Advanced Microscopy Course 2014

Lecture 5:

Basic Image Processing

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* Dominic Waithe, Lecture 17: Applied Image Analysis and Matlab *

Basic Image Processing

- What is a digital image?
- What makes a good image?

  Correct image acquisition

  Signal to Noise

  Resolution and Sampling

- The basics of image processing

  Golden rules of image processing

  Conceptual Hierarchy of Image Processing

  Low Level Processing:
  - Display (Figures)
  - Filtering

  Mid - Level Processing:
  - Segmentation
  - Spectral unmixing

  High -Level Processing / **Analysis**:
  - Colocalisation
  - Tracking
  - Statistics

What is a digital image?

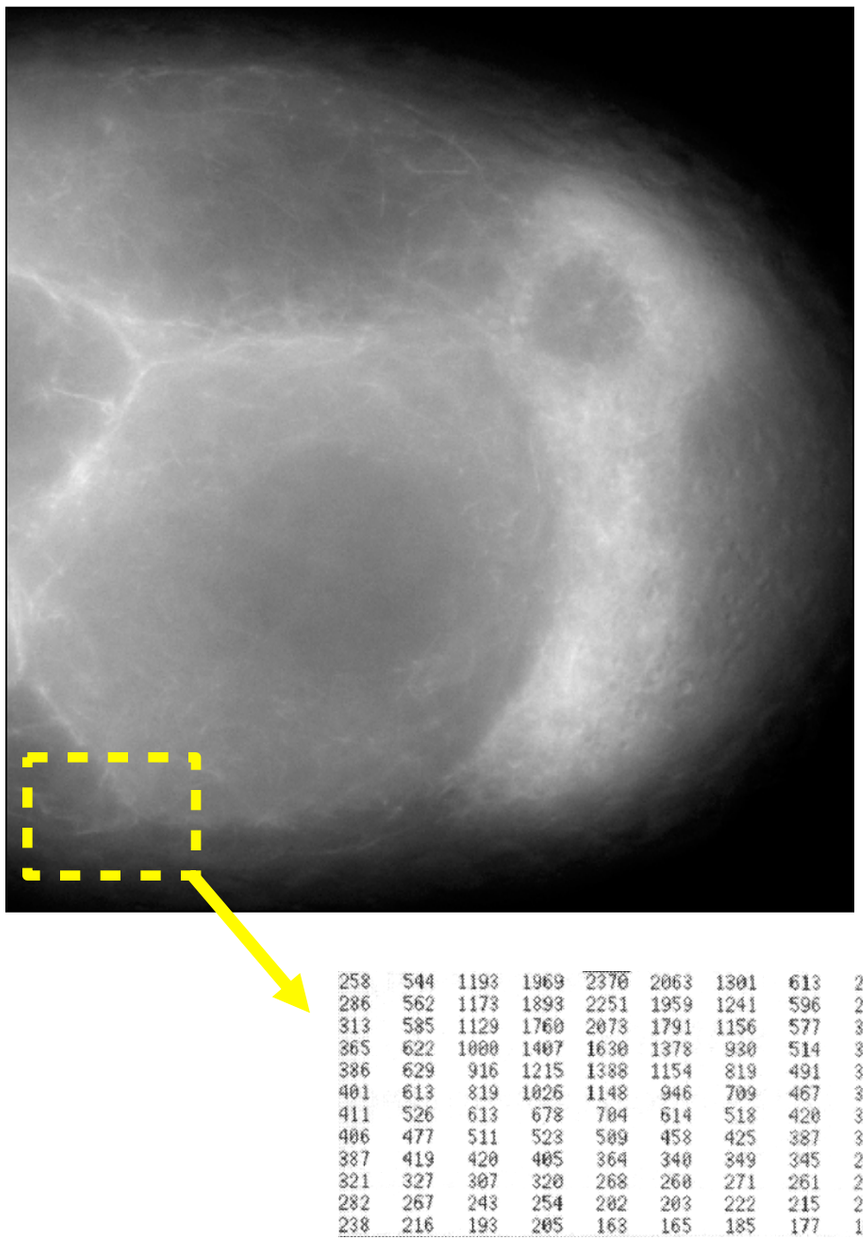
http://en.wikipedia.org/wiki/Money_for_Nothing_(song)

What is a digital image?

An image represents the output of the optics and detector of the imaging system

image ≠ object

image = object ⊗ PSF



258	544	1193	1969	2370	2063	1301	613	263
286	562	1173	1893	2251	1959	1241	596	278
313	585	1129	1760	2073	1791	1156	577	300
365	622	1000	1407	1630	1378	930	514	357
386	629	916	1215	1388	1154	819	491	373
401	613	819	1026	1148	946	709	467	372
411	526	613	678	704	614	518	420	328
406	477	511	523	509	458	425	387	307
387	419	420	405	364	340	349	345	284
321	327	307	320	268	260	271	261	245
282	267	243	254	202	203	222	215	217
238	216	193	205	163	165	185	177	186

- **A digital image is a numerical array: elements = pixels or voxels with:**
  - **defined size (sampling resolution)**
  - **defined no. of grey levels (bit depth)**

- **In addition to "useful" signal there is:**
  - **dark signal from the detector**
  - **autofluorescence (background)**
  - **statistical noise of photon detection**

- **Details are detected within the limitations of:**
  - **the imaging optics**
  - **the sampling rate (pixel size)**
  - **the statistical noise**
  - **the sample contrast / detector dynamic range**

Image Parameters - what to record (= image metadata)

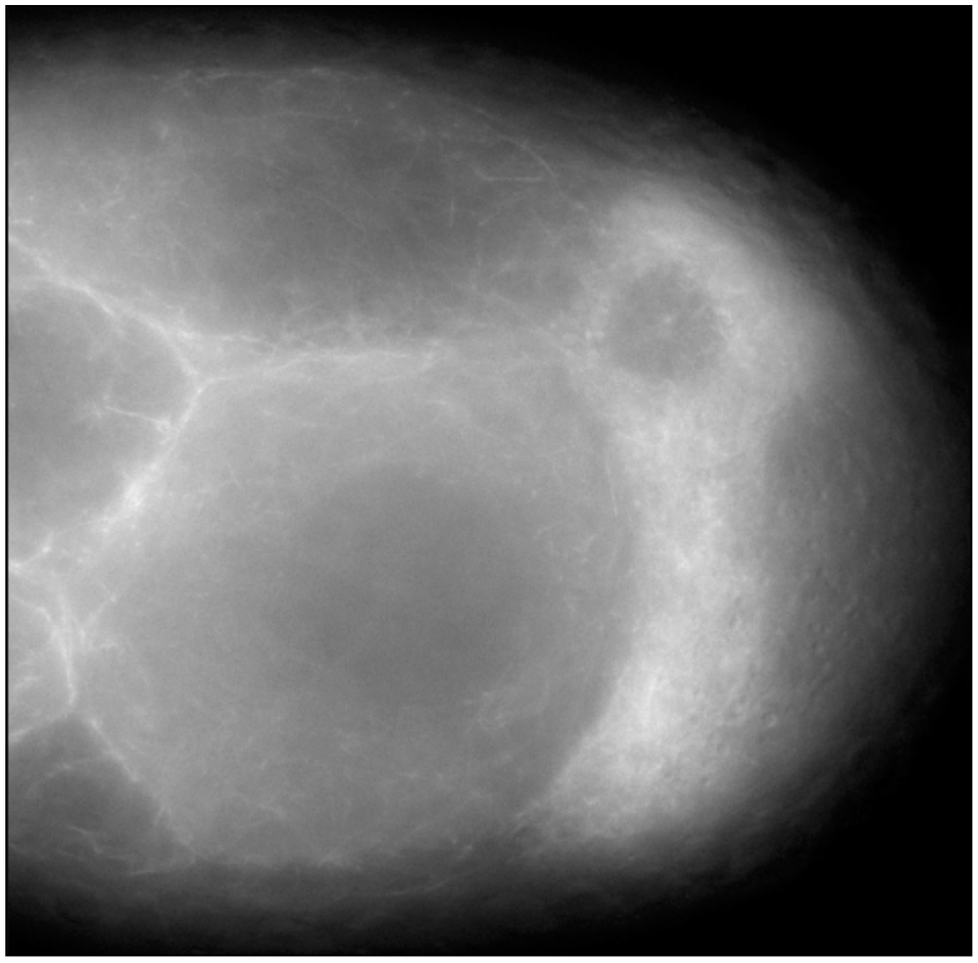
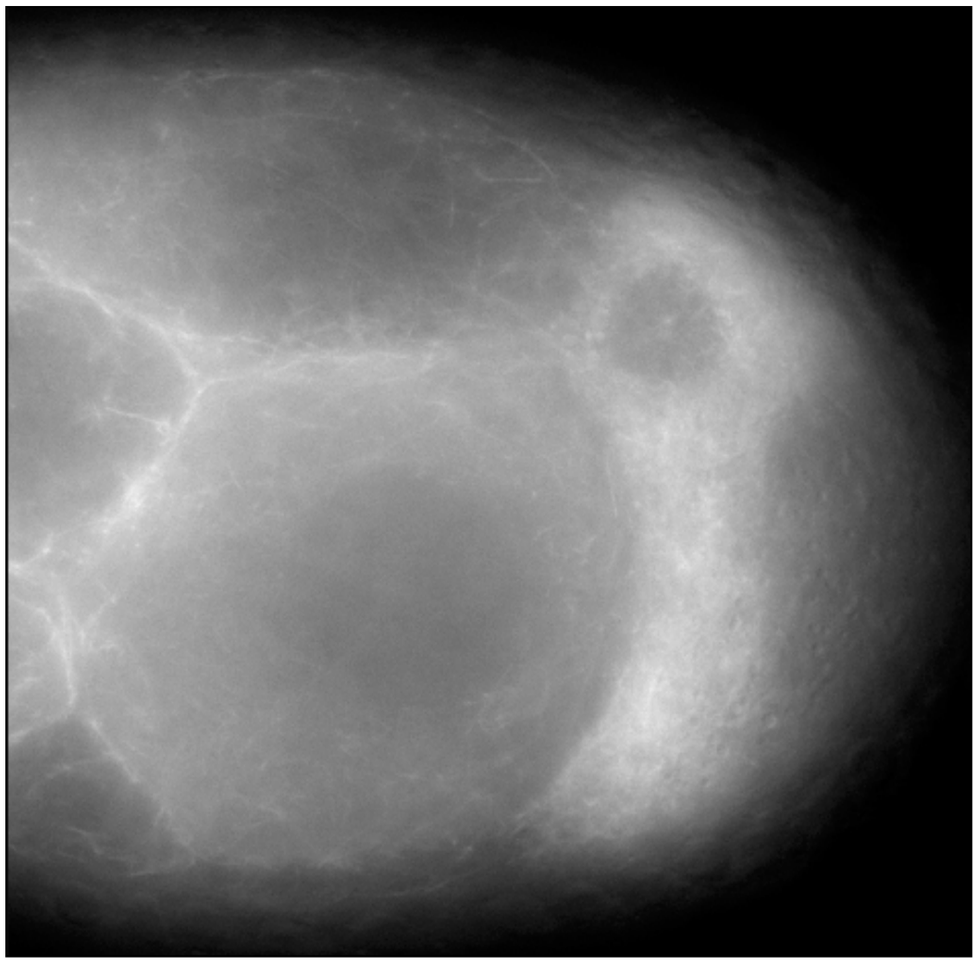


Image Parameters - what to record (= image metadata)



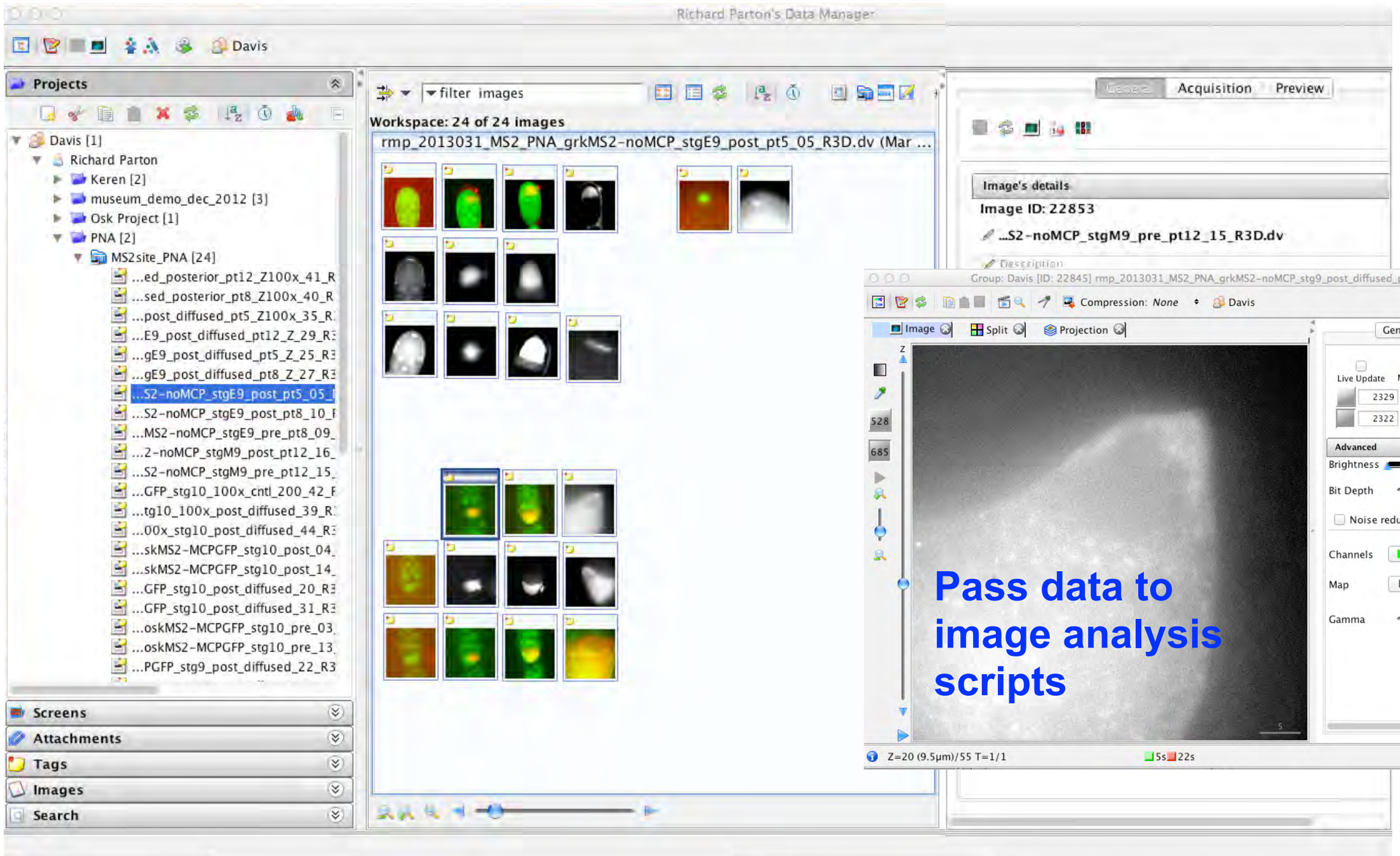
Wide-field fluorescence 490 ex 520 em
X60 1.2 WI
xy 212 nm; 60 (z step 200 nm)
Bin 2x2
250 ms exposure
Contrast stretched to fill 8 bit display
Tau-GFP Oocyte

- **Type of imaging** – Wide-field fluorescence
- **Excitation and Emission** wavelengths 490/520
- **Optics used** – x60 NA 1.2 water immersion
- Image **pixel dimensions** – 212x212 (x200) nm
- **Depth** or Dynamic range – 8 bit; 256 greys
- Any **processing** performed
  - 12 bit to 8 bit conversion
  - contrast adjustment
- **Display parameters** - range 0-255, grey scale
  - gamma = 1
- **The Biology** - *Drosophila* stage 8 egg chamber
  - Tau GFP, labelling microtubules

OME - Open Microscopy Environment

* Douglas Russell, Lecture 18: Image Management *

- **Purpose: Supporting Metadata Management for Microscopy**
  - **avoids problems of image formats**
  - **archiving and retrieval**
  - **data sharing**



What makes a good image?

Adapted From - http://www.go4costumes.com/products/Black-High-Afro-Wig

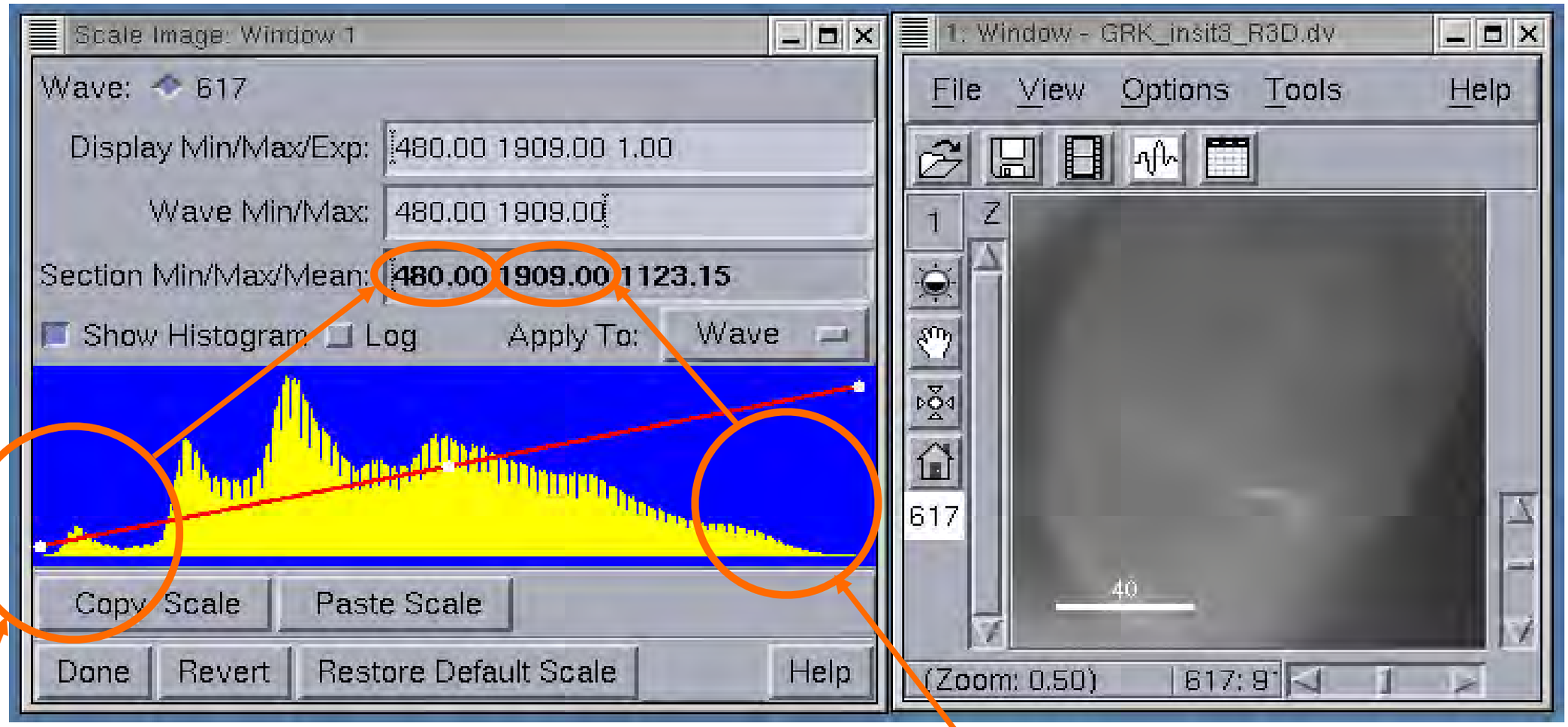
Correct Image Acquisition

- **The system must be correctly set up and aligned**
  - **PSF verification (beads)** *Practical 2*
- **The specimen should not cause undue optical aberration**
  - **mounting / appropriate optics**
- **Avoid underflow and overflow but fill the dynamic range**

  - **use a colour LUT**
  - **beware of auto-intensity scaling**
- **Take a dark signal image and/or background**
  - **– Dark subtraction processing**
- **Be aware of XYZ optical resolution of the system and sample appropriately**
  - **PSF of the imaging system** *Nyquist*
  - **Pixel (voxel in 3D) size in the image**
- **Take care with signal to noise limitations**
  - **collect enough light: integrate, average**

Correct image acquisition

- **Use histogram analysis to ensure you collect enough light in the features of interest**



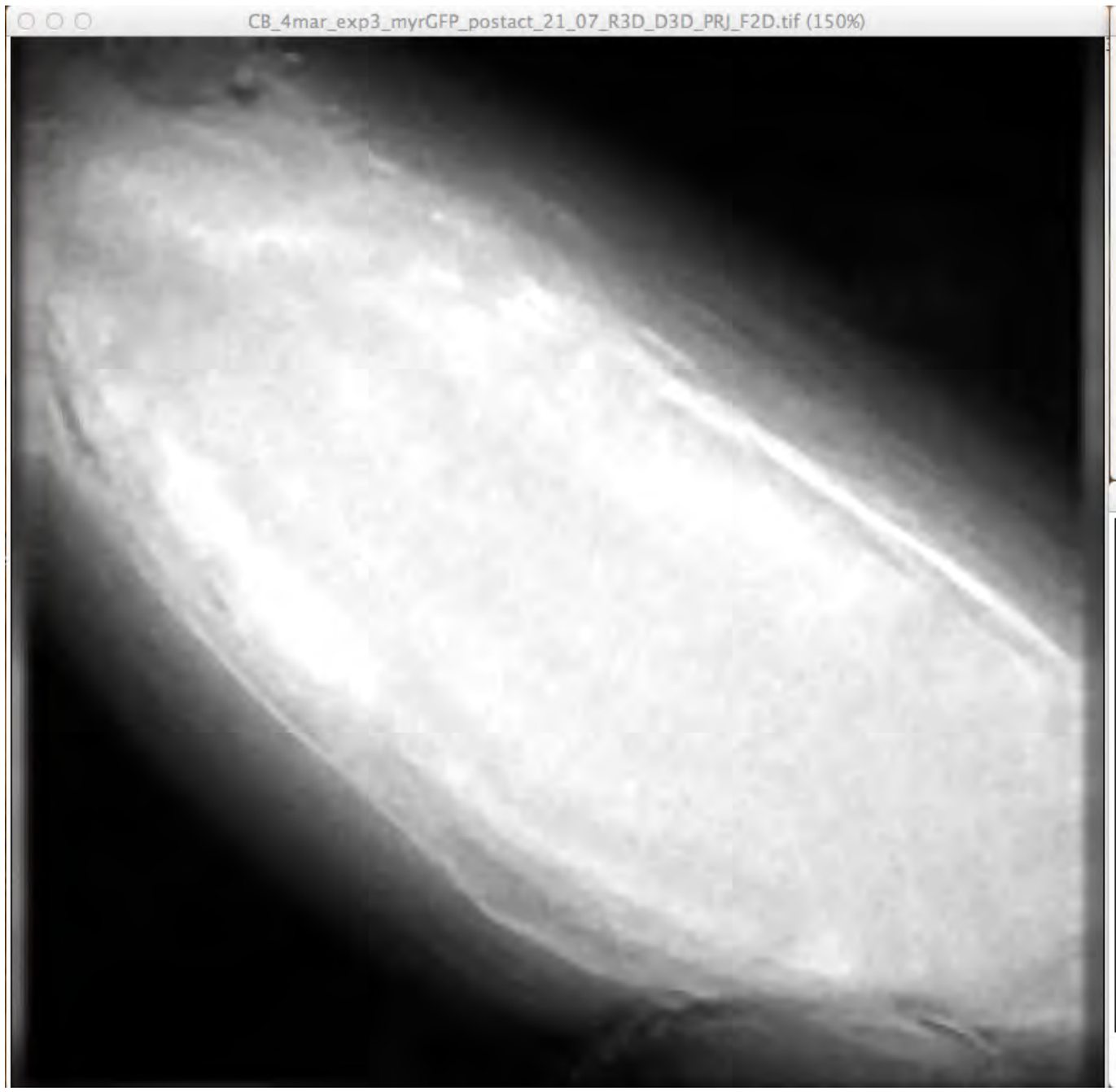
mostly camera offset, dark signal and background

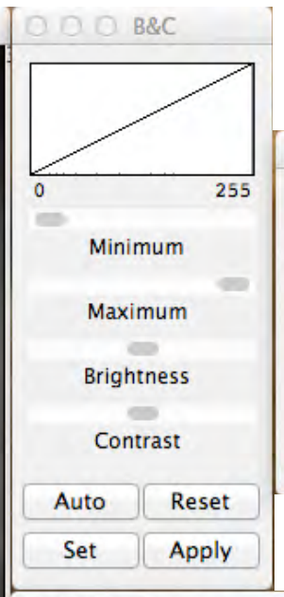
max value should be below detector saturation = avoid saturation

