



## Abstract

Kinetic analyses define the change of a specific parameter over time, such as the change in intracellular calcium concentration that occurs when lymphocytes are activated. This change in calcium concentration is detected using fluorescent calcium-binding dyes.

FlowJo's powerful Kinetics Platform calculates and displays time-dependent data. To enable kinetic analyses, parameters such as time and the ratio of two fluorescent parameters can be calculated by FlowJo. When you launch the Kinetics Platform, FlowJo brings up a graph window that is specially designed for time series analysis. In this window, you define what type of calculation to show as a function of time, which parameter to display, and the time-slices over which to calculate statistics. These statistics (average, peak, slope, peak time, etc.) are calculated for the entire sample and for any time-slice within a sample. In addition, the transformed kinetics data are easily exported to any spreadsheet application for further analysis.

As with all analysis functions in FlowJo, the customized kinetic analysis created for one subset can be copied to any other gated subset within the same or different samples, or even to entire groups of samples. In addition, the Kinetics graphs can be overlaid in the Layout Editor to compare responses between subsets or samples.

## General Information

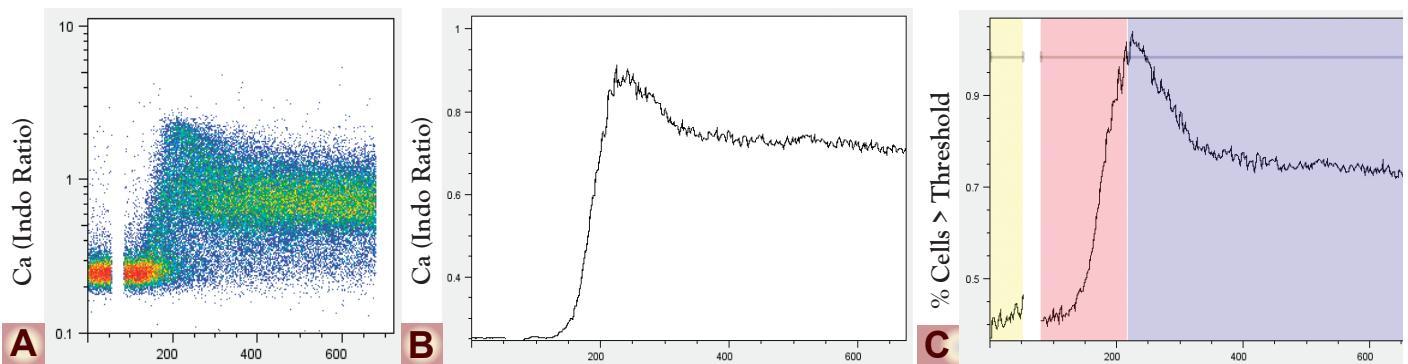


Figure 1. Calcium flux of peripheral-blood naïve CD4 T cells. a) Dot plot displaying the ratio of Indo-1 fluorescence vs. time b) The median Indo-1 fluorescence ratio as a function of time c) The fraction of responding cells as a function of time

Figure 1 shows three different displays of calcium flux data from human CD4 naïve T cells stimulated with anti-CD3 antibodies. Each graph display provides unique information about the data.

Figure 1A is a dot plot of the calcium-binding dye fluorescence vs. time for each cell in the sample. Although the response is visible, the large number of dots tends to obscure the trend and often prevents comparison of two different samples.

Figure 1B summarizes the same data by displaying the median fluorescence value of the cells at each time point. Thus, the data are simplified to a single line that clearly conveys the response of the cell population and facilitates comparison between multiple samples. You can view the median, mean, or geometric mean of a parameter as a function of time to view the change in the population's central tendency.

