

# Content

---

Description .....	2
Usage .....	2
Voltration Wizard.....	3
1.    Rainbow Beads + Unstained Cells.....	4
2.    Single Stained .....	5
Results Window .....	6
Report .....	9
Appendix - Algorithm .....	11

## Description

---

The plugin allows the user to determine the best voltage for acquisition that will optimize the stain index (SI) of cells stained with a reference reagent for each detector (see the Appendix - Algorithm for more information on the calculation).

The plugin allows the user work with different types of samples, to perform the analysis:

The user can use rainbow beads + unstained cells to determine the best voltage (we will see in Rainbow Beads + Unstained Cells part)

or

The user can use other configurations, like unstained cells + single beads, or stained cells where determine the negative and positive gate or a mixture of them and define a custom analysis (we will see in Single Stained part)

## Usage

---

You need to create the plugin node in any population/sample of the workspace by clicking the option in menu Plugins/Voltration.

In case you want to perform the analysis on a specific population, you must create the plugin node on that population.

And after configuring the voltration plugin settings (See Voltration Wizard for more details) the user can inspect the results by clicking on the plugin node created above (See Results Window for more details).

# Voltration Wizard

---

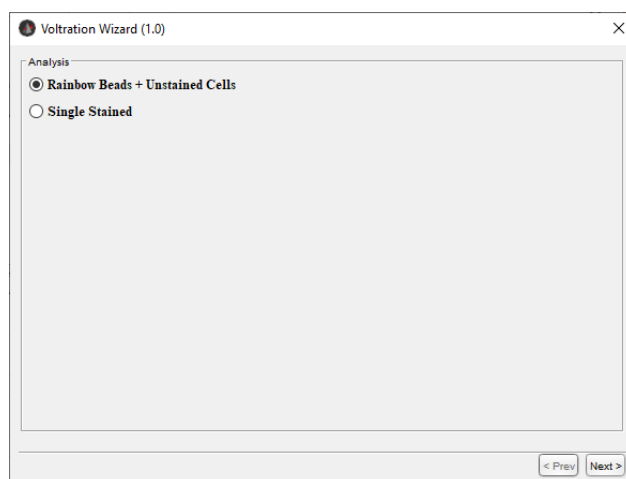


Figure 1 First wizard step

This first step will allow you to choose the type of analysis. There are two possible options:

1. Rainbow Beads + Unstained Cells
2. Single Stained

Depending on the option selected the next step will let you enter different information.

## 1. Rainbow Beads + Unstained Cells

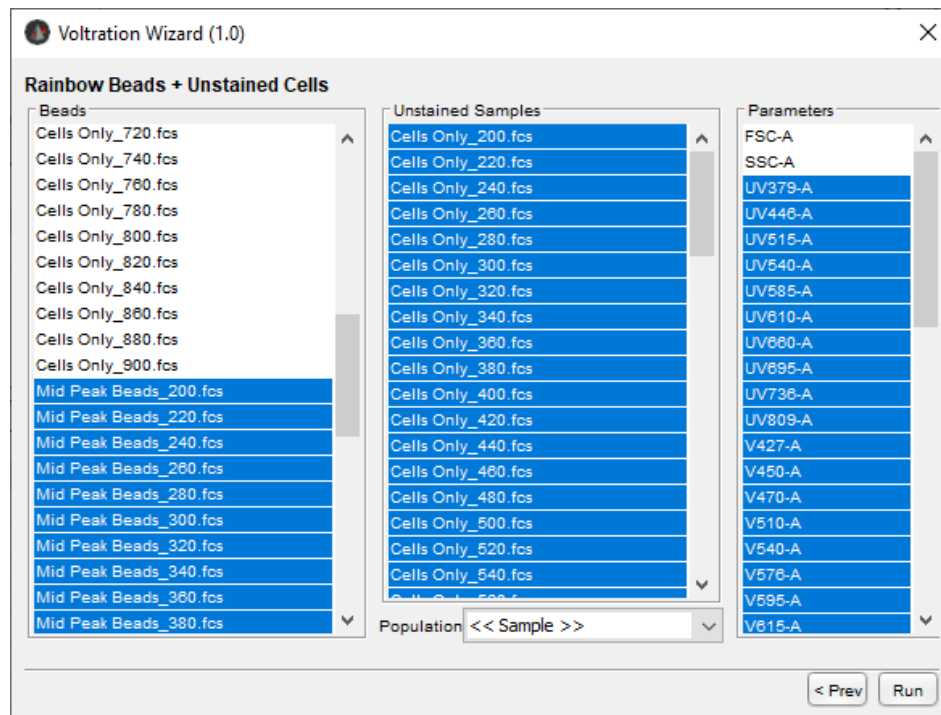


Figure 2 Rainbow beads step.

