GWYDDION TIPS FOR 83-411

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1. Introduction

This document describes a variety of ways to process AFM images using Gwyddion. These are methods which are likely to be useful for analysis of the data that are acquired in the Advanced Bio-Engineering Lab course (83411). This is an open-ended project, and methods will be added as they are developed by the students and staff in the course. You will find this material most helpful if you use it together with the Gwyddion User Guide.

2. Background Leveling

The sample in the AFM is never precisely perpendicular to the tip. As a result, the topological scan of the sample will include a wedge (that is, the sample will be higher at one end). In addition, the lateral (XY) scanner pivots the sample about an axis. Therefore, there is a quadratic component to the scan. We are usually interested in small structures, whose topological maps ride on top of the background. To properly visualize and analyze the objects of interest, the background must be corrected, generally by subtraction from the image.

Gwyddion includes many methods for removing background. These include:

(1) *Data Process>Level>Plane Level...*, which implements mean plane subtraction. This will eliminate the wedge, but not the quadratic component of the background.
(2) *Data Process>Level>Polynomial Background...* extends this to estimate a nonplanar background.
(3) *Data Process>Level>Flatten Base...* attempts to fit the background without including objects in its estimate.

There are other methods in *Data Process>Level*. In many cases they can be combined with a mask to work only on areas inside (or outside) the mask (see below about Data Process¿Mask). Figure 1 shows an example of leveling, and Figure 2 shows 3D renderings of the images in Figure 1.

3. Masks

It is often useful to specify specific regions within an image.
Data Process>Mask>Mark With... can do this. This can be especially useful after leveling. Figure 3 shows an example of masks created with this tool. *Data Process>Mask* includes many operations that can used to modify masks. Figure 3b was created by inverting the mask in Figure 3a. Arbitrary masks can be created manually with the mask editor tool.

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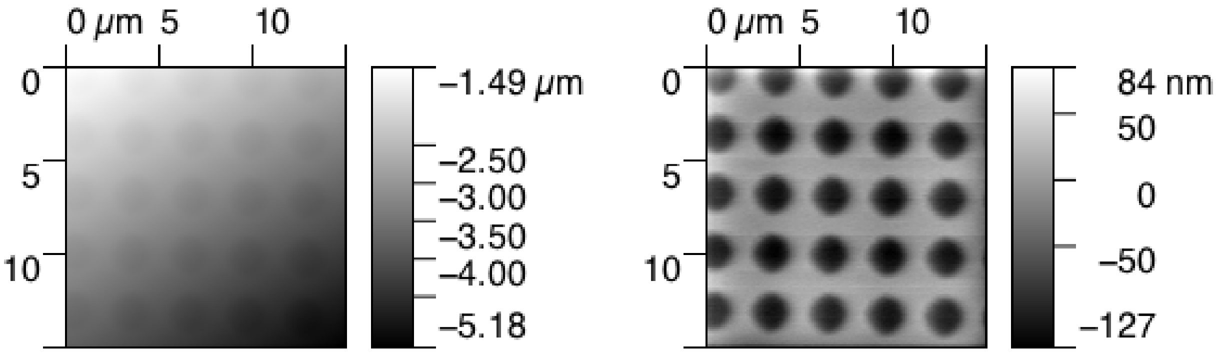
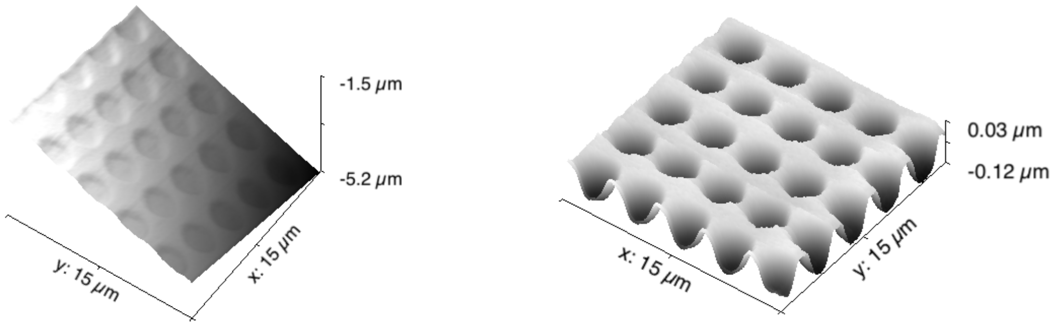


(A) (B)

FIGURE 1. SHS-1 area A (3 µm grid) (a) raw image (b) after Flatten Base leveling.



