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Surface Roughness Analysis for Atomic Force Microscope using Python

CME 502 Final Project Presentation

5/4/2023

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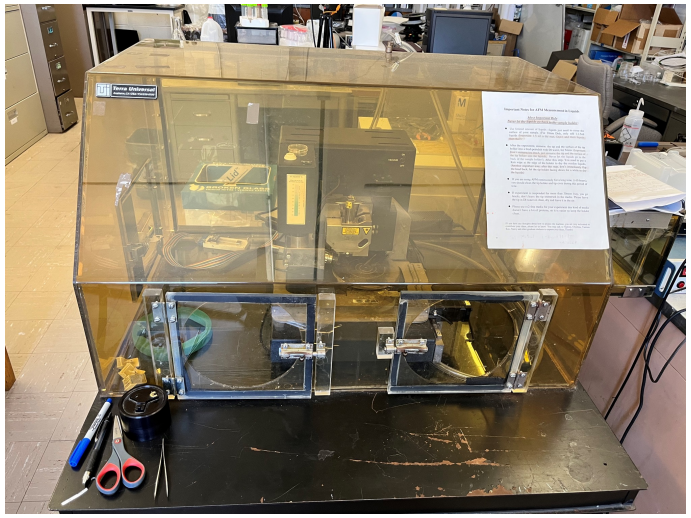
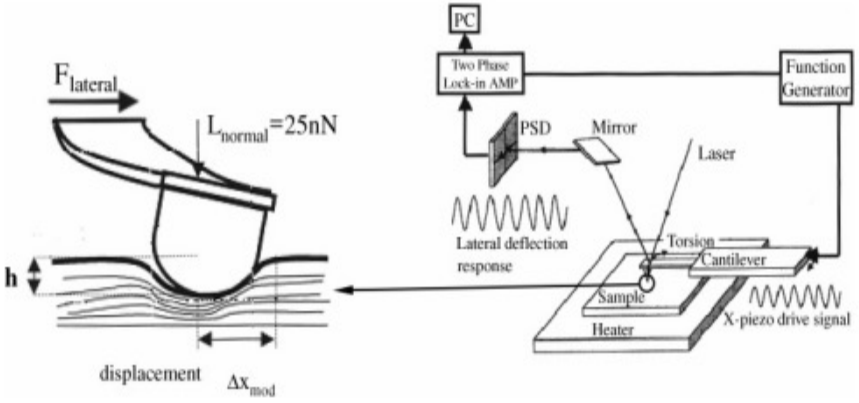
Introduction

- Atomic force microscopy (AFM)
  - AFM is a widely used surface analysis technique for investigating nanoscale structure of materials.
  - Our lab has utilized a Bruker AFM for over 40 years and has been used in numerous research projects.
    - Explore the growth of cells on polymers scaffold
    - Examine the effects of polymer additives on perovskite grain boundary theory
    - Investigate the interaction between polymer thin films at the interface
- Surface roughness is an important parameter in characterizing the texture and visual qualities of a material's surface





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Problem

- Bruker's NanoScope Analysis is a powerful software for processing AFM data
  - But unfortunately, it was only installed on certain outdated computers in out lab
  - The software has been discontinued and is no longer available for download
  - Does not support MAC OS

System Requirements

Required
2GHz CPU, 1GB RAM
50GB Hard Drive
Windows XP, Vista, Windows 7
Minimum 800x600 monitor

Recommended
3GHz CPU, 2GB RAM
50GB Hard Drive
Windows XP, Vista, Windows 7
Minimum 1280x1024 monitor

You can download a copy of NanoScope Analysis from ftp://Release:Z3Analysis91@sboftp.bruker-nano.com. Once the software is installed, hit the F1 key or click on the blue button with the question mark on the toolbar for the online manual. A list of major new features is available under the "Help" menu – click on "What's New…"

No longer available for download

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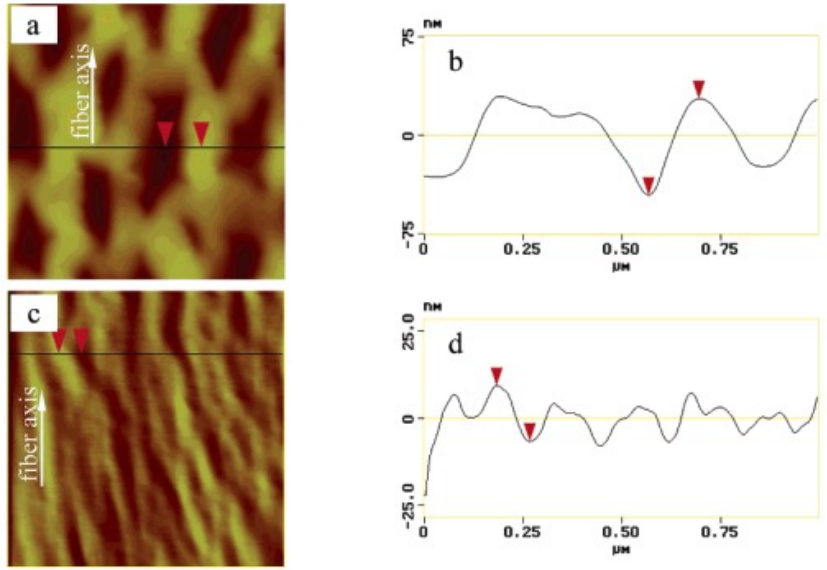
Propose solution

