Moxi GO - No-Wash-Lyse Immuno-Labeling of Whole Blood

Overview:

This procedure applies to the immunolabeling (e.g. anti-CD marker) of white blood cells (WBC's or leukocytes) isolated from a peripheral whole blood sample by 1.) Labeling the sample with an appropriate antibody and 2.) Lysing the residual red blood cells (RBC's) with a no-wash lyse approach. The protocol has been optimized for running the assays on the Moxi GO instrument

Reagents:

- Purified Water (Milli Q or Distilled)
- BD FACS (10X) Lysing Solution (BD, Ref#249202)
- Cell Staining Buffer, E.g.
  - BioLegend Cat #420201

  OR
  - PBS + 0.5% BSA + 0.1% Azide
- Compatible fluorophore conjugated antibody
  - ***561nm/LP Filter (538nm or 488nm GO systems)*** - Phycoerythrin (PE/R-PE) labeled antibody (e.g. PE anti-human CD4 - BioLegend cat # 300507) – *Note: Substitute Antibodies that are compatible with 532nm excitation and 561nm/LP emission can also be used instead of PE.*
  - ***525/45nm Filter (488nm GO ONLY)*** – FITC or Alexa Fluor 488 labeled antibody
- Moxi Cyte Flow Reagent (Orflo MXA079/MXA080)

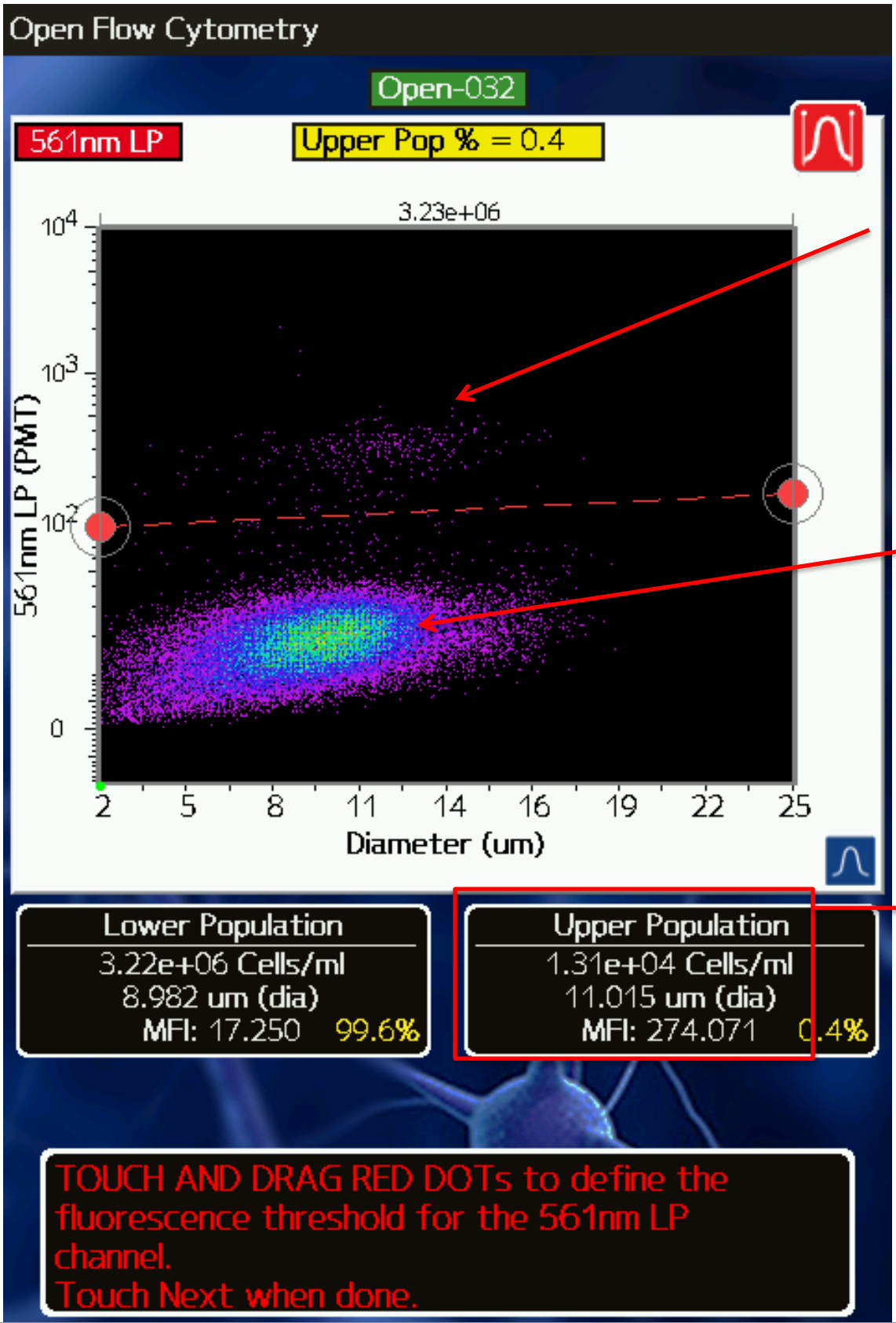
Pre-Prep:

- 1X Lyse Solution
  - Warm water and BD FACS Lysing Solution to room temperature
  - Dilute BD FACS lyse buffer to 1x, E.g.
    - Add 9ml of purified water to a 15mL centrifuge tube
    - Add 1ml of 10x BD FACS Lyse to the above tube
- Antibodies
  - It is recommended that, for a given manufacturer's antibody, an antibody concentration titration be performed with the protocol outlined below to determine the optimal concentration.
  - Recommended antibody concentrations on the Moxi GO are typically lower than the manufacturer-specified per-test recommendations when using a no-wash lyse approach to labeling.
  - When in doubt, 1µg/ml (final antibody concentration in blood) is a good target.
  - Measuring smaller quantities of Ab, might require dilution of stock Ab into staining buffer.

Protocol

1. Obtain a fresh blood sample into an anti-coagulant (e.g. Sodium Citrate or Heparin)-treated tube.

2. Pre-measure antibody/antibodies volume into micro-centrifuge tube (and label accordingly) – Note: Prepare Isotype and compensation controls as necessary (CellSimple Only)
3. Add 50µL of blood
4. Vortex gently (i.e. setting #3) to mix
5. Incubate for 15min (room temperature: 20-25°C, dark)
6. Add 450µL of BD FACS Lyse Reagent (Note: use 1X FACSLyse dilution as prepared above in the pre-prep section)
7. Vortex gently (i.e. setting #3) to mix
8. Incubate for 15min (room temperature: 20-25°C, dark)
9. Dilute 3x (minimum) in PBS (e.g. 1000µL Staining Buffer or PBS to 500µL of labeled/lysed blood)
10. Add 20µL/ml Moxi Cyte Flow Reagent and invert 10x to mix (e.g. add 30µL flow reagent to 1500µL PBS-diluted sample) – Note total dilution factor is 30.6x with above recommendations (assuming 1µl Ab test volume)
11. Analyze immediately with a Flow Cytometers, e.g. using the "Open Flow Cytometry" on the Moxi GO.
    a. Use "Open Flow Cytometry"
    b. Fluorescent gain = MEDIUM
    c. Example output for PE anti-human CD4 (OKT4) is shown below.



CD4+ Cells

Residual RBC's (mainly) + Leukocytes

Relevant Statistics