Doublet Discrimination

UWCCC Flow Cytometry Laboratory
https://cancer.wisc.edu/research/resources/flow/

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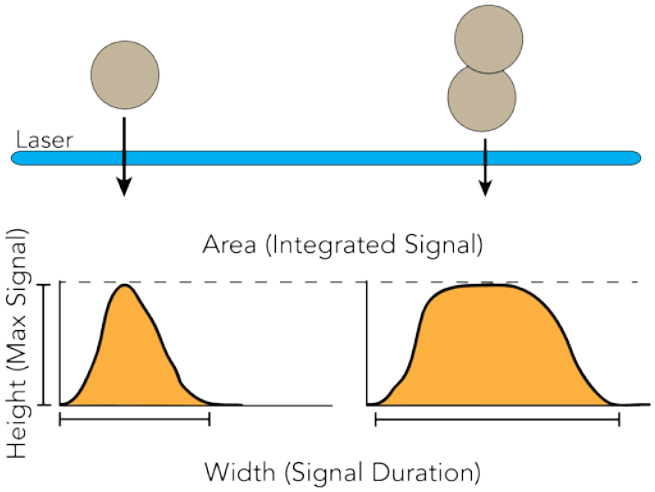
Doublet Discrimination

Good sample preparation practices are critical for flow cytometry. Filtering removes large aggregates that may otherwise clog the cytometer, but doublets and small multiplets will pass through and remain in the sample. Each clump passes through the lasers and is interpreted as one event with the combined properties of all cells in the group. These composite events must be excluded during analysis.

Voltage Pulses and Signal Processing

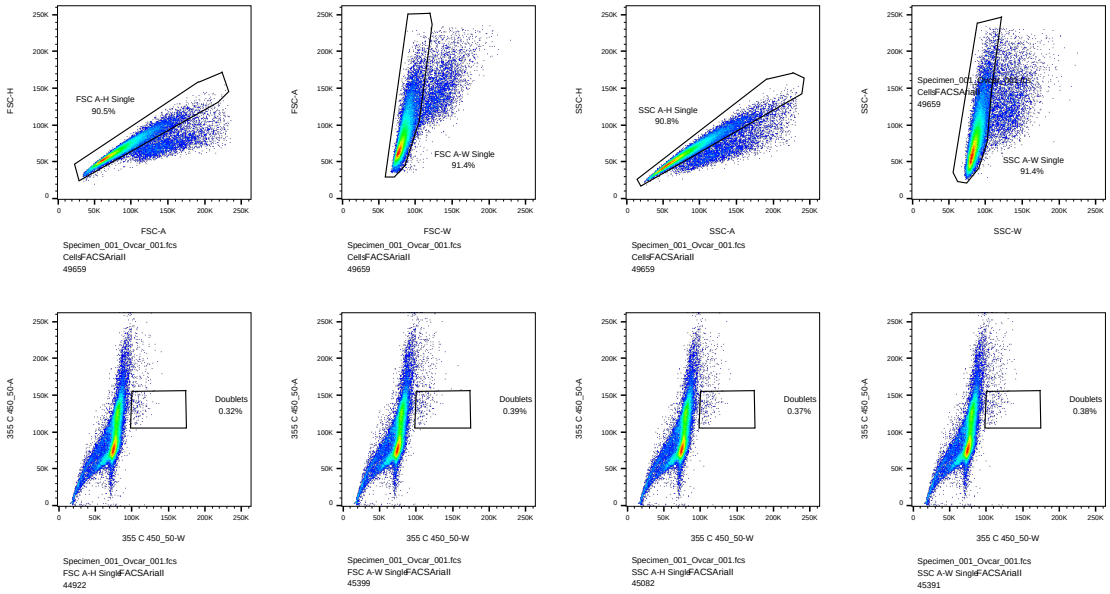
As a particle passes through the laser, photons are converted to current by the photomultiplier tube (PMT) and a voltage pulse is generated. The voltage pulse has three characteristics: area, height, and width. The area is overall amount of fluorescence associated with an event, the height is the peak signal, and the width is signal duration (somewhat analogous to time of flight.) The two different ways to tell single events from multiple particles are plotting width vs area, or plotting area vs height.



