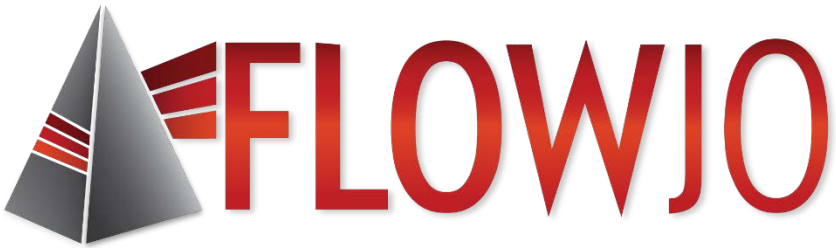
Cell Cycle, Proliferation, Kinetics analysis in FlowJo

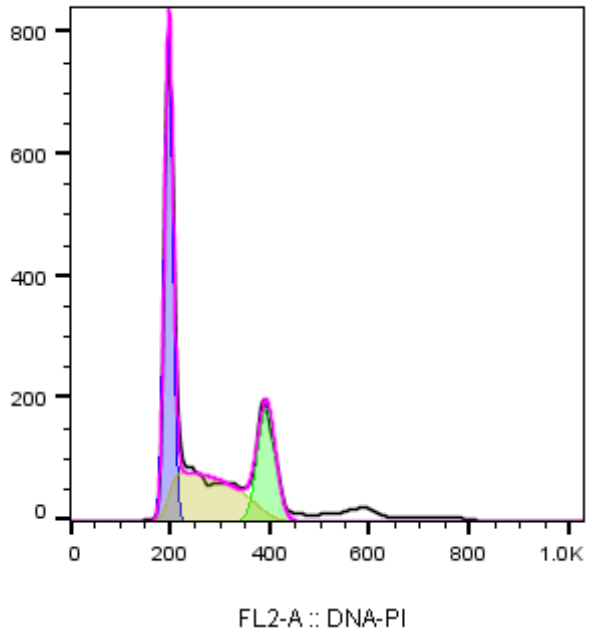


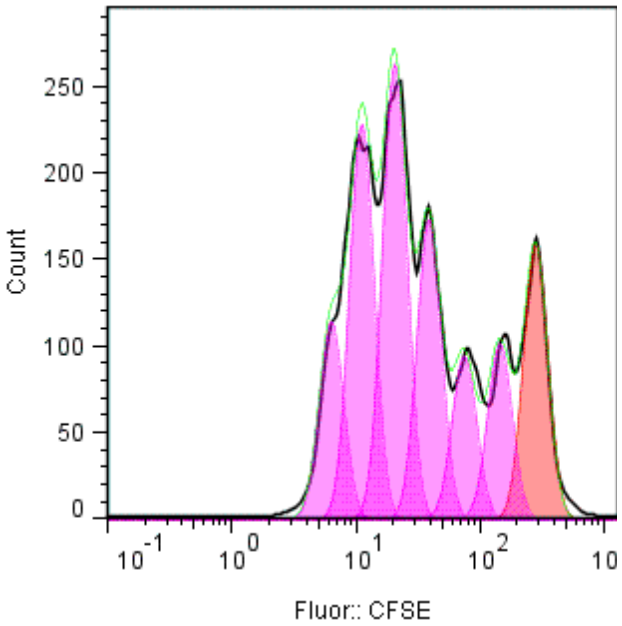
產品專員 蔣明涵 Michelle
techsupport@gtbiotech.com.tw

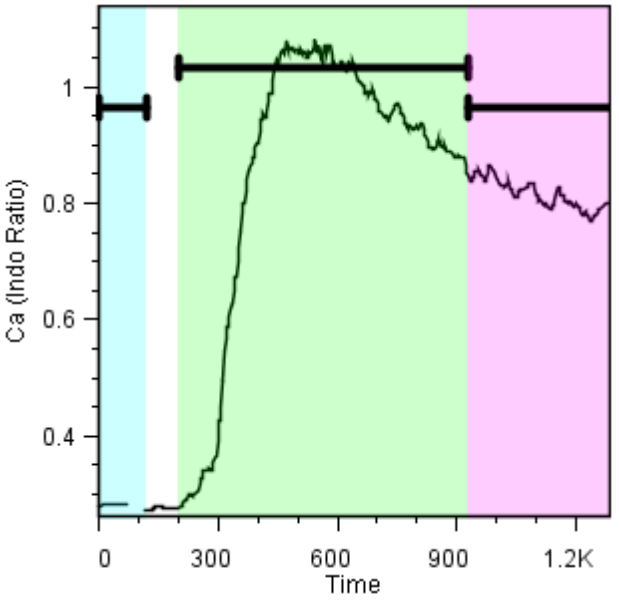
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Outline

- Cell Cycle Analysis
- Proliferation Analysis
- Kinetics Analysis (Calcium flux)



