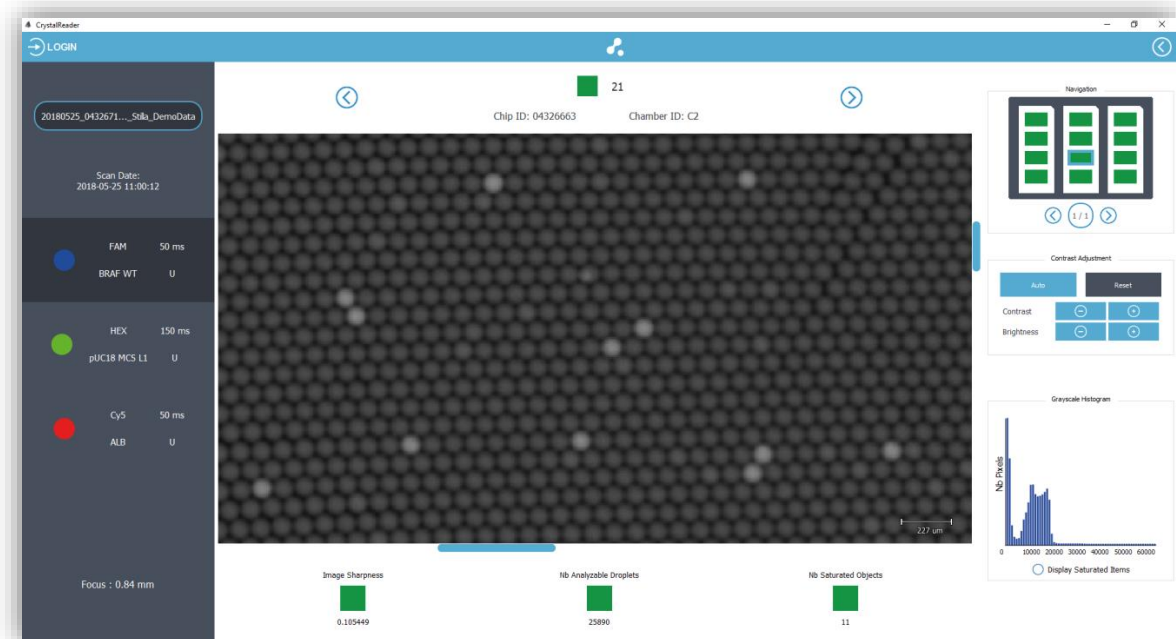




# Crystal Reader™ for the Naica™ System



## User Manual v2.0

## TECHNICAL SUPPORT

For help and technical advice, please contact the Technical Support Department at Stilla Technologies. The Technical Support department is open Monday to Friday, 9:30 AM - 6:30 PM Paris time and closed on French bank holidays.

Phone: +33-982-374-747

Email: [support@stilla.fr](mailto:support@stilla.fr)

Online technical support is also available [www.stillatechnologies.com/technical-support/](http://www.stillatechnologies.com/technical-support/)

## LEGAL NOTICES

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage or retrieval system, without permission in writing from Stilla Technologies.

Stilla Technologies reserves the right to modify its products and services at any time. Information contained in this user manual is subject to change without notice. Stilla Technologies assumes no liability for errors, or for any damages resulting from the use of this information.

All elements of the Naica system and its uses are covered by US and corresponding non-US patent claims owned by or under license to Stilla Technologies (Patents US9,452,432 ; US9,133,009 ; US9,816,133 | Patent Applications US15/568,399 ). Purchase of this product includes a limited, non-transferable right under such intellectual property for use of the product for internal research only. No rights are granted for diagnostic uses.

No right under any other patent claim and no right to perform commercial services of any kind, including without limitation, reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.

Further information on purchasing licenses may be obtained by contacting Stilla Technologies, 1 Mail du Professeur Georges Mathé, 94800 Villejuif, France. It is the responsibility of the purchaser/end user to acquire any additional intellectual property rights that may be required.

## TRADEMARKS

Crystal Digital™ PCR, Crystal Miner™, Crystal Reader™ and Naica™ are registered trademarks of Stilla Technologies. All other trademarks are the sole property of their respective owners.

## COPYRIGHT & INTENDED USE

Copyright 2018, Stilla Technologies. All rights reserved.

**For Research Use Only. Not for use in diagnostic procedures.**

# Contents

I.	Introduction .....	4
1.	Purpose of the document .....	4
2.	What is Crystal Reader? .....	4
3.	Intended use .....	4
II.	Context & Prerequisite .....	5
1.	Definition of an experiment .....	5
2.	Prerequisite for using Crystal Reader software .....	5
III.	Workflow of Experiment Acquisition .....	6
1.	Step (1) - Create a new experiment & Define experiment context .....	6
a)	Name your experiment .....	8
b)	Load preset parameters .....	8
c)	Edit experiment details .....	9
d)	Check scanning parameters .....	11
2.	Step (2) - Select the chambers to scan & Define chamber context .....	12
3.	Step (4) - Place the chips & Enter chip IDs .....	16
4.	Step (5) - Launch the scan .....	17
5.	Step (6) - Check image quality .....	19
IV.	Advanced Functionalities .....	25
1.	How to get the software version? .....	25
2.	How to rescan existing chambers? How to scan new ones? .....	25
a)	How to rescan all the chambers of the experiment? .....	26
b)	How to scan additional chambers? .....	26
c)	How to rescan only a subset of chambers? .....	26
3.	Settings: non-logged user .....	27
4.	Settings: logged user with "Basic" profile .....	28
5.	Settings: logged user with "Manager" profile .....	28
6.	How to perform focus calibration? .....	30
V.	Maintenance and Technical Support .....	33
VI.	Troubleshooting .....	34
VII.	License Information .....	35
1.	Crystal Reader license .....	35
2.	Third-party licenses .....	35

## I. Introduction

### 1. Purpose of the document

This document is the specific “Crystal Reader” user manual. It provides all detailed information for the use of the “Crystal Reader” software application which is part of the Naica™ System.

Please refer to the general user manual named “Naica Crystal Digital PCR System” for complete information about the use of the materials and equipment composing the Naica™ System.

### 2. What is Crystal Reader?

The Crystal Reader™ software component of the Naica™ System is dedicated to the image acquisition step in the Crystal Digital™ PCR workflow.

The Crystal Reader software:

- Allows to define the context of the current experiment or to plan the next ones
- Controls the Prism3™ instrument for the acquisition of the fluorescence images of the Naica chips
- Applies a pre-analysis treatment to the acquired images to provide a first quality control and prepare the next experiment analysis step performed by the Crystal Miner™ software

### 3. Intended use

The Naica system is a laboratory instrument for use by qualified personnel in a climate-controlled environment. The Naica system is intended for high-resolution genetic analysis based on digital PCR.

The Naica system is intended to be used for research use only, not for diagnostic procedures.

## II. Context & Prerequisite

### 1. Definition of an experiment

An experiment is a set of chambers which:

- Belong to unique Stilla chips (defined by a unique ID)
- Share the same experimental conditions, i.e.:
  - the same primers and the same probes (which implies the same fluorophores and the same targets)
  - the same Master Mix
- Have been imaged at 6.7 microns / pixel by the Prism3 instrument with the same scanning parameters, i.e.:
  - the same exposure times (in ms) for each of the 3 LEDs
  - the same focus value (in mm)

Once the experiment is scanned by the Prism3 instrument, the Crystal Reader software automatically records it as a “Naica Crystal Experiment” in a ZIP format called “.ncx”. This generated .ncx file contains all the chamber images in 16 bits TIFF format, as well as all the contextual and pre-analysis data. The indicative size for the .ncx file is: 60 MB / chamber.

### 2. Prerequisite for using Crystal Reader software

The image acquisition step can be performed either on the fixed PC provided by Stilla or on any other fixed PC which has the following specifications:

- **Operating System:** Windows 10 in 64 bits
- **Disk storage:** 1 TB (allowing to store ~1000 .ncx experiments of 12 chambers each)
- **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
- Computer tower case & motherboard should be compatible with the addition of [a PCI express card "Expansion card USB 3.0"](#), equivalent to the PEXUSB3S25 model, on the rear side of the PC. This USB 3.0 extension card allows efficient USB communication between the PC and the Prism3 instrument.

### III. Workflow of Experiment Acquisition

The Prism3 instrument should be switched on (green light on front side) and correctly connected to the PC (the gray USB cable should be plugged on the “PRISM3”-labeled ports located on the rear side of the PC and on the rear side of the Prism3 instrument). The PC should be turned on (default password is “admin” for “admin” user).

- Double-click on the desktop icon to launch the Crystal Reader application.



Fig. 1 - Launch icon

The 6 main steps of the experiment imaging workflow using Crystal Reader software are as follows:

- (1) Create a new experiment & Define experiment context
- (2) Select the chambers to scan & Enter chip IDs
- (3) Define chamber context
- (4) Clean & Place the chips
- (5) Launch the scan
- (6) Check image quality

#### 1. Step (1) - Create a new experiment & Define experiment context

- When the software is started, the HOME page is displayed:

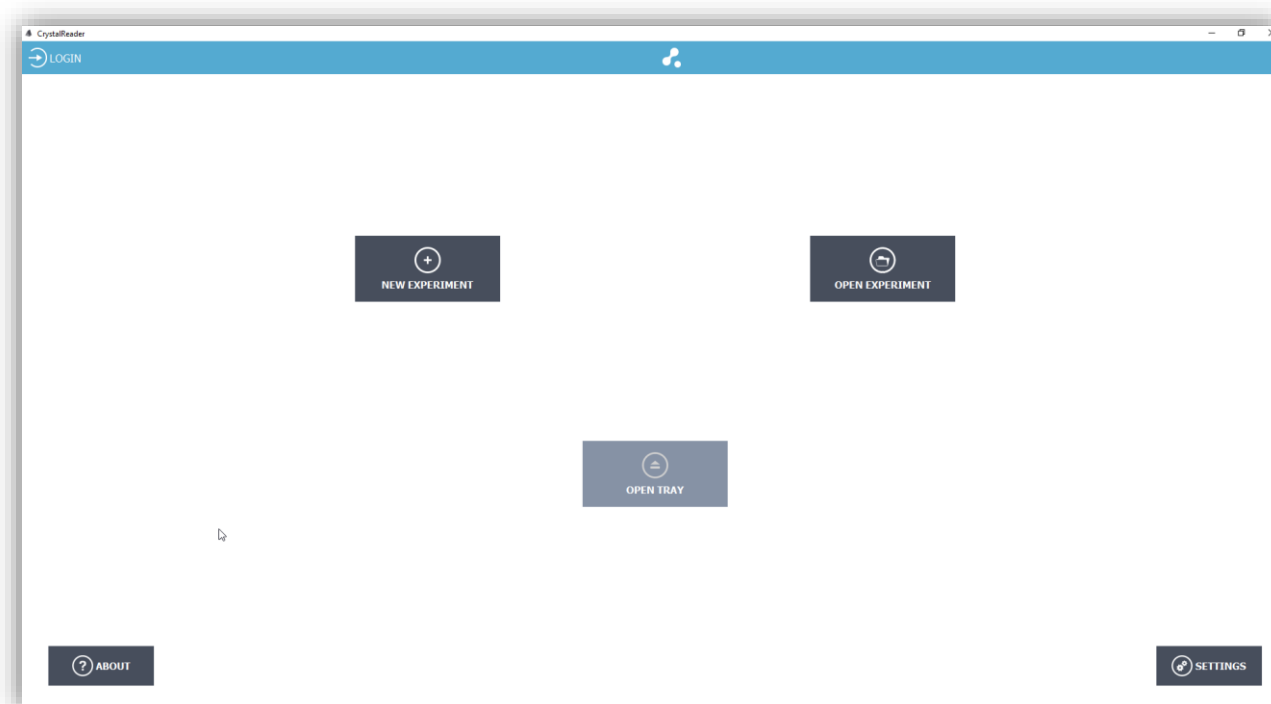
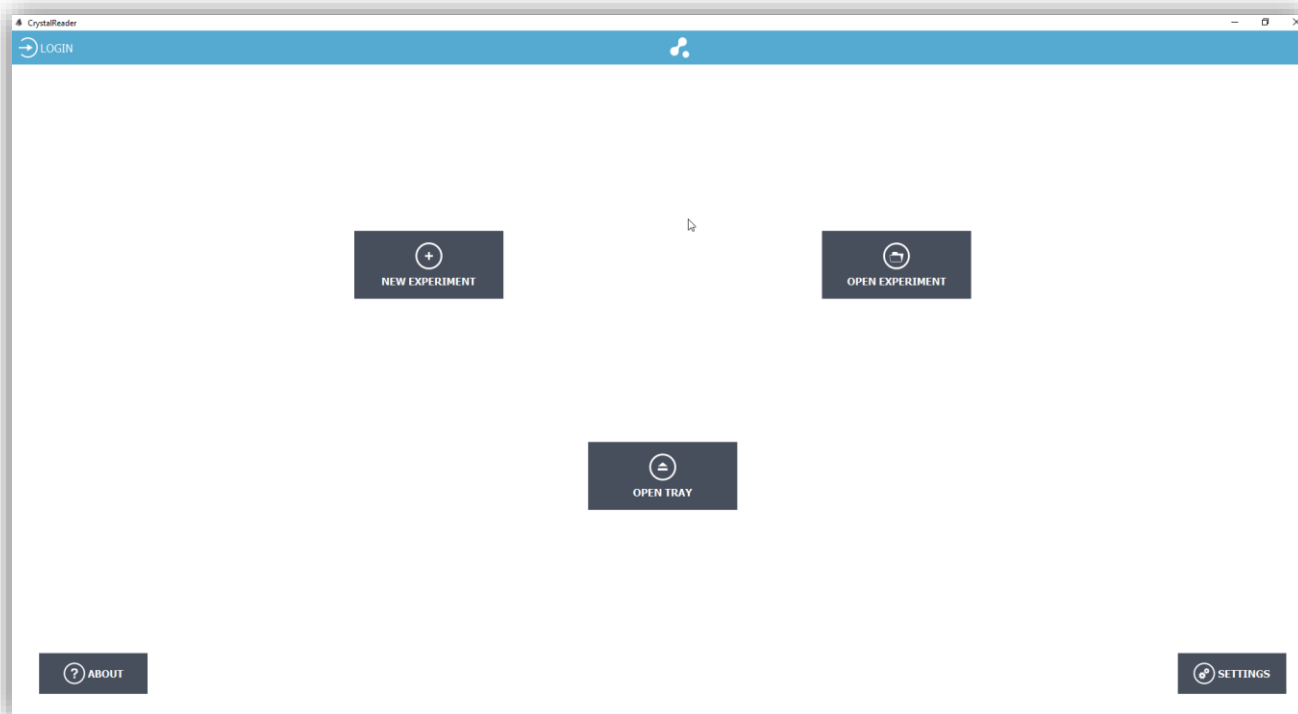


