Panel Design Tool Documentation

Panel Design Tool, Cytometry Facility, University Zürich
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The panel used in the examples in this documentation is the T-Cell panel from the Human Immunology Project Consortium.

Panel Design Tool – Overview

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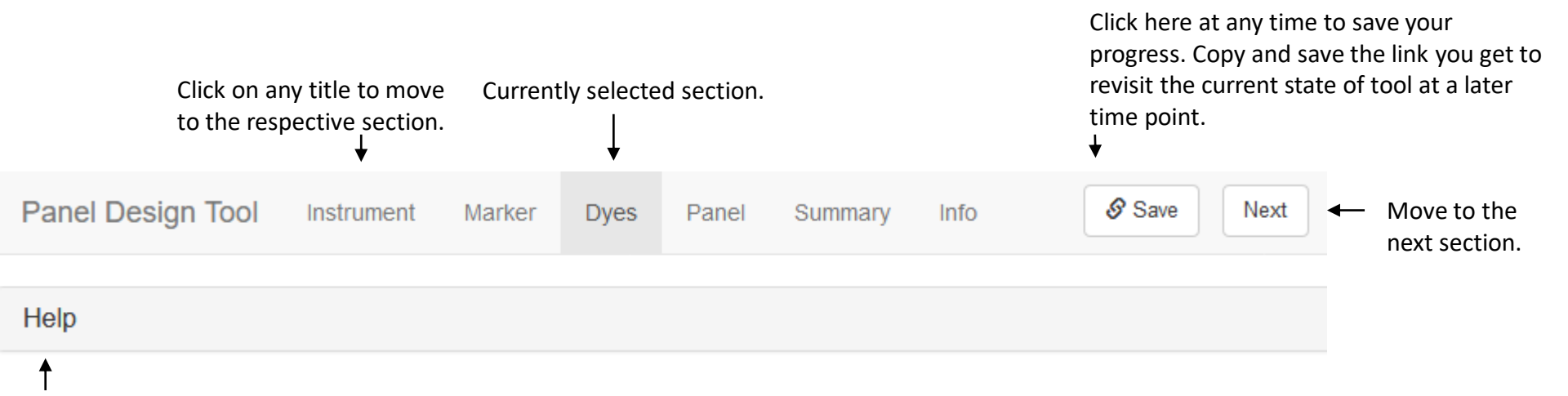
The Panel Design Tool supports you with your panel design for conventional flow cytometry. Based on your panel you can explorer different marker – dye combinations in the various plot. The tool incorporates different markers, their expression levels and cell populations they are expressed on.

All plots are based on calculated estimates and thus allow you to get a first impression on possible design and resolution issues in your panel before you start your first experiments.

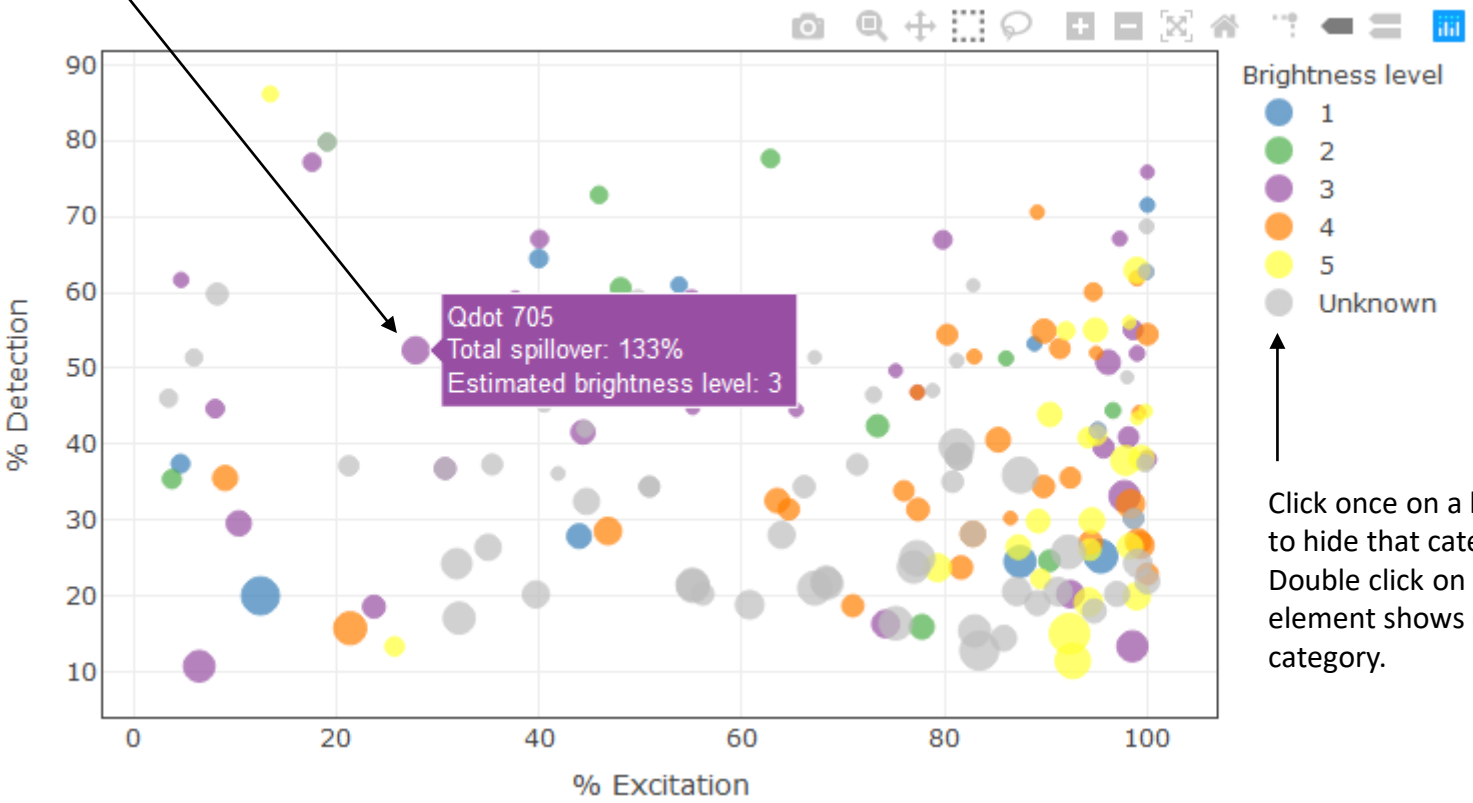
- **Instrument**: Here you select the instrument you want to work with.
- **Marker**: Provide the information about which markers and populations your panel contains. This step is optional but allows for more visualization options of your panel.
- **Dyes**: Explore how different dyes perform on your instrument of choice and generate a pre-selection of the dyes that you want to use for your panel design (optional).
- **Panel**: Here you explore different marker – dye combinations to optimize your panel resolution with the right assignments. All the panel performance plots are in this section.
- **Summary**: Generate a printable summary of your panel.
- **Info**: Information on the development and the spectra sources.



Most plots in the PDT are interactive plotly plots.

Hover the mouse over a data point or plot tile to get more information.

Use the toolbar to zoom in and out, select data points and export the plot as an image.



Click once on a legend element to hide that category. Double click on a legend element shows only that category.

Instrument – Selection

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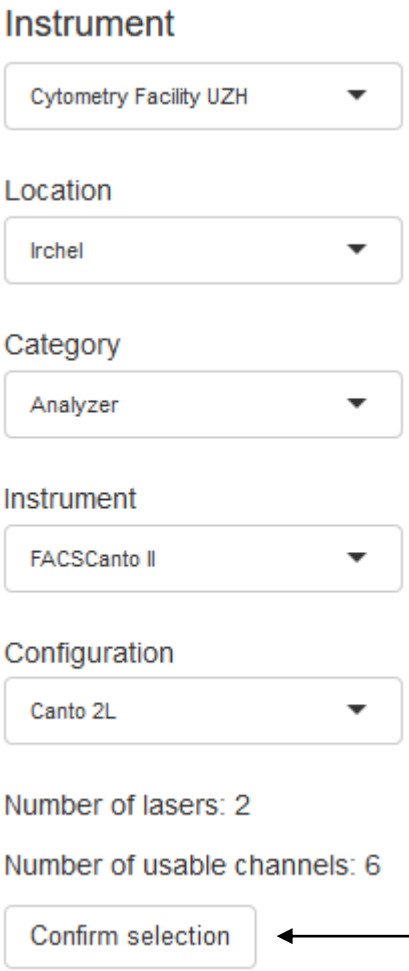


Instruments at the UZH Cytometry Facility or upload custom configuration.

Choose working location (Irchel, USZ or Schlieren).

Choose instrument class (analyzer or sorter).

Choose instrument type.

Choose instrument configuration. These can be either different options on a single machine or different instruments of the same type at the same location.

Summary info on number of excitation lasers and fluorescence detection channels.

After choosing the right instrument, confirm your selection.

Instrument – Optical Configurations

Preview of the instrument configuration.

