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A comparison of DNA quantitation by image and flow cytometry

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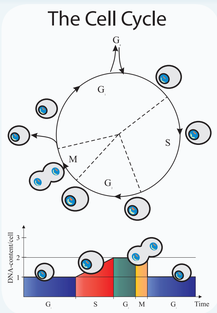
Abstract

The most common approach for determining the cell cycle stage is based on quantification of cellular DNA content. DNA content can be determined using fluorescent DNA-selective stains that exhibit emission signals proportional to DNA mass. DNA staining is typically performed on cells permeabilzed with either non-ionic detegents or alcohol fixation. Traditionally, flow cytometry has been the method of choice for analysing cell cycle distribution and, currently, stands as the gold standard.

We have employed an image based system, NucleoCounter NC-3000, for quantifying DNA content of different mammalian cell lines stained with DAPI. NC-3000 demonstrated accurate and precise determination of cell cycle stages compared to flow cytometric analyses (BD LSRII).

Introduction

The cell cycle represents the most fundamental and important process in eukarotic cells. Being an ordered set of events, culminating in cell growth and division into two daughter cells, the cell cycle is tightly regulated by defined temporal and spatial expression, localization and destruction of several cell cycle regulators. Cyclins and cyclin-dependent kinases (CDK) are major control switches for the cell cycle, causing the cell to move from G_1 to S or from G_2 to M phases. In a given population, cells will be distributed among three major phases of cell cycle: G_1 /G_0 phase (one set of paired chromosomes per cell), S phase (DNA synthesis with variable amount of DNA), and G_2/M phase (two sets of paired chromosomes per cell, prior to cell division).
There are a number of ways to study cell cycle progression and regulation. Here, we present a comparison study between image and flow cytometry using cells permeabilized by either alcohol fixation or acid lysis.



Methods

Cell Preparation:
Cell lines: U2OS (human osteosarcoma cell line, ECACC #92022711), CHO (Chinese hamster ovary cell line, ECACC #85050302) and Jurkat (human leukemia T cell line, ATCC #CRL-2570) cells.
Suspension cell line (Jurkat) was grown to a density of 5x10^5 cells/ml in RPMI + 6% FCS. The cell sample was divided into 6 T-flasks and half of the flasks were supplemented with 10 µM camptothecin (CPT). After 16 hours of incubation the CPT-treated and untreated cells were harvested and analysed for DNA content. Each of the samples were analysed in duplicates. Adherent cell lines (U2OS and CHO) were grown to 90% confluency in RPMI + 6% FCS. Cells were harvested by trypsination and the samples were divided into 6 T-flasks. The T-flasks were incubated approximately 24 hours and at 75% confluency half of the T-flasks (3 for each cell line) were supplemented with 10 µM camptothecin (CPT). After further 16 hours of incubation CPT-treated and untreated cells were harvested and analysed for DNA content. Each of the samples were analysed in duplicates.

Cell Staining:
Cells were permeabilized and DAPI stained using two different methods. In method 1 (Fixed Cell Cycle Assay), cells are permeabilized by alcohol fixation and after washing with PBS cells are stained with DAPI. In method 2 (2-Step Cell Cycle Assay), cells are permeabilized by acid lysis and DAPI stained without any prior washing steps. For more details about the two cell cycle assays please refer to www.chemometec.com.

Instrumentation:
Parallel cell cycle analyses were performed on identical samples by traditional flow cytometry and image cytometry. Standard flow cytometry was performed using a BD LSRII cytometer (BD Biosciences) configured with a 405 nm laser. 10.000 single cells were acquired collecting the DAPI blue fluorescence in the pacific blue channel. Image cytometry was performed using a NucleoCounter NC-3000 (Chemometec). 10.000 single cells were acquired using either the “Fixed Cell Cycle Assay” or the “2-Step Cell Cycle Assay”. For more details please refer to www.chemometec.com

Data analysis:
To facilitate a direct comparison data obtained with the two different cytometers were exported in FCS format and analyzed using FlowJo (Treestar). DNA content and cell cycle distribution for 10.000 single cells was automatically quantified and analyzed using the cell cycle-modelling algorithm Watson Pragmatic , version 7.6.5. Each data point in the columns shown below represents the average of 6 samples (three independent samples analysed in duplicate).