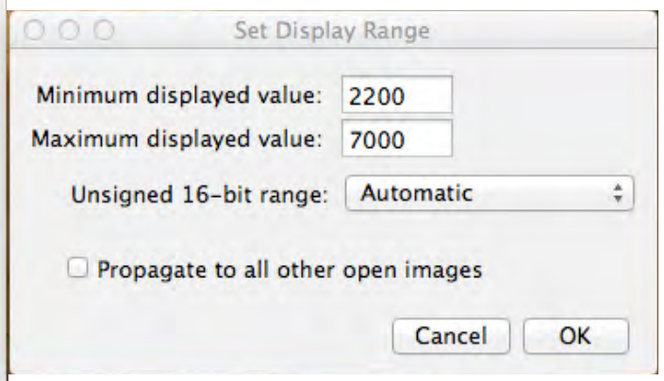
- **NOTE - the brightness of the display is not the best indicator of image signal**
- **Make good use of the dynamic range:8-bit = 255, 12-bit = 4095, 16 bit = 65535**

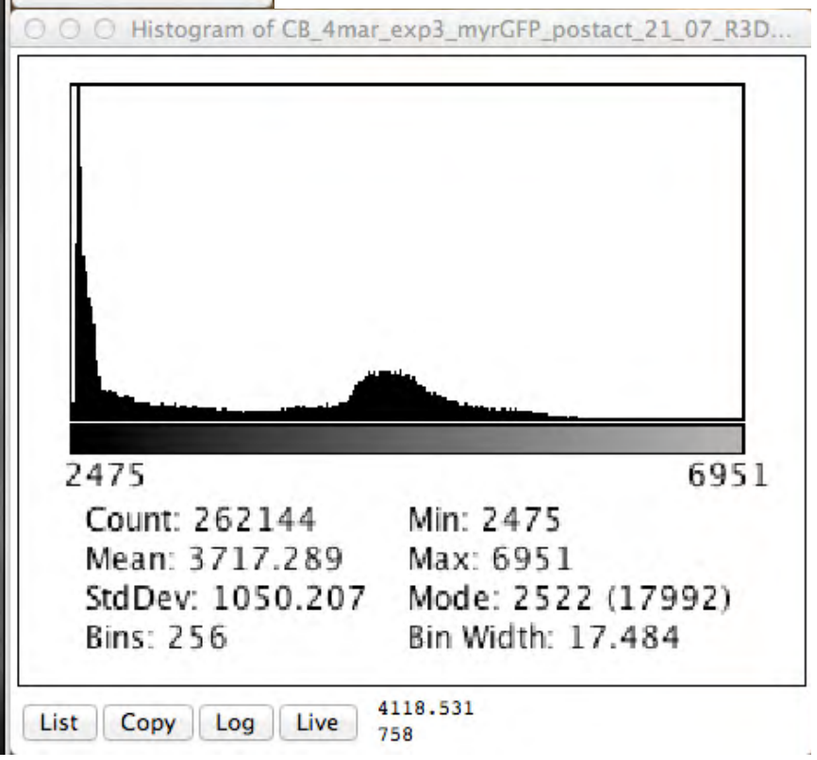
Correct image acquisition

Fiji









Noise / Signal to Noise (S/N)

http://rogewu.comyr.com/brad-pitt-meet-joe-black-wiki.php

Signal to Noise - definitions:

- **One of the most important limitations to image quality and image processing**

$$\text{S:N ratio} = \frac{\text{Signal}}{\text{Variation in the signal}} \qquad \frac{\text{mean}}{\text{S.D.}}$$

- **Noise is NOT background, auto-fluorescence or dark signal**
- **Good image data has a high S:N ratio (>4)**
- **Fundamental limit = Poisson distributed statistics of photon detection (shot noise)**

$$\text{Poisson distributed variation} \qquad \text{S:N ratio} = \frac{n}{\sqrt{n}}$$

- **Statistics of photon counting dictate the minimum useful signal**

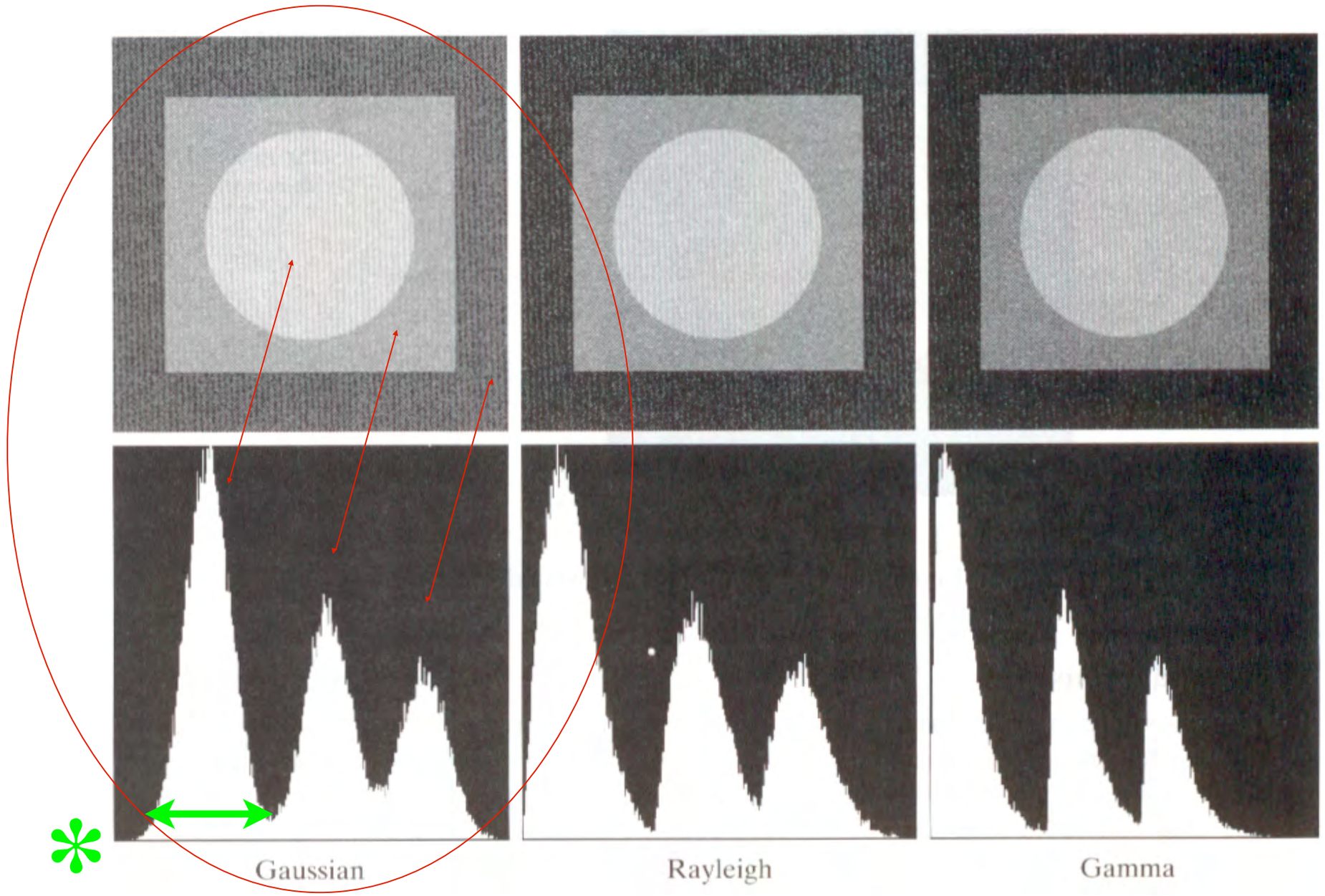
Average signal = 9, S:N ratio = 3
Average signal = 100, S:N ratio = 10
Average signal = 10,000, S:N ratio = 100

A meaningful difference in intensity needs to be at least three times the noise level

- **Additional sources of noise from digitisation, detector readout, thermal noise.**

Signal to noise
- noise types

Most commonly used noise model for image processing = Gaussian



Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

How to deal with signal to noise

Acquisition

- **Use sensitive, high dynamic range, low noise detectors: cooled CCD, EMCCD**
- **Count as many photons as possible:**

  Bright dyes
  Good excitation / emission
  Integration time (accumulation or averaging)

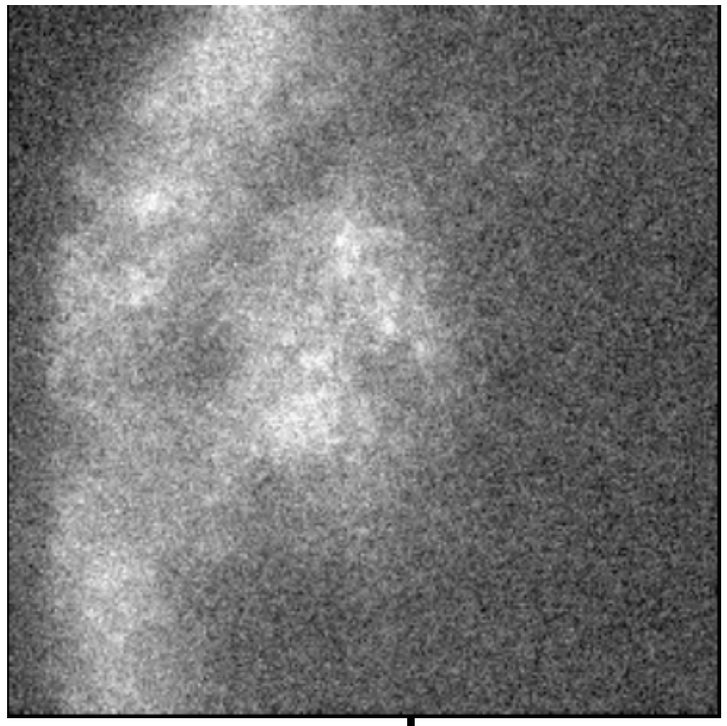
Post Acquisition

- **Image averaging**
- **Noise reduction filtering using a spatial filtering mask – 3x3 median filter**
- **Noise reduction filtering in the frequency domain – Fourier bandpass filter**

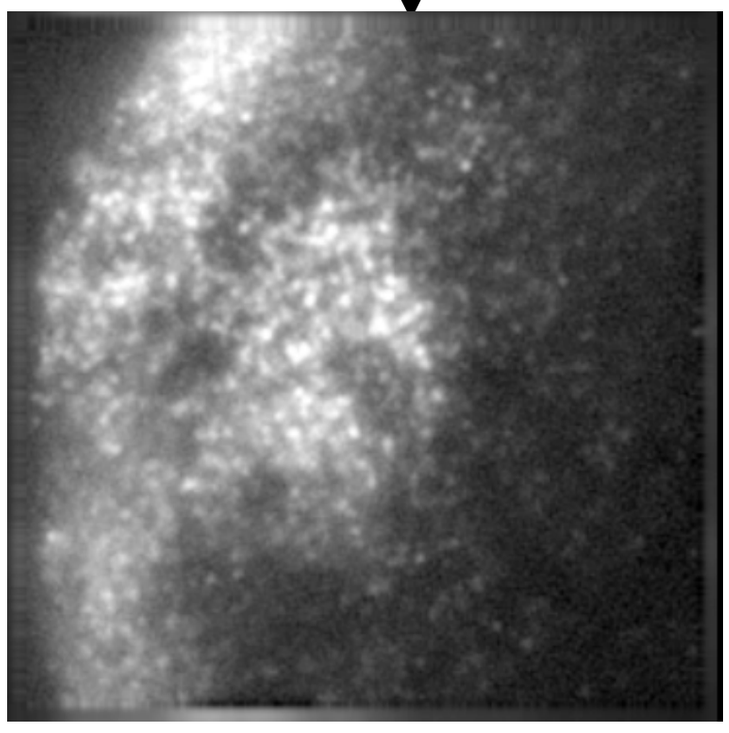
Improving signal to noise

increased signal increases S/N = improved contrast

noisy image (scaled)



5x integration time



increased number of photons counted

improved S/N

ImageJ

Avoid propagating noise

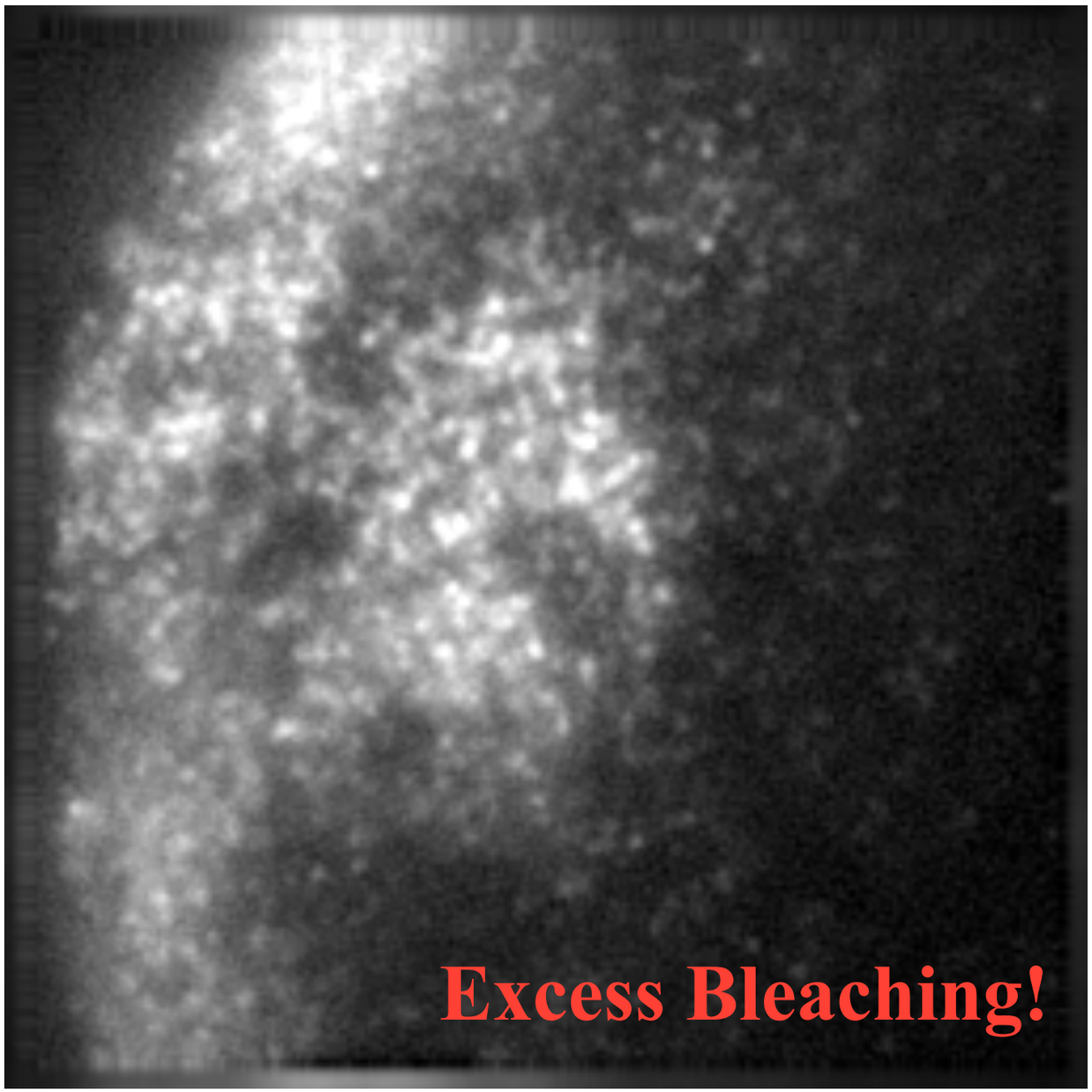
- **Noise is additive:**
  SO subtracting one noisy image from another propagates noise

  THEREFORE
  Subtract an AVERAGE signal to avoid noise propagation
  Or
  Where the signal is non-uniform across the field subtract a 4x AVERAGED image to avoid noise propagation

http://www.dpcorner.com/topics/

Detail Preserving Denoising Algorithms

10 ms; 50% power;
projected 4Z; 100T



grk MS2(12), MCP-GFP 7Z, 3stacks/s

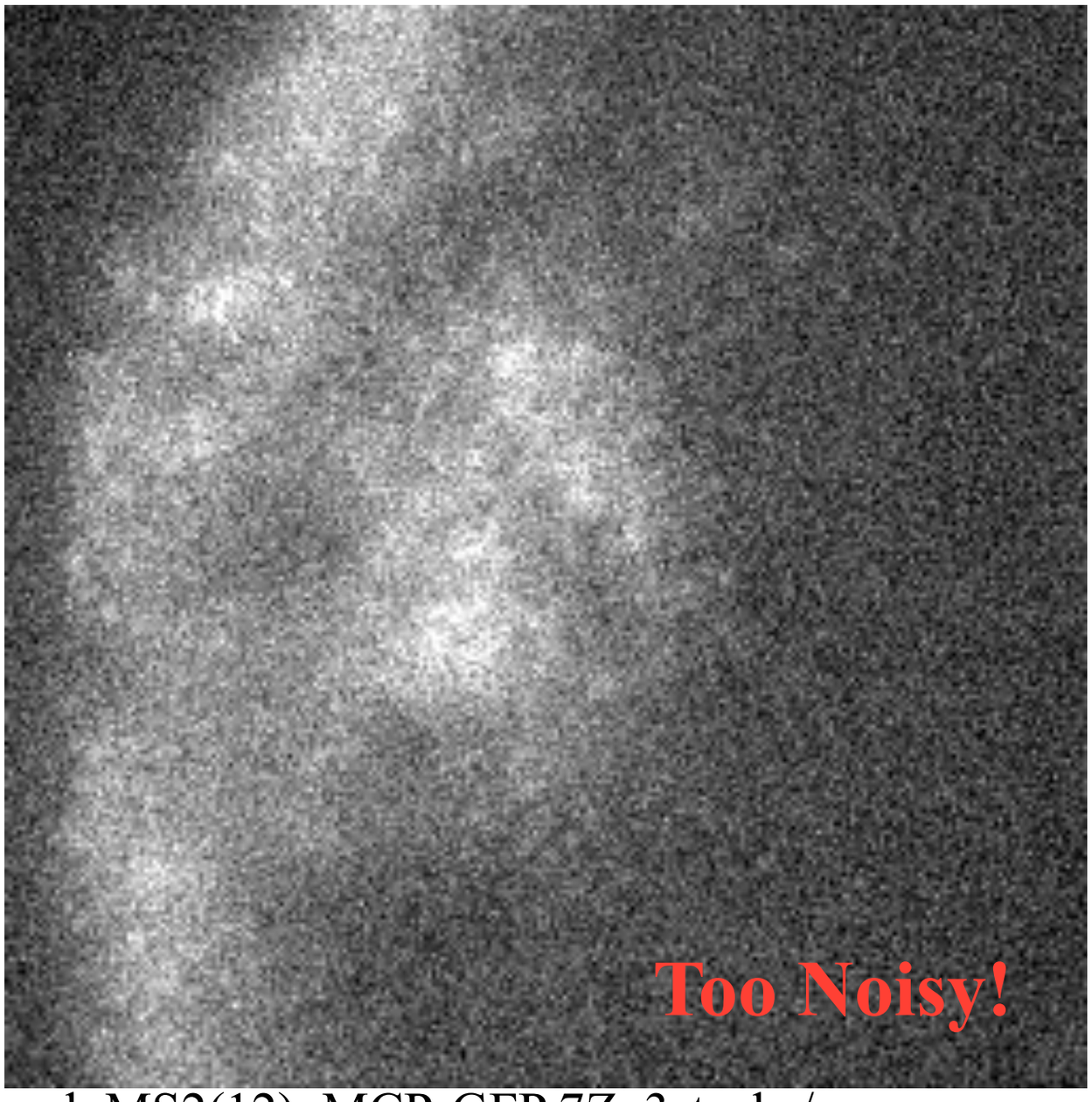
* Collaboration with Graeme Ball

Detail Preserving Denoising Algorithms

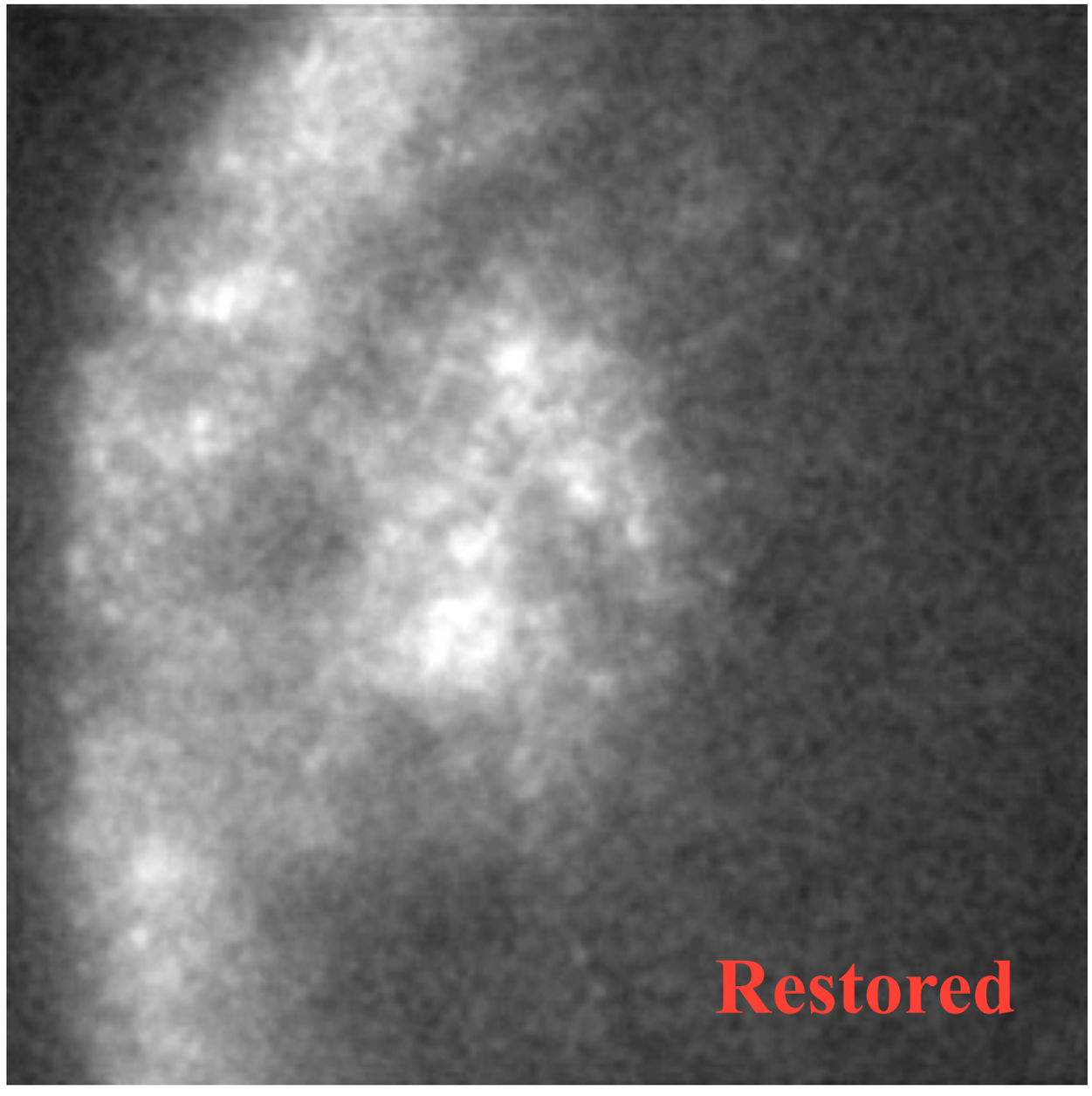
Patch-Based Denoising Kervrann and Boulanger

Boulanger, J., Kervrann, C., Bouthemy, P., Elbau, P., Sibarita, J.-B., & Salamero, J. (2010)

