NucleoView™ NC-200™
Software User’s Guide

P/N 991-0204
Revision 1.12
Caution!
This software must be operated as described in this User’s Guide and documents referred to herein. Please read the entire guide and referred documents before attempting to use this software.

Contacting support
Technical information including product literature and answers to questions regarding the operation of the NucleoView™ NC-200™ not covered in this document and referred documents is available through the following:

- For e-mail support, send questions to NucleoCounter® NC-200™ Technical Support on the address support@chemometec.com
- Check out the FAQ section under support at www.chemometec.com
- To speak with a Technical Support Specialist, call (+45) 48 13 10 20.

Please note the NucleoCounter® NC-200™ serial number and have it available when contacting ChemoMetec A/S for support. The NucleoCounter® NC-200™ serial number is found on the label affixed to the bottom of the instrument. The version number of the NucleoView™ NC-200™ software shall also be noted, this can be found on the Help – About menu item in the NucleoView™ NC-200™ software.

Sales and ordering information
For sales assistance with NucleoCounter® NC-200™ or the NucleoView™ NC-200™ software, to place an order for a NucleoCounter® NC-200™ or consumables, call (+45) 48 13 10 20, fax (+45) 48 13 10 21, or send e-mail to sales@chemometec.com

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Quick Guide

1. Install the NucleoView™ NC-200™ software and the NucleoCounter® NC-200™ instrument as described in the documentation on the USB stick containing the NucleoView™ NC-200™ software.

2. Start the NucleoView™ NC-200™ software by double-clicking on the NucleoView™ NC-200™ icon on the desktop.

3. Wait while the NucleoCounter® NC-200™ instrument initializes (you will hear motors working). When the LED on the instrument turns green the instrument is ready.

4. Click the button (below the ‘F3’ button) and select the desired protocol.

5. Click the button to see the application note for the selected protocol. Check that you have all the materials needed for this protocol (cassettes, reagents ...).

6. Fill the cassette with the sample and place it in the cassette fixture.

7. Optional: Write a descriptive text in the Sample ID field and enter a user name in the Operator field.

8. Press the ‘Run’ button. A few moments later the results of the analysis will be displayed.

9. Repeat steps 6 to 8 to perform more analyses of the same kind or repeat from step 4 to start a new analysis.
Installation Guide

Installation Overview

Important: You must be logged on as system administrator to install the NucleoView™ NC-200™ system components.

In a first time installation, you will typically install both the NucleoView™ NC-200™ software and one or more NucleoCounter® NC-200™ instrument specific configuration data. This involves installation and activation of various USB drivers on one or more USB ports.

To uninstall the software follow the described Un-installation procedure.

Minimum Computer Requirements and Preparations before Installation

Following PC requirements must be fulfilled before you can perform the installation:

   The Windows operating system must not be virtualized.
2. To install the NucleoView™ application, you need to log on with administrator rights.
3. At least 2 GB RAM and 10GB free disc space is recommended.
4. At least 2.0 GHz clock rate.
5. At least one USB 3.0 or USB 2.0 port must be available.
6. Screen size minimum 1024 x 768 pixels.
   OBS: Screen size much greater than this will result in windows which the user may find too small and difficult to read. If this happens, the screen settings must be configured to a lower resolution.

To perform the installation you will also need the NucleoCounter® NC-200™ instrument and the NC-200™ Package which is comprised of a power supply, a USB cable and the USB stick containing the software.

IMPORTANT: Make sure the serial number printed on the instrument matches the serial number on the USB stick.

Installing the NucleoView™ Software and NucleoCounter® Instrument

This section describes the software and instrument installation on Windows 10.

Installation of the software and instrument typically takes 5-8 minutes.

1. See the previous section about minimum computer specifications and preparations.
2. Ensure that the NucleoCounter® NC-200™ instrument is NOT connected to the PC.
3. IMPORTANT: Log on with administrator rights for this installation session.
4. Insert the USB stick holding the software.

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1 If a USB 3 port is used, it must have proper USB 2.0 support (all modern USB 3 ports does have this).
5. Windows will detect the USB stick and present an AutoPlay window if AutoPlay is enabled. Select the *Open folder to view files* option.

![AutoPlay window](image)

If AutoPlay is not enabled open the contents of the USB stick via Windows Explorer.

6. The directory contents on the USB stick will be shown. Double-click on the *Install NucleoView NC-200™ X.X.X.X* file (the version number e.g. 1.0.22.0 will vary depending on your installation) to launch the installation program.

*Note: It is the .exe file you should double-click, not the .bin file. The .exe file also has the installation icon associated in contrast to the icon for the .bin file.*

![File Explorer](image)

7. If a window with the text *‘Do you want to allow the following program from an unknown publisher to make changes to this computer?’* appears, click ‘Yes’.
8. After a few seconds the ‘Welcome to the NucleoView NC-200™ Install Wizard’ window will appear. Click ‘Next’.

![Welcome to the NucleoView NC-200™ Install Wizard](image)

9. On the Prerequisites window you must check the check box to confirm that you have disconnected all NucleoCounter® instruments from the PC. Click ‘Next’.

![Prerequisites window](image)
10. On the ‘Ready to install NucleoView NC-200™’ window you will be given options to install or upgrade the NucleoView™ NC-200™ software itself, to install configuration data for specific instrument(s) and to browse for instrument configuration data which are stored elsewhere. Check the appropriate options and click ‘Next’.

11. On the License Agreement window read the license agreement. To proceed with the installation you will have to select ‘I accept the agreement’ and click ‘Next’.
12. On the ‘Select Additional Tasks’ window check the desired options and click ‘Next’.
   Checking the ‘Associate ChemoMetec Files with the NucleoView application’ will associate the
   image files *.cm and protocol files *.cmsx with the NucleoView™ application making it possible to
   open the files directly by double-clicking.


   The ‘Installing’ window will appear and show progress of extraction and copying of files.
   During the installation some windows may appear to pause, this is most likely due to installation of
   redistributable files from Microsoft framework. Please wait for these files to install and do not close
   the window. This process may take several minutes.
   You may also see a command prompt window briefly appear, reporting about installation of a
driver.

Installation of FTDI CDM divers may appear.

Select ‘Extract’ on the FTDI CDM Drivers window.

Select ‘Next’ on the Device Driver Installation Wizard window.

Select ‘I accept this agreement’ and click ‘Next’.
Click ‘Finish’ to complete the FTDI driver installation.


Optional: To avoid this window during future software upgrades check the box Always trust software from “IDS Imaging Development Systems GmbH”.


Optional: To avoid this window during future software upgrades check the box Always trust software from “MATRIX VISION GmbH”.

17. On the ‘Completing the NucleoView NC-200™ Install Wizard’ window you may optionally deselect the check box if you only wish to install the software without attaching the instrument.
Click ‘Finished’.

18. On the ‘Completing the NucleoView NC-200™ Install Wizard’ window you may select the option to restart the PC later. The Software requires a restart of the PC to operate correctly. Make sure to login with the same user after restart.

Click ‘Finish’.

19. After installation of the NucleoView™ NC-200™ software two new shortcuts have been placed on your desktop. One is the NucleoCounter® NC-200™ instrument icon used to launch the NucleoView™ NC-200™ software, the other is a shortcut to the data folder where NucleoView™ NC-200™ stores application data.

![NucleoView™ NC-200™ shortcuts]

After restart of the PC the NucleoView™ NC-200™ software has successfully been installed.

The NucleoView NC-200™ program will now automatically be started and it will guide you through the instrument installation procedure, if the check mark for ‘Install NucleoCounter® NC-200™ instrument after Install has finished’ was checked.
Note: Depending on file versions and previous install history, a reboot of the computer may be requested before the NucleoView™ NC-200™ program is launched. If a reboot is needed, it is essential that you log on using the same administrator user as used during the above installation.

1. NucleoView™ NC-200™ is now launched in installation guided mode. Click ‘OK’ to continue with installation of the NucleoCounter® NC-200™ Instrument.

2. Follow the on-screen instructions for the installation of the NucleoCounter® NC-200™ Instrument using the USB cable and power supply. Note that the instrument icon in the upper left corner of the application bar is red while no instrument is attached.

3. You will now see a series of pop-up messages from the Windows operating system informing about driver installations. Among those are:
   - A USB hub inside the NucleoCounter® NC-200™ instrument
   - FTDI device driver installed
   - The camera

When the NucleoView™ NC-200™ software detects and has initialized the instrument, the previous red icon changes to true life colors indicating the instrument has been successfully installed.
4. NucleoView™ NC-200™ will display a message box stating successful installation of the instrument, and recommends that you continue installing this instrument on the remaining USB ports on the PC. Click ‘Yes’ if you wish to do so.

