Prism6 & Crystal Reader software for the naica® system

User Manual
Prism6 H24000.3/H24000.6
Crystal Reader software v3.0.7.3

TECHNICAL SUPPORT

For help and technical advice, please contact the Technical Support Department at Stilla Technologies. The Technical Support Department:
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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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I. Table of Contents

II. Introduction

1. Purpose of the document

This document provides general information for the use of the Prism6 instrument and its Crystal Reader software for Crystal Digital PCR™. The Prism6 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/technical-resources/naica-system-prism6/

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 (**Fig. 1**). The sample is first flowed through a network of microchannels and partitioned into a large 2D array of 15,000 to 30,000 individual droplets (Sapphire chips) and 10,000 to 20,000 droplets (Opal chips), also called a droplet crystal. PCR is then performed within the chips and the 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.

Sample loading	Generation of droplet crystal and PCR	Reading and analysis
		
Prepare the sample for the reaction mix. Stilla® 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, seal with the provided caps.	Place the prepared chips into the Geode. Launch the combined partitioning and amplification program: droplet crystals are generated from each sample and PCR amplification is performed immediately after crystal generation.	After PCR, transfer the chips to the Prism6 instrument. Set up the read-out using Crystal Reader software for data acquisition of droplet crystals using up to 6 fluorescent channels (Blue, Teal, Green, Yellow, Red and Infra-Red). Image analysis and data extraction are performed using the Crystal Miner software.

Figure 1: Overview of Crystal Digital PCR™ Workflow.

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 Prism6, which enables imaging of the droplet crystals in up to 6 detection channels. The Crystal Reader software is used to control and set up Crystal Digital PCR™ experiments on the Prism6 instrument.

The Crystal Reader software functions as the user interface to set up the experiment for the Prism6 and 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 Prism6 instrument for the acquisition of the fluorescence images of Sapphire or Opal chips.
- Applies pre-analysis treatments to the acquired images and provides a first quality control in preparation for the detailed experiment analysis performed by the Crystal Miner software.

The Crystal Miner software is used to extract data from the images acquired using the Prism6 and to calculate the absolute concentrations of the targeted nucleic acids. The software is pre-installed on the Prism6 delivered as part of the naica® system.

The naica® system performs Crystal Digital PCR™ within microfluidic chips (Sapphire chips & Opal chips). The naica® PCR MIX reagents are recommended for use 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 chips, Opal chips, 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 should be trained by a Stilla Technologies representative.

In general, Crystal Digital PCR™ can be performed with all types of DNA sample types 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 might influence sample compatibility for digital PCR applications as well. For application-specific references, please visit the website.

The Prism6 instrument is intended for use by professional users trained in molecular biological techniques and the operation of the Prism6 instrument.

The Prism6 instrument is part of the naica® system. The naica® system is intended for Research Use Only. Not for use in diagnostic procedures.

a) Prism6 packaging:

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
- Prism6
- Sapphire chips
- Opal chips
- Crystal Reader software
- Crystal Miner software
- naica® PCR MIX reagents:
  - naica® PCR MIX (Stilla Technologies, France)
  - naica® multiplex PCR MIX(Stilla Technologies, France).

III. Materials and Equipment

- Prism6 instrument (H24000.3/H24000.6)
  - 1 tray holder for Sapphire chips
  - 1 tray holder for Opal chips
  - 1 tray holder for generic 96 wells microtiter plates
- Prism6 peripheral box
  - Power cords
  - Monitor
  - Monitor USB cable
  - Mini display port cable
  - USB mouse
  - USB keyboard
  - naica® system accessory box
- Blue envelope
  - naica® system mouse pad

Prism6 safety Information:
• The following warning labels are located on the Prism6 instrument:

	**Caution - the possibility of pinch risk** Please ensure to correctly insert the tray holders onto the movable tray of the Prism6 instrument to avoid pinch risk. Avoid any manual operation of the Prism6 instrument flap to access the movable tray to avoid pinch risk.
	**Caution - optical radiation** High power light sources used for fluorescence imaging. Do not stare directly at the beam. Do not open the Prism6 instrument flap manually while the Prism6 instrument is switched on. Do not open the Prism6 instrument flap manually while the Prism6 instrument is in operation. Do not stare into the light beam when using a handheld bar code scanner.

	**Grounding point** This point is connected to the earth. Use this connection if grounding is required.
	**Caution – attention required** ○ The Prism6 instrument must be placed on a stable surface that is clean and vibration-free. ○ Do not block the area in front of the Prism6 instrument and ensure that the movable tray has sufficient space to move while the Prism6 instrument flap is opening and closing. ○ A sufficient clearance area around the Prism6 instrument is required to allow access to the main power switch. ○ A 10cm (4 inches) clearance must be left at the back of the device to ensure proper ventilation. ○ Before transportation, the respective tray holder (Sapphire chips & Opal chips) must be removed.

- Additional warning for the Prism6 instrument

	**Warning- heavy object** Do not try to lift the Prism6 instrument alone. Two persons are required to lift the Prism6 instrument.

- General safety instructions for the Prism6 instrument:

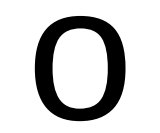
○ Please ensure that the Prism6 instrument is operated as instructed in the provided User Manuals. User Manuals are subjected to changes. The latest version can be accessed at the Technical Resources webpage.
○ Stilla Technologies cannot be held responsible for any damages or injuries arising from improper operation.
○ Do not use the Prism6 instrument if any parts are broken, chipped, rusty, or if the power cables are damaged in any way.
○ Do not open the housing of the Prism6 instrument if not explicitly instructed by a Stilla Technologies representative.
○ Opening the Prism6 instrument housing may breach any warranty for the Prism6 instrument.
○ Only operate the Prism6 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 Prism6 instrument.
○ For the Prism6 instrument, the mains supply source must meet the national regulatory requirements.
○ The power cord of the Prism6 instrument must be connected to a wall outlet with a grounded conductor.
○ For the Prism6 instrument, the mains voltage must correspond to the range given in the product specification.

b) Prism6 labeling

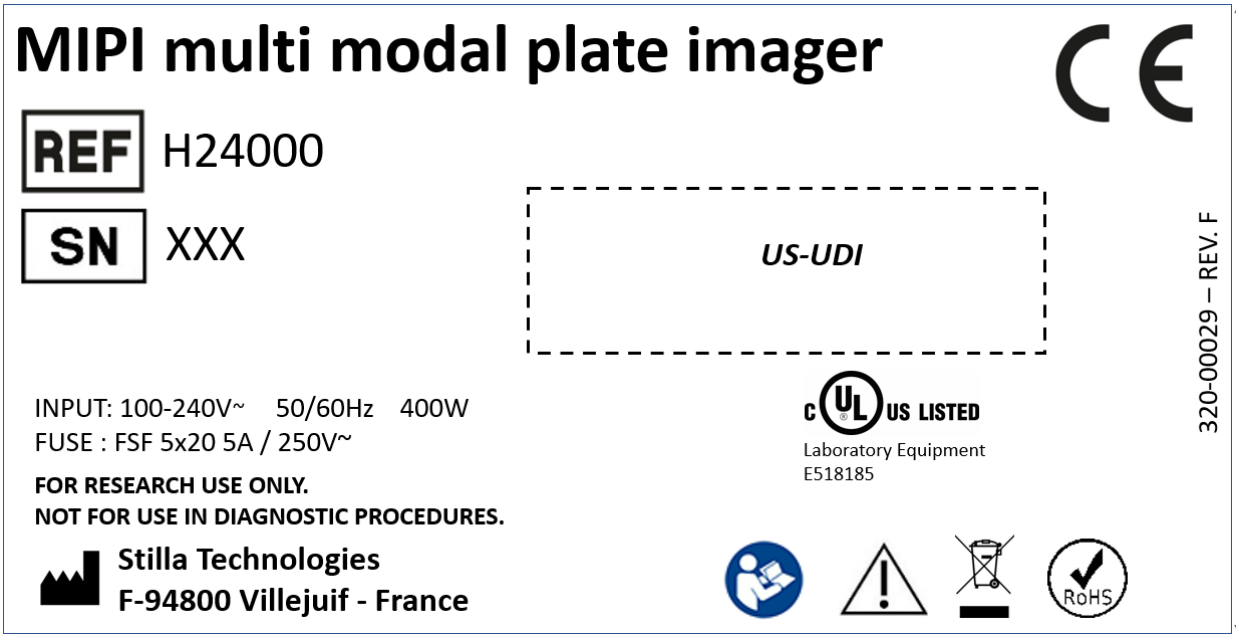
- Keep liquids away from the Prism6 instrument; avoid percolation of liquids inside the Prism6 instrument.
- For optimal use of the Prism6 instruments, the room temperature must be between 15°C and 30°C.
- Samples can be infectious or cause other damage to health. Safety regulations issued for the handling of sample material in the laboratory must be followed by wearing the proper Personal Protective Equipment (e.g., gloves and protective clothing).
- For instrument cleaning and decontamination follow the provided instructions. Do not use any other cleaning agents than specified.
- For instrument shipment only follow the detailed packaging and shipment procedures provided by Stilla Technologies. Do 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.

- Labeling symbols present on the Prism6 instrument

	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 requirements of the applicable EC directives).
	Power on.
	Power off.
	Protective conductor terminal.

- Labels present on the Prism6 instrument



d) Prism6 instrument specifications

To view the declaration of conformity certificate, please visit the Technical Resources webpage.

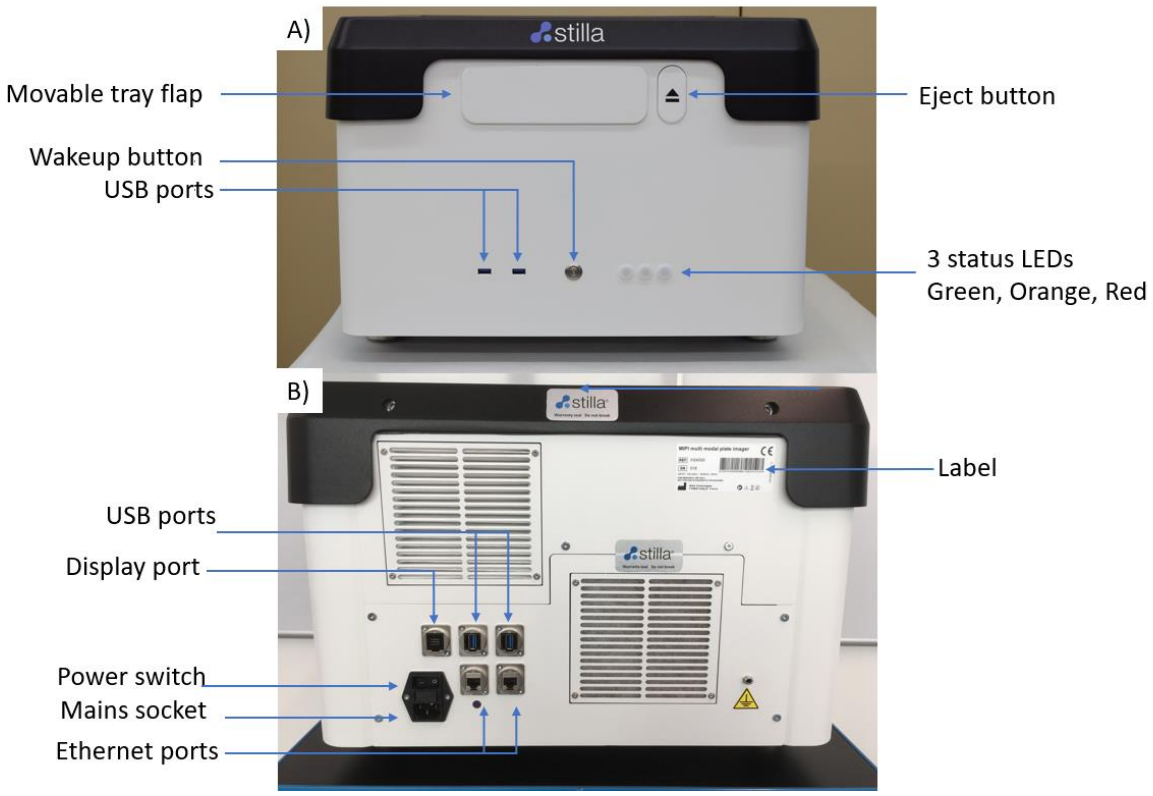


Figure 1: A) Front panel of the Prism6 instrument. B) Back panel of the Prism6 instrument.

Fig. 1 A front panel of the Prism6 instrument:

- a flap where chips are loaded
- an eject button to eject the movable tray from the Prism6
- 3 status LEDs (Green Orange, Red)
    - Green: Prism6 instrument ready for operation
    - Orange: Prism6 instrument busy (instrument initialization, instrument operation)
    - Red: Prism6 instrument in failure mode
- 2 USB 3.0 ports for data transfer
  Note: Differences in USB key compatibility with the Prism6 front panel USB ports can occur. Stilla Technologies recommends to use a USB key with a maximum capacity of 8Gb.The compatibility of individual USB keys needs to be validated by the end user for functional data transfer.
- 1 soft power button to turn on the instrument if it has been powered up beforehand

Fig. 1 B back panel of the Prism6 instrument:

- 2 USB 3.0 ports
- 1 display port to connect the Prism6 monitor
- 2 ethernet ports
- 1 label
- 1 socket for the mains
- 1 power switch

Technical specifications	
Instrument dimensions & weight	
Instrument dimensions (W x D x H in mm)	514 x 564 x 365

e) Prism6 installation instructions

Instrument weight	23 kg	
Sample throughput capacity & scanning times		
Sample throughput capacity per run	Up to 3 chips / run: Sapphire chips: up to 12 samples / run Opal chips: up to 48 samples / run	
Scan time per sample	~ 3-4 min / sample for Sapphire chips < 2 min / sample for Opal chips	
Scan time per run	Depending on the number of LED selected: 3-color scanning: 30 min/run for 3 Sapphire chips 60 min/run for 3 Opal chip 6-color scanning: 45 min/run for 3 Sapphire chips 80 min/run for 3 Opal chips	
Specifications for chip re-scanning	Up to 3 scans of the same chip within 48h	
Imaging system		
LED	**Excitation wavelengths [nm]**	**Emission wavelengths [nm]**
Blue	445-490	503-537
Teal	504-526	527-551
Green	540-560	566-597
Yellow	562-588	598-642
Red	623-643	650-684
Infrared	675-698	704-755
Recommended fluorophores*	FAM, Yakima Yellow®, Atto550, ROX, Cy®5, Atto700/Cy®5.5	
Result data format	16bit TIFF grayscale, CSV spreadsheet, XML files	
Ambient condition		
Pollution degree	2	
Operation		
Environmental conditions	Clean indoor laboratory	
Temperature	+15°C to +30°C	
Relative humidity	20% to 90% non-condensing	
Altitude	Operating at max. 1000m above sea level	
Barometric pressure	795 hPa to 1060 hPa	
Noise	<45 dB at a distance of 1 meter	
Electrical		
AC input	100-240V~, 50/60Hz	
Installation category	II	
Input mains fluctuation	Max. 10%	
Input power	Max. 400W	

*Additional fluorophores within the specified wavelengths can be used, fluorophore selection requires validation depending on the digital PCR assay design.

- **<u>Operating requirements</u>**

Proper infrastructure requirements for the Prism6 installation:
- Clean laboratory environment
- Sturdy surface, for supporting up to 40 kg
- A minimum distance of 10 cm to neighboring objects around the Prism6 instrument
- Room temperature between 15°C and 30°C

- **<u>Installation and performance validation</u>**

The initial naica® system installation, including the Prism6 instrument, is always executed by a Stilla Technologies' Service Specialist. The installation includes the performance validation of Stilla Technologies Crystal Digital PCR™ specifications for all components of the naica® system, including the Prism6 instrument. At the end of the naica® system installation, Stilla Technologies

provides a certification guaranteeing the adequate naica® system installation and validation. The certification is required to release the naica® system for end-user operation.

IV. Operating the Prism6 instrument

a. General considerations and prerequisite for using Crystal Reader software

The experiment analysis step can be performed either directly on the naica® system or on any other PC which fulfills the following specifications for optimal Crystal Reader software performance:

- Operating System: Windows 10 in 64 bits
- RAM: at least 16 GB
- Processors: Intel Core i5 or higher, at least 2 cores of 2 GHz or higher
- Graphical Card: recommended (equivalent to NVIDIA GeForce GT 1030 or higher)
- Screen resolution: at least 1920 x 1080; aspect ratio 16:9

To download the latest version of the Crystal Reader software to install it on another PC, visit the Technical Resources webpage.

