

# Tutorial



**Ryan Fuierer, PhD**  
Asylum Research Oxford  
Instruments

[ryan.fuierer@oxinst.com](mailto:ryan.fuierer@oxinst.com)

## Introduction to AFM Image Processing for Asylum Research AFMs

## *Because we want to make a measurement:*

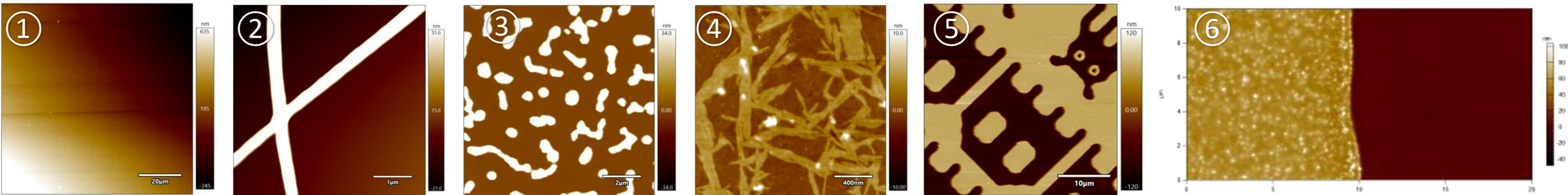
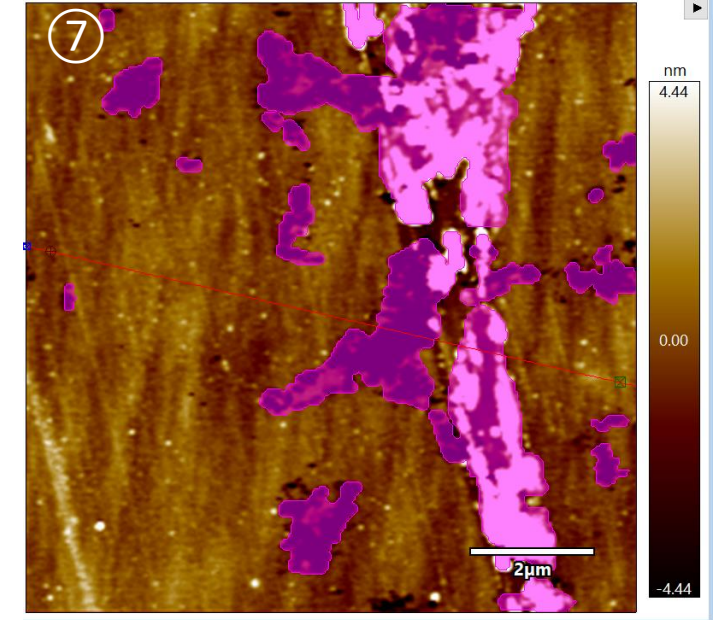
- Image analysis for three types of measurements
  - Critical dimension in XY
  - Step height
  - Roughness
- Image processing
  - It's a necessary evil
  - Principle of least damage
  - Alternatives to processing
  - Polynomial subtraction
    - Planefit
    - Flatten

# Outline

- Opening files
- Introduction to Display Window
- Processing Considerations
- The **Analysis Panel** (Line sections, Roughness, Histograms)
- The **Modify Panel** (Flatten, PlaneFit, Mask, Erase)
- Argyle **3D/4D rendering**

## Examples:

- 1) Tilted Plane (Planefit, Flatten, line section, Roughness)
- 2) Al<sub>2</sub>O<sub>3</sub> Fibers (Masking, Flattening, Line section, Roughness,)
- 3) Molla's Sample (Masking, Flattening)
- 4) MoS<sub>2</sub> (iterative masking, Histogram)
- 5) Calibration Grating (magic Mask Pits)
- 6) Polymer Step edge (Planefitting, Line section)
- 7) RF Challenge Sample (Masking Challenge)



# Available Resources

## Manuals

<https://oxinst.sharepoint.com/sites/OIARServices>

Use Help Menus to Right of parameter setvars/ buttons

Igor Getting Started Tutorial (Help → Getting Started)

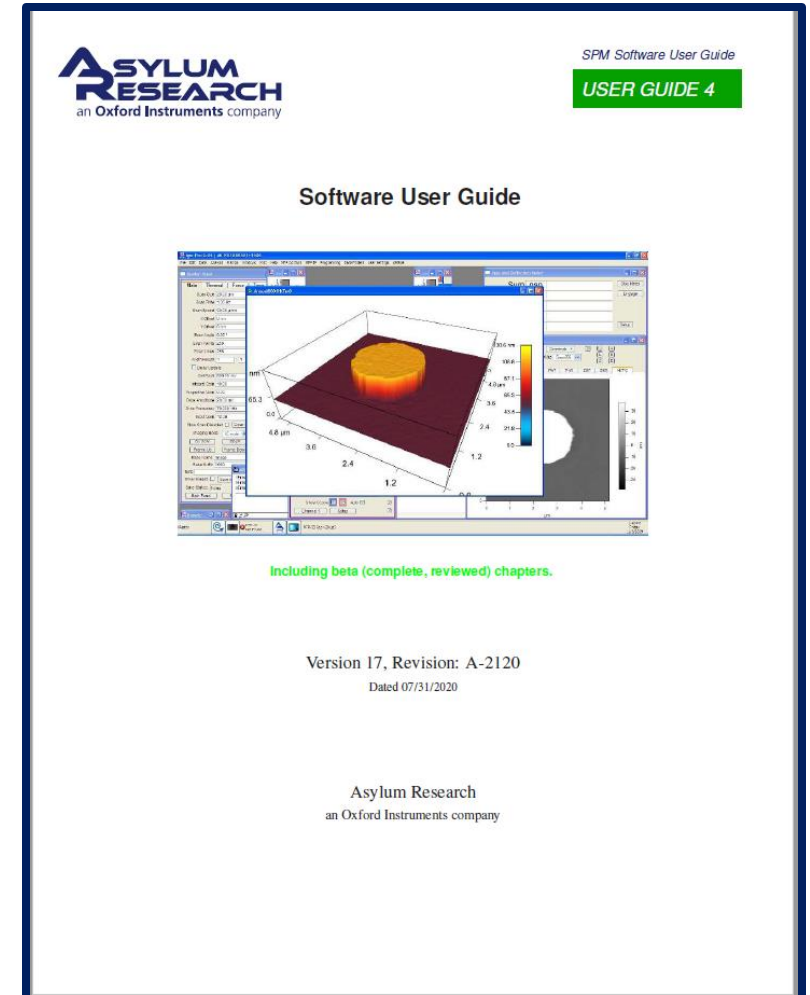
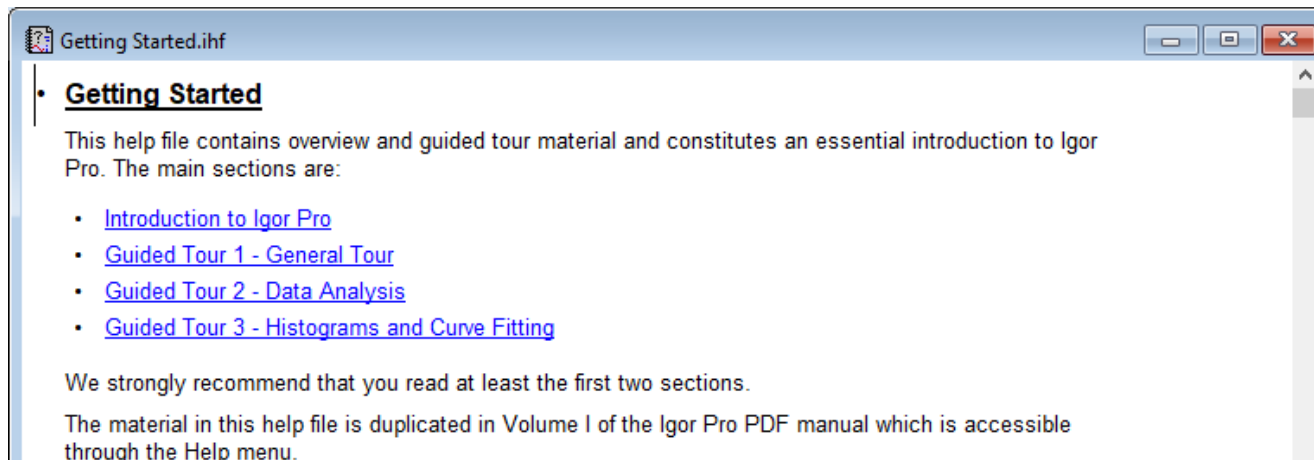
Ctrl A- return to full view

Ctrl I – brings up cursors

Ctrl T- brings up tool box

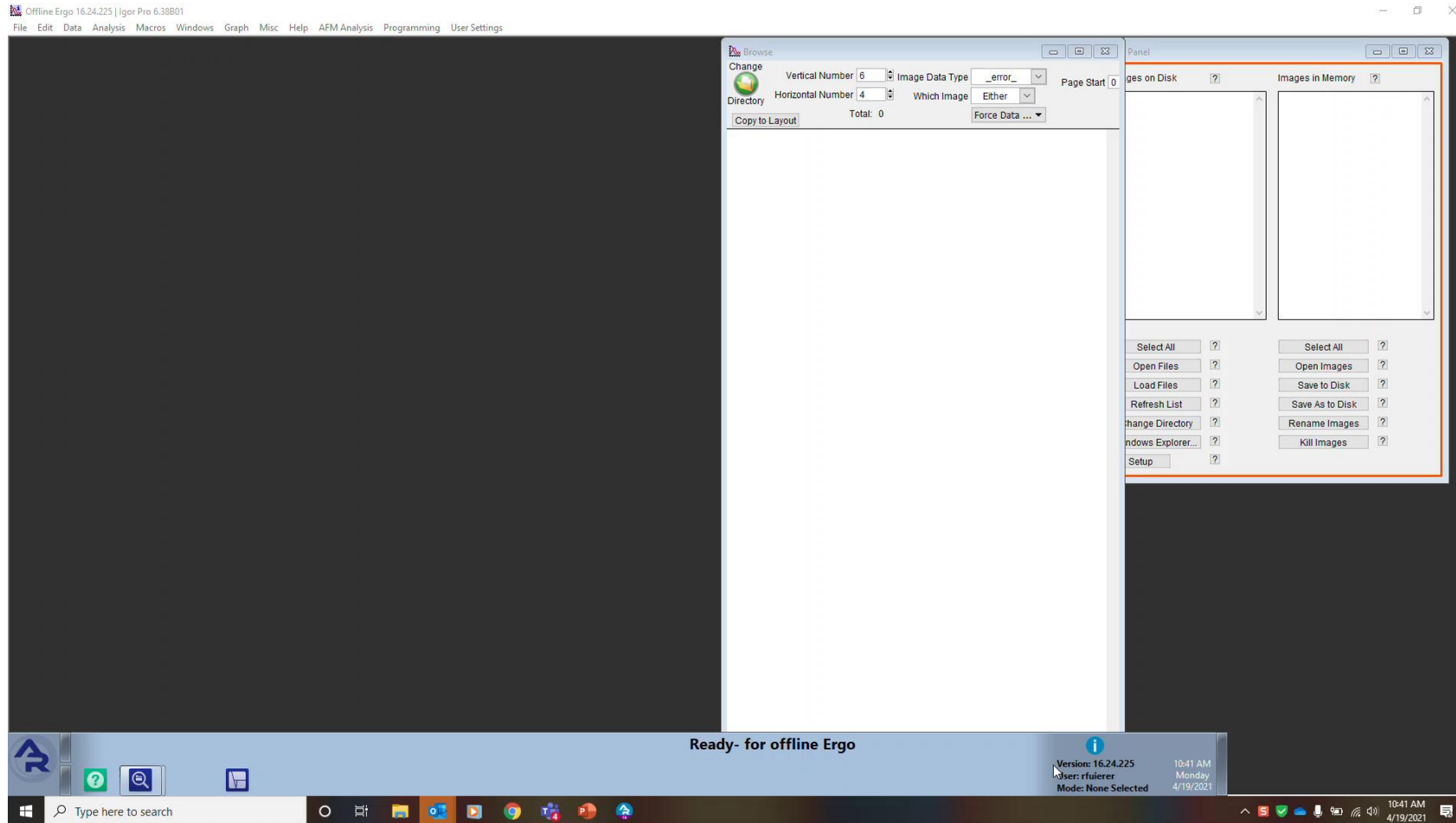
Argyle Lite: Stand alone 3D&4D viewer

<https://support.asylumresearch.com/filedata/fetch?id=19894>



# Opening a File:

AFM Analysis → Browse Saved Data (Brings UP Browse Panel)  
Brings up **Browse & List** Panels



Play Movie

# The Display Window Anatomy

**Auto Z Range**

**Z Range**

**Offset in Z Range**

**Image Layer Scroll**

**Commonly Used Functions/Panels**

**AFM Channels**

**Z Color Tables**

**Commands**

- D Display Panel
- M Modify Panel
- A Analyze Panel
- L List Panel
- Ex Extract Layer
- In Insert Layer
- Delete Layer
- N Show Note
- Image Name -- Show
- Change User Names
- S Save Image
- Save As ...
- Save Then Kill Image
- Kill Image
- Tf Data TIFF
- TIFF Export 1x
- TIFF Export 2x
- TIFF export 4x
- ASCII Export
- Ly Export to Layout
- Set Layout Note Fields
- Nanolindenter Tip Area Func
- Crop Image
- Help
- Classic Appearance

**Params**

N Ex In Tf S

ScanRate:  
0.10 Hz

PointsLines:  
512 x 512

ImagingMode:  
AC Mode

ImageNote:

Date:  
Jul 6, 2020  
1:31 PM

XOffset:  
0 nm

Yoffset:  
0 nm

Select Params

Range 880.00 nm

Offset 195.00 nm

Color Bar

Commands

3D Ly A L M D Fr

Auto ColorMap Mud

HtR AmR PhR ZSR

nm

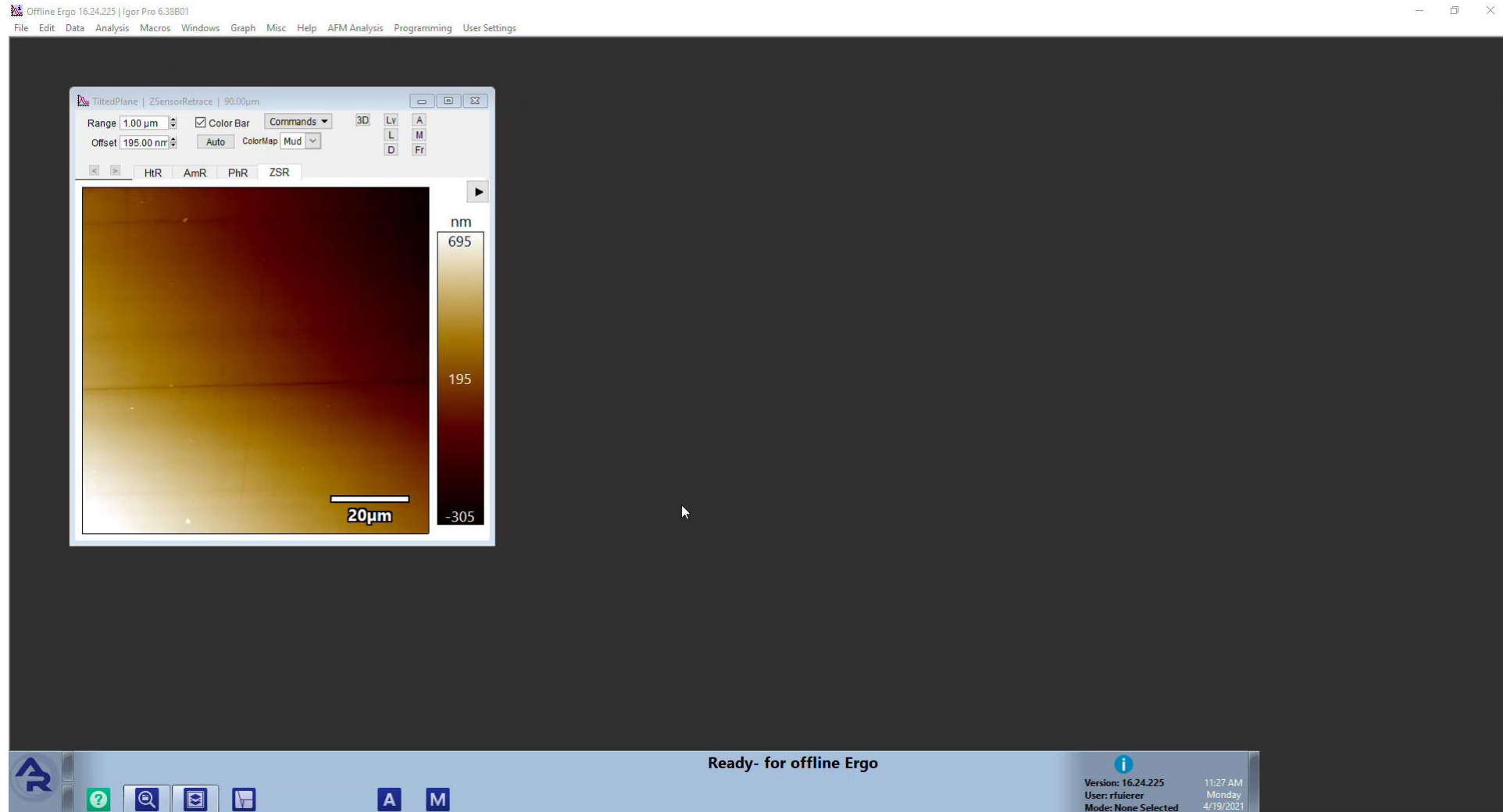
635

195

-245

20µm

# The Display Window Anatomy



Play Movie



# The Analysis Panel

**Roughness** SubTab- Stats on Roughness values

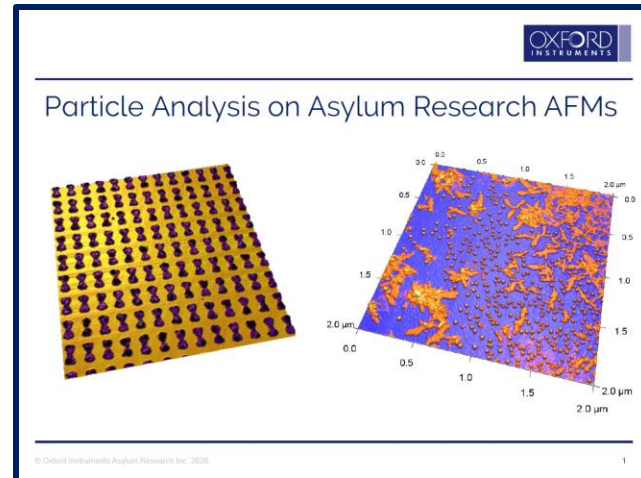
**Section** SubTab- all things Line Sections

**Histogram** Sub tab- creates histograms of data pixels

**Particle Analysis**- counts shapes relative to a threshold masking plane.

-Author File upon request

- <https://youtu.be/SJbj7picaI0>



Analyze Panel

Roughness Section Histogram Particle Analysis

Al2O3\_fibers | ZSensorRetrace | 5.00µm

Calculate Roughness

Mask Make Reset

Box Size 1.00 µm

X:Y 1:1

Y Offset 500.00 nm

X Offset 500.00 nm

Stats	Full Image	Masked Image
Sdev [Rq]	38.686 nm	9.241 nm
Adev [Ra]	25.430 nm	7.200 nm
Max	182.631 nm	30.505 nm
Min	-35.879 nm	-35.879 nm
Avg	0.000 m	-14.366 nm
Skew	2.23	0.666
Kurt	3.97	2.22
Percent	100.0%	86.3%
Area	26.1 µm²	21.9 µm²
Area %	4.225%	1.896%
Volume	0 m³	-0.31 µm³

Export Roughness

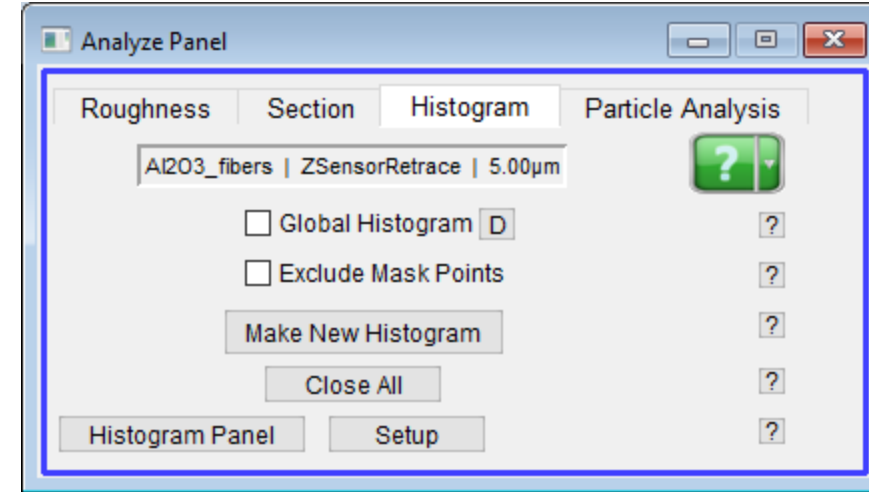
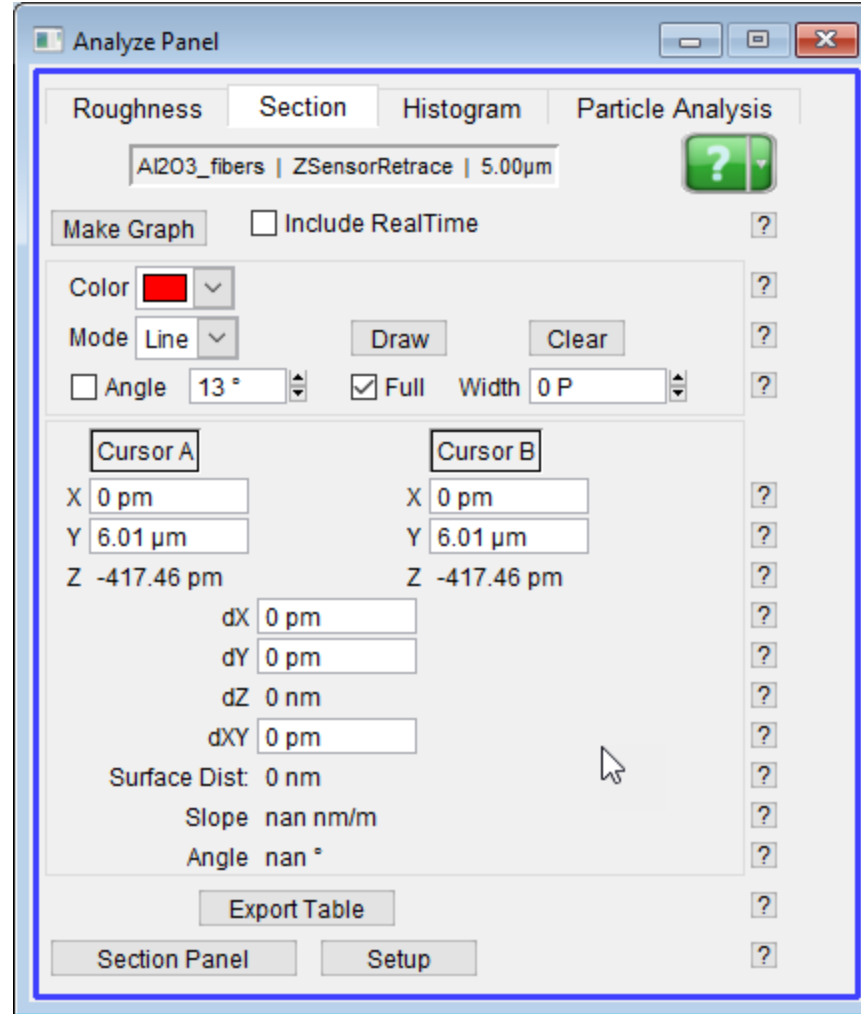
Roughness Panel Setup



# The Analysis Panel

## Section SubTab:

- Linear Line sections (offline & *real time*)
- Free Hand Line Sections (Offline only)
- Averaging lines
- Defined angle sections
- Display multiple sections
- Calculates line cursor differentials



## Histogram SubTab:

- Display histogram data channel
- Overlay multiple histograms
- Simple binning & fitting



# The Modify Panel

**Flatten** SubTab- Controls all flattening

**Planefit** SubTab- Controls all Planefitting

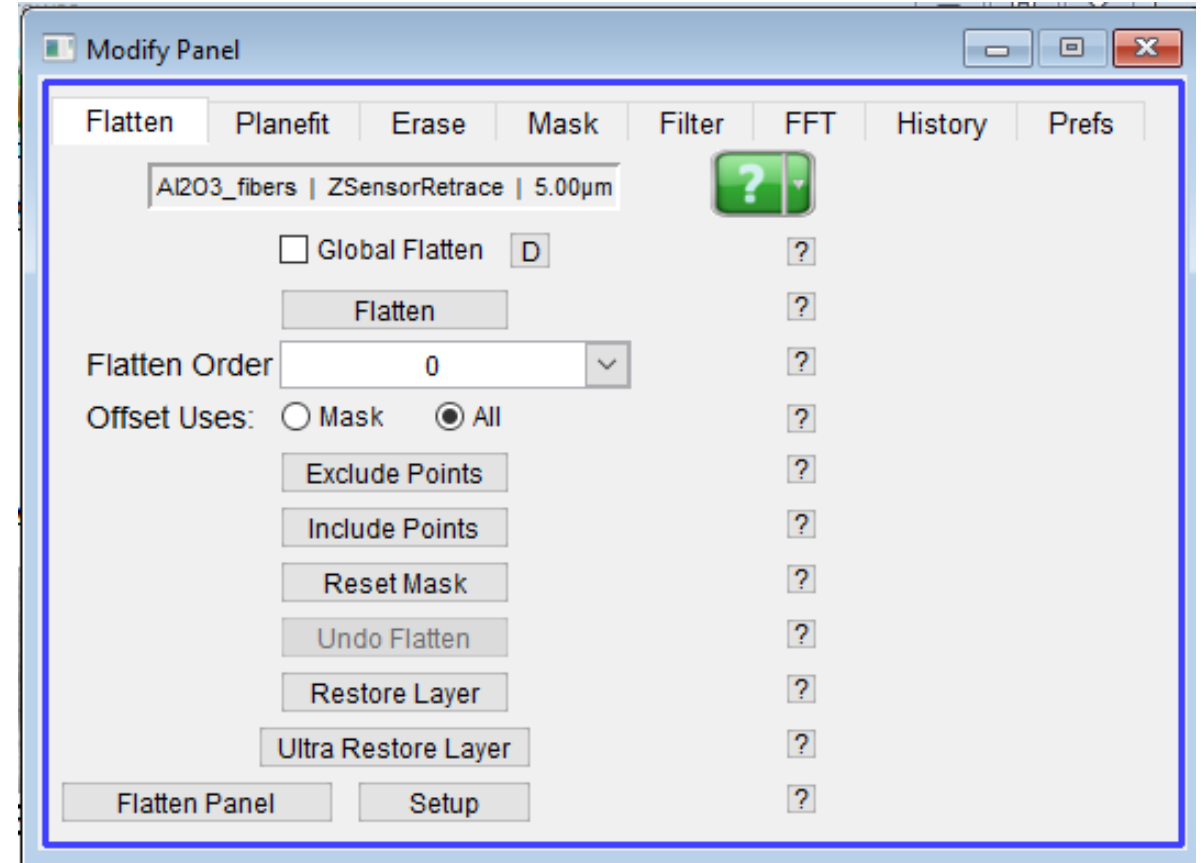
**Erase** Sub tab- Erase lines here.

