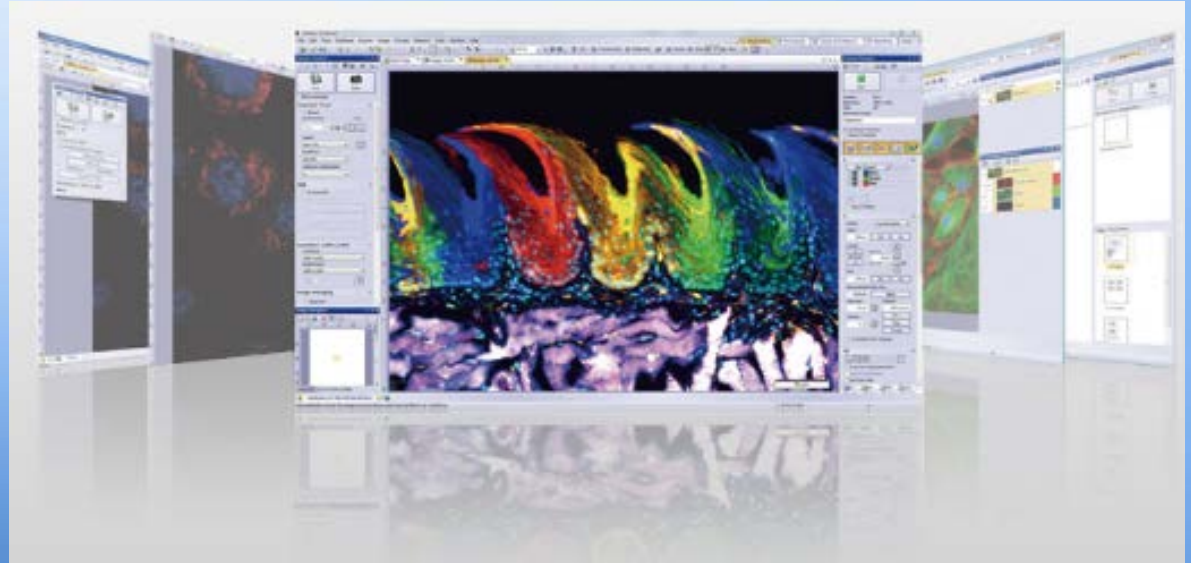


OLYMPUS

Sheng Le MBBS PhD
Product Manager
Olympus Australia



25th Jul 2019

WIMR

Dimension
cellSens





Understand parameters




Structure and parameters of the images

- Formats (VSI, TIF, TIFF, JPEG...)
- Layers (multichannel image, over view, ROIs)
- Channels (FL,BF....)
- Colour (Colour image, monochrome image, pseudo colour)
- Intensity
- Resolution (dpi, ppi..)




Structure and parameters of the images


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- New ▶
- Open ▶
-  Close Ctrl+W
-  Close All Ctrl+Alt+W

-  Save Ctrl+S
-  Save As... Ctrl+Shift+S
- Export to OME...
- Export Image...**
-  Batch Convert...

- Export to ▶

-  Page Setup...
-  Print Preview
-  Print... Ctrl+P

-  Send E-mail...

- Recent Files ▶






- Exit Alt+F4


Export Image ? X

Select layers




Layer	Size (Pixels)	Data Type	File Format (Compression)	Status
<input checked="" type="checkbox"/> 20x	29789 x 29566	16 bit Grayscale	*.tif (None)	OK
<input type="checkbox"/> Overview	17080 x 7482	16 bit Grayscale	*.tif (None)	OK
<input type="checkbox"/> Label	7700 x 9187	24 bit RGB Color	*.tif (None)	OK

Layer settings (20x)






Select channels:

Channel
<input checked="" type="checkbox"/>  TX Red-Tri
<input checked="" type="checkbox"/>  FITC-Tri
<input checked="" type="checkbox"/>  DAPI-Tri

Select frames:

Axis	From	To	Step	Selection	Split
Channel	1	3	1	3/3	No

Output: Raw data Settings...

Write OME meta data to XML-file

File type: Tagged Image File Format (*.tif) Options...

Compression: None

Export to folder

[Empty Folder Name]

