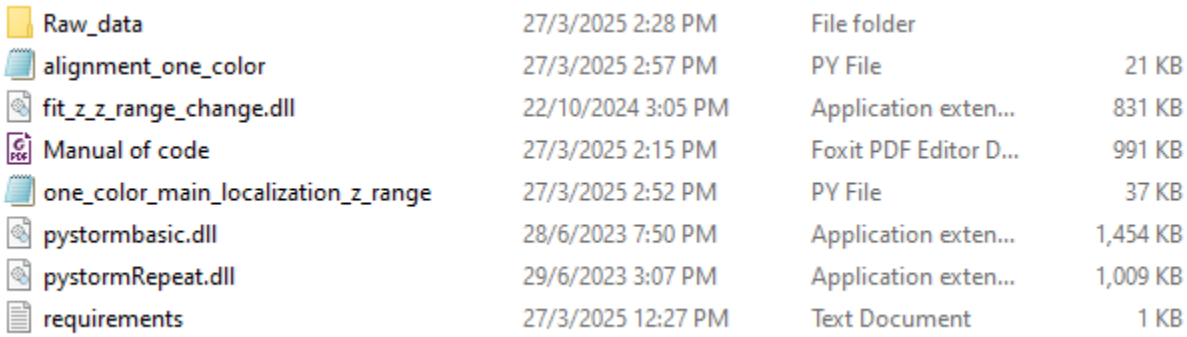
SiLM Code User Manual

1. File introduction



Raw_data	27/3/2025 2:28 PM	File folder	
alignment_one_color	27/3/2025 2:57 PM	PY File	21 KB
fit_z_z_range_change.dll	22/10/2024 3:05 PM	Application exten...	831 KB
Manual of code	27/3/2025 2:15 PM	Foxit PDF Editor D...	991 KB
one_color_main_localization_z_range	27/3/2025 2:52 PM	PY File	37 KB
pystormbasic.dll	28/6/2023 7:50 PM	Application exten...	1,454 KB
pystormRepeat.dll	29/6/2023 3:07 PM	Application exten...	1,009 KB
requirements	27/3/2025 12:27 PM	Text Document	1 KB

Raw_data: code test data (Calibration_200frames.dat: three-channel calibration raw data; Acquisition_1000frames.dat: 1000frames test raw data; Processed result: the result of processed result) can be found from https://zenodo.org/records/15487082

alignment_one_color.py: three-channel alignment program (using Calibration_200frames.dat); one_color_main_localization_z_range.py: 3D localization main program (using Acquisition_1000frames.dat).

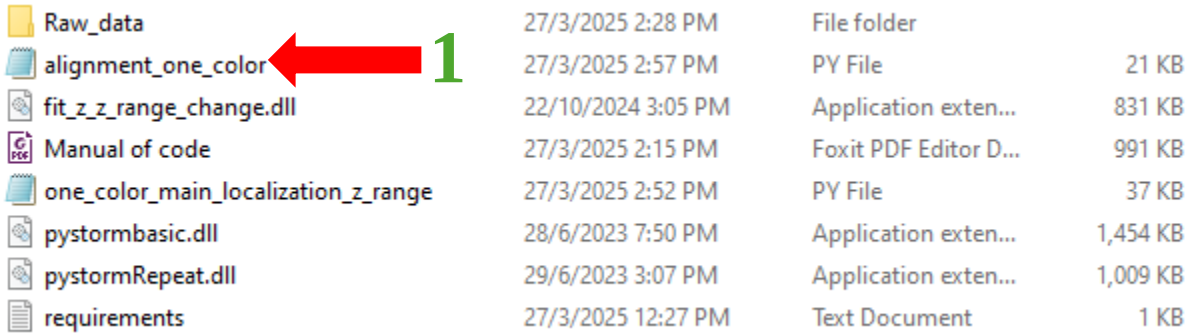
requirements.txt: The version of the library file, python version, CUDA version, and Windows SDK version.

dll file: The CUDA file that is called in the program.

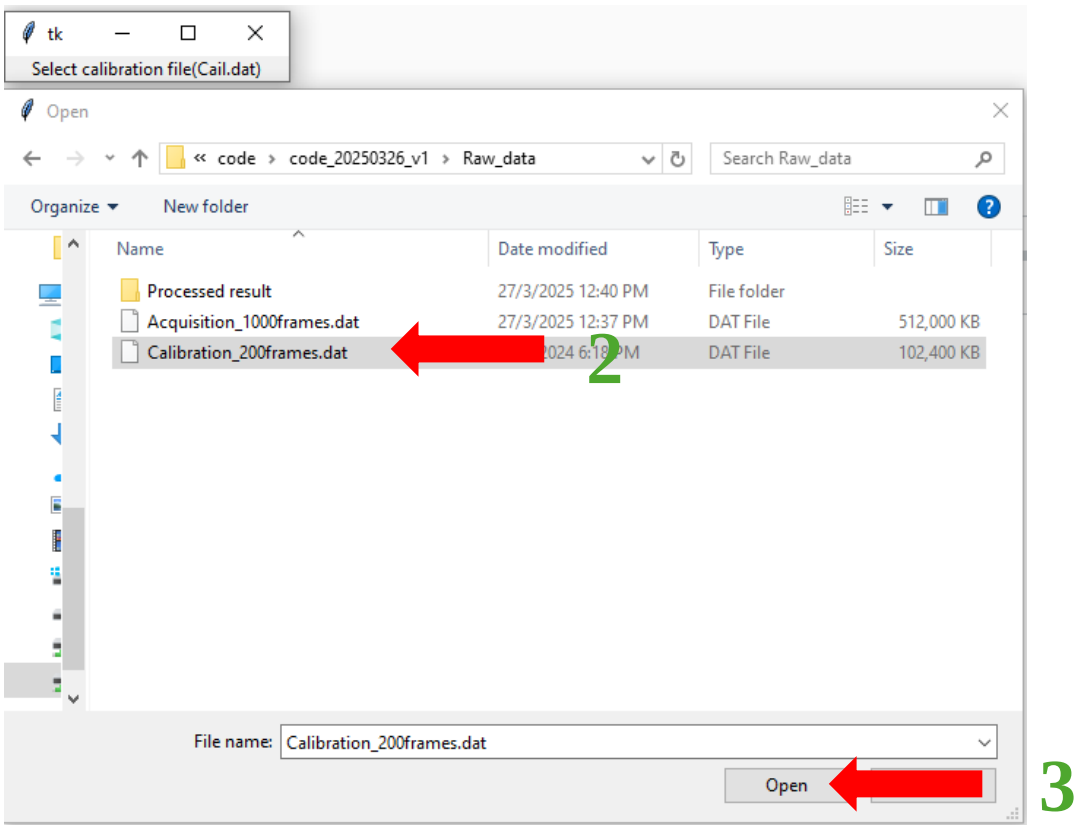
We split the code into modules with comments so that users can read and modify it.

The crop size of each channel is 180x180 pixels, and the peak detection window size is 7x7, which can be modified by users according to their own experimental needs.