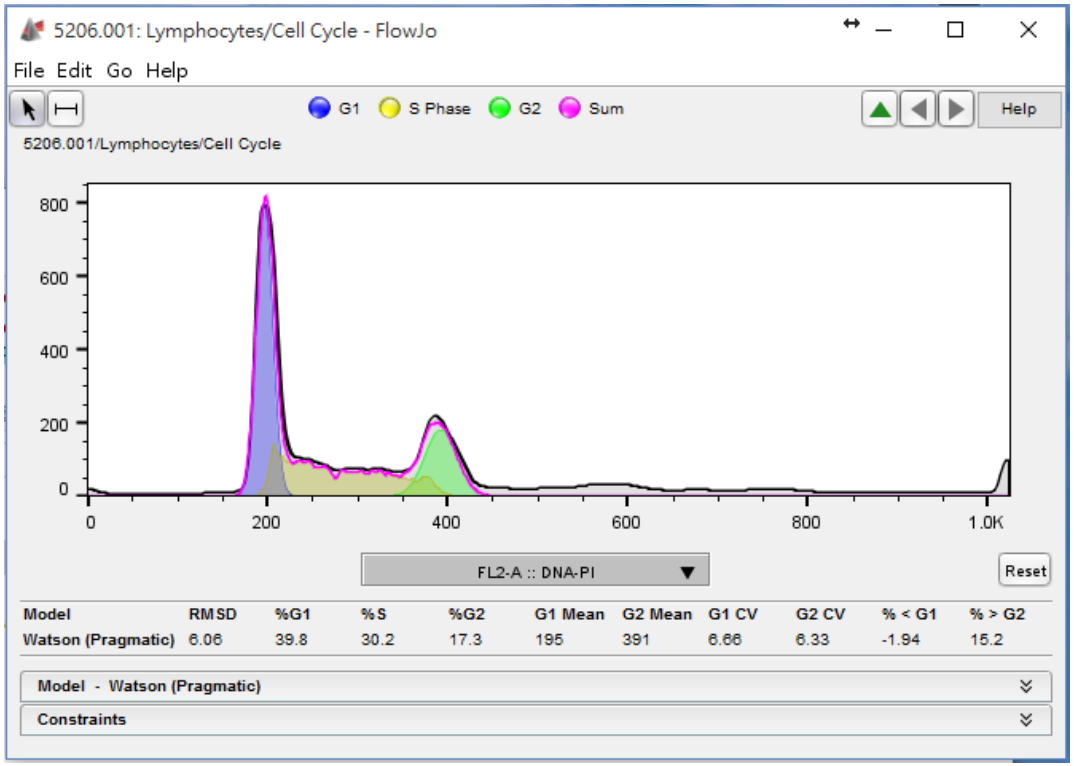




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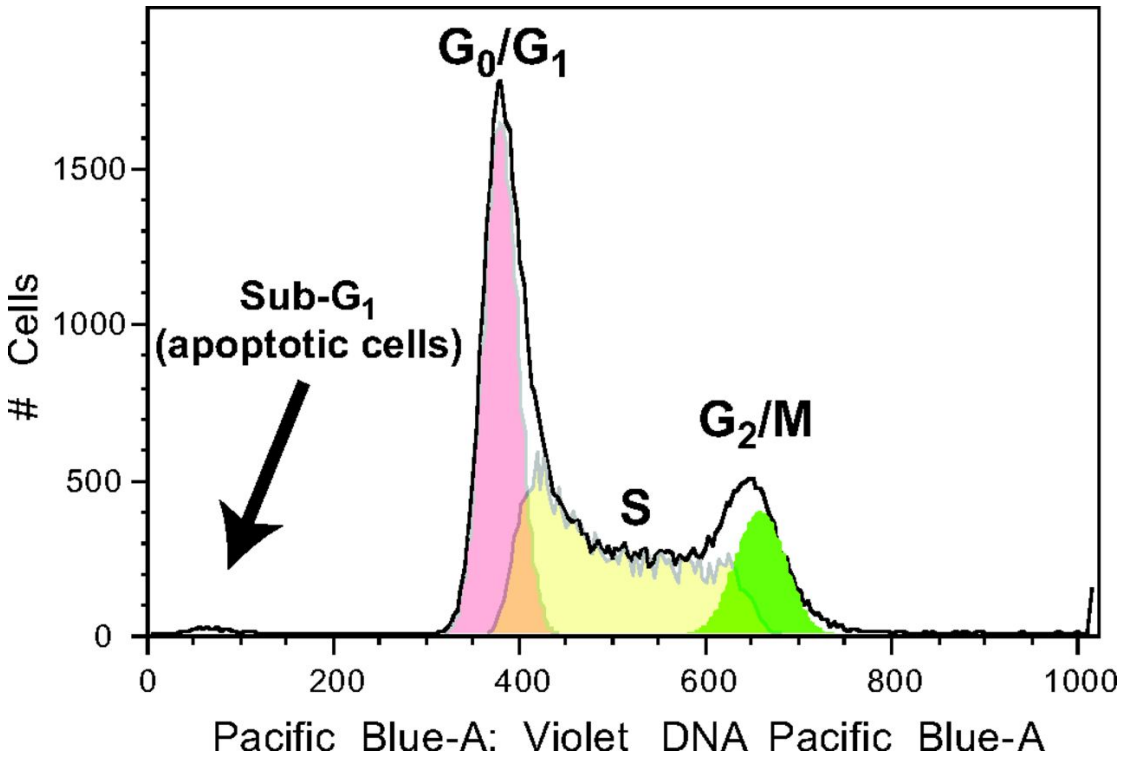
Cell Cycle Analysis

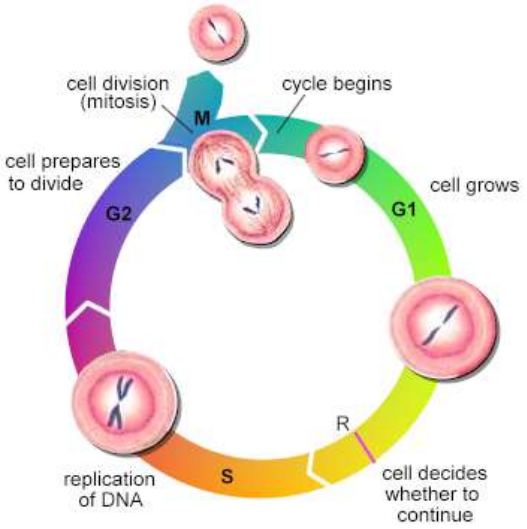
- The Cell Cycle platform allows 1D modeling of cell cycle phases based on DNA content
- V10 has 1D Watson and Dean-Jett-Fox models, similar to FlowJo V9 (Mac) or V7 (Windows)

