

Preparation of digital images for publication

Pixel size; Image resolution; 8, 12, 14 or 16-bit depth; Printing resolution; 300 dpi

Oldřich Benada

Electron Microscopy Group

Laboratory of Molecular Structure Characterization

INSTITUTE OF MICROBIOLOGY, CAS, v.v.i.

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142 20 Praha 4 - Krč

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October 11, 2024



OME Metadata - Ferritin_kontrola_01.tif

Key	Value
BiosPerPixel	16
DimensionOrder	XYZCT
IsInterleaved	false
isRGB	false
LittleEndian	true
PixelType	uint16
Series 0 Name	Ferritin_kontrola_01
SizeX	1
SizeY	1
SizeZ	1280
SizeT	1024
SizeZ	1
Acquisition date	2012-11-22T10:41:00
Artist	
BiosPerSample	16
Camera name	MegaView
Channel name	MegaView 1 Snapshot
Compression	Uncompressed
Image name	Ferritin_kontrola_01
Image length	1024
ImageWidth	1280
Instrument Make	
Location	/home/olga/smb/6-A/117/C6/04/0
Magnification	6400.0
MetadataPhotometricInterpretation	Monochrome
Nanometers per pixel 0	9.821637998127912
Nanometers per pixel (Y)	0.821637998127912
NumberOfPixels	1
NumberOFChannels	1
PhotometricInterpretation	BlackIsZero
PlanarConfiguration	Chunky
ResolutionUnit	None
SamplesPerChannel	unsigned Integer
SamplesPerPixel	1
Software	analySIS 3.2



Image recording in electron microscopy

Human eye and Visual perception

An introduction: What to do with our hardware?

Digital image size: pixels, DPI, resolution, etc.

Digital image resolution: spatial vs. lateral

Scalebars, Arrows and Lettering

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What is an anaglyph?

Human binocular vision - stereopsis

Stereo pairs

Stereo pairs in SEM

Stereo pairs: A toy or serious tool in scientific imaging?

Resolution

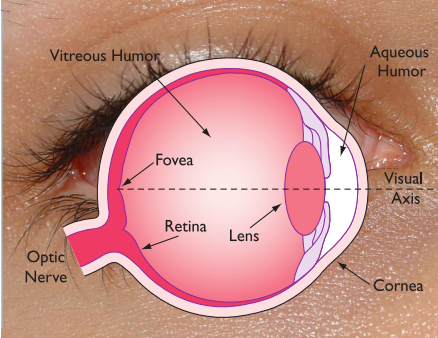
Ability to record and reproduce details of certain size



Human eye resolution
1-2/60 degree

Reading distance
 $\approx d = 25 - 30 \text{ cm}$

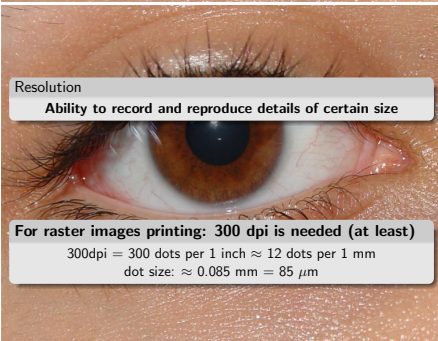
Smallest
resolvable detail
 $\approx 80 - 160 \mu\text{m}$



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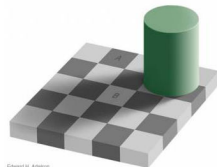
Smallest
resolvable detail
 $\approx 80 - 160 \mu\text{m}$

For raster images printing: 300 dpi is needed (at least)
300dpi = 300 dots per 1 inch $\approx 12 \text{ dots per } 1 \text{ mm}$
dot size: $\approx 0.085 \text{ mm} = 85 \mu\text{m}$

Human vision is not perfect

Persci

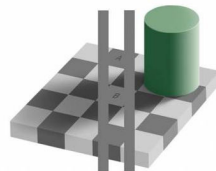
Checkershadow Proof



Edward H. Adelson

The original image of the illusion

The squares marked A and B are the same shade of gray, yet they appear different.



