AFM image processing and analysis

All measurements and measurement techniques are prone to artefacts. In AFM imaging, these artefacts are sometimes easy to spot and sometimes very difficult. Some artefacts can be easily avoided, if the user knows what to look for and knows the source of the artefact. A few artefacts are unavoidable, but knowing that they exist in an image helps to avoid misinterpreting them as genuine image features. This means that recognizing image artefacts is very important for the AFM user.

Therefore, some image processing is usually necessary before viewing or analyzing any AFM image. All processing is done with the aim of clarifying the data obtained during measurement. In other words, the purpose is to make it easier to measure and observe the features that have been measured.

Because image processing operations can also introduce artefacts in the image, they should only be used when necessary! Which processing operations will be used depends highly on the image and the properties we want to measure.

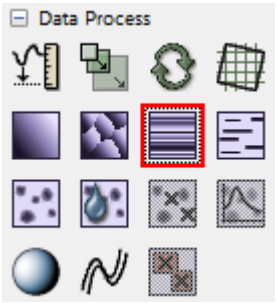
For the processing of the AFM images we will use ***Gwyddion***, which is free and open source software for SPM (*Scanning Probe Microscopy*) data visualization and analysis. All information about this software can be found at http://gwyddion.net

1. Levelling

Various types of image levelling operations are usually the first processing operations carried out on the AFM image data and often the only processing step used on AFM image data.

1.1. Line median matching

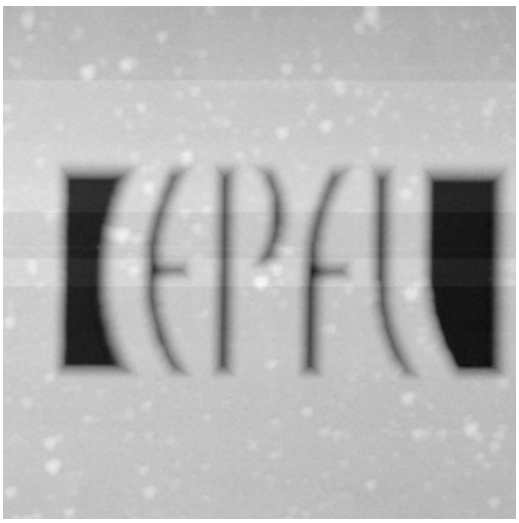
During imaging it can sometimes happen that cantilever change the level of interaction with sample or some mechanical disturbance can cause shift in the laser beam deflection. This will cause the jumps in the AFM height image on the slow scanning axis. That is why it is sometimes necessary to first do the line by line height median matching.



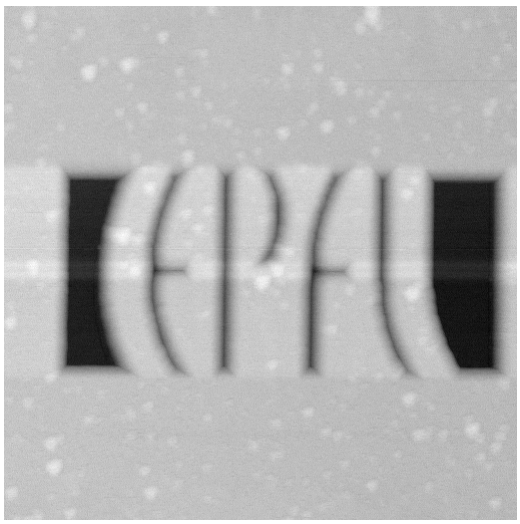
To do this in Gwyddion go to

Data Process -> Correct lines by matching height median

This will do the height line median matching for all horizontal lines of the AFM image.



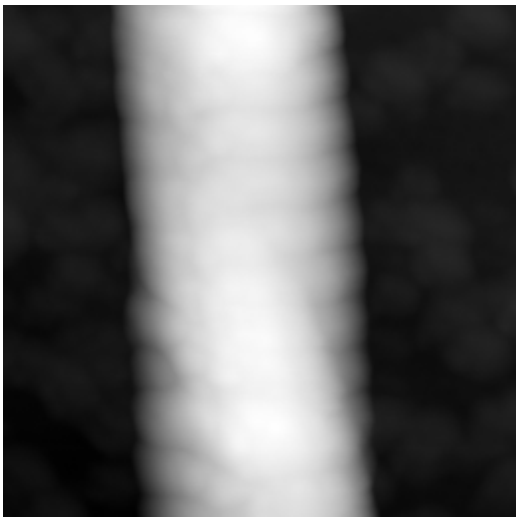
Before



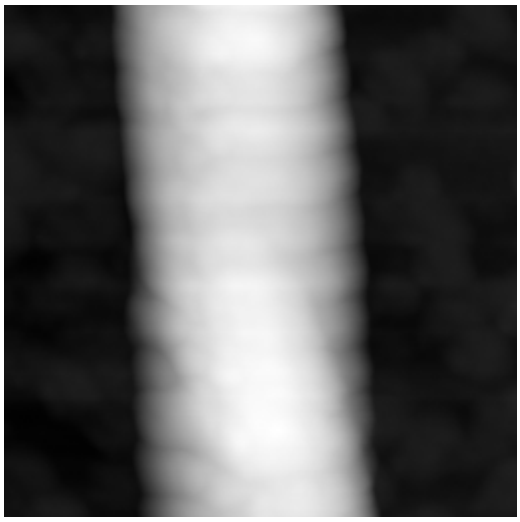
After

Image 1. AFM image of the EPFL logo grating before and after the line by line height median subtraction operation

