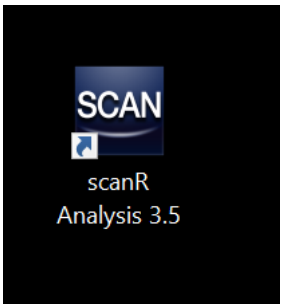
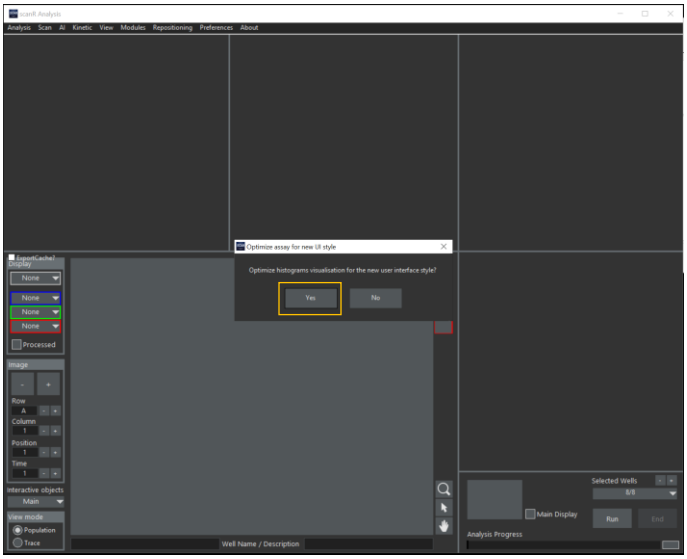
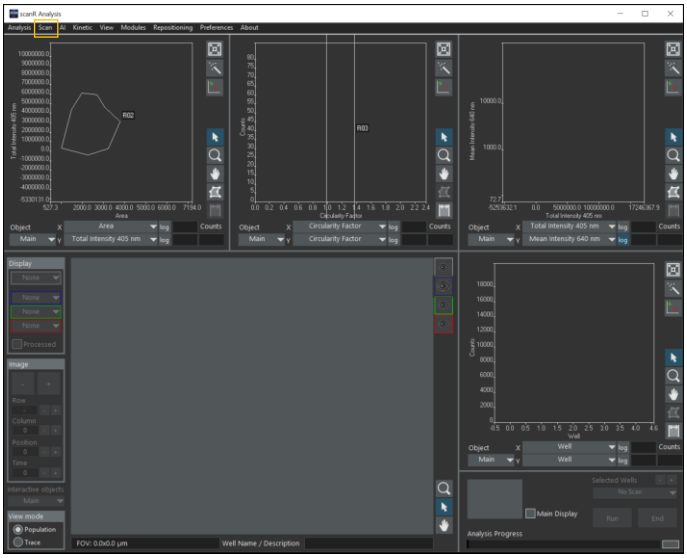
Šárka
Janscak's group