Fig. 2 - Step (1): Home page

You may notice that the “OPEN TRAY” button is deactivated. This happens when the connection with the Prism3 instrument is establishing. After a few seconds, the scanner is successfully recognized and all the instrument control buttons are enabled (including the “OPEN TRAY” button):

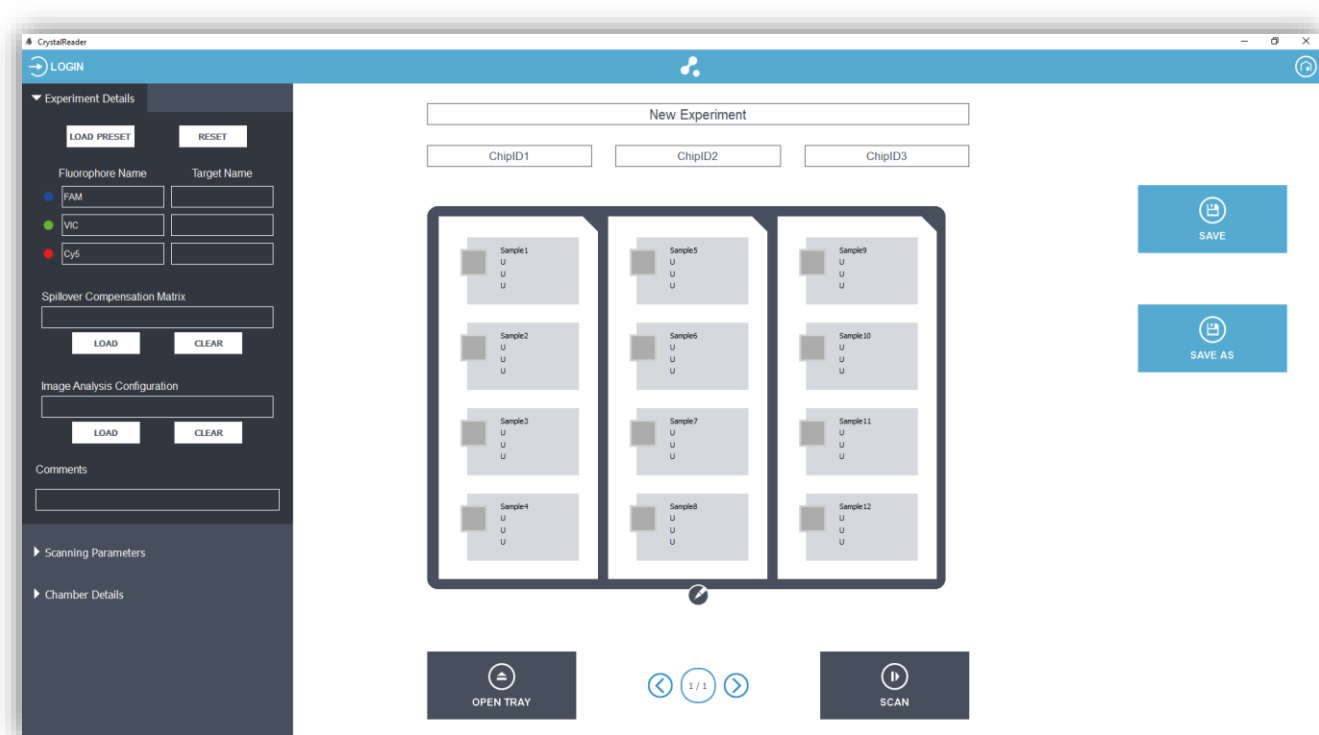


*Fig. 3 - Step (1): Home page when the scanner is successfully connected*

The Home page displays the following buttons:

- LOGIN: log in to make the user preference applied
- NEW EXPERIMENT: create a new experiment
- OPEN EXPERIMENT: open an existing .ncx experiment file
- OPEN TRAY / CLOSE TRAY: open / close the tray of the Prism3 instrument to place the chips on the chip holder
- ABOUT: see the information about the software and the list of third-party licenses
- SETTINGS: edit user preferences, software and instrument settings
- Cross icon (top right corner): quit the software

- Click on “NEW EXPERIMENT”, then the Scan page is displayed:



*Fig. 4 - Step (1): Scan page for a new experiment;  
the “Experiment Details” form can be edited*

## a) Name your experiment

- Enter the “Experiment Name” in the upper field.

Note: it is recommended to use traceability rule for experiment naming (e.g. date prefix, chip IDs, user initials, objective of the experiment).

## b) Load preset parameters

- If all your experiment parameters have been previously saved as a template, click on the “LOAD PRESET” button on top left and select your .ncx template to directly load them.

A pop-up window will ask you if you want to apply the template parameters to “Experiment Details” and “Scanning Parameters” only, or also to the “Chamber Details” (i.e. the configuration of all your samples).

Note that a .ncx template can be created by simply saving an experiment, and that [any compensation matrix file or image analysis configuration file included in this template will be automatically applied](#) once the template is loaded (see the next paragraphs explaining these files).



The default .ncx templates provided by Stilla are stored in “C:\Program Files\Stilla\CrystalReader\templates”.

They include the following templates for DNA and RNA analysis experiments using EvaGreen® dye or TaqMan probes:

- For DNA & EvaGreen® experiments:
  - Test experiment: “DNA\_analysis\_with\_Evagreen\_dye.ncx”
  - LOB Control experiment for Limit of Blank determination based on 36 negative control chambers: “DNA\_analysis\_with\_Evagreen\_dye\_LOB.ncx”
- For DNA TaqMan experiments:
  - Test experiment: “DNA\_analysis\_with\_Taqman\_probes.ncx”
  - LOB Control experiment for Limit of Blank determination based on 36 negative control chambers: “DNA\_analysis\_with\_Taqman\_probes\_LOB.ncx”
  - MonoColor Control experiment for fluorescence spillover compensation based on 1 negative control chamber and 3 mono-color control chambers: “DNA\_analysis\_with\_Taqman\_probes\_monocolor\_controls.ncx”
- For RNA & EvaGreen® experiments:
  - Test experiment: “RNA\_analysis\_with\_Evagreen\_dye.ncx”
  - LOB Control experiment for Limit of Blank determination based on 36 negative control chambers: “RNA\_analysis\_with\_Evagreen\_dye\_LOB.ncx”
- For RNA & TaqMan experiments:
  - Test experiment: “RNA\_analysis\_with\_Taqman\_probes.ncx”
  - LOB Control experiment for Limit of Blank determination based on 36 negative control chambers: “RNA\_analysis\_with\_Taqman\_probes\_LOB.ncx”
  - MonoColor Control experiment for fluorescence spillover compensation based on 1 negative control chamber and 3 mono-color control chambers: “RNA\_analysis\_with\_Taqman\_probes\_monocolor\_controls.ncx”

### c) Edit experiment details

- Go to the “Experiment Details” form in the left panel, then enter for each detection channel (“Blue”, “Green” and “Red” LED):
  - the name of the fluorophore that is mainly excited by the LED
  - The name of the target associated with this fluorophore
- If the “Spillover Compensation Matrix” field is empty: you have the possibility to load an already created spillover compensation matrix (using the matrix computation tool of Crystal Miner

software) by clicking in the “Load” button in the “Compensation Matrix” widget and selecting the corresponding .ncm file.

Note that the loaded compensation matrix should correspond to the conditions of your experiment (see Crystal Miner User Manual for more explanations).

If a compensation matrix is loaded, the fluorescence spillover will be automatically applied at experiment opening 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 (Master Mix used) and the scanning parameters (exposure times of the LEDs).*

- 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 largest amount of experiments you will prepare. This file is the following:

“C:\Program Files\Stilla\CrystalMiner\config\ AnalysisConfiguration\_GenericTemplate.yaml”

If you have defined another .yaml configuration file to suit a specific analysis from a previous experiment loaded and analyzed with Crystal Miner software, you may select the file here to automatically perform the image analysis with this configuration.

**CAUTION:**

*Please do not edit the default image analysis configuration file provided with Crystal Miner, it is advised to work on copies of the file. Do not try to perform this operation without reading the Crystal Miner User Manual.*

The default .yaml image analysis configuration files provided by Stilla is stored in “C:\Program Files\Stilla\CrystalMiner\config”.

They include these 2 configurations files for DNA and RNA analysis experiments:

- For all DNA experiments:

“AnalysisConfiguration\_GenericTemplate.yaml”

- For RNA experiments using the XLT mix:

“AnalysisConfiguration\_GenericTemplate\_mix-XLT.yaml”

- Finally, the “Comments” field can be used to store any information you wish to record about the experiment you are preparing.

