

Analysis with acquired images from FV1000 and VS120

What we can do with cellSens?

What is image analysis?

Image acquisition

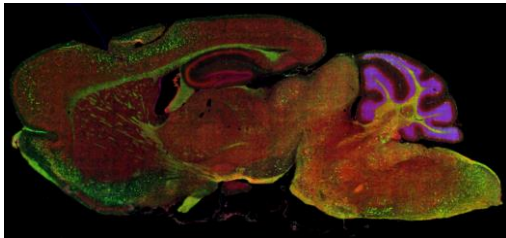


Image processing

Image filters
Background subtraction
Deconvolution
Channel combine
Projection

Image analysis

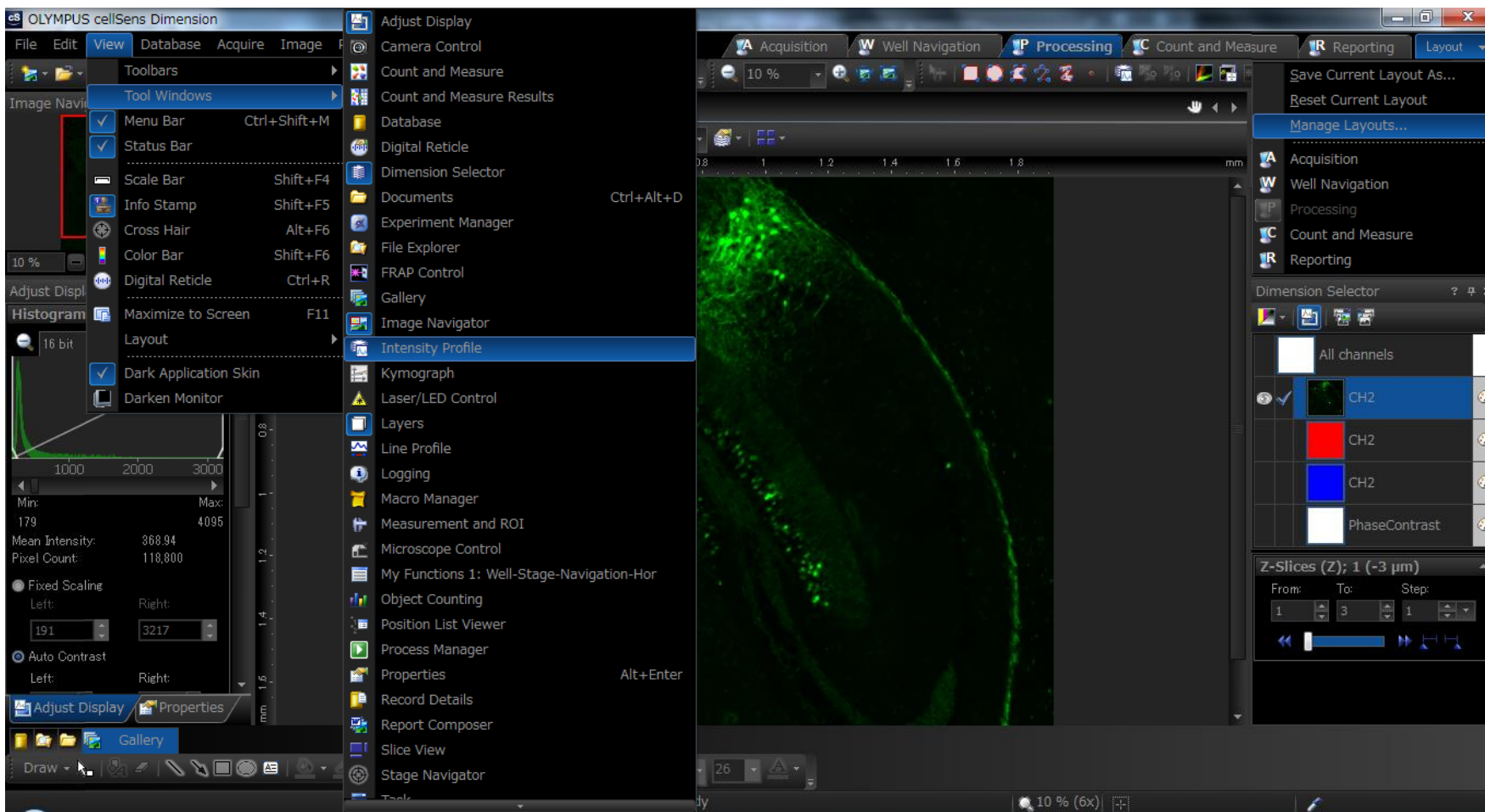
Measurement
Intensity profile
Co-localization
Ratio/FRET
FRAP
Kymograph



Data analysis
Statics analysis

cellSens dimension, is a powerful software for image analysis.

cellSens layout

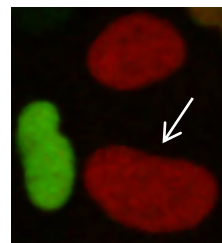


Case 1: Measurement

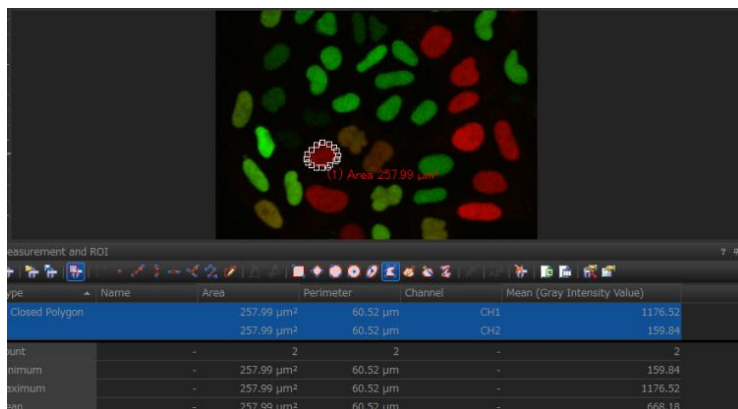


I want to know how big this nuclear is.

I want to know the intensity of this nuclear.

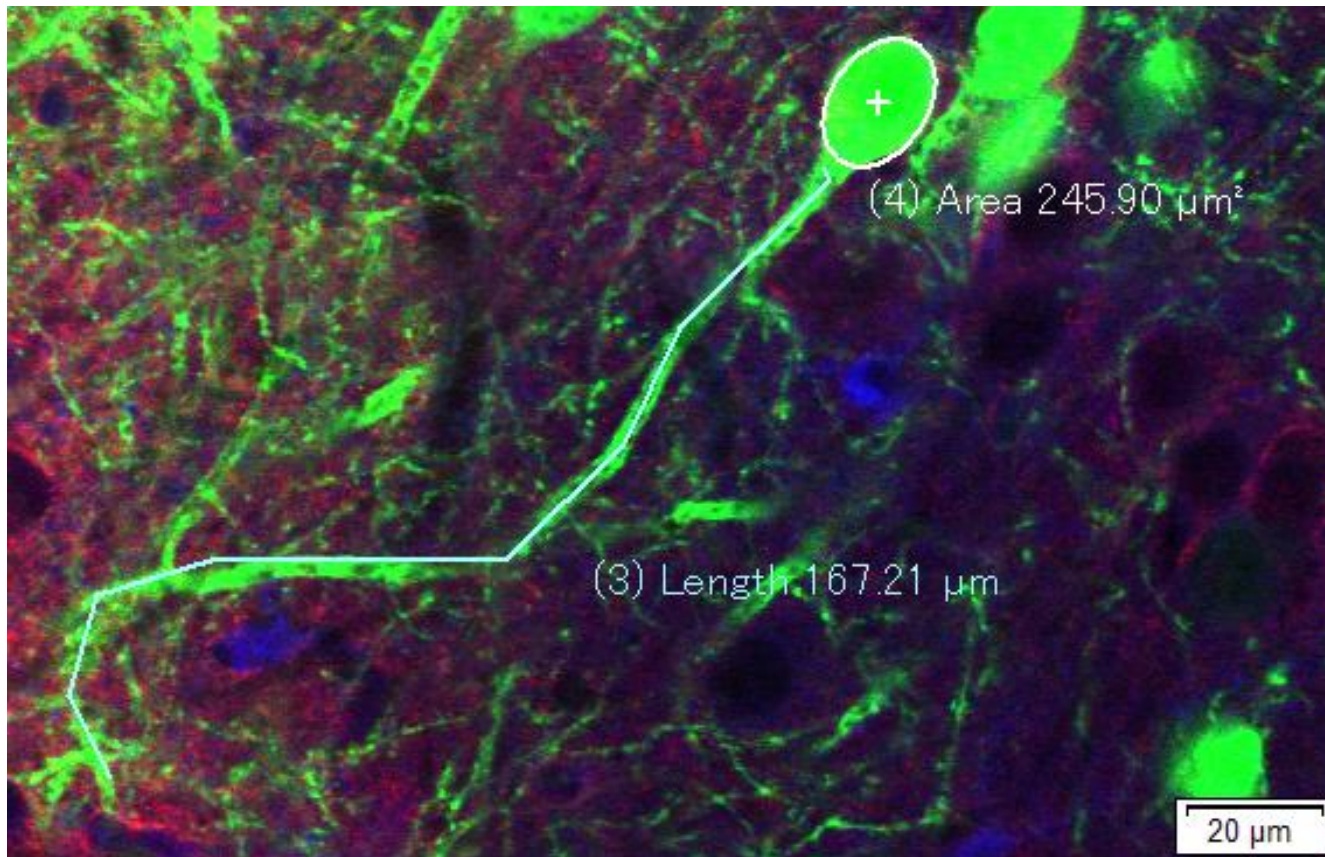


You may use measurement for that.



Basic Measurement

1. Set Measurement objects
2. Edit Measurement objects
3. Select Measurements
4. Export measurement results to Excel





<Operation>

1. Select an Image
2. Open "Measurement and ROI" tool window
Menu bar "View" >> "Tool windows" >> "Measurement and ROI"
3. Select "Measurement and ROI" tab in the tool window.
4. Select a Measurement Object
5. Set the object in selected image (click/move/right-click)
6. Confirm the result displayed in the tool window.