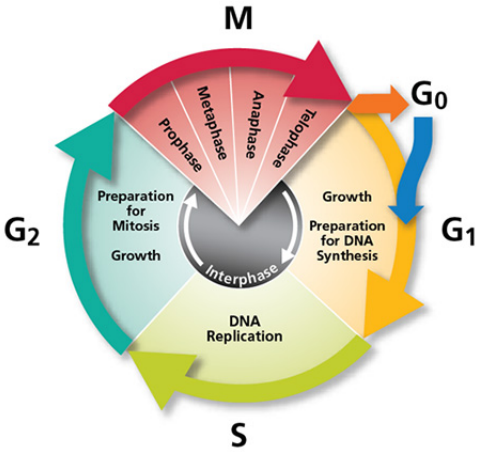


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Cell Cycle Analysis



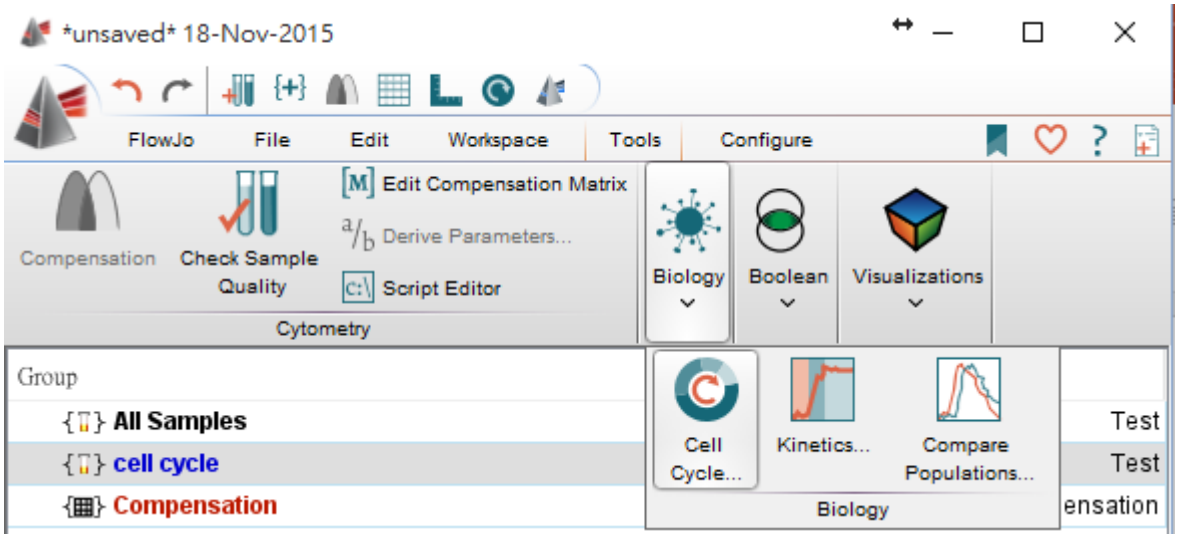




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Pre-Processing

- Removing doublets, dead cells, and debris
- Tools->Biology-> Cell Cycle

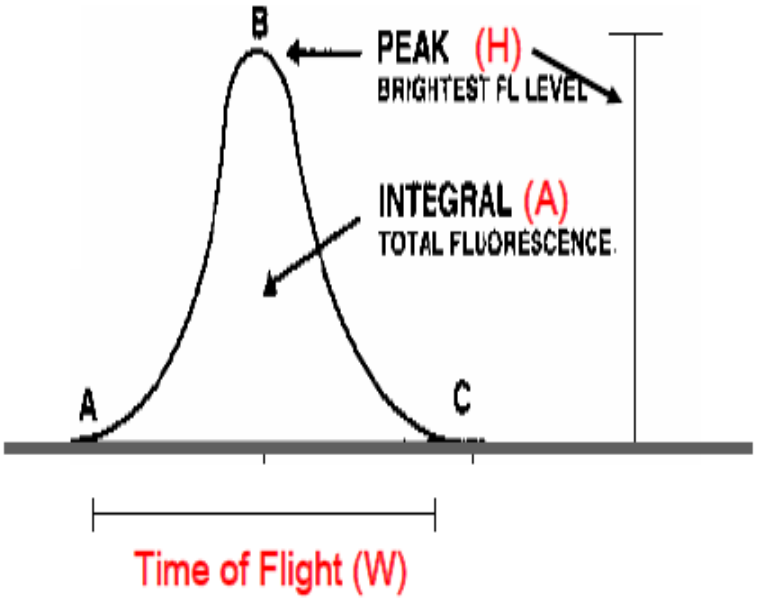


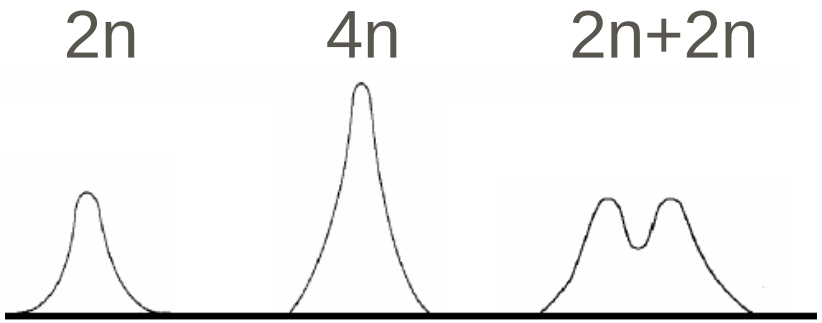
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Doublets Discrimination

What are doublets and why are they harmful?

- Doublets are two cells stick together
- Doublets can lead to higher background and false positive and especially harmful for cell cycle analysis.

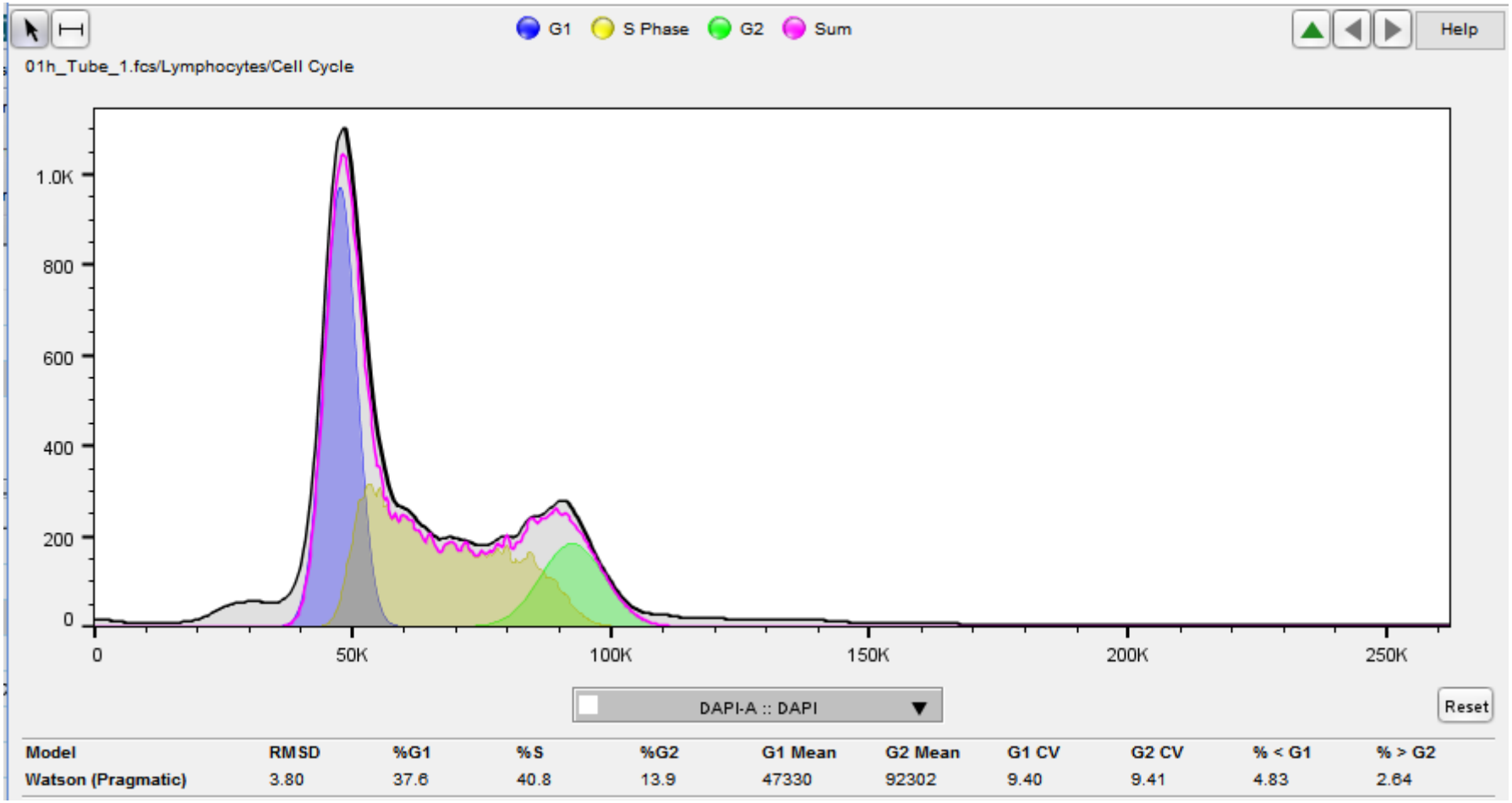




	2n	4n	2n+2n
H	1	2	1
A	1	2	2
W	1	1	2

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Platform



Model	RMSD	%G1	%S	%G2	G1 Mean	G2 Mean	G1 CV	G2 CV	% < G1	% > G2
Watson (Pragmatic)	3.80	37.6	40.8	13.9	47330	92302	9.40	9.41	4.83	2.64

