Technical Overview

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Absolute Counting Using an Agilent NovoCyte Flow Cytometer

Introduction

This technical overview describes how to obtain cell counts using an Agilent NovoCyte flow cytometer. The method described can be used for general live cell counting, but can also be modified to obtain cell counts with any experimental design.

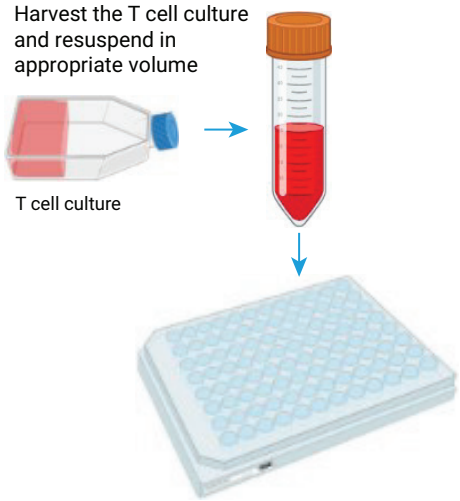

Figure 1. Resuspension and plating of a T cell culture.

Materials

- Agilent NovoCyte flow cytometer (with or without an autosampler)
- Agilent NovoExpress acquisition and analysis software
- Sample(s) to count
- Desired viability or cell labeling reagents
- Labware (plates or tubes) compatible with NovoCyte flow cytometers (12 × 75 mm tubes, 24-, 48-, 96-, or 384-well plates)
- Appropriate personal protective equipment (PPE)

The example described in this overview will focus on obtaining live, cellular counts using 7-AAD, a viability dye, to exclude any dead cells. The NovoCyte flow cytometer can obtain cell counts with any sample run through the instrument using multiple types of labware. If a different sample preparation is used, proceed to Step 2.

Step 1

Prepare sample material to be counted. Add 50 µL viability staining solution (7-AAD, Biolegend Cat # 420404), which is 5 µL of 7-AAD in 0.5 mL staining buffer (Biolegend Cat. no. 420201) plus 50 µL of cell suspension to a 96 well plate. Prepare samples in replicates as desired for counting. If choosing to include a viability dye or other markers, make sure to include appropriate controls for accurate gating.

Step 2

1. Open the NovoExpress software and log in.
2. Click **Plate Manager** and select the plate type (Figure 2A).
3. Add samples to the plate manager in their respective positions, and rename samples if desired.
4. Verify that the Absolute Count setting is selected (Figure 2B).

