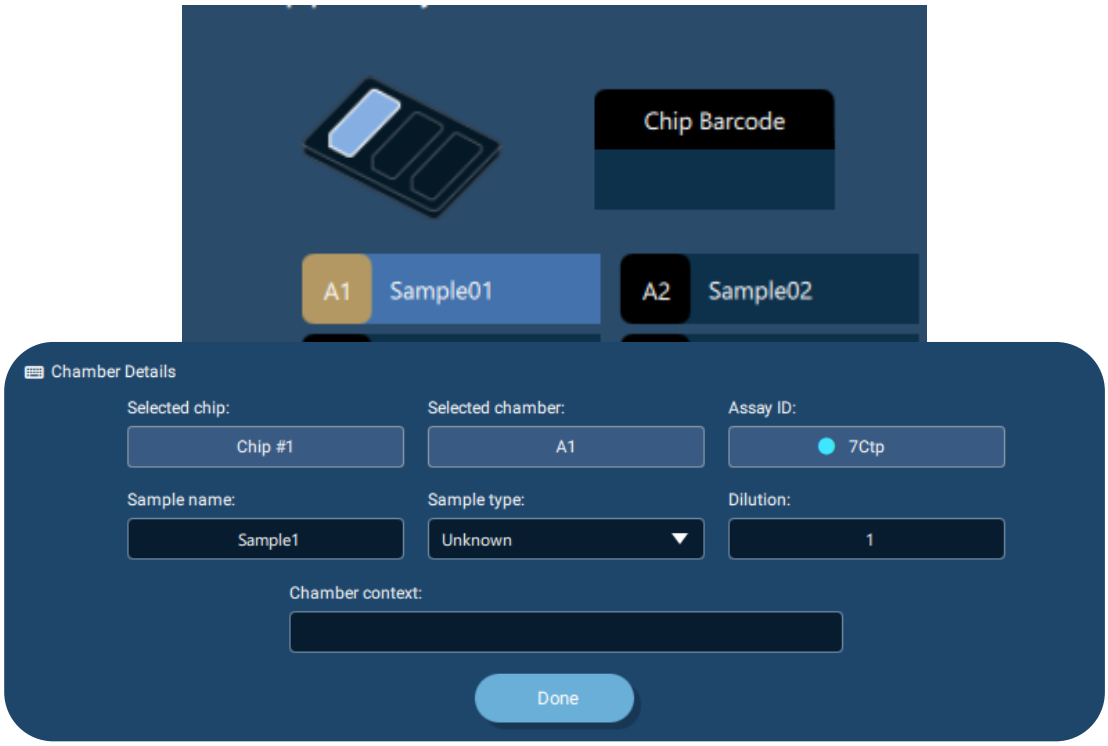
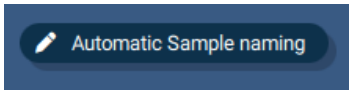


Figure 32: Chamber editor

The sample name can also be manually edited by double-clicking on the sample field of the chamber in the Chip plate Layout.

For repetitive sample naming structures, Nio® Reader software offers the possibility to automatically name all the chambers of the experiment following a customizable nomenclature. In the ‘Sample Settings’ menu, the ‘Automatic Sample Naming’ button opens the editor to define the naming nomenclature to follow.



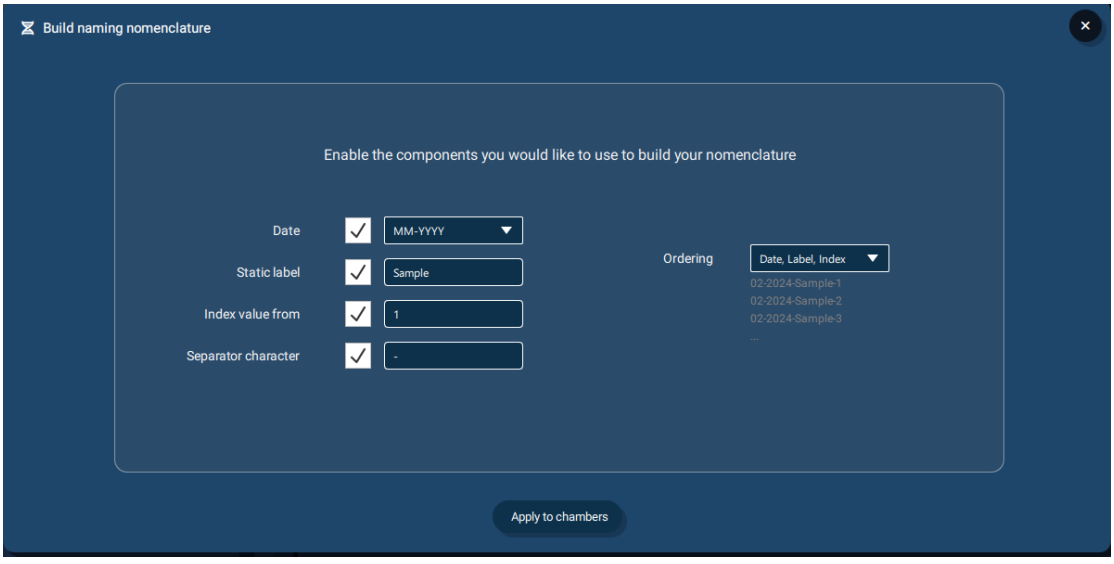


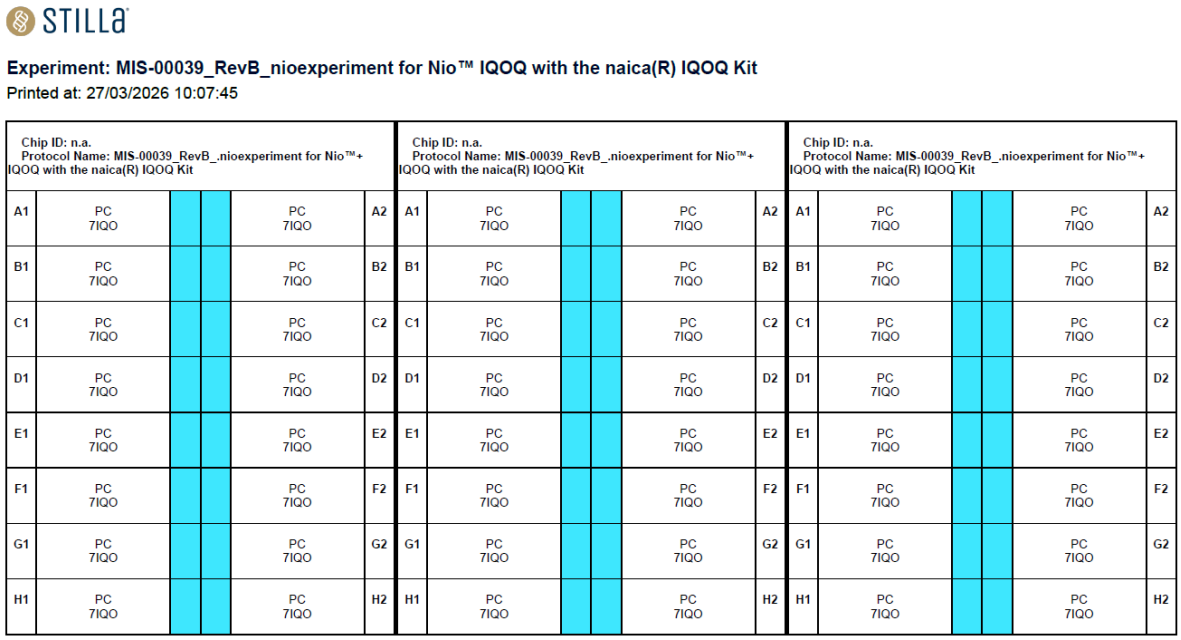
Figure 33: the automatic sample naming editor

The editor allows to define multiple rules to personalize the final naming such as:

- Add a date: multiple formats are available
- Add a static sample name
- Add an index value: possibility to choose the start value
- Add the separator character to use
- Choose the ordering of those different rules
- Preview of the future generated name available

It is the responsibility of the user to check the validity of the generated sample names applied to the chambers.

Once the definition of the experiment is finished, it is possible to print the defined layout of by clicking on the button “Print” on the top-right corner of the Digital Experiments page. This will generate a printable Pdf file.



STILLA

Experiment: MIS-00039_RevB_nioexperiment for Nio™ IQOQ with the naica(R) IQOQ Kit

Printed at: 27/03/2026 10:07:45

Chip ID: n.a. Protocol Name: MIS-00039_RevB_.nioexperiment for Nio™+ IQOQ with the naica(R) IQOQ Kit						Chip ID: n.a. Protocol Name: MIS-00039_RevB_.nioexperiment for Nio™+ IQOQ with the naica(R) IQOQ Kit						Chip ID: n.a. Protocol Name: MIS-00039_RevB_.nioexperiment for Nio™+ IQOQ with the naica(R) IQOQ Kit					
A1	PC 7IQO			PC 7IQO	A2	A1	PC 7IQO			PC 7IQO	A2	A1	PC 7IQO			PC 7IQO	A2
B1	PC 7IQO			PC 7IQO	B2	B1	PC 7IQO			PC 7IQO	B2	B1	PC 7IQO			PC 7IQO	B2
C1	PC 7IQO			PC 7IQO	C2	C1	PC 7IQO			PC 7IQO	C2	C1	PC 7IQO			PC 7IQO	C2
D1	PC 7IQO			PC 7IQO	D2	D1	PC 7IQO			PC 7IQO	D2	D1	PC 7IQO			PC 7IQO	D2
E1	PC 7IQO			PC 7IQO	E2	E1	PC 7IQO			PC 7IQO	E2	E1	PC 7IQO			PC 7IQO	E2
F1	PC 7IQO			PC 7IQO	F2	F1	PC 7IQO			PC 7IQO	F2	F1	PC 7IQO			PC 7IQO	F2
G1	PC 7IQO			PC 7IQO	G2	G1	PC 7IQO			PC 7IQO	G2	G1	PC 7IQO			PC 7IQO	G2
H1	PC 7IQO			PC 7IQO	H2	H1	PC 7IQO			PC 7IQO	H2	H1	PC 7IQO			PC 7IQO	H2

Figure 34: Example of a printed experiment

Warning:

- .nioexperiment, .niorotocol and .nioassay files shall only be edited with the Nio® Reader software.
- Users without Admin rights shall not access the Program data directory to avoid modifying the temporary run data.

9.3.7 Runs

The Runs page allows to run experiments on the Nio® Digital instrument and control their status. This is the only step where the Nio® Digital PCR instrument is mandatory, all the other previous steps can be done on a personal computer.

Note that some features and buttons are disabled when Nio® Reader software is not running on the Nio® Digital PCR instrument (remote connection). The actions related to instrument handling (shutdown/unloading/acknowledging feature failures) are disabled as well as run data interaction (loading, unloading, etc.). These actions need to be performed when Nio® Reader software runs on the instrument.

By clicking on Plan & Start, the Experiment selection window opens:

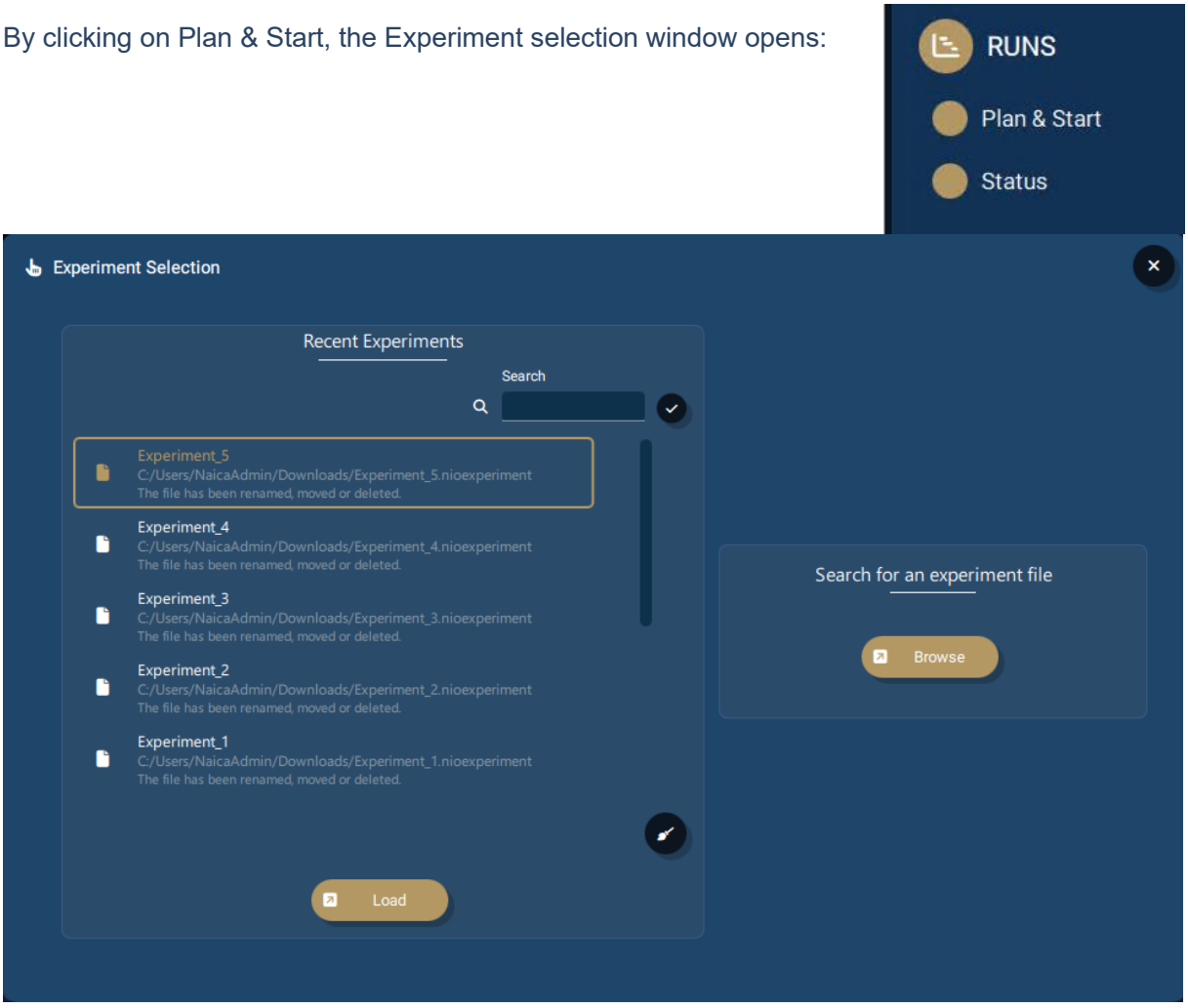
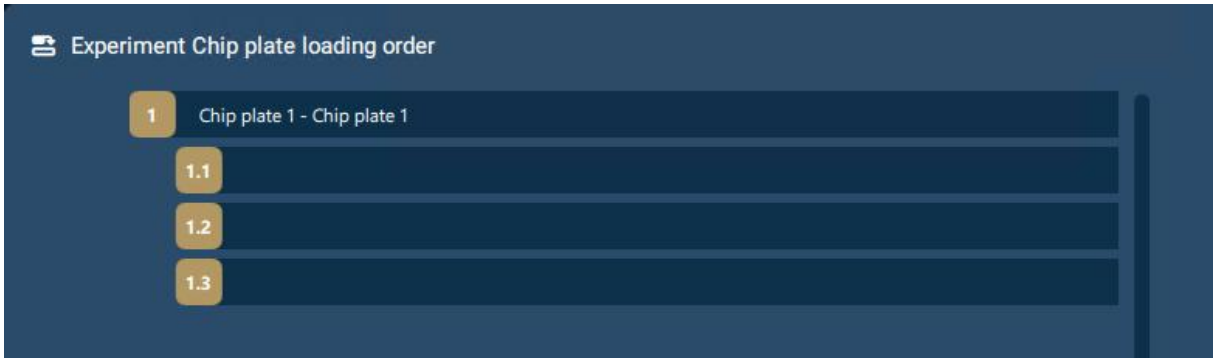


Figure 37: Digital Experiment Selection

It allows selecting the experiment to launch either from the recent experiments or by browsing to find a specific experiment.

Once the experiment is selected, follow the instruction provided on the right panel “Instructions”, to continue the workflow.

Before loading the chip plate, a visualization of what is expected in the chip plate under the “Experiment Chip plate loading order” is displayed.



In this example, the chip plate 1 is composed of 3 chips without a specified barcode, it will be read by the barcode reader during the loading of the chips through the front panel.

The chip plate loading and unloading menu is also available to retrieve some chips from previous experiments. The instrument gives its current occupation status.

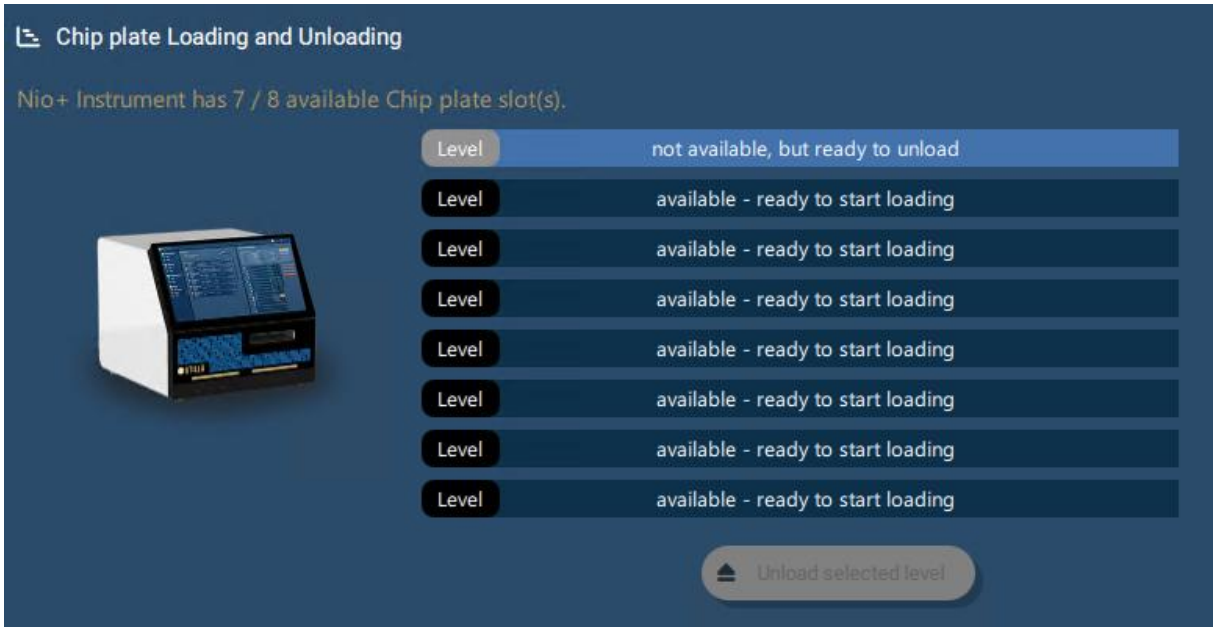


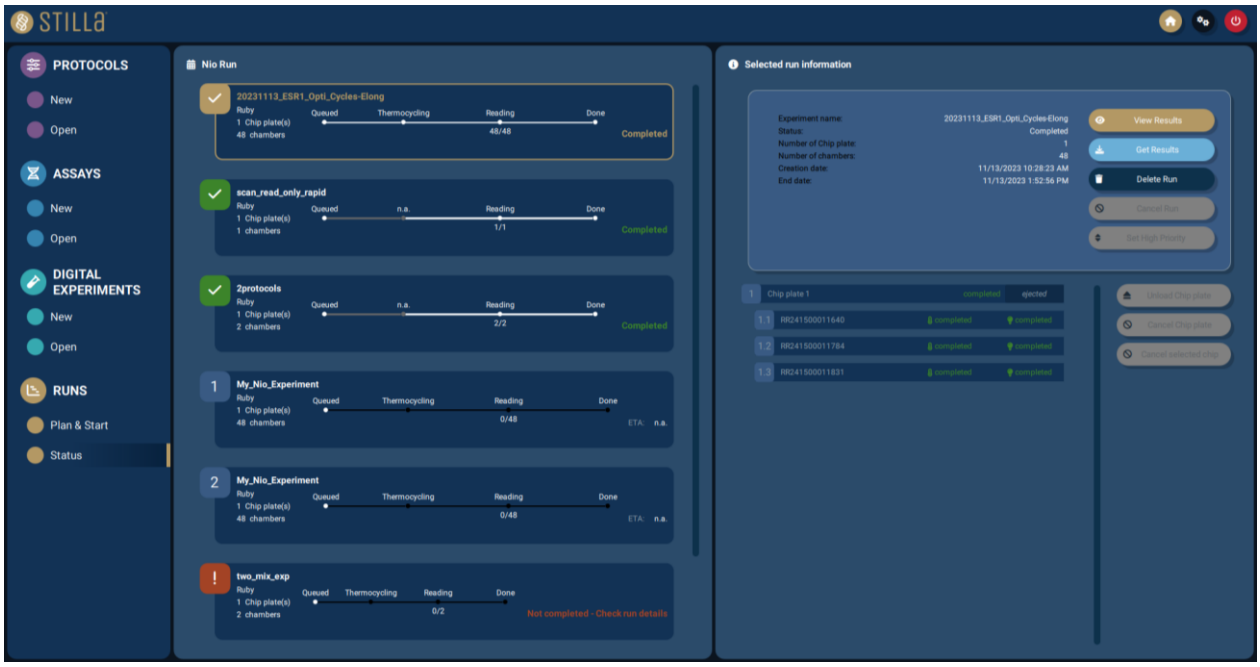
Figure 35: Chip Plate loading and unloading menu. Example for the Nio®+ configuration

Once the chips are loaded, the chip plate display shows the recognized barcodes for each chip and their respective positions.

Click on start run and go the status page.

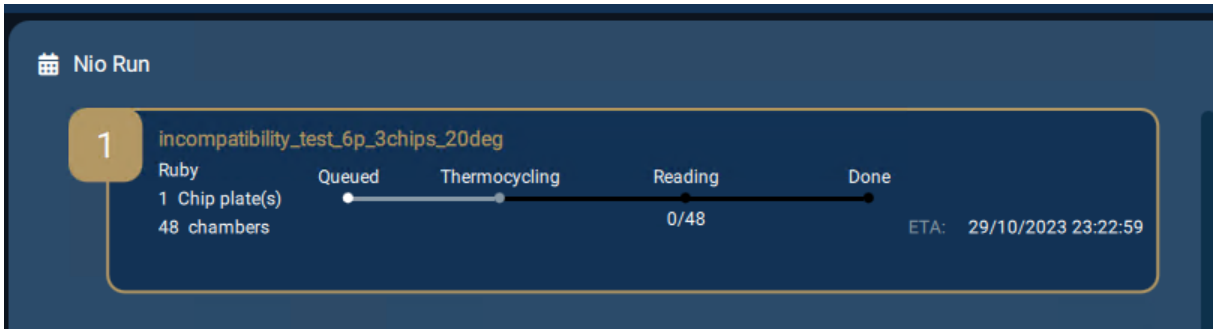
Note that it is also possible to directly enter chips in the instrument. As no experiment is associated, the Nio® Reader software will request to associate an existing experiment to those chips, and it will start automatically once the chips are in the instrument.

The status page allows to know the progress and status of each chip for up to 8 chip plates in the Nio®+ configuration, 4 chip plates in the Nio® configuration and one plate in the Nio® E configuration.

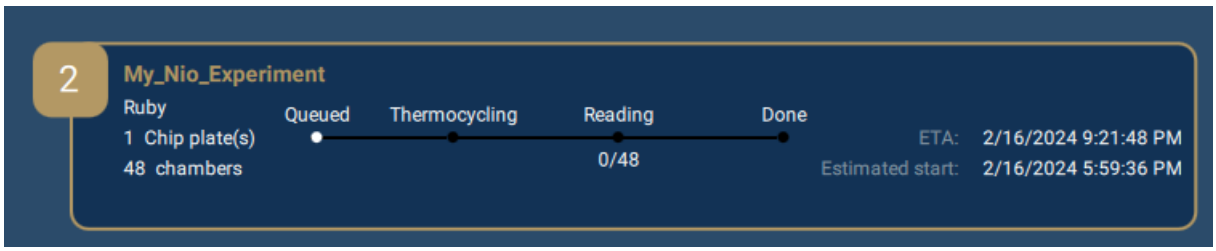


Under “Nio® Run” a progress bar for each experiment will display different status:

- Queued: the chips are inside the instrument and will be processed as soon as possible by the instrument without need of external intervention.
- Thermocycling: the partition, thermocycling and release step are in progress.
- Reading: scan in progress, the counter will increase each time a chamber is dully read and ready to be visually checked.
- Done the experiment is completed.
- ETA: Estimated time of arrival is the moment where data are available for download.



