

BASIC FLOW CYTOMETRY GLOSSARY AND CELL CYCLE ANALYSIS TERMINOLOGY

Amplifier Electronic component of a flow cytometer that increases the signal by an adjustable factor.

Aneuploid Having an abnormal number of chromosomes. Aneuploid cells may also have an abnormal DNA content.

Average The mean value, which is the total amount divided by the number of contributors.

Channel The measured value of a parameter, representing the signal intensity of an event after amplification. On an analog cytometer, to appear on a plot, data for an event must fall into one of either 256 channels (0-255) or one of 1024 channels (0-1023) depending on the resolution of the plot. On a digital cytometer channel resolution is 262,144 individual channels.

Compensation An electronic calculation that removes signal overlap which the optical system cannot remove. Fluorescence compensation works for specific pairs of fluorescent parameters; for example, FL1 and FL2, or between all fluorescence parameters that are measured in a polychromatic experiment.

Cursor A highlight appearing in a data field that indicates you can modify this field, on a dot plot, a crosshair for drawing a polygon gate, on histograms: a line separating regions on single parameter histograms that are treated statistically separate. See marker.

%CV Percent coefficient of variation is a measure of peak distribution. The percent coefficient of variation is the standard deviation of the peak divided by the mean channel number of the peak, times 100.

Data file A file that contains list mode data.

Diploid Having the normal number of chromosome pairs for non-reproductive, mammalian cells. The amount of DNA in diploid cells defines the normal DNA content for the species.

Disk Directory List of data files on a storage device.

DNA Index The ratio of GO/G1 peak channel in a DNA histogram of the experimental sample to the GO/G1 peak channel of the reference sample, when normal human diploid cells or nuclei are the reference. This is a measure of DNA aneuploidy, or abnormal DNA content.

Dot Plot A two parameter data graph used for acquisition and analysis. Each dot on the display represents one event that the flow cytometer analyzed.

Doublet Two particles stuck together, which a flow cytometer records as one larger event. Particles may also occur in triplets and higher associations.

Event A unit of data representing one particle or cell. An event has a relative intensity value and channel number for each parameter.

Filter An optical device used to attenuate particular wavelengths or frequencies while passing others.

Fluorescence A property of molecules to absorb light at one wavelength and emit light at a longer wavelength. Flow cytometers detect Fluorescence emission at a 90 degree angle to the exciting light beam.

Forward Scatter (FSC) A parameter measuring light scattered less than 10 degrees as a cell passes through the laser beam. The FSC measurement is related to cell size.

G0 Phase of cell cycle designating cells that are quiescent and have not yet entered the growth cycle. Normal cells in this phase have exactly one set of chromosome pairs.

G1 Phase of cells cycle in which cells are committed to division. These cells have the same number of chromosomes and the same amount of DNA as G0 phase cells. They are about to enter S phase where they synthesize DNA.

G2 Phase of cells cycle in which proliferating cells have duplicated their DNA and formed two sets of chromosome pairs, in preparation for division. G2 follows the S phase and precedes the M (mitosis) phase.

Gain An adjustment that modifies amplifier responses. Increasing the gain increases the electronic pulse (and the relative fluorescence intensity value and the channel value) in response to a light signal.

Gate A boundary that defines a subset or sub-population of events. Gates are set by drawing boundaries around the subsets on data plots (dot plots or histograms). Use gates either for data acquisition or analysis. Inclusive gates select only the events that fall within (and on) the boundary. Exclusive gates select only the events that fall outside of the boundary.

Live gate Gate through which any data from the flow cytometer/computer parallel interface must pass before acquisition. If the acquisition criteria are set in such a way that only data within a gate are stored, any data outside the gate does not enter the computer.
Analysis: Gates used in analyzing the data. Data which fall outside the analysis gate bounds remain in memory but are not included in analysis.

Gate Set A collection of gates that limits data acquisition or analysis. Each parameter can only be used once in a gate set. Gating trees allow for the selection of subsets of cells that fulfill certain criteria and typically use the "AND" Boolean operator.

Gating The process of drawing a gate boundary on a dot plot or histogram with arrow keys or a mouse. Also refers to applying a gate set to data.

Haploid Having one set of single chromosomes. Reproductive cells, like eggs and sperm, are haploid.

Histogram A data plot of a single parameter. This displays either the relative fluorescence intensity value or the channel number on the x axis and the number of events on the y axis.

Linear scale The scale on which the output is directly proportional to the input. When the control is set at Linear, the voltage measured is directly proportional to the channel into which the event falls.

Linearity The flow cytometer's ability to accurately measure DNA content.

List Mode Data File File containing all measured parameters for each event, in sequence.

Logarithmic scale The scale on which the values increase logarithmically. This scale is used when the instrument is set to LOG.

M Phase of the cell cycle, mitosis, during which a cell divides into two. This precedes G₀/G₁ and follows G₂. M phase and G₂ cells contain the same amount of DNA and the same number of chromosomes. In normal cells, this is the tetraploid number (4N) of chromosomes.

Marker A line separating regions on single parameter histograms that are treated statistically separate. See cursor.

Model The mathematical method of DNA data analysis.

Operator An individual who can operate a flow cytometer and is supposed to produce data that investigators like.

Parameter A measurement of light intensity, either scattered or emitted fluorescence, from a particle as it passes through a laser beam.

Photodiode A semiconductor device used to detect light and generate an electrical current. Typically used in forward scatter (FSC) detection.

Photomultiplier A photodetector, with adjustable voltage, that translates optical tube (PMT) signals into electrical current. PMT detectors are used for SSC and fluorescence parameters. Increasing the PMT voltage, increases the output signal for a given amount of light.

Ploidy The number of chromosomes present in the cell. The amount of DNA in a cell gives an indication of the ploidy, but is not directly proportional. See Haploid, Diploid, and Aneuploid.

Pulse The electronic signal generated by a particle (cell or nucleus) in the flow cytometer.

Rate The number of events per second processed by the computer.

Reference Sample A sample of known DNA content mixed with an experimental sample to provide an internal standard.

S Phase of the cell cycle, DNA synthesis, in which the cell duplicates its DNA.

Scale The maximum number of events displayed for a channel in a histogram. A low scale number magnifies the data.

Setup Manual or automatic adjustments of flow cytometer settings for consistent performance using stable beads or fixed biological preparations.

Side Scatter (SSC) Also called 90° scatter or right angle scatter. Light scattered at a 90 degree angle as a cell passes through the laser beam. This measurement is related to the internal granularity or complexity of a particle.

Singlet A single particle (cell or nucleus), which a flow cytometer records as one event.

Threshold The lowest channel number for a selected parameter for which an event may be recorded. The flow cytometer sends to the computer only events above the threshold level.