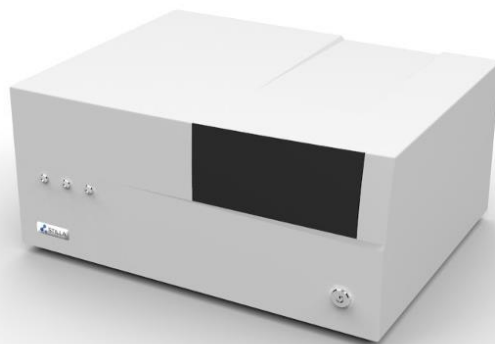




**Prism3
&
Crystal Reader software
for the
3-color naica® system**



**User Manual
Prism3 H22000
Crystal Reader software v4.0
Sapphire Chip & Ruby Chip**

TECHNICAL SUPPORT

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Closed on French bank holidays.

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Online Technical Support is also available at: www.stillatechnologies.com/technical-support/

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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 Prism3 instrument and the Crystal Reader software for Crystal Digital PCR™. The Prism3 workflow, using the Crystal Reader software and the different hardware components, is 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 the 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.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/>

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. The sample is first flowed through a network of microchannels and partitioned into a large 2D array of 12,900 to 28,500 individual droplets (Sapphire Chip)¹ and 10,000 to 17,000 droplets (Ruby Chip)², also called a Droplet Crystal. PCR is then performed within the chips and the Droplet Crystal is imaged to reveal the droplets that contain amplified targets. The last step consists of counting the number of these 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 data you can trust.

¹ 12,900 to 25,800 for naica® multiplex PCR MIX and naica® PCR MIX
15,000 to 30,000 for qScript™ XLT One-Step RT-qPCR ToughMix®

² naica® multiplex PCR MIX and naica® PCR MIX & qScript™ XLT One-Step RT-qPCR ToughMix®




SAMPLE LOADING	GENERATING DROPLET CRYSTAL AND PCR	READING AND ANALYSIS
		
<p>Prepare the sample for the reaction mix. Stilla Technologies recommends the use of the naica® PCR MIX reagents, specifically developed for Crystal Digital PCR™. Load the reaction mix into the wells of the selected chip, and seal with the provided caps.</p>	<p>Place the prepared chips into the Geode. Launch the combined partitioning and amplification program; Droplets are generated by partitioning of each sample and PCR amplification is performed immediately after Droplet Crystal generation.</p>	<p>After PCR, transfer the chips to the Prism3 instrument. Set up the read-out using Crystal Reader software for data acquisition of Droplet Crystal using up to 3 fluorescent channels (Blue, Green, and Red). Image analysis and data extraction are performed using the Crystal Miner software.</p>

Figure 1: Overview of Crystal Digital PCR™ Workflow.

1.3. Intended use of the naica® system

The naica® system for Crystal Digital PCR™ is composed of two instruments: the Geode, which performs droplet generation and amplification, and the Prism3, which enables imaging of the Droplet Crystals. The Crystal Reader software is used to control and set up Crystal Digital PCR™ experiments on the Prism3 instrument.

The Crystal Reader software functions as the user interface to set up the experiment for the Prism3 and it is dedicated to the image acquisition in the Crystal Digital PCR™ workflow.

The Crystal 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 Prism3 instrument for the acquisition of the fluorescence images of Sapphire Chip or Ruby Chip.
- 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 Crystal Miner software.

The Crystal Miner software is used to extract data from the images acquired using the Prism3 instrument and to calculate the absolute concentrations of the targeted nucleic acids.

Crystal Reader and Crystal Miner software is pre-installed on the naica® PC delivered with the naica® system.

The naica® system performs Crystal Digital PCR™ within microfluidic chips (Sapphire Chip & Ruby Chip).

The naica® PCR MIX reagents are recommended to achieve optimal Crystal Digital PCR™ performance on the naica® system.

For detailed instructions for the Geode and the Crystal Miner software, please refer to the respective User Manual. For detailed instructions for Sapphire Chip, Ruby Chip, naica® PCR MIX, and naica® multiplex PCR MIX refer to the respective Instruction for Use (IFU).

The naica® system is a laboratory instrument to be used by qualified personnel in a controlled environment. Before using the naica® system, the user must be trained by a Stilla Technologies representative.

In general, Crystal Digital PCR™ can be performed with all types of DNA sample on the naica® system. However, individual sample-type compatibility for digital PCR applications may require a dedicated assay validation by the end-user. Please note that the extraction method used and sample purity can also influence sample compatibility for digital PCR applications.

The Prism3 instrument and Crystal Reader software is intended for use by professional users trained in molecular biological techniques and in the operation of the Prism3 instrument and Crystal Reader software.

The Prism3 instrument and Crystal Reader software is part of the naica® system. The naica® system is intended for Research Use Only. Not for use in diagnostic procedures.

1.4. Citing the naica® system in scientific publications, presentations, seminars, etc.

To cite the use of the naica® system use:

Crystal Digital PCR™ (Stilla Technologies, France)

naica® system (Stilla Technologies, France)

naica® system component names:

- Geode
- Prism3
- Sapphire Chip
- Ruby Chip
- Crystal Reader software
- Crystal Miner software
- naica® PCR MIX reagents:
 - naica® PCR MIX (Stilla Technologies, France)
 - naica® multiplex PCR MIX (Stilla Technologies, France).

2. Materials and Equipment

2.1. Prism3 packaging

- Prism3 instrument (H22000):
 - 1 SAPPHIRE CHIP HOLDER H20001
 - 1 OPAL CHIP HOLDER H20002³
 - Two transportation lock screws
 - Certificate of Conformity

Note: The 2 screws for securing the Prism3 instrument for transportation should not be discarded. In case of an instrument return, the screws are essential for shipment of the instrument to not breach any warranty claims.

- Prism3 peripheral box:
 - Cable for Prism3 instrument (H22000)
 - Cable for naica® PC
 - Cable for Monitor
 - naica® PC
 - Keyboard
 - Mouse
 - Mousepad
 - External USB Barcode Scanner
 - Precision Wipes (Kimtech™ Science, 7552, 1 ply, 213x114 mm)
 - Antistatic wetted wipes (ACL Staticide®, Reference: SW12)
 - USB key

Note - Replacement Precision Wipes must be purchased using the reference - Precision Wipes (Kimtech™ Science, 7552, 1 ply, 213x114 mm) from global standard laboratory suppliers

Note - Replacement Antistatic wetted wipes (ACL Staticide®, Reference: SW12) can be ordered through Stilla Technologies using product reference 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](#)





³ The OPAL CHIP HOLDER H20002 is compatible to be used with Ruby Chip

2.2. Safety instructions for the naica® system

Before the first use of the naica® system, please read the User Manual entirely. Special notice should be taken of the following.

2.3. Prism3 instrument safety information

- The following warning labels are located on the Prism6 instrument:

SYMBOL	MEANING
	<p>Caution of dangerous voltage Please ensure that the voltage indicated on the back of the device exactly matches your local electrical supply.</p>
	<p>Caution- liquids Ensure that no liquids can enter the device. Sample must be loaded outside of the Geode.</p>
	<p>Caution- environment The ventilation of the device must not be covered.</p>
	<p>Caution- use If the device is used in a manner not described in this manual, safety may be compromised.</p>
	<p>Caution- the risk of pinching The lid of the Geode should be closed with caution before starting a run. Do not try to open the Geode lid during a run. Do not manipulate the Geode lid handle during a run. Always check that the internal pressure of the chamber is below 20 mbars before opening the lid.</p>
	<p>Protective conductor terminal</p>











General safety instructions for the Prism3 instrument:


- The Prism3 instrument must be placed on a stable surface that is clean and vibration-free.
- No objects should be placed on top of the Prism3 instrument.
- The Prism3 instrument should not be exposed to direct sunlight.

- Please ensure that the Prism3 instrument is operated as instructed in the User Manuals. User Manuals are subjected to changes. The latest version can be accessed at the Technical Resources webpage.
- Stilla Technologies cannot be held responsible for any damage or injuries arising from improper operation.
- Do not use the Prism3 instrument if any parts are broken, chipped, rusty, or if the power cables are damaged in any way.
- Do not open the Prism3 instrument lid manually while the Prism3 instrument is switched on.
- Do not open the housing of the Prism3 instrument unless explicitly instructed by a Stilla Technologies representative.
- Opening the Prism3 instrument housing may breach any warranty claims for the Prism3 instrument.
- Only operate the Prism 3 instrument with the provided and specified detachable main supply cords. Do not replace the components with non-specified cords.
- Do not attempt any repairs or alterations except as expressly described in this User Manual or as instructed by a Technical Support Representative.
- Always disconnect the instruments from their power source before cleaning or moving the Prism3 instrument.
- For the Prism3 instrument, the mains supply source must meet the national regulatory requirements.
- The power cord of the Prism3 instrument must be connected to a wall outlet with a grounded conductor.
- For the Prism3 instrument, the mains voltage must correspond to the range given in the product specification.
- Mains supply voltage fluctuations 90%.
- Keep liquids away from the Prism3 instrument; avoid percolation of liquids inside the Prism3 instrument.
- For optimal use of the Prism3 instrument, the room temperature must be between 15°C and 25°C.
- For optimal performance, ensure to operate the Prism3 instrument in a dust-free environment.
- Do not block the area in front of the Prism3 instrument and ensure that the tray has sufficient space to move while the Prism3 instrument door is opening and closing.
- A sufficient clearance area around the Prism3 instrument is required to allow access to the main power switch.
- Samples can be infectious or cause other damage to health. Safety regulations issued for the handling of sample material in the laboratory must be followed by wearing the proper Personal Protective Equipment (e.g., gloves and protective clothing). For detailed instructions, please refer to Decontamination instructions.
- For instrument cleaning and decontamination follow the provided instructions. Do not use any other cleaning agents than specified.
- For instrument shipment, follow only the detailed packaging and shipment procedures provided by Stilla Technologies. Only package and ship the instrument when instructed by a Stilla Technologies representative.
- Regular instrument service maintenance is recommended to ensure optimal system performance at all times. Please contact Stilla Technologies for the respective service offers.

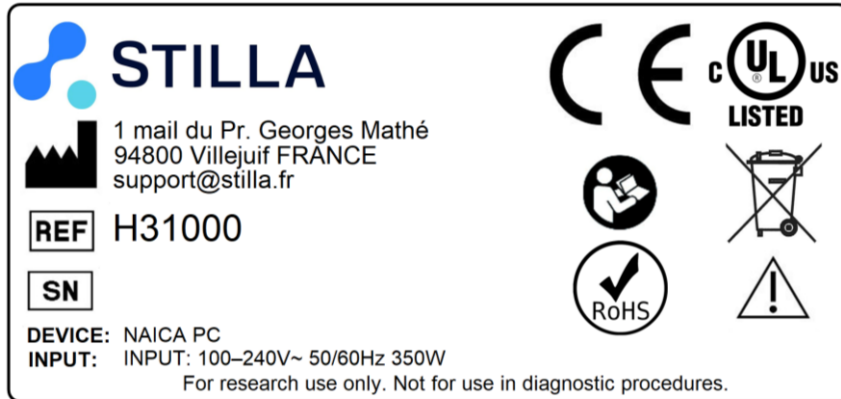
2.4. Prism3 instrument labeling

Labeling symbols present on the Prism3 instrument

SYMBOL	MEANING
	Manufacturer.
	Product reference (part number).
	Product serial number.
	Read the User Manual before using the product.
	Caution: documentation must be consulted in all cases where this symbol is marked. Using the product without applying the instructions explained in the User Manual may result in personal injury or damage the equipment and facilities.
	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).
 	The product should be disposed of in an appropriate recovery and recycling structure.
	CE marking (manufacturer's declaration that the product meets the essential requirements of the applicable EC directives).

	Indicates that the product was tested and has met the certification requirements for electrical products (CSA Group certification mark).
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Label of the naica® PC



2.5. Prism3 declaration of conformity

To view the declaration of conformity certificate, please visit the Technical Resources webpage <https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/>

2.6. Prism3 instrument specifications

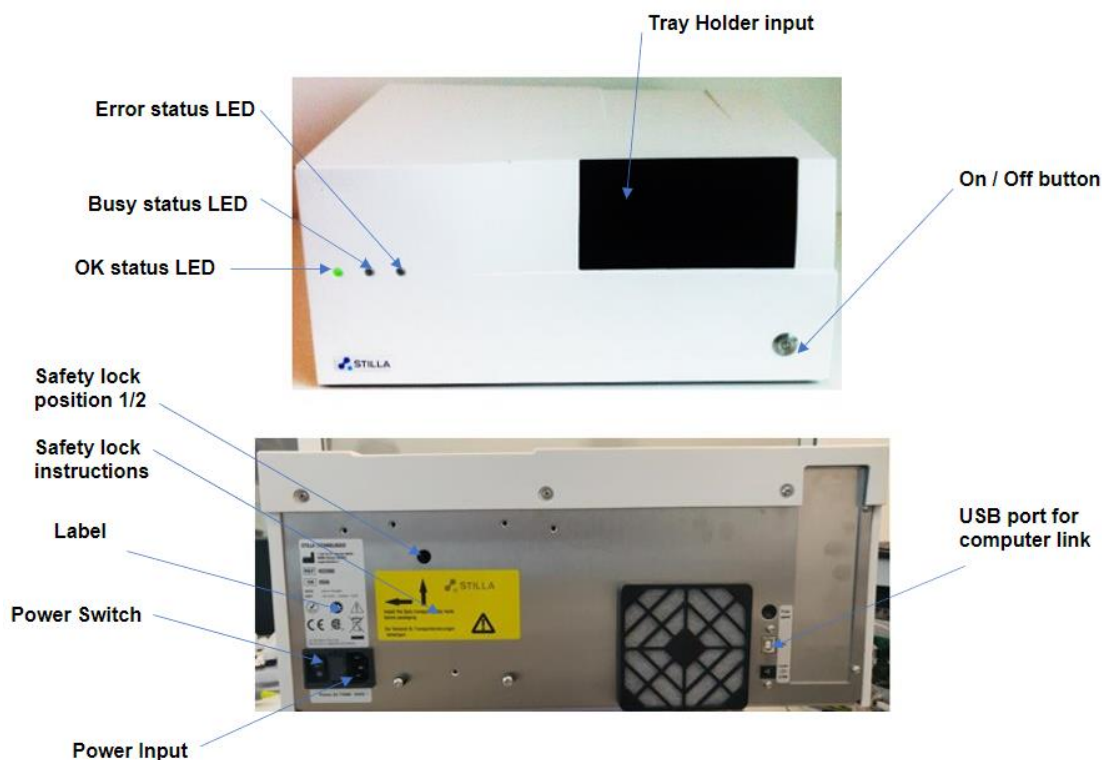


Figure 2: Front and back view of the Prism3 instrument.

GENERAL CHARACTERISTICS	
Capacity	Up to 3 chips /run Sapphire Chip: up to 12 samples / run Ruby Chip: up to 48 samples / run
Scan time	~ 50s/sample for Sapphire Chip; ~ 25s/sample for Ruby Chip
Connection	USB port and USB cable for connection to a separate PC
Dimensions (W x D x H)	44 x 34 x 21 cm
Weight	15 kg
Power supply	AC input: 100-240V~, 50/60Hz / 150 W
IMAGING SYSTEM	
Sample illumination	High power LEDs (blue, green, red)
Excitation wavelengths (single-band)	415 - 480 nm (blue) // 530 - 550 nm (green) // 630 - 640 nm (red)
Detection wavelengths (triple band)	495 - 515 nm // 560 - 610 nm // 655 - 720 nm
Validated fluorophores	FAM, fluorescein// Cy®3, HEX // Cy®5
Image resolution	6.7 µm/pixel
Field of view	6.5 x 6.5 mm
Depth of field	150 µm
Camera pixel resolution	1.3 MPixel

Pixel Capacity	5000 e- (Single-pixel), 20K e- (2x2 pixel-binning)
Result data format	16bit TIFF grayscale, CSV spreadsheet, XML files
AMBIENT CONDITION	
Pollution degree	2
OPERATION	
Environmental conditions	Indoor use only
Temperature	+15°C to +25°C
Relative humidity	10% to 75% non-condensing
Altitude	Operating at max. 1000m above sea level
Barometric pressure	795 hPa to 1060 hPa
STORAGE	
Temperature	+5°C to +40°C
Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa
Waiting time until operation	8h
SHIPPING	
Temperature	-40°C to +60°C
Relative humidity	10% to 95% non-condensing
Barometric pressure	700 hPa to 1060 hPa
Waiting time until operation	24h
FAN FILTER	
Replacement fan filter	Fandis Part No.: F92/MR
ELECTRICAL	
AC input	100-240V~, 50/60Hz
Installation category	II
Input mains fluctuation	Max. 10%
Input power	Max. 150W
Fuse type and rating	Two fuses, 5x20mm, T4AH/250V~ (4A slow-blow)

2.7. Prism3 installation instructions

2.7.1. Operating requirements

Proper infrastructure requirements for the Prism3 installation:

- A clean laboratory environment
- A sturdy surface, for supporting from 20 to 40 kg
- A minimum distance of 10 cm from neighboring objects around the Prism3 instrument
- Room temperature between 15°C and 25°C

2.7.2. Installation and performance validation

The initial naica® system installation, including the Prism3 instrument, must always be executed by a Stilla Technologies representative. The naica® system installation and operational qualification according to Stilla Technologies specifications will be documented with a certificate. The certification is required to release the naica® system for customer operation.

3. Operating the Prism3 instrument

3.1. General considerations and prerequisites for using Crystal Reader software

The Crystal Reader software is pre-installed on the naica® PC for Prism3 instrument operation. Future software version updates for Crystal Reader software are possible. Stilla Technologies will communicate about any required and available software update.

To download the latest version of the Crystal Reader software, visit the Technical Resources webpage: <https://www.stillatechnologies.com/digital-pcr/naica-system-support/technical-resources/>

Note: 2 GB of storage is required for software installation.

Note: Only install the required Windows 10 Operating System security updates for the naica® system. DO NOT update full Windows 10 Operating System updates as they may have a negative impact on Crystal Reader & Crystal Miner software robustness and stability. Contact Technical Support with any questions.

The officially supported scanning templates for the Crystal Reader software are located on the Prism3 instrument by default at, "C:\Program Files\Stilla\CrystalReader\templates".

Stilla Technologies does not recommend modifying or deleting any of the default scanning templates provided. Instructions to create a new scanning template from the default scanning templates provided are detailed below in section: Create a new experiment by selecting the scanning template file

All scanning templates are named as described below:

FileType_InstrumentName_ChipType_PCR-Mix_Name_AssayIndicator_version.ncx

3.2. Prism3 instrument initialization

The Prism3 instrument is operated through the naica® PC. The monitor, keyboard, and mouse are connected to the naica® PC. The naica® PC is connected to the back panel of the Prism3 instrument.


