ZOI - Zone Of Influence Segmentation for ZEN 2.5 (blue edition)

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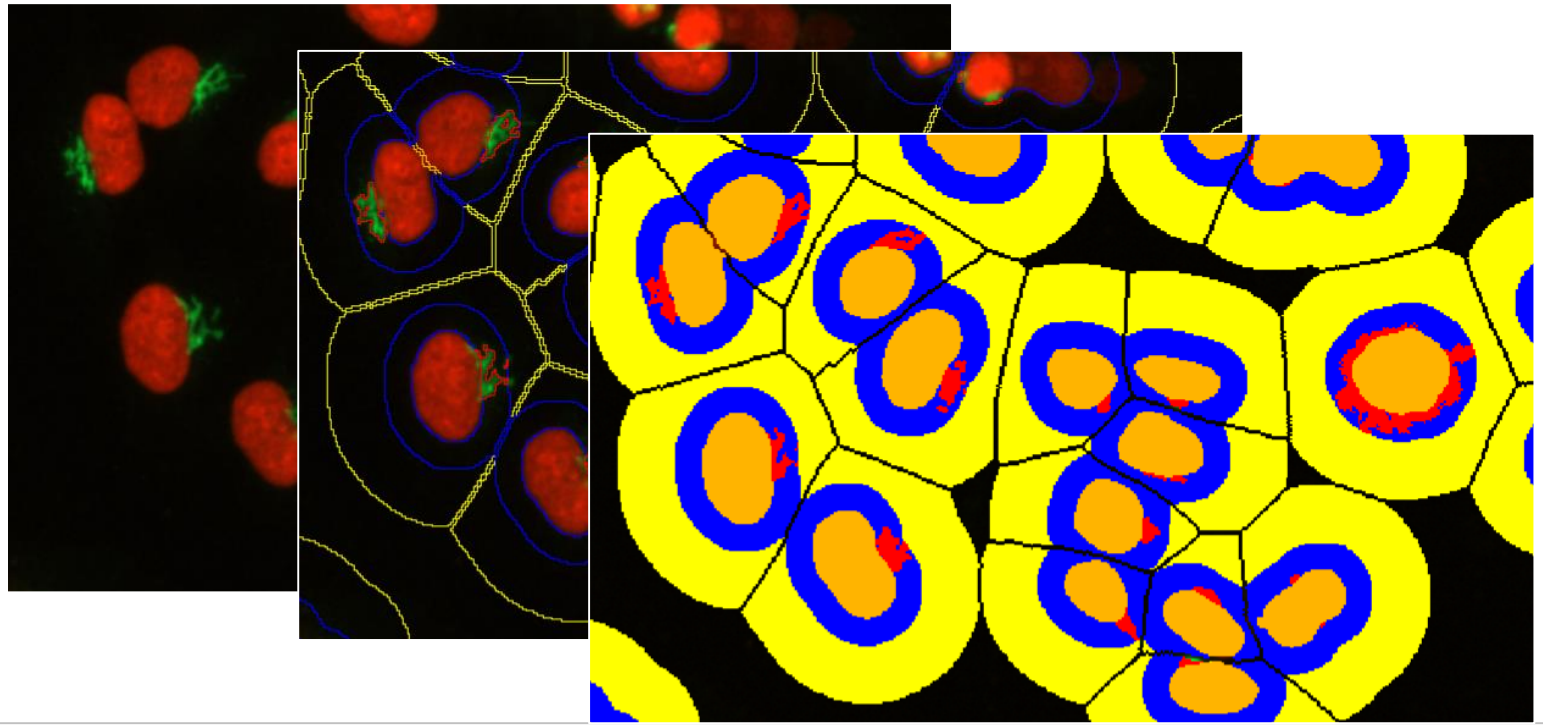
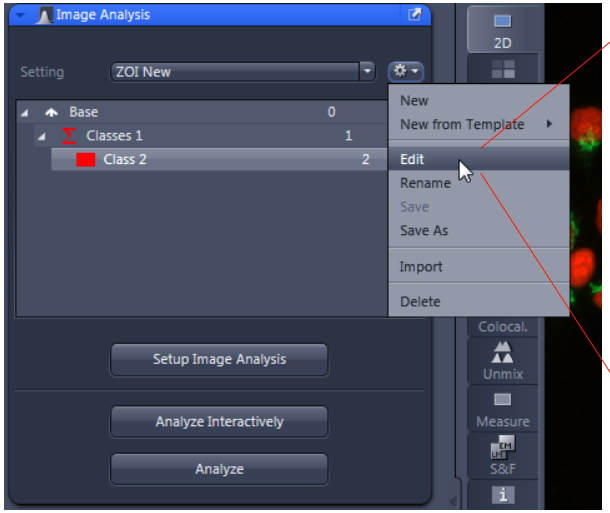
Typical ZOI Applications

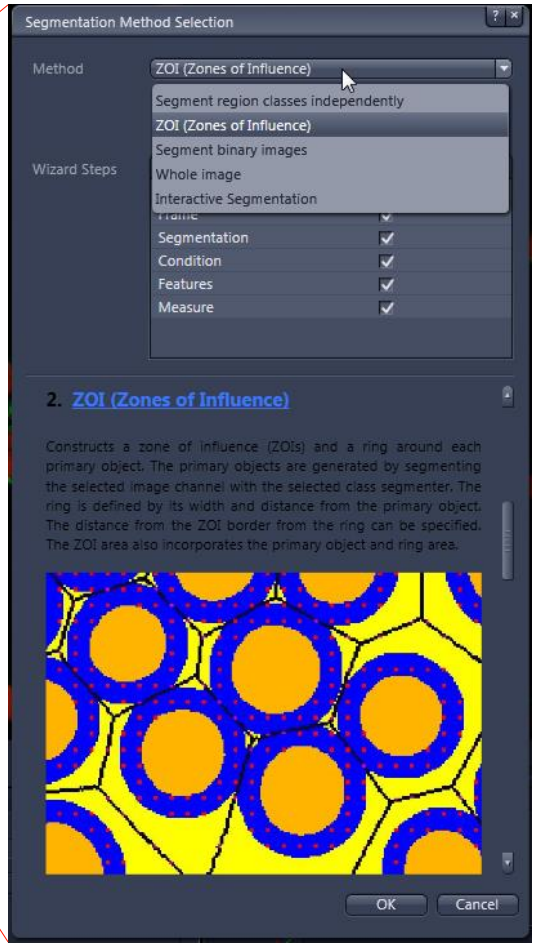
Applications in cell biology, drug discovery, in-vitro assays, endpoint assays, e.g.:

- Cytoplasm-Nucleus Translocation
- Protein Localization
- Count sub-objects inside and outside of the primary object
  (e.g. vesicles, PML bodies,…)



