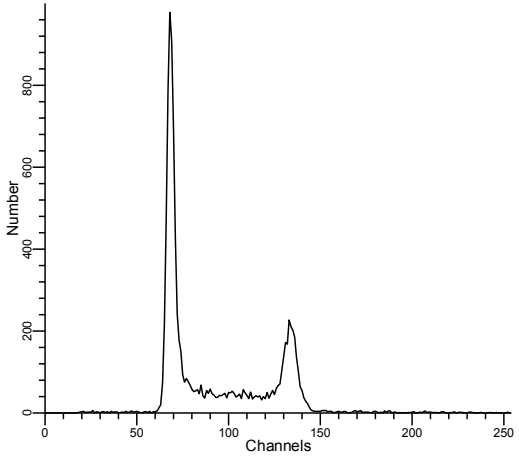
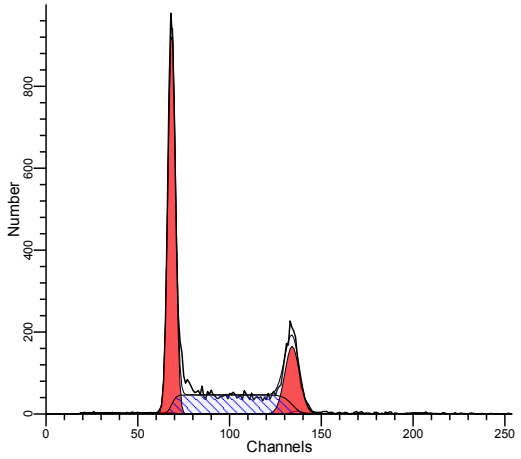
DNA Analysis by Flow Cytometry





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CRL Flow Cytometry Lab.
University of California, Berkeley
June, 2008

Definitions

Ploidy- Measurement of the number of chromosomes in a cell.

Diploid- 2n number of chromosomes.

Haploid- Half the normal 2n number of chromosomes.

Hyperdiploid- More than the normal 2n chromosomes.

Hypodiploid- Less than the normal 2n chromosomes.

Tetraploid- Double the normal number of 2n chromosomes.

Aneuploid- An abnormal number of chromosomes.

DNA Probes

	Name	Excitation	Emission
*	Propidium Iodide(PI)	536nm(488nm Laser)	623nm
	DRAQ5	536nm(488nm,633nm Lasers)	680nm
*	Sytox Green	503nm(488 laser)	531nm
*	7AAD	551nm(488, 561 lasers)	660nm
	DAPI	359nm(UV laser)	461nm
	Hoechst	346nm(UV laser)	460nm

DNA probes are stoichiometric. The fluorescence intensity is proportional to the amount of DNA in the cell or nuclei.

* These dyes can also bind to RNA. RNAase have to be added.

DRAQ5, DAPI and Hoechst bind specifically to A-T nucleic bases. These are also known as vital stains.

There are three basic technical approaches to DNA content analysis by flow cytometry.

1. One method involves permeabilizing and fixing cells (ethanol fixation) followed by the addition of DNA fluorochrome cocktails containing RNAses(PI, Sytox Green, etc.).
2. Another method permeabilizes cells with detergents.
3. Live cell DNA staining (supravital staining) using reagents like Hoechst, DAPI or DRAQ 5.

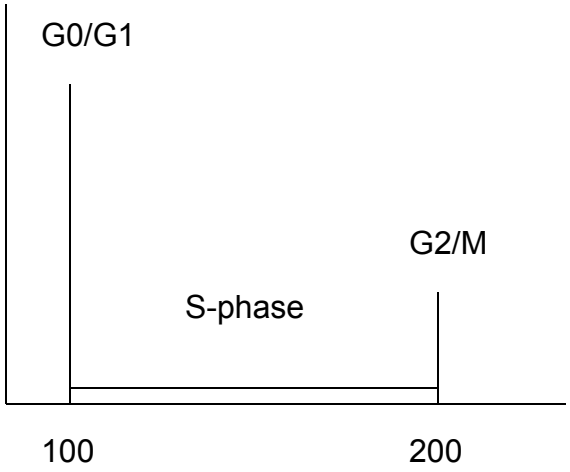
The first method permits prolonged sample storage but has higher cell loss due to adherance. CV's are higher.

The second method offers the advantage of lower CV's (more accurate DNA measurents). One disadvantage, samples have to be analysed within 1 hour after preparation.