Currently selected instrument configuration (after pressing Confirm selection)

Optical configuration

FACSCanto II

Excitation	LP	BP	Active	Channel
488	735	780/60	TRUE	Blue 780/60
488	655	670	TRUE	Blue 670
488	610	None	FALSE	None
488	556	585/42	TRUE	Blue 585/42
488	502	530/30	TRUE	Blue 530/30
488		488/10	FALSE	SSC
640	735	780/60	TRUE	Red 780/60
640	685	None	FALSE	None
640		660/20	TRUE	Red 660/20

Each row is one detection channel →

Selected instrument

LSRFortessa

Excitation	LP	BP	Active	Channel
405	735	800/50	TRUE	Vio 800/50
405	685	710/40	TRUE	Vio 710/40
405	630	670/30	TRUE	Vio 670/30
405	600	610/20	TRUE	Vio 610/20
405	505	525/50	TRUE	Vio 525/50
405		450/50	TRUE	Vio 450/50
488	685	710/50	TRUE	Blue 710/50
488	505	530/30	TRUE	Blue 530/30
488		488/10	FALSE	SSC
561	750	780/60	TRUE	YG 780/60
561	685	710/50	TRUE	YG 710/50
561	635	670/30	TRUE	YG 670/30
561	600	610/20	TRUE	YG 610/20
561		586/15	TRUE	YG 586/15
640	750	780/60	TRUE	Red 780/60
640	690	730/45	TRUE	Red 730/45
640		670/14	TRUE	Red 670/14

Excitation: Wavelength of excitation laser (nm).

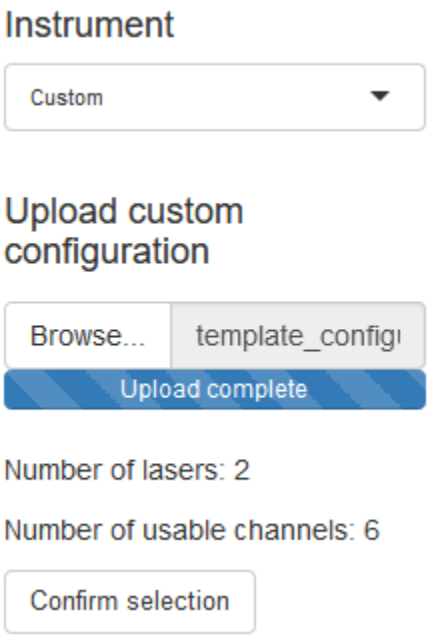
LP: Wavelength of long pass filter (nm).

BP: Wavelength of filter window middle followed be window width (nm). If only one number is given, a second LP is installed instead of a BP.

Active: Can this channel be used in the software for fluorescence detection (yes/no).

Channel: Channel name.

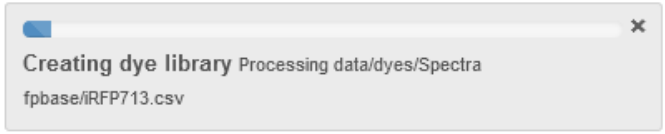
Instrument – Custom Configurations



By choosing custom, you can upload your customized optical configuration or the configurations of other instruments.

To upload the configuration, click Browse… and select the configuration on your hard-drive.

Processing all dyes for your custom configuration will take several seconds:



Uploaded configurations are only temporary and not visible to anyone else. Save the state of the tool to get a link that contains your uploaded configuration.

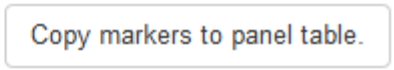
Currently, only Excel workbooks are accepted as uploads. The Excel table must follow this layout:

	A	B	C	D	E	F	G	H	I
1	Excitation	LP	BP	Active	Name	Instrument	Configuration	Location	Class
2	488	735	780/60	TRUE	Blue 780/60	FACSCanto II	Standard	Irchel	Analyzer
3	488	655	670	TRUE	Blue 670	FACSCanto II	Standard	Irchel	Analyzer
4	488	610	None	FALSE	None	FACSCanto II	Standard	Irchel	Analyzer
5	488	556	585/42	TRUE	Blue 585/42	FACSCanto II	Standard	Irchel	Analyzer
6	488	502	530/30	TRUE	Blue 530/30	FACSCanto II	Standard	Irchel	Analyzer
7	640	735	780/60	TRUE	Red 780/60	FACSCanto II	Standard	Irchel	Analyzer
8	640	685	None	FALSE	None	FACSCanto II	Standard	Irchel	Analyzer
9	640		660/20	TRUE	Red 660/20	FACSCanto II	Standard	Irchel	Analyzer

You can download an example file from the Help section of the PDT.

Marker – Upload

Upload marker table



To upload a marker and population table, click Browse… and select the file on your hard-drive.

Currently, only Excel workbooks are accepted as uploads. The Excel table must follow this layout:

	A	B	C	D	E	F	G
1	Population	CD3	CD4	CD8	CD38	CD45RA	CCR7
2	T Cell	+					
3	CD4+ T Cell	+	+	-			
4	Activated CD4+ T Cell	+	+	-	+		
5	Naive CD4+ T Cell	+	+	-		+	+
6	Central memory CD4+ T Cell	+	+	-		-	+
7	Effector memory CD4+ T Cel	+	+	-		-	-
8	Effector CD4+ T Cell	+	+	-		+	-

The first column (Population) contains the names of the cell populations. The other columns have the marker names as title.

+ indicates that the population is positive for that marker, - that it is negative.

Marker – Marker Network

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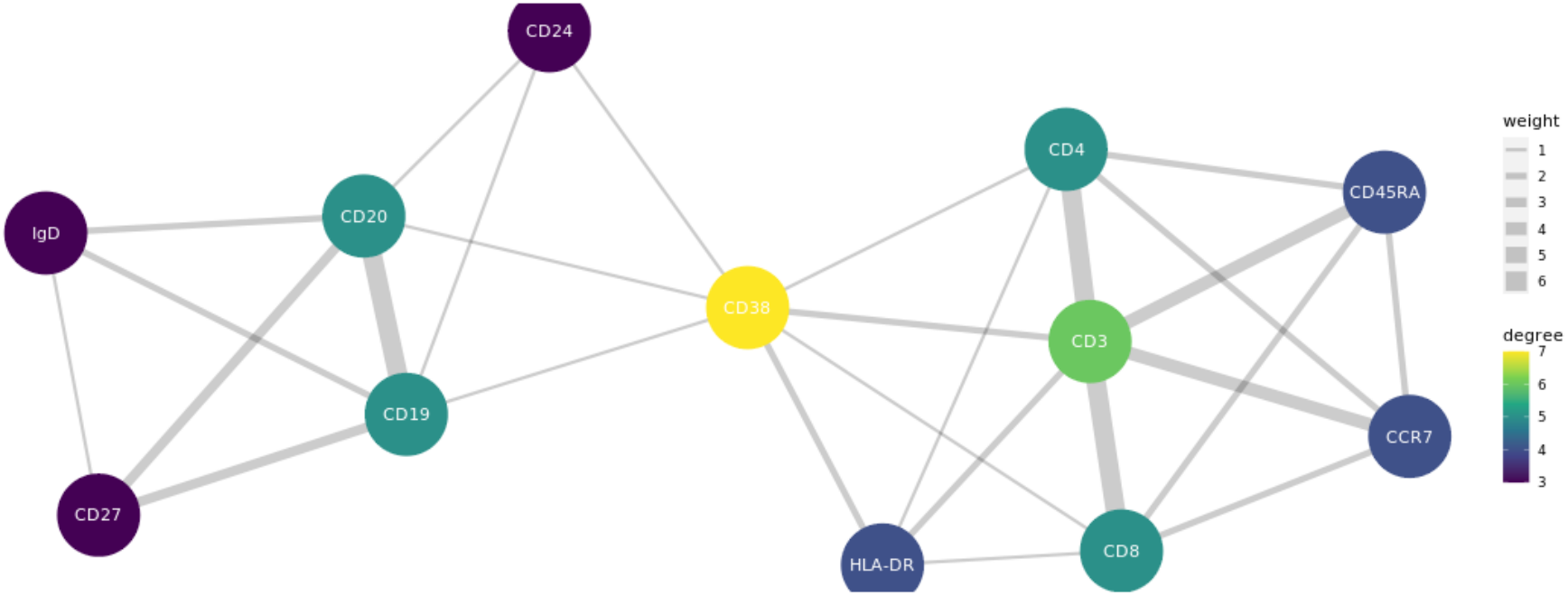
A bipartite network is created from the uploaded marker and population table. The projection for the markers is plotted. The plot shows how the different markers are connected in terms of co-expression on different populations.



Degree (color): Number of times the marker is co-expressed with other markers. Example: CD38 is co-expressed with 7 other markers.

Weight (connection width): Number of populations the connected markers are co-expressed on. Example: CD3 is co-expressed together with CD8 on 6 different populations.