Creating an Image Analysis Setting using ZOI



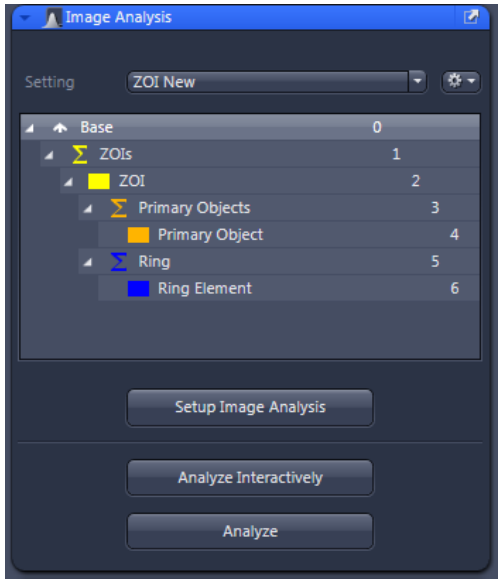


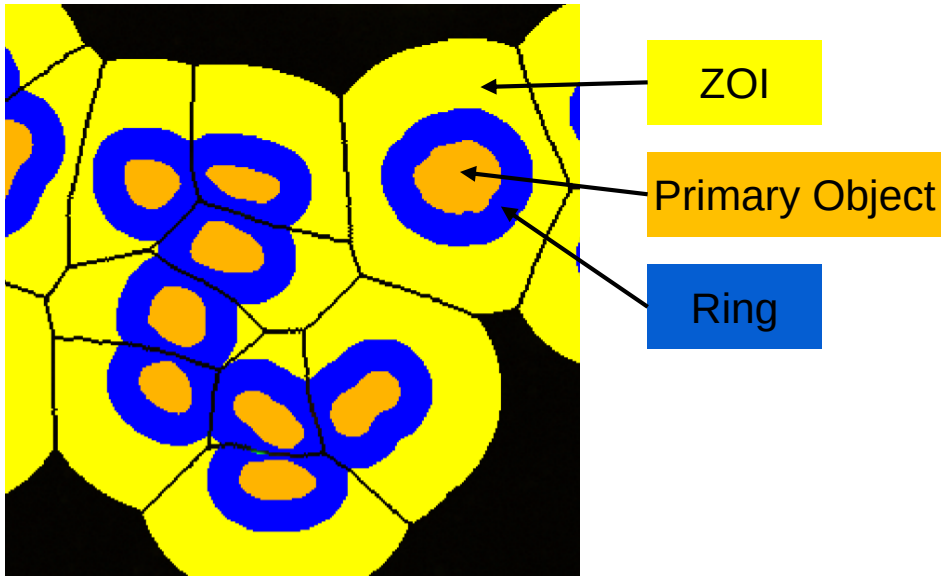
Create a new Image Analysis Setting and choose ZOI (Zones of Influence) as Segmentation Method

Set up an Image Analysis Setting using ZOI

The ZOI-method will create the necessary classes automatically:

- **ZOIs/ZOI**: the area (zone of influence) that is attributed to the primary objects
- **Primary Objects/Primary Object**: the objects that identify the cell (e.g. nuclei)
- **Ring/Ring Element**: automatically generated around each primary object to measure parameters or to detect sub-objects





Set up an Image Analysis Setting using ZOI

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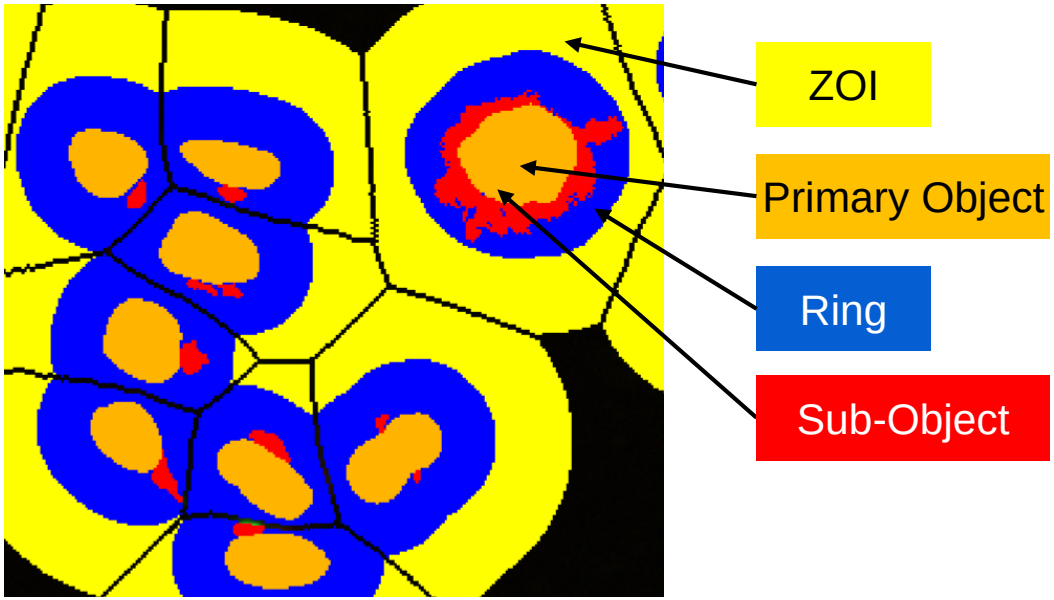
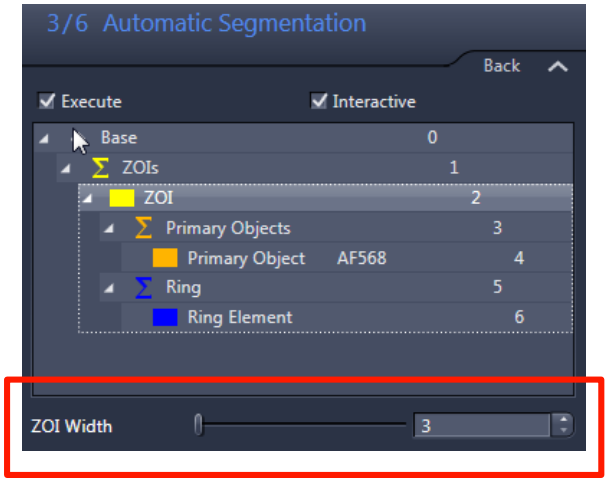
- **ZOIs/ZOI**: the area (zone of influence) that is attributed to the primary objects
- **Primary Objects/Primary Object**: the objects that identify the cell (e.g. nuclei)
- **Ring/Ring Element**: automatically generated around each primary object to measure parameters or to detect sub-objects
- It is possible to add another sub-object below the Ring

