Image Analysis for Scanning Microscopy

UNIVERSITY OF LIVERPOOL

Dr Steve Barrett

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Introduction

Scanning Microscopy

- Image File Formats
- Image Display
- Spatial Calibration

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- Rank Filters
- Fourier Filters

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- Particle Analysis
- Specialist Solutions

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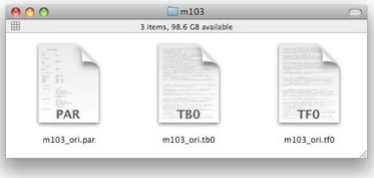
Scanning Microscopy

Before an image can be processed or analysed, the data must be loaded from a file.

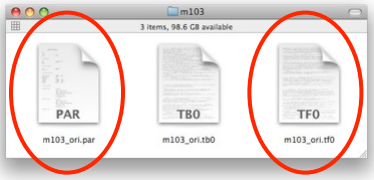


SPM manufacturers have not been able to agree on a common file format for the image data and, crucially, the data acquisition parameters. As a result, image analysis software provided by a manufacturer will open only images created by that manufacturer's system.

Scanning Microscopy

Software that is written to handle SPM images must be able to identify...

Where the image data is stored...
How the image data is stored...
Where the parameters are stored...
How the parameters are stored...



For some manufacturer the image data values and the acquisition parameters are stored in separate files, whereas for others the data and parameters are mixed together.

Scanning Microscopy

The image data (z values for an SPM image) can be stored in a variety of formats:

8-bit, 12-bit, 16-bit, 32-bit or 64-bit integer values
32-bit, 48-bit or 64-bit real (floating point) values

```
0006FC00: 05 13 05 20 05 40 05 3A 05 3C 05 47 05 45 05 3D
0006FC10: 05 38 05 34 05 28 05 2C 05 33 05 2D 05 4E 05 51
0006FC20: 05 4C 05 5B 05 56 05 44 05 30 05 1C 05 07 04 F3
0006FC30: 04 DD 04 C8 04 B4 04 9F 04 8A 04 75 04 60 04 4B
0006FC40: 04 37 04 21 04 0C 03 F8 03 E3 03 CD 03 B9 03 A3
0006FC50: 03 8E 03 7A 03 65 03 50 03 3B 03 26 03 11 02 FC
0006FC60: 02 E7 02 D2 02 BD 02 A8 02 93 02 7F 02 6A 02 55
0006FC70: 02 40 02 2B 02 16 02 02 01 EE 01 D9 01 C4 01 AF
0006FC80: 01 9A 01 85 01 71 01 5C 01 47 01 32 01 1D 01 09
0006FC90: 00 F4 00 DF 00 CA 00 B5 00 A0 00 8B 00 76 00 61
0006FCA0: 00 4C 00 37 00 22 00 0D FF FA FF E5 FF D0 FF BB
0006FCB0: FF A6 FF 90 FF 7C FF 67 FF 51 FF 3C FF 29 FF 1C
0006FCC0: FF 18 FF 18 FF 13 FF 0E FF 07 FF 03 FF 03 FF 07
0006FCD0: FF 0B FF 0C FF 0A FF 07 FF 03 FF 00 FF 07 FF 0B
0006FCE0: FF 07 FF 01 FE FD FE FC FE FA FE F7 FE F6 FE F4
0006FCF0: FE F0 FE F4 FE FB FF 01 FF 03 FE FF FE FA FE F5
0006FD00: FE F3 FE F4 FE F2 FE F0 FE F0 FE F1 FE ED FE EA
0006FD10: FE E2 FE DA FE D5 FE D8 FE DC FE DE FE DC FE DD
0006FD20: FE DA FE D3 FE CA FE C0 FE BD FE C8 FE D0 FE D0
0006FD30: FE CF FE D1 FE D5 FE D2 FE CC FE C4 FE C5 FE CB
0006FD40: FE CD FE C9 FE C4 FE C0 FE BD FE BB FE BB FE B9
0006FD50: FE BA FE BC FE BA FE B8 FE B9 FE BB FE BA FE B5
0006FD60: FE B3 FE B3 FE B1 FE AF FE AD FE A9 FE A3 FE A4
0006FD70: FE A7 FE A4 FE A0 FE 9B FE 9A FE 99 FE 9B FE 9E
0006FD80: FE 9E FE 9D FE 9A FE 95 FE 91 FE 8F FE 89 FE 88
0006FD90: FE 8B FE 8E FE 8E FE 8D FE 8D FE 8E FE 8F FE 8D
```


Scanning Microscopy

The data acquisition parameters can be stored as text or binary.