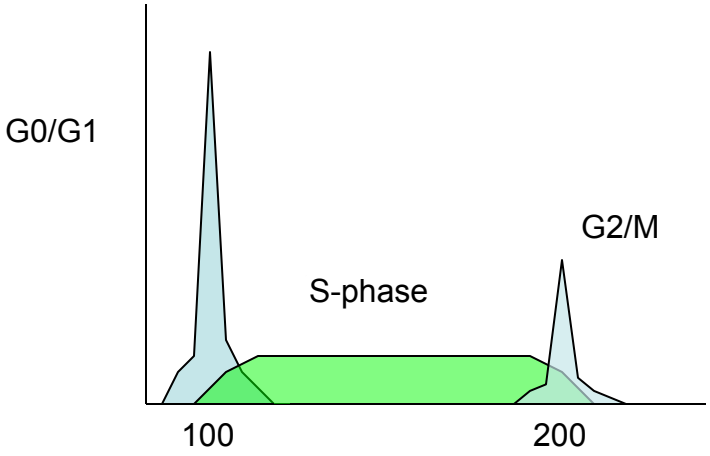
Method 3 does not work for all cell types. Some cells have very active P-Glycoptotein pumps which remove the dyes from cells.

The DNA Histogram

DNA probes intercalate between the bases in double stranded nucleic acids. The areas under each peak correspond to different phases of the cell cycle.



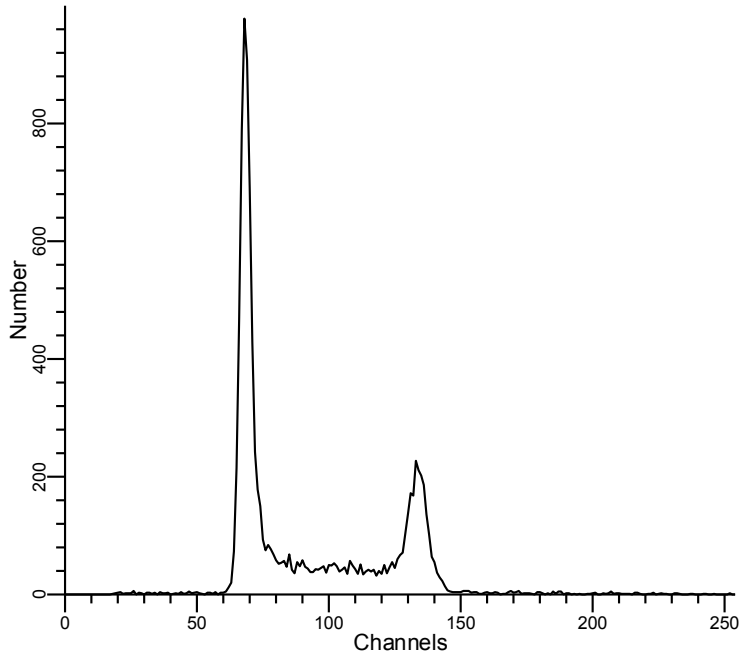
Theoretical Model of DNA Fluorescence Distribution Histogram.



Actual DNA Fluorescence Distribution Histogram as measured by flow cytometry.

DNA Flow Cytometry Data

Raw Data



Notice that the G0/G1 and the G2M peaks have a gaussian distribution.

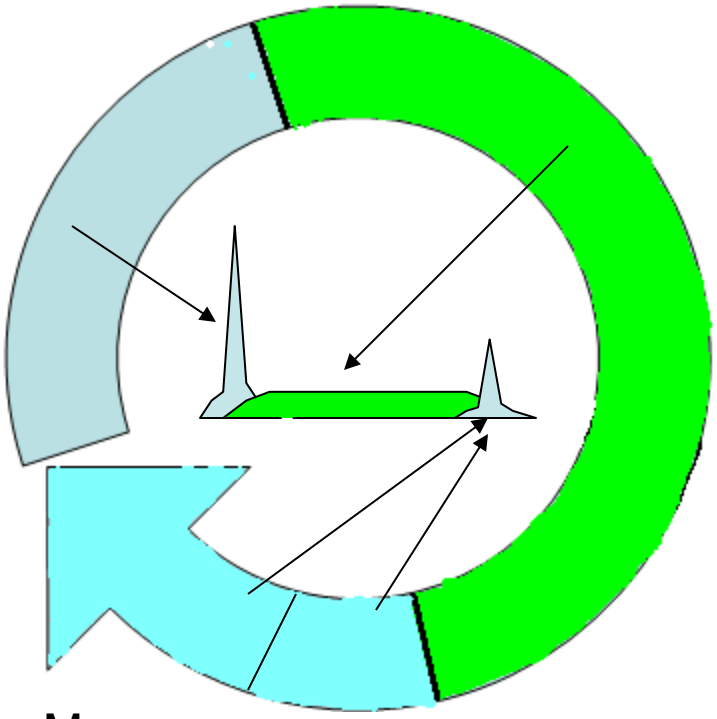
Also, the S-phase peak is characteristically non-gaussian.

