OLYMPUS

Your Vision, Our Future

Analysis with acquired images from FV1000 and VS120

What we can do with cellSens?

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WMRI, 14th June, 2018

What is image analysis?

Image acquisition

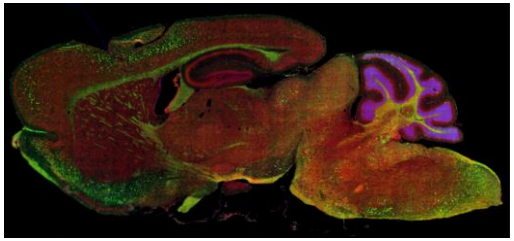


Image processing

- Image filters
- Background subtraction
- Deconvolution
- Channel combine
- Projection

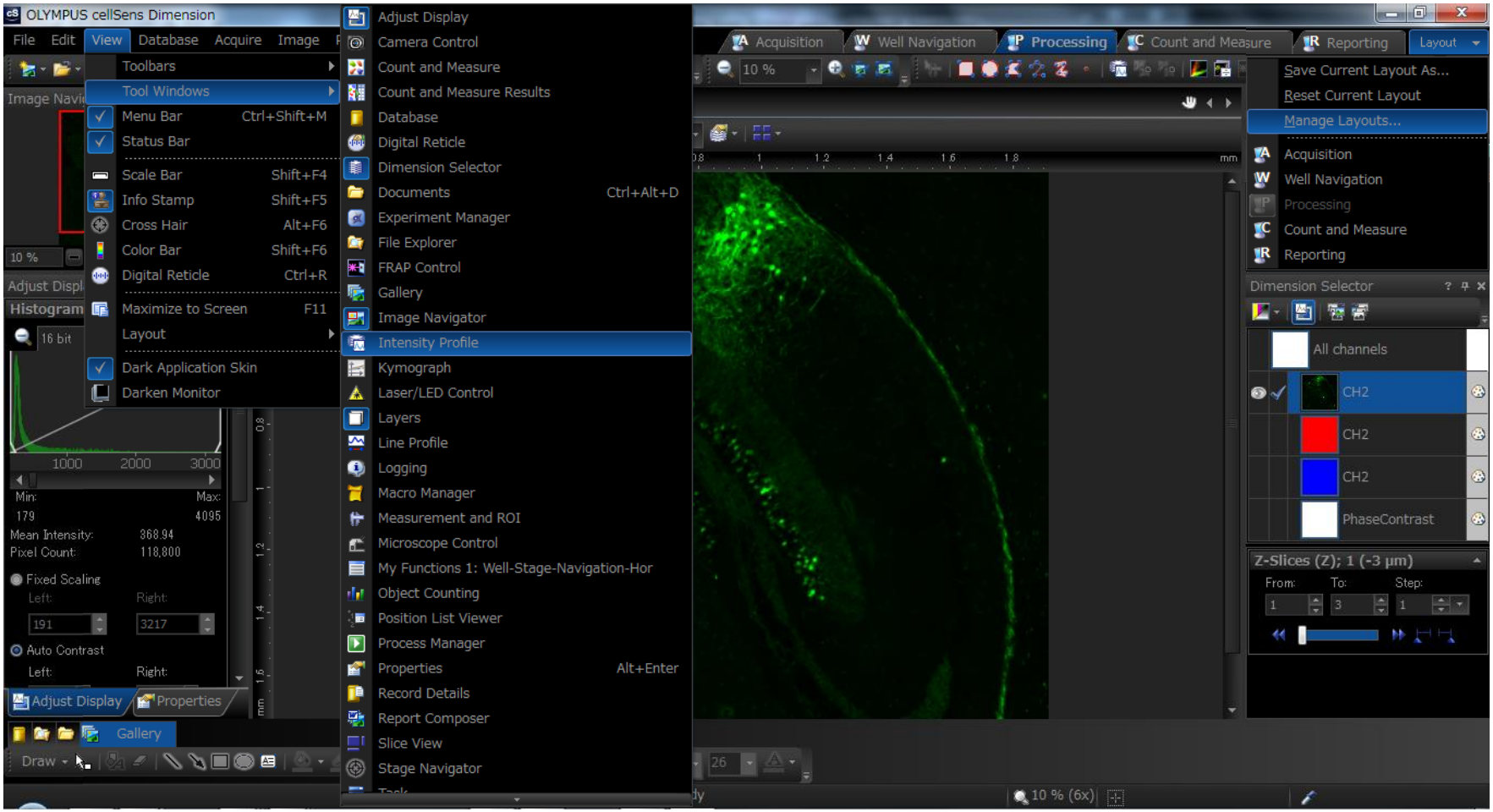
Image analysis

- Measurement
- Intensity profile
- Co-localization
- Ratio/FRET
- FRAP
- Kymograph

Data analysis
Statics analysis

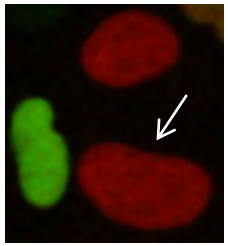
cellSens dimension, is a powerful software for image analysis.

cellSens layout

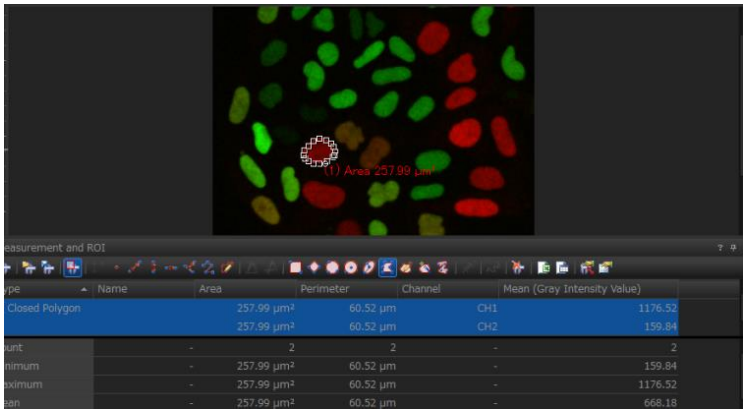


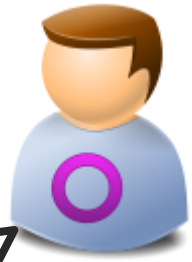
Case 1: Measurement

I want to know how big this nuclear is.



I want to know the intensity of this nuclear.





You may use measurement for that.

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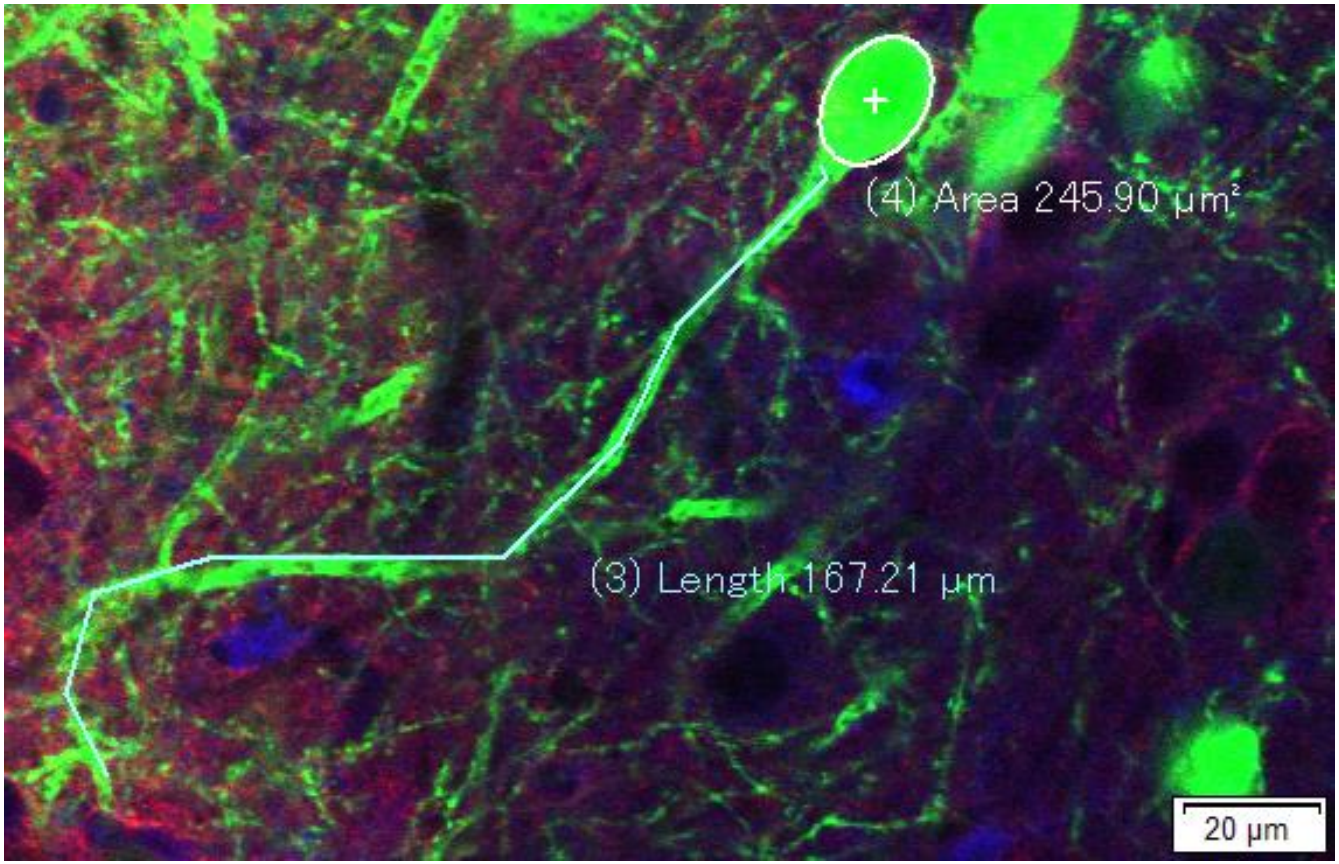
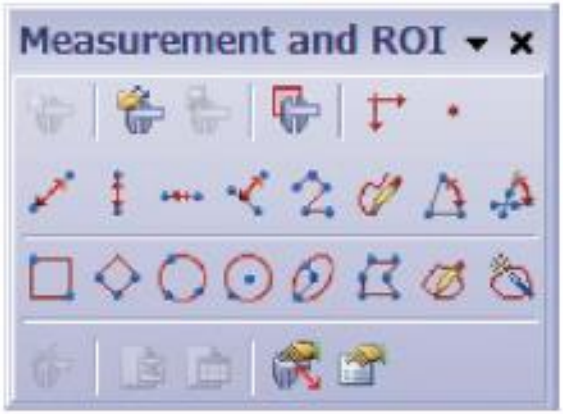
Basic Measurement

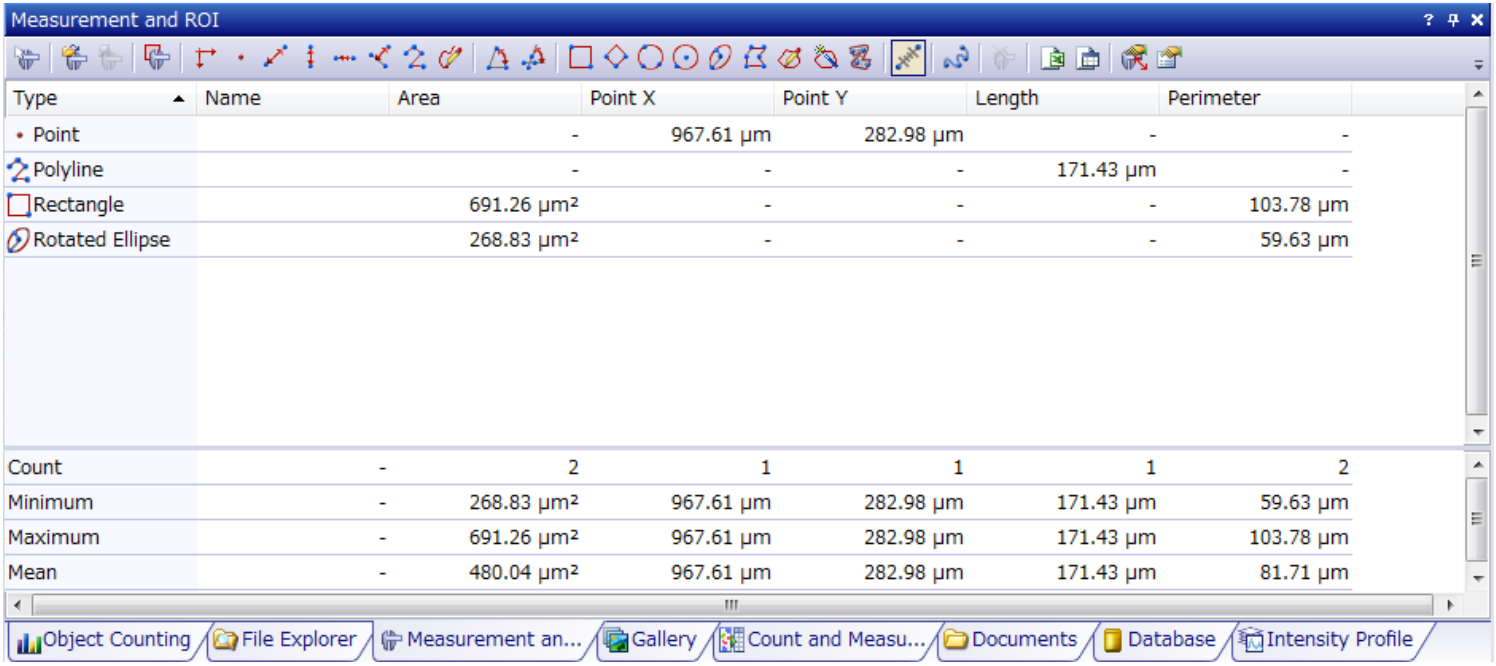
1. Set Measurement objects
2. Edit Measurement objects
3. Select Measurements
4. Export measurement results to Excel





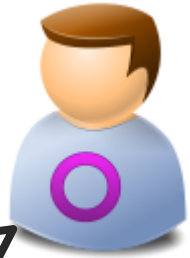
<Operation>

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.

