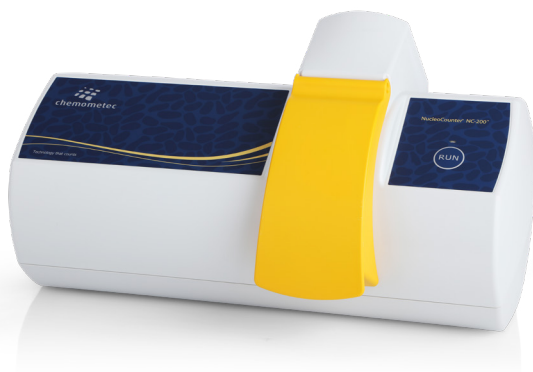


Technology that counts



Quick Guide

to the easiest cell count ever
using Reagent A100 + Reagent B



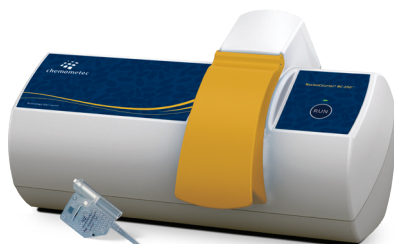
NucleoCounter® NC-200™

How to get started

Dear NucleoCounter® Customer,

Thank you for purchasing the NucleoCounter® NC-200™ which offers the easiest one-step viability and cell count ever.

The NucleoCounter® NC-200™ Concept



- ✓ One step viability and cell count
- ✓ No pre-treatment
- ✓ Fast and easy operation
- ✓ Safe sample handling and disposal
- ✓ Excellent reproducibility
- ✓ User adaptable counting protocols
- ✓ 21 CFR Part 11 ready



NucleoView™ Software
Included on a USB stick for an unlimited number of installations.



Via1-Cassettes™
For one-step viability and cell count. Contains DAPI and Acridine Orange.



OPTIONAL **Reagent A100 + Reagent B**
Can be used for disaggregation of aggregating cells.



OPTIONAL **NC-200™ laptop stand**
Can be used to minimize footprint and facilitates even easier operation.

How to get started - eight easy steps to install the NucleoCounter® NC-200™

- 1** Unpack the NucleoCounter® NC-200™ instrument and plug it in the mains outlet. Do NOT connect the USB cable to the PC.
- 2** Make sure that there are full administrator rights on the PC during the installation of the NucleoView™ NC-200™ software.
- 3** Insert the USB stick in the PC and open the "Install_Guide.html" file for detailed installation instructions.
- 4** Open the "Install NucleoView NC-200 X.X.X.X.exe" file (the Xs indicate the version number e.g. 1.4.3.0). ⚠ Do NOT open the .bin file.
- 5** Follow the instructions on the screen. After the software installation it will be required to restart the PC.
- 6** Open the NucleoView™ NC-200™ software by double clicking on the NucleoCounter® NC-200™ icon on the desktop to continue the installation of the instrument.
- 7** Follow the on-screen instructions to complete the installation of the instrument.
- 8** The NucleoCounter® NC-200™ is ready to use when the LED indicator light on the instrument turns green.

For detailed instructions, please read the User Guide.

NucleoCounter® NC-200™

Service Plan

ChemoMetec is dedicated to keeping your instruments and software qualified, validated and up to date. With our Service Plan, have peace of mind knowing that every year you will receive outstanding, direct support for your instrument.

Servicing your NucleoCounter® NC-200™ instrument regularly will help ensure that you are staying GMP compliant. For more information, please contact us.

For GMP Compliance & Priority Support

- Priority access to technical and scientific specialists.
- Enhanced warranty covers all defects/breakdowns and includes free repairs.
- On-site service check with service kit including visits and instrument validation from our Field Application Scientists/service-technicians.

Service Plan features

Priority access to specialists

Our experienced Field Application Scientists (FAS) are first line support, and will address your case as a top priority. Our FAS are certified and expertly trained to identify, isolate, and resolve even the most challenging issues. You have direct access to our FAS during office hours. After hours, you can reach out via service@chemometec.com to resolve any issues and your local FAS will respond in a timely manner.

