









SiLM Code User Manual

1. File introduction

 Raw_data	27/3/2025 2:28 PM	File folder	
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 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
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Raw_data: code test data (Calibration_200frames.dat: three-channel calibration raw data; Acquisition_1000frames.dat: Original 1000frames test raw data; Processed result: The result of processed result)

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

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

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

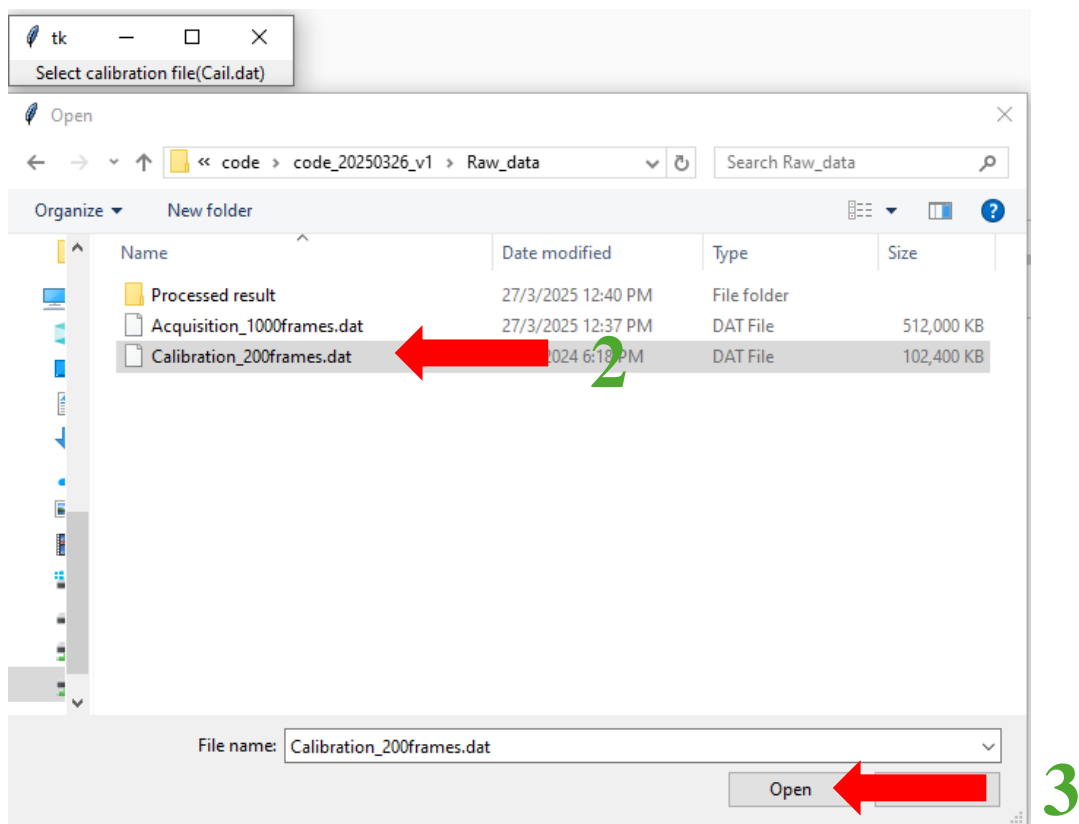
We split the code into modules with comments so that users can read and modify it.