- The goal of this project is to develop an AFM image analysis program that can calculate the surface roughness average, Ra, using python
  - Ra is the arithmetic average of the absolute values of the profile heights over the evaluation length
  - Two modes: line Ra & area Ra
- AFM output data is a topography picture → extract the depth values from the picture
  - Calculate roughness average, Ra using eqn 1.

$$R_a = \frac{1}{L}\int_0^L |Z(x)|dx \text{ (eqn 1.)}$$

Where Z(x) is the function that describes the surface profile analyzed in terms of height (Z) and position (x) of the sample over the evaluating length "L"



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Overall workflow

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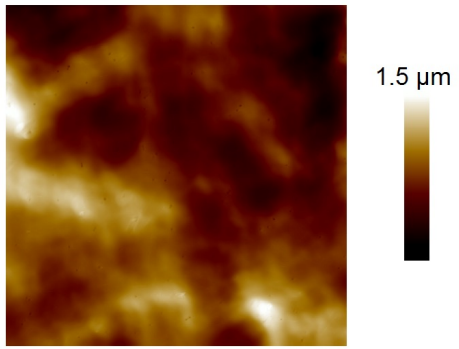
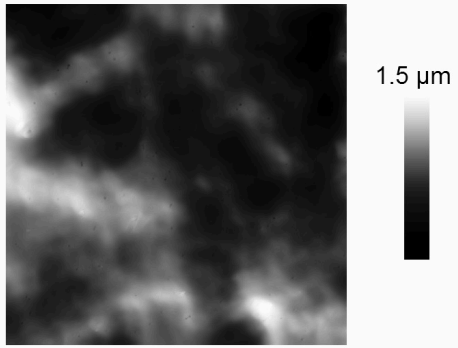
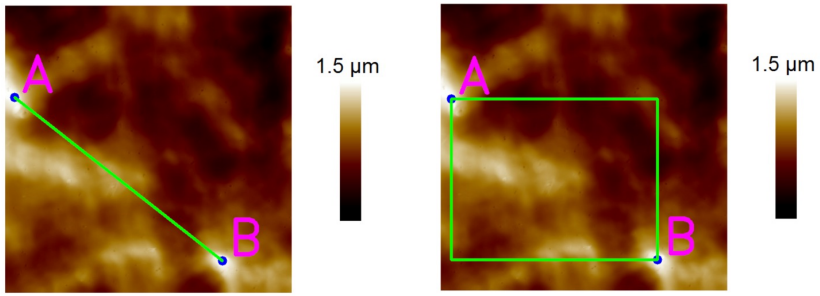
User input



Read image & Convert to grayscale

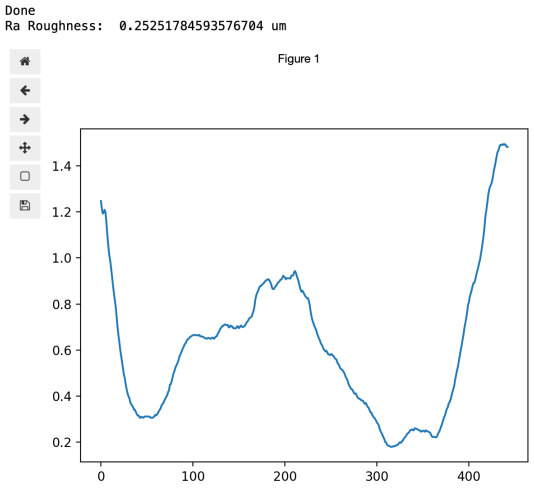


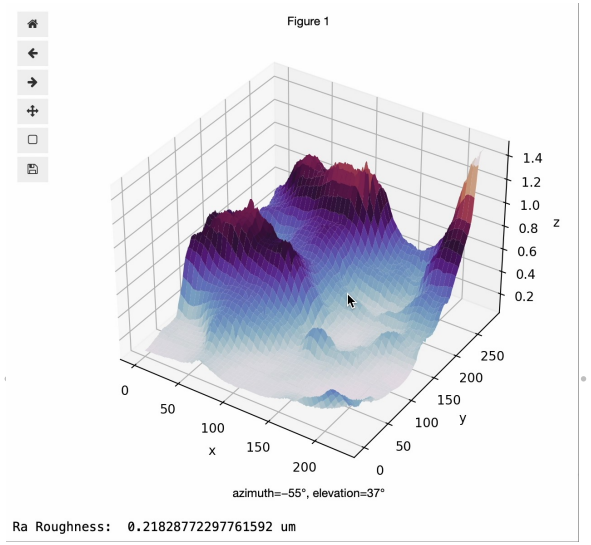
Select region of interested



Press the "return" key on keyboard

Output the Ra value and a height profile





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Method – Python modules

```
import cv2 # module import name for opencv
import numpy as np
from scipy import interpolate
import matplotlib.pyplot as plt
# generates an ipywidget that renders plots in a control
%matplotlib widget
from mpl_toolkits.mplot3d import Axes3D # 3D graphing
```

- cv2 → opencv
  - cv2.rmread → read an image
- Numpy → mathematical operations on arrays
- Interpolate → linear interpolation
- Matplotlib → 2d plotting for heights profile
- Axes3D → 3d graphing for heights profile

