Technology that counts

chemometec

NucleoView NC-3000™ Software User's Guide

P/N 991-3003
Revision 1.0



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NucleoView NC-3000™

P/N 991-3003 (English)
Revision 1.0
August, 2013

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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-3000™ not covered in this document and referred documents is available through the following:

- For e-mail support, send questions to NucleoCounter® NC-3000™ 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-3000™ serial number and have it available when contacting ChemoMetec A/S for support. The NucleoCounter® NC-3000™ serial number is found on the label affixed to the rear side of the instrument. The version number of the NucleoView NC-3000™ software shall also be noted, this can be found on the Help – About menu item in the NucleoView NC-3000™ software.

Sales and ordering information

For sales assistance with NucleoCounter® NC-3000™ or the NucleoView NC-3000™ software, to place an order for a NucleoCounter® NC-3000™ 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-3000™ software and the NucleoCounter® NC-3000™ instrument as described in the documentation on the USB stick containing the NucleoView NC-3000™ software.

2. Start the NucleoView NC-3000™ software by double-clicking on the NucleoView NC-3000™ icon on the desktop.

3. Wait while the NucleoCounter® NC-3000™ instrument initializes. The slide tray can be heard aligning during this process. When the LED on the instrument turns green, the instrument is ready to use.

4. Click the button (below the '*F3*' button) and select the desired protocol.

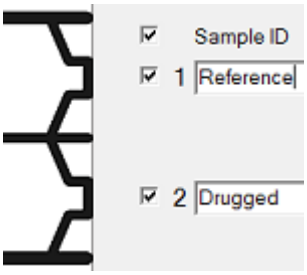
5. Click the button to see the application note for the selected protocol.
   Check that you have all the materials needed for this protocol (slides, reagents ...).

6. Mix your cell sample, add potential solution(s) and load a Cassette, A2 or A8 side.





7. Eject the sample tray and insert the slide or cassette.



8. Select the chambers to be analyzed by clicking in the checkboxes.

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

10. Press the '*Run*' button. RUN
    The analysis will now be started and the results of the analysis will be displayed a few moments later.

    Repeat steps 6 to 10 to perform the same protocol again on a new sample or repeat from step 4 to start a new type of analysis.

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Installation Guide

Installation Overview

Important: You must be logged on as system administrator to install the NucleoView NC-3000™ system components.

In a first time installation, you will typically install both the NucleoView NC-3000™ software and one or more NucleoCounter® NC-3000™ 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:

1. Operating System: Windows 8, Windows 7 or Windows XP with service pack 3.
   The Windows operating system must not be virtualized.
   Note: Windows Vista is not supported!
2. To install the NucleoView™ application, you need to log on with administrator rights.
3. At least 4 GB RAM and 10GB free disc space is recommended.
4. At least 2.0 GHz clock rate.
5. At least one USB 2.0 port must be available.
6. Screen size minimum 1366 x 768 pixels.

To perform the installation you will also need the NucleoCounter® NC-3000™ instrument and the NC-3000™ 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 7. For other versions of Windows there will be minor variations, but as a general rule, simply accept all the default settings as they are presented.

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-3000™ 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.

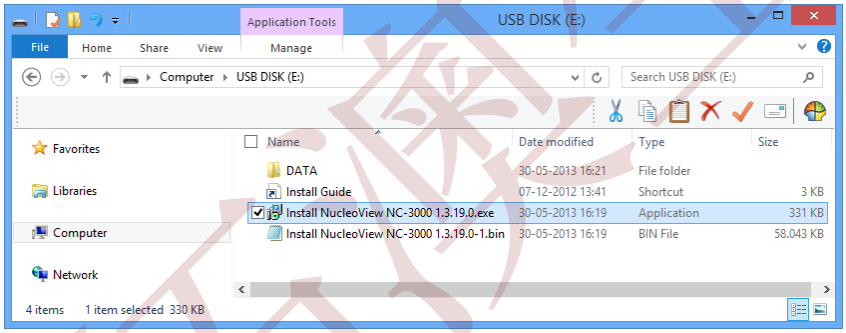
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5. Windows will detect the USB stick and present an AutoPlay window if AutoPlay is enabled. Select the *Open folder to view files* option.



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

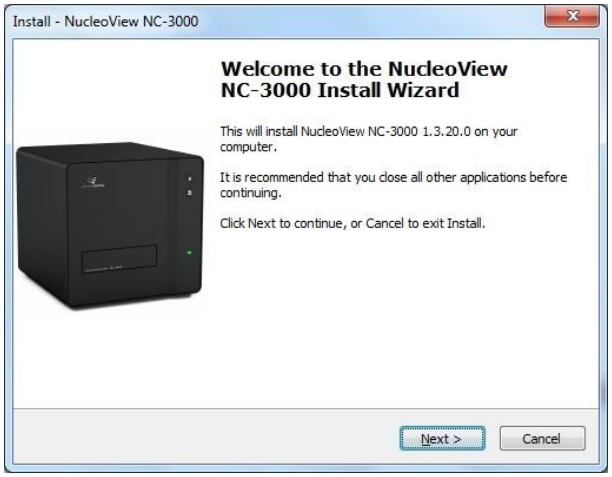
6. The directory contents on the USB stick will be shown. Double-click on the *Install NucleoView NC-3000™ X.X.X.X* file (the version number e.g. 1.3.19.0 will vary depending on your installation) to launch the installation program.
Note*: It is the .exe file you should double-click, not the .bin file. The .exe file also has the installation icon associated in contrast to the icon for the .bin file.*



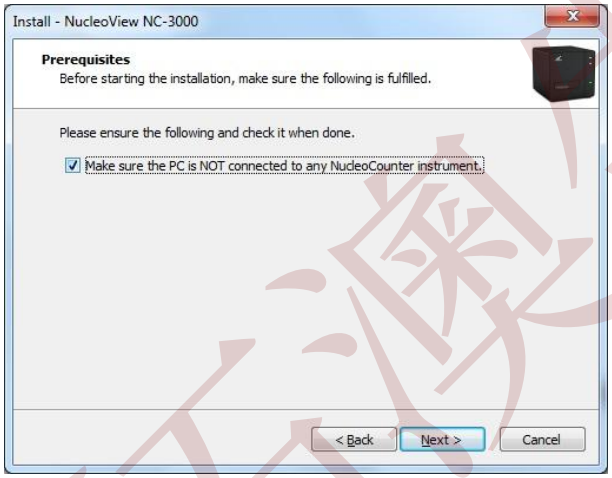
7. If a window with the text '*Do you want to allow the following program from an unknown publisher to make changes to this computer?*' appears, click '*Yes*'.

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8. After a few seconds the '*Welcome to the NucleoView NC-3000™ Install Wizard*' window will appear. Click '*Next*'.



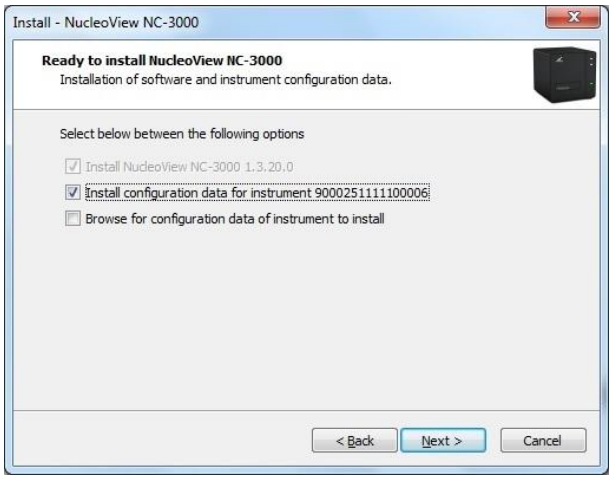
9. On the *Prerequisites* window you must check the check box to confirm that you have disconnected all NucleoCounter® instruments from the PC.
Click '*Next*'.



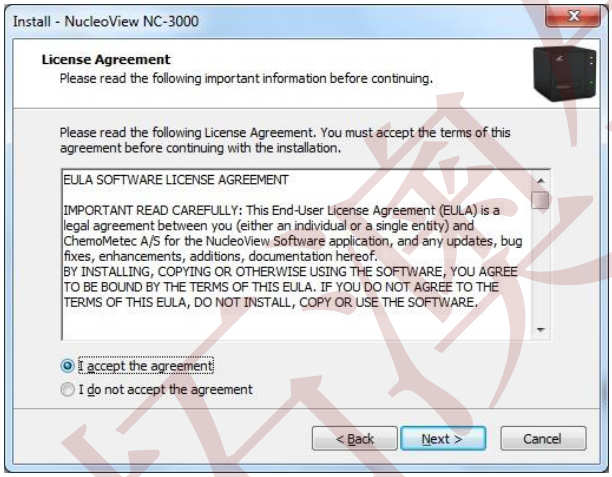
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10. On the '*Ready to install NucleoView NC-3000™*' window you will be given options to install or upgrade the NucleoView NC-3000™ software itself, to install configuration data for specific instrument(s) and to browse for instrument configuration data which are stored elsewhere. Check the appropriate options and click '*Next*'.

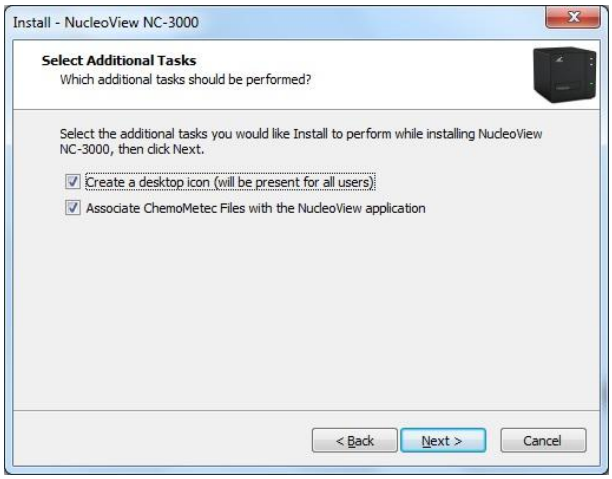


11. On the *License Agreement* window read the license agreement. To proceed with the installation you will have to select '*I accept the agreement*' and click '*Next*'.