When the run is in “Queued” state, an estimated starting time is provided:



By selecting a specific experiment, more information is displayed on the "Selected run information” panel:

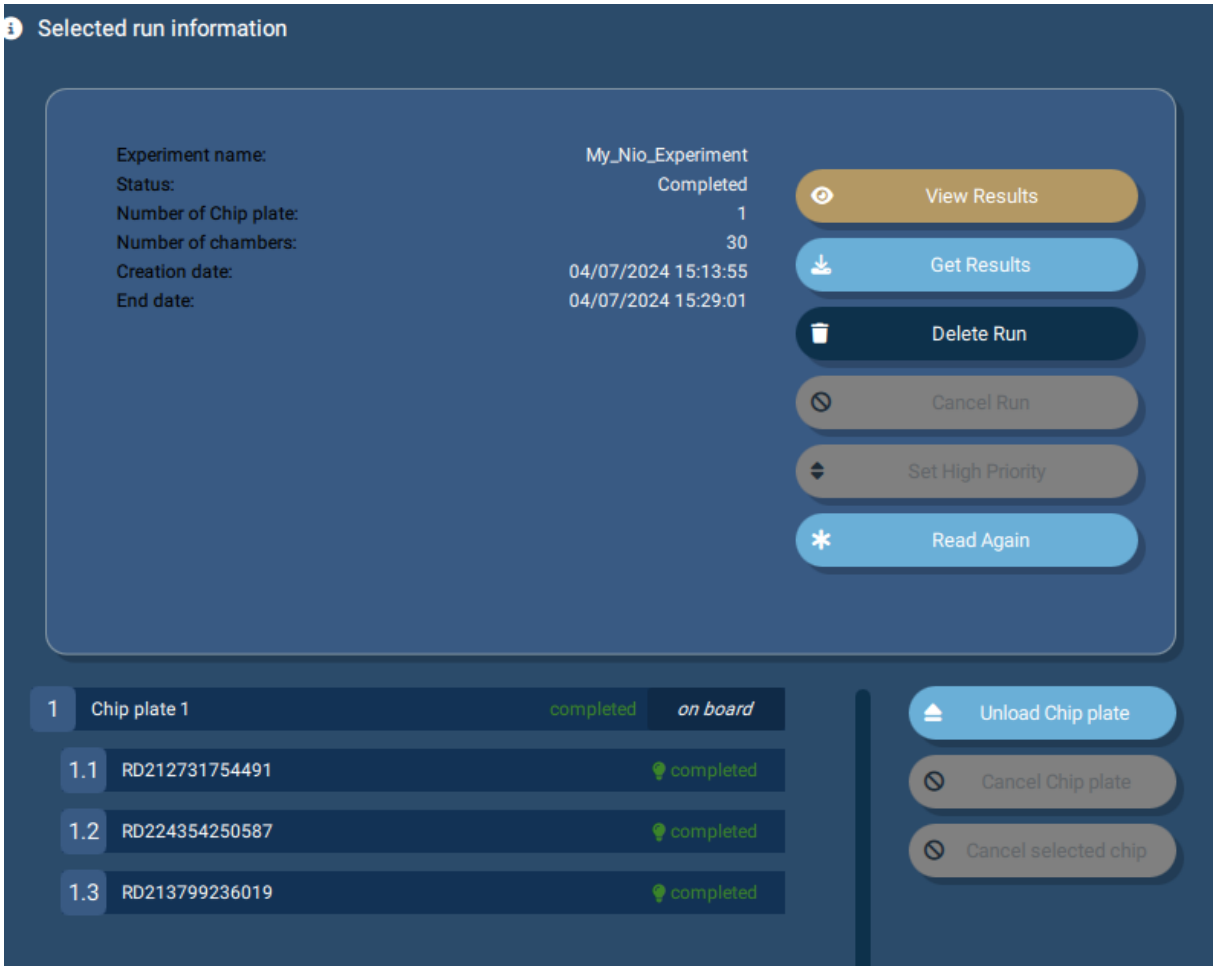
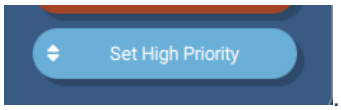


Figure 36: Run information

There are two levels of priority for chip plates in the Nio®+ and Nio® instruments: Normal or High.

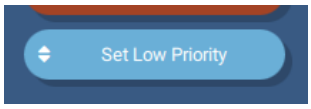
If multiple experiments are queued, it is possible to modify the order in which they will be processed and force a given experiment to start next with the button “Set High Priority” 

. This feature is available only while the experiments are in the queue. When the processing has started, order becomes the processing starting order, high priority experiment started after a normal priority one does not overtake it.

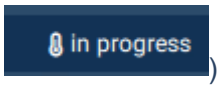
Setting high priority means that the order of the queued experiment is modified, and that the chip plate with high priority will be processed before the rest of the queued experiment.

It is possible to set multiple chip plates as high priority, in this case those chip plates will be processed in the order they entered in the instrument but before any other normal status chip plate.

Note: that modifying the priority will impact the ETA of the chip plate, the one with high priority as well as the one from any other queued experiment.

A high priority chip plate can be switched back to normal priority status by clicking on the “Set Low Priority” button. When doing so, these chip plates will be processed according to their entering order in the instrument. 

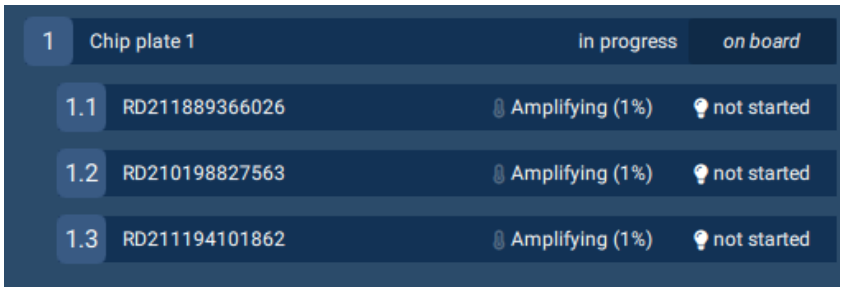
The chip plate is indicated as in progress and on board of the instrument.

Each chip is in progress for either thermocycling (blinking thermometer followed by in progress 

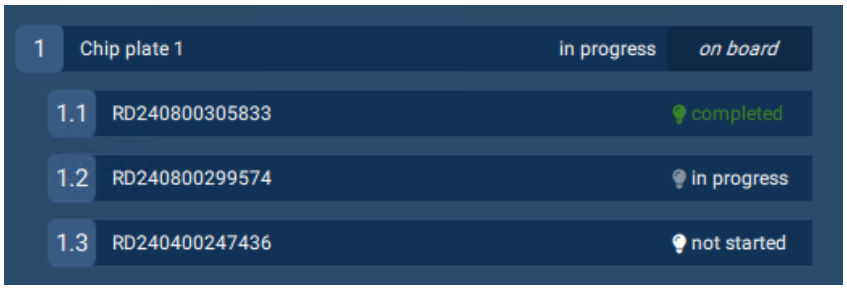
) or scan (blinking light bulb followed by in progress 

).

Some details about the thermocycling part are also provided:

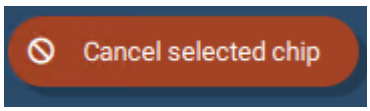


The status will evolve according to the advancement of the run for example.



In this run, the 1st chip is processed, the 2nd one is in progress for scanning and the last one is waiting to be scanned.

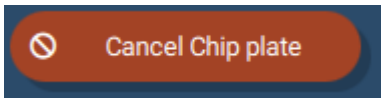
It is possible to cancel a run at any time, either for one chip or for the whole chip plate.

To cancel a single chip, select the specific chip and click on 

.

Warning: Canceling a chip during thermocycling will stop the thermal plate from heating/cooling, thus it will end the PCR and the chambers of the canceled chips will not be scanned.

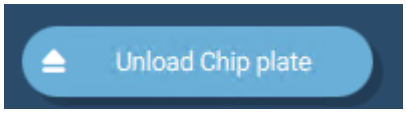
When canceling just at the reading step, it is always possible to rescan later on, as the PCR step proceeded already.

To cancel the run for the whole chip plate, select the chip plate and click on: 

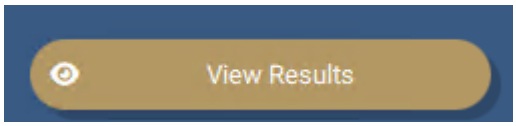
Warning: The consequences of cancelling a complete chip plate is the same as a single chip, except that when canceling a complete chip plate, the instrument will automatically come back to atmospheric pressure, remove the chips from the thermocycling and allow to retrieve the chips. When canceling only one chip, the instrument will wait until all the remaining chips of the chip plate are done to allow to retrieve the chips.

The cancellation is not immediate. When canceling a run, wait until the instrument allows to unload chips.

Once a run or a cancellation is completed, it is possible to retrieve the chips by clicking on:



for the selected run.

As soon as the first chamber of the run is scanned, the "View results" button activates. 

Clicking on it will open the “Overview” panel and the outcome of all already processed chambers of a chip plate can be visualized.

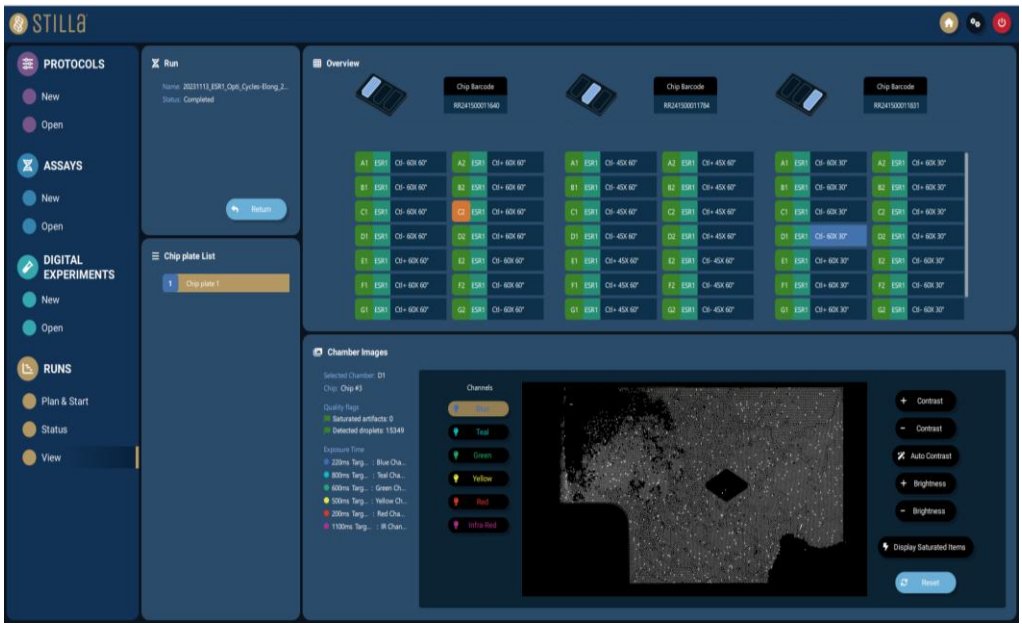


Figure 37: Nio® Reader software View Page

For each chamber scanned, two quality indicators are displayed in the “Overview” panel, based on the number of saturated artefacts and the number of droplets detected in the chamber. Each indicator can be either green, yellow or green but in the shape of an eye.

Figure 38: Example of the quality indicators status for a given chamber

If both indicators are green, the quality flag of the chamber is green. If one indicator is yellow, the quality flag of the chamber is yellow. If one of the indicators is a green eye, an eye icon is displayed on the green flag.

A green flag indicates that all quality indicators are within expected specifications.

A green flag with an eye icon on it means that Stilla Technologies recommends inspecting visually the droplet crystals to decide whether the result should be taken into account. Refer to the Ruby Chip troubleshooting section for more details.

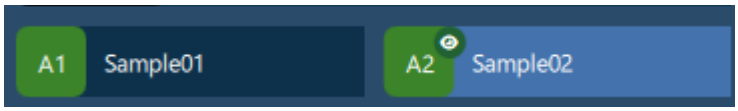
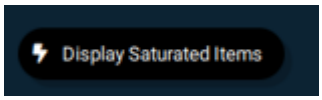


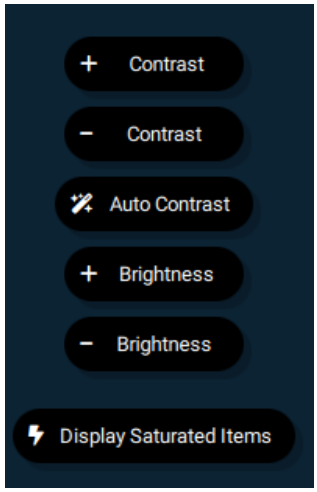
Figure 39: Example of a chamber with a green flag and another one with a green flag marked with an eye icon.

A yellow flag indicates that there is either too many saturated artefacts on the picture or there are less than 1000 detected droplets in the chamber. When dust particles cause saturated artefacts, Stilla Technologies recommends cleaning the bottom of the Ruby Chip with a dust free tissue and read the chambers again (see below for instructions on the re-readd function).

If the flag is orange because of saturated artifacts, it means that there are too many saturated pixels in the image. It can be linked to a too long exposure time. It is possible to visualize which pixel is saturated by clicking on 

Any saturated pixel will highlight on orange.

When inspecting visually the chambers, it is possible to zoom in or out in the droplet image and change the contrast or brightness of the image by clicking on the corresponding buttons. Use the "Auto Contrast" button to adjust the contrast of the image automatically.

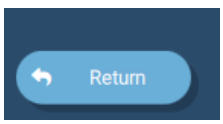


By clicking on the "Get Result" button, when running Nio® Reader software on the instrument, the results will be assembled into a .niodata file, automatically copied to the output folder. By default, the output folder is C:\Users\Public\Stilla\NiReaderResults. One user can specify a custom path in the Settings menu. The customized path is set at the system level, meaning that it will be set for all the users. In a case where several users can use different path for data saving, verify the output path in the Settings page prior to data extraction after clicking on "Get Result' button. The user is free to copy/move the .niodata file from the default or specified folder to another location for post processing. When connected remotely to the instrument, the .niodata files are downloaded to the chosen path in the Settings menu.

Note: the results can be split in several .niodata files depending on the way protocols and assays are assigned to the chips or chambers in the experiments. Refer to section 9.4 for more details.

After downloading the result of a completed run, as well as for cancelled runs, it is recommended to click on the "Delete Run" button to clean the Nio® Reader Server data for the selected run. This action will not impact the data saved either in the default folder (C:\Users\Public\Stilla\NioReaderResults) or in the specified output folder, nor any other copies.

Keeping too many run data in the instrument's memory will impact its performance. The Nio® Digital PCR instrument stores .nioresults files for 8 or more runs. To ensure optimal operation of the system, please consider deleting the run results after retrieving and storing them. The system's drive available space is under 40Go. Please consider clearing space to ensure an optimal processing. A warning message will be displayed to remind the user to clean the memory.

The "Return" button allows to return to the precedent menu 

Warning: .niodata file is generated by Nio® Reader software and needs to be opened in Nio® Analyzer software for further analysis. The user shall not try by any mean to open/modify the file with another application.

Whenever a run has been performed entirely, it is possible to read the chambers again thanks to the “Read Again” button.

This feature can be used to replace the scanned images of a run by reading again the chambers of a subset of chambers in the run (using the same exposures times set in the experiment’s protocol(s).

When clicking on the “Read again” button, a popup is displayed asking to select the chambers to be rescanned. Pending the chambers are selected and the corresponding chips are detected in the Nio® instrument, it is possible to click on the ‘Perform Reading’ button to launch or queue the rescan experiment (depending on the next slot available in the instrument).

Note: Ruby Chip chambers can be scanned up to three times within 48 hours from the initial scanning.

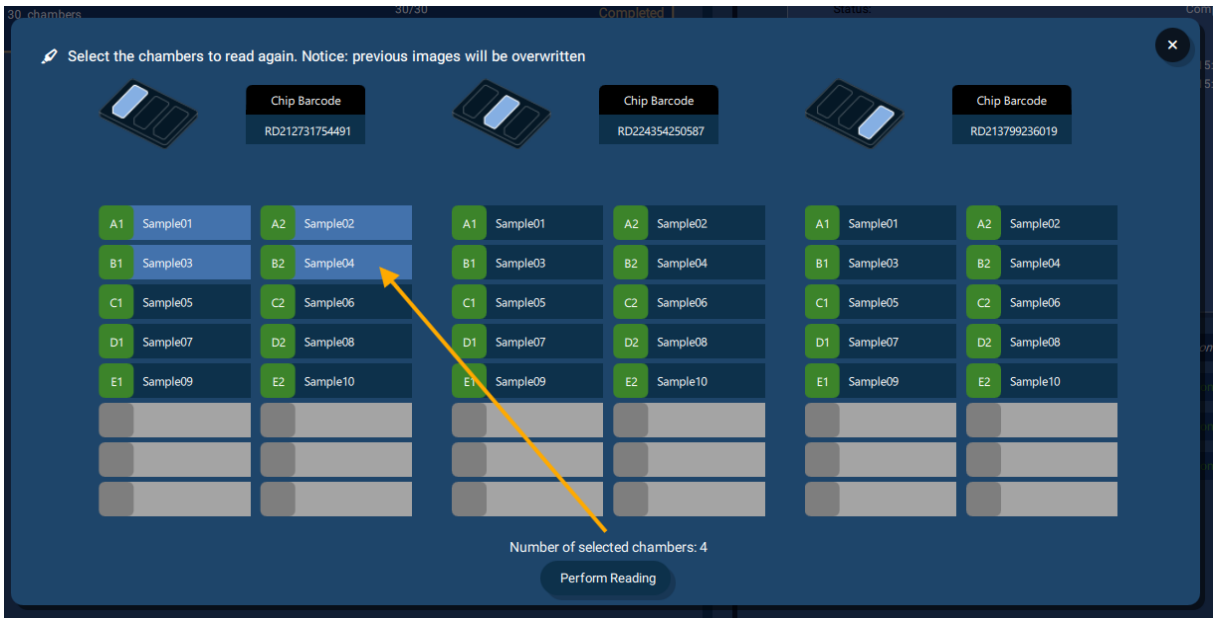


Figure 40: Display of the re-read menu

A 'Completed' (or 'Failed') run thus becomes either 'Queued' or 'Running'.

As soon as a chamber is re-read, the reading result becomes visible in the "View Result" page. Whenever this run is completed, the "Get Result" button enables and the .niodata file contains the re-read data.

9.4 Composite experiments

To fully leverage the high throughput capacity and the high flexibility of the Nio® Digital PCR instrument, it is possible to mix different protocols and/or assays within the chips from the same experiment:

- It is possible to assign different protocols with different scanning conditions to chips from the same chip plate.
- It is possible to assign different assays to different chambers from the same chip

Note: assays and protocols are linked together via the list of activated channels. Thus, all the assays set to chambers of the same chip should match the channels configuration of the protocol's reading program assigned to that chip.

An experiment containing multiple protocols and/or assays is referred to as a "Composite Experiment" and it can gather chambers with different experimental parameters such as mix type, target names, fluorophores used etc. However, to be analyzed together by the Nio® Analyzer software, the chambers from a given experiment must share the following common experimental parameters: mix type, activated channels, exposure times of the activated channels, target names, fluorophores, thresholding mode, defined populations and compensation matrix. This is why, the results from a composite experiment can be split into several .niodata files, based on the similarities between its protocols and/or assays characteristics. In other words, for composite experiments, clicking on the "Get results" button can lead to the generation of several .niodata files depending on the Protocols and Assays layout. Each .niodata will contain chambers sharing the same protocol with the same assay.

The split is done first at the protocol level and then at the assay level.

Note: if only the PCR program varies between two protocols; the output .niodata file will not be split into two files. This allows for example the post-processing of an assay-optimization experiment where different annealing temperature are assessed within the same chip plate. If any other characteristics of the protocol differs (mix type, scanning parameters), then data will be split.

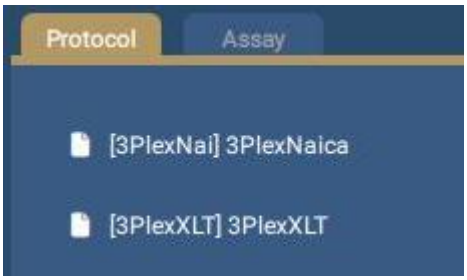
When Nio® Reader software splits the data into several .niodata files, a specific nomenclature is applied to name them according to the layout of the chip plate defined at the experiment level.

As an example, let's consider a composite experiment designed as follows:

	Chip1		Chip2		Chip3	
	Column1	Column2	Column1	Column2	Column1	Column2
Protocol	1	1	1	1	2	2
Assay	1	2	1	1	1	1

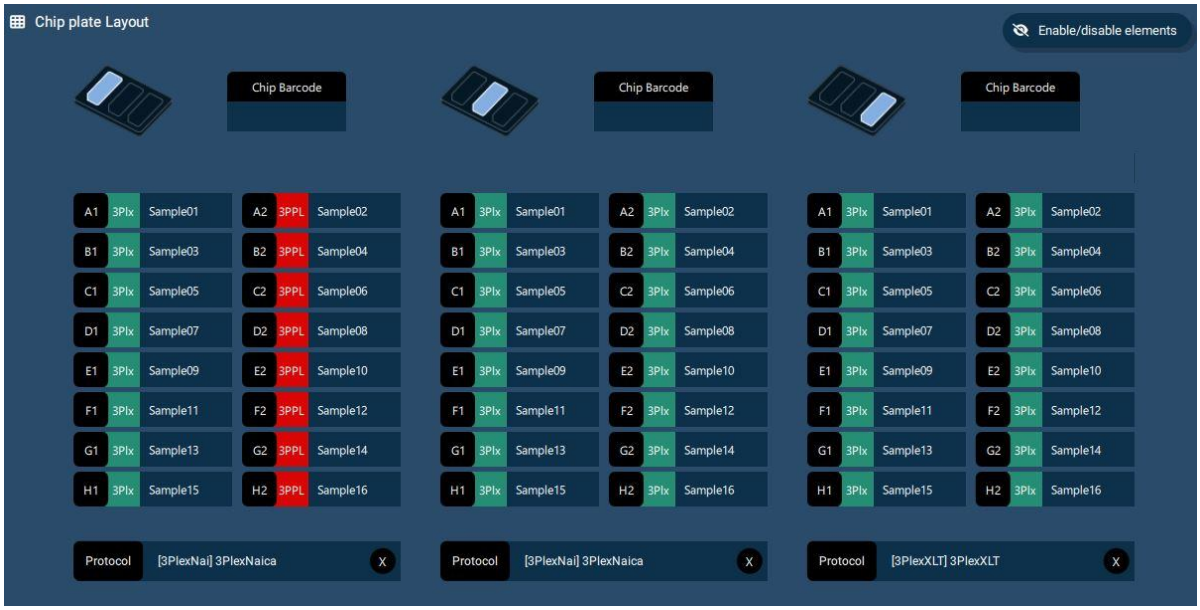
Where:

- Protocol 1 is called "3Plex naica" and used the tag "3PlexNai".
- Protocol 2 is called "3PlexXLT" and used the tag "3PlexXLT".
- The difference between protocol 1 and 2 is the mix type which qualifies it for a split (as it will impact the analysis by Nio® Analyzer). If the difference would have been only number of PCR cycles for example, data would not have been split.
- When loading these protocols in the "Digital experiments" menu, the protocol's tag is displayed before their file names.



- Assay 1 is called "3Plx", its tag is "3Plx" and is assigned the color green.
- Assay 2 is called 3PPL", its tag is "3PPL" and is assigned the color red .
- When loading these assays in the "Digital experiments" menu, the assay's tag is displayed:

When assigning the protocols and assays to the chips, the assay tag and its assigned color are displayed in each chamber assigned. The protocols' tags are displayed bellow the chips.

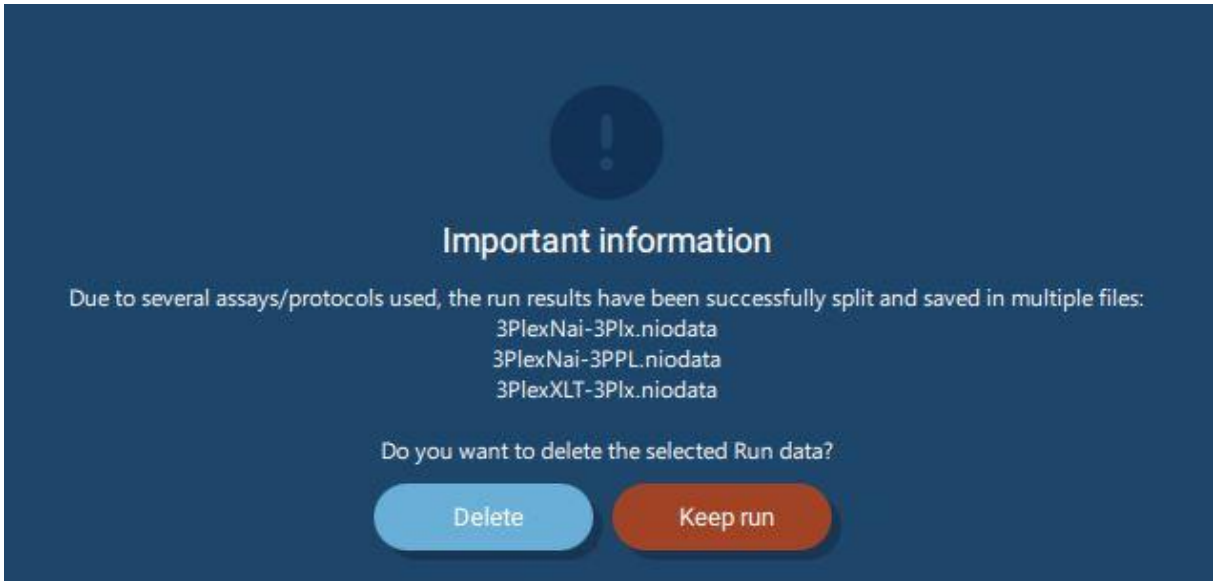


Upon “Get Results” click: the run data are browsed and chambers using the same pairs of protocol and assay will be gathered and embedded in the same .niodata output file. With such a configuration each pair will produce one .niodata file and the user will retrieve as many files as necessary to get all the results.

