

Introduction into image processing with Fiji

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

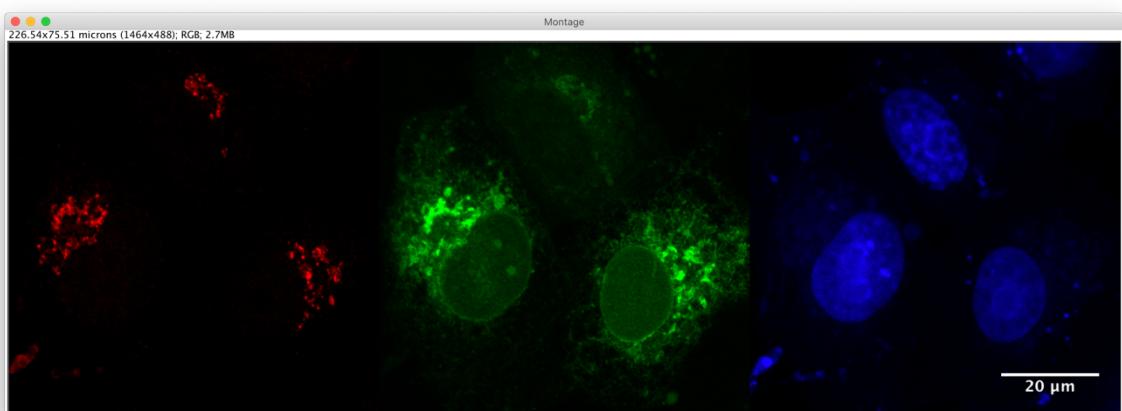
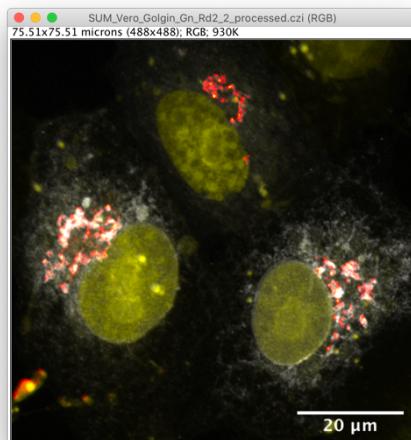


- Look at <https://imagej.net/learn/> to get a basic introduction
- Aspects that will be discussed during the course:
 - Main GUI/Interface
 - Open/Import images
 - General image manipulation
 - Stacks
 - LUTs
 - Simple measurements
 - Plugins and where to find them
 - Macros
- Other software that will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

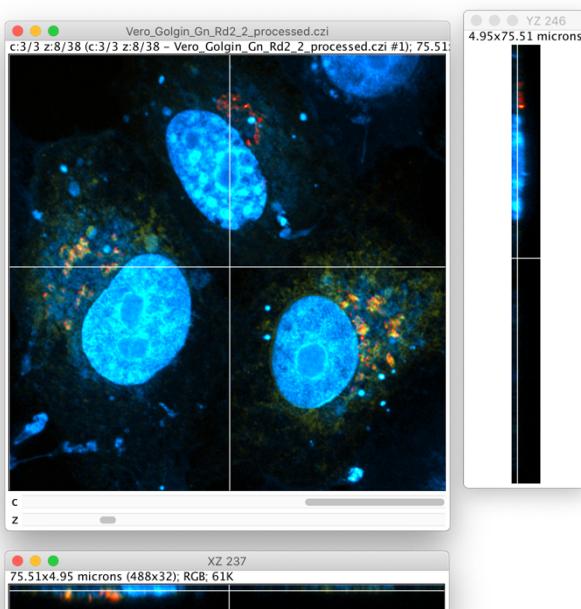
Exercise 1:

- 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>Sum slices
- Image>Stacks>Make Montage

