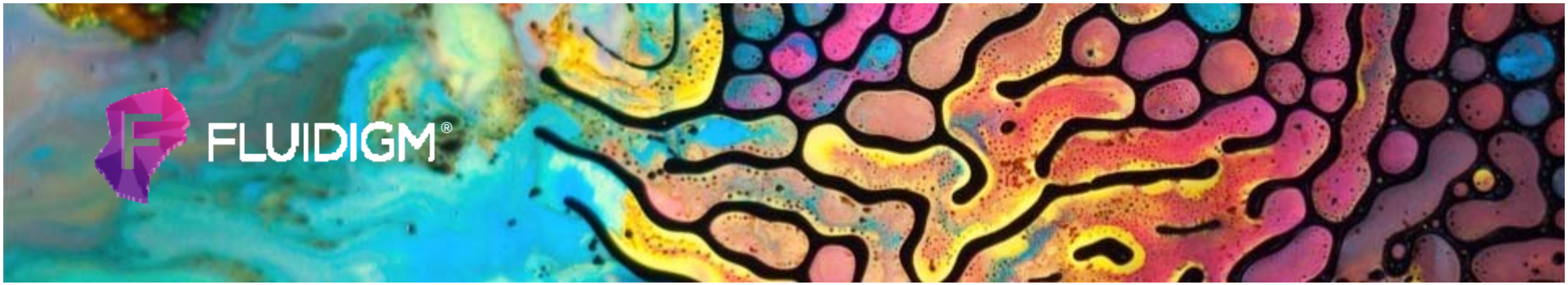
Recommendations for Use of Gaussian Discrimination Parameters



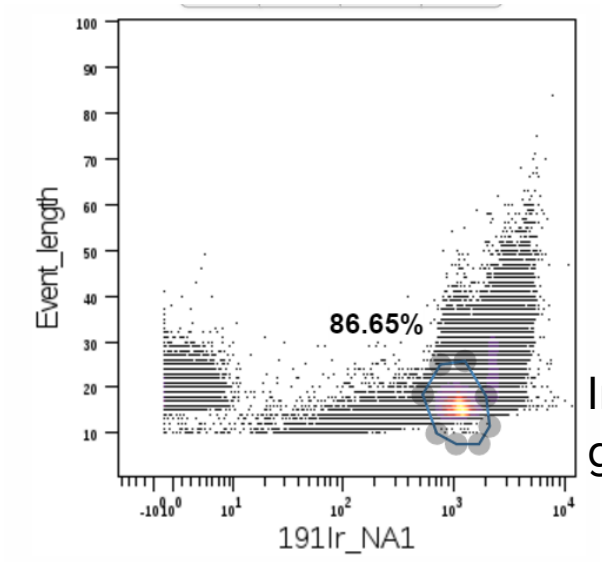
General Doublet Discrimination Recommendations

- Make changes in Event Length scaling for Helios data to make gating easier
  - Try log or arcsinh scale
  - Change scale from default of 256 if using a linear scale

- Another doublet discrimination strategy which does use the Gaussian Discrimination parameters utilizes CD45 and can be used for leukocyte data

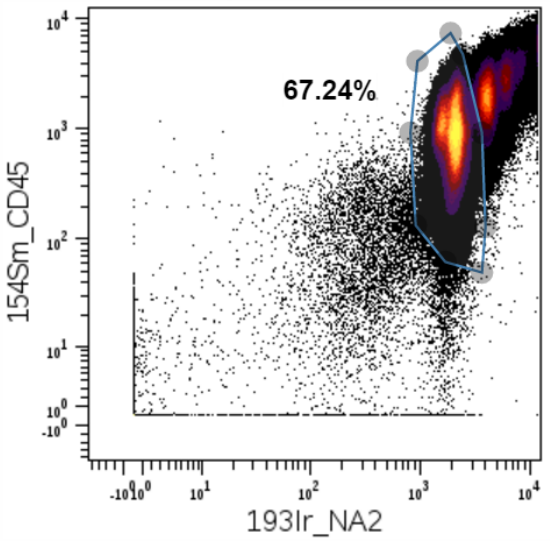
CD45 based doublet discrimination

191Ir vs Event Length

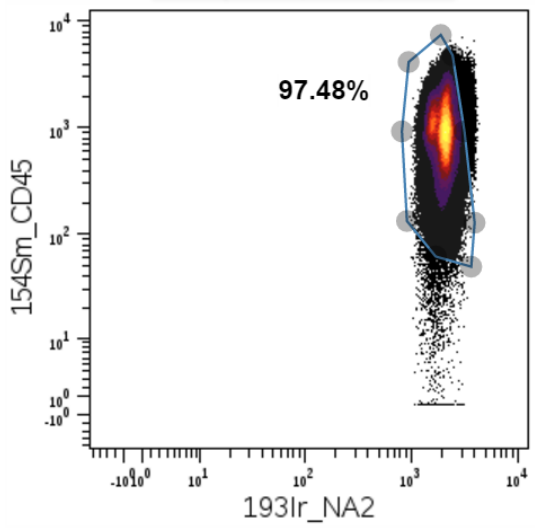


Initial singlets gate

193Ir vs CD45



Ungated for better visualization to draw gate



Gate on singlets to visualize excluded doublets.

