

## Guide for colocalization\_calculator.py algorithm

Author: Austin Chiappetta, achiapp2@jh.edu

Description: this algorithm determines which spots from two separate channels are colocalized based on the maximum colocalization distance given. It requires the x and y pixel to nm conversion factors (e.g., images taken with the 63x objective lens have x and y pixel to nm conversion factors of 102.4 nm/pixel in both directions) and the zstep in nanometers, as well as the processed\_data.txt files generated by using spot\_calling\_batch\_processing.py. The algorithm generates a tab-separated values file with the data describing each pair of colocalized spots (x coordinates, y coordinates, z coordinates, intensities, number of molecules quantified in each spot, and the distances between the pairs of spots) which can be used for further downstream analysis. The algorithm also generates pie charts and histograms comparing colocalized and non-colocalized spots in each channel.

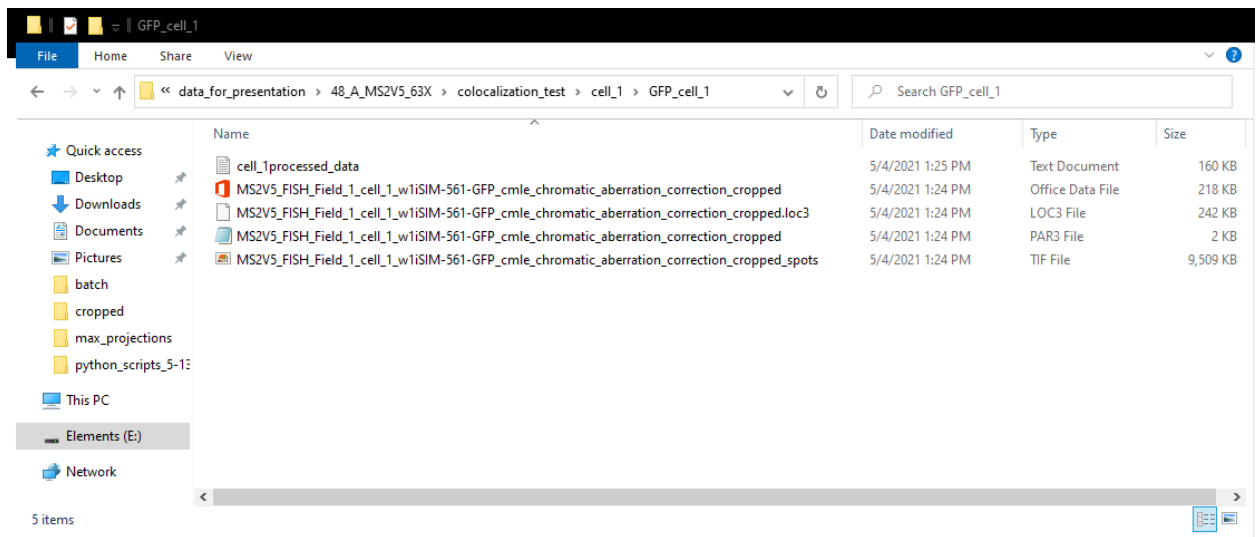
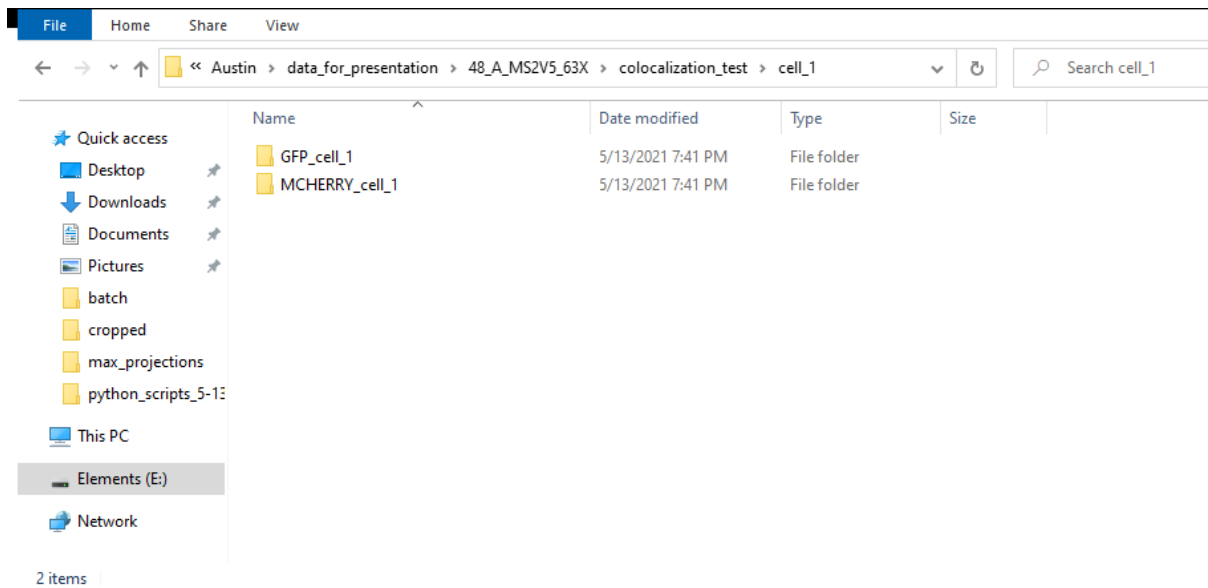
Function call usage: in the command line, type: python colocalization\_calculator.py /PATH/TO/FOLDER/CONTAINING/DATA/SUBFOLDERS/ xpixel\_to\_nm\_conversion ypixel\_to\_nm\_conversion zstep max\_distance\_threshold