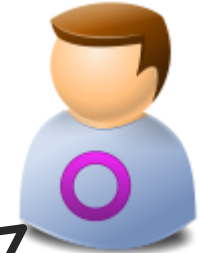
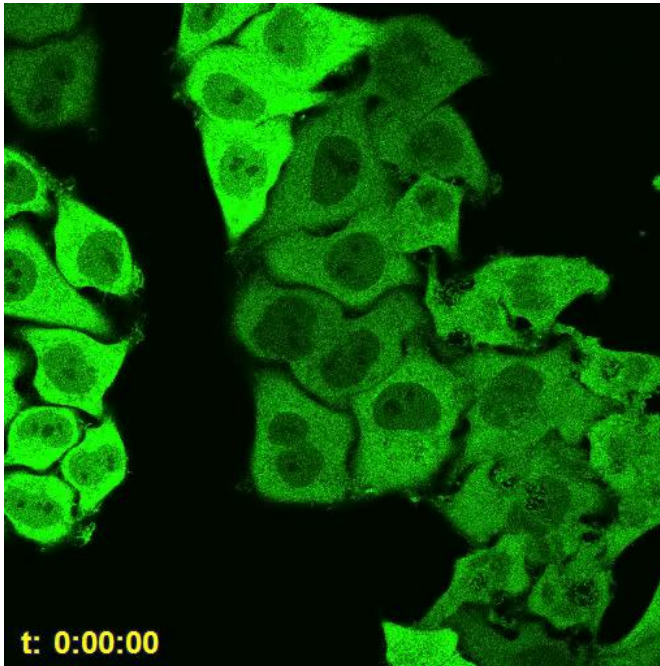
Type	Name	Area	Point X	Point Y	Length	Perimeter
• Point		-	967.61 μm	282.98 μm	-	-
⚙ Polyline		-	-	-	171.43 μm	-
□ Rectangle		691.26 μm^2	-	-	-	103.78 μm
⊗ Rotated Ellipse		268.83 μm^2	-	-	-	59.63 μm
Count		-	2	1	1	1
Minimum		-	268.83 μm^2	967.61 μm	282.98 μm	171.43 μm
Maximum		-	691.26 μm^2	967.61 μm	282.98 μm	103.78 μm
Mean		-	480.04 μm^2	967.61 μm	282.98 μm	171.43 μm

Taskbar: Object Counting | File Explorer | Measurement an... | Gallery | Count and Measu... | Documents | Database | Intensity Profile

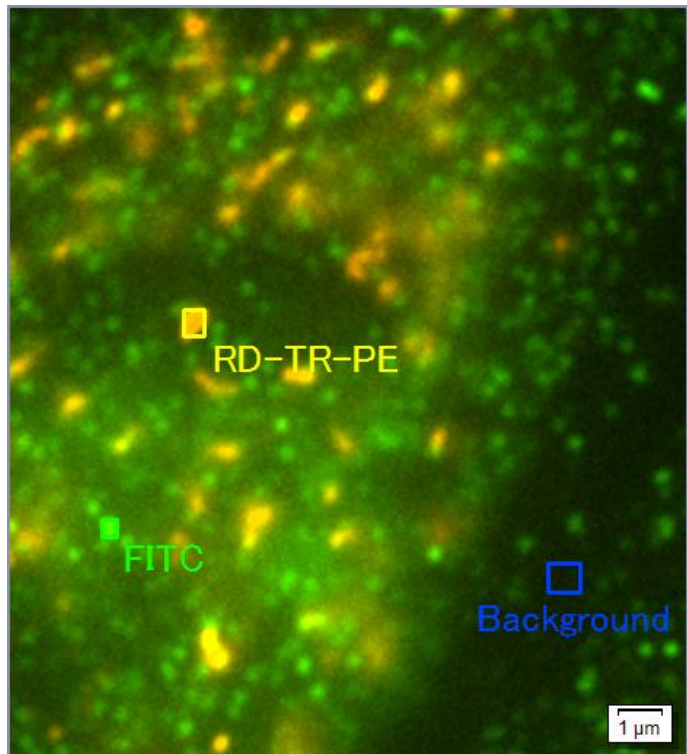
Case 2: Intensity profile



I want to analyze
this movie.



Do you mean the
intensity changes in
the movie?



<Operation>

1. Select an image.
2. Set ROIs to the image
(Better to set one region for background)
3. Click “Intensity Profile” button in Life Science Applications toolbar

Menu bar “View” >> “Toolbars” >> “Life Science Applications”

Or select “Intensity Profile”

Menu bar “Measure” >> “Intensity Profile”

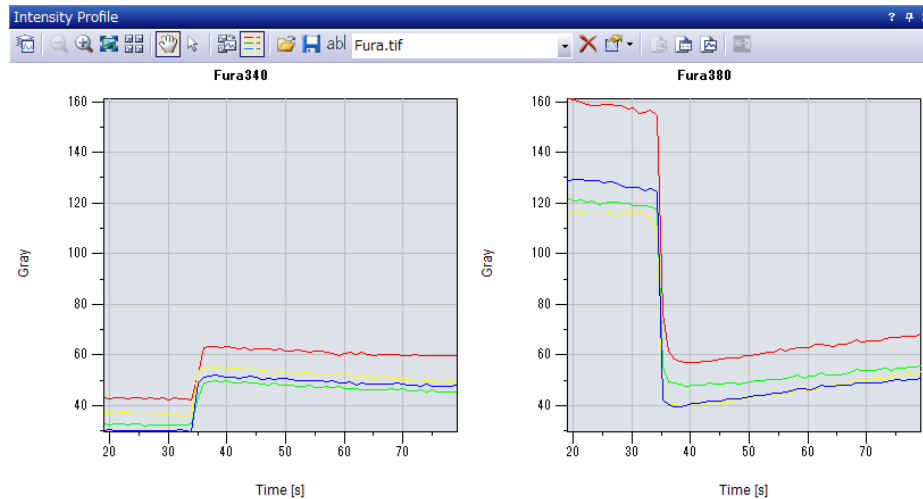
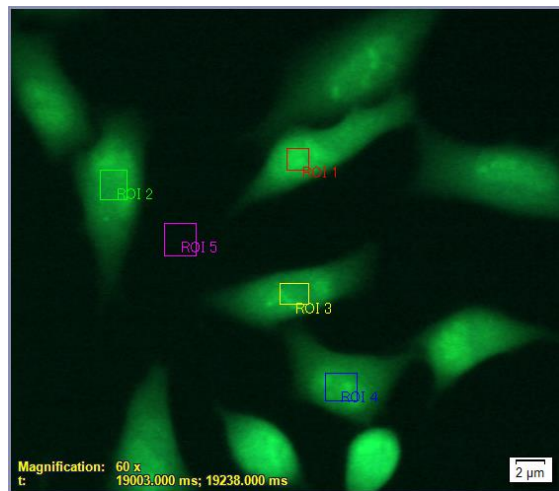
Or

Menu bar “View” >> “Tool Windows” >> “Intensity Profile”

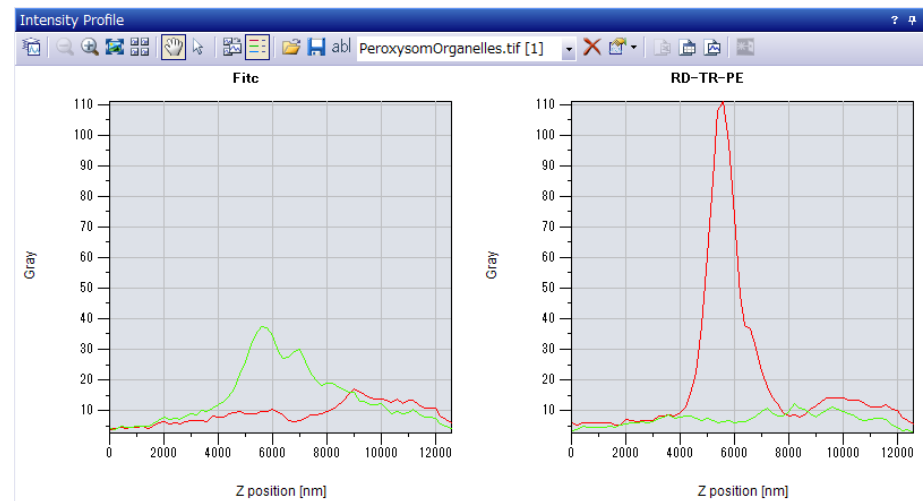
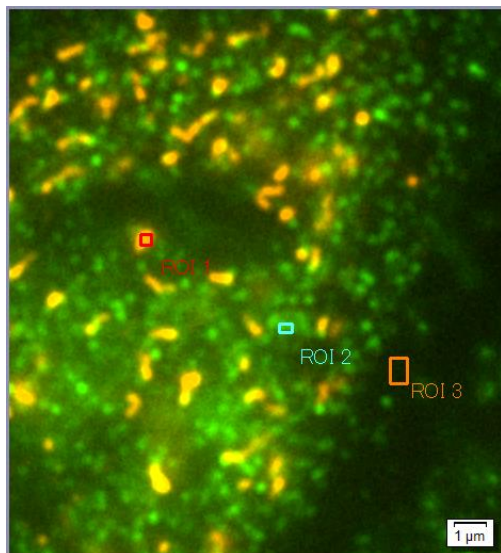
Life Science Applications toolbar



Time



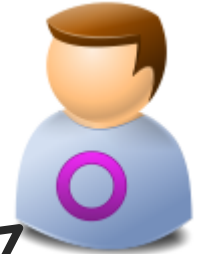
Z stack



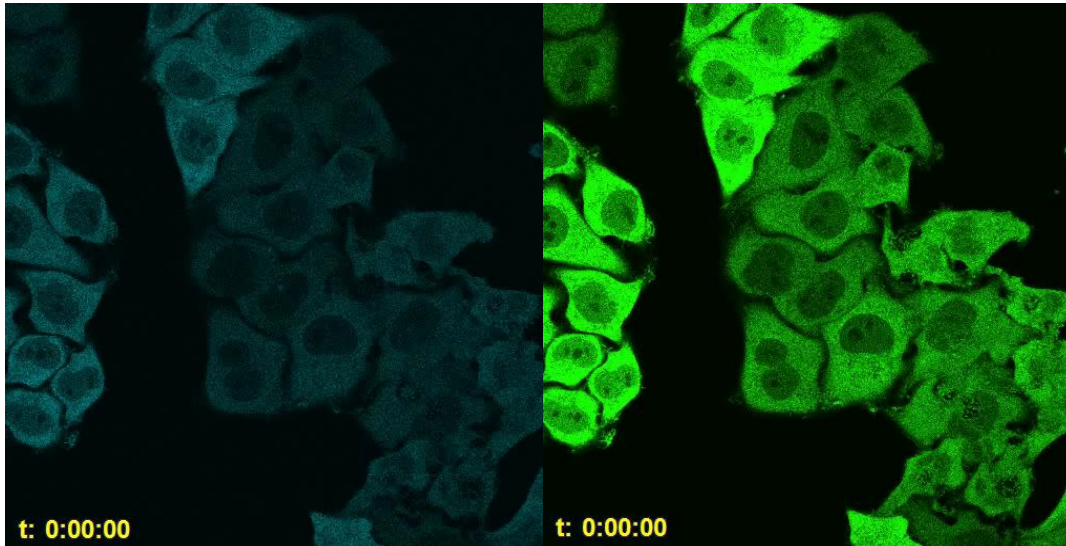
Case 3: Ratio

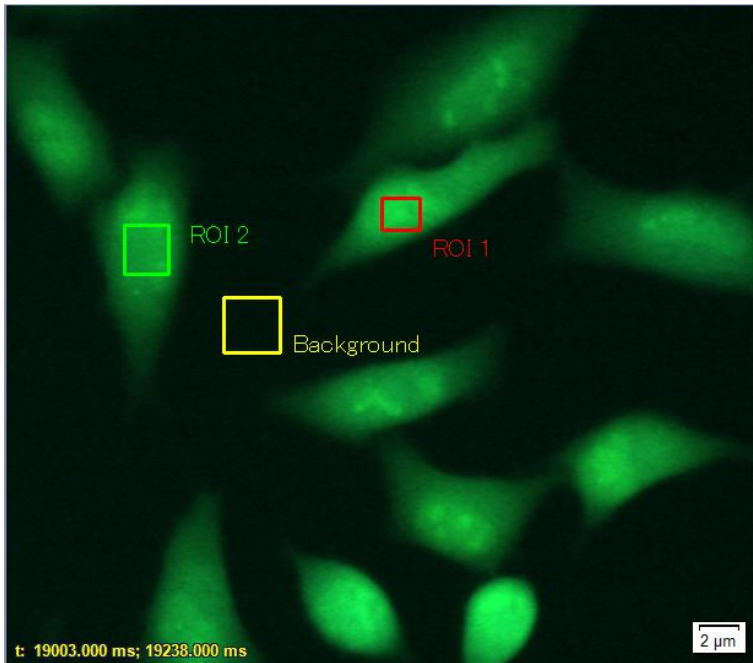


I want to analyze this movie.