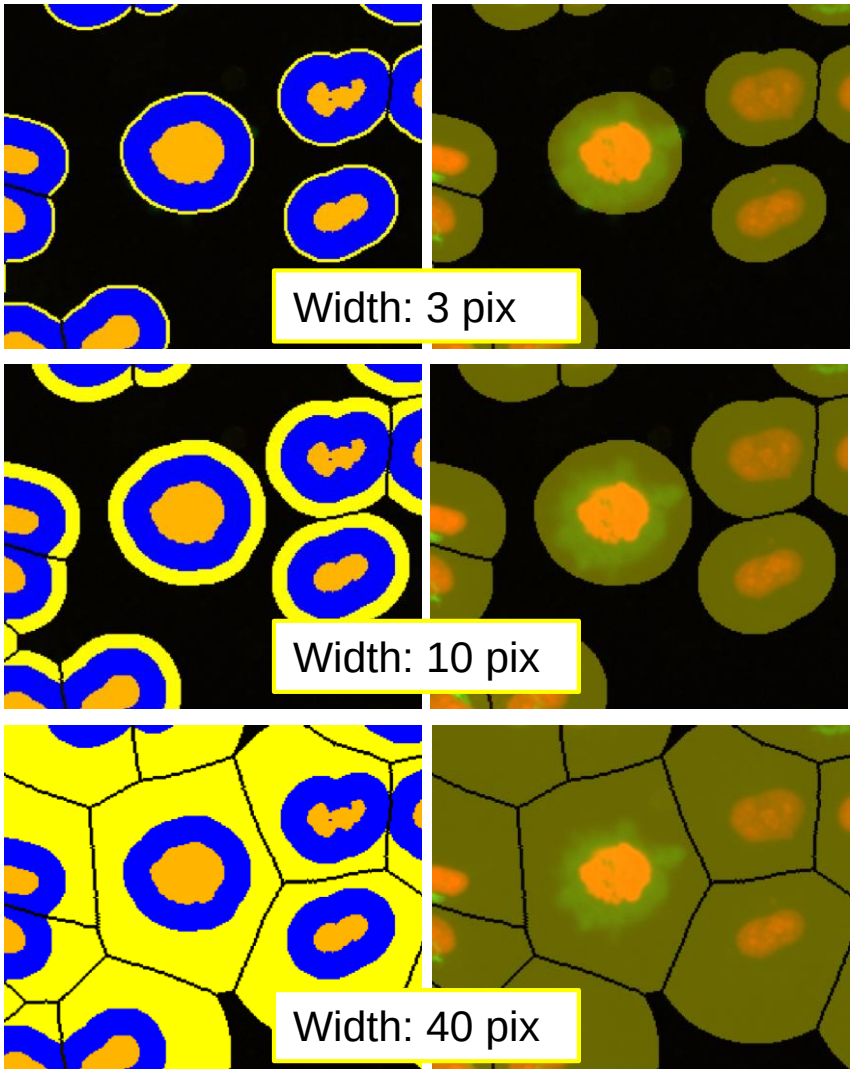


Adjust ZOI Parameters

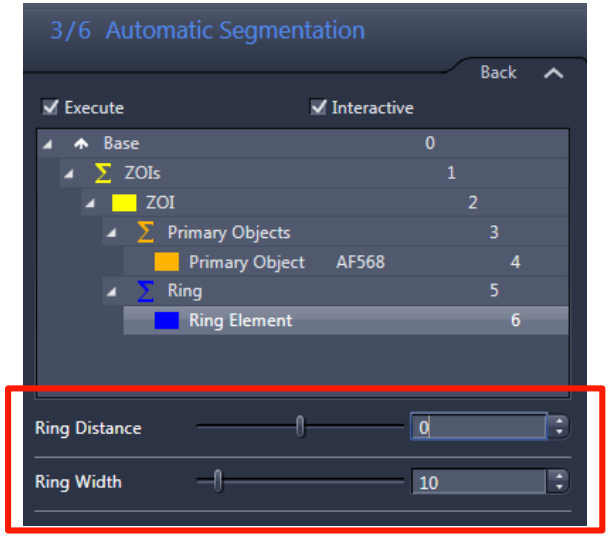


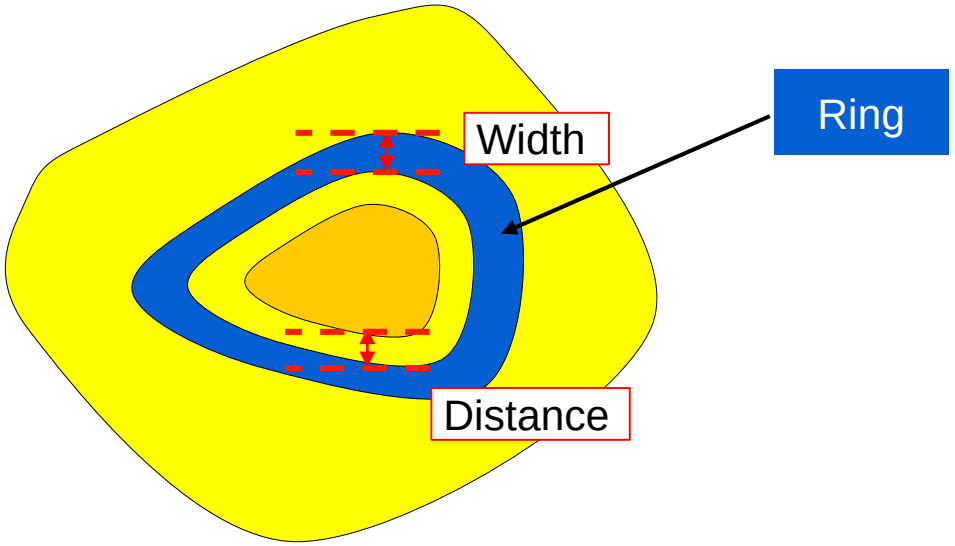
Adjust ZOI Width

- The ZOI covers the whole area including Ring and Primary Object
- Minimum ZOI Width is 3 pixel larger than either the Ring or the Primary Object (depending on which one is larger)



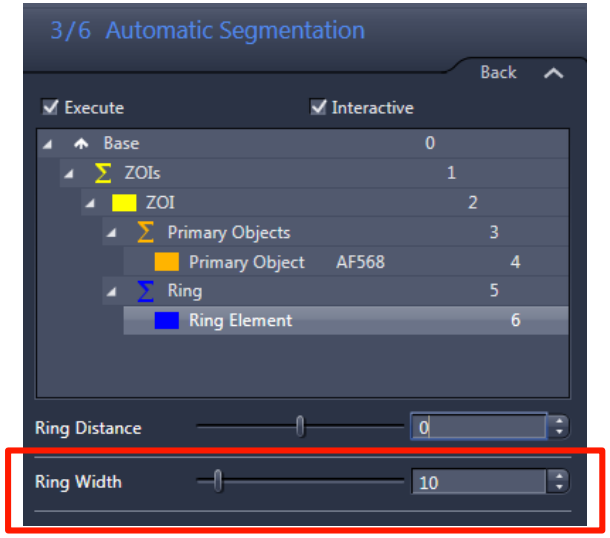
Adjust Ring Parameters

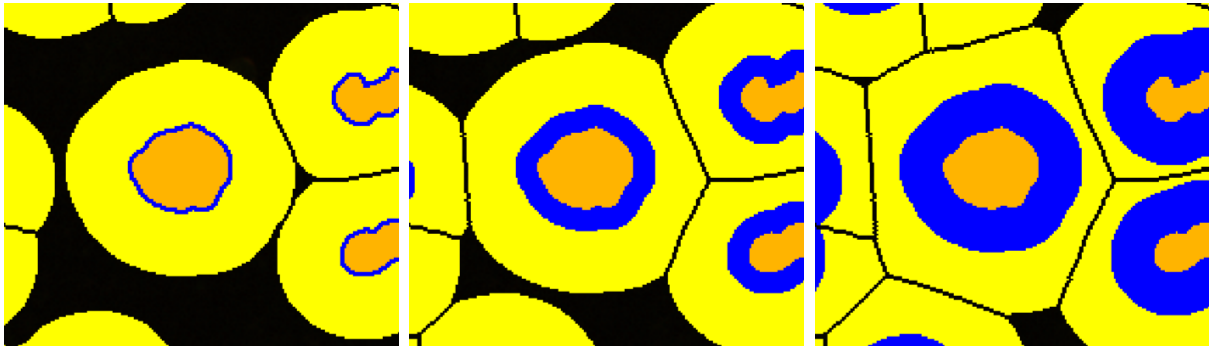




Flexible definition of the Ring Distance and Ring Width

Adjust Ring Parameters





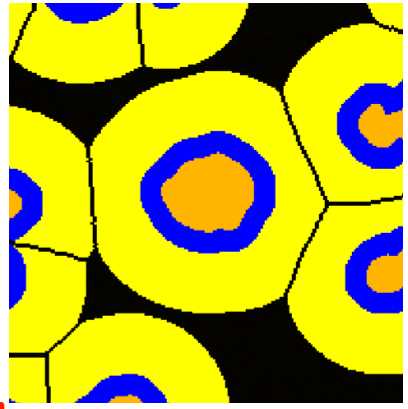
Width: 2

Width: 10

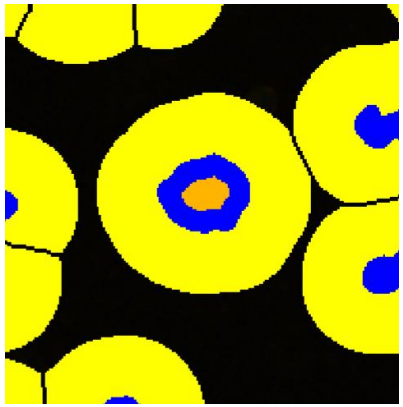
Width: 20

Flexible definition of the Ring Width (in pixel)

