Cyto 2019
Program: #314
Board: #B176

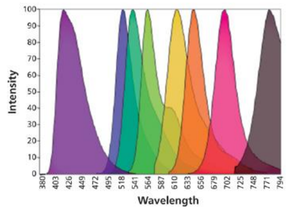
Comparing Fluorescent Flow and Mass Cytometry Optimization and Implementation Workflows

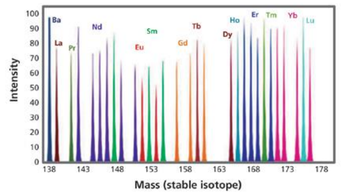
Terry Wightman[1], Julia Houk[1], Ravi Misra[2], Meghann O'Brien[1], Gloria Pryhuber[2], Matthew Cochran[1,2], Wojciech Wojciechowski[1,2], and Timothy Bushnell[1,2]
1. Flow Cytometry Shared Resource Laboratory, University of Rochester Medical Center, Rochester, NY 14642
2. Department of Pediatrics, University of Rochester, Rochester, NY, USA.

URMC Shared Resource Laboratories

Introduction

Flow cytometry is a powerful tool for the examination of cells and biological processes at the whole cell level. Traditional fluorescent flow cytometry (FFC) relies on fluorescent markers as a reporter. These can be limited due to cellular properties (autofluorescence), availability of fluorochromes and compensation related issues. The development of time of flight cytometry, (mass cytometry [MC], or 'CyTOF') sought to eliminate some of the limitations of FFC while expanding the number of parameters that can be measured simultaneously.





The URMC FCR sought to compare the two techniques in practical terms such as time (preparation of sample and acquisition), cell recovery, and cost of experiments (reagents, staff and instrument costs) to help guide our userbase in choosing which technique was best for their needs.

For this work we partnered with the LungMAP project to help them achieve their goal of maximizing the information learned from precious lung samples. To create a single 32 marker MC panel we took an existing 17 marker FFC panel and combined it with 2 additional panels including a number of "wish list" markers that had not previously been acquired for this project due to the rare nature of these samples. Overall, while there were differences in the processing and instrument time for these two techniques, the total time was similar. Likewise, there were differences in the cell recovery between the two techniques, but overall the end recoveries were approximately equal. The outcome of this work is that the LungMAP project is going forward with a 32 marker MC panel for future work which includes all of the desired targets previously not accessible.

In the end, with all other factors being equal, the final recommendation for which technique to use will be based on the sample scarcity, autofluorescent issues, and target molecules.

Workflow

Overall, the workflows for FFC and MC are very similar. Cells are processed and stained before sample acquisition. Since in MC acquisition, traditional scatter parameters are lost, the use of the intercalators 191Ir and 193Ir are used to identify cell clouds. Even with this extra step, processing of MC samples takes about 50% less time than the equivalent three FFC panels.

Sample acquisition of the three FFC panels takes approximately twice as long as for the MC panel. This is due, in part, to the multiple tubes for compensation that must be collected.

It should be noted that the quality of the MC sample cleanliness is critical parameter that those new to the technology need to keep in mind. Since any antibody that is not washed away will be detected by the CyTOF, investigators must be more vigilant with the sample processing.

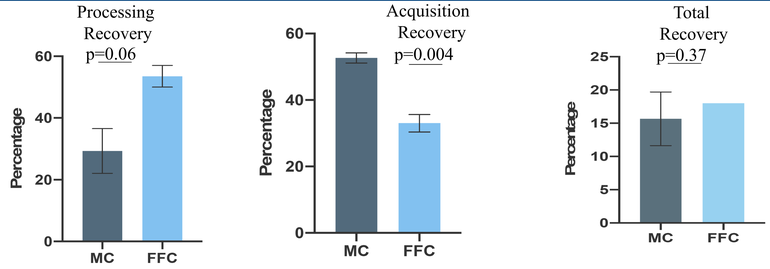
Efforts not included in the experimental workflow include:

FFC
- Panel design – more complex
  - 3 panels with overlapping markers
  - TH1 (17), TH2 (15), EOS (12)
- Antibody titration - 2 days
- Voltage optimization – 2 days

MC
- Panel design – easy
  - 1 panels (32 parameters)
- Custom conjugations ~1 day
- Custom titration ~ 1 day

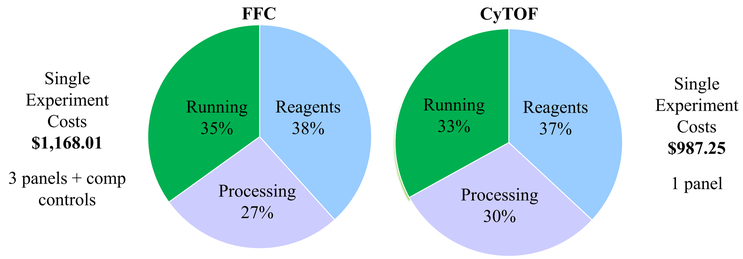
Note: These may be one time efforts depending on the scale of the project so are not considered recurring costs.