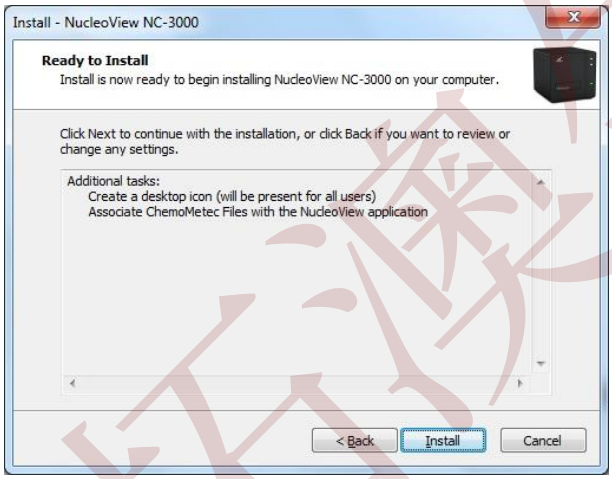


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12. On the '*Select Additional Tasks'* window check the desired options and click '*Next*'.
    Checking the '*Associate ChemoMetec Files with the NucleoView application'* will associate the image files *.cm and protocol files *.cmsx with the NucleoView application making it possible to open the files directly by double-clicking.



13. On the '*Ready to install'* window click '*Install'*.



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.

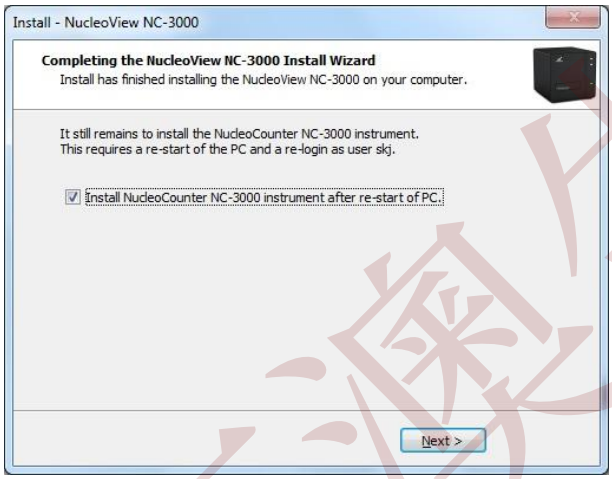
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14. On the '*Window Security*' window click *Install*.

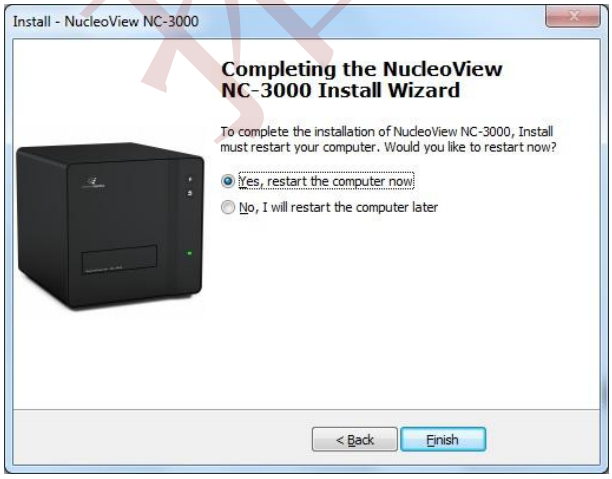
    Optional: To avoid this window during future software upgrades check the box *Always trust software from Lumenera Corporation*.



15. On the '*Completing the NucleoView NC-3000™ Install Wizard'* window you may optionally deselect the check box if you only wish to install the software without attaching the instrument.
    Click '*Finished*'.



16. On the '*Completing the NucleoView NC-3000™ 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*'.



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17. After installation of the NucleoView NC-3000™ software two new shortcuts have been placed on your desktop. One is the NucleoCounter® NC-3000™ instrument icon used to launch the NucleoView NC-3000™ software, the other is a shortcut to the data folder where NucleoView NC-3000™ stores application data.

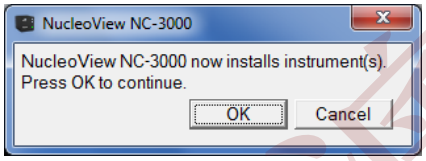


After restart of the PC the NucleoView NC-3000™ software has successfully been installed.

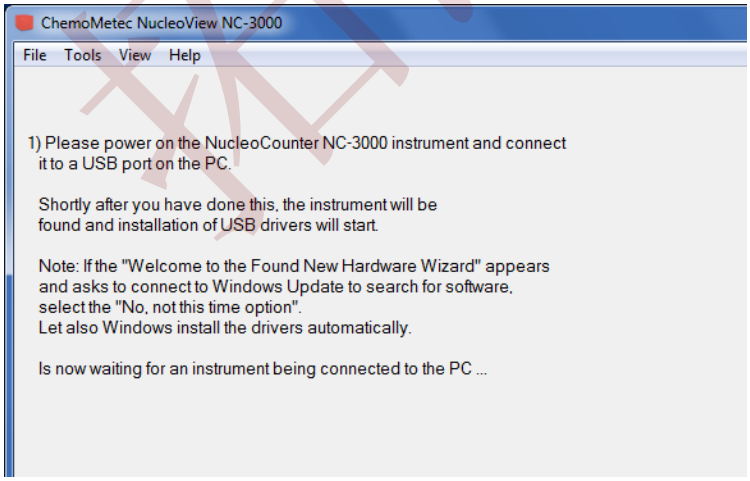
The NucleoView NC-3000™ program will now automatically be started and it will guide you through the instrument installation procedure, if the check mark for 'Install NucleoCounter® NC-3000™ instrument after Install has finished' was checked.

Note: Depending on file versions and previous install history, a reboot of the computer may be requested before the NucleoView NC-3000™ 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-3000™ is now launched in installation guided mode. Click '*OK*' to continue with installation of the NucleoCounter® NC-3000™ Instrument.



2. Follow the on-screen instructions for the installation of the NucleoCounter® NC-3000™ 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:

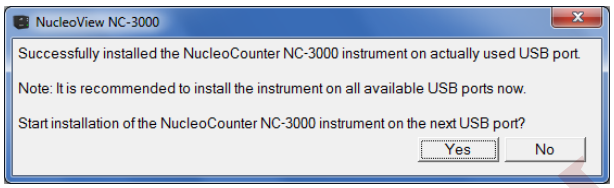
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- A USB hub inside the NucleoCounter® NC-3000™ instrument
- FTDI device driver installed
- The camera

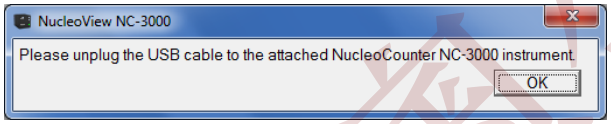
Due to the intentional and very fast reconfiguration of the camera, you may see an error message from Windows, which is not of any consequence. Click *Close* on any of these pop up messages if needed.

When the NucleoView NC-3000™ 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-3000™ 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.

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.

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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-3000™ 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-3000™ Software and NucleoCounter® NC-3000™ Instrument' and the NucleoView NC-3000™ 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-3000™ instruments, use the USB stick holding the appropriate NucleoView NC-3000™ software release and follow the procedure in the previous section describing installation of the software. The NucleoView NC-3000™ 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-3000™ 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-3000™ 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-3000->Uninstall NucleoView NC-3000* in the *Start Menu*. This may be required if you wish to downgrade to an earlier version of NucleoView NC-3000™.

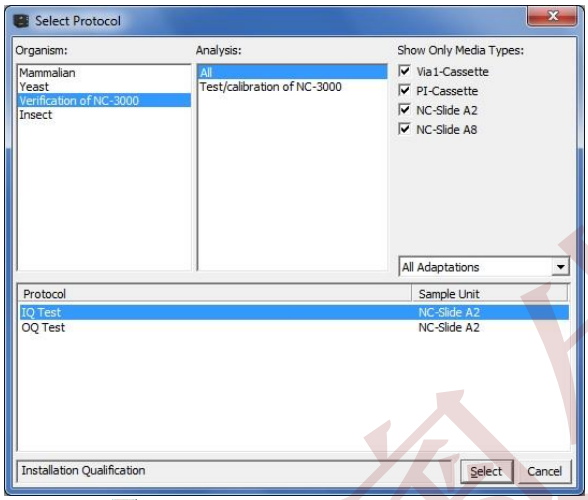
Validation with IQ, OQ and PQ protocols

After installation/upgrade of the NucleoView NC-3000™ software and/or NucleoCounter® NC-3000™ instrument and after transportation of the NucleoCounter® NC-3000™ 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.

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Installation Qualification (IQ Test)

1. Start the NucleoView NC-3000™ software by double-clicking the icon on the desktop.
2. Wait while the NucleoCounter® NC-3000™ 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-3000" and "Test/calibration of NC-3000". 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).



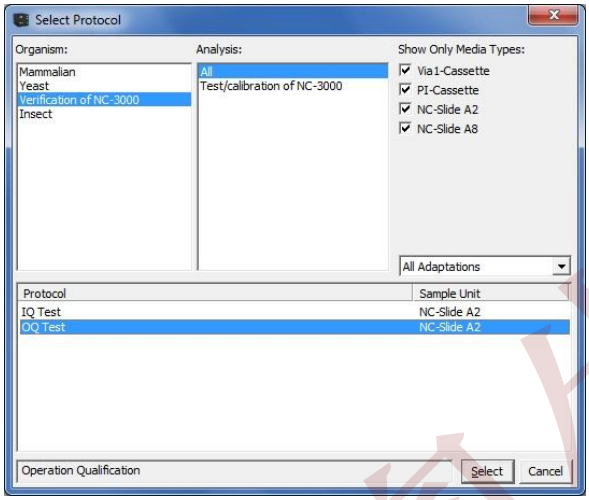
5. Click the button to see the application note for the selected protocol.
6. Follow the instructions on screen and in the application note to finalize the IQ Test.