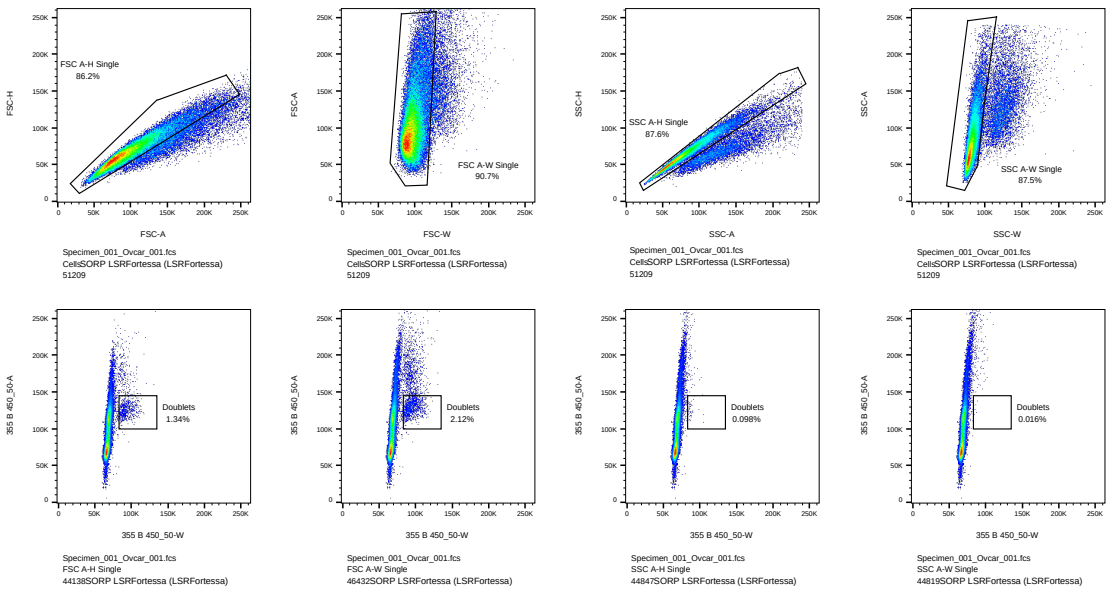
Which Do I Choose?

Technically, the choice of area-width or area-height is determined by the geometry of the laser spot at the flow cell. A narrower beam geometry lends itself to area-width discrimination because it's extremely unlikely that the entire doublet could be illuminated at the same time. With a wider beam spot, the likelihood of this occurrence increases and makes the area-height plot more reliable. **In practice, it is best to collect area, height, and width for both forward and side scatter so that you can see which one looks best for your particular sample and instrument**. To give you a feel for how this may look on the UWCCC flow cytometers, I ran a sample of OVCAR cells fixed and stained with DAPI. I gated each sample for cells, then followed up with either an area-height or an area-width gate off of either forward or side scatter. I took that "singlet" population and plotted cell cycle area-width to look for doublets that made it through my singlet gate. For the sorter, the choice of parameter and plot made very little difference our ability to distinguish singlet from aggregate events. The LSRII and Fortessa did best when using area-height off of the side scatter. The MACSQuant was fairly consistent, with the exception of forward scatter area-width performing less well than the others.

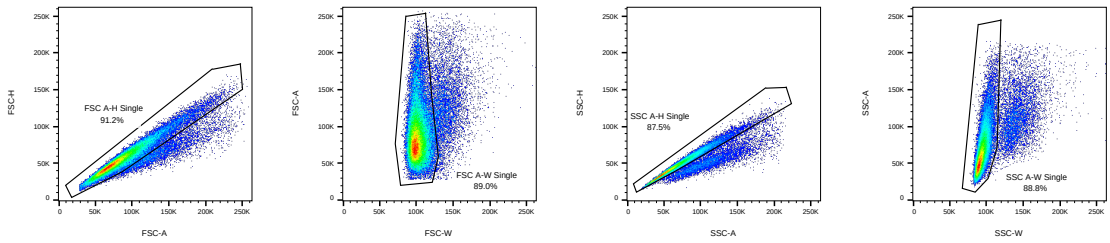
Jill, BD FACSAria II

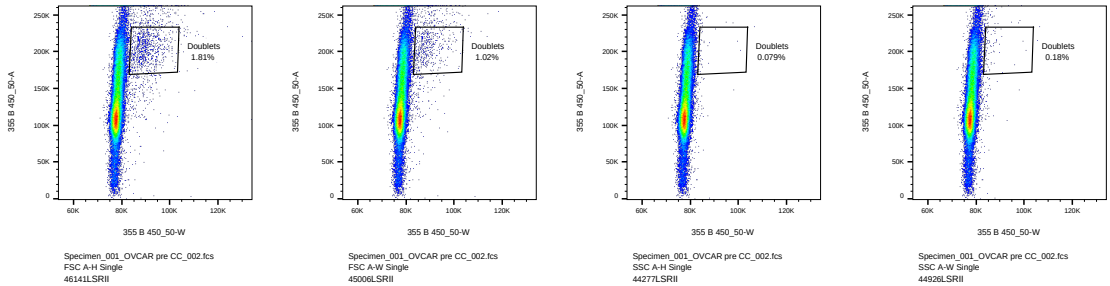


LSRFortessa



LSRII





MACSQuant