10 ms; 1% power;
projected 4Z; 100T



dn_Iter2_P3



grk MS2(12), MCP-GFP 7Z, 3stacks/s

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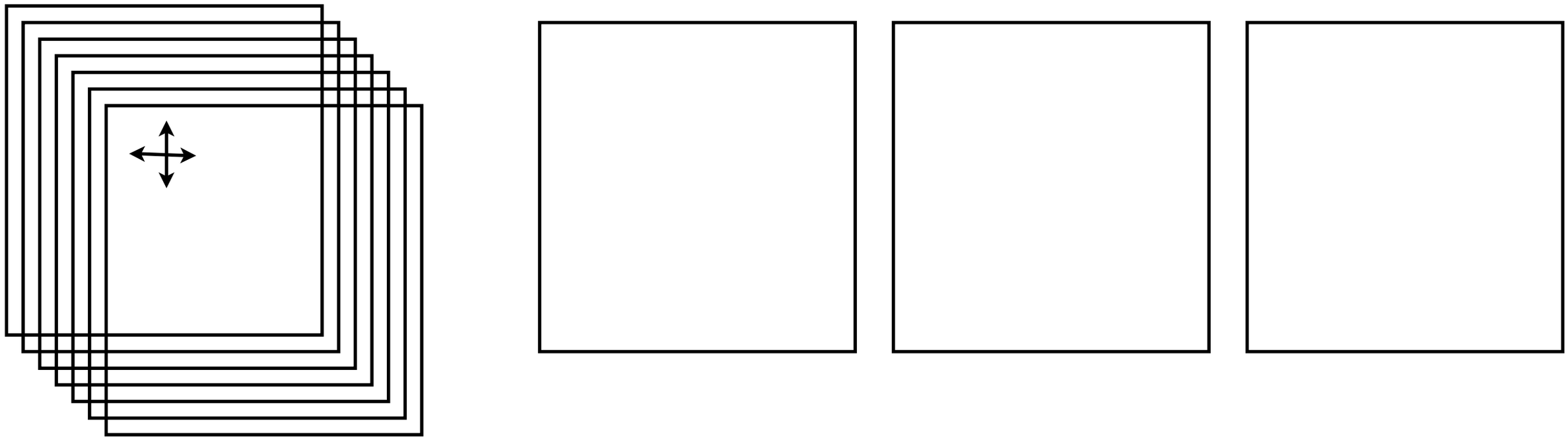
Detail Preserving Denoising Algorithms

* effectively averaging redundant data across a data set



XY redundancy at least 3 x 3 pixels = 9 (multiplies for each Z frame)

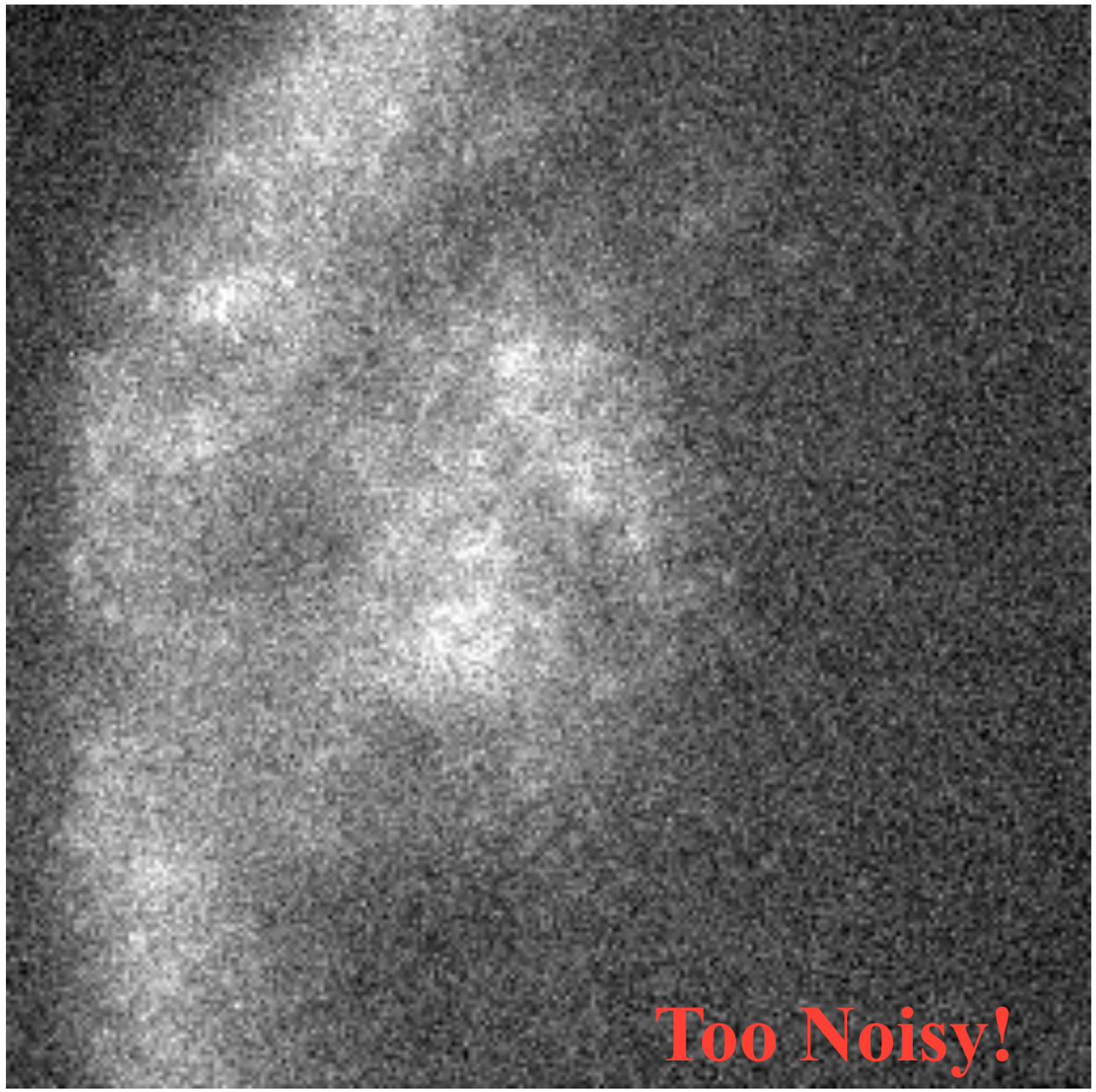
Z redundancy 3 pixels (Z >5 required for deconvolution)

t redundancy 3 to 100 pixels (depends upon events and temporal sampling)

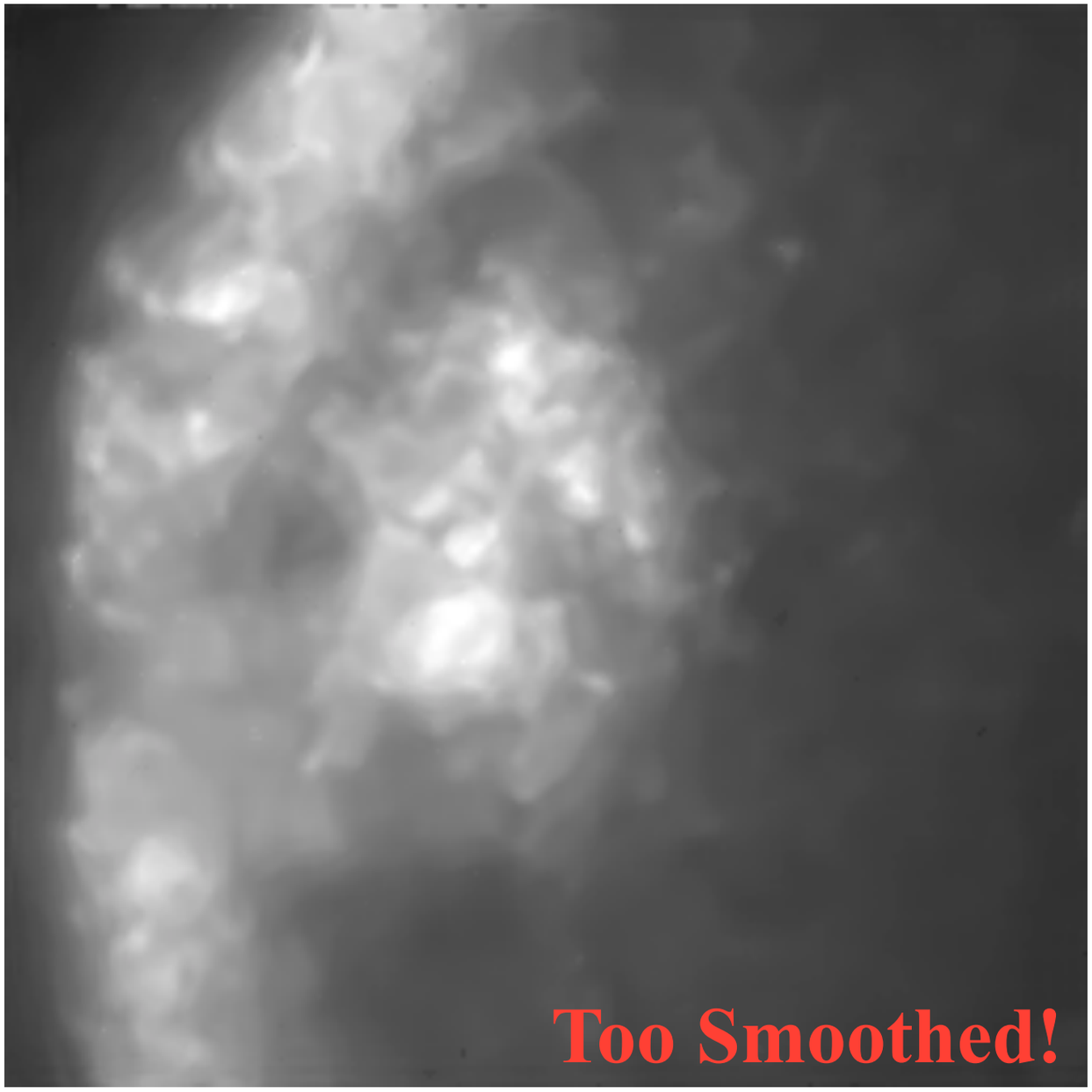
* root [(3 x 3) x7z x 9t] = 24 times noise reduction
* root [(5 x 5) x7z x 25t] = 66 times noise reduction

Detail Preserving Denoising Algorithms: oversmoothing

10 ms; 10% power;
projected 4Z; 100T



dn_Iter5_P3



grk MS2(12), MCP-GFP 7Z, 3stacks/s

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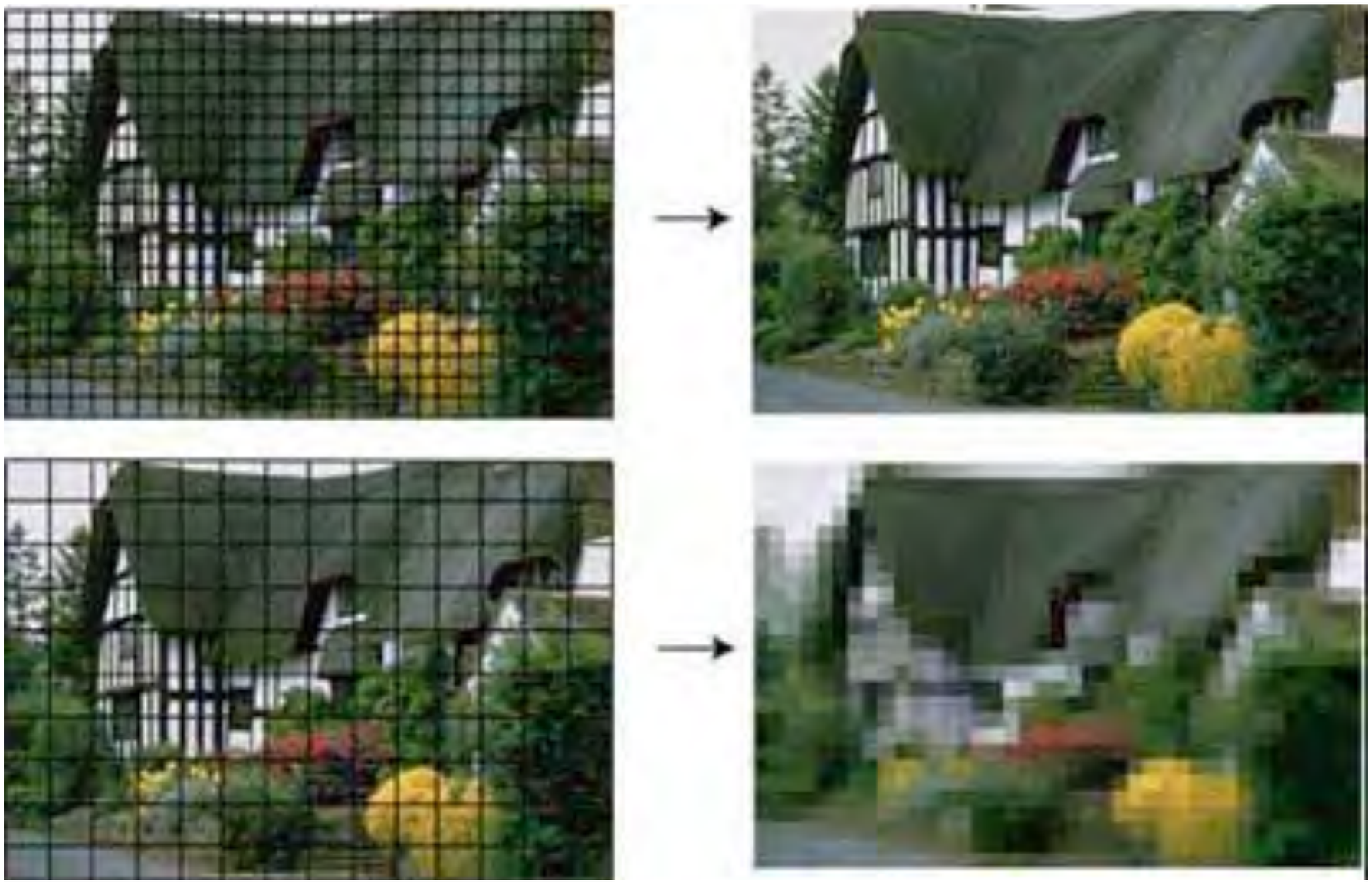
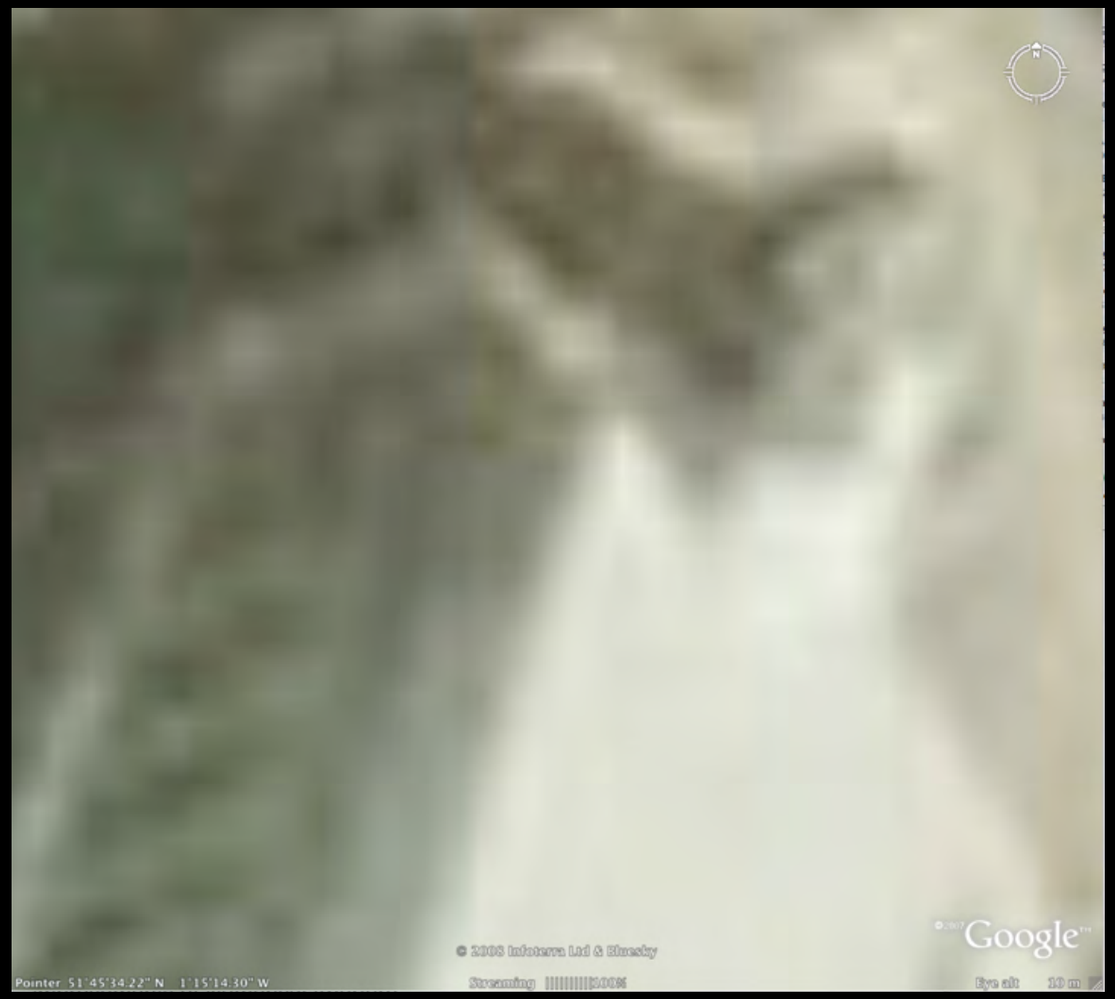
Resolution and Sampling



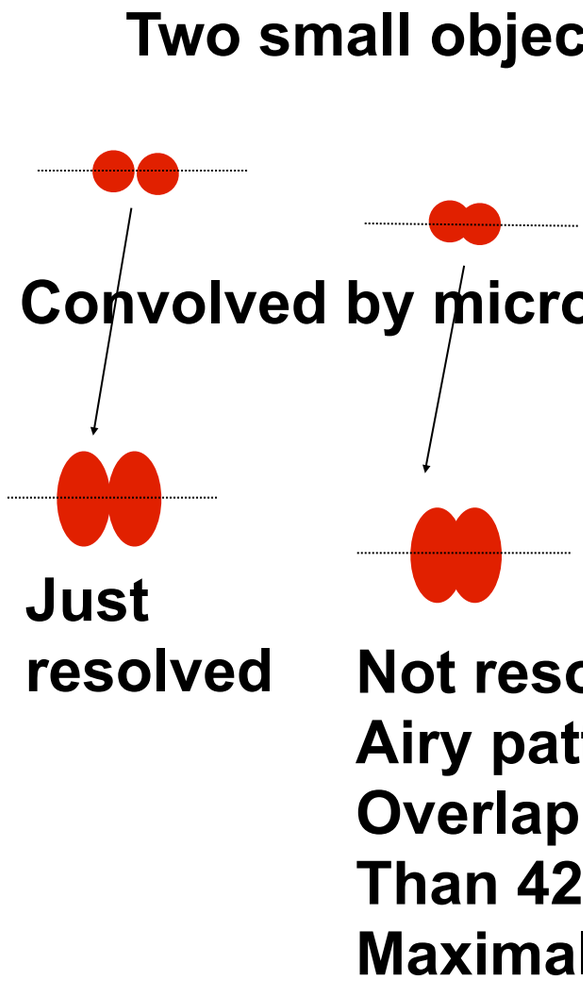
Resolution .....the ability to see small stuff

Magnifying is not enough:



.....resolution is limited

Optical resolution: The Rayleigh Criterion

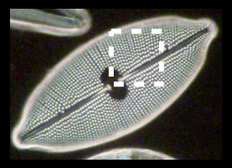


resolution limit $\approx \lambda_{em}/2$

XY resolution ~ 200 nm
Z resolution ~ 500 nm

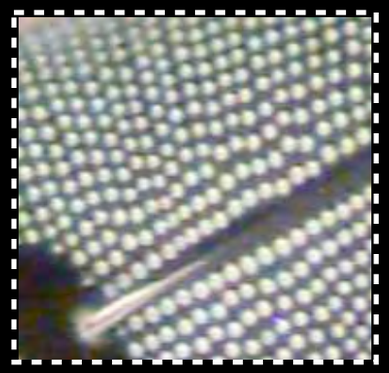
Resolution .....Magnification and Sampling

Specimen
Fine Detail

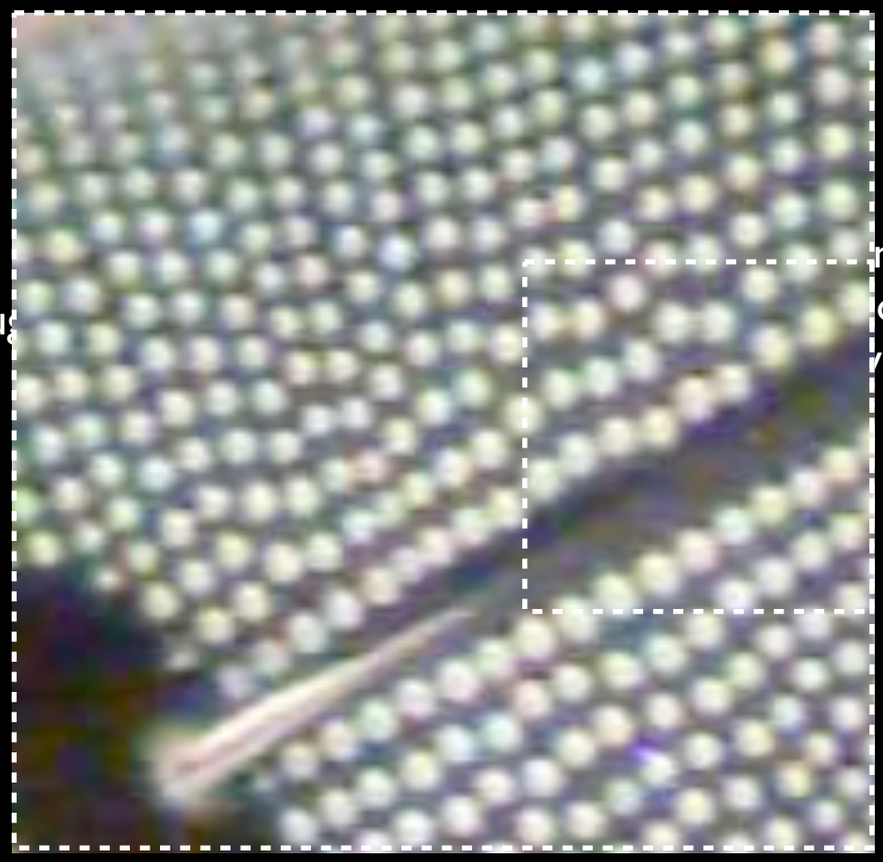


Detail imaged
by microscope

*magnification
*optical resolution



too magnified enough



not resolved
wide field of view

crop

auxiliary magnification to match image to detector

to match image to detector

Oversampling
Empty magnification
All resolvable detail recorded
Blurred image
Limited field of view

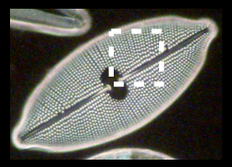
What is the optimum magnification.....?