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

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

The original configuration and calibration files for the Crystal Miner software are located on the Prism6 instrument by default at ”C:\Program Files\Stilla\CrystalReader\config”. Stilla Technologies does not recommend reading, modifying or deleting any of the following files:

- AnalysisConfiguration_GenericTemplate.yaml
- AnalysisConfiguration_GenericTemplate_Classical.yaml
- AnalysisConfiguration_GenericTemplate_mix-XLT.yaml
- AnalysisConfiguration_GenericTemplate_Opal.yaml
- AnalysisConfiguration_GenericTemplate_Opal_Classical.yaml
- AnalysisConfiguration_naica_multiplex_PCR_MIX_Prism3.yaml
- AnalysisConfiguration_naica_PCR_MIX_Prism3.yaml
- AnalysisConfiguration_prism_opal_naica-mix-Classical.yaml
- AnalysisConfiguration_prism_opal_naica-mix.yaml
- AnalysisConfiguration_prism_sapphire_naica-mix.yaml

b. Prism6 instrument initialization

The Prism6 instrument is operated through the monitor, keyboard, and mouse connected to the back panel of the Prism6 instrument.
To turn on and initialize the Prism6 instrument, and log into the Crystal Reader software, follow these steps:

1. Switch on the Prism6 instrument.
   First, switch on the power button located at the back of the Prism6, then press the wake-up power button located on the front panel of the Prism6 instrument. Wait for instrument initialization. The left-most light on the front LED panel of the Prism6 instrument lights up green.

2. Turn on the Monitor of the Prism6 instrument. With instrument initialization, the Microsoft Windows Login window appears. The default password is “admin” for “admin” user. Refer to Microsoft Windows User Manual regarding account features.

3. 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.

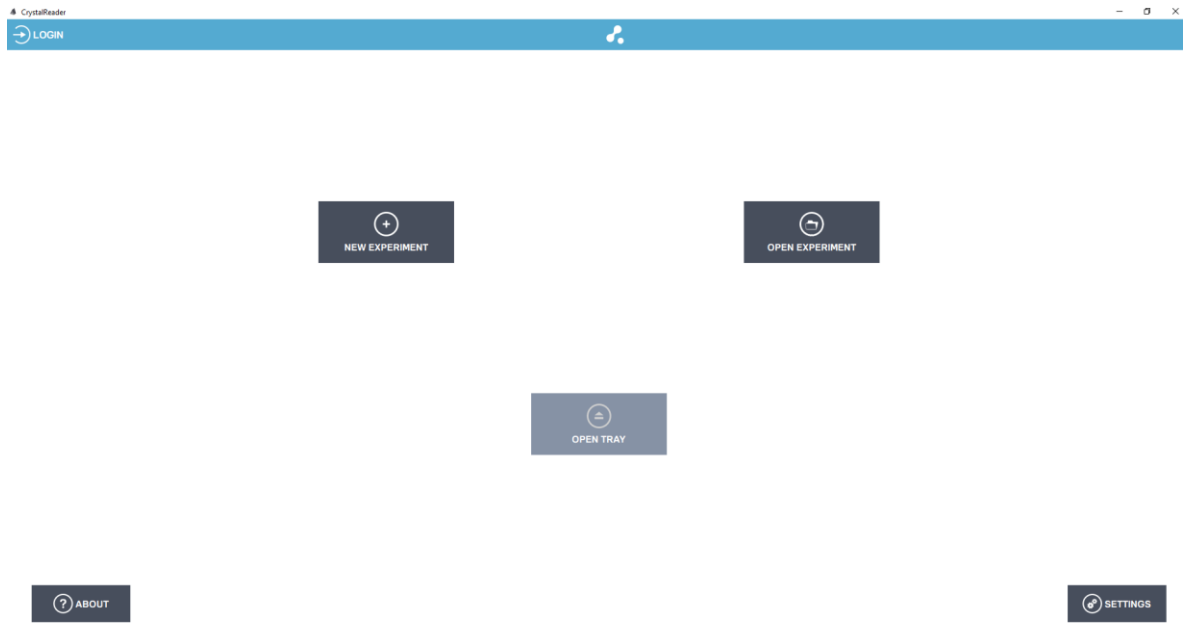


Figure 2: Crystal Reader software homepage with disabled “OPEN TRAY” button during Prism6 initialization or operation mode.

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 Opal 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 Prism6 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 Prism6 instrument settings. Please refer to How to edit the Settings for different user profiles section for detailed instructions.

4. Eject the moveable tray for chip positioning

a) Press the “OPEN TRAY” button on the Crystal Reader software homepage.
b) Alternatively, press the “Eject” button located at the front of the Prism6 instrument (Fig 1).

Wait until the moveable tray is fully ejected before placing the chips for scanning.

Figure 3: Fully ejected position of the movable tray. In this position, the moveable tray is ready to be loaded with the tray holder for chip scanning.

CAUTION! Do not manually open the Prism6 instrument flap. Do not manually engage with the movable tray. Do not manually pull out the moveable tray any further.

c. Instructions for chip positioning

Note: Prior to chip positioning for scanning read the respective Sapphire chip and Opal chip Instructions for Use for detailed instructions on how to clean the bottom surface of each chip and for automatic chip ID barcode reading using a handheld USB barcode reader.

Figure 4 -: Sapphire tray holder (left) & Opal tray holder (right)

Three kinds of tray holders are provided with each Prism6 instrument:

- Opal tray holder – H24001, to be used with Opal chips only
- Sapphire tray holder – H24002, to be used with Sapphire chips only

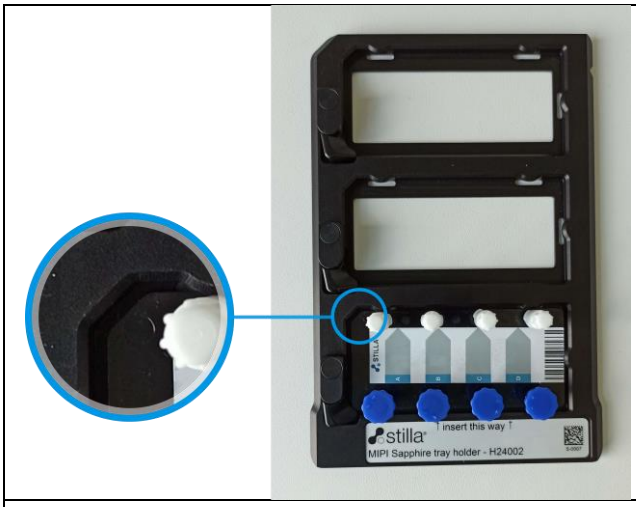
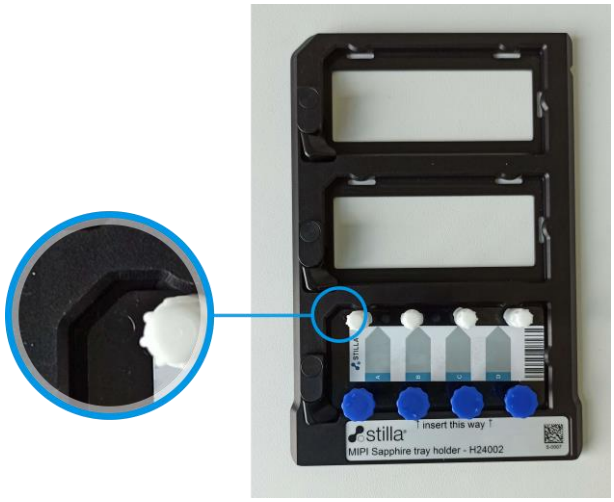
- 96 well plate tray holder – H24003, to be used for Prism6 instrument service maintenance operation only. Only to be used by Stilla Technologies Service Specialist.

Up to 3 chips can be fitted into one tray holder for scanning at a time.
The direction for insertion of the tray holders onto the moveable tray is indicated on the individual tray holder labels. Stilla Technologies recommends cleaning all tray holders occasionally with 70% ethanol solution, rinse with water using a lint-free wipe.

Position one of the chips (Sapphire chips or Opal chips) in the individual slots of the corresponding tray holder (Sapphire tray holder or Opal tray holder). Check for correct positioning. The respective chips should fit perfectly into the individual slots.

Instructions to position the chips in the tray holder.

<table>
<tr><td colspan="2">Example provided for Sapphire chips and Sapphire tray holder only. Similar instructions apply for Opal chip placement in Opal tray holder. Differences are indicated in the provided text description with the respective image instructions.</td></tr>
<tr><td> 
</td><td>Place the tray holder on a clean surface.</td></tr>
<tr><td> 
</td><td>Release the chip clamp by rotating it away from the slot.</td></tr>
</table>

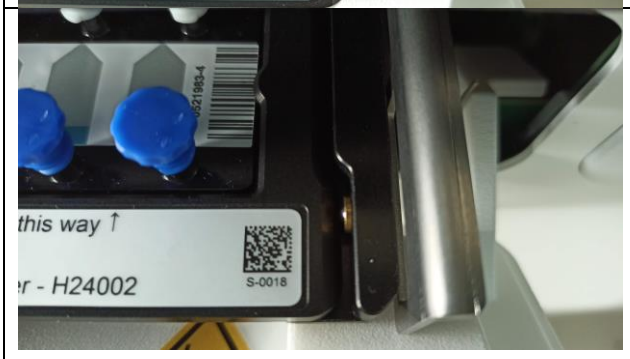
	Insert the chip in the tray holder. Each type of chip has a poka-yoke corner matching the respective tray holder. **Note :** - Sapphire tray holder - top-left corner - Opal tray holder - bottom-right corner
	Ensure the respective chip type is pushed against the top-right corner of the slot

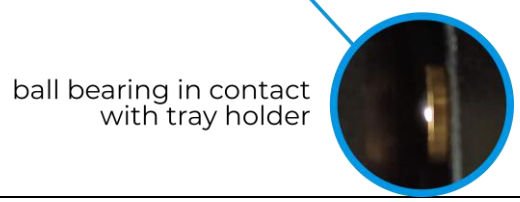
	Correct positioning of a chip in the tray holder.
	Incorrect positioning of a chip in the tray holder. The chip is lying at an angle in the tray holder and is not well attached to the top-right corner of the tray holder.
Repeat the described chip positioning operation for all the tray holder slots to be used.	

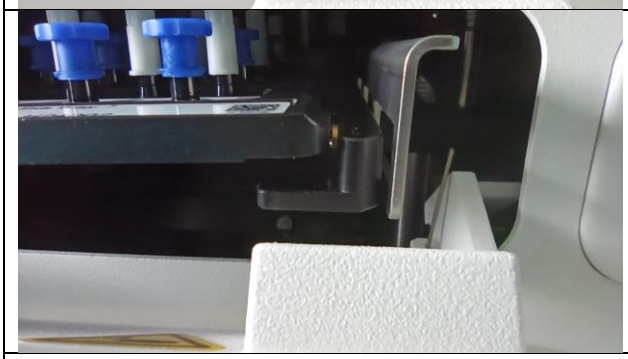
Note: *Make sure to use the specific Sapphire tray holder or Opal tray holder with the respective chip types only. Do not operate Sapphire chips on Opal tray holder. Do not operate Opal chips on Sapphire tray holder. Do not mix chip types on one tray holder. The tray holder is clearly identifiable by the tray holder label*.

Instructions for loading the tray holder in the movable tray of the Prism6 instrument:

Example provided for Sapphire chips and Sapphire tray holder only. Identical instructions apply for Opal tray holder placement.

<table>
<tr><td> 
</td><td>Place the tray holder on the movable tray</td></tr>
<tr><td> 
</td><td>Gently push the tray holder towards the back of the moveable tray.</td></tr>
<tr><td> 


</td><td>The tray holder is correctly positioned after the spring-loaded ball bearing (blue circle) is set in position with a distinct click.</td></tr>
<tr><td colspan="2">Ensure the tray holder is lying flat on the movable tray.</td></tr>
</table>

	Correct placement of the tray holder on the moveable tray.
	Incorrect placement of the tray holder on the moveable tray.
Retract the moveable tray for chip scanning. a) Press the “CLOSE TRAY” button on the Crystal Reader software homepage. b) Alternatively, press the “Eject” button located at the front of the Prism6 instrument (Fig 1). Ensure that the moveable tray is fully retracted and the Prism6 instrument flap is fully closed before chip proceeding for chip scanning.	

Note: In case the tray holder, one or multiple chips or part of chips enter the Prism6 instrument interior, turn off the Prism6 instrument immediately and contact Technical Support.

d. Instructions for chip scanning

Crystal Reader software is the operating system of the Prism6 instrument to set up the experimental conditions for image acquisition.

e. Definition of a Crystal Digital PCR™ experiment

An experiment is 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 chips or Opal chips). Chip types cannot be mixed in a single experiment for image acquisition with the Prism6 instrument.
- The samples of the individual experiment were prepared using the same PCR mastermix and the same PCR program for partitioning and amplification with the Geode.
- The same scanning parameters, embedded files and the experimental details will be applied to all samples for an individual experiment during a Prism6 run, i.e.,:
  - the same exposure times (in ms) for each of the 6 LEDs;
  - the same focus value (in mm)
  - Fluorophores and targets names
  - Image analysis configuration
  - Optional:
    - Spillover compensation matrix
    - Plots configuration
    - Analyzing configuration

If different scanning parameters or experimental details 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.

f. Experimental set-up with Crystal Reader software

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

(1) Create a new experiment by selecting the scanning template file
(2) Confirm or modify the experiment context in the Experiment Edition page
(3) Define chamber details
(4) Launch the experiment scan
(5) Evaluate scanning performance

Overview of the Edit experiment page where the experiment set up will be done:

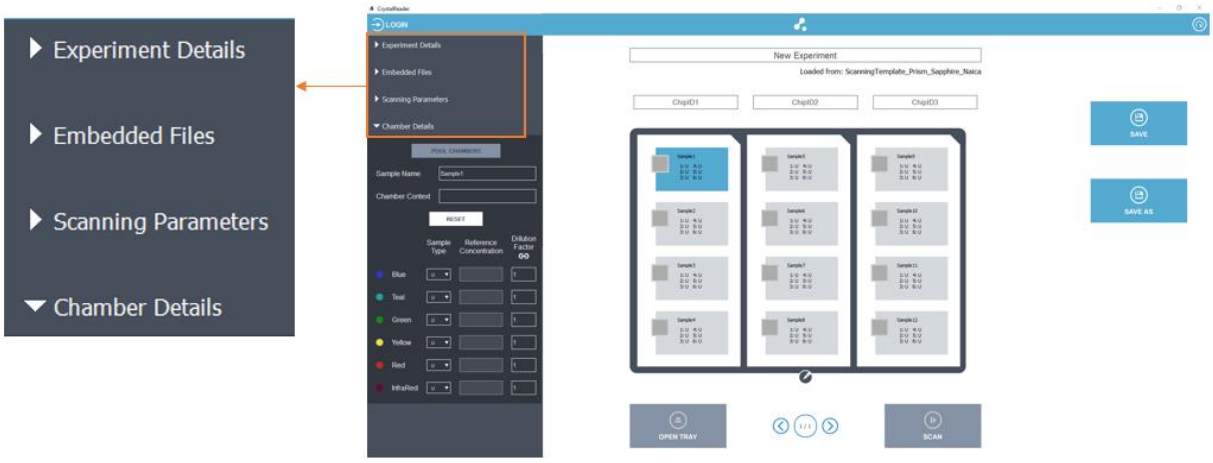


Figure 5: The Crystal Reader new experiment screen. The example visualizes the experimental set up for a Sapphire chip experiment. The categories on the left panel can be edited for each experiment.

1. Step (1) - Create a new experiment & define the experiment context:

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

It consists of loading a ".ncx" as an empty experiment and the user is then invited 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_Prism6_SapphireChip_naica-multiplex-PCR-MIX_Taqman_v1.0.ncx
- For an experiment using the naica® PCR MIX compatible with EvaGreen® fluorescent intercalating dye and the Opal chip select
  ScanningTemplate_Prism6_OpalChip_naica-PCR-MIX_Evagreen_v1.0.ncx.

  Note: Stilla Technologies does not recommend modifying the provided file templates. 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.

