Further instructions for protocol step 3.4.1:

1. Convert flow cytometric raw data into the CLOCCS input format

2. The CLOCCS\_alignment conda environment should be activated already. If not activate the conda environment by entering the following command in the terminal:

> conda activate CLOCCS\_Alignment

3. Open the conversion notebook by typing the following commands in the terminal in the cloccs\_alignment repo folder:

> jupyter notebook CLOCCS\Convert\_FCS\_CLOCCS.ipynb

4. Run the first cell to load the utilities files.

5. The notebook contains two examples for condition 1 and condition 2. To perform the conversion on user data, run the function flow\_cytometry\_CLOCCS\_file\_from\_fcs, but substituting own user input information as described below.

flow\_cytometry\_CLOCCS\_file\_from\_fcs(name, input\_raw\_dir, channel, output\_file\_path = None, cleaned\_lower=None, cleaned\_upper = None)

5.1. Substitute a string name for the output file for name

5.2. Substitute a string path to the input directory containing the .fcs files for input\_raw\_dir

5.3. Substitute a string of the channel to be used for the CLOCCS output file for channel

5.4. Optionally, set output\_file\_path to a string containing the desired output path for the output file. If set to None, the file will generate in the current folder.

5.5. Optionally set the lower limit for cleaning. If none, no lower limit will be set. If set to a value, points below the value will be set to zero for cleaning and an additional cleaned output file will be generated.

5.6. Optionally set the upper limit for cleaning. If none, no upper limit will be set. If set to a value, points above the value will be set to zero for cleaning and an additional cleaned output file will be generated.