5. You will then be prompted to unplug the USB cable from the USB port on the PC. Do this and click ‘OK’.

6. When NucleoView™ detects that the instrument has been disconnected, the icon will again turn red, and you will be prompted to connect the instrument to another USB port on the PC.

7. Repeat the procedure described to install the instrument on the remaining USB ports on the PC.

8. If you are installing more than one instrument on the same PC, you must repeat the USB installation for all instruments on all USB ports, but note that only one instrument must be attached at any time.
9. Make sure that virus protection and other programs do not affect disc operations on the ChemoMetec data folder. Otherwise sharing violations may occur when a program such as a virus protection program is scanning a cm file while NucleoView™ is trying to access the same file. In virus protection programs disable scanning of the ChemoMetec data folder and its sub folders: C:\Users\Public\Documents\ChemoMetec. Furthermore, if alternative folders are selected in the options in NucleoView™, also disable scanning of these folders

**Installing Licenses and Protocols**

Follow the procedure described in the Licenses section to install licenses and protocols. This is not needed during a normal installation procedure, as licenses and protocols are installed automatically.

**Software Upgrade**

New software releases are typically distributed via our web page or on USB sticks and are installed as described below. Collected data (images), settings and installed licenses will be preserved.

1. **IMPORTANT:** Log on with administrator rights for this upgrade session.
2. Disconnect any NucleoCounter® NC-200™ instrument connected to the PC.
3. Insert the USB stick holding the software upgrade or download and unpack the software upgrade.
4. Continue as described in the previous section ‘Installing the NucleoView NC-200™ Software and NucleoCounter® NC-200™ Instrument’ and the NucleoView™ NC-200™ Install Wizard will now guide you through the remaining part of the upgrade.

**Installing a new NucleoCounter® Instrument**

To install one or more new NucleoCounter® NC-200™ instruments, use the USB stick holding the appropriate NucleoView™ NC-200™ software release and follow the procedure in the previous section describing installation of the software. The NucleoView™ NC-200™ Install Wizard will guide you through the remaining part of the upgrade. While following this procedure, you will be given the option to specify which NucleoCounter® NC-200™ devices you want installed (identified by their serial numbers). Be sure to check the boxes which specify the new serial number(s) you want installed.

You will also be given the option to browse for and select instrument data stored elsewhere.

**IMPORTANT:** You may install multiple instruments on the same PC, but be aware that NucleoView™ NC-200™ is only able to operate one instrument at the time. You must never try to operate with more than one instrument as behavior may not be correct.
Uninstall NucleoView™ Software

Either uninstall via Add/Remove Programs in the Control Panel, or run the un-installer via the ChemoMetec ->NucleoView NC-200->Uninstall NucleoView NC-200 in the Start Menu. This may be required if you wish to downgrade to an earlier version of NucleoView™ NC-200™.

Validation with IQ, OQ and PQ protocols

After installation/upgrade of the NucleoView™ NC-200™ software and/or NucleoCounter® NC-200™ instrument and after transportation of the NucleoCounter® NC-200™ instrument to a new location, it is recommended that you perform validation by running the Installation Qualification (IQ Test), Operation Qualification (OQ Test) and Performance Qualification (PQ Test) protocols as described below.
**Installation Qualification (IQ Test)**

1. Start the NucleoView™ NC-200™ software by double-clicking the icon on the desktop.
2. Wait while the NucleoCounter® NC-200™ initialize. During this time, the motors may be heard positioning.
3. Click the button (just below the F3 button in the right side of the main window) to launch the Select Protocol window.
4. In the Select Protocol window choose "Verification of NC-200" and "Test/calibration of NC-200". Select the "IQ Test" protocol in the list box in the lower half of the window (make sure that the NC-Slide A2 checkbox is checked).

![Select Protocol Window]

5. Click the button to see the application note for the selected protocol.
6. Follow the instructions on screen and in the application note to finalize the IQ Test.
**Operation Qualification (OQ Test)**

1. Start the NucleoView™ NC-200™ software by double-clicking the icon on the desktop.
2. Wait while the NC-200™ instrument is initializing. During this time, the motors may be heard positioning.
3. Click the button (just below the F3 button in the right side of the main window) to launch the Select Protocol window.
4. In the Select Protocol window choose "Verification of NC-200" and "Test/calibration of NC-200". Select the "OQ Test" protocol in the list box in the lower half of the window (make sure that the NC-Slide A2 checkbox is checked).

![Select Protocol window]

5. Click the button to see the application note for the selected protocol. Check that you have all accessories needed for this protocol available (Cassette, Slides, beads).
6. Follow the instructions on screen and in the application note to finalize the OQ Test.
Performance Qualification (PQ Test)

1. Check that you have a 912-3003 NC Test Kit 3 (Cassette, beads).
2. Start the NucleoView™ NC-200™ software by double-clicking the icon on the desktop.
3. Wait while the NC-200™ instrument is initializing. During this time, the motors may be heard positioning.
4. Click the button to launch the Select Protocol window.
5. In the Select Protocol window choose "Mammalian" and "Total Count and Viability". In the list box in the lower half of the window, select the "Viability and Cell Count Assay" protocol.
6. Follow the instructions the 912-3003 NC Test Kit 3 to finalize the PQ Test.
Main Window

Menu Line: See the Menu Line section below for a more detailed description of content.

Image Tab Page: If a file is selected, the image is shown on the left hand side of the main window. Zooming in and out on the image may be done by using the scroll wheel with the mouse pointer inside the image area. Zooming may also be achieved by using the + or - buttons. To zoom in on a tablet PC, touch the screen with two fingers and then slide them apart. To zoom out, drag your fingers together. Pan with the ‘arrow’ buttons or by dragging with the mouse.

Right-click options: Right-click on the image to get a context menu offering the following options:

- **Copy Bitmap Image:** Copies the displayed image area to the clipboard.
- **Track Position:** Displays a bar in the top or bottom of the image, where the x and y position is displayed together with the pixel values for different channels in the picture. The format is X-position, Y-position: Pixel value Channel 0  Pixel value Channel 1  Pixel value Channel 2  … This feature can be disabled again by right-clicking on the image and selecting Track Position.
- **Image Scaling:** Opens the Image Scaling window, where the presentation of the image scale in the different channels can be modified. See the Image Scaling section for further details.

Main Window Controls:

1. **Image selection controls.** These controls are used to select which image to show when a file holding more than one image is selected. Images are numbered 1, 2 and upwards.

2. **Channel color buttons.** Colors assigned to each channel. Click on a channel color field to enable/disable the display of the respective channel.
3. Overlay control. After an analysis is successfully completed, the ‘overlay’ button is enabled. Left-click on this button to enable or disable the display of an Image Overlay, highlighting the cells included in the analysis. See the Image Overlay section for more details.

4. Result Tab Page: A detailed overview of the results of the active file is displayed when the result tab page in the upper left corner is selected. Right-clicking the results will bring up a context menu with options for copying or printing the displayed results. See the Analysis Results section for more details.

5. Volume input fields: Located below the image are the relevant volume controls for the selected protocol. Right-click in the area below and to the right of the ‘Sample ID’ to change from volumes to parts. Prior to starting an analysis, the volumes or parts used can be edited and the dilution or concentration of the sample will be taken into account in the final results. Upon selecting a protocol, volumes present in the volume input fields are those recommended for the assay. The recommended volumes may be reloaded by clicking on the ‘reload’ button (or ). If the volume of the sample or dilution is changed and a solution input field is present it will be updated with the volume or part recommended. If the user changes the volume or part in the solution input field, it will not be updated anymore. Entering a negative number in the dilution input field represents that the sample has been concentrated. The sum of the sample and dilution is required to be a positive number greater than 0.0001. The first multiplication factor input field is defined as the sample divided by the sum of the sample and dilution. Entering a number greater than 1 in this input field represents a dilution of the sample, whereas entering a number less than 1 represents a concentration of the sample. Subsequently, the volume input fields will automatically be updated with the recommended volumes or parts. The second multiplication factor (right of the first editable multiplication factor) is not editable and is defined as the sample divided by the sum of all volumes or parts.

6. Browse Files: The upper right region of the main window shows the currently selected data folder and files within it. Left-click files in the selected folder to browse the results. See the Browse Analysis Results section for more details.