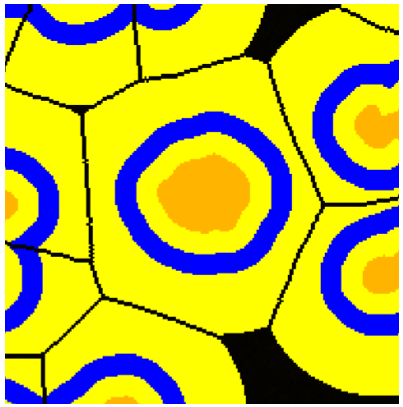
Adjust Ring Parameters



Distance: 0



Distance: -10

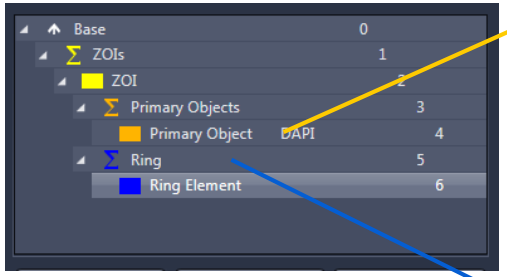


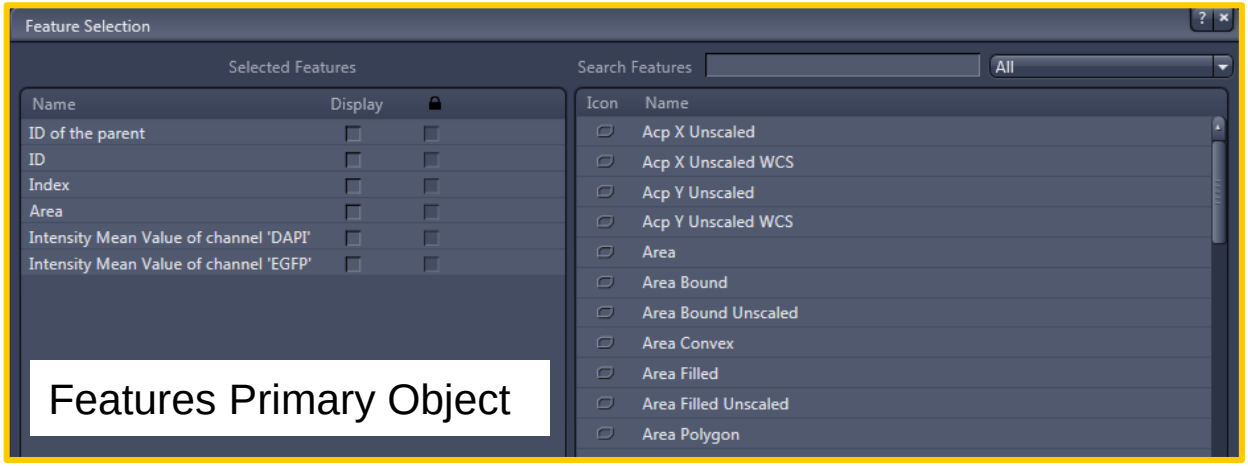
Distance: +10

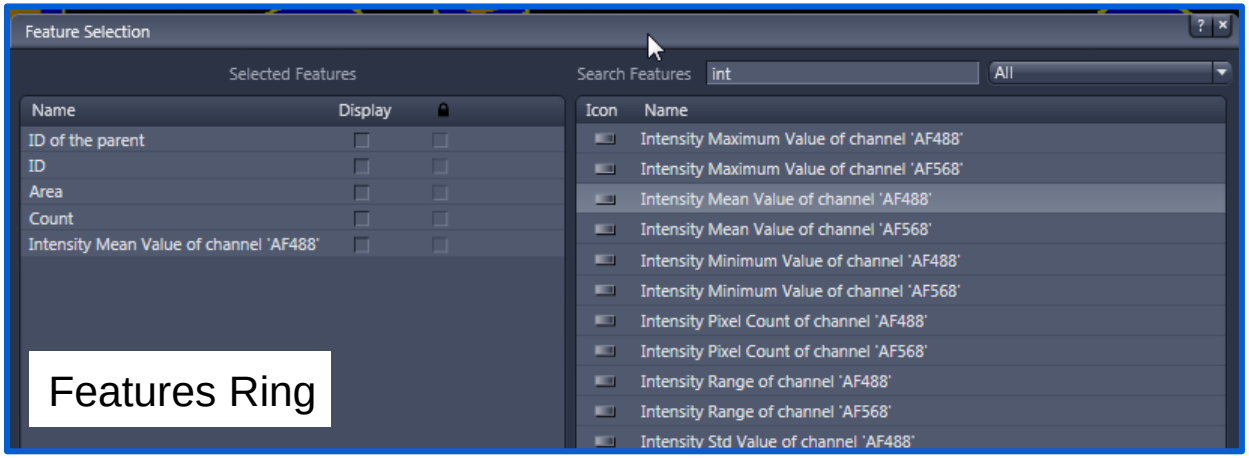
Flexible definition of the Ring Distance (in pixel)
The Ring Distance is measured from the outer border of the Primary Object

- Ring Distance = 0 : Ring starts at the border or the Primary Object
- Ring Distance > 0 : Ring starts outside of the Primary Object
- Ring Distance < 0 : Ring starts inside of the Primary Object

Feature Selection

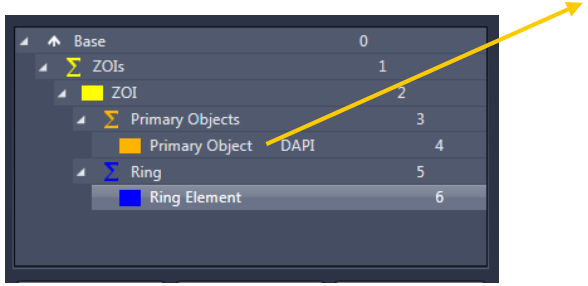


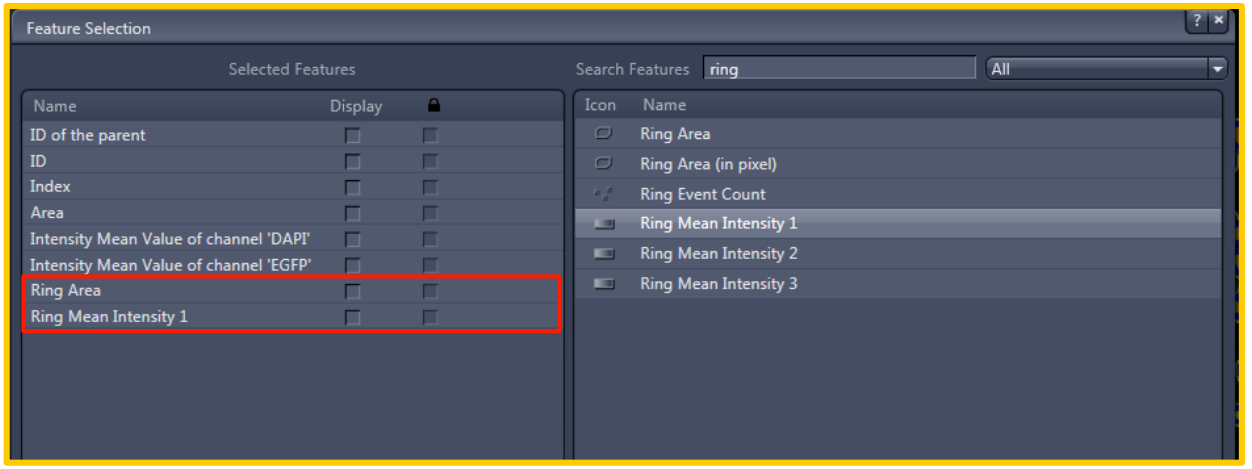




Attribute Features to Primary Object and Ring.