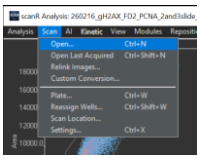


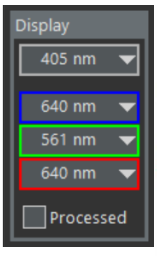



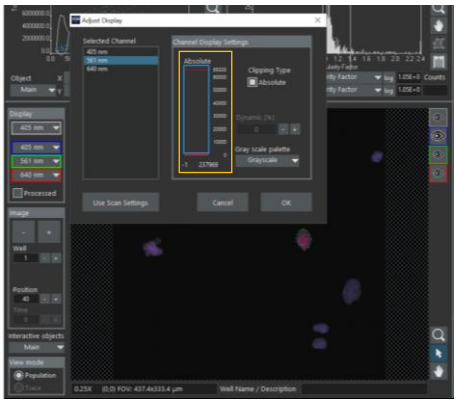


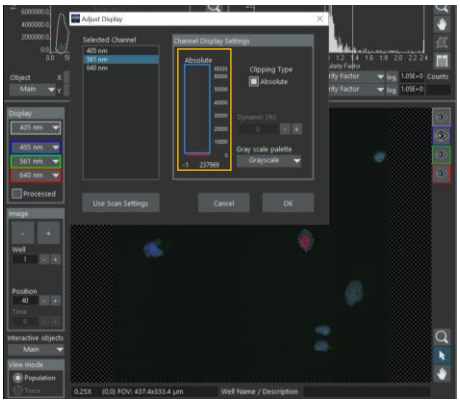
Load data from microscope
Scan → Open … (Ctrl + N) → find Folder → experiment_descriptor.xml

Or open previous analysis (to apply previous settings)
Analysis → Open







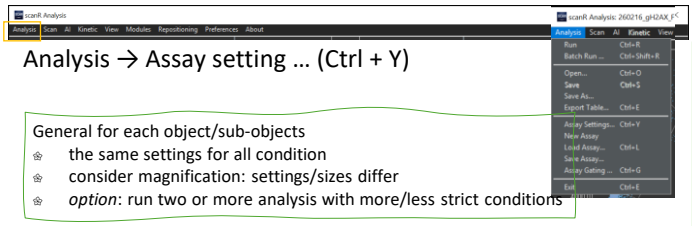
***Display***

- colors can be combined e.g. blue + red = magenta

View → Adjust Display

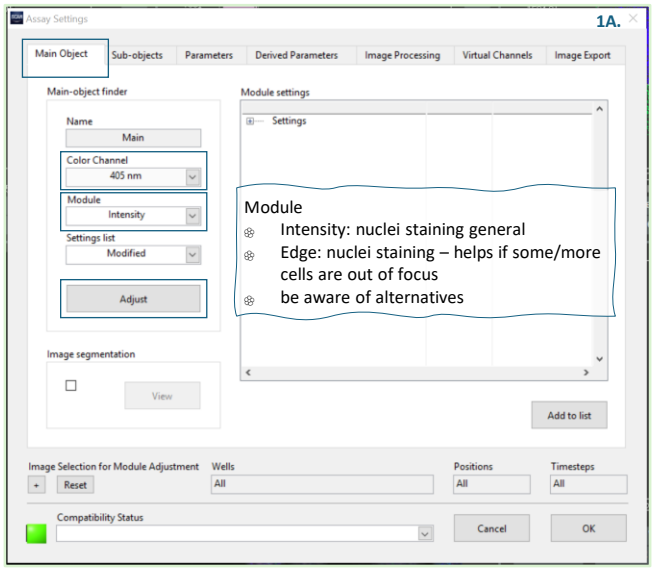
- Adjust intensity (usually it is necessary to go very low)
- it has to be done for each channel separately

- can be done also later (for gating)

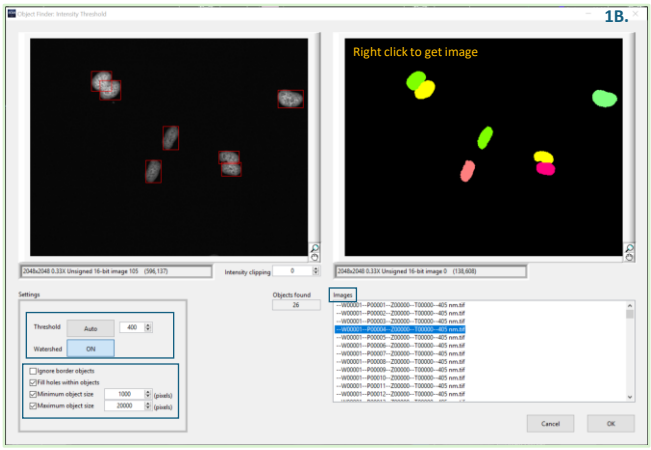


# Analysis → Assay setting … (Ctrl + Y)

General for each object/sub-objects
- the same settings for all condition
- consider magnification: settings/sizes differ
- *option*: run two or more analysis with more/less strict conditions




