ExoView® Analyzer

Software User Guide

For the most recent version of this User Guide, please see
https://nanoviewbio.atlassian.net/servicedesk/customer/portal/1/article/1137541121

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About

ExoView® Analyzer is our platform for analyzing, aggregating, and exporting the large amounts of data which can be captured with ExoView. ExoView Analyzer enables complete characterization of all particles detected in the interferometry and fluorescence channels. This includes analysis of particle size, fluorescence intensity, colocalization, and more. Data from one or more ExoView chips can be analyzed on a rich variety of plots. Plots can be easily saved to a browser-based report, which conveniently enables downloading of plots and raw data.

Requirements

ExoView Analyzer can be run on a 64-bit Windows 10 PC, with a recommended 16GB of memory. For viewing the exported data report, an updated version of Google Chrome is recommended.

First Look

ExoView Analyzer is divided into four interactive sections—(1) a data control panel, (2) a data navigation bar, (3) a plotting area with attached controllers, (4) a panel for building reports, and (5) a log which records progress through the program.

1. The **data control panel** contains three sequential workflow buttons (**Setup**, **QC**, and **Analysis**) which change the actions available to the user.
    - **Setup** encompasses loading data, entering metadata, and matching isotype and capture probes.
    - **QC** (Quality Control) walks through disabling outlier spots, and setting cutoffs for particle detection
    - **Analysis** enables data browsing and plotting.

2. The **navigation bar** provides controls to select a chip, a probe, and/or a spot for viewing. The **heatmap** button returns to a whole experiment heatmap view.

3. The **plotting panel** shows quantitative data views.

4. The **reporting panel** provides resources for exporting data.

5. The **logging panel** shows informative messages when moving through the software .

Setup

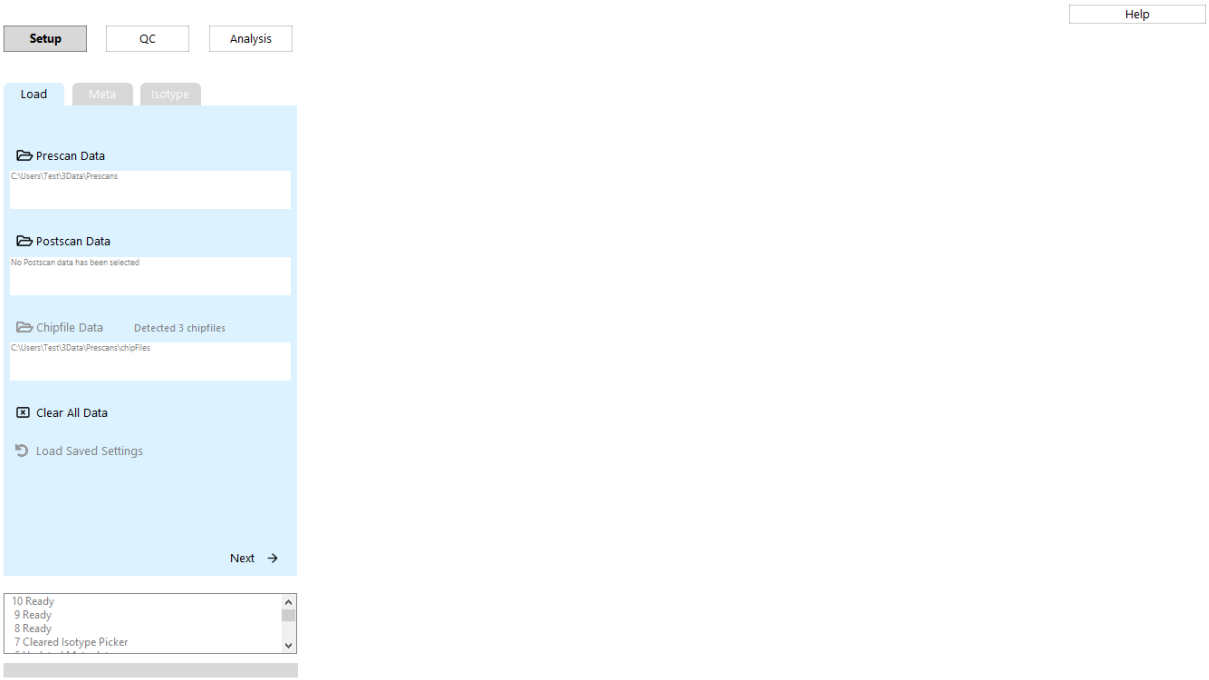
The **Setup** workflow is the initial starting point for any ExoView analysis. This workflow contains three panels: **Load**, **Meta**, and **Isotype**.

- The **Load** panel is used to load single-scan (prescan-only) or multi-scan (prescan and postscan) data. If viewing single-scan data, the folder path should be entered in the **Prescan Data** field.
- The **Meta** panel is used to enter sample-specific labels for chips and staining probes and include chips and probes in the analysis.
- The **Isotype** panel is used to identify and match isotype control and capture probes. In nearly all cases, this panel will auto-populate.

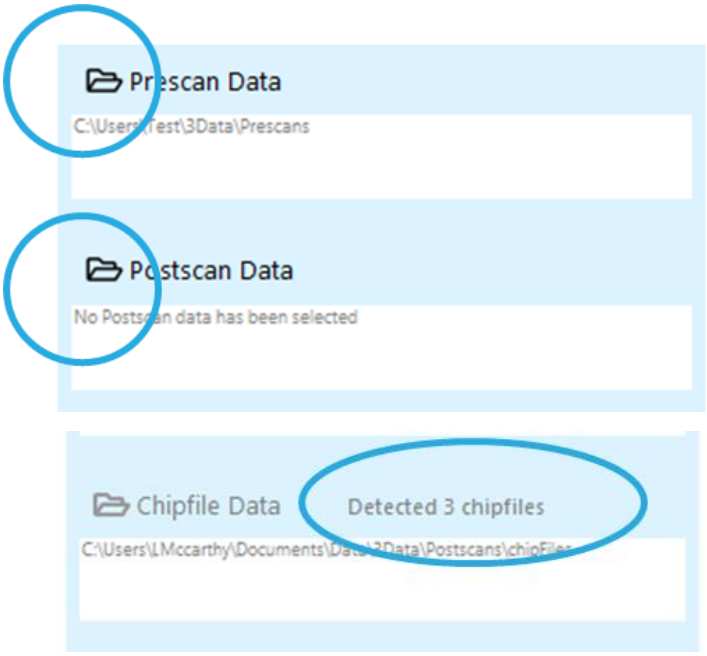
Saving of the **Setup** and **QC** user-data is allowed for multi-scan data.

Load

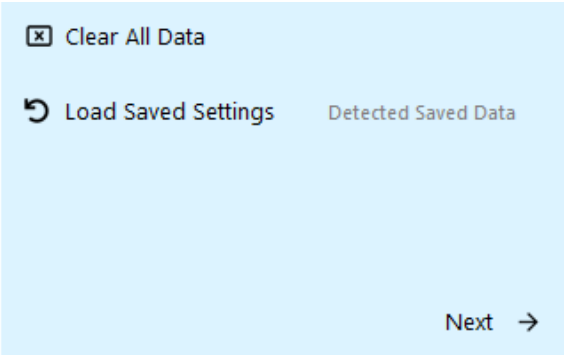
1. Double-click the ExoView Analyzer icon to launch. At first, only the **Load** panel is visible.



2. To start viewing your data, select a Prescan folder and a Postscan folder by clicking the folder icons. Only one set of prescan-postscan folders can be loaded at a time. ExoView Analyzer will automatically detect chip configuration data, the number of chipfiles in the folder will populate to the right of the "Chipfile Data" title.

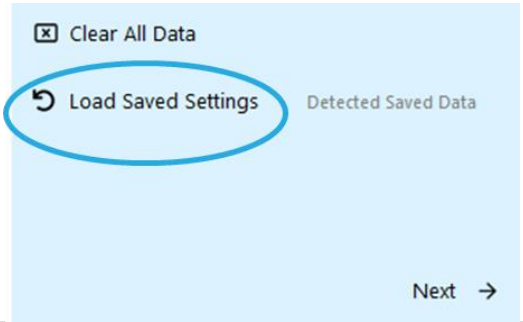


3. When ExoView data has been selected, the "Next" button will be enabled to advance. Press "Next" to advance, or "Clear All Data" to select new folders.



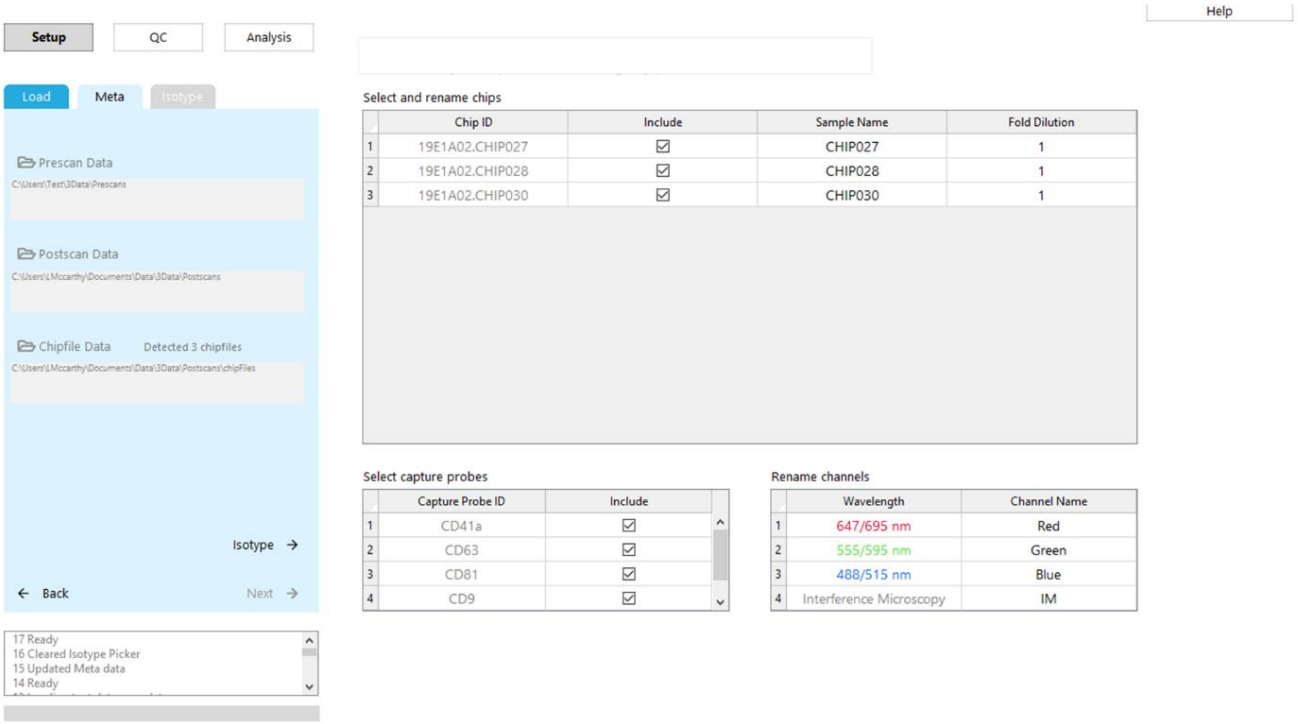
Reload

- If data has been saved, the "Load Saved Settings" button can be pressed to restore the previously entered **Setup** and **QC** selections. This option does not restore the report or plot settings such as color contrast.



Meta

ExoView data consists of one or more chips containing one or more capture probes that can be loaded together as an experiment. Up to sixteen chips can be analyzed simultaneously. Currently, ExoView Analyzer supports analysis of chips that share a set of capture probes.



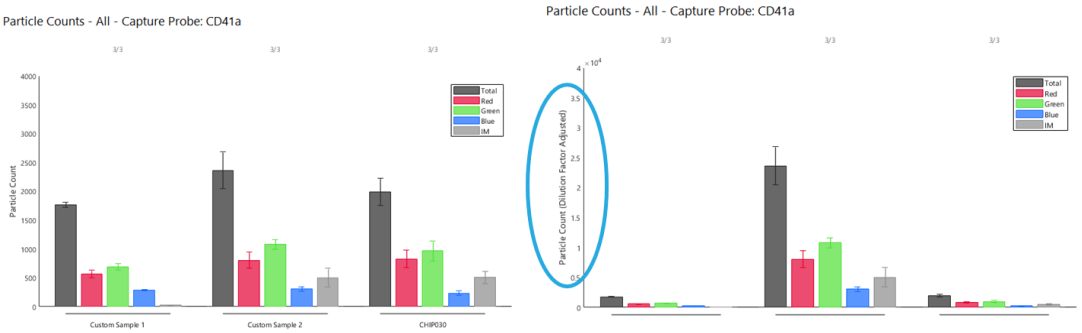
- Chips can be excluded from analysis by unchecking the corresponding "Include" checkbox. Sample names can be assigned to chips by using the corresponding "Sample Name" cell. Chip names cannot be duplicated and are limited to 16 characters or under. Special characters are not recommended.

Select and rename chips

	Chip ID	Include	Sample Name	Fold Dilution
1	19E1A02.CHIP027	☑	Custom Sample 1	1
2	19E1A02.CHIP028	☑	Custom Sample 2	1
3	19E1A02.CHIP030	☑	CHIP030	1

- Entering a "Fold Dilution" will automatically apply dilution adjustment to all plots and calculations in the Analysis portion of the software. When relevant, dilution adjustment is annotated on figures, as seen below.

	Chip ID	Include	Sample Name	Fold Dilution
1	19E1A02.CHIP027	☑	Custom Sample 1	1
2	19E1A02.CHIP028	☑	Custom Sample 2	10
3	19E1A02.CHIP030	☑	CHIP030	1



- Capture probes can be included or excluded from analysis, as seen below.

Select capture probes

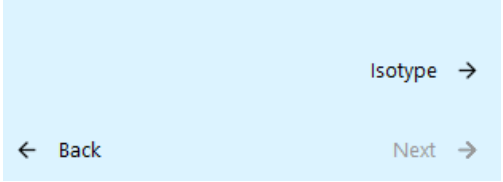
	Capture Probe ID	Include
1	CD41a	☑
2	CD63	☐
3	CD81	☑
4	CD9	☑

- The staining probe used in each excitation channel can be renamed by selecting a cell under "Channel Name" and entering a new name. Channel names cannot be duplicated and are limited to 16 characters or under. Special characters are not recommended.