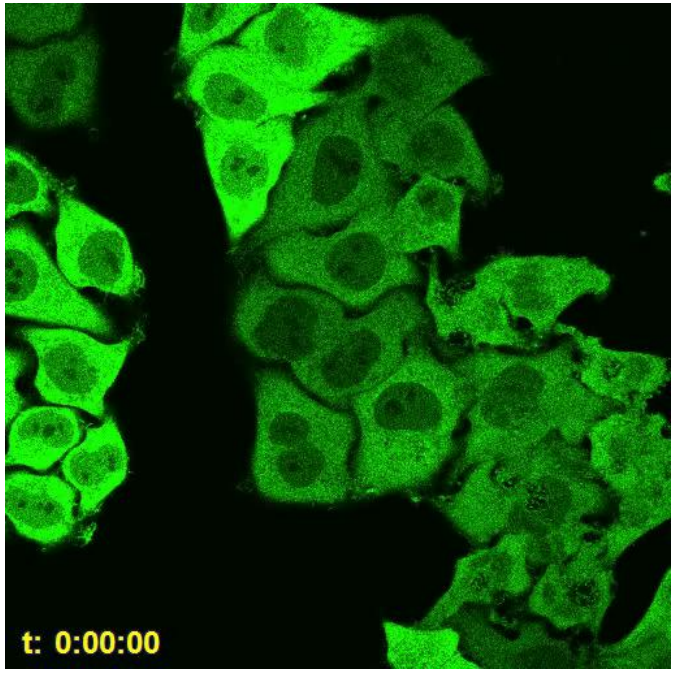


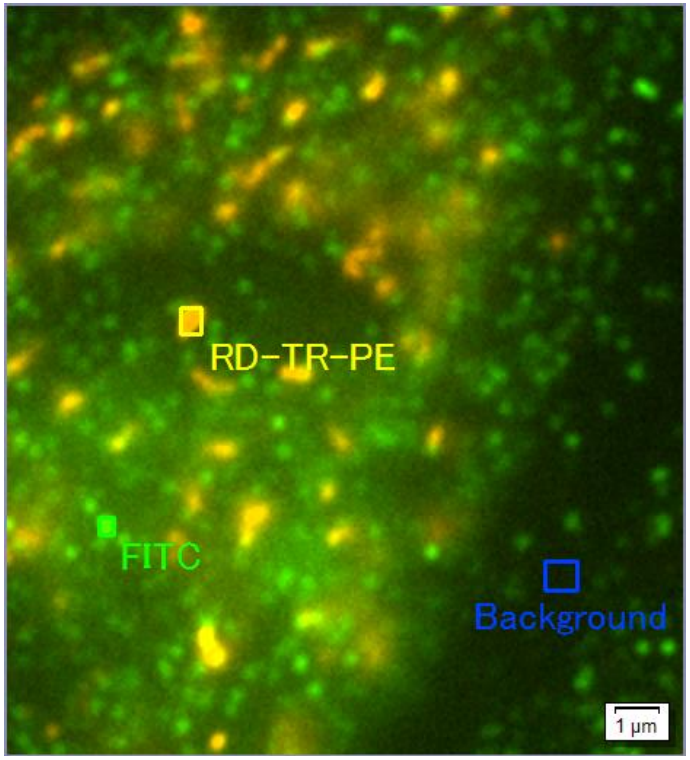
Case 2: Intensity profile

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”

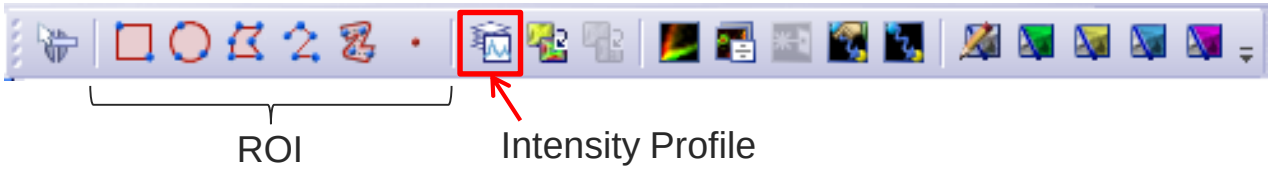
   Or select “Intensity Profile“

   Menu bar “Measure” >> “Intensity Profile”

   Or

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

Life Science Applications toolbar



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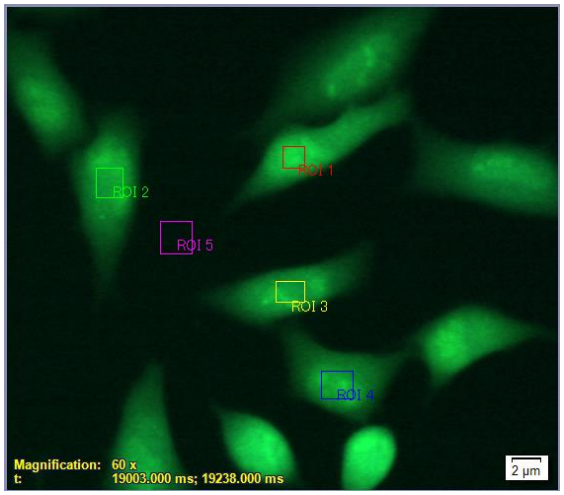
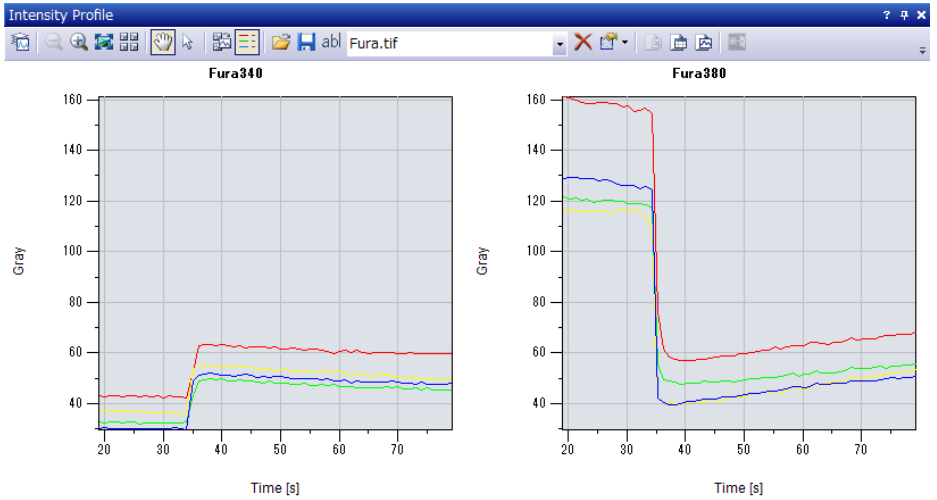
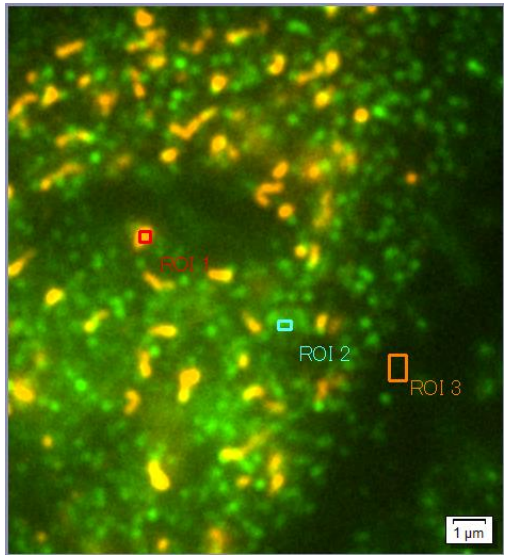
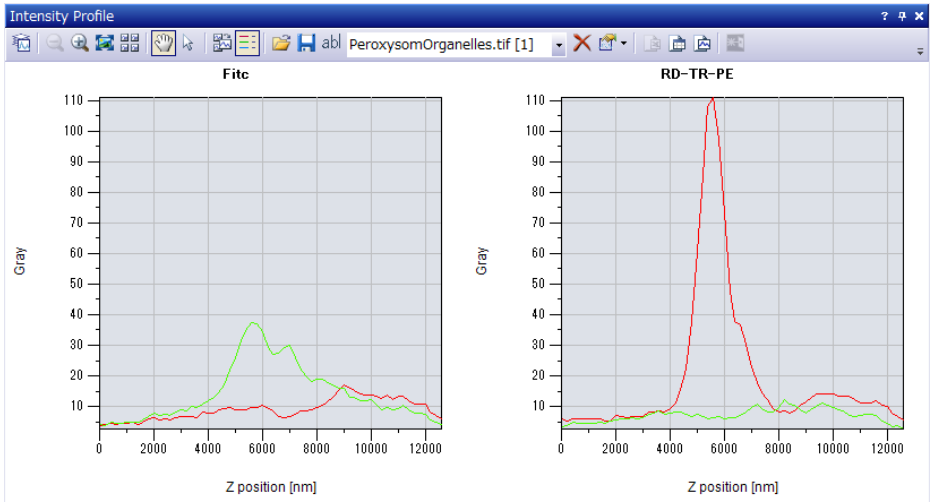
Time





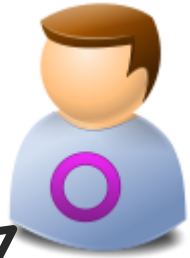
Z stack



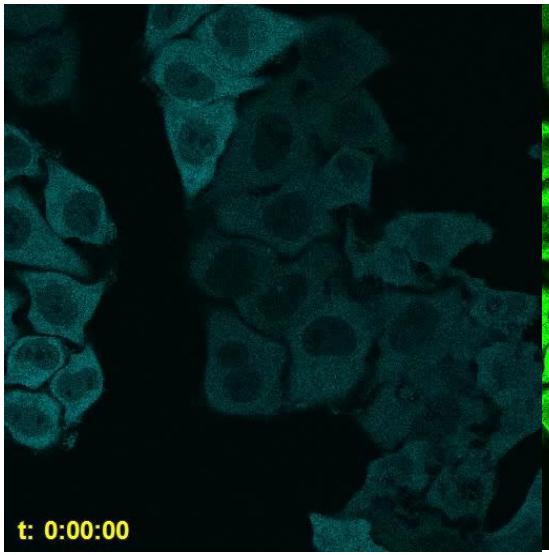


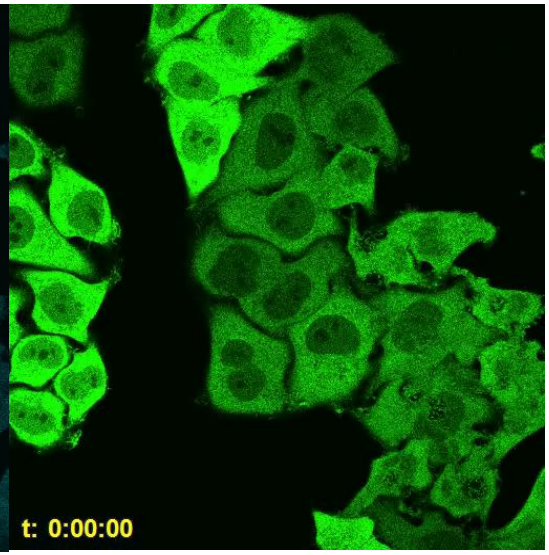
Case 3: Ratio

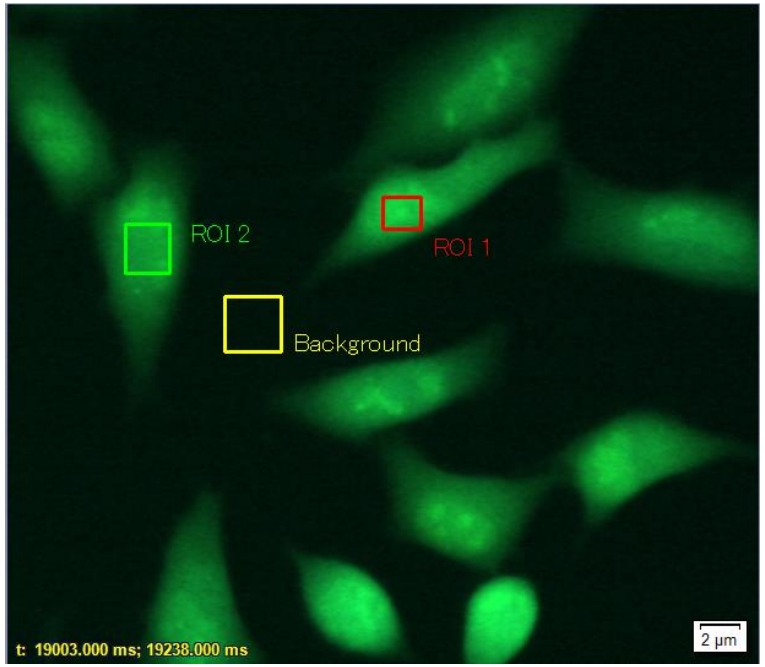
I want to analyze this movie.



