The axial to radial transformation of 2D localizations for LBR-C

Section Outline

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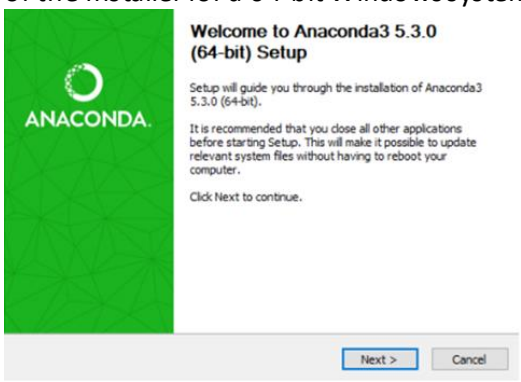
Section I

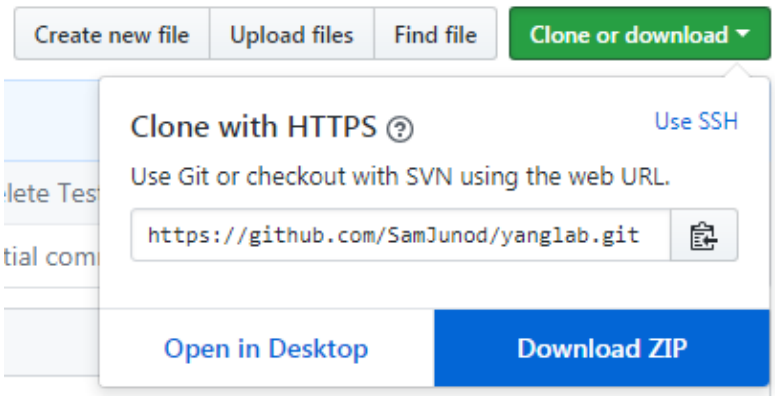
Software Download

Python 3

1) Install Python 3
    i. Since these scripts make use of several Python 3 libraries (tkinter, csv, random, os, sys, numpy, scipy, and math), the simplest way to install Python 3 with these required libraries is through the Anaconda distribution of Python 3 which can be found here (https://www.anaconda.com/download/).
    ii. Download the appropriate installer for your operating system (32-bit or 64-bit - Windows or Mac)
    iii. Once the installer has finished downloading, open it and follow the click-through instructions to install the Anaconda distribution of Python 3. An example of the first step of the installer for a 64-bit Windows system is shown below.



2) Download the script files
    i. Follow the URL link below.
        i. https://github.com/SamJunod/yanglab
    ii. Select the "Clone or Download" button and download the ZIP file. Extract the ZIP file to your preferred file location.



MatLab

1) Go to MatLab website, MATLAB - MathWorks - MATLAB & Simulink, and download the MatLab software.
    i. URL: https://www.mathworks.com/products/matlab.html

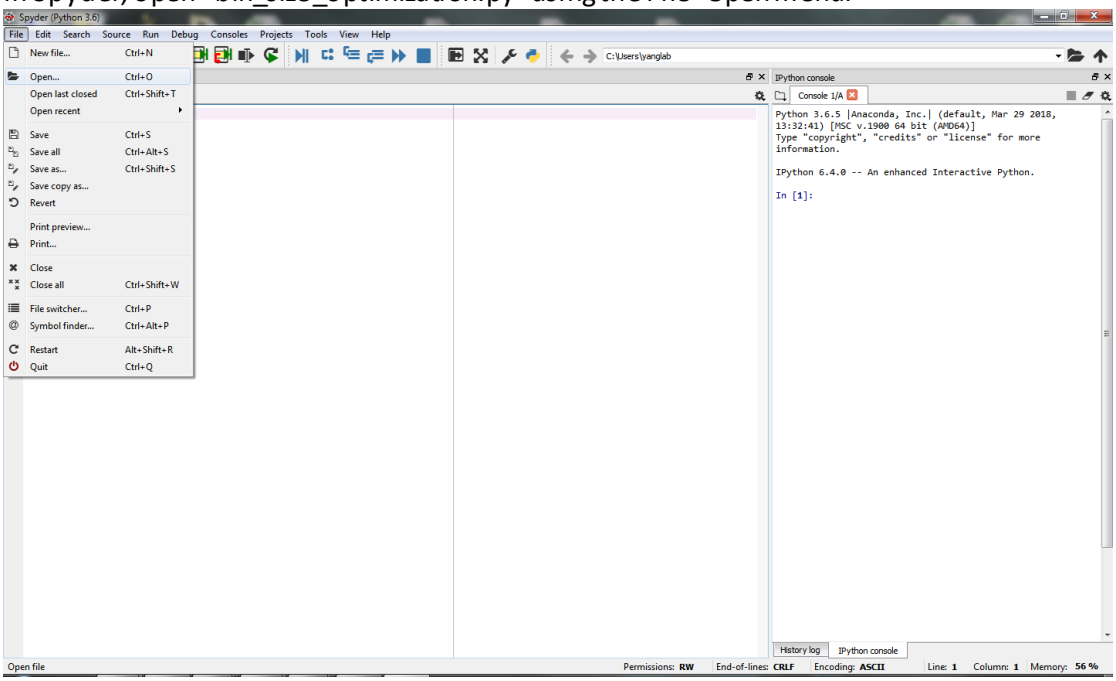
OriginLab

2) Go to OriginLab website and download the Origin software.
    i. URL: https://www.originlab.com/demodownload.aspx