Important parameters for estimating the various phases of cell cycle include:

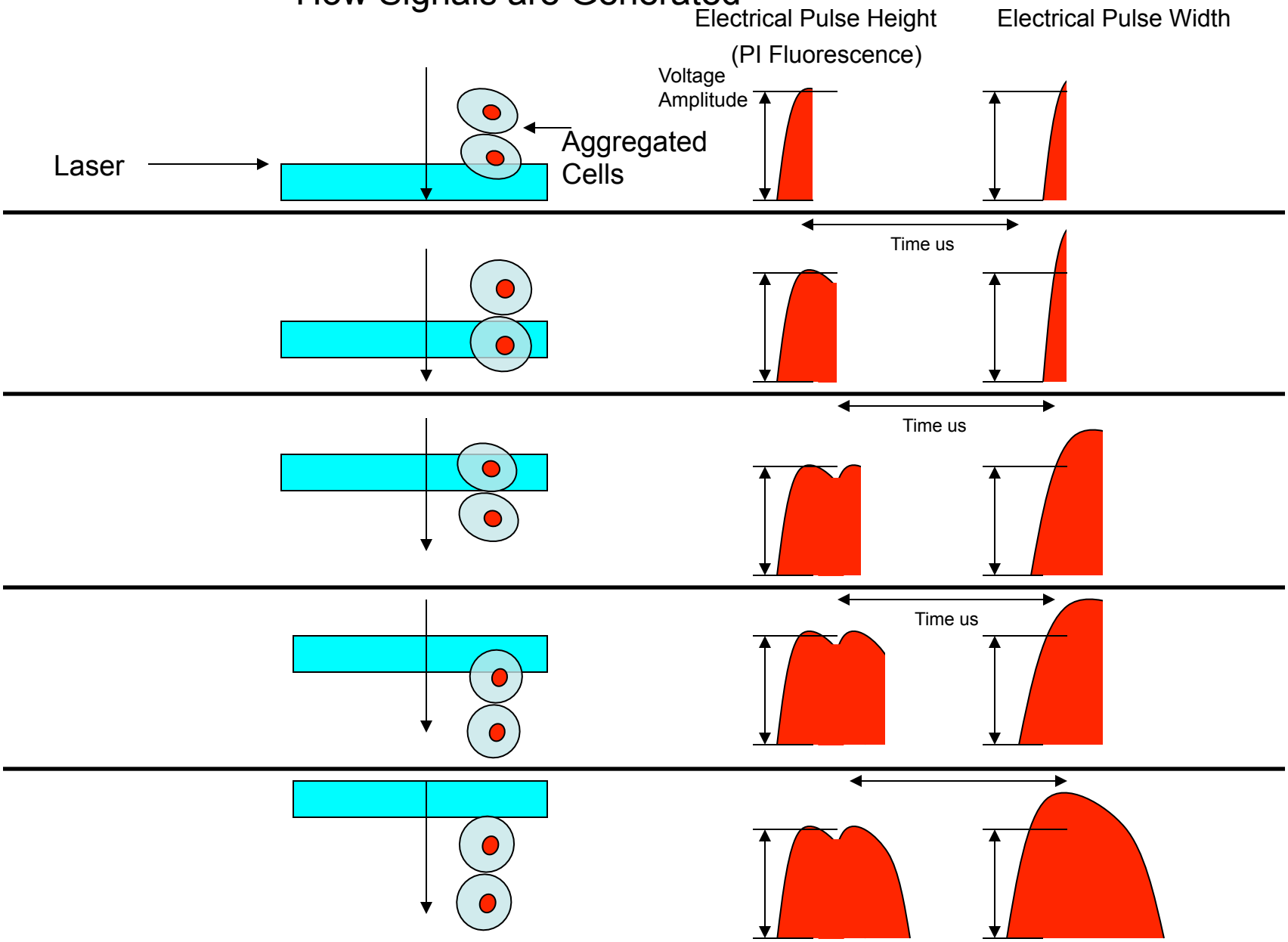
Tight areas for the G0/G1 and G2M peaks. The areas under the peaks are measured by the coefficient of variance (CV).