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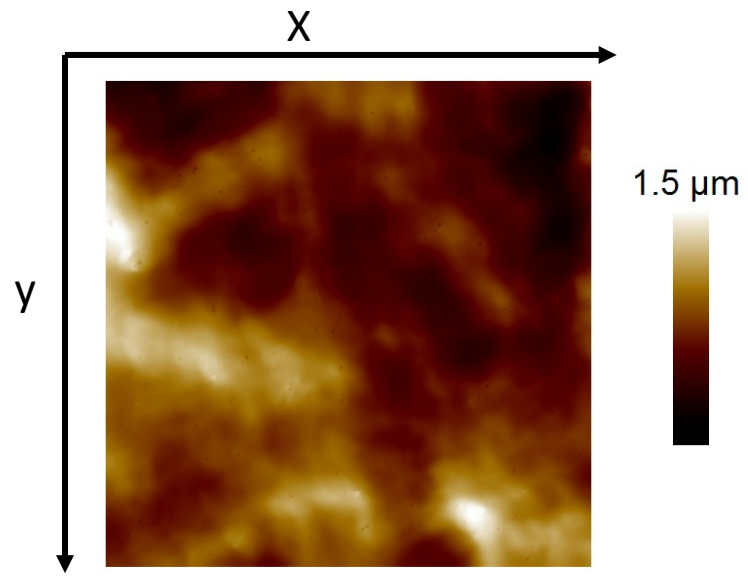
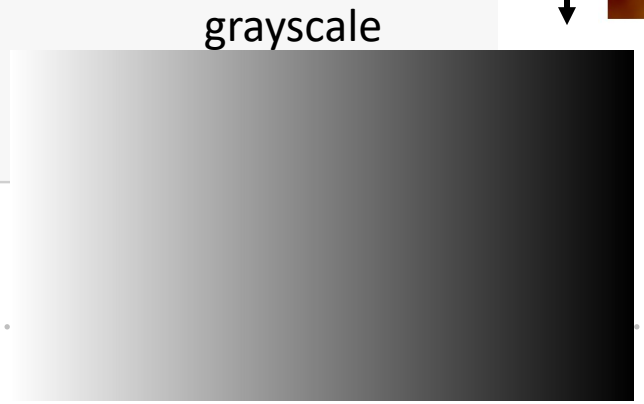
Method – User inputs & image read

```
########## INPUT ##########

imageName = 'pla3d0325.002_1'+'.png'

high = 1.5 # unit micron
low = 0  # unit micron

mode = 'line' #line or area


################################################
# image reading
img = cv2.imread('/Users/huitingluo/CME502_Final_Project/AFM images/'+imageName)

# convert to GrayScale image
imgGray = cv2.cvtColor(img, cv2.COLOR_BGR2GRAY)

# ratio of scale bar and the range of grayscale values
ratio = (high-low)/(255-0)
```





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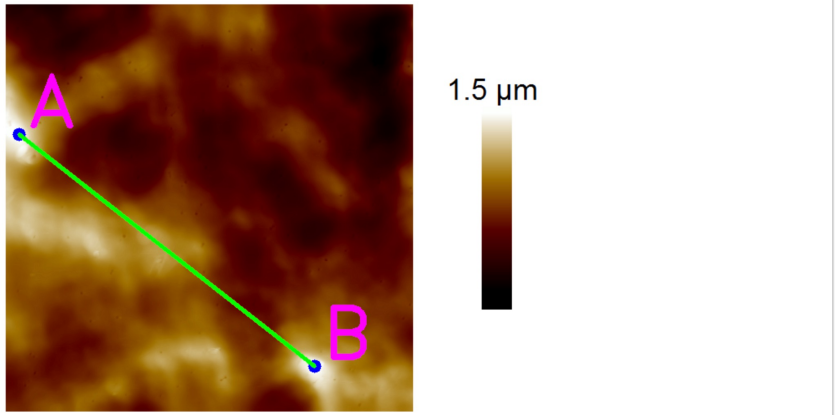
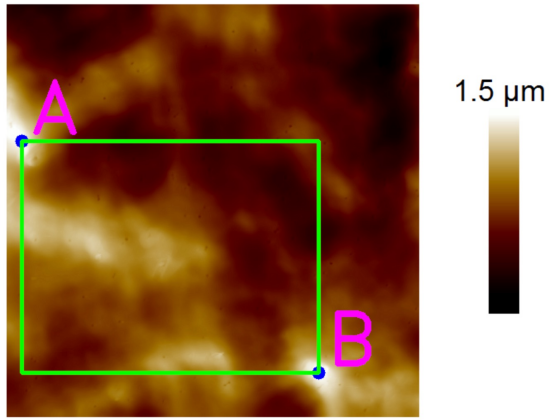
Method – points selection






```python
# Function: Pick Starting & ending points
def Pick_two_points(image, *args):
    global counter
    counter = 0
    # Create point matrix get coordinates of mouse click on image
    point_matrix = np.zeros((2,2),int)
    clone = image.copy()
    img = image.copy()
    def mousePoints(event,x,y,flags,params):
        global counter
        # Left button click
        if event == cv2.EVENT_LBUTTONDOWN: # indicates that the left mouse button is down
            if counter <2:
                point_matrix[counter] = x,y
                counter = counter + 1

        for x in range (0,2):
            #Draw circle on image with mouse click
            cv2.circle(img,(point_matrix[x][0],point_matrix[x][1]),8,(255,0,0),cv2.FILLED)
            cv2.putText(img,
                        'A' if x==0 else 'B',
                        (point_matrix[x][0]+10,point_matrix[x][1]-10),
                        cv2.FONT_HERSHEY_DUPLEX,
                        3.0,
                        (255,0,255),
                        3
                       )
            if counter == 2:
                # assign starting & ending points
                starting_x = point_matrix[0][0]
                starting_y = point_matrix[0][1]

                ending_x = point_matrix[1][0]
                ending_y = point_matrix[1][1]


                if 'line' in args:
                    # Draw line between starting & ending points
                    ImgWithMarker=cv2.line(img, (starting_x, starting_y), (ending_x, ending_y), (0, 255, 0), 3)
                if 'area' in args:
                # Draw rectangle for area of interest
                    ImgWithMarker=cv2.rectangle(img, (starting_x, starting_y), (ending_x, ending_y), (0, 255, 0), 3)
```