(A) (B)

FIGURE 2. 3D rendering of the image in Figure 1 (a) raw image (b) after Flatten Base leveling. Note the difference in the vertical scale.

We might not want to include the edges of a region in the masked area. Erode and dilate (as well as other morphological operations) can be used to modify the masks. Figure 3c shows the mask of Figure 3a eroded, in order to exclude the edges of the wells of the grid, and Figure 3d shows the mask of Figure 3b eroded, in order to include only the central areas of the grid.

The statistical quantities tool can be used to measure averages and variations (among other statistical parameters) for either the whole image, the area within the mask, or the area outside of the mask. Figure 4 shows the output of the statistical quantities tool for the dilated masks in Figure 3. The average height of the background is -2.4 nm, while the average height on the inside is -94.0 nm. The SHS-1 test target label states that the height is 104.1 nm. Therefore, we can conclude that the Z-calibration should be corrected by about $104/94 = 1.106$ or about 11%.

4. Exporting images to ImageJ/Fiji

AFM images are saved as "wsf" files, which consist entirely of text characters. They begin with metadata that contain a variety of instrument parameters, followed by lines which contain the image values (eg height in the case of Z_DRIVE) , and end with the line "User Comment:". *File>SaveAs...* has options for saving these images in many formats, with or without the scalebars, metadata, etc. A typical wsf file, produced by the TT-AFM, is shown in Figure 5. I often prefer to process images using the Fiji distribution of Imagej. The simplest way to export the images into Fiji is to edit the wsf image, and