**Mask** SubTab- mask layers to include or exclude data in some processing calculation

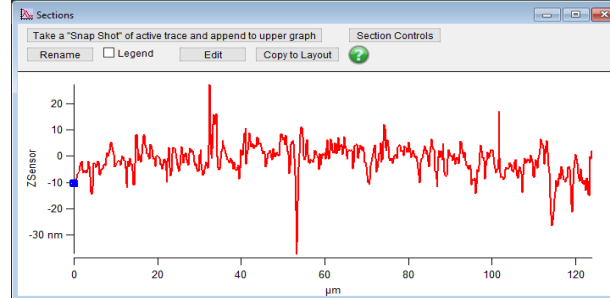
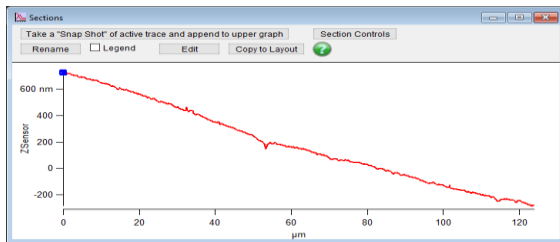
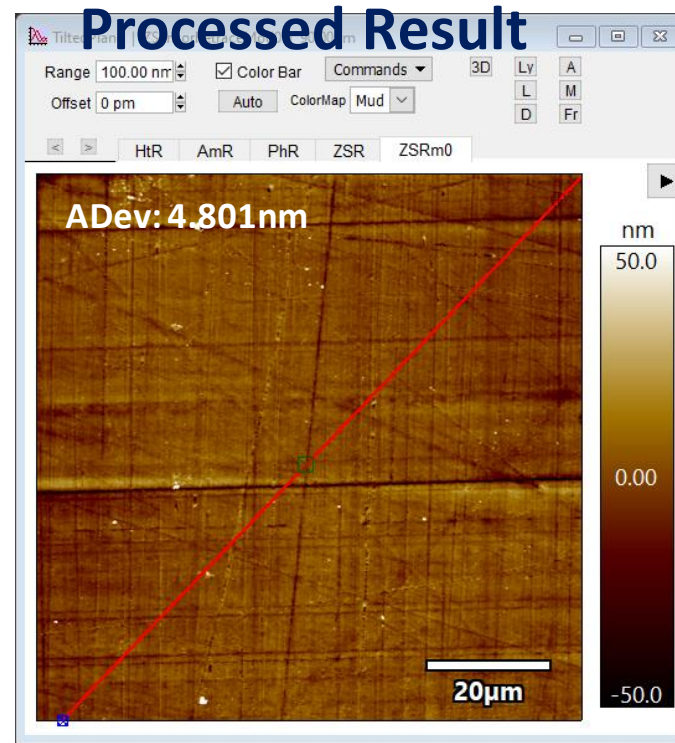
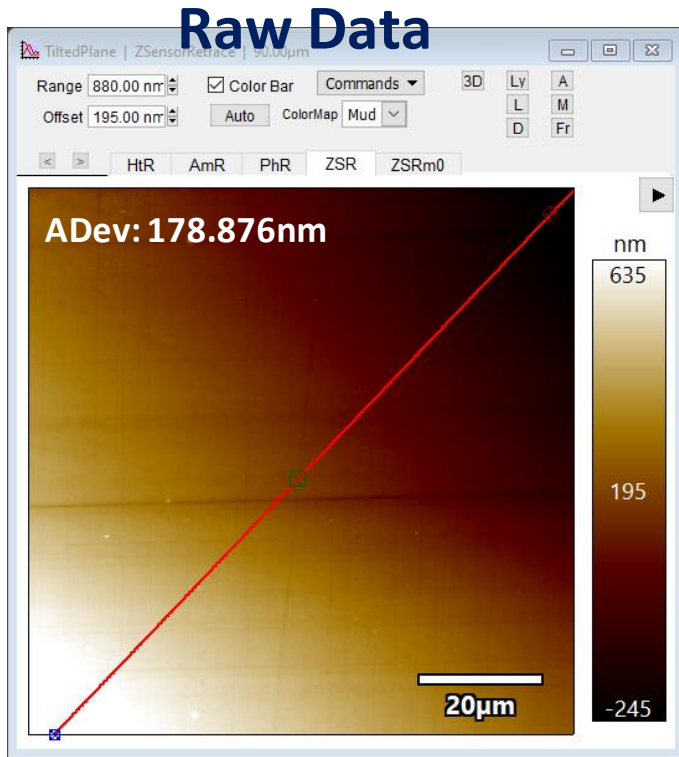
## General Precautions:

Try to keep modifications to lower order processes (0,1<sup>st</sup>).

**Masking features will be the differentiator**



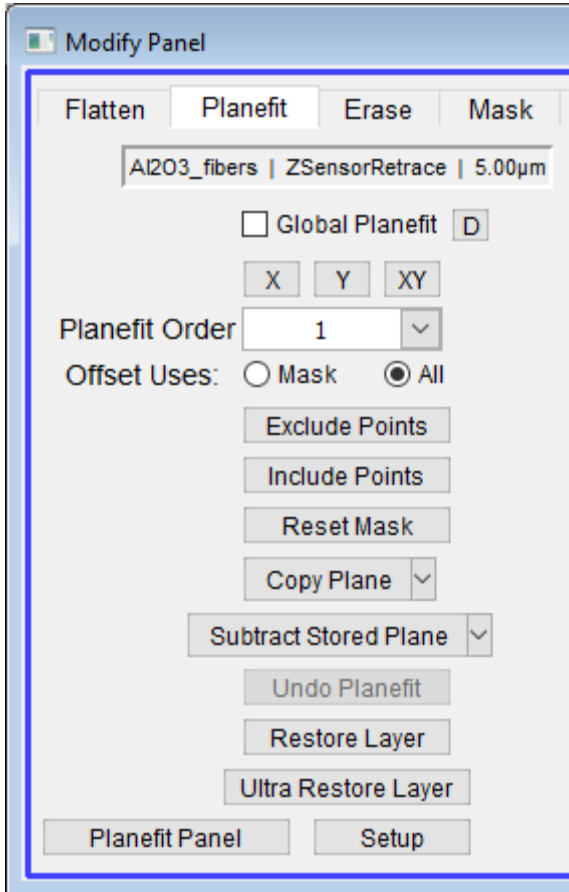
# Image 1: Flattening & PlaneFit



File: TiltedPlane  
DataType: ZSensorRetrace  
Date: 2020-07-06  
ImageNote:  
ImagingMode: AC Mode  
ScanLines: 512  
ScanPoints: 512  
ScanRate: 0.10 Hz  
ScanSize: 90.00 µm  
Time: 1:31:27 PM



# The Modify Panel



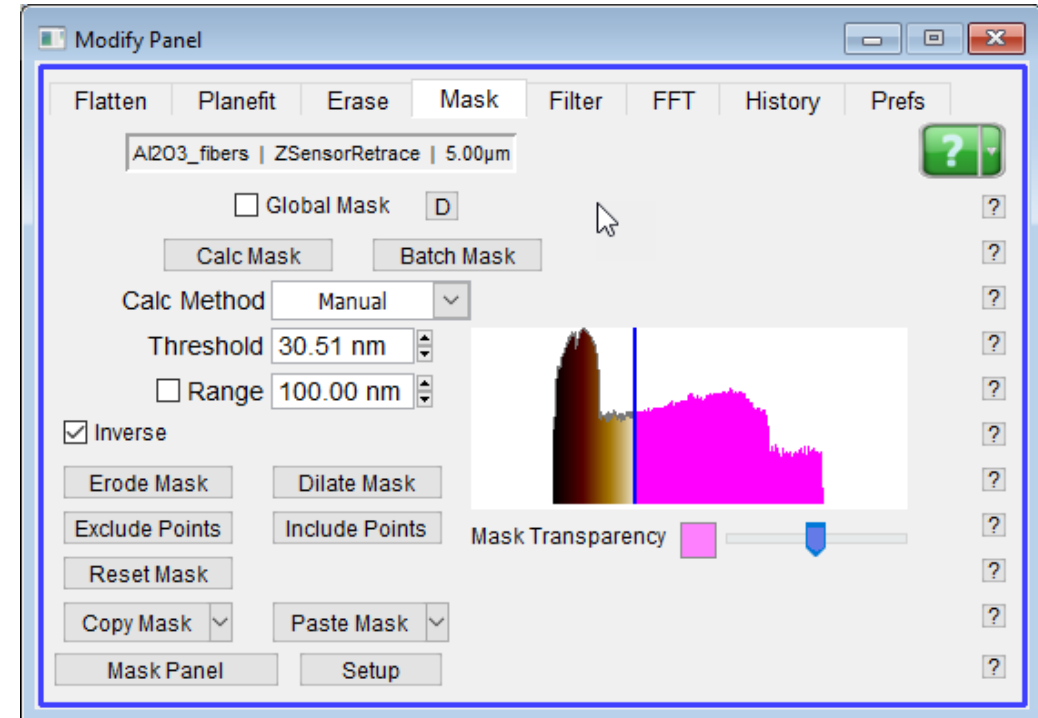
## Plane Fitting-

0,1<sup>st</sup> order.

Mask or full image

## Masking-

- Many approaches
- Erode/ Dilate Mask
- Copy/ paste Masks



## Inverse Mask

This flips the mask so that excluded points become the included points.

## Keep processing to minimum

Sometimes Image Processing is a necessary evil

Offers more variables and potential artifacts into image *after processing*

Why Use it?- sometimes need to isolate part of image

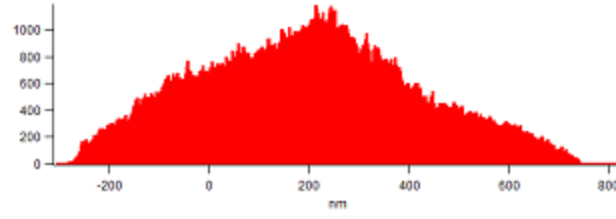
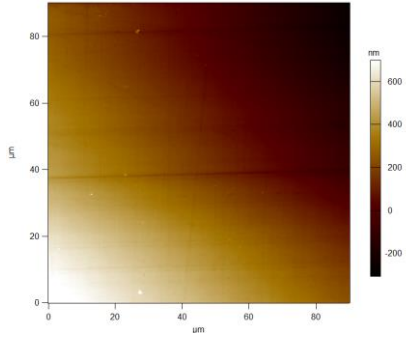
- Planefitting less invasive than Flatten
- Try to keep processing orders 'smaller'- 0 & 1 order
- Use masking for excluding processing artifacts
- Avoid filtering and erasing lines
- only use processing when it makes your measurement better

- Select a different image from your imaging session
- Make sure your sample is free of particles to avoid transients
- Try a different scan rate; tap harder (amplitude)
- Thermally equilibrate the AFM after loading the sample;
- Protect the tip and sample from air currents inside the acoustic hood
- Avoid using the first image after engaging on the surface (particularly bad for the Height channel)
- Consider using the most stable AFM you can

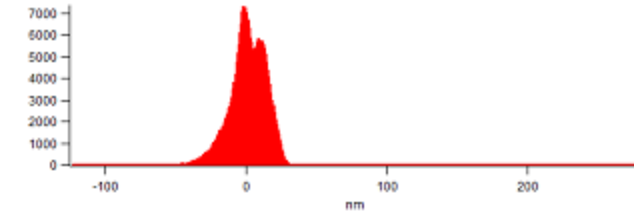
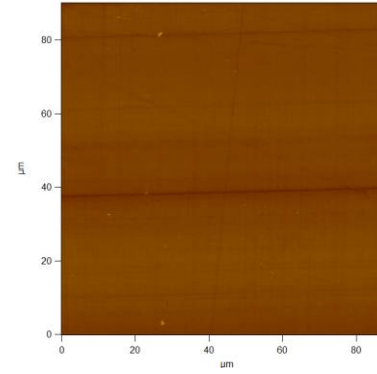
**Not always possible! Sometimes the cost in time and/or money is too great.**

# PlaneFitting (XY): Order Examples

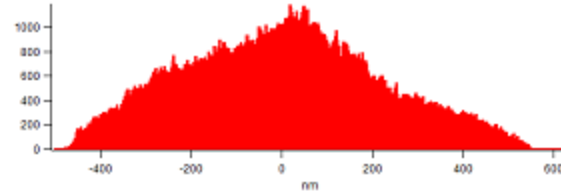
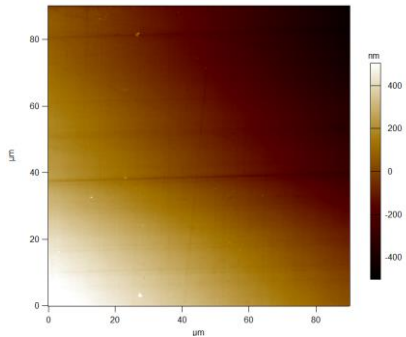
RAW



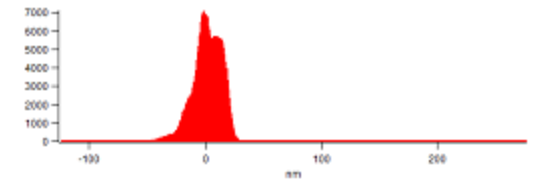
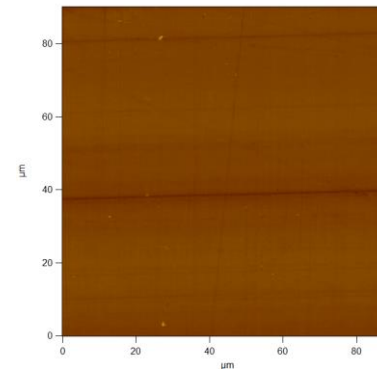
2ND



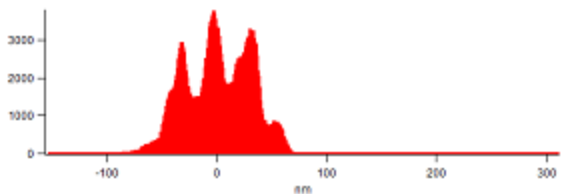
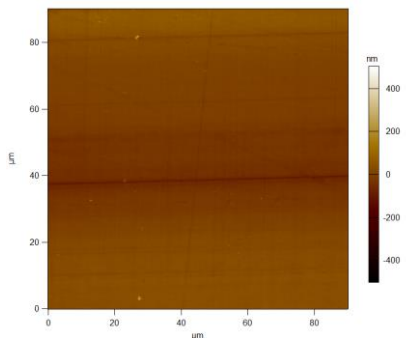
ZERO



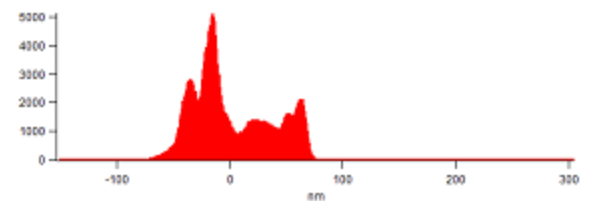
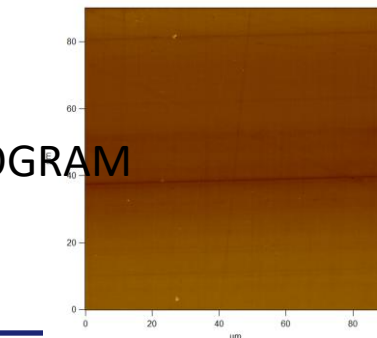
3RD  
THIRD



FIRST



HISTOGRAM



# PlaneFitting (XY): Order Examples

Y

X



		0 order	1 <sup>st</sup> order	2 <sup>nd</sup> Order	3 <sup>rd</sup> Order
X					
Y					
XY					

# Image 1: Flattening & PlaneFit

Offline Ergo 16.24.225 | Igor Pro 6.39801  
File Edit Data Analysis Macros Windows Graph Misc Help AFM Analysis Programming User Settings