The original image plus two stripes

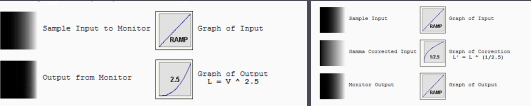
By joining the squares marked A and B with two vertical stripes of the same shade of gray, it becomes apparent that both squares are the same.

Hardware calibration - setting the gamma

Gamma

Describes the nonlinear relationship between the pixel levels in your computer and the luminance of your monitor - WEB links:

- Norman Koren PHOTOGRAPHY ePaperPress
- Wikipedia - Gamma correction
- EIZO monitor test



Hardware calibration - setting the gamma

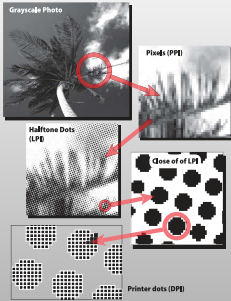
Gamma

Describes the nonlinear relationship between the pixel levels in your computer and the luminance of your monitor - WEB links:

- Norman Koren PHOTOGRAPHY ePaperPress
- Wikipedia - Gamma correction
- EIZO monitor test

Gamma (γ)

An ideal case $L = B + x.C$
Real world $L = B + x^\gamma.C$
L = luminance of imaging point
B = brightness of the monitor screen (black level)
C = contrast of monitor screen
x = signal from graphic card normalized to 1; For 8-bits monitor (range 0-255): x = (signal value)/255



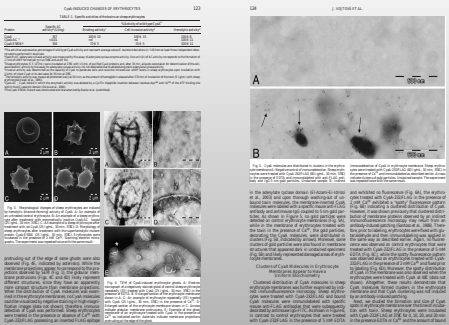
Digital image size: pixels, DPI, resolution, etc.

David Creamer:
Understanding Resolution
and the meaning of DPI, PPI, SPI, & LPI. (2012)

DPI = Dots per inch = Units used to measure the resolution of a printer.

LPI = Lines per inch = The offset printing 'lines' or dots per inch in a halftone or line screen.

PPI = Pixels per inch = the number of pixels per inch in screen/scanner file terms.



Scientific journal
Text area dimensions

1/2 printed column
≈ 42.5 mm

One printed column
≈ 85 mm

Two printed columns
≈ 180 mm

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O. Benada (Institute of Microbiology) Preparation of digital images for publication October 11, 2024 21 / 78

Digital Image Sampling Frequency

Object

5 nm pixel size

2.5 nm pixel size

1.25 nm pixel size

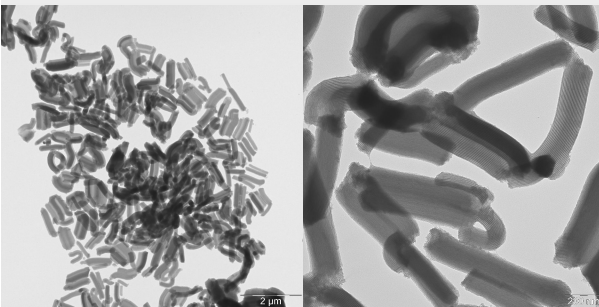
2.5 nm pixel size

Author: Salurambo
<https://commons.wikimedia.org/wiki/File:Airy-3d.svg>

Source: <https://photographylife.com/what-is-diffraction-in-photography/>

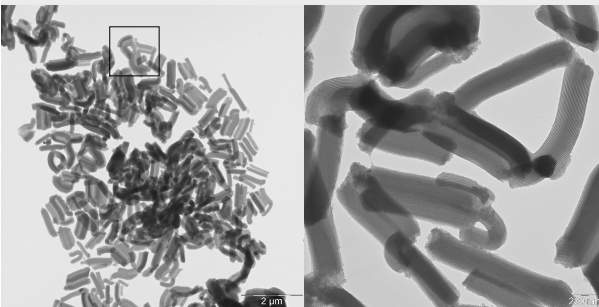
Primary magnification: 10.5 k , Pixel size: 4.8 nm