Results

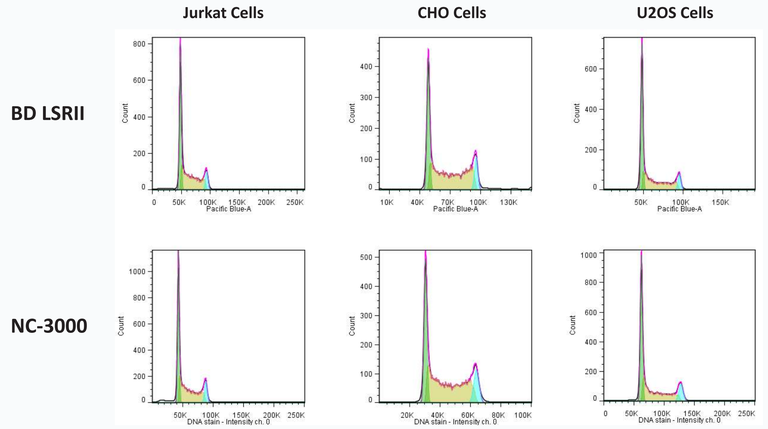


Fig. 1. Representative examples of DNA histograms otained from BD LSRII and NC-3000
Exponentially growing Jurkat, CHO and U2OS cells were permeabilized by alcohol fixation, stained with DAPI and analyzed for DNA content by flow cytometry (upper row) and image cytometry (lower row). Acquired data were analyzed as described in the Methods section. Green, yellow and cyan area of histograms represent, respectively, G_1, S and G_2/M cells.

For all three cell lines the DNA content histograms obtained with the image cytometer resemble those acquired with the flow cytometer. In all cases the histograms display narrow G_1 peaks with low coefficient of variance (CV).

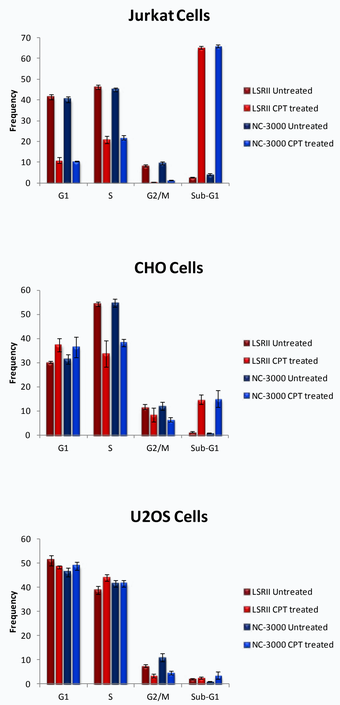


Fig. 2. Comparison of image cytometry cell cycle analysis and flow cytometric analysis using alcohol fixed cells. Concordance between NC-3000 and BD LSRII on three different mammalian cell lines
Exponentially growing cells (untreated) and camptothecin treated cells (CPT treated) were permeabilized by alcohol fixation, stained with DAPI and analyzed for DNA content by flow cytometry (reddish columns) and image cytometry (bluish columns). Acquired data were analyzed as described in the Methods section. Columns depict the percentage of the population determined to occupy each stage of the cell cycle. Sub-G_1 represents cells with less than 2C DNA content. Each column represents the mean of 6 samples (three independent samples analyzed in duplicate). Standard deviation is indicated by an error bar.

For all three cell lines there is a high degree of concordance between cell cycle distributions measured by flow cytometry and image cytometry. In general, the accuracy and precision of the NC-3000 is comparable with BD LSRII. Thus, in the untreated Jurkat samples the fraction of G_1 cells is measured to 41.5±1.2% by BD LSRII and 40.3±1.2% by NC-3000. Furthermore, in the CTP treated Jurkat samples the fraction of G_1 cells is quantified to 10.8±1.4% by BD LSRII and 10.4±0.2% by NC-3000. Good concordance is also observed for the other two investigated cell types.