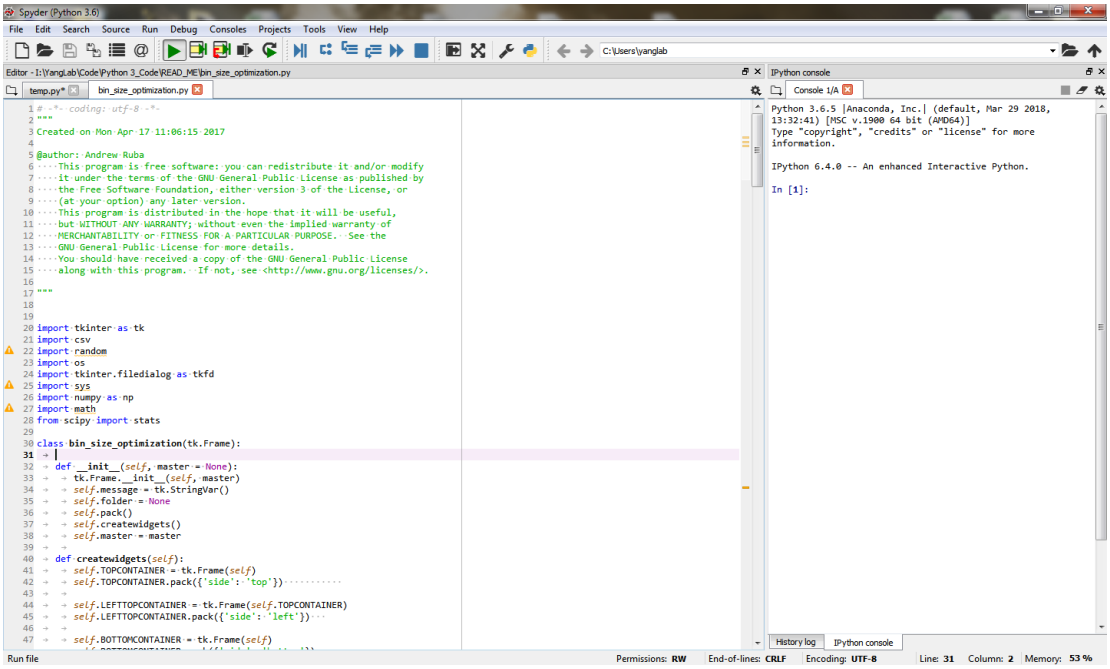
Section II
Bin Optimization

1) Packaged in the Anaconda distribution of Python 3 is an integrated development environment for Python 3 called "Spyder." Open Spyder– it should have an icon on your desktop or an icon in the program list under the start menu for Windows.

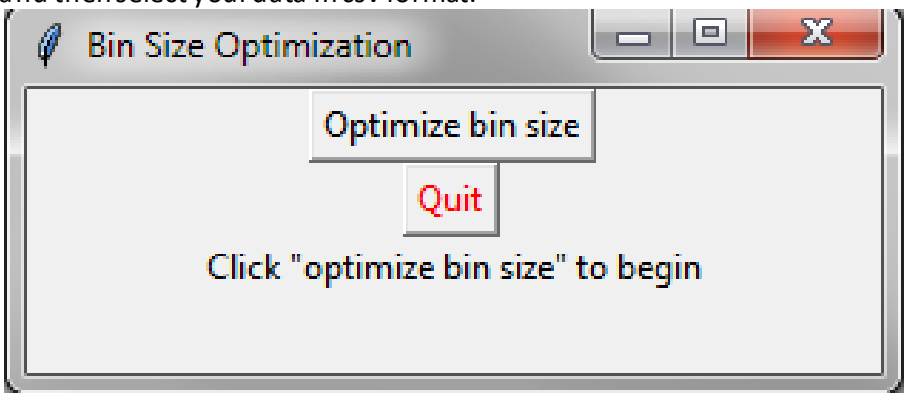
2) In Spyder, open "bin_size_optimization.py" using the File>Open menu.



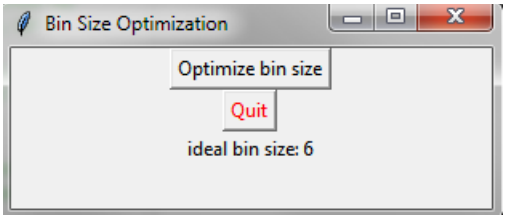
3) Then click the green "Run file" arrow in the toolbar.



4) Once the script begins to run, a pop-up window will appear. Select the Optimize bin size button and then select your data in csv format.



5) After the data is selected the script will run and output the ideal bin size. For LBR-C, the ideal bin size is 6.