#### d) Check scanning parameters

- Click on the “Scanning Parameters” form to potentially edit the exposure time of each excitation LED (in ms):

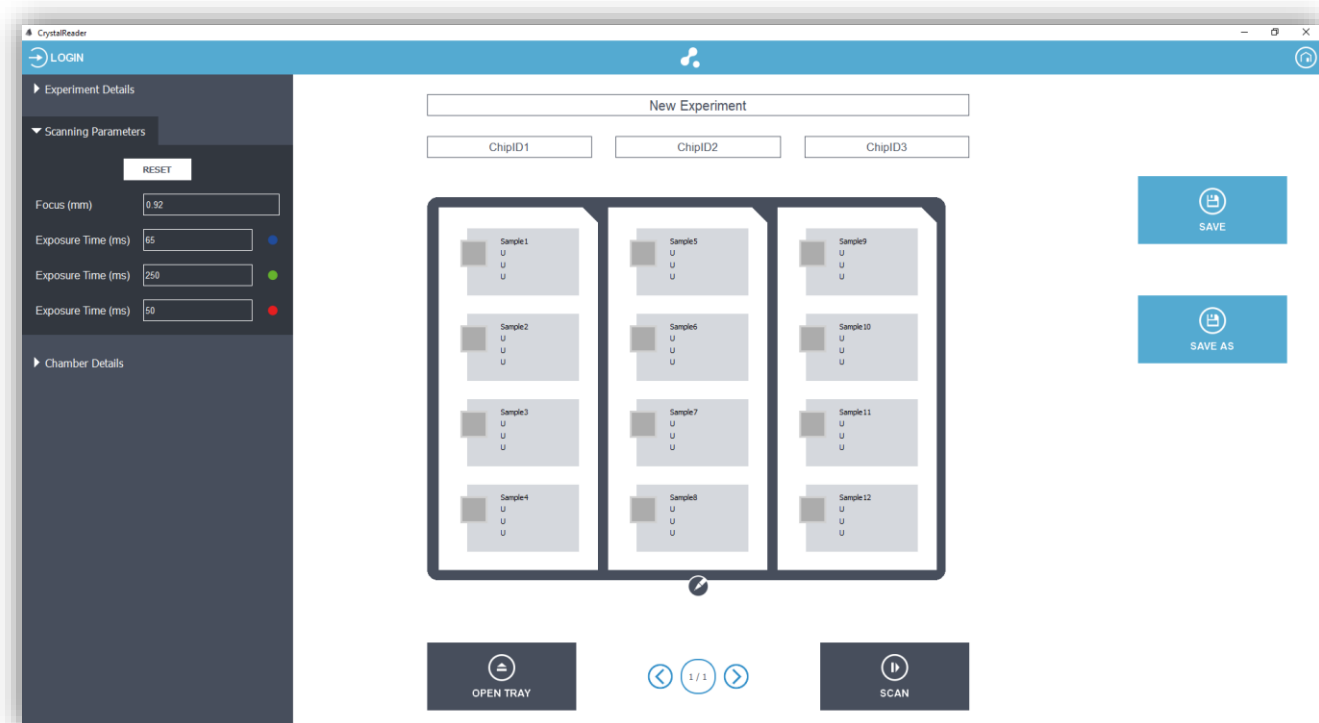


Fig. 5 - Step (1): Edit “Scanning Parameters” form


- 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). Some quality flags and tools will be given in the following “Chamber Exploration” chapter.

Note that:

- The focus value (representing the optimal z-distance of the imaging plane in mm) cannot be edited as it can only be calibrated in the “SETTINGS” menu by a user having a “Manager” account (for more details, see the section Advanced Functionalities > Settings)
- When at least one chamber of the experiment has been scanned in the experiment, the LED exposure times shouldn't be edited anymore in order to ensure that all the chambers of the experiment share the same scanning parameters.  
If you try to change an exposure value, you will be asked if you wish to proceed and erase the images of the already scanned chambers.

- To find the optimal LED exposure times for new experimental conditions, you may create a control experiment in which you activate only one chamber and perform a few scans until finding an optimal set of exposure times.

## 2. Step (2) - Select the chambers to scan & Define chamber context

- Click on the pencil icon  (located below the chip holder layout) in order to modify the selection of the chips & chambers to be scanned (by default the 12 chambers of a chip holder are selected)

Clicking on cross icons will deactivate the object (chamber, chip, chip holder)

Clicking on “+” blue icons will activate the object (chamber, chip, chip holder)

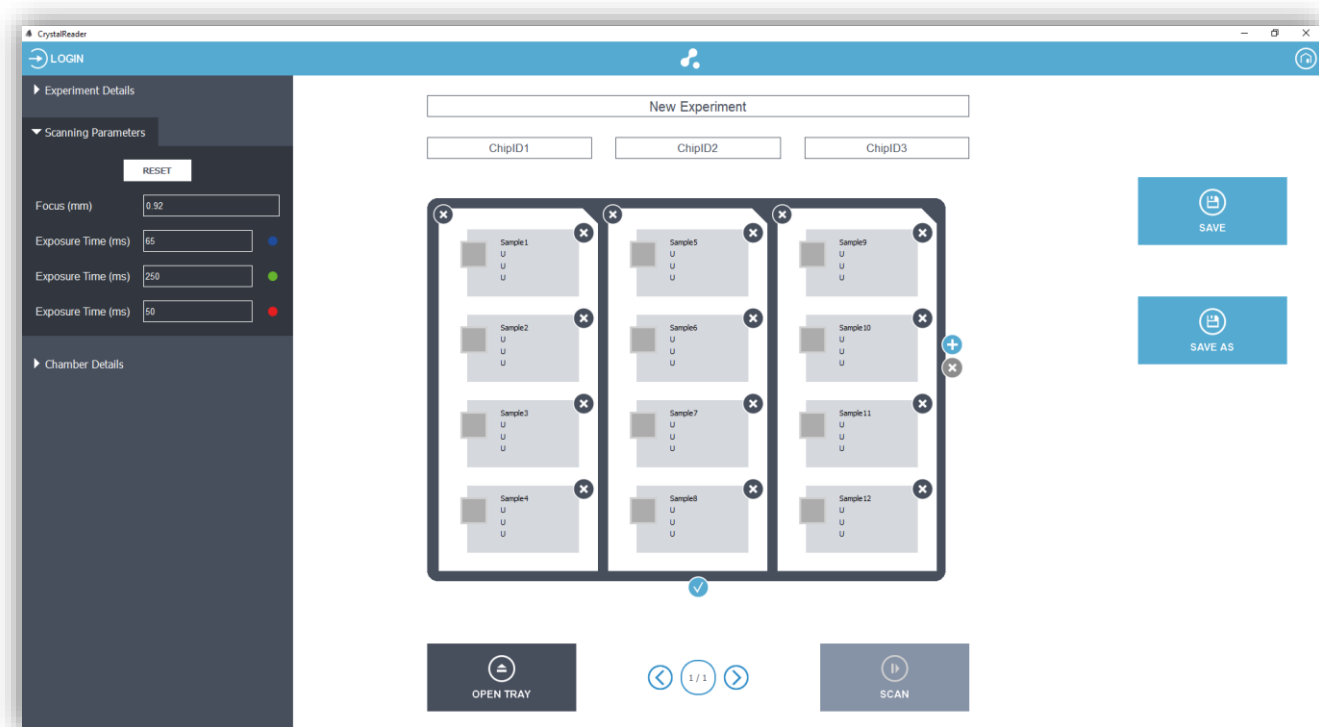


Fig. 6 - Step (2): Edit chamber selection

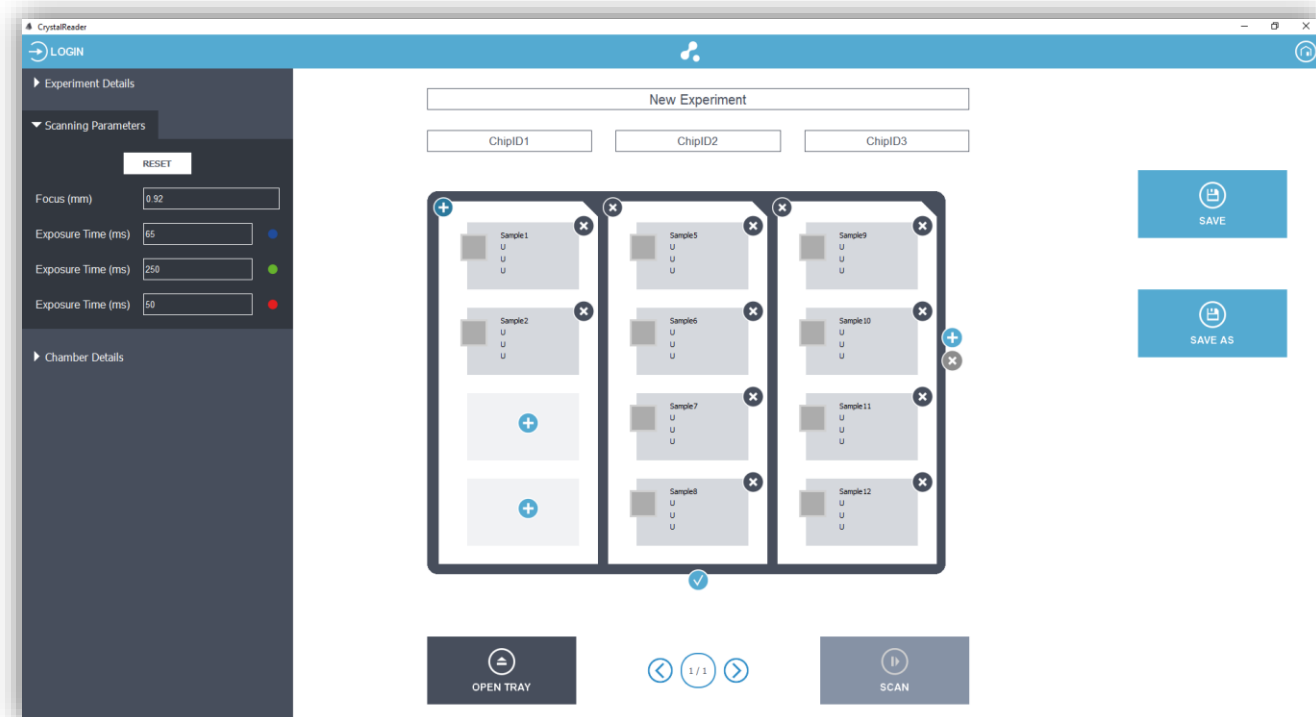



Fig. 7 - Step (2): Click on the cross icons to deactivate objects; click on the “+” icons to activate objects

- If you want to add another chip holder, click on the  button on the right side of the plate. 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.