Recovery



Results of cell recovery experiments (n=3) are shown above. For processing recovery calculations, cells were counted before and sample processing to determine the percentage recovery. The run recovery was based on the number of recorded cells divided by the number of input cells . The overall recovery was the product of these two numbers. While there were significant differences between the recoveries at the two steps, overall there was not a significant difference in total recovery observed. (unpaired T-test, α=0.05 using GraphPad Prism 8.1.1)

Costs



One consideration in deciding between FFC and MC is the cost to perform each experiment. To determine the comparable costs, three variables were captured:

- Reagent Costs – the cost for antibodies and specialized reagents for each assay
- Processing costs – the cost of standard reagents, laboratory supplies and technician time to label samples
- Acquisition costs – the cost for acquisition of the data including technician time.

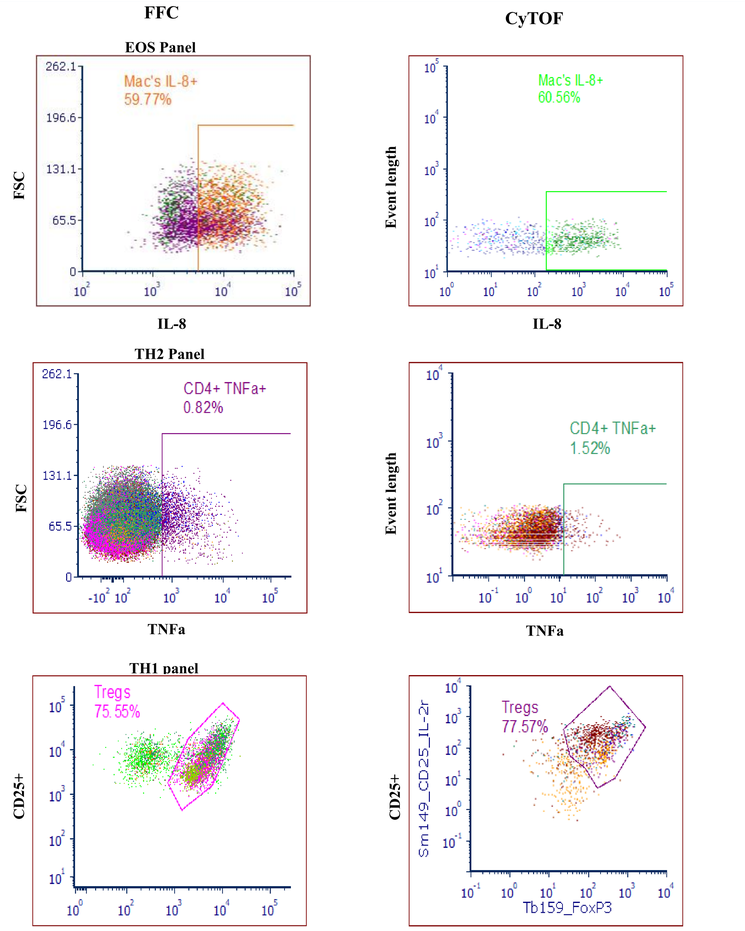
All rates were based on full cost (i.e. no discounts) and the published internal rates for the URMC FCR.

As can be seen from the two pie charts, each of these variables contributes approximately the same percentage to the total cost of the experiment. FFC is slightly higher cost than MC, due in part, to the longer instrument time and additional samples (compensation controls) that must be captured for proper analysis.

Note: these costs do not include FMO controls which may be required for proper data interpretation, depending on the panel.

	Block	L/D	Surface Stain	Fix/Perm	Intracellular Stain	IR Staining	Sample Acquisition
FFC							
Processing Time	20 min	20 min	25 min	20 min	35 min	0 min	
Incubation Time	16 min	36 min	86 min	42 min	92 min	0 min	240 min
Total Time for 3 Panels							632 min
MC							
Processing Time	6 min	6 min	9 min	8 min	13 min	11 min	
Incubation Time	16 min	11 min	86 min	42 min	92 min	61 min	120 min
Total Time for 1 panel							481 min

Example Plots



Manual gating was performed in FCS Express v6 following a standard gating protocol including removing doublets, flow anomalies, and dead cells. Gates were set based on an unstimulated control. Similar populations were identified between the one MC panel and the three FFC panels.

Conclusions

The focus of this project was to compare FFC and MC for the LungMap project, which has as its goal maximizing the information learned from precious lung samples. While the workflows between FFC and MC are similar, MC has several advantages and is the correct choice for the LungMAP project:

- The ability to increase the number of parameters while not sacrificing detection ability thus obtaining more data and relationships with a single sample rather than splitting a precious tissue sample into multiple panels.
- Autofluorescence, which is a concern in the lung tissue, is not a factor in MC making it especially good for the intracellular targeting needed.
- MC is slightly less expensive and time consuming in this case.
- While not considered for this project, barcoding in MC can be beneficial and new developments will offer flexibility and the opportunity for this technique to apply to a greater variety of applications.
- This work shows that MC is comparable to FFC and in some cases can be preferable.

Acknowledgements

This Poster was supported NHLBI grant U01-HL-122700 (G. Deutsch, T. Mariana and G. Pryhuber) and Institutional Support of the Flow Cytometry Shared Resource