Image Analysis

The Basics

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Introduction

Image Processing

Kernel Filters
Rank Filters
Fourier Filters

Image Analysis

Fourier Techniques
Particle Analysis
Specialist Solutions

Image Display

An image is a 2-dimensional collection of pixel values that can be displayed or printed by assigning a shade of grey (or colour) to every pixel value.

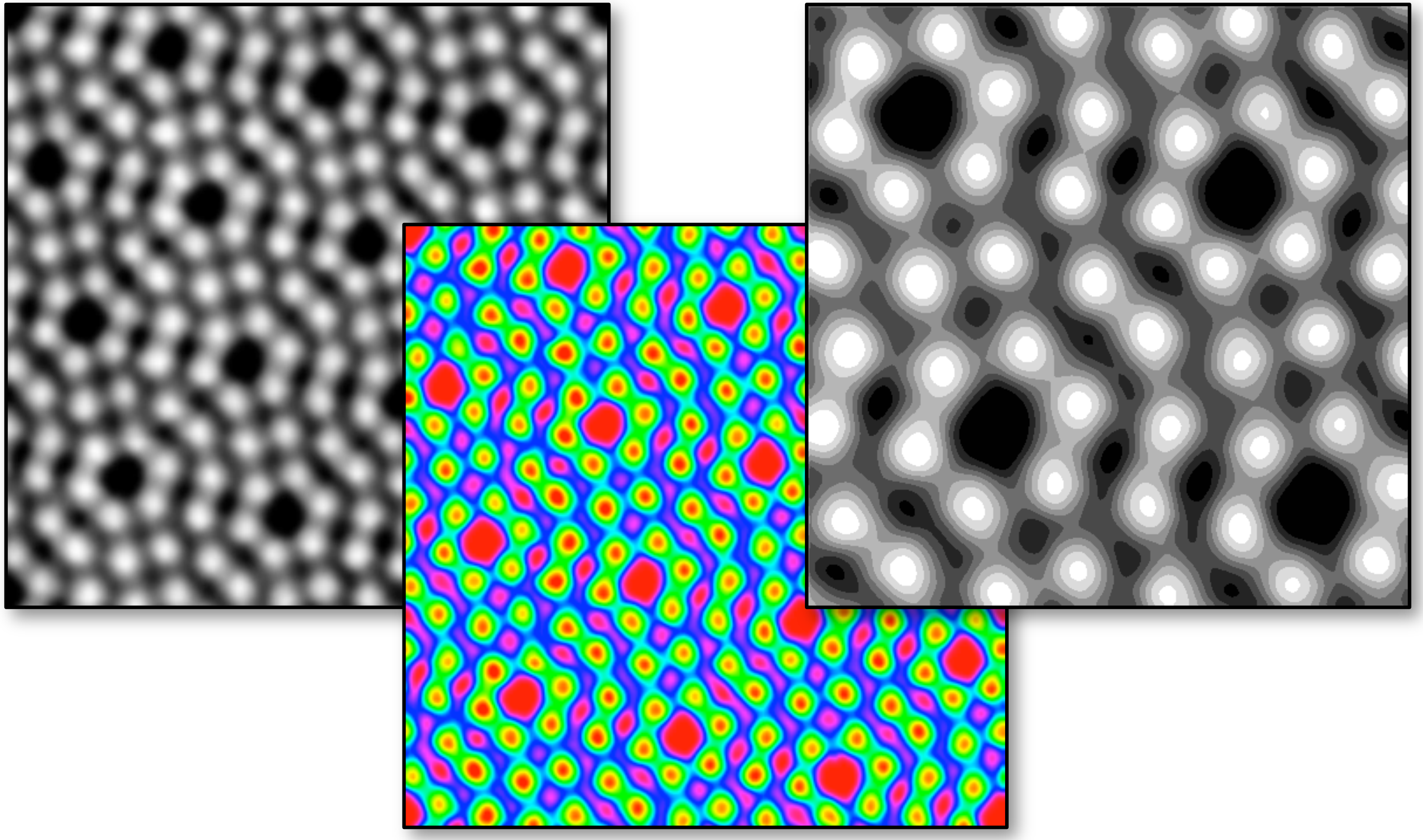

Image Display

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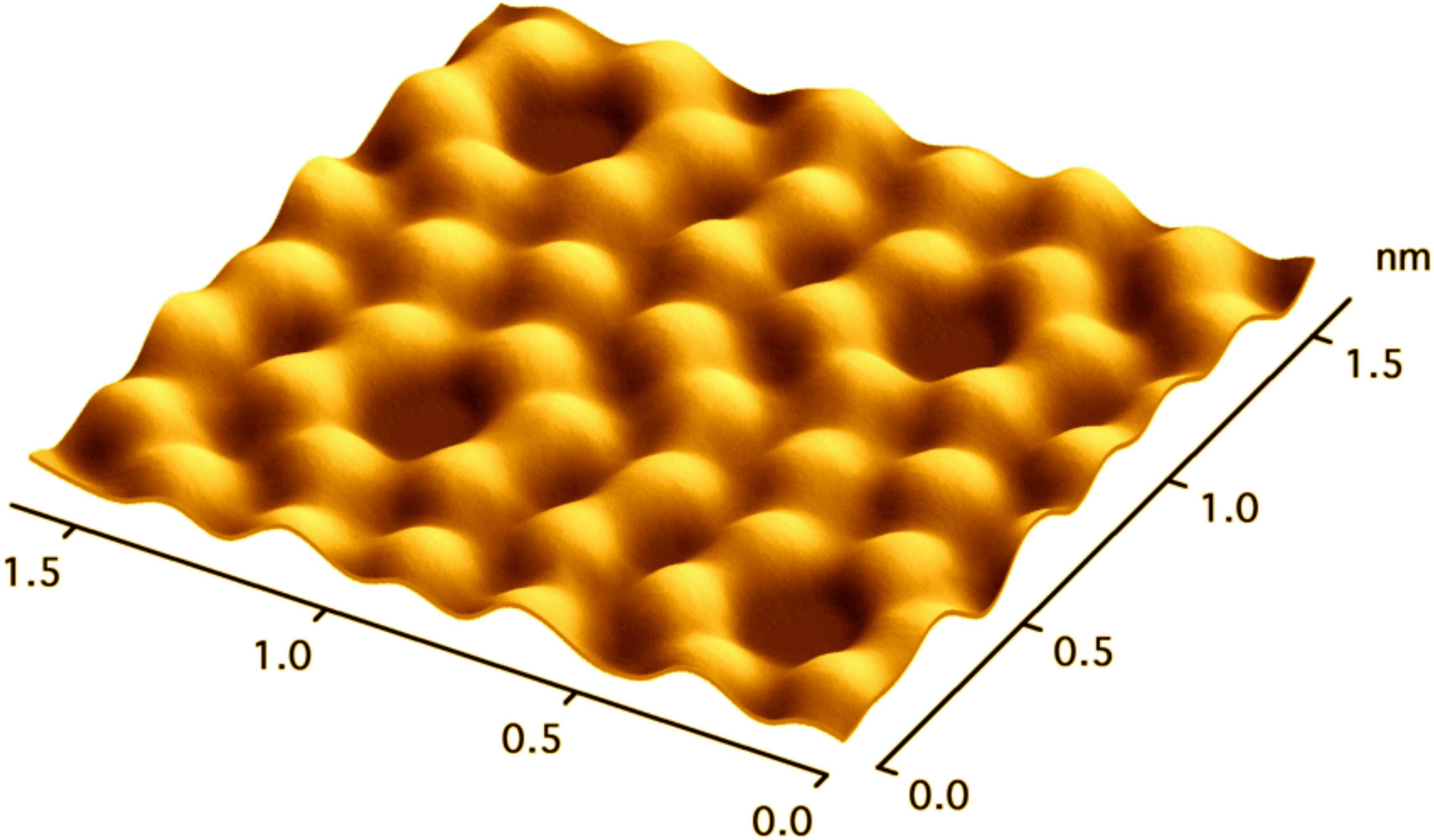
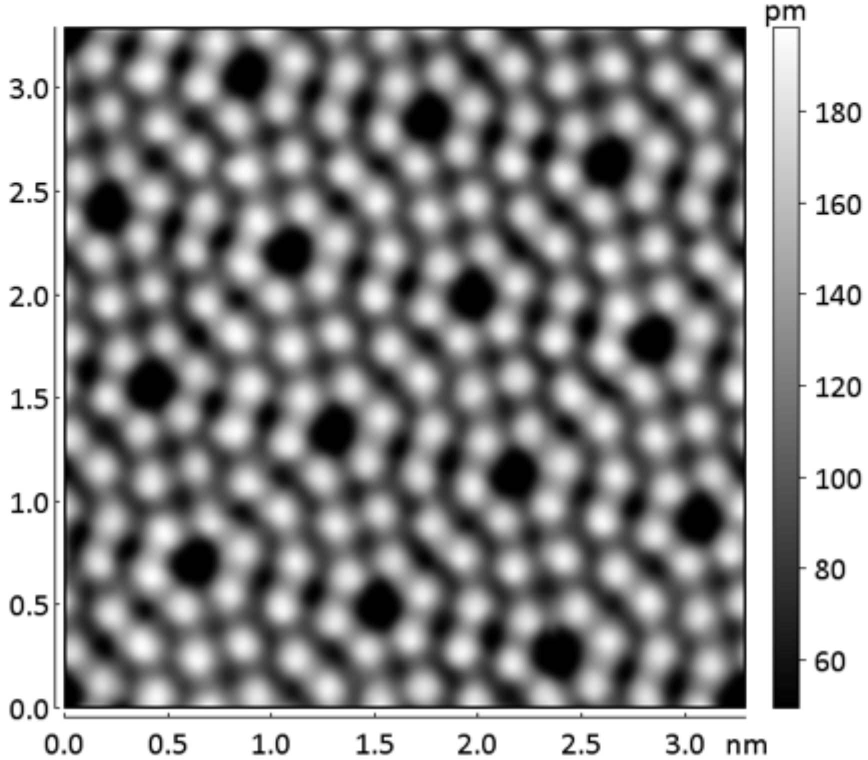


