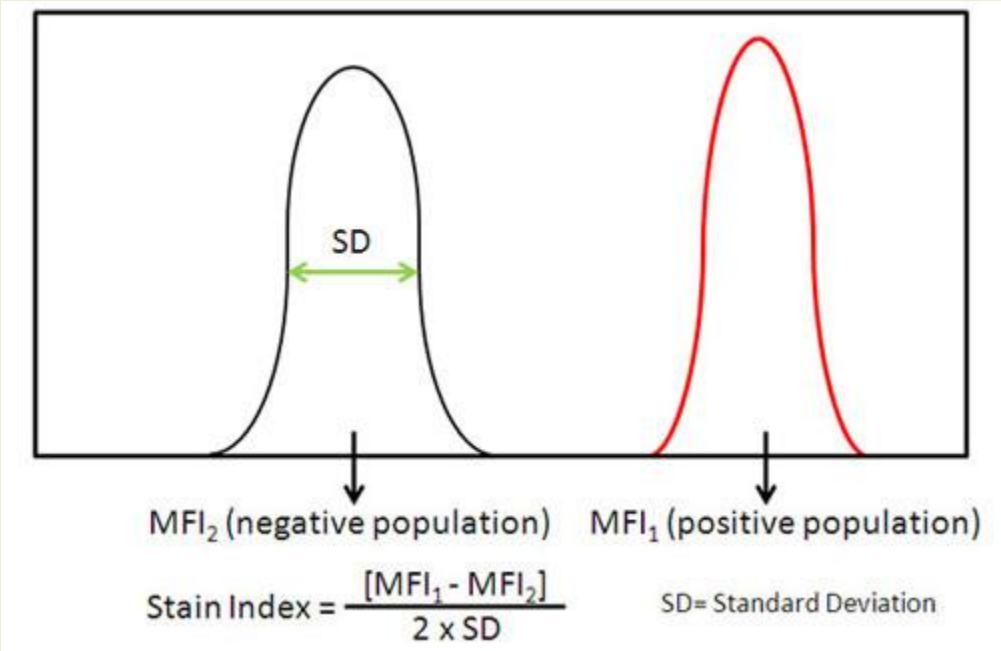
Stain index:

Stain index is a measure of resolution sensitivity (the ability to resolve a dim positive signal from background). It depends upon the difference between positive and background peak medians (D) and the spread of the background peak (W). The stain index is a metric that captures both of these factors.

Scientists have agreed that the overall brightness of a fluorophore can be estimated by the relationship between the positive and the negative (background) signals. In turn, the background in a particular detector is affected by **Signal intensity, Autofluorescence of the sample, The amount of non-specific staining, Electronic noise of the instrument, Potential contribution of other fluorophores when co-staining**.



So, the brightness of the different fluorophores can only be relative due to the multiple variables affecting their performance. These specific factors, among others, can significantly affect the stain index of any fluorophore ,Antibody clone and quality, Fluorophore purity and quality, Degree of labeling, or fluorophore to protein ratio (f:p ratio), Laser line and power, Longpass and bandpass filters used, Target cells used. Since conjugation processes and f:p ratios can vary from company to company, it is important to understand that SI values can also vary accordingly.

The experiment done in our lab to find out the stain index of the flurochrome which we are using. For this purpose we have use CD45 conjugates which are generally using in our lab(Beckman Coulter and Biolegend). Used control sample for staining and follow the same surface staining procedure in which lyse stain wash was done.

Post staining the sample run on Gallios with some fix events on a pre form protocol but during acquisition also check any spilling and spectral overlap problem or voltage issue to get the accurate results.

The lmd's can be seen on gallios software or any other analysis software (kaluza)Beacause gallios software don't have option for standard deviation so prefer to use some analysis software otherwise standard deviation can also be calculate by geometric mean and coefficient of variation(cv). Formulae available on internet or SD=2*GM *CV

After all this calculation the stain index of different flurochrome is as under shown in table form Gallios machine. This result may vary from machine to machine and also due to lab work and handling but more or less the ranking may little variate. other factor is the antibody storage and handling condition and it clone also have some effect on it . so this is the ranking which we have found in our lab.

Stain Index Ranking

laser	FC	emission	excitation	PMT	SI RANK
488nm	**AF 488**	495	519	FL1	**11**
488nm	**FITC**	493	525	FL1	**9**
488nm	**PE**	496, 565	575	FL2	**2**
488nm	**ECD**	496, 565	613	FL3	**8**
488nm	**PC5**	496, 565	670	FL4	**NA**
488nm	**PC5.5**	496, 565	690	FL4	**1**
488nm	**PC7**	496, 565	774	FL5	**3**
633nm	**APC**	645	660	FL6	**7**
633nm	**AF 700**	696	719	FL7	**4**
633nm	**AF647**	650	668	FL6	**10**
633nm	**APC750**	650	774	FL8	**12**
405nm	**KO**	398	528	FL9	**13**
405nm	**PB**	410	555	FL10	**14**
405nm	**BV421**	405	421	FL9	**6**
405nm	**BV 510**	405	510	FL10	**5**

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