1. Check that the naica® PC is correctly connected to the Prism3 instrument: the grey USB cable should be plugged in the “PRISM3”-labeled ports located at the rear of the naica® PC and the Prism3 instrument.
2. Turn on the naica® PC that controls the Prism3 (default password is “admin” for “admin” user and “guest” for “Guest User” user).
3. Switch on the Prism3 instrument.
 - First, switch on the power button located at the back of the Prism3, then turn on the power button in the front. Wait for initialization (the left-most light on the front LED panel of the Prism3 should be green, Figure 2).
 - Note: Ensure that the storage rack for the chips is clean and free from dust particles and correctly placed in the tray (error-proof cut-out upper-righthand corner). Clean both chip holders occasionally with ethanol or water and a Precision Wipe.
4. Launch the Crystal Reader software by clicking on the “Crystal Reader icon”  located on the desktop. When the software is launched, the Crystal Reader software homepage is displayed.



Figure 3: Crystal Reader software homepage with disabled “OPEN TRAY” button during Prism3 initialization or operation mode.

The Crystal Reader software homepage displays the following buttons:

- LOGIN: log in to apply user preference.
- NEW EXPERIMENT: create a new experiment

- Embedded drop-down menu for chip-specific experimental templates
 - New Sapphire Chip experiment
 - New Ruby Chip experiment
- OPEN EXPERIMENT: open a previously saved experiment file to use identical experimental settings.
- OPEN TRAY/CLOSE TRAY: engagement button to open/close the Prism3 instrument flap to eject/retract the movable tray.
 - If the movable tray is fully retracted, this button displays “OPEN TRAY”.
 - If the movable tray is fully ejected, this button displays “CLOSE TRAY”.
 - If the moveable tray is in initialization mode or in operation the button is greyed out and inactive for engagements.
- ABOUT: detailed information about the Crystal Reader software.
- SETTINGS: edit user preferences, Crystal Reader software, and Prism3 instrument settings. Please refer to How to edit the Settings for different user profiles section for detailed instructions

3.3. Prism3 instrument availability

Only one instance of Crystal Reader can operate the Prism3 instrument at a time. When Crystal Reader is started, the application takes control of the instrument and locks the access for other Crystal Reader instances. To unlock the access of the instrument, the Crystal Reader instance using the instrument must be closed.

Note: Always close Crystal Reader after scanning your experiment to make the Prism3 instrument available for the next user.

If another Crystal Reader instance is running when Crystal Reader is started, the software will detect that the Prism6 instrument is unavailable. Crystal Reader will display a warning message while opening and the scan button will be disabled. Until the instrument is made available, it will only be possible to view and edit parameters to prepare the next experiment.

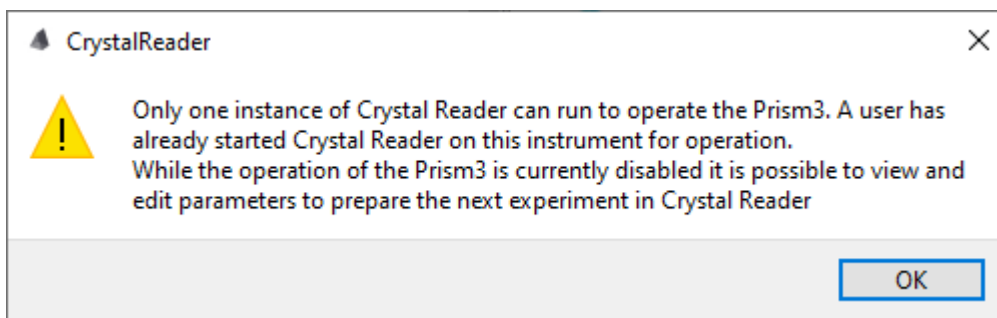


Figure 4 Warning message at Crystal Reader launch when the Prism3 instrument is unavailable

To take control of an instrument that is locked, first make sure that the Prism3 instrument is not in use by another user. The Crystal Reader software instance holding the Prism3 instrument resource can be running on the current Windows user session or in another user session. Connect to the user session running Crystal Reader and close the software. Restart Crystal Reader and check that the instrument has connected successfully.

3.4. Instructions for chip positioning

Note: Prior to chip positioning for scanning, read the respective Sapphire Chip and Ruby Chip Instructions for Use.

Eject the moveable tray for chip positioning

- Click on “OPEN TRAY” to open the tray of the Prism3 instrument.

The “OPEN TRAY” button is greyed out at the launch of the Crystal Reader software. This can happen when the connection with the Prism3 instrument is being established. After a few seconds of launching, the scanner is recognized, and all the instrument control buttons are enabled (including the “OPEN TRAY” button):

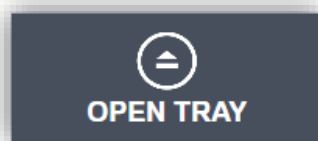


Figure 5: The “OPEN TRAY” button to open the tray of the Prism3 instrument. Once the tray is opened, this button becomes “CLOSE TRAY”.

CAUTION!

Make sure that the chip holder is clean and free from dust particles (clean it occasionally with ethanol or water) and correctly placed on the tray (error-proof cut-out upper-righthand corner for Sapphire Chip and bottom-left for Ruby Chip).

- Place the chips in the slots of the chip holder and check correct positioning (the chips should fit perfectly into the slots, with the diagonal error-proofing on the top). Up to 3 chips can be scanned at a time.
- Enter the chip barcodes in the corresponding chip ID fields (from left to right slot). If available, use a USB barcode reader to gain time and avoid typing mistakes.

CAUTION!

Make sure that chip barcodes (8 digits) are correctly entered with respect to the position of the chip on the tray.

- Click on “CLOSE TRAY” to close the tray of the Prism3 instrument.

Note: Make sure to use the respective Sapphire Chip holder or Ruby Chip holder with the specific chips. Do not mix chip types on one chip holder. Follow the markings on the chip holders to ensure the correct chip holder is used.



Figure 6: Positioning of the Sapphire Chip (left) and Ruby Chip (right) in the chip holder of the Prism3. The chip holder should be correctly oriented with error-proof cut-out upper-right hand corner for the Sapphire Chip and bottom-left for Ruby Chip (arrows).

Note - For Ruby Chip operation use the OPAL CHIP HOLDER H20002.

- Position the respective chip holder on the tray of the Prism3 instrument
 - SAPPHIRE CHIP HOLDER - H20001 for Sapphire Chip
 - OPAL CHIP HOLDER - H20002 for Ruby Chip
- Retract the movable tray from the Prism3 by pressing the “Eject” button on the instrument or the “CLOSE TRAY” button in the Crystal Reader software.

3.5. Definition of a Crystal Digital PCR™ experiment

An experiment is defined as being a set of samples processed during an individual Crystal Digital PCR™ workflow on the naica® system:

- The samples of an individual experiment were all processed on the same respective chip type (Sapphire Chip or Ruby Chip). Chip types cannot be mixed in a single experiment for image acquisition on the Prism3 instrument
- The samples of the individual experiment were prepared using the same PCR mastermix and the same PCR program for partitioning and amplification in the Geode.
- The same scanning parameters and the experimental details will be applied to all samples for an individual experiment during a Prism3 run, i.e.,
 - the same exposure times (in ms) for each of the 3 LEDs;
 - the same focus value (in mm).

Once an experiment is scanned by the Prism3, the Crystal Reader software automatically records the data in a ZIP format in a “.ncx” file (naica® crystal experiment). This generated “.ncx” file contains all the chamber images as well as all the contextual and pre-analysis data.

The indicative sizes of an individual “.ncx” file is:

Chip type (3-plex data)	.ncx file size / chamber
Sapphire Chip	57 +/- 1 MB
Ruby Chip	24 +/- 0 MB

3.6. Experimental set-up with Crystal Reader software

The 5 main steps of the experimental set up for chip scanning using Crystal Reader software are as follows:

1. Create a new experiment by selecting the scanning template file
2. Confirm or modify the experiment context
3. Select the chambers to scan and define chamber details
4. Launch the experiment scan
5. Check chamber image quality

3.7. Step 1 - Create a new experiment by selecting the scanning template file:

Each experiment starts with the selection of the scanning template (".ncx" format) for an experiment. The scanning template contains all the information and components required to set up an experiment consistently and quickly

Just load a ".ncx" as an empty experiment (Figure 7) and the user is then instructed to modify or add the data corresponding to the experiment.

Information that can be included in an ".ncx" template:

- Experiment details
- Scanning Parameters
- Embedded files:
 - Image Analysis Configuration file (".yaml"), corresponding to a set of chip type, scanner type and mix type
 - Spillover Compensation Matrix (".ncm")
 - Crystal Miner Analysis Configuration file (".nca") containing the zoning (thresholds or polygons) and populations settings
 - Crystal Miner Plot configuration file (".ncp") containing favorite views and dotplot settings.
- Chamber details

Different default scanning templates are available based on the parameters of the experiment:

- Chip type
- PCR mastermix

All scanning templates and analysis configuration files are named as described below:

FileType_InstrumentName_ChipType_PCR-Mix-Name_AssayIndicator_version.ncx

Examples:

- For an experiment using the naica® multiplex PCR MIX compatible with TaqMan® probe and the Sapphire Chip select:
ScanningTemplate_Prism3_SapphireChip_naica-multiplex-PCR-MIX_Taqman_v2.0.ncx
- For an experiment using the naica® PCR MIX compatible with EvaGreen® fluorescent intercalating dye and the Ruby Chip select
ScanningTemplate_Prism3_RubyChip_naica-PCR-MIX_Evagreen_v1.0.ncx.

Note: Stilla Technologies does not recommend modifying the provided original template files. Always use the “save as” function to ensure to keep the original template files. If a modification of any template file is required, please contact the Technical Support.

- On the Crystal Reader software homepage click on “NEW EXPERIMENT”. A pop-up window appears for scanning template selection (Figure 7).

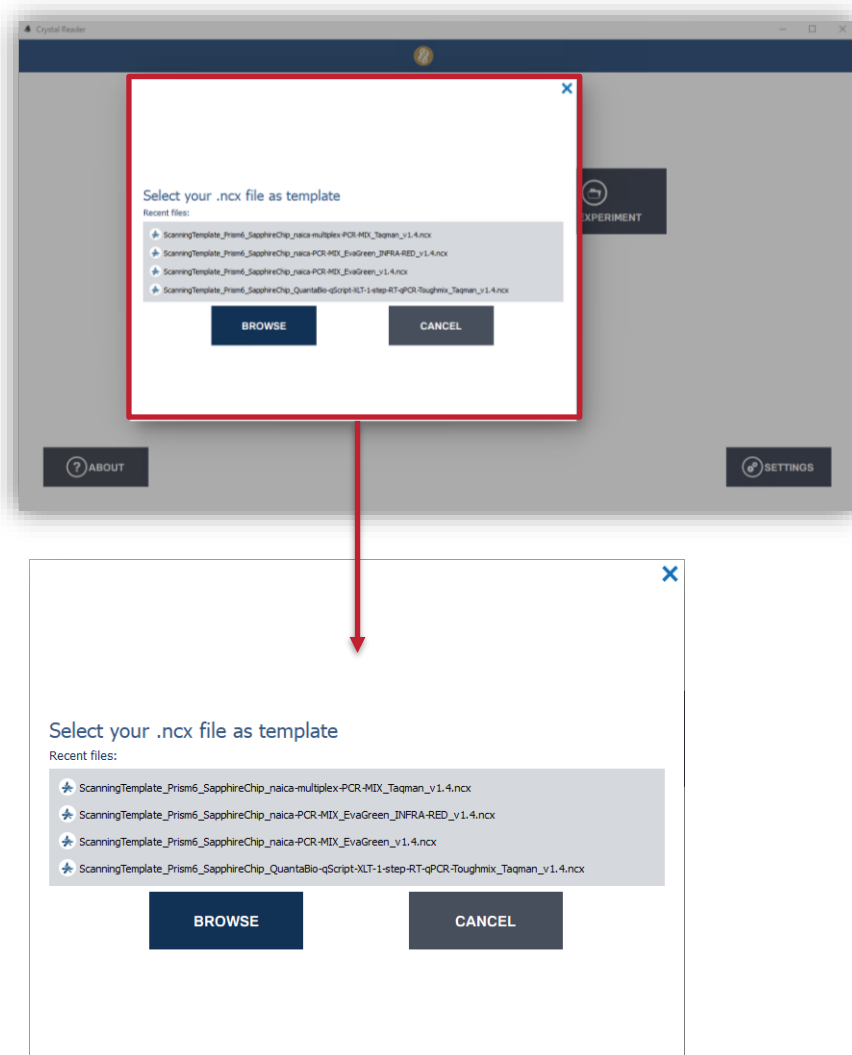


Figure 7: Crystal Reader software prompt to select the scanning template (“.ncx” file) for the experiment.

Recently used scanning templates are provided for direct selection from the displayed menu “Recent files”.

To select a different scanning template, proceed to “BROWSE” and select the respective scanning template from the set of default scanning templates provided at the location: **C:\Program Files\Stilla\CrystalReader\templates.**

Different default scanning templates are available based on the parameters of the experiment:

- Chip type
- PCR mastermix

All scanning templates and analysis configuration files are named as described below:

FileType_InstrumentName_ChipType_PCR-Mix-Name_AssayIndicator_version.ncx

Examples:

- For an experiment, using the naica® multiplex PCR MIX, compatible with TaqMan® probe and the Sapphire Chip select:
ScanningTemplate_Prism6_SapphireChip_naica-multiplex-PCR-MIX_Taqman_v1.5.ncx
- For an experiment using the naica® PCR MIX compatible with EvaGreen® fluorescent intercalating dye (with droplet recognition in the blue channel) and the Ruby Chip select: ScanningTemplate_Prism6_RubyChip_naica-PCR-MIX_Evagreen_v1.1.ncx.

Note: Stilla Technologies does not recommend modifying the provided original template files. Always use the “save as” function to ensure to keep the original template files. If a modification of any template file is required, please contact the Technical Support.

After selecting the scanning template, the user is prompted to choose to use the default sample details (i.e. samples are simply named “Sample 1” to “Sample 12” for Sapphire Chip and “Sample 1” to “Sample 48” for Ruby Chip) or load the chamber details that are embedded in the scanning template (Figure 8). This feature provides the user a convenient way to set up chamber settings that will be run routinely.

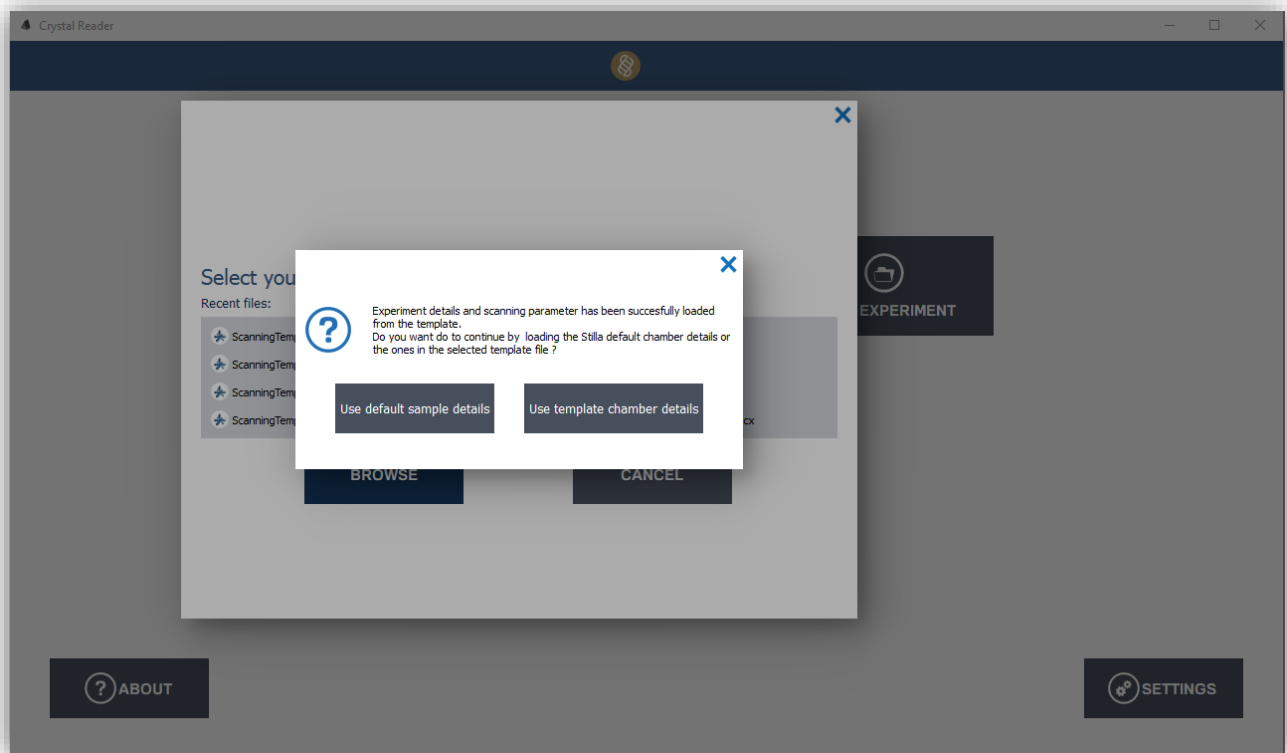


Figure 8: After the template has been loaded a pop-up window prompts the chamber details configuration that the user can load.