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Operation Qualification (OQ Test)

1. Start the NucleoView NC-3000™ software by double-clicking the icon on the desktop.
2. Wait while the NC-3000™ 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-3000" and "Test/calibration of NC-3000". 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).



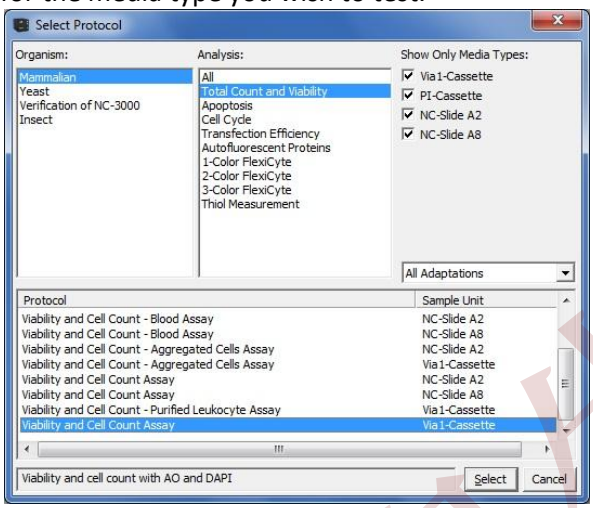
5. Click the button to see the application note for the selected protocol.
   Check that you have all accessories needed for this protocol available (Cassette, Slides, beads).
6. Follow the instructions on screen and in the application note to finalize the OQ Test.

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Performance Qualification (PQ Test)

1. Check that you have a 912-3003 NC Test Kit 3 (Cassette/Slides, beads).
2. Start the NucleoView NC-3000™ software by double-clicking the icon on the desktop.
3. Wait while the NC-3000™ 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 for the media type you wish to test.

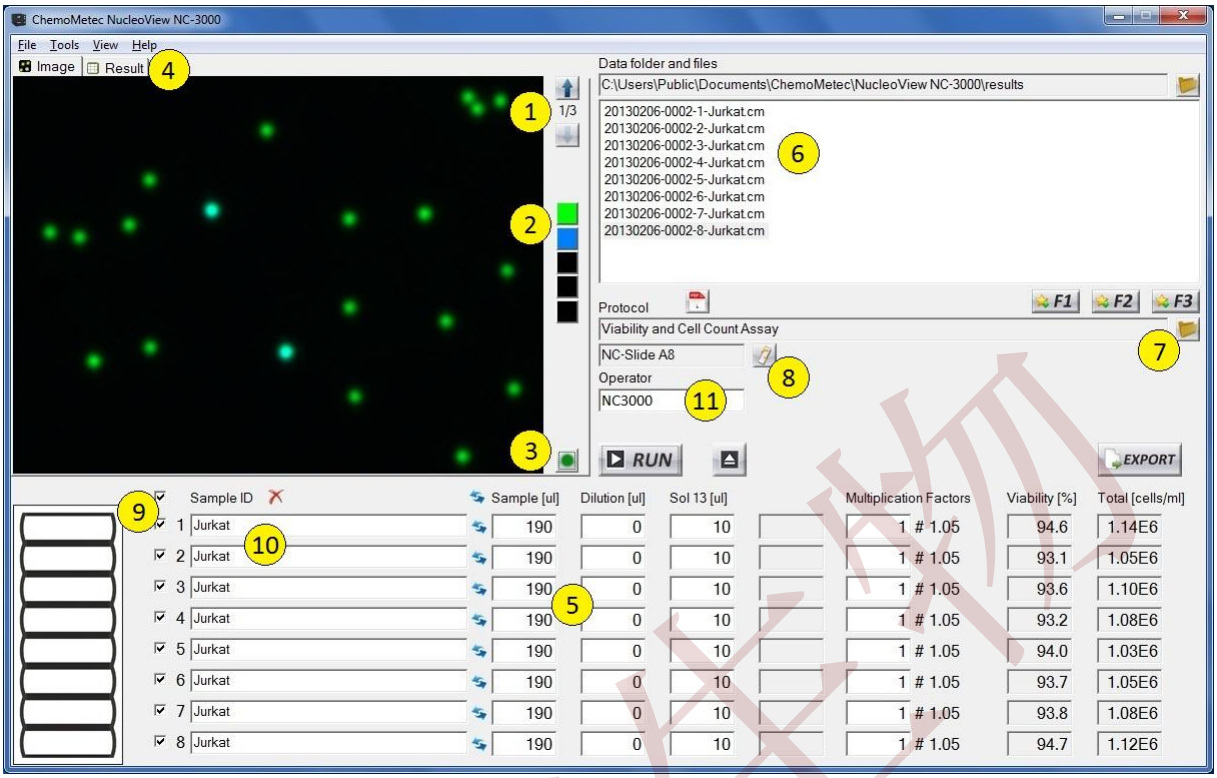


6. Follow the instructions the 912-3003 NC Test Kit 3 to finalize the PQ Test.

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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.

Image Controls: The control icons on the right side of the image area are used to interact with the image. The functionalities are:

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.

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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. Media Type: The slide type for the selected protocol is displayed in the box below the protocol box. If

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both NC-Slide A2™ and NC-Slide A8™ types are available for the selected protocol the slide type can be changed by left-clicking on the button.

9. Chamber Selection: The chambers in which to perform the analysis can be selected by clicking the checkboxes next to the chamber.

10. Sample ID: A description of the sample can be entered in the sample ID input fields prior to starting the analysis. The entered sample IDs can be deleted by pressing the button. 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

11. 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.

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.

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

This button inserts or ejects the sample tray.

EXPORT This button allows the user to export the results for the shown files as a CSV file.

Menu Line

File -> Import Package: Imports files into the NucleoView NC-3000™ software. This will typically be a zip-file containing license, protocol and documentation files, or individual *.cmsx, *.cmsu and *.pdf files.

File -> Export Data: Opens a window where the acquired data from an analysis can be exported in ACS or FCS file formats. See the Export Data section for more details.

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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 -> Protocol Adaption Editor: Opens the Protocol Adaption Editor which is used to create user adapted versions of protocols released with NucleoView NC-3000™ software versions prior to version 2.0.0.0. The menu item will not be available in most cases.

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 -> Options: Opens the options dialog where the result folder can be changed, user login settings enabled, 21 CFR Part 11 can be turned on or off (See details in 21 CFR Part 11 section) and image compression options can be set. 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-3000™ software user's guide.

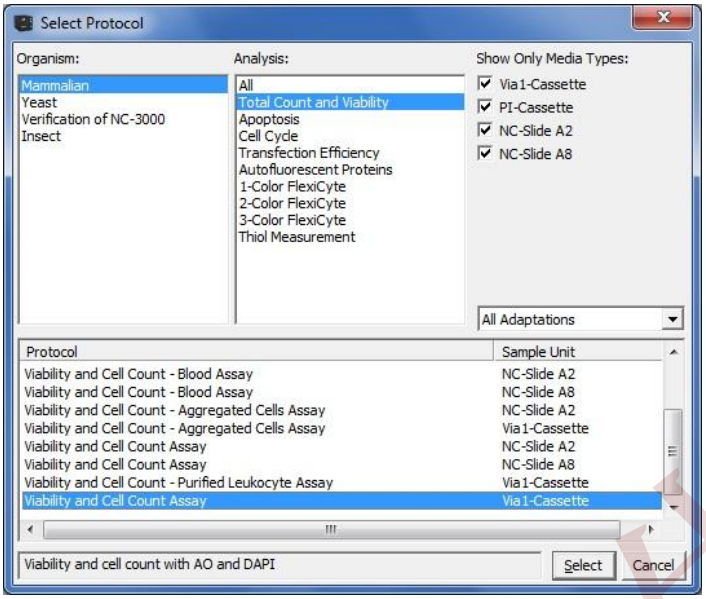
Help -> Instrument Users Guide: Opens the NucleoView NC-3000™ Instrument user's guide.

Help -> About: Displays the About NucleoView NC-3000™ window that contains the software version number and information about the connected NucleoCounter® NC-3000™.

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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:



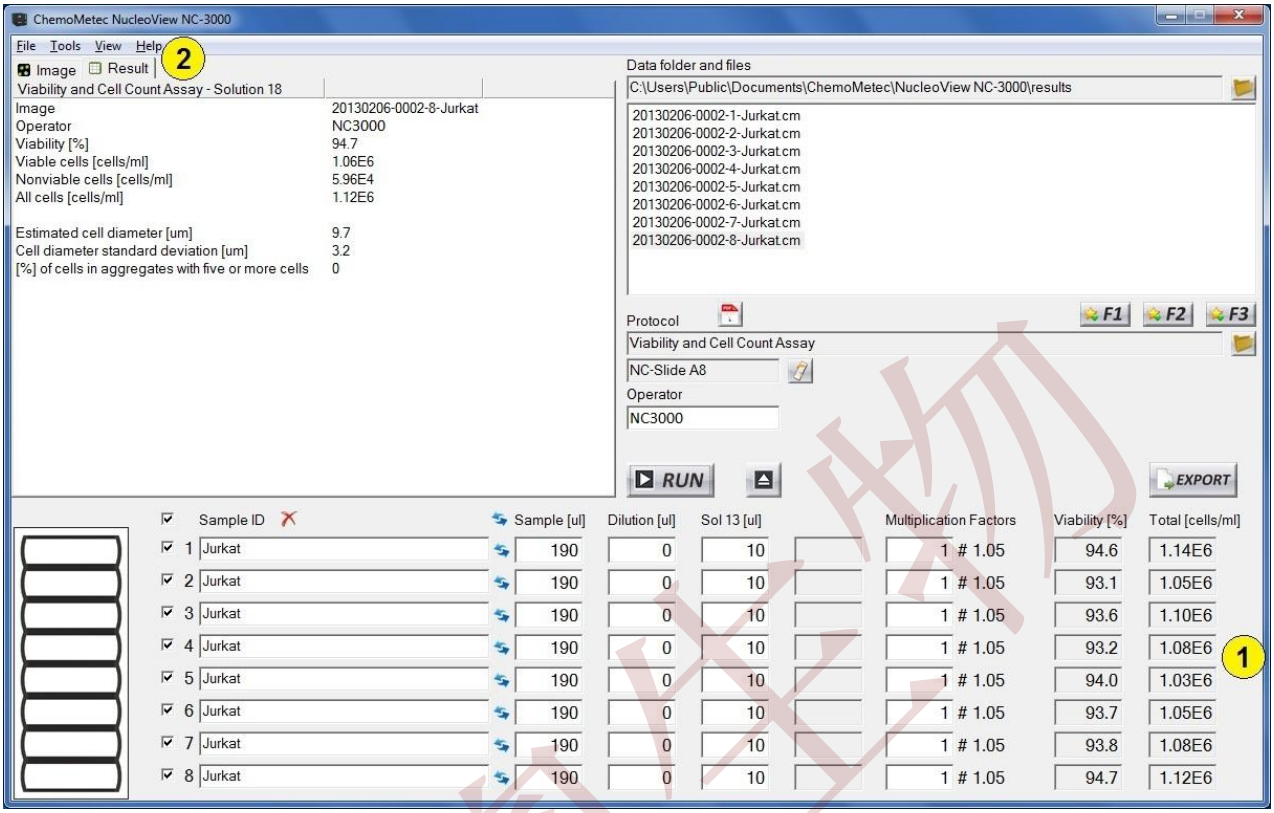
Select type of '*Organism'*, type of '*Analysis'* and preferred '*Media Types'* to see which protocols are available for this in the current installation.
Select the protocol which supports the chosen sample unit (media type) and click '*Select'*.

