Digital Images

Bi/BE177: Principles of Modern Microscopy

Vikas Trivedi

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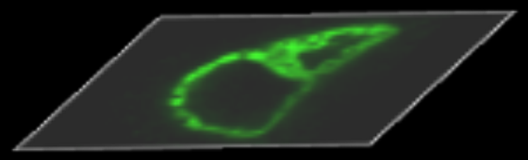
California Institute of Technology

Outline



Digital image formation

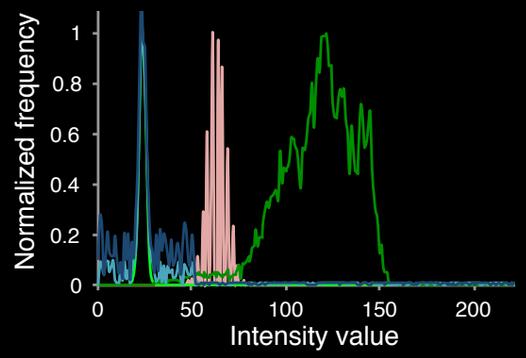


Image properties

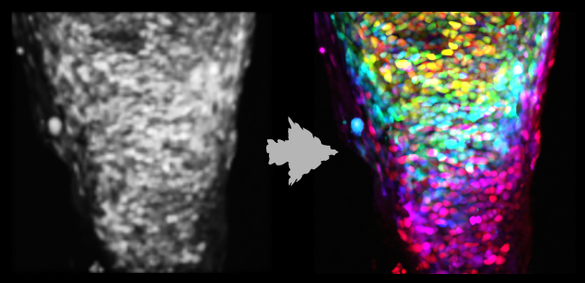
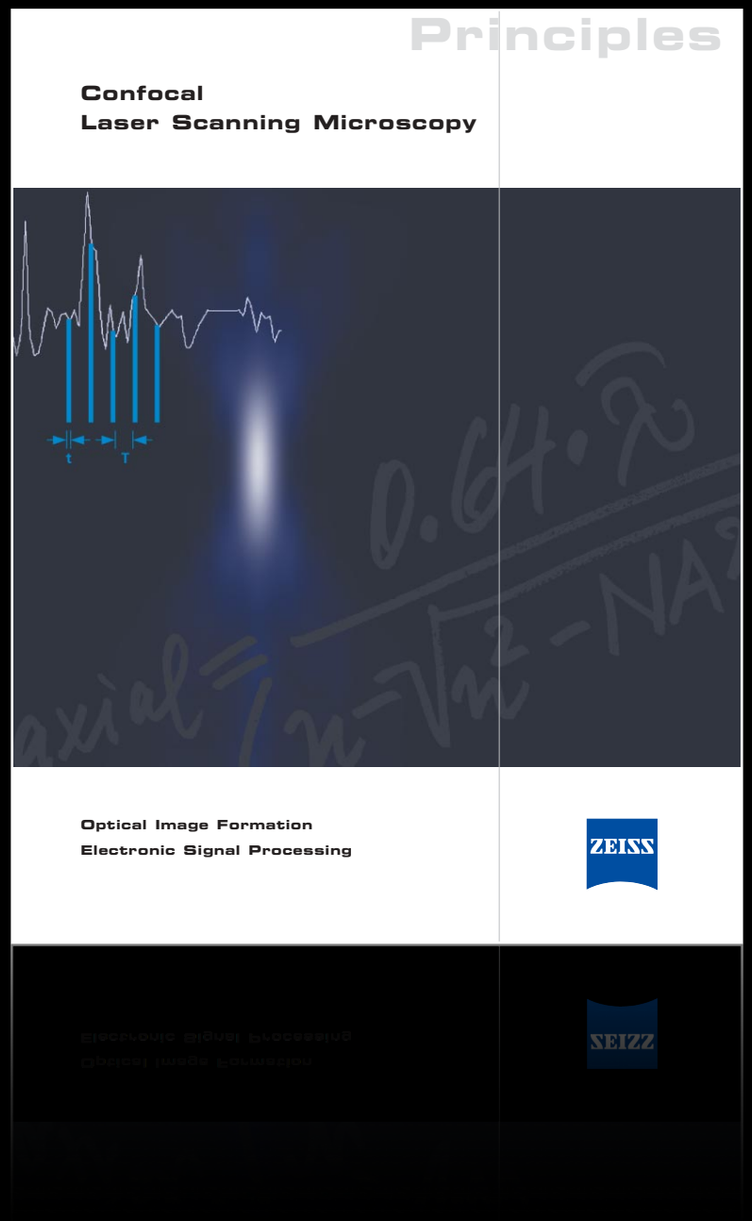


Image processing

Available softwares

Resources


Principles
Confocal
Laser Scanning Microscopy
Optical Image Formation
Electronic Signal Processing
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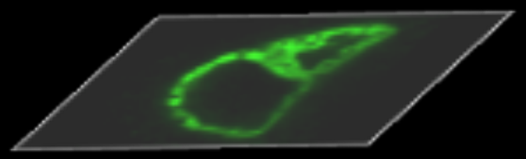
Physics of Light & Color
Microscopy Basic Concepts
Special Techniques
Fluorescence Microscopy
Confocal Microscopy
A broad range of fluorescent protein genetic variants have been developed over the past several years that feature fluorescence emission spectral profiles spanning almost the entire visible light spectrum.
Olympus BioScapes Contest
Fluorescent Proteins Intro
Physics of Light and Color
Total Internal Reflection
Fundamentals of FRET
Confocal Microscopy
Microscope Basics

Outline



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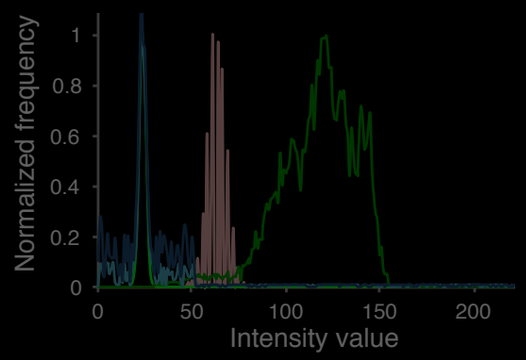

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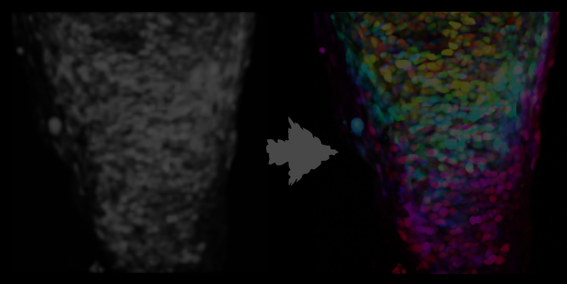


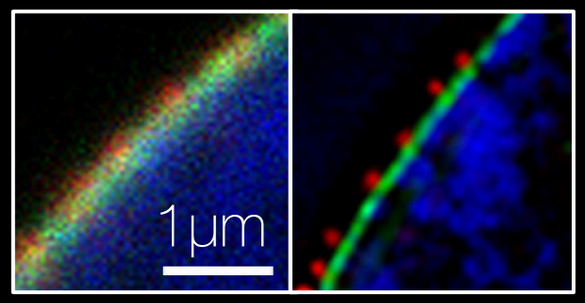
Image processing

Available softwares

Microscopy as a compromise

Competition between different performance parameters

Resolution



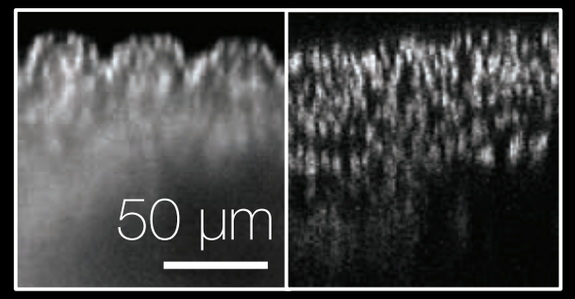
Schermelleh L., *et al* (2008) (*Science)*

Trade off

Trade off

Trade off

Depth

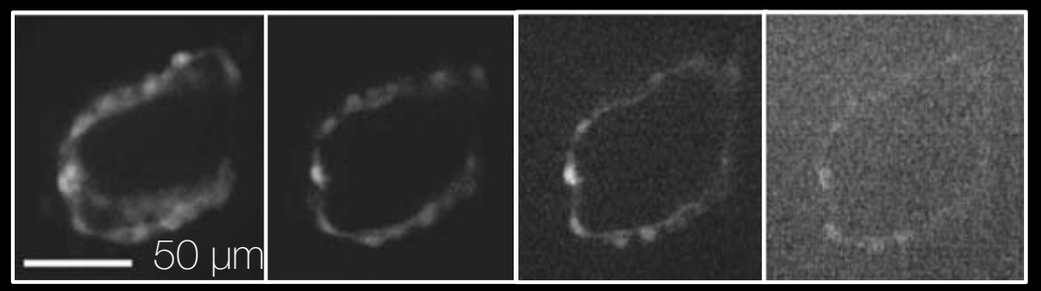


excitation direction

Truong T.V., *et al* (2011) (*Nature Methods)*

Speed

Frame rate : 2.5 10 35 104



Vermot J., *et al* (2008) (*HFSP Journal)*

Slide adapted from Dr. Thai V. Truong

Microscopy in biological context

Competition between different performance parameters

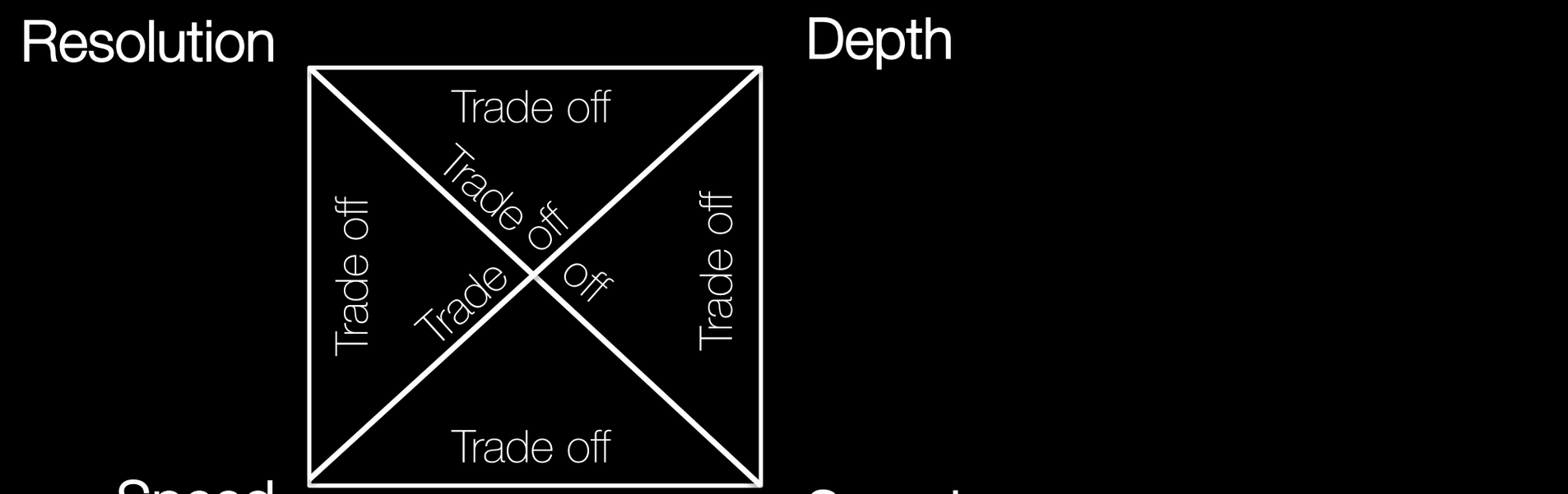
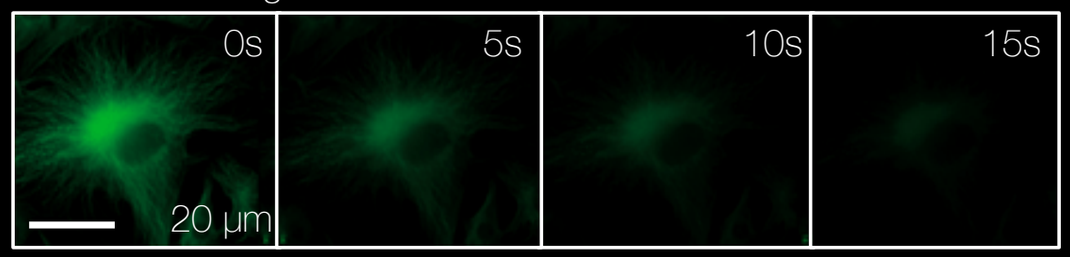


Photo-bleaching



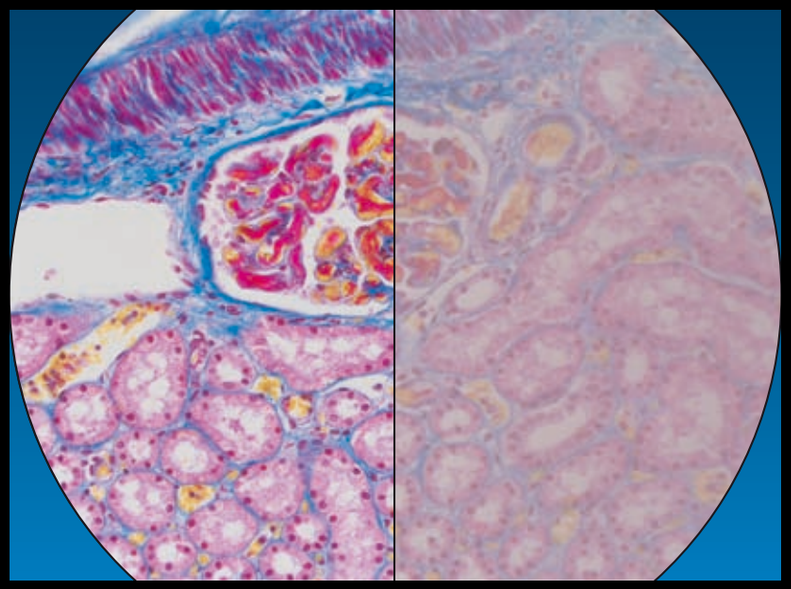
Life technologies, Invitrogen

Photo-toxicity

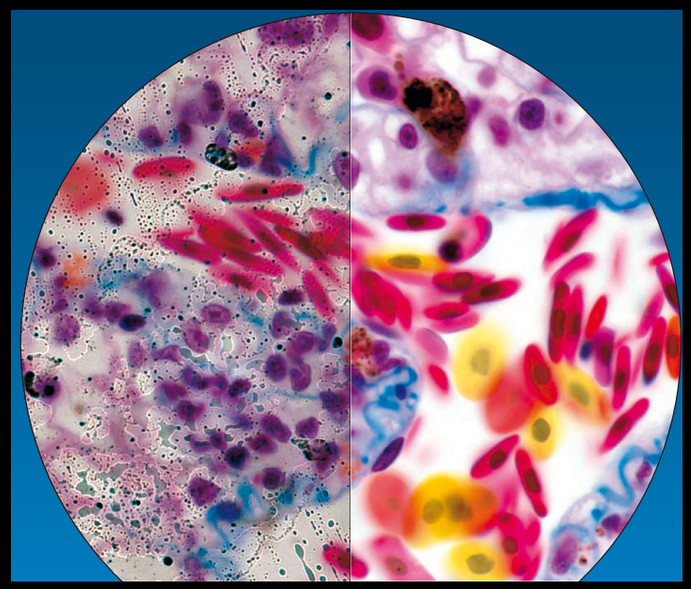
Always best to have the best image settings

-better to optimize acquisition than rely on post-acquisition processing

Something really simple :-)