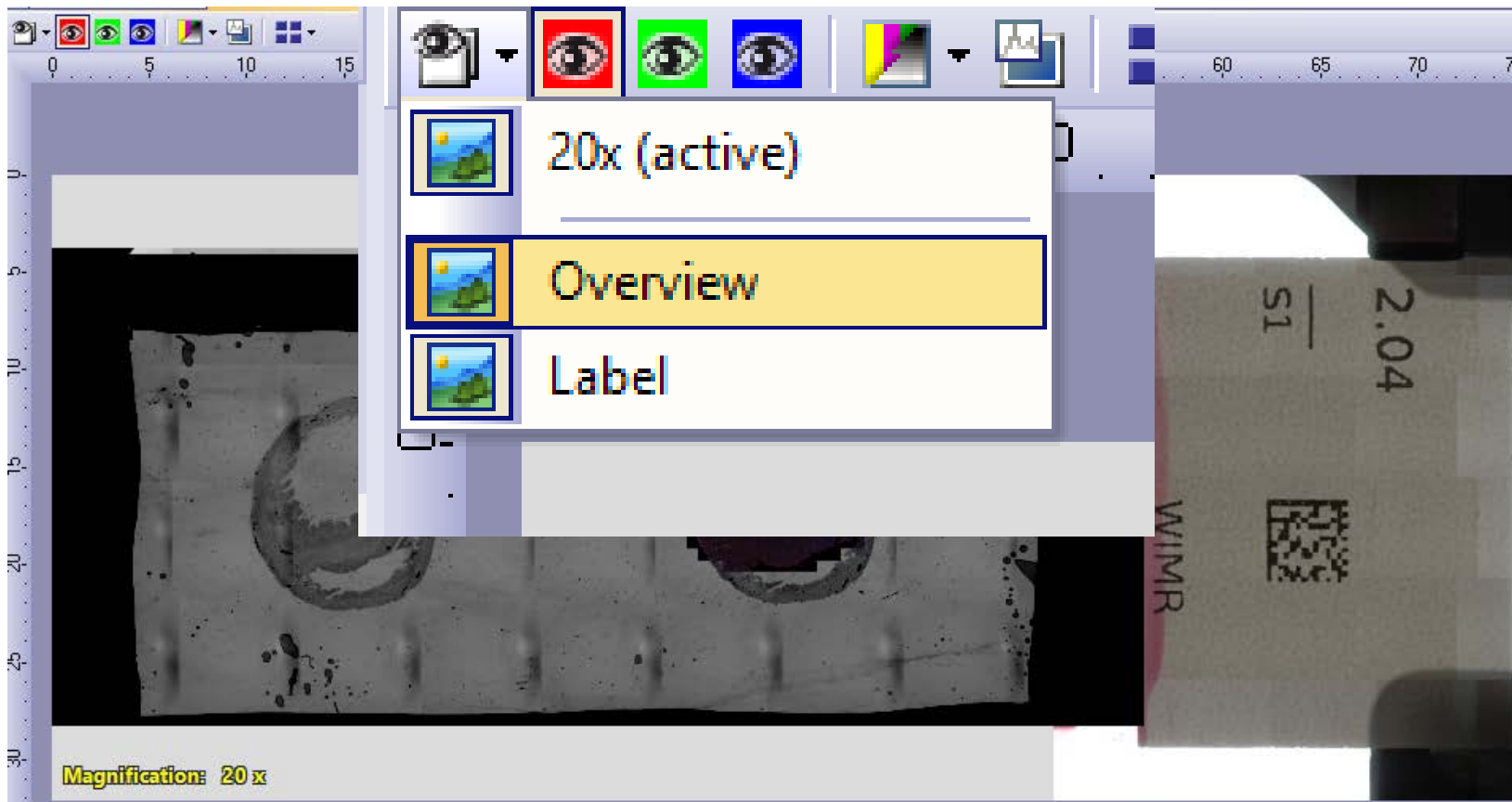
Select...

Overwrite existing files

OK
Cancel

Structure and parameters of the images

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Dimension Selector

	All channels	
	TX Red-Tri	
<input checked="" type="checkbox"/>	FITC-Tri	
	DAPI-Tri	

Position Navig... Camera Control Dimension Sel...

Count and Measure

1 Manual Threshold...

2

Start Page Image_012.04.vsi*

27 28 29 30 31 32 33 34 35 36

Colors

Standard Custom

Colors:

OK Cancel

New

Current

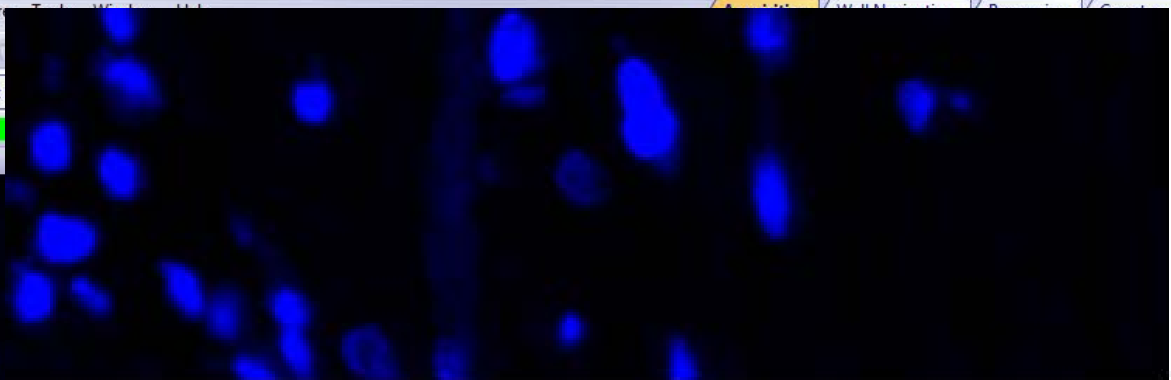
Structure and parameters of the images

- Formats (VSI, TIF, TIFF, JPEG...)
- Layers (multichannel image, over view, ROIs)
- Channels (FL,BF....)
- Colour (Colour image, monochrome image, pseudo colour)
- **Intensity**
- Resolution (dpi, ppi..)

Dimension Selector

All channels		
	TX Red-Tri	17,32
	FITC-Tri	17,34
	DAPI-Tri	17,36

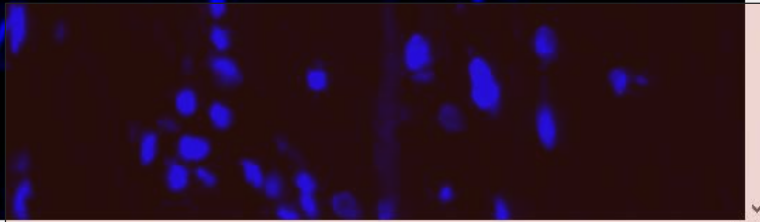
17,32
17,34
17,36
17,38
17,4
17,42
17,44
17,46
mm



