

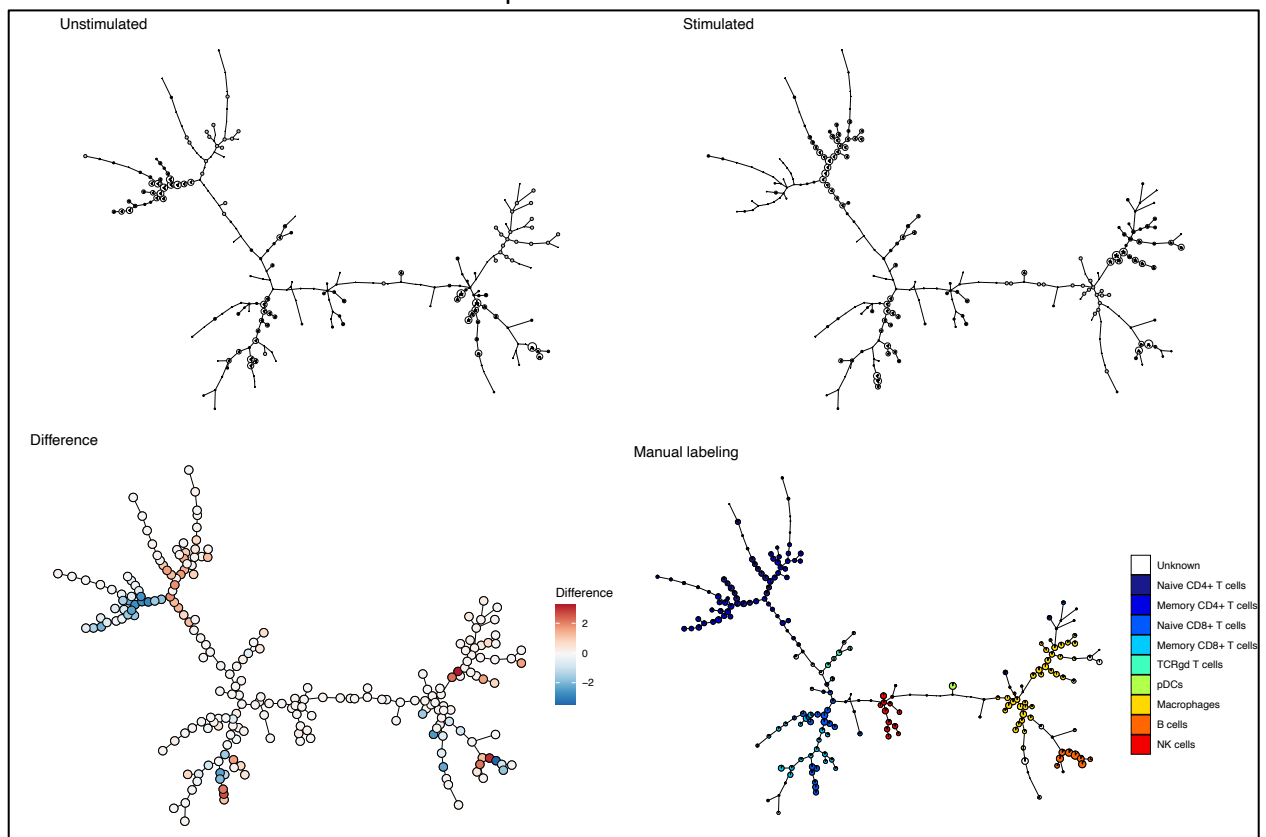
## Additional Exercise

### Data background

This dataset was used in the CytoNorm paper (<https://doi.org/10.1002/cyto.a.23904>). These are whole blood samples from 2 healthy volunteers, unstimulated or stimulated with IFN $\alpha$  and LPS. Today, we will look at the effect of the stimulation on the immune profile.

### End goal

- A plot with two FlowSOM trees where we can see the difference between unstimulated and stimulated of one patient.
- A plot where the color of the FlowSOM tree corresponds to the difference between unstimulated and stimulated for once patient.



- The fourth plot, showing the manual labeling, is not the objective of this exercise. The code to obtain this figure will be provided afterwards.

### Steps

- Create directories to store preprocessed data, quality control results, RDS objects and results. Be careful to not overwrite any previous objects.
- Channels of interest are already listed in the R script
- The data is located in Data/AdditionalExercise
- Since we are working with mass cytometry now, there are some implications for the preprocessing:

- Do not remove the margin events
- There is no need to compensate the data
- Apply an arcsinh transformation (instead of a logicle in the workshop). Use a cofactor of 5 ( $b = 1/5$ ).  
Hint: look at the `arcsinhTransform` function and use this to compute your `transformList` object.
- In PeacoQC, the `remove_zeros` argument is recommended to be set to `TRUE` for mass cytometry data.
- Make use of the manual gating to select the CD45+ CD66- events. The lines of code to do this, are already in the script.
- Use all four FCS files to compute a FlowSOM object with 225 clusters and 15 meta-clusters
- Investigate your FlowSOM object with for example the `FlowSOMmary`
- Now, compare the stimulated and unstimulated file for one patient. Do this by mapping the file to the FlowSOM model (Hint: look at the `NewData` function). Afterwards, retrieve the cluster percentages per newly computed FlowSOM tree and use these to calculate the difference between the two stimulation conditions.
- Visualize your results, e.g. with the `PlotStars` and `PlotVariable` functions (hint: in `PlotStars`, you can explore using `list_insteadof_ggarrange = TRUE` if you'd like to combine multiple plots yourself without repeating the legend)