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

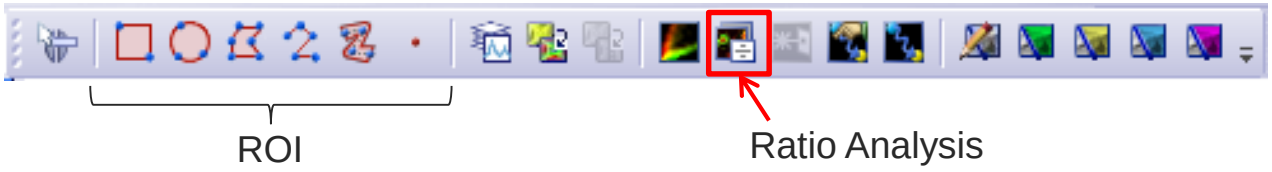
   Menu bar “View” >> “Toolbars” >> “Life Science Applications”

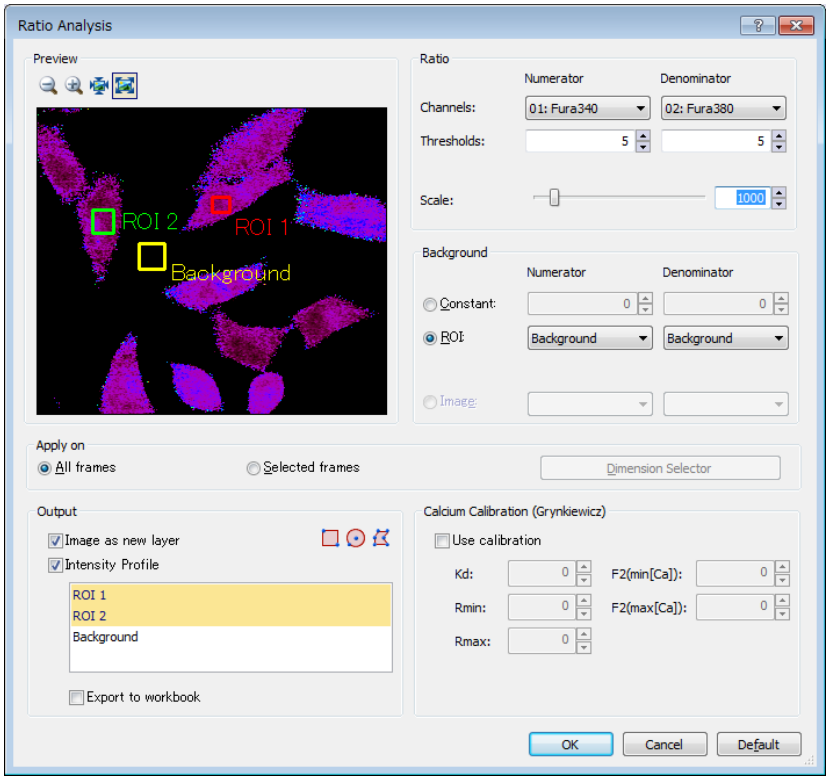
   Or

   Select “Ratio Analysis“

   Menu bar “Measure” >> “Ratio Analysis”

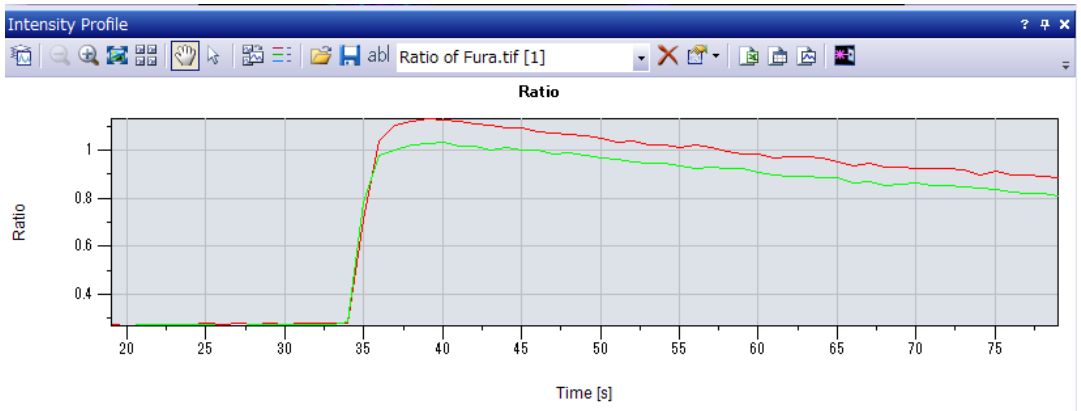
Life Science Applications toolbar



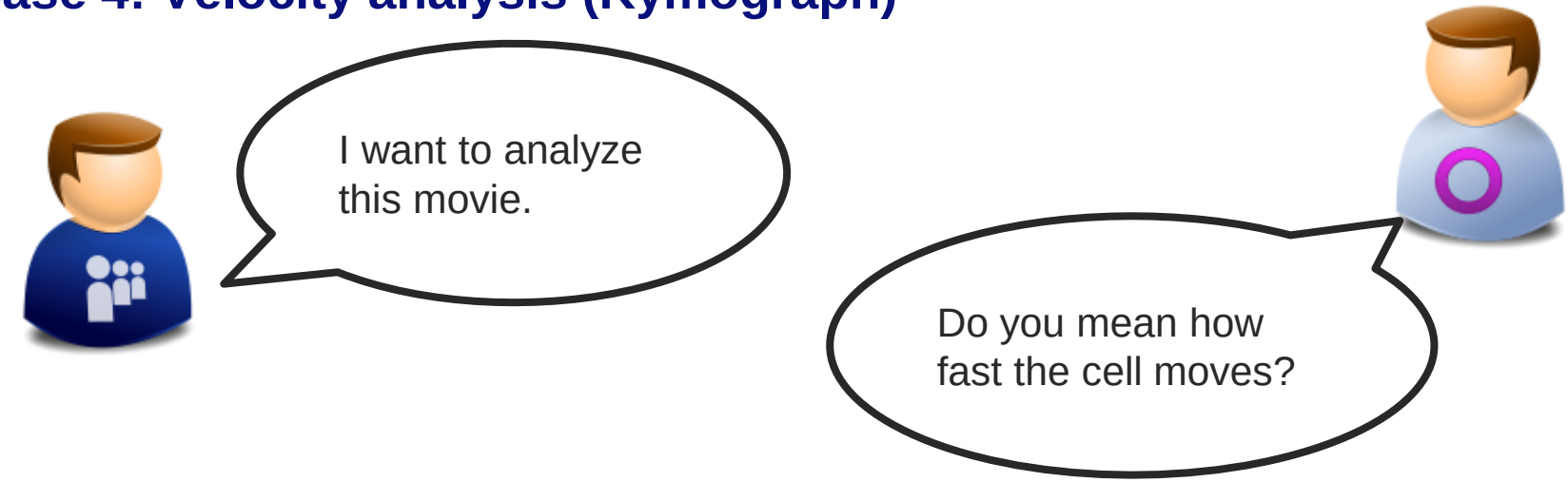


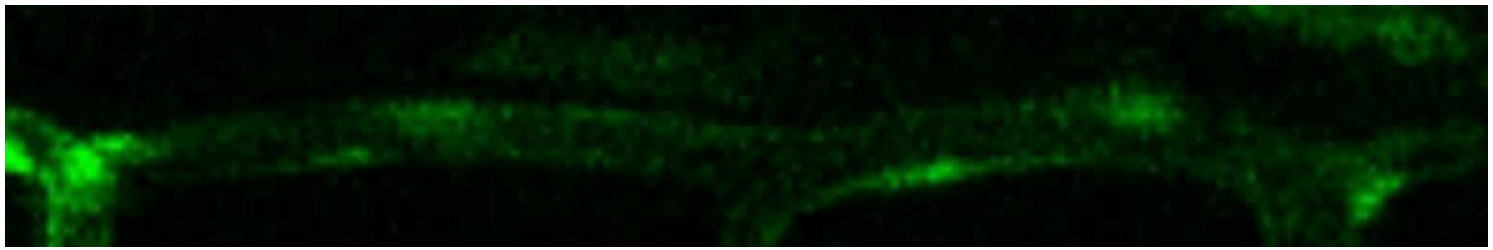
<Operation>

1. 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)
2. Click “OK” button.

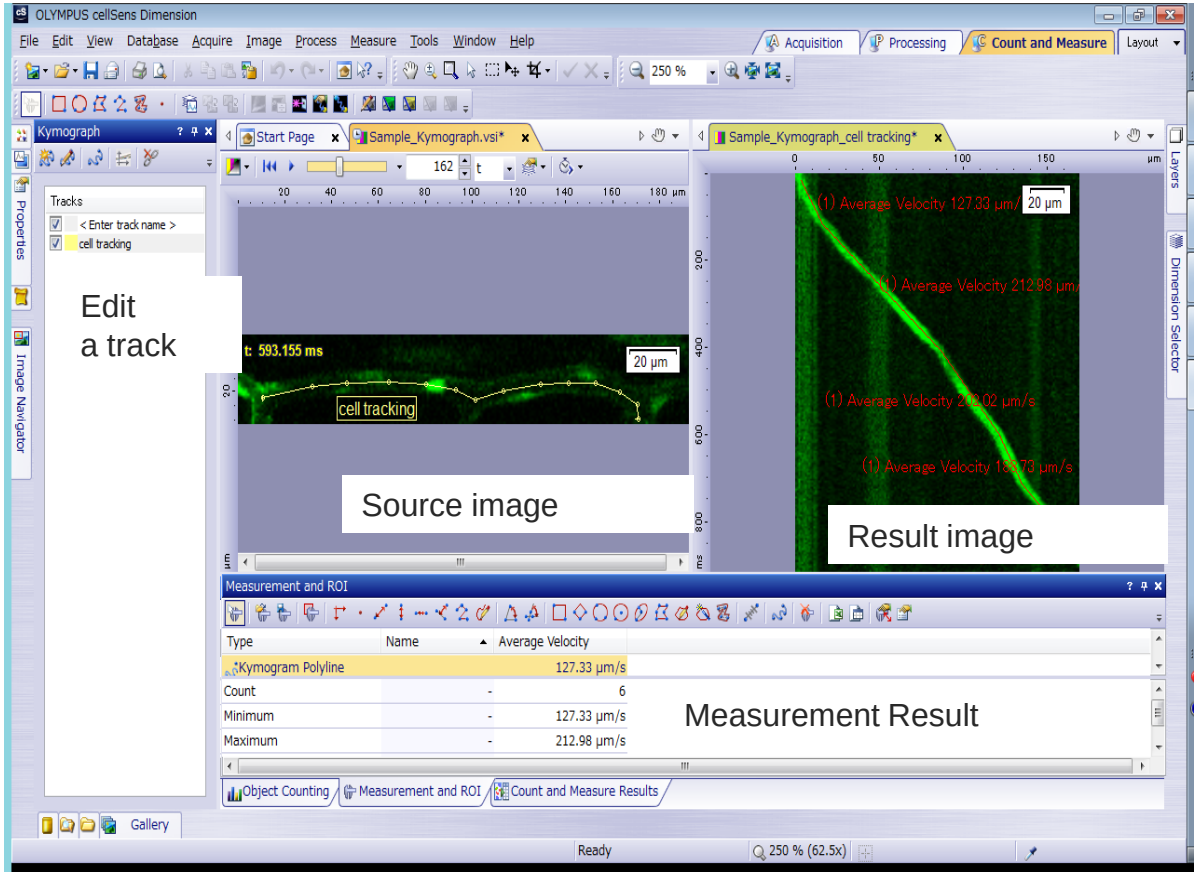


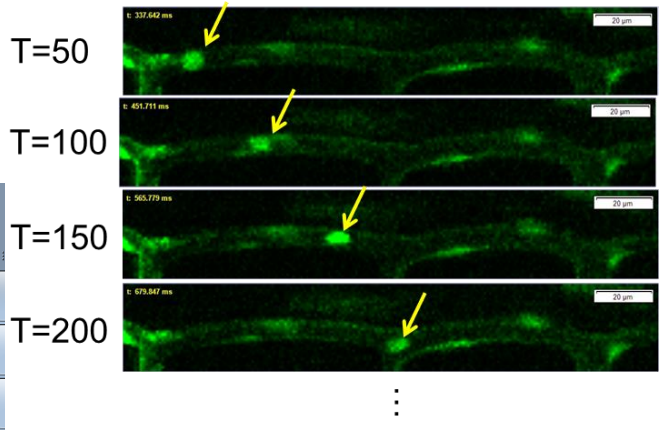
Case 4: Velocity analysis (Kymograph)



