

Introduction into image processing with Fiji

- Please download Fiji at <https://fiji.sc/>

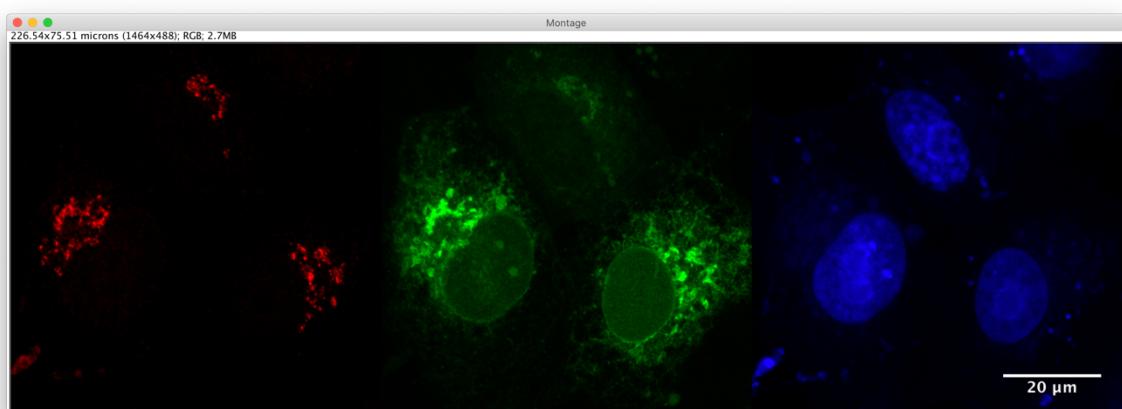
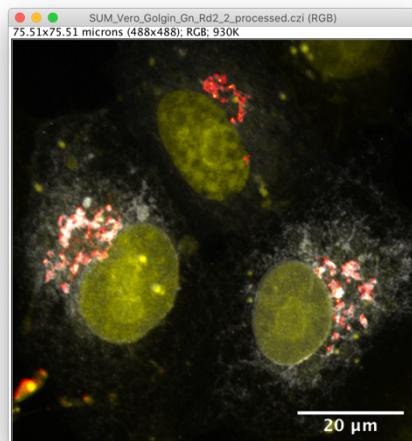


- Look at <https://imagej.net/learn/> to get a basic introduction
- Aspects that will be discussed during the course:
 - Main GUI/Interface
 - Open/Import images
 - General image manipulation
 - Stacks
 - LUTs
 - Simple measurements
 - Plugins and where to find them
 - Macros
- Other software that will be shown in demo examples:
 - Cellprofiler
 - Python
 - Matlab

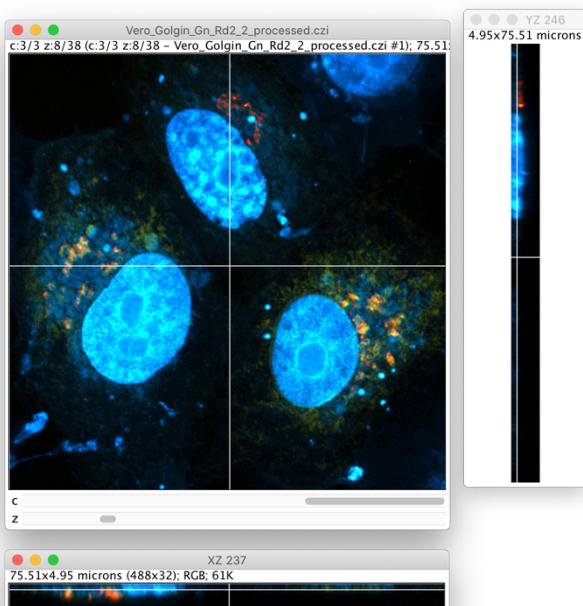
Exercise 1:

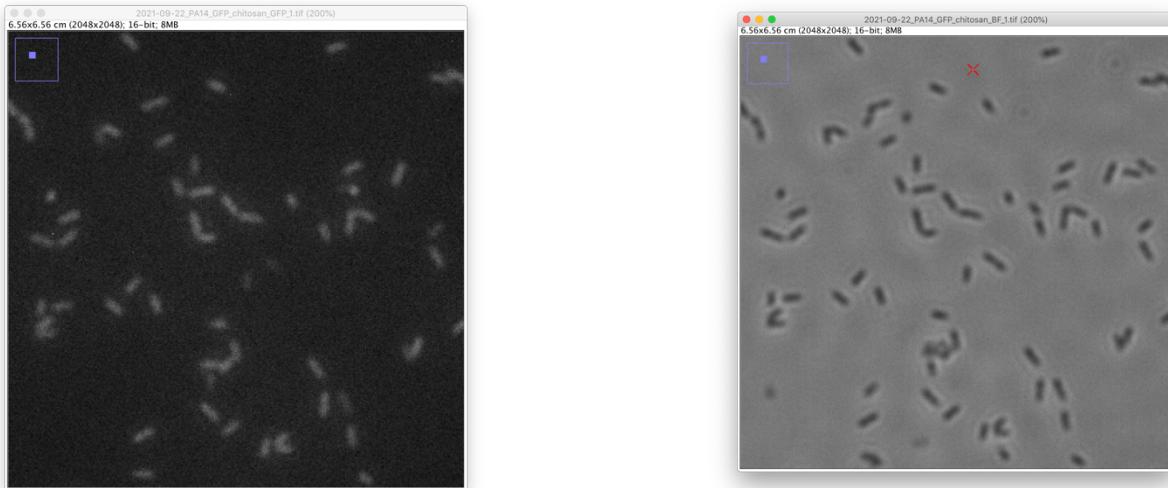
- 1) Basic Fiji functions
- 2) Composite images
- 3) Simple Macro

- Open Vero_Golgin_Gn_Rd2_2_processed.czi
- Image>Adjust>Brightness and Contrast
- Image>Color>Channels Tool
- Image>Stacks>Z Project
- Image>Stacks>Make Montage