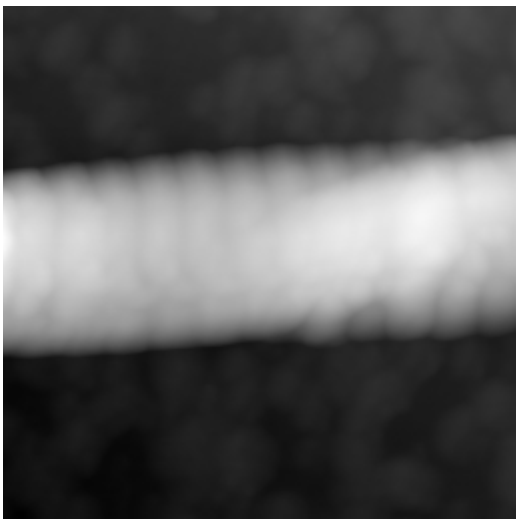
On the images above, you can observe that, while this operation did some successful leveling on the upper part of the image, it introduced new leveling artifacts on the border areas of EPFL logo. So this operation should not be used on the images where there is a horizontal sample with large height difference with respect to the background. Misuse of this operation could also be explained on the example of vertically and horizontally imaged collagen fiber, on the images below.



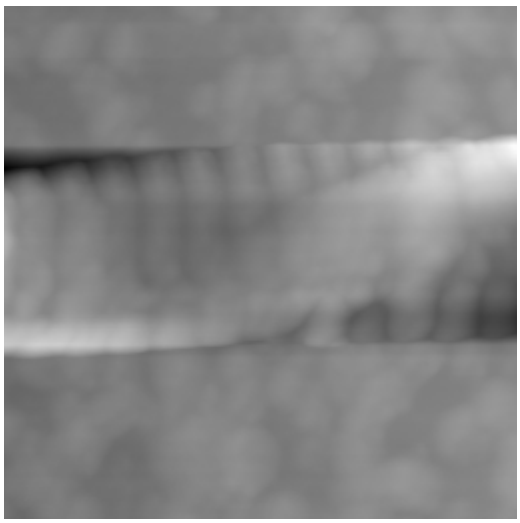
Collagen fiber, vertically imaged, before line median matching operation



Collagen fiber, vertically imaged, after line median matching operation ☺



Collagen fiber, horizontally imaged, before line median matching operation



Collagen fiber, horizontally imaged, after line median matching operation ☹

Image 2. AFM images of vertically and horizontally imaged collagen fiber, before and after the line by line height median matching operation

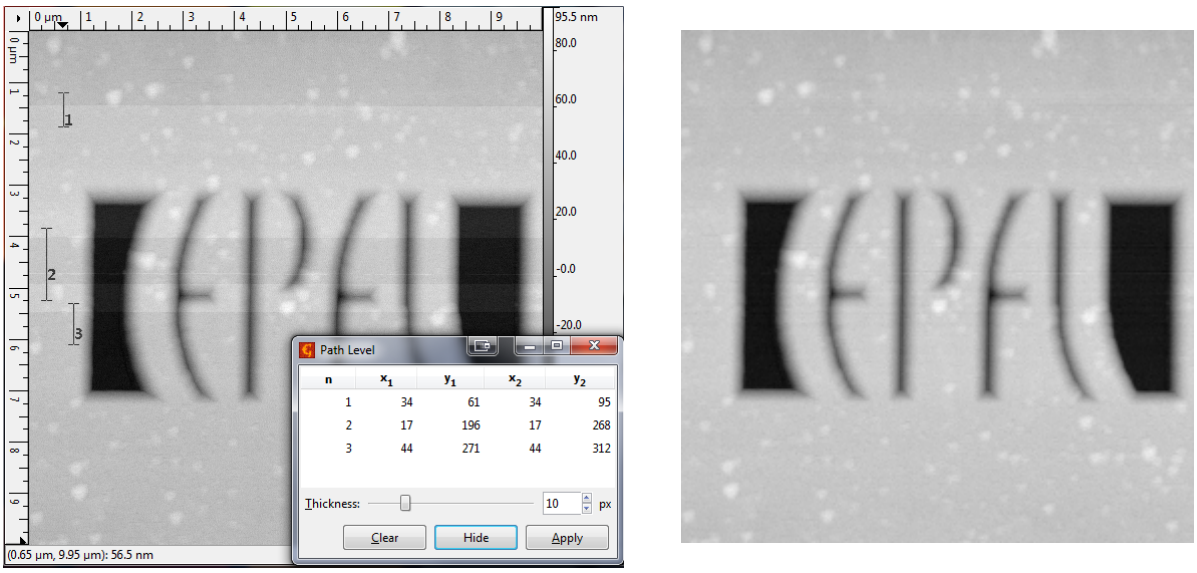
In the case of horizontally imaged collagen fiber, we lost the height information of our collagen sample by doing the line median matching operation!

In the case of the artefacts on the EPFL logo image, these can be better removed by using the **Tools->Level rows using intersections with given lines**. With this operation we can actually select on which part of the image we want to apply line median matching operation. This is shown in the images below.