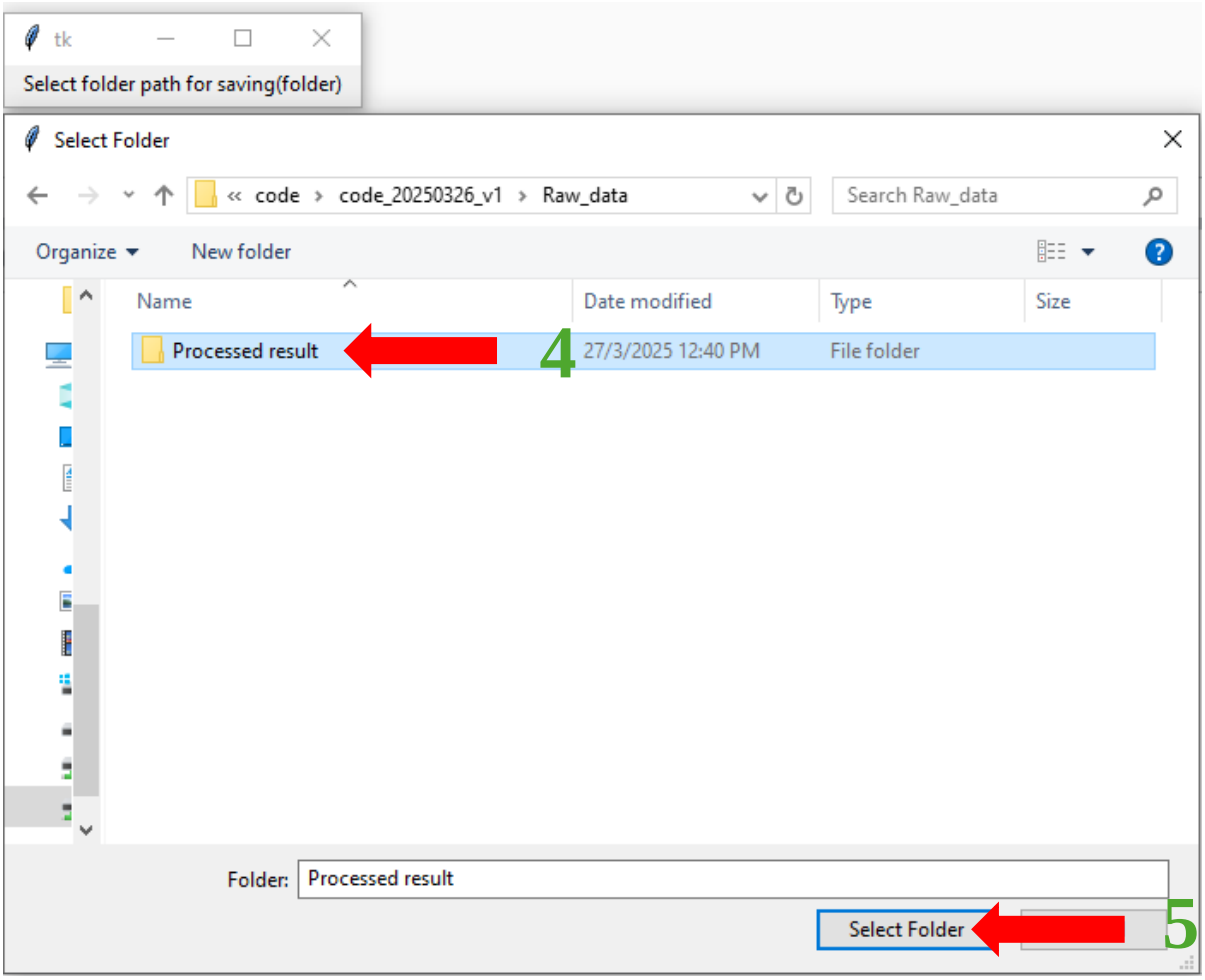
1. Alignment program runs



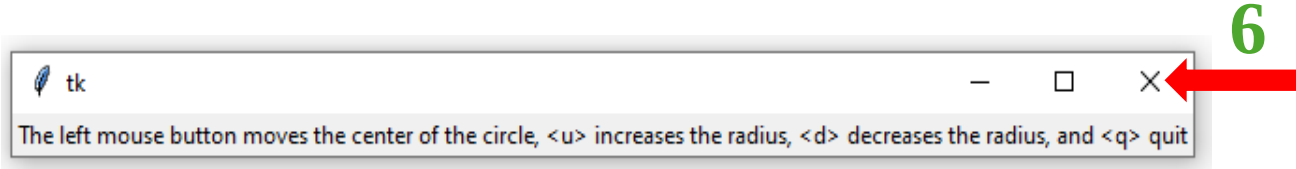
1.1 Select Calibration_200frames.dat for three-channel alignment.



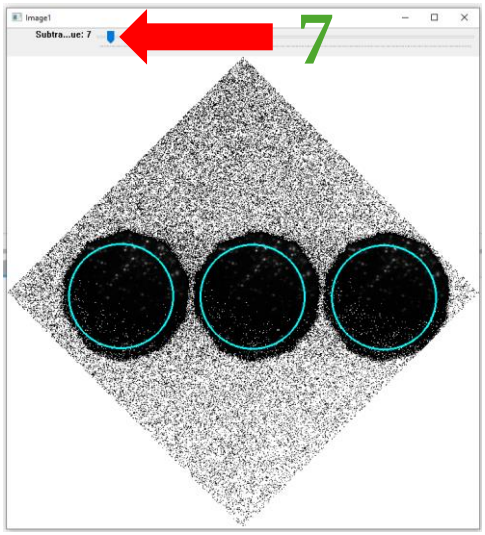
1.2 Select a folder to save the alignment parameters calculated later.

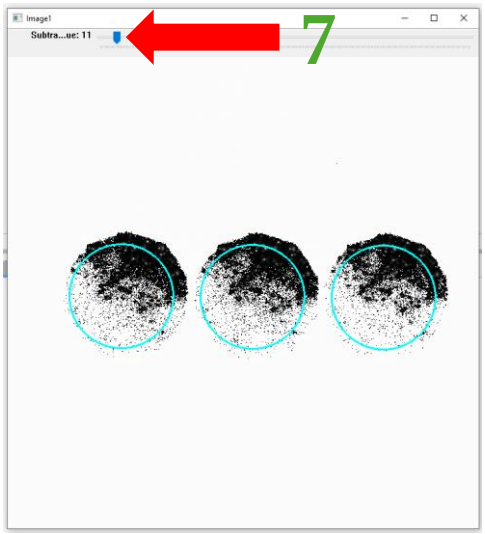


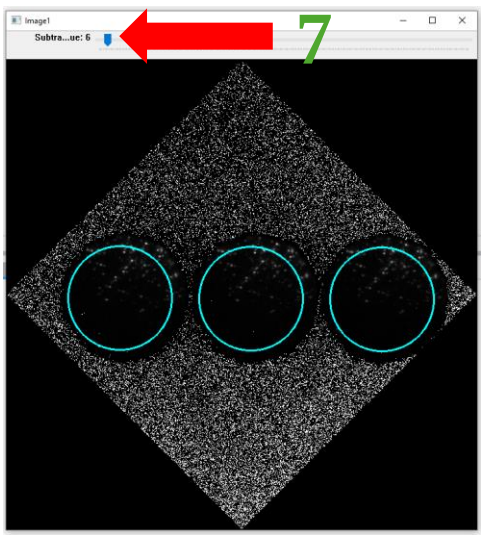
1.3 Rough alignment operation instructions: “left mouse clicking" changes the position of the circle, the "u" key increases the circle radius, the "d" key decreases the circle radius, and the "q" key finishes this step.



