Flow Cytometry Lab Meeting at Rutgers

Meeting Date: 12/06/2022

Flow Cytometry Lab:

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Meeting Minutes and

1. We discussed gating strategies. We asked Dr. Cervelli if she creates gates using a single sample or data pulled from multiple/all samples and then applies them to all samples. Jessica said sometimes she would use a single control sample to create gates and then apply them to other samples but more often she would have to draw different gates for each sample (within the same experiment). Davit noted that this would complicate the comparison as we would be comparing different subpopulations. Dr. Rancourt explained that applying the same gating templates to all samples might not work because characteristics of the same cell type (say, macrophages) can be vastly different from person to person. For example, the size of macrophages can be many times different in people living near pollutants compared to people who live in cleaner environments. NOTE: Javier thinks that this might be at least in part resolved by normalizing the readouts.
2. We asked Dr. Cervelli about the fluorochromes (dyes), specifically, if they order the fluorochromes for specific proteins or if they bind dyes with antibodies on their own. Dr. Rancourt said that it is possible but technically challenging and many things can go wrong. Instead, companies that have fluorochromes have selections of colors for each protein, i.e., fluorochromes are ordered already bonded to antigens. E.g., if you want to mark CD45+ protein (surface marker for immune cells), you will go to the supplier's website and select a color for the CD45+ that you want. NOTE: Dr. Cervelli explained that she chooses the colors so that they are maximally separated (dissimilar), and no confusion arises. We need to learn more about it.
3. Dr. Cervelli shared several links to flow cytometry analytical tools and suppliers' websites that list fluorochromes etc. Both, Dr. Cervelli and Dr. Rancourt offered their support so we can ask them for additional resources (but we should do our own lit search first). They are using [Kaluza](http://kaluza/) software for .FCS file processing and visualization but [FlowJo](https://nam02.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.bdbiosciences.com%2Fen-us%2Fproducts%2Fsoftware%2Fflowjo-v10-software&data=05%7C01%7Cmd1518%40stat.rutgers.edu%7Cdc119b86e33c41ad695e08dad8dae942%7Cb92d2b234d35447093ff69aca6632ffe%7C1%7C0%7C638060731975970475%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C3000%7C%7C%7C&sdata=cqEOScI0FuCTuMjpfqbE1PylE%2BCdVs3laRzyXFXEpw4%3D&reserved=0) is another popular choice. There are also some open-source alternatives (we need to research that).
4. For forward scatter and side scatter, height represents the peak of the signal, width represents the time it takes to go in and out, and area is the area under the curve.
5. Doublets can be bound together in any shape, vertical, horizontal, or any other direction.
6. We would always need to exclude doublets from the data, as well as debris. That is also mostly the case for dead cells unless they are the cells of interest.
7. Dyes are chosen based on the experiment. There are many factors that need to be considered prior to choosing the dyes, specifically when we have multiple channels so that the overlap(spillover) is minimized.
8. Gating is always arbitrary, there is no specific threshold for the process.
9. The dataset contains controlled values as well. They provide the data for each fluorochrome as well as all combined, and that is how they can know about the spillover and do the compensation process.
10. Dr. Cervelli thought we should not get the negative values, but Dr. Rancourt said it is possible at times after doing the compensation (confirming what Mahan read online regarding negative values).
11. It is possible for the experiment to be not well designed, and as a result, we get a dataset that is not meaningful for analysis (the data might contain too much debris for example). When the gating is being done manually such cases can be recognized and taken care of accordingly. That might be a struggle for automated gating strategies.
12. Dr. Rancourt and Dr. Cervelli suggest performing the analysis on a basic dataset where no complication is present and grow it from there.
13. ***Backgating*** is the process when after the gating is done, they check on the original graphs (prior to gating) where their gated cells appear.

At this point, we have a lot of background information and a lot of flow cytometry sites. We propose to do the following:

1. Write a comprehensive review paper on the flow data analysis breaking it down by instruments, assay type (e.g., phenotyping, apoptosis, etc.), and analytical tools. Write pros and cons and identify gaps. This should lead to:
2. Paper on the new method to identify differentially populated regions, i.e., everything we discussed so far.