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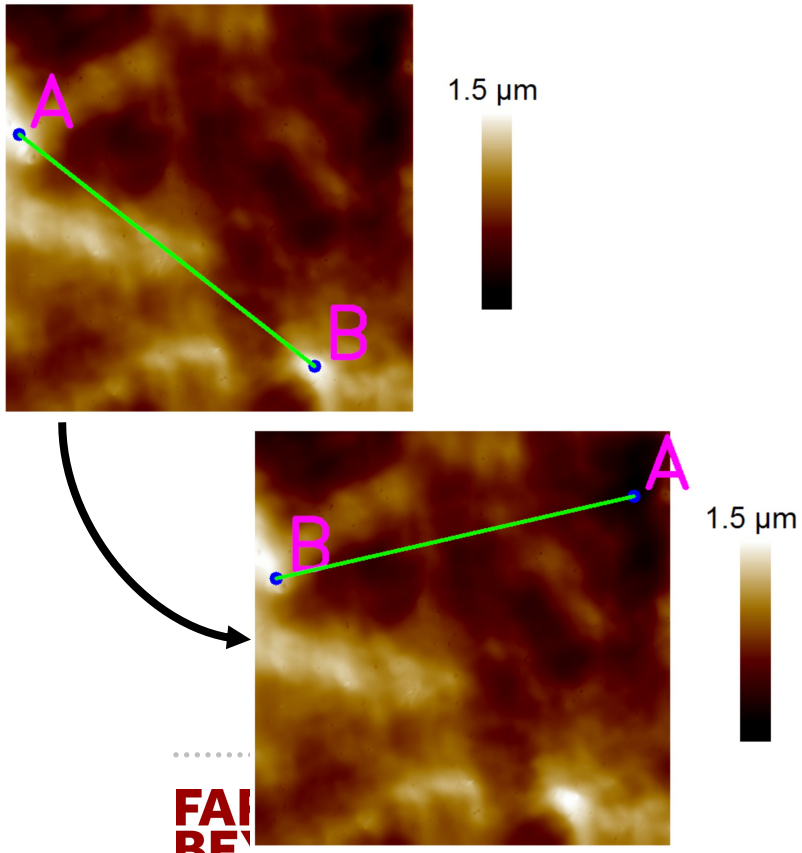
Method – points selection




```
  # keep the image window active
    while True:
        cv2.namedWindow('AFM', cv2.WINDOW_NORMAL)
        # Mouse click event on original image
#        cv2.resizeWindow('AFM', image.shape[1], image.shape[0])
        cv2.setMouseCallback('AFM', mousePoints)
        cv2.imshow('AFM', img)
        key = cv2.waitKey(1) & 0xFF # maintain output window until user presses a key

         # if the 'r' key is pressed, <<<reset>>>> the region
        if key == ord("r") and counter ==2:
            counter = 0
            point_matrix = np.zeros((2,2),int)
            img = clone.copy()
        # if the 'enter' key is pressed, break from the loop
        elif key == ord("\r"):
            break

    print('Done')
#     cv2.destroyAllWindows()
#     cv2.waitKey(1) #???? should distroy  or not
                                          #confirm selection
    return point_matrix

#################################################################
```