Module
- Intensity: nuclei staining general
- Edge: nuclei staining – helps if some/more cells are out of focus
- be aware of alternatives



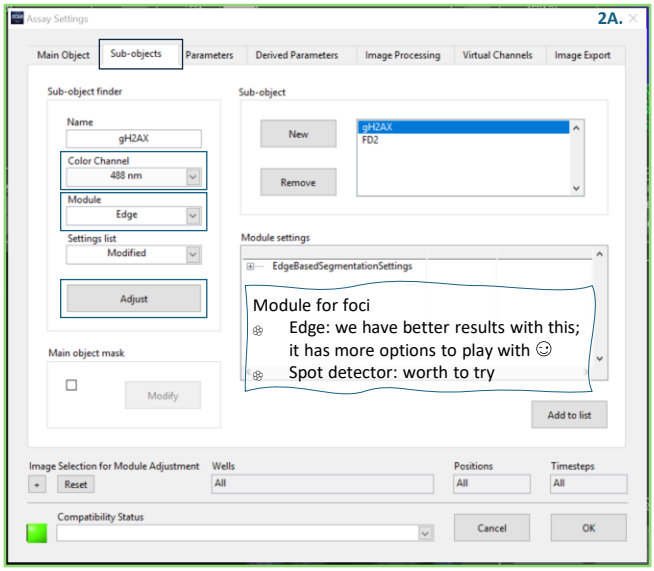

Threshold
- intensity o signal against background

Ignore borders
- optional, can be gated out

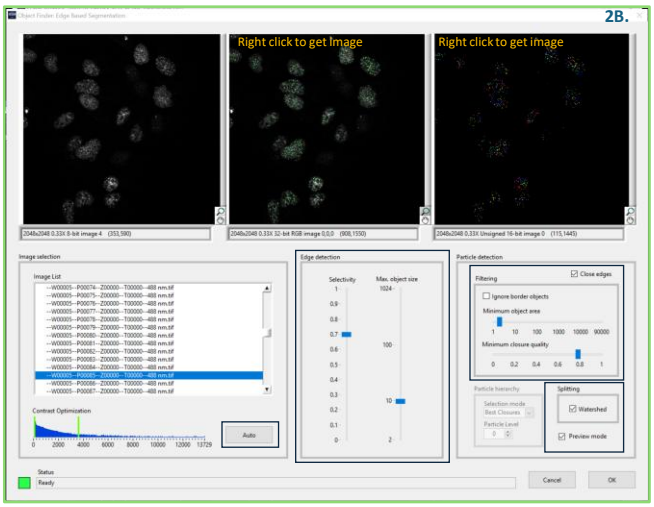
Watershed ON
- to separate object close or together

Minimum and Maximum object size
- to find almost all cells – it can be excluded later in gating

Images
W0000X … X … individual well/cover slip
P0000X … image
- go thought a few images and different treatments/ctrl to see, if settings are fine for all conditions

