Image Processing for Microscopy

1. Scientific image processing fundamentals
2. Acquiring images
3. Considerations when taking electron microscopy images
4. Correcting defects (noise etc.)
5. Image enhancement in the spatial domain
   (on image pixels)

6. Processing images in frequency space (in Fourier space)
7. Extraction of (quantitative) information:
   Segmentation and thresholding

8. Image measurements
9. Correlation, classification, identification and matching

Software Practice: ImageJ [or Fiji]

IMAGEJ

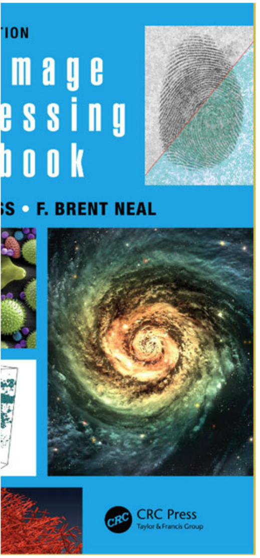
An open platform for scientific image analysis

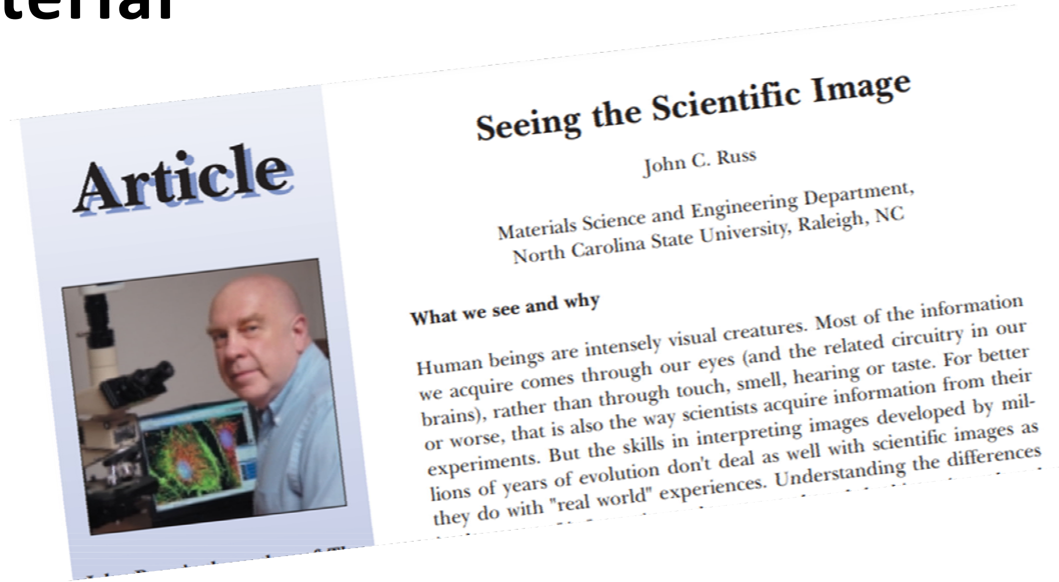
http://imagej.net/ImageJ2

Image Processing Lectures

- Today: lecture part 1
- Part 2: 2 hours of lectures
  & 1 hour of introduction to ImageJ/Fiji, installation of Fiji on your computer !
- Part 3: 3 hours of practicals with Fiji, questions etc.

Reading Material




Article

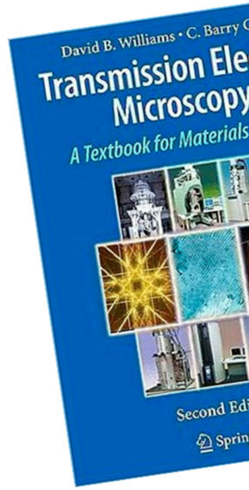
Seeing the Scientific Image

John C. Russ

Materials Science and Engineering Department,
North Carolina State University, Raleigh, NC

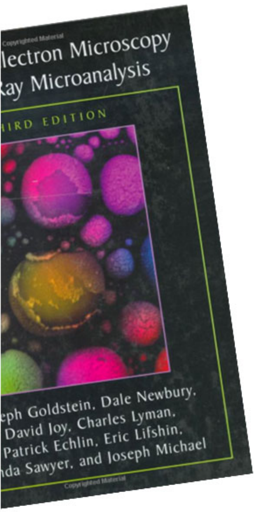
What we see and why

Human beings are intensely visual creatures. Most of the information we acquire comes through our eyes (and the related circuitry in our brains), rather than through touch, smell, hearing or taste. For better or worse, that is also the way scientists acquire information from their experiments. But the skills in interpreting images developed by millions of years of evolution don't deal as well with scientific images as they do with "real world" experiences. Understanding the differences

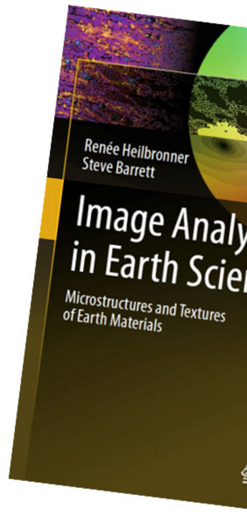


Main Resources

- Seeing the scientific image, article as supporting material on moodle
- The **Image Processing Handbook** by John Russ and Brent Neal, CRC Press - 7th edition – Can be found ONLINE at the EPFL library

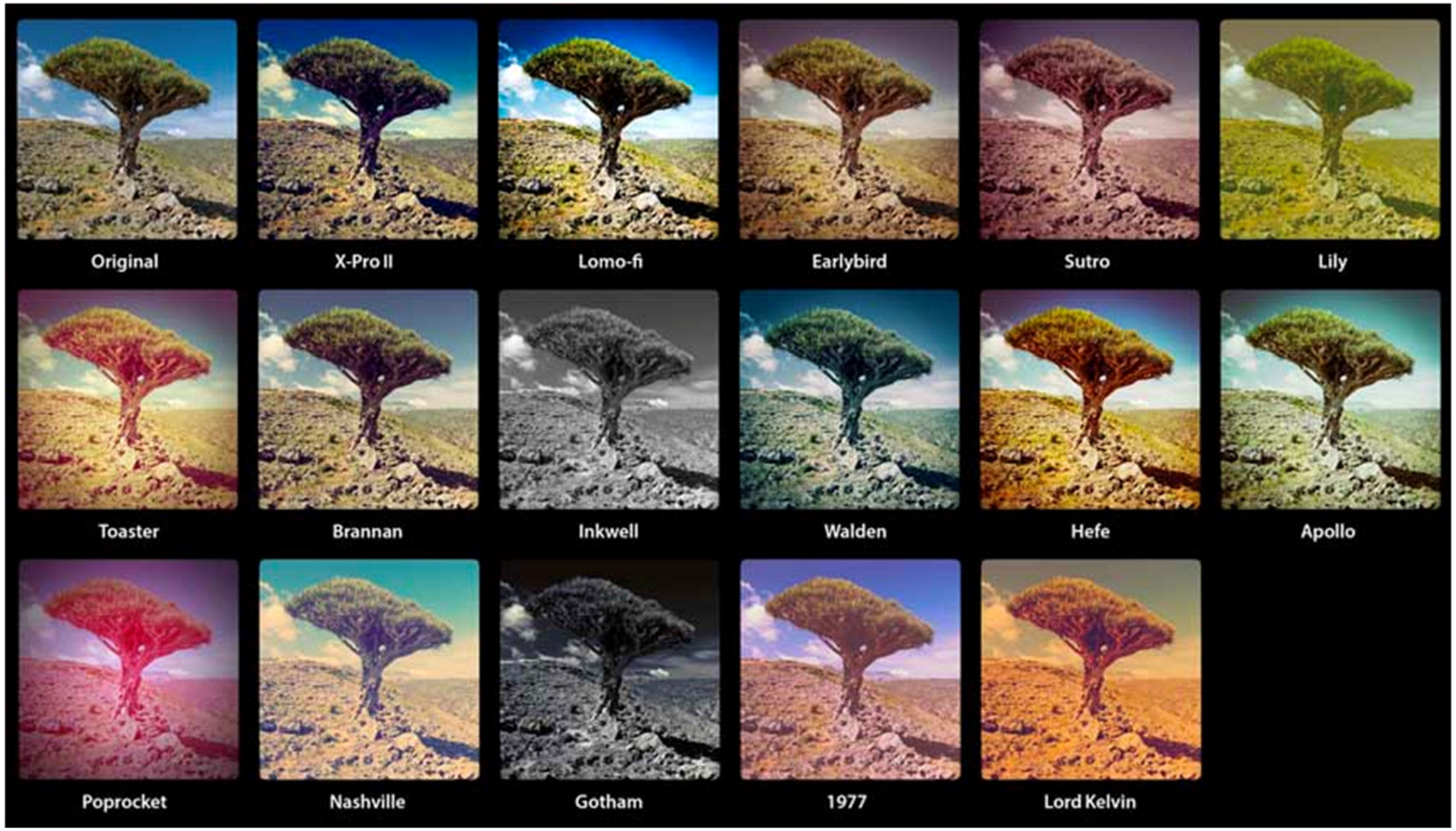


- Chapter 4 & Chapter 7 in Scanning Electron Microscopy and X-Ray Microanalysis by Joseph Goldstein et al., Springer – 3rd Edition – Hardcopy at the Library & at CIME library
- Chapter 7 in Transmission Electron Microscopy by David Williams and Barry Carter, Springer – Hardcopy at the Library & at CIME Library
- Image Analysis in Earth Sciences, Microstructures and Textures of Earth Sciences by Renne Heilbronn & Steve Barett, Springer 2014 – ONLINE at the EPFL library



1. Image Processing Fundamentals

Do you use image processing?



Two main purposes:

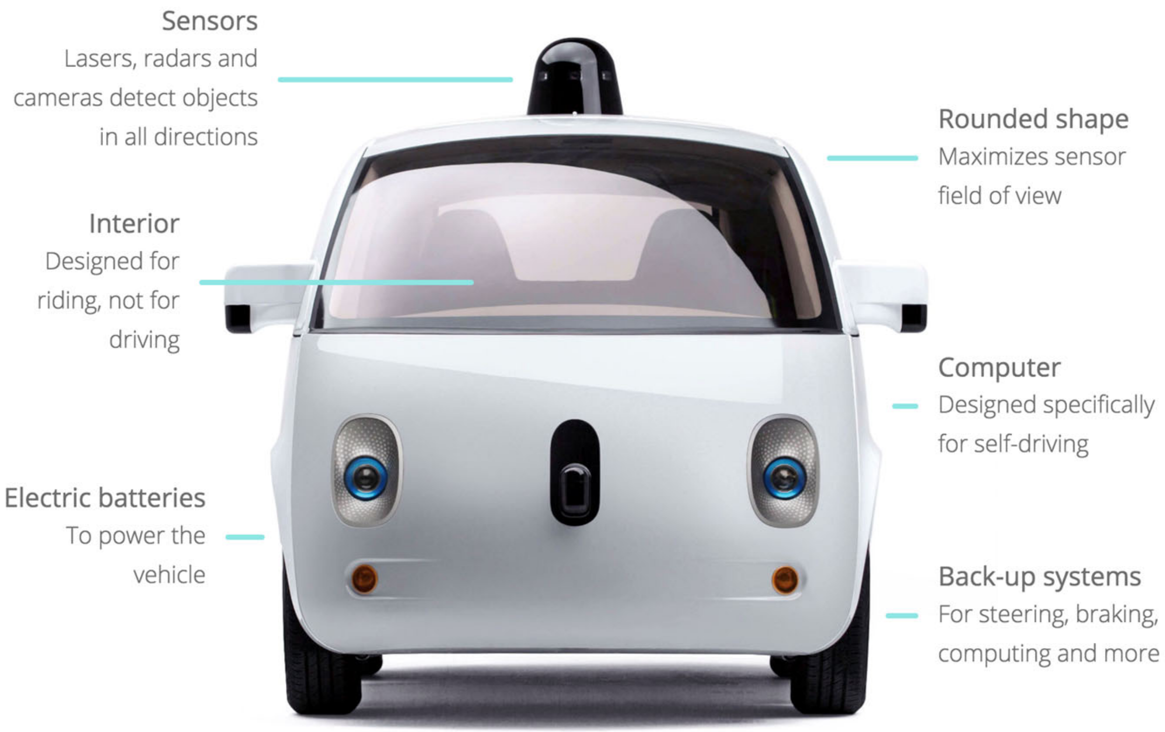
1. improving the visual appearance of images for a human observer, including their printing and transmission

Do you use image processing?

Face-ID

Google

self-driving car project



Two main purposes:

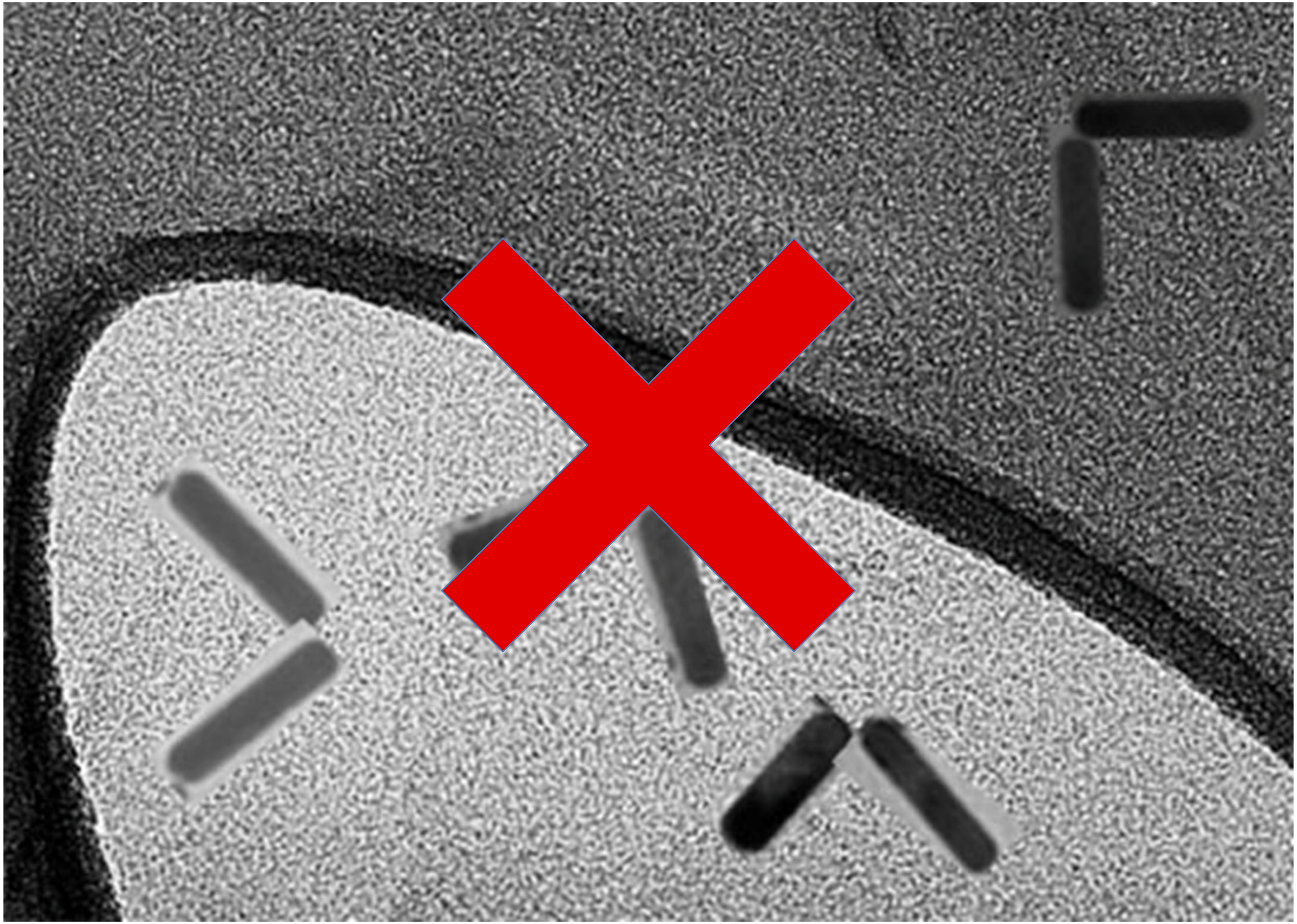
1. improving the visual appearance of images for a human observer, including their printing and transmission
2. preparing images for the measurement and/or *real-time* analysis of the features and structures that they reveal

A word of caution

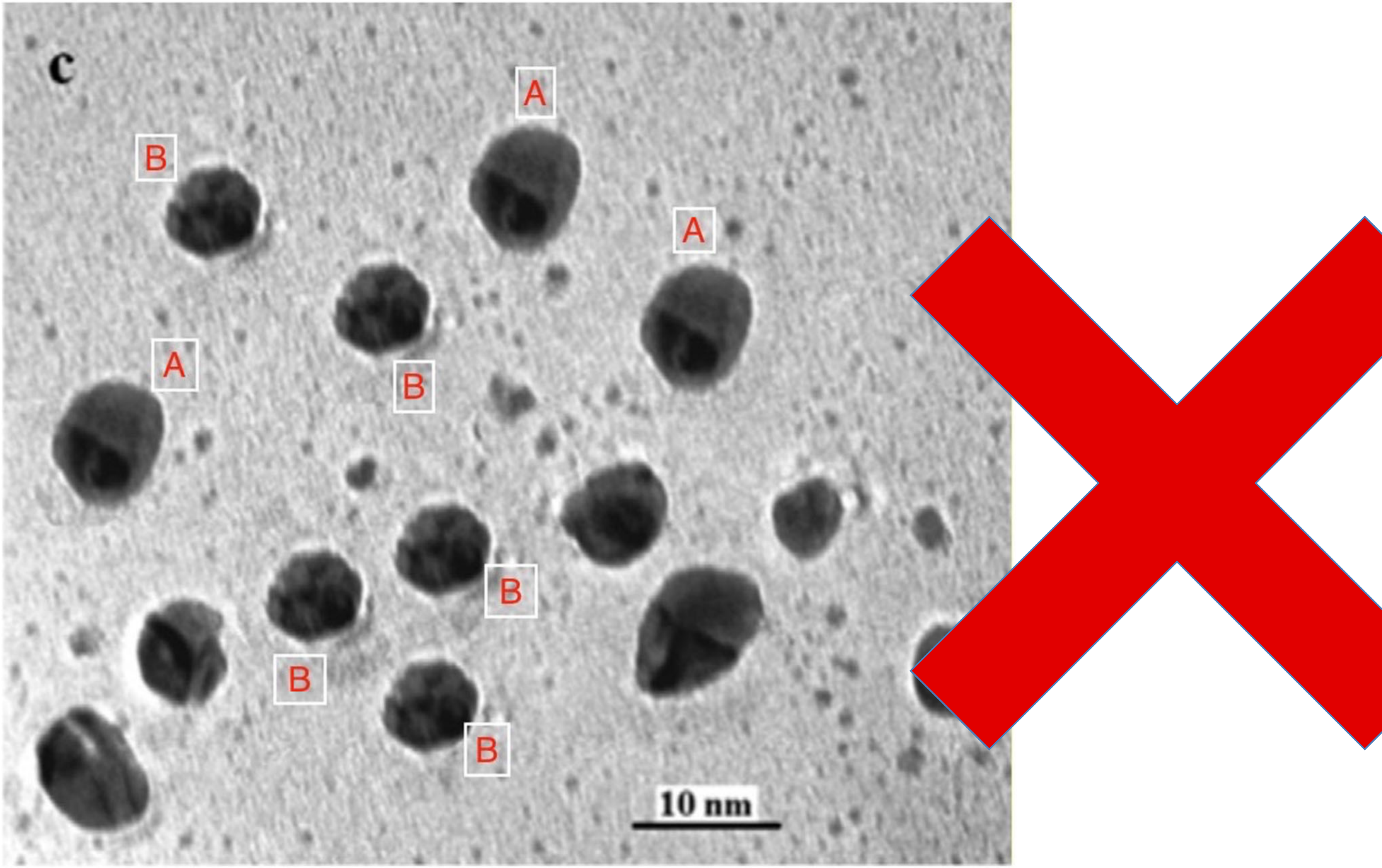
A very real concern for everyone involved in scientific imaging, is the question of what constitutes proper and appropriate processing,
and what constitutes unethical or even fraudulent manipulation

Anything that alters an image to create a false impression on the part of the viewer is wrong

1. Always store a permanent copy of the original image along with relevant data on its acquisition: OpenData, OpenScience !
2. Carefully document whatever steps are taken to process the image and generally to report those steps when the processed image is published !
Image processing has to be reproducible !



Examples of what image processing is NOT: 1.Particles



Published in Spectrochemica Acta

Oops…

"Your X-ray showed a broken rib, but we fixed it with Photoshop."

Image processing for Microscopy

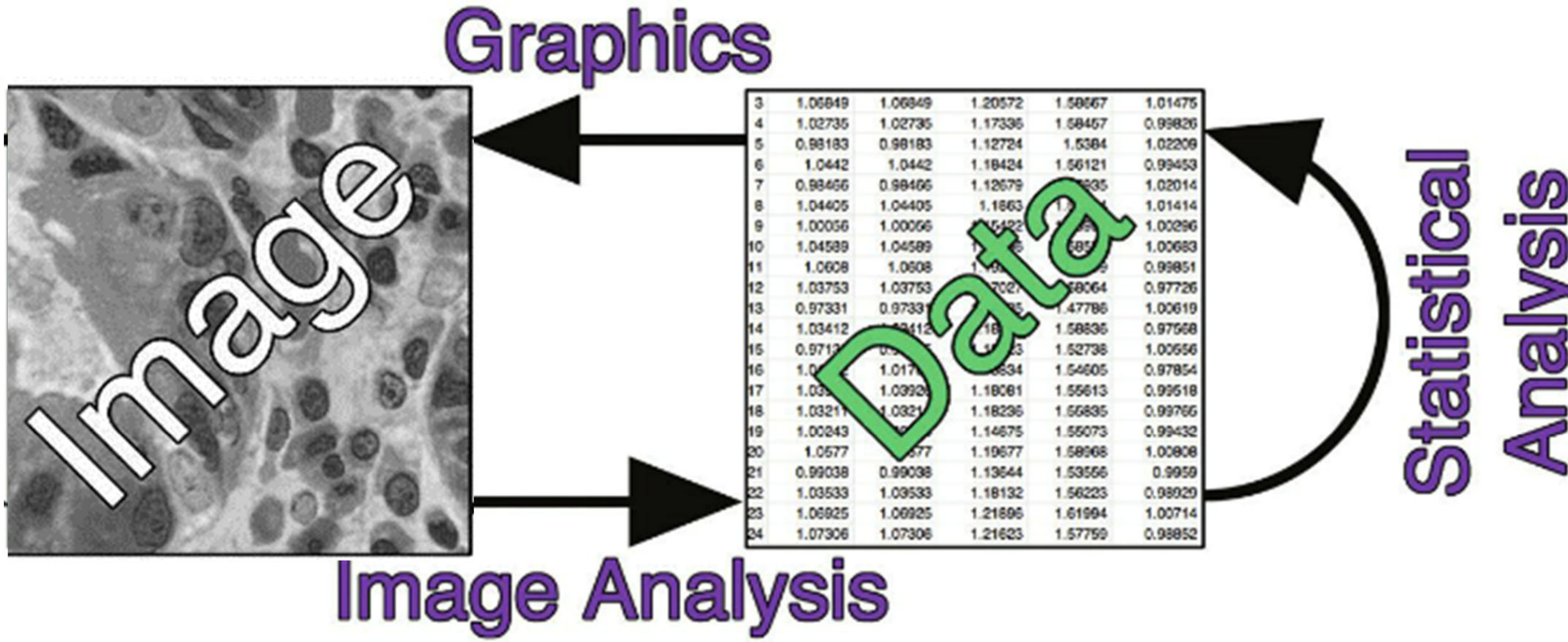
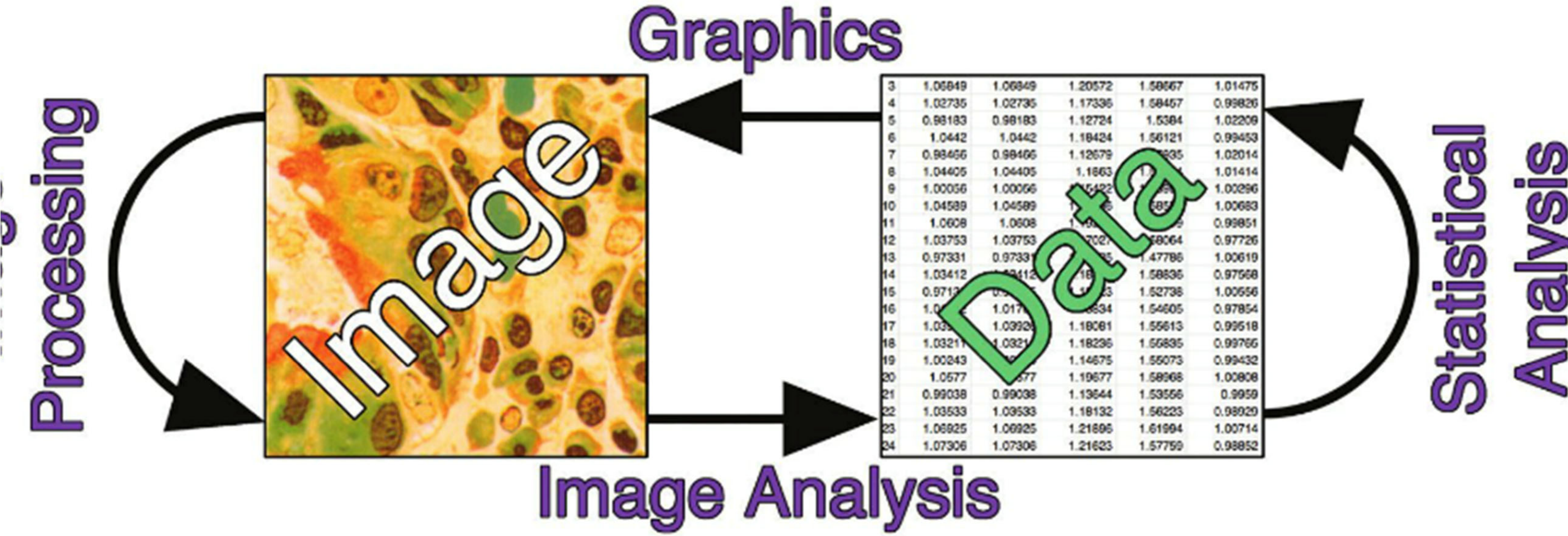
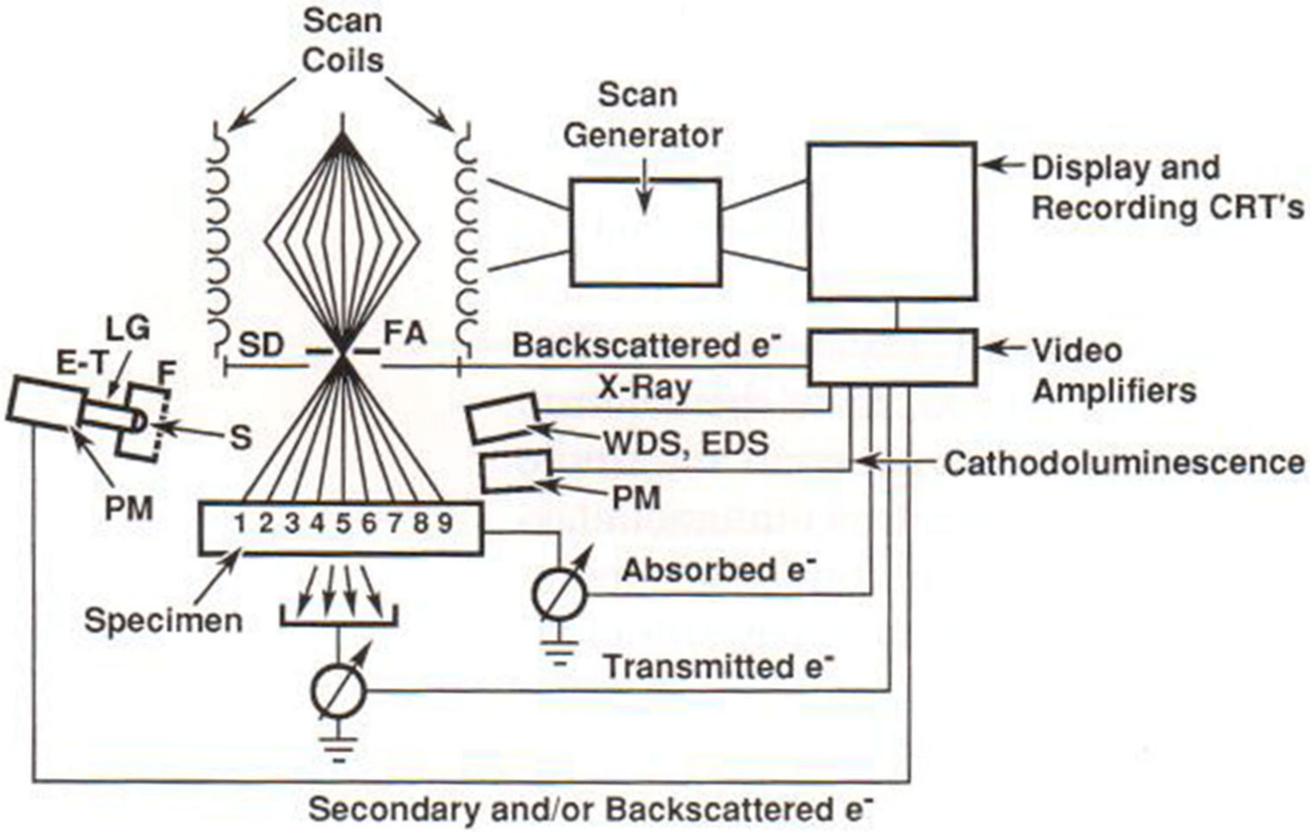


