

## Application note No. 201. Rev. 1.3

# NucleoCounter® NC-200™

## Mammalian Cells - Viability and Cell Count using the Via1-Cassette™

### Product description

The NucleoCounter® NC-200™ system enables the user to perform automated cell counting and analyses of a broad range of mammalian cells.

### Application

The Via1-Cassette™ used together with the NucleoCounter® NC-200™ facilitates determination of viability and concentration of cell suspensions by measuring cell counts (total and non-viable) per volume in one simple step. Red blood cells and/or hemoglobin present in cell samples quench fluorescence light resulting in reduced cell counts. Contact ChemoMetec for more details if such samples are to be analyzed.

### Introduction

In order to determine viability and cell concentration, a sample containing cells in suspension is drawn into the Via1-Cassette™. The inside of the Via1-Cassette™ is coated with two different dyes, staining the entire population of cells and the non-viable cells, respectively. The volume of each Via1-Cassette™ has been calibrated to give a high precision of the resulting count.

The Via1-Cassette™ is placed in the NucleoCounter® NC-200™ where cell concentration and viability are determined.

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### Procedures

If the cell line to be investigated is adherent or semi-adherent, then start by getting all cells into suspension using the preferred method of your laboratory (e.g. trypsin/EDTA treatment). Although NucleoCounter® NC-200™ is able to count aggregated cells the accuracy is higher for single cell suspensions. If higher precision in cell count is needed when analysing a cell line with **aggregation** we recommend using the counting application for aggregating cells.

### Materials needed

- Cells to be counted
  - Via1-Cassette™
1. The cell suspension is mixed to obtain a homogenous suspension. Load a cell sample by inserting the tip of the Via1-Cassette™ into the cell suspension and pressing the piston.
  2. Immediately place the loaded Via1-Cassette™ in the NucleoCounter® NC-200™, select the “**Viability and Cell Count Assay**” and press RUN.

After approximately 1 minute the viability (in percent) and the concentrations (cells/ml) of the total cell count are displayed in the user interface together with the cell diameter. Moreover, in the result tab page the count of viable and non-viable cells and depending on the used method an approximation of the cell diameter (in  $\mu\text{m}$ ), provided together with information about cell aggregation (“clumpiness”).

### Note

To assure reliable results, it is recommended that the cell concentration of the counted cell suspension should be in the range of  $5 \cdot 10^4$  cells/ml to  $5 \cdot 10^6$  cells/ml. If the cell concentration is above  $5 \cdot 10^6$  cells/ml, the cell suspension can be diluted with growth media or PBS to achieve the desired concentration. The diluted cell sample is then treated as described above. By inserting the value for the dilution volume in the dilution field in the user interface the returned cell concentration is for the original cell sample.

If the concentration of cells is below  $5 \cdot 10^4$  cells/ml then the cell concentration may be increased by centrifugation followed by resuspension of the pellet using growth media or PBS. The resuspended cell sample is then treated as

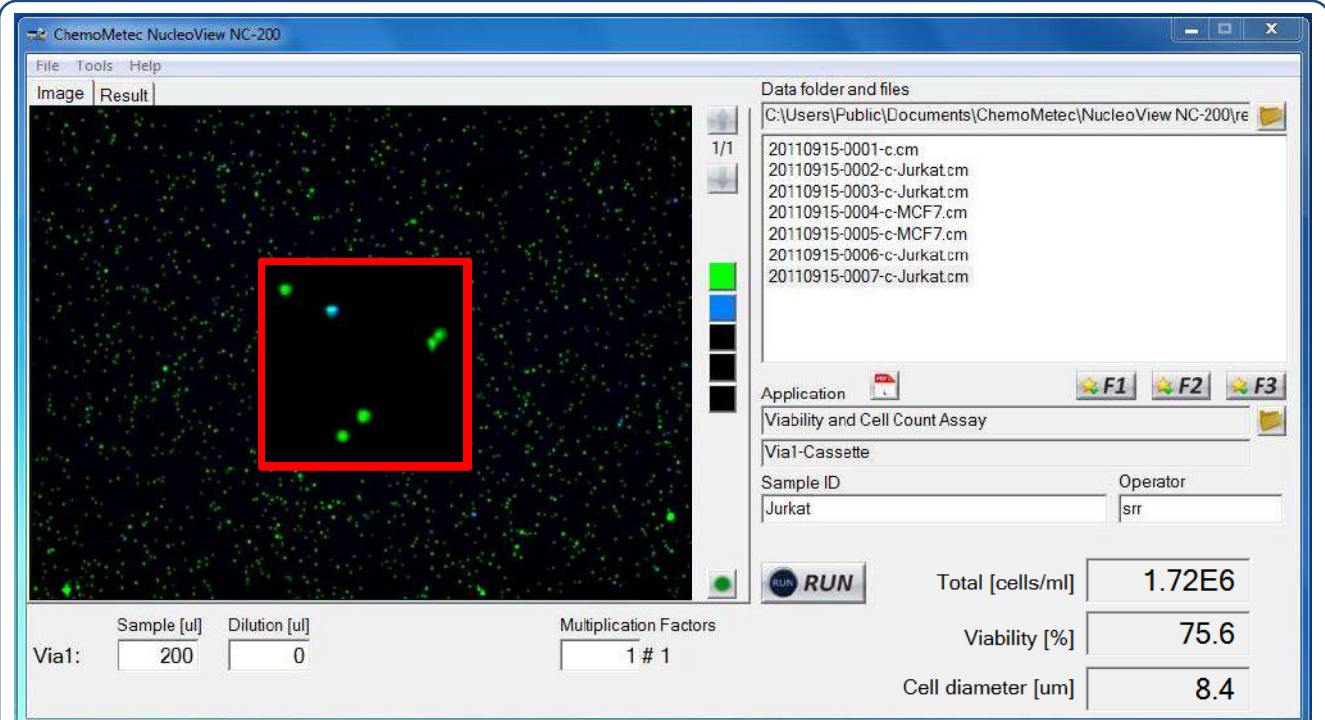
described in the procedure. By inserting a negative value representing the volume removed from the sample in the dilution field in the user interface the returned cell concentration is for the original cell sample.