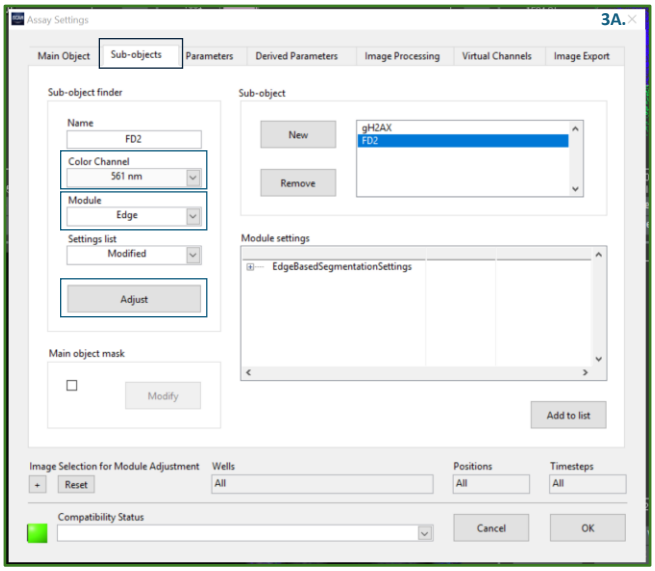


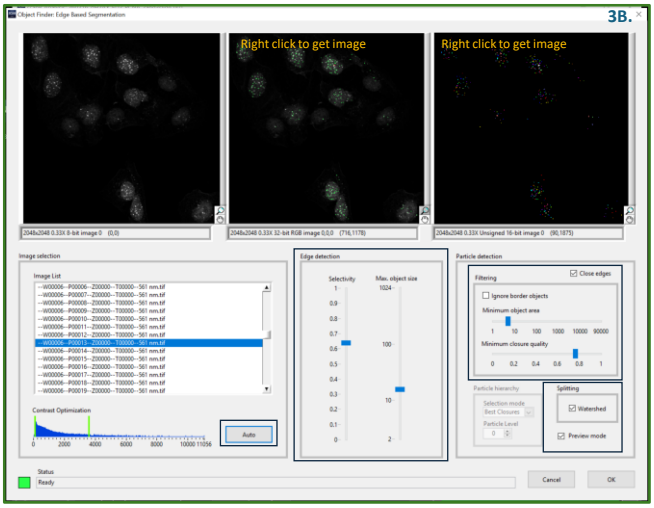

Module for foci
- Edge: we have better results with this; it has more options to play with ☺
- Spot detector: worth to try



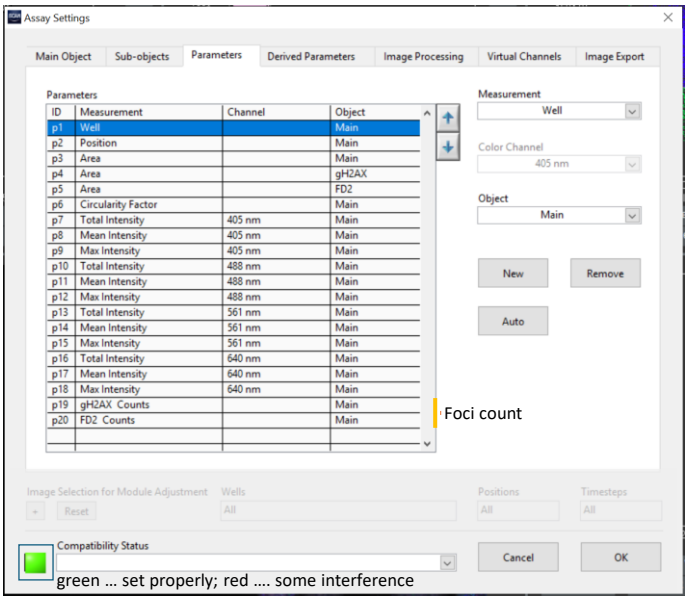

Auto
- set it to see, but also adjust brightness manually – same range for all condition

Edge & particle detection
- Adjust based on desired objects
- tip : zoom in: to be sure, which foci are masked