Enhanced warranty including free repairs

This warranty covers defects or breakdowns caused by wrong installation, handling and storage, unintended use, lack of reasonable maintenance or operation by the Buyer, Buyer's agent or representative. This warranty also covers all costs for transportation of instruments to and from our facilities for repair.

On-site service check with service kit

Our FAS will perform instrument maintenance and IQ/OQ/PQ testing on-site approximately 9-11 months after installation and every 12 months thereafter. ChemoMetec will contact the customer at least one month before the planned service check to organize the visit. We provide test kits for the on-site service check.

How to Perform Total Cell Count and Viability of Heavily Aggregated Cells

- 1 Select the protocol from the menu 'Viability and Cell Count - A100 and B Assay'.
- 2 **Optional:** Enter Sample ID and Operator Name.
- 3 **Retain an aliquot of at least 200 µl of the original cell suspension for analysis in step 5.** Add equal volumes of **Reagent A100** and your cell sample to a microcentrifuge tube and mix. For example, to 100 µl of cell suspension add 100 µl of **Reagent A100**. Afterwards add one volume of **Reagent B** to the mixture of cell suspension and **Reagent A100**. To 200 µl of the mixture of cell suspension and **Reagent A100** add 100 µl of **Reagent B** and mix by pipetting i.e. Cell Sample: **Reagent A100: B**, should be mixed in a ratio of 1:1:1.
- 4 Immediately load your sample into a Via1-Cassette™ and press 'RUN'.
- 5 After analysis, follow the on-screen instructions and load a sample of your cells NOT treated with **Reagent A100** and **B** into a new Via1-Cassette™ and press 'RUN'.

! Compensation for the sample dilution with **Reagent A100** and **B** is automatically performed by the software. However, if the proportions are different from those above, please add the correct volumes in the input fields under the image window prior to running your assay. The entered volumes are used for calculating the cell concentration in the original cell sample.

ChemoMetec NucleoView NC-200

File Tools View Help

Image Result

chemometec

NucleoView® NC-200™

Data folder and files

Users\Public\Documents\ChemoMetec\NucleoView NC-200\results

20140509-0001-c-Viability and Cell Count Assay - Jurkat.cm
 20140509-0002-c-Viability and Cell Count Assay - MCF7.cm
 20140509-0003-c-Viability and Cell Count - Blood Assay.cm
 20140509-0004-c-Viability and Cell Count - Aggregated.cm
 20140509-0005-c-Count of Aggregated Cells - MCF7.cm

Protocol

Via1-Cassette

Sample ID

Viability and Cell Count Assay - Jurkat

Operator (Login ID)

user

RUN

Total cells/ml

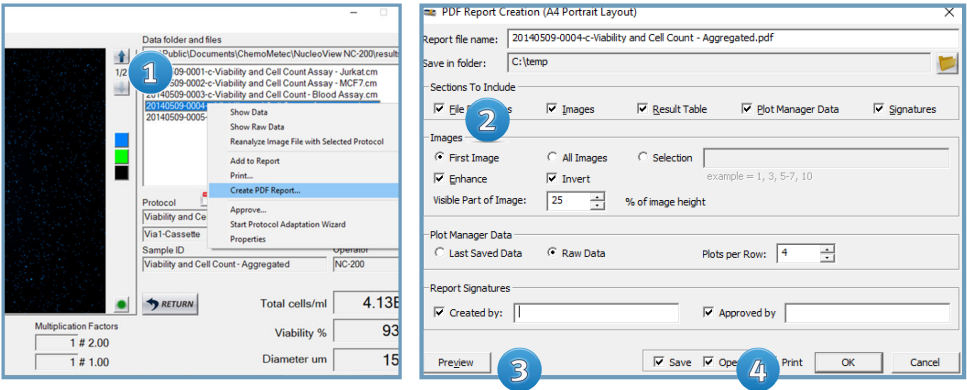
Viability %

Diameter um

	Sample [ul]	Dilution [ul]	B [ul]	Multiplication Factors
1: Total	100	0	100	1 # 3.00
2: Dead	200	0		1 # 1.00