Image processing for Microscopy



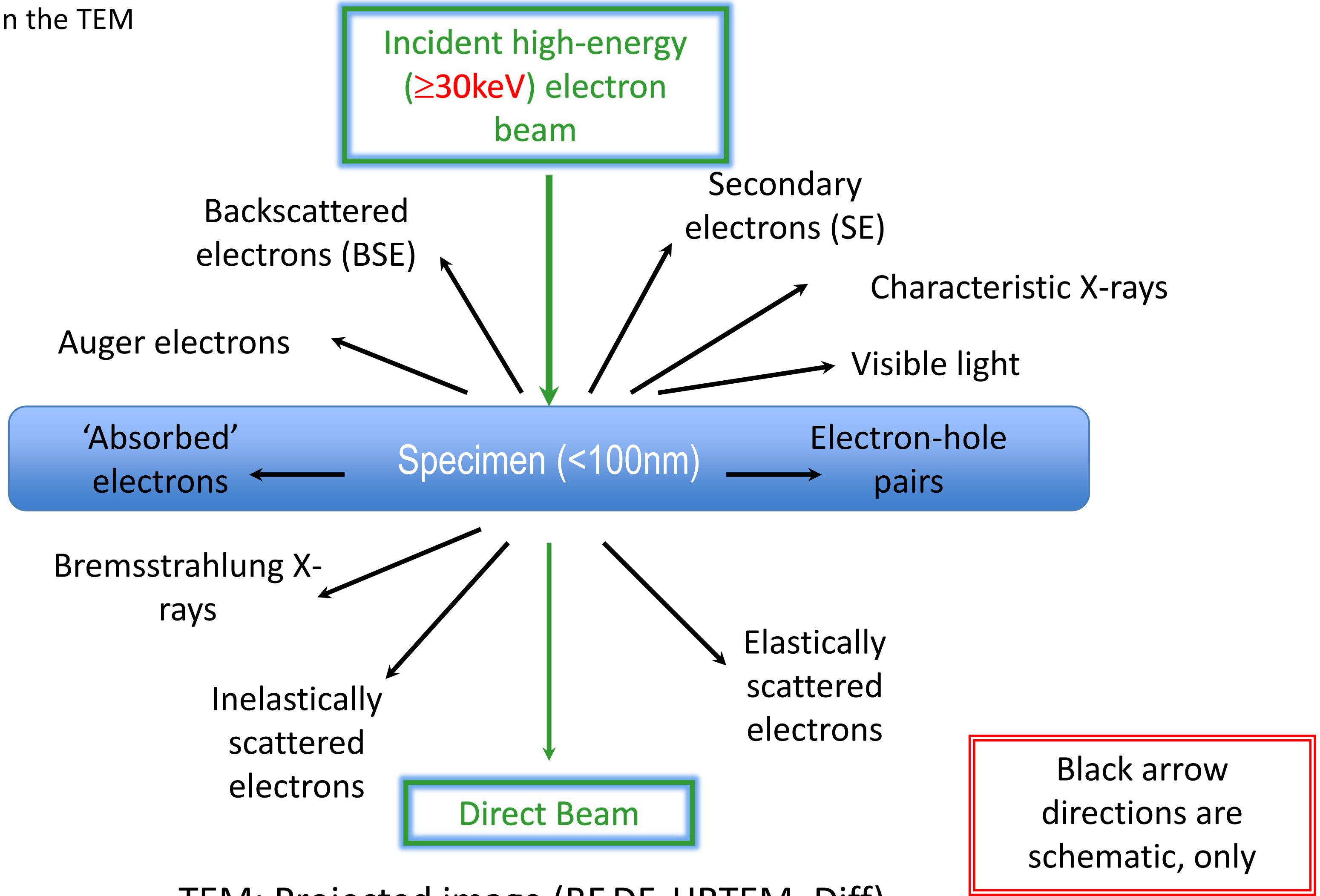
2. Acquiring Images:
SEM (Scanning Electron Microscopy)

Schematic illustration of the scanning system of the SEM with the available signals



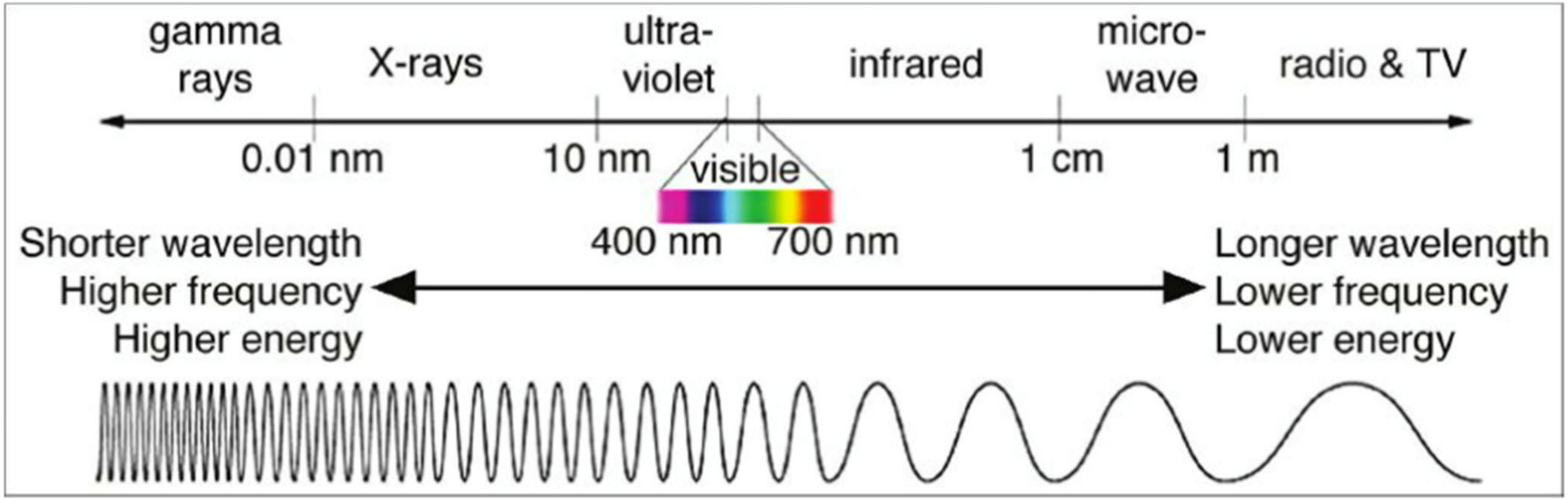
2. Acquiring Images:
TEM (Transmission Electron Microscopy)

Available signals in the TEM



TEM: Projected image (BF,DF, HRTEM, Diff)
STEM: Detector (BF, DF, HAADF)

Electrons are not visible to the human eye



Accelerating voltage kV	Velocity m/s	Uncorrected λ nm	Relativistic λ nm
1	1.873e+7	0.03879	0.03877
5	4.163e+7	0.01735	0.01730
10	5.846e+7	0.01227	0.01221
25	9.049e+7	0.00776	0.00766
50	1.237e+8	0.00549	0.00536
100	1.644e+8	0.00388	0.00370
200	2.085e+8	0.00274	0.00251
300	2.328e+8	0.00224	0.00197
400	2.482e+8	0.00194	0.00164
1,000	2.821e+8	0.00123	0.00087

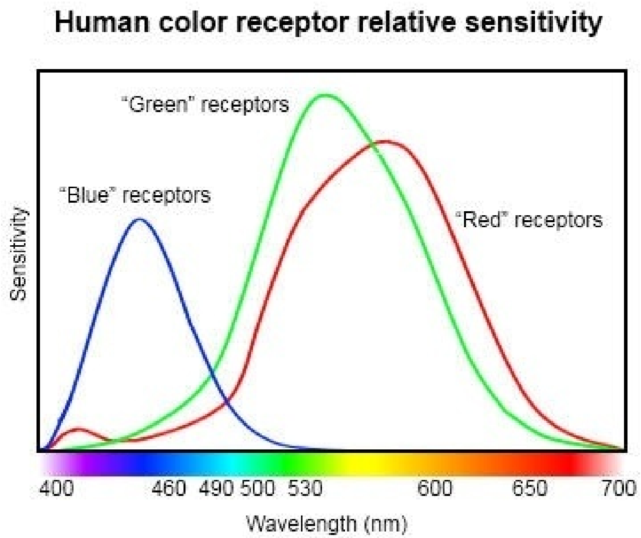


Image Processing in Microscopes

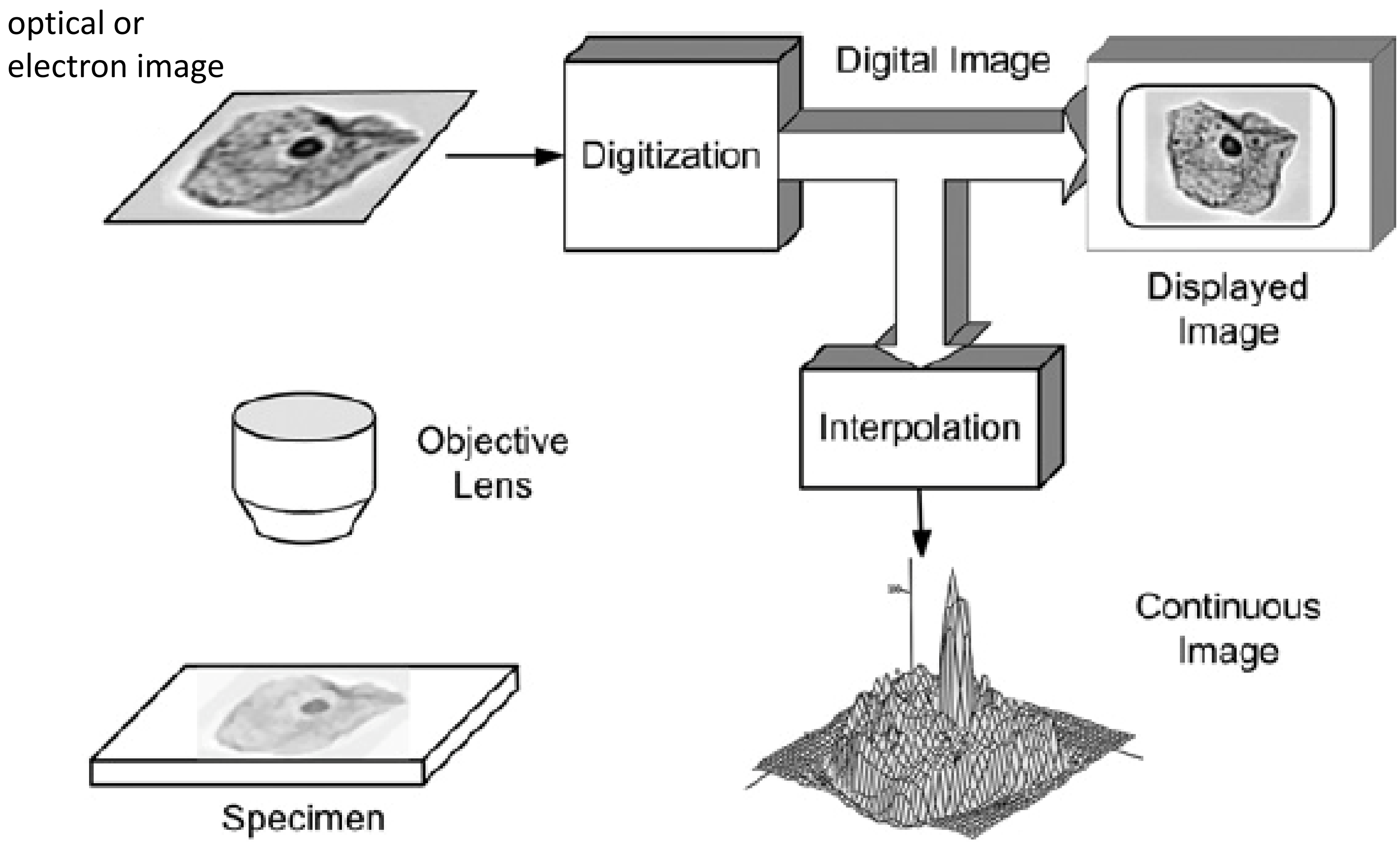
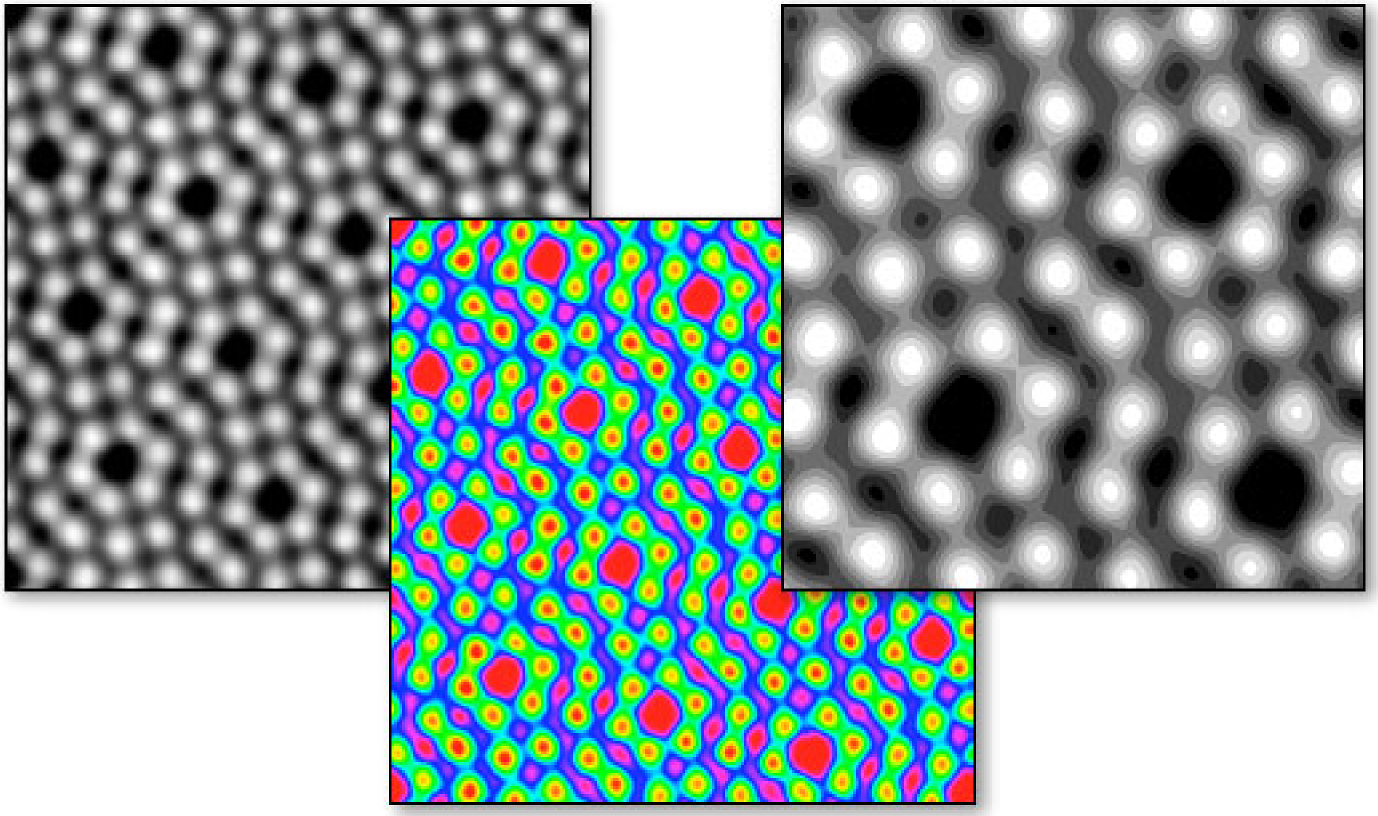


Image Display

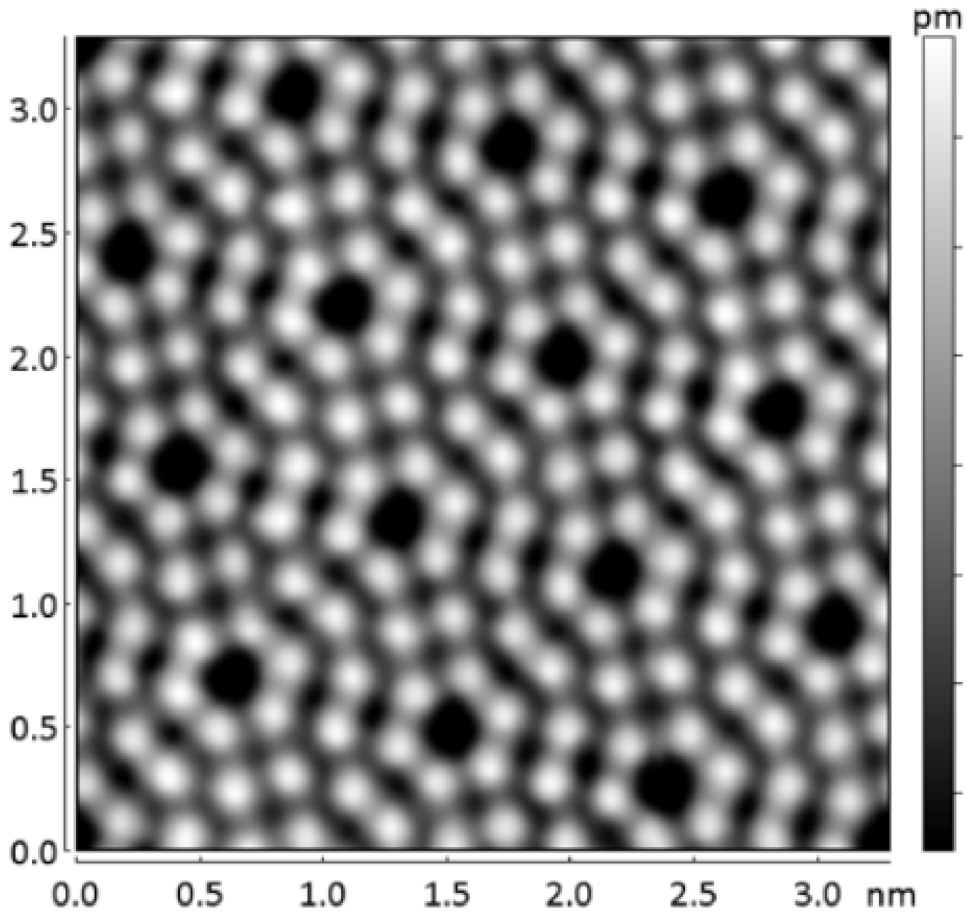
An image is a 2 dimensional collection of pixel values that can be displayed or printed by assigning a shade of grey or color to every pixel value.

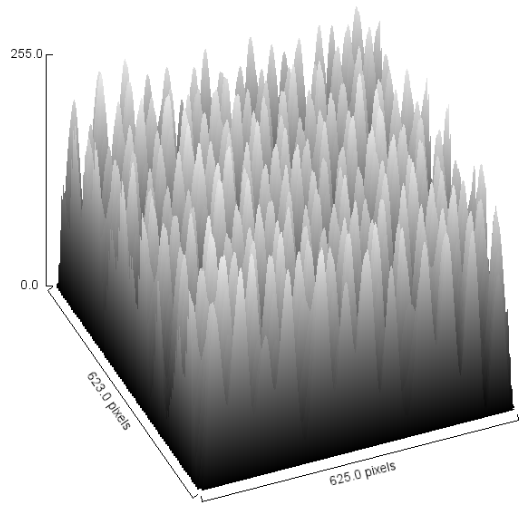


For meaningful results, the spatial calibration of the image must be known

Calibration

For meaningful results, the spatial calibration of the image must be known



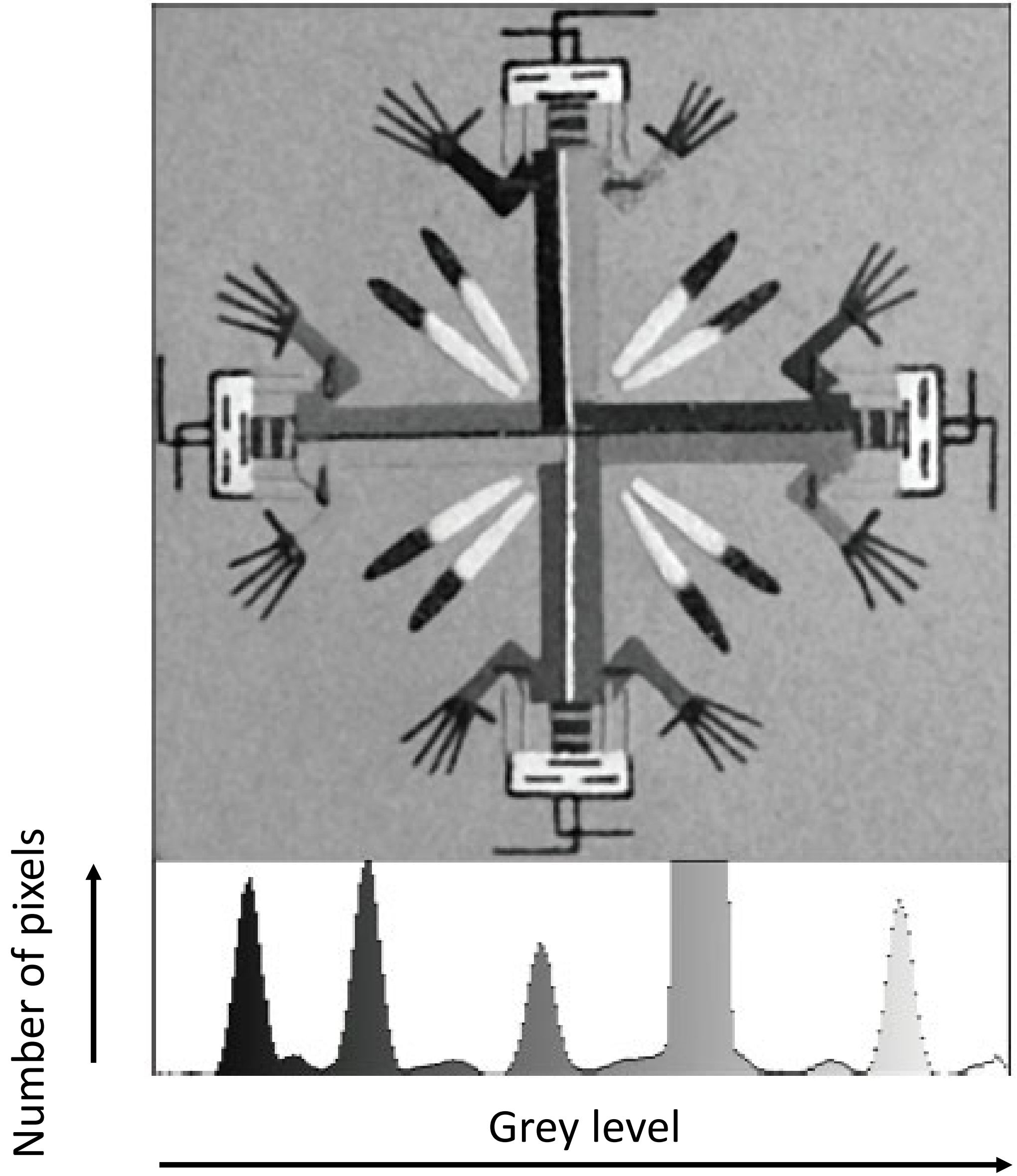


Horizontal scale: lateral dimensions
Vertical scale: height (surface)

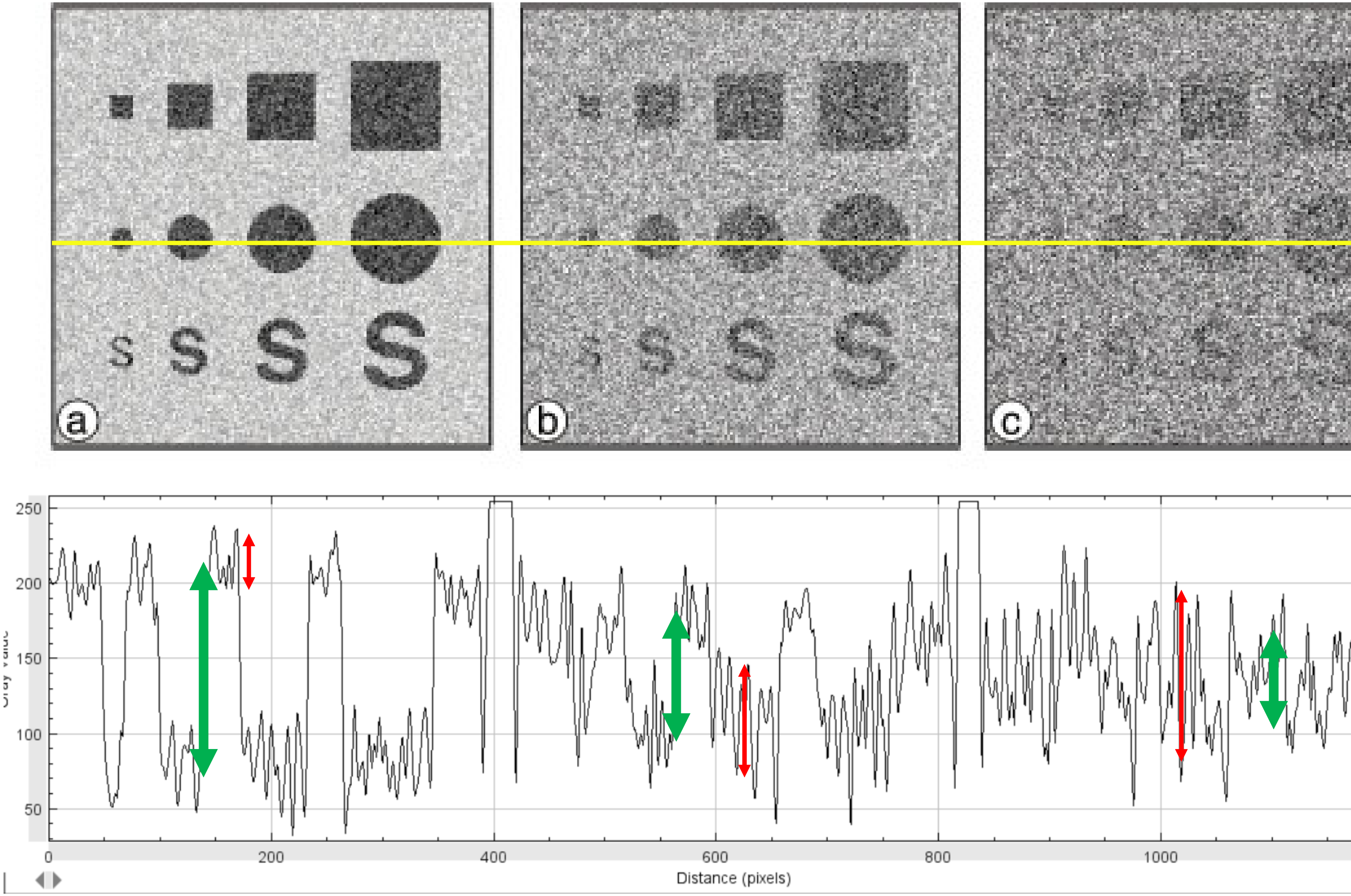
Example: Scanning Tunnelling Microscope

The information on the image: Histogram

monochrome (grayscale)
age and its **brightness histogram**,
hich plots the number of pixels with
ch brightness value. The counts in
ch peak correspond to the areas of
e regions of the image



Noise



Features on a noisy background:

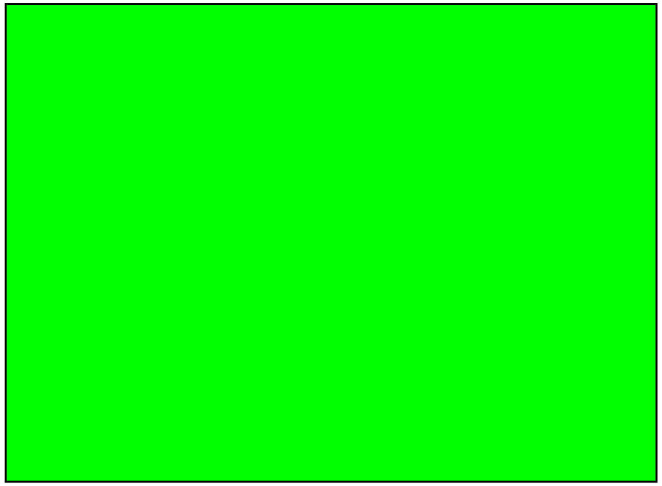
(a) High signal to noise (S/N)

(b) Medium signal to noise ratio

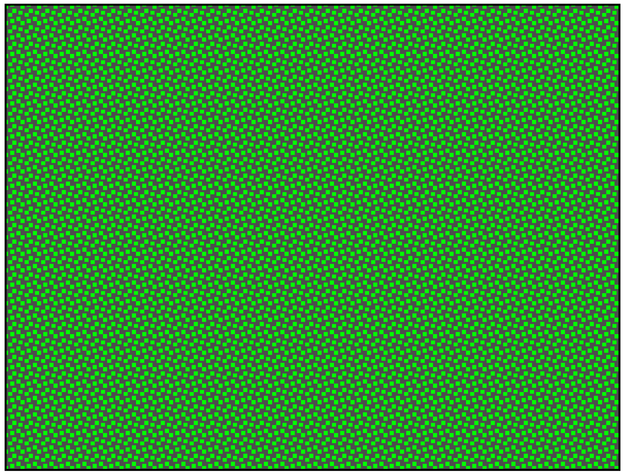
(c) Low signal to noise ratio

What is an image?

