Cell Area for 10x

Pipeline logic

1. Roughly recognize what is a signal coming from cardiomyocytes and use it for mask (Primary object: `cells`)

2. Identify nuclei in the sarcomere signal masked image (Primary object: `CM_nuclei`)
3. Merge close-by nuclei as one object (SplitOrMergeObjects: `CM_nuclei_merged`)
4. Segment sarcomere signal channel into cells based on the merged nuclei (Secondary object: `cells_final`)
5. Now there are 3 different objects

- nucleus (Primary object)
- cytoplasm (Tertiary object)
- cell (Secondary object)

7. Measure YAP1 signal in nucleus, cytoplasm, and cell
8. Save image for checking cell segmentation:

- Left: Sarcomeric signal overlayed with `CM_merged_nucleicells_final‘` outlines
- Right: YAP1 signal overlayed with `CM_merged_nuclei` and `cells_final` outlines
- object numbers

7. Save files with measurements, set that all measurements are exported

Reproducible structure of the project

1. `source` folder contains all the images
2. `output_<group-name>` folders where output from different pipelines go
3. `CP-<Pipeline-ID>_<group-specification>` one or more pipelines with clearly indicated what group was which pipeline used on

Pipeline Quality control

1. Go throgh the exported overlays and check for segmentation
2. If there are some really badly segmented cells (more then 5% per image) remove them from the measurment files

- create a filter file:
  - first column for image number, second column for object number to be removed
  - in case of all to be removed write `all` in second column
  - save and run the filterCP script on the files to remove the files

3. Add `QC-passed` to all `output` folders which were checked
4. Check the number of columns

- `cells_final.csv` (BJ last column)
- `CM_nuclei_merged.csv` (BL last column)

Post processing

1. Stack all the `cells_final.csv` and `CM_nuclei_merged.csv`

- `csvstack -g *cells*final*.csv > cells_final.csv`
- `csvstack -g *CM*merged*.csv > CM_nuclei_merged.csv`

2. Select the columns for intensities and ratios in the `CM_nuclei_merged.csv` and add them to the end of `cells_final.csv` `selectR -c 1,40,47,63 CM_nuclei_merged.csv`

3. Rename the columns to:
    - `YAP_nucleus_Int-Int`
    - `YAP_nucleus_Mean-Int`
    - `YAP_ratio_Int-Int`
    - `YAP_ratio_Mean-Int`
    - `scale_umperpx` (fill in 0.55 for all)
    - `Area_Scaled` (0.55*0.55* AreaShape_Area)
4. Create the columns for the experimental conditions and groups (new columns 2-4, manually fill in the values)

- `ExpNo`
- `ExpGroup`
- `Coating`

5. save as `data_final_all-cols.csv`

Data analysis

1. Select only the relevant columns from this file

```
selectR -c 1-4,7,6,42,49,69-72 data_final_all-cols.csv
```

2. rename to `data_final_selected-cols.csv`
3. Run descriptive statistics

```
summaryR -c 2-4 data_final_selected-cols.csv
```

4. Weed out the non-necessary columns

```
selectR -c 1-4,10-12,15-17,20-22,25-27,30-32,35-37,45-47 summary_*.csv
```

Save as `summary_statistics.csv`

5. In excel sort the files by ExpGroup, Coating, ExpNo, this way you can copy into the grouped graph

- select grouped graph
- replicates = 3
- Group A -fill `FN1`, Group B fill `FN10`
- In rows put `WT`, `mock`, `full-YAP`,`dPDZ`
- use `ctrl`+`shift`+`T` to transpose the values when copying

Example files

Pipeline (works on version 4.2.7)

pipeline

Output files

Output cells_final.csv file with measurment data

Post-Processing

Output cells_final.csv file with all images and objects

Example Filter file removes first object in image “1” and all objects in image “2”