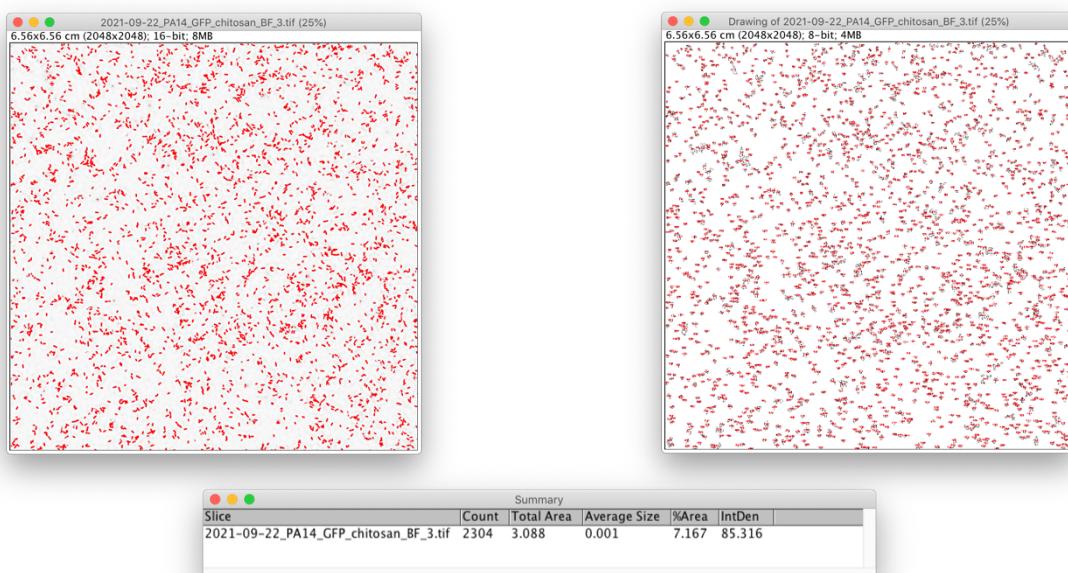


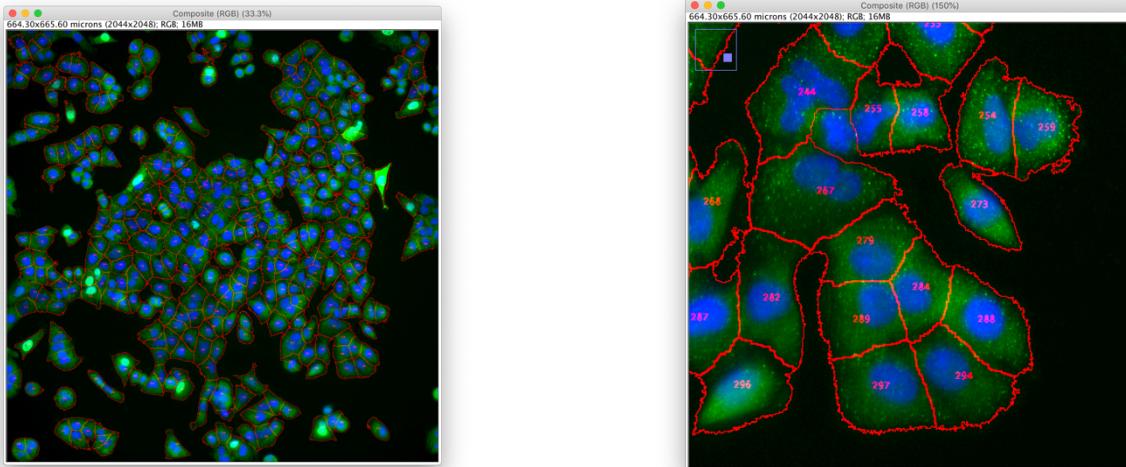
- Play around with LUTs Image>Lookup Tables
- Image>Stacks>Orthogonal Views
- *Put the first 5 commands into a macro.*



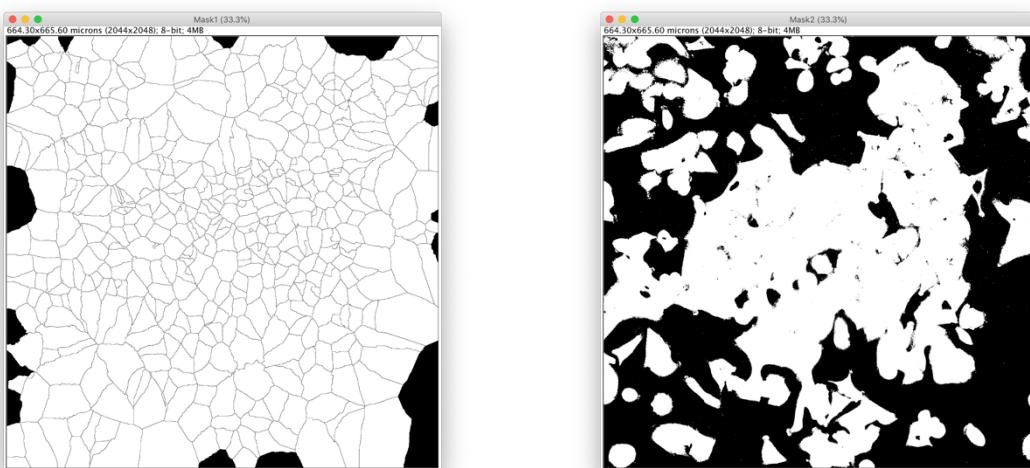
Exercise 2:**Counting bacterial cells**

- Open the image 2021-09-22_PA14_GFP_chitosan_BF_1.nd2 and 2021-09-22_PA14_GFP_chitosan_GFP_1
- Tip: Analyze>Syncronize Windows
- Select the BF image
- Process>Subtract background
- Image>Adjust>Threshold
- Choose Threshold manually or use automatic threshold (i.e. Otsu), don't press Apply
- Analyze>Analyze Particles
- *Optional: Press 'Apply' after thresholding, then Process>Binary>Watershed*
- *Try it out with the GFP channel.*