Spatial Calibration

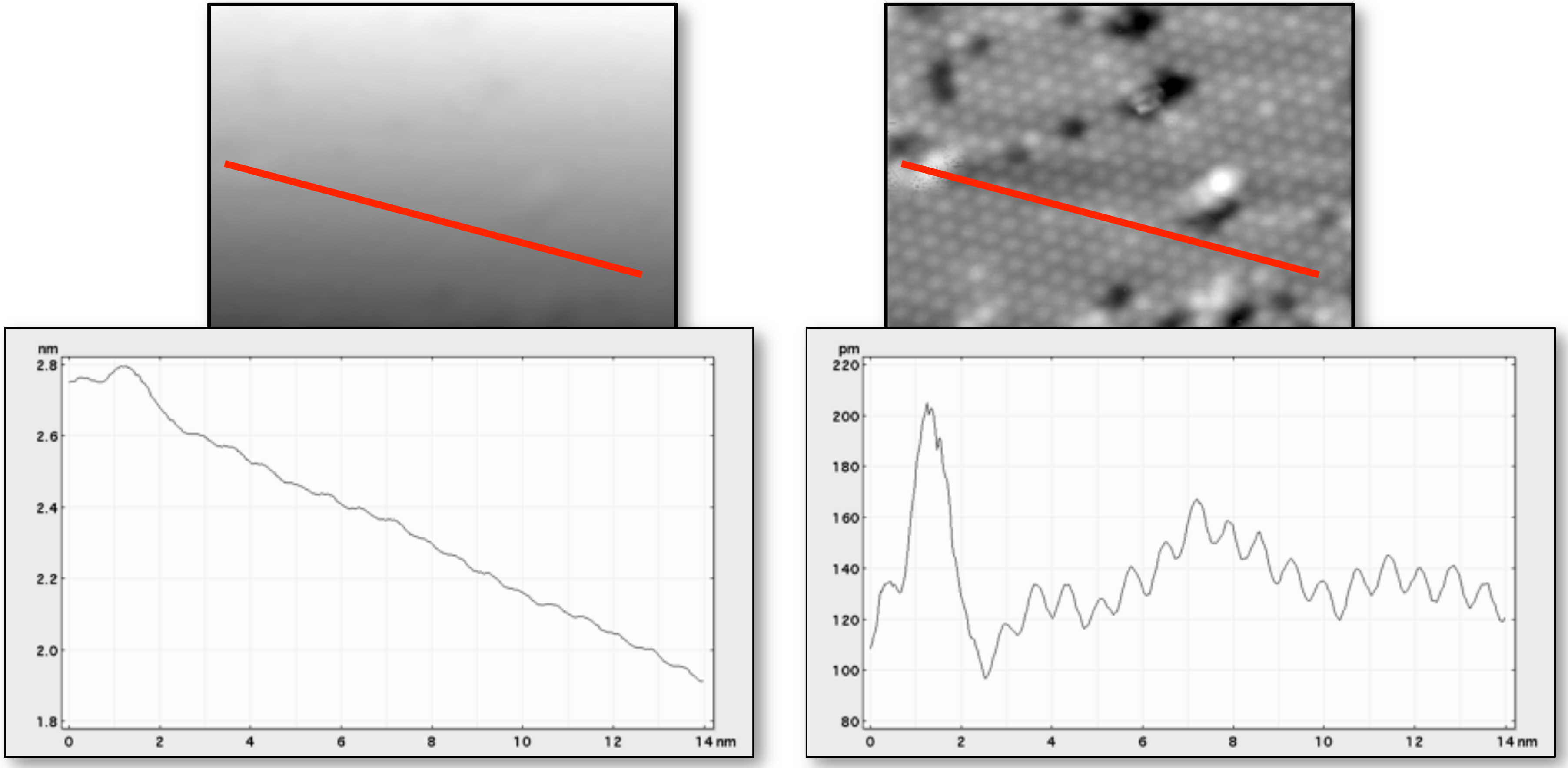
For image analysis to produce meaningful results, the spatial calibration of the image must be known. If the data acquisition parameters can be read from the image (or parameter) file then the spatial calibration of the image can be determined.



For simplicity and clarity, spatial calibration will not be indicated on subsequent images.

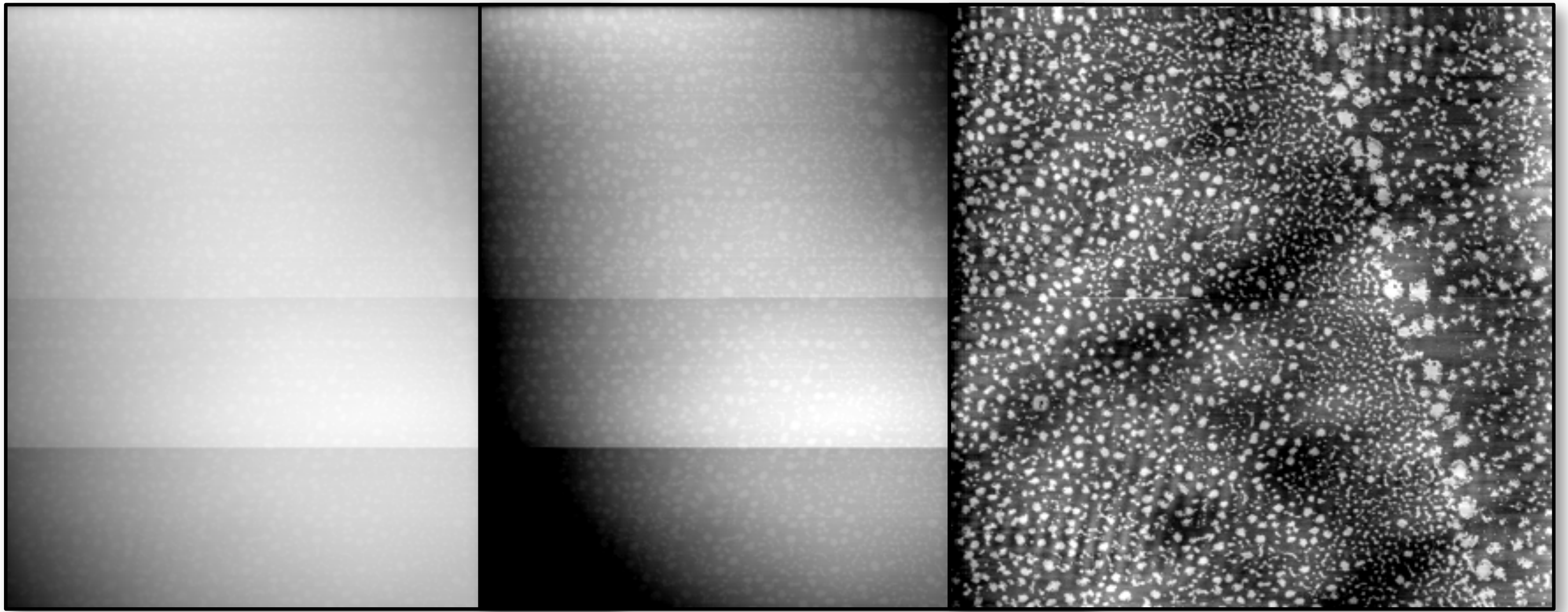
SPM Image Display

With Scanning Probe Microscope images there is no guarantee that the sample surface is level (so that the z values of the image are, on average, the same across the image).



SPM Image Display

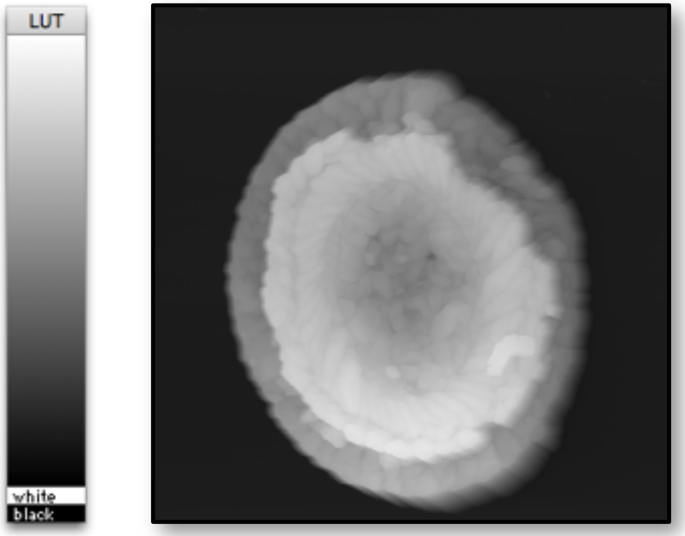
By treating each scan line of an SPM image independently, anomalous jumps in the apparent height of the image (produced, for example, in STMs by abrupt changes in the tunnelling conditions) can be corrected for.



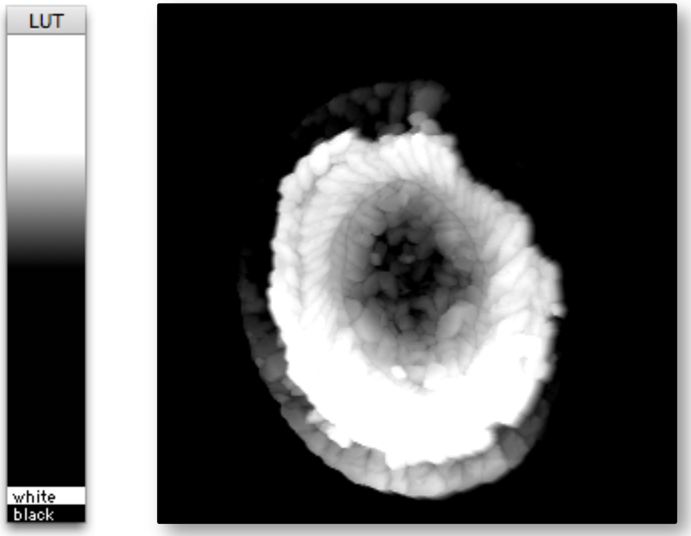
Raw image Compensated for tilt Line-by-line compensation

Image processing means changing all or some of the pixel values in an image, usually with the aim of making some feature(s) of the image more easily ‘visible’.

The most trivial example would include changing the colour used to represent each pixel value — the look-up table (LUT).