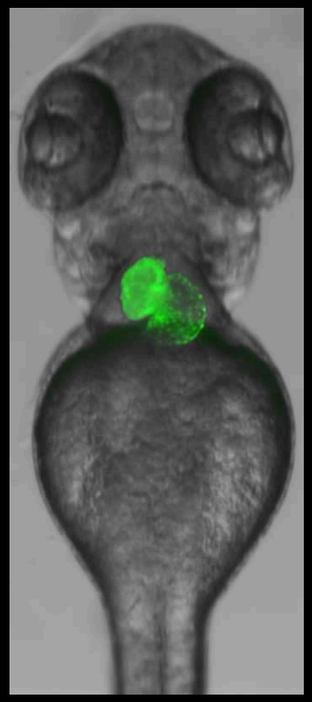
Soiled Lens

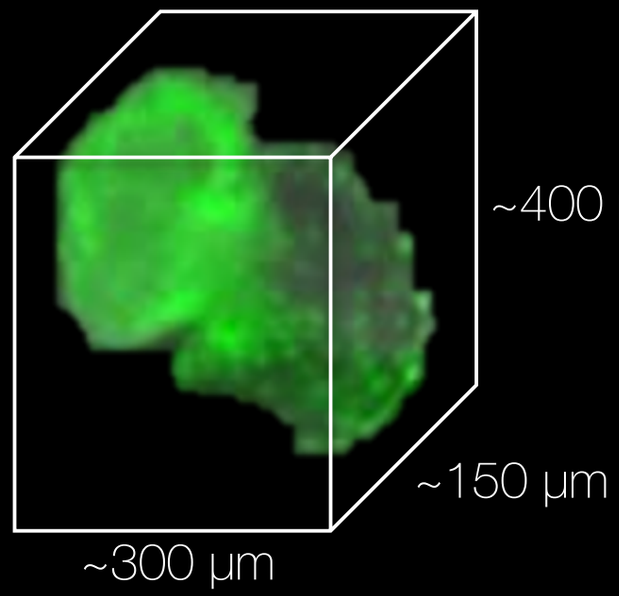


Soiled Camera and dirt

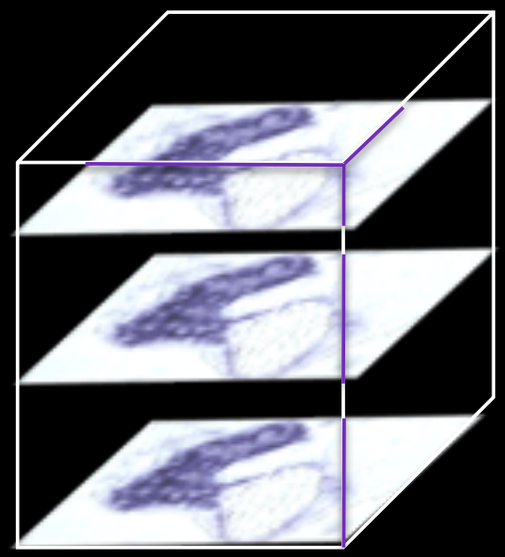
Images : Carl Zeiss

Building up a 3D image

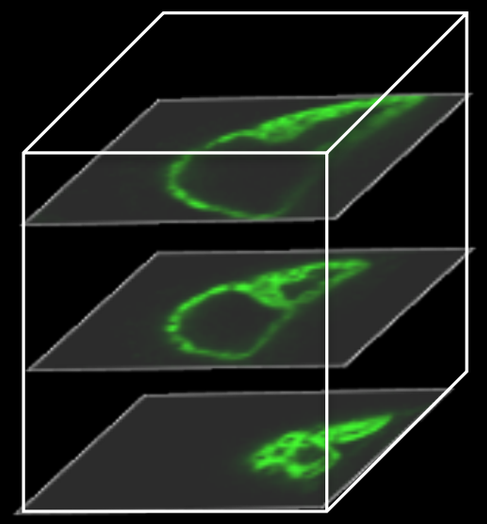




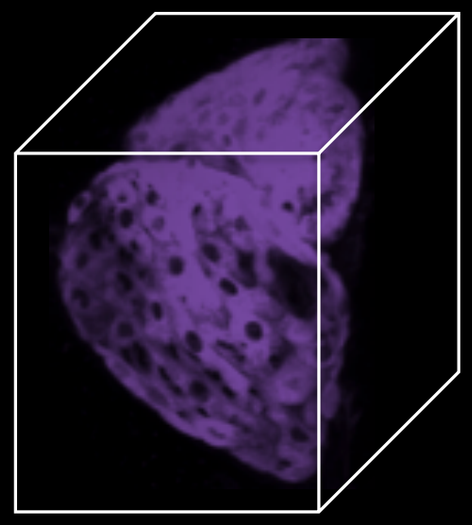
Physical sectioning
(invasive)

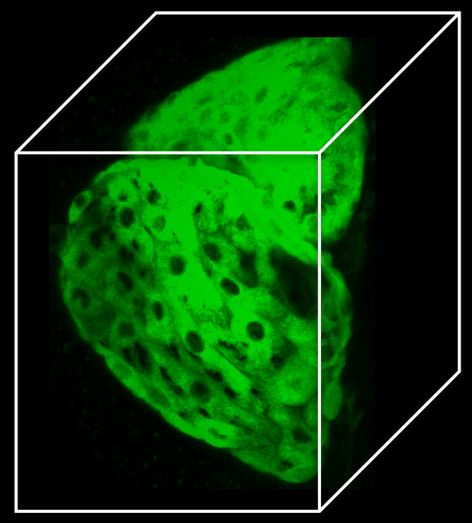


Optical sectioning
(non-invasive)



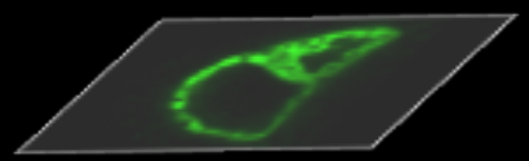
Poss et al, (2002) (*Science*)

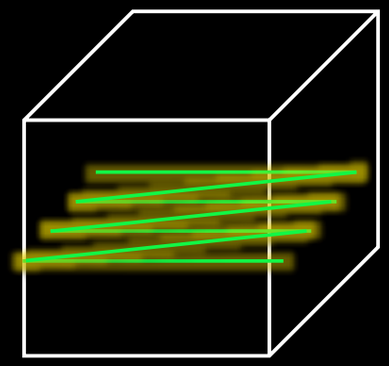




Building up a 2D image

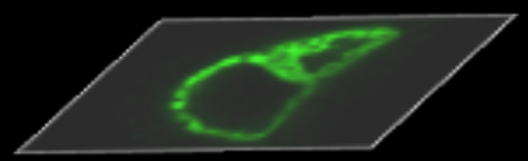
1D detectors (PMT, GaAsP, APD)

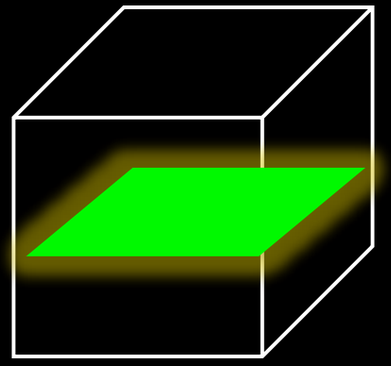




Raster scan to
form 2D images

2D detectors (CCD, CMOS, sCMOS)

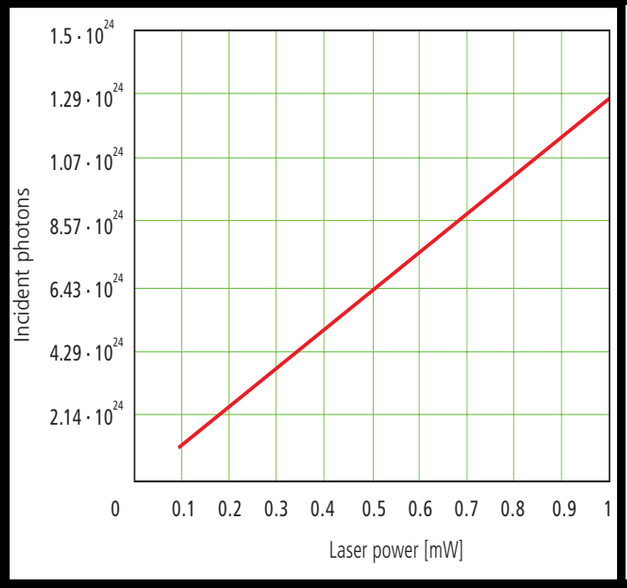


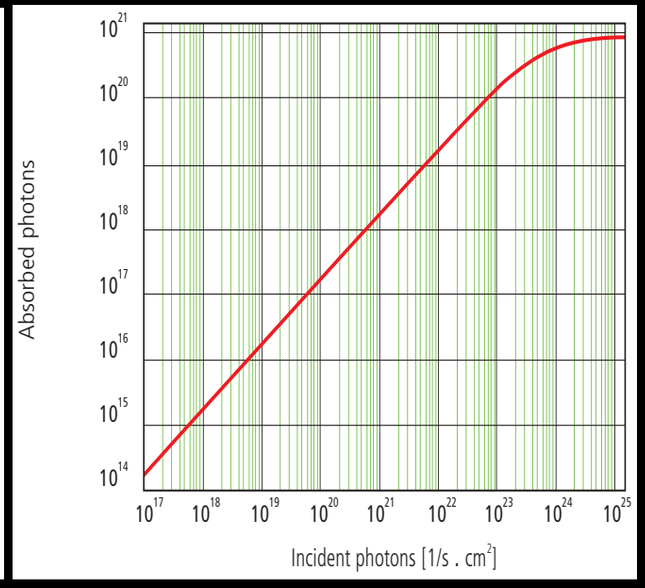


2D illumination

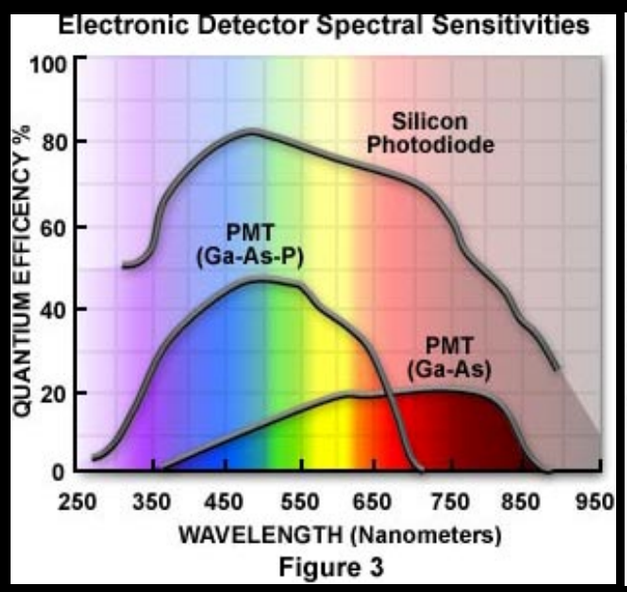
Imaging at the right microscope setting is crucial