## Viability

The viability is calculated as follows:

$$\% \text{ viability} = \frac{C_t - C_{nv}}{C_t} \cdot 100\%$$

<b>% viability</b>	The percentage of viable cells in the cell suspension
<b>C<sub>t</sub></b>	The total concentration of cells
<b>C<sub>nv</sub></b>	The concentration of non-viable cells



The screenshot shows the ChemoMetec NucleoView NC-200 software interface. The main window displays a fluorescence image of Jurkat cells, with a red box highlighting a region. The interface includes a menu bar (File, Tools, Help), a toolbar (Image, Result), and a data folder list. The application is 'Viability and Cell Count Assay' using a 'Via1-Cassette'. The sample ID is 'Jurkat' and the operator is 'srr'. The results displayed are: Total [cells/ml] = 1.72E6, Viability [%] = 75.6, and Cell diameter [um] = 8.4. Input fields for Sample [ul] (200), Dilution [ul] (0), and Multiplication Factors (1 # 1) are also visible.

Determination of count and viability of Jurkat cells. The cells were harvested, loaded into a Via1-Cassette™ and analyzed using the Viability and Cell Count Assay. The image acquired is shown (left). The total cell population is stained with acridine orange and appears green while non-viable cells are stained with DAPI that appear blue. An insert shows a close up of a part of the image. The results are presented at the bottom right and extended results are presented in the result tab page.

## Troubleshooting

### Inaccurate and imprecise counting:

When setting up a new cell line it is important to inspect that the cell line is counted correctly. The cells included in the total count can be marked by clicking on the overlay button in the bottom right corner of the image. Visual inspect the image to evaluate in the vast majority of the cells has been counted correctly. If this is not the case right click on the image file in question and choose "Show Counting Gates in Plot Manager". Inspect the gates displayed in the Plot Manager. If the gating is inappropriate adapt the gate(s) to cover the cell population (do not include debris and very large objects) using the Protocol Adaptation Wizard and save the changes to a new protocol. Note that the user is responsible for defining appropriate gating of the particular cell line.

### Unexpectedly high viability:

Cells that have been dead for a long period of time contain degraded nucleic acid. The dyes used in this assay primarily binds to nucleic acid and consequently the fluorescent signals from dead cells may be exceptionally dim. Very dim signals can be observed in cells that, for example, have been growing for a long period of time in a bioreactor and in some suspension cell lines. Dim signals can be detected by enabling method 2 in the Protocol Adaptation Wizard. Note that method 2 increases the possibility of counting foreign objects and bubbles as cells. Furthermore, be aware that when cells die they will at some point break up to smaller parts and that these cell remnants can be counted at multiple cells. The procedure used in method 2 does not allow for analysis of the cell area, diameter and the aggregation of cells.

### **Handling and storage**

For handling and storage of ChemoMetec instruments and reagents, cassettes and NC-Slides refer to the corresponding product documentation. For other reagents refer to the material data sheet from the manufacturer of the reagents and chemicals.

### **Warnings and precautions**

For safe handling and disposal of the ChemoMetec reagents, cassettes and NC-slides refer to the corresponding product documentation and the NucleoCounter® NC-200™ user's guide. For other reagents refer to the safety data sheet from the manufacturer of the reagents and chemicals required for this protocol. Wear suitable eye protection and protective clothes and gloves when handling biologically active materials.

### **Limitations**

The NucleoCounter® NC-200™ system is FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE. The results presented by the NucleoCounter® NC-200™ system depend on correct use of the reagents, Cassettes and the NucleoCounter® NC-200™ instrument and might depend on the type of cells being analyzed. Refer to the NucleoCounter® NC-200™ user's guide for instructions and limitations.

### **Liability disclaimer**

This application note is for RESEARCH PURPOSES ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual experienced in handling potentially hazardous chemicals. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. ChemoMetec A/S shall not be held liable for any damage resulting from handling or contact with the above product.

### **Product disclaimer**

ChemoMetec A/S reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

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