

Image Processing

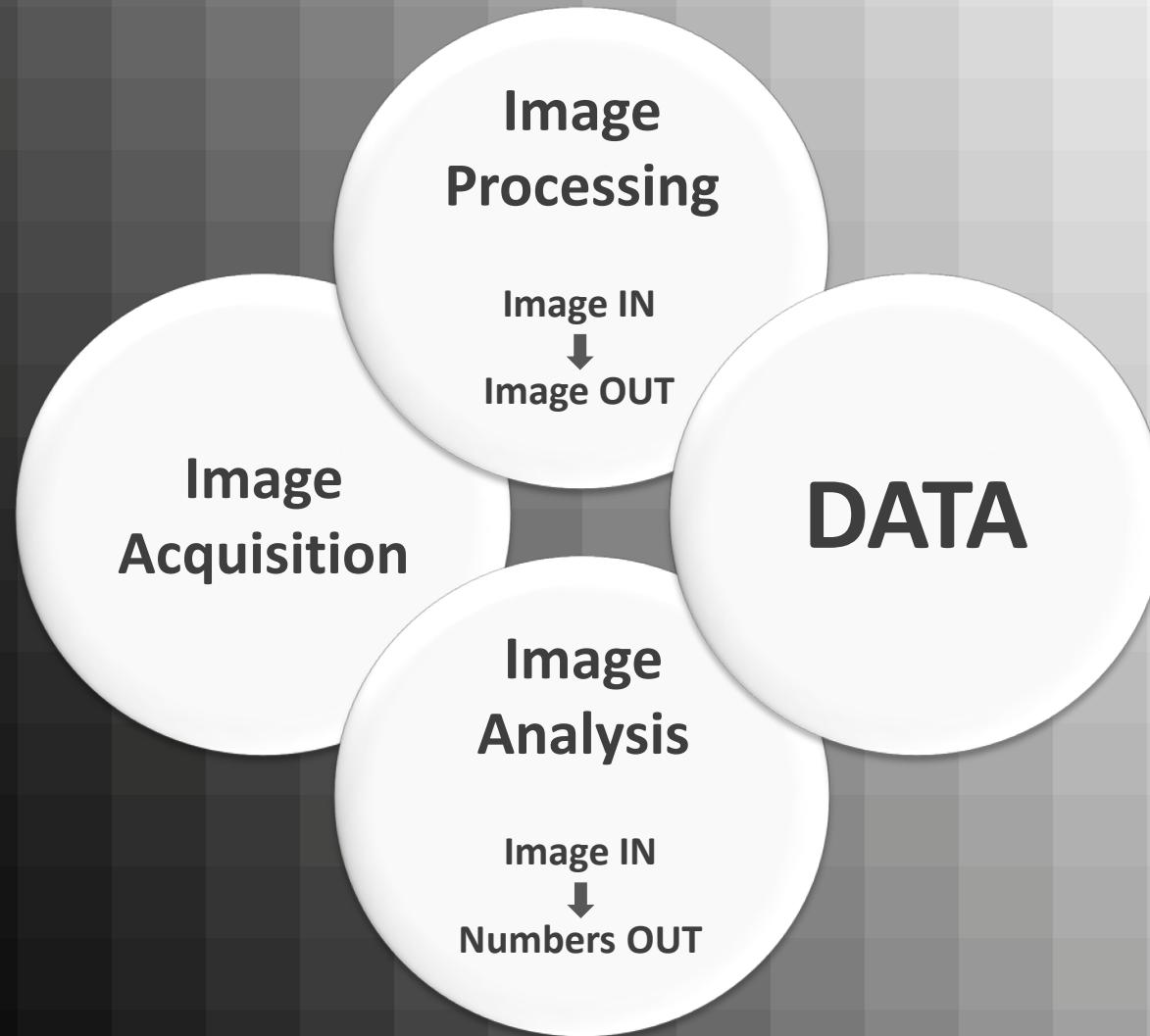
Image IN
↓
Image OUT

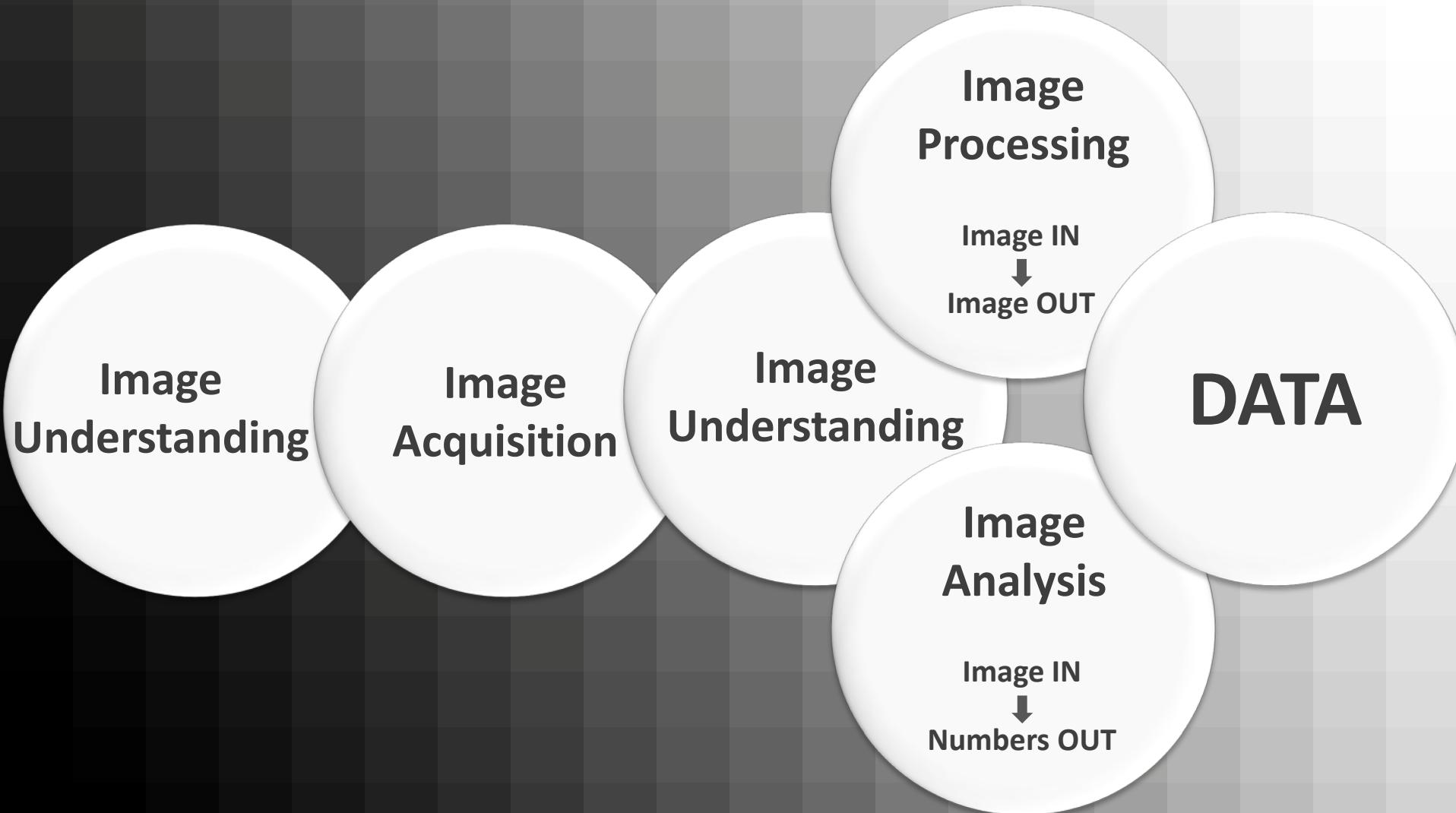
Image Acquisition

Image Understanding

Image Analysis

Image IN
↓
Numbers OUT





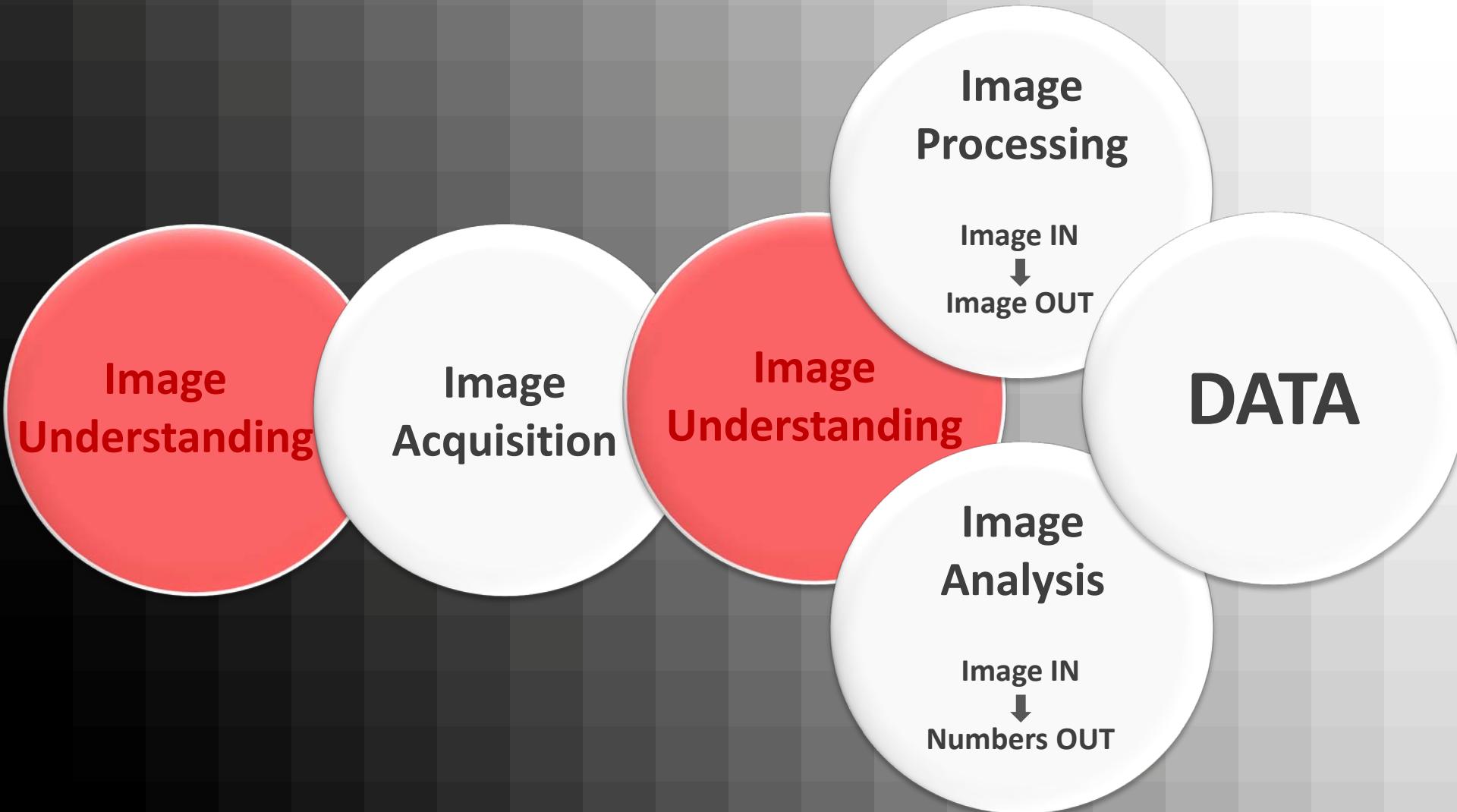
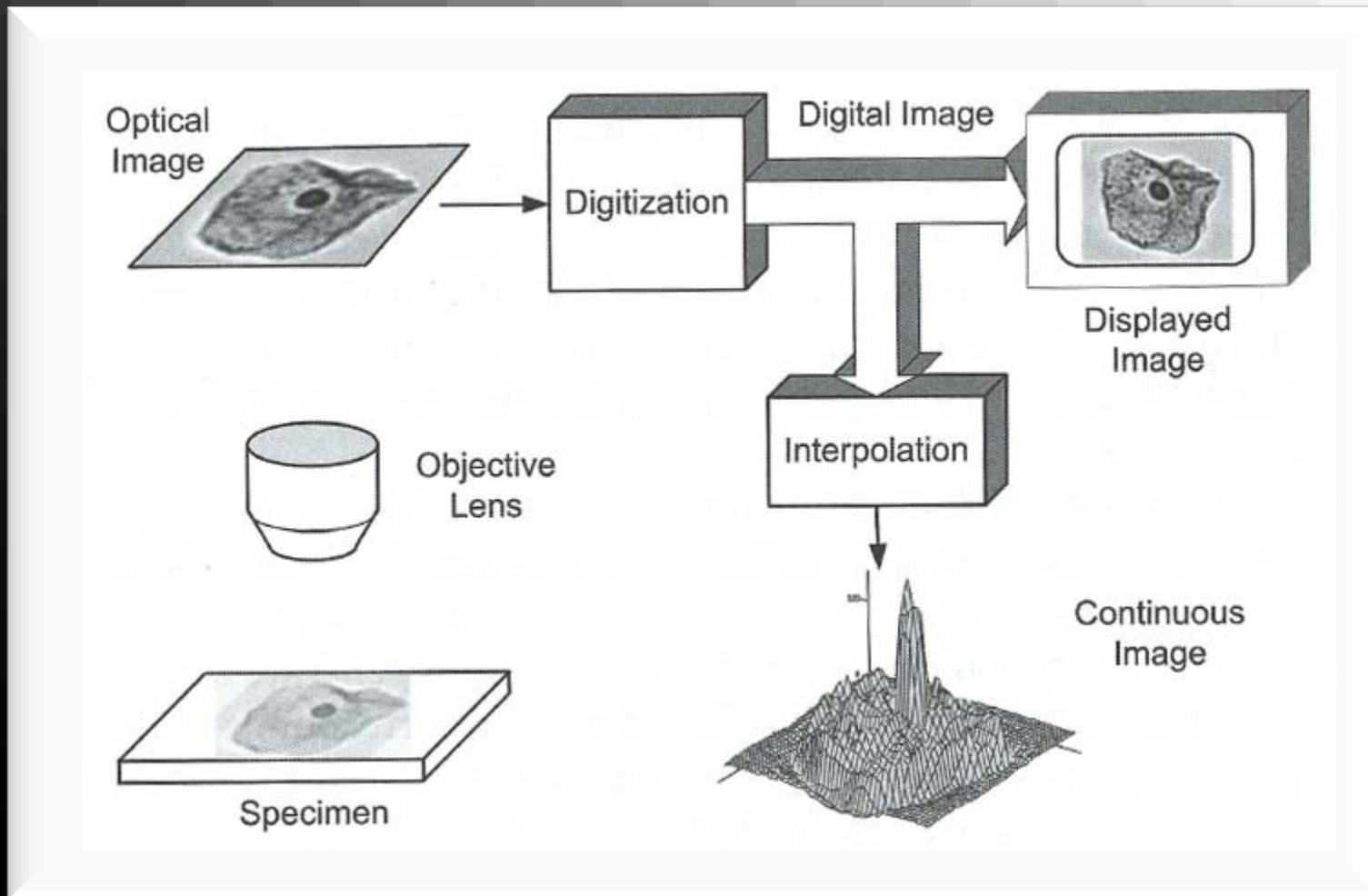


Image Understanding Basics

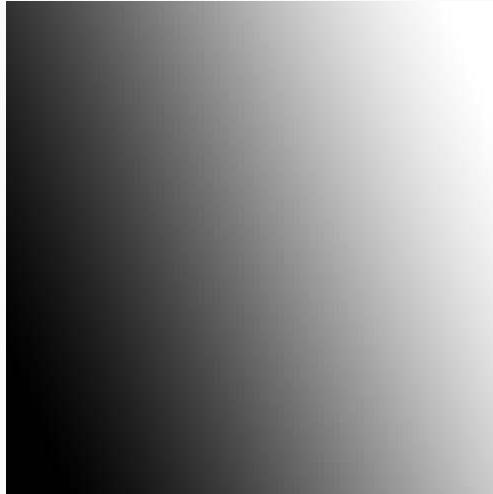
- What is an image?
 - How is an image defined?
 - Dimensions
 - How is an image stored?
 - File Formats
 - How to display gray values?
 - Histograms in an image
 - How to display gray values?
 - Color Look Up Table
- ## How to...?