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Parameter

- RMSD: Root Mean Square Deviation, the measure of quality of the fit.
- %G1: 2N
- %S: 2N~4N
- %G2: 4N
- %<G1: <2N
- %>G2: >4N
- G1 Mean
- G2 Mean
- G1 CV: coefficient of variation
- G2 CV: coefficient of variation

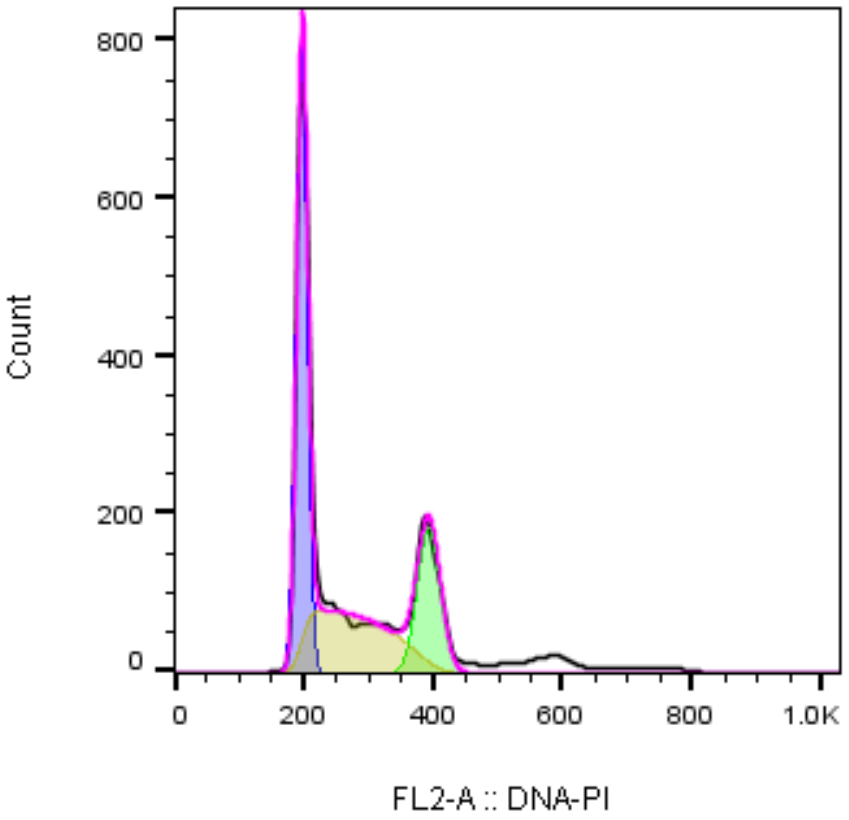
Model	RMSD	%G1	%S	%G2	G1 Mean	G2 Mean	G1 CV	G2 CV	% < G1	% > G2
Watson (Pragmatic)	6.06	39.8	30.2	17.3	195	391	6.66	6.33	-1.94	15.2

Model - Watson (Pragmatic)

Constraints

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Results

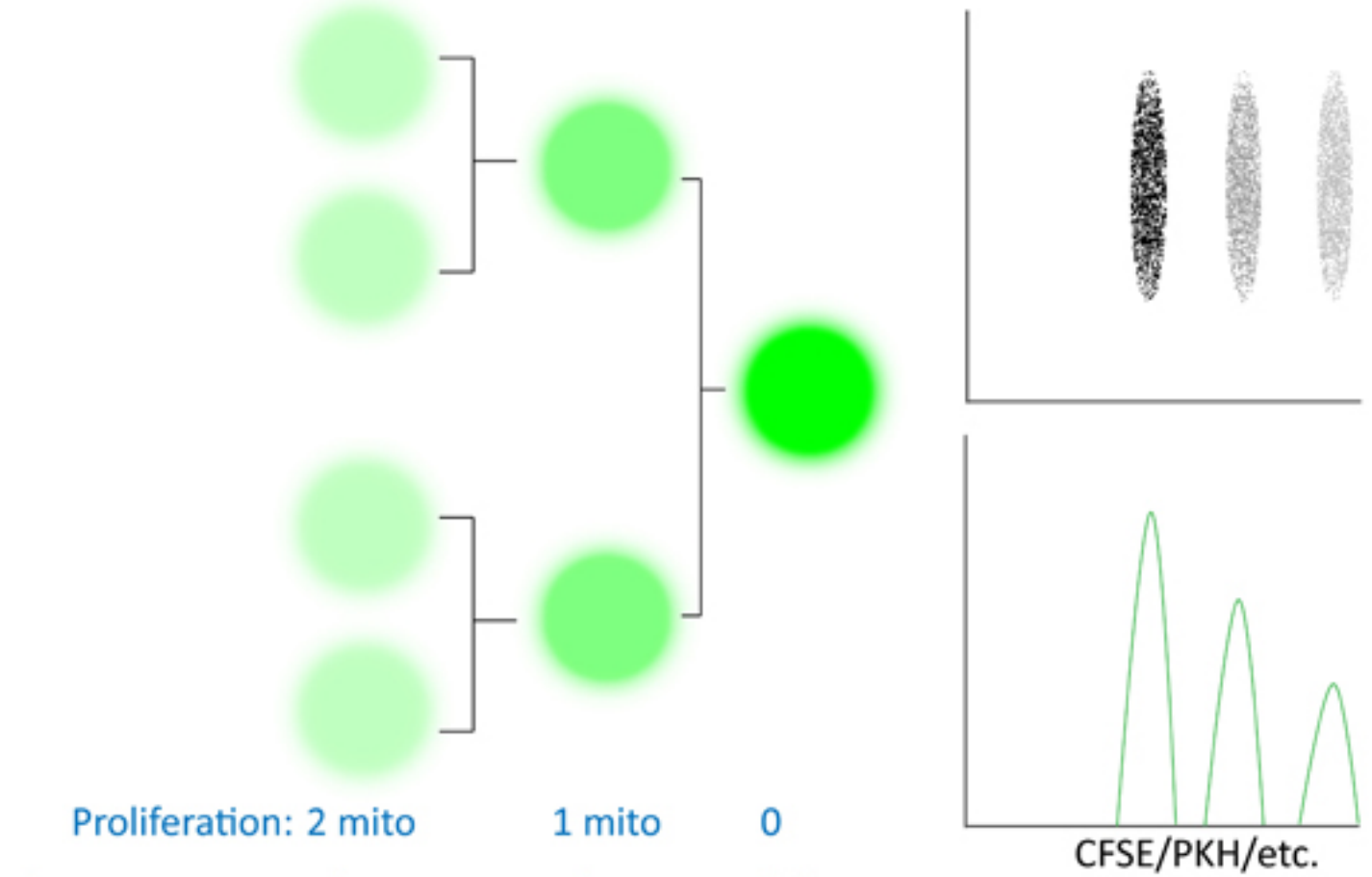


RMSD : 4.58
%G1 : 40.5
%S : 29.1
%G2 : 19.4
G1 Mean : 196
G2 Mean : 391
G1 CV : 6.23
G2 CV : 6.43
% less G1 : -0.89
% greater G2 : 10.6