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

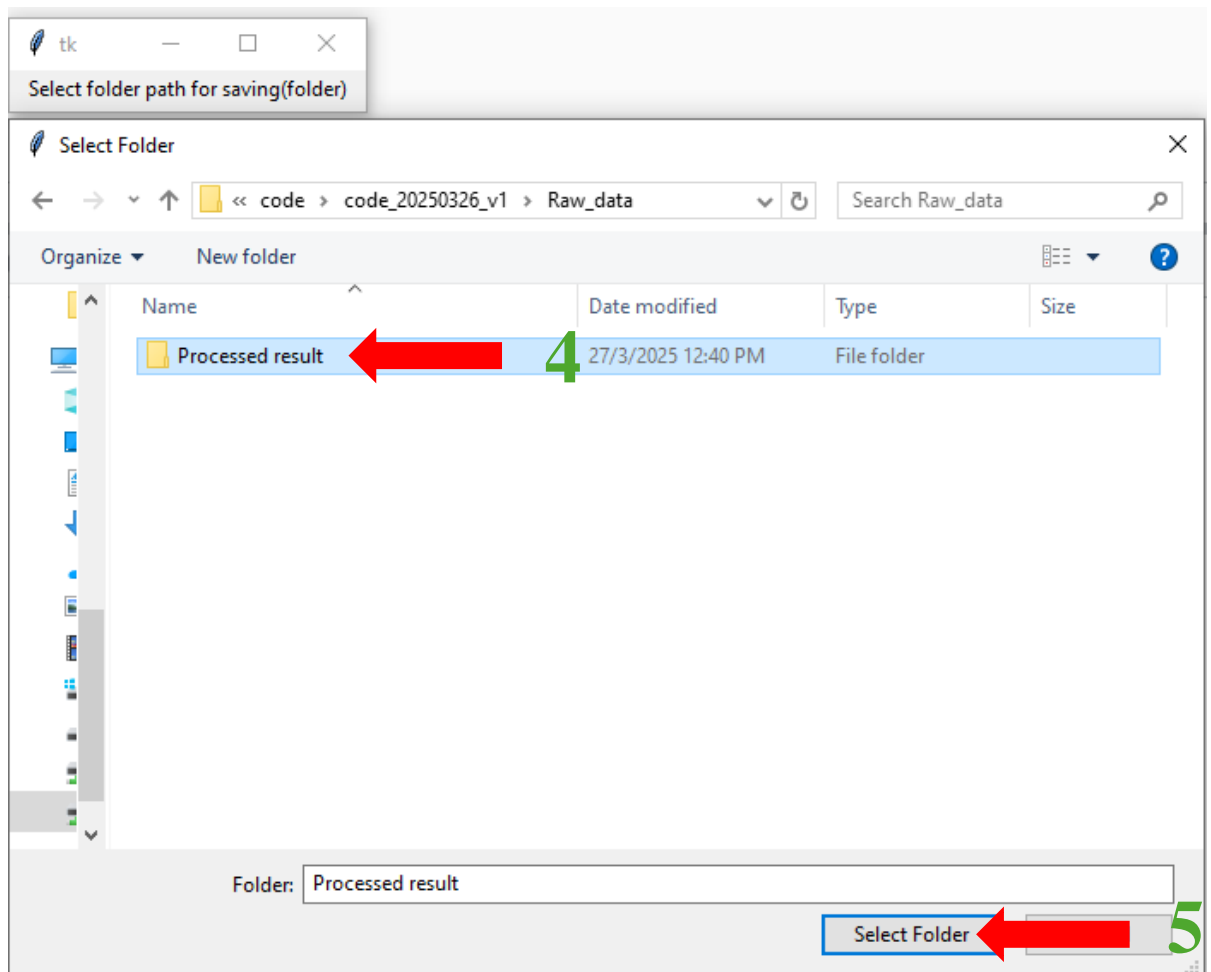
1. Alignment program runs

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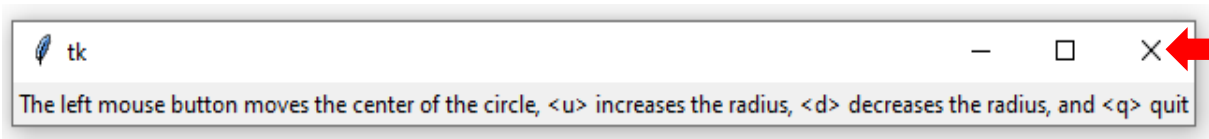
1.1 Select Calibration_200frames.dat for three-channel alignment.



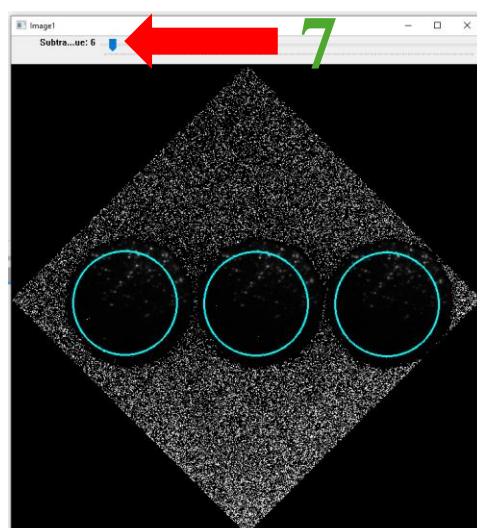
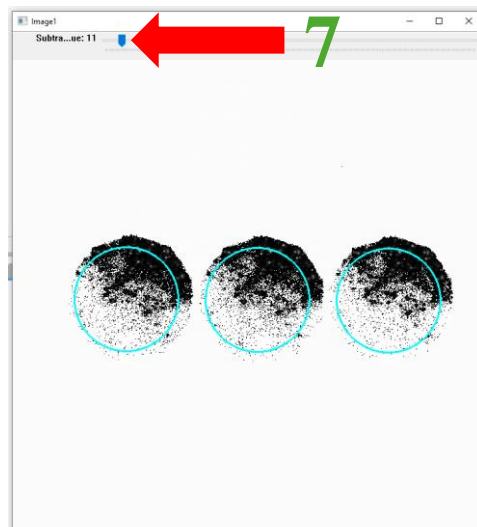
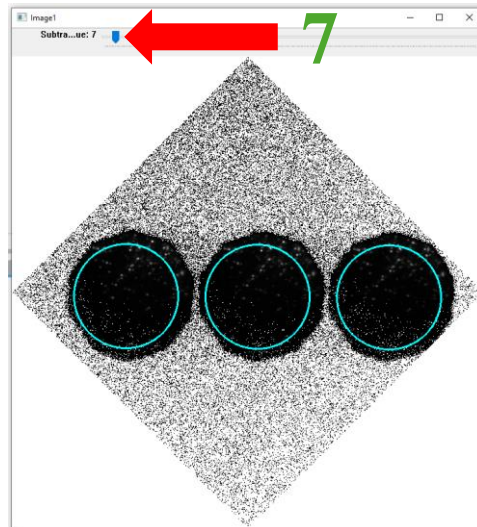
1.2 Select a folder to save the alignment parameters calculated later.



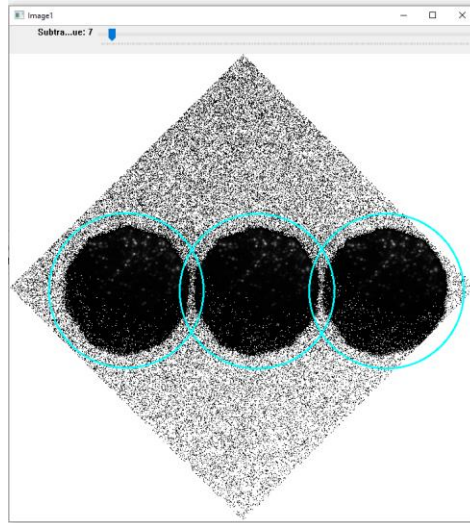
1.3 Rough alignment operation instructions: Left mouse click to change the position of the circle, the "u" key increases the circle radius, the "d" key decreases the circle radius, and the "q" key ends this step.