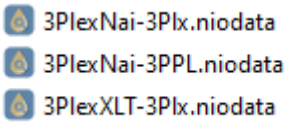
In the example here, Nio® Reader software will split the data into three .niodata files as illustrated by the gold, blue and red colors below:

	Chip1		Chip2		Chip3	
	Column1	Column2	Column1	Column2	Column1	Column2
Protocol	1	1	1	1	2	2
Assay	1	2	1	1	1	1

The pop-up window below displays to inform the user of the split.



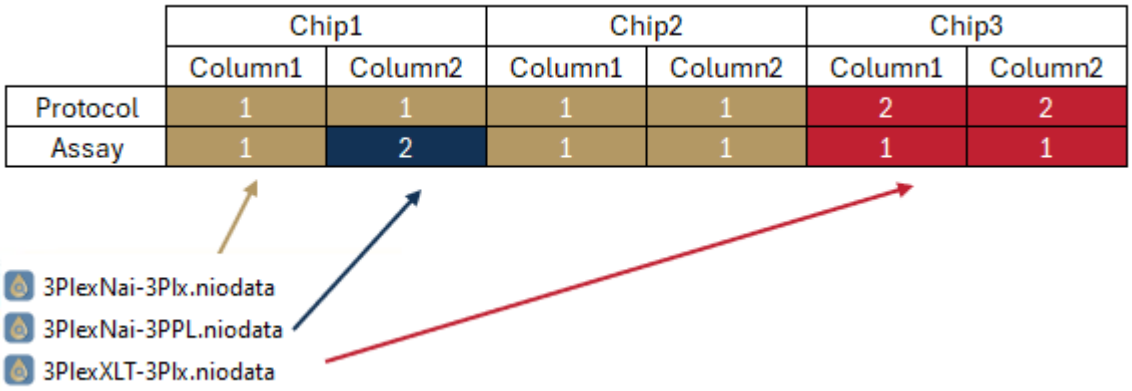
In the .nioresults folder, the 3 .niodata items are generated with the following names:



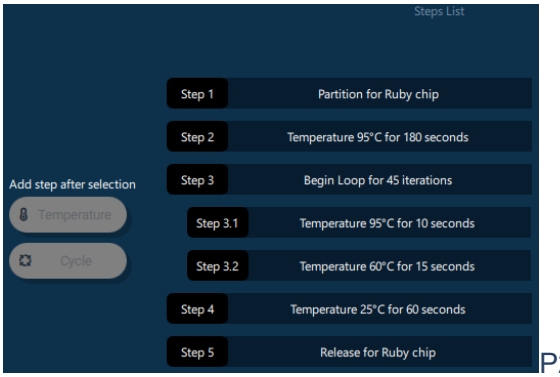
The assay name is directly visible in the split .niodata name whereas, as mentioned above, the protocol name is replaced by its tag.

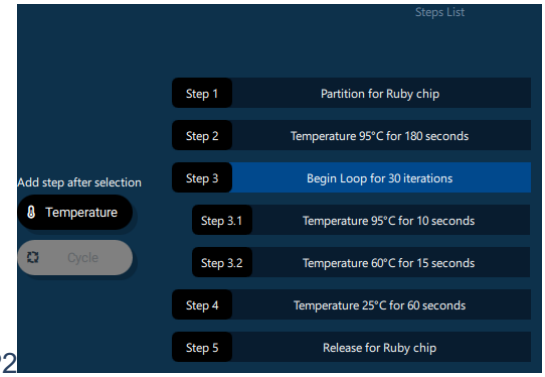
In our example, “3PlexNai” corresponds to Protocol1 (“3plex naica”) and “3PlexXLT” corresponds to Protocol 2 (“3PlexXLT”).

Then, Protocol 1 is paired with two different assays within the left chip (Chip 1), an extra split is done, resulting in the third .niodata file : “3PlexXLT-3Plx”.

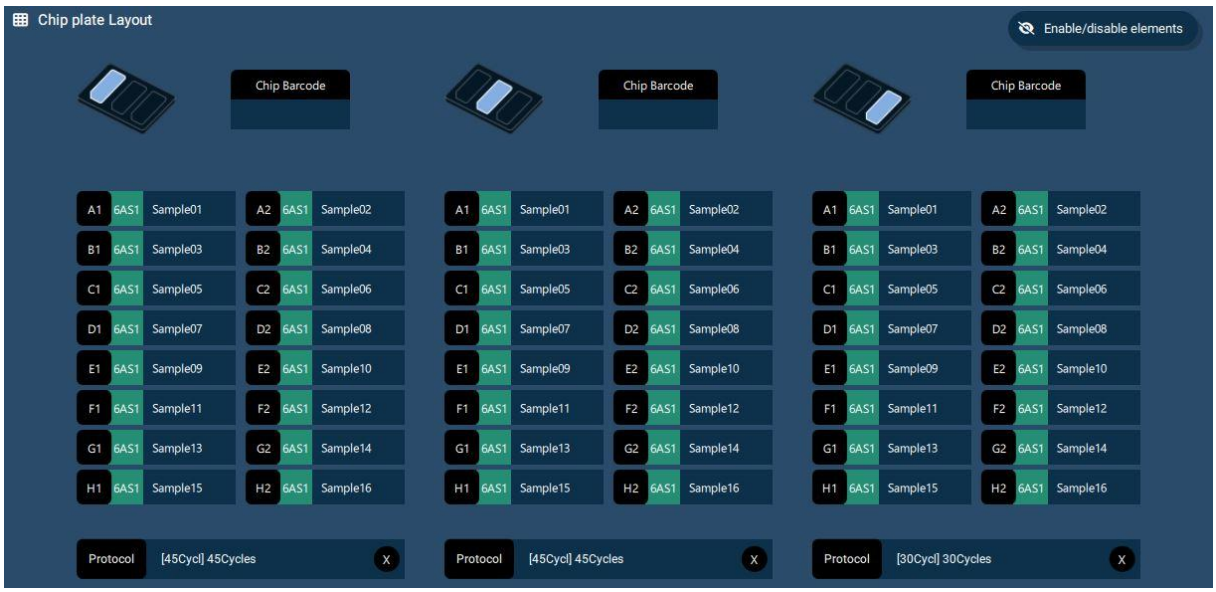


In the next example below, Protocols 1 and 2 differ by the number of PCR cycles in the PCR program and the same assay “6AS1” is assigned to every chamber of the experiment:

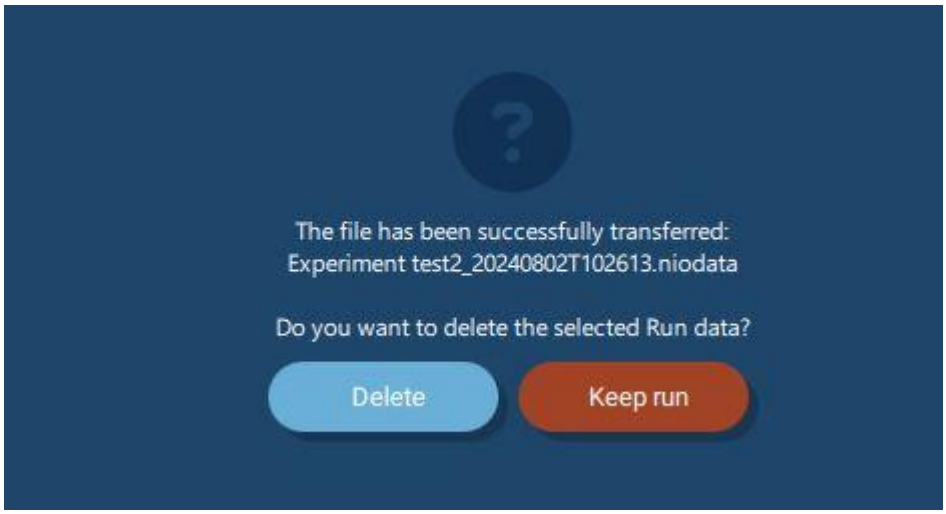
P1 

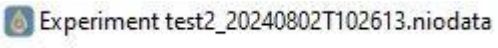
P2 

P1 is saved as “45Cycles.nioprotocols” with the tag “45Cycles” and P2 is saved as “30Ccycles.nioprotocols” with the tag “30Cycles”.



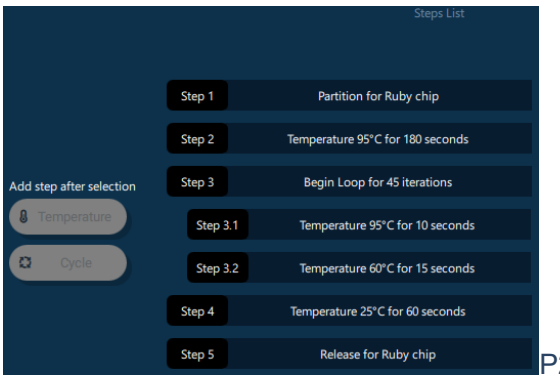
In this case, Nio® Reader software will not split the data. The following pop-up displays after clicking on the “Get results” button:



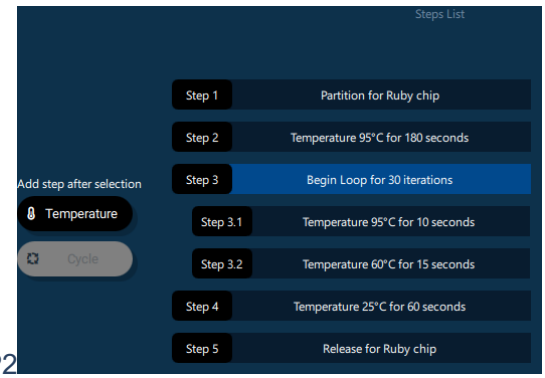
The generated file will be displayed as follow:  As there is no data split, the protocols’ tags nor the assay tag is displayed .niodata files names are built out of the name of the experiment file and a timestamp.

In the last example, Protocols 1 and 2 still differ by the number PCR cycles in the PCR program but two assays, “6AS1” and “6AS2”, are assigned to a given subset of chambers within the chip plate.

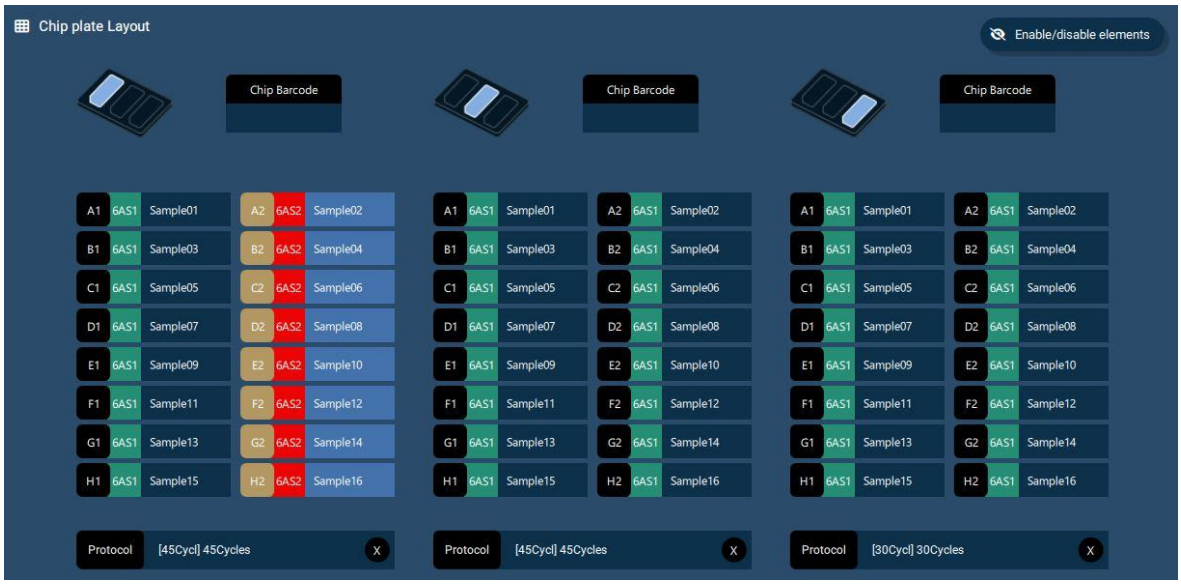
P1



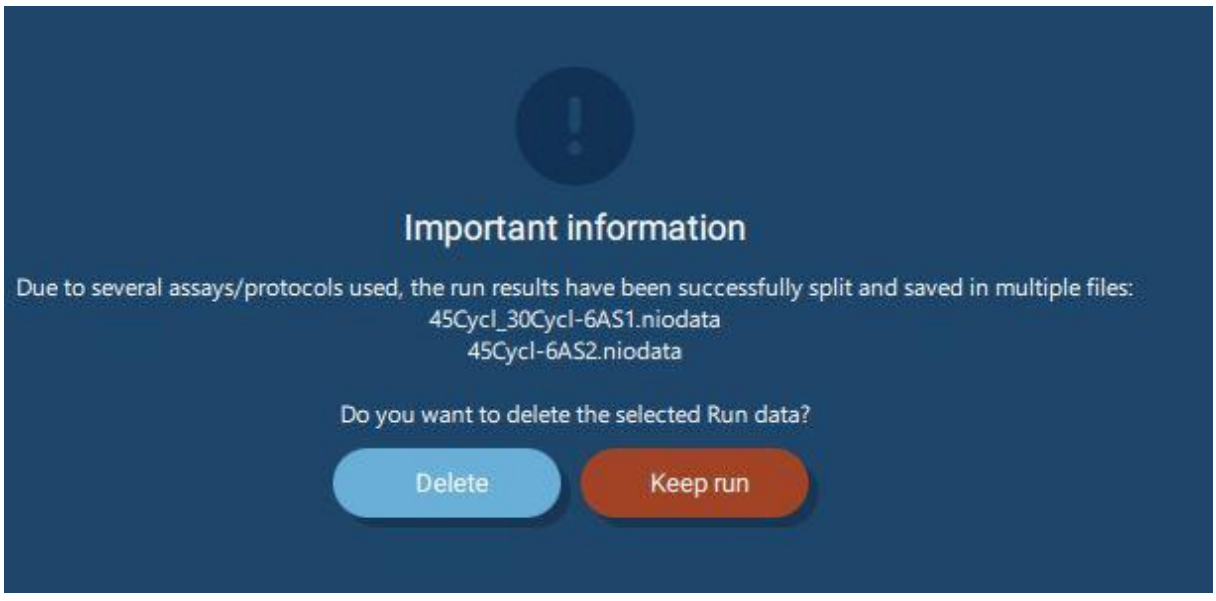
P2



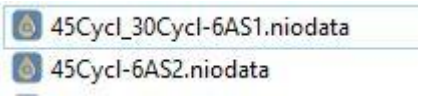
P1 is saved as “45Cycles.nioprotocols” with the tag “45Cycles” and P2 is saved as “30Ccycles.nioprotocols” with the tag “30Cycles”.



After clicking on the “Get results” button, Nio® Reader software will split the data as shown in the pop-up window below:



The generated files will display as follow:



9.5 Chip plate Loading

Only chip plates must be loaded into the Nio® Digital PCR instrument. Never attempt to directly load a chip consumable without a chip plate.

Each chip plate can be loaded with up to 3 individual chip consumables. Each chip must be placed into an individual chip plate chip area. Do not stack multiple chips on top of each other for chip plate loading. To load a chip plate in the Nio® Digital PCR, start by entirely defining its corresponding experiment and launch the run. The Plan and start page will display the instructions about chip plate loading as described previously.

The chips are delivered in boxes of 12, arranged in a stack of 4 chip plates containing 3 chips each. Chip plates serve both as packaging and is processed in the Nio® Digital PCR. In case of need to process less than three chips, it is possible to reuse a chip plate up to three times, but it is necessary to clean it before reuse, following the Instruction for Use of the chips (MKT-00132_IFU_RubyChip_RUO).

Before entering the chip plate in the loading area, please check that the loading area is available (loading status line above the area continuously illuminated in white) and wait for the GUI confirmation.

The loading of a chip plate requires to present it in the right direction as shown on the picture below.

The chip plate needs to be pushed until it reaches the stop position. The Nio® Digital PCR will detect the full loading of the chip plate. Once the chip plate has reached the stop position and is correctly detected by the Nio® Digital PCR, the loading status light will start blinking. The Nio® Digital PCR will start processing the chip plate according to the specified experiment parameters.

Figure 41: Chip Plate loading

Chip plate loading warnings.

- Two chip plates shouldn't be loaded in a row. It is mandatory to wait for the full loading by the instrument (materialized by the loading status line blinking stop) before starting a new loading action. The loading of two successive chip plates can move the position of a chip plate already stored in the instrument and cause a crash of the loading module.
- No other object should be entered inside the loading area and a chip plate shouldn't be inserted in the wrong orientation (see picture below). This could also cause a crash of the loading module.
- A chip plate entered in the loading area shouldn't be manually withdrawn by the user. If a chip plate needs to be retrieved, the proper procedure is to let the instrument load it inside its storage area and ask for a chip plate unloading.
- No chip plate should be loaded while an unloading has started. It is mandatory to wait for the availability of the loading area (no blinking of the loading status line) to start any loading action to prevent from damaging the instrument.
- An already processed chip shall never be entered into the instrument for a thermocycling program.
- Never attempt to load a Chip Plate with a broken pin (Figure 43) inside the Nio® Digital PCR instrument.

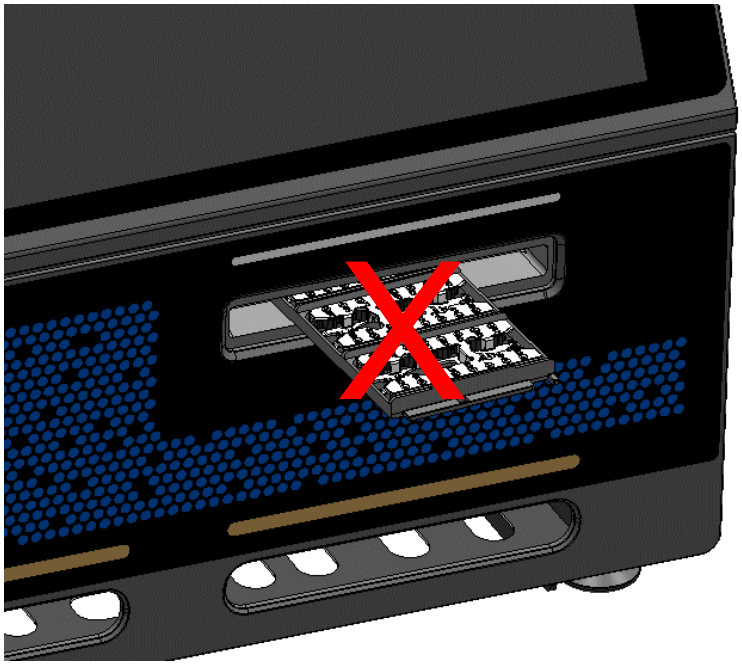


Figure 43: Wrong orientation of Chip Plate loading

Figure 42 : Damaged Chip plate with broken pin

Loading of chips with altered barcodes

The Nio® Digital PCR instrument provides automated barcode reading for all three chip consumable positions within a chip plate.

During the loading phase of a chip plate, the barcode readers will detect the presence of all chip consumables and identify their barcodes. This will allow to easily associate a chip with its experiment and guarantee the traceability of the process and the result.

In case of a chip with an unreadable barcode, it is possible to manually enter the barcode of a chip and associate it to a position in the chip plate and to an experiment. The instrument will not be able to check the format and information related to the manually entered barcode. It is therefore the User responsibility to control the exactness of the data. The chip will be processed normally but the Nio® Instrument will not be able to check if no mistake has been made during the process and if the good result is associated to the chip by reading the barcode during the unloading phase. It will also be the User responsibility to check if the chip unloaded is in the position in the chip plate announced on the GUI and in the right orientation.

When the Nio® Digital PCR detect a missing barcode, it will eject the chip plate to offer to the User the possibility to:

- Control the position of expected chips in the chip plate (position and orientation)
- Correct the position and/or orientation of a chip in the chip plate if a mistake has been detected
- Manually enter the barcode of a chip if no mistake has been detected.

In any case, the User will have to fully retrieve the chip plate before loading it back inside the instrument to allow the Nio® Digital PCR to correctly load it inside its storage position.

9.6 Nio® Digital PCR running

It is highly recommended not to use any other instrument or realize actions near the Nio® Digital PCR that could result in shocks or vibrations to the instrument while it is running and more specifically imaging chips.

9.7 Chip plate unloading

To unload a Chip plate from the Nio® Digital PCR, start by requesting the chip plate unloading on the GUI.

Once the unloading is validated, the status line of the loading area will start blinking and the Nio® Digital PCR will proceed to the unloading. It will push the chip plate from the storage position to the retrieve position, 13mm on the outside of the front door. The chip plate movement needs to be over before pulling it to have a better grab (loading status line blinking stop).

Figure 44: Chip Plate unloading

Once the chip plate is retrieved, the unloading action will be considered finished by the instrument and the loading area status line will turn off.

10 Regulatory Mode

An overview of the supported 21 CFR Part 11 requirements fulfilled using the Nio® Reader software and Nio® Analyzer software in Regulatory mode is provided in the Nio® Analyzer user manual.

In the following, “Nio® software suite Regulatory” refers to either Nio® Reader software or Nio® Analyzer software or both in regulatory mode.

The naica® Data Service software functions as the Nio® system user account manager and controls the compliance of all Nio® system operations with respect to 21 CFR Part 11 regulation.

10.1 Installation options

There are three different ways to install the Nio® software suite Regulatory at a customer’s organization, depending on the individual customer’s requirements and infrastructures. All three options require their own specific set of parameters to be put in place. The different options are detailed below.

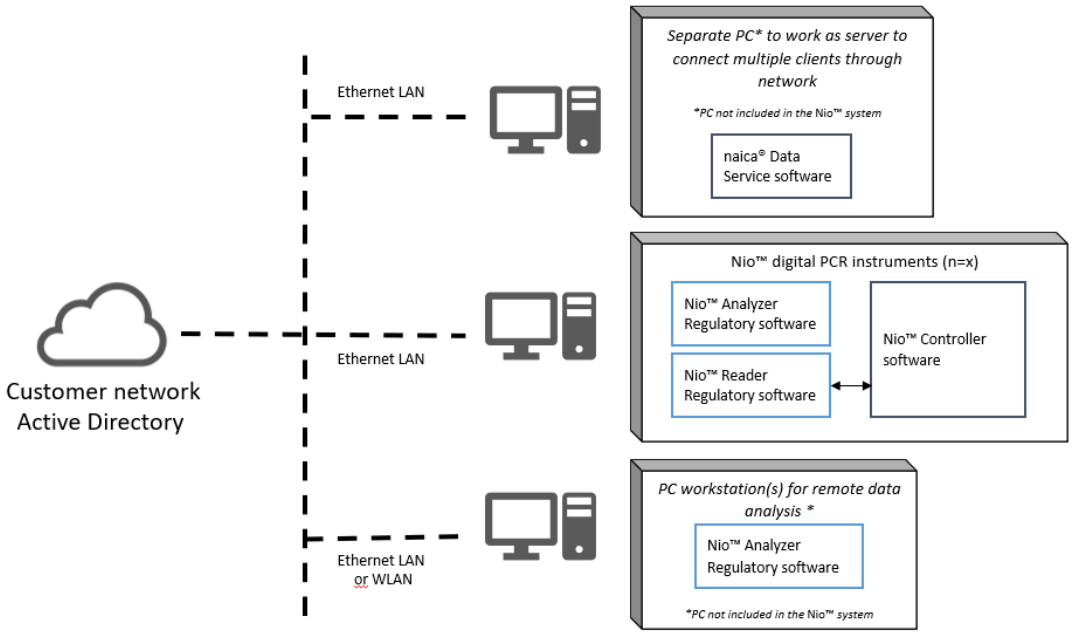
10.1.1 Active Directory user authentication & customer hosted PC as server

Recommended installation set up for Nio® software suite Regulatory

Utilize the customer´s organization’s Microsoft Windows Active Directory user authentication.

