

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_nuclei` and `cells_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 through the exported overlays and check for segmentation
2. If there are some really badly segmented cells (more than 5% per image) remove them from the measurement 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 (0.550.55 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

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A1 fx Σ = 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”

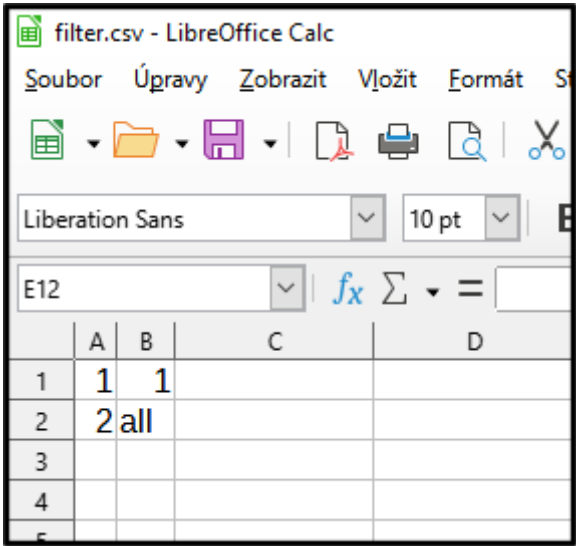


Figure 3: Example: filter.csv file

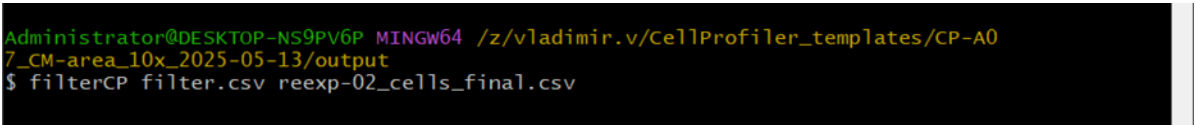


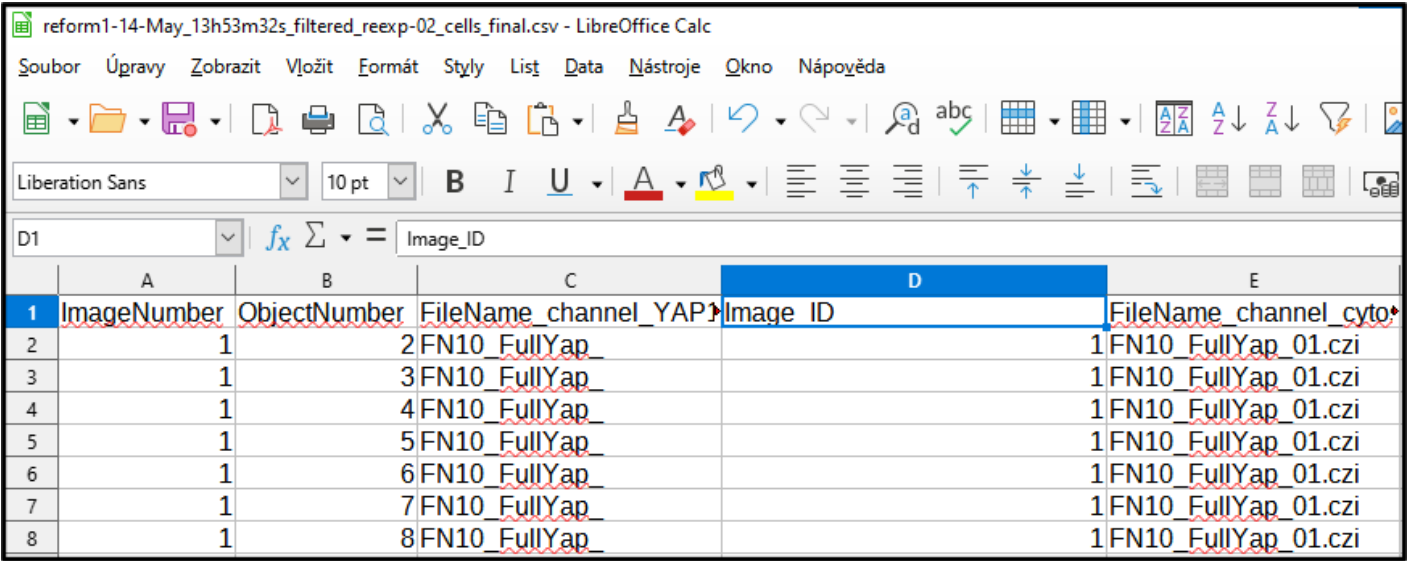
Figure 4: Example: filter command

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

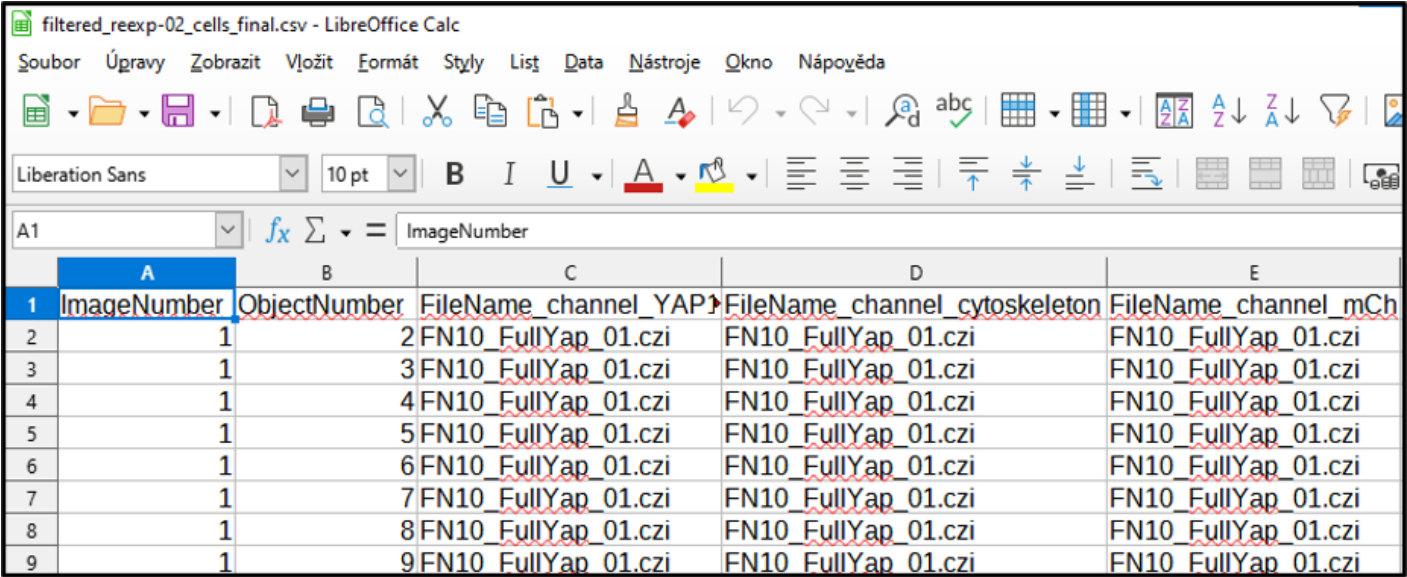
Split the name of the file into an experimental condition and image number