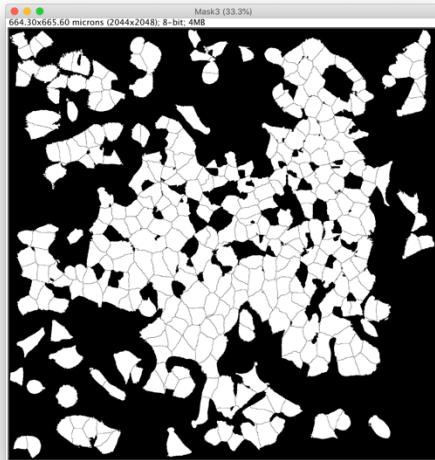
Exercise 3:**Cell segmentation**

- Open the image A549_20x_6h_not-inf_NP.nd2
- Split the image stack into images
- Select the DAPI channel showing the nuclei
- Use Process>Find maxima to identify the cells
 - Find the best noise tolerance
 - Select Segmented Particles
 - Exclude edge maxima
- Rename the result Mask1; Image>Rename

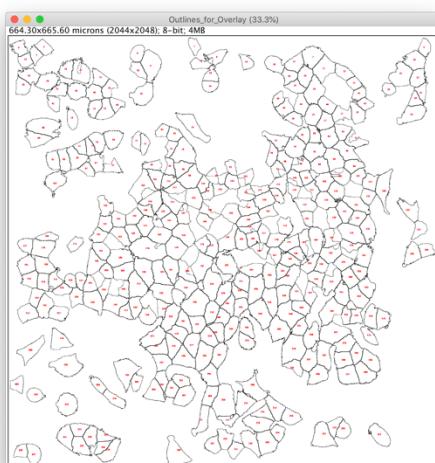


- Select the NP channel
- Define a threshold using Image>Adjust>Threshold
- Process>Smooth before you apply the threshold

- Rename the result Mask2; Image>Rename
- Combine the two masks using Process>Image Calculator
 - Mask1 > AND > Mask2
- Rename the result Mask3; Image>Rename



- Remove small fragments using Analyze > Analyze Particles
 - Exclude on edges
 - Show Mask
- Invert LUT (BG is black)
- Process > Binary > Fill Holes
- Analyze > Set Measurements
 - Redirect to original data
- Analyze > Analyze Particles
 - Display Outlines
 - Display Results
 - Summarize

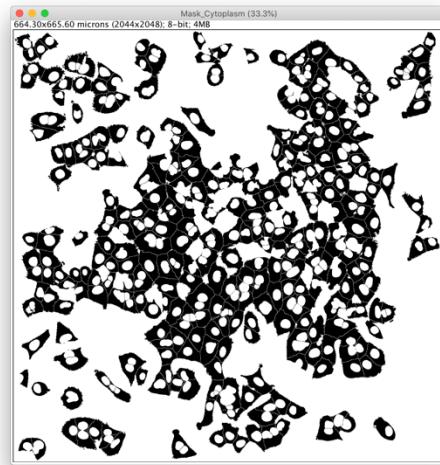
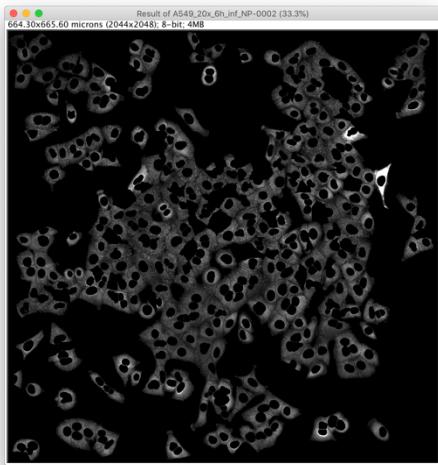


Resources:

<https://www.youtube.com/watch?v=agejuH8ebMc&t=1208s>

<https://www.youtube.com/watch?v=82N-eIPqnwM>

Add a nuclear mask:

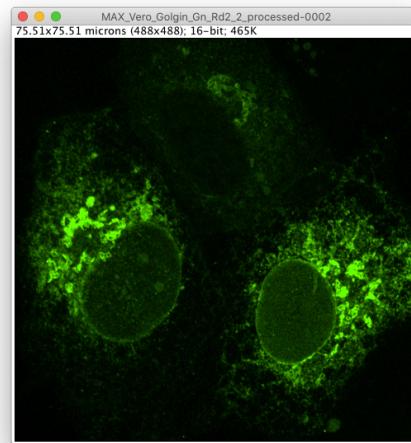
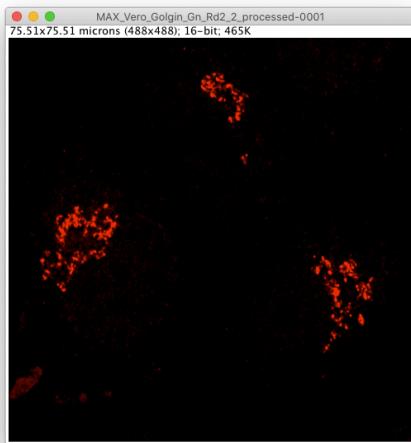