Do you mean you want to analyze the ratio change in this movie?





<Operation>

1. Select an image (For example, select “Fura.tif “.)
2. Set ROIs to the image.
(Better to set one region for background)
3. Click “Ratio Analysis“ button in Life Science Applications toolbar

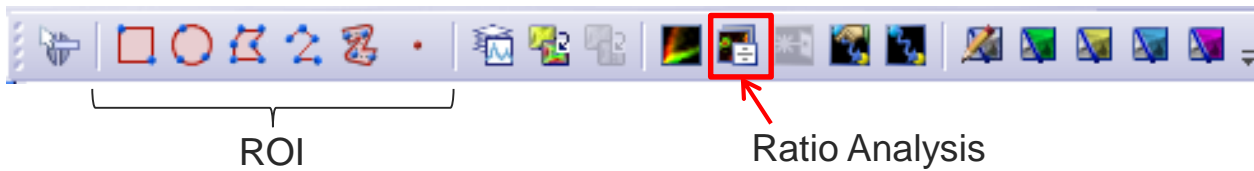
**Menu bar “View” >> “Toolbars” >>
“Life Science Applications”**

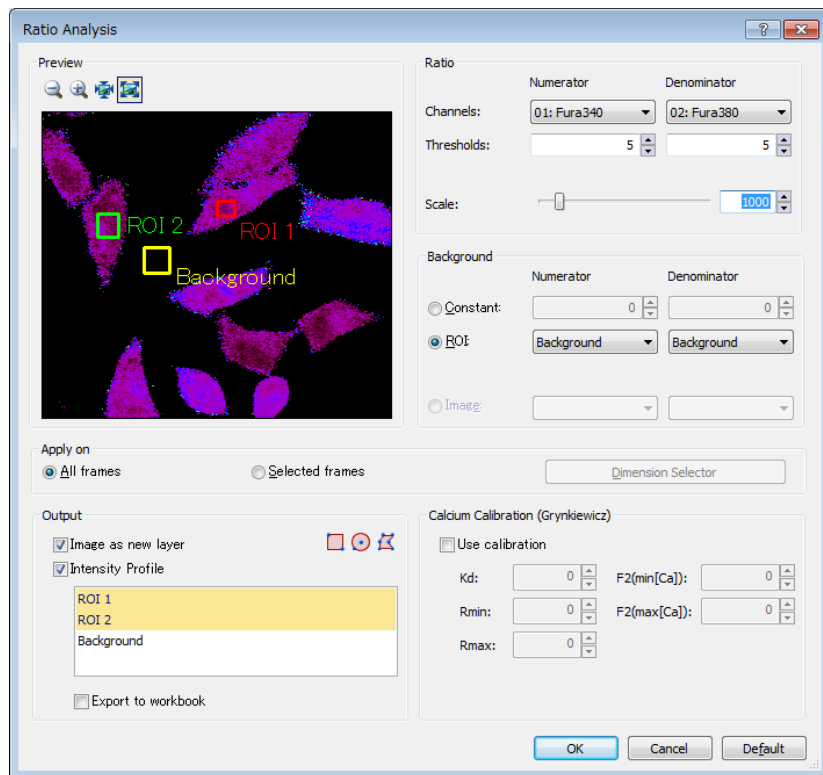
Or

Select “Ratio Analysis“

Menu bar “Measure” >> “Ratio Analysis”

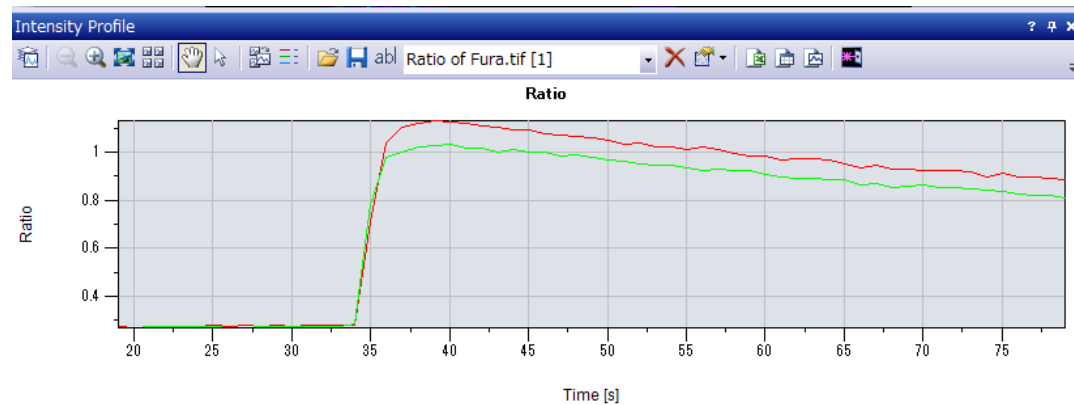
Life Science Applications toolbar





<Operation>

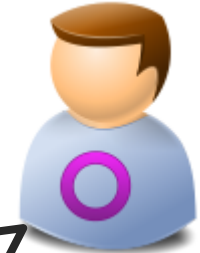
- In Ratio Analysis dialog, set following parameters.
 - Ratio
 - Numerator (ex. Fura340 channel)
 - Denominator (ex. Fura380 channel)
 - Thresholds (recommend: 5) 0 - 65535
 - Scale (recommend:1000) 1 - 10000
 - Background
 - Select a ROI for background.
 - Output
 - Image as new layer :Check
 - Intensity Profile : Check
(Turn background ROI not active)
- Click “OK” button.



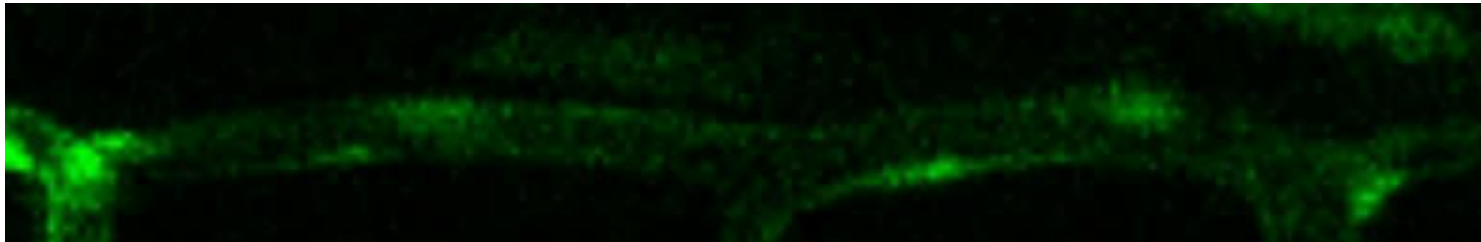
Case 4: Velocity analysis (Kymograph)



I want to analyze this movie.



Do you mean how fast the cell moves?

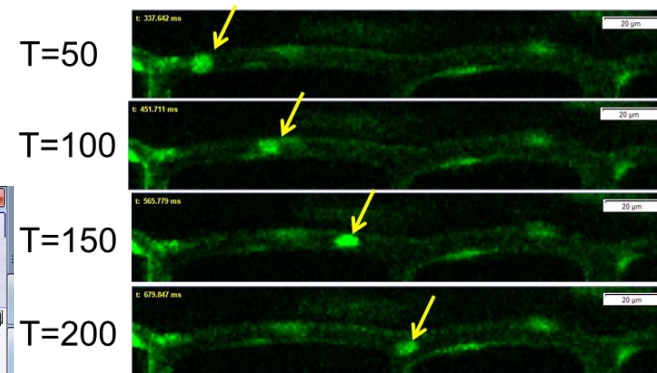


Kymograph

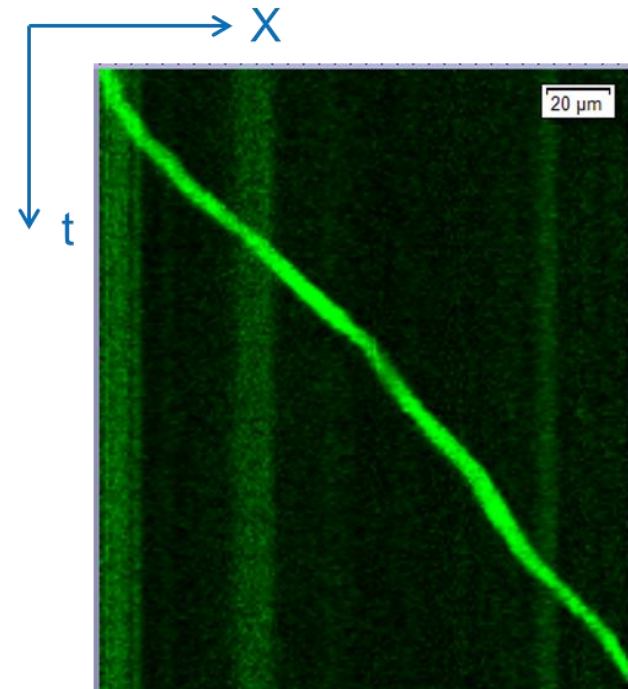
The screenshot shows the OLYMPUS cellSens Dimension software interface. The main window displays a kymograph with a green track. A white box labeled "Source image" points to the original frame, and another white box labeled "Result image" points to the processed kymograph. The "Measurement and ROI" window at the bottom shows the following data:

Type	Name	Average Velocity
Kymogram Polyline		127.33 $\mu\text{m/s}$
Count		6
Minimum		127.33 $\mu\text{m/s}$
Maximum		212.98 $\mu\text{m/s}$

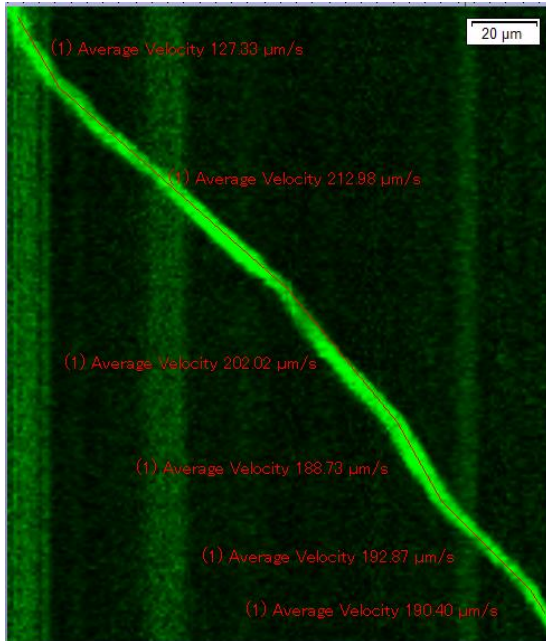
Other visible text in the interface includes "Edit a track" and "Measurement Result".



Result: Kymogram



Kymograph



<Operation> : how to measure the velocity.