Low intensity $I(x,y) = I_o = 0$



High intensity $I(x,y) = I_o \neq 0$



$I(x,y) = f(x,y)$

In order to get **information**, need <u>variations</u> in intensity from one point to another, i.e.,

Definition of CONTRAST

$$C = [I_2(x_2,y_2) - I_1(x_1,y_1)] / I_1(x_1,y_1)$$

Contrast vs. Intensity (Brightness)

High

Low

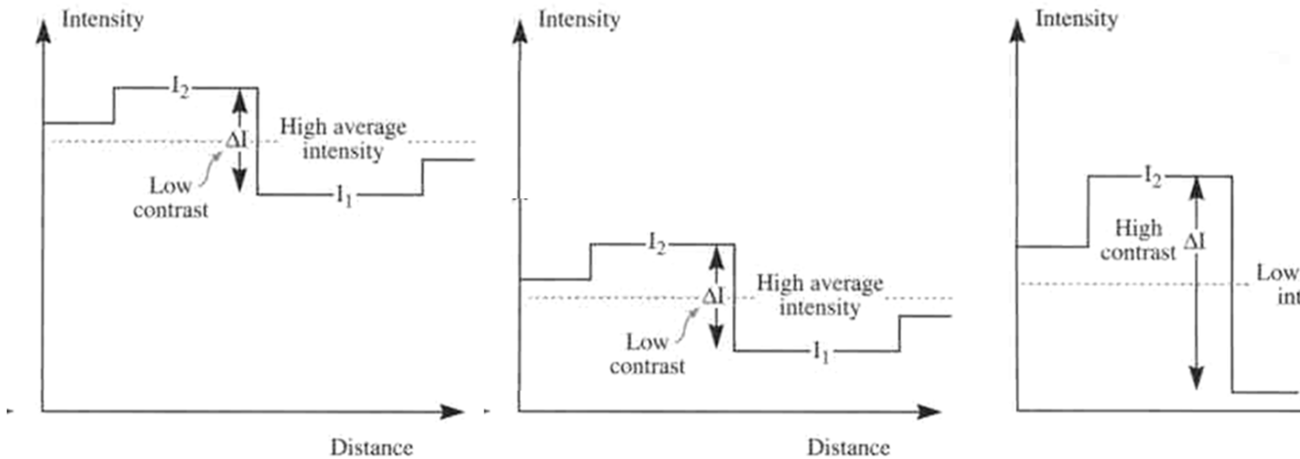
contrast

Intensity/Brightness

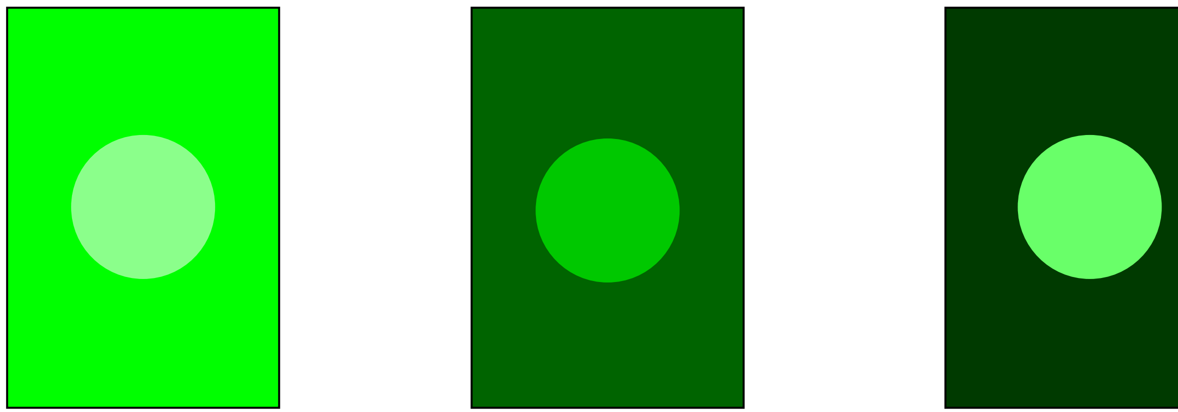
Low

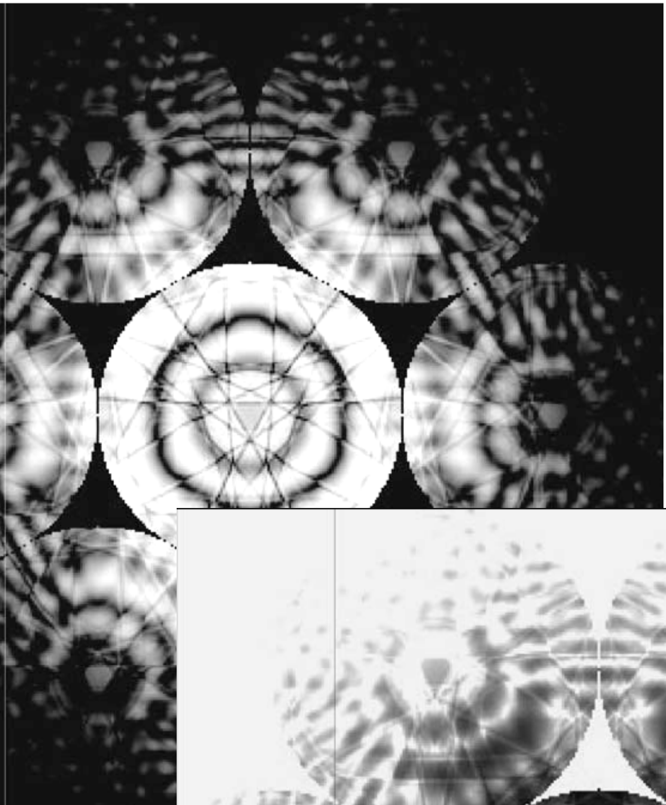
High

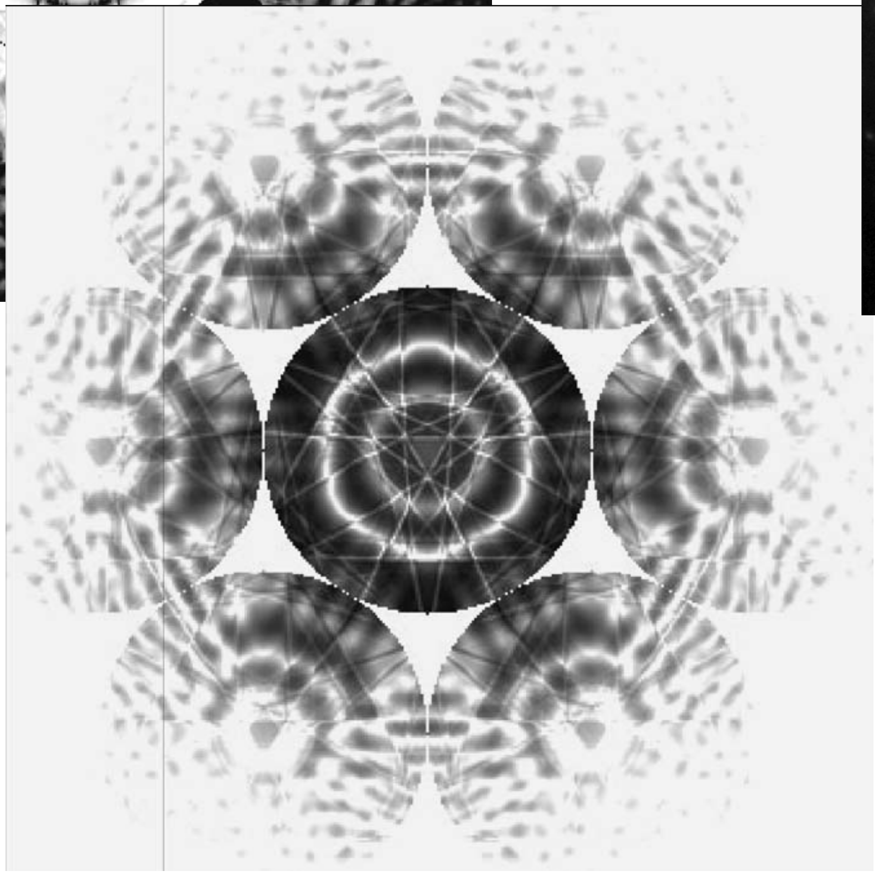
Contrast vs. Intensity (Brightness)



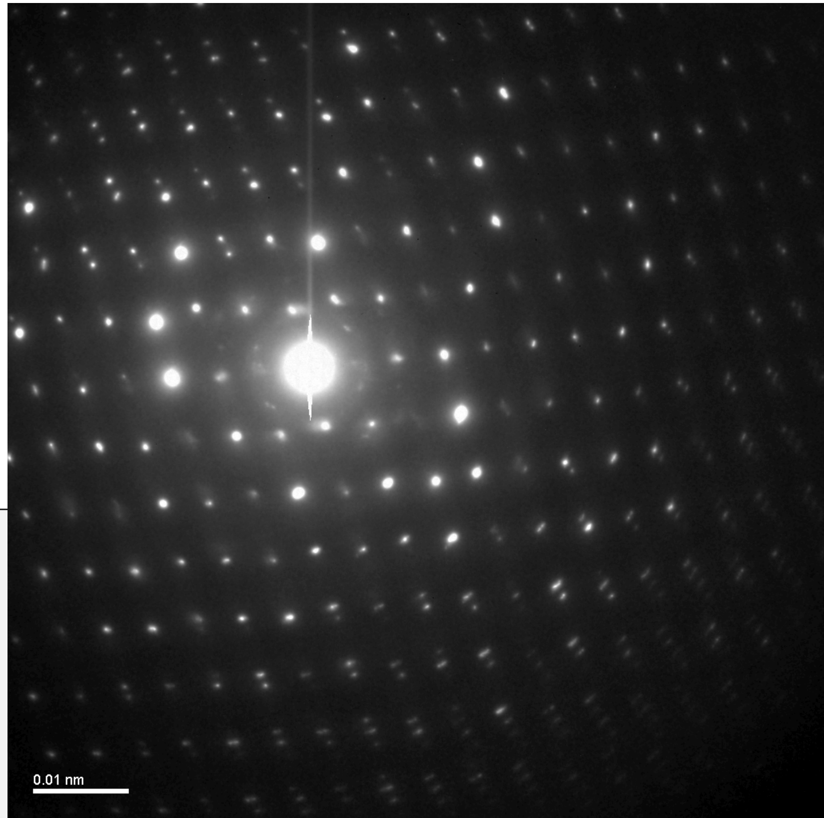
- ariations in Intensity with Position in the bject
- se Full Dynamic Range of Scale: Don't hrow Away Information!
- enerally Better off with Lower Overall ntensity
  - Its easier to see small changes in intensity on a low background signal
  - Leads to Higher Contrast
- an Boost Contrast Electronically







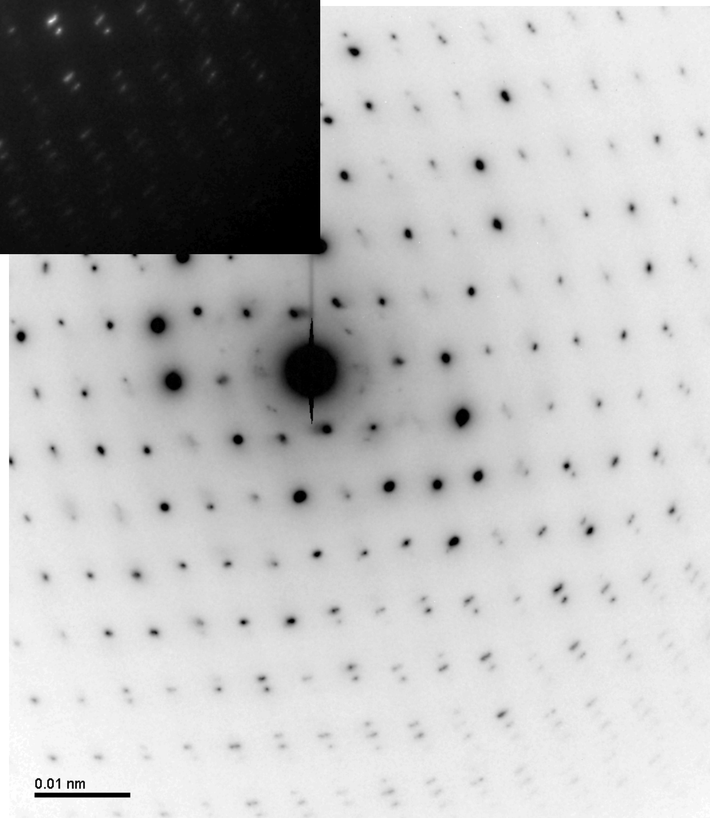
D



$C = [I_2(x_2,y_2) - I_1(x_1,y_1)]$ /

$C = \Delta I/I_0$

SAED



Contrast vs. Intensity (Brightness)

tment during live acquisition:
ne scan and observe intensity profile

ronically shifts the signal up/down
nsity, Brightness

:
ches the signal (contrast)

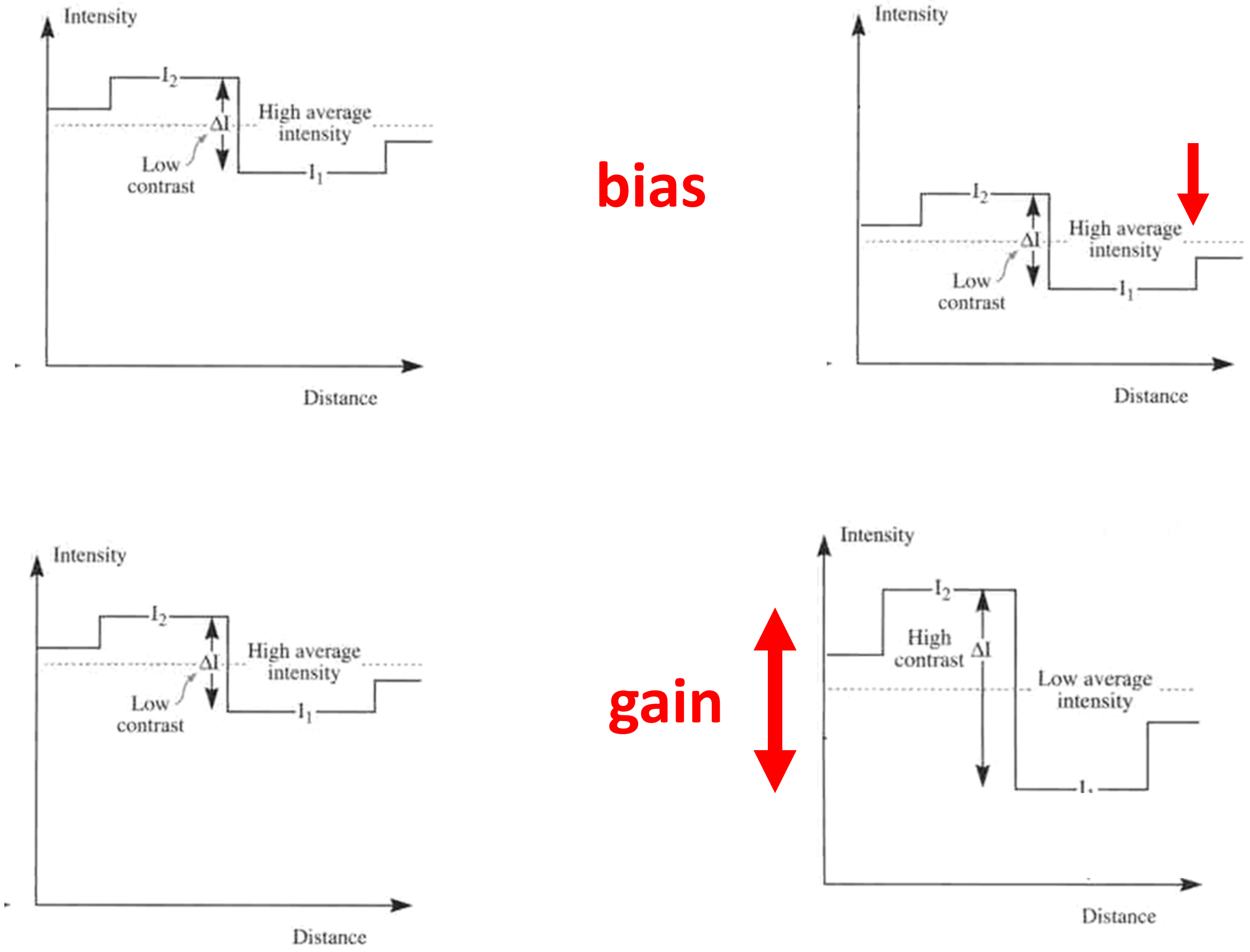


Image Formation: Line Scans

The information flow consists of the scan location in x-y space and a corresponding set of intensities from the detection device that monitor the beam-specimen interactions

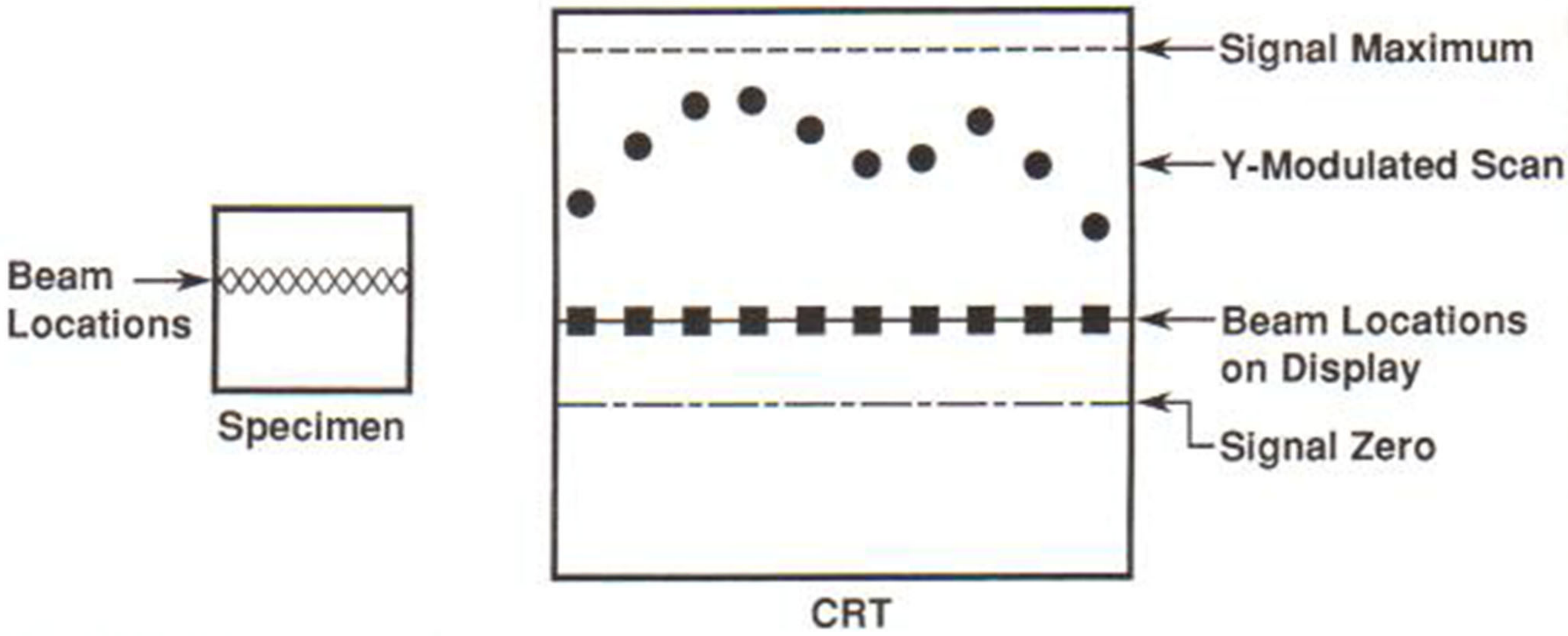


Image Formation: Area Scans

The information flow consists of the scan location in x-y space and a corresponding set of intensities from the detection device that monitor the beam-specimen interactions

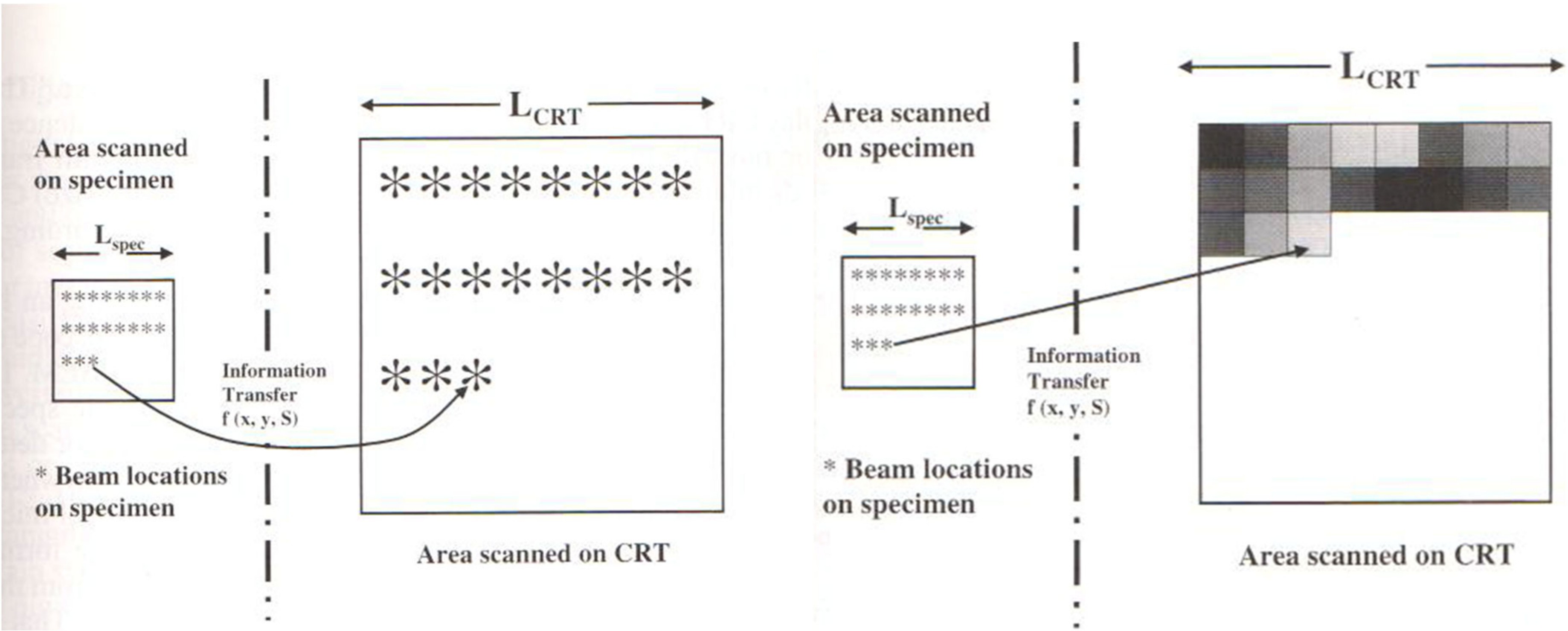
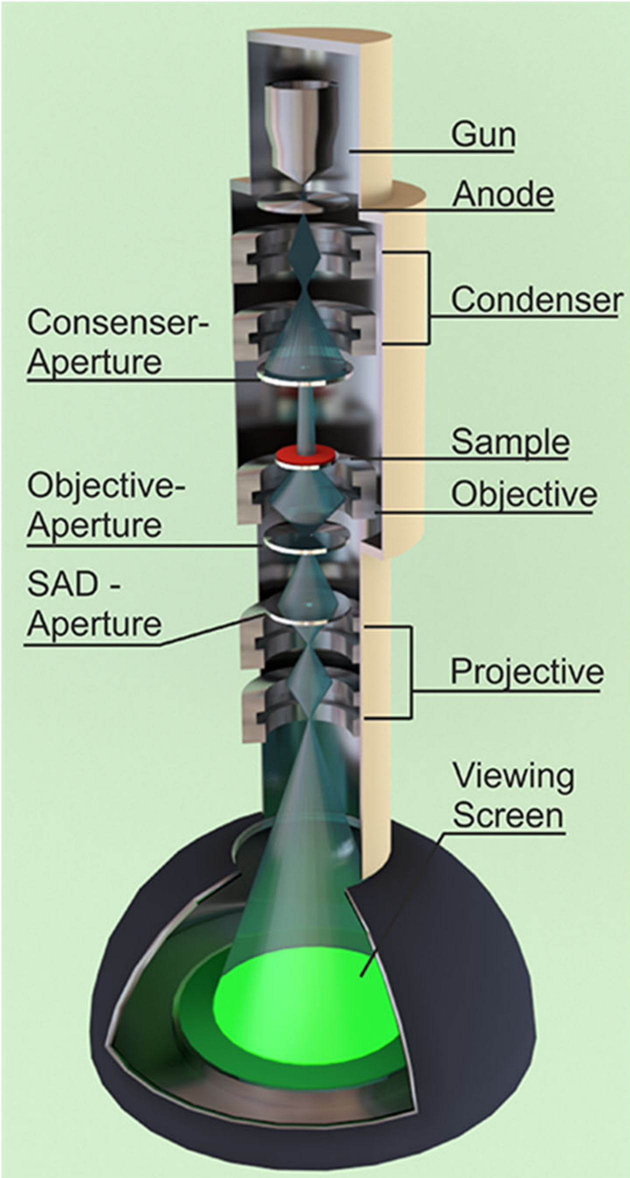


Image Formation: Parallel Illumination



All (x,y) values are acquired (recorded) at the same time (in parallel).

