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NATIONAL CENTER OF COMPETENCE IN RESEARCH

Introduction to ImageJ session 1: Basics

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adolphe merkle institute
excellence in pure and applied nanoscience

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FNSNF
SWISS NATIONAL SCIENCE FOUNDATION

Administration

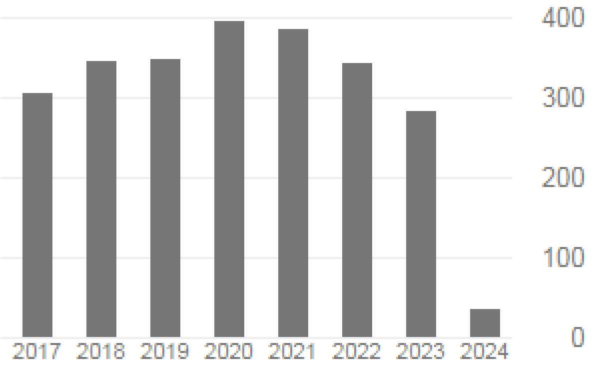
Who am I?

- PhD in structural biology (University of Bern)
- PostDoc in 3D image quantification
- 2 years at Max Planck Institute of Biochemistry, Munich, Germany
- At AMI since 2012
- Author or co-author on 80+ scientific papers
    - >3500 citations
    - h-index: 30
    - i10-index: 45

- 20 years of experience with scientific images (2D, 3D and 4D: LSM, SEM, TEM, FIB, etc…)
- 20 years of experience with imageJ and Fiji

Cited by VIEW ALL

	All	Since 2019
Citations	3523	1792
h-index	30	24
i10-index	45	41

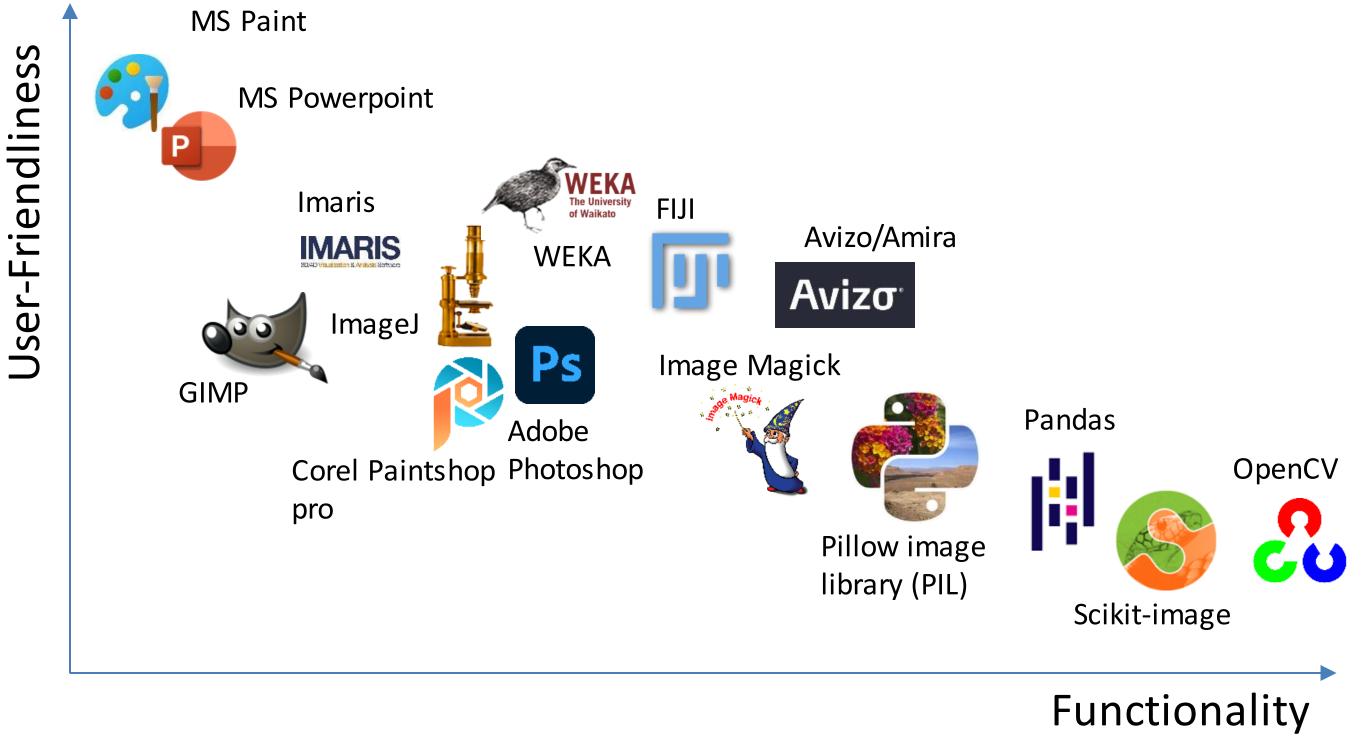


Administration

- How far can go with FIJI
- use key ImageJ options correctly
- I want to know precisely what I am doing
- Exploit the capacity of ImageJ/FIJI
- To improve own data / pictures
- Determine the size of objects and size distribution
- Distribution size of my nano-particles
- Learning how scripting works
- (Nano)-particle size distribution and size analysis
- Count cell number in a more objective way

Administration

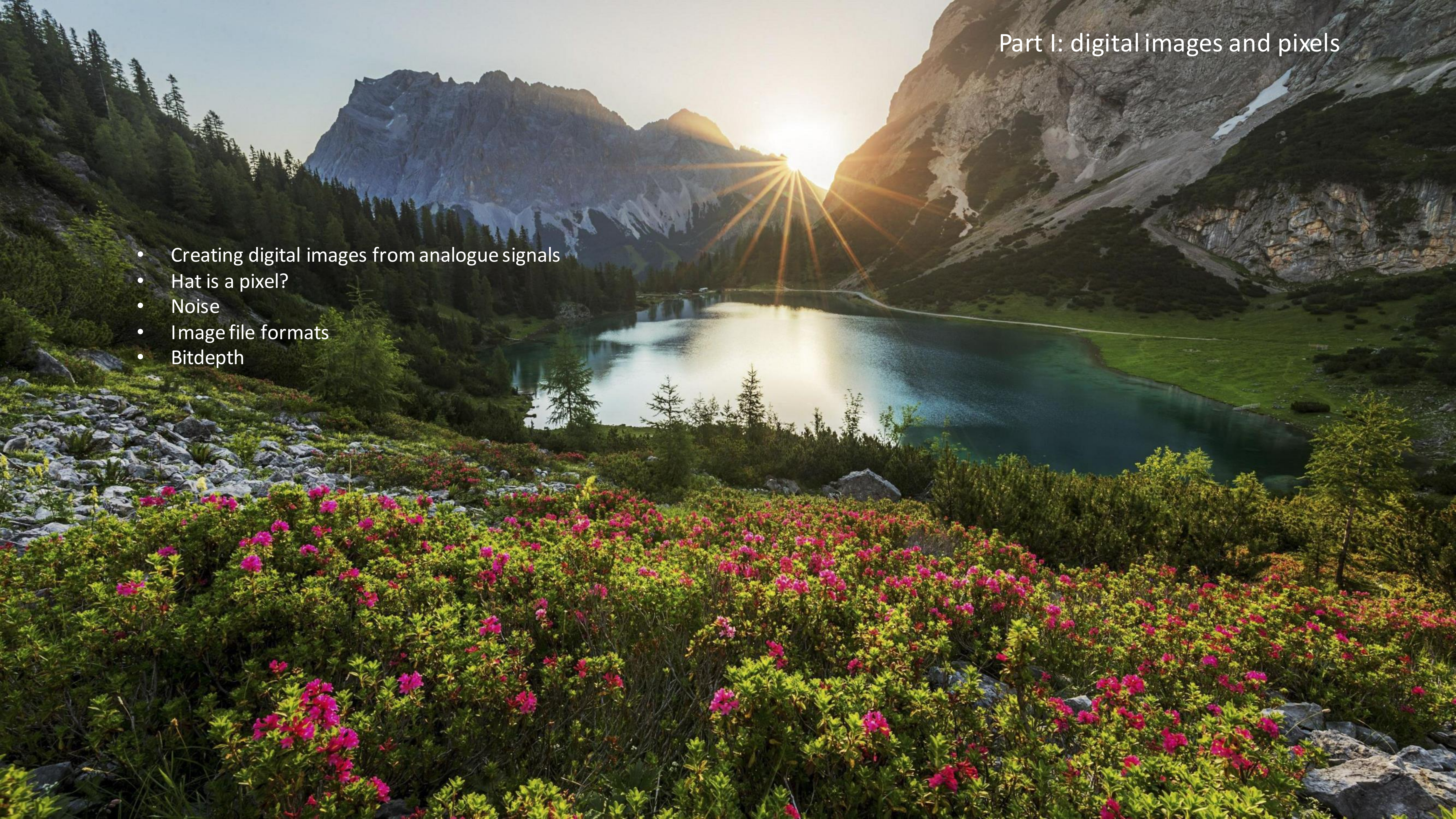
Why ImageJ (in fact: FIJI)?



FIJI (FIJI is just imageJ)

- Graphical user interface
- Extendable with plugins
- Designed for scientific data
- Broad functionalities
- Scripting possibilities

Part I: digital images and pixels

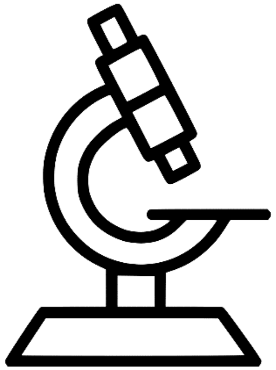


- Creating digital images from analogue signals
- Hat is a pixel?
- Noise
- Image file formats
- Bitdepth

Image processing

Image processing starts with recording good images

Sample preparation



Imaging

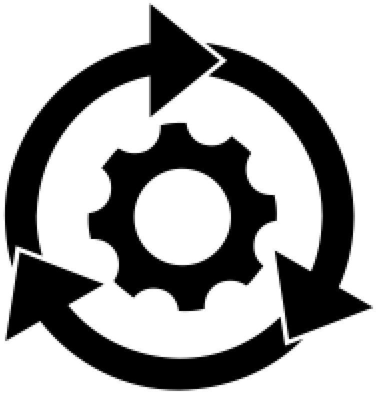


Image processing

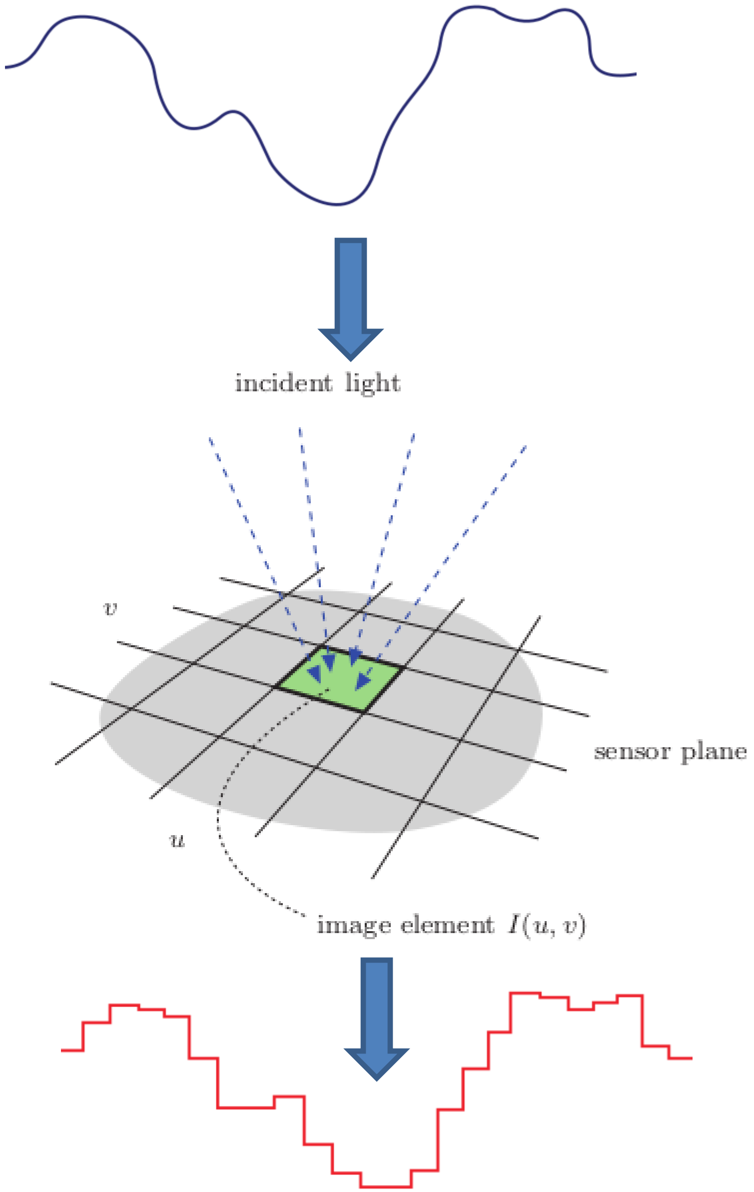
Central idea:

GIGO

Garbage in, garbage out

Good image in ➔ maybe better image out
Bad image in ➔ never a good image out

Going digital: Continuous to discrete function

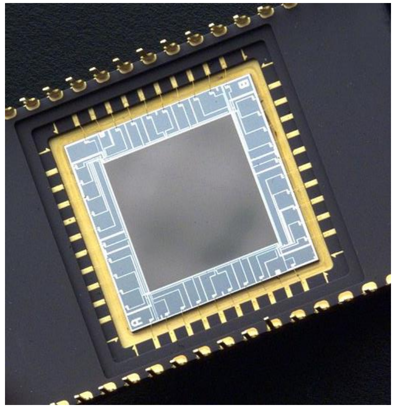


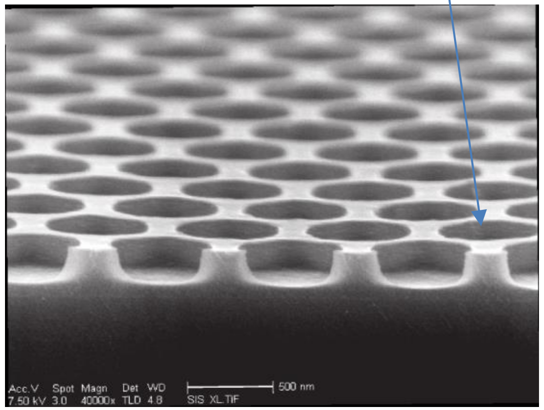
1. Spatial sampling
2. Time sampling
3. Analog to digital conversion

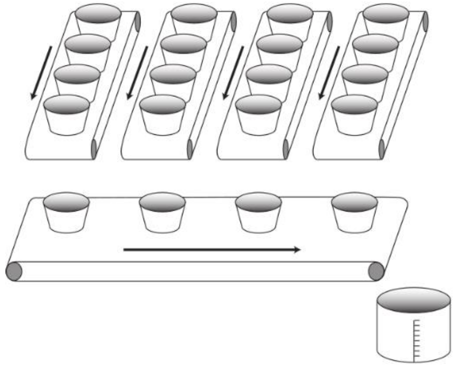
What is a pixel - Cameras and detectors

Photodiode (or well, or element)

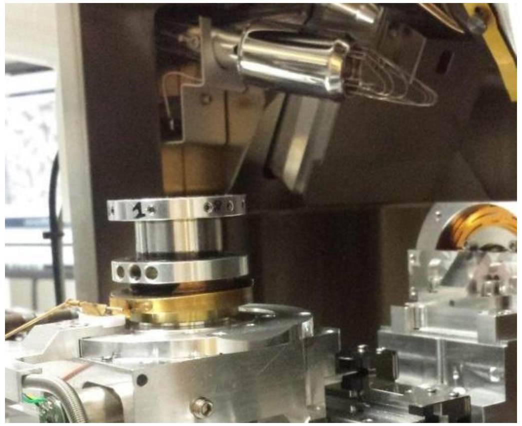
Camera
(e.g LM, TEM)

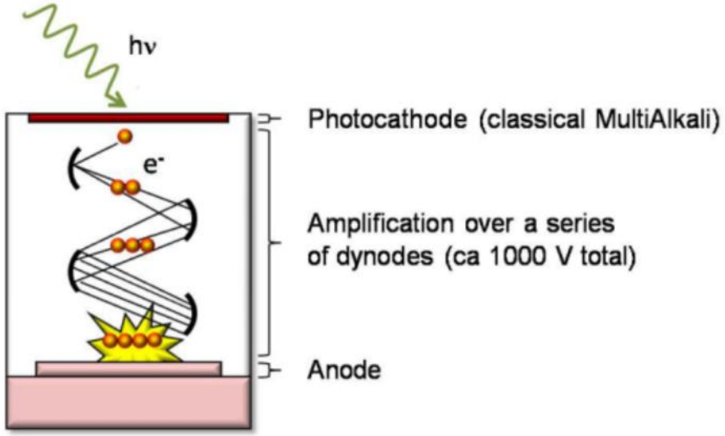






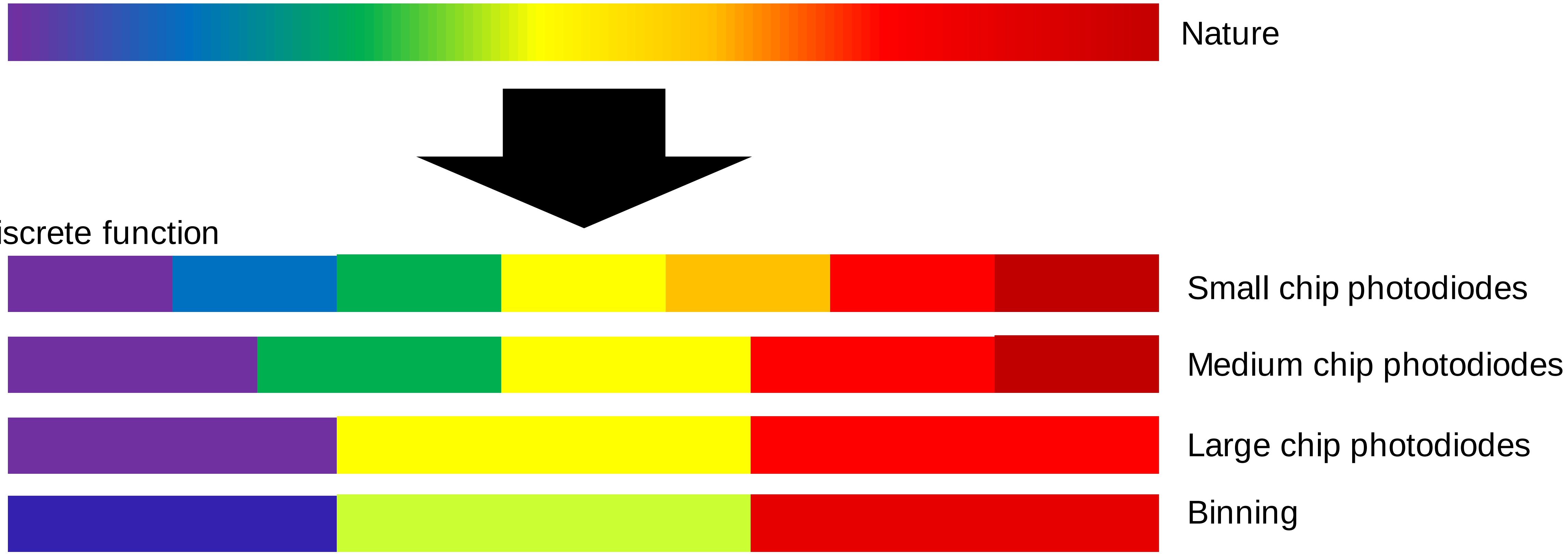
Detector
(e.g LSM, SEM, STEM)





Step 1: spatial sampling

Continuous function

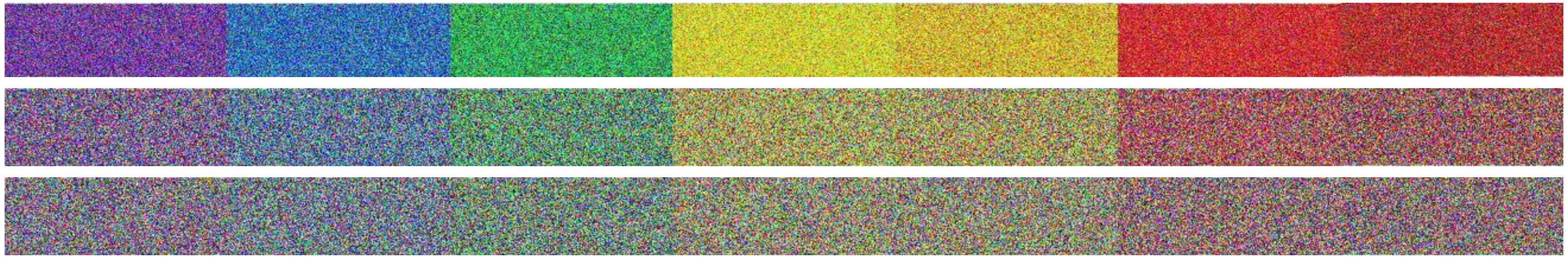


1. The **continuous** (=infinite) light distribution is *spatially sampled* to a **discrete** function
   - ➔ Conversion from continuous to a discrete function (artefacts: e.g. moiré)
   - ➔ Sensor geometry is relevant (e.g. binning)
   - ➔ According to Nyquist-Shannon theorem

Step 2: temporal sampling

Discrete function

Discrete function, time domain sampled



Long exposure time, high SNR

Medium exposure time

Short exposure time, low SNR

2. This resulting discrete function is *sampled in the time domain* (=1 still image)

➔ Exposure time (the regular time interval) is relevant, **signal to noise ratio** (SNR), shot noise

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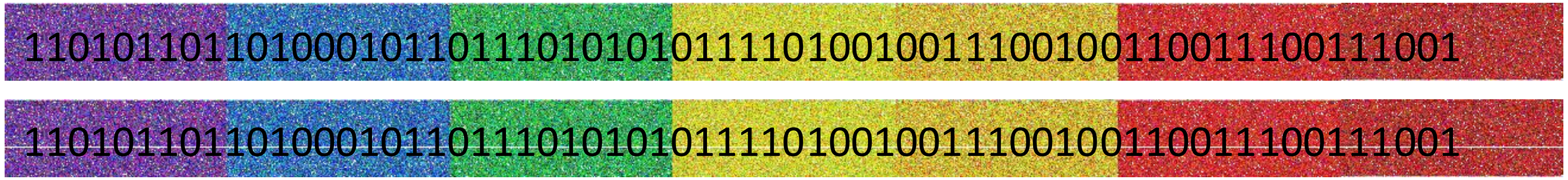
Step 3: analog to digital conversion

Discrete function, time domain sampled

Discrete function, time domain sampled



Salt-&-pepper noise, read noise

Scan faults, jitter, periodic noise

3. The resulting values are *quantitized* to a finite set of numeric values

➔ Conversion from photons (or electrons, X-rays,...) to electric charge (= analog to digital converter)

➔ charge is converted to a **range of integers**

➔ hot pixels, jitter, periodic noise

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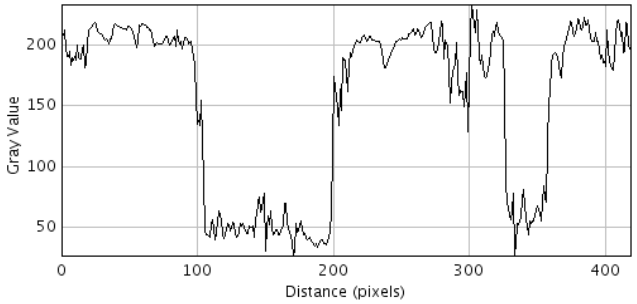
Going digital – what is a digital image?

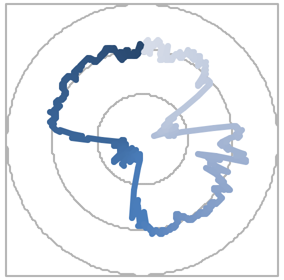
A digital image is an ordered, rectilinear array (or grid) of **integers (numbers: 0,1,2,3…)**.
Each element (=number) in the grid is also known as a picture element or 'Pixel'

Spectrum

1 dimensional array

208
209
213
198
191
191
195
184
190
188
191
188
200

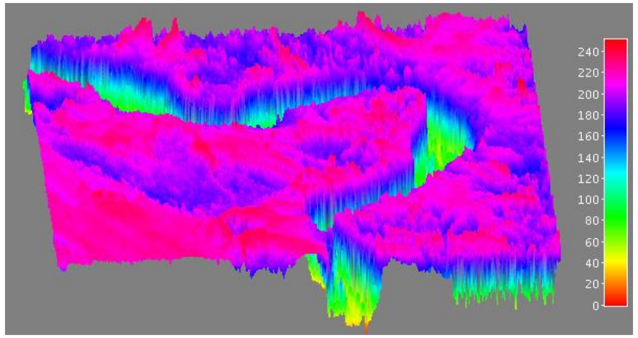




Image

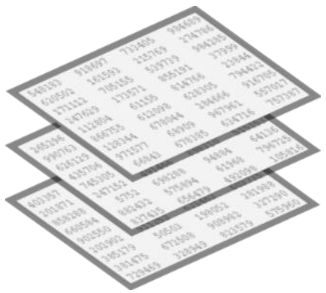
2 dimensional array

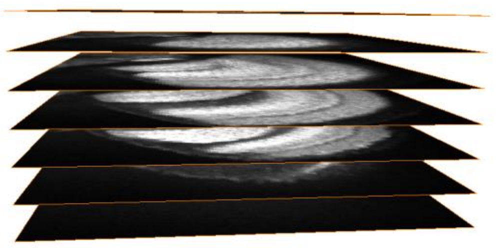
82	72	78	86	65	41
157	144	167	188	201	19
185	191	195	188	188	19
193	195	195	191	189	17
173	170	181	192	194	19
210	214	206	202	203	20
237	224	221	230	232	22
183	180	190	188	192	18
178	170	159	187	195	18
167	164	170	186	192	18
159	162	164	184	170	16
180	172	165	172	185	17
193	180	196	195	185	17
167	184	182	183	180	17
195	191	182	189	195	18
183	188	184	183	174	16
191	196	185	179	188	19



Stacks

3D array
(= volume stack or video





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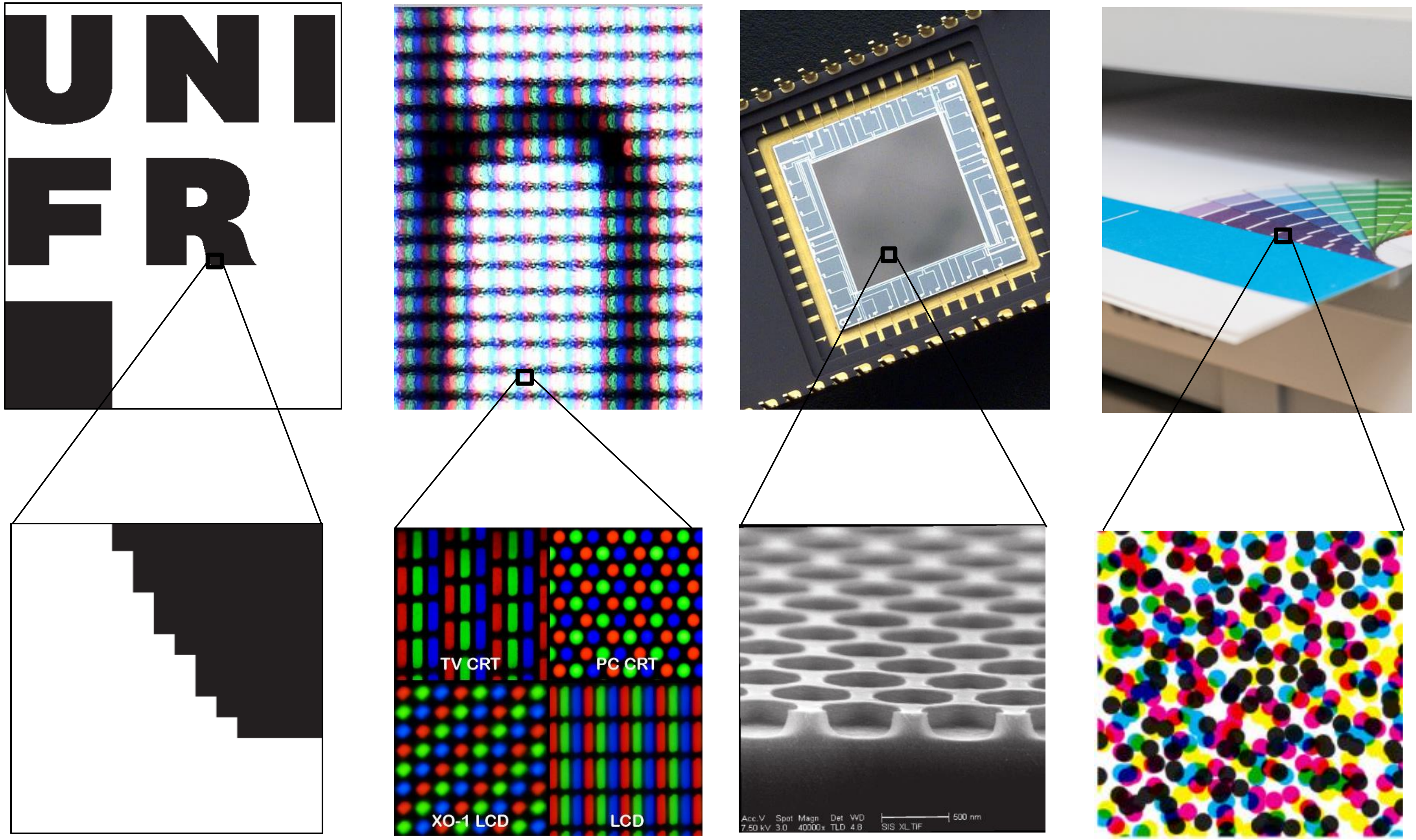
What is a pixel? – it depends on the context

Digital image Pixels	Display Triades	Camera chip Wells	Printed output Dots



Pixel = 'Pic(ture) + el(ement)'

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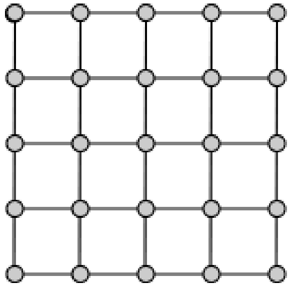
What is a pixel – the little square model

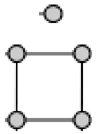
Problem: how to convert a continuous spectrum (nature) to a discrete set of intensities (digital)

Thought experiment

Consider a grid:



What are the pixels?



The corners? 5x5 grid?

The squares? 4x4 grid?

Where is the center of a pixel?

'Integerists'

Pixel (i, j) corresponding to square $(\mathrm{x}, \mathrm{y}) \rightarrow i - 0.5 \leq x \leq i + 0.5, j - 0.5 \leq y \leq j + 0.5$

A pixel is a point sample
(it exists only at **one point***)

…re $(\mathrm{x}, \mathrm{y}) \rightarrow i - 1 \leq x \leq i + 1, j - 1 \leq y \leq j + 1$

*For color pixels, there might be 3 or even 4 values at that point

An image = an array on point samples (Shannon sampling theorem!)

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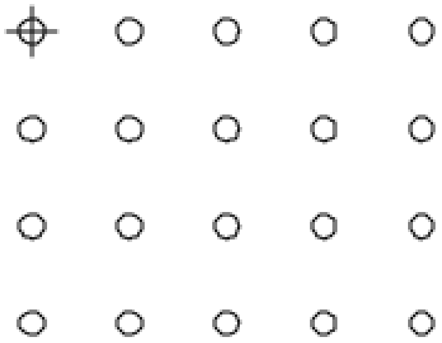
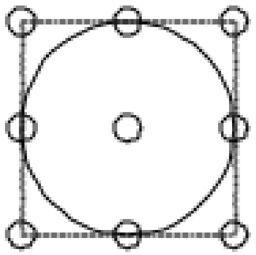
What is a pixel – the reconstruction filter

Problem: how to convert a continuous spectrum (nature) to a discrete set of intensities (digital)



A 5x4 image



The footprint of a ”reconstruction filter” (e.g. beam)

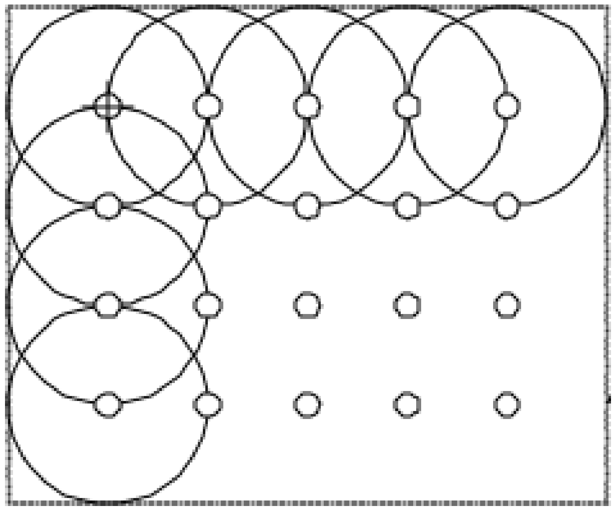
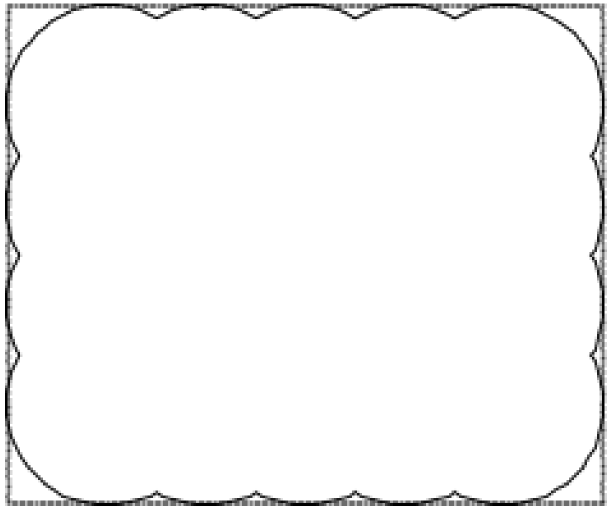


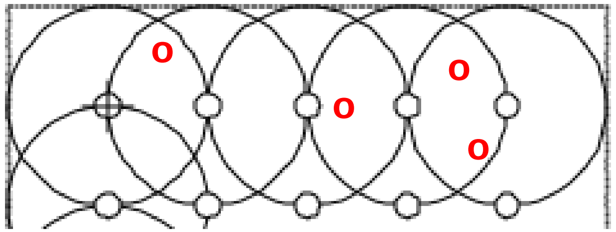
Image under construction



Recorded image

At no point, a square is involved!

What is a pixel – the point sample model

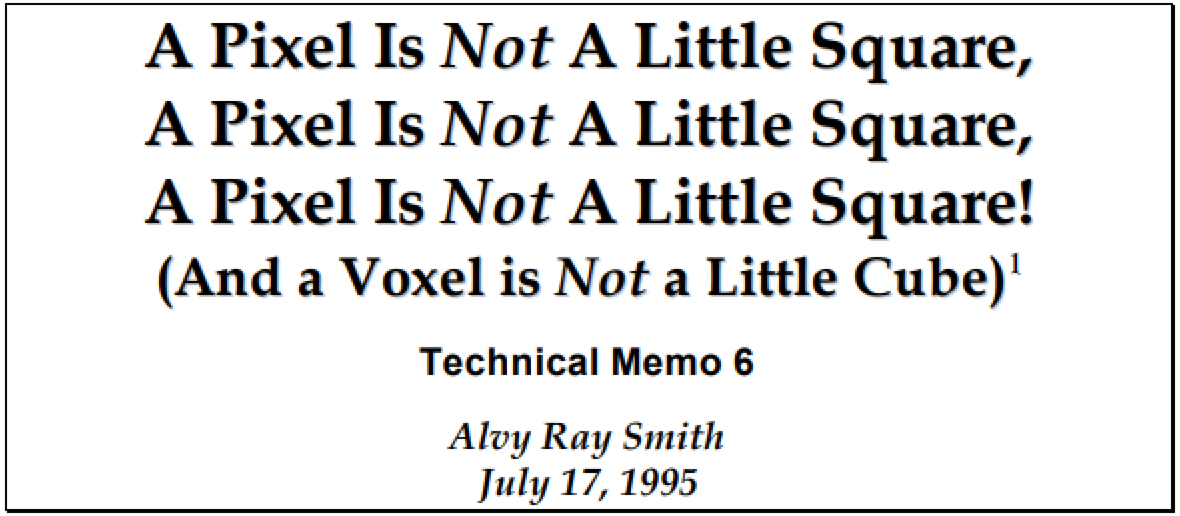


Q: What about the information (O) below the Shannon limit?

A: we simply do not know!
But you could try to guess. This is called **Reconstruction or interpolation**

Nature is continuous (has a value at every position), a digital image is a finite set of measurements: information will be lost.

Using the point sample model, we include the notion that information has been lost, and can talk about the best ways to measure that loss



A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* a Little Cube)[1]

Technical Memo 6

Alvy Ray Smith
July 17, 1995

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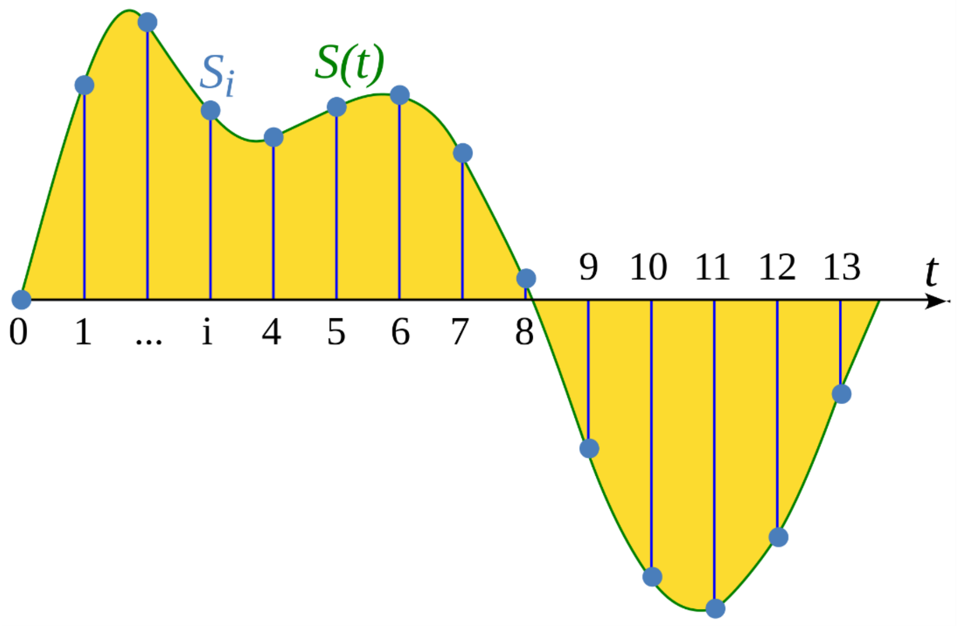
Going digital: the sampling theorem

Sampling =
process of converting continuous signal (time and space) into a sequence of values (a discrete function).



Shannon Sampling Theorem
If a function S(t) contains no frequencies higher than B hertz, it is completely determined by giving its ordinates at a series of points spaced 1/(2B) seconds apart

Shannon Sampling Theorem - revisted
If a series of discrete values has been sampled 1/(2B) apart, it does not contain information smaller than B hertz.

Shannon Sampling Theorem - applied
If an image has been recorded at a resolution of e.g.100 nm, it does not contain information smaller than 200 nm.

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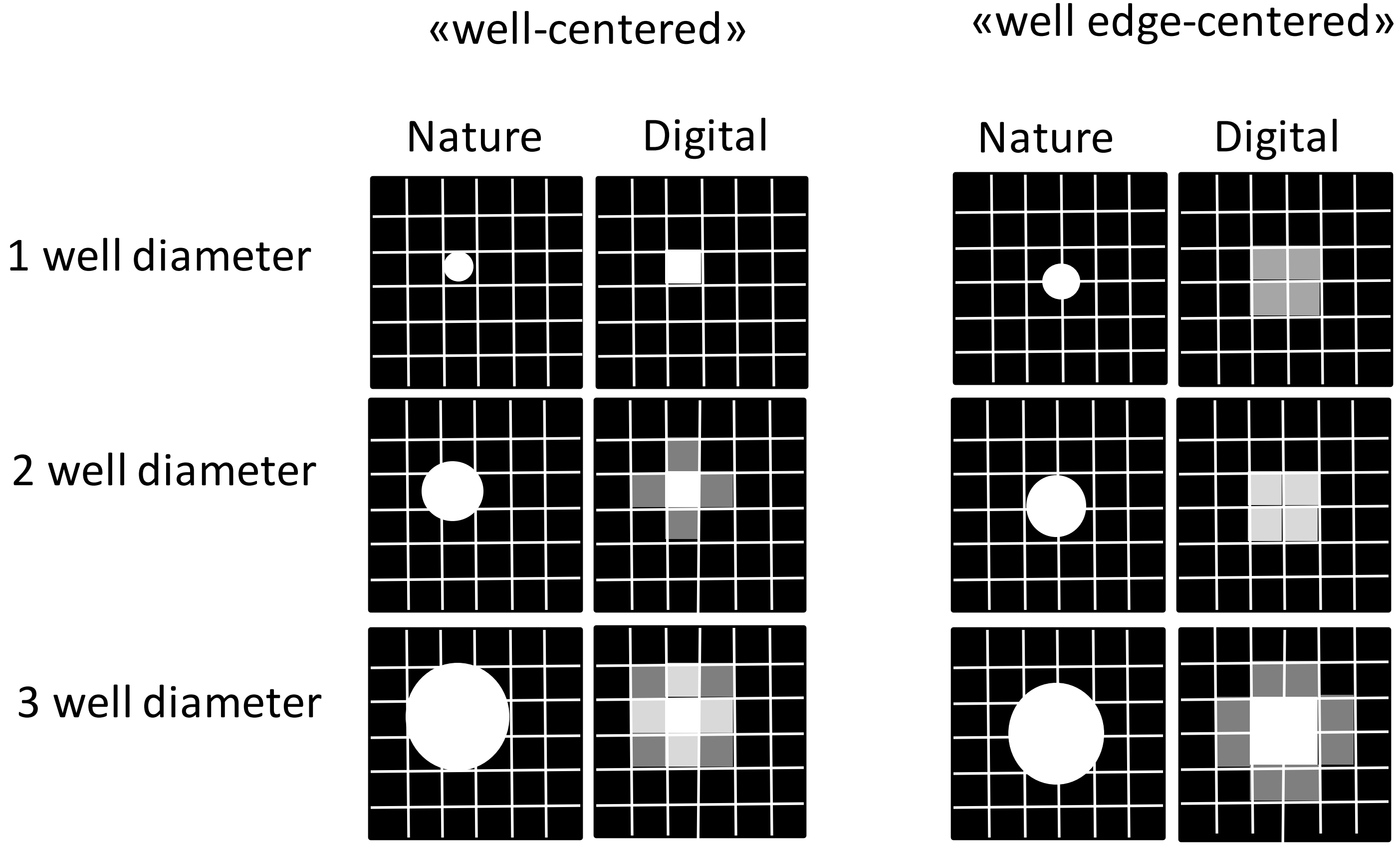
What is a pixel: Nyquist theorem

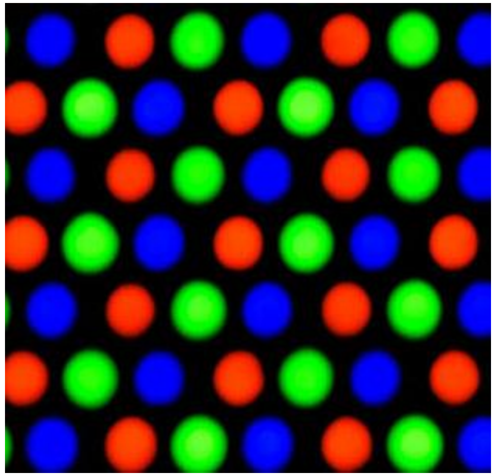


Nyquist's theorem ("howto convert analog to digital"):
the frequency of the digital sample should be twice that of the analog frequency

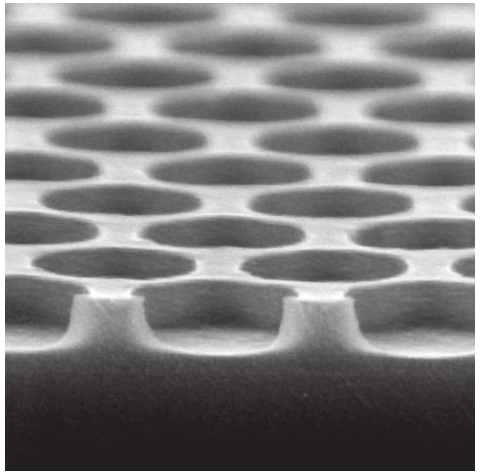
For digital images:
= a "sampling rate" of 2 wells relative to the object image size.

What is a pixel

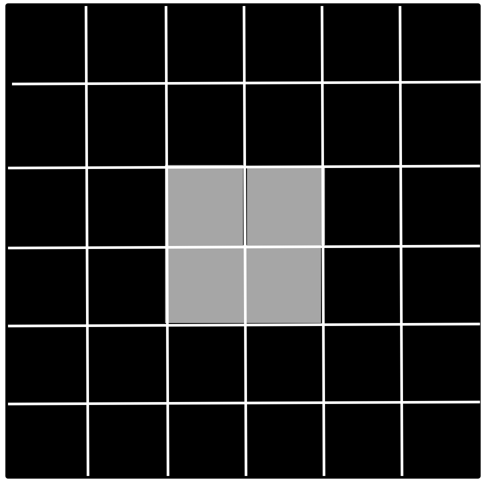
Something in a display
Triade dimensions



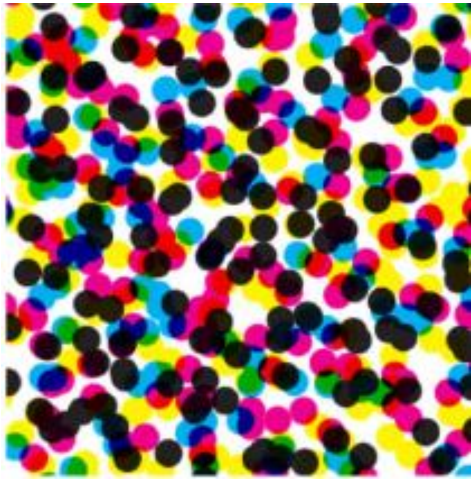
Something in a camera/detector
Photodiode dimensions



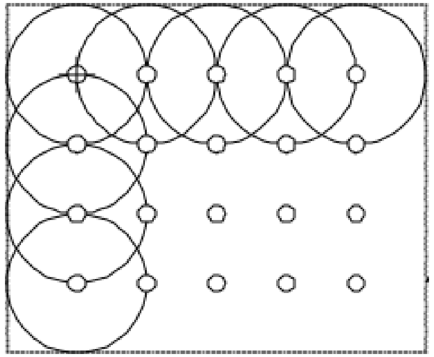
A picture element
Pixel size



A dot of printer ink
Dots per inch



Pixel concept
=Point sampling



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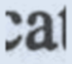
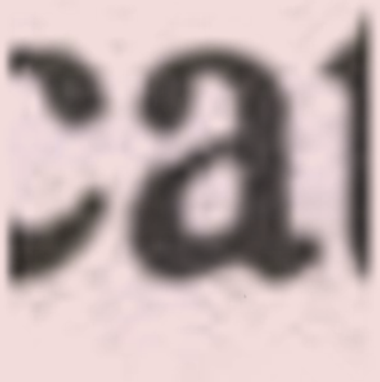
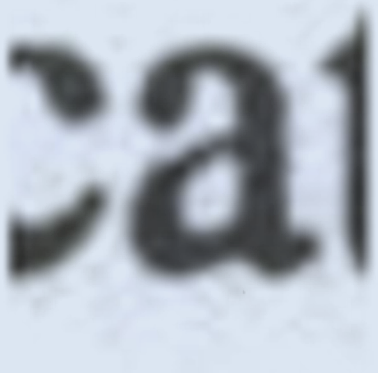
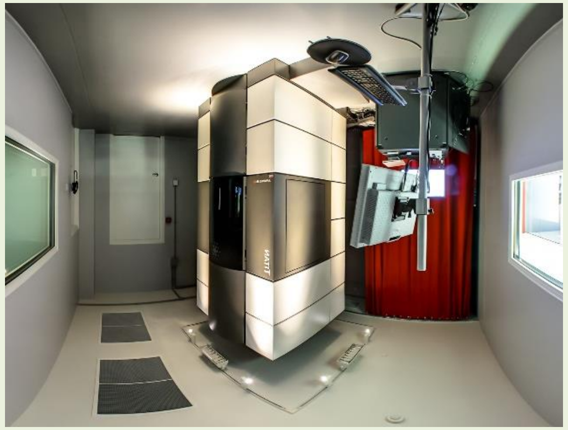
What is a resolution? And what is magnification?

	Magnification	Resolution	Resolving power
Low			
High			
Assessment	Easy	Very difficult	

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Noise

Additive noise		Multiplicative noise	

Gaussian noise

Sum of all natural sources forming a normal distribution (=Gaussian distribution).

- **Johnson–Nyquist noise** (thermal vibrations of atoms in conductors)
- **Black-body radiation** (radiation from the earth or other objects)
- **Sensor noise** (when not recording at 0 K)
- **Electronic circuit noise** (impedance in electronic cables)
- **kTC noise** (Effects of capacitors)

FFT filtering, Gaussian smoothing.

Poisson (shot) noise

Caused by quantum effects due to the movement of discrete, quantized, packets

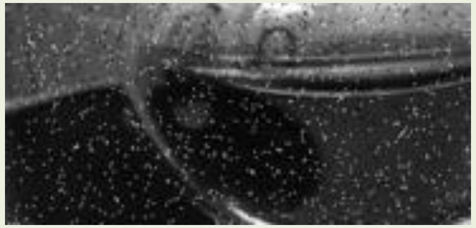
- In the source (light)
- In the electronics (electric current).
- Dark shot noise: shot noise from the dark leakage current in the image sensor

high intensity, exposure times
Correct with bias images

Salt and pepper noise

Caused by errors. Typically B/W distribution

- During data transmission
- Failure in a memory cell
- Analog-to-digital converter errors
- Occasionally in TEM (X-rays)
- In camera-based systems: dead pixels



Median filtering

Repetitive noise

caused by electrical interference during the image capturing process.

Signal deconvolution in reciprocal space

Intermezzo - how does a computer work?

Examples of decimal (=10 digits) counting
- begins with 1 digit (rightmost digit or first digit)
- When all available symbols are exhausted:
  - the least significant digit is reset to *0*,
  - the next digit (one position to the left) is incremented (=overflow*)*

```
 0   1   2   3   4   5   6   7   8   9
10  11  12  13  14  15  16  17  18  19
```

Examples of binary (=2 digits) counting
- begins with 1 digit (rightmost digit or first digit)
- When all available symbols are exhausted:
  - the least significant digit is reset to *0*,
  - the next digit (one position to the left) is incremented (=overflow*)*

```
 0     1    10    11   100   101   110   111
(0)   (1)   (2)   (3)   (4)   (5)   (6)   (7)
```

Computer = on/off
= binary digits ('bit')

1 Bit

8 Bits = 1 byte

1024 bytes = 1 KB		KB = Kilobyte	
1024 KB = 1 MB		MB = Megabyte	
1024 MB = 1 GB		GB = Gigabyte	
1024 GB = 1 TB		TB = Terabyte	
1024 TB = 1 PB		PB = Petabyte	

From numbers to text
- ASCII: American Standard Code for Information Interchange
- List of numbers with defined characters

Dec	Char	Dec	Char	Dec	Char
32	SPACE	64	@	96	`
33	!	65	A	97	a
34	"	66	B	98	b
35	#	67	C	99	c
36	$	68	D	100	d
37	%	69	E	101	e
38	&	70	F	102	f
39	'	71	G	103	g
40	(	72	H	104	h
41	)	73	I	105	i
42	*	74	J	106	j
43	+	75	K	107	k
44	,	76	L	108	l
45	-	77	M	109	m
46	.	78	N	110	n
47	/	79	O	111	o
48	0	80	P	112	p
49	1	81	Q	113	q
50	2	82	R	114	r
51	3				
52	4				
53	5				
54	6				
55	7				
56	8				
57	9				
58	:				
59	;	91	[	123	{
60	<	92	\	124	|
61	=	93	]	125	}
62	>	94	^	126	~
63	?	95	_	127	DEL

'AMI'
(dec): 65 77 73
(bin): 1000001 1001101 1001001

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File formats

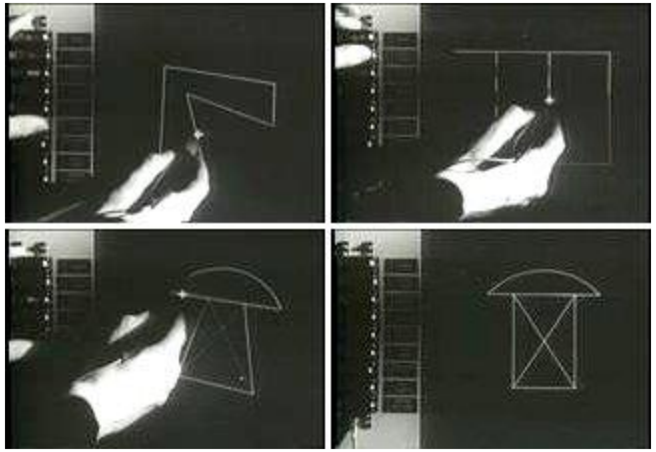
From concept: 3 different approaches:

1. Vector graphics formats
2. Raster graphics formats
3. Hierarchical file formats

File formats: Vector graphics

Formats svg, wmf, eps, pdf, cdr, ai, …

Origin 1963, with SketchPad, the PhD thesis of Ivan Sutherland

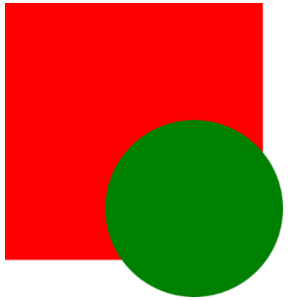


Concept text file (usually xml style) with information on *objects* to be drawn

Example

```
<rect
      y="11.25"
      x="5.6696429"
      height="34.017857"
      width="34.017857"
      id="rect4518"
      style="fill:#ff0000;fill-opacity:1;stroke:none;stroke-
width:0.52999997;stroke-linejoin:round;stroke-
miterlimit:10;stroke-dasharray:none" />

<circle
      r="11.717261"
      cy="38.464279"
      cx="30.616072"
      id="path4522"
      style="fill:#008000;fill-opacity:1;stroke:none;stroke-
```

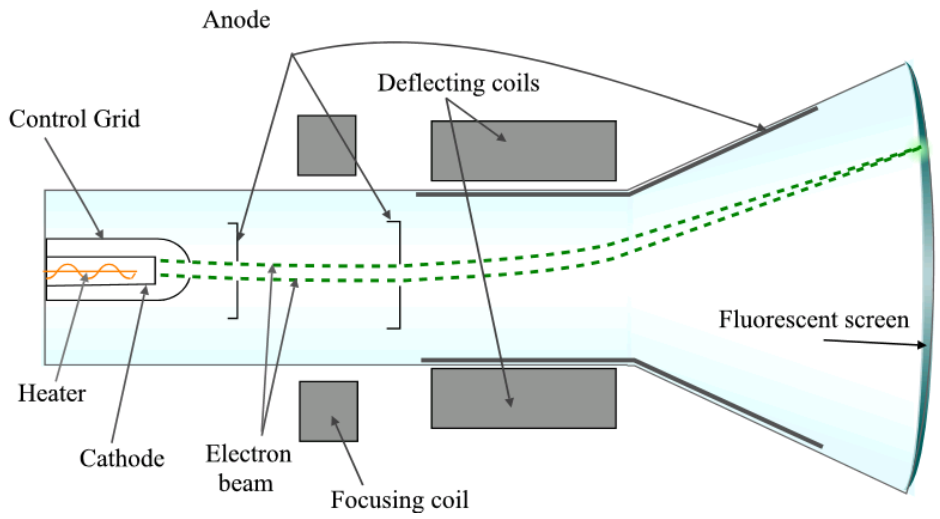


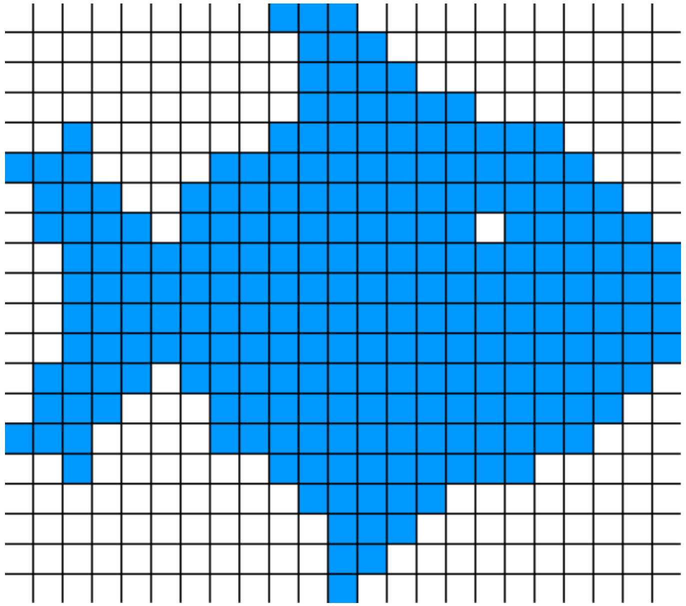
Important for Overlays!!

File formats: Raster graphics

Formats tif, jpg, png, bmp, webP, psb, …

Origin raster scan of cathode ray tubes (i.e. early TV screens)
'Raster' comes from rastrum (Lat.) = to scrape





Concept Tesselation (tiling) of a 2D plane with a value for every cell ('pixel')

File formats: Raster graphics – Headers

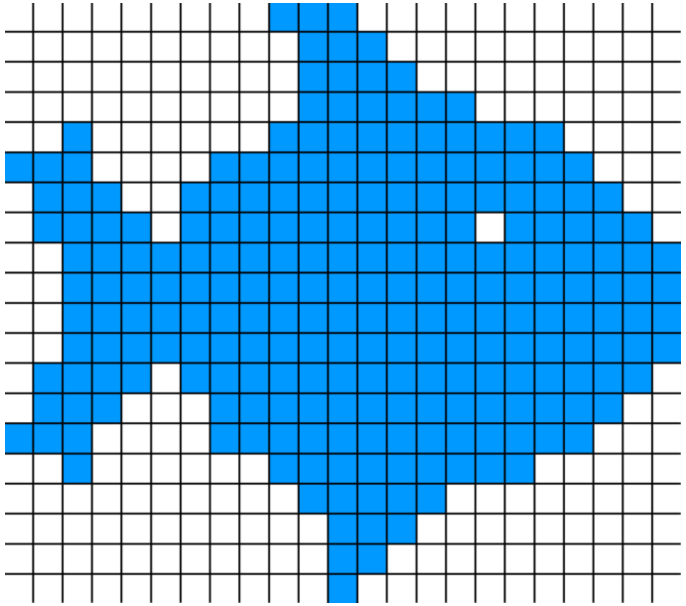
Concept Tesselation (tiling) of a 2D plane with a value for every cell (‘pixel’)

Problem Metadata: How large is the image? When was it made? But also:
e.g. medical data (patient information)
e.g. scientific data (experiment information)
e.g. consumer data (GPS coordinates, time and date)

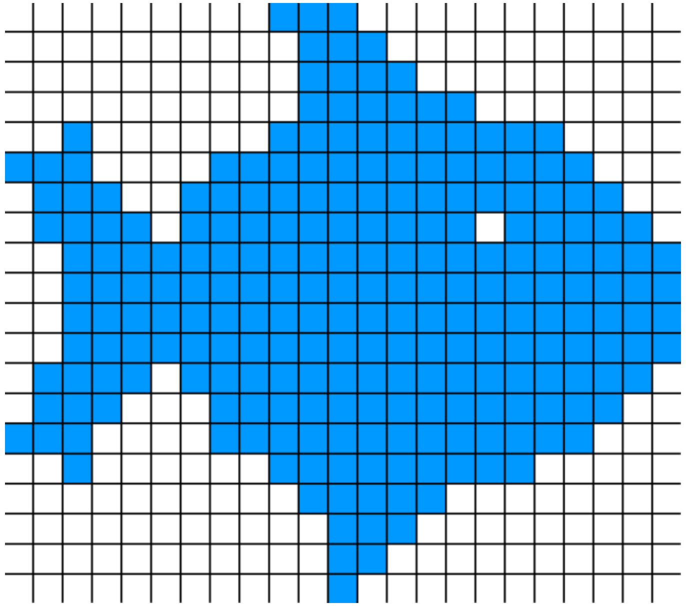
Solution Header
contains height and width of the raster (=image) +lots of other data

Example

Header	01110100 (ASCII, 116 = T) 01101001 (ASCII, 105 = I) 01100110 (ASCII, 102 = F) 00000100 (width = 4 px) 00000001 (heigth = 1 px)
data	00000000 00110010 01100100 10010110



File formats: Raster graphics – Compression

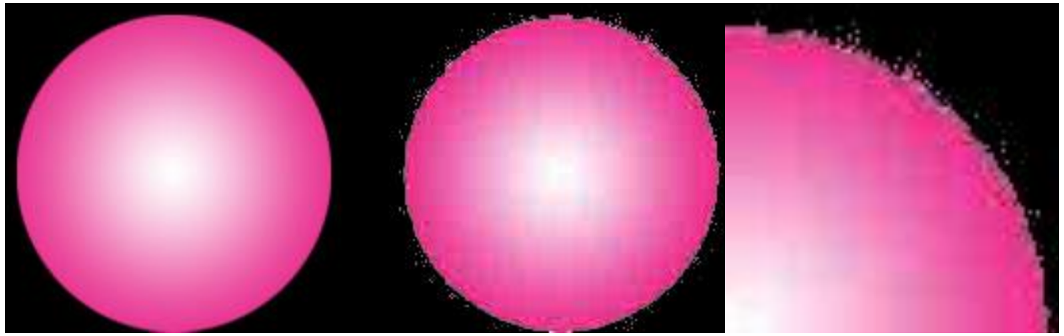


Concept Tesselation (tiling) of a 2D plane with a value for every cell ('pixel')

Problem Data gets really big

Solution Compression. There are 2 compression concepts:

- lossy (ie. The original data is approximated, but not restored)
e.g. jpg -> compression artefacts



- lossless (the original data can be restored)
e.g. Run Length Encoding (RLE), Lempel Ziv (LZx, uses lookups)

Original data

```
00000000
00110010
01100100
10010110
```

32 bits

RLE compressed

```
8x0
2x0 2x1 2x0 10
0 2x1 2x0 1 2x0
1 2x0 10 2x1 0
```

29 bits

LZ compressed

```
A = 00
B = 11
C = 10
4xA
A B A C
0 B A 1 A
1 A C B 0
```

38 bits

```
Repeated to get 1024x1024:
Original 8 Mb
RLE      7.25 Mb (-10%)
LZ       7 bytes (!) (-99.99999%)
```

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File formats: Hierarchical data formats (HDF)

Formats	h5
Origin	1987 by the Graphics Foundations Task Force. Around 1992, NASA investigated 15 different file formats for use in the Earth Observing System (EOS) project and settled for HDF.
Concept	Container of multiple, heterogenous data (including really big datasets), with metadata
Some advantages	- really really big datasets possible (e.g. tomography data) - hierarchical data (e.g. google maps) - heterogenous data (e.g. EDX spectra with images) - slices: only part of an image can be read (e.g. google maps) - embedded coding (allows advanced compression techniques)

File formats: raster graphics: bit depth

The range of the values a pixel can take is called the bit depth

2^n = number of shades
n = bitdepth

1 bit (=black and white, =binary image) — 2 shades — **Binary (e.g. Thresholding!)**

8 bit (=common consumer cameras) — 256 shades

~9 bit (=human eye) — ~500 shades

12 bit (=middle range scientific cameras) — 4096 shades

16 bit (=high range scientific cameras) — 65536 shades

32 bit (=only computational) — 4 294 967 296 shades

Grayscale (8 bit to 32 bit)

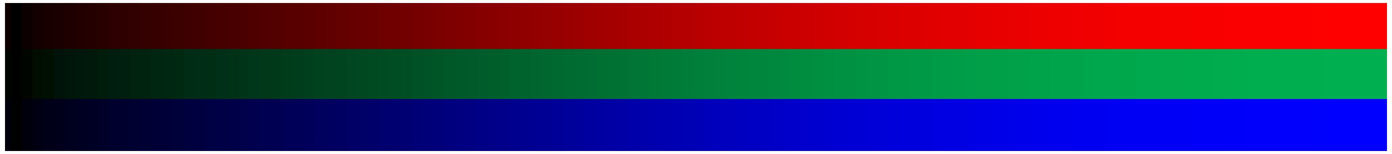
Signed (e.g. Signed 16bit). -32768 → +32768 — **Special**

File formats: raster graphics - bit depth & colour

The range of the values a Pixel can take is called the bit depth

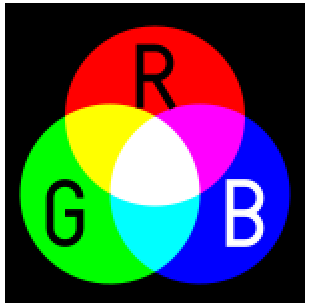
2^n = number of shades
n = bitdepth

RGB (=3 channels of each 8 bit = 24 bit) 256 x 256 x 256 colours



RGBA (=4 channels of each 8 bit = 32 bit) 256 x 256 x 256 colours x 256 Alpha

Additive color mixing (Displays)

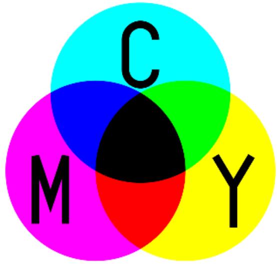


48 bit RGB (= 3 channels of each 16 bit = 48 bit)

Computational

CMYK (=4 channels of each 8 bit = 32 bit) 256 x 256x 256x 256 colours

Subtractive color mixing (pigments, Printers)



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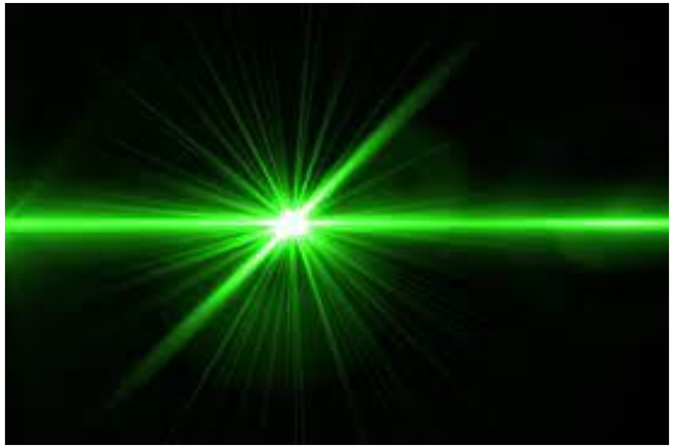
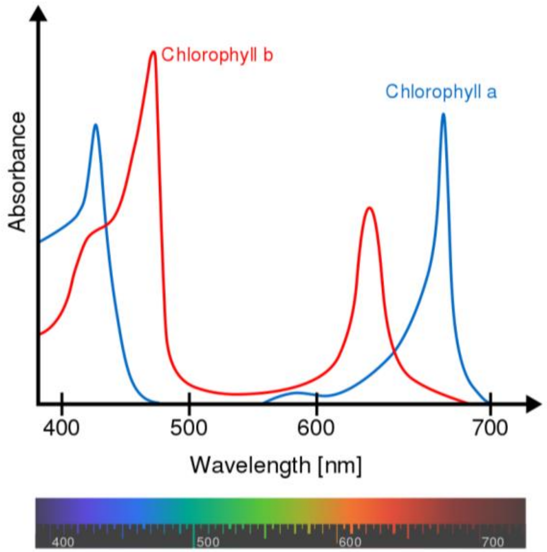
Note: light sources and dyes/pigments

Additive color mixing
Light sources



A green laser is green because it emits green photons

Subtractive color mixing
Dyes and pigments



A leaf is green because it absorbs all white light except the green photons (which it reflects)

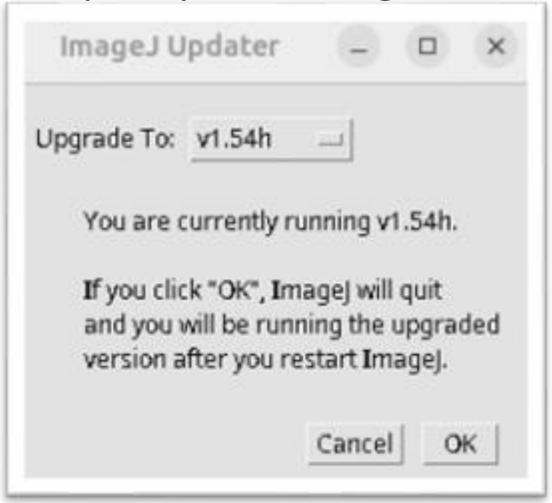
Image processing

Part II: How to open your data?

- Updating ImageJ/FIJI
- Native file formats
- Repositories
- FIJI import
- Plugins
- Raw import

0. Update FIJI

Help > update ImageJ…



Start ImageJ, then Help > About ImageJ…

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1. How to open your data in FIJI?

5 Possibilities:

- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)

1. Native file formats

5 Possibilities:

- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)

Format	1 bit	8 bit	16 bit	32 bit	RGB	stacks	Hyper-stacks	Compression
TIFF	✔	✔	✔	✔	✔	✔	✔	Lossless
GIF	✔	✔			✔	✔		Lossless
JPEG		✔			✔			lossy
PNG	✔	✔	✔					Lossless
DICOM	✔	✔	✔	✔	✔	✔	✔	Lossless
BMP	✔	✔			✔			Lossless
PGM	✔	✔	✔		✔			Lossless
FITS	✔	✔	✔		✔			Lossless
AVI	✔	✔			✔	✔		Lossless

TIFF is the 'default' format of ImageJ (OME-TIFF)
DICOM is a standard popular in the medical imaging community
FITS Flexible Image Transport System: adopted by the astronomical community
PGM: Portable GrayMap
AVI container format, only uncompressed AVIs supported
Red: no header information

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Native and non-native file formats

EXERCISE 1

Try to open example 1 A, B, D (HDR) and D (.SER)

File > open…

Or drag and drop the icon from the folder onto ImageJ/FIJI

Does it work?

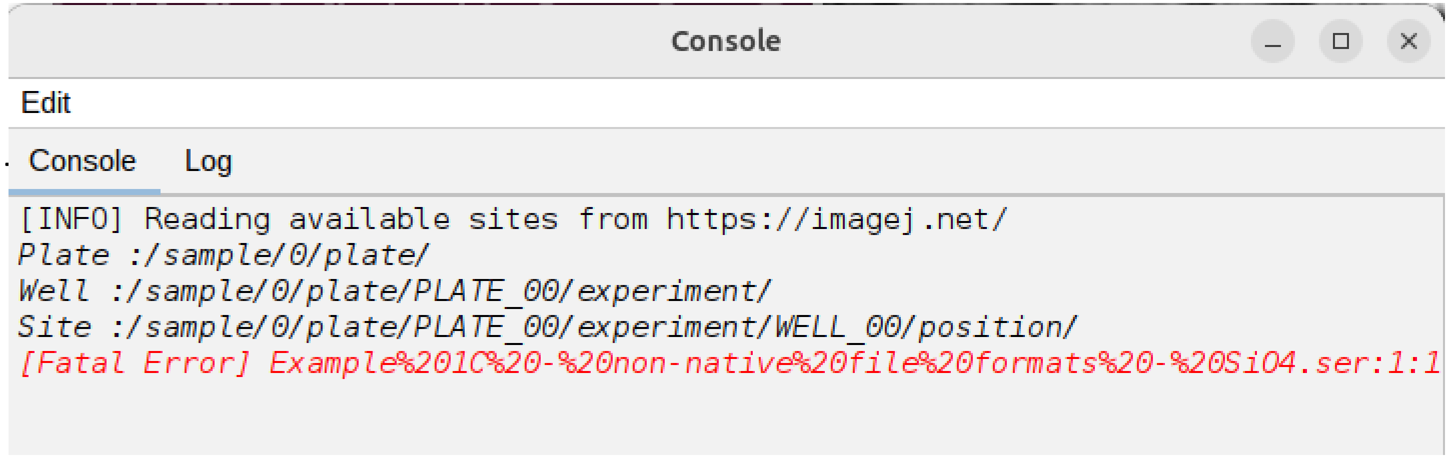
Native and non-native file formats

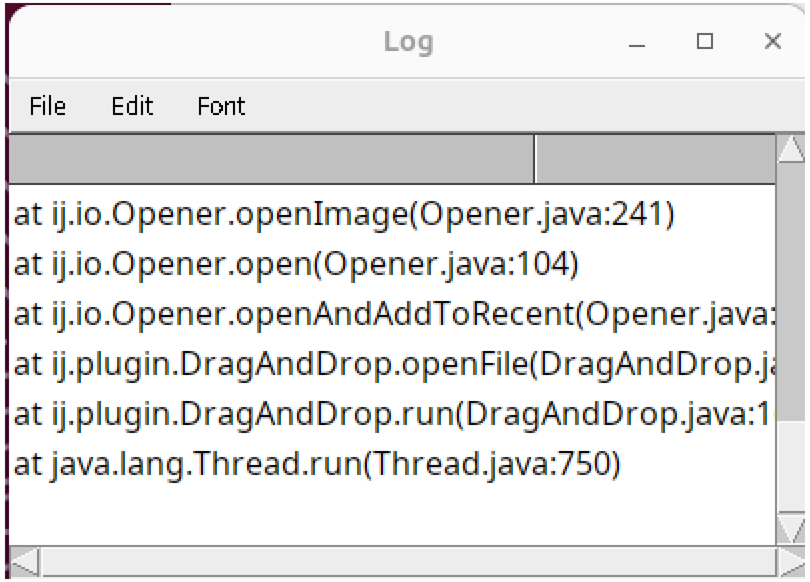
EXERCISE 1

Try to open example 1 A, B, D (HDR) and D (.SER)

File > open…

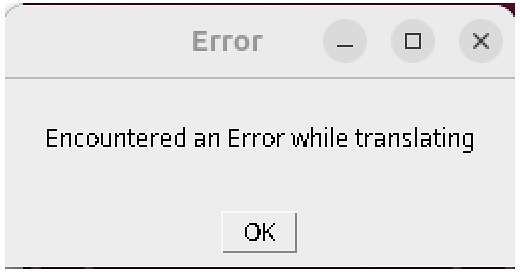
Or drag and drop the icon from





Does it work?

No… Only Example 1A and 1B open (or maybe not even 1B)



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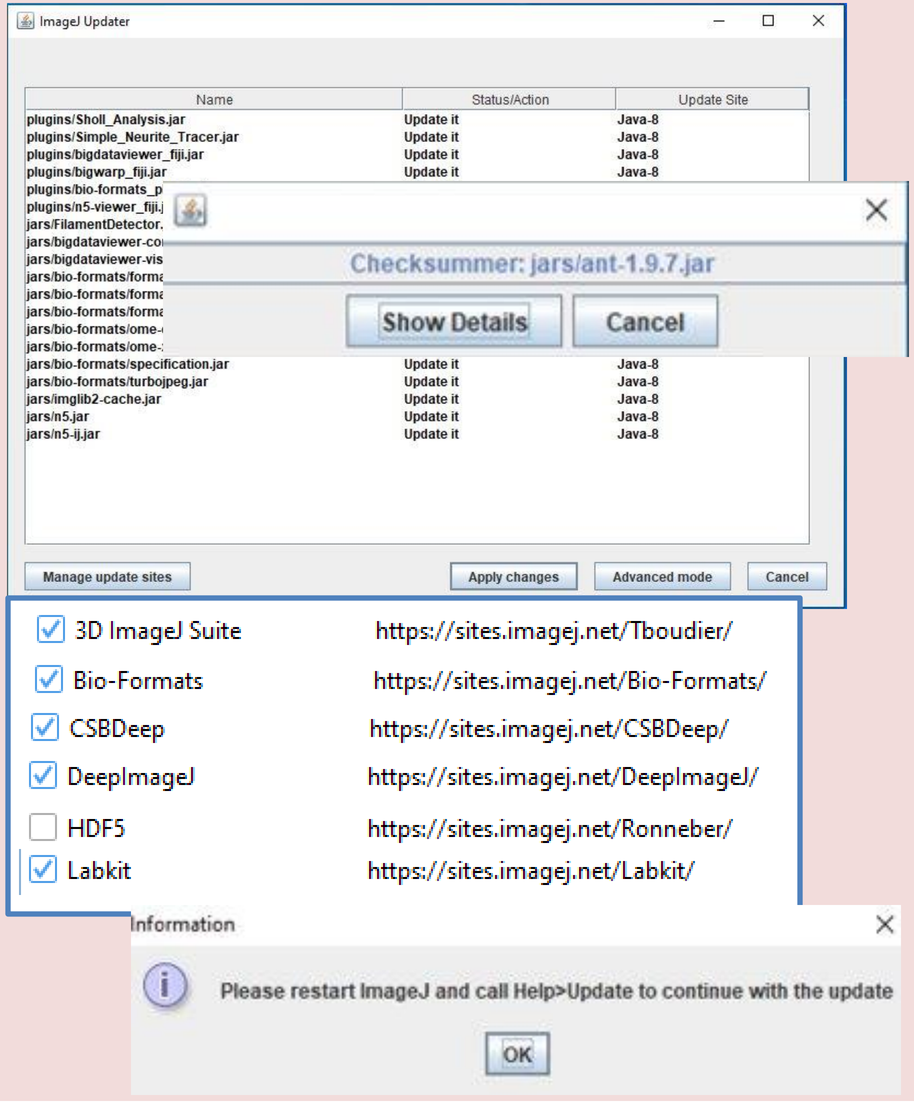
2. Using a plugin from the repository

5 Possibilities:

- Native file formats
- **Use a repository**
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)

Help > update…

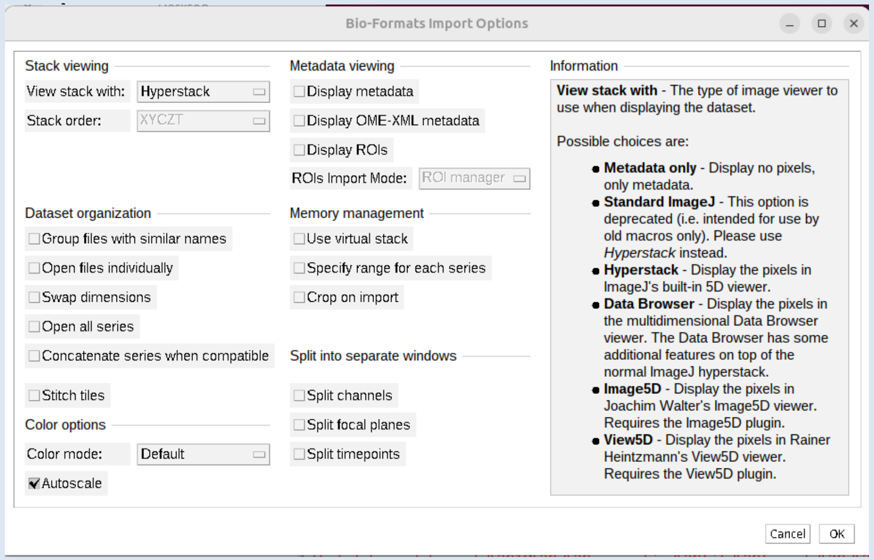


Plugins > Import > Bio-Formats > Bio-Formats Importer

EXERCISE 1

Try to open example 1B with the Bio-Formats plugin

In the dialog: point to the file (Example



Click OK

2. Using a plugin from the repository

5 Possibilities:

- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)

Update lists / repositories

ImageJ
Fiji
3D ImageJ Suite
3Dscript
ActogramJ
AICJanelia
Angiogenesis
AngioTool
Archipelago
AxoNet
BACMMAN
BAR
BaSiC
BigDataProcessor
BIG-EPFL
BigStitcher
BigVolumeViewer Demo
Bio-Formats
Biomat
Biomedgroup
BioVoxxel
Blind Analysis Tools
BoneJ
CALM
CAMDU
CATS
CellTrackingChallenge
CIP
CircleSkinner
clij
clij2
clijx-assistant
clijx-assistant-extensions
ClearVolume
CMCI-EMBL
CMP-BIA tools
CMTK Registration
Colocalization by Cross Correlation
Colour Deconvolution2
Cookbook

Bio-formats repository

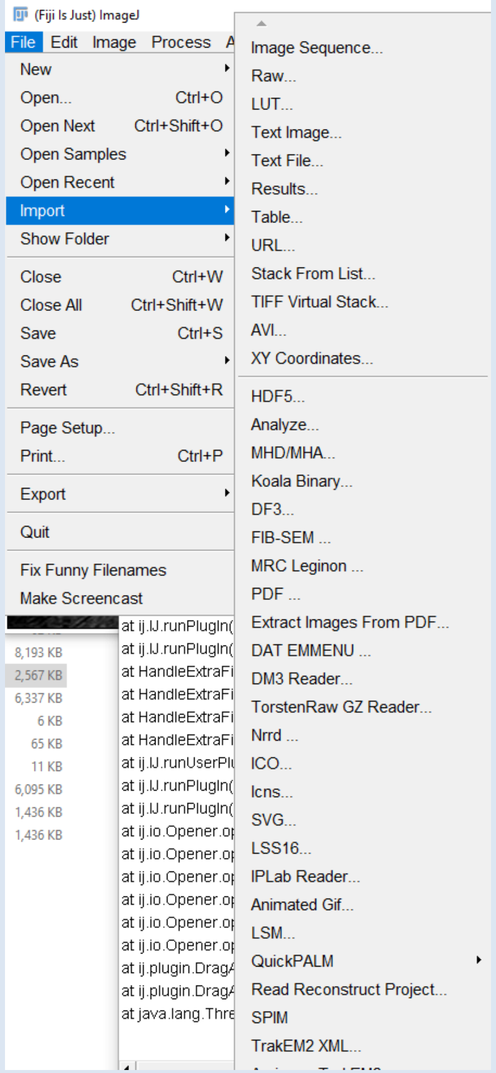
3i SlideBook
AIM
AVI (Audio Video Interleave)
Adobe Photoshop PSD
Alicona 3D
Amersham Biosciences Gel
Amira Mesh
Amnis FlowSight
Analyze 7.5
Andor Bio-Imaging Division (ABD) TIFF
Animated PNG
Aperio AFI
Aperio SVS TIFF
Applied Precision CellWorX
Axon Raw Format
BD Pathway
Becker & Hickl SPCImage
Bio-Rad Gel
Bio-Rad PIC
Bio-Rad SCN
Bitplane Imaris
Bruker MRI
Burleigh
Canon DNG
CellH5
CellVoyager
Cellomics
DICOM
DeltaVision
ECAT7
EPS (Encapsulated PostScript)
Evotec/PerkinElmer Opera Flex
FEI
FEI TIFF
Gatan Digital Micrograph
Gatan Digital Micrograph 2
Hamamatsu Aquacosmos NAF
Hamamatsu HIS
Hamamatsu VMS
Hamamatsu ndpi

3. Using a Fiji resident plugin (Import)

5 Possibilities:

- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)

File > Import >

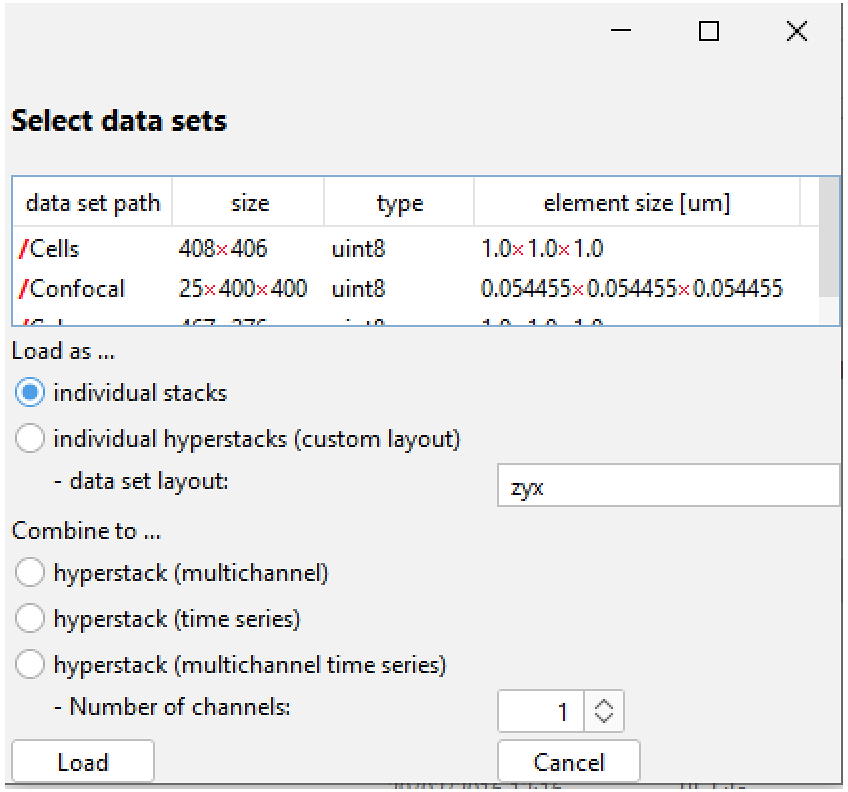


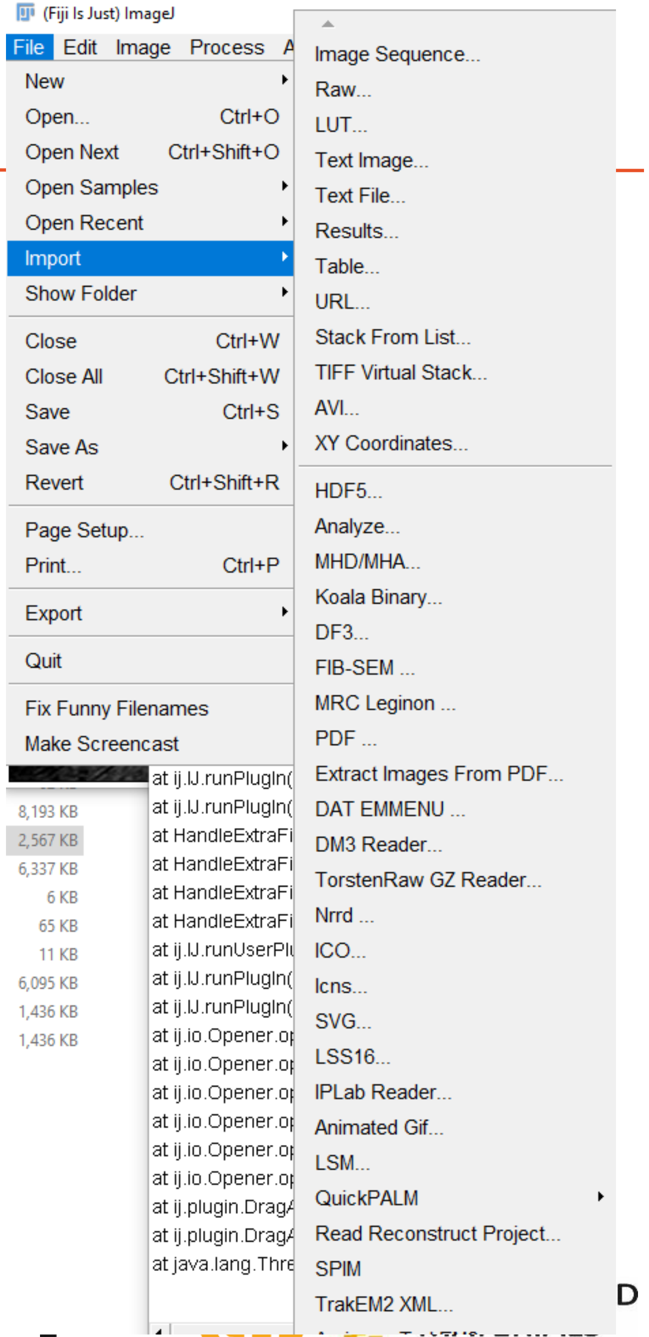
3. Using a Fiji resident plugin (Import)

EXERCISE 1
Try to open example 1D (HDR)

File > Import > HDF5
Point to the file example 1C

The file contains 3 datasets (!)
- Select /Cells in data set path
- load as 'Individual stacks'
- Click 'Load'

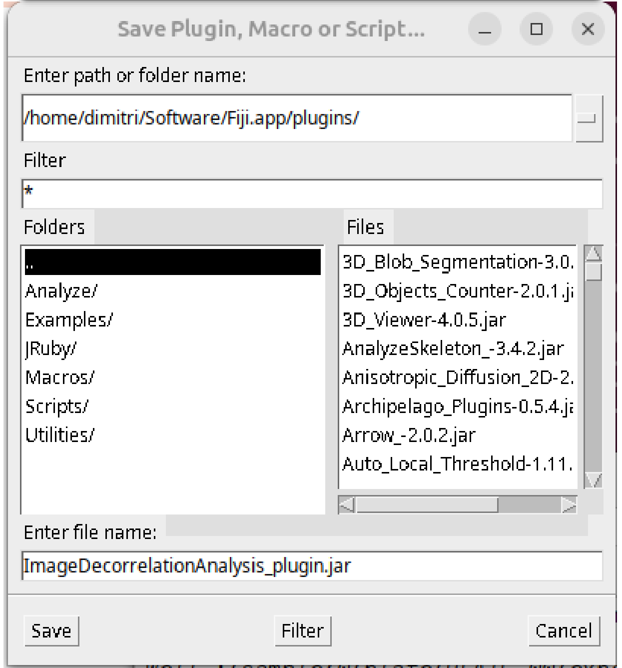




4. Using a plugin from the internet

5 Possibilities:

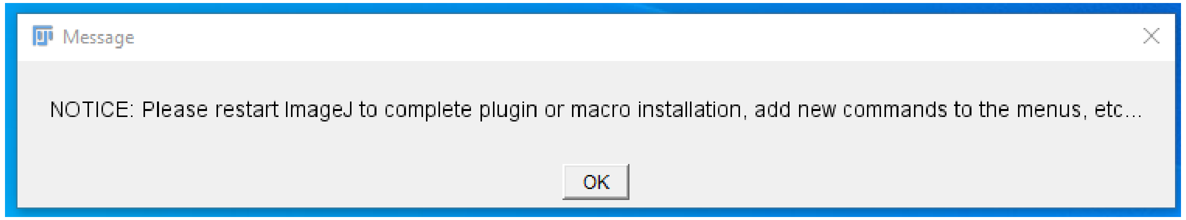
- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)



Installing a plugin from a downloaded .jar file (https://imagej.nih.gov/ij//plugins/tia-reader.html)

3 ways of installing the jar file (it is also provided):

1. Drag and drop the file onto Imagej (and save in the plugin folder)
2. Plugins > install plugin… and point to the .jar file
3. Copy the .jar file into the plugins folder of your FIJI folder



In any case: **Restart FIJI** (close it and start it again)

Find the new functionality under Plugins > Input / output > Tia Reader

ASCII header format
SPE Images
PICT, Targa using Jimi
Biorad Z-Series
Leica SP multi-channel stacks
QuickTime
Jimi Stack Writer
AVI Writer
JMF Reader
Animated Gif
PDS Images
AVI Reader
LSM Reader (Zeiss LSM confocal microscopes)
Quess RAW
IPLab Reader
Excel Writer
Multi FDF
VFF Opener
OpenSIF (opens Andor SIF files)
EXIF Reader
Bruker NMR
Zeiss ZVI Reader
ISAC images (e.g., Fuji BAS scanners)
Gatan DM3 Reader
Deltavision Opener
Nanoscope AFM files
NIfTI Input/Output
UNC format images
PDF Writer
Leica SP2 TIFF Sequence
EPS (Encapsulated PostScript) Writer
PerkinElmer Reader
Nikon ND2 Reader (Windows only)
TIA Reader (FEI/Emispec .ser files)
Heyex Raw Files (imports Heidelberg Spectrali
CINE File Reader (opens Phantom High Speed
MRI File Manager (reads Bruker MRI spectrom

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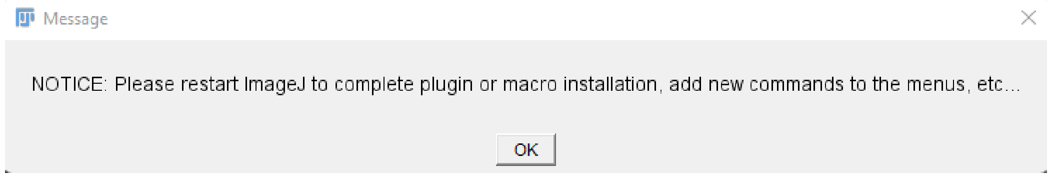
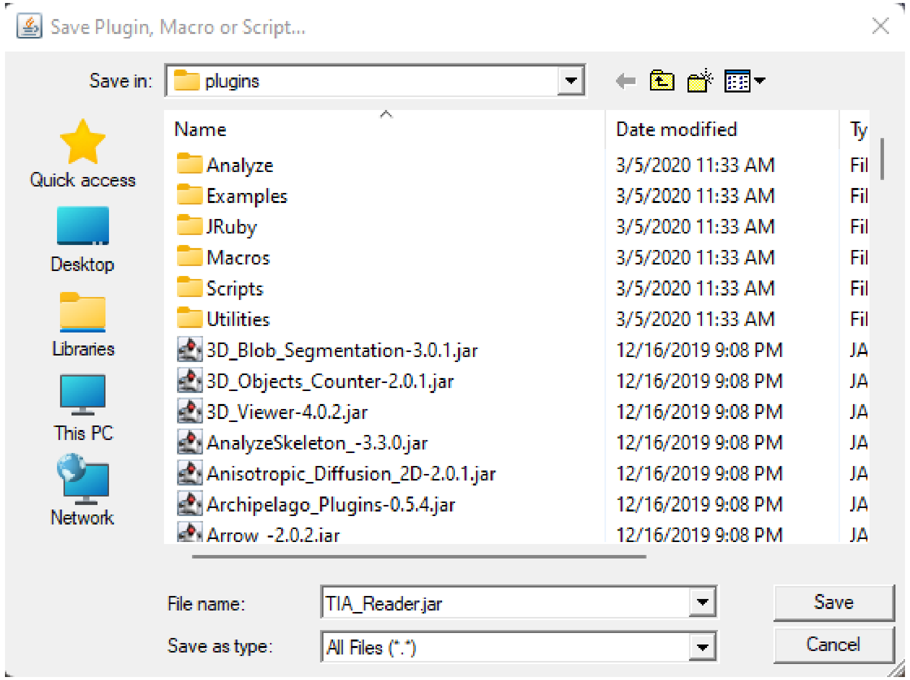
4. Using a plugin from the internet

EXERCISE 1
Install the TIA reader plugin (TIA_Reader.jar) if you have not done so
Then try to open Example 1D, the .ser file, using plugins > input/output > TIA reader

Installing a plugin from a downloaded .jar file

- drag and drop the .jar file onto Imagej or click Plugins > install plugin...
- Save it in the plugins folder of your FIJI folder





- Restart FIJI
- Then find the new functionality under
  Plugins > **Input / output** > Tia Reader
- Plugins > Input / output > Tia Reader
- Point to the .ser file
- Click 'Open'

Does it work?

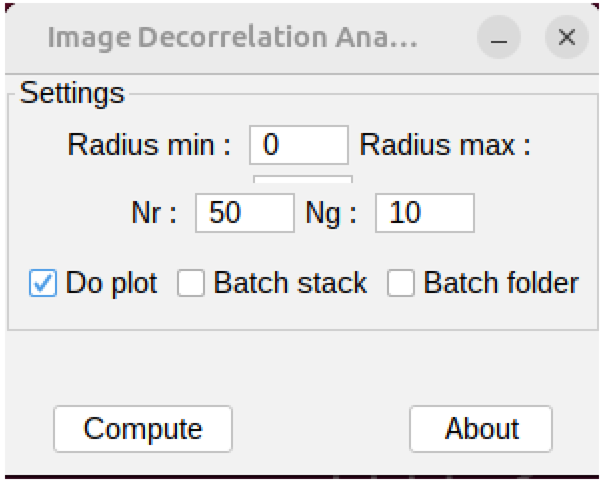
https://imagej.nih.gov/ij//plugins/tia-reader.html

4. Using a plugin from the internet (short resolution intermezzo)

EXERCISE 1

Install the image decorrelation plugin as well (ImageDecorrelationAnalysis_plugin.jar).

- Open Example 1B – Header – Cells.lsm with the Bio-Formats plugin (Plugins > Bio-Formats > Bio-Formats Importer)
- Run the Image Decorrelation plugin on the blue channel (Plugins > Image Decorrelation Analysis)

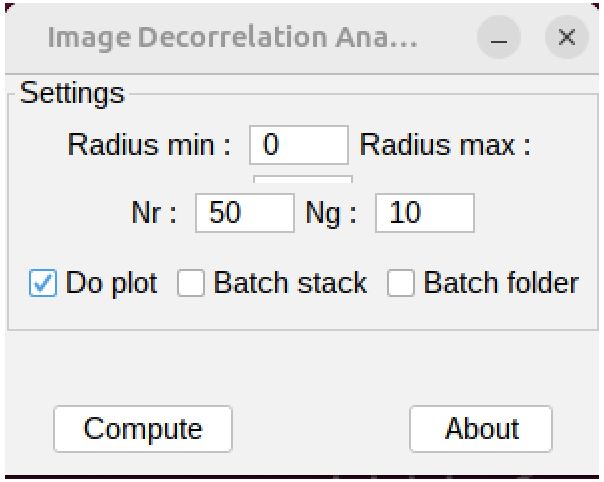


4. Using a plugin from the internet (short resolution intermezzo)

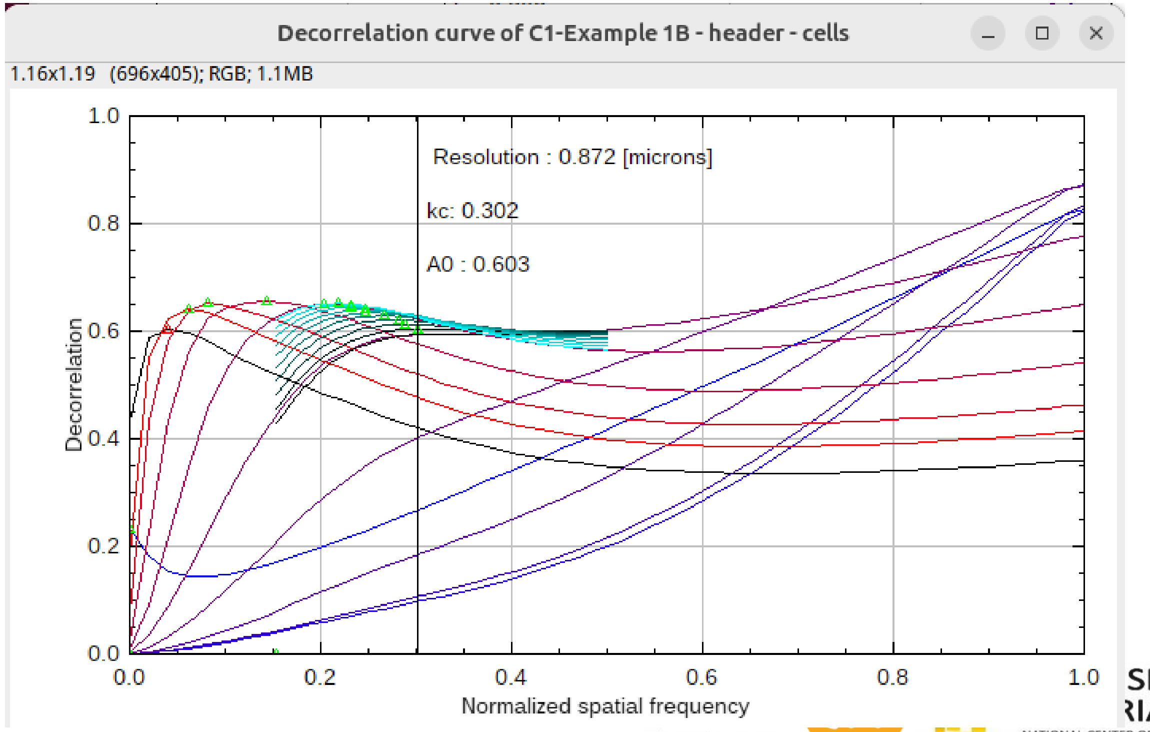
EXERCISE 1

Install the image decorrelation plugin as well (ImageDecorrelationAnalysis_plugin.jar).

- Open Example 1B – Header – Cells.lsm with the Bio-Formats plugin (Plugins > Bio-Formats > Bio-Formats Importer)
- Run the Image Decorrelation plugin on the blue channel (Plugins > Image Decorrelation Analysis)



Label	Res.	units
C1-Example 1B - header - cells	0.872	[microns]



5. RAW import

5 Possibilities:

- Native file formats
- Use a repository
- Use Import (Fiji only!)
- Use a plugin
- Through RAW import (raster graphics only)


```
0000   49 49 2a 00 10 00 00 00  43 52 02 00 14 b7 00 00 | II*.....CR...... | TIFF header [CR2 header]
0010   11 00                                            | ..               | IFD0 entries
0012   00 01 03 00 01 00 00 00  40 14 00 00             | ........@...     | IFD0-00 ImageWidth
001e   01 01 03 00 01 00 00 00  80 0d 00 00             | ............     | IFD0-01 ImageHeight
002a   02 01 03 00 03 00 00 00  e2 00 00 00             | ............     | IFD0-02 BitsPerSample
0036   03 01 03 00 01 00 00 00  06 00 00 00             | ............     | IFD0-03 Compression
0042   0f 01 02 00 06 00 00 00  e8 00 00 00             | ............     | IFD0-04 Make
004e   10 01 02 00 0e 00 00 00  ee 00 00 00             | ............     | IFD0-05 Model
005a   11 01 04 00 01 00 00 00  64 1b 01 00             | ........d...     | IFD0-06 PreviewImageStart
0066   12 01 03 00 01 00 00 00  01 00 00 00             | ............     | IFD0-07 Orientation
0072   17 01 04 00 01 00 00 00  b2 83 21 00             | ..........!.     | IFD0-08 PreviewImageLength
007e   1a 01 05 00 01 00 00 00  0e 01 00 00             | ............     | IFD0-09 XResolution
008a   1b 01 05 00 01 00 00 00  16 01 00 00             | ............     | IFD0-10 YResolution
0096   28 01 03 00 01 00 00 00  02 00 00 00             | (...........     | IFD0-11 ResolutionUnit
00a2   32 01 02 00 14 00 00 00  1e 01 00 00             | 2...........     | IFD0-12 ModifyDate
00ae   3b 01 02 00 01 00 00 00  00 00 00 00             | ;...........     | IFD0-13 Artist
00ba   bc 02 01 00 00 20 00 00  74 b7 00 00             | ..... ..t...     | IFD0-14 ApplicationNotes
00c6   98 82 02 00 01 00 00 00  00 00 00 00             | ............     | IFD0-15 Copyright
00d2   69 87 04 00 01 00 00 00  b2 01 00 00             | i...........     | IFD0-16 ExifOffset
00de                                              3e b6 |               >. | Next IFD
```

Total file size (in bytes) = Header size + Image data size

5. RAW import

3 Possibilities:

- Native file formats
- Use a plugin
- **Through RAW import (raster graphics only)**

Header

Image 1 - data

01110100 (ASCII, 116 = T)
01101001 (ASCII, 105 = I)
01100110 (ASCII, 102 = F)
00000100 (width = 4 px)
00000001 (height = 1 px)

00000000
00110010
01100100
10010110

Header: 5 bytes (TIF, 4x1 pixel image)

Data: 4 bytes

Total file size: 9 bytes

Total file size (in bytes) = Header size + Image data size

5. RAW import

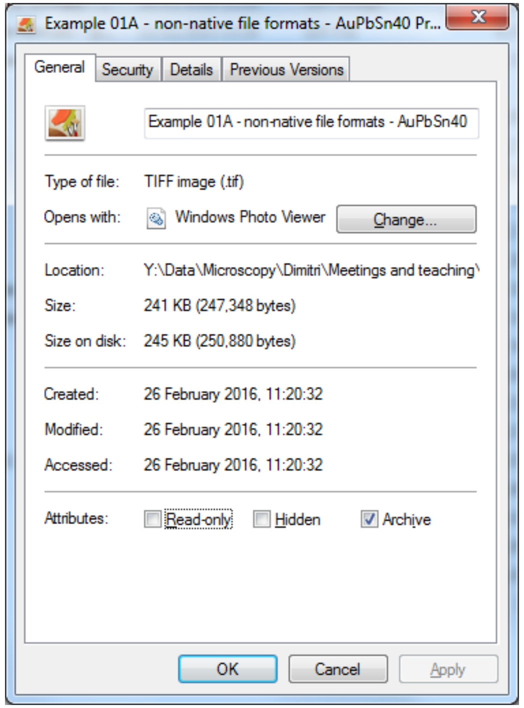
EXERCISE 1

Import through RAW (file > import > Raw…) of
Example 1A, the .tif file and Example 1D, the .SER file

Idea: we will only read the DATA – and jump over the HEADER

Example 1A

1. A priori information: Camera size = 600x412 px x 8 bit depth
2. Use your operating system to find the file size (in bytes) of Example 1A



Size: 241 KB (247,348 bytes)

Size on disk: 245 KB (250,880 bytes)

Example 1D

Camera size: Veleta at AMI (2048 x 2048, 16 bit)

Use your operating system to find the file size

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5. RAW import

EXERCISE 1
Import through RAW (file > import > Raw…)
Example 1A, the .tif file
Example 1D, the .ser file

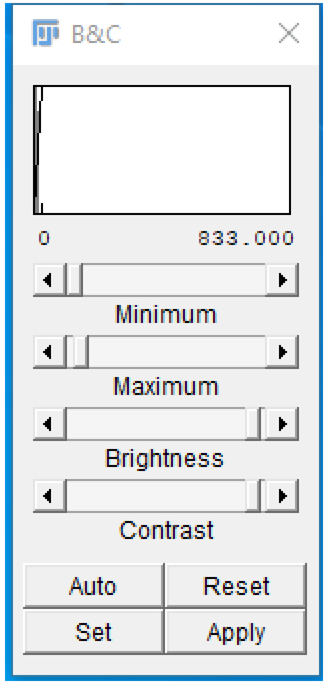
Idea: we will only read the DATA – and jump over the HEADER

Example 1A
1. Camera size: 600x412 px x 8 bit
2. 247 348 bytes

Example 1D
1. Camera size: Veleta at AMI (2048 x 2048, 16 bit)
2. 8 388 754 bytes

File > import > RAW...

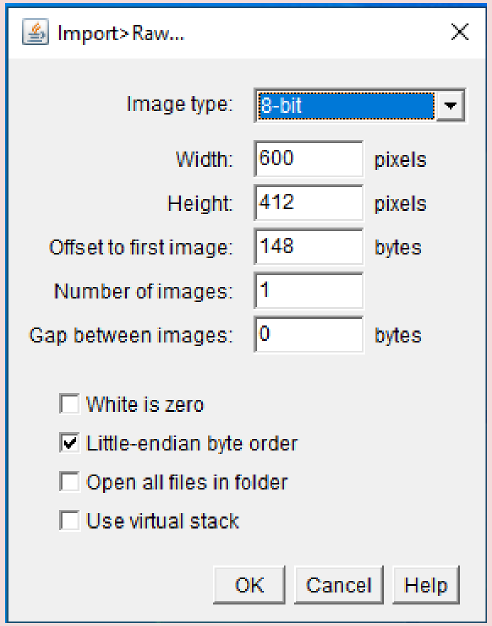
If your image is black, update brightness contrast:
Image > adjust > Brightness / contrast…
And click auto, or set (between 100 and 450)



5. RAW import

1. Data size: 600 x 412 = 247 200 bytes
2. File size : 247 348 bytes

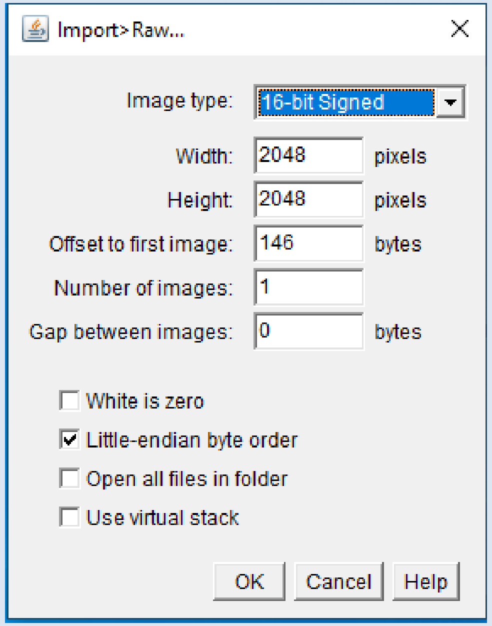
Therefore: header is 247 348 – 247 200 = 148 bytes



Endian: big-endian is often an "Apple thing"

1. Data size: 2048 x 2048 x 2 = 8 388 608 bytes (**why x 2??**)
2. File size : 8 388 754 bytes

Therefore: header is 8 388 754 – 8 388 608 = 146 bytes



Header

Files may (must) contain meta data (additional, useful information about the image)
F.A.I.R. Principles (https://www.go-fair.org/**), open source data**

Open Example 1D using File > **import > RAW...**
Select Image > Show info...

Title: Example 1D - non-native file formats - SiO4.ser
Width: 2048 pixels
Height: 2048 pixels
Size: 8MB
Pixel size: 1x1 pixel^2
ID: -77
Bits per pixel: 16 (signed)
Display range: 100 - 450
No threshold

Open Example 1D **using the TIA Plugin** >
Select Image > Show info...

Title: /home/.../Example 1D - non-native file formats.ser
Width: **8.3029 microns** (2048)
Height: **8.3029 microns** (2048)
Size: 8MB
Resolution: 246.6609 pixels per microns
Pixel size: **0.0041x0.0041** microns^2
ID: -63
Bits per pixel: 16 (unsigned, grayscale LUT)
Display range: 0-922
Pixel value range: 0-922
Image: 1/1 (1) No threshold
...

Summary: Howto open your data in FIJI

Native file formats
Use TIF whenever possible
Forget JPEG (has no header)
Metadata properly imported

Repositories
500+ scientific file formats available through Repositories.
Metadata is (very very often) imported
E.g. Zeiss, Leica, Olympus, Nikon, FEI, ...

FIJI Import
Not under plugins, but a bit hidden under file > import

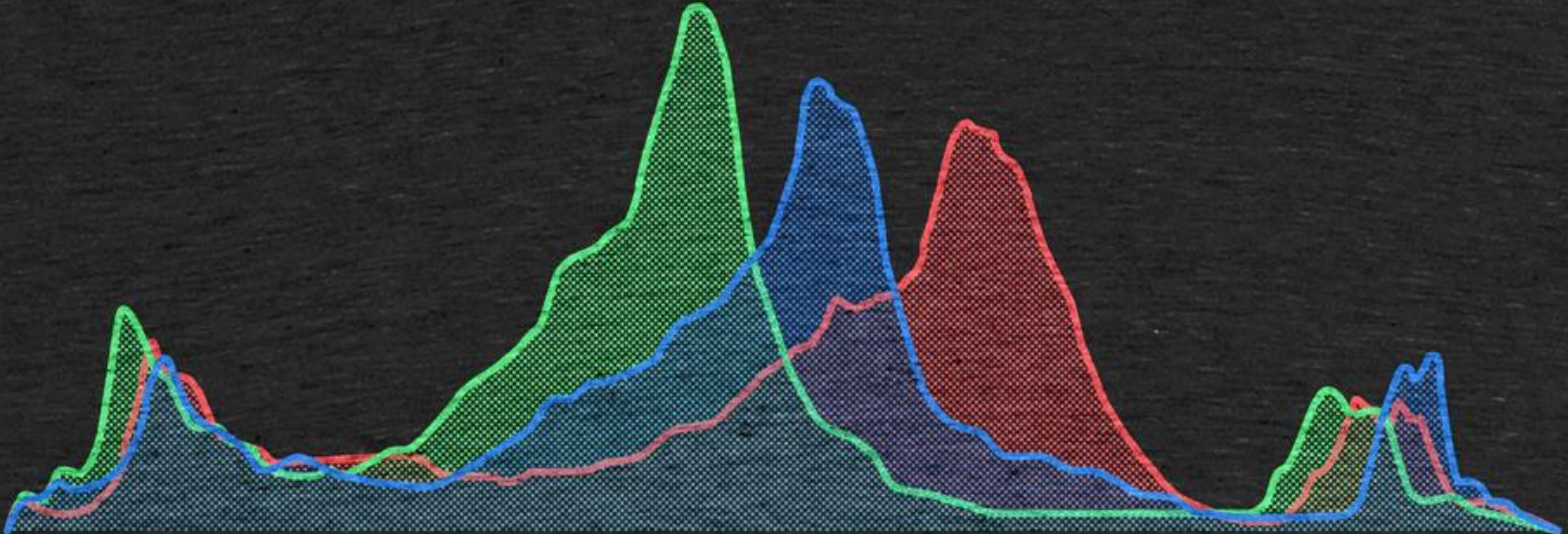
Install a plugin
Using the repositories or from the internet

Raw import
Last resort, if everything else fails
Typically: *a priori* information about the file needed (dimension, bit depth)
Opens only the image data. No metadata.

Metadata is a love note to the future

Part III: Histograms

- Histograms of grayscale image
- Color images
- Histogram normalization and histogram equalization



Histograms

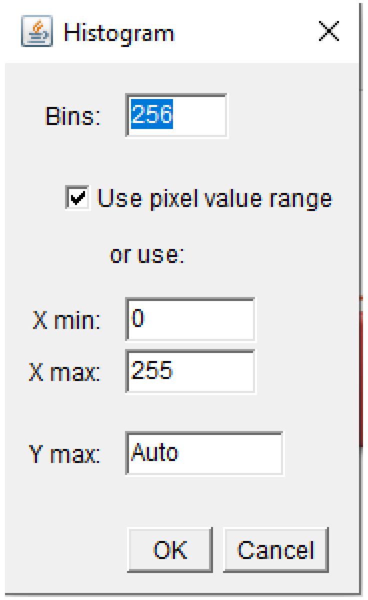
EXCERCISE 2
Open Example 1A and produce a histogram

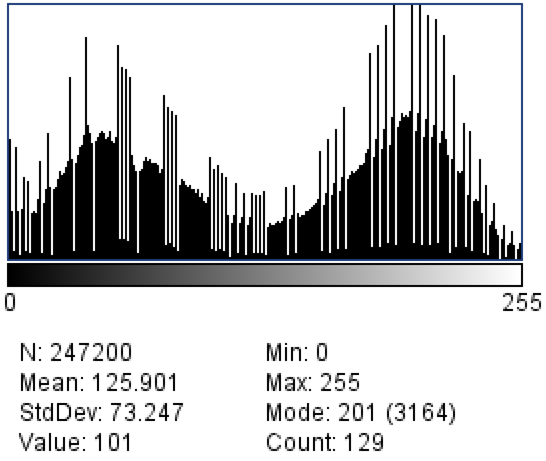
Histogram
a representation of the distribution of numerical data.
Pearson, K. (1895)

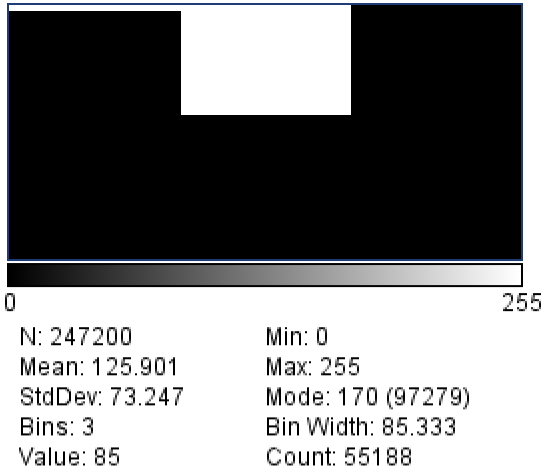
The intensity distribution of the image (= it plots the number of pixel for each intensity or tonal value)

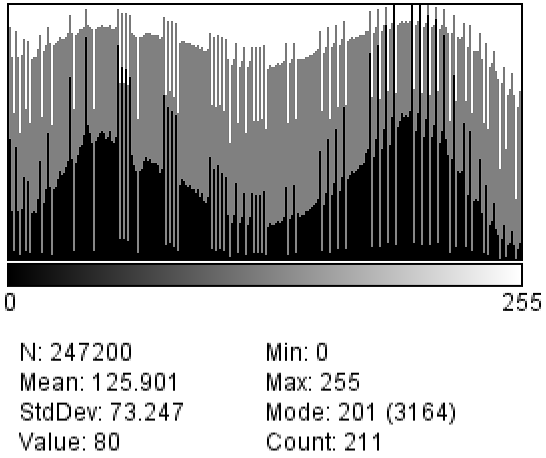
Analyze > histogram (or CTRL+H)

- What can you deduce from the histogram?
- Try: List, Copy, Log, Live
- Save the histogram itself to a TIFF?
- Also try CTRL+ALT+H









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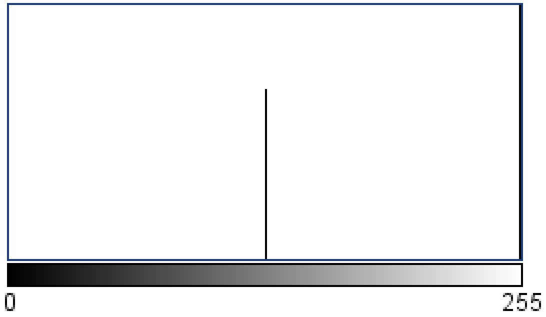
Histograms

EXCERCISE 2
Open Example 2A and 2B and look at the image

- Do you see a difference between the images?
- Check the histograms
- Do you see a difference between the histograms?

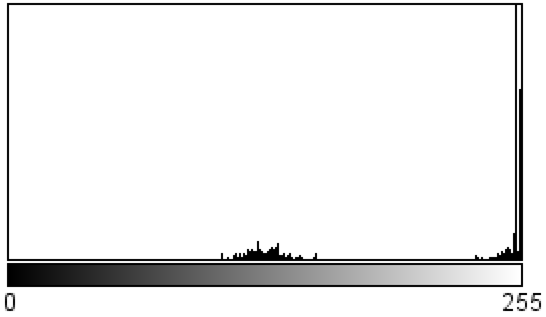
Histograms

EXCERCISE 2
Open Example 2A and 2B and look at the image



N: 65536
Mean: 247.269
StdDev: 30.036
Value: 23

Min: 128
Max: 255
Mode: 255 (61272)
Count: 0



N: 65536
Mean: 245.270
StdDev: 29.543
Value: 134

Min: 88
Max: 255
Mode: 253 (57340)
Count: 219

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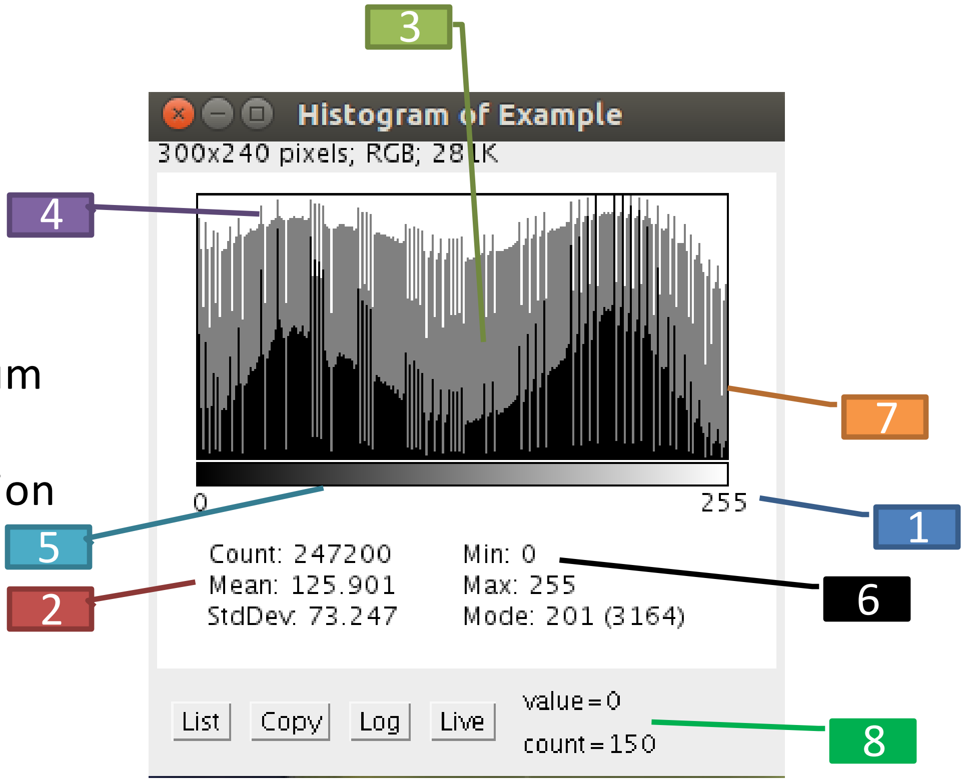
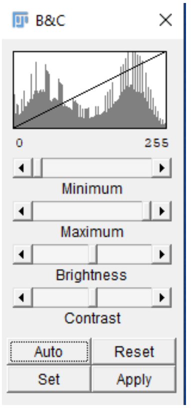
Histograms

What can you deduce from the histogram?

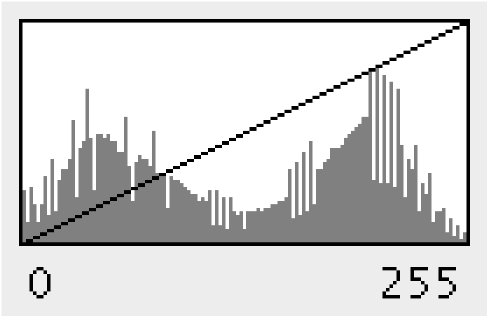
- 1. Bitdepth: 8 bit image (ranging from 0 to 255)
- 2. Pixel value distribution:
  Mean pixel value, variance of the intensity,
  Min, Max & modal
- 3. Type of distribution: e.g. bimodal, exponential, …
- 4. Spikes: image normalization or equalization occurred (see later)
- 5. Contrast and Lookup table (see later): the range between maximum and minimum (in this case: 255)
- 6. Dynamic range: the number of distinct pixel values. Eg. Compression will affect the dynamic range
- 7. Overillumination effects
- 8. The intensity at each grayscale value



Histograms → Linear transfer function



Find the contrast brightness window (Image > adjust > brightness/contrast)



Each image intensity value is mapped to its corresponding screen value:
The linear transfer function is running from bottom left to top right

The linear transfer function
assigns every original pixel value a new value on a linear scale. The endpoints of the function determine what value is white, and what black

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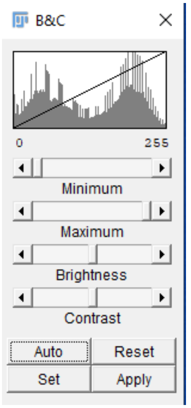
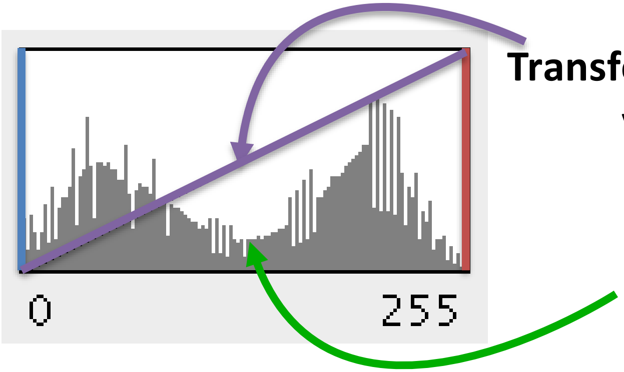
Histograms → Linear transfer function



Find the contrast brightness window (Image > adjust > brightness/contrast)



Transfer function: Attribute of visual perception

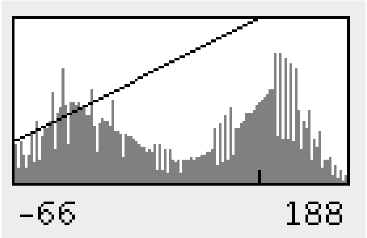
Histogram: Attribute of the image

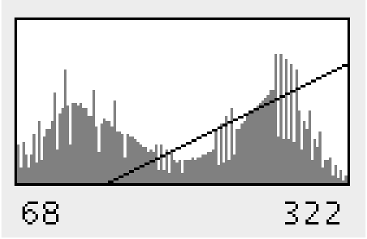
Pixels to the left of TF_{min} = black
Pixels to the right of TF_{max} = white
Pixels > TF_{min} and < TF_{max} = gray (linearly)

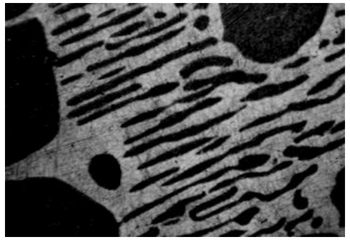
KEY: Changing the transfer function does not alter your data, only the way it is depicted

Brightness

The transfer function is shifted

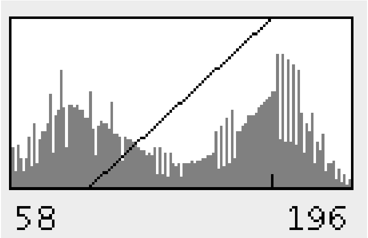


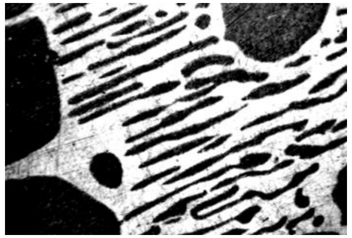


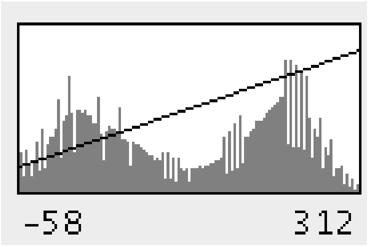


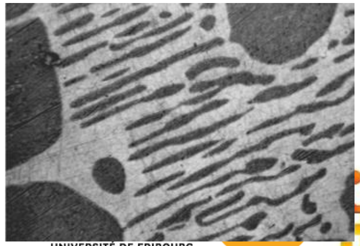
Contrast

The transfer function is tilted









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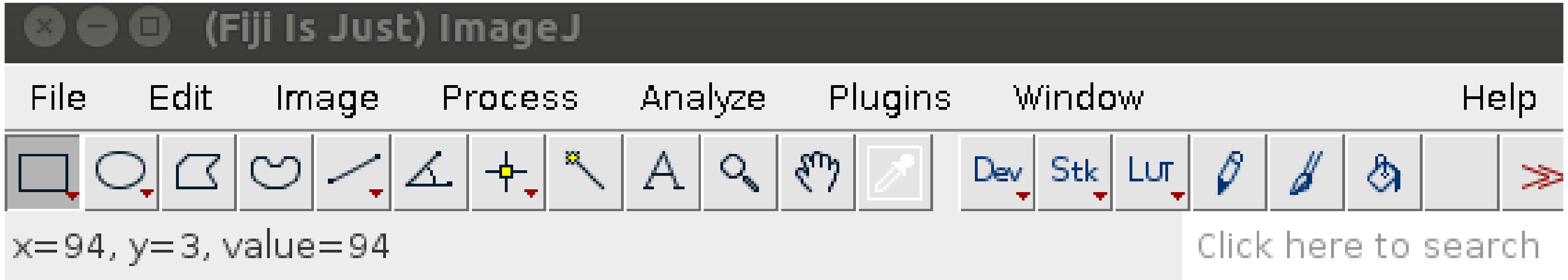
Histograms

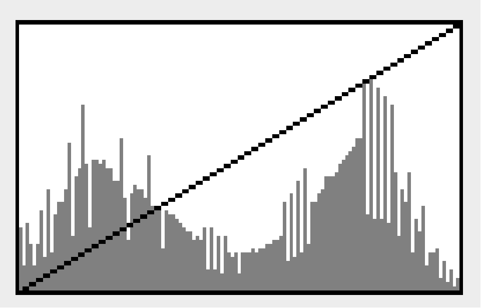
EXCERCISE 3
Load Example 3 – GrayscaleLUT, and read out the grayscale values in the ImageJ statusbar.

- Use (+ to zoom in, - to zoom out)
- Hoover with the mouse over the image and check the status bar in ImageJ
- Image > adjust > Brightness/Contrast (CTRl+Shift+C)
- Make a histogram of this image. How do you interpret the histogram?
- Play around with the Minimum and Maximum in the transfer function (Image > Adjust> Brightness/Constrast). Check the effect on the values.
- Do you delete information?



Histograms: other lookup tables (LUTs)

82	72	78	86	65	41
157	144	167	188	201	19
185	191	195	188	188	19
193	195	195	191	189	17
173	170	181	192	194	19
210	214	206	202	203	20
237	224	221	230	232	22
183	180	190	188	192	18
178	170	159	187	195	18
167	164	170	186	192	18
159	162				
180	172				
193	180				
167	184				
195	191				
183	188				



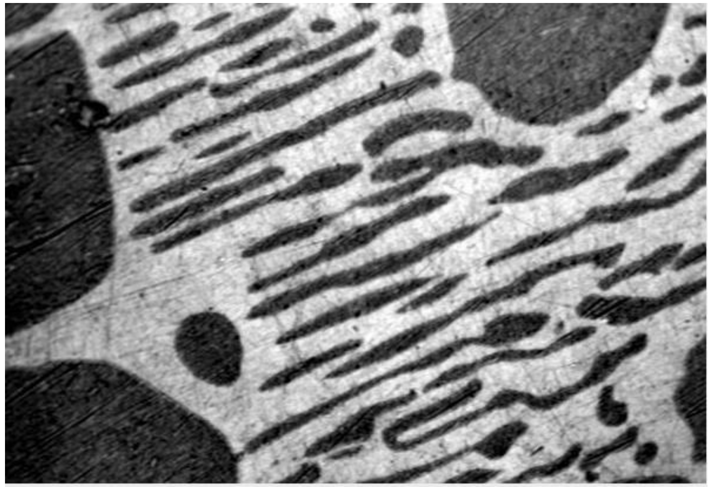
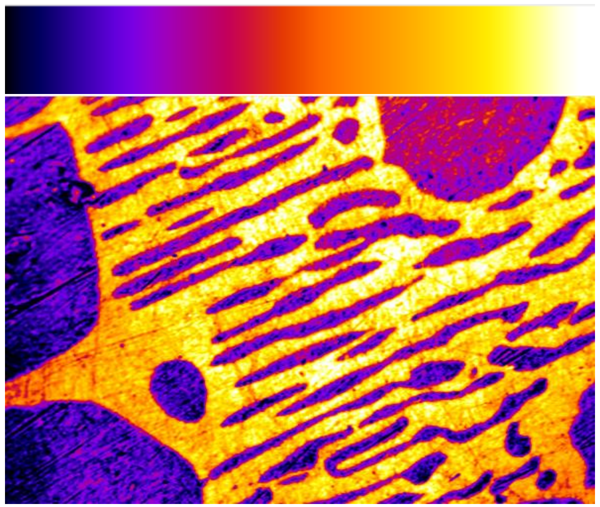
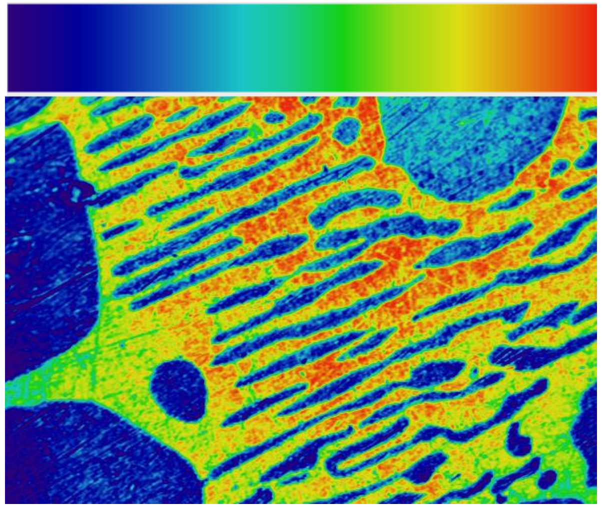
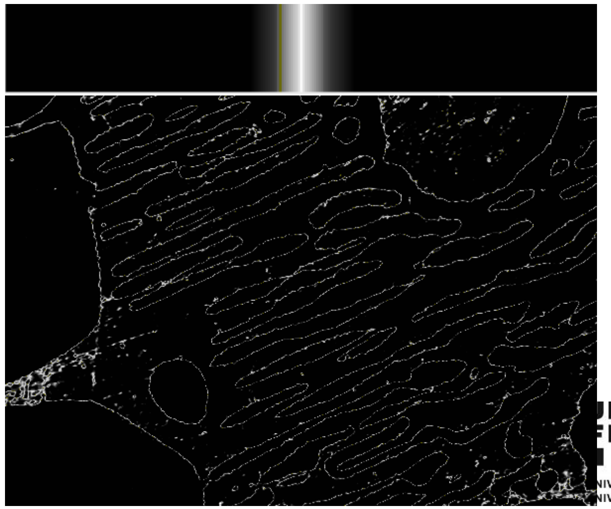


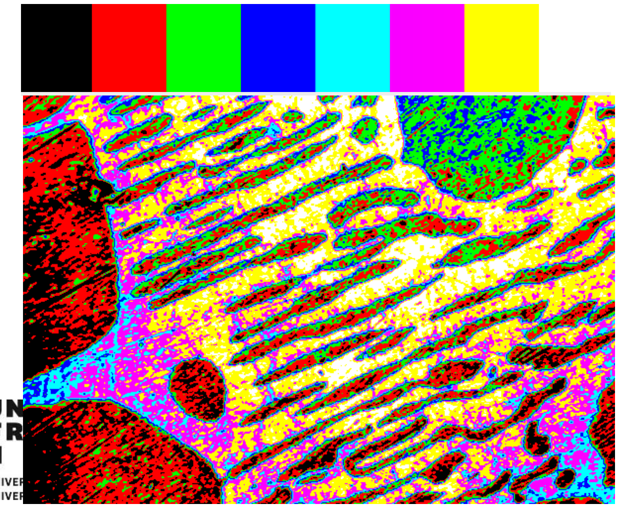
Image = 2D array of numbers

Grayscale = giving a graphical meaning to these Look-Up Tables.
But Grayscale is just one of these Look-Up tables!









Histograms: other lookup tables (LUTs)

EXCERCISE 3
Load Example 3 or Example 1A and try different LUTs

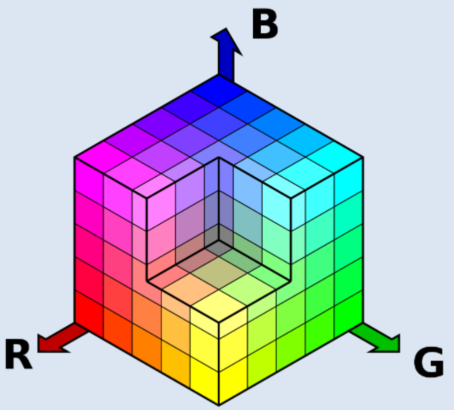
- Image > Lookup Tables > ...
- Image > Color > Show LUT
- Change brightness and contrast with some exotic LUTs

- You can make your own LUT using Image > color > Edit LUT

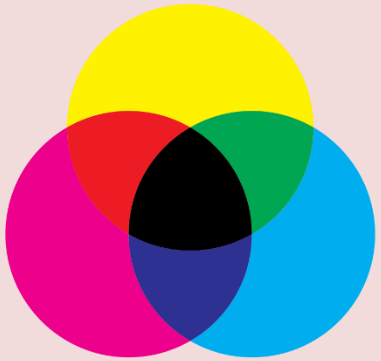
Histograms: color model

A color model
is a method of describing a color. Color models can be represented as tuples of numbers, typically as three or four values or color components.

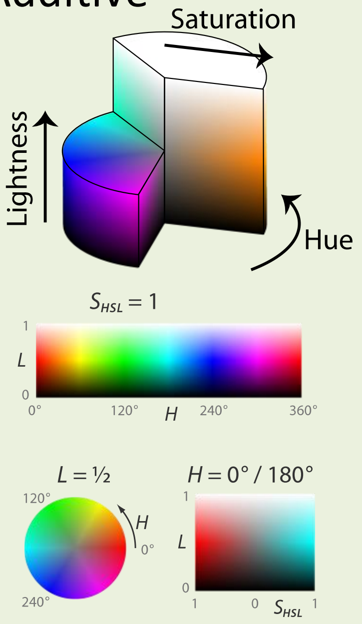
RGB
- 3 values: Red, green & blue
- Additive



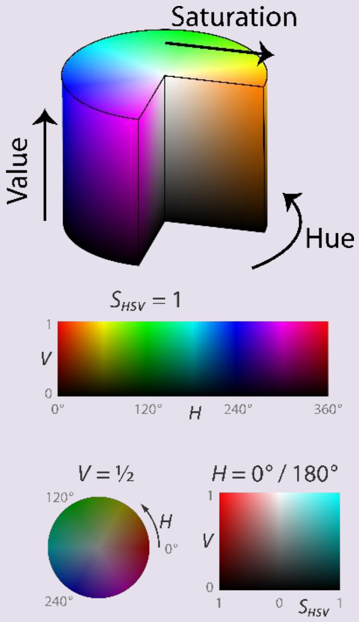
CMYK
- 4 values: Cyan, Mangenta, Yellow and black
- Subtractive



HSL
- 3 values: Hue, Saturation and Lightness
- Additive

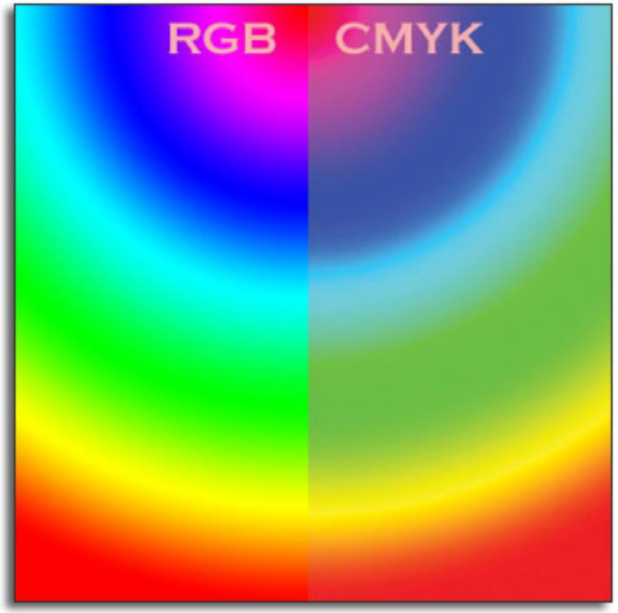


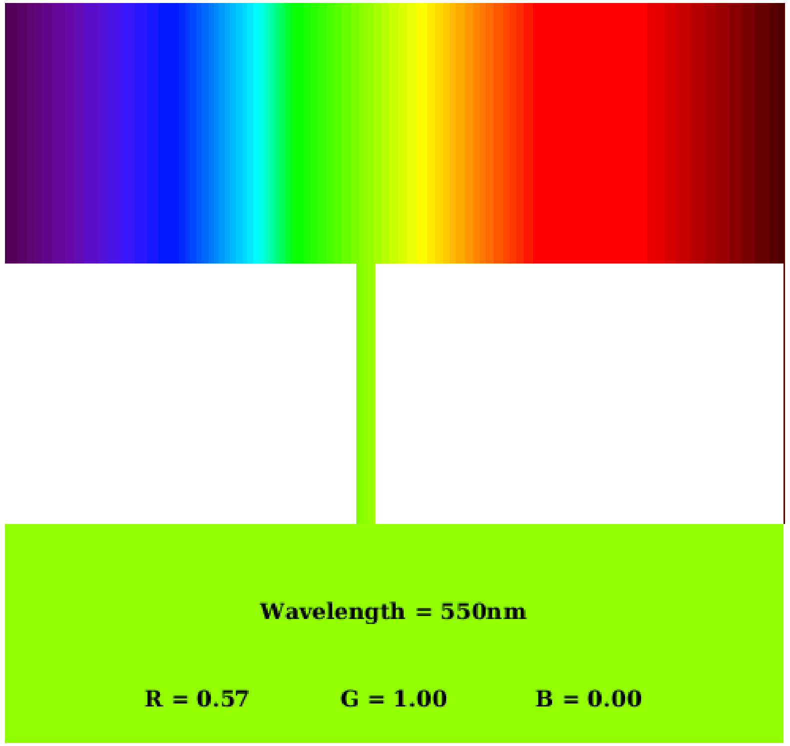
HSV
- 3 values: Hue, Saturation and Value
- Additive



Histograms: color space

A color space
is a way of mapping real colors to the color model's particular values. the goal having reproducible, unambiguous representations of color – whether such representation entails an analog or a digital representation.





Based on human perception
CIE 1931 XY
CIEUVW

Uniform color spaces CIELUV
CIELAB
HSLuv

RGB primaries (for CRT & LED displays)
sRGB (created by HP & Microsoft for on line use)
Adobe RGB (designed for CMYK color)
Adobe Wide Gamut RGB (wider range than sRGB: 77.6% vs 50.6%)
Rec. 2020 (used for HDR-TV)

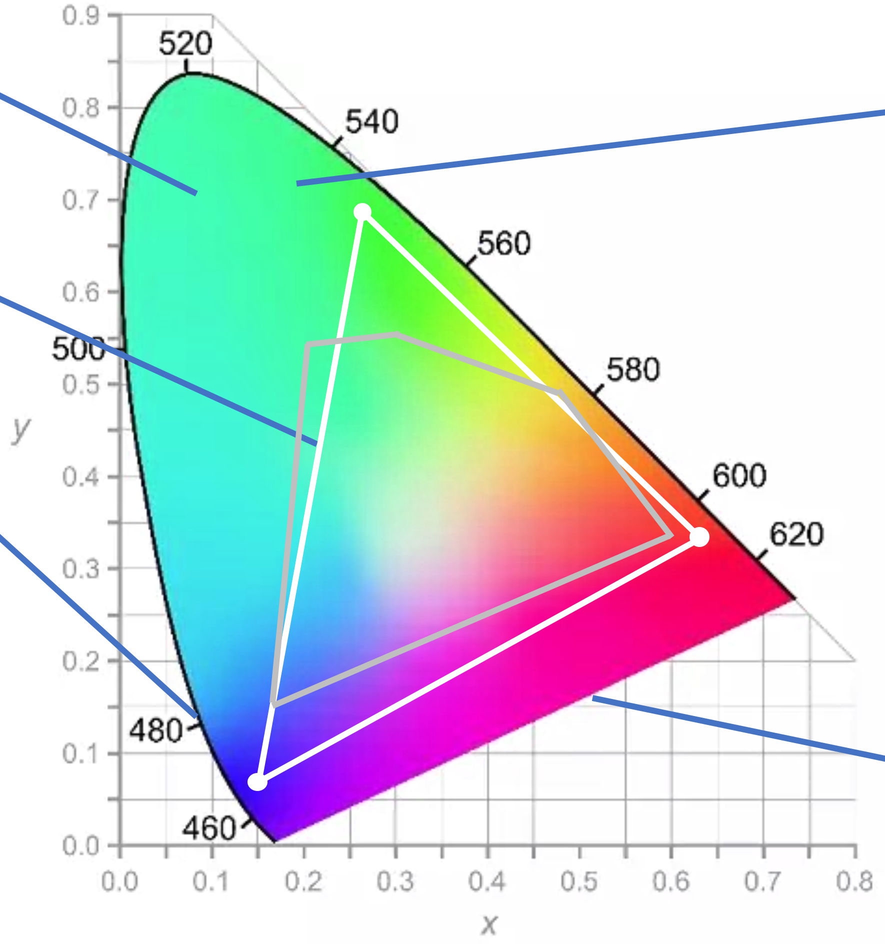
Histograms: the color gamut

The color gamut
The entire range of colors and tones achievable by an imaging system (eyes, printer, display)

Inside the diagram:
All colors visible to the average human eye (= CIELAB color space)

Color mixing:
The colors along any line between two points can be made by mixing the colors at the end points

Spectral locus:
The edge of the diagram represents pure monochromatic light (single wavelength, in nm)



Color gamut:
Subset of colors that can be represented by mixing the colors at it's corners

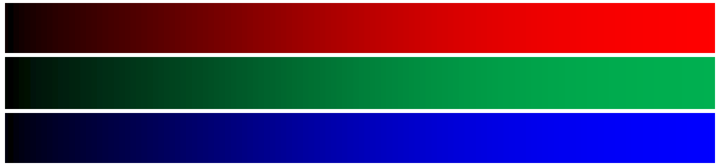
White: sRGB color space
Gray: color space defined by CMYK

Line of purples:
Non-monochromatic saturated colors (cannot be represented by a single wavelength)

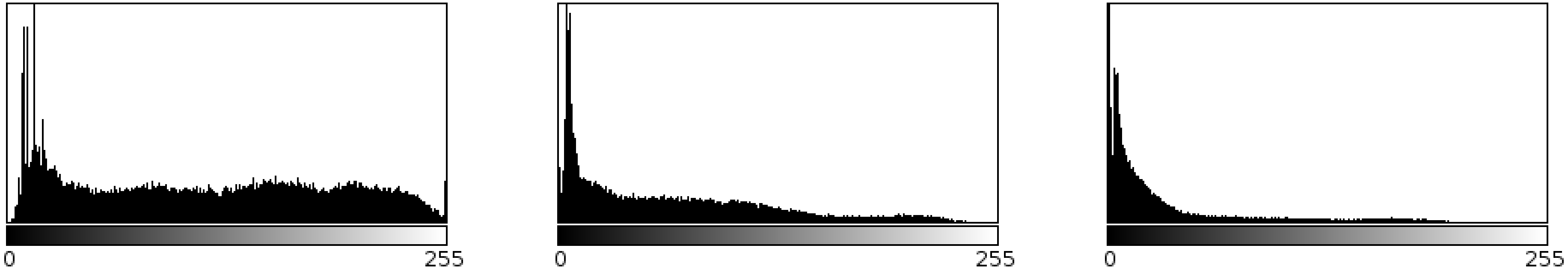
CIE 1931 XY colorogram

Histograms: color images

- Color image = 3 grayscale images combined (=composite).
- with a red LUT
- With a green LUT
- With a blue LUT

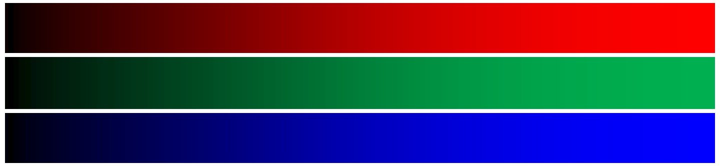


They are also called RGB images, or 24 bit images (=3x8 bit)



Histograms: color images

- Color image = 3 grayscale images combined (composite).
- with a red LUT
- With a green LUT
- With a blue LUT



They are also called RGB images, or 24 bit images (=3x8 bit)

Header

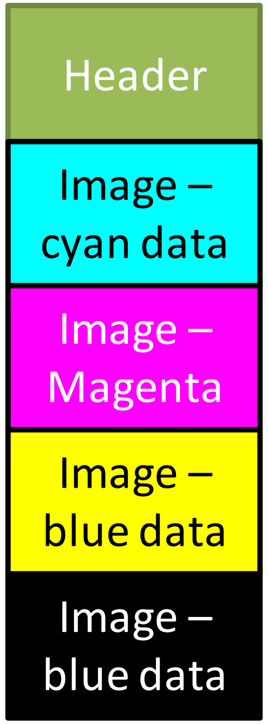
Image – red data

Image – green data

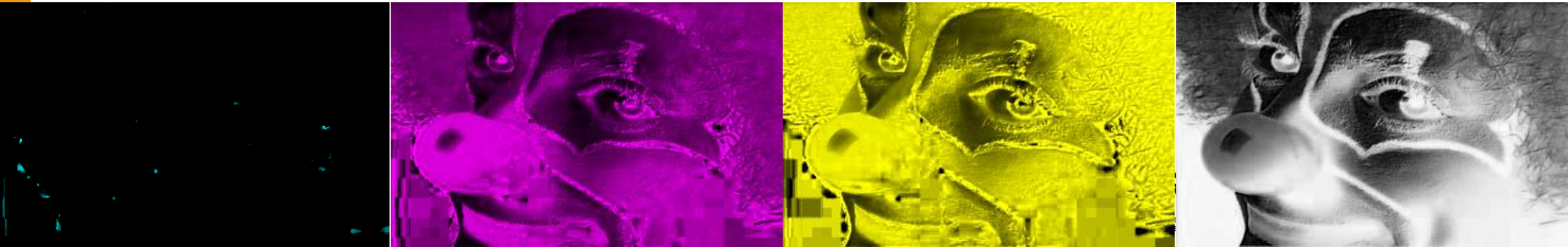
Image – blue data

Histograms: color images

- Color image = 4 grayscale images combined (composite).
- with a cyan LUT
- With a magenta LUT
- With a yellow LUT
- With a black LUT



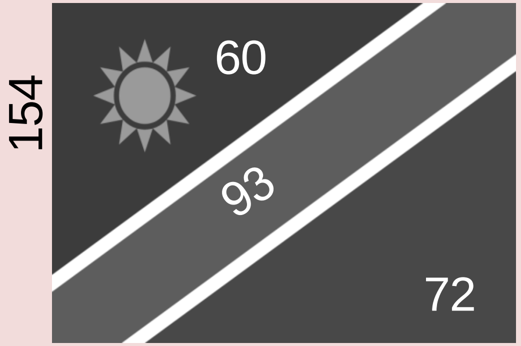
They are also called CMYK images, or 32 bit color images (=4x8 bit)

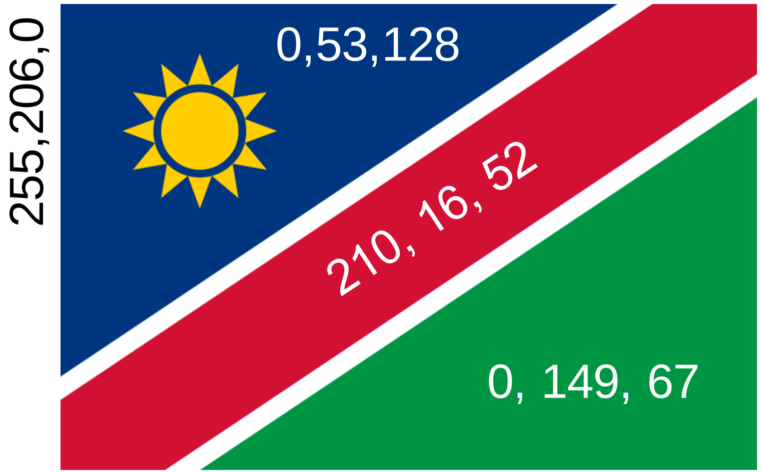


Converting color to grayscale

Direct conversion

$$8bit = \frac{Red + Green + Blue}{3}$$

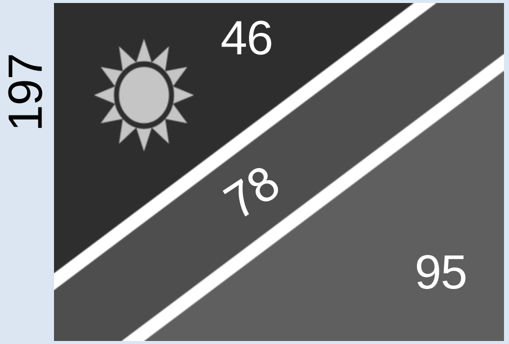




Edit > Options > Conversions…

Weighted conversion

$$8bit = 29.9\%Red + 58.7\%Green + 11.4\%Blue$$



Converting formats, saving data

EXCERCISE 4
Load the clown test image and convert it to another format

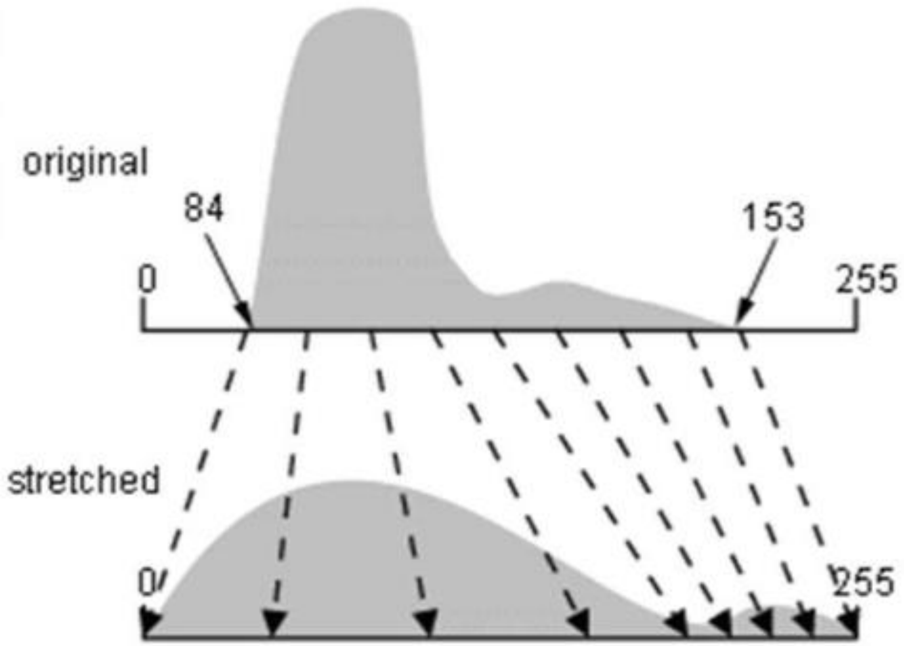
- File > Open Samples > Clown
- Image > Type
- Change the type to e.g. 8-bit and check what happened to the size
- Can you change back to RGB (without Edit > undo, off course)?
- Save as 8-bit, 16-bit and RGB TIFF. Compare file sizes

Bit depth	Channels	Filesize (bytes)	Data	Header
8 bit	1	64 236 bytes	64 000 bytes (=320x200x1)	236
16 bit	2	128 254 bytes	128 000 bytes (=320x200x2)	254
RGB	3	192 166 bytes	192 000 bytes (=320x200x3)	166
32 bit	4	256 266 bytes	256 000 bytes (=320x200x4)	266
L*a*b (3x32 bit)	3x4	768 672 bytes	768 000 bytes (320x200x4x3)	672

- Convert to CMYK: https://imagej.nih.gov/ij/plugins/cmyk/index.html

Histogram normalization, histogram equalization

Goal: to use the entire range of intensities in the histogram



Solutions:

- Histogram normalization (histogram stretching)
- Histogram equalization

WARNING:

1. GIGO
2. We will now **change the actual data**, not longer only the transfer function.

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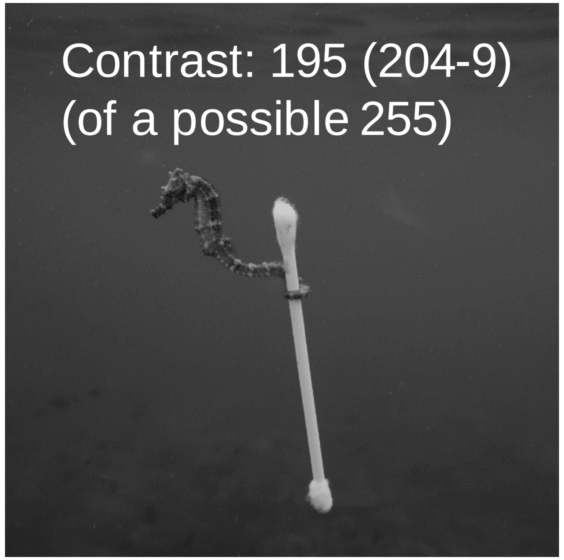
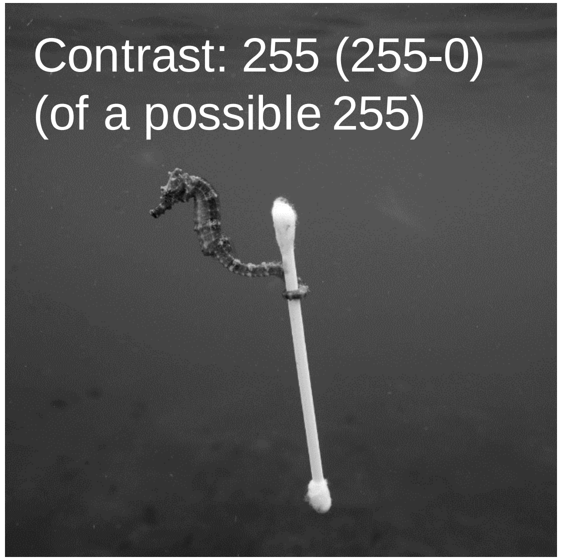
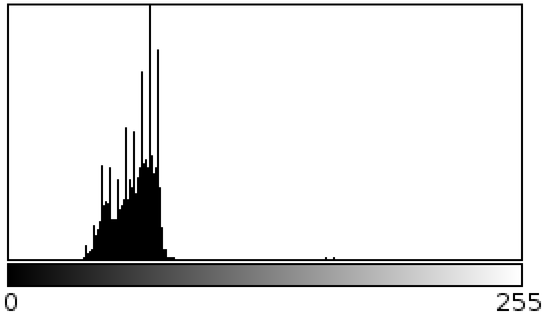
Histogram normalization

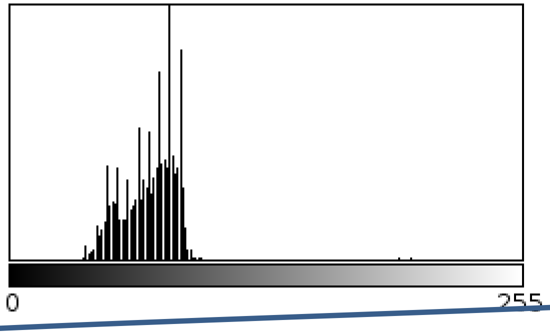




Contrast: the range between the maximum and minimum intensity in the image



Count: 1210000
Mean: 63.619
StdDev: 15.943
Min: 9
Max: 204
Mode: 70 (101022)



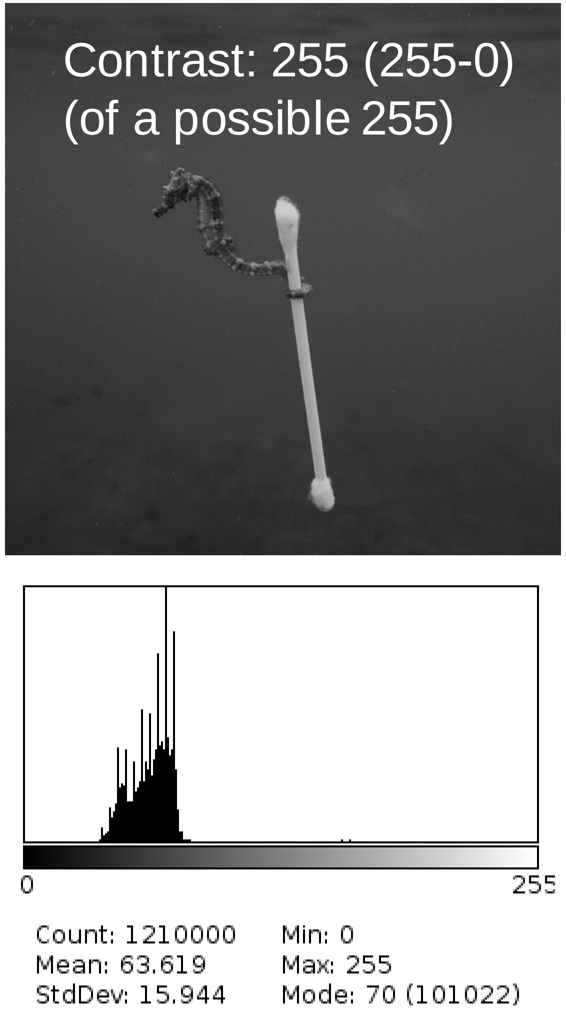
Count: 1210000
Mean: 70.985
StdDev: 20.861
Min: 0
Max: 255
Mode: 79 (101022)

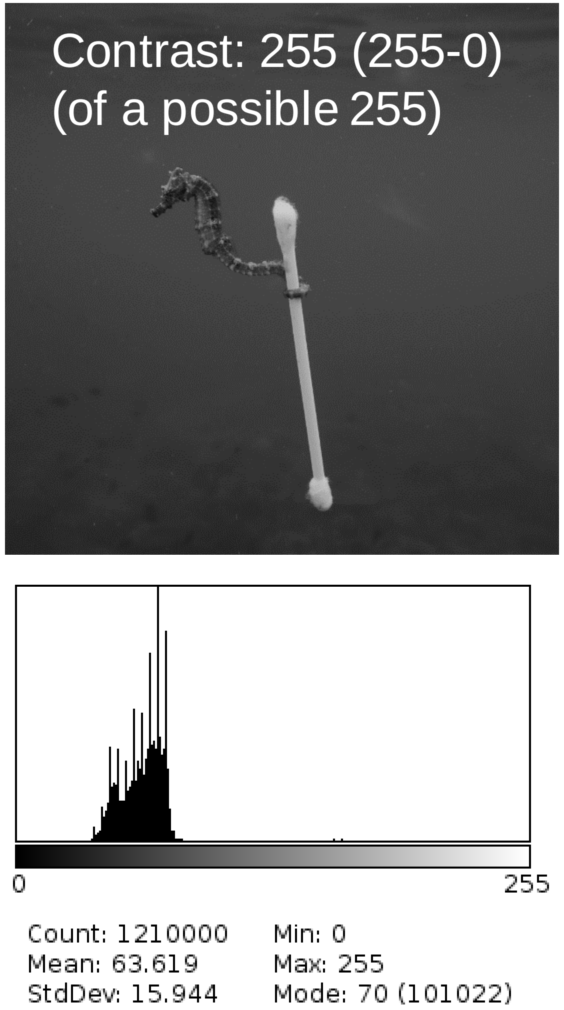
$$g(x,y) = \frac{f(x,y) - I_{min}}{I_{max} - I_{min}} \cdot 2^n$$

$$g(x,y) = \frac{f(x,y) - 9}{204 - 9} \cdot 256$$

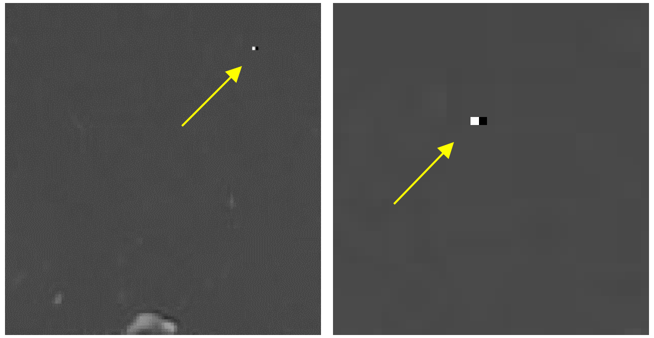
Histogram normalization: attention!

Problem: hot/cold pixels

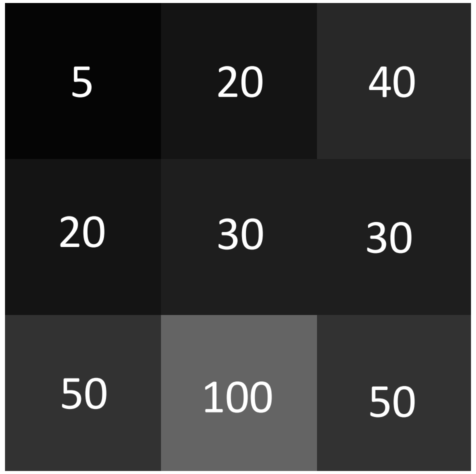




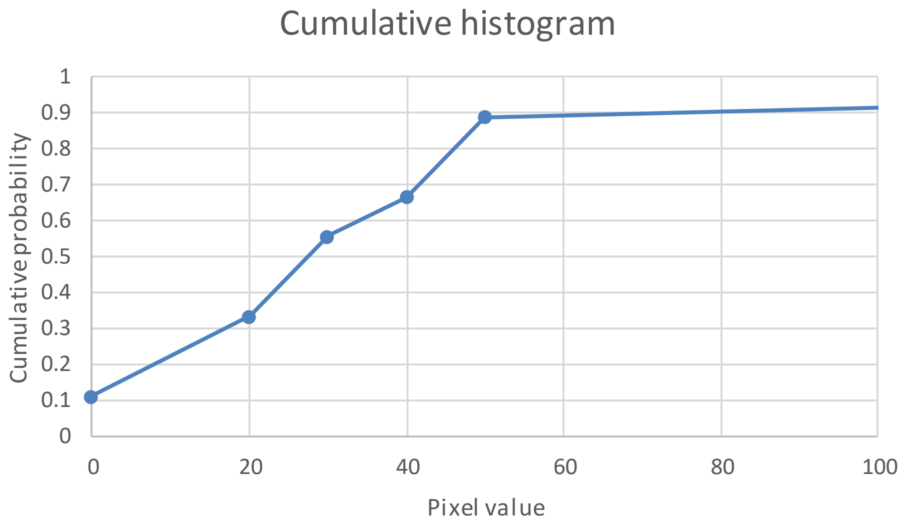
$$g(x,y) = \frac{f(x,y) - I_{min}}{I_{max} - I_{min}} \cdot 2^n$$



Histogram equalization



Values	Occurrence	PMF*	CDF*
5	1	0.111	0.111
20	2	0.222	0.333
30	2	0.222	0.555
40	1	0.111	0.666
50	2	0.222	0.888
100	1	0.111	1
	Total = 9	Total = 1	

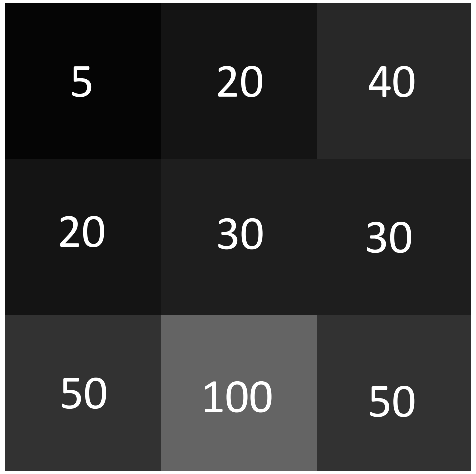


= the normalization of the cumulative histogram of the image

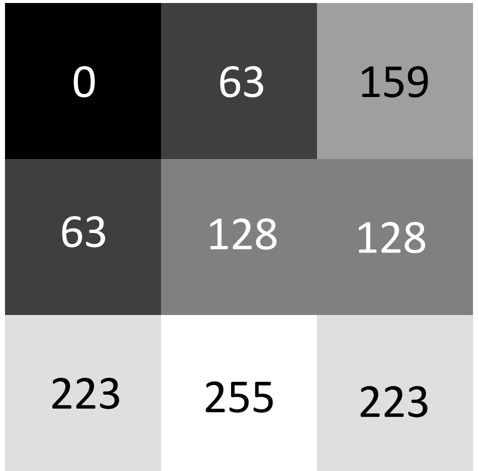
*PMF = Probability mass function
*CDF = Cumulative distribution function = cumulative histogram

Histogram equalization

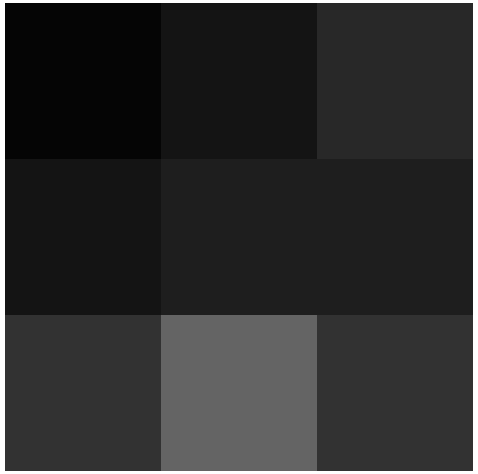
Note to the not-so-mathematically-inclined:
This is exactly the same formula as 3 slides back, but now we use the newly created cumulative histogram instead of the histogram



Values	CDF*	CDF norm	8 bit
5	0.111	0	0
20	0.333	0.25	63
30	0.555	0.5	128
40	0.666	0.625	159
50	0.888	0.875	223
100	1	1	255

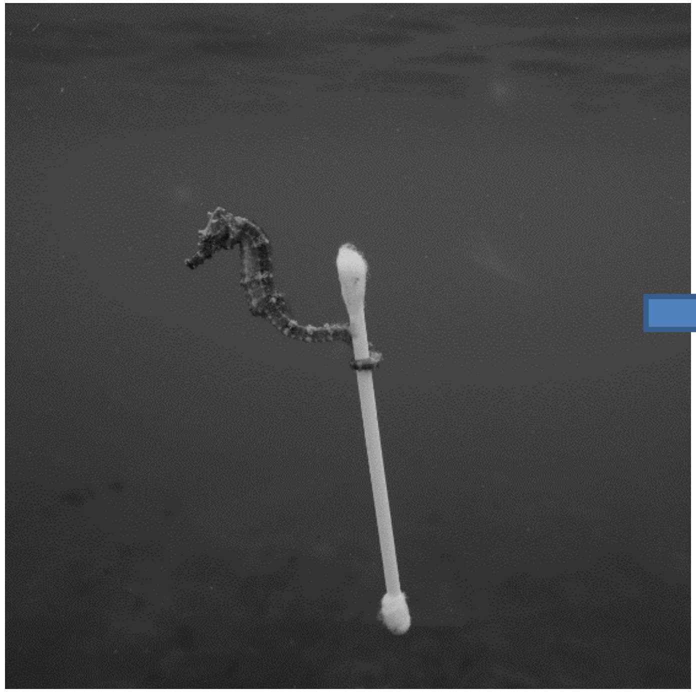


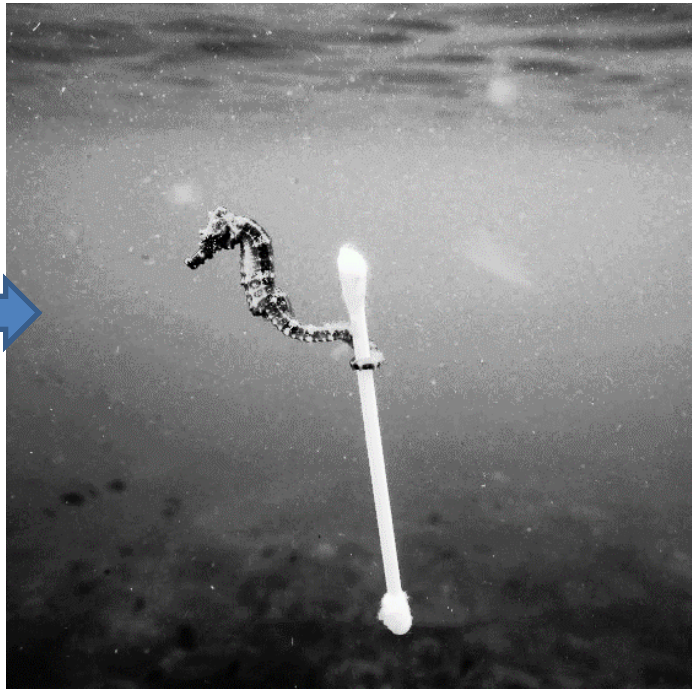
= the normalization of the cumulative histogram of the image

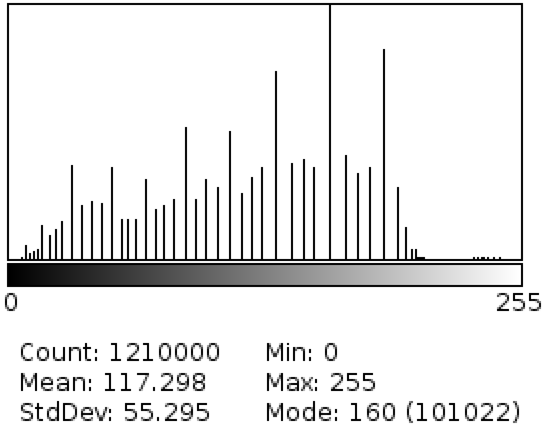


*PMF = Probability mass function
*CDF = Cumulative distribution function = cumulative histogram

Histogram equalization: attention!







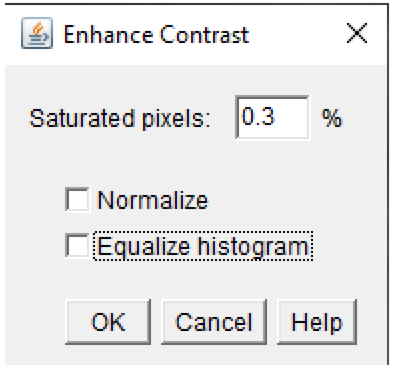
<u>Watch out with Histogram equalization!</u>

- Unrealistic artefacts in 8 bit (grayscale images)
- Image gradients in images with low depth (it will further reduce dynamic range)
- Undesired effects when histogram is not continuous

Histogram equalization: attention!

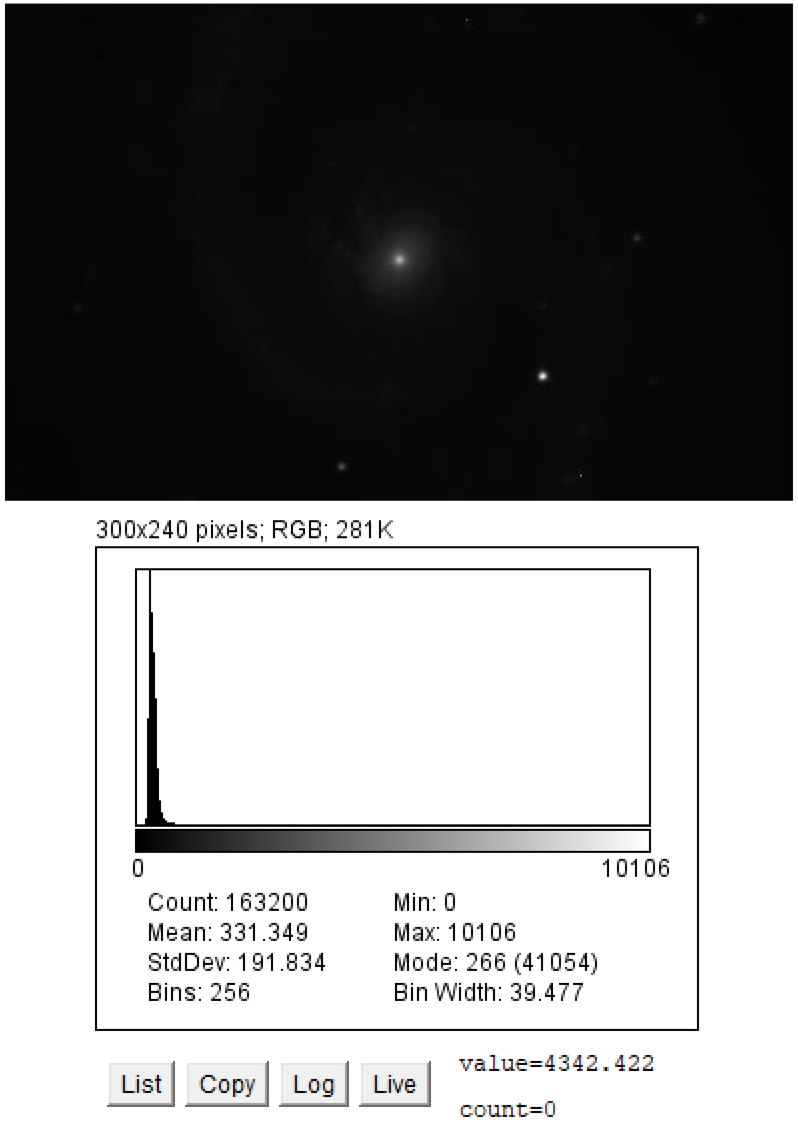
EXCERCISE 5
Open Example 5B.tif and try the histogram normalization and histogram equalization

Process > Enhance contrast…
Amount of Saturated pixels: default = 0.3% (but play with it)
(usually, you want this very low)

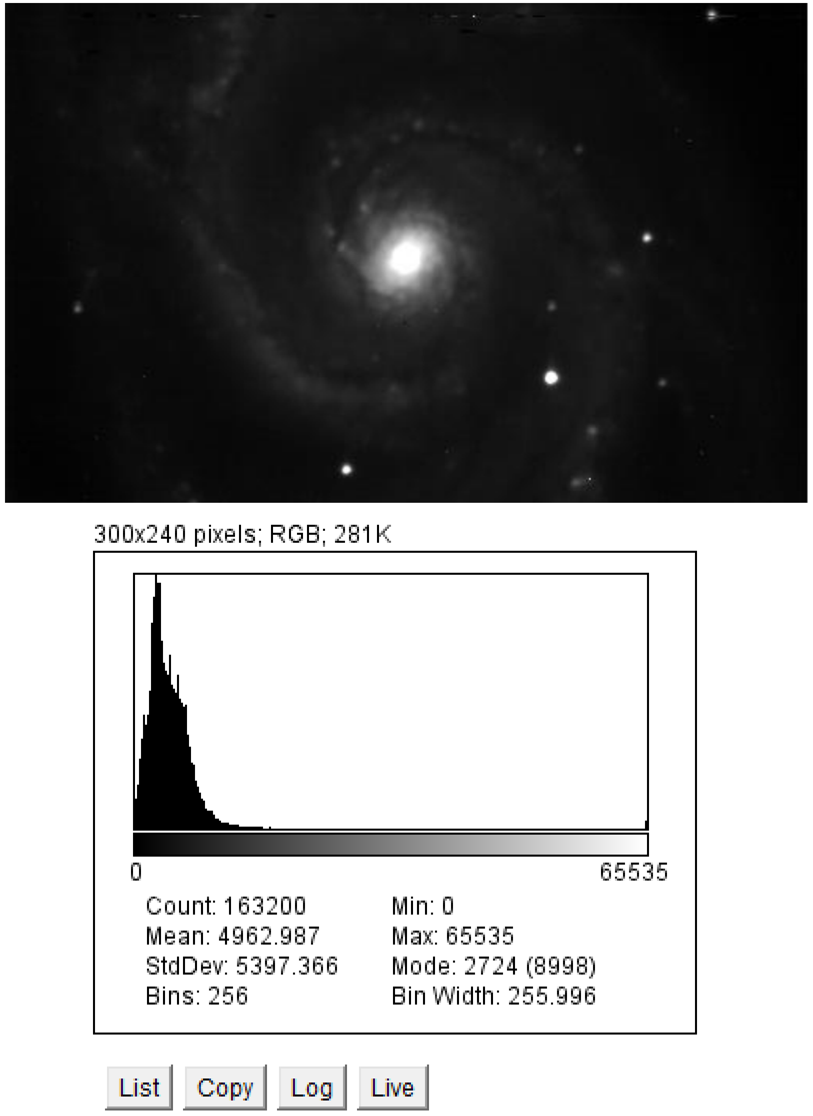


Histogram equalization: attention!

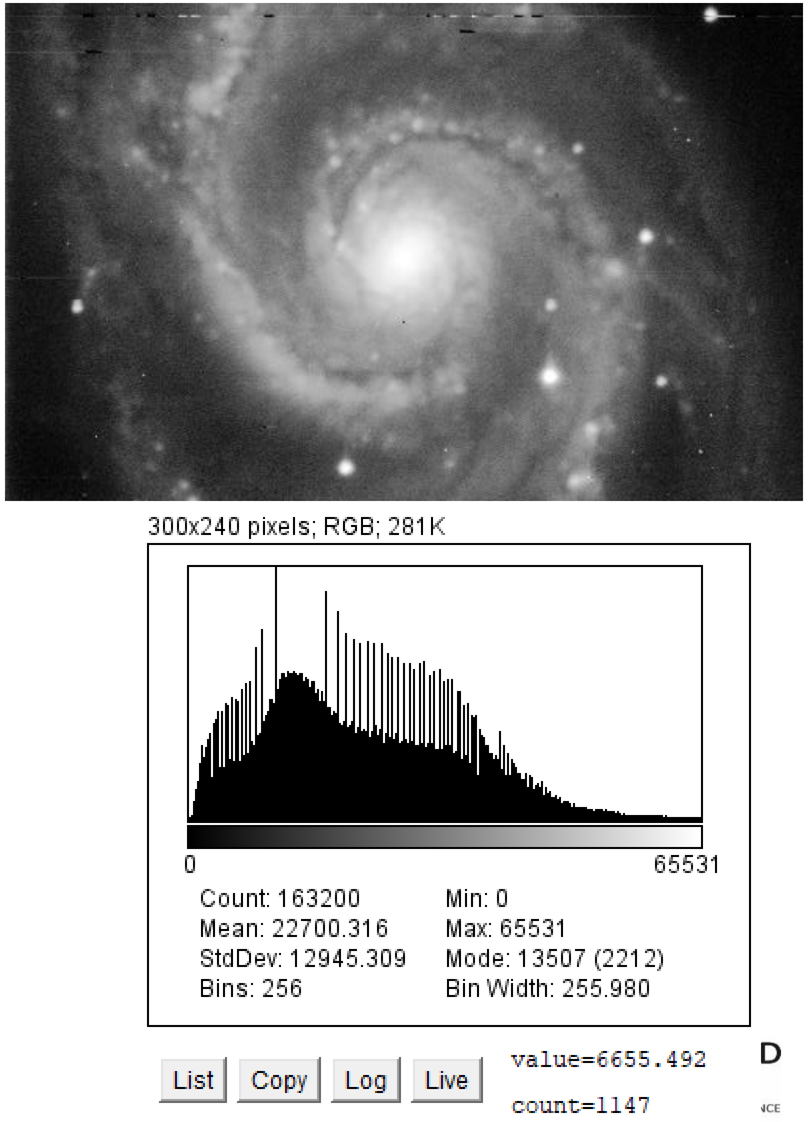
Example (Galaxy M51)



Histogram normalization



Histogram equalization



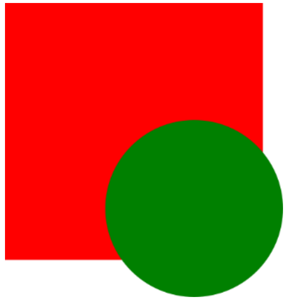
Part IV: Overlays and preparation for publication

- Overlays
- Annotations and scale bars
- Preparing figures for publications
- Inkscape
- FigureJ

Overlays

Overlays are **vector graphics**: non-active, non-destructive selections displayed 'over' the rastered graphics data.

Overlay selections are
- Mathematically-defined **paths** (=not rastered), not affected by scaling, i.e., do not become pixelated.
- Overlays are **saved in the header** (e.g. of tif images), and do not need to be saved externally.
- Examples: Scalebars, annotations, …



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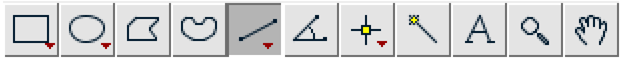
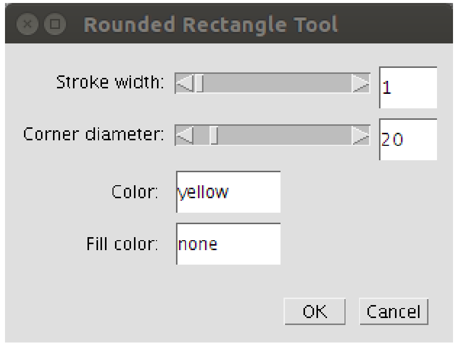
Selections

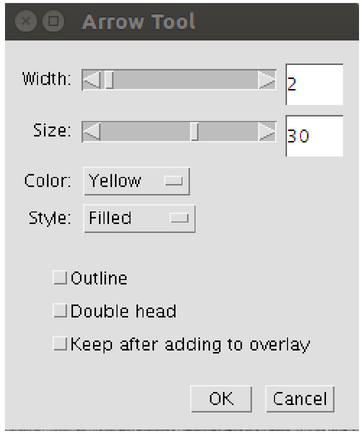
EXCERCISE 6

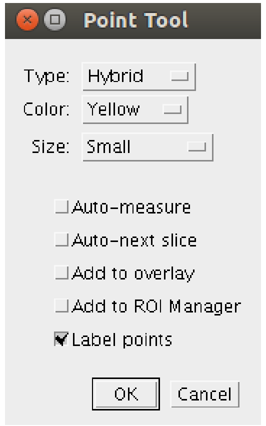
Open Example 4 – Manual scale

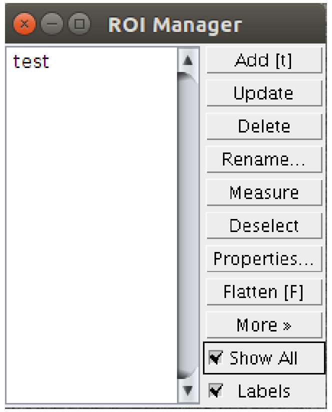


- Select the line selection and draw lines on the image. Check the status bar. Length? Unit of length?
- Try out the other selection options
- Right-click the icons with a red triangle at the bottom right.
- Use Analyze > Tools > ROI manager to
  - Add selections, rename them and remove them
  - Save selections to a file and open them again









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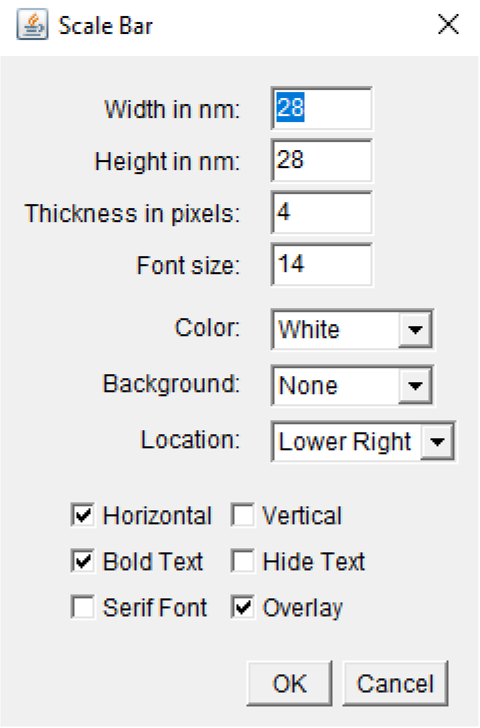
Scale bars

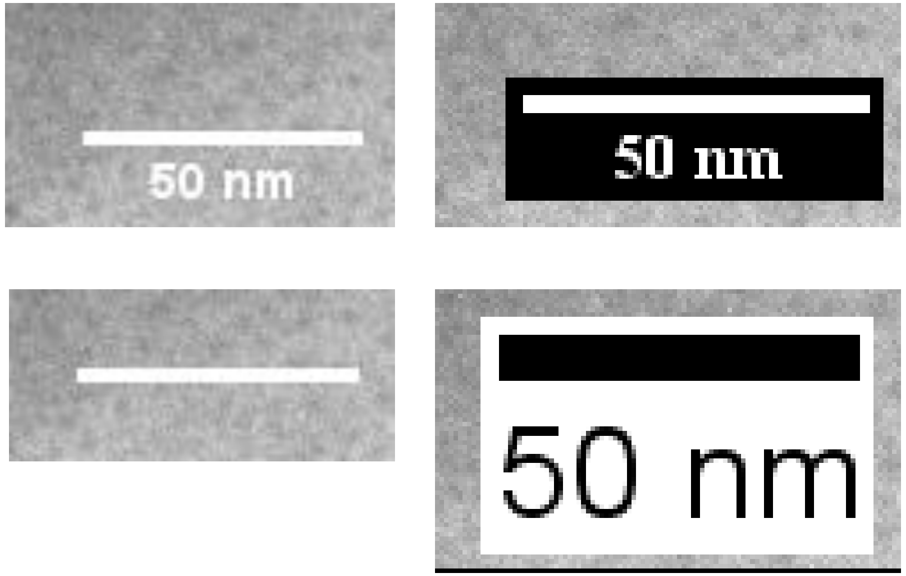
EXCERCISE 6

Add a scale bar to Example 1D (the .ser file)

- Open Example 1D (Plugins > Input-Output > TIA reader)
- Check the info in the header: CTRL + i (or Image > Show info …)
- Analyze > Tools > Scale bar…

Width: 8.3029 microns (2048)
Height: 8.3029 microns (2048)
Size: 8MB
Resolution: 246.6609 pixels per microns
Pixel size: 0.0041x0.0041 microns^2





Scale bars

EXCERCISE 6

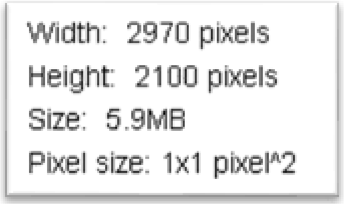
Try to repeat for Example 4 – Manual scale. Retrieve the scale and add a scale bar to a cropped version of Example 4

- Open Example 4
- Image > show info…

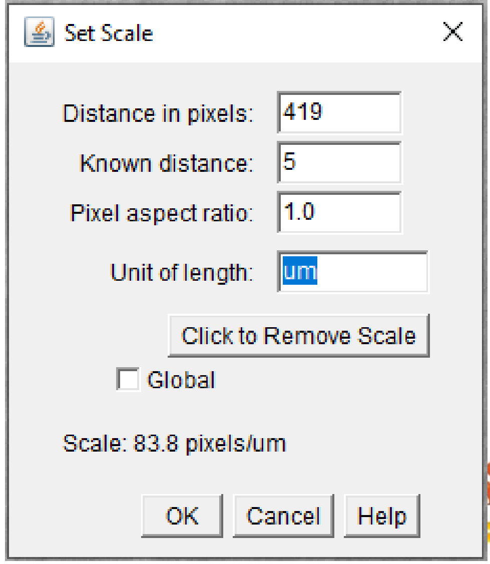
Scale bars

EXCERCISE 6
Try to repeat for Example 4 – Manual scale. Retrieve the scale and add a scale bar to a cropped version of Example 4

- Open Example 4
- Image > show info… The pixel size is NOT calibrated →
- But! There is a scale bar burnt into the rastered image.
- Draw a line exactly the length of the burnt-in scalebar



- Use Analyze > Set Scale
- The length of the line (in pixels) will be automatically filled in
- Enter the known distance (1) and the unit (um, there is no μ)
- (Global will set this scale to all open images)
- Click OK. Your image is now calibrated (Check the info!)



Now add the overlay scalebar

- Analyze > Tools > Scale bar…
- Note: the burnt-in scale cannot be removed, only cropped out

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Annotations

EXCERCISE 7
Open Example 4 – Manual scale and annotate the image

Arrows, lines, text

- Image > annotate > Arrow..

Accepting the annotation

- Press CRTL+B to make the annotation in overlay (recommended)
- Press CTRL+D to **d**raw the annotation in the image (burn in: convert vector to pixels)

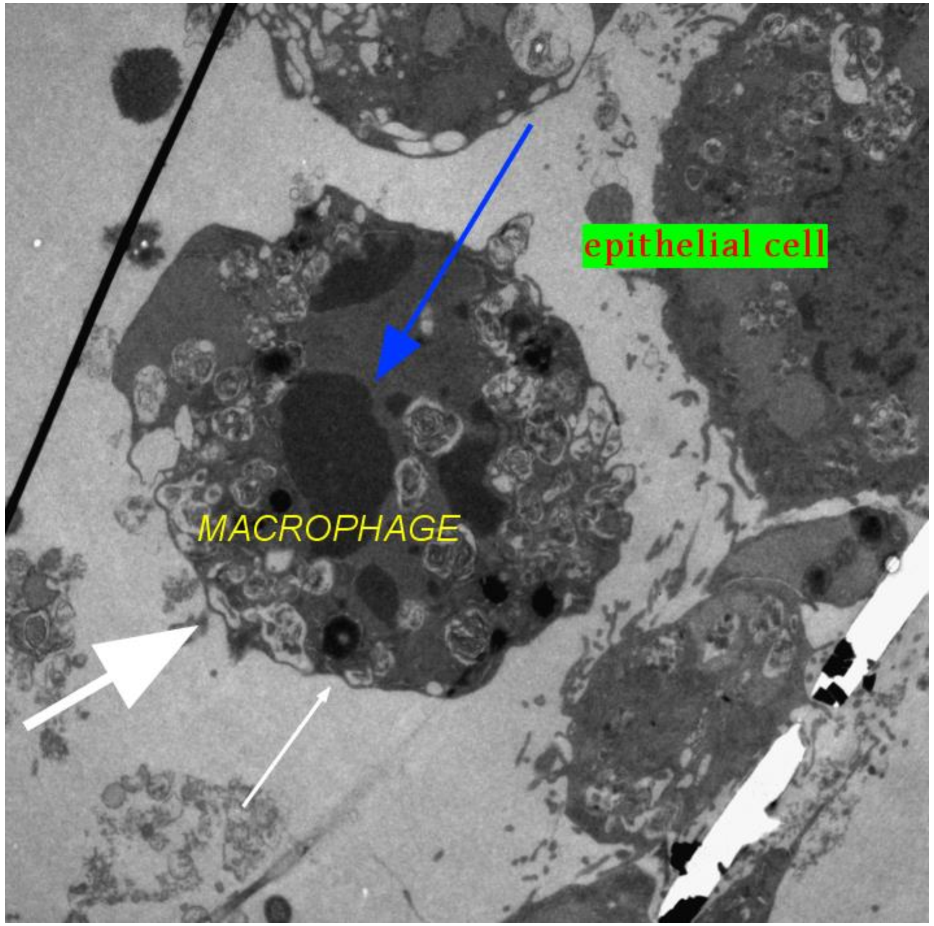
Note: ImageJ/FIJI is not great for annotations

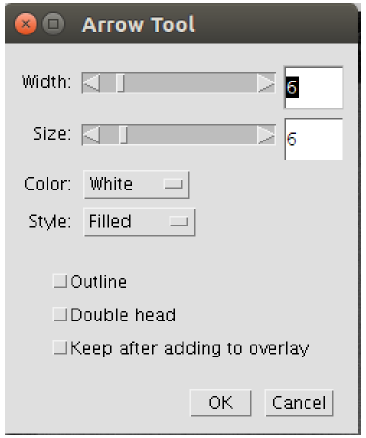
Illustrator, Affinity designer, Inkscape, CorelDraw, … are superior tools for vector graphics design.

Annotations

EXCERCISE 7

Open Example 4- Manual scale and annotate the image





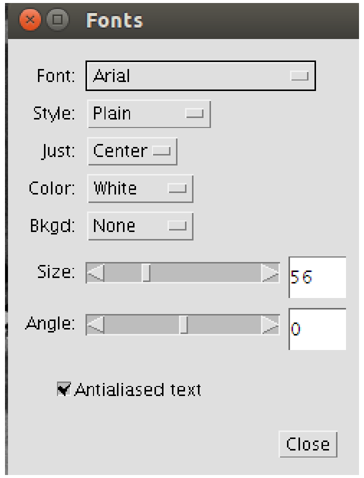


Image > Overlay > List elements

Type	X	Y	Width	Height	Color	Fill	LWidth
Straight Line	396	1070	139	189	white	none	6
Straight Line	58	977	273	164	white	none	16
Straight Line	602	204	245	400	#0036ff	none	8
Text	320	795	422	71	yellow	none	0
Text	923	360	378	68	red	green	0

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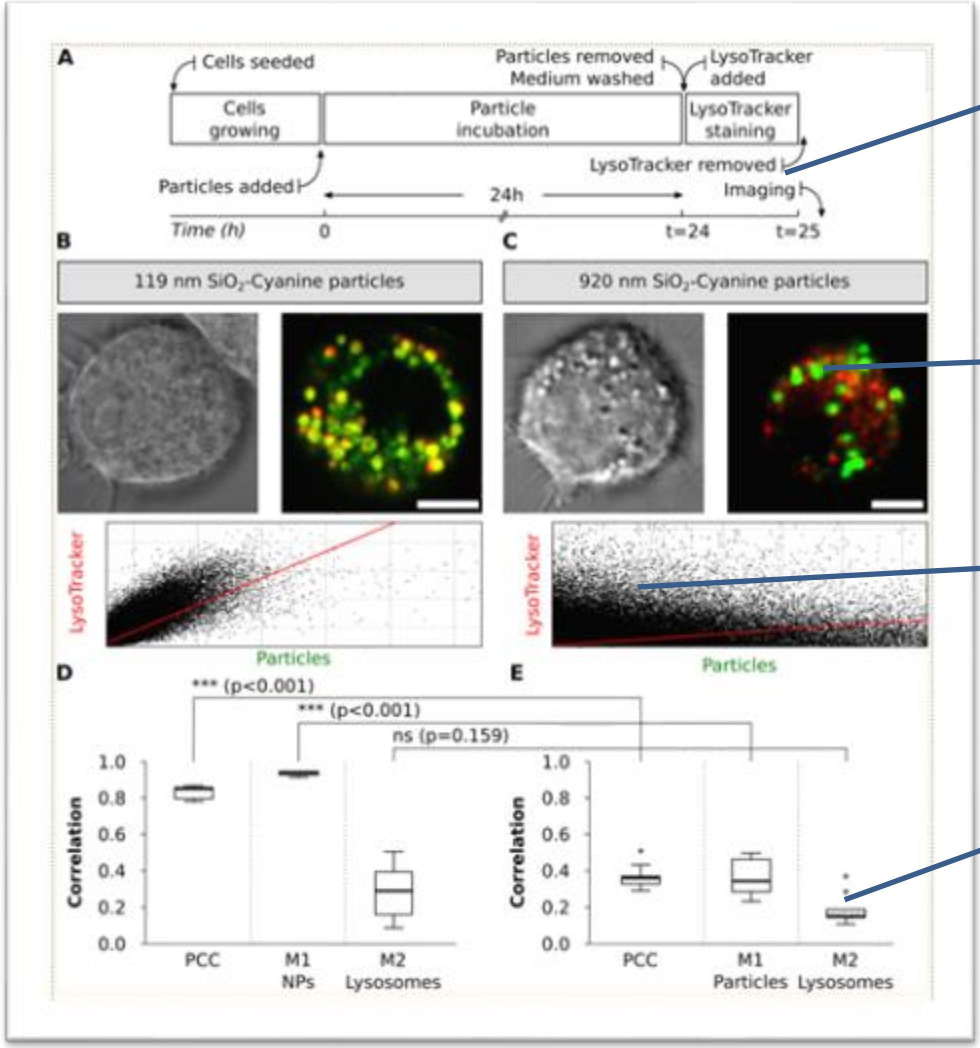
Preparing for publication - semantics

Some semantics:



Line art

Image

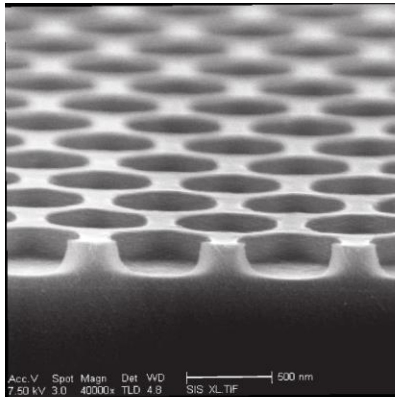
Graph or plot

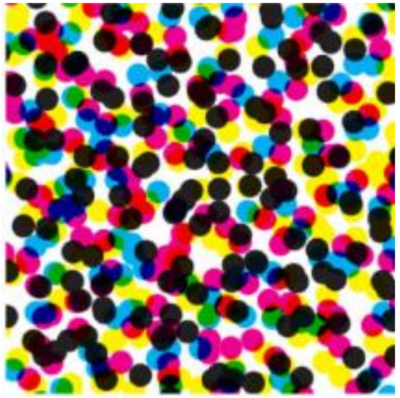
Line art

Figure

Preparing for publication - DPI

Images: from camera to printer: PPI and DPI





Dots per inch (DPI)
is a measure of spatial dot density in printing, in particular the number of individual dots that can be placed in a line within the span of 1 inch (2.54 cm).

Points per inch (PPI)
is the same but with concerning electronic displays. Not relevant here.

Rule of thumb: what is the resolution I need to publish my data?

Images: at least 300 DPI, but 600 DPI has been requested
Line art: higher: 1200 DPI, but I have seen 2400 DPI requests
Graphs: 600 DPI and up

Low resolution

High resolution

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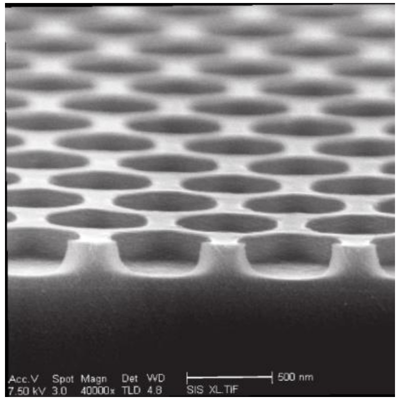
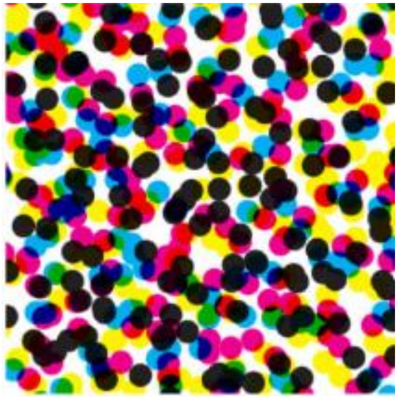
Preparing for publication - example

Images: from camera to printer: PPI and DPI





Info:
1 inch = 2.54 cm
Expected Image width in print: 10 cm
Expected printed resolution: 300 DPI

Question: how many pixels wide does my digital picture have to be?

Calculation:

Width (printer) = $\frac{10\ cm}{2.54 \frac{cm}{inch}}$ = 3.937 inch

at 300 dots per inch = 3.9737 inch x 300 $\frac{dots}{inch}$ = 1181 dots

Result:
The image must be 1181 pixels wide to get a 10 cm wide figure at 300 DPI

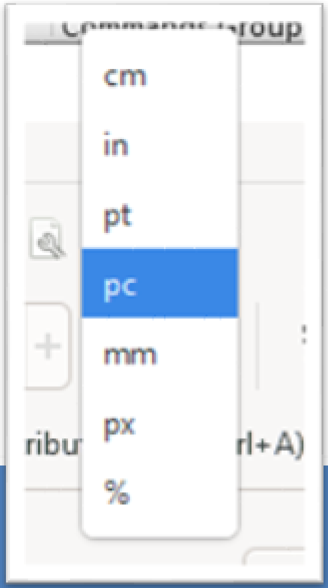
Dots per inch (DPI)
is a measure of spatial dot density in printing, in particular the number of individual dots that can be placed in a line within the span of 1 inch (2.54 cm).

Resizing and cropping
What happens with my image during resizing? Am I allowed to crop an image?
==> See lecture 2 Advanced Image processing

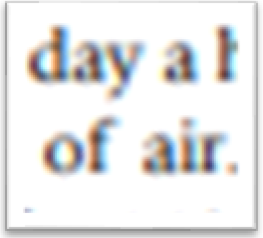
Preparing for publication - pica

Publishers and printers work often with PICA (pc) and POINTS (pt).

- 12 points (pt) — 1 pica (pc)
- 1 pc — 1/6 of an inch
- 1 pc — 4.23333 mm
- 1 pt — 0.35277 mm
- Text height — 1 pica (12 points)
- Text width (page) — 41 pica
- Column width (2 / page) — 20 pica
- Central space — 1 pica



Info
Expected Image width in print: 20 pc
Expected printed resolution: 300 DPI
Question: how many pixels wide does my digital picture have to be?
Calculation:
20 pc = 20/6 inch = 3.333 inch (= 8.333 cm)
Width (printer) = 3.333 inch
at 300 dots per inch = 3.333 inch x 300 $\frac{dots}{inch}$ = 1000 dots
Result:
The image must be 1000 pixels wide to get a columnwide figure at 300 DPI

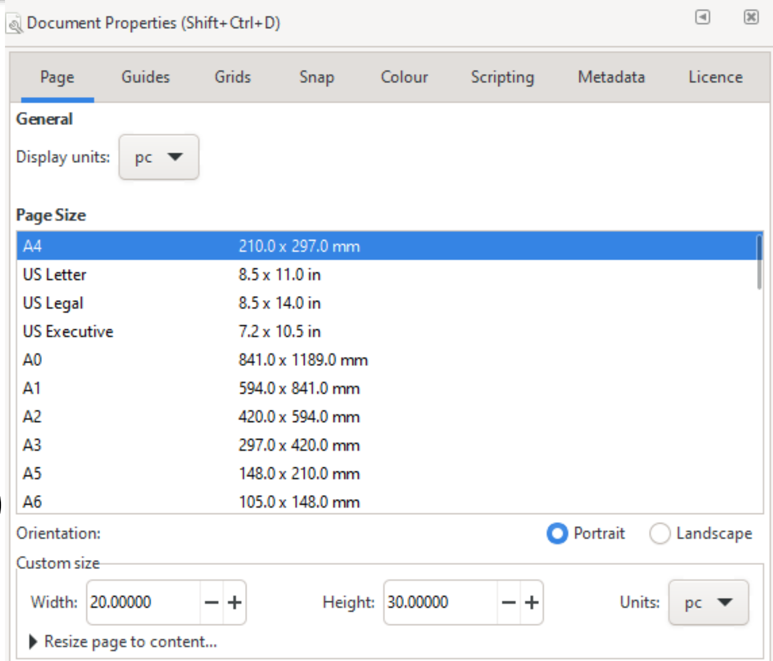


1 pica (12 points)

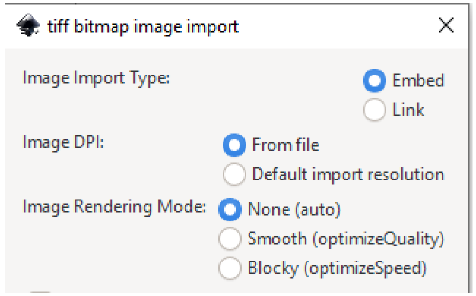
Preparing for publication – Example using InkScape

EXCERCISE 8

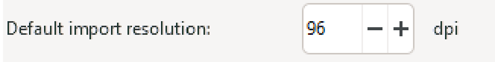
Create a 300 dpi, 20 pica PNG figure containing Example 4 in Inkscape



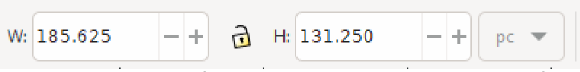
1. **Start the software (inkscape.org)**
2. **Adjust the window size**
   File > Document properties
   Set Display units to pica (pc) and custom size to 20 pc width
   Close the Document properties window
3. **Import the image**

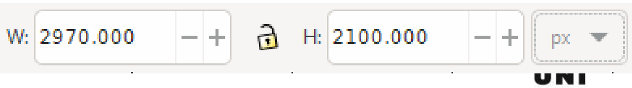


   File > Import… and point to Example 4
   In the import dialog box: do not change anything
   (the default is 96 dpi = 96 PPI = screen resolution)
   Edit > Preferences > Imported Images



4. **Set the image dimensions**
   Left tool bar: make sure you selected the black pointer
   Select the image, you can find the dimensions in the horizontal toolbar



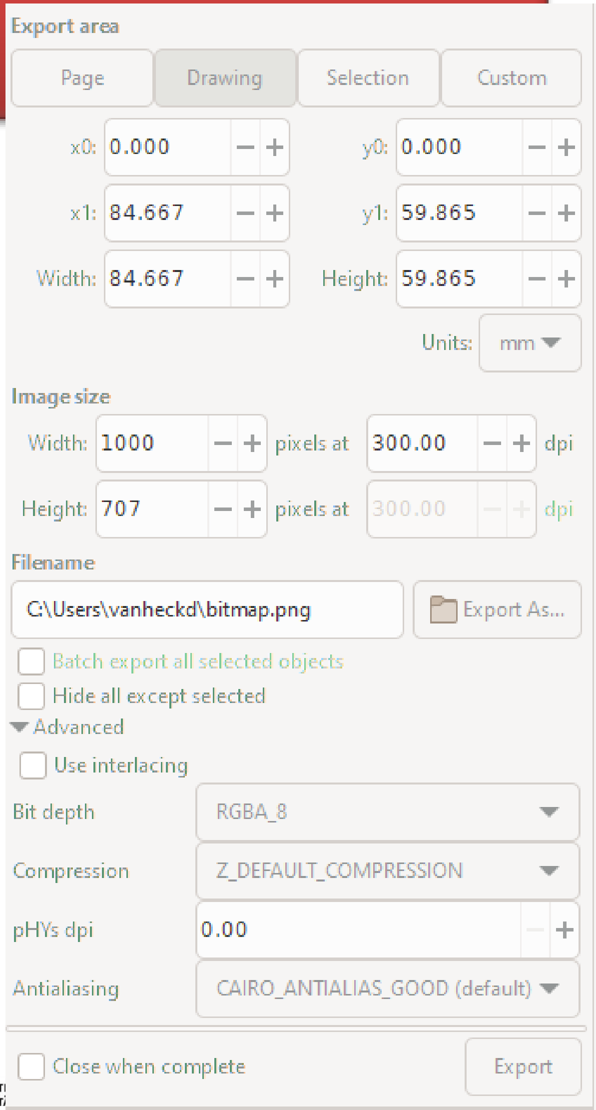


   Try out different units (px, mm, in, pc, …)

Preparing for publication – Example using InkScape

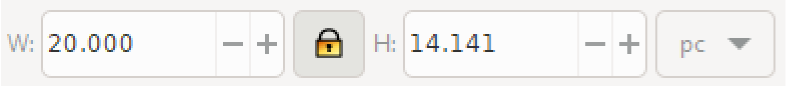
EXCERCISE 8

Create a 300 dpi, 20 pica PNG figure containing Example 4 in Inkscape

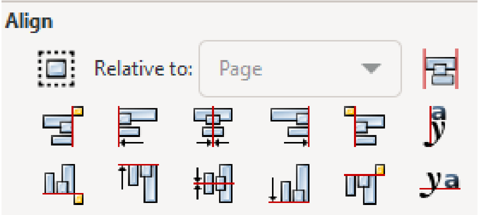


5. <u>**Scale the image**</u>

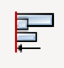
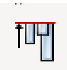
   With the lock ticked (keeps W/H ratio constant), change W to 20 pc



6. <u>**Align and distribute**</u>



   Object > Align and distribute… you get new possibilities on the right side of the screen

   Align relative to: Page and click  and then  (order is irrelevant)

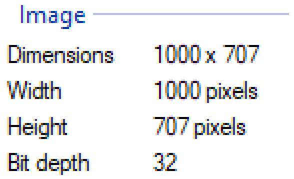
   The image is now aligned to your 20 pc wide page

7. <u>**Add annotations**</u>
8. <u>**Export to PNG**</u>

   File > Export (you get a new window on the right)

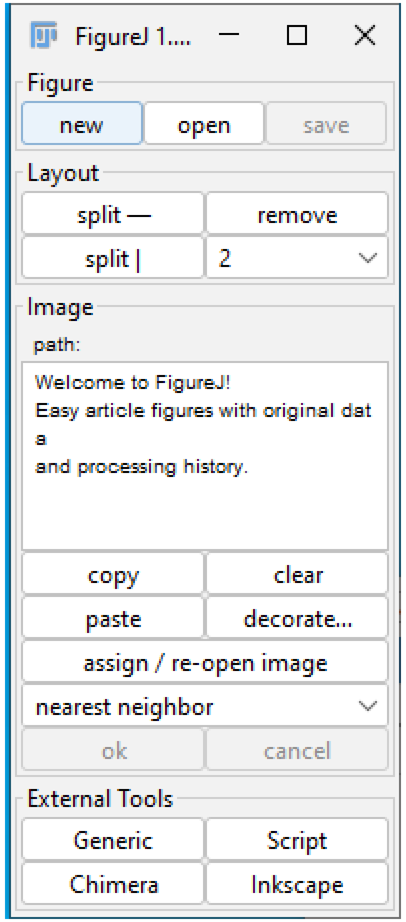
   Set the Image size … pixels at to 300 DPI. Click Export.

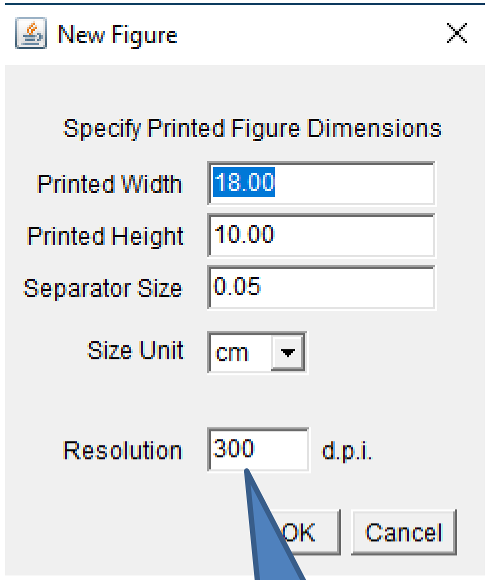
   Check its dimensions in your Operating system



Preparing for publication

FigureJ: attempt to create Figure panels for scientific publications in ImageJ
Plugin download: add the IBMP-CNRS, ImageScience and BioFormats repository, then restart.
Plugin start: Plugins> FigureJ > new





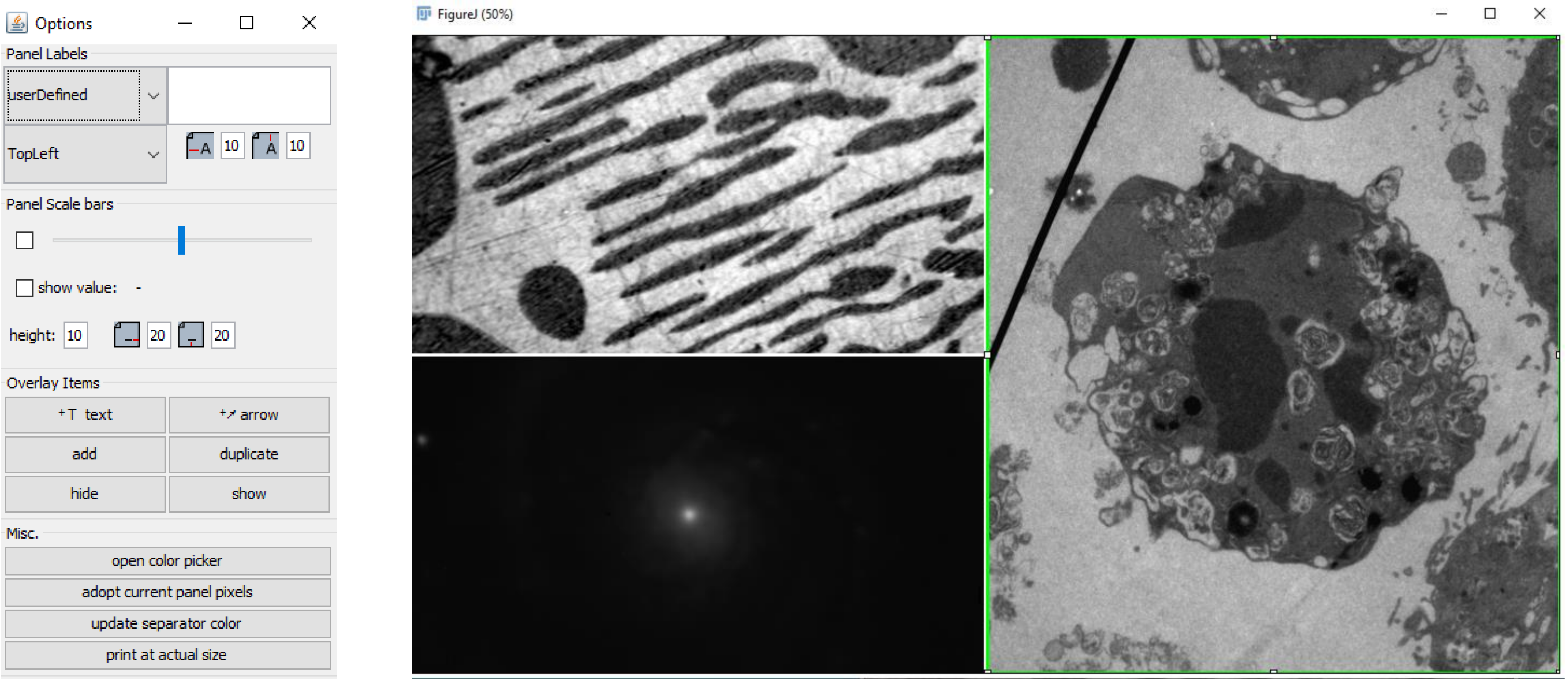
Set the resolution
in DPI at the start

Advantage
FigureJ takes care of the DPI conversion of your images

https://imagejdocu.tudor.lu/plugin/utilities/figurej/start

MUTTERER, J., & ZINCK, E. (2013). Quick-and-clean article figures with FigureJ. *Journal of Microscopy*, *252*(1), 89–91.

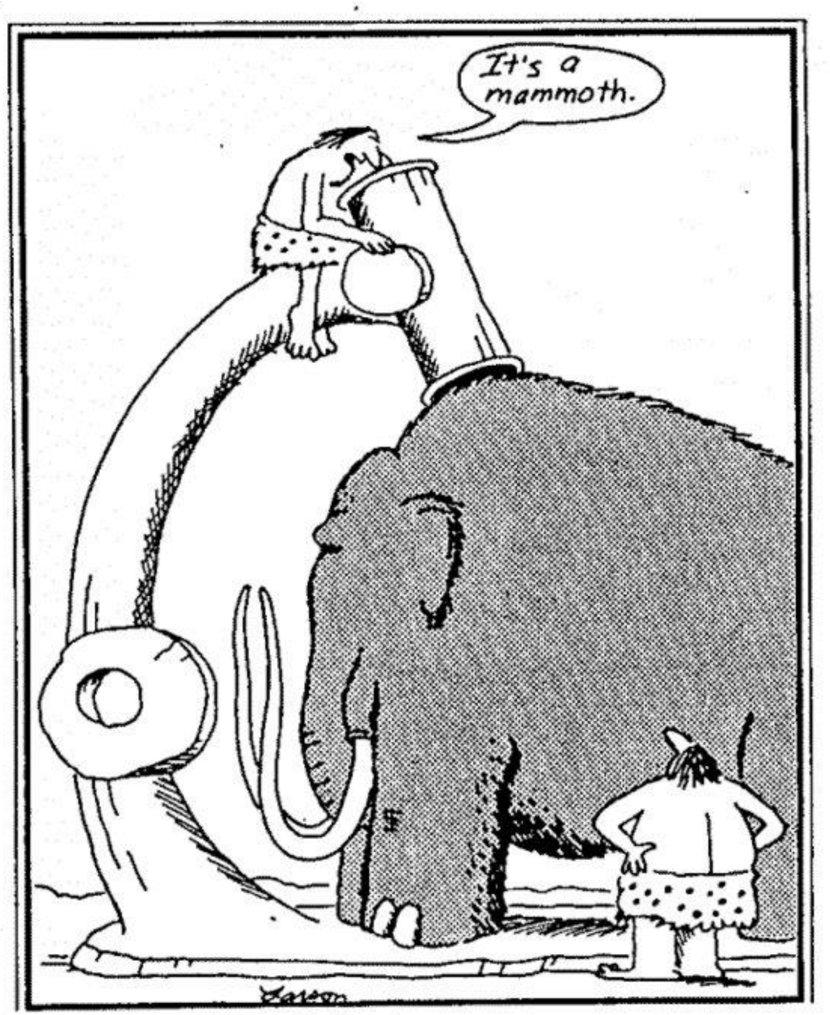
Annotations: FigureJ



Finished!

✓ Congratulations,

You finished Part I, Basics!



Early microscope