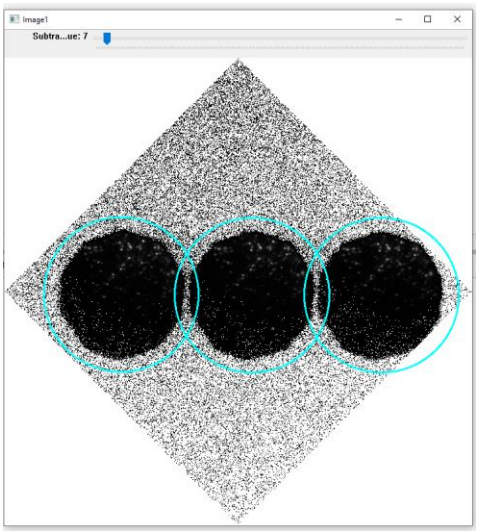
1.3 Rough alignment operation instructions: Adjusting the slider position can adjust the contrast to help rough alignment.







1.3 Rough alignment operation instructions



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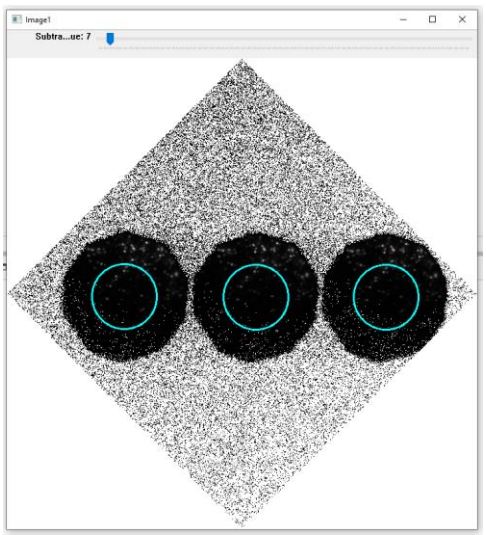
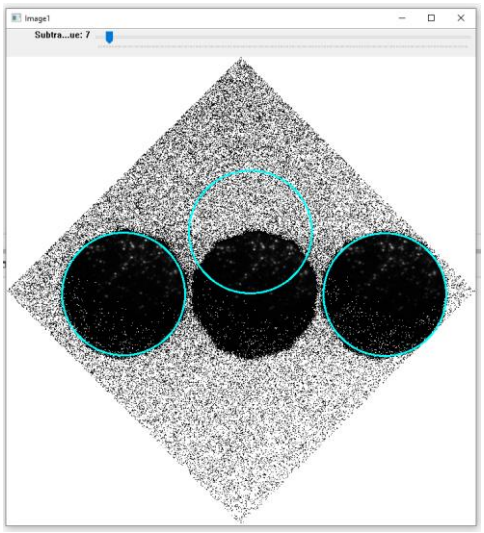
The "u" key increases the circle radius



8

The "d" key decreases the circle radius



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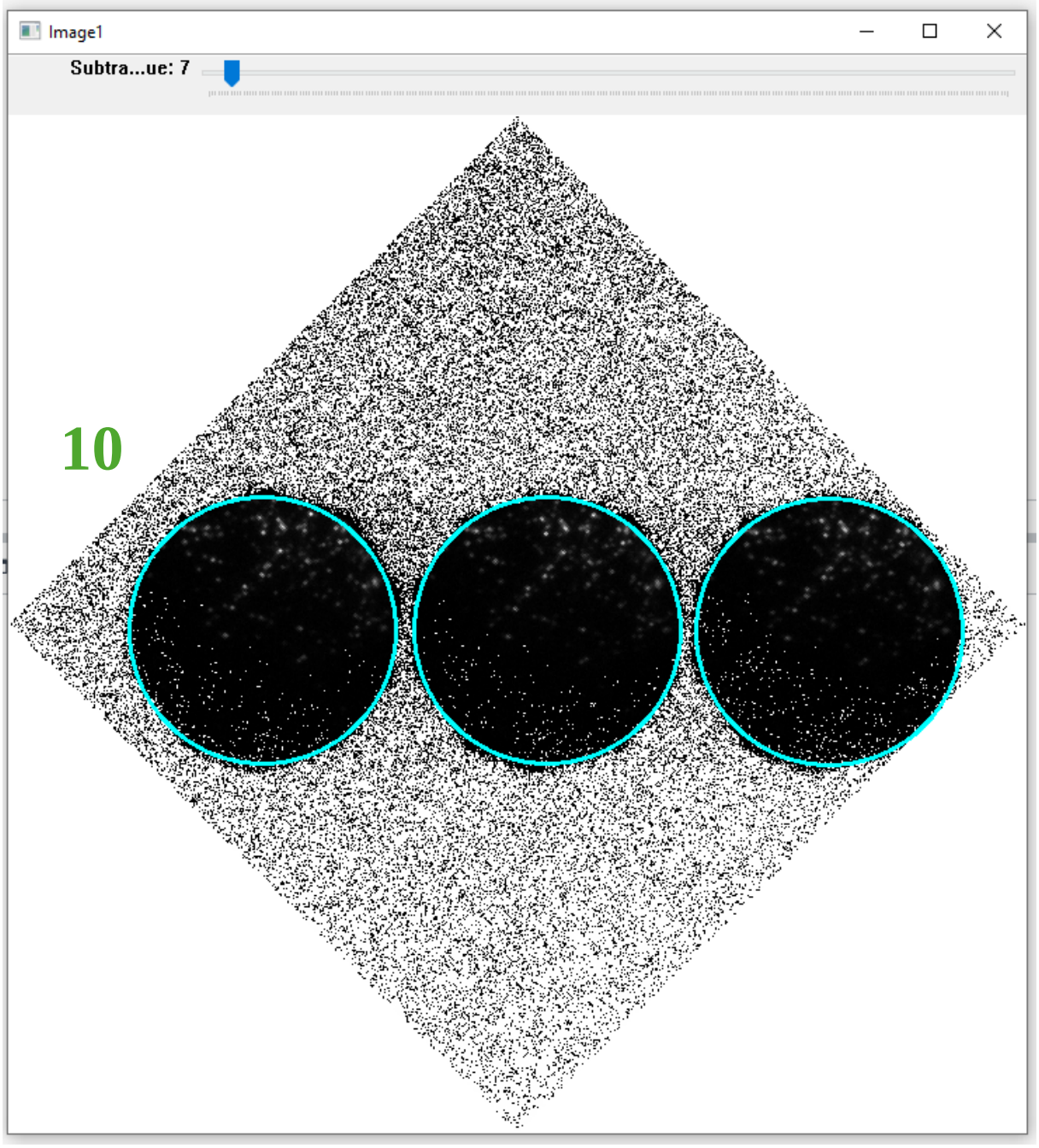
“left mouse clicking” changes the position of the circle

1.3 Rough alignment operation instructions: Match the three circles to the three channels, and then press the "q" key to start the fine alignment.



1.4 Fine alignment : Through iterative calculation, the program obtains the parameters of the fine alignment of the three channels and saves parameters as "Calibration_200frames_calibration_para.pkl".