7. Select Protocol: The instrument can perform different analyses depending on the protocol selected. To select the intended protocol, click the button. This will open the protocol selection window. When the intended protocol is selected, clicking the button will open the application note for the selected protocol. The selected protocol can be attached to the ‘favorite’ buttons by right-clicking on a ‘favorite’ button (F1, F2 or F3) and choosing ‘Attach current protocol to button’. A favorite protocol can then be loaded by clicking the ‘favorite’ button or pressing F1, F2 or F3 on the keyboard. See the Selecting a Protocol section for more details.

8. Sample ID: A description of the sample can be entered in the sample ID input field prior to starting the analysis. The entered sample ID will be part of the file name. The file name consists of the date – a consecutive number – a number for the chamber number or a c for cassette – and the entry in the sample ID input field.
The sample ID can be changed after an analysis has been completed by right-clicking on the files and selecting Properties. In the window that appears in the General tab page the Sample ID can be changed in the according input field.

The sample ID cannot include the following characters: < > \ : / ½ § ´ ~ | ? „ “ £ µ

9. **Operator**: The name or initials of the individual that performs the assay can be entered in the operator input field prior to starting the analysis. When browsing files the operator field will display which operator was entered when the image file was created.

The operator cannot include the following character: #

**Current Run and Next Run**: The lower right part of the main window holds the main results of the active image analysis (from the last run or the file that is selected in the browse list). Detailed results are available by left-clicking on the result tab page in the upper left corner on the main window. After running a protocol, the input fields will display the entries for the previous analysis and the input fields will be enabled for entering new entries for the **Next Run**.

This button starts the analysis according to the selected protocol. During the first part of the analysis an ‘Analysis in Progress’ image will be displayed.

This button returns to ‘acquisition mode’ to run a new analysis, when the instrument is connected.

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**Menu Line**

*File* -> *Import Package*: Imports files into the NucleoView™ NC-200™ software. This will typically be a zip-file containing license, protocol and documentation files, or individual *.cms*, *.cmsu* and *.pdf* files.

*File* -> *Save Image File As*: Opens a window where the selected image can be saved as a TIFF or Bitmap file.

*File* -> *Exit*: Shuts down the program.

*Tools* -> *Plot Manager*: Opens a new Plot Manager window which can be used for advanced post processing of CM-files. See the [Plot Manager](#) section for more details.

*Tools* -> *Protocol Adaptation Wizard*: Opens the Protocol Adaptation Wizard which is used to create user adapted versions of protocols. See the [Protocol Adaptation Wizard](#) section for more details.

*Tools* -> *Report Generator*: Opens the File Browser and the Report Generator which are used to create collected results in a table or for data export of one or several data files. See the [Report Generator](#) section for more details.

*Tools* -> *Create PDF Report*: Opens the PDF Report dialog that is used to create and print PDF reports for files. See the [PDF Report](#) section for further details.

*Tools* -> *Options*: Opens the options dialog. See the [Options](#) section for more details.
View -> Event Logs: Opens a window where log files of all operations made by the user are recorded (See details in Event Log part of the 21 CFR Part 11 section).

View -> License File: Opens the license file. See License, Protocol and Documentation Installation for more details.

View -> Application Log: Opens the NucleoView™ log file window. This file may be useful for ChemoMetec if support is needed.

Help -> Software Users Guide: Opens the NucleoView™ NC-200™ software user’s guide.

Help -> Instrument Users Guide: Opens the NucleoView™ NC-200™ Instrument user’s guide.

Help -> About: Displays the About NucleoView™ NC-200™ window that contains the software version number and information about the connected NucleoCounter® NC-200™.
Selecting a Protocol

Press the ‘select protocol’ button (placed just below the F3 button in the right side of the Main window) to open the Select Protocol window:

Select type of ‘Organism’, type of ‘Analysis’ and preferred ‘Media Types’ to see which protocols are available for this in the current installation.

Select the protocol which supports the chosen sample unit (media type) and click ‘Select’.

If the current installation holds any user adapted protocols, these will be listed indented under their respective master protocols.

Via right-clicking on a protocol you can select to see details about the protocol or to see the protocol application note. For a user adapted protocol, it is further possible by right-click to export the protocol adaptation for copying to another PC or to delete it.

After export of the protocol on one PC it can be imported to another PC that have the NucleoView™ NC-200™ software installed by choosing File -> Import and locating the exported file. Import of a protocol with an identical name of an already existing protocol will result in two protocols with identical names. Therefore, it is recommended to rename or delete an old adapted protocol before importing the new protocol.

When ‘Hide Protocols setup access’ under Tools -> Authorization -> User Permissions is enabled for the current user, an extra button ‘Hide Protocols …’ is shown just above the drop down box. Pressing this button will launch a ‘Hide Protocols’ window, where it is possible to limit the number of protocols being shown during daily use. Creating new cm-files with hidden protocols is NOT possible, but they can still run on already existing cm-files when run via Show Raw Data, Reanalyze Image File with Selected Protocol, and Protocol Adaptation Wizard operations are also NOT influenced by the protocol being hidden.
Analysis Results

When running a protocol the main results (1) will be displayed on the user interface and detailed results will be displayed in the result tab (2). The different files can be opened by selection in the browse list in the upper right part of the main window.

**Image File (*.cm), Post Processing File (*.cmpp) and Plot Manager File (*.cmpm)**

**Image Files (*.cm)**
- Hold all primary analysis data (picture, instrument ID, settings, protocol used etc.)
- Have up to 5 channels per image
- Use a ChemoMetec proprietary file format with extension `cm`
- Are named `yyyyymmdd-####-##.*.cm` (year-month-day-number)–{c for cassette}–{sample ID}.cm
- Are placed in *Today's Directory* named `yyyyymmdd` (year-month-day)
- *Today's Directory* is created automatically in *Results Directory*
- *Results Directory* default location set during installation is typically:
  C:\Users\Public\Documents\ChemoMetec\NucleoView NC-200\results
  The location may be changed by the user via menu entry *Tools->Options*

**Post Processing File (*.cmpp)**
- Holds post processing results for a specific *.cm image file corresponding to one row in a *Plot Manager*
- Has the same file name as its corresponding image file (*.cm file), but with extension ‘cmpp’
- Is placed in the same directory as its corresponding image file
- It may also be saved in the *Master files* directory under the *Results Directory*
• Using the Protocol Adaptation Wizard to add user specified post processing to user adapted protocols results in special “cmsu_* .cmpp” files which are saved in the Master files directory under the Results Directory.

• Be careful during disk maintenance that you do not delete files in the Master files directory which are still needed for e.g. user adapted protocols.

Plot Manager File (*.cmpm)

• Holds a listing of loaded post processing files (*.cmpm)

• Is placed in the PM files directory under the Results Directory

Plot Manager

The Plot Manager performs post processing on cell populations and can show results from multiple images in thumbnail sized scatter plots and histograms. See the Plot Manager section for more details.
Browse Analysis Results

In the upper right corner launches the File Browser to browse images from a specified date range (day, week, month or year).

Return to ‘acquisition mode’ to run a new analysis, when the instrument is connected.

File list:
- Click on a file in the data folder to browse the results of a previously acquired file. The file will be opened and the image will be displayed.
- Pan and zoom settings are preserved from the previously selected file. The extended results for the selected file will be shown in the result tab page. Furthermore, the protocol, media type, sample ID, operator and volumes will be shown for the selected file.
- Right-click options: Right-click on a file to get a context menu offering the following options:
  - Show Data: Opens the selected file in Plot Manager with the saved plots defined by the user. However, if no plots have been saved, then plots defined by the protocol will be displayed.
  - Show Raw Data: Opens the selected file in Plot Manager with the plots defined by the protocol.
  - Reanalyze Image File with Selected Protocol: Reanalyzes the image file with the protocol selected in the acquisition mode and displays the results of this analysis. Be aware that the selected protocol is chosen in acquisition mode (when the Run button is displayed). Afterwards when a file is selected in the browse file list the protocol name that the file was
acquired with is displayed (when the Return button is displayed), but the selected protocol will still be the protocol chosen in acquisition mode.

- **Add to Report**: Adds the file to the Report Generator from where the data can be copied into a spreadsheet program.
- **Print**: Prints the embedded data for the file.
- **Print with Plots**: Prints the embedded data for the file together with the original plots for the file.
- **Create PDF Report**: Opens the PDF Report dialog, where a PDF report can be created and printed for the selected file.
- **Approve**: Option to approve the data for the file in connection with 21 CFR Part 11. See the Approval part in the 21 CFR Part 11 section for further details.
- **Start Protocol Adaptation Wizard**: Starts the Protocol Adaptation Wizard for the protocol that the file has been acquired with.
- **Properties**: Opens the Properties window for the file.
File Browser

Select a date, a week, a month or a year on the left side of the File Browser. The right side is then populated with all images from the selected period. The first column lists file names, and the following columns list further properties of each file.