The panel used in this examples is a combination of the T-Cell and B-Cell panel from the Human Immunology Project Consortium.

Marker – Population and Markers Table

Displays the table that has been uploaded.

Population	CD3	CD4	CD8	CD38	CD45RA	CCR7	HLA-DR	CD19	CD20	CD27	IgD	CD24
T Cell	+							-	-			
CD4+ T Cell	+	+	-					-	-			
Activated CD4+ T Cell	+	+	-	+			+	-	-			
Naive CD4+ T Cell	+	+	-		+	+		-	-			
Central memory CD4+ T Cell	+	+	-		-	+		-	-			
Effector memory CD4+ T Cell	+	+	-		-	-		-	-			
Effector CD4+ T Cell	+	+	-		+	-		-	-			
CD8+ T Cell	+	-	+					-	-			
Activated CD8+ T Cell	+	-	+	+			+	-	-			
Naive CD8+ T Cell	+	-	+		+	+		-	-			
Central memory CD8+ T Cell	+	-	+		-	+		-	-			
Effector memory CD8+ T Cell	+	-	+		-	-		-	-			
Effector CD8+ T Cell	+	-	+		+	-		-	-			
B Cell	-							+	+			
Naive B Cell	-							+	+	-	+	
Memory B Cell	-							+	+	+		
Memory B Cell IgD+	-							+	+	+	+	
Memory B Cell IgD-	-							+	+	+	-	
Transitional B Cell	-			+				+	+			+

Marker – Co-Expressed Markers

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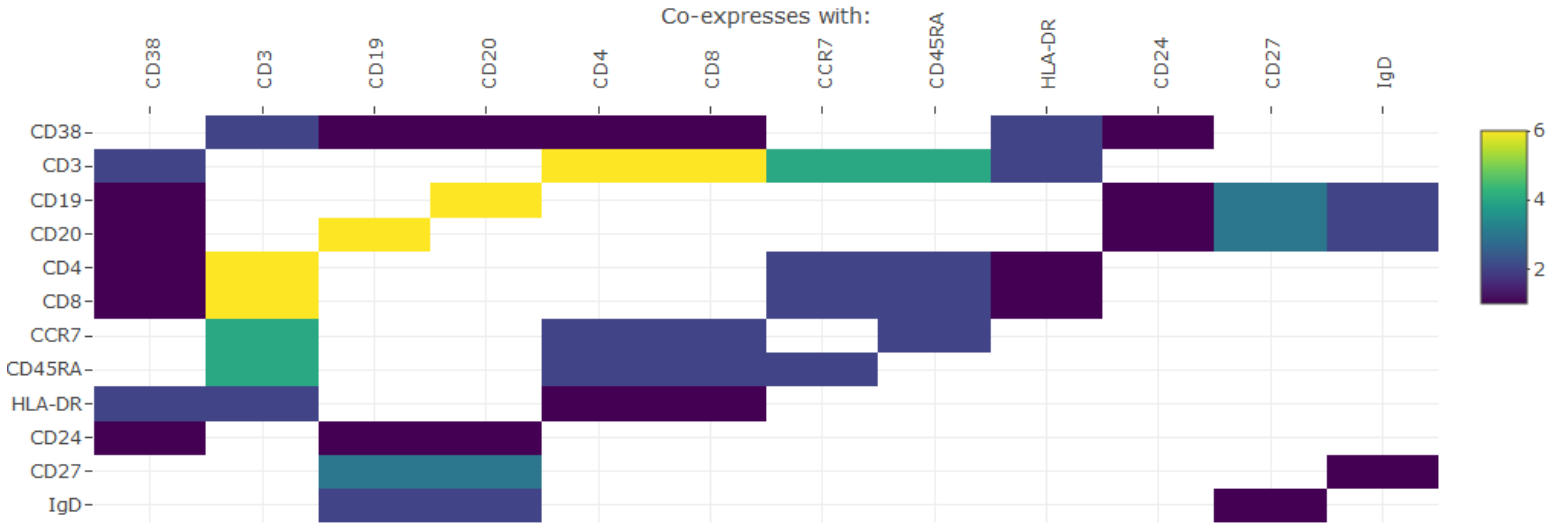
The heat map shows which markers are co-expressed together with which other markers and on how many different populations.



Spillover from co-expressed markers will lead to broadening of the positive population.

Marker – Markers in Negative Population

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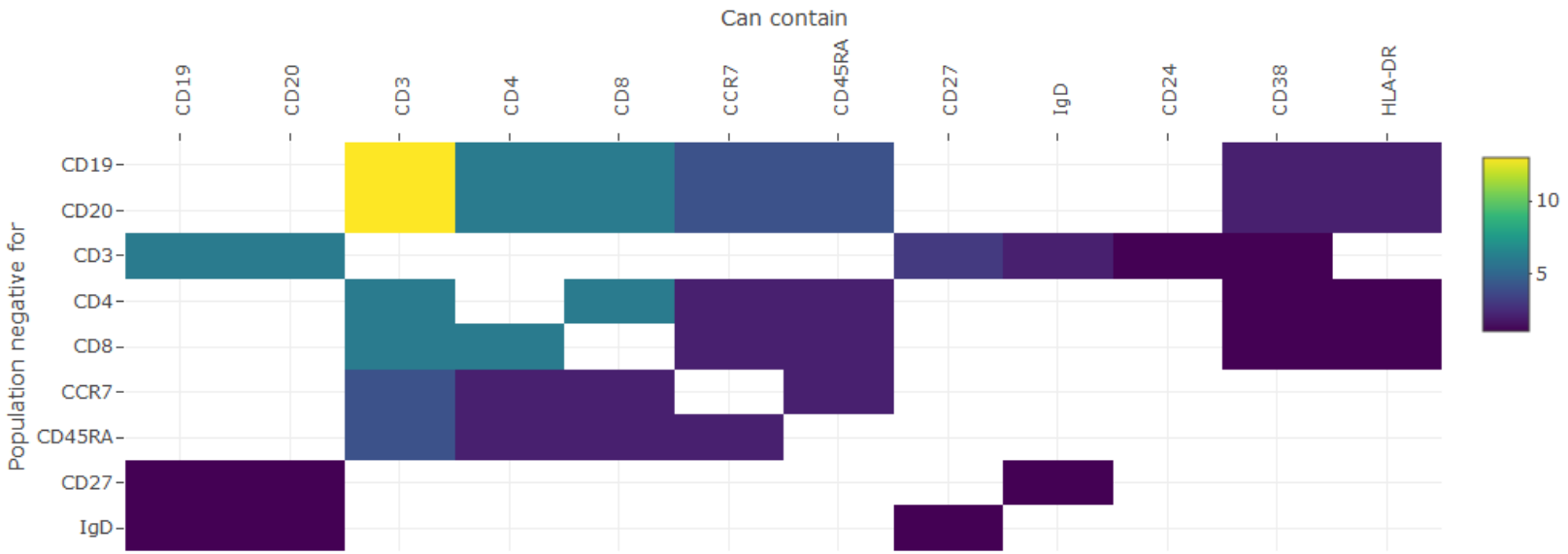
The heat map shows which markers are potentially in the negative population for a given marker and on how many populations.



Spillover of the markers in the negative population into the positive marker channel will broaden the negative population.

Dyes – General Notes

In this section, you can make an optional preselection of the dyes that you want to use during the panel design later on.

- All values are estimates based on calculations, not measurements!
  - Calculations are based on dye spectra provided by the manufacturers and idealized functions for the optical filters.
  - Note that the provided spectra sometimes do not cover the whole used wavelength range. Therefore the calculations will miss spillover in regions where we have no excitation and/or emission information for a dye. The issues are usually lacking excitation data in the UV range and lacking emission data in the near IR range.
  - For spillover across lasers, equal excitation power (in terms of photon flux, not wattage!) is assumed for each laser.
  - Instrument background is not taken into account during the calculations.

- Only dyes in the database that are excited to at least 1% by the instrument are available for selection.
- Spectra of tandem dyes can differ strongly between batches and manufacturers.
- Brightness level information was aggregated from information provided by the manufacturers. As this information is usually instrument specific, the brightness levels stated here should be treated as very rough estimates.
- Based on published staining index values, we assume a brightness difference of factor 10 between the lowest brightness level (1) and the highest (5).
- A low value in "% Excitation" or "% Detection" does not necessarily mean that the dye cannot be used on the instrument. Usability also depend on the dye brightness, labelling abundance and expression level.
  For example, DAPI is only excited with an efficiency of 8% on the LSRFortessa. But because of the large amounts of DNA in the cell, a DAPI staining can still produce a very bright signal on that instrument.