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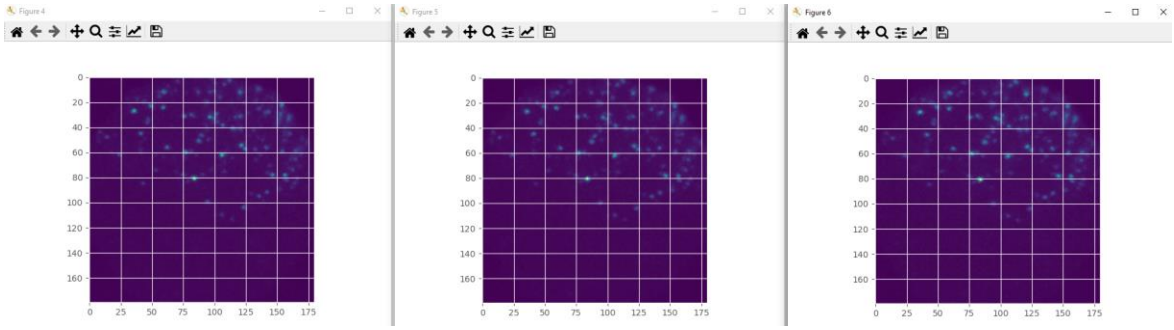
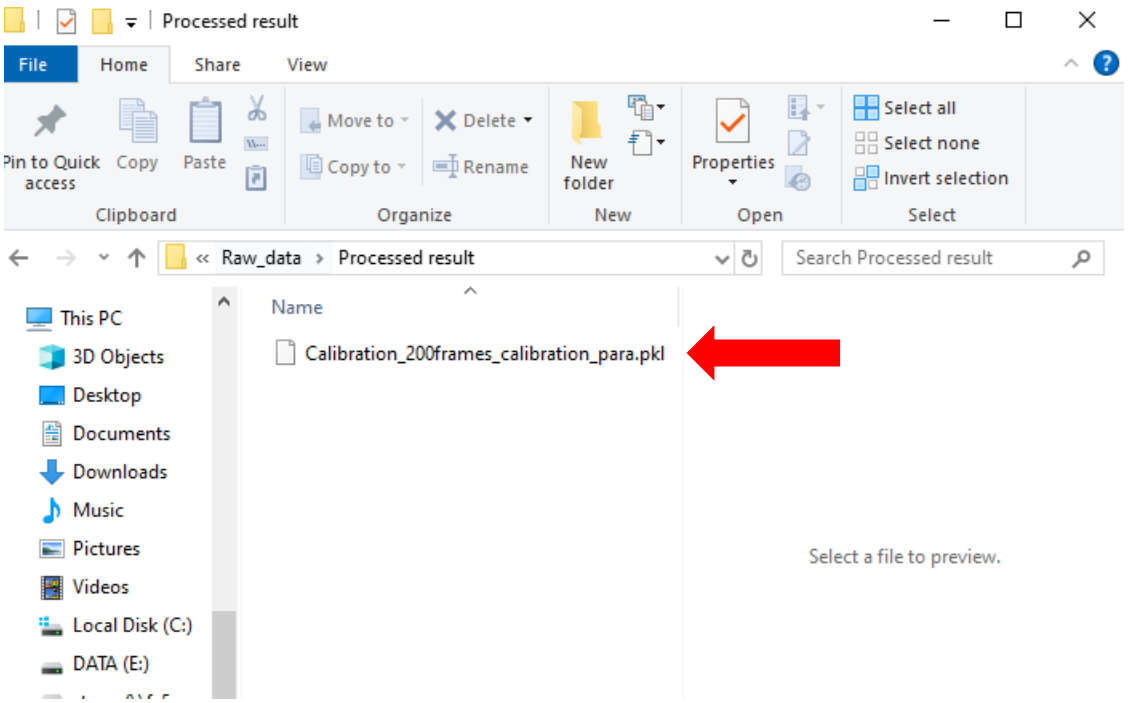
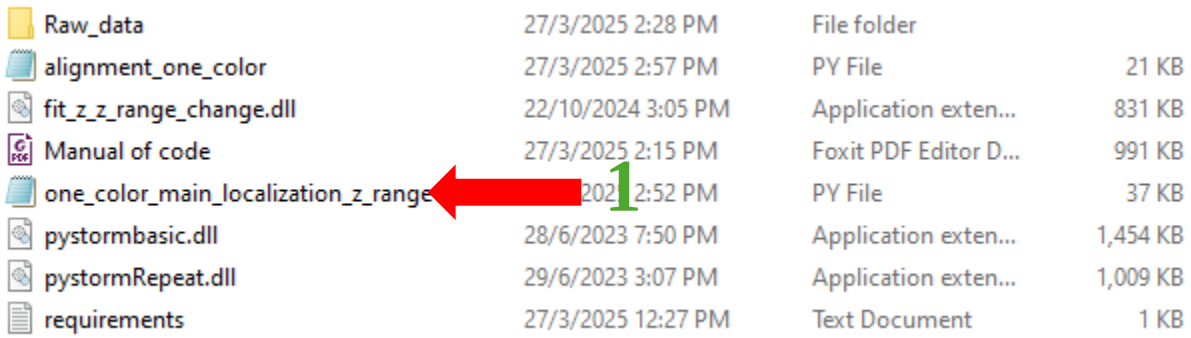