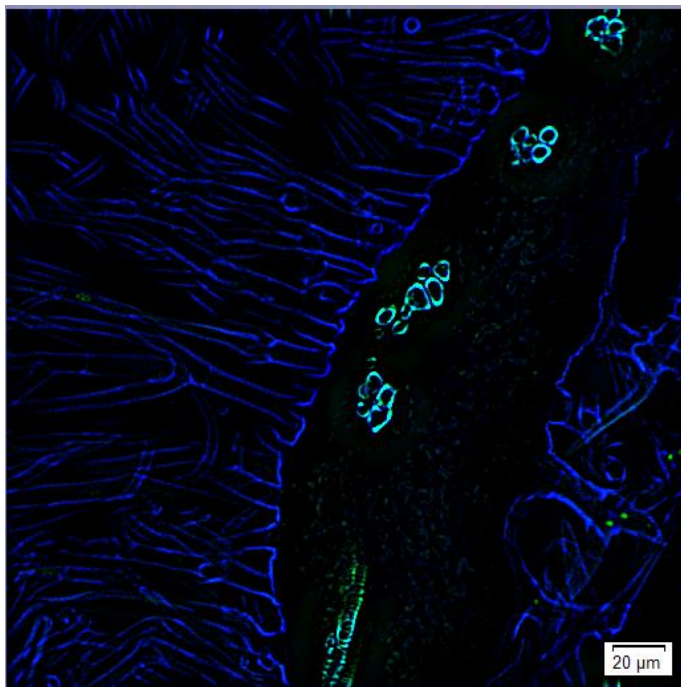
1. Click “Kymogram Polyline” button in Measurement and ROI tool window.
Menu bar “View” >> “Tool windows” >> “Measurement and ROI”
2. On the Kymogram image, track a line by clicking (left and finally right click).
3. Click “Select Measurements” button in Measurement and ROI tool window. And then select **the following measurements** as you like.
4. Check the measurement results.

Measurement and ROI					
Type	Name	Sum of Object Displacement (Time)	Current Object Displacement (Time)	Average Velocity	Current Velocity
Kymogram Polyline		0.13 s	0.13 s	183.31 μm/s	183.31 μm/s
		0.21 s	0.09 s	222.96 μm/s	281.40 μm/s
		0.35 s	0.13 s	225.12 μm/s	228.61 μm/s
		0.46 s	0.11 s	209.59 μm/s	160.40 μm/s
Count	-	8	8	8	8
Minimum	-	0.13 s	0.05 s	183.31 μm/s	116.65 μm/s
Maximum	-	0.83 s	0.13 s	225.12 μm/s	281.40 μm/s
Mean	-	0.50 s	0.10 s	204.53 μm/s	190.10 μm/s

Case 5: Colocalization



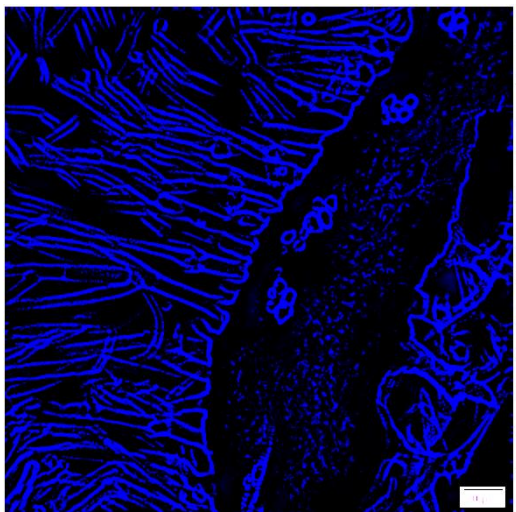
I want to analyze this image.



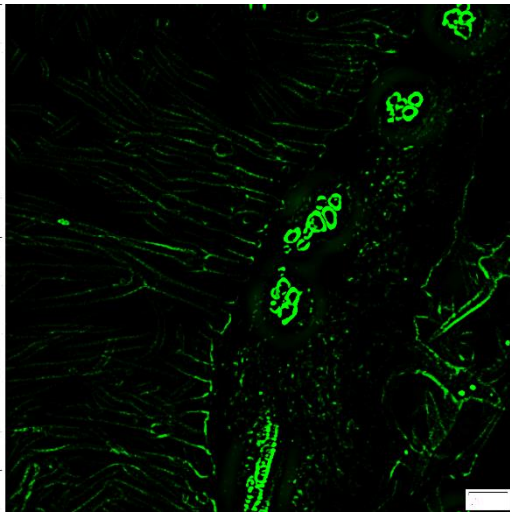
Do you mean pixel intensity spatial correlation analysis and Pearson's correlation coefficient?

Colocalization

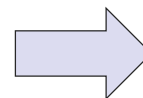
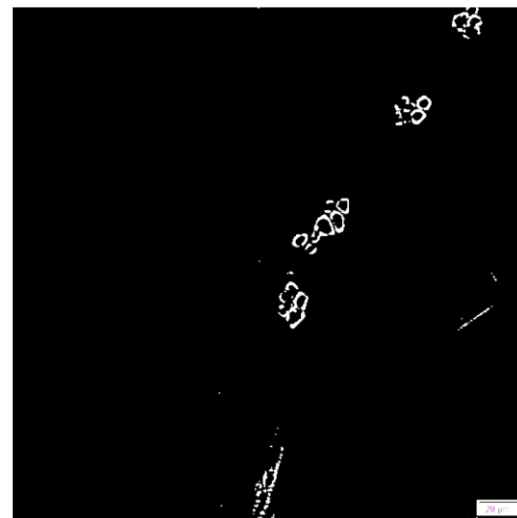
CHS1



CHS2



Colocalization (CHS1/CHS2)



Sample image "Lavender.tif"

<Operation>

1. Select an multi-channel image.
2. Click "Colocalization" button in Life Science Applications toolbar

Menu bar "View" >>

"Toolbars" >>

"Life Science Applications"

Or

Select "Colocalization".

Menu bar "Measure" >>

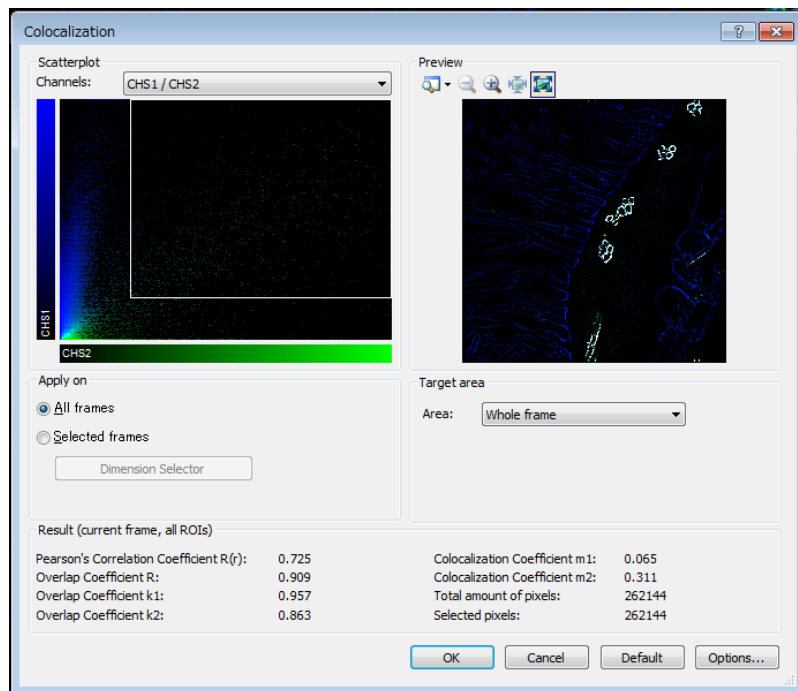
"Colocalization"

Life Science Applications toolbar



ROI

Colocalization



<Operation>

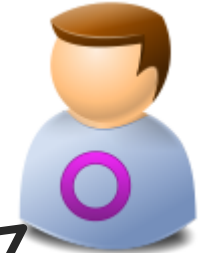
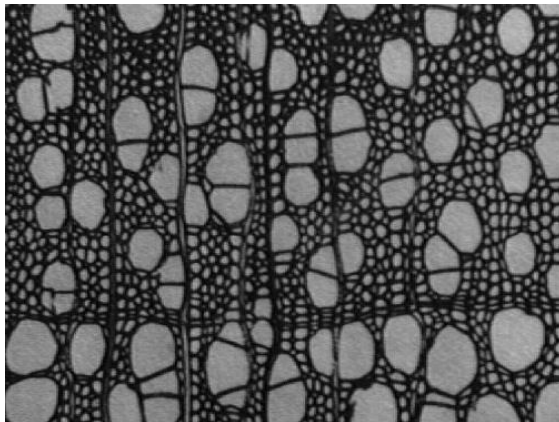
1. In Colocalization dialog box, set followings
 - Channels in Scatterplot field
 - ✓ Select channels
 - Preview
 - ✓ Select displayed image.
 - Apply on frames
 - ✓ Basically select “All frames”
 - Target area
 - ✓ Select area
 - ✓ Whole area
 - ✓ ROI
 - ✓ Segmentation
2. Click “option” button and select output format (image / workbook)
3. Click “OK” button.

	Range	Meaning
Pearson's Correlation Coefficient	$-1 \leq R(r) \leq 1$	1 : complete match of the displays in both channels -1: No match of the displays in both channels
Overlap Coefficient R	$0 < R < 1$	The closer the value is to 1, the stronger the colocalization is.
Overlap Coefficient k1/k2	$0 < k1 < 1$ $0 < k2 < 1$	
Colocalization Coefficient m1/m2	$0 < m1 < 1$ $0 < m2 < 1$	

Case 6: count and measure



I want to know the size holes there are in this image.



Manual measurements are quite hard, so let's try with count & measure.

Count and Measure

1. Measurement

1. Class measurement
2. ROI measurement
3. Each object measurement

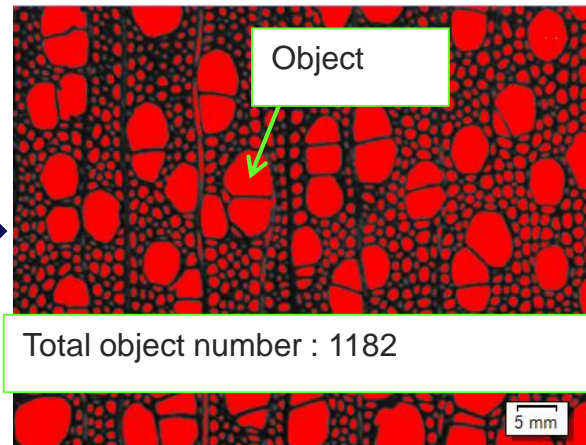
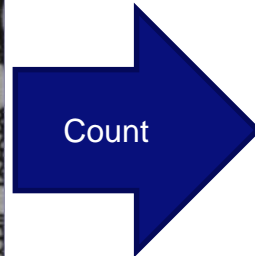
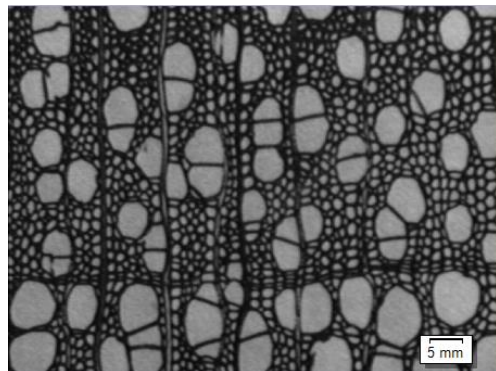
2. Select objects