PDF reports

1 Right-click on file to create a PDF report.

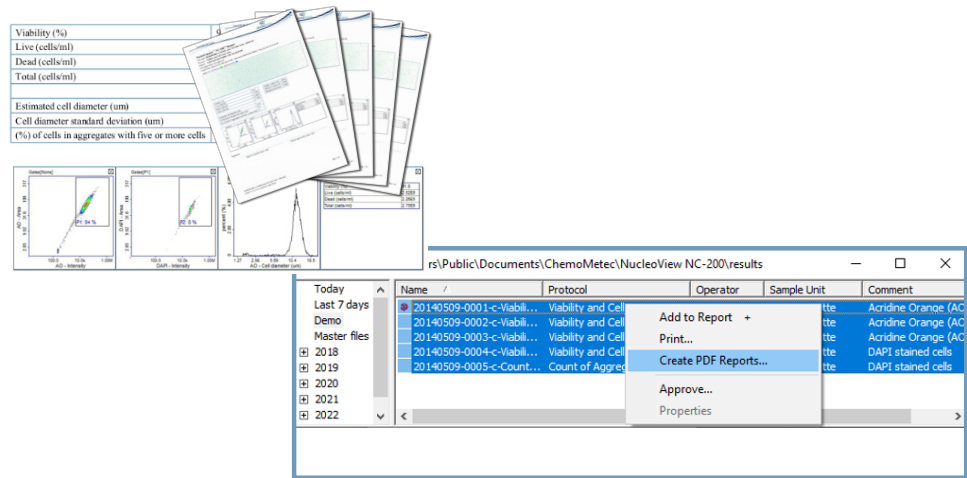


2 Select the parameters that will be visible on the report, and the properties of the parameters.

3 Optional: Preview your result.

4 Save and/or print your report to the default printer.

Tip: Batch exports can be done from the NucleoView™ File Browser.

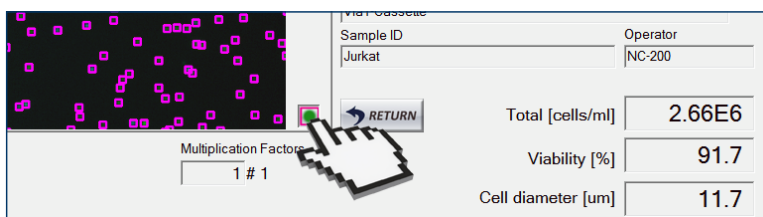


Optional: Visual Inspection of Counting Gates

Option One (Preferred)

Click the green dot in the right-hand corner of the image window.

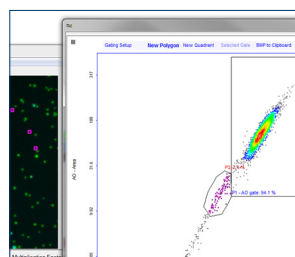
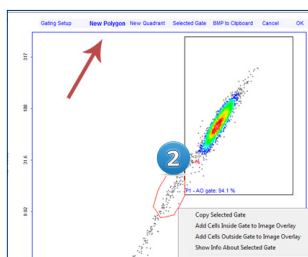
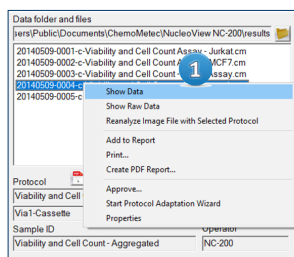
This activates the image overlay function indicating all the events in the total cell count. The mouse scroll button allows the user to zoom in at the cursor position. Cells will be framed by a pink square.



Option Two (Advanced)

- 1 Right-click on the sample file name in the 'Data folders and files' window and select 'Show Raw Data'. A new window will open displaying scatter plots and histograms of event intensity and area for the appropriate channels (Acridine Orange and DAPI). It is important that the center of the population, most usually seen as a colored region on the scatter plot, is included in the counting gate.
- 2 To check distinct cell populations, create a new polygon around the particular cell population.
- 3 Right-click inside of the newly formed polygon and select 'Add Cells Inside Gate to Image Overlay'. This activates the image overlay function indicating all the events visually to determine the validity of their inclusion or exclusion from the final counting result.