When

- Taking photographs
- Taking optical microscopy images
- Taking transmission electron microscopy images (shown is a TEM column with parallel illumination hitting a florescent screen)

Detectors vs. Cameras

pixel of a
digital
camera

Definitions (from Cambridge Dictionary)

Detector: a device used to find particular substances or things, or measure their level

Camera: a device for taking photographs or making films or television programs

Digital Camera: a type of camera that records images that can be look at on a computer

in earlier days, images were taken in photographic film
analogic recording

Basic difference

The signal from any electronic detector can be digitized and electronically manipulated prior to display, in a way that is impossible with analog images (images from film cameras or negatives)

Detector Properties: The Quantum Efficiency

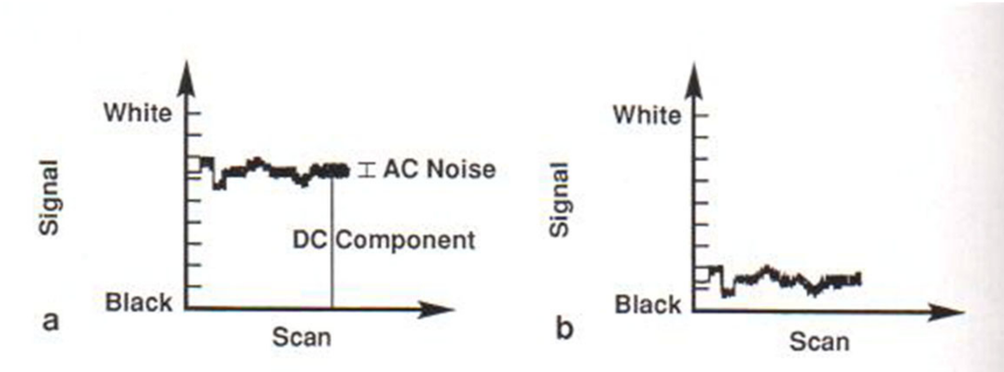
To compare the properties of detection and recording devices, we use the concept of **detection quantum efficiency (DQE). For linear response detectors:**

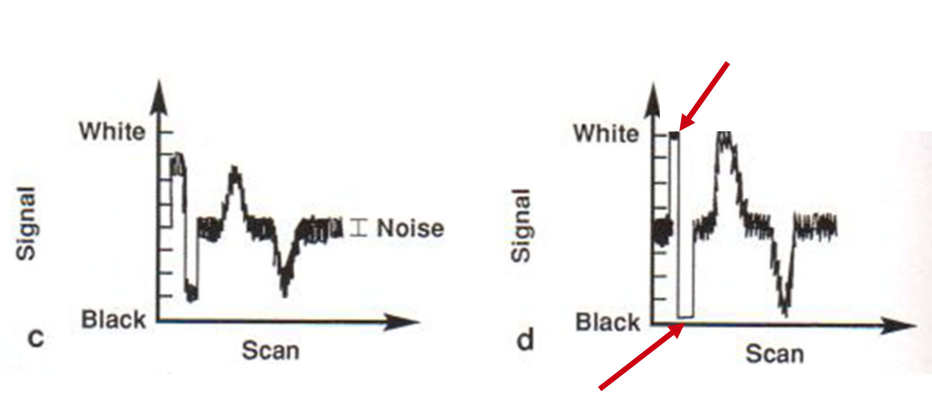
$$DQE = \frac{\left(\frac{S_{out}}{N_{out}}\right)^2}{\left(\frac{S_{in}}{N_{in}}\right)^2}$$

where S/N is the signal to noise ratio of the output or input signal

A perfect detector has a DQE of 1, but all practical detectors have a DQE<1

- any detector adds a certain amount of (electronic) noise





weak signal amplified
noise amplified

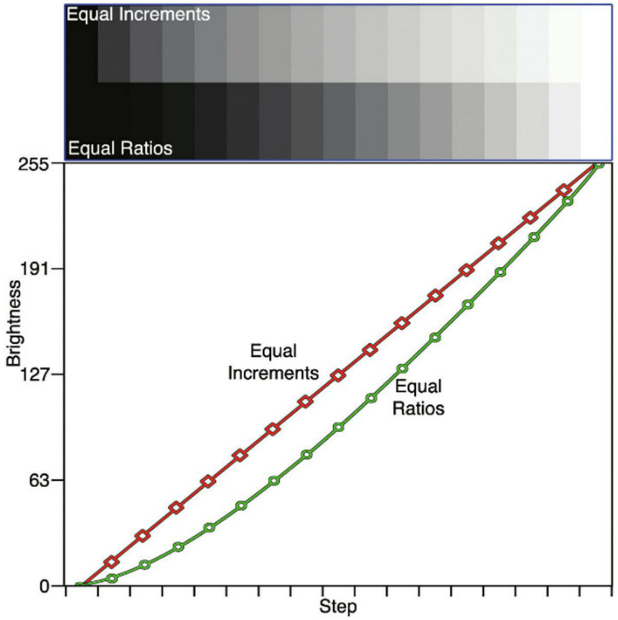
too much amplified
signal cropped (cut)

Detector Properties… More

Linear response & Dynamic range

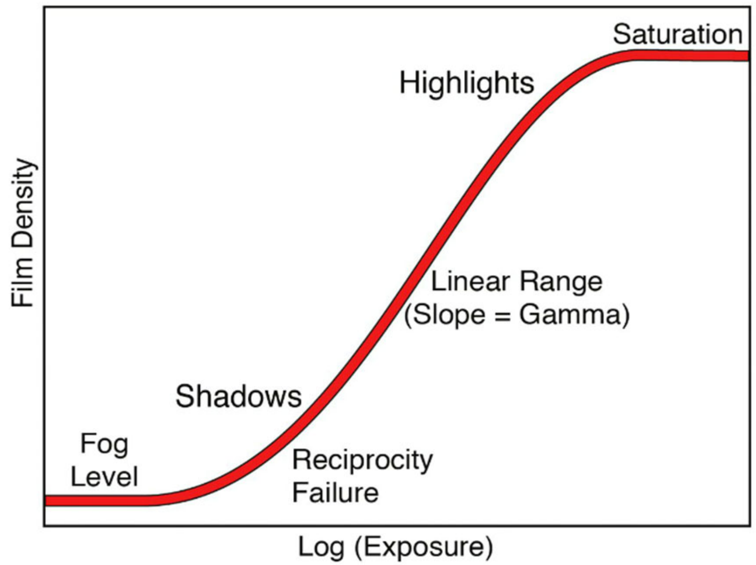
Response of different detectors

output



input

Response of photographic film

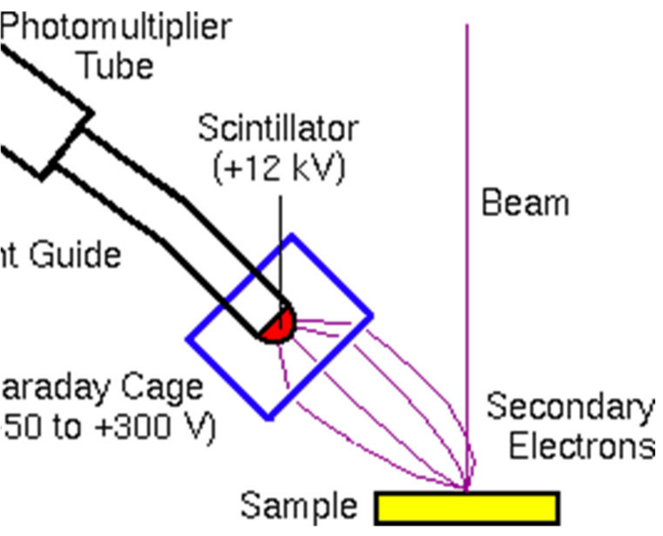


A detector generally meets the linearity condition for only a limited range of the input signal level. There are two effects that define the boundaries of this dynamic range

1. At very low levels of the input signal, the detector’s output is largely dominated by noise
2. At high levels of the input signal, the detector’s output signal no longer increases proportional to its input signal, and the detector therefore does not meet the linearity condition.

Linear vs Logarithmic Response

Secondary Electron Detectors in SEM



Everhart-Thornley Detector

Also Through The Lens (or In-Lens) Detector

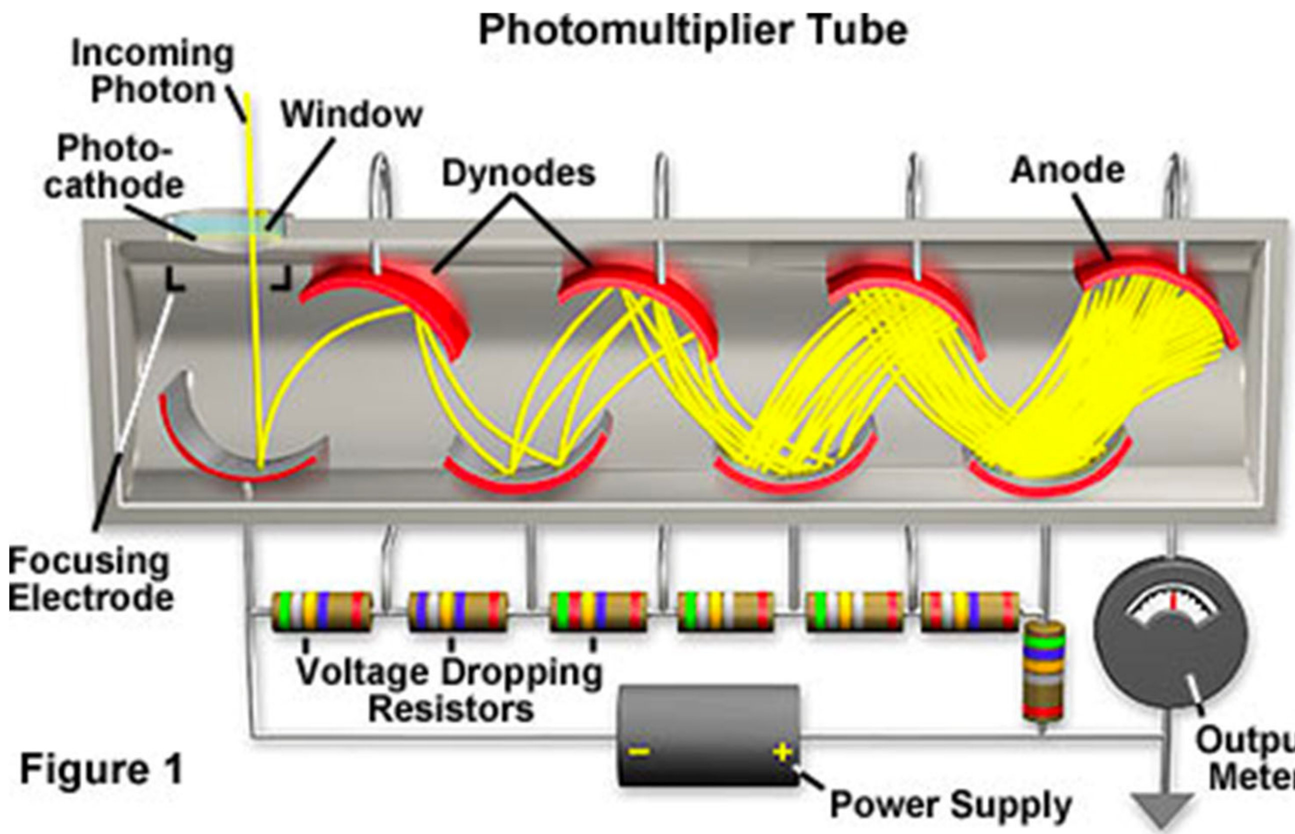
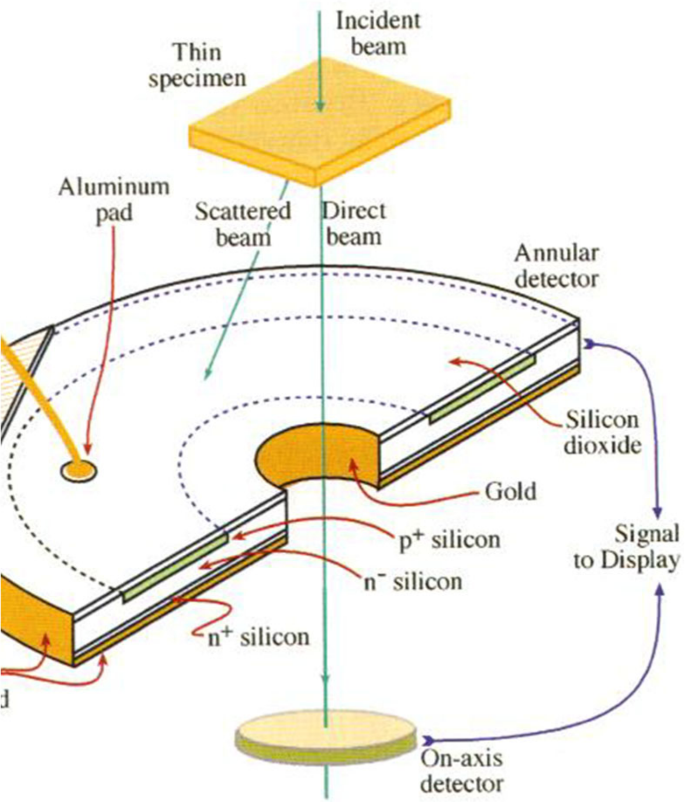
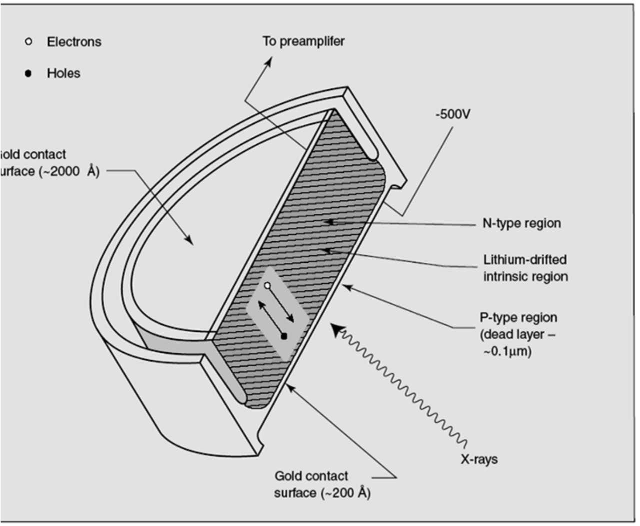


Figure 1

- Linear & fast response
- High gain
- **Poor quantum efficiency (DQE)**

Solid-State Detectors



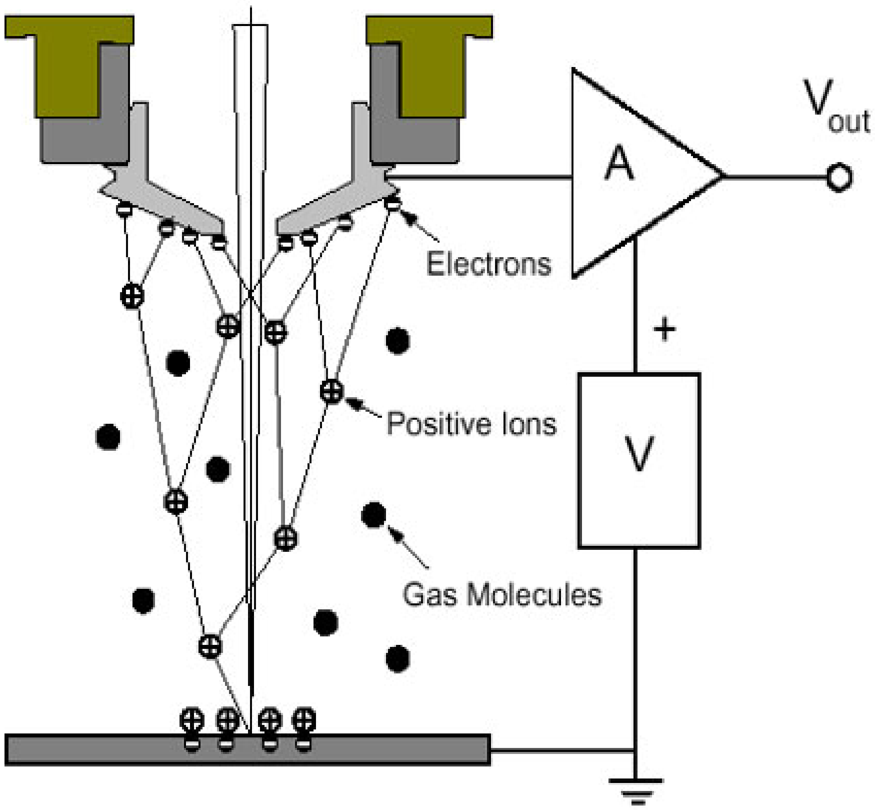


For:

- Backscattered Electron detectors
- Xray Detectors
- Scanning Transmission Electron Microscopy Detectors (high angle annular dark field (HAADF), bright field, etc.

- Electrons amplified by high voltage and impact ionization
- **High DQE**
- Cannot be run too fast, cannot record too many electrons at the same time

Secondary Electron Detectors in ESEM

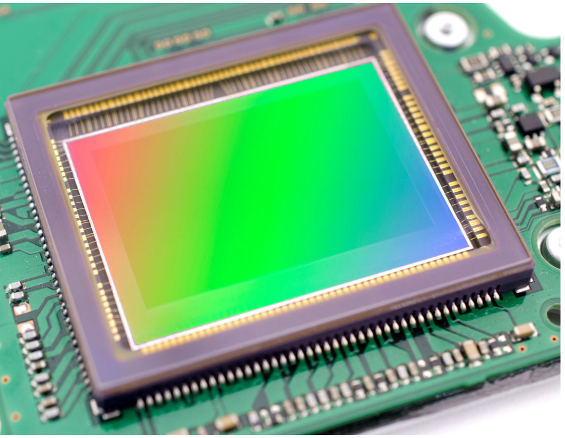


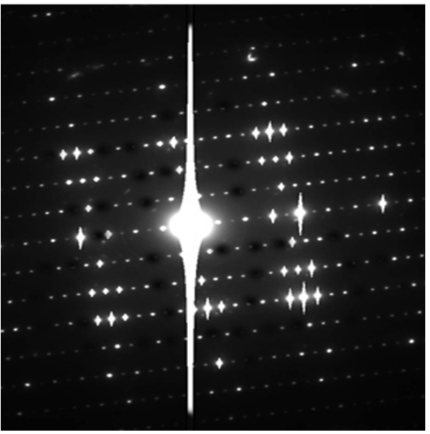
Amplification in native environment

- User has full control over the amplification conditions
- High noise
- **Low DQE**

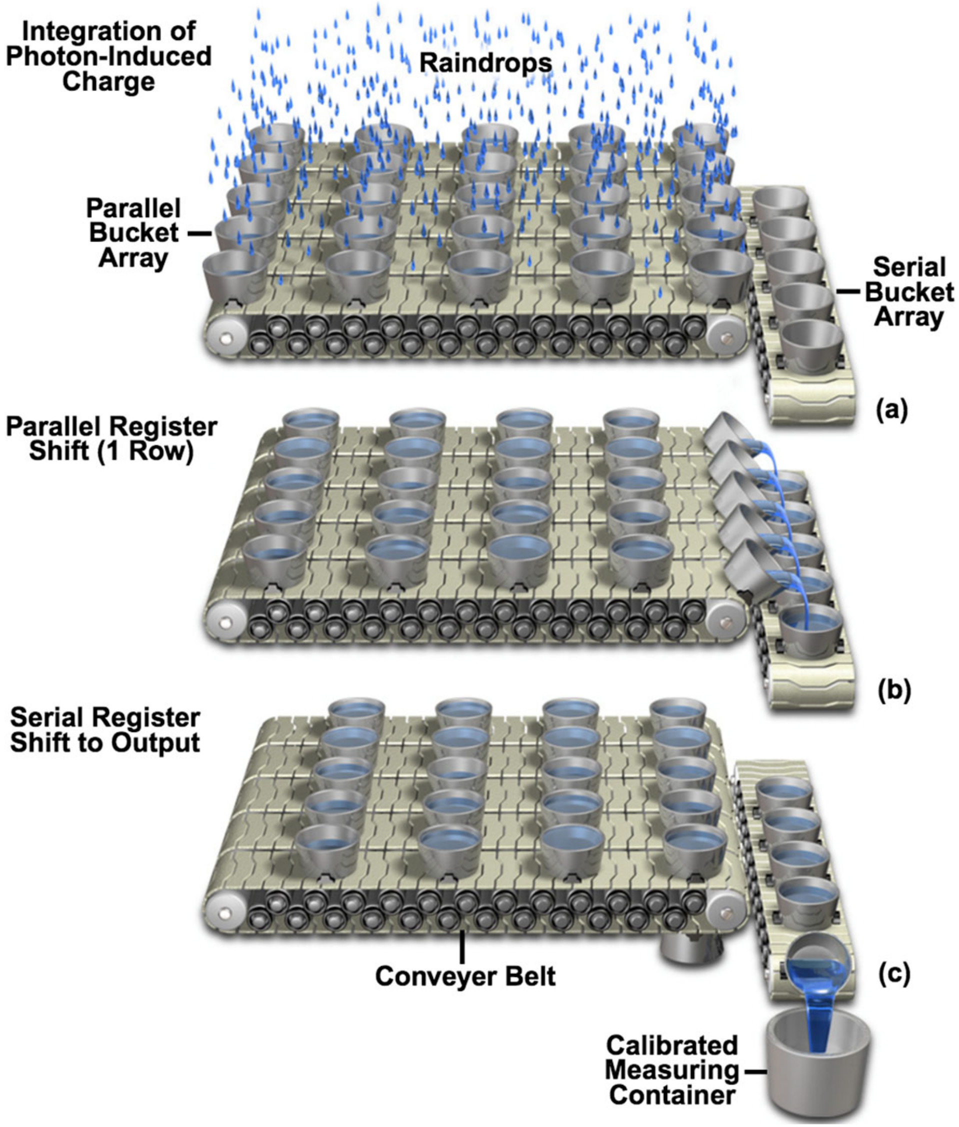
Cameras: Charged Couple Device (CCD)

They are metal-insulator-silicon devices that store charge generated by light or electron beams





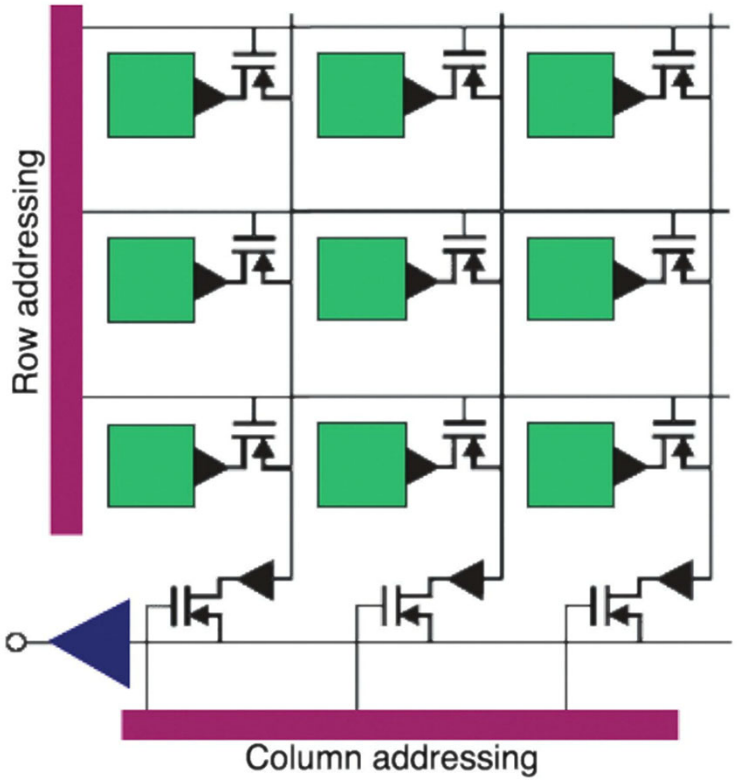
“blooming” artefact:
overspill of charges



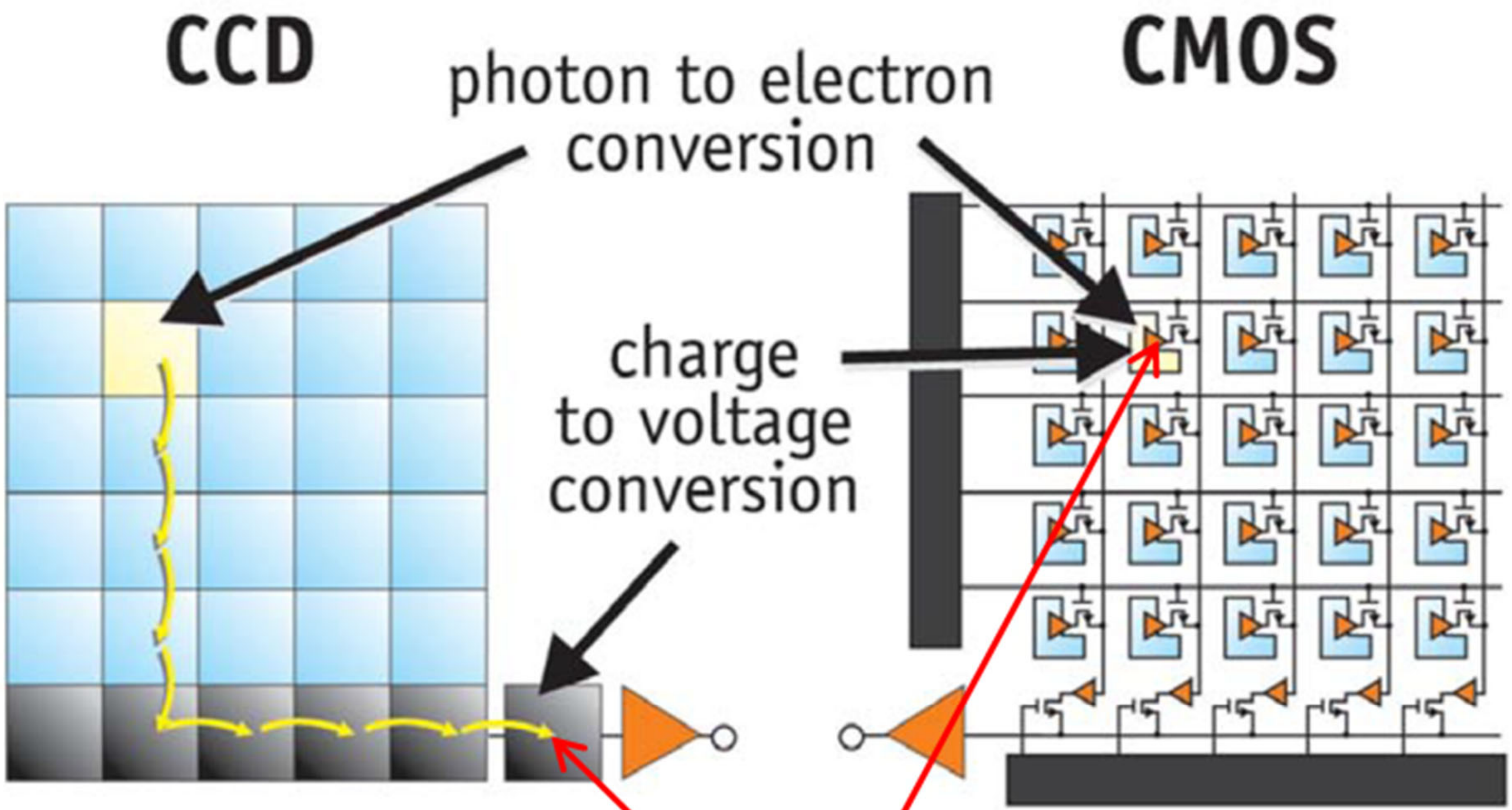
- **Single read-out amplifier**
- **Transfers charge**
- **Slow**
- **High signal-to-nois ratio**

Cameras: Complimentary Metal-Oxide Semiconductors (CMOS)

The conversion of light phonons to electrons functions the same way as in the CCD chip. The difference start with the way the signal is read out. Any pixel in the image that be read out directly, addressing a pixel by row and column as in a memory chip



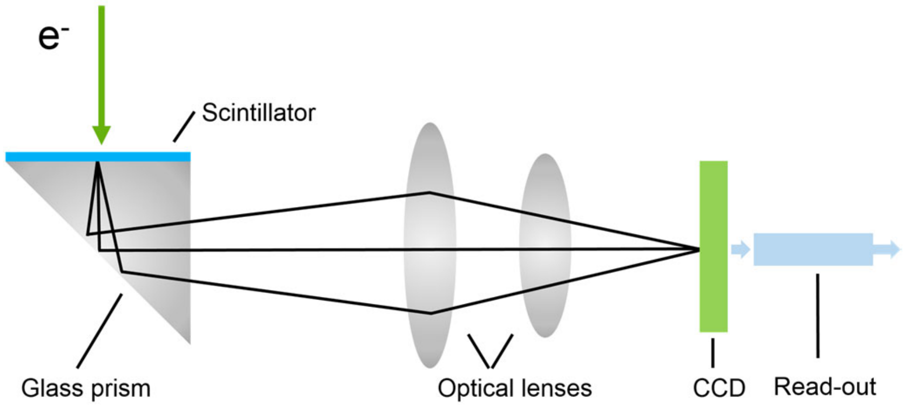
- **Each pixel has an amplifier**
- **Transfers voltage**
- **Fast**
- **Noisy**

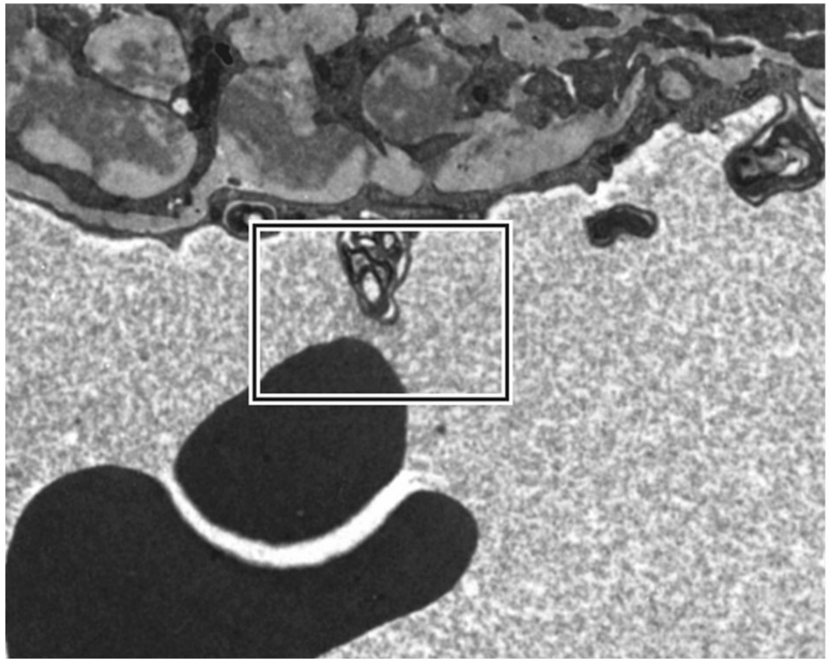


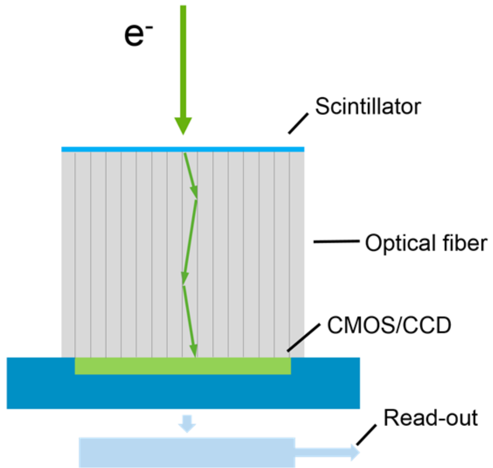
CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.

