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





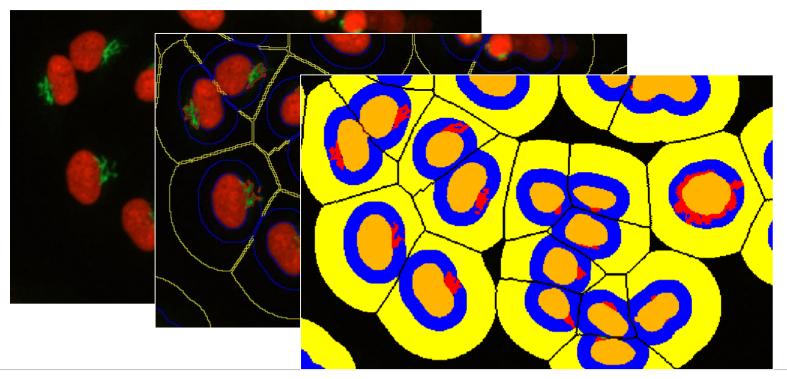
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Product Manager
2018-07-02

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

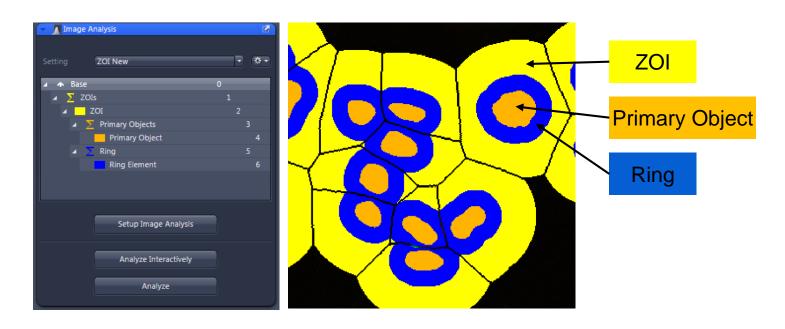


Set up an Image Analysis Setting using ZOI



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



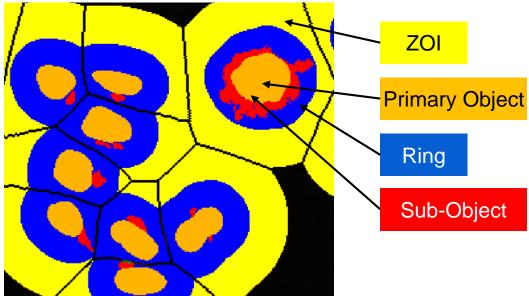
Set up an Image Analysis Setting using ZOI



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- 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