1. Threshold setting
2. Object Filtering

3. Edit an object

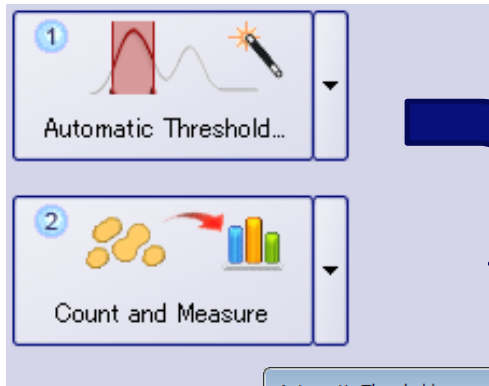
4. Application

1. Fucci cell-counting



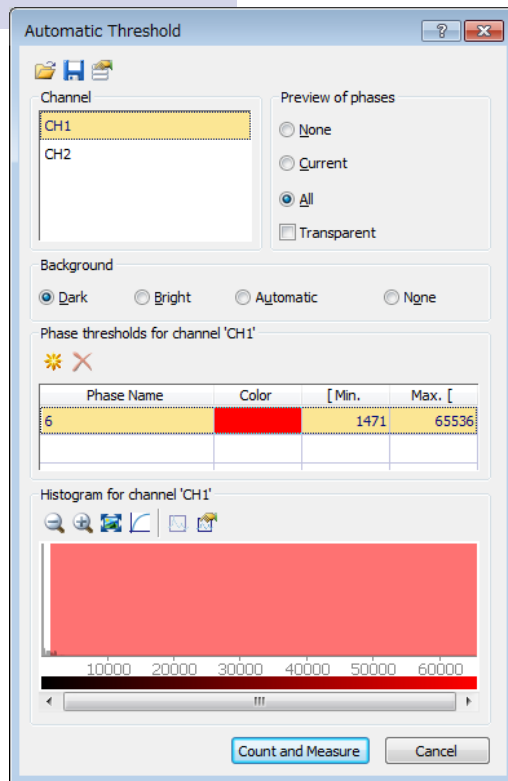
Class: All objects
Object: Each region which is separated by a threshold

1-1. Class Measurement - Operation -



<Operation>

1. Select "Count and Measure" tab
2. Select an image.
3. Click "Threshold" button in Count and measure tool window. (Automatic / manual / HSV)
Menu bar "View" >> "Tool windows" >> "Count and Measure"
4. Set some parameters in the dialog box. (channel, phase thresholds, background)
5. Click "Count and Measure" button in the dialog or in Count and Measure tool window.
6. Check "Class Measurements" tab or "Class Histogram" tab in Count and Measure Results tool window.



Menu bar "View" >> "Tool windows" >> "Count and Measure Results"

1-1. Class Measurement - Result Image -

The screenshot displays the OLYMPUS cellSens Dimension software interface. The main window shows a wood vessel image with red segmentation. The 'Count and Measure' panel on the left includes 'Automatic Threshold...' and 'Count and Measure' options. A red box highlights the 'Object Count' section, which shows 'Total: 1002' and 'In filter ranges: 1002'. A white box labeled 'Count number' points to the 'Total' value. The 'Count and Measure Results' panel at the bottom is also highlighted with a red box and contains a table with the following data:

Object Class	Sum (Area) [mm ²]	Area Fraction ROI [%]	Relative Object Count [%]	Mean (Mean (Gray Intensity Value))	Mean (Mean (Color Intensity Value))
1	1723.81	41.75	100.00	-	-
2					
3					
4					
5					
Count in filter ranges		1	1		0
Mean		41.75	100.00		
Standard Deviation					

A white box labeled 'Count Results (only sum area)' points to the 'Sum (Area) [mm²]' column in the table. The software interface also shows a '5 mm' scale bar in the bottom right corner of the image area.

1-2. ROI Measurement - Operation-

The diagram illustrates the sequence of operations. Step 1 shows the 'Automatic Threshold' window, and Step 2 shows the 'Count and Measure' window. A large blue arrow indicates the transition from step 1 to step 2.



<Operation>

1. Select "Count and Measure" tab
2. Select an image.
3. Set ROI(one or more) on the image
4. Click "Threshold" button in Count and measure tool window.
(Automatic / manual / HSV)

Menu bar "View" >> "Tool windows" >> "Count and Measure"

5. Set some parameters in the dialog box.
(channel, phase thresholds, background)
6. Click "Count and Measure in ROI" button in Count and Measure tool window.
7. Check "ROI Measurements" tab or "ROI Histogram" tab in Count and Measure Results tool window.

Menu bar "View" >> "Tool windows" >> "Count and Measure Results"

The screenshot shows the 'Automatic Threshold' dialog box. It includes a 'Channel' list with 'CH1' selected, a 'Preview of phases' section with 'All' selected, a 'Background' section with 'Dark' selected, and a 'Phase thresholds for channel 'CH1'' table.

Phase Name	Color	[Min.	Max. [
6		1471	65536

Below the table is a 'Histogram for channel 'CH1'' with a red bar chart and a scale from 0 to 60000. At the bottom are 'Count and Measure' and 'Cancel' buttons.

1-2. ROI Measurement - Result Image-

The screenshot displays the OLYMPUS cellSens Dimension software interface. The main window shows a grayscale image of wood vessels with two regions of interest (ROI 1 and ROI 2) highlighted in red. The software is in the 'Count and Measure' mode. The 'Object Count' panel shows a total count of 616 objects, with 616 objects in filter ranges. The 'Count and Measure Results' panel shows a bar chart of 'Sum (Area) [mm²]' for ROI 1 (436.53) and ROI 2 (469.87). The 'Object Count' panel is highlighted with a red box, and the 'Count and Measure Results' panel is also highlighted with a red box. A white box labeled 'Count number' points to the 'Object Count' panel, and another white box labeled 'Count Results' points to the 'Count and Measure Results' panel.

Object Count

Total:	616
In filter ranges:	616

Count and Measure Results

ROI	Sum (Area) [mm²]
ROI 1	436.53
ROI 2	469.87

1-3. Object Measurement

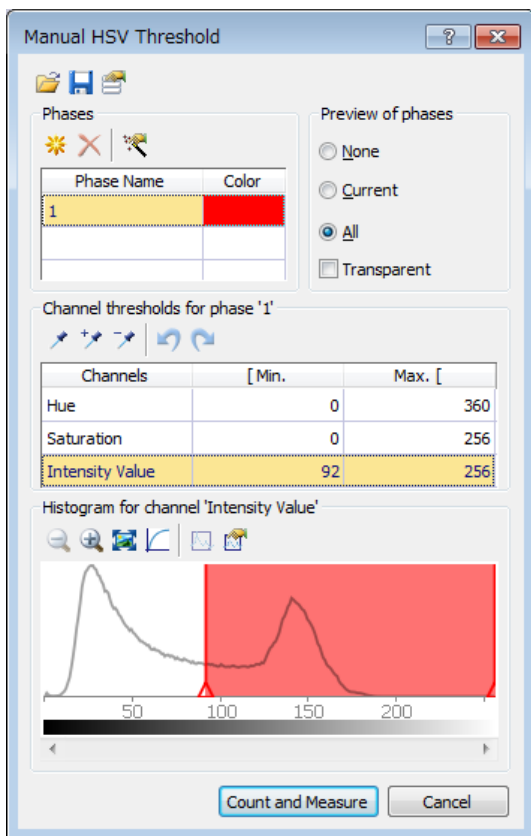
The screenshot displays the OLYMPUS cellSens Dimension software interface. The main window shows a microscopic image of wood vessels with a red grid overlay. A red arrow points from a selected object in the image to the 'Object Measurements' tab in the 'Count and Measure Results' window. The table below shows the measurement data for the selected object (ID 360).

Object ID	Object Class	Area [mm ²]	Perimeter [mm]	Mean (Radius) [mm]	Mean (Gray Intensity Value)	Mean (Color Intensity Value)	Shape F
357	1	0.61	2.78	0.43	-	122.92	
358	1	0.68	2.94	0.45	-	115.32	
359	1	0.80	3.28	0.49	-	117.65	
360	1	25.54	19.99	2.83	-	136.48	
361	1	1.09	3.88	0.57	-	123.75	
Count in filter ranges	-	-	1002	1002	0	1002	
Mean	-	1.72	3.30	0.48	-	112.43	
Standard Deviation	-	5.06	3.95	0.54	-	11.79	

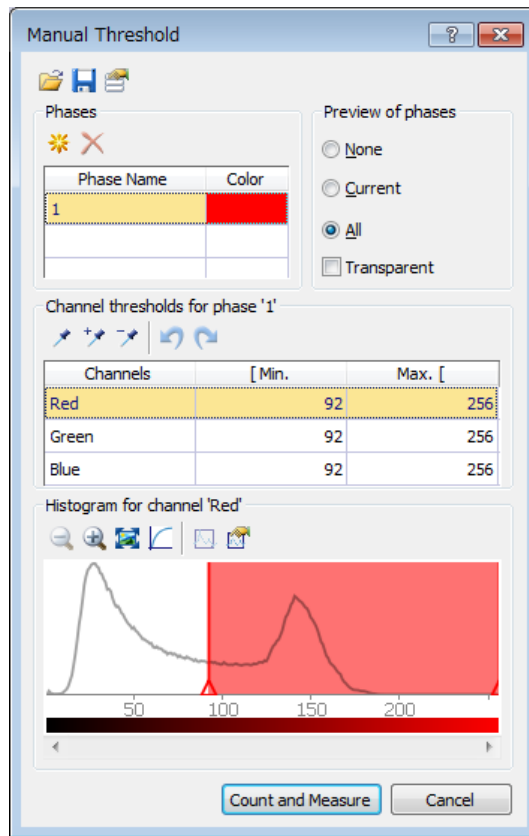
When selecting an object on an image, The measurement result of the object is active in “Object Measurements” tab in Count and measure Results tool window

2-1. Threshold setting

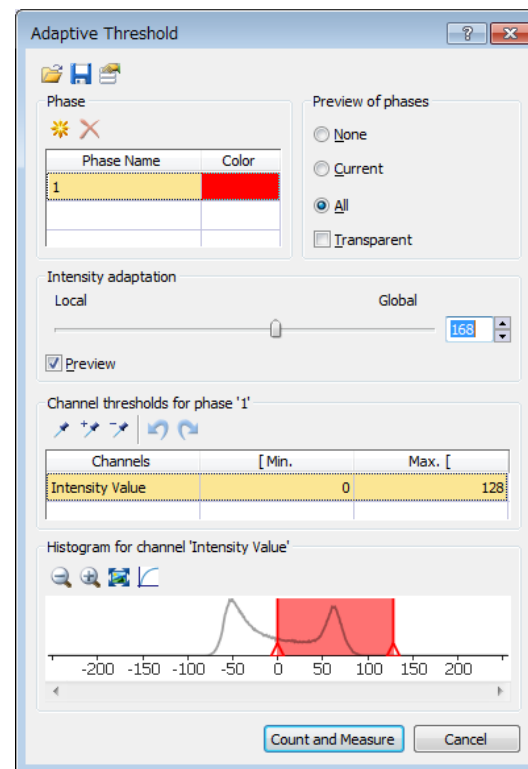
Threshold is set by Intensity, RGB or HSV(Hue, Saturation, Intensity Value) value.