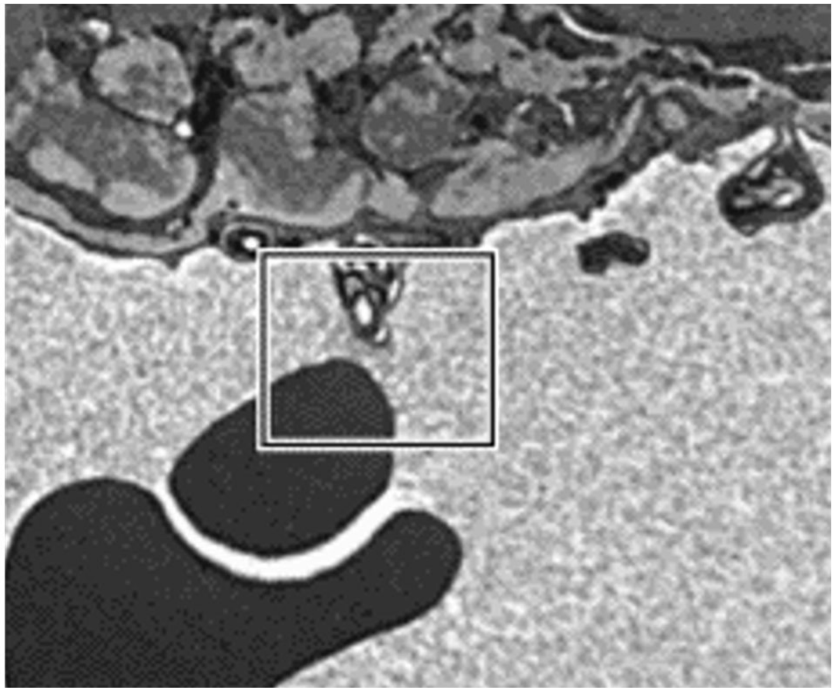
Read-out noise generated

Signal transfer in CCD/CMOS







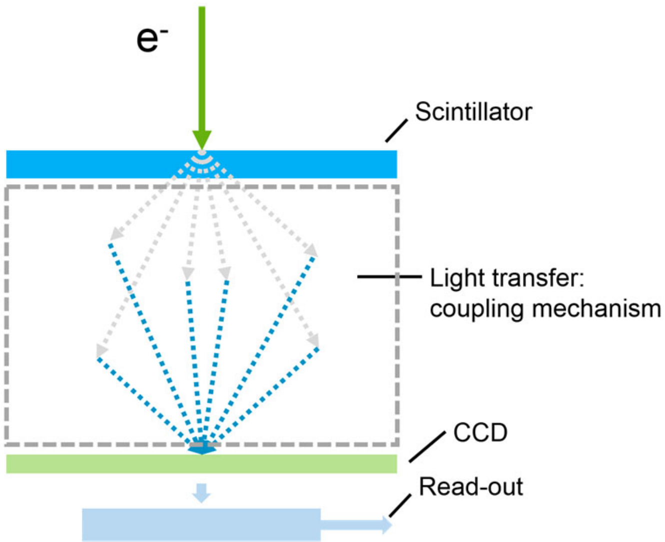


Conversion of electrons into visible light
Scintillator, phosphorous screens

Image detection in electron microscopy

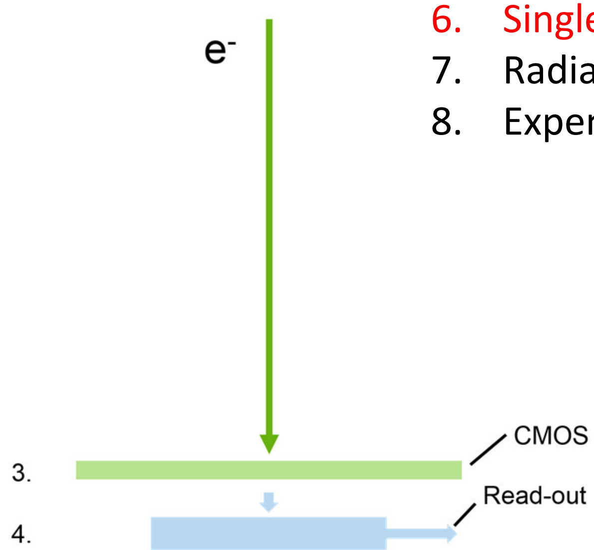
Conventionally

1. Convert electron to signal (light)
2. Transfer signal
3. Detect signal with sensor
4. Electronically transfer signal and read out to form signal



Direct Electron Detection

1. -
2. -
3. Detect signal (electron) with sensor
4. Electronically transfer signal and read out to form signal
5. No spreading of signal
6. Single electron detection
7. Radiation damage !
8. Expensive (400-1000 kFr.)



3. Considerations when Taking Images, resolution

Microscope Resolution is the minimum distance between two points or lines where they can be distinguished. Resolution is determined by the wavelength of the illuminating beam and the aperture of the collecting lens (numerical aperture.), aberrations in optics…

Computer image spatial resolution (Image Definition) is measured (usually) in dots per inch (dpi) or pixels per inch

Grayscale resolution (Dynamic Range) is a binary measure of the number of possible gray values. Most image processing and image capture programs express grayscale in 8-bit format or 2e8 (256) levels of gray. For compariso our eyes can distinguish only about 32 levels of gray.

Color resolution on a computer monitor is usually a binary measure of 256 (2^8) possible values of each of R, G, and B. This results is a total of over 16 million colors (256x256x256), and is referred to as 24-bit color.

Printing resolution is determined by the halftone screen frequency measured in lines per inch (lpi). This is a reflection of the size of the small, solid color dots in any printed image. A typical halftone screen frequency for newspapers is 65-85 lpi. Magazines print at 133 lpi, 600 dpi laser printers at 85 lpi, and high-quality publications pr at 150-175 lpi.

Capturing Images: Image Digitalization

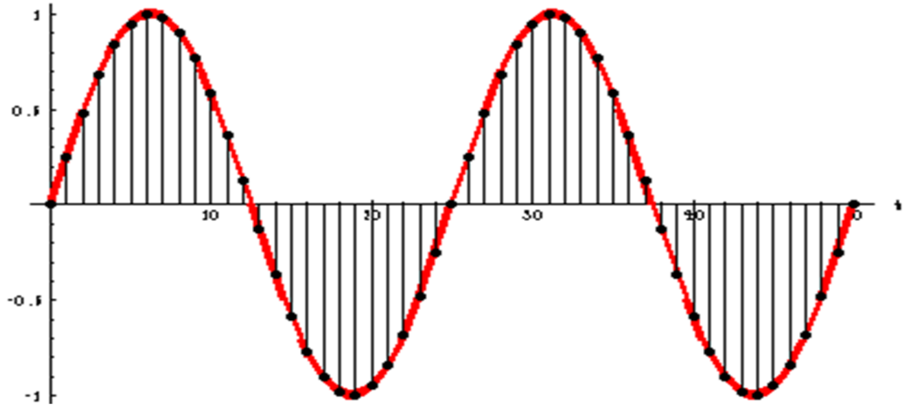
In order to get images into a form, computers can **manipulate** they must be modified from their analog form (continuous gradients) into a digital form (step gradients) using the technique of **sampling.**

Sampling: A technique used to record analog information by recording periodic snapshots. If the sampling rate is fast enough, the eye cannot discern the gaps between each snapshot when they are played back. This is the principle behind motion pictures. Sampling is the key technique used to digitize analog information such as sound, photographs, and images.

Nyquist sampling (f) = d/2, where d=the smallest object, or highest frequency

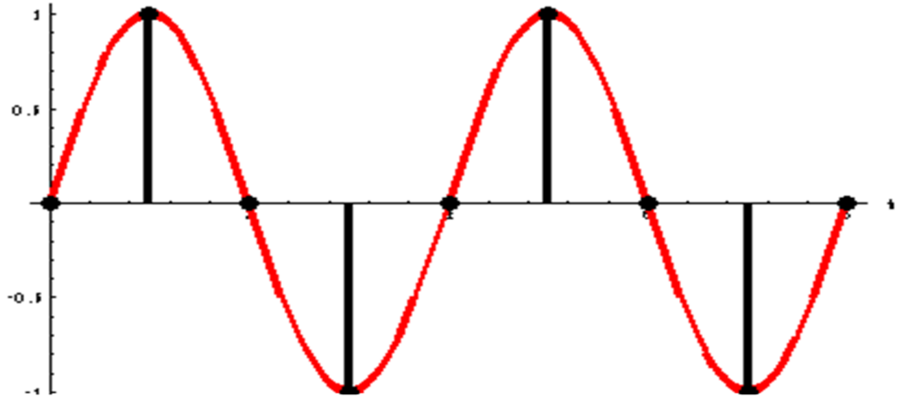
The Nyquist Theorem states that in order to adequately reproduce a signal it should be periodically sampled at a rate of at least 2x the highest frequency you wish to record. With images, frequency is related to structure size. Small structures are said to have a high frequency. Thus, the imaging sample rate (or pixel) size should be 1/2 the size of the smallest object you wish to record.

Sampling Examples



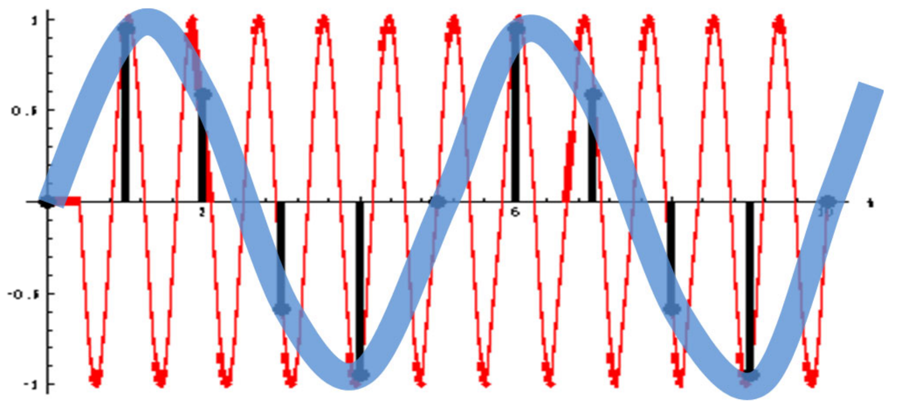
Oversampling

A High Sampling Rate = much greater than 2X the highest frequency. This is 'Oversampling' that, while not "bad" will take time and create a large digital file



Nyquist Sampling Rate

The minimum sample rate that captures the "essence" of the analog information



Undersampled

Low sampling rate produces results that report false information about the analog data; which does not represent the original. This phenomenon is called aliasing

Red: real curve Blue: sampled curve shape

Sampling (pixel size <-> object size)