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

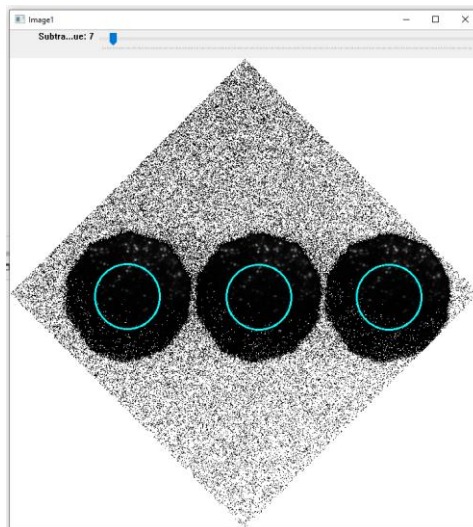


1.3 Rough alignment operation instructions



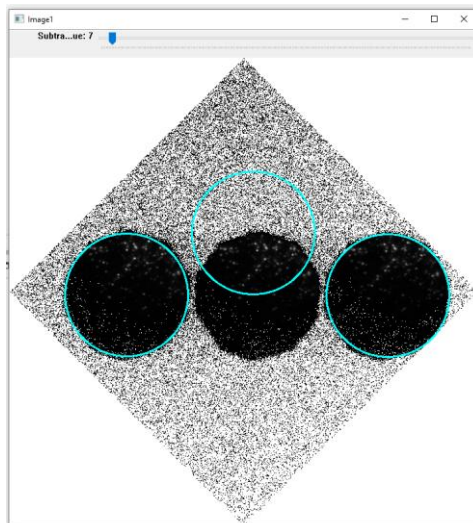
8

The "u" key increases the circle radius



8

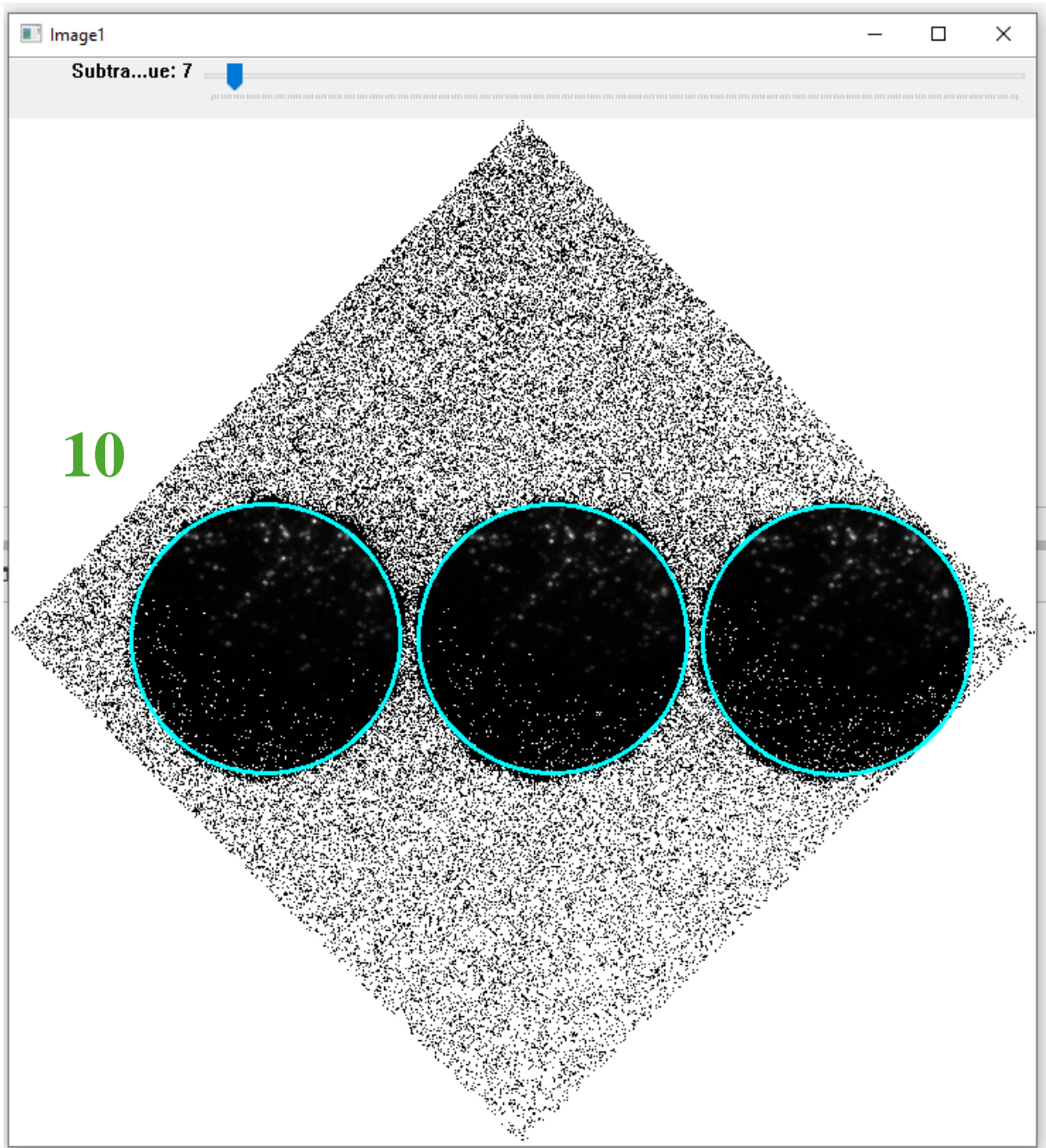
The "d" key decreases the circle radius