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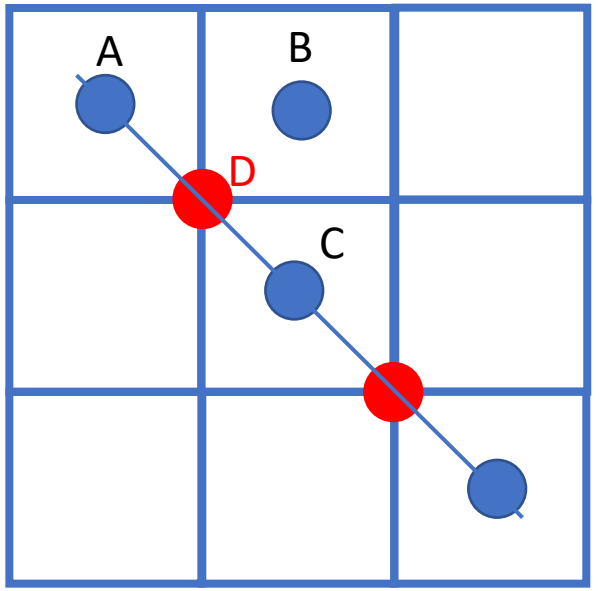
Method – calculate line Ra

```
StartEndPoints  = Pick_two_points(img, mode)

start_x = StartEndPoints[0][0]
start_y = StartEndPoints[0][1]
end_x = StartEndPoints[1][0]
end_y = StartEndPoints[1][1]

d_x = abs(end_x - start_x) # d_x : number of pixels at x axis
d_y = abs(end_y - start_y) # d_y : number of pixels at y axis

L = int(np.sqrt(d_x**2+d_y**2)+1) # L: Number of pixels in the line

z_p = imgGray[min(start_y,end_y):max(start_y,end_y), min(start_x,end_x):max(start_x,end_x)]
z = high-ratio*(255-z_p)
# z = imgGray[min(start_y,end_y):max(start_y,end_y), min(start_x,end_x):max(start_x,end_x)] # start & end
#z = zg*100/255+50

if mode == 'line': # line interpolation

    f = interpolate.interp2d(np.arange(z.shape[1]), np.arange(z.shape[0]),  z, kind='linear')
    #f = interpolate.interp2d(x, y, z, kind='linear')

    Xnew = np.linspace(0,z.shape[1],L)
    Ynew = np.linspace(0,z.shape[0],L)
    Znew = f(Xnew,Ynew)
    if (start_x-end_x)*(start_y-end_y)<0:
        Z_dial = np.fliplr(Znew).diagonal()
        if start_y>end_y:
            Z_dial = Z_dial[::-1]
    else:
        Z_dial = np.diagonal(Znew)
    if start_x > end_x and start_y>end_y:
        Z_dial = Z_dial[::-1]
    plt.plot(Z_dial)

    Ra = np.sum(np.abs(Z_dial-np.mean(Z_dial)))/Z_dial.size
```

Linear interpolation



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Method – calculate area Ra

```
elif mode == 'area':
    x = np.arange(z.shape[1])
    y = np.arange(z.shape[0])
    X,Y = np.meshgrid(x,y)
    fig = plt.figure()
    ax = Axes3D(fig)
    ax.plot_surface(X, Y, z, cmap=plt.get_cmap('twilight'))
    ax.set_title("3d")
    ax.set_xlabel("x")
    ax.set_ylabel("y")
    ax.set_zlabel("z")
    #plt.savefig('3D.png') # save fig in the Main Folder
    plt.show()


    Ra = np.sum(np.abs(z-np.mean(z)))/z.size

print('Ra Roughness: ', Ra, 'um')
```

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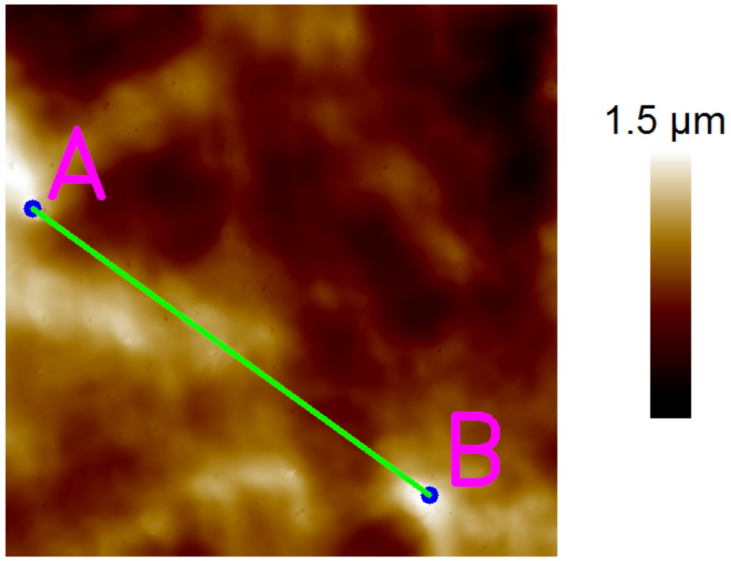
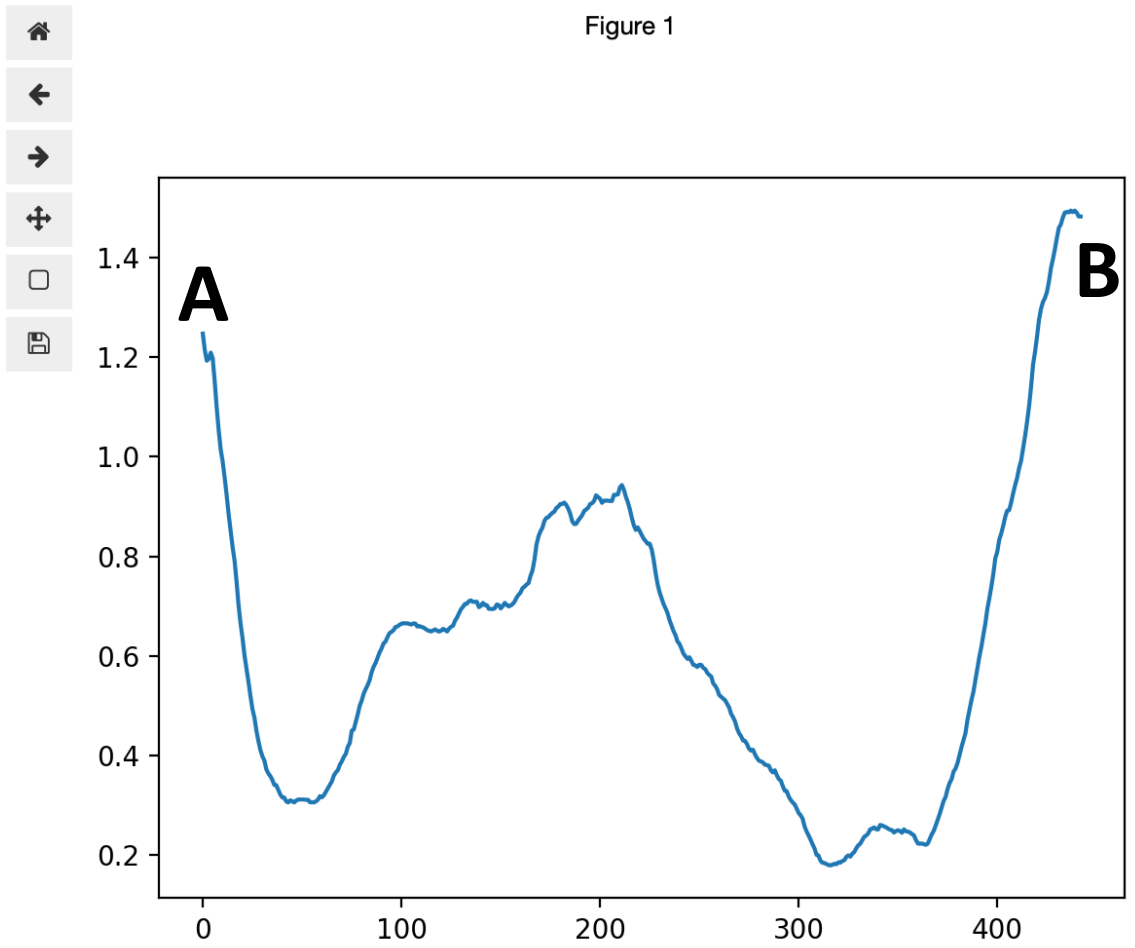
Result – line Ra




```
Done
Ra Roughness:  0.25251784593576704 um
```