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-3000™ 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.

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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 yyyymmdd-####-#-*.cm (year-month-day-number)-(chamber number or c for cassette)-(sample ID).cm
- Are placed in *Today's Directory* named yyyymmdd (year-month-day)
- *Today's Directory* is created automatically in *Results Directory*
- *Results Directory* default location set during installation is typically:
  Windows XP:
  C:\Documents and Settings\All Users\Shared Documents\ChemoMetec\NucleoView NC-3000\results
  Windows 7 and 8:
  C:\Users\Public\Documents\ChemoMetec\NucleoView NC-3000\results

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The location may be changed by the user via menu entry *Tools->Options*

Post Processing File (*.cmpp)

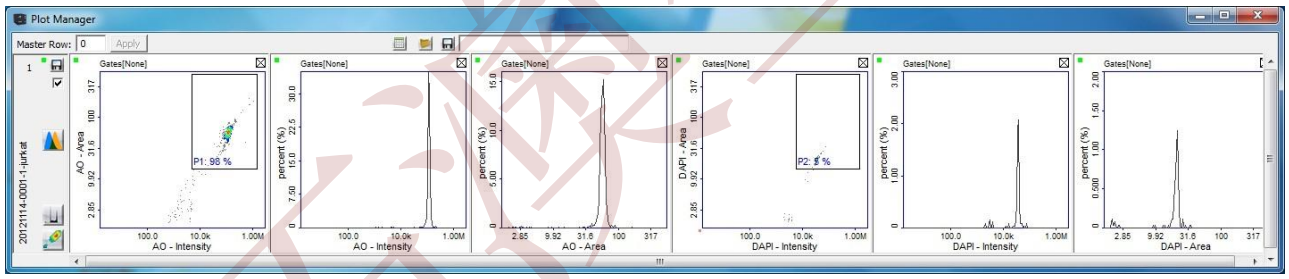
- Holds post processing results for a specific *.cm image file corresponding to one row in a Plot Manager
- Has the same file name as its corresponding image file (*.cm file), but with extension 'cmpp'
- Is placed in the same directory as its corresponding image file
- It may also be saved in the *Master 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*

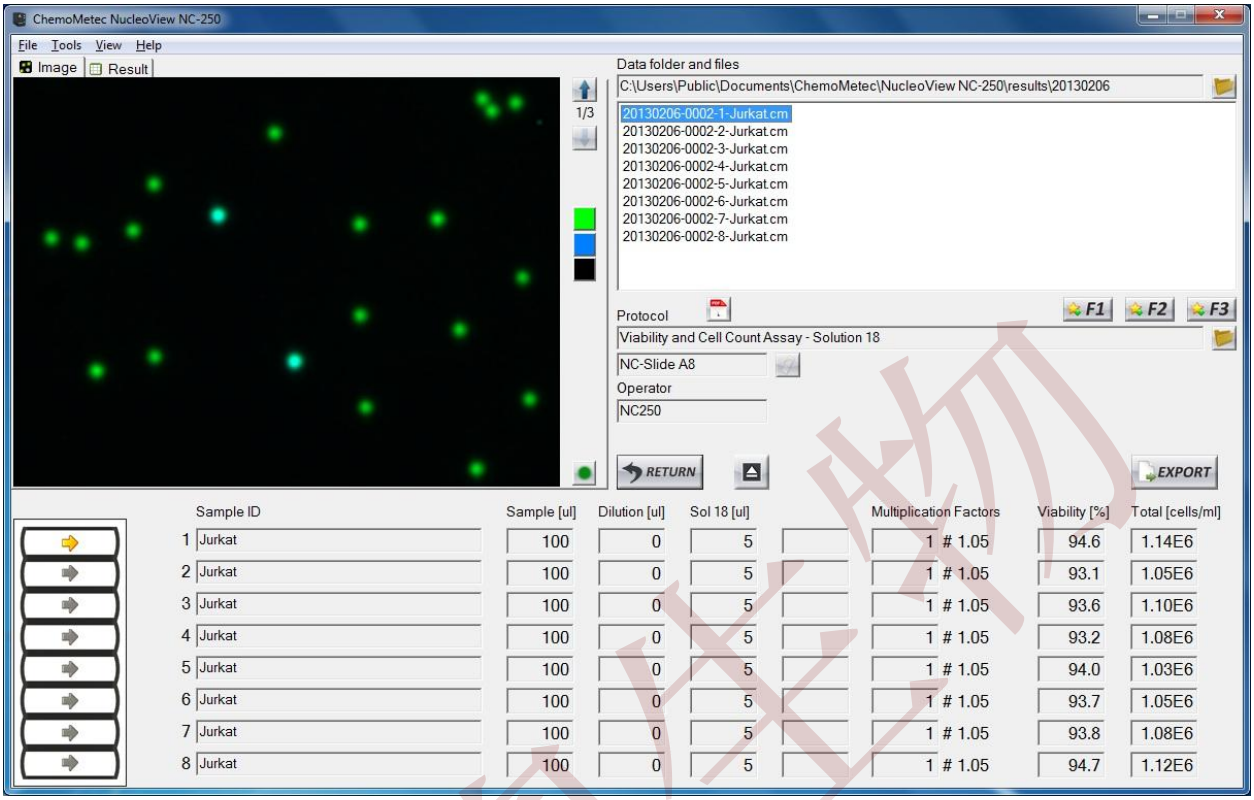
Plot Manager

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.



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Browse Analysis Results



Buttons

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

 Marks the row for the selected file for which the image and extended results are displayed.

 Click on this button to select the file acquired in this chamber.

 Return to '*acquisition mode*' to run a new analysis, when the instrument is connected.

 This button inserts or ejects the sample tray.

 This button allows the user to export the results for all the files for the shown slide or cassette as a CSV file.

File list:

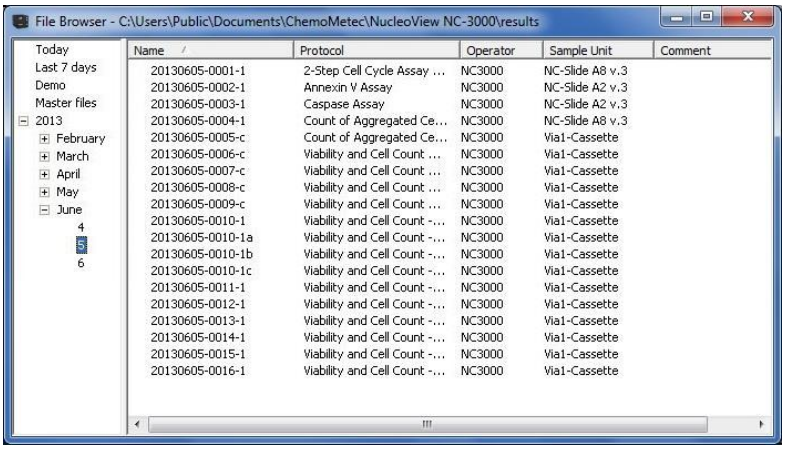
- Click on a file in the data folder to browse the results of a previously acquired file. The file will be opened and the image will be displayed. Optionally files acquired within the same slide may be selected by clicking on the arrow in the desired chamber.

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- 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. This option is only available for images acquired with NucleoView NC-3000™ software version 2.0.0.0 or newer.
    - *Show Raw Data*: Opens the selected file in Plot Manager with the plots defined by the protocol. This option is only available for images acquired with NucleoView NC-3000™ software version 2.0.0.0 or newer.
    - *Show Embedded Image Analysis Results*: Displays the original results of the image file. This option is only available for images acquired with NucleoView NC-3000™ software versions prior to version 2.0.0.0.
    - *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.
    - *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.
    - *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.

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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.

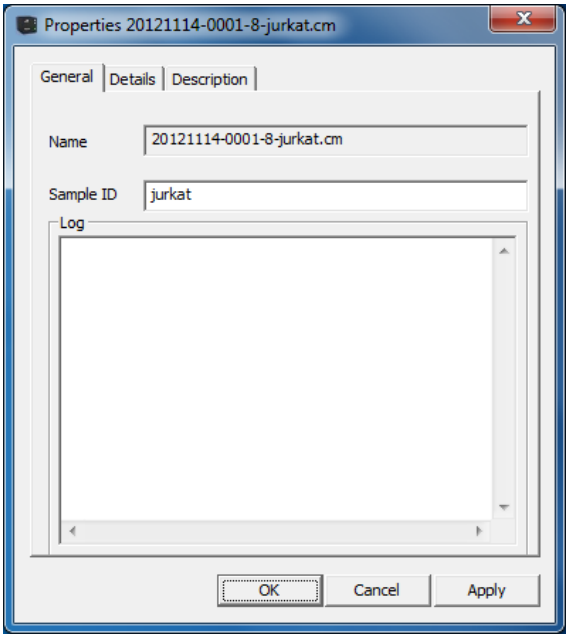
Right-click options: Right-click on one or more selected files to get a context menu offering the following options:

- *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.
- *Batch Export Data*: Opens a window where the acquired data from an analysis can be exported in ACS or FCS file formats. Be aware that multiple files can only be exported together if they have been acquired with the same protocol. See the section for more details.
- *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.

