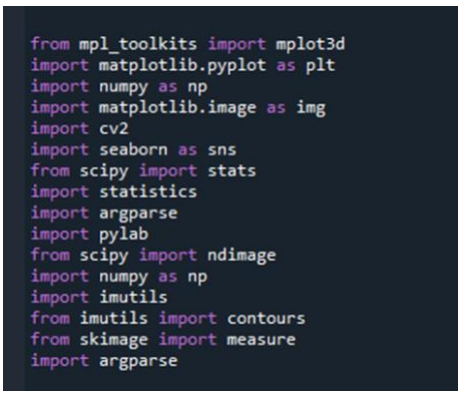
SOP AFM AutoDetect- X Y Coordinate Extraction

1. Software Requirements – Python, Anaconda IDE, and all the required libraries such as numpy, opencv, argparse as well as the libraries mentioned in the image below:



```
from mpl_toolkits import mplot3d
import matplotlib.pyplot as plt
import numpy as np
import matplotlib.image as img
import cv2
import seaborn as sns
from scipy import stats
import statistics
import argparse
import pylab
from scipy import ndimage
import numpy as np
import imutils
from imutils import contours
from skimage import measure
import argparse
```

To download these libraries, use the following commands:
Numpy→ pip install numpy
OpenCv→ pip install opencv-python
Argparse→ pip install argparse

NOTE – for any library do “pip install <library name>

2. Create a folder named “AFM_Autodetect” in Desktop and place all the python files required for xy coordinate extraction and finding height which include: slider.py, height_finder.py and brightest_point_coordinates_nm.py.

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3. Image as JPEG – After receiving the AFM (.ibw) file, remove the scale on the image and save it as a .jpg file.

*It is important to make sure the image has **no scale** as the program will count the scalebar as a particle.*

4. ASCII Text - Repeat the above step for an ASCII XYZ file of the image and save it as .txt in the AFM_Autodetect folder- this file is required for the conversion from pixels to nanometers (nm).

To acquire an ASCII XYZ file, you must open the .ibw file of the image in WSxM and save as type ASCII XYZ files (*txt).

Place the files from the steps above in the “AFM_Autodetect” folder.

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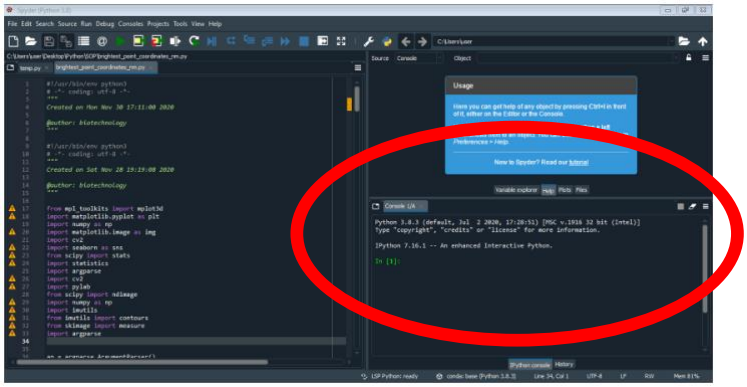
5. Spyder (anaconda 3)- In your search bar, look for Spyder and run the application. The image below displays the appearance of the application. The circled area is the terminal where you will be inserting commands.



6. Set Directory – Go to the terminal (IPythonconsole) and set your current directory as "AFM_Autodetect" folder.

This can be achieved by typing the command "cd" in front of the folder you would like to access.

For example: cd Desktop (will bring you to desktop), cd AFM_Autodetect (will set your current directory). This step must be done in order*

- After, check to see if the above step was executed properly by typing "pwd" in terminal and this command will show your current location.

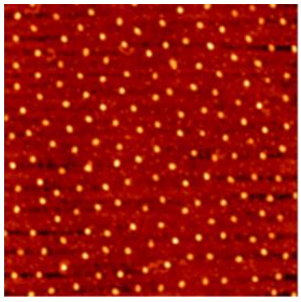
Your current location should be in "AFM_Autodetect" folder.

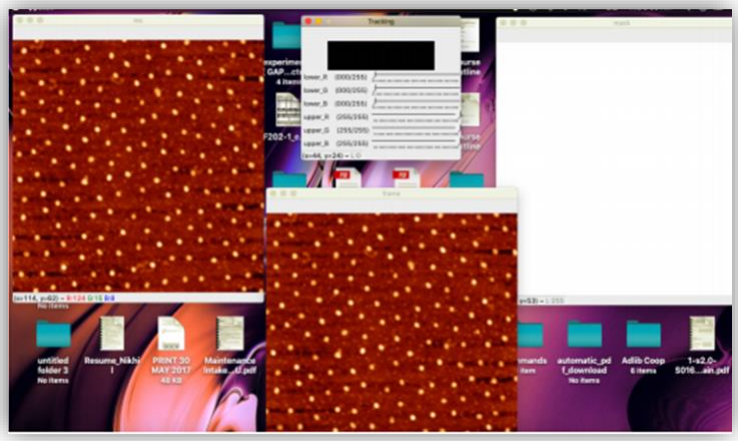
To check to see if you have the proper files in the folder, you can type the command "ls" - This will show you every file in the folder.