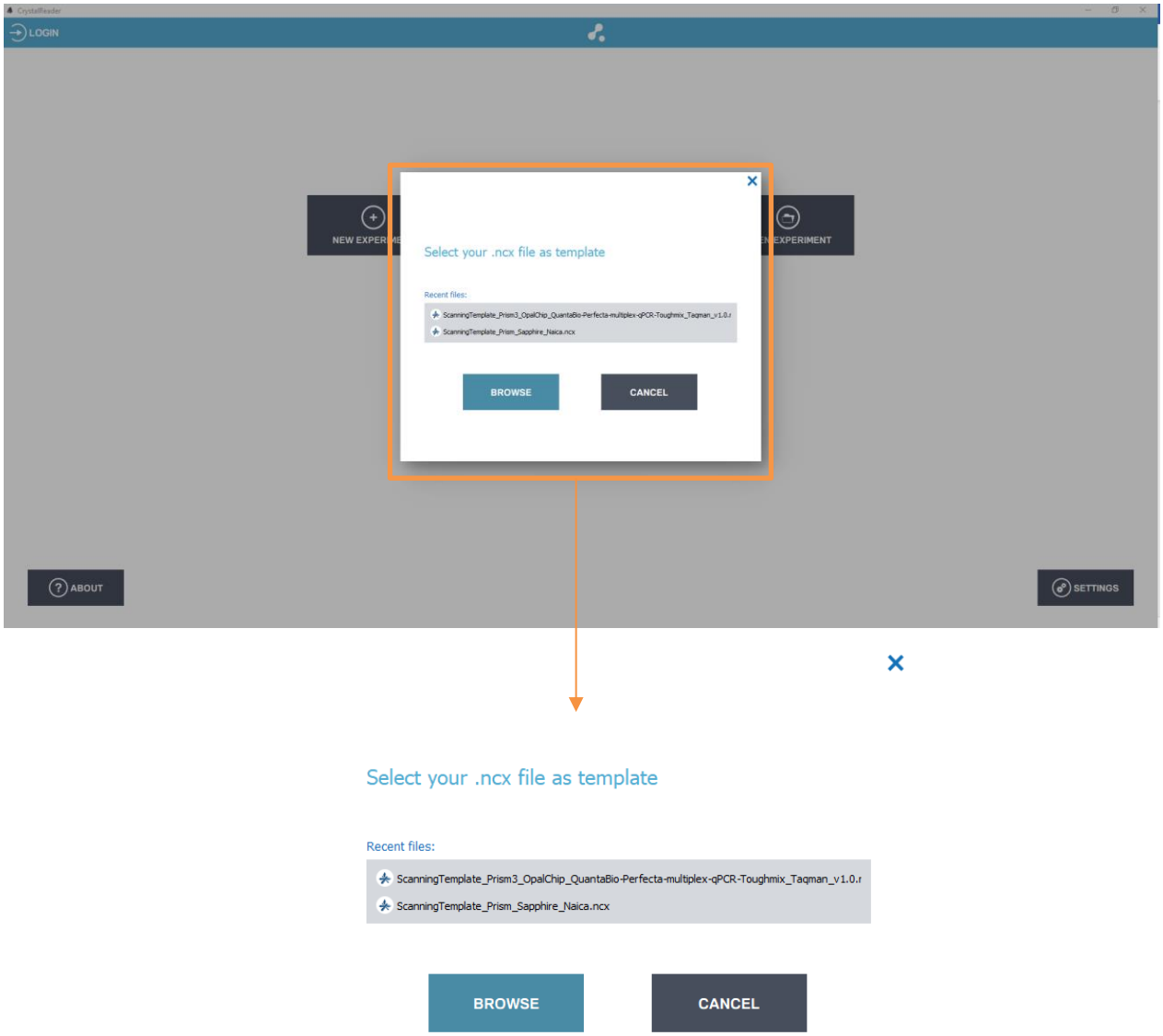


Figure 6: Crystal Reader software prompt to select the respective scanning templates (“.ncx” format) for the experiment.

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**. The user can use the chamber details default settings or load the chamber details that are embedded in the scanning emplate. This

feature will provide the user a faster way to set up an experiment context that will be run several times.

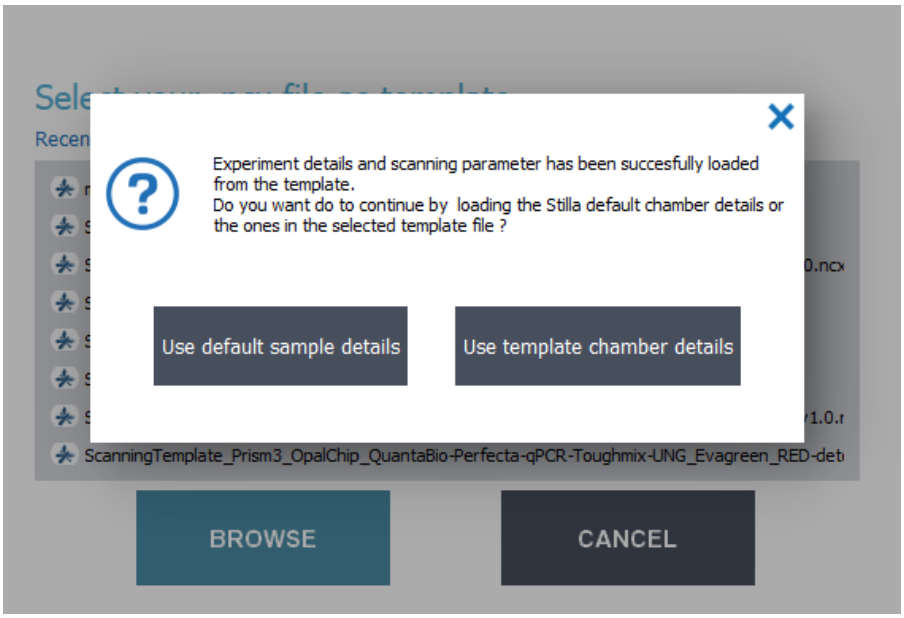


Figure 7: Crystal Reader software pop-up window prompt the chamber details configuration that the user can load.

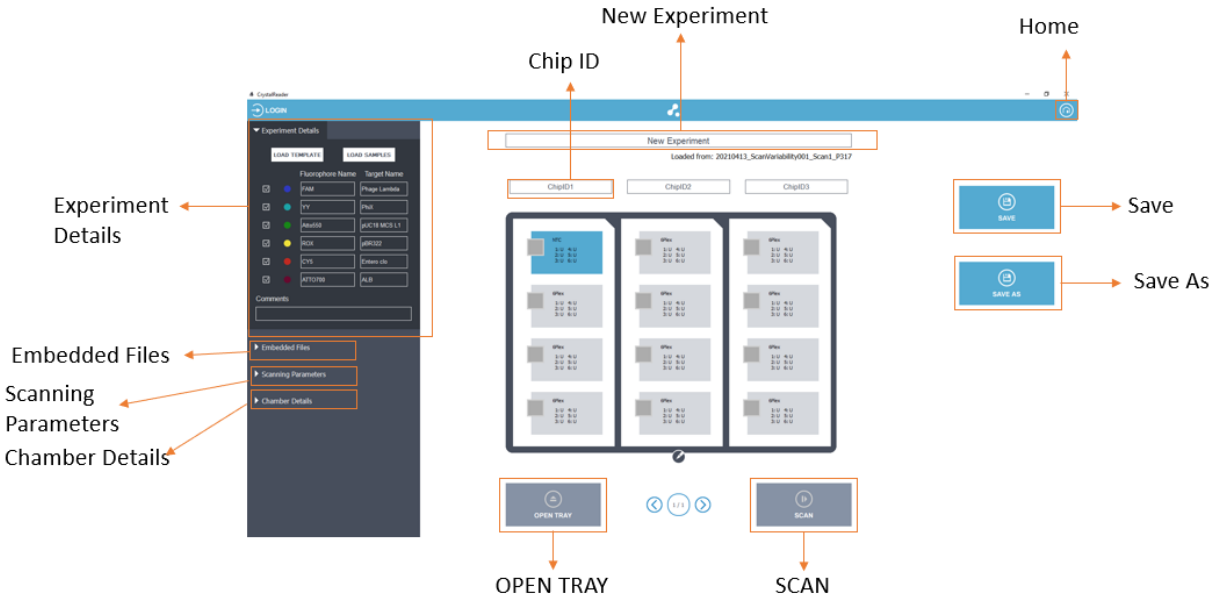


Figure 8: The Crystal Reader software home page for the Sapphire chips.

a) Name the experiment:

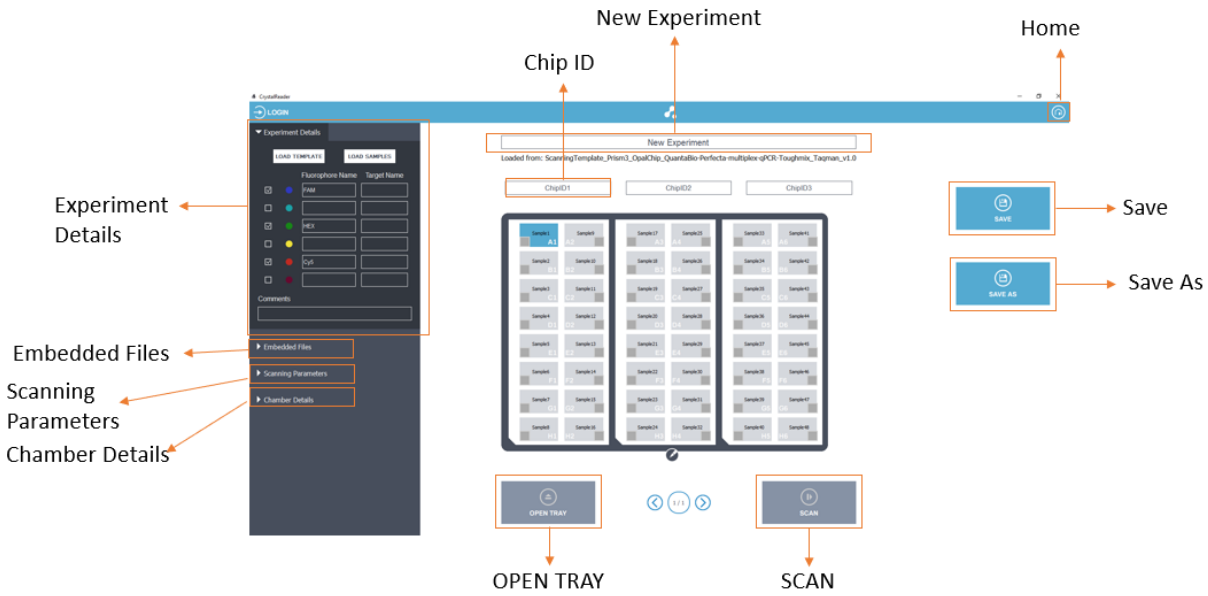


Figure 9: The Crystal Reader software home page for the Opal chips.

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

- Enter the experiment name in the “New Experiment” 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_6plex).

Figure 10: Enter Experimental Details in the “New Experiment” interface.

b) Edit experiment details:

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

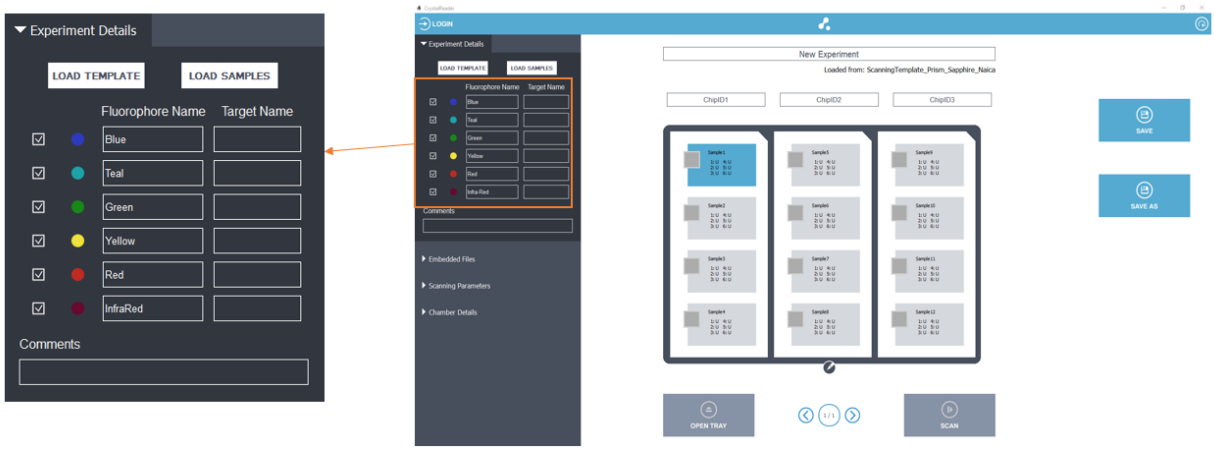


Figure 11: Enter fluorophore and target names in the “Experiment Details” interface.

- Go to the “Experiment Details” button in the left panel, then enter for each detection channel (“Blue”, “Teal”, Green”, “Yellow” “Red” and “Infra-Red” LED):
  - 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 from scanning.
  - Selecting a minimum of three detection channels, including blue are mandatory (it is the main detection channel).

Note: Ensure to select the appropriate detection channel for the experiment to avoid any false-positive results. In case of selection of a wrong detection channel, re-scan the chips.

- Go to “Embedded Files” selection menu and select the desired:

The following files are (or can be) embedded into the experiment file:

- The Image Analysis Configuration File (mandatory)
- The Spillover Compensation Matrix (optional)
- The Plots configurations (optional)
- The Analysis Configuration (optional)

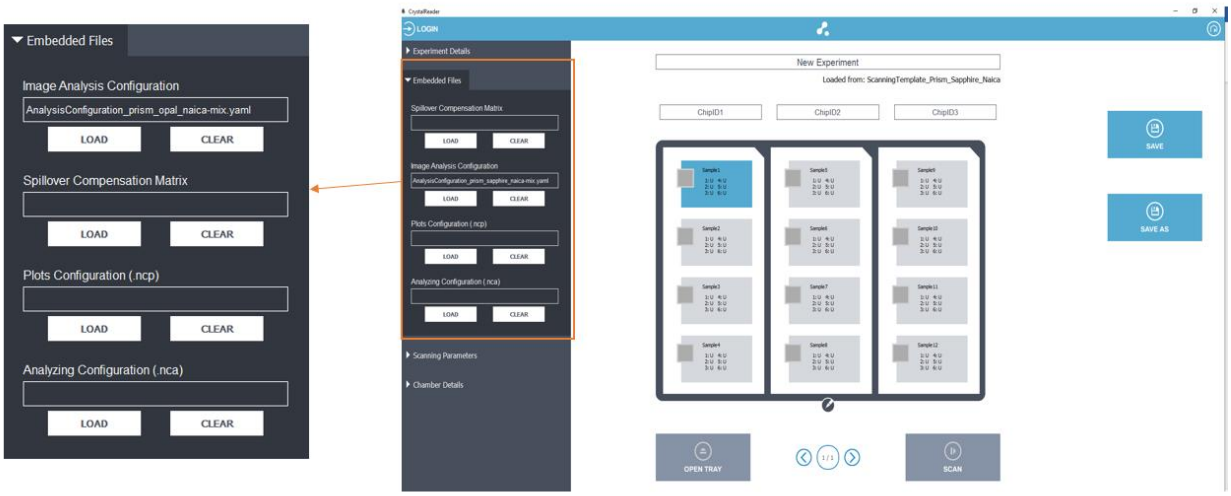


Figure 12: Select the relevant "Embedded Files" for the experiment.

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.

*Note**:** The embedded file selection menu is about supported files that can be pre-loaded in the Crystal Reader user interface. The only mandatory file is the Image Analysis Configuration File. The other files are optional. These files are loaded and then used in the Crystal Miner software and provide a smoother user experience.*

The Crystal Miner files (".ncm", ".ncp", ".nca") can be imported and exported to the Crystal Miner software, please refer to the Crystal Miner software User Manual for additional information.

Additional details about the files:

- The "Image Analysis Configuration" field is an advanced setting. If this field is empty, the software will automatically use the default image analysis configuration file that is optimal for most experiments prepared by the user. The specific files for different applications are available on the Monitor at this location by default: "C:\Program Files\Stilla\CrystalMiner\config".

- "Spillover Compensation Matrix"- If the "Spillover Compensation Matrix" field is empty: it is possible to load an already created spillover compensation matrix (using the matrix computation tool of the Crystal Miner software) by clicking on the "Load" button in the "Spillover Compensation Matrix" widget and selecting the corresponding ".ncm" file.

Note: The loaded spillover compensation matrix should correspond to the conditions of the experiment (please see the Crystal Miner software User Manual for more details).
If a spillover compensation matrix is loaded, the fluorescence spillover will be automatically applied at the launch of an experiment in the Crystal Miner software.

CAUTION!
The spillover compensation matrix is experiment-dependent as it depends on the set of fluorophores used but it may also depend on the biological setup (mastermix used) and the scanning parameters (exposure times of the LEDs).

c) Check the scanning parameters:

- The "Image Analysis Configuration" field is an advanced setting.