Chess board pattern 5x5 pixels

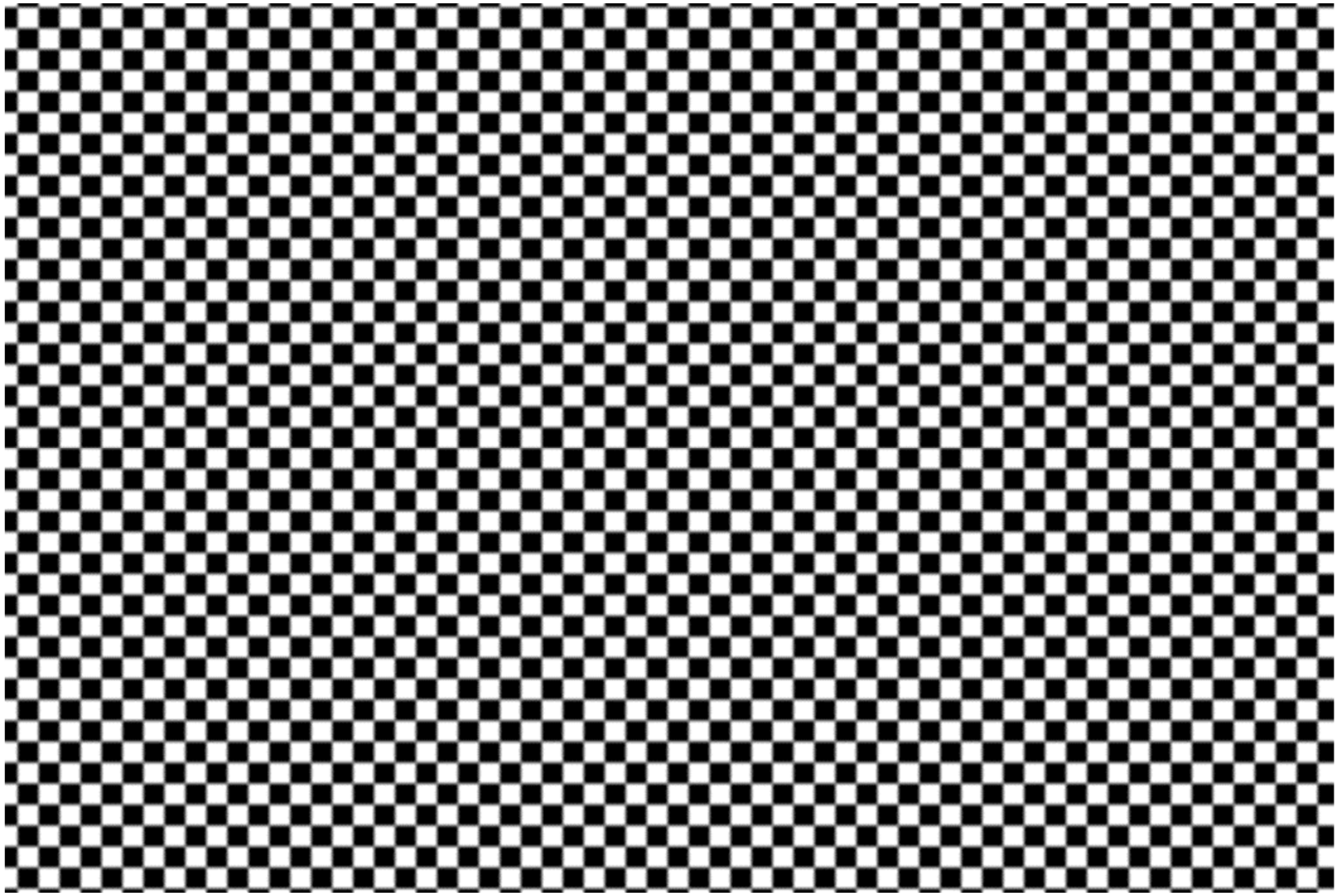


Image pixels size: 5 pixel / square

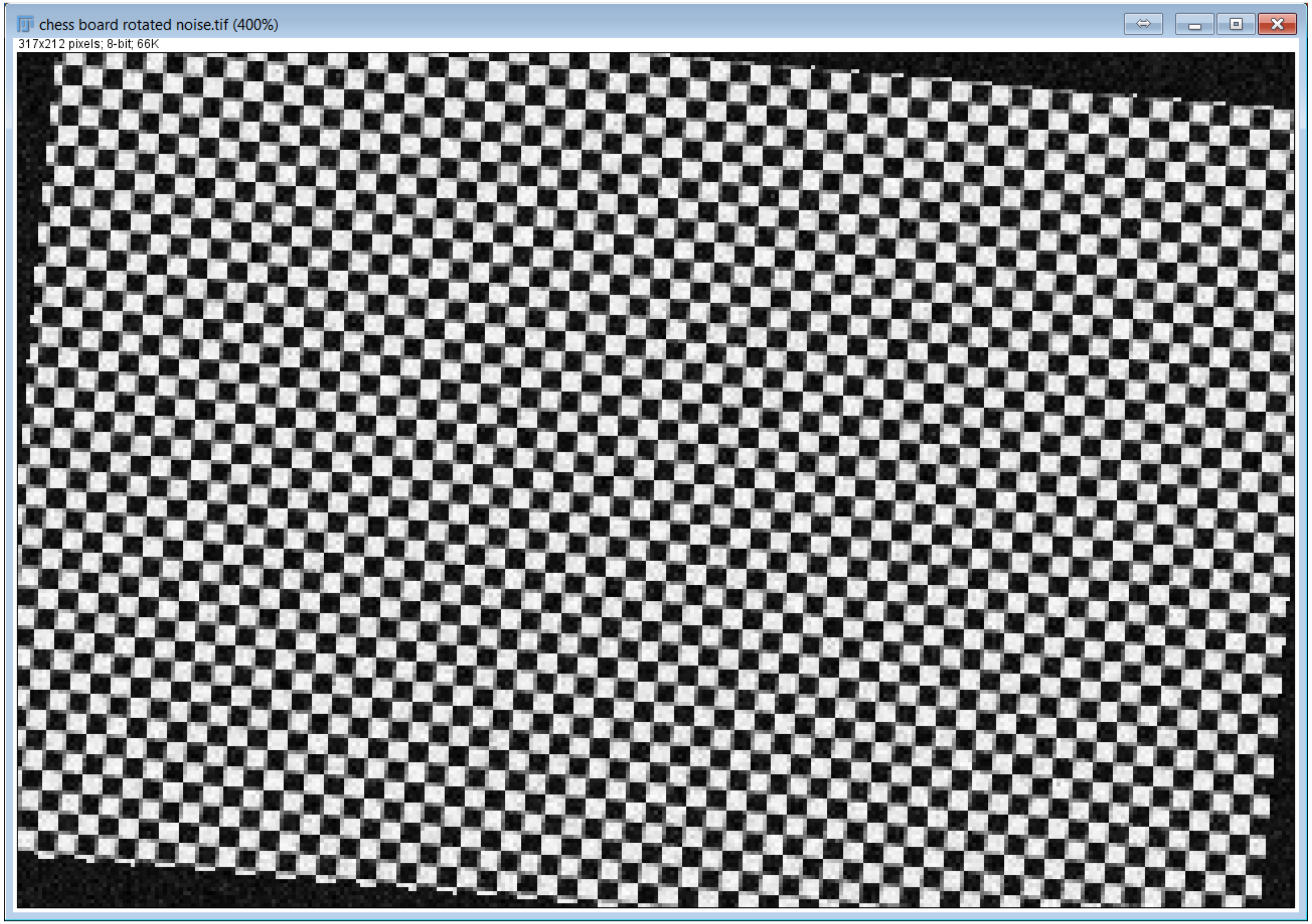


Image pixels size: 5 pixel / square

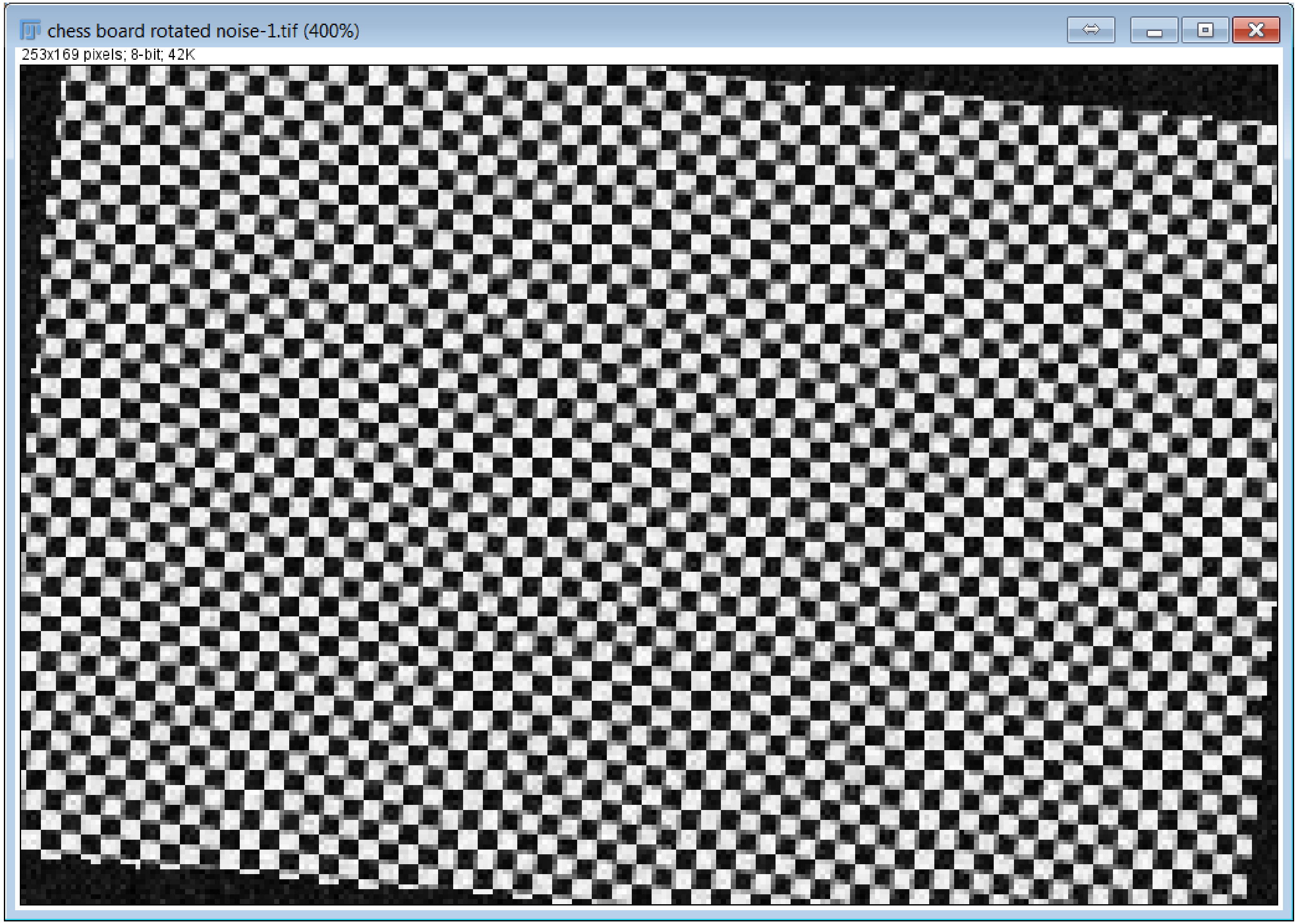


Image pixels size: 4 pixel / square

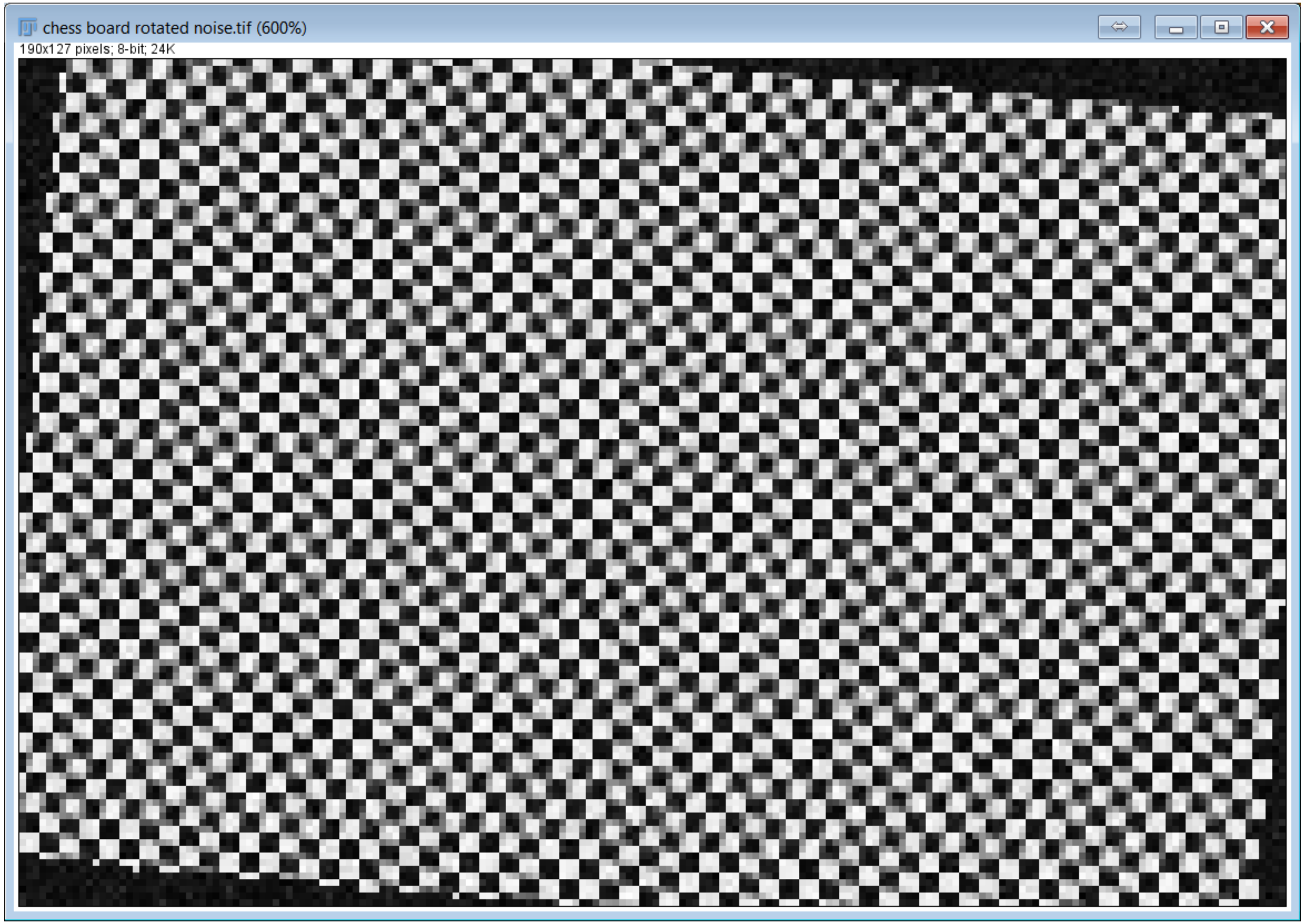


Image pixels size: 3 pixel / square

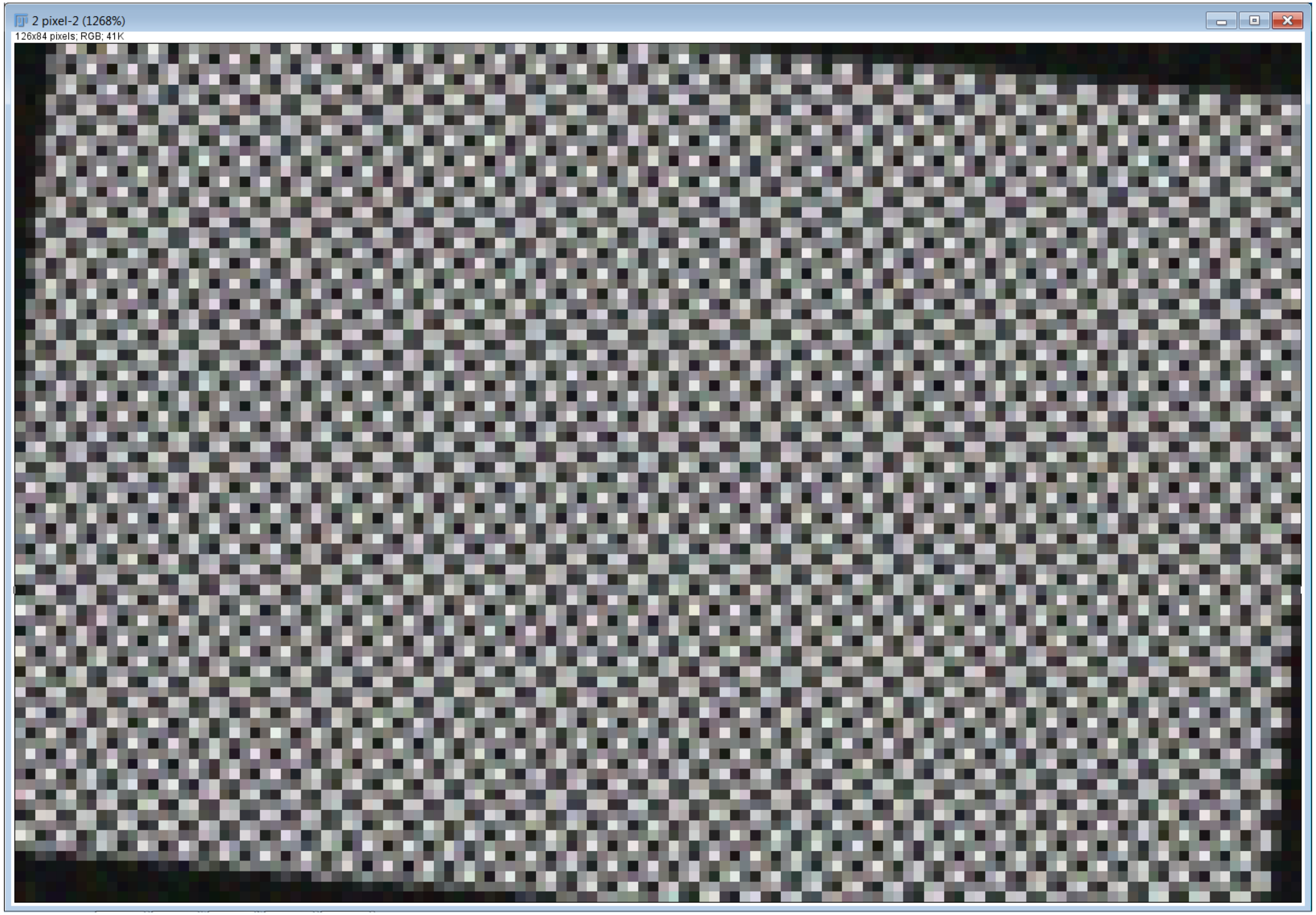


Image pixels size: 2 pixel / square

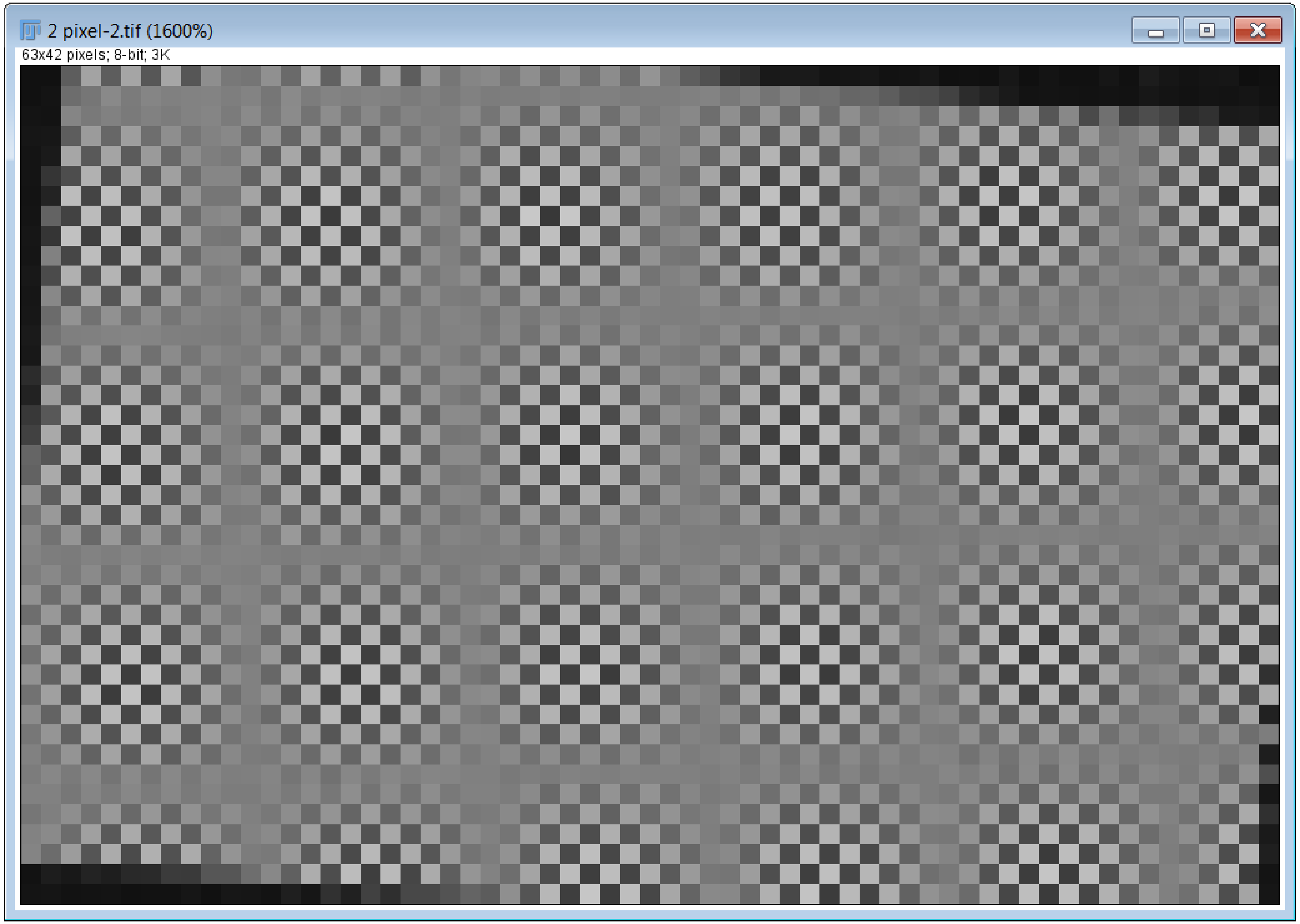


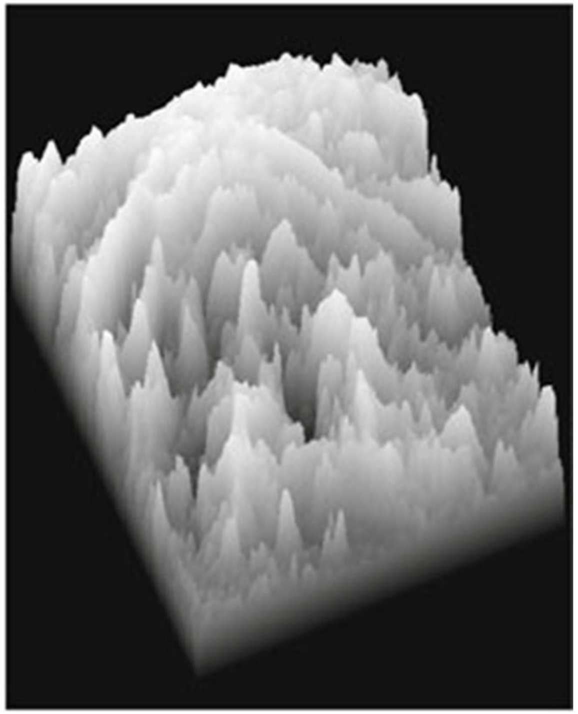
Image pixels size: 1 pixel / square

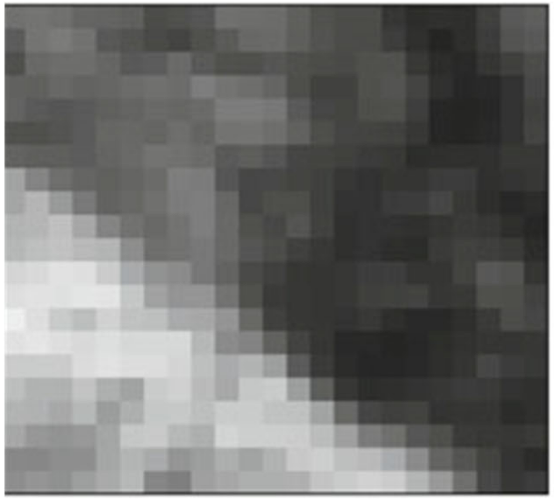
Sampling artefact (aliasing effect) : interference pattern

Going back to basic concepts

Image Processing: **Changing images into numbers and manipulating the numbers**

Grey scale image	«hill plot» of data
Zoomed area	Pixel values as numbers





134	131	135	146	148	151	173	168	161	135	137	139	152	167	173	174	198
155	131	124	133	158	165	176	180	168	156	143	141	154	165	172	175	188
168	142	138	137	145	158	155	141	157	167	169	161	169	172	176	168	167
150	151	143	149	145	139	135	139	122	132	137	146	171	184	184	168	164
148	157	162	157	151	147	141	144	140	123	123	128	157	178	181	171	170
167	169	164	156	145	143	146	137	146	132	130	137	150	171	180	181	184
148	152	152	155	147	141	132	134	140	157	153	163	179	181	185	191	195
87	116	136	146	151	146	140	121	124	162	178	177	181	185	198	202	199
85	69	94	126	148	143	135	124	121	144	178	171	171	186	202	203	191
71	69	60	82	116	133	142	132	121	142	169	177	164	184	205	202	203
67	61	57	65	70	107	136	133	127	142	162	174	186	195	205	203	209
40	36	28	31	48	78	102	109	127	149	176	185	198	200	198	190	189
31	28	30	26	27	54	88	100	98	132	169	193	196	199	197	187	185
18	31	50	62	43	58	61	78	82	99	143	186	196	199	203	195	208
28	25	31	37	32	38	38	39	67	108	117	150	182	198	215	213	221
48	49	48	34	28	33	33	40	61	79	71	90	156	192	212	218	217
61	72	72	57	75	86	50	44	64	81	54	50	91	153	197	209	207
71	73	81	67	92	75	54	53	61	50	49	58	59	86	144	179	187
82	97	101	96	85	54	51	68	75	42	47	47	48	63	89	124	136
112	113	107	105	84	70	78	98	90	76	86	72	51	47	61	65	78
105	98	103	91	80	68	114	120	89	82	76	76	74	56	50	43	48

Pixels, Bits and Bytes

Pixel is the **picture element**, a finite space where the analog signal is deposited to put it in digital form. It is commor
or the pixel to be **square**, equal dimensions in horizontal and vertical dimensions, as it helps with image processing
fterwards

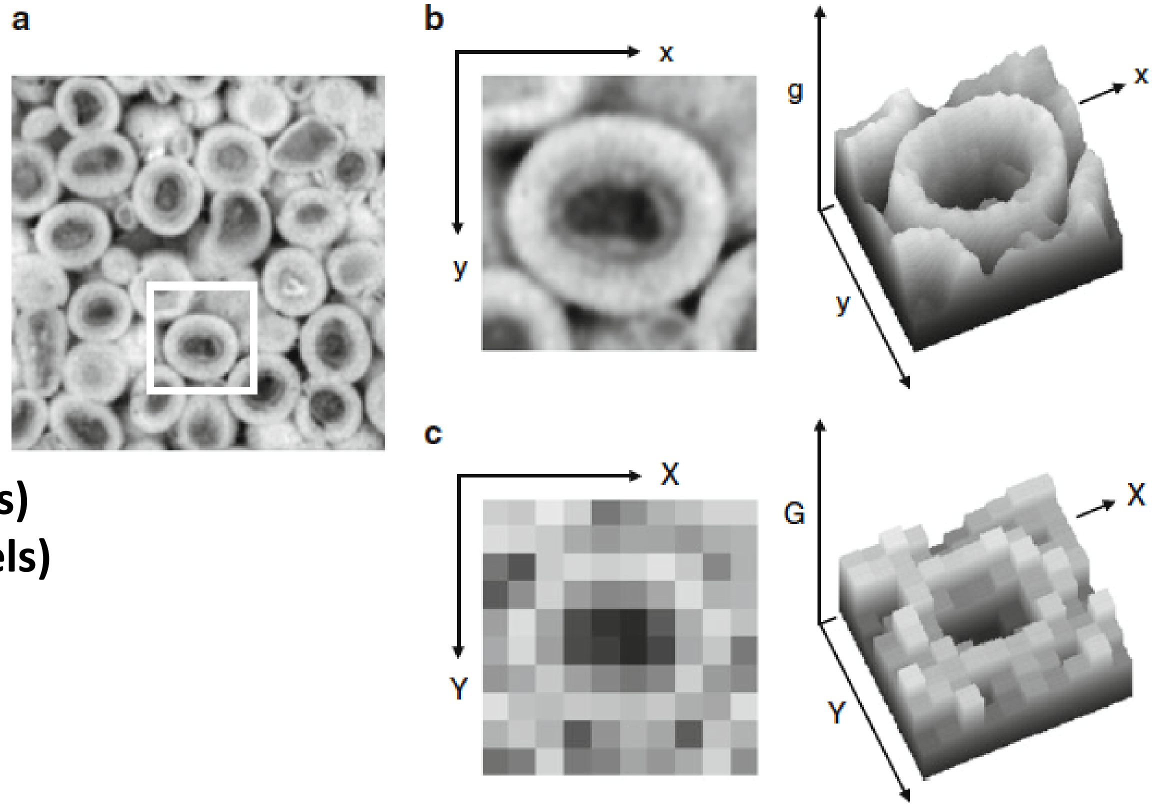
n the computer, numbers are stored as **bits and bytes**. A bit can assume the value 0 or 1, i.e., two different values.
ight bits in a row ,**1 byte**, can assume 2^8 = 256 different values between 0 and 255. Using 12 bits or 16 bits to
ecord gray values, it is possible to represent 2^{12} = 4,096 or 2^{16} = 65,536 different gray values, respectively.

lpful to remember:

yte = 8 bits
B (kilobyte) = 1024 bytes
2^{10} bits

x 1k (camera) = 1024 x 1024 pixels (= 1 Megapixels)
x 2k (camera) = 2048 x 2048 pixels (= 4 Megapixels)

nning 2 : reducing the total number of pixels by 2x
nning 3 : reducing the total number of pixels by 3x



Example: Pixel Size

256 Steps - appears visually to be continuous

128 Steps

64 Steps

32 Steps

16 Steps

Example: Gray Levels

32 grey levels

16 grey levels

8 grey levels

4 grey levels

4. Correcting Defects (post-processing)

Image processing operations and procedures that can be applied to correct some of the defects or shortcomings in as-acquired images that may occur due to imperfect detectors, limitations of the optics, inadequate or nonuniform illumination, or an undesirable viewpoint.

These are corrections applied after the image has been digitized and stored and therefore are unable to deliver the highest quality result that could be achieved by optimizing or correcting the acquisition process in the first place. Nothing can replace a “perfect” image !

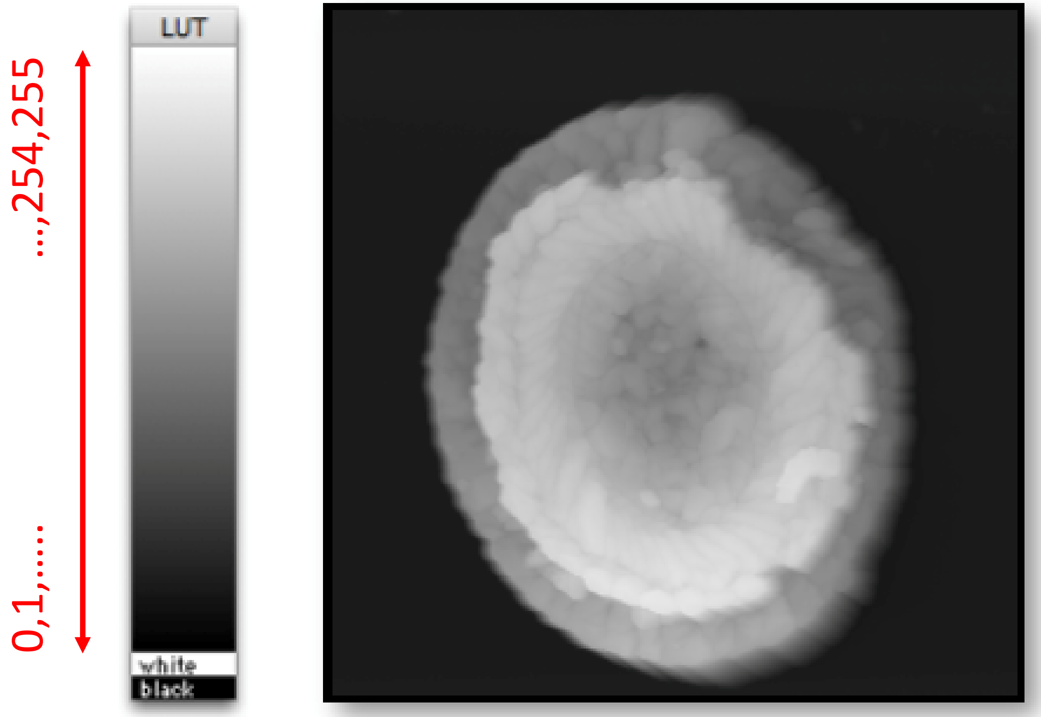
However, acquiring the optimum quality image can be impractical – reasons

- low dose imaging – noisy
- intensity variations – irregularities of surfaces
- outliers – x-rays, dead pixels

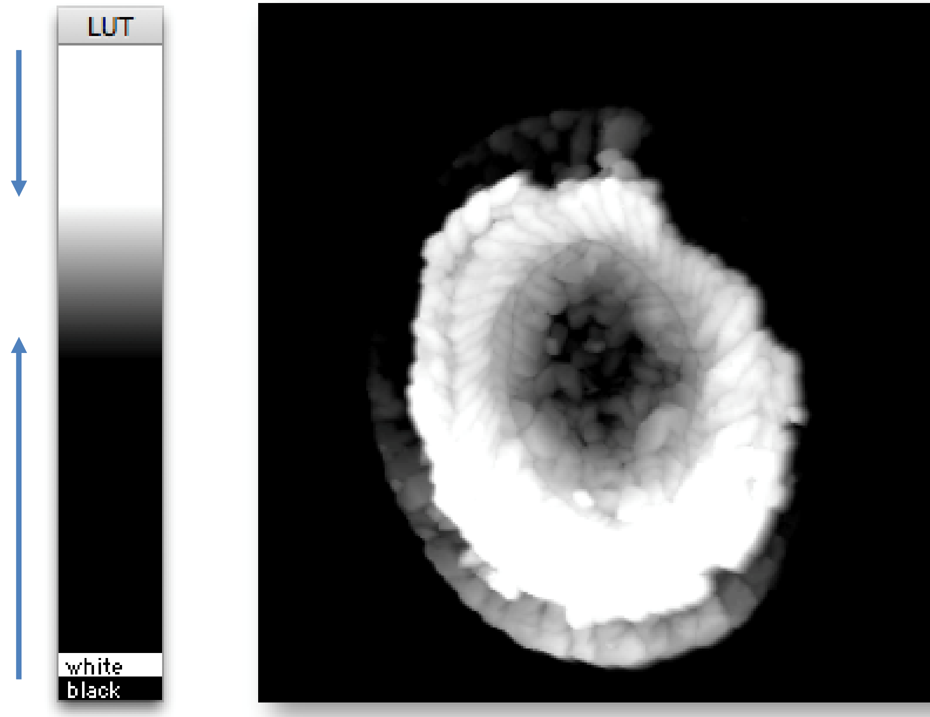
Color Adjustments: Look Up Table (LUT)

Image processing refers changing all or some of the pixel values in an image, usually with the aim of making some features of the image more easily “visible”

The easiest way would be to change the color used to represent each pixel value – the **Look-Up Table (LUT)**
= remapping of grey scale levels



default greyscale

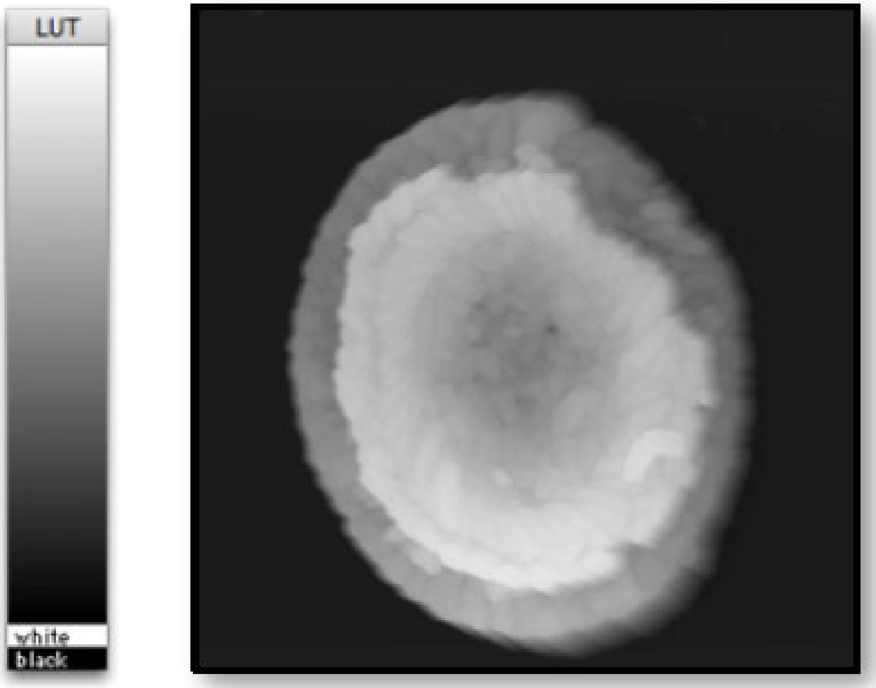


increased contrast

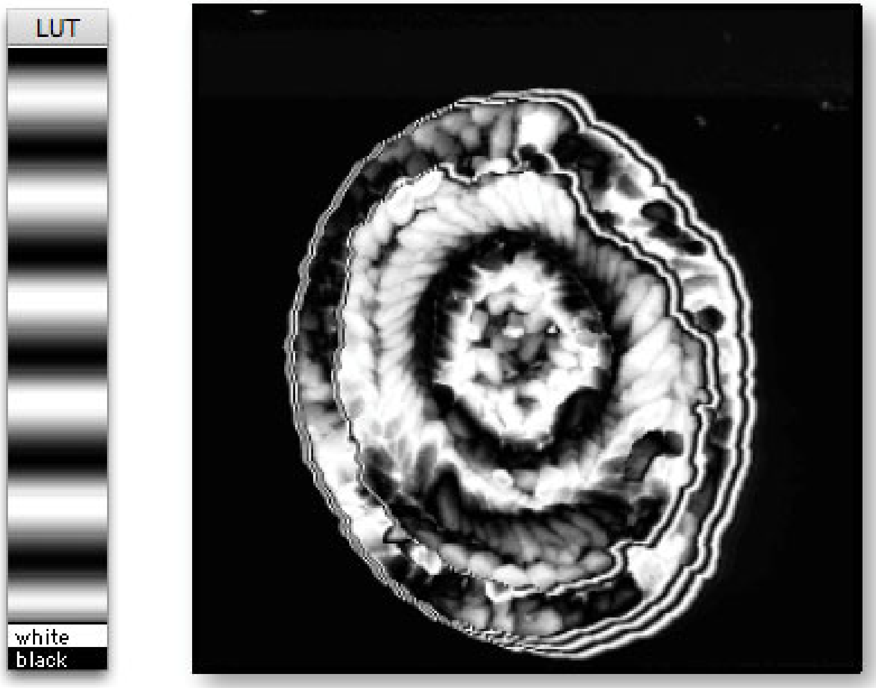
Color Adjustments

Both linear and non-linear scales can be used. A non-linear mapping between the pixel value and displayed color can reveal unexpected features

Always have a idea of what you expect to see – do some research beforehand



default greyscale

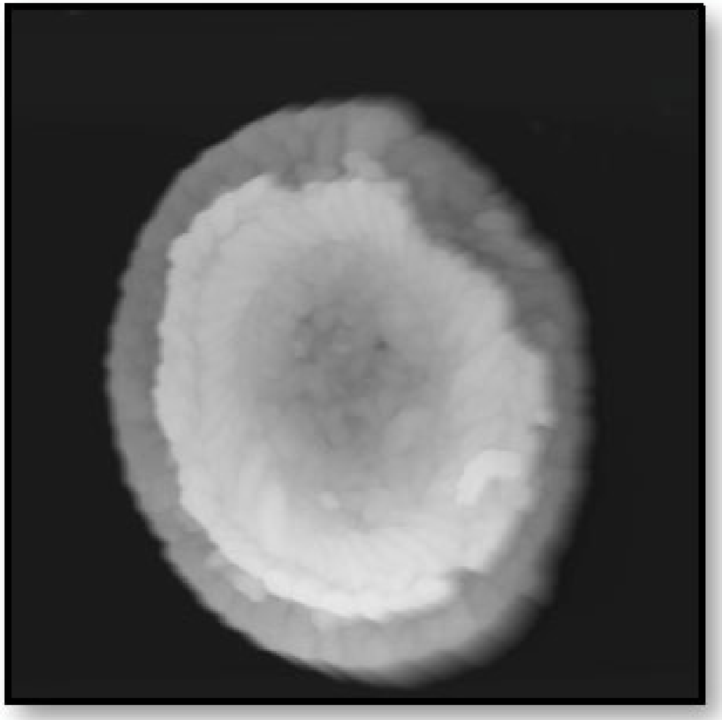


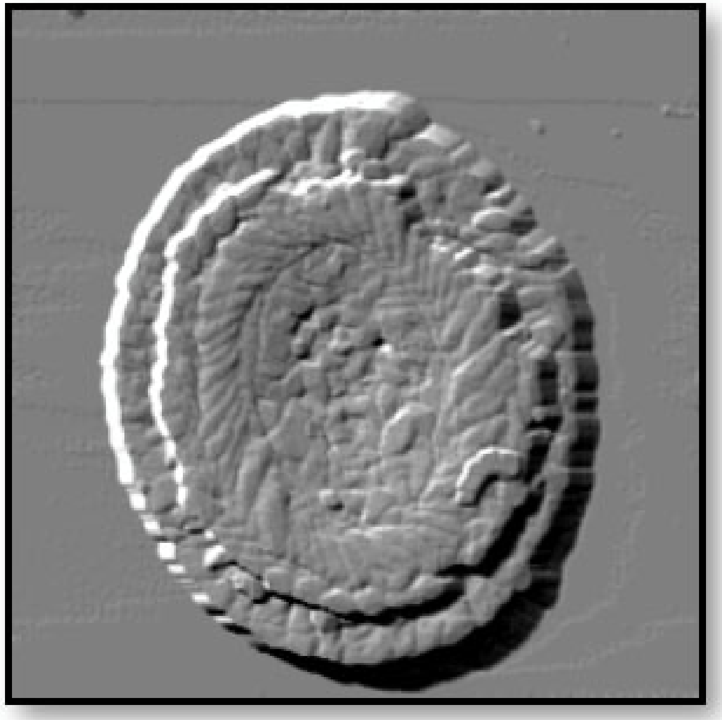
zebra greyscale

Color Adjustments

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

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





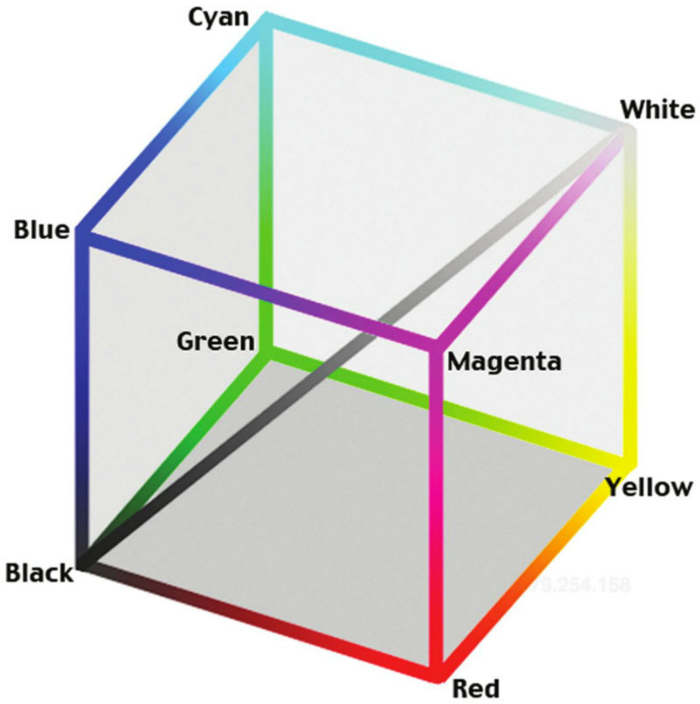
greys $\rightarrow$ z values

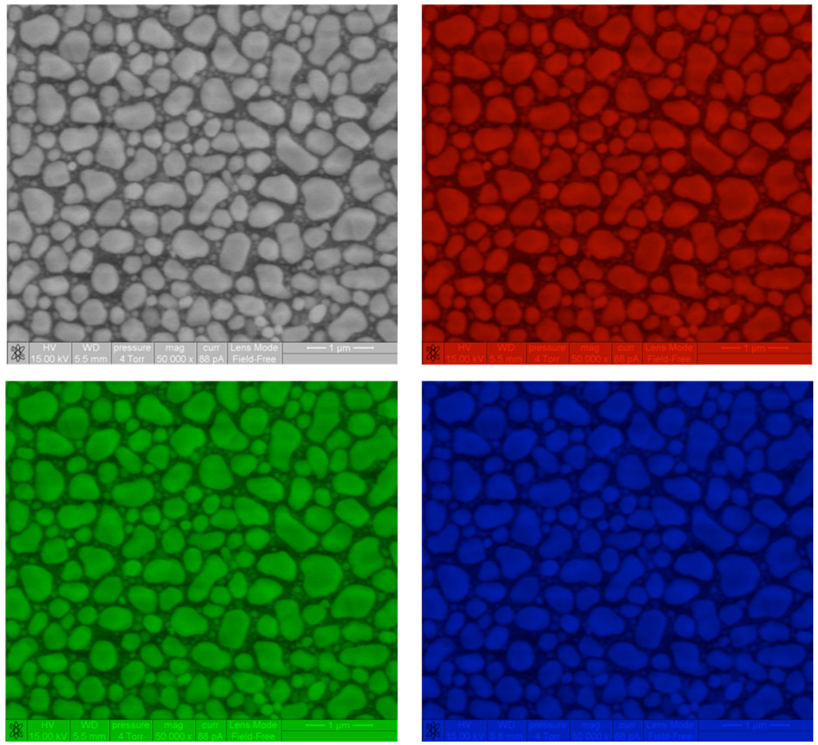
greys $\rightarrow$ $^{\partial z}/_{\partial x}$ values