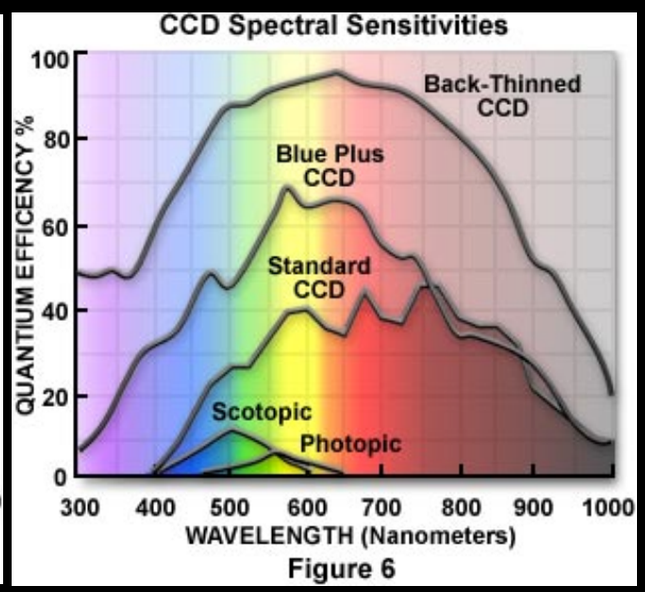
Suitable laser power





Suitable detector with a good quantum efficiency

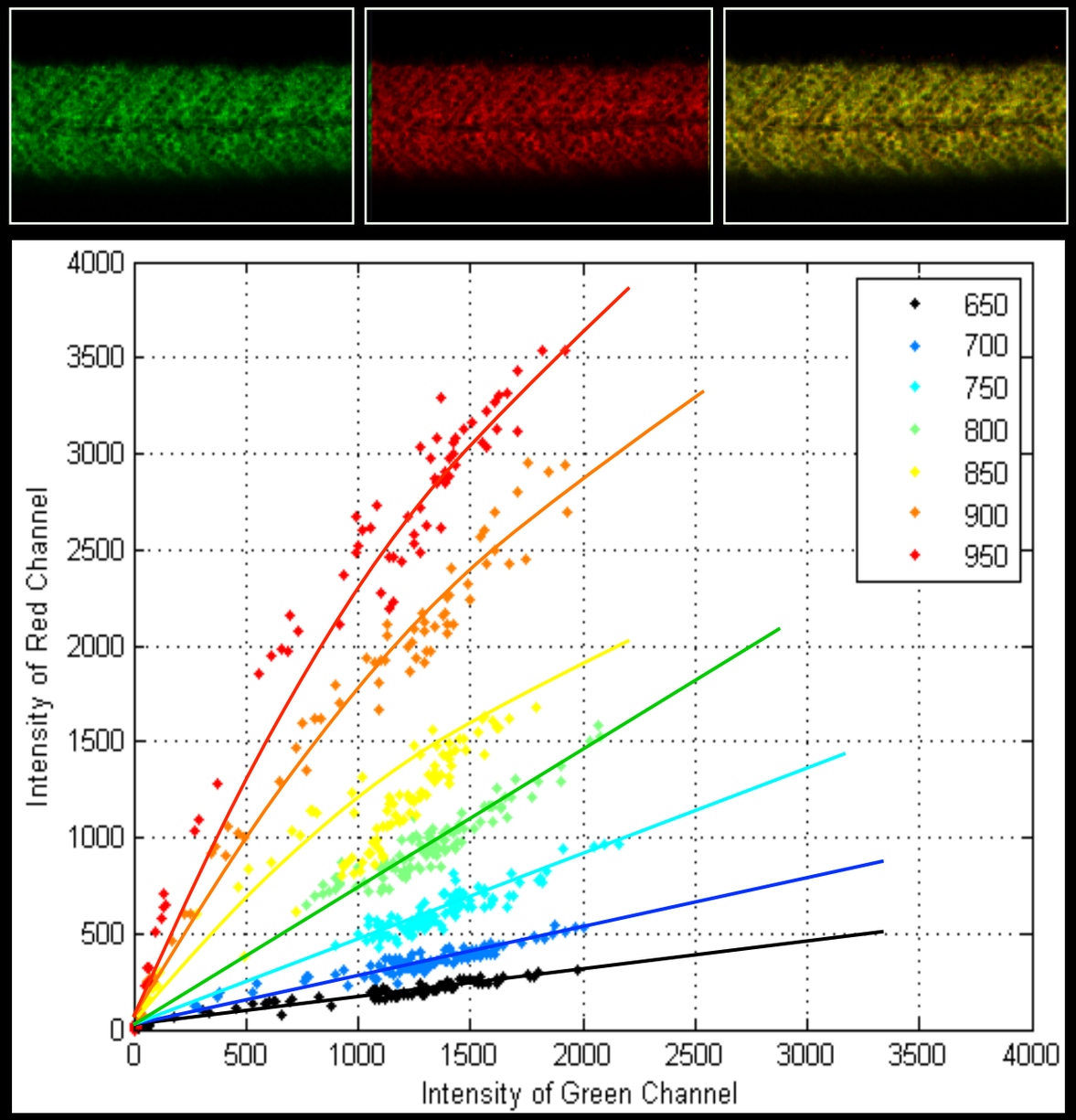




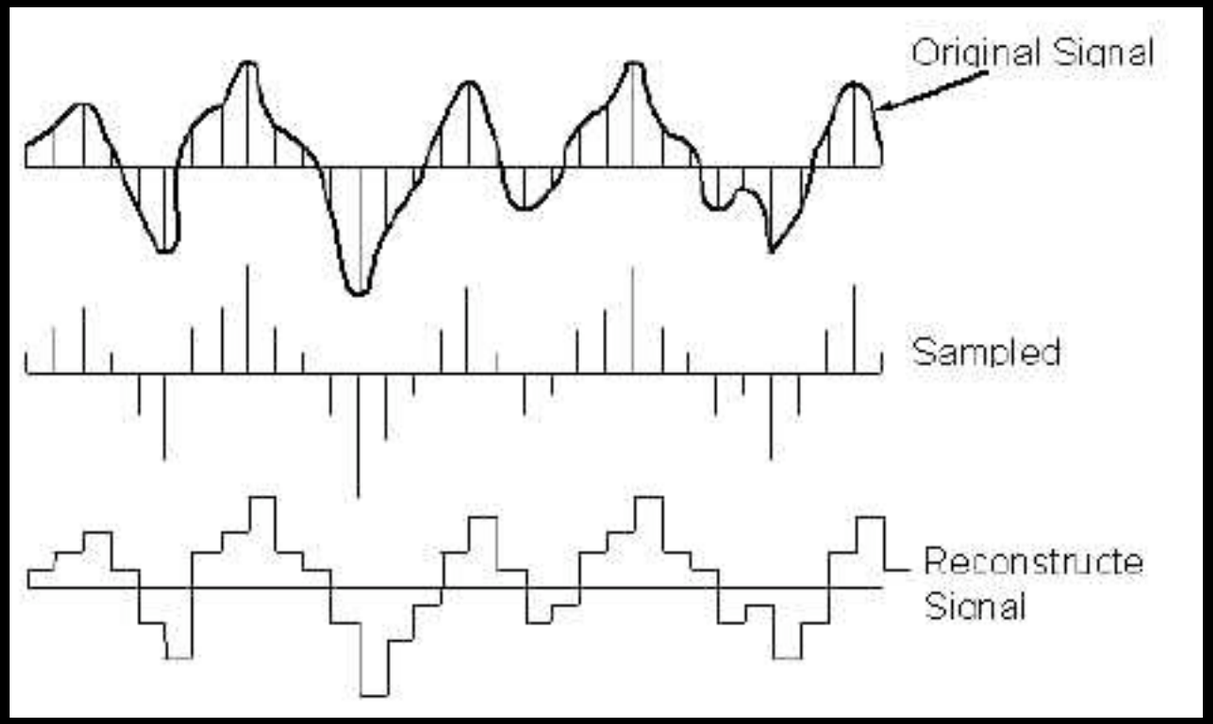
Images : Carl Zeiss

Imaging at the right microscope setting is crucial

Suitable detector gain



Digitization



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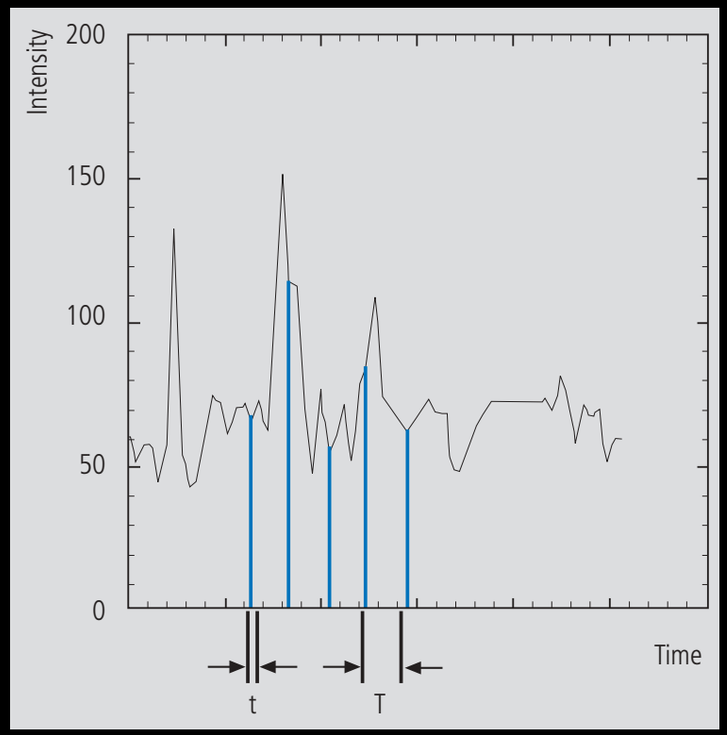
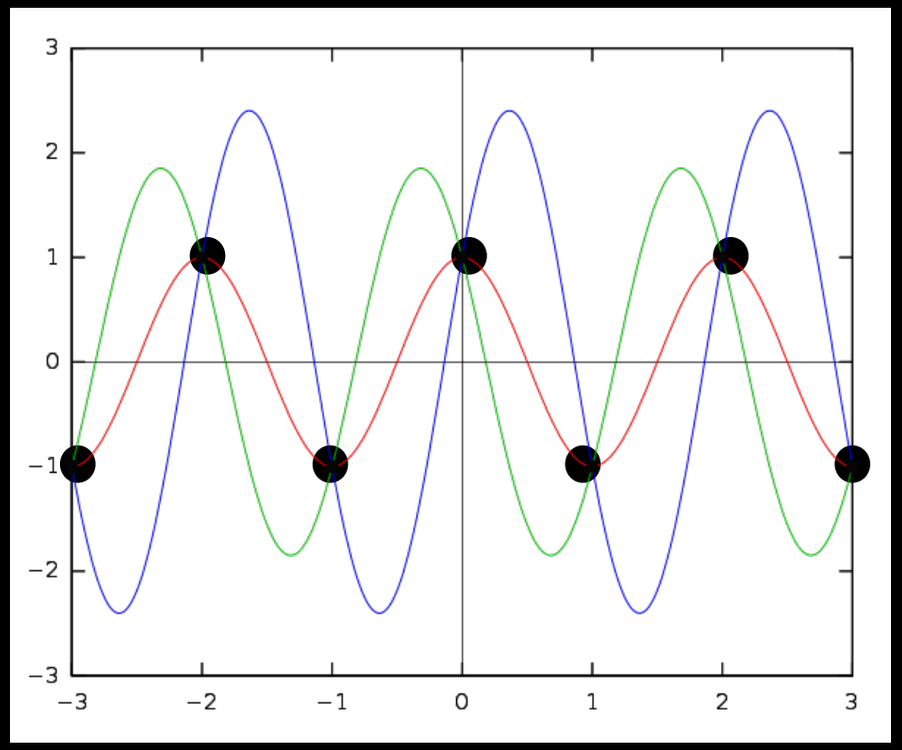
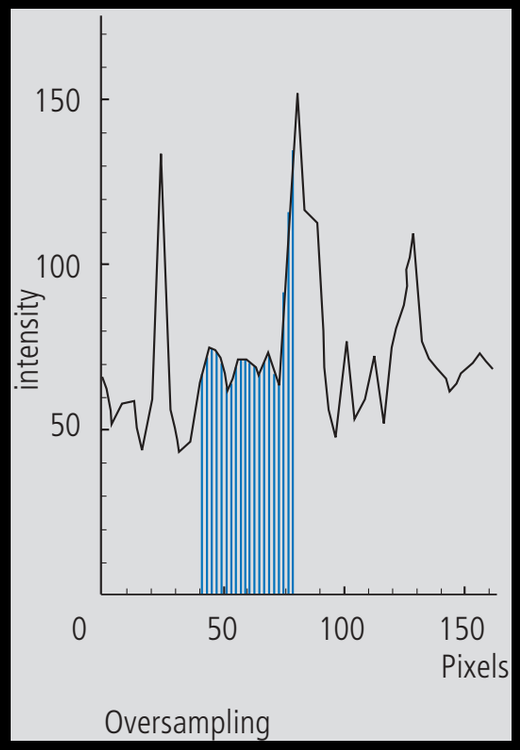


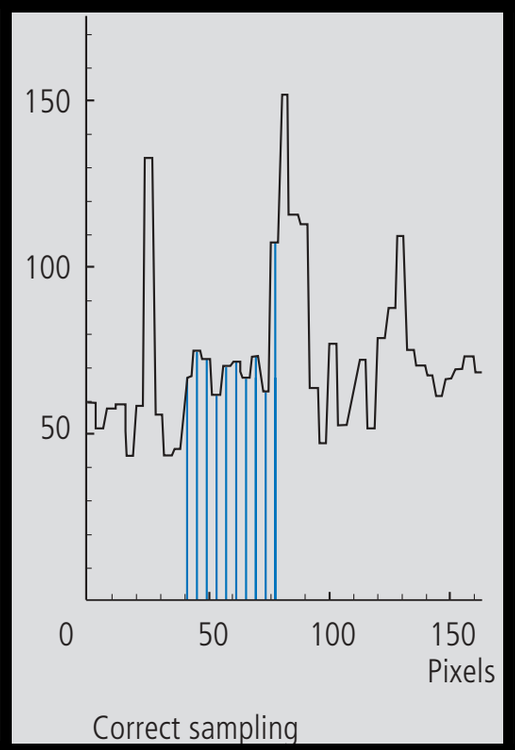
Carl Zeiss

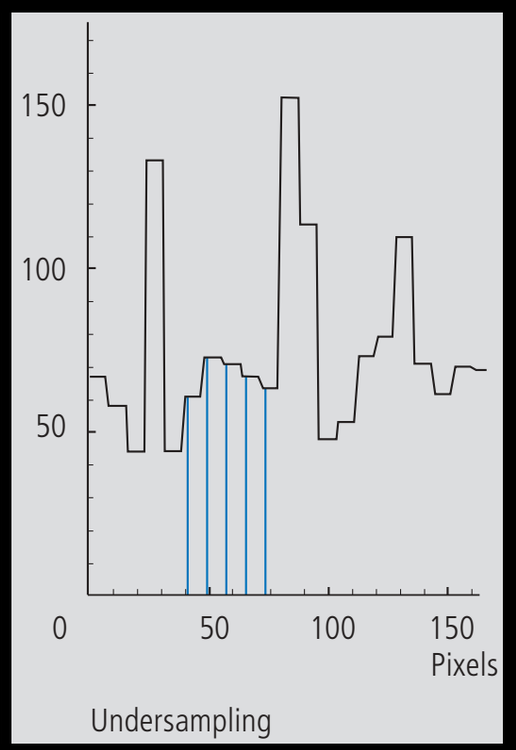
Correct Sampling



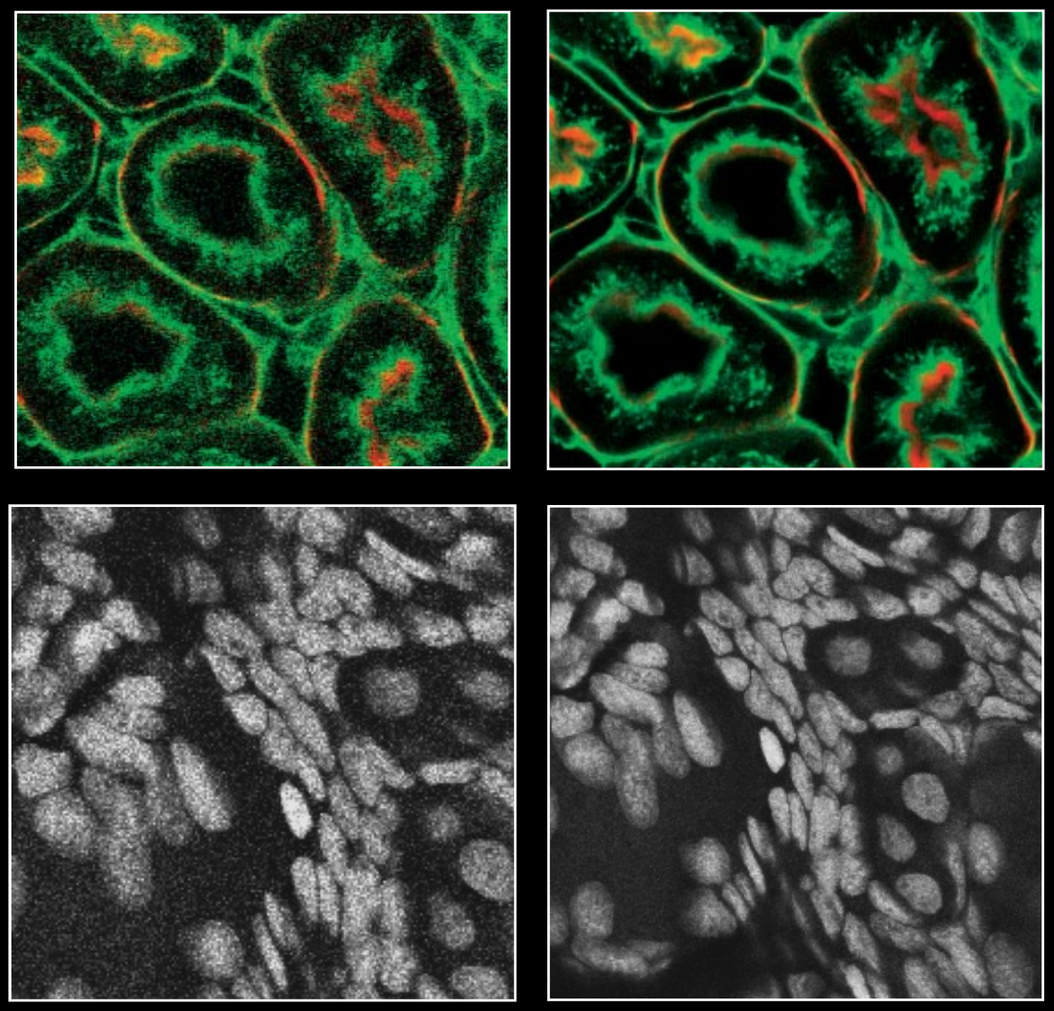
Wikipedia







Pixel dwell-time



Outline

Digital image formation

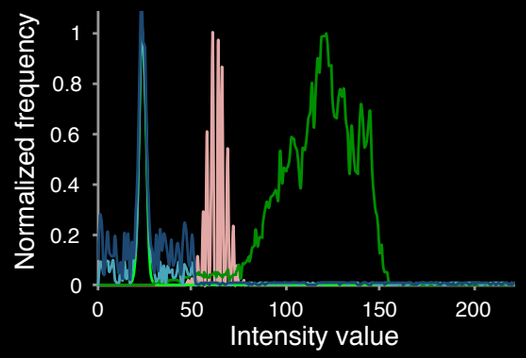


Image properties

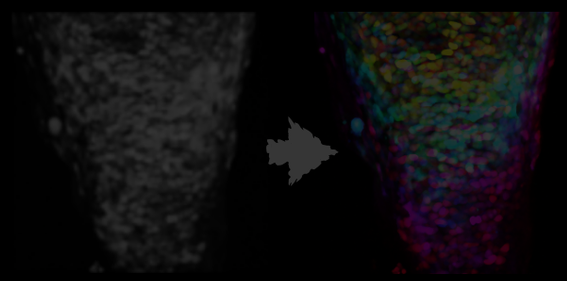
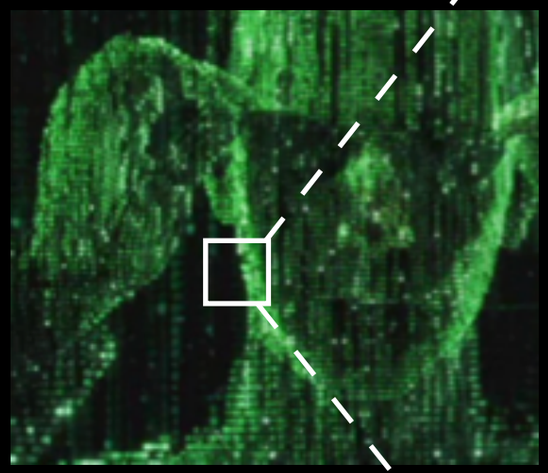


Image processing

Available softwares

Image is a matrix of numbers



			230	221	
			250	203	
			240	202	
				220	200
					230

Gray values

How high can the numbers be ?