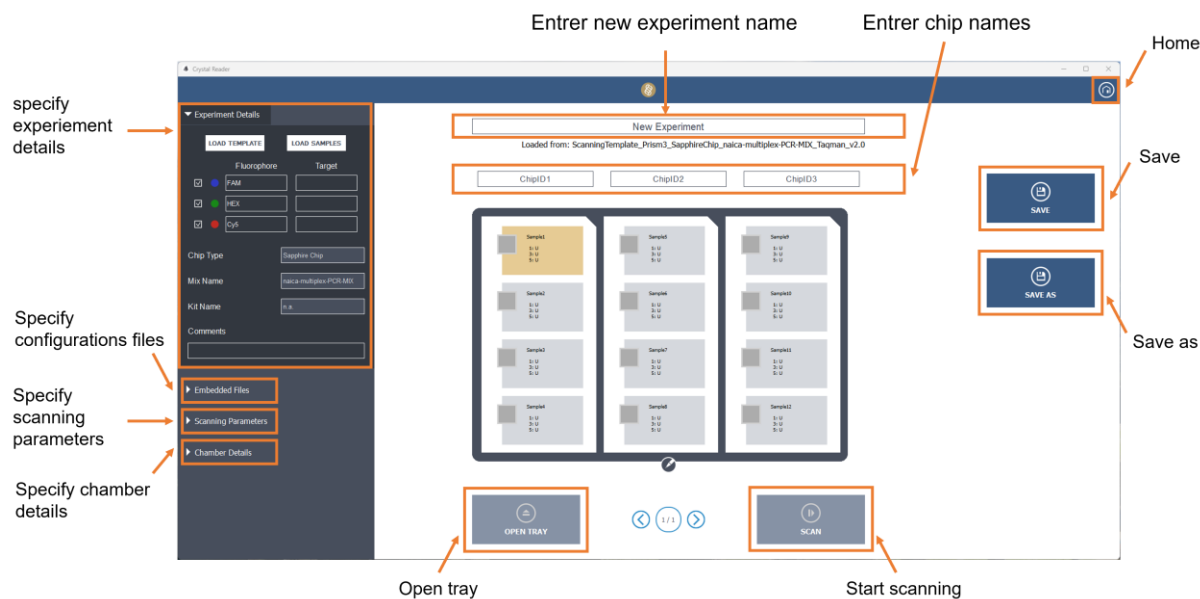


Figure 9: The Crystal Reader software home page for the Sapphire Chip.

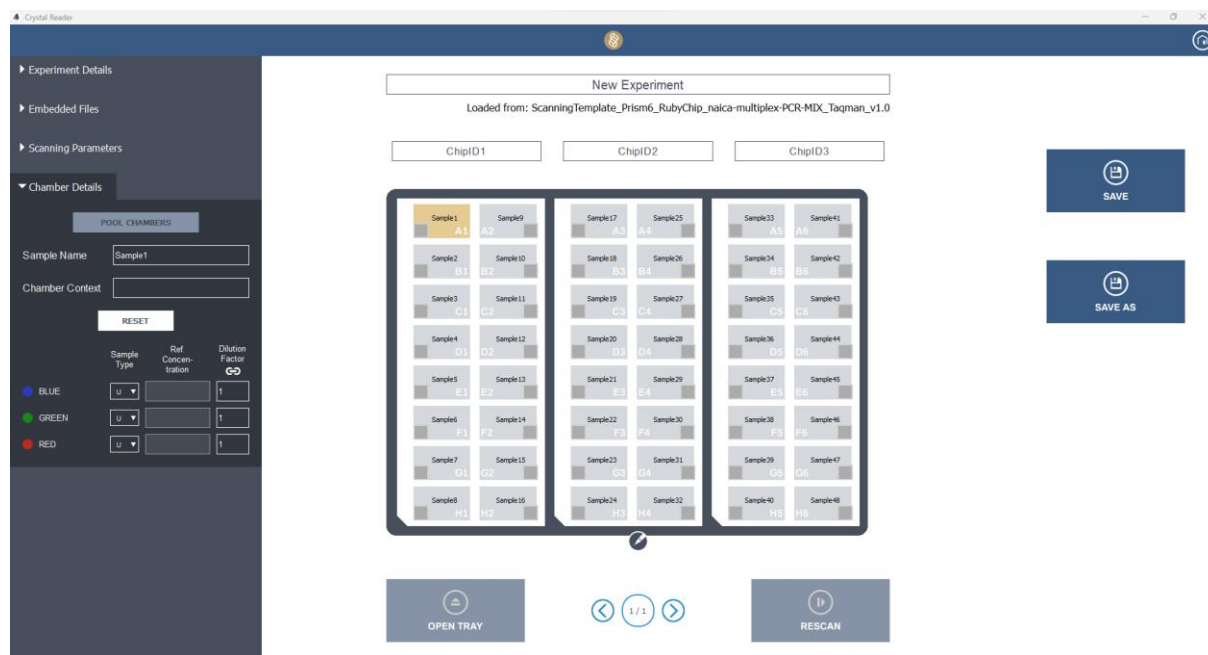


Figure 4: The Crystal Reader software home page for the Ruby Chip.

The Experiment Details, Embedded Files, Scanning Parameters and Chamber Details are pre-filled according to the selected scanning template; some settings can be checked and modified if needed at the next step.

3.8. Step 2 - Confirm or modify the experiment context

Note: All subsequent sections provide instructions based on the Sapphire Chip as default but can be applied for the Ruby Chip.

3.8.1. Name the experiment

Enter the “Experiment Name” in the upper field.

Note: Stilla Technologies recommends establishing traceability rule for experiment naming (e.g., date prefix, chip IDs, user initials, objective of the experiment; 20210507_04527983-04568924-04698435_ABC_valid_3plex).

3.8.2. Enter the Chip IDs

The chip ID is a unique identifier that contains traceability information. It is located on the chip box label in both text and QR code formats. Stilla Technologies strongly recommends entering the chip IDs for each experiment. To do so, select Chip ID1, Chip ID2 or Chip ID3 according to the position of the chip in the tray holder and scan the QR code of the corresponding chip with the external barcode reader provided with the naica® system.

The Chip ID can also be manually written, although this is more error prone.

3.8.3. Edit experiment details

In the left panel, drop down the “Experiment Details” menu (Figure 11).

Use the “LOAD TEMPLATE” button to load a new scanning template.

Use the “LOAD SAMPLES” button to load new samples information (see section “Step 3” for more details about samples information).

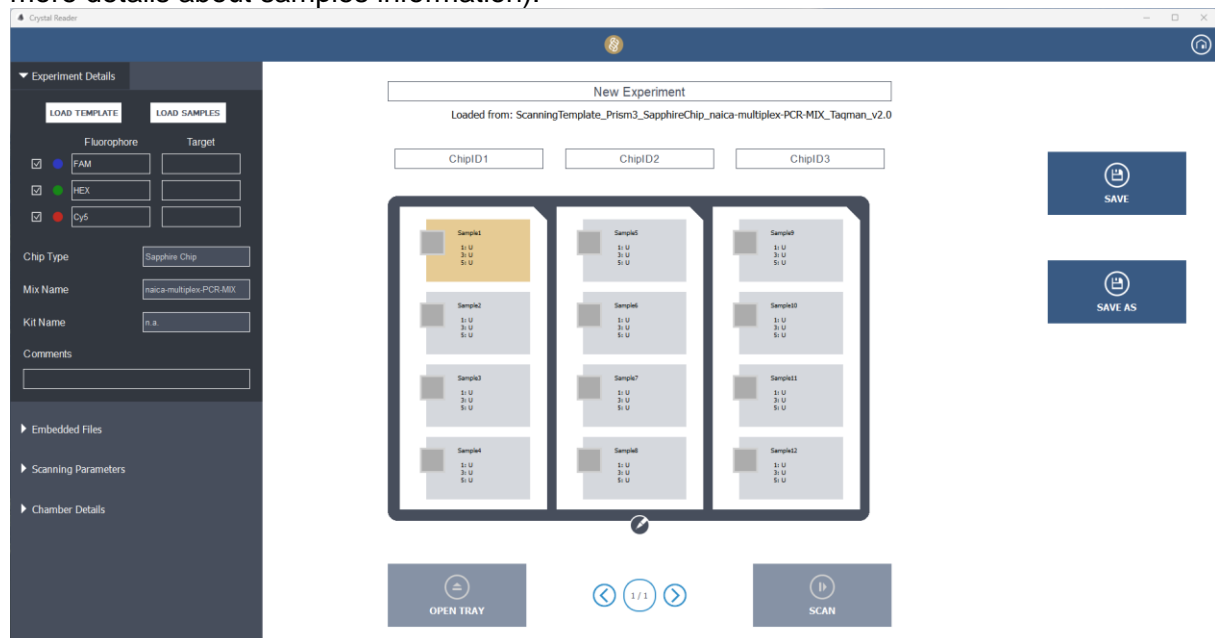


Figure 5: Enter Experimental Details in the “New Experiment” interface.

For each detection channel (“Blue”, “Green” and “Red” LED) enter the following:

- the name of the fluorophore that is mainly excited by the LED.
- the name of the target associated with this fluorophore.
- Select/unselect the checkbox to include/exclude a detection channel for/from scanning.

It is mandatory to include the blue channel, as it is the main detection channel.

Note: Ensure to select the appropriate detection channel for the experiment to avoid any false-positive results. If the wrong detection channel was selected, re-scan the chips.

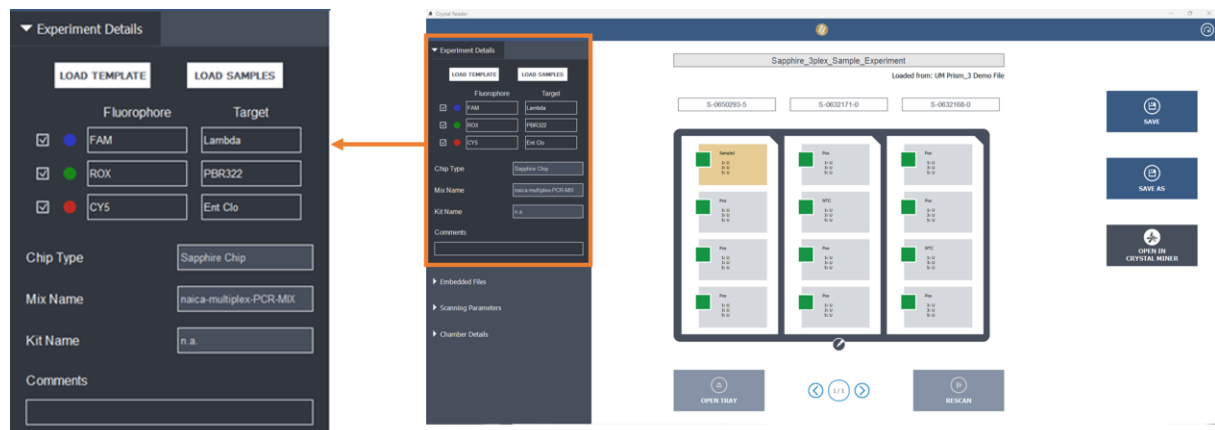


Figure 12: Enter fluorophore and target names in the “Experiment Details” interface.

The “Comments” field can be used to add any information pertaining to the experimental record.

3.8.4. Check the Embedded Files

The embedded file selection menu concerns the supported files that can be pre-loaded in the Crystal Reader software user interface. The only mandatory file is the Image Analysis Configuration File (.yaml) which defines the set of chip type, scanner type and mix type.

The three other files are optional:

- The Spillover Compensation Matrix (.ncm)
- The Plots configurations (.ncp) containing favorite views and the settings of dot-plots.
- The Analysis Configuration (.nca) containing the zoning (thresholds or polygons) and populations settings.

When these files are loaded in Crystal Reader software, they will be saved in the .ncx file together with the raw data. When opening the .ncx file in Crystal Miner software, the display settings of the histograms plots, 1D and 2D dot-plots will be pre-set from the .ncp file and the thresholds and populations will be pre-defined from the .nca file. This is especially useful for routine use cases and allows the user to directly access the quantification without having to reset the display and population settings for every analysis.

Please refer to the Crystal Miner software User Manual for additional information.

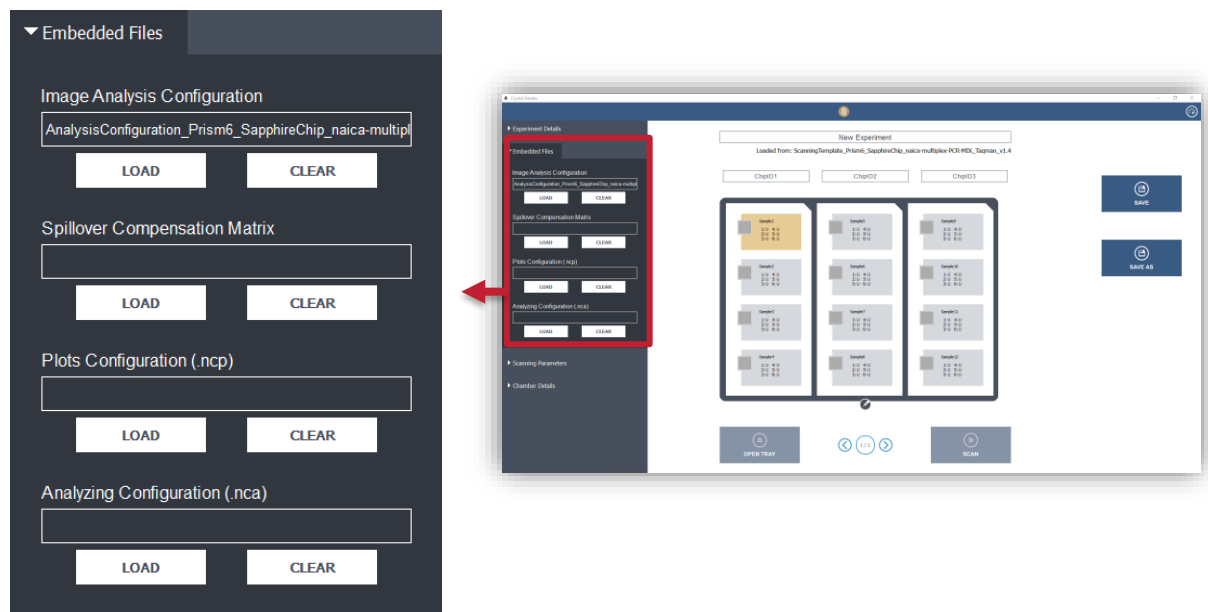


Figure 13: Select the relevant “Embedded Files” for the experiment.

Use the LOAD button to add a file (e.g. to add a spillover compensation matrix if the scanning template does not contain one).

Use the CLEAR button to remove a file if needed.

If the “Spillover Compensation Matrix” field is empty, it is possible to load a spillover compensation matrix that has been created for a previous experiment with the Crystal Miner software matrix computation tool. To load a pre-existing matrix just click on the “Load” button in the “Spillover Compensation Matrix” widget and select the desired “.ncm” file.

If a spillover compensation matrix is loaded, the fluorescence spillover will be automatically applied when opening the data later in the Crystal Miner software.

CAUTION!

The spillover compensation matrix (.ncm), is specific to the set of fluorophores used. However, the matrix may also depend on the biological setup (mastermix used and PCR program) and the scanning parameters (exposure times of the LEDs). When changing an assay parameter or a scanning parameter, Stilla Technologies recommends to either redefine the compensation matrix or to verify that any compensation matrix defined for the previous parameters still provides optimal spillover compensation before validating its use on the assay with the new parameters.

Please refer to the Crystal Miner software User Manual for additional information about spillover compensation.

Note: The Image Analysis Configuration File (“.yaml”) is mandatory in the experiment. When selecting a scanning template provided by Stilla®, an Image Analysis Configuration File is already set for the experiment.

3.8.5. Check the scanning parameters

Check the scanning parameters

Click on the “Scanning Parameters” drop-down to edit the focus value and exposure time of each excitation LED (in ms):

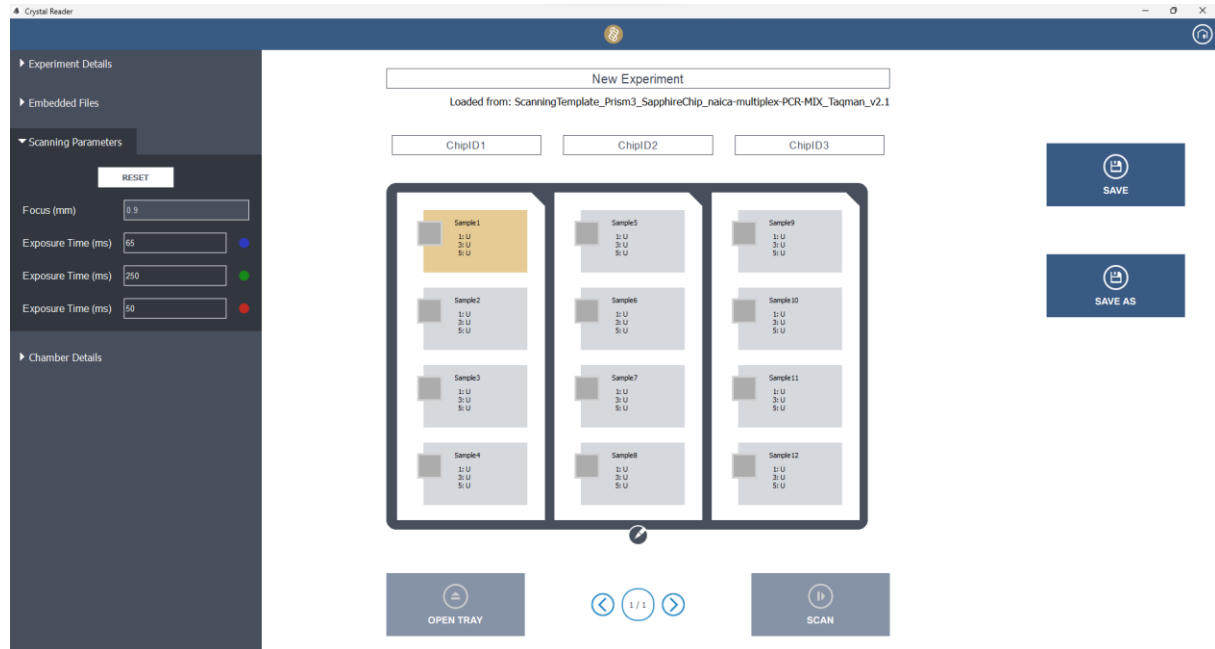


Figure 14: The “Scanning Parameters” menu.




Focus value

- The focus value is a set value based on the original instrument calibration and it does not need to be modified.
- The focus value represents the optimal z-distance of the imaging plane in mm.

Exposure times

- When a scanning template is selected, the default exposure times from the template will be displayed
- Exposure times can be modified to optimize experimental settings. Only positive integer values between 1 and 5000 ms must be used for exposure times.
- Increasing or decreasing the exposure time of an LED will correspondingly increase or decrease the fluorescence intensity value of the droplets in the image in that detection channel.
- The exposure time of each LED should be high enough to discriminate between negative and positive droplets in the detection channel, but not so high that it saturates the positive droplets in the detection channel (intensity saturation incurs the loss of quantitative information).
- To find the optimal LED exposure times for new experimental conditions, the user may create a control experiment in which only one chamber is activated. In this case a few scans should be run until an optimal set of exposure times is found.
- When at least one chamber of the experiment has been scanned in the experiment, the LED exposure time should no longer be edited. This will ensure that all the chambers of the experiment share the same scanning parameters. If the user tries to change an exposure value, the software will give a prompt, asking if the user wishes to proceed to erase the images of the already scanned chambers.