Color Adjustments

All images in microscopy are taken in grey levels, however, you can also apply color to them if needed

The are many color spaces (chromaticity diagrams) – the most commonly used is Red – Green – Blue (RGB)





The neutral greyscale axis is running diagonally from black to white and has equal amounts of red, green, and b

Noise reduction “kernel” filters

he term noise refers to random fluctuations in pixel values that arise from the characteristics of the image acquisitio

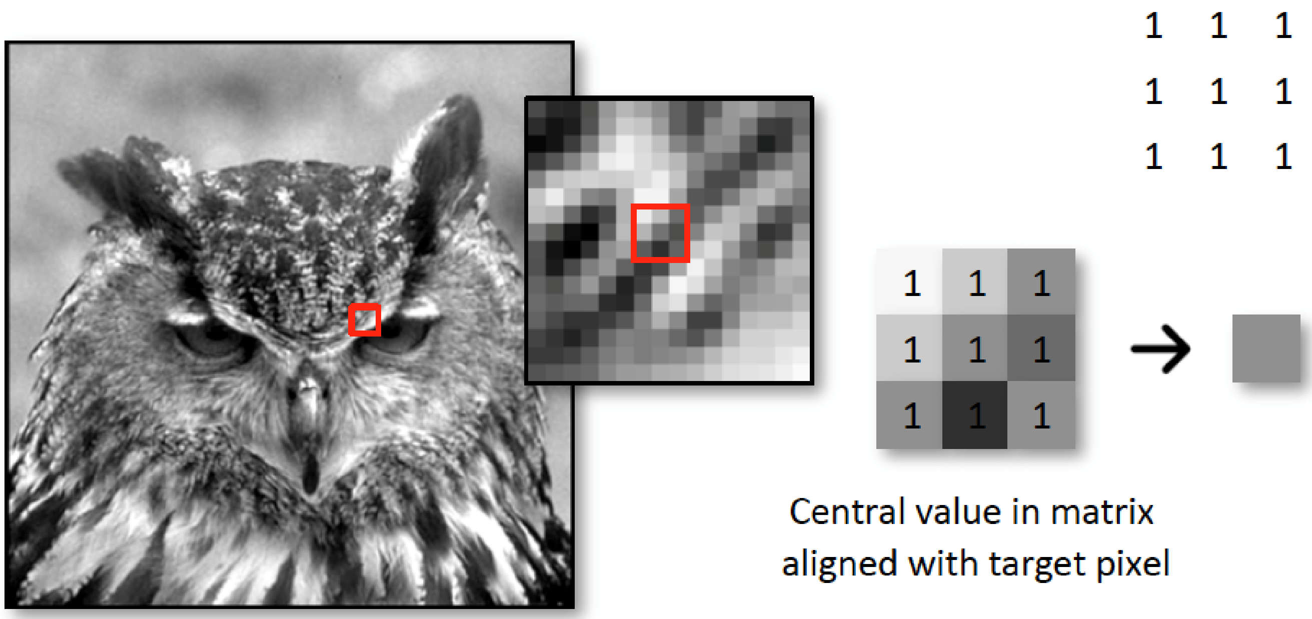
his includes

the **statistical** variations in the number of electrons that are **emitted** (and hitting the specimen),

the number of electrons each sample **produces**, plus thermal electrons,

the effects of capacitance and other **electronic** sources of variation in **amplification** and **digitization** process.

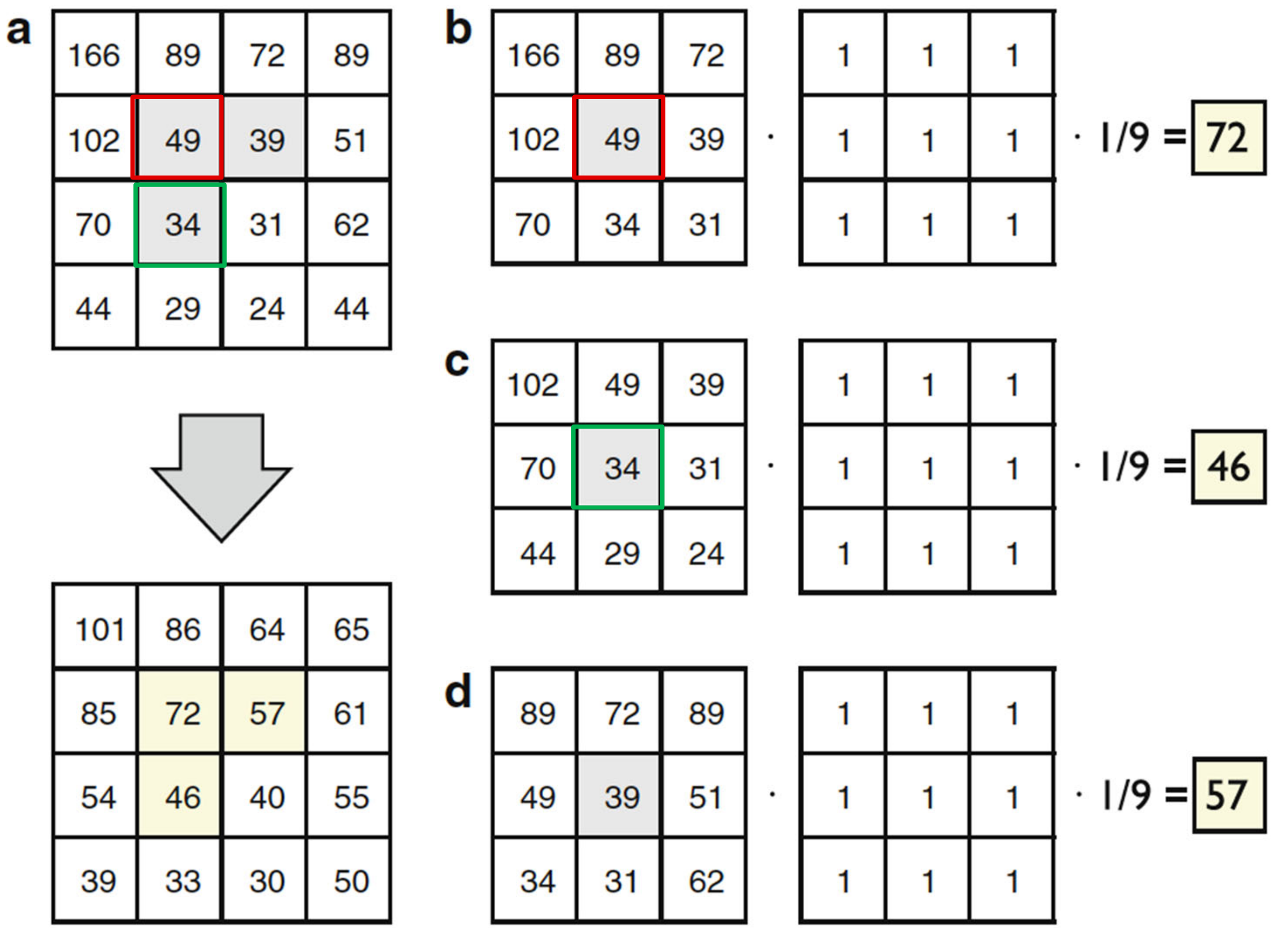
e-noising is often carried out using a **kernel filter** which uses a n x n matrix of elements. The kernel matrix is applied
very pixel in the image in turn



Central value in matrix
aligned with target pixel

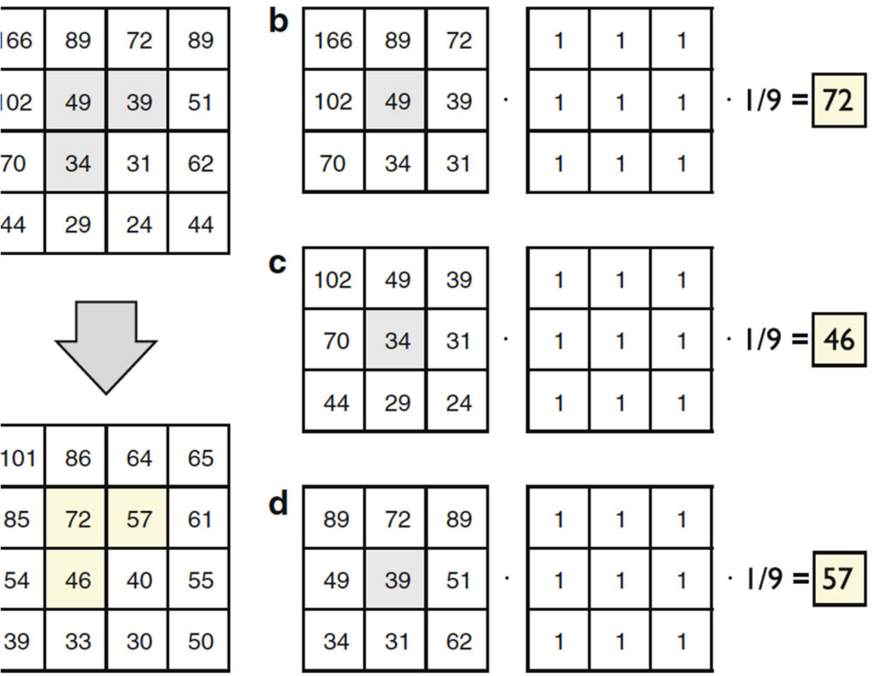
Neighborhood Averaging

The most common way to accomplish neighborhood averaging is to replace each pixel with the average of itself and its neighbors – sum of the pixel values in a region multiplied by an array of numeric weights



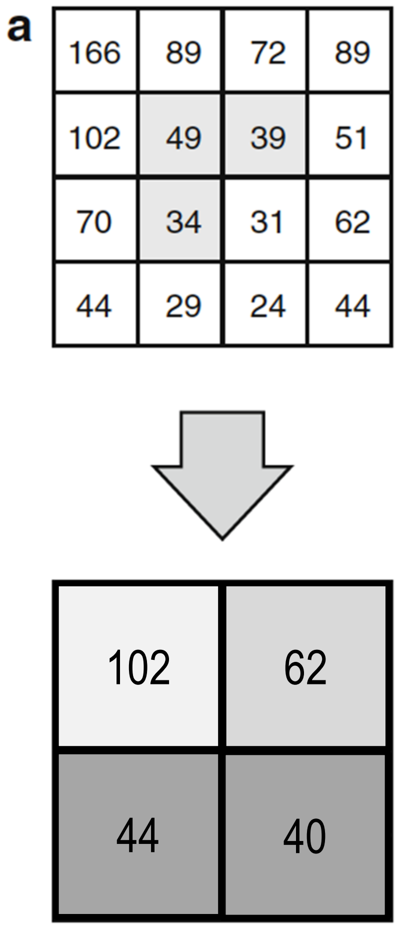
Neighborhood Averaging vs Binning

ighbourhood averaging with 3x3 kernel



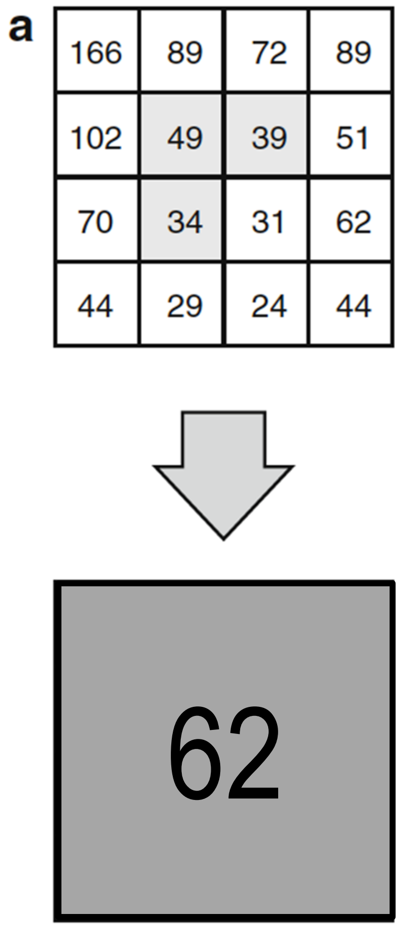
number of pixels unchanged

Binning 2



Reduced number of pixels

Binning 4



Reduced number of pixels

eighborhood
eraging

3x3 binning
(=resampling)

Kernel of 3x3

$$\begin{bmatrix} 1 & 1 & 1 \\ 1 & {\color{red}1} & 1 \\ 1 & 1 & 1 \end{bmatrix}$$

ernel of 7x7

$$\begin{bmatrix} 0 & 0 & 1 & 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 1 & 1 & {\color{red}1} & 1 & 1 & 1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 0 & 1 & 1 & 1 & 1 & 1 & 0 \\ 0 & 0 & 1 & 1 & 1 & 0 & 0 \end{bmatrix}$$

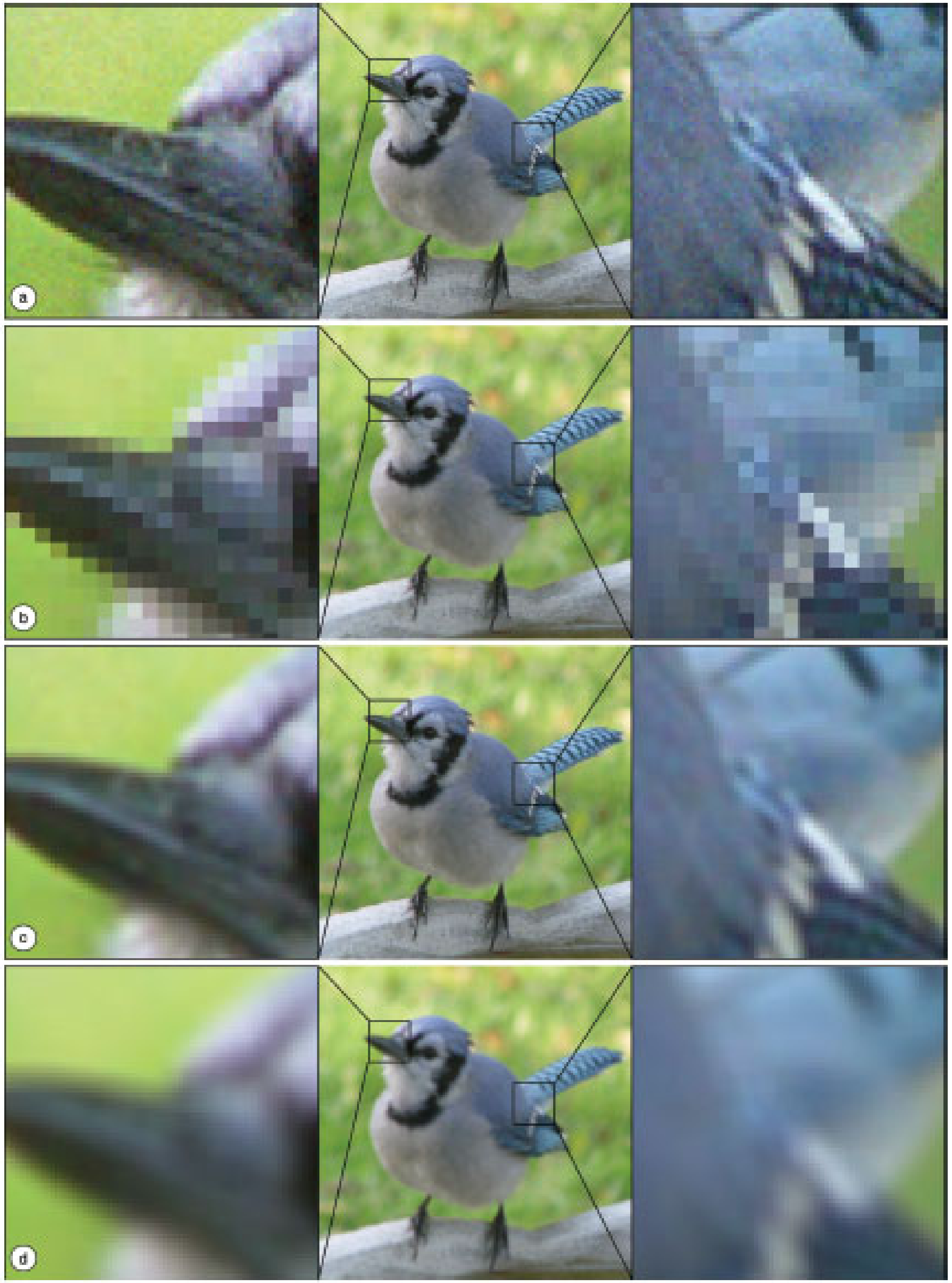
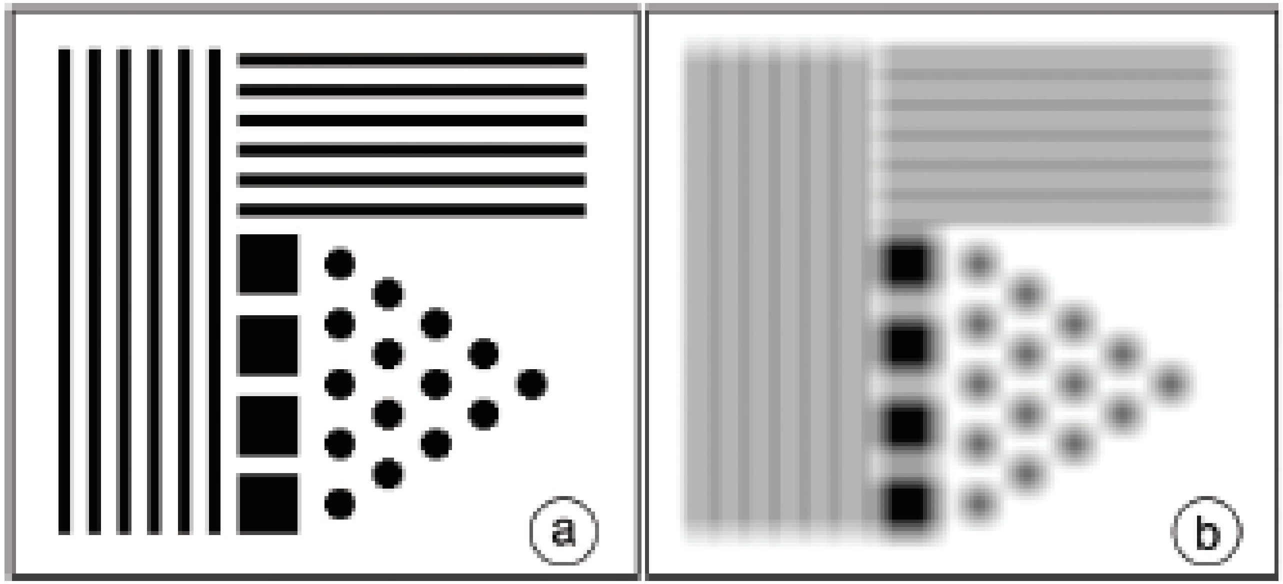


Figure 4.14 Noise reduction by averaging: (a) original image; (b) averaging in 3 × 3 blocks; (c) 3 × 3 averaging around each pixel; (d) 7 × 7 averaging around each pixel.

Apparent features -> artifacts

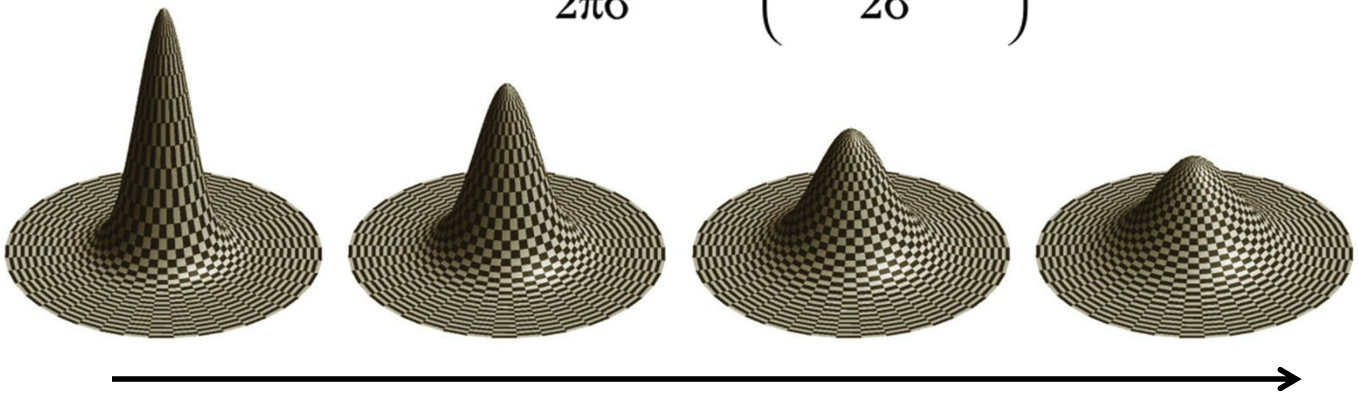


If the kernel size and shape becomes similar to the features in the image filtering artifacts can occur: aliasing / anti-aliasing

Gaussian Smoothing

This is a set of weights that approximates the profile of a Gaussian function along any row, column, or diagonal through the center

$$G(x, y, \sigma) = \frac{1}{2\pi\sigma^2} \cdot \exp\left(\frac{-\left(x^2 + y^2\right)}{2\sigma^2} \right)$$

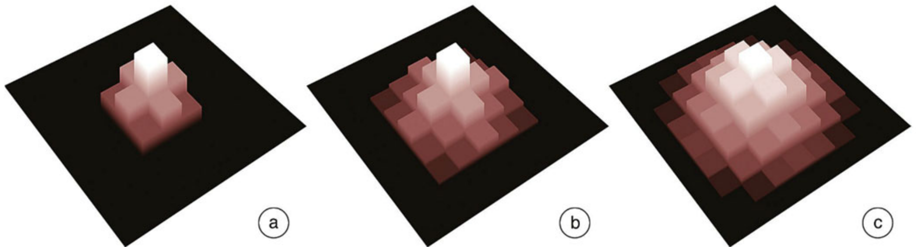


increasing sigma

i.e. **smoothing proceeds with a weighted average convolution**

$$\begin{bmatrix} 1 & 2 & 1 \\ 2 & {\color{red}4} & 2 \\ 1 & 2 & 1 \end{bmatrix}$$

3x3 kernel



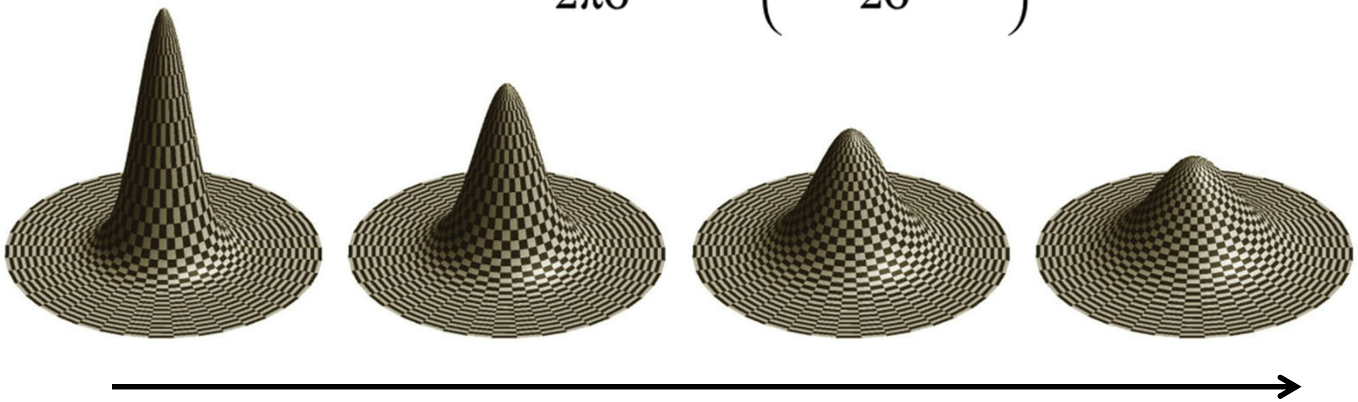
5x5 kernel

7x7 kernel

Gaussian Smoothing

This is a set of weights that approximates the profile of a Gaussian function along any row, column, or diagona
through the center

$$G(x, y, \sigma) = \frac{1}{2\pi\sigma^2} \cdot \exp\left(\frac{-\left(x^2 + y^2\right)}{2\sigma^2} \right)$$



increasing sigma

In ImageJ for the Gaussian Blur filter -> 'Radius' means the radius of decay to exp(-0.5) ~ 61%
i.e, the standard deviation, sigma (in pixels).