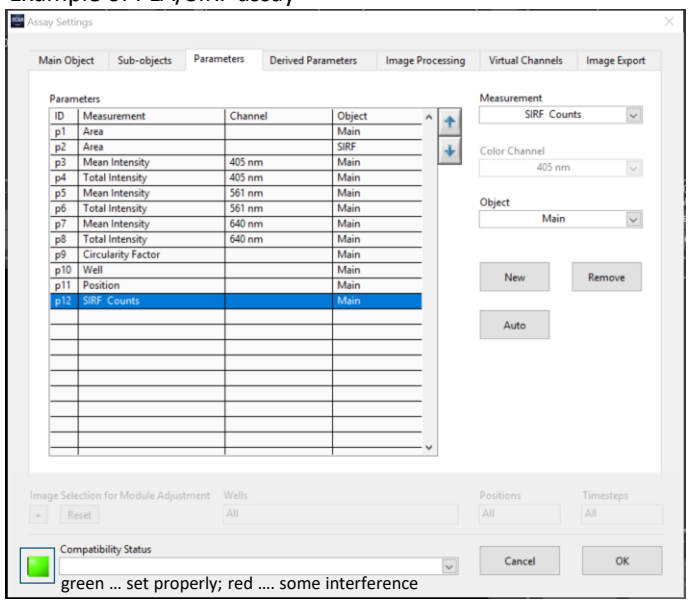
Foci



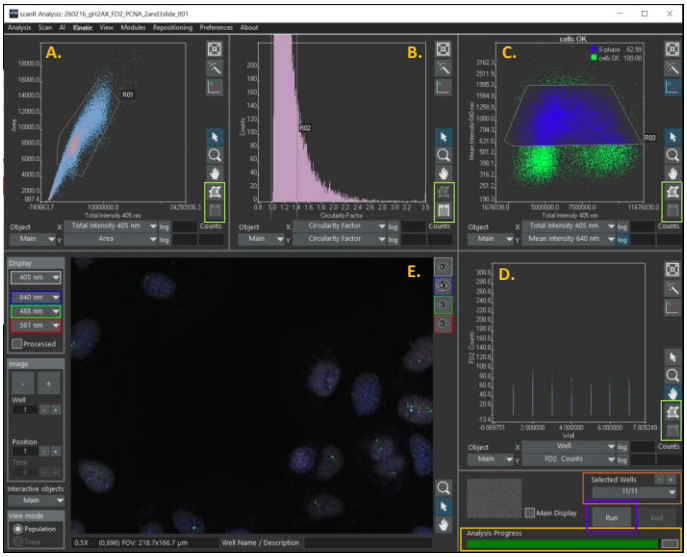

Foci count

green … set properly; red …. some interference

Example of PLA/SIRF assay




green … set properly; red …. some interference




once Parameters set → OK → **Run** analysis

Gates drawing

- double click by mouse to close gate (“circular” ones)

***Gating***

***A:*** selection of cells

- exclude debris (down) or doublets (up) etc.

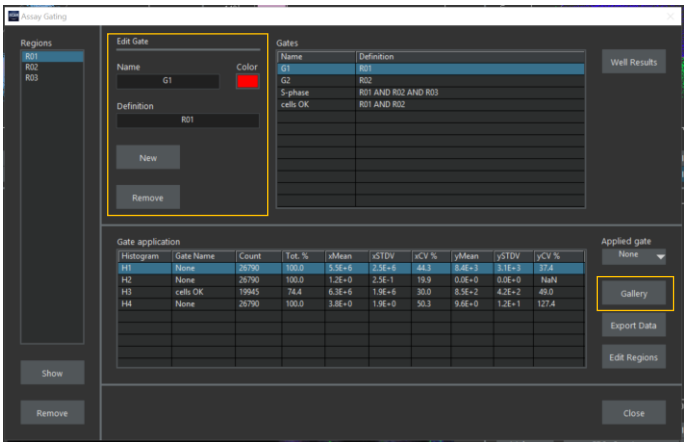
***B.*** selection of circular cells (base on circularity factor)

***C.*** Cell cycle (X: Total intensity DAPI / Y: Mean intensity PCNA/EdU)

***D.*** Results: condition (Y axis) / well (X axis)

- Selected wells – enables to see individual conditions
- clicking on the individual points in each plots show the image of object in the view window (***E***)

Analysis → Assay Gating …




***Applying gating***

Analysis → Assay Gating …

- define gates; it has to be written properly (capital letters)
- apply gating: right click on plot (A – C) → select desired gate

Export Gallery

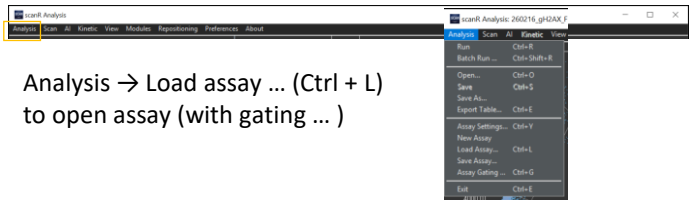
- according active widow/field

*option*: right click on well in plot D.