100 % (20x) 34928.49 ; 17420.84 DAPI-Tri: 5500

Magnification: 20 x

35 35,02 35,04 mm



Dimension Selector

- All channels
- Channel 1
- Channel 2
- Channel 3
- Channel 4

Adjust Display

Histogram

Zoomed

Min: 0 Max: 27125

Mean Intensity: 1817.69

Pixel Count: 88,773

Fixed Scaling

Left: 927 Right: 2999

Auto Contrast

Left: 0.1 % Right: 0.1 %

Histogram of all frames

Exclude spikes in histogram

Apply Default

Display Enhancement

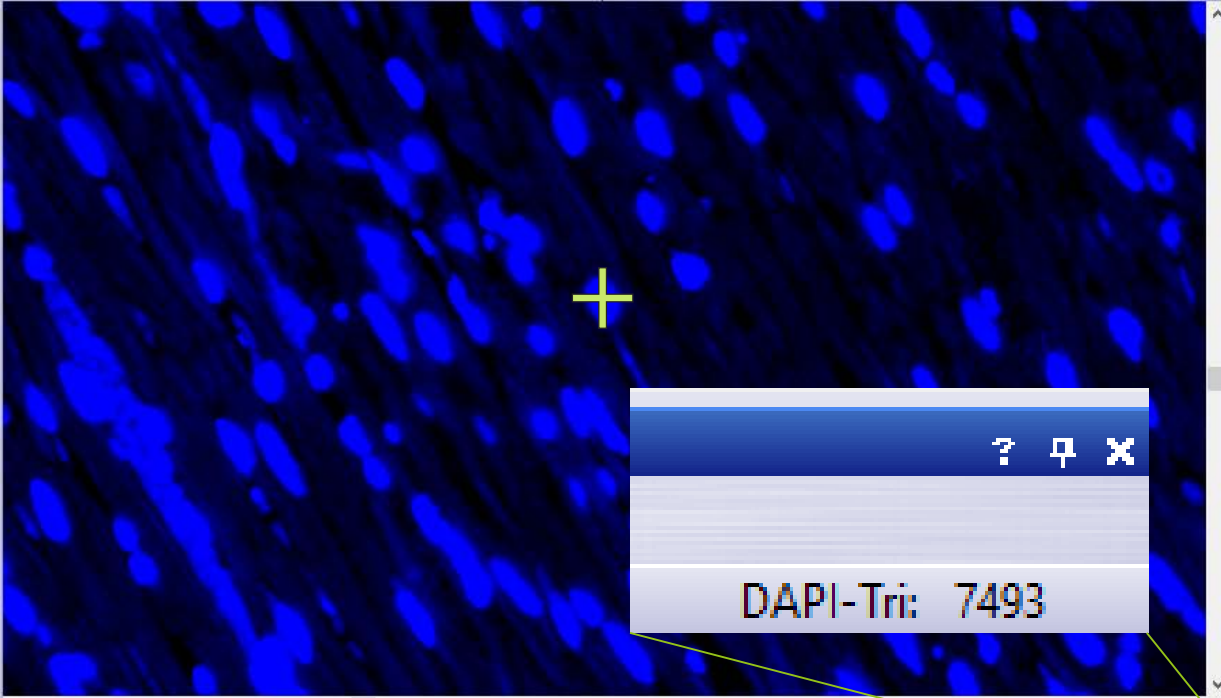
Brightness: 50

Contrast: 50

Gamma: 1.0

Snap-23_ORG.tif

4 34.42 34.44 34.46 34.48 34.5 34.52 34.54 34.56 34.58 34.6 34.62 34.64 34.66 mm