Attribute Ring-Features to Primary Object



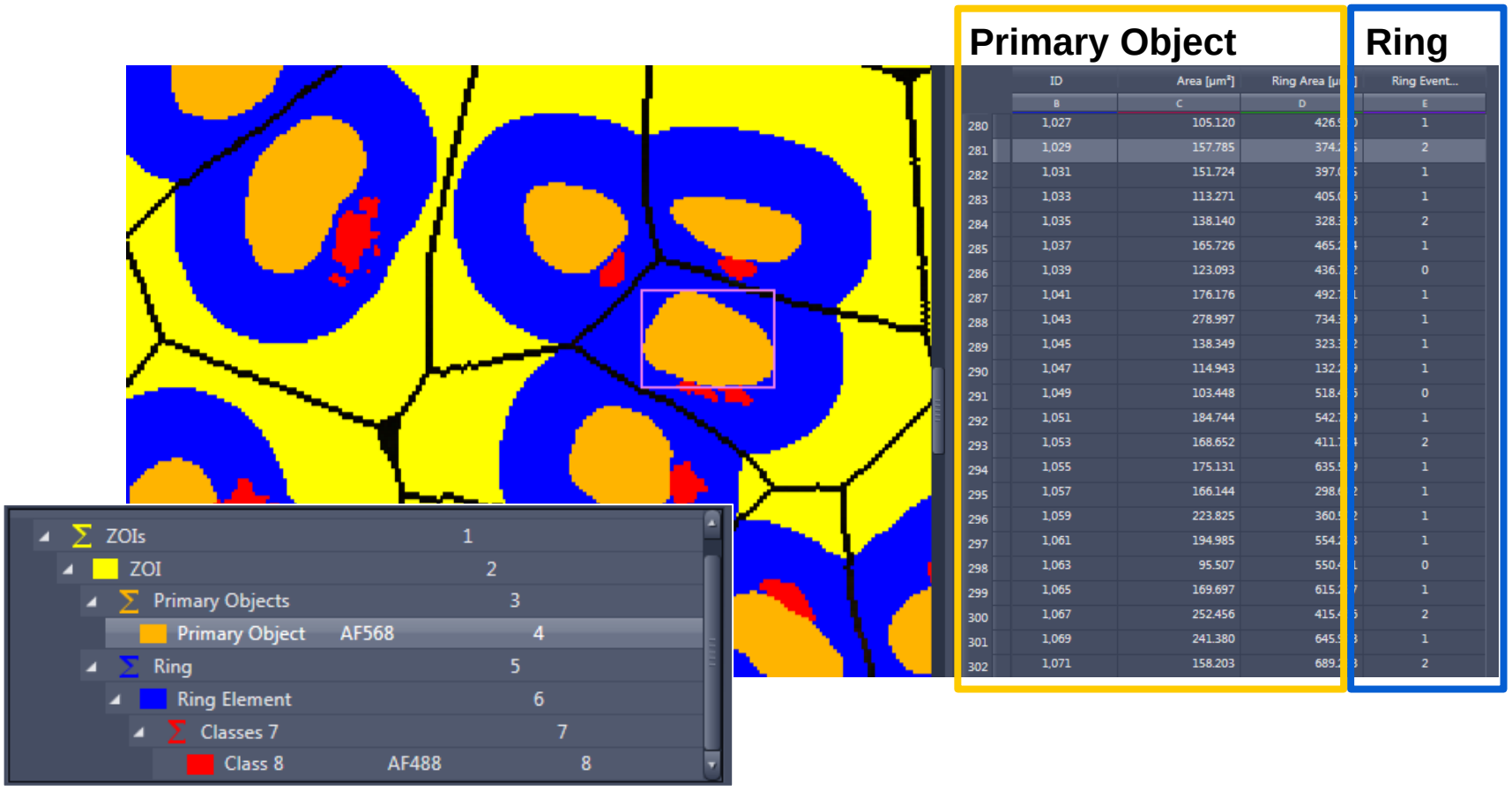


Add Ring-Features to the Primary Object

A fixed set of features can be added to the Primary Object:

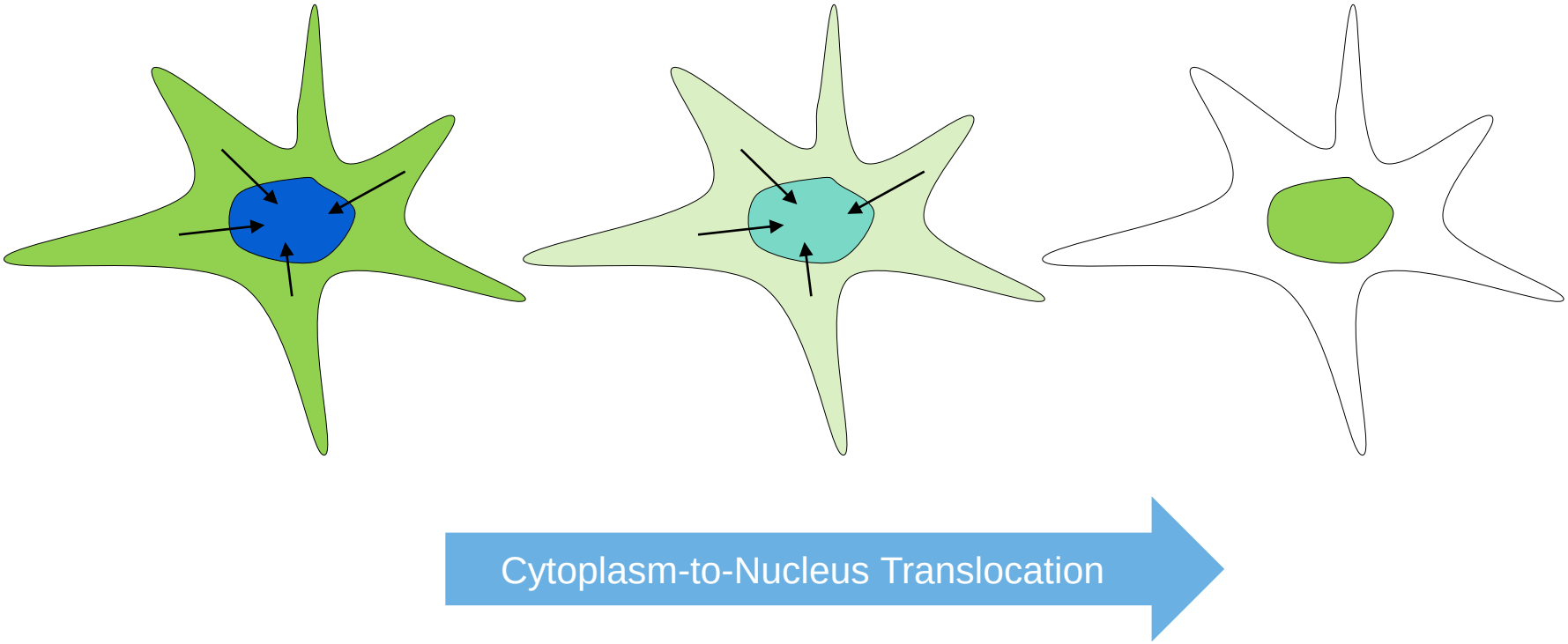
- Ring Area
- Ring Event Count
  (number of sub-objects detected on the Ring)
- Ring Mean Intensity
  (up to three channels can be used)

Results



The resulting feature list for the Primary Object also contains the selected features of the Ring (e.g. number of Sub-Objects (red) detected on the Ring)

Application Example: Translocation Assay



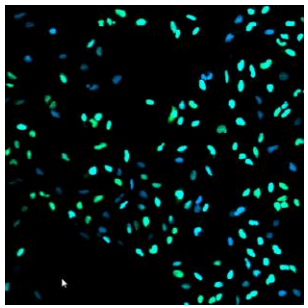
Human U2OS cells cytoplasm–nucleus translocation

- 96-well plate, human osteosarcoma cells (U2OS), nuclei stained with DRAQ
- Cytoplasm to nucleus translocation of the Forkhead (FKHR-EGFP) fusion protein
- In proliferating cells, FKHR is localized in the cytoplasm (constantly moving into the nucleus, but is transported out again by export proteins).
- Upon inhibition of nuclear export, FKHR accumulates in the nucleus
- Export is inhibited by blocking PI3 kinase / PKB with Wortmannin or LY294002.

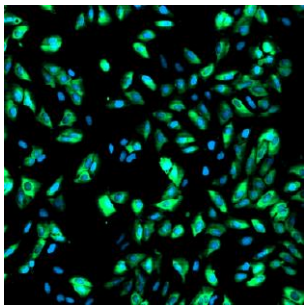
96-well plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg. Ctrl	39688	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
B	Neg. Ctrl	empty	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
C	Neg. Ctrl	empty	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
D	Neg. Ctrl	empty	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
E	Pos. Ctrl	empty	0.31	0.63	1.25	2.5	5	10	20	40	80	Neg. Ctrl
F	Pos. Ctrl	empty	0.31	0.63	1.25	2.5	5	10	20	40	80	Neg. Ctrl
G	Pos. Ctrl	empty	0.31	0.63	1.25	2.5	5	10	20	40	80	Neg. Ctrl
H	Pos. Ctrl	empty	0.31	0.63	1.25	2.5	5	10	20	40	80	Neg. Ctrl