In this step it is necessary to select before running:

- Rainbow Bead Sample Files (one per voltage).
- Unstained samples (one per voltage to be analyzed) and optionally the negative population that needs to be present in each unstained sample.
- The parameters (by default all detectors will be selected).

## 2. Single Stained

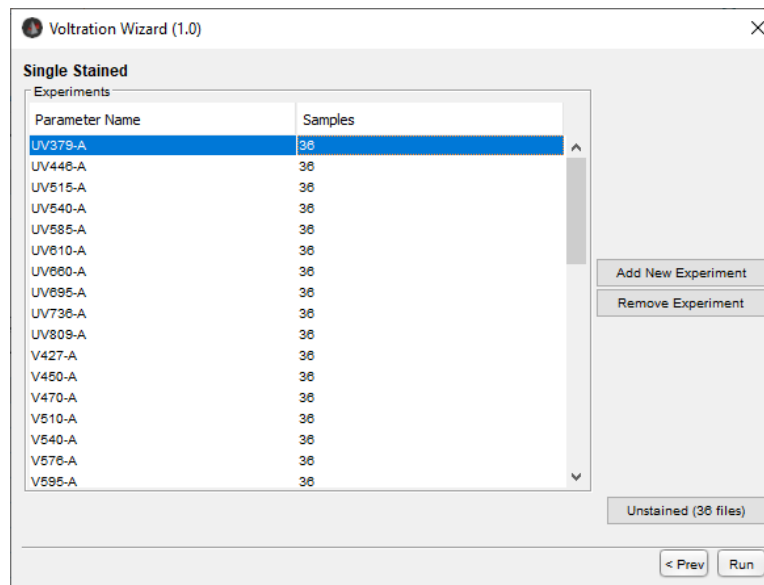


Figure 3 Single Stained step.

In this step you need to create the experiments to run, one per detector.

You can add a new experiment by clicking the "Add New Experiment" button or remove them by clicking the "Remove Experiment" button.

When you add a new experiment, you must first define the detector we are going to analyze. And in general, an experiment is defined by the positive samples (each acquired at different Voltration settings on the detector we are analyzing) and, optionally, unstained samples.