If this field is empty, the software will automatically use the default Image Analysis Configuration File that is optimal for the majority of experiments prepared by the user. The specific files for different applications are available on the Monitor at this location by default: "C:\Program Files\Stilla\CrystalMiner\config"

- In the "Plots Configuration", the user can load the Crystal Miner plots configuration here to have it ready to use in the ".ncx" file after the scan. Please see Crystal Miner documentation about ".ncp" files that embed the plots viewing configuration.

- In the "Analyzing Configuration" the user can load the Crystal Miner plots configuration here to have it ready to use in the ".ncx" file after scan. Please see Crystal Miner software documentation about the ".nca" files that embeds the threshold or polygons settings, and the population configuration.

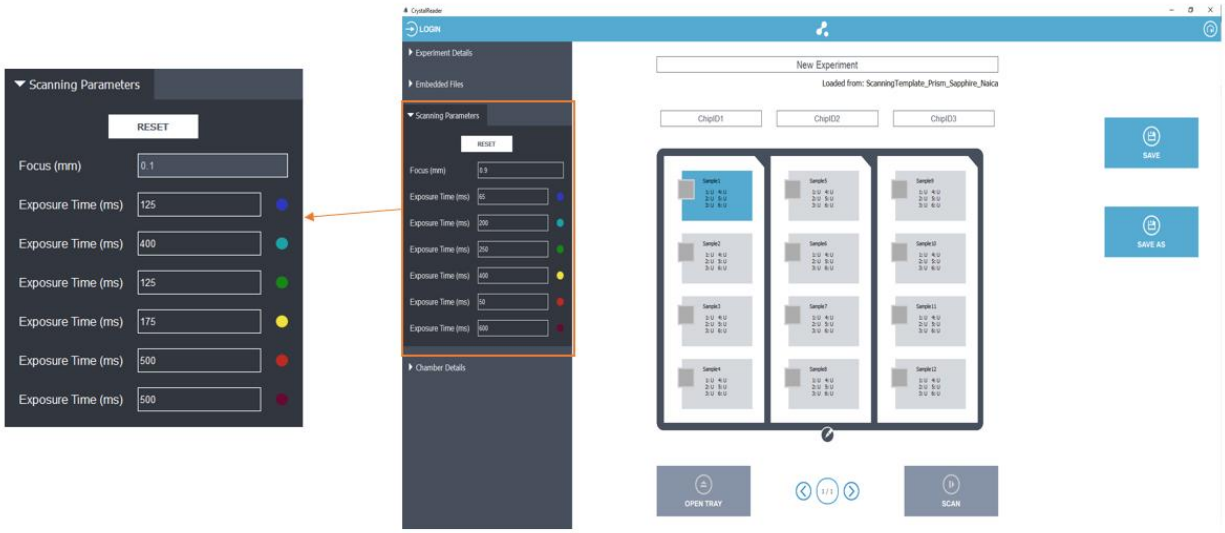


Figure 13: The "Scanning Parameters" menu.

- Click on the "Scanning Parameters" drop down to edit the exposure time of each excitation LED (in ms):

Focus value

- The focus value representing the optimal z-distance of the imaging plane in mm.
- The focus value is a set value based on the original instrument calibration that do not need to be modified.

Exposure times

- With the selection of a respective scanning template file default exposure times will be displayed
- Exposure times can be modified to optimize experimental settings
- Increasing (resp. decreasing) the exposure time of a LED will increase (resp. decrease) the fluorescence intensity value of the droplets in the image associated with the detection channel.
- The exposure time of each LED should be sufficiently high to discriminate between negative and positive droplets in the detection channel, but not too high in order to avoid the saturation of positive droplets in the detection channel (intensity saturation implies 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, and a few scans are 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 not be further edited to 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 prompt to ask if the user wishes to proceed and erase the images of the already scanned chambers.

2. Step (2 and 3) - Select the chambers to scan & Define chamber context:

- 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 chips and 48 chambers for Opal chips 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).

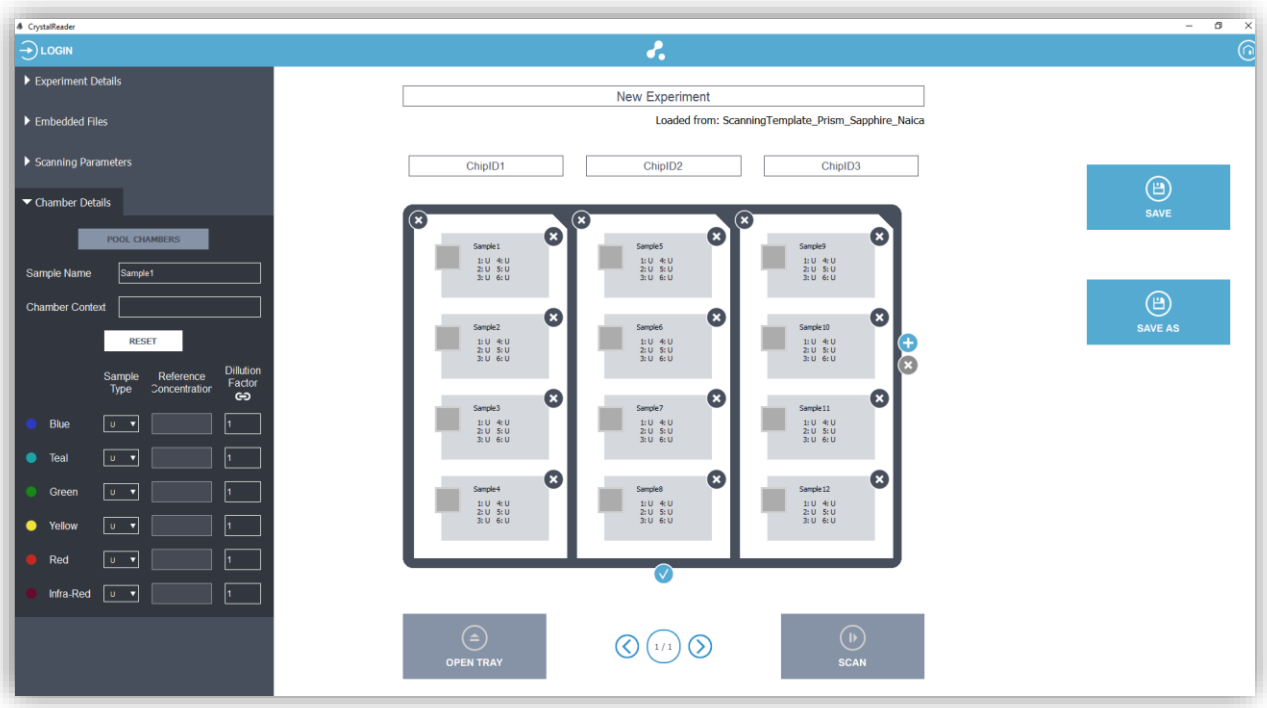


Figure 14: Click on the cross icons to deactivate objects click on the “plus icon” to activate objects.

- 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 at the bottom (**Fig 13**).

Figure 15: Chip holder counter.

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

The Chambers Details panel:

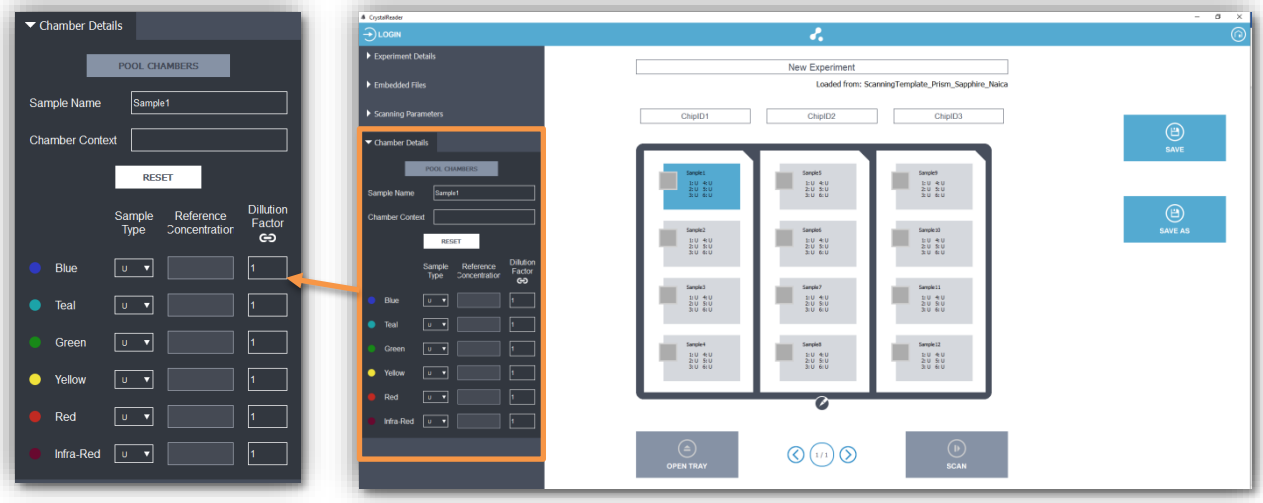


Figure 16: The "Chamber Details" form.

Enter the details of a given chamber, first click on the chamber in the rack layout (chamber rectangle becomes blue), proceed to edit the "Chamber Details" form **(Fig. 14**).

- "Sample Name": to identify sample loaded in the chamber.
  A 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):
  - "Sample Type" (see details below).
  - "Reference Concentration" if the Sample Type is "S" (meaning that the target concentration is known): the expected concentration (in copies/µL).
  - "Dilution Factor" (e.g., 10 for a 10-fold dilution from the stock sample).

Then, edit the type of the sample for each given channel, the possible values are:

- U: unknown
- S: Standard (i.e., the stock concentration is known for the channel of interest and should be entered in the next field in copies/µL)
- 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)

For the dilution factor, in case 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 chips and 7 µL of reaction mix for Opal chips.

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. To unlink the dilution values among the channels, click on the “link icon” .

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

The chamber details are displayed on the chamber button of the Tray Holder:

The sample ID and sample types per channel are displayed on the chamber button.

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

3. 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.

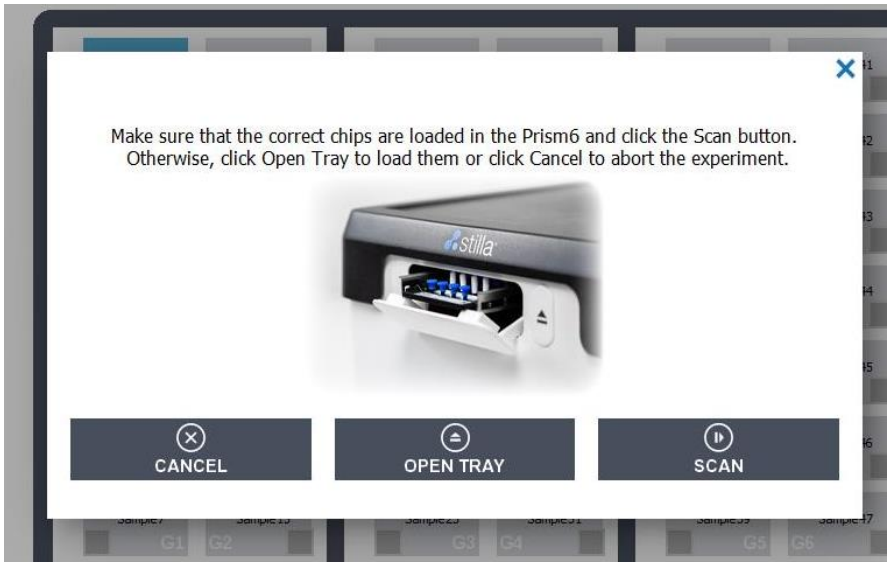


Figure 17: Pop-up confirmation before launching the scan.

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.

CAUTION!

Do not push the power button on the front panel of the Prism6 instrument while it is still in operation. This would cause a shutdown of the instrument and displays a risk of data being lost and a consequent requirement to re-scan the chips.

a) Check chamber image quality

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 have been selected for the scan, the scanning process is split in several steps:
  - Once the current chip holder is completely scanned, a pop-up window is displayed and invites you 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 scan.

Already scanned chambers are displayed on the Crystal Reader software scan page interface for a visual pre-analysis of chamber images while the scanning is still in progress. A quality flag is added in the chamber rectangle as soon as it is ready to be explored for pre-analysis:

-  “Green” quality flag indicates scanning finished without remarks of quality parameters.
-  “Yellow and Green” quality flag indicates scanning finished with remarks for some quality parameters.
-  “Yellow” quality flag indicates scanning finished with remarks for major quality parameters.

When the image of the last chamber of the experiment is acquired, the following message is displayed:

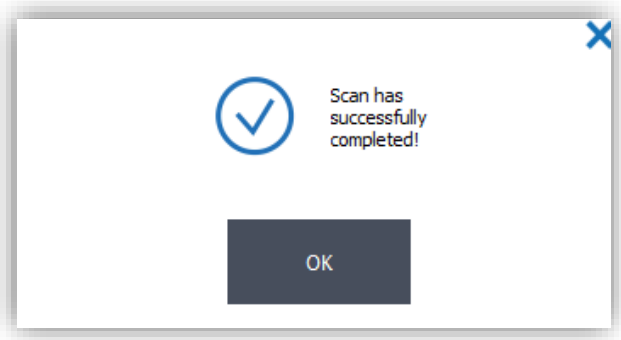


Figure 18: Pop-up indicating completed scan.

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

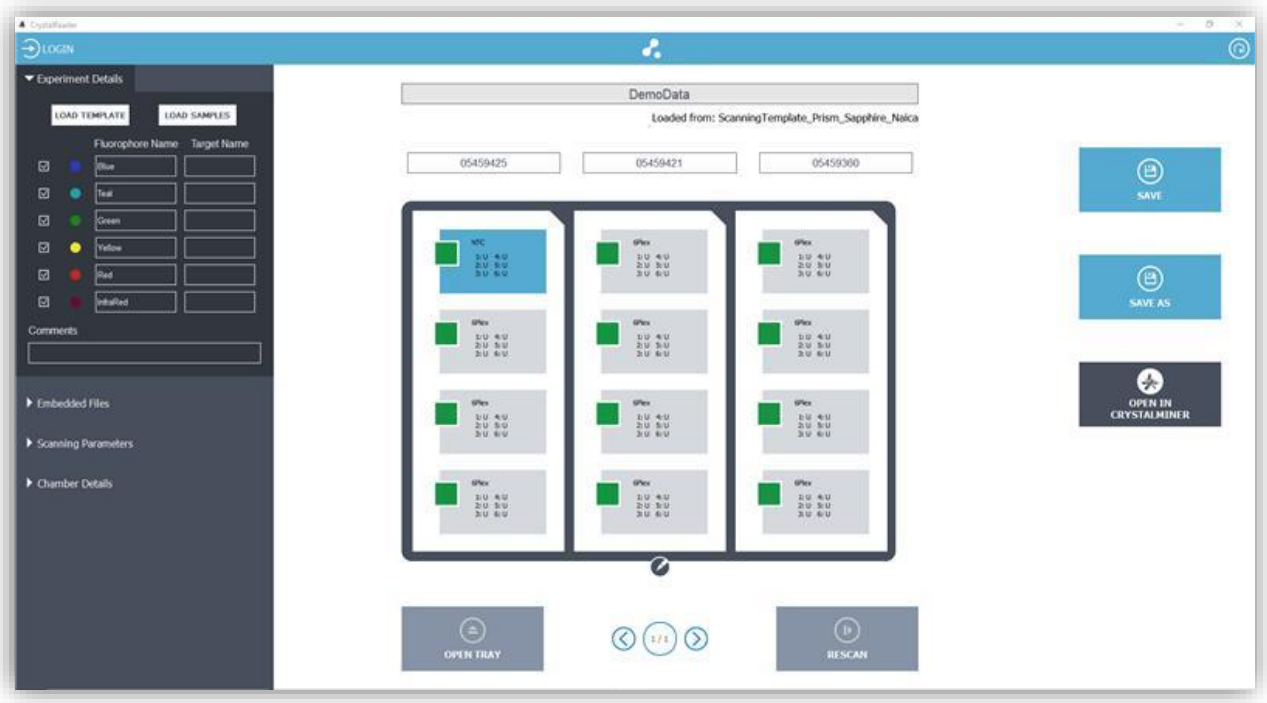


Figure 19: Example of a Sapphire chip experiment layout with all green quality flags at the end of the scan.

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".

Default output directory is:
"%USERPROFILE%\Documents\Stilla\CrystalReader"

Note: Quality flags only apply to the Blue LED channel. Stilla technologies recommends to visually inspect all the other the LED channels.