Dyes – Dye Selection Plot

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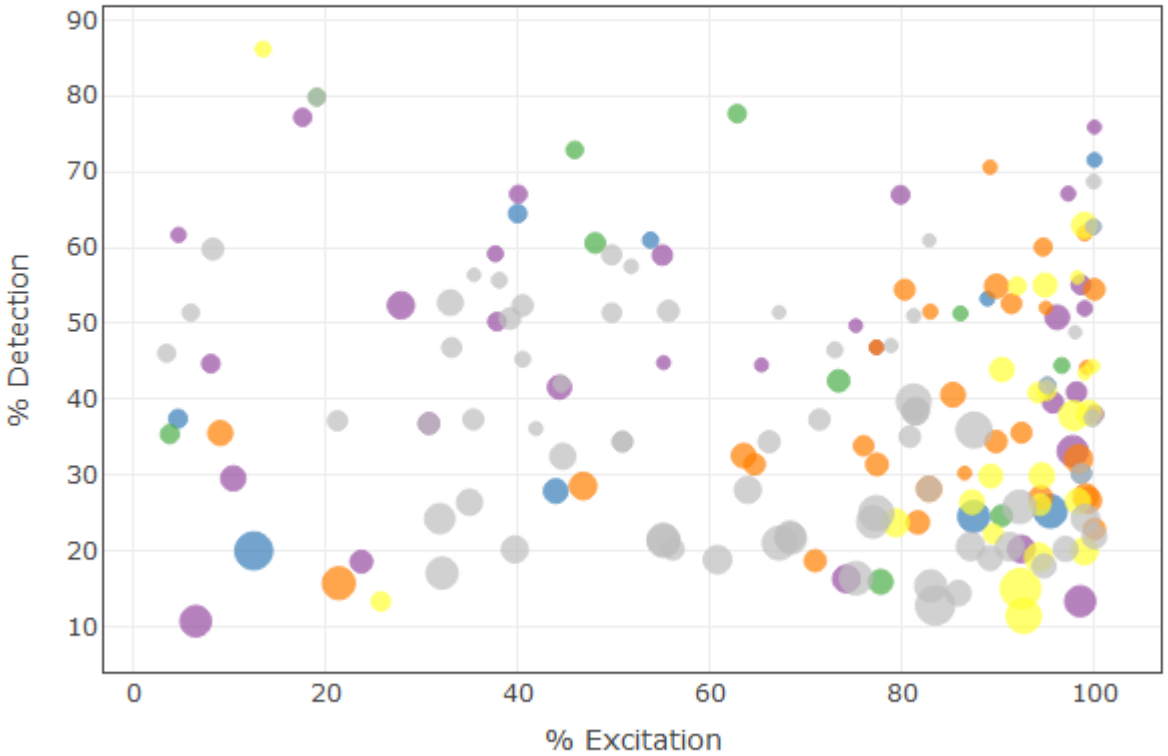
The plot shows how well a given dye is excited and how much of its emission is collected by the instrument.

All channels ← Select to see either all dyes or only those for a specific channel.



Dot **size** indicates how much spillover the dye produces in total. Dot **color** indicates the brightness level.

To select dyes in the plot:

- Use the rectangle or lasso tool to select dyes.
- Hold shift to create multiple selection regions.
- Double click in the plot to clear all selections.
- Show/hide dyes in the plot (will not affect current selections):
- Click on a brightness level in the legend to hide/show the corresponding level.
- Double-click on a brightness level in the legend to only show that level.

Add selected Dyes to "Dye pre-selection"

Add the selected dyes to the selection table.

Dyes – Dye Selection Table

To add a dye to the selection table, click on the row. Multiple dyes can be selected at once. The table is fully searchable and can be sorted by every column.

Example: to find the brightest dyes for the Blue 530/30 channel, search for Blue 530 and then sort by brightness.

Search:

Show 10 entries

	Dye	Channel	Brightness	% Total Spillover	% Excitation	% Detection	Spectral Data Source
1	ATTO390	Vio 450/50	Unknown	71	81	35	ATTO-TEC
2	ATTO488	Blue 530/30	Unknown	3	67	51	ATTO-TEC
3	ATTO594	YG 610/20	Unknown	176	32	24	ATTO-TEC
4	ATTO633	Red 670/14	Unknown	222	75	16	ATTO-TEC
5	ATTO655	Red 730/45	Unknown	228	67	21	ATTO-TEC
6	ATTO680	Red 730/45	Unknown	119	45	32	ATTO-TEC
7	ATTO700	Red 730/45	Unknown	70	39	51	ATTO-TEC
8	APC-Cy7	Red 780/60	2	41	63	78	BD Biosciences
9	APC-H7	Red 780/60	1	42	40	64	BD Biosciences
10	APC-R700	Red 730/45	4	103	85	41	BD Biosciences

Showing 1 to 10 of 174 entries

Previous 1 2 3 4 5 … 18 Next

Channel: The instrument channel where most of the fluorescence emission is expected to be collected for the dye.

Brightness: The dye brightness in levels from 1 (dim) to 5 (bright).

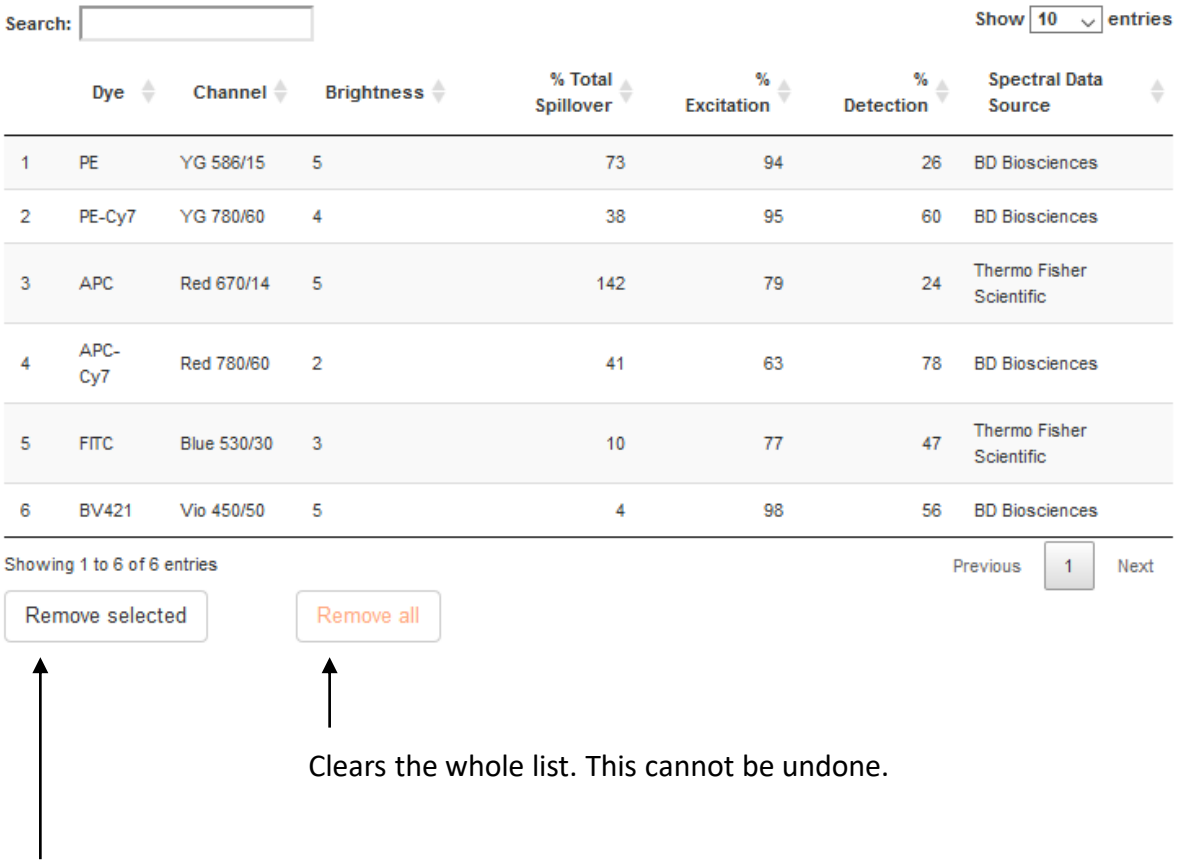
% Total Spillover: The sum of spillover produced by this dye into the other instrument channels.

% Excitation: How well the dye is excited by the instrument relative to its absorption maximum.

% Detection: How much of the total dye fluorescence is collected by the channel for that dye.

Dyes – Selected Dyes Table

This table shows all dyes currently in the pre-selection list. It displays the same information as the dye selection table.