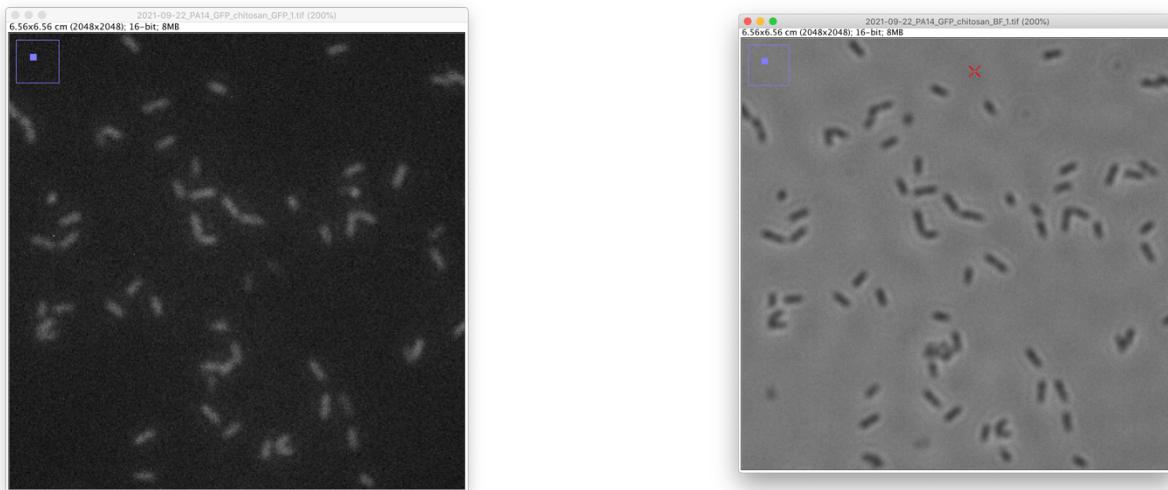


- Play around with LUTs Image>Lookup Tables
- Image>Stacks>Orthogonal Views
- *Put the first 5 commands into a macro.*
- Plugins>Macros>Record

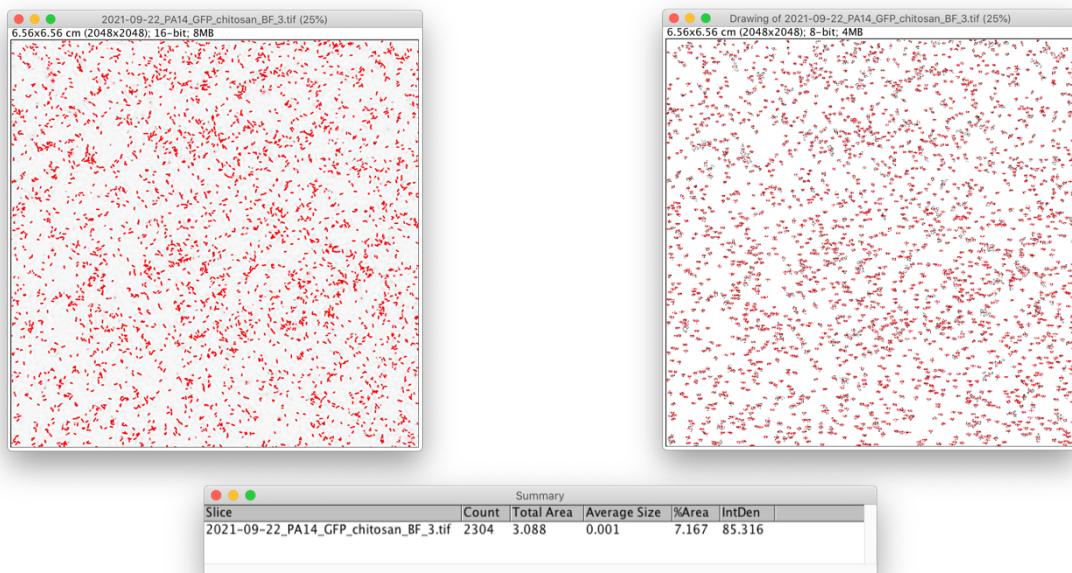


Exercise 2:

Counting bacterial cells

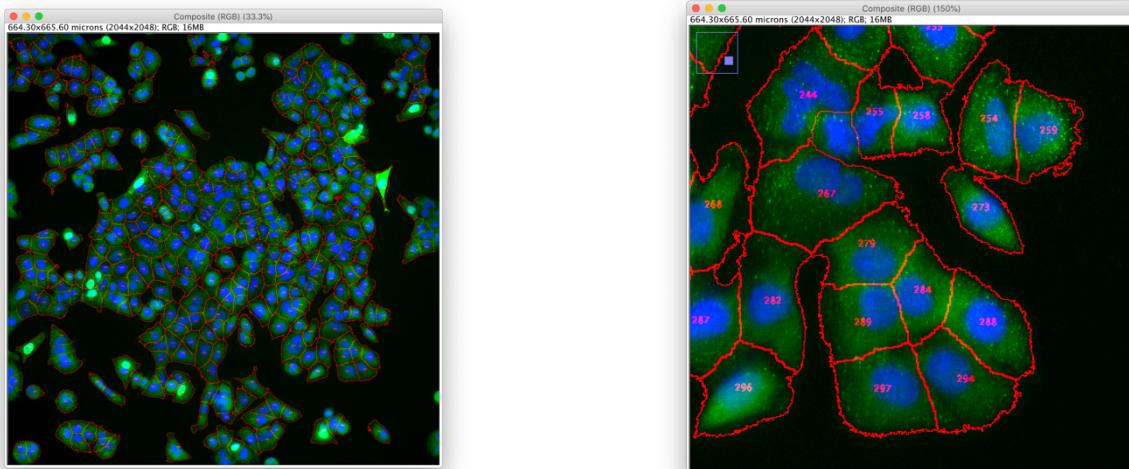


- Open the image 2021-09-22_PA14_GFP_chitosan_BF_1.nd2 and 2021-09-22_PA14_GFP_chitosan_GFP_1
- Tip: Analyze>Tools>Synchronize Windows
- Select the BF image
- Process>Subtract background
- Image>Adjust>Threshold (Select B&W, Light Background)
- Choose Threshold manually or use automatic threshold (i.e. Otsu), don't press Apply
- Analyze>Analyze Particles (Tip: show: Outlines, choose a minimum size)
 - Click Summarize
- *Optional: Press 'Apply' after thresholding, then Process>Binary>Watershed*
- *Try it out with the GFP channel.*

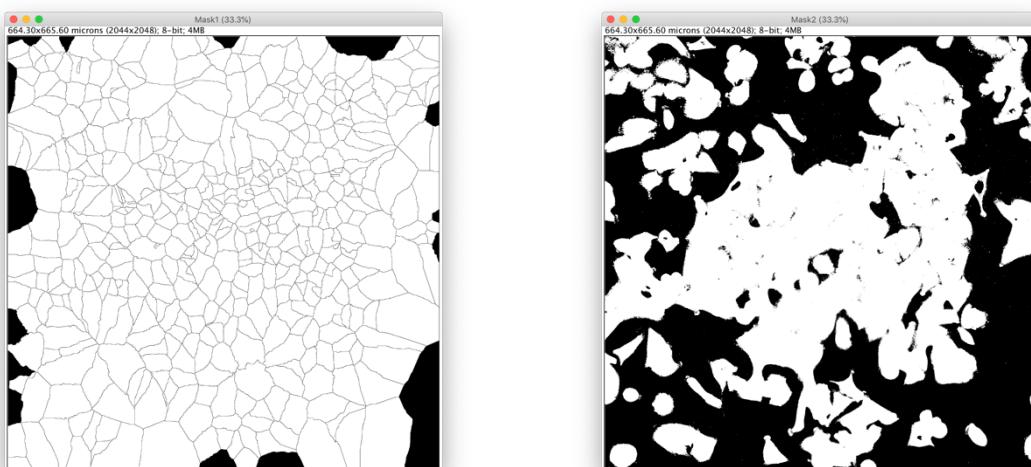


Exercise 3:

Cell segmentation

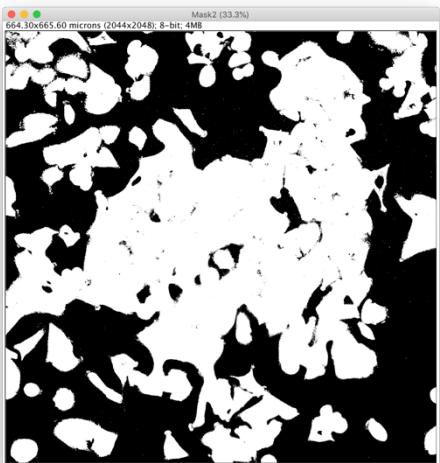


- Open the image A549_20x_6h_inf_NP.nd2
- Split the image stack into images
- Select the DAPI channel showing the nuclei
- Use Process>Find maxima to identify the cells
 - Find the best Prominence
 - Select Segmented Particles
 - Exclude edge maxima
- Rename the result Mask1; Image>Rename; Image>LUT>Invert LUT