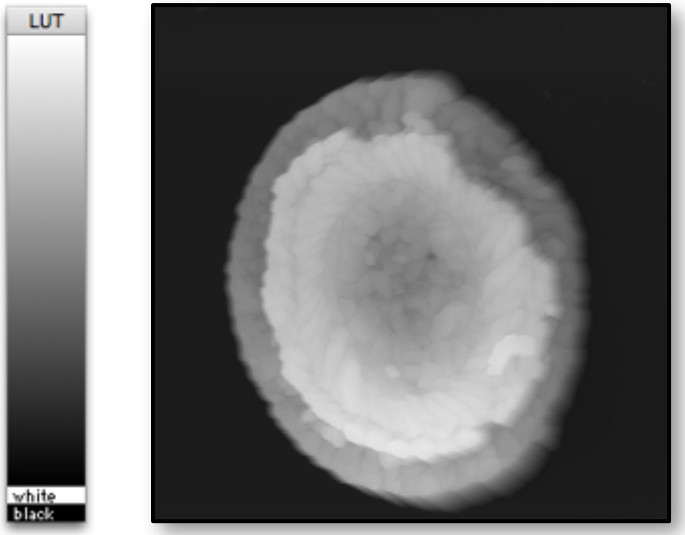
default greyscale



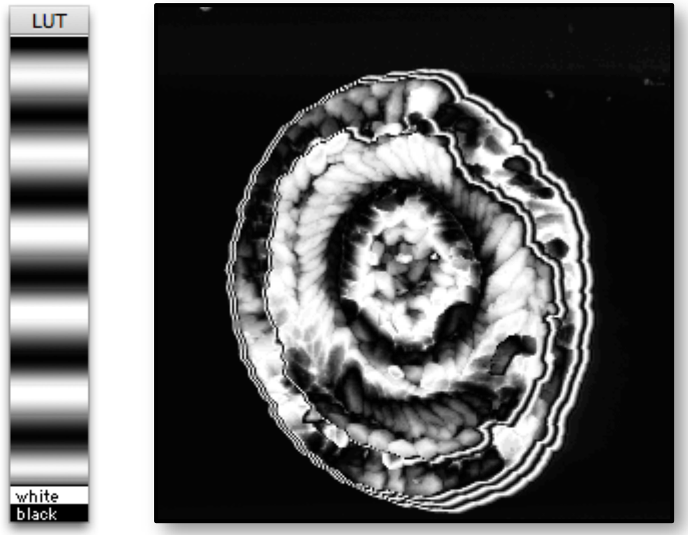
increased contrast

Image Processing

The LUT does not have to be a linear, or even monotonic. A non-linear mapping between pixel value and displayed colour can often reveal unexpected detail in the image.



default greyscale

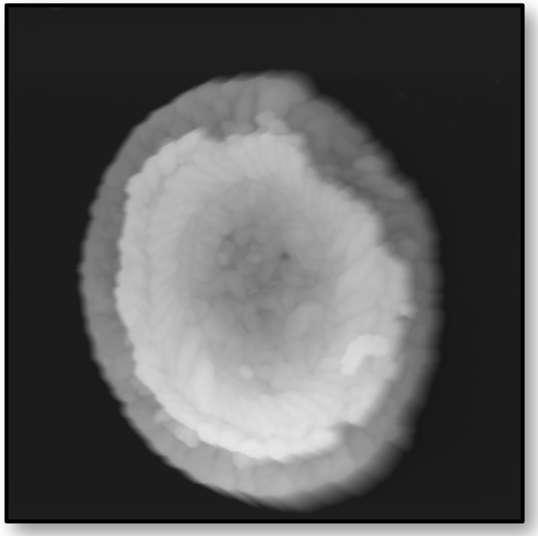


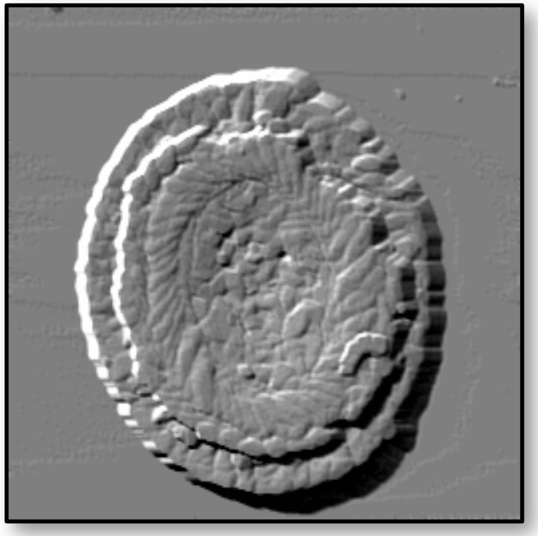
zebra greyscale

Image Processing

Changing the LUT is reversible, as it is only the mapping between pixel values and display colours that is changed.

Taking a differential – replacing each pixel with the value of the local differential of the surface with respect to some direction – is irreversible in the sense that integrating doesn't (necessarily) get you your original image back.



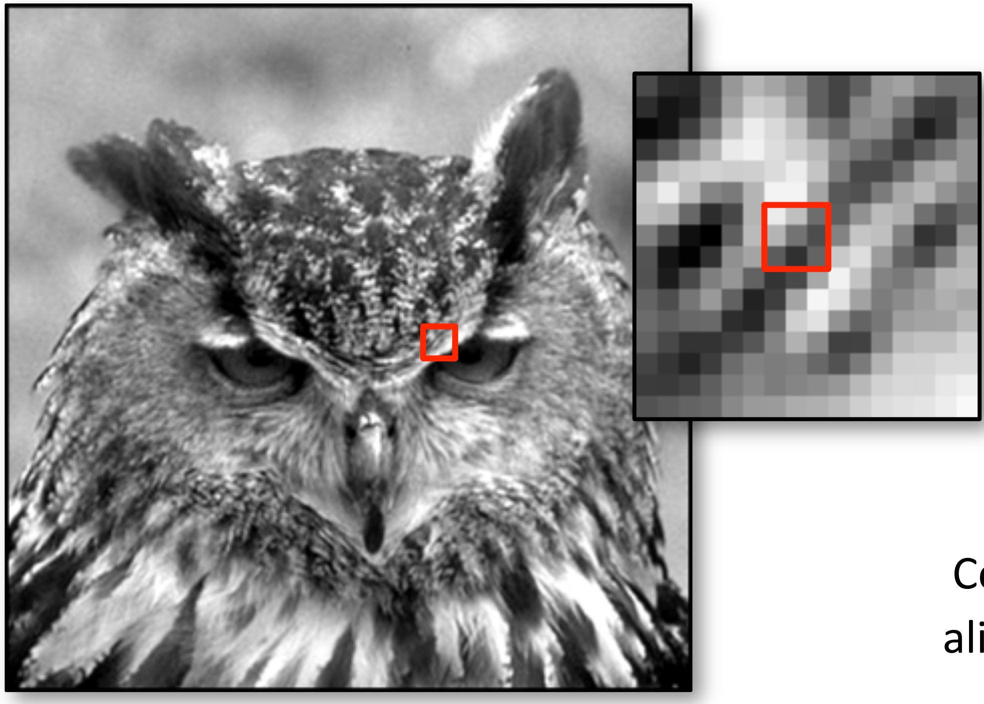


greys $\rightarrow$ z values

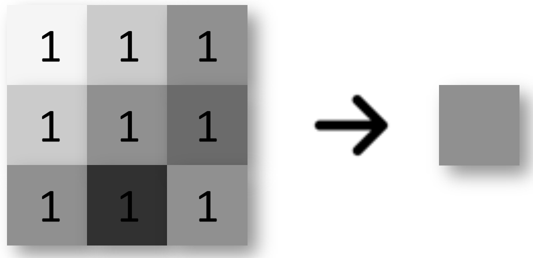
greys $\rightarrow$ $^{\partial z}/_{\partial x}$ values

Kernel Filters

Processing is often carried out using a kernel filter which uses an $n \times n$ matrix of numbers. The kernel matrix is applied to every pixel in an image in turn.



$$\begin{matrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{matrix}$$

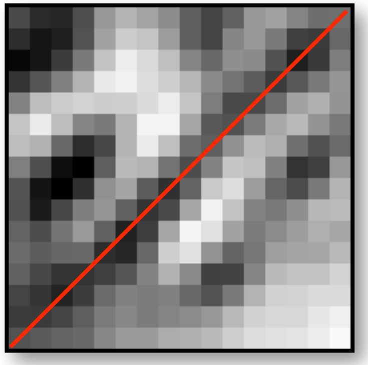


Central value in matrix aligned with target pixel

The elements of the kernel matrix are multiplication values that are applied to a target pixel and its neighbouring pixels. The target pixel is replaced with the normalised sum of these products, and then the process is repeated for the next (overlapping) set of pixels.