CV= (SD/Mean channel #) X 100. A lower CV results in less overlap between the G0/G1 and S, and, G2M and S. A CV lower than 8 is considered good.

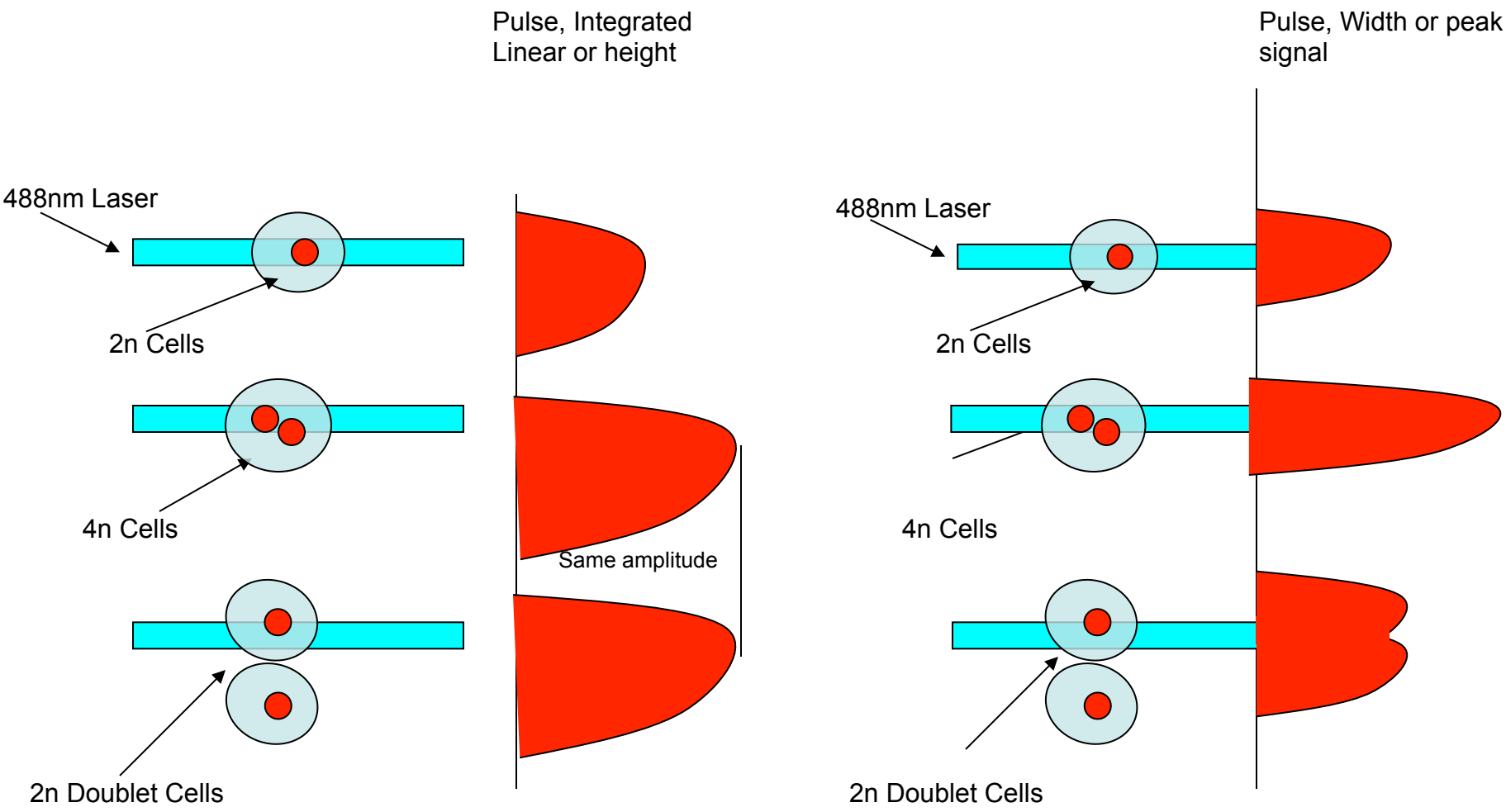
The DNA histogram identifies cells in various stages of the cell cycle.