Rename channels

	Wavelength	Channel Name
1	647/695 nm	Red
2	555/595 nm	Custom Name 1
3	488/515 nm	Blue
4	Interference Microscopy	IM

- When selections have been finished on this panel, press "Isotype" at the bottom of the leftmost panel to proceed to select Isotype controls. Once controls have been selected once, this step can be skipped by pressing "Next" instead. If controls are already included in the chipfile, they will be assigned automatically and this step can be skipped.



Isotype

ExoView Analyzer allows for isotype controls to be identified and matched with the correct capture probes. The isotype controls are used to set the FL cutoffs during the **QC** (see below). On the **Isotype** tab, capture probes can be paired with their appropriate isotype controls.

In nearly all cases, the selection and pairing of isotype controls auto-populates from the chipfile data.

- Click on a probe name to select it as an isotype control. The columns that appear can be used to match probes with controls. In the example shown, the capture probes CD41a, CD63, CD81 and CD9 have all be matched with the MIgG isotype control.

- The "Launch Advanced" option should be used when there are more than 12 capture probes or more than 2 different isotype controls. Clicking this button will popup an expanded isotype matching tool.

- Press the "Next" button to proceed to the QC portion of the software.

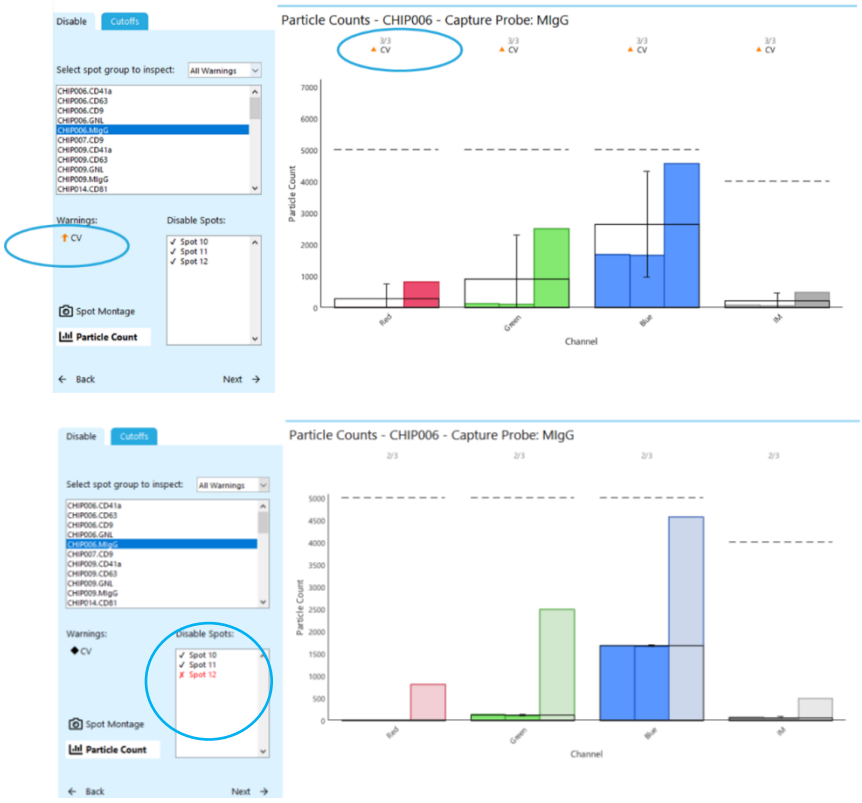
QC

The ExoView QC process improves data quality through disabling any problematic capture probe spots and setting fluorescence cutoffs based on isotype control probes. Disabling problematic spots helps reduce data variability, and setting fluorescence cutoffs improves the detection of real particles.

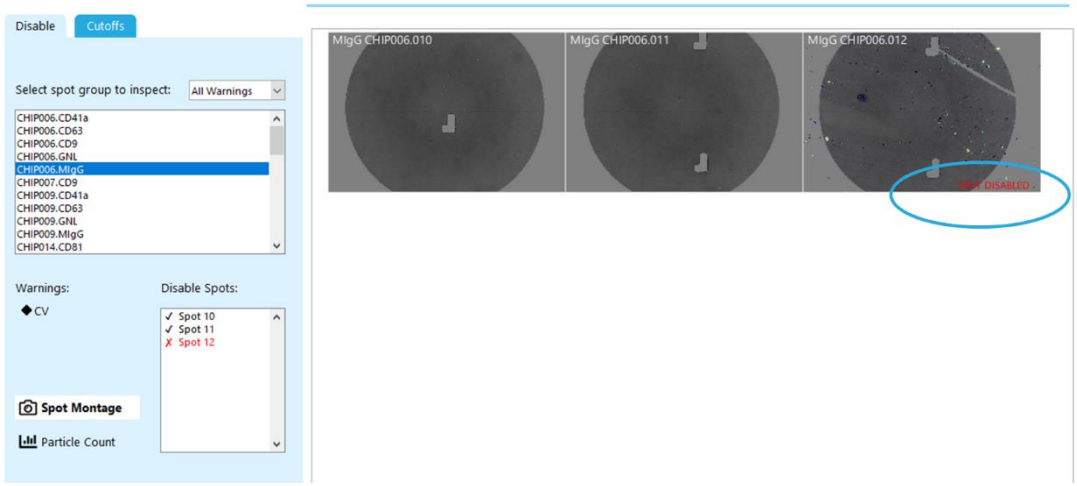
Disable

Chip and sample handling can introduce artifacts onto the spots that can increase data variability. These artifacts include: scratches from tweezer handling, non-uniformity of binding from improper handling or washing, and saturated counts from improper sample incubation. ExoView chips contain multiple technical replicates so that these issues can be accounted for during data analysis. Outlier spots in each capture probe group can be disabled to address these issues.

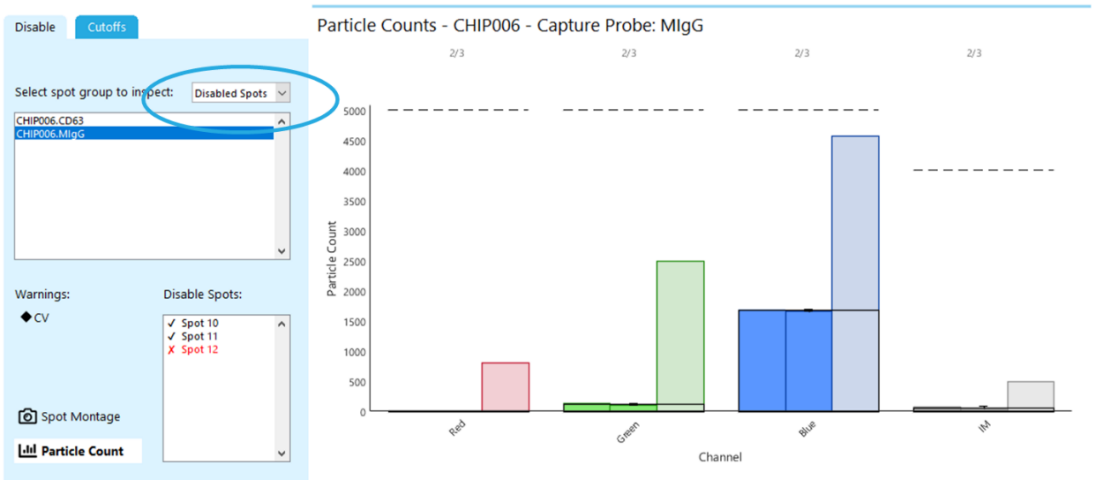
- ExoView Analyzer automatically flags capture probe spot groups with high coefficient of variation (CV) above 20% or high particle count (above the upper limit of quantification). Flagged spot groups are listed in the "Disable" tab. These groups can be visually inspected using the **Spot Montage** or analytically inspected using the **Particle Count**.
- The **Disable Spots** list shows the spots that belong to a selected spot group. Clicking on any of the spot names will disable that spot, which excludes it from all subsequent analysis.
- In the example below, a high CV warning is marked as resolved after deselecting a single spot.



- Viewing the "Spot Montage" allows for a simple visual confirmation of the disabled spot. Below, it is apparent that a scratch on the surface of the chip has contributed to a high variation in particle count relative to the two other spots in this group.



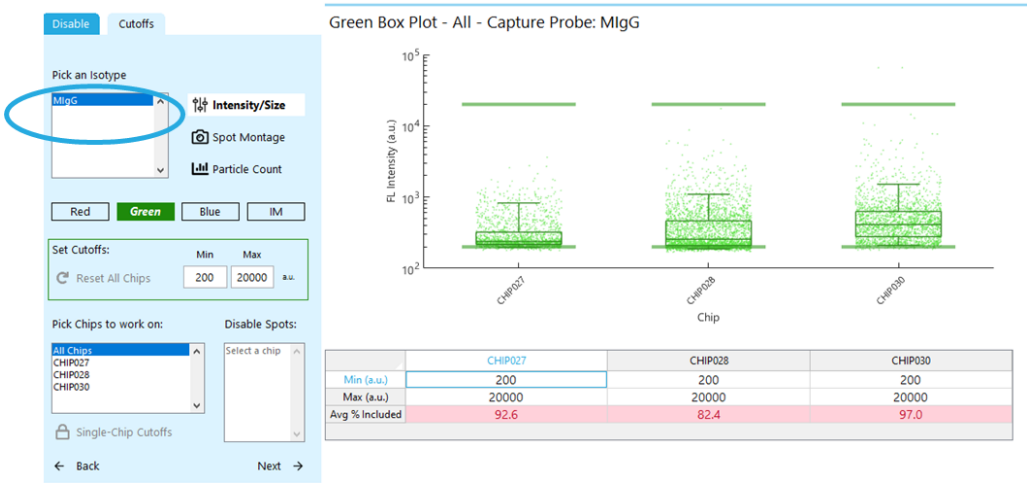
- The dropdown menu on the **Disable** tab can be used to filter the list of spot groups shown for selection. Below the dropdown selection is set to “Disabled Spots” which shows all the spot groups that contain a disabled spot.



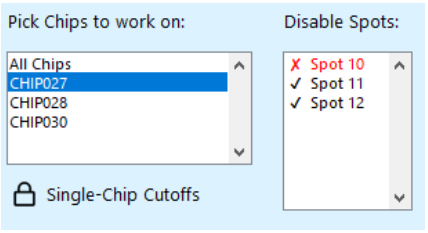
Cutoffs

The **Cutoffs** tab enables setting isotype control-based cutoffs for the experiment. Any particles detected outside the range (below the min value or above the max value) are removed from the analysis. A color-coded "Traffic Light" system is included to give visual assistance with cutoff adjustment.

- When selecting a fluorescence channel, by default a summary of all chips is shown in the form of a boxplot. The isotype control under review is shown in the top-left corner of the panel.



- The table underneath the plot shows the Minimum and Maximum intensity cutoffs per chip, as well as the percentage of particles that are included under the current cutoff settings.
- If the "Avg % Included" for an individual chip turns Yellow or Red, that indicates that too many particles are being detected on the isotype control spot. First, we need to determine if one of the spots contains an artifact and should be disabled. After that, if there are still too many particles detected, then we can adjust the cutoffs.
- High count control spots can be visually inspected using the **Spot Montage.** Signs to look out for are aggregates, gradients, or large streaks of particles on an otherwise homogenous background. If any damage is detected, select the corresponding chip from the menu in the bottom-left corner, and then select the damaged spot to disable it.
- If after disabling any problematic spots, there are still too many particles detected on the isotype control spots, the cutoffs can be adjusted.



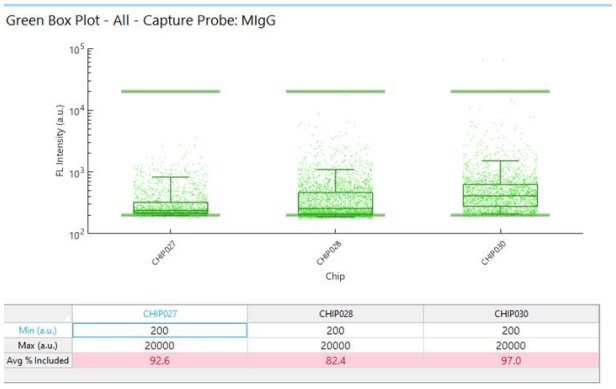
- As a prompt to make adjustments to the cutoff settings, a "Traffic Light" aide highlights spot groups which may be problematic. Adjusting cutoffs in response to these warnings, while not required, can help avoid false positives in particle counts when conducting downstream analyses.

- When adjusting cutoffs, both the table underneath the plot and the text entry box will highlight to reflect whether the new cutoffs are set within reason. The table below shows default warning ranges for minimum cutoff values. To balance control values without overcorrecting, we recommend avoiding values in the Red range.

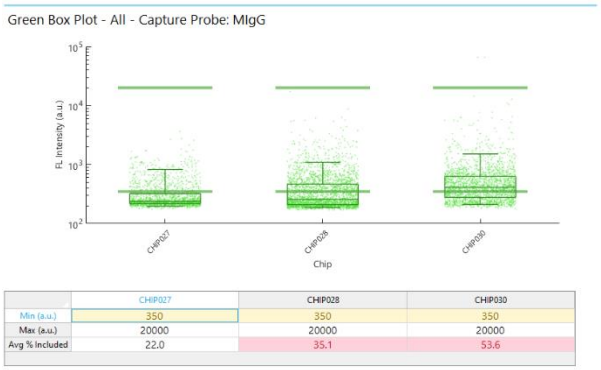
Default Cutoff Intensity Warning Ranges

	Neutral	Yellow	Red
Red 647 Channel	0-300	301-500	>500
Green 555 Channel	0-300	301-500	>500
Blue 488 Channel	0-500	501-750	>750

- Once spot images have been reviewed and spots with artifacts have been disabled, if a Yellow or Red highlight is still present then the channel cutoffs can now be adjusted by increasing the Minimum intensity value. The Maximum intensity is set by default to 20,000 a.u. for all channels and typically does not require any adjustment.