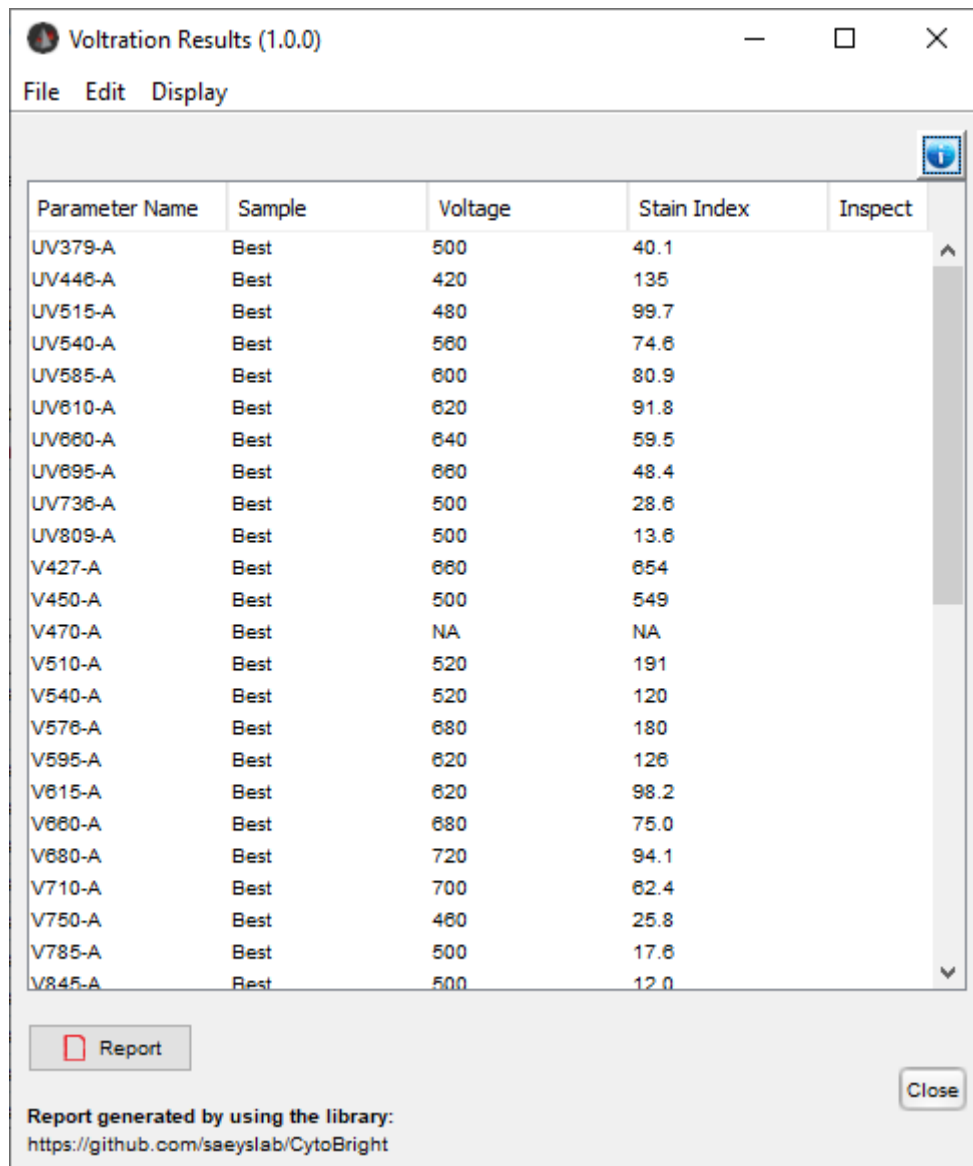
The user can choose the positive samples the first time when adding a new experiment or can change the selected samples by clicking the "Samples" column.

The unstained samples are the same for all the experiments defined in this window. They can be selected by clicking the Unstained button. In case the user doesn't define the negative samples, the negative population to calculate the SI will be inferred from the positive samples.


In the end, the experiments present in the table will be executed after clicking the "Run" button.

NOTE: The first time you go to this step, by default for each parameter with a defined fluorescence (\$PnS) an experiment will be created with all samples containing that fluorescence.

## Results Window



Parameter Name	Sample	Voltage	Stain Index	Inspect
UV379-A	Best	500	40.1	
UV446-A	Best	420	135	
UV515-A	Best	480	99.7	
UV540-A	Best	560	74.6	
UV585-A	Best	600	80.9	
UV610-A	Best	620	91.8	
UV660-A	Best	640	59.5	
UV695-A	Best	660	48.4	
UV736-A	Best	500	28.6	
UV809-A	Best	500	13.6	
V427-A	Best	660	654	
V450-A	Best	500	549	
V470-A	Best	NA	NA	
V510-A	Best	520	191	
V540-A	Best	520	120	
V576-A	Best	680	180	
V595-A	Best	620	126	
V615-A	Best	620	98.2	
V660-A	Best	680	75.0	
V680-A	Best	720	94.1	
V710-A	Best	700	62.4	
V750-A	Best	460	25.8	
V785-A	Best	500	17.6	
V845-A	Best	500	12.0	

 Report

Report generated by using the library:  
<https://github.com/saeyslab/CytoBright>

Close

Figure 4 Results window.

For each detector, the results window shows the best Voltage and the Stain Index (SI).

You can see more details by using the “Info” icon button on the right or using the menu Display/Show Details:

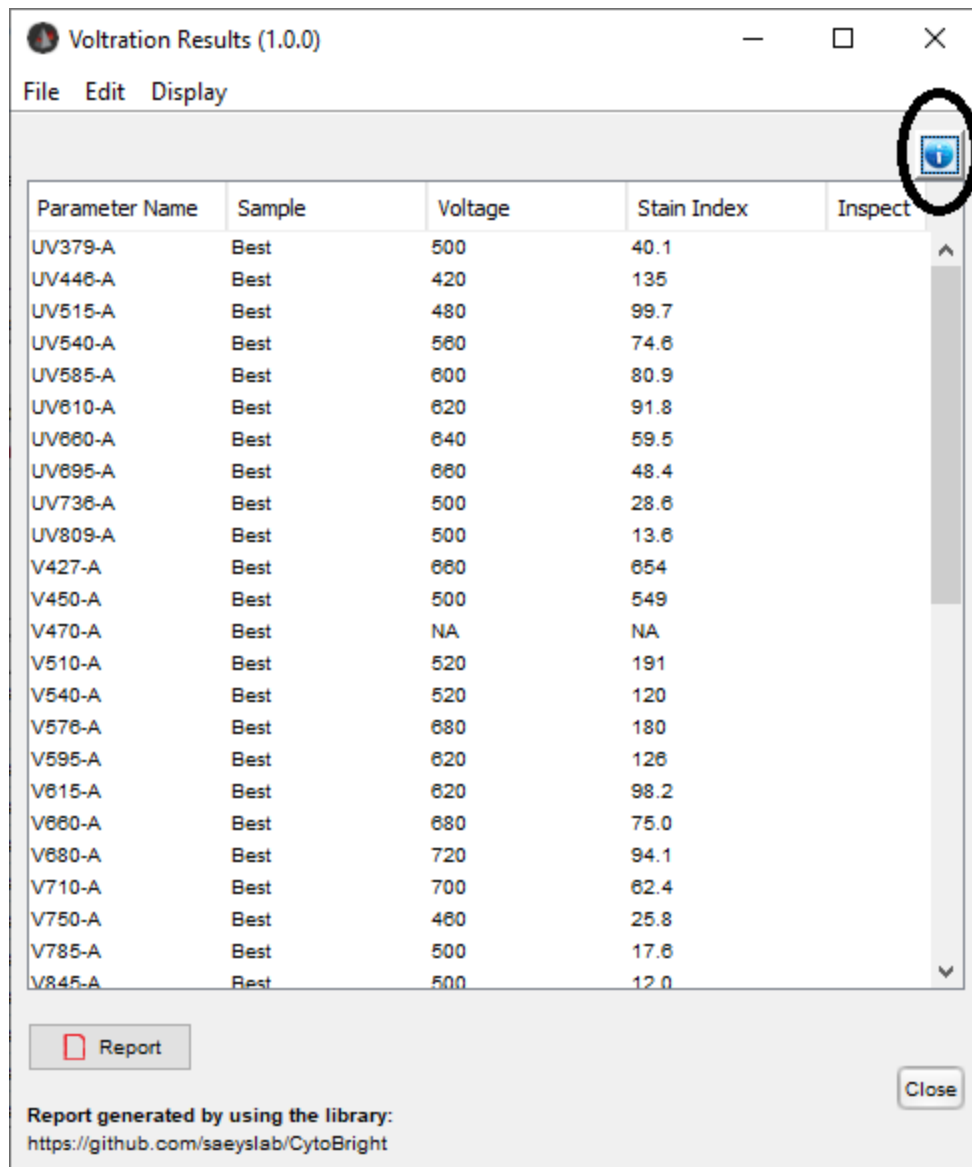


Figure 5 Showing details in the results window.

In the details we see the different samples, voltages, the SI and the best sample marked with an asterisk (\*).

By clicking the magnifying glass icon in the "Inspect" column, you will be able to see in the population tree the selection strategy selected to determine the different populations and finally the SI.

If you click on the magnifying glass icon again, the created populations will be deleted.

NOTE: The magnifying glass icon only appears if the unstained population is not selected. In case the unstained ones are selected, the plugin considers that it is not necessary to create new populations and that they are all in the analysis tree.

The "Report" button opens a PDF to see different graphs with the relevant information (see the

Report part for more details).

- Menu options:
  - *File*
    - *Add Results to DIVA settings file*: Option to include the current results in a DIVA settings file. Steps:

1. First you need to select the DIVA settings file (csv).
2. And after doing that the results are saved inside that file in Voltage column:

BD FACSDiva Software Version 9.8													
User Name:	Administrator												
Export Time:	19-JAN-2024-12:25:03												
Cytometer Type:	LSRFortessa												
Experiment Name:	Experiment_044												
Specimen Name:													
Tube Name:													
Record Date:													
Cytometer Settings Name	Threshold Operator:		Auto Compens	Compensatio	Flow Rate	Scales Recor	Use Auto Scale						
Cytometer Settings		2	false	false		0	false	true					
Compensation Matrix													
	48	1	0	0	0	0	0	0	0	0	0	0	0
	0												
Parameters	Type	Data Min	Data Max	Raw Data Ind	Linear Data In	Log Data Ind	Final Data Inc	Is Log	Is Quantitate	Is Enabled			
FSC-A		30	0	262143	1	1	99	1	false	false	true		
SSC-A		30	0	262143	2	2	100	2	false	false	true		
UV379-A		30	0	54 185	3	3	101	101	true	false	true		
UV446-A		30	0	54 185	4	4	102	102	true	false	true		
UV515-A		30	0	54 185	5	5	103	103	true	false	true		
UV540-A		30	0	54 185	6	6	104	104	true	false	true		
UV585-A		30	0	54 185	7	7	105	105	true	false	true		
UV610-A		30	0	54 185	8	8	106	106	true	false	true		
UV660-A		30	0	54 185	9	9	107	107	true	false	true		
...													
Cytometer Parameters	Fluorophore	Voltage	Target	Brightnes	Rat Quantitation	Threshold	Threshold En	Labels Only	Can Be Com	BiExponential	Computed Bil		
FSC-A	FSC	250	3	1	NaN	5000	True	False	False	0	0		
SSC-A	SSC	300	3	1	NaN	5000	False	False	False	0	0		
UV379-A	UV379	535	3	1	NaN	5000	False	False	True	0	0		
UV446-A	UV446	482	3	1	NaN	5000	False	False	True	0	0		
UV515-A	UV515	527	3	1	NaN	5000	False	False	True	0	0		
UV540-A	UV540	605	3	1	NaN	5000	False	False	True	0	0		
UV585-A	UV585	594	3	1	NaN	5000	False	False	True	0	0		
UV610-A	UV610	618	3	1	NaN	5000	False	False	True	0	0		
UV660-A	UV660	614	3	1	NaN	5000	False	False	True	0	0		
UV695-A	UV695	621	3	1	NaN	5000	False	False	True	0	0		
UV736-A	UV736	520	3	1	NaN	5000	False	False	True	0	0		
UV809-A	UV809	527	3	1	NaN	5000	False	False	True	0	0		
V427-A	V427	583	3	1	NaN	5000	False	False	True	0	0		
V450-A	V450	534	3	1	NaN	5000	False	False	True	0	0		
V470-A	V470	594	3	1	NaN	5000	False	False	True	0	0		
V510-A	V510	610	3	1	NaN	5000	False	False	True	0	0		
V540-A	V540	580	3	1	NaN	5000	False	False	True	0	0		
V576-A	V576	628	3	1	NaN	5000	False	False	True	0	0		
V595-A	V595	597	3	1	NaN	5000	False	False	True	0	0		
V615-A	V615	594	3	1	NaN	5000	False	False	True	0	0		

- Close: close this window.
- *Edit*
  - *Reset Results*: to clear the results and go to the initial Voltration Wizard step
  - *Copy Content*: copies the table to the clipboard as text.
- *Display*
  - *Show Details*: same option that previous “Info” button.



# Report

Special mention here to Sofie Van Gassen and her library [GitHub - saeyslab/CytoBright](https://github.com/saeyslab/CytoBright), which was a source of inspiration for creating the plugin and it is used to present the results in Report.

Description: For each parameter there is a page in the report dedicated to it.