For color image



For all image

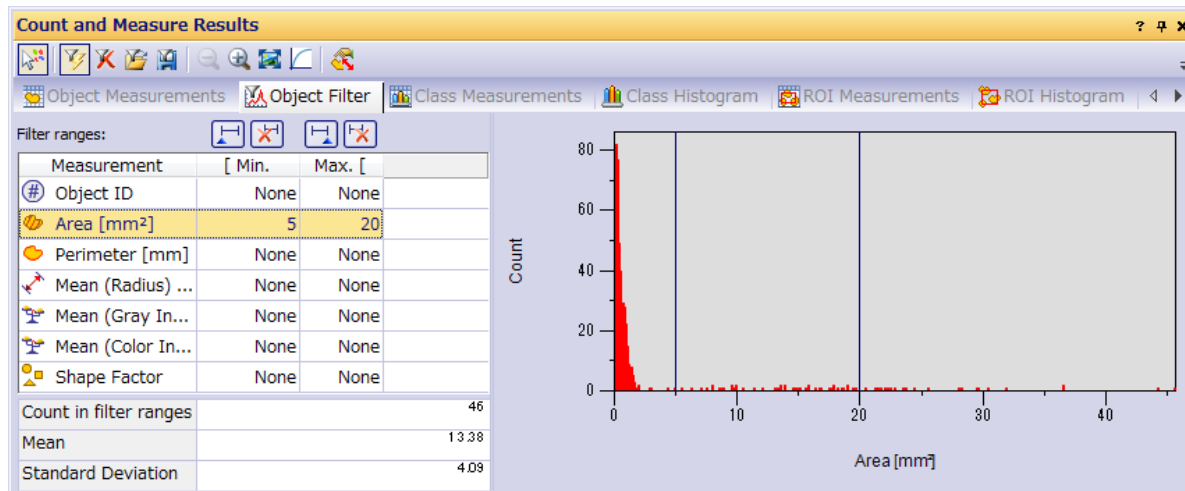


For images which has uneven brightness background (shading)

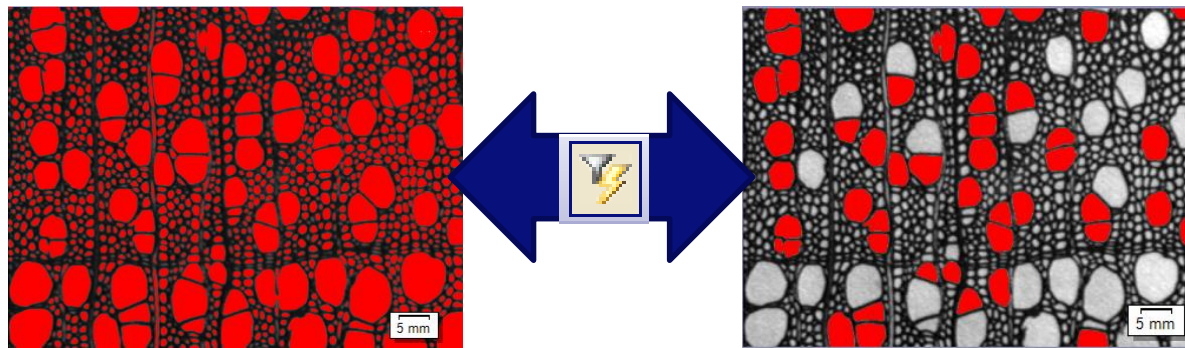
2-2. Object Filter

After counting, objects filter is available. The filter items are following.

By “Toggle Object Filter” button in the tool window, switching of filter on/off is available.



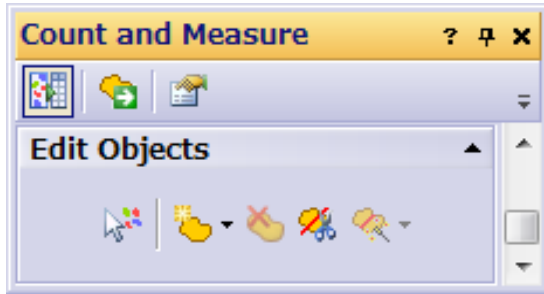
- Object ID
- Area
- Mean (Radius)
- Mean (Gray Intensity)
- Mean (Color Intensity)
- Shape Factor (Roundness)








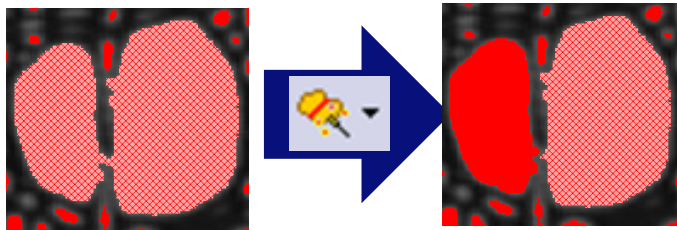
Count :1002

Count :46

3. Edit objects



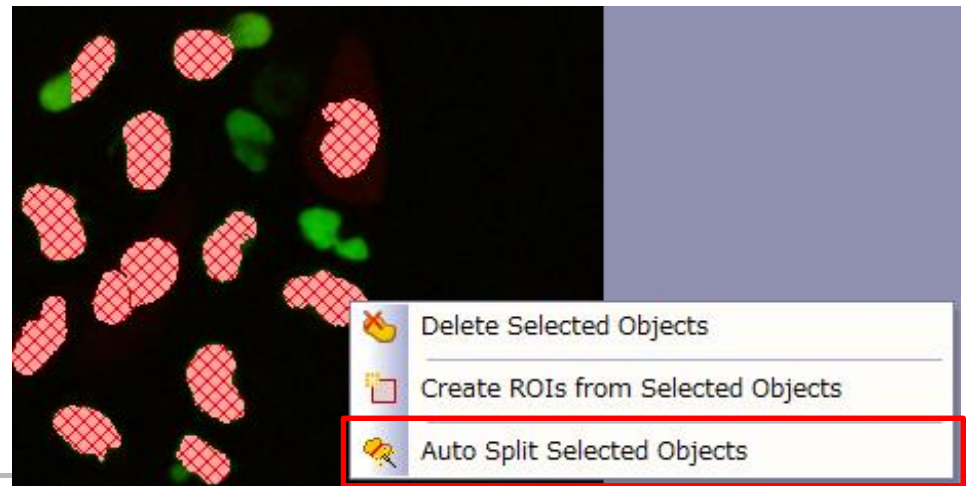
	Allows selection of detected objects.
	Create a new object
	Deleted selected objects
	Manually split objects
	Automatic split objects



After detection of objects by counting, we can edit the objects one by one.

[Point]

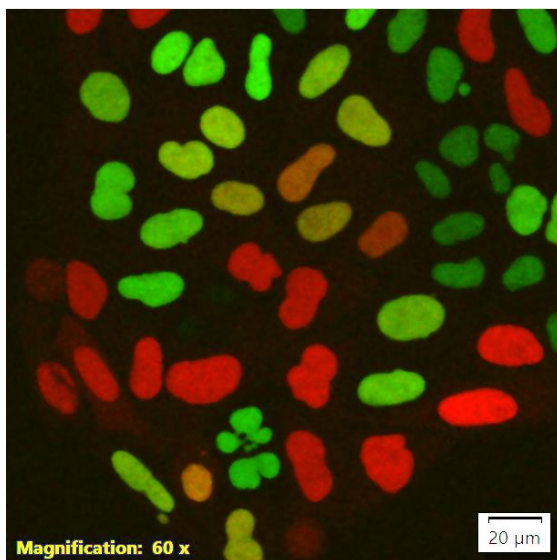
If you want to split objects to all objects automatically, after select one object in the image, push “**Ctrl + A**” on the keyboard. And then click “Automatic split objects” button or right-click and select “Auto Split Selected Objects”.



Case 7: Object Counting (Touch Counting)



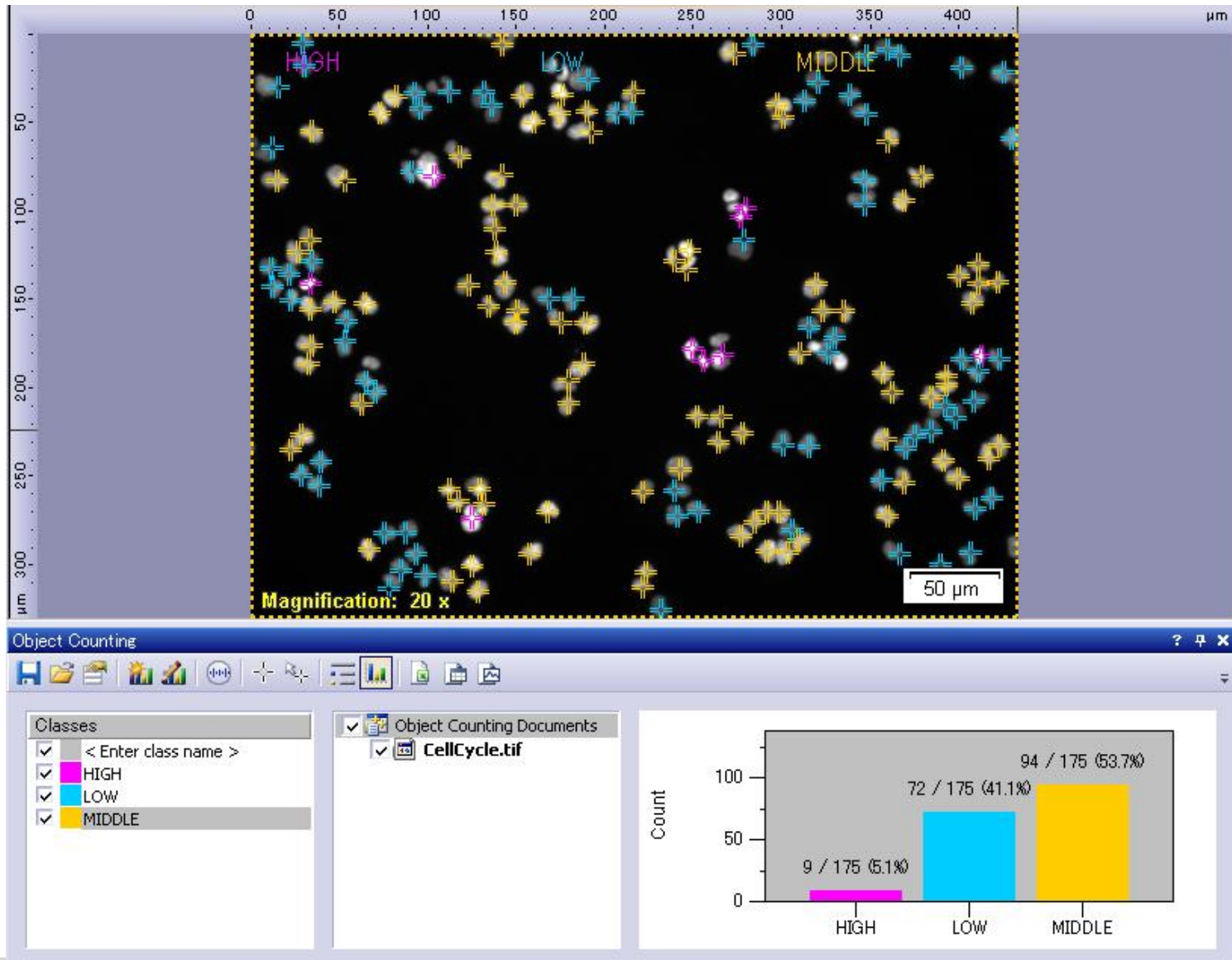
I want to know the how many cells at different phase of mitosis cycle there are in this image.



We can count objects manually in your images by clicking on the objects. You can define different classes and assign the objects to those classes.



Object Counting

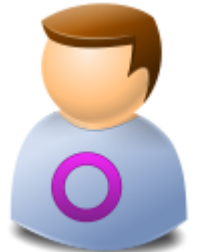


Case 8: Macro Recording

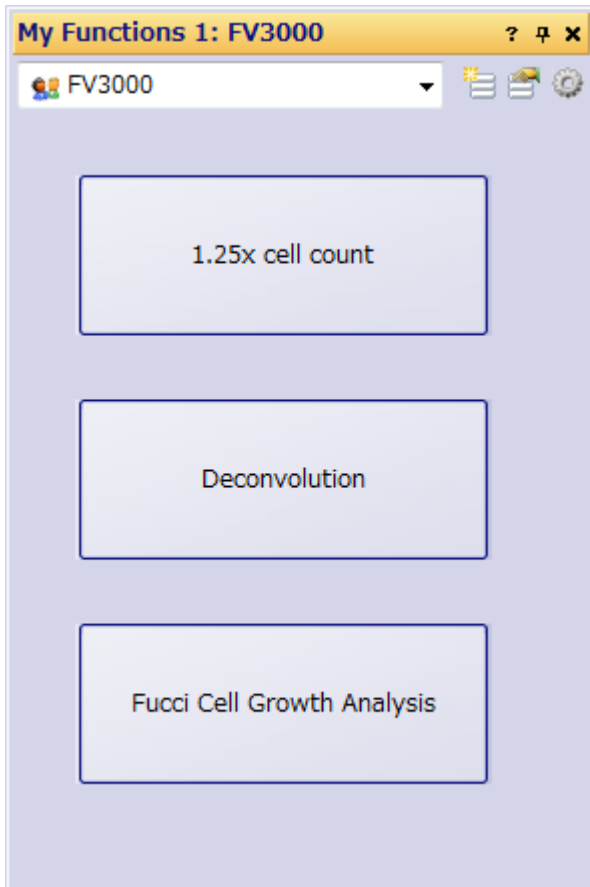


I am working for same processing/analysis for several images.