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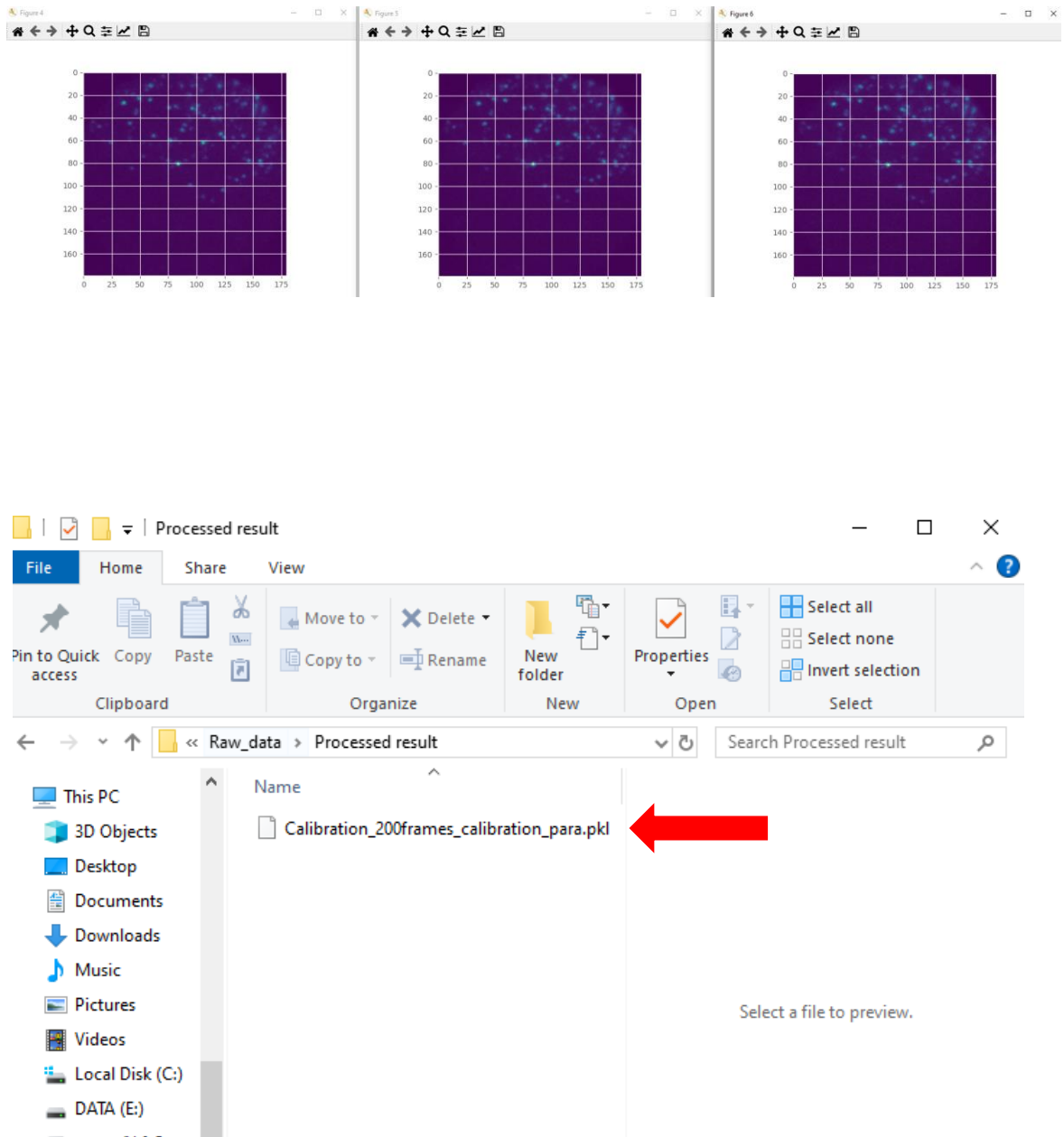
Left mouse click to change the position of the circle

1.3 Rough alignment operation instructions: Finally 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 them as "Calibration_200frames_calibration_para.pkl".

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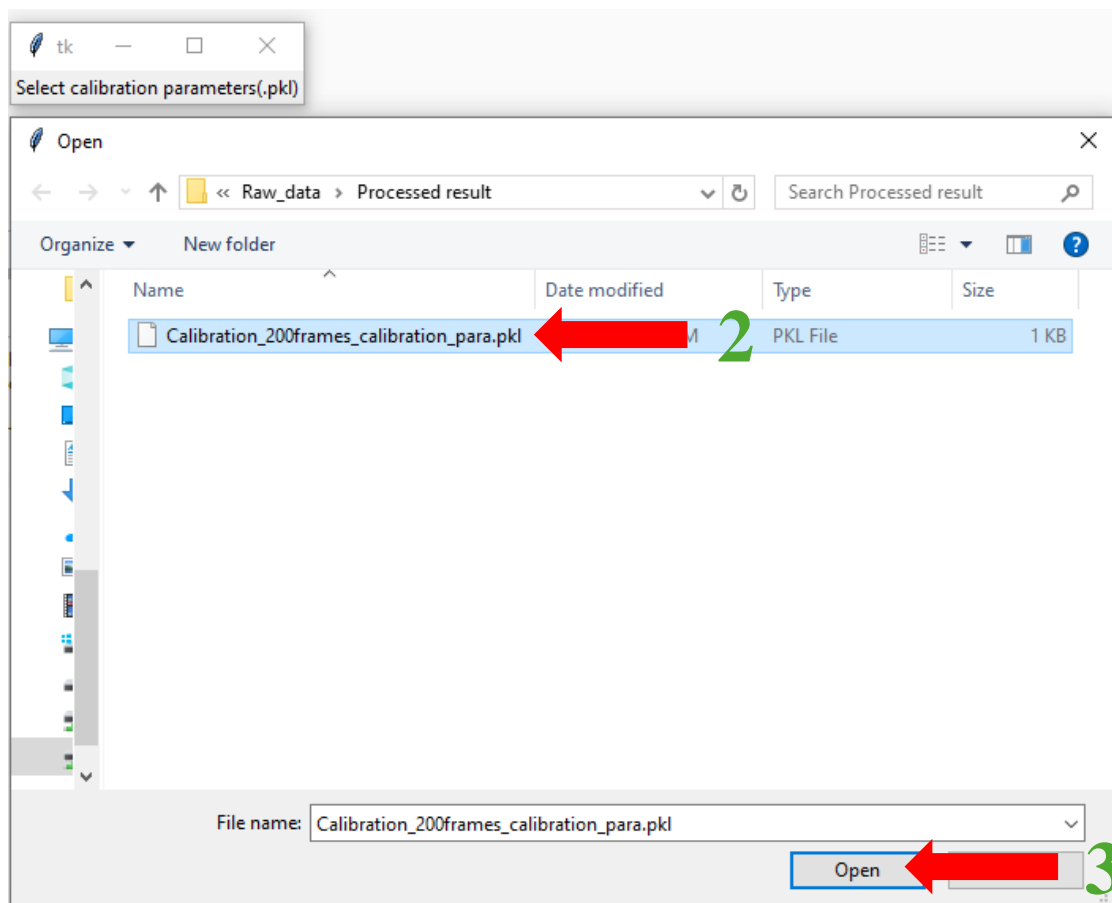


