

## **Supporting information 6: Particle size distribution of extracellular vesicles from “Removal of platelets from blood plasma to improve the quality of extracellular vesicle research”**

The aim of Supporting information 6 is to show the particle size distributions of unfiltered and filtered platelet-, and erythrocyte-derived extracellular vesicle (EV) samples. Particle size distributions were processed using a custom-build software (MATLAB R2020b).

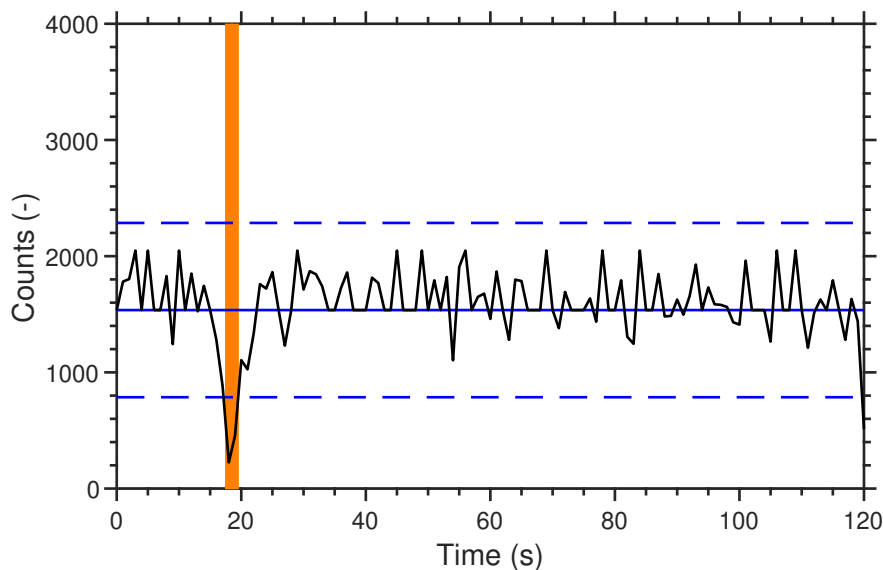
For each sample the count rate stability is shown as counts (-) vs. time (s). Furthermore, the side scattering cross section ( $\text{nm}^2$ ) vs. the fluorescence in Allophycocyanin (APC) or Phycoerythrin (PE) Molecules of Equivalent Soluble Fluorochrome (MESF) is shown in the next plot. The particle size distribution is shown as the concentration of the corresponding EV subtype ( $\text{mL}^{-1}$ ) vs. the diameter (nm) based on side scatter. A bin size of 50 nm was used. The EV size distribution shows the number of particles that exceed the side scatter threshold and the fluorescence gate. Details about the flow cytometry experiment and analysis can be found in Supporting information 4.

The size distributions show that filtration effectively removes platelets while having a minimal effect on the concentration and size-distribution of EVs detected by flow cytometry.

**Table 1. Sample list**

<b>ID</b>	<b>Sample name</b>	<b>Antibody</b>
Exp31_2spinEVs1_20x_ CD61-APC_CD235a- PE_A12_0.fcs	Unfiltered EV sample 1	CD61-APC, CD235a-PE
Exp31_2spinEVs2_20x_ CD61-APC_CD235a- PE_B1_0.fcs	Unfiltered EV sample 2	CD61-APC, CD235a-PE
Exp31_2spinEVs3_20x_ CD61-APC_CD235a- PE_B2_0.fcs	Unfiltered EV sample 1	CD61-APC, CD235a-PE
Exp031_FilterEVs1_ 20x_CD61-APC_CD235a- PE_B4_0.fcs	Filtered EV sample 1	CD61-APC, CD235a-PE
Exp031_FilterEVs2_ 20x_CD61-APC_CD235a- PE_B4_0.fcs	Filtered EV sample 2	CD61-APC, CD235a-PE
Exp031_FilterEVs3_ 20x_CD61-APC_CD235a- PE_B6_0.fcs	Filtered EV sample 3	CD61-APC, CD235a-PE

Falcon - Apply gates  
 Exp31\_2spinEVs1\_20x\_CD61-APC\_CD235a-PE\_A12\_0.fcs



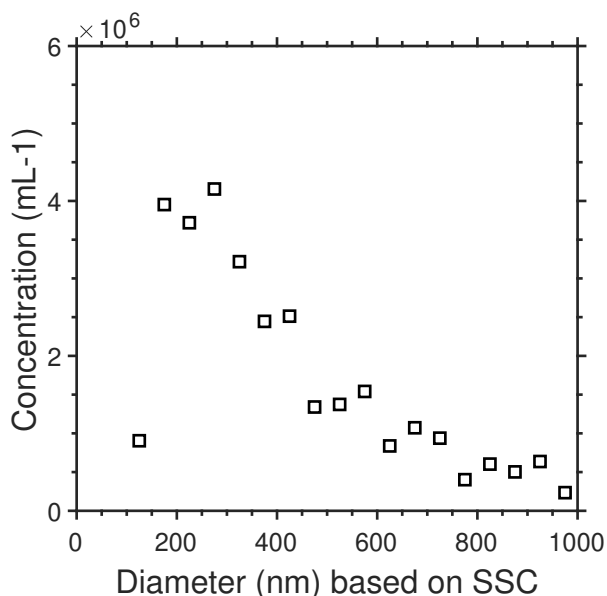
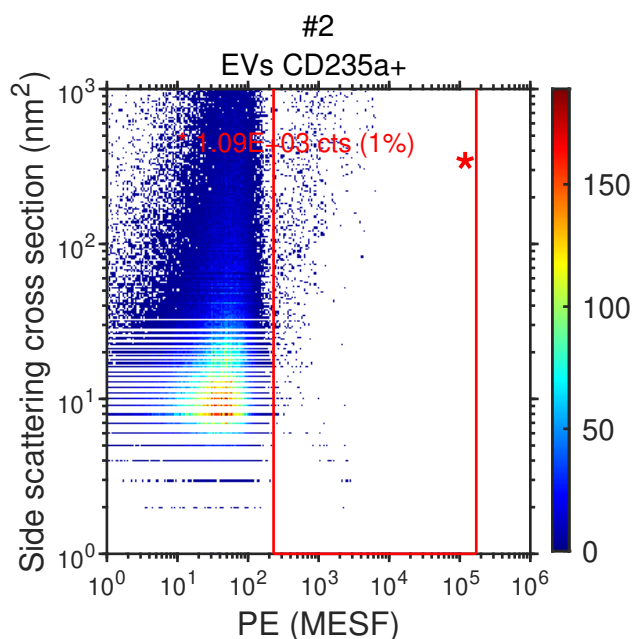
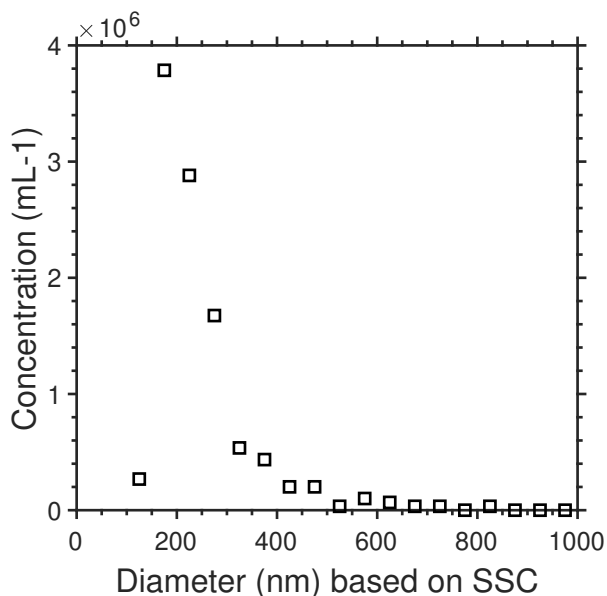
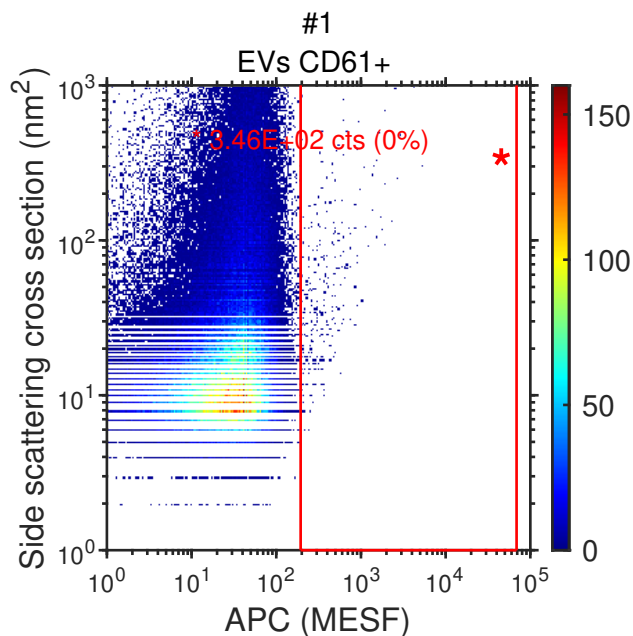
█ Excluded  
- - - Upper boundary  
- - - Lower boundary  
— Median  
— Data

All:

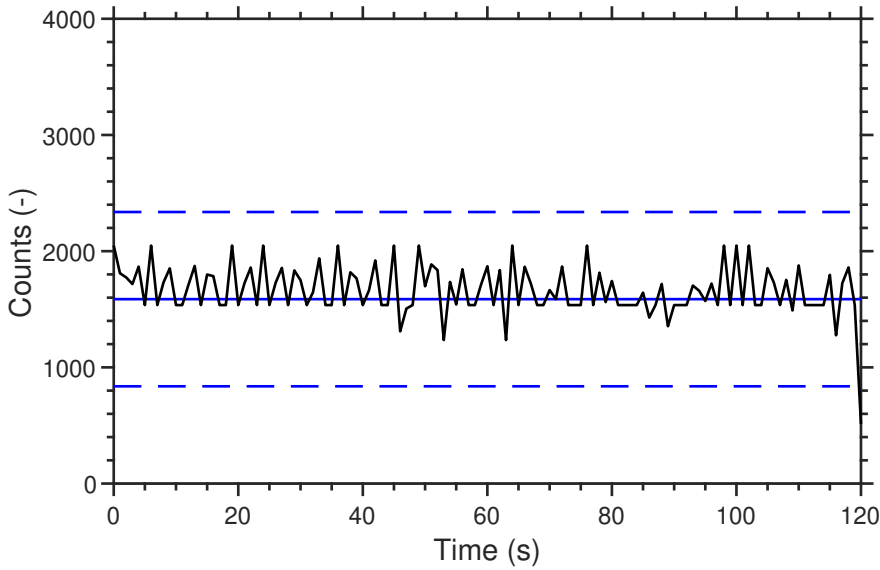
Total counts = 191765  
 Median count rate = 1536 cnts/s  
 CV count rate = 20 %

White area only:

Count rate range = 786 - 2286 events/s  
 Included = 98 %  
 Total counts = 191083  
 Median count rate = 1536 cnts/s  
 CV count rate = 17 %



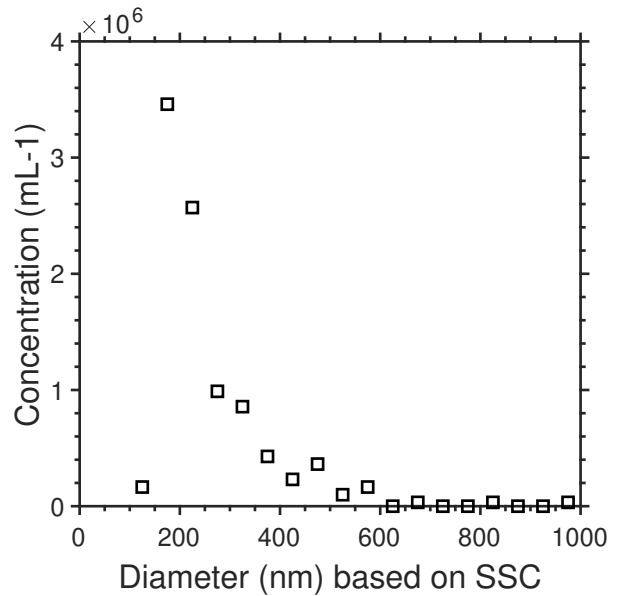
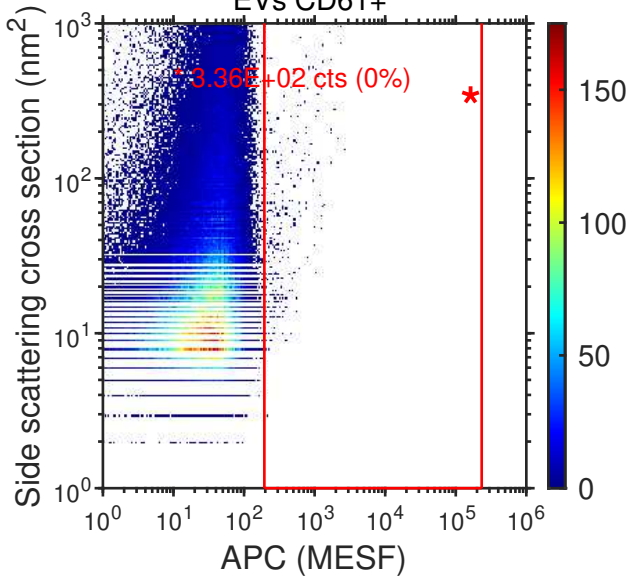
Falcon - Apply gates  
 Exp31\_2spinEVs2\_20x\_CD61-APC\_CD235a-PE\_B1\_0.fcs



Excluded  
 Upper boundary  
 Lower boundary  
 Median  
 Data  
 All:  
 Total counts = 201472  
 Median count rate = 1587 cnts/s  
 CV count rate = 14 %  
 White area only:  
 Count rate range = 837 - 2337 events/s  
 Included = 100 %  
 Total counts = 201472  
 Median count rate = 1587 cnts/s  
 CV count rate = 14 %

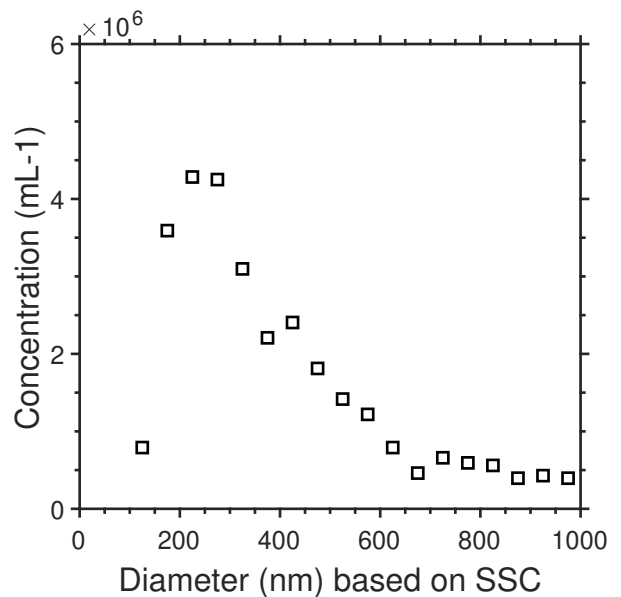
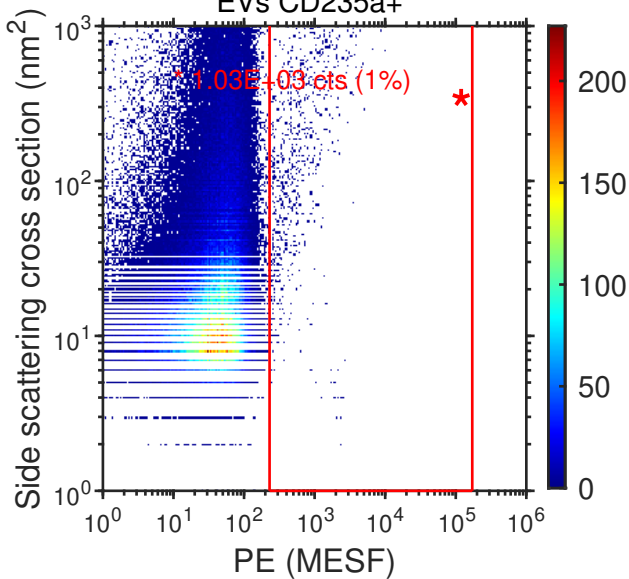
#1

EVs CD61+

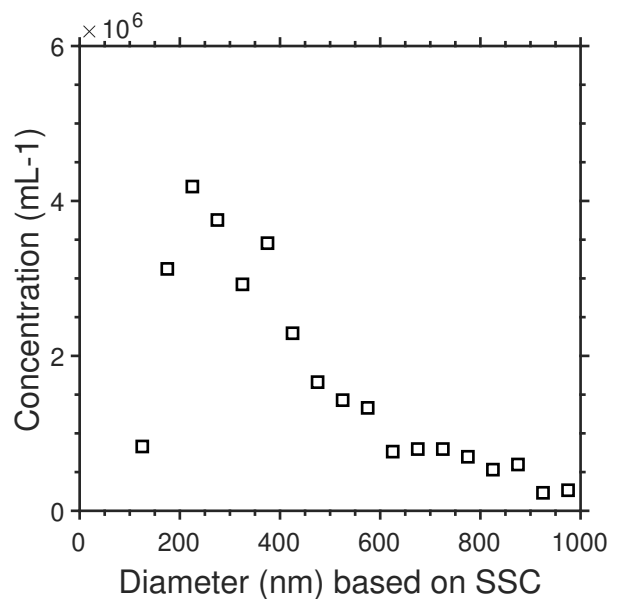
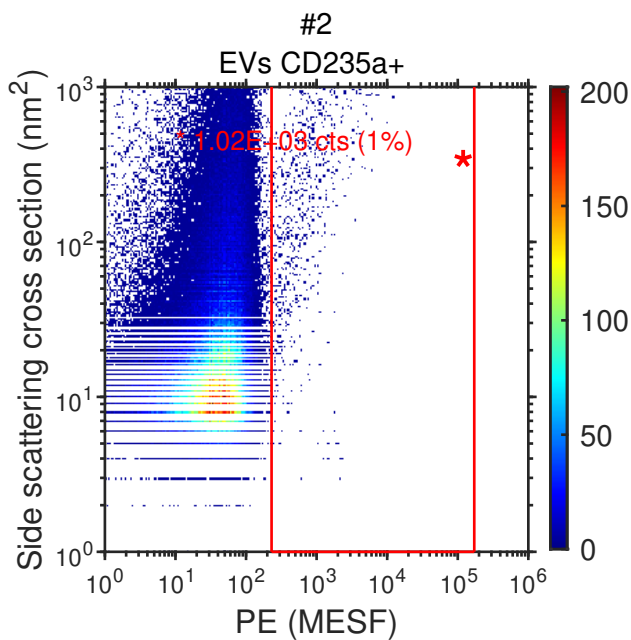
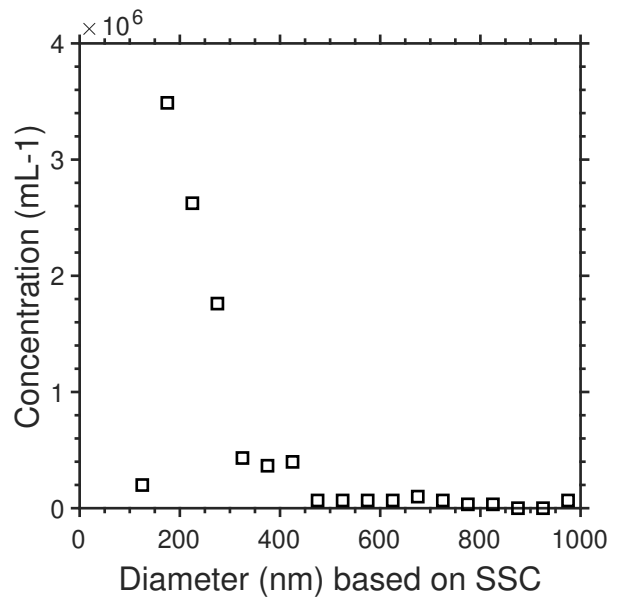
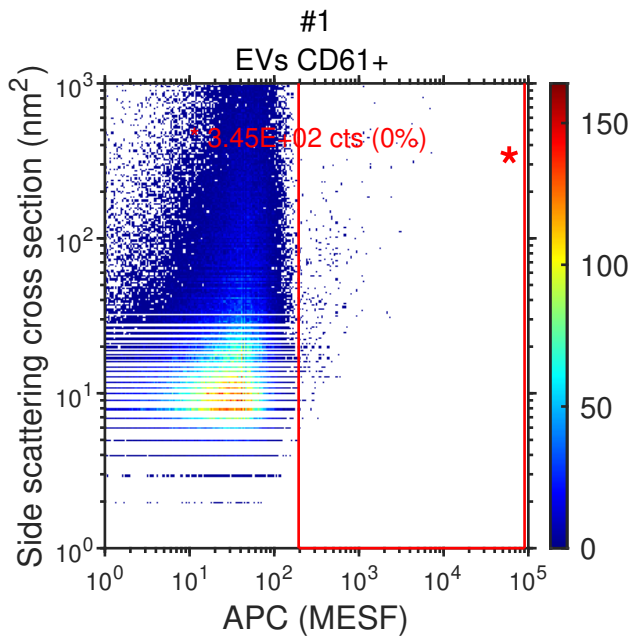
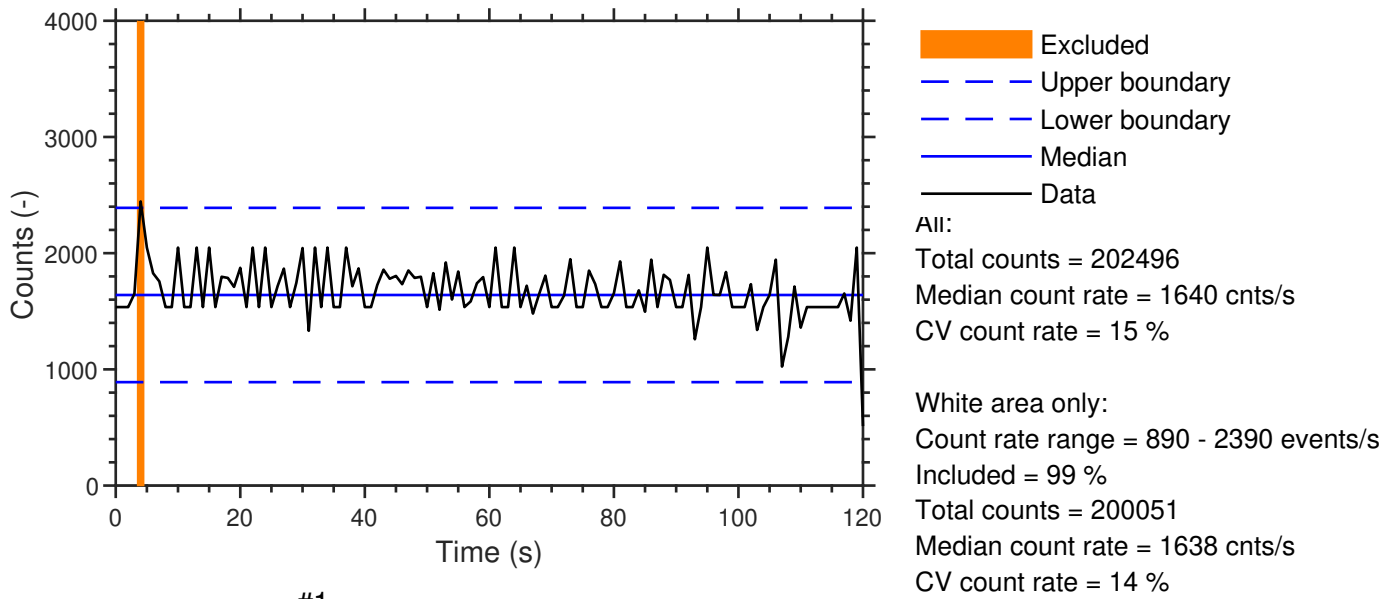


#2

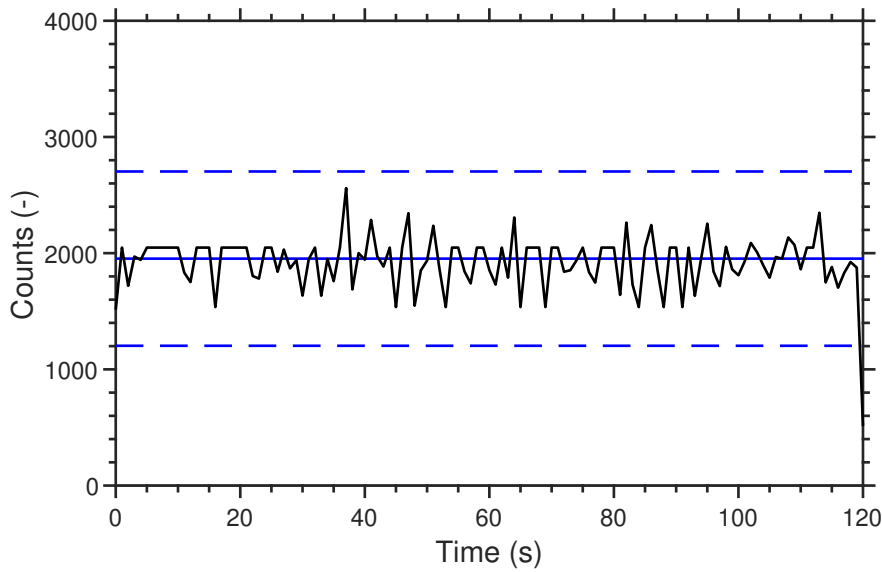
EVs CD235a+



Falcon - Apply gates  
 Exp31\_2spinEVs3\_20x\_CD61-APC\_CD235a-PE\_B2\_0.fcs



Falcon - Apply gates  
 Exp031\_FilterEVs1\_20x\_CD61-APC\_CD235a-PE\_B4\_0.fcs



Excluded  
 Upper boundary  
 Lower boundary  
 Median  
 Data

All:

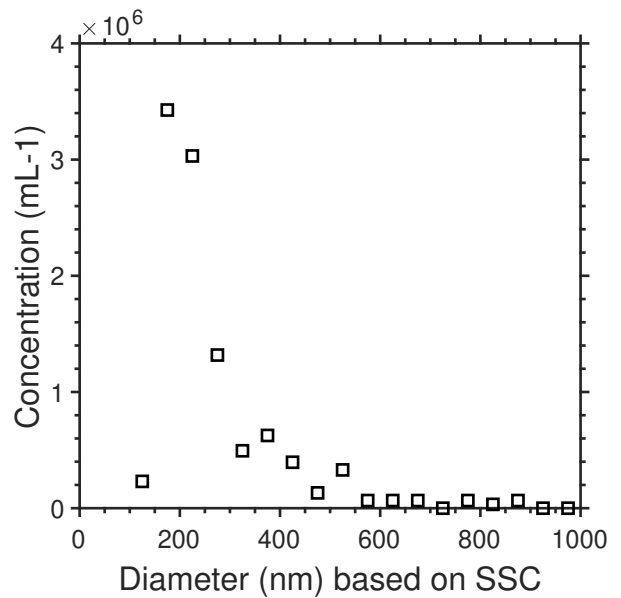
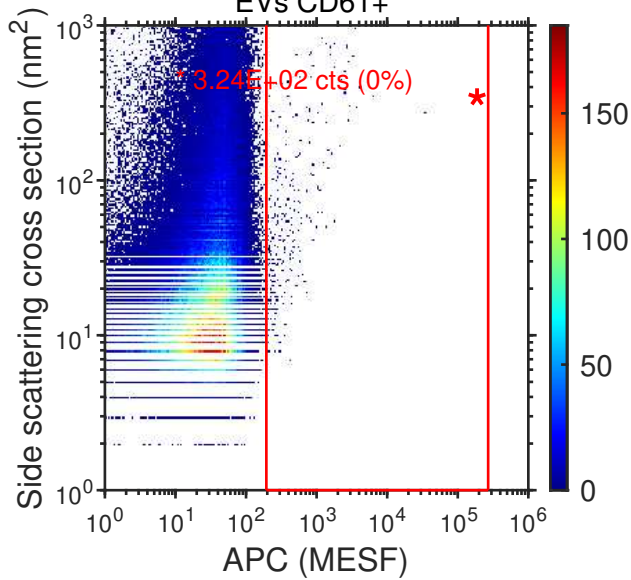
Total counts = 232137  
 Median count rate = 1953 cnts/s  
 CV count rate = 12 %

White area only:

Count rate range = 1203 - 2703 events/s  
 Included = 100 %  
 Total counts = 232137  
 Median count rate = 1953 cnts/s  
 CV count rate = 12 %

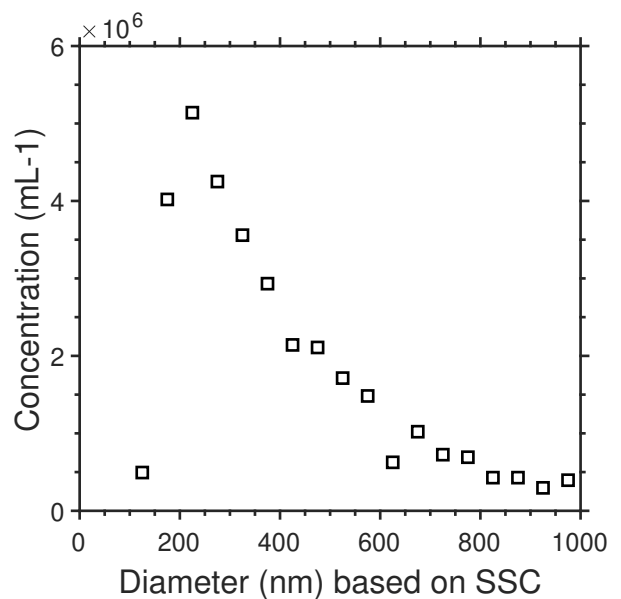
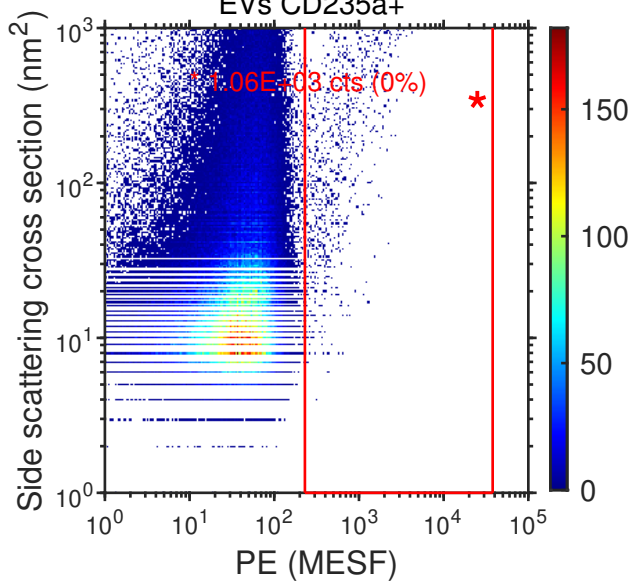
#1

EVs CD61+

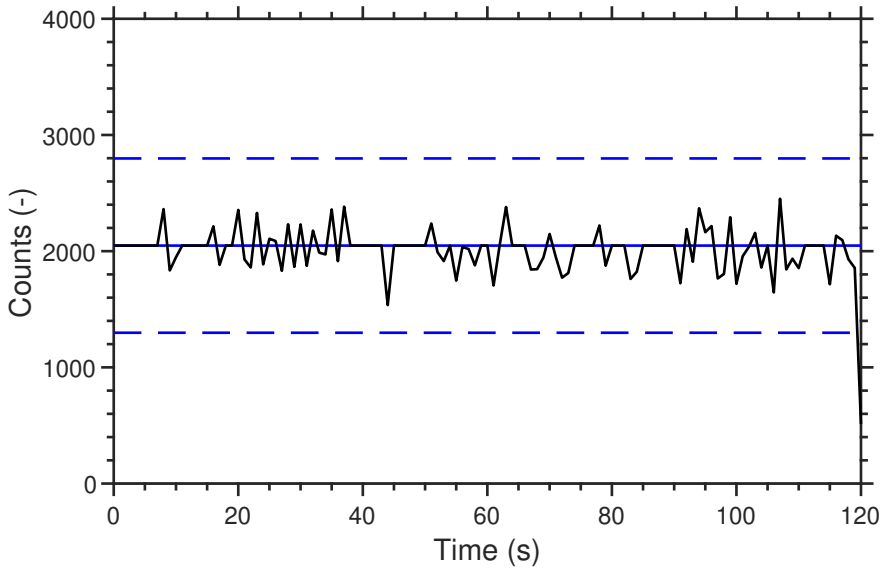


#2

EVs CD235a+



Falcon - Apply gates  
 Exp031\_FilterEVs2\_20x\_CD61-APC\_CD235a-PE\_B5\_0.fcs

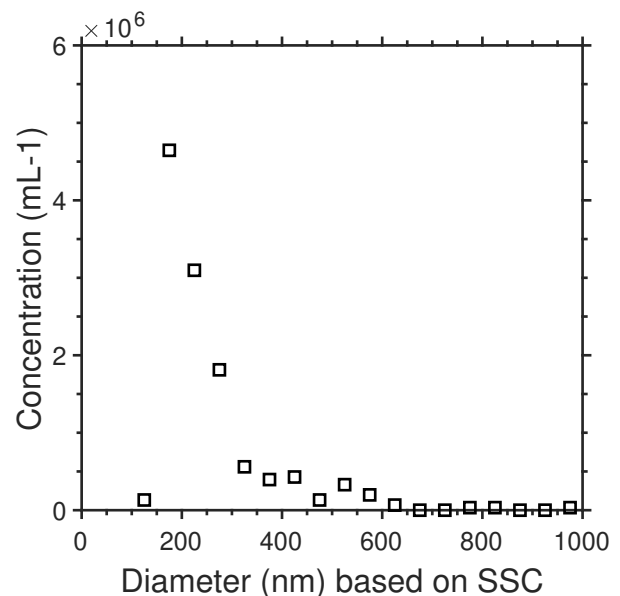
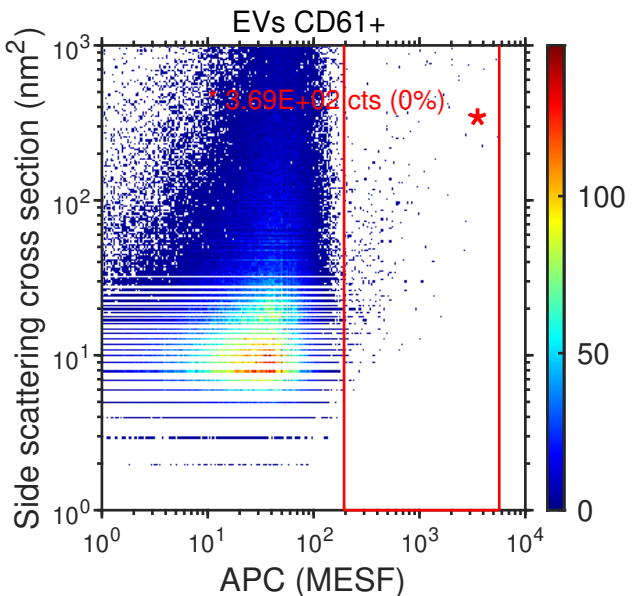


Excluded  
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 Lower boundary  
 Median  
 Data

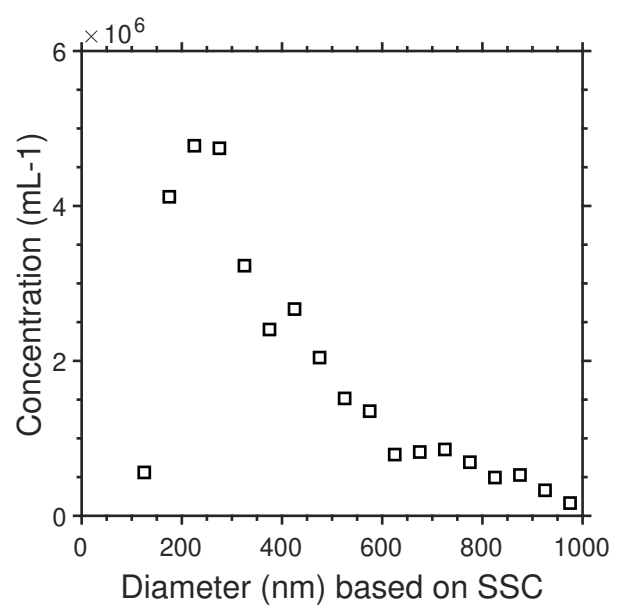
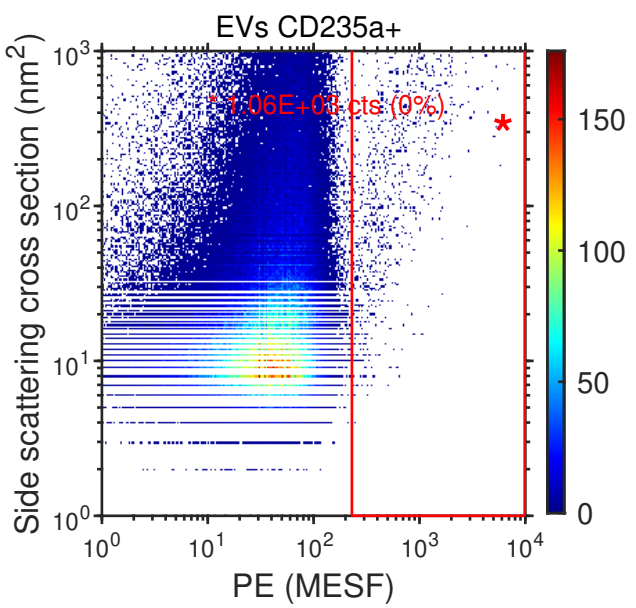
All:  
 Total counts = 242688  
 Median count rate = 2048 cnts/s  
 CV count rate = 10 %

White area only:  
 Count rate range = 1298 - 2798 events/s  
 Included = 100 %  
 Total counts = 242688  
 Median count rate = 2048 cnts/s  
 CV count rate = 10 %

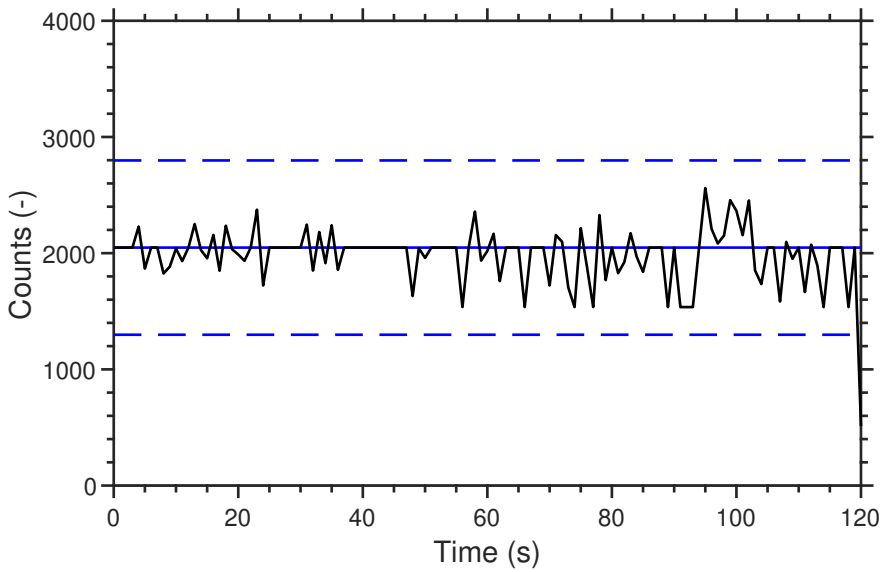
#1



#2



Falcon - Apply gates  
 Exp031\_FilterEVs3\_20x\_CD61-APC\_CD235a-PE\_B6\_0.fcs



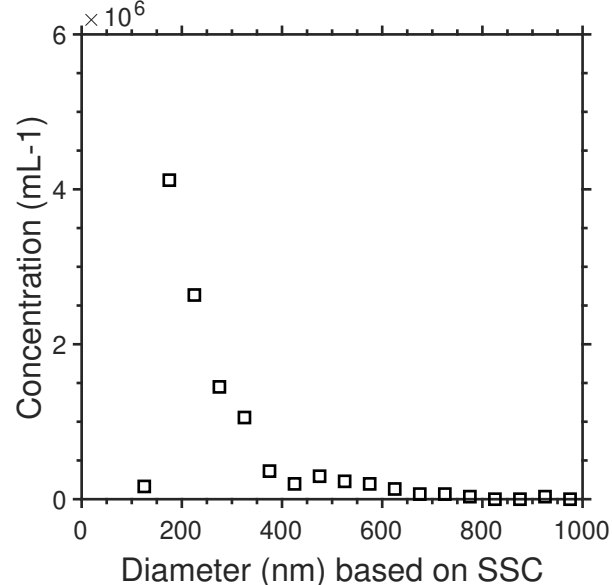
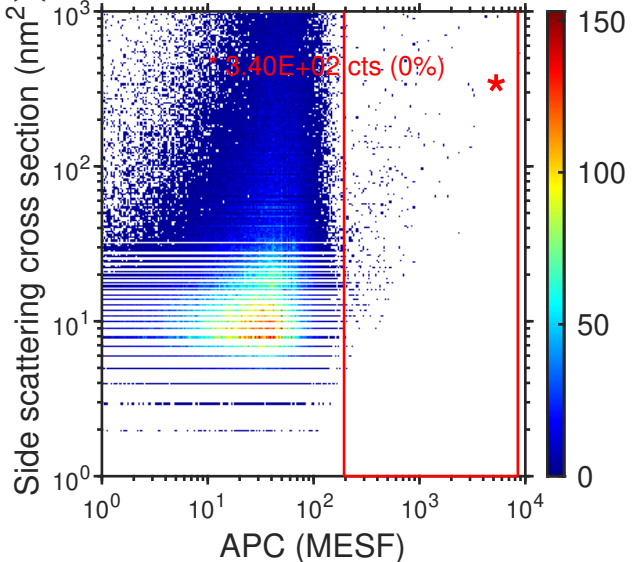
Excluded  
 Upper boundary  
 Lower boundary  
 Median  
 Data

All:  
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 Median count rate = 2048 cnts/s  
 CV count rate = 12 %

White area only:  
 Count rate range = 1298 - 2798 events/s  
 Included = 100 %  
 Total counts = 239616  
 Median count rate = 2048 cnts/s  
 CV count rate = 12 %

#1

EVs CD61+



#2

EVs CD235a+