Omicron parameter file for SPM data

```
...
...
Field X Size in nm   : 150.000    ;[nm]
Field Y Size in nm   : 150.000    ;[nm]
Image Size in X      : 512
Image Size in Y      : 512
Increment X          : 0.292969   ;[nm]
Increment Y          : 0.292969   ;[nm]
Scan Angle           : 0.000000   ;[Degree]
X Offset             : 677.447    ;[nm]
Y Offset             : 1502.63    ;[nm]
...
...
```

RHK Technology SPM parameters

```
00000000: AA 00 53 00 54 00 69 00 4D 00 61 00 67 00 65 00
00000010: 20 00 30 00 30 00 34 00 2E 00 30 00 30 00 32 00
00000020: 20 00 31 00 00 00 0E 00 01 00 00 00 26 00 00 00
00000030: 00 00 00 00 07 00 00 00 00 00 00 00 00 00 00 00
00000040: 84 01 00 00 01 00 00 00 00 00 00 00 00 00 00 00
00000050: 00 00 00 00 E4 EB DB 02 10 06 00 00 00 00 00 00
00000060: 00 00 00 00 33 93 29 3C 00 00 80 3F 77 CC AB 2A
00000070: 00 00 00 00 4D FD FF BF 00 00 00 00 00 00 00 00
00000080: 17 B7 D1 38 C0 1C 80 3F D6 BC 74 2F 00 00 00 00
00000090: F2 CC 1B 1E AF 7F 29 4F 92 B5 D1 57 3F 53 5E B3
000000A0: 01 00 00 00 00 00 00 00 00 00 00 00 08 00 43 00
000000B0: 75 00 72 00 72 00 65 00 6E 00 74 00 20 00 00 00
000000C0: 00 00 1F 00 55 00 56 00 20 00 6F 00 66 00 66 00
000000D0: 2C 00 20 00 53 00 54 00 53 00 2C 00 20 00 31 00
000000E0: 2E 00 30 00 56 00 2C 00 20 00 30 00 2E 00 31 00
000000F0: 37 00 6E 00 41 00 2C 00 2D 00 32 00 7E 00 32 00
00000100: 56 00 31 00 43 00 3A 00 5C 00 52 00 48 00 4B 00
00000110: 5C 00 64 00 61 00 74 00 61 00 5C 00 43 00 68 00
00000120: 72 00 69 00 73 00 5C 00 75 00 68 00 76 00 5C 00
00000130: 61 00 70 00 72 00 69 00 6C 00 30 00 38 00 5C 00
00000140: 41 00 75 00 31 00 35 00 30 00 30 00 30 00 31 00
00000150: 30 00 34 00 30 00 38 00 30 00 30 00 33 00 2E 00
00000160: 53 00 4D 00 33 00 08 00 30 00 34 00 2F 00 31 00
00000170: 37 00 2F 00 30 00 38 00 08 00 31 00 35 00 3A 00
00000180: 30 00 37 00 3A 00 30 00 38 00 01 00 56 00 01 00
00000190: 20 00 01 00 41 00 00 00 00 00 00 00 04 00 30 00
```

The result is a bewildering number of permutations of image file format...

Scanning Microscopy

SXM manufacturers A–N

		Parameters				Image Data Format						
Manufacturer	**System**	Text	Bin	Head	Foot	Int	Real	8	16	32	+	+/-
Asylum Research	MFP-3D	•	•	•	•		•			•		•
Burleigh	ISTM		•	•	•	•			•		•	
Digital Instruments	NanoScope II-IV	•		•		•			•			•
DME	Rasterscope		•	•		•			•			•
DME	Surface Data File	•		•	•	•				•		•
Gatan	DigitalMicrograph	•			•	•			•		•	
JEOL	WinSem		•			•		•			•	
JEOL	WinSPM		•	•		•			•			•
JEOL	JSM	•		•		•		•			•	
JPK Instruments	SPM		•	•		•			•		•	
Klocke	Atomikro		•	•		•		•	•		•	
Leica	TCS	•			•	•		•			•	
LEO	SEM		•	•		•		•			•	
Molecular Imaging	PicoScan	•		•		•			•		•	
NanoMagnetics	SPMSIF		•	•	•	•			•		•	
Nanonics Imaging	Quartz		•	•		•			•		•	
Nanonis	SPM	•		•		•			•	•		•
Nanosurf	easyScan	•		•		•		•	•			•
Nanotec Electronica	WSxM	•		•		•			•			•