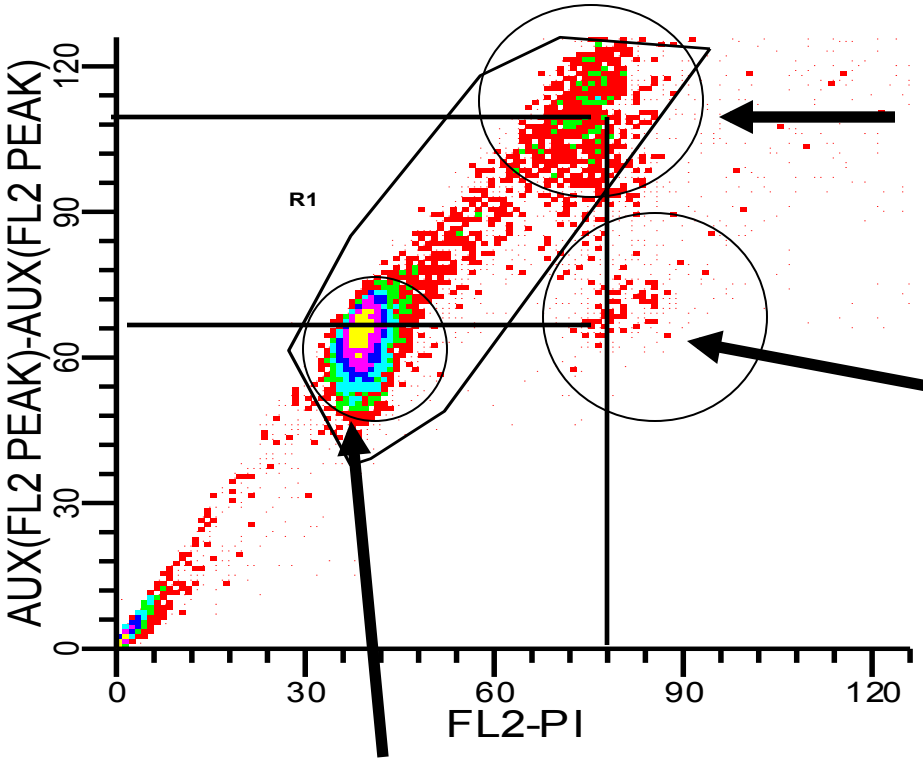
How Signals are Generated

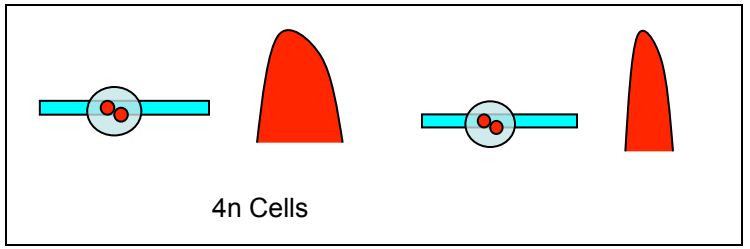


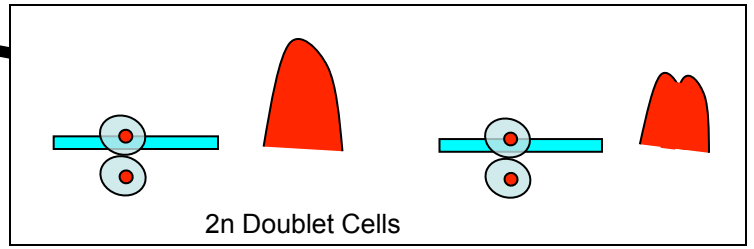
Aggregate Discrimination

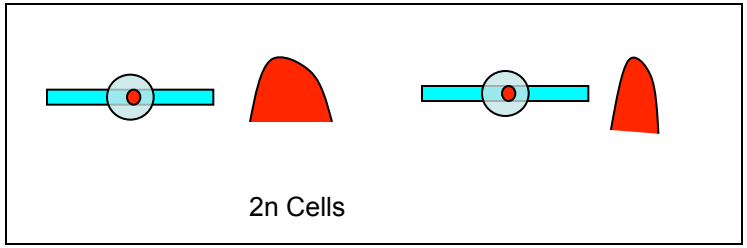


Aggregate Discrimination Dot Plot