To do this in Gwyddion go to

Tools->Level rows using intersection with given lines

Select the lines on which you would want to do the line median subtraction and click **Apply**.



Before After

Image 3. AFM image of the EPFL logo grating before and after the line by line height median matching operation on specific areas

1.2. 1st order plane levelling

If the background in the image (such as the substrate on which the sample was deposited) has considerable tilt in it, the change in height of the background will mask the changes in height associated with the sample. This effect is shown in the image below.

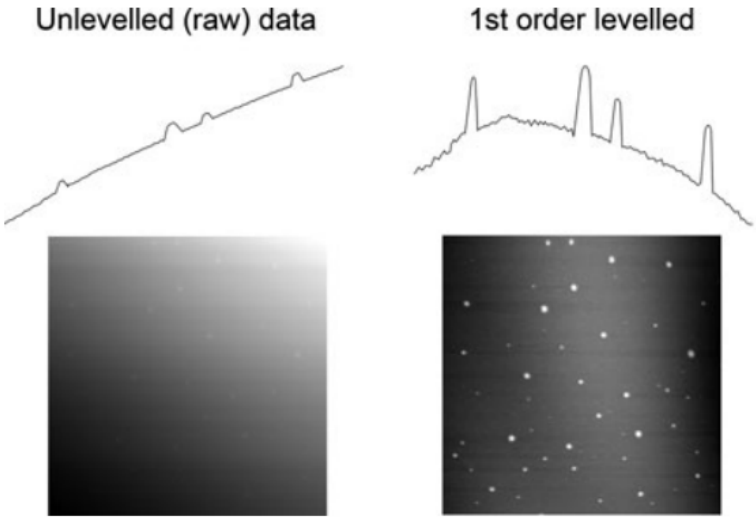
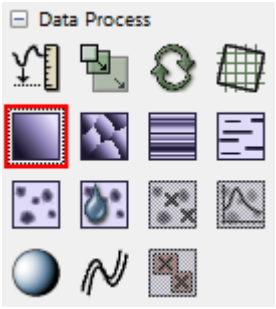


Image 4. AFM image before and after background tilt removal

This effect can be removed in two ways:

1.2.1. Plane subtraction



In order to do the plane subtraction, in Gwyddion go to

Data process->Level data by mean plane subtraction

By clicking on it, Gwyddion will automatically calculate the background plane and subtract it from the image.

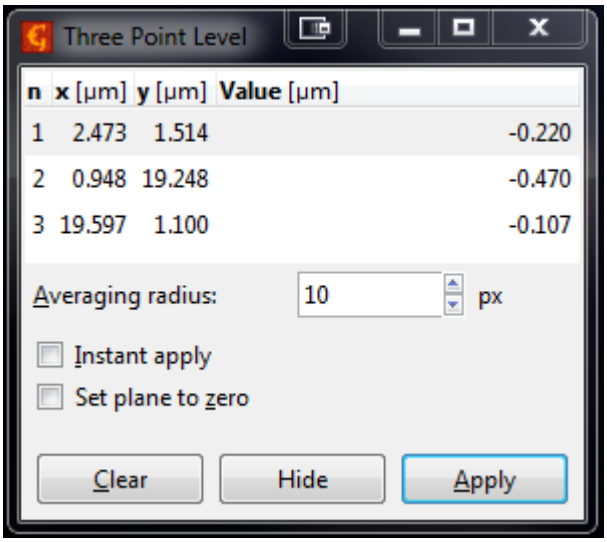
This operation should not be applied if the sample has large height difference with respect to the background because it will not properly flatten the background tilt!

1.2.2. Three-point fitting

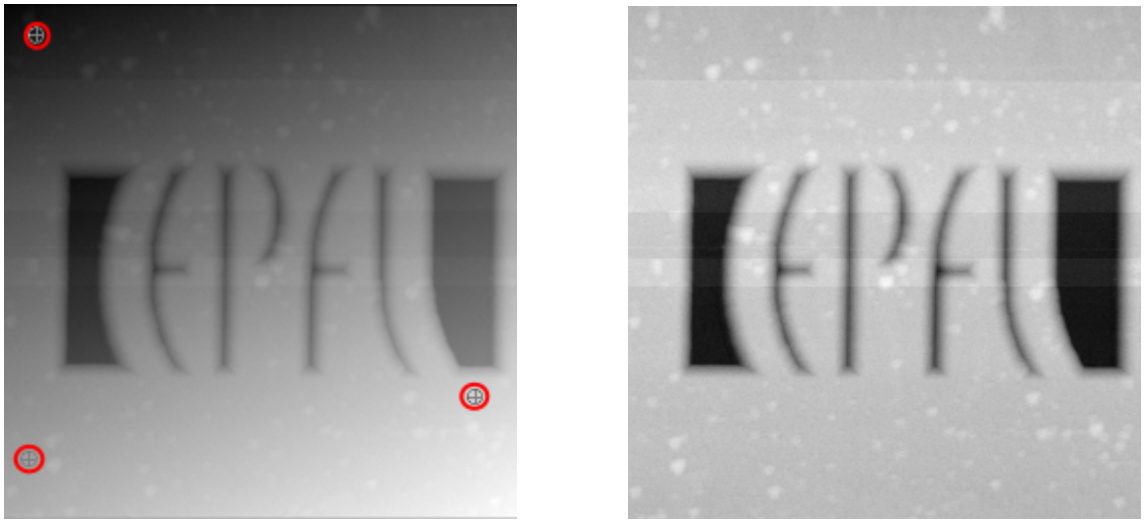
This procedure is similar to plane subtraction, but is a rather more 'manual' and often more accurate approach. In this method, the AFM user identifies three points on the image to define the plane to subtract.