Scanning Microscopy

SXM manufacturers N–Z

		Parameters				Image Data Format						
Manufacturer	**System**	Text	Bin	Head	Foot	Int	Real	8	16	32	+	+/-
Noran	Vantage	•	•	•		•		•			•	
NT-MDT	SPM		•	•		•			•			•
Omicron	Pre-SCALA		•			•			•		•	
Omicron	SCALA	•				•			•			•
Oxford Instruments	TOPSystem 3		•	•		•			•			•
Park Scientific Instr	HFS-LIF	•	•	•		•			•			•
Park Scientific Instr	HDF		•	•		•			•			•
Philips	SEM		•	•		•		•			•	
Quesant Instruments	SPM		•	•	•	•			•		•	
RHK Technology	SPM-32	•		•		•	•	•	•	•	•	•
RHK Technology	XPM Pro		•	•		•				•		•
Seiko Instruments	SPI		•	•		•			•		•	
SPECS	STM 150 Aarhus		•	•		•			•			•
TopoMetrix	SPMLab		•	•		•			•		•	
Unisoku	SPM	•				•			•		•	
Vacuum Generators	SAM		•	•		•				•		•
WA Technology	Pre-TOPSystem		•	•		•		•			•	
Zeiss	LSM		•	•		•		•			•	

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.

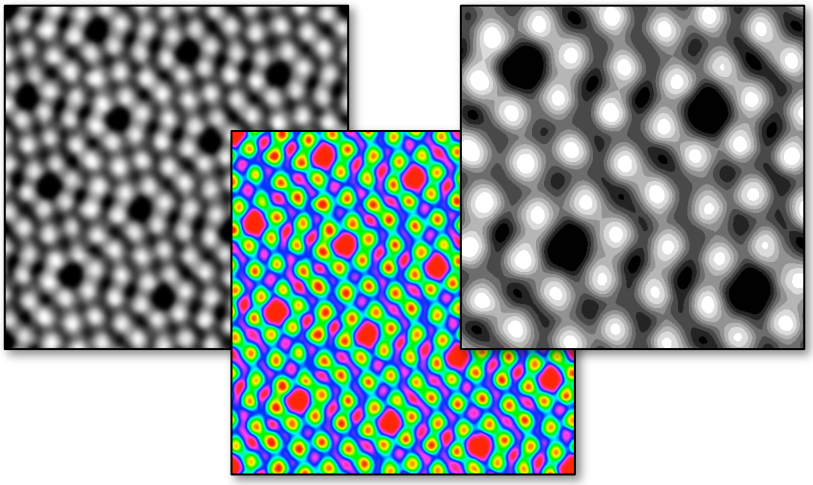


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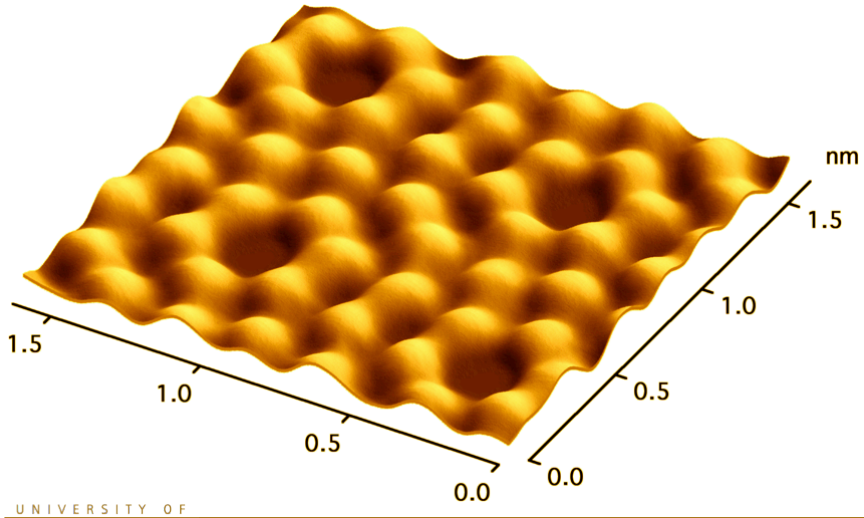
Image Display

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.



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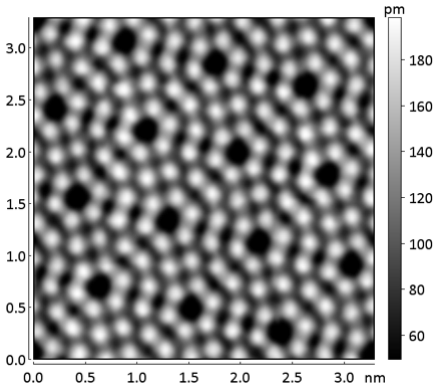
Spatial Calibration

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.