TiltedPlane | ZSensorRetrace | 90.00µm  
Range 880.00 nm  
Offset 195.00 nm  
Color Bar Auto ColorMap Mud  
Commands 3D LY A L M D Fr

nm  
635  
195  
-245  
20µm

Analyze Panel  
Roughness Section Histogram Particle Analysis  
TiltedPlane | ZSensorRetrace | 90.00µm  
Make Graph  Include RealTime  
Color ■  
Mode Free Hand Draw Clear  
Edit Width 0 P  
Cursor A Cursor B  
X 7.14 µm X 7.14 µm  
Y 9.86 µm Y 9.86 µm  
Z -17.05 nm Z -17.05 nm  
dX 0 pm  
dY 0 pm  
dZ 0 nm  
dXY 0 pm  
Surface Dist: 0 nm  
Slope nan nm/m  
Angle nan °

Modify Panel  
Flatten PlaneFit Erase Mask Filter FFT History Prefs  
TiltedPlane | ZSensorRetrace | 90.00µm  
 Global PlaneFit  
X Y XY  
PlaneFit Order 1  
Offset Uses:  Mask  All  
Exclude Points  
Include Points  
Reset Mask  
Copy Plane  
Subtract Stored Plane  
Undo PlaneFit  
Restore Layer  
Ultra Restore Layer  
PlaneFit Panel Setup

Ready- for offline Ergo  
Version: 16.24.225 3:35 PM  
User: rfueller Tuesday  
Mode: None Selected 4/20/2021

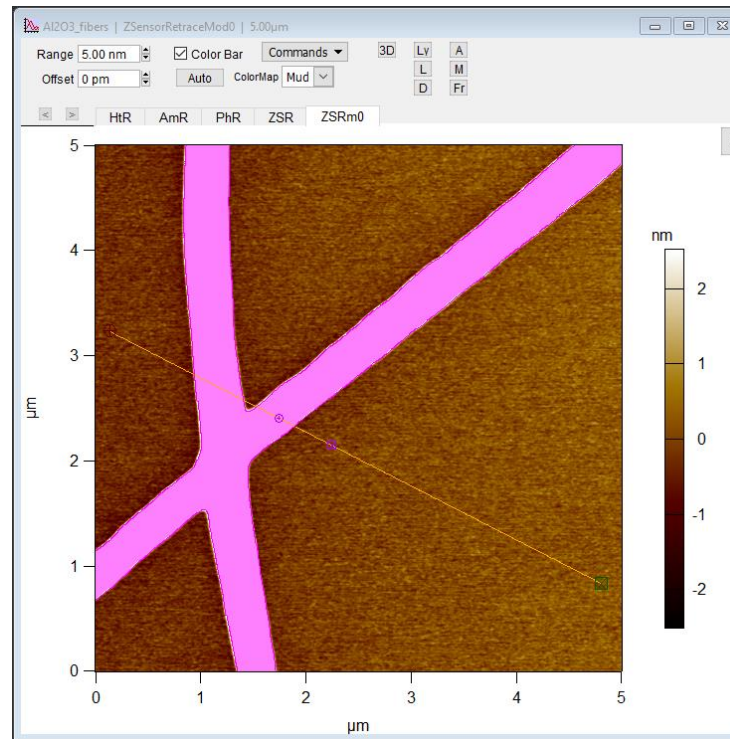
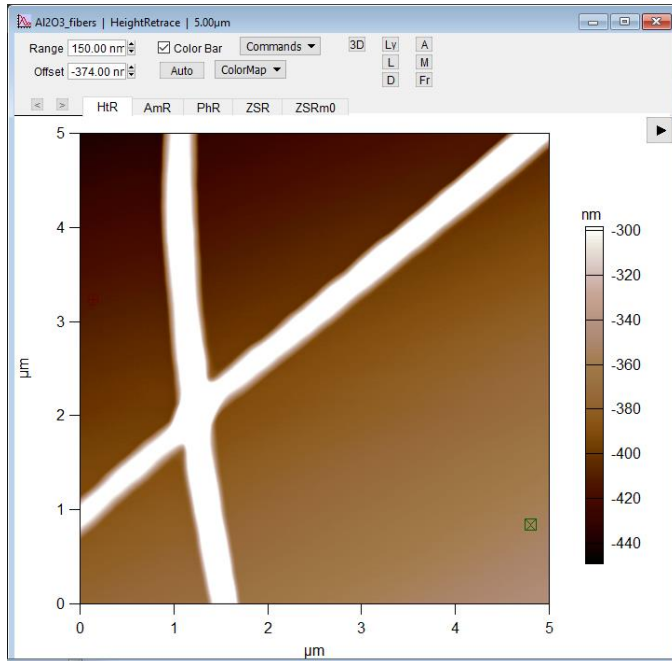
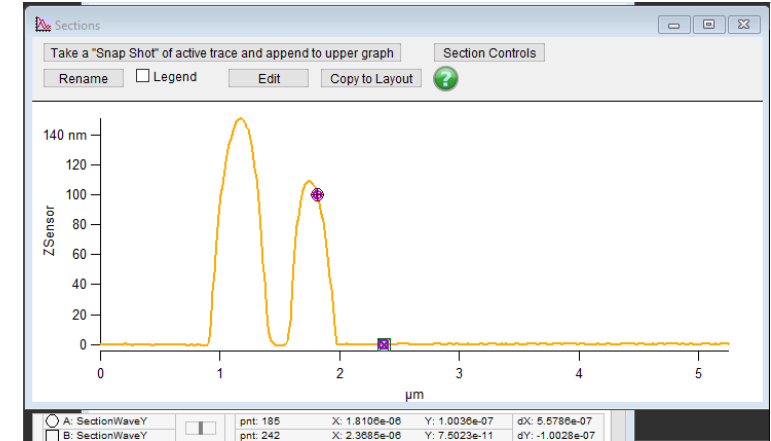
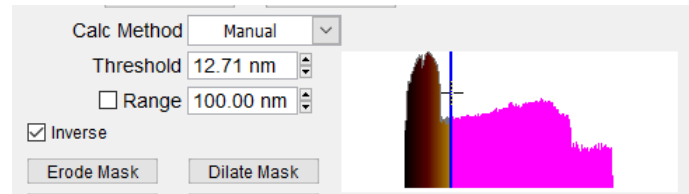


Play Movie

# Image 2: Al<sub>2</sub>O<sub>3</sub> Fibers

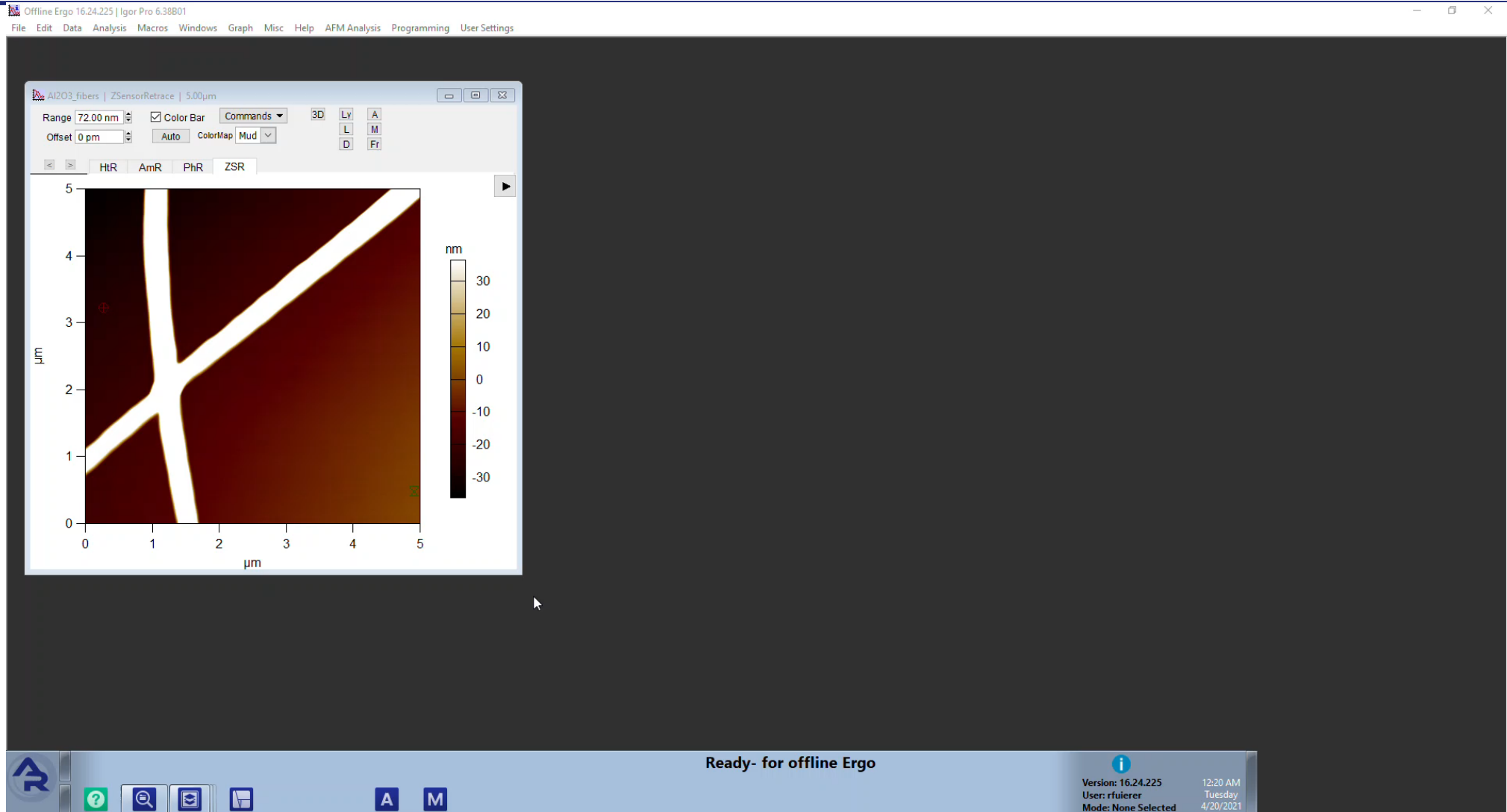
Recipe:

- 1) Manual Mask (12.71nm)
- 2) Dilate (1x)
- 3) 1<sup>st</sup> order  $\odot$ Mask Planefit
- 4) 0 or 1<sup>st</sup> order  $\odot$  Mask flatten



Stats	Full Image	Masked Image
Sdev [Rq]	39.366 nm	374.052 µm
Adev [Ra]	26.450 nm	273.351 µm
Max	201.104 nm	3.714 nm
Min	-1.537 nm	-1.537 nm
Avg	15.846 nm	318.889 zm
Skew	2.39	1.56
Kurt	4.34	10.7
Percent	100.0%	82.9%
Area	26.1 µm <sup>2</sup>	20.6 µm <sup>2</sup>
Area %	4.224%	0.07547%
Volume	0.4 µm <sup>3</sup>	0.0066 nm <sup>3</sup>

# Image 2: Al<sub>2</sub>O<sub>3</sub> Fibers

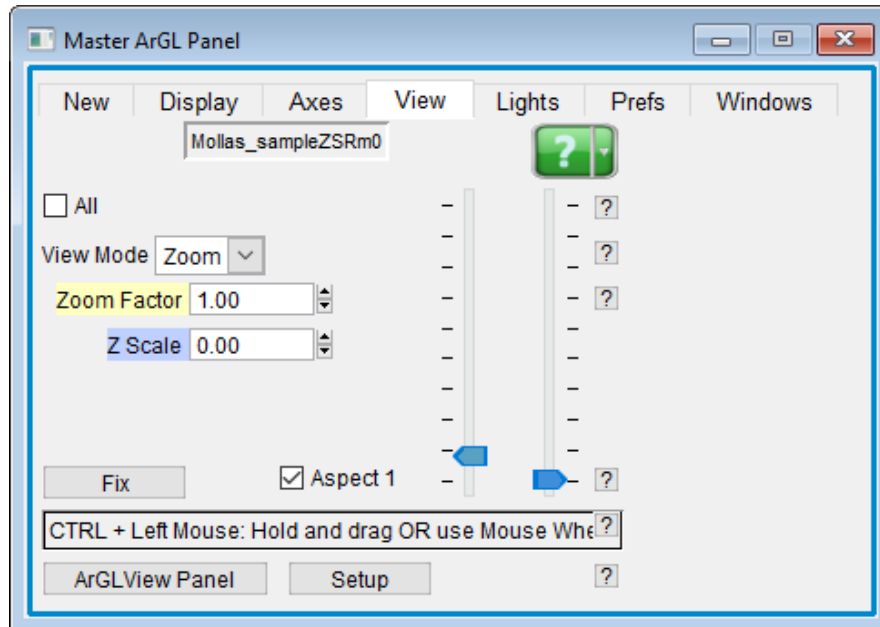


Play Movie



# ARgyle 3D Rendering

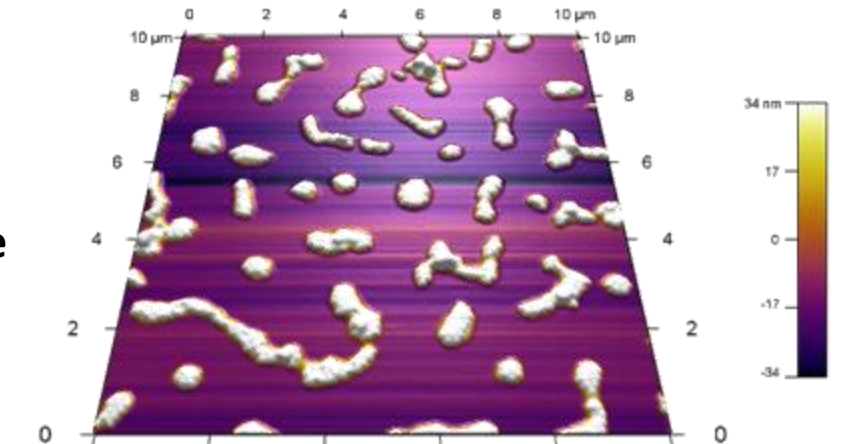
Use specular lighting to monitor processing changes