Filter command

Cells_final.csv file after filtering

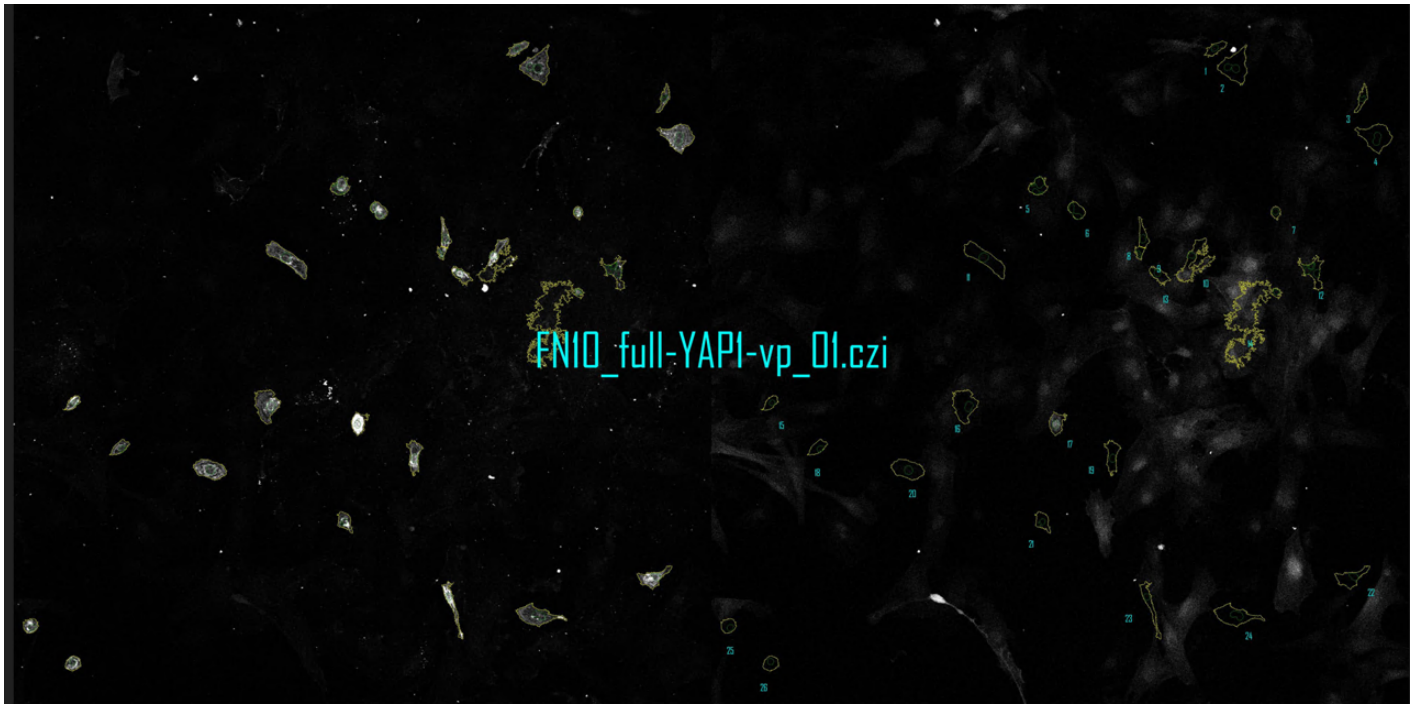


Figure 1: example-output-image_01

reexp-02_cells_final.csv - LibreOffice Calc

Soubor Úpravy Zobrazit Vložit Formát Styly List Data Nástroje Okno Nápověda

Liberation Sans 10 pt

A1 ImageNumber

	A	B	C	D	E
1	ImageNumber	ObjectNumber	FileName_channel_YAP1	FileName_channel_cytoskeleton	FileName_channel_mCh
2	1	1	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
3	1	2	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
4	1	3	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
5	1	4	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
6	1	5	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
7	1	6	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
8	1	7	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
9	1	8	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
10	1	9	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
11	1	10	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
12	1	11	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi

Figure 2: “Example: cells_final.csv file”

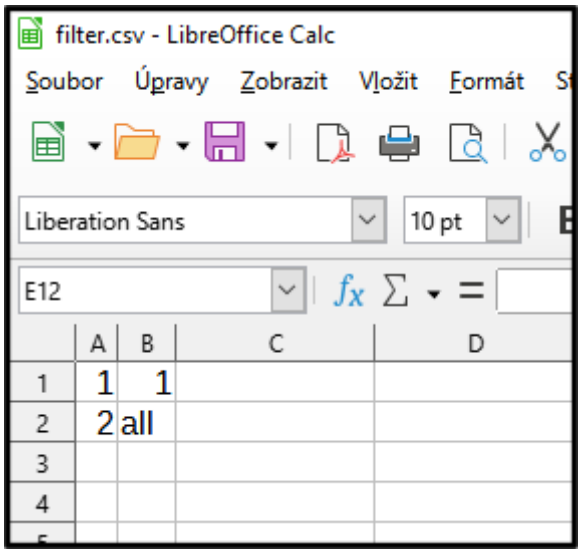


Figure 3: Example: filter.csv file

```
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A0
7_CM-area_10x_2025-05-13/output
$ filterCP filter.csv reexp-02_cells_final.csv
```

