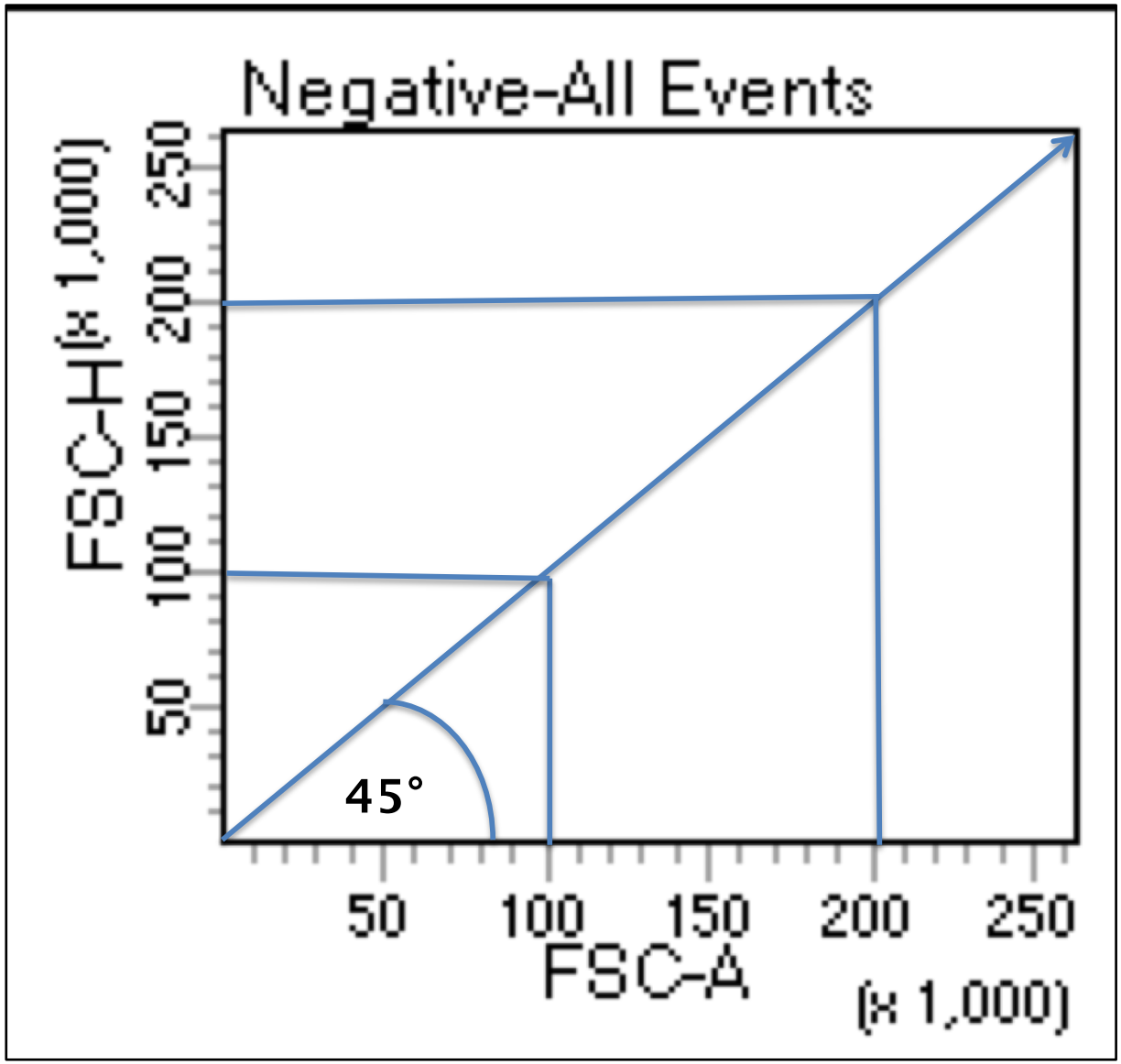
FSC-A Scaling

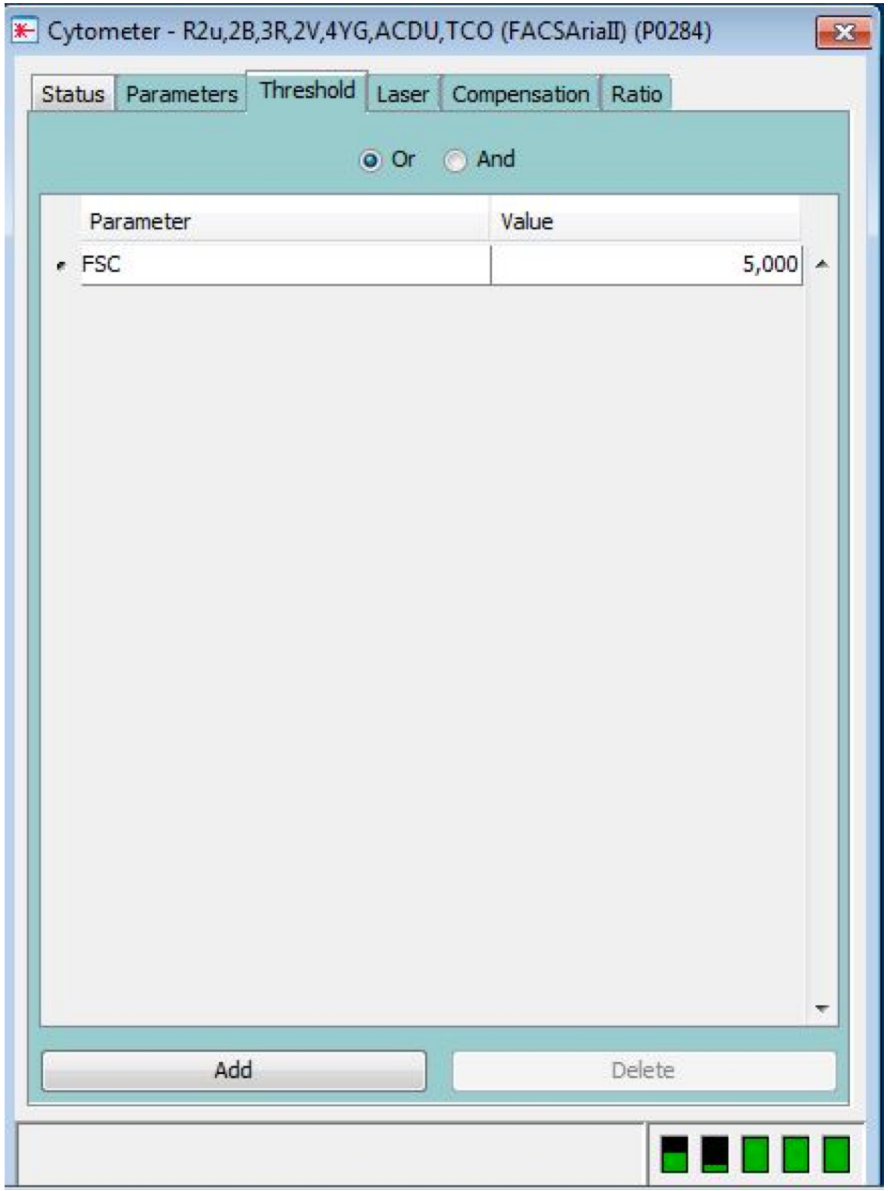
Area and Height of FSC pulses must be the same.



WHY?

The FSC threshold value is based on the FSC pulse height. NOT area

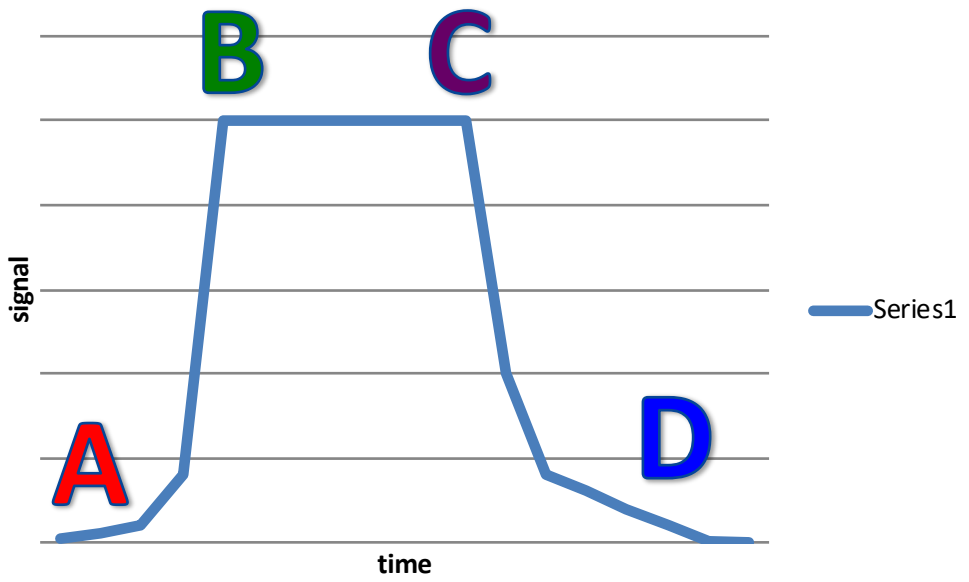
- The value of 5000 is not relevant on the FSC-A axis since FSC-A ≠ FSC-H by default

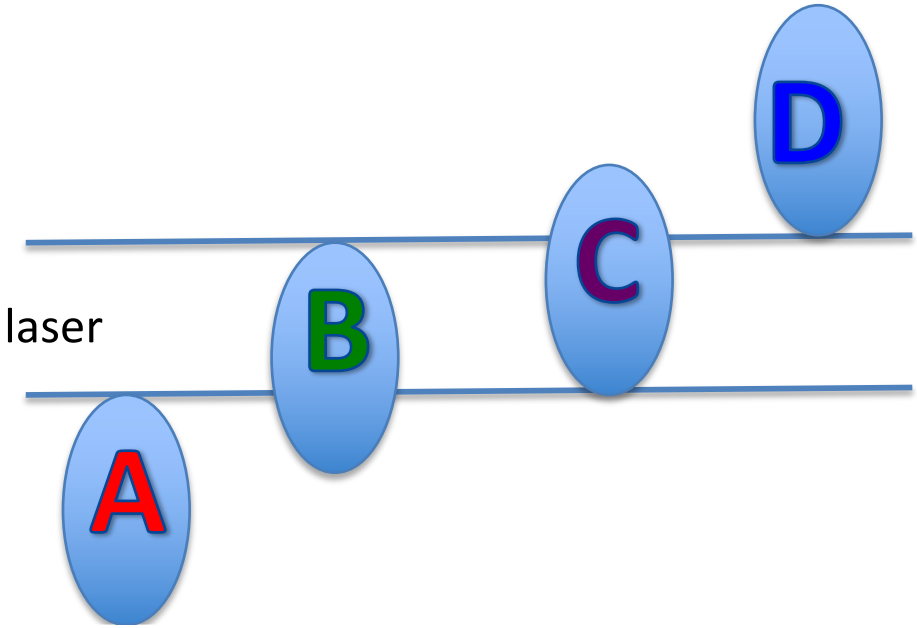


WHY does FSC-A≠ FSC-H?

- The laser is only 8 um high and the cell is larger than the 8 um
- EXCEPTION, these cells are not larger than 8um: bacteria, yeast and RBC’s or platelets

- The pulse flattens out on top because the cell is larger than the laser.
- Without being fully illuminated the FSC-H pulse can never reach its true maximum
- FSC-A will always be equal to the full FSC value of the cell, therefore FSC-A> FSC-H IF the cell is larger than the laser

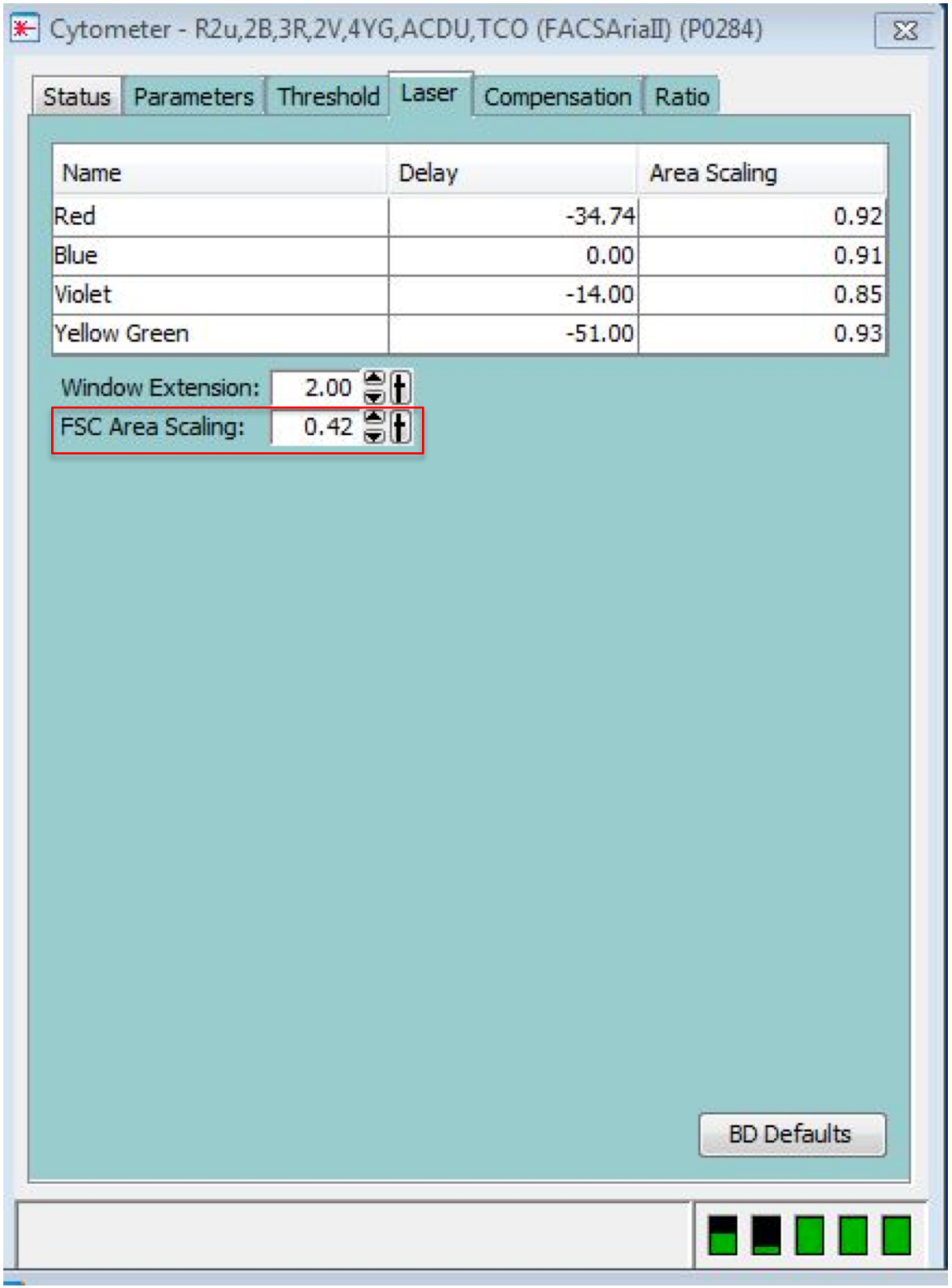


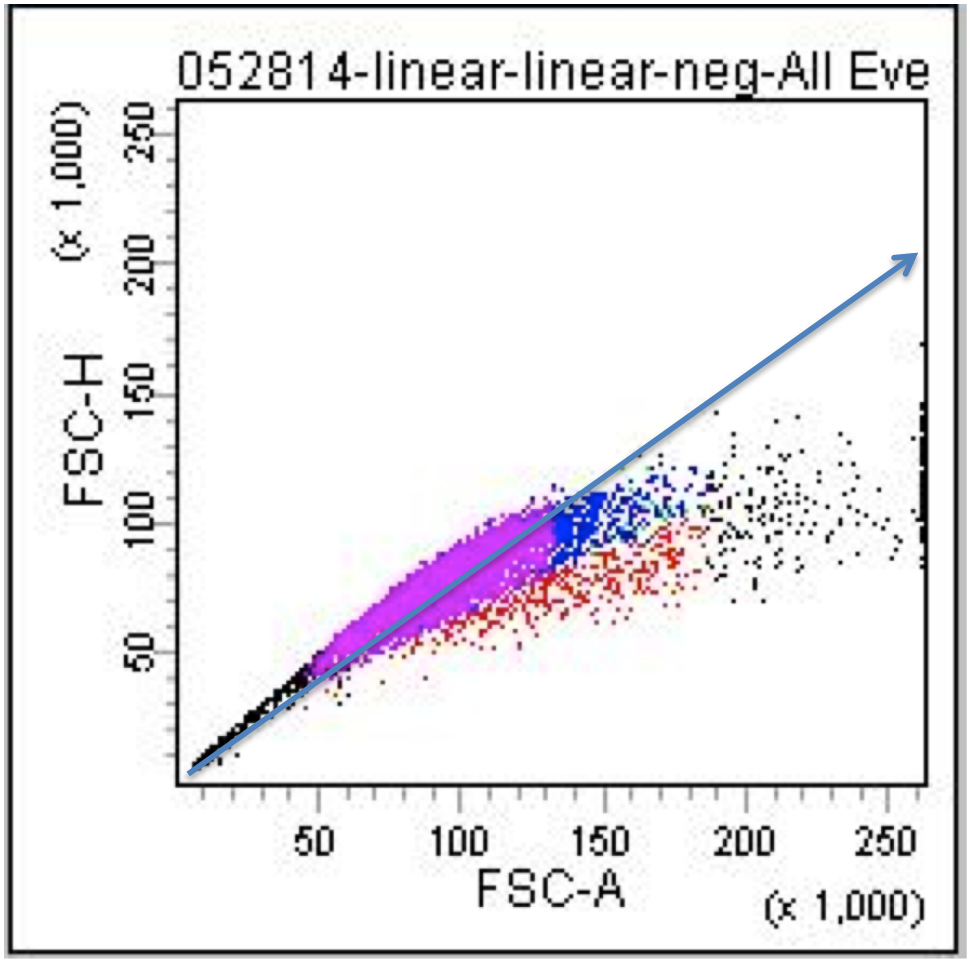


You must multiply the FSC-A by some number less than 1 to make the FSC-A = FSC-H

HOW?

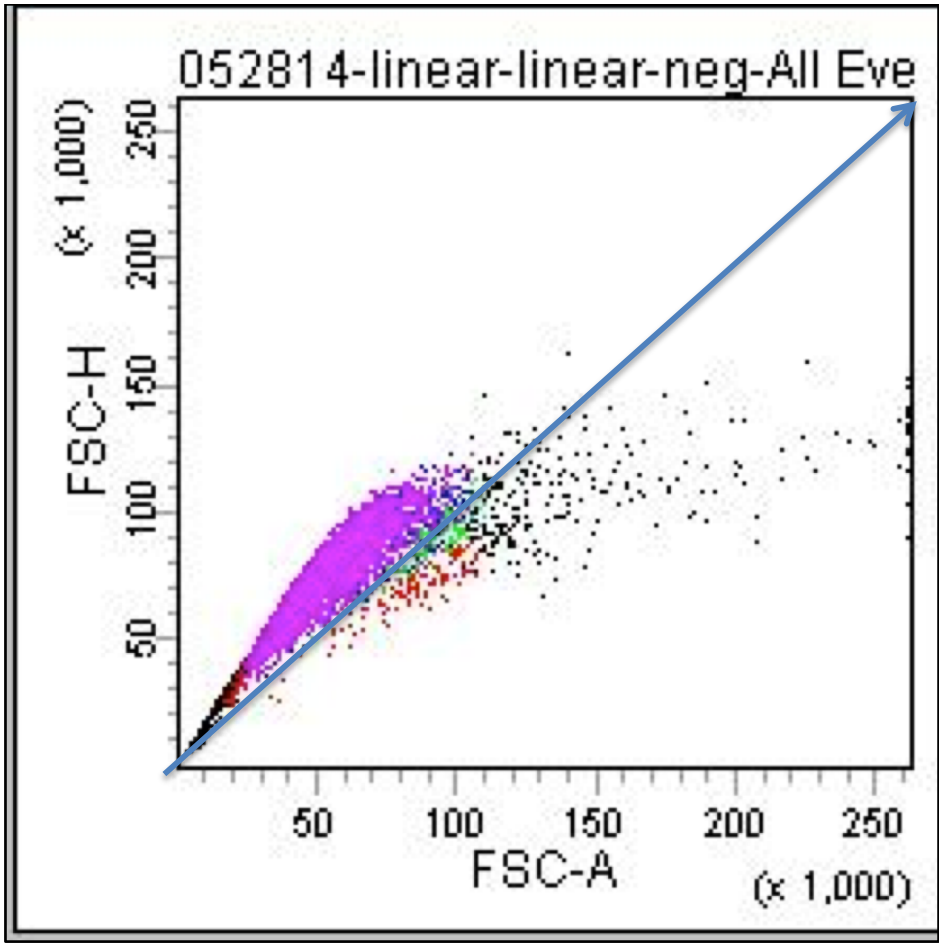
Adjust the FSC-A Scaling number so the cells fall on a 45° angle on a FSC-A vs. FSC-H plot





BEFORE
FSC Area Scaling adjustment

FSC Area Scaling= 0.75



AFTER
FSC Area Scaling adjustment

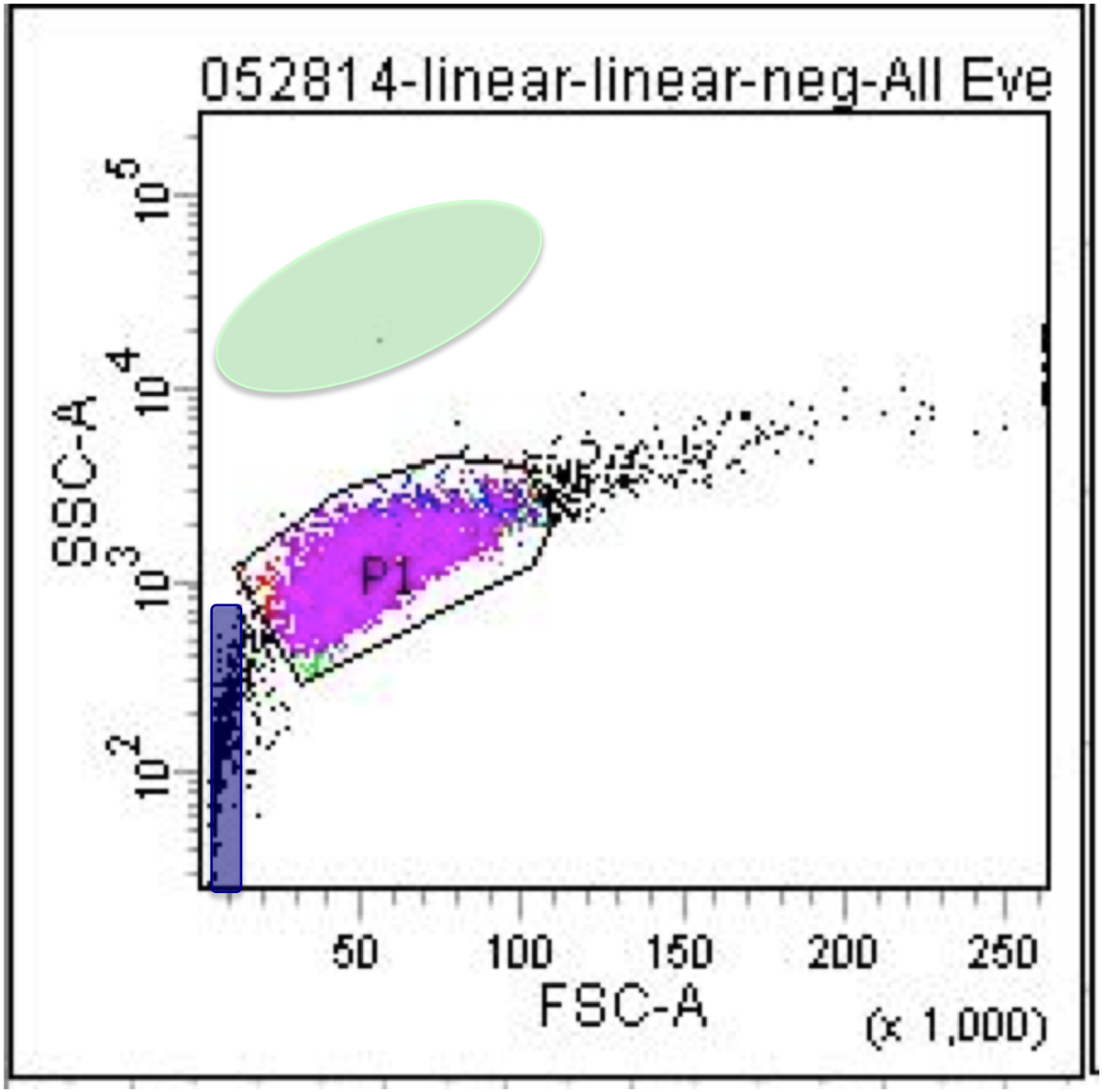
FSC Area Scaling= 0.42

Now the threshold value of 5000 has relevance on a FSC-A vs. SSC-A plot

= debris

P1= live cells

= dead cells



In conclusion…

- FSC threshold is based on the FSC-H
- Adjust the FSC-A using the FSC-A Scaling under cytometer window> laser tab
- So FSC-A= FSC-H by placing the cells on a 45° angle on a FSC-A vs. FSC-H plot