Image Types

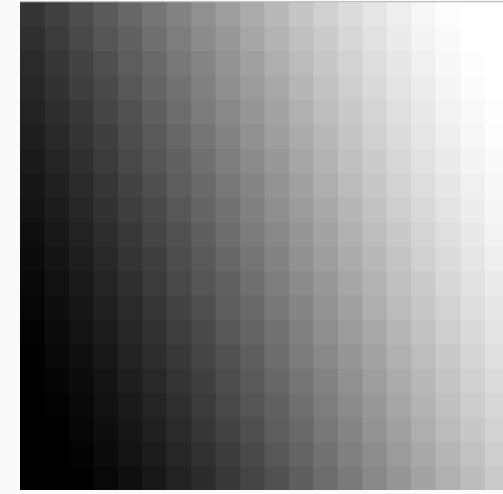


Digital Image

Continuous Light



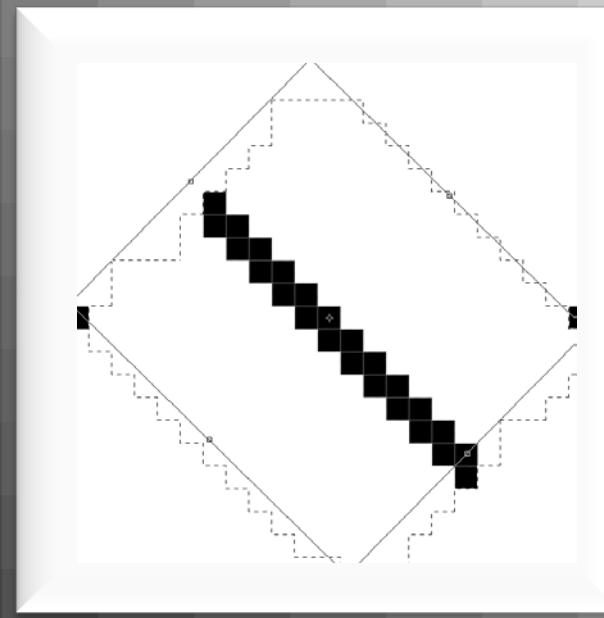
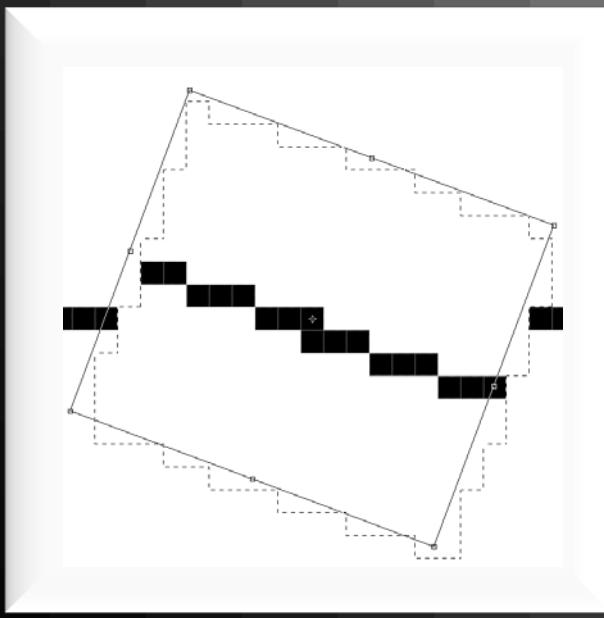
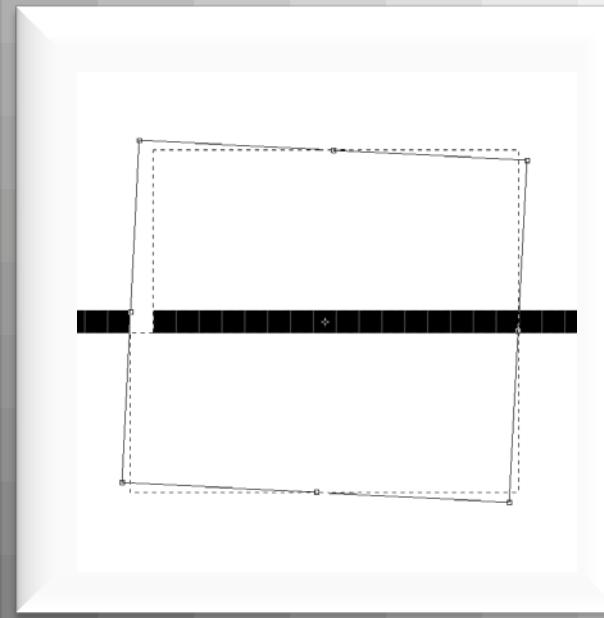
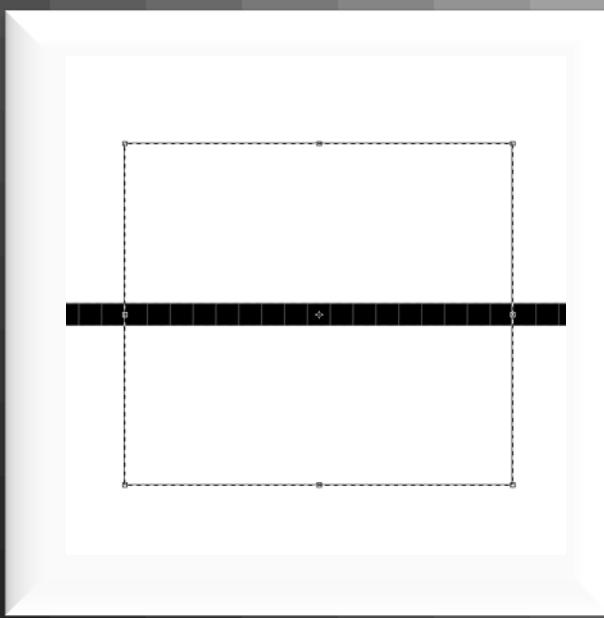
Spatial Sampling/Temporal Sampling



Digitalization

52	64	77	90	103	238	246	253	255	255
47	59	72	85	97	235	243	250	255	255
...
...
...
...
...
0	0	3	11	19	155	168	181	193	206
0	0	1	8	15	149	163	176	188	202

Digital Image



Digital Image

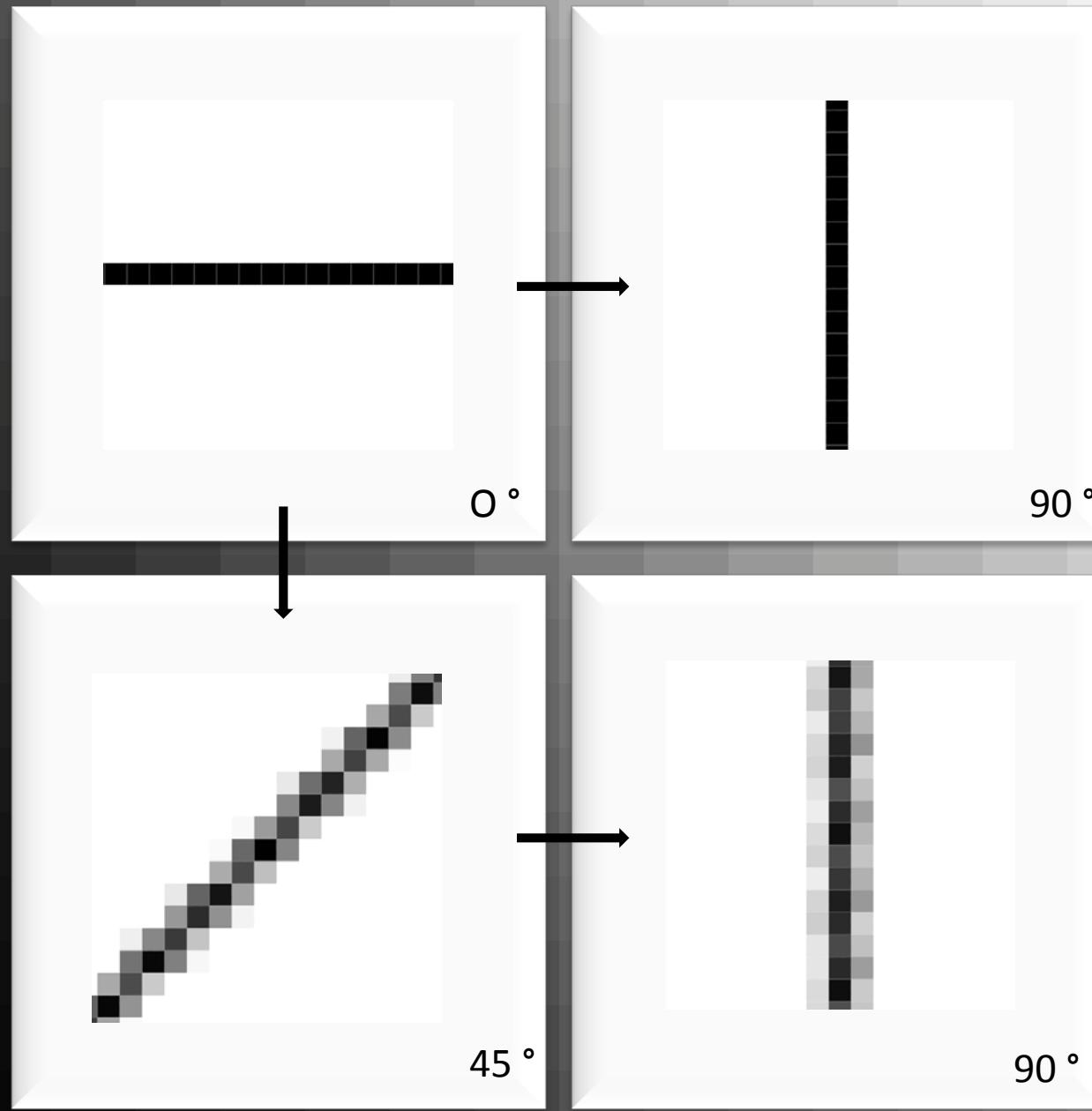


Image Dimension

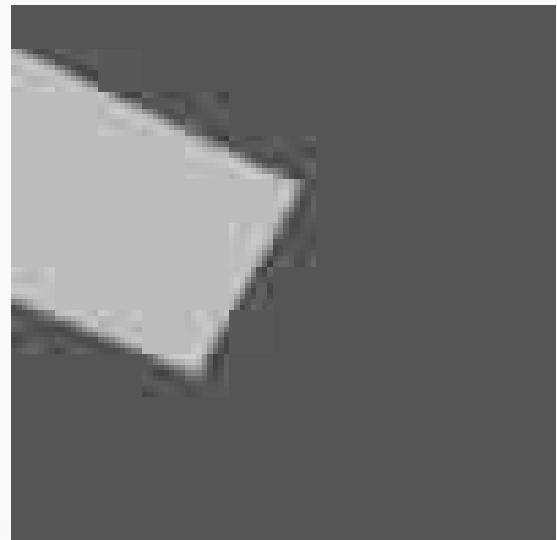
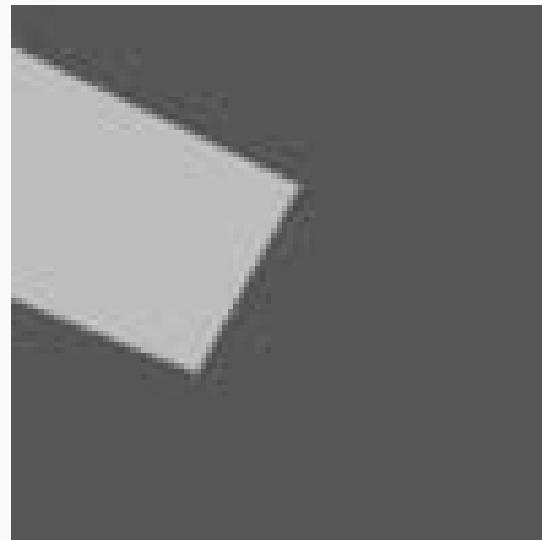
- **Size:** width (number of columns) x height (number of rows)
- **Resolution:** amount of pixel per unit e.g. dot per inch (dpi)
- **Coordinates:** position of the pixel in the image – origin left/upper corner
- **Pixel Value:** intensity of the pixel – can have 2^k values k = bit depth (resolution)

Grayscale:	1 channel	1bit	2^1	0 ... 1
		8bit	2^8	0 ... 255
		12bit	2^{12}	0 ... 4095
		(14bit)	2^{14}	0 ... 16384)
		16bit	2^{16}	0 ... 65535
Color:	3 channel	24bit	$3 * 8$	$(0 \dots 255)^3$
		36bit	$3 * 12$	$(0 \dots 4096)^3$
		48bit	$3 * 16$	$(0 \dots 65535)^3$

File Formats

- **Tiff:** **Tagged Image File Format**
universal and flexible
Can handle images and data within one file, it consists of a Image File Header and image objects - image data and information in ,tags'
Tags can vary: *Thousands of Incompatible File Formats*
Can be a container for various images (jpg, float comma, etc.)
max. 4GB (bigger BigTiff)

- **JPEG:** **Joint Photographic Experts Group**
Standardized compression method – lossy!
JPEG File Interchange Format = JFIF



File Formats

- **ICS:** **Image Cytometry Standard**
image data and parameters: Text header File: *.ics & Image File *.ids
ICS2: header & image in *.ics file
8 or 16 bit integer, floating point and floating point complex data
multi-dimensional data
- **GIF:** **Graphics Interchange Format**
Limited to 8-bit, grayscale or color-indices
Can be animated
- **PNG:** **Portable Network Graphics**
Grayscale up to 16-bit
Color up to 3 x 16-bit
Index-color up to 256 colors
Lossless compression – only one image
- **Microscope specific formats**
LIF, LEI, LSM, ZVI, STK, OIF, dv, r3d, PIC, IPL, OIB, OIF, ND2 . . .

File Formats – Compression

Loss-less compression

- Writes exactly the same data in a smaller file
- Think winzip
- Tends not to actually help that much - doesn't save that much space and takes time

Lossy compression

- Makes a smaller file that looks pretty much the same but changes the actual image
- Various formats (e.g. JPEG) and extents of compression
- Great for the web, holiday photos etc
- NEVER use for quantification

Overview

- What is an image?
- How is an image defined?
 - Dimension
- How is an image stored?
 - File Formats
- How to interpret gray values in an image?
 - Histogram
- How to display gray values?
 - Color Look Up Table

Histogram

The image - **histogram** shows the distribution of intensity values within an image.