3.9. Step 3 - Select the chambers to scan & define chamber details

- Click on the “pencil icon”  (located below the chip holder display) to modify the selection of the chips & chambers to be scanned (by default the 12 chambers for the Sapphire Chip and 48 chambers for Ruby chip are selected).
- Clicking on the “cross icon”  will deactivate the object (chamber, chip, chip holder).
- Clicking on the “plus icon”  will activate the object (chamber, chip, chip holder).

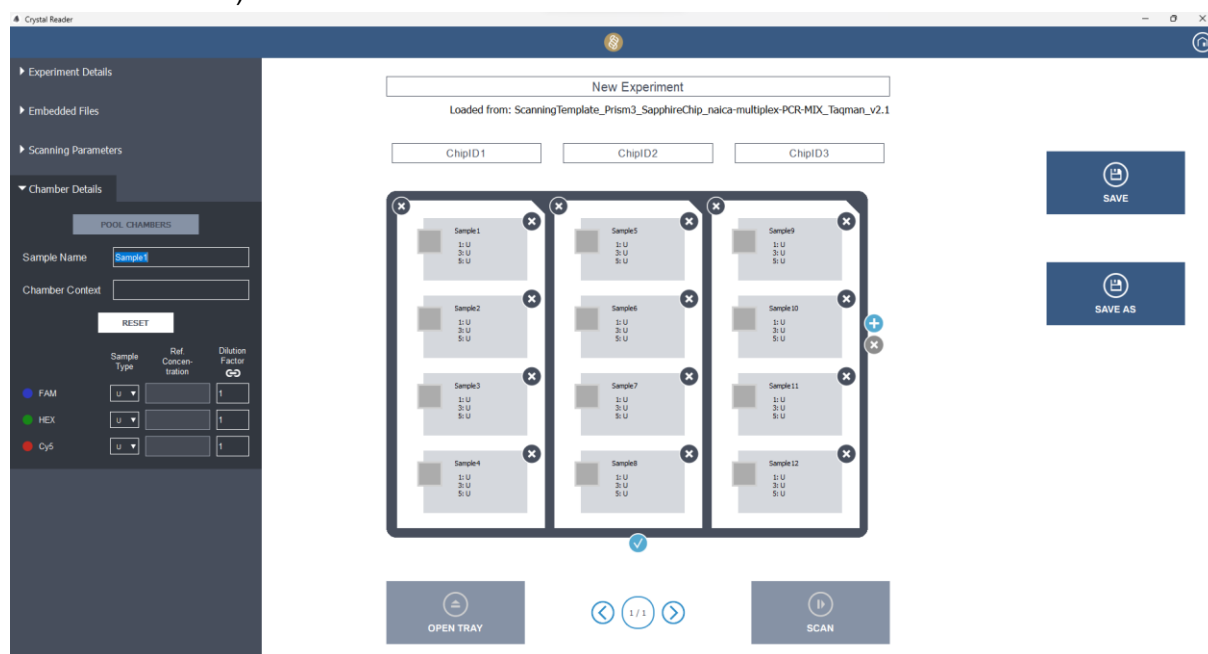


Figure 15: Click on the cross icons to deactivate objects; click on the “plus icon”  to activate objects.



- If an additional chip holder is required, click on the “plus icon”  on the right side of the chip holder display. The chip holder count will be increased.
- To navigate from one chip holder to another, click on the left or right arrows around the chip holder counter on the bottom (**Figure**).



Figure 16: Chip holder counter.

- Click on the “validation icon”  (located below the chip holder layout) to validate the selected chambers.

3.9.1. The Chamber Details panel

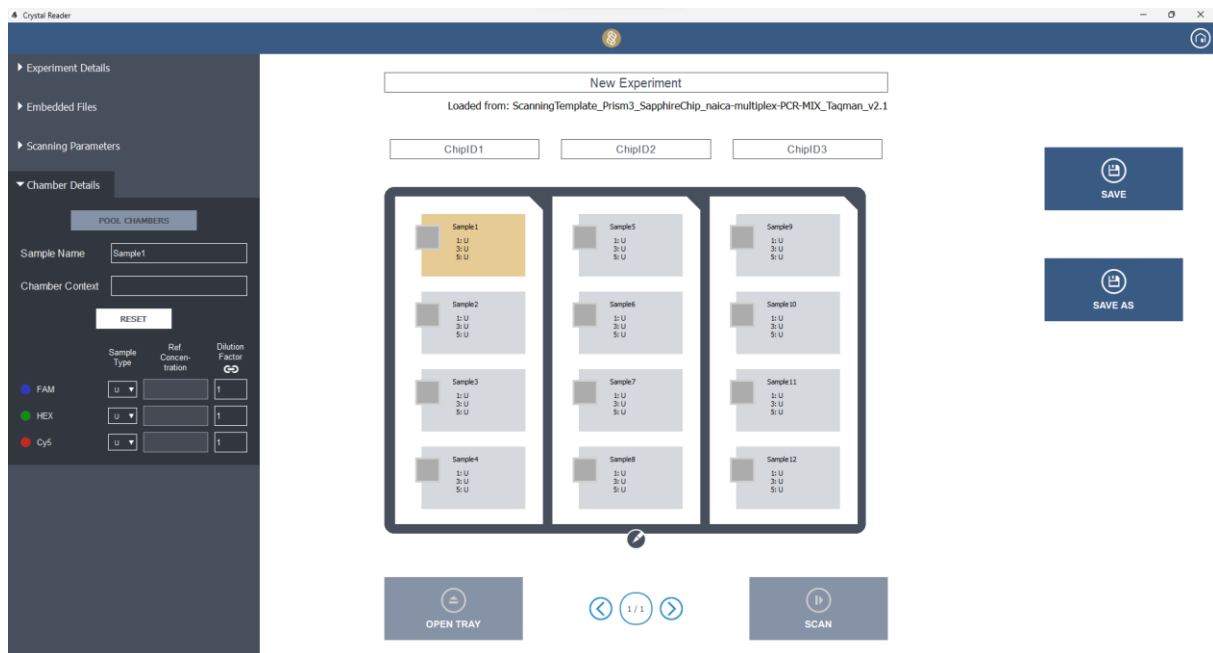



Figure 17: The “Chamber Details” form.

To enter the details of a given chamber; first click on the chamber in the rack layout (chamber rectangle becomes golden), then proceed to edit the “Chamber Details”:

- “Sample Name”: to identify sample loaded in the chamber.
- The USB barcode reader can be used to autofill the chip code to gain time.
- “Chamber Context” (optional): to enter additional information to make the scan data unique (useful if scanning the chamber multiple times).
- For each detection channel (“Blue”, “Teal”, Green”, “Yellow” “Red” and “Infra-Red” LED) enter:
 - “Sample Type” (see details below).
 - “Reference Concentration”. The Sample Type “S” stands for Standard. It is used when a sample of known concentration is processed. In that case, it is possible to edit the expected concentration, in cp/μL. By doing so, both the expected concentration and the measured concentration will be displayed close to each other in the Results page in Crystal Miner software.
 - “Dilution Factor” (e.g., 10 for a 10-fold dilution from the stock sample).
 - By default, the same dilution factor is applied to every channel. To make the dilution values independent from one channel to another, click on the “unlink icon” . Clicking again on this icon will reset the default configuration.
 - If no value is specifically added, the final concentration in copies/μL will correspond to the number of DNA copies for each μL in the whole 25 μL of reaction mix for Sapphire Chip or 7 μL of reaction mix for Ruby Chip.
 - If, for example, the volume of DNA solution is 4 μL out of 25 μL of reaction mix, it could be useful to insert a dilution factor of 6.25 (25 μL/4 μL) to obtain the effective concentration of copies/μL in the 4 μL of the initial sample.

- Then, edit the type of the sample for each given channel, the possible values are:
 - U: Unknown
 - S: Standard. This type is used when a sample with a known concentration is run. In that case, it is possible to edit the expected concentration in the “reference Concentration” field (in copies/μL). This is not mandatory but by doing so, the expected concentration will display next to the measured concentration in the results page in Crystal Miner software.
 - N: Negative control (i.e., there are only negative droplets for the channel of interest)
 - P: Positive control (i.e., there are positive droplets for the channel of interest)

Note: It is possible to navigate between the chambers using the arrows of the keyboard by pressing the “ALT” key. This method may help to save some time editing the chamber context.

- The chamber details are displayed on the chamber button of the chips’ layout:
- The sample ID and sample types per channel are displayed on the chamber in the chips’ layout.

Note: Before the scan is completed, the flag button is grayed and deactivated. The user will be able to click on this button once the chamber has been scanned and analyzed.

The same “Experimental Details”, “Embedded Files” and “Scanning Parameters” will be applied to all samples for an individual experiment during a Prism3 run (see the previous three sections for more details).

If different “Experimental Details” or “Scanning Parameters” are to be applied to the same set of samples for an experiment, chips can be scanned up to three times within 48 hours following the first scan.

3.10. Step 4 - Launch the scan

Click on the “SCAN” button, then validate the path to save scanned data (as a “.ncx” file) and confirm the loading of the first chip holder to launch the scan (Figure 18).

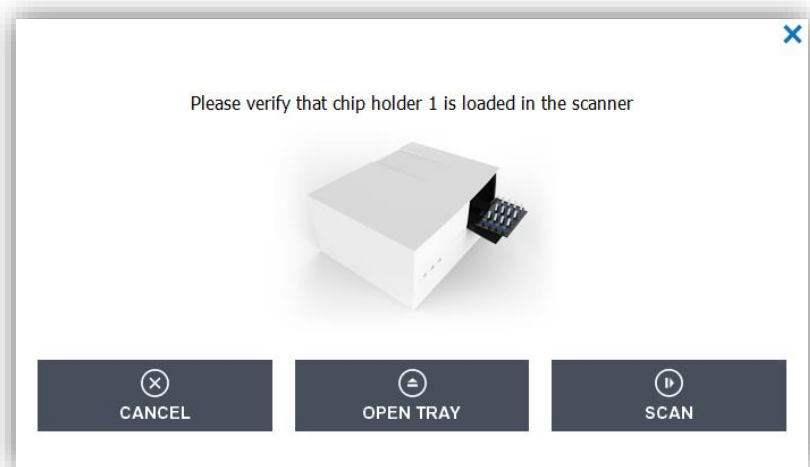


Figure 18: Pop-up requesting confirmation that the chip holder has been placed in the Prism3 before launching the scan.

Click on “Continue Scanning” to confirm the loading of the next chip holder to launch the next scan.

If the chips to be scanned are loaded in the scanner, click on “OK”, else click on “CANCEL” and go back to the previous steps.

Once the scan is processing, a progress bar with the estimated remaining time is displayed.

It is possible to cancel the scan at any time, but there is a risk of losing some scanned data.

Editing the experiment context is deactivated during the scan process.

- If several chip holders have been selected for the scan, the scanning process is split into several steps:
 - Once the current chip holder is scanned, a pop-up window is displayed that instructs to load the next chips.
 - Click on “OPEN TRAY”, place the next chips to be scanned, then click on “CLOSE TRAY”.
 - Click on “Continue Scanning” to confirm the loading of the next chip holder to launch the next scan.

When saving an “.ncx” file, the available disk space is checked and the user is warned if the available disk space is less than 10 GB (Figure 19).

If the chips to be scanned are loaded in the scanner, click on “OK”. Otherwise click on “CLOSE” and go back to the previous steps.

Once the scan is processing, a progress bar with the estimated remaining time is displayed.

CAUTION!

Do not push the power button on the front panel of the Prism3 instrument while it is still in operation. This would cause the instrument to shut down with a risk of data being lost, necessitating re-scanning of the chips.

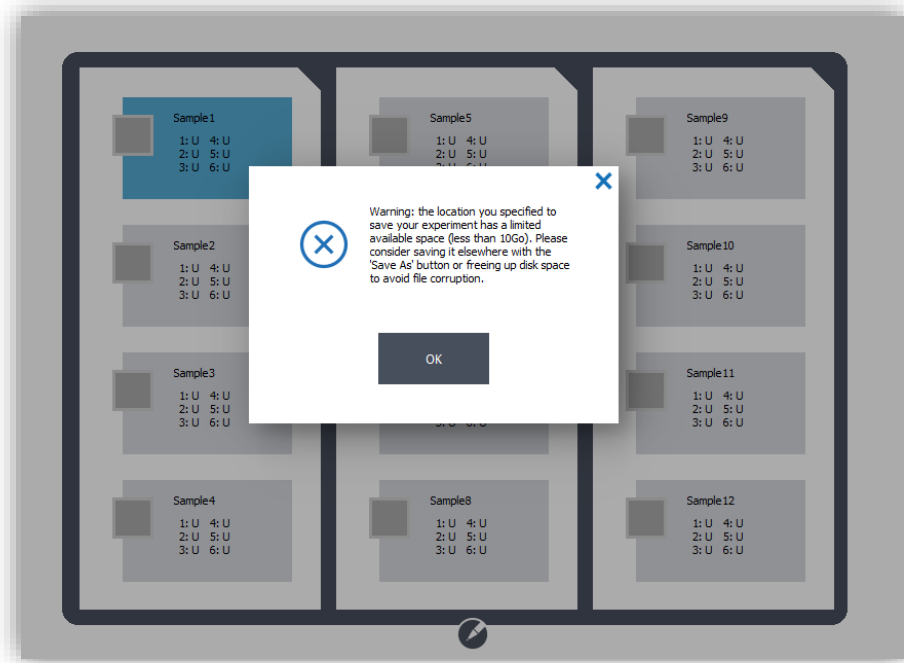


Figure 19: Pop-up warning about low disk storage space

The scan can be canceled at any time, but you might lose some scanned data. Editing the experiment context is deactivated during the scan process.

- If several tray holders are to be scanned sequentially as part of a larger experiment, the scanning process is split into several steps:
 - Once the current chip holder is completely scanned, a pop-up window is displayed that instructs to load the next chips.
 - Click on “OPEN TRAY”, place the next chips to be scanned, then click on “CLOSE TRAY”.

3.10.1. Check chamber image quality

As the individual chambers are scanned, the quality flags are displayed on the Crystal Reader software scan page.

A quality flag can be green or yellow.

Chamber Quality Flag

Chamber Quality Flag

A chamber quality flag is displayed next to each chamber name on the top left side of the application window. This chamber quality flag provides visual feedback for chamber quality control. Chamber level: the chamber flag corresponds to the lowest quality flag, among the criteria computed for the chamber.

There are 2 types of chamber quality flags:



- “Green” quality flag indicates that the chamber and its picture are good quality.



- “Yellow” quality flag indicates that the that the software detected major alteration of the analyzed chamber quality parameters. Visual inspection of the Droplet Crystal is required to determine if the experiment may have to be repeated.
- For a visual pre-analysis, while the scanning is still in progress, clicking on one of these quality flags will display the corresponding chamber page.
- In case of non-green flags, a visual inspection of the chamber must be performed in every channel, directly in Crystal Reader software. This is because re-scanning with adjusted Scanning Parameters may suffice to improve the quality of the picture and get a green flag.
- For further details on quality flags, refer to the section 3.6.3 Check image quality
- When the image of the last chamber of the experiment is acquired, the following message is displayed:

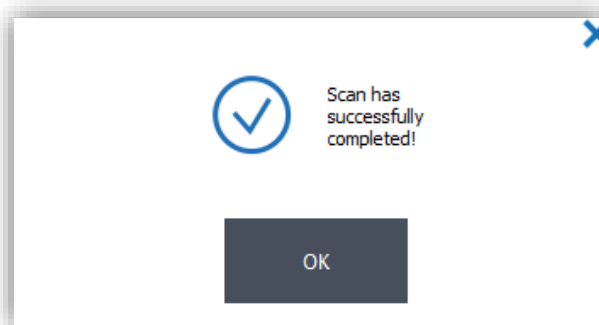


Figure 20: Pop-up notifying scan completion.

- Click “OK” to display all results of the scanned experiment.

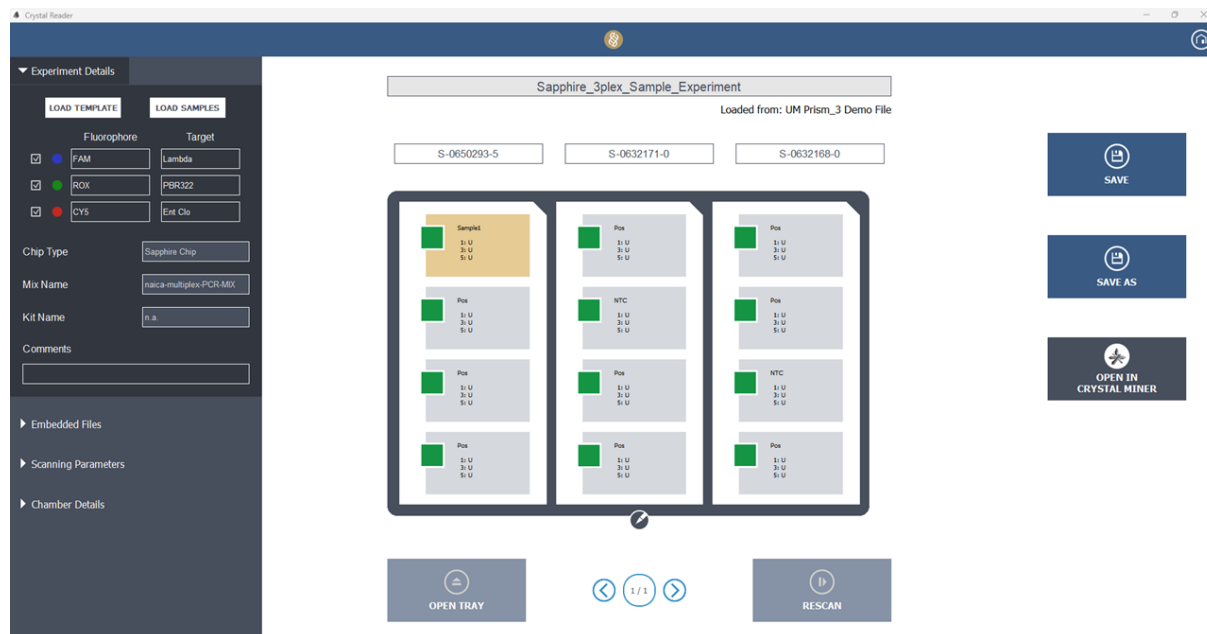


Figure 21: Example of a Sapphire Chip experiment layout with all green quality flags at the end of the scan.