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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.

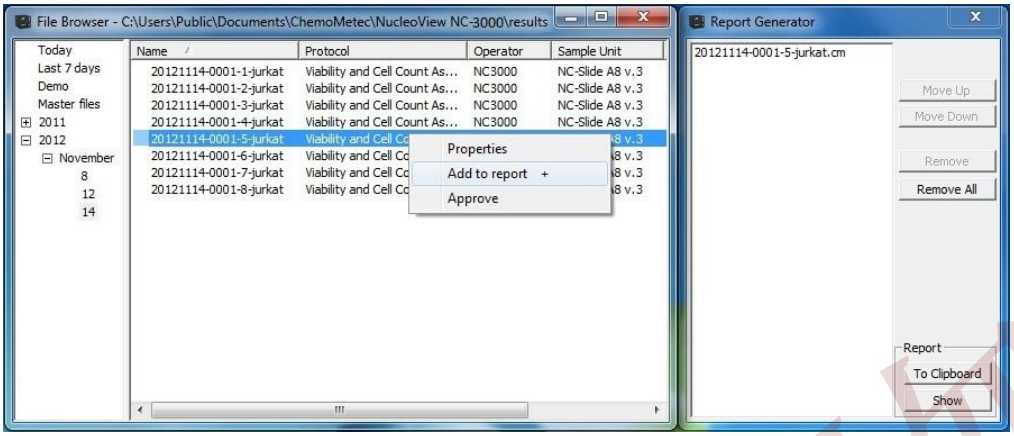
- *Details* that contain information about the image file.

- *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.

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Report Generator



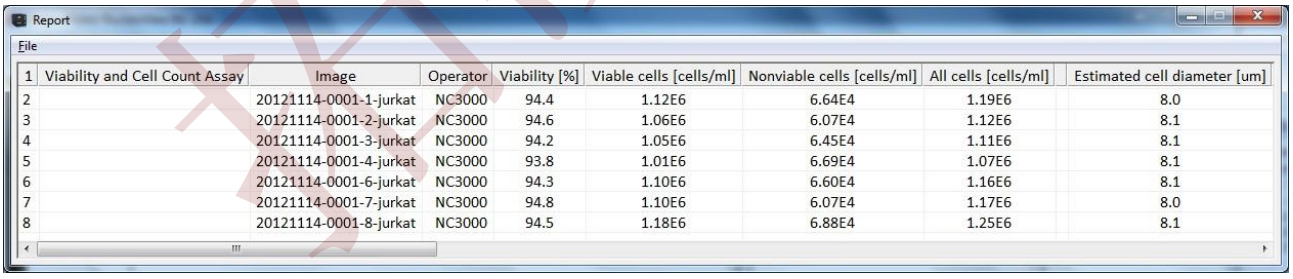
The Report Generator may be launched in several ways; from the File Browser window by right-clicking on one or more selected files and selecting '*Add to report*'; from the Browse File list in the main user interface right-clicking on a file and selecting '*Add to report*'; or via the menu entry '*Tools->Report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



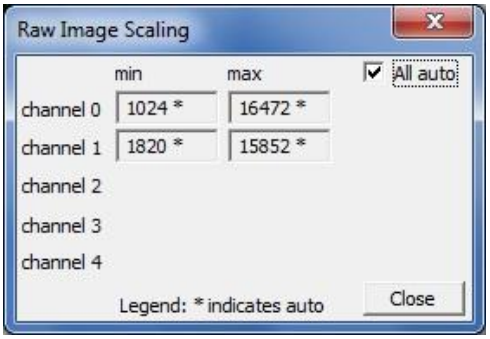
1	Viability and Cell Count Assay	Image	Operator	Viability [%]	Viable cells [cells/ml]	Nonviable cells [cells/ml]	All cells [cells/ml]	Estimated cell diameter [um]
2		20121114-0001-1-jurkat	NC3000	94.4	1.12E6	6.64E4	1.19E6	8.0
3		20121114-0001-2-jurkat	NC3000	94.6	1.06E6	6.07E4	1.12E6	8.1
4		20121114-0001-3-jurkat	NC3000	94.2	1.05E6	6.45E4	1.11E6	8.1
5		20121114-0001-4-jurkat	NC3000	93.8	1.01E6	6.69E4	1.07E6	8.1
6		20121114-0001-6-jurkat	NC3000	94.3	1.10E6	6.60E4	1.16E6	8.1
7		20121114-0001-7-jurkat	NC3000	94.8	1.10E6	6.07E4	1.17E6	8.0
8		20121114-0001-8-jurkat	NC3000	94.5	1.18E6	6.88E4	1.25E6	8.1

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.

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Image Scaling

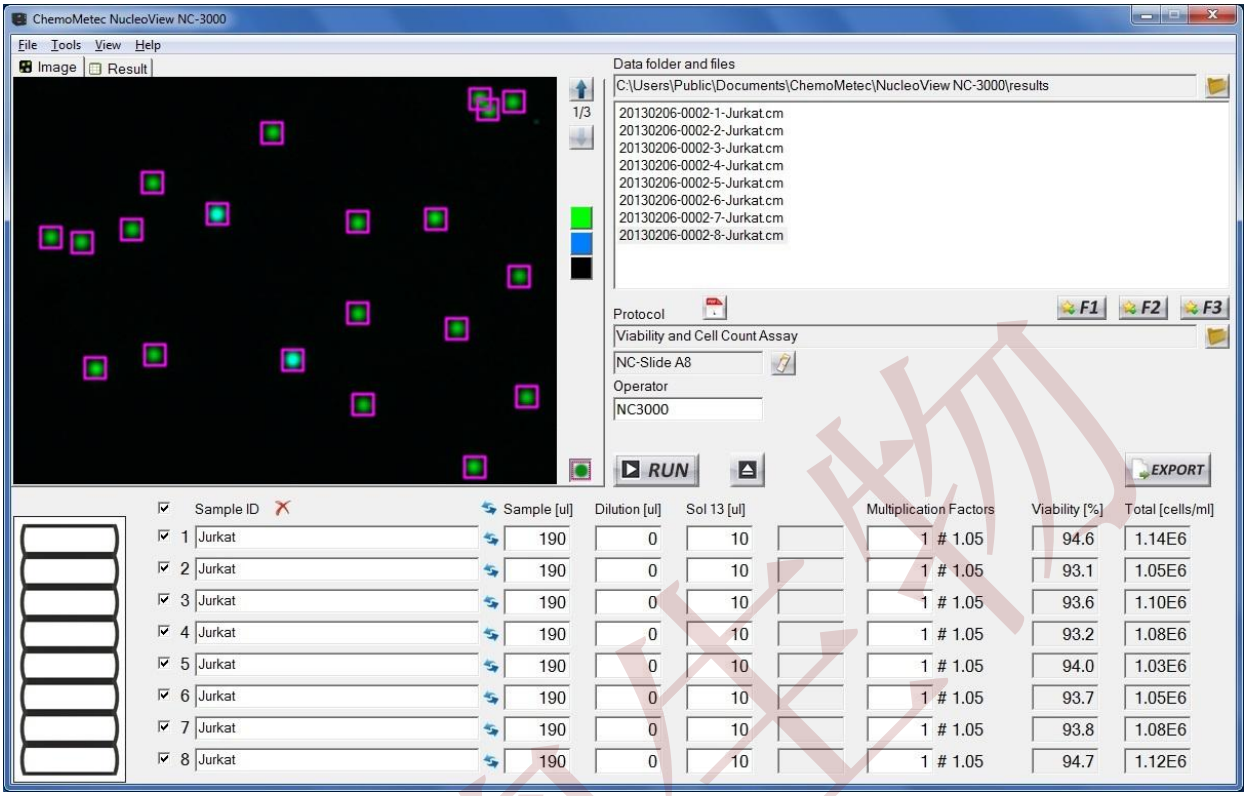


When the image is presented on the screen, the color intensity (pixel intensity values) is scaled according to the channel scaling values.
For each channel, pixel values inside the specified input range (min to max) are scaled linear to the output range 0 to 255 in color intensity.
If a * symbol is appended to a specified value automatic scaling will be enabled. The *All auto* check box may be used to enable automatic scaling on all values.
To increase color contrast you may typically enter a lower max value, e.g. 50% of the channels max value. This will often give a better result when making a bitmap export.

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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*'.