2. 3D localization program runs

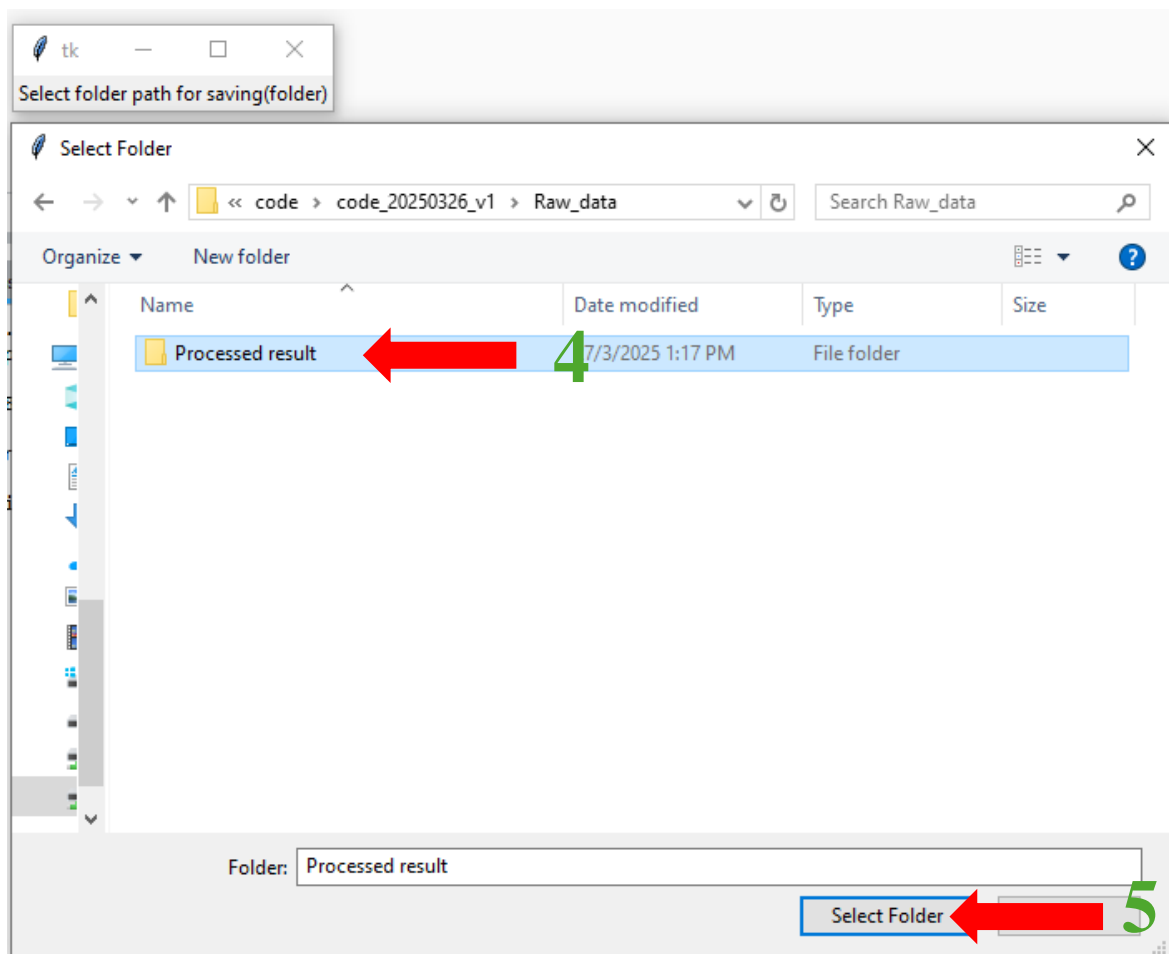
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After the parameters of the fine alignment are obtained, the 3D localization program is run to obtain the three-dimensional coordinates of the molecule.

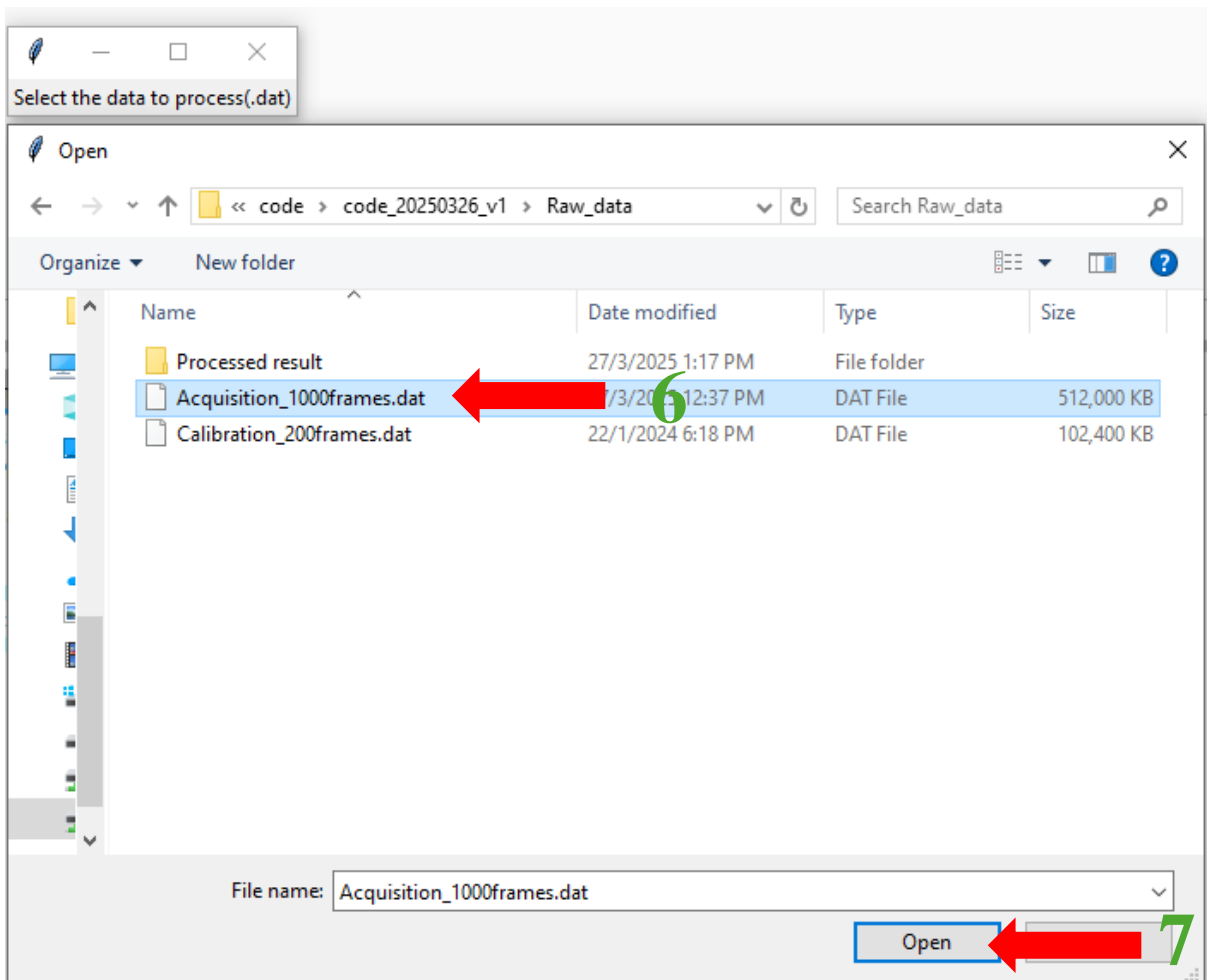
2.1 Select Calibration_200frames_calibration_para.pkl to load the parameters of the fine alignment.