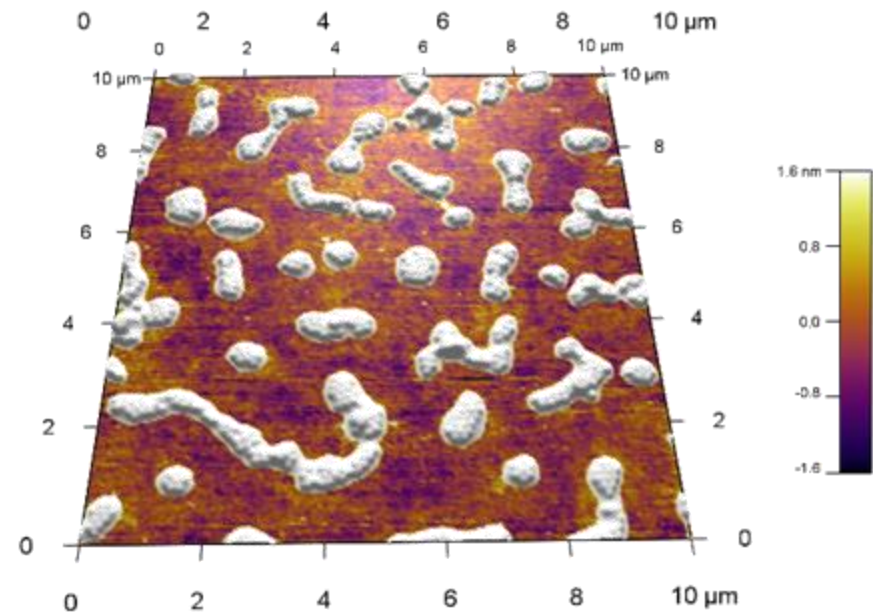
## HotKeys:

- Left Mouse + Cursor- rotate light
- Right Mouse + Cursor: rotate sample
- Ctrl+cursor: Zoom
- Shift + cursor: Move image around

Before



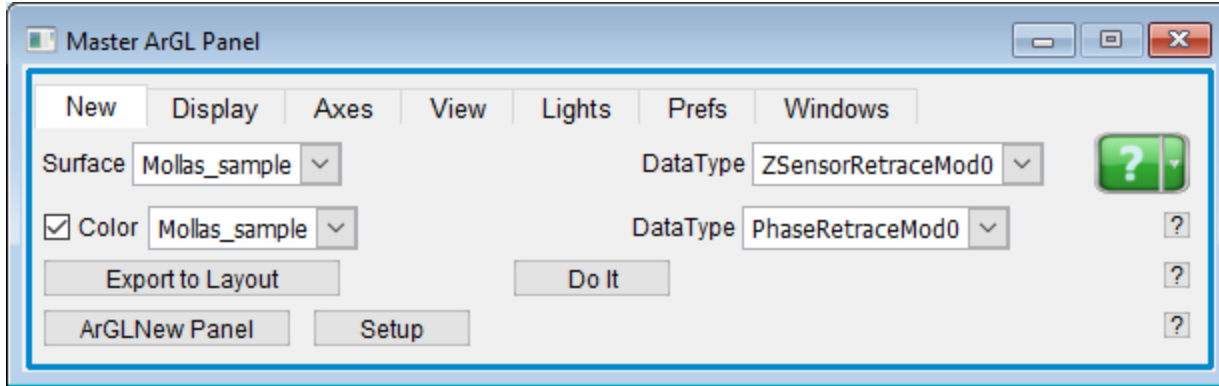
After



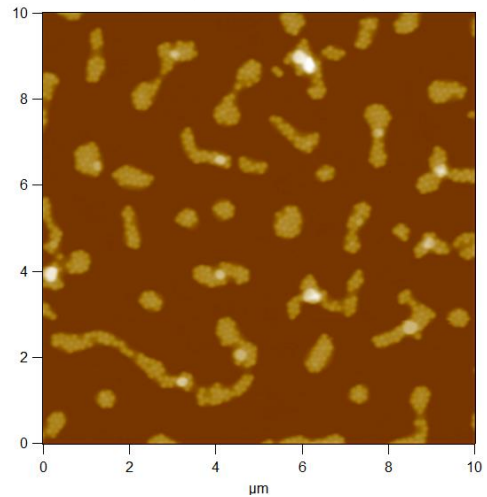


# ARgyle 4D Rendering

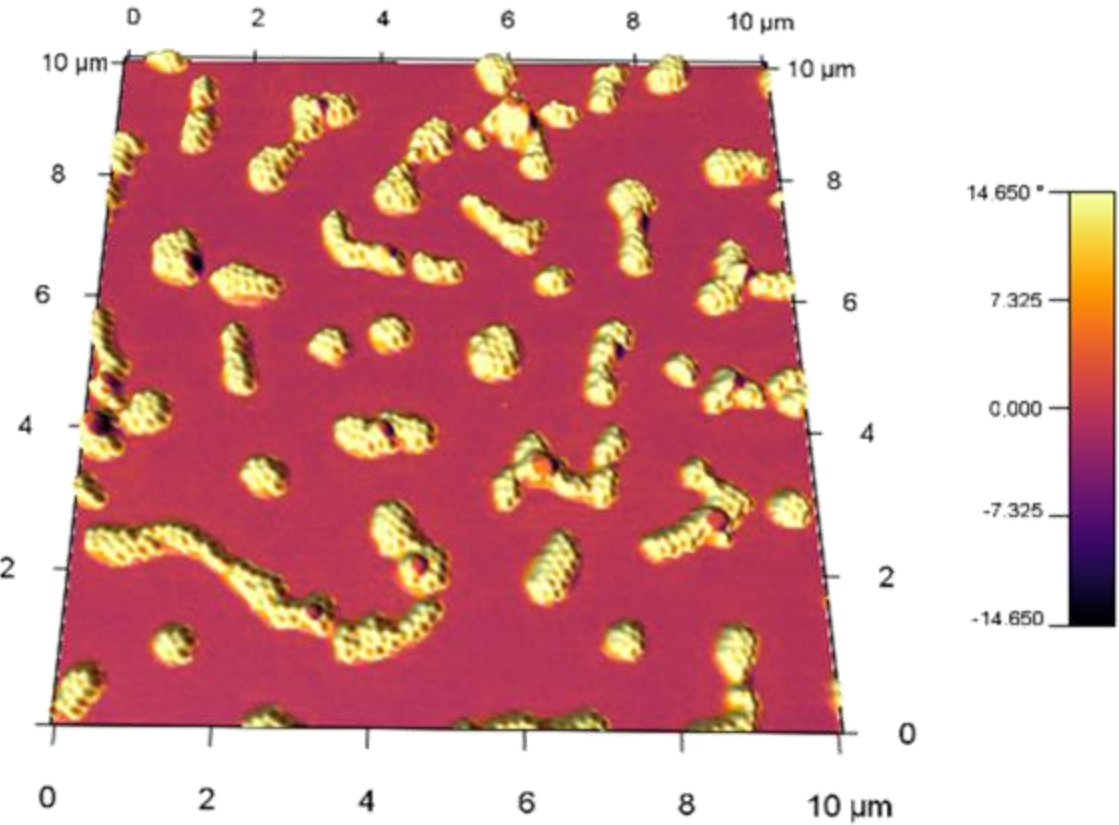
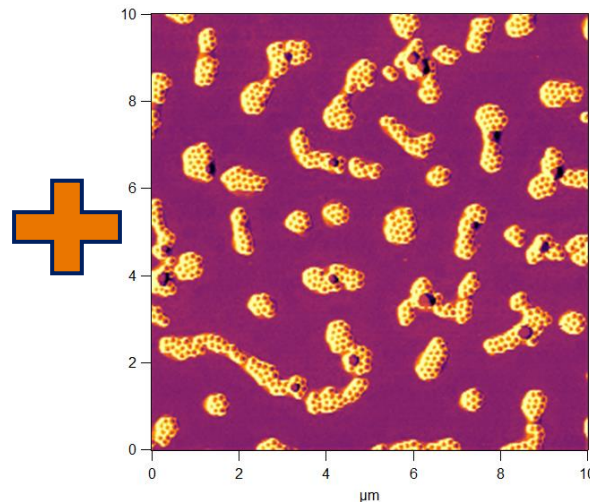
Menu: AFM Analysis → 3D Surface Plots



Topography



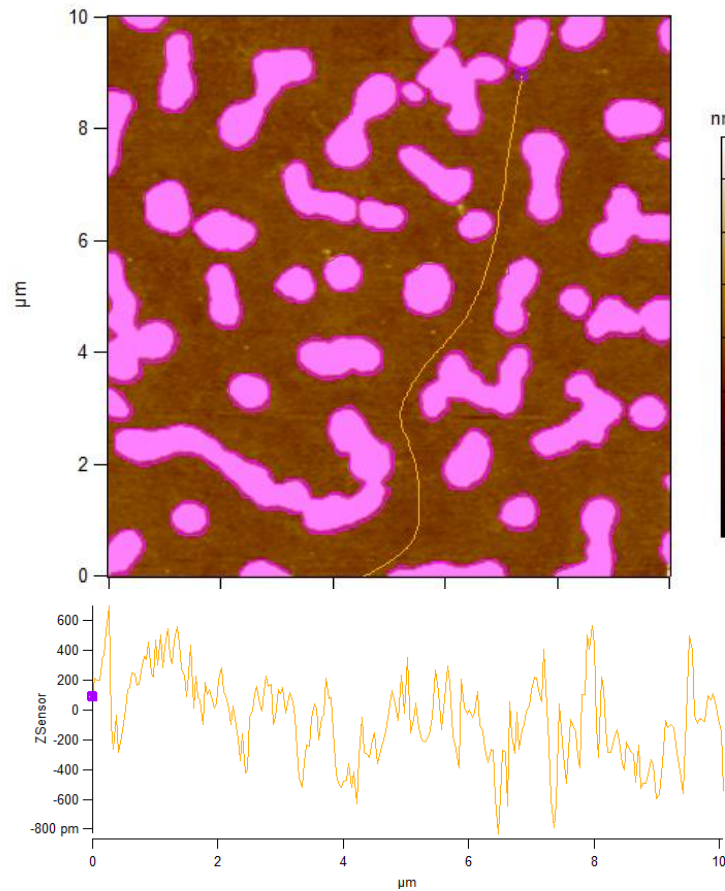
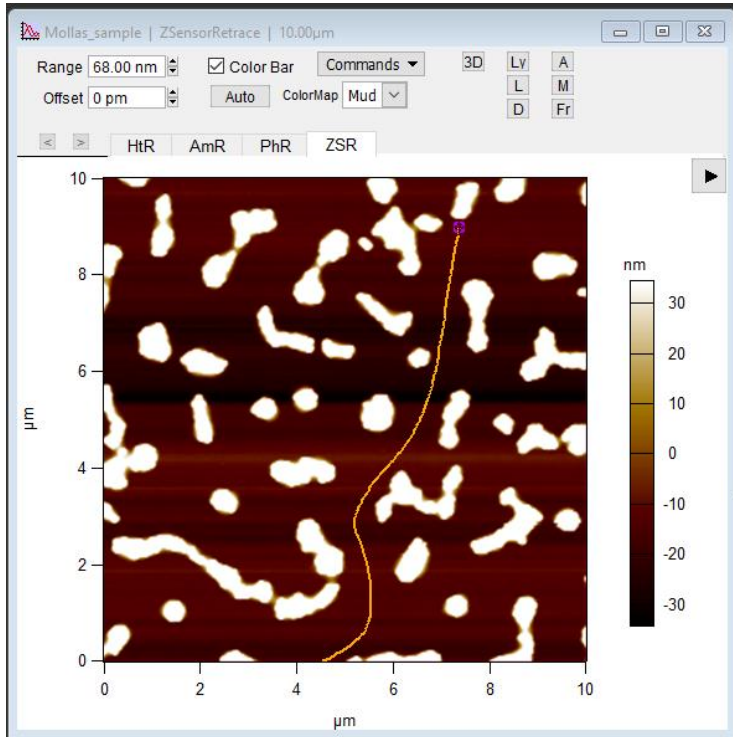
AC Phase



# Image 3: Molla's Sample

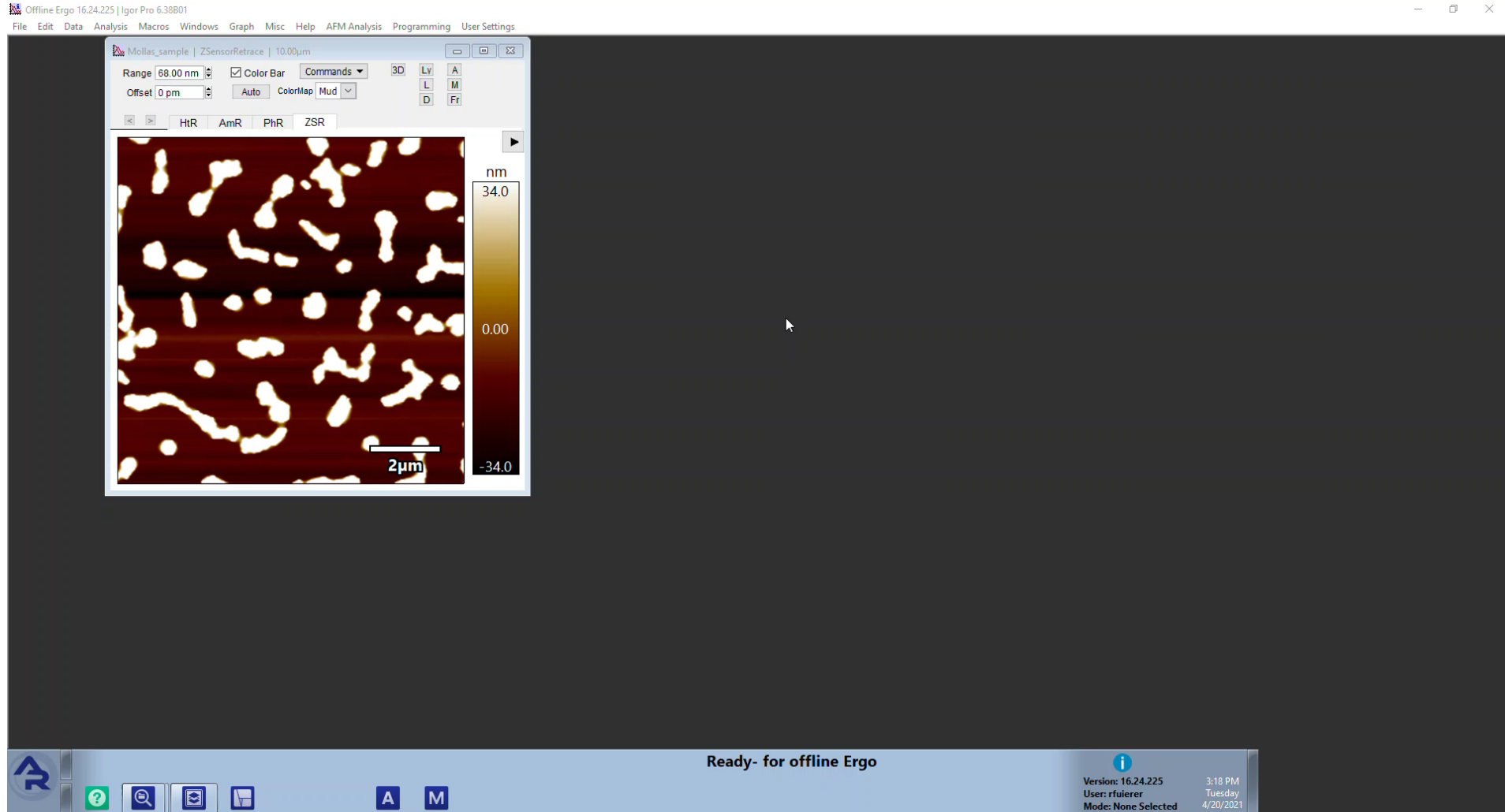
Recipe:

- 1) Magic Mask (2.66nm)
- 2) Dilate Mask
- 3) 1<sup>st</sup> Order Flat



Stats	Full Image	Masked Image
Sdev [Rq]	37.236 nm	346.147 μm
Adev [Ra]	26.513 nm	255.217 μm
Max	280.413 nm	11.896 nm
Min	-1.826 nm	-1.826 nm
Avg	17.201 nm	71.053 μm
Skew	2.28	2.67
Kurt	5.01	59.2
Percent	100.0%	69.1%
Area	104.8 μm <sup>2</sup>	67.3 μm <sup>2</sup>
Area %	4.758%	0.005431%
Volume	1.7 μm <sup>3</sup>	0.005 nm <sup>3</sup>

# Image 3: Molla Sample

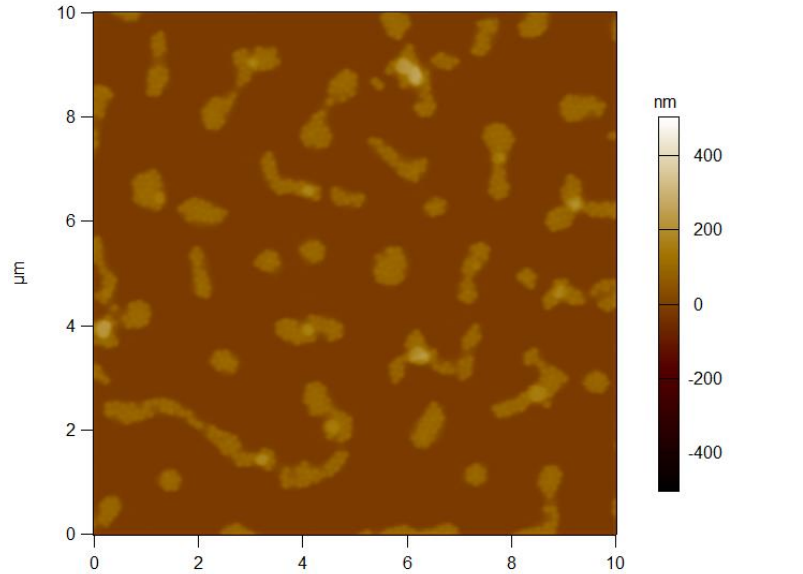


Play Movie

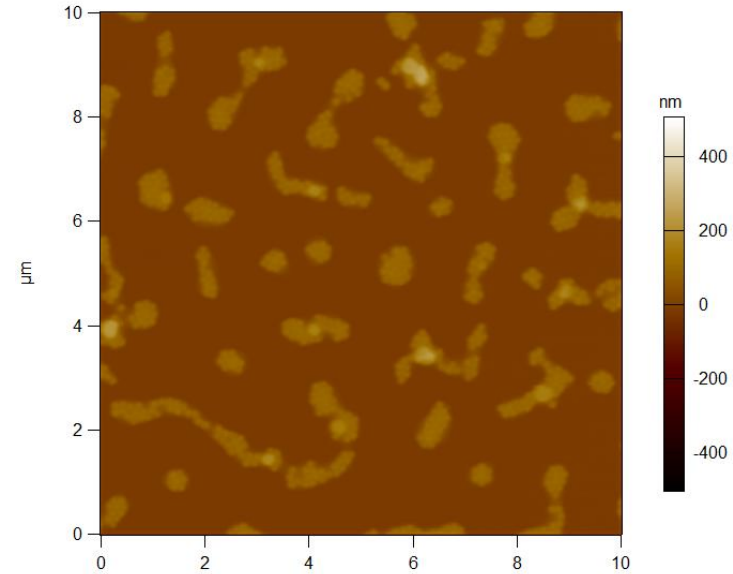
# Flattening Conveniences

2D Z Sensor  
Retrace image

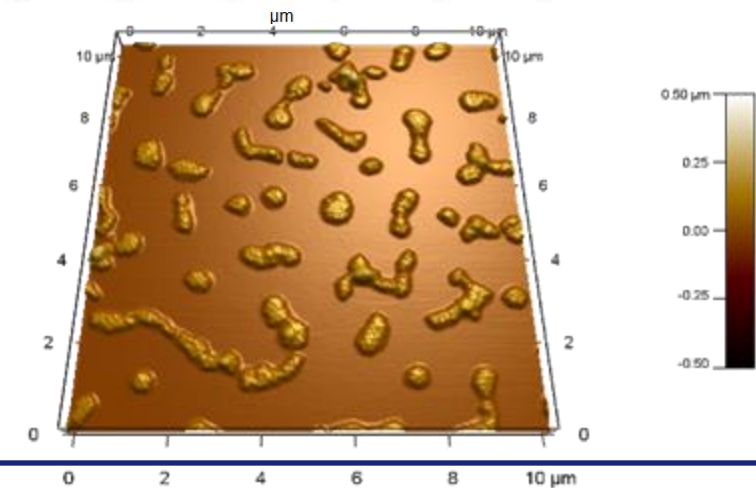
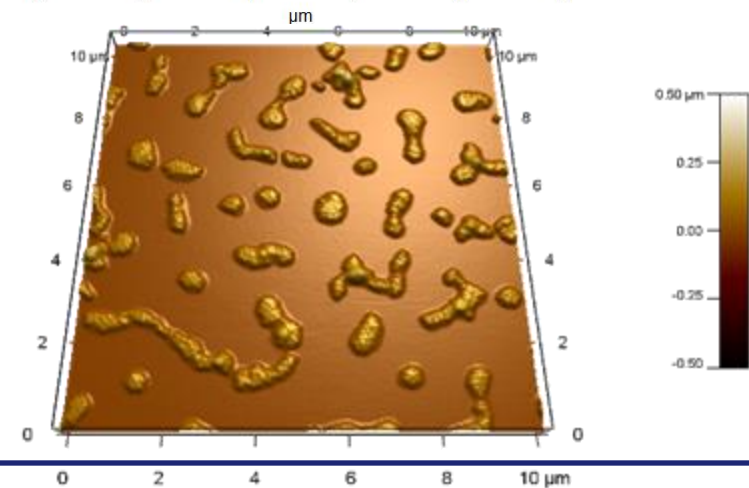
### Magic Mask Flatten Only



### Histogram Flatten Only

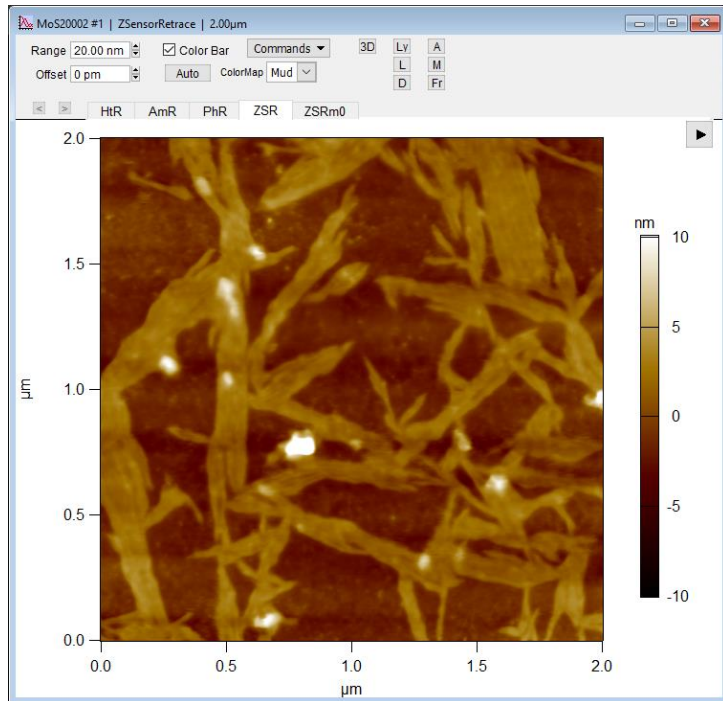


Argyle 3D  
Rendering

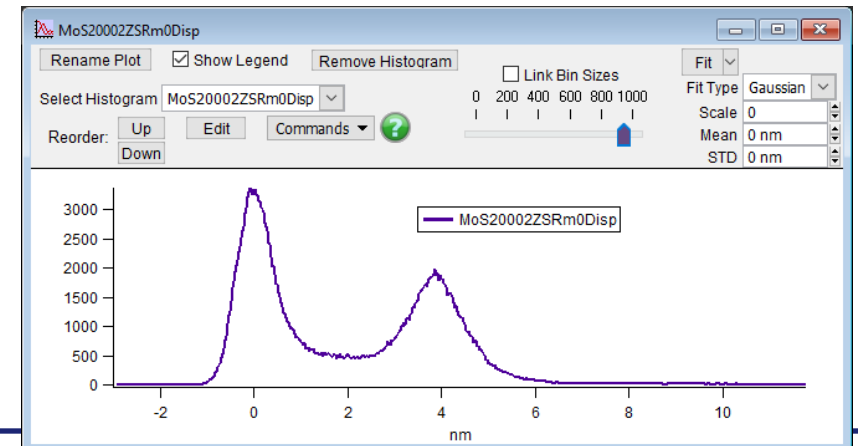
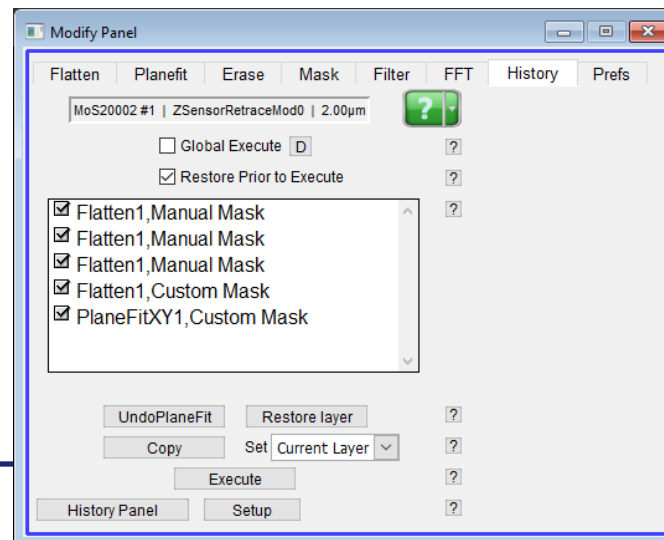
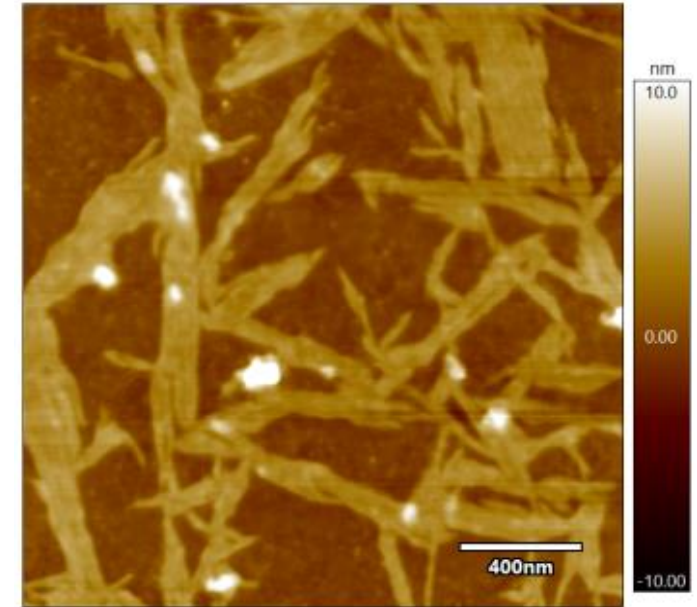
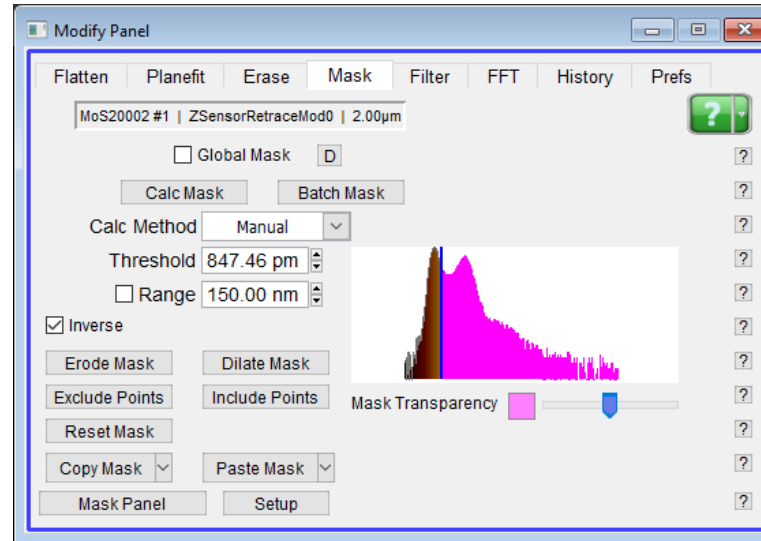