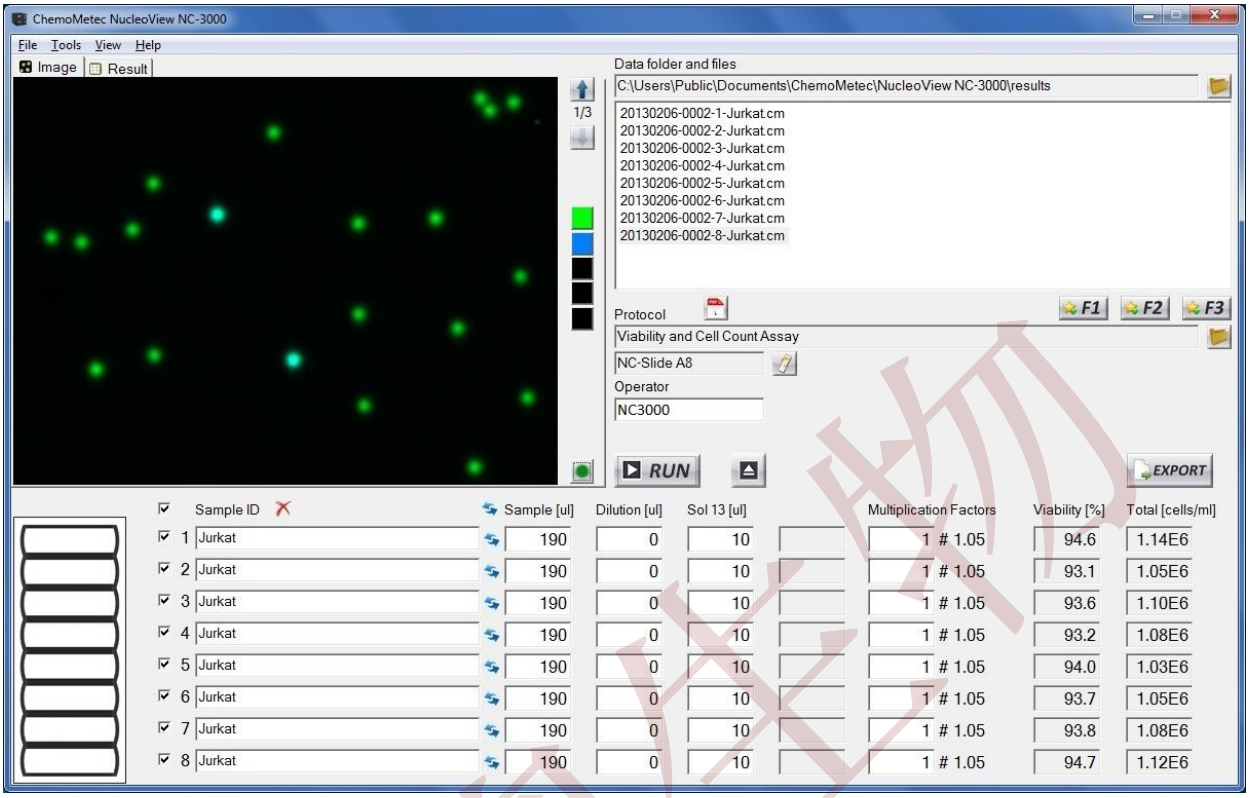
The cells are marked with squares and this overlay can be toggled on and off by clicking the button. When one of the two overlay functions has been used it is possible to choose cells in the Main Window by holding the Ctrl button down and clicking on individual cells in the image. This will mark the 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.

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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 for each chamber. 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.

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Protocols

To perform a particular analysis, the appropriate protocol must be used. This involves selecting and running the corresponding protocol on the NucleoView NC-3000™ software while following the guidelines in the protocol as described in the Quick Guide section.

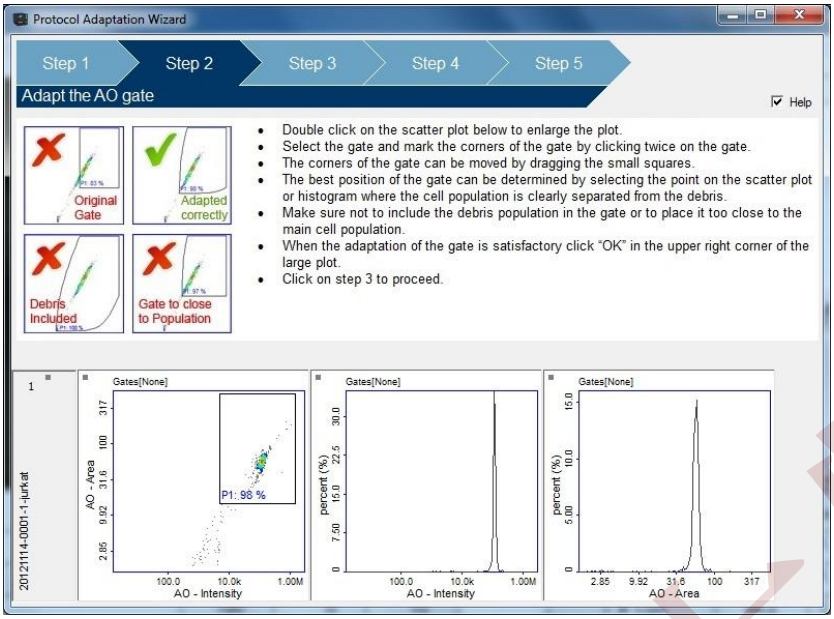
Each instrument requires a unique license to run a protocol. Via the menu entry *View -> License File* you can see a list of the installed Licenses.

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.

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Protocol Adaptation Wizard

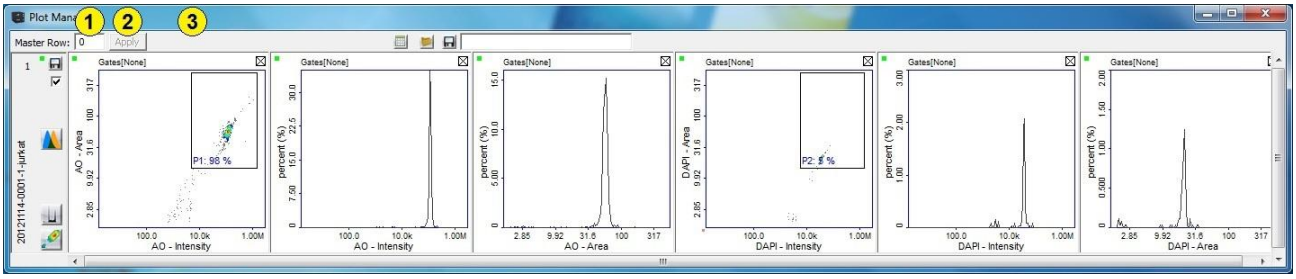


The Protocol Adaptation Wizard will guide you through adapting different parameters and saving the adapted protocol as a user adapted protocol. A saved protocol may be locked by the user to prevent further changes. A locked protocol can be unlocked again to overwrite with new user adaptations. A new 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. The Protocol Adaptation Wizard behaves in many ways like the Plot Manager, therefore can the section on the Plot Manager be read for at better understanding of details.

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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 and histograms 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

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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.

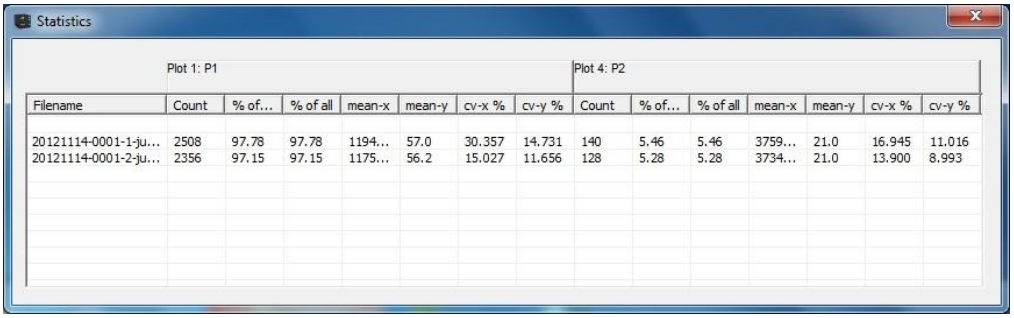
Layout Editing

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

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

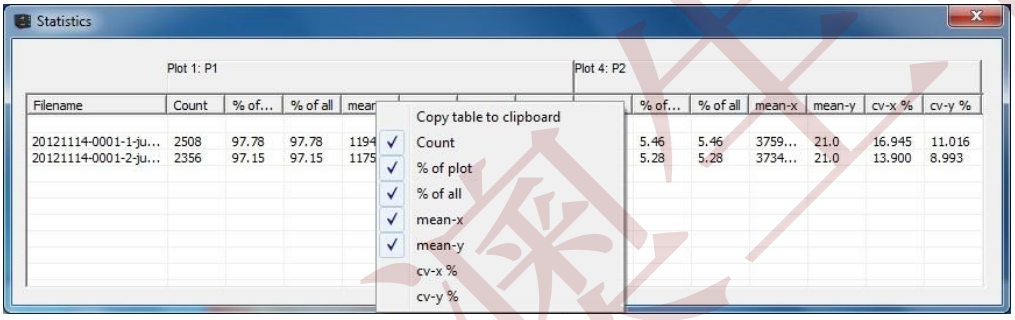
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Plot Statistics



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 to copy table contents to clipboard which can be directly pasted into a spreadsheet program.

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.

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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 histogram'* button to add a new Histogram Plot to the end of this row.


Click the '*add scatter plot'* button to add a new Scatter Plot to the end of this row.


Click on this button to open the Compensation Window.

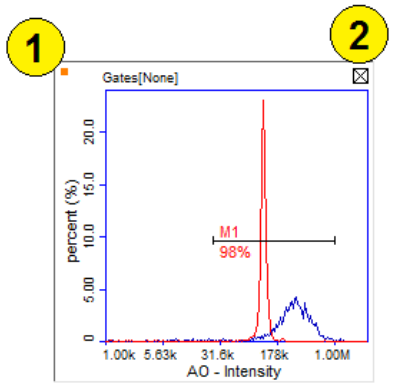
Right-click options: Right click on the row window to see the options:

- *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.

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- *Remove Row:* Removes this row from the Plot Manger, re-numbers subsequent rows and moves the subsequent rows up.
- *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. ☒ 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.

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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.

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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.

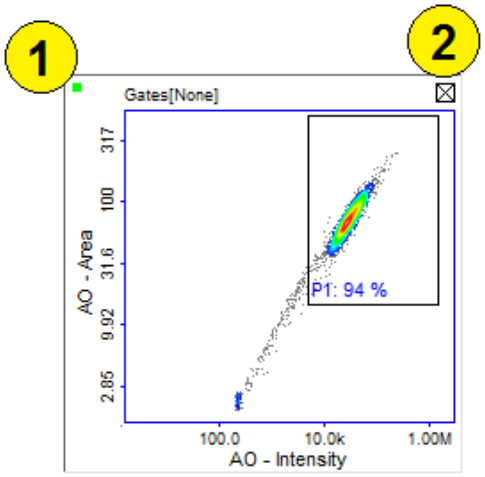
Note: Adding cells to the image overlay is cumulative when it is done from the same plot. When adding cells to the image overlay from another plot, the overlay is first cleared. Consequently if you use this to select both inside and outside cells you may assume that all cells in the image should be marked with an enclosing square. This is however, not always the case. If certain cells have been excluded from the cell

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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. ☒ 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.

