

Introduction into image processing with Fiji

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

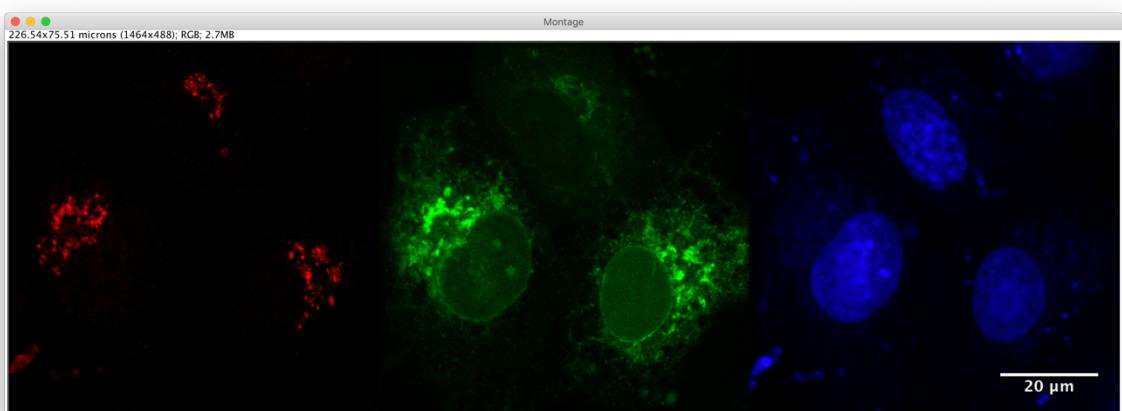
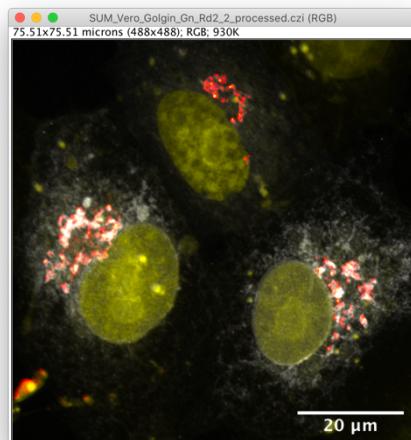


- Look at <https://imagej.net/learn/> to get a basic introduction
- Aspects that will be discussed during the course:
 - Main GUI/Interface
 - Open/Import images
 - General image manipulation
 - Stacks
 - LUTs
 - Simple measurements
 - Plugins and where to find them
 - Macros
- Other software that will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

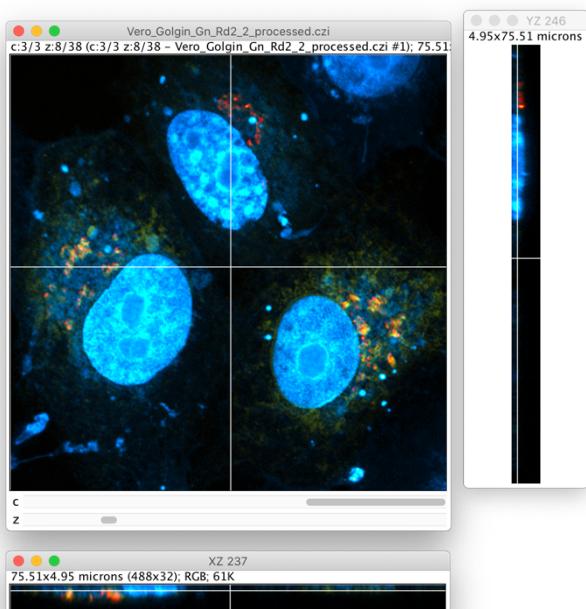
Exercise 1:

- 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

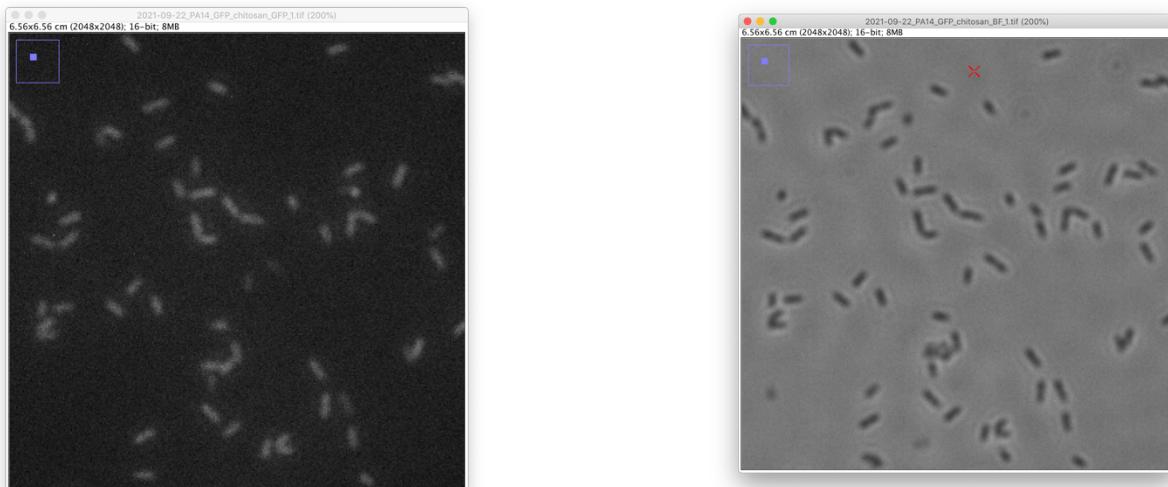


- Play around with LUTs Image>Lookup Tables
- Image>Stacks>Orthogonal Views
- *Put the first 5 commands into a macro.*

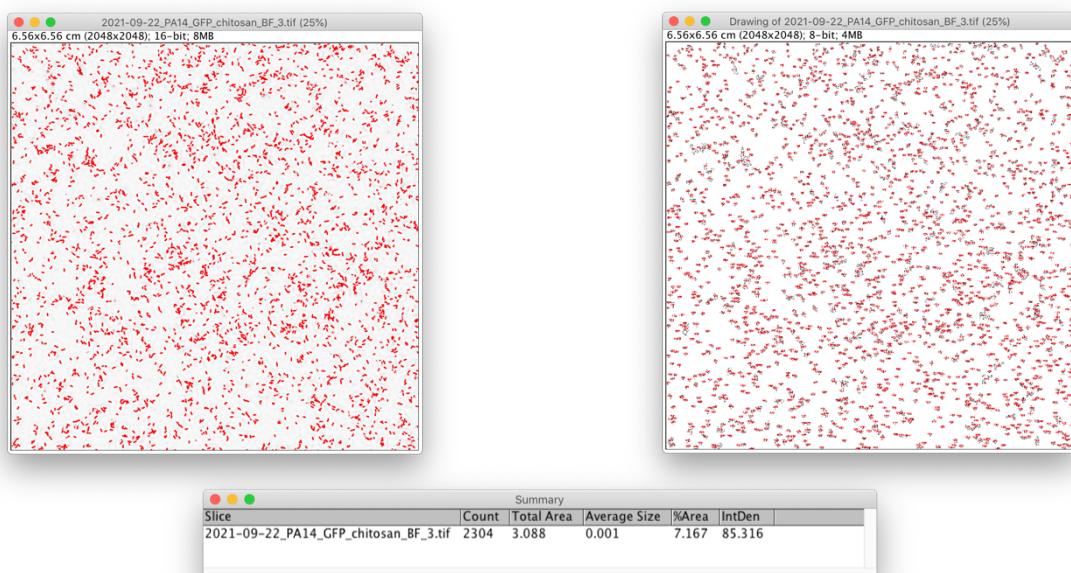


Exercise 2:

Counting bacterial cells

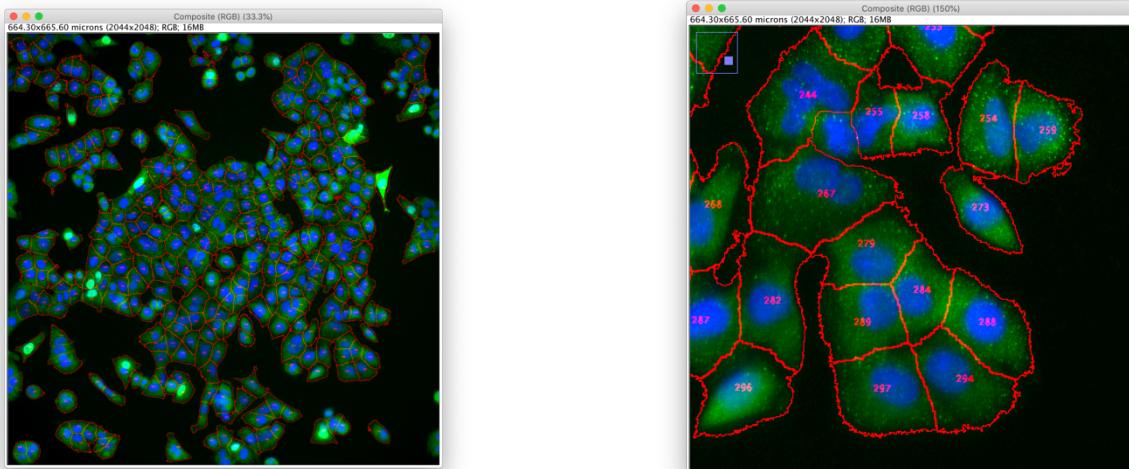


- Open the image 2021-09-22_PA14_GFP_chitosan_BF_1.nd2 and 2021-09-22_PA14_GFP_chitosan_GFP_1
- Tip: Analyze>Syncronize Windows
- Select the BF image
- Process>Subtract background
- Image>Adjust>Threshold
- Choose Threshold manually or use automatic threshold (i.e. Otsu), don't press Apply
- Analyze>Analyze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

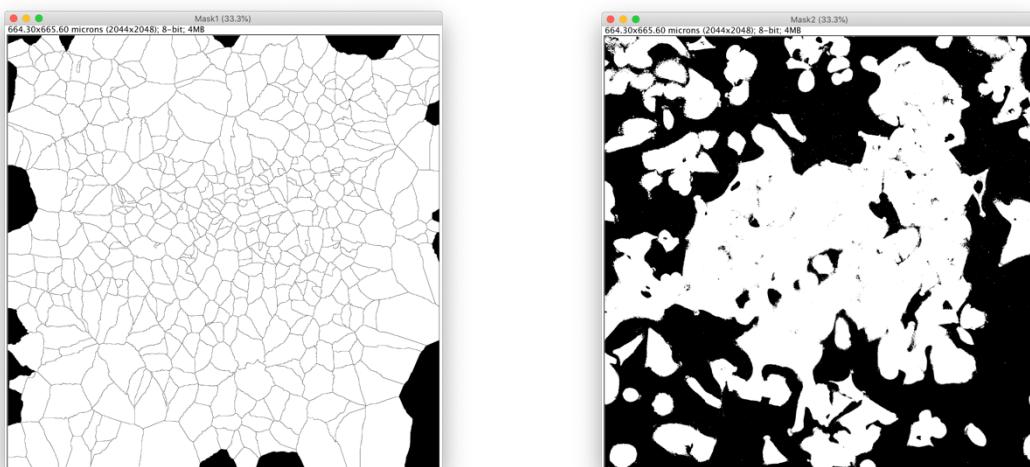


Exercise 3:

Cell segmentation



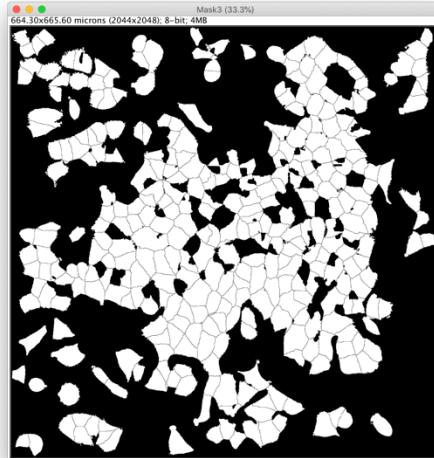
- Open the image A549_20x_6h_not-inf_NP.nd2
- Split the image stack into images
- Select the DAPI channel showing the nuclei
- Use Process>Find maxima to identify the cells
 - Find the best noise tolerance
 - Select Segmented Particles
 - Exclude edge maxima
- Rename the result Mask1; Image>Rename



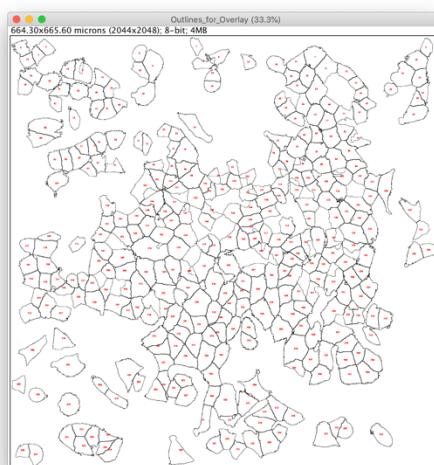
- Select the NP channel
- Define a threshold using Image>Adjust>Threshold
- Process>Smooth before you apply the threshold

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- 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



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Resources:

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

Add a nuclear mask:



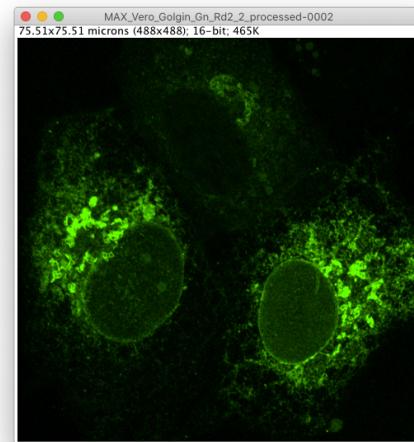
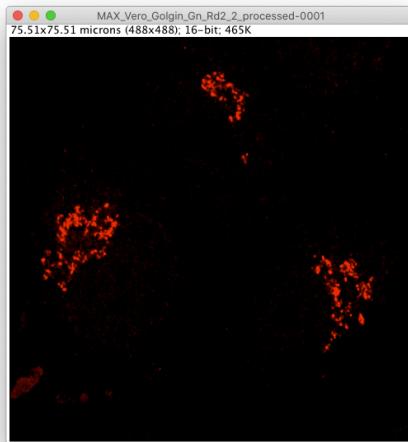
- Select the DAPI channel showing the nuclei
- Define a threshold using Image>Adjust>Threshold
- Process > Binary > Watershed to segment joined nuclei
- Rename the result Mask_Nuclei; Image>Rename

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

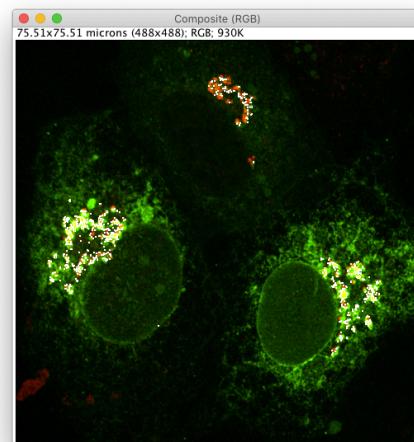
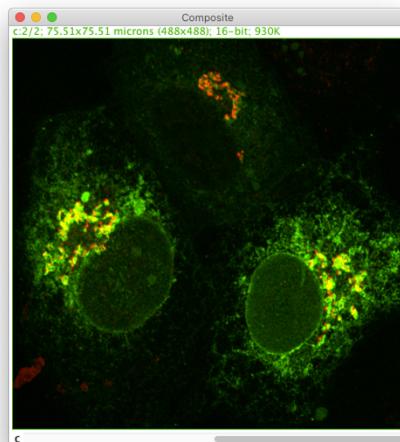
Exercise 4:

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



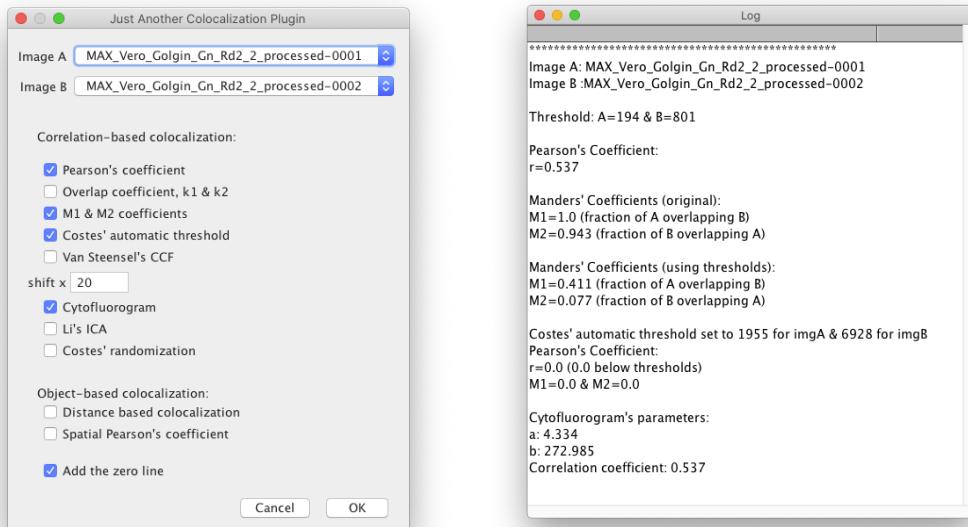
- Image > Type > RGB
- Color Threshold
- Select yellow region, Press Select
- Analyze > Measure
- Go back to Color Threshold and select the whole cell
- Measure again
- 4% colocalization

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

Exercise 5:

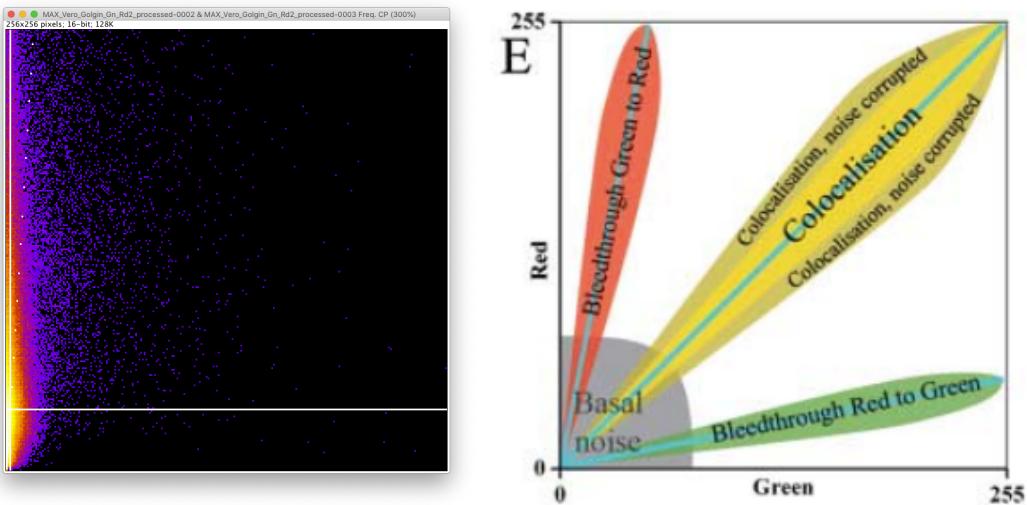
Quantify colocalization using the plugin JACoP (Pixel-based colocalization)