You should have the following files:

- slider.py
- brightest_point_coordinates_nm.py.
- JPEG file of image
- ASCII text file of image

Figure1 will be the example used in this procedure.



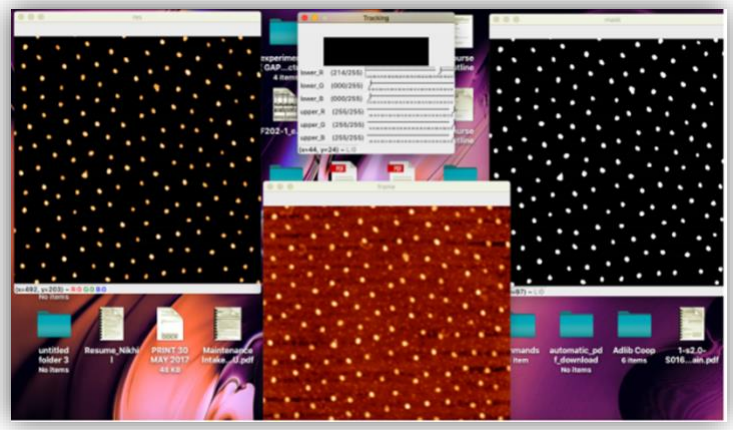


7. Creating “mask_data” and “black_white” – to create both image_md and image_bw, run the following command in the terminal:

The program should display three image windows and one contrast tracking bar. An example is shown below.

run slider.py --image figure1.jpg

This setup can be used to create “mask_data” and “black_white” images. After using tracker you will get an output as shown in the image below. *Try to separate the particles as best as possible*



Save the mask image as “mask_data.png” and res image as “black_white.png” by just simply pressing CTRL+S and saving them in the folder “AFM_Autodetect”

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8. Coordinate Extraction:

You must have the following files in the proper folder for this next step:

- brightest_point_coordinates_nm.py.
- ASCII text file of image
- black_white.png
- mask_data.png

To find the brightest points of the image, use this command:

The result will appear similar to the image below including total particles and all coordinates. You will also obtain a CSV file in the AFM_Autodetect folder named “coordinates.cvs”

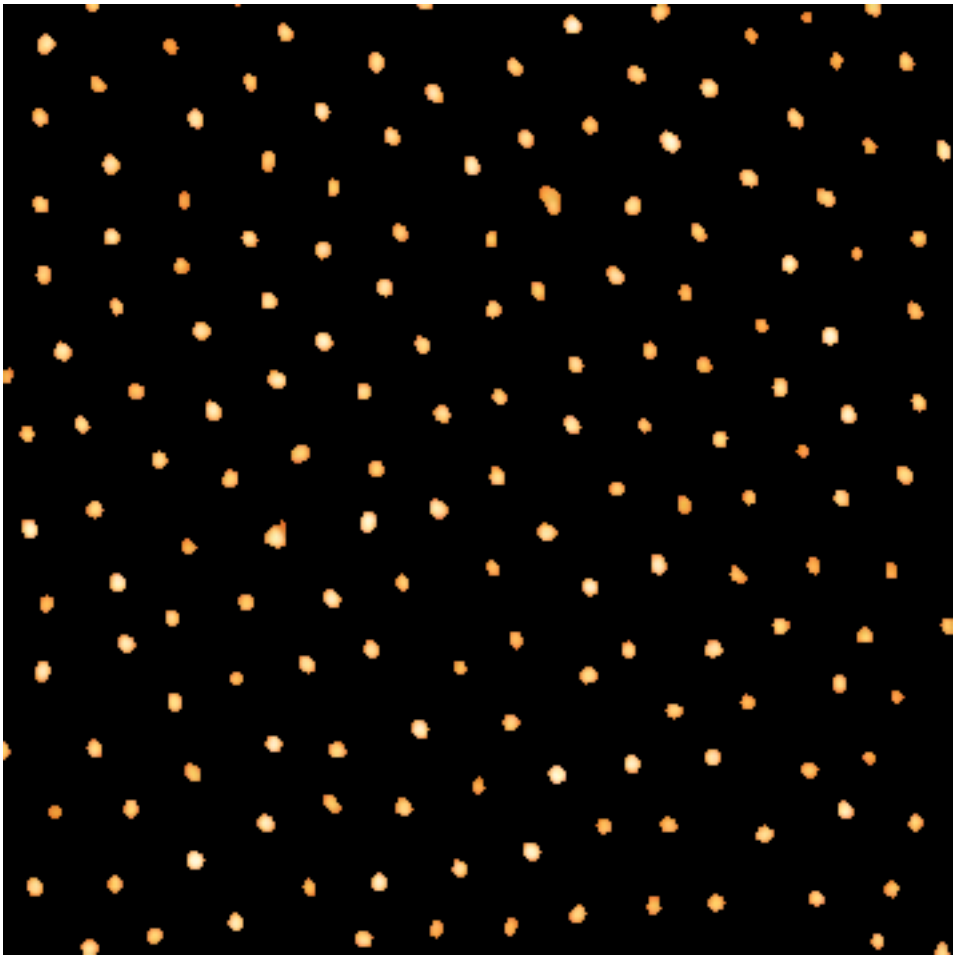
run brightest_point_coordinates_nm.py --file figure1.txt --image_bw black_white.png --image_md mask_data.png