Wortmannin in nM LY294.002 in µM



negative



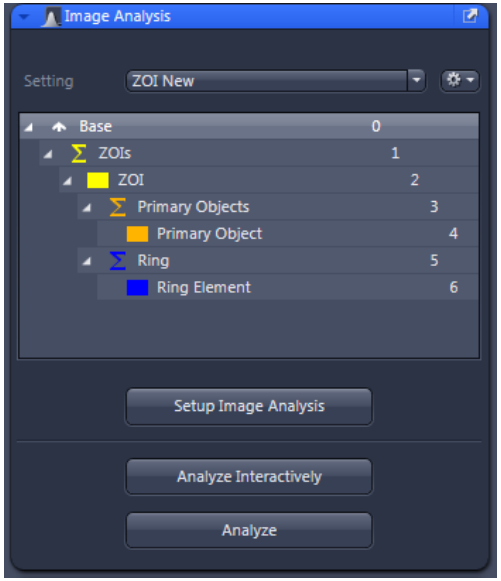
positive

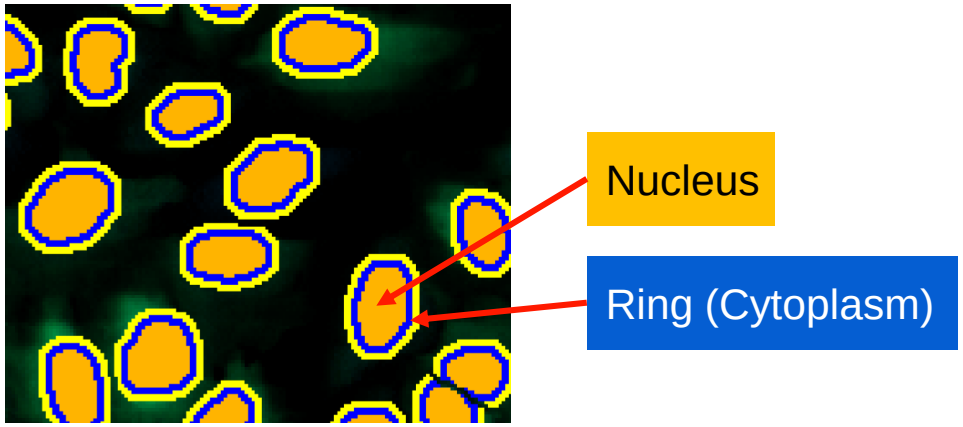
Data set BBBC013v1 by Ilya Ravkin, available from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

Human U2OS cells cytoplasm–nucleus translocation

ZOI – Segmentation:

- Segmentation based on the DRAQ-channel (nuclei)
- The Ring is used to measure the EGFP signal in the cytoplasm



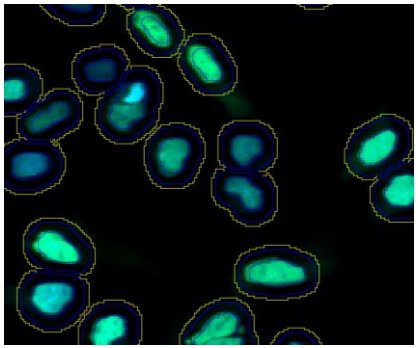


Data set BBBC013v1 by Ilya Ravkin, available from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

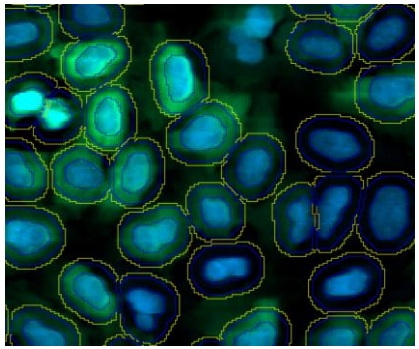
Human U2OS cells cytoplasm–nucleus translocation

ZOI – Segmentation:

- Segmentation based on the DRAQ-channel (nuclei)
- The Ring is used to measure the EGFP signal in the cytoplasm
- Determine measurement features for nuclei and ring around the nuclei:
  - Nucleus Mean Intensity EGFP
  - Ring Mean Intensity EGFP



negative

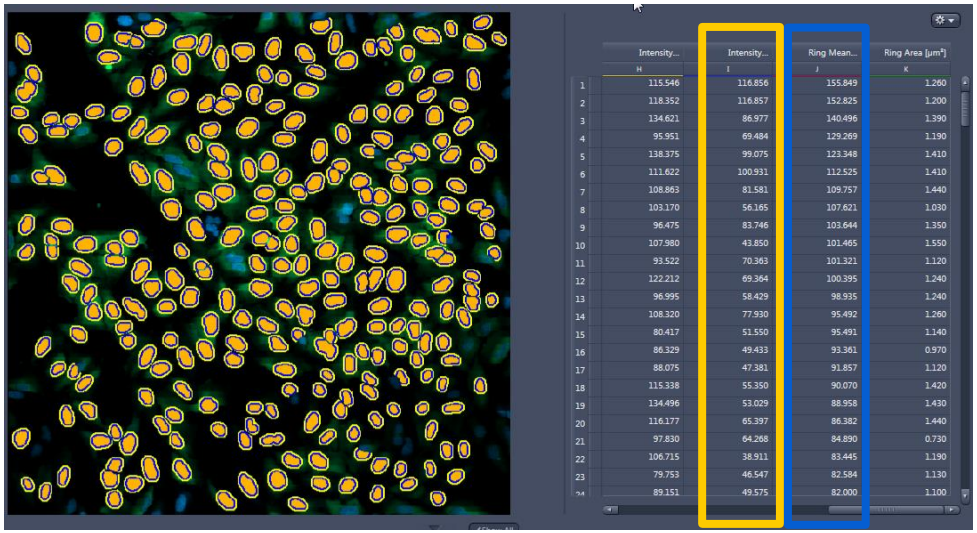


positive

Data set BBBC013v1 by Ilya Ravkin, available from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

Human U2OS cells cytoplasm–nucleus translocation

- Calculate the Translocation-Ratio (T) for each cell
- Calculate the mean value for each well
  (e.g. via OAD and Python)



$$T = \frac{I_{\text{Mean, Nucleus}}}{I_{\text{Mean, Ring}}}$$

Data set BBBC013v1 by Ilya Ravkin, available from the Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012]

OAD Script for automatization

Tasks to perform:

1. Load load the image file (*.csv) and image anlaysis setting (*.czias)
2. Run the image analysis
3. Extract the image analysis results as *.csv
4. Start the python script (test_wellplate_from_ZEN.PY)

5. Read in data
6. Calculate the translocation Ratio
7. Generate heatmaps for different features (e.g. Translocation Ratio)
8. Save heatmaps as PNG files

9. Load PNG files in ZEN

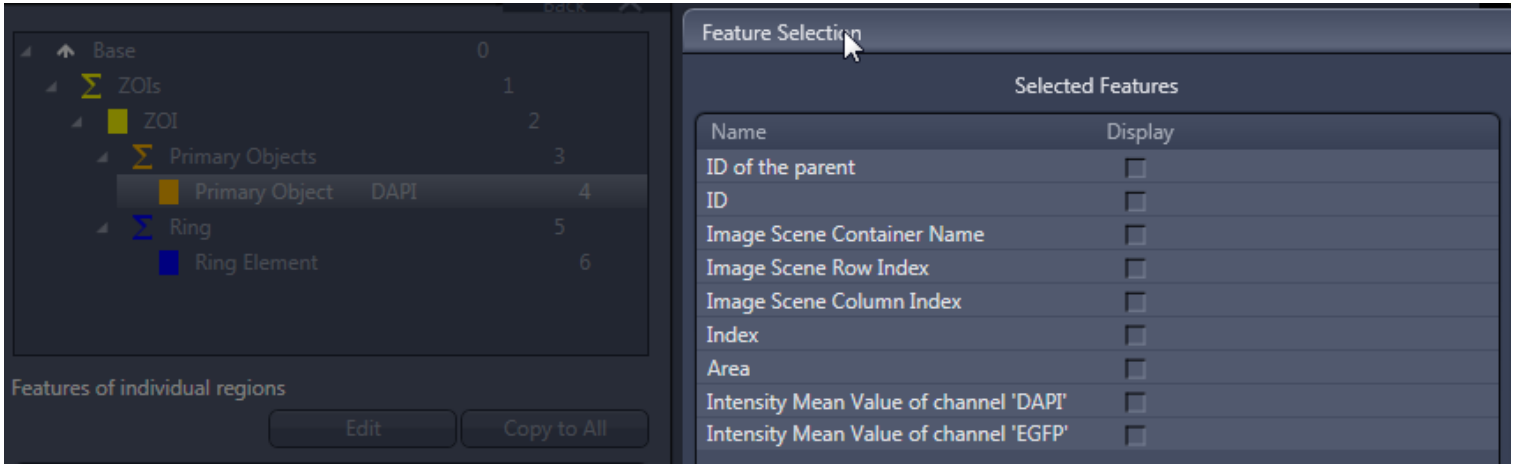
OAD Script

```
from System.Diagnostics import Process
from System.IO import File, Path, Directory
import time

# define the external plot script or tool
pythonexe =  r'C:\Anaconda3\python.exe'
script = r'C:\...\test_wellplate_from_ZEN.PY'


# load image and add it to ZEN
image_to_analyze = r'C:\...\Translocation_comb_96_5ms.czi'
image = Zen.Application.LoadImage(image_to_analyze)
Zen.Application.Documents.Add(image)

# get the image path
outputpath = Path.GetDirectoryName(image_to_analyze)
resultname = Path.GetFileNameWithoutExtension(image.Name)
```