Stilla Technologies recommends to first continue to check the scanning performance by looking at the image quality. Image quality can either be inspected directly in Crystal Reader software or after opening the Crystal Miner software application.

Following, the button "Open in Crystal Miner" can be used to automatically launch the Crystal Miner software for the scanned experiment:

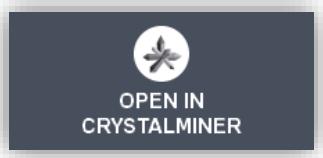


Figure 20: Button to open the scanned experiment with Crystal Miner software.

4. Step (5) - Check image quality

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

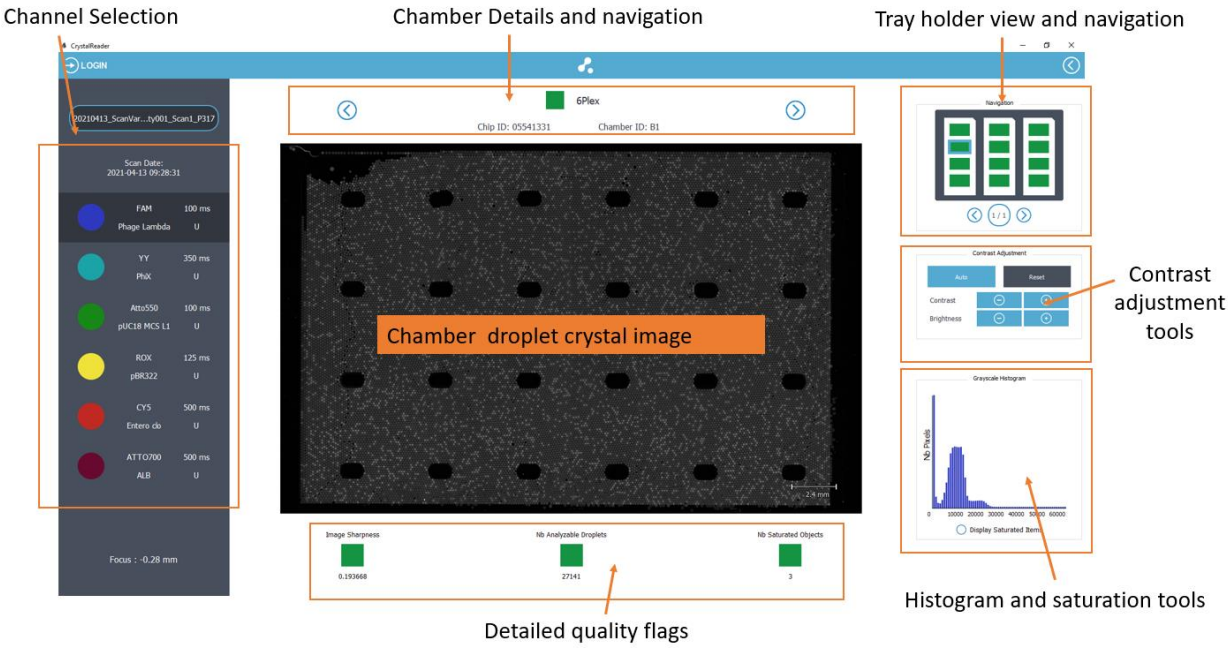


Figure 21: *Overview of a Sapphire chip chamber image in the "Blue Channel" detection channel in the Crystal Reader software*.

To explore the images of a chamber:

- Click on the LED icons (on the left panel) to change the acquisition channel.
- Use the mouse scroll to zoom in/out in the image.
- Use the left mouse click to translate the image.
- Click on the "Auto" button to automatically adjust the image contrast.
- Click on the "Reset" button to reset the image contrast.
- 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 thanks to the addition of the reference fluorophore (e.g., fluorescein). If necessary, click on the "Auto" button to adjust the image contrast.

CAUTION!
The negative droplets should be visible in all Blue channel images, otherwise, experiment analysis cannot be performed.

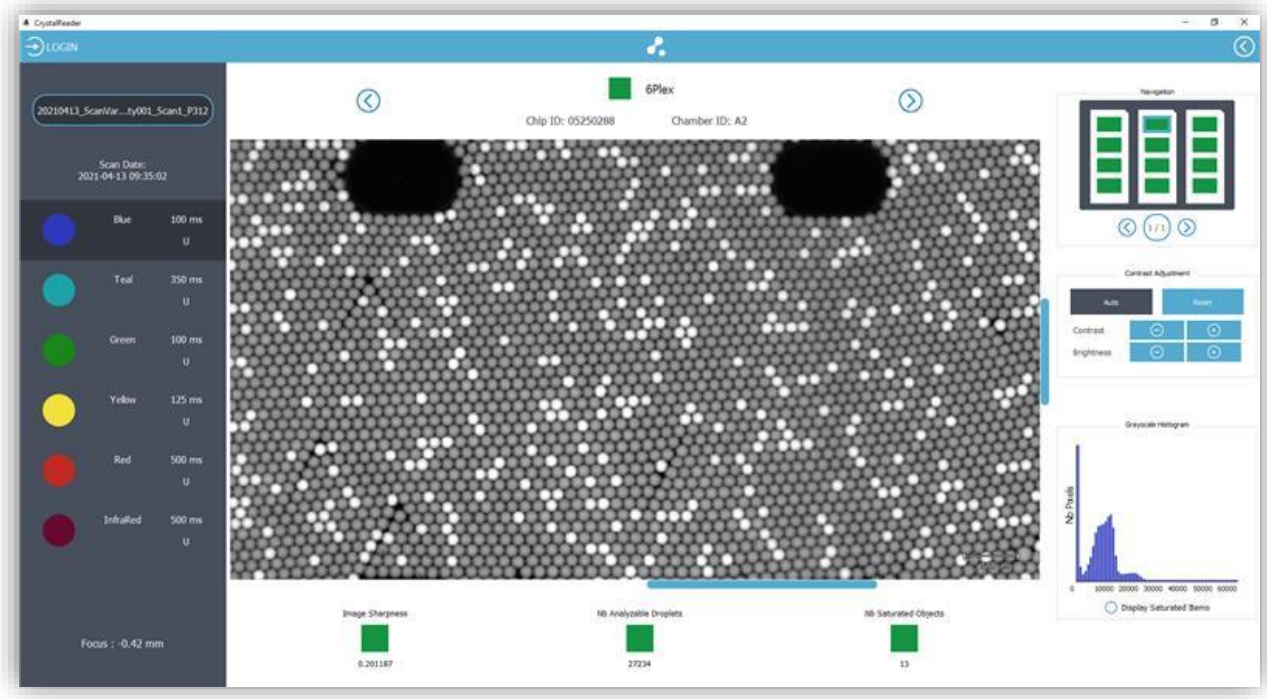


Figure 22: Zoom-in into a chamber image in the Blue detection channel (all negative droplets should be visible in the Blue channel image) in the Crystal Reader software.

- The Grayscale Histogram tool (bottom right) might help you to appreciate the distribution of pixel intensities expressed in RFU (Relative Fluorescence Unit), where 0 RFU indicates black and $2^{16} - 1 = 65535$ RFU indicates saturation.

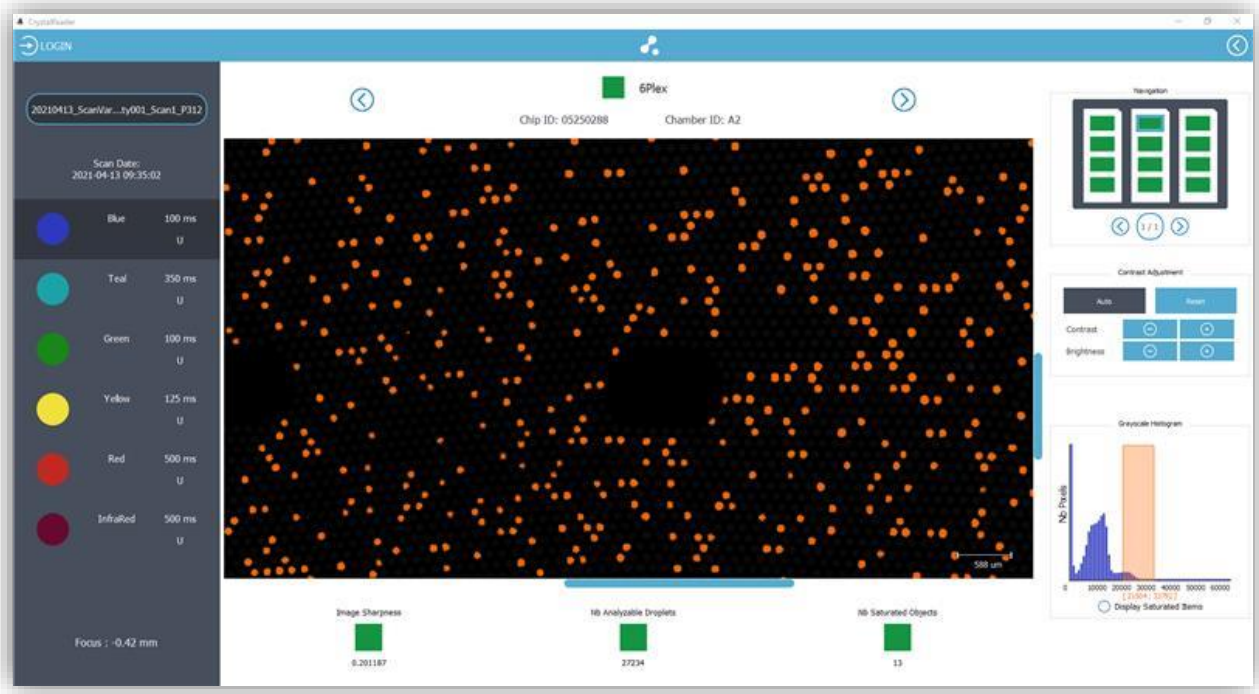


Figure 23: Step (5)- Use the histogram to place intensity cursors and reveals the pixels corresponding to the RFU interval in the Crystal Reader software.

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, which reveals the positive droplet population:

This tool helps to detect time exposure issues and provides an indication of separability between the negative and positive droplet populations.

- Check the saturated objects in each detection channel by checking the box “Display Saturation Map”. The saturated pixels are displayed in orange color:

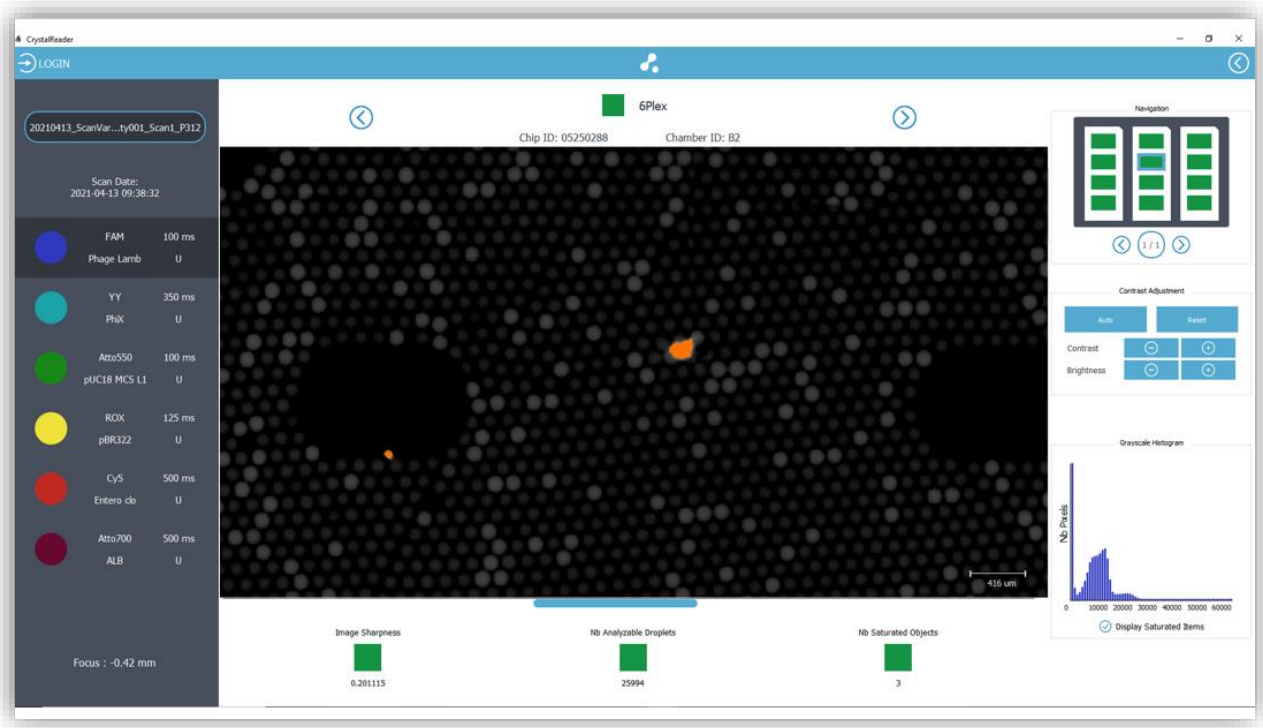


Figure 24: Overlay of the saturation map in the Blue detection channel in the Crystal Reader software (saturated pixels are displayed in orange color). In this case, it is a dust particle.

CAUTION!

Ensure that all the pixels located in the droplets are not saturated in any channel, otherwise, the saturated droplets will be considered as not analyzable by the software and will be rejected from the analysis. If necessary, decrease the exposure time of the channel and rescan the chamber.

- Perform quality control:
  The quality flags are automatically computed and provided at 2 levels:
  - Chamber level: the chamber flag corresponds to the worst quality flag at the criterion level, among the criteria computed for the chamber.
  - Criterion level: 3 specific flags are used.
    - “Image Sharpness”: the higher the score the better the sharpness in chamber images.

      Note: 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 chamber is well placed).

- "Number of analyzable droplets": the higher the number the better the confidence in predicted concentration results.

  Note: Saturated droplets and droplets covered by artifacts are not analyzable. They can be avoided by checking that exposure times are not too high, and the chamber is cleaned up.

- "Number of saturated objects": the lower the number the better the chamber quality.

  Note: 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, and the chamber is cleaned up.

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 rescan).

If some of the chambers need to be cleaned to improve the image quality, the chips should be rescanned for the 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 1 time on the length directly on a cleaning wipe (delivered with the respective chips) 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 Prism6 instrument off, click on "Open tray"/"Close tray" to remove the chips. Proceed by pressing on the power button located in the front (the green light becomes orange) for a few seconds. .

V. Advanced Functionalities

1. How to rescan existing chambers? How to scan new ones?

- Click on the RESCAN button
  One of 3 options can be chosen from:
  - a) Rescan all the chambers of the experiment.
  - b) Scan some new chambers or chips and keep the images of the already scanned chambers.
  - c) Rescan only specific chambers of the experiment and keep the images of the other chambers.

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.

b) How to scan only a selected set of chambers in the experiment?

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:

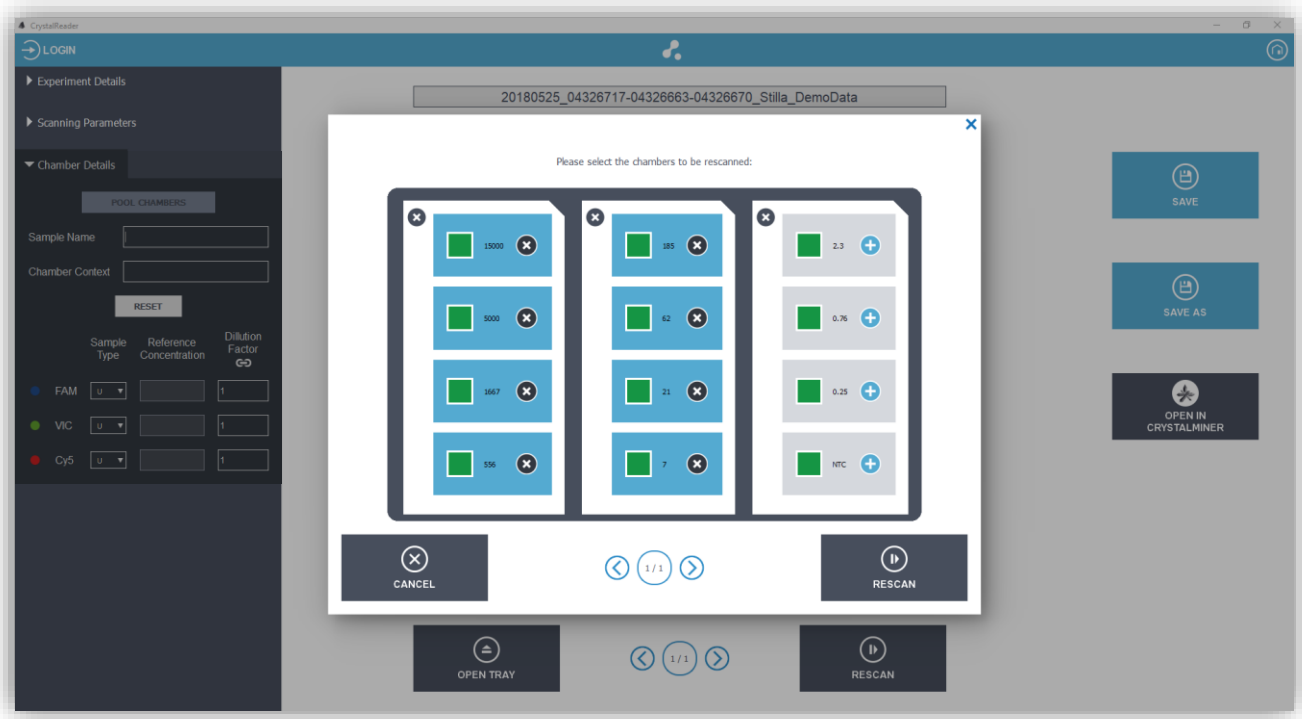


Figure 25: Default rescan window (chambers to be rescanned are highlighted in blue color) in the Crystal Reader software.