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

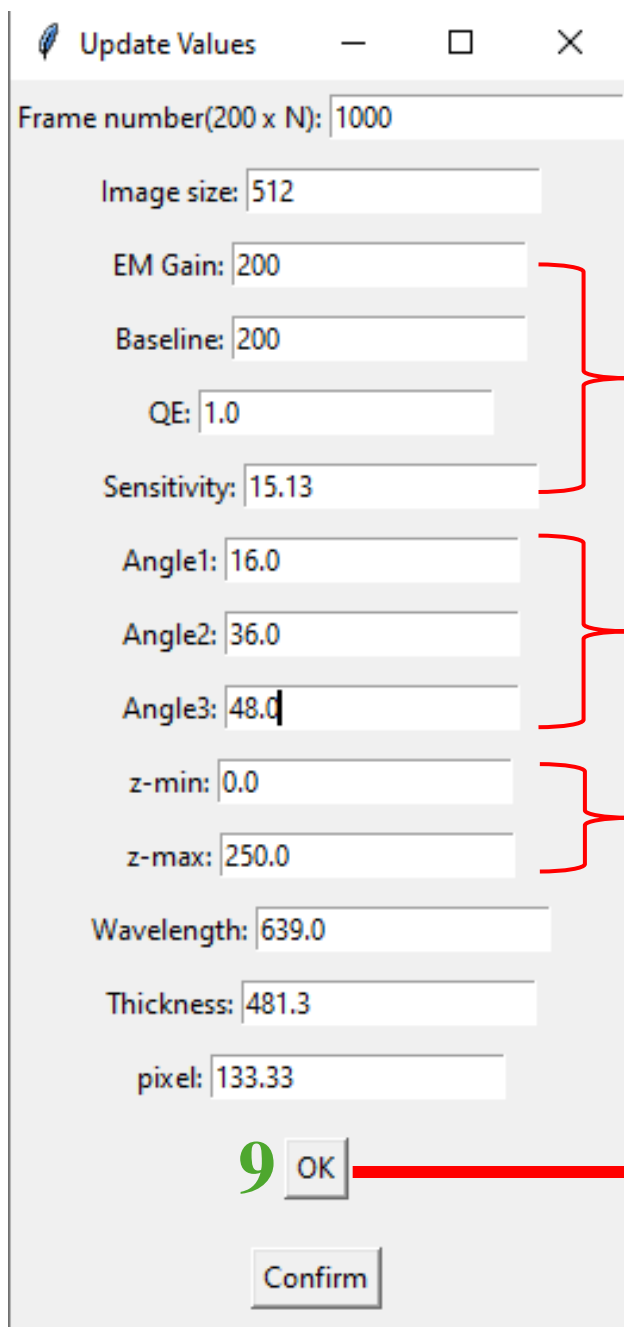


2.3 Select the data you want to process.



2.4 Update acquisition parameter

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The 'Update Values' dialog box contains the following parameters:

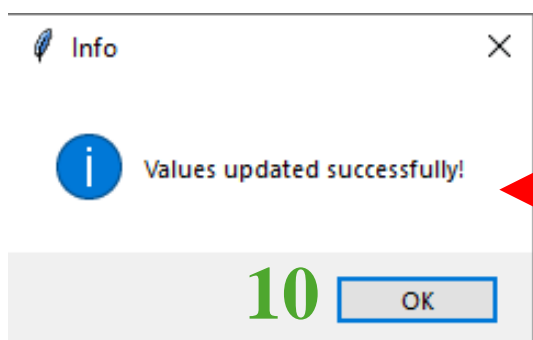
- Frame number(200 x N): 1000
- Image size: 512
- EM Gain: 200
- Baseline: 200
- QE: 1.0
- Sensitivity: 15.13
- Angle1: 16.0
- Angle2: 36.0
- Angle3: 48.0
- z-min: 0.0
- z-max: 250.0
- Wavelength: 639.0
- Thickness: 481.3
- pixel: 133.33

Annotations on the right side of the dialog box:

- The number of frames acquired
- The size of one frame image
- Camera parameter (grouped with EM Gain, Baseline, QE, and Sensitivity)
- The three angles of the laser used (grouped with Angle1, Angle2, and Angle3)
- Axial position fitting range (grouped with z-min and z-max)
- Wavelength of laser
- Thickness of Silicon Wafer
- Camera pixel size

Buttons at the bottom: OK, Confirm.

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The 'Info' dialog box displays the message: "Values updated successfully!"