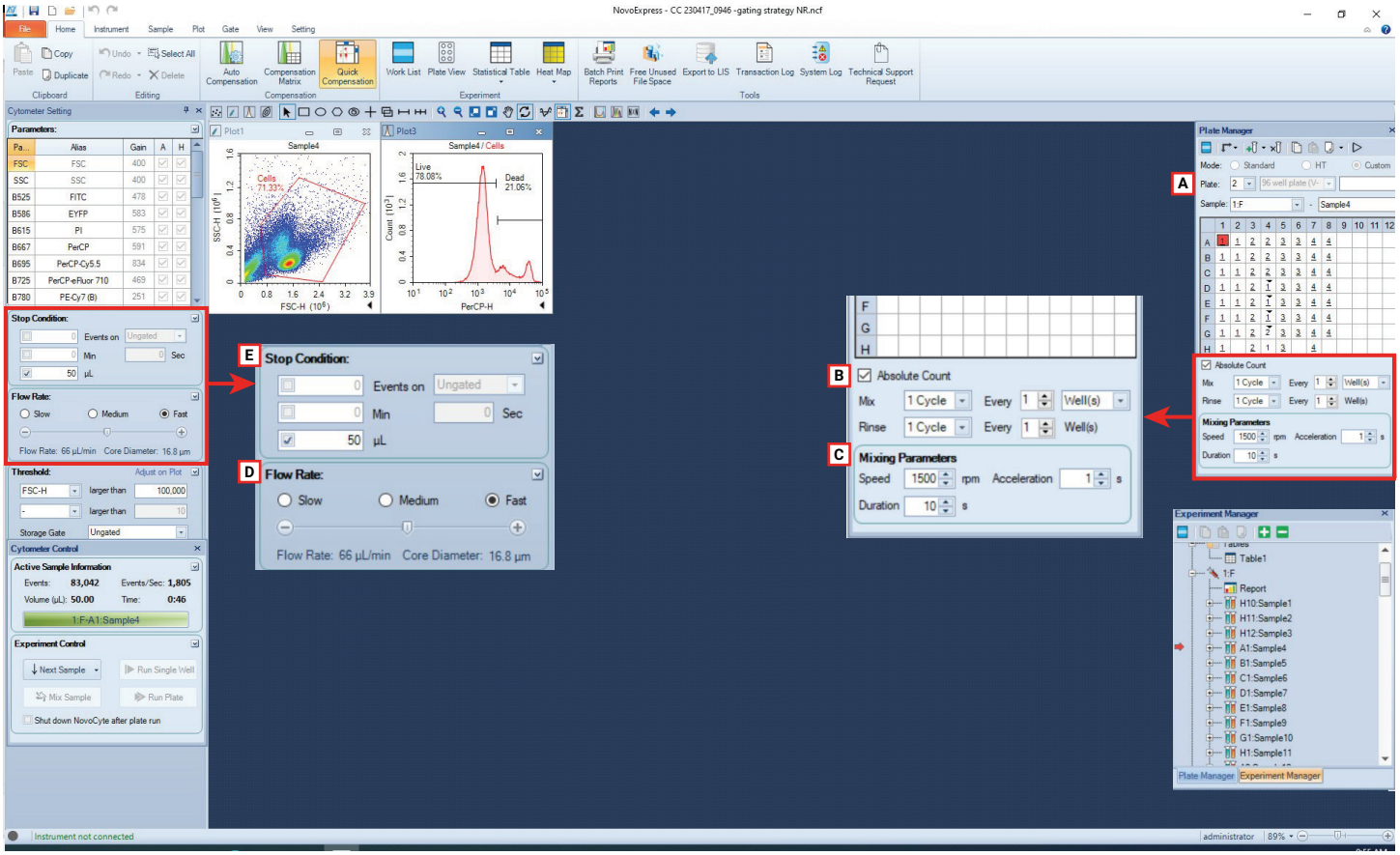

Figure 2. Setting up the experiment template and cytometer settings.

5. Including at least one mix and one rinse cycle between sampling of each well is recommended (users will need to independently verify that mixing speed and duration (Figure 2C) is appropriate for the plate type and sample volumes to avoid splashing or spills).

Step 3

Set up the cytometer settings.

1. Select the appropriate channel for fluorescence detection.
2. Set your desired Flow Rate (Figure 2D), and set Stop Condition to 50 µL (Figure 2E).
3. To verify the correct Absolute Count setting, click the Sample tab, then **Absolute Count Setting** (Figure 3A). The Absolute Count Setting window will open (Figure 3B).
4. Drag and drop the current sample onto the Specimen name to apply these settings to all the samples.
5. If a sample dilution was made, it can be entered under Dilution Factor. The absolute cell count will automatically reflect that dilution factor if **Apply to All Samples in the Same Specimen** is selected.
6. Verify that the correct units are selected under Absolute Count Unit.