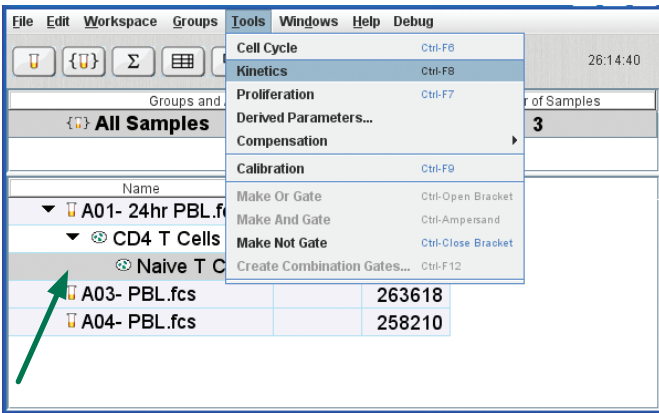
Figure 1C shows another useful measure, the percentage of cells that are responding to the stimulus. This statistic is calculated by setting a threshold value and computing the percentage of cells with a fluorescence value above this threshold. The baseline value can be either an absolute fluorescence value or a statistic computed on a time-slice within the data. Generally, the baseline is derived from the events collected prior to stimulation. In addition, statistics such as median and mean can be calculated for these responding cells.



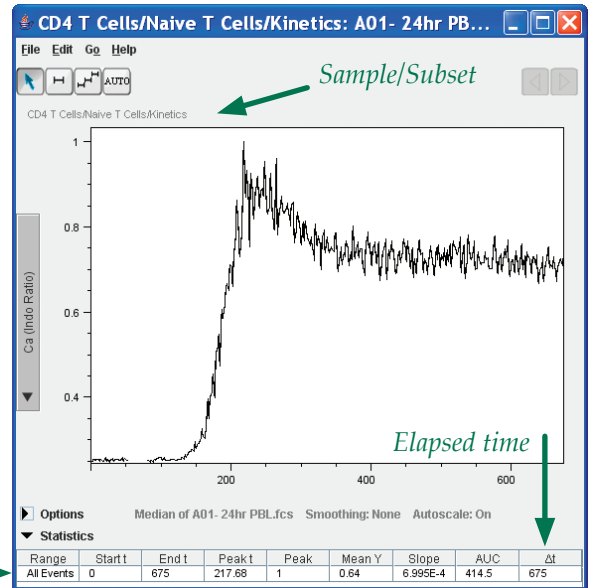
# Kinetics

## Procedure

**1** Launch the Kinetics platform. Click once to select the sample or subpopulation in the workspace you would like to analyze. Under the **Tools** menu, select **Kinetics**.



Kinetics Analyses are performed only on this selected population.



Name of Time-slice

Start and end time

Time of peak value

Peak value (scaled units)

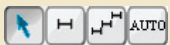
Average value over time-slice

Linear least squares

Area under the curve

**2** Choose graph display options.

Create single or multiple time-slices or automatically generate a set of slices. (See Step 4.)



- ForSc
- Ca (Indo Ratio)
- Fluor
- PhyEry
- Cy5PE
- Time

Select parameter to analyze.

Open options.

Median, Mean, and Geometric mean display the central tendency of the data.

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

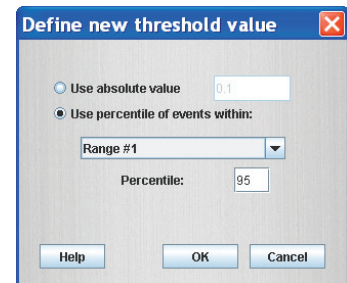
% of cells over Threshold displays the fraction of responding cells.

(for cells > T) displays the statistic for cells over threshold.

**3** Set Threshold.

Select a Threshold value to calculate the fraction of responding cells.