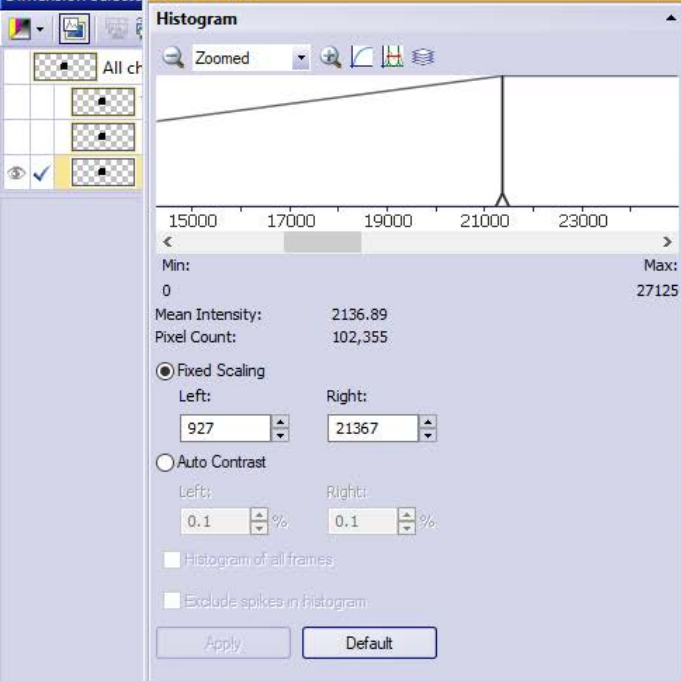
DAPI-Tri: 7493

Camera Control

Gallery

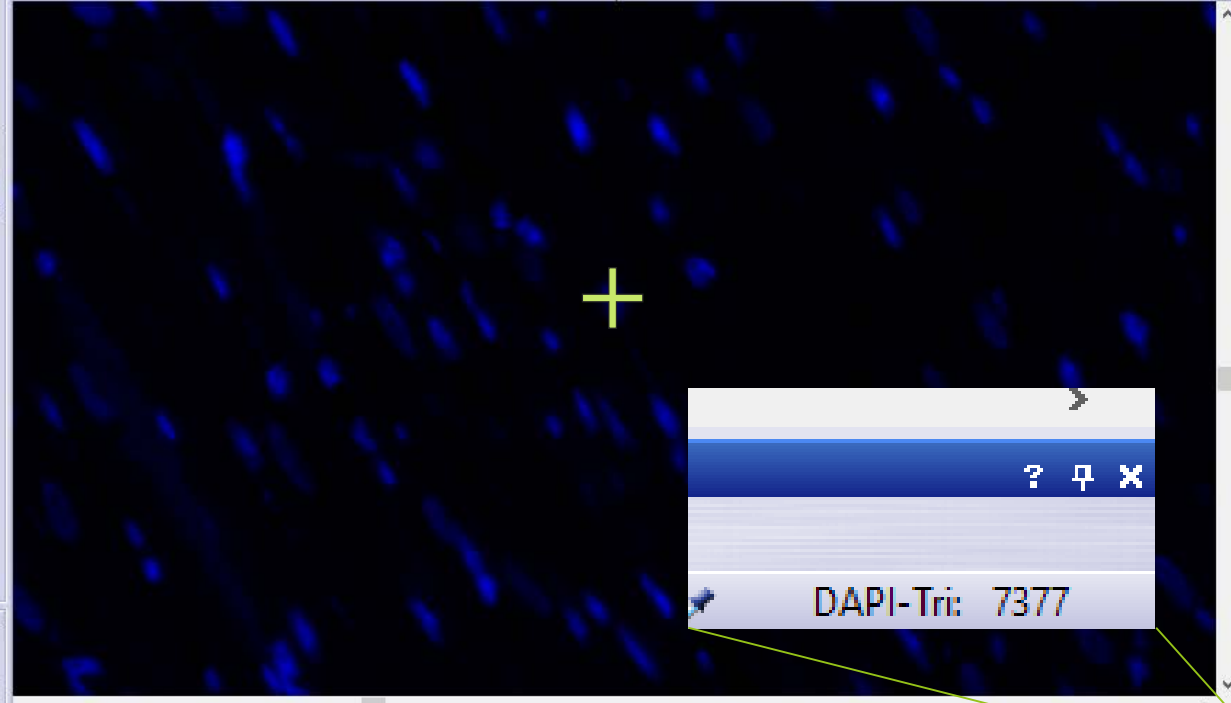
Ready

Adjust Display



Snap-23_ORG.tif

34.4 34.42 34.44 34.46 34.48 34.5 34.52 34.54 34.56 34.58 34.6 34.62 34.64 34.66 mm



DAPI-Trit: 7377

Camera Control

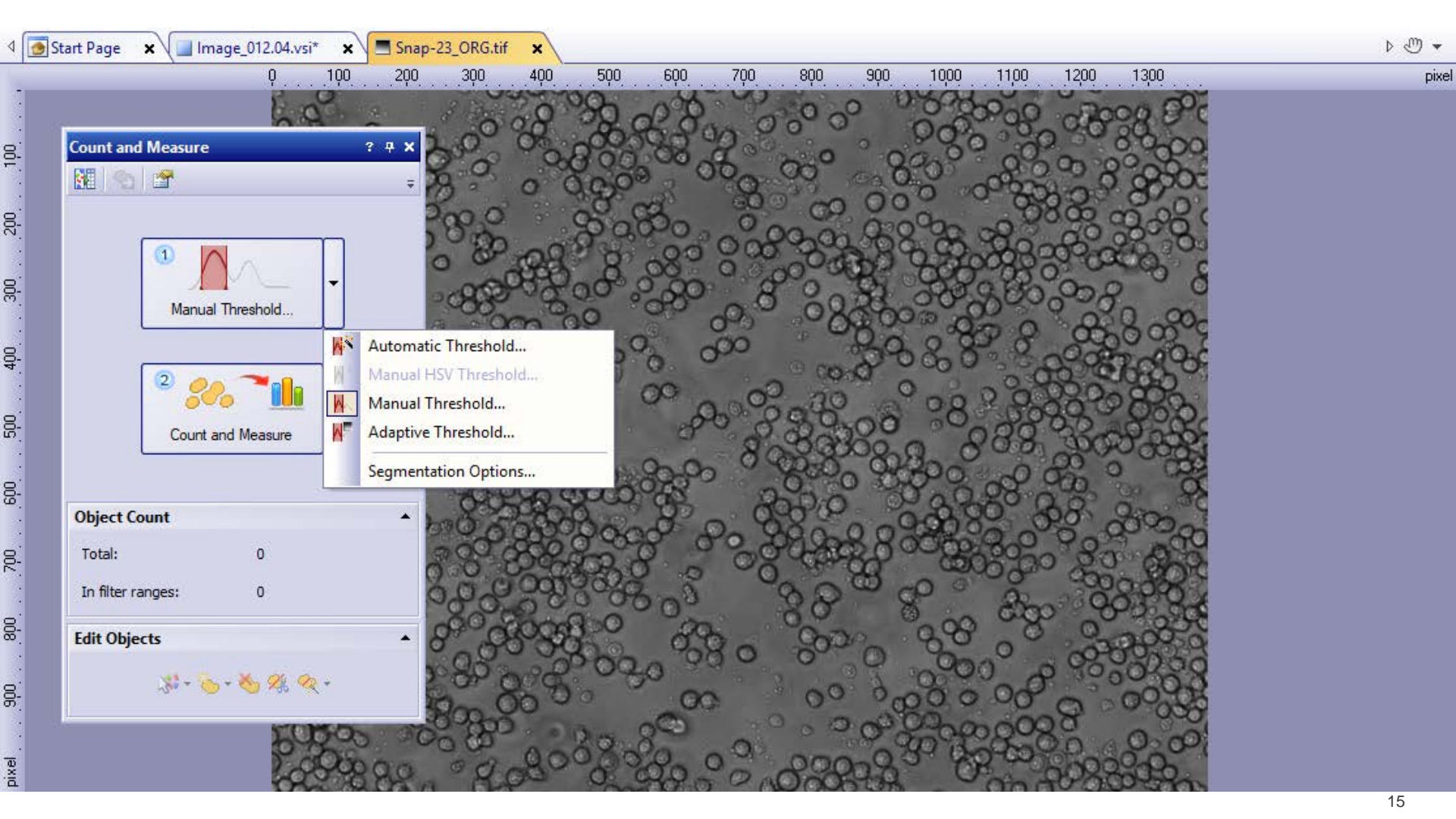
Gallery

Ready

100% (20x) 34539.64 ; 17568.24 DAPI-Trit: 7377



Strategies to analyze your image



Count and Measure

1 Manual Threshold...

2 Count and Measure

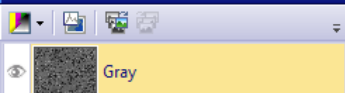
- Automatic Threshold...
- Manual HSV Threshold...
- Manual Threshold...
- Adaptive Threshold...
- Segmentation Options...

