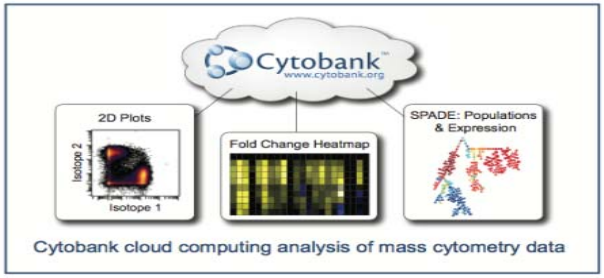
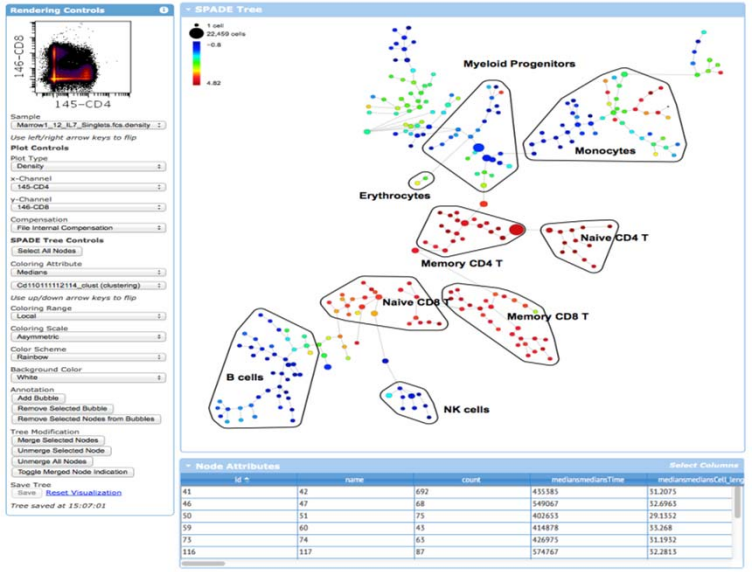
Cytometry Data Analysis

DVS Cytobank

- http://dvs.cytobank.org/
- Analysis toolbox designed for Mass Cytometry
  - histograms and bivariate plots
  - SPADE
  - heat maps and dose response curves
- Cloud-based
  - analysis anywhere you have internet access
  - share data securely with collaborators
  - data backed up
- Demo datasets and tutorials
- Cytobank Support
  - Strong scientific, cytometric and bioinformatic background
  - help with experimental design

