Cellometer Image
Cytometry for Cell Cycle Analysis
Importance of Cell Cycle Research

• Oncology:
  • Since cancer cells often undergo abnormal cell division and proliferation, it is important to understand the cell cycle of those cells.

• Pharmacology:
  • Studies involving characterization of pharmacological reagents and their effect on cell cycle regulation
  • Development of drugs to arrest normal cell cycling during chemotherapy treatment to reduce side effects

• Cell/Molecular Biology:
  • To understand molecular and biological mechanisms, it is essential to examine their role in cell cycle.
What is Cell Cycle?

Cell cycle is defined as “the period between successive divisions of a cell.” Standard cell cycle of eukaryotic cells is divided into 4 phases.

1. **G1 (G=Gap) phase.** Cells are preparing for DNA replication. At this stage the cells contain about the same amount of DNA. (diploid (2n))

2. **S (S=Synthesis) phase.** Replication of nuclear DNA occurs at this stage. During S phase there is a broad distribution of DNA content between G1 and G2 phase. The DNA content is doubled by the end of this phase. (2n to 4n)

3. **G2 (G=Gap) phase.** Replication of the DNA is complete, and the cell is growing and preparing for division. At this stage, the cells contain twice the number of chromosomes (4n) that are found in G1.

4. **M (M=Mitosis) phase.** Cell division.
Cell Cycle Detection Methods

There are multiple methods and protocols for detecting DNA content during cell cycle:

**Radioactivity Measurement**
- **3H-thymidine** – Radioactive labeling of thymidine. Upon DNA replication, the radiolabeled thymidine is incorporated into new DNA.

**Fluorescence-Based Detection**
- Live cell measurement
  - **Hoechst 33342** – Is cell permeable and therefore do not require cell fixation. Binds to the minor groove of double-stranded DNA.

- Fixed cell measurement
  - **Bromodeoxyuridine (BrdU)** – A thymidine analog, is incorporated into the genome during the S-phase of the cell cycle. Detected using anti-BrdU antibodies.
  - **DAPI** – Binds to the AT rich regions of the DNA. Poor live cell permeability and therefore often used post-fixing.
  - **Propidium Iodide (PI)** – A membrane exclusion dye and an intercalating agent that stains the cellular genome upon cell fixation.
Labeling DNA with propidium iodide (PI) allows for fluorescence-based analysis of cell cycle.

- This assay generates a cell population histogram (on left) in respect to PI fluorescence intensity.
- Amount of PI fluorescence intensity is correlated to the amount of DNA within each cell.
- Since the amount of DNA doubles ($2n \rightarrow 4n$) between G1 and G2 phases, the amount of fluorescence intensity of the cell population also doubles.
In this cell cycle experiment, cells were treated with Nocodazole, a compound designed to arrest the cell cycle at the G₂M Phase.
Sample Preparation for PI Cell Cycle Analysis

1. Collect cells (control and treated)
2. Fix for 15 min
3. Stain with PI Cell Cycle Reagent (40 min)
4. Re-suspend in final volume of PBS
5. Analyze using Vision CBA
Cellometer Image Cytometry Procedure
Analyze Cells in Just a Few Steps

1. Pipette 20uL of cells into counting chamber
2. Insert chamber
3. Select Assay & enter Sample ID
4. Click count to image and view cell count, concentration, and diameter
5. View bright field and fluorescent cell images
6. With Vision CBA, click export to view data plots

Assay: CBA_Cell Cycle-PI660nm
Cell Type F1: CBA_Cell Cycle PI 660nm
Sample ID: Cell Cycle Treated
Dilution Factor: 1.00

Results:
<table>
<thead>
<tr>
<th>Total Count</th>
<th>Concentration</th>
<th>Mean Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>10063 cells</td>
<td>1.41X10^4 cells/mL</td>
<td>7.8 microns</td>
</tr>
</tbody>
</table>
PI Cell Cycle: Jurkat Cells

Sample Information
Cell type: T lymphocyte
Disease: acute T cell leukemia
Type: non adherent
Untreated control

Cell Pop Gated |
Sub G0 0.2%  |
G0/G1 62.1% |
S 17.1%    |
G2/M 15.4% |

Bright Field Image
Propidium Iodide Image
Nocodazole-Induced Cell Cycle Arrest

Nocodazole [0.004 - 0.1 µg/ml] Dose Response
Cells arrested at G₂/M Phase

<table>
<thead>
<tr>
<th>Phase</th>
<th>Control</th>
<th>Nocodazole (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Sub G₁</td>
<td>6.96%</td>
<td>6.71%</td>
</tr>
<tr>
<td>G₀/G₁</td>
<td>53.12%</td>
<td>53.69%</td>
</tr>
<tr>
<td>S</td>
<td>15.00%</td>
<td>17.26%</td>
</tr>
<tr>
<td>G₂/M</td>
<td>14.35%</td>
<td>12.35%</td>
</tr>
</tbody>
</table>
Cellometer and Flow Cytometry Correlation

% of Cells Arrested at G₂/M Phase

Excellent correlation between Cellometer Vision CBA and flow results

<table>
<thead>
<tr>
<th>Phase</th>
<th>0.004 μg/ml</th>
<th>0.02 μg/ml</th>
<th>0.1 μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₂/M</td>
<td>12.35%</td>
<td>41.40%</td>
<td>47.78%</td>
</tr>
<tr>
<td>G₂/M</td>
<td>15.25%</td>
<td>39.10%</td>
<td>43.10%</td>
</tr>
</tbody>
</table>
PI Cell Cycle: PC-3 Cells

Sample Information
Organ: prostate
Cell Type: epithelial
Disease: adenocarcinoma
Type: adherent
Untreated control
PI Cell Cycle: HeLa Cells

Sample Information
Organ: cervix
Cell Type: epithelial
Disease: adenocarcinoma
Type: adherent
Untreated control

Legend
- Red: Sub G1
- Blue: G0G1
- Green: S
- Green: G2M

Bright Field Image

Propidium Iodide Image

Nexcelom Bioscience
Cellometer® Simply Counted
PI Cell Cycle: PANC-1 Cells

Sample Information
Organ: pancreas
Cell Type: epithelial
Diseases: epithelioid carcinoma
Type: adherent
Untreated control

[Graph showing cell cycle distribution]

Bright Field Image

Propidium Iodide Image
Sample Information
Organ: colon
Cell Type: epithelial
Diseases: colorectal adenocarcinoma
Type: adherent
Untreated control
Sample Information
PC3 cells: Untreated control
Organ: prostate
Cell Type: epithelial
Disease: adenocarcinoma
Type: adherent
The Cellometer can be utilized to effectively measure and analyze the cell cycle of mammalian cells.

There is an excellent correlation between the Cellometer results and flow cytometry results.

Cellometer may be used to examine a wide variety of mammalian cells.