Fig. 8 - Step (2): Chip holder counter

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

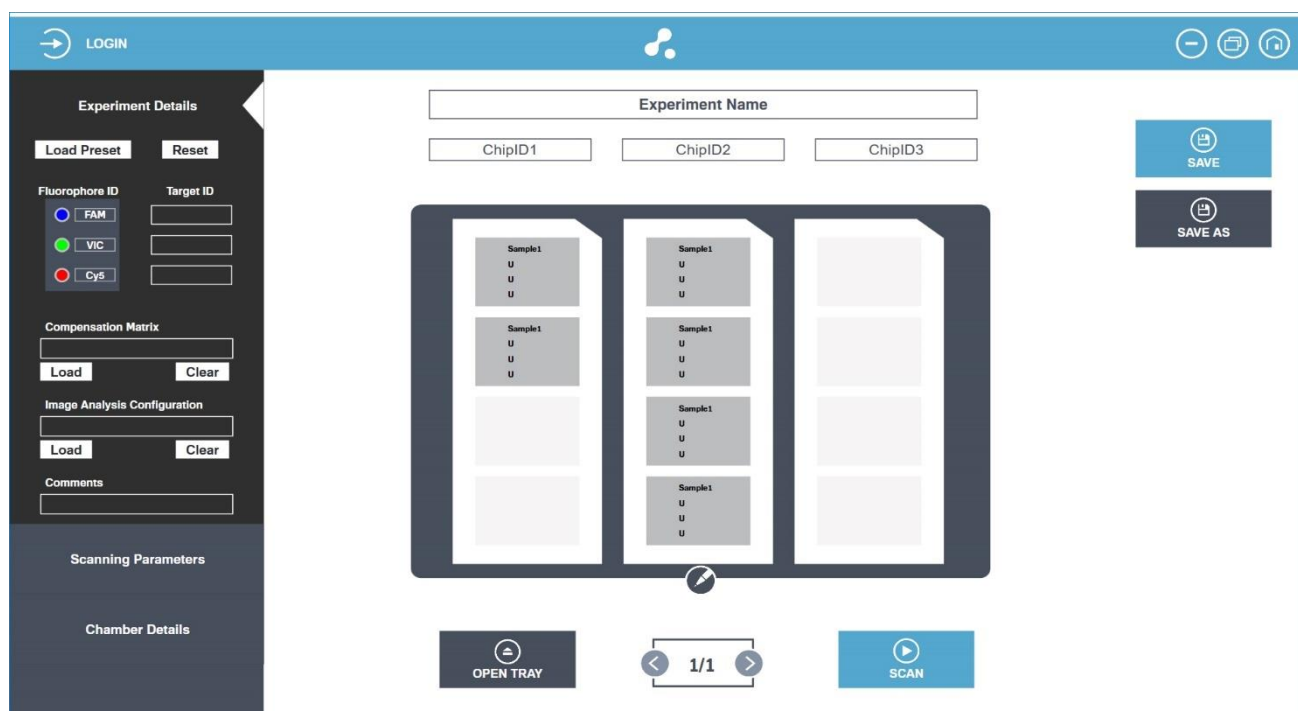
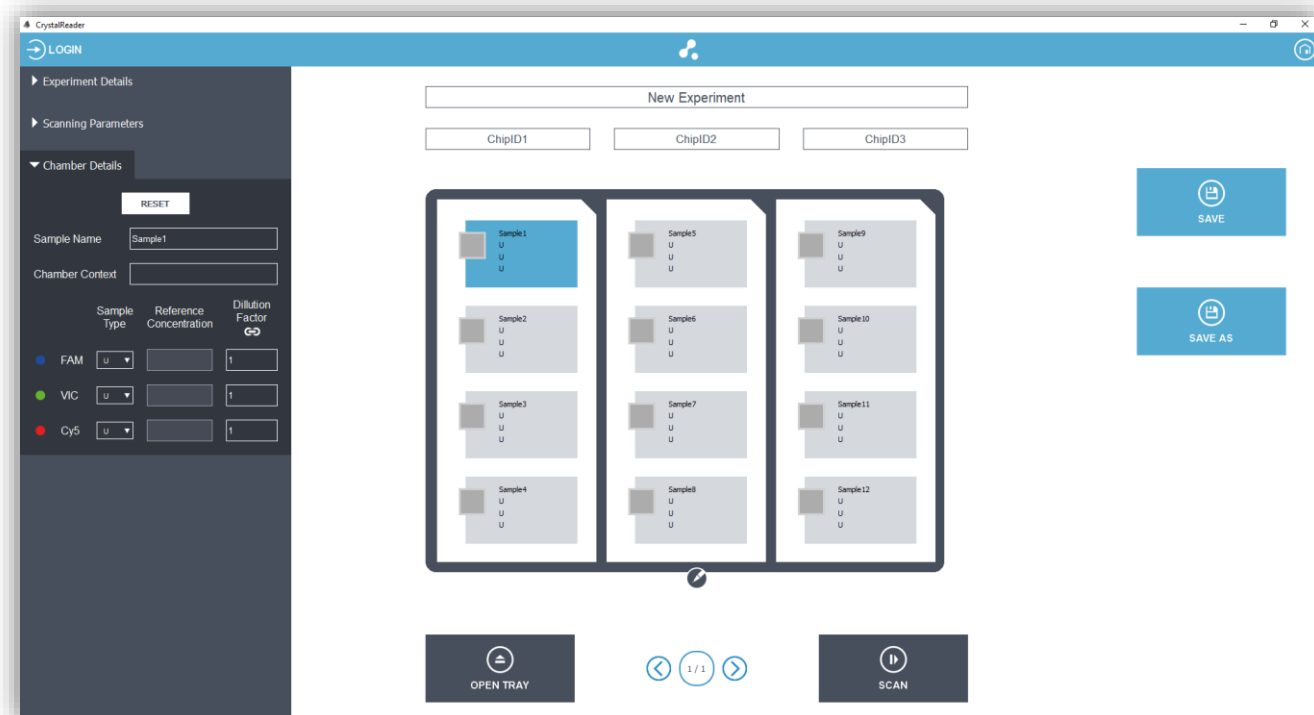


Fig. 9 - Step (2): The chamber selection is validated

- In the chip holder layout, click in the chamber for which you want to edit the chamber details (the chamber rectangle becomes blue and the “Chamber Details” form opens up), then you can edit the “Chamber Details” form:
  - “Sample Name”: to identify sample loaded in the chamber
  - “Chamber Context” (optional): to add extra information making the scan data unique (useful if scanning the chamber multiple times)
  - For each detection channel (“Blue”, “Green” and “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 /  $\mu\text{L}$ )
    - “Dilution Factor” (e.g. 10 for a 10-fold dilution from the stock sample)



*Fig. 10 - Step (3): Edit “Chamber Details” form associated with one or multiple chamber(s) highlighted in blue*

This form should be filled for each chamber of your experiment.

If the chamber details are common to multiple chambers, use “Ctrl+Click” or “Shit+Click” to select multiple chambers in the chip holder layout, then edit their details.

- Possible values for sample type in a given channel are:
  - U: Unknown (default value)
  - N: Negative control (i.e. there are no targets for the channel of interest)
  - P: Positive control (i.e. there are targets for the channel of interest)
  - S: Standard (i.e. the stock concentration is known for the channel of interest and should be entered in the next field in copies /  $\mu$ L)
- For the dilution factor, entering the value “10” means that the sample has been diluted 10 times from the stock. To unlink the dilution values among the channels, click on the “link” icon.

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

### 3. Step (4) - Place the chips & Enter chip IDs

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

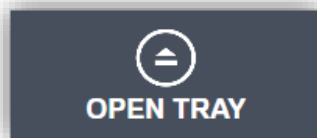


Fig. 11 - Step (4): “OPEN TRAY” button to open the tray.  
Once the tray is opened, this button becomes “CLOSE TRAY”.

#### 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-hand corner).*

- Clean the bottom surface of each chip using a lint-free wipe to remove any dust that may be present. Keep the chip horizontal during cleaning.
- 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 mistake-proofing on the top).

You can scan up to 3 chips at a time.



Fig. 12 - Step (4): Positioning of the chips in the chip holder of the Prism3 instrument  
with the diagonal mistake-proofing on the top right

#### CAUTION:

*Make sure to clean the chips prior to placing them in the slots of the chip holder.*

- Enter the chip barcodes in the corresponding chip ID fields (from left to right slot).

If available, use the barcode scanner to gain time and avoid typing mistakes.



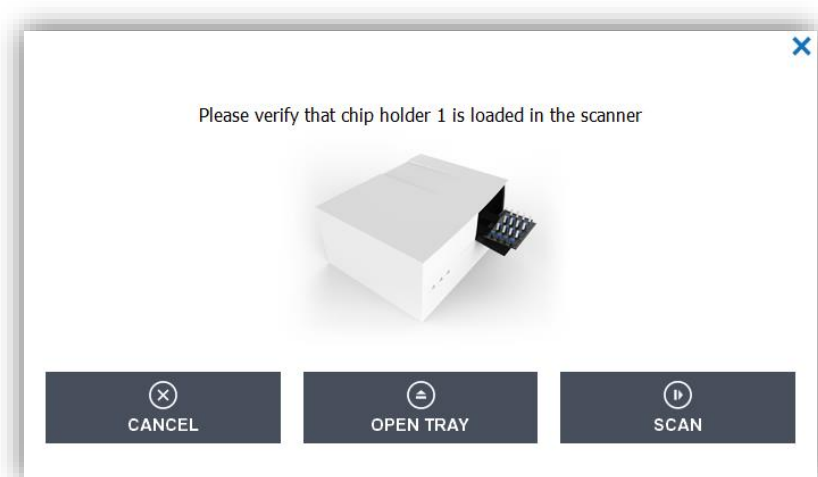
**CAUTION:**

***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

**4. Step (5) - Launch the scan**

- Click on the “SCAN” button, then confirm the loading of the first chip holder to launch the scan



*Fig. 13 - Step (5): 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. You may cancel your scan at any time, but you might lose some scanned data.

Editing the experiment context is deactivated during the scan process.

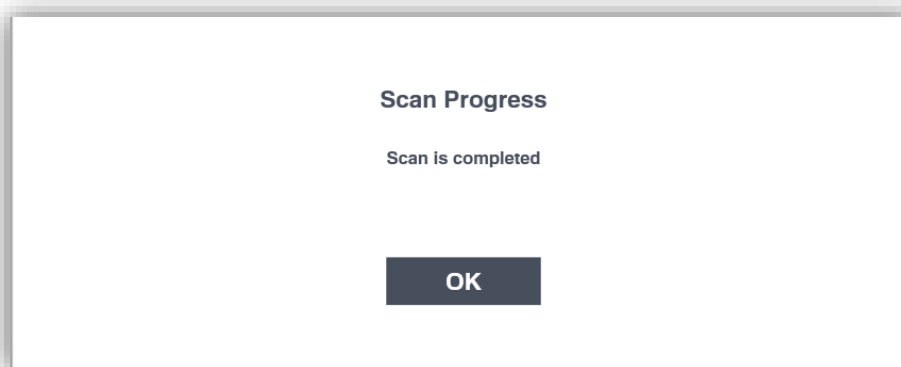
- If several chip holders have been selected for the scan, the scanning process is split in several steps:
  - Once the current chip holder is completely scanned, a pop-up window is displayed and invites you to load the next chips
  - Click on “OPEN TRAY”, place the next chips to be scanned, then click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

During the scanning process, the already scanned images are pre-analysed in parallel and a quality flag is added in the chamber rectangle as soon as it is ready to be explored:

-  "Green" quality flag indicates high quality
-  "Yellow and Green" quality flag indicates moderate quality
-  "Yellow" quality flag indicates low quality

Before the scanning process is over, it is already possible explore the images of a chamber by clicking on its flag.

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



*Fig. 14 - Step (5): Scan is completed*

- Click on "OK" to see the results of the scanning process

Note that 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: "C:\Users\<User>\Documents\Stilla\CrystalReader"

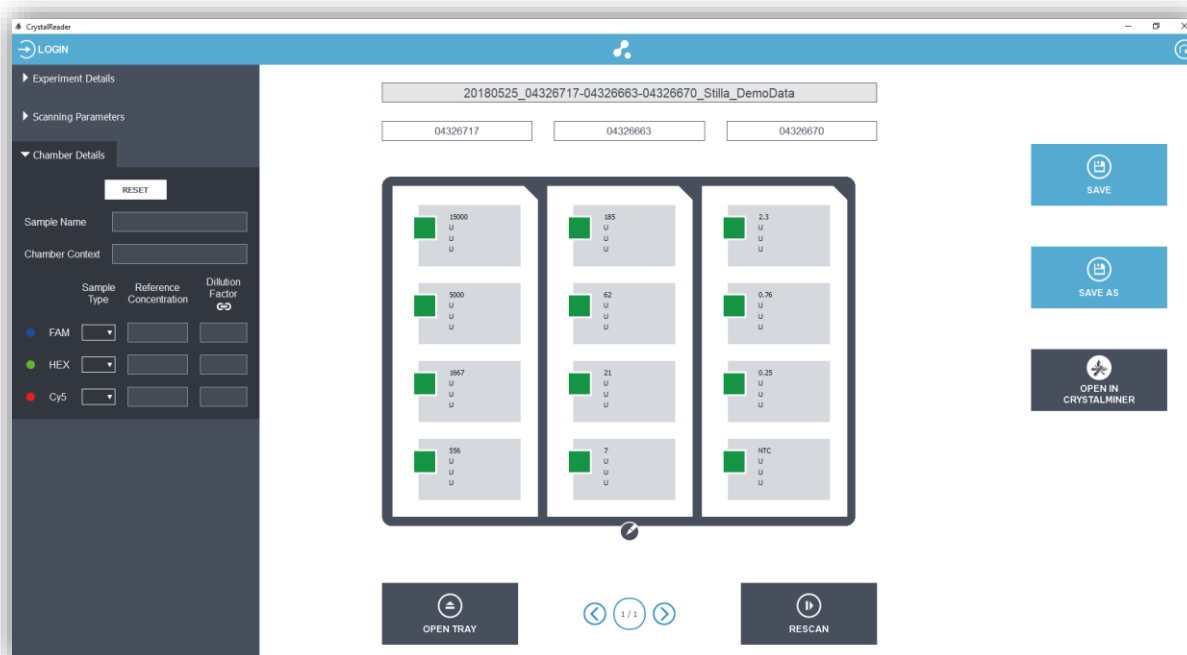


Fig. 15 - Step (5): Example of chip holder layout with quality flags at the end of the scan

