Flow Cytometry Workshop:

Section III:
DNA Content Analysis

Beckman Coulter Taiwan Branch

Tim Yang
DNA Content Analysis—common sense

- DNA content analysis is one of the oldest Flow applications.
- Using appropriate dye to label DNA and then measuring the fluorescence by flow cytometry
- DNA dyes using on flow cytometry:
  - Propidium Iodide (PI)
  - 7-AAD
  - Acridine Orange (AO)
  - DAPI
  - Hoechst 33342, 33258
  - Vybrant DyeCycle
- Familiar applications using this technology:
  - Cell cycle analysis
  - Ploidy analysis
  - DNA index analysis
  - Apoptosis sub-G1 analysis

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Application 1: cell cycle analysis

Cells contain different **DNA content through cell cycle**
Application 1: cell cycle analysis

Fig. 2. Schematic illustration of the generation of a DNA distribution.
(a): Distribution of cells around the cell cycle for an asynchronous population. 
(b): Illustration of the relation between DNA content and cell cycle position for an asynchronous population. 
(c): DNA distribution of the hypothetical population illustrated in (b). 
(d): Broadened DNA distribution for the hypothetical distribution in (c). Broadening may result from variability in cell staining or in fluorescence measurement.
Application 1: cell cycle analysis

Stained with PI, analyzed by Flow

Cell Numbers

DNA content or FL intensity

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Application 1: cell cycle analysis

Fluorescence Intensity

# of Events

G₀-G₁ %

S %

G₂-M %

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Application 1: cell cycle analysis

Cell cycle (PI stained) + mataphase specific protein (phospho-histon-H3)
Application 1: cell cycle analysis

BromodeoxyUridine (BrdU) Incorporation

Control (left) versus drug treated (right) effect on cell cycle

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Application 2: ploidy analysis

- **Hyperdiploid**: greater than the normal 2n number of chromosomes
- **Hypodiploid**: Less than the normal 2n number of chromosomes
- **Tetraploidy**: Containing double the number of chromosomes
Application 3: DNA index analysis

DNA Index = \frac{A_0 \text{ Mean}}{G_0 \text{ Mean}}

= \frac{330}{220} = 1.5

Fig. 3  Flow cytometric DNA histogram of a human malignant melanoma showing a diploid cell population at channel No. 200 and two aneuploid cell lines with DNA indices of 1.08 and 1.14.
Application 4: apoptosis (sub–G0/G1) analysis

DNA Content Measurement: Sub-G0/G1

[Graphs showing DNA content distribution for control and drug-treated samples]

Control

Drug treated
The process: cell stained with PI, gating the major population from SS/FS plot and display gated cell on fluorescent (FL2 or FL3) histogram.

But........PI usually bring the doublet problem........

How to gate out the doublet cell before FL plot display??

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The signaling process by Flow

There are 3 kinds of signals could be measured

In FACSan and FACSCalibur, we used to used ‘H’ as the major parameter
In FC500, XL, and FACSCanto, we used to used ‘A’ as the major parameter

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To measure the signals among singlets and doublet:

- Peak (H) 1 2 1
- Integral (A) 1 2 2
- TOF (W) 1 1 2

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Doublet discrimination: method 1, using H and A

<table>
<thead>
<tr>
<th></th>
<th>Peak (H)</th>
<th>Integral (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2N</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4N</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2N + 2N</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

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Doublet discrimination: \textit{method 1, using H and A}

The histogram is gated to exclude debris and doublets. For DNA, doublets are detected by collecting peak versus integrated signals.

You could use this method on XL, FC500, FACSCanto, Cyan…….

\textit{Tim Yang}
Doublet discrimination: method 2, using H, A and H/A

<table>
<thead>
<tr>
<th></th>
<th>2N</th>
<th>4N</th>
<th>2N + 2N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak (H)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Integral (A)</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Ratio (H/A)</td>
<td>1</td>
<td>1</td>
<td>½ or &lt;1</td>
</tr>
</tbody>
</table>

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Doublet discrimination: method 2, using H, A and H/A

Histogram Gating to Exclude Doublets

The histogram is gated to exclude debris and doublets. For DNA, doublets are detected by collecting ratio versus integrated signals.

You could use this method on XL, FC500.......
Doublet discrimination: method 3, using A and W

Integral (A)  1  2  2
Tim of Flight (W)  1  1  2

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Doublet discrimination: method 3, using A and W

You could use this method on FACScan, FACSCalibur, FACSCanto…….

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So... the real process are.................
Cell cycle analysis: real practice on FC500

1. Parameters selection

![Parameter Selection diagram](image-url)
2. Plots Creation

3. Instrument Setup:

Run Control Cell Staining with PI:

調整 FS、SS、FL3 Lin(Int)、FL3 Peak Voltage & Gain
Final Result

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Other critical issues:

1. The proficiency of sample preparation: the CV of G0G1 phase

Solution:
Other critical issues:

2. How to resolve GoG1 / S / G2M phase?

Manually

By Software:
Phoenix MultiCycle or Verity Mofit
Some Flow relative web-sites:

Purdue University Cytometry Laboratories
http://www.cyto.purdue.edu/

Purdue University Cytometry Laboratories' E-mail Archive
http://www.cyto.purdue.edu/hmarchiv/cytomail.htm

陽明大學儀器中心及 Flow 討論區
http://140.129.64.205/Xoops224/modules/sign_up/

NCI ETI Branch Flow Cytometry Core Laboratory
http://home.ncifcrf.gov/ccr/flowcore/index.htm

Invitrogen’s Fluorescence Spectra Viewer
http://probes.invitrogen.com/resources/spectraviewer/