- Increase the Minimum cutoff by 10 to 20 unit increments until the "Avg % Included" cell for each chip is either Yellow or no longer highlighted. If all Red values have now been removed, complete setting cutoffs for all channels and then move to the Analysis step.



	CHIP027	CHIP028	CHIP030
Min (a.u.)	350	350	350
Max (a.u.)	20000	20000	20000
Avg % Included	22.0	35.1	53.6

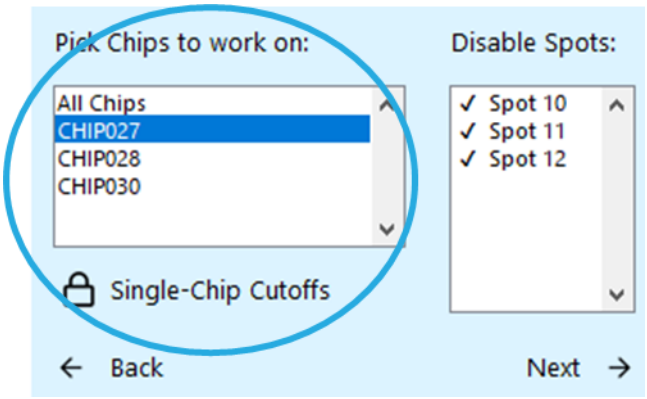
- If the Minimum cutoffs value and the "Avg % Included" both become highlighted in Red, it is likely that the data selected for analysis has non-standard properties, such as unusually high isotype particle background or low antigen expression. We recommend reaching out to NanoView support via the ExoView Analyzer portal, which can be found here:

  https://nanoviewbio.atlassian.net/servicedesk/customer/portal/1/article/1137541121

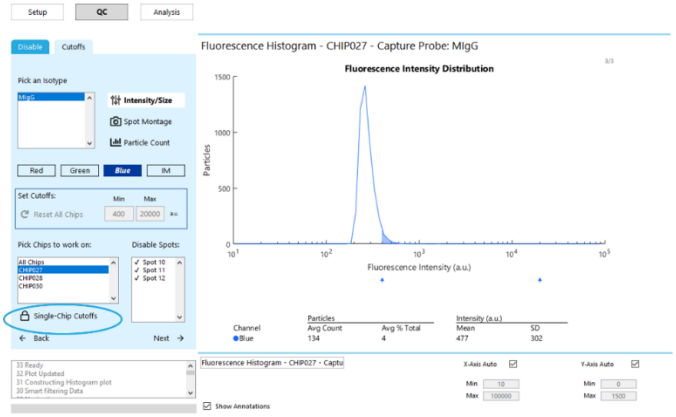
	CHIP027	CHIP028	CHIP030
Min (a.u.)	425	425	425
Max (a.u.)	20000	20000	20000
Avg % Included	16.6	27.1	40.6

Advanced Cutoff Adjustment

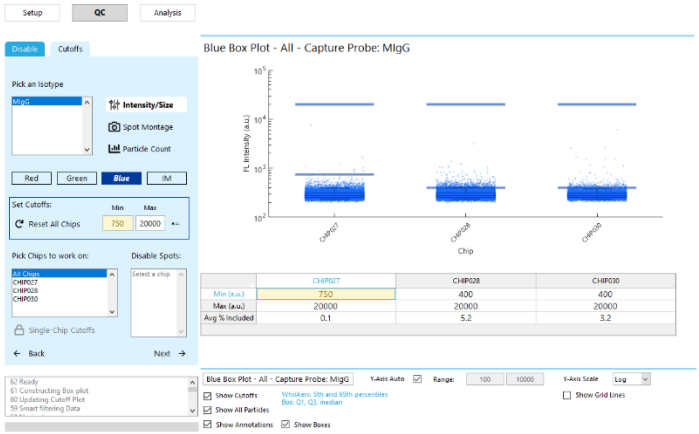
- Cutoffs can also be adjusted individually for each chip in the loaded data. To do so, first select the chip to be adjusted from the list of chips. We recommend that single-chip cutoffs are only used when all chips are not part of the same experiment.



- After selecting a chip, press the "Single-Chip Cutoffs" button to unlock cutoff adjustment for that chip.



- Selecting "All Chips" again shows that only a single cutoff has been adjusted. Adjusting the cutoff value when "All Chips" is selected will overwrite all single-chip cutoffs.



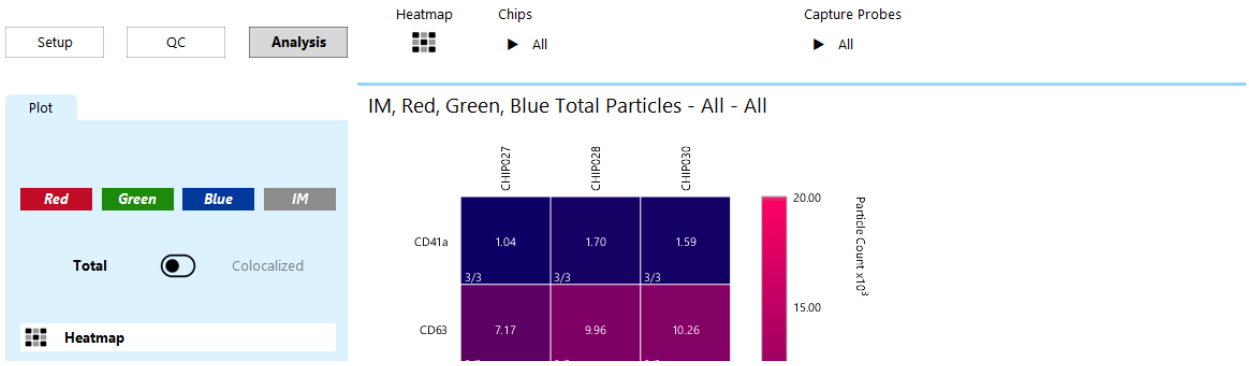
Analysis

After QC completion, the **Analysis** section allows for viewing plotted data and calculated metrics at a high-level experimental view or a more in-depth analysis of individual sample or capture probe groups. Available analysis includes counts, intensity distributions, size distributions, colocalization analysis, and images.

The plot type and type of data that is aggregated on any given plot can be controlled on the "Plot" tab. The available plots for any subset of data are enabled based on the channels selected for analysis, and on the navigation bar.

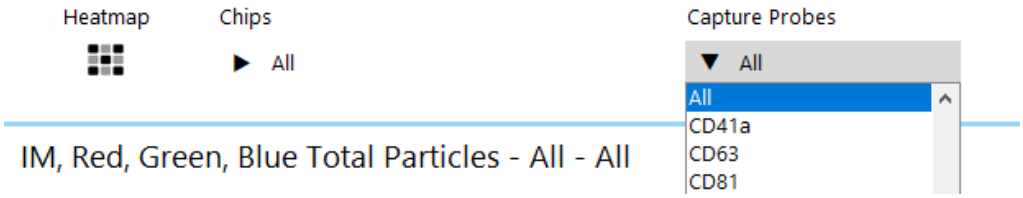
Data Navigation

- After the QC process has been completed, the Analysis portion of the software is enabled. The first plot shown is a Heatmap which captures all Chips and all Capture Probes included in the loaded data.
- The key component to navigating through the data is the Navigation Bar, located at the top of the window.

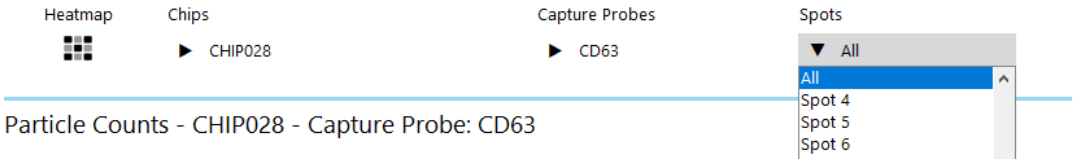


- Select a chip from the "Chips" dropdown menu to go to a single-chip, all-capture probes view of the data.

- Select a probe from the "Capture Probes" dropdown menu to go to a single-probe, all-chip view of the data.



- To view data for a single capture probe spot, select a single chip and a single capture probe group. The "Spots" dropdown menu will populate with the spots available to view.



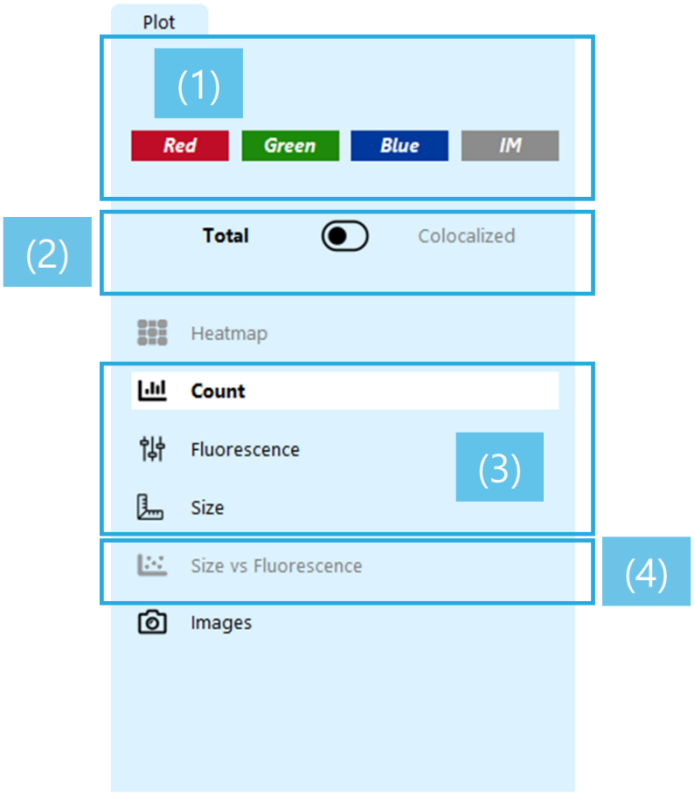
- Press the "Heatmap" button at any time to return home to the heatmap.

1. To include or exclude channels from analysis, use the color-coded channel buttons.

2. To swap between counting based on total particles or colocalized particles, use the toggle switch.

3. To view a plot available at the current navigation level, use the plot buttons.

4. To view any plots with disabled buttons, change the navigation level via the Navigation Bar. For example, pressing the "Heatmap" button to return home will enable the "Heatmap" button.



- For an in-depth view of all available plots, their availability, and their controls, see the "Guide to Plot Controls" section.

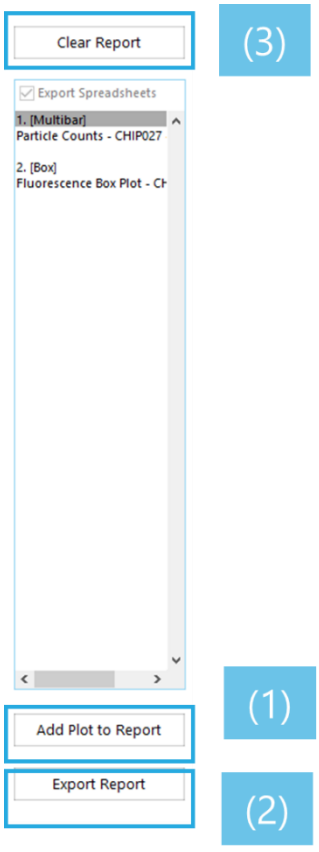
Export

At any point when a plot is visible, the plot data can be exported to a standard browser-based report that includes the plot image, related metadata, and formatted spreadsheets for quick data review and sharing. The report can be printed to PDF for streamlined sharing.

1. Press the "Add Plot to Report" (1) button to add the currently viewed plot to the report. The plot types and names are populated in the list of plots to export.

2. Press "Export to Report" to select the export directory (2).

3. At any time, the "Clear Report" (3) button can be pressed to remove all plots from the current list.
   The "Export Spreadsheets" checkbox below "Clear Report" determines whether.xlsx data exports are included in the report. Unchecking it decreases the time needed to save a plot, but means that raw data will not be available on export. This option can only be changed before any plots have been added.

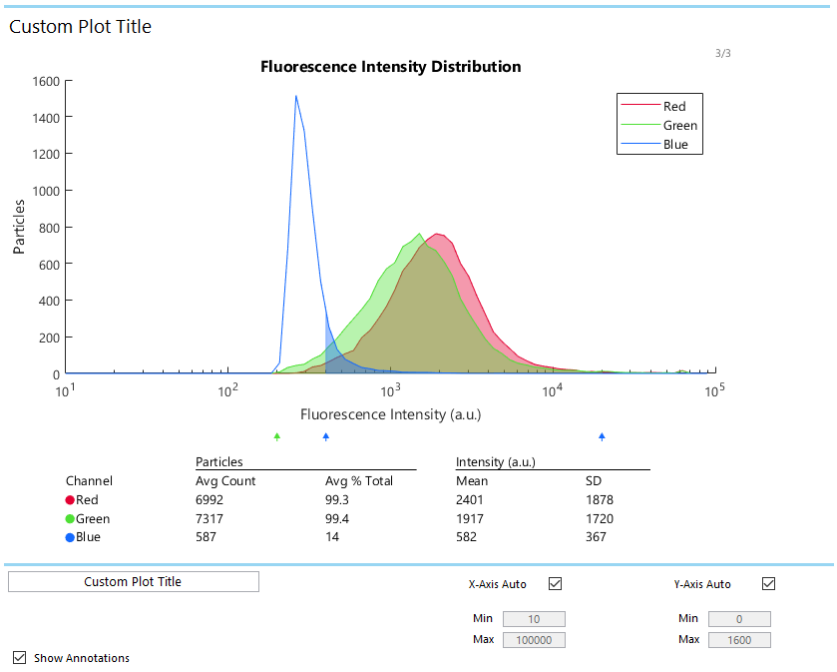


For an in-depth guide to the in-browser report, see the "Guide to Data Export" section.