Utilize the customer’s local area network (LAN) to host the naica® Data Service on a separate computer to work as a server, to which multiple Nio® Digital PCR instruments and or other workstations, such as office PCs, can be connected.

The naica® Data Service must be connected through a network to all Nio® Digital PCR instruments and all other user workstations running Nio® software suite Regulatory.



In this installation set-up (separate PC working as a server), multiple simultaneous communication sessions may be initiated with the naica® Data Service.

- The customer must ensure the IT department has established a separate network hosted PC fulfilling all specified requirements to work as a server for the installation of the naica® Data Service (see section 10.2.1.1).
- The customer must ensure that the IT department will provide all required information about the separate PC working as a server (IP address / port number).
- The customer must ensure that Microsoft Windows Active Directory user authentication can be configured on all Nio® Digital PCR instruments and all other user workstations running Nio® software suite Regulatory.
- The customer must ensure that their IT department will provide a Nio® Digital PCR guest account within the Microsoft Windows Active Directory user authentication. They must ensure to record and document the guest account login details (user ID & password).
- The guest account is required by the Stilla Technologies representative. This account will allow for the installation qualification and operation qualification (IQOQ) and service maintenance routines executed on the Nio® Digital PCR under 21 CFR Part 11 conditions.
- If the guest account cannot be provided by the customer's IT department, the IQOQ procedure as well as any future service maintenance routines cannot be tracked and traced within the 21 CFR Part 11 configuration and will be documented separately by the Stilla Technologies representative.

The customer must ensure that their IT department administrator will be available on-site the day of the installation, as administration privileges are required for the 21 CFR Part 11 Nio® software suite Regulatory installation.

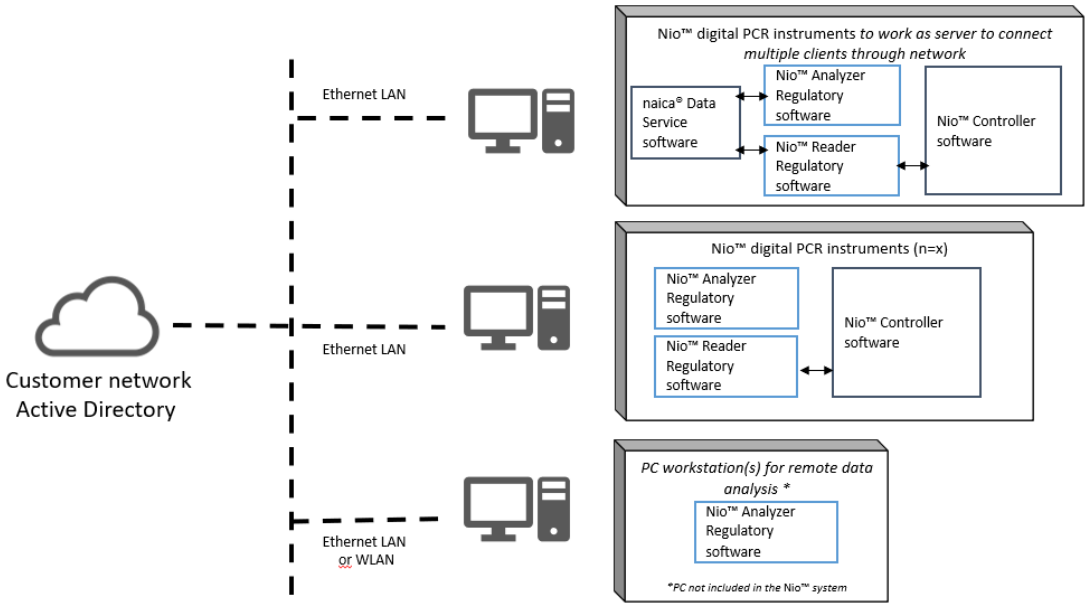
10.1.2 Active Directory user authentication & Nio® Digital PCR instrument as server

Limitation of this installation set-up:

The Nio® Digital PCR instrument, where the naica® Data Service is hosted, must always remain powered on. Each time this instrument is powered off, the naica® Data Service will not be available for any Nio® Reader software Regulatory and Nio® Analyzer software Regulatory applications.

Utilize the customer´s organization's Microsoft Windows Active Directory user authentication. Utilize the customers local area network (LAN) to host the naica® Data Service on the Nio® Digital PCR instrument. In this configuration, the Nio® Digital PCR instrument will act as a server; multiple Nio® Digital PCR instruments and/or other workstations, such as office PCs, can be connected to it through the network.

The naica® Data Service will communicate with Nio® Reader software Regulatory and Nio® Analyzer software Regulatory over the network.



In this installation set-up (Nio® digital PCR instrument working as a server), multiple simultaneous communication sessions may be initiated with the naica® Data Service.

- The customer must ensure that their IT department will provide all required information about the Nio® Digital PCR instrument working as a server (IP address / port number).
- The customer must ensure that Microsoft Windows Active Directory user authentication can be configured on all Nio® Digital PCR instruments and all other user workstations running Nio® Analyzer software Regulatory.
    - The customer must ensure that their IT department will provide a Nio® Digital PCR instrument guest account within the Microsoft Windows Active Directory user authentication. They must ensure to record and document the guest account login details (user ID & password).
- The guest account is required by the Stilla Technologies representative. This account will allow for the installation qualification and operation qualification (IQOQ) and service maintenance routines executed on the Nio® Digital PCR instrument under 21 CFR Part 11 conditions.
    - If the guest account cannot be provided by the customer's IT department, the IQOQ procedure, as well as any future service maintenance routines, cannot be tracked and traced within the 21 CFR Part 11 configuration, and will be documented separately by the Stilla Technologies representative.
- The customer must ensure that their IT department administrator will be available on-site the day of the installation, as administration privileges are required for the 21 CFR Part 11 Nio® software suite Regulatory installation.

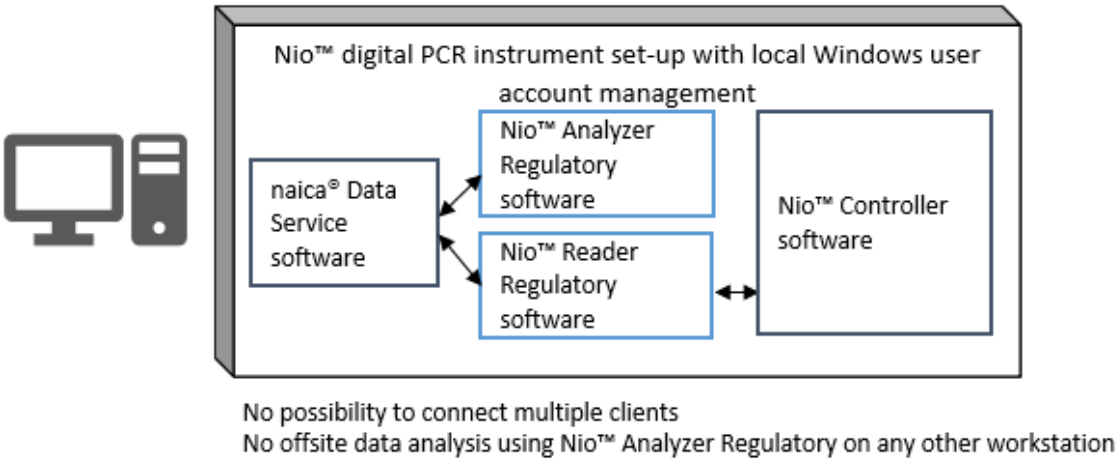
10.1.3 Local Windows user authentication & set up on single Nio® Digital PCR instrument

Limitation of this installation set-up:

This installation is not recommended for multi-instrument installations, as each instrument is independent and must have its own naica® Data Service installed.

Each user must prepare and analyze Crystal Digital PCR® experiments directly on the Nio® Digital PCR instrument. It will not be possible to open experiments on any other workstation or any other Nio® Digital PCR instrument offsite the configured 21 CFR Part 11 Nio® Digital PCR instrument hosting the naica® Data Service.

Utilize local naica® system Windows user account authentication and selected single Nio® Digital PCR instrument to host naica® Data Service without any network integration. With this installation configuration the client will be responsible for managing and administering the individual local Nio® Digital PCR Windows user account authentication.



- Local Windows user account authentication must be created for each 21 CFR Part 11 Nio® Digital PCR instrument individually.
- Custom standard experiments will not be recognized across the different 21 CFR Part 11 Nio® Digital PCR within the same facility. The standard experiments must be imported individually on each Nio® Digital PCR instrument.
    - Sharing experiments across different 21 CFR Part 11 Nio® Digital PCR instruments is prohibited.

This installation set-up can be managed by the Stilla Technologies representative and does not require support of a customer IT department administrator.

10.2 Installation instructions

10.2.1 naica® Data Service installation configuration

This procedure documents the installation process of the naica® Data Service including the Microsoft Windows Active Directory user authentication. The customer must ensure an IT department administrator is available to assist the 21 CFR Part 11 Nio® software suite Regulatory installation.

The naica® Data Service software is used to administrate Nio® Digital PCR user management, audit trails and electronic document signature capabilities to the software applications Nio® Reader software Regulatory and Nio® Analyzer software Regulatory.

The naica® Data Service software is a network connected application that must be installed, in addition to Nio® Reader software Regulatory and Nio® Analyzer software Regulatory applications, to ensure 21 CFR Part 11 compliance.

The naica® Data Service application can either be installed on a separate PC to work as a server or directly on the Nio® Digital PCR instrument.

After installation and setup, all user actions are logged and documented in the Nio® data file (.niodata) audit trails as well as in the naica® Data Service audit trails.

10.2.1.1 Nio® software suite Regulatory requirements

Prerequisites for using the naica® Data Service

The naica® Data Service software can run either directly on the Nio® Digital PCR PC or on any other PC which fulfills the following specifications for optimal performance:

- Operating System: Windows 10 and Windows 11 in 64 bits
- RAM: at least 16 GB
- Processors: Intel Core i5 or higher, at least 2 cores of 2 GHz or higher
- Screen resolution: at least 1920 x 1080; aspect ratio 16:9
- Network: any wired or wireless networking environment.

naica® Data Service software installer can be downloaded on Stilla Technologies' website (https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/).

Note: 2 GB of disk space is required for software installation.

For integration of the naica® system to the customer's network or installation of customer specific antivirus applications, it is important to ensure that access to the following naica® system directories is not prevented:

- C:\ProgramData\Stilla
- C:\Program Files\Stilla
- %USERPROFILE%\Stilla
- %USERPROFILE%\AppData\Local\Stilla

➢ **Microsoft Windows Active Directory user authentication service**

The naica® Data Service uses the Microsoft Windows Active Directory user authentication feature to authenticate users. It is recommended to follow Microsoft password policy to ensure accounts are secure against password attacks.

If the client's IT infrastructure allows the integration of the Nio® Digital PCR in Microsoft Windows Active Directory domain for user authentication, the naica® Data Service can be installed:

- on a separate PC to work as a server

- on a Nio® Digital PCR instrument to work as a server

The Nio® Reader software Regulatory and Nio® Analyzer software Regulatory applications must be configured to communicate with the naica® Data Service over the network.

If Microsoft Windows Active Directory user authentication is not available, it is possible to configure the naica® Data Service to use local Microsoft Windows accounts for user authentication. In this case, the Nio® Reader software Regulatory and Nio® Analyzer software Regulatory applications must be installed and used on the same Nio® Digital PCR instrument where the naica® Data Service is installed.

➢ **naica® Data Service security**

The separate PC to work as a server must be configured such that only the IT administrator has administration privileges to access the installation directory on the naica® Data Service.

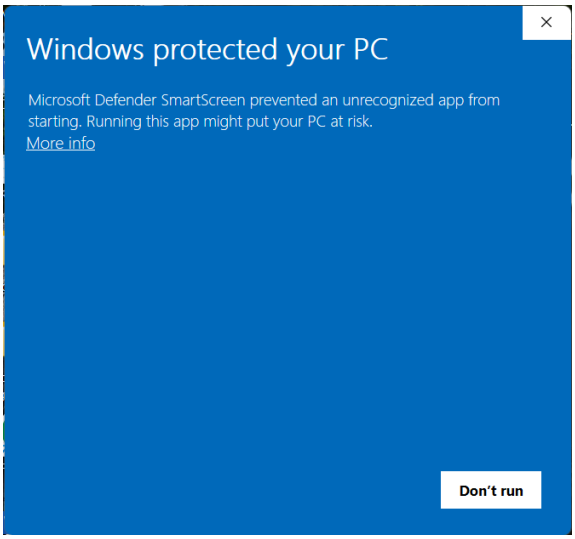
Other Nio® Digital PCR users shall not have “write” or “execute” rights on the installation directory of the naica® Data Service.

This configuration is mandatory to guarantee the security of the Nio® software suite Regulatory. It is the customer’s responsibility to ensure the set-up configuration as described for the naica® Data Service.

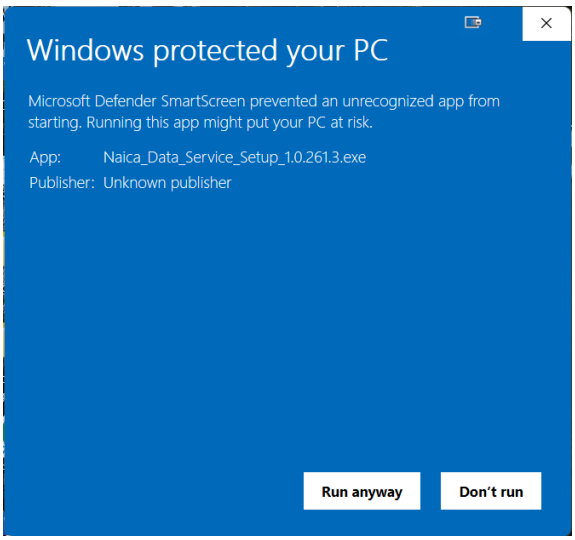
10.2.1.2 naica® Data Service download and installation

The installation of the naica® Data Service, on separate PC to work as a server, should be executed with IT administration privileges. Naica® Data Service software installer can be downloaded from Stilla Technologies website’s Technical Resources page after customer login (https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/).

1. Download the file to the PC and double-click to start the installation process.

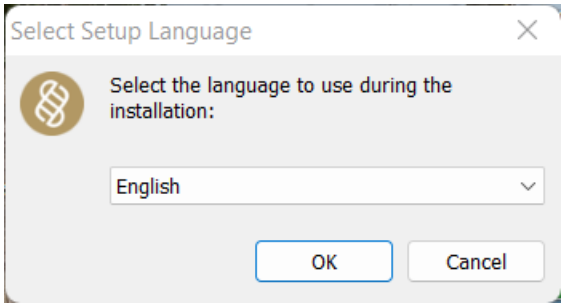


2. Click « More info » in the Microsoft Defender SmartScreen dialog.

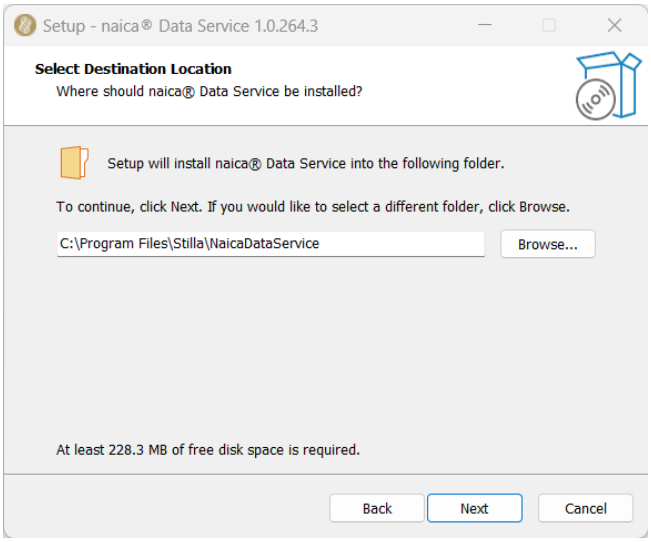


3. Click « Run anyway » in the Microsoft Defender SmartScreen dialog to allow the installer to execute the installer.

4. Click « Yes » in the Windows User Account Control Dialog asking to allow changes to the device.

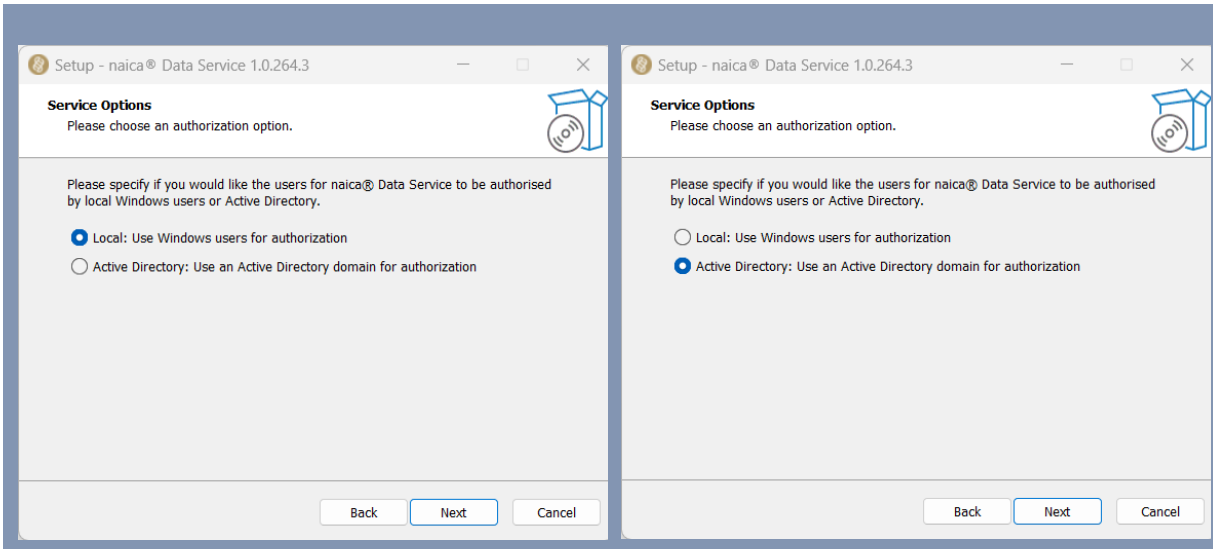


5. Select the language to use during the installation.
6. Click « Next ».



7. Select the preferred installation location for naica® Data Service software.
8. Click « Next » to install in the default installation directory

9. Click « Browse » to choose a different Destination Location for the naica® Data Service.



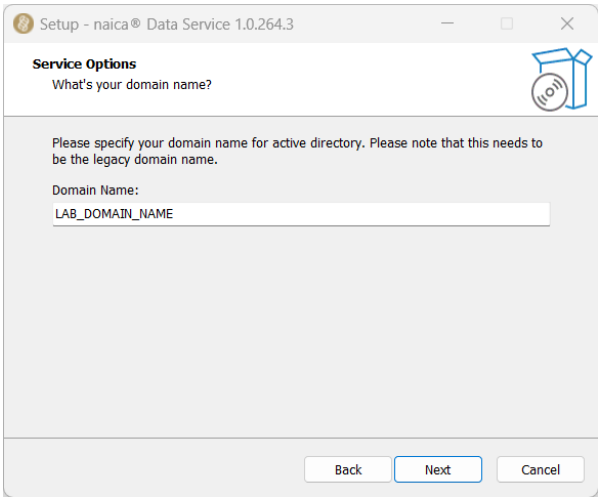
10. Check the preferred installation option for local Windows user authorization (left, section 2.3) or Active Directory domain for authorization (right, sections 2.1 & 2.2).

- For Section 2.3 (Local Windows user authentication)

  If local authentication is selected, the naica® Data Service must be installed on an individual naica® system instrument and all 21 CFR Part 11 naica® system Pro software applications can only be used locally. Local Windows user accounts must be administrated by the customer.

- Sections 2.1 and 2.2 (Active Directory user authentication)

  Stilla Technologies recommends the installation supported by the Microsoft Windows Active Directory user authentication to benefit from a centralized customer organization user authentication as well as to allow multiple clients access to all 21 CFR Part 11 naica® system Pro software applications.

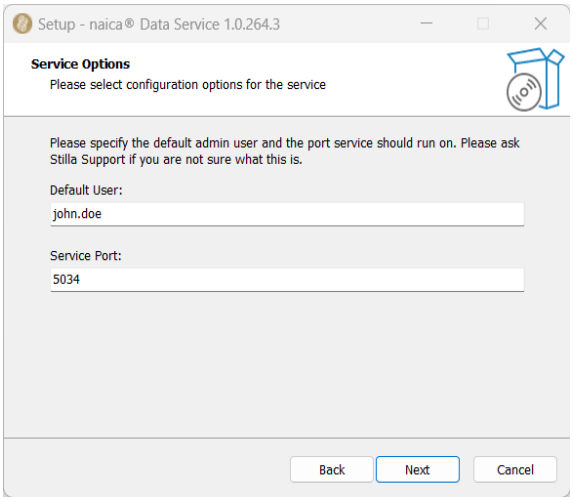


11. Specify the domain name.

If the naica® Data Service is to be used with Local Windows accounts (section 2.3), there is no need to provide the domain name.

If the naica® Data Service is to be used with Microsoft Windows Active Directory user authentication (sections 2.1 & 2.2), the customer specific domain name needs to be configured.

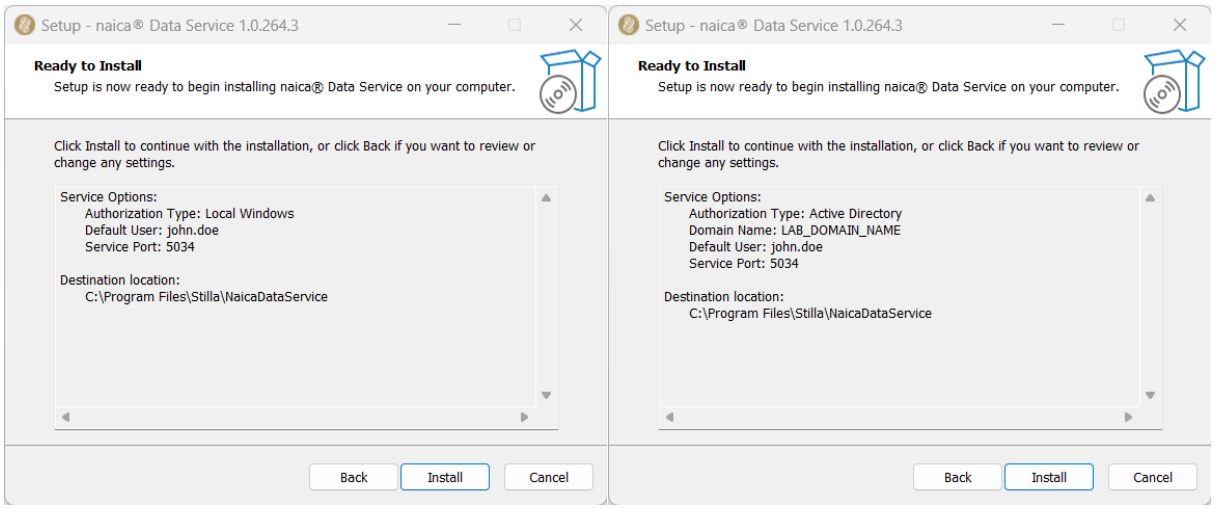
The naica® Data Service installer automatically detects the domain name. Make sure the correct domain name is displayed. Modify if required.