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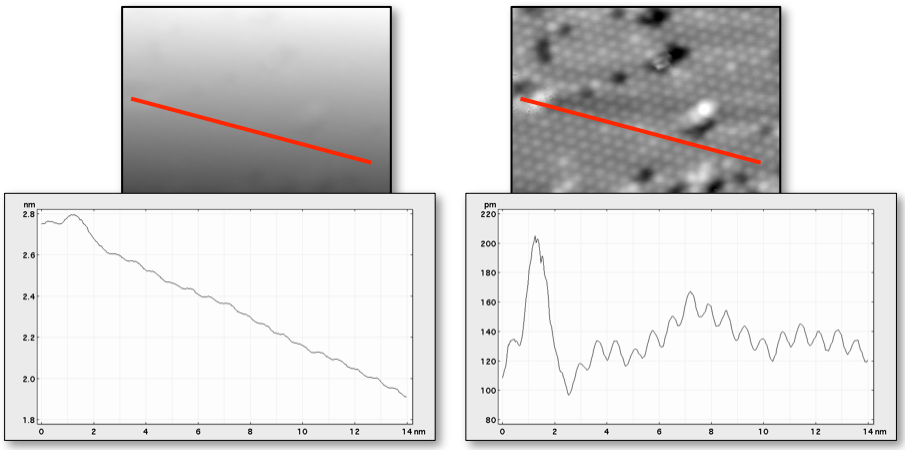
SPM Image Display

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).



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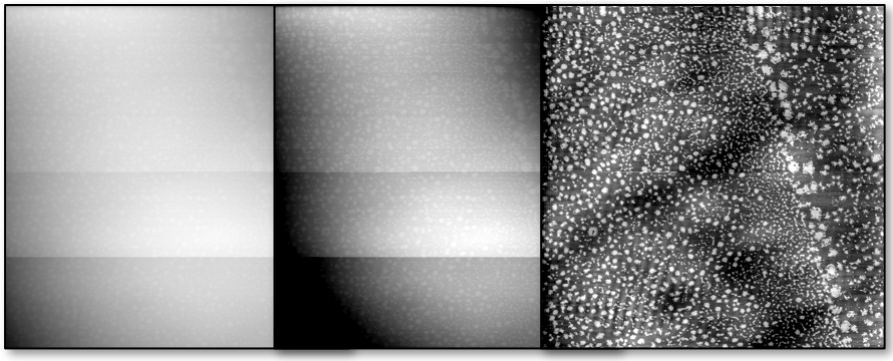
SPM Image Display

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.

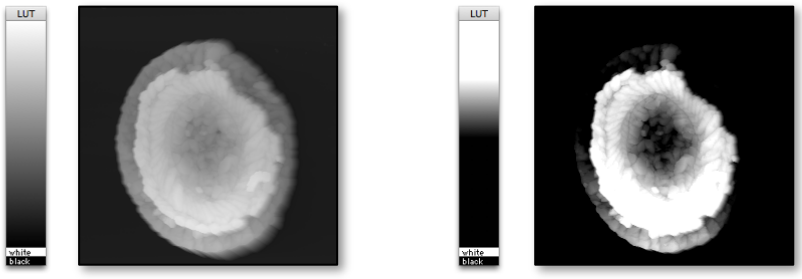


Raw image | Compensated for tilt | Line-by-line compensation

Image Processing

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'.

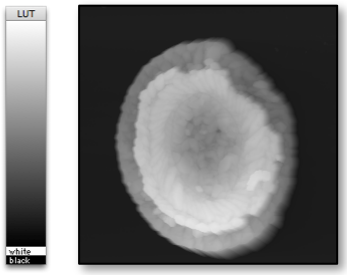
The most trivial example would include changing the colour used to represent each pixel value — the look-up table (LUT).



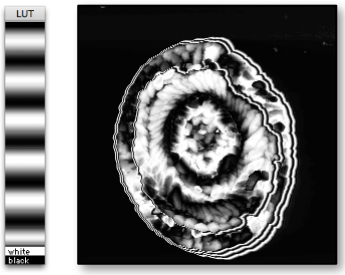
default greyscale | increased contrast

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

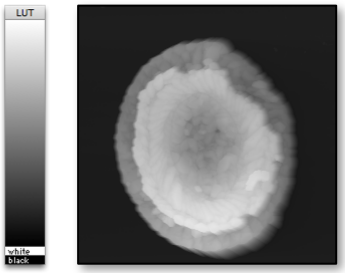


zebra greyscale

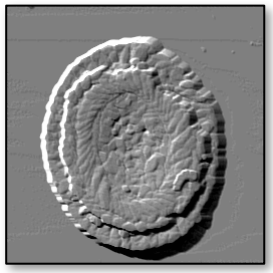
Image Processing

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.



