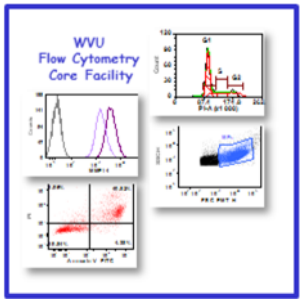
# WVU FLOW CYTOMETRY CORE FACILITY

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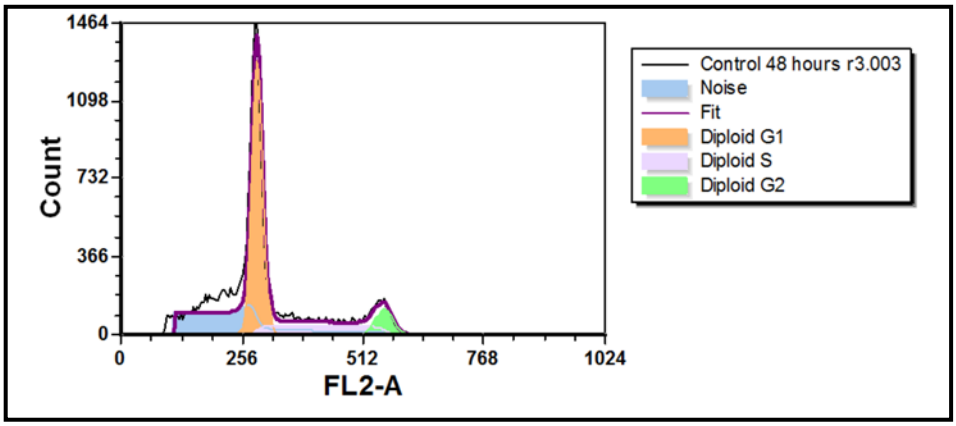
## Assay Highlight: Cell Cycle Analysis




A common assay run on flow cytometers is for cell cycle analysis. Cell cycle assays allow investigators to measure the DNA content (ploidy) of a cell along with determining the percent of cells in $G_0/G_1$, S and $G_2$/M stages of the cell cycle. The assay can also detect sub-G1 cells indicative of an apoptotic population. However, to confirm whether the sub-G1 cells are indeed apoptotic cells, other assays such as Annexin V binding or measuring caspase activity need to be performed.

Cell cycle assays use unique dyes that work by intercalating into the DNA of a cell. Table 1 lists some of the common dyes used in cell cycle assays. The most common dye used for this assay is propidium iodide (PI). This dye intercalates into double stranded DNA (dsDNA) as well as RNA. When using PI, DNAse free RNAse A is used to degrade the cellular RNA so that PI only intercalates into the dsDNA.

**Table 1. List of Dyes used in Cell Cycle Assays**

| Dye | Binding Specificity |
|---|---|
| Propidium Iodide | dsDNA & RNA |
| 7-Aminoactinomycin D (7-AAD) | dsDNA (G/C rich) |
| Ethidium Bromide | dsDNA, ssDNA & RNA |
| Vybrant®Dye Cycle™ | dsDNA |
| Hoechst 33342 | dsDNA (A/T rich) |
| Dapi | dsDNA (A/T rich) |
| Chromomycin | dsDNA (G/C rich) |

The key to obtaining good cell cycle data is in the sample preparation and fixation. Overall, while the basic cell cycle assay is not technically demanding, there are a few key things to keep in mind. First, prior to fixation, it is important to make sure the cells are in a single cell suspensions. Once the cells are fixed, you cannot separate them.

## Inside this Issue



**Facility Location:**
2160 HSCN
Phone:304-293-6273
e-mail: flowcore@hsc.wvu.edu

**Hours of operation:**
9:30 am to 5:00 pm, M-F

After hours access for experienced users by prior approval from Kathy Brundage

Contact Kathy at:

kbrundage@hsc.wvu.edu

Although the common flow cytometry fixative paraformaldehyde can be used to fix cells for cell cycle analysis it is not the best choice. For best results, ice cold 70% ethanol should be used. Cells fixed with paraformaldehyde tend to have higher CVs for the $G_0/G_1$ and $G_2/M$ stages of the cell cycle than those fixed with ethanol. Higher CVs result in data that do not fit the cell cycle models as well as data with small CVs. The minimum fixation time is overnight. Fixed cells can be stored at 4°C or –20°C for up to a year. When the investigator is ready to analyze the cells, all they have to do is wash away the ethanol, permeabilize the cells using PBS containing Tween-20, treat with RNAse A (if necessary) and add the dye. After a short incubation (15 minutes), the cells are ready to be analyzed on the flow cytometer. For best results, a minimum of 30,000 events should be collected on low speed (each tube takes approximately 2 min to run).