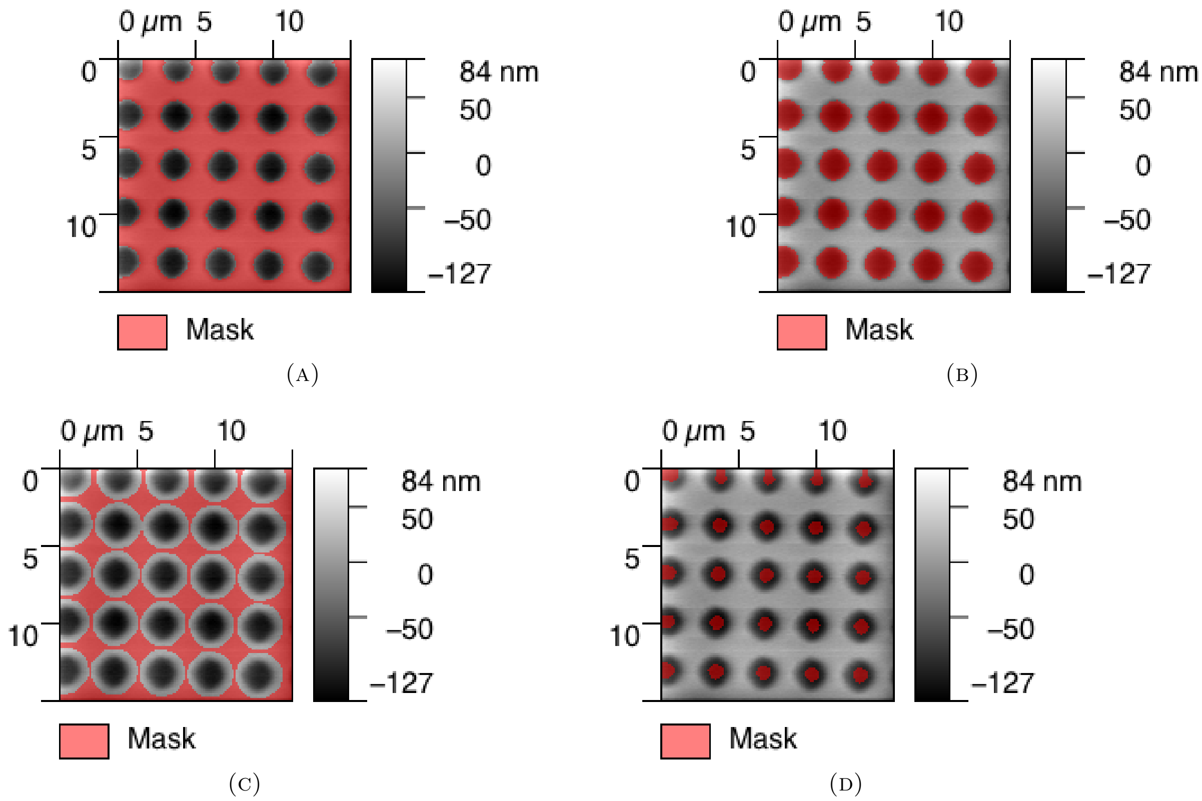


Figure 3. SHS-1 area A (3 µm grid) (a) background masked; (b) foreground masked by inverting the background mask; (c) background masked and eroded; (d) foreground masked (by inverting the background mask) and then eroded.

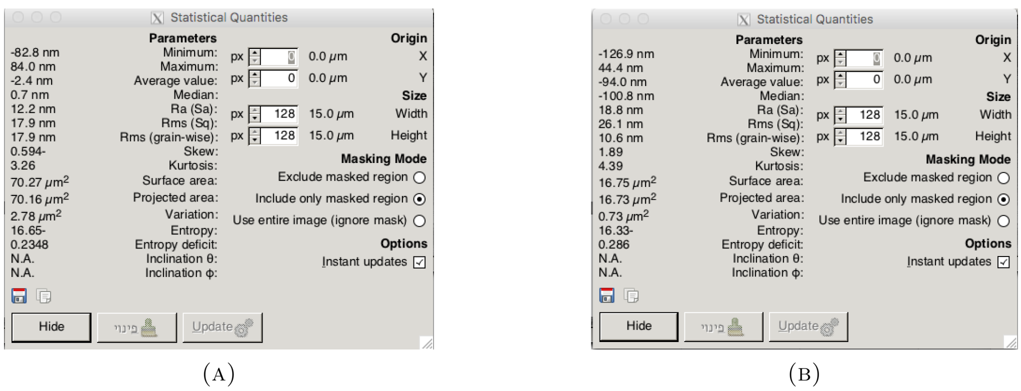


Figure 4. Screenshots of the pop-up window produced by the statistical quantities tool. (a) Results for the mask in Figure 3c; (b) results for mask in Figure 3d.

remove the metadata and User Comment line. This edited image should be saved under a different name so as not to lose the original image with the metadata. The edited image can be imported to ImageJ using *File>Import>Text Image...* . The result will be a 32bit floating point image with the correct height data. The XY calibration can be restored using the *Image>Properties...* dialog, The calibration

```
Pixels in X:     256
Lines in Y:      256
X Range:         2.000
Y Range:         2.000
Z Calibration:   0.9000
X Calibration:   0.5220
Y Calibration:   0.5060
X Offset:        5.000
Y Offset:        5.000
Rotation(∞):     0
Scan Rate(Hz):   1.00
Scan Type:       Vibrating
Vpp:     0.120
Phase:   92.09
Demod Gain :     4dB
Selected (kHz) :         170.29
Display Type:    Z_ERR
Samples/Pixel:   25
XY HV Gain:      15
Z HV Gain:       3
Z Sensor Gain:   640
X GPID:          100.000000000 %, 256, 4096, 0
Y GPID:          100.000000000 %, 256, 4096, 0
Z GPID:          1.0000, 190, 1500, 0
Setpoint:        920

-0.01483693      -0.01371827      -0.01249792      -0.02358279      ...
<256x266 matrix of floating point numbers>

User Comment:
```

FIGURE 5. wsf file format. The file is a text file, with a header that stores instrument parameters, image values as floating point numbers (stored as text), and an unused User Comment field at the end. In this image, most of the 256×256 floating point numbers have been deleted to keep the figure size reasonable.

can be computed as

$$X_{cal} = X\_Range/Pixels\_in\_X \tag{1a}$$

$$Y_{cal} = Y\_Range/Lines\_in\_Y \tag{1b}$$

where X_Range and Y_Range are the width and height of the image in physical units (usually microns), respectively, $Pixels\_in\_X$ and $Lines\_in\_Y$ are the width and height, respectively, of the image in pixels (typically 128×128, 256×256 or 512×512, but height and width might be different if the scan was stopped before completion), and X_{cal} and Y_{cal} are the calibration in physical units/pixel (typically microns/pixel) that should be entered into the properties menu that *Image>Properties...* displays. The parameters on the right-hand-side of Equations 1a and 1b are found in the image file metadata (see Figure 5).