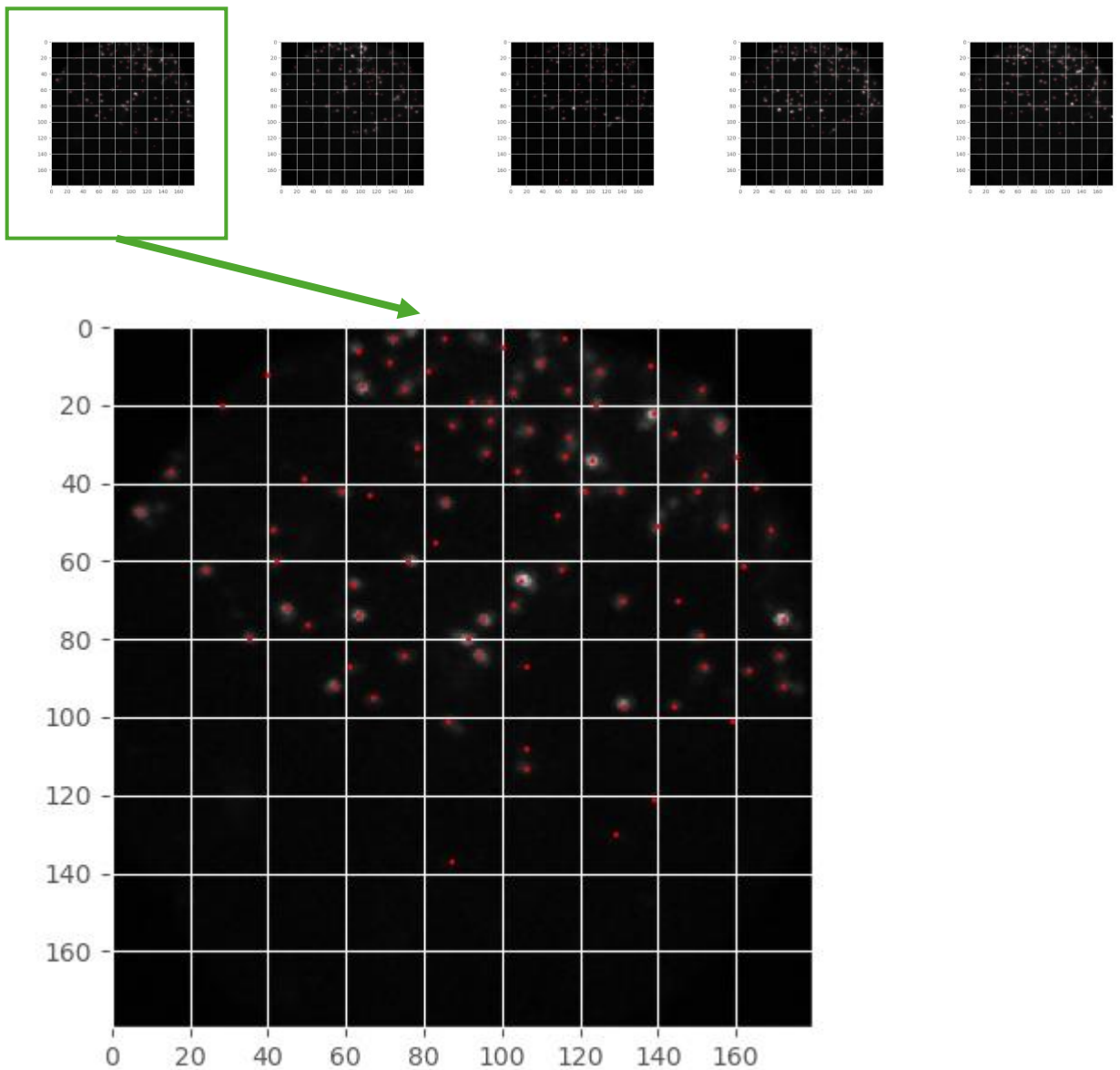
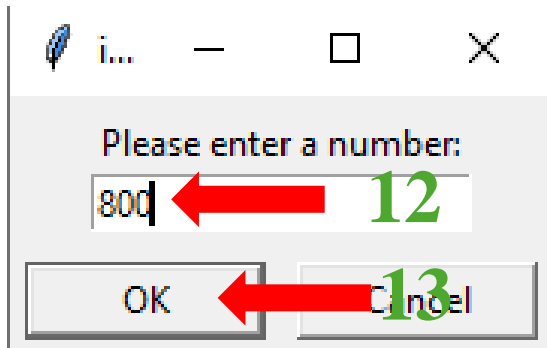
Buttons: OK

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Click "Confirm" to the next step

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2.5 Enter parameters to preview peak selection.



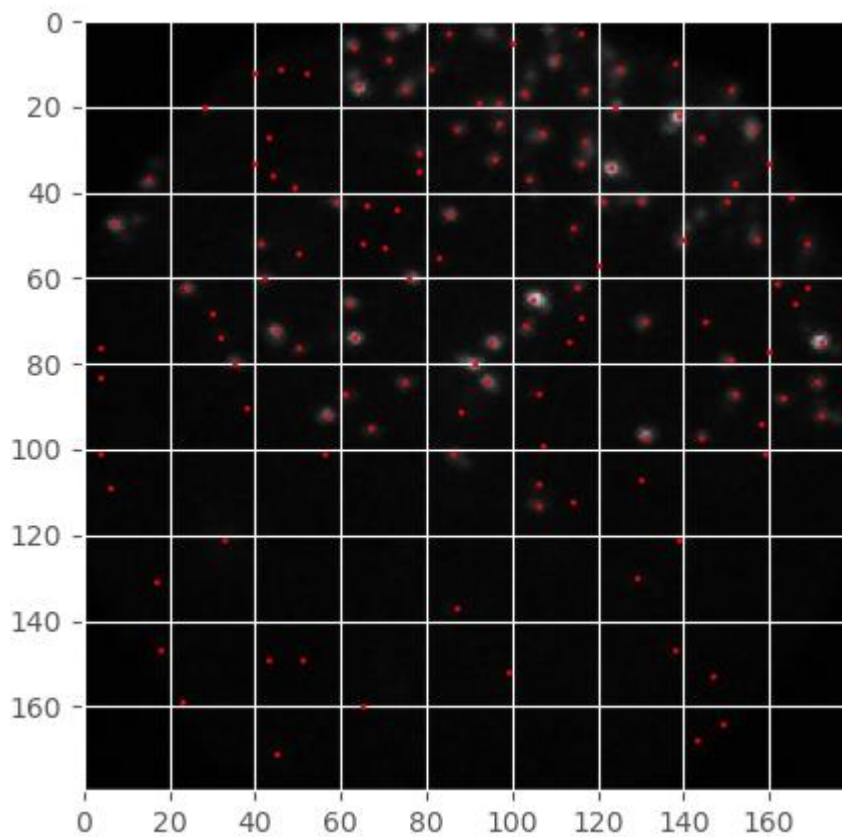
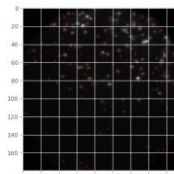
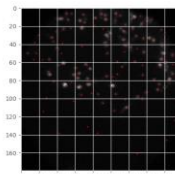
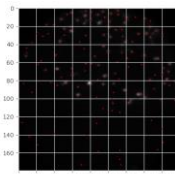
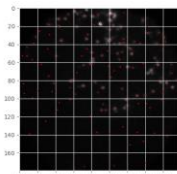
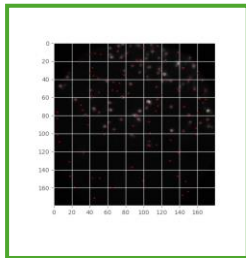
2.5 Enter parameters to preview peak selection.