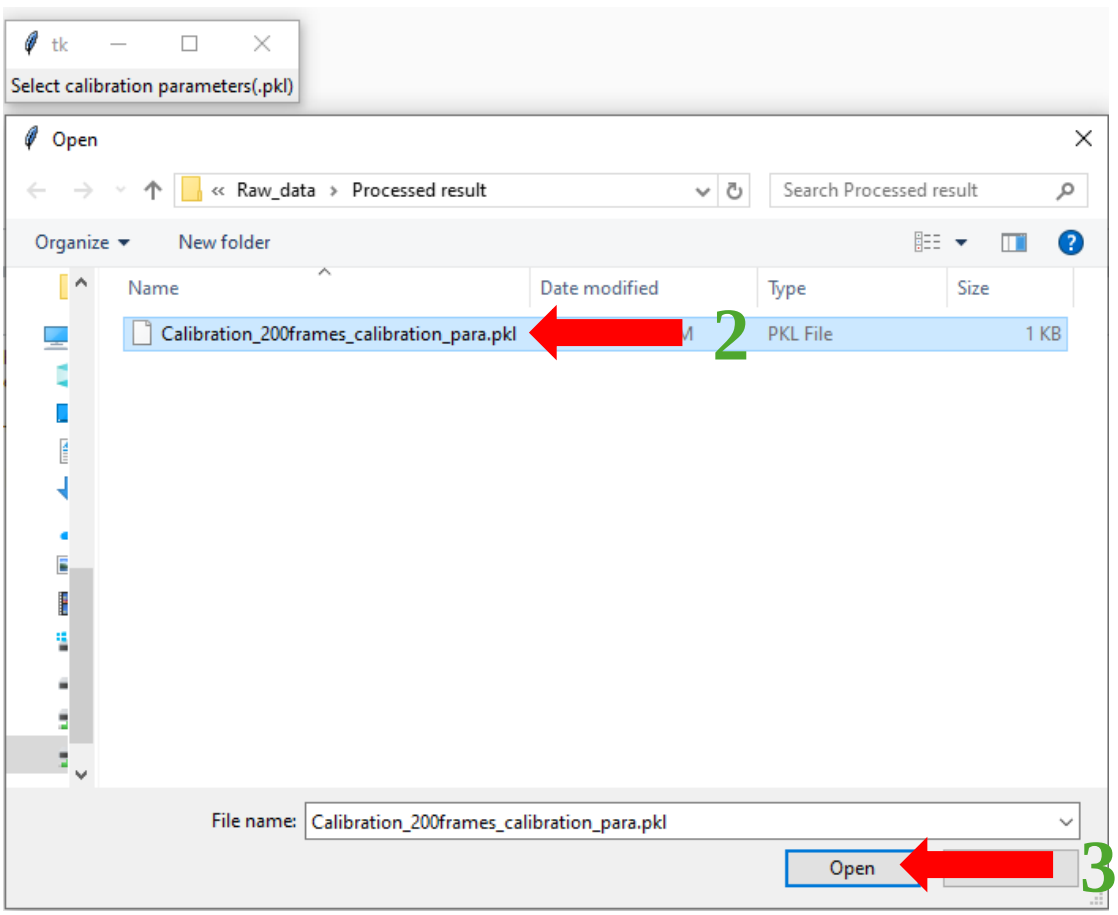


2. 3D localization program runs

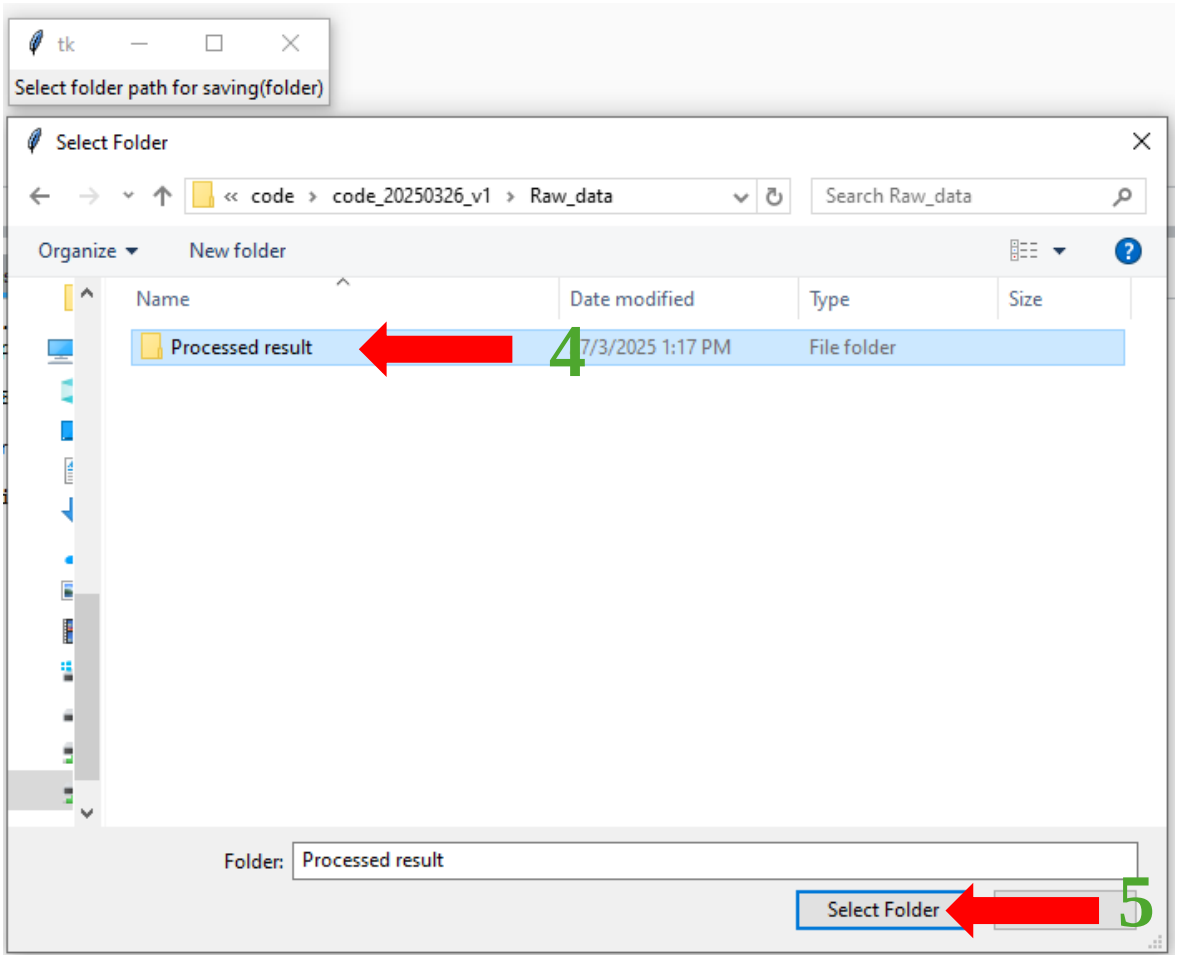


After the parameters of the fine alignment are obtained, the 3D localization program is run to obtain the three-dimensional coordinates of the molecule.

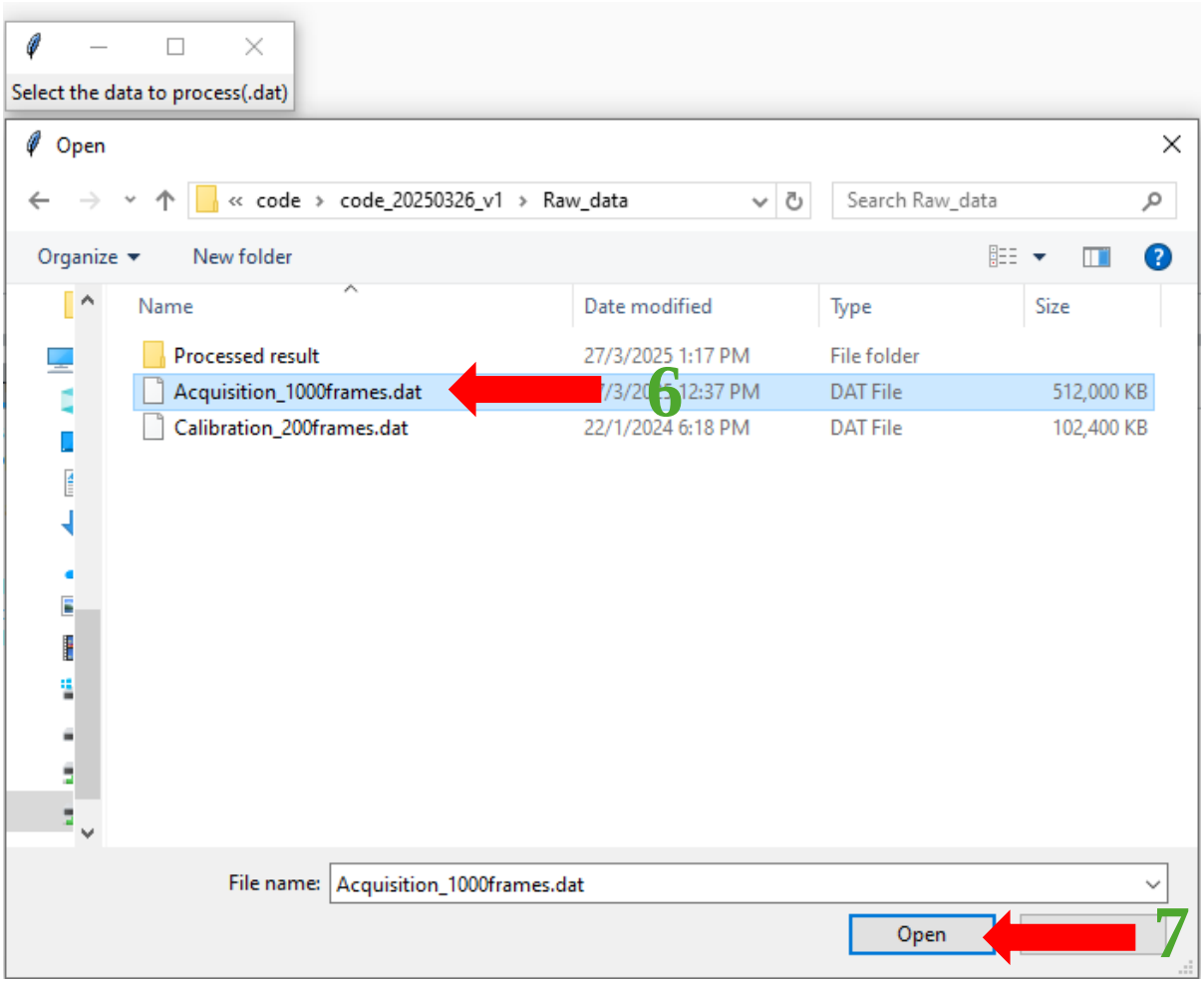
2.1 Select Calibration_200frames_calibration_para.pkl to load the parameters of the fine alignment.