5. FFT Filtering

Our AFM currently suffers from periodic noise, that was recently found to be mechanical in origin, and amplified by the large mount of the optical microscope. This provides an opportunity to demonstrate the use of 2D FFT filtering remove (or at least attenuate) this noise. The 2D FFT filtering functions can be found in *Data Process>Correct Data>2D FFT Filtering...*

Figure 6 shows an example of this process. The image in Figure 6a includes periodic spatial noise. The FFT of this image appears in Figure 6b. The part of the FFT under the dark vertical bars was suppressed, and the inverse FFT of this filtered spectrum appears in Figure 6c. The periodic noise is greatly suppressed. The periodic noise is the inverse FFT of the part of the spectrum that is under the dark bars. This is shown in Figure 6d.

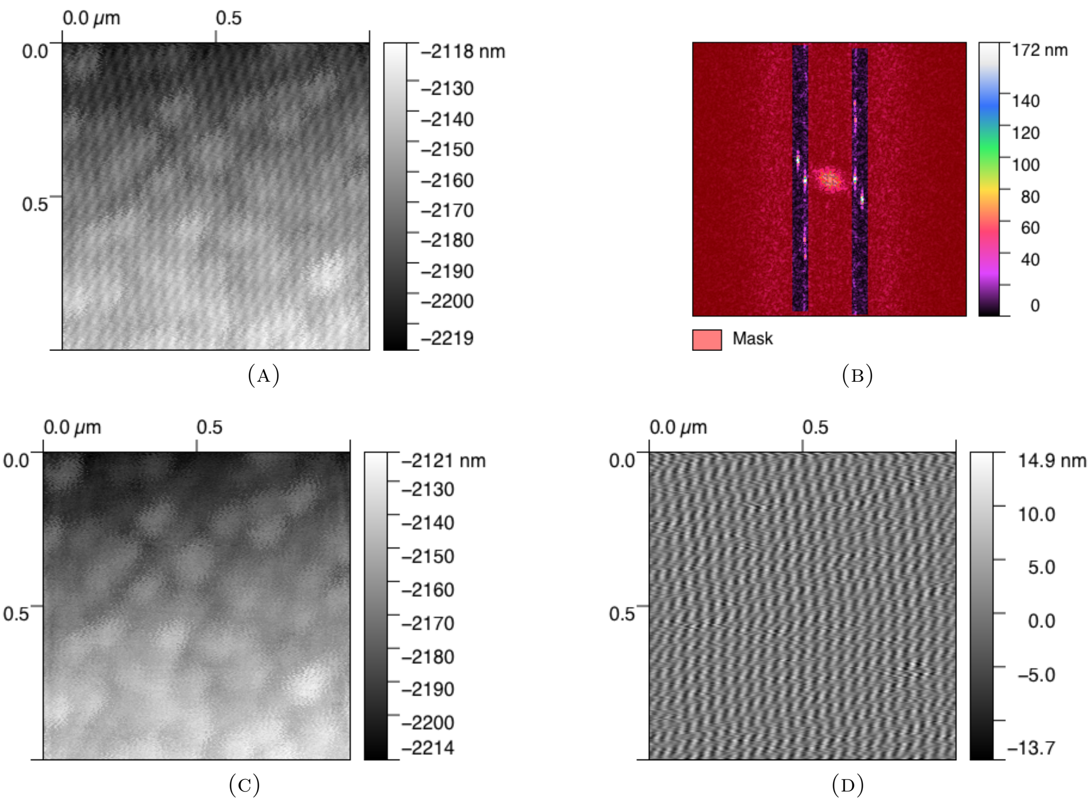
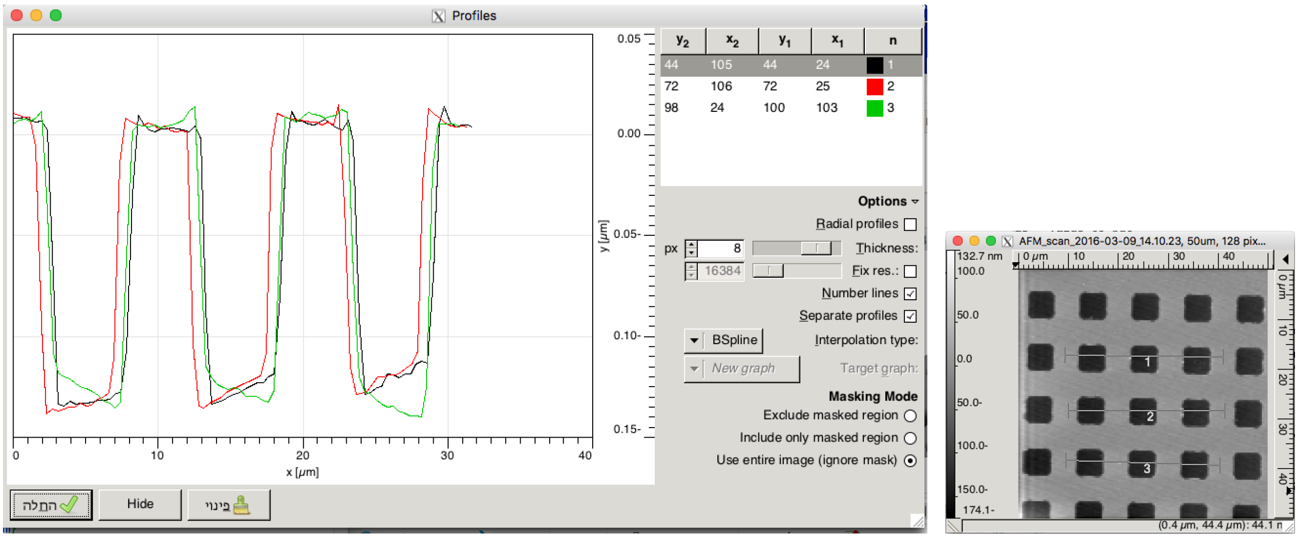