Set New Threshold



Absolute fluorescence or Relative fluorescence (based on a time-slice as defined in the next step)

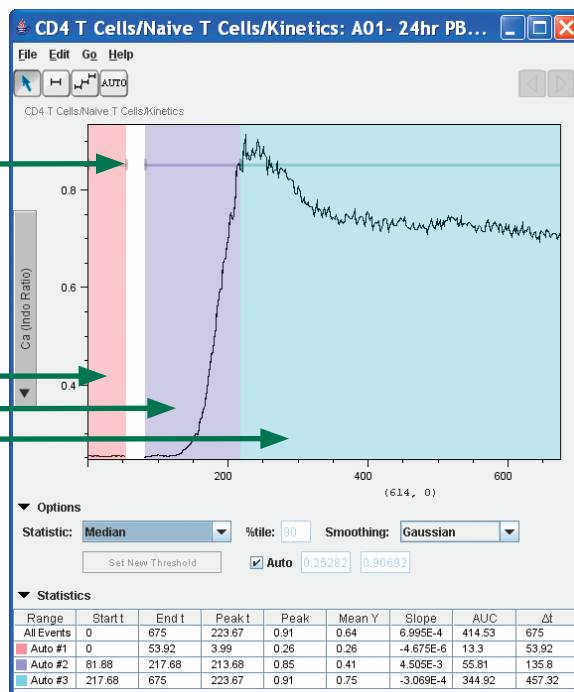
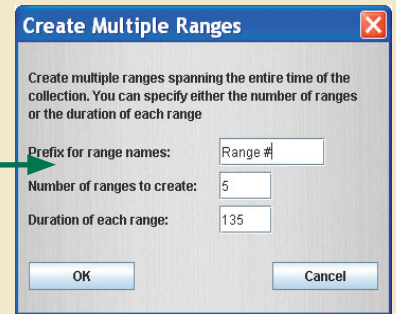
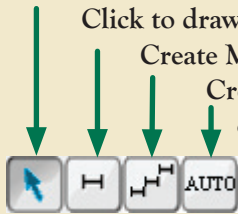
## 4 Create time-slices.

Click to select and edit a time-slice.

Click to draw a new time-slice with the mouse.

Create Multiple Ranges creates a series of equal time-slices.

Creates three time-slices: one before the stimulation break, one as the ratio rises to a peak, and the third after the peak.



Click and drag the gray handles to adjust start and end times. Values update in the table below.

Background  
Response  
Resolution

Statistics displayed in the table are calculated for individual time-slices as well as for all cells.

## 6 Generate a table of statistics in the Table Editor.

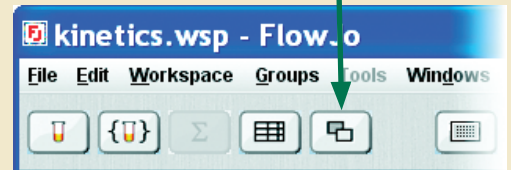
Drag the Kinetics node to the Table Editor. Press the Make Table button.



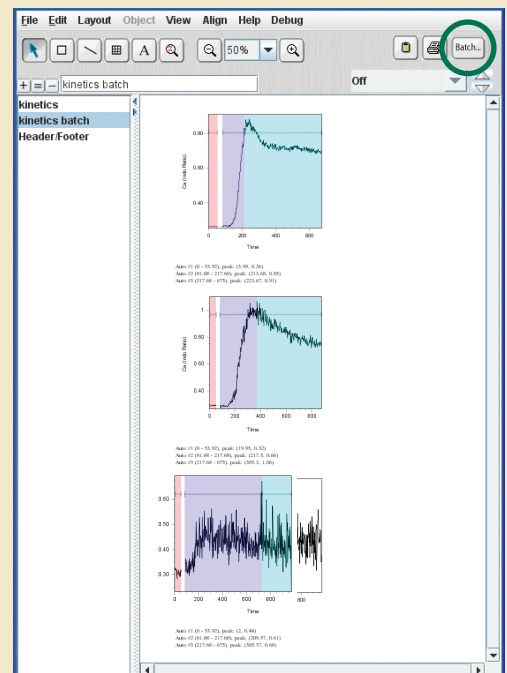
	A	B	C	D	E	F	G	H	I
Samples	Start t	End t	Peak t	Peak	Mean Y	Slope	AUC	Δt	
A01- 24hr PBL.fcs	0.0	675.0	217.7	1.1	0.7	0.0	452.8	675.0	
A03- PBL.fcs	0.0	872.0	305.3	1.1	0.8	0.0	635.2	872.0	
A04- PBL.fcs	0.0	972.0	718.5	0.7	0.5	0.0	484.7	972.0	
Mean	0.0	839.7	413.8	1.0	0.7	0.0	524.2	839.7	
Standard Deviation	0.0	151.1	267.5	0.2	0.2	0.0	97.4	151.1	