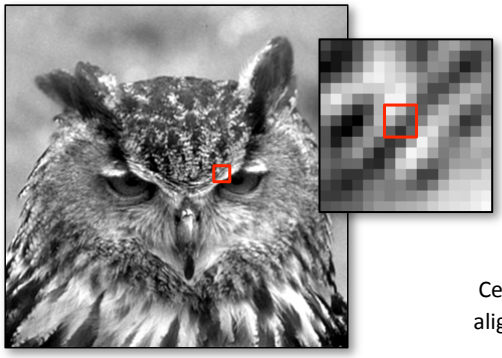
greys → z values



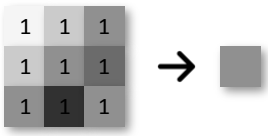
greys → $\partial z / \partial x$ values

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.

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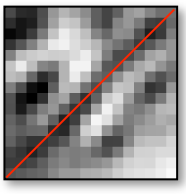
Kernel Filters

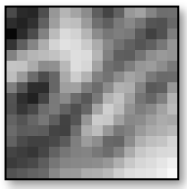
The simplest kernel filters use 3 x 3 matrices...



original image

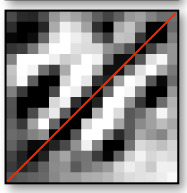
$$\begin{matrix} 1 & 1 & 1 \\ 1 & 4 & 1 \\ 1 & 1 & 1 \end{matrix}$$

smooth



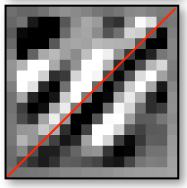
$$\begin{matrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{matrix}$$

sharpen



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

gradient



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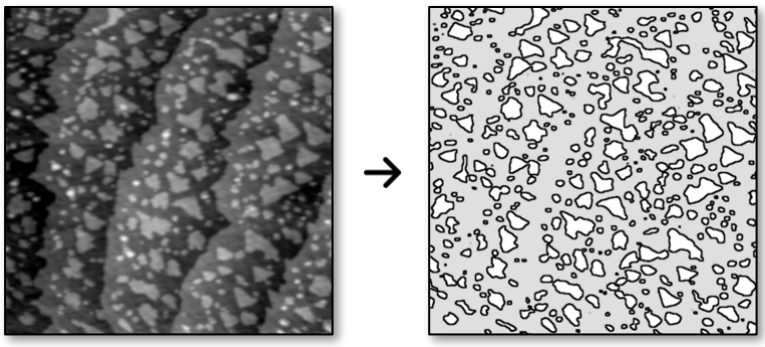
Kernel Filters

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.]

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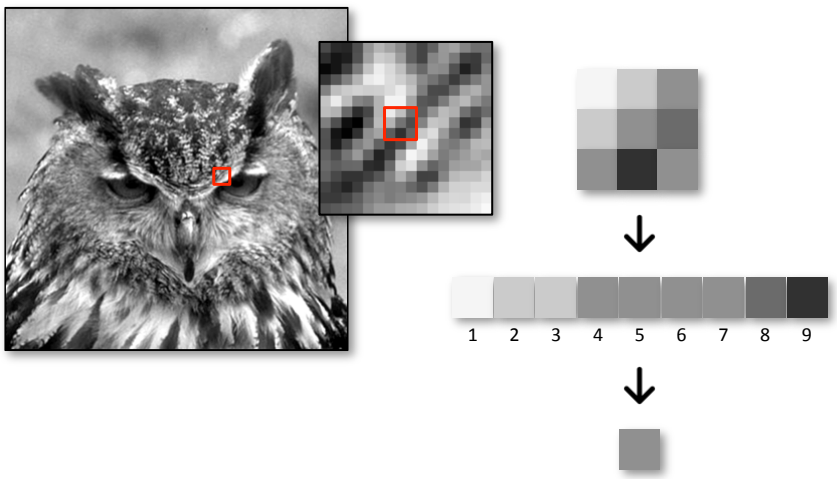
Rank Filters

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.



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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.