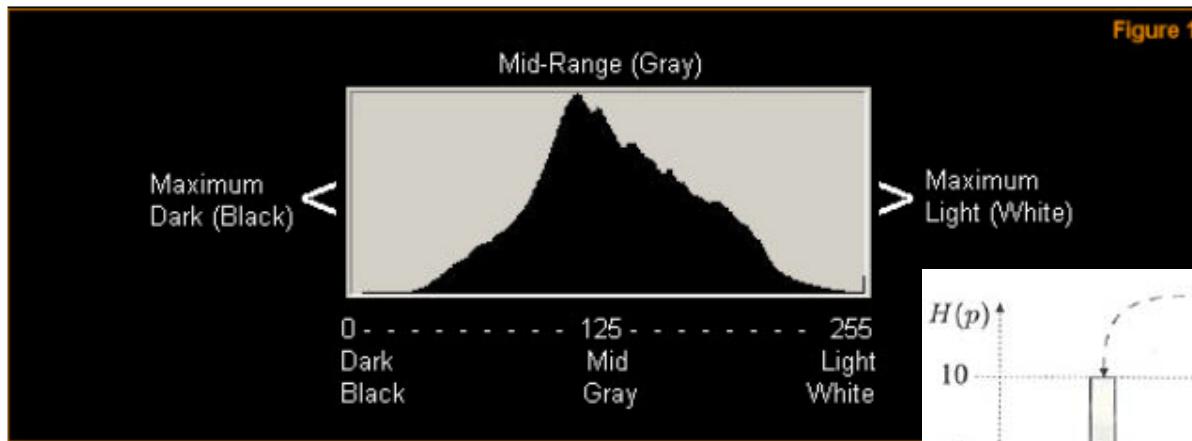
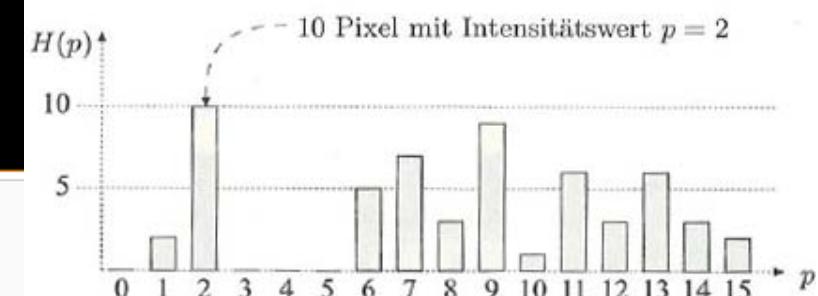


Figure 1



$H(p)$	0	2	10	0	0	0	5	7	3	9	1	6	3	6	3	2
p	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

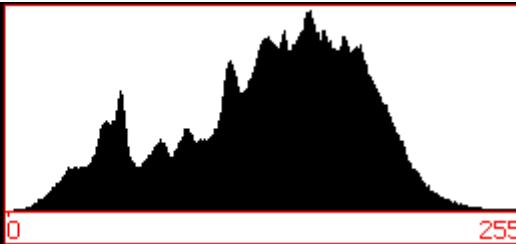
Shows fast and compact:

- exposure (over- and under-exposure)
- contrast
- dynamics & dynamic range
- image manipulation

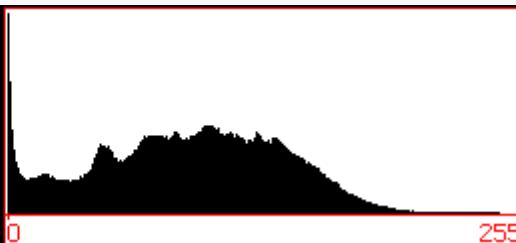
Does now show:

- localization of intensities within the image

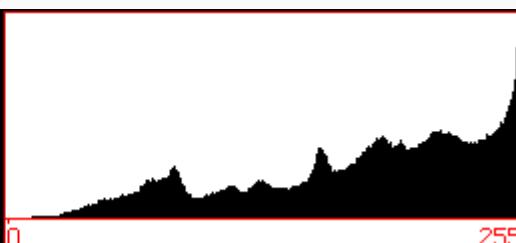
Histogram – Exposure



Correctly exposed image



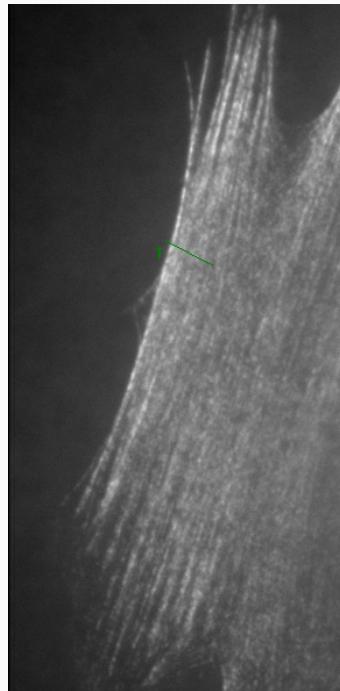
Underexposed image



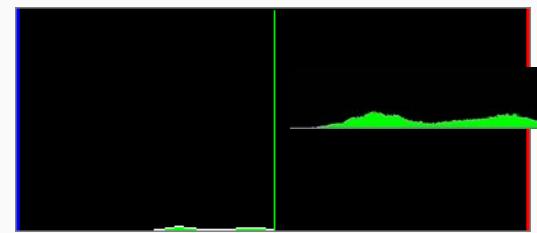
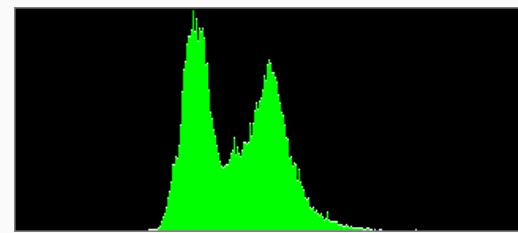
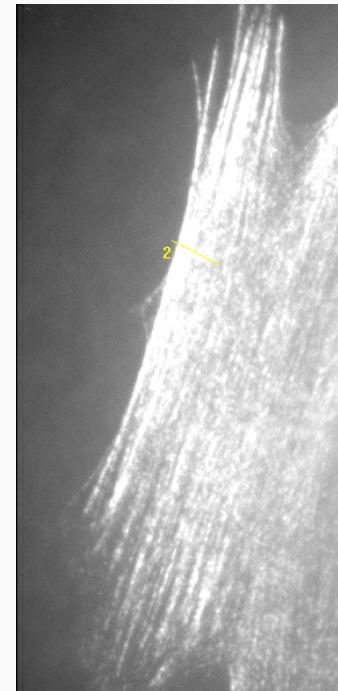
Overexposed image

Histogram – Exposure

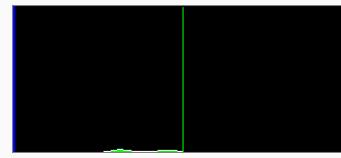
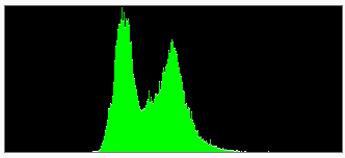
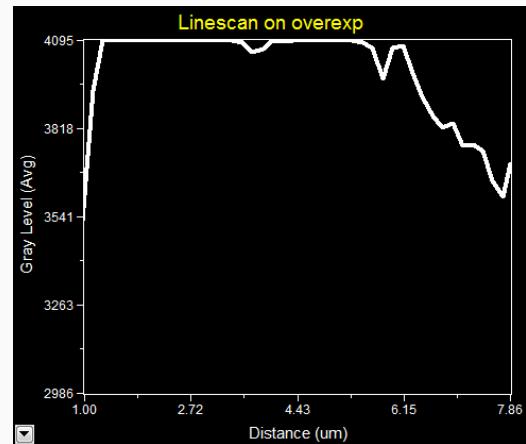
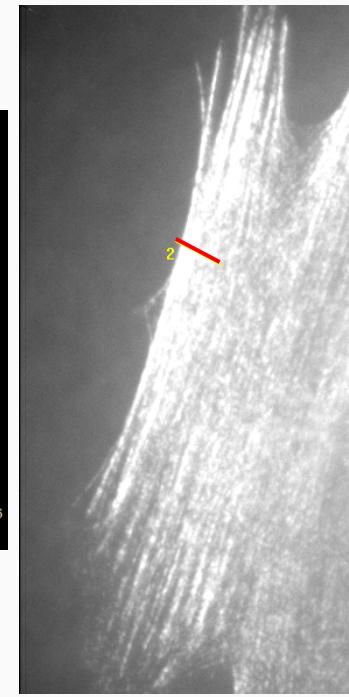
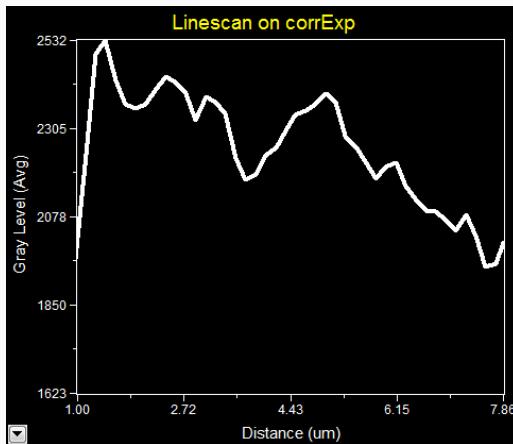
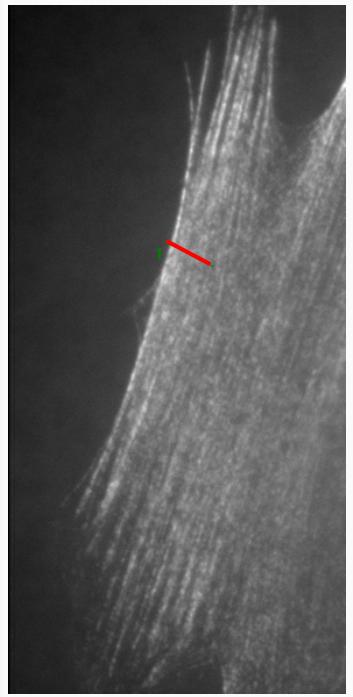
Correctly exposed image



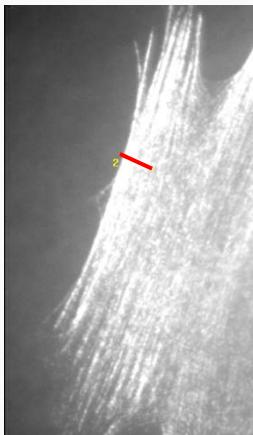
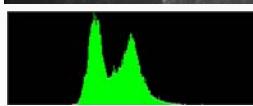
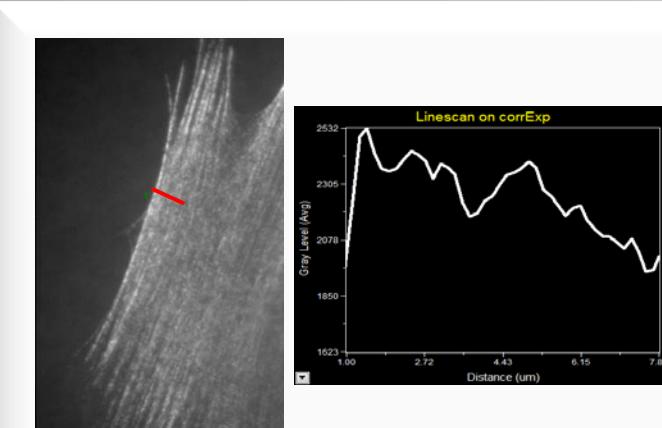
Overexposed image



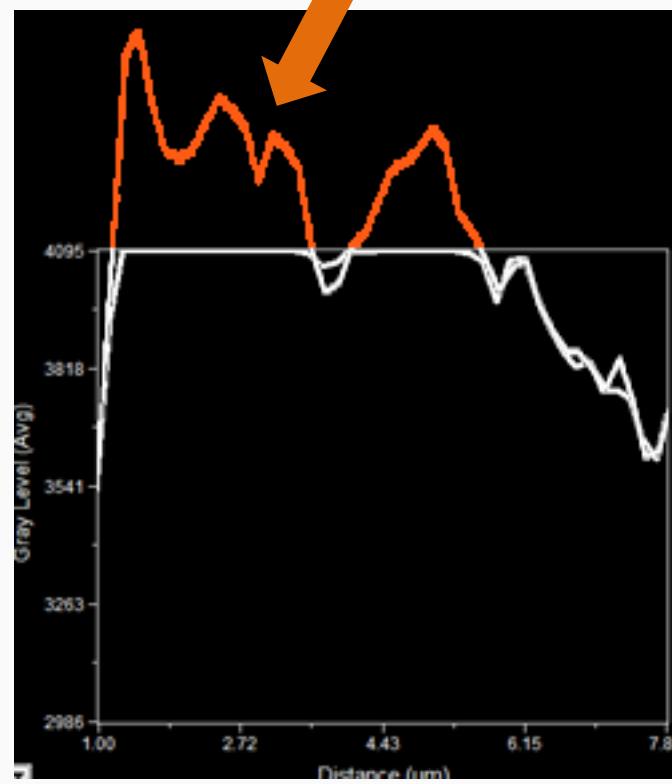
Histogram – Exposure



Histogram – Exposure



Bye Bye Data!!!



Histogram – Contrast

Contrast = the difference in visual properties that makes an object distinguishable from other objects and the background

Contrast = the interval of intensities that is effectively used within an image
= Difference between minimal and maximal pixel value

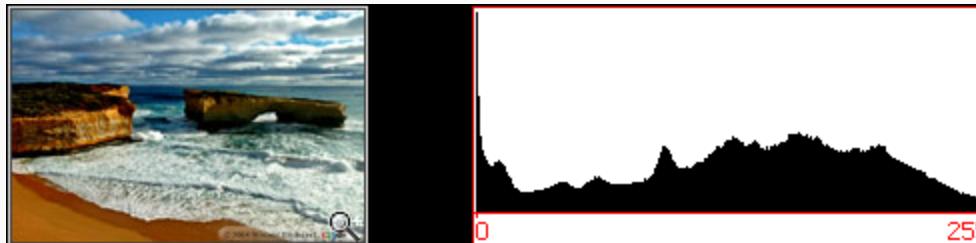


Image with too much contrast

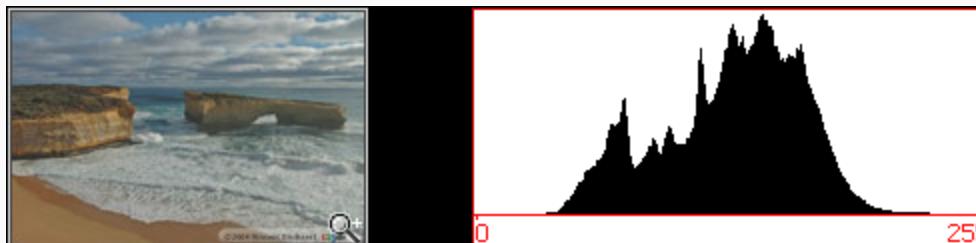
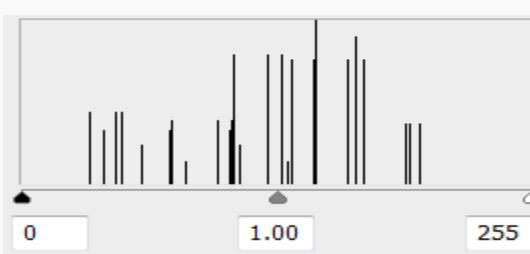
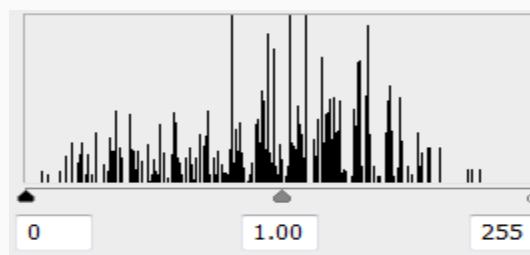
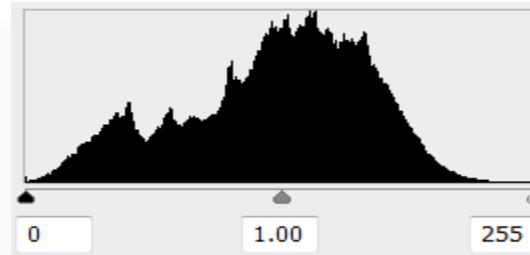


Image with too little contrast

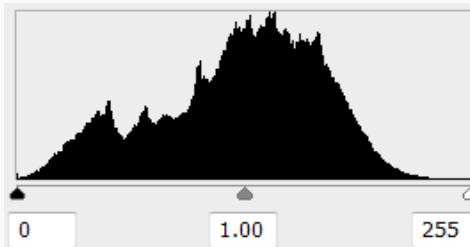
Histogram - dynamics & dynamic range

Dynamics = number of different pixel values used within an image



Histogram - dynamics & dynamic range

Dynamic range = the ratio between the largest and smallest possible values of a changeable quantity.