For optimal imaging: magnification must match the resolution to the detector

Diatom image: http://www.micromagnus.net

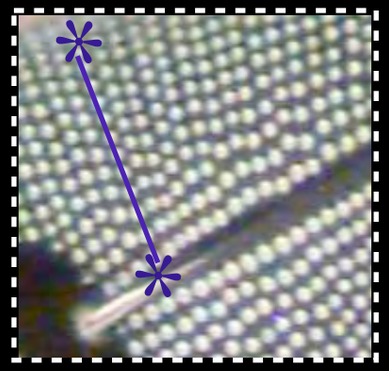
Resolution .....Magnification and Sampling

Specimen
Fine Detail

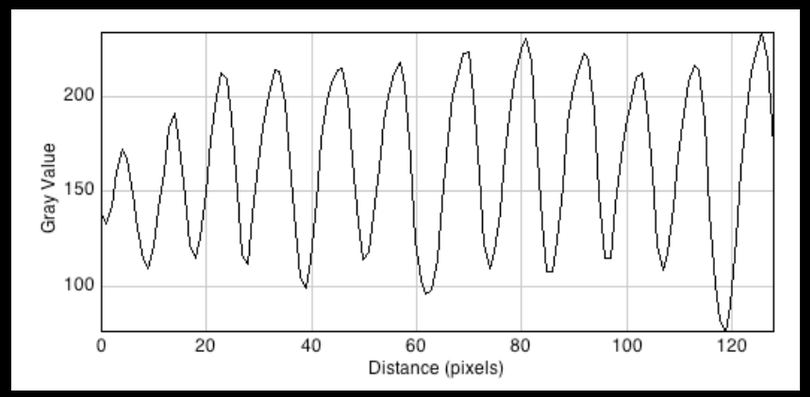


Detail imaged
by microscope

*magnification
*optical resolution



intensity profile ~ a sine wave

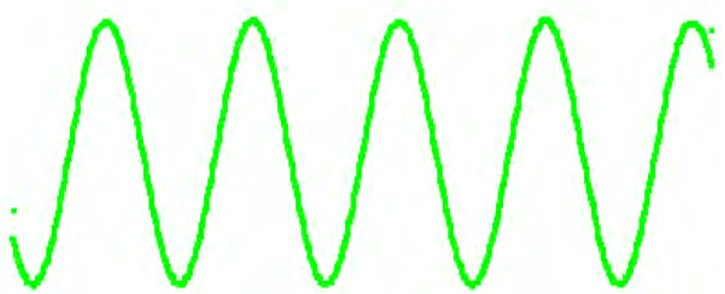


Optimum = 2.3 times per cycle

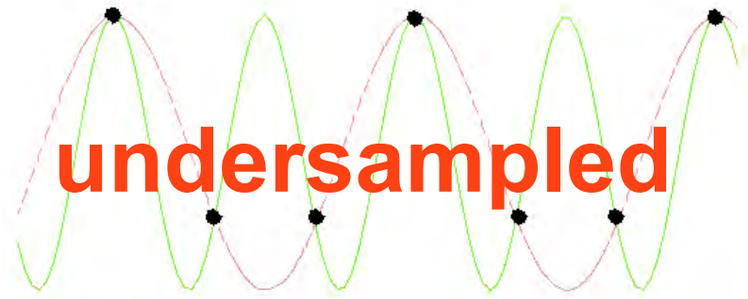
= Nyquist sampling

Diatom image: http://www.micromagnus.net/index_micro.html

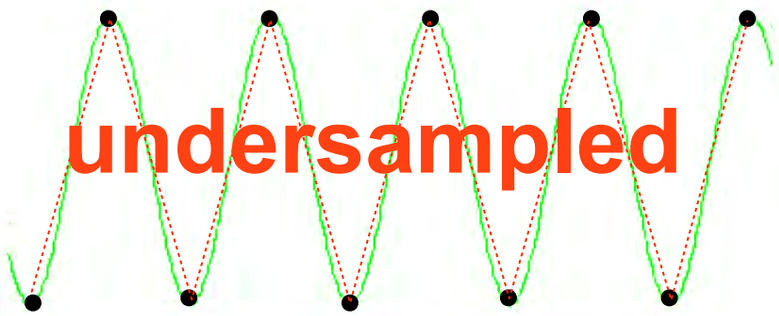
sampling a sine wave



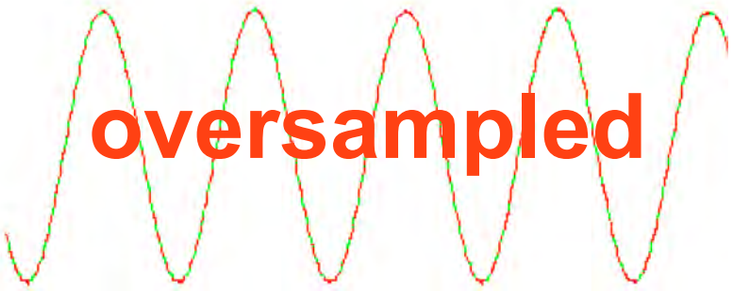
Sampling 1.5 times per cycle



Sampling 2.0 times per cycle



Sampling many times per cycle



Resolution .....Magnification and Sampling

For optimal imaging the magnification must match the resolution to the detector (eye or camera).....

optimal total mag X resolvable distance = detector element size

Considering Fluorescence imaging x100 objective; 1.4 Na; 520 nm emission,.....

total mag	resolvable distance	Detector Element
x100 objective X x1.0 Aux mag	1.22 x **λ520**/ 2Na	Camera pixel element = 6.6 um (/~3 taking into account Nyquist)

Rearranging to find the optimum magnification ....

$$\text{optimal total mag} = \text{Na} \times \frac{2 \times \text{Detector Element}}{1.22 \times 520\ \text{nm}} \approx \text{ideal pixel size} \sim 80\ \text{nm}$$

$$1.4 \times \frac{6600\ \text{nm} \times 2 \times 3}{1.22 \times 520} \approx \mathbf{87 \times mag}$$

x100 obj = GOOD SAMPLING

Resolution: sampling

Theoretical Axial Resolution (em 525 nm)	Appropriate Sampling According to Nyquist theorem - at least half the size	Pixel size on Delta Vision
1.4 oil = 229 nm	Nyquist ~ sample at 0.100 um/pixel	x100 = 0.063 um/pixel
1.35 oil = 237 nm	Nyquist – sample at 0.103 um/pixel	x60 = 0.106 um/pixel
1.2 water = 267 nm	Nyquist – sample at 0.116 um/pixel	x40 = 0.158 um/pixel
0.75 air = 427 nm	Nyquist – sample at 0.186 um/pixel	x20 = 0.317 um/pixel

- **Undersampling limits the data available**

Interactive tutorial – http://www.microscopyu.com/tutorials/java/digitalimaging/pixelcalculator/

Resolution, contrast, noise

- **Noise limits the contrast which limits the details that can be resolved**
  = Noise limits resolution

Resolution, contrast, noise

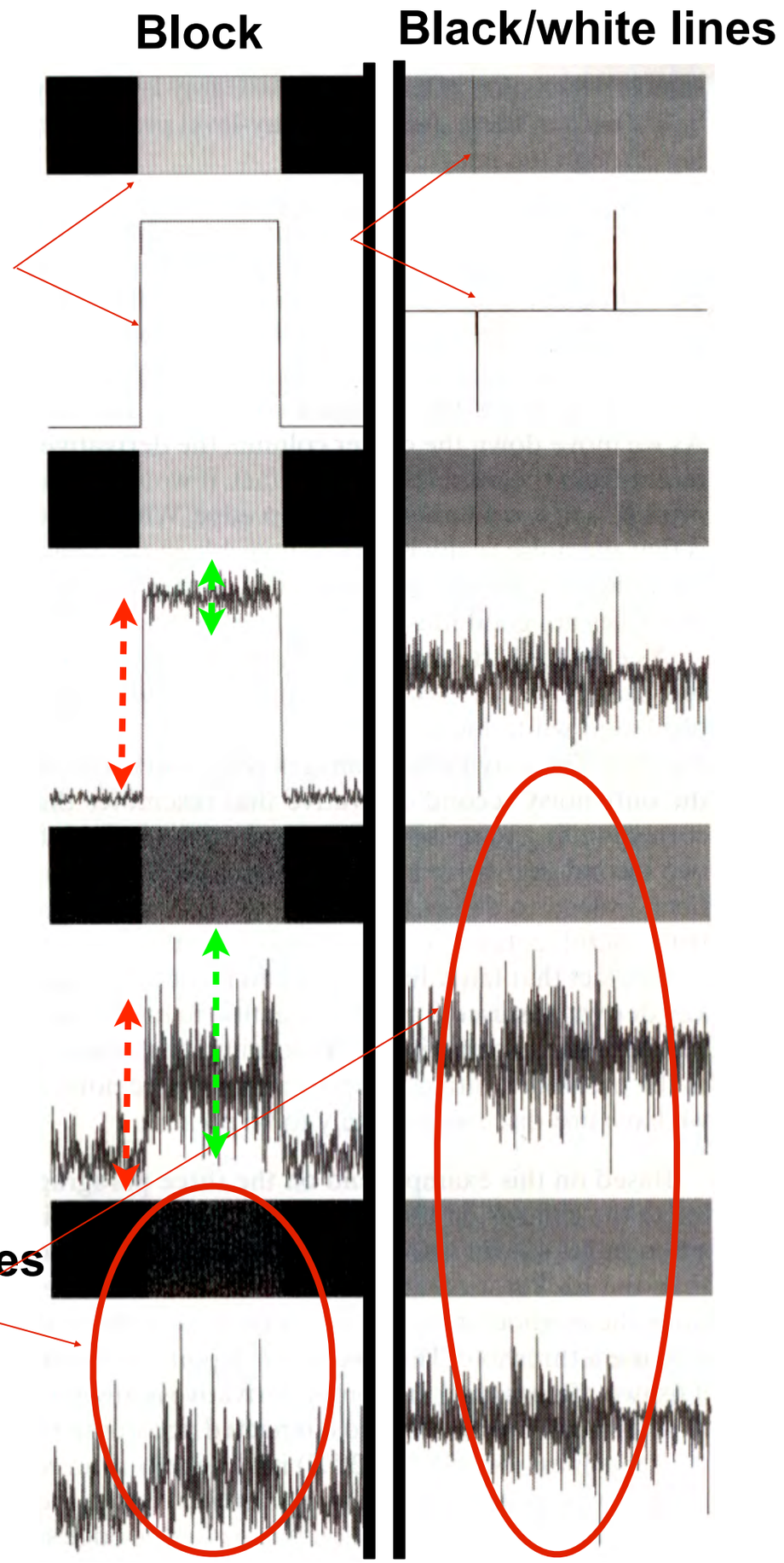
The **difference between signal and background** must be at least 3X the **noise** to be detectable

Boundaries and lines easily resolved in the absence of noise

Increasing levels of Gaussian noise

Decreasing S/N

Boundaries and lines no longer resolved



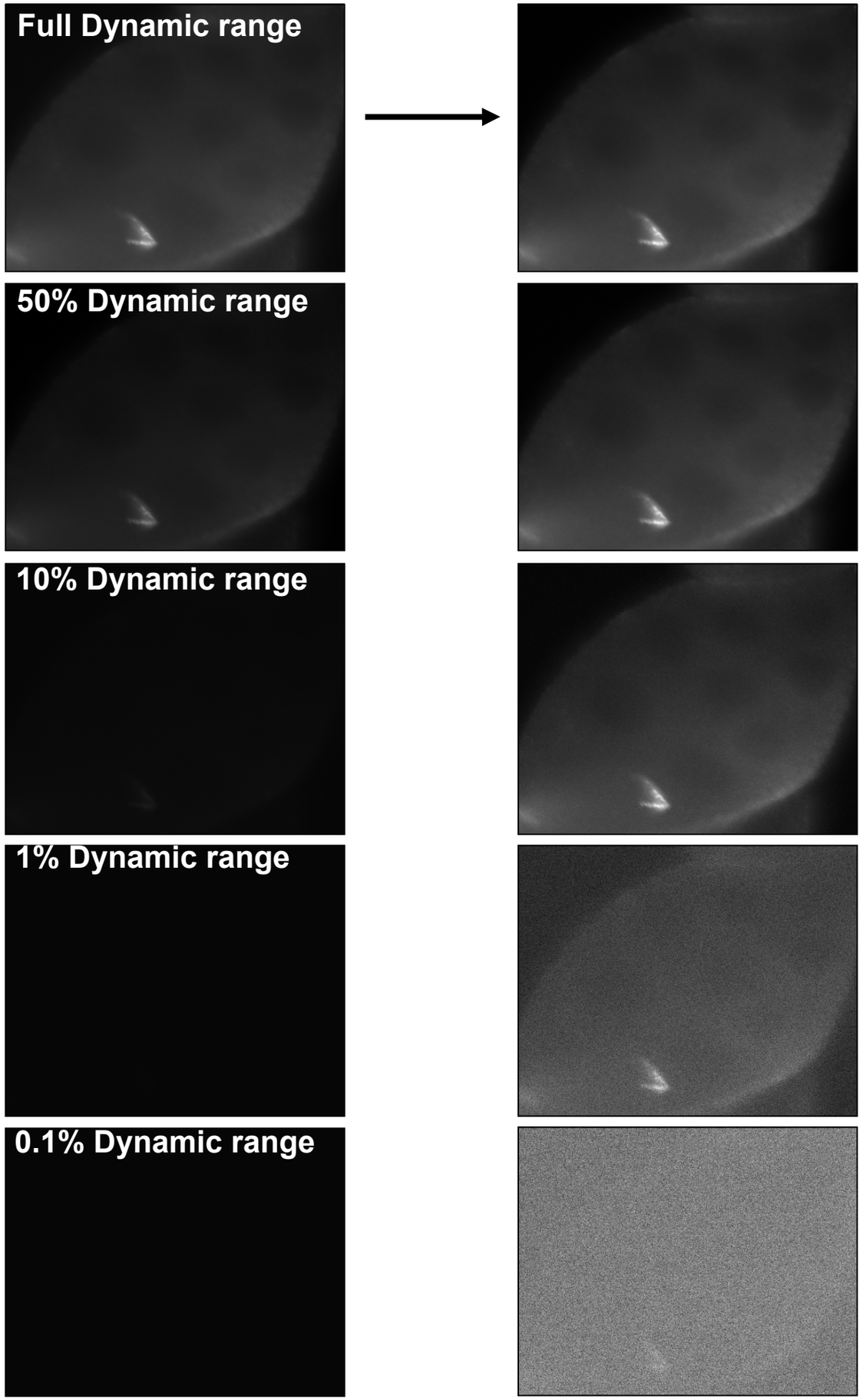
Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

Resolution
Contrast
noise

Image series collected by decreasing the excitation lamp intensity from 100% to 50%, 10%, 1%, and 0.1%

Scaled 0 - 4095

Auto Intensity Scaled display



Decreasing S/N

Increasingly Noisy looking

Decreasing Image quality

Cannot resolve

softwoRx API

The basics of image processing

"I want you to make me the fairest of them all."

Remember what makes a good image

- **Good image data has a high S:N ratio (count more photons)**

- **Correctly sampled to reproduce the optical resolution (pixel = optical resolution/2)**

- **Avoid aberrations (sample prep / choice of objective / technique)**
  - **spherical aberration (SA)**
  - **motion blur**
  - **bad system alignment**

- **Correctly annotated (Metadata retained)**

Image Processing is NOT a substitute for a good image

http://mrsofmr.files.wordpress.com/2011/10/picofjohnny.jpg

http://rogewu.comyr.com/brad-pitt-meet-joe-black-wiki.php

Golden Rules of Image Processing

- **Always retain the original data**

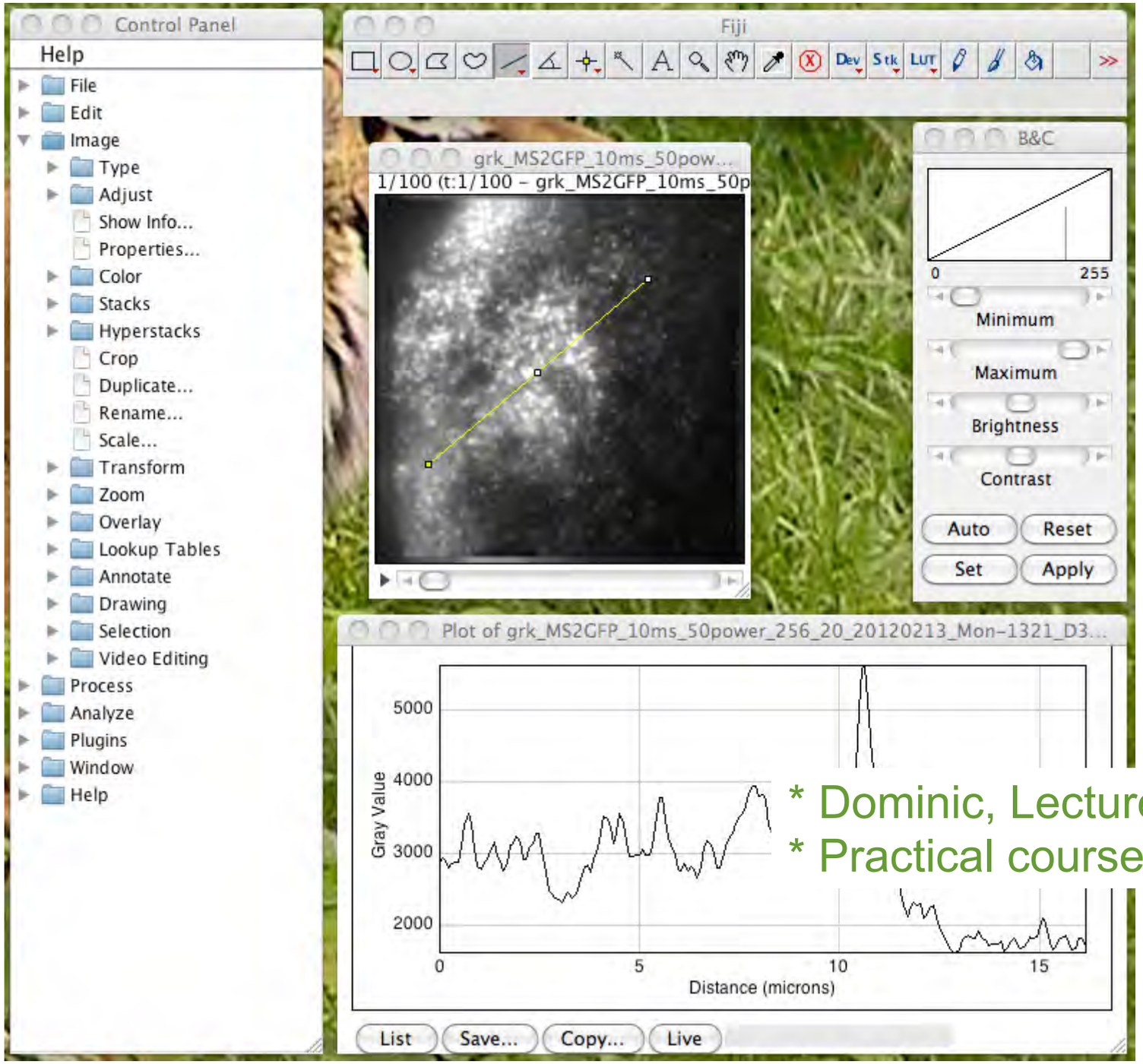
- **Do not corrupt the integrity of the original data through processing:**
  Processing should NOT generate data not present in the original image

- **Images are arrays of numerical data and should be given appropriate consideration.**

- **Always record and report all processing steps.**

Fiji = Image J http://fiji.sc/wiki/index.php/Fiji (http://rsb.info.nih.gov/ij/)

- **Fiji is FREE and works on MAC, PC and linux**
- **Consists of a core program and plugins**
- **Uses Loci Bioformats to convert between file types**



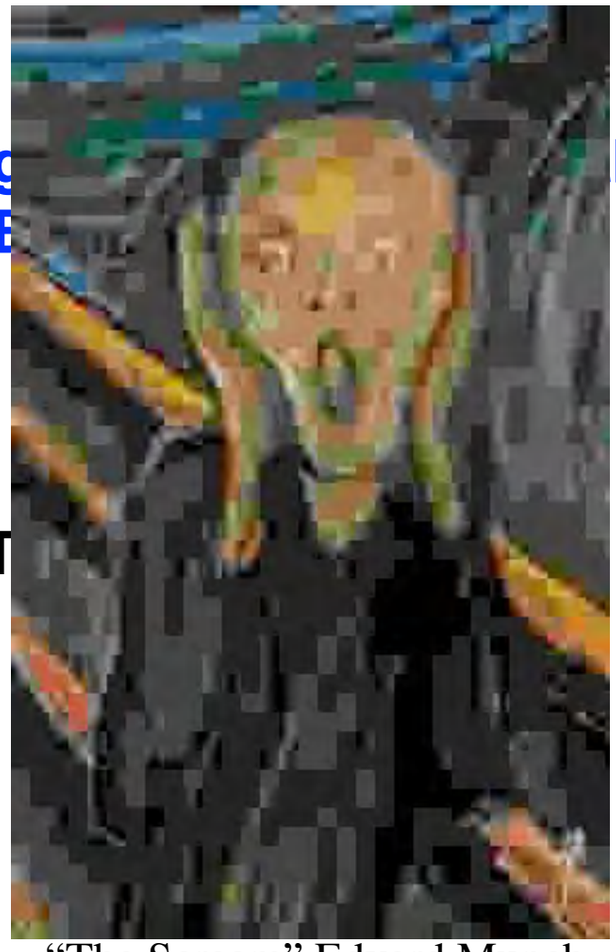
Fiji Is Just ImageJ

For users, Fiji is easy to install and has an automatic update function, bundles a lot of plugins and offers comprehensive documentation.

* Dominic, Lecture 17: Applied Image Analysis *
* Practical course - Demo of Fiji plugins and macros *

Do not corrupt the integrity of the original data

- **Retain your original data in its original file format and original metadata associations**
- **Consider OMERO for data archiving (lecture 18 - OME, bioformats)**
  http://loci.wisc.edu/software/bio-formats
- **Ideally use Uncompressed TIF (tagged image file format) for processed data**
- **AVOID compressed file formats when processing: JPEG, PSD, PDF, compressed TIF…This will cause data corruption and loss**
- **Most data is collected as single channel grey scale imag h**
  Avoid saving primary image data in colour formats (RGE
- **Avoid repeated inter-conversions of file formats**
- **Prepare figures for publication in Adobe Photoshop in T**
  and annotate in Adobe Illustrator



"The Scream" Edvard Munch

Conceptual Hierarchy of image processing

- **Low - level processing** = Image enhancement (most common)
- **Mid - level processing** = Features and attributes extracted
- **High - level processing = Analysis** = Interpretation of images

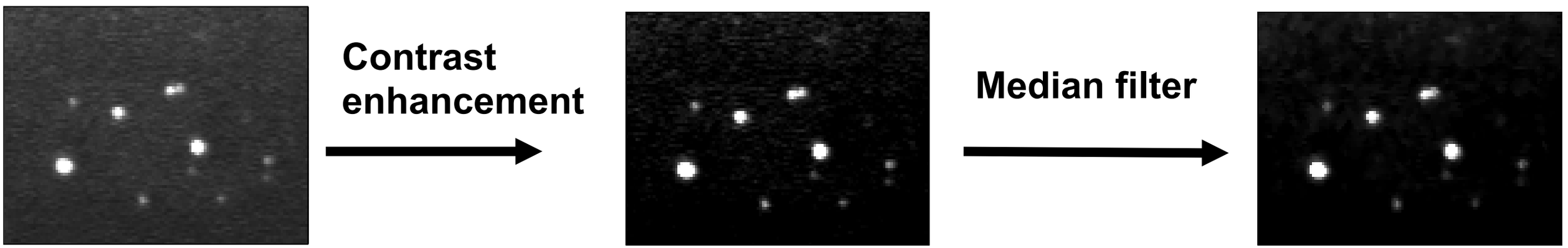
Gonzales & Woods (2002). Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

LOW LEVEL Processing

- Visual enhancement
- Subjective = looks better
- **Input is an image, output is an image**

- Enhancement Filters

- Adjustments for image Display / Making figures



LOW LEVEL Processing - Image Restoration

- **"Restoration" = attempting to reverse distortions of the object which arise during image capture**

*** Flat-field correction - correcting uneven illumination**

*** Deblurring = Deconvolution, with or without PSF, unsharp mask**

*** Simple filtering - post acquisition increase in S/N e.g. by averaging**

* Dominic, Lecture 17: Applied Image Analysis *

*** Normalization - intensity of each time-point scaled to correct bleaching (or flicker)**

*** Denoising - post acquisition increase in S/N without loss of resolution**

*** Image registration - alignment of multiple channels for co-localisation analysis**

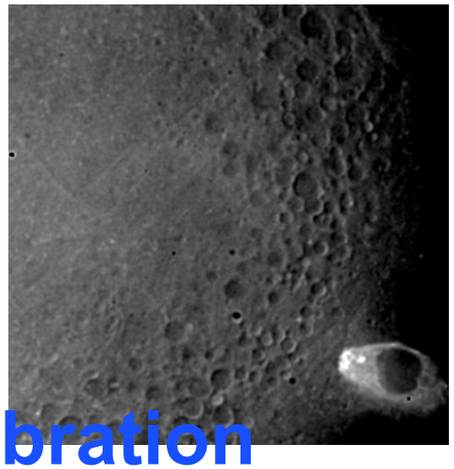
LOW LEVEL Processing - “Background” Correction