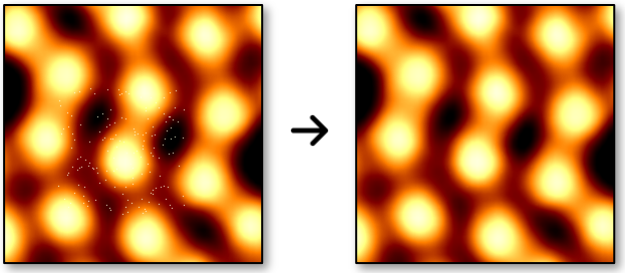
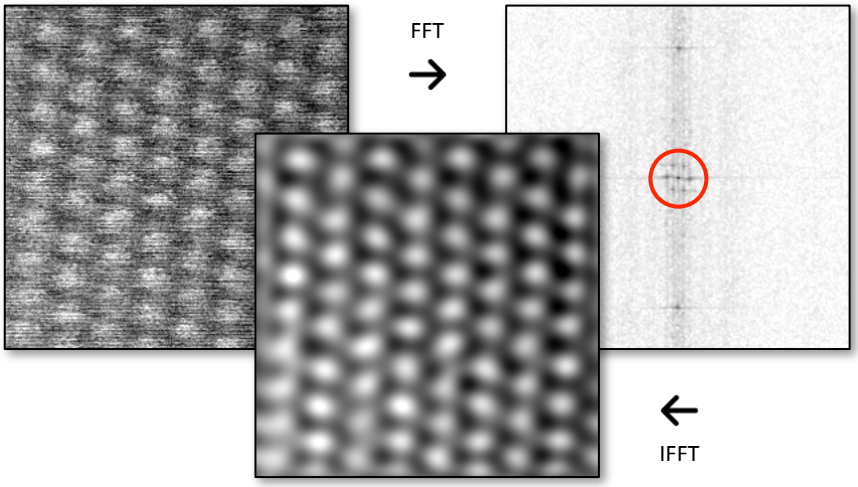