A red dot in front of the file name indicates that the file has been modified by a user after it was created or that the data was acquired with an unlocked user adapted protocol. Modifications may be that a log entry has been added to the file, or that the file has been renamed.

Double-click on a file in the list to open it in the **Main Window**.

Multiple files may be selected by holding down Shift or Control and selecting files by left clicking. One option is to add the selected files to an open **Plot Manager** by drag and dropping the selected files to the **Plot Manager**.

**Right-click options**: Right-click on one or more selected files to get a context menu offering the following options:

- **Properties**: Opens the **Properties** window for the file.
- **Add to Report**: Adds the file to the **Report Generator**.
- **Print**: Prints the embedded data for the file.
- **Print with Plots**: Prints the embedded data for the file together with the plots saved for the file.
- **Create PDF Report**: Opens the **PDF Report** dialog, where PDF reports can be created and printed for one or multiple files.
- **Approve**: Option to approve the data for the file in connection with 21 CFR Part 11. See the **Approval** part in the **21 CFR Part 11** section for further details.

Right-click the header row in the right side of the browser and select ‘**Columns set-up**’ to specify which properties are to be listed.
File Properties

The file properties window contains three or four tab pages:

- **General** with the file name and the sample ID. The sample ID can be edited after an analysis has been completed. In the Log field the user can optionally enter comments about the image file that will be logged in the file. NucleoView™ will automatically create entries in the log when a user changes Sample ID and when a user approves the cm-file.

- **Details** that contain information about the image file.

- **Description** where a description of the actual image channel is displayed together with a potential comment about the image channel. The channel number can be changed by clicking the arrow up or down buttons.

- **Post Processing** which describes details about the post processing performed for the *.cm file. This tab page is only present when a corresponding *.cmpp post processing file has been saved in the Plot Manager.
Report Generator

The Report Generator may be launched in several ways; from the File Browser window by right-clicking on one or more selected files and selecting ‘Add to report’; from the Browse File list in the main user interface right-clicking on a file and selecting ‘Add to report’; or via the menu entry ‘Tools->Report Generator’. It is used to generate a report for the files added to the Report Generator.

Multiple files may be added to the Report Generator by selecting files while holding down shift or control and selecting ‘Add to report’ after right clicking on the multiple file selection.

Edit the file list using the four buttons in the upper right side of the Report Generator window.

Click the ‘To Clipboard’ button to create a report on the clipboard, ready for pasting into a spreadsheet program.

Click the ‘Show’ button to show the created Report.

Right click options on the shown report:

- **Copy Result to Clipboard**: Copies the displayed results to the clipboard.
- **Save Result to csv-file**: Brings up a dialog from where a csv file of the displayed results can be saved.
- **Create Transposed Results**: Opens a new window where the results have been transposed.
Image Scaling

When an image is presented on the screen, the color intensity (pixel intensity values) will by default be shown with automatic scaling and automated estimation of the background. For each channel, pixel values will be depicted in the range 0 to 255 in color intensity.

The automatic scaling and the automated estimation of the background can simultaneously be turned off by clicking Auto Scale and the automated estimation of the background can be turned off by clicking Auto Bkg, when Auto Scale is enabled. Note that disabling the automated background estimation may increase the depicted background in the shown image dramatically. When Auto Scale is disabled values can be entered for min and max values to alter the depicted range. Remember to delete the * symbol to avoid automatic scaling. Contrary, If a * symbol is appended to a specified value automatic scaling will be enabled.

The Enhance function will depict low pixel values brighter. Disabling the Enhance function will display the image linear scaled.

The Invert function will depict the image inverted so the background appears white instead of black while the colors of each channel is attempted to be preserved.

To increase color contrast you may estimate the background level by right clicking on the image and selecting Track Position. Holding the arrow over a background area of the image the pixel values in the background can be estimated for all channels. The values in track position are defined as following: X position separated by a comma Y position, then following the semicolon the Intensity values for each channel is written separated by spaces e.g. 700, 500: 214 1476 15 will mean that the pixel the arrow is pointing at will have the X position 700, the Y position 500, the intensity in channel 0 will be 214, the intensity in channel 1 will be 1476 and the intensity in channel 2 will be 15. These values can be entered for the min value for each channel when Auto Scale is disabled. This will often give a better result when making a bitmap export.
Image Overlay

An image overlay may be enabled with the button near the right bottom corner of the image. It performs two kinds of cell marking on the displayed image:

- When an analysis is completed successfully, cells that are included in the analysis are marked with a square.
- When a large scatter plot or a large histogram is open in the Plot Manager the cells inside or outside the selected marker or gate respectively, can be marked with the overlay squares by selecting ‘Add Cells Inside Selected Gate to Image Overlay’ or ‘Add Cells Outside Selected Gate to Image Overlay’.

The cells are marked with squares and this overlay can be toggled on and off by clicking the button. When one of the two overlay functions has been used it is possible to choose cells in the Main Window by holding the Ctrl button down and clicking on individual cells in the image. This will mark the last selected cell with a yellow square and previously selected cells with a pink square. Selected cells will be marked in Large Scatter Plots.

The selected cells for the image overlay can be unselected by double clicking in the image, by right clicking the button or the large plot and selecting ‘Delete Image Overlay’ or by right clicking on a large plot and selecting ‘Delete Image Overlay’.

See the Large Scatter Plot for details about polygons and cell gating. See the Large Histogram Plot for details about markers and cell gating.
Next Analysis

When a run is completed, the main result is displayed at the bottom right of the user interface and detailed results are displayed in the result tab page.

**Input fields:** The user may optionally enter sample IDs before each sample is run. The sample ID will be used as last part of the image file name for all image files shot for the respective protocol. The user may optionally change the operator or the volumes used for the next sample run.

When the input fields have been filled out, press the ‘Run’ button to start the next analysis. All the settings will be transferred to the results.
Protocols

To perform a particular analysis, the appropriate protocol must be used. This involves selecting and running the corresponding protocol on the NucleoView™ NC-200™ software while following the guidelines in the protocol as described in the Quick Guide section.

Each instrument requires a unique license to run a protocol. Via the menu entry View -> License File you can see a list of the installed Licenses.

All protocols are user adaptable. The menu entry Tools -> Protocol Adaptation Wizard opens the Protocol Adaptation Wizard window, which is used to create user adapted versions of protocols.
The Protocol Adaptation Wizard will guide you through adapting different parameters and saving the adapted protocol as a user adapted protocol. A saved protocol may be locked by the user to prevent further changes. A locked protocol can be unlocked again to overwrite with new user adaptations. A new adapted protocol, based on a locked protocol, can be saved by choosing the save as function. All lock and unlock events are logged in the adapted protocol file.

The Protocol Adaptation Wizard can also be used for applying a master post processing to the analysis results. Hence if a predefined set of markers, polygons and/or quadrants should be applied to new data acquisitions it can be setup to do so automatically via the Protocol Adaptation Wizard for analytical assays. Please follow the guide in each step in the Protocol Adaptation Wizard. This guide is visible when then Help checkbox is checked.

In the case that a counting gate needs to be adapted the gate can be adapted in the Plot Manager and saved in the file. Afterwards right click on the saved file in the Main Window and select ‘Start Protocol Adaptation Wizard’, follow the instructions and save the adapted protocol.
Plot Manager

The Plot Manager is used for evaluation and post processing of cell populations found in one or more image files.

Results are displayed in scatter plots, histograms and table plots arranged in rows and columns, where each row comprises results for one particular image file. The Plot Manager is specifically suited for batch processing of several image files as it can replicate processing from one ‘Master’ row to all selected rows.

1. **Master Row**: Enter the number of the row that is to be used as the Master row that functions like a template.

2. **Apply**: This button replicates all processing from the Master row to all selected rows. Note that a blank area will appear in target rows if the channel defined in the master row does not exist in the file for the target row or if the parameter defined in the master row does not exist in the file for the target row.

3. **Info field**: The area to the right of the ‘Apply’ button displays information regarding the plot coordinates and number of cells where the pointer is located in the Plot Manager.

This button launches a **Statistics** window which lists detailed gating statistics for all visible polygons and markers.

This button launches a window to add Plot Manager files (*.cmpm) or post processing files (*.cmpp) to the Plot Manager. Each added image file will be appended as one new row.