## 5. Step (6) - Check image quality

- Click on the quality flag of a chamber to explore the chamber images

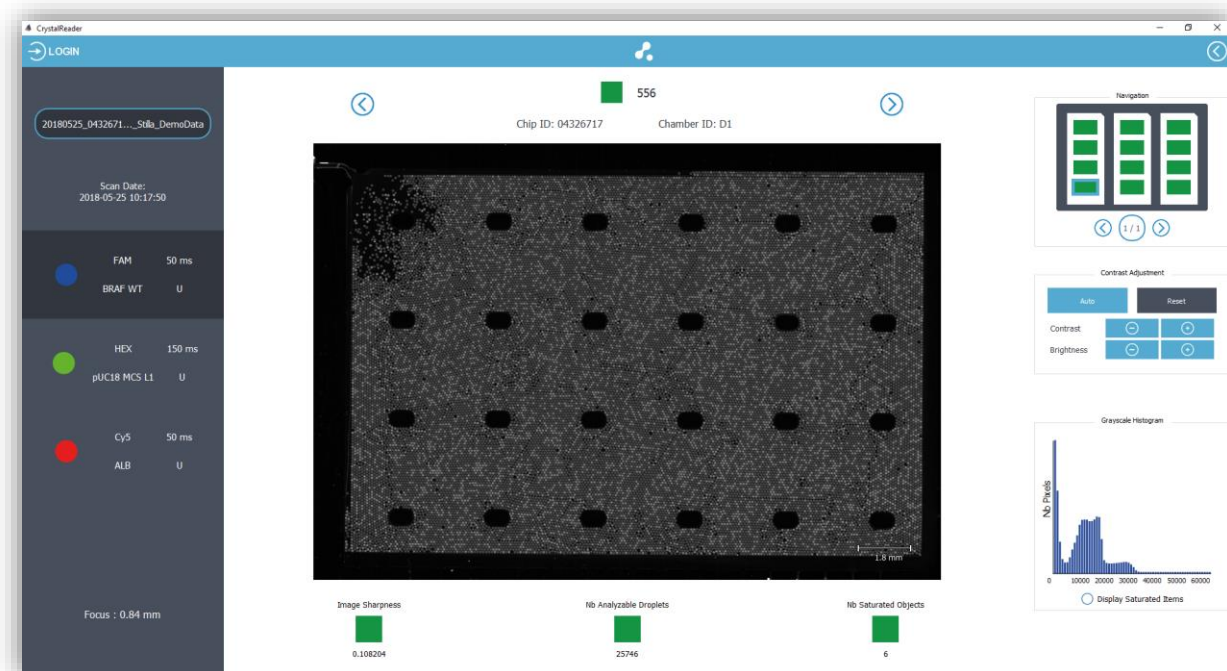


Fig. 16 - Step (6): Overview of a chamber image in the “Blue” detection channel

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 top right (click on the rectangle of a chamber to explore it)
  - The left / right arrow buttons on 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 fluorophore).

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 is not possible.***

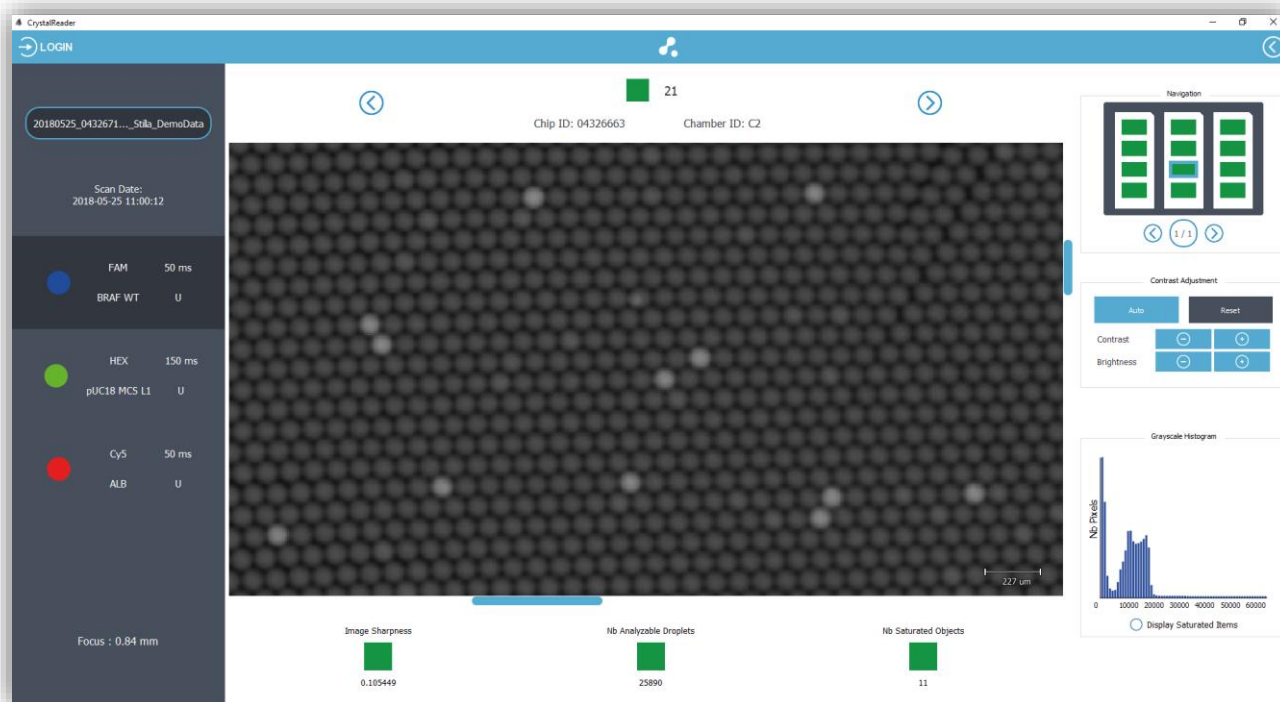


Fig. 17 - Step (6): Zoom in a chamber image in the Blue detection channel (all negative droplets should be visible in the Blue channel image)

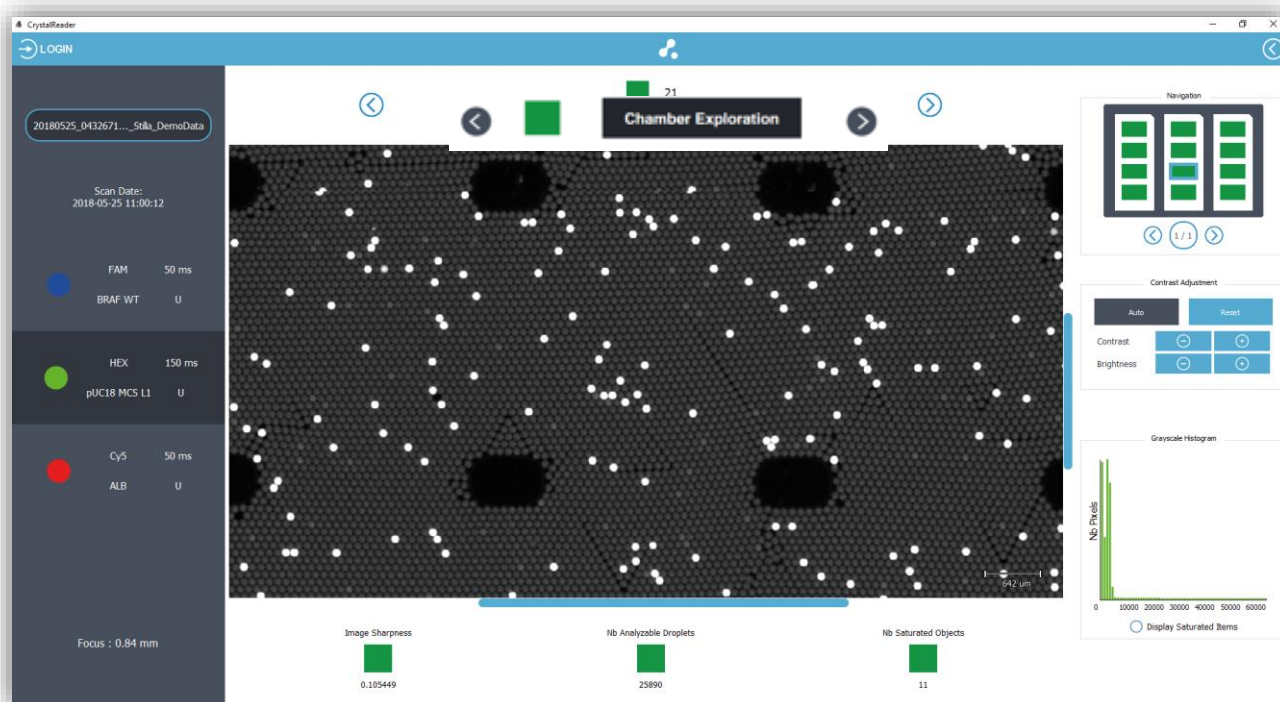
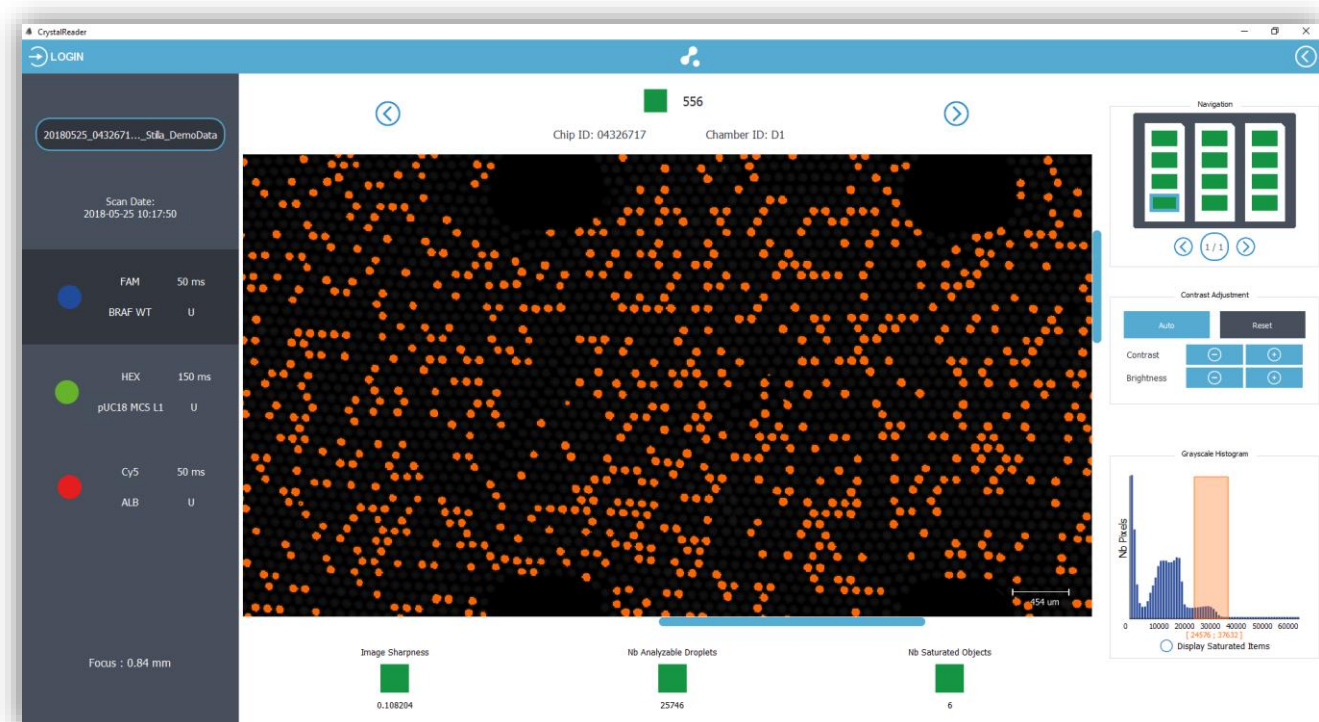


Fig. 18 - Step (6): Zoom in a chamber image in the Green detection channel with the "Auto" contrast

- The Grayscale Histogram tool (on 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.