Output of the reformatting script

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

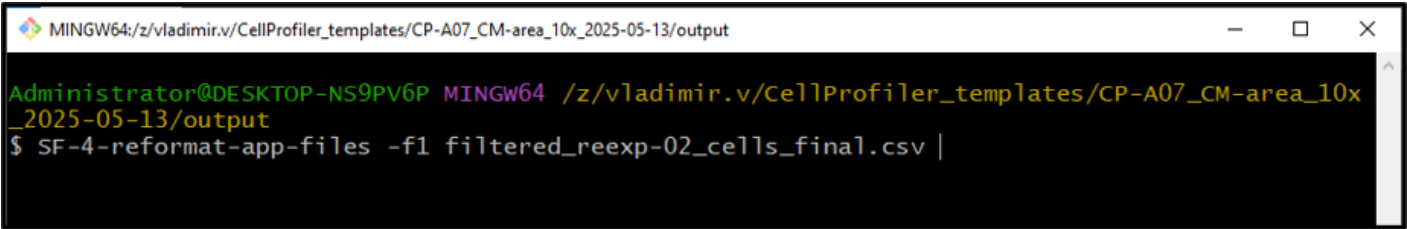


Get the names of columns to select only the relevant ones using csvcut -n <file>



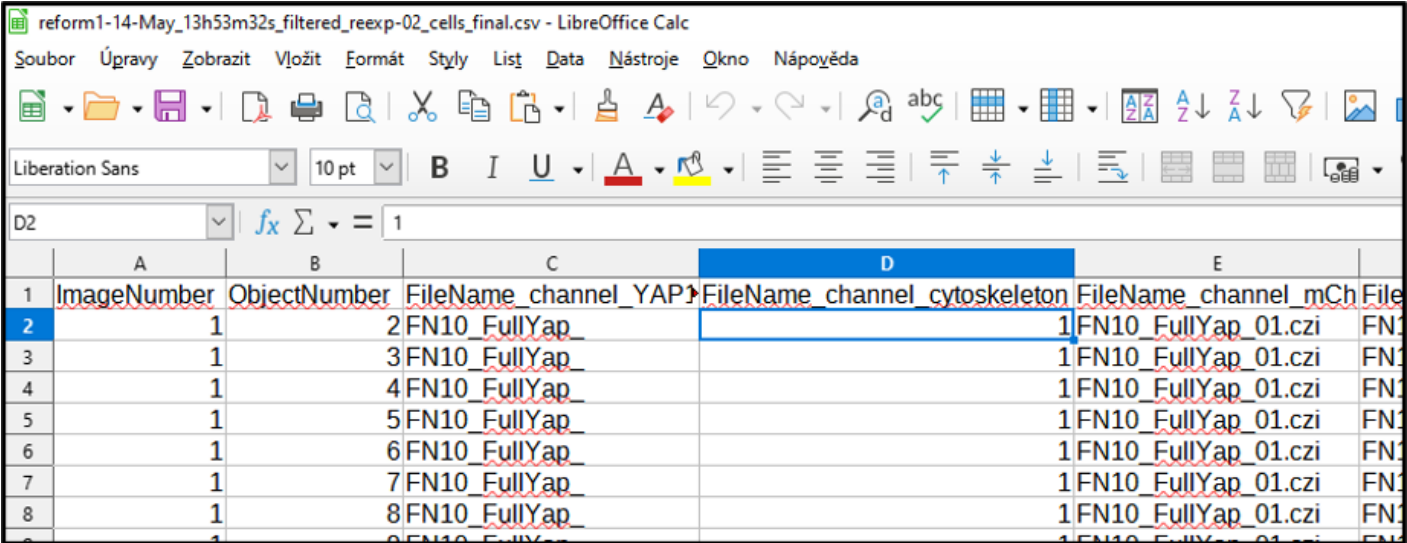
	A	B	C	D	E
1	ImageNumber	ObjectNumber	FileName_channel_YAP	FileName_channel_cytoskeleton	FileName_channel_mCh
2	1	2	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
3	1	3	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
4	1	4	FN10_FullYap_01.czi	FN10_FullYap_01.czi	FN10_FullYap_01.czi
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



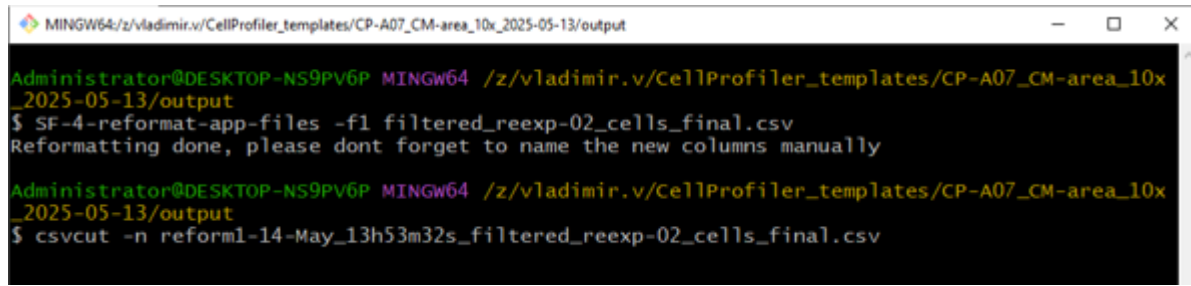
```
MINGW64:/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ SF-4-reformat-app-files -fl filtered_reexp-02_cells_final.csv |
```