FIGURE 6. Tipchecker scan (a) unfiltered image, 256×256; (b) FFT of the Tipchecker image. The dark vertical bars are the parts of the FFT that will be suppressed; (c) Filtered image – inverse FFT with the areas under the vertical bars removed; (d) Periodic noise – inverse FFT of the the vertical bars.

6. Line profiles and lengths

Line intensity profiles can be drawn using the line profile tool. Figure 7 shows the interface for this tool together with an image which shows the lines along which the profiles were measured. The thickness parameter that appears in the menu (in this case set to 8 pixels) is the thickness of the line along which the profile is measured. For example, each point of the profiles in Figure 7 is an average of 8 pixels perpendicular to the line along which the profile was measured. The profiles can be extracted to



(A) (B)

FIGURE 7. Line profile tool: (a) Line profile tool menu, with three profiles drawn; (b) Image from which the profiles were measured. The short vertical lines at the ends of the horizontal lines show the thickness of the lines along which the profiles are measured (in this case 8 pixels).

individual graphs or to a single graph, and the data can be saved as images or a list of numbers. A variety of processing and export options for graphs can be found in the various *Graph* submenus. See the User's Guide for more details.

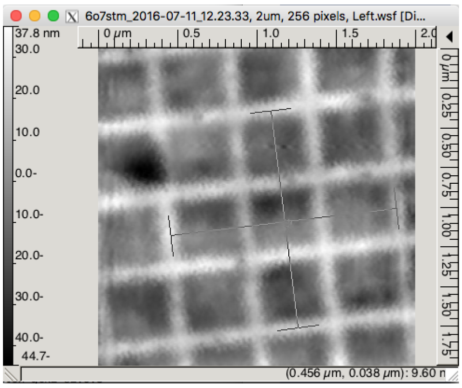
The distance tool is closely related to the line profile tool. The lines drawn with the line profile tool can be measured with the distance tool.

When the image contains a regular array of objects (as is the case for many of our calibration targets, such as the SHS-1 that appears in this document), then the thickness parameter in the line profile tool can be set to a very large value, which makes it easy for the user to align the line profile with the grid. The distance tool can then be used to record the length of the line.