In order to do the three-point fitting, in Gwyddion go to

Tools -> Level data by fitting the plane through a three points



Set **Averaging radius** of the points to 10 px. Now select 3 points in image background, as far away from each other as possible and click **Apply**. Image should now be levelled. Click **Clear** to remove the points and close **Three point level** window.



Before After

Image 4. AFM image before and after three-points plane fitting

1.3. Higher order levelling

A common problem in AFM images is scanner bow. It occurs mainly in instruments that use tube scanners, and is caused by a swinging motion of the free end of the scanner. This leads to a curve in the image plane as shown in image below.

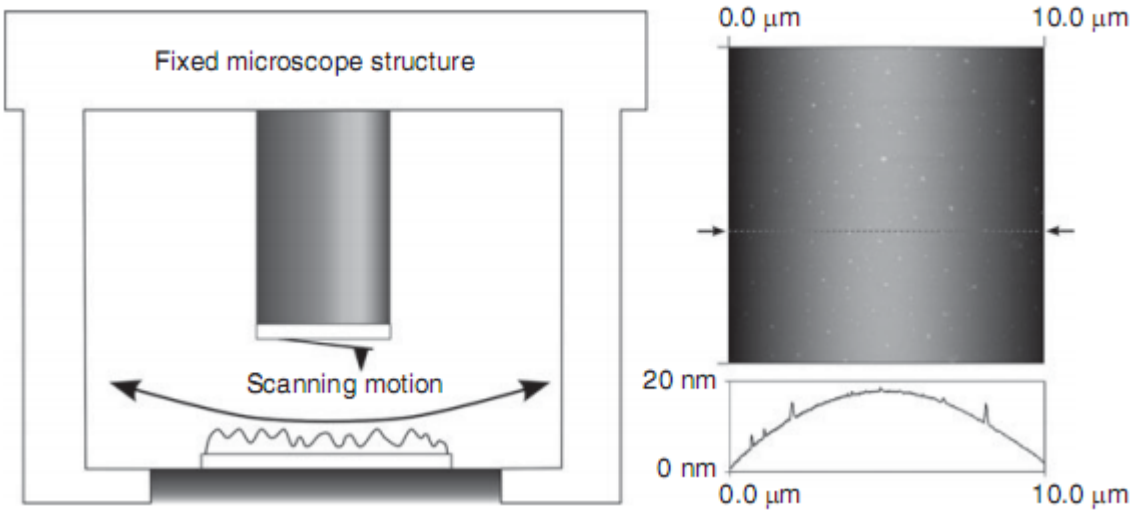
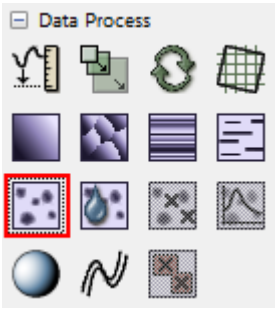


Image 5. Effect of scanner bow on the AFM image

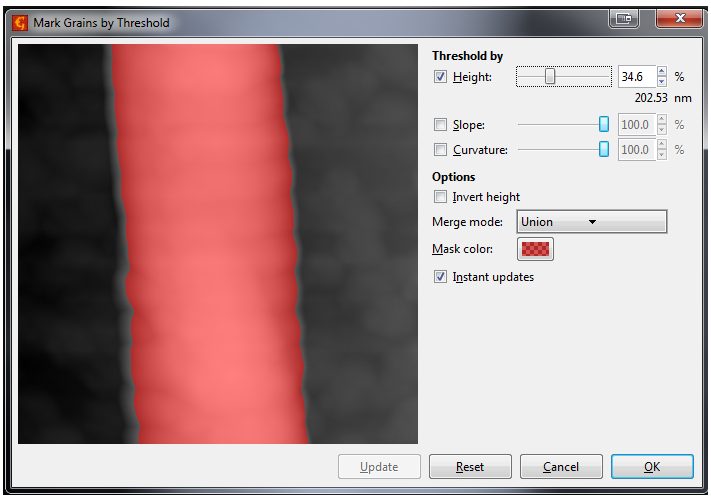
In order to remove this curve and similar image distortions that can arise from AFM system, or the background tilt - if the background is not perfectly flat, we will use higher order polynomial two-dimensional curve fitting. This processing works well where the background (on which the sample is deposited) is really flat, and does not include any curvature. Typical examples of this case would include nanoparticles, micro-organisms on glass slides, or individual molecules on mica.

If AFM microscope uses a flexure scanner instead of a tube scanner, scanner bow will not be an issue.

But, before doing this step, we would like to select our sample to exclude it from the two-dimensional curve fitting, because we want to do the 2D curve fitting only on the sample background.