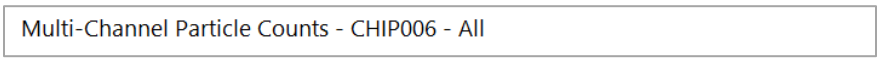
Guide to Plot Controls

Shared Controls

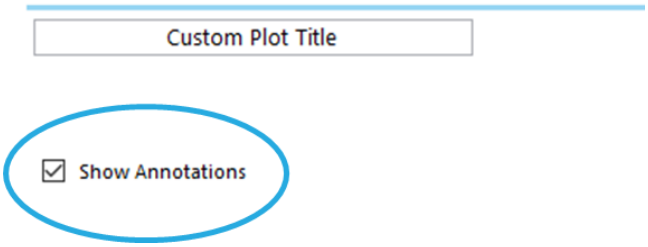
- **Title Bar** – plots, with the exception of images, have a custom title bar which is shown at the top of the plot. This title is shown while viewing the plot in the program and also shown on the export. The title can be edited using the box just beneath the plot on the top-left of the plot controls. Typing into this field dynamically updates the plot title.



- The default title for a plot is assigned based on the data that is currently being viewed in the following format: *Plot Information – Chips – Probes – Spots.* For example, the plot below shows particle counts for all probes present on CHIP006. Since no spots are selected, the spot portion is blank.



- **Annotations Checkbox** – supplemental information such as number of disabled spots can be toggled on and off on most plots.



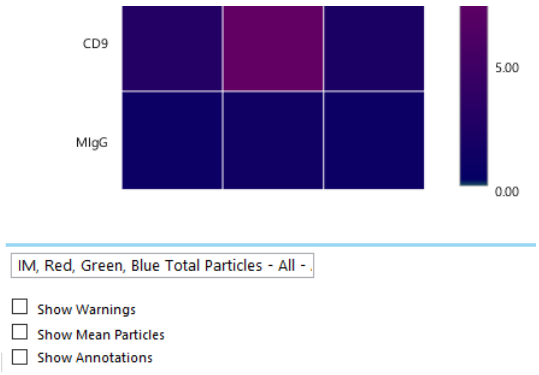
Heatmap

- The **Heatmap** shows particle counts for all included chips and probes and is the landing point for the **Analysis** view. Counts are shown as the mean across all spots for each group.

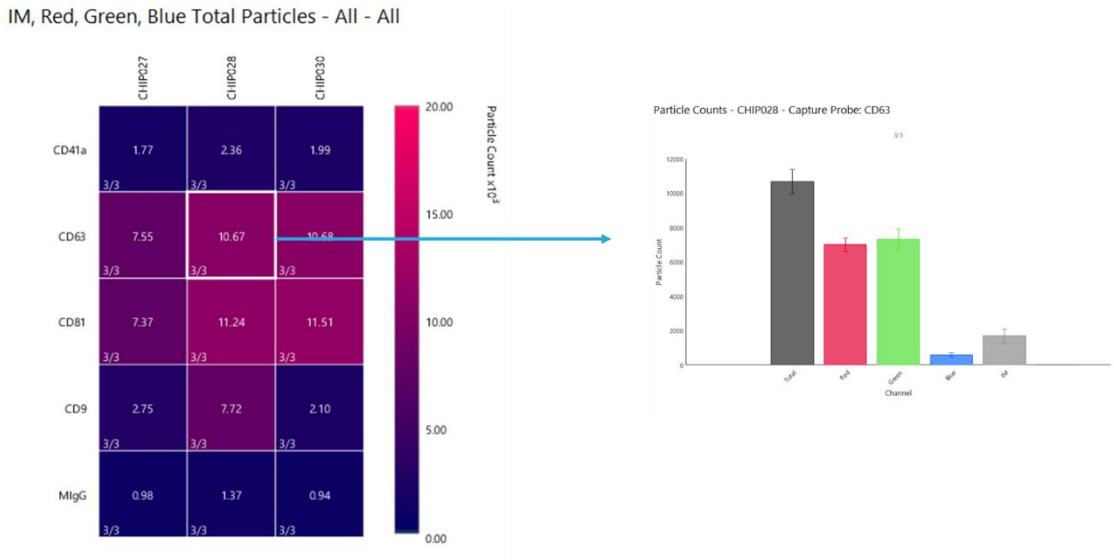
- Plot color contrast can be adjusted via the slider bar on the bottom right.

- Checking "Show Warnings" shows or hides warning flags associated with any given group of spots.

- Checking "Show Mean Particles" shows or hides the mean particle count across spots.



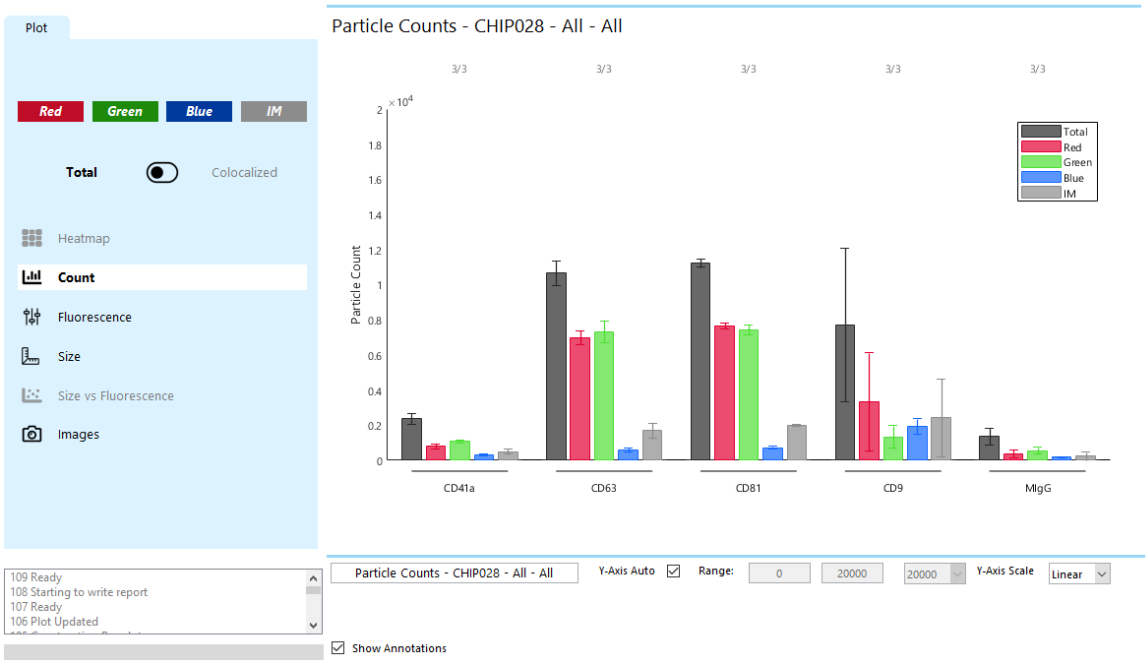
- As an alternative to the navigation bar, the Heatmap can be used to navigate to a subset of data. A specific set of capture probes, a specific chip, or a specific capture probe group on a specific chip can be selected by clicking on the appropriate location in the Heatmap.



Count

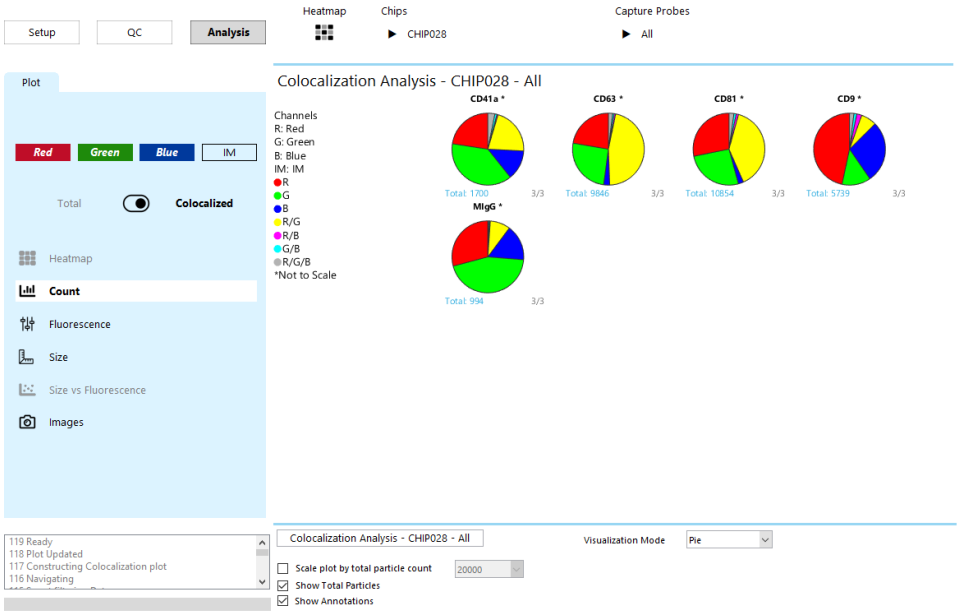
Particle Counts

- The **Multi-Channel Bar Plot** shows mean and standard deviation of particle counts for all selected channels. Annotations, y-axis range, and y-axis scale (linear or log) can be adjusted in the controller below the plot. Renamed channels will populate in the legend, which can be clicked and dragged around the plot area.
    - On this plot, the 'Total' bar represents total unique particles, while the bar for each channel counts any particle tagged with a given staining probe. For example, the 'Red' bar below counts particles which are positive in the Red channel but could be either positive or negative for Green and Blue.

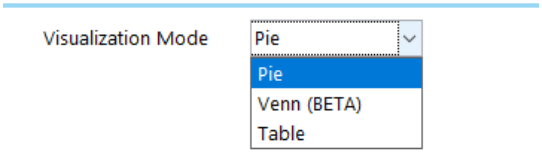


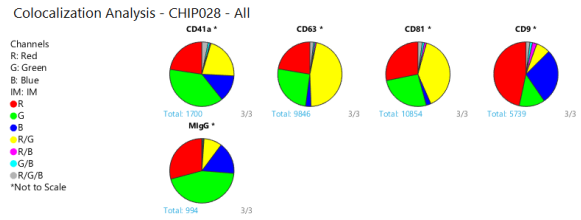
Colocalization Analysis

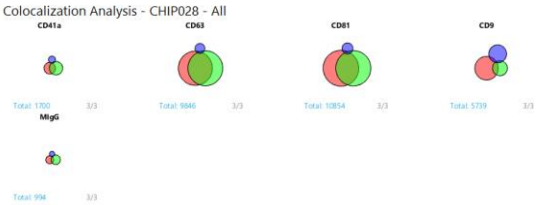
- When **Colocalized** is selected, the colocalization analysis view is displayed. Colocalization counts are aggregated based on the currently selected channels, and ignore deselected channels. Below, the counts for **Red** correspond to single-positive particles that are detected in the Red fluorescence channel only, **Yellow** corresponds to **Red/Green** double-positive particles, **Gray** correspond to **Red/Green/Blue** triple-positive particles.

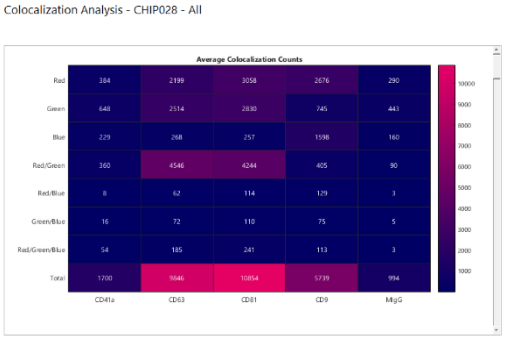


- The selected channels can be viewed as a series of pie charts, a series of Venn diagrams, or a data table by selecting the option via a dropdown menu.

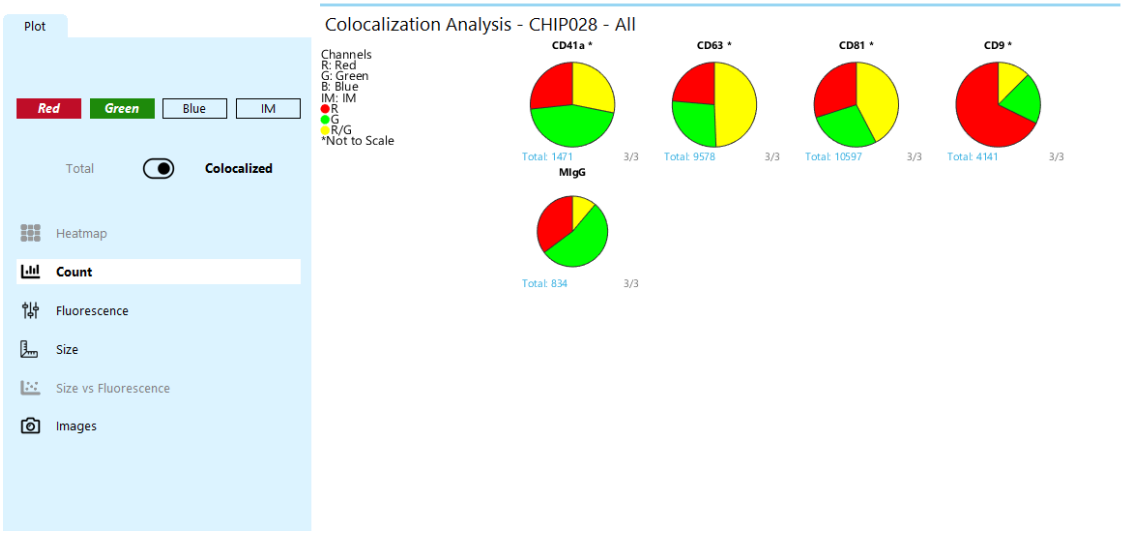




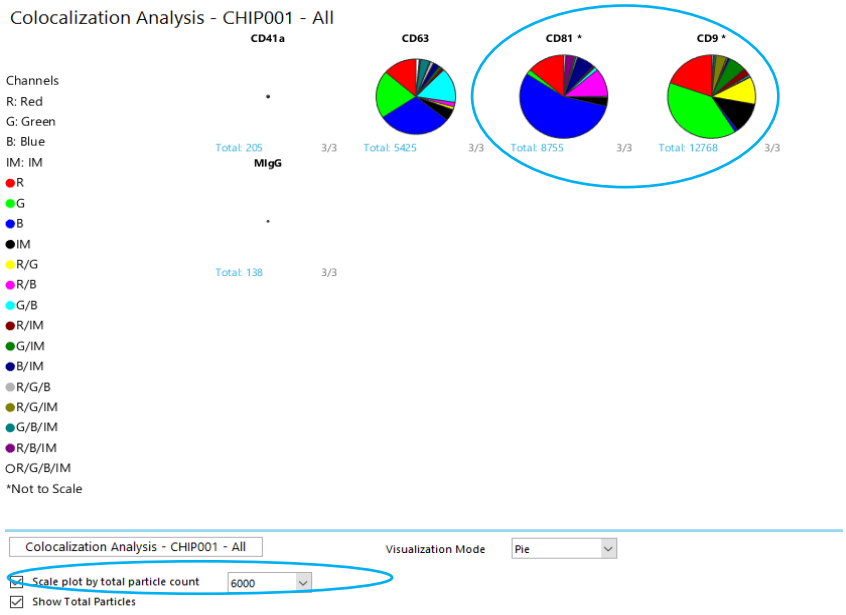




- Deselecting a channel ignores it in the colocalization analysis. Below is a plot where Blue and IM channels are deselected.
    - The counts for **Red** correspond to particles that are detected in the Red fluorescence and **not** the Green fluorescence channel, but can have any values for Blue or IM.
    - The counts for **Green** correspond to the particles that are detected in the Green fluorescence channel and **not** the Red fluorescence channel, but can have any values for Blue or IM.
    - The counts for **Red/Green** correspond to the particles that are detected in **both Green** and **Red** fluorescence channels, but can have any values for Blue or IM.