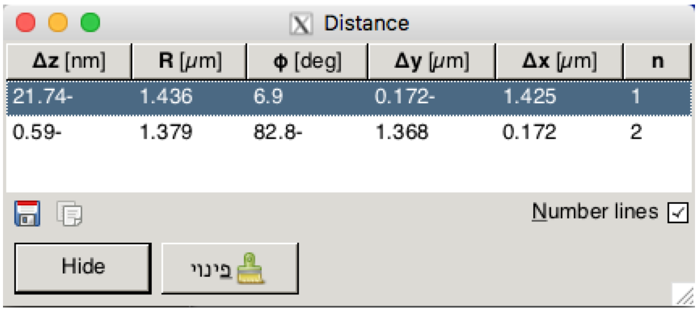
7. Lateral (XY) scanner calibration with a grid that is not aligned to the scanner axes

Calibration grids are used to calibrate the scanner. If the grid has a well defined height, then it can be used to calibrate the Z-piezo as well. Consider the waffle pattern shown in Figure 8. This grid is specified as having 2100lines/mm, which corresponds to a period of 476.2 nm. The two lines that are overlaid on the image of the grid each cover three periods, and should be $3\times476.2\,\text{nm} = 1428.6\,\text{nm}$. The measurement window that appears in Figure 8 demonstrates two problems that appear to be caused by incorrect calibration of the scanner:

(1) The lengths are not as expected.
(2) The lengths are not equal, which means that the errors in the two piezos are not equal.



(A)



(B)

FIGURE 8. Line profile tool: (a) Waffle test pattern with two line selections shown; (b) Measurements of these two lines, including total length R, Δx, Δy, and the angles relative to horizontal.

Our goal is to determine the correction factors for the X and Y axes of the scanner. These correction factors are defined in Equations 2a and 2b.

$$\Delta x_{true} = a \times \Delta x_{measured} \tag{2a}$$

$$\Delta y_{true} = b \times \Delta y_{measured} \tag{2b}$$

where Δx_{true} and Δy_{true} are the true distances which the scanner moved, $\Delta x_{measured}$ and $\Delta y_{measured}$ are the distances that the instrument reported, and a and b are factors which correct these reported distance to produce the true distances. Equations 3a and 3a are two equations that can determine a and b.

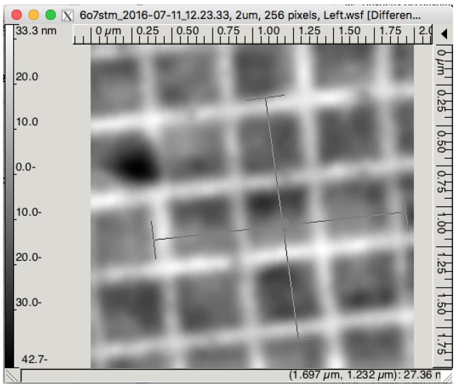
$$P^2 = a^2 \times \Delta x^2_{1measured} + b^2 \times \Delta y^2_{2measured} \tag{3a}$$

$$P^2 = a^2 \times \Delta x^2_{2measured} + b^2 \times \Delta y^2_{2measured} \tag{3b}$$

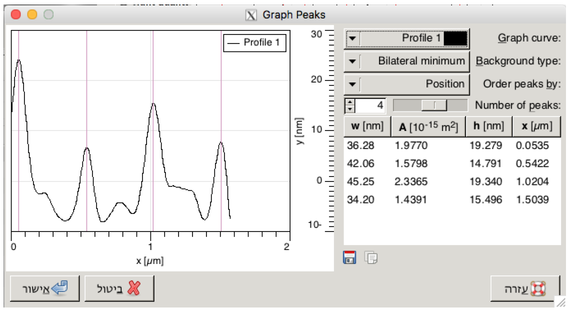
where P is the known spatial period, $\Delta x_{1measured}$ and $\Delta y_{1measured}$ are the displacements measured in one of the line selections, and $\Delta x_{2measured}$ and $\Delta y_{2measured}$ are the displacements measured in the second line selection. For the image in Figure 8a, $a = 0.978$ and $b = 1.042$.

In Figure 8a, the edges of the lines were positioned manually. We can improve on this by extracting the line profiles and using *Graph>Find Peaks...* to locate the peaks in the waffle pattern, as illustrated in Figure 9. The image was smoothed with the mean filter that is provided in the basic filters tool, so that local noise would not influence the peak position.