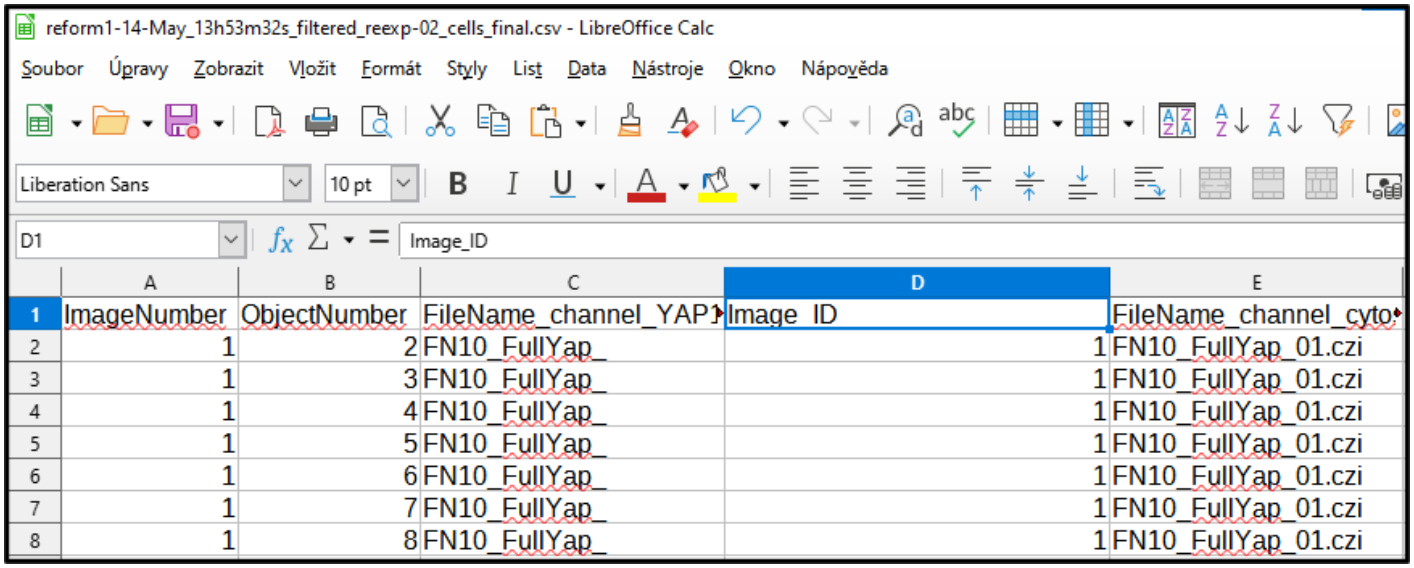
Figure 4: Example: filter command

This filter removes first object in image 1 and all objects in image 2

Split the name of the file into an experimental condition and image number

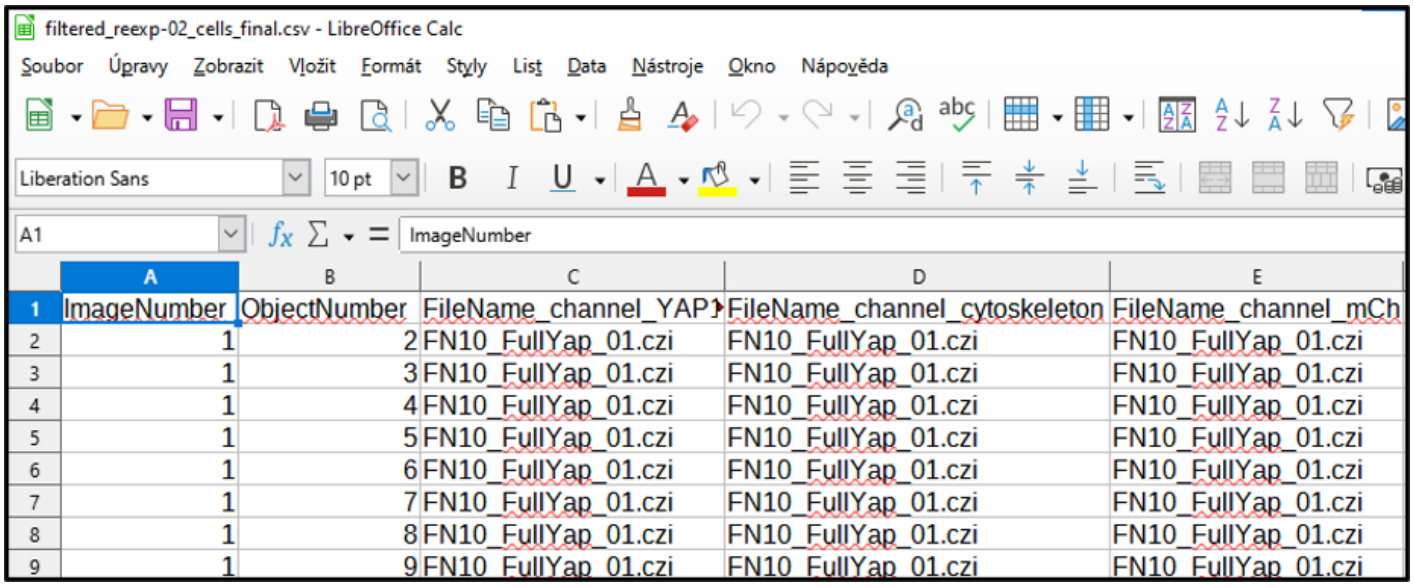
Output of the reformatting script

Add a new column name to the Image number split from the name



	A	B	C	D	E
1	ImageNumber	ObjectNumber	FileName_channel_YAP1	Image_ID	FileName_channel_cyto
2	1	2	FN10_FullYap_	1	FN10_FullYap_01.czi
3	1	3	FN10_FullYap_	1	FN10_FullYap_01.czi
4	1	4	FN10_FullYap_	1	FN10_FullYap_01.czi
5	1	5	FN10_FullYap_	1	FN10_FullYap_01.czi
6	1	6	FN10_FullYap_	1	FN10_FullYap_01.czi
7	1	7	FN10_FullYap_	1	FN10_FullYap_01.czi
8	1	8	FN10_FullYap_	1	FN10_FullYap_01.czi

Get the names of columns to select only the relevant ones using `csvcut -n <file>`


filtered_reexp-02_cells_final.csv - LibreOffice Calc