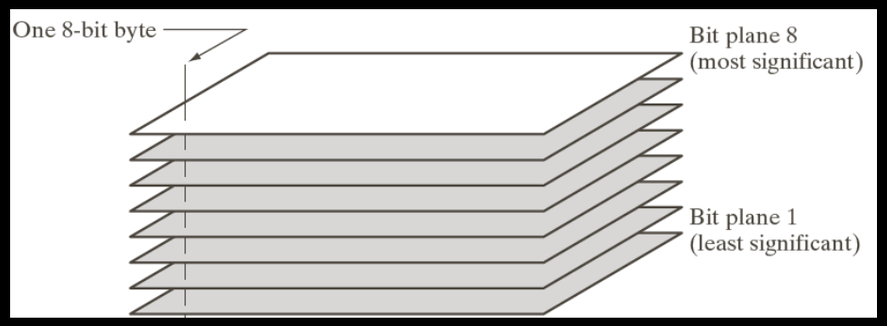
			230	221	
			250	203	
			240	202	
				220	200
					230

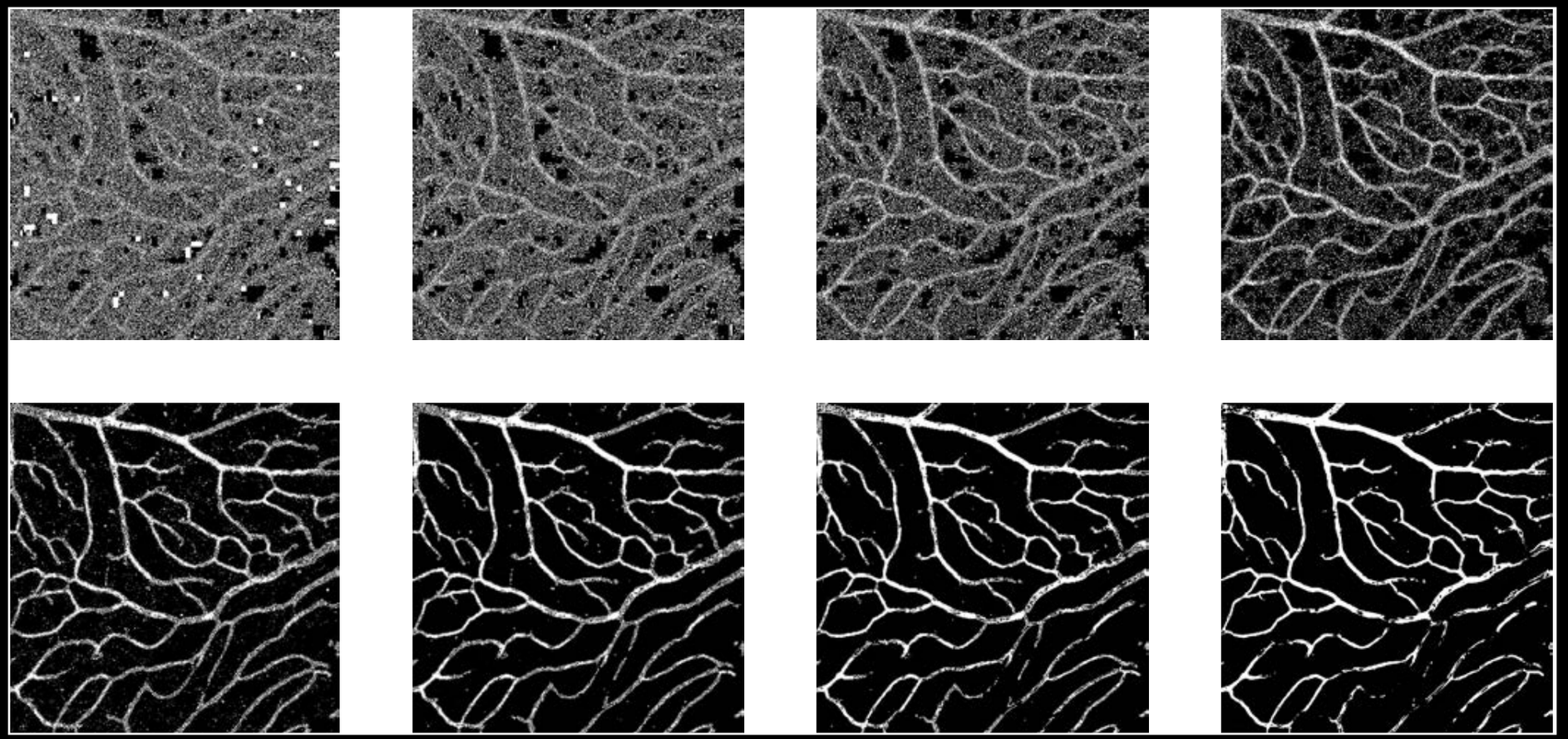
$$(0 - 11)_{binary} = (0 - 1x2^1 + 1x2^0)_{decimal}$$
$$= (0 - 3)_{decimal}$$

0 -1	1-bit	0 - 1
0 -11	2-bit	0 - 3
:	:	:
:	:	:
0 -11111111	8-bit	0 - 255
:	:	:
0 -111111111111	12-bit	0 - 4095
:	:	:
0 -1111111111111111	16-bit	0 - 65535

Dynamic Range

Image bit-depth





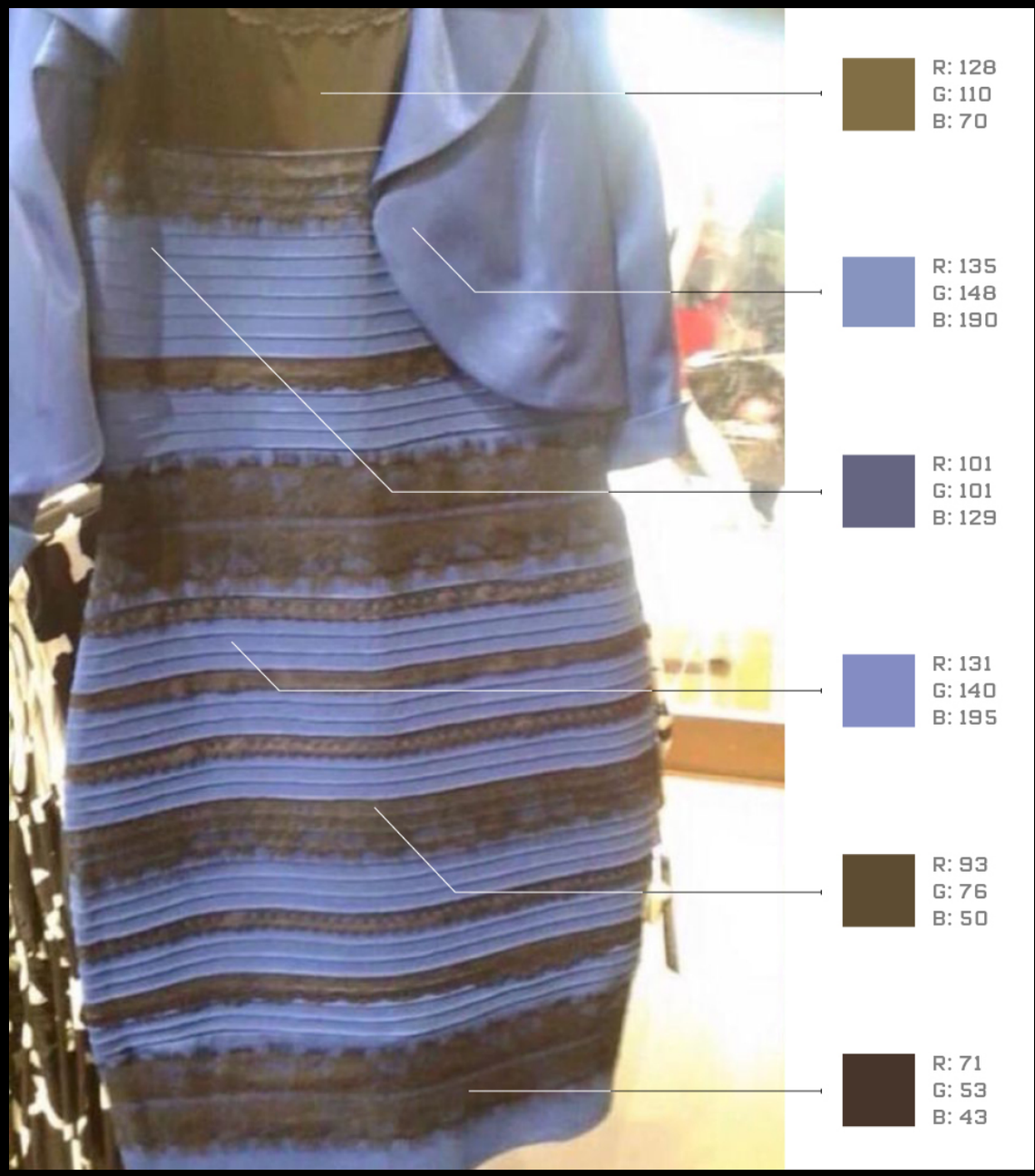
Slide Courtesy : Dr. Alphan Altinok

Colored Images

R

G

B



http://www.wired.com/wp-content/uploads/2015/02/Unknown.png

Colored Images

A

B

C

ABC

BAC

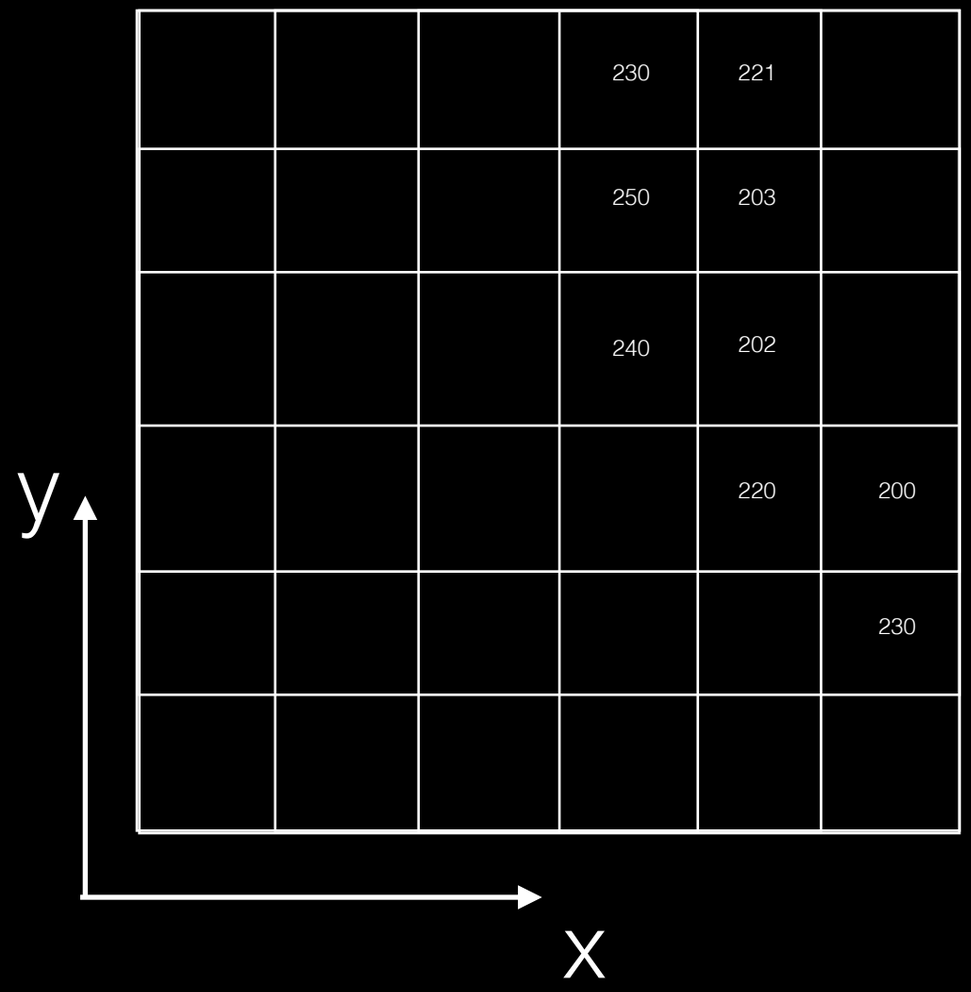
CBA

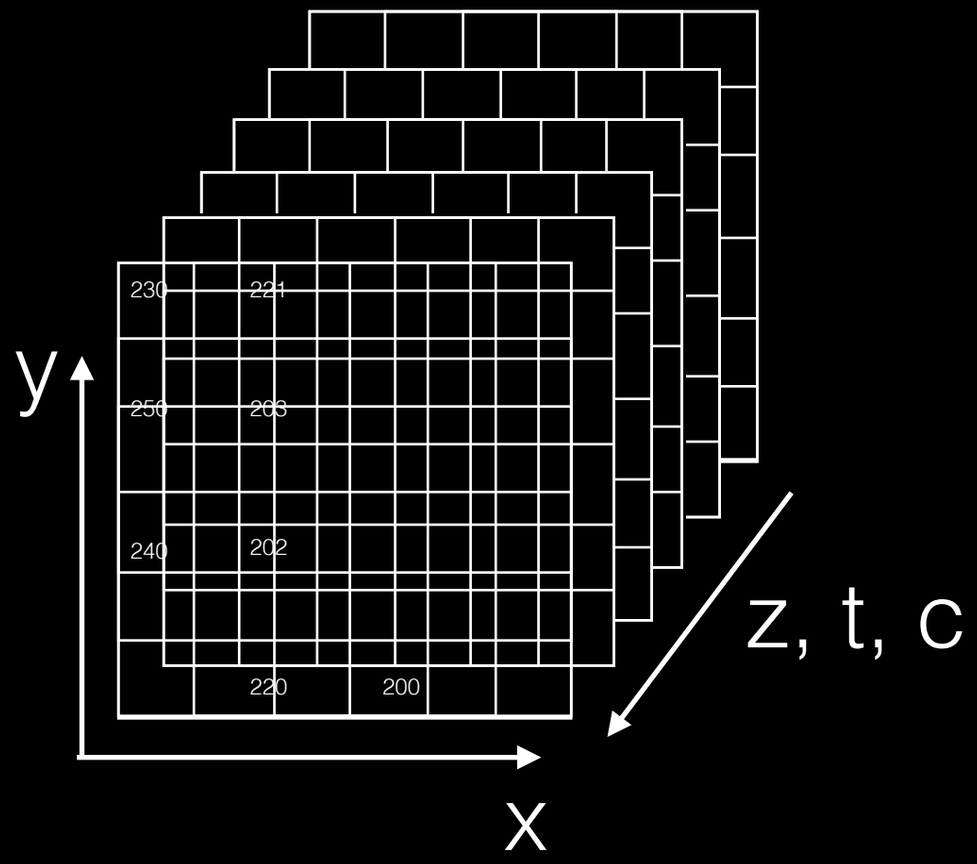
ACB

BCA

CAB

Stacks and Hyper-stacks





Meta-data

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ColorType	Indexed
BitsPerSample	16
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PhotometricInterpretation	RGB Palette
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MinSampleValue	0
Binning	1
PixelResolutionUnitX	microns
PixelResolutionUnitY	microns
PixelResolutionUnitZ	microns
PixelResolutionX	0.155370
PixelResolutionY	0.155370
PixelResolutionZ	4.754000

- information about image content
- acquisition parameters
- manufacturer-specific data

Slide Courtesy : Dr. Alphan Altinok

Outline

Digital image formation

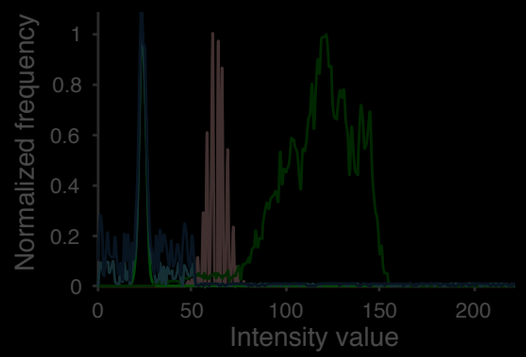


Image properties

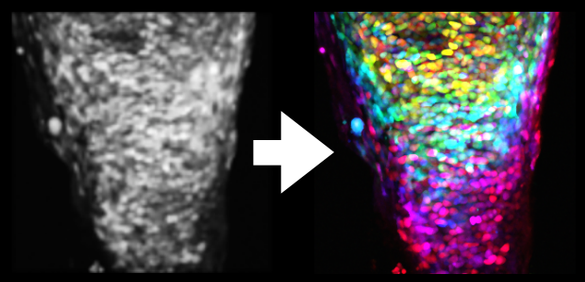
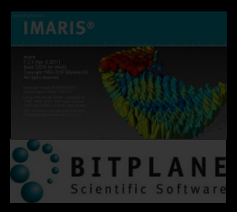
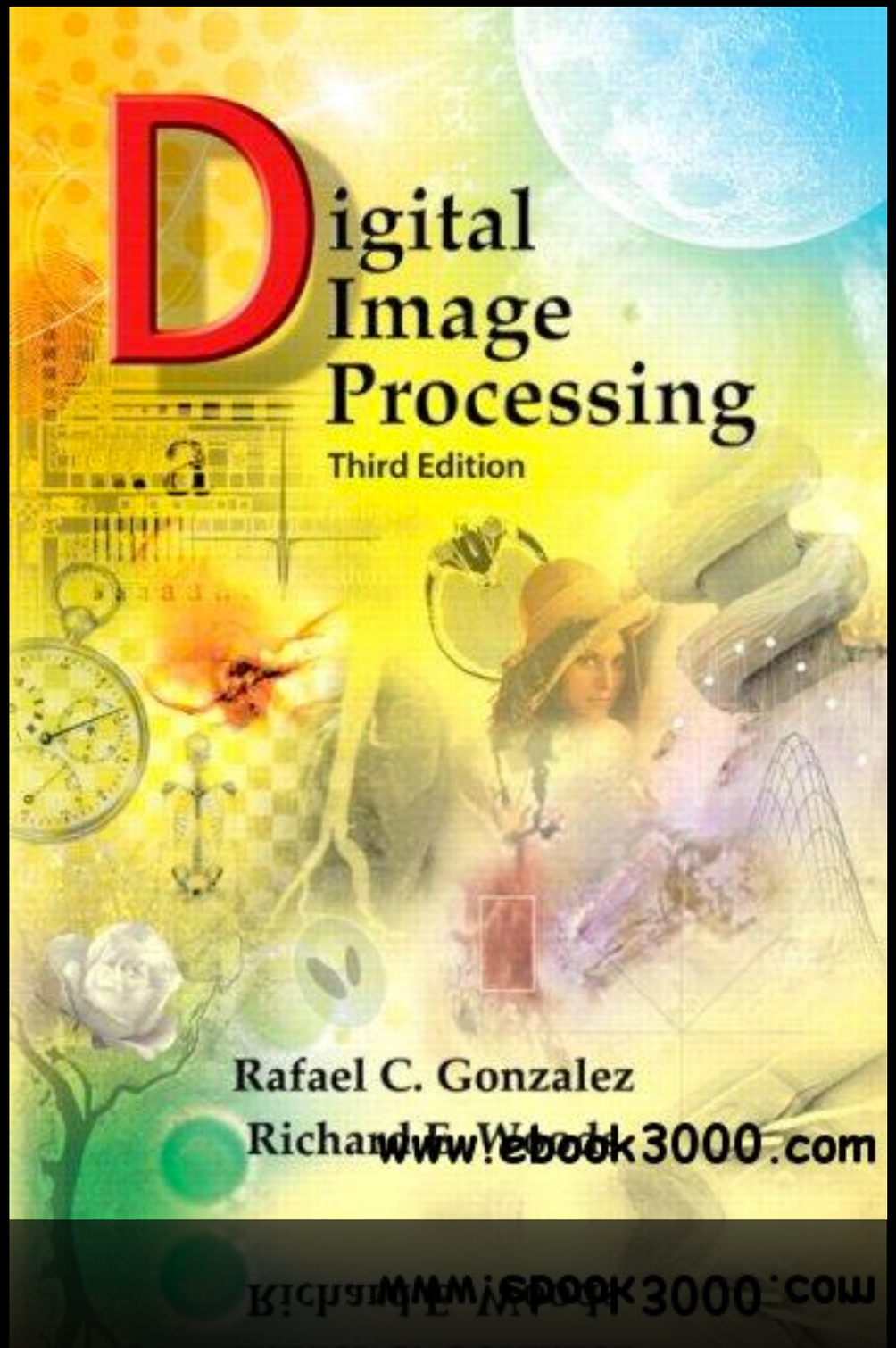