Kernel Filters

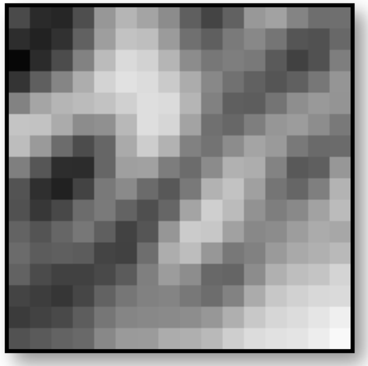
The simplest kernel filters use 3 x 3 matrices...



original image

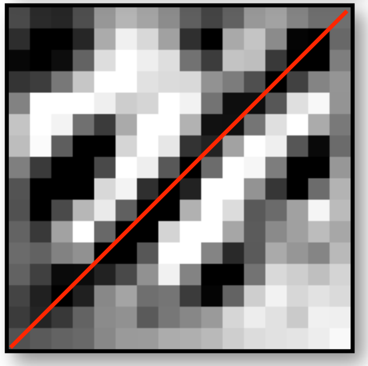
$$\begin{matrix} 1 & 1 & 1 \\ 1 & 4 & 1 \\ 1 & 1 & 1 \end{matrix}$$

smooth



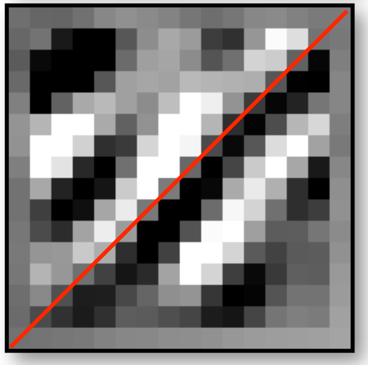
$$\begin{matrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{matrix}$$

sharpen



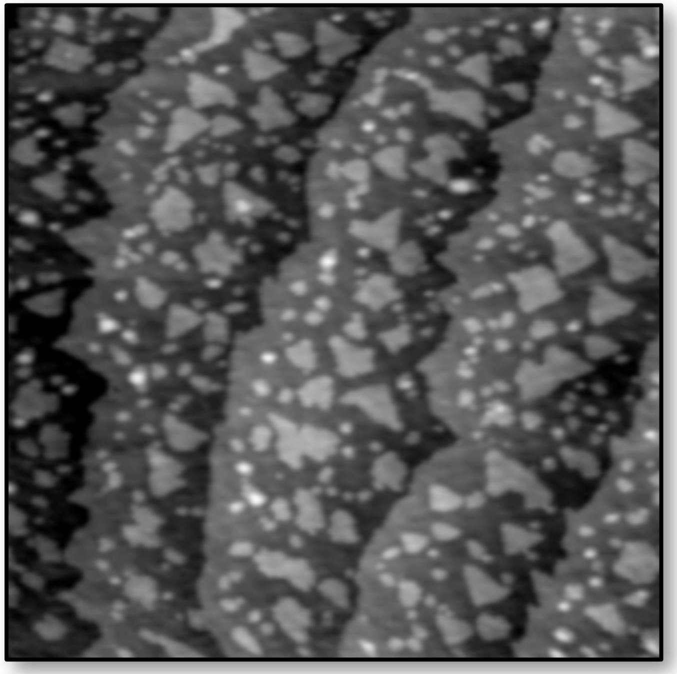
$$\begin{matrix} -2 & -1 & 0 \\ -1 & 0 & 1 \\ 0 & 1 & 2 \end{matrix}$$

gradient

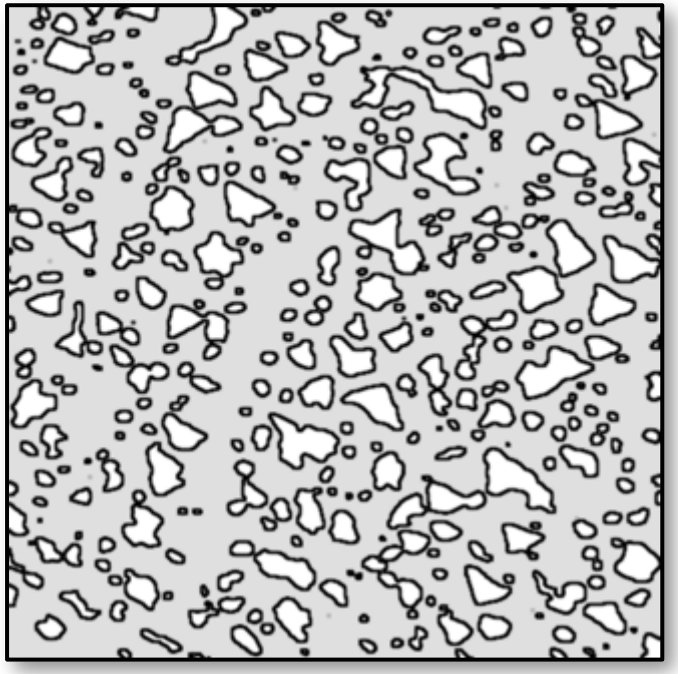


Kernel Filters

An edge detection filter (or Sobel filter) does exactly what it says it does.



original image

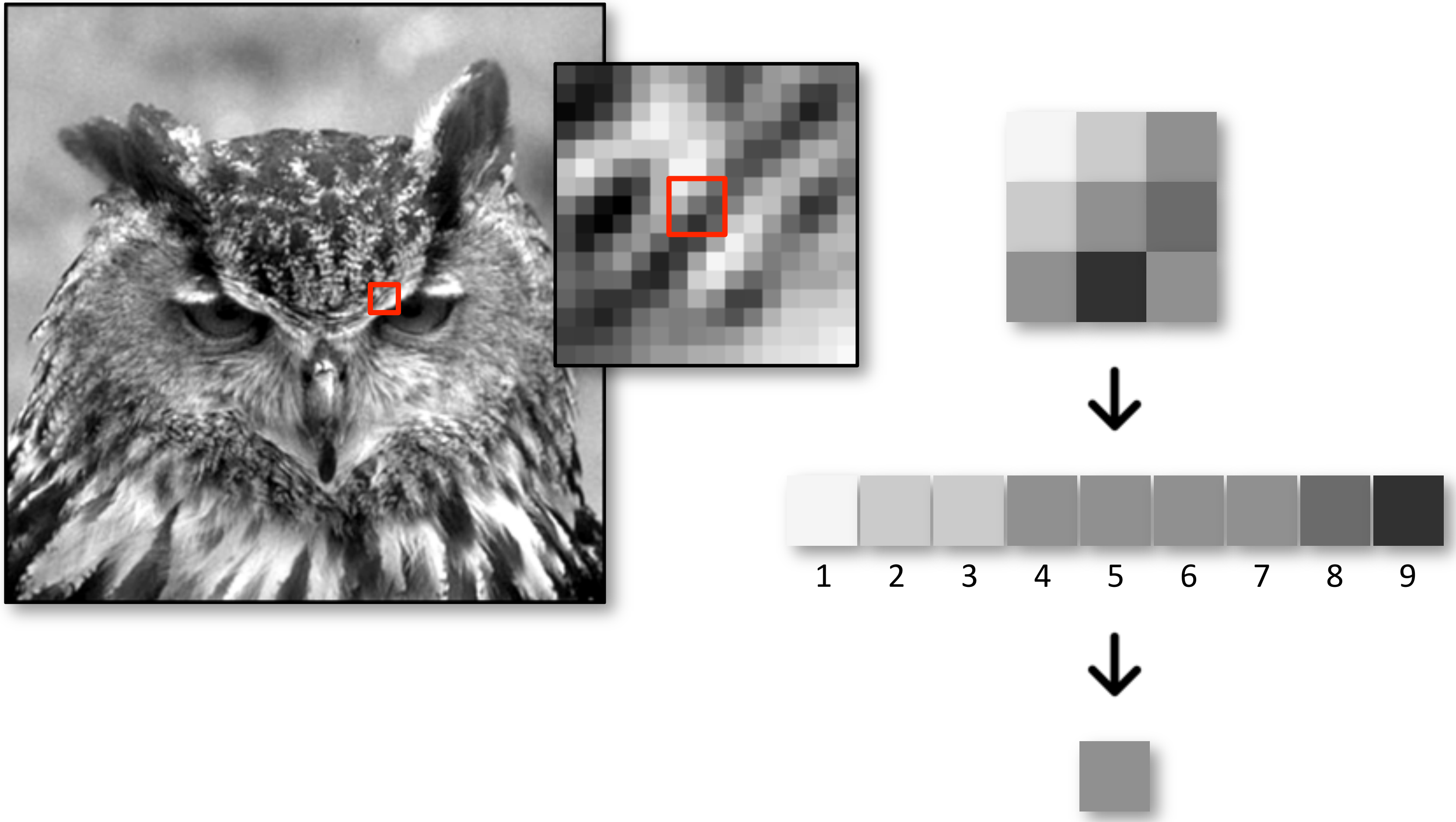


Sobel filtered

[Note that the image was pre-processed so that the filter picked out the islands clearly. Also, the filtered image had the contrast increased and the background grey filled in.]

Rank Filters

Processing using a rank filter uses the same idea of an $n \times n$ neighbourhood of pixel values, but without the matrix of numbers used with kernel filters.



Rank Filters

Rank filters can be used to remove isolated pixels that are significantly brighter or darker than their neighbours. A median filter can be a very effective noise reduction filter.

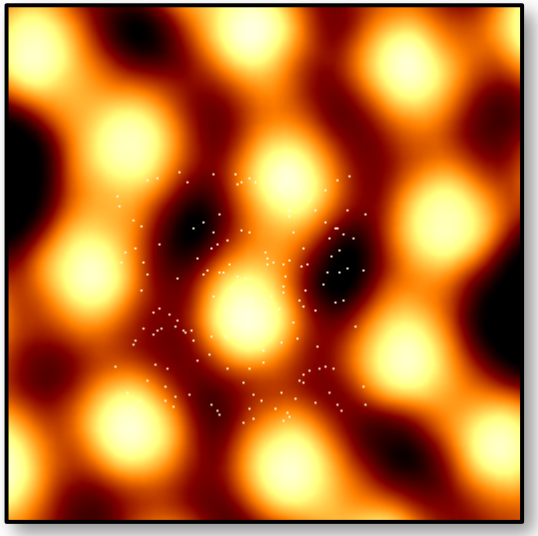
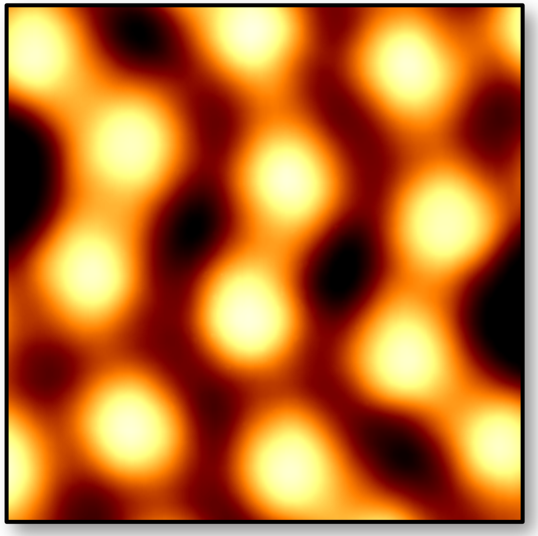