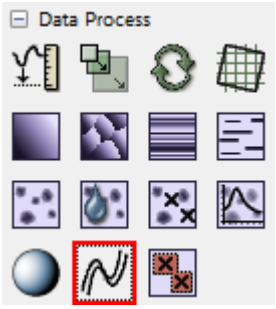


To do this in Gwyddion go to
Data Process -> Mark grains by threshold

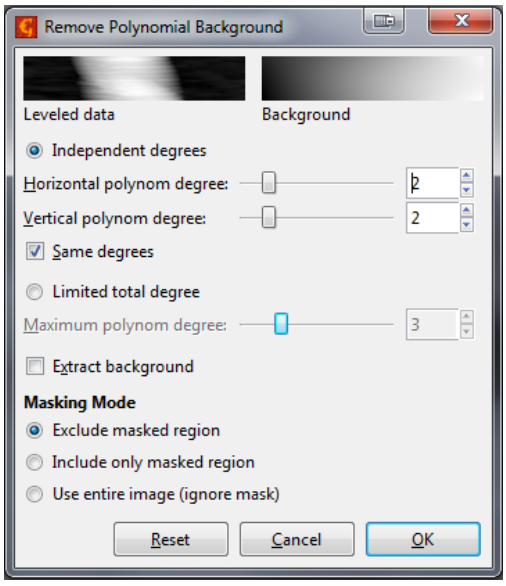


Check **Threshold by**: **Height** and change the percentage of the **Height** parameter until the entire sample is selected, then click **OK**

Now we will do the higher order polynomial two-dimensional curve fitting.



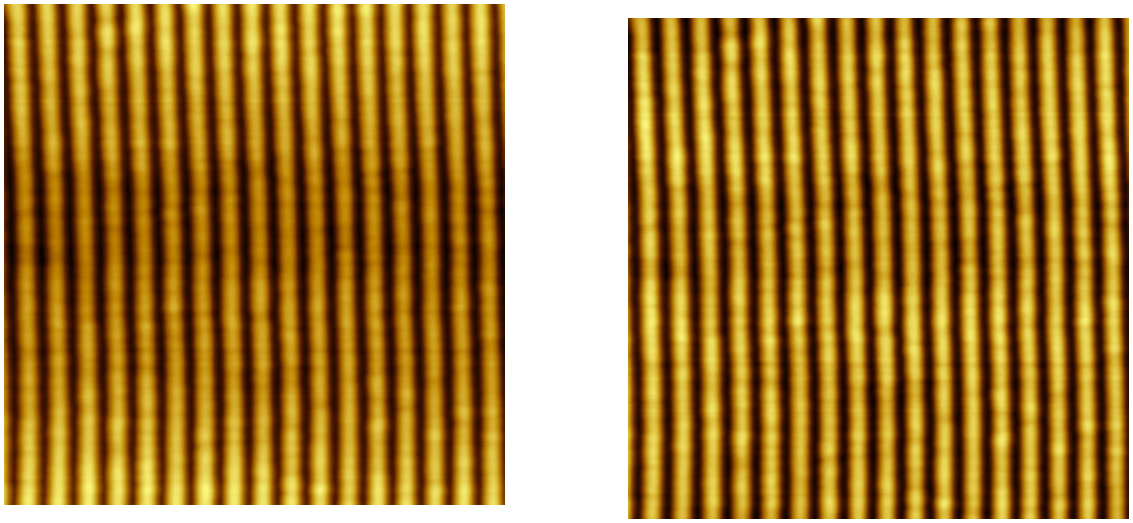
To do this in Gwyddion go to
Data Process -> Remove polynomial background



Set value for **Horizontal polynom degree** and **Vertical polynom degree** parameters (2-3).

In the **Masking Mode** part check the **Exclude masked region** option. This will exclude our previously selected sample from the 2D curve fitting.

Click **OK**.



Before After

Image 6. AFM image before and after higher order 2D curve levelling

After this is done, to deselect the sample selection, again go to **Data Process -> Mark grains by threshold**, uncheck the **Threshold by**: **Height** and click **OK**.

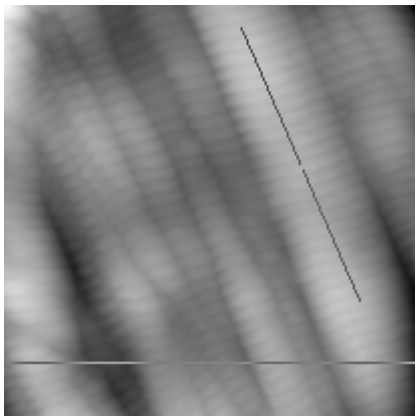
2. Extracting profiles

Many times it is necessary to extract line profiles from the image in order to examine certain sample characteristics. Since it is usually difficult to measure dimensions directly from AFM images, line profiles are usually extracted in order to measure dimensions from the AFM images. Gwyddion allows the user to arbitrarily define lines to be extracted, and these can be at any angle.