A customer recently shared this strategy that he has used with success.

Gaussian Discriminations Channels

- Four channels derived from a Gaussian fit to each event transient are included with the events collected in branches 6.3 and higher of the CyTOF Software.

- These channels can be used discriminate between events and non-events (e.g., debris, doublets).

- The names for the Gaussian discrimination (GD) channels are:
    - Center
    - Offset
    - Width
    - Residual

Gaussian Fit

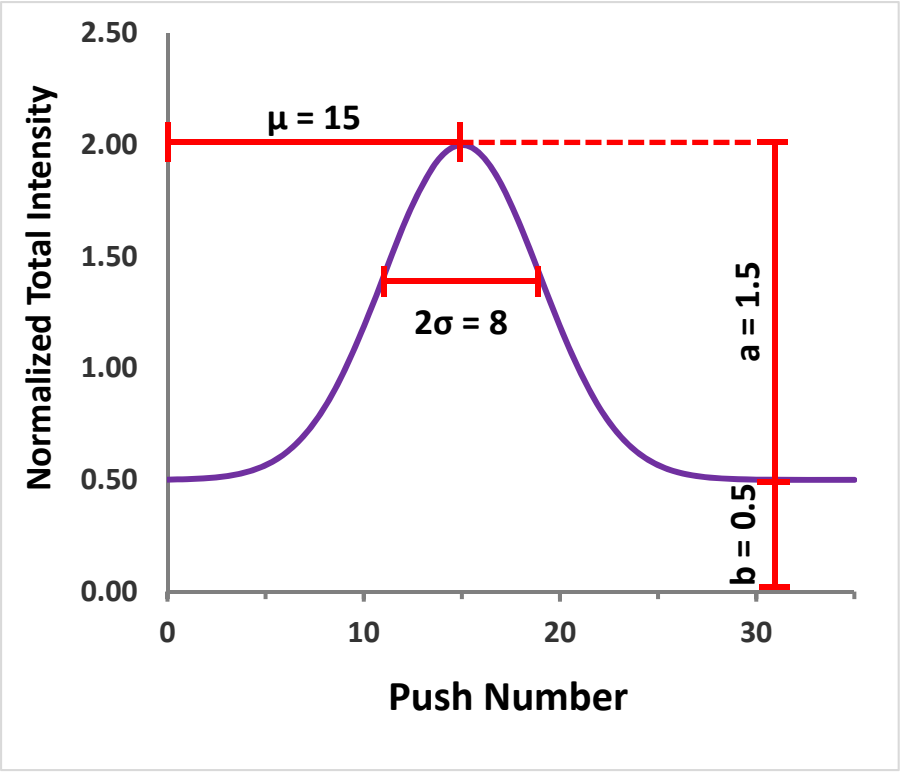
The normalized event transients are analyzed using the equation:

$$f(x) = ae^{-(x-\mu)^2/2\sigma^2} + b$$

for a Gaussian or "*bell shape*" curve, where:

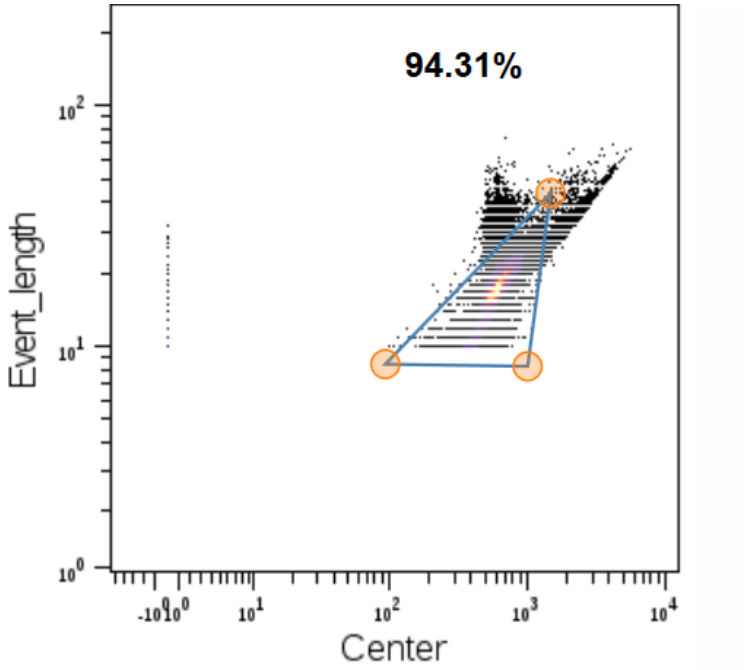
- **x** is the push number
- **a** is the amplitude
- **b** is the ***offset***
- **μ** is the mean
- **σ** is the standard deviation

- The difference between the fitting curve and the normalized transient is the ***residual***.