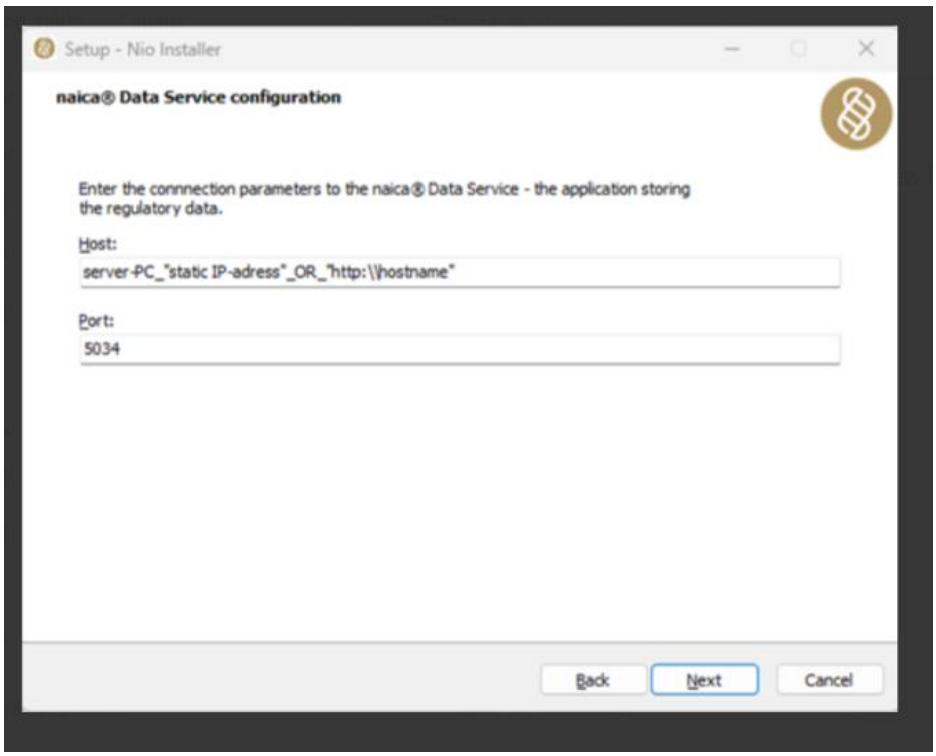


12. Specify the default Administrator user and configure the service port number.

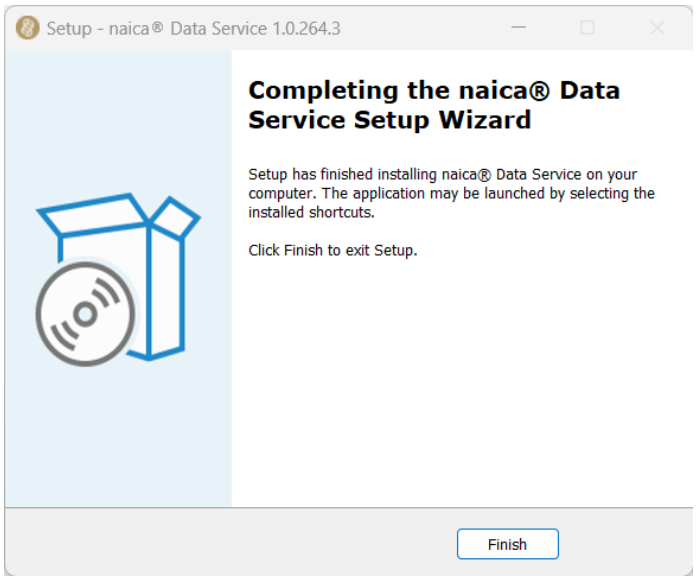
The naica® Data Service installer automatically detects the current user's Microsoft Windows Active Directory account ID as the default user. Make sure the correct spelling for the Default User is detected. Correct manually if required. At this stage, it is recommended to use the username of the IT Administrator from the customer's organization as the default user.

By default, the Service Port is set to « 5034 ». The Service Port can be configured to another value. The PC that is selected to work as a server to host the naica® Data Service application must allow TCP connections on the selected port.





13. Review all specified settings in the « Ready to Install » dialog box.
14. Click on « Back » to edit settings.
15. Click on « Install » to complete the naica® Data Service installation with all specified settings.



16. Click on « Finish » to exit the naica® Data Service setup.

After successful installation, the naica® Data Service is automatically activated to run on the configured PC.

Note: The PC that is selected to work as a server to host the naica® Data Service application must always be turned on to ensure the 21 CFR Part 11 compliant communication between Nio® Digital PCR instruments, as well as with any separate workstations. The PC hosting the naica® Data Service must not use hibernation to ensure the proper execution of the service software.

10.2.1.3 Nio® Reader software Regulatory installation

The Nio® Reader software Regulatory installer must be executed to install the corresponding application and configure the connection with the naica® Data Service-hosting PC.

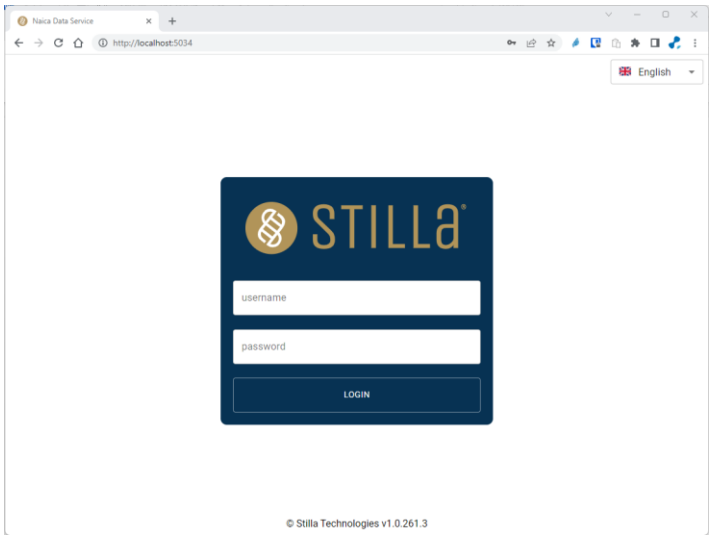
Nio® Reader software Regulatory must be installed on every Nio® Digital PCR instrument.

In addition, it can be installed on additional workstations meeting the specified minimal technical requirements (Section 10.2.1.1) to allow experiment preparation using Nio® Reader software Regulatory offsite the Nio® Digital PCR instrument.

Follow instructions provided in section 7.3.1 and select the Regulatory option to install Nio® Reader software Regulatory.

10.2.1.4 naica® Data Service web interface configuration

1. To configure the naica® Data Service web interface, open a web browser and enter the specific naica® Data Service URL: http://<server_ip_or_dns>:<port_number>.
    a. For network integrated installation options (Sections 10.1.1 & 10.1.2) the IT administrator must replace the internet protocol address (IP) or Domain Name Server address (DNS) and the port number, according to the naica® Data Service configuration. It is important to configure the selected server to allow HTTP connections on the naica® Data Service port. To facilitate naica® Data Service's interface connections, it is recommended to associate a Domain Name Server (DNS) address to the server.
    b. For the local installation option (Section 10.1.3), the default parameters to configure the naica® Data Service web interface are:
        - http://localhost:5034 (DNS)
2. It is recommended to create a bookmark for the naica® Data Service web interface URL in the web browser, to support easy access to the naica® Data Service web interface, without the need to enter the specific complete naica® Data Service URL.



3. The IT administrator must log in to naica® Data Service using their individual Microsoft Windows Active Directory user authentication (username and password) to create the first 21 CFR Part 11 Nio® Digital PCR account.

- This first 21 CFR Part 11 Nio® Digital PCR account must be a “Lab Manager” account, to allow the creation of additional user accounts, independent of any additional support from the individual IT administrator Microsoft Windows Active Directory user authentication.
- This first 21 CFR Part 11 Nio® Digital PCR account must be selected among the Nio® Digital PCR users.

For instructions to create and manage 21 CFR Part 11 Nio® Digital PCR user accounts and user roles, refer to Section 10.3.

10.3 naica® Data Service roles and permission administration

10.3.1 naica® Data Service Permissions

The naica® Data Service software introduces the notion of Permissions. Each User Role grants assigned users access to a set of predefined permissions for the Nio® Reader software and Nio® Analyzer software applications.

The permission settings can be administrated through the Roles / Permission tab on the naica® Data Service user interface. All available Permissions are displayed in a list format, where granted permissions are marked with an activated check-box symbol for a defined User Role. Permissions not granted for a User Role display an unchecked box.

The available permissions to select for Nio® Reader software are (refer to section 9 for specific instructions about the features listed below):

1. Open Protocol.
2. Edit Protocol.
3. Open Assay.
4. Edit Assay.
5. Create an experiment from scratch (“blank experiment”).
6. Create a Standard experiment.
7. Import assays and/or protocols to an experiment.
8. Remove assays and/or protocols from an experiment.
9. Start Run.

   The «Start Run » permission allows the user to run experiments with Nio® Reader software Regulatory. The granted «Start Run» permission includes the ability to scan experiments, re-scan experiments as well as save experiments.

   Note: To be able to scan an experiment, users with the “Start Run” permissions, but without the permissions to create experiments (5 and 6) must use existing experiments previously registered by a user with a such permissions in the naica® Data service software.

10. Set an experiment as High Priority.
11. Cancel runs from other users.
12. Download data from other users’ runs.
13. Delete other users’ runs.
14. Manage users.

    The «Manage Users» permission allows the user to manage User Roles within the naica® Data Service application. Only a person with the granted permission «Manage Users» will be able to create new User Roles, or to modify and delete existing User Roles and Permissions.

Only a person with the granted permission «Manage Users» will be able to activate a defined User Role to a new Nio® Digital PCR user or manage / administrate all created Users.

It is the customer's responsibility to define who is responsible for managing roles and permissions in naica® Data Service software and to ensure that only authorized personal is granted with the “Manage Users” permission.

10.3.2 naica® Data Service Roles

The naica® Data Service software introduces the notion of User Role.

The User Roles can be administrated through the Roles / Permission tab on the naica® Data Service user interface.

All available User Roles are listed in the available drop-down menu in the Roles / Permission tab on the naica® Data Service user interface.

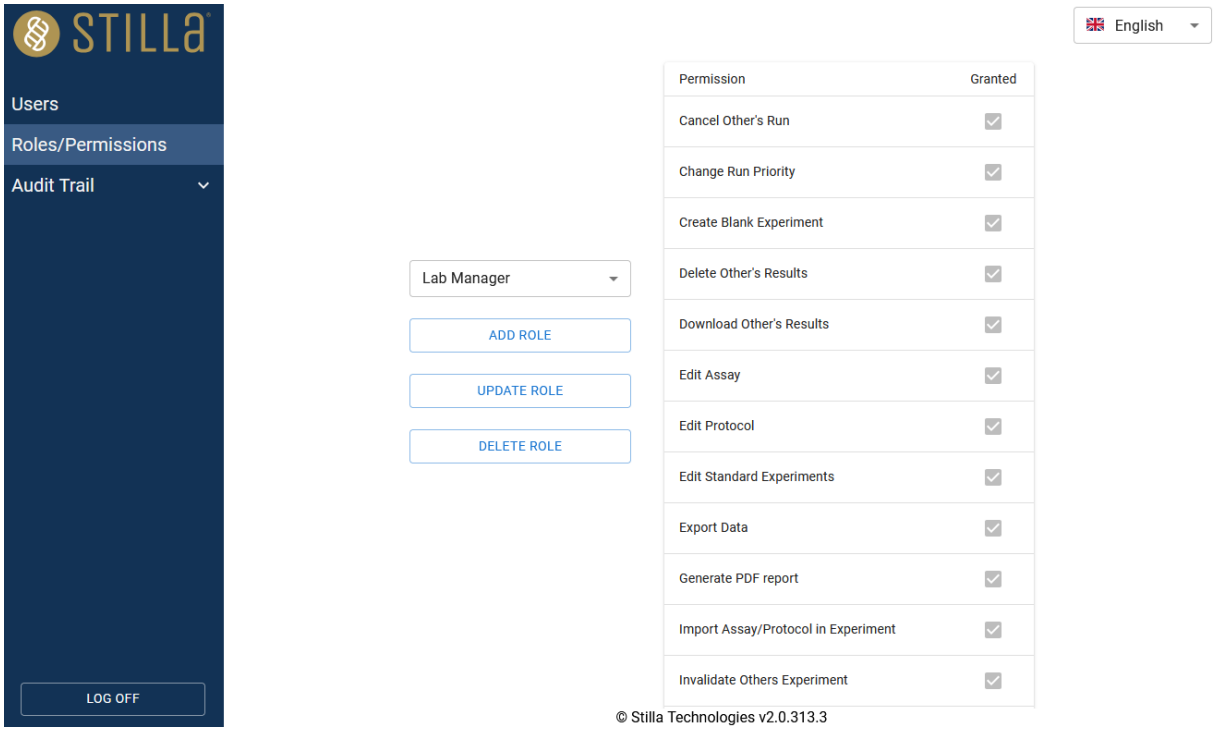
Each User Role defines what naica® Data Service permissions are granted to the assigned users. The User Role manages access to a set of predefined permissions for both Nio® Reader and Nio® Analyzer software applications.

10.3.3 Default User Roles

The naica® Data Service installation provides two predefined default User Roles.

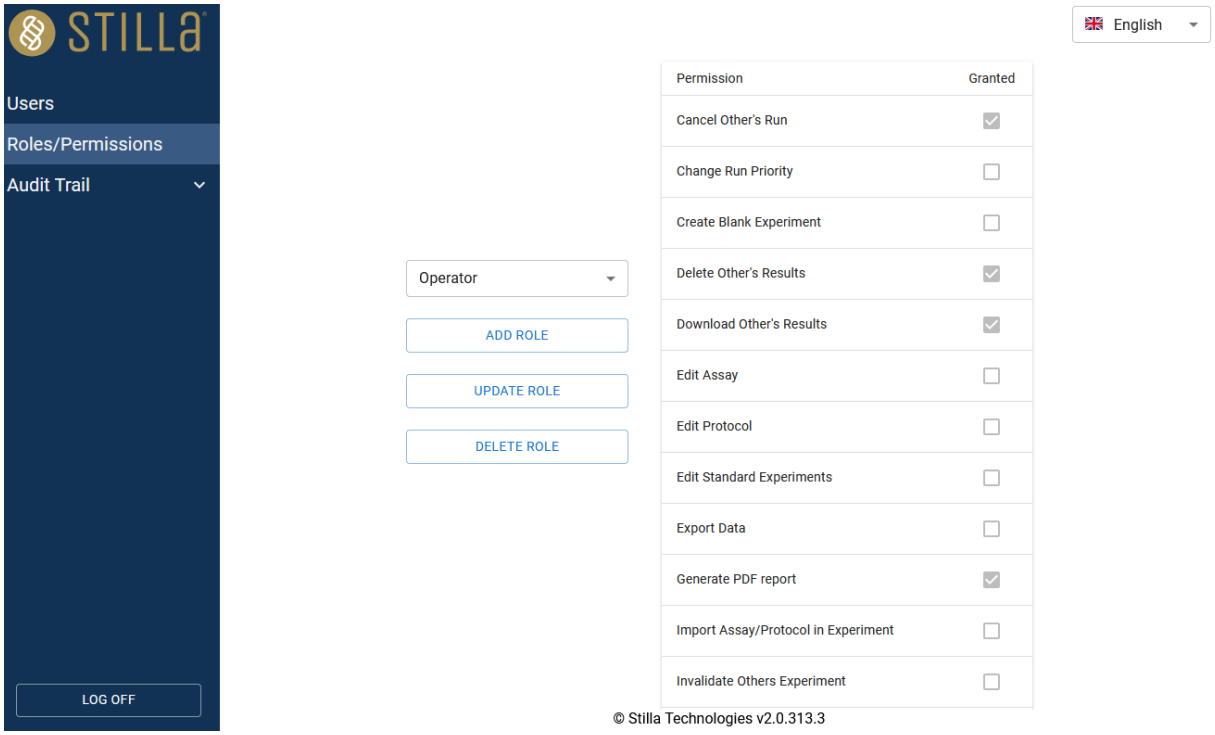
10.3.3.1 Lab Manager

With the Lab Manager User Role, all available permissions are granted for Nio® Reader software and Nio® Analyzer software.



10.3.3.2 Operator

With the Operator User Role, only limited permissions are granted for Nio® Reader software and Nio® Analyzer software.



An Operator User Role will have permissions to:

- Generate PDF report
- Run experiments
- Validate experiments

The Operator User Role is intended to have access only to validated standard experiment files included in the customer's SOP that are released for routine operation after Lab Manager validation.

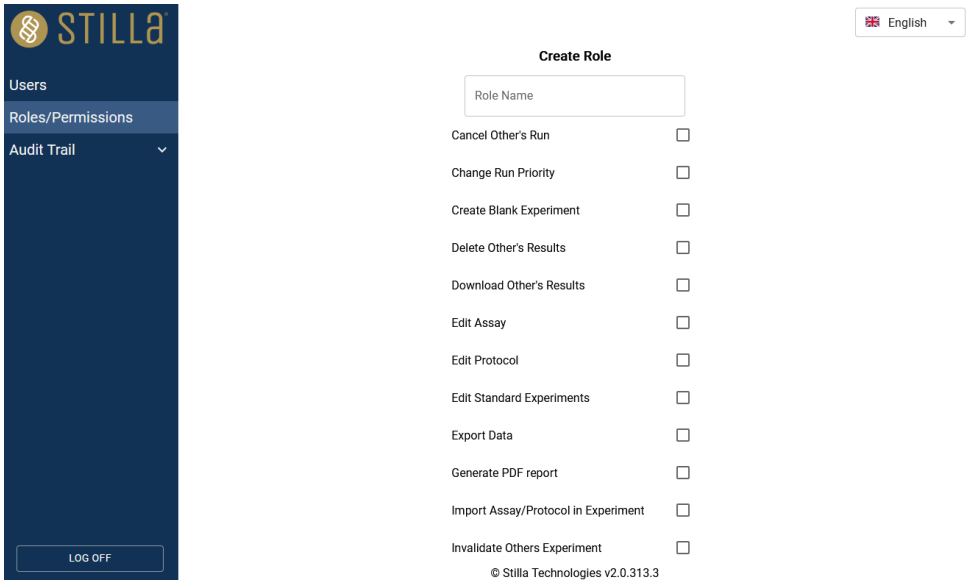
10.3.4 Administration of customized User Roles

Customized User Roles can be created as described below.

10.3.4.1 **Add Role**

To add a new User Role select "Add Role".

Enter the Role Name and select all permissions that are to be included in the customized User Role. Confirm the creation of the new User Role by acknowledging "Create Role". The newly created User Role will then be listed in the User Role drop down menu in the Roles / Permission tab.



10.3.4.2 Update an existing Role

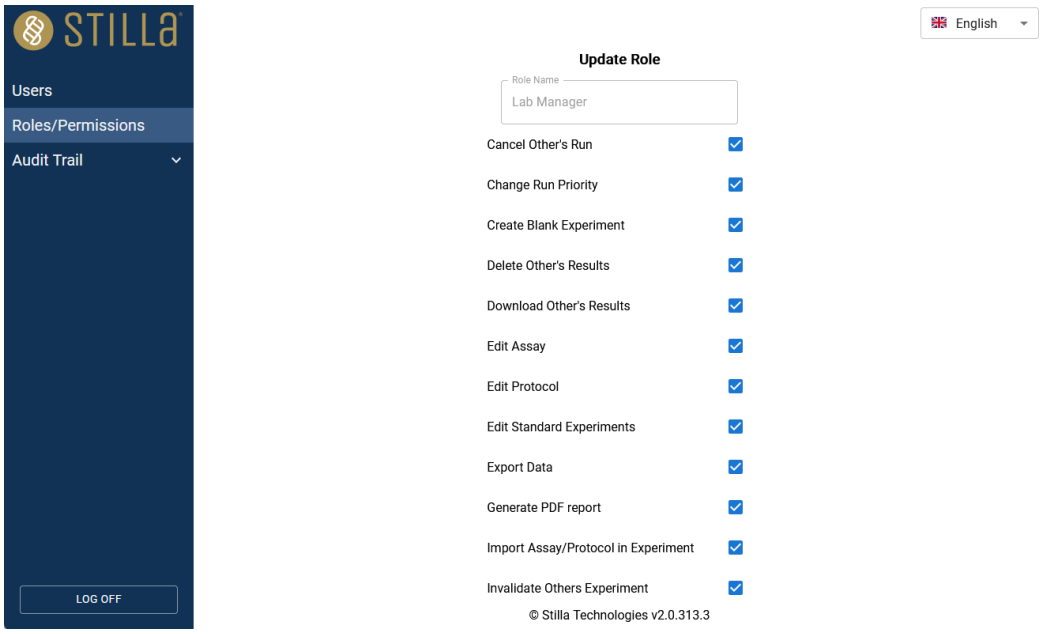
Click on the **Update Role** button to update any existing User Role.

Modify the Role Name.

Modify the User Role permission by activating or deactivating permission settings.

Confirm the modifications of the updated User Role by acknowledging “Update Role”.

The updated User Role will then be listed in the User Role dropdown menu in the Roles / Permission tab.



Updated roles / permission settings will be activated for assigned users at the next login to Nio® Reader software and Nio® Analyzer software.

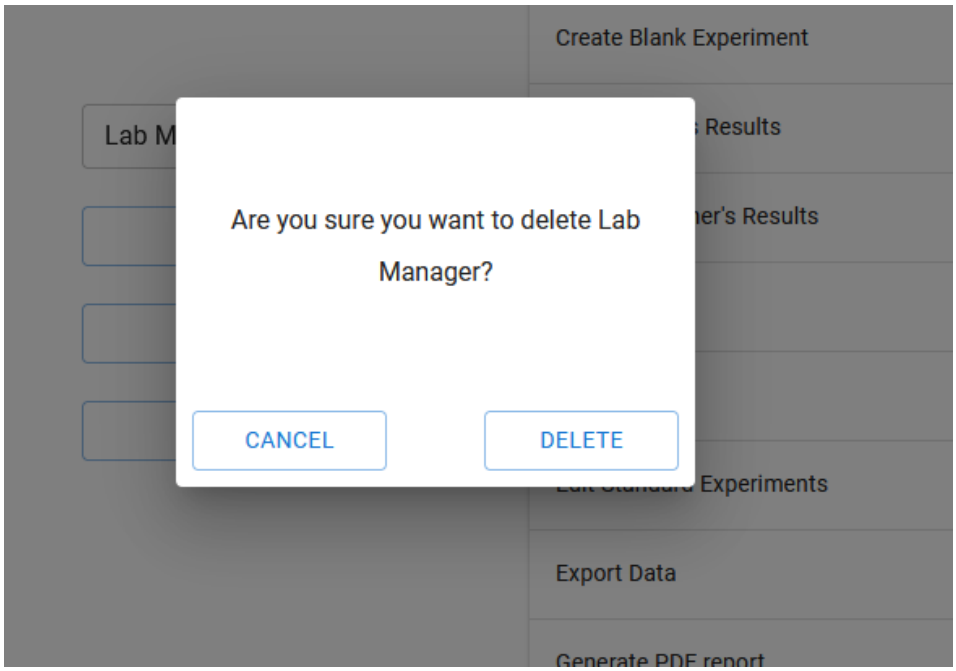
10.3.4.3 Delete an existing Role

***Note**: User Roles that are assigned to at least one User cannot be deleted.*

Click on the **Delete Role** button to delete any existing User Role.

A warning message will appear. Confirm the deletion of the selected User Role by acknowledging “Delete”.

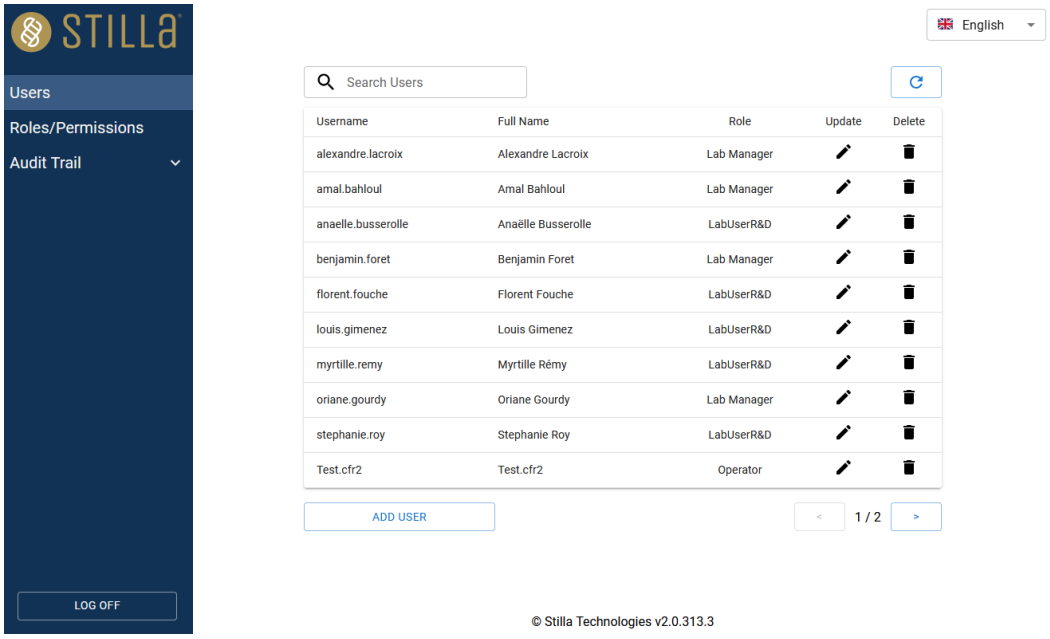
The deleted User Role will then no longer be listed in the User Role dropdown menu in the Roles / Permission tab.



10.3.5 naica® Data Service User management

For 21 CFR Part 11 compliance it is obligatory to ensure that only authorized individuals have access to naica® Data Service.

Therefore, all users are obliged to log in to the naica® Data Service using individual user authentication credentials (username and password).



The Users menu displays the list of all naica® Data Service user accounts within the customer's organization. All listed naica® Data Service user accounts will be able to log in to all 21 CFR Part 11 Nio® Digital PCR software applications.