- The option is available to view pie charts either at a fixed size or scaled by total particle count (shown in blue text). An asterisk by any given sample name means that the plot is either not scaled, or cannot be correctly scaled because the total counts are above the user-entered maximum particle count to scale against. To the right, CD81 and CD9 show counts above the user-entered max value of 6000.

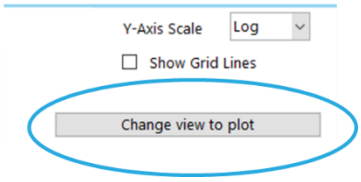


Fluorescence

Fluorescence Box Plot

- The **Fluorescence Box Plot** shows cutoffs, 5th and 95th percentiles, Q1, Q3, and the median of particle fluorescence intensity for all fluorescence channels enabled. This plot can be viewed when Navigation is set to one Chip and all Probes, or one Probe and all Chips.

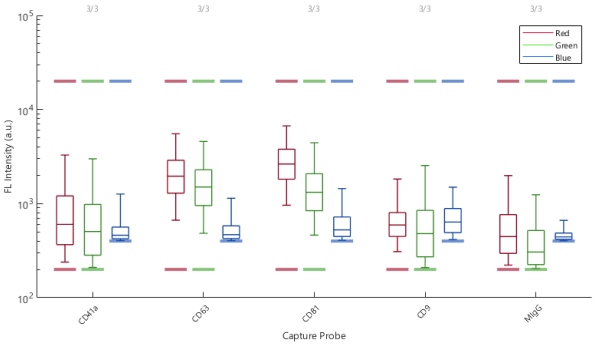
- Metrics are first viewable in a tabular format for quick reference, and can be switched to a visual boxplot, as shown below.



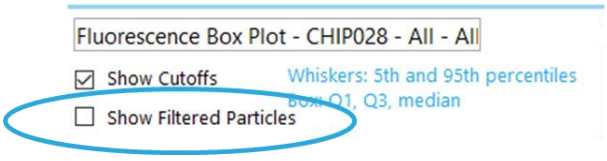
Fluorescence Box Plot - CHIP028 - All - All

	CD41a	CD63	CD81	CD9	MIgG
Channel	Red	Red	Red	Red	Red
Median (a.u.)	602	1956	2636	593	447
Mean (a.u.)	1100	2401	3114	778	725
SD (a.u.)	1550	1878	2109	826	937
Channel	Green	Green	Green	Green	Green
Median (a.u.)	505	1501	1319	480	306
Mean (a.u.)	931	1917	1778	813	482
SD (a.u.)	1482	1720	1750	1140	638
Channel	Blue	Blue	Blue	Blue	Blue
Median (a.u.)	461	468	526	637	443
Mean (a.u.)	588	582	678	764	477
SD (a.u.)	392	367	465	456	142

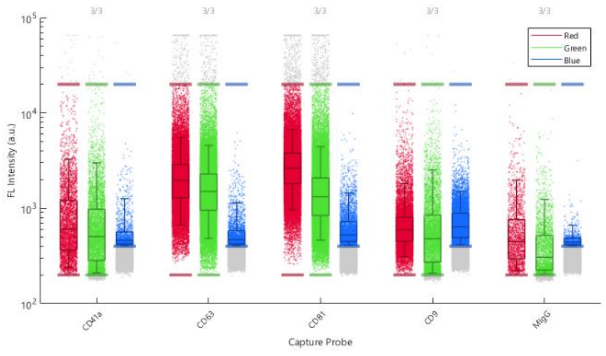
Fluorescence Box Plot - CHIP028 - All - All



- The boxplot has an option to display the scatter of individual particles, as shown below.

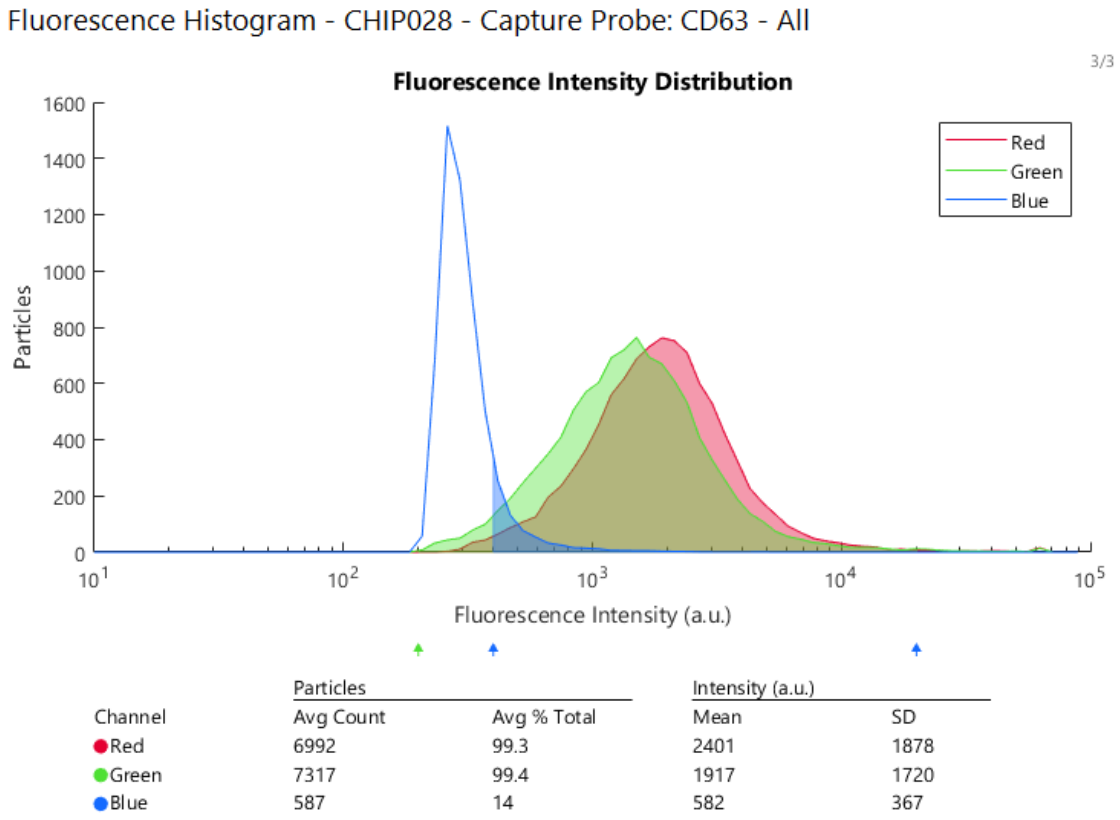


Fluorescence Box Plot - CHIP028 - All - All



Fluorescence Histogram

- The **Fluorescence Histogram** shows histograms of particle count binned by fluorescence intensity (a.u.). This plot can be viewed when Navigation is set to one Chip and one Probe. The shaded portion represents the particles included for analysis after cutoffs have been applied. The table below the plot shows mean count and % of total particles across capture probe spots, as well as mean and standard deviation of fluorescence intensity across capture probe spots.



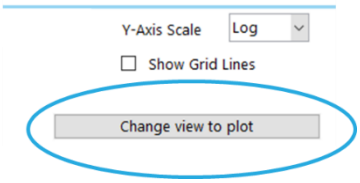
	Particles		Intensity (a.u.)	
Channel	Avg Count	Avg % Total	Mean	SD
Red	6992	99.3	2401	1878
Green	7317	99.4	1917	1720
Blue	587	14	582	367

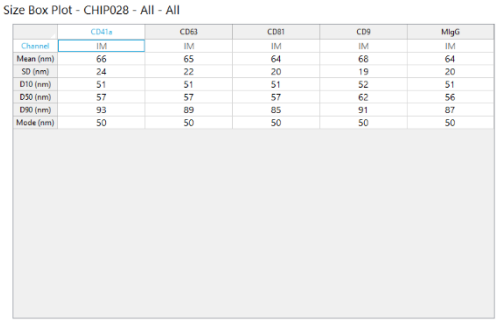
Size

Size Box Plot

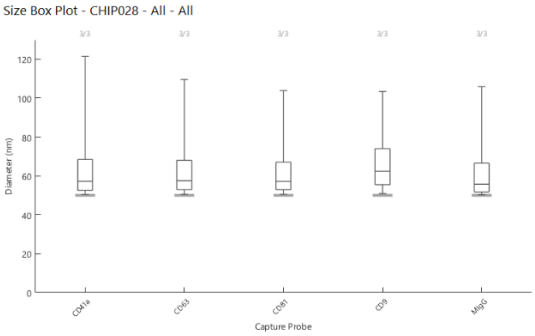
- The **Size Box Plot** shows cutoffs, 5th and 95th percentiles, Q1, Q3, and the median of particle diameter. The option to plot every particle is also available. This plot can be viewed when Navigation is set to one Chip and all Probes, or one Probe and all Chips.

- Metrics are first viewable in a tabular format for quick reference, and can be switched to a visual boxplot, as shown below.

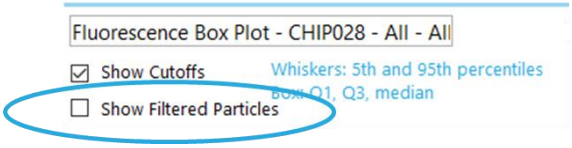


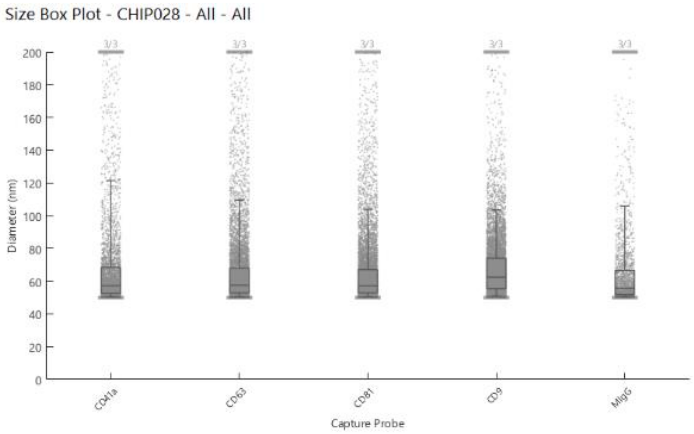


	CD41a	CD63	CD81	CD9	MIgG
Channel	IM	IM	IM	IM	IM
Mean (nm)	66	65	64	68	64
SD (nm)	24	22	20	19	20
D10 (nm)	51	51	51	52	51
D50 (nm)	57	57	57	62	56
D90 (nm)	93	89	85	91	87
Mode (nm)	50	50	50	50	50



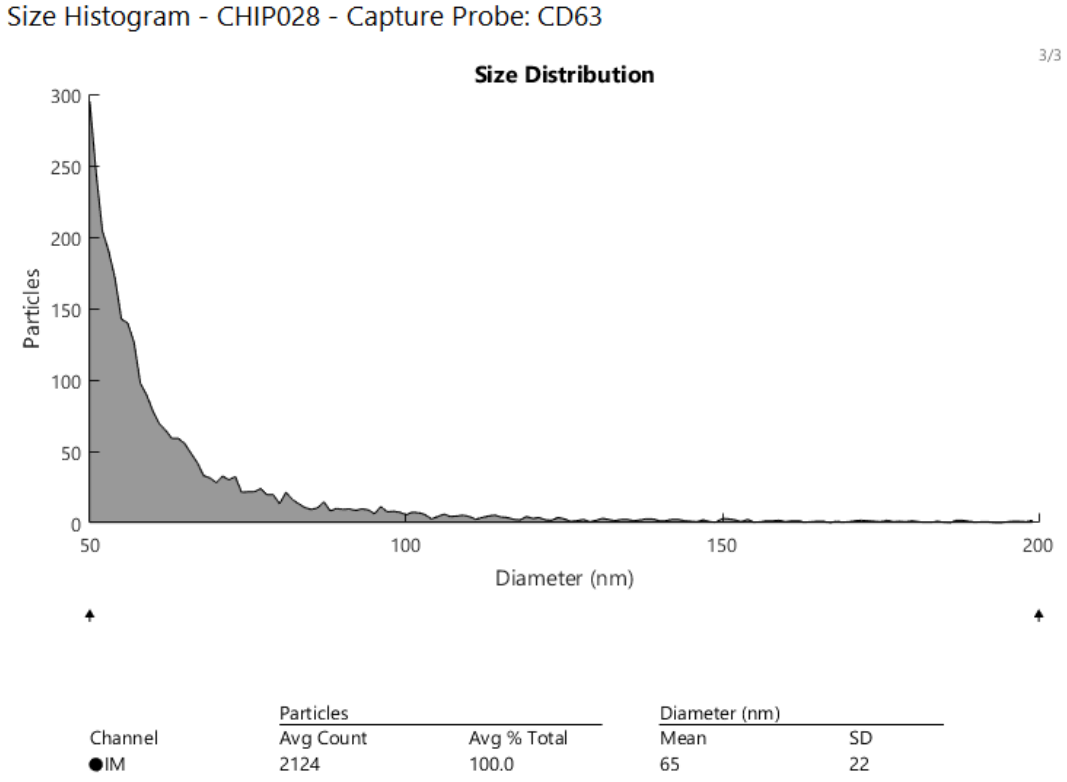
- The boxplot has an option to display the scatter of individual particles, as shown below.