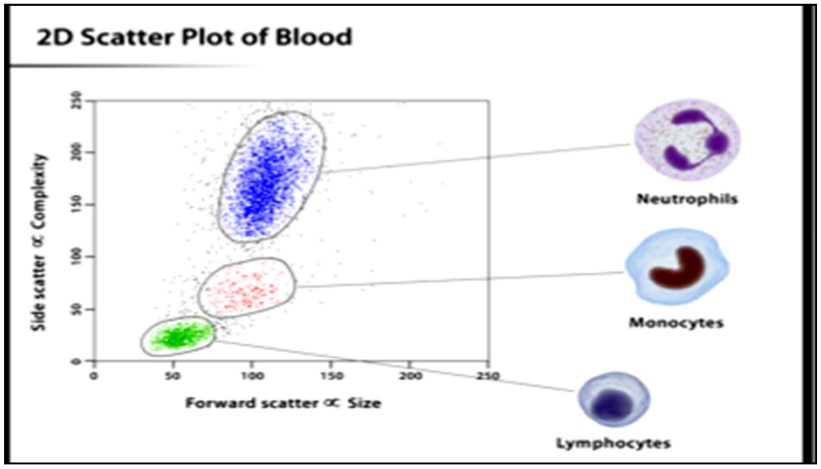


Cytobank cloud computing analysis of mass cytometry data

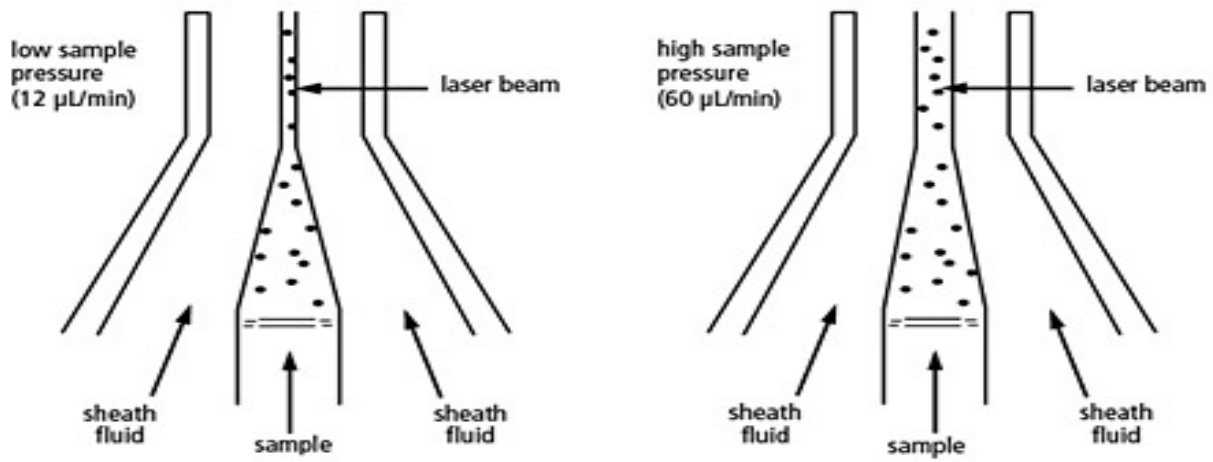


Gating

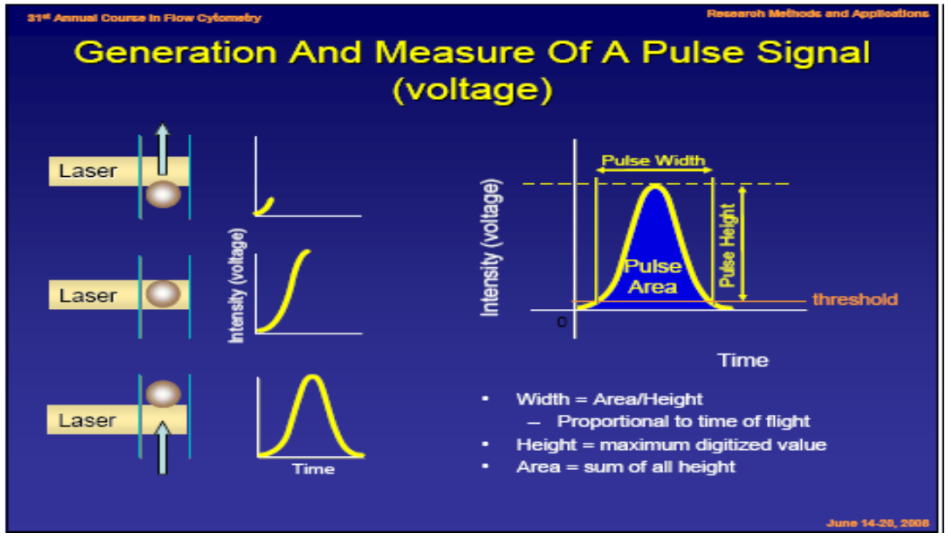
FSC vs. SSC—Cells vs. Debris