# Image 4: MoS2

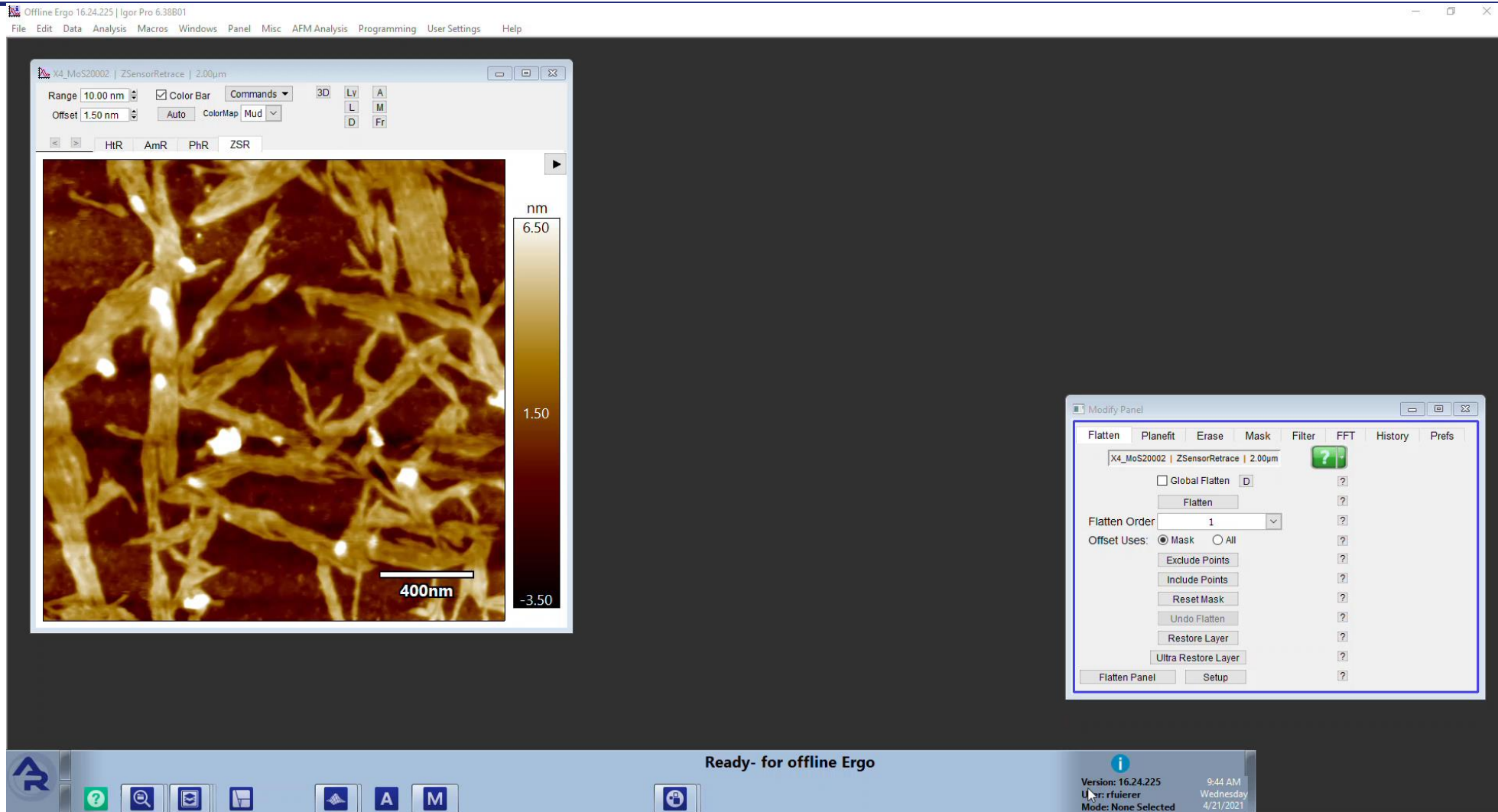
## Iterative Manual Masking/ 1<sup>st</sup> Order Flat cycle (3)



Raw Z Topo

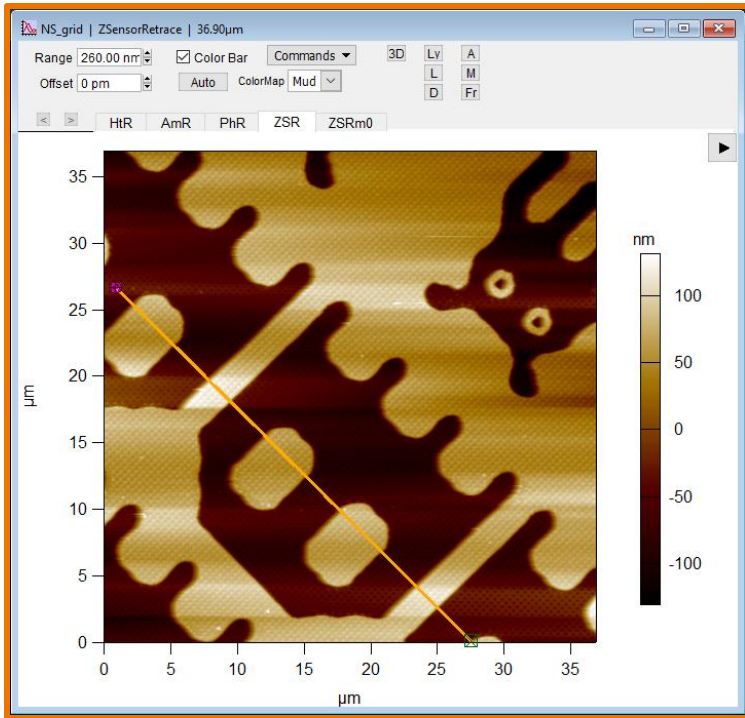
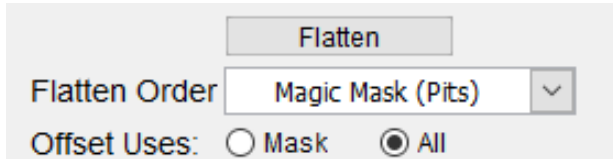


# Image 4: MoS2

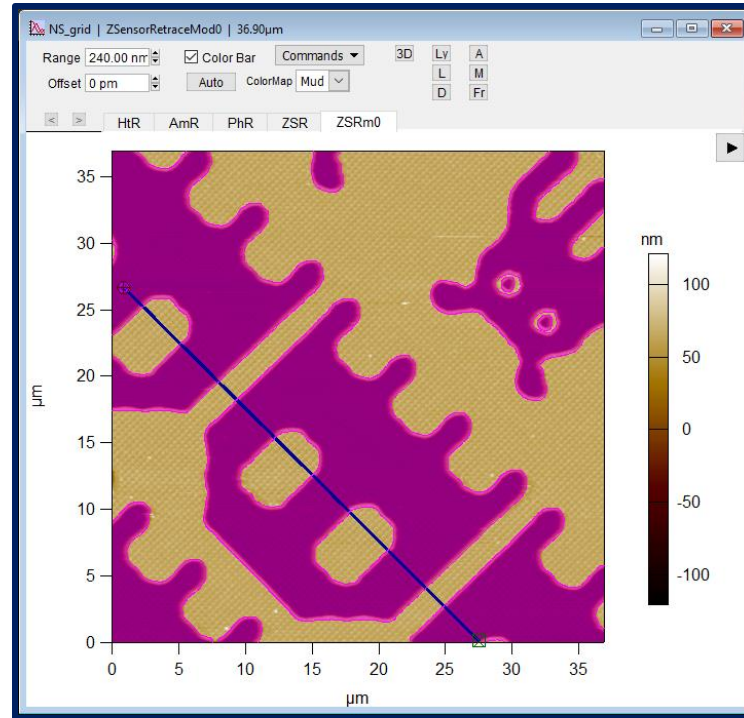


Play Movie

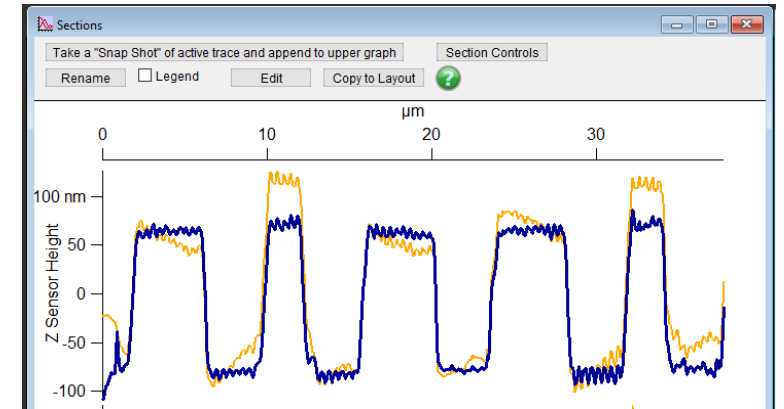
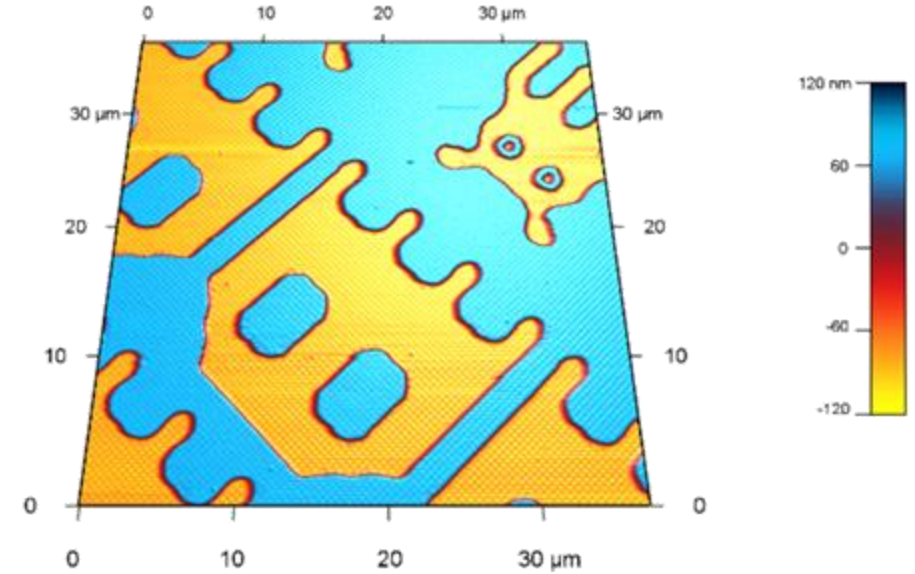
# Image 5: Calibration Grating



Before



After



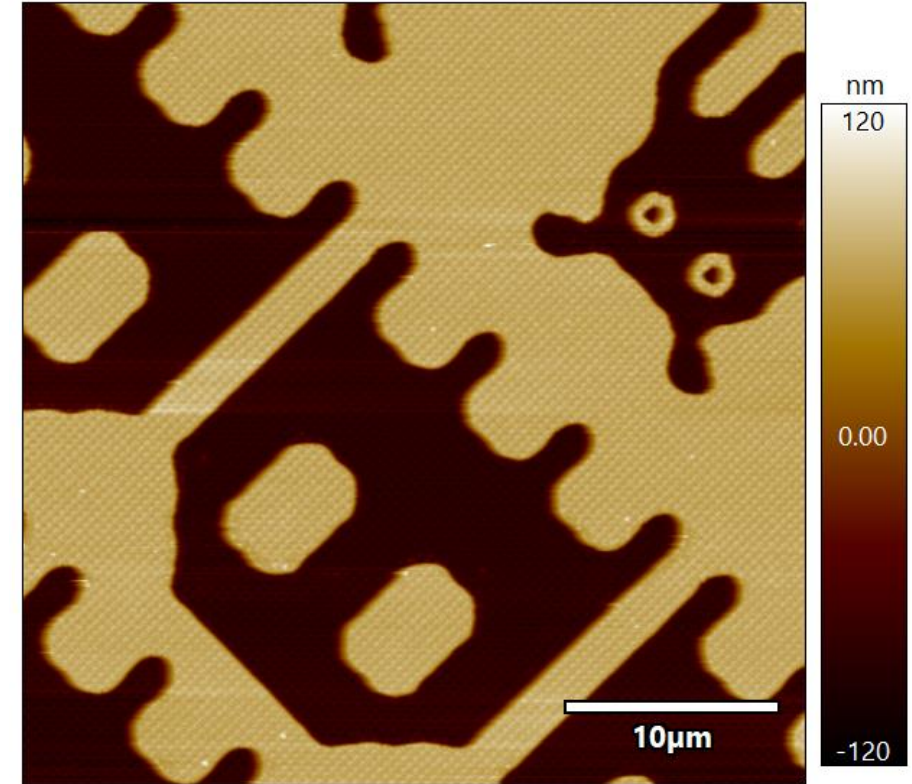
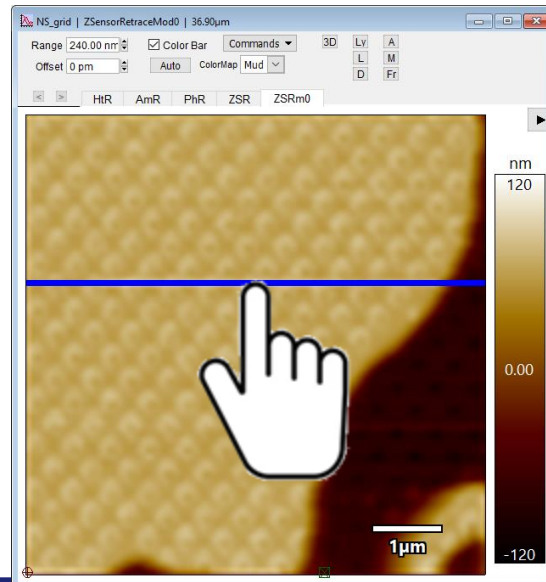
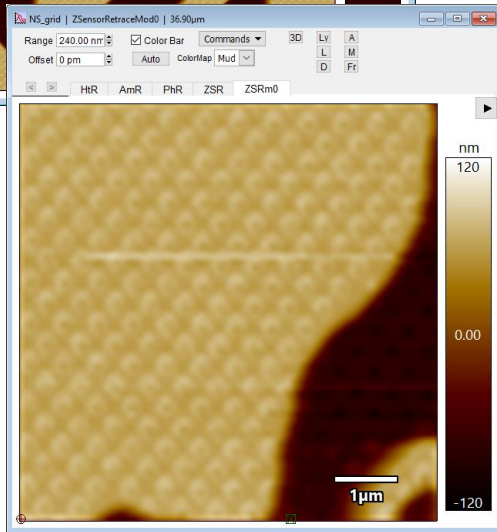
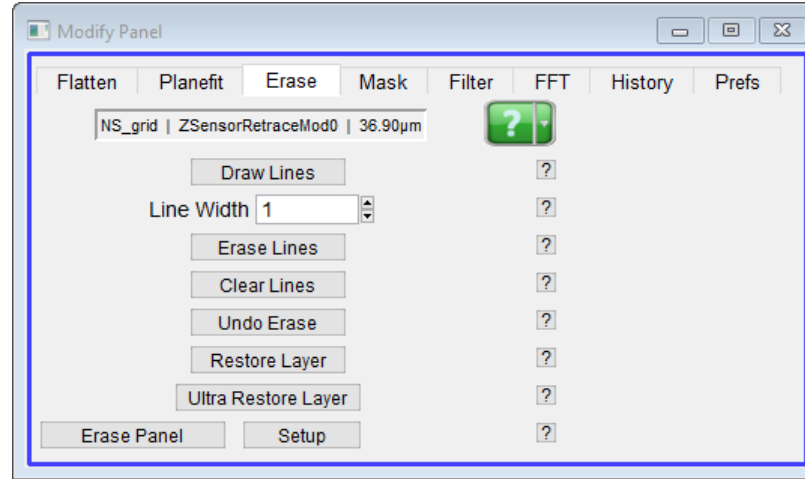
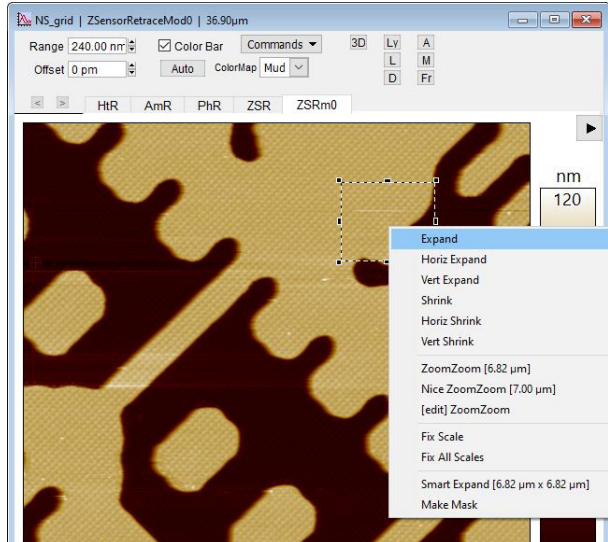
# Image 5: Calibration Grating

The screenshot displays the Igor Pro interface for an AFM image analysis. The main window shows a color-coded AFM image of a calibration grating with a 10 μm scale bar. The image is overlaid with a grid. The 'Analyze Panel' is open, showing the 'Roughness' section with parameters for 'NS\_grd | ZSensorRetrace | 36.90 μm'. The 'Modify Panel' is also open, showing the 'Flatten' section with options for 'Global Flatten', 'Flatten Order', and 'Offset Uses'. The status bar at the bottom indicates 'Ready- for offline Ergo' and provides system information: Version: 16.24.225, User: rfuierer, Mode: None Selected, 2:10 PM Tuesday 4/20/2021.