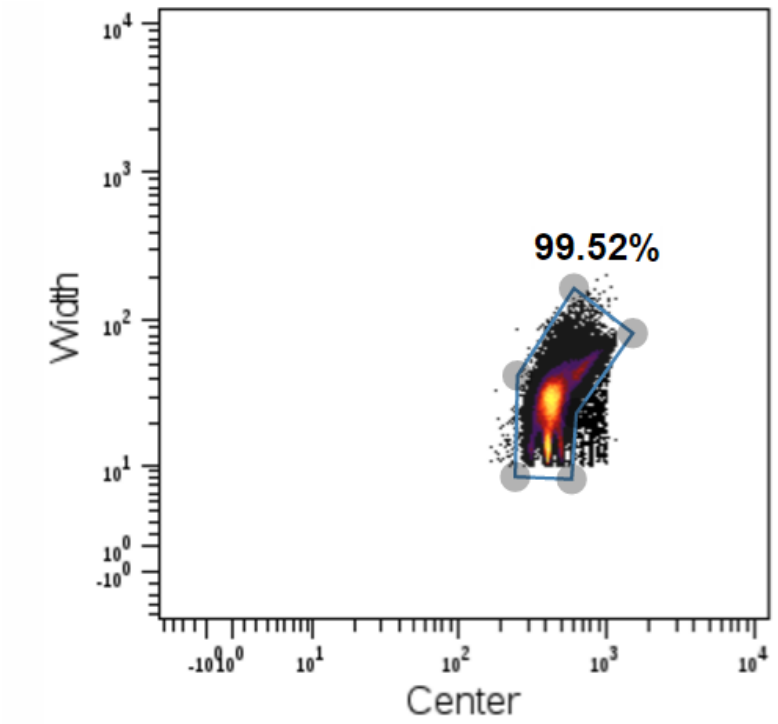
Gating with Gaussian Channels (Helios)

1) Center to Event Length Plot



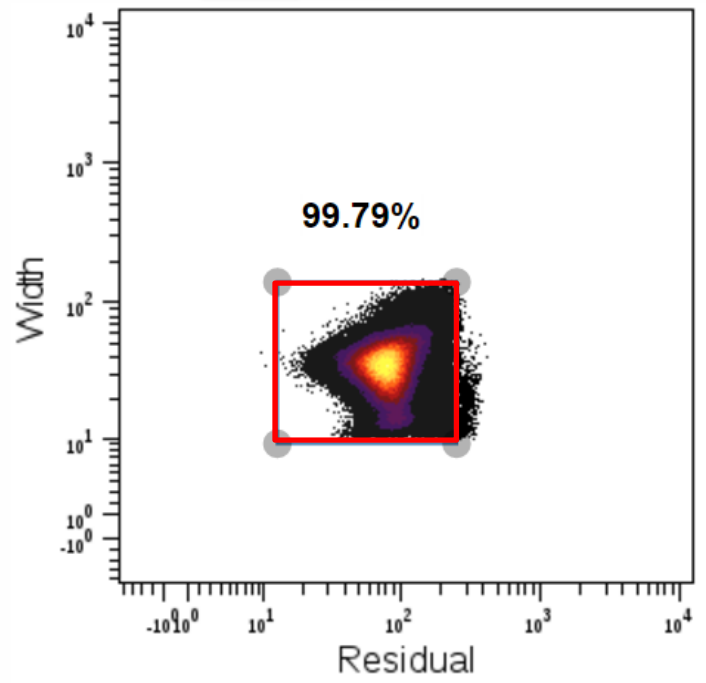
- Select Event Length and Center channels
- Change scaling for Event Length to Arcsinh or Log
- Select *Contour - Color By Density* from the Plot Type menu in Cytobank.
- If necessary, reduce “Percent per Contour” and “Outliers Start At” to 1.
- Draw a triangular gate over the main population.

2) Center to Width Plot



- Select Center and Width channels
- Select *Dot - Color By Density* from the Plot Type dropdown menu in Cytobank.
- Draw an L-shape gate over the main population.

3) Width to Residual Plot



- Select Width and Residual channels.
- Draw an square gate over the main population.
- This gate will remove events with high residuals
- This gate needs to be tailored to each file

4) Verify Gates