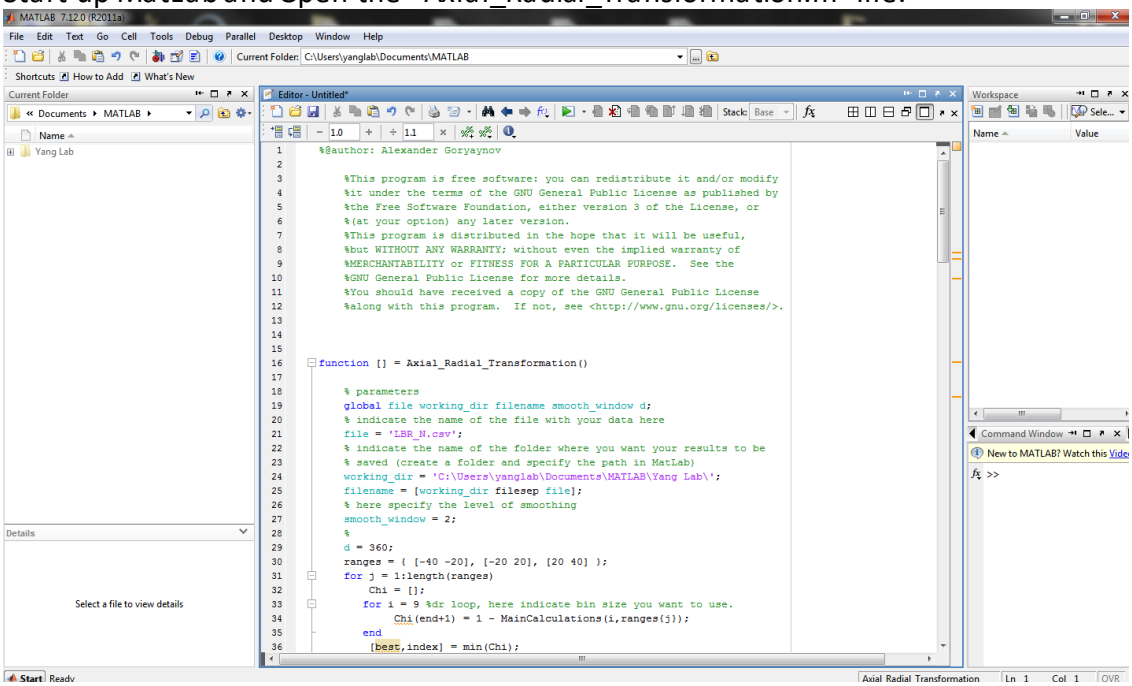


6) Select the "Quit" button to close the pop-up window.

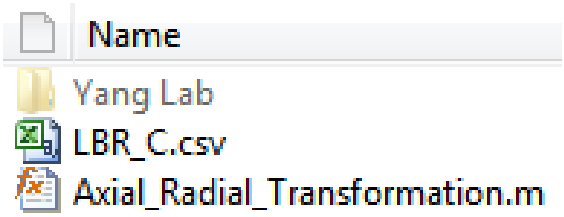
Section III

Axial to Radial Transformation

1) Start-up MatLab and Open the "Axial_Radial_Transformation.m" file.



2) The MatLab script (Axial_Radial_Transformation.m) and the 2D localizations (LBR_C.csv) will have to be in the same folder.



3) In the MatLab script, under the Editor box, make the following edits:

   i. File name (line 21, file = '***file name.csv***';)

```
20        % indicate the name of the file with your data here
21        file = 'LBR_C.csv';
```

   ii. Working directory(line 24, working_dir = '***folder location of data***';)

```
22        % indicate the name of the folder where you want your results to be
23        % saved (create a folder and specify the path in MatLab)
24 -      working_dir = 'C:\Users\yanglab\Documents\MATLAB\Yang Lab\';
```

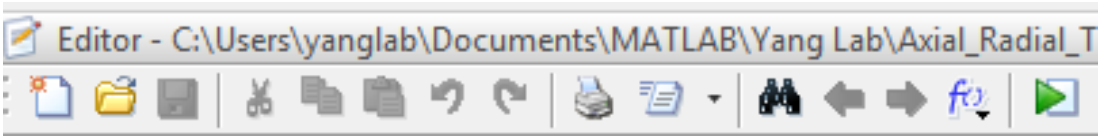
   iii. Axial ranges (line 30, ranges ={ [***axial ranges***] , [***for data***] };

```
30 -        ranges = { [-40 -20], [-20 20], [20 40] };
```

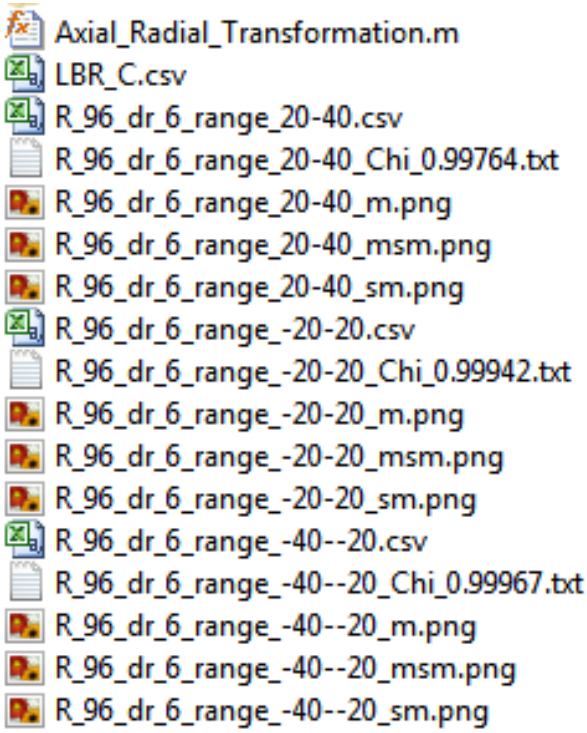
   iv. Binsize (line 33, for i = ***optimal bin size***). This is the bin size determined from section II.

```
33 -        for i = 6 %dr loop, here indicate bin size you want to use.
```

4) Select "Save" and run the script by selecting the green arrow in the toolbar under the "Editor" box.