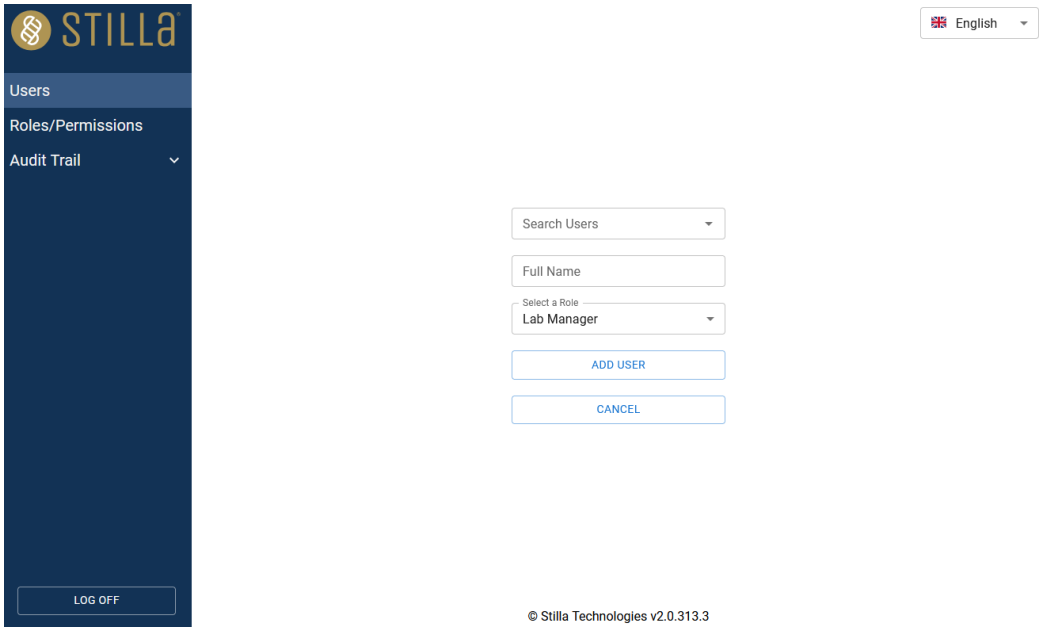
Only naica® Data Service User Roles with the permission «Manage Users» can administrate the Users menu to Update, Delete or Add Users.

To modify an existing naica® Data Service user account, click on the respective icon.

Update 	To update a naica® Data Service user account with a different User Role.
Delete 	To delete a naica® Data Service user account. A warning message will request to confirm the deletion of the selected naica® Data Service user account.

Proceed as follows to create a new naica® Data Service user account:

1. Click on «Add USER».



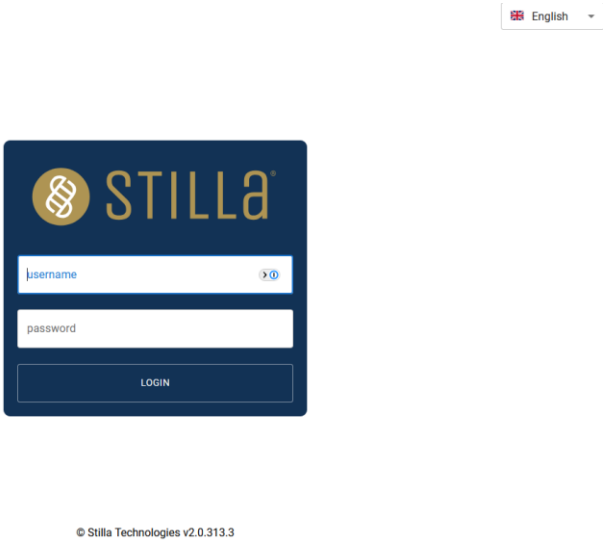
2. Enter the Microsoft Windows username of the new user in the «Search Users» field.

Note: Based on the installation setup in the customer's organization, this Microsoft Windows specific username will either depend on:

- the customer organization's Microsoft Windows Active Directory user authentication (Sections 10.1.1& 10.1.2)
- local Nio® Digital PCR specific Microsoft Windows user authentication (Section 10.1.3)

The naica® Data Service will search all available Microsoft Windows accounts that match the provided username.

3. From the provided list select the correct username.
4. The naica® Data Service will automatically complete the field «Full Name» with information from the Microsoft Windows account.
5. From the available drop-down menu «Select a Role », assign a User Role for the naica® Data Service user account.
6. Confirm the creation of the naica® Data Service user account with «Add User», after validating that all user specific information is correct.
7. The confirmed user account is thereby activated, and the user can log into the naica® Data Service application using their individual Microsoft Windows authentication credentials (username and password) in the web interface.



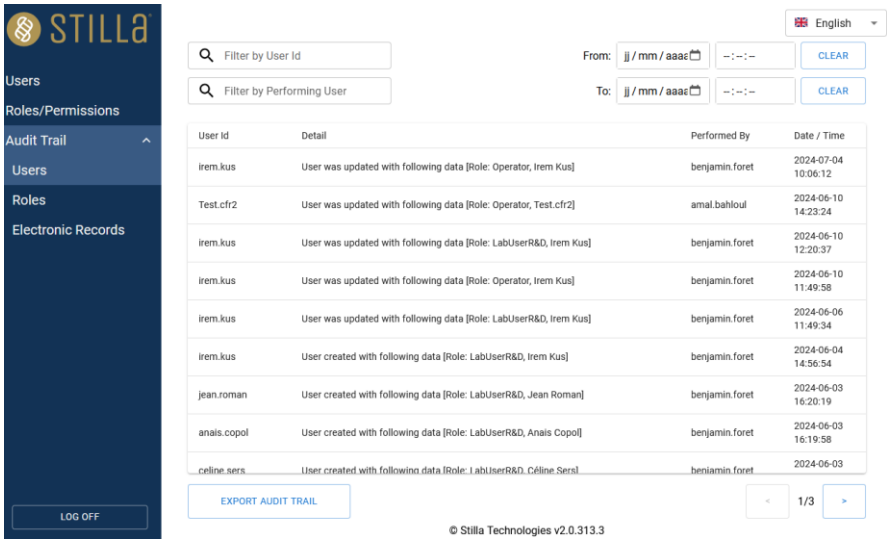
Once logged in to naica® Data Service, any user not executing active events, will automatically be logged-out by the application, to ensure compliance with 21 CFR Part 11. To log in again the naica® Data Service web interface login screen will be presented.

10.3.6 naica® Data Service Audit Trail

The naica® Data Service Audit Trail provides an automatically generated, time-stamped electronic record that allows to document the history of events related to the creation or modification of electronic records, based on individual naica® Data Service user accounts.

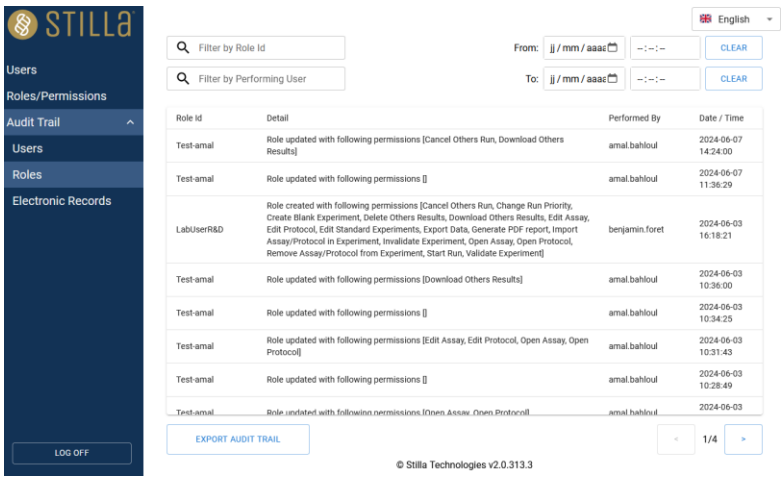
Three naica® Data Service Audit Trail categories are available:

8. Users

9. 

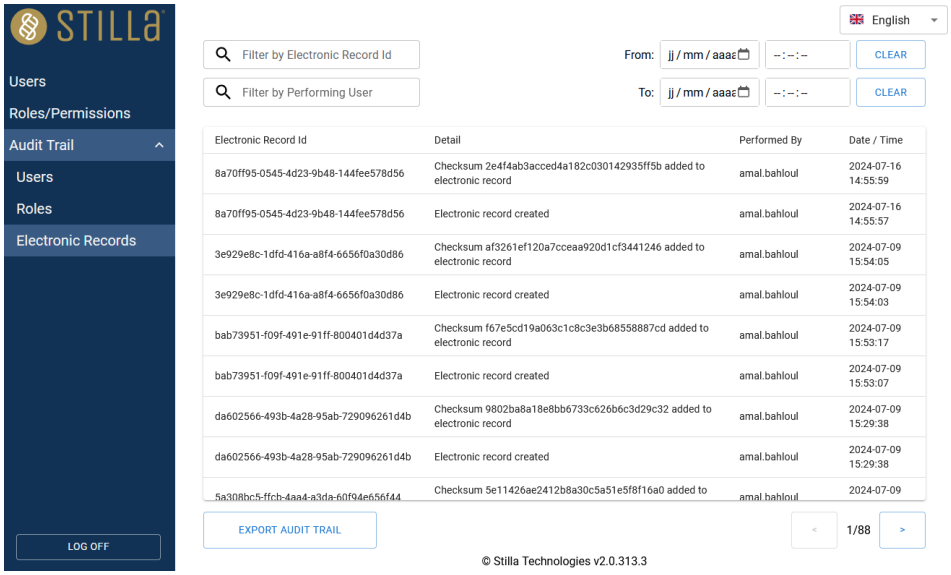
   The naica® Data Service Audit Trail «Users» tab documents the event history of naica® Data Service user account management. The naica® Data Service Audit Trail «Users» can be filtered by «User Id», the naica® Data Service user account affected by the recorded event.

10. Roles



The naica® Data Service Audit Trail «Roles» documents the event history of naica® Data Service User Role management. The «Roles» space can be filtered by «Role Id», the naica® Data Service User Role affected by the recorded event.

11. Electronic Records



The naica® Data Service Audit Trail «Electronic Records» documents the event history of any Nio® Digital PCR file. The naica® Data Service Audit Trail «Electronic Records» can be filtered by «Electronic Record Id». The electronic record Id is a unique ID created by the naica® Data Service to identify the event history of any Nio® Digital PCR file. The electronic record checksum is a condensed version of the Nio® Digital PCR file that is required by naica® Data Service to verify the file integrity.

It is important to perform regular back-ups of the naica® Data Service Audit Trail database. The database file is stored in “C:\Program Files\Stilla\NaicaDataService\Naica.db”. It is the responsibility of the customers organization to establish and maintain a backup routine for the naica® Data Service Audit Trail database.

All naica® Data Service Audit Trail categories can be filtered by « Date & Time ». To produce consistent Audit Trails, all computers running 21 CFR Part 11 Nio® software suite Regulatory applications must be configured with the correct date and time. It is the customers’ organization’s responsibility to ensure the correct date and time settings are configured.

All naica® Data Service Audit Trail categories can be filtered by «Performing User», the naica® Data Service user account executing the recorded naica® Data Service event.

All naica® Data Service Audit Trail categories can be exported as a PDF document by clicking «EXPORT AUDIT TRAIL».

10.4 Nio® Reader specific features in Regulatory mode

This section details the 21 CFR Part 11 specific software features of the Nio® Reader software Regulatory. All general software features common to both Nio® Reader software Standard and Regulatory modes are described in section 9.

10.4.1 Individual User Authentication

For 21 CFR Part 11 compliance it is obligatory to ensure that only authorized individuals have access to Nio® Reader software Regulatory. Therefore, all users are obliged to log in to Nio® Reader software Regulatory using individual user authentication credentials (username and password).

A failed login attempt can come from using incorrect login credentials.

Note: Following several incorrect entries for user login credentials the naica® Data Service account can be locked. Only an IT administrator can reset the user's Windows account to be activated again.

Failed log-in attempts can also be due to a connectivity issue to the naica® Data Service. Please contact the respective IT department to ensure that naica® Data Service connection is provided.

If the user's session expires, the Nio® Reader software Regulatory will request a new authentication. Any attempt to avoid the user authentication will automatically exit the software, which may lead to unsaved changes.

Automatic log-out is managed through the individual organization's setup for the Microsoft Windows user account settings. However, it is recommended to always proactively close all Nio® Reader software Regulatory if the application is not in use.

Note: The login attempt can fail if the underlying Windows authentication is denied or if the software loses the connection to the naica® Data Service.

10.4.2 Experiment Audit Trail

The Experiment Audit Trail records all edition events in Nio® Reader software Regulatory and Nio® Analyzer software Regulatory applications for an experiment file. Please refer to the Nio® Analyzer software user manual for more details about the content of the experiment audit trail.

11 Troubleshooting

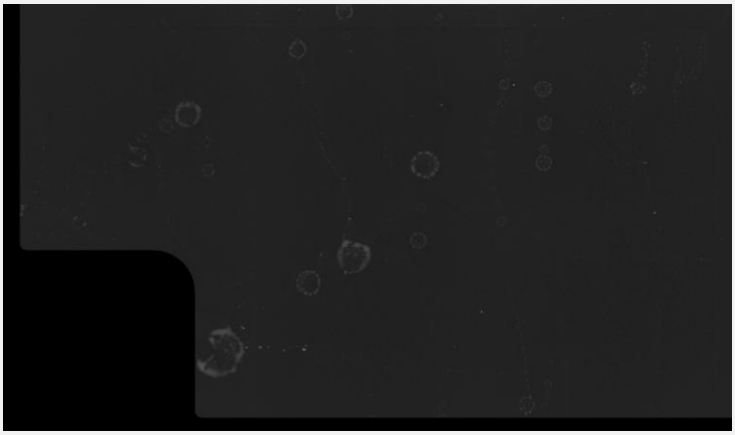
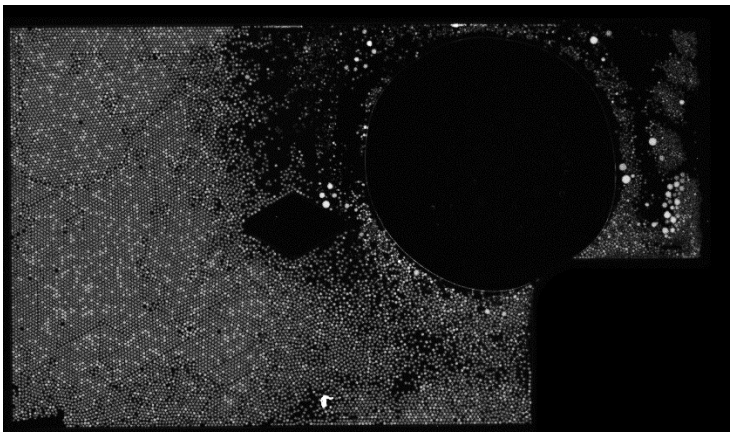
This section provides information about what to do if an error occurs and recommendations for avoiding or correcting them. In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

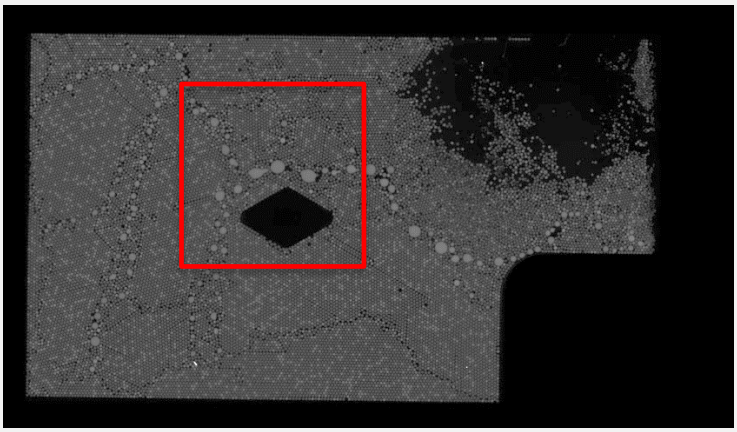
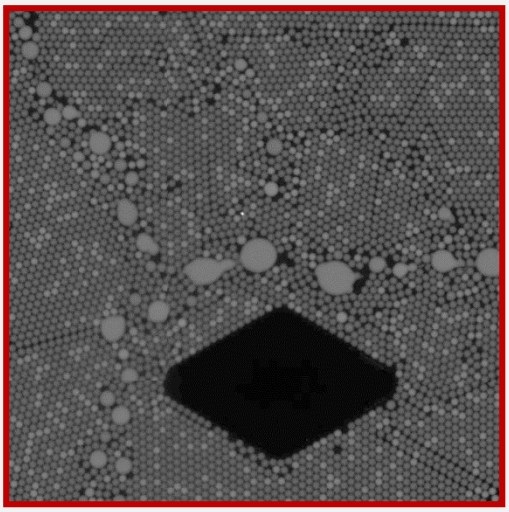
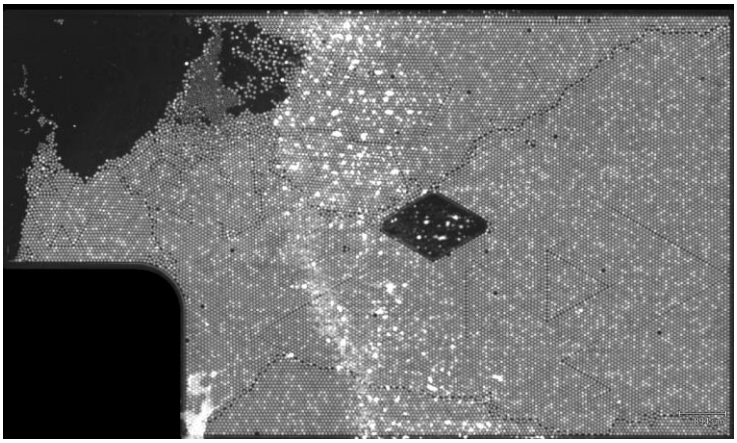
11.1 Contact Technical support

When you encounter a problem, ensure that you have the following information:

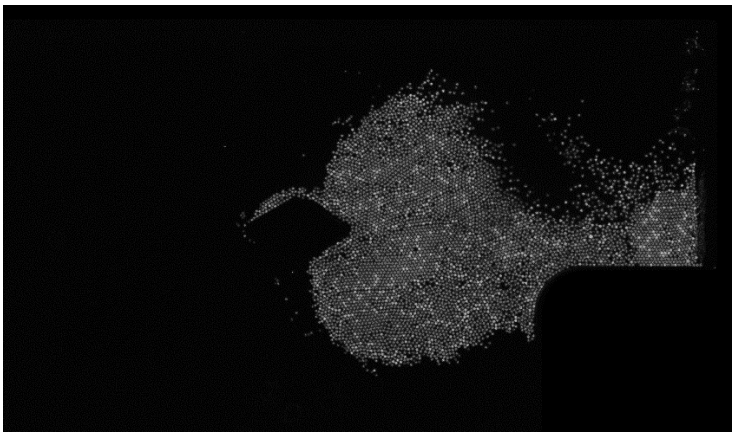
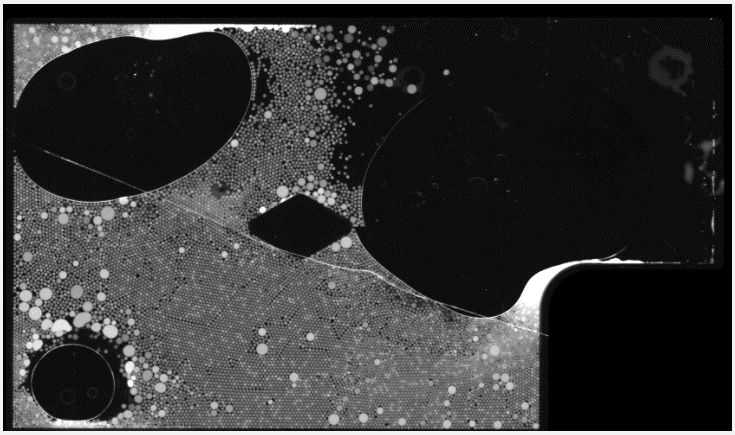
- Instrument Serial number and software version.
- Sample material.
- Logs and Niodata.

11.2 Ruby Chip

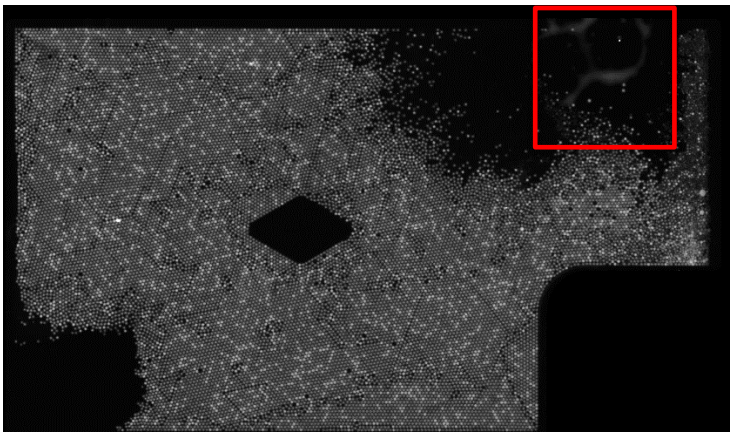
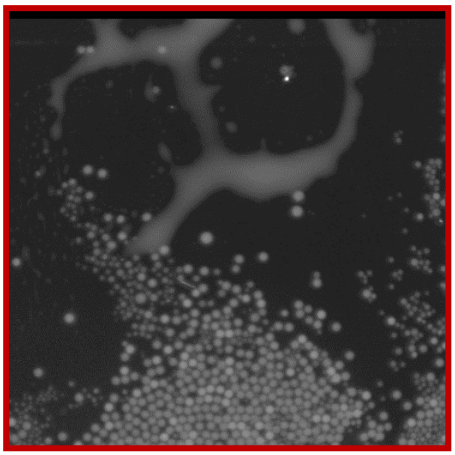
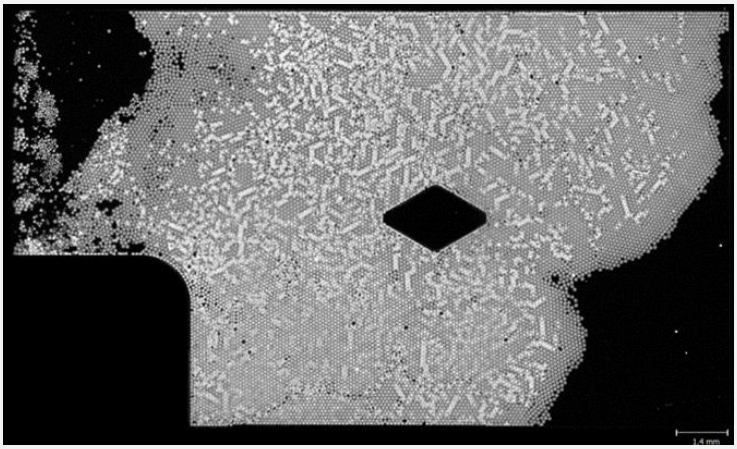
OBSERVATION	POSSIBLE CAUSE	RECOMMENDATION
Empty Chamber 	The sample was not pipetted in the correct well. The sample was ejected from a tip that was introduced too deep (and could touch the bottom of the well) or insufficiently deep (i.e. it could have been at the air-oil interface). When pipetting with a multichannel pipet, the 8 tips were not perfectly horizontal, hence some of them were either above the oil or touching the bottom of the well.	Take stock of the numbering on the wells to ensure that samples are always pipetted in the correct well. When pipetting your sample, stabilize the pipet tip about 1mm above the bottom of the well just by lifting it slightly from the bottom of the well (i.e. neither in contact with the bottom of the well, nor out with the oil) before ejecting your sample from the tip For more information about how to properly pipet your sample in the Ruby Chip, please refer to the IFU for Ruby Chip.
Bubble 	Small bubbles can sometimes occur during the PCR run, due to heat and pressure cycles. If you observe big and frequent bubbles, the inflated bag containing the Ruby Chip might have been damaged during transport.	It is recommended to check that the inflated pouch is still airtight upon reception.
Electrocoalescence	The procedure using the antistatic wipes was omitted or	Apply the antistatic wipes on all the chips as described in the IFU for the chips.