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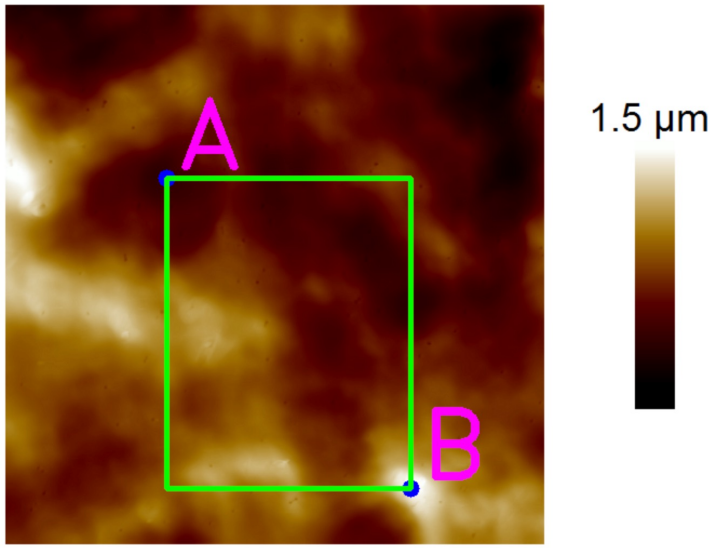
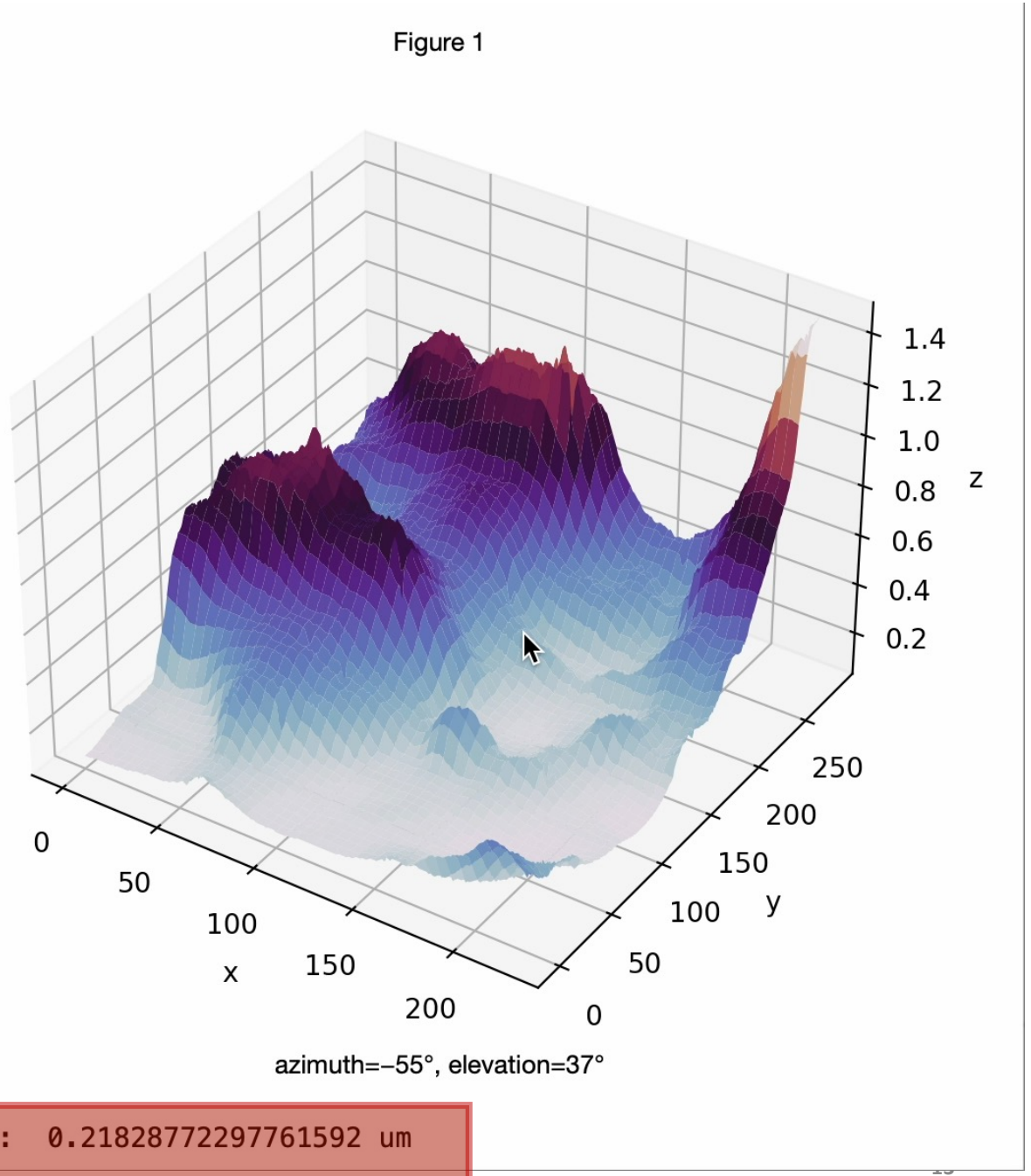
Result – area Ra