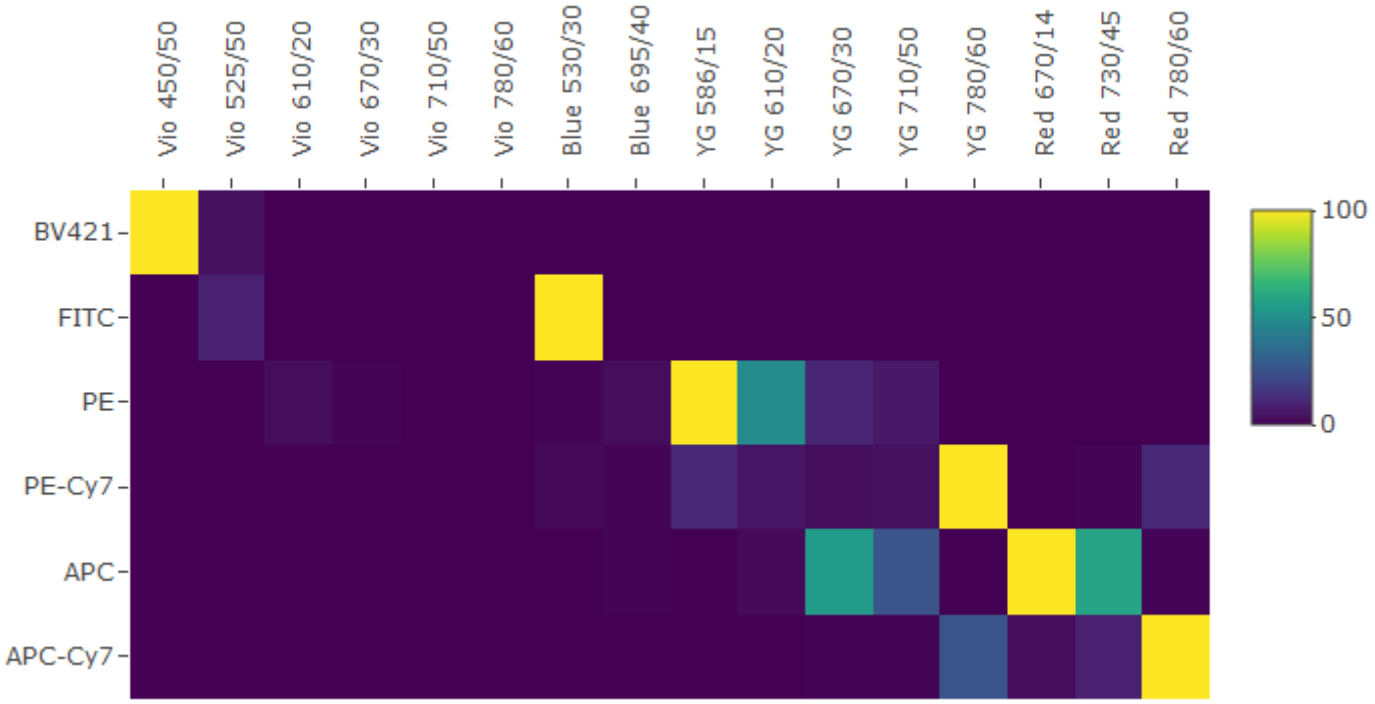


Clears the whole list. This cannot be undone.

Removes selected dyes from the list.

Dyes – Dye Spillover

The heat map shows the calculated spillover coefficients (values in %) of the selected dyes for all instrument channels.



Hover the mouse over the tiles to see the calculated spillover coefficients.

Dyes – Dye Similarities

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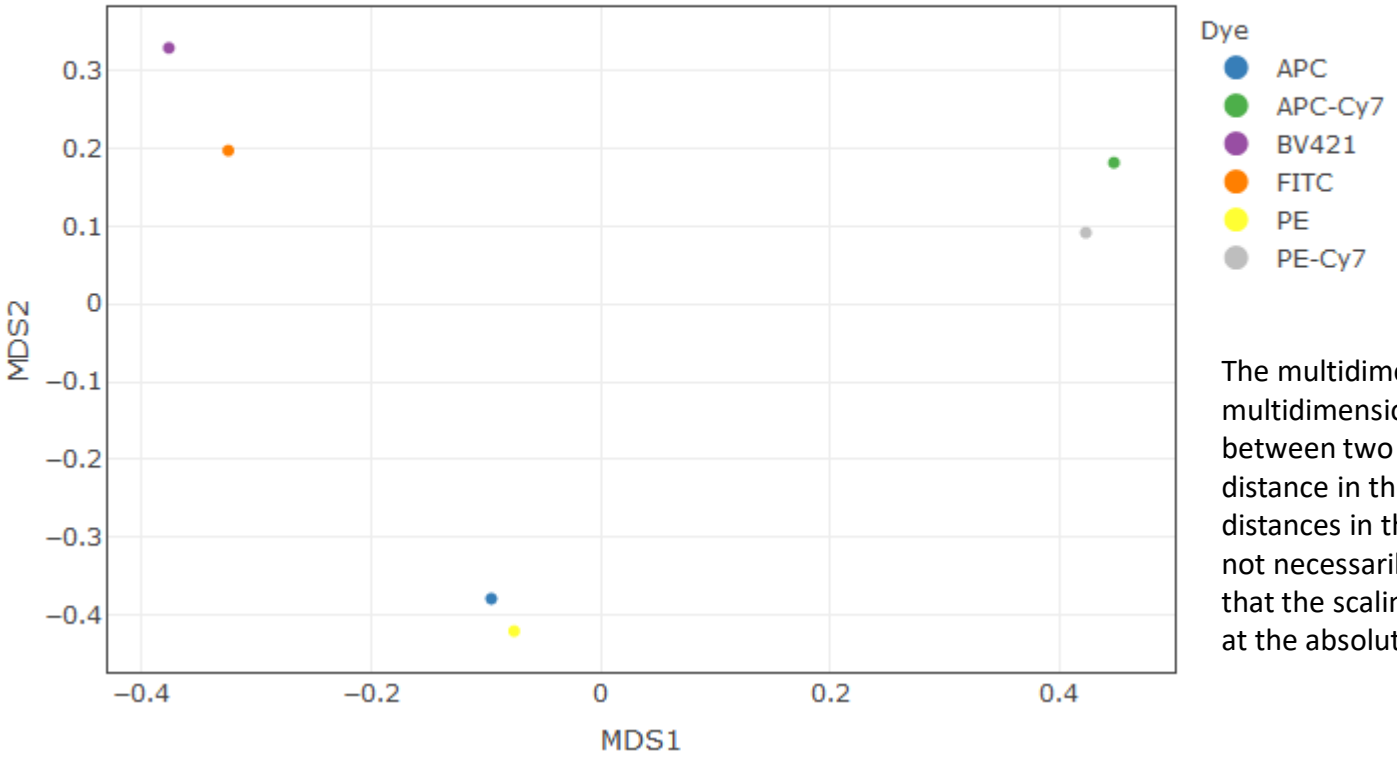
Shows the similarity of the selected according to the chosen distance metric.

Cosine angular distance

The distance metric can be chosen from cosine angular (spectral overlap), Chebishev (gating) and Euclidian (clustering) distance. When choosing cosine angular distance, the distance is calculated as 1 - the cosine angular similarity.



The multidimensional distances are plotted in a multidimensional scaling plot. A large distance between two points in the plot represents a large distance in the multidimensional space. But the distances in the MDS1 and MDS2 coordinate are not necessarily directly comparable. Also note that the scaling of the plot is dynamic, have a look at the absolute values on the coordinates.

To determine how similar two dyes are in terms of total spillover, the cosine angular distance is the most informative. The Chebishev and Euclidian distance are more useful when comparing the actual signal levels for markers or populations. The calculated distance looks at the overall similarity and is thus non-directional.

The distances in the plot are relative and depend on the dye selection. Different dye combinations will result in different absolute distances in the MDS map.

Panel – General Notes

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In this section you make the marker – dye assignments to create your panel.

Consider the notes for the Dye section to understand the limits of the calculations!

Marker-dye assignments can be made in two ways:

- Per channel: Choose which dye to use in which instrument channel and then add the marker.
- Per marker: Directly pair markers and dyes

If you have uploaded a marker table and copied the entries to the panel section, the markers will be in the "Marker" tab.

Expression and brightness levels:

- Expression levels can be set from 1 (low) to 5 (high), and a factor of 100 is assumed between the lowest and highest level.
- Expression levels are modeled as fixed values, no distributions can be chosen at the moment.
- Brightness levels can be set from 1 (low) to 5 (high), and a factor of 10 is assumed between the lowest and highest level.
- The brightness value is only a very rough estimate.

Panel – Marker Assignment – Channels

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Dye lists

All dyes ← Work with either all dyes or the ones you selected before in the Dyes section.

Copy to Marker tab ← Copies all entries to the Marker tab. This action overwrites any previous entries in the Marker tab.

Channel	Dye	Brightness	Marker	Expression
Vio 450/50	BD Horizon V450	1	CD3	3
Vio 525/50	BD Horizon V500	1	HLA-DR	3
Vio 610/20	None	1	Marker name	1
Vio 670/30	None	1	Marker name	1
Vio 710/50	None	1	Marker name	1
Vio 780/60	None	1	Marker name	1
Blue 530/30	None	1	Marker name	1
Blue 695/40	PerCP-Cy5.5	3	CD4	5
YG 586/15	PE	5	CCR7	1
YG 610/20	None	1	Marker name	1
YG 670/30	None	1	Marker name	1
YG 710/50	None	1	Marker name	1
YG 780/60	PE-Cy7	4	CD45RA	5
Red 670/14	APC	5	CD38	3
Red 730/45	None	1	Marker name	1
Red 780/60	APC-H7	1	CD8	5

Select the dye you want to measure in a given channel. Only dyes having this channel as main channel are shown.

