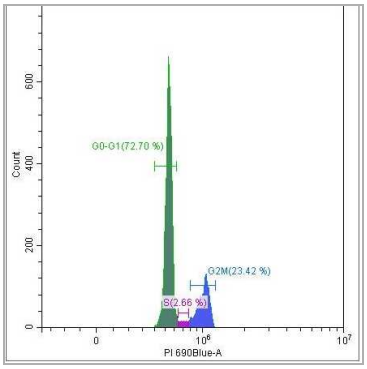

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We use this protocol and it's working.

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Basic Instructions for Running Cell Cycle Analysis with Propidium Iodide on the Beckman Coulter Cytoflex S or Other Cytometers

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ABSTRACT

Basic instructions for performing Cell Cycle Analysis on the Beckman Coulter Cytoflex S, or any similar flow cytometry analyzer in tube mode. This protocol does not cover sample prep. For advice on the preparation of samples for cell cycle analysis, a suggested reference is Diamond, R. A., & DeMaggio, S. (2000). *In Living Color: Protocols in Flow Cytometry and Cell Sorting*. Springer Lab Manual, pg 361

GUIDELINES

This protocol assumes that the researcher has a basic knowledge of flow cytometry and has been trained to use the CytoFLEX S analyzer and the CytExpert software. This protocol can be adapted for use with other flow cytometry platforms and software programs.

MATERIALS

Your choice of 1.5ml Eppendorf tubes or 12x75 Falcon Tubes (polypropylene or polystyrene) for loading samples in tube mode
30-35um mesh filter caps for troubleshooting (options include Falcon™ 352235, or Sysmex 04-0042-2316)
Cytoflex S or other flow cytometer
Data analysis software

BEFORE START INSTRUCTIONS

Check the configuration of your Cytoflex S (or other flow cytometry analyzer) to ensure that the instrument is equipped with the lasers and filters you need. Do this before preparing any samples or purchasing any reagents or supplies for your experiment. This is a best practice for any type of experiment, especially when using shared instrumentation.

Instructions

1 On the day of the experiment, once you are at the instrument with your negative control, PI positive control, and prepared experimental samples, set up your template in the CytoFLEX flow cytometer's CytExpert software for the appropriate filter set for the stain that you are using for Cell Cycle Analysis (for PI, you may view using the 610/20 on the Yellow Laser or the 690/50 on the blue laser on a standard Cytoflex S).

1.1 Add the following plots to the template: A dot-plot of FSC-A vs. SSC-A, a dot-plot of PI-A vs PI-H, a histogram with PI-A on the X axis, and a plot with PI versus Time to monitor the stability of the flow rate. PI on Y axis, Time on X axis.

1.2 Set the flow rate to “Slow” 10uL/min for the entire experiment for best results.

2 Run the negative control to identify the population of interest.

2.1 Adjust the plot axes so that all events are visible in the FSC-A vs SSC-A dot-plot, then draw a gate around the population of interest. The gating shown is more conservative - you may want to expand the gate for your cells due to the possibility of some cells having abnormal phenotypes