- **Requires dark image, flat field image and detector calibration**

- **Detector offset / background**
  subtract averaged dark value or image

- **Uneven illumination**
  correct using a flat field image

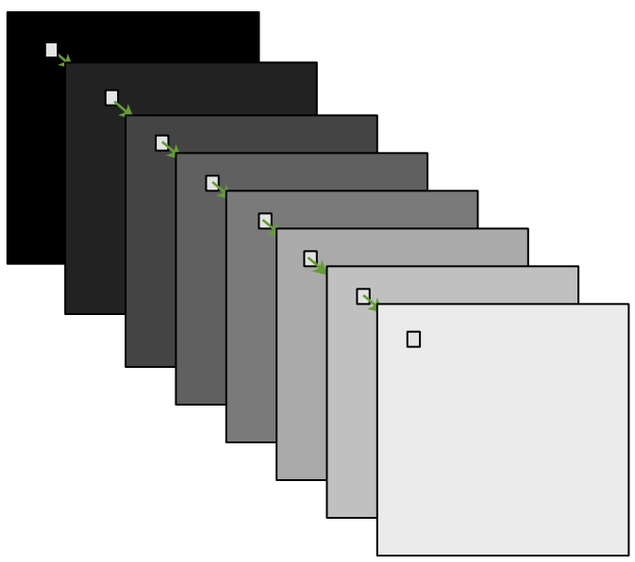
original image

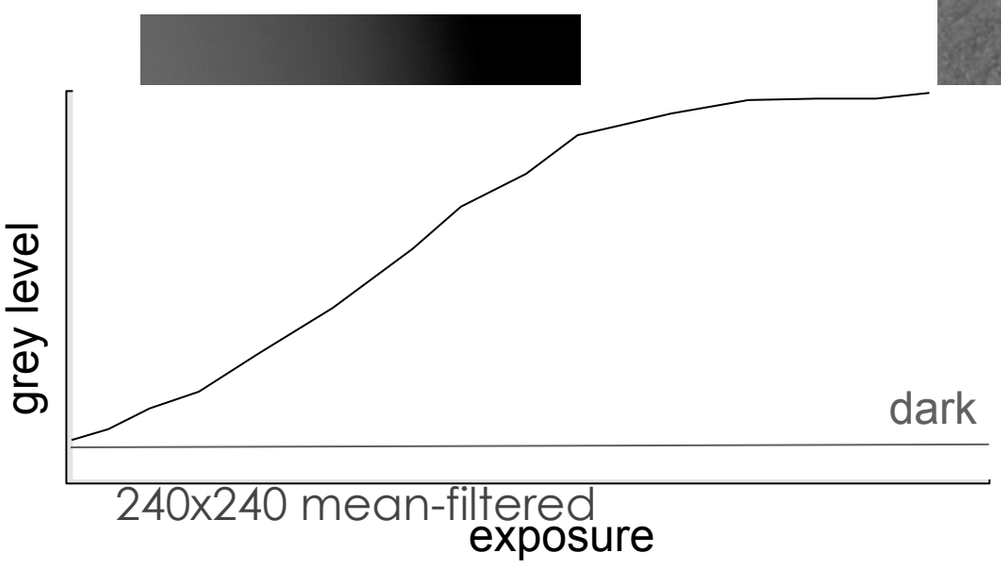


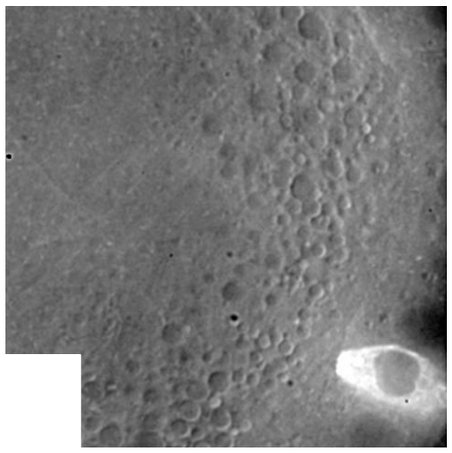
pseudo-flat field

original/mean-filtered * mean value

- **Uneven detector response**
  normalise using a detector calibration
  mapping the response of each pixel



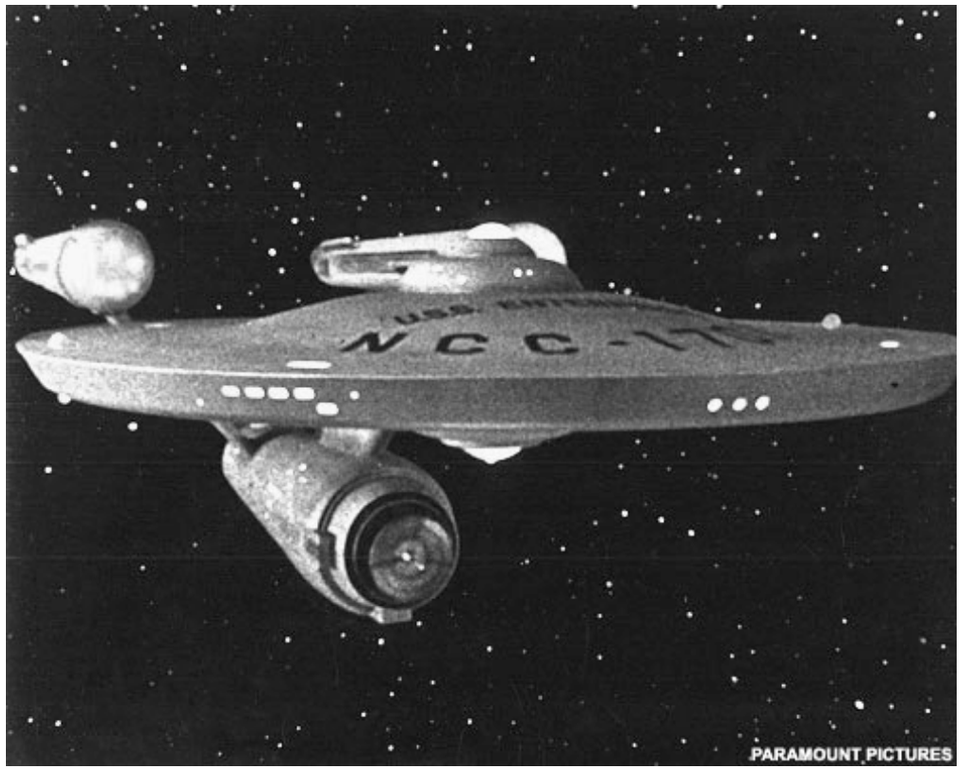




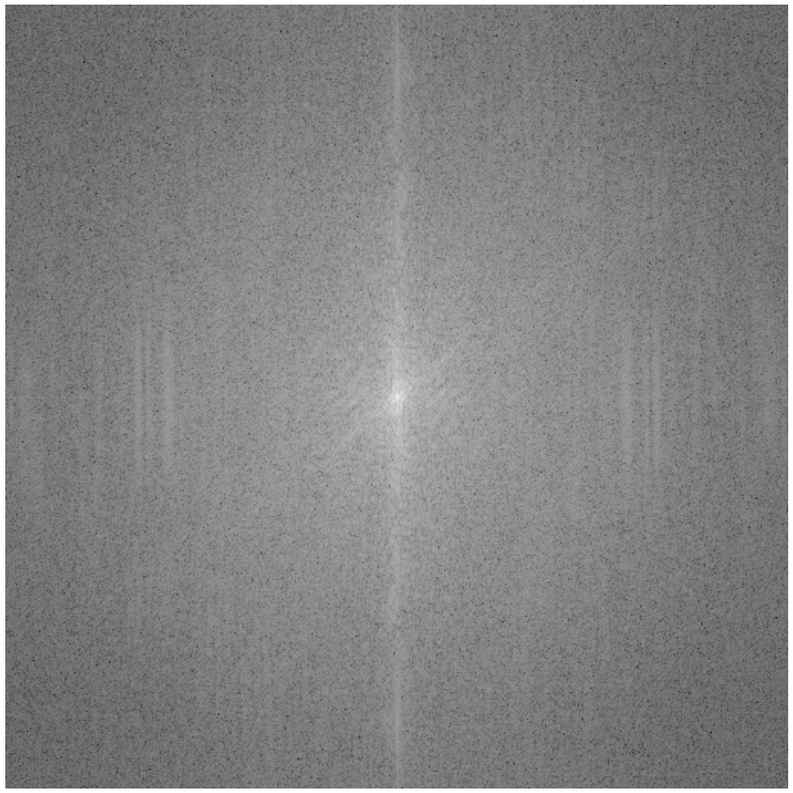
eudo-corrected

LOW LEVEL Processing - Simple filtering

Processing in Real Space or Frequency Space



Fourier Transform →



Example:

- Noise reduction filtering using a spatial filtering mask – 3x3 median filter
- Noise reduction filtering in the frequency domain – Fourier bandpass filter

LOW LEVEL Processing - Simple filter, spatial domain

- **Real space - pixel by pixel**

e.g. Noise reduction filtering using a 3x3 median filter

MEDIAN 3x3:
Replaces value of a pixel by the median grey scale value of the ranked values of the 9 neighbourhood pixels.

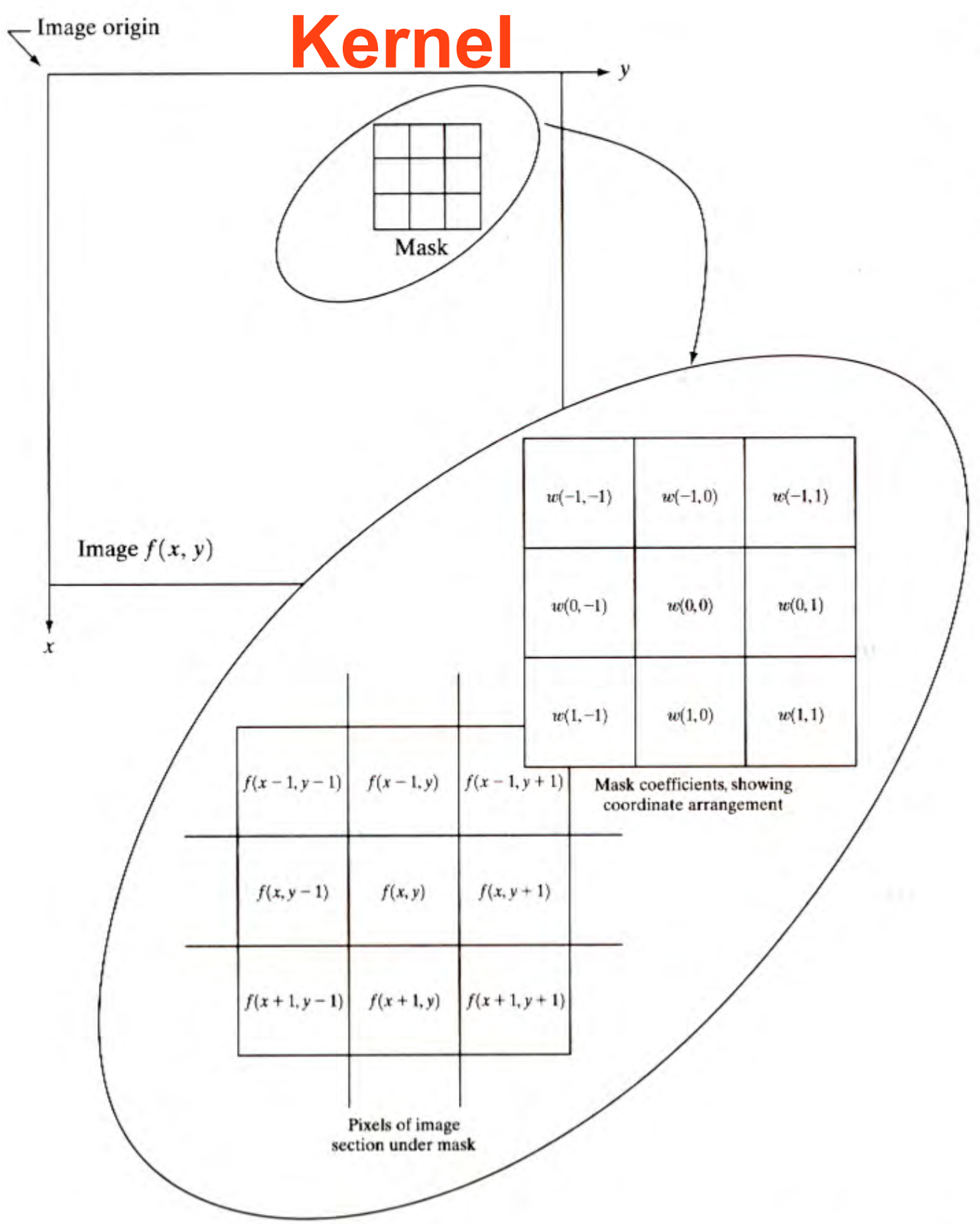


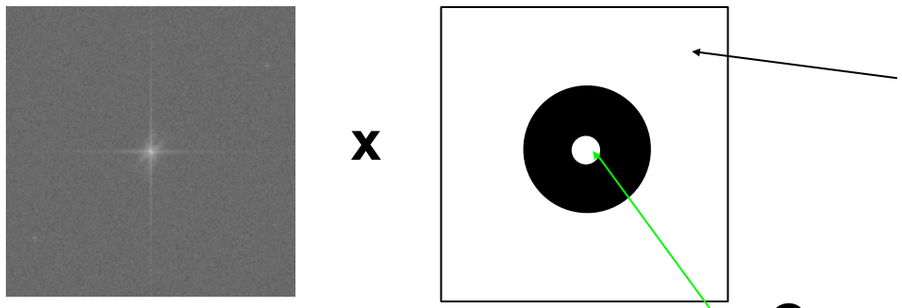
FIGURE 3.32 The mechanics of spatial filtering. The magnified drawing shows a 3×3 mask and the image section directly under it; the image section is shown displaced out from under the mask for ease of readability.

Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

LOW LEVEL Processing - Simple filter, Fourier domain

- **Frequency domain - images converted to Fourier space**

  e.g. Noise reduction using a low pass or band pass filter



x

Fourier "mask" selectively eliminates the highest frequencies = noise and very fine detail.

Can also eliminate large expanses of even intensity - low frequencies

Works on the whole image at once

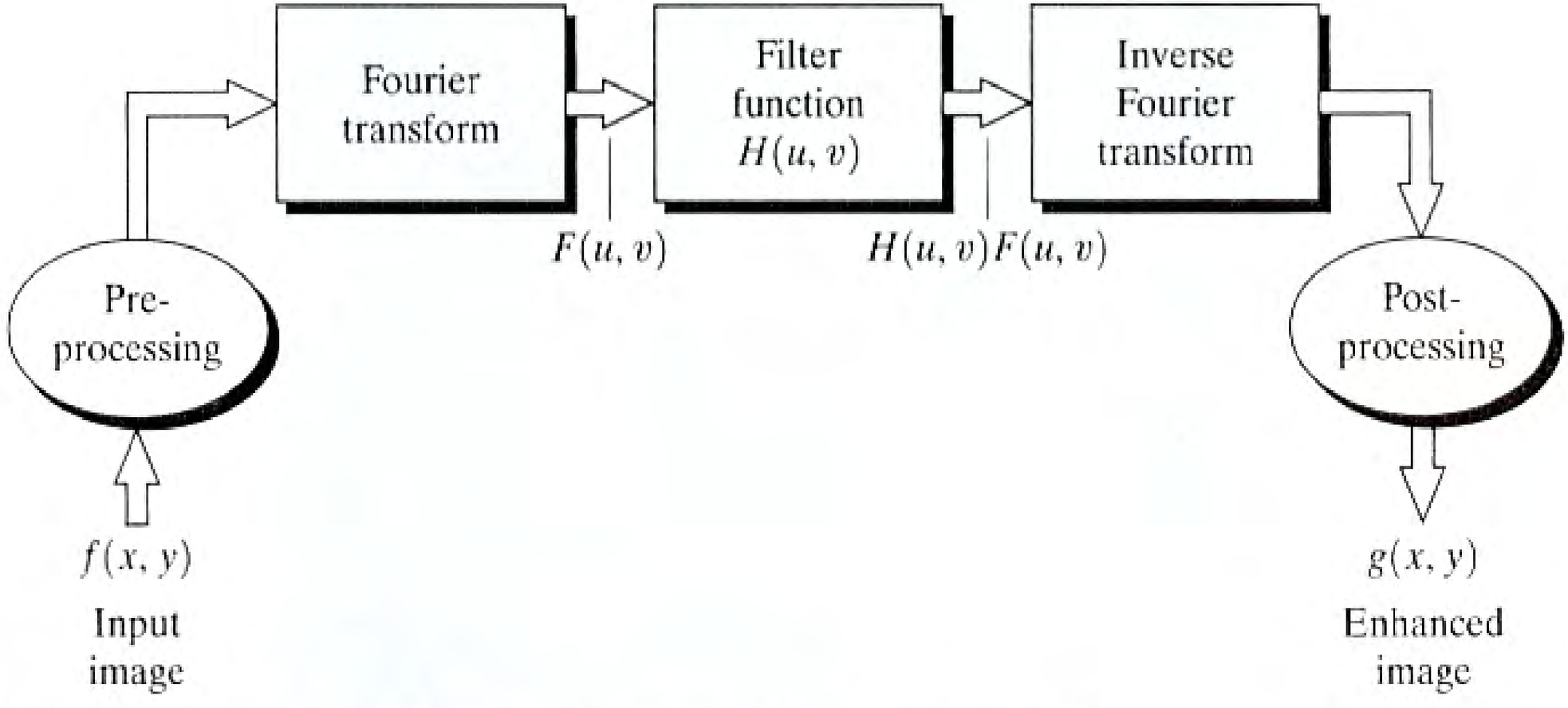


FIGURE 4.5 Basic steps for filtering in the frequency domain.

Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

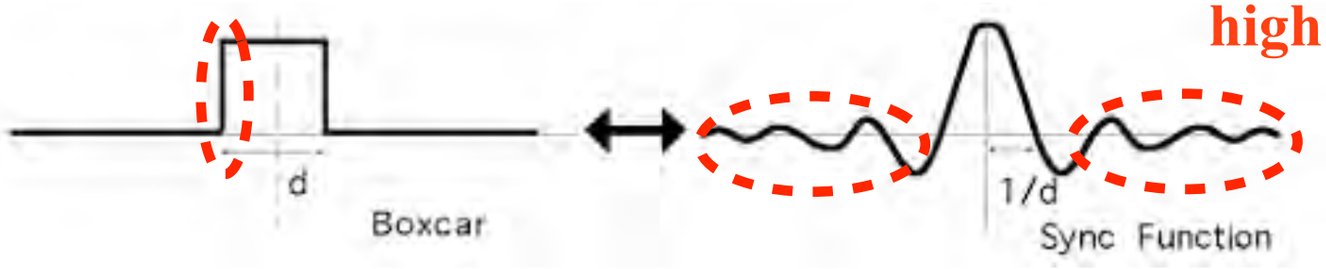
Fourier Space = Frequency space
= Reciprocal space
= K space

Data is broken down into its "frequency" components

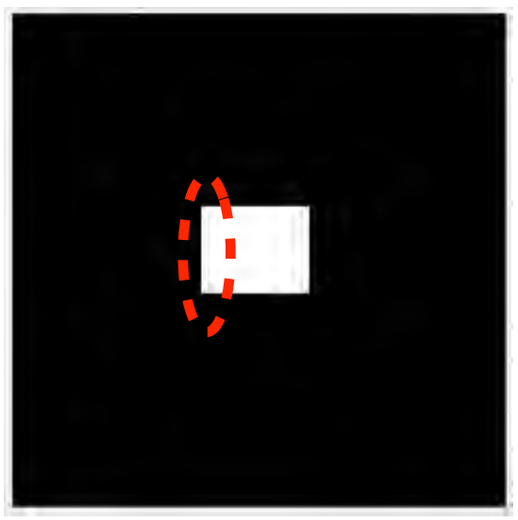
real space **reciprocal space**

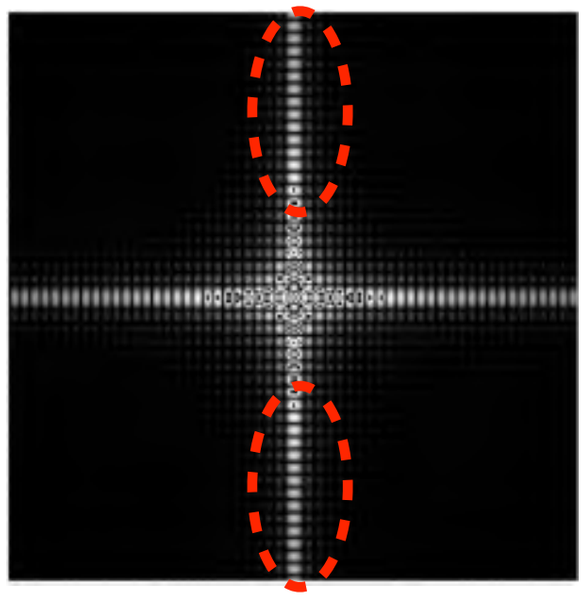
fine detail = high frequencies

1D case



2D case





fine detail = high frequencies

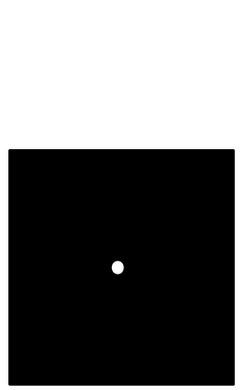
LOW LEVEL Processing - Deconvolution

- **Blur = out of focus information**

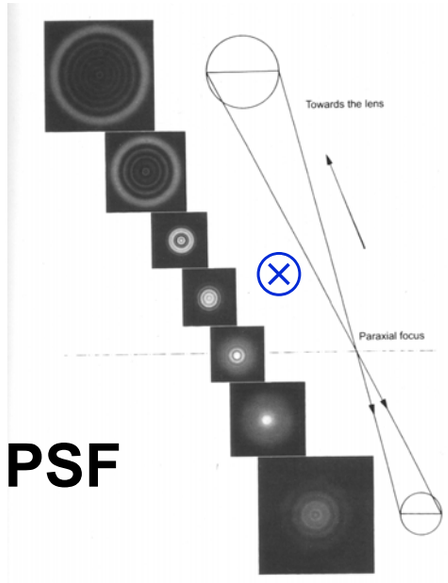
An image represents the output of the optics and detector of the imaging system

image ≠ object

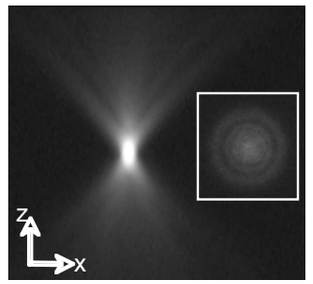
image = object ⊗ PSF



object

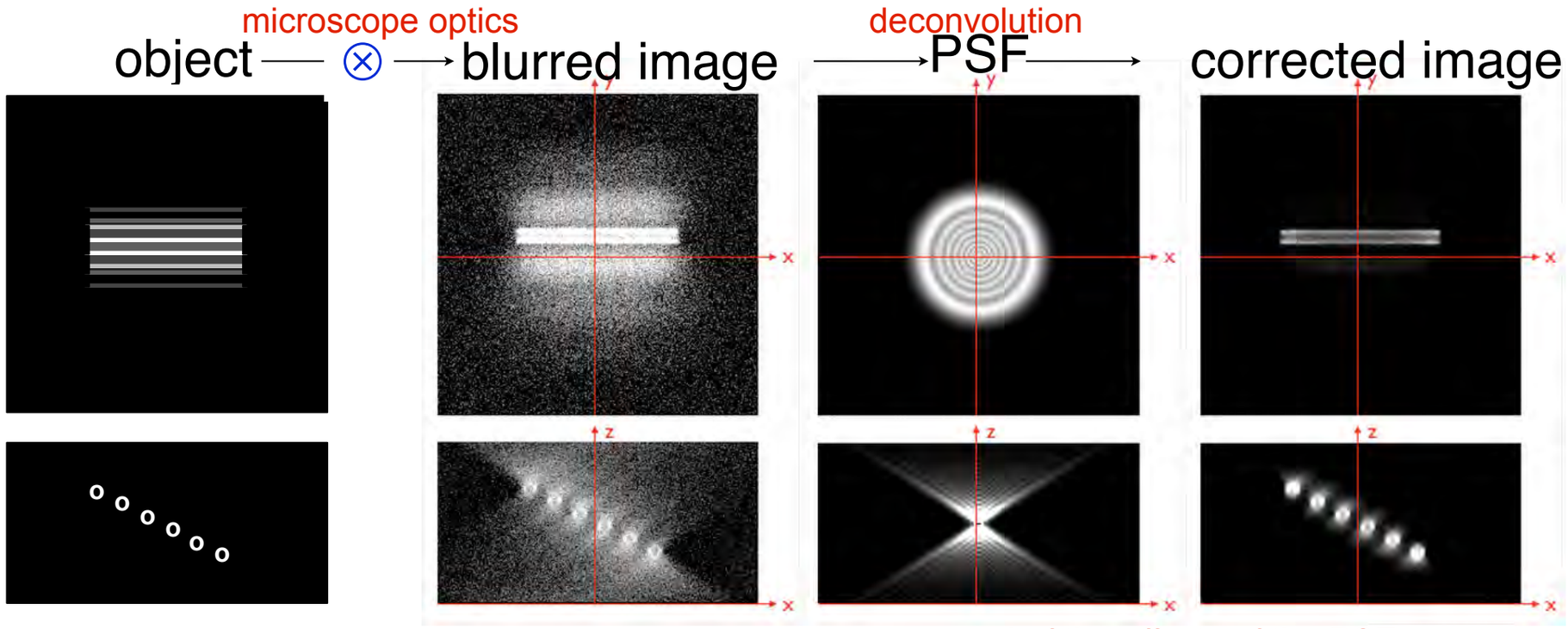


PSF



image

- **Deblurring ≈ Deconvolution = out of focus information removed or reassigned**



reversal of the effects of the PSF

Different classes of deconvolution

- **Deblurring**: nearest neighbours / no neighbours / unsharp mask
  Not true deconvolution, subtractive (throws away light)
  Quick and easy