 i... ☐ ☒

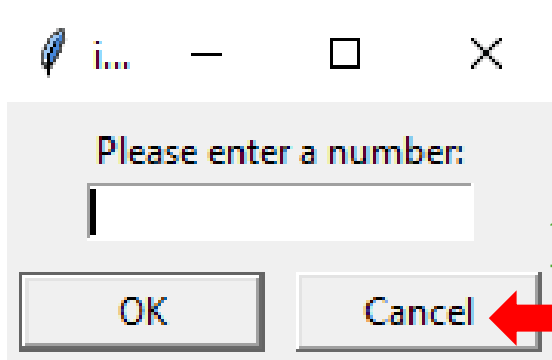
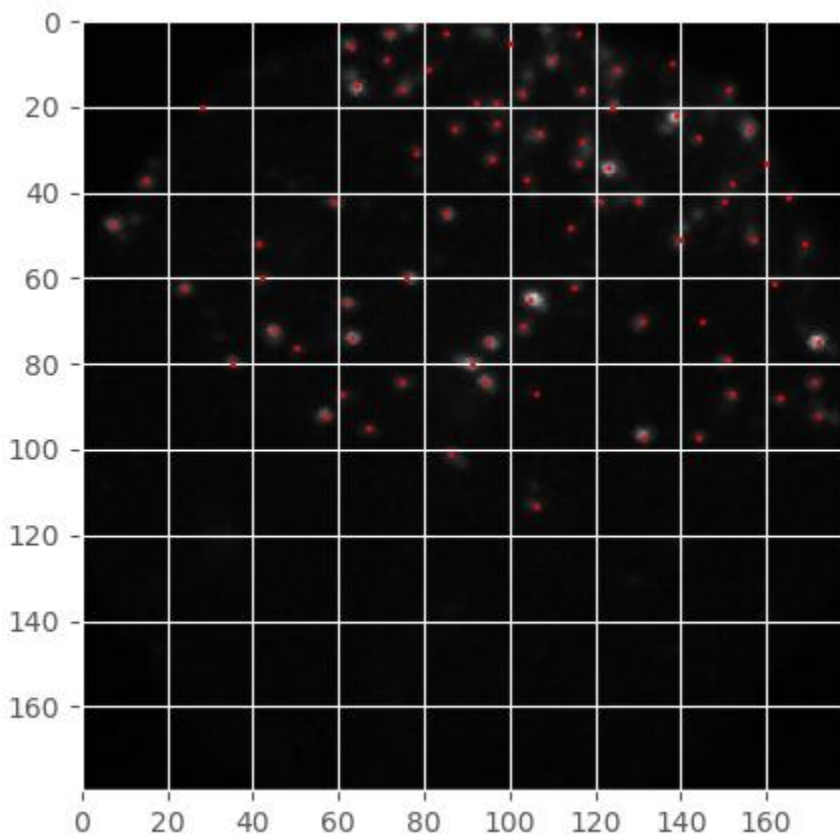
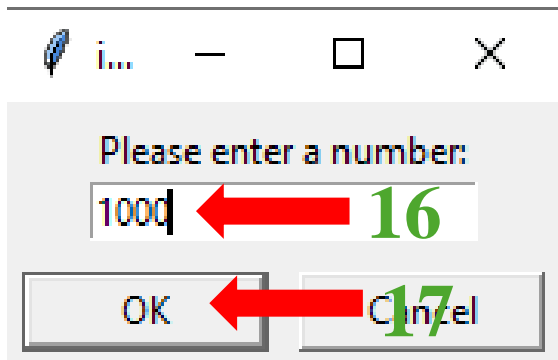
Please enter a number:

200 ← 14

OK ← 15 Cancel



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.

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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 in the "Acquisition_1000frames_mlefit_frame_x_y_z_crlbxy_crlbz_sigma_y_N_bg_N1_N2_N3_0120250327_1358.npy" under folder " Processed result ".

points - NumPy object array

	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	1895.04	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

Format Resize ☒ Background color Save and Close Close

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.

- Acquisition_1000frames_mlefit_frame_x_y_z_crlbx_crlby_crlbz_sigma_y_N_bg_PALM.hdf5
- Acquisition_1000frames_mlefit_frame_x_y_z_crlbx_crlby_crlbz_sigma_y_N_bg_PALM
- Acquisition_1000frames_mlefit_frame_x_y_z_crlbxy_crlbz_sigma_y_N_bg_N1_N2_N3_0120250327_1358
- Acquisition_1000frames_mlefit_frame_x_y_z_crlbxy_crlbz_sigma_y_N_bg_N1_N2_N3_0120250327_1358.npy
- Acquisition_1000frames100_spots_image
- Calibration_200frames_calibration_para.pkl