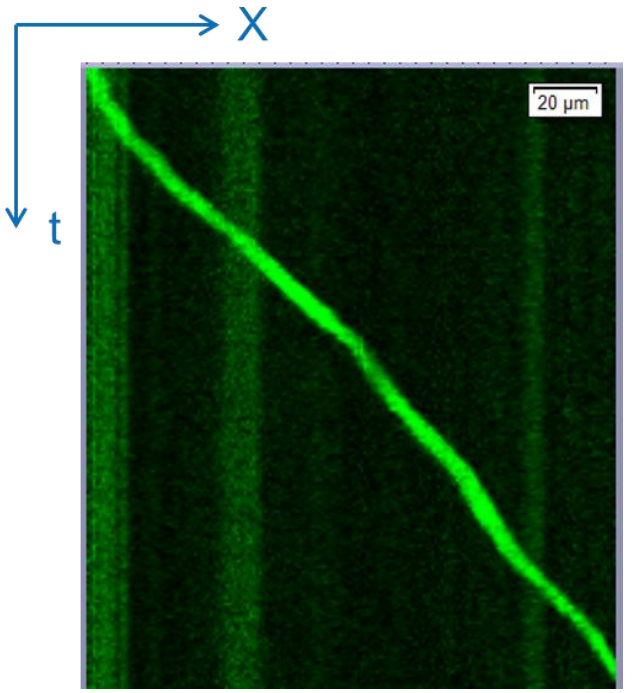


Kymograph

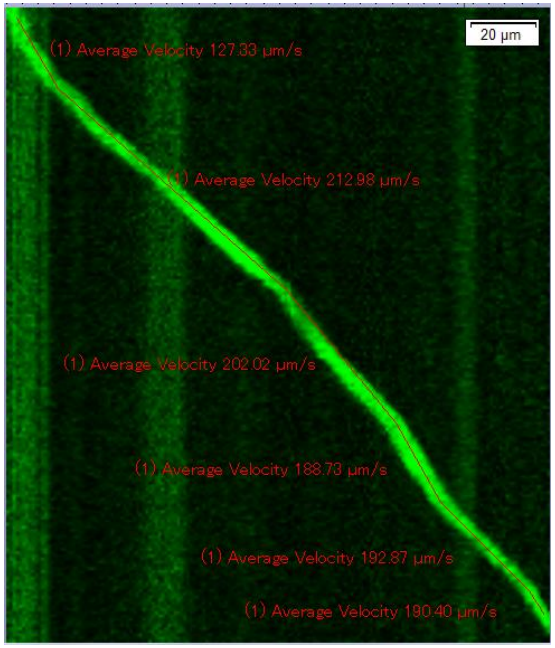




Result: Kymogram

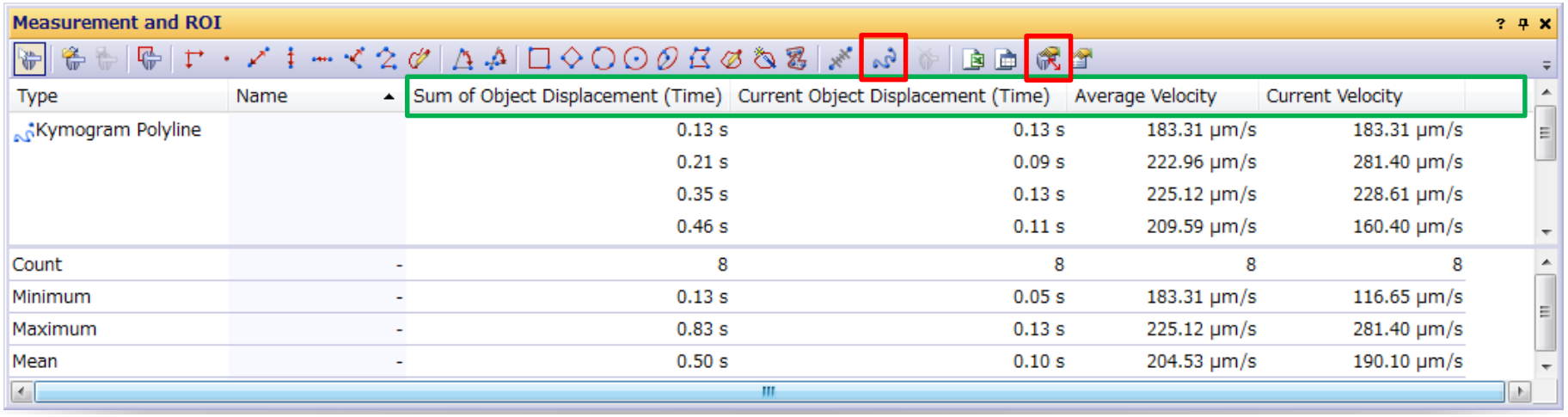


Kymograph



<Operation> : how to measure the velocity.

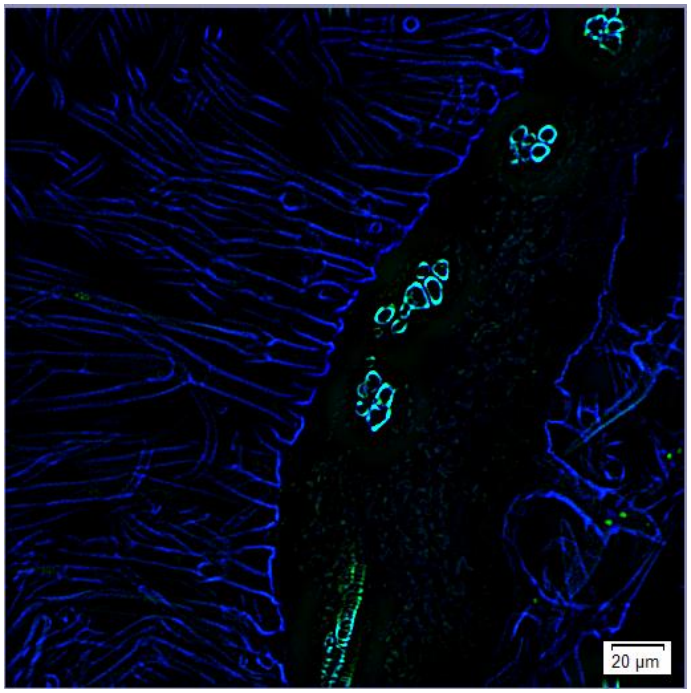
1. Click “Kymogram Polyline” button in Measurement and ROI tool window.
   Menu bar “View” >> “Tool windows” >> “Measurement and ROI”
2. On the Kymogram image, track a line by clicking (left and finally right click).
3. Click “Select Measurements” button in Measurement and ROI tool window. And then select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

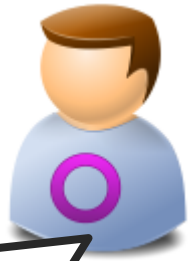


Type	Name	Sum of Object Displacement (Time)	Current Object Displacement (Time)	Average Velocity	Current Velocity
Kymogram Polyline		0.13 s	0.13 s	183.31 µm/s	183.31 µm/s
		0.21 s	0.09 s	222.96 µm/s	281.40 µm/s
		0.35 s	0.13 s	225.12 µm/s	228.61 µm/s
		0.46 s	0.11 s	209.59 µm/s	160.40 µm/s
Count	-	8	8	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.

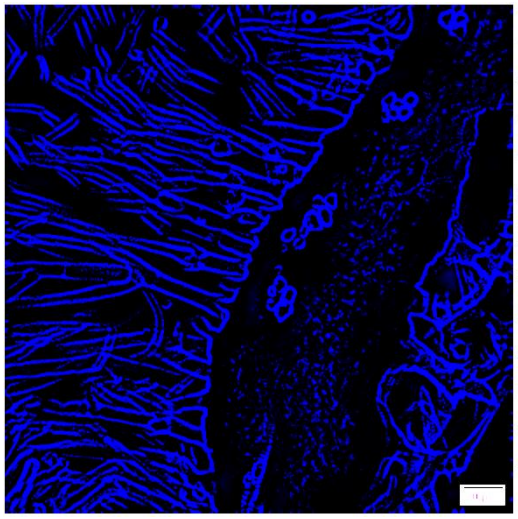




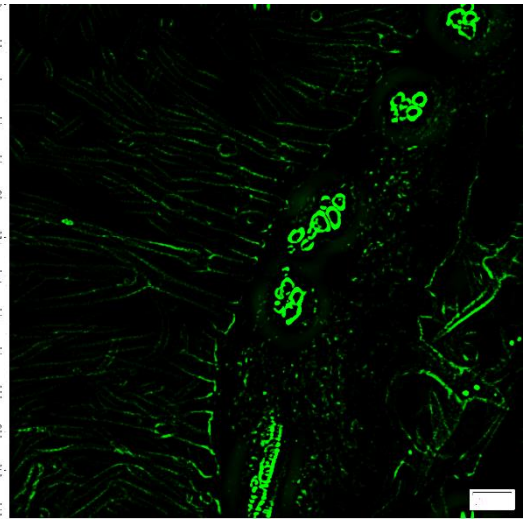
Do you mean pixel intensity spatial correlation analysis and Pearson's correlation coefficient?

Colocalization

CHS1

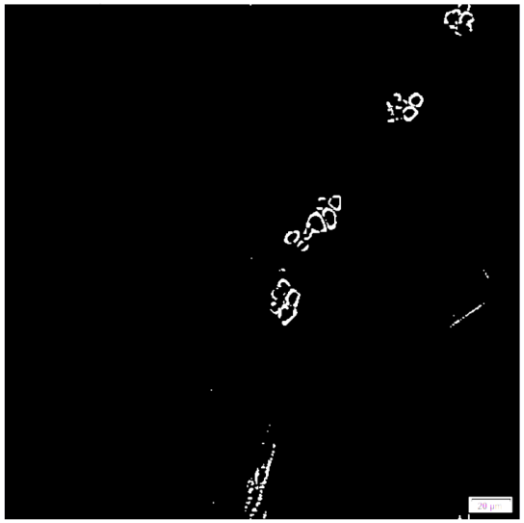


CHS2



Sample image “Lavender.tif”

Colocalization (CHS1/CHS2)



<Operation>

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

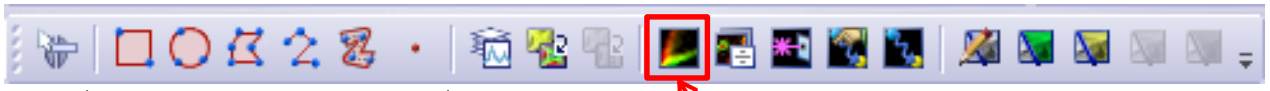
   Menu bar “View” >> “Toolbars” >> “Life Science Applications”

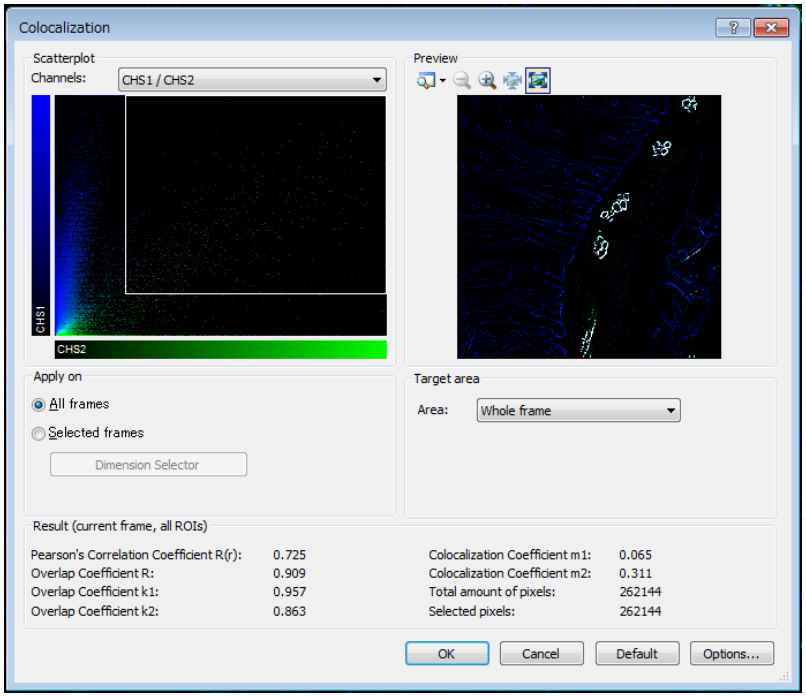
   Or

   Select “Colocalization“.

   Menu bar “Measure” >> “Colocalization“

Life Science Applications toolbar





<Operation>

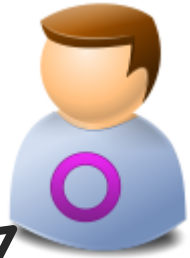
1. In Colocalization dialog box, set followings
   - Channels in Scatterplot field
     - ✓ Select channels
   - Preview
     - ✓ Select displayed image.
   - Apply on frames
     - ✓ Basically select “All frames”
   - Target area
     - ✓ Select area
       - ✓ Whole area
       - ✓ ROI
       - ✓ Segmentation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

	Range	Meaning
Pearson’s Correlation Coefficient	-1 ≦ R(r) ≦ 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	

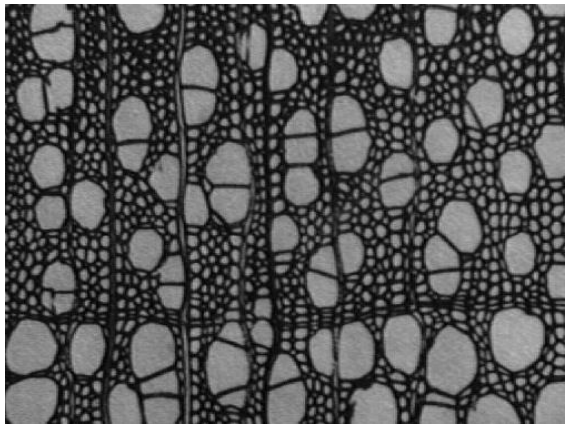
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3074624/

Case 6: count and measure

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

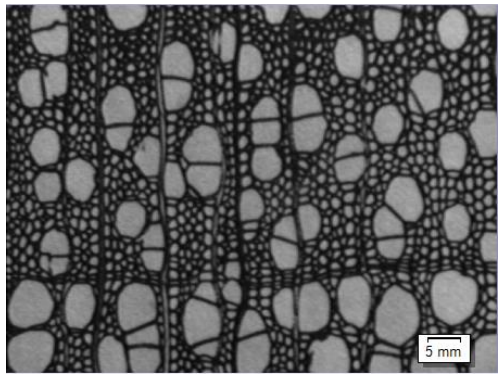


Manual measurements are quite hard, so let's try with count & measure.