5206.001
Cell Cycle
11046

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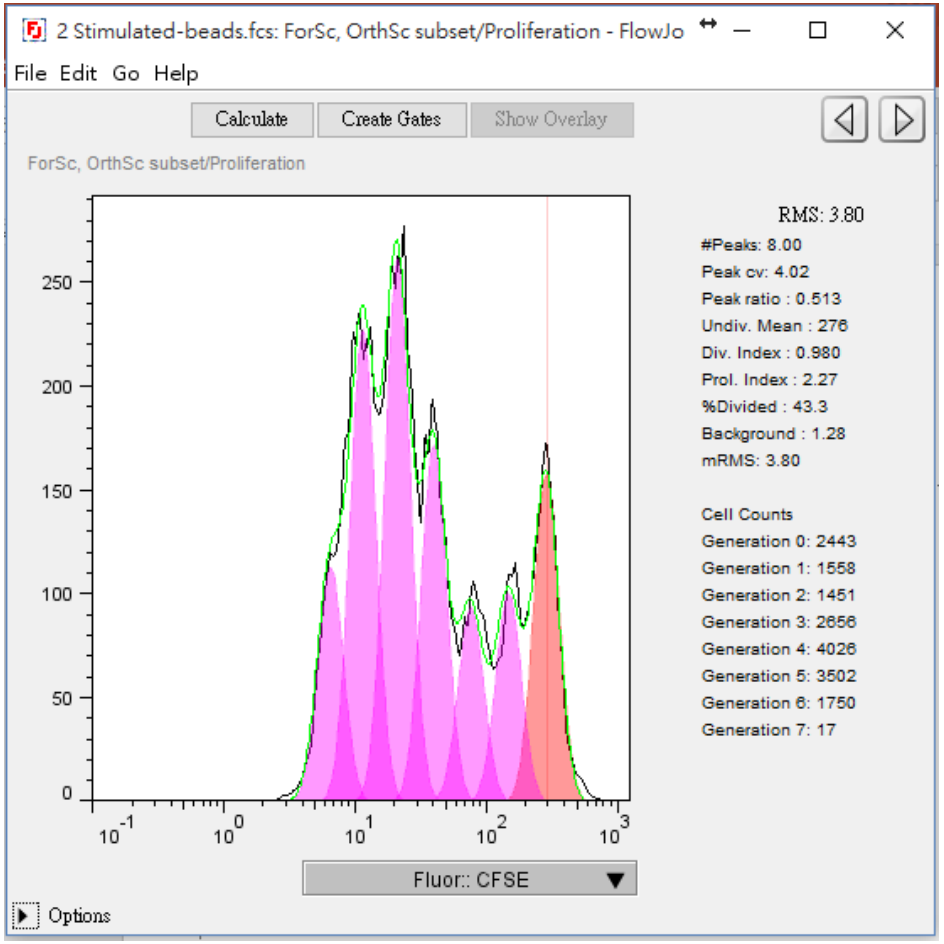
Proliferation



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Proliferation

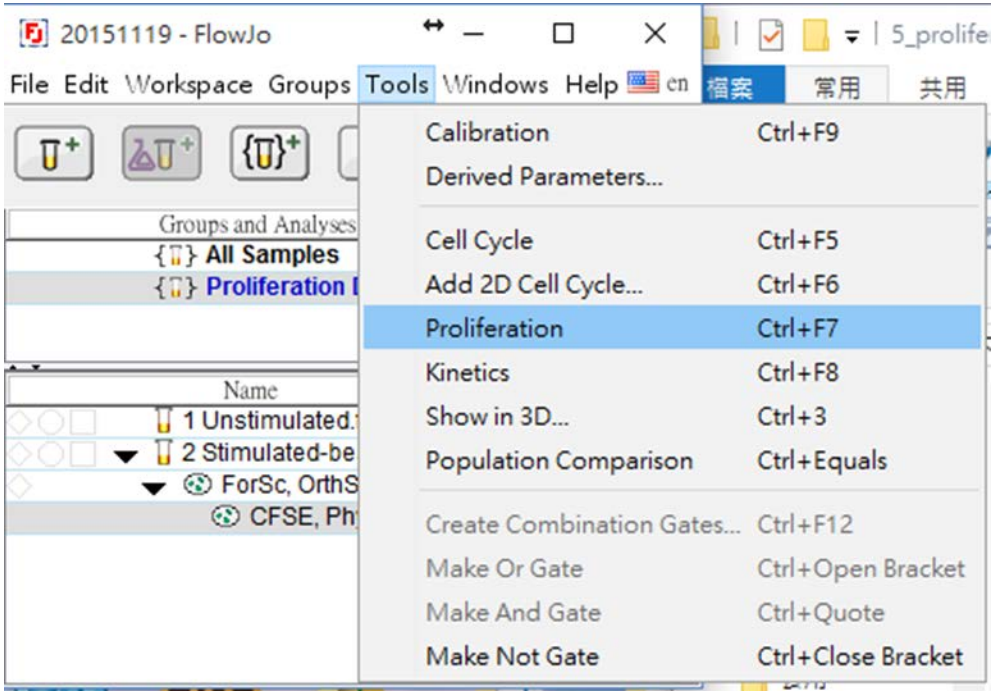
- Please use V7.6.5 (windows) or V9 to analyze proliferation



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Pre-Processing

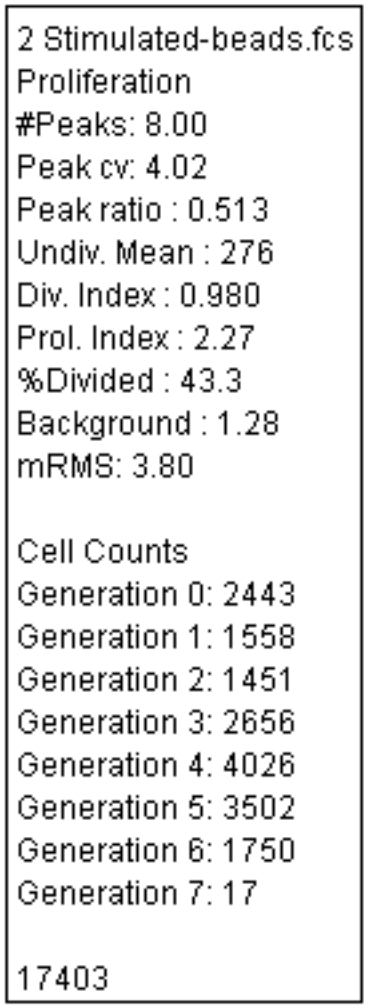
- Removing doublets, dead cells, and debris
- Tools-> Proliferation



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Parameter

- **#Peaks**: Peak number
- **Peak ratio**: the ratio of fluorescence between subsequent peaks. The value should be close to 0.5
- **Undivided Mea**n: intensity of original populations
- **Division Index**: the average number of cell divisions undergone by a responding cell.
- **Proliferation Index**: the total number of divisions divided by the number of cells that went into division
- **% Divided** (Precursor frequency): The percentage of divided cells from the original sample.