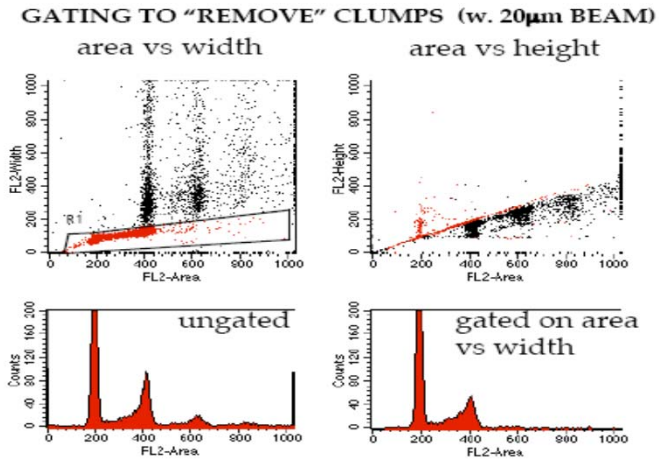


Why go slow? Effect of flow rate



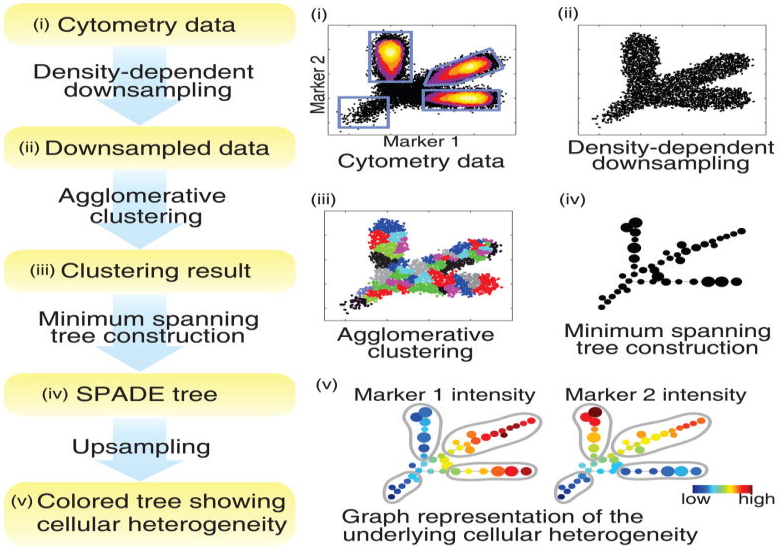
Importance of doublet exclusion





Advanced Methods

- e.g. SPADE (spanning-tree progression analysis of density-normalized events)