We have a macro function to record the procedure and apply to the different samples. And we can assign the macro to the icon, so you can start with 1 click for the procedure.



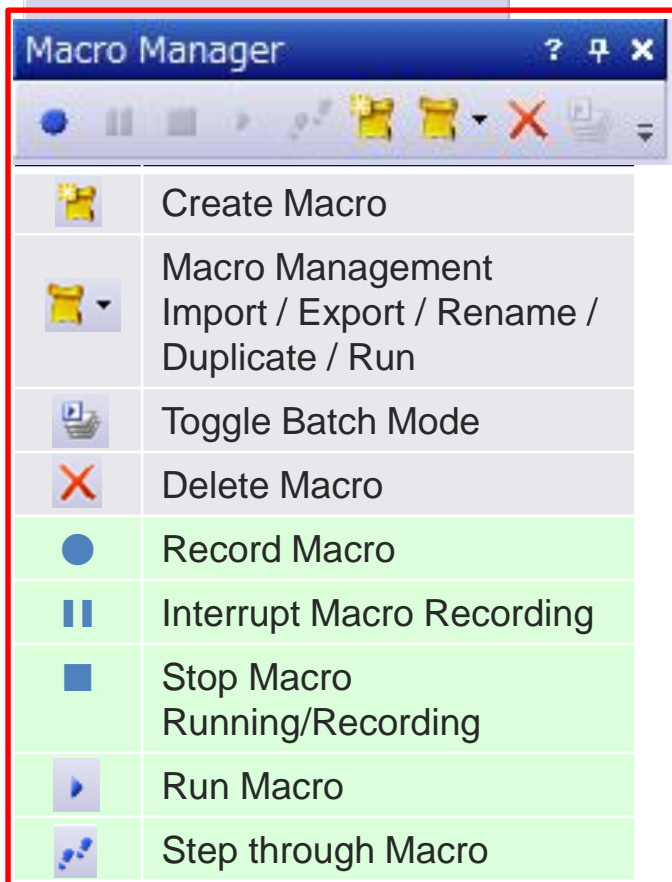
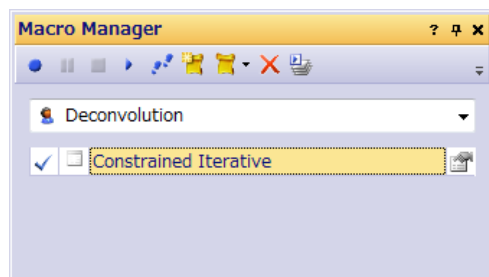
One Click Macro Capability



cellSens Macro function allows to run multiple processing by one click

<How to make this>

1. Create a macro in Macro Manger tool window
2. Create a button in My Functions tool window



<Operation>

1. Click “Create Macro” button in Macro Manager tool window
Menu bar “View” >> “Tool windows” >> “Macro Manager”
2. Put a name and click “OK” button. Then the macro recording will be started.
3. Operate an image processing or an image analysis
4. If click “interrupt macro Recording” button, we can have a break until click “Record macro”
5. If the operation is finished, click “Stop Macro” button.
6. If click “Run macro” or “Step through Macro”, we can check whether the macro could work correctly.

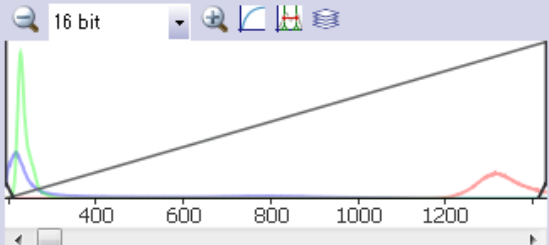
Overview of other Image Processing

Adjust Display

Adjust Display ? [] x

Histogram

16 bit [] [] [] []



Min: 179 Max: 4095

Mean Intensity: 610.81
Pixel Count: 1,329,714

Fixed Scaling
Left: 194 Right: 1431

Auto Contrast
Left: 0.1 % Right: 0.1 %

Histogram of all frames
 Exclude spikes in histogram

Apply Default

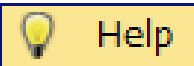
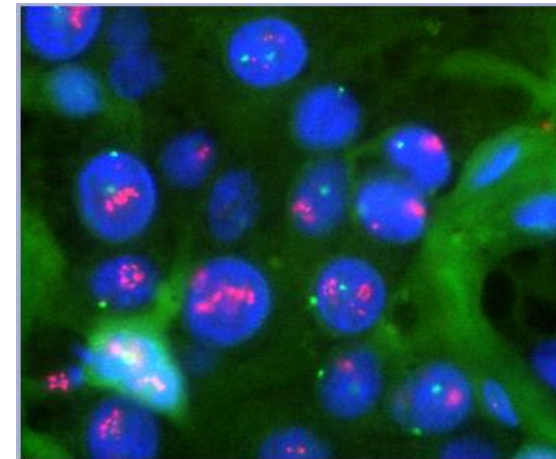
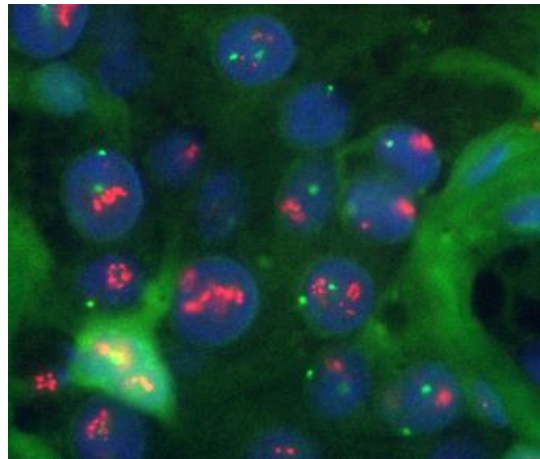
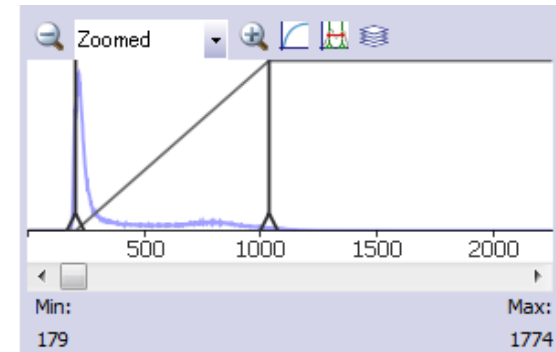
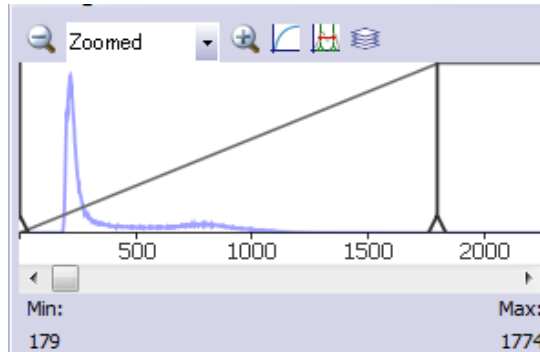
Display Enhancement

Brightness: [] 50 []

Contrast: [] 50 []

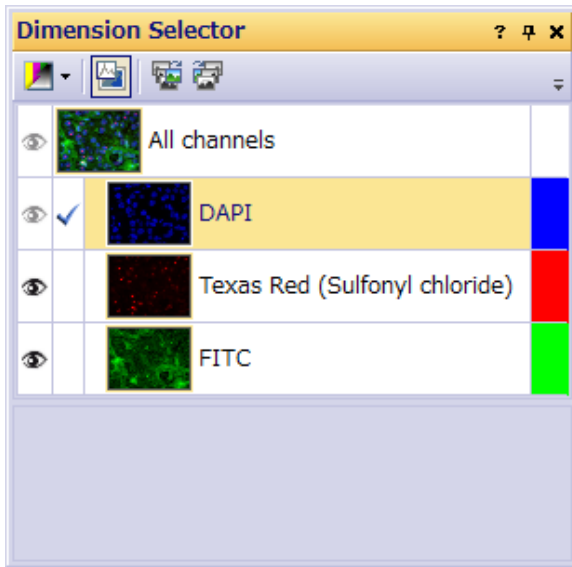
Gamma: [] 1 []

Default



Please note that you do **not** change the actual image data when you customize the display. The image will only be displayed differently on your monitor. The settings for the display will be saved along with the image, provided you save the image in the TIF or VSI format.