Object Count

Total:	0
In filter ranges:	0

Edit Objects

Dimension Selector



Start Page x Image_012.04.vsi* x

Count and Measure

Manual Threshold...

Count and Measure

Object Count

Total:	0
In filter ranges:	0

Edit Objects

Measurement and ROI

OLYMPUS cellSens Dimension Online Help

Hide Back Forward Home Print

Contents Index Search

Type in the keyword to find:

threshold

List Topics

Select Topic to display:

- Adaptive threshold values for gray-value images
- Adaptive threshold values for RGB images
- Adjusting the isovalue
- Adjusting the opacity curve
- Automatic threshold value for gray-value images
- Automatic threshold values for RGB images
- Blending settings
- Carrying out a Ratio Analysis
- Carrying out an automatic image analysis
- Carrying out an automatic image analysis on ROIs
- Carrying out an intensity adjustment
- Confuency Checker - Calibration - Calibration settings
- Confuency Checker - Calibration - Cell Size
- Confuency Checker - Calibration - Confuency
- Confuency Checker - Calibration - Count: Threshold
- Define Detection Options - Segmentation
- Dialog box - Automatic Threshold Comput...
- Dialog box - Colocalization
- Dialog box - FRAP Analysis
- Dialog box - FRET Analysis
- Dialog box - Ratio Analysis
- Dialog box - Ratio Analysis
- EFI Processing - Advanced Settings
- Enhancing the segmentation

See also

- [Carrying out an automatic image analysis](#)
- [Carrying out an intensity adjustment](#)

(1) Intensity adaptation

The **Adaptive Threshold** dialog box, enables you to process the image before the **threshold** values are set. You define this preprocessing, in the **Intensity adaptation** group.

What is an intensity adjustment?

When an intensity adjustment is carried out, the different intensities in the image are mutually adjusted. To do this, the mean intensity of the adjoining pixels is determined for each pixel. This mean intensity is then subtracted from the pixel's intensity. When, for instance, the image shows light objects against a dark background, the equally light objects will also appear equally light, after the correction has taken place.

WIMR WORKSHOP 2019.pptx - PowerPoint

WIMR WORKSHOP 2019.pptx...

... image before the intensity adjustment.

... when the intensity adjustment has been carried out, the objects equally light.

Image Process Measure Tools Window Help

Acquisition Well Navigation Processing Count and Measure Reporting Layout

23_ORG.tif x 66% 20x Transmitted Reflected BF GFP

400 500 600 700 800 900 1000 1100 1200 1300 pixel

Adaptive Threshold

Channel: Gray

Preview of phases: None Current All Transparent

Intensity adaptation: Local Global 36

Preview

Phase thresholds for channel 'Gray'

Phase Name	Color	[Min.	Max. [
1	Red	-9070	-425

Histogram for channel 'Gray'

Count and Measure Cancel

on Selec... Measurement and ROI

Q 66% (0.66x)

Count and Measure ? [maximize] [close]

1 Adaptive Threshold...

2 Count and Measure

Object Count

Total:	285
In filter ranges:	285

Edit Objects

- Count and Measure
- Count and Measure on ROI
- New ROI
- Detection Options...**
- Classification Options...
- Adjust Current Classification...

Options ? [close]

- Measure
- Tracking
- Environment
- Simple Layout
- Images
- Scale Bar
- Color Bar
- Info Stamp
- Cross Hair
- Measurement and ROI
- Image Export Filter
- Image Import Filter
- Report Composer
- Digital Reticle
- Workbook
- 3D
- Voxel View
- Slice View
- Count and Measure
 - General
 - Segmentation
 - Detection**
 - Measurements
 - Classification
 - Display
 - Results
 - Position Navigator

Pixel connectivity

Adjacent borders (4) Include diagonals (8)