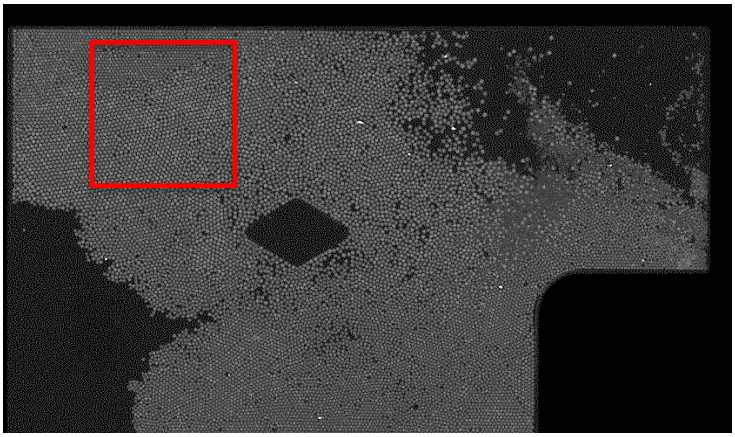
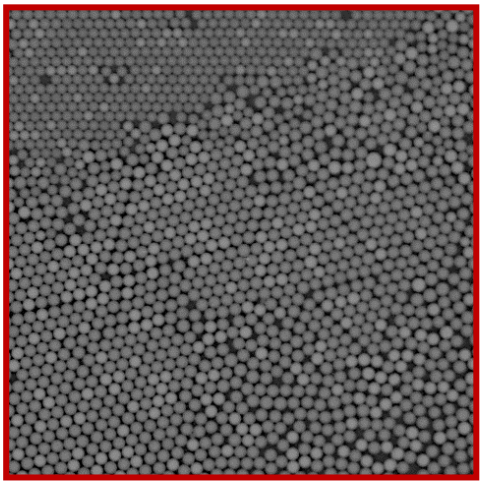
 	not properly performed.	In case of further questions or support, please contact Technical Support
Traces of antistatic product 	The presence of residual antistatic product under the chip generates artifacts in the blue channel.	After applying the antistatic treatment, wipe off the excess of antistatic product with a piece of Precision Wipe according the IFU instruction.

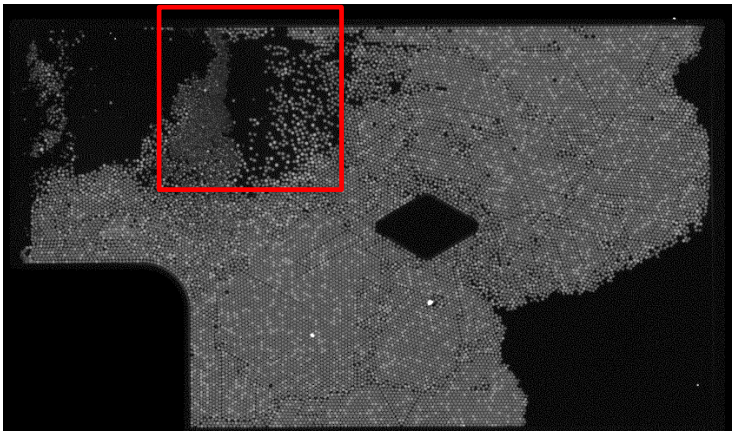
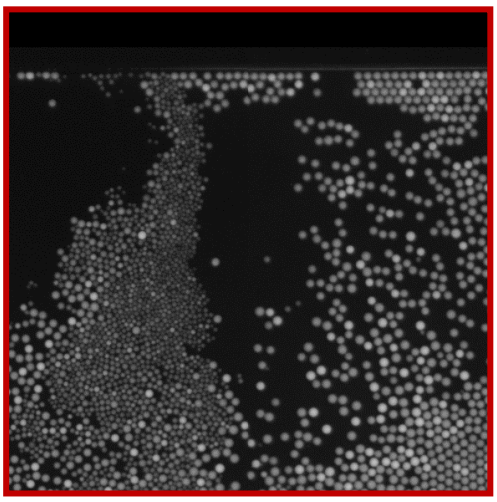
Low number of droplets	**The sample was ejected from a tip that was introduced too deep (and could touch the bottom of the well) or insufficiently**	**When pipetting your sample, stabilize the pipet tip about 1mm above the bottom of the well just by lifting it slightly from the bottom of the well (i.e. neither in contact with the bottom of the**

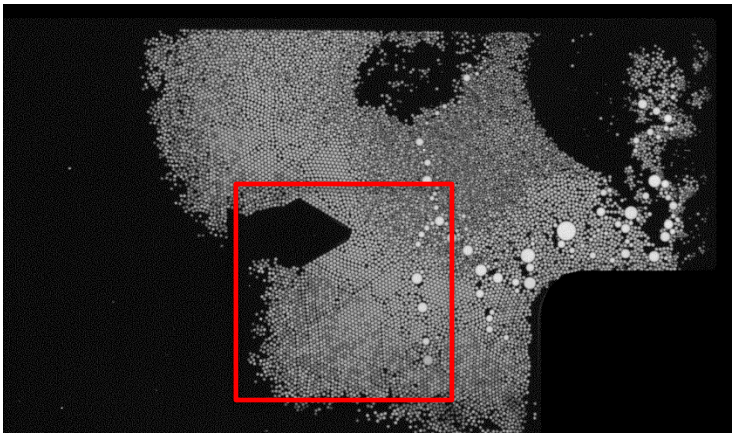
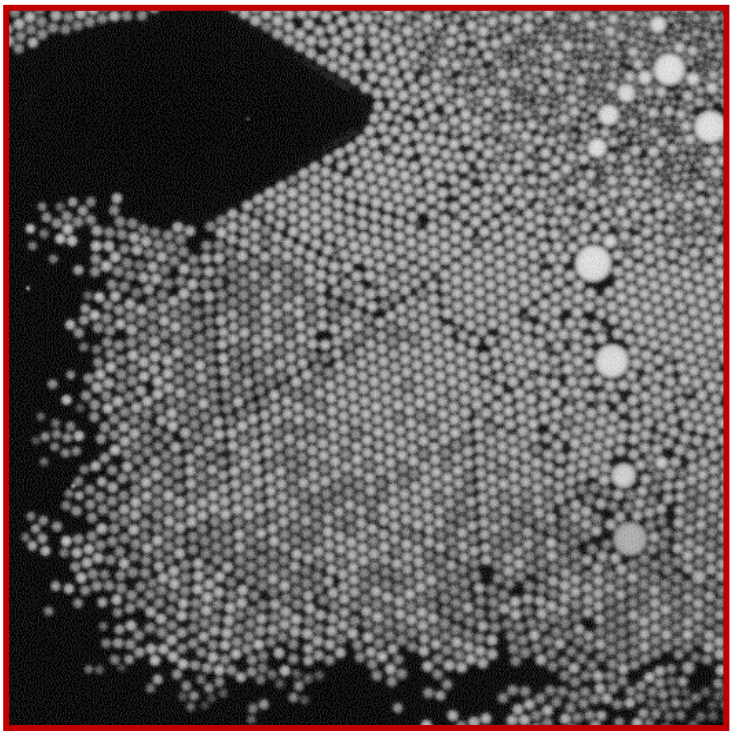
	**deep (i.e. it could have been at the air-oil interface).** **When pipetting with a multichannel pipet, the 8 tips were not perfectly horizontal, hence some of them were either above the oil or touching the bottom of the well.**	**well, nor out with the oil) before ejecting your sample from the tip** **For more information about how to properly pipet your sample in the Ruby Chip, please refer to the IFU for Ruby Chip.**
Tension on transparent foil leading to oil traces 	Incorrect pipette tips have been used to pierce the Ruby Chip Not all the 16 wells were pierced before launching the PCR program. The heating plate was not clean, and a dust particle generated tension on the transparent foil.	Only use 10µL and 20µL universal pipette tips as specified in the IFU. Always pierce all 16 wells of each chip, even when some wells will be left empty, before launching the PCR program. The impacted chamber(s) can be rescanned if needed. If there is oil leaking, wipe the chip with the antistatic wipe and then the Kimwipe, JUST ONCE, as before loading the chip and rescan the impacted chamber(s).

Oil Traces	**The chip contained oil that could condense outside of the wells in normal conditions of use.**	**Oil will evaporate, there is no need to clean inside the instrument. Stilla recommends a yearly preventive maintenance by a Stilla Technologies or Stilla's distributor technical support member. The thermocyclers are cleaned during this intervention. Contact your local**

 		**Stilla Technologies representative or more information about maintenance contracts.**
Wetting Droplets 	A higher concentration of qScript XLT 1-Step RT-qPCR ToughMix may cause droplet to wet the surface of the chamber rather than staying spherical during RT-PCR experiments.	These droplets are excluded during the analysis. Use the recommended XLT mix concentration (1 x). Avoid leaving the RT-PCR mix too long in the open air (e.g. in a microplate) before injection into the chip.

Bigger Droplets  	**The cycling of temperature and pressure could cause, in some rare cases, the presence of these artifacts.**	**This population of droplets is excluded during the analysis. The software recognizes the different sizes of the droplets and considers them as artifacts.** **This phenomenon does not normally affect the output of the assay.**

Small Droplets  	**The cycling of temperature and pressure could cause, in some rare cases, the presence of these artifacts.**	**This population of droplets is excluded during the analysis. The software recognizes the different sizes of the droplets and considers them as artifacts.** **This phenomenon does not normally affect the output of the assay.**

Non homogenous distribution of positives  	**A part of the sample was injected in the chamber after the PCR cycles had begun. Hence, they were amplified in bulk before being partitioned and they present a higher ratio of positive droplets.** **The possible reasons for this late injection are:** **The sample was ejected from a tip that was introduced too deep (and could touch the bottom of the well) or insufficiently deep (i.e. it could have been at the air-oil interface).** **When pipetting with a multichannel pipet, the 8 tips were not perfectly horizontal, hence some of them were either above the oil or touching the bottom of the well.**	**When pipetting your sample, stabilize the pipet tip about 1mm above the bottom of the well, by just lifting it slightly from the bottom of the well (i.e. neither in contact with the bottom of the well, nor outwith the oil) before ejecting your sample from the tip** **For more information about how to properly pipet your sample in the Ruby Chip, please refer to the IFU for Ruby Chip.**

11.3 Instrument troubleshooting

The error messages of the instrument will be displayed on the top right corner of the Nio® Reader software.

As long as the instrument works without error, the GUI will only display on the top right corner the “Home” and “Shutdown/Restart” buttons.

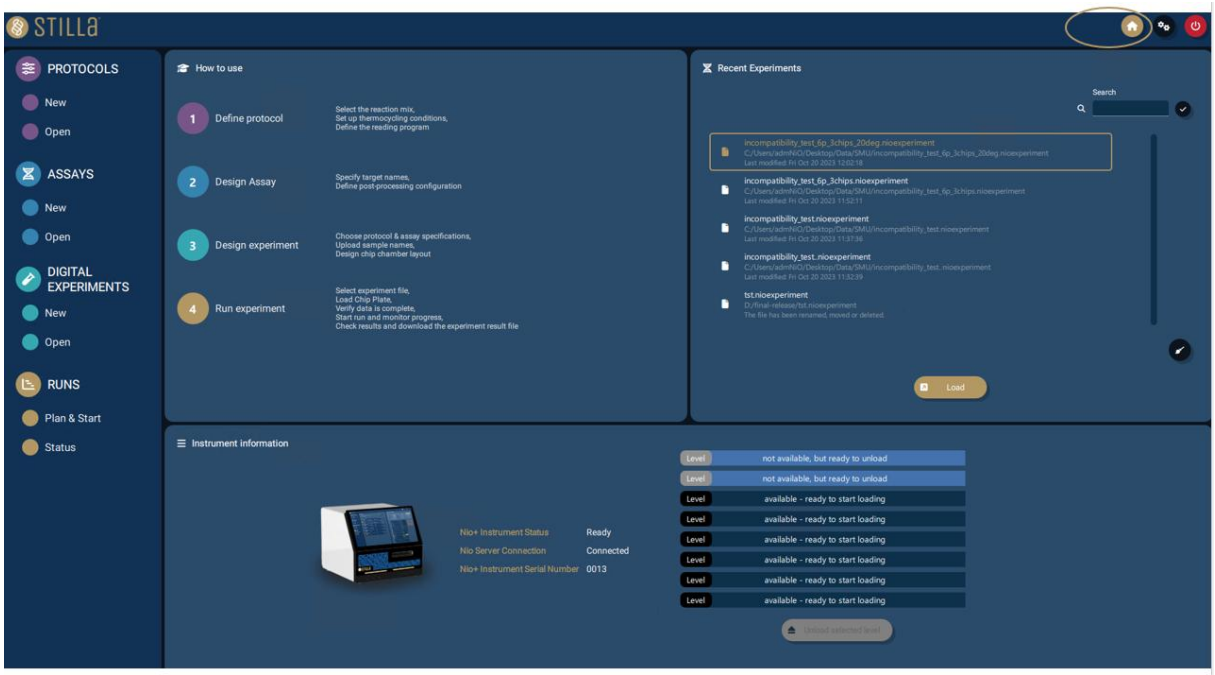


Figure 45: Nio® Reader software normal status

All error messages will be displayed in this same area with the apparition of an error icon.

Figure 46: Nio® Reader software error message icon

To have more information on an error, click on the Error icon.

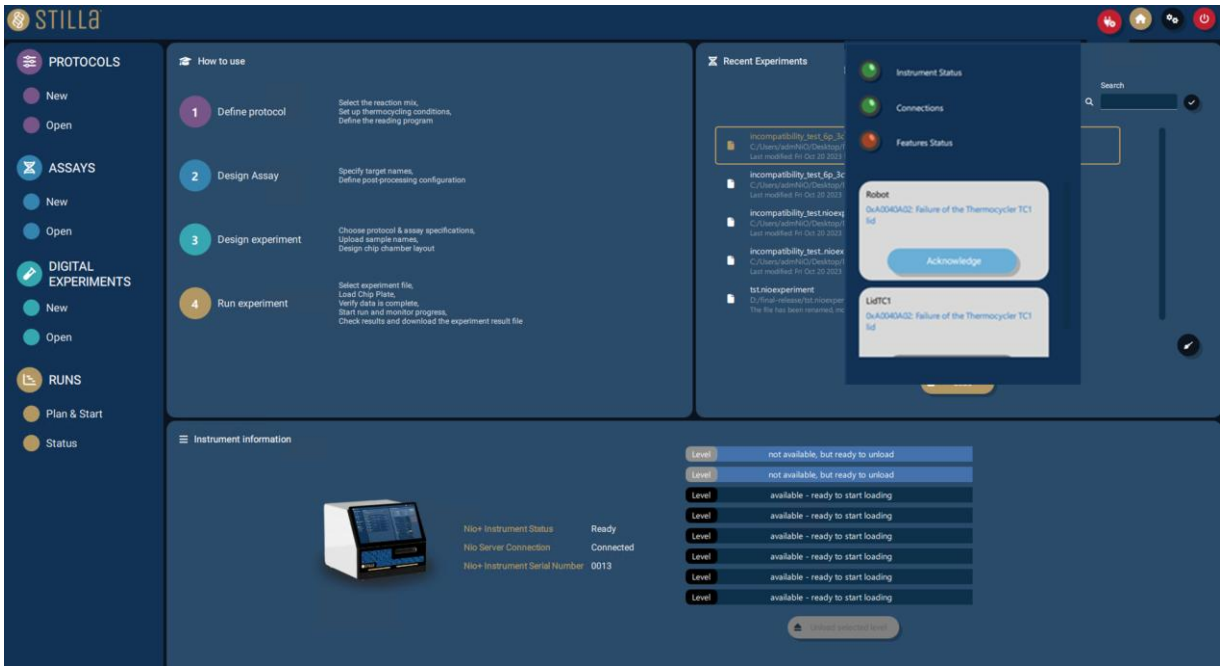


Figure 47: Nio® Reader software errors display

Most errors will be acknowledgeable by clicking on the Acknowledge button. If doing so, the instrument will try to solve the error itself. Please wait up to 2 minutes to allow the instrument to solve the error.

If the problem persists, contact Technical Support.

The following table lists the recommendations for the errors that require a specific user behavior:

Error code	Description	Recommendations
0xA0000502 **0xA0050A01** **0xA0050A02** **0xA0050A04** **0xA0060A01** **0xA0060A02** **0xA0060A04** **0xA0070A01** **0xA0070A02** **0xA0070A04**	Loading Module positioning error	In case of power breakdown, the instrument can be in a state where it needs a user intervention to be restarted. In this case, or unexpected stop of the complete instrument, execute the procedures described in the following chapters : 10.3.1. Door opening 10.3.2. Loading module unblocking 10.3.3. Closing the door In other cases, try to acknowledge and contact Technical support if the error persists.
0xA0000503	Unexpected object insertion in Loading area	An object other than a chip plate has been introduced in the Loading area, remove it in order to suppress the error
0xA0000504	Insertion of 2 chip plates at the same time	Remove the second chip plate from the Loading area

Error code	Description	Recommendations
0xA0000913	Missing chip barcode during the unloading of a chip plate	Wait that the chip plate is fully ejected before pulling on it to retrieve it. Wait that the loading area status line stops blinking before trying to retrieve the chip plate. Check the presence of the chip in the unloaded chip plate. If the chip is missing, contact Technical support.
0xA0000914	Software failure resulting in inversion of chips results	Unload all chips inside the instrument and invalidate all results
0xA00A0301 **0xA00A0302**	Robotic error during lid opening	Execute the procedure described the chapter 10.3.1. Door opening And close the Thermocyclers lids.

11.3.1 Door opening

Requirements: Hex Allen wrench 3mm

Always wear glasses, masks, gloves, and laboratory coat when accessing inside the Nio® Digital PCR instrument.

Procedure:

- If necessary, power on the Nio® Digital PCR instrument using the power switch at the rear of the instrument.
- If necessary, start up the Nio® Digital PCR using the start-up button on the Nio® Digital PCR front door.
- When the starting up and initialization of the instrument is complete (status lines blinking stop), request a shutdown of the instrument and select “Open service door after shutdown” before confirming Shutdown.

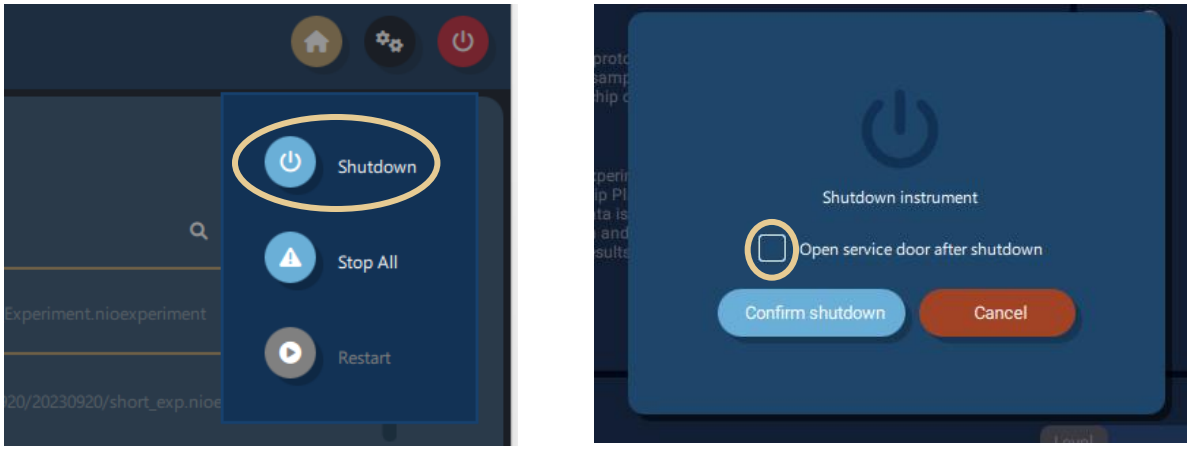


Figure 48: Shutdown Nio® Reader software with door opening

- Wait for the safe shutdown (status lines and turned off) and the door opening by the instrument (unlocking of the magnetic lock).

- Shut down the Nio® Reader software.
- Shut down the instrument through the windows shutdown.
- Power off the instrument using the power switch at the rear of the instrument and unplug the instrument from the mains.
- Unscrew the safety screw with the 3mm hex Allen wrench behind the screen panel at the top of the door, while holding the front door with one hand.

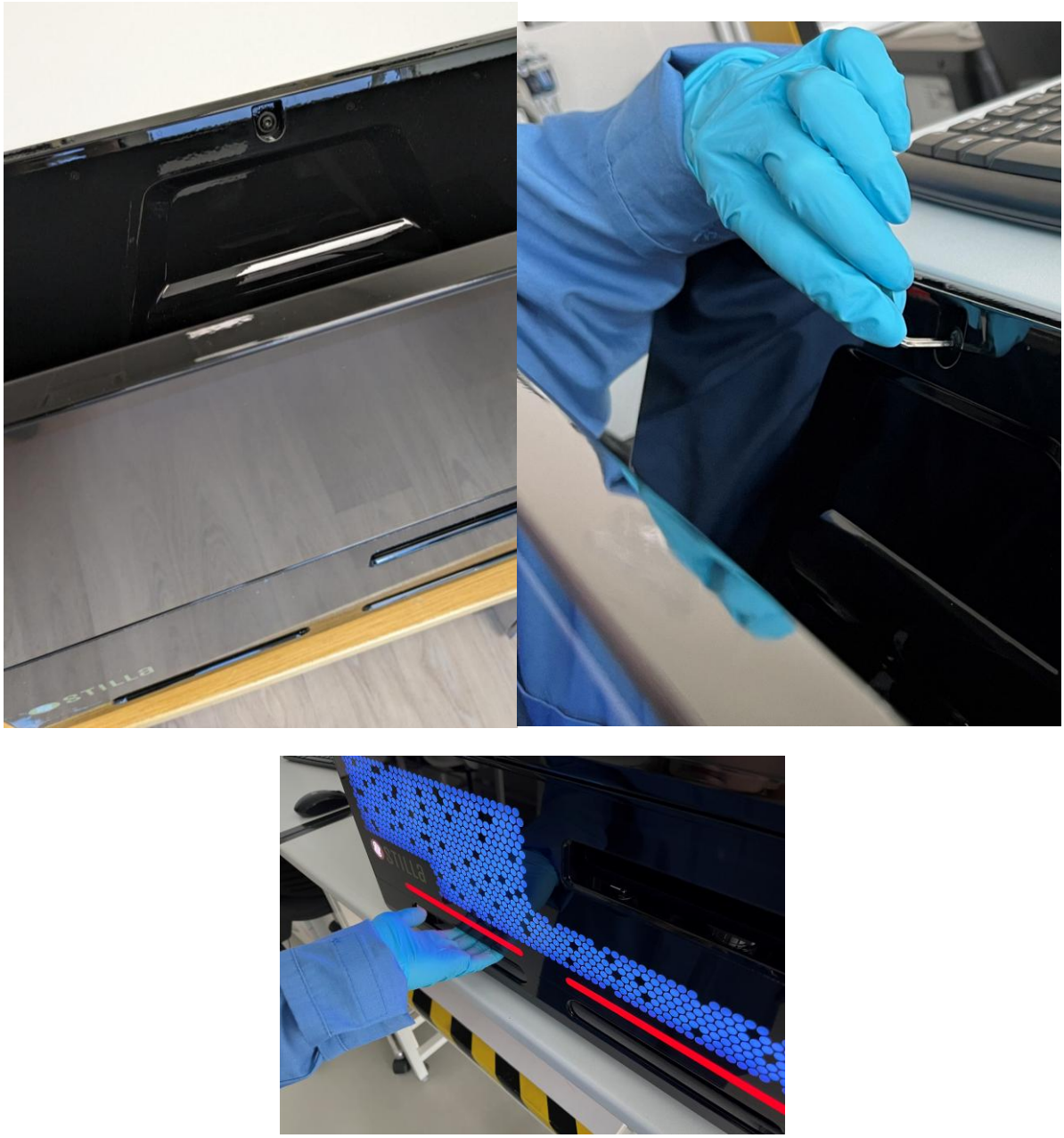


Figure 49: Opening the door. On top left, location of door lock screw. On the top right, illustration of lock screw unscrewing. On bottom, holding of the door while unscrewing.

- Open the door by placing the door in this position. A mechanical stop will stabilize the opened position.

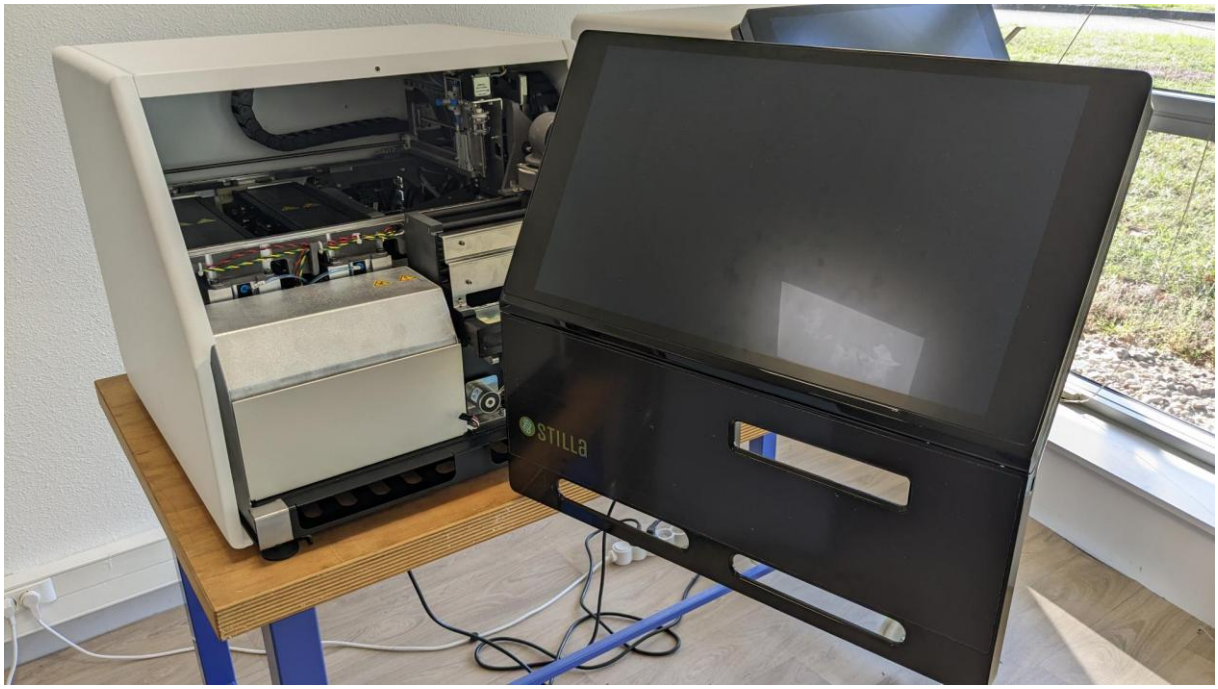


Figure 50: Nio® Digital PCR with door open

11.3.2 Loading module unblocking

Procedure:

1. In case a chip plate is locked in the fork, manipulate gently the lift up and down and the fork back and forth to remove the chip plate from the fork. There will be a little bit of resistance for each element, try to move it a bit if it resists too much, please contact Stilla Technologies' support.