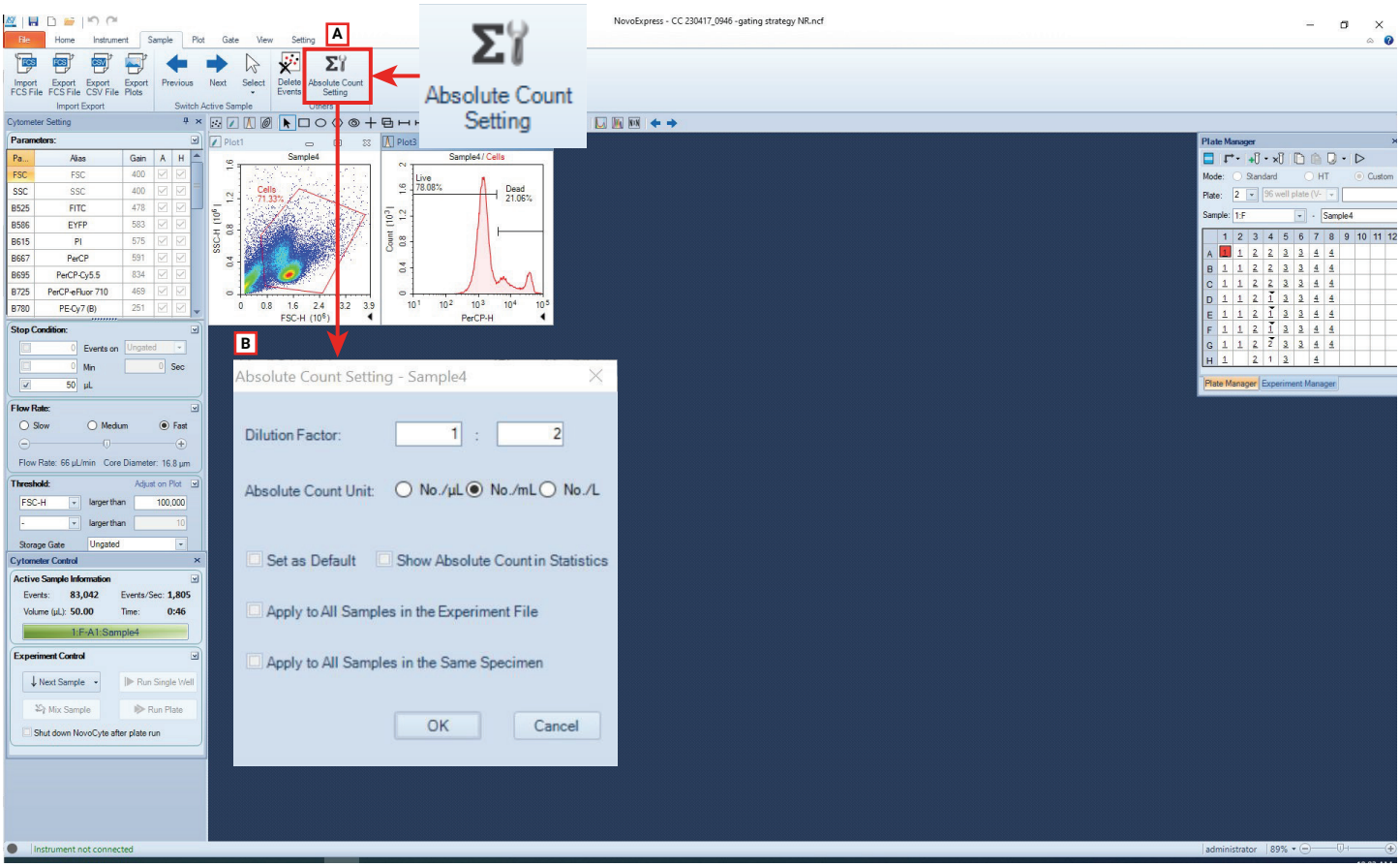

Figure 3. Setting up the absolute cell count feature.

Step 4

1. Acquire your sample(s).
2. Gate your cells of interest (i.e., exclude debris).
   Note: gating strategies and specific plots used may be different depending on individual experiments or preferences.
3. Display the gated cells in another plot to identify live or dead cells (Figure 4); draw gates on populations of interest (i.e., live cells). Gates are determined by experimental controls.
4. Gate the T cells based on forward and side scatter properties. As shown in Figure 5, the red gate is placed around the T cell population. Debris is usually collected at the left corner of the density plot with lowest level of side and forward scatter.
5. Set up a gating strategy for live and dead cells using the histogram plot (Figure 6).