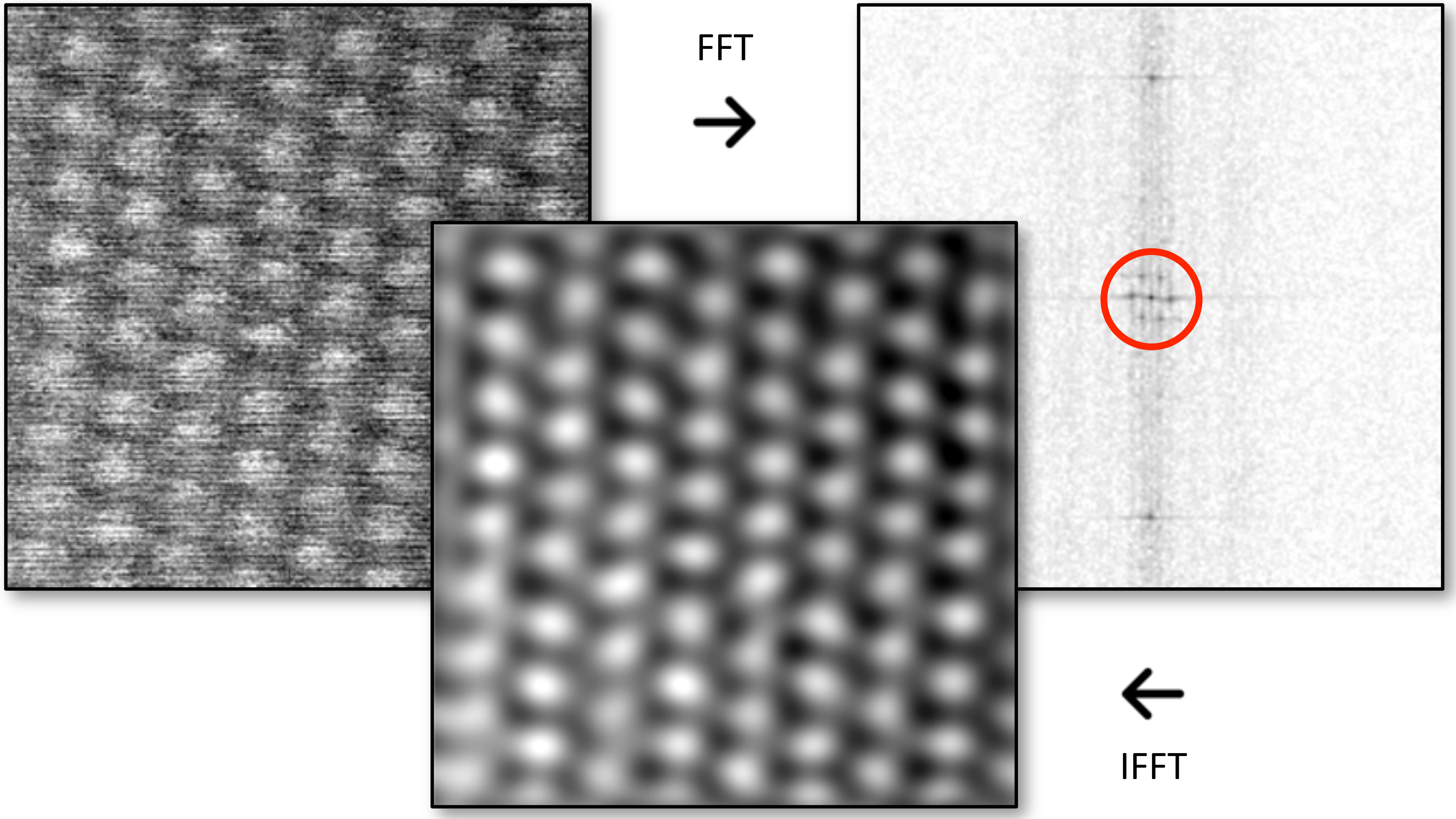
image + artificial noise



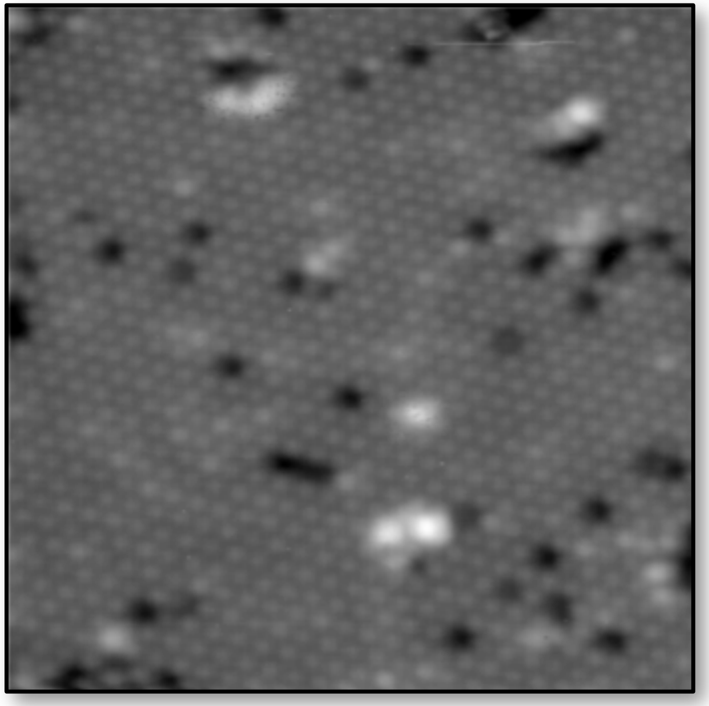
median filtered

Fourier Filters

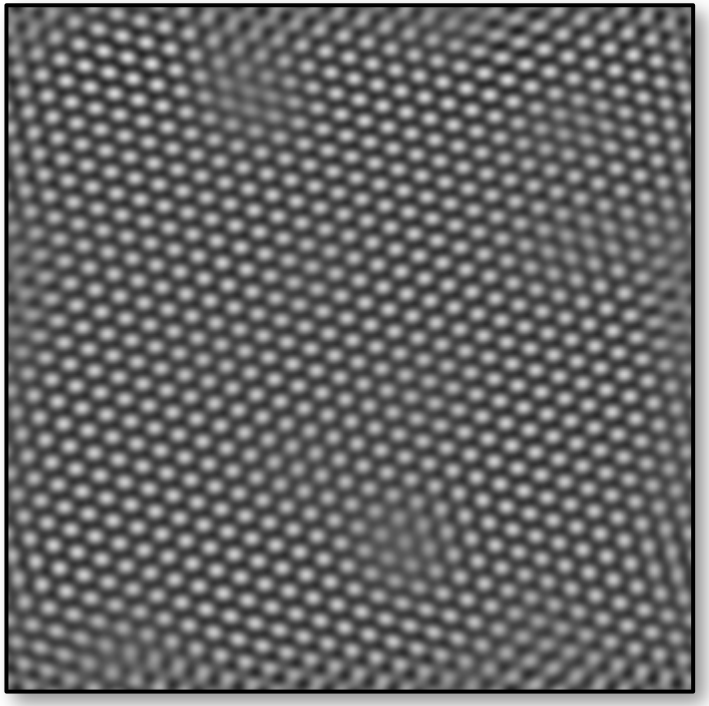
Processing using Fourier filters involves calculation of the image's frequency components.



Here's an easy way to 'clean' a surface.