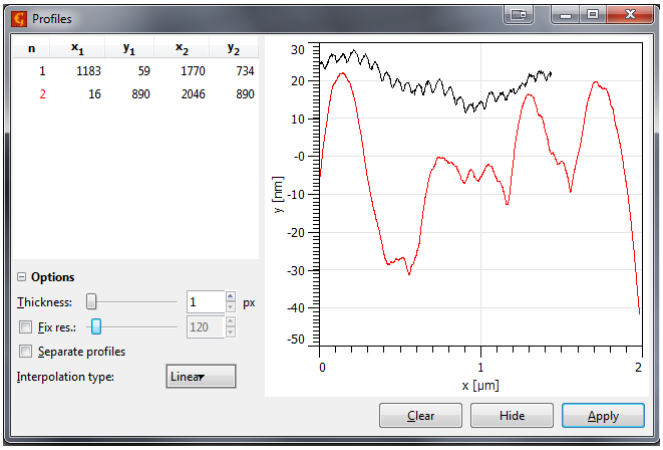
To do this in Gwyddion go to

Data Process -> Extract profiles

Clicking once on this button external window will open to show line profiles.



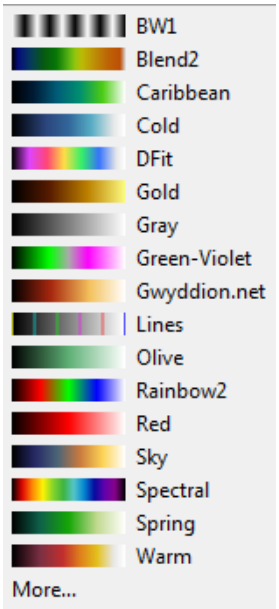
Draw a line using mouse. If you hold **SHIFT** button while doing this, lines will be drawn only under certain angles (use this to draw horizontal and vertical lines). You can draw more than one line.



In the external window you will get line profiles.

3. Color palettes

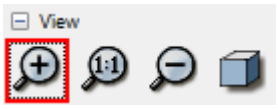
The color palette used to display an AFM image can be selected to make the image seem more visually compelling. In some cases, selecting a specialized color pallet can help with visualizing certain aspects of an image.

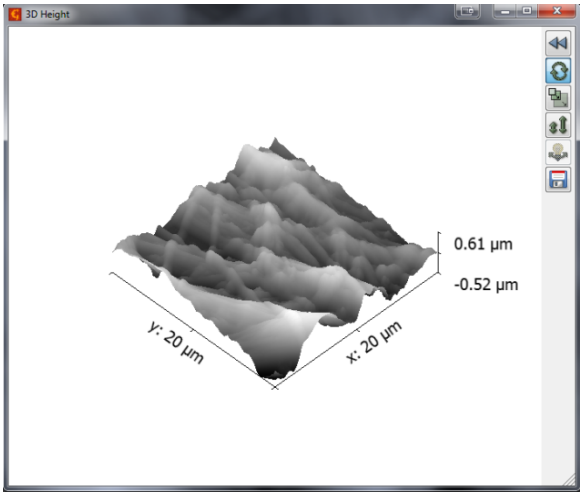


In Gwyddion you can change the color palette by right clicking with mouse on the colorbar located on the right side of an AFM image.

4. 3D Data representation

AFM height data is inherently three dimensional (3-D). However, the standard method of rendering AFM data shows a two-dimensional (2-D) image, using a color scale to represent height information. This is not a normal way for humans to see shapes, and can make interpretation difficult. In particular, for viewers unused to AFM data, it can be difficult to determine which features are higher than others, etc. One way to overcome this is to render the height information as a pseudo-three-dimensional image. The ability to display images that show what we want is vital, to make use of AFM data.





To present AFM image in 3D, in Gwyddion go to

View->Display a 3D view of data

This will present AFM image in 3D. On the right, there is a menu to process 3D image.

Use this button to enter advanced menu with possibilities such as choosing a color palette, choosing whether to show labels and axis, choosing type of lighting etc.

Use this button to rotate 3D AFM image.

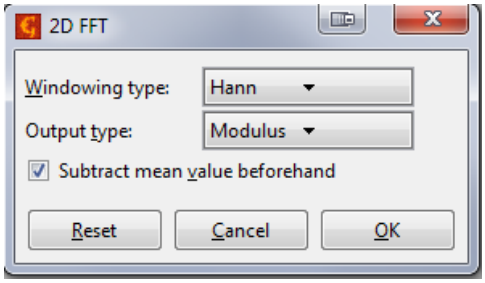
If you selected the **Lighting** type of image presentation in advanced menu, use this button to change the spot of the light source.

Use this button to save 3D image.

5. 2D FFT Analysis

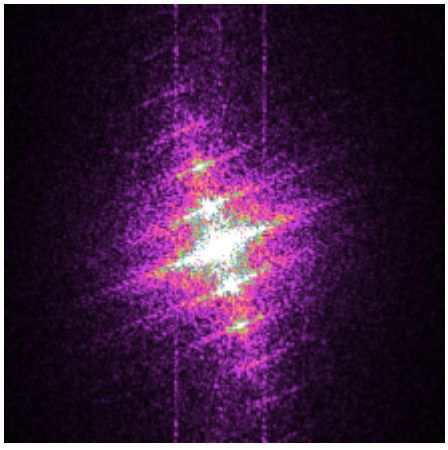
In AFM image processing and analysis, a two-dimensional Fourier transform is an operation that converts the AFM image from the spatial domain, into the frequency, or more correctly, the wavelength domain. This is carried out by a mathematical operation known as a fast Fourier transform, so is sometimes also known as FFT analysis. When transformed into Fourier space, the image will show features in terms of wavelength (or frequency). This is particularly useful to identify any repeating patterns in the image. For instance, 2D FFT can be used to calculate period of atomic lattice parameters, depth of corrugations on the collagen fibers etc. The Fourier transform can also be used to identify the frequency of noise in an image and to remove it.