Image processing



Available softwares

Resources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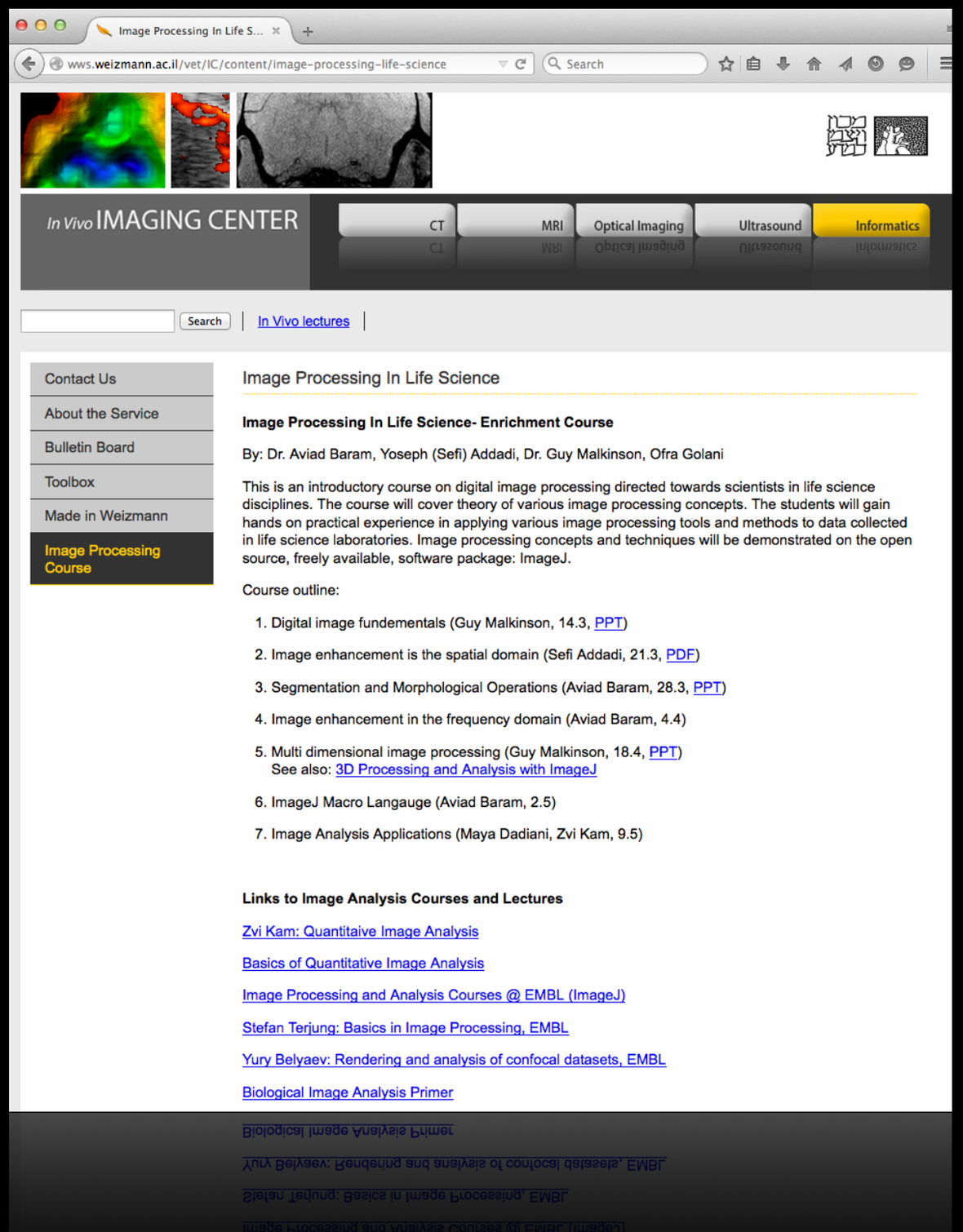
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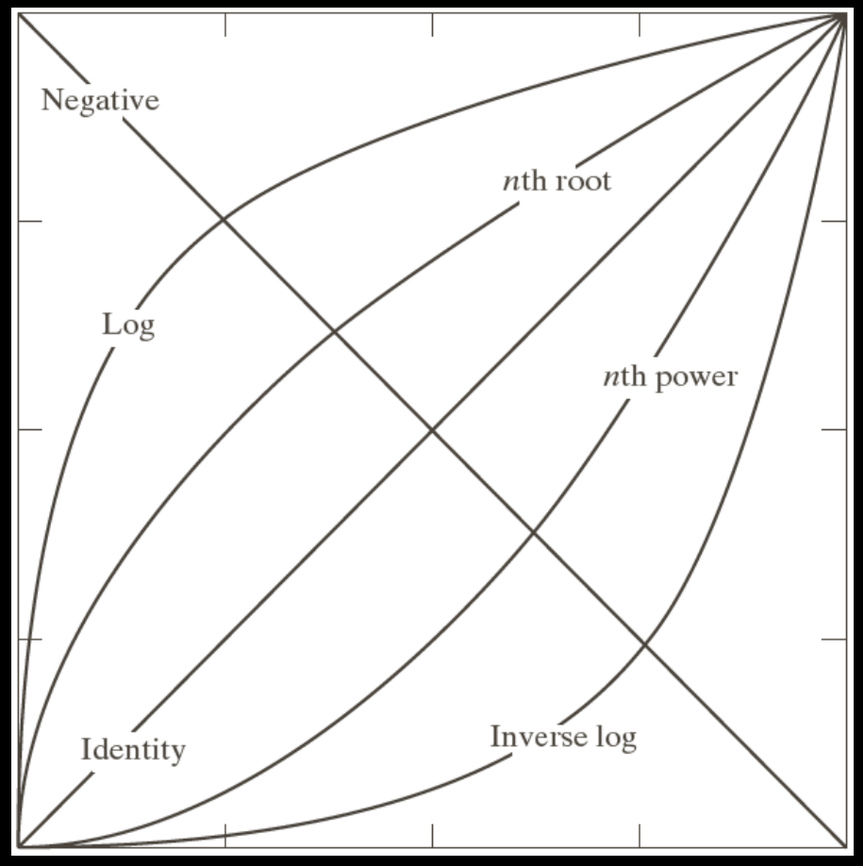
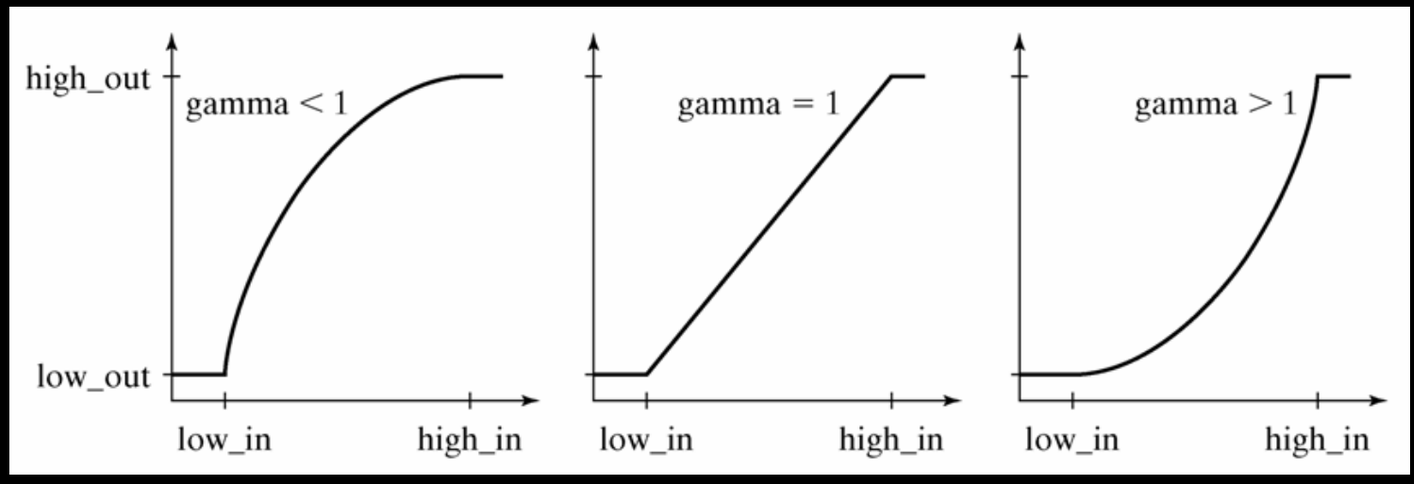
Image Processing Course
Image Processing In Life Science
Image Processing In Life Science- Enrichment Course
By: Dr. Aviad Baram, Yoseph (Sefi) Addadi, Dr. Guy Malkinson, Ofra Golani
This is an introductory course on digital image processing directed towards scientists in life science disciplines. The course will cover theory of various image processing concepts. The students will gain hands on practical experience in applying various image processing tools and methods to data collected in life science laboratories. Image processing concepts and techniques will be demonstrated on the open source, freely available, software package: ImageJ.
Course outline:
1. Digital image fundementals (Guy Malkinson, 14.3, PPT)
2. Image enhancement is the spatial domain (Sefi Addadi, 21.3, PDF)
3. Segmentation and Morphological Operations (Aviad Baram, 28.3, PPT)
4. Image enhancement in the frequency domain (Aviad Baram, 4.4)
5. Multi dimensional image processing (Guy Malkinson, 18.4, PPT)
See also: 3D Processing and Analysis with ImageJ
6. ImageJ Macro Langauge (Aviad Baram, 2.5)
7. Image Analysis Applications (Maya Dadiani, Zvi Kam, 9.5)
Links to Image Analysis Courses and Lectures
Zvi Kam: Quantitaive Image Analysis
Basics of Quantitative Image Analysis
Image Processing and Analysis Courses @ EMBL (ImageJ)
Stefan Terjung: Basics in Image Processing, EMBL
Yury Belyaev: Rendering and analysis of confocal datasets, EMBL
Biological Image Analysis Primer

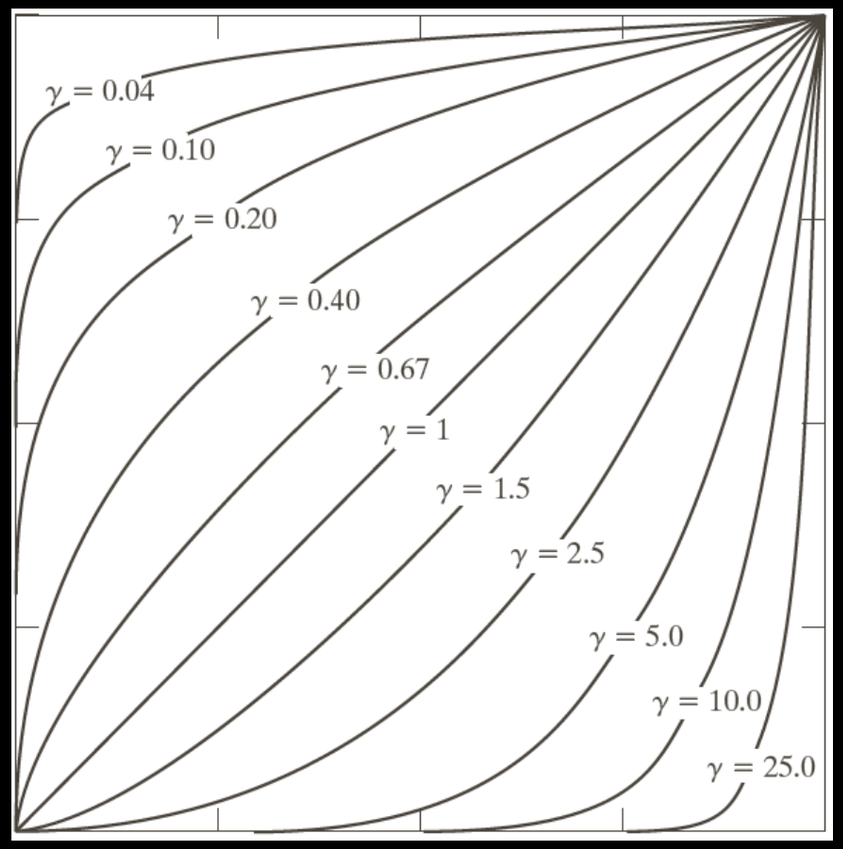
Contrast adjustment

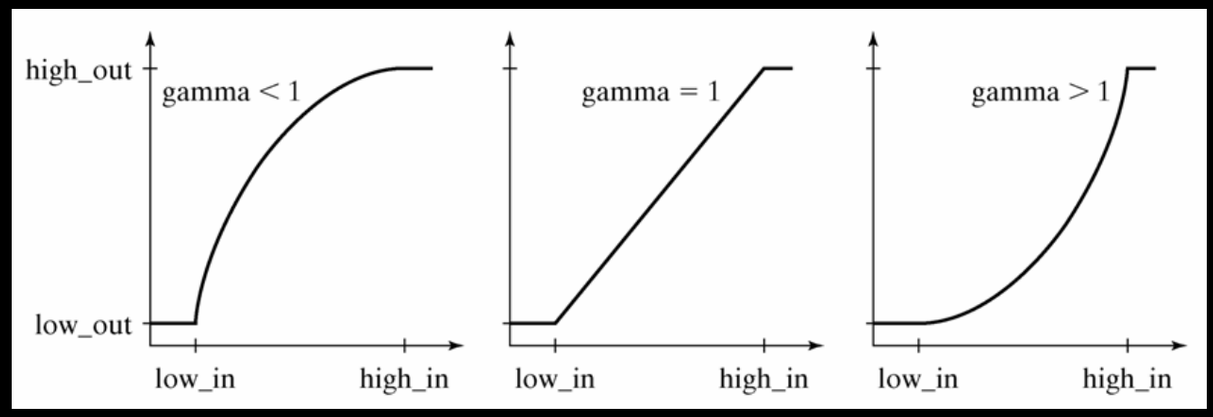


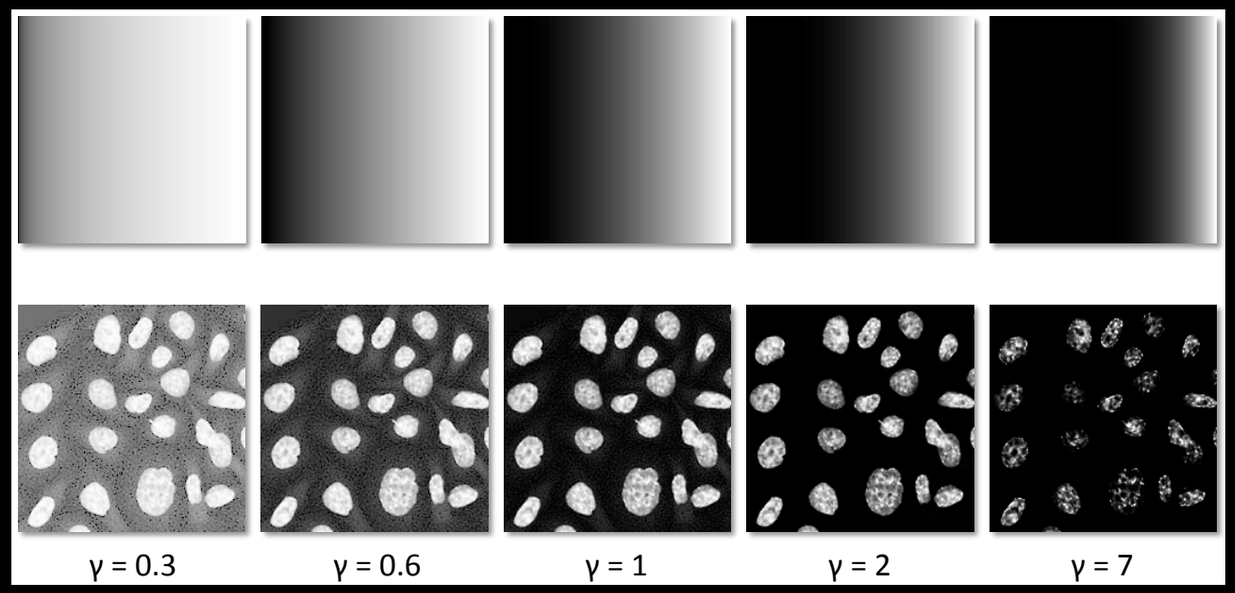


Slide Courtesy : Dr. Alphan Altinok

Contrast adjustment







Output = c. (Input Image)^(gamma)