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

For this scenario: click on the validation icon, and the scan will start.

For this scenario: unselect the previously scanned chambers and activate the new ones to be scanned.

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

2. How to edit the Settings for different user profiles

- From the HOME page, click on the “SETTINGS” button.
  The Settings page is displayed:

b) Settings: logged user with “Basic” profile

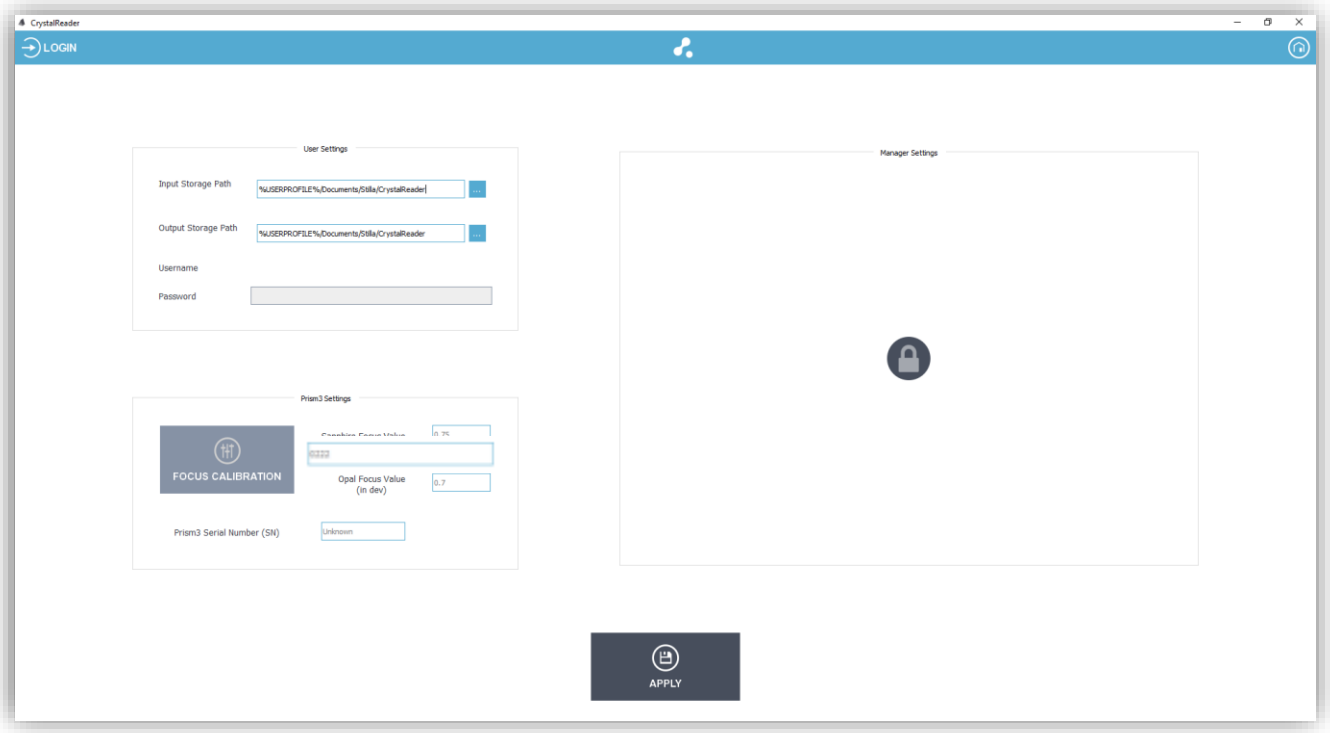


Figure 26: Settings page for a non-logged user in the Crystal Reader software.

- Description of the fields:
  - User Settings:
    - Input Storage Path: default storage path to select the experiment templates (i.e., the “.ncx” files which may be loaded in the “Experiment Details” form), the spillover compensation matrices (i.e., the “.ncm” files which may be loaded for fluorescence spillover compensation in the “Experiment Details” form), and the image analysis Configurations (i.e., the “.yaml” files which may be loaded for image analysis Configuration in the “Experiment Details” form).
    - Output Storage Path: default storage path to save the “.ncx” experiment files
    - Username: chosen username
    - Password: chosen password

  - Prism6 Settings:
    - Focus Value (mm): z-distance to the imaging plane used for image acquisition (set by the user).
    - Prism6 Serial Number (SN): unique serial number of the Prism6 (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)

Note: If the Crystal Reader software is operated without logging in, only the paths to output and input storage can be modified, and this configuration will not be saved for the next session of the Crystal Reader software.

c) Settings: logged user with “Manager” profile Unless modified after default software installation, a default “Basic” account is created with the login “guest” and the password “guest”.

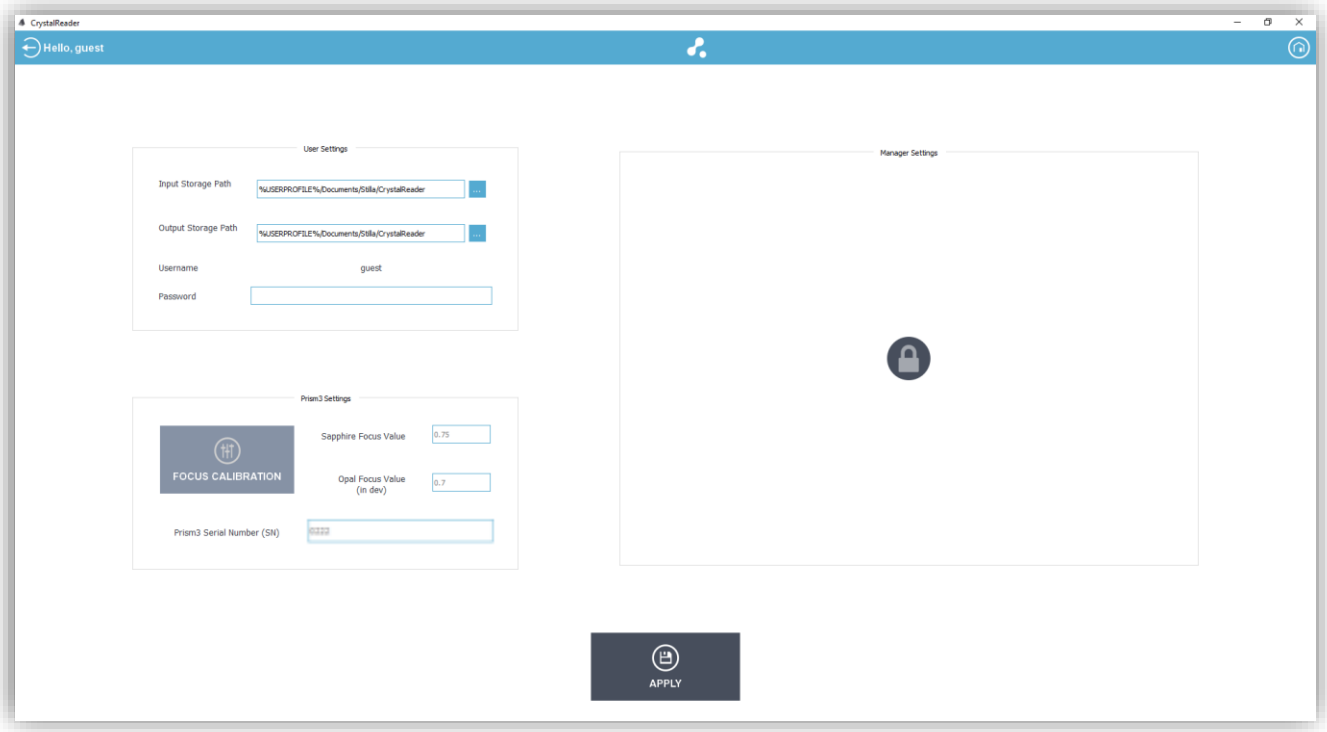


Figure 27: Settings page for a logged user in the Crystal Reader software.

A logged user has the same rights as a non-logged one, except that his/her user preferences for the input/output storage directories will be saved even after quitting Crystal Reader software. He/she is also allowed to modify his/her password.

Unless modified after software installation, a default “Manager” account is created with the login “manager” and the password “manager” (it is recommended to change this password).3

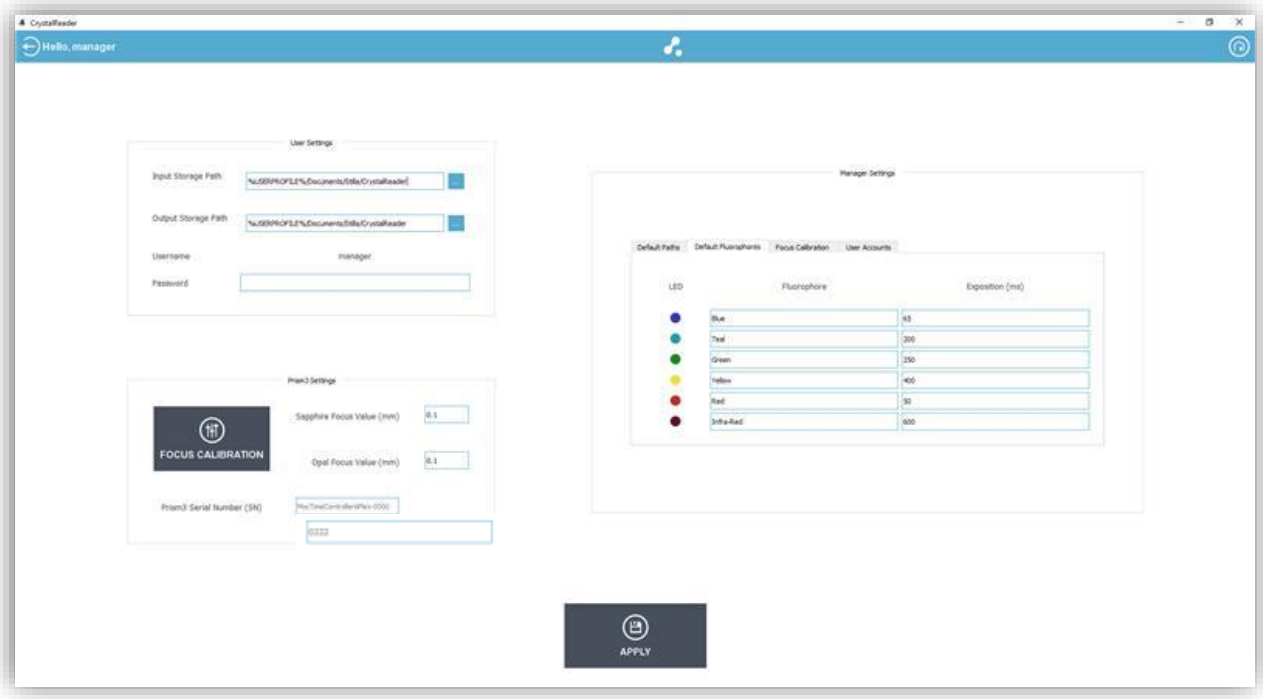


Figure 28: Settings page for the logged manager in the Crystal Reader software.

A user with the “Manager” profile has the same rights as a “Basic” user and has the following additional rights:

- Modify the “Focus Value” used for experiment scanning, either manually or via the “Focus Calibration” functionality, for each chip model.
- Create or remove user accounts.
- Modify the default output and input directory paths used for new user accounts and non-logged users.
- Modify the password of user accounts.
- Modify the profile of user accounts (“Basic” or “Manager” profile).
- Modify the default values for LED exposure times.
- Modify the default fluorophore names.
- Modify the default min, max, and step values for the z-values to be tested during the “Focus Calibration”, for all chip models.

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

3. How to perform focus calibration?

The focus calibration is an advanced functionality that can only be performed by the “Manager” user. This feature automatically computes the optimal acquisition focus value, at which the image sharpness of the droplet regions is the highest.

- Assuming that the user is already logged with the “Manager” account and that a chip with a chamber of interest is already inserted in the Prism6 instrument, access the SETTINGS menu, click on the “Focus calibration” button, then select the chip model of interest (Sapphire chip or Opal chip).

  Note:
  - *For optimal focus calibration on the Sapphire chip, it is recommended to use at least one chip that includes a chamber with good quality droplet crystal and to place this chip on the middle slot of the chip holder (i.e., slot 2).*
  - *For optimal focus calibration on the Opal chip, it is recommended to use three chips including chambers with good quality droplet crystal 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.

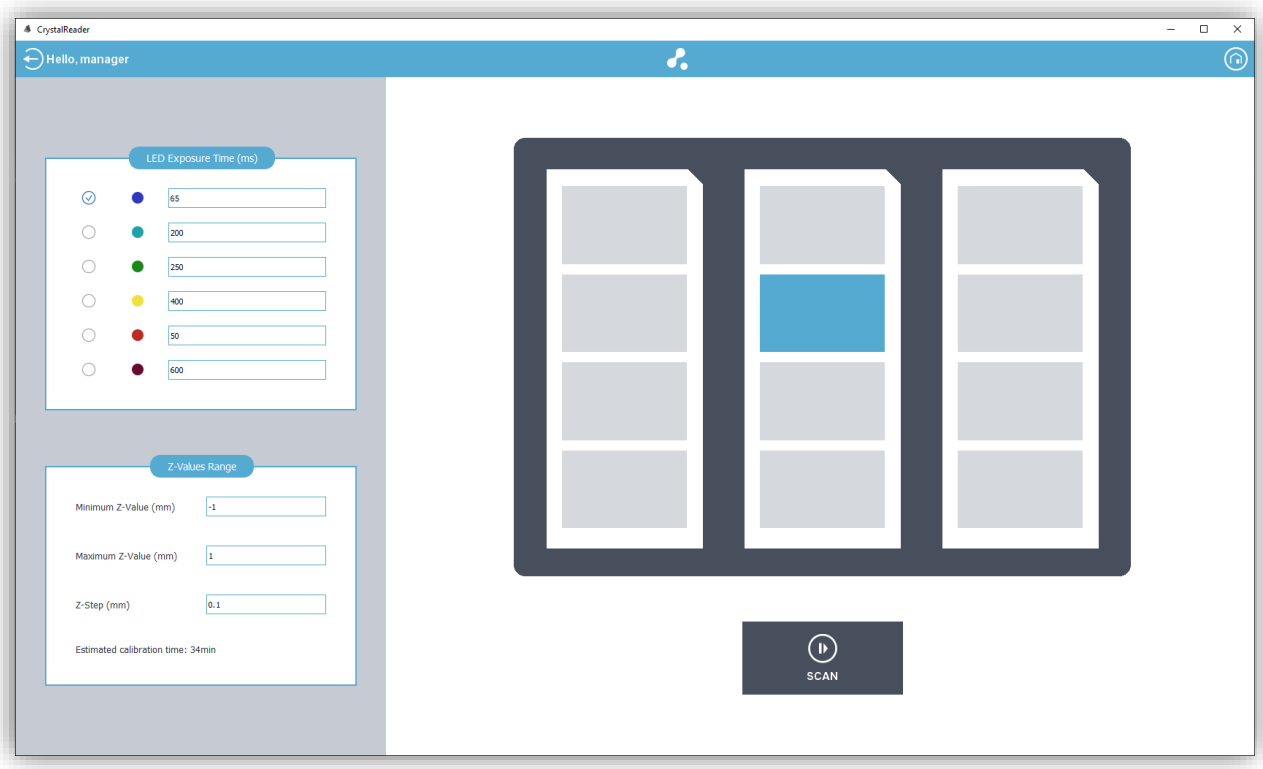


Figure 29: Example of selection of one chamber to calibrate the focus for the Sapphire chip in the Crystal Reader software.