- **Image restoration**: (inverse filter) constrained iterative algorithms
  True deconvolution, light is re-assigned to its point of origin.
  Can use measured (empirical), theoretical or derived (blind) PSF

The PSF and OTF

Microscope Image	=	object	⊗	PSF

But in Fourier space (or frequency space)

FT microscope Image	=	FT object (“true” image)	X	FT PSF

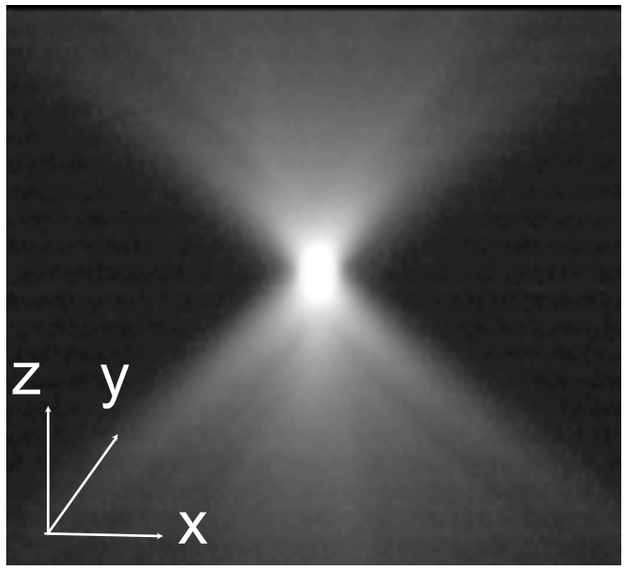
- **Calculations for deconvolution are done in Fourier space (simpler / faster)**
- **The PSF is Fourier transformed to the OTF (optical transfer function)**
- **The inverse calculation to obtain a “true” image = a linear inverse filter**

The PSF and OTF

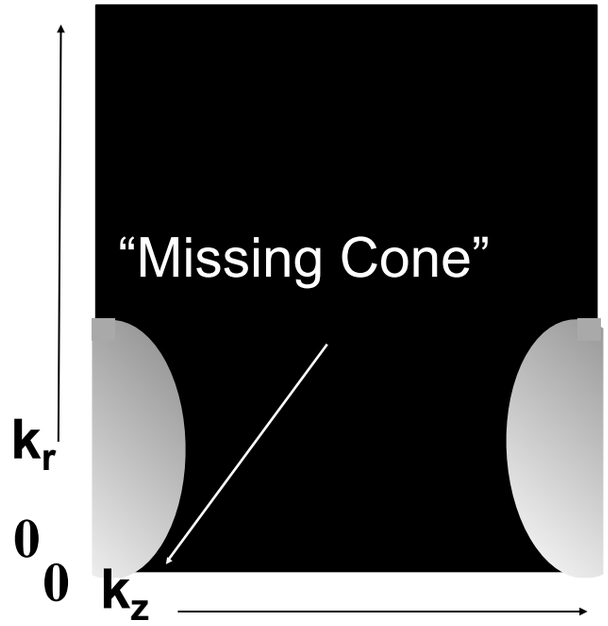
Real space

Frequency space

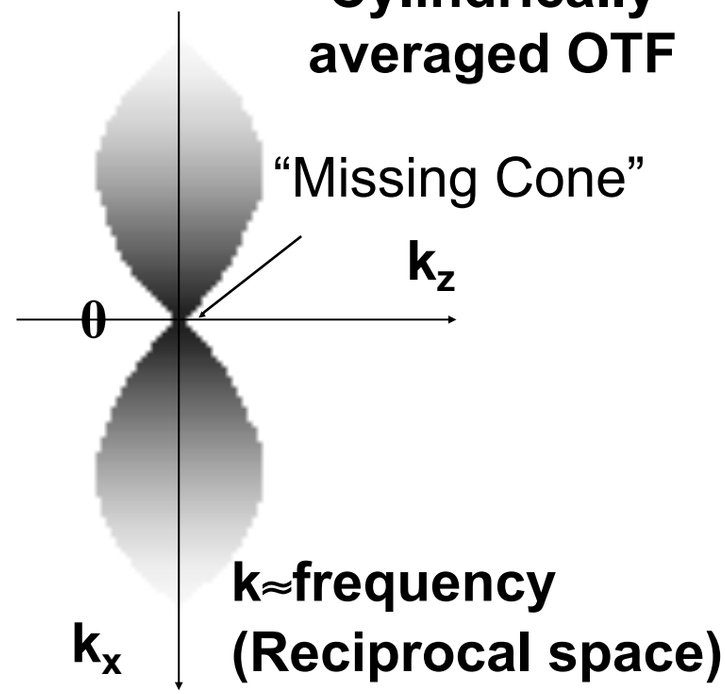
3D - PSF



OTF image (API, softWoRx)

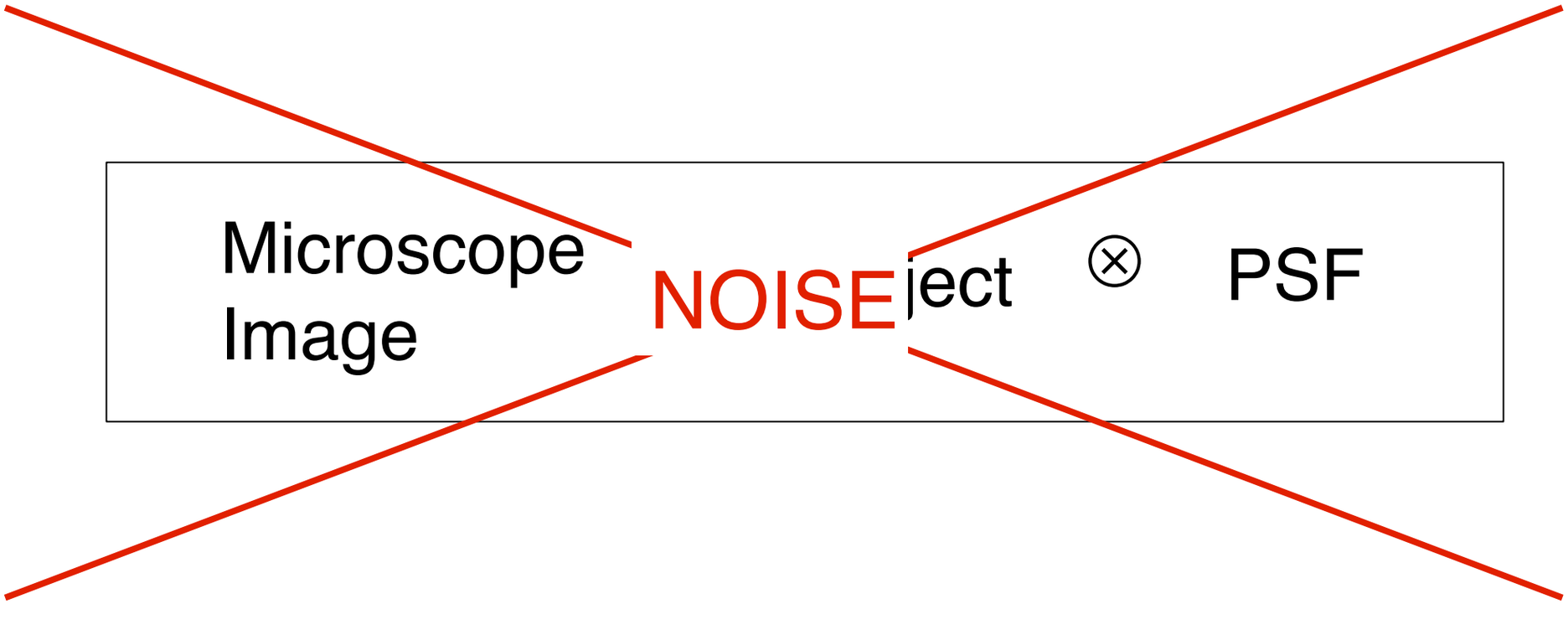


Cylindrically averaged OTF



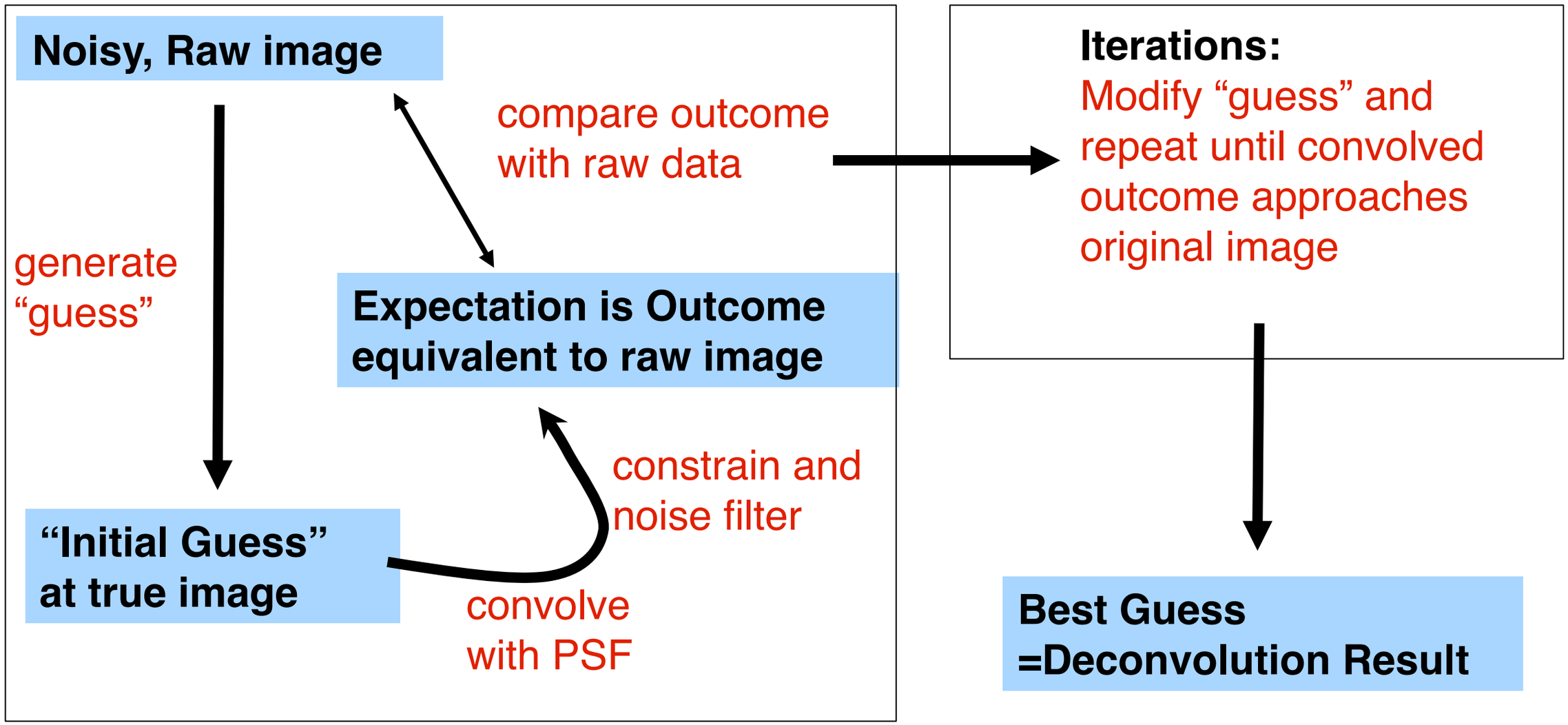
- To simplify deconvolution calculations the OTF is often simplified by assuming radial symmetry for the PSF
- The “missing cone” represents missing frequency information not collected by an objective with a limited NA (not all light can be gathered)

Deconvolution and Noise



- **Much of the complexity of deconvolution is a direct consequence of having to deal with “noise” inherent in image data**

Constrained iterative deconvolution



Data requirements for deconvolution

- **Ensure that the imaging system is correctly set up, aligned and calibrated (pixel sizes).**

- **Reduce aberrations if possible:**
  Sample preparation and correction of optics
  Collect more light / average images
  Aim for a high S/N in image data.

- **Make sure that images are collected according to the Nyquist sampling criteria (pixel size in XY and Z step).**

- **Collect sufficient image planes in Z.**
  (2D data can be deconvolved but lacks Z information so restoration is limited.)

- **Minimise lamp flicker between Z sections.**
  (corrected for on the DV system)

- **Avoid motion blur from live specimens.**
  (short exposure times)

Has deconvolution worked?

- **Should look sharper and more contrasted and not excessively noisy**
- **Should NOT "invent" features not visible in the original data**
- **Be wary of very small punctate features**
- **Be wary of "ringing" artefacts - dark circles round structures**

LOW LEVEL Processing - Making Figures

cartoonstock

LOW LEVEL Processing - Figure Making Guidelines

- **Carry out all processing and analysis of images before making figures by using pixel based (raster) programs:**

- **Handling of images (tif files) for figures should use pixel based (raster) programs**

Arranging multi-panel figures
Cropping, resizing, rotating

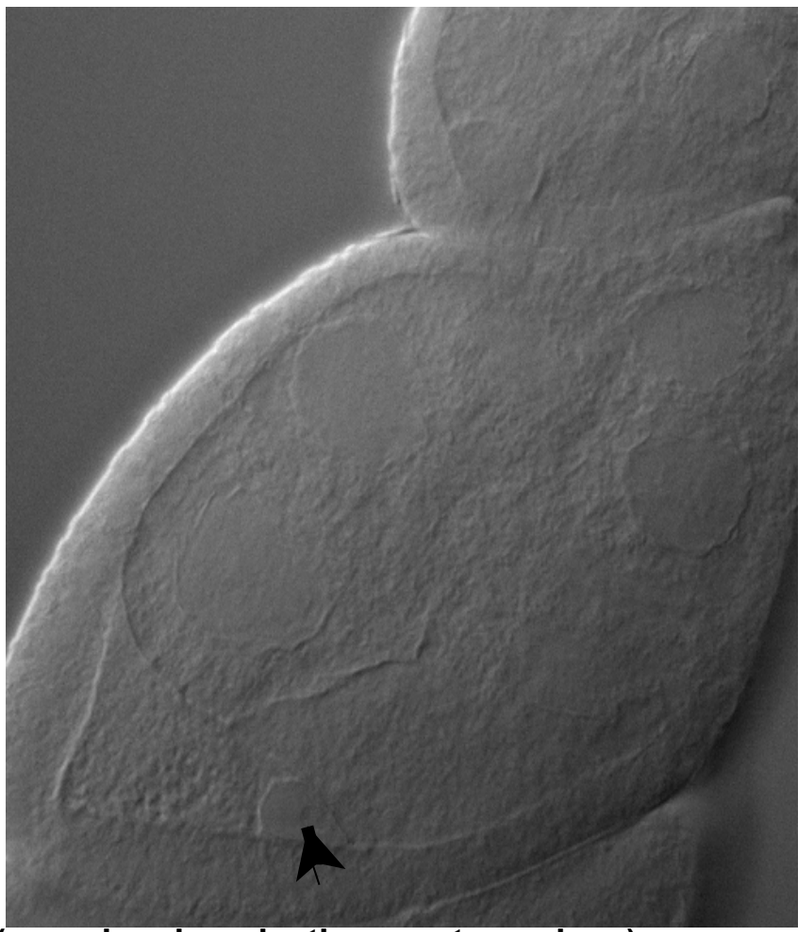
Before using vector graphics based programs

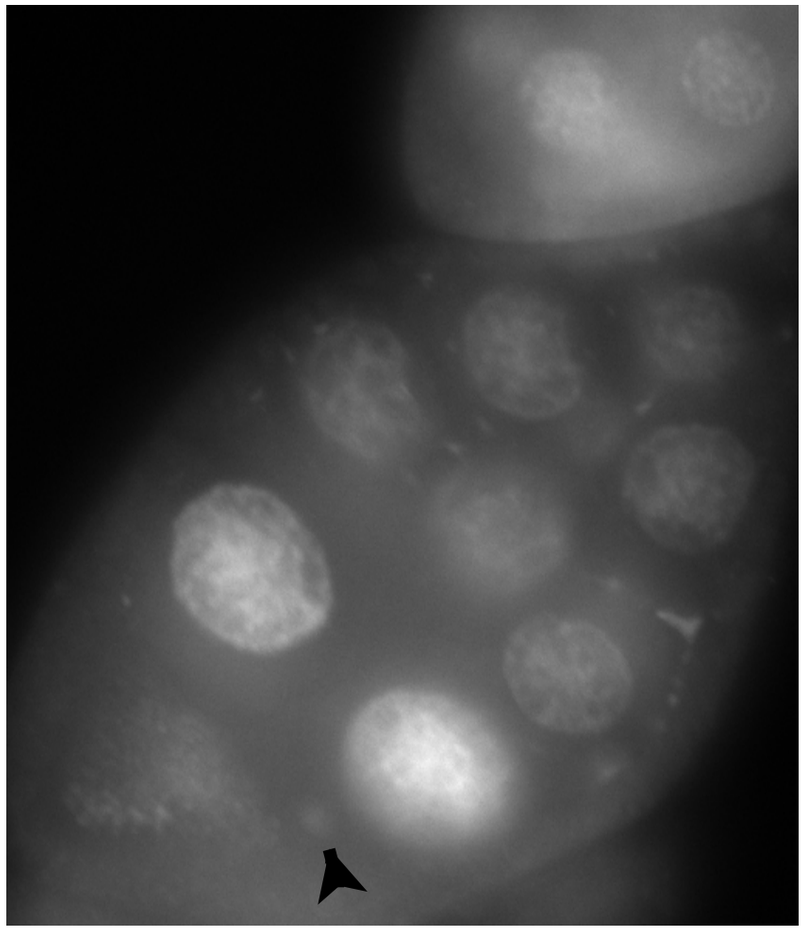
Adding lettering, arrows, charts diagrams....

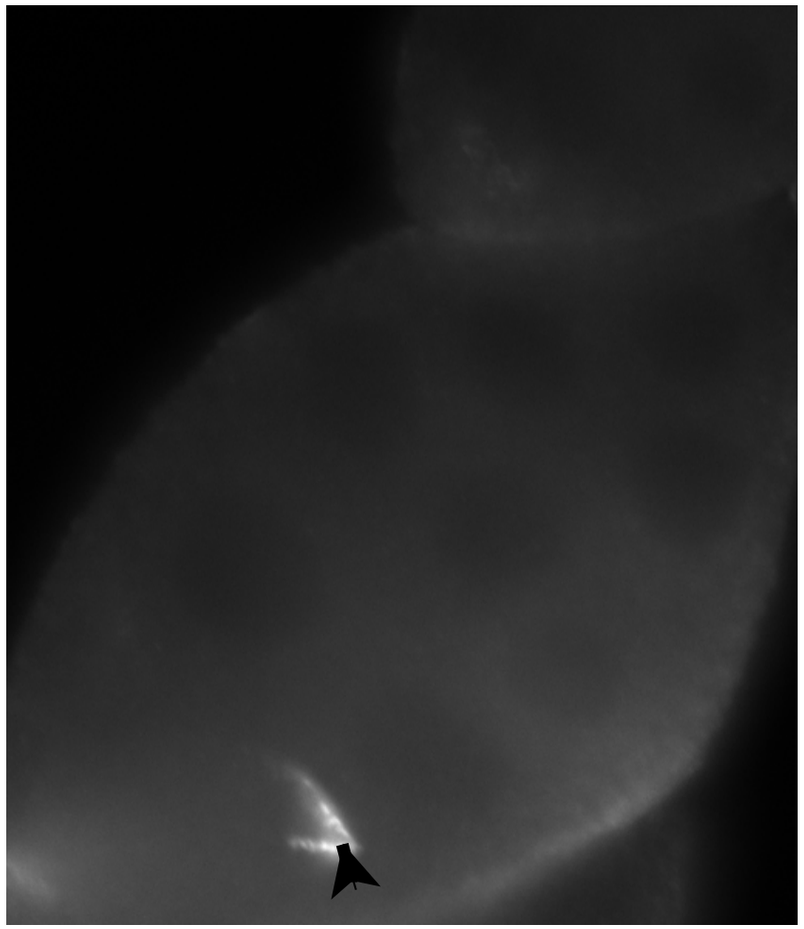
- **Do not prepare figures in powerpoint or keynote or by screen capture**
- **Understand what happens when you resize an image**
- **Be consistent with processing steps, especially contrasting**

LOW LEVEL Processing - Display, Grey Scale

DIC/DAPI/Grk in situ - grey scale images





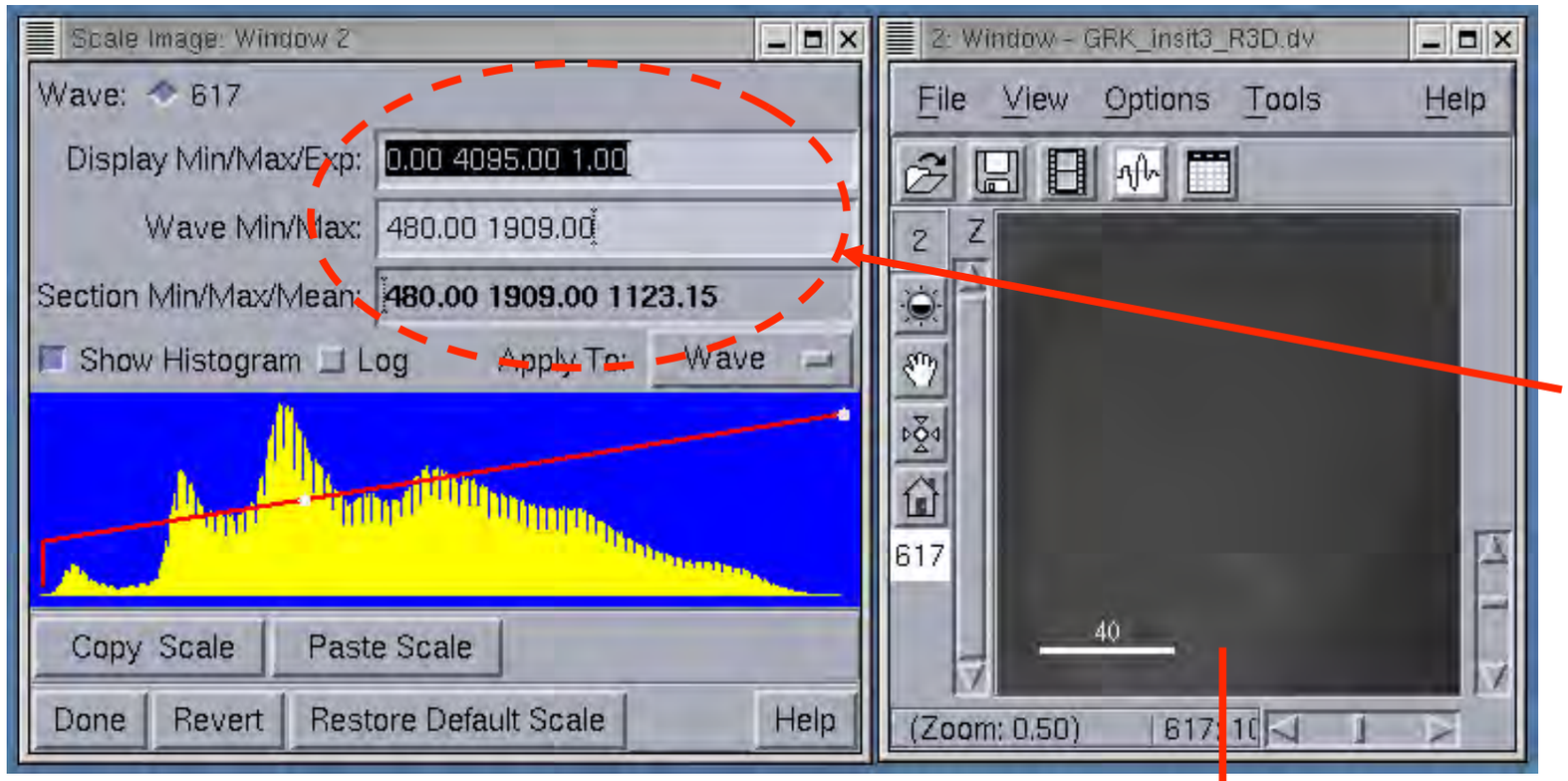


(arrowhead marks the oocyte nucleus)

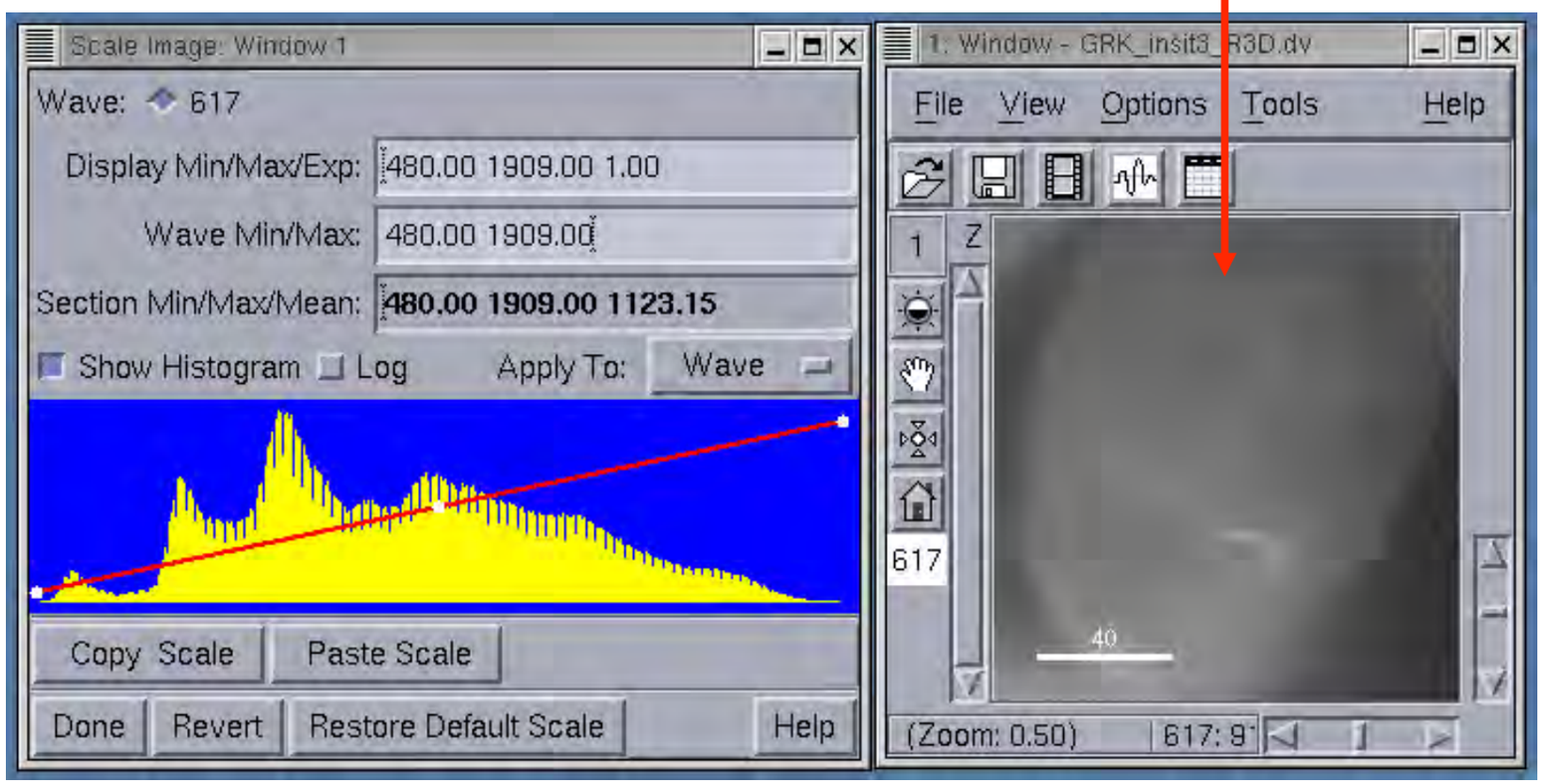
- For viewing and display use grey scale scale images to see fine detail.