Note: the folder path can be either the absolute path or the relative path, and the path must contain forward slashes ("/") instead of back slashes.

**VERY important!** Note that none of the folders in the path can contain spaces! Please use underscore (" \_ ") instead of spaces.

### Step-by-step tutorial:

1. Organize the processed\_data.txt files generated by the spot\_calling\_batch\_processing.py algorithm into **two** separate subfolders with descriptive titles as shown below. The subfolder names are used in generating the output figures so make sure it is descriptive.



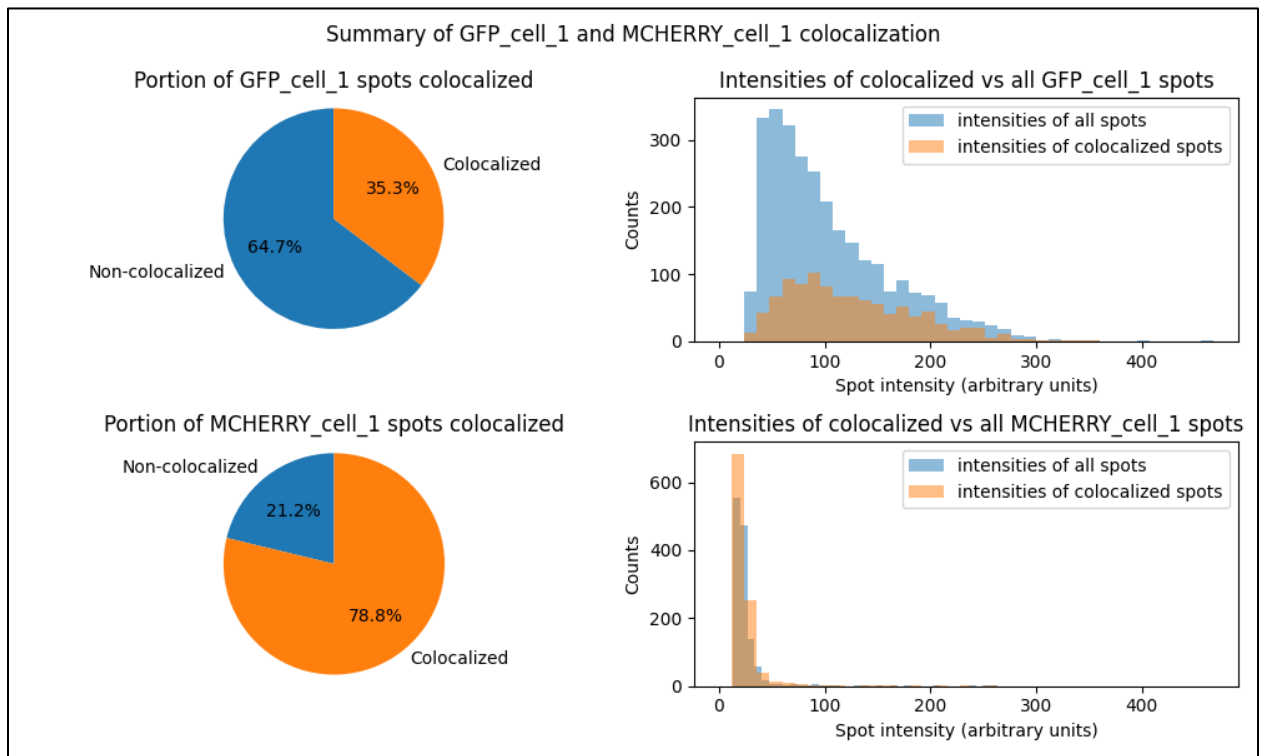
2. In the command line, navigate to the directory which contains the python script (If using Windows Git Bash, you can right click in the folder and select "Git Bash Here").
3. Type "python spot\_calling\_threshold\_analysis.py  