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



	A	B	C	D	E	F
1	ImageNumber	ObjectNumber	FileName_channel_YAP	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
9	1	9	FN10_FullYap_	1	FN10_FullYap_01.czi	FN1

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



```
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ SF-4-reformat-app-files -f1 filtered_reexp-02_cells_final.csv
Reformatting done, please dont forget to name the new columns manually

Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ csvcut -n reform1-14-May_13h53m32s_filtered_reexp-02_cells_final.csv
```

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

```

MINGW64/z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ csvcut -n reform1-14-May_13h53m32s_filtered_reexp-02_cells_final.csv
1: ImageNumber
2: ObjectNumber
3: FileName_channel_YAP1
4: Image_ID
5: FileName_channel_cytoskeleton
6: FileName_channel_mCh
7: FileName_channel_nucleus
8: PathName_channel_YAP1
9: PathName_channel_cytoskeleton
10: PathName_channel_mCh
11: PathName_channel_nucleus
12: AreaShape_Area
13: AreaShape_BoundingBoxArea
14: AreaShape_BoundingBoxMaximum_X
15: AreaShape_BoundingBoxMaximum_Y
16: AreaShape_BoundingBoxMinimum_X
17: AreaShape_BoundingBoxMinimum_Y
18: AreaShape_Center_X
19: AreaShape_Center_Y
20: AreaShape_Compactness
21: AreaShape_ConvexArea
22: AreaShape_Eccentricity
23: AreaShape_EquivalentDiameter
24: AreaShape_EulerNumber
25: AreaShape_Extent
26: AreaShape_FormFactor
27: AreaShape_MajorAxisLength
28: AreaShape_MaxFeretDiameter
29: AreaShape_MaximumRadius
30: AreaShape_MeanRadius
31: AreaShape_MedianRadius
32: AreaShape_MinFeretDiameter
33: AreaShape_MinorAxisLength
34: AreaShape_Orientation
35: AreaShape_Perimeter
36: AreaShape_Solidity
37: Intensity_IntegratedIntensityEdge_channel_YAP1
38: Intensity_IntegratedIntensity_channel_YAP1
39: Intensity_LowerQuartileIntensity_channel_YAP1
40: Intensity_MADIntensity_channel_YAP1
41: Intensity_MassDisplacement_channel_YAP1
42: Intensity_MaxIntensityEdge_channel_YAP1
43: Intensity_MaxIntensity_channel_YAP1
44: Intensity_MinIntensityEdge_channel_YAP1
45: Intensity_MeanIntensity_channel_YAP1
46: Intensity_MedianIntensity_channel_YAP1
47: Intensity_MinIntensityEdge_channel_YAP1
48: Intensity_MinIntensity_channel_YAP1
49: Intensity_StdIntensityEdge_channel_YAP1
50: Intensity_StdIntensity_channel_YAP1
51: Intensity_UpperQuartileIntensity_channel_YAP1
52: Location_CenterMassIntensity_X_channel_YAP1
53: Location_CenterMassIntensity_Y_channel_YAP1
54: Location_CenterMassIntensity_Z_channel_YAP1
55: Location_Center_X
56: Location_Center_Y
57: Location_Center_Z
58: Location_MaxIntensity_X_channel_YAP1
59: Location_MaxIntensity_Y_channel_YAP1
60: Location_MaxIntensity_Z_channel_YAP1
61: Number_Object_Number
62: Parent_CM_nuclei_merged