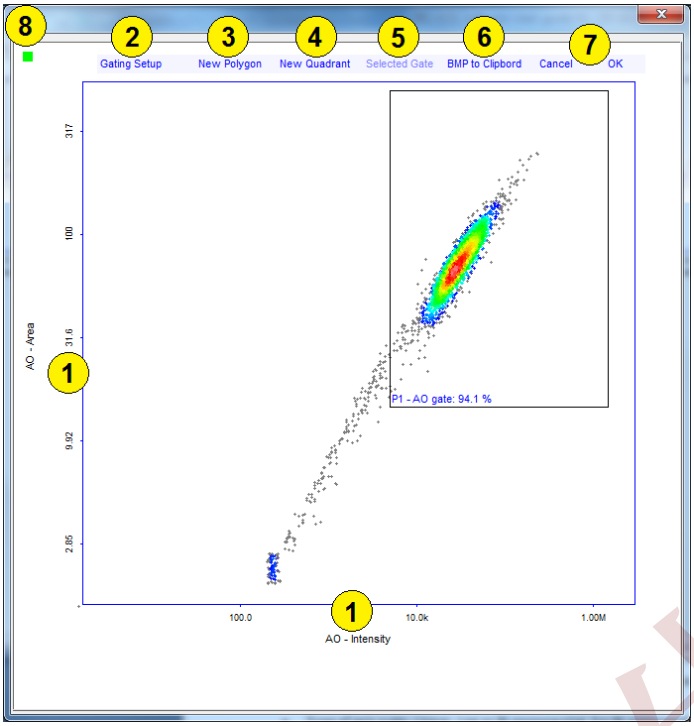
Double-click: To launch the Large Scatter plot double-click in the plot area. In the large scatter plot you can define axis parameters, polygons, quadrants and gating settings.

Right-click: Shows the context menu offering the following options:

- Show Large Plot (dbl-click)
- Copy BMP to Clipboard

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Large Scatter Plot in Plot Manager

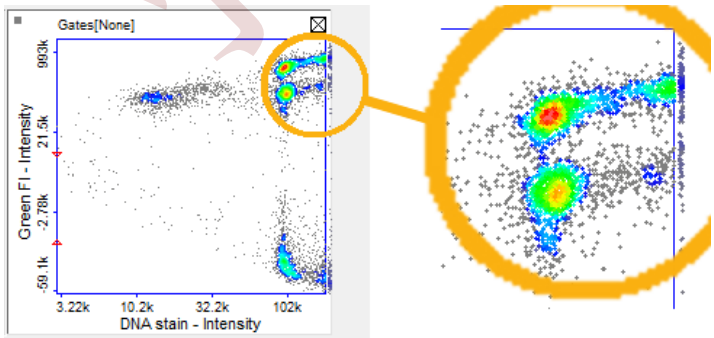


The large scatter plot is used to set axis parameters, add or edit polygons and quadrants and define gating settings.

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.

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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.

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- *Show Info About Selected Gate*: Displays a new window with information of how many cells are outside and inside the gate in each image.

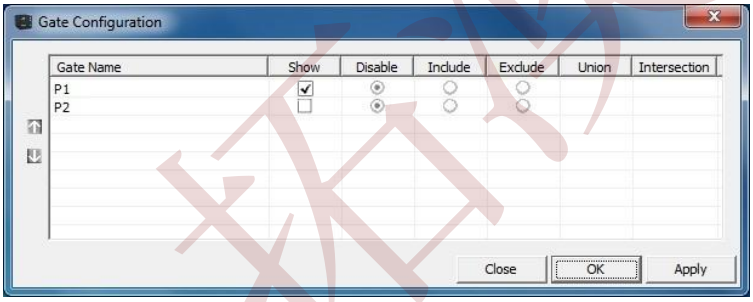
Depending on the state of the large scatter plot, some of the above options may be dimmed or be replaced with an alternative option.

Note: Adding cells to the image overlay is cumulative when it is done from the same plot. When adding cells to the image overlay from another plot, the overlay is first cleared. 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.

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.

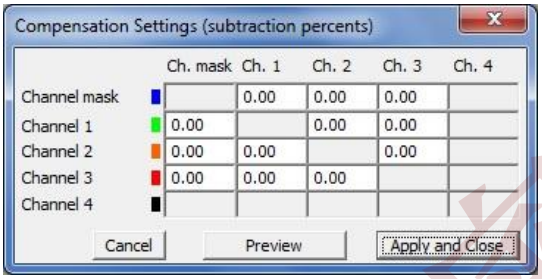
To disable the showing of a gate click to remove the checkmark in the show column.

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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).

Compensation Window



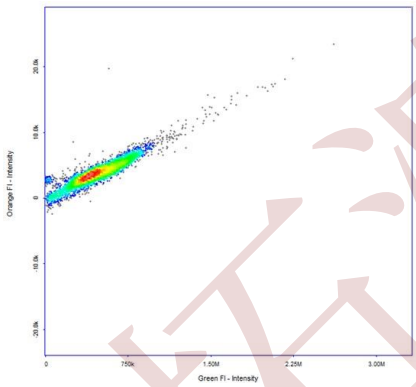
The Compensation Window is used to compensate for spectral overlap of fluorophores into neighboring channels.
For each channel it is possible to enter a subtraction percentage value to be used when compensating cell parameter values for spectral overlap.

Even though fluorophores are designated a particular color, they emit light over a range of wavelengths. The color associated with a particular fluorophore refers to the wavelength where it has maximum fluorescence, however the wavelengths where it has weaker emission may spillover into a neighboring channel giving a false increase in signal in this channel. For example, green fluorescent protein (GFP) is detected in the green channel in the NC-3000™, however the emission from GFP can be weakly detected in the orange channel. To compensate for this fluorescent spillover into neighboring channels, a compensation factor is set for each fluorophore and channel. Guidelines for approximate compensation factors to be applied for commonly used fluorophores is listed in the FlexiCyte™ application note but the exact value should be determined as described below.

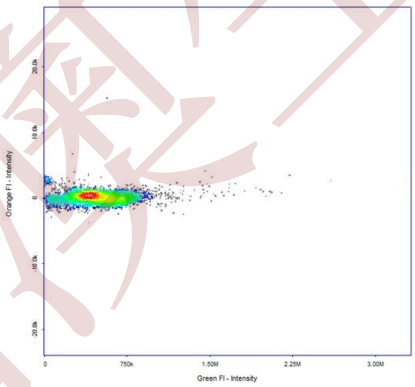
1. Load a sample labeled with a single fluorophore of interest and the blue DNA masking fluorophore i.e. DAPI or Hoechst 33342 into the NC-3000™.
2. Run the sample using the FlexiCyte™ protocol.

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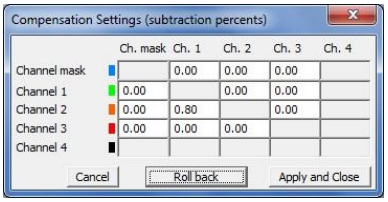
3. In Plot Manager, open a new scatter plot by clicking on the scatter plot icon on the left-hand side of the data row.
4. Double-click the small scatter plot to open the large scatter plot in editing mode. Change the x-axis to the color of the fluorophore and the y-axis to the color of the neighboring channel. The axis settings for both axes should be ‘Linear’ and 'Intensity'. Click 'OK'. *Tip: Change the y-axis range so that the cell population is centered on the y-axis.*
5. If there is spillover of fluorescence between the channels the cell population will be placed diagonally across the graph area. If there is no spillover, the cell population will be placed horizontally across the graph area.
6. Open the compensation matrix in Plot Manager by clicking on the icon on the left-hand side of the data row.
7. Enter an estimated compensation factor into the matrix box associated with the two channels of interest and click 'Preview'. Inspect the cell population to ascertain that cell population is spread horizontally across the graph area. If the compensation factor is incorrect, click 'Roll back' enter a new value and inspect the cell population again. Repeat this procedure until the correct compensation factor is ascertained and click 'Apply'.
8. Repeat steps 1-7 for each individual fluorophore.
9. If multiple samples have been analyzed, enter the row number of the sample with the corrected compensation factor into the 'Master Row' box situated in the top left-hand corner of Plot Manager and click 'Apply'. This will replicate the compensation in all other samples selected in Plot Manager.



Scatter plot of GFP expressing cells showing spillover of green fluorescence into the orange channel. An increase in orange fluorescence is seen as green fluorescence increases.



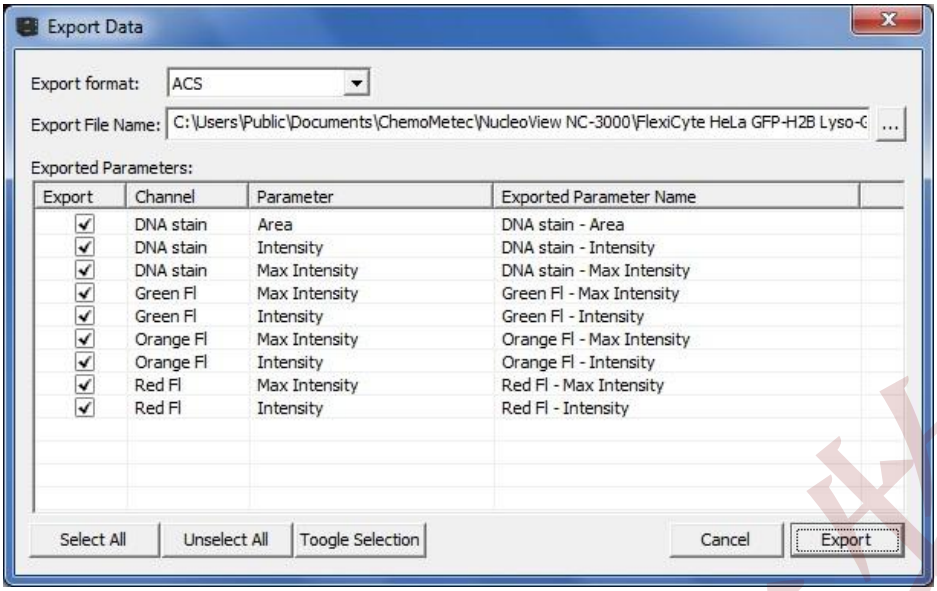
Scatter plot showing GFP fluorescence in the green and orange channel after compensation. Note that there is no increase in orange fluorescence as green fluorescence increases.



