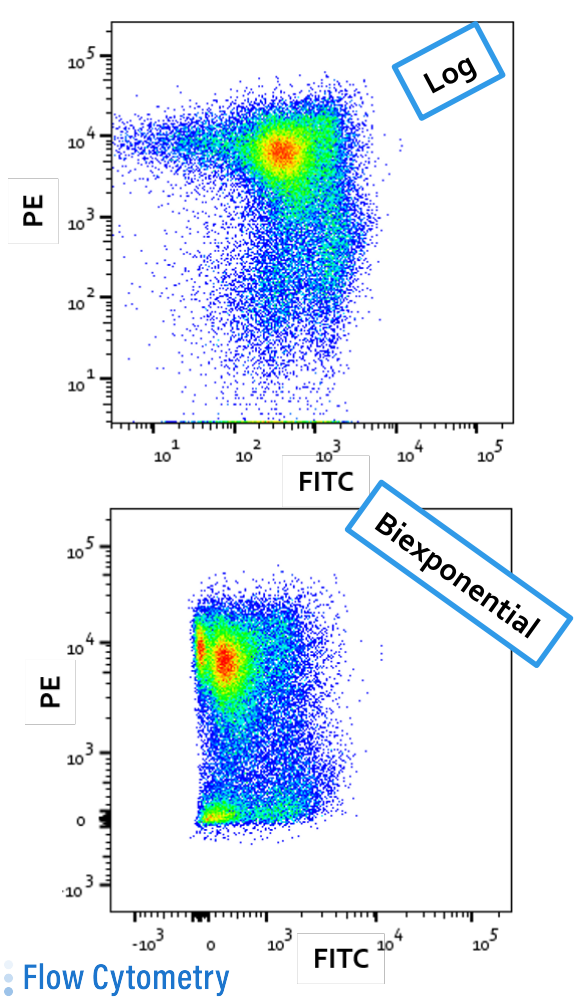
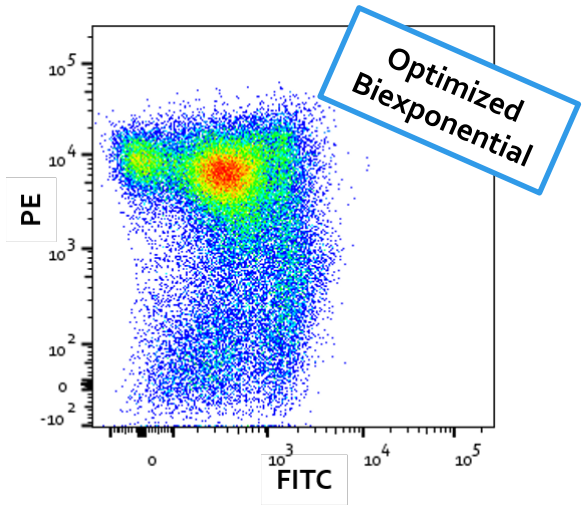
Flow Post-its
Kathy Daniels

# Scaling Matters

September 2020

In flow cytometry, the most common data scaling methods are **linear** and **logarithmic.** When presenting data where there is a wide range of fluorescent signal, data is typically displayed in log so all events can be visualized. Often, when analyzing data in log scale, there can be a large portion of events stacked against the axis at zero. Through **biexponential log transformation,** we can visualize the distribution of these negative events around zero in a better way.




## Comparison of scaling across same sample

**Log:** Negative events are against the axis and populations are not well resolved.

**Biexponential:** Default biex transformation no longer shows events on the axis, but the data is compressed together .

**Optimized Biexponential:** Clear distribution of all populations, allowing for best visualization and optimal conditions for gating.

## Helpful Hints

- Scaling transformation does not alter the data, it simply changes the way the data is visualized.
- Distribution of events around or below zero can be a result of baseline restoration or compensation.
- Biexponential scaling needs to be optimized for better visualization.
- Incorrect scaling of data can hide poorly compensated or unmixed data.
- Scaling adjustments made in acquisition software do not carry over into analysis software.
- Other methods of log transformation can include Hyperlog and ArcSinh.


Useful read: Herzenberg LA, Tung J, Moore WA, Herzenberg LA, Parks DR. Interpreting flow cytometry data: a guide for the perplexed. Nat Immunol. 2006 Jul;7(7):681-5. (link)