Data Visualization

Zooming
Adjust the plot view to display the data points well centered. AmphaSoft offers several options:

- Autozoom: Use the Zoom button in the axis section on the lower left part of the measurement tab
- Zoom using a fixed aspect ratio by scrolling the mouse wheel. Move view by dragging
- Define axis limits in the axis section on the lower left part of the measurement tab

Density Coloring
Density coloring is used to show how close data points are.

- Move the mouse cursor into a plot and change the coloring by pressing ALT + scrolling the mouse wheel

Point Size
The point size can be adjusted. For measurements with many data points, a smaller point size is recommended.

- Move the mouse cursor into a plot and change the point size by pressing CTRL + scrolling the mouse wheel

Gating
Gating is a method to quantify points in scatter plots.

- 4 different gate types can be selected, either in the AmphaSoft menu under Tools > Gating, or using the 4 icons in the menu bar.
- Gates can be placed by mouse click: Select the desired gate type and position it in the plot with a left click. For polygon gates you can define the corner positions with left clicks. Close the polygon with a double click. For repositioning, left click on a gate and drag it. Select a gate by left clicking on it (lines become red) and press delete to remove it.
- Copy gates to the whole workspace by right clicking into the plot and selecting Gating > Apply to > All. If you want to apply it only to a selection of measurements, select the measurements first using CTRL + clicks on the respective measurements in the Navigation panel, then right click into the plot > Gating > Apply to > Selected.

Gating types and gate statistics

- **Polygon**
  - 388 data points are inside polygon P1
  - This corresponds to 3.65 % of all data points

- **Vertical**
  - 5647 data points are on the right side (R) of the vertical gate.
  - This corresponds to 53.08 % of all data points

- **Cross**
  - 355 data points are in the lower left (LL) quadrant of the cross gate.
  - This corresponds to 3.34 % of all data points
Hiding debris

With Impedance Flow Cytometry, particles are analyzed based on their resistive properties in an alternating current electric field. Every particle above a certain size and resistivity is detected. Impurities in a sample, such as debris microparticles, can lead to additional point clouds in the scatter plots. Such clouds can be hidden to obtain correct gating statistics.

- Draw a polygon gate around the debris cloud. Select the polygon by left clicking on it.
- Right click into the polygon and select Gating > Hide > Cells. Hidden cells are grey. They are not counted.

Where to find debris

- Usually debris particles are smaller than the cells. Therefore they can be found at lower impedance amplitudes than the cell clouds, i.e. at the bottom of the plot.
- Debris often consists of inorganic material or cell fragments. Therefore it is usually below the dead cell cloud.

Attention

- Samples containing small deformed or shrunk cells also have low amplitude populations below the dead cell cloud. Depending on the type of analysis, these cells should be included and not hidden.
- You can use a microscope to determine whether you have a population of significantly smaller cells and adjust your gating strategy accordingly.

Reporting

AmphaSoft can report your measurement results in various forms.

- Click on Measurements in the Navigation panel to open the Worklist.
- Click on Report. Select the report type (*.csv or *.html) at the bottom of the navigation view (Save as type). If required, change the directory. Click Save.
- You can change the layout of the report using the Layout dropdown in the Worklist. The three available layouts are Comparison, Measurement and Row.
  - The Comparison layout creates a report section containing all the instrument settings and another section with all the results.
  - The Measurement layout reports settings and results of each measurement together in one section.
  - The Row report (*.csv only) combines all the settings and results of one measurement in one row. This report type is useful for customized downstream processing of larger datasets and merging of such reports.
- You can consolidate *.csv row reports using the Multi-Report button. Select the workspaces to be consolidated in the navigation view and click Choose.

Tip: If you don’t want to report all the measurements, click on Settings (+/-) in the bottom part of the Worklist. Select Report from the list of settings to display the report column in the Worklist. Enter a 0 (zero) in the report column to exclude a measurement.