The data demonstrate that NC-3000 allows for accurate and quantitative investigations of subtle alterations in the cell cycle

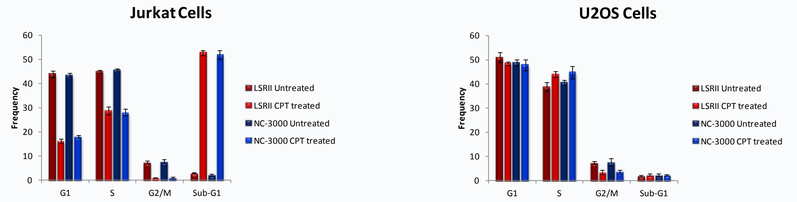


Fig. 3. Comparison of image cytometry cell cycle analysis and flow cytometric analysis using acid lysed cells. Concordance between NC-3000 and BD LSRII on Jurkat and U2OS cells
Exponentially growing cells (untreated) and camptothecin treated cells (CPT treated) were permeabilized by acid lysis, stained with DAPI and analyzed for DNA content by flow cytometry (reddish columns) and image cytometry (bluish columns). Acquired data were analyzed as described in the Methods section. Columns depict the percentage of the population determined to occupy each stage of the cell cycle. Each column represents the mean of 6 samples (three independent samples analyzed in duplicate). Standard deviation is indicated by an error bar.
As observed with alcohol fixed cells, the two cytometric methods provided similar cell cycle distributions using acid lysed cells. For both Jurkat and U2OS cells there are a high degree of concordance between cell cycle distributions measured by flow cytometry and image cytometry. In general, the accuracy and precision of the NC-3000 is comparable with BD LSRII. For example, for the untreated Jurkat samples the fraction of S-phase cells is measured to 44.9±0.6% by BD LSRII and 45.5±0.7% by NC-3000. Furthermore, for the untreated U2OS samples the fraction of S-phase cells is quantified to 38.8±1.8% by BD LSRII and 40.5±1.1% by NC-3000.

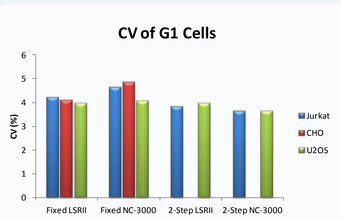


Fig. 4. Comparison of mean coefficient variation (CV) of the G_1 peaks measured by flow and image cytometry
Exponentially growing cells were permeabilized by either alcohol (Fixed) or acid lysis (2-Step) and parallely analysed by flow cytometry (LSRII) and image cytometry (NC-3000). Acquired data were analyzed as described in the Methods section. Columns depict the mean coefficient variation (CV) of the G_1 peaks obtained from 6 samples (three independent samples analyzed in duplicate). Note: no data for acid lysed CHO cells are available.
The two cytometric systems provided relatively similar CVs of the G_1 peaks. Compared to NC-3000, BD LSRII produced somewhat lower CVs on cells fixed with alcohol. In contrast, NC-3000 yielded lower CVs on cells lysed with acid than BD LSRII. In general, cells lysed with acid provided lower CVs than cells fixed with alcohol.

Conclusions

Dysregulation of the cell cycle is a distinct characteristic of cancerous cells and numerous chemotherapeutic drugs target cell cycle progression. In order to perform robust cell cycle analysis and to detect genomic abnormalities, such as aneuploidy, systems providing accurate and precise quantification of DNA content is required. Currently, flow cytometry stands as the gold standard for quantification of cellular DNA content.

In this study, we have compared an image cytometer, NucleoCounter NC-3000, and a flow cytometer, BD LSRII, with respect to quantifying DNA content and, thus, determining cell cycle distributions. The obtained data demonstrate that NC-3000 is highly accurate and precise for quantification cellular DNA content when compared with BD LSRII. Thus, we found a high degree of concordance between cell cycle distributions measured by the two cytometric systems. We have furthermore compared two different methods for permeabilzation cells prior to DNA staining. In method 1, cells are permeabilized by conventional alcohol fixation. In method 2, a new rapid approach is employed where cells are lysed with a combination of mild acid and non-ionic detergent. The presented results show that the new acid lysis method allows for accurate quantification of DNA content. In general, cells lysed with acid provided lower coefficient variation (CV) of the G_1 peak than cells fixed with alcohol.
In conclusion, cell cycle distributions quantified by image cytometry is accurate and precise when compared with flow cytometry, with the advantages of cost effectiveness, robustness and potential for morphological confirmation of the measured objects.