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:



*Fig. 19 - Step (6): Use the histogram to place intensity cursors and reveals the pixels corresponding to your RFU interval*

This tool helps you 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:

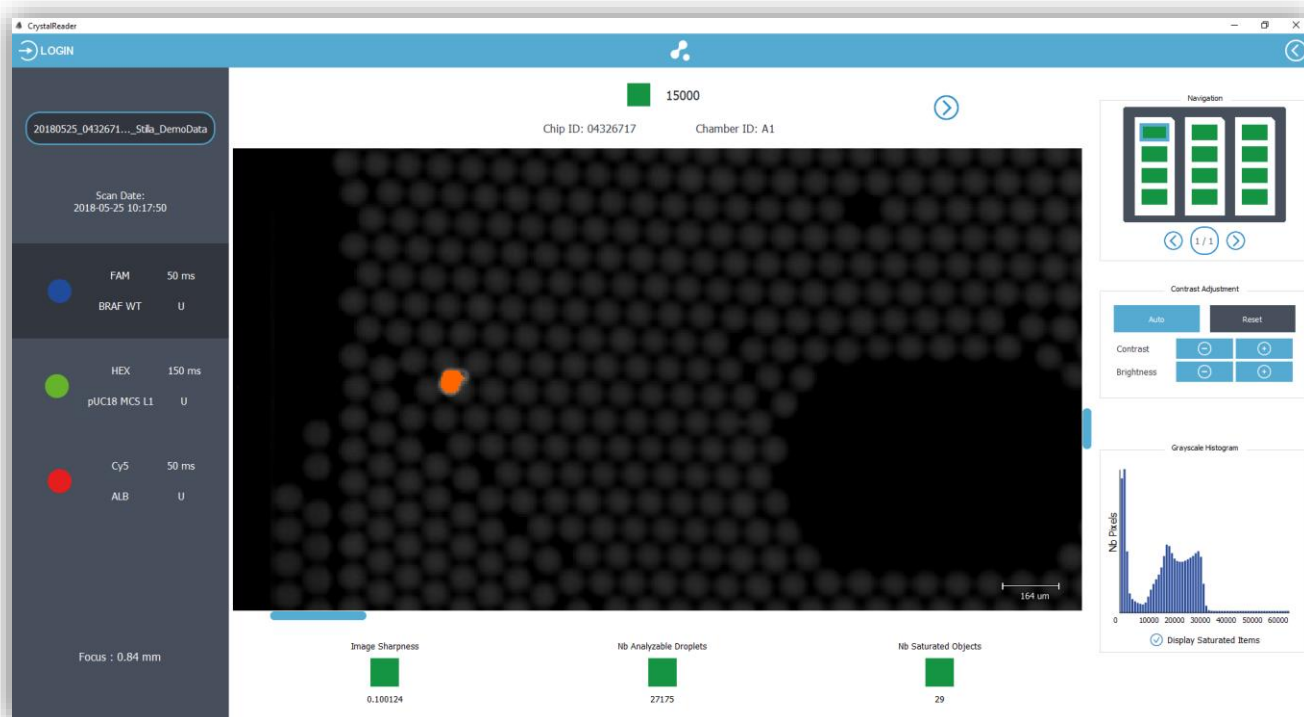


Fig. 20 - Step (6): Overlay of the saturation map in the Blue detection channel (saturated pixels are displayed in orange color). In this case it is a dust particle.

### 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 analysable 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 that a yellow flag may be caused by an out-of-focus issue (this can be corrected by checking that chip holder is clean and chamber is well placed)

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

Note that saturated droplets and droplets covered by artefacts are not analyzable. They can be avoided by checking that exposure times are not too high and chamber is cleaned up.

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

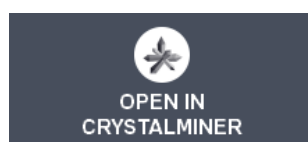
Note that saturated objects are either saturated droplets caused by too high exposure time or saturated artefacts. They can be avoided by checking that exposure times are not too high and chamber is cleaned up.

If some exposure times need to be modified in order to improve the image quality, you will need to rescan the whole experiment (see the section “Advanced Functionalities” for more details about chamber rescan).

If some chamber needs to be cleaned in order to improve the image quality, you will need to partially rescan the experiment (see the section “Advanced Functionalities” for more details about chamber rescan).

- The saved experiment can be now analyzed with Crystal Miner software (please refer to Crystal Miner Software User Manual for details).

You can use the button “Open in Crystal Miner” to automatically run the software on your scanned experiment:



*Fig. 21 - Button to open the scanned experiment with Crystal Miner software*

- To turn off the Prism3 instrument, press a few seconds on the front power button (the green light becomes orange), then switch off the back power button.



## IV. Advanced Functionalities

### 1. How to get 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:

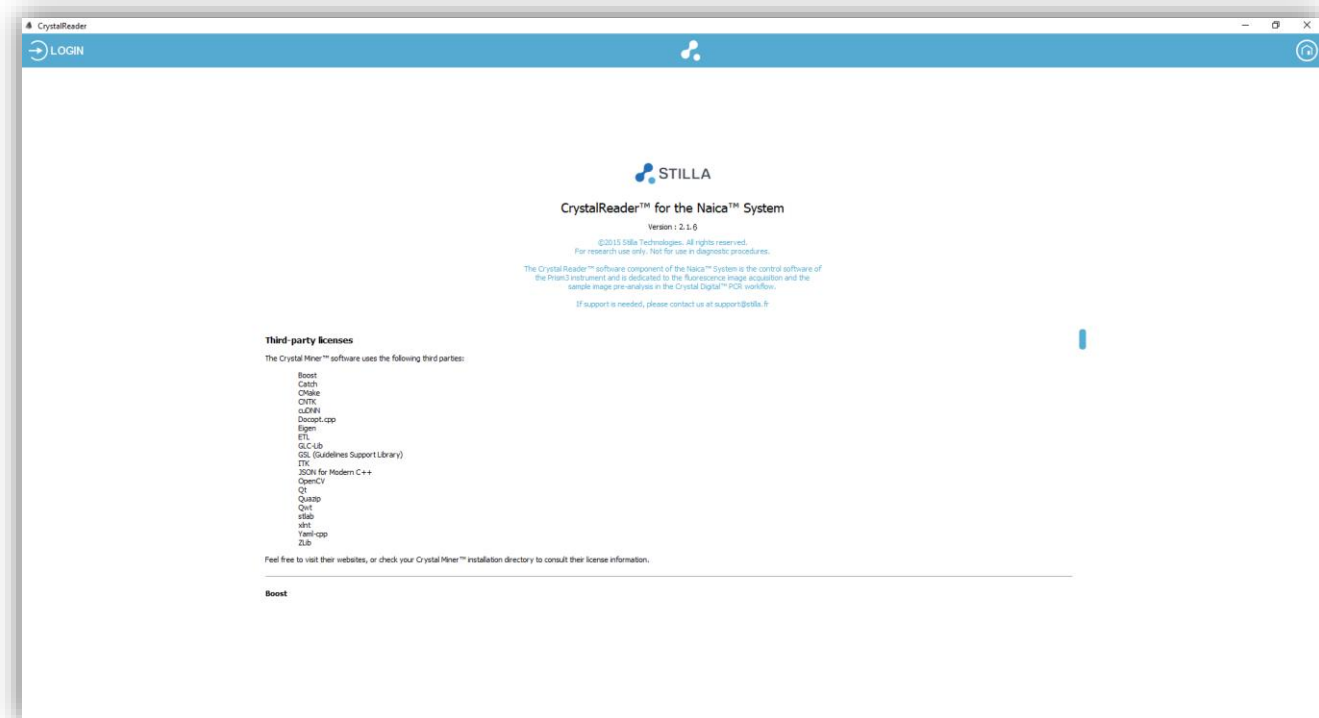


Fig. 22 - About page

### 2. How to rescan existing chambers? How to scan new ones?

- Click on the RESCAN button

You may face 3 scenarios:

- 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

Whatever scenario you choose, if there is at least one already scanned chamber when you click on the “SCAN” button, this window will be displayed:

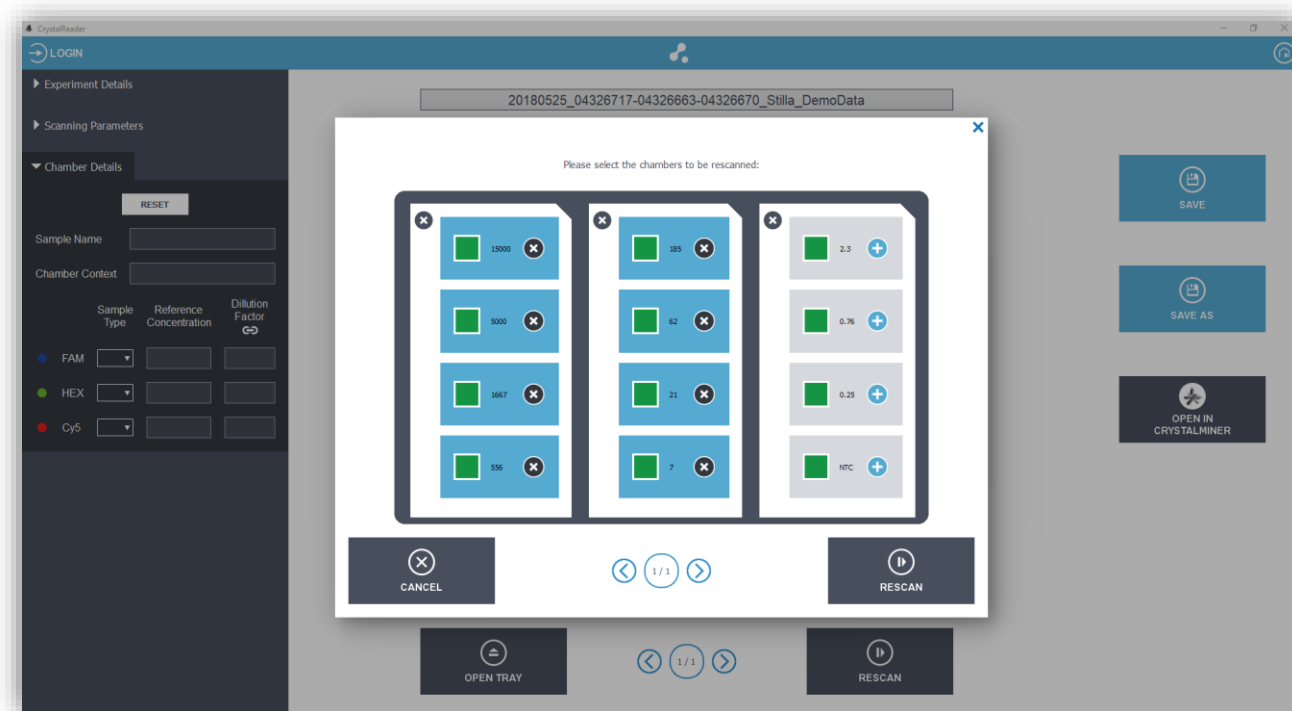


Fig. 23 - Default rescan window (chambers to be rescanned are highlighted in blue color)

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

#### a) How to rescan all the chambers of the experiment?

For this scenario: [click on the validation icon](#), and the scan will start

#### b) How to scan additional chambers?

For this scenario: [unselect your previously scanned chambers, and activate the new ones to be scanned](#)

#### c) 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 you wish to add to your experiment](#)

### 3. Settings: non-logged user

- From the HOME page, click on the “SETTINGS” button.