```

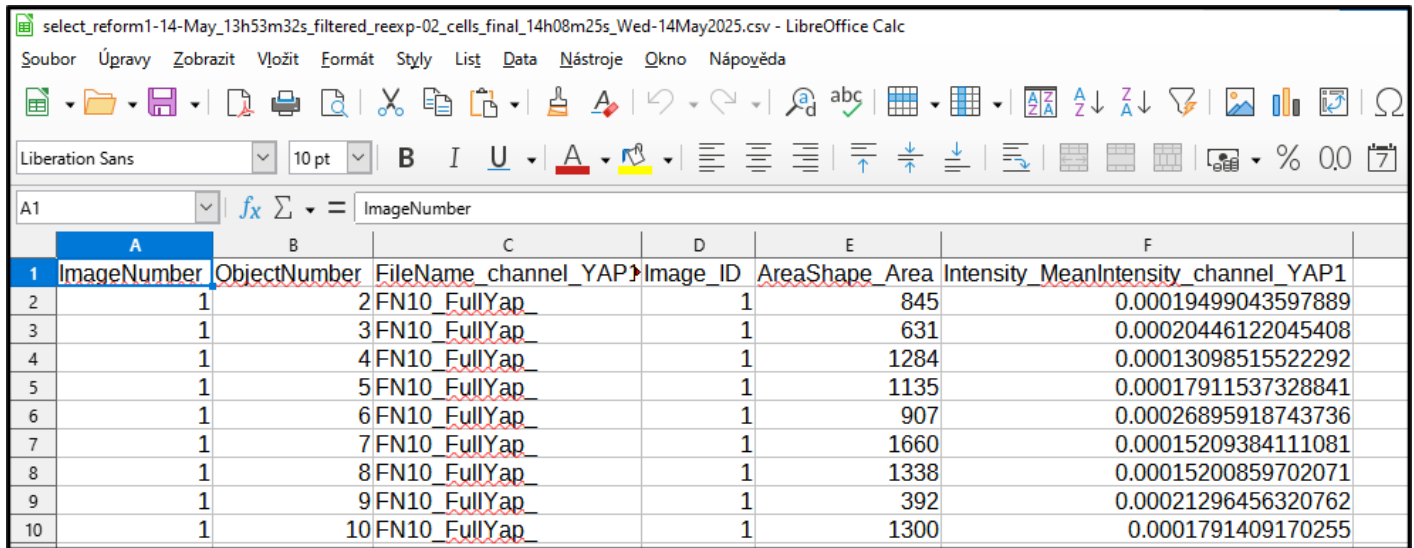
Save the relevant columns into a new file using `select.R -c N,M-O <file>`

```

MINGW64/z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ selectR -c 1-4,12,45 reform1-14-May_13h53m32s_filtered_reexp-02_cells_final.csv |