Dynamic range of a camera is the amount of electrons that can be held in a well (pixel)

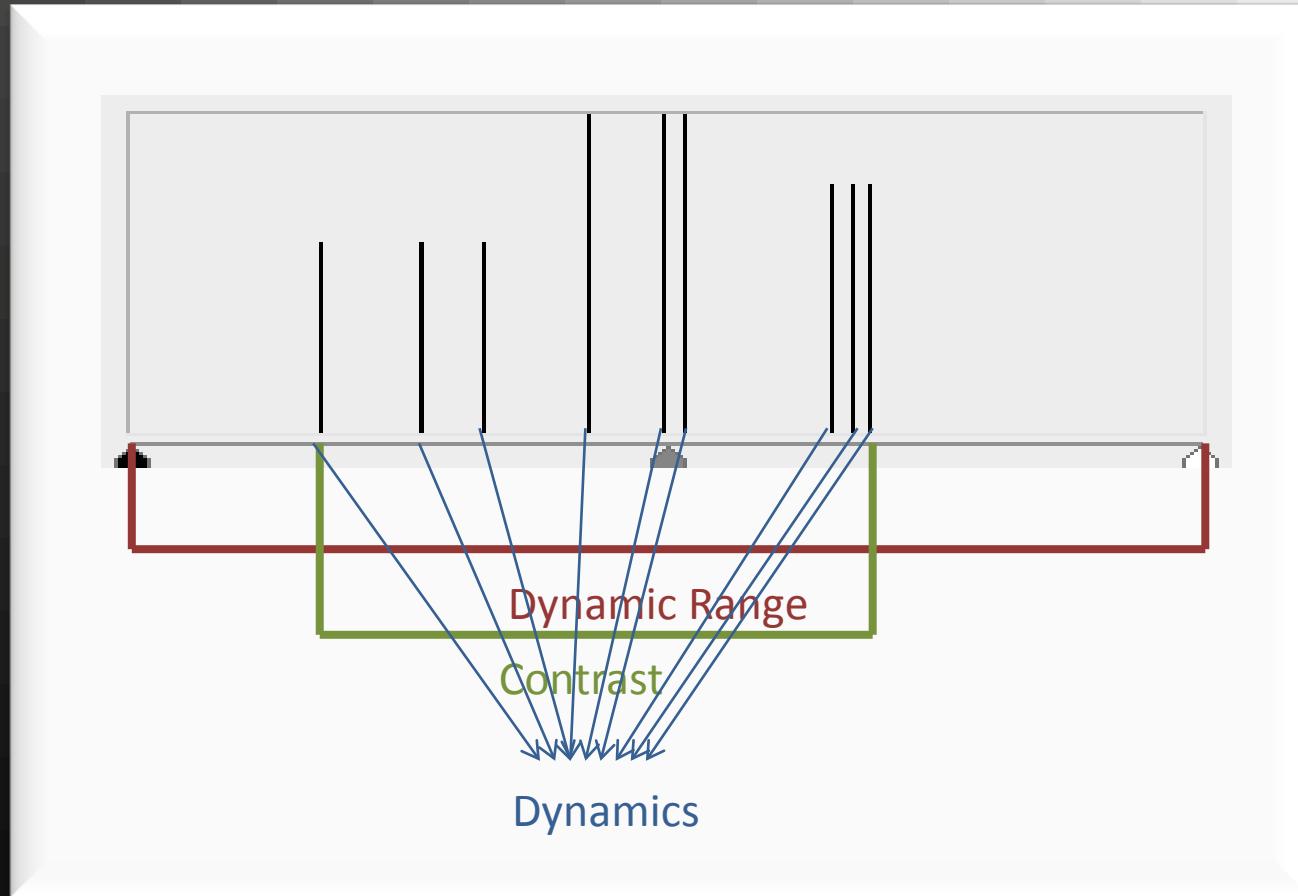


A/D – converter



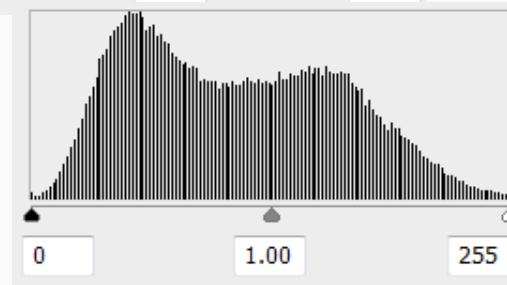
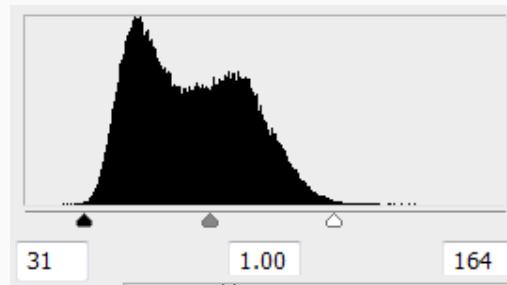
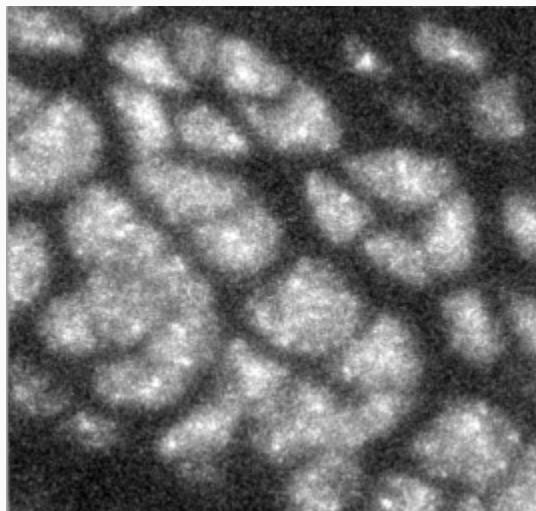
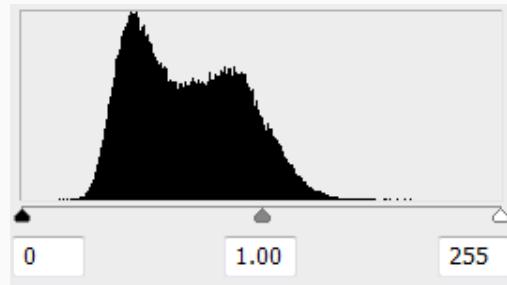
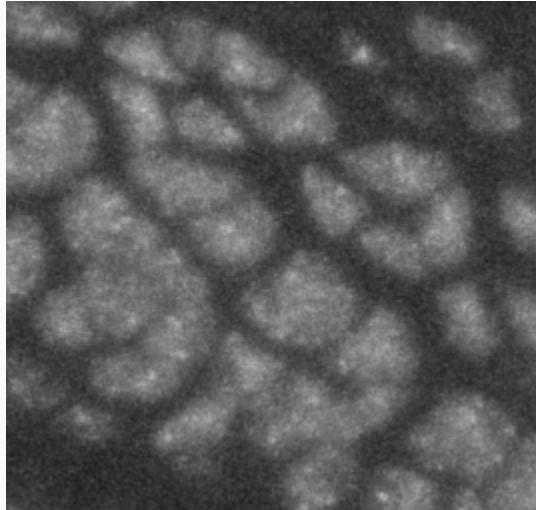
Dynamic range of an image is the used bit-depth

Histogram – contrast/dynamics & dynamic range



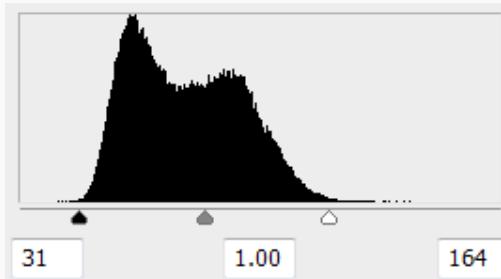
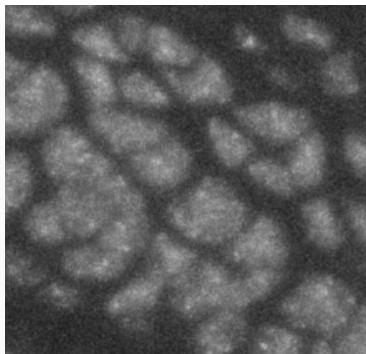
Histogram – contrast & dynamics

The contrast of an image can be increased – the dynamics cannot!

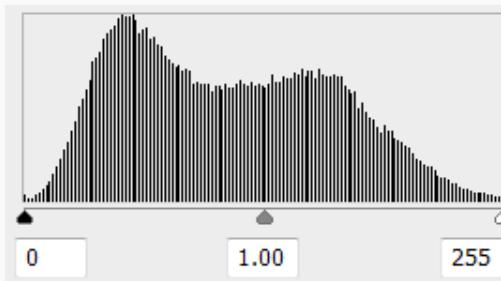
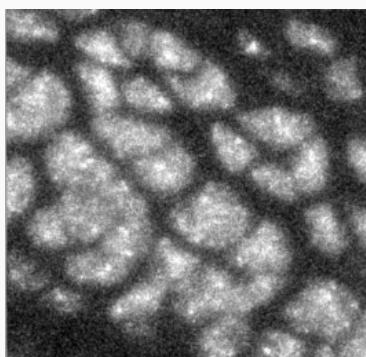


Use higher bit-depth to
avoid quality losses
after image processing

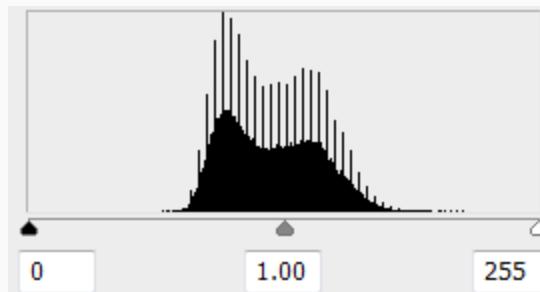
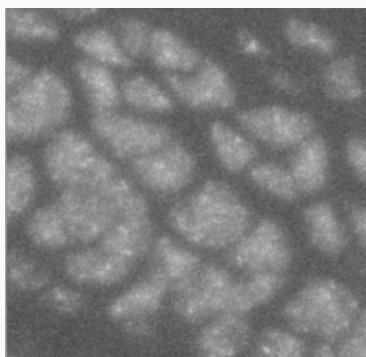
Histogram – image manipulation



original

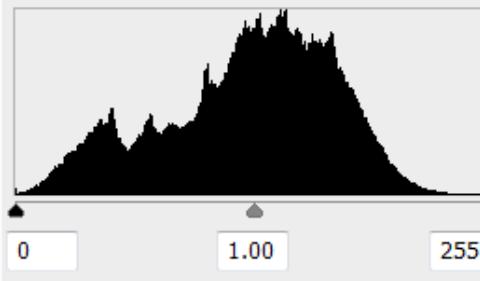


contrast enhancement

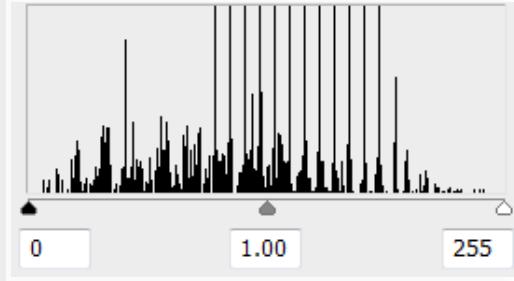


contrast reduction

Histogram – image compression

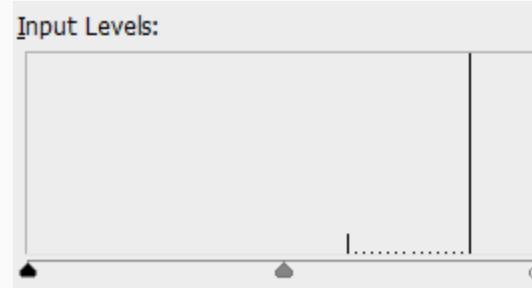


original



gif compression

Histogram



original

Histogram

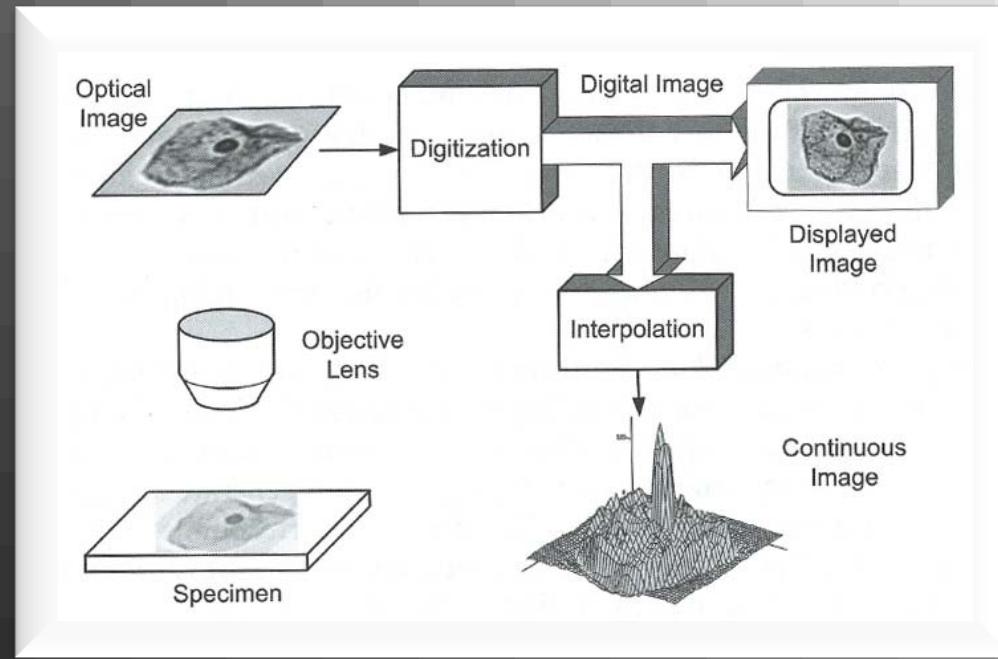


jpeg compression

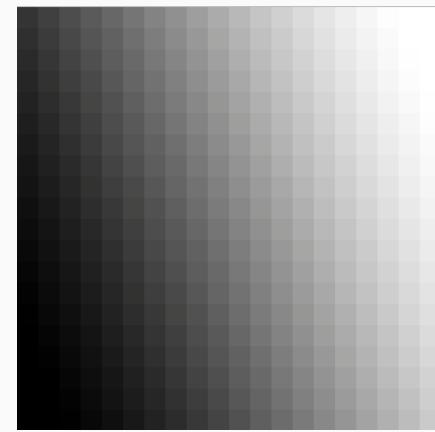
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Look Up Table



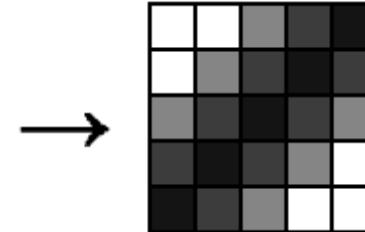
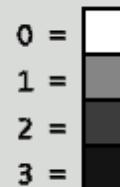
52	64	77	90	103	238	246	253	255	255
47	59	72	85	97	235	243	250	255	255
...
...
...
...
...
0	0	3	11	19	155	168	181	193	206
0	0	1	8	15	149	163	176	188	202



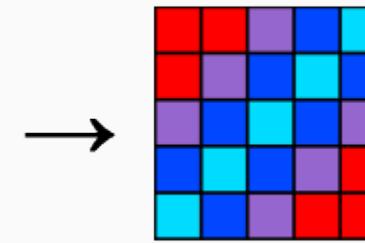
Look Up Table

A **colour look-up table' (CLUT)** is a mechanism used to transform a range of input colors into another range of colours.

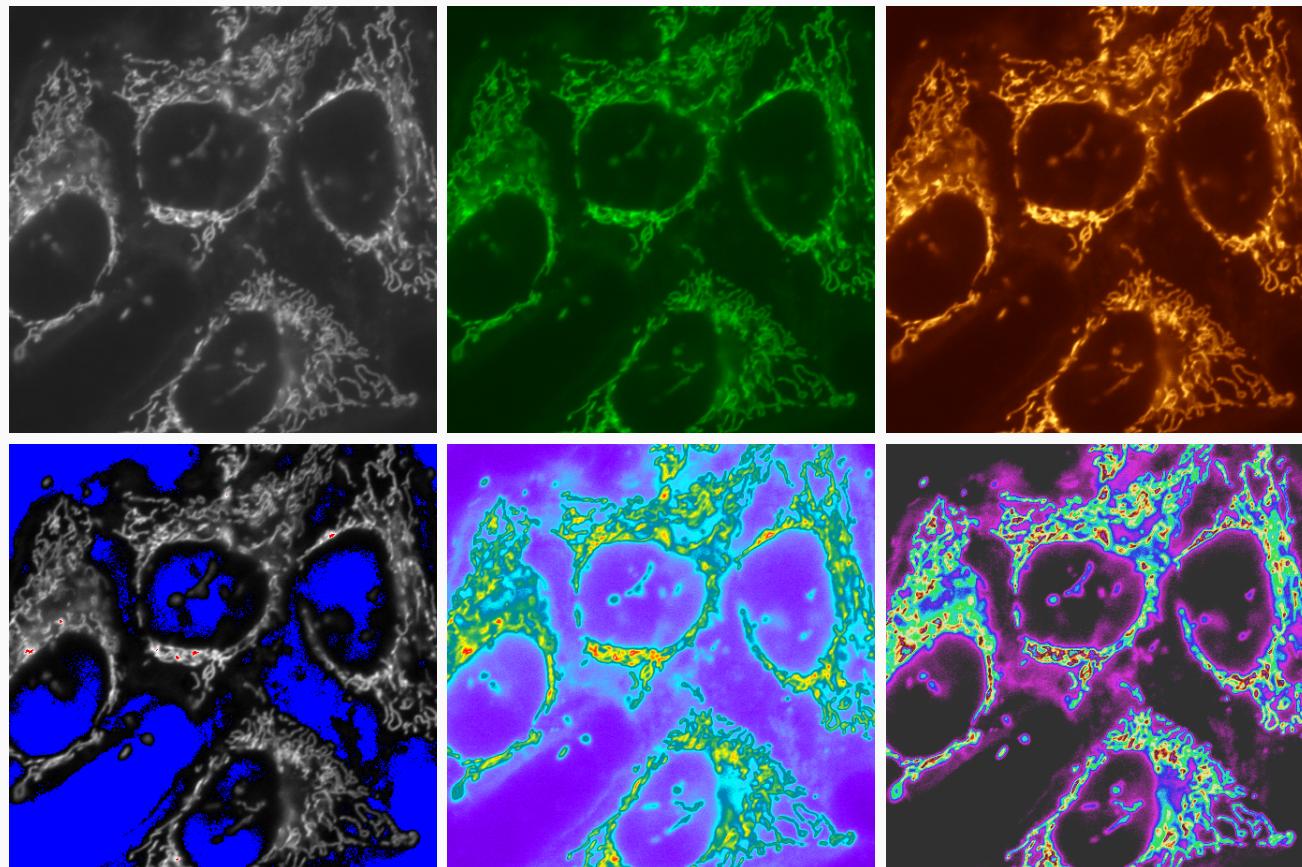
0	0	1	2	3
0	1	2	3	2
1	2	3	2	1
2	3	2	1	0
3	2	1	0	0



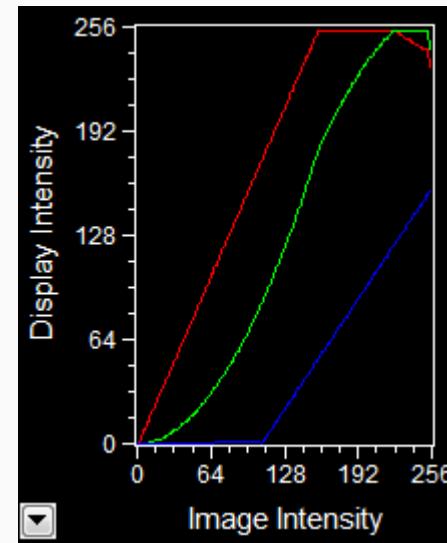
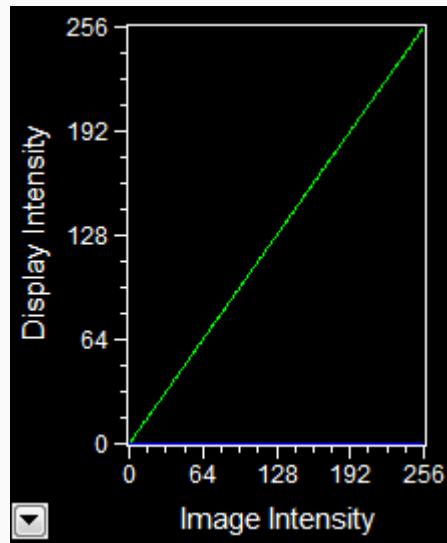
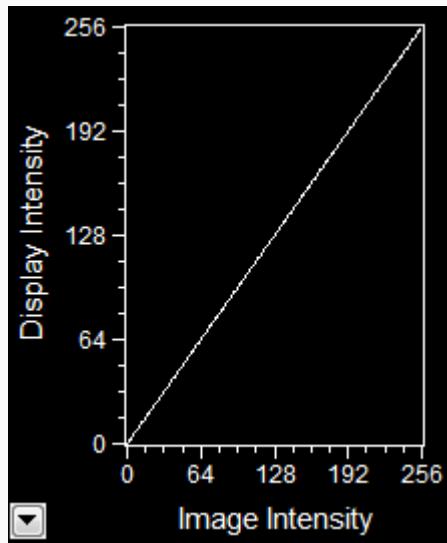
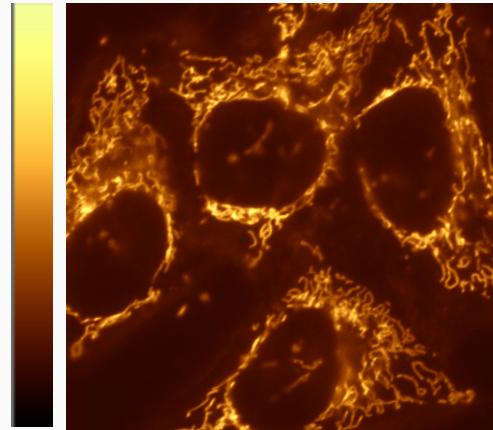
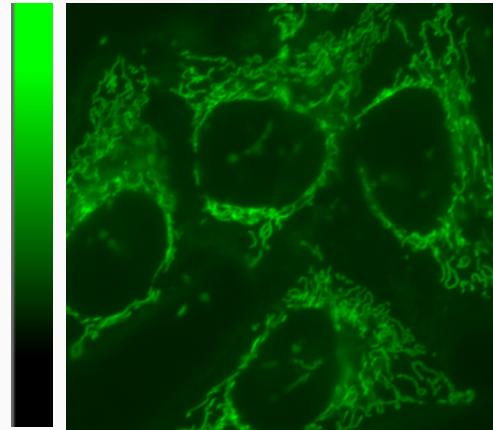
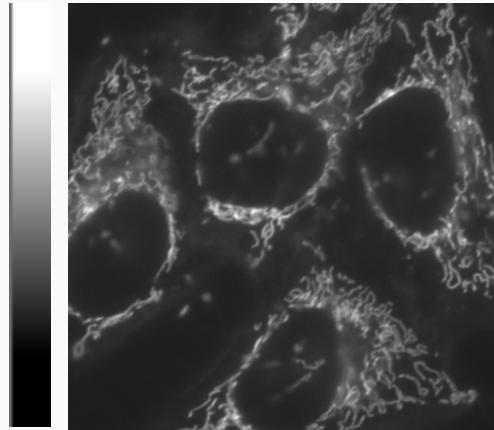
0	0	1	2	3
0	1	2	3	2
1	2	3	2	1
2	3	2	1	0
3	2	1	0	0



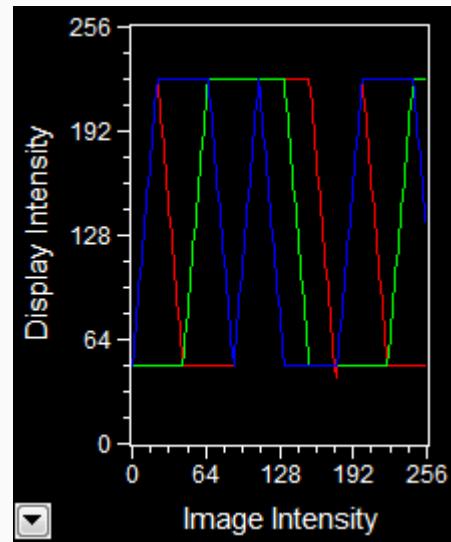
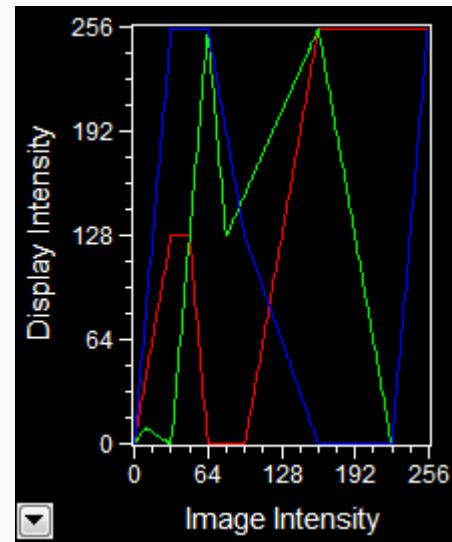
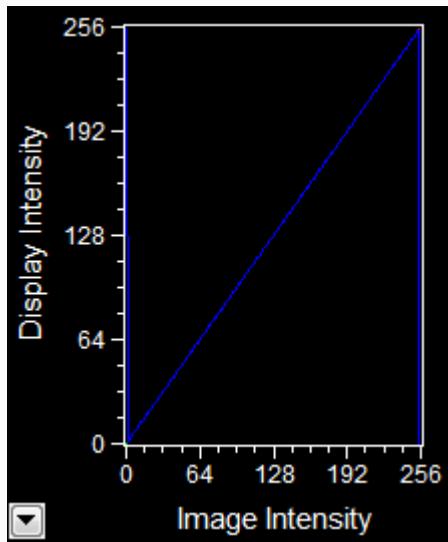
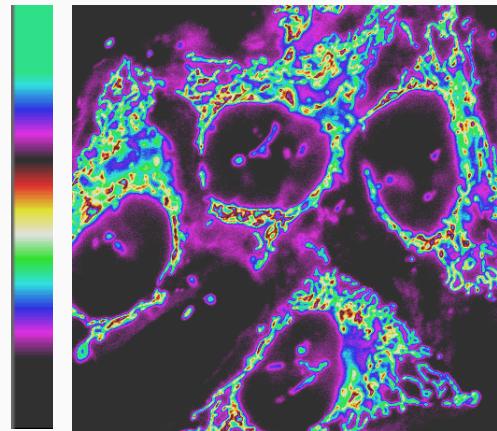
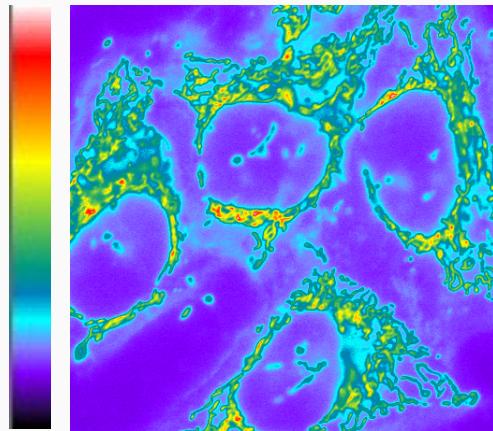
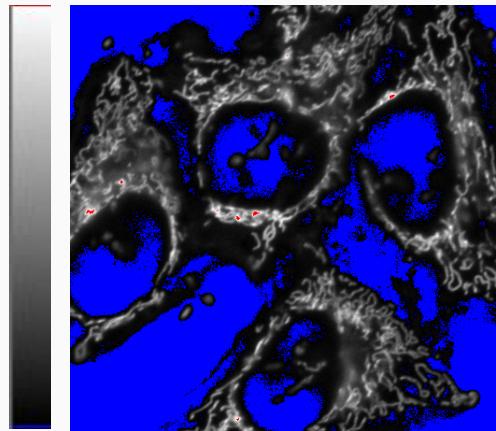
Look Up Table



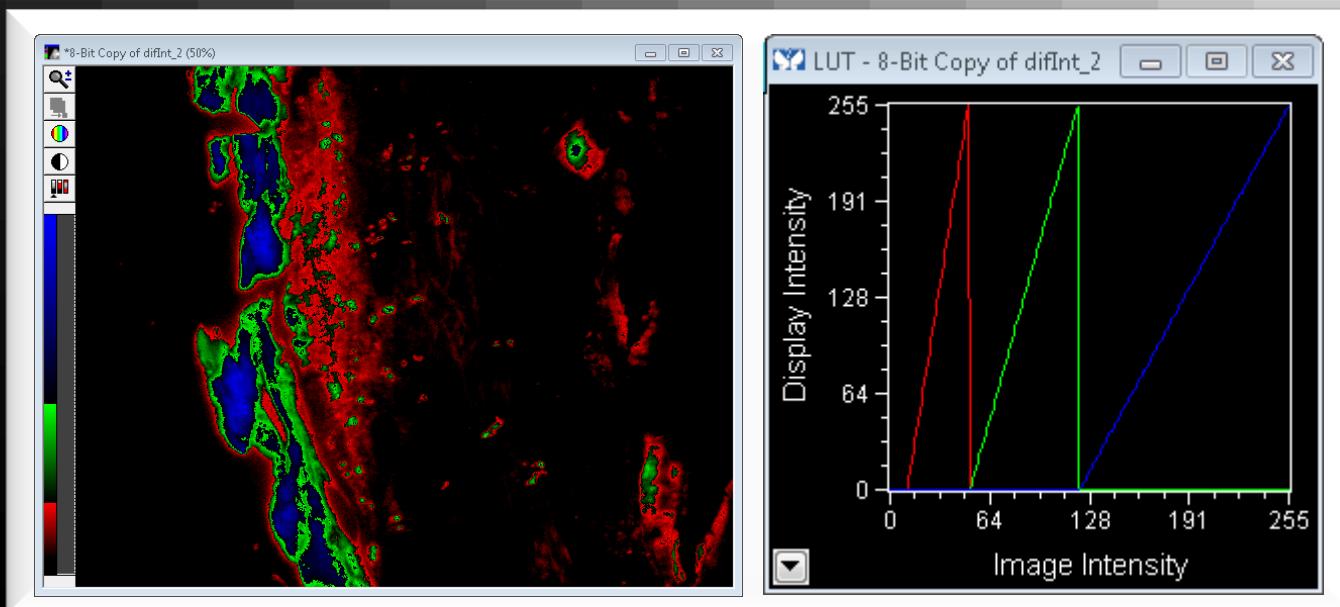
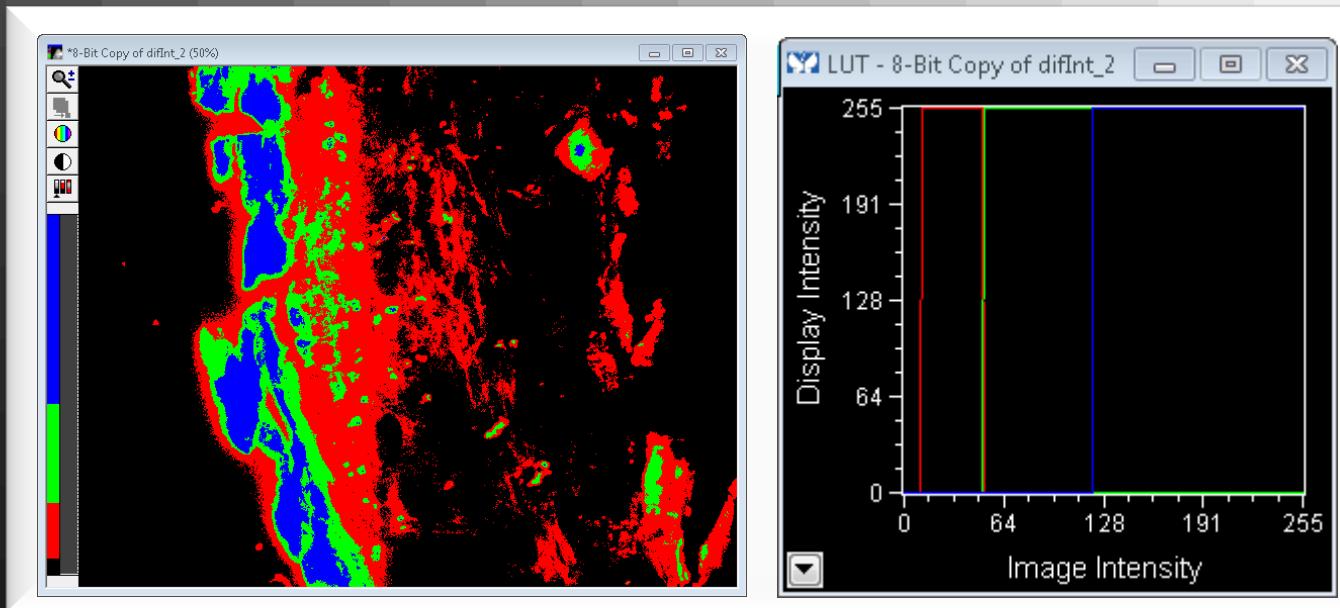
Look Up Table



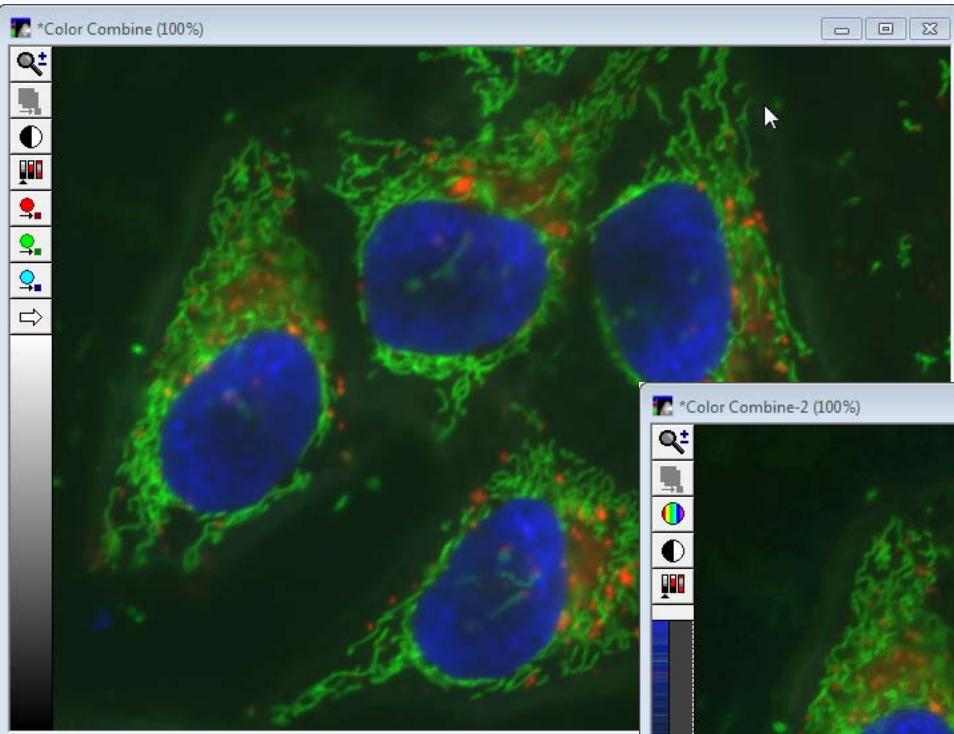
Look Up Table



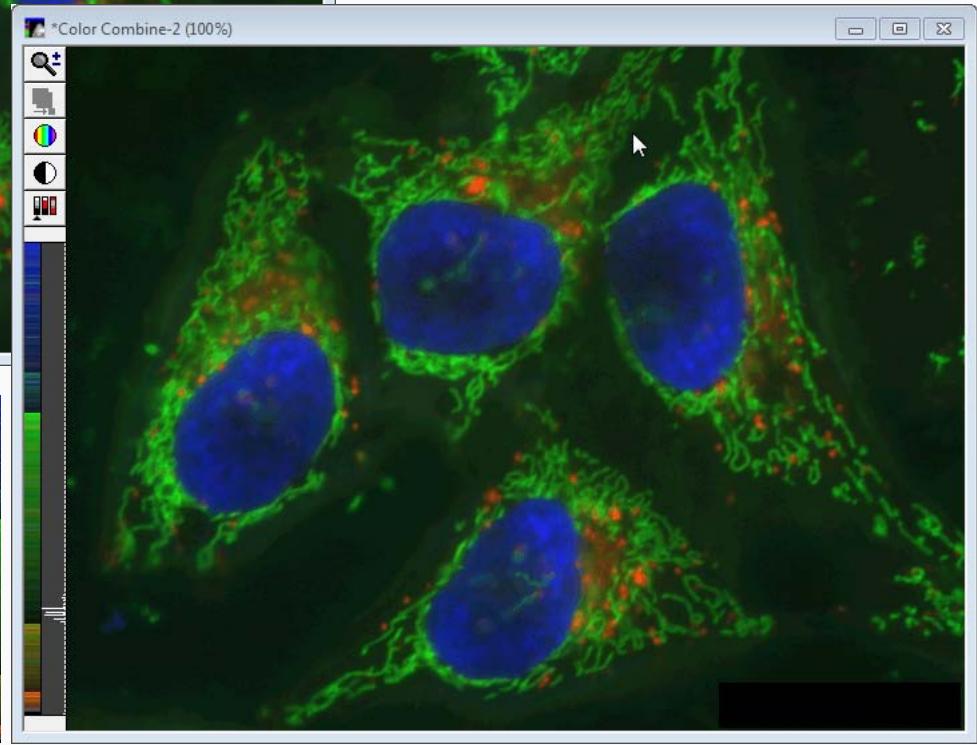
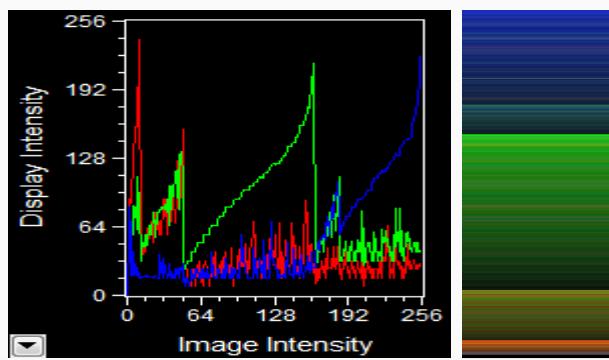
Look Up Table



Look Up Table



Index CLUT –
show 3x8 – 24-bit in 8-bit



Overview

- What is an image?
- How is an image defined?

Dimension

- How is an image stored?

File Formats

- How to interpret gray values in an image?

Histogram

- How to display gray values?

Color Look Up Table

How to...?

Programs used in the course



Fiji Fiji Is Just ImageJ—batteries included

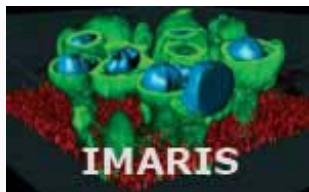
- freeware / open-source
- Fiji includes ImageJ, JDK, Java 3D, Set of plugins in coherent menu structure
- platform independent
- 500+ plugins available



MetaMorph®

MetaMorph

- Commercial software from Molecular Devices
- acquisition software on wide-field systems
- installed on computers in BioOptics Office
- wide variety of functions
- easy to automate/batch process



Imaris

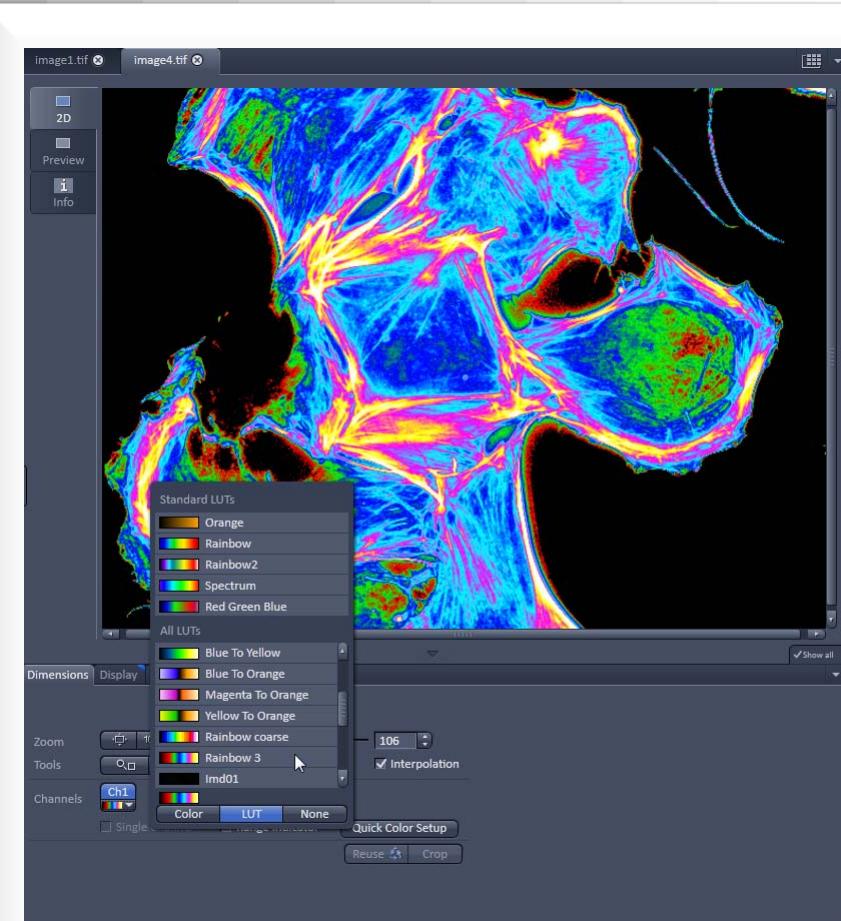
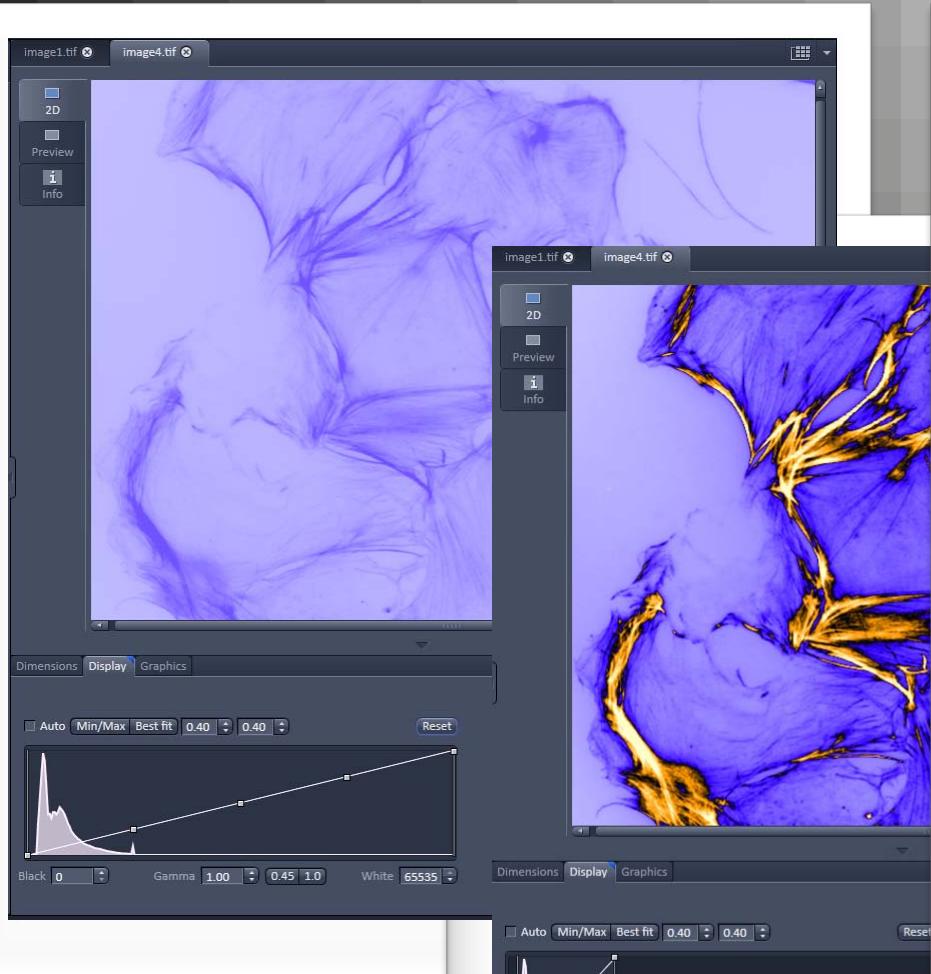
- Commercial software from Bitplane/Andor
- easy to use software for 3D rendering
- installed on one computer in BioOptics Office



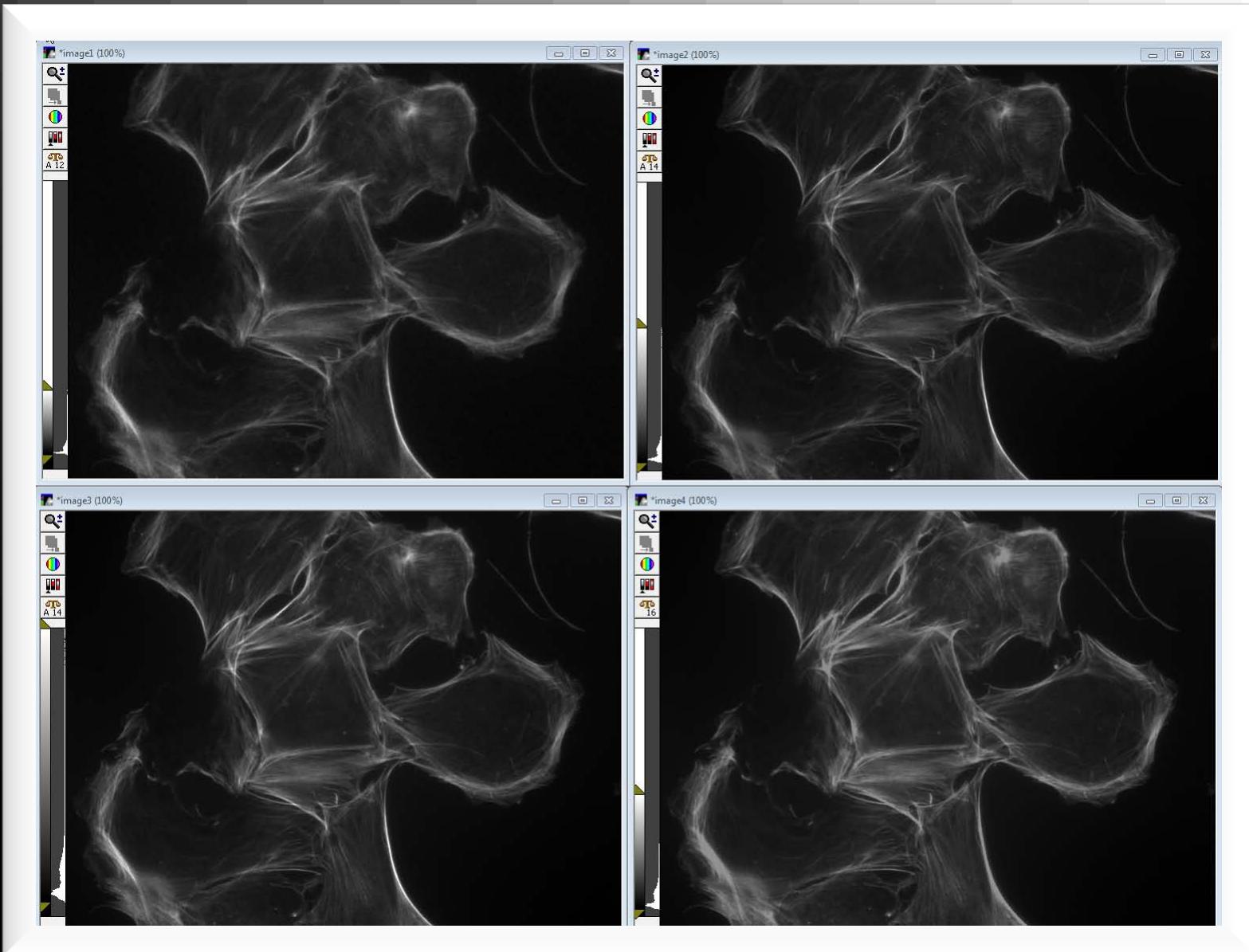
ZEN

- Commercial software from Zeiss
- confocal acquisition software

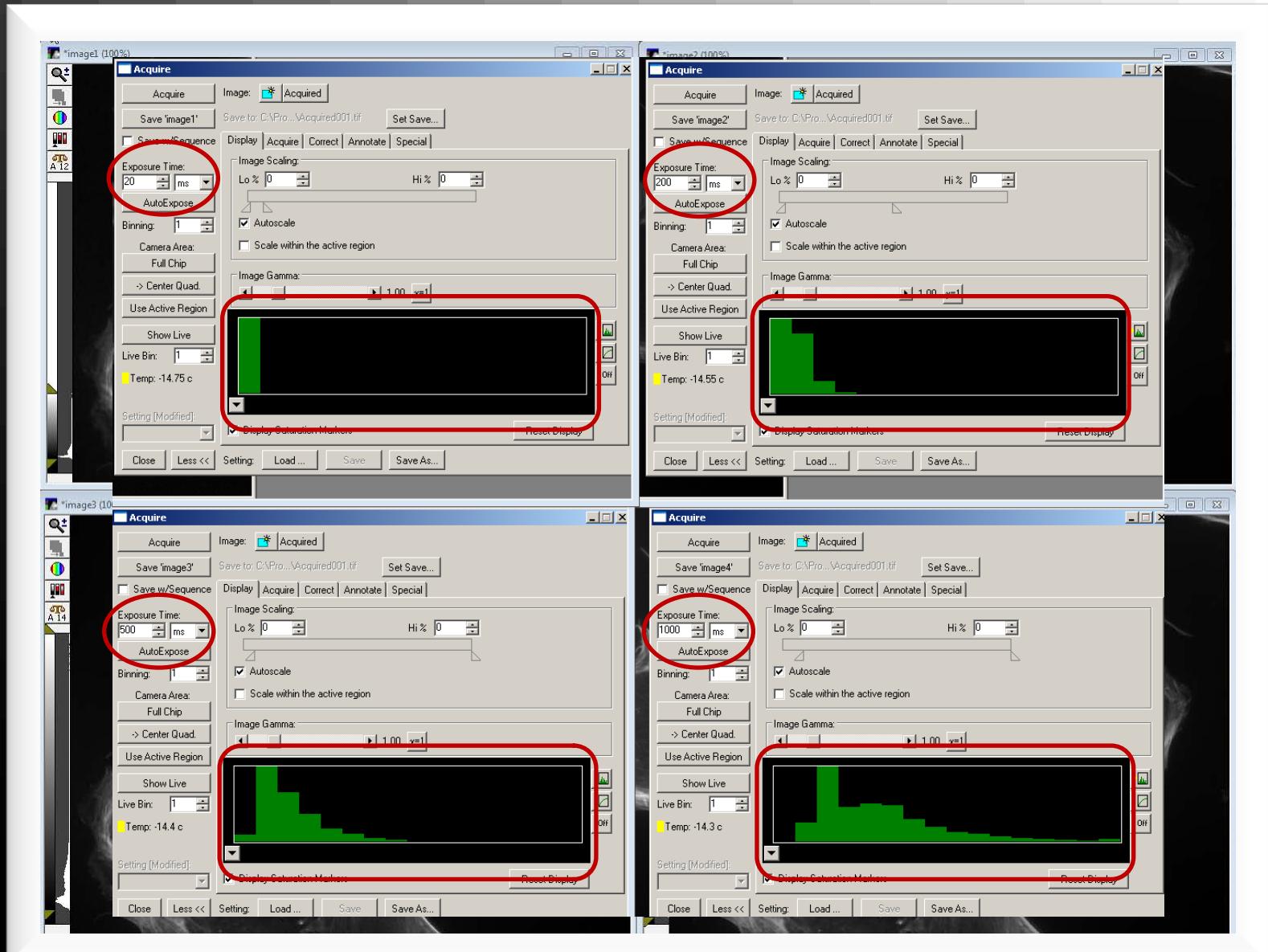
ZEN



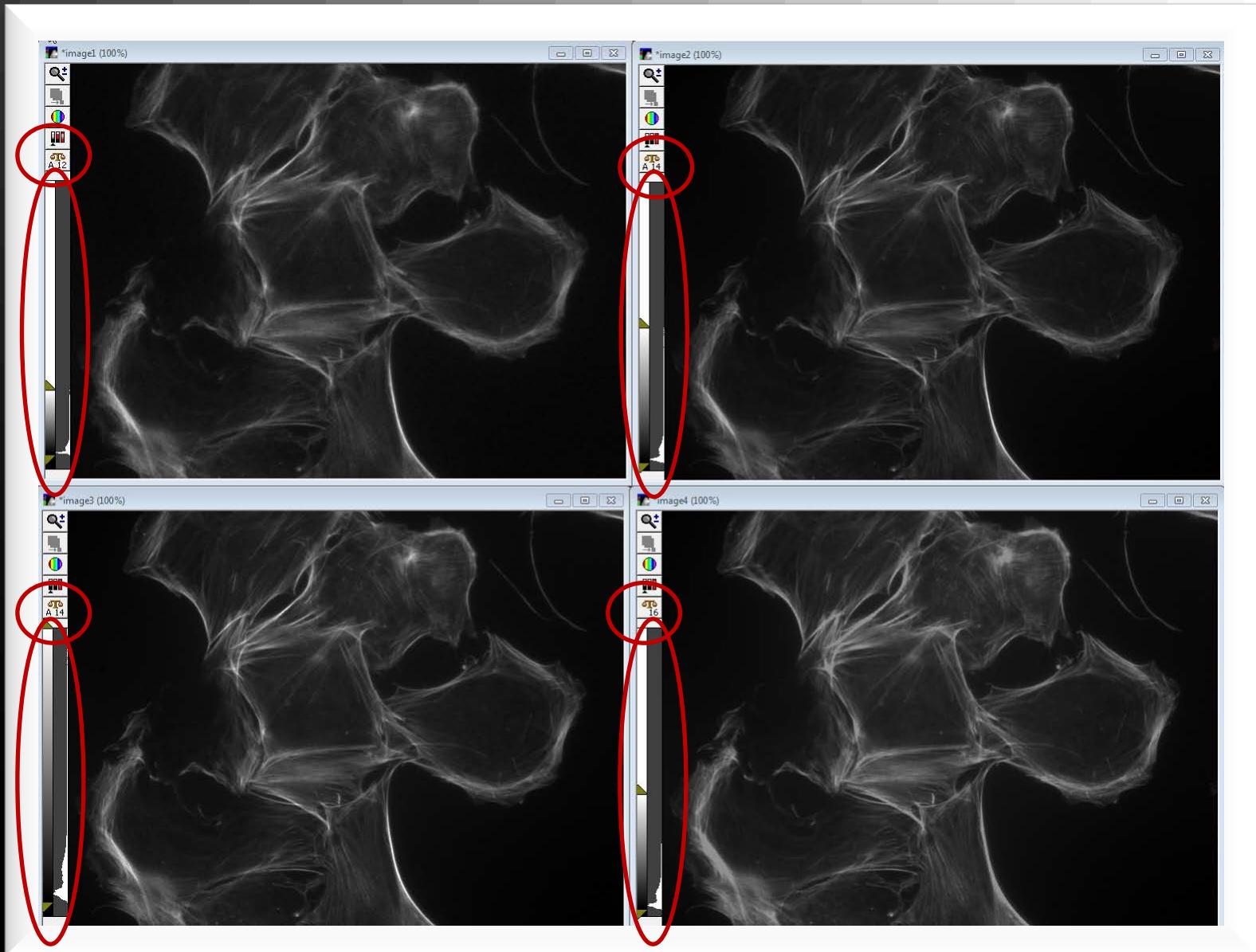
MetaMorph - Acquisition



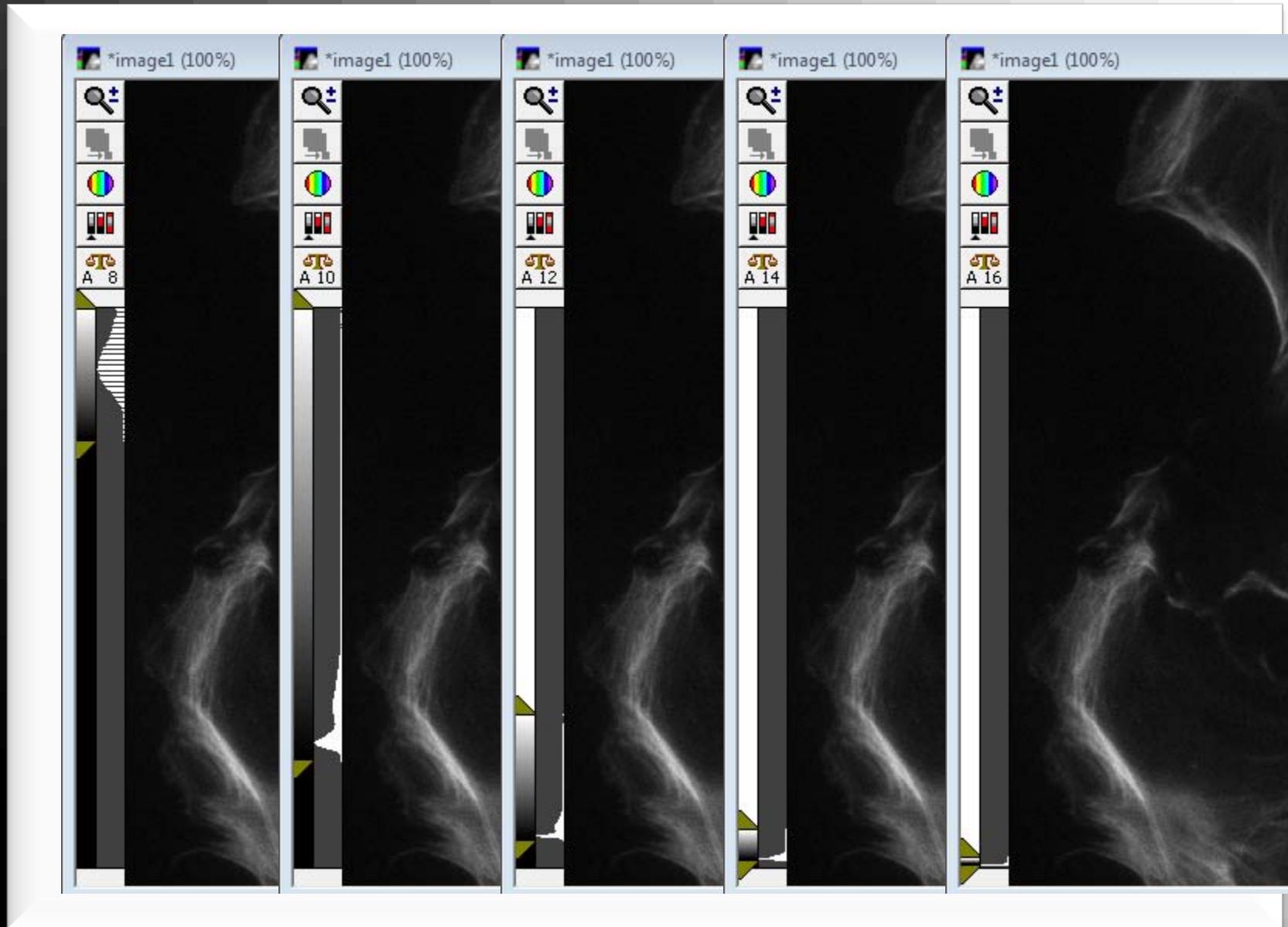
MetaMorph - Acquisition



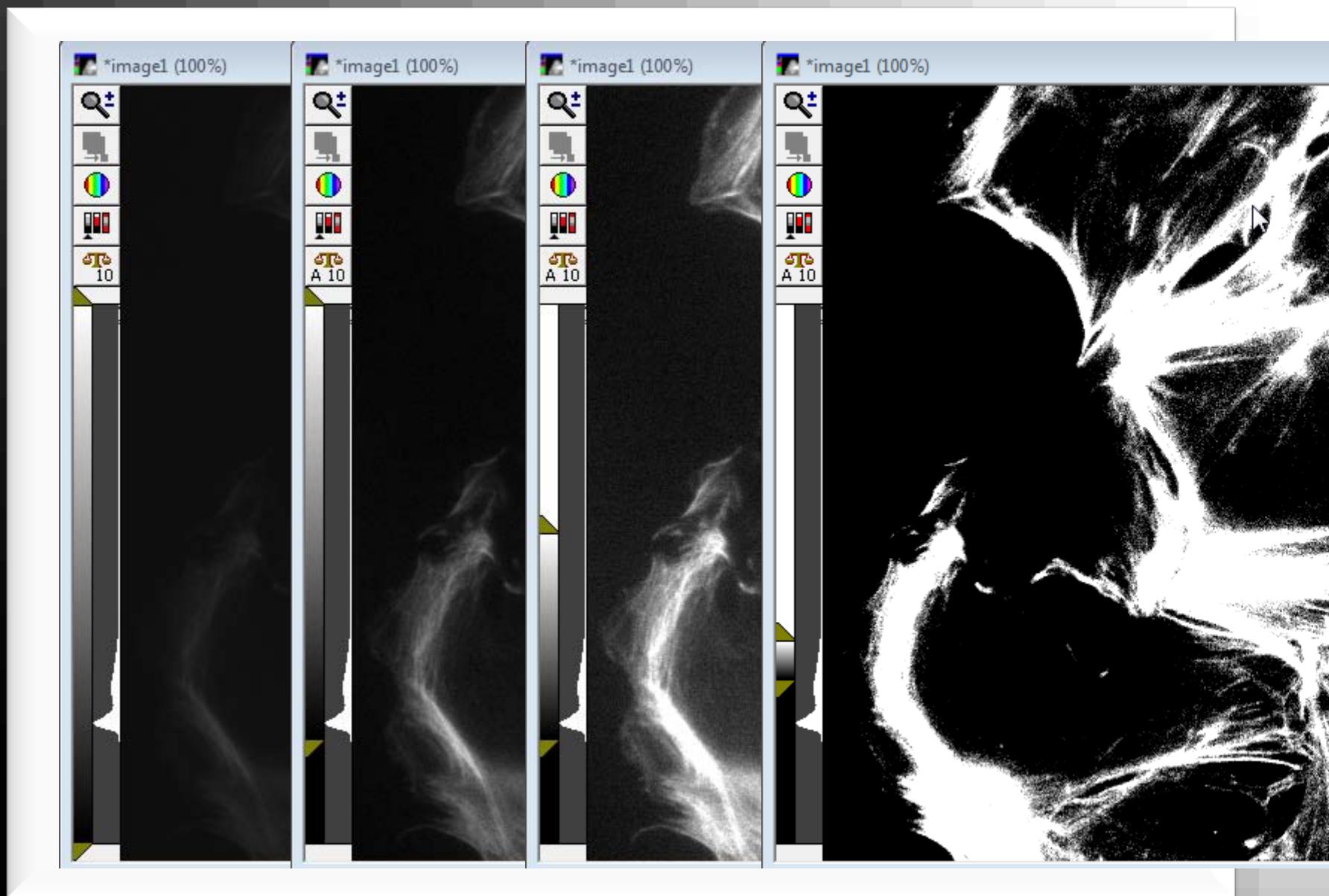
MetaMorph - Acquisition



MetaMorph - Acquisition



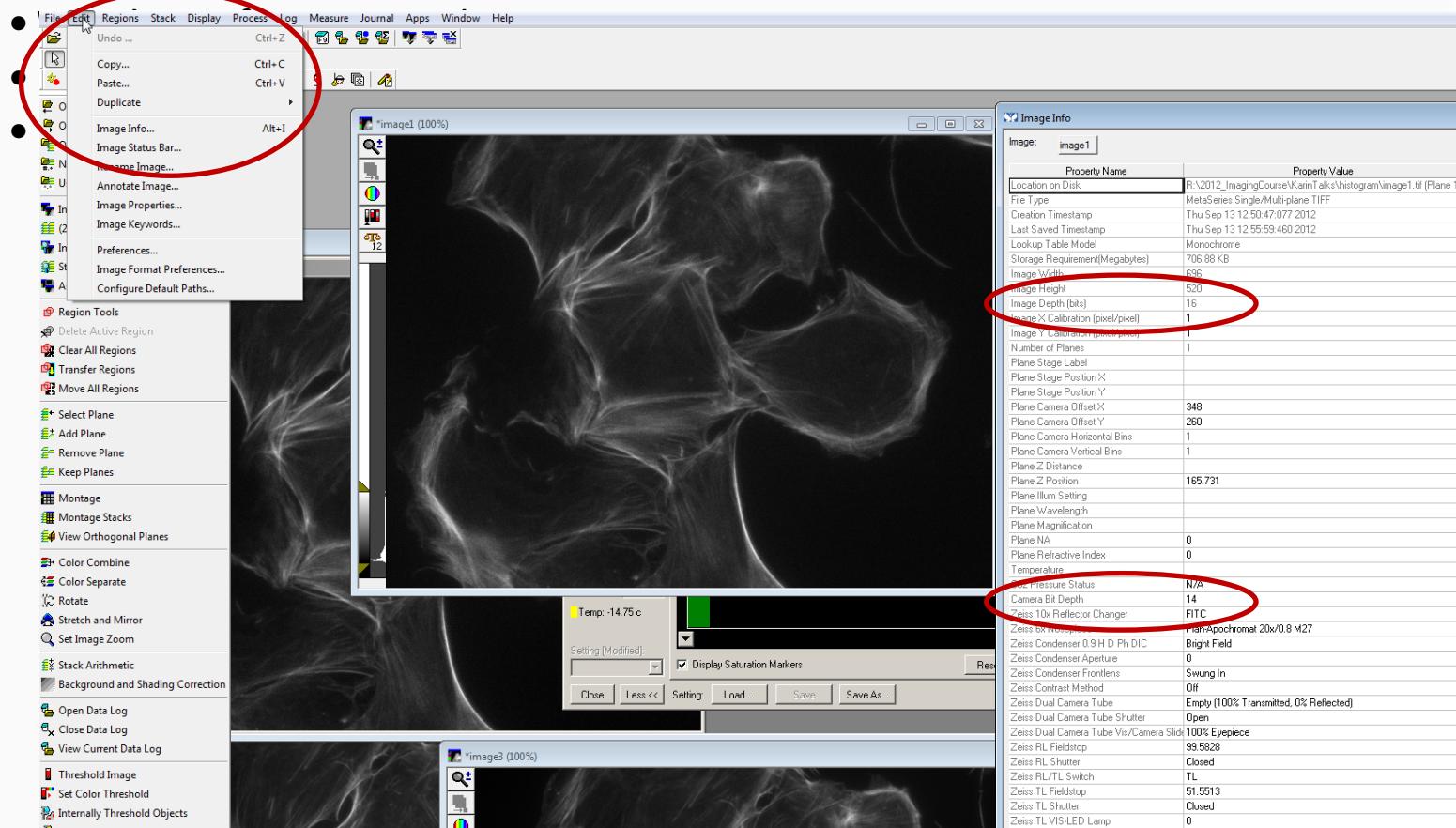
MetaMorph - Acquisition



MetaMorph - Acquisition

Take Home Message

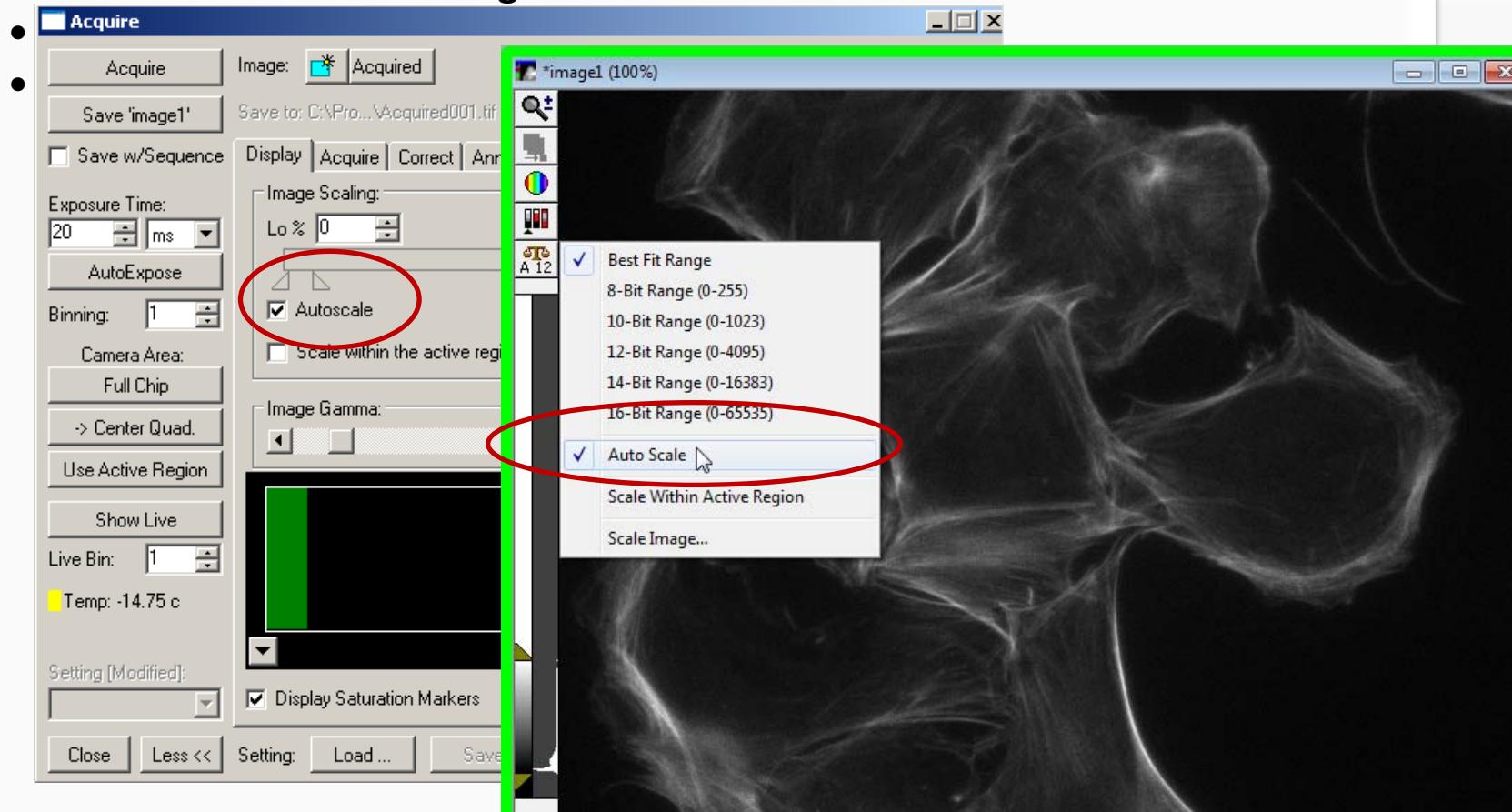
- Know the bit depth of your camera



MetaMorph - Acquisition

Take Home Message

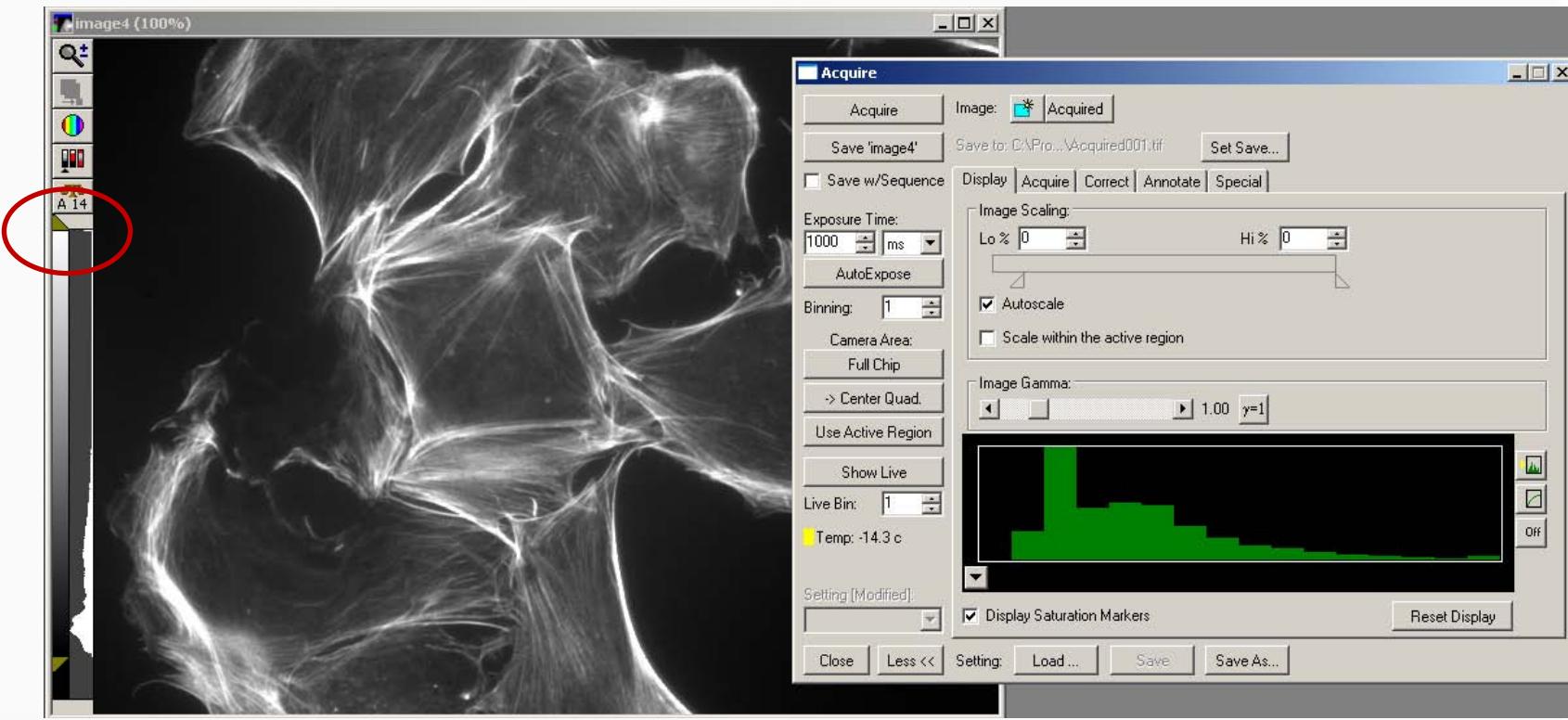
- Know the bit depth of your camera
- **Watch out for Auto-scaling**



MetaMorph - Acquisition

Take Home Message

- Know the bit depth of your camera
- Watch out for Auto-scaling
- **Use complete dynamic range**
- **Do not overexpose image**



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Exercise

Exercise

- open image 1_Basics.tif
- convert to an 8-bit image with a maximum Intensity of 125
- display in a way that all values below 50 are shown in red with increasing intensity, above 100 in blue and in-between in green

Take Home Massage

Take Home Massage

- Know the bit depth of your camera
- Keep raw data image
- be careful on conversion of bit-depth
- Understand and take in account the difference of image information and display
- watch histogram
- never do measurements on image processed in a way that intensities are altered