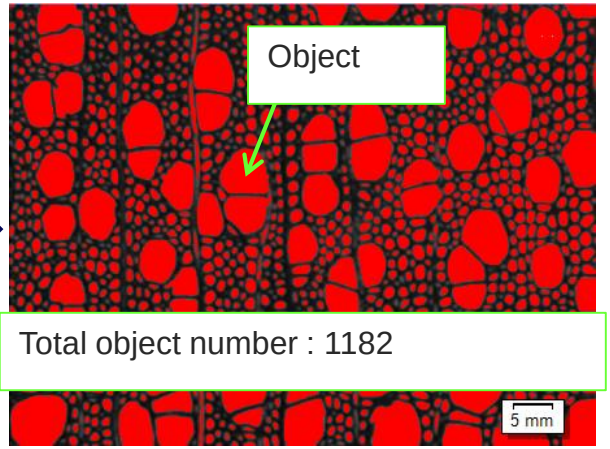


Count and Measure

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

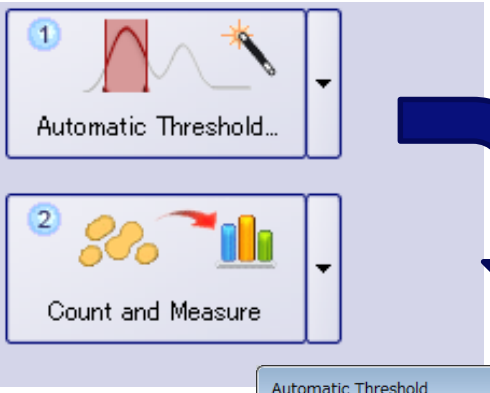


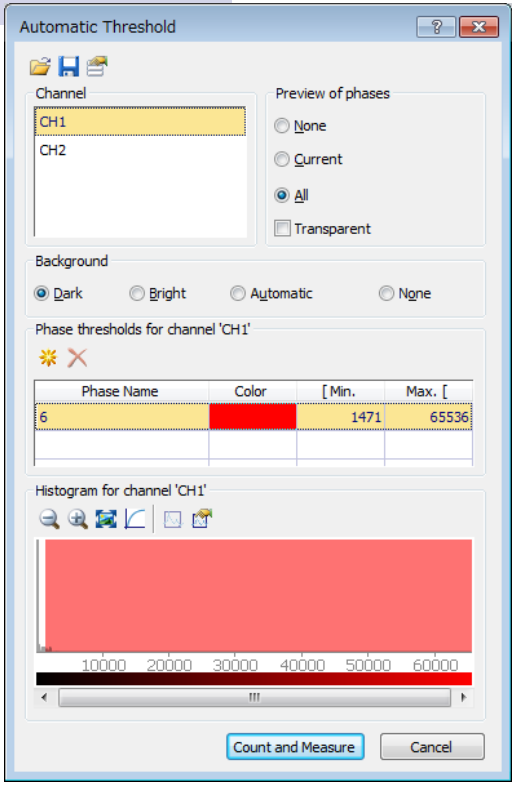
Count



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

1-1. Class Measurement - Operation -

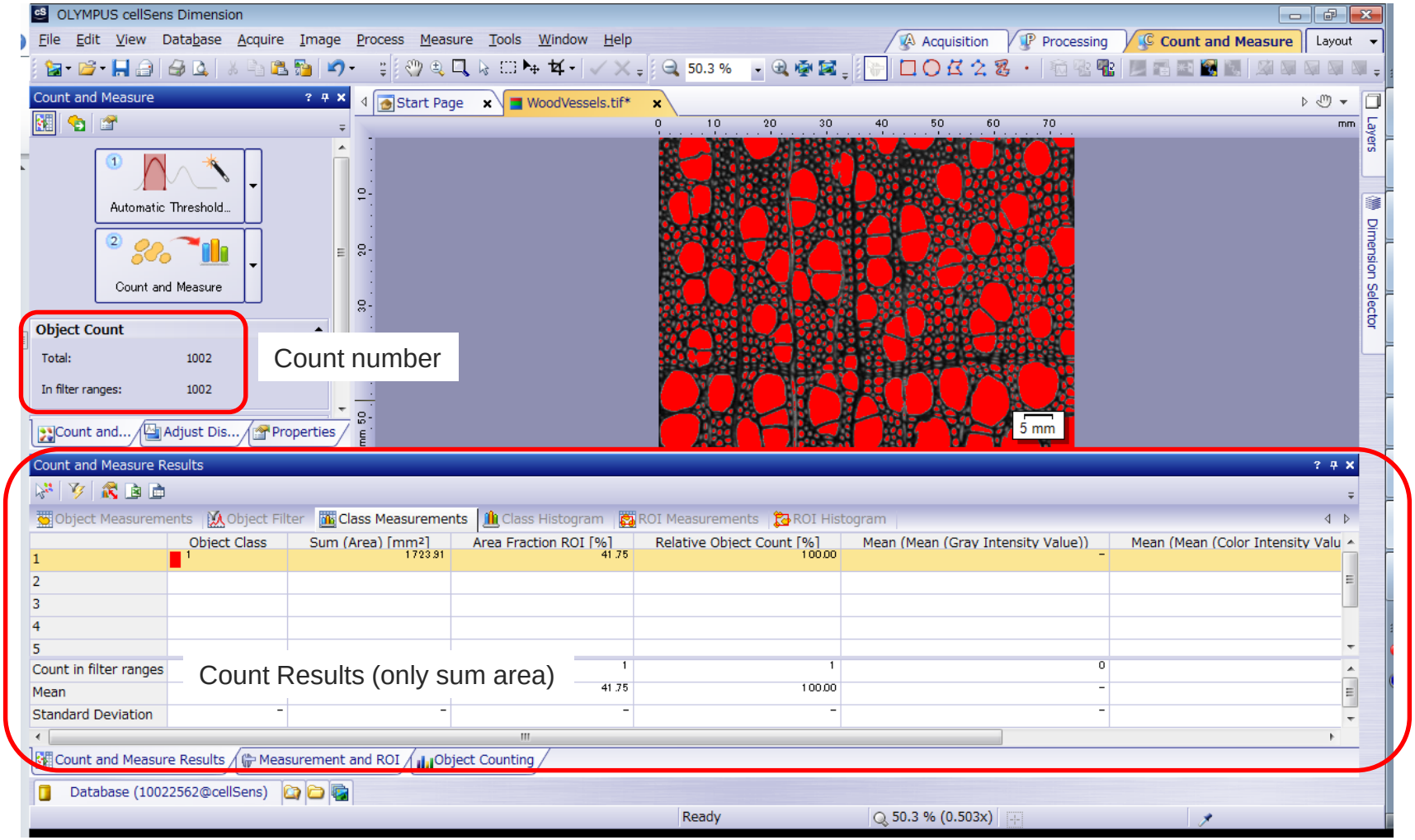




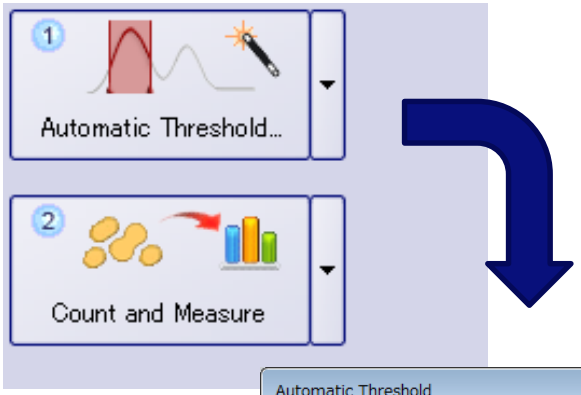
<Operation>

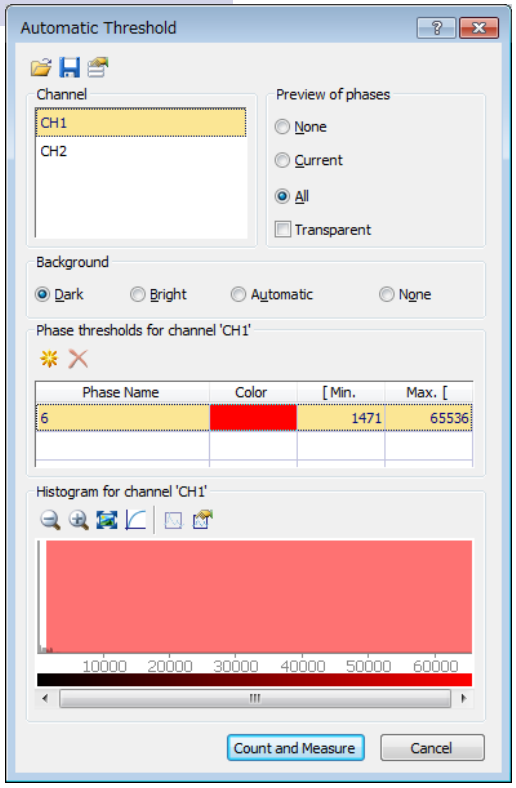
1. Select “Count and Measure” tab
2. Select an image.
3. Click “Threshold” button in Count and measure tool window. (Automatic / manual / HSV)
   Menu bar “View” >> “Tool windows” >> “Count and Measure”
4. Set some parameters in the dialog box.
   (channel, phase thresholds, background)
5. Click “Count 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 -



1-2. ROI Measurement - Operation-





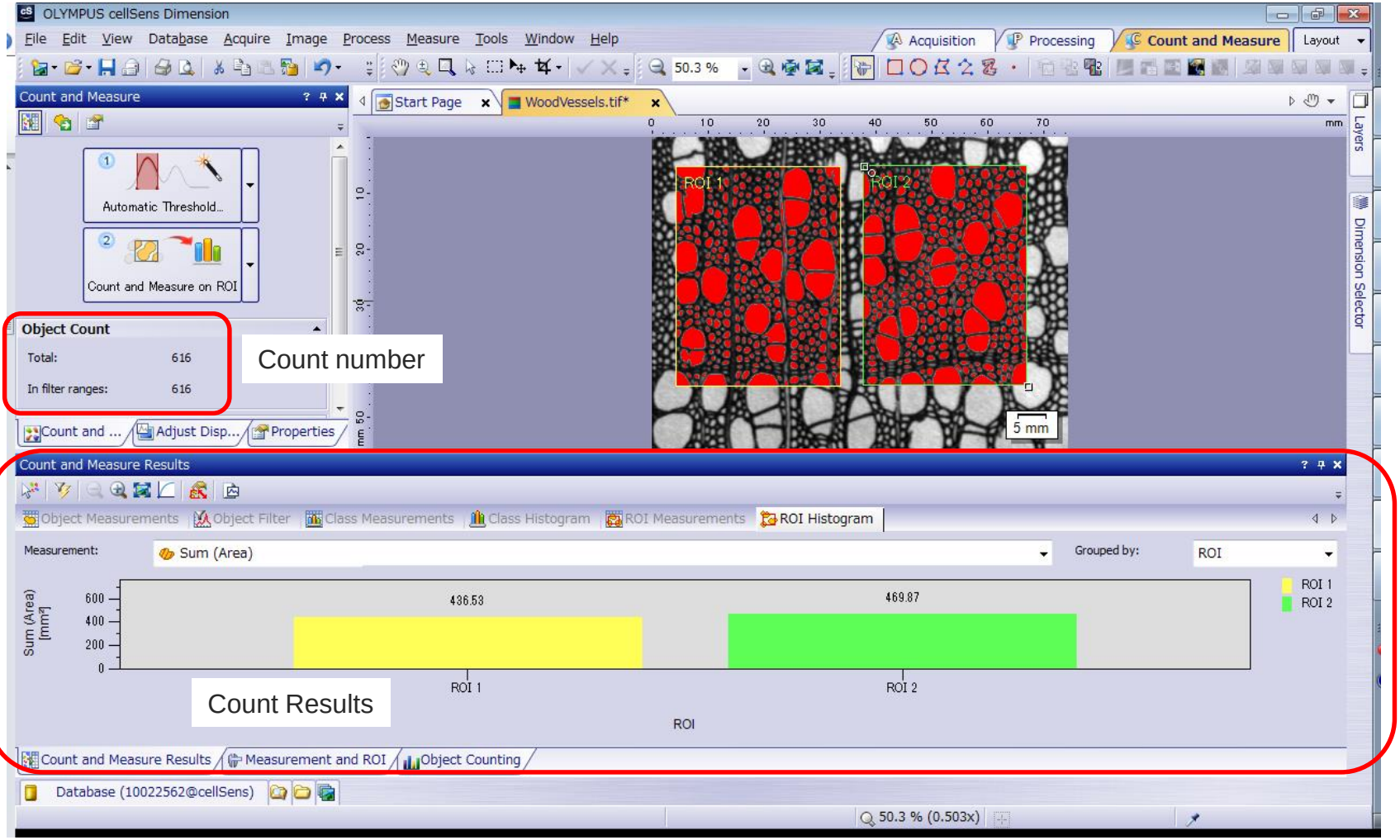
<Operation>

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)