The brightness is set automatically (if available) but can still be adjusted (1 – 5).

Enter the marker name.

Choose the marker expression level (1 – 5).

Panel – Marker Assignment – Marker

Copies all entries to the Channels tab. This action overwrites any previous entries in the Channels tab.
Signal plots are only updated after copying the assignments to the Channels tab!
Only rows for which a marker name has been set are copied.

Copy to Channels tab Plots are only updated after copying assingment to Channels tab.

Marker	Expression	Dye	Brightness	Show all brightness.	Hide unknown brightness.	Show all dyes.
CD3	3	BD Horizon V450	1	☐	☐	☐
CD4	5	PerCP-Cy5.5	3	☑	☐	☐
CD8	5	APC-H7	1	☑	☐	☐
CD38	3	APC	5	☑	☐	☐
CD45RA	5	PE-Cy7	4	☑	☐	☐
CCR7	1	PE	5	☐	☐	☐
HLA-DR	3	BD Horizon V500	1	☐	☐	☐

Enter the marker name (if not automatically filled out already by uploading a marker table in the "Marker" section).

Choose the marker expression level (1 – 5).

Assign a dye to the marker. By default, only pre-selected dyes with a brightness matching the chosen expression level will be shown.
Of dyes sharing the same main channel, only one can be selected per panel.

The brightness is set automatically (if available) but can still be adjusted (1 – 5).

Control which dyes are available for selection.

Panel – Signal Plots – Overview

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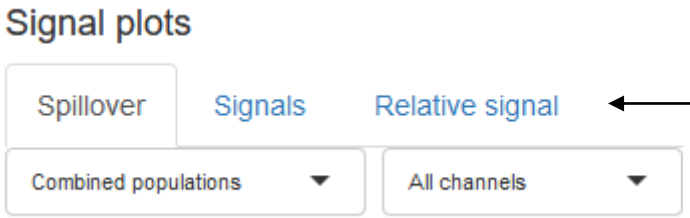
The signal plots show you the calculated features of your panel.



Choose from spillover, signals or relative signals (see below).

Values for the markers can be shown either over all populations or for individual populations (if a marker table has been uploaded). Calculations can be done either for all instrument channels or only for the currently used ones (i.e. channels used by the dyes in your current panel).

Spillover

- Predicted spillover coefficients.
- Dye brightness and expression levels are **not** taken into account.
- Useful if you have no information on dye brightness or marker expression levels.

Signals

- Predicted signals (spillover coefficients * dye brightness * marker expression).
- Useful to compare actual signal levels of markers and to estimate how well populations can be differentiated.

Relative signals

- Predicted signal divided by the sum of spillover signals in this channel. “How much of the total observed signal is your signal of interest?”
- Useful to estimate the resolution loss due to spillover collected in a channel.

Panel – Signal Plots – Spillover

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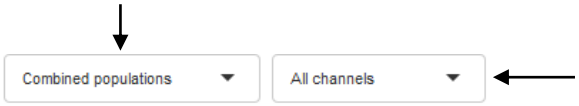
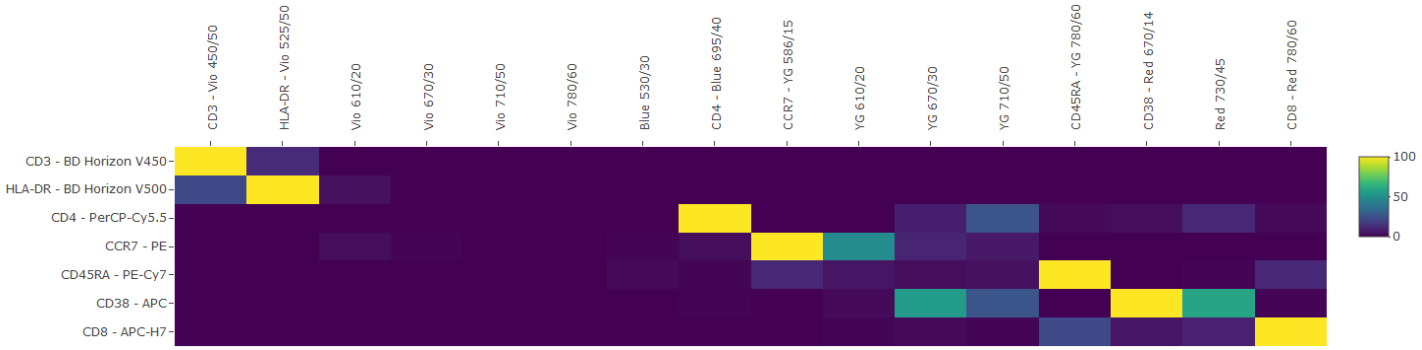
The calculated spillover coefficients can be shown either for all or for individual populations (if a marker table has been uploaded).



Display the spillover coefficients for all instrument channels or only for the currently used ones (i.e. channels used by the dyes in your current panel).

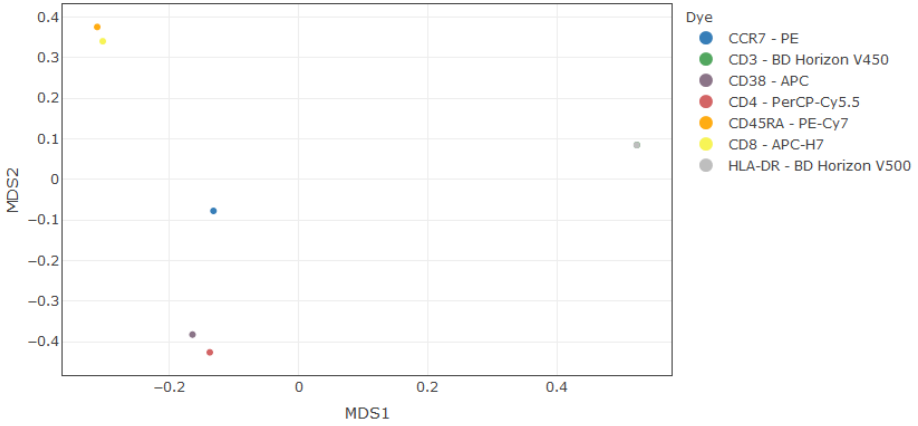
Spillover coefficient heat map:



Spillover coefficients are given in %. Dyes are in the rows, and instrument channels in the columns.

All % values can be compared when switching to the table view.

Spillover coefficient distance plot:



See also the description in the Dyes section.

Note that the spillover coefficients do not reflect the actual signal levels and thus won't tell you what impact the generated spillover will have on the resolution in your panel.

By definition, the set brightness and expression levels have no influence on the spillover coefficients.

Panel – Signal Plots – Signals – Heat Maps

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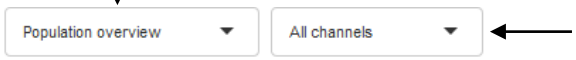
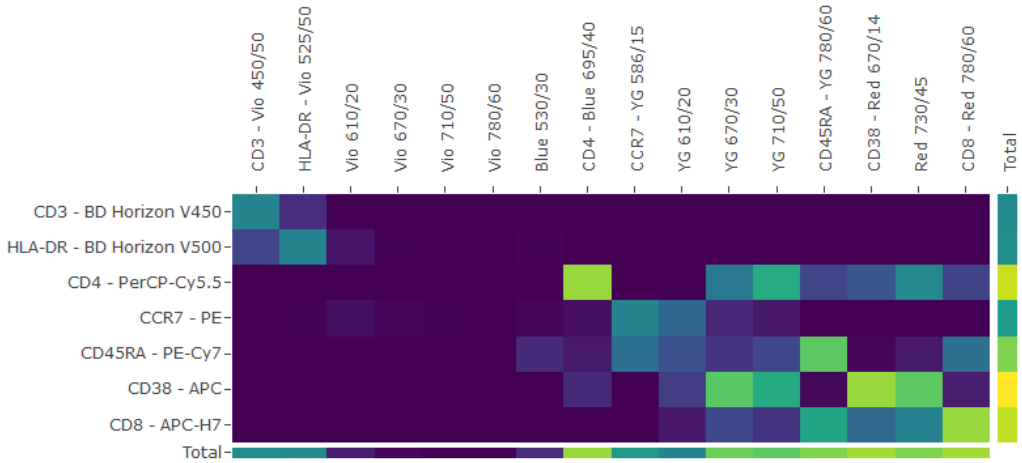
The calculated signals can be shown either for all or for individual populations as well as in a population overview (if a marker table has been uploaded). By choosing "Population overview", you will see the total signal generated by the stained population.