5) The program will write several items to the folder selected in the MatLab script.



6) Open the csv files created by the program. The values within each of the csv file are the raw results from the MatLab script. The file name of the csv document corresponds to the total radius of the program, the bin size(dr), and the range of the axial segment.

7) The csv file will have 3 columns of radial information (histogram count, raw matrix equation values, and smoothed matrix equation values). Copy the third column (smoothed matrix equation values) to a separate excel file.

	A	B	C
1	21	-1.1869	-1.1869
2	23	0.97271	0.75969
3	22.667	2.4933	0.68325
4	18.667	-1.4162	0.19648
5	23	-0.48759	-0.41475
6	27.667	0.65957	1.2208
7	31	3.4905	2.207
8	25.667	2.4708	2.2779
9	22	0.87243	1.5557
10	23.667	1.3237	1.3769
11	25	1.9344	2.2327
12	24.333	3.4399	2.4597
13	16.667	2.0047	2.4583
14	13	1.9302	1.8781
15	8	1.6994	1.3779
16	2	0.50395	0.73446
17	0	0	0

	A	B	C
1	(-40 to -20)	(-20 to 20)	(20 to 40)
2			
3	-1.1869		
4	0.75969		
5	0.68325		
6	0.19648		
7	-0.41475		
8	1.2208		
9	2.207		
10	2.2779		
11	1.5557		
12	1.3769		
13	2.2327		
14	2.4597		
15	2.4583		
16	1.8781		
17	1.3779		
18	0.73446		
19	0		

8) Repeat the last step for the other axial ranges from the other excel files.

	A	B	C
1	(-40 to -20)	(-20 to 20)	(20 to 40)
2			
3	-1.1869	-0.89177	-1.1508
4	0.75969	-0.85905	0.2399
5	0.68325	-0.73929	0.64383
6	0.19648	0.070089	0.74101
7	-0.41475	1.9312	0.4396
8	1.2208	4.8701	1.1513
9	2.207	6.5832	0.64912
10	2.2779	6.3345	0.7658
11	1.5557	3.8766	0.18354
12	1.3769	1.6434	1.106
13	2.2327	0.86393	1.5809
14	2.4597	1.1673	1.8684
15	2.4583	1.6952	1.3469
16	1.8781	1.3457	0.76825
17	1.3779	1.7469	1.6748
18	0.73446	1.1665	1.4493
19	0	0	0

9) These values will have to be normalized to the number of bins in each subsection. For instance, with a bin size of 6nm the section that ranges from -40nm to -20nm is comprised of 3.33 subregions (bins). This value is calculated by taking the number the length of the axial segment (20nm) and dividing by the bin size (6nm). Likewise, the subsection that ranges from -20nm to 20nm is comprised of 6.66 subregions, and the subsection that ranges from 20nm to 40nm is comprised of 3.33 subregions. The values

in the -40nm to -20nm column will be divided by 20/6. The data from -20nm to 20nm is divided by 40/6, and the data from 20nm to 40nm is divided by 20/6.

(-40 to -20)	(-20 to 20)	(20 to 40)
-0.35607	-0.133766	-0.34524
0.227907	-0.128858	0.07197
0.204975	-0.110894	0.193149
0.058944	0.0105134	0.222303
-0.124425	0.28968	0.13188
0.36624	0.730515	0.34539
0.6621	0.98748	0.194736
0.68337	0.950175	0.22974
0.46671	0.58149	0.055062
0.41307	0.24651	0.3318
0.66981	0.1295895	0.47427
0.73791	0.175095	0.56052
0.73749	0.25428	0.40407
0.56343	0.201855	0.230475
0.41337	0.262035	0.50244
0.220338	0.174975	0.43479
0	0	0

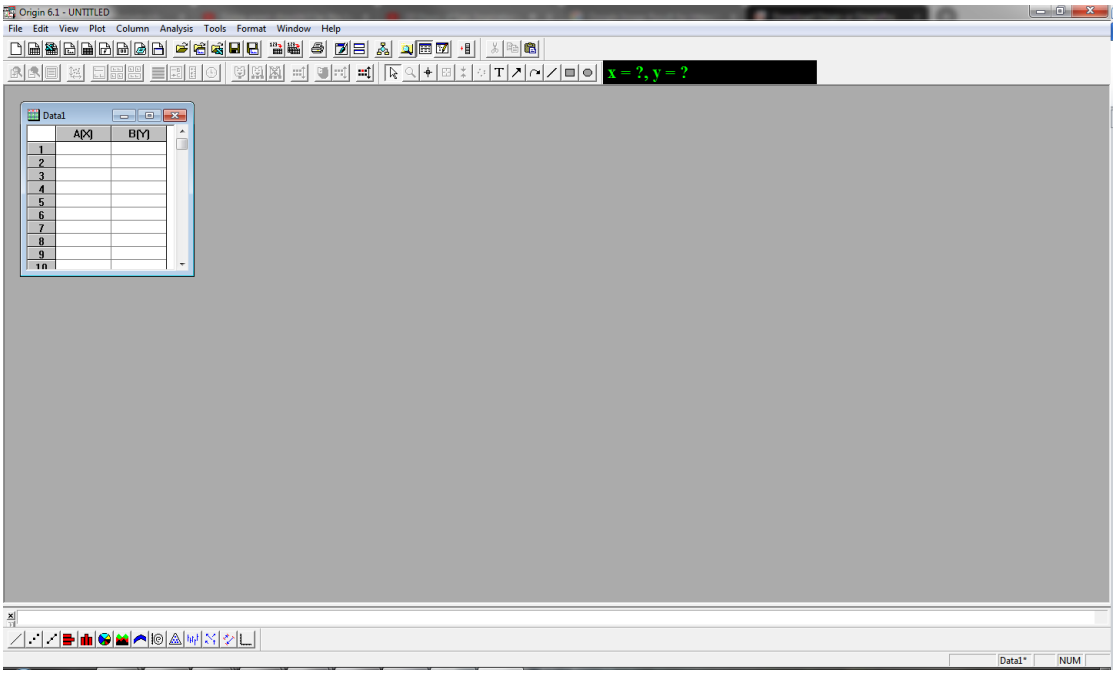
10) Find the maximum value of the whole data set.