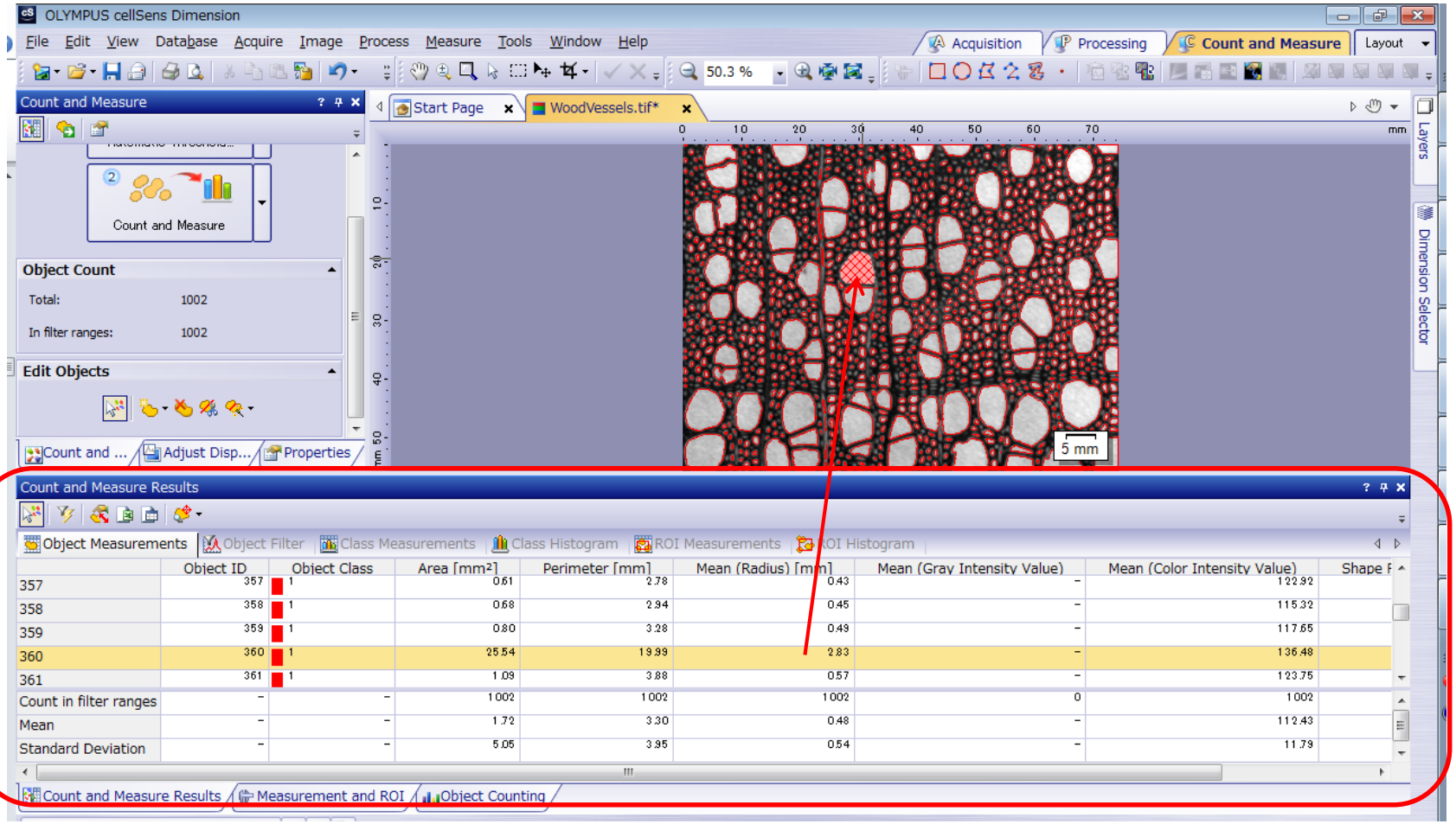
   Menu bar “View” >> “Tool windows” >> “Count and Measure”
5. Set some parameters in the dialog box. (channel, phase thresholds, background)
6. Click “Count and Measure in ROI” button in Count and Measure tool window.
7. Check “ROI Measurements” tab or “ROI Histogram” tab in Count and Measure Results tool window.

   Menu bar “View” >> “Tool windows” >> “Count and Measure Results”

1-2. ROI Measurement - Result Image-



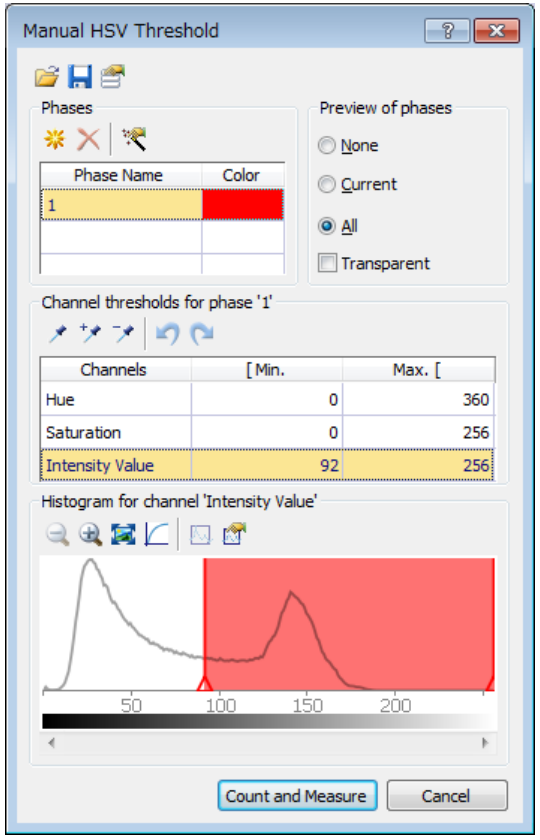
1-3. Object Measurement



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

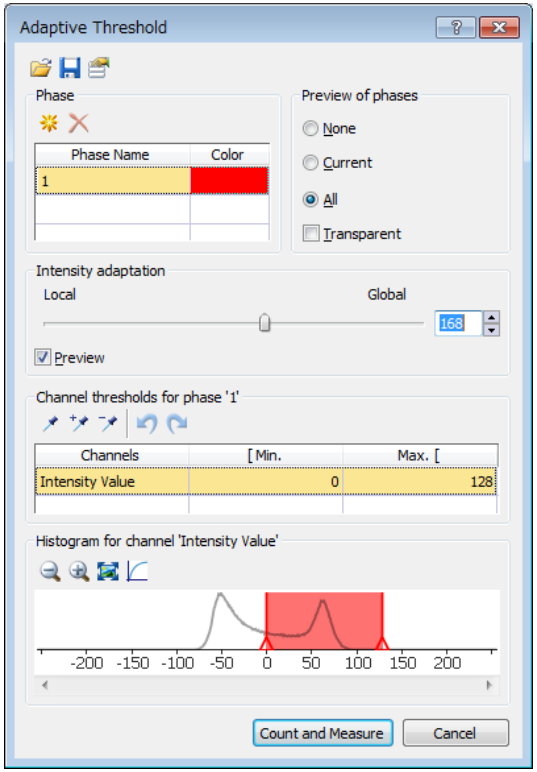
2-1. Threshold setting

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



For color image

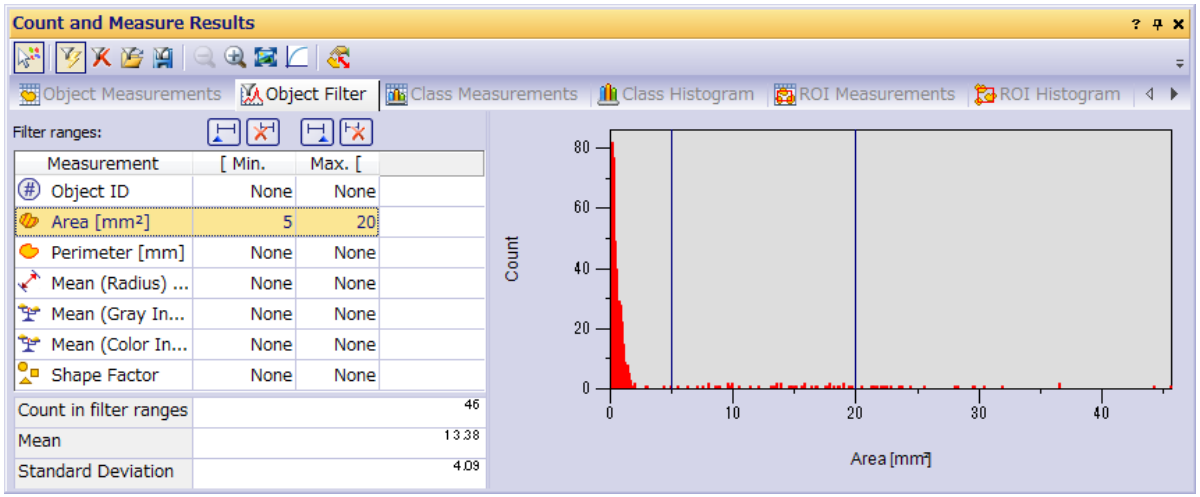
For all image



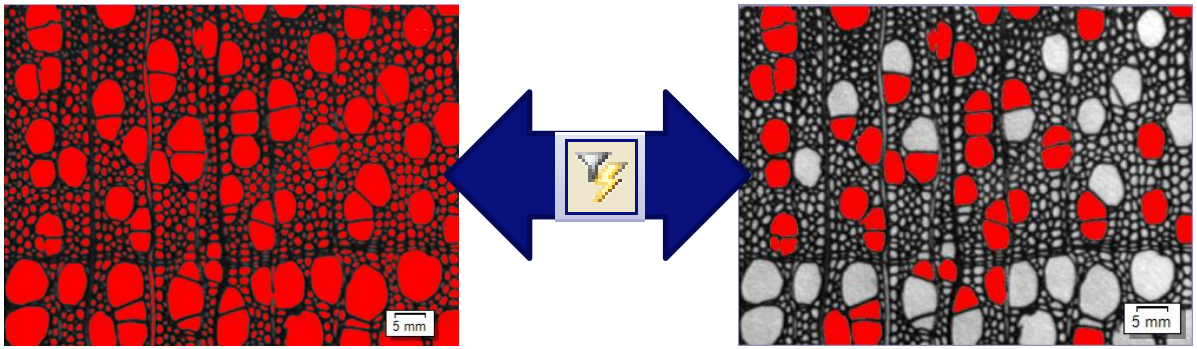
For images which has uneven brightness background (shading)

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.



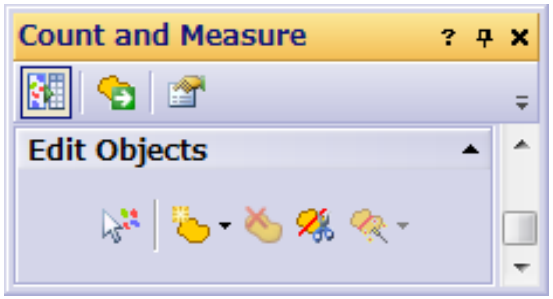
- Object ID
- Area
- Mean (Radius)
- Mean (Gray Intensity)
  Mean (Color Intensity)
- Shape Factor (Roundness)



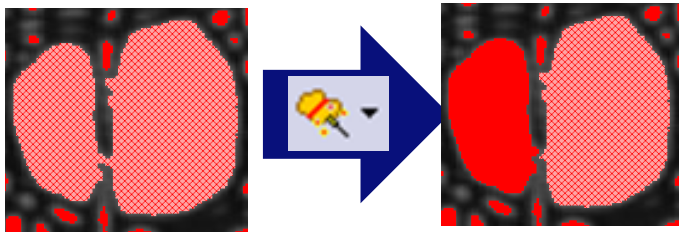
Count :1002

Count :46

3. Edit objects

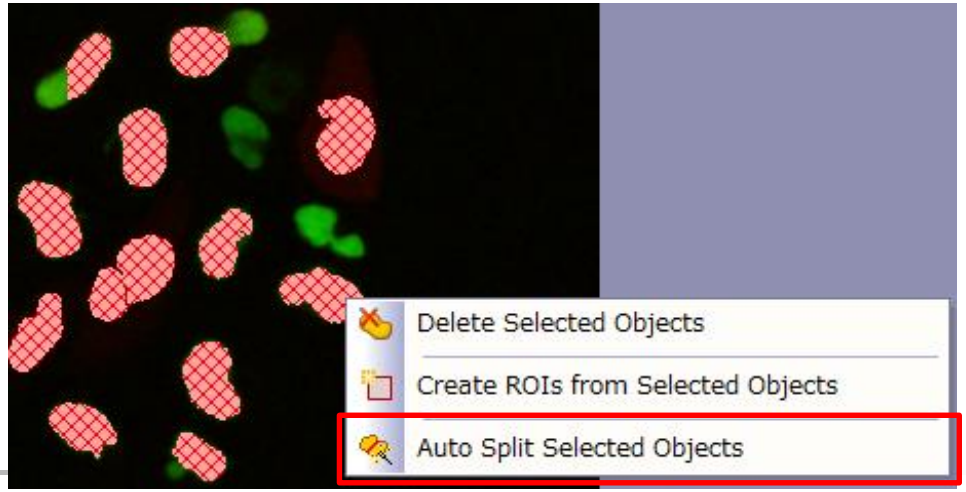


	Allows selection of detected objects.
	Create a new object
	Deleted selected objects
	Manually split objects
	Automatic split objects



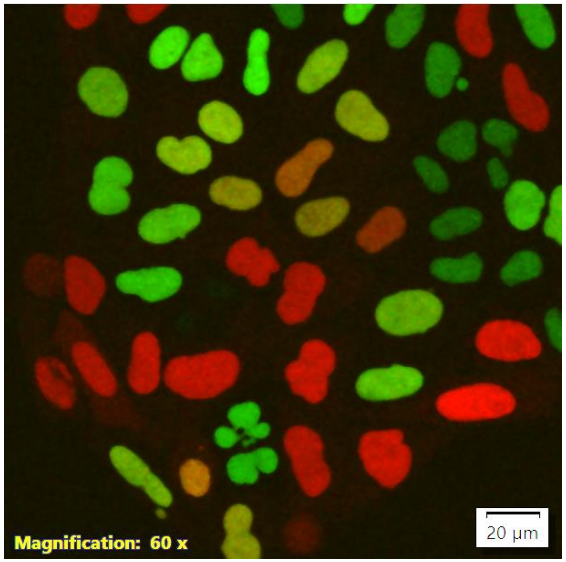
After detection of objects by counting, we can edit the objects one by one.

[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".