- Select the NP channel (green channel)
- Define a threshold using Image>Adjust>Threshold (Dark background)
- Process>Smooth before you apply the threshold

- Image>LUT>Invert LUT (Mask2)



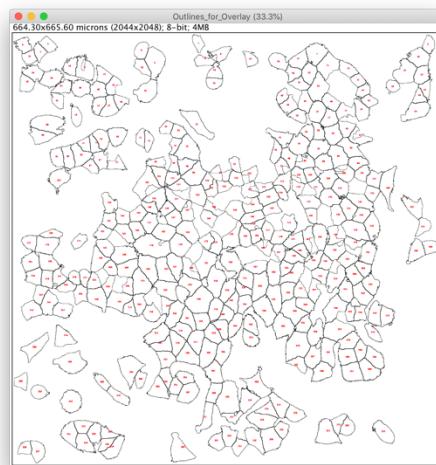
- Rename the result Mask2; Image>Rename
- Combine the two masks using Process>Image Calculator
 - Mask1 > AND > Mask2
- Rename the result Mask3; Image>Rename
- Edit>Invert (Mask3)



- Remove small fragments using Analyze > Analyze Particles
 - Exclude on edges
 - Show Mask
- Process > Binary > Fill Holes
- Analyze > Set Measurements
 - Redirect to original data
- Analyze > Analyze Particles
 - Display Outlines
 - Display Results

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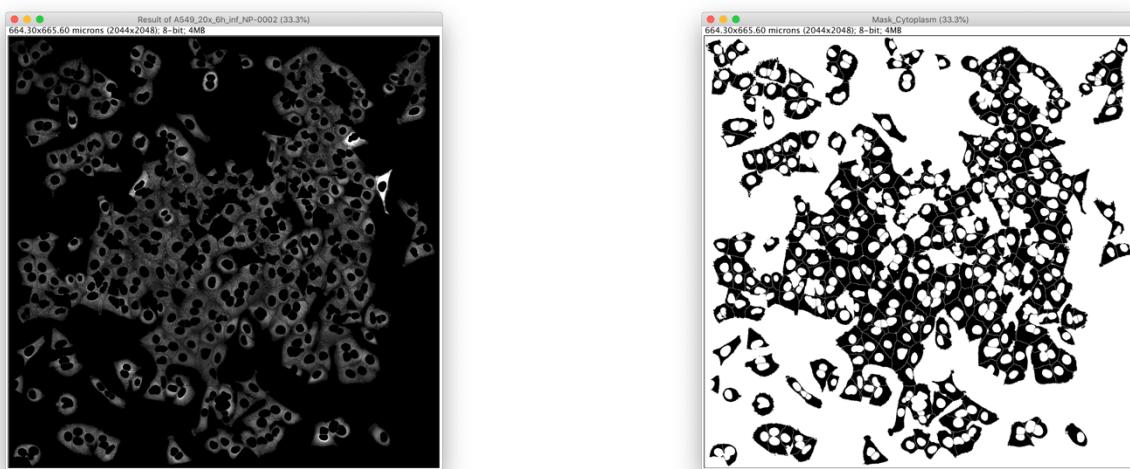
- Summarize



Resources:

<https://www.youtube.com/watch?v=agejuH8ebMc&t=1208s>
<https://www.youtube.com/watch?v=82N-eIPqnwM>

Add a nuclear mask:



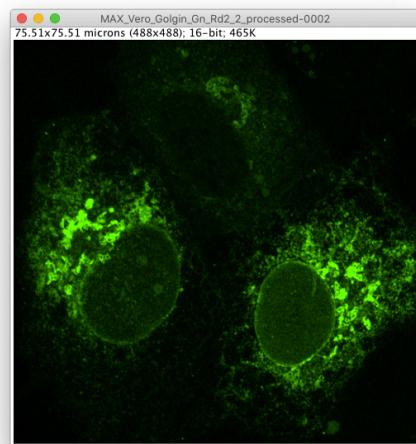
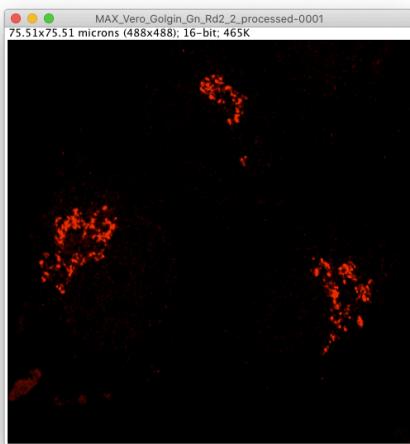
- Select the DAPI channel showing the nuclei
- Define a threshold using Image>Adjust>Threshold
- Process > Binary > Watershed to segment joined nuclei
- Rename the result Mask_Nuclei; Image>Rename

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

Exercise 4:

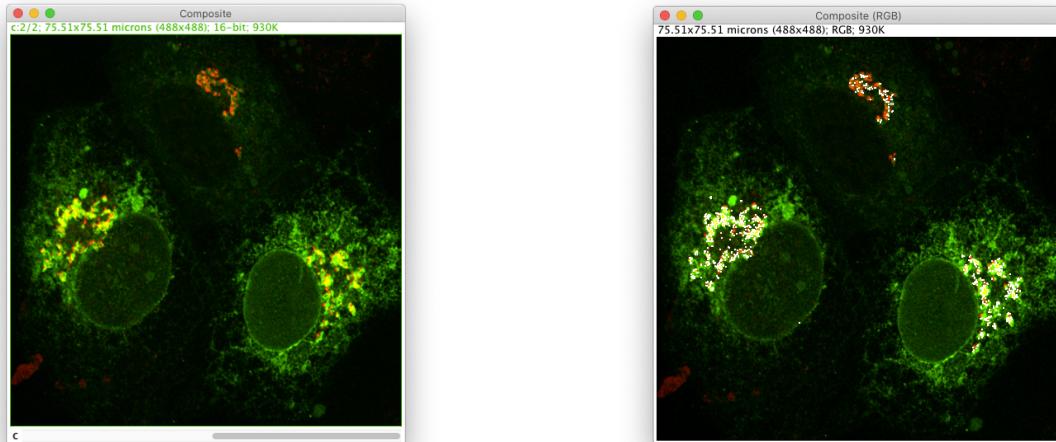
Quantify colocalization (Color-based colocalization)

- 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



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- Image > Color > Merge Channels



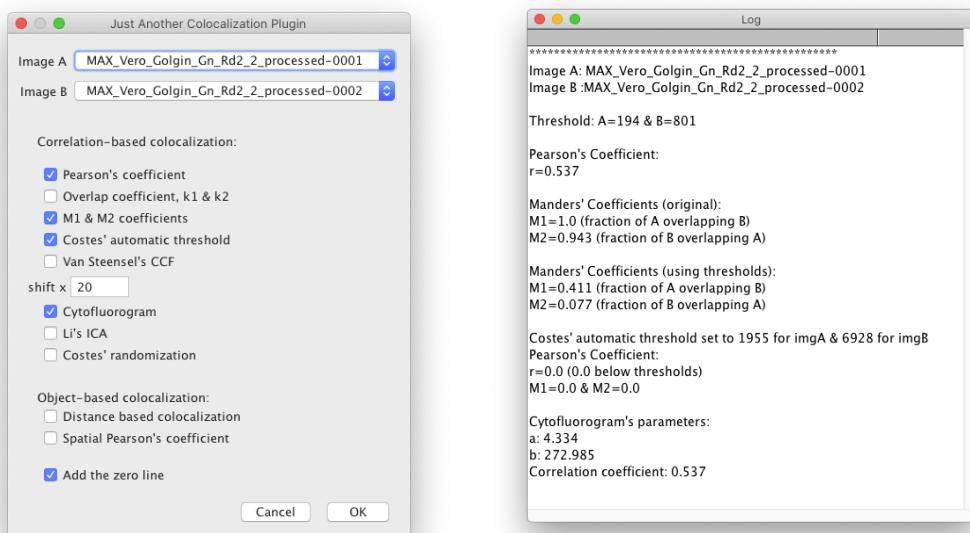
- Image > Type > RGB
- Color Threshold
- Select yellow region, Press Select
- Analyze > Measure
- Go back to Color Threshold and select the whole cell
- Measure again
- 4% colocalization