(-40 to -20)	(-20 to 20)	(20 to 40)
-0.35607	-0.133766	-0.34524
0.227907	-0.128858	0.07197
0.204975	-0.110894	0.193149
0.058944	0.0105134	0.222303
-0.124425	0.28968	0.13188
0.36624	0.730515	0.34539
0.6621	0.98748	0.194736
0.68337	0.950175	0.22974
0.46671	0.58149	0.055062
0.41307	0.24651	0.3318
0.66981	0.1295895	0.47427
0.73791	0.175095	0.56052
0.73749	0.25428	0.40407
0.56343	0.201855	0.230475
0.41337	0.262035	0.50244
0.220338	0.174975	0.43479
0	0	0
	Max	**0.98748**

11) Normalize the values in each column with the maximum value by dividing each value by the maximum value. For LBR-C, this maximum value is 0.98748.

(-40 to -20)	(-20 to 20)	(20 to 40)
-0.360585	-0.13546	-0.34962
0.2307966	-0.13049	0.072882
0.2075738	-0.1123	0.195598
0.0596913	0.010647	0.225122
-0.126003	0.293353	0.133552
0.3708835	0.739777	0.349769
0.6704946	1	0.197205
0.6920343	0.962222	0.232653
0.4726273	0.588863	0.05576
0.4183072	0.249635	0.336007
0.6783023	0.131233	0.480283
0.7472658	0.177315	0.567627
0.7468404	0.257504	0.409193
0.5705736	0.204414	0.233397
0.418611	0.265357	0.50881
0.2231316	0.177193	0.440303
0	0	0

12) To determine the peak density at different radial distances, you will then transfer the data set into Origin.

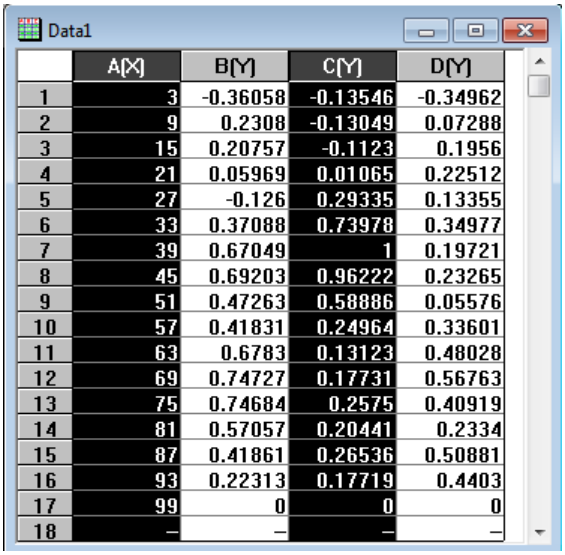


13) Copy the dataset into a new worksheet. With the radial dimension values as (X) and the density values as (Y).

Data1

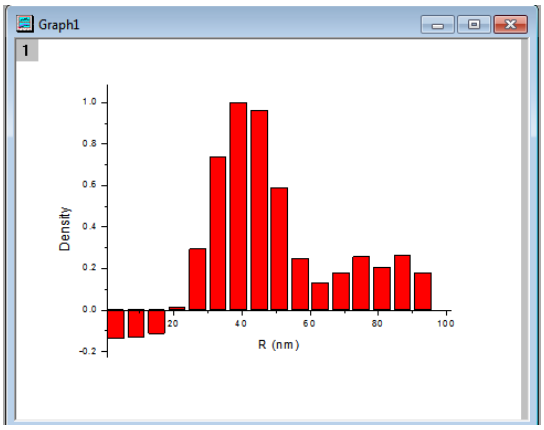
	A(X)	B(Y)	C(Y)	D(Y)
1	3	-0.36058	-0.13546	-0.34962
2	9	0.2308	-0.13049	0.07288
3	15	0.20757	-0.1123	0.1956
4	21	0.05969	0.01065	0.22512
5	27	-0.126	0.29335	0.13355
6	33	0.37088	0.73978	0.34977
7	39	0.67049	1	0.19721
8	45	0.69203	0.96222	0.23265
9	51	0.47263	0.58886	0.05576
10	57	0.41831	0.24964	0.33601
11	63	0.6783	0.13123	0.48028
12	69	0.74727	0.17731	0.56763
13	75	0.74684	0.2575	0.40919
14	81	0.57057	0.20441	0.2334
15	87	0.41861	0.26536	0.50881
16	93	0.22313	0.17719	0.4403
17	99	0	0	0
18	–	–	–	–

14) Select the column containing the radial dimension values (For this example, A(X)) and then while holding the "Ctrl" button on the keyboard select one of the density value columns (For this example, B(Y), or C(Y), or D(Y)).