LOW LEVEL Processing - Display, Brightness/Contrast

- **Brightness and contrast - Enhancing details which are too close in grey level to be easily discernible.**



Better to set your contrast by numbers to make the operation more systematic
0-4095 is full range for 12 bit



Auto-intensity scaling by softWoRx scales the display to fill the dynamic range

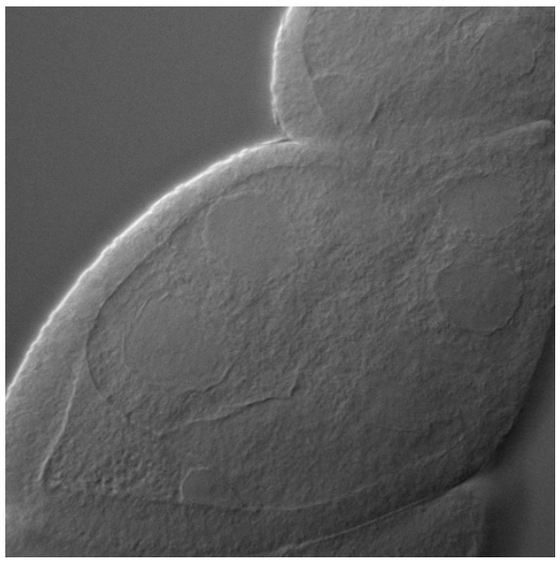
softwoRx; API

LOW LEVEL Processing - Display, Bit Depth (levels)

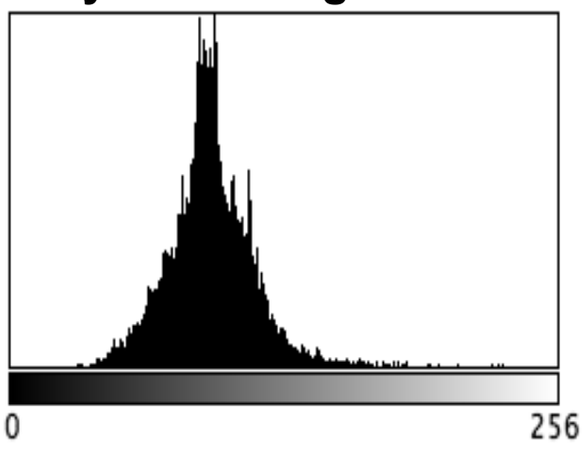
- **"grey levels" = the number of discrete values in an image**
- **Dynamic range = the number of possible grey levels**
- **Eye has limited ability to distinguish grey levels/colours**
  Above 32 grey levels images look smooth - 16 and below grey levels eye perceives objectionable banding = false contours.

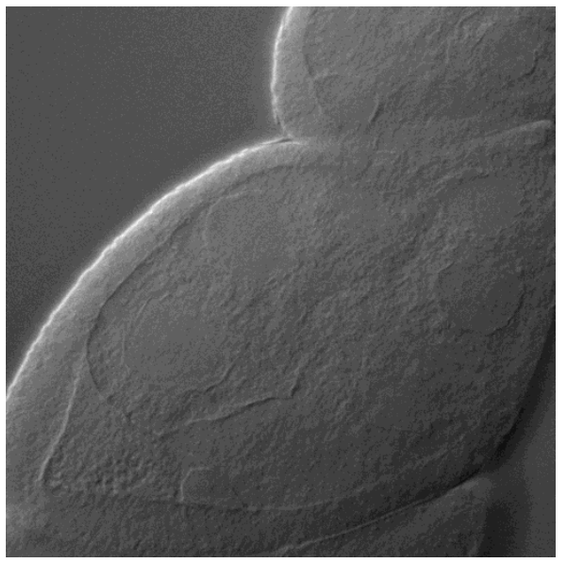
Imaging detectors:
8 bit = 2^8 = 256 grey levels
12 bit = 2^{12} = 4096 grey levels
16 bit = 2^{16} = 65536 grey levels

False contouring due to insufficient grey levels ⟶

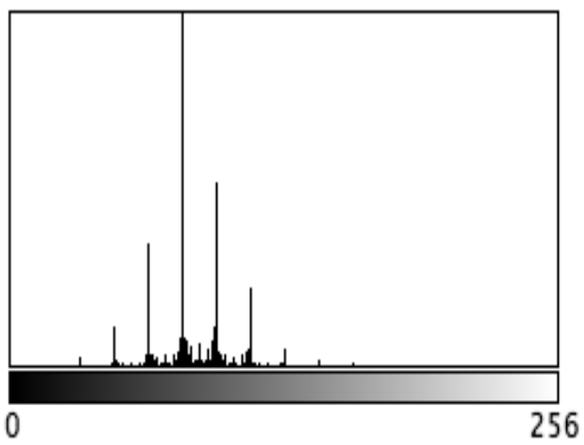


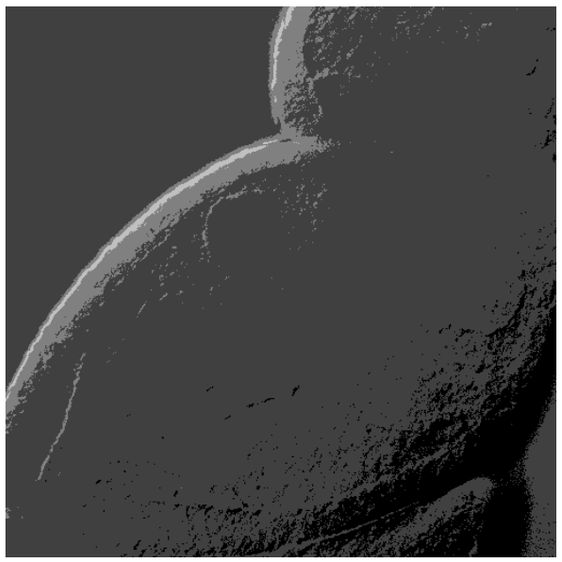
8 bit - 256 greys
dynamic range filled



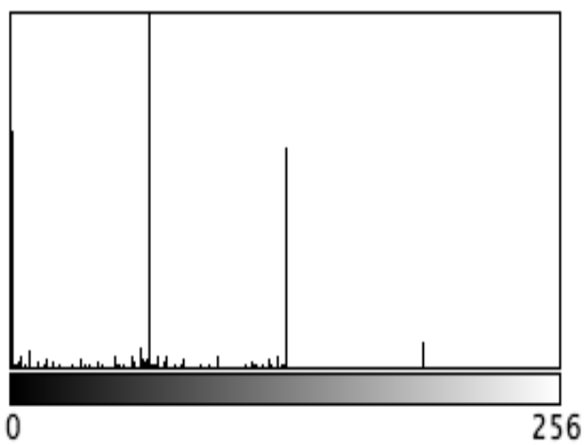


16 greys

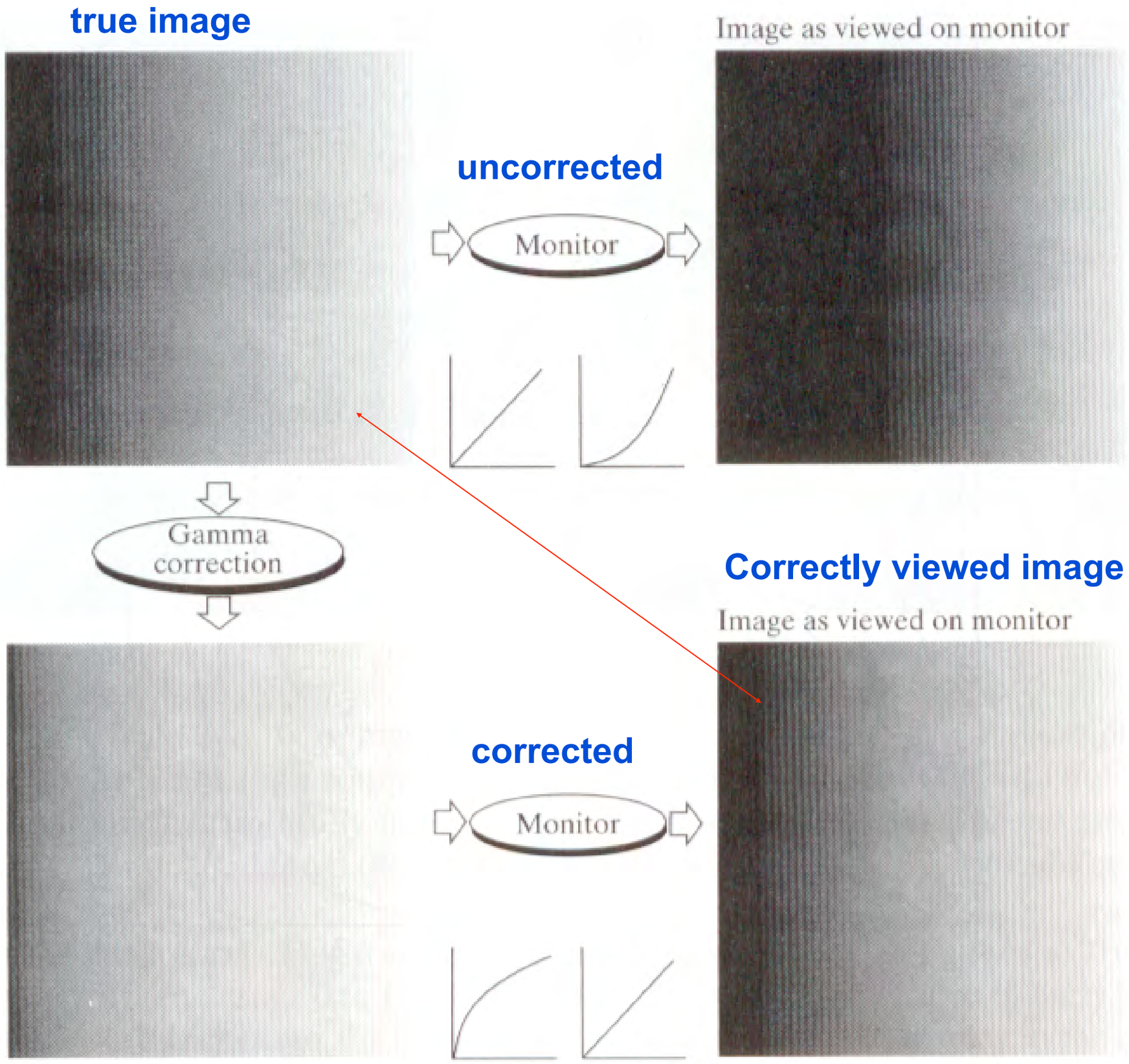




4 greys



LOW LEVEL Processing - Display, Using Gamma

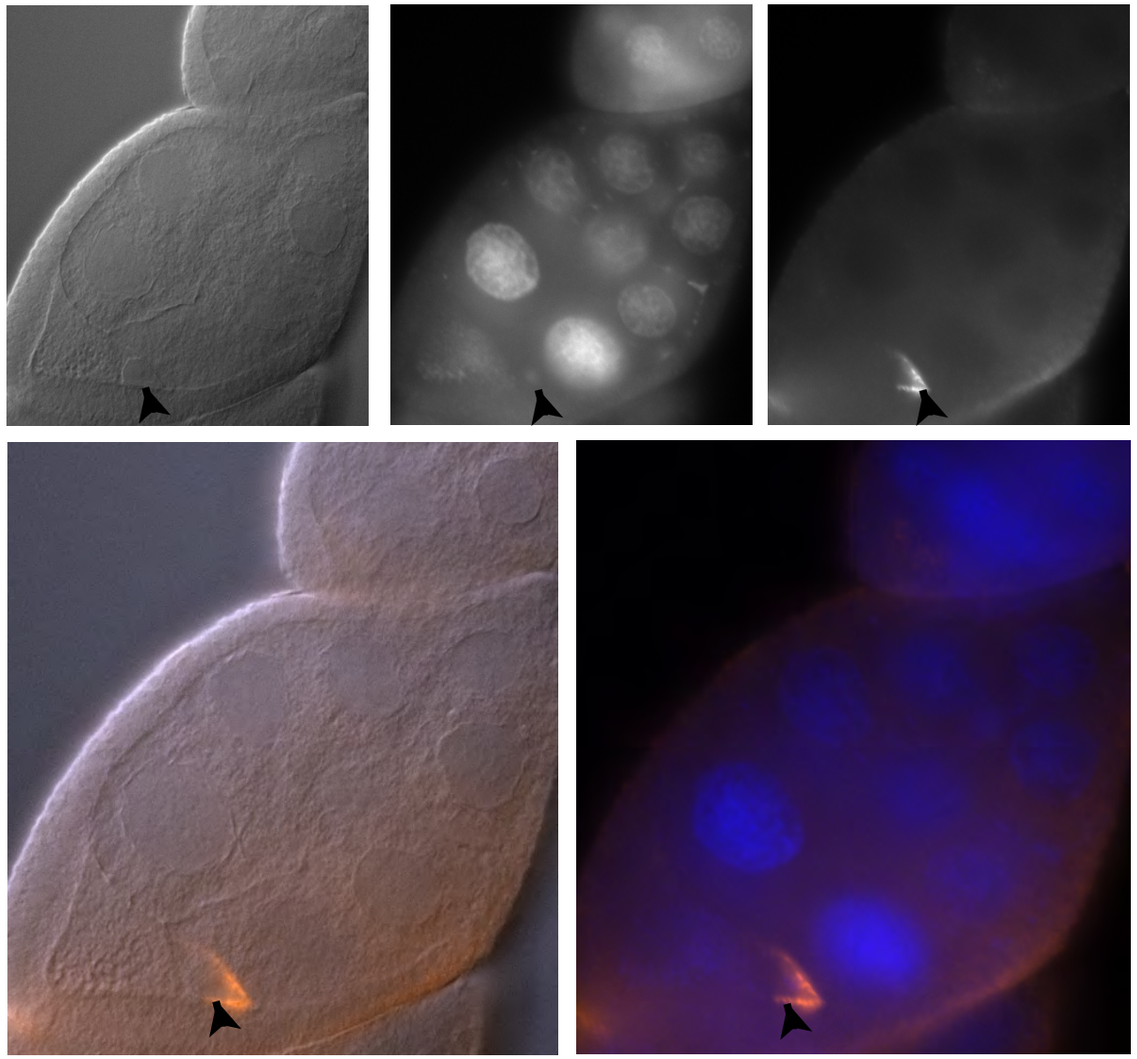


Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.

LOW LEVEL Processing - Display, Colour

DIC/DAPI/Grk in situ - grey scale images, colour blended, additive overlays

- Look-Up Tables (LUTs) map intensity to color

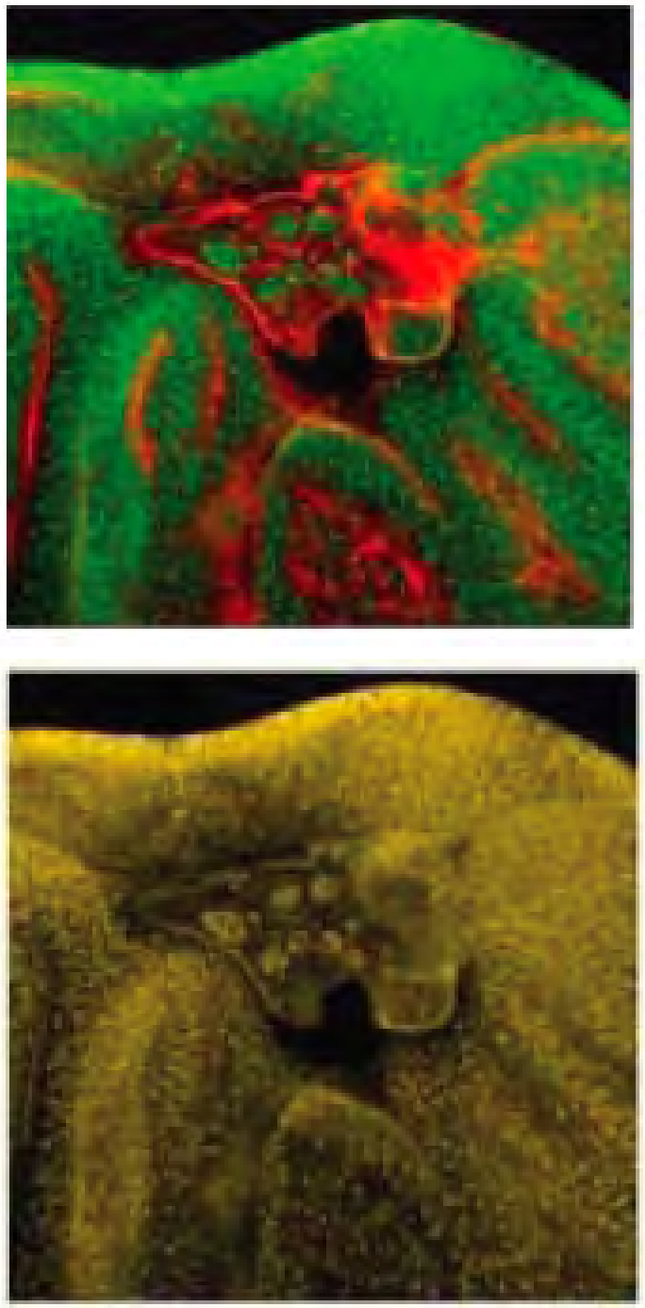


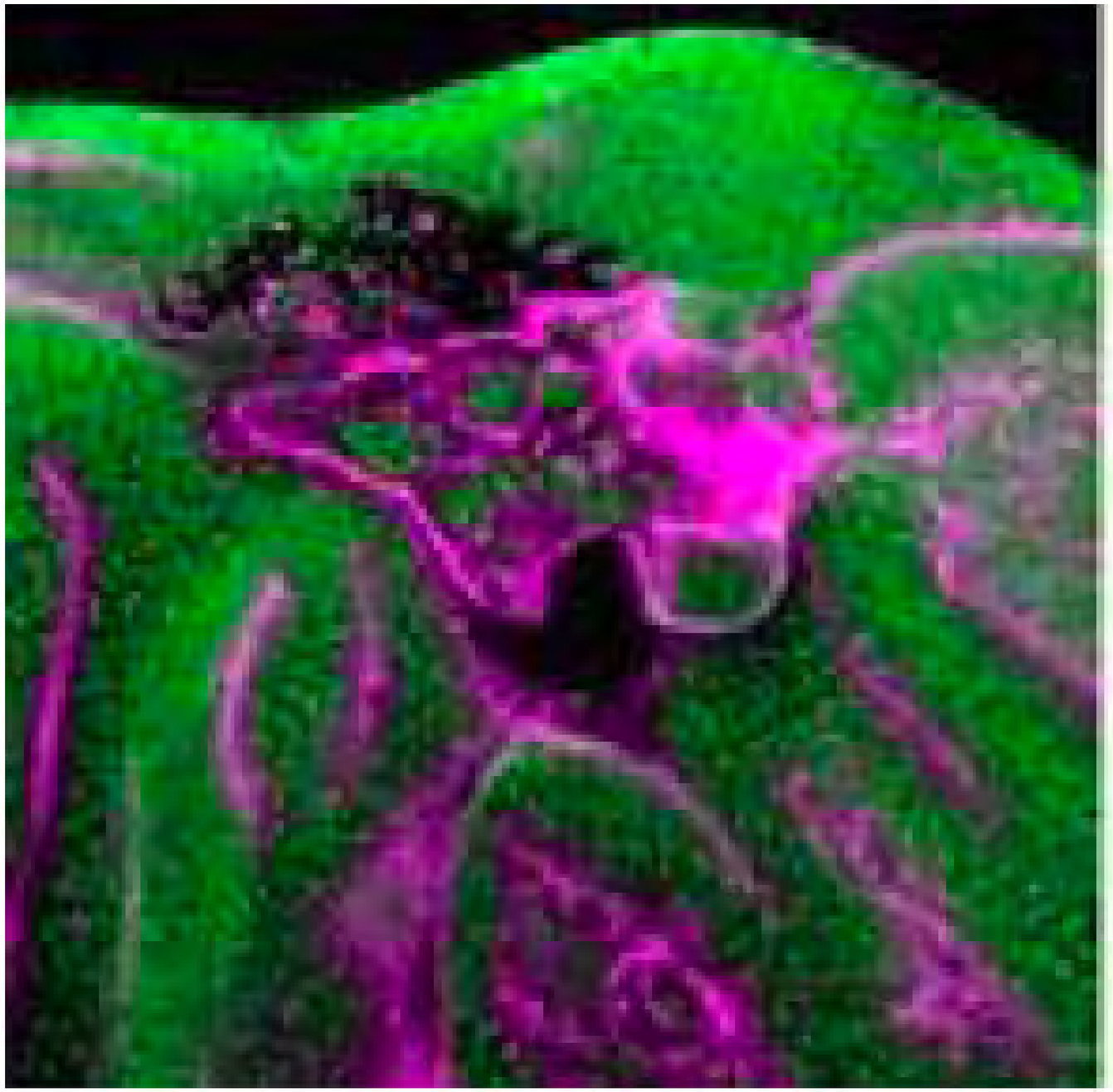
(arrowhead marks the oocyte nucleus)

- **Colour should be used for highlighting particular intensity differences / co-localisation.**
- **For publication show greyscale images alongside colour overlays.**

LOW LEVEL Processing - Display, Colour

- Consider colour blind friendly colours: magenta, green





LOW LEVEL Processing - Display, Making Movies

- Movie formats: .avi; .mov; (.mpeg)
- Considerations:

  image quality vs movie size (use of compression).
  speed of play (frames / second).
  speed of play on the computer may be slowed by large movies

- ImageJ

  Will open many file formats and export straight to .avi or .mov.,
  also can open tif image series (image001.tif; image002.tif; etc)
  restack and export to .avi

- Quicktime Pro 7

  Can open tif image series and export to .avi or .mov
  Can interconvert movie formats.
  Has a range of compression options for .mov

Bad Imaging Practices

Do not corrupt the integrity of the original data

Examples of Bad Imaging Practices

Rossner & Yamada (2004). What's in a picture? The temptation of image manipulation. J. Cell Biology 166: 11–15.