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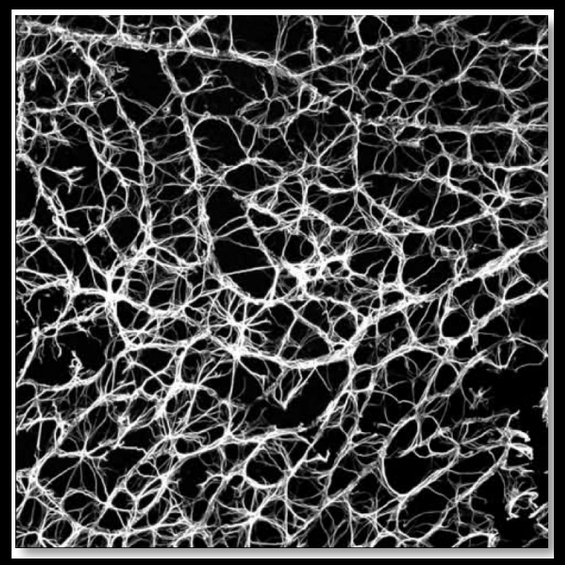
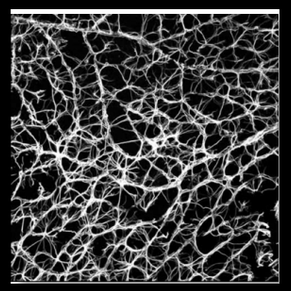
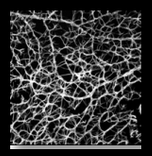
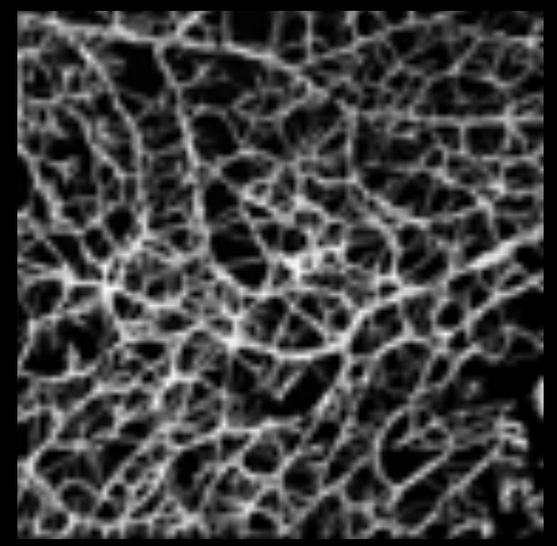
Binning









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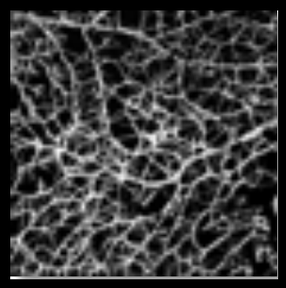
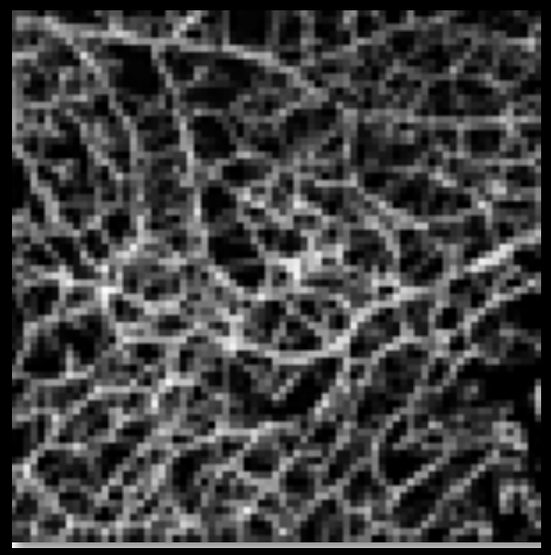
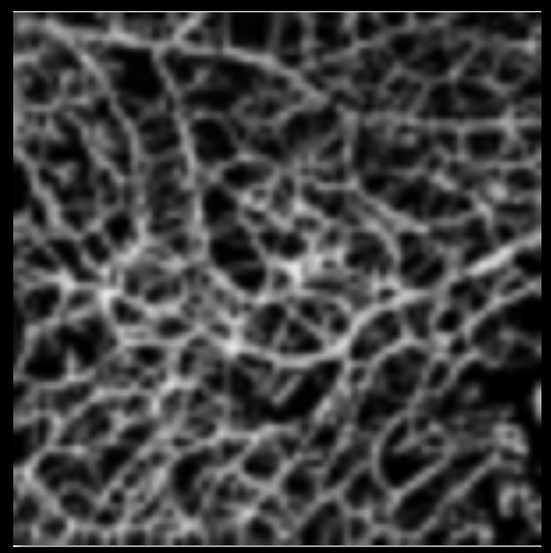
Interpolation

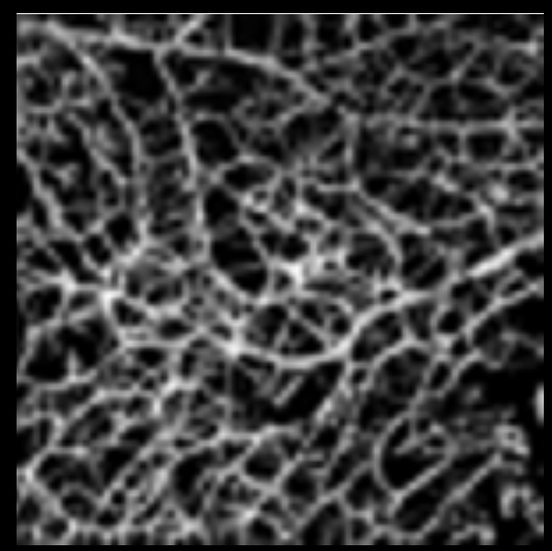




<u>nearest neighbor</u>
impose a finer grid, pick nearest pixel in original



<u>bilinear</u>
weighted average of 2 x 2 neighbors



<u>bicubic</u>

weighted average of 4 x 4 neighbors

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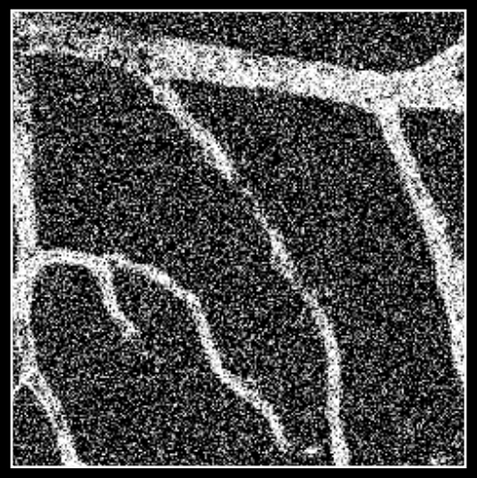
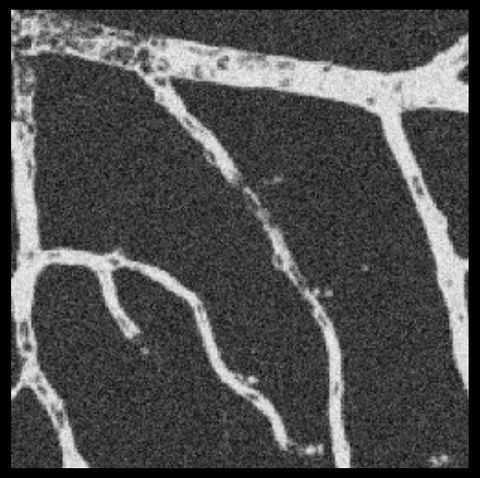
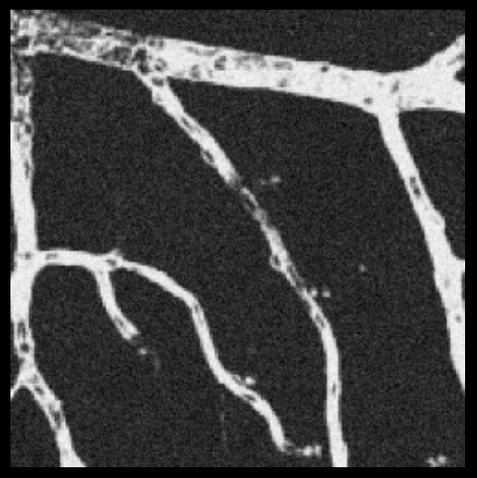
Average



single frame



average of 20



average of 100

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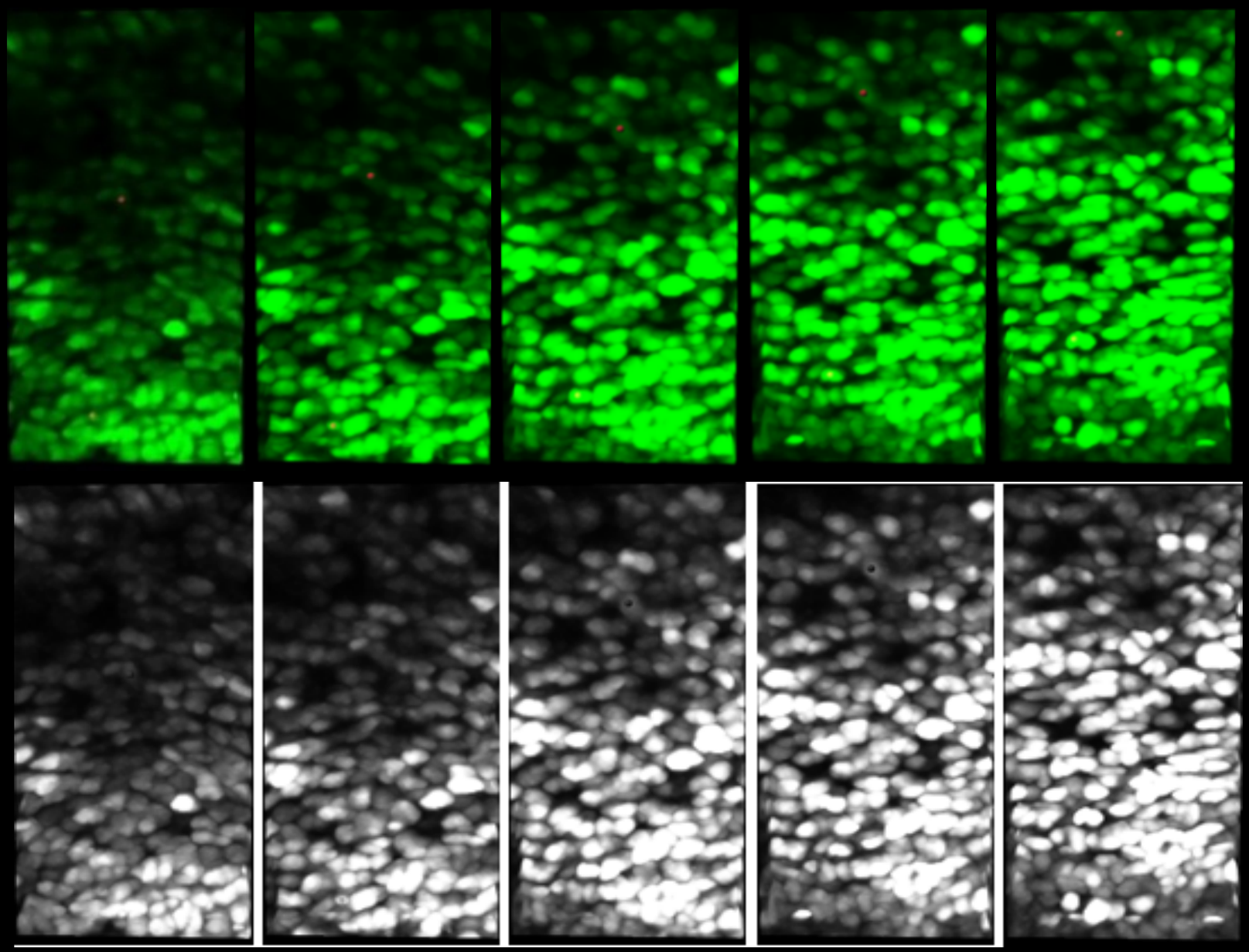
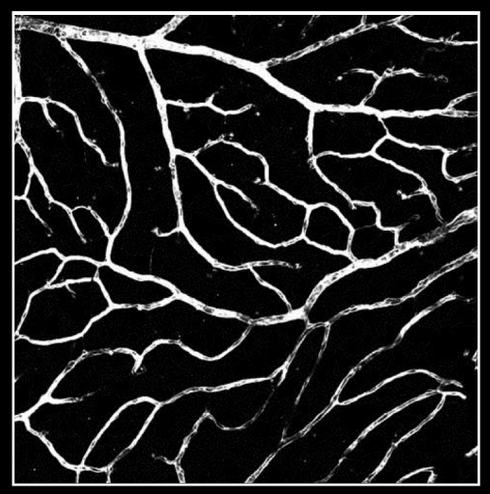
Binary images (thresholding)



Mask



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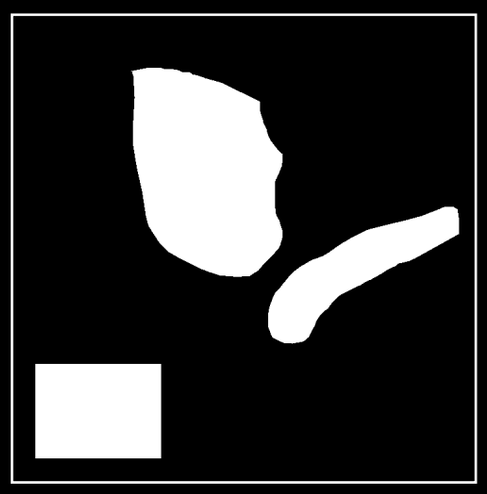

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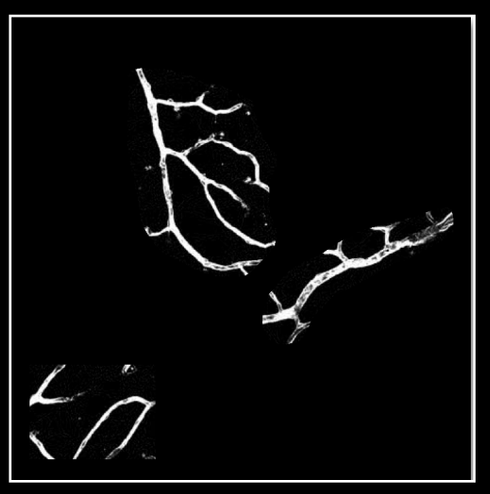