2.2 Select a folder to save three-dimensional coordinates of the molecule calculated later.



2.3 Select the data you want to process.



2.4 Update acquisition parameter

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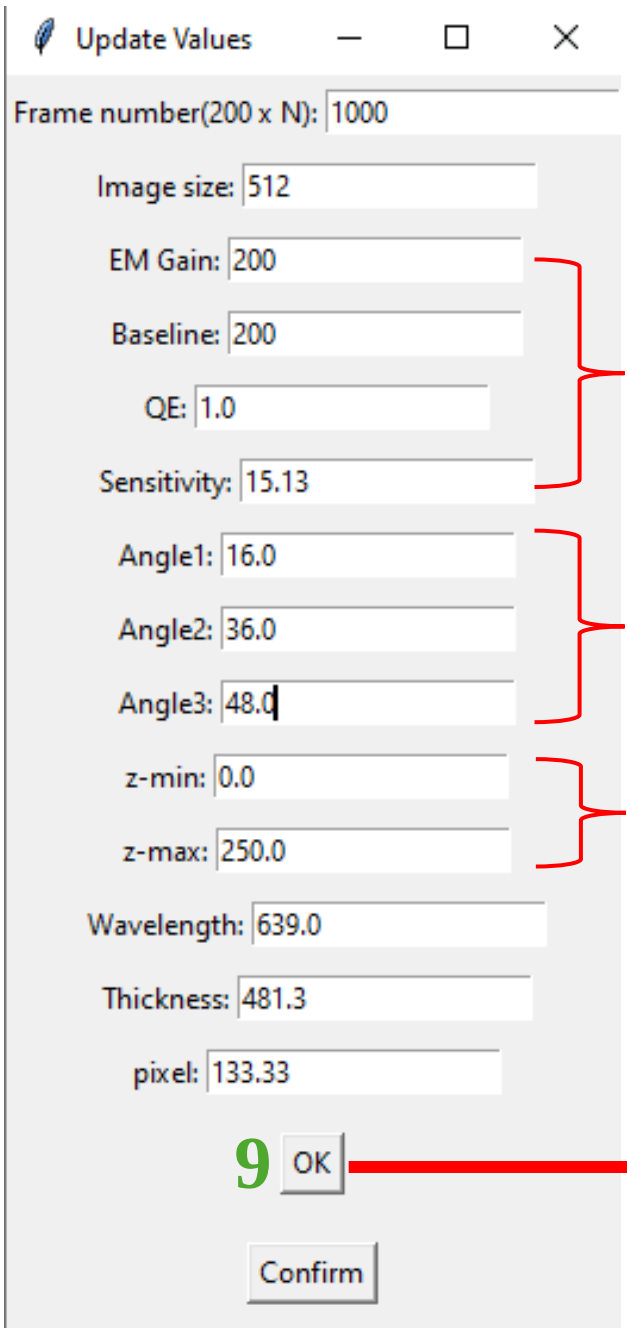