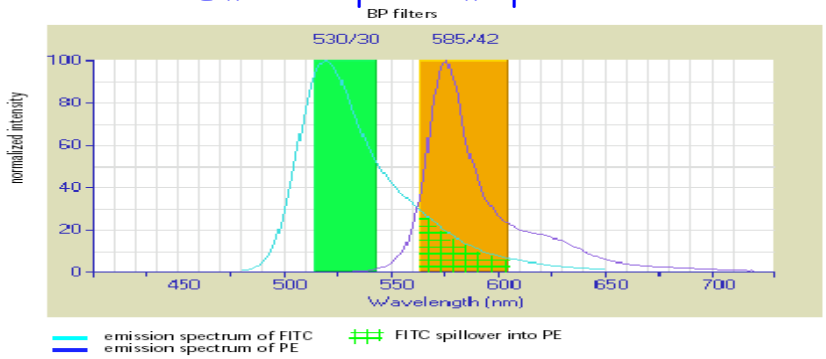


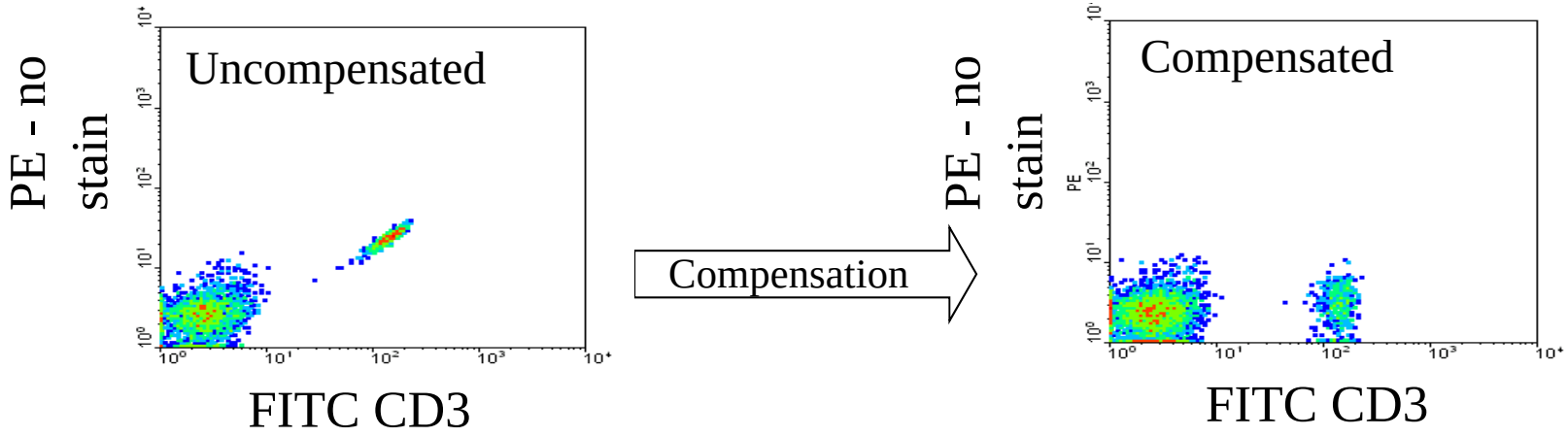
Nat Biotechnol. 2011 October 2; 29(10): 886–891.

Compensation

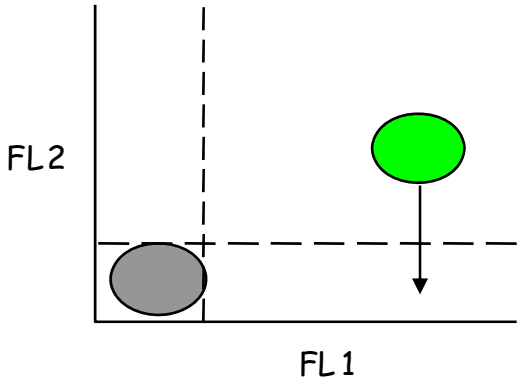
Compensation –Why?

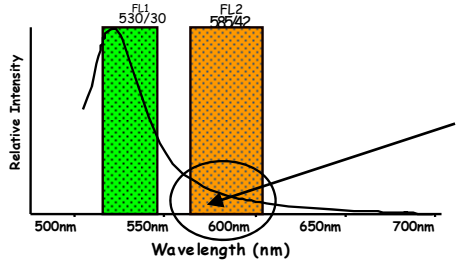
Emission Spectrum Spill over

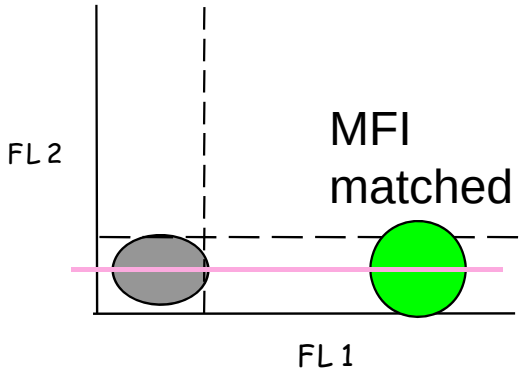




Example: FITC into PE compensation







- What % of the signal in FL2 derives from FL1?
- Subtract FL2 signal from FL1 channel until the MFI (median fluorescence intensity) of the positive matches that of the negative

Quantification

- Gating, gating, gating
- Proper compensation if applicable
- For DNA
  - On a linear scale
- For antibodies
  - Titration—make sure that the cellular target is limiting, not the antibody
    - Stringent requirements for antibody quality and specificity
- Frequency of cells in different gates
  - Requires acquisition of sufficient cell number and proper treatment of all the above considerations
- Intensity of staining in different gates
  - Outliers can severely distort the mean, therefore often the median is used or outliers are removed from analyses