Soubor Úpravy Zobrazit Vložit Formát Styly List Data Nástroje Okno Nápověda

Liberation Sans 10 pt

A1 ImageNumber

	A	B	C	D	E
1	ImageNumber	ObjectNumber	FileName_channel_YAP1	FileName_channel_cytoskeleton	FileName_channel_mCh
2	1	2	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
3	1	3	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
4	1	4	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
5	1	5	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
6	1	6	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
7	1	7	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
8	1	8	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
9	1	9	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi

Figure 5: Example: cells_final before separating image number

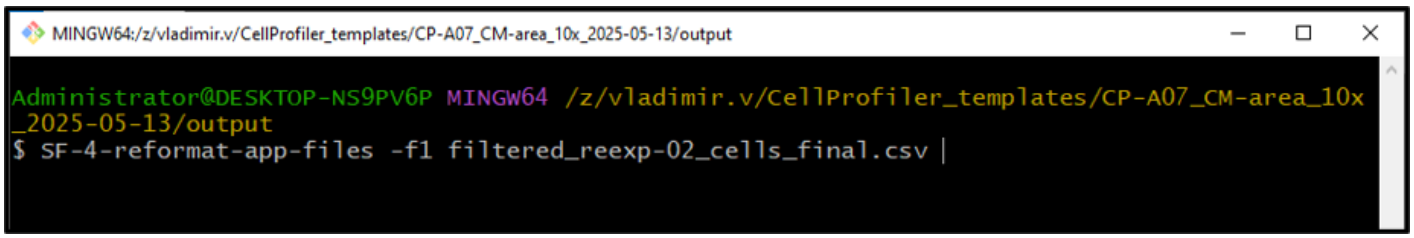
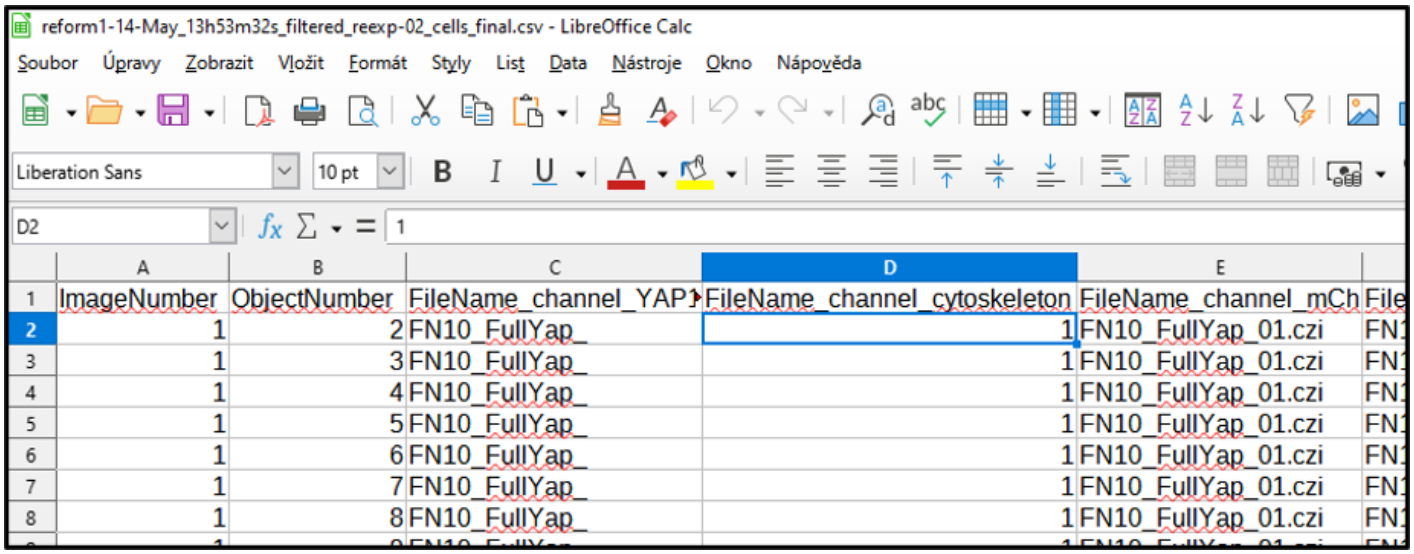