Detection area

Frame ROI

Borders - frame

Truncate Exclude

Borders - ROI

Truncate Exclude Include

Options

Minimum object size: pixel

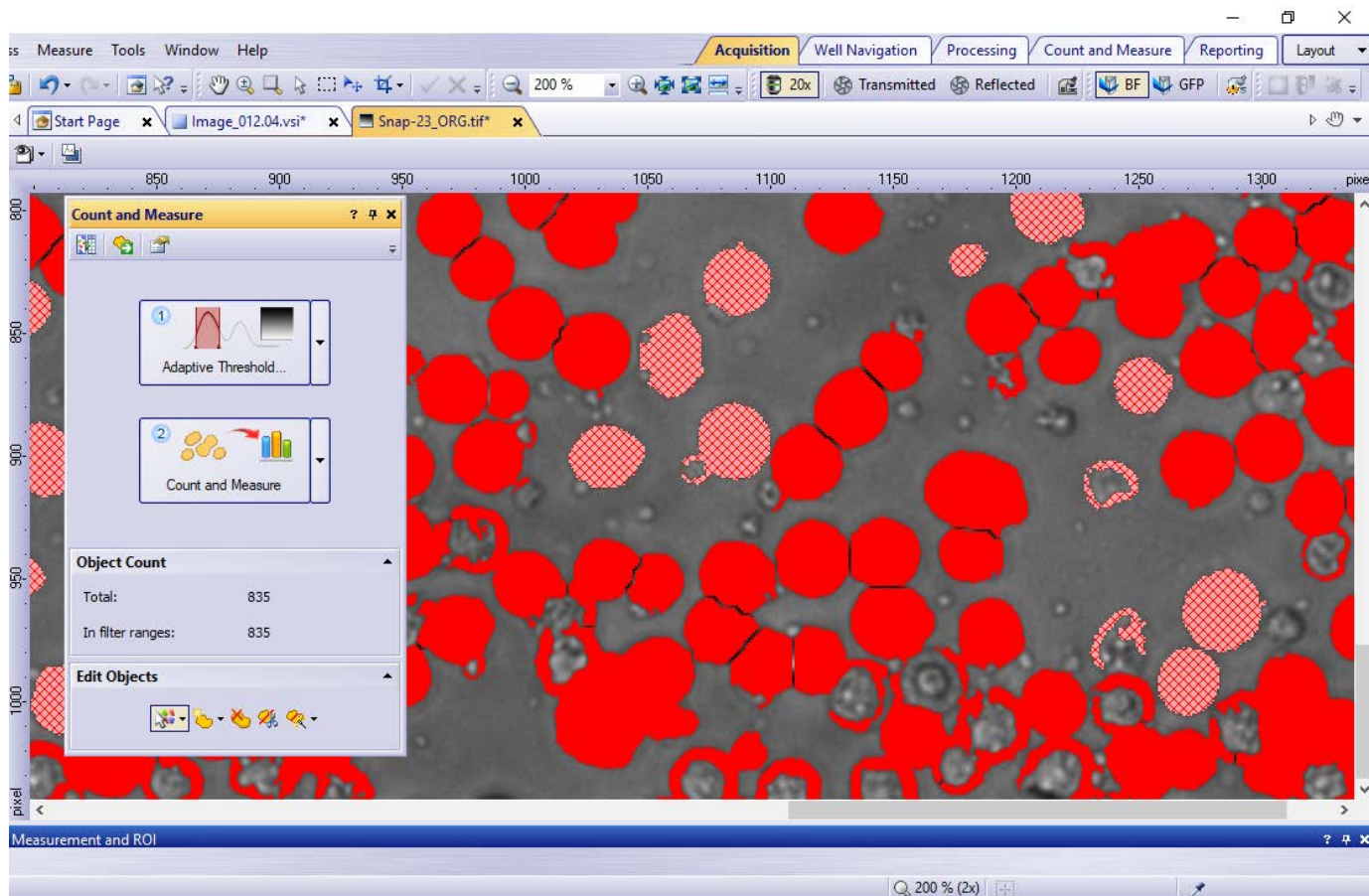
Fill holes

Limit hole size

Maximum hole size: pixel

Get from Active Image

OK Cancel



Auto split
(Watershed)

The screenshot shows the Olympus software interface. On the left, the 'Count and Measure Results' panel displays a table with the following data:

Measurement	[Min.	Max. ^	
Elongation	None	Ni	
Sphericity	None	Ni	
Hole Count [#]	None	Ni	
Aspect Ratio	None	Ni	
Count in filter ranges			835
Mean			1.36
Standard Deviation			0.35

The 'Select Object Measurements' dialog box is open, showing a list of available object measurements. The 'Area' measurement is selected. A preview window shows an orange object with the text 'The area of the object (excluding holes)'. Below the preview, there are checkboxes for 'Measure on convex hull' and 'Measure the orientation'. At the bottom of the dialog, there is an 'Add Area' button and a list of 'Measurements computed for all objects' including Area, Perimeter, Mean (Radius), Mean (Gray Intensity Value), Mean (Color Intensity Value), and Shape Factor.

Select your parameters

Use filters to narrow down your target

The screenshot displays an image analysis software interface. At the top, there are three tabs: 'Start Page', 'Image_012.04.vsi*', and 'Snap-23_ORG.tif*'. Below the tabs is a coordinate grid with x-axis labels from 400 to 1300 and y-axis labels from 600 to 700. The main image area shows a grayscale micrograph of cells with red circles overlaid on them, representing a filtered selection. Below the image is a 'Count and Measure Results' panel. This panel includes a toolbar with icons for various functions and a menu bar with options: 'Object Measurements', 'Object Filter', 'Class Measurements', 'Class Histogram', 'ROI Measurements', and 'ROI Histogram'. The 'Object Filter' menu is currently active, showing a table of filter ranges for various measurements. The 'Area [pixel²]' measurement is highlighted, with a minimum value of 373.8 and a maximum value of 977.25. Below the table, summary statistics are shown: 'Count in filter ranges' is 373, 'Mean' is 604.35, and 'Standard Deviation' is 111.05. To the right of the table is a histogram showing the distribution of the filtered objects' areas. The x-axis is labeled 'Area [pixel²]' and ranges from 0 to 7000. The y-axis is labeled 'Count' and ranges from 0 to 15. The histogram shows a peak count of approximately 15 at an area of about 500 pixel², with a long tail extending to the right.