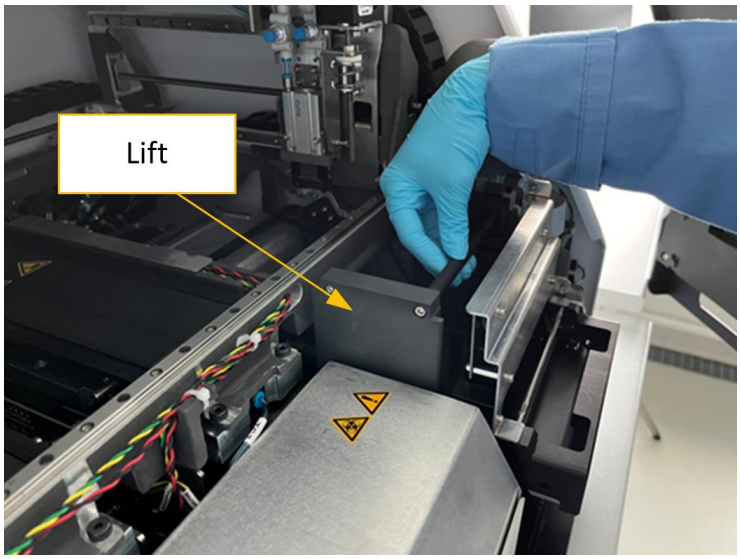


Figure 51: Nio® Digital PCR Lift

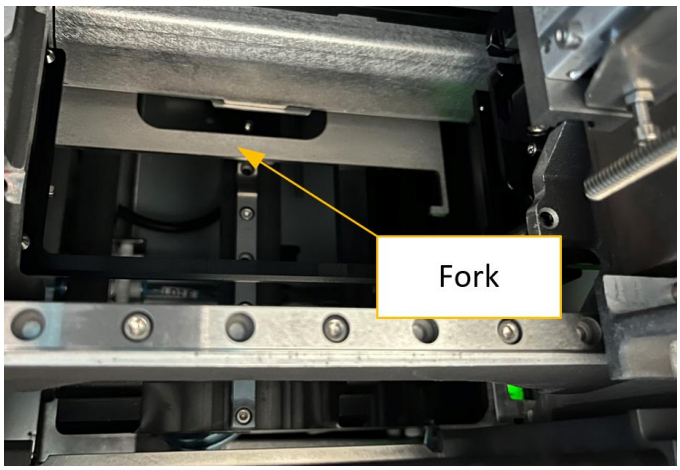


Figure 52: Nio® Digital PCR Fork

2. Position the fork at its initialization position by pushing it gently in its back position and it blocks completely.

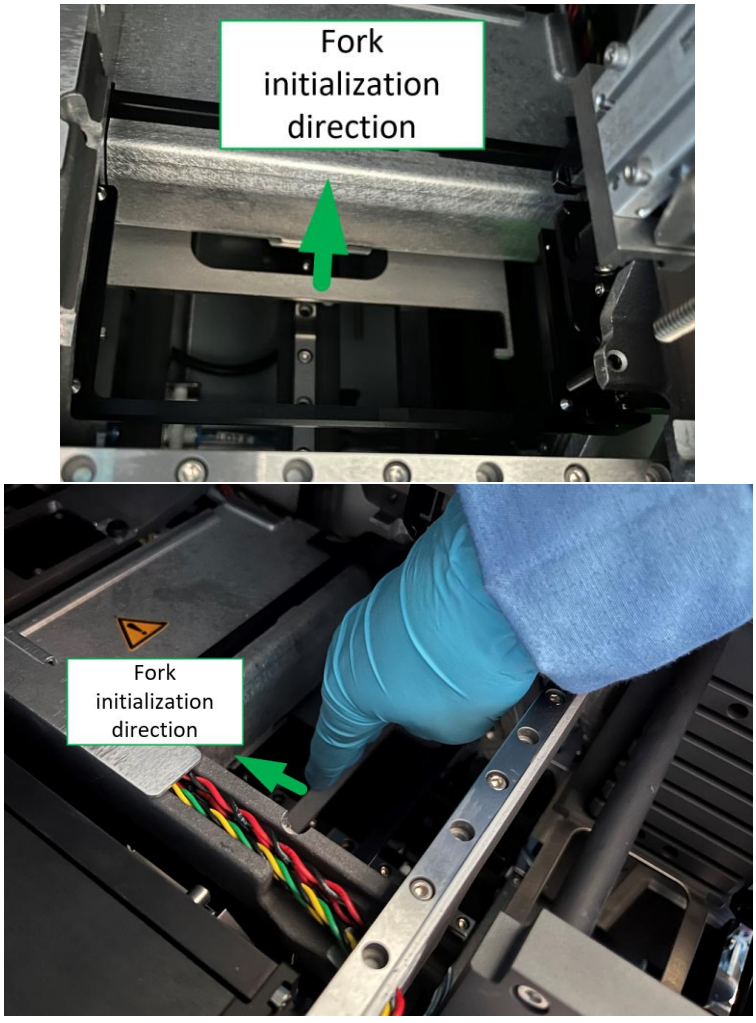


Figure 53: Nio® Digital PCR Fork initialization direction. On the top, view from the above. On the bottom, view from the side.

3. Position the basket at its initialization position by pulling it gently in its top position.

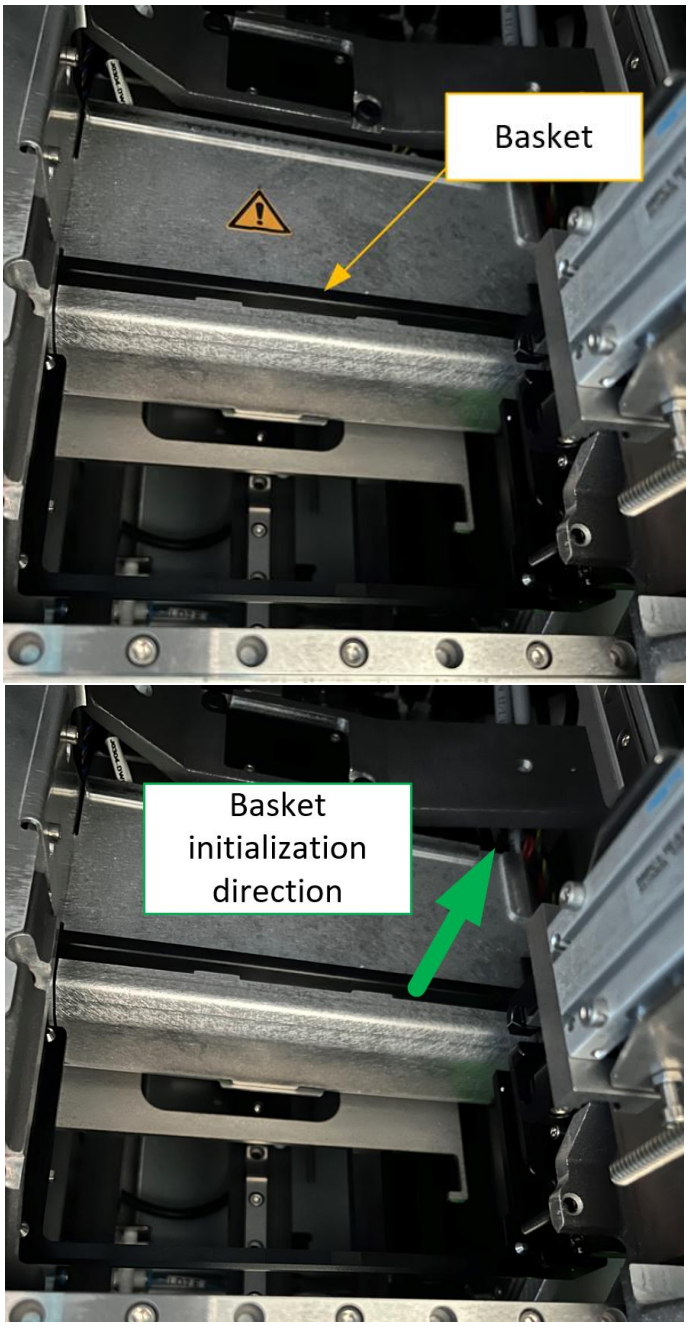


Figure 54: Nio® Digital PCR Basket. On the top, Basket view. On the bottom, Basket initialization direction.

4. Follow the procedure 10.2.3 Closing the door before doing the restart of the instrument. Ask the unloading of all chip plates from the instrument.

11.3.3 Closing the door

- Push back the door until the magnetic lock secures the closing position by lifting slightly the door. Grab the door by the two openings at the bottom to close it more easily.

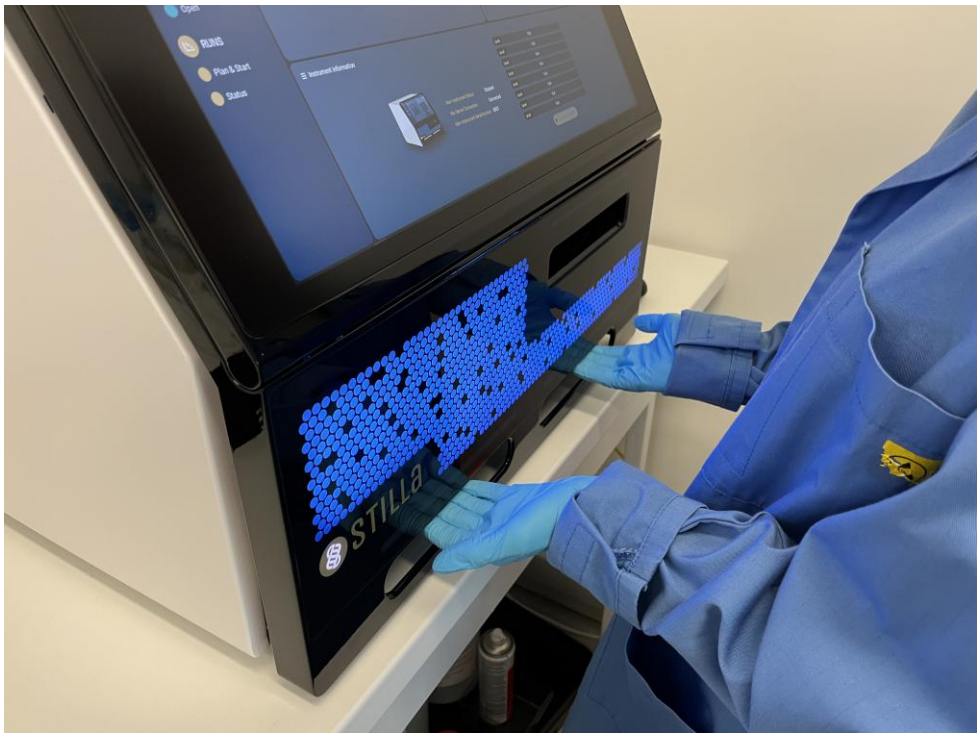


Figure 55: Closing the door.

- Place and fasten the security screw at the top of the door behind the screen cover.
- Fasten the screw until being in contact with the door and keep turning by a quarter of turn to secure the fastening.

11.3.4 Temperature warnings and errors

The instrument shall be placed in a controlled temperature environment to properly work.

The instrument internal temperature is monitored, and warnings and errors will be displayed if it exceeds defined values.

At first, a warning will be displayed to inform of the increase of the internal temperature. This warning will not have any impact on the instrument work but shall be interpreted as an increase of the ambient temperature of the room that will need to be decreased.

Figure 56: Nio® Reader software temperature warning.

The second level will be a temperature error that will forbid all new launches of experiments. This error can't be acknowledged and will disappear when the internal temperature decreases. The Nio® Reader software will offer the possibility to stop all the chips processing.

Figure 57: Nio® Reader software temperature error.

11.3.5 Instrument not ready

If a problem appears during instrument initialization, a red “Instrument Status” will be displayed.

Figure 58: Instrument status error.

Perform a restart of Nio® Reader software to attempt a new initialization. In case the problem persists, contact Stilla Technologies Support.

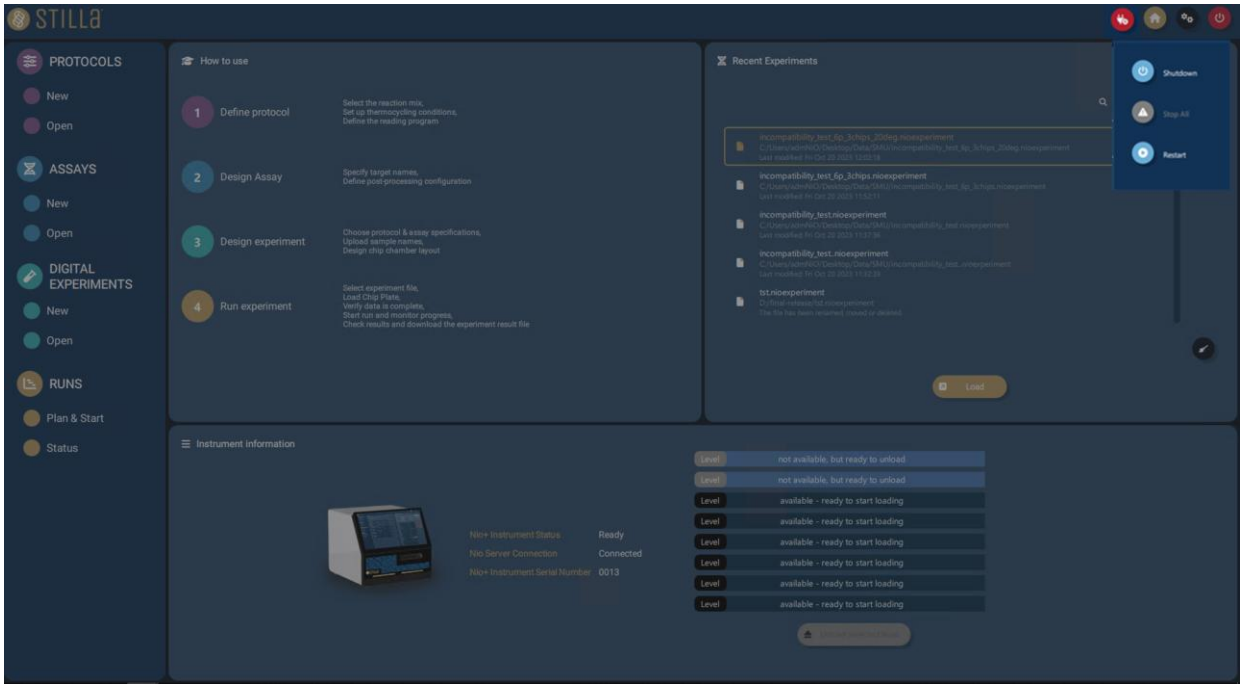


Figure 59: Nio® Reader software restart.

11.3.6 Automatic and repeated restarts of the instrument

In case the instrument restarts automatically after the shutdown of the Windows session, try to shut down again Nio® Reader software, then the Windows session. If the instrument restarts automatically again, wait for the Windows session login page to appear, then turn off the power switch at the back of the instrument. Contact Stilla Technologies Support if the phenomenon occurs in a regular way.

11.3.7 Error while launching an experiment file from a Local Area Network

When attempting to create a run from an experiment file located on a Local Area Network requiring credentials different that the current Nio® Digital PCR Windows session, an error might occur. This pop up will be displayed on the screen:

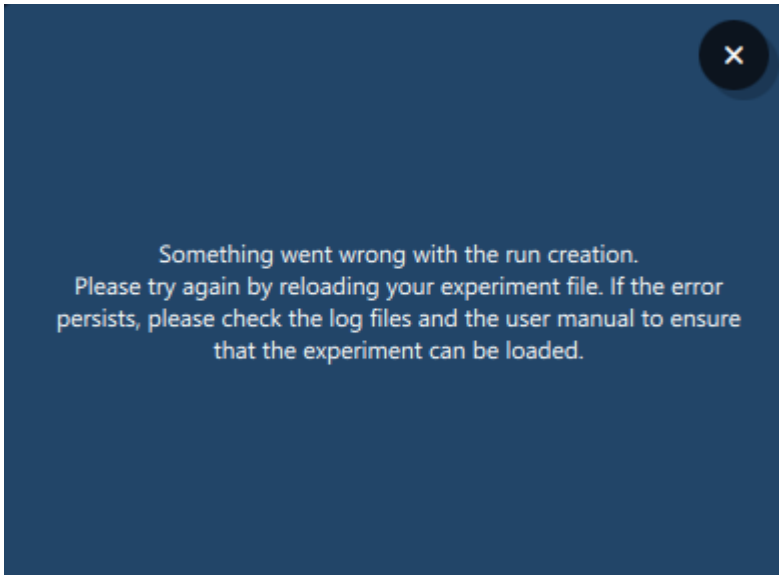
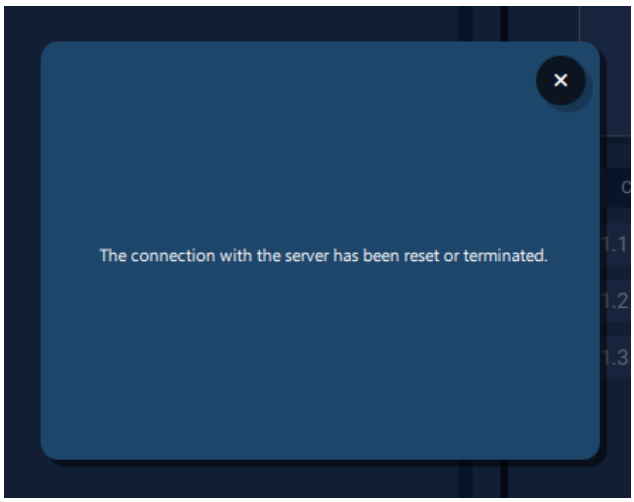


Figure 60: Nio® Reader software experiment launch error when attempting to run from Local Area Network with different credentials.

In this case, create a copy of the experiment file in a local folder on the Nio® Digital PCR, then plan a run from this file instead from the one on the Local Area Network.

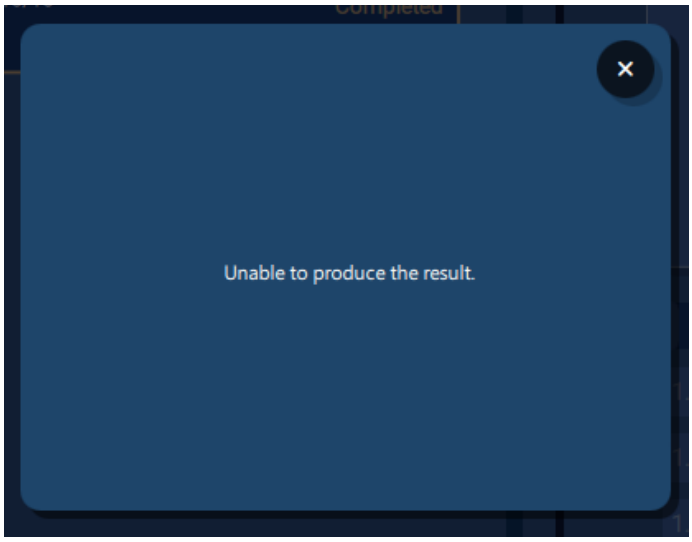
In case of regular use of file on a Local Area Network, contact Stilla Technologies Support.

11.3.8 Error when getting the run result from a remote computer.



The download of a run from a distant computer can fail if the network connection is unstable or if the network connection is too slow. In this case, download the run results from the instrument.

11.3.9 Error when getting the run result locally on the instrument



When getting the Run results if this error message appears it is recommended to :

- Try a second time to get the results
- Check the disk storage to ensure that the disk is not saturated.

12 Cleaning, preventive maintenance and disposal

In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

12.1 Cleaning and decontamination of the Nio® Digital PCR

The regular standard instrument cleaning routines only concerns the front door and housing (including touch screen).

The cleaning of the Nio® Digital PCR shall only be performed with the instrument powered off and completely unplugged.

Start by requesting a safe shutdown. When the Nio® Digital PCR is turned off, power it off by using the power switch at the rear of the instrument and unplug it.

Once the Nio® Digital PCR is powered off, the cleaning operations can be performed.

Material necessary for the cleaning / decontamination procedure:

- Gloves
- Glasses
- Mask
- Laboratory coat

Use a lint free tissue soaked with one of the following cleaning agents listed below and air-dried for the external surface of the Nio® Digital PCR. In case of an accidental spill of liquid on the instrument, absorb the liquid with and then clean the instrument, using one of the following cleaning agents and air-dry.

- 10% diluted Bleach
- Diluted Sodium Hydroxide (e.g., RNAase away)
- Lab grade Water
- 70% Lab grade Ethanol
- Isopropanol.

Cleaning/decontaminating the Front door and housing:

Moisten a lint free tissue with the disinfectant/decontaminant cleaner specified above and pass it through the external surfaces of the instrument, paying attention to avoid the power connector.

Warnings:

- Never try to clean other parts of the instrument, specifically internal parts including the optical module, to avoid altering the instrument functioning.
- Always power off and unplug the Nio® before performing its cleaning to ensure the user protection and avoid turning it on accidentally.
- Never directly spray any cleaning agents inside the instrument.
- Do not use spray bottles to spray cleaning liquids onto surfaces of the Nio® Digital PCR.
- Follow manufacturer's safety instruction for handling cleaning agents.

12.2 Nio® Digital PCR preventive maintenance

The Nio® E / Nio® / Nio®+ preventive maintenance includes air filter replacement and internal cleaning, to be performed by a Bio-Rad representative. The recommended preventive maintenance frequency is once a year. Contact local Bio-Rad representative to schedule the preventive maintenance.

The maintenance of the Nio® shall only be performed by a field service engineer trained by Bio-Rad Laboratories. Only a trained and habilitated user can access inside the Nio® Digital PCR instrument.

12.3 Disposal

The disposal of the Nio® Digital PCR at the end of the product's life should comply with the current legislation, in force in the country of use, regarding electrical and electronic waste.

The Nio® Digital PCR must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

13 Maintenance and Technical Support

In the following, the instrument is referred to as Nio® Digital PCR and this applies whatever the instrument's configuration (Nio® E, Nio® or Nio®+) unless otherwise specifically stated.

Maintenance operations of the Nio® Digital PCR should be executed by a Bio-Rad Field Safety Engineer during a visit on-site or by the return of the device to Bio-Rad Laboratory premises. Bio-Rad Laboratory cannot be held responsible for any intervention or modification done by the user on devices of the Nio® Digital PCR.

For technical questions or any issue regarding instrument or software contact us:

Region	Country	Email	Phone
AMER	USA	support@bio-rad.com	support@bio-rad.com
AMER	Canada	support@bio-rad.com	1-800-361-1808
APAC	Japan	life_ps_jp@bio-rad.com	03-6404-0331
APAC	India	cts_india@bio-rad.com	1800-103-1224, 1800-180-1224, +91 9873177477
APAC	Singapore	ctssingapore@bio-rad.com	6424 0262
APAC	Australia	techsupport.anzlsg@bio-rad.com	1-800-224-345
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China	China	callcenter_china@bio-rad.com	86-400-820-3630
EMEA	Germany	cts-ce@bio-rad.com	00 800 00 24 67 23
EMEA	Austria	cts-ce@bio-rad.com	00 800 00 24 67 23
EMEA	Switzerland	cts-ce@bio-rad.com	00 800 00 24 67 23
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EMEA	Finland	techsupport.nordic@bio-rad.com	00 800 00 24 67 23
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EMEA	Belgium	cts.benelux@bio-rad.com	00 800 00 24 67 23
EMEA	Netherlands	cts.benelux@bio-rad.com	00 800 00 24 67 23

14 Software license Information

Information regarding license is provided in Stilla Technologies Terms and conditions of Sale.

Nio® Reader software license

For Research Use Only.

Not for use in diagnostic procedures.

15 Warranty

Information regarding license is provided in Stilla Technologies Terms and conditions of Sale.

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