Figure 6: The script for separating image number from image name


reform1-14-May_13h53m32s_filtered_reexp-02_cells_final.csv - LibreOffice Calc

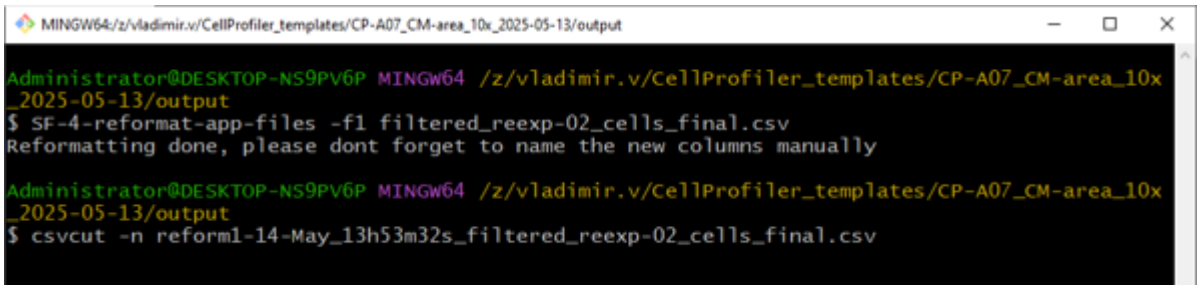
Soubor Úpravy Zobrazit Vložit Formát Styly List Data Nástroje Okno Nápověda