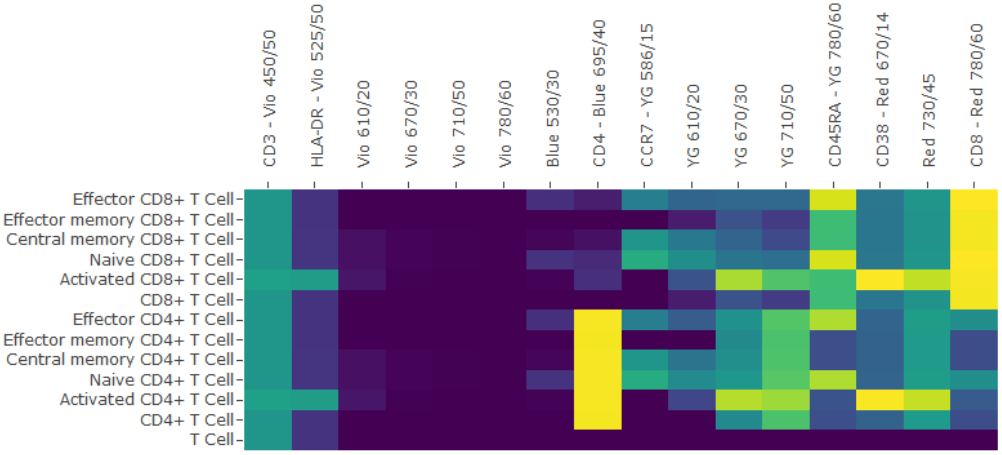
Display the signals for all instrument channels or only for the currently used ones (i.e. channels used by the dyes in your current panel).



Combined populations heat map:

Dyes/markers are in the rows, and instrument channels in columns. The last row (Total) shows the total signal collect in a given channel. The last column (Total) shows the total signal generated on the instrument by the dye/marker combination.

Use to check how much signal a marker-dye combination produces in the individual channels and in total.

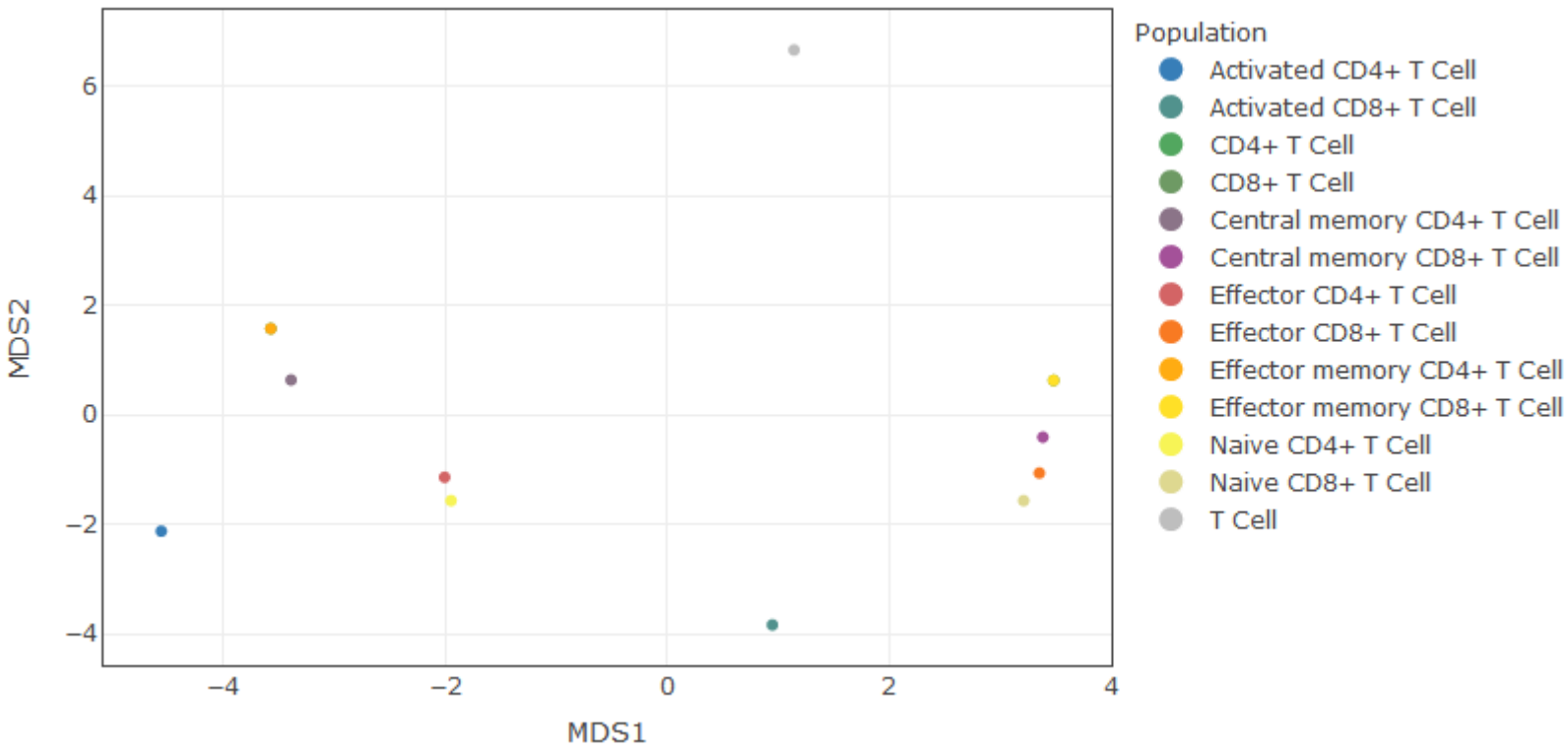


Population overview heat map:

Shows the total signal generated by the individual populations.

Populations are in the rows, and instrument channels in the columns.

When adding new markers to populations, avoid channels that already receive large amounts of signal. Use these channels for markers expressed on different populations.



See also the description in the Dyes section.

The distances here indicate how similar the dye/marker combinations are.

When displaying the population overview, you get an estimate on how well the populations can be separated. Chebishev distance (shows the largest difference in any marker signal) reflects (simplistic) manual gating, while cosine angular and Euclidian distance are more relevant to clustering.

Panel – Signal Plots – Relative Signals – Heat Maps

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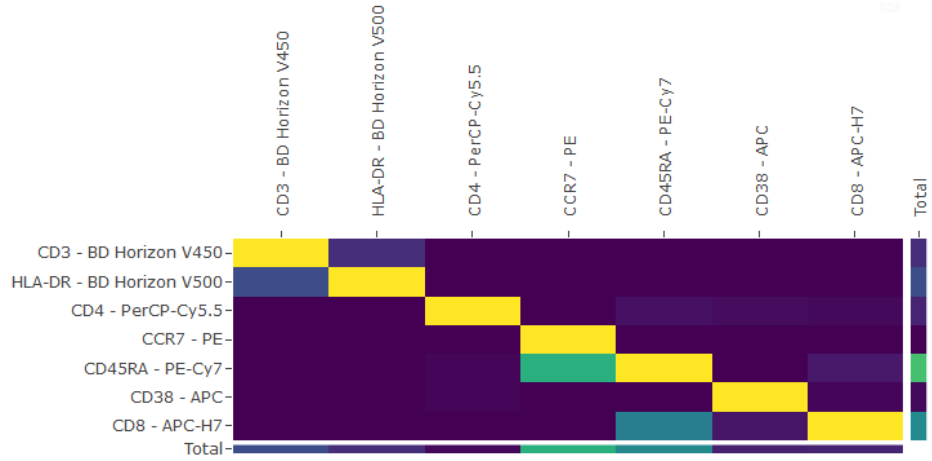
Relative signal is the signal generated by spillover divided by the signal that we intend to measure in a channel (e.g. for the Blue 530/30 channel: the spillover signal generated by PE divided by the FITC signal). The higher the ratio, the higher the contribution of "foreign" signal to the channel and the larger the impact on resolution.

Population overview

The relative signals can be shown either for all or for individual populations as well as in a population overview (if a marker table has been uploaded). By choosing "Population overview", you will see the total relative signal for one marker on a given population.

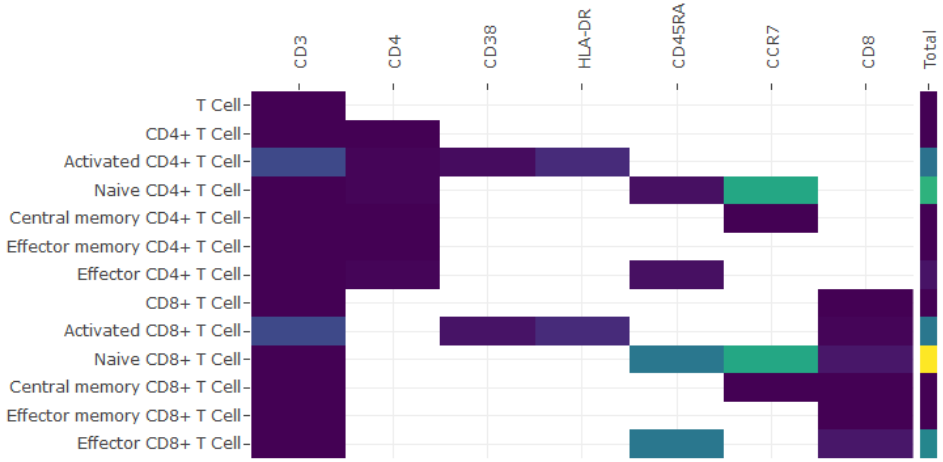


When displayed for the combined populations, this heat map can be compared to the spillover spreading matrix.