Compensation matrix adjusted for 0.8% signal spillover from the GFP into the orange channel.

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Export Data



In the menu line *File->Export Data* cell data may be exported to file formats which are compatible with third party software such as *FCS Express*.

Format Specification:
Select export format FCS for export according to ISAC 'Data File Standard for Flow Cytometry, Version FCS3.0' format.
Select export format ACS for export compatible with FCS Express 4 Image Cytometry.
OBS: To see images in FCS Express 4 Image, make sure that the *Load Images* option in under *Edit Preferences->Data Loading->ICE format* in FCS Express 4 Image is checked.

Parameter Specification:
The number of exportable parameters depends on the protocol used to create the *.cm image file.

Multiple image files acquired with the same protocol can be batch exported by opening the File Browser, selecting the desired files by holding Ctrl or Shift down and clicking the files, right clicking and selecting 'Batch Export Data'.

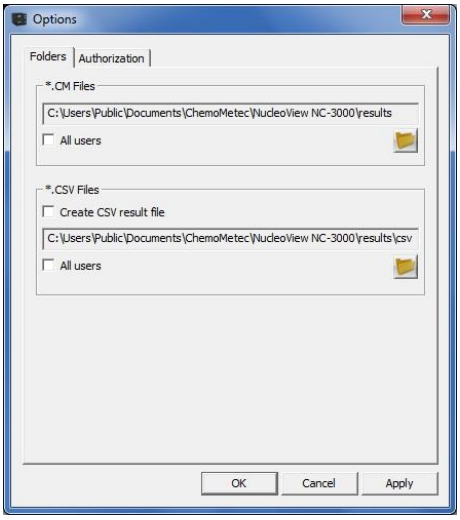
Bitmap Export

Before exporting a bitmap for presentation or printing, increasing the contrast via the Image Scaling window often yields better visualization.

Right-click on the image in the Main Window and select *copy to clipboard (bmp image, all channels)*. From here it can be pasted into other applications or documents.

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Options



The options dialog has the following tab pages:

- *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 completed 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.

- *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 enable under this tab and do not require activation of 21 CFR part 11 mode. See the 21 CFR part 11 section for more details.

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21 CFR Part 11

The U.S. Food and Drug Administration has defined regulations for electronic records and electronics signatures in Part 11 of Code of Federal Regulations Title 21, in short here termed 21 CFR Part 11.

The NucleoView NC-3000™ application can be set into a restricted mode, so the user via the NucleoView NC-3000™ 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 NC-3000™ application.
Using the 21 CFR Part 11 requires a specific license.

Requirements on the Microsoft Windows Computer System

It is not within the scope of this document to describe in details how to make the computer system, on which the NucleoView NC-3000™ application is running, 21 CFR Part 11 compatible. Normally this can be done by installing and configuring third party software on the computer system.

Apart from installing and configuring a third party software application on the computer, and activating the 21 CFR Part 11 mode in the NucleoView NC-3000™ application, the following setup is needed by the NucleoView NC-3000™ application itself:

1. Three types of groups need to be defined (either locally on the computer or in the domain the user logs on to). The groups must be named *NucleoViewAdmin*, *NucleoViewSuperVisor* and *NucleoViewUser*. Any user logging on to the computer system and using the NucleoView NC-3000™ application must be part of one of those groups.
2. All users using the NucleoView NC-3000™ application also need the security policy *Impersonate a client after* authentication enabled. It is recommended to enable the Impersonate a client after authentication for the three groups NucleoViewAdmin, NucleoViewSuperVisor and NucleoViewUser.
3. A default printer must be defined for each user on the computer as the user can not select nor change printer destination. And this default printer must not print to a file; it must be a real printer.

Ask the local IT department for how to setup the above three listed items.

NucleoView™ User Groups

Any user logged onto the computer and starting the NucleoView NC-3000™ application must be member of one of the groups NucleoViewAdmin, NucleoViewSuperVisor and NucleoViewUser.
The NucleoViewAdmin users have full rights in the NucleoView NC-3000™ application, e.g. the user may switch 21 CFR Part 11 mode on or off, thus breaking the 21 CFR Part 11 compliance.

The NucleoViewSuperVisor and NucleoViewUser users have the same general rights in the NucleoView NC-

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3000™ application, and are restricted in their use of the application. Running the NucleoView NC-3000™ application as one of those users makes it impossible to break the 21 CFR Part 11 compliance.

Approval

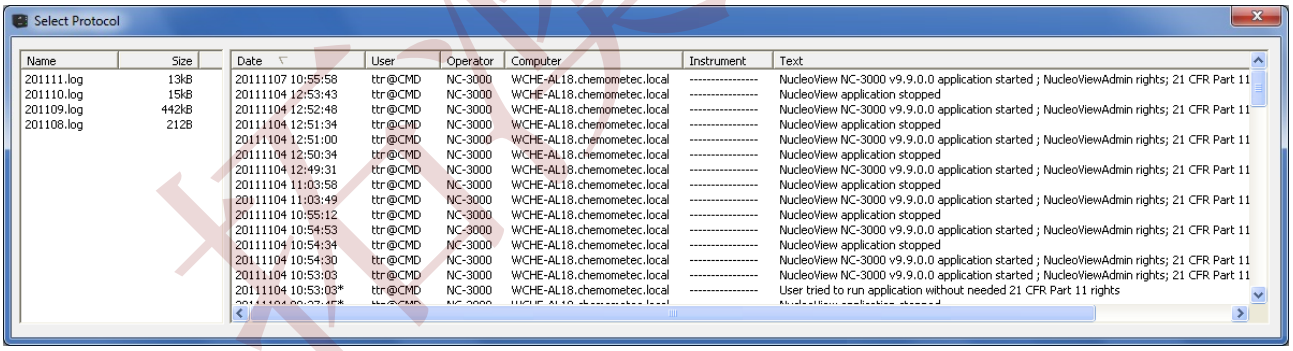
All results can be approved by a user in a user group that is allowed to approve data.
The group right to approve data can be setup in the Tools->Options menu in the tab 21 CFR Part 11. Also it is possible to setup whether a user should be allowed to self approve.

Event Log

The event logs created by the NucleoView NC-3000™ application are always stored in the current results folder. Changing this folder will create a new event log file in the newly set results folder.
It is recommended to backup the event logs, archived and aged according to the same rules as e.g. the results created by the NucleoView NC-3000™ application itself. See Maintenance and Backup for details.

Apart from securing that the user cannot tamper with measured results in the NucleoView NC-3000™ application in 21 CFR Part 11 mode, all operations changing the behavior of the NucleoView NC-3000™ application, all new files created etc. are logged in the event log system. The user needs to be part of the NucleoViewAdmin group to see the event log.

The NucleoViewAdmin user can launch the event log viewer via the main menu *View -> Event Logs*. This will bring up the window shown below:



To the left is shown a list of existing event log files, one for each month. Selecting one of these will bring up the content of this event log file to be shown in the right window. Clicking on one of the headers in the right window will sort the content according to the selected column either ascending or descending.

Note that in the date column, some of the entries have a small * marked. This indicates that the NucleoView NC-3000™ application has recorded this event happened when the application was not in 21 CFR Part 11 mode.

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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-3000™ 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-3000™ instrument (identified by serial number). A license may have an expiry date.

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Maintenance and Backup

NucleoView™ Software Maintenance

A two-channel image taken in an A8 slide with NucleoView NC-3000™ is approximately 15 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-3000™ 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>.log* like *201205.log* indicating a log file from May 2012.

The NucleoView NC-3000™ 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-3000™ system.

The NucleoCounter® NC-3000™ 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.
The outer surfaces of the NucleoCounter® NC-3000™ instrument may also be cleaned with 96-100% pure ethanol if needed.

It is good practice to insert the sample tray, whenever the instrument is not being used, to prevent dust build up on the internal surfaces.

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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-3000™ 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.

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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.*

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Trouble Shooting

Installation The NucleoView NC-3000™ software or the NucleoCounter® NC-3000™ instrument is not installed correctly.	The user must be logged on with administrator rights during installation. When connecting the NucleoCounter® NC-3000™ instrument to a previously unused USB port, the NucleoView NC-3000™ 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.
NucleoView NC-3000™ Issues (Software)	
Protocol file is invalid	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.
Connection error	The NucleoCounter® NC-3000™ instrument icon in the upper left corner of the main window stays red after NucleoView NC-3000™ has been launched, i.e. the NucleoView NC-3000™ 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.
Cassette	
No cassette is found	There is no cassette in the sample tray. Place a cassette which has been correctly loaded into the sample tray.
Non-valid cassette	A used cassette has been placed in the sample tray. Remove the used cassette and replace with a new correctly loaded cassette.
Flow detection error	The flow has failed to reach the flow detection point within given time limits. Too little sample volume or bubbles have been loaded into the cassette. Replace cassette with a new, correctly loaded cassette.

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NucleoCounter® instrument	
The sample tray is ejected and the instrument is on standby	The connection between the NucleoCounter® NC-3000™ and NucleoView NC-3000™ software has been terminated in an inappropriate way while the tray was ejected. Restart NucleoView NC-3000™ and the tray is automatically inserted.
The instrument gives abnormal sounds	An actuator motor might be blocked. Inspect that the sample tray movement has not been blocked by any external objects, then check that there is no internal blocking, e.g. by a wrongly located cassette. Remove any blocking devices and restart the NucleoView NC-3000™ software. If issues persist contact ChemoMetec A/S or local representative.

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).

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