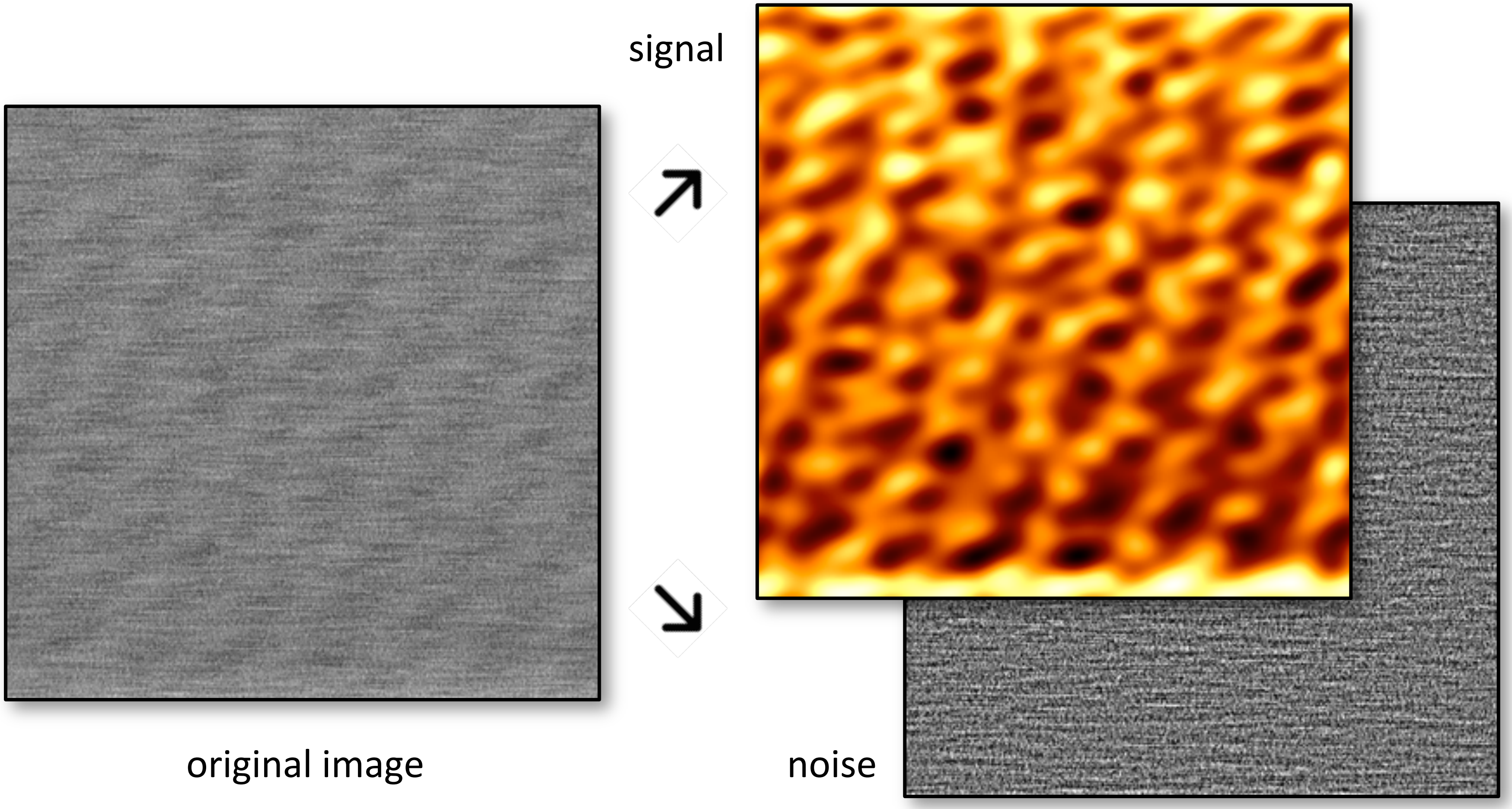
original image



Fourier filtered

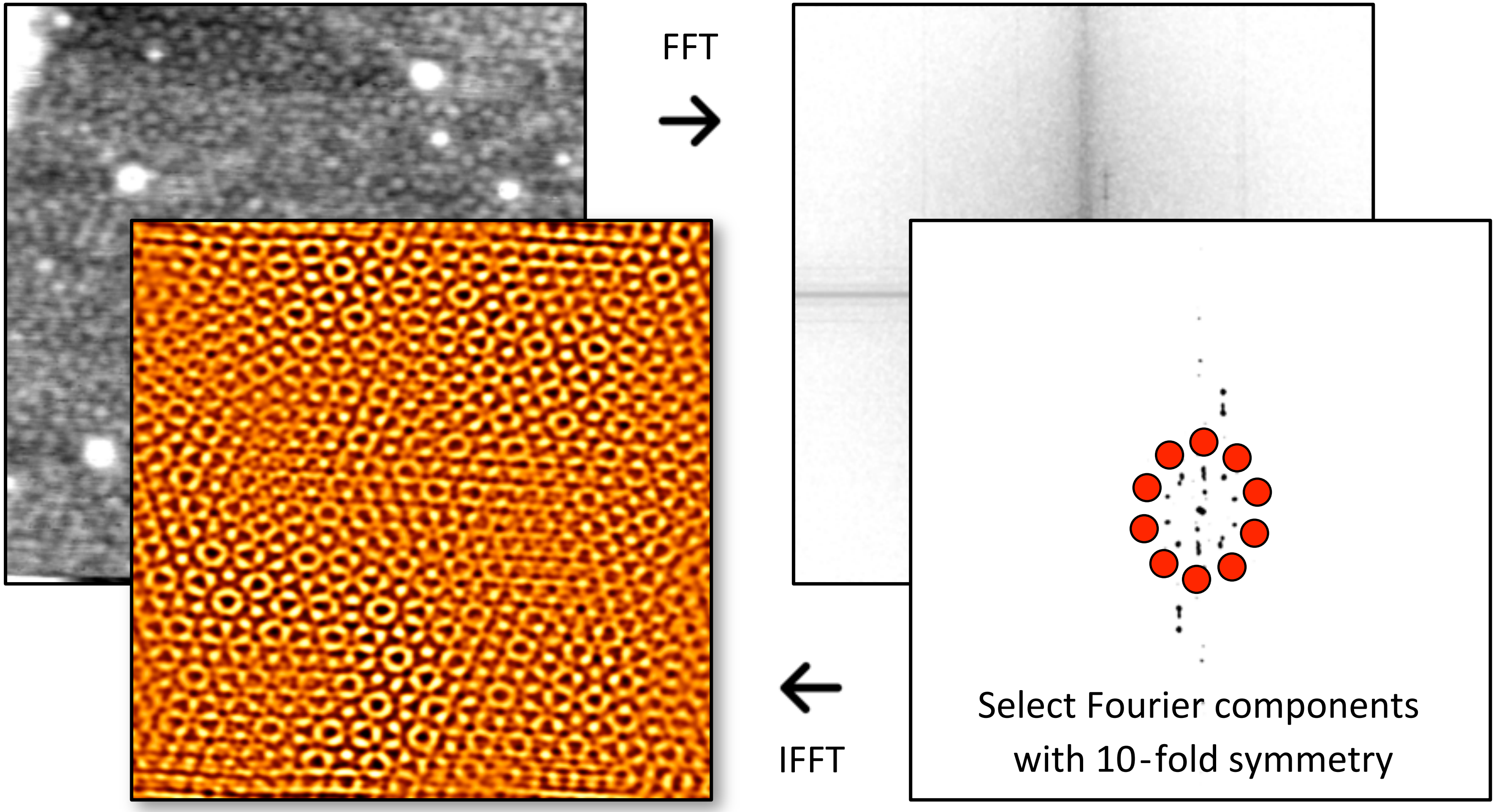
Fourier Filters

Even when the signal–to–noise ratio is lousy, Fourier filtering can extract the signal.



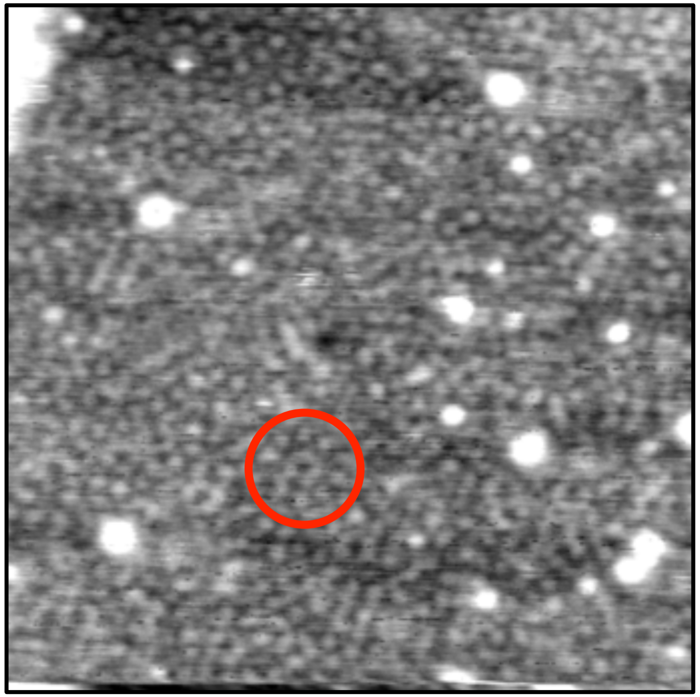
Fourier Filters

Fourier filters can be even more powerful if the symmetry of the surface is exploited.

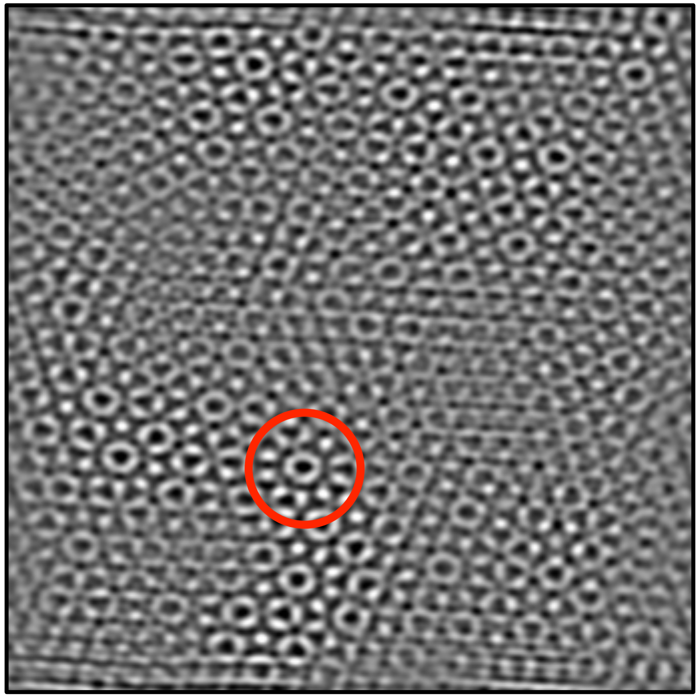


Fourier Filters

Although features lacking 10-fold rotational symmetry (such as the randomly dispersed contamination) have been effectively removed from the image, the correspondence between the original image and the Fourier filtered image can still be seen.



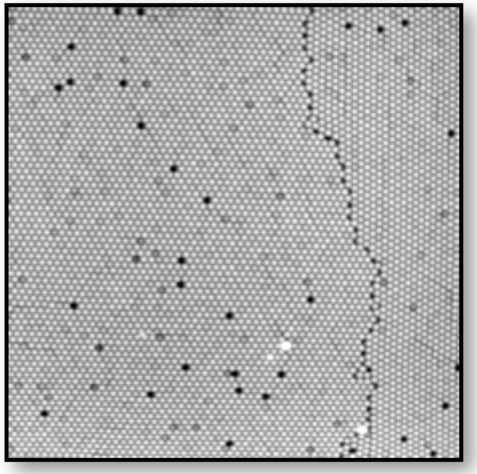
original image



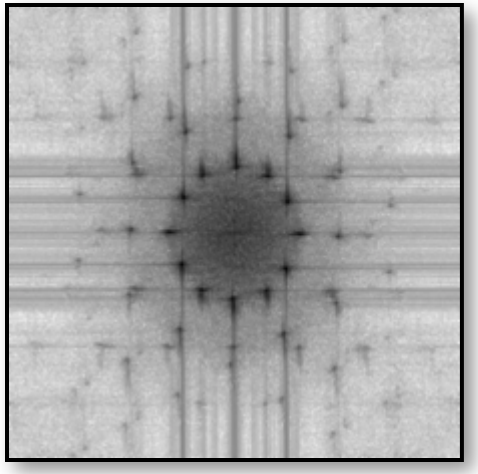
Fourier filtered

Image analysis means extracting quantitative information that is derived from the pixel values in an image.