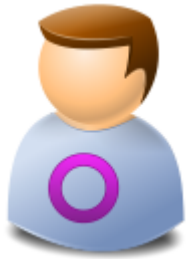


Case 7: Object Counting (Touch Counting)

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

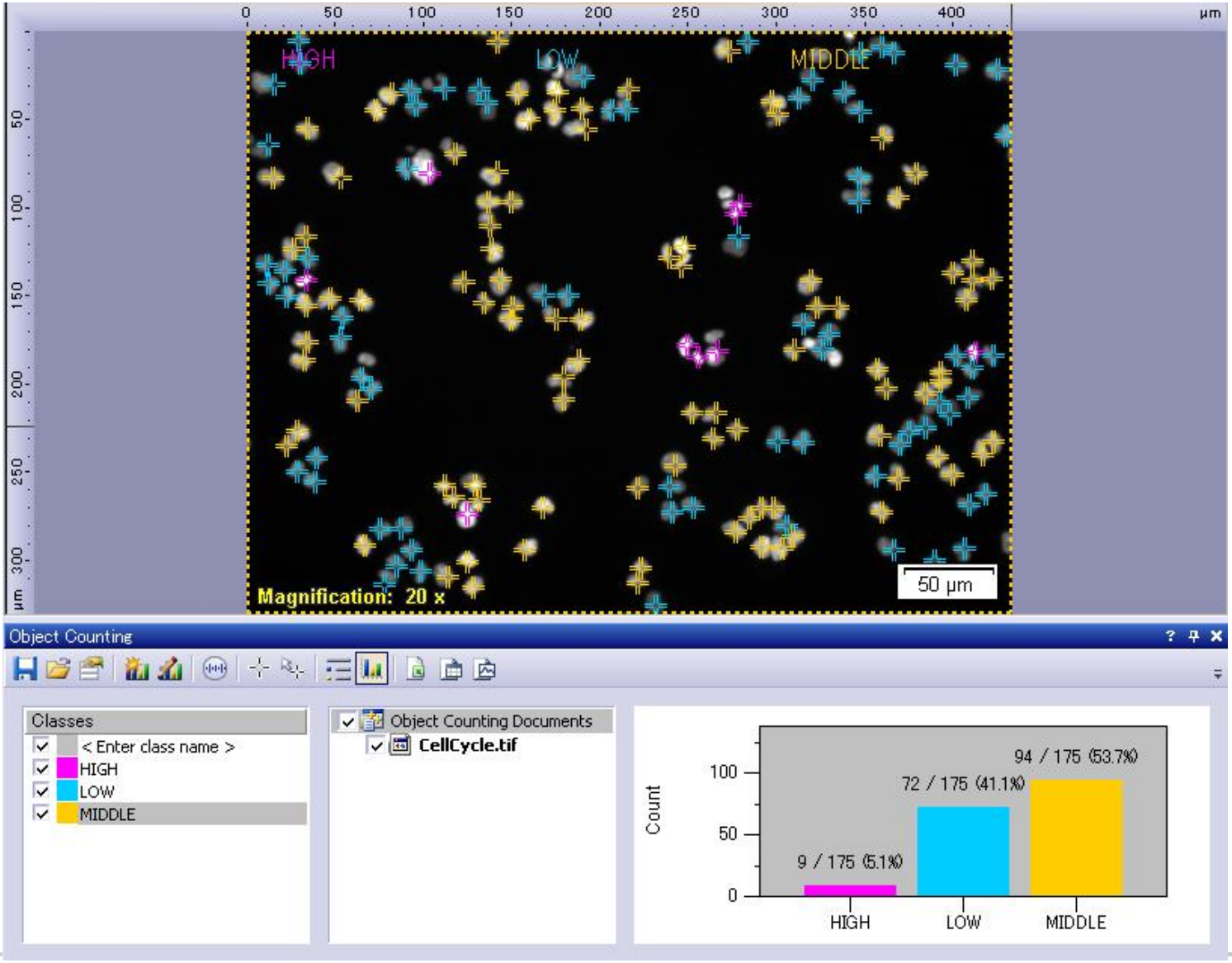


We can count objects manually in your images by clicking on the objects. You can define different classes and assign the objects to those classes.



OLYMPUS

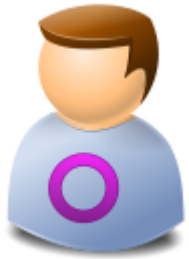
Object Counting



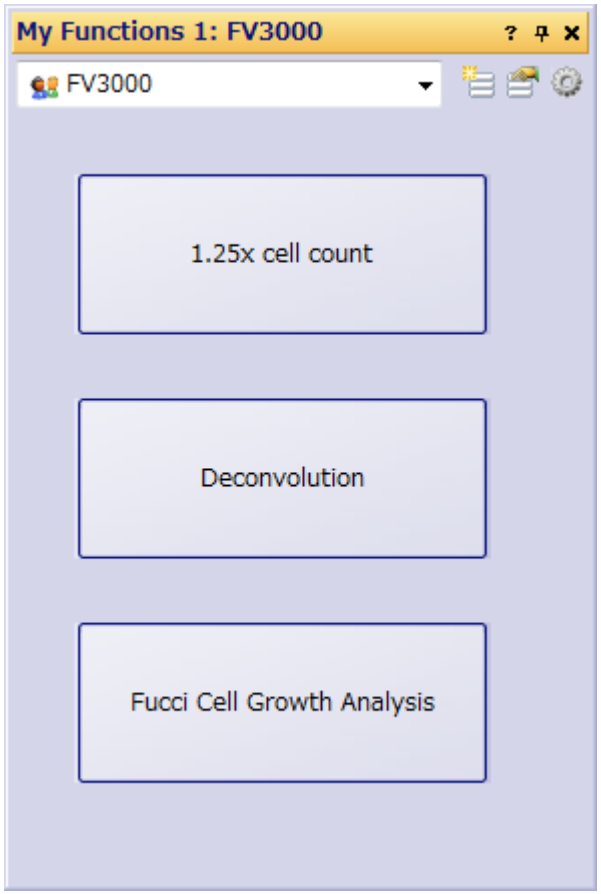
Case 8: Macro Recording

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.



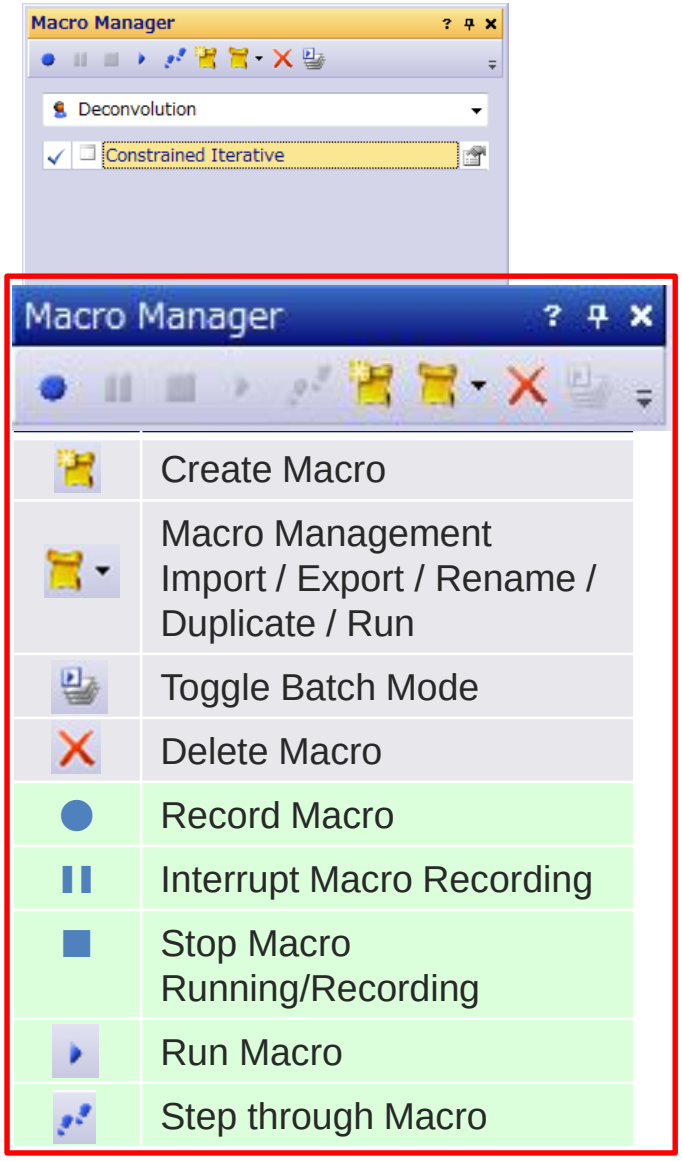
One Click Macro Capability



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

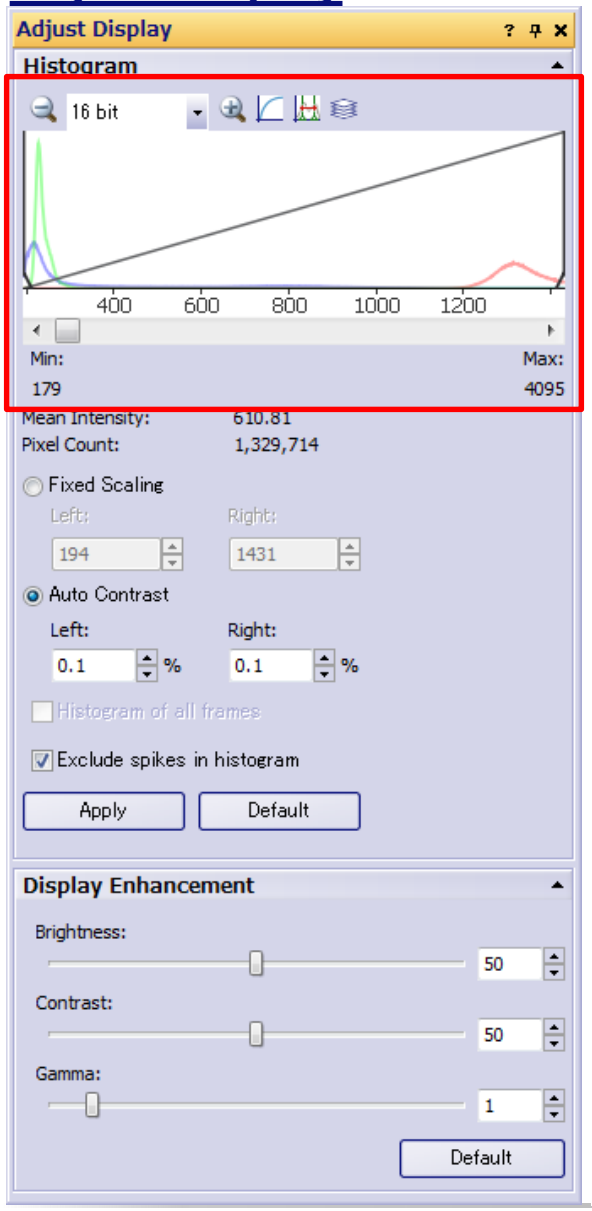


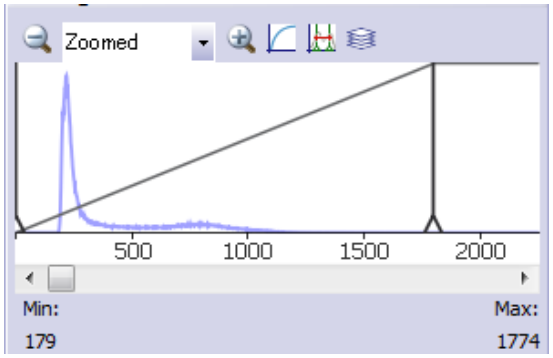
<Operation>

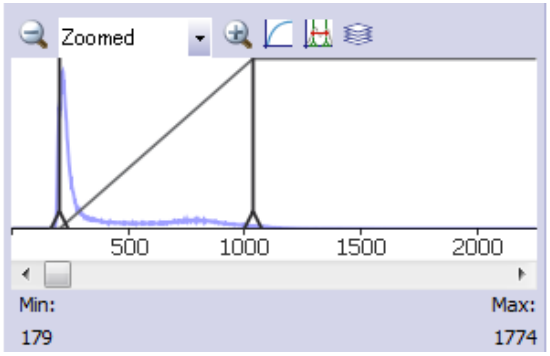
1. Click “Create Macro” button in Macro Manager tool window
   Menu bar “View” >> “Tool windows” >> “Macro Manager”
2. Put a name and click “OK” button. Then the macro recording will be started.
3. Operate an image processing or an image analysis
4. If click “interrupt macro Recording” button, we can 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.

