

Cell Sorting Matching (CSM) Versus Other Gating Strategies

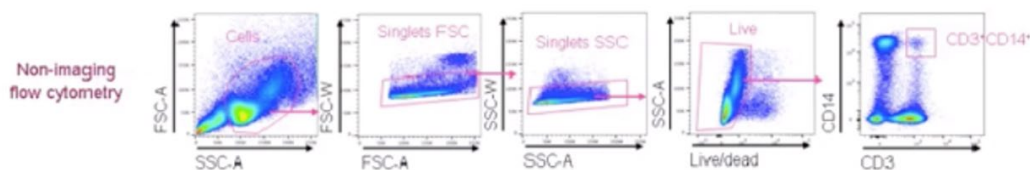
The gating strategy one uses can be important especially when attempting to compare data between two different technologies like non-imaging versus imaging flow cytometry. In the webinar “ImageStream® and the challenge of distinguishing cell-cell complexes from singlet cells in flow cytometry”, the cell sorting matching (CSM) gating strategy is found to be more similar to the traditional non-imaging flow cytometry gating strategy than the optimal imaging (OPT) gating strategy is. The OPT gating strategy has the best discrimination of doublets. However, if you wish to compare your data with data from a non-imaging cytometer, you should use the CSM gating strategy. With the CSM gating strategy, you can look for populations that may be cell-cell complexes that fail to be eliminated with the doublet discrimination steps used in the non-imaging flow cytometry gating strategy but are eliminated with the OPT gating strategy.

You can see instructions for the different gating strategies below including instructions for the traditional imaging gating strategy. This gating strategy was not included in the webinar and differs from the OPT gating strategy by gating on the focused cell population first using the Gradient Root Mean Squared (Gradient RMS) of BF instead of the cell population using the Bright Field (BF) area and SSC intensity.

A) Traditional Non-Imaging Flow Cytometry Gating Strategy

1. Create a FSC-A versus SSC-A plot and gate on the cells.
2. Create a FSC-A versus FSC-W plot (could also use a FSC-H versus FSC-W plot), choose to only show the cells gate on the plot, and gate on the single cells.
3. Create a SSC-A versus SSC-W plot (could also use a SSC-H versus SSC-W plot), choose to only show the FSC single cells gate on the plot, and gate on the single cells.
4. If applicable, create a live/dead stain-A versus SSC-A plot, choose to only show the SSC single cells gate on the plot, and gate on the live cells.
5. Continue with gating strategy necessary for individual experiment.

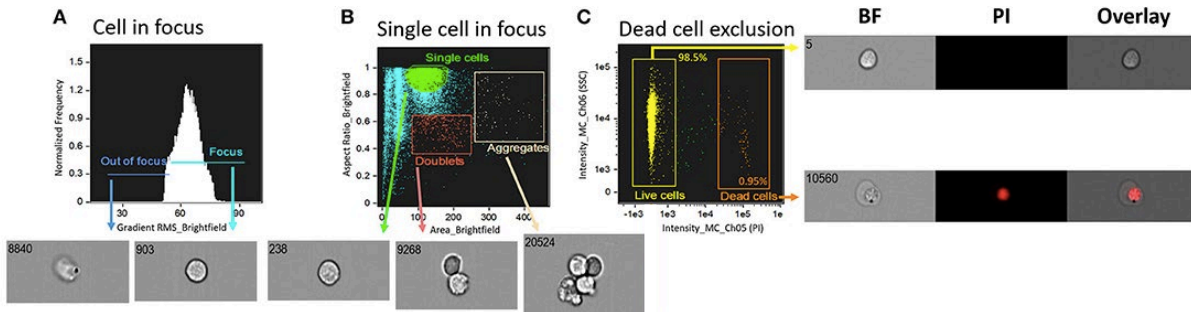
Note: You can see a double positive population (CD3+ CD14+). This population in fact contains doublets.



B) Traditional Imaging Gating Strategy

1. Create a 1-parameter histogram with Gradient Root Mean Squared (Gradient RMS) of Bright Field (BF). Gate on the focused cells.

2. Create a BF area versus BF aspect ratio plot. Only show the focused cells gate on the plot and gate on the single cells.
3. If applicable, create a live/dead stain intensity versus a SSC intensity plot (could also use a live/dead stain intensity versus a BF area plot). Choose to only show the single cells gate on the plot and gate on the live cells.
4. Continue with gating strategy necessary for individual experiment.

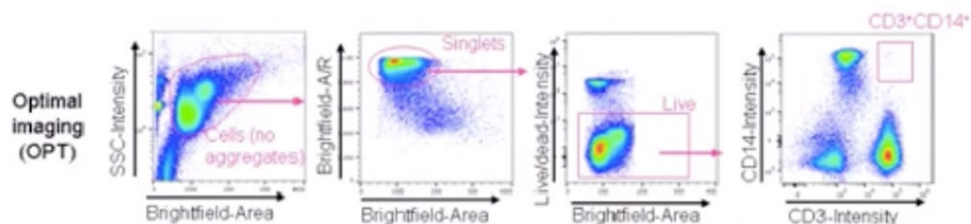


*Analysis wizards on the ImageStream will have you complete steps 1 and 2 as part of the different wizards.

C) Optimal Imaging (OPT) Gating Strategy

1. Must record SSC parameter when acquiring samples.
2. Create a Brightfield (BF) area versus SSC intensity plot. Gate on the cells and gate out the aggregates.
3. Create a BF area versus BF aspect ratio plot. Only show the cells gate on the plot and gate on the single cells.
4. If applicable, create a BF area versus live/dead stain intensity plot. Choose to only show the single cells gate on the plot and gate on the live cells.
5. Continue with gating strategy necessary for individual experiment.

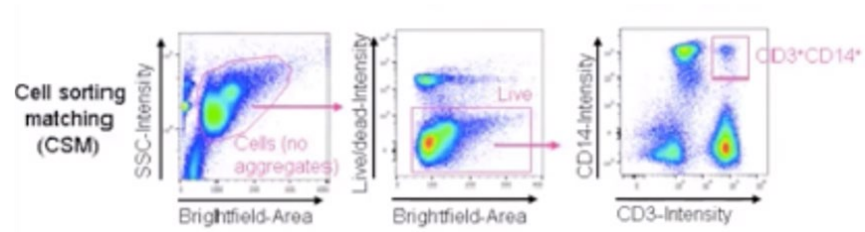
Note: You cannot see a double positive population (CD3+ CD14+).



D) Cell Sorting Matching (CSM) Gating Strategy

1. Must record SSC parameter when acquiring samples.
2. Create a Brightfield (BF) area versus SSC intensity plot. Gate on the cells and gate out the aggregates.
3. If applicable, create a BF area versus live/dead stain intensity plot. Choose to only show the cells gate on the plot and gate on the live cells.

4. Continue with gating strategy necessary for individual experiment.
Note: You can see a double positive population (CD3+ CD14+).



*Traditional non-imaging flow, OPT, and CSM gating strategy Images from webinar "ImageStream® and the challenge of distinguishing cell-cell complexes from singlet cells in flow cytometry" given by Julie Burel and originally aired on April 7, 2020.
**Traditional imaging gating strategy image from Figure 1 in article "Imaging Flow Cytometry Protocols for Examining Phagocytosis of Microplastics and Bioparticles by Immune Cells of Aquatic Animals" by Youngjin Park, Isabel S. Abihssira-García, Sebastian Thalmann, Geert F. Wiegertjes, Daniel R. Barreda, Pål A. Olsvik and Viswanath Kiron and published in Front. Immunol., 18 February 2020.