image + artificial noise | median filtered

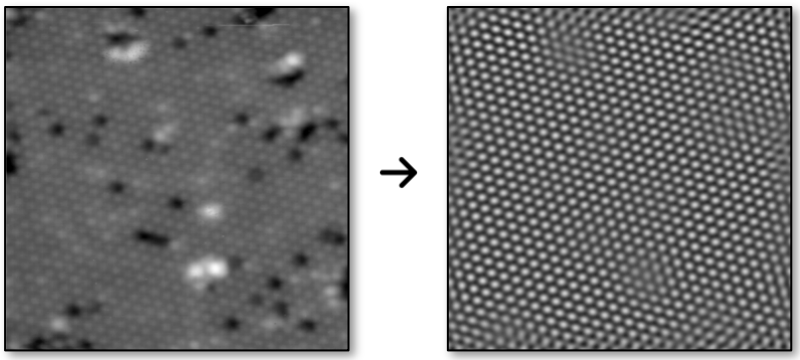
Fourier Filters

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



Fourier Filters

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

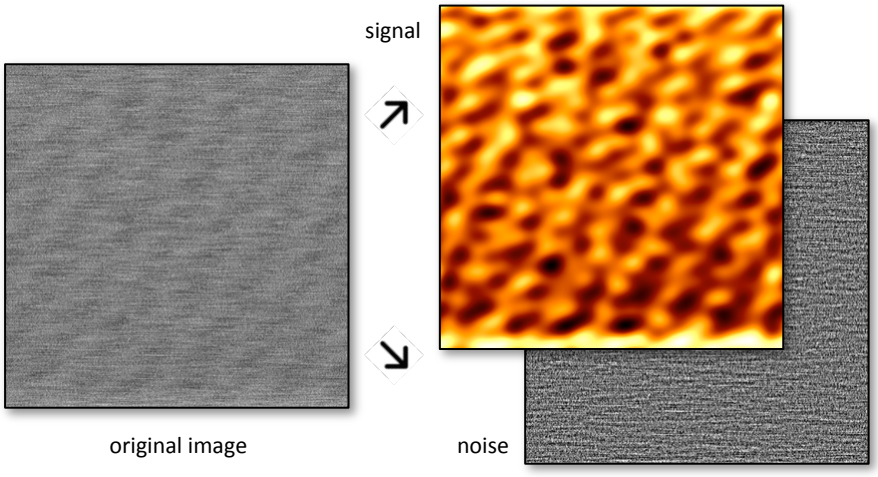


original image

Fourier filtered

Fourier Filters

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

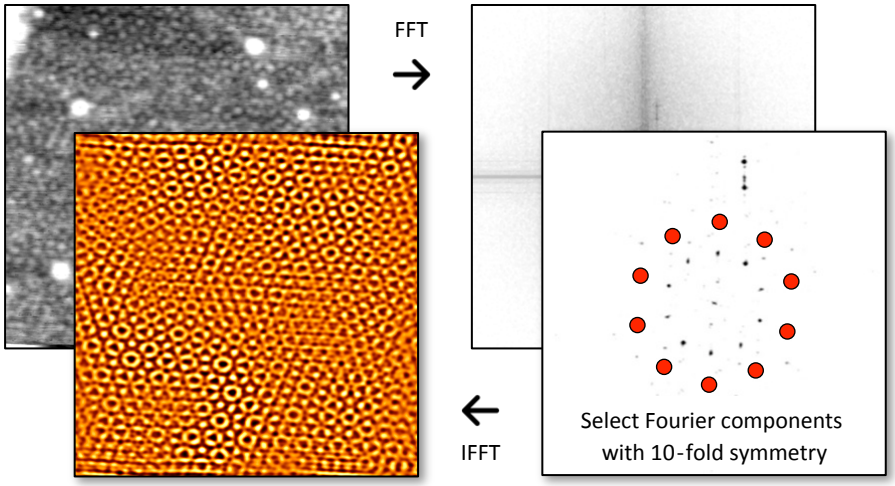


original image

noise

Fourier Filters

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



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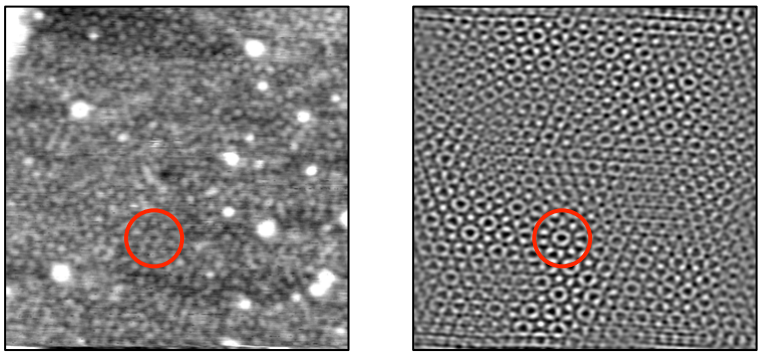
Fourier Filters

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

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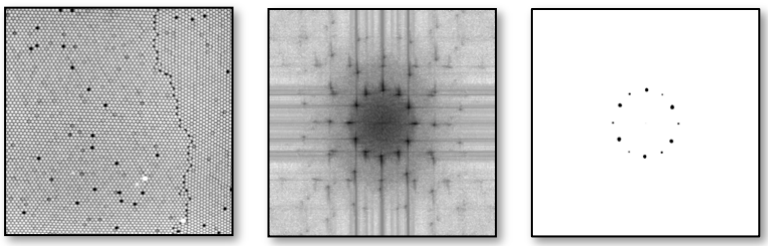
Image Analysis

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.

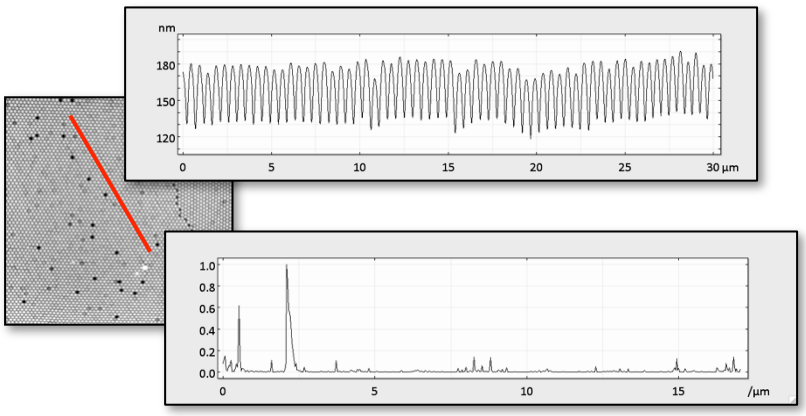


original image | Fourier transform | strongest components

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

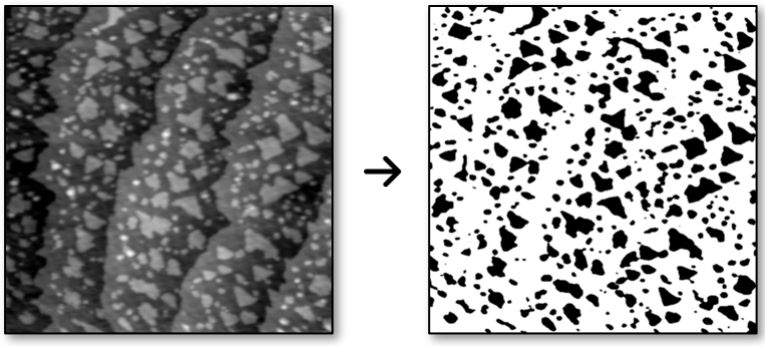
Image Analysis

Taking a profile (section) from an image allows a one–dimensional FFT of the profile data, providing corrugation and modulation values.