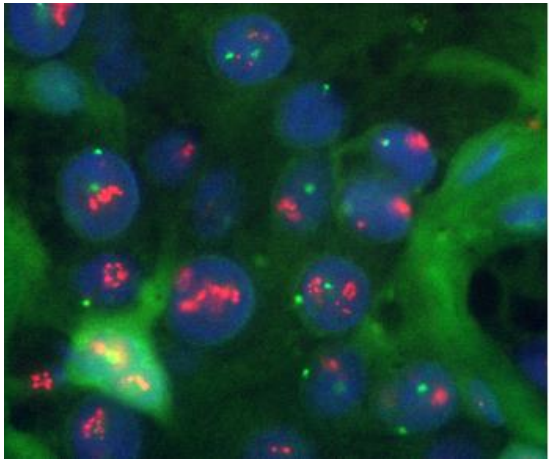
Overview of other Image Processing

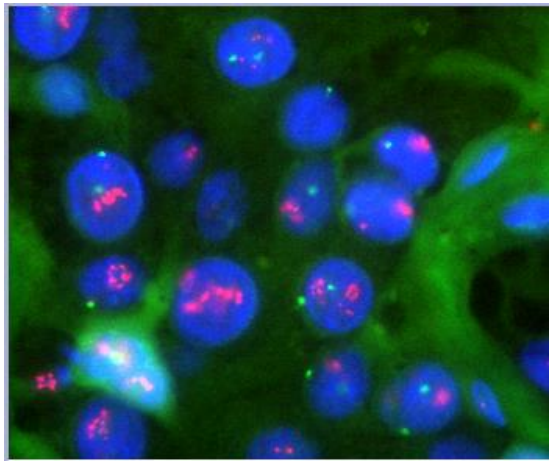
Adjust Display







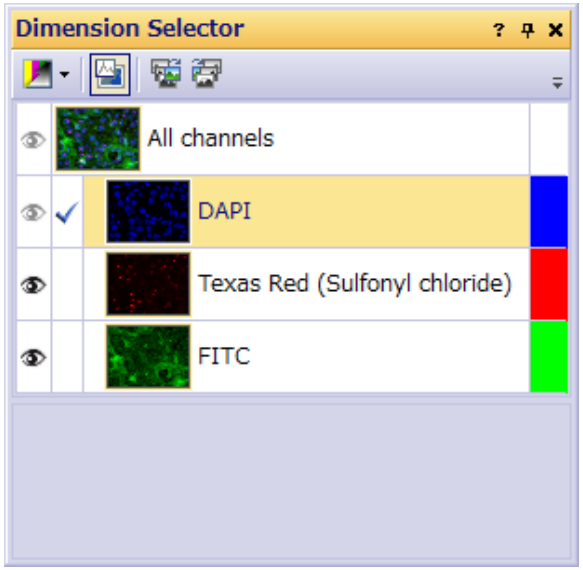




Help

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

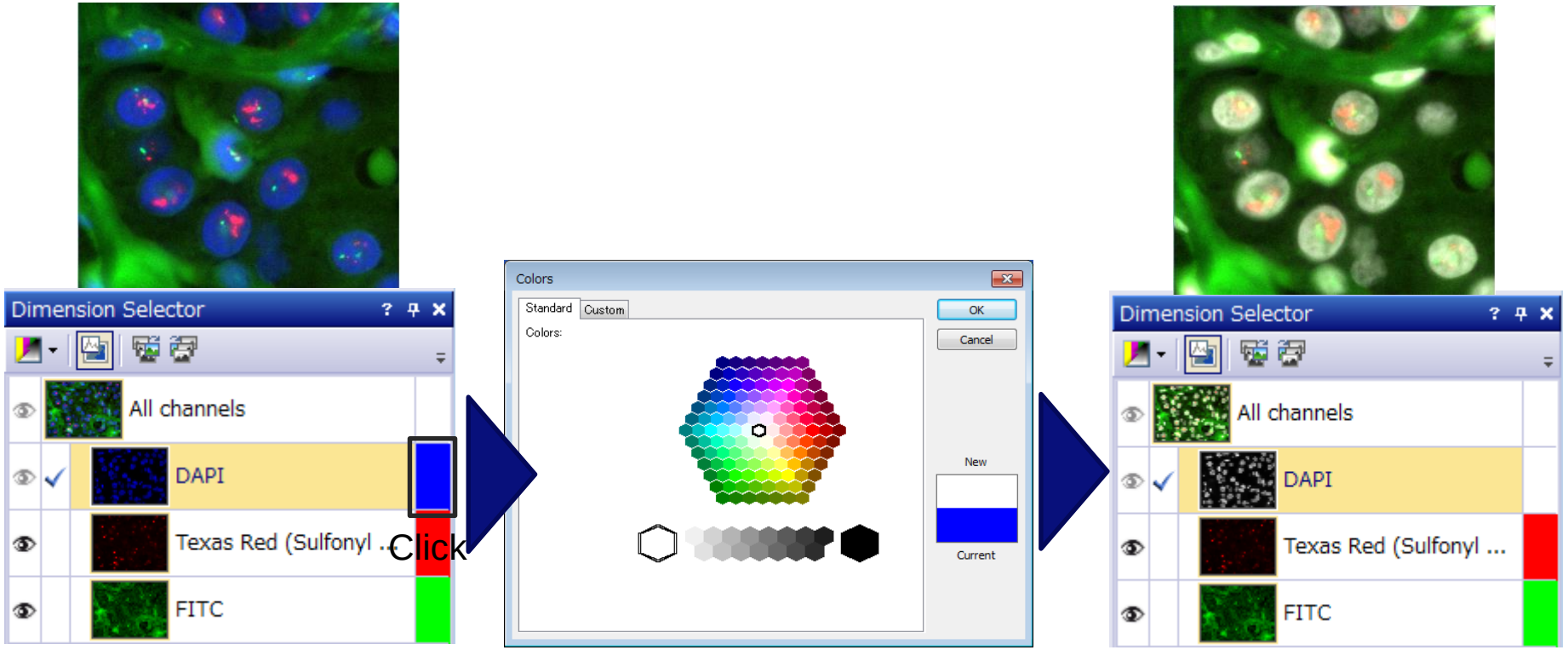
     Enter the minimum and maximum values you want, directly in the Left and Right fields.
   - In "Auto Contrast"

     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

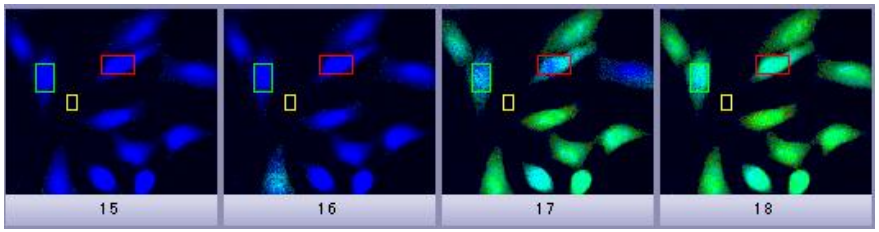


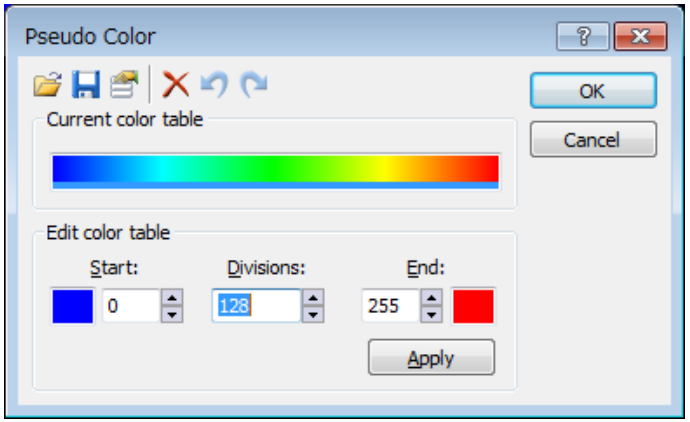
<Operation>

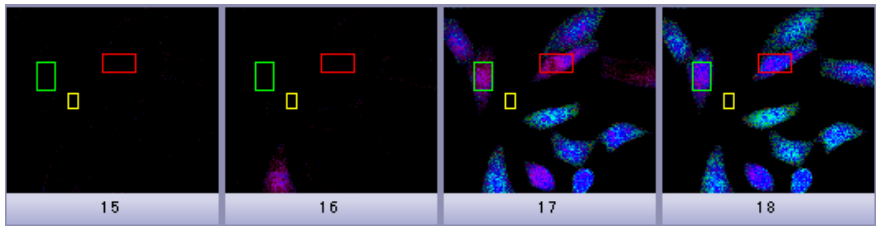
1. Open Dimension Selector tool window
   Menu bar “View” >> “Tool Windows” >> “Adjust Display”
2. Click color field which is next to the channels name
3. Select one of the colors from the palette on the standard or custom tab.
4. Click OK button.

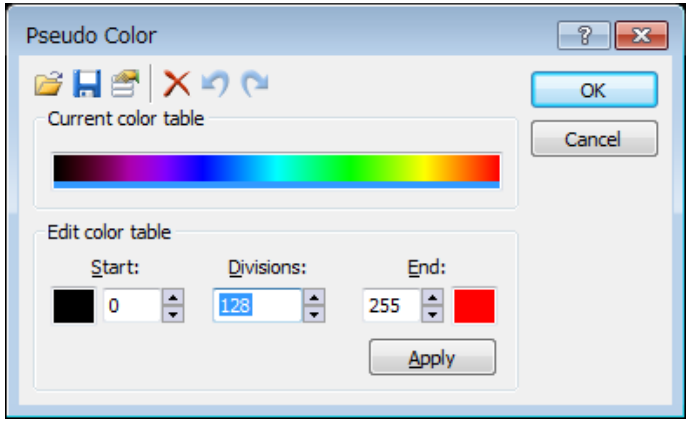
Edit Pseudo color

Change color for ratio image









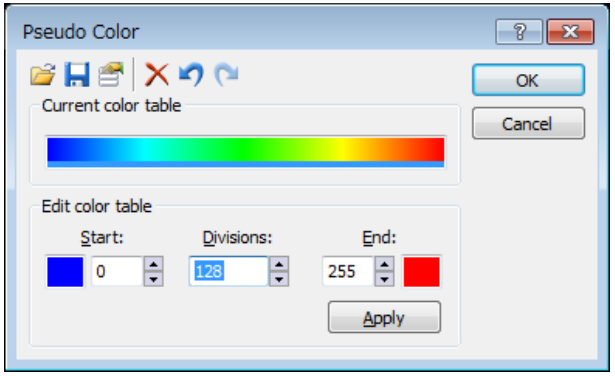
Help

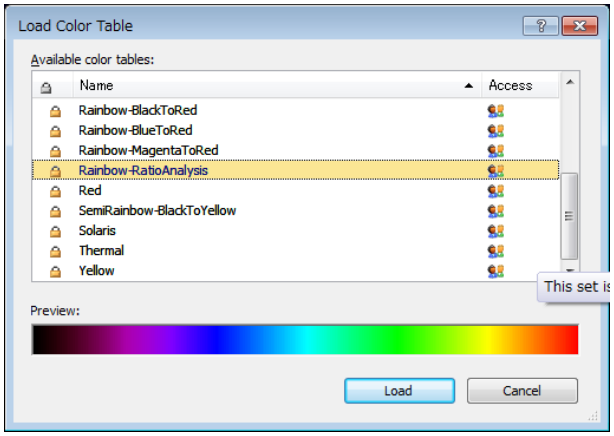
Prerequisite: This command is only available when the active document is a gray-value image.

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

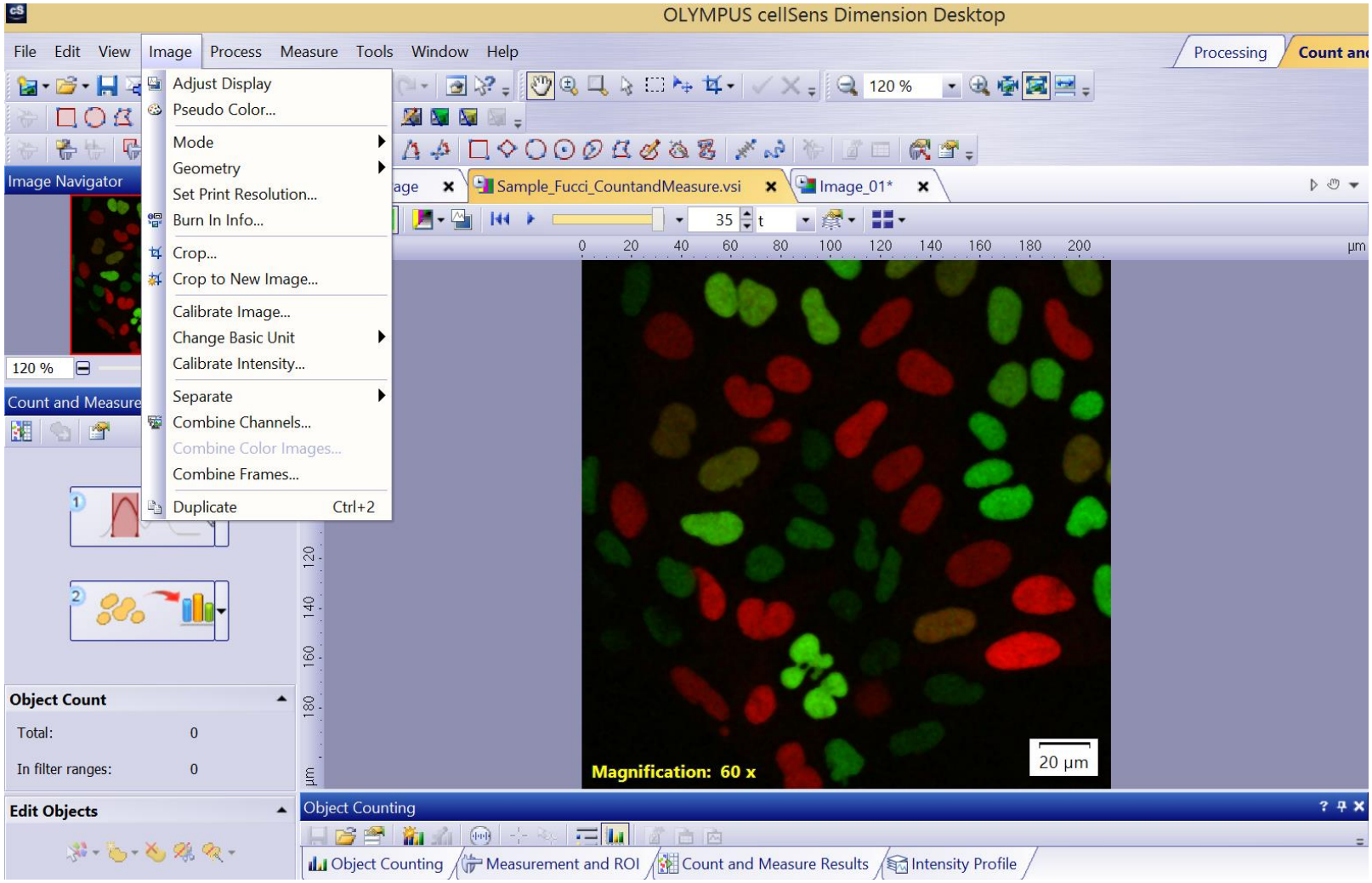




<Operation>

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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