The number of frames acquired

The size of one frame image

Camera parameter

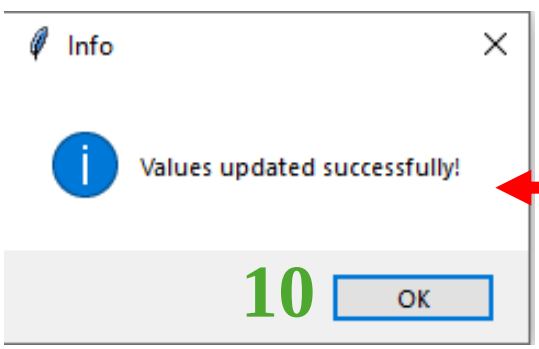
The three angles of the laser used

Axial position fitting range

Wavelength of laser

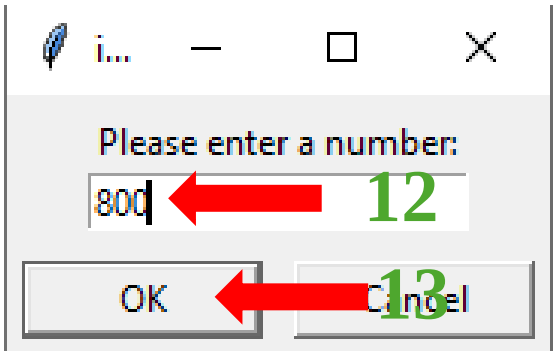
Thickness of Silicon Wafer

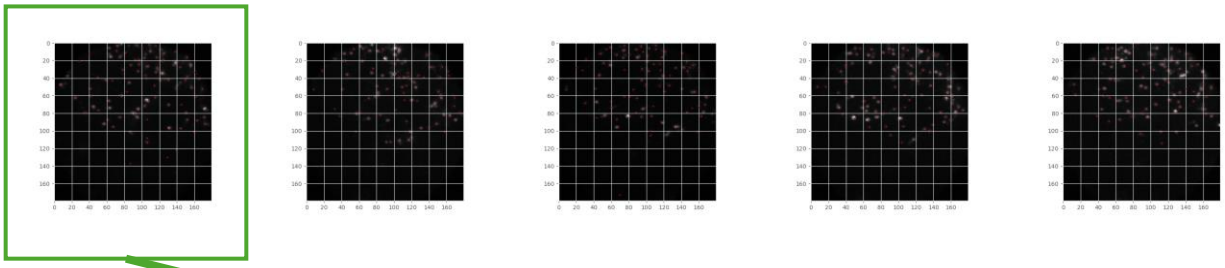
Camera pixel size

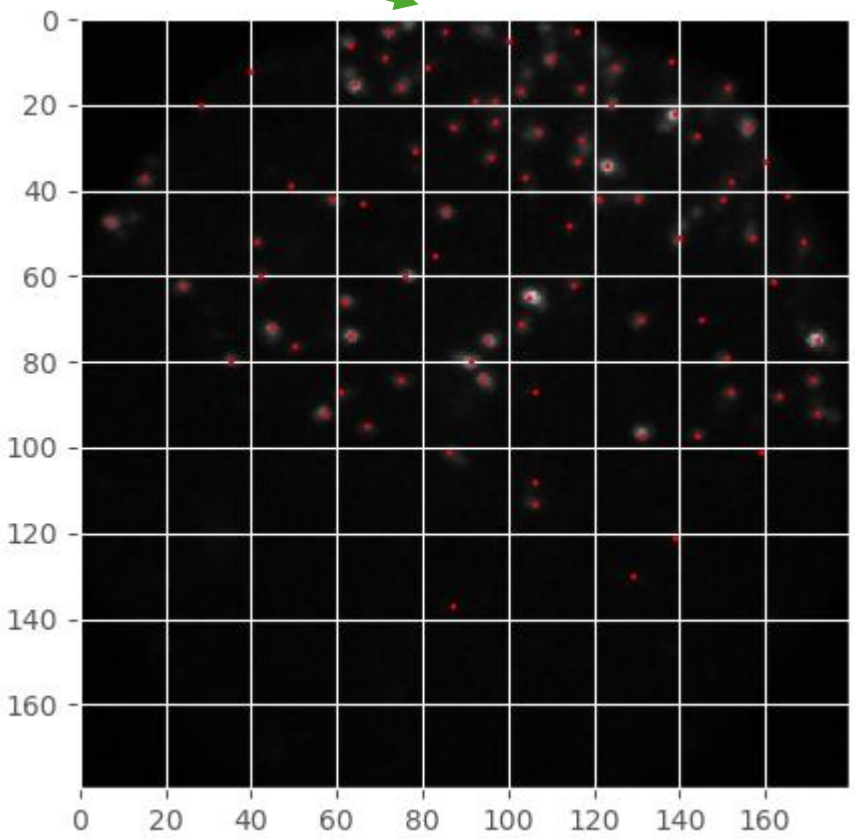


Click "Confirm" to the next step **11**

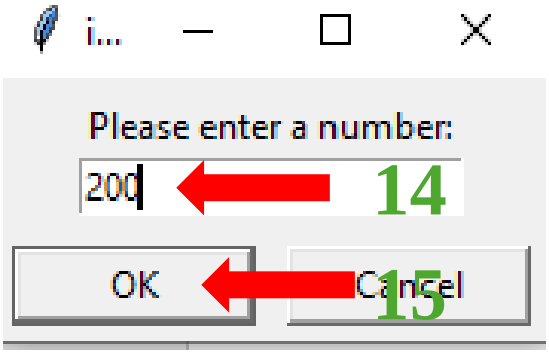
2.5 Enter parameters to preview peak selection.

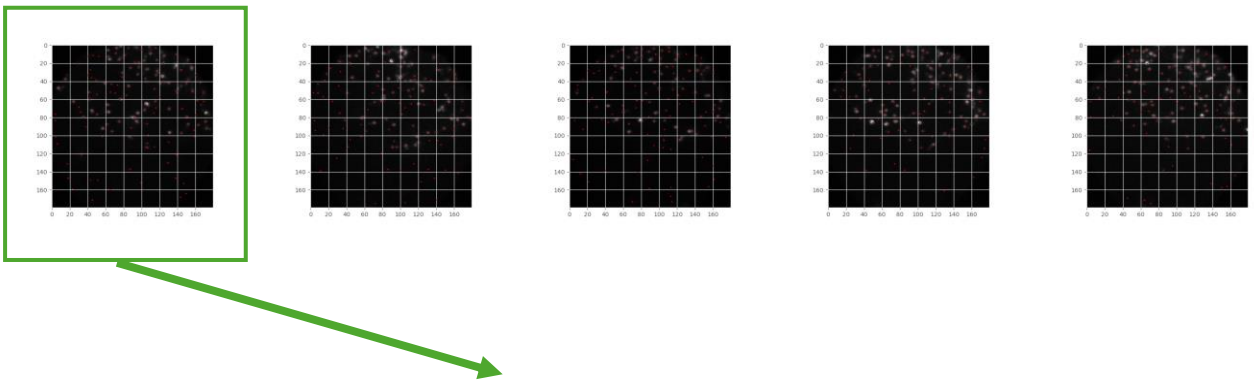


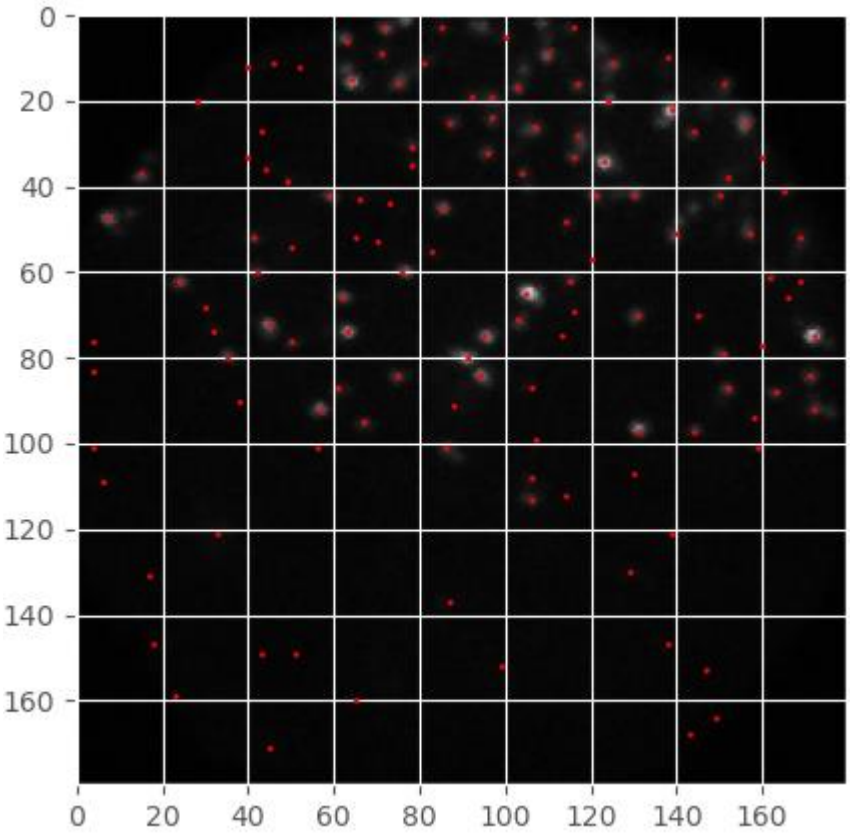




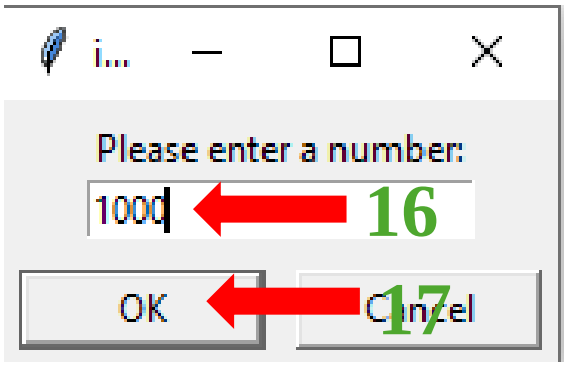
2.5 Enter parameters to preview peak selection.