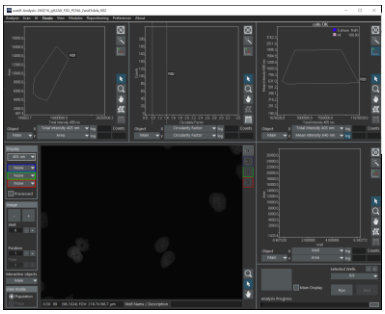
***Finish analysis***

- save assay: .say
- save analysis: .sca
- export analysis (data): _Main.txt
  - before export be sure everything is saved !!!
  - it will export all, once _Main is exported close ScanR analysis software from Task manager (it can take long time)

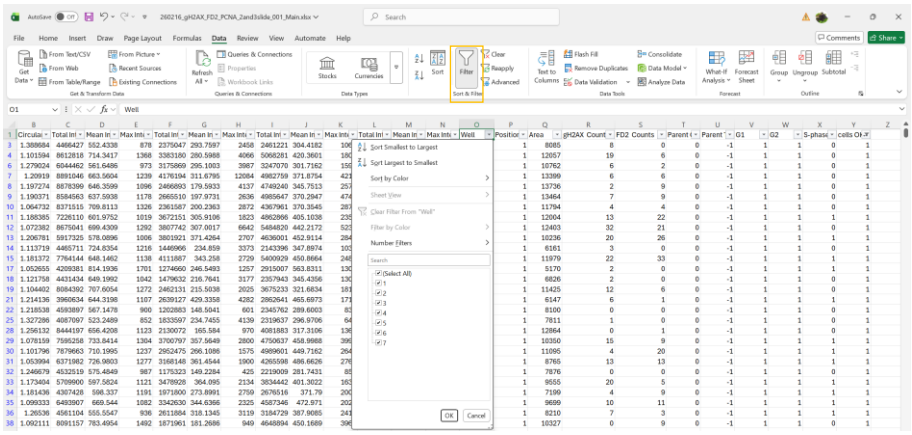

***Note: Loading saved assay (.say)***



Analysis → Load assay … (Ctrl + L)
to open assay (with gating … )



***Note: Example of further data processing (.txt)***



Open _Main.txt file in Excel

Apply filters
Well … condition

Tip: Freeze top row (view)

Copy individual values for each condition to a new sheet

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 260216 gH2AX_FD2_PCNA_003 | | | | | | | | |
| 2 | NT | | CPT | | CDDP | | MMC | | |
| 3 | well 1 | | well 2 | | well 3 | | well 5 | | |
| 4 | | | | | | | | | |
| 5 | | | | | | | | | |
| 6 | | 17.59587 | | 52.93338 | | 23.65166 | | 43.20109 | |
| 7 | | 3677 | | 5284 | | 4042 | | 3655 | |
| 8 | | 647 | | 2797 | | 956 | | 1579 | |
| 9 | 7.994017 | 16.48001 | 43.71631 | 33.64269 | 30.52251 | 19.30529 | 39.67469 | 27.43256 | |
| 10 | H2AX foci | FD2 foci C | H2AX foci | FD2 foci C | H2AX foci | FD2 foci C | H2AX foci | FD2 foci Counts | |
| 11 | 15 | 26 | 35 | 28 | 29 | 14 | 79 | 38 | |
| 12 | 26 | 21 | 46 | 69 | 6 | 17 | 41 | 45 | |
| 13 | 9 | 20 | 0 | 1 | 7 | 11 | 26 | 26 | |

***Example***
count FD2 foci >30

=D8/D7*100
=COUNT(D11:D140002)
=COUNTIF(D11:D19002,">30")

***Important !!!***
Save as excel file, otherwise _Main.txt will be overwritten