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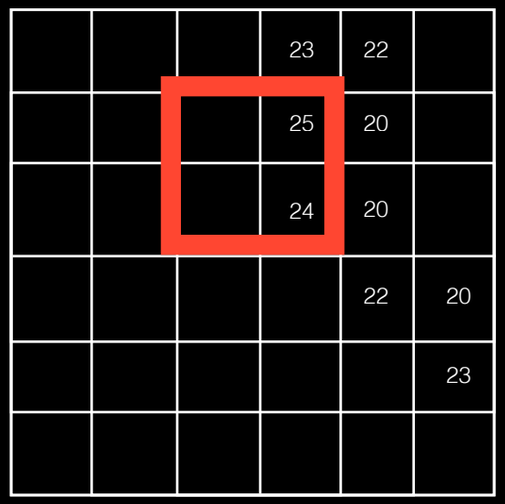
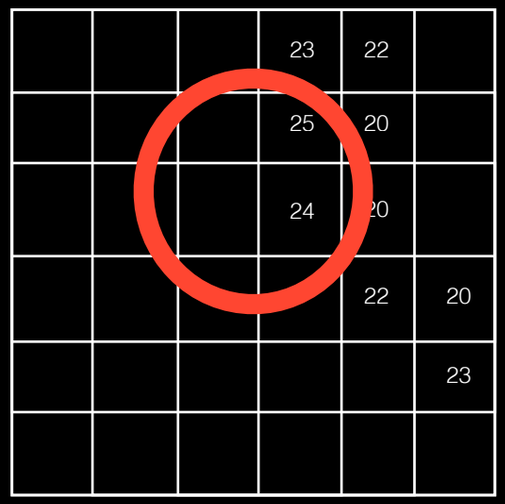
Spatial filtering

Smoothing

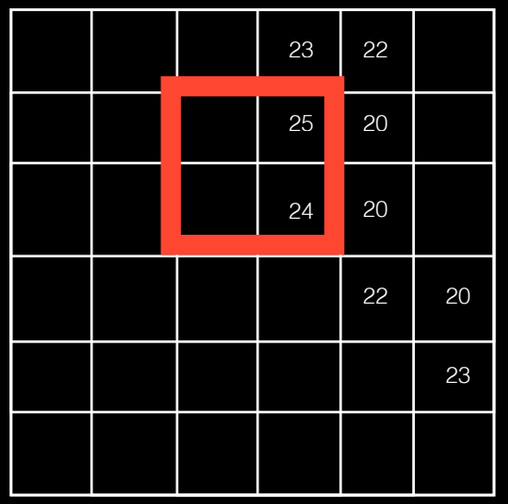
Average (Mean)



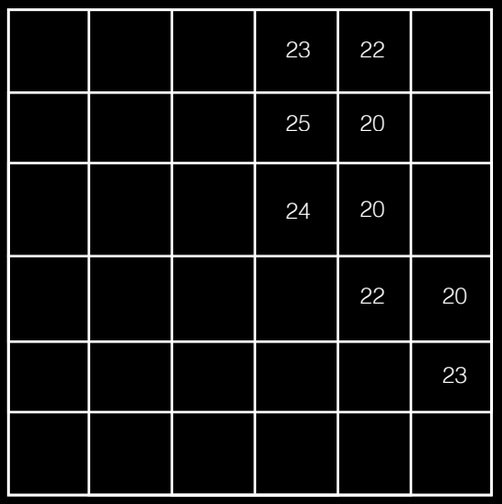
Disk



Median



Gaussian



Spatial filtering

Derivatives

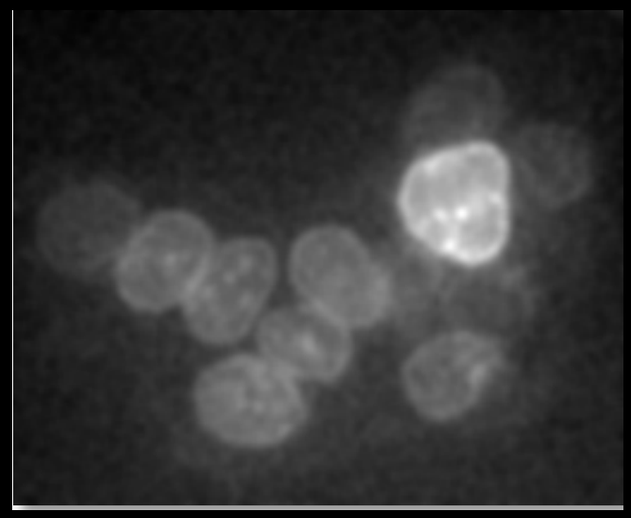
$$\frac{\partial f}{\partial x} = f(x+1) - f(x)$$

$$\frac{\partial^2 f}{\partial x^2} = f(x+1) + f(x-1) - 2f(x)$$

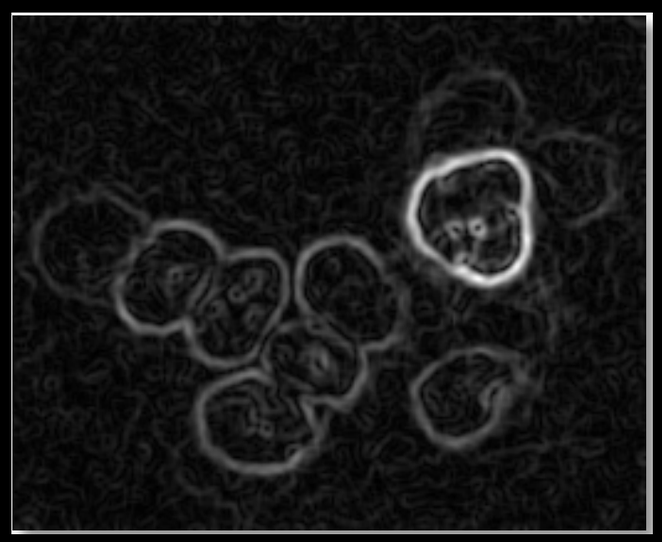
$$\nabla^2 f = \frac{\partial^2 f}{\partial x^2} + \frac{\partial^2 f}{\partial y^2}$$

$$\frac{\partial^2 f}{\partial x^2} = f(x+1,y) + f(x-1,y) - 2(x,y)$$

$$\frac{\partial^2 f}{\partial y^2} = f(x,y+1) + f(x,y-1) - 2(x,y)$$

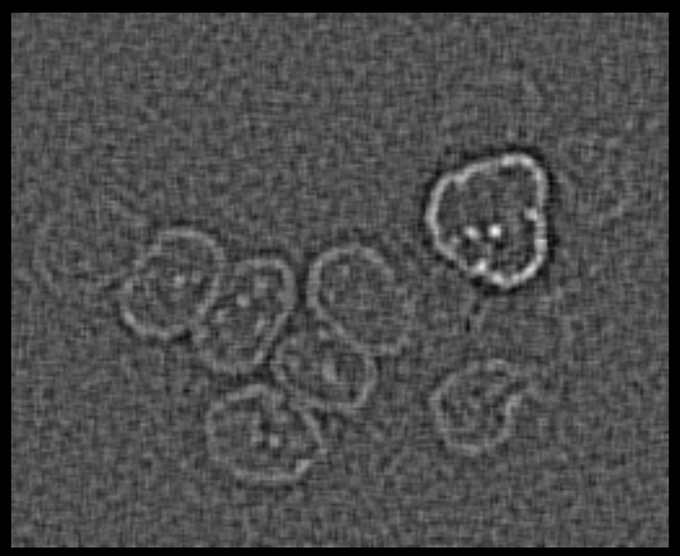


Gradient



$$\left|\frac{\partial f}{\partial x}\right| + \left|\frac{\partial f}{\partial y}\right|$$

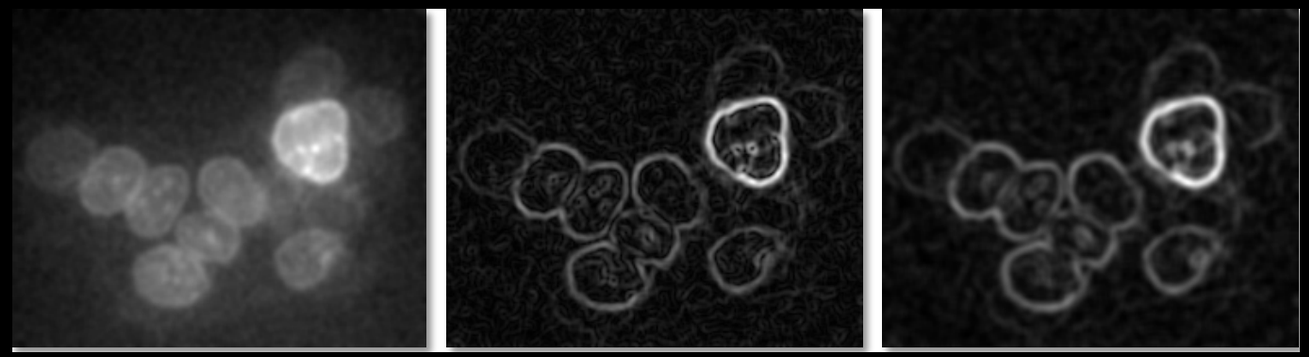
Laplacian

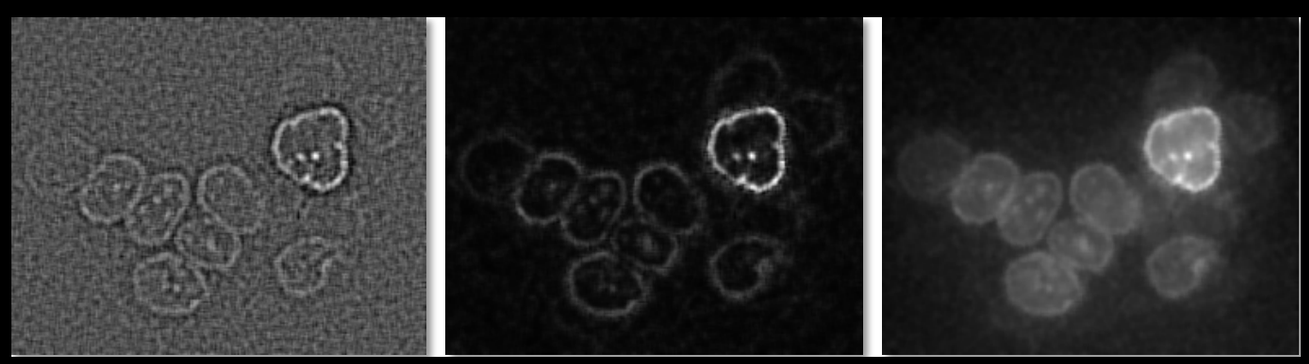


$$\nabla^2 f(x,y)$$

LoG

Combined filtering





Edge detection and segmentation

Slide Courtesy : Dr. Alphan Altinok

Image similarity

Image differences

Slide Courtesy : Dr. Alphan Altinok

Image similarity

Image correlation

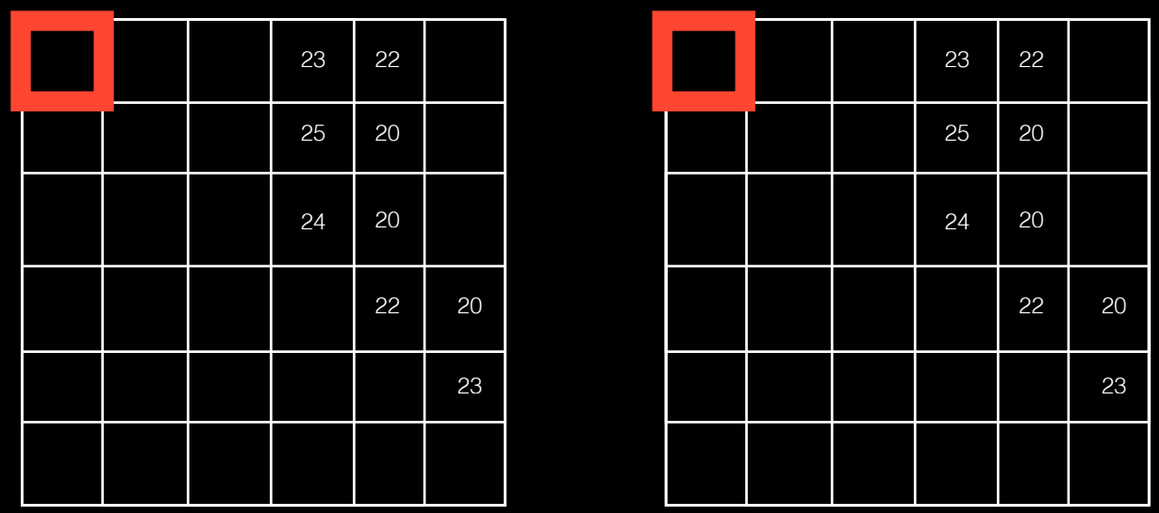
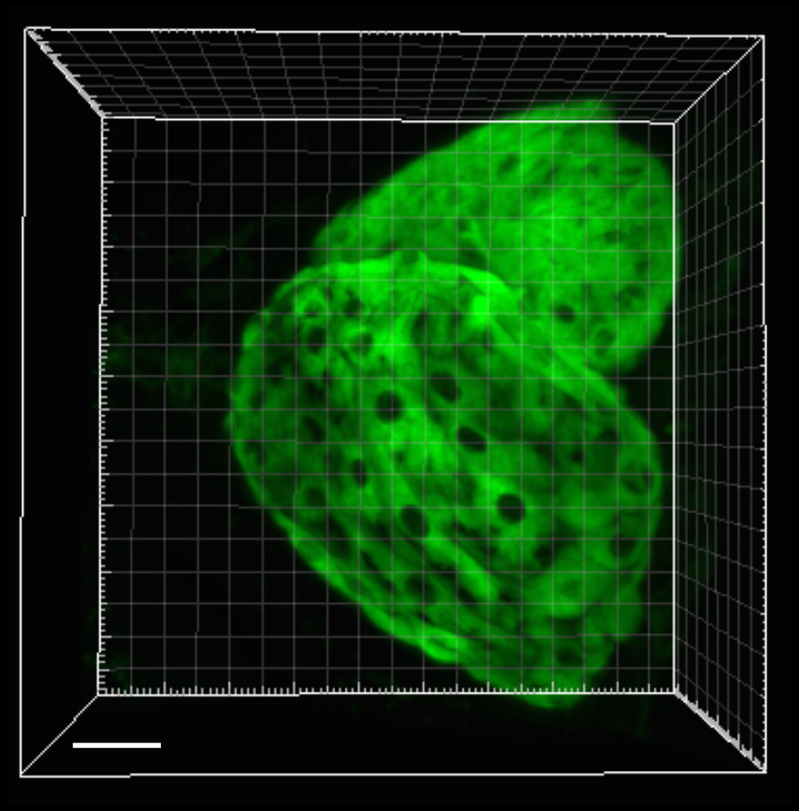
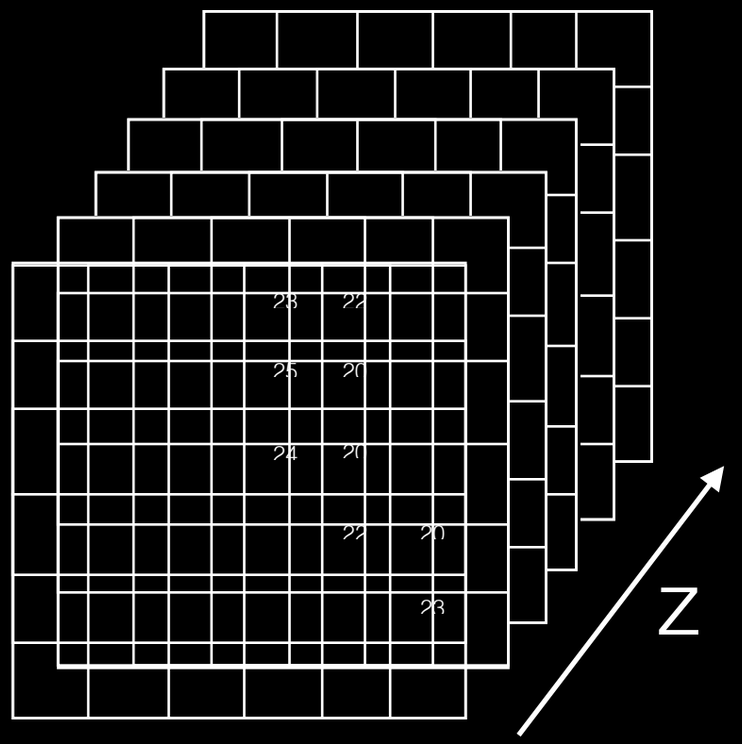
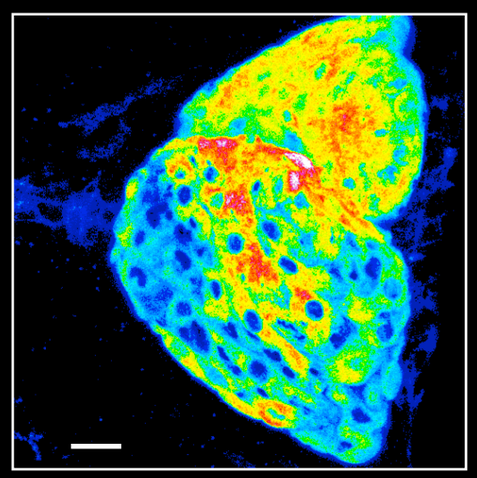