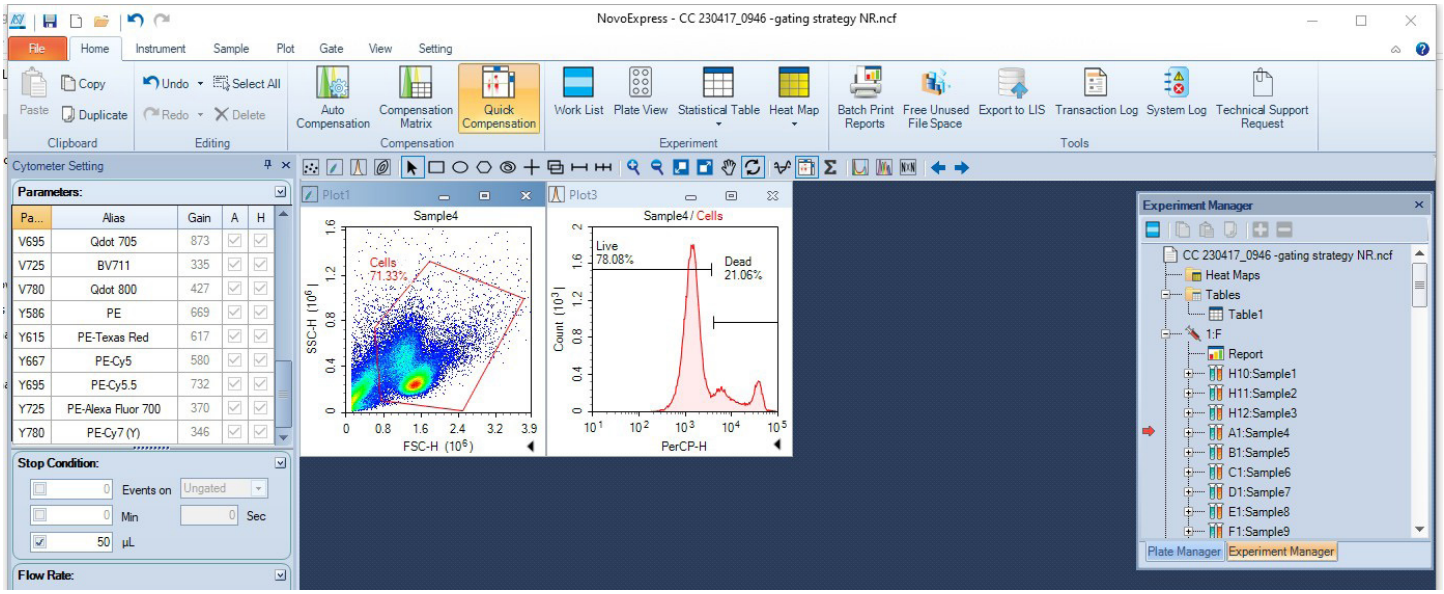

Figure 4. Setting up gating strategy 1 based on FSC and SSC.

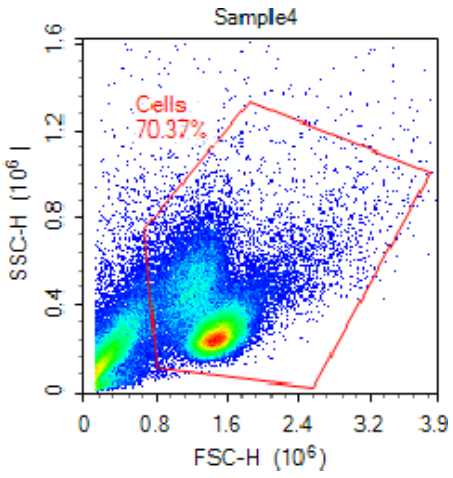

Figure 5. Gating strategy 1: forward and side scatter density plots.

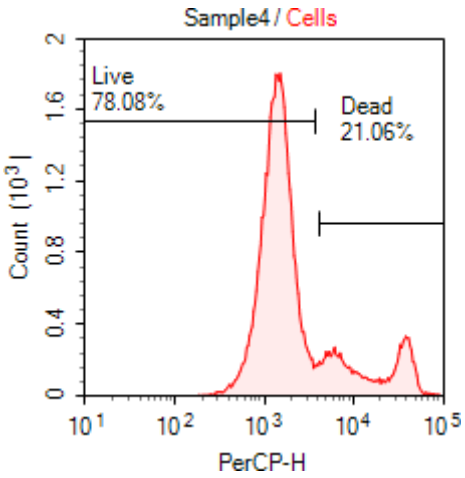

Figure 6. Gating strategy 2: single parameter histogram identifying viable cells.

6. Open the statistical table by clicking the icon under the Home tab (Figure 7A). A new window will open, which will be a table with columns (Figure 7B); users can customize and add or delete specific statistics to be displayed (Figure 7C).
7. Add columns to show gating parameters as mentioned in the previous step and as shown in Figures 7C and 7D. Customize the statistical table by adding or deleting gating parameters. It is recommended to add columns to get absolute cell counts on gates: Cells, Live, and Dead. All these steps are shown in the next steps in detail.
8. In the Add Column window, select **Abs. Count** under the Statistics tab then select **Cells**, **Dead**, and **Live** under the Gate tab (Figure 7D). Click **Add**. A statistical table will open in a new window, as shown in Figure 8.
9. The absolute count for live, viable cells is represented in units of cells/mL. The units for volume can be changed to µL, mL, or L by modifying the Absolute Count Settings (Figure 3).