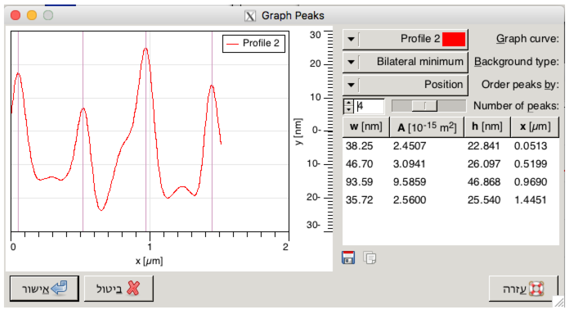
7.1. Determination of lateral calibration with Lattice Parameters. Gwyddion has a Measure Latice function (*Data Process>Measure Features>Lattice...*) that can be used to find the lateral distances between the features of the grid patterns This measure makes maximal use of the available data, but it does not provide information on uniformity. Figure 10 shows the output of the lattice measurement for an HS100MG test target, measured over a grid with 5 µm pitch. Ideally, the grid should be oriented parallel to the scan axes, is the case in Figure 10. However, it is difficult at best to get the orientation exactly correct, and it is also not necessary. The projections of the distances can be used to correct for the angles and separate the errors in X and Y.



(A)



(B)



(C)

FIGURE 9. Line profile tool: (a) Waffle test pattern, smoothed, with two line selections shown; (b) Profile of the nearly horizontal line, showing 4 peaks; (c) Profile of the nearly vertical line, showing 4 peaks; The image was smoothed with the mean filter that is provided in the basic filters tool. The filter size was set to eight. Note that the line profile have been extended to include the entire white border, so that a peak would be found.

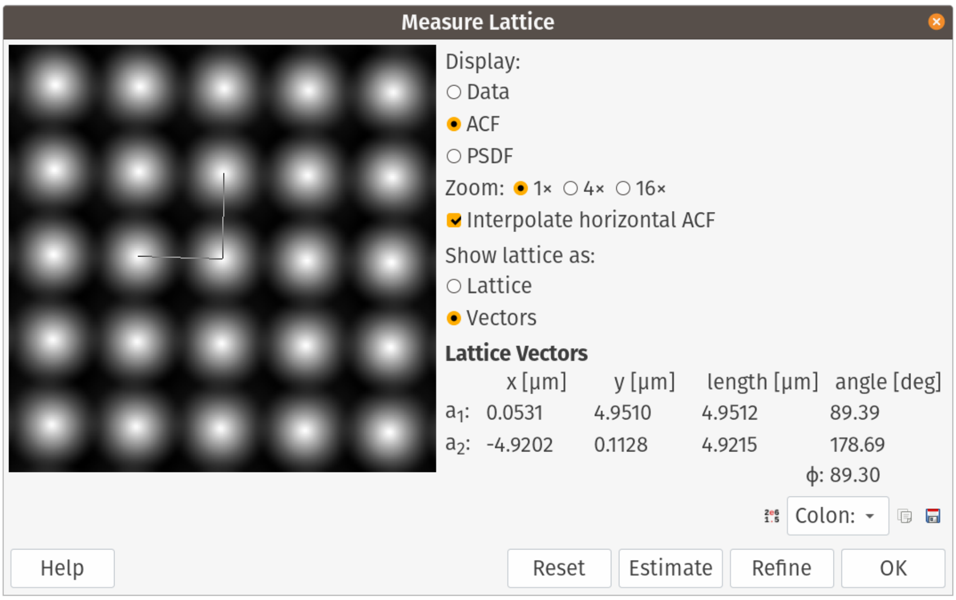


FIGURE 10. Lattice parameters for an HS100MG test target.The specified pitch for this area is 5 µm. The measured length is within 1-2% of the specification.

8. SiC-STEP calibration sample (SiC/1.5)

The SiC/1.5 calibration sample povides a series of 1.5 nm steps in a cleaved crystal of silicon carbide. The sample is a staircase, which means that it cannot be leveled with a global leveling operation.

Figure 11a shows a raw image of an SiC/1,5 target. Figure 11b shows the image after leveling with a global 5^{th} order polynomial. The line profile in Figure 11d shows that steps create an easily identified transition, but the steps "droop" and no longer appear as steps. Figure 11c shows image (b) after plane leveling in the masked area only. Figure 11e shows the line profile across the steps in the image shown in 11c. The staircase is seen, although there is still a bit of droop. The mask and lines along which profiles were taken are shown on the images.