- Select the DAPI channel showing the nuclei
- Define a threshold using Image>Adjust>Threshold
- Process > Binary > Watershed to segment joined nuclei
- Rename the result Mask_Nuclei; Image>Rename
-

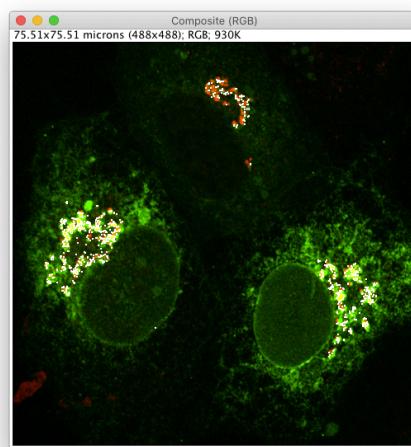
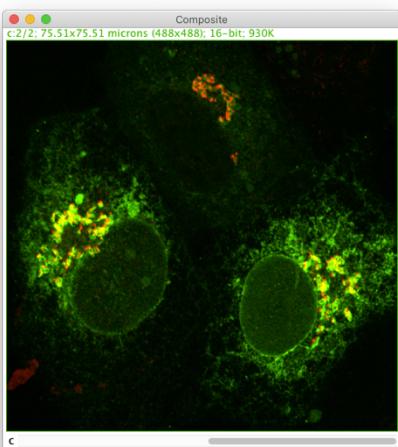
Resource: <https://www.youtube.com/watch?v=yEbFOGAjoXI>

Exercise 4:**Quantify colocalization (Pixel-based colocalization)**

- Open the image Vero_Golgin_Gn_Rd2_2_processed.czi
- Image > Stacks > Z Project > Max Intensity
- Image > Stacks > Stack to Images
- Tip: Auto adjust Brightness and Contrast



- Image > Color > Merge Channels

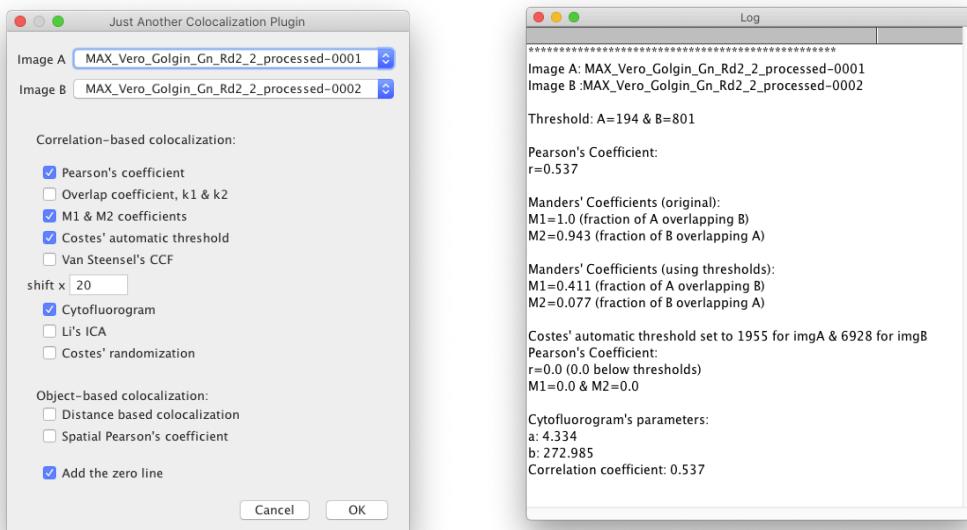


- Image > Type > RGB
- Color Threshold
- Select yellow region, Press Select
- Analyze > Measure
- Go back to Color Threshold and select the whole cell
- Measure again
- 4% colocalization