```

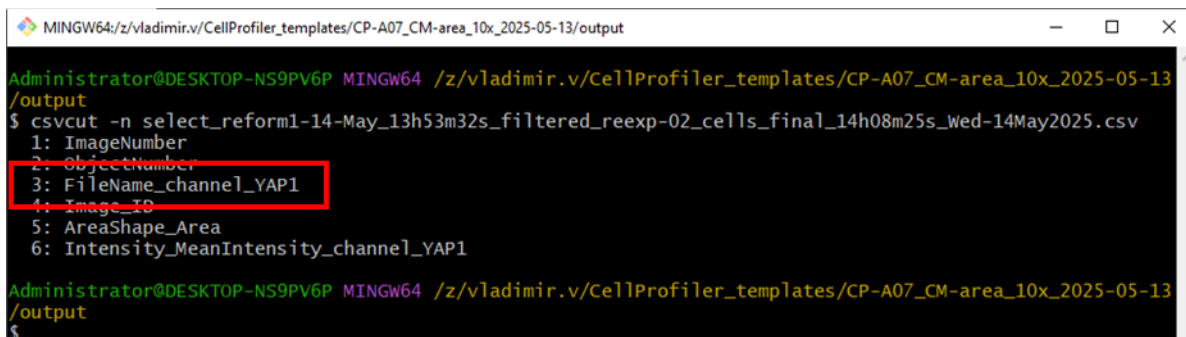
Check the columns got selected



	A	B	C	D	E	F
	ImageNumber	ObjectNumber	FileName_channel_YAP1	Image_ID	AreaShape_Area	Intensity_MeanIntensity_channel_YAP1
1	1	2	FN10_FullYap	1	845	0.00019499043597889
2	1	3	FN10_FullYap	1	631	0.00020446122045408
3	1	4	FN10_FullYap	1	1284	0.00013098515522292
4	1	5	FN10_FullYap	1	1135	0.00017911537328841
5	1	6	FN10_FullYap	1	907	0.00026895918743736
6	1	7	FN10_FullYap	1	1660	0.00015209384111081
7	1	8	FN10_FullYap	1	1338	0.00015200859702071
8	1	9	FN10_FullYap	1	392	0.00021296456320762
9	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>`



```

Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ csvcut -n select_reform1-14-May_13h53m32s_filtered_reexp-02_cells_final_14h08m25s_Wed-14May2025.csv
1: ImageNumber
2: ObjectNumber
3: FileName_channel_YAP1
4: Image_ID
5: AreaShape_Area
6: Intensity_MeanIntensity_channel_YAP1
Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$

```

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



```

Administrator@DESKTOP-NS9PV6P MINGW64 /z/vladimir.v/CellProfiler_templates/CP-A07_CM-area_10x_2025-05-13/output
$ summaryR -c 3 select_reform1-14-May_13h53m32s_filtered_reexp-02_cells_final_14h08m25s_Wed-14May2025.csv

```

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

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S12 \sum =

	A	Q	R	S	
1	FileName_channel_YAP1	AreaShape_Area_mean	AreaShape_Area_sd	AreaShape_Area_N	AreaS
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					

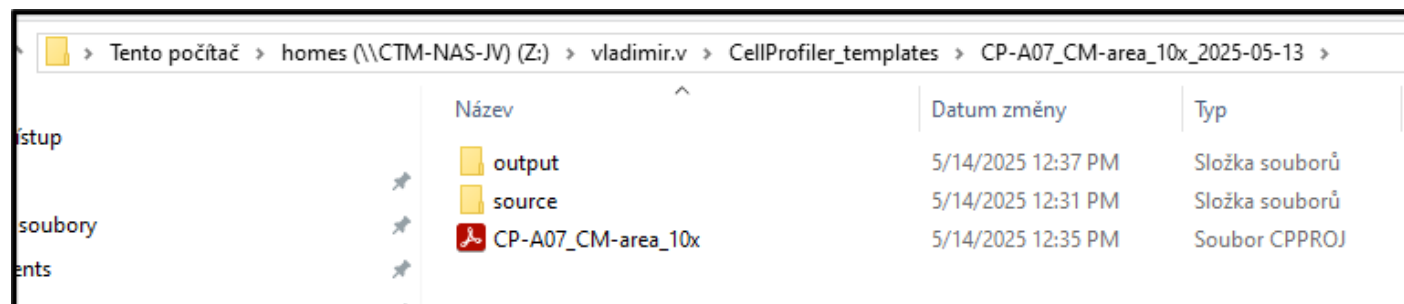
Acquisition setting

Image Dimensions	
Channels	4
Scaling (per Pixel)	0.55 μ m x 0.55 μ m
Image Size (Pixels)	2048 x 2048
Image Size (Scaled)	1.13 mm x 1.13 mm
Bit Depth	12 Bit
Image Center Position	X: -32.12 mm, Y: -4.89 mm
Stage Position	X: -32.12 mm, Y: -4.89 mm
ROI Center Offset	X: 0.00 μ m, Y: 0.00 μ m

Resources

Cell Profiler Versions

Pipeline location on Leica computer



	Název	Datum změny	Typ
Ústup	output	5/14/2025 12:37 PM	Složka souborů
soubory	source	5/14/2025 12:31 PM	Složka souborů
ents	CP-A07_CM-area_10x	5/14/2025 12:35 PM	Soubor CPPROJ

Tutorial on cell profiler