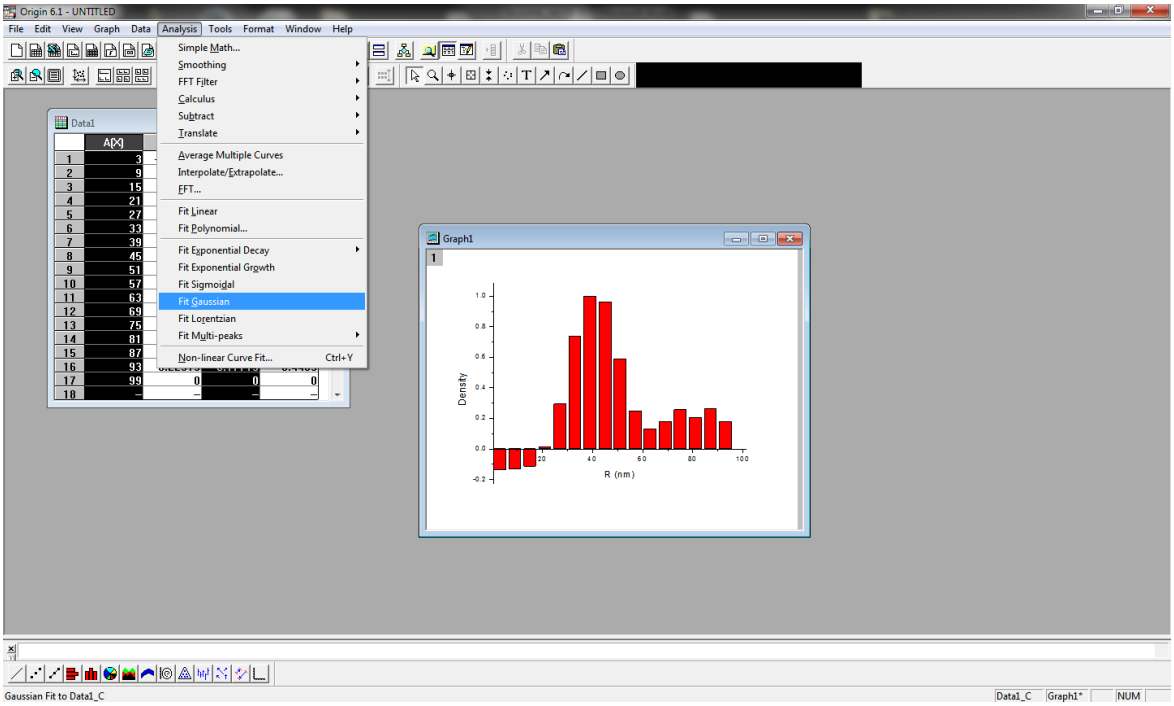


15) Then select the red vertical column graph button on the toolbar at the bottom of the Origin program.

16) After selecting the vertical graph column a graph will appear.

