**READ ME   
COSMX ANALYSIS- 3244**

**All files can be found in the directory: /export/jessie1/ac715y/3244**

**The analysis begins with the standard workflow including:**

1. Meta data parsing
2. Clustering
3. Dimensionality reduction
4. Integration
5. Automated annotation with single R
6. Manual annotation

Files:

1. ‘seurat\_obj\_integrated.rds’
2. ‘cosmx\_plots’ (umaps, heatmap, spatial plots)
3. ‘markers.csv’
4. ‘cosmx\_standard\_workflow.R’

**Pseudobulk analysis per cell type (‘Top Marker for Cell Typing’):**

1. ‘pseudobulk\_final.R’
2. ‘pseudobulk\_functions.R’
3. SearchLight2 results for each cell type

**/export/jessie1/ac715y/3244/tsv\_output:**

1. Contains all cell type pseudobulk matrices and the corresponding DE tables as well as the sample sheet.
2. It also contains the top markers.tsv

**I attempted to automate running searchlight for all cell types at once using snakemake:**

Recreate the environment using:

cd **/export/jessie1/ac715y/3244/Searchlight2/software**

conda env create -f environment.yml

conda activate snakemake

**/export/jessie1/ac715y/3244/Searchlight2/software/config.yaml**

**/export/jessie1/ac715y/3244/ Searchlight2/software/Snakefile\_1**

**snakemake -s Snakefile\_1**

**Selecting regions of interest:**

\*Since there were two replicates for each fuso\_pos sample, I separated all 28 samples using C1, C2, C3 identifiers in the format - sampleID\_C1/2/3):

C1- fuso\_neg

C2- fuso\_pos

C3-fuso\_pos

Files needed:

* centroids1.csv
* selector.py
* draw\_circ.py

1. Run the draw\_circ.py script.

Since the centroids represent the position of the center of mass of each segmented cell, the x\_max and y\_max are just the max values of X and Y and the center\_x and center\_y is computed by calculating the mean of X and Y as the mean represents the location of the center of mass of the entire distribution of cells.

1. The script will automatically save the cells in each circle excluding the previous to separate files per sample, so all that needs to be specified is the sample directory.

Example:  
C:/Users/AakankshaChoudhary/OneDrive/Desktop/cosmx/INC0046\_C2/my\_regions