- 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
 - Pearsons 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

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

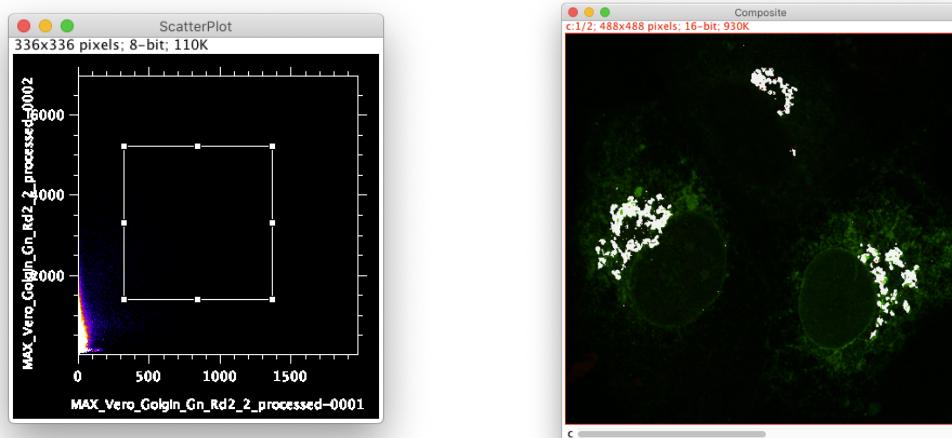


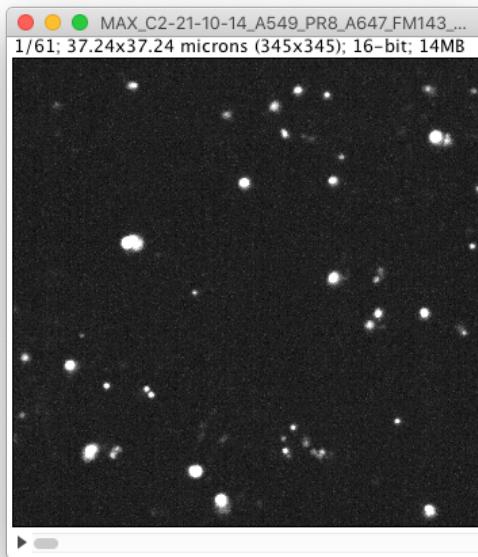
Figure E from:

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.

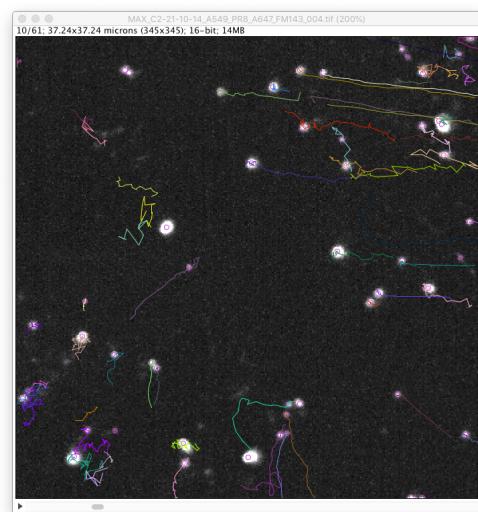
Exercise 6:

Single-particle tracking

- Open the image MAX_C2-21-10-14_A549_PR8_A647_FM143_004.tif
- 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" |
|----|-------|--------|--------|---------|-----------|----------------|
| 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 | 15289 | -222.85 | 4540.6 | -202.52 |

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

Filter: Apply Restrict to ROI

Post-processing history: - Reset

Preview Defaults Plot histogram Visualization Import Export

- 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