The Settings page is displayed:

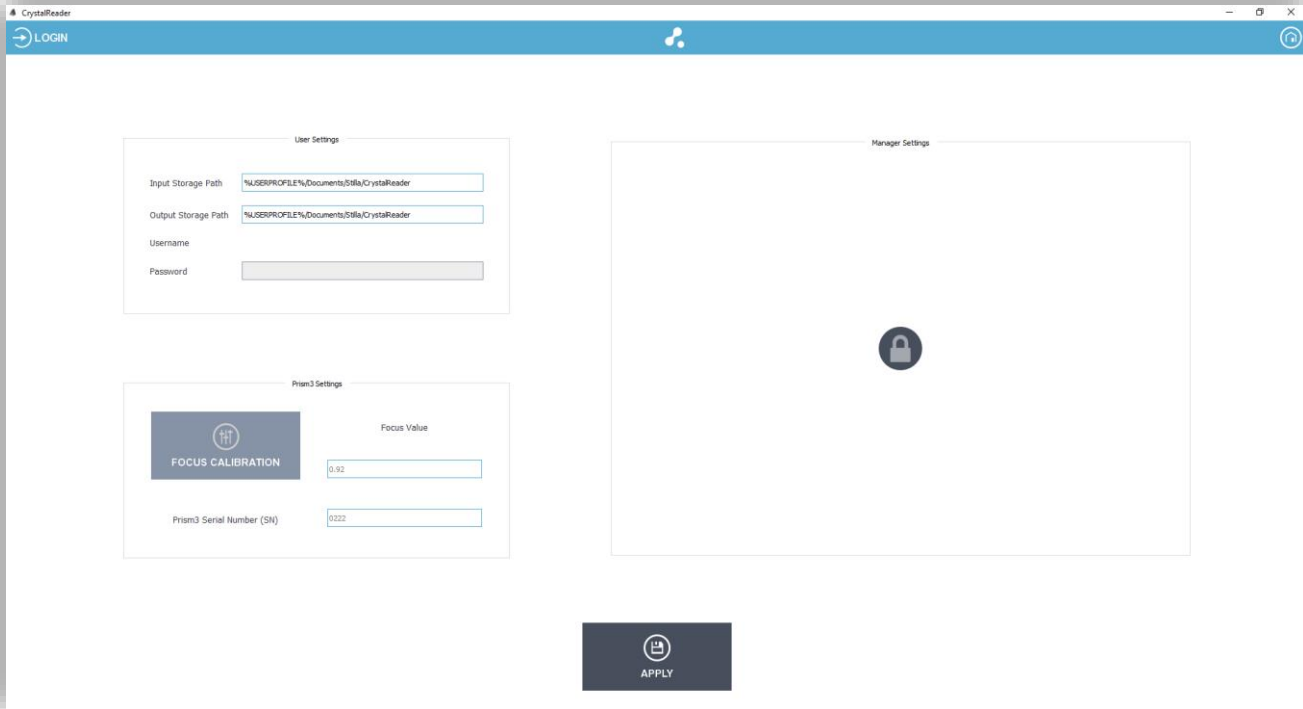


Fig. 24 - Settings page for non-logged user

- 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 your .ncx experiment files
- **Username:** your username
- **Password:** your password

- Prism3 Settings:

- **Focus Value (mm):** z-distance to the imaging plane used for image acquisition (set by the lab manager)
- **Prism3 Serial Number (SN):** unique serial number of the Prism3 instrument (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 you use Crystal Reader without log-in, you will be only able to modify the paths to output and input storage, and this configuration will not be saved the next time you launch Crystal Reader software.

#### 4. Settings: logged user with “Basic” profile

Unless modified after software installation, a default “Basic” account is created with the login “guest” and the password “guest”.

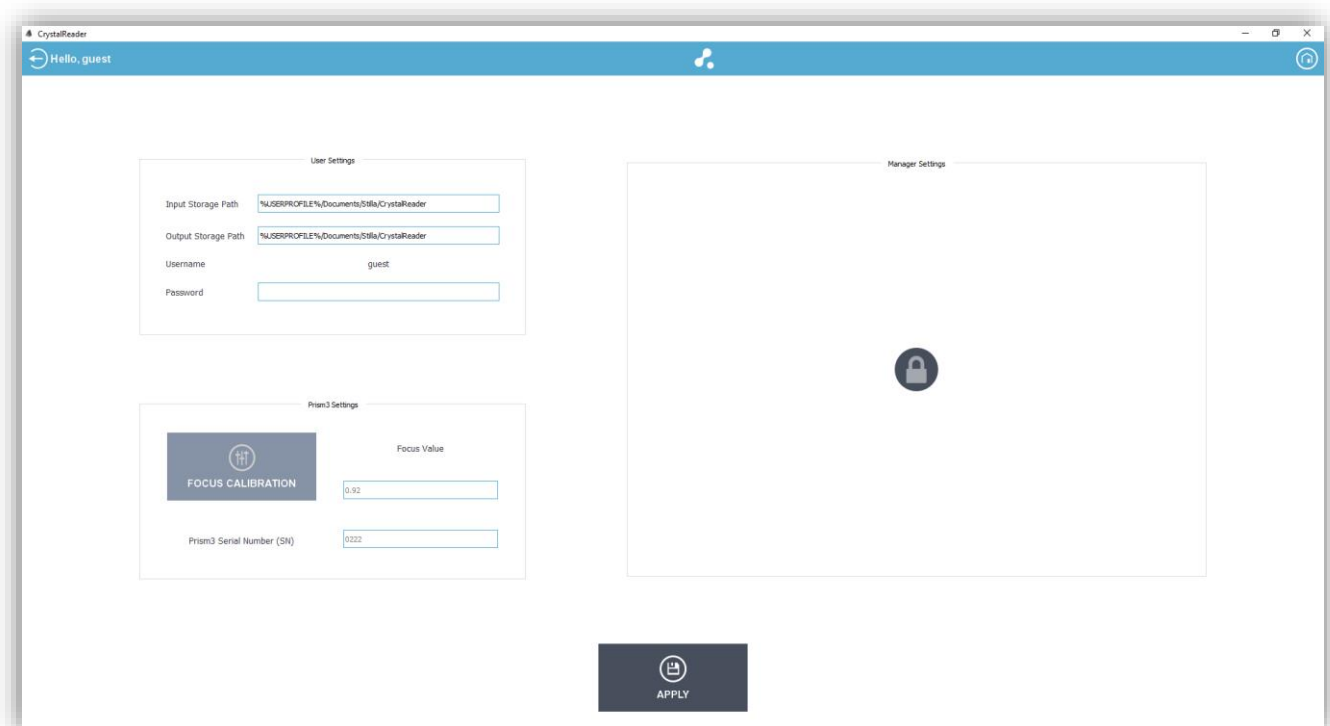


Fig. 25 - Settings page for logged user

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.

#### 5. Settings: logged user with “Manager” profile

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).

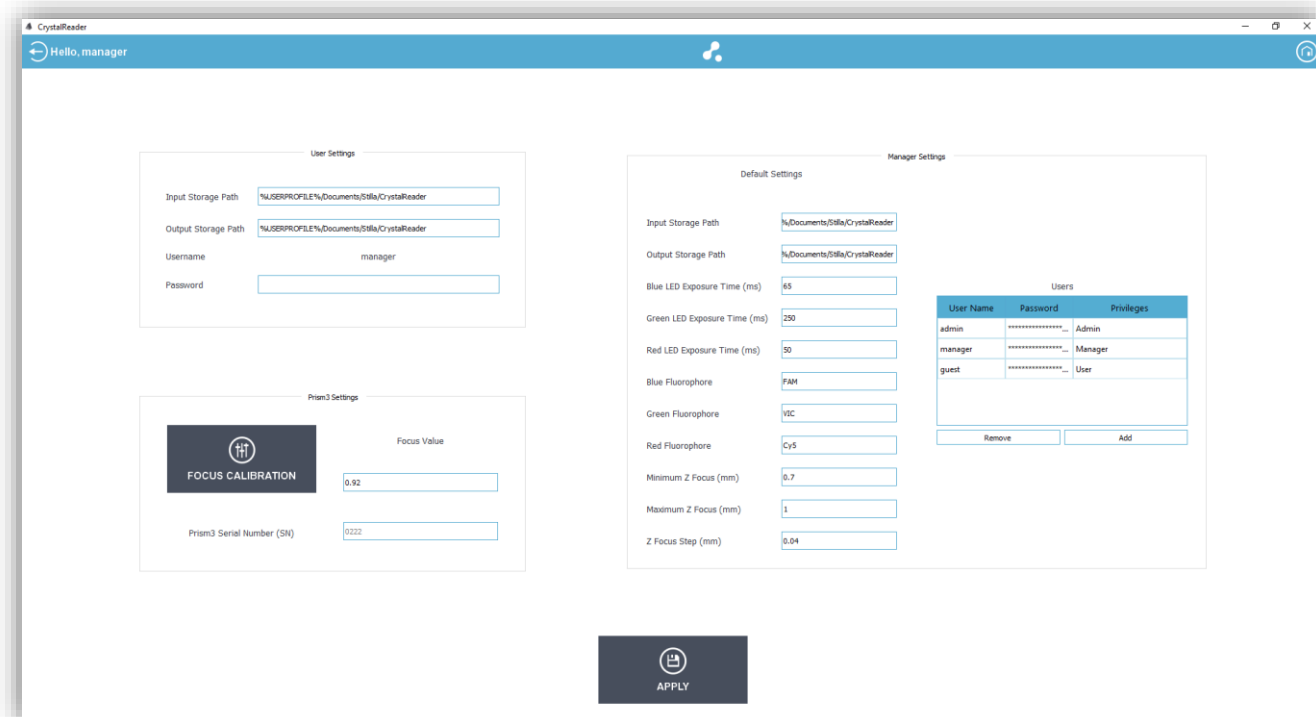


Fig. 26 - Settings page for logged manager

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.
  - 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”
- You may use “%USERPROFILE%” in the path fields: this is a Windows environment variable which 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 purpose, 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).

## 6. How to perform focus calibration?

The focus calibration is an advanced functionality which 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 you are already logged with your “Manager” account, and that a chip with a chamber of interest is already inserted in the Prism3 instrument, go to the [SETTINGS](#) menu and click on the “Focus calibration” button.

Note: For optimal focus calibration, it is recommended to use a chip that includes chamber with good quality droplet crystal and to place this chip on the middle slot of the chip holder (i.e. slot 2).

- [Select your chamber on interest](#) (i.e. the chamber on which you want to calibrate the optimal acquisition focus) by clicking on the chamber rectangle in the chip holder layout (the rectangle becomes blue).