Two exceptions exist: Adding a *.cmpp file from the **Master files** directory or when a *.cmpm file holds a master row. In these cases the file will be added to row number zero (i.e. as upper most row). In case row zero already holds an image data file, the new file will be appended as one new row.

Note, it is also possible to load image files (*.cm) and processed plot manger files (*.cmpp) into the Plot Manager by dragging them from either the **Browse** list in the main window or from the **File Browser**.
There are two ‘save’ buttons in the Plot Manager. The ‘save’ button exists for each row and is placed in the Row window in the left part of the row. This button saves the post processing data i.e. gates, polygon, quadrants and graphs associated with the individual sample. The second type of ‘save’ button at the top of the window is always present (beside the button) and saves the current Plot Manager contents to a *.cmpm file placed in a PM Files folder. This requires that all rows in the Plot Manager have been saved. All unsaved rows may be saved by right clicking in the gray area in the top of the Plot Manager. The file name is user defined via the edit field to the right of the ‘save’ button.

Three types of files may be added to Plot Manager with the following effects:

1. **Image files**: These are image files (*.cm) on which no data processing, referred to as post processing, has been performed. If no previous post processing has been saved, the new row will only show a row window in the left side of the Plot Manager window. If previous post processing has been saved for this image, this post processing will also be shown in the added row. See the description about the row window for details about which cell population data is loaded.

2. **Post processing files**: A post processing file (*.cmpp) holds post processing results for a particular image file and has the same file name and is placed in the same location as the associated image file. The *.cmpp file location may alternatively be in a special folder named Master Files which is accessible via the image browser. Such Master files are used when replication of post processing to other data files is needed. Using the Protocol Adaptation Wizard to add user specified post processing to a user adapted protocol results in special “cmsu_*.cmpp” files.

3. **Plot Manager files**: Plot manager files *.cmpm holds data for a complete plot manager session, i.e. for any number of images.

**Right-click option**: Right-click in the gray area in the top of the Plot Manager to see the option:

- **Show hidden rows**: Toggles between showing and hiding rows marked as hidden.
- **Save unsaved rows**: Saves all unsaved rows.

**Layout Editing**

Please read the Row Window section for further details on how to use the Plot Manager.

Scatter plots, histograms or table plots can be added to the Plot Manager by left-clicking the, or buttons respectively.
Plot Statistics

The Statistics window lists detailed gating statistics for all visible polygons and markers.

The above image shows all the available sub-columns. Right-click on a column header to edit which sub-columns to display.

If several different alias names has been set for a gate the gate alias name presented in the statistics will be for the first row with an alias name. Example: if P1 has been named ‘live cells’ in row 1 and ‘GFP’ in row 2, then the statistics window will show ‘P1 live cells’.

Right-click in the table offers an option, ‘Copy table to clipboard’, to copy table contents to clipboard which can be directly pasted into a spreadsheet program.

It also offers an option, ‘Copy table to clipboard (extended)’, which copies both the gate content and the content of all Plot Tables to the clipboard. This option enables bulk data reanalysis of data from Plot Manager.

Row Window in Plot Manager
The Plot Manager Row Window is used as row header for the post processing of a specific image file. It is always placed in the left side of the Plot Manager window.

**Row number:** The row number is shown in the upper left corner.

**Data source indicator:** The data source of the cell population in this row is shown with a small colored square to the left of the ‘save’ button:

1. **Grey:** Indicates that the cell population has been loaded from data embedded in the image file, i.e. data which was obtained when the image was originally acquired and analyzed by running a protocol.
2. **Green:** Indicates that the data has been loaded from the main window, and that the main window still holds the image file in memory. This is referred to as *Main data (synchronized).* This population is loaded instead of embedded data if the main window holds cell population data for that particular image file at the time when the row is added to the Plot Manager.
3. **Orange:** Same as for green, but the main window no longer holds the same image file in memory. This is referred to as *Main data (not synchronized).*

The ‘save’ button saves the post processing results in a *.cmpp file which is automatically given the same name and same location as the *.cm image file.

**Note:** Any already existing post processing result for this CM-file will be overwritten.

For all analytical assays a *.cmpp files is saved automatically when running the analysis, hence it is not possible to do further post processing or saving of data in 21CFR Part 11 mode since this will overwrite existing data.

To get saved *.cmpp files with data analysis in 21 CFRP part 11 mode, it is advised to use adapted protocol containing a master template for the analysis of data.

**File name:** The name of the image file is shown with vertical font in the left side of the row window.

**Check box:** Check the check box under the ‘save’ button if you want the row to be a target for the apply master function.

- Click the ‘add scatter plot’ button to add a new Scatter Plot to the end of this row.
- Click the ‘add histogram’ button to add a new Histogram Plot to the end of this row.
- Click the ‘add table plot’ button to add a new Table Plot to the end of this row.

**Right-click options:** Right click on the row window to see the options:

- **Show Image:** Loads the rows corresponding image file (cm file) into the Main Window.
- **Save As Master:** Saves this rows post processing as a Master for post processing. The saved master can be loaded into subsequent Plot Manager sessions where the post processing can be replicated to other rows.
- **Reload Post Processing:** Reloads the last saved post processing of the file.
- **Move Row Up:** Swaps row position and number with the previous visible row.
- **Move Row Down:** Swaps row position and number with the next visible row.
- **Remove Row**: Removes this row from the Plot Manager, re-numbers subsequent rows and moves the subsequent rows up.
- **Hidden Row**: Toggles the hidden state of the selected row.
- **Show Hidden Rows**: Toggles between showing and hiding rows marked as hidden.
- **Properties**: Displays the [File Properties](#) dialog for the file corresponding to the selected row.

### Histogram Plot in Plot Manager

The histogram plot is used to plot a user selectable parameter together with user defined marker sets which marks, gates and counts cell populations. The histogram above shows a histogram for the rows own cell population (red) plus an overlay with a histogram from another row (blue).

1. **Data source indicator**: A small colored square in the upper left corner indicates the source of the cell population (see further details in the section [Row Window](#) in Plot Manager).

2. ![Delete Plot Button](delete.png) Click the ‘delete plot’ button to remove a plot from the row. This will leave an empty area in which you may, via right-click, insert another plot or simply delete the area.

**Double-click**: To launch the [Large Histogram](#) plot, double-click in the plot area. In the large histogram plot you can define axis parameters, markers and gating settings.

**Right-click**: Shows the context menu offering the following options:
- Show Large Plot (dbl-click)
- Copy BMP to Clipboard
- Copy Histogram
- Paste Histogram

**Copy Histogram** and **Paste Histogram** options may be used to insert and overlay histograms from other histogram plots.
Large Histogram Plot in Plot Manager

The large histogram plot is used to set axis parameters, edit marker sets, define gating settings and add overlay histograms.

1. **Axis Settings**: By clicking on the x-axis it is possible to select type of axis, image channel, parameter type and axis limits. By clicking on the y-axis it is possible to select between *count* and *percent*, and by clicking on ‘*Max*’ the axis max limit can be set.
   - Type of axis: Linear, Log or Bi-exponential. For Bi-exponential it is possible to edit the *below zero* scale value.
   - Image channel: Names of the available channels.
   - Parameter types: The available parameters depends on the protocol used to generate the *.*cm file.

2. **Gating Setup**: Launches the *Gate Configuration* window, where gating settings can be configured for this plot.

3. **New Marker**: Creates a new marker.

4. **Selected Gate**: Edit the selected marker.
   - Move with Arrow Keys
   - Move Left End with Arrow Keys
   - Move Right End with Arrow Keys
   - Copy to Clipboard
   - Delete

5. **BMP to Clipboard**: Copies the large plot to the clipboard to paste the bitmap image into a preferred program.
6. **Cancel**: Closes the large plot without saving the changes.
   **OK**: Closes the large plot and saves the changes.

7. **Line type and colors**: These may be set with the controls in the upper right corner of the plot window after an additional histogram has been overlaid to an existing histogram.

8. **Data source indicator**: A small colored square in the upper left corner of the plot area indicates the source of the cell population (see further details in the section *Row Window* in Plot Manager).

**Right-click options**: Right-click inside the plot area when no markers are selected to get a context menu offering the following options:

- **Paste Marker**: Inserts a copied marker into the histogram plot.
- **Show Gate Counts**: Toggles between displaying counts and percents for the gates.
- **Copy Histogram**: Copies the histogram curve.
- **Paste Histogram**: Inserts a copied histogram curve into the histogram plot.
- **Delete Image Overlay**: Unselects cells added to the *Image Overlay*.