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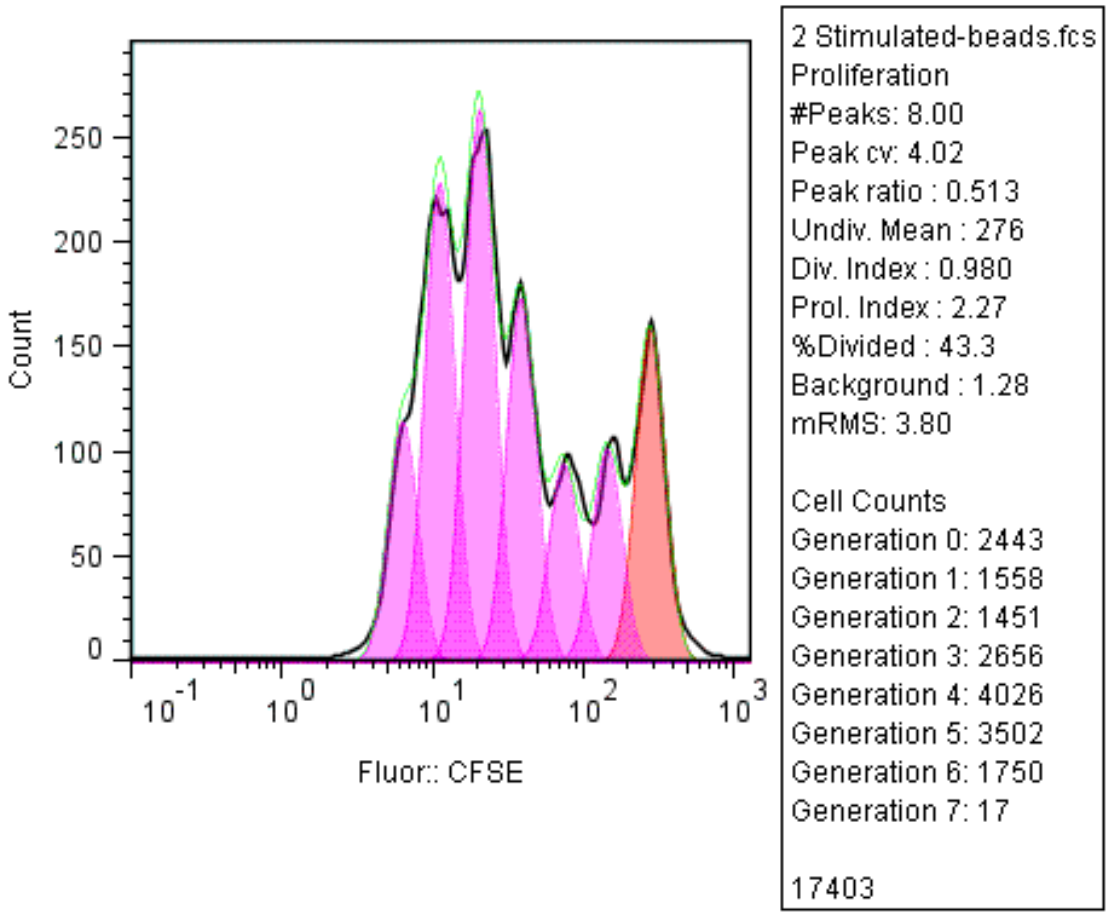
Example of how Proliferation Index and Division Index would be calculated

G0 = 15888 G1 = 32922 G2 = 13647 G3 = 897

- The number of cells at start of culture:
  15888 + (32922/2) + (13647/4) + (897/8) = 35872.875
- The total number of divisions:
  (32922/2)*1 + (13647/4)*2 + (897/8)*3 = 23620.875
- The number of cells that went into division:
  35872.875 - 15888 = 19984.875
- Division Index:
  23620.875 / 35872.875 = 0.66
- Proliferation Index:
  23620.875 / 19984.875 = 1.18

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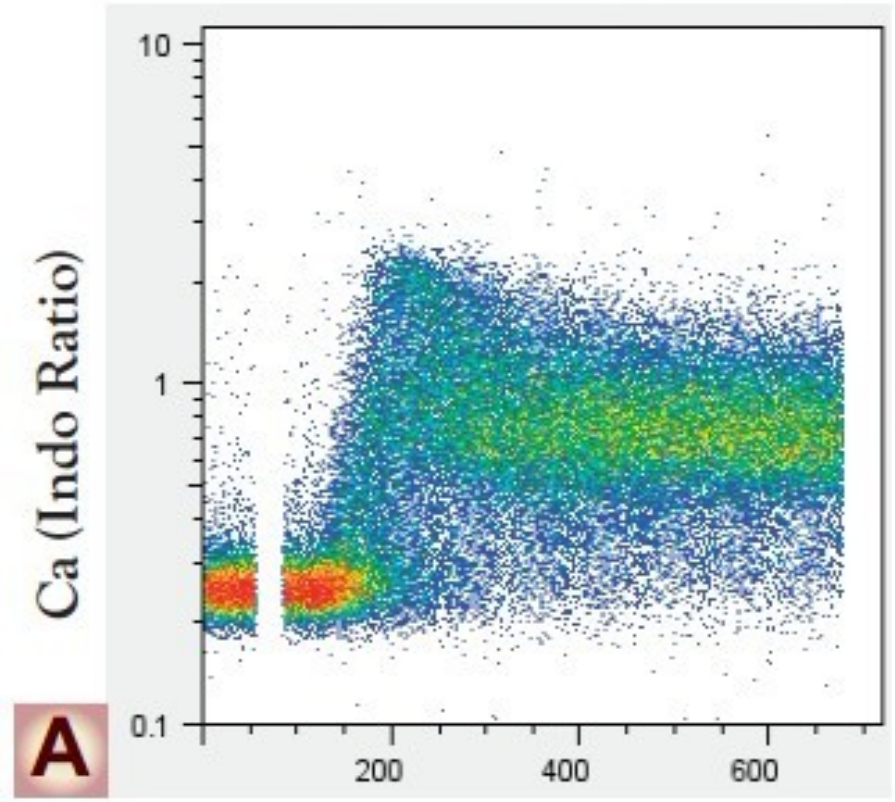
Results



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Kinetics (Calcium flux analysis)

- Kinetics analysis define the change of a specific parameter overt **time**.
- Ex: The change in intracellular calcium concentration when lymphocytes are activated.
- Ratiometric Calcium Dyes: Indo-1, Fluo-4, Fura RED