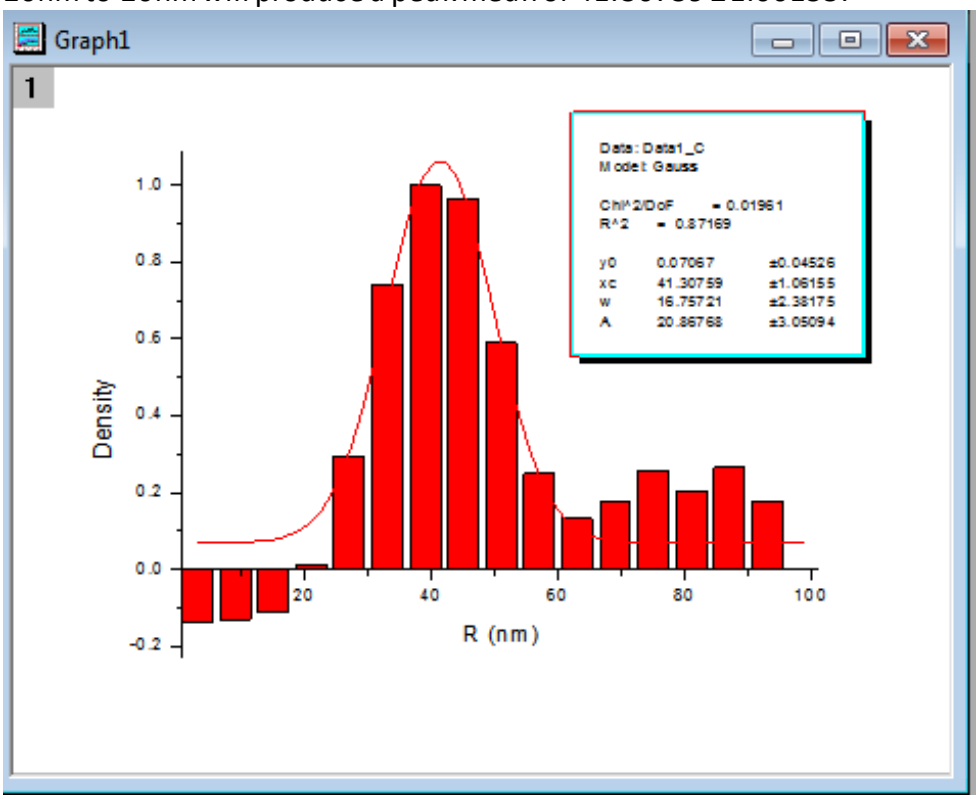


17) Select the graph, then on the toolbar Analysis>Fit Gaussian



18) Origin will fit a Gaussian to the selected graph. The xc will be the mean of the peak density distribution. This value is the transport route with largest density distribution within that axial range. The density distribution of LBR-C, within the axial range of -

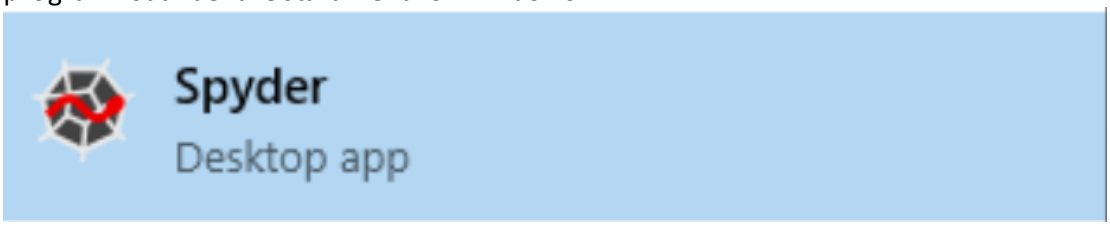
20nm to 20nm will produce a peak mean of 41.30759 ± 1.06155.