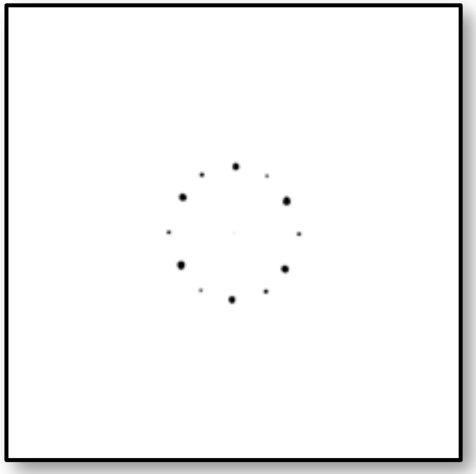
Rather than being used as an intermediate step in image processing, the FFT can be a valuable source of quantitative information.



original image



Fourier transform

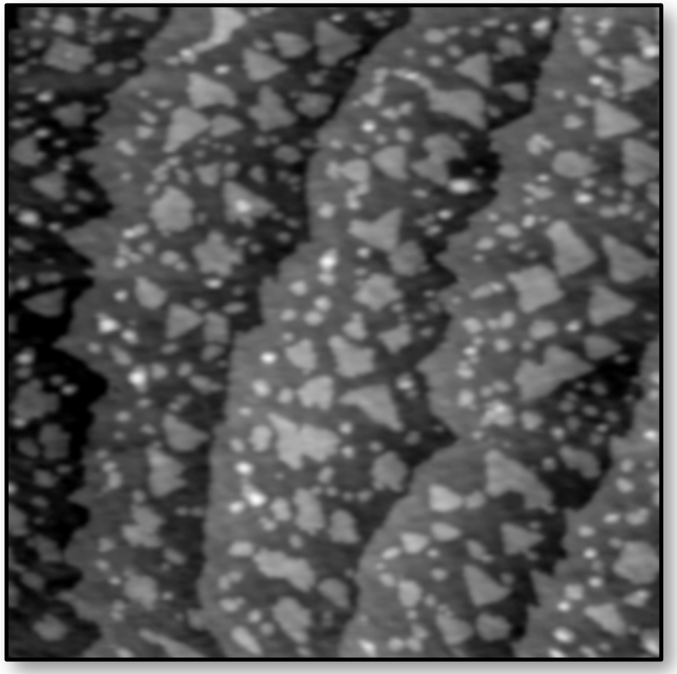


strongest components

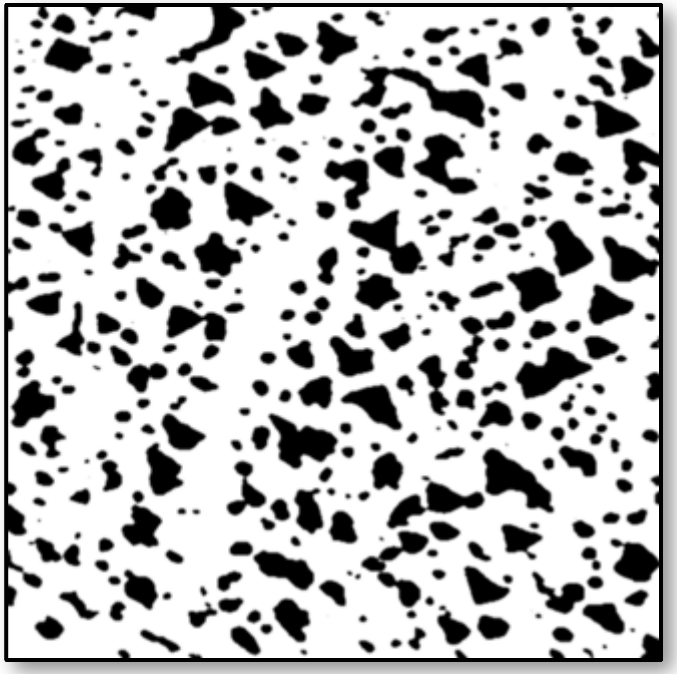
The FFT maxima occur at a spatial frequency of 2.33 per µm ($\rightarrow$ period = 0.43 µm).

Particle Analysis

The name particle analysis should not be taken too literally. The 'particles' can be any features that can be separated from the background by thresholding.



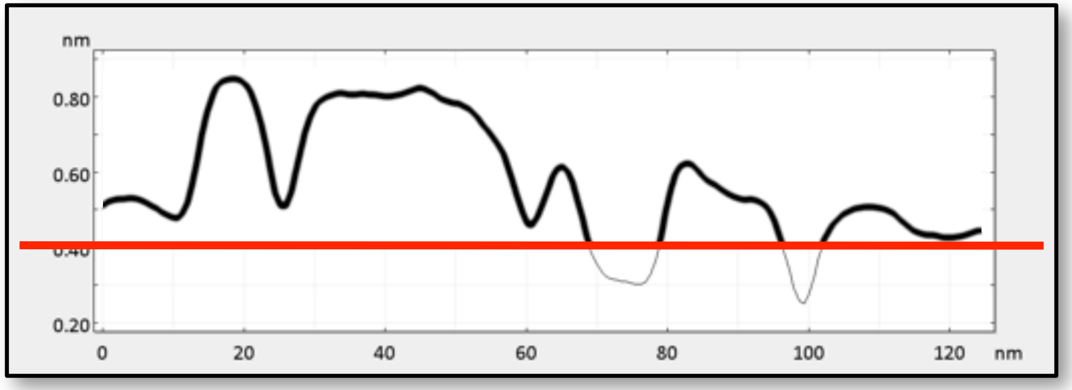
original image

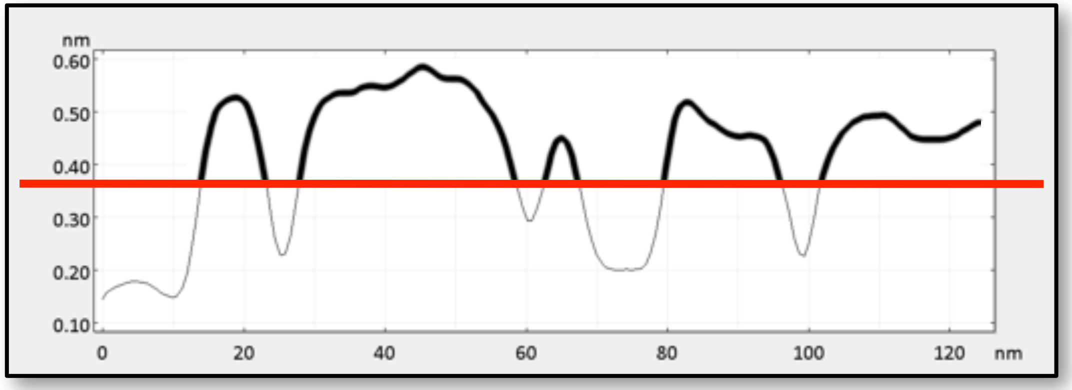


thresholded

Note that an image may require pre-processing to ensure that the intensity (height) of features of interest are above a threshold and all 'background clutter' is below.

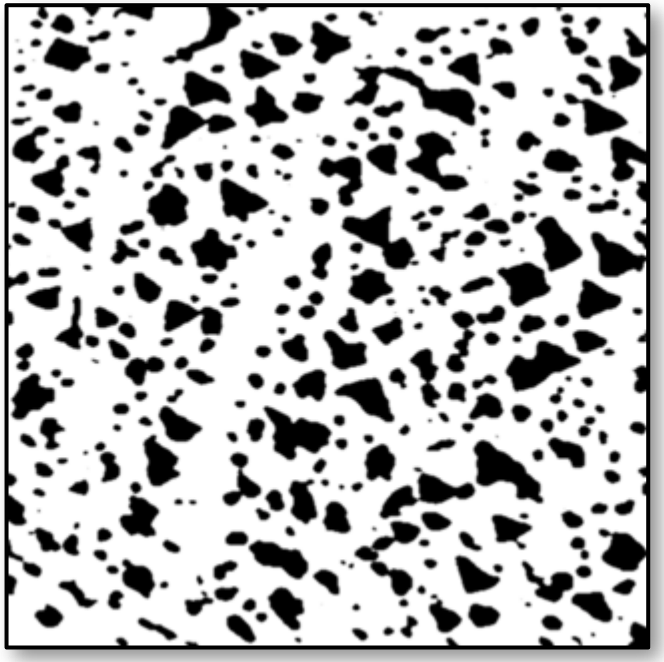
Thresholding will not work effectively if the features of interest are on a varying background.





Setting the threshold at the appropriate level may require some care.