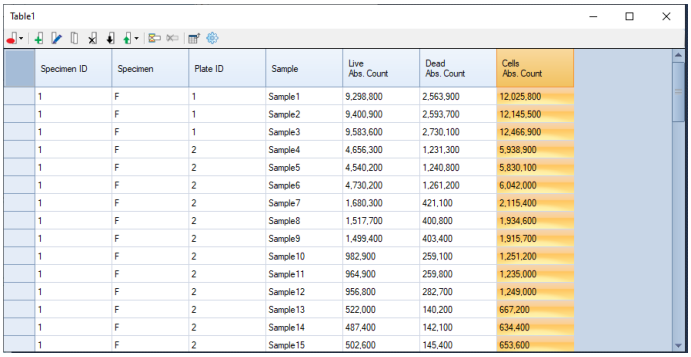

Figure 8. Complete table with absolute counts – both live and dead.

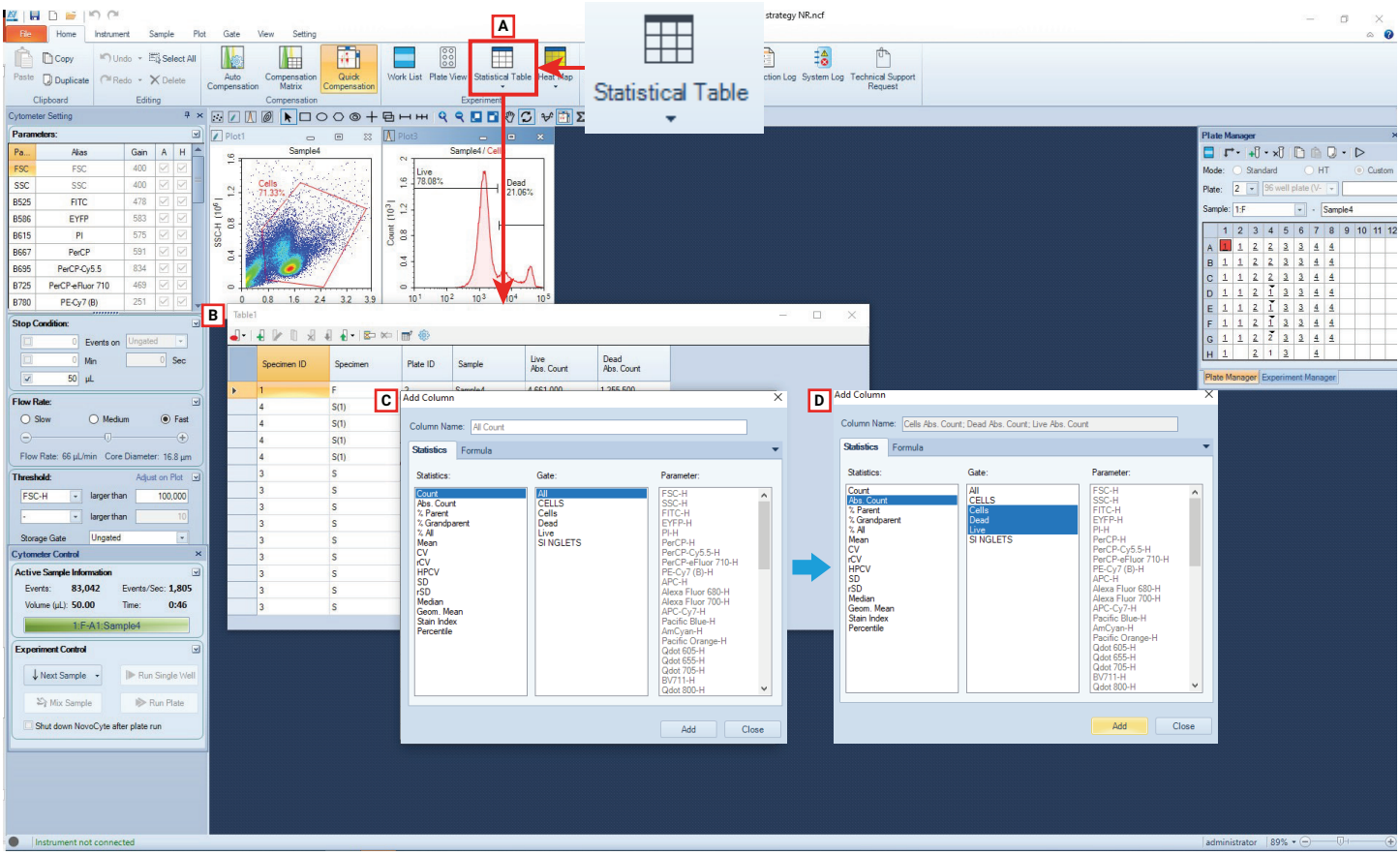

Figure 7. Setting up the template to get absolute counts.

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