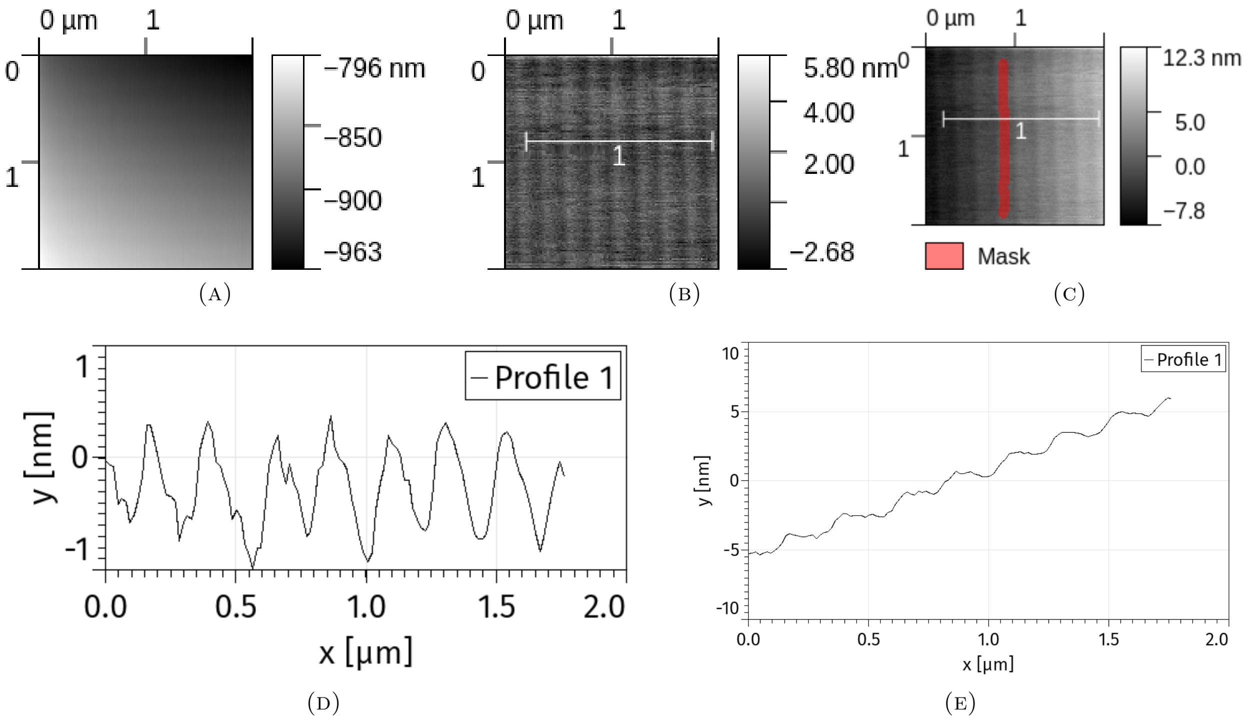


Figure 11. SiC scan (a) rawimage, 128×128; (b) Image (a) after polynomail level (5^{th} order).; (c) Image (b) after plane level applied only to the masked region, indicated in red; (d)line profile of (b). Notice that he staircase is pulled down so that the stairs all appear at the same height. (e)line profile of (c). Now the staircase is seen, although there is still a bit of droop.

A study of the SiC targets that was done using the lab's AFMs can be found here. This document demonstrates the use of a relatively new tool in Gwyddion called `Fit terraces...` that enables one to estimate terrace spacing for imperfectly leveled images.

9. Processing AFM images of Gwyddion output with ImageJ/Fiji

Images that have been processed within Gwyddion (eq, using the various leveling or filtering algorithms that Gwyddion includes) can be exported for further processing within ImageJ/Fiji. However, the lateral and height scaling is not preserved when exporting the values in PNG or TIFF formats. This section includes links to two Python scripts that can allow such images to be read into ImageJ together with the spatial calibration.

(1) A script to read the wsf files generated by the AFMWorkshop TT-AFM can be found here.

(2) Image files that have been processed within Gwyddion (eg, with level, Fourier filtering, or other operations within *Data Process...*) can be saved as ASCII image files. In this case, the actual height values in nm are preserved. If the information comment header is included, then the saved images will have four lines of metadata that include the width and height in µm, the channel label, and the units. A script that can read these files, and extract the metadata (if present) can be found here. The script will prompt for the image dimensions (in µm) if the metadata are not present.

10. Things I would like to know

Here are some things that will be added to this document when we figure out how to do them.

(1) annotate the images with either text or shapes
(2) automatically measure the distances between a collection of points or objects.
(3) Change the color of selections. This would make the line selections in Figure 8 and Figure 9 visible.