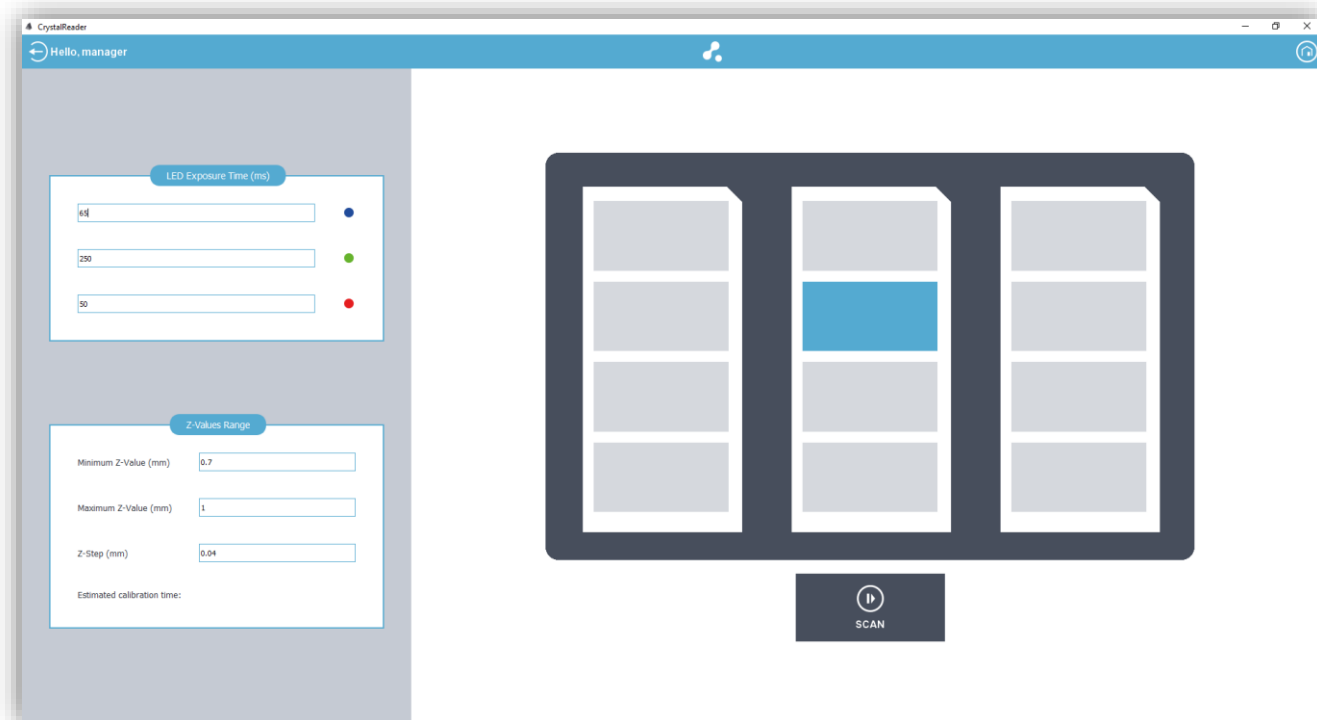
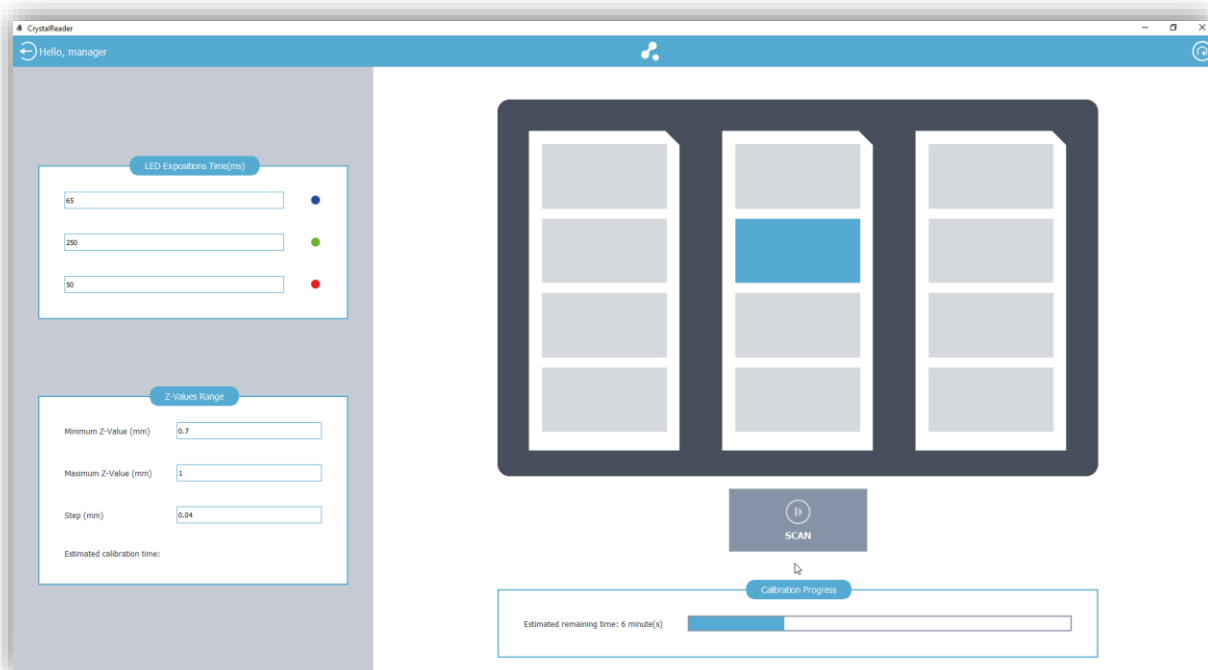


Fig. 27 - Selection of the chamber to calibrate the focus

- Click on the validation icon on the bottom to launch the focus calibration process.

- A progress bar is displayed with the estimated remaining time:



*Fig. 28 - The focus calibration is in progress*

- At the end of the focus calibration process, a result page is displayed, showing the value of the optimal focus (in mm) providing the highest image sharpness score, and the chamber image acquired at this optimal focus. A table and its corresponding graph will help you to check or adjust the z-value that provides the best result:

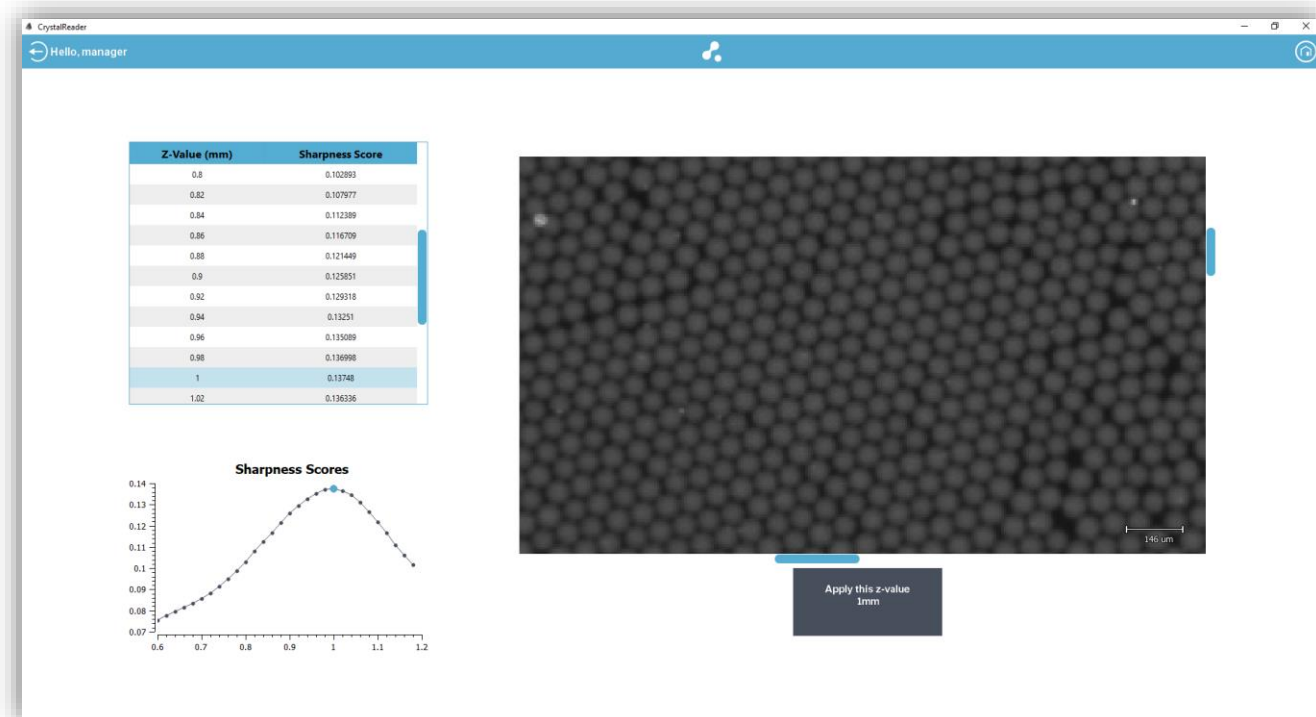


Fig. 29 - Result of the focus calibration

- You may select the other tested focus values by clicking on them in the table, in order to check the sharpness of the chamber images acquired at different focus values.
- You 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.

To cancel, click on the “Home” button on top right.



## V. Maintenance and Technical Support

For technical questions or dysfunction, refer to [www.stillatechnologies.com](http://www.stillatechnologies.com) or contact us directly at [support@stilla.fr](mailto:support@stilla.fr).

Tel: (+33) 9 82 27 47 47

Opening hours: 9h30-18h30 (GMT+2) on working days

***We will make our best to answer as promptly as possible.***

To enable remote software maintenance on the PC provided by Stilla, check that the PC 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 [support@stilla.fr](mailto:support@stilla.fr).



*Fig. 30 - Desktop icon of the “Team Viewer” application for remote software maintenance*

Please prepare the following information 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:

“C:/Users/<UserName> /Stilla/CrystalReader”

## VI. Troubleshooting

Observations	Recommended action
a) A red light appears in 3 <sup>rd</sup> position on the front side of the Prism3 instrument	Restart the Prism3 instrument (switch off and on the rear power button, then press on the front power button)
b) The Crystal Reader application does not respond anymore	Go to "Control Panel" > "Task Manager" and kill the Crystal Reader process, then start it again
c) The Crystal Reader application does not control the Prism3 instrument anymore (e.g. impossible to open / close the tray, or to scan)	<ul style="list-style-type: none"> <li>Check that no red light appears in 3<sup>rd</sup> position on the front side of the Prism3 instrument (else see a)).</li> <li>Check that the PC is correctly connected to the Prism3 instrument (the gray USB cable should be plugged on the "PRISM3"-labelled port of the PCI express card located on the rear side of the PC and on the "PC"-labelled port located on the rear side of the Prism3 instrument), and restart the PC.</li> </ul>
d) The Prism3 scanner is not recognized at all by the PC	<ul style="list-style-type: none"> <li>Go to "Control Panel" &gt; "Device Manager" &gt; "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).</li> <li>If the problem persists, try to reboot the PC</li> </ul>
e) The Crystal Reader software sends an error message when it is started, saying that a "msvcpxxx.dll" library is missing	<p>Download and install libraries "Redistributable Visual C++ for Visual Studio 2017" . You may use this link:  <a href="https://aka.ms/vs/15/release/vc_redist.x64.exe">https://aka.ms/vs/15/release/vc_redist.x64.exe</a></p> <p><b>Warning:</b> please check that you are downloading the x64 processor version of the redistributables</p>
f) Unstable connection between the Prism3 and the Naica PC	Check that the scanner is ON and correctly connected to the PC (the original USB cable should be plugged on the "PRISM3"-labelled port of the PCI express card located on the rear side of the PC and on the USB port located on the rear side of the Prism3 instrument). Then, restart the Crystal Reader software. If the issue persists, reboot the PC.
g) Image acquisition issue (Black or non-existent flag)	Re-scan the concerned chamber
h) Non optimal image assembly in a chamber	Re-scan the concerned chamber

If these observations persist, contact the technical support:

Email : [support@stilla.fr](mailto:support@stilla.fr); Phone: (+33)0982274747

## VII. License Information

### 1. Crystal Reader license

*©2015 Stilla Technologies. All rights reserved.  
For research use only. Not for use in diagnostic procedures.*

### 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++
- OpenCV
- Python
- Qt
- Quazip
- Qwt
- stlab
- xInt
- Yaml-cpp
- ZLib

Feel free to visit their websites, or check your Crystal Reader and Crystal Miner installation directory to consult their license information (by default in “C:\Program Files\Stilla\CrystalMiner\licenses”).

Note that the license information of all the third-part software components is also accessible in the Crystal Reader application, by clicking in “About” button in the Home page.

# Crystal Reader™ for the Naica™ System

---

Stilla Technologies  
Villejuif BioPark  
1, Mail du Professeur Georges Mathé  
94800 Villejuif, FRANCE  
Phone: (+33)0982295050

For sale information please  
contact our local office or refer to our web site:  
[www.stillatechnologies.com](http://www.stillatechnologies.com)