## 5 Generate a graphical report in the Layout Editor.

Open the Layout Editor and drag the Kinetics node to the Layout Editor.



Generate a batch layout to display graphs from all samples.





## Hints

- Kinetics require that there be a parameter in the data named **Time**. If FlowJo doesn't find a time parameter, it will use the event number, which, assuming a constant flow rate, corresponds to time.
- Often the parameter you want to see across time is the ratio between two collected signals. For example, the fluorescent emission of the calcium-binding dye Indo-1 is detected in two separate channels, and the ratio of these emissions correlates with calcium concentration [1]. Create a *derived parameter* to plot the ratio of two collected parameters.
- Note that the ratio of the Indo-1 fluorescences is not linearly correlated to calcium concentration. This correlation must be determined using external reference standards [2].
- The Kinetic data platform summarizes the fluorescence of each cell vs. time. The sample is divided into bins, based on time. FlowJo calculates the total fluorescence of the cells in each bin. The bins can be thought of as a non-overlapping set of narrow gates that divide the data into time-slices (usually 1 - 2 seconds each).
- Displaying your data as a mean, median, and geometric mean shows the central tendency of the data, whereas a percentile shows the tendency of any chosen fraction.
- To determine the fraction of responding cells, you need to set a baseline level or threshold. When you select the **% of cells over Threshold** option, you

also need to enter a fluorescence value above which a cell is deemed to be responding. This threshold can be either an absolute number or a percentile computed from a time-slice you have defined in the Kinetics tool. (Create a time-slice by clicking and dragging over the desired region in the Graph window.)

- Normally the background time-slice is based on events collected prior to stimulus. The advantage of defining the threshold based on the background events is that the background level may be different from one sample to the next. A relative baseline value will adjust itself properly when you copy the kinetic analysis to other samples.
- To edit the threshold to more accurately display the data, press the **Set New Threshold** button.
- The 95<sup>th</sup> percentile of the background population can be used as a starting point to calculate the level of background fluorescence. Prior to stimulus it is common to find about 5% of cells fluxing calcium spontaneously.
- The final four statistics in the function menu are calculated only for the responding cells. The mean, median, geometric mean, and percentile are displayed for the cells with a value over the designated threshold.
- The platform's **Edit** menu offers the ability to copy the kinetics plot alone, the plot including gates, the statistics in text form, or the Time/Value Series in text form.

## Links and References

1. June CH, Rabinovitch PS. **Flow cytometric measurement of intracellular ionized calcium in single cells with indo-1 and uo-3.** *Methods Cell Biol.* ; 33:37-58 1990.
2. Schepers E, Vanholder R, et al. **Flow cytometric calcium flux assay: Evaluation of cytoplasmic calcium kinetics in whole blood leukocytes.** *J ImmunMeth.* Vol 348, 1-2, 31 August 2009, p 74-82.
3. Kaposi et al. **Cytometry-acquired calcium-flux data analysis in activated lymphocytes.** *Cytometry A.* 2008 Mar; 73(3):246-53.
4. Walshe C, Cragg M, et al. **Induction of cytosolic calcium flux by CD20 is dependent upon B Cell antigen receptor signaling.** *J. Biol. Chem.* 2008 283: 16971-16984.



FlowJo is a product of Tree Star, Inc.

[www.flowjo.com](http://www.flowjo.com)

1- 541-201-0022

email: [flowjo@treestar.com](mailto:flowjo@treestar.com)

TN.Kinetics.20100423