- Note: At the end of the scanning process, the experiment is automatically saved in “.ncx” format in the user-defined output directory under the name: “<ExperimentName>.ncx”.
- The default output directory is: “%USERPROFILE%\Documents\Stilla\CystalReader”

Stilla Technologies recommends initially checking the scanning performance by inspecting the image quality. Image quality for the scanned experiment can either be directly inspected in Crystal Reader software or later in the Crystal Miner software after clicking on the “OPEN IN ” button:

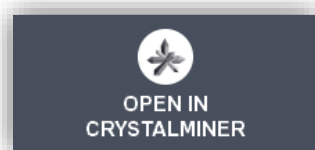


Figure 22: Button to open the scanned experiment with Crystal Miner software.

3.11. Step 5 - Check image quality

Click on the quality flag of a chamber to explore the respective chamber image in detail.

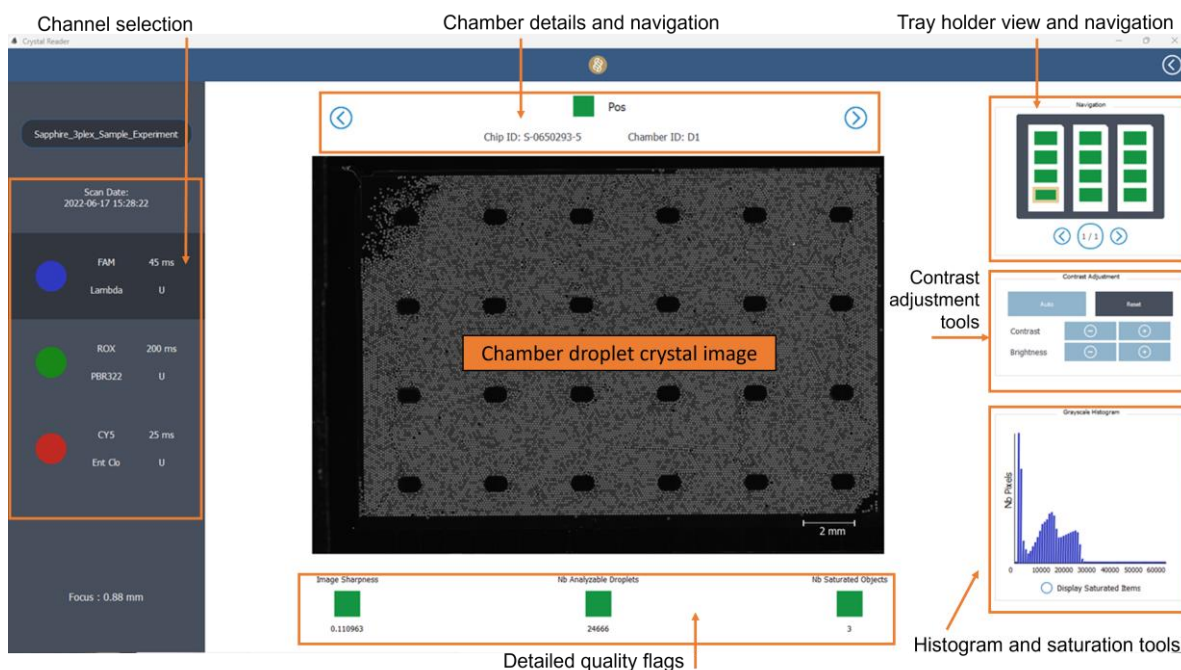


Figure 23: Overview of a Sapphire Chip chamber image in the “Blue” detection channel in the Crystal Reader software.

To explore the images of a chamber:

- Click on the LED icons (on the left panel) to select the acquisition channel.
 - I. Use the mouse scroll to zoom in or out in the image.
 - Left mouse click and drag to span the image.
 - II. Click on the “Auto” button to automatically adjust the image contrast.
 - Click on the “Reset” button to reset the image contrast.
 - III. It is possible to navigate across the chambers using:
 - the chip holder layout on the top right (click on the rectangle of a chamber to explore it)
 - The left/right arrow buttons, on the top, to explore the previous/next chamber.
 - The left/right/top/left arrows of the keyboard to explore the chamber in the specified direction.
 - Check that the negative droplets are visible in the “Blue Channel” image via the reference fluorophore (e.g., fluorescein). If necessary, click on the “Auto” button to adjust the image contrast.

CAUTION!

For every chamber, the negative droplets should be visible in the Blue channel image for the experiment to be analyzable.

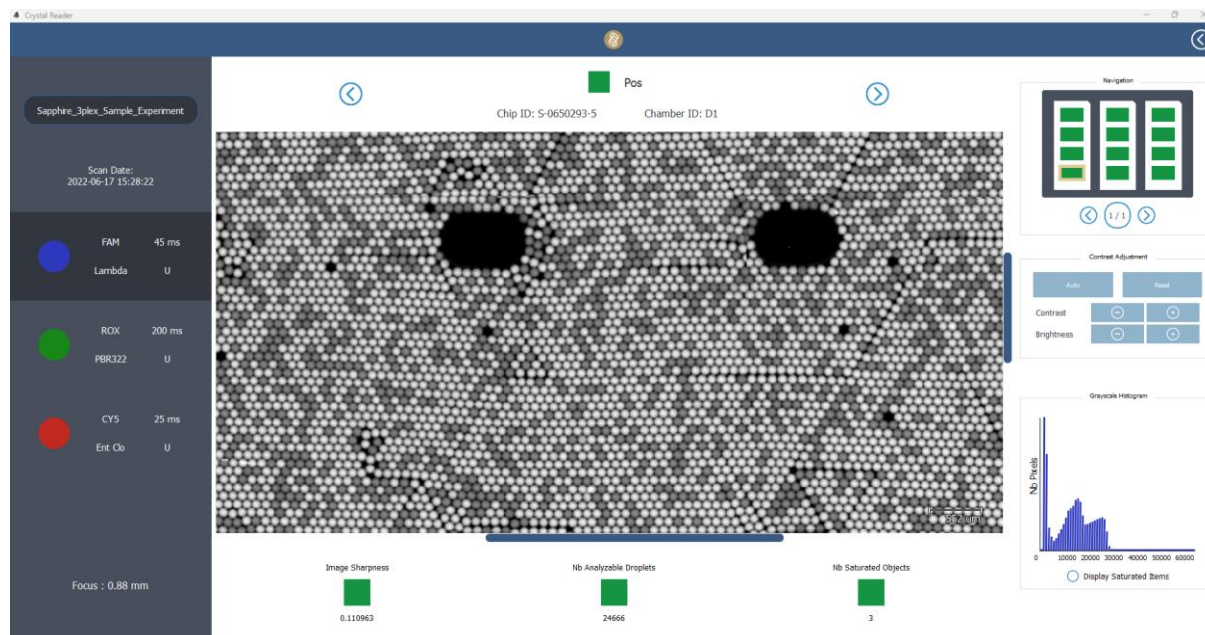


Figure 24: Zoom into a chamber image in the Blue detection channel (all negative droplets should be visible in the Blue channel image) in the Crystal Reader software.

- The Grayscale Histogram tool (bottom right) helps understand the distribution of pixel intensities expressed in RFUs (Relative Fluorescence Units), where zero RFUs means black and $2^{16} - 1 = 65,535$ RFUs indicates saturation.
- By dragging & dropping in the histogram (with the left mouse button) you can set the minimum and maximum intensity cursors and draw an interval. The pixels with RFU values included in this interval will be highlighted in orange color in the chamber image.
- In the following example we select the interval 24576-37632 RFU. This interval is the selected orange band on the histogram that reveals the positive droplet population in the crystal image.

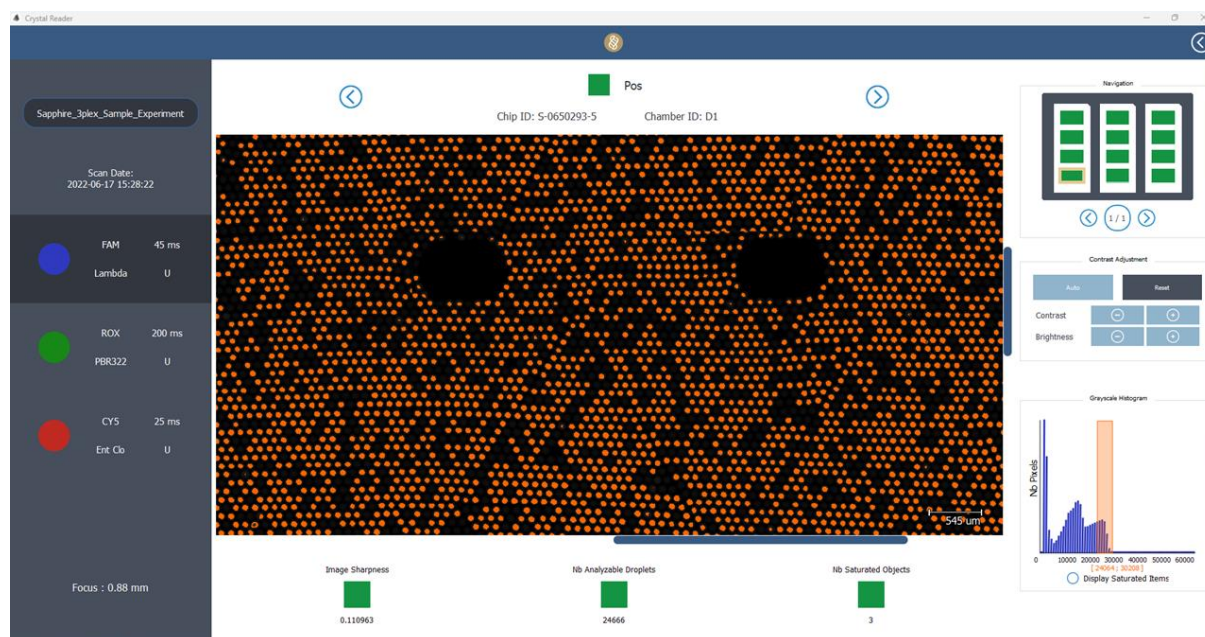


Figure 25: Zoom in a chamber image in the Green detection channel with the “Auto” contrast in the Crystal Reader software.

Refer to the “Perform Quality Control” paragraph below for more details about the “Display Saturated Items” checkbox.

3.11.1. Perform quality control:

- Performing the quality control of a given chamber consists first in checking its quality flag. The quality flag of a given chamber is automatically computed based on three criteria which are themselves also displayed in the form of quality flags. The three criteria are:
 - The “Image Sharpness” which reflects the sharpness of the image. A yellow flag may be caused by an out-of-focus issue. This can be corrected by checking that the chip holder is clean and the chip is flat in the tray holder. If the instrument is still out of focus after checking this, contact customer support for assistance, to run the focus calibration procedure.
 - “Number of analyzable droplets” which corresponds to the total number of droplets included in the digital PCR computation, after image analysis of the chamber by Crystal Reader software. The higher the number the better the precision of the concentration results.
 - “Number of saturated items” which are pixels whose fluorescence intensity is above 65,535 RFU. These pixels are automatically excluded from the analysis. By checking the “Display Saturated Items” box under the Grayscale Histogram tool, the saturated pixels are highlighted in orange color in the Droplet Crystal image (Figure 26). Counted saturated objects are either saturated droplets caused by a high exposure time or saturated artifacts located inside the chamber. They can be avoided by checking that exposure times are not too high.



Figure 26: Overlay of the saturation map in the Blue detection channel in the Crystal Reader software (saturated pixels are displayed in orange color). In this case, they are dust particles.

CAUTION!

Ensure that none of the saturated pixels are located in positive droplets, in any channel. Otherwise, these positive droplets will be excluded from the analysis by the software. When positive droplets saturate in a given channel, it means that the

chamber(s) has(have) been over-exposed. The exposure time of the corresponding channel must be reduced before rescanning the experiment.

- If exposure times need to be modified to improve the image quality, the chips should be rescanned (see the section “Advanced Functionalities” for more details about chamber rescanning).
- If the underside of a chip needs to be cleaned to improve the image quality of some chambers, the chip should be rescanned for these respective chambers (see the section “Advanced Functionalities” for more details about chamber rescan).
- In case of artifacts, such as dust (yellow flag for the number of saturated objects not due to saturation of droplets), clean the back surface of the chip by slowly sliding it once along the length of a Precision Wipe and rescan.

The saved experiment can be now analyzed with Crystal Miner software (please refer to Crystal Miner software User Manual available on the Technical Resources webpage for details).

- Before turning the Prism3 instrument off, click on “Open tray”/ “Close tray” to remove the chips. Then press the power button located at the front.

4. Advanced Functionalities

4.1. How to rescan existing chambers and how to scan new ones

Once a scan is finished, the SCAN button becomes a RESCAN button. It is then possible to, either:

- a) Rescan all the chambers of the experiment.
- b) Rescan only specific chambers of the experiment and keep the images of the other chambers.

To do so, Click on the RESCAN button

Note: If different scanning parameters or experimental details are to be applied to the same set of samples for an experiment, the chips can be scanned up to three times within 48 hours following the first scan.

Irrespective of the option chosen, if there is at least one already scanned chamber when the “SCAN” button is clicked, this window will be displayed:

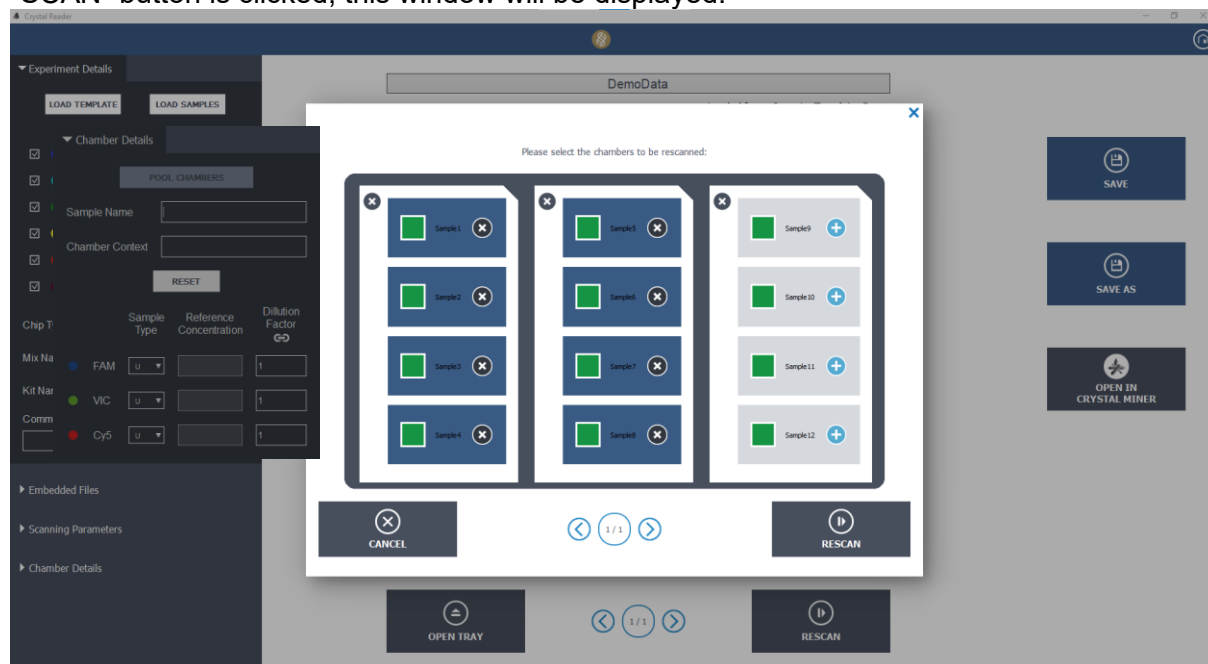


Figure 27: Default rescan window (chambers to be rescanned are highlighted in blue color).

In the default configuration, all the previously scanned chambers are selected for rescanning (i.e., they are highlighted in blue color).

4.1.1. How to rescan all the chambers of the experiment

- For this scenario: click on the Rescan button, and the scan will start.

4.1.2. How to rescan only a subset of chambers

- For this scenario: unselect the previously scanned chambers to be kept and click on the new ones desired to be added to the experiment.

4.2. How to edit the Prism3 settings

To access the settings panel, click on the “SETTINGS” button from the HOME page in Crystal Reader software. The settings window will display different editable fields.

The following settings can be edited:

- Prism3 instrument settings:
 - Focus Value (mm): z-distance to the imaging plane used for image acquisition. If it is necessary to reset the focus settings, please contact Technical Support for guidance.
 - Prism3 Serial Number (SN): unique serial number of the Prism3 (set by Stilla Technologies).
 - “Focus Calibration” button: advanced functionality to automatically adjust the focus value for the image acquisition process (available only for the lab manager).
- System Settings:
 - Default paths: default output and input directory paths.

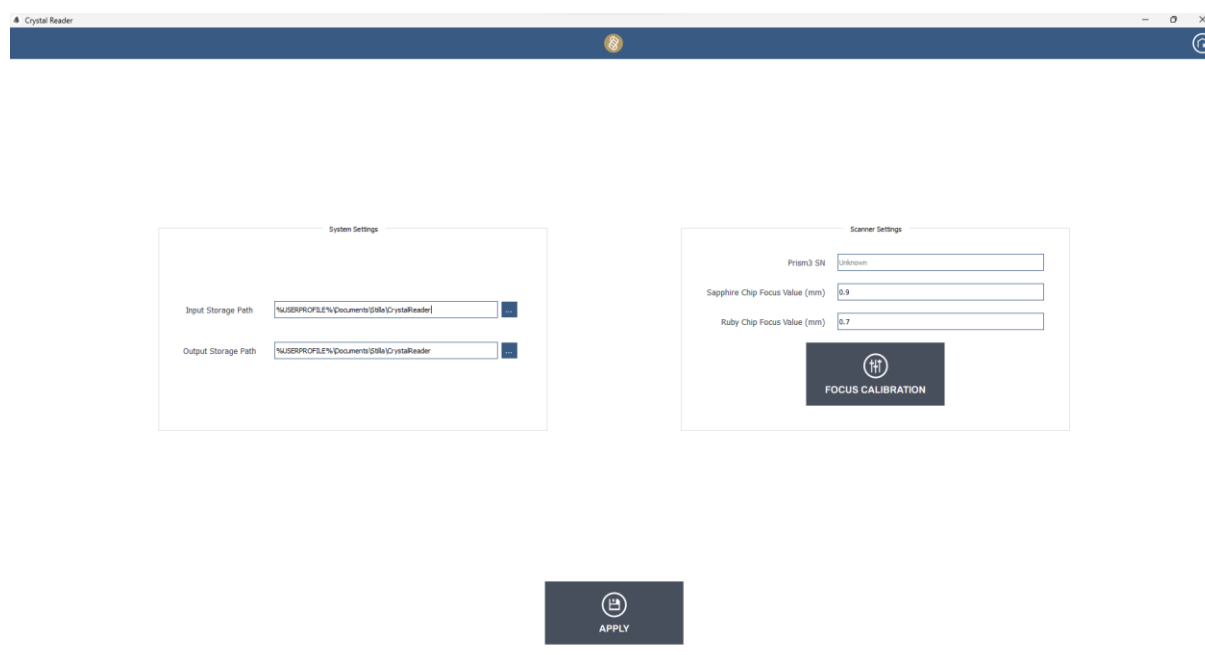


Figure 28: Settings page in the Crystal Reader software.