Measurement	[Min.	Max. [
Area [pixel ²]	373.8	977.25
Perimeter [pixel]	None	None
Mean (Radius) ...	None	None
Mean (Gray Int...	None	None
Mean (Color In...	None	None

Count in filter ranges: 373
 Mean: 604.35
 Standard Deviation: 111.05

Set Classification

OLYMPUS cellSens Dimension

File Edit View Acquire Image Process Measure Tools Window Help

Acquisition Well Navigation Processing Count and Measure Reporting Layout

Count and Measure

Start Page Image_012.04.vsi Snap-23_ORG.tif

Count and Measure Results

Object Measurements Object Filter Class Measurements Class Histogram ROI Measurements ROI Histogram

Filter ranges:

Measurement	[Min.	Max. [
Convexity	0.82	None
Elongation	None	None
Sphericity	None	None
Hole Count [#]	None	None
Aspect Ratio	None	None

Count in filter ranges: 538

Mean: 0.93

Standard Deviation: 0.04

Count and Measure Results

Count

Convexity

Ready

100 % (1x)

View and export your data

OLYMPUS cellSens Dimension

File Edit View Acquire Tools Window Help

Acquisition Well Navigation Processing Count and Measure Reporting Layout

Count and Measure Start Page Image_012.04.vsi* Snap-23_ORG.tif* Count and Measure of Snap-23_ORG.tif*

	Object Class	Sum (Area) [pixel ²]	Area Fractio...	Relative O...	Mean (M...	Mean (...	Mean (S...	Object Count [#]
1	Class 1	9614.00	0.67	8.36	9467.31	-	0.69	45
2	Class 2	299108.00	20.72	86.62	9199.28	-	0.73	466
3								
4								
5								
6								
-	Count	2	2	2	2	-	2	2
-	Minimum	9614.00	0.67	8.36	9199.28	-	0.69	45
-	Maximum	299108.00	20.72	86.62	9467.31	-	0.73	466
-	Mean	154361.00	10.69	47.49	9333.29	-	0.71	255.50

Object Measurements Class Measurements

Count and Measure Results

	Object Class	Sum (Area) [pix...	Area F
1			
2			
3			
4			
5			
6			
7			
8			

Object Count

Total: 0

In filter ranges: 0

Edit Objects

Count in filter ranges: - 0

Mean: -

Standard Deviation: -

Export to Excel

This PC

Organize

This PC

Folders (7)

- 3D Objects
- Desktop
- Documents
- Downloads
- ...

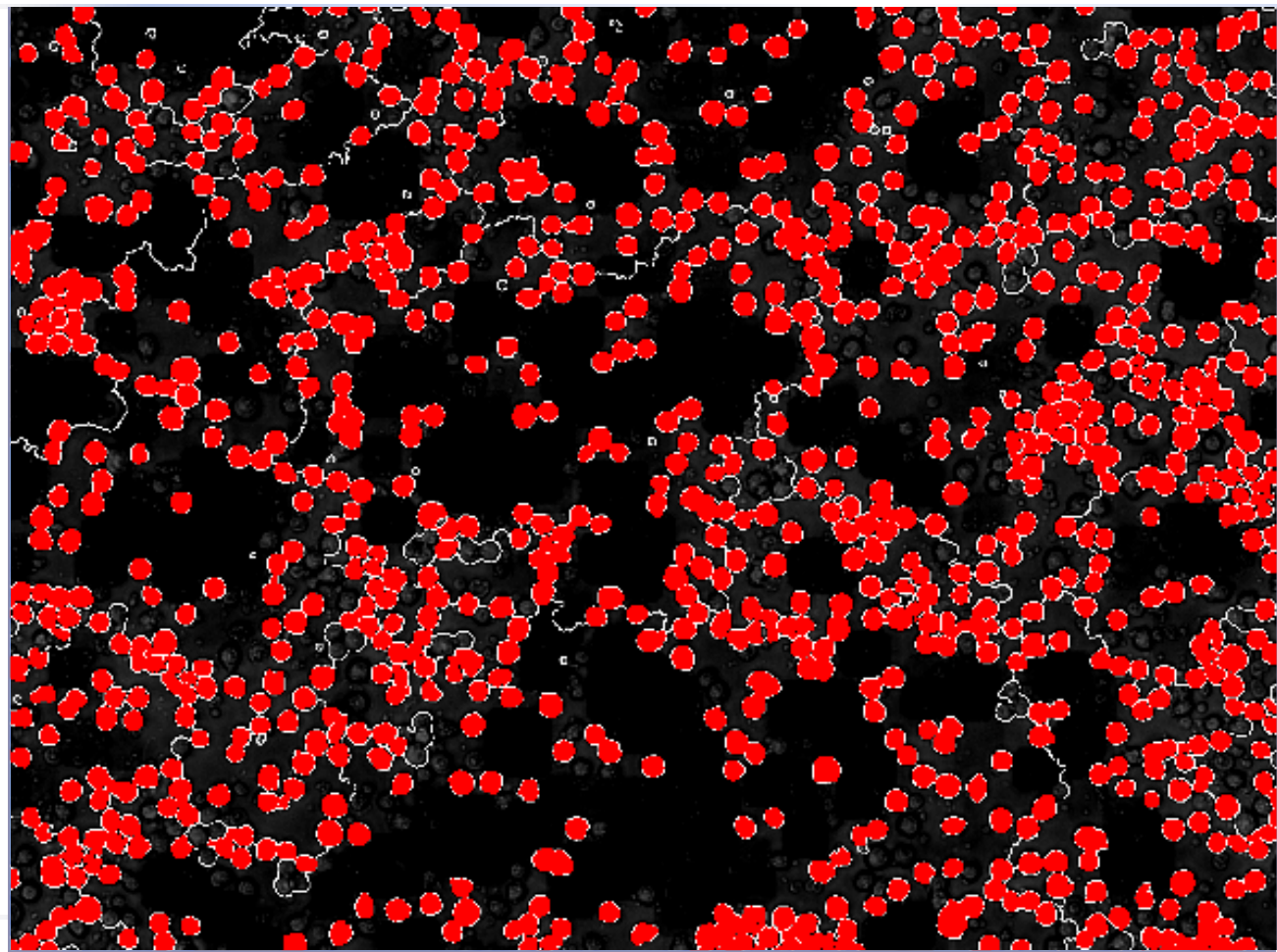
File name: Count and Measure of Snap-23_ORG.tif.xlsx