PATH/TO/FOLDER/CONTAINING/THRESHOLD/SUBFOLDERS/ xpixel\_to\_nm\_conversion,  
ypixel\_to\_nm\_conversion, zstep, max\_distance\_threshold" (all one line, without the quotation marks and commas) into the terminal.

```

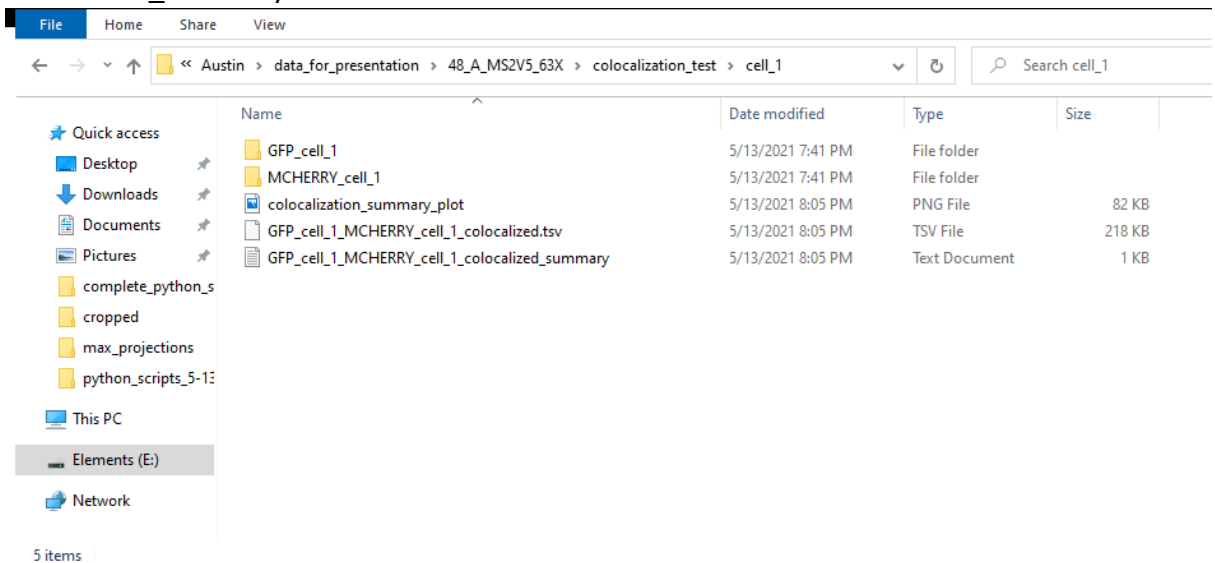
MINGW64:/e/Austin/complete_python_scripts
achiapp2@BIOL-TT-7070-M MINGW64 /e/Austin/complete_python_scripts
$ python colocalization_calculator.py /e/Austin/data_for_presentation/48_A_MS2V5_63X/colocalization_test/cell_1 102.4 102.4 250 500

```

- Press Enter.
- After about 30 seconds, an image of the colocalization plot (Summary of <channel\_1> and <channel\_2> colocalization) will be generated and pop-up automatically.



- Upon refreshing the folder after closing the plot window, you will find that the plot has been saved to that subfolder, as well as a colocalized.tsv file and a colocalized\_summary.txt file.