Delete the polygon and the image overlay by right-clicking and selecting 'Delete Image Overlay'.



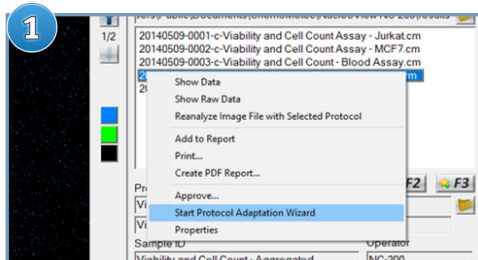
Only if required:

Create your own protocol with adjusted counting gates

- 1 Perform the desired type of assay on a sample of the cells to be analyzed.
In the Tools menu select **Protocol Adaptation Wizard** or right-click on the desired file and select 'Start Protocol Adaption Wizard'.
- 2 Follow the instructions in the **Protocol Adaptation Wizard** to create your own adapted protocol.

⚠ The viability and cell count results will not be adjusted for the image file used for the adaption of counting gates. New results can only be obtained by running a new sample with the new gating protocol.

1

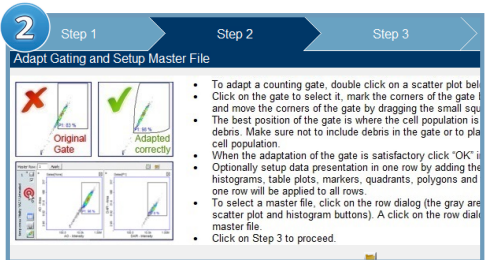
A screenshot of a software interface showing a list of files on the left and a menu on the right. The menu has options like 'Show Data', 'Show Raw Data', 'Reanalyze Image File with Selected Protocol', 'Add to Report', 'Print...', 'Create PDF Report...', 'Approve...', 'Start Protocol Adaptation Wizard' (highlighted in blue), 'Properties', and 'Operator'. The file list includes '20140509-0001-c-Viability and Cell Count Assay - Jurkat.cm', '20140509-0002-c-Viability and Cell Count Assay - MCF7.cm', and '20140509-0003-c-Viability and Cell Count - Blood Assay.cm'.

2

Step 1

Step 2

Step 3

A screenshot of a dialog box titled 'Adapt Gating and Setup Master File'. It has three tabs: 'Step 1', 'Step 2', and 'Step 3'. The 'Step 1' tab is active. It shows two scatter plots side-by-side. The left plot is labeled 'Original Gate' and has a red 'X' over it. The right plot is labeled 'Adapted correctly' and has a green checkmark over it. Below the plots, there are instructions: 'To adapt a counting gate, double click on a scatter plot below and move the corners of the gate by dragging the small squares. The best position of the gate is where the cell population is debris. Make sure not to include debris in the gate or to place cell population.' Below this, there are more instructions: 'When the adaptation of the gate is satisfactory click "OK" if optionally setup data presentation in one row by adding the histograms, table plots, markers, quadrants, polygons and one row will be applied to all rows.' Below that, there are instructions: 'To select a master file, click on the row dialog (the gray are scatter plot and histogram buttons). A click on the row dialog master file.' Below that, there are instructions: 'Click on Step 3 to proceed.'

Additional Resources

Go to www.chemometec.com to find:

- Documentation
- Safety Data Sheet
- Application notes
- Certificates of analysis
- Videos etc.

Consumables:

Item no.	Description
941-0012	Via1-Cassettes™, 1 box (100 pcs)
910-3010	Solution 10
910-3017	Solution 17
910-0003	Reagent A100 - Lysis Buffer, 500 ml
910-0002	Reagent B - Stabilizing Buffer, 500 ml
929-0012	Laptop Stand II for NC-200™ series



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