Primary magnification: 64 k , Pixel size: 0.8 nm

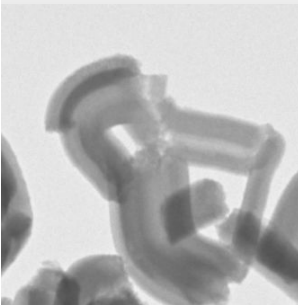


Primary magnification: 10.5 k , Pixel size: 4.8 nm

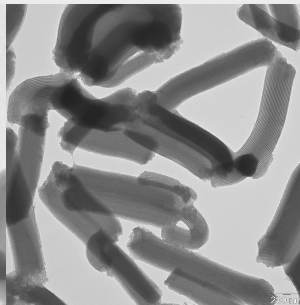
Primary magnification: 64 k , Pixel size: 0.8 nm



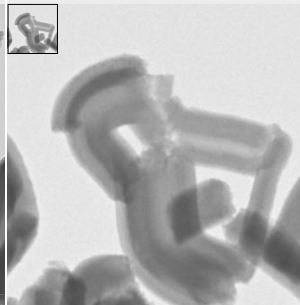
Primary magnification: 10.5 k , Pixel size: 4.8 nm



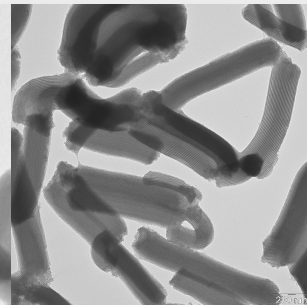
Primary magnification: 64 k , Pixel size: 0.8 nm



Primary magnification: 10.5 k , Pixel size: 4.8 nm



Primary magnification: 64 k , Pixel size: 0.8 nm



Insert primary magnification: 10.5 k , Zoom: 6 , PNG



Insert primary magnification: 10.5 k , Zoom: 6 , JPG

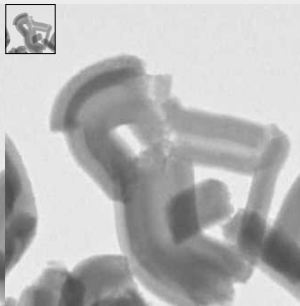
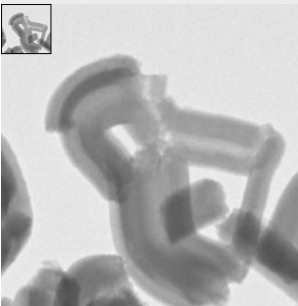


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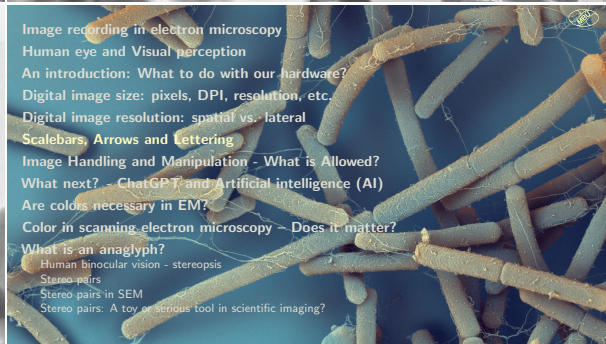
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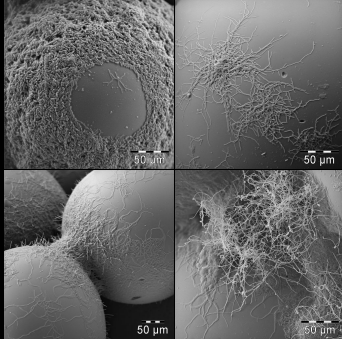


Where to Place 1m Scalebar?