Size Histogram

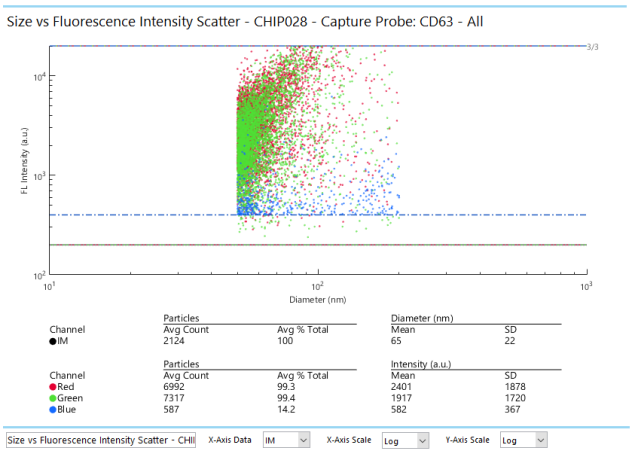
- The **Size Histogram** shows histograms of particle count binned by diameter (nm). The table below the plot shows the mean count and mean % total of particles included across capture probe spots in analysis, as well as the mean and standard deviation of particle diameters across spots.

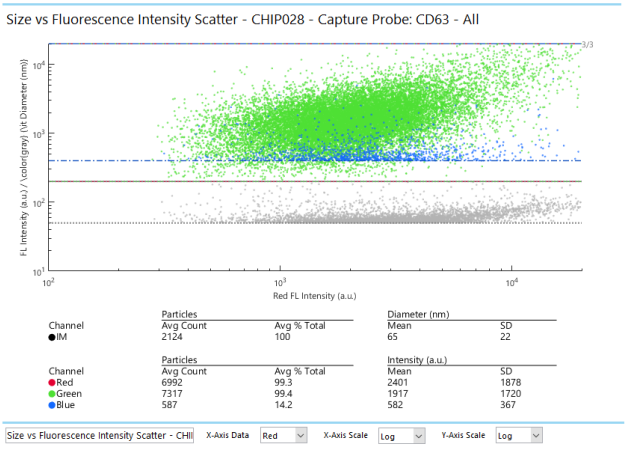


	Particles		Diameter (nm)	
Channel	Avg Count	Avg % Total	Mean	SD
●IM	2124	100.0	65	22

Size vs Fluorescence

- The **Size vs Fluorescence** plot shows a scatter plot of particles plotted by their fluorescence intensities, or by their fluorescence intensities relative to diameter. Data shown on the x-axis and axis scale (log or
- linear) can be adjusted on the lower controller.





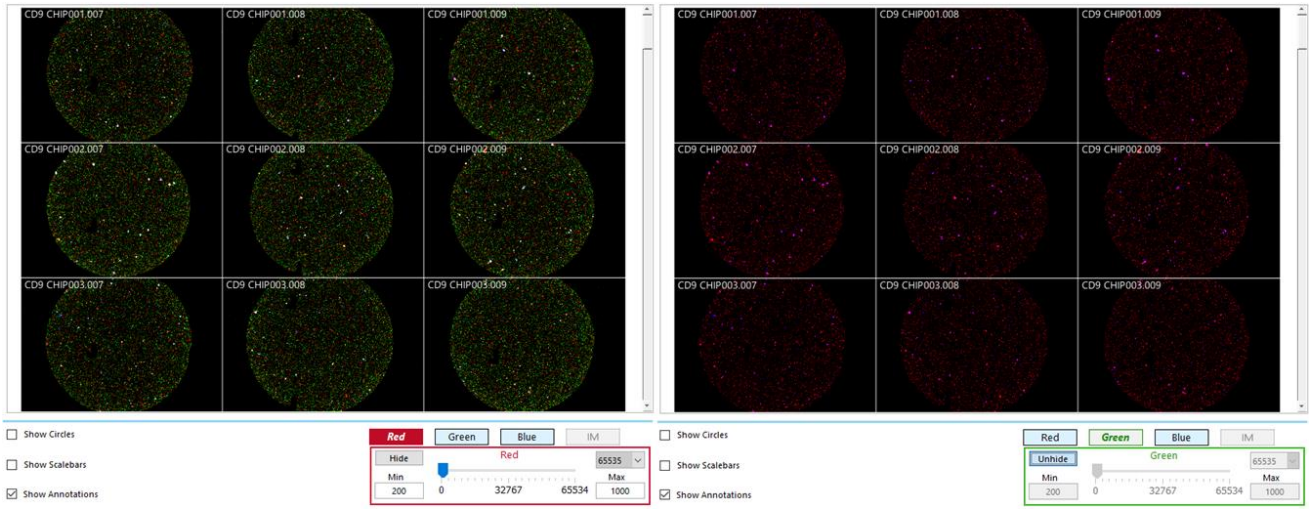
Images

Image plots allow for easy review of individual capture probe spots. Beneath the image view, on the right hand side of the plot controls, is the image display controller. This controller allows for each scanned channel to be made visible or invisible, and for image contrast to be adjusted.

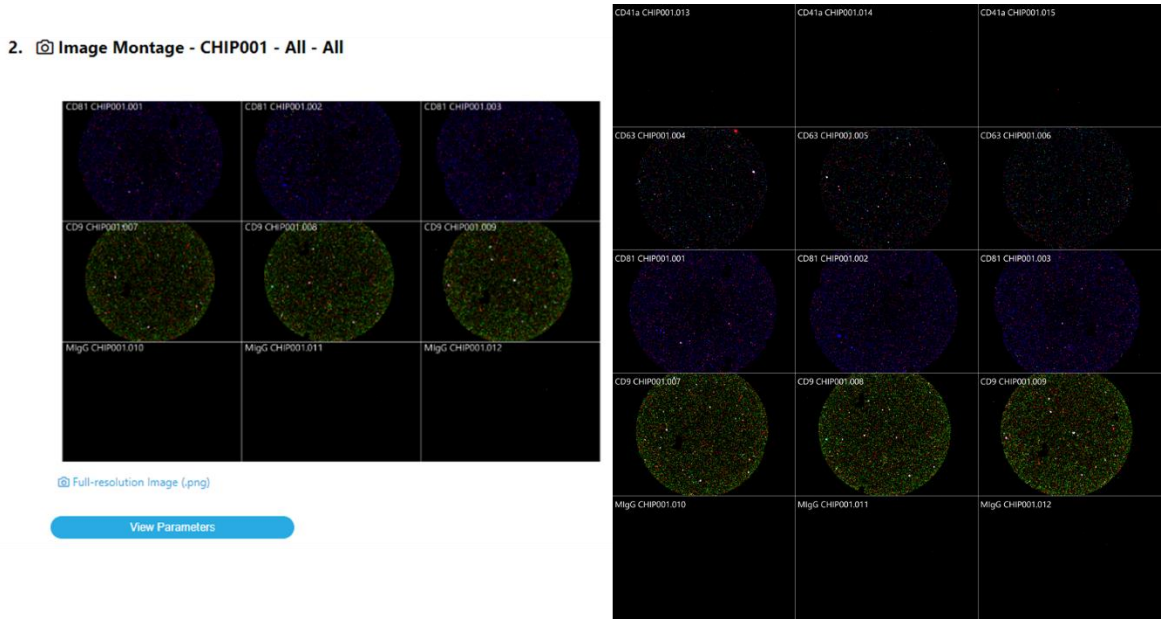
Multi-Spot Montage

- At the "one-Chip, all-Probes", the "all-Chips, one-Probe", or the "one-Chip, one-Probe, all-Spots" levels, an image montage can be viewed. As an alternative to using the Navigation Bar, an image on the Montage can be clicked to navigate to the one-Chip, one-Probe, one-Spot composite image.

- On export, both a preview image (left), which shows exactly the view on screen, and a full-size PNG

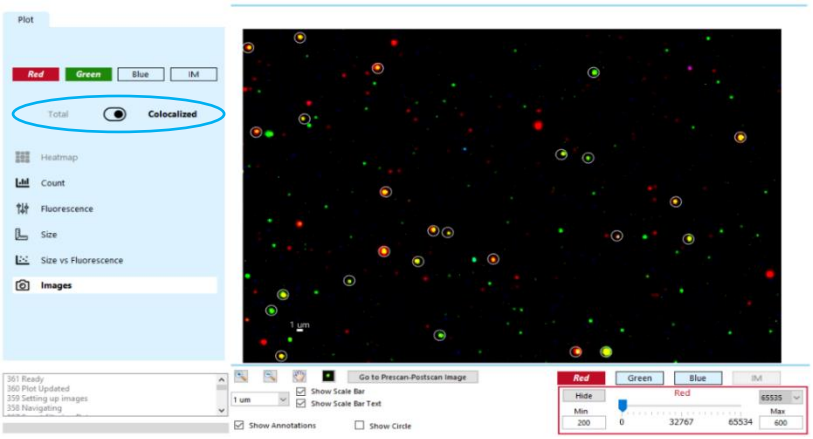


(right), which shows all spots at full resolution, are provided.

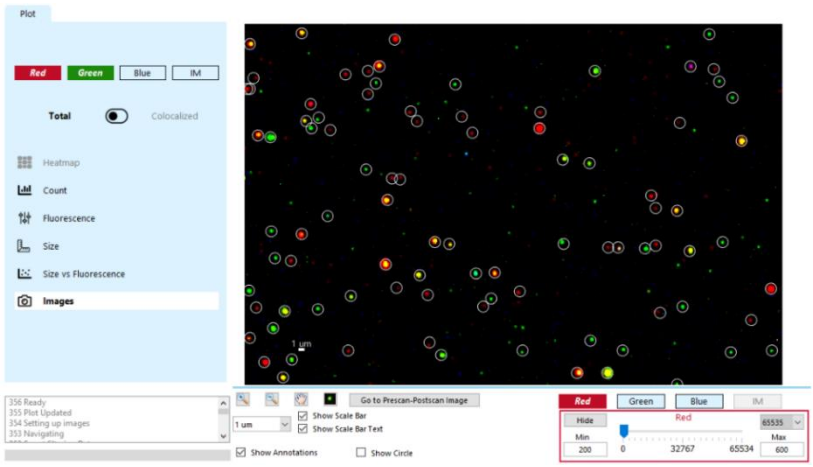


Single-spot Composite

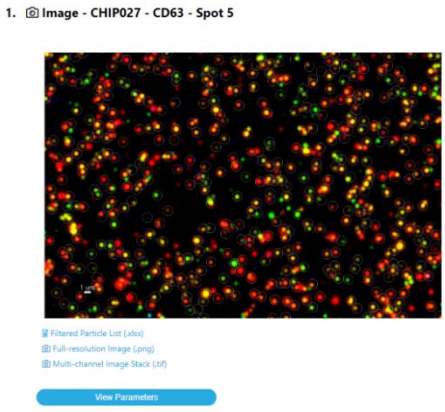
- The single-spot Composite Image allows spot images to be zoomed and panned. An additional option enables the circling of particles. To the right, we see circling of Red-Green colocalized particles (top), compared with circling of all particles labeled in Red and/or Green (bottom). The circling mode is controlled by the Total vs Colocalized toggle switch, which is circled.

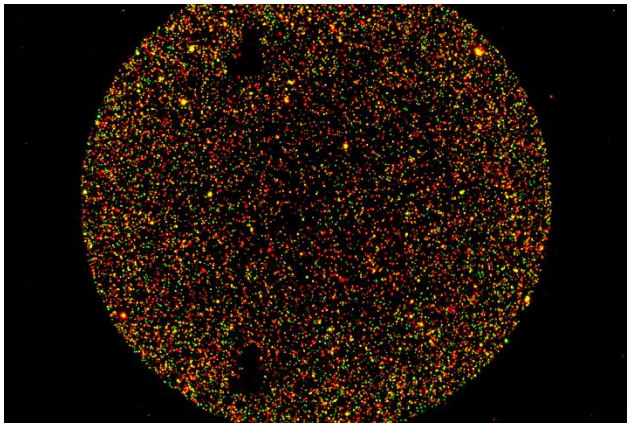


- The slider controller on the bottom-right of the plot does not change the particle circling, only the channels visible in the image.



- On export, a preview image, full-resolution .png, and full-resolution .tif are provided.