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

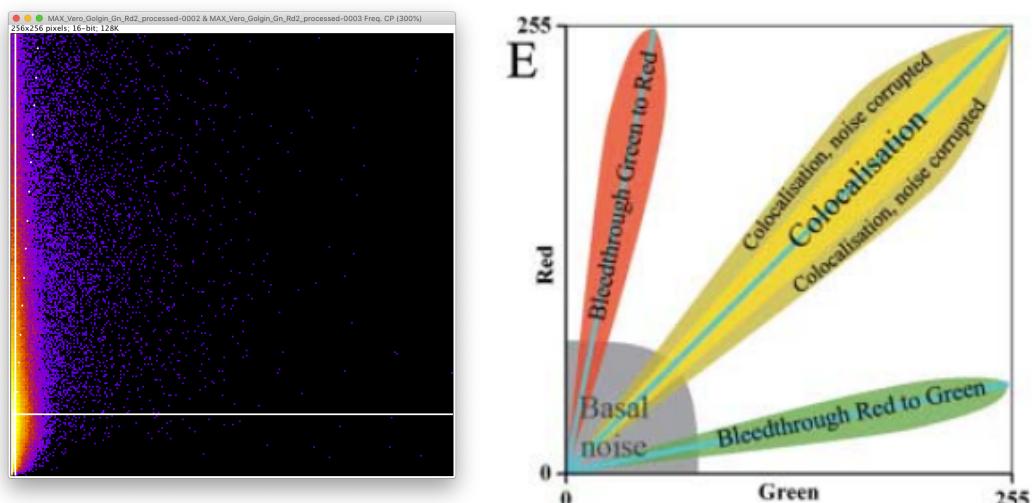
Exercise 5:

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)

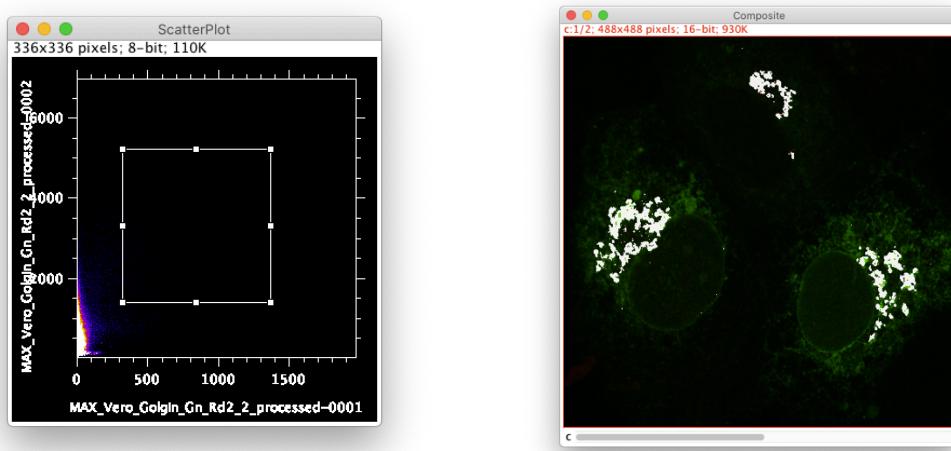


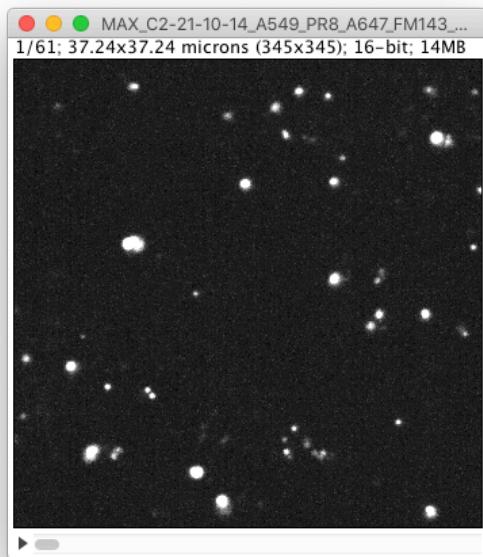
Figure E from:

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.