Signal producing dyes/markers are in the rows, and the receiving dye/marker channels in the columns.

The last row (Total) shows the total signal collect relative to the main signal in a given channel.

The last column (Total) shows the total relative signal generated by a dye/marker combination.



In the population overview, the total relative spillover for each marker is displayed for each population.

Here you can spot directly, which maker might have a resolution problem on which population.

Panel – Signal Plots – Relative Signals – Distance Plot

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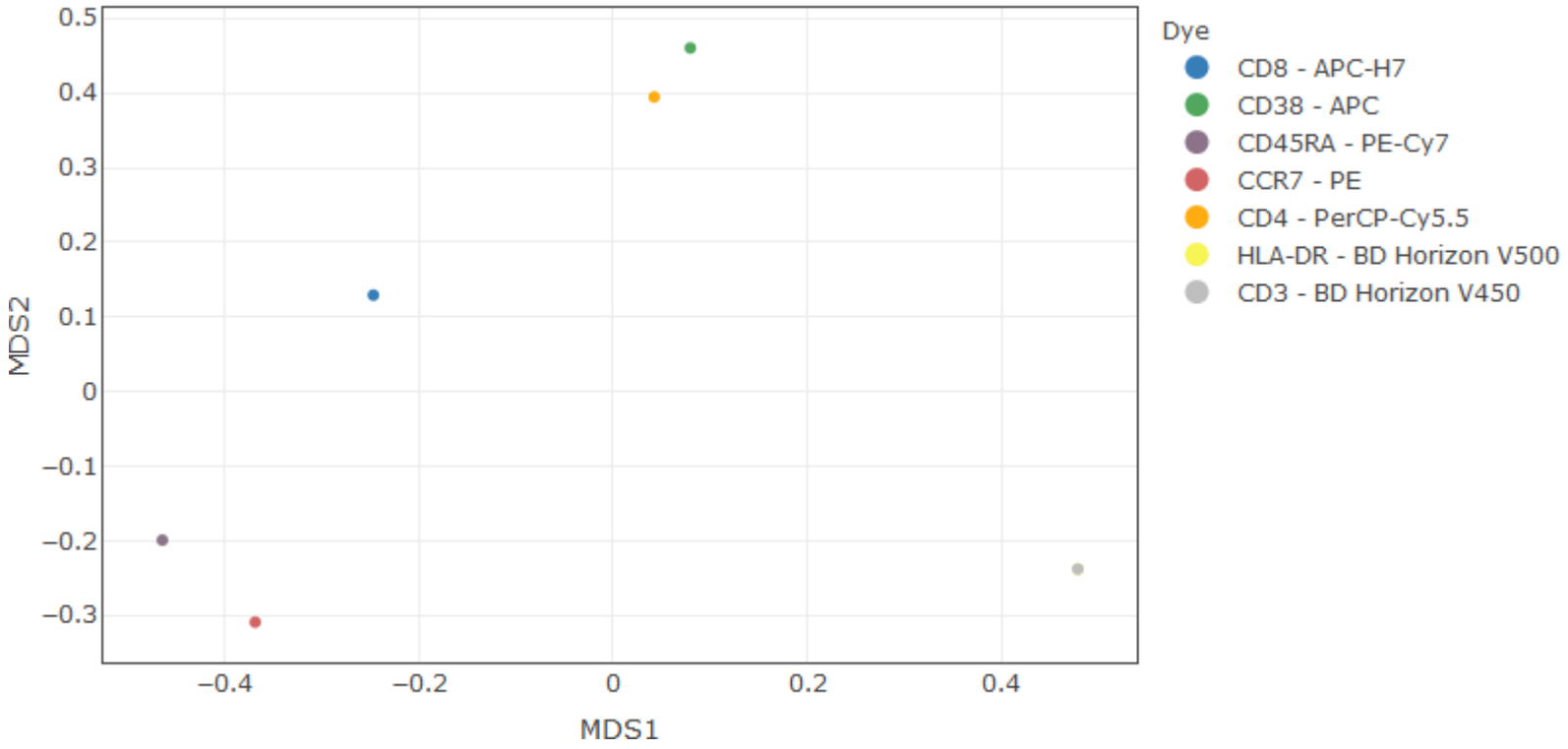


See also the description in the Dyes section.

For the combined populations, the distance map with cosine angular or Euclidian distance can highlight the outlier dye/marker combinations (better and worse than average).

Summary – Download

Give your panel a name.

Download a printable HMTL summary of your panel.

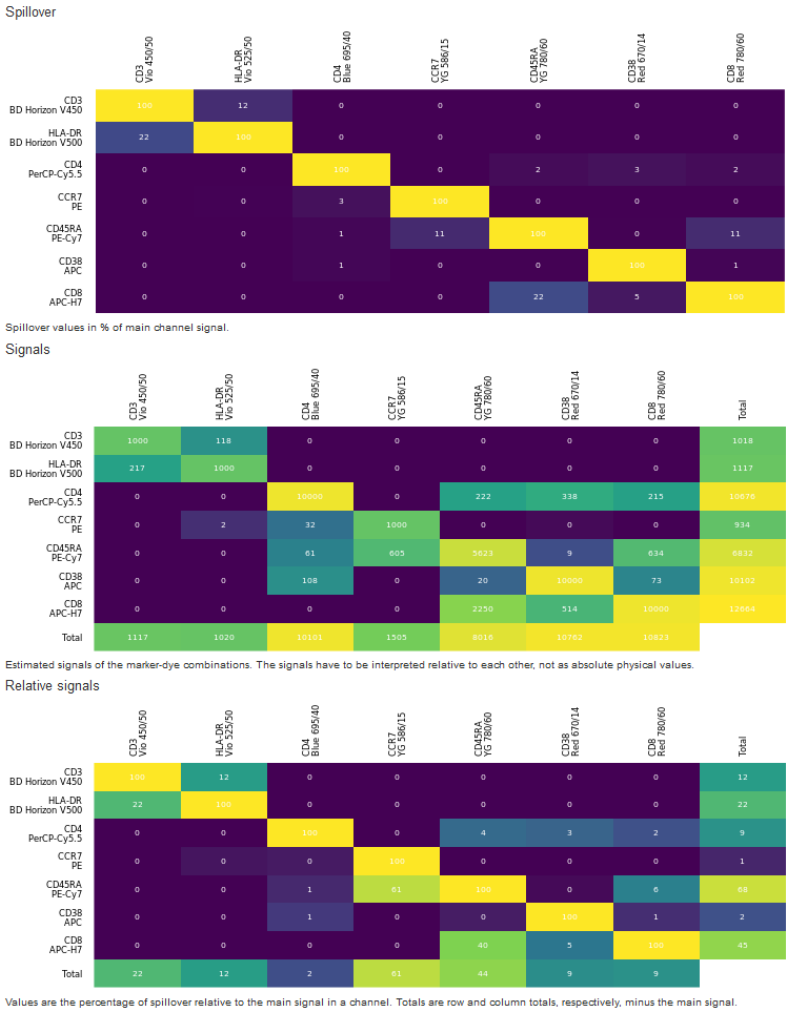
Test panel

Download Summary

Marker	Expression	Dye	Brightness	Channel
CD3	Level 3	BD Horizon V450	Level 1	Vio 450/50
HLA-DR	Level 3	BD Horizon V500	Level 1	Vio 525/50
CD4	Level 4	PerCP-Cy5.5	Level 3	Blue 695/40
CCR7	Level 1	PE	Level 5	YG 586/15
CD45RA	Level 3	PE-Cy7	Level 4	YG 780/60
CD38	Level 3	APC	Level 5	Red 670/14
CD8	Level 5	APC-H7	Level 1	Red 780/60

Panels generated with the help of this tool will have many uncertainties (e.g. due to incomplete spectral information, the rough estimates for brightness and expression levels etc.).
It should be regarded as a starting point only and needs to be verified by experimental data!

The summary contains non-interactive plots that can be printed.



The following companies have kindly granted us permission to use their spectra in the Panel Design Tool:

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- BD Bioscience
- BioLegend
- biostatus
- Miltenyi Biotec
- Thermo Fisher Scientific
  All spectra from Thermo Fisher Scientific used with permission from Thermo Fisher Scientific copyrighted 2020.

All fluorescent protein spectra are taken from the excellent FPbase.

See the "Source" column in the dye tables in the Dyes section for the source of the respective spectra.

ALL SPECTRA ARE PROVIDED WITHOUT ANY WARRANTY.

Contact us at info@cytometry.uzh.ch if the dyes that you want to use are not yet included in the database.