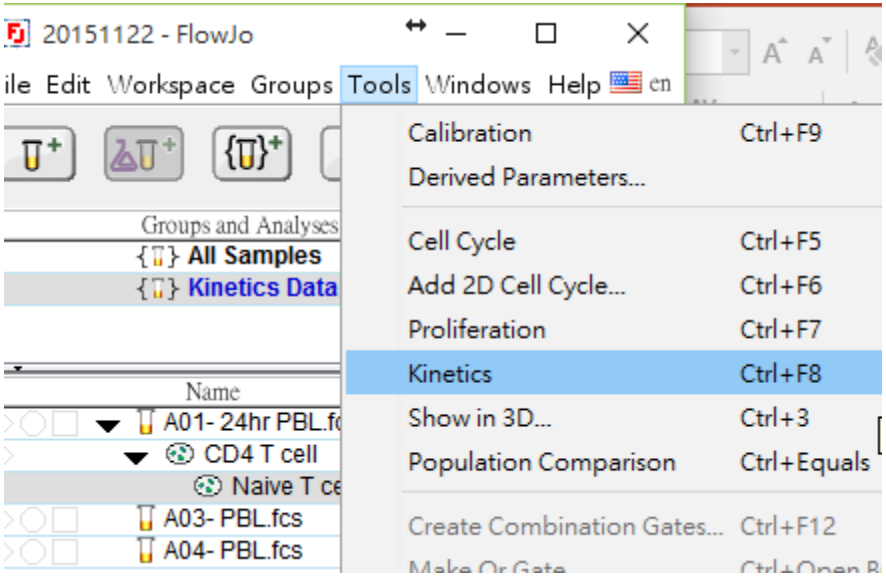


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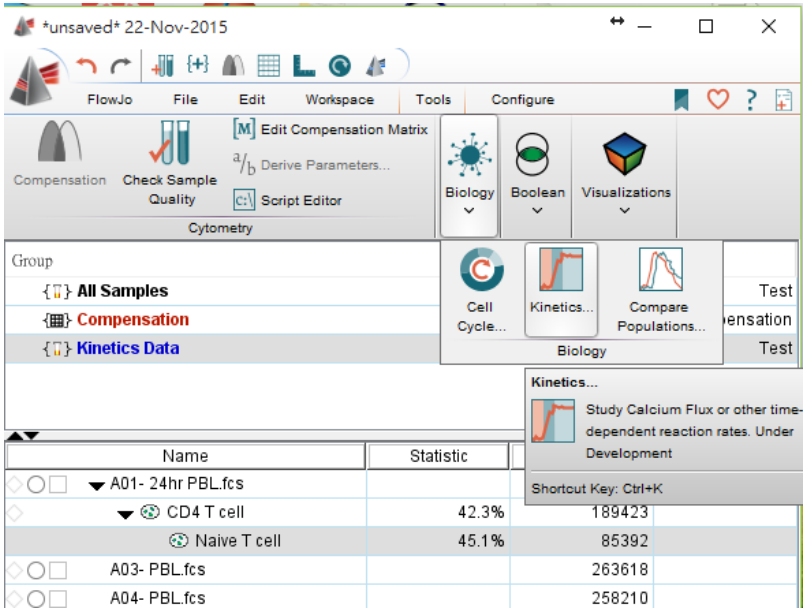
Pre-Processing

- Removing doublets, dead cells, and debris
- Gating response cells. (EX: Naïve T cells)
- Tools-> Kinetics

V7



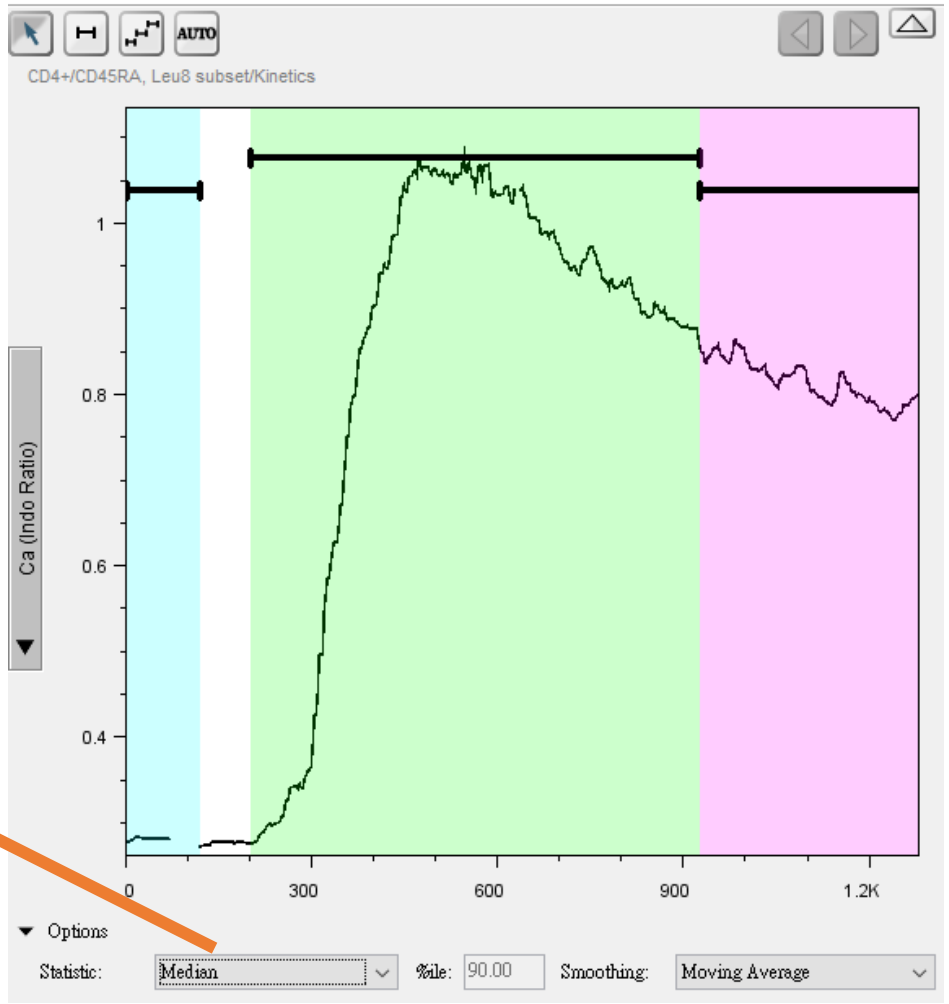
V10



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Parameter

- Median
- Mean
- Geometric Mean
- Percentile
- ---
- % of cells over Threshold T
- Median (for cells > T)
- Mean (for cells > T)
- Geom. mean (for cells > T)
- Percentile (for cells > T)



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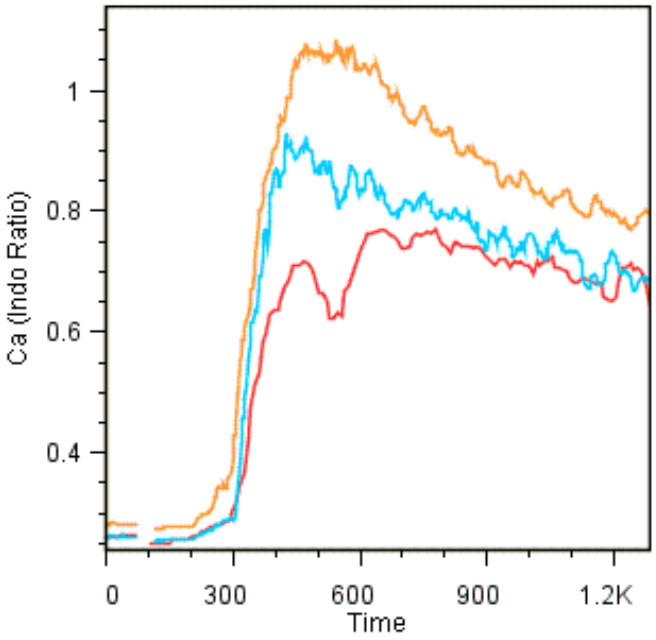
Parameter