Depending on the state of the large scatter plot, some of the above options may be dimmed or be replaced with an alternative option.

**Right-click options**: Right-click inside the plot area when a marker is selected to get a context menu offering the following options:

- **Paste Marker**: Inserts a copied marker into the histogram plot.
- **Copy Selected Gate**: Copies the selected gate.
- **Copy Histogram**: Copies the histogram curve.
- **Paste Histogram**: Inserts a copied histogram curve into the histogram plot.
- **Add cells inside marker to image overlay**: Selecting this option will add cells inside the selected marker to the image overlay, causing these cells to be marked with a non-filled, enclosing square.
- **Add cells outside marker to image overlay**: Selecting this option will add cells outside the selected marker to the image overlay, causing these cells to be marked with a non-filled, enclosing square.
- **Delete Image Overlay**: Unselects cells added to the *Image Overlay*.

Depending on the state of the large histogram plot, some of the above options may be dimmed or not present.

**Note**: Adding cells to the image overlay is cumulative. Consequently if you use this to select both inside and outside cells you may assume that all cells in the image should be marked with an enclosing square. This is however, not always the case. If certain cells have been excluded from the cell population, e.g. all non-
single cells, then these are not part of the histogram, and therefore will not be included in the selected cells list.

*Editing a Marker:* A marker can be moved when the mouse curser is above the marker and is marked with the move symbol. The left or right end of a marker can be moved when the mouse curser is above the end of a marker and is marked with the move symbol.

**Scatter Plot in Plot Manager**

The scatter plot is used to plot two user selectable parameters against each other together with protocol- or user defined polygons or quadrants which marks, gates and counts cell populations.

1. **Data source indicator:** A small colored square in the upper left corner indicates the source of the cell population (see further details in the section *Row Window* in Plot Manager).

2. **Successfully delete plot:** Click the ‘delete plot’ button to remove a plot from the row. This will leave an empty area in which you may, via right-click, insert another plot or simply delete the area.

*Double-click:* To launch the Large Scatter plot double-click in the plot area. In the large scatter plot you can define axis parameters, polygons, quadrants and gating settings.

*Right-click:* Shows the context menu offering the following options:
- Show Large Plot (dbl-click)
- Copy BMP to Clipboard
**Large Scatter Plot in Plot Manager**

The large scatter plot is used to set axis parameters, add or edit polygons and quadrants and define gating settings.

1. **Axis Settings:** By clicking on either the y-axis or x-axis it is possible to select type of axis scale, image channel, parameter type and axis limits.
   - Type of axis: Linear, Log or Bi-exponential. For Bi-exponential it is possible to edit the below zero scale value.
   - Image channel: Names of the available channels.
   - Parameter types: The available parameters depends on the protocol used to generate the *.cm file.

The scatter plot below shows an example where the maximum x-axis limit has been set lower than the measured maximum.

Note how cells outside the plot area are plotted in the margins, visualizing that the current x-axis max limit setting causes cells to fall outside the plot area.
2. **Gating Setup**: Launches the **Gate Configuration** window, where gating settings can be configured for this plot.

3. **New Polygon**: Creates a new polygon. Click in the plot area to add points in the polygon. Click in the gray area around the plot to delete the last point in the polygon. Click on the starting point to close the polygon.

4. **New Quadrant**: Creates a new quadrant. Click in the plot area to add the center point of the quadrant. Optionally click on the quadrant and drag center- and end points. Click away from the quadrant to unselect and finish quadrant editing.

5. **Selected Gate**: Edit the selected gate.
   - Set Alias Name
   - Show Info
   - Copy to Clipboard
   - Delete

6. **BMP to Clipboard**: Copies the large plot to the clipboard to paste the bitmap image into a preferred program.

7. **Cancel**: Closes the large plot without saving the changes to the row.
   **OK**: Closes the large plot and saves the changes to the row.

8. **Data source indicator**: A small colored square in the upper left corner of the plot area indicates the source of the cell population (see further details in the section **Row Window** in Plot Manager).

**Right-Click Options**: Right-click inside the plot area when no gates are selected to get a context menu offering the following options:

- **Paste Gate**: Paste a polygon- or quadrant gate copied from another scatter plot.
- **Show Gate Counts**: Toggles between displaying counts and percents for the gates.
- **Delete Image Overlay**: Unselects cells added to the **Image Overlay**.

Depending on the state of the large scatter plot, some of the above options may be dimmed or be replaced with an alternative option, e.g. **Show gate counts** can toggle with **Show gate counts in %**.

**Right-Click Options**: Right-click inside the plot area when a gate is selected to get a context menu offering the following options:

- **Copy Selected Gate**: Copies the selected gate.
- **Add Cells Inside Gate to Image Overlay**: Selecting this option will add cells inside the selected gate to the image overlay (only cells that are shown in the plot), causing these cells to be marked with a non-filled enclosing square.
- **Add Cells Outside Gate to Image Overlay**: Selecting this option will add cells outside the selected gate to the image overlay (only cells that are shown in the plot), causing these cells to be marked with a non-filled enclosing square.
- **Delete Image Overlay**: Unselects cells added to the **Image Overlay**.
- **Show Info About Selected Gate**: Displays a new window with information of how many cells are outside and inside the gate in each image.

  Depending on the state of the large scatter plot, some of the above options may be dimmed or be replaced with an alternative option.

**Note**: Adding cells to the image overlay is cumulative. Consequently, if you use this to select cells both inside and outside the gate you may assume that all cells in the image should be marked with an enclosing square. This however, is not always the case. If certain cells have been excluded from the cell population, e.g. all non-single cells, then these are not part of the scatter plot, and therefore will not be included in the selected cells list.

**Editing a Polygon**: First select the polygon you want to edit by clicking close to one of the sides. Then select it again to get into ‘polygon editing mode’ indicated by red squares on the polygon corners. In this mode it is possible to remove points or add new points and to drag points to new positions. Further details are described in the light gray area surrounding the plot.

**Editing a Quadrant**: First select the quadrant you want to edit by clicking close to one of the lines. Then select it again to get into ‘quadrant editing mode’ indicated by red squares on the quadrant lines. In this mode it is possible to drag points to new positions. Further details are described in the light gray area surrounding the plot.

**Table Plot in Plot Manager**

Table plots are used for presenting data. Example: The percent of cells inside a gate can be presented and acceptance criteria set up so that the output may show for example failed or OK in the red and green font, respectively.

1. **Data source indicator**: A small colored square in the upper left corner indicates the source of the cell population (see further details in the section Row Window in Plot Manager).
2.  ☒ Click the ‘delete plot’ button to remove a plot from the row. This will leave an empty area in which you may, via right-click, insert another plot or simply delete the area.

Double-click: To launch the Large Table Plot double-click in the plot area.

Right-click: Shows the context menu offering the following options:
- Show Large Plot (dbl-click)
- Copy BMP to Clipboard

**Large Table Plot in Plot Manager**

![Large Table Plot](image)

The large table plot is used to add tables and edit the contents.

1. **New Table:** Creates a new table. Click in the plot area to place the upper left corner of the table and select the number of row and columns of the table.
2. **BMP to Clipboard:** Copies the large plot to the clipboard to paste the bitmap image into a preferred program.
3. **Cancel:** Closes the large plot without saving the changes to the row.
   **OK:** Closes the large plot and saves the changes to the row.
4. **Data source indicator:** A small colored square in the upper left corner of the plot area indicates the source of the cell population (see further details in the section Row Window in Plot Manager).

**Right-Click Options:** Right-click inside a table cell to get a context menu offering the following options:
• **Insert Formula ...**: Selecting Insert formula writes a = that indicates that a number or a calculation will be written in the selected table cell. Subsequently, “Insert value ...” can be selected to insert a gate parameter or a protocol variable. Selecting gate parameter guides in steps to select the desired gate and parameter like % of plot or mean for the x-axis parameter. Protocol variables are values like volumes that are defined for each file when it was acquired with the protocol.

Two or multiple gate parameters and protocol variables can be inserted in the same table cell together with calculation expressions like + - * / . See Calculation Expressions below for more details.

• **Format Cell ...**: Opens the Format Cell dialog that has two tab pages:
  - **Number**:
    - The number of decimal places after the separator can be defined.
    - Scientific notation can be selected.
    - A 1000 separator can be enabled.
    - A symbol can be defined like %.
  - **Font**:
    - The font can be set to be value controlled. A low and a high threshold can be set. If only one threshold is needed the value can be set to the same for both the high and low. The color of the font for the different threshold can be selected and an alternative text to be written instead of the value in the table cell.