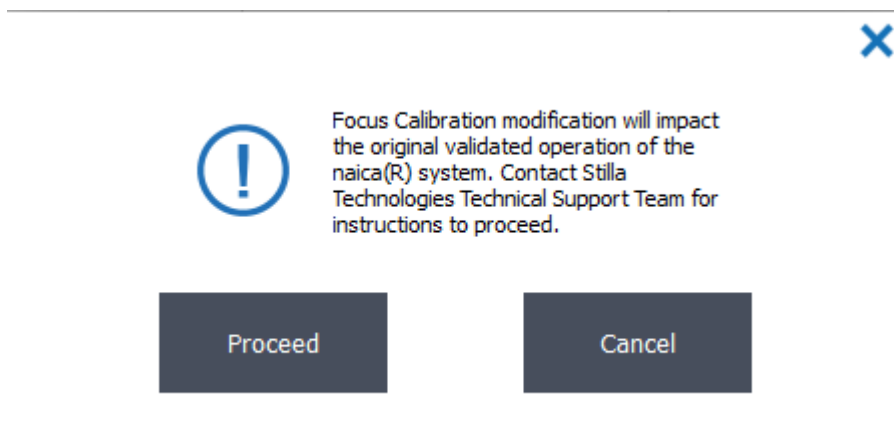
- A user can:
 - Modify the “Focus Value” used for experiment scanning in the “Scanner Settings”, either manually or via the “Focus Calibration” functionality (see next section), for each chip model (3).
 - Modify the default output and input directory paths. System preferences for the input/output storage directories (1 and 2) will be saved after quitting Crystal Reader

- In the path fields, “%USERPROFILE%” can be used; this is a Windows environment variable that redirects to the path of the current Windows session user.
- For example: if the user is logged into Windows with the account “John”, %USERPROFILE% has the following value: “C:\Users\John”
- For calibration purposes, it is possible to perform an automated estimation of the optimal acquisition focus (i.e., optimal z-value) by clicking on the “Focus Calibration” button (see next section).

4.3. How to perform focus calibration

The focus calibration is an advanced feature.

Caution!
It is strongly recommended not to alter focus settings.
If there is a requirement to reset the focus settings, please contact Technical Support .



- Assuming that a chip with a chamber of interest is already inserted in the Prism3 instrument, access the SETTINGS menu, click on the “Focus calibration” button, confirm, then select the chip model of interest (Sapphire Chip or Ruby Chip).

Note: For optimal focus calibration on the Sapphire Chip, it is recommended to use at least one chip that includes a chamber with a good quality Droplet Crystal and to place this chip on the middle slot of the chip holder (i.e., slot 2).

Note: For optimal focus calibration on the Ruby Chip, it is recommended to use three chips including chambers with good quality Droplet Crystals in positions A, D, and H.

Select the chamber(s) of interest (i.e., the chamber for which the optimal acquisition focus needs to be calibrated) by clicking on the chamber rectangle in the chip holder layout (the rectangle becomes blue). It is also possible to modify the scanning times, as well as the min, max, and step values for the z-values to be tested.

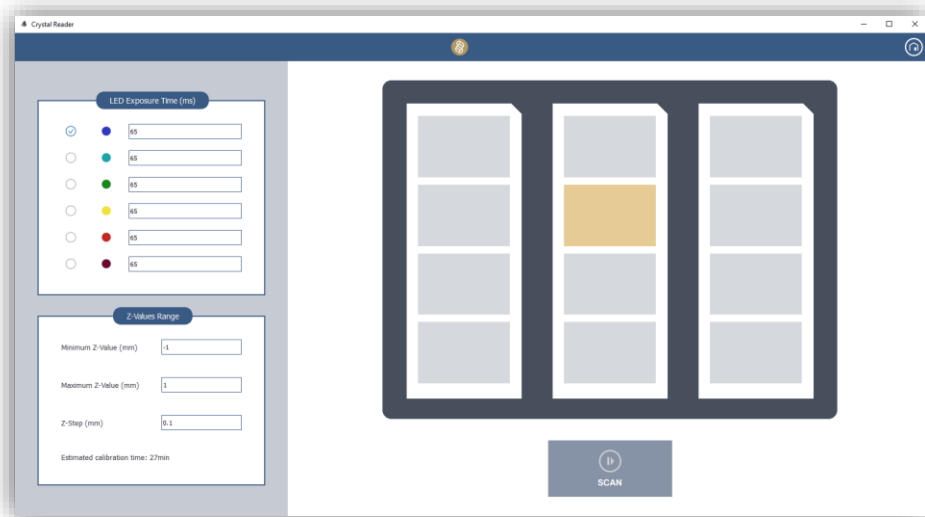


Figure 29: Example of selection of one chamber to calibrate the focus for the Sapphire Chip in the Crystal Reader software.

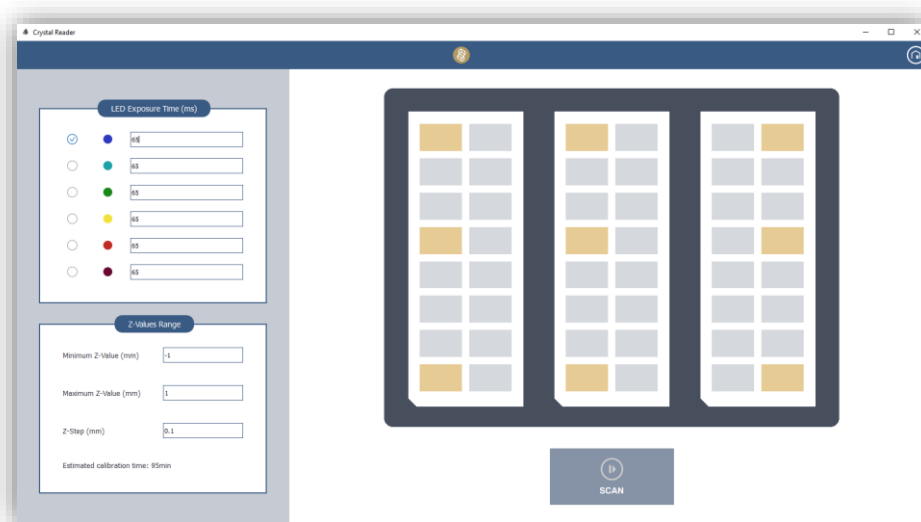


Figure 30: Example of selection of nine chambers to calibrate the focus for the Ruby Chip in the Crystal Reader software.

- Click on the “SCAN” button at the bottom to launch the focus calibration process.
- A progress bar is then displayed with the estimated remaining time.
- At the end of the focus calibration process, a result page is displayed, showing the value of the optimal focus (in mm). This value maximizes the average image sharpness score among all the selected chambers. The result page also shows the chamber image of the first selected chamber at this optimal focus.
- A table with all the computed sharpness scores for each selected chamber and each tested z-value is also provided, together with a graph showing the sharpness score for each chamber as a function of the tested z-value.
- The “manager” user may select the other tested focus values by clicking on them in the table, to check the sharpness of the chamber images acquired at different focus values.
- Once the optimal focus value has been obtained from the calibration experiment, please contact Technical Support for confirmation. The user may keep the optimal focus value or select a different focus value, then click on “Apply this z-value” to apply this new focus value to all the next scanning processes for the chip model of interest.
- To cancel, click on the “Home” button on the top right.

4.4. How to Pool/Unpool Chambers

An additional feature, where users can pool multiple selected chambers containing the same sample, called “POOLING CHAMBERS”, is available in the Crystal Reader software. Pooling a set of chambers in which the same sample has been loaded increases both detection sensitivity and quantification precision. Indeed, by considering each set of pooled chambers as one larger chamber, this pooling strategy allows to increase the analyzed volume.

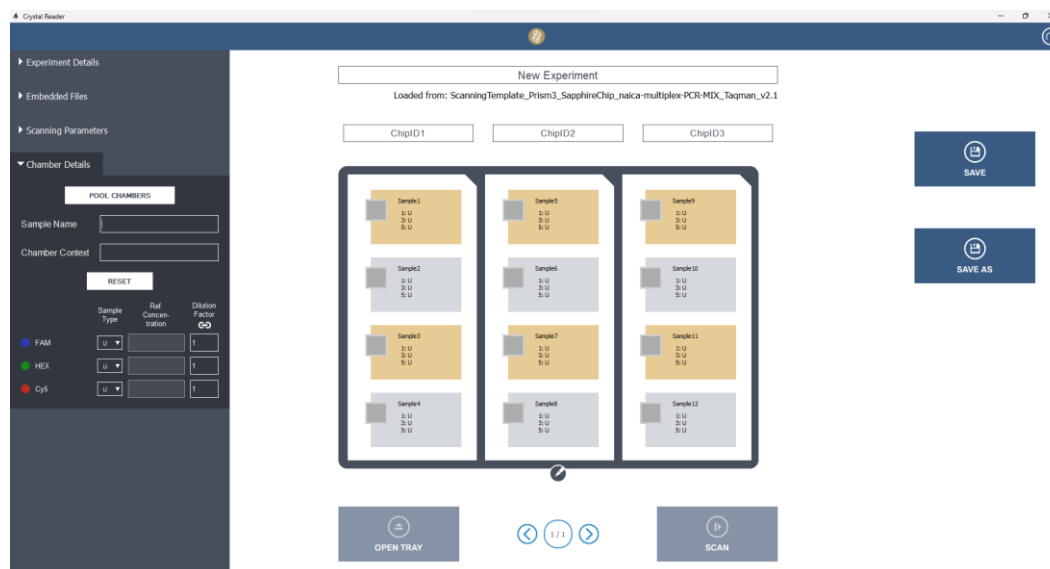


Figure 31: Pooling feature of the Crystal Reader software Chamber Details section.

CAUTION!

The chamber pooling functionality should only be used under the assumption that pure replicates have been loaded in the pooled chambers (e.g., the same sample has been loaded).

- If pooling (resp. unpooling) a set of chambers in which the same sample has been loaded is required, simply select these chambers in the chip layout using “Ctrl+Click” or “Shift+Click”, and then click on the “POOL CHAMBERS” button (resp. “UNPOOL CHAMBERS” button) in the “Chamber Details” tab (Figure).

All chambers pooled together will automatically share the same “Pool ID” (displayed as an incremented number in the chip layout), as well as the same “sample name”, “chamber context” and, for each detection channel, the pooled samples will have the same: “sample type”, “reference concentration” and “dilution factor”.

Note: If pooled chambers have not been defined before scanning, it is still possible to pool the chambers after scanning, or during data analysis using the Crystal Miner software (see Crystal Miner software User Manual for more details).

5. Maintenance and Technical Support

Maintenance operations of the naica® system should be executed by a Stilla Technologies Technical Specialist during a visit on-site or by a return shipment of the device to Stilla Technologies premises. Stilla Technologies cannot be held responsible for any intervention or modification done by the user on devices of the naica® system. Before the intervention, we will request the user to decontaminate the instrument following instructions detailed in the Decontamination Protocol and to thereafter fill a Decontamination Certificate; both these documents are provided by a Stilla Technologies Service Specialist.

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

For customers from Europe, China and Africa:
Monday to Friday, 9:30 AM - 6:30 PM, Central European Time (CET).
Closed on french bank holidays.

Phone: (+33) 9 82 27 47 47
Email: support@stilla.fr.


For customers from America, Asia (without China) and Oceania:
Monday to Friday, 8:00 AM – 6:00 PM, Eastern Standard Time (EST).
Closed on American bank holidays.

Phone: 1-833-888-0150 ext. 1
Email: support@stilla-inc.com

Online Technical Support is also available at: www.stillatechnologies.com/technical-support/

We will try our best to answer as promptly as possible.

If applicable, Stilla Technologies offers to engage for remote troubleshooting by using the shared desktop application “Team Viewer”, which is installed on the naica® PC.

To enable remote software maintenance on the naica® PC provided by Stilla Technologies, check that the naica® PC is turned on and connected to the internet. Then, double-click on the “Team Viewer” desktop icon  and send both the user ID & password information by email to the Technical Support Team.

Please ensure to provide the following information when contacting the respective service & support team:

- The Crystal Reader software version number, which is available in “About” in the Home page
- The log files of the experiments which have been generated in the directory: “%USERPROFILE%\Stilla\CystalReader”

5.1. Cleaning and Decontamination

Please see below for maintenance-related instructions for the Prism3.

For optimal performance, it is recommended to limit the contact of dust particles with the naica® system.

All naica® system devices should be switched off and unplugged before cleaning and decontamination operations. Make sure there are no chips left in the instruments prior performing cleaning and decontamination procedures.

External parts of the Prism3 and the naica® PC delivered, can be cleaned using a cloth soaked with an alcohol-based solution (e.g., Phagospray) and air-dried. Monthly or when necessary, clean the chip holder of the Prism3 using a dust remover. In case of an accidental spill of liquid on devices, absorb and then clean using water or an alcohol-based solution (e.g., Phagospray) and air-dry. When disinfection is required, please contact Technical Support.

When decontamination for return shipments is required, please contact Technical Support for the respective protocol and instructions: Material necessary for the decontamination procedure:

- Gloves;
- Safety Glasses;
- Mask;
- Laboratory coat;
- Hydroalcoholic disinfectant solution for device surfaces commonly used for biological and medical devices (e.g. Phagospray).
- Decontaminant solution for device surfaces used for biological and medical devices, specifically targeting nucleases and DNA contamination (e.g. RNase away).
- Note: Bleach is not recommended; if its use is unavoidable, be sure to abundantly rinse with water after the bleach treatment.
- Standard laboratory paper towels.

5.2. Disposal

- Disposal of naica® equipment

The disposal of the Prism3 and the naica® PC 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.

5.3. To ship the instrument back to Stilla®

If shipping the Prism3 instrument and the naica® PC is required, use all the original packaging and cables provided upon reception of the naica® system.

No chip (Sapphire Chip/Ruby Chip) must be left inside the Prism3.
The original double packaging and protective foam should be used for the Prism3.

To prepare the Prism3 instrument and naica® PC for shipment, proceed carefully as follows:

6. Remove the, Sapphire Chip/Ruby Chip, holder from the Prism3 instrument and ensure that no chips remain in the Prism3 instrument.
7. Switch off the power switch located at the back of the Prism3 instrument, disconnect the power supply cable, disconnect the mouse, keyboard, and naica® PC from the Prism3 instrument.
8. Open the front lid and push the motorized stage manually into the back panel right position. The threads should now be in the correct position to insert the lock screws.
9. Lock the motorized stage by tightly screwing the 2 knurled screws into the openings on the right and the back panel side of the housing.
10. Repack the Prism3 instrument into the original packaging in the same way it was delivered (contact Technical Support for detailed instructions for packing the instrument); please include in the packaging the power supply cable, the USB cable, and the chip holder.
11. For detailed packaging instructions, please refer to the packaging procedures available at the Technical Resources webpage.

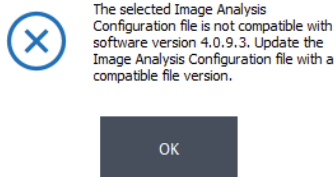
6. Troubleshooting

6.1. Updated Prism3_SapphireChip scanning templates

All default scanning templates have been improved and will automatically update with the v4.0 installers.

3-color naica® system users will benefit from:

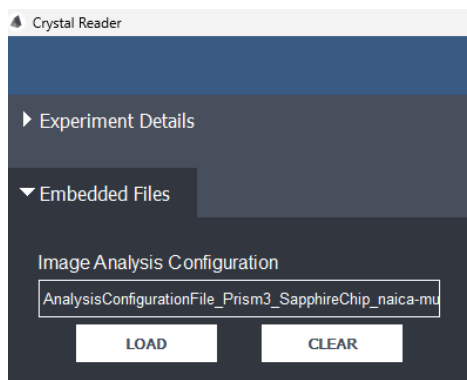
- Improved recognition of droplets, including better exclusion of smaller droplets and wetted droplets.



Note! For the 3-color naica® system all Prism3_Sapphire Chip scanning templates released with previous software versions become incompatible with the new available software v4.0. Only ScanningTemplate_Prism3_SapphireChip files with the ending “_v2.1.ncx” will be compatible with software version 4.0

The error message will appear if an incompatible ScanningTemplate_Prism3_SapphireChip file is used. To retrieve compatibility with previously customized scanning template files there are two options:

1. Starting with new software v4.0 ScanningTemplate file
 - Open Crystal Reader
 - Go to “New Experiment” and browse a new software v4.0 default ScanningTemplate file
 - All default ScanningTemplate files are available at C:\Program Files\Stilla\CystalReader\templates
 - Modify all experiment parameters according to the specific assay
 - “Save” or “Save as” experiment file in Crystal Reader
 - To save as new Scanning Template file open the experiment file in Crystal Miner
 - Go to “I/O” and “Save as template (.ncx)”.
2. Updating previously customized (v3.1 or below) ScanningTemplate files



- Open Crystal Reader
- Open the previously customized ScanningTemplate file
- Go to the “Embedded Files” section and load the respective new software v4.0 Image Analysis Configuration file (.yaml).
 - All Image Analysis Configuration files are available at C:\Program Files\Stilla\CystalMiner\config
- “Save” or “Save as” experiment file in Crystal Reader
- To save as new Scanning Template file open the experiment file in Crystal Miner
- Go to “I/O” and “Save as template (.ncx)”.