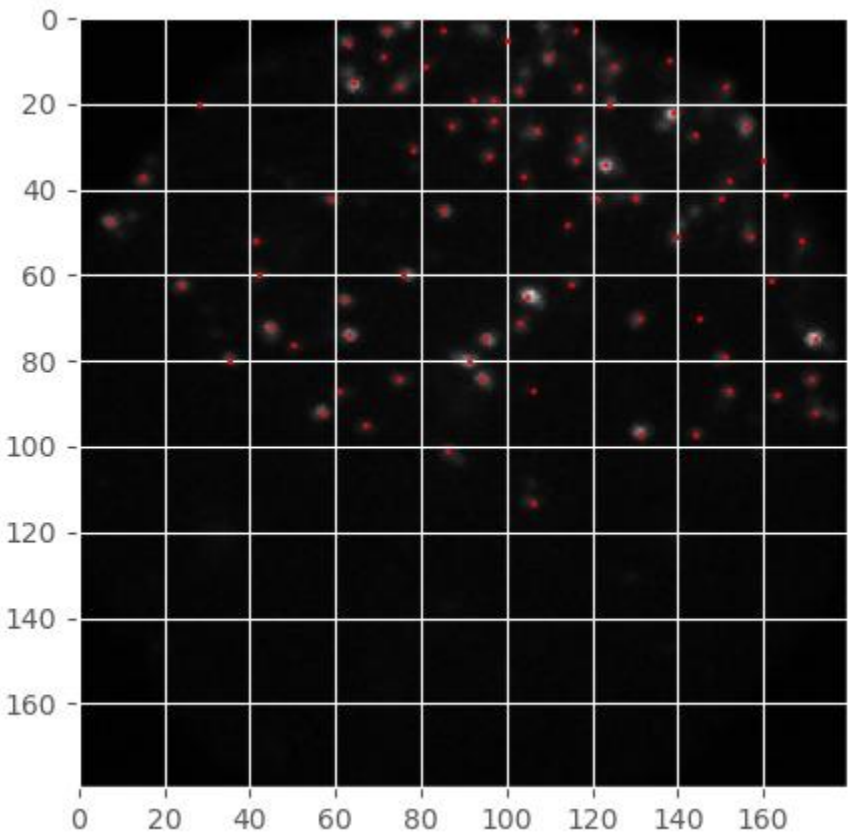


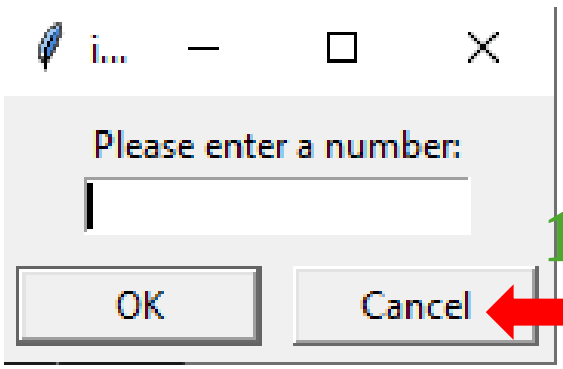




2.5 Enter parameters to preview peak selection.







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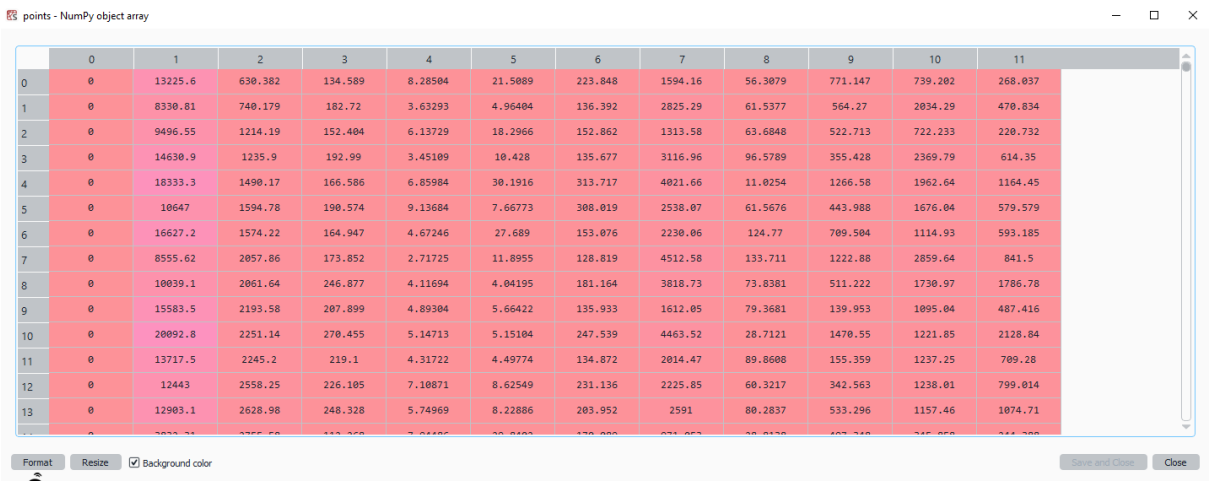
Click "Cancel" to proceed to the next step

2.6 Obtain the three-dimensional coordinates of the molecule.

After a while of waiting, we will get the three-dimensional coordinates of the molecules, which are saved in the "points" numpy matrix and finally wrote in the "Acquisition_1000frames_mlefit_frame_x_y_z_crlbxy_crlbz_sigmax_y_N_bg_N1_N2_N3_0120250327_1358.npy" under the folder " Processed result ".



	0	1	2	3	4	5	6	7	8	9	10	11
0	0	13225.6	630.382	134.589	8.28504	21.5089	223.848	1594.16	56.3079	771.147	739.202	268.037
1	0	8330.81	740.179	182.72	3.63293	4.96404	136.392	2825.29	61.5377	564.27	2034.29	470.834
2	0	9496.55	1214.19	152.404	6.13729	18.2966	152.862	1313.58	63.6848	522.713	722.233	220.732
3	0	14630.9	1235.9	192.99	3.45109	10.428	135.677	3116.96	96.5789	355.428	2369.79	614.35
4	0	18333.3	1490.17	166.586	6.85984	30.1916	313.717	4021.66	11.0254	1266.58	1962.64	1164.45
5	0	10647	1594.78	190.574	9.13684	7.66773	308.019	2538.07	61.5676	443.988	1676.04	579.579
6	0	16627.2	1574.22	164.947	4.67246	27.689	153.076	2230.06	124.77	709.504	1114.93	593.185
7	0	8555.62	2057.86	173.852	2.71725	11.8955	128.819	4512.58	133.711	1222.88	2859.64	841.5
8	0	10039.1	2061.64	246.877	4.11694	4.04195	181.164	3818.73	73.8381	511.222	1730.97	1786.78
9	0	15583.5	2193.58	207.899	4.89304	5.66422	135.933	1612.05	79.3681	139.953	1095.04	487.416
10	0	20092.8	2251.14	270.455	5.14713	5.15104	247.539	4463.52	28.7121	1470.55	1221.85	2128.84
11	0	13717.5	2245.2	219.1	4.31722	4.49774	134.872	2014.47	89.8608	155.359	1237.25	709.28
12	0	12443	2558.25	226.105	7.10871	8.62549	231.136	2225.85	60.3217	342.563	1238.01	799.014
13	0	12903.1	2628.98	248.328	5.74969	8.22886	203.952	2591	80.2837	533.296	1157.46	1074.71

Each column is “Frame number", “x (nm)“ , “y (nm)" , “z (nm)" , “CRLB_xy (nm)" , “CRLB_z (nm)" , “Sigma_xy (nm)" , “Total photon number" , “Background" , “Photon number of channel 1" , “Photon number of channel 2" and “Photon number of channel 3" from left to right.