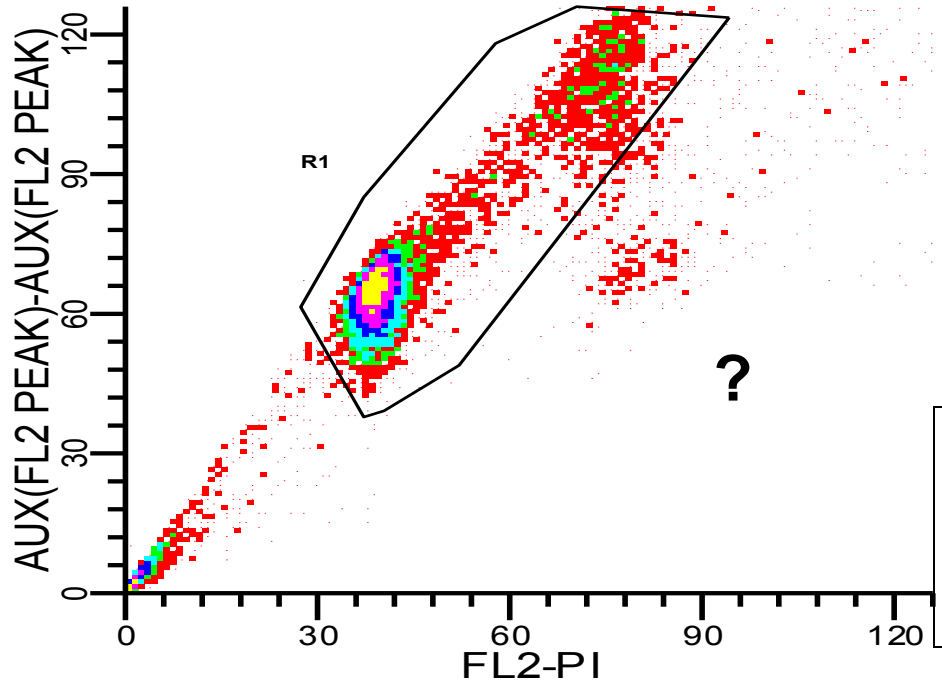


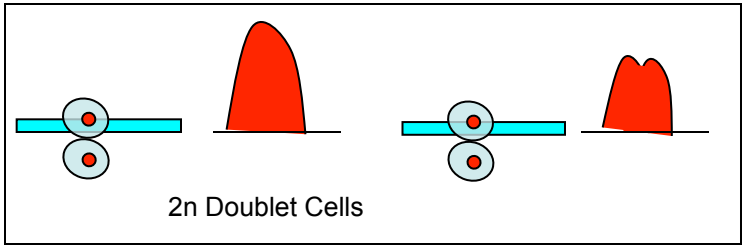


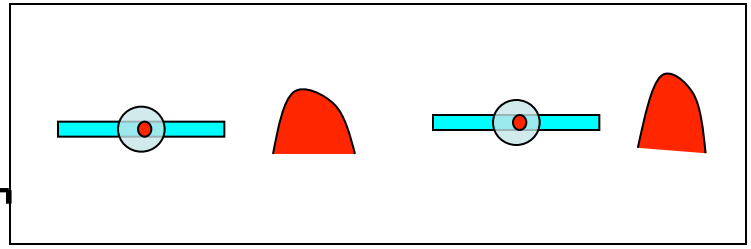




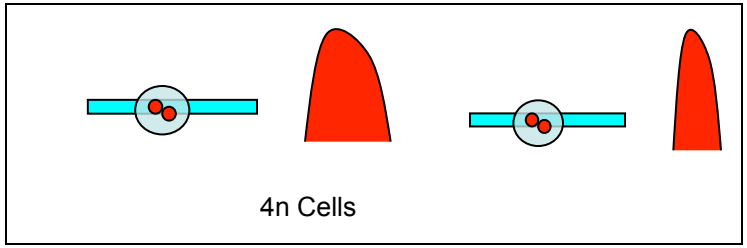
Aggregate Discrimination Dot Plot







2n Cells