Cell cycle analysis is one of the few assays that requires fluorescent detection on a linear scale instead of a log/bi-exponential scale. The reason for this is because cell cycle analysis involves measuring the DNA content of a cell. When a cell is in the $G_0/G_1$ cell cycle stage its DNA content is considered 2C (2 copies of each chromosome) while a cell in $G_2/M$ cell cycle stage has a DNA content that is doubled and is considered 4C (4 copies of each chromosome). So the cells in $G_2/M$ will at a maximum only be twice as bright as the cells in $G_0//G_1$. This small change in fluorescence would be lost if a log/bi-exponential scale is used.

To obtain reportable data, the FCS files obtained on the flow cytometers have to be analyzed using software specifically designed to interpret cell cycle files. The facility uses FCS Express 4 software for this purpose. The software puts the data through a series of algorithms to best fit your data to one of 6 cell cycle models. The box on the left and the one below are examples of data that can be obtained using this software. For more details on how to use the FCS Express 4 software to analyze cell cycle data turn to page 3 of the Newsletter.

If you are interested in learning more about cell cycle analysis please contact the WVU Flow Cytometry Core Facility for assistance.

**Box 1. DNA Experiment Statistics**

**Interpretation**

MultiCycle suggestions (a guideline only):
No abnormal DNA content is observed.
The diploid %S=22.3, %G2=9.56
The S Phase confidence is good

**Experiment Statistics**

Chi sq: 5.60
BAD: 16.79
Number of cells: 23626.00
Number of cycles: 1.00
Cycle fit model: 1 Cycle

**Box 2. DNA Cycle Statistics**

| Cycle | G1 Mean | G1 CV | %G1 | G2 Mean | G2 CV | %G2 | %S | G2/G1 | %Total | B.A.D. |
|---|---|---|---|---|---|---|---|---|---|---|
| Diploid | 289.42 | 4.26 | 68.13 | 559.49 | 3.38 | 9.56 | 22.31 | 1.93 | 100.00 | 16.79 |

**To log in and reserve a flow cytometer, AutoMACS, MSD or analysis computer, please point your browser to the following URL**

CORES
Core Ordering & Reporting Enterprise System
https://cores-wvu.mis.vanderbilt.edu/login.cfm

## Instruments in this facility

**FACSAria Cell Sorter/Analyser**

**Operator: Facility Staff**

**Lasers:** 488 nm Sapphire(SS)
633 nm HeNe
407 nm Violet(SS)

**Detection Parameters:**

Forward Scatter, Side Scatter, simultaneous detection of up to 11 fluorochromes

**Applications:**

Cell Sorting (Aseptic)
Cell phenotyping
Cell Viability
FISH, FRET, SPA

**FACSCalibur Analyser**

**Operator: User**

**Lasers:** 488 nm Argon
633 nm Red Diode

**Detection Parameters:**

Forward Scatter, Side Scatter, simultaneous detection of up to 4 fuorochromes

**Applications:**

Cell phenotyping
Cell cycle analysis

**AutoMACS Magnetic Bead separator**

**Operator: User**

**Application:**

single marker (extracellular) sorting, depletion sorting

**LSR Fortessa**

**Operator: User**

**Lasers:** 405 nm OBIS LX
488 nm Sapphire (SS)
561 nm Sapphire (SS)
628 nm OEM

**Detection Parameters:**

Forward Scatter, Side Scatter, simultaneous detection of up to 12 fluorochromes

**Applications:**

Cell phenotyping
Cell Viability
Cell Cycle analysis
FISH, FRET, SPA

# Analysis Tips - Cell Cycle Analysis

In FCS Express 4, cell cycle analysis is done using a multi-cycle histogram. Below is an outline of the steps required to analyze the data.