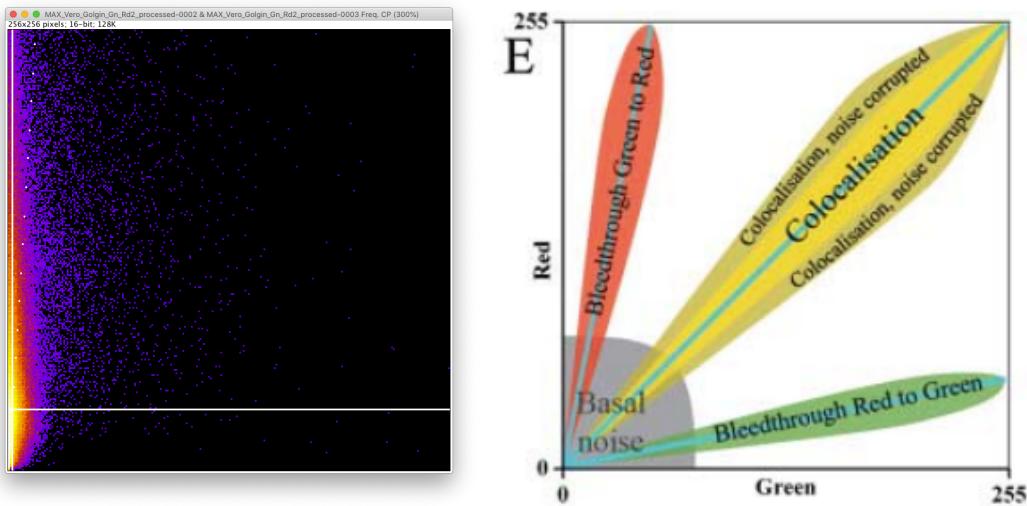
	Area	X	Y	IntDen	RawIntDen
1	27	33	38	3242	135411
2	685	40	42	32807	1370092

Exercise 5:**Quantify colocalization using the plugin JACoP (Pixel-based colocalization)**

- Go back to the red and the green image
- Download the JACoP plugin
 - https://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:jacop_2.0:just_another_colocalization_plugin:start
- Start the Plugin
- Set up the input parameters



- Adjust threshold for both images
- Ok
- Pearson's coefficient: -1(exclude) : 0 (random) : 1 (perfect colocalization)
- Manders coefficients range between 0 : 1
- There are many other plugins out there. Try out Analyze > Colocalization > Colocalization Threshold



- There are many other plugins out there. Try out Analyze > Colocalization Finder
- Play with the ScatterPlot (Cytofluorogram)

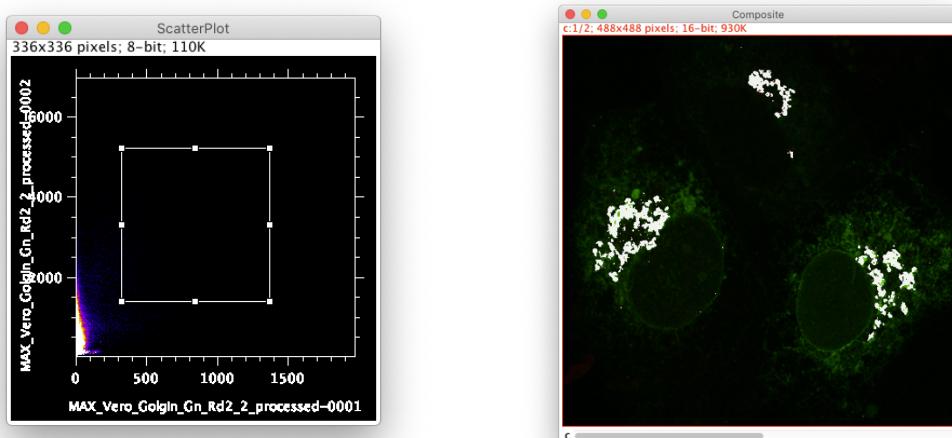


Figure E from:

Bolte, S. and Cordelières, F.P., 2006. A guided tour into subcellular colocalization analysis in light microscopy. *Journal of microscopy*, 224(3), pp.213-232.