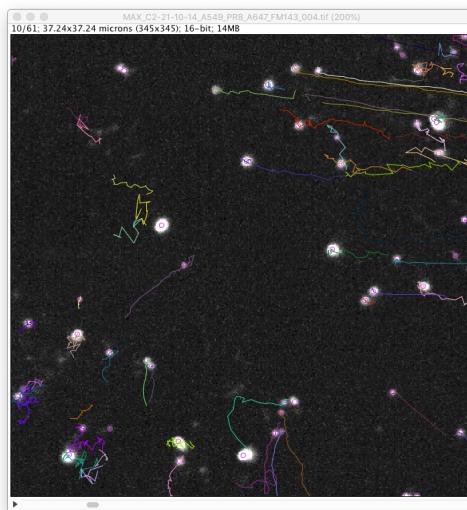
Exercise 6:

Single-particle tracking

- Open the image MAX_C2-21-10-14_A549_PR8_A647_FM143_004.tiff
- Image>Adjust>Brightness and Contrast
- Run the movie and follow the particles by eye
- Plugins>Tracking>TrackMate
- Follow the instructions of the GUI



- Generate particle trajectories using Trackmate
- Filter and proceed with tracks longer than 10 s
- Save tracks as xml file
- Save overlay as movie



Exercise 7:

Generate a STORM reconstruction

- Plugins>ThunderStorm>Import Cos7_MT_A647_2_1_locs_ROI_2_DC_forTS.csv

ThunderSTORM: results							
id	frame	x [nm]	y [nm]	z [nm]	intensity	loglikelihood ^a	
1	1001	35293	21680	-116.01	3772.4	-327.28	
2	1001	36776	19983	-142.16	3857.9	-351.95	
3	1001	37462	18167	-231.99	3269.9	-310.49	
4	1001	37559	11394	-249.28	3651.8	-282.97	
5	1001	40730	15502	-344.86	3796.3	-399.42	
6	1001	43127	20124	-223.25	4564.5	-278.8	
7	1001	50032	15307	-136.66	3902.6	-271.05	
8	1002	35279	21676	-126.66	3735	-285.61	
9	1002	37559	11388	-276.09	3848.6	-264.39	
10	1002	43113	20120	-185.28	2811.7	-539.92	
11	1002	50029	15301	-161.14	4164.8	-265.16	
12	1003	35276	21680	-223.98	3860.6	-281.79	
13	1003	37559	11410	-206.82	3529.9	-295.32	
14	1003	43898	23250	-56.915	2544.7	-420.57	
15	1003	50020	15304	-166.4	4208.2	-272.73	
16	1004	35287	21679	-145.33	2813.3	-312.19	
17	1004	37388	13946	-36.271	2998.3	-456.94	
18	1004	37554	11398	-165.95	3430.9	-277.12	
19	1004	42885	18252	168.23	4905.7	-292.15	
20	1004	50020	15299	-137.49	3537.3	-247.79	
21	1005	37394	13942	-54.619	2733.3	-496.98	
22	1005	37464	18166	-243.63	2882.2	-284.04	
23	1005	37550	11409	-353.33	4319.8	-340.86	
24	1005	37842	12724	-256.53	2383.5	-508.2	
25	1005	40807	21768	-121.67	3519.4	-315.67	
26	1005	50044	15304	-183.12	3869.5	-279.61	
27	1006	37468	18163	-201.22	2700	-288.92	
28	1006	37559	11299	-410.49	6150.9	-362.76	
29	1006	37838	12711	-245.97	3415.9	-522.01	
30	1006	40808	21782	-156.4	4322.7	-324.56	
31	1006	43513	22801	-278.96	3848.6	-350.55	
32	1006	50040	15280	-722.85	4540.6	-202.62	

Filter Density filter Remove duplicates Merging Drift correction Z-stage offset

Filter:

Post-processing history: -

Preview

- Plot histogram of intensity
- Filter out localization with < 3000 photons
- Render a super-resolved image using “Visualization”
 - Try 2D with different pixel sizes, e.g. 5 nm vs 50 nm
 - Try 3D rendering