Liberation Sans 10 pt

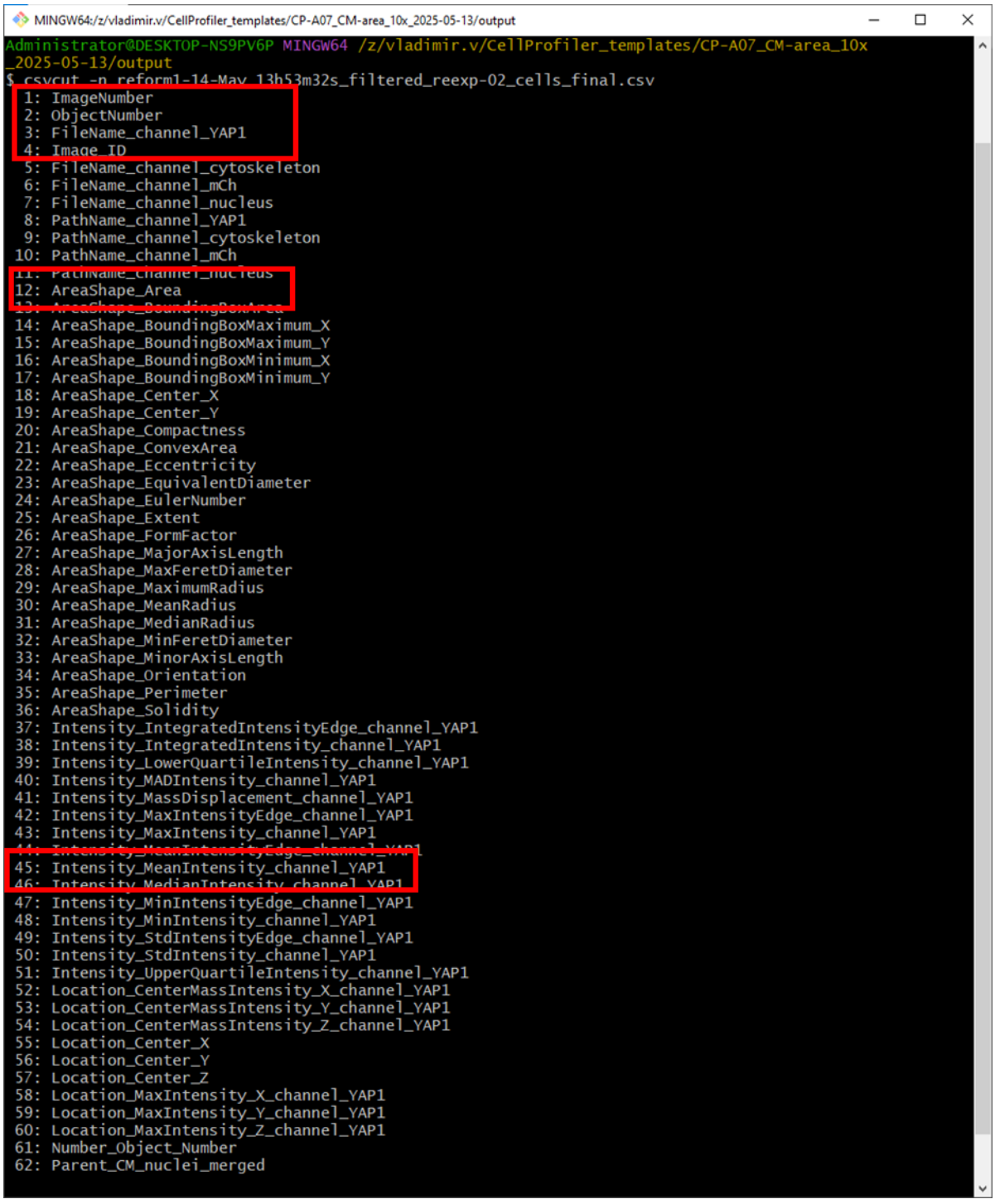
D2 1

	A	B	C	D	E	
1	ImageNumber	ObjectNumber	FileName_channel_YAP1	FileName_channel_cytoskeleton	FileName_channel_mCh	File
2	1	2	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
3	1	3	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
4	1	4	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
5	1	5	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
6	1	6	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
7	1	7	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1
8	1	8	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1

Figure 7: Example: cells_final.csv with new column with image number separated

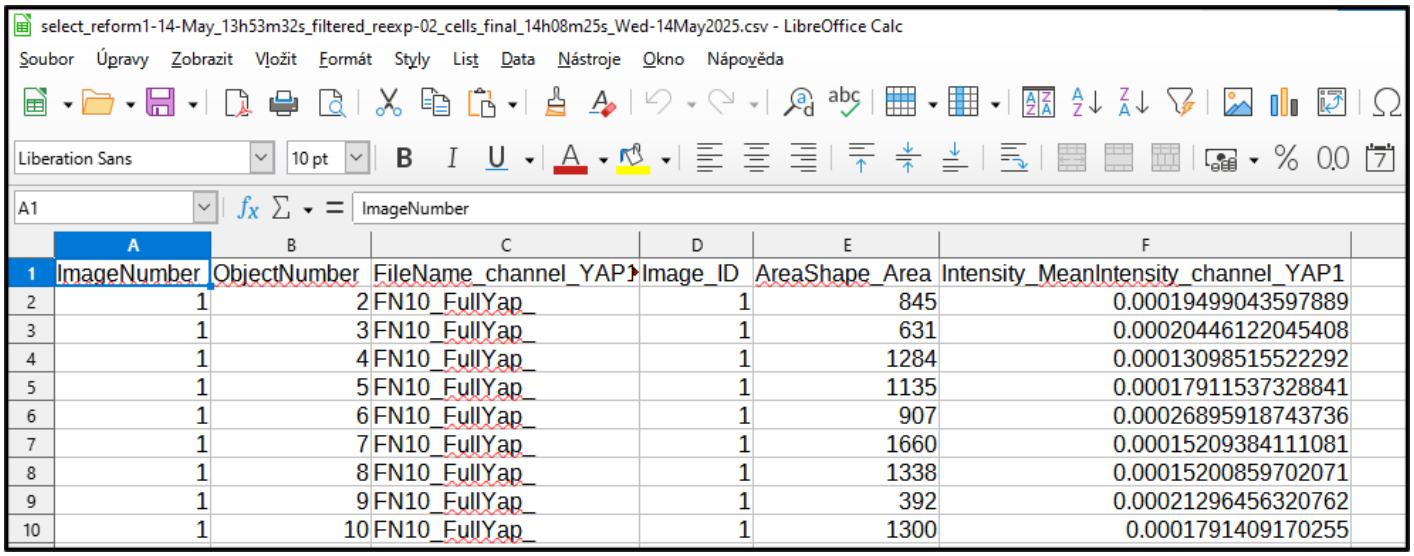


Decide which columns are relevant for further analysis (highlighted in red)



Save the relevant columns into a new file using `select.R -c N,M-O <file>`

Check the columns got selected


select_reform1-14-May_13h53m32s_filtered_reexp-02_cells_final_14h08m25s_Wed-14May2025.csv - LibreOffice Calc