OAD Script: Run Image Analysis

Features defined in the image analysis setting:
ID parent | ID | Image Scene container Name | Image Scene Row | Image Scene Column | Index # | Area | NucMeanDapi | NucMeanGFP | RingMeanGFP | RingArea

```
# Load image analysis setting and perform image anlaysis
iasfilename = r'C:\...\Image Analysis Settings\Translocation_2.czias'
ias = ZenImageAnalysisSetting()
ias.Load(iasfilename)
Zen.Analyzing.Analyze(image,ias)
```



OAD Script: Write CSV File

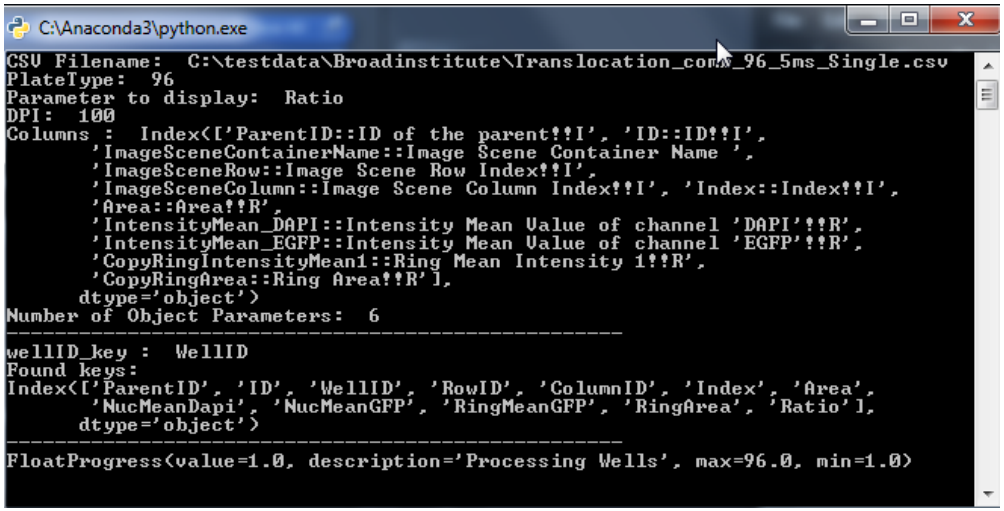
```
# For ZOI-Image Analysis Settings need to get the results for the Primary Objects
# Create data list with results for each primary object
table_single = Zen.Analyzing.CreateRegionTable(image, "Primary Object")
#Zen.Application.Documents.Add(table_single)

# Save both data list as CSV files
table_single_filename = Path.Combine(outputpath,  resultname + '_Single.csv')
table_single.Save(table_single_filename)

# close the image and image analysis setting
image.Close()
ias.Close()
```

	A	B	C	D	E	F	G	H	I	J	K	L
1	ParentID::ID	ID::ID!!I	ImageScene	ImageScene	ImageScene	Index::Index	Area::Area!!	IntensityMe	IntensityMe	CopyRingInt	CopyRingArea::Ring Area!!R	
2							µm²	Gray	Gray	Gray	µm²	
3	269	270	A1	1	1	1	2.64	133.337121	88.0530303	141.167832	1.43	
4	271	272	A1	1	1	2	1.84	79.2880435	0	0.02962963	1.35	
5	273	274	A1	1	1	3	3.04	168.177632	9.81907895	9.22058824	1.36	
6	275	276	A1	1	1	4	2.81	106.231317	38.7935943	46.6071429	1.96	
7	277	278	A1	1	1	5	2.47	146.546559	52.7773279	65.0347222	1.44	
8	279	280	A1	1	1	6	2.01	96.6865672	33.4079602	68.3777778	1.35	
9	281	282	A1	1	1	7	1.58	94.3734177	18.7468354	28.1842105	1.14	
10	283	284	A1	1	1	8	1.81	87.6574586	31.0607735	53.5683453	1.39	
11	285	286	A1	1	1	9	2.25	114.826667	0	0	1.36	
12	287	288	A1	1	1	10	2.72	95.4669118	0.11397059	0.625	1.44	
13	289	290	A1	1	1	11	1.95	96.4564103	13.2974359	22.0735294	1.36	
14	291	292	A1	1	1	12	2.23	104.753363	0.07623318	0.10218978	1.37	

OAD Script: Start Python with Parameters

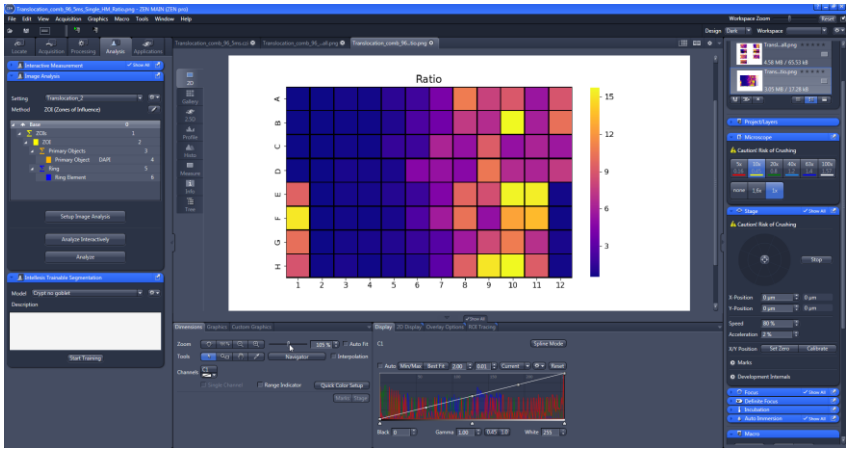
```
# define the actual CSV file and the parameters
csvfile = Path.Combine(outputpath, table_single_filename)

# this depends on the actual CZIAS and the import of the CSV table in python
#parameter2display = 'CellCount'
parameter2display = 'Ratio'
params = ' -f ' + csvfile + ' -w 96' + ' -p ' + parameter2display + ' -sp False -dpi 100 -
xlsx True'

# start the data display script as an external application
Process.Start(script, params)
```

