Imaging and Flow Cytometry Core

Cell-cell interaction

- **Imaging channels selection (for blue laser-responsive fluorochromes only):**

Image channel	Bandpass filter	Recommended dyes
IMG 1 (535)	534/46 (511-557)	FITC, BB515
IMG 2 (600)	600/60 (570-630)	PE, RB613, RB545
IMG 3 (790)	788/225 (676-900)	PE-Cy7, RB780, RB744

In the absence of imaging channels spectral unmixing, **IMG 1** and **IMG 3** are the recommended detectors to be used simultaneously.

- **Reference staining protocol for PBMC**

1. Dispense a 100 µL aliquot of PBMC suspension (approximately 10^6 cells per tube) into each labelled tube for single-color compensation controls for each fluorochrome, using the same antibody concentrations as the full stain panel.
2. Prepare FMO (Fluorescence Minus One) controls for the **IMG1** and **IMG3** imaging channels to facilitate proper gating.
3. Incubate all tubes for 20 minutes at room temperature, protected from light.
4. Wash the cells by adding 2 mL of stain buffer to each tube. Centrifuge at 1,500 rpm for 5 minutes.
5. Carefully aspirate the supernatant, avoiding the cell pellet. Vortex the pellet briefly and repeat the wash by adding another 2 mL of Stain Buffer.
6. Repeat centrifugation at 1,500 rpm for 5 minutes. Aspirate the supernatant, taking care not to disturb the pellet.
7. **For fixation:**
    a. Add 0.5 mL of cold (2–8 °C) 1% paraformaldehyde (PFA) in PBS solution to each tube and vortex gently.
    b. Store stained and fixed cells (and compensation beads, if applicable) at 2–8 °C, protected from light, for a minimum of 30 minutes and a maximum of 1 week prior to acquisition.
8. **For live cell analysis (if not fixing):**

   Resuspend the final pellet in 0.5 mL of PBS containing 1% FBS for acquisition.
9. Filter the sample using a 70 µm cell strainer with a 100 µm nozzle or using a 100 µm cell strainer with a 130 µm nozzle.

Aspects affect the adhesion between the cell-cell interaction

1. Vigorous pipetting up/down

2. RBC lysis buffer

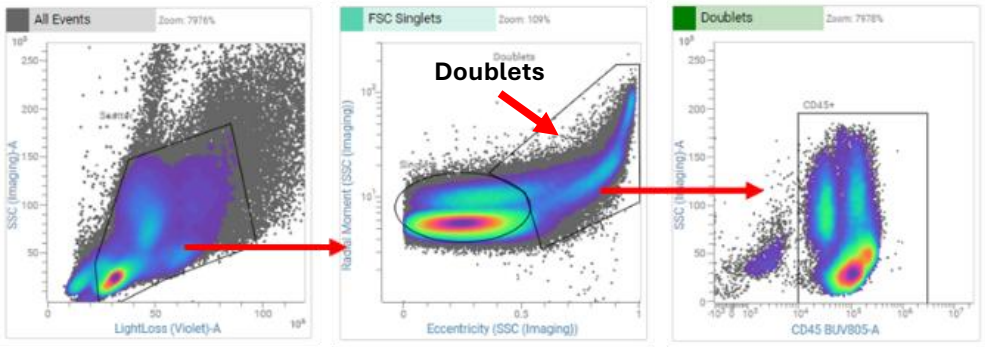
3. High EDTA concentrations

4. Fixation or not

- **Gating strategy from S8**
  (e.g.: interaction between CD3 T cells and CD19 B cells)

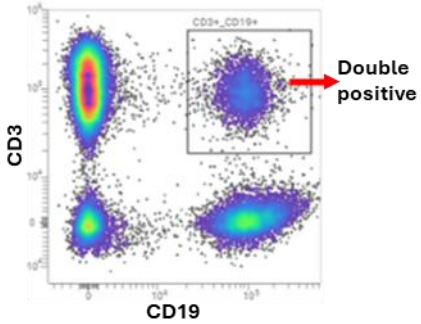
1. Gate the target population excluding the debris from SSC (imaging)-A *vs* Lightloss (Violet)-A

2. Gate the "**Doublets**" population with increasing Eccentricity and Radial Moment



3. (Optional) Gate the $CD45^+$ population from doublets.

4. Gate the double positive population, e.g.: $CD3^+CD19^+$



5. From the $CD3^{+}CD19^{+}$ population, set the plot with Correlation (CD3/CD19) *vs* Radial Moment (Lightloss (imaging)). The population with low correlation and low radial moment might be the cells with interactions.