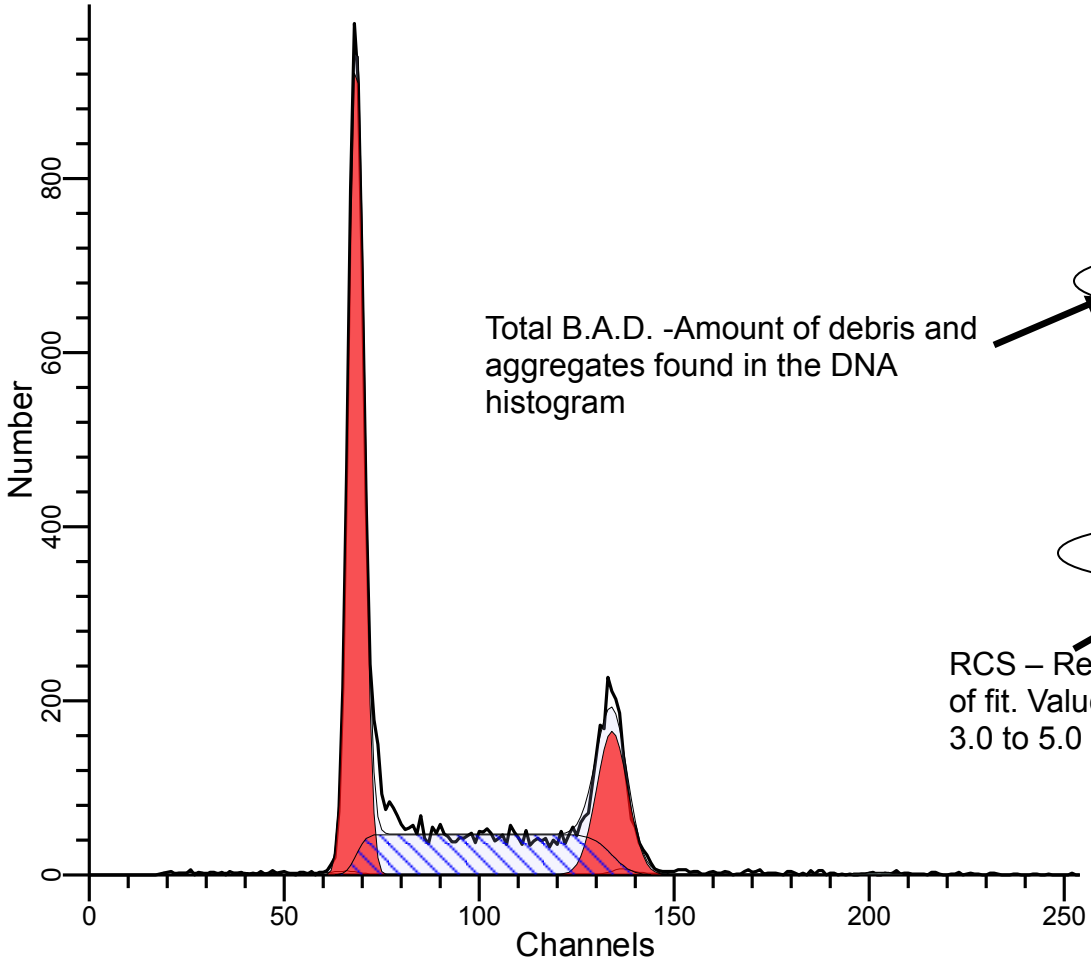
Normal Cell Cycle

File analyzed: Z0043202.H02
Date analyzed: 12-Nov-2008
Model: 1DA0n_DSF
Analysis type: Manual analysis

Diploid: 100.00 %
Dip G1: 50.41 % at 68.45
Dip G2: 17.43 % at 134.08
Dip S: 32.17 % G2/G1: 1.96
%CV: 2.90

