Flow cytometric analysis of a limited number of cells using the Agilent 2100 bioanalyzer

Application

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Introduction

In recent years the demand for higher throughput, the need for reduced reagent costs and the increasing work with primary cells in research has very much limited the absolute number of cells available for many cytometric analyses. While some techniques based on fluorescence microscopy use low cell numbers they lack automation and sensitivity. Traditional flow cytometry works at relatively high flow rates and typical cell volumes for analysis can range from hundreds of microliters to millilitres. The associated staining protocols have high cell and reagent consumption. Cell loss during preparation may account for 10 – 80 % of the original cell count. The Agilent 2100 bioanalyzer performs simple flow cytometric analysis using microfluidic technology[1,2]. The chip platform requires smaller sample volumes and permits unique on-chip staining protocols optimized for primary cells analysis[3]. The Agilent 2100 bioanalyzer and on-chip staining help to minimize sample consumption, sample handling and reagent consumption, while increasing automation and ease of use. The Agilent 2100 bioanalyzer has recently been described for single cell analysis of mammalian cells, yeast and fungal spores[4], showing the inherent flexibility and handling advantages of microfluidic technologies. This Application Note describes how we determined the minimum number of cells required to obtain meaningful data for a given experiment, using on-chip staining.

Experiments and results

To determine the number of cells required to maintain consistent reproducible results, decreasing concentrations of Jurkat cells were stained for the CD3 surface marker. All staining was performed on-chip as previously described[5]. The Agilent 2100 bioanalyzer requires two-color staining of cells for data analysis with blue and red detection channels. Calcein AM (Molecular Probes, Eugene, Or, USA) was used to determine the live cell population in the blue channel. Cells were counterstained with APC labeled anti-CD3 antibodies (BD, Franklin Lakes, NJ USA) in the red channel. As a negative control Jurkat cells were stained with Calcein and APC-conjugated anti-CD86 antibodies (CD86 is not expressed in non-activated lymphocytes). According to the on-chip staining procedure, Jurkat cells were resuspended in CB (Cell Buffer is an isobuoyant buffer included in the Cell Fluorescence LabChip® kit). The samples varied in cell concentration from 2 x 10^6 to 6 x 10^4 cells/mL. We prepared two solutions with 10-µM Calcein-AM and 1:40 of each labeled antibody in CB. A 10-µL volume of each cell suspension was loaded on a cell chip together with 0.5-µL of the respective CB solution for staining. After vortexing for one minute, chips were stacked and incubated in the dark for 20 minutes at room temperature. Follow-