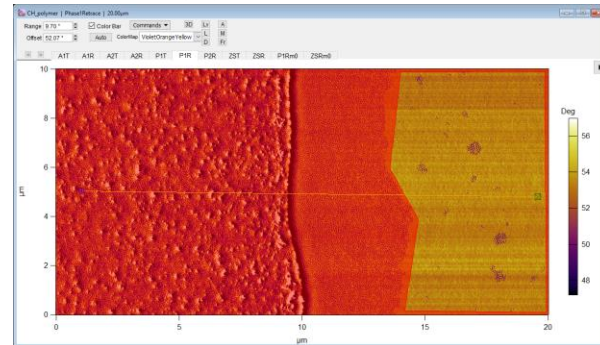
Play Movie

# Image 5: Erasing lines

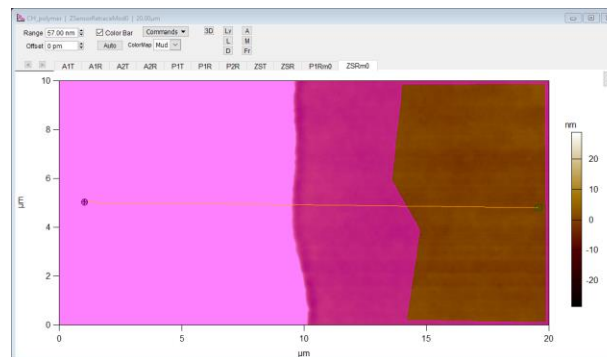
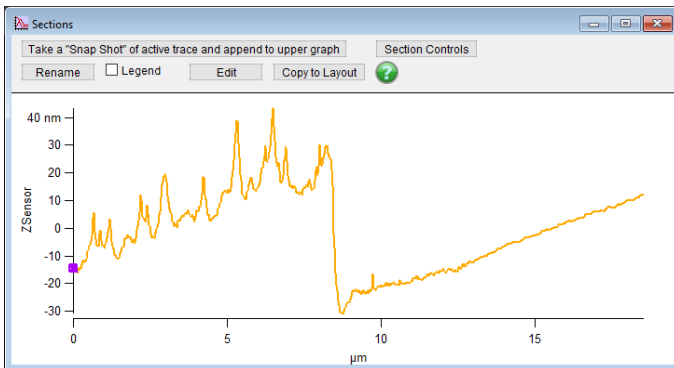
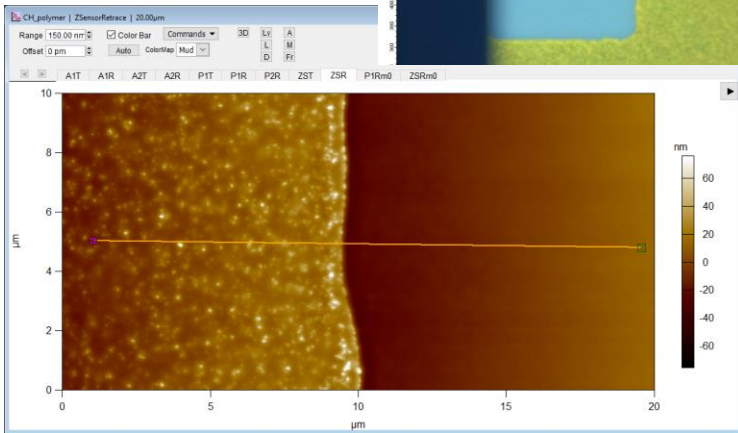
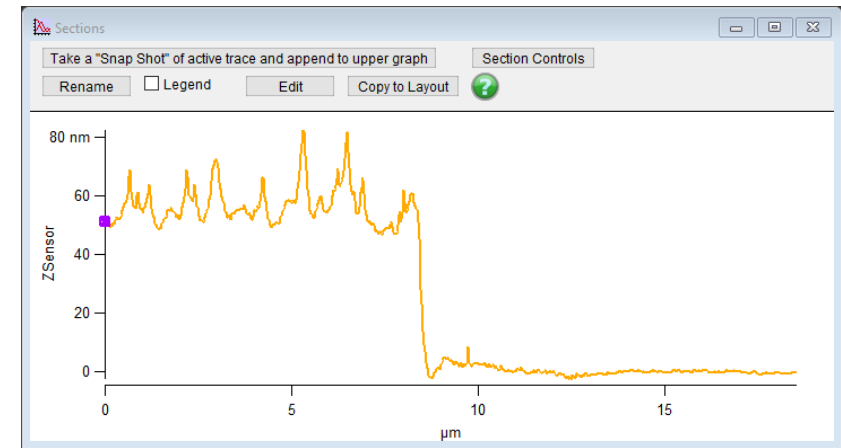
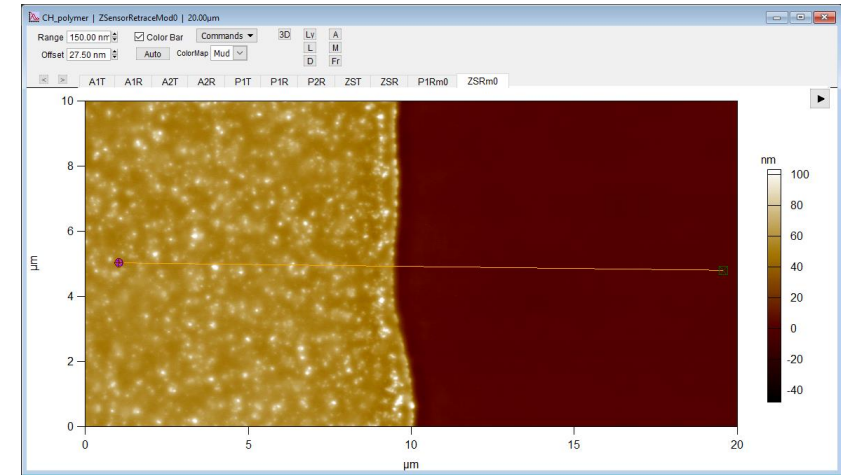


# Image 6: PMMA on Au Step Height

Build Include Points Mask on Phase; Copy Mask



Paste Mask on ZSR, 1<sup>st</sup> order Planefit XY.



# Image 6: PMMA on Au Step Height

The screenshot displays the Offline Ergo software interface. The main window shows an AFM image of PMMA on Au step height. The image is color-coded by height, with a scale bar on the right ranging from -28.5 nm to 28.5 nm. A 4 μm scale bar is visible at the bottom of the image. The software title bar indicates 'Offline Ergo 16.24.225 | Igor Pro 6.38B01'. The menu bar includes File, Edit, Data, Analysis, Macros, Windows, Graph, Misc, Help, AFM Analysis, Programming, and User Settings. The main window has a toolbar with buttons for A1T, A1R, A2T, A2R, P1T, P1R, P2R, ZST, and ZSR. The image shows a bright, textured region on the left and a darker, smoother region on the right, separated by a vertical step. The 'Analyze Panel' is open on the right, showing the 'Roughness' tab. It includes a 'Make Graph' button, a color selection dropdown (red), and a mode dropdown (Line). The 'Cursor A' and 'Cursor B' coordinates are displayed: X: 4.21 μm, Y: 0 pm, Z: 724.94 nm. The 'Modify Panel' is also open, showing the 'Flatten' tab. It includes a 'Global Flatten' checkbox, a 'Flatten' button, and a 'Flatten Order' dropdown set to 1. The 'Offset Uses' section has radio buttons for 'Mask' (selected) and 'All'. Other buttons include 'Exclude Points', 'Include Points', 'Reset Mask', 'Undo Flatten', 'Restore Layer', and 'Ultra Restore Layer'. The bottom status bar shows 'Ready- for offline Ergo', the version '16.24.225', the user 'rfuierer', and the date 'Tuesday 4/20/2021'.



Play Movie

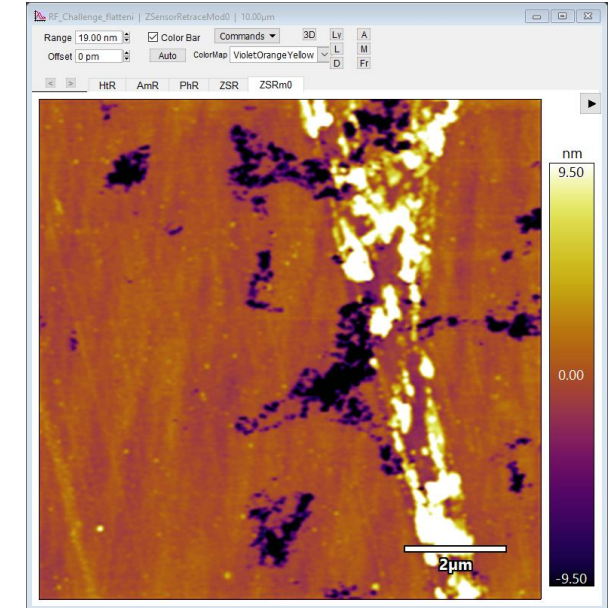
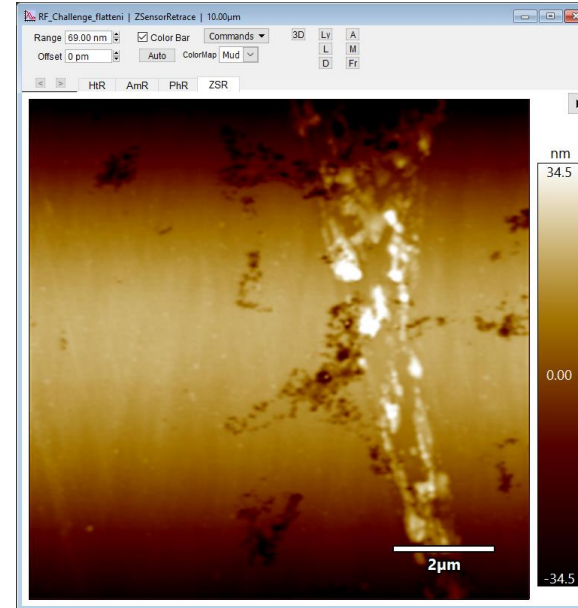
# Image 7: RF's Challenge Sample

No idea what the Challenge samples material.

- 'Magic Mask Flatten' *fails*

- Above & below substrate features give standard processing some artefacts.

- Make (subjective) mask with slider bar to include the higher features, then iteratively exclude areas created with freehand tool under 'exclude points' operation.



# Image 7: RF's Challenge Sample

The screenshot displays the Oxford Instruments software interface for processing AFM data. The main window shows a color-coded AFM image of a sample with a 2 μm scale bar. The image's vertical axis is labeled in nanometers (nm), ranging from -34.5 to 34.5. A color bar on the right indicates the height scale. The software interface includes several panels:

- Modify Panel:** Contains options for 'Flatten', 'PlaneFit', 'Erase', 'Mask', 'Filter', 'FFT', 'History', and 'Prefs'. The 'Mask' section is active, showing a 'Global Mask' checkbox, 'Calc Mask' and 'Batch Mask' buttons, 'Calc Method' set to 'Manual', a 'Threshold' of 6.10 nm, and a 'Range' of 100.00 nm. There is also an 'Inverse' checkbox, 'Erode Mask', 'Dilate Mask', 'Exclude Points', 'Include Points', 'Reset Mask', 'Copy Mask', and 'Paste Mask' options. A 'Mask Transparency' slider is visible.
- Analyze Panel:** Features tabs for 'Roughness', 'Section', 'Histogram', and 'Particle Analysis'. The 'Histogram' tab is selected, showing a 'Global Histogram' checkbox, 'Exclude Mask Points' checkbox, and a 'Make New Histogram' button. There are also 'Close All' and 'Histogram Panel' buttons.
- Master ArGL Panel:** Includes tabs for 'New', 'Display', 'Axes', 'View', 'Lights', 'Prefs', and 'Windows'. The 'Lights' tab is active, showing a 'Shiny Factor' of 50.00% and an 'ArGLLight Panel' button.

The bottom status bar indicates the system is 'Ready- for offline', the version is 16.24.225, the user is rfuierer, and the mode is OfflineImage. The date and time are 8:13 PM Thursday 4/22/2021.



Play Movie

- AFM is for measurements
- Think about what measurement you want to make
  - Have you defined completely what the measurement is?
  - Have you considered the factors that affect it?
- **Use ZSensor, not Height, for Z in most cases**
- Be aware of how processing affects your measurement
- Do the least damage possible to your data
- Planefit modifies the whole image at once
  - Good for slope, form, bow
- Flatten modifies each line separately
  - More destructive than planefit
  - Sometimes necessary to correct stuff that happened in the time-domain
- When comparing two images, use the same processing.