Note that formatting a table cell only has a visible effect when the table cell is defined as a value (by writing = or selecting insert formula).

**Right-Click Options**: Right-click when the cursor is placed on the lines of the table to get a context menu offering the following options:

• **Copy Table Content**: Copies the content of the selected table to the clipboard for inserting in a spreadsheet program.

**Right-Click Options**: Right-click when the cursor is below or above a column and an arrow points to the column to get a context menu offering the following options:

• **Insert Column to the Left**: Inserts a new column to the left of the selected column.
• **Insert Column to the Right**: Inserts a new column to the right of the selected column.
• **Delete Column**: Deletes the selected column.

**Right-Click Options**: Right-click when the cursor is right or left of a row and an arrow points to the row to get a context menu offering the following options.
• **Insert Row Above:** Inserts a new row above the selected row.
• **Insert Row Below:** Inserts a new row below the selected row.
• **Delete Row:** Deletes the selected row.

**Editing a table:** The table can be moved when the mouse cursor is above the lines of the table and is marked with the move symbol •. The vertical lines in a table can be moved when the mouse cursor is above the end of a marker and is marked with the move symbol ‣.

**Calculation Expressions:**

```
+ Addition
- Subtraction
* Multiplication
/ Division
Sqrt(x) Square root of x
Ln(x) Natural logarithm of x
Log(x) Logarithm of x
Exp(x) Natural exponential function of x
Power(x,y) x to the power of y
Round(x) Rounds x to the nearest integers
Floor(x) Largest integer less than or equal to x
Ceil(x) Smallest integer that is not less than x
```

**Plot Manager Gate Configuration**

The table in the ‘Gate Configuration’ window is used to configure settings for polygons, quadrants and marker sets for the plot from which the gate configuration window was launched.

The table lists all gates (polygons, quadrants and markers) which have been defined in the associated row. Note that gates marked as shown will only be shown in the plot, if the conditions for this are fulfilled, i.e. markers are only shown in histogram plots, and polygons are only shown in scatter plots. Furthermore the axis parameters of the plot must be identical to the axis parameters for the gate.
To disable the showing of a gate click to remove the checkmark in the show column.

Gate configuration:

- **Disable**: This option disables gating for the selected gate.
- **Include**: This option results in display of all events inside the selected gate.
- **Exclude**: This option results in display of all events outside the selected gate.
- **Union**: This option is available when two gates have been set to either **Include** or **Exclude**. Selecting this option results in display of the sum of all events displayed for the two gates (included or excluded).
- **Intersection**: This option is available when two gates have been set to either **Include** or **Exclude**. Selecting this option results in display of the events displayed for both of the two gates (included or excluded).
PDF Report

In the menu line File->Create PDF Report data may be saved to a PDF file for the selected file. The PDF report functionality is also available by right clicking on a file in the file list in the main window.

The report file name can be set and the location where to save the PDF file can be selected. Note that the extension of the file name must be ‘.pdf’.

The different sections to include in the PDF report can be selected. For some sections the setup can be specified. For the image section the first image, all images or a selection of images can be selected. Furthermore, the images can be selected to be enhanced and inverted, making the cells in the images clearer and inverting the background from black to white, respectively. For PDF reports intended for printing it is recommended to select inverted images for better visualization and to save black ink. In the Plot Manager section it can be selected to include the last saved data from the plot manager or the original raw data from when the cm file was created. In addition, it can be specified how many plots may be shown per row before wrapping to next row. When specifying more than 2 plots per row the plot size is reduced accordingly. Finally, signatures for the person that has created the report and the person to approve the report can be specified and which of the signatures to include.

The preview button allows the user to see the preview of the PDF report. This preview will not be saved.

The PDF report can be saved to the specified location and automatically opened if chosen. The PDF can be printed to the default printer if one is installed. Note that it is optional to save the PDF report, if it is only sought to print the report.

The selections will be remembered for the next PDF report creation.

PDF report for multiple files may created by opening the File Browser, selecting the files by left clicking while holding down Shift or Control and finally selecting Create PDF Reports after right clicking. This will open the PDF report dialog in a state where a prefix can be specified for the file names. The full PDF file name cannot be specified when multiple files are selected. The selected options for what to include in the PDF report will be applied for all selected files.
Options

The options dialog has the following tab pages:

- **General**: The software can be set to require a sample ID to be written for all selected chambers, before an analysis can be started.

  When *Always show Login ID in Operator field* is checked, the Main Window Operator field shows Login ID and is read-only. In case the Login ID contains any hash characters (#), these will each be replaced by “_hash_”. In 21CFR11 mode it is only NucleoViewAdmin who may change this setting.

  Image settings for the image display can be set to enhance and invert. The *Enhance* function will depict low pixel values brighter. Disabling the *Enhance* function will display the image linear scaled. The *Invert* function will depict the image inverted so the background appears white instead of black while the colors of each channel is attempted to be preserved.

- **Folders**: The location of the result file folder can be set by the user and the new folder selection can be applied for all users. Please note that changing the result folder location to a network location is not recommended, as this may cause slow reading of the relatively large image files depending on the speed of the network. It may also cause problems with saving new images during image acquisition, if access to the network location is limited.  

  A copy of the results without the image can be saved as a csv file for every complete run of a protocol. The location for the folder, where to save the csv files, can be set by the user and the new folder selection can be applied for all users.

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2 If a user defined folder is set, but not available, the software will:

- When 21 CFR Part 11 is disabled:
  - Warn the user and temporary use the default folders.
- When 21 CFR Part 11 is enabled:
  - NucleoViewUser and NucleoViewSupervisor group members cannot access the software, but instead present a warning asking them to contact a NucleoViewAdmin.
  - Event log entries will be written in the default folder.
  - NucleoViewAdmin are given a warning when logging in, and software uses the default results folder.
  - Event log entries will be written in the default folder.
PDF Report: A PDF report can be saved and/or printed for every completed run of a protocol. The location for the folder, where the PDF reports will be saved can be set and the folder selection can be applied for all users. The default folder for saving PDF report is in the NucleoView™ NC-200™ folder on the desktop in the subfolder \results\PDF reports.  

Saved PDF reports will be named with the same file name as the cm file name with a pdf extension and can be selected to be automatically opened. The PDF can be printed to the default printer if one is installed. This will result in a printed report for every completed run of a protocol. Note that it is optional to save the PDF report, if it is only sought to print the report.

The different sections to include in the PDF report can be selected. For some sections the setup can be specified. For the image section the first image or all images can be selected. Furthermore, the images can be selected to be enhanced and inverted, making the cells in the images clearer and inverting the background from black to white, respectively. For PDF reports intended for printing it is recommended to select inverted images for better visualization and to save black ink. In the Plot Manager section it can be selected to include the last saved data from the plot manager or the original raw data from when the cm file was created. In addition, it can be specified how many plots may be shown per row before wrapping to next row. When specifying more than 2 plots per row the plot size is reduced accordingly. Finally, signatures for the person that has created the report and the person to approve the report can be specified and which of the signatures to include.

The paper size can be specified to be A4 or letter.

Authorization: The 21 CFR Part 11 mode can be enabled or disabled if this license has been purchased. User rights to approve results can be set. User login options can also be enabled under this tab and do not require activation of 21 CFR part 11 mode. See the 21 CFR part 11 section for more details.

Log in is mandatory at NucleoView™ start up if 'User login needed when starting' is enabled. Windows user credentials are used to log into NucleoView™. The User Name will be recorded in image- and event log files. If the 'User login needed when starting' option is disabled, the User Name used to log on to Windows will be reported instead.

User Permissions are in the above picture shown with default settings. Sample ID renaming is as default allowed, but can – individually for each of the NucleoView™ user groups be prevented, optionally with a 15 minutes grace period where the user can change Sample ID up to 15 minues after the cm-file was created. Access to change which protocols are hidden is as default disabled, i.e. the ‘Hide Protocols...’ button on the ‘Select Protocol’ window is not visible. To make the ‘Hide Protocols...’

3 ‘User Name’ is also referred to as ‘Login ID’ or ‘User’ in NucleoView.
button visible for any of the NucleoView™ user groups, set the corresponding check box.
21 CFR Part 11

The US regulatory agency Food and Drug Administration (FDA) have issued the 21 Code of Federal Regulation 21 part 11; Electronic Records, Electronic Signatures (21 CFR part 11). In short, the 21 CFR part 11 defines the FDA acceptance criteria for use of electronic records and electronic signatures as equal to paper records with handwritten signatures.