→Jupiter Notebook



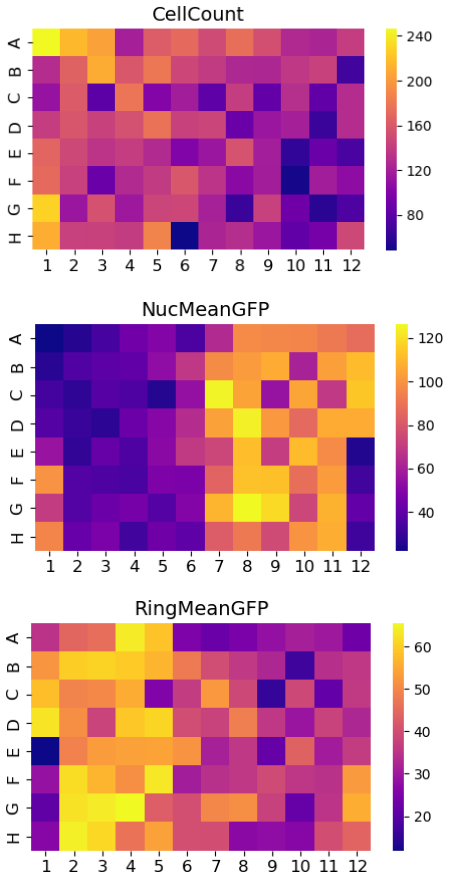
OAD Script: Load PNG Files

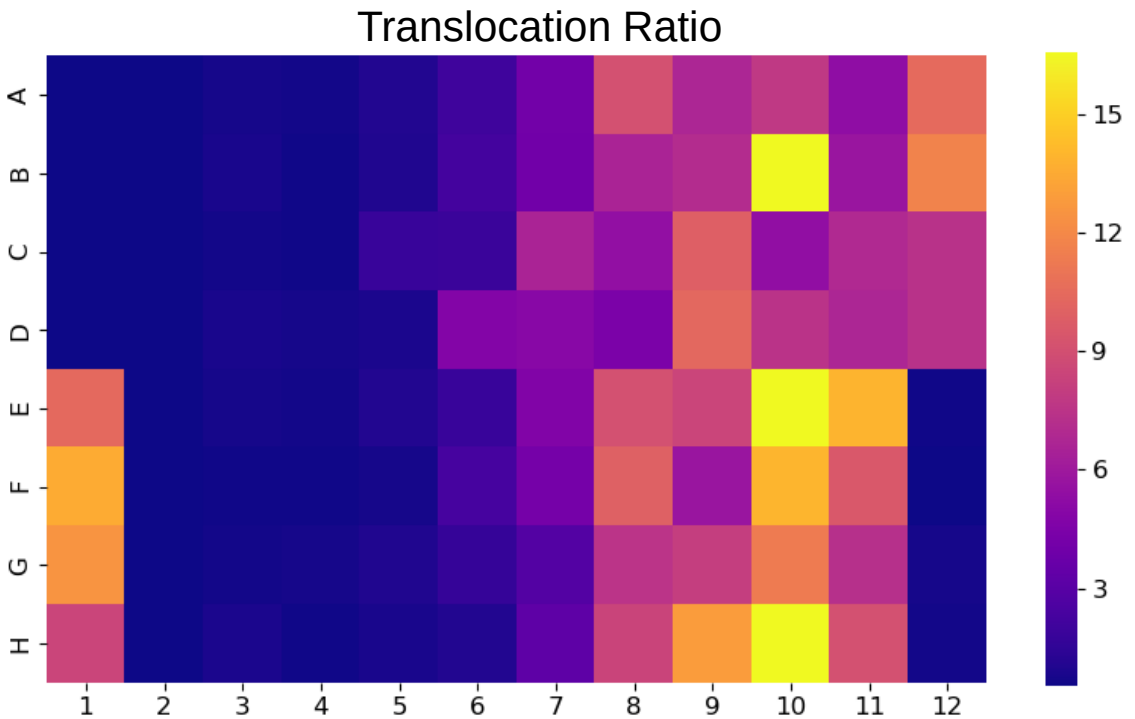
```
# define filenames of PNG files
savename_all =  Path.Combine(Path.GetDirectoryName(image_to_analyze),
Path.GetFileNameWithoutExtension(image_to_analyze) + '_Single_HM_all.png')
savename_single = Path.Combine(Path.GetDirectoryName(image_to_analyze),
Path.GetFileNameWithoutExtension(image_to_analyze) + '_Single_HM_' + parameter2display +
'.png')
print 'Showing saved figure in ZEN.'

# Check if fileneame exists, and Load images in ZEN
if File.Exists(savename_all):
    plotfigure1 = Zen.Application.LoadImage(savename_all, False)
    plotfigure2 = Zen.Application.LoadImage(savename_single, False)
    Zen.Application.Documents.Add(plotfigure1)
    Zen.Application.Documents.Add(plotfigure2)
else:
    print 'Saved figure not found.'

print 'Done.'
```



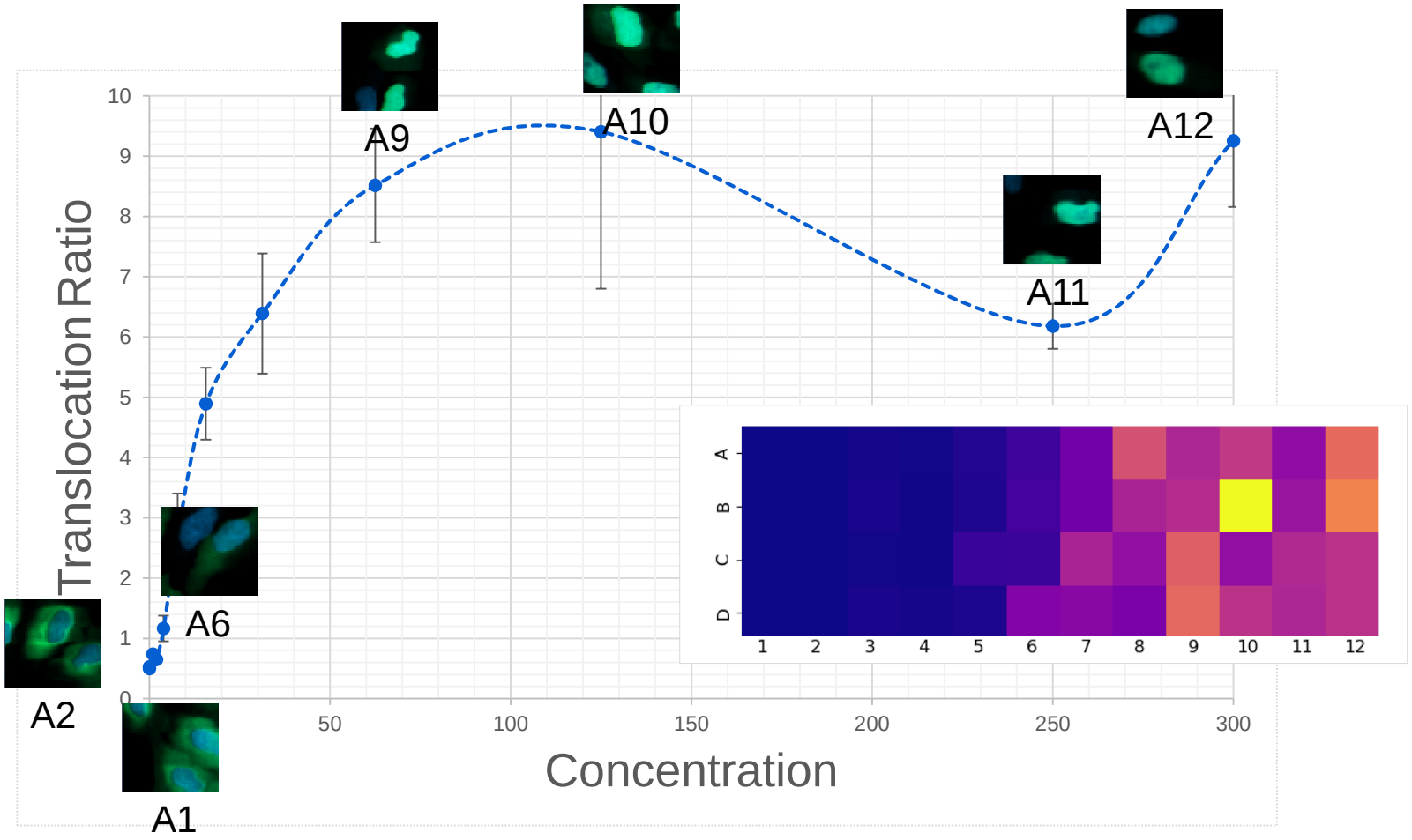
Results (heatmap)

Extract relevant features and plot them as a heatmap for the 96 well plate (e.g. via OAD and Python)





Results (mean translocation ratio)



- Plot results (e.g. via Excel)
- Mean translocation ratio (for rows A to D)

Technical Details

The ZOI Segmentation Method is part of the Image Analysis Module.
The ZOI Segmentation Method is available from ZEN 2.5 (blue edition)

More information on how to set up an Image Analysis Setting using the Zone of Influence Segmentation Method can be found in the ZEN (blue edition) Online Help in chapters:

- Measuring Mean Fluorescence Intensity on a Ring around the Primary Object
- Counting the number of Objects in a Ring around the Nucleus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