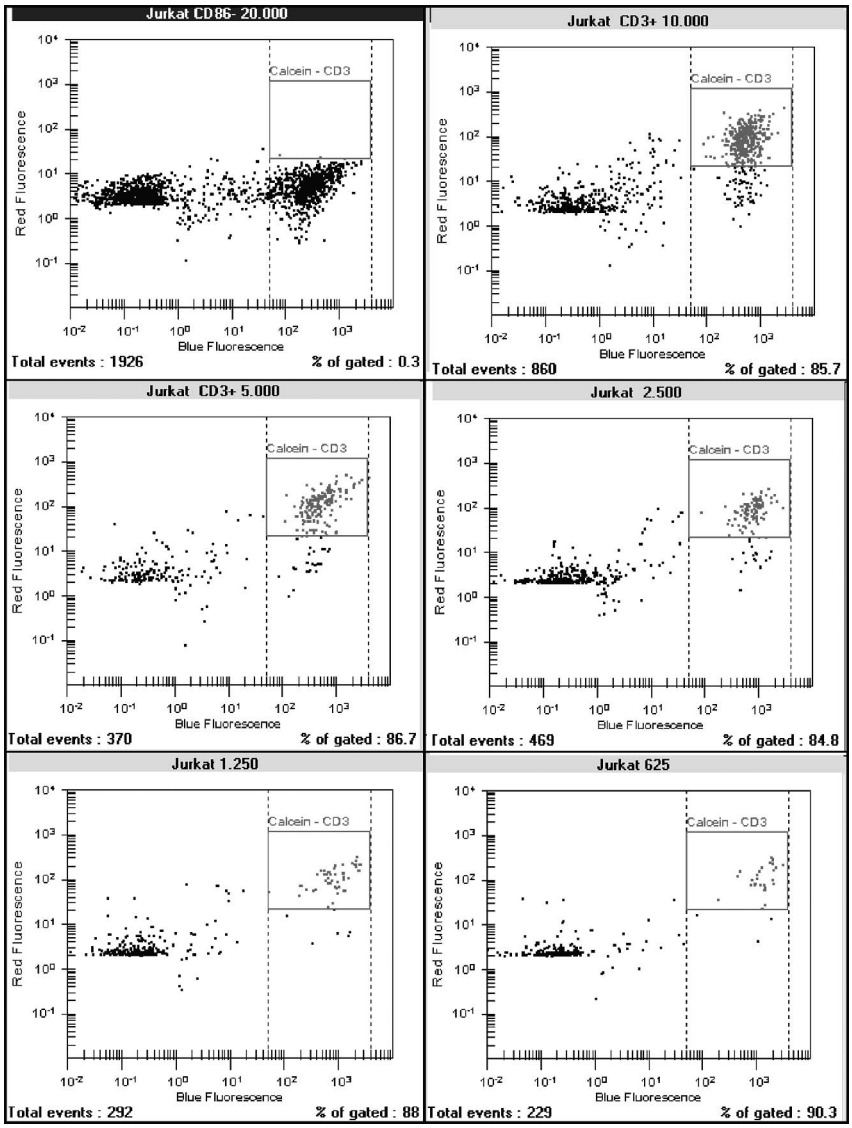
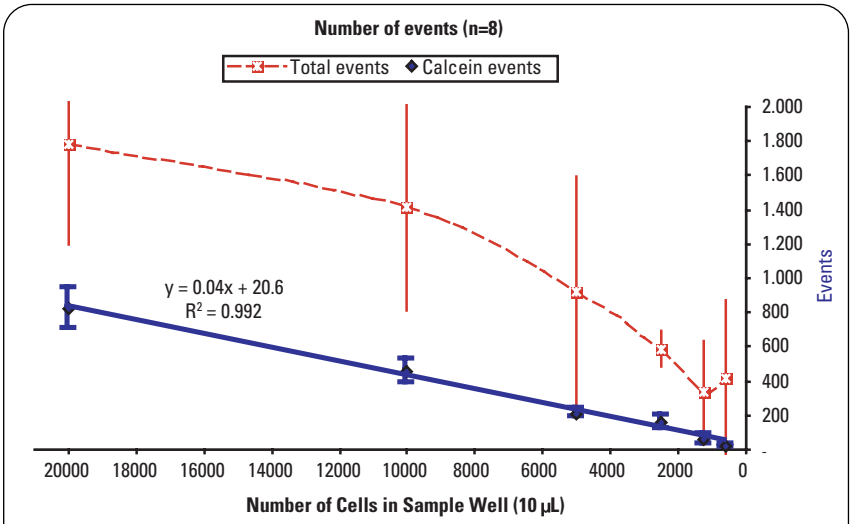


Figure 1
Dot plot view showing little effect of reduced number of cells per sample in percentage of CD3 positive cells. Jurkat cells (sample 2 – 6) stained with Calcein (blue) and APC-anti CD3 antibody (red). A negative control was used for region setting (sample 1).

ing incubation the chip was again gently vortexed (1000 rpm) for one minute. The chip was then loaded and analyzed on the Agilent 2100 bioanalyzer. The data in the table of figure 2 summarizes the detected percentage of CD3 positive cells (cells stained with viability dye and positive for the CD3 receptor) against the total number of cells loaded in a sample well. It shows that reducing the number of cells in the sample has little effect on the percentage of cell population being determined as CD3 positive. The result from samples with 2500 cells load onto the chip indicates that this small number of cells was enough for appropriate quantitation.

In figure 2 the correlated number of total events and live cells as determined by Calcein staining is plotted. The Calcein staining results show a linear correlation between detected live cells and cell consumption. Total event numbers are affected by cell debris, dye aggregates and other particles in suspension.

In a similar experiment, primary normal human dermal fibroblasts (NDHF) were used. NDHF were successfully stained on-chip with Calcein and APC labeled antibodies against HLA-A, -B, -C down to 625 cells per sample (figure 3). About 100 % of the live, Calcein stained cells were found to be HLA-positive in all samples with different cells consumption.



Cells	Live-CD3+	STD(n=4)
20.000	83,7%	3,5%
10.000	85,6%	4,1%
5.000	87,7%	4,2%
2.500	84,0%	3,0%
1.250	89,8%	6,5%
625	90,0%	9,3%

Figure 2
Number of live cells detected maintains a linear correlation with the number of cells in the well but the total number of events is biased by cell debris, unbound dye aggregates and suspension particle. Summary table shows mean percentages with standard deviation values (n=4).