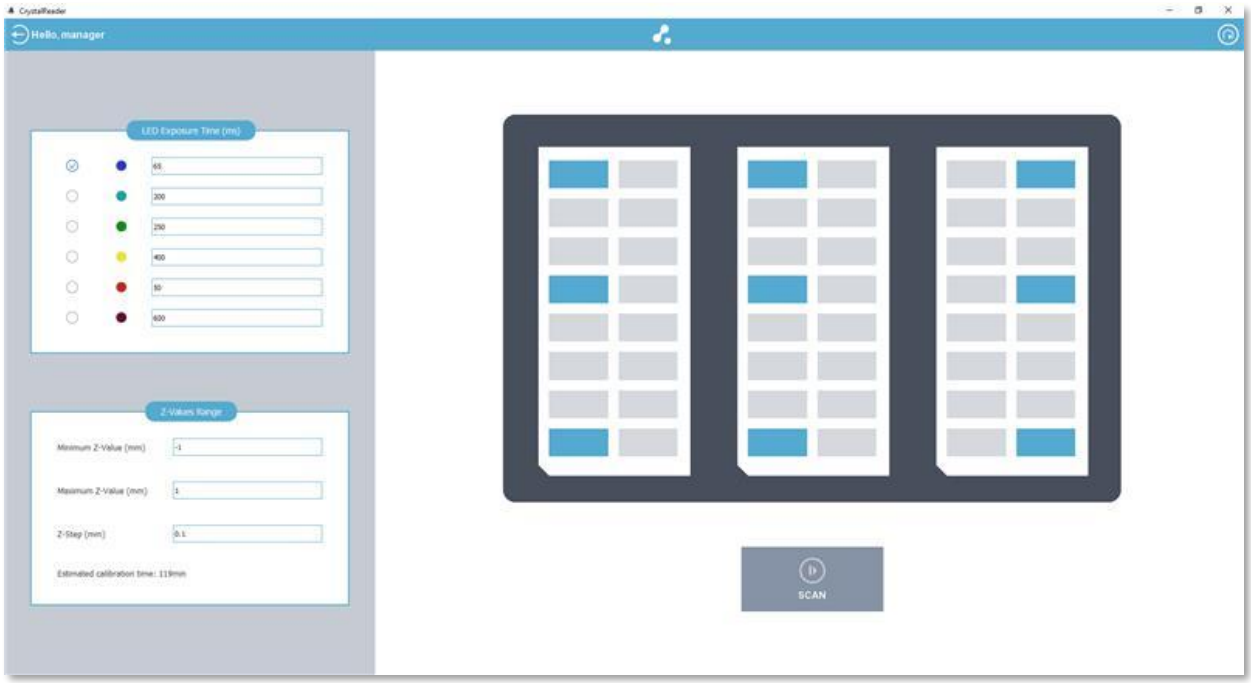


Figure 30: Example of selection of nine chambers to calibrate the focus for the Opal chip in the Crystal Reader software.

- Click on the “SCAN” button on 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) which maximizes the average value of the image sharpness score among all the selected chambers, as well as 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 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.
- 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.

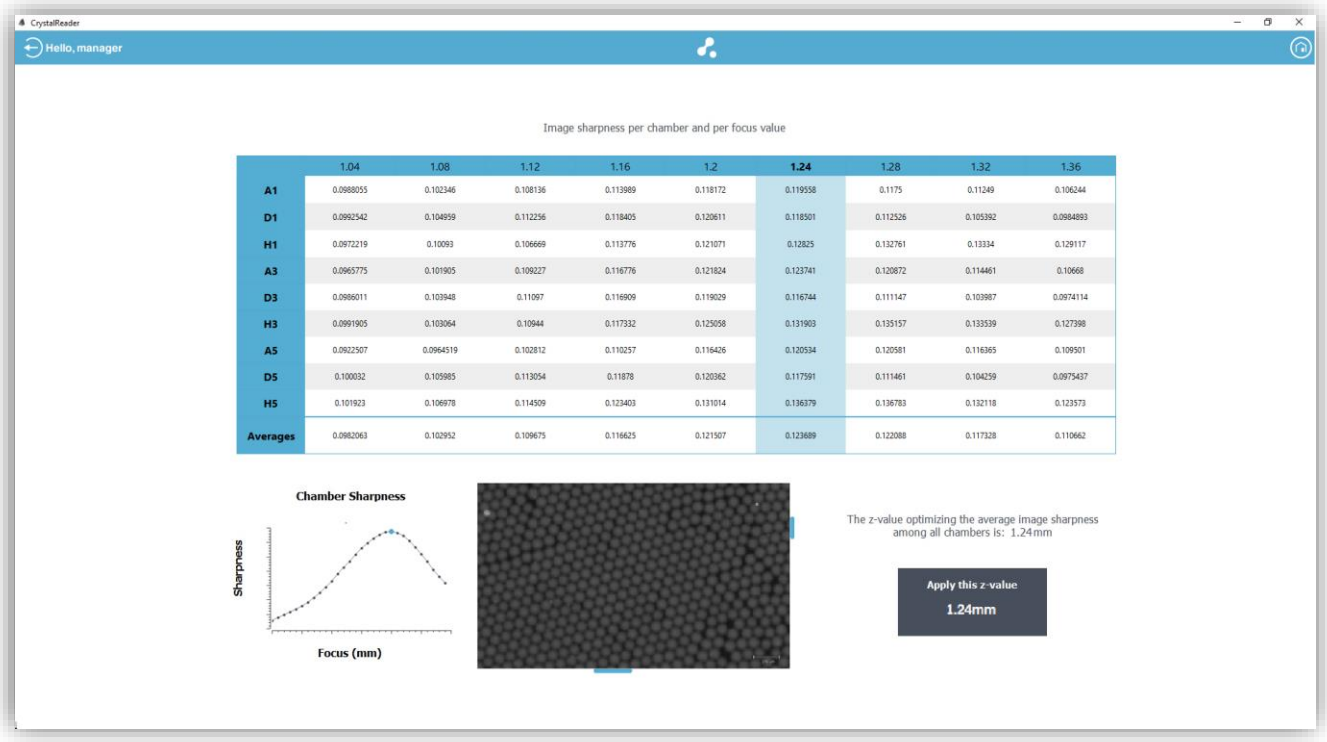


Figure 31: Example of the result of focus calibration for the Opal chip in the Crystal Reader software.

4. How to Pool/Unpool Chambers:

Figure 32: Pooling feature of the Crystal Reader software.

Figure 33: Detailed view of a chamber in the Crystal Reader software.

An additional feature where users can pool multiple selected chambers containing the same sample called the “POOLING CHAMBERS” is available in the Crystal Reader software. Pooling a set of chambers in which the same sample has been loaded allows to gain 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.
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 (**Fig. 14**).
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 same “sample type”, “reference concentration” and “dilution factor”.

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).

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).

VI. Maintenance and Technical Support

a) Cleaning

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, refer to www.stillatechnologies.com or contact us:

For European Customers:
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 North American Customers:
Monday to Friday, 8:00 AM – 6:00 PM, 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 Prism6 monitor.

To enable remote software maintenance on the Prism6 monitor provided by Stilla Technologies, check that the Prism6 monitor is turned on and connected to the Internet. Then, double-click on the “Team Viewer” desktop icon and send both your ID & password information by email to the Technical Support Team. Please have the following information ready for remote maintenance:

- The software version, which is available in “About” in the Home page
- The log files which have been generated in the directory: “%USERPROFILE%\Stilla\CrystalReader”

Please see below for maintenance-related instructions for the Prism6 instrument.

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 before cleaning and decontamination operations.

External parts of the Prism6 instrument and the monitor 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 Prism6 instrument 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. Ensure to clean the Prism6 instrument to prevent any

d) Decontamination

marks on the housing or the benchtop. When disinfection is required, please contact Technical Support.

Material necessary for the decontamination procedure:

- Gloves;
- Glasses;
- Mask;
- Laboratory coat;
- Hydroalcoholic disinfectant solution for device surfaces commonly used for biological and medical devices (Ex. Phagospray). Decontaminant solution for device's surfaces used for biological and medical devices, specifically targeting nucleases and DNA contaminations (Ex. RNAse away). Bleach is not recommended; if its use is unavoidable, be sure to abundantly rinse with water after the bleach treatment.

Wipes. For the detailed procedure for decontamination of the Geode, please view the Decontamination Protocol.

- *Disposal of naica® system equipment*

The disposal of the Prism6 at the end of the product's life should comply with the current legislation in force in the country of use regarding electrical and electronic waste.

The Prism6 instrument should be packed and shipped back to Stilla® only after a Stilla Technologies Service Specialist has deemed it necessary. If shipping the Prism6 is required, use all the original packaging and cables provided upon reception of the naica® system. To prepare the Prism6 instrument for shipment, please contact Technical Support for detailed instructions.

Ensure that no chips (Sapphire chip/Opal chip) and tray holders (Sapphire tray holder / Opal tray holder) are left inside the Prism6 instrument for shipment.

d) Disposal of the instrument back to Stilla®

VII. Troubleshooting

Display Screen Error Messages

Prism6 and Crystal Reader software:

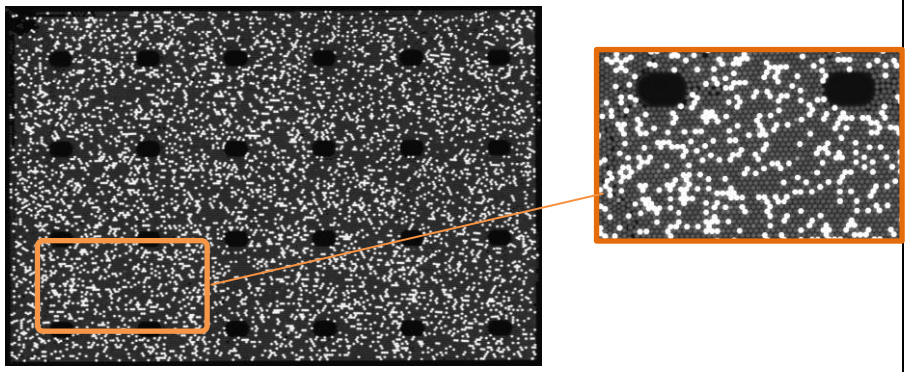
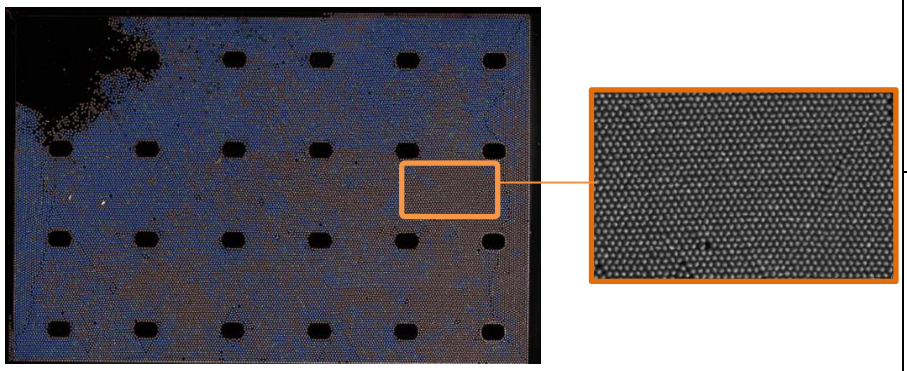
Description error message	Recommendations
A red light appears in the third position at the front of the Prism6 instrument	Reboot the Prism6 instrument (press the power button in the front for a few seconds until the Prism6 instrument shuts down, then press again to start it). Restart Crystal Reader software.
The Crystal Reader software application stops responding	Go to “Control Panel” > “Task Manager” and stop the Crystal Reader process, then start it again.
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)
Error message: It is not possible to deactivate the blue channel, it is used for droplet detection.	Ensure to select at least 3 detection channels, including the Blue channel.
Error: it is necessary to scan three channels, including the blue one, to perform a scan	
"Error message: Prism6 instrument configuration not applicable with detection channel selection."	Depending on the Prism6 instrument configuration, up to 6 detection channels are available. H24000.3: 3-color Prism6 configuration with the detection in Blue, Green, and Red channels. H24000.6: 6-color Prism6 configuration with detection in Blue, teal, Green, Yellow, Red and Infra-red channels. Please contact the Technical Support to enable detection channels, if applicable.
Error: the current Image Analysis Configuration File (.yaml) is not designed to be used on this scanner type. Please ensure the consistency between the “.yaml” file and the experiment settings.	Please use the scanning templates provided by Stilla Technologies accessible at https://www.stillatechnologies.com/technical-resources/naica-system-prism6/. Please ensure the consistency between the “.yaml” file and the experiment settings when editing “.yaml” files.
Error: the selected Crystal Miner Plot configuration file (“.ncp”) uses different channels than the ones activated.	Please use the scanning templates provided by Stilla Technologies accessible at https://www.stillatechnologies.com/technical-resources/naica-system-prism6/. Please ensure the consistency between the “.ncp” file and the experiment settings.

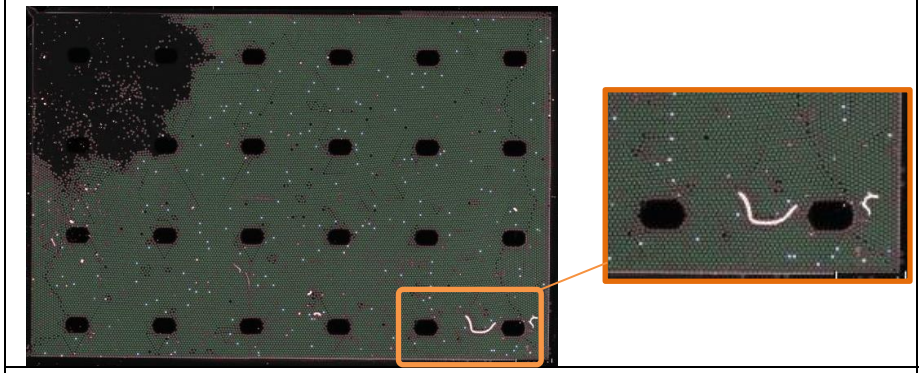
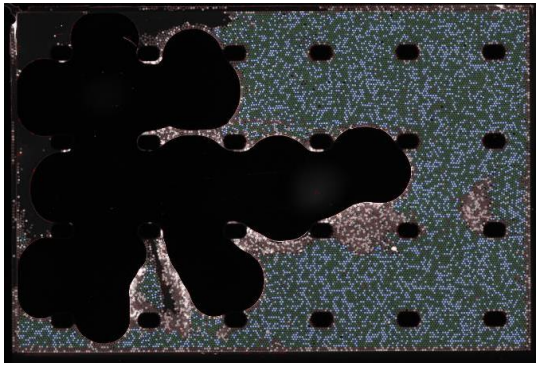
Error: the selected Spillover Compensation Matrix file (“.ncm”) uses a different number of channels than the " "current experiment. Please check the consistency of these elements.	Please use the scanning templates provided by Stilla Technologies accessible at https://www.stillatechnologies.com/technical-resources/naica-system-prism6/.

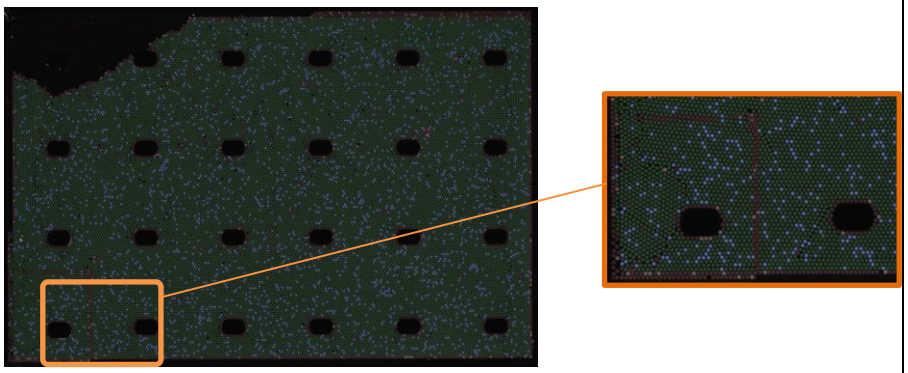
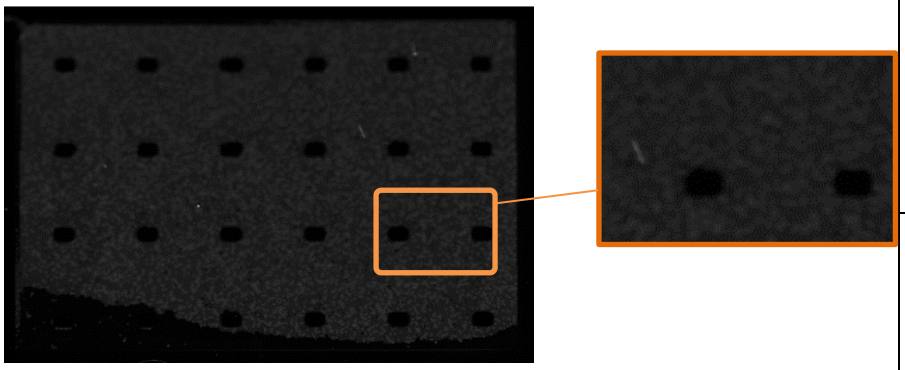
If these observations persist, please contact Technical Support.