Total S-Phase: 32.17 %
Total B.A.D.: 1.15 %

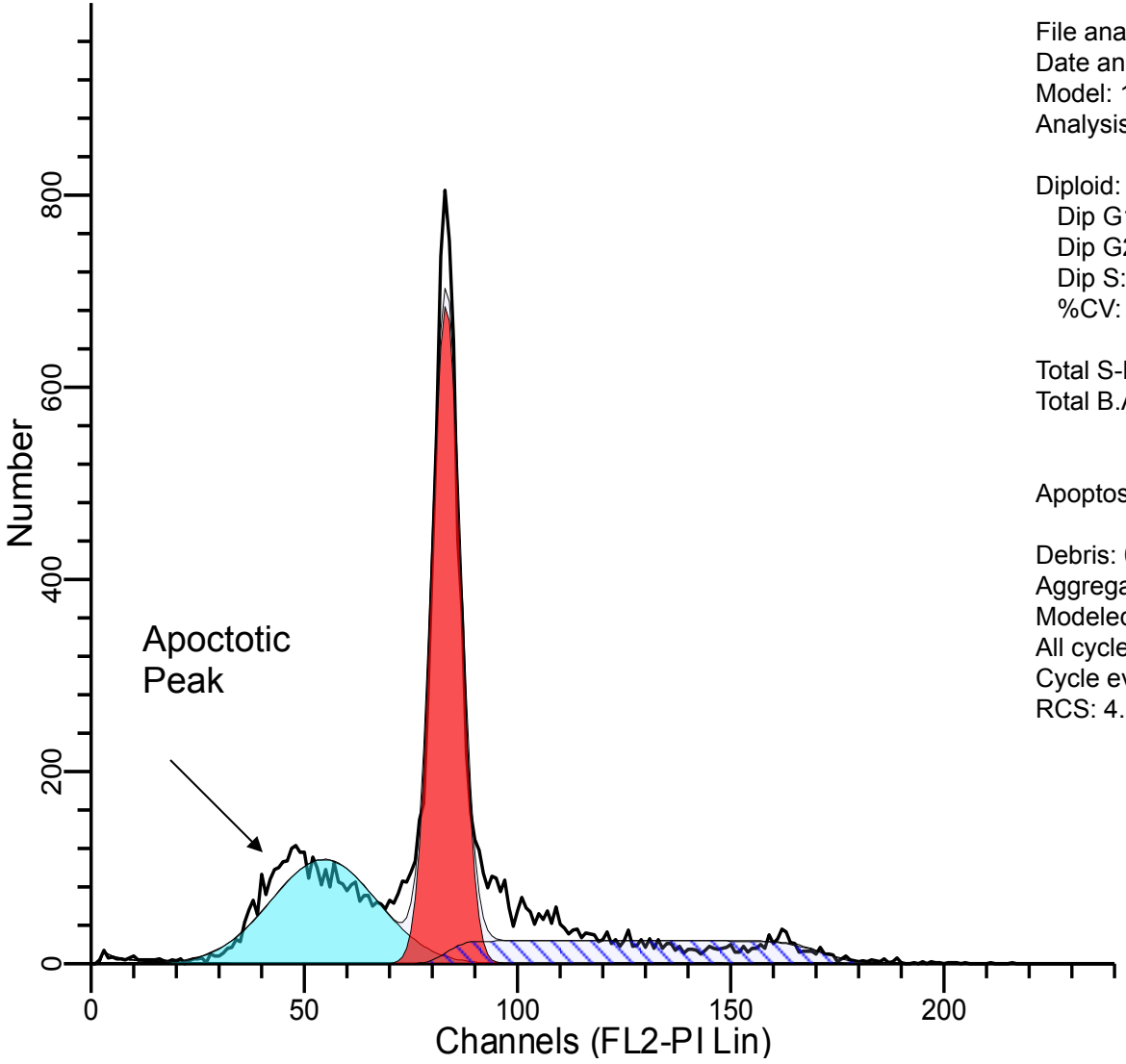
Debris: 2.06 %
Aggregates: 1.65 %
Modeled events: 9767
All cycle events: 9404
Cycle events per channel: 141
RCS: 2.155



Total B.A.D. -Amount of debris and aggregates found in the DNA histogram

RCS – Reduced chi-square, a measure of goodness of fit. Values from 1.0 to 3.0 are considered good, 3.0 to 5.0 fair, values larger than 5.0 are poor.

Apoptosis



File analyzed: G0169300.LMD
Date analyzed: 17-Jun-2008
Model: 1DA0A_DSF
Analysis type: Manual analysis

Diploid: 100.00 %
Dip G1: 73.72 % at 83.24
Dip G2: 0.00 % at 171.00
Dip S: 26.28 % G2/G1: 2.05
%CV: 4.03

Total S-Phase: 26.28 %
Total B.A.D.: 0.71 %

Apoptosis: 29.89 % Mean: 54.60

Debris: 0.81 %
Aggregates: 0.54 %
Modeled events: 11377
All cycle events: 7850
Cycle events per channel: 88
RCS: 4.958