Discussion and conclusion

The recommended number of cells for detecting subpopulations as low as 10 % of the total cell population is 20,000 cells per sample for most Agilent 2100 bioanalyzer cell applications. However, when cell availability is limited, useful experimental information can still be obtained with fewer cells per sample using the on-chip staining protocol. We have shown that as little as 2500 cells per sample are sufficient to provide similar data for this experiment.

The Agilent 2100 bioanalyzer continues to provide high accuracy and reproducibility with cell counts ten times lower than the recommended cell concentrations. To further assist in the measurement of dilute cell samples, it is possible to extend the data acquisition up to six times longer than the default settings when a single sample is analyzed on a cell chip, expanding the usability of the system for flow cytometric analysis of scarce samples that could not be routinely analyzed by traditional flow cytometry instrumentation.

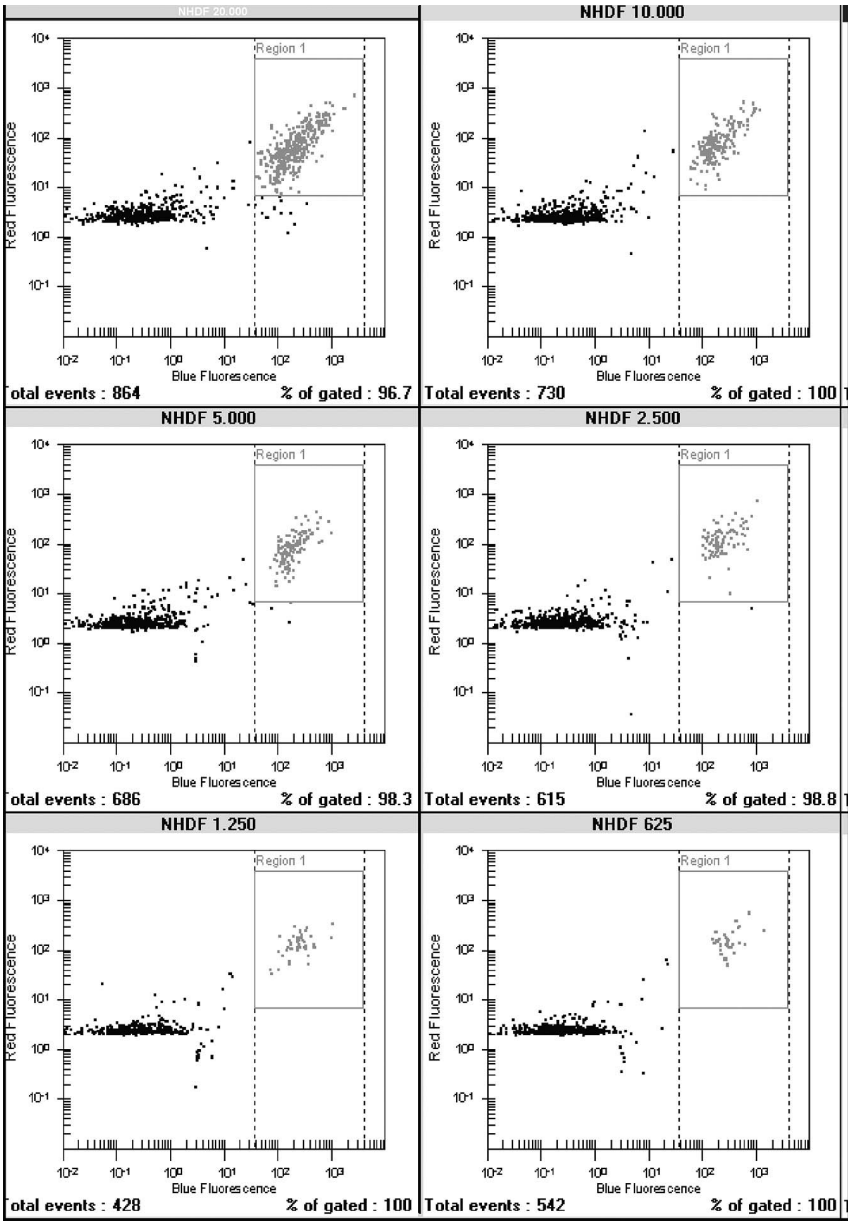


Figure 3
Dot plot chip overview showing no effect of reduced number of cells per sample in the qualitative determination of NDHF cells. Almost 100 % of the live NDHF cells were determined positive by APC labelled anti-HLA antibodies. Data kindly provided by Samuel Chan, Caliper Technologies, Mountain View, CA, USA.

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