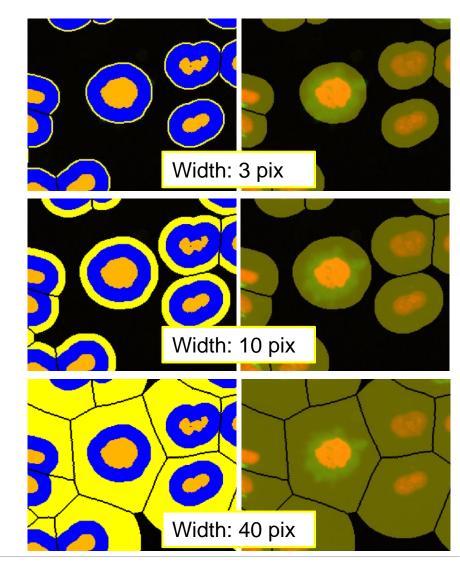
Adjust ZOI Parameters





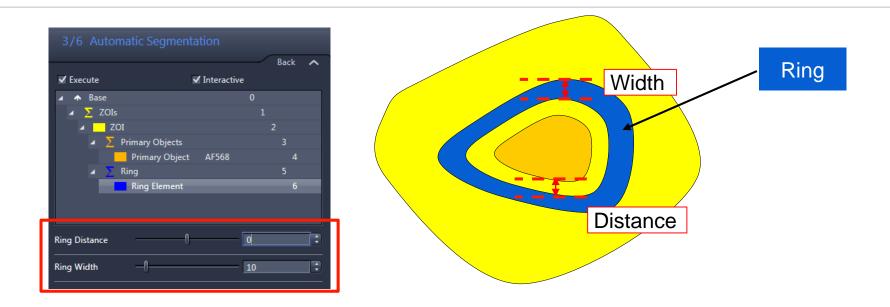
Adjust ZOI Width

- 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

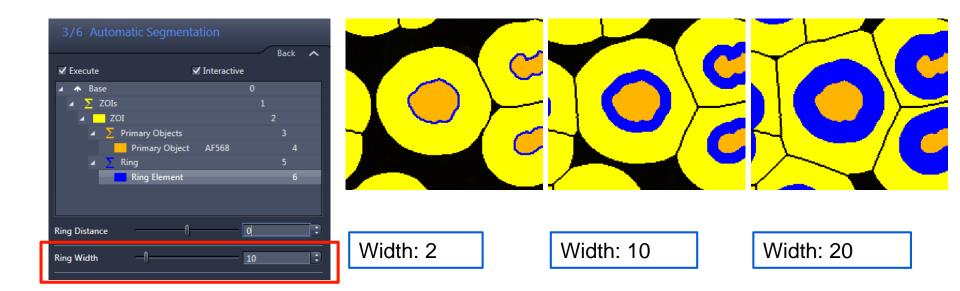




Flexible definition of the Ring Distance and Ring Width

Adjust Ring Parameters

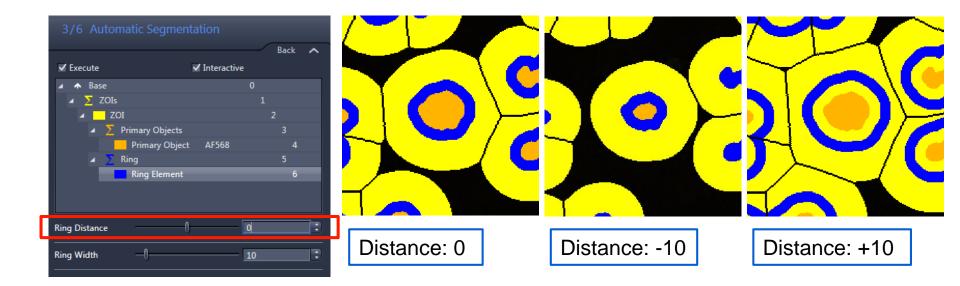




Flexible definition of the Ring Width (in pixel)

Adjust Ring Parameters





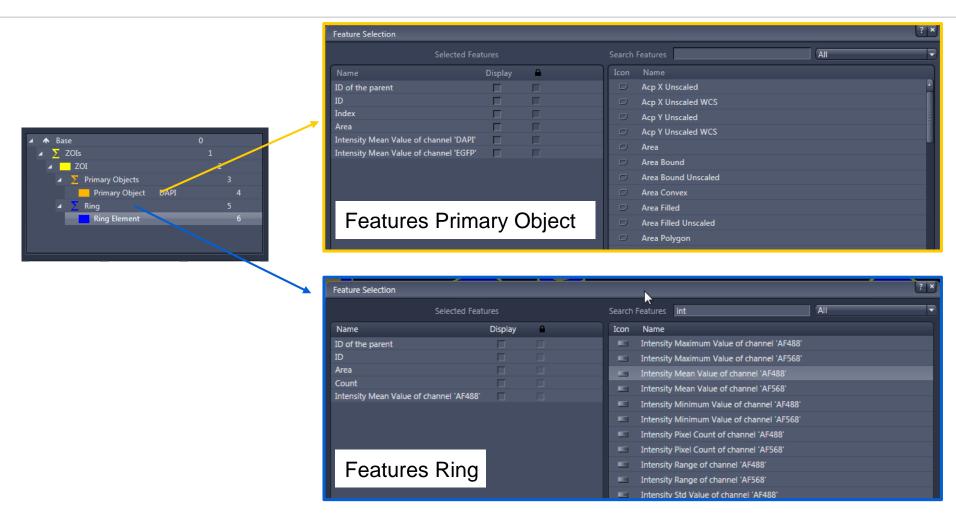
Flexible definition of the Ring Distance (in pixel)

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



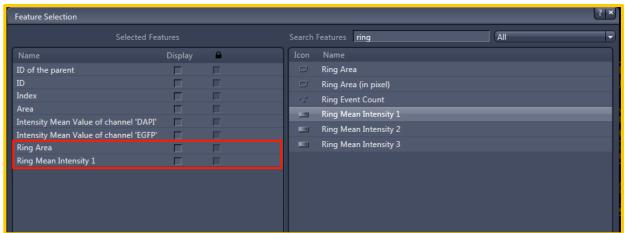


Attribute Features to Primary Object and Ring.