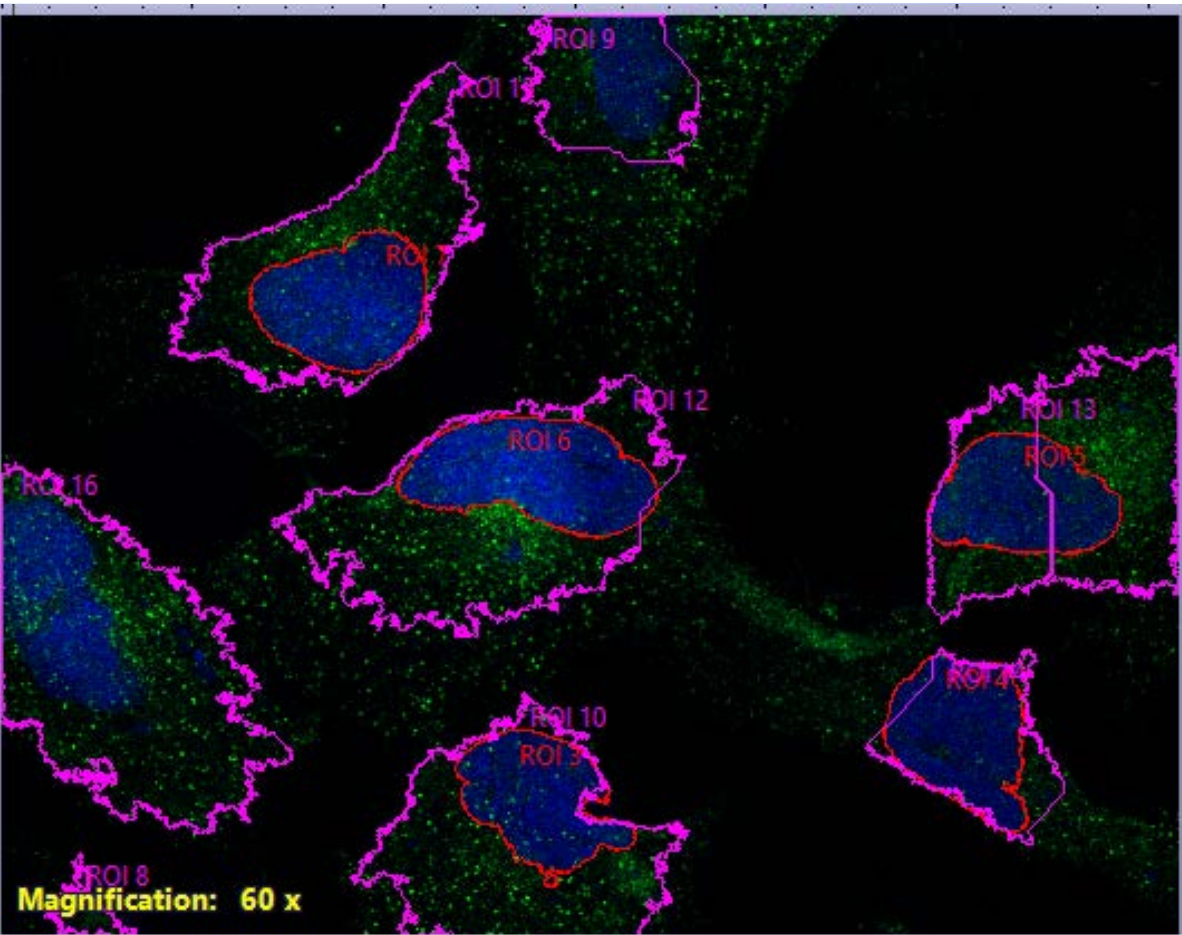
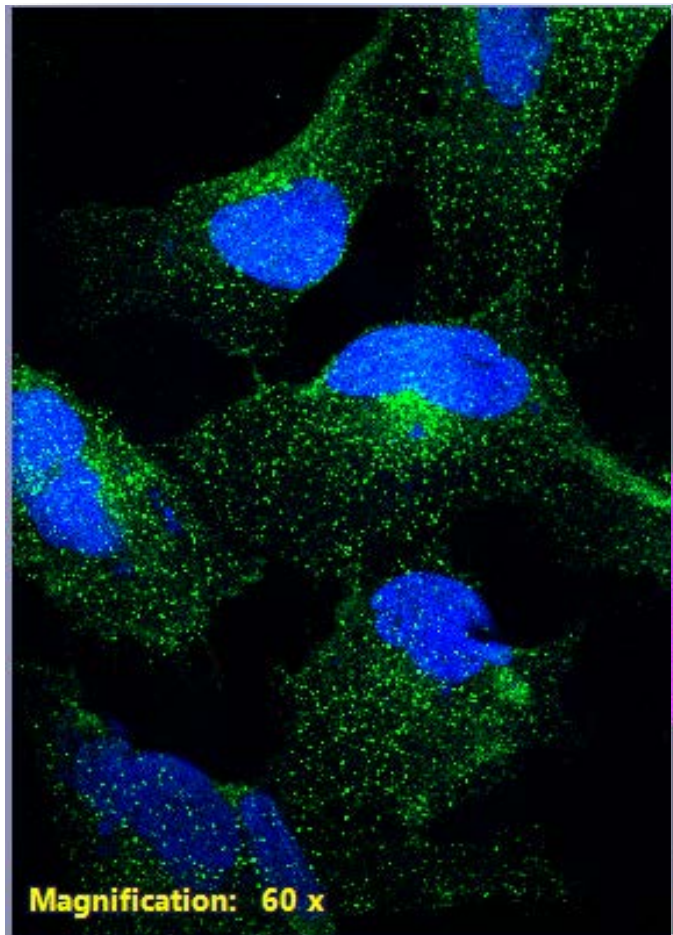
Save as type: Excel Sheet (*.xlsx)

Save Cancel

Camera... Dimensi... Count a... Count and Measure Results Measurement and ROI

Gallery







Thank you.