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Flow Cytometer Fluorescence Voltration for FCMPASS V.2

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol to perform flow cytometer voltration to identify optimal detector settings for small particle analysis. Data acquired from this protocol are compatible with semi-automated analysis tools built into FCMPASS software.

Protocol materials

- 8-peak rainbow beads **SpheroTech**
- DPBS **Thermo Fisher Scientific** **Catalog #**14190144
- 5 mL Round-bottom tube **Corning** **Catalog #**352052
- 8-peak rainbow beads **SpheroTech**
- 5 mL Round-bottom tube **Corning** **Catalog #**352052

Troubleshooting

Sample preparation

10s

1 Vortex 8-peak rainbow beads **SpheroTech** bottle on a high setting for 00:00:05 . 5s

2 Pipette 500 µL of DPBS **Thermo Fisher Scientific Catalog #**14190144 to two 5 mL Round-bottom tube **Corning Catalog #**352052 . Label one tube 'DPBS" and the second tube 'Beads'.

> Note
>
> An observation from our protocol development is that it is important not to use a low protein binding tube for this step, as it can result in excess unbound fluor from the beads creating background noise increases leading to excessive event rate.

3 Add 3 drops of 8-peak rainbow beads **SpheroTech** to the 'Beads' 5 mL Round-bottom tube **Corning Catalog #**352052 and vortex for r 00:00:05 . 5s

Cytometer Setup

4 Ensure cytometer is clean and that -Height and -Area statistics are set to be collected on all parameters and that all parameters are on.

5 Create a pseudocolor plot with FSC-A on the X-Axis and (488 nm) B-SSC-A on the Y-Axis and make sure both parameters are being plotted on a linear-scale.

6 Create a histogram plot with (405 nm) V-SSC-H on the X-Axis and make sure it is plotted on a log-scale.

7 Set the cytometer triggering threshold to (405 nm) V-SSC-H. **All samples should be acquired with the lowest flow rate, typically ~10-15 µL min^{-1}.**

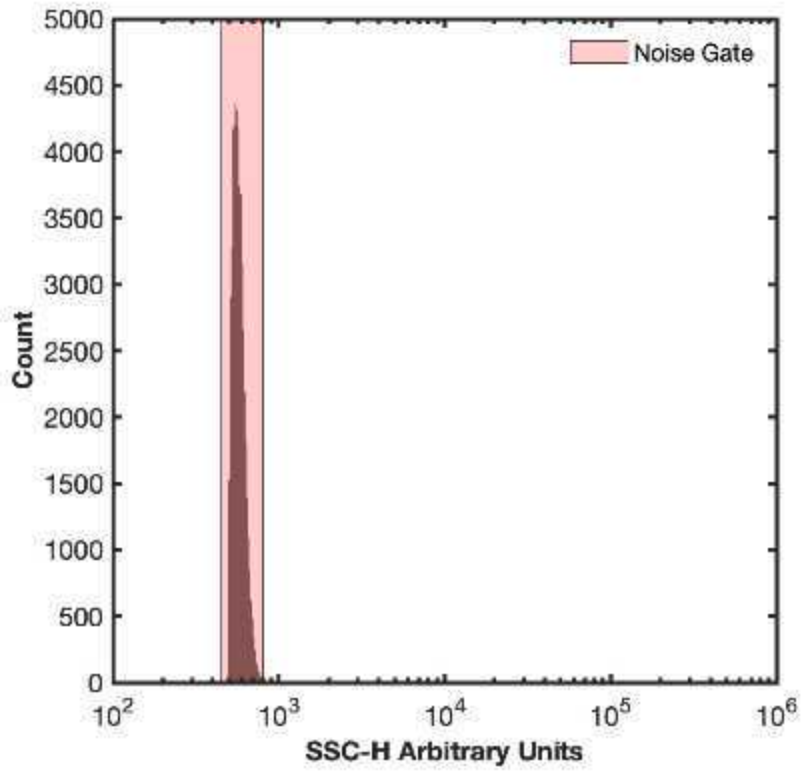
Note

Cytometer Voltage/Gain and threshold settings are subjective due to their dependency on alignment, and the scatter filters in place, amongst other variables. The following are guide values to start with and may need adjustment for optimal acquisition.

Beckman Coulter, CytoFLEX [405 nm OD0 filter, 488 nm OD2 filter]
- Threshold V-SSC-H = 1000;
- V-SSC Gain = 200
- FSC Gain = 100
- B-SSC Gain = 100

Cytek Bioscience, Aurora [405 nm OD0 filter, 488 nm OD2 filter]
- Threshold V-SSC-H = 1000;
- V-SSC Gain = 600
- FSC Gain = 70
- B-SSC Gain = 70

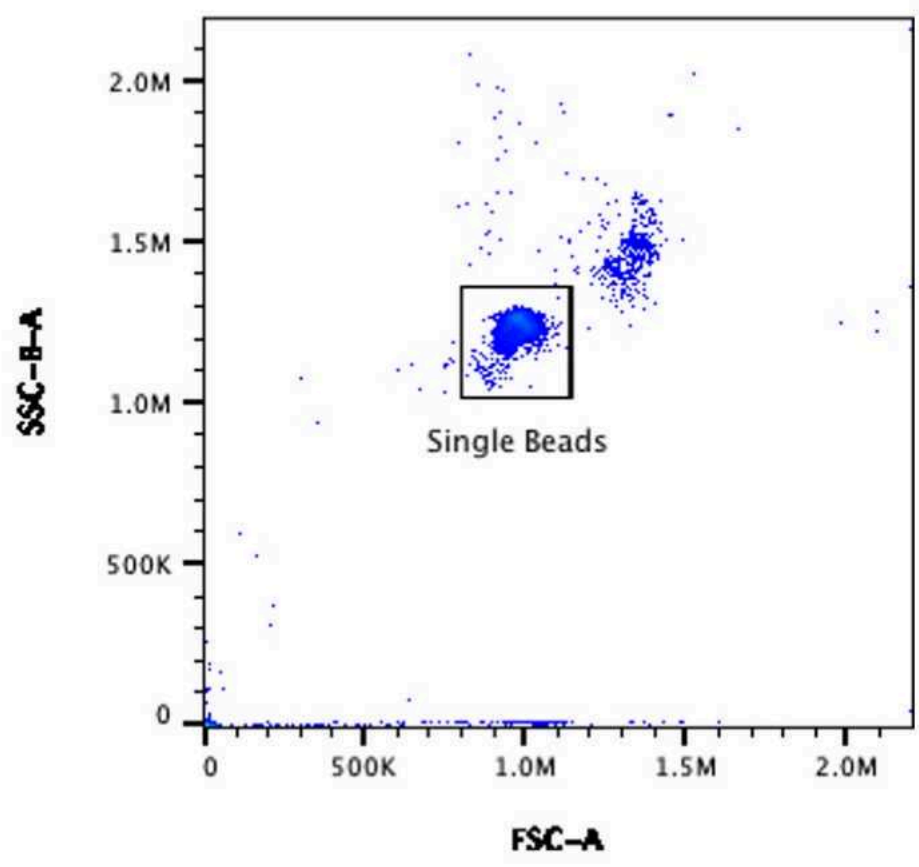
8 Acquire the DPBS tube while viewing the histogram plot from go to step #6. Adjust the detector gain or trigger threshold until the instrument noise is being **acquired at ~1000 events/sec.** The instrument noise floor is distinct from detected background events in sheath as it has a sharp increase. In a system with debris there may be a tail that elongates out of this this sharp peak.



Example of threshold on the instrument noise floor

8.1 Recording this noise is not necessary as this step is identifying optimal settings.

9 Acquire the 'Beads' tube from go to step #3 . Using the plot from go to step #5 adjust the FSC and B-SSC gain until the single bead population is clearly visible and can be easily gated from the doublet population to the top right of it. Use the Violet SSC trigger settings identified in go to step #8



Example of clearly resolved singlet bead population

10 Creating a gate around the single bead population named 'Gate 1';

11 Adjust the stopping criteria of the instrument to record until 10,000 events are acquired on 'Gate 1' drawn in go to step #10 .

Performing Voltration

12 Voltration can now be performed by recording the 'Beads' tube at multiple fluorescent detector gains, leaving the trigger threshold and light scatter gains consistent. It is recommended that a recording of at least 10 fluorescent detector settings is taken. Including more increments within a voltration will result in being more confident of the subsequent optimal detector settings. Acquisition Template.xlsx

Note

For flow cytometers with avalanche photodiodes its is recommended that the fluorescent detector settings have more incrementation at lower gains than higher gains while for instruments with photomultiplier tubes they should be spaced evenly. See template for example of settings for each tube analyses

Example Gain Voltration for CytoFLEX & Aurora

1. 100
2. 200
3. 300
4. 400
5. 500
6. 750
7. 1000
8. 1250
9. 1500
10. 2000
11. 2500
12. 3000

12.1 To ensure accurate data analysis, the brightest bead must be visible on at least two of the selected gains.