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Scalebars, Arrows and Lettering in SEM



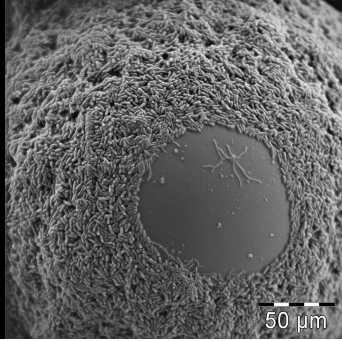
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Where to Place 500 μm Scalebar?

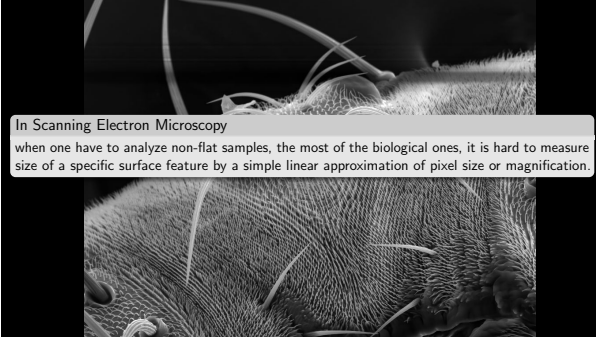


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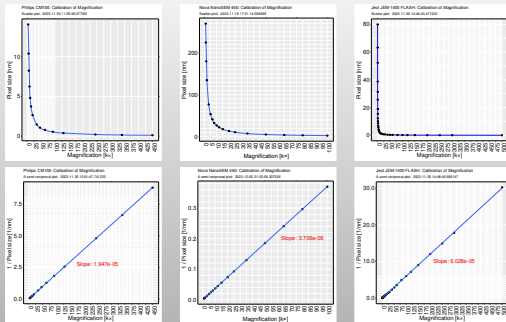
Scalebars, Arrows and Lettering in SEM



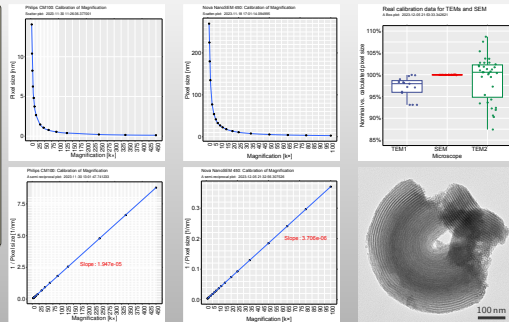
© 2018 ERM Group # Laboratory of Molecular Structure Characterization MREI #P Dr. https://mreui.com



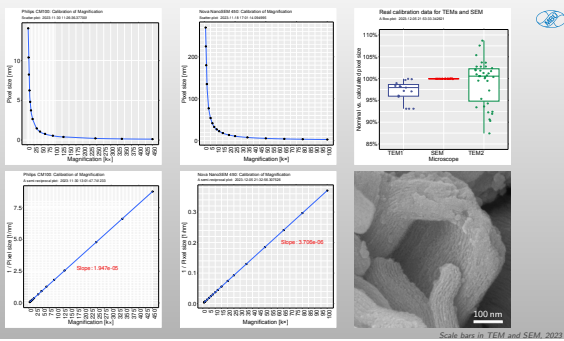
TEM1		TEM2		SEM	
Magnification	Pixel size [nm]	Magnification	Pixel size [nm]	Magnification	Pixel size [nm]
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4600	10.395	1200	12.225	1200	224.83
5800	8.244	1500	10.101	1500	179.86
7900	6.248	2000	8.696	2000	134.9
10500	4.807	2500	6.826	3500	77.08
13500	3.733	3000	5.747	5000	53.96
19000	2.648	4000	4.283	6500	41.51
34000	1.466	5000	3.448	8000	32.72
46000	1.070	6000	2.817	10000	26.98
64000	0.780	8000	2.105	12000	22.48
92000	0.549	10000	1.718	15000	17.99
130000	0.391	12000	1.437	20000	13.49
245000	0.209	15000	1.139	25000	10.79
340000	0.151	20000	0.855	35000	7.71
450000	0.114	25000	0.722	50000	5.4
		30000	0.608	65000	4.15
		40000	0.442	80000	3.37
		50000	0.348	100000	2.7
		60000	0.283		
		80000	0.204		
		100000	0.167		



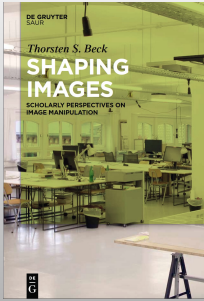
Scale bars in TEM and SEM, 2023



Scale bars in TEM and SEM, 2023



Digital Images and Misconduct



Digital Images and Misconduct

The Rockefeller University Press has established 4 basic guidelines

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
- Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the whole image and as long as they do not obscure, eliminate, or misrepresent any information present in the original.
- The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the text of the figure legend.
- If the original data cannot be produced by an author when asked to provide it, acceptance of the manuscript may be revoked.

Software gamma correction

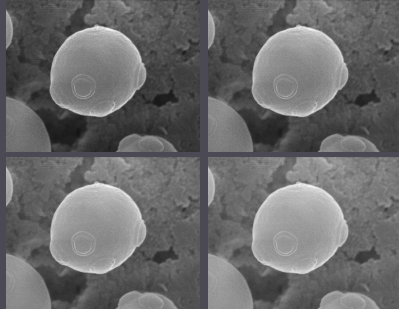


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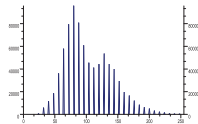
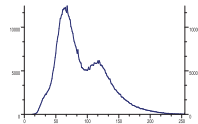
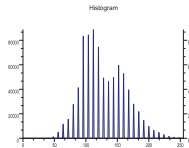
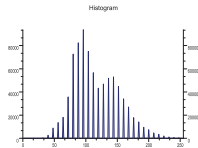
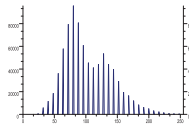
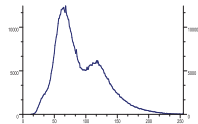
Stereo pairs: A toy or serious tool in scientific imaging?

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October 11, 2024

36 / 78



If the gamma adjustment is performed on the image by software, it leads to information loss.

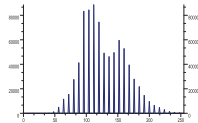
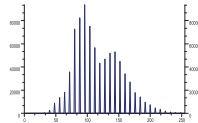
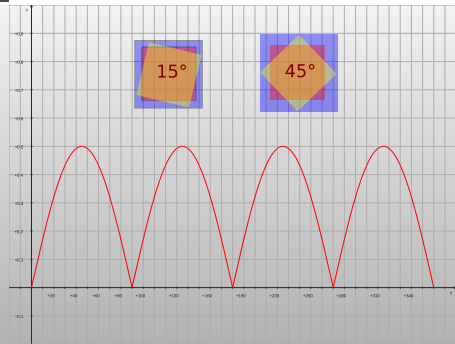
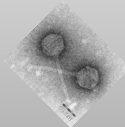
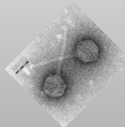
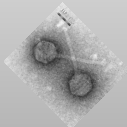
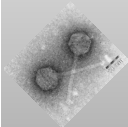


Image rotation

Lossless vs. Lossy






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O. Benada (Institute of Microbiology) Preparation of digital images for publication October 11, 2024 45 / 78

nature in 🐦 f

Science and the new age of AI



Updated 10 October 2023

Credit: Carlo Cadenas Link: <https://www.nature.com/immersive/d41586-023-03017-2/index.html>



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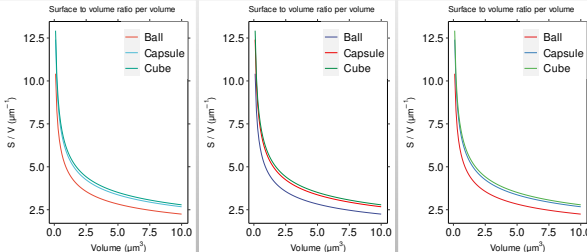
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O. Benada (Institute of Microbiology) Preparation of digital images for publication October 11, 2024 48 / 78

Scientific palettes ggsci: npg, aaas, brewer1 <https://nanx.me/ggsci/articles/ggsci.html> 



O. Benada (Institute of Microbiology) Preparation of digital images for publication October 11, 2024 48 / 78

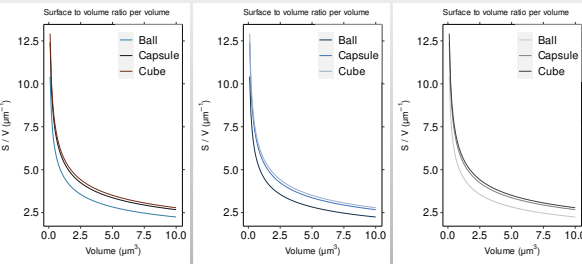
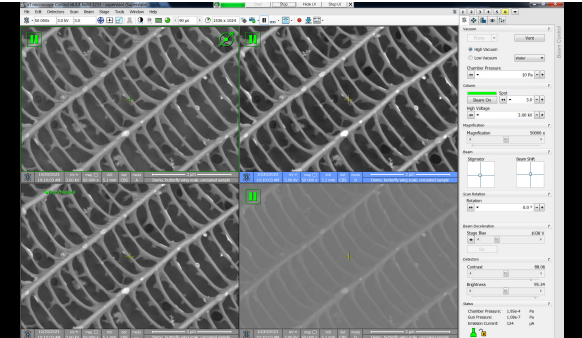
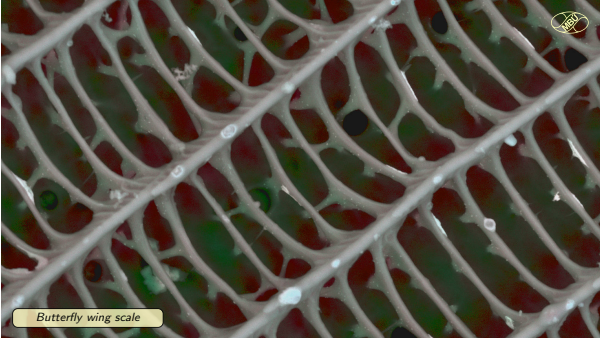
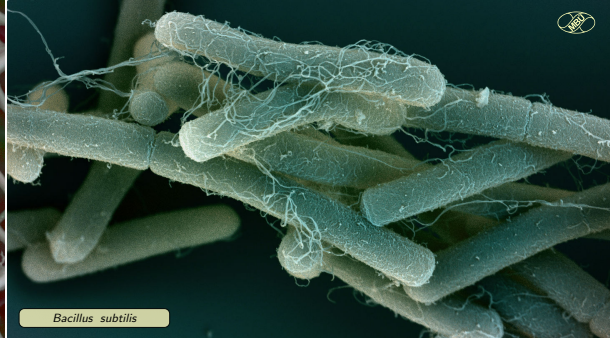


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Butterfly wing scale



Bacillus subtilis



Lysing *Bacillus subtilis* cells



Thank you
for your attention

Soil biofilm