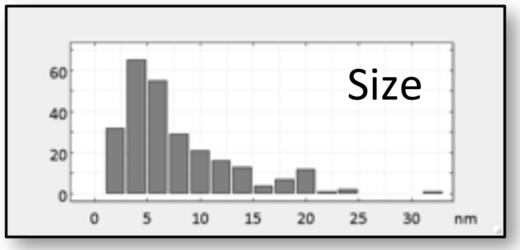
Particle Analysis

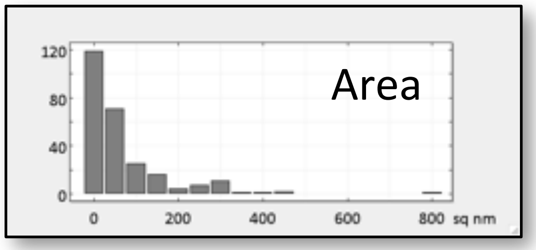


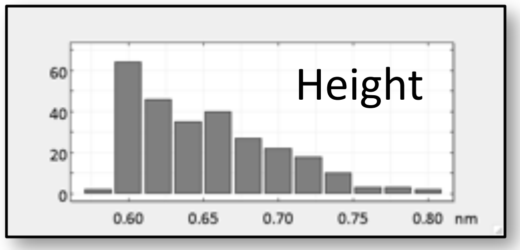
	Area	Mean	StDv	X	Y	Len	Majr	Minr	Angle	Min	Max
1.	324.38	0.74	0.07	109.15	329.47	105.67	34.59	11.94	43.0	0.59	0.91
2.	6.86	0.63	0.02	135.40	341.15	11.54	4.65	1.88	9.0	0.59	0.65
3.	5.39	0.59	0.01	294.51	341.03	8.74	3.33	2.06	177.5	0.59	0.60
4.	4.90	0.61	0.02	340.76	339.57	12.36	5.79	1.08	94.9	0.59	0.65
5.	1.47	0.59	0.00	43.40	340.90	4.37	2.37	0.79	0.0	0.59	0.59
6.	10.29	0.62	0.03	129.07	339.20	11.13	4.40	2.98	172.8	0.59	0.67
7.	24.50	0.67	0.05	298.94	335.79	17.89	6.72	4.64	170.9	0.59	0.77
8.	5.88	0.65	0.04	172.26	336.17	8.74	3.63	2.06	175.2	0.59	0.70
9.	48.02	0.70	0.08	312.58	332.72	27.79	9.18	6.66	54.0	0.59	0.87
10.	40.67	0.64	0.03	28.80	332.96	24.07	8.05	6.43	5.1	0.59	0.68
11.	34.30	0.73	0.09	279.41	332.26	21.44	7.83	5.57	20.0	0.59	0.89
12.	6.37	0.61	0.01	252.92	333.85	9.73	4.17	1.95	13.0	0.59	0.63
13.	1.96	0.60	0.01	14.70	333.02	4.95	1.91	1.30	0.0	0.59	0.61
14.	5.39	0.63	0.03	264.09	331.23	8.33	3.33	2.06	2.5	0.59	0.67
15.	21.56	0.65	0.04	298.71	329.05	16.08	5.95	4.61	149.8	0.59	0.73
16.	5.39	0.60	0.01	233.04	329.57	8.33	3.49	1.97	13.3	0.59	0.62
17.	96.04	0.73	0.12	335.38	321.78	46.50	15.97	7.66	118.2	0.59	1.00
18.	44.59	0.65	0.03	223.54	326.75	24.82	9.36	6.06	175.6	0.59	0.70
19.	19.60	0.61	0.02	144.71	325.83	18.88	7.72	3.23	169.3	0.59	0.64
...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...
...	...	...	...	...	...	...	...	...	...	...	...

Mean nearest neighbour distance = 13 ± 5 nm

Nearest neighbour lies in azimuthal direction 83° (anisotropy = 0.19)

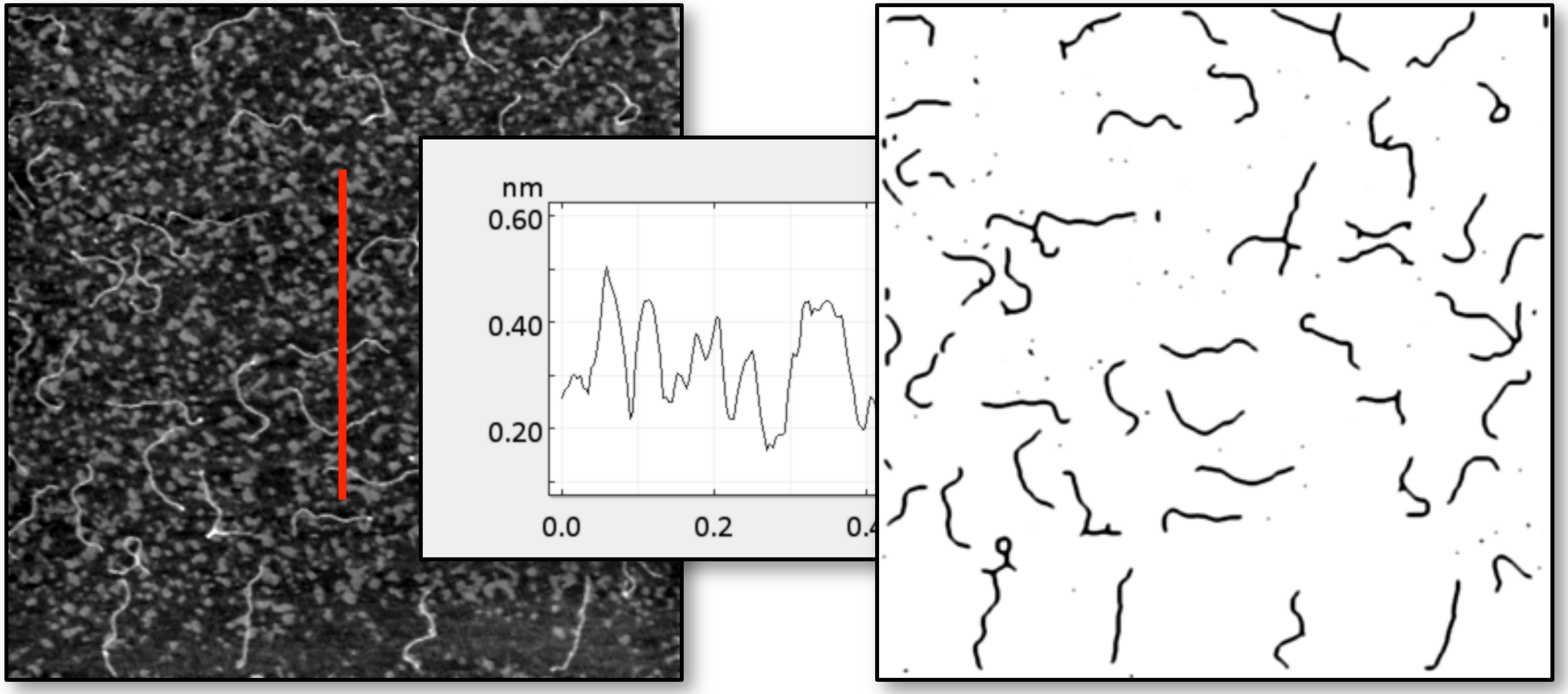






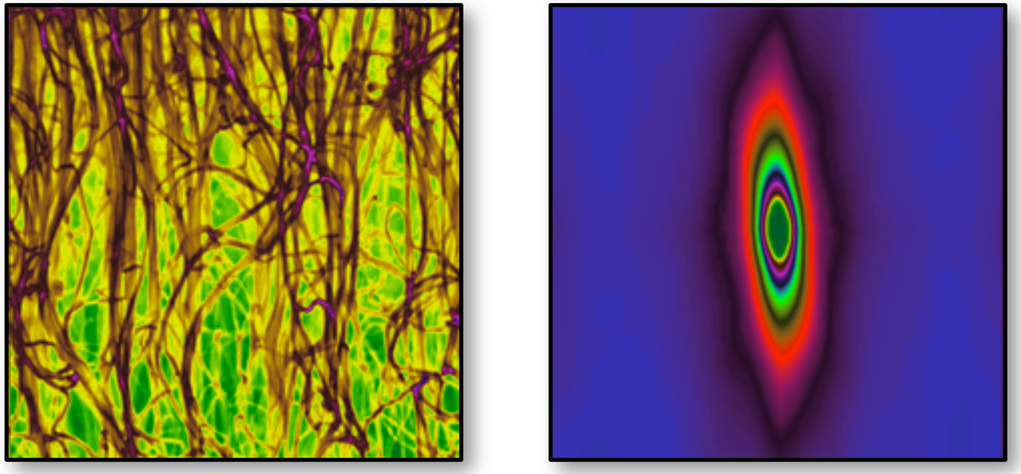
Specialist Analysis

When "seeing the wood for the trees", or in this case the adsorbate for the substrate, computers can find the task much harder than an eye/brain combination.

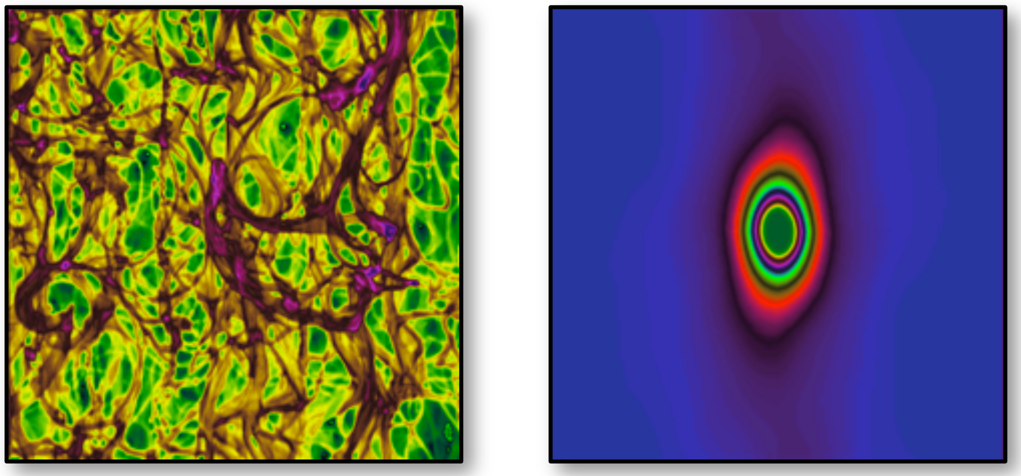


The property of the DNA strands that allows them to be separated from the background clutter is their curvature (the second differential of height wrt transverse distance).

Can the extent of ‘entanglement’ be quantified?



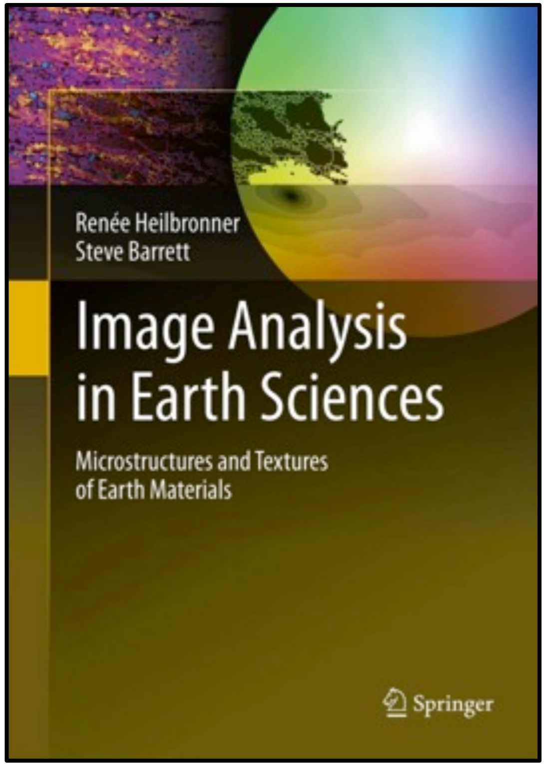
1.18



0.25

If you'd like to know more about the principles behind digital image processing and image analysis, or maybe you want to get your teeth into some of the maths, then try…

The examples used have been drawn from the field of earth sciences rather than medical sciences, but the same principles apply.



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