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.

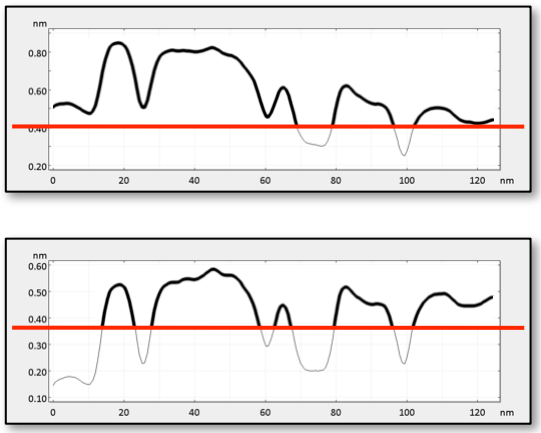


original image thresholded

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.

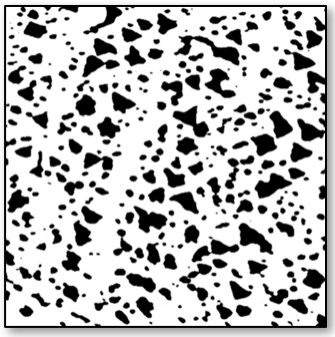
Thresholding

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.

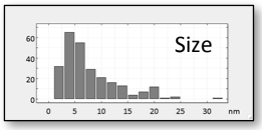
Particle Analysis

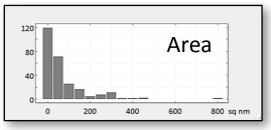


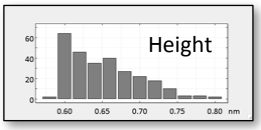
	Area	Mean	StDv	X	Y	Len	Majr	Minr	Angle	Min	Max
1.	324.38	0.74	0.07	109.15	329.47	105.67	34.59	11.94	43.0	0.59	0.91
2.	6.86	0.63	0.02	135.40	341.15	11.54	4.65	1.88	9.0	0.59	0.65
3.	5.39	0.59	0.01	294.51	341.03	8.74	3.33	2.06	177.5	0.59	0.60
4.	4.90	0.61	0.02	340.76	339.57	12.36	5.79	1.08	94.9	0.59	0.65
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)







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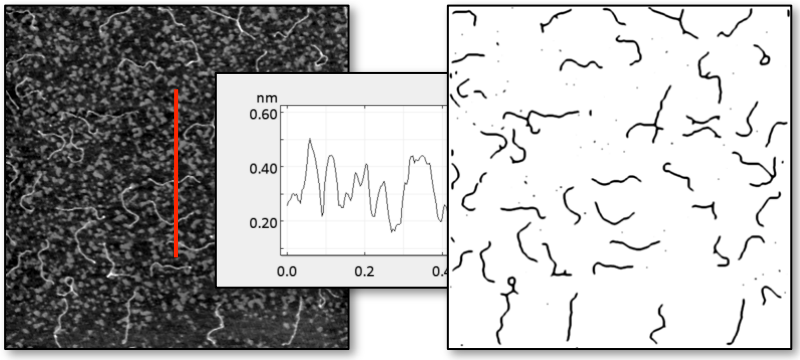
Specialist Analysis

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).

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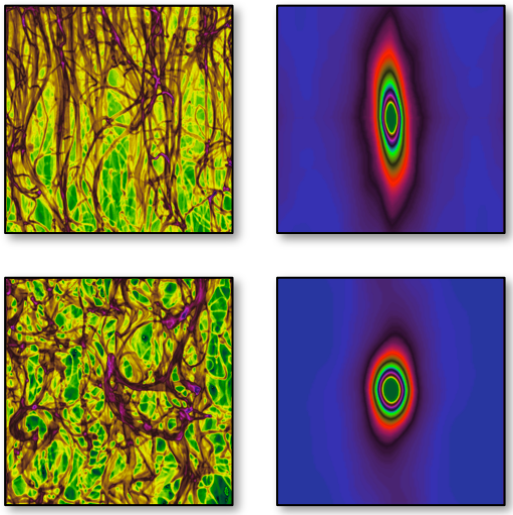
Specialist Analysis

Can the extent of 'entanglement' be quantified?



1.18

0.25

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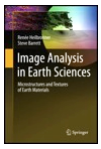
MIASMA

PrinCIPia



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