GFP\_cell\_1\_MCHERRY\_cell\_1\_colocalized\_summary - Notepad

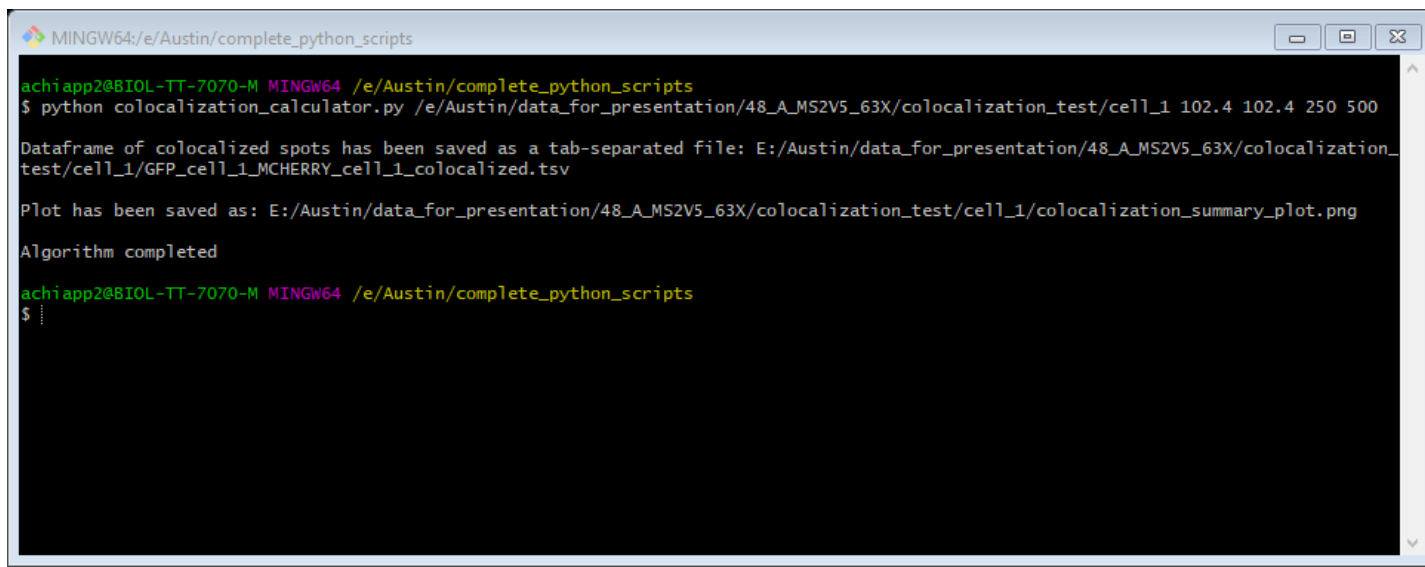
File Edit Format View Help

Total number of GFP\_cell\_1 spots: 2888

Total number of MCHERRY\_cell\_1 spots: 1295

Number of colocalized spots: 1020

GFP_cell_1_MCHERRY_cell_1_colocalized - Excel														Austin Chiappetta			
File Home Insert Page Layout Formulas Data Review View Help Tell me what you want to do																Share	
Clipboard Font Alignment Number Styles Cells Editing																	
	A	B	C	D	E	F	G	H	I	J	K	L	M				
1	GFP_cell_1_x_pixel	GFP_cell_1_y_pixel	GFP_cell_1_z_pixel	GFP_cell_1_x	GFP_cell_1_y	GFP_cell_1_z	GFP_cell_1_intensity	GFP_cell_1_scaled intensity	GFP_cell_1_number of molecules quantified	MCHERRY_cell_1_x_pixel	MCHERRY_cell_1_y_pixel	MCHERRY_cell_1_z_pixel	MCHERRY_cell_1_intensity				
2	329.49676	286.78906	17.280386	33740.46822	29367.19974	4320.0965	132149.25	132.14925	2	329.26871	286.80245	17.245806	3371.14925				
3	348.25633	161.33444	14.532987	35661.44819	16520.64666	3633.24675	181419.37	181.41937	3	348.61051	161.4576	14.580274	35697.14937				
4	319.06605	169.40731	12.77245	32672.36352	17347.30854	3193.1125	94788.191	94.788191	2	319.3114	169.3439	12.94631	32697.1125				
5	275.99534	300.00965	18.771747	28261.92282	30720.98816	4692.93675	54842.299	54.842299	1	276.30057	299.9612	18.960621	28293.93675				
6	421.77421	220.51914	14.090683	43189.6791	22581.15994	3522.67075	139286.43	139.28643	2	421.646	219.94663	14.116368	4317.67075				
7	373.04331	206.85624	13.391914	38199.63494	21182.07898	3347.9785	84475.203	84.475203	1	372.66796	206.98728	13.184718	3816.07898				
8	339.06925	241.68818	14.139227	34720.6912	24748.86963	3534.80675	169131.03	169.13103	3	339.45646	241.6547	13.907331	3476.86963				
9	247.18648	317.19442	19.025925	25311.89555	32480.70861	4756.48125	41125.729	41.125729	1	247.5219	316.71026	19.189678	25346.729				
10	377.27311	174.29438	12.792449	38632.76646	17847.74451	3198.11225	66865.091	66.865091	1	376.58215	174.04474	12.828311	38562.11225				
11	353.58044	192.97776	17.754607	36206.63706	19760.92262	4438.65175	137835.16	137.83516	2	354.11555	192.78808	17.989731	36261.92262				
12	297.71859	188.70955	14.743417	30486.38362	19323.85792	3685.85425	215633.26	215.63326	4	297.17345	189.19864	14.906756	30430.85425				
13	343.30058	189.84374	12.551364	35153.97939	19439.99898	3137.841	230262.7	230.2627	4	343.15764	189.62965	12.226296	35139.99898				
14	289.66426	160.88103	15.016144	29661.62022	16474.21747	3754.036	244227.7	244.2277	4	289.52209	160.14247	14.862921	29647.62022				
15	387.16772	209.33543	14.050782	39645.97453	21435.94803	3512.6955	221386.35	221.38635	4	387.37086	208.65366	13.855829	39666.97453				
16	294.43613	192.96454	13.21724	30150.25971	19759.5689	3304.31	104058.27	104.05827	2	294.43341	193.31603	12.889457	30149.5689				
17	364.42225	284.41701	15.534186	37316.8384	29124.30182	3883.5465	136121.91	136.12191	2	363.68874	284.45874	15.254029	37260.30182				
18	387.47159	247.24204	14.156383	39677.09082	25317.5849	3539.09575	181722.02	181.72202	3	387.65194	247.00254	13.801254	39695.09575				
19	393.91141	205.32774	15.261556	40336.52838	21025.56058	3815.389	228836.09	228.83609	4	393.10633	205.69502	15.147645	40254.56058				
20	265.96524	214.89323	17.826575	27234.84058	22005.06675	4456.64375	67193.253	67.193253	1	266.47561	215.56729	17.995095	27287.06675				
21	342.77519	185.38474	13.587956	35100.17946	18983.39738	3396.989	212149.1	212.1491	4	343.05504	185.29969	13.973552	3512.39738				
22	355.32575	186.48796	13.504109	36385.3568	19096.3671	3376.02725	263254.67	263.25467	4	355.79599	186.72594	13.855746	36433.3671				
23	309.27458	212.5887	17.980051	31669.71699	21769.08288	4495.01275	112074.56	112.07456	2	309.15008	213.58708	18.017642	31656.08288				
24	379.42249	270.02845	14.082565	38852.86298	27650.91328	3520.64125	245249.3	245.2493	4	378.45713	270.32693	14.017666	38754.91328				
25	291.81642	193.30651	14.908204	29882.00141	19794.58662	3727.051	230901.95	230.90195	4	292.36012	194.07876	15.075437	29937.58662				
26	397.52639	230.35809	14.251924	40706.70234	23588.66842	3562.981	206819.76	206.81981	3	398.44592	230.5024	14.065403	40800.66842				
27	372.00277	278.37332	16.861094	38093.08365	28505.42797	4215.2735	131174.76	131.17476	2	371.20512	277.72845	16.711763	38011.42797				
28	378.04607	199.43545	14.803833	38711.91757	20422.19008	3700.95825	149369.24	149.36924	2	378.23869	198.70452	15.153346	38731.95825				
29	285.85455	153.5786	16.680325	29271.50592	15726.44864	4170.08125	173654.39	173.65439	3	285.83504	152.44255	16.7166	2926.50592				
30	323.60177	273.6755	13.79328	33136.82125	28024.3712	3448.32	114396.63	114.39663	2	323.51373	274.8121	13.781625	33127.82125				
31	335.57219	282.47995	17.915048	34362.59226	28925.94688	4478.762	110425.41	110.42541	2	334.79786	281.68881	18.042982	34283.94688				



```
MINGW64:/e/Austin/complete_python_scripts
achiapp2@BIOL-TT-7070-M MINGW64 /e/Austin/complete_python_scripts
$ python colocalization_calculator.py /e/Austin/data_for_presentation/48_A_MS2V5_63X/colocalization_test/cell_1 102.4 102.4 250 500

Dataframe of colocalized spots has been saved as a tab-separated file: E:/Austin/data_for_presentation/48_A_MS2V5_63X/colocalization_test/cell_1/GFP_cell_1_MCHERRY_cell_1_colocalized.tsv

Plot has been saved as: E:/Austin/data_for_presentation/48_A_MS2V5_63X/colocalization_test/cell_1/colocalization_summary_plot.png

Algorithm completed

achiapp2@BIOL-TT-7070-M MINGW64 /e/Austin/complete_python_scripts
$ .....
```

7. The end.