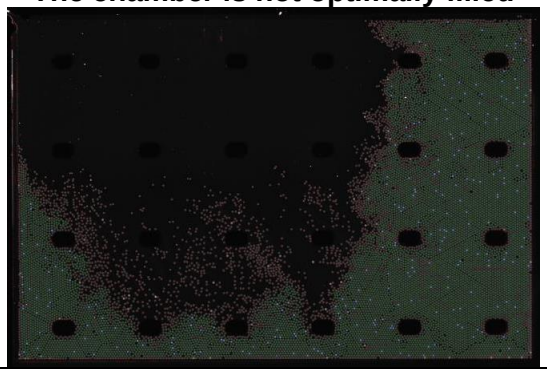
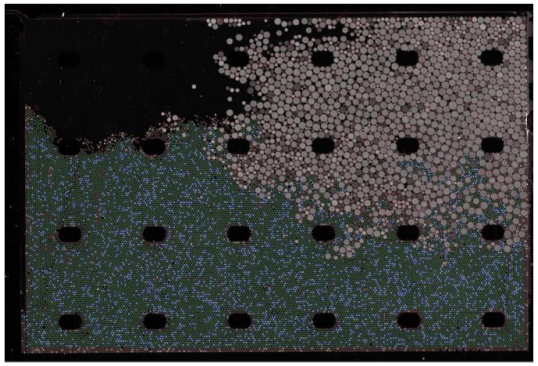
a) For Sapphire chips		
Observations	**Possible cause**	**Recommendations**
Any or few analyzable droplets No air bubble	The partitioning step did not occur	Check that the crucial partitioning step is included in the PCR program used (1st step). If the problem persists, please extract the live and system logs (refer to the section "How to use the Geode Software User Interface" in the Geode User Manual and contact the Technical Support.
	Problem during the pressure release step	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 the Technical Support.
		Make sure the experiment was not interrupted.
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 lint-free wipe before the loading step.
Droplet overlapping (typically the bottom right corner of the chamber)	There are too many droplets within the chamber	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.
	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.	Check that the appropriate amount of reference fluorophore (e.g., fluorescein at 100 nM) was included in the reaction mix.
Presence of droplets of different size in the chamber.	Some microchannels are clogged.	Check the sample preparation conditions and/or the PCR mix for precipitates. Contact the 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 lint-free 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 the Technical Support.

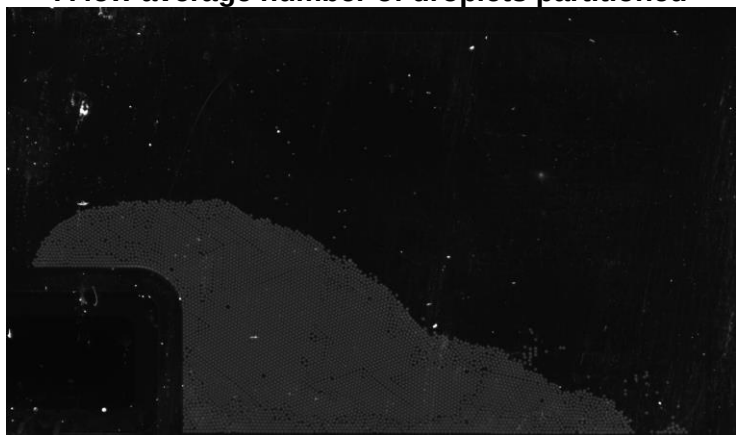
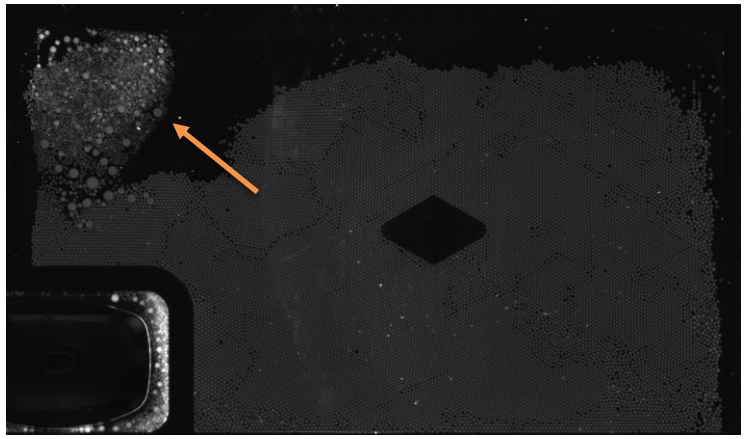
Presence of an out of focus sub-image.	Autofocus failed during scanning the sub-image	Rescan the concerned chamber.
Oversegmentation or undersegmentation of droplets.	There are two or more droplets (crosses) instead of one (oversegmentation) or one droplet instead of two or more (undersegmentation) which may lead to false positive or false negative results.	Manually inspect the droplets, in case of red crosses, the software automatically rejests the droplets, no action is required. However, in case the crosses are green, then manual exclusion of the crosses is necessary.

Observation	Possible cause	Recommendation
Droplets with a saturated fluorescence level. 	The exposure time is too long for at least one channel	Saturated droplets have an intensity equal to 2^{16} = 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.
Shiny aggregates within droplets 	The PCR mix can aggregate when it has been heated up for too long	Optimize the PCR conditions: decreasing denaturation times and/or the PCR master mix concentration with a scan-rescan. It is also possible to use the Template PCR program "FAST-Template Stilla PCR 45 cycles.js ".
	Aggregates can also occur depending on testing conditions	Rescan the chip immediately after the first scan.

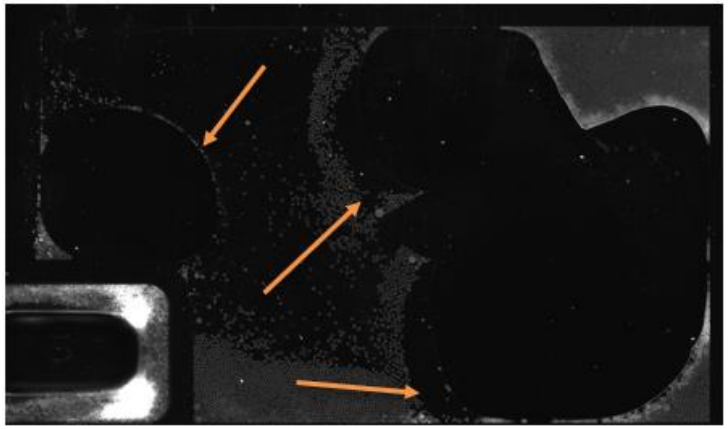
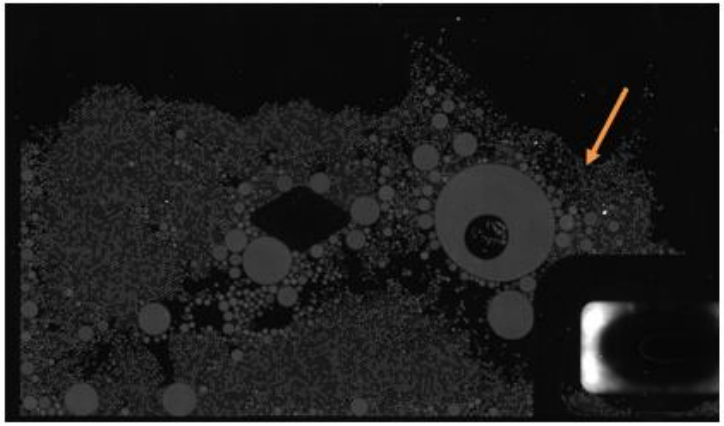
Shiny dust particles on the external film of the chip. 	Presence of dust particles on the chip foil	Clean the foil with lint-free wipe and re-scan the concerned chamber.
Large air bubbles in the chamber 	Air was injected into the chamber during partitioning	Make sure to pipet 25 µL of the reaction mix. The volume should reach the top of the inlet. Make sure to not introduce air bubbles at the top of the inlet port or between oil and PCR mix during the pipetting step. Check the expiry date of the used chips listed on the box. Make sure the white PCR caps are on the inlet port during PCR.
Image stitching event	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.	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 chips and Opal chips.

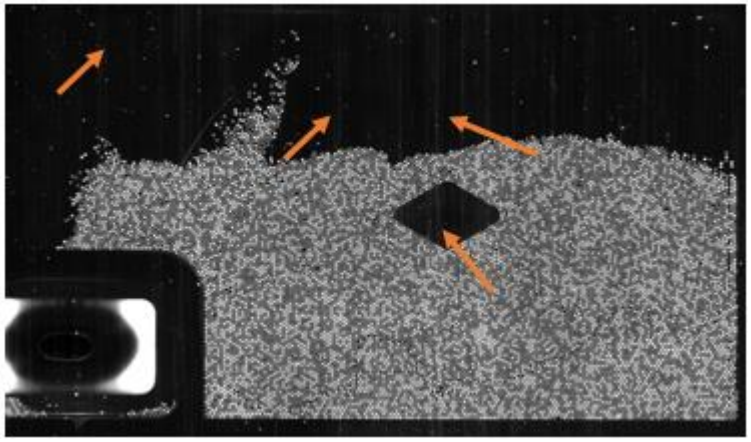
		
The whole chamber is out of focus 	The chip and/or the holder is not well positioned	Verify that the chip and/or chip holder is positioned flat. A foreign particle might be present between the chip and the holder, forcing the chip to be tilted. Please clean the foil of the chip and the holder with a lint-free wipe and re-scan the concerned chamber.
	The focus value is not well calibrated	Refer to the How to perform focus calibration? section and re-scan the concerned chamber.

The chamber is not optimally filled 	The sample volume loaded in the chamber was low.	The chamber is still analyzable as the partitioning step was successful. Depending on the assay, it is the user discretion if the number of analyzable droplets is sufficient.
Presence of different sizes of droplets in the chamber (polydispersity) 	The procedure using the antistatic spray was omitted or not properly performed.	The chamber is still analyzable as the partitioning step was successful. Depending on the assay, it is the user discretion if the number of analyzable droplets is sufficient. Please refer to the IFU for the Sapphire chips and the Opal chips for this step.

b) For Opal chips		
Observation	**Possible cause**	**Recommendation**
A low average number of droplets partitioned 	During the loading of the chips, multiple droplets could have been generated in the inlet port. Those droplets were probably not merged properly using the electrostatic pen.	Make sure that the electrostatic pen is working. For more information about how to use the electrostatic pen with the opal chips, please refer to the IFU for Opal chips.
	The Geode is not tilted enough (front-back axis) or is tilted in the wrong direction.	Contact Technical Support if unsure about the correct positioning of the Geode.
The small and defined population of droplets with different sizes. (The population is often positioned close to the chip injectors, and it is composed of droplets often smaller than the normal ones) 	The normal cycling of temperature and pressure could cause, in some rare cases, the presence of these artifacts.	This population of droplets is excluded during the analysis. The software recognizes the different sizes of the droplets and considers them as artifacts. This phenomenon usually does not affect the output of the assay.

Empty chambers/non-injected chambers. 	The Opal chips were not placed following the recommendations provided in the IFU.	Refer to the IFU for correct placement of the Opal chip in the Geode before starting a new run.
	The Opal chip PCR caps were not properly placed on the chip.	If the Opal chip PCR caps are not properly placed on the Opal chips, a lower amount of mix (or any amount of mix) will enter the chamber. Refer to the IFU for instructions to properly place the Opal chip PCR caps.
	During the loading of the chips, potential multiple droplets could have generated. Those droplets were probably not merged properly using the electrostatic pen.	Make sure that the electrostatic pen is working. For more information about how to use the electrostatic pen with the opal chips, refer to the IFU for Opal chips.
	The partitioning step did not occur.	Check that the crucial partitioning step is included in the PCR program used. If the problem persists, contact the Technical Support.
	The droplet was not correctly positioned in the Opal chip well.	Ensure to follow the troubleshooting steps for correct positioning of the droplets described in the IFU.

Air bubbles in the chambers. 	The Opal chip PCR caps are not properly set on the chip.	If the Opal chip PCR caps are not properly clipped on the Opal chips, a lower volume of mix (or any volume of mix) will enter the chamber. Refer to the IFU for instructions to properly place the Opal chip PCR caps.
	The inflated bag containing the Opal chips was not opened upon arrival at the customer site, upon reception.	It is recommended to open the inflated pouch upon reception. If the inflated pouch containing the chips was not opened upon reception and the chips are still under the expiry date, it is recommended to open the inflated pouch and to keep the chips at atmospheric pressure for at least 2 weeks before using them.
Presence of a large droplet coalescence area in the chamber 	The procedure using the antistatic spray was omitted or not properly performed.	Spray the antistatic spray on all the chips as described in the protocol "*How to avoid electrocoalescence*" provided during the installation or available upon request from the Technical Support.

Visible traces on the chamber 	The traces could be due to the partial drying of the antistatic spray.	Repeat the procedure of cleaning the Opal chip foil with a lint-free tissue sprayed with the antistatic spray. Ensure to completely dry the chip and rescan.

VIII. Software license Information

1. Crystal Reader software license

For Research Use Only.
Not for use in diagnostic procedures unless specified otherwise by domestic registration.

2. How to view the software version?

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

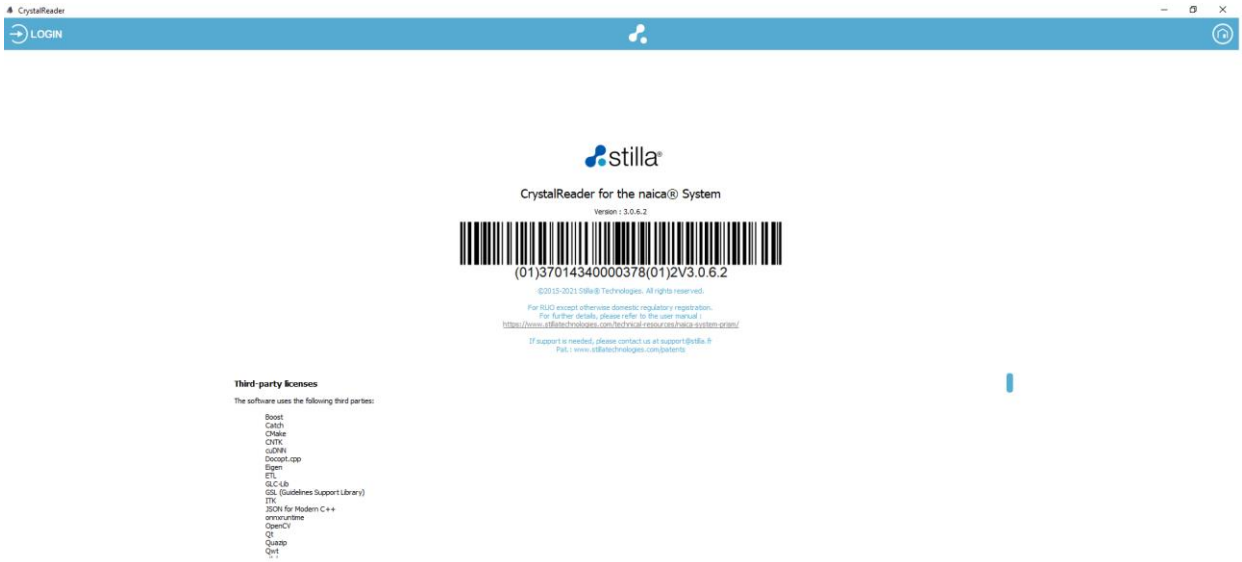


Figure 34: The “About” page of the Crystal Reader software.

3. 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
- xlnt
- 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 on the “About” button on the Home page.

Prism6 and Crystal Reader software for the naica® system

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