**Setting up the Analysis page:**

1. Open a new layout page.
2. Insert a Color Dot Plot (FSC-A vs SSC-A)
3. Draw a gate around the cell population to be analyzed and label it "Cells"
4. Insert a second Color Dot Plot showing the fluorescent dye the cells were stained with (example PI-W vs PI-A)
5. Apply the gate "Cells" drawn on the (FSC-A vs SSC-A) dot plot to this second plot
6. Draw a gate around the single cell population on the second (PI-W vs PI-A) dot plot
7. Label the new gate "Singlets"
8. Insert a Multi-cycle histogram and apply the gate "Singlets"

**Cell Cycle Analysis:**

1. Click on the "Multicycle" tab on the top menu
2. Click on the "AutoFit" tab icon
3. To obtain the DNA Model Statistics, right click on the Multicycle histogram → Statistics → DNA Model Summary. It should look like this:

| Model | Dip %G2 | Dip %S | Chis |
|---|---|---|---|
| SL S0 | 9.56 | 22.31 | 5.60 |
| SL CL S0 | 0.00 | 17.11 | 8.30 |
| +G2/G1 Fixed | 6.10 | 25.38 | 6.78 |
| +Aggregates | 4.49 | 23.01 | 6.65 |
| +S Order = 1 | 10.92 | 22.24 | 5.36 |
| +CVs Fixed | 11.66 | 21.78 | 5.45 |

4. The model that has the lowest Chis is the cell cycle model that your data fits best.
5. Click on the appropriate model (the one with the best Chis) on the tool bar. This will re-analyze the data using the selected model
6. To obtain the Experiment statistics, click on Multicycle histogram → Statistics → DNA Experiment Statistics (example: page2 Box1)
7. To obtain the Cycle statistics click on the Multicycle histogram → Statistics → DNA Cycle Statistics (example: page 2 Box 2)

**Critical Aspects of Cell Cycle Analysis:**

1. Cell number: minimum of 30,000 events in cell cycle; after excluding aggregates and debris
2. CVs – on standards <3%; on fresh tissues/ cells <8%; paraffin derived <10%
3. Number of histogram channels: lowest G1 population should be in channel > 50
4. Histogram range: should be 1/10$^{th}$ of G1 peak to 6x of G1 peak
5. Histogram linearity: check often using standards
6. DNA content standards: best choice is normal counterpart to the neoplastic cells
7. For most cells G2/G1 ratio usually 1.9 – 2.0.
8. BAD: Background Aggregates and Debris
   a. quantitative measure of aggregates and debris
   b. used as one indicator of cell cycle fit reliability
   c. BAD should be < 20%

**Note to users:**

***Please acknowledge the WVU Flow Cytometry Core Facility when reporting your flow cytometry data, using the appropriate phrase(s):***

**FACSAria users**: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number RR020866 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

**LSRFortessa users**: Flow Cytometry experiments were performed in the West Virginia University Flow Cytometry Core Facility, which is supported by the National Institutes of Health equipment grant number S10OD016165 and the Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant numbers P30GM103488 (CoBRE) and P20GM103434 (INBRE).

## *What's New*

**Check out our redesigned website with its new features including:**

1. **Clickable link to the CORES scheduling/billing system**
2. **Training guidelines for New users**
3. **Printable laser & filter configurations for all instruments**
4. **Archived newsletters**
5. **Useful links**

**The WVU Flow Cytometry Core Facility staff would like to thank everyone who took the time to fill out our annual Flow Cytometry Core Facility survey. Your feedback is greatly appreciated!!!!!**

**Upcoming Events:**

| | | |
|---|---|---|
| **April 3** | **Spring Holiday** | **Facility Closed** |
| **May 25** | **Memorial Day** | |
| **July 3** | **Independence Day** | |
| **June 17—July 6** | **Kathy Out of Lab** | **Facility open to experienced users,** |

*Fee Schedule (2014-2015 hourly rates)*

| | WVU user | Non-WVU user |
|---|---|---|
| **Data acquisition by User** | **$33.00** | **$48.84** |
| **Facility assistance for data acquisition** | **$17.00** | **$25.16** |
| **Data acquisition by Facility Staff** | **$50.00** | **$74.00** |
| **Sorting** | **$74.00** | **$109.52** |
| **Data Analysis (unassisted)** | **0.00** | **0.00** |
| **Data Analysis by Facility Staff** | **$50.00** | **$74.00** |

### New User Guide

Hands-on training for FACSCaliber and LSRFortessa is **mandatory** for all new users and must be scheduled by consultation with facility director.

Sorting as well as data acquisition on FACSAria is by facility staff only.

Training will initiate with user's first experiment. Due to the complexity of the instruments and software, facility staff will fully assist with the acquisition of the first dataset and will continue with additional assistance on a "needs" basis until users are comfortable operating the instrument on their own.