The NucleoView™ NC-200™ application can be set into a restricted mode, so the user via the NucleoView™ application itself can not violate the 21 CFR part 11 regulations. This means that on a computer system that is 21 CFR part 11 compatible, it is possible to keep the compliancy even after installation and use of the NucleoView™ application.

Using the 21 CFR part 11 requires a specific license that should be purchased in addition to the NucleoCounter NC-200™ instrument and NucleoView™ NC-200™ software.

Please refer to the document Guide for 21 CFR part 11 on NucleoView™ NC-200™ (991-0211) for a description of the operation of NucleoView™ NC-200™ in 21 CFR part 11 mode and how the 21 CFR part 11 scripts are activated.

Also refer to the document NucleoCounter® NC-200™, NucleoView™ NC-200™ Software and Code of Federal Regulation 21 Part 11; Electronic Records, Electronic Signatures (994-0208) for a description of what approach ChemoMetec A/S has taken to meet each relevant section in the 21 CFR part 11 guidelines.

Event Log

The Event Log function creates an audit trail of all user activity within the NucleoView™ NC-200 Software.

Please refer to the document Guide for 21 CFR part 11 on NucleoView™ NC-200™ (991-0211) for a description of the Event Log function and viewer.
License, Protocol and Documentation Installation

To run a given protocol, the corresponding protocol-, license- and documentation-files must have been imported. This is normally performed when the NucleoView™ NC-200™ software is installed, but it may also be done any time later as described in the next section.

Install licenses, protocols and protocol documentation as follows:

1. In the menu select File->Import Package
2. In the file selection browser pop up window, browse to and select the file you want to install.
   This will typically be a zip-file containing multiple license, protocol and documentation files, or it may be e.g. individual *.cmsx, *.cmsup and *.pdf files.

If you at any later time want to install extra licenses and protocols, this is done using the same procedure. The new elements will simply be added to the existing collection.

Important: Do not edit license files or protocol files manually as this will invalidate the files.

Select View -> License File to see a list of installed licenses.

A license is typically valid for a particular NucleoCounter® NC-200™ instrument (identified by serial number). A license may have an expiry date.
Maintenance and Backup

**NucleoView™ Software Maintenance**

A two-channel image taken with NucleoView™ NC-200™ is approximately 12 MB in size. Therefore, for example performing 50 viability and cell count assays each work day will over a month accumulate approximately 16 GB. The user must hence backup results and free up disc space in good time to ensure trouble free operation. Each time a protocol run is started, NucleoView™ tests for sufficient disk space and if there is not enough, it aborts the protocol with an error message to the user. See [Analysis Results](#) for further details about file types and locations.

The NucleoView™ NC-200™ constantly records all operations in event log files. These event log files should be handled in the same manner as the image files with respect to backing up and freeing up disc space. The event log files are always stored in the current results folder. One file is created for each month, and files are named according to the rule `<year><month>.logx` like `201610.logx` indicating a log file from October 2016.

The NucleoView™ NC-200™ software including standard protocols is typically delivered on a USB stick, and protocols and licenses are distributed as zip-files via e-mail, USB stick or other media. The user must keep appropriate backup of these zip-files in order to be able to make a new installation in case re-installation of the software is required.

**NucleoCounter® Instrument Maintenance**

ChemoMetec A/S provides specific Installation Qualification (IQ), Operation Qualification (OQ) and Performance Qualification (PQ) protocols and associated test kits, which may be used to verify proper operation of the NucleoView™ and NucleoCounter® NC-200™ system.

The NucleoCounter® NC-200™ instrument can be cleaned in case it has been contaminated with sample fluids, dust or other spillage. Use dry cleaning swabs for cleaning, and only if this is not enough, use 96-100% pure ethanol. Under no circumstances should the inside of the cassette fixture tried to be cleaned. The outer surfaces of the NucleoCounter® NC-200™ instrument may also be cleaned with 96-100% pure ethanol if needed.

It is good practice to put the large lid down, whenever the instrument is not being used, to prevent dust build up on the internal surfaces. Also avoid splashing sample fluids into the interior of the instruments, as cleaning of these parts should be done only by ChemoMetec A/S.
How To

How to Get Context Sensitive Help

While having focus on a control on a specific window, press F1 (not applicable on the Main Window). This will launch the software manual on the relevant page.

How to Verify Correct Operation of the NucleoCounter® Instrument after Transportation

The NucleoCounter® NC-200™ Instrument is a very robust device which handles shipping well, provided it is packaged correctly in the original shipping box. In case of extreme physical stress, it may however suffer internal damage and/or misalignment.

After transportation it is recommended to run the Installation Qualification (IQ) protocol. This protocol will inspect critical internal alignment and report any changes.

The Operation Qualification (OQ) protocol verifies correct operation of the instrument.
Keyboard Shortcuts

Image
+                  Zoom in
-                  Zoom out
Arrow keys        Pan up, down, left or right in displayed image

Application Selection
F1                  Quick selection of protocol attached to favorite F1 button
F2                  Quick selection of protocol attached to favorite F2 button
F3                  Quick selection of protocol attached to favorite F3 button

Note: Shortcut usability depends on actual focus.
## Trouble Shooting

<table>
<thead>
<tr>
<th>Installation</th>
<th>The NucleoView™ NC-200™ software or the NucleoCounter® NC-200™ instrument is not installed correctly.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The user must be logged on with administrator rights during installation.</td>
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<tr>
<td></td>
<td>When connecting the NucleoCounter® NC-200™ instrument to a previously unused USB port, the NucleoView™ NC-200™ software may need to reinstall the camera drivers and will prompt the user to do so. This operation may need administrator rights, so either use another USB port where the camera has already been installed, or log on with administrator rights and complete the camera installation as described in Installation of Instrument.</td>
</tr>
<tr>
<td></td>
<td>During connection to the instrument, you may see an error message from Windows, which is not of any consequence. Click Close on any of these pop up messages if needed.</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>NucleoView™ NC-200™ Issues (Software)</th>
<th>Protocol file is invalid</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>The license file is corrupted, missing or does not include a license for the selected protocol. The appropriate license must be installed as described in the Licenses section.</td>
</tr>
</tbody>
</table>

| Connection error | The NucleoCounter® NC-200™ instrument icon in the upper left corner of the main window stays red after NucleoView™ NC-200™ has been launched, i.e. the NucleoView™ NC-200™ software does not connect to the instrument. This may be caused by an error in the USB connection. To inspect and solve this, open the Control Panel and launch System. Select the Hardware tab and click Device Manger. In case of a USB driver error there will be a USB item marked with a yellow dot containing an exclamation mark. Select properties for this item and select the driver tab. Select Update Driver and let the system select a driver automatically. |

<table>
<thead>
<tr>
<th>Cassette</th>
<th>No cassette is found</th>
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<tbody>
<tr>
<td></td>
<td>This may be because either no cassette is in the instrument or the cassette is placed incorrectly. In the latter case, remove the cassette and re-insert it.</td>
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</tbody>
</table>

| Non-valid cassette | Typically the user has placed an already used cassette in the instrument. Simply replace with an unused cassette which has been correctly loaded. |

<table>
<thead>
<tr>
<th>Flow detection error</th>
<th>The flow has failed to reach the flow detection point within given time limits. A common cause is that too small a volume of sample</th>
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</thead>
<tbody>
<tr>
<td>NucleoCounter® instrument</td>
<td>The instrument gives abnormal sounds</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Typically the piston motor has been blocked by a cassette that has not been correctly inserted. Stop the run by removing the power supply. Reconnect the power supply and remove the cassette and re-insert it again before starting a new run. If issues persist contact ChemoMetec A/S or local representative.</td>
</tr>
</tbody>
</table>

solution has been loaded into the cassette. Simply place a cassette which has been correctly loaded. Another typical cause is that the user has placed an already used cassette in the instrument.

Check also on-line the known issues section on the ChemoMetec homepage for up-to-date information (URL: http://www.chemometec.com/en-GB/Support/Software_downloads/Known-Issues.aspx).
Appendix A: Description of changes from latest revision
This chapter describes changes from latest revision of this User’s guide.

A.1 New:

A.2 Changes:

• Front Page
  o Revision 1.12
• Page 2
  o Revision 1.12
  o June 2020
• Page 7 and 63
  o References to older Windows OS versions removed
• Plot Statistics (page 41)
  o Added Copy table to clipboard (extended) option.
• Options (page 55)
  o Added Always show Login ID in Operator field.

A.3 Removed: