Termite Nuclei Dissociation and FACS Sorting

02/06/2023-06/06/2023

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Experimental Overview

Goals:

1. Dissociate a decent number of nuclei from termites.
2. Remove termite debris from dissociated nuclei.
3. Achieve a concentration of nuclei appropriate for 10x Genomics sc sequencing (approx 700-1200 cells/uL)

Method:

1. Nuclei dissociation of termite nuclei using homogenisation and resuspension buffer solutions, grinding, centrifugation and straining.
2. FACS sorting using DAPI and the BD FACSAria III to collect nuclei and remove debris.

Results:

1. Current homogenisation and resuspension buffer solutions, grinding, centrifugation and straining protocol dissociates nuclei from termites.
2. FACS sorting with the BD FACSAria III collected 100K DAPI stained nuclei per run, from a sample containing 6-7 termites, and removed debris from nuclei. Resulting FACS plot looks similar to the plot of single nuclei from Drosophila tissues.
3. Concentration of nuclei was achieved by centrifugation.

Below I will briefly outline the protocol we used, and our results, including the FACS plots and images of nuclei under epifluorescent microscope. Please find our excel lab protocol attached to the email.

1. ***Nuclei Dissociation***

Twelve *N. walkeri* soldiers and twelve workers were placed on a dissection dish and were cut into small pieces using tweezers and scissors. Approximately six termites (soldiers and workers were moved to separate tubes) where each moved into one 1.5-mL tube containing 1000 µL of chilled homogenization buffer (Nuclease Free water, 250 mM Sucrose (nuclease free), 10 mM Tris (pH 8.0), 25 mM KCl, 5 mM MgCl2, 0.1% Triton-x 100 and 0.1 mM DTT). The tissue was then homogenized using a pellet pestle, by twisting the pestle approximately 20 times. The nuclei were centrifuged at 1000g for 10 min at 4 °C, and the supernatant was discarded. The pellet was then resuspended in 1000 µL of resuspension buffer (PBS and 0.5% BSA). The nuclei were then strained using 100 and 35 µm cell strainers (see Result 1). After the previous two steps, and after FACS sorting (see Result 2), 10 µL of the nuclei was stained with DAPI (1:1000) and visualized

under an epifluorescent microscope. Images of the cells were taken using an Iphone (1), and microscope in the DAPI (2) and brightfield (3) channels.

2. FACS Sorting

FACS sorting was performed on the BD FACSAria III. Approximately 100 µL of nuclei suspension was run as an unstained control (see Result 3). DAPI was added (1:1000) to the remaining nuclei and was captured into a FACS tube containing 50 µL of PBS, excluding debris and doublets, and sorting was run until (1) there was no sample left (Tube 2) or 100K events had occurred (Tube 1, 3 and 4). (see Result 3). Subsequently, the concentration of nuclei was determined with a hemocytometer and counting the number under a 20× objective of an epifluorescence microscope (see Result 2). The nuclei were centrifuged at 1000g for 6 min at 4 °C, and approx. 600 µL of supernatant was discarded. The pellet was resuspended in the remaining supernatant.

Sort Settings:

Experiment : CG20230606 | Sort Report | Report Date : 2023.06.06 at 16:39:24
Specimen : Specimen_001 | Device : 2 Tube
Tube : T4-W2 | User ID : catgat
Sort Layout : Sort Layout_001 | Cytometer : FACSAriaIII (1)
Application : FACSDiva Version 8.0.1

Sort Settings

Sort Setup	100 micron	Precision	Purity
Frequency	31.1	Yield Mask	32
Amplitude	4.2	Purity Mask	32
Phase	0.00	Phase Mask	0
Drop Delay	28.52	Single Cell	Off
Attenuation	Off	Plates Voltage	4,500
Sweet Spot	On	Voltage Centering	200
First Drop	220	Sheath Pressure	20.00
Target Gap	10		

Side Stream Voltage (%)

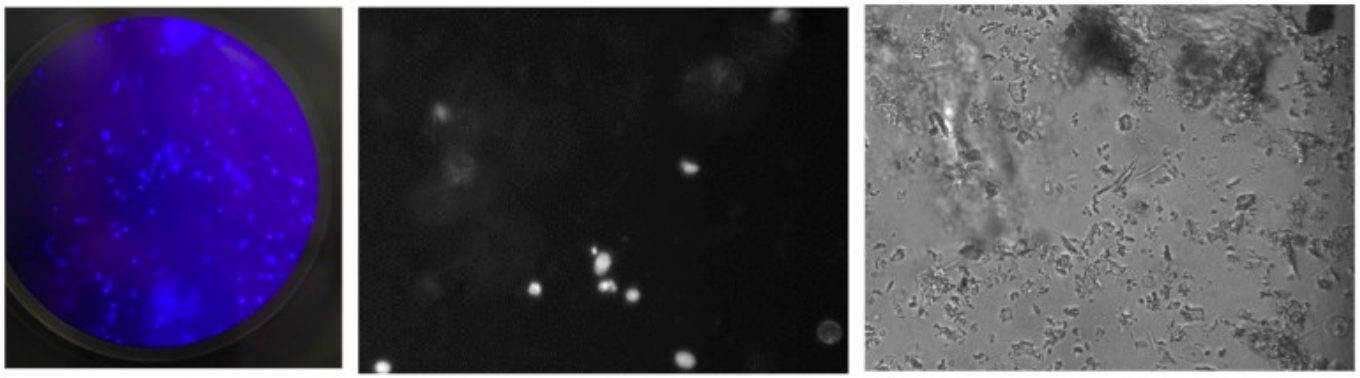
Far Left	Left	Right	Far Right
0.00	32.00	0.00	0.00

Neighboring Drop Charge (%)

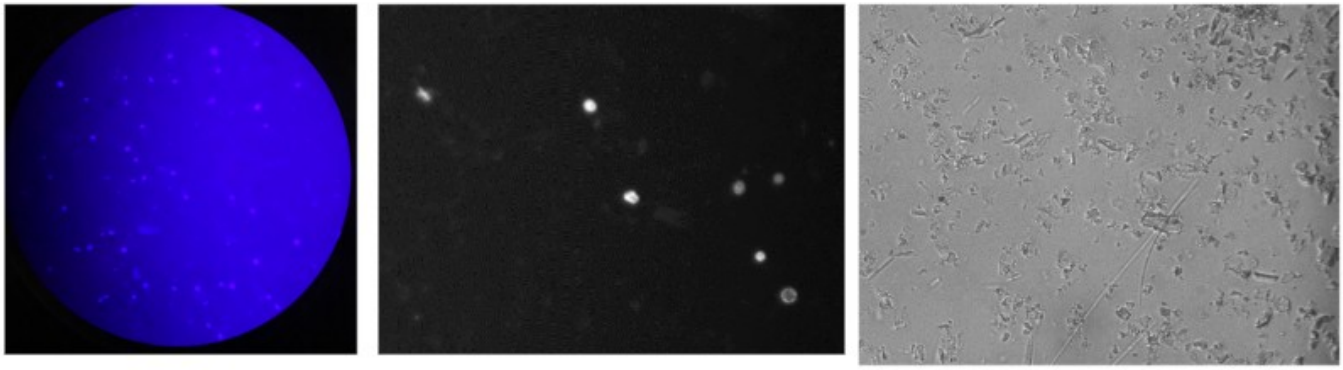
2nd	3rd	4th
15.00	6.00	1.00

Results

1. **Nuclei after Straining**
    a. **100 µm cell strainer**

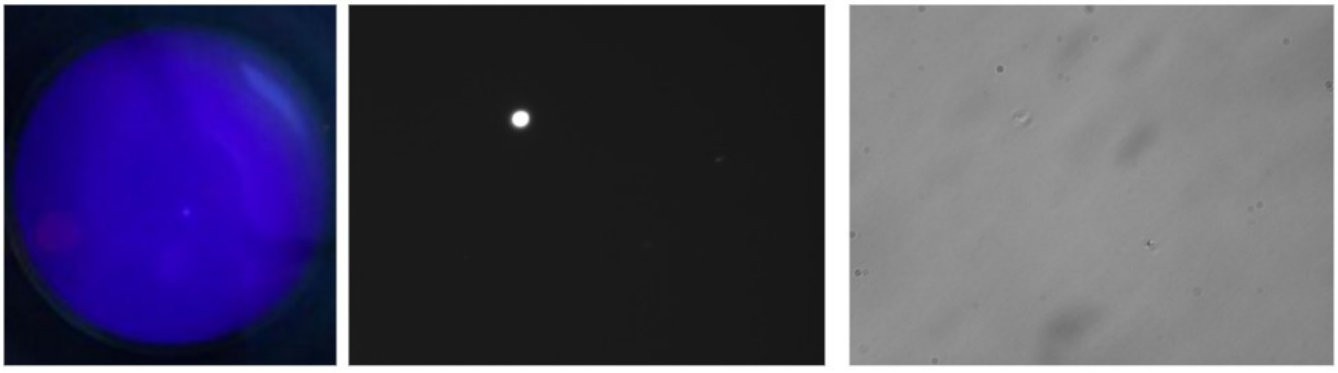


b. 35 µm cell strainer

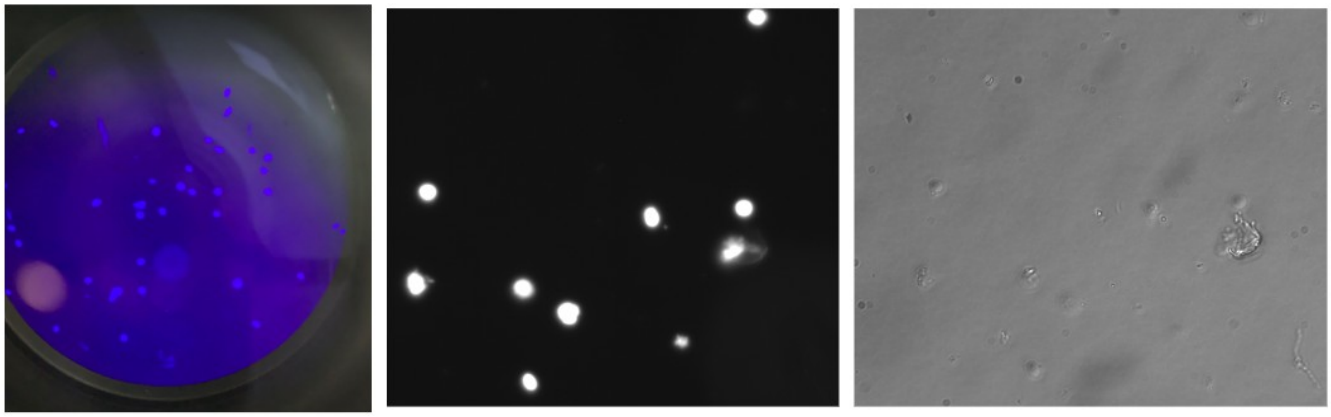


2. Nuclei After FACS

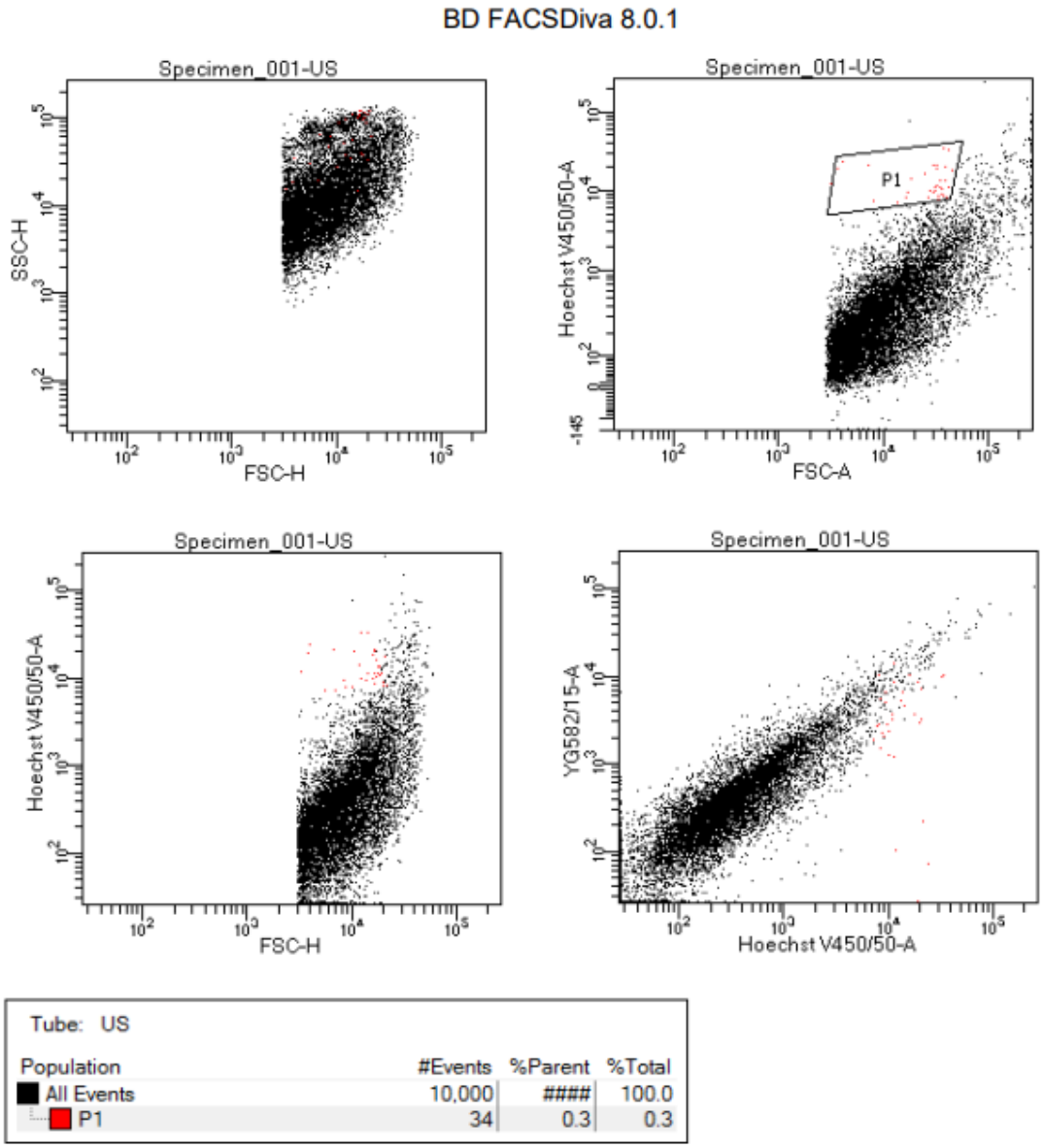
a. Before Concentration



b. After Concentration



3. **FACS Plots**
    a. **Unstained Control, 02/06/2023**



Tube: US

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	34	0.3	0.3

b. Tube 1 (soldiers), 06/06/2023

c. **Single nuclei from Drosophila tissues**

i. Source: https://star-protocols.cell.com/protocols/1669

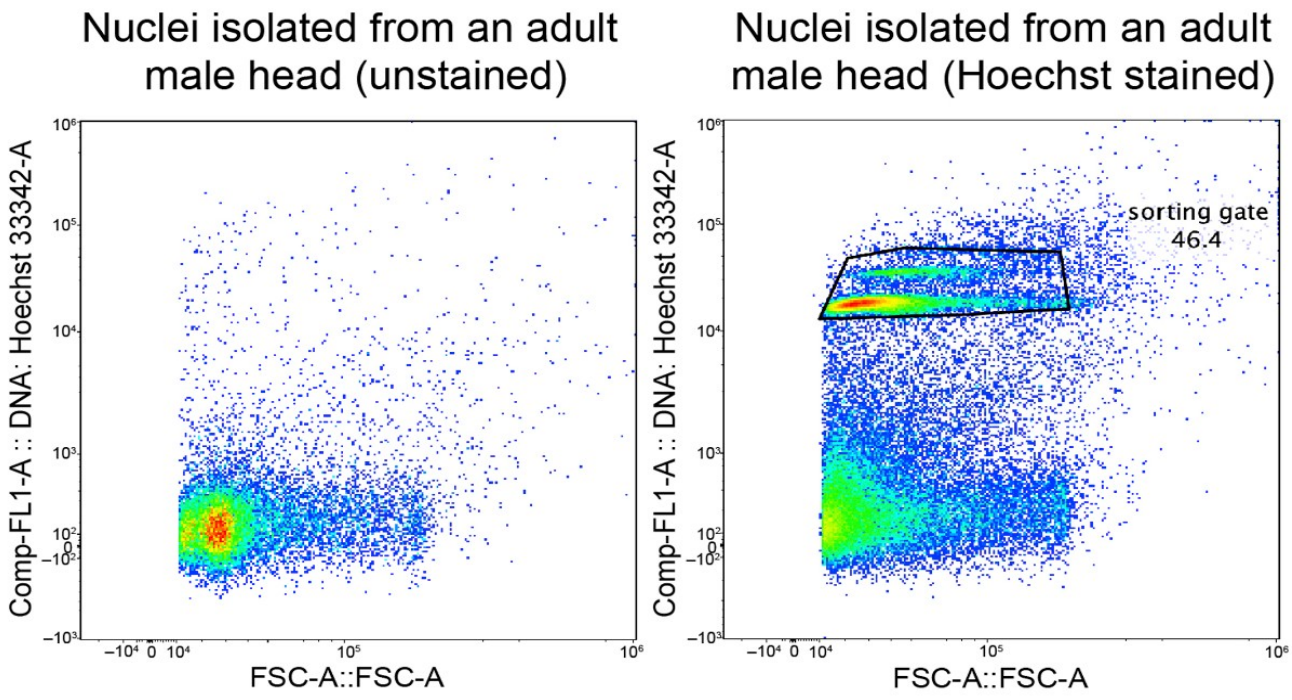
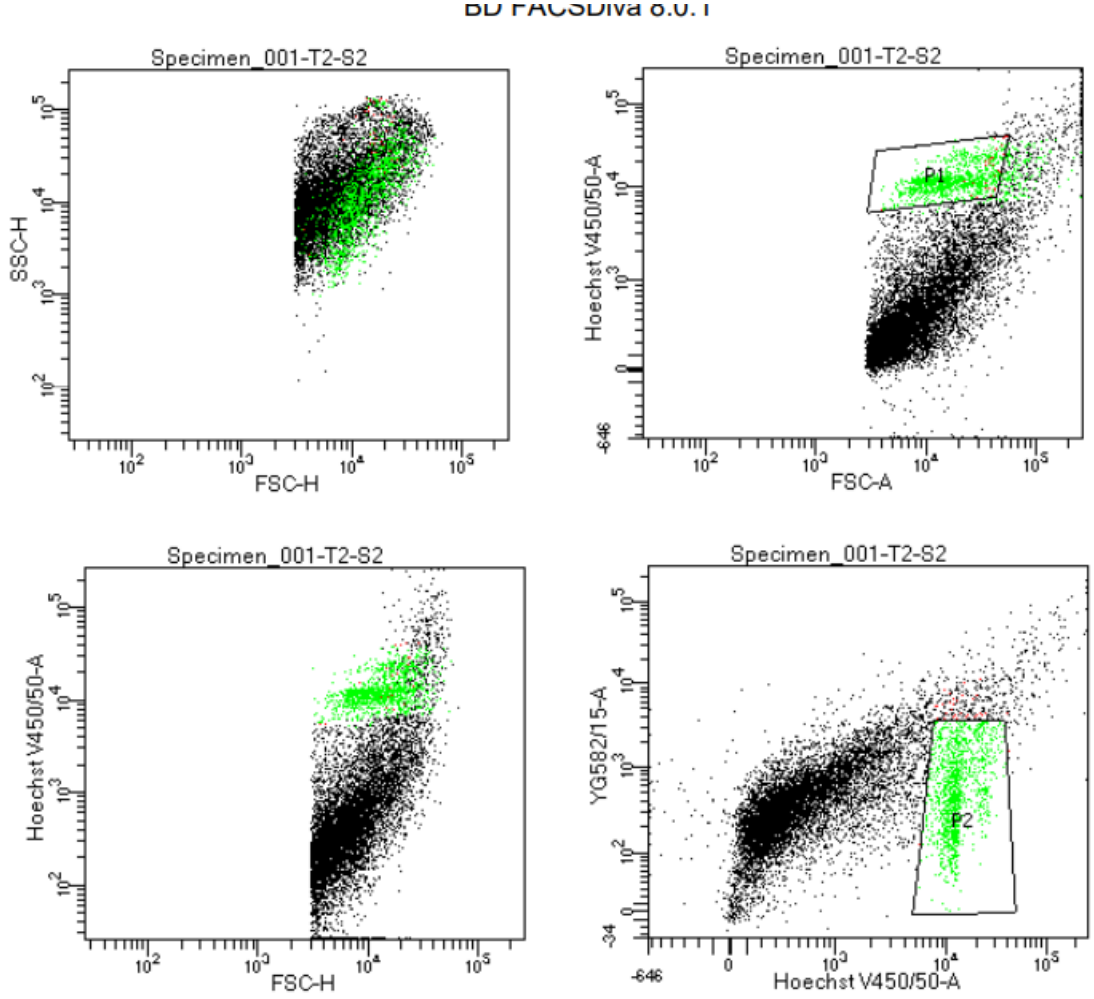


Figure 2. Representative FACS plots of nuclei from adult fly heads

Left: plot of unstained nuclei. Right: plot of nuclei stained with Hoechst. The two populations of nuclei in the polygon are sorted for snRNA-seq.

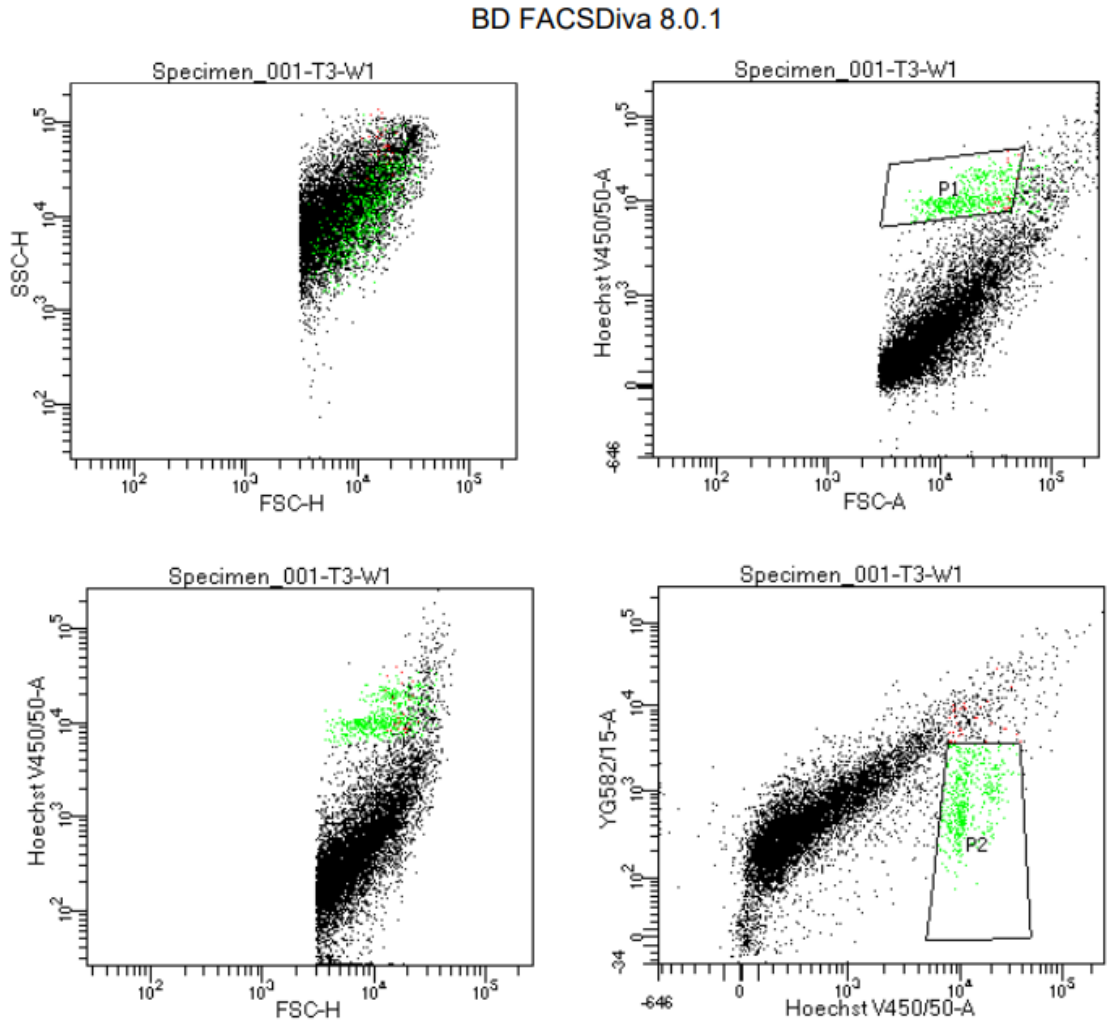
d. Tube 2 (soldiers), 06/06/2023



Tube: T2-S2

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	1,131	11.3	11.3
P2	1,276	12.8	12.8

e. Tube 3 (workers), 06/06/2023

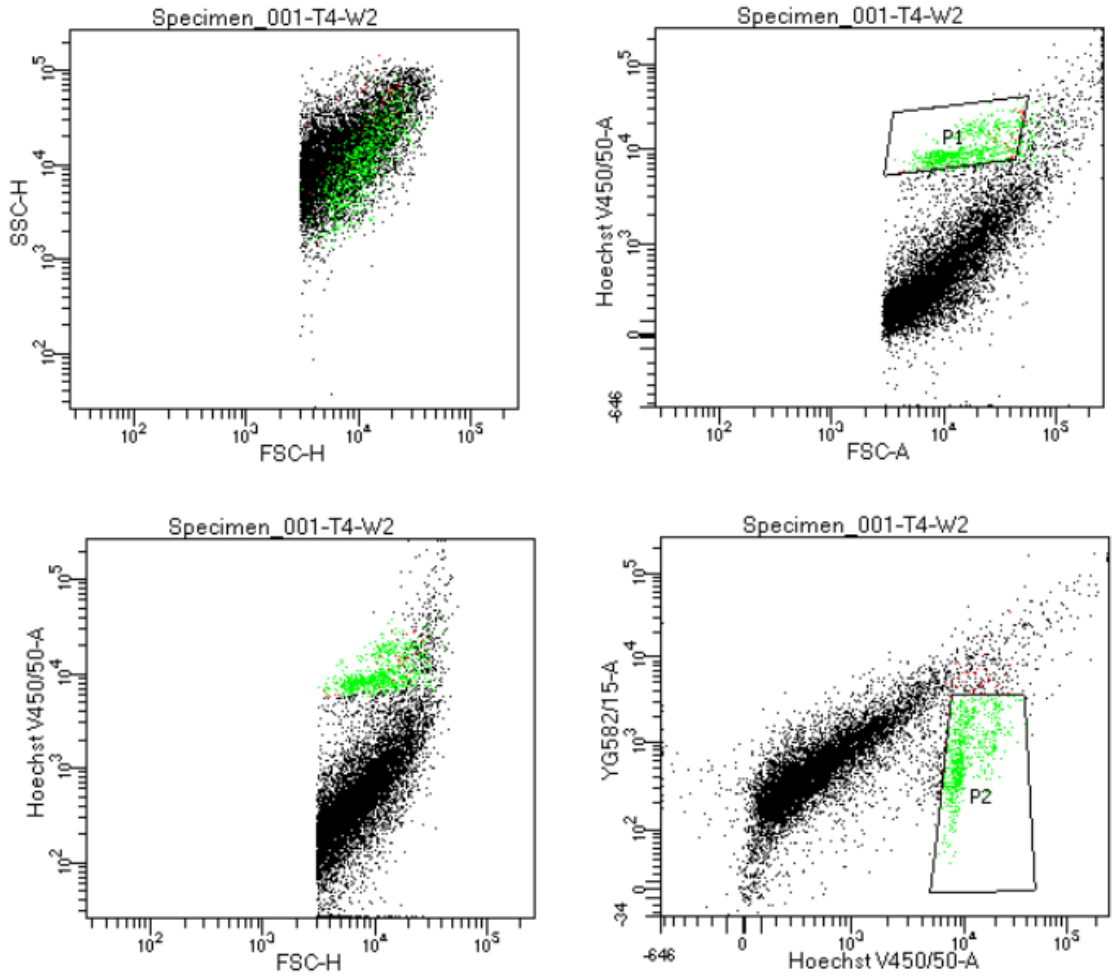


Tube: T3-W1

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	491	4.9	4.9
P2	516	5.2	5.2

f. Tube 4 (workers) 06/06/2023

BD FACSDiva 8.0.1



Tube: T4-W2

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
P1	673	6.7	6.7
P2	709	7.1	7.1