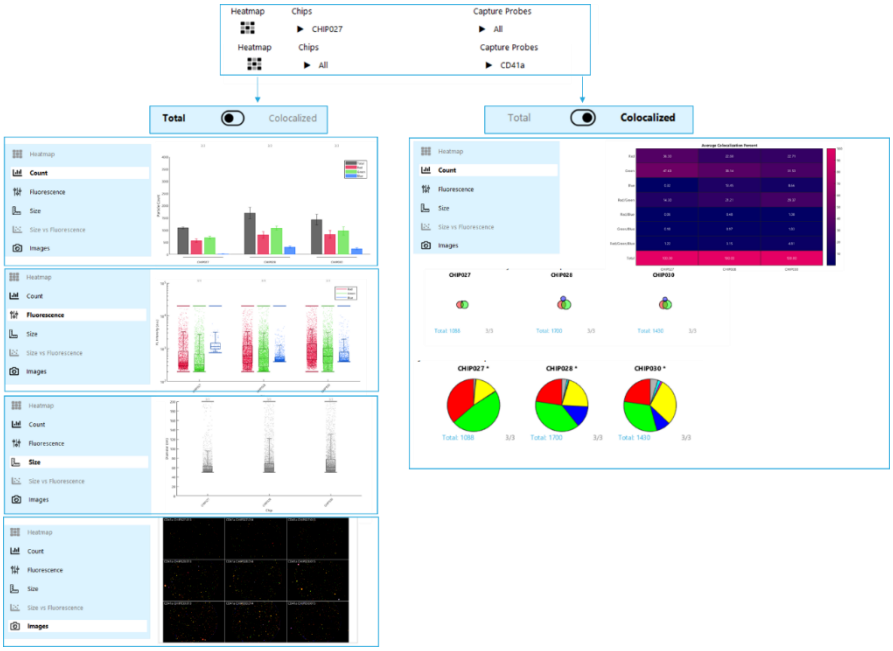


Plot Navigation Quick Guide

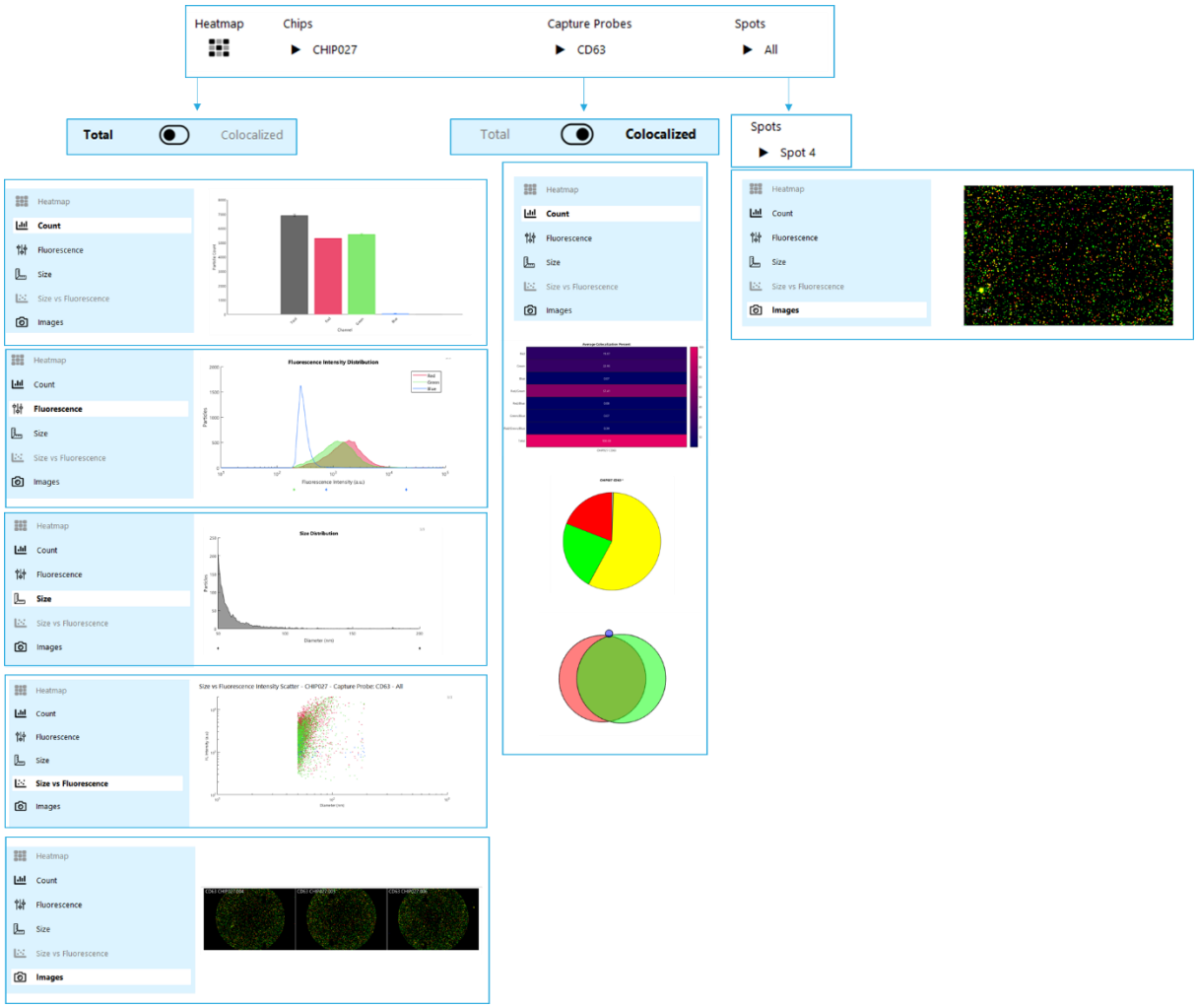
- ExoView Analyzer provides quick views for large amounts of data at different levels of specificity. Below, a summary is provided of how to reach each plot in the software.

- At the "All Chips, All Probes" level a Heatmap of Total or Colocalized counts for all selected channels is available.

- At the "All Chips, One Probe" level or the "All Probes, One Chip" level, a multi-channel bar plot, boxplots, and image plots can be viewed when "Total" is selected. If "Colocalized" is selected, a unique colocalization view is displayed under the "Count" plot type. Other plots remain the same.



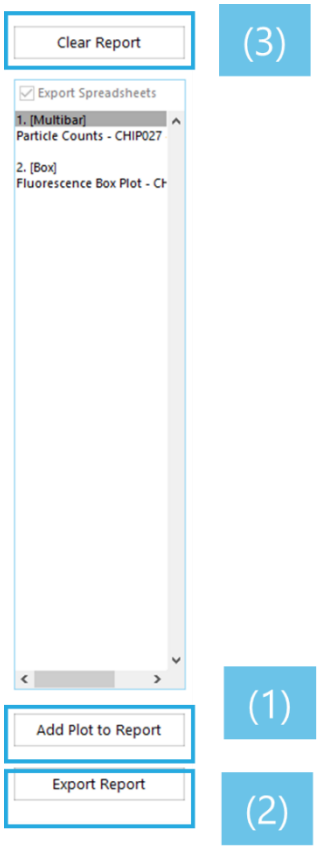
- At the "One Chip, One Probe, All Spots" level, the "Count" plot displays a multi-channel bar plot, the "Fluorescence" and "Size" options display histograms, and the "Size vs Fluorescence" plot option, which displays a scatter plot, is enabled. Selecting "Colocalized" displays a colocalization analysis for the single capture probe spot group selected.
    - Selecting a single spot from the "Spots" dropdown and viewing the “Image” shows a single image view that can be zoomed, panned, or have particles circled. For more details view the "Single-spot Composite" section.



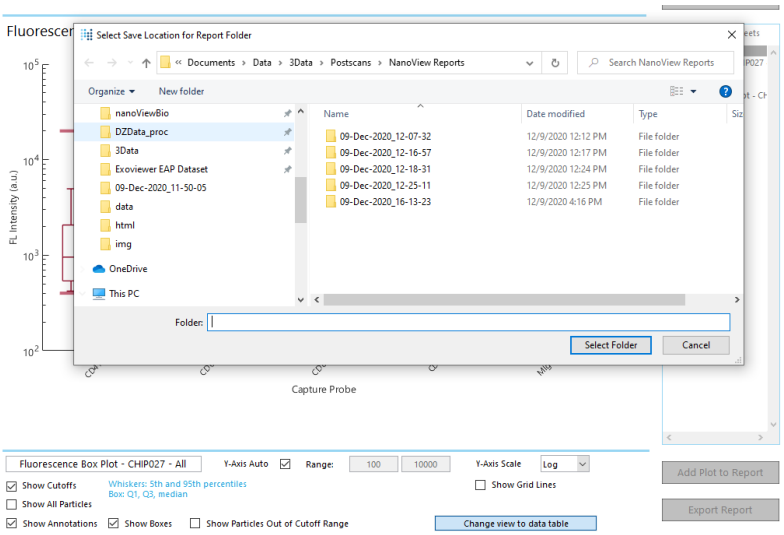
Guide to Data Export

At any point when a plot is visible, the plot data can be exported to a standard browser-based report that includes the plot image, related metadata, and formatted spreadsheets for quick data review and sharing. The report can be printed to PDF for streamlined sharing.

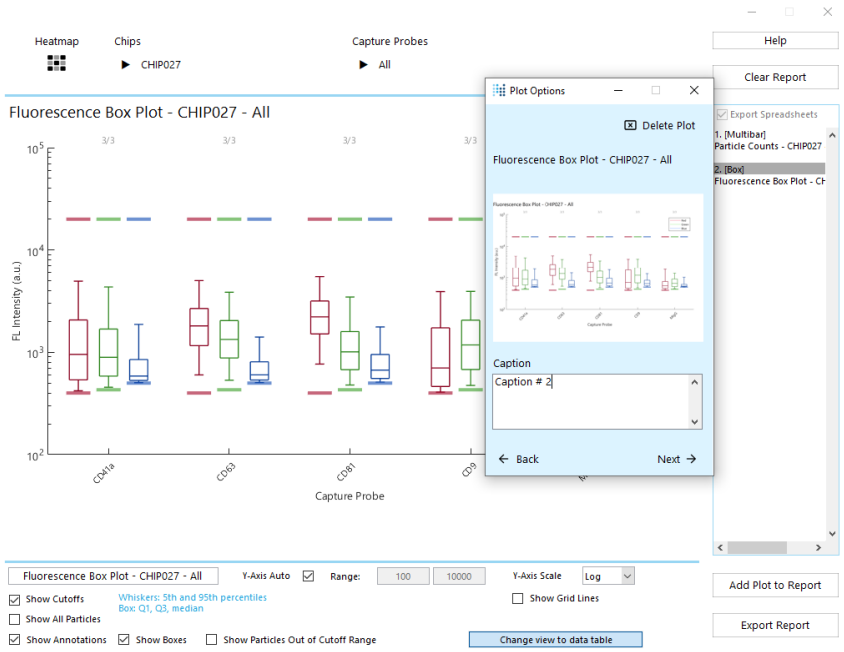
- At any point where a plot is visible, its data can be exported to a standard browser-based report.
- Press the "Add Plot to Report" (1) button to add the currently viewed plot to the report. The plot types and names are populated in the list of plots to export.
- Press "Export to Report" to select the export directory (2). At any time, the "Clear Report" (3) button can be pressed to remove all plots from the current list.
- The "Export Spreadsheets" checkbox determines whether.xlsx data exports are included in the report. Unchecking it decreases the time needed to save a plot, but means that raw data will not be available on export. This option can only be changed before any plots have been added.

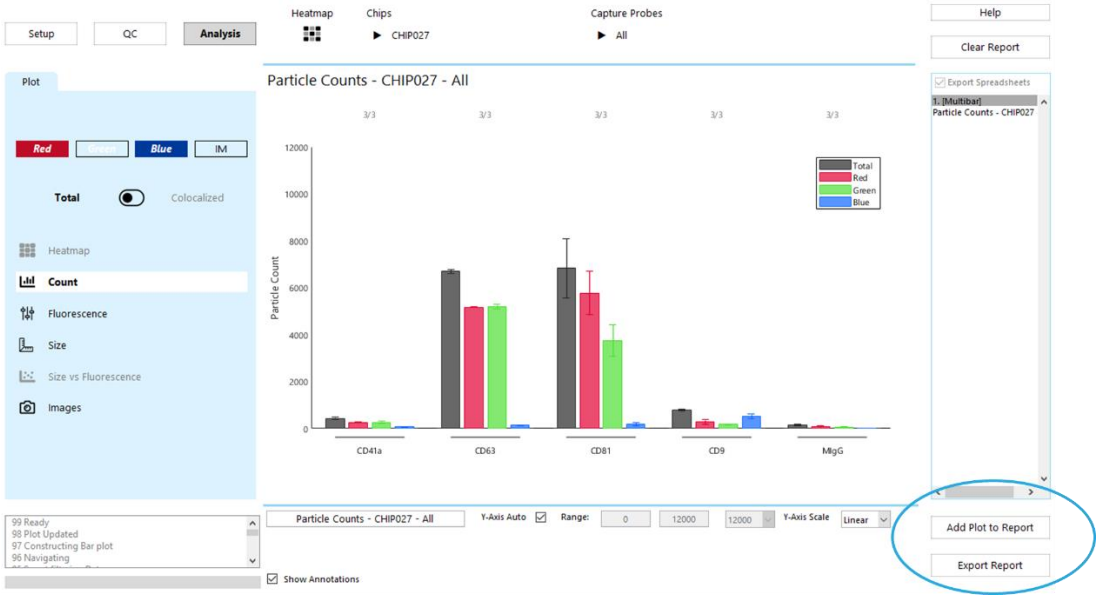


- On export, a Windows prompt appears that can be used to select the save directory. The default location is the Postscan Data folder, under the directory "NanoView Reports". The export will be packaged into a folder labeled with the date and time of export inside of the selected folder.

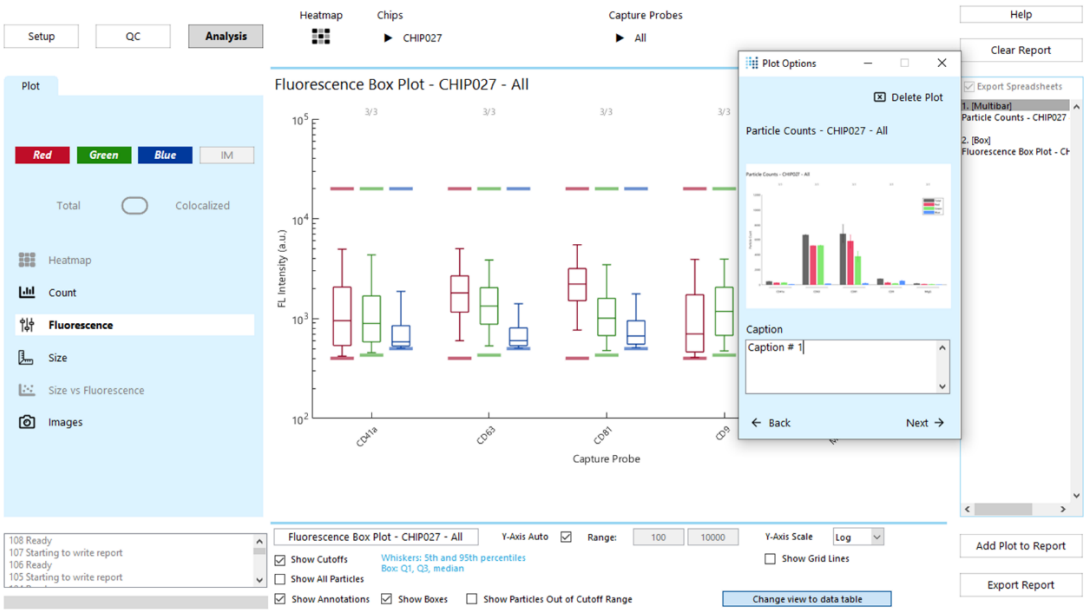


- Clicking an item on the report panel pops up the Plot Options window. The window shows a preview of the plot and provides options to add or modify plot captions or remove plots from the report.