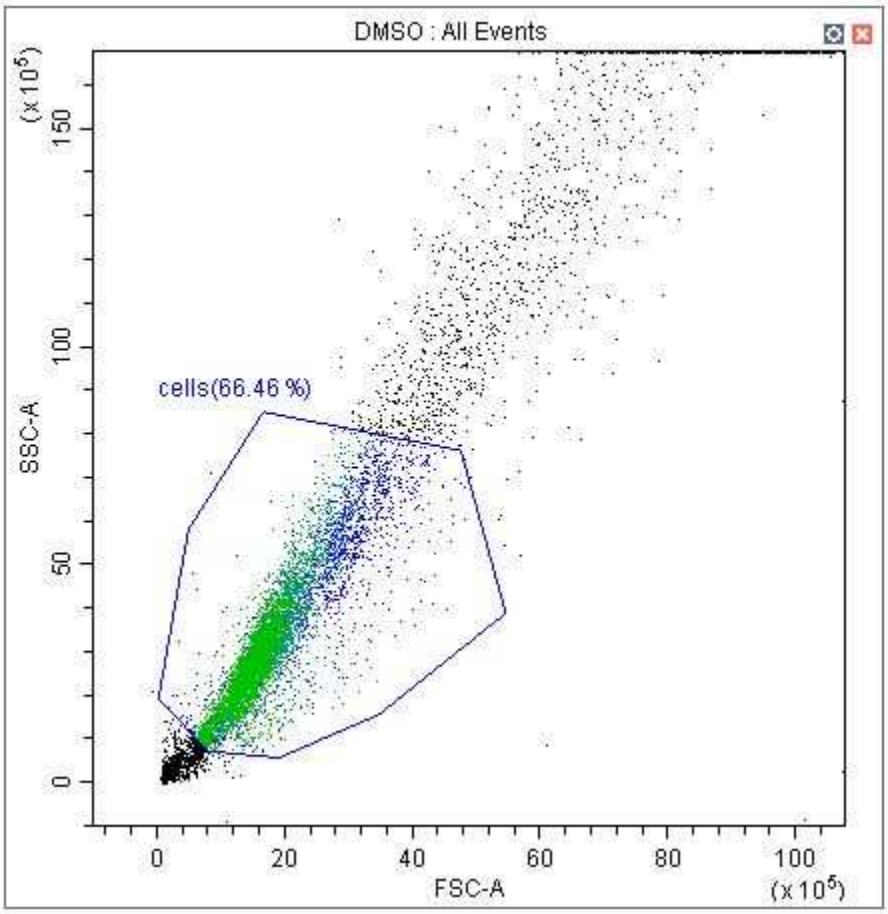

Figure 1: Example of Human U2OS Cancer Cells FSC/SSC plot using linear scale. Courtesy of Eunny Bae, Campbell Lab, 2022

2.2 In the PI-A vs PI-H dot plot, show only the events in the gate set on the population of interest. Then, draw a gate around the singlet events only (single cells). Using this method, singlet events are presented in a diagonal pattern and doublets will appear above the main diagonal population. Doublet discrimination is very subjective. But generally, using Height versus Area, the doublets will appear with higher values outside of the main population as shown.

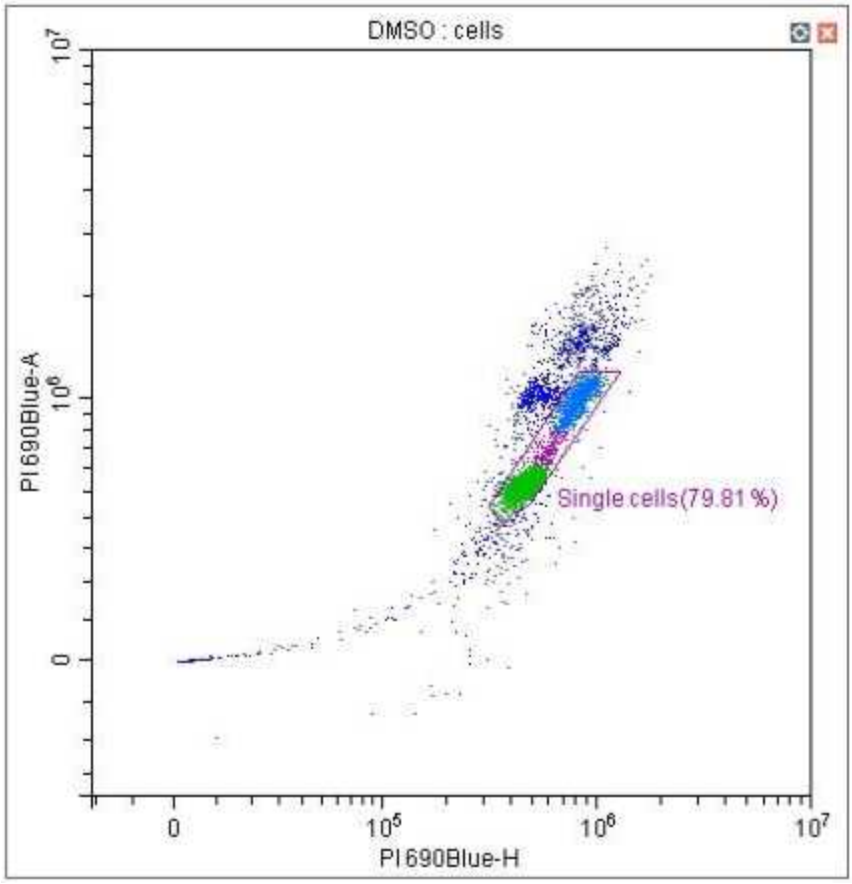

Figure 2: It is necessary to gate on single cells that are positive for the cell cycle stain. On the CytoFlex instrument, you must use PI-H vs. PI-A