To do the 2D FFT in Gwyddion first open an image you want to apply it to. 2D FFT image will be much clearer if it is applied to the squared image (equal number of pixels on horizontal and vertical axis). So, to do this first go to **Data Process->Basic Operations->Square Samples**. Then do the 2D FFT by going to **Data Process->Integral Transforms->2D FFT**.



External window will be opened to set FFT parameters. You can leave default parameters and click **OK**.

You can click on the **View->Zoom in** button to zoom into 2D FFT image details.



Use on this image the **Extract profile** tool (which has already been explained), to determine period and spatial frequency of the measured pattern (e.g. period of the collagen corrugations).

Gwyddion file handling

Opening file:

Go to **File->Open** and then find the directory of the file you want to open. Once you click on the file, on al the images that the file contain will be shown on the right.

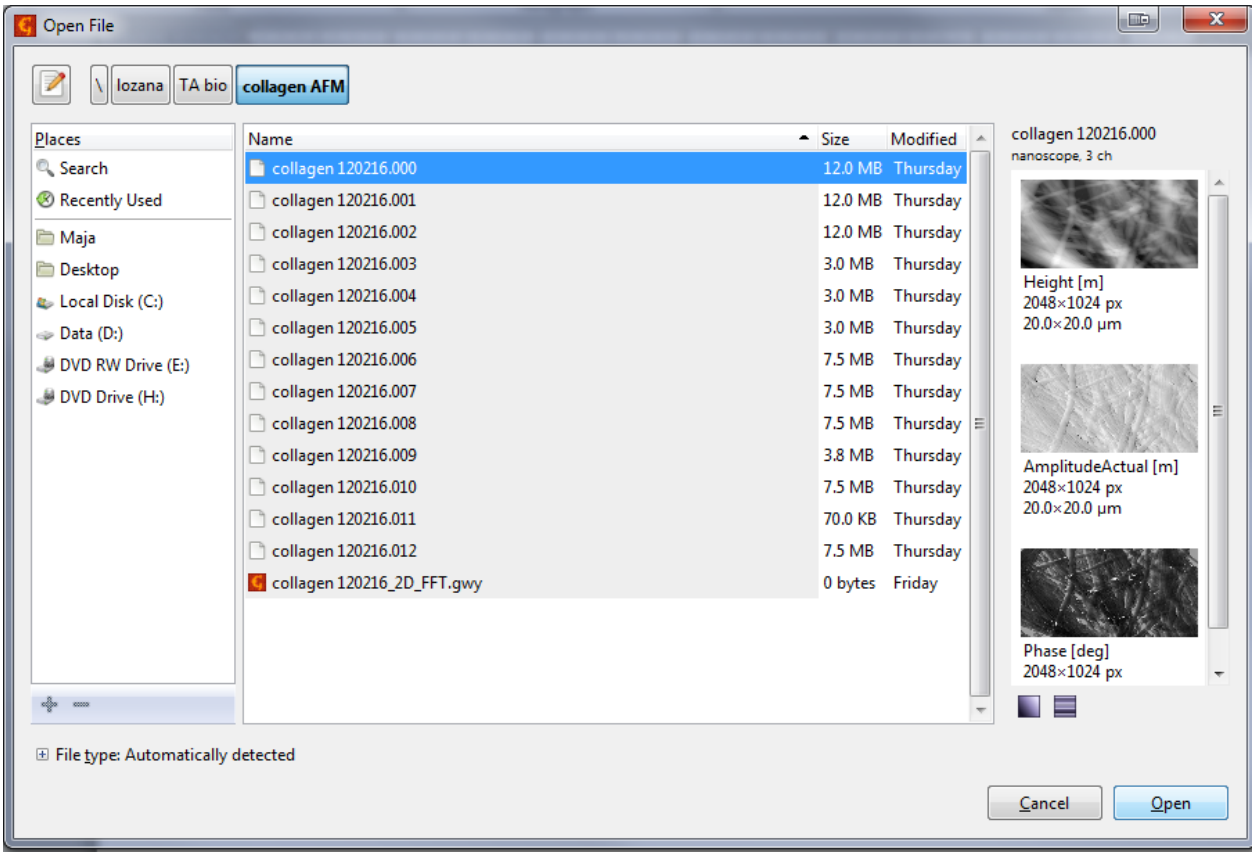


Image 7. Opening file in Gwyddion

Once the file is opened, to open/close desired images, that you wish to process and analyze go to **Meta->Show Data Browser**. The images that are checked will be opened. But be aware that if you uncheck all of them – the opened file close!