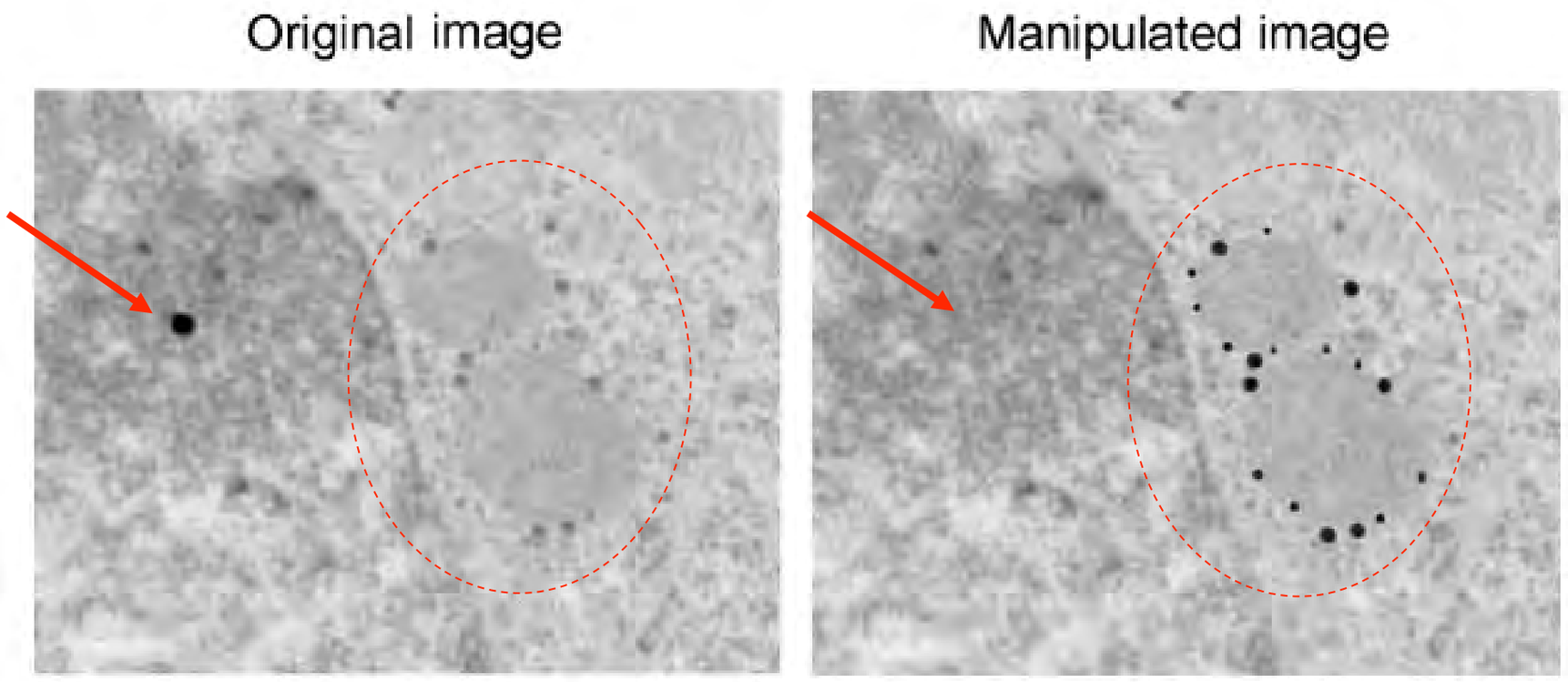
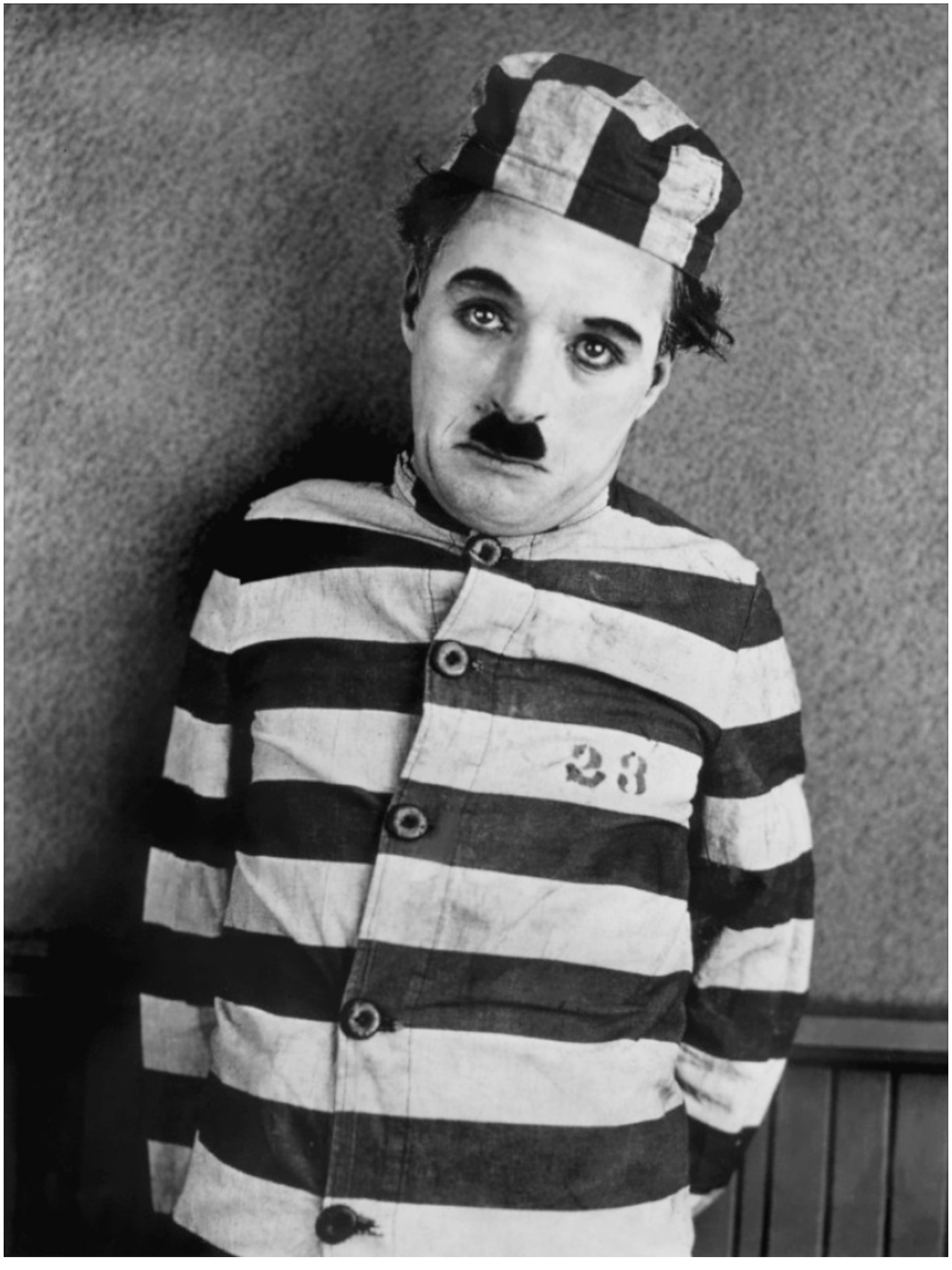


Figure 5. **Misrepresentation of immunogold data.** The gold particles, which were actually present in the original (left), have been enhanced in the manipulated image (right). Note also that the background dot in the original data has been removed in the manipulated image.

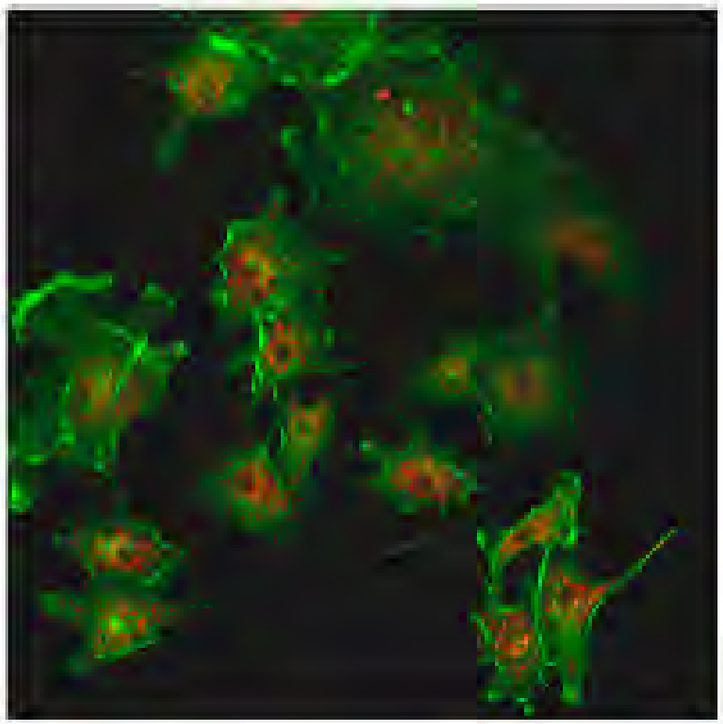
- **BAD: manipulated but does not alter interpretation**
- **VERY BAD: Changes interpretation with intention to defraud**
- **Adjustments necessary to reveal a feature ALREADY PRESENT in the original data are acceptable if they can be justified**

THEY HAVE WAYS OF FINDING OUT WHAT YOU DID!



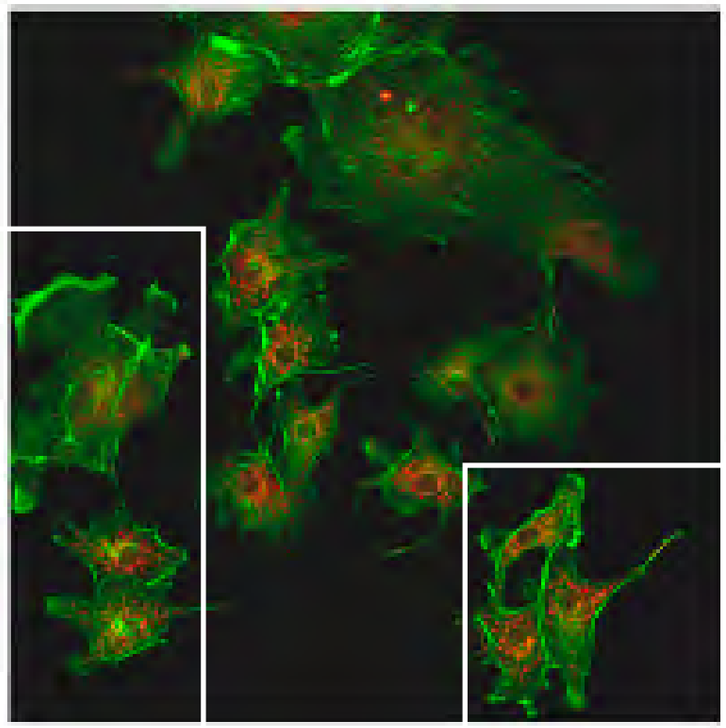
http://www.rottentomatoes.com/quiz/charlie-chaplin-fun-facts-1177891/

Manipulated image

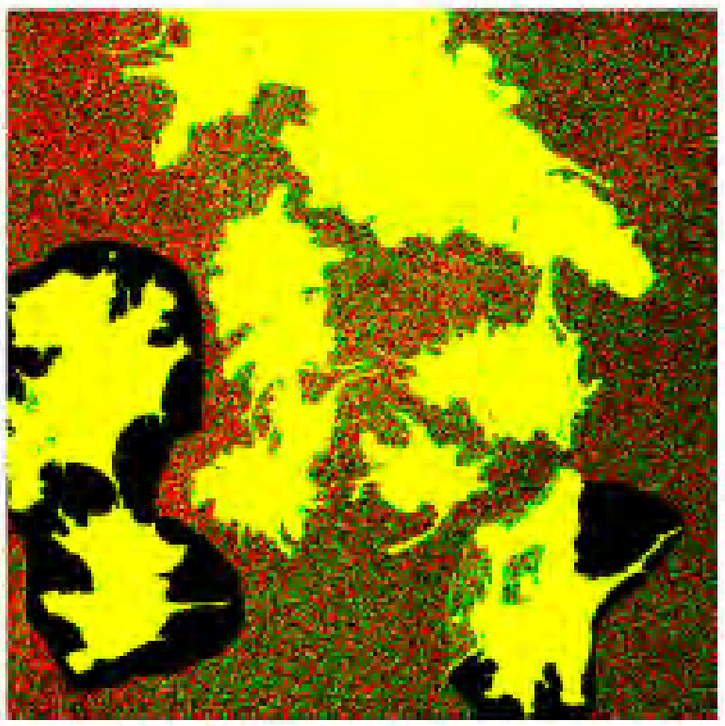


- **Miss-representation of cell population within an oberved field**
- **VERY BAD: Changes interpretation with intention to defraud**

Correct:



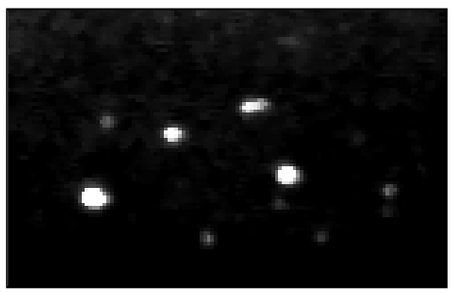
Manipulation revealed by contrast adjustment



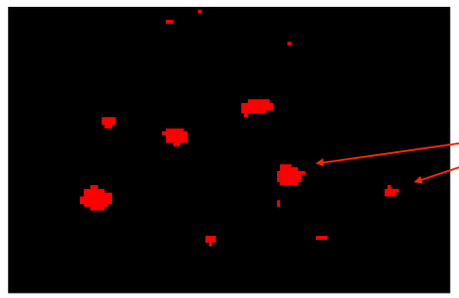
Rossner & Yamada (2004). What's in a picture? The temptation of image manipulation. J. Cell Biology 166: 11–15.

MID LEVEL Processing

- Input is an image, output is an attribute extracted from the image



Segmentation

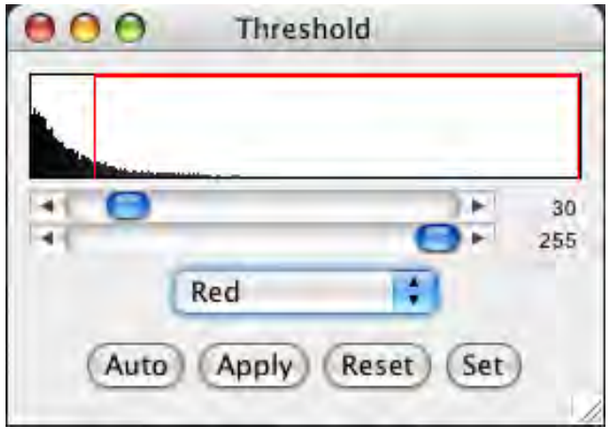


Objects defined

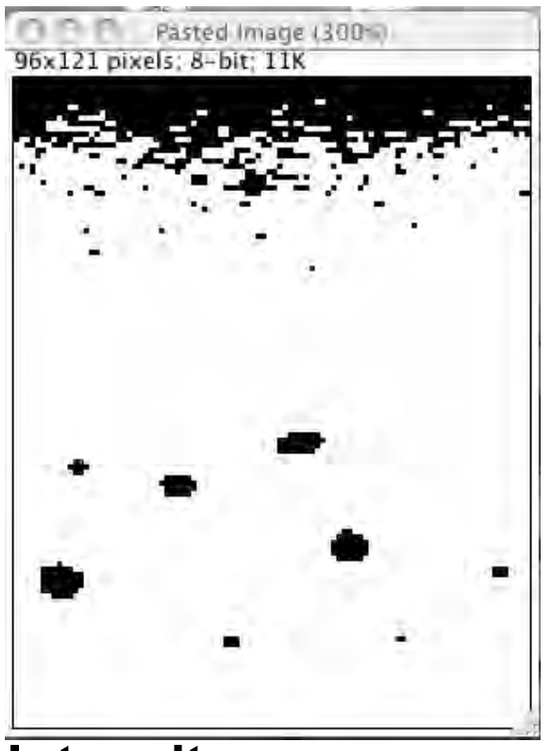
Number of objects
Positions of objects
Size of objects

MID LEVEL Processing - simple segmentation

Segment by intensity

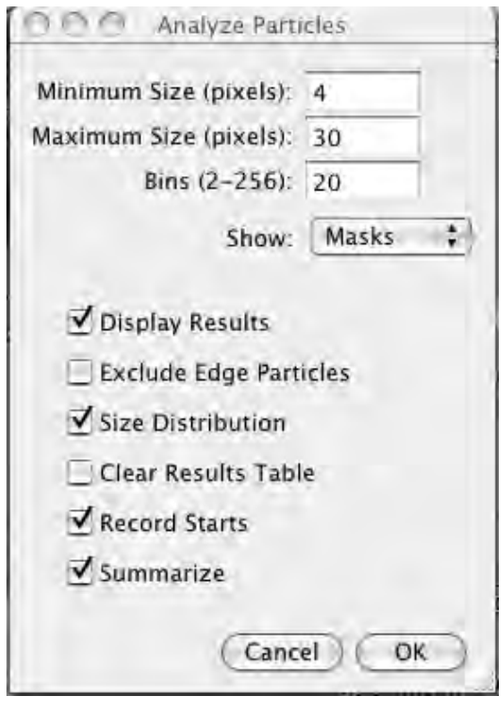


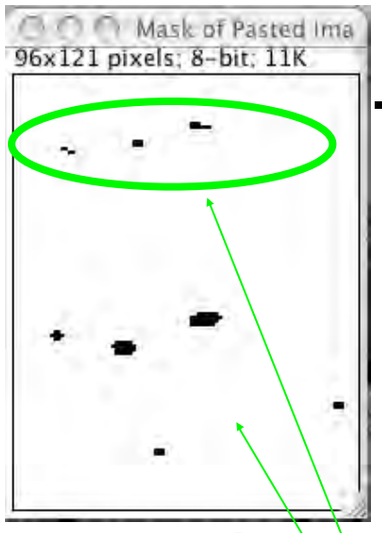
problem region



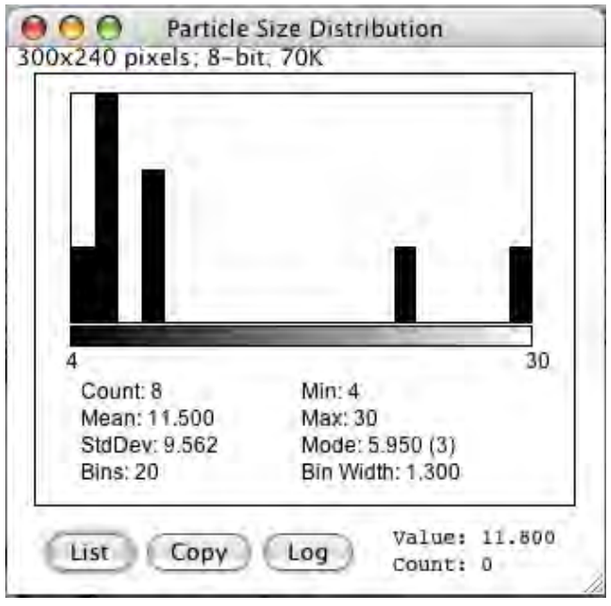
Intensity segmented binary image

Segment again by area size





mask defining regions



attributes

Some particles lost - some artificially created

ImageJ

The human brain is still one of the best segmentation tools

But

Subjective not objective!

http://www.websitepulse.com/blog/uploads/man_with_magnifying_glass.jpg

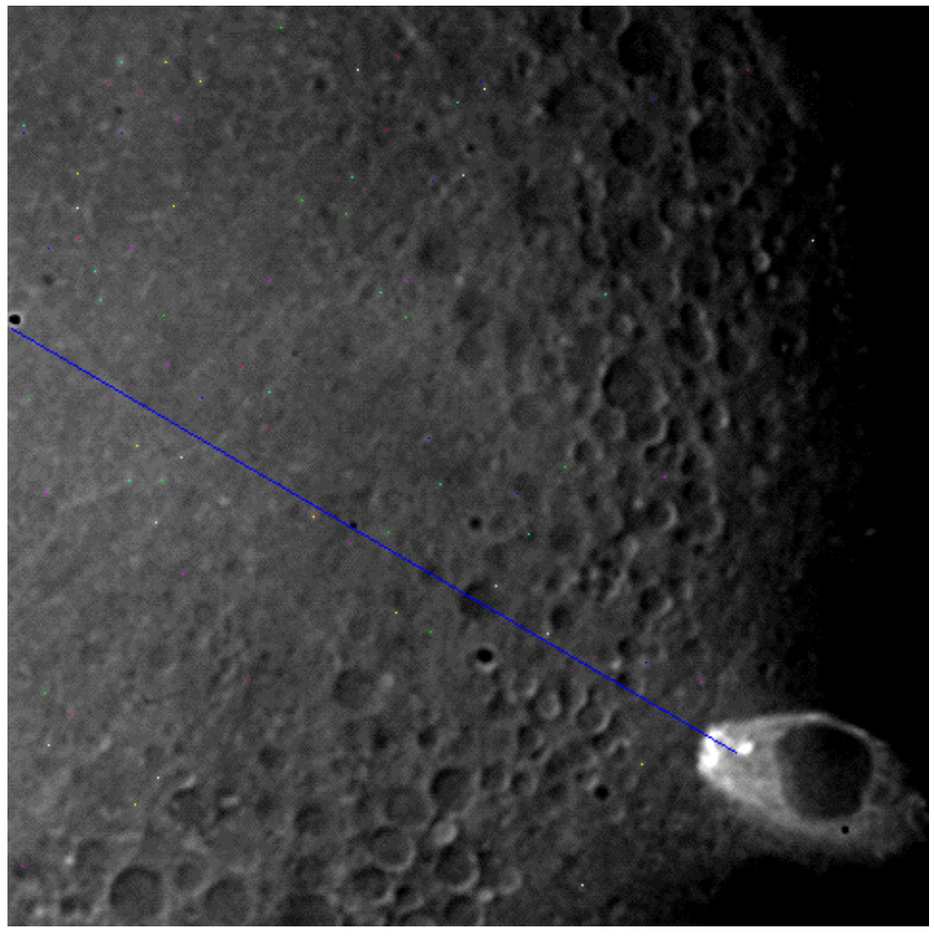
Automated segmentation desirable for data quality and sanity

* Dominic, Lecture 17: Applied Image Analysis *
* Practical course - Demo of Fiji plugins and macros *

HIGH LEVEL Processing = Image Analysis

- Outputs are interpretation (making sense)

Automated Particle tracking



Particle velocities

X cent	y cent	dist	Δt	Velocity

Particle trajectories
Particle velocities
Run lengths
Directionality
Net directionality

HIGH LEVEL Processing = Image Analysis

* **MACHINE LEARNING** Dominic, Lecture 17: AppliedImage Analysis *
* Practical course - **Scripting and Automation**: Demo of Fiji plugins and macros *

http://graemeeyre.info/?p=1154

END

http://www.warrenphotographic.co.uk/03360-silver-spotted-cat-looking-back

Reference Material

Fundamentals of light microscopy and electronic imaging
Douglas B. Murphy. Wiley-Liss 2001
ISBN 0-471-25391-X
http://www.biology.uoc.gr/courses/BIOL493/documents/book.pdf
* Very good general book for understanding the principles of microscopy.

Gonzales & Woods, 2002. Digital Image Processing 2nd Ed. Prentice-Hall Inc, USA.
* Excellent book to get into image processing, there is also a version of the book which takes you through the use of Matlab for image processing

References for denoising:

Boulanger, J., Kervrann, C., Bouthemy, P., Elbau, P., Sibarita, J.-B., & Salamero, J. (2010). Patch-based nonlocal functional for denoising fluorescence microscopy image sequences. IEEE Transactions on Medical Imaging, 29(2), 442–454. doi:10.1109/TMI.2009.2033991

Carlton, P. M., Boulanger, J., Kervrann, C., Sibarita, J.-B., Salamero, J., Gordon-Messer, S., et al. (2010). Fast live simultaneous multiwavelength four-dimensional optical microscopy. Proceedings of the National Academy of Sciences of the United States of America, 107(37), 16016–16022. doi:10.1073/pnas.1004037107