Range	Start t	End t	Peak t	Peak	Mean Y	Slope	AUC	dt
All Events	0	671	183	0.92	0.64	8.803E-4	315.94	671
Auto #1	0	51.92	32.07	0.26	0.26	5.628E-6	10.2	51.92
Auto #2	79.88	239.64	182.59	0.92	0.63	4.821E-3	101.13	159.76
Auto #3	239.64	878.69	246.49	0.78	0.73	-2.448E-4	199.26	639.05

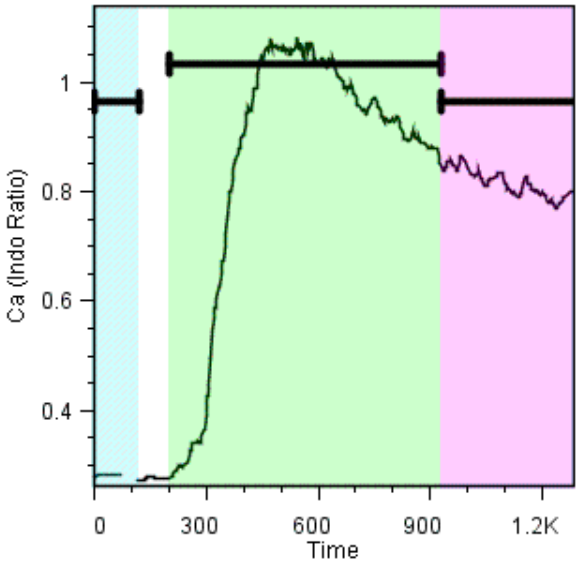
- **Start t**: The time in seconds that a time range begins.
- **End t**: The ending time in seconds of a time range.
- **Peak t**: The time that the maximum Y-axis value occurred at for the specific time range noted.
- **Peak**: The magnitude of the Y-axis value at its maximum for the specific time range noted.
- **Mean Y**: The mean of the Y-axis value for the time range noted.
- **Slope**: The gain or loss of intensity over the duration of the time range, for the calculated linear regression line of the data in this range
- **AUC**: Area under the curve.
- **dt**: Delta time, or the change in time. End t – Start t.

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Result



	Population Name
	Kinetics
	Kinetics
	Kinetics

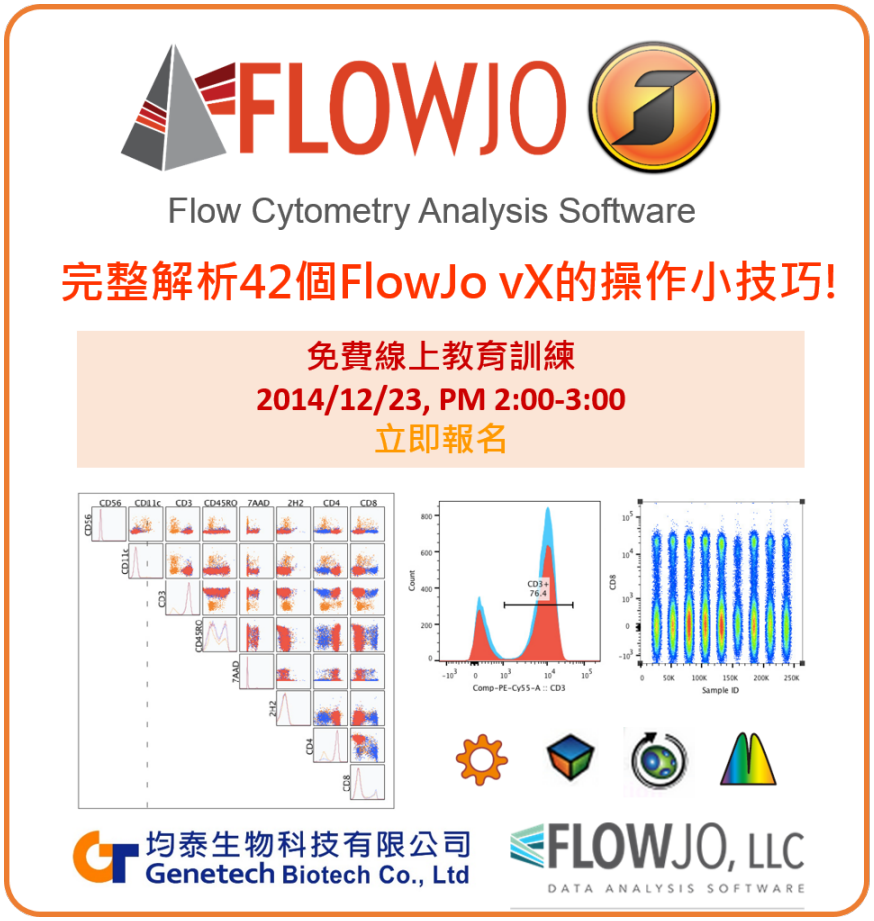


A03- PBL.fcs
Kinetics
Auto #1 (0 - 48), peak: (7.02, 0.29)
Auto #2 (80 - 370), peak: (218.09, 1.08)
Auto #3 (370 - 1478), peak: (391.68, 0.86)

55974

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- 均泰生技提供每兩個月一次FlowJo免費線上教育訓練
- 活動訊息請洽均泰官網或均泰FB粉絲團



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FLOWJO

- Youtube
- WebEx

FlowJo VX Advanced Tutorial



FlowJo Advanced Tutorial: Welcome to FlowJo (1 of 24)
影片上傳者：FlowJoLLC Media
觀看次數：4,733 1 年前



FlowJo Advanced Tutorial: Welcome to FlowJo -...
影片上傳者：FlowJoLLC Media
觀看次數：2,413 1 年前



FlowJo Advanced Tutorial Lesson 1: Workspaces an...
影片上傳者：FlowJoLLC Media
觀看次數：3,192 1 年前

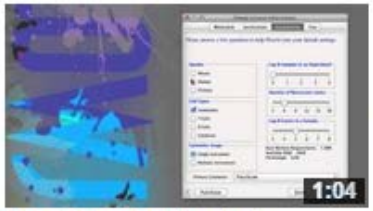


FlowJo Advanced Tutorial Lesson 1 (continued):...
影片上傳者：FlowJoLLC Media
觀看次數：2,101 1 年前

FlowJo VX Basic Tutorial



FlowJo Basic Tutorial: Introduction (1 of 13)
影片上傳者：FlowJoLLC Media
觀看次數：19,115 1 年前



FlowJo Basic Tutorial: Getting Started (2 of 13)
影片上傳者：FlowJoLLC Media
觀看次數：6,532 1 年前



FlowJo Basic Tutorial Lesson 1: The Workspace...
影片上傳者：FlowJoLLC Media
觀看次數：11,396 1 年前



FlowJo Basic Tutorial Lesson 1 Continued:...
影片上傳者：FlowJoLLC Media
觀看次數：5,970 1 年前

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