Soubor Úpravy Zobrazit Vložit Formát Styly List Data Nástroje Okno Nápověda

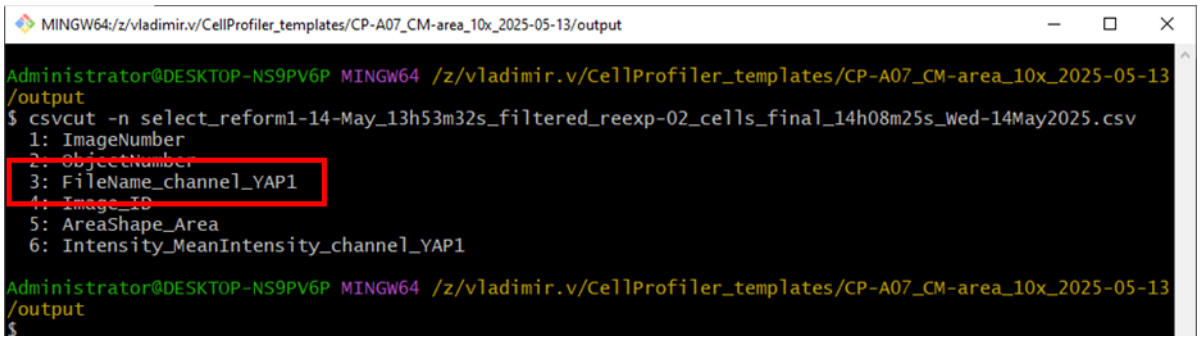
Liberation Sans 10 pt

A1 ImageNumber

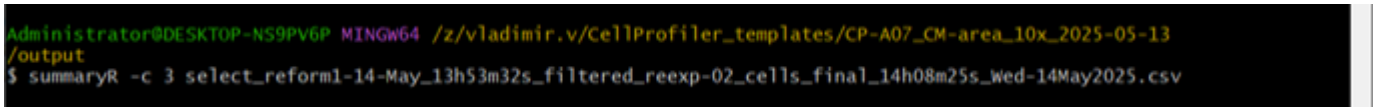
	A	B	C	D	E	F
1	ImageNumber	ObjectNumber	FileName_channel_YAP1	Image_ID	AreaShape_Area	Intensity_MeanIntensity_channel_YAP1
2	1	2	FN10_FullYap_	1	845	0.00019499043597889
3	1	3	FN10_FullYap_	1	631	0.00020446122045408
4	1	4	FN10_FullYap_	1	1284	0.00013098515522292
5	1	5	FN10_FullYap_	1	1135	0.00017911537328841
6	1	6	FN10_FullYap_	1	907	0.00026895918743736
7	1	7	FN10_FullYap_	1	1660	0.00015209384111081
8	1	8	FN10_FullYap_	1	1338	0.00015200859702071
9	1	9	FN10_FullYap_	1	392	0.00021296456320762
10	1	10	FN10_FullYap_	1	1300	0.0001791409170255

To get the final descriptive statistics (aka summary), select what columns describe the experimental condition (here it is the the column number 3)

First check the number of columns using `csvcut -n <file>`

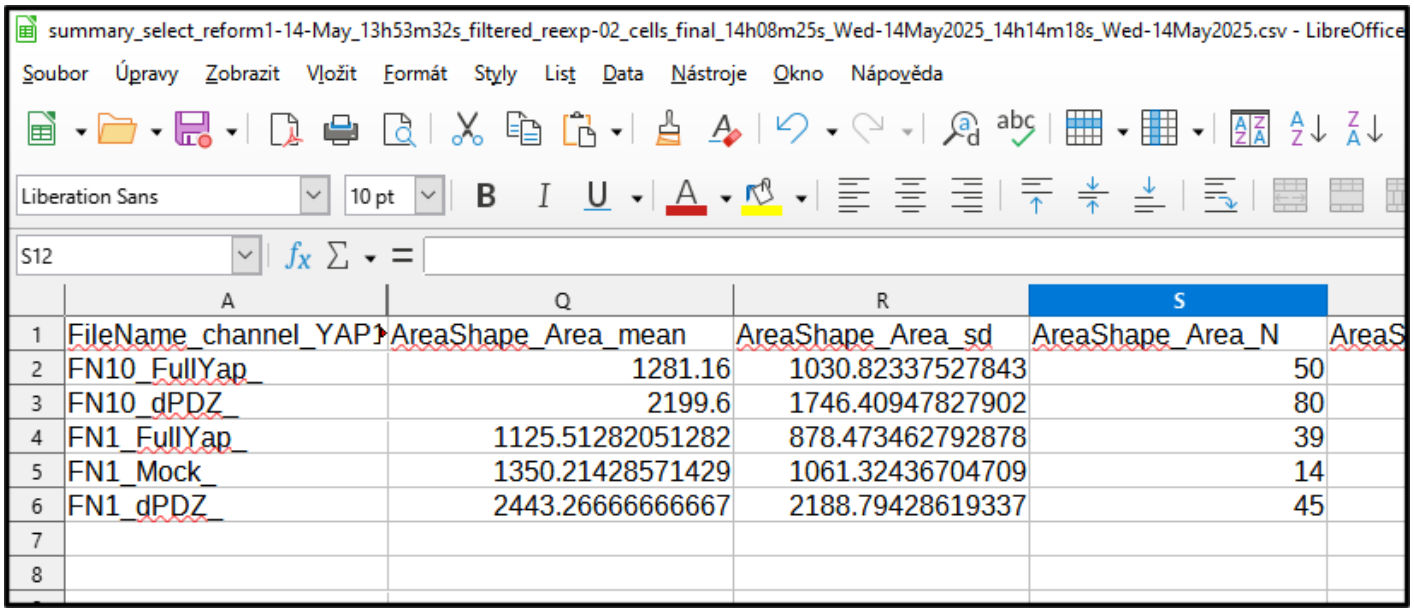


Make a summary statistics using the `summaryR -c <file>` targeting the third columns