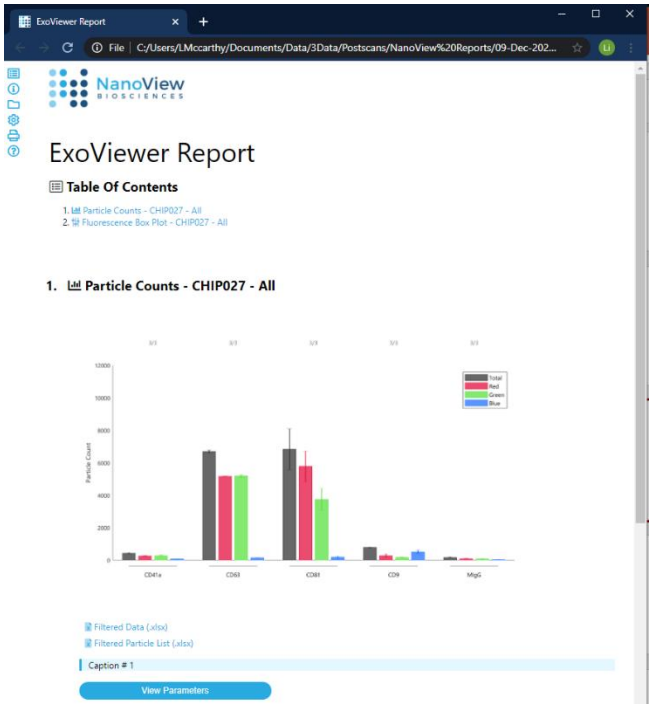




- Once a plot has been added to the Report, it can be removed or have a caption added at any time by clicking its name in the list and interacting with the Plot Options window.



- Once exported, the Report will automatically open in your web browser. We recommend using an updated version of Google Chrome to view the report.

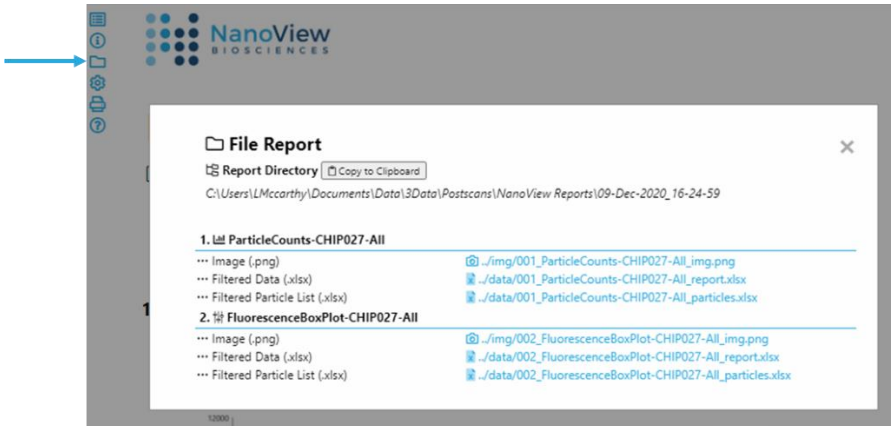


- Below, we see that plot titles have been automatically populated from the in-app report panel (left) to the report table of contents (right). Exported plots are listed in the clickable Table of Contents.

- A list of icons to the left of the window allows quick access to report functionality. These icons remain in the same convenient location even as the report is scrolled.
    - **Table of contents –** click to pop open the same table of contents as the top of the page.

    - **Report Summary –** click to open information such as original paths to data sources and creation dates.

- **File Report –** click to view direct links to all data contained within the report.

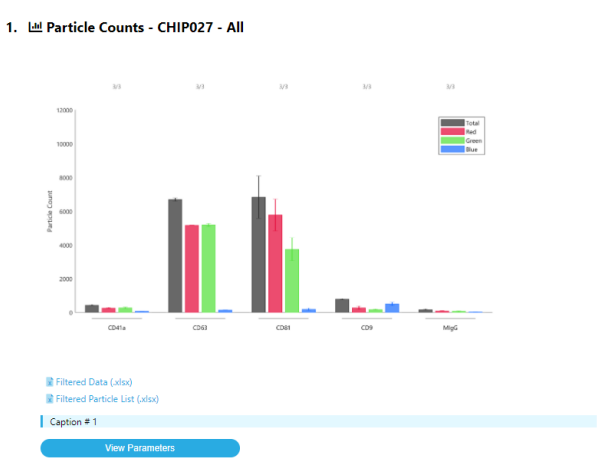


- **Settings –** click to access additional display settings.

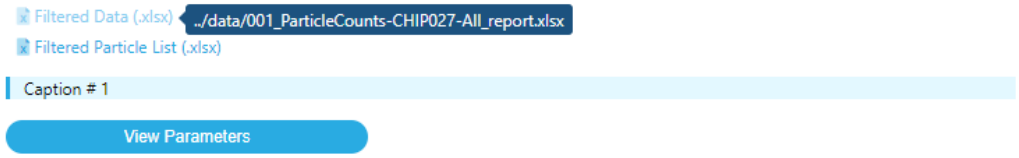
- **Print –** click to show an optimally-formatted report to print or save as .pdf.

- **Support –** click to show additional links for support.

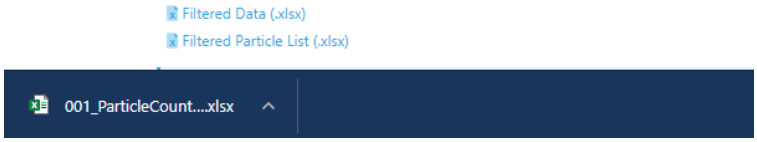
- A set of additional tools to view exported data is available beneath every plot. Selecting "View Parameters" pops open a list of cutoffs and controls.



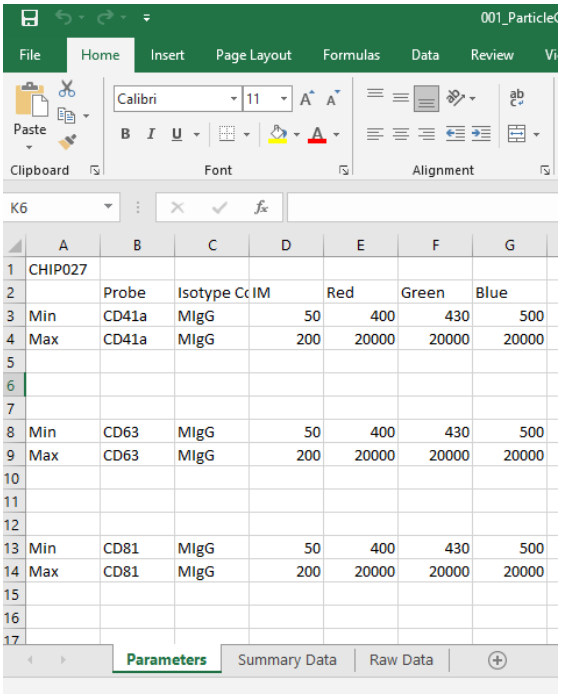
- Most plots provide a custom "Filtered Data" export, which allows download of the Excel Workbook (.xlsx) data corresponding to the information on the plot, and which can be used to recreate the plot in a different software. Plots typically also include a "Filtered Particle List", which includes the particle lists used to build the plot view.



- Selecting one of these options allows your browser to download the corresponding .xlsx file.



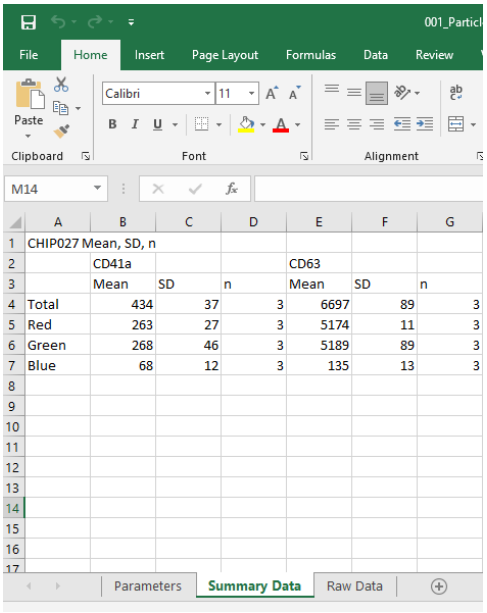
- Both the "Filtered Data" and "Filtered Particle List" files will always include a Parameters tab which summarizes the chips, capture probes, channels, and cutoffs, similarly to the "View Parameters" section.



	A	B	C	D	E	F	G
1	CHIP027						
2		Probe	Isotype Cc	IM	Red	Green	Blue
3	Min	CD41a	MIgG	50	400	430	500
4	Max	CD41a	MIgG	200	20000	20000	20000
5							
6							
7							
8	Min	CD63	MIgG	50	400	430	500
9	Max	CD63	MIgG	200	20000	20000	20000
10							
11							
12							
13	Min	CD81	MIgG	50	400	430	500
14	Max	CD81	MIgG	200	20000	20000	20000
15							
16							
17							

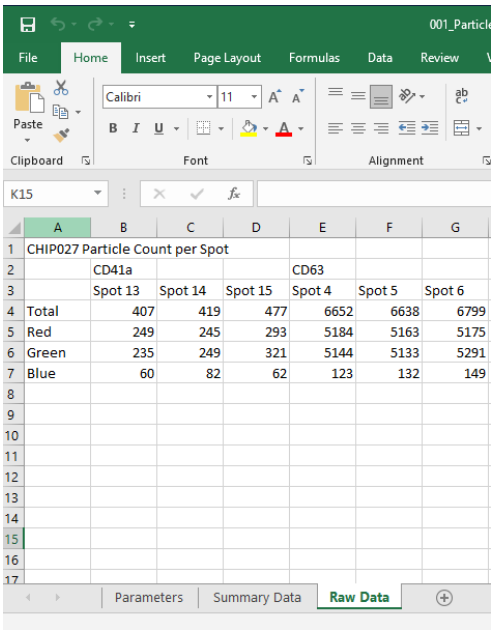
Parameters | Summary Data | Raw Data

- Depending on the export, separate tabs are available to represent the data shown in the plot. Below, "Summary Data" (left) shows the aggregated spot group values used to create the plot view, whereas "Raw Data" (right) shows the individual spot values used to calculate the aggregated values.



	A	B	C	D	E	F	G
1	CHIP027 Mean, SD, n						
2		CD41a			CD63		
3		Mean	SD	n	Mean	SD	n
4	Total	434	37	3	6697	89	3
5	Red	263	27	3	5174	11	3
6	Green	268	46	3	5189	89	3
7	Blue	68	12	3	135	13	3
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							

Parameters | Summary Data | Raw Data



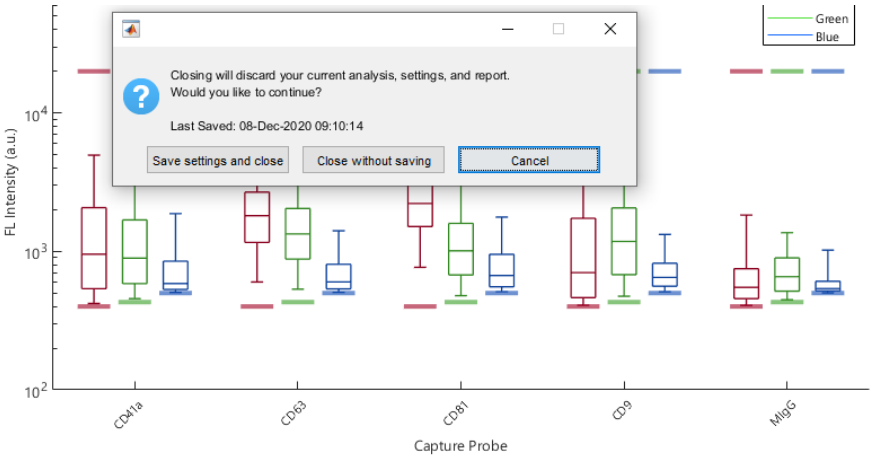
	A	B	C	D	E	F	G
1	CHIP027 Particle Count per Spot						
2		CD41a			CD63		
3		Spot 13	Spot 14	Spot 15	Spot 4	Spot 5	Spot 6
4	Total	407	419	477	6652	6638	6799
5	Red	249	245	293	5184	5163	5175
6	Green	235	249	321	5144	5133	5291
7	Blue	60	82	62	123	132	149
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							

Parameters | Summary Data | Raw Data

Finishing Up

After reaching the **Analysis** portion of the program, the **Setup** and **QC** selections (load, meta, isotype, disable, cutoff) can be safely saved at any time to reload at a later point. **Any reports generated will not be saved and should be exported before exiting.**

- Exiting the app pops up a window with an option to save the experiment settings. Press "Yes" to save, "No" to discard the most recent settings and close, or "Cancel" to return to the program.



- Saved settings are associated with the Postscan folder, and can be reloaded the next time you open the folder with ExoView Analyzer. To restore saved data, follow the instructions under the "Setup" section.

Help

To reach out for any additional help, please visit the ExoView Analyzer user portal at https://nanoviewbio.atlassian.net/servicedesk/customer/portal/1/article/1137541121.

If you are submitting a support request, please include your ExoView Analyzer version, whether you are running ExoView Analyzer on your ExoView control computer or personal desktop, and when your ExoView dataset was acquired. This information will enable us to efficiently assist you.