Adjust Display

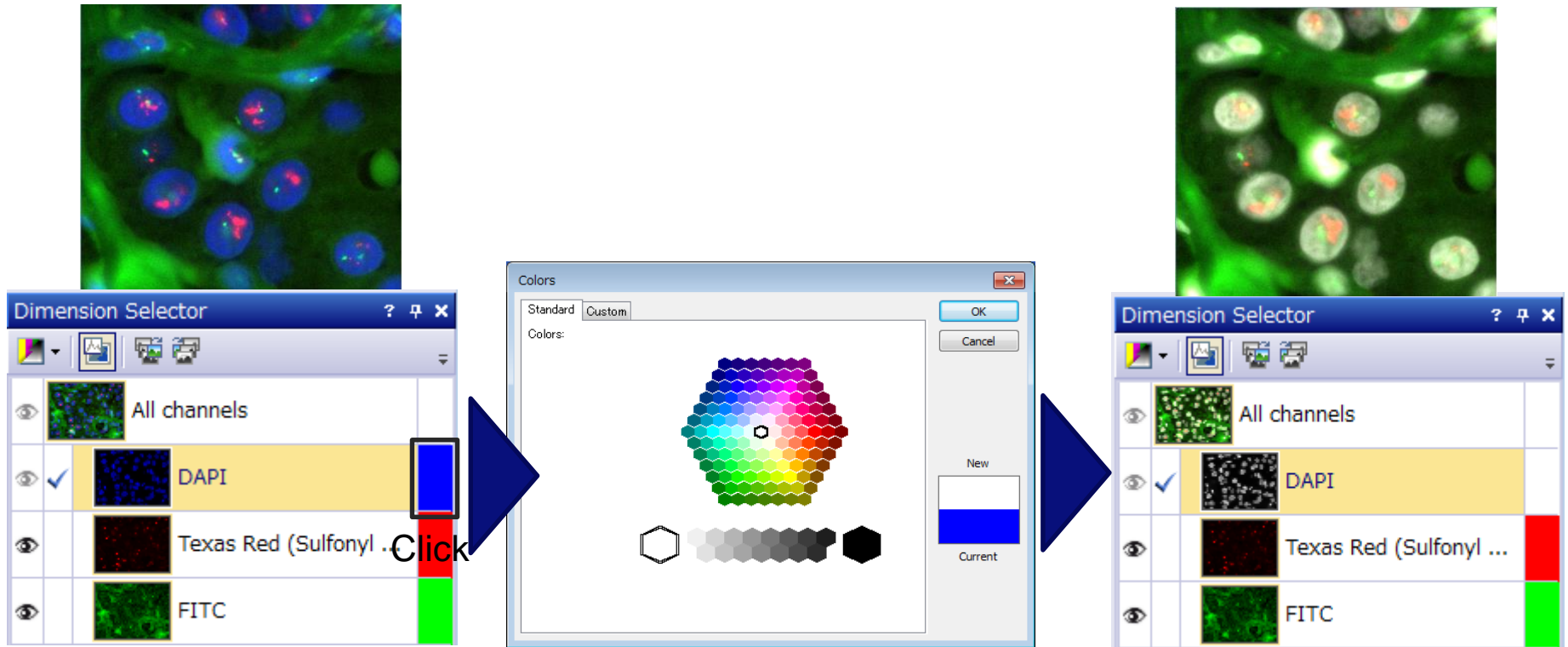


<Operation>

1. Select an Image
2. Open 2 tool windows
 - Dimension Selector tool window
Menu bar “View” >> “Tool Windows” >> “Adjust Display”
 - Adjust Display tool window
Menu bar “View” >> “Tool Windows” >> “Dimension Selector”
3. Select channel in Dimension Selector
4. Adjust Histogram in Adjust display
 - Select Fixed scaling or Auto Contrast
 - In “Fixed scaling”,
Click and drag the 2 vertical lines (Min or Max) from Histogram itself
Enter the minimum and maximum values you want, directly in the Left and Right fields.
 - In “Auto Contrast”
Define limits in the Left and Right fields.
Click and drag the 2 vertical lines (Min or Max) from Histogram itself.
5. Click Apply button
6. Click the Default button if you want to return to the default setting.

Edit Pseudo color

Change color for each channel of multi-channel image

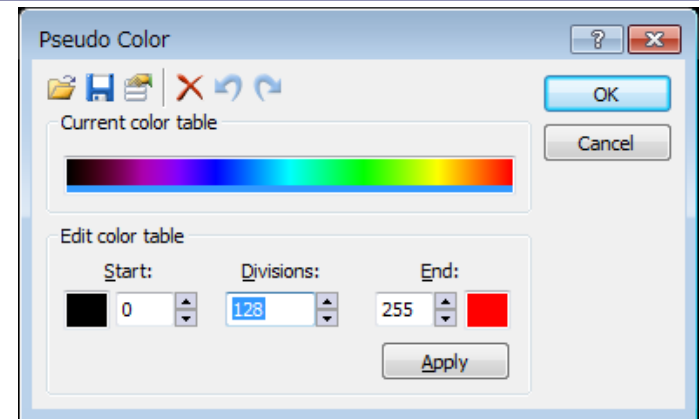
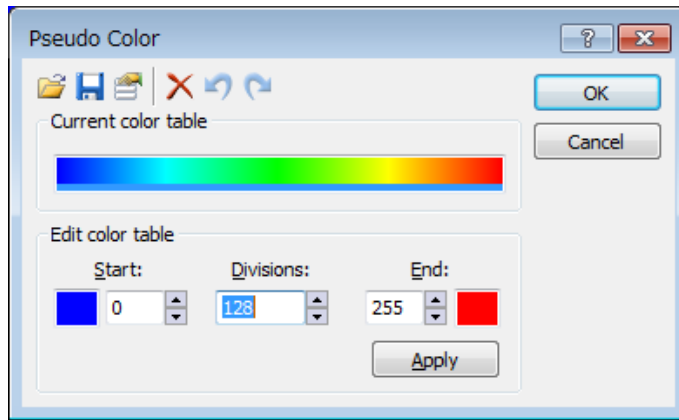
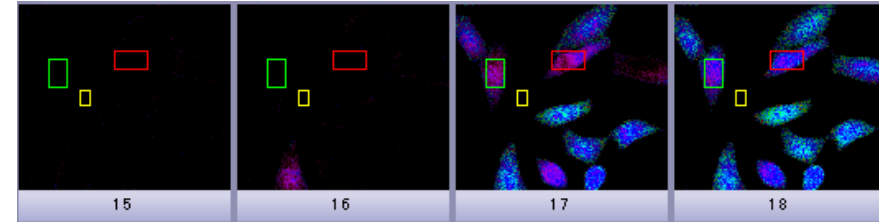
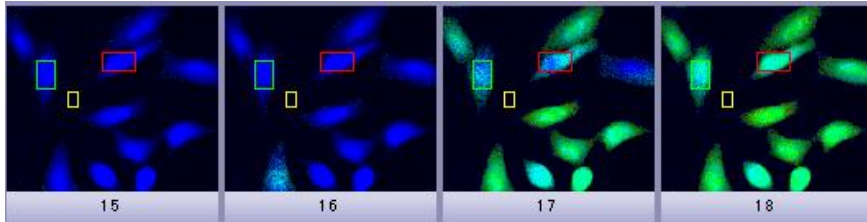


<Operation>

1. Open Dimension Selector tool window
Menu bar “View” >> “Tool Windows” >> “Adjust Display”
2. Click color field which is next to the channels name
3. Select one of the colors from the palette on the standard or custom tab.
4. Click OK button.

Edit Pseudo color


Change color for ratio image



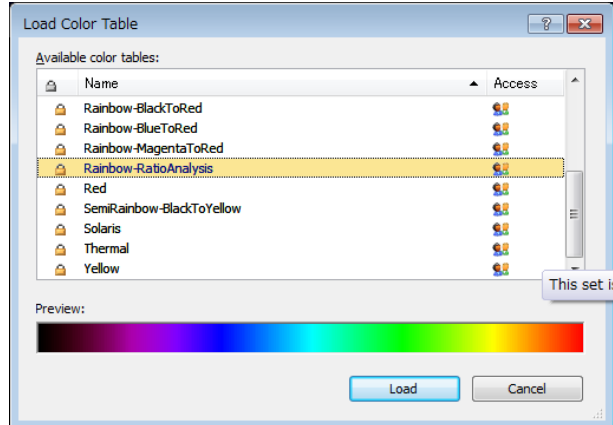
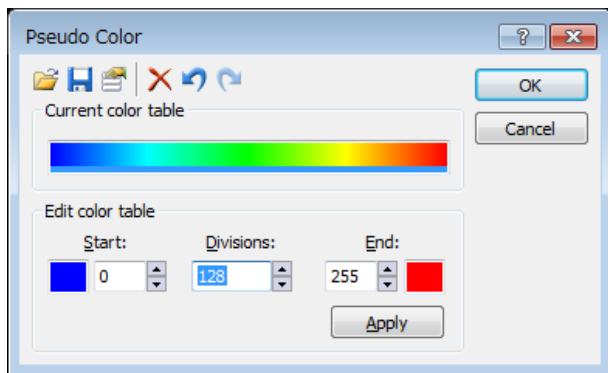
 Help

Prerequisite: This command is only available when the active document is a gray-value image.


Note: You don't change any image data when you use this function. The image will only be displayed differently on your monitor. The color table will be saved along with the image, if you save the image in the TIF or VSI format.

 **Multi-dimensional images** can be made up of gray-value images. You can also display these multi-dimensional images with a color table. This is especially valid for multi-channel images. All of the frames will then be displayed with the same color table.

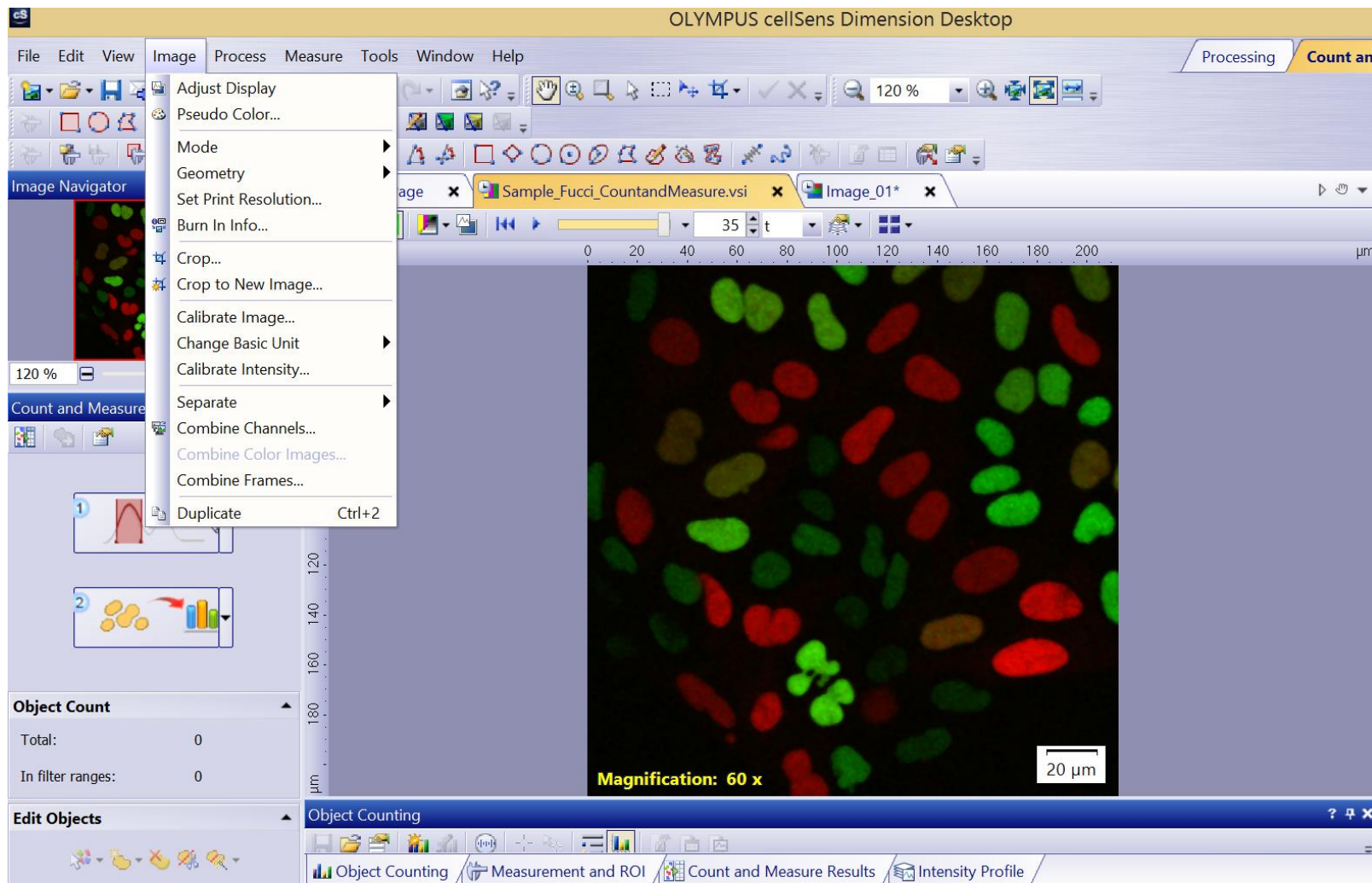
Edit Pseudo color



<Operation>

1. Select an Image
2. Open Pseudo Color dialog box
Menu bar “Image” >> “Pseudo Color”
3. Click “ Load Color Table” button in the dialog box.
4. Select one of the color tables.
5. Click Load button in “Load Color Table” dialog box.
6. Click OK button in “Pseudo Color” dialog box.

Other processing tools in Menu bar “Image” >>



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