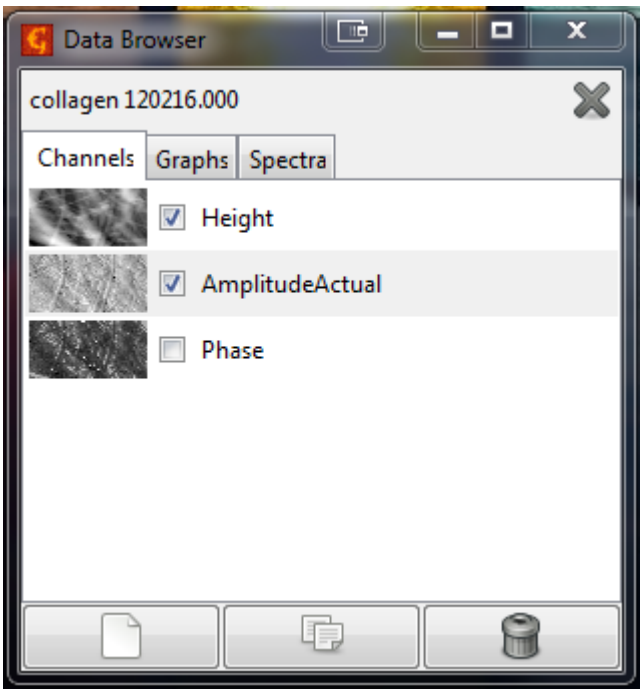


Image 8. Opening images within the file in Gwyddion

Saving file:

To save the file (containing all the images) go to **File->Save as**. Find the folder you wish to save your file in. Then, at the bottom of the window chose the proper extension of the file **.gwy**. On the top give the desired name to your file, but make sure to type it with the extension, e.g. **name_of_the_file.gwy**. Then click **Save**. You can also save just single image, by choosing one of the image formats, but also make sure to type the desired image name with the proper extension (e.g. **name_of_the_file.png**).

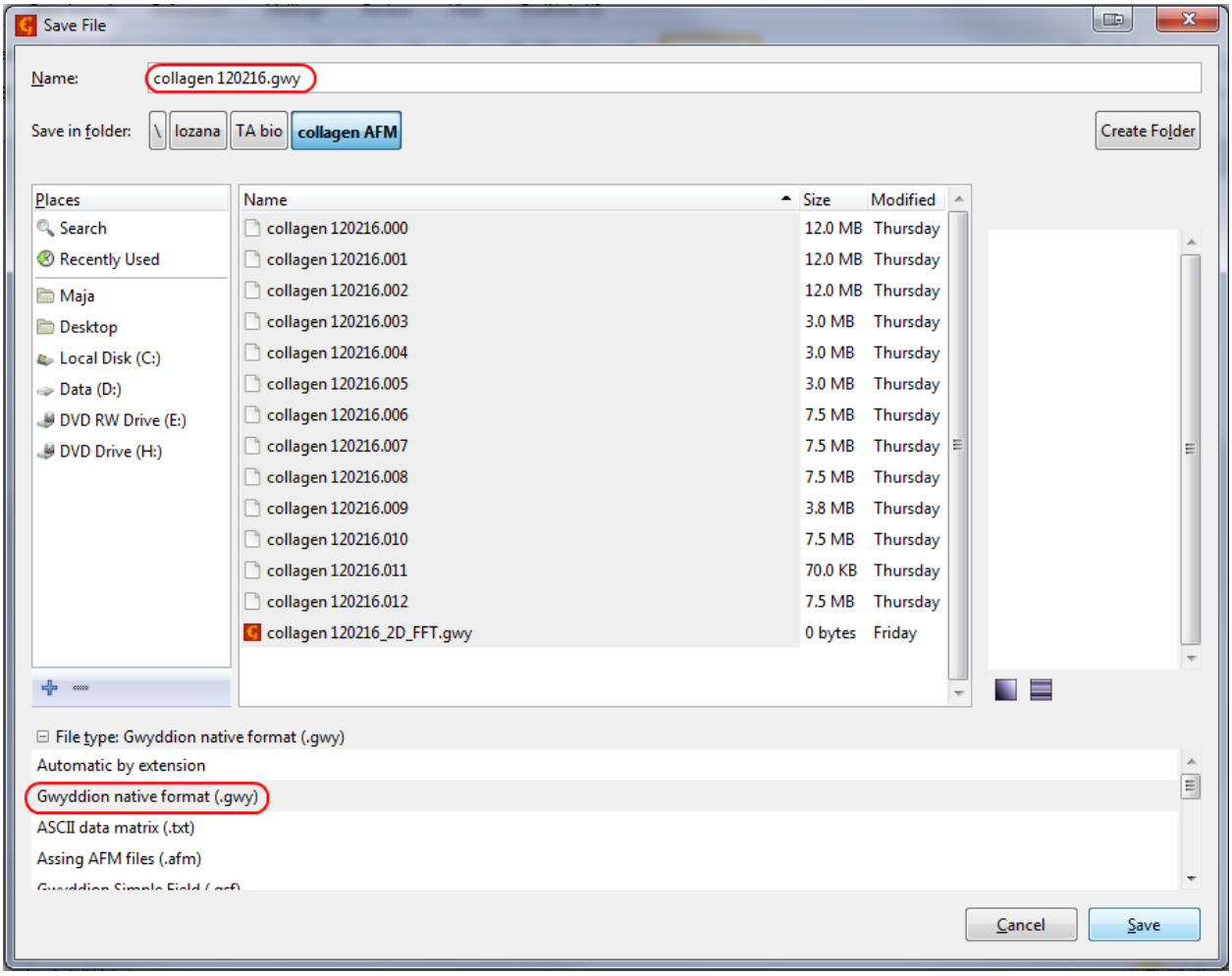


Image 9. Saving file in Gwyddion