Ra Roughness: 0.21828772297761592 um

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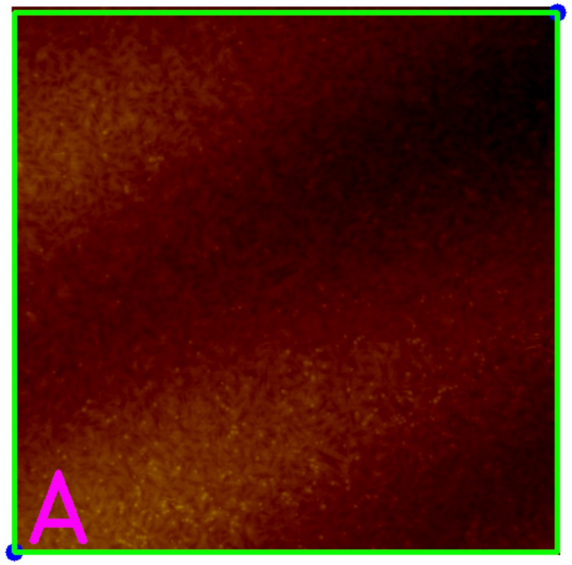
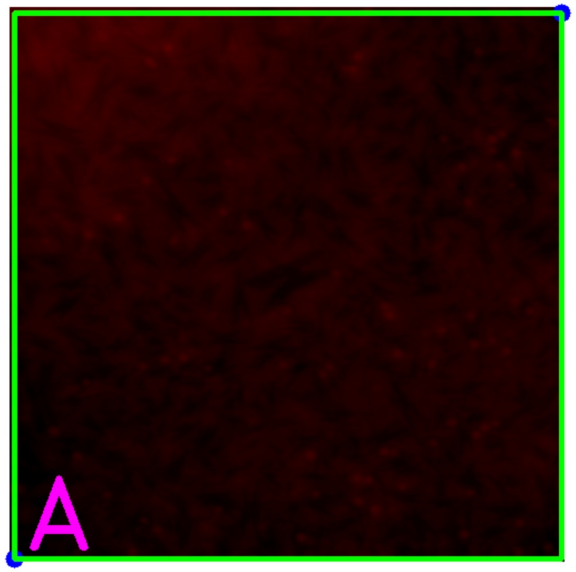
Research: effect of coated substrate for cell growth – spincast coated PLA & 3d printed PLA