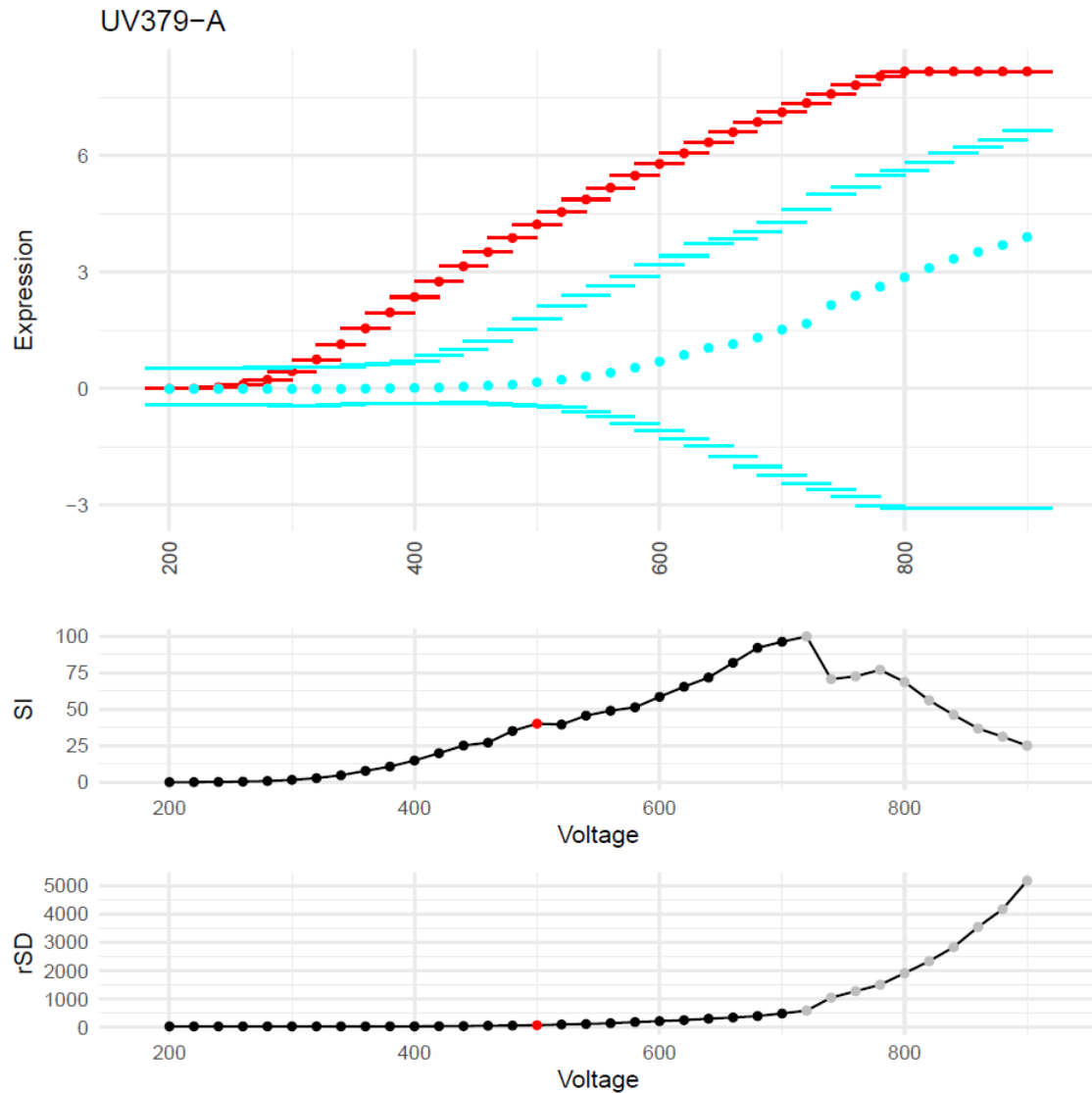


Figure 6 Report Page

The Report Page has three different graphs that display the voltage on the X axis:

- Expression Graph: Graph with the expression level in a ArcSinh scale in Y Axis. Legend:
  - A red dot with the MFI of the positive population.
  - A cyan dot with the MFI of the negative population.
  - Two cyan lines that mark the limits of the negative population.
- Stain Index (SI) Graph. Legend:
  - The red dot is the selected voltage.
  - The gray dots are the voltages discarded because the number of off-scale cells is greater than 1%.

- Robust Standard Deviation (rSD) Graph. Legend:
  - The red dot is the selected voltage.
  - The gray dots are the voltages discarded because the number of off-scale cells is greater than 1%

# Appendix - Algorithm

---

Glossary of terms:

- **MFI:** Median Fluorescence Intensity
- **rSD:** Robust Standard Deviation
- **Positive Population:** Is the group of cells that have a high fluorescence intensity due to the binding of the antibody.
- **Negative Population:** Is the group of cells that have a low fluorescence intensity due to the lack of binding.
- **SI:** Stain Index. The value is calculated as follows:

$$SI = \frac{MFI_{positive\_population} - MFI_{negative\_population}}{2 \times rSD_{negative\_population}}$$

Using different samples acquired with different voltages and identified the positive and negative population, we calculated the SI for each voltage.

And we determine the optimal voltage following these rules:

- The maximum SI.
- The rSD of the negative population at that voltage is not greater than 3 x rSD' (using as rSD' the rSD of the negative population of the first voltage).
- No more than a 1% of the cells in the positive population out of scale.