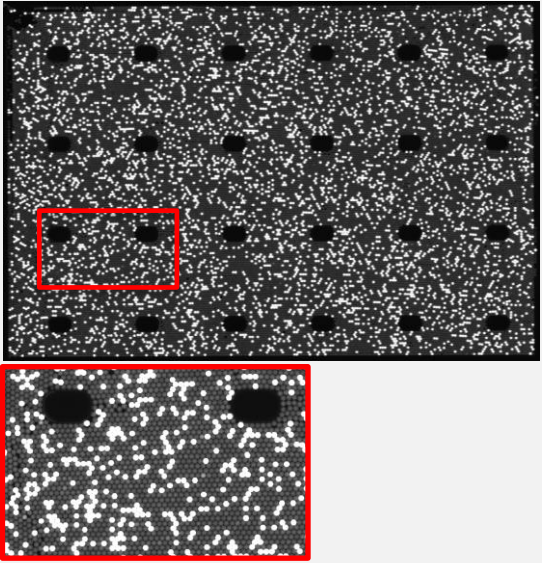
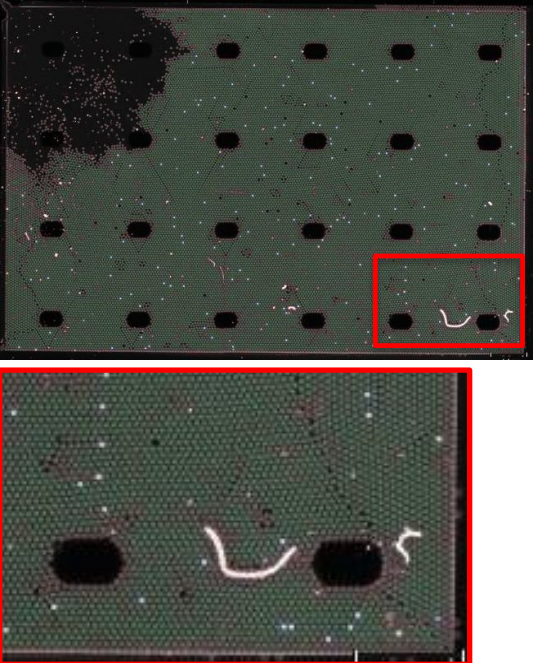
6.2. Prism3 and Crystal Reader software

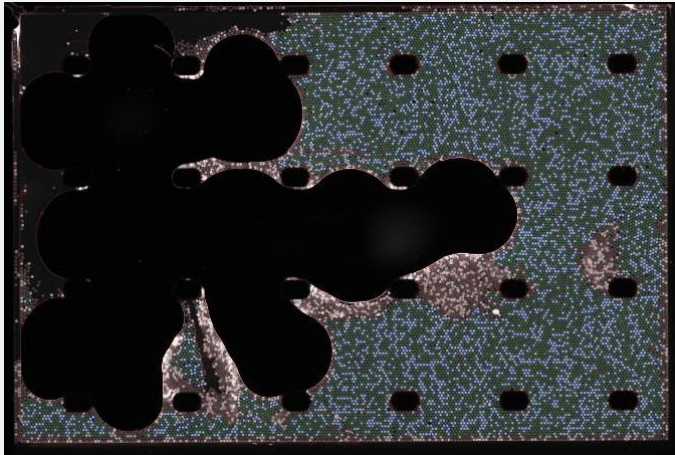
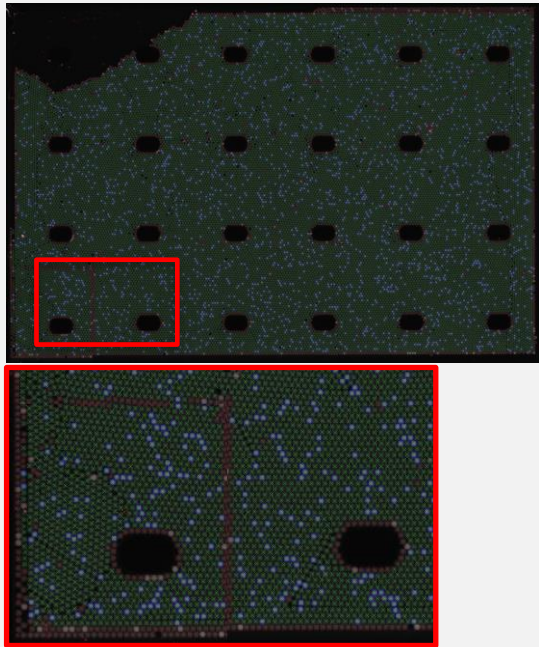
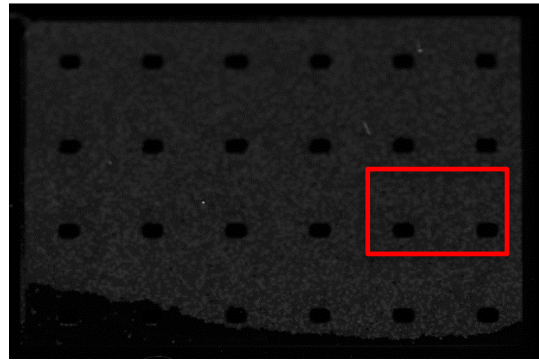
DISPLAY SCREEN ERROR MESSAGE	RECOMMENDATIONS
“Please check that the scanner is connected”	Check that the Prism3 instrument is turned on and correctly connected to the naica® PC (the gray USB cable should be plugged on the “PRISM3”-labeled port of the PCI express card located at the back panel of the naica® PC and on the USB port located at the back panel of the Prism3 instrument). Then, restart the Crystal Reader software. If the issue persists, reboot the naica® PC.
A red light appears in the third position of the front panel of the Prism 3 instrument	Reboot the Prism3 instrument (press the power button on the front for a few seconds until the Prism3 instrument shuts down, then press again to start it). Restart the Crystal Reader software. If the issue persists, reboot the naica® PC.
The Prism3 reader is not detected by the naica® PC	Go to “Control Panel” > “Device Manager” > “Universal Serial Bus controllers”, then check if there is a warning symbol on the PCI card driver named “Renesas USB 3.0 eXtensible”. In this case, right-click on this driver and try to deactivate and reactivate it (or to update it). If the problem persists, try to reboot the naica® PC.
The Crystal Reader software application stops responding	Go to “Control Panel” > “Task Manager” and kill the Crystal Reader process, then start it again.
The Crystal Reader software application no longer controls the Prism3 anymore (e.g., impossible to open/ close the tray, or to scan)	Check that no red light appears in the 3 rd position of the front panel of the Prism3. Otherwise check the scanner is connected (see above). Check that the naica® PC is correctly connected to the Prism3 (the gray USB cable should be plugged into the “PRISM3”-labeled port of the PCI express card located at the rear of the PC and on the “PC”-labeled port located at the rear panel of the Prism3), then close the Crystal Reader application, reboot the Prism3, and launch the Crystal Reader application.
Unstable connection between the Prism3 and the naica® PC	Check that the scanner is “ON” and correctly connected to the naica® PC (the original USB cable should be plugged in the “PRISM3”-labeled port of the PCI express card located at the rear panel of the naica® PC and in the USB port located at the rear panel of the Prism3), then close the Crystal Reader software application, reboot the Prism3, and launch the Crystal Reader software application. If the issue persists, reboot the naica® PC.
Image acquisition issue (Black or non-existent flag)	Re-scan the concerned chamber
Non-optimal image assembly in a chamber	Re-scan the concerned chamber
Error message: Unable to scan chamber(s)	Re-scan the non-scanned chamber(s)

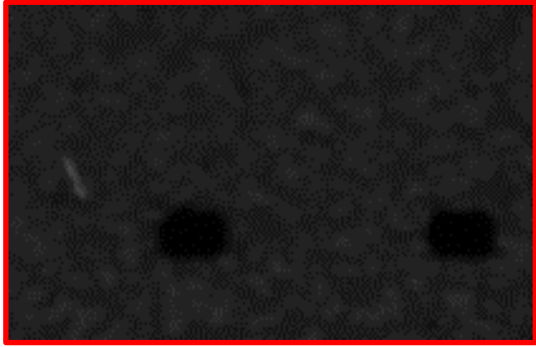
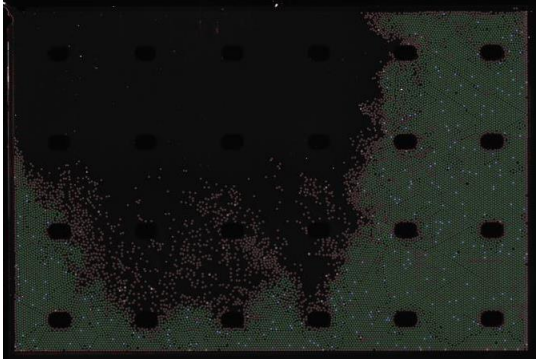
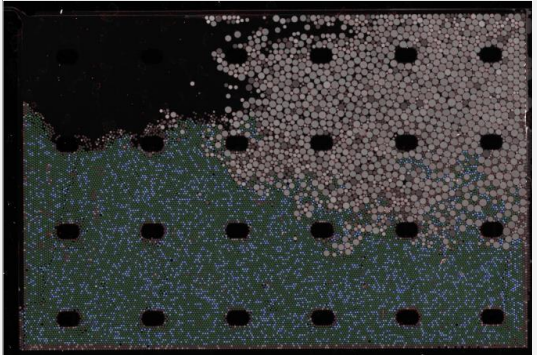
6.3. Sapphire Chip

OBSERVATION	POSSIBLE CAUSE	RECOMMENDATIONS
No, or few, analyzable droplets No air bubble	The partitioning step did not occur	<p>Check that the crucial partitioning step is included in the PCR program used (1st step). If the problem persists, please extract the live and system logs (refer to the section “How to use the Geode Software User Interface” in the Geode User Manual and contact Technical Support.</p>
	Problem during the pressure release step	<p>Check that the crucial pressure release step is included in the PCR program used (last step). If the problem persists, please extract the live and system logs (refer to the section “How to use the Geode Software User Interface” in the Geode User Manual and contact Technical Support.</p>
		<p>Make sure the experiment was not interrupted.</p>
Presence of a large droplet coalescence area in the chamber	Droplet Crystals may be sensitive to static shocks	Clean the foil of the chip with an anti-static spray and a Precision Wipe before the loading step.
Droplets overlapping (typically in the bottom right corner of the chamber)	There are too many droplets within the chamber	<p>Stacked droplets are considered artifacts and thus excluded from the analysis. To relax the crystal, bring back the chips to the Geode. In the menu, select Template > Protocols and launch the program named “Sapphire Protocol Unpacking Droplets”. After the reading step, the neural network of Crystal Miner should recognize more droplets.</p>
	Droplets are too soft (i.e., the surface tension is too low) The chip has been tilted for a few minutes	
The droplets in the chamber have low background fluorescence	Droplets are not detected.	<p>Check that the appropriate amount of reference fluorophore (e.g., fluorescein at 100 nM) was included in the reaction mix.</p>

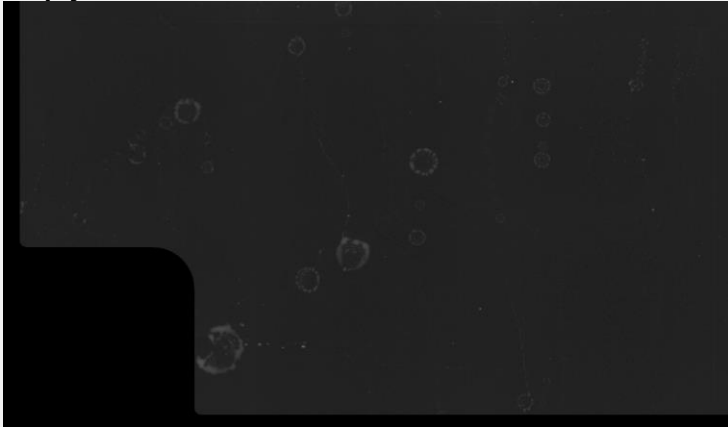
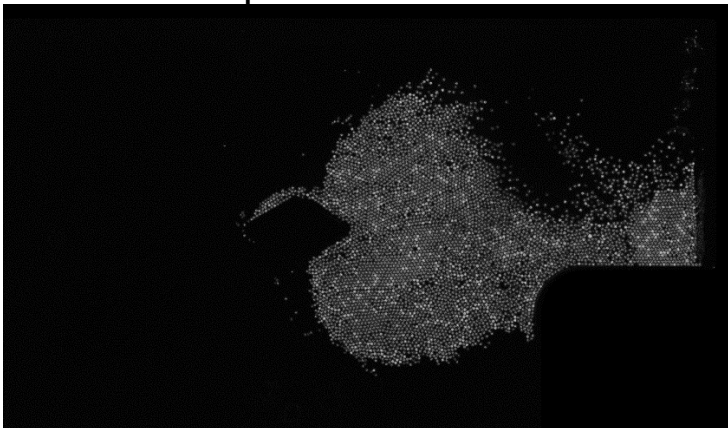
Presence of droplets of different sizes in the chamber.	Some microchannels are clogged.	Check the sample preparation conditions and/or the PCR mix for precipitates. Contact Technical Support for further information.
	There was a slight static shock on the chip	Clean the foil of the chip with an anti-static spray and a Precision Wipe before the loading step.
While using EvaGreen®, random exclusion of droplets.	The fluorophore EvaGreen® usually provides basic fluorescent background, necessary for droplet detection. In rare cases, this would not be sufficient.	Contact Technical Support.
Presence of an out of focus sub-image.	Autofocus failed during sub-image scanning	Rescan the concerned chamber.
Oversegmentation or undersegmentation of droplets.	There are two or more droplets (crosses) instead of one (oversegmentation) or one droplet instead of two or more (undersegmentation) which may lead to false positive or false negative results.	Manually inspect the droplets, in case of red crosses, the software automatically rejects the droplets, no action is required. However, if the crosses are green, then manual exclusion of the crosses is necessary.

OBSERVATION	POSSIBLE CAUSE	RECOMMENDATION
<p>Droplets with a saturated fluorescence level (saturation is indicated by the quality flags).</p> 	<p>The exposure time is too long for at least one channel</p>	<p>Saturated droplets have an intensity equal to $2^{16} - 1 = 65535$ RFU in at least one channel. They can be visible by clicking on "Display Saturation Map". Decrease the exposure time (usually by a factor 2) set for the detection channel where saturation is observed and re-scan the experiment.</p>
<p>Shiny dust particles on the external film of the chip.</p> 	<p>Presence of dust particles on the chip foil</p>	<p>Clean the foil with a Precision Wipe and re-scan the concerned chamber.</p>
<p>Large air bubbles in the chamber</p>	<p>Air was injected into the chamber during partitioning</p>	<p>Ensure to pipet 25 µL of the reaction mix. The volume should reach the top of the inlet.</p> <p>Be sure to not introduce air bubbles at the top of the inlet port or between oil</p>

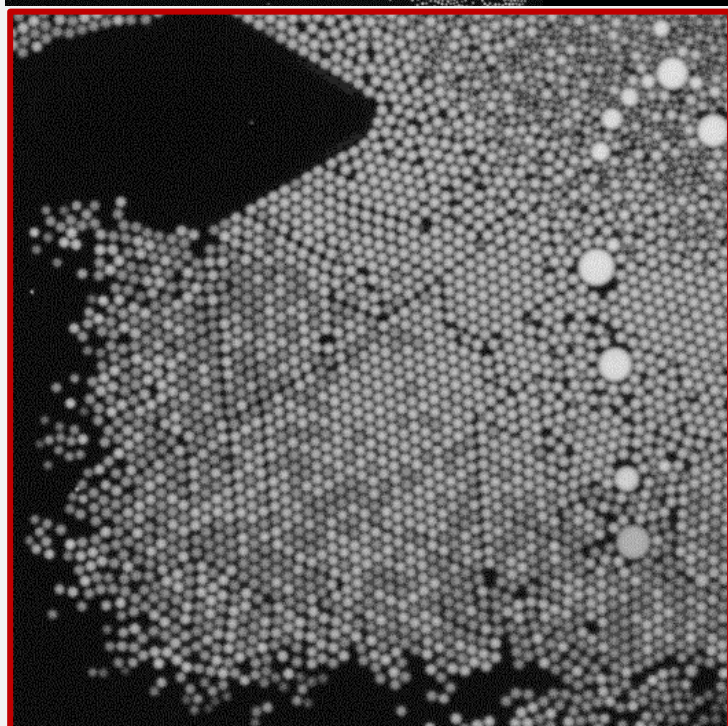
		<p>and PCR mix during the pipetting step.</p> <p>Check the expiry date of the used chips listed on the box.</p> <p>Make sure the white PCR caps are on the inlet port during PCR.</p>
<p>Image stitching event</p> 	<p>The Crystal Reader software stitches several images of the chamber taken subsequently into one final image. Droplets along the stitching lines are occasionally excluded to avoid over/under quantification of the final droplet numbers.</p>	<p>No action is required. Occasional image stitching events are expected with Crystal Reader software imaging. Image stitching events can occur with both chip types- Sapphire Chip and Ruby Chip.</p>
<p>The whole chamber is out of focus</p> 	<p>The chip and/or the holder is not well positioned</p>	<p>Verify that the chip and/or chip holder is positioned flat. A foreign particle might be present between the chip and the holder, forcing the chip to be tilted. Please clean the foil of the chip and the holder with a Precision Wipe and re-scan the concerned chamber.</p>

	<p>The focus value is not well calibrated</p>	<p>Please contact the Technical Support team for assistance.</p>
<p>The chamber is not optimally filled</p> 	<p>The sample volume loaded in the chamber was low.</p>	<p>The chamber is still analyzable as the partitioning step was successful. Depending on the assay, it is at the user's discretion whether the number of analyzable droplets is sufficient.</p>
<p>Different sizes of droplets present in the chamber (polydispersity)</p> 	<p>The procedure using the antistatic treatment was omitted or not properly performed.</p>	<p>The chamber is still analyzable as the partitioning step was successful. Depending on the assay, it is at the user's discretion whether the number of analyzable droplets is sufficient. Please refer to the IFU, for the Sapphire Chip or the Ruby Chip, for this step.</p>

6.4. Ruby Chip

OBSERVATION	POSSIBLE CAUSE	RECOMMENDATION
<p>Empty Chamber</p> 	<p>The sample was not pipetted in the correct well.</p> <p>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).</p> <p>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.</p>	<p>Take stock of the numbering on the wells to ensure that samples are always pipetted in the correct well.</p> <p>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 outwith the oil) before ejecting your sample from the tip</p> <p>For more information about how to properly pipet your sample in the Ruby Chip, please refer to the IFU for Ruby Chip.</p>
<p>Low number of droplets</p> 	<p>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).</p> <p>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.</p>	<p>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</p> <p>For more information about how to properly pipet your sample in the Ruby Chip, please refer to the IFU for Ruby Chip.</p>

Non homogenous distribution of positives



A part of the sample was injected in the chamber after the PCR cycles have begun. Hence, they have been amplified in bulk before being partitioned and they present a higher ratio of positive droplets.