Spincast sample 1



Ra = 0.0496 um

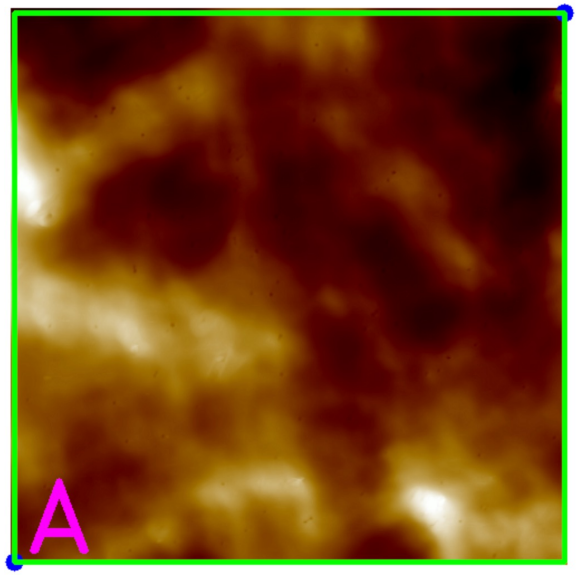
Spincast sample 2



Ra = 0.0106 um

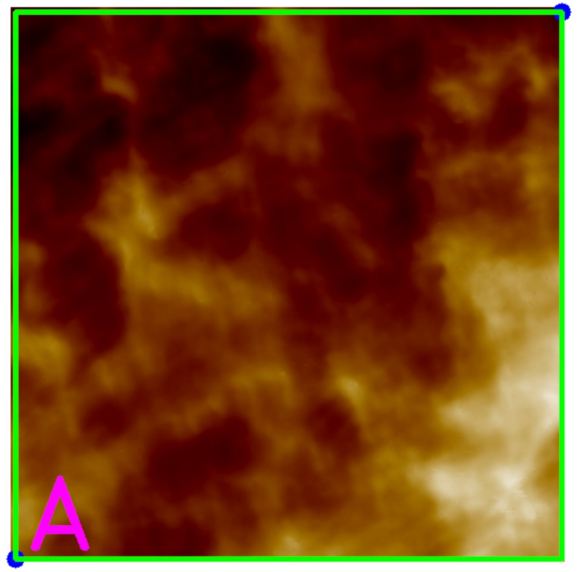
Flat

3d printed sample 1



Ra = 0.2276 um

3d printed sample 2



Ra = 0.4109 um

Rough

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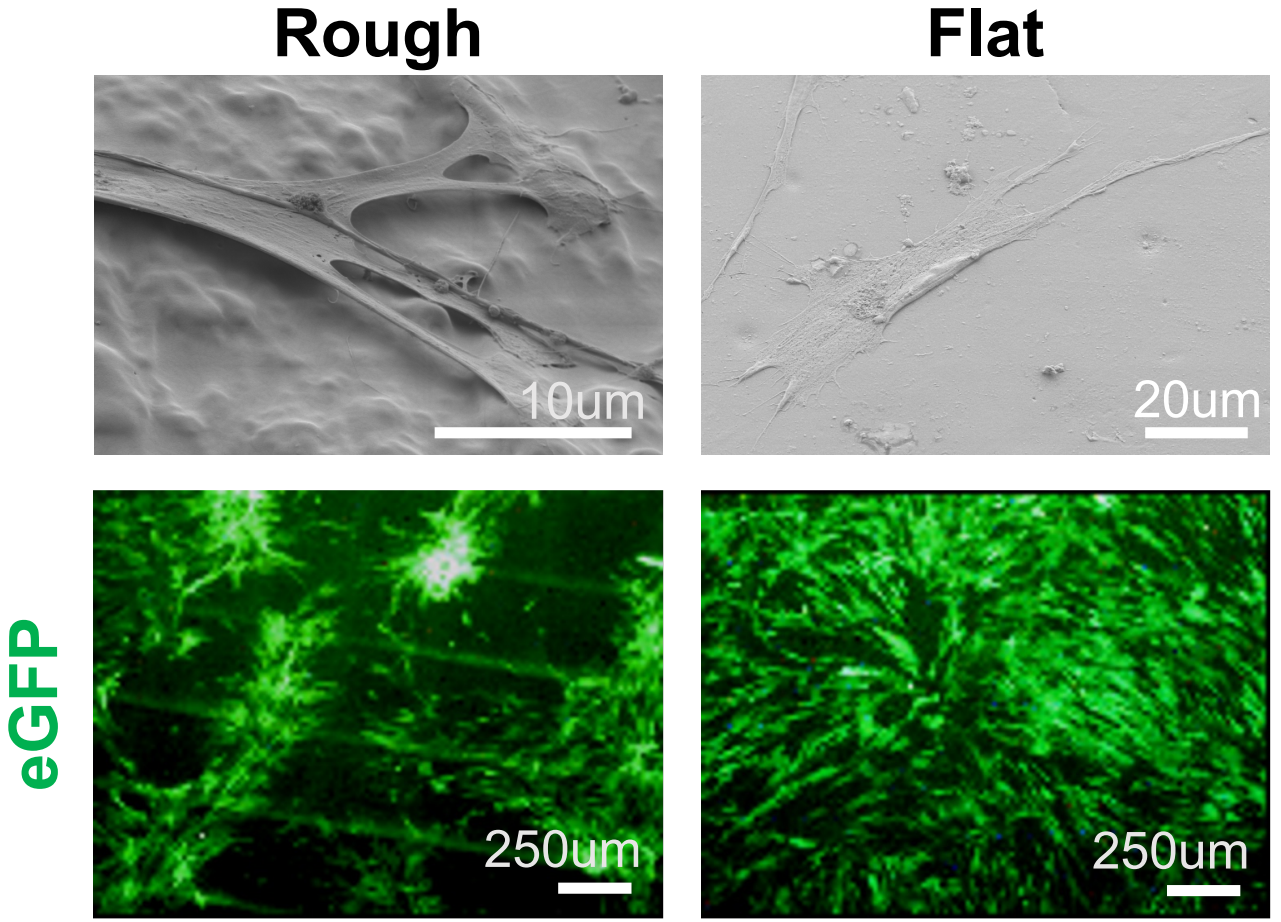
Research: effect of coated substrate for cell growth – spincast coated PLA & 3d printed PLA



Cells grow better in a flat surface

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Conclusion

- The program of surface roughness analysis for AFM is developed
- Some advantage of the program
  - Beneficial for all group members in our lab
  - Both window and Mac users friendly
  - Free for everyone
  - Analyze the surface roughness of any region of interested
    - In nanoScope, image cropping is needed to get the area Ra of an interested area, and the cropping can only be square. Only horizontal line Ra is avaliable