In practice, the Gaussian distribution is effectively zero more than about three standard deviations from the mean
and so the kernel is truncated at this point
For example, for a Gaussian with sigma equal to 1, the kernel is approximated by a 7x7 matrix

7x7 smoothing kernel with Gaussian weights

0.0	0.00307	0.00676	0.00860	0.00676	0.00307	0.0
0.00307	0.01106	0.02355	0.02991	0.02355	0.01106	0.00307
0.00676	0.02355	0.04937	0.06350	0.04937	0.02355	0.00676
0.00860	0.02991	0.06350	0.08152	0.06350	0.02991	0.00860
0.00676	0.02355	0.04937	0.06350	0.04937	0.02355	0.00676
0.00307	0.01106	0.02355	0.02991	0.02355	0.01106	0.00307
0.0	0.00307	0.00676	0.00860	0.00676	0.00307	0.0

For example, for a Gaussian with sigma equal to 1, the kernel is approximated by a 7x7 matrix

Discrete approximations of Gaussian kernels
3x3, 5x5, 7x7

1/16

1	2	1
2	4	2
1	2	1

1/273

1	4	7	4	1
4	16	26	16	4
7	26	41	26	7
4	16	26	16	4
1	4	7	4	1

1/1003

0	0	1	2	1	0	0
0	3	13	22	13	3	0
1	13	59	97	59	13	1
2	22	97	159	97	22	2
1	13	59	97	59	13	1
0	3	13	22	13	3	0
0	0	1	2	1	0	0

One dimensional convolutions of Gaussian blur

$$\begin{bmatrix}1\\2\\1\end{bmatrix} \text{ and } \begin{bmatrix}1 & 2 & 1\end{bmatrix}$$

$$\begin{bmatrix}1\\2\\4\\2\\1\end{bmatrix} \text{ and } \begin{bmatrix}1 & 2 & 4 & 2 & 1\end{bmatrix}$$

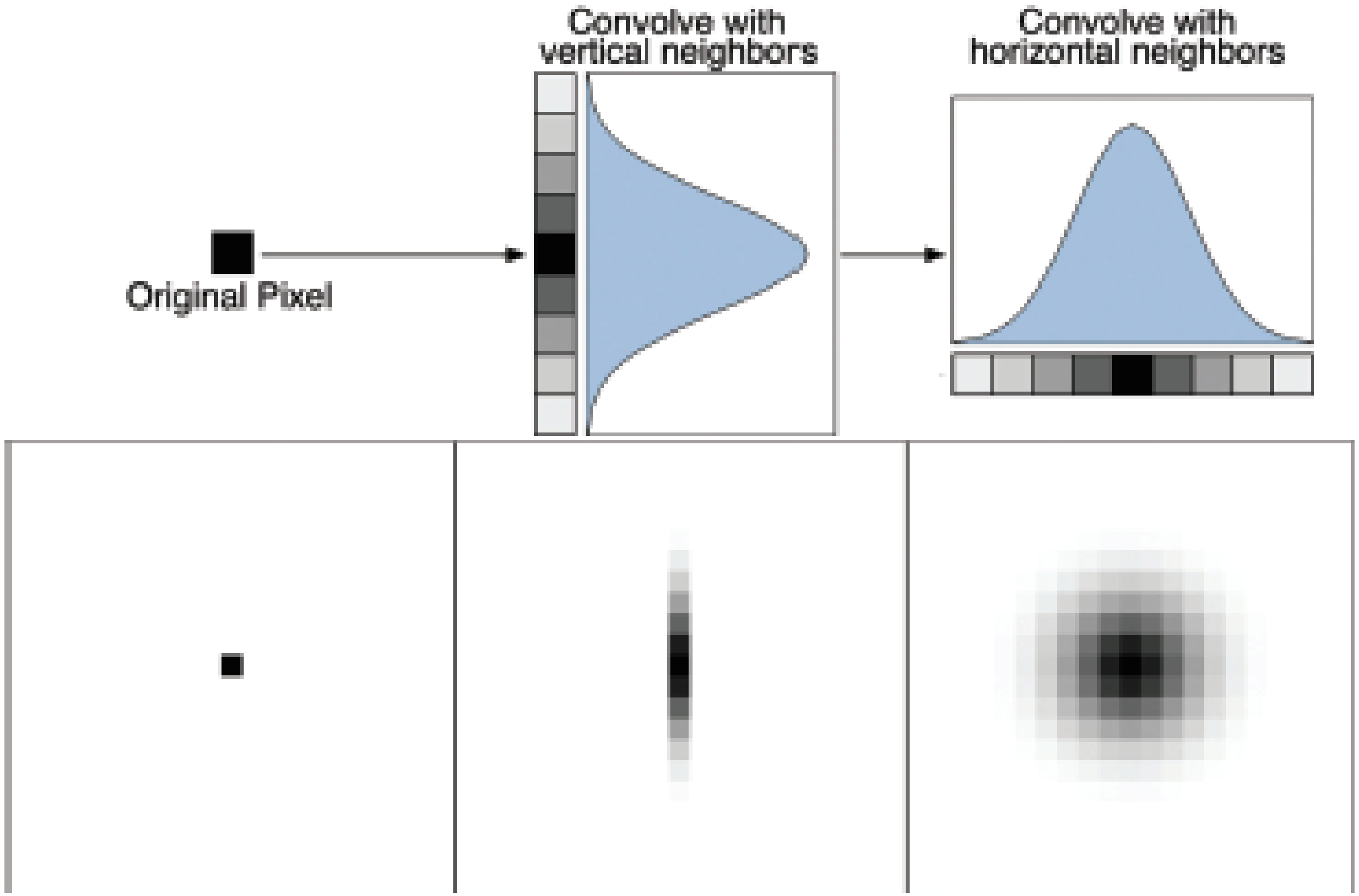


Figure 4.17 Performing a Gaussian smooth by applying two one-dimensional convolutions.

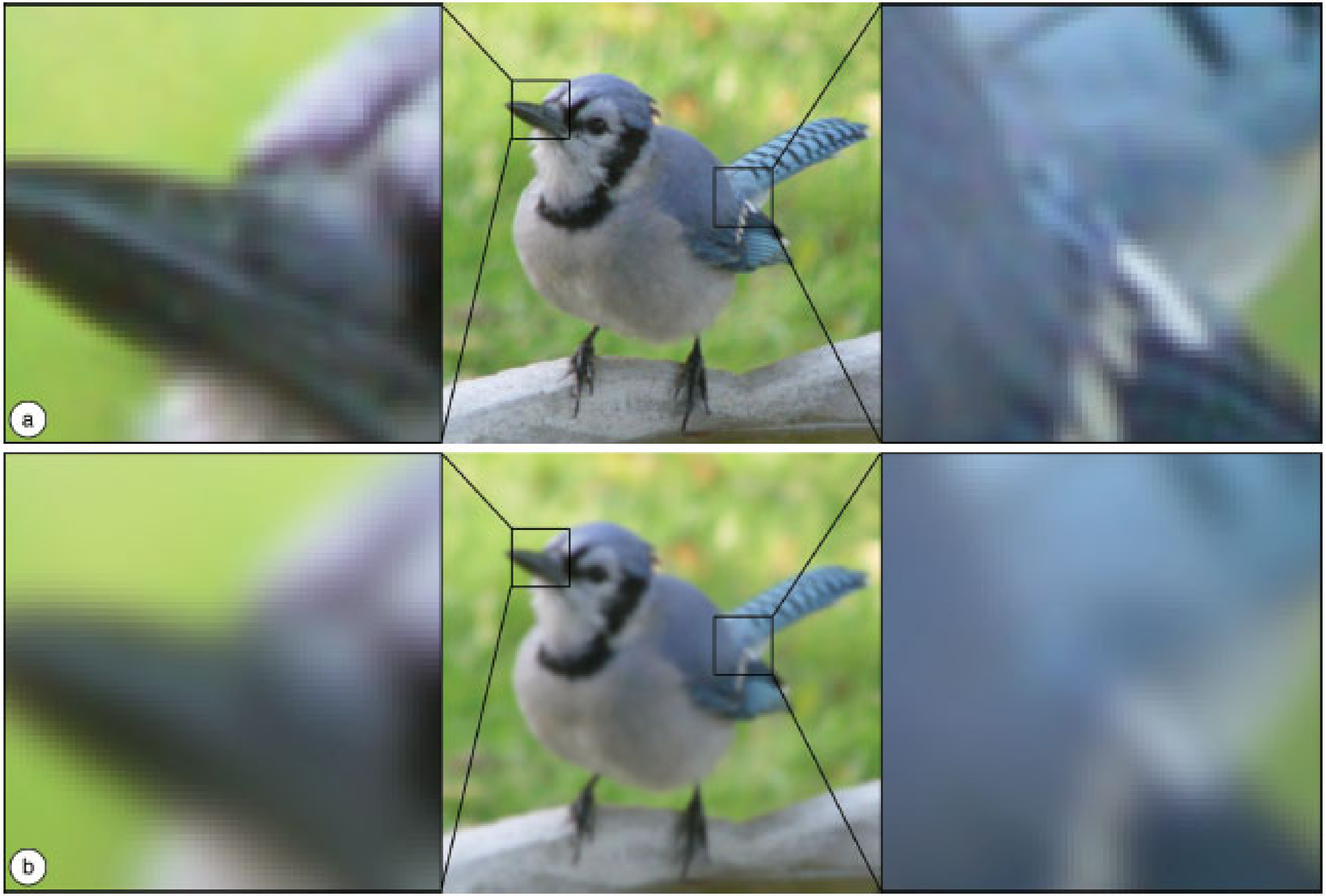
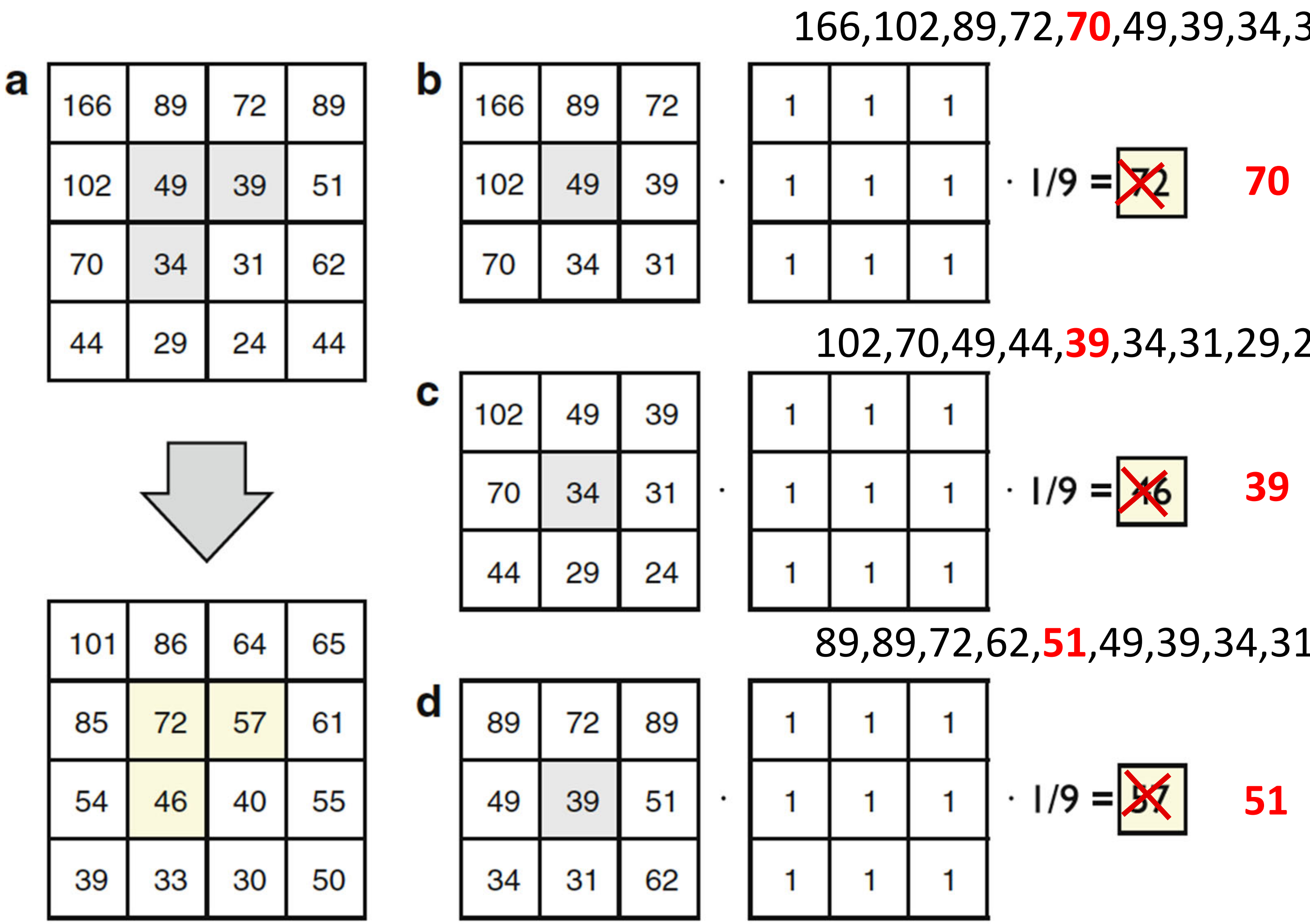


Figure 4.18 *Gaussian smoothing of the image from* ***Figure 4.14a****: (a) standard deviation = 1.0 pixels; (b) standard deviation = 3.0 pixels.*

Neighborhood Averaging vs Median

The most common way to accomplish neighborhood averaging is to replace each pixel with the average of itself and its neighbors. Calculated value !

Median: replace the central pixel value by the central value of an ordered array of all values in the neighbourhood. Replace with a existing (most central) value.

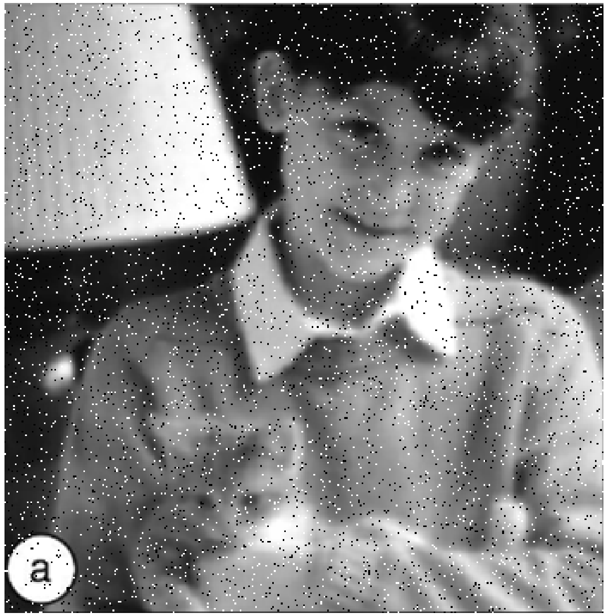


Neighborhood Ranking – Median Filtering

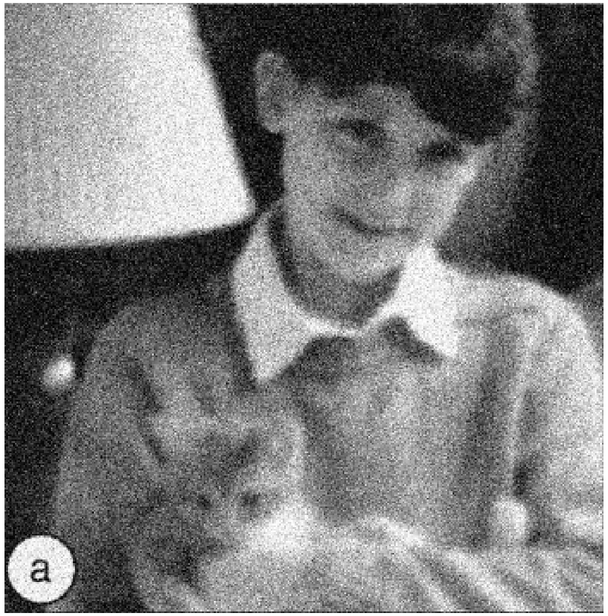
Smoothing filters reduce random noise, but the underlying assumption is that all of the pixels in the neighborhood represent multiple samples of the same value -> that they belong to the same structure or object – but what happens at the edges or boundaries?

- Ranking of pixel according to their brightness level in their neighborhood
- The median value is used as the new value for the central pixel

The median filter can dramatically reduce the noise if it is “speckle” noise.



Salt and pepper
(black and white pixels)



Random noise

Neighborhood Ranking – Median Filtering

Smoothing filters reduce random noise, but the underlying assumption is that all of the pixels in the neighborhood represent multiple samples of the same value -> that they belong to the same structure or object – but what happens at the edges or boundaries?

- Ranking of pixel according to their brightness level in their neighborhood
- The median value is used as the new value for the central pixel

The median filter can dramatically reduce the noise if it is “speckle” noise.

Salt and pepper & median

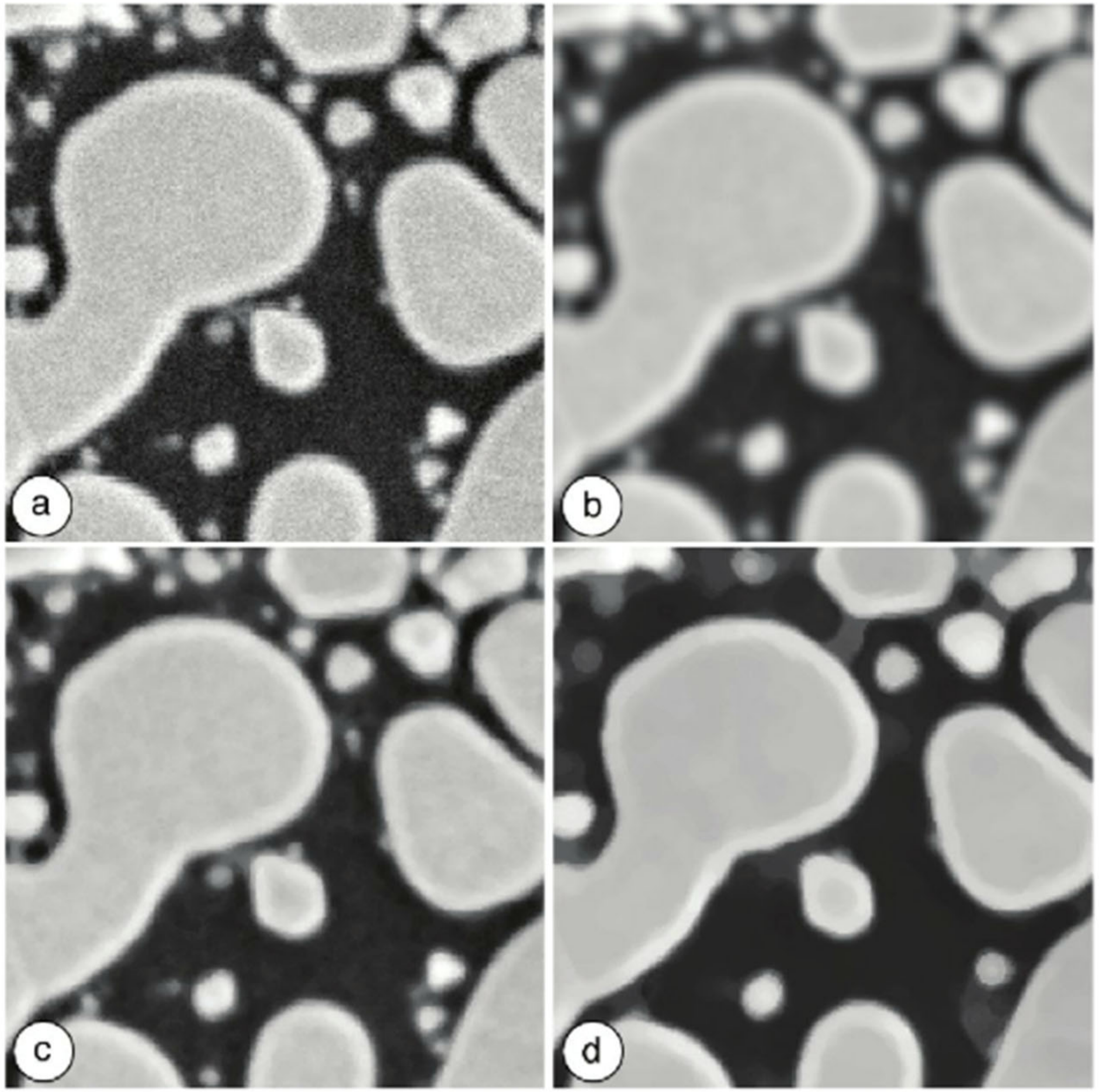
Random noise & median

Very efficient in removing extreme outliners (black or white pixels)

Gaussian Smoothing vs Median Filtering

SEM image

Gaussian
sigma=2 pixels



Median
radius=2pixels

Median
radius=15pixels

Median filters preserve **edges** in general better than gaussian or averaging filters!

Iterative Median (several steps)

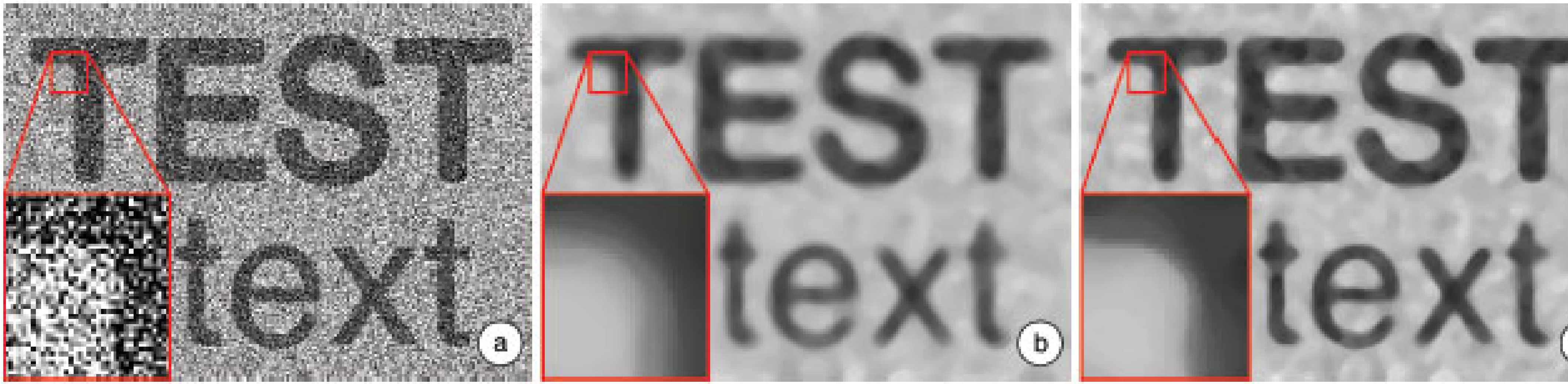


Figure 4.24 Iterated median filtering: (a) original noisy image; (b) iterated median using a sin large (7 pixel radius) neighborhood; (c) iterated median using a sequence of 3, 5, and 7 pixel rad neighborhoods.

- Fine details are erased and large regions take on the same brightness
- This is also called contouring

- First one to improve signal-to-noise and second one (larger) to demarcate the boundaries

Hybrid Median (or corner-preserving median)

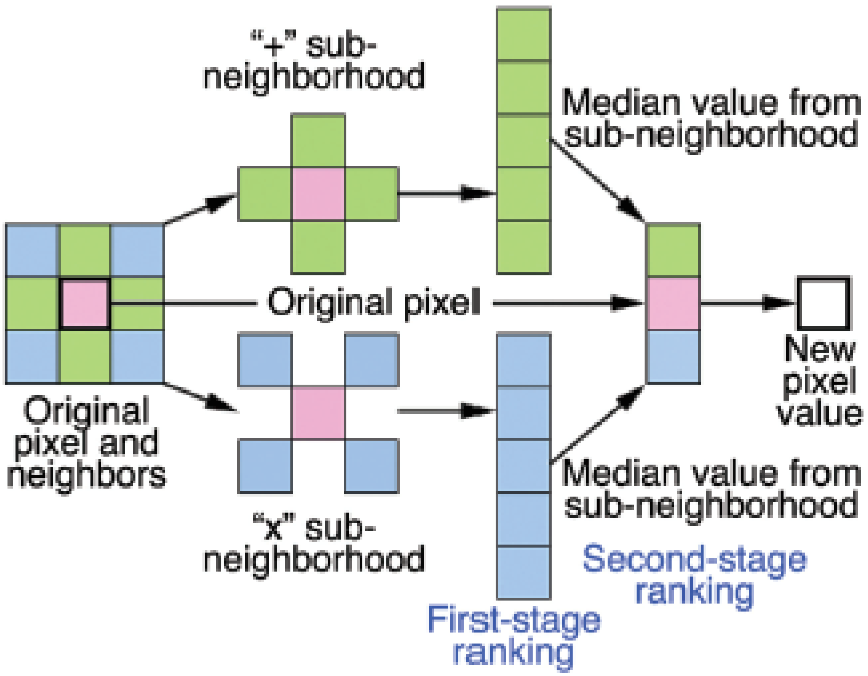
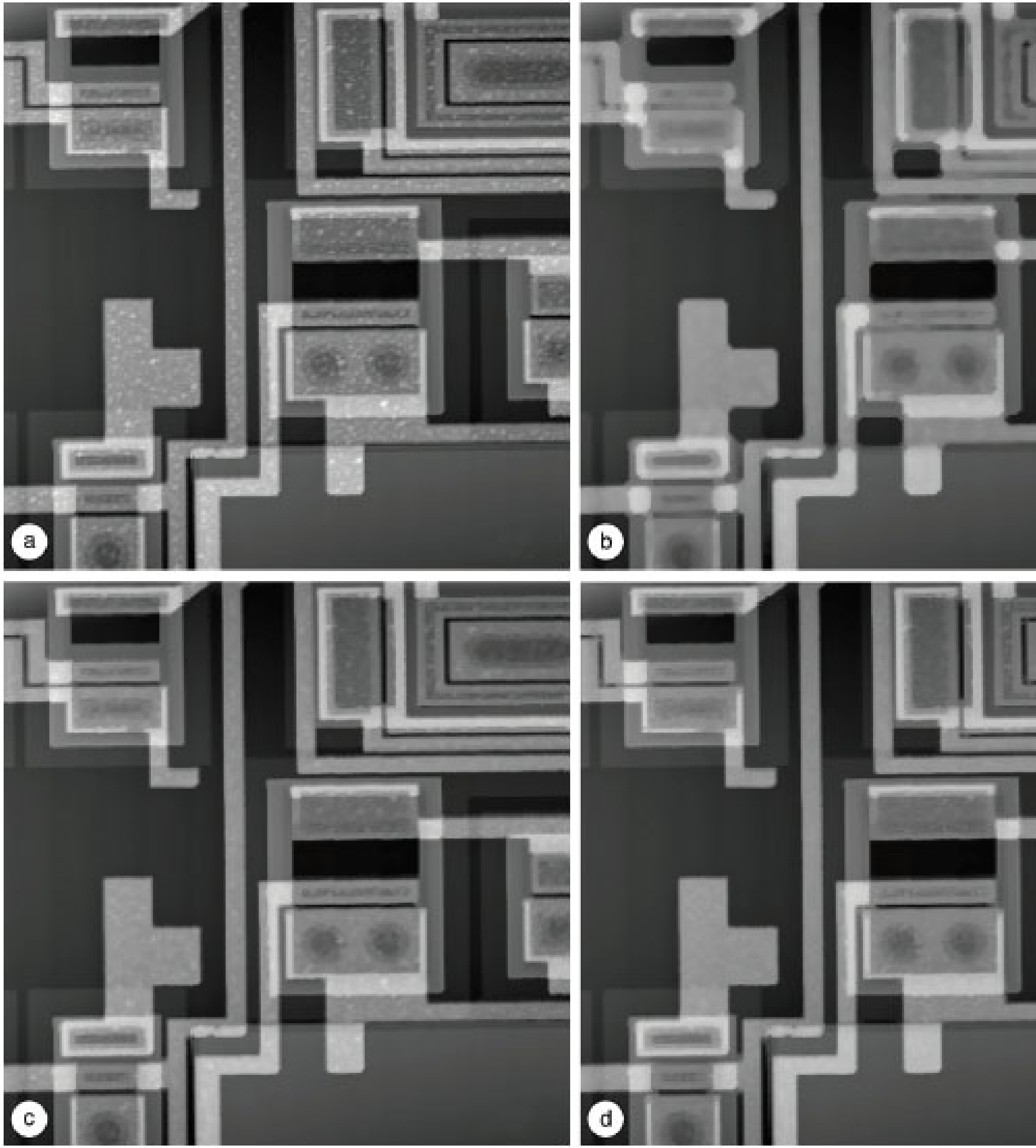


Figure 4.30 *Diagram of neighborhood pixels used in the 3 × 3 hybrid median filter. Both groups include the central pixel and are ranked separately. The median of each group and the central pixel are then ranked again to select the final median value.*

- Multistep ranking median
- Preserves lines and corners that are erased or rounded by the conventional median

Original
Radius 2 median: rounded corners
Radius 2 hybrid median: preservation of corners
Radius 4 hybrid median



Several Other Defect/Noise Removal Methods

Conditional neighborhoods: excludes pixels from the summation or from the ranking if they are very different from original central pixel

Interpolation: filling the region of defects using adjacent values – does not always match the surrounding

Maximum entropy: alter the pixel brightness values to maximize the entropy in the image based on prior knowledg on the image formation process

Maximum likelihood: it assumes that the boundaries between regions should be sharp – whichever region has the lowest variance is taken to represent the region to which the central pixel should belong and it is assigned the mea value from that region

Also, adjustments form non-uniform illumination, background correction and image alignment can be performed