2.3 In the histogram with PI-A on the X axis, show only the events in the singlet gate.

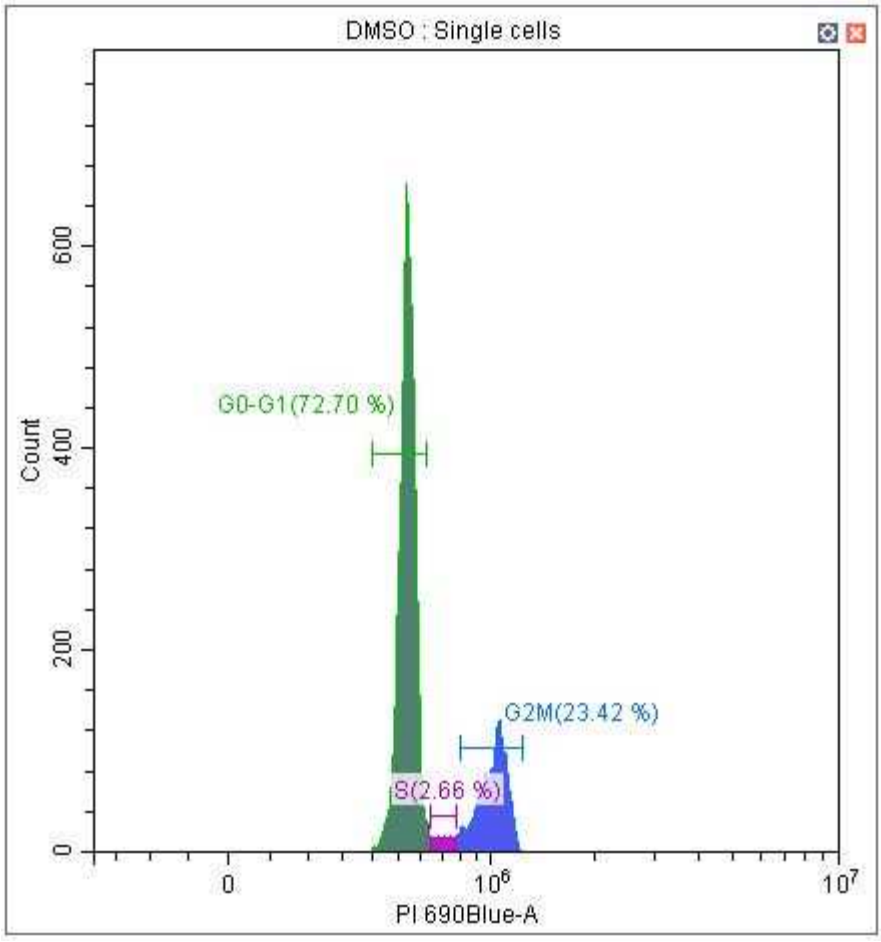

Figure 3: Example of histogram with PI positive control cells in various stages of the cell cycle. This plot only includes the Single Cells as gated on the doublet plot.

3 Remove the negative control and then place the PI control sample in the sample carrier. Click "Run"

3.1 Troubleshooting: In the time plot, gate out any perturbations in the flow for subsequent analysis if they are minor.

- If the perturbations appear to be major or constant, there are a few possibilities:

If they appear to be sample-related (aggregation, clogging, etc), perform sample prep optimization as needed for future runs. For the current run, you can try re-filtering your sample through a 30-35um mesh if the sample has been sitting for longer than 15 minutes.

- If it appears to be instrument related, it may be a result of peristaltic pump tubing that needs to be replaced, perform the necessary maintenance and/or seek assistance in doing so. It could also be that the laser is failing, in which case this would require a service call.

4 Adjust the gains on the PI channel until the G1 peak MFI is at 2 x 10^6. This will keep the peak for the G2/M on scale and ensure good resolution between G2/M and the G1 peaks.

5 Once the gains are set appropriately, stop running the PI control sample. Set the preferred stopping rules for recording your FCS files. With the new gain settings, you will need to record your negative control. Record a fresh copy of your PI control sample. Then, you may proceed with recording your experimental samples.

6 The FCS files are automatically saved when you click "Record." You can then batch export your data to PDFs using the CytExpert software for your records. You may import your FCS files for further analysis into your preferred software program.