When executing the program for multiple images, to avoid confusion, you may want to isolate them into separate folders within the AFM_Autodetect and set the directory further to that specific folder. All steps mentioned above must take place in that folder. Be sure to include all necessary files.

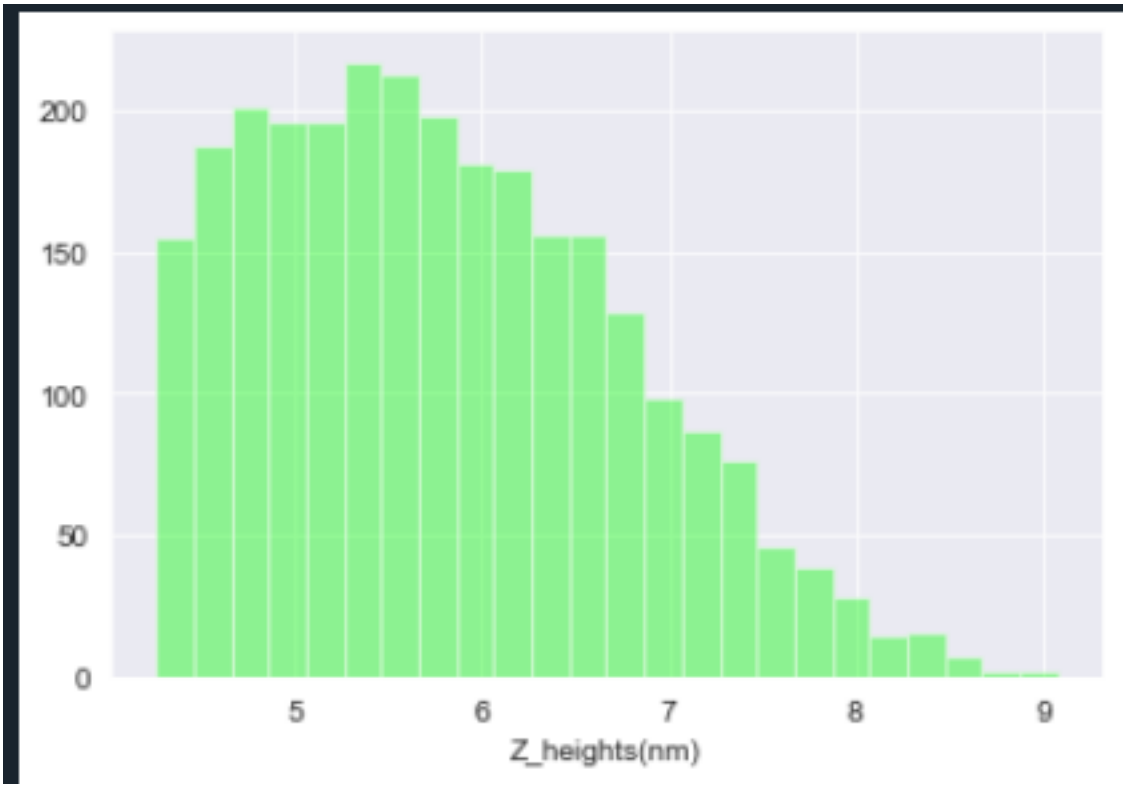
For height_finder.py use

1. **use command (run height_finder.py --file <ascii text file> --md_image <masked data image>**

NOTE - "< masked data image > " this is an image which looks like the image under



You result will be a histogram and two text file ("wanted_heights.txt" # these are the height with background and "wanted_heights_corrected.txt", 'w' # these are the corrected heights, without background)



Please refer the article attached for the full description of application