Attribute Ring-Features to Primary Object







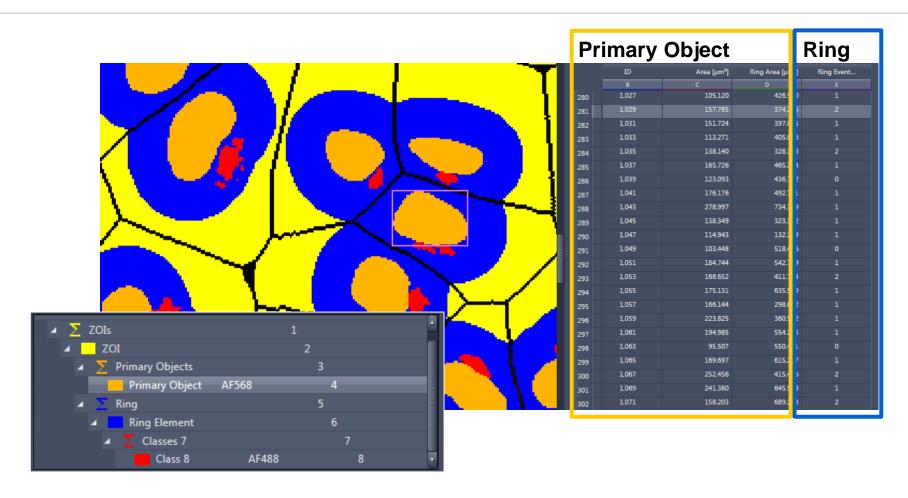
Add Ring-Features to the Primary Object

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

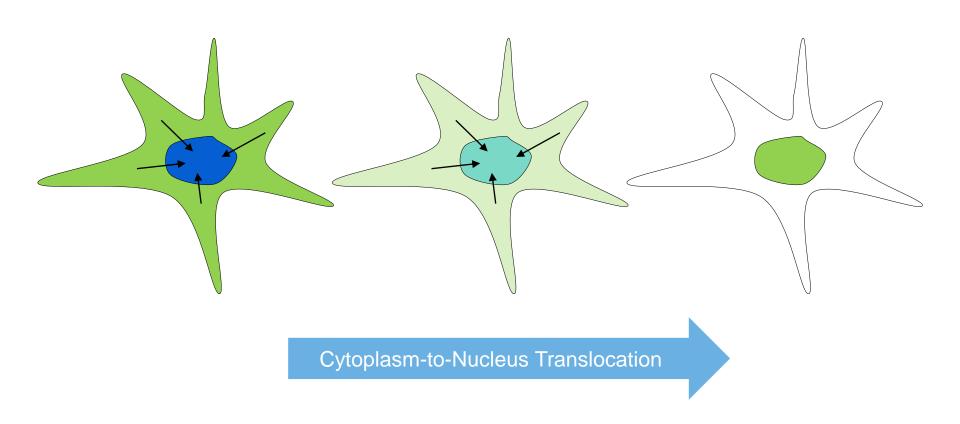




The resulting feature list for the Primary Object also contains the selected features of the Ring (e.g. number of Sub-Objects (red) detected on the Ring)

Application Example: Translocation Assay



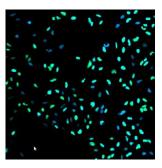


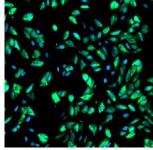


- 96-well plate, human osteosarcoma cells (U2OS), nuclei stained with DRAQ
- Cytoplasm to nucleus translocation of the Forkhead (FKHR-EGFP) fusion protein
- In proliferating cells, FKHR is localized in the cytoplasm (constantly moving into the nucleus, but is transported out again by export proteins).
- 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
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	1	2	3	4	5	6	7	8	9	10	11	12
Α	Neg. Ctrl	39688	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
В	Neg. Ctrl	empty	0.977	1.95	3.91	7.81	15.63	31.25	62.5	125	250	Pos. Ctrl
С	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
Н	Pos. Ctrl	empty	0.31	0.63	1.25	2.5	5	10	20	40	80	Neg. Ctrl
	Wortmannin	in nM		LY294	.002 i	n μM						





negative

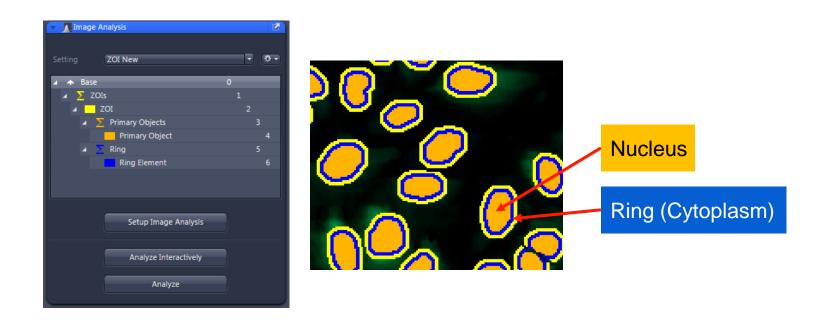
positive

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



ZOI - Segmentation:

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

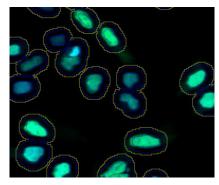


Data set <u>BBBC013v1</u> by Ilya Ravkin, available from the Broad Bioimage Benchmark Collection [<u>Ljosa et al., Nature Methods, 2012</u>]

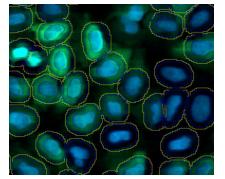


ZOI – Segmentation:

- 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

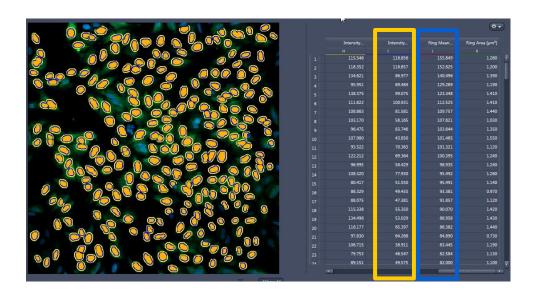


positive

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



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



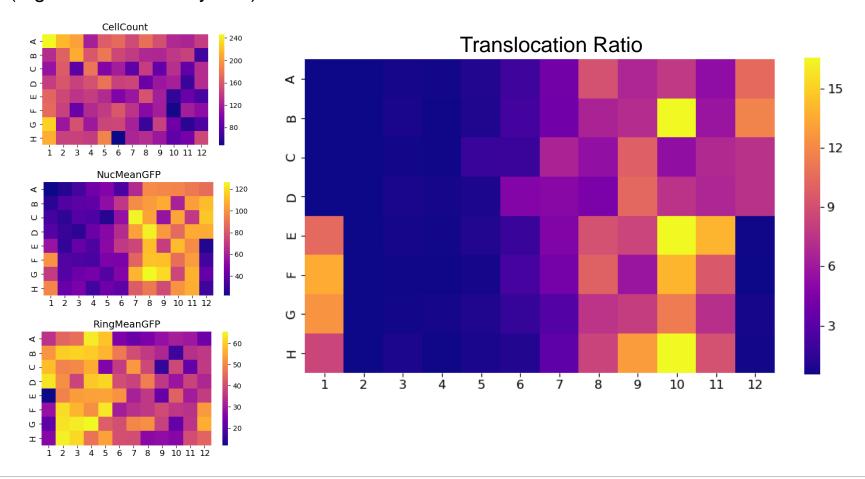
$$T = \frac{I_{\text{Mean, Nucleus}}}{I_{\text{Mean, Ring}}}$$

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

Results (heatmap)

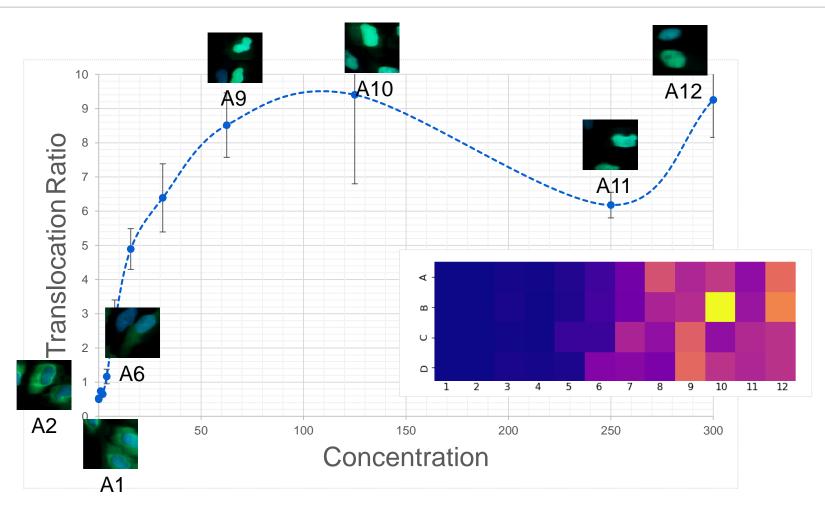


Extract relevant features and plot them as a heatmap for the 96 well plate (e.g. via OAD and Python)



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 a 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