The possible reasons for this late injection are:

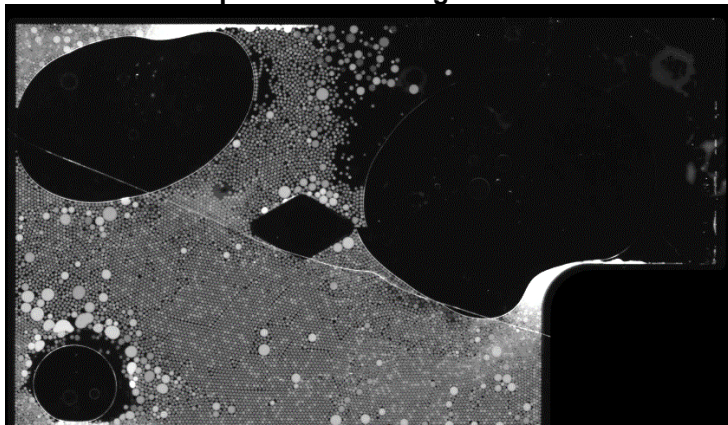
The sample was ejected from a tip that was introduced too deep (and could touch the bottom of the well) or insufficiently deep (i.e. it could have been at the air-oil interface).

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 just by 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.

Tension on transparent foil leading to oil traces



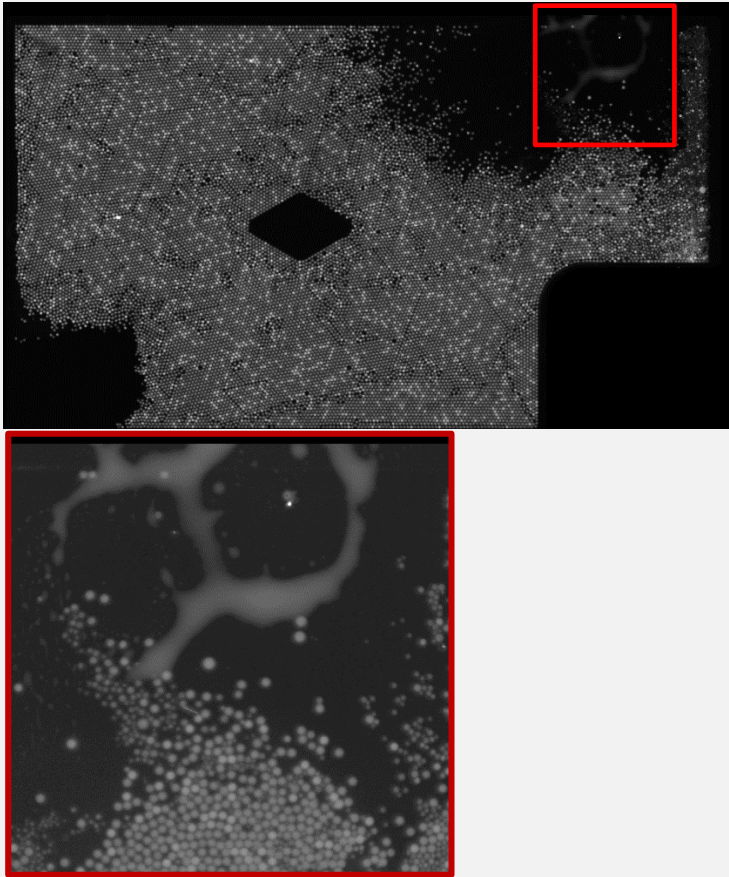
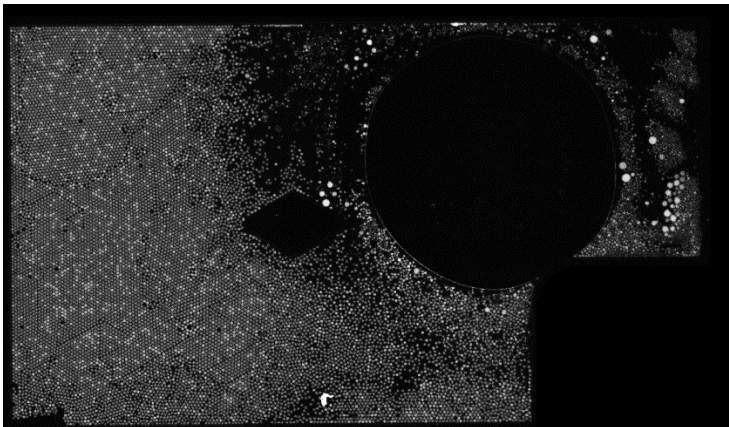
Incorrect pipette tips have been used to pierce the Ruby Chip. Not all the 16 wells were pierced before launching the PCR program.

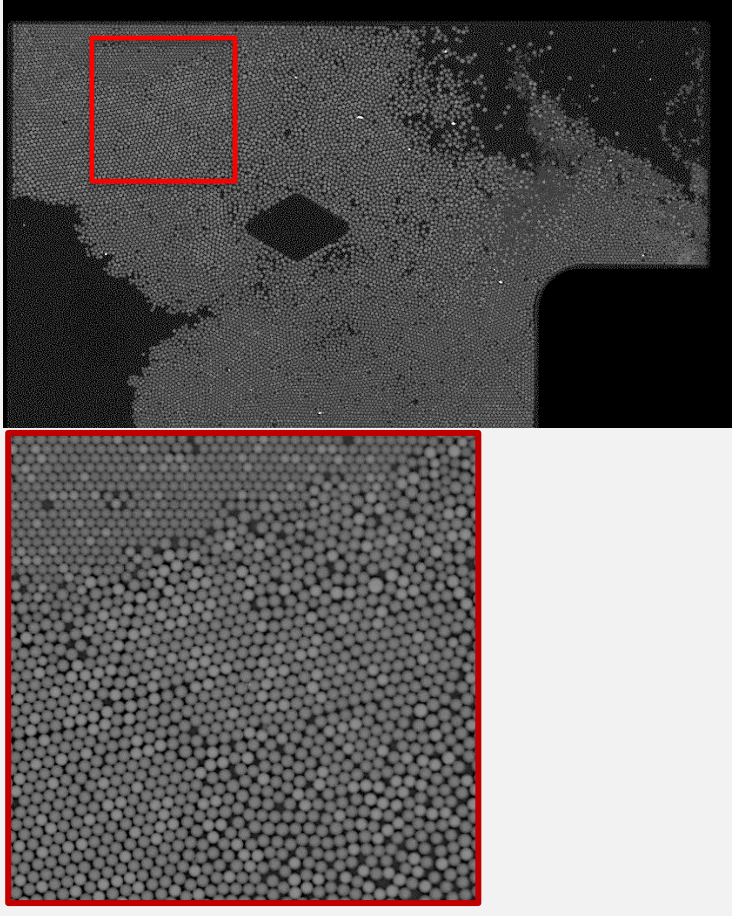
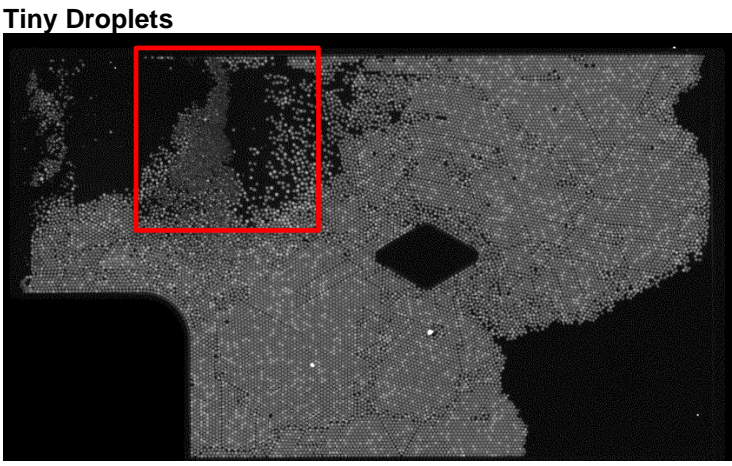
The heating plate was not clean and a dust particle generated

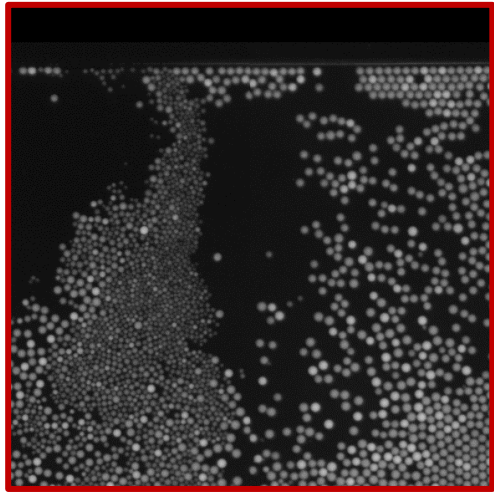
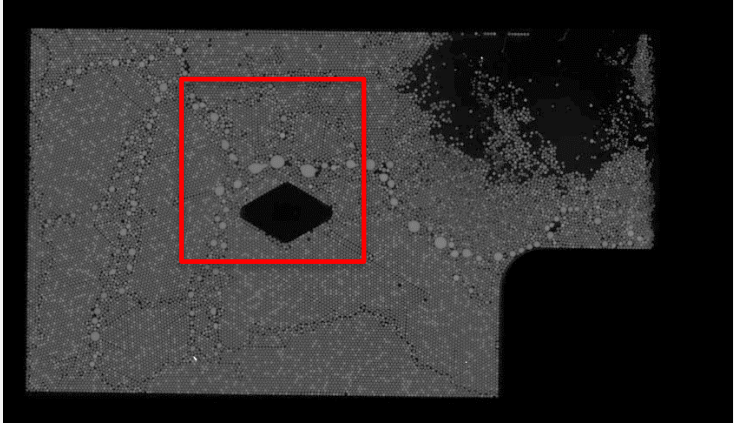
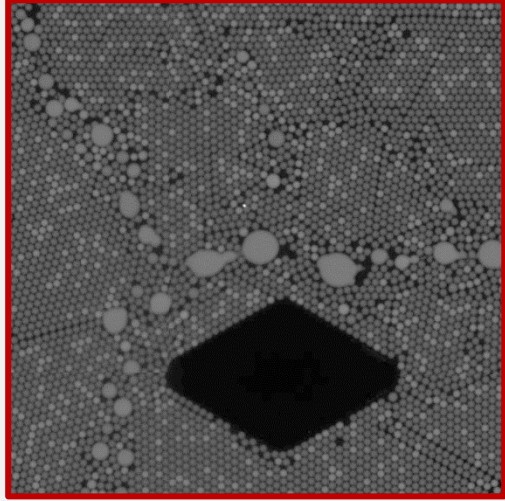
Only use 10µL and 20µL universal pipette tips as specified in the IFU.

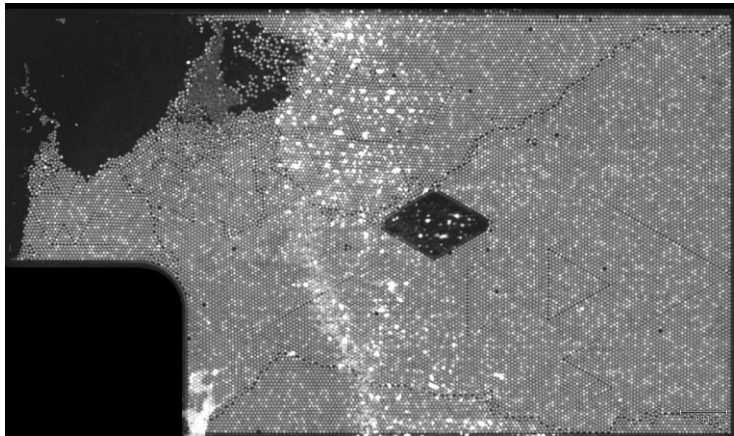
Always pierce all 16 wells of each chip, even when some wells will be left empty, before launching the PCR program.

Keep the heating plate clean from oil and dust. Clean traces of oil on a part of the Geode.

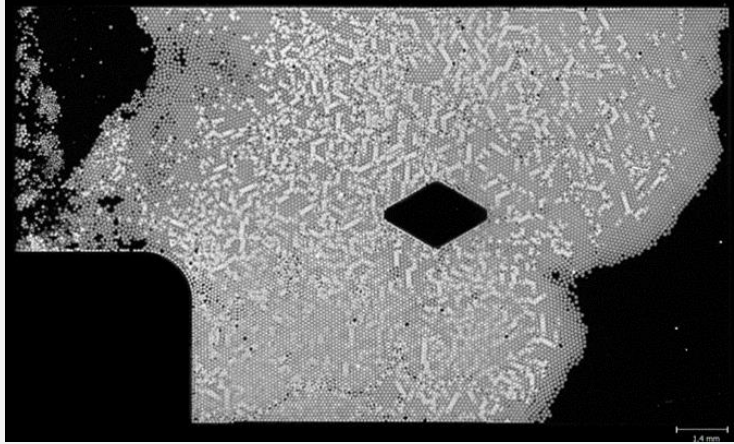
	<p>tension on the transparent foil.</p>	<p>For more information about how to properly clean the Geode, please refer to the cleaning and decontamination standard procedure</p>
<p>Oil Traces</p> 	<p>The chip contained oil that could condense outside of the wells in normal conditions of use.</p>	<p>Keep the heating plate clean from oil and dust. Clean traces of oil from any part of the Geode.</p> <p>For more information about how to properly clean the Geode, please refer to the cleaning and decontamination standard procedure.</p>
<p>Bubble</p> 	<p>Small bubbles can sometimes occur during the PCR run, due to heat and pressure cycles.</p> <p>If you observe big and frequent bubbles, the inflated bag containing the Ruby Chip might have been damaged during transport.</p>	<p>It is recommended to check that the inflated pouch is still airtight upon reception.</p>
<p>Bigger Droplets</p>	<p>The cycling of temperature and pressure could cause, in some rare cases, the</p>	<p>This population of droplets is excluded during the analysis. The software recognizes the</p>

	<p>presence of these artifacts.</p>	<p>different sizes of the droplets and considers them as artifacts. This phenomenon does not normally affect the output of the assay.</p>
<p>Tiny Droplets</p> 	<p>The cycling of temperature and pressure could cause, in some rare cases, the presence of these artifacts.</p>	<p>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.</p>

		
<p>Electrocoalescence</p>  	<p>The procedure using the antistatic wipes was omitted or not properly performed.</p>	<p>Apply the antistatic wipes on all the chips as described in the IFU for the chips. In case of further questions or support, please contact Technical Support</p>
<p>Traces of antistatic product</p>	<p>The presence of residual antistatic product under the chip generates artifacts in the blue channel.</p>	<p>After applying the antistatic treatment, wipe off the excess of antistatic product with a piece of Precision Wipe according the IFU instruction.</p>



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.

7. Software license information

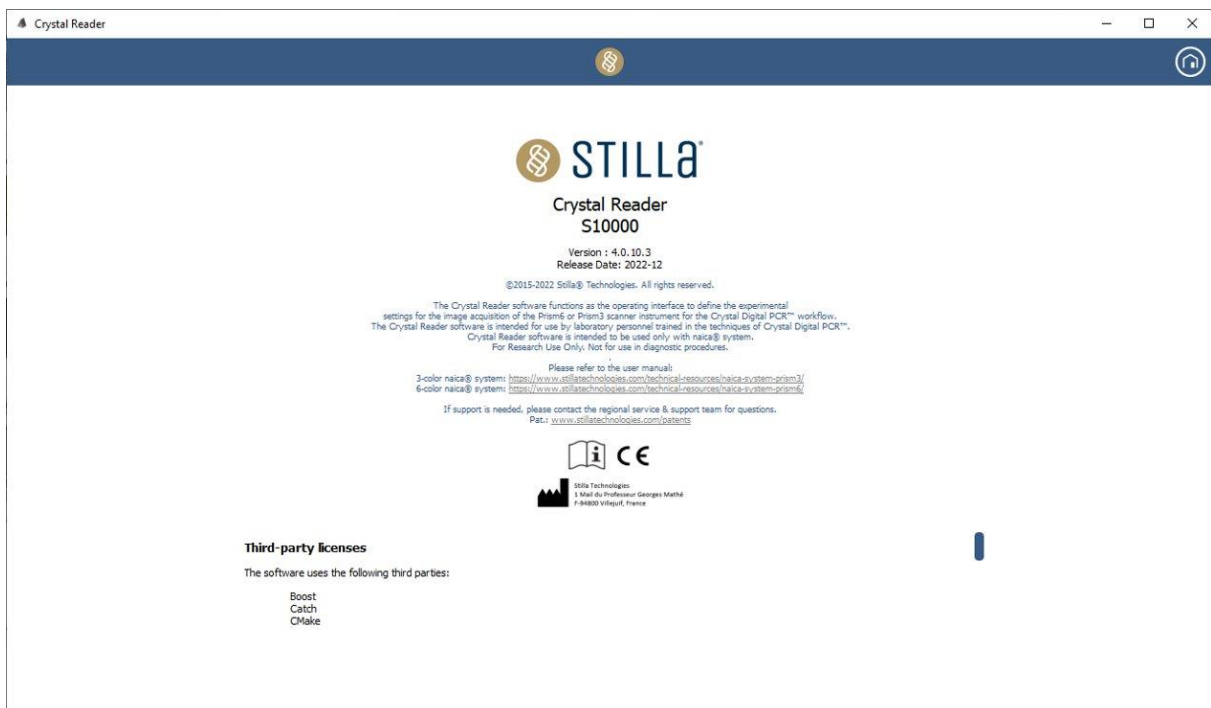
*Crystal Reader software license
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For Research Use Only.

Not for use in diagnostic procedures unless specified otherwise by domestic registration.

7.1. How to view the software version

From the HOME page, click on the “ABOUT” button. The software version will be displayed as well as the third-party license information:



The “About” page of the Crystal Reader software

7.2. Third-party licenses

The Crystal Reader software uses the following third-party software components:

- Boost
- Catch
- CMake
- CNTK
- cuDNN
- Docopt.cpp
- Eigen
- ETL
- GLC-Lib
- GSL (Guidelines Support Library)
- ITK
- JSON for Modern C++
- onnxruntime
- OpenCV
- Python
- Qt
- Quazip
- Qwt
- stlab
- xInt
- Yaml-cpp
- ZLib

To view the license information, please visit the third-party websites or check the Crystal Reader software and Crystal Miner software installation directory (by default in “C:\Program Files\Stilla\CrystalMiner\licenses”).

Note: The license information of all the third-party software components is also accessible in the Crystal Reader software, by clicking in “About” button on the Home page.

Prism3 and Crystal Reader software for the 3-color naica® system

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