The output file contains statistics for each numerical column

In first, last, mean, sd and N is calculated for each group


summary_select_reform1-14-May_13h53m32s_filtered_reexp-02_cells_final_14h08m25s_Wed-14May2025_14h14m18s_Wed-14May2025.csv - LibreOffice

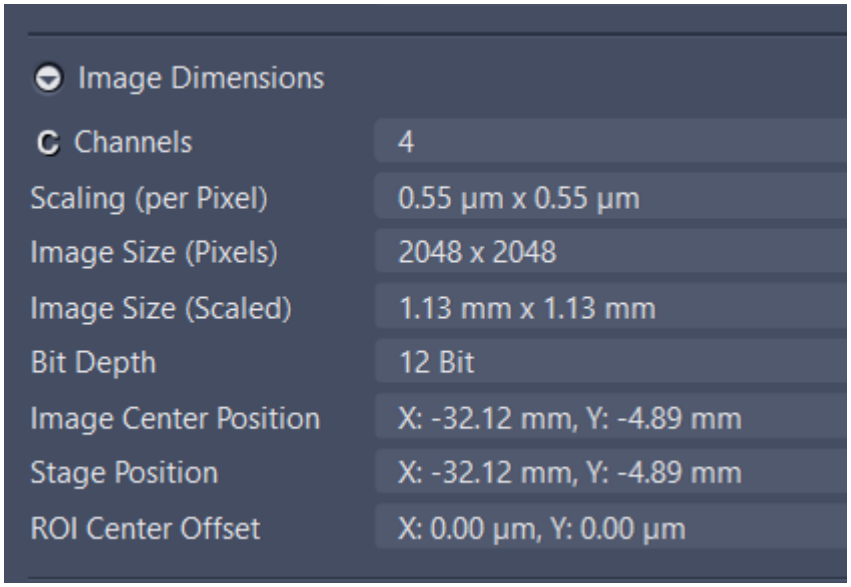
Soubor Úpravy Zobrazit Vložit Formát Styly List Data Nástroje Okno Nápověda

Liberation Sans 10 pt

S12

	A	Q	R	S
1	FileName_channel_YAP1	AreaShape_Area_mean	AreaShape_Area_sd	AreaShape_Area_N
2	FN10_FullYap_	1281.16	1030.82337527843	50
3	FN10_dPDZ_	2199.6	1746.40947827902	80
4	FN1_FullYap_	1125.51282051282	878.473462792878	39
5	FN1_Mock_	1350.21428571429	1061.32436704709	14
6	FN1_dPDZ_	2443.26666666667	2188.79428619337	45
7				
8				

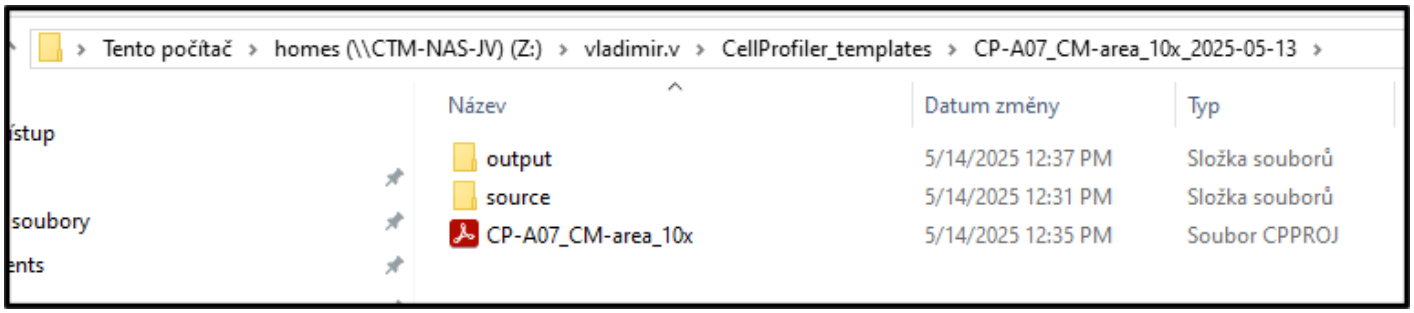
Acquisition setting



Resources

Cell Profiler Versions

Pipeline location on Leica computer



Tutorial on cell profiler