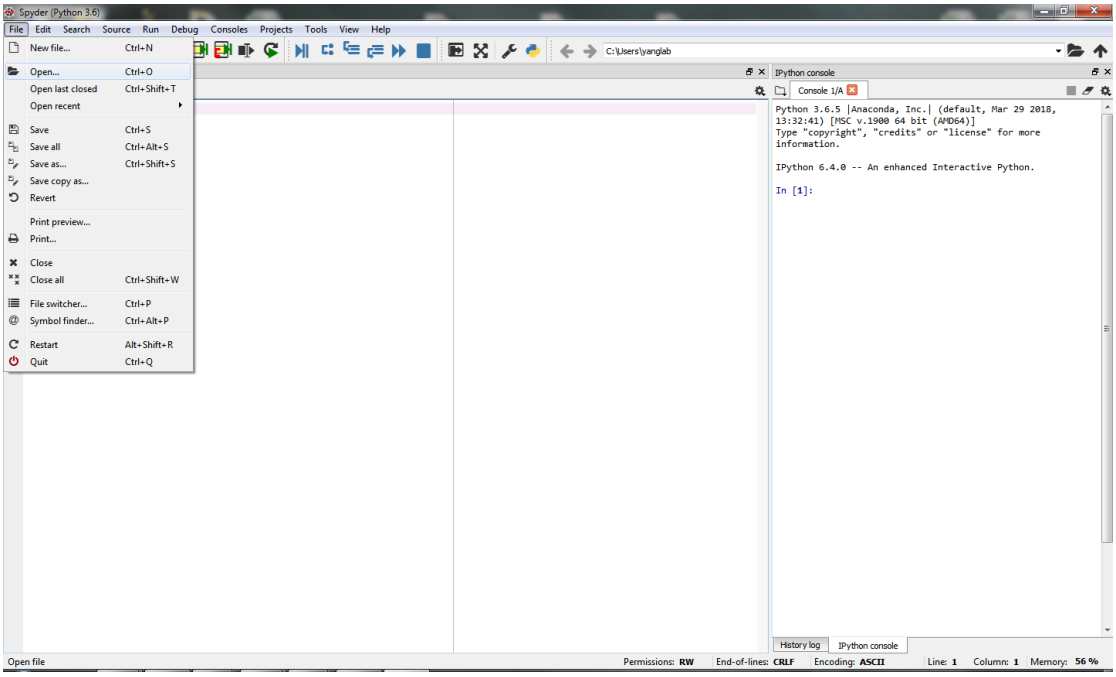
Section V

Point Number Simulation

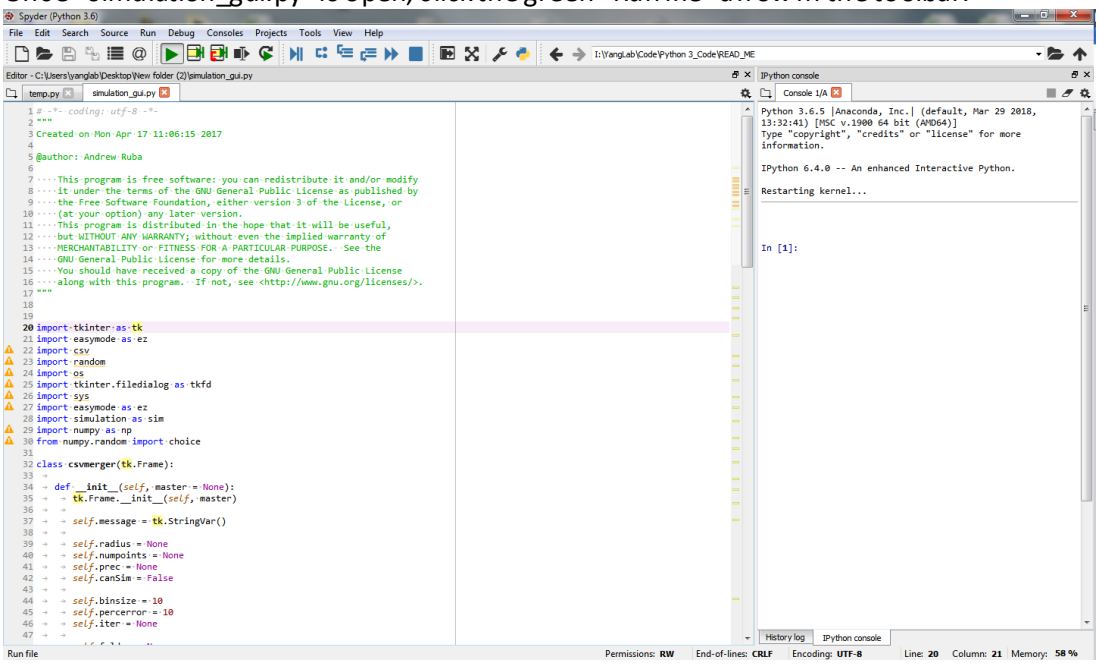
1) Similarly to section II, Open Spyder – it should have an icon on your desktop or an icon in the program list under the start menu for Windows.



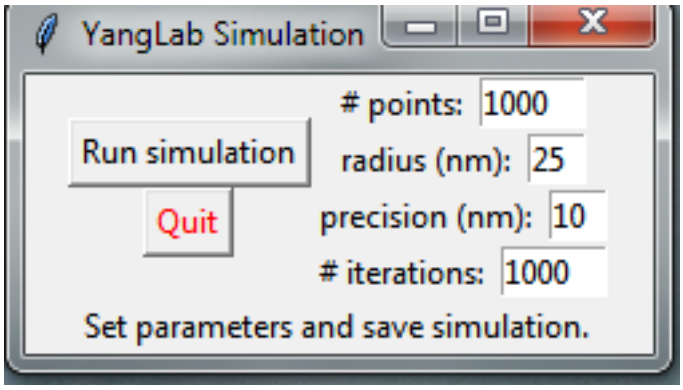
2) Once Spyder starts up, open "simulation_gui.py" using the File>Open menu.



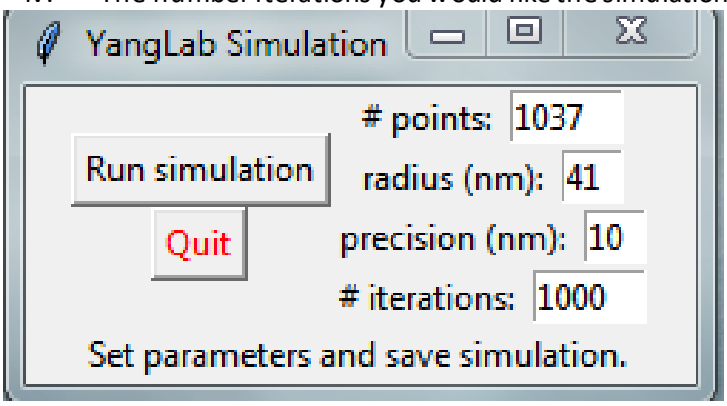
3) Once "simulation_gui.py" is open, click the green "Run file" arrow in the toolbar.



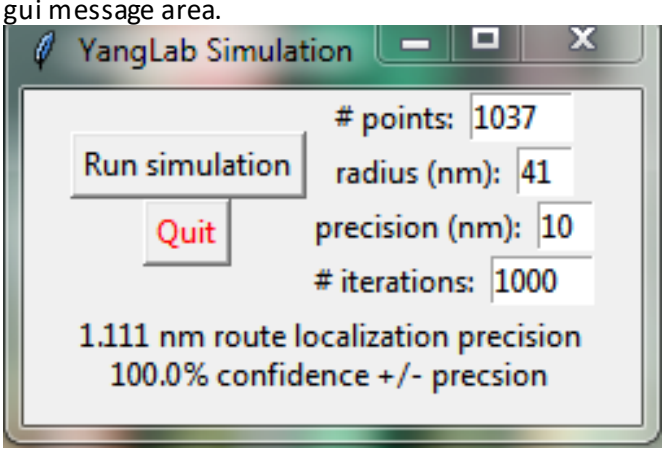
4) Once the script begins to run, a pop-up window will appear.



5) Input the following values;
   i. The number of points (***# points:***).
   ii. The peak radius found from Origin as explained in section III (***radius (nm):***).
   iii. The precision of the your single-molecule localizations (**precision (nm):**).
   iv. The number iterations you would like the simulation to run (**# iterations:**).



6) Click "Run simulation" after entering the proper integer values for the simulation parameters you would like to run. The optimal bin size will be dynamically calculated according to the parameters and the reproducibility rate and route localization precision will be written into the gui message area.



7) Select the "Quit" button to close the pop-up window.