Image convolution

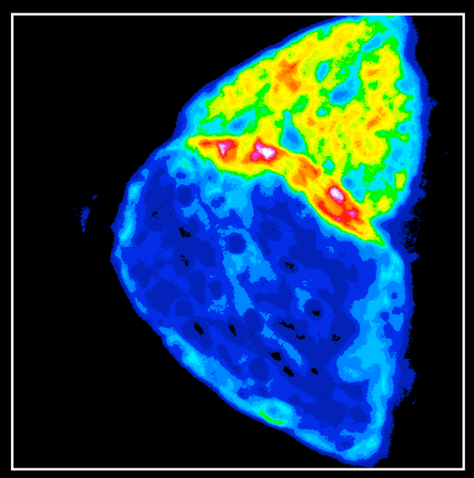
Intensity Projections



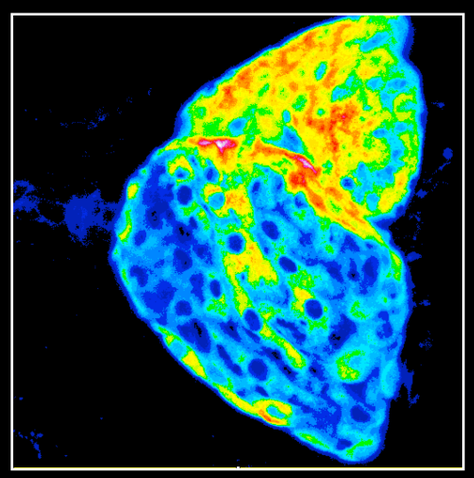




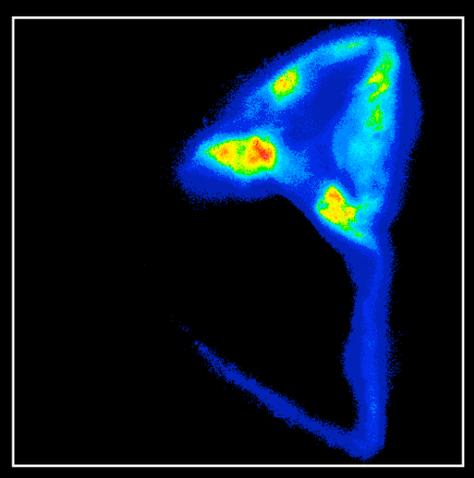
Maximum



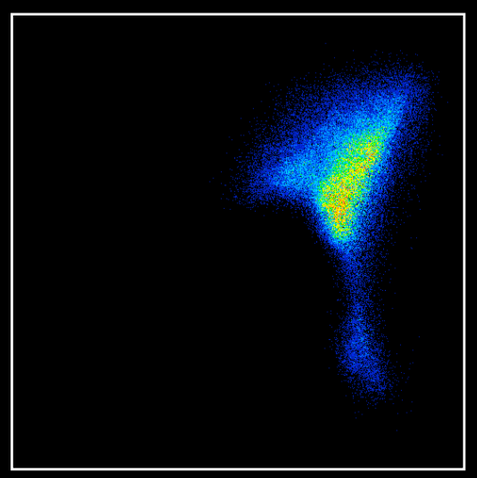
Average



Standard Dev.



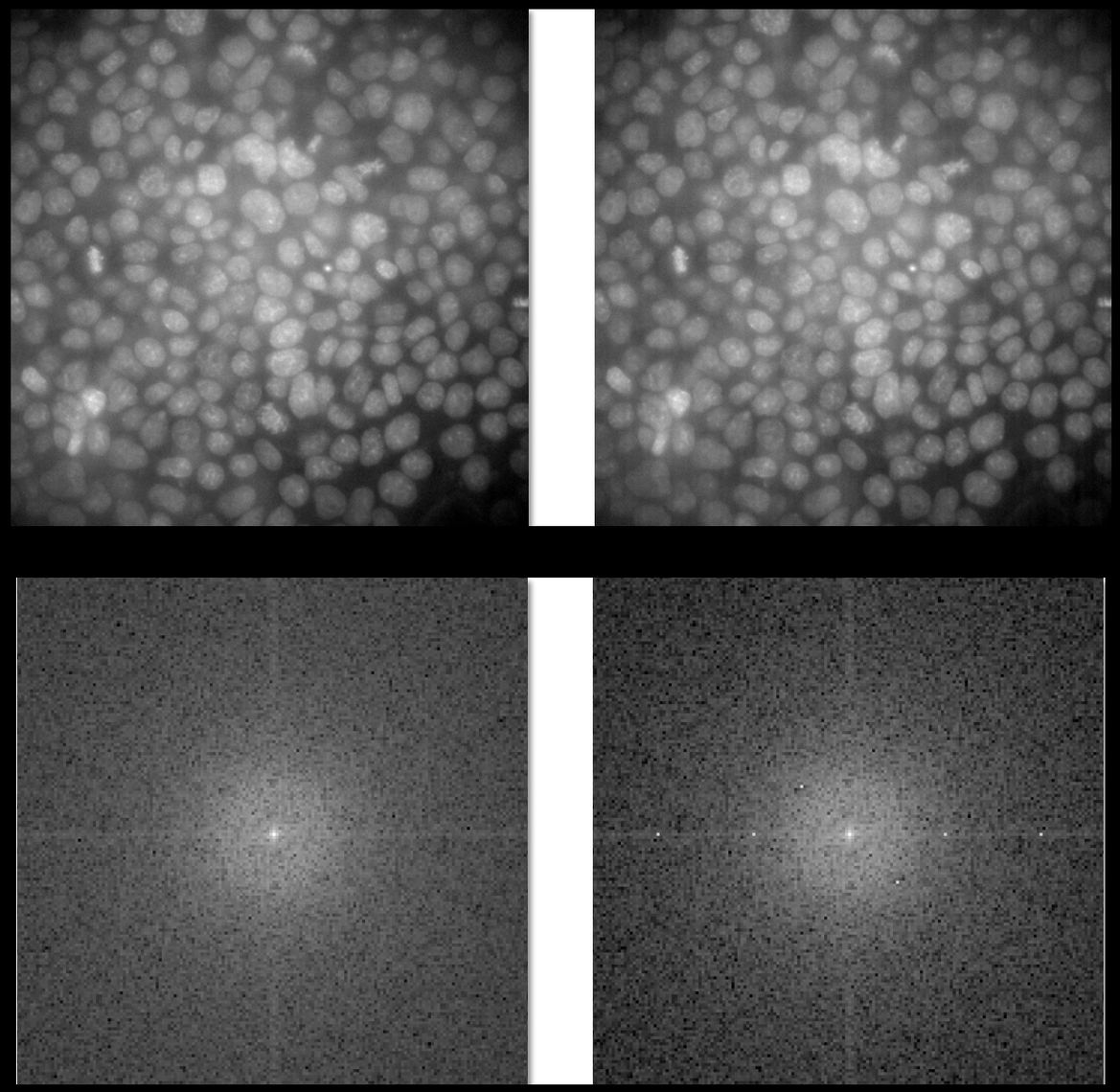
Median

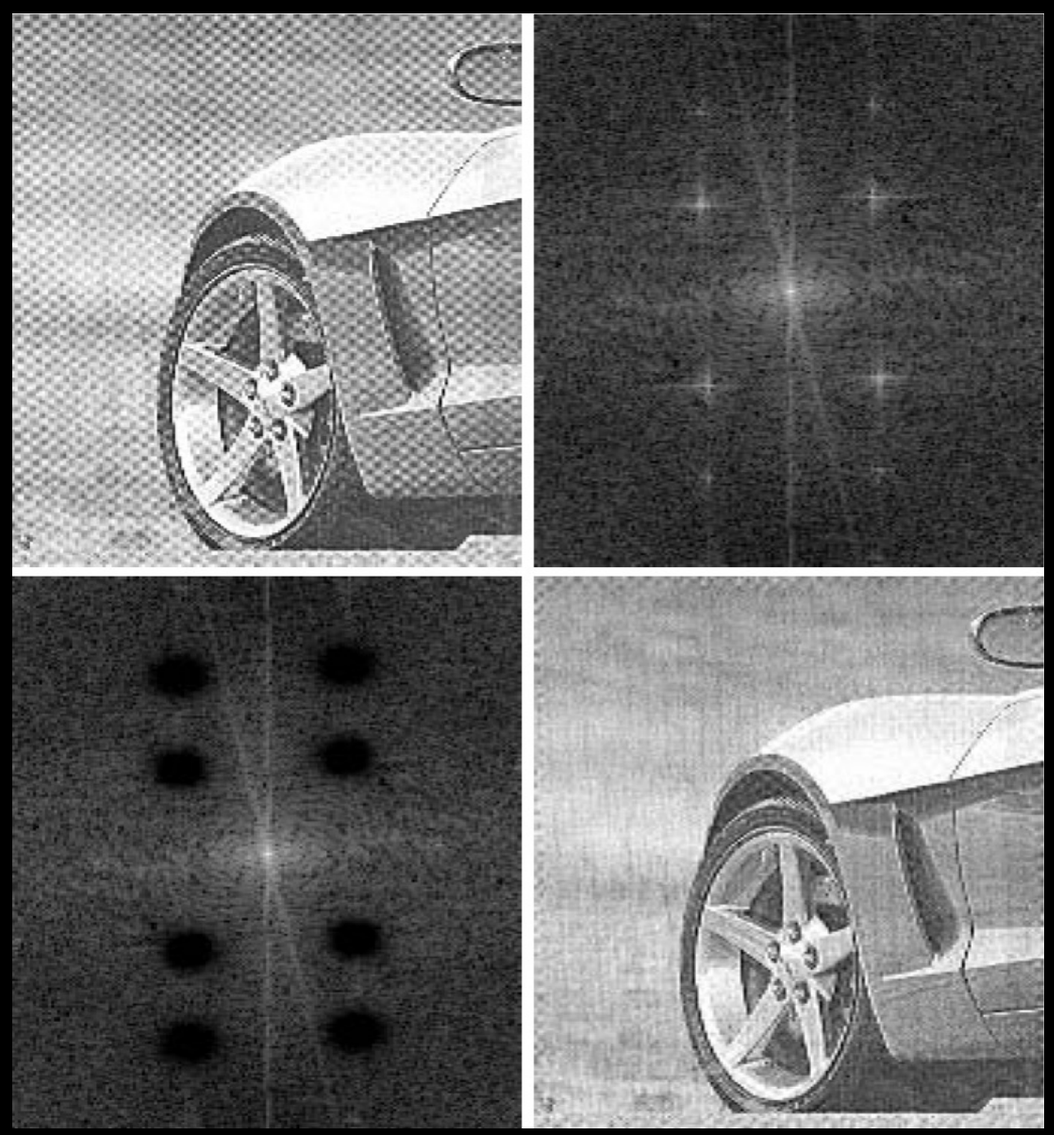


Minimum

Image transforms

Fourier transform





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Outline

Digital image formation

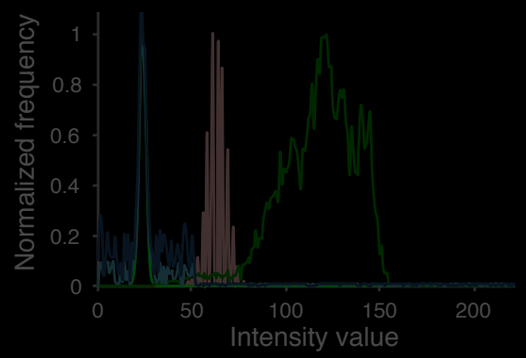


Image properties

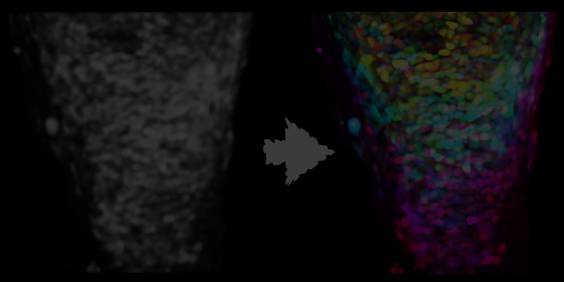
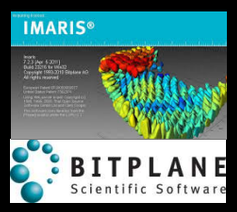


Image processing

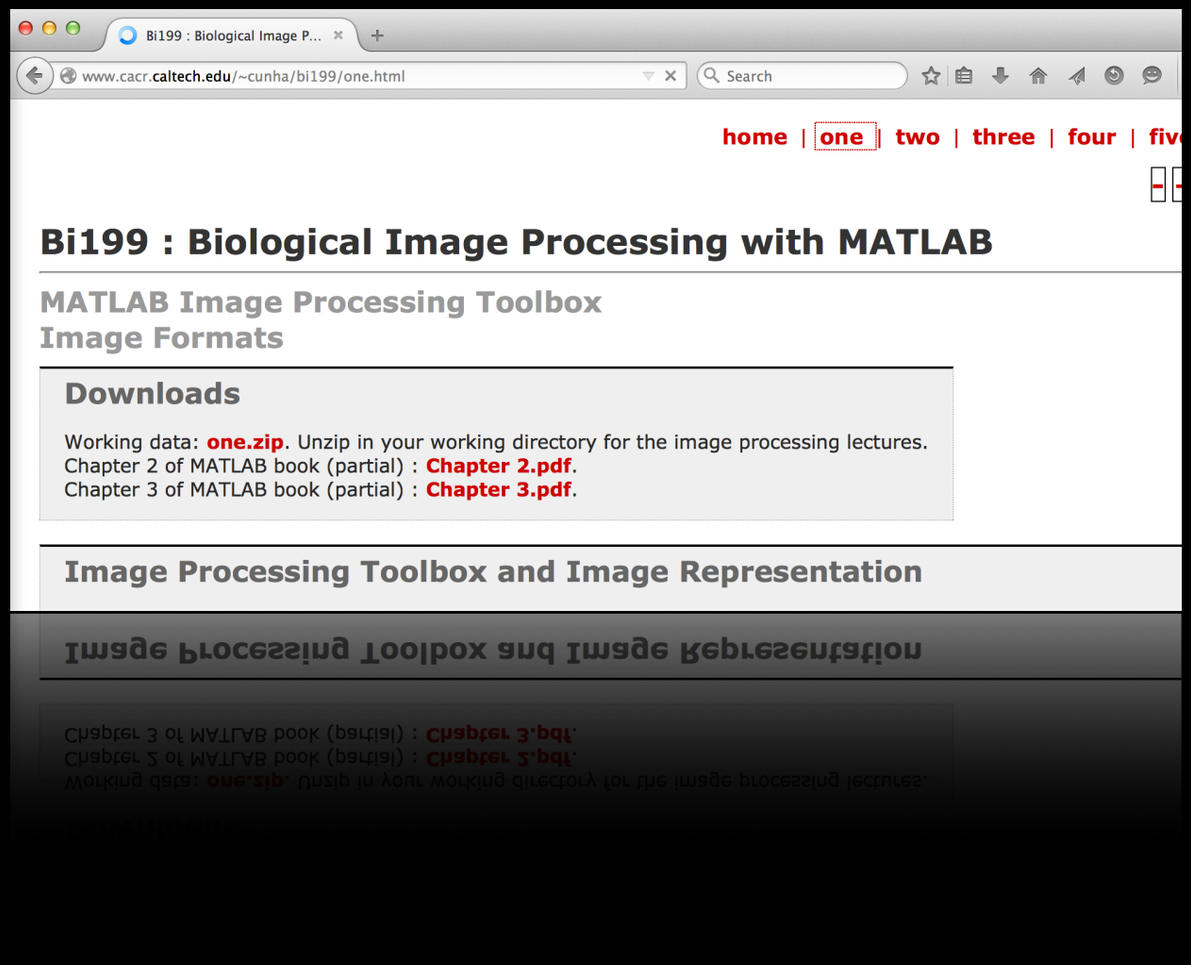


Available softwares

Resources @ Caltech

Bi 270. **Special Topics in Biology**. *Units to be arranged*; *first*, *second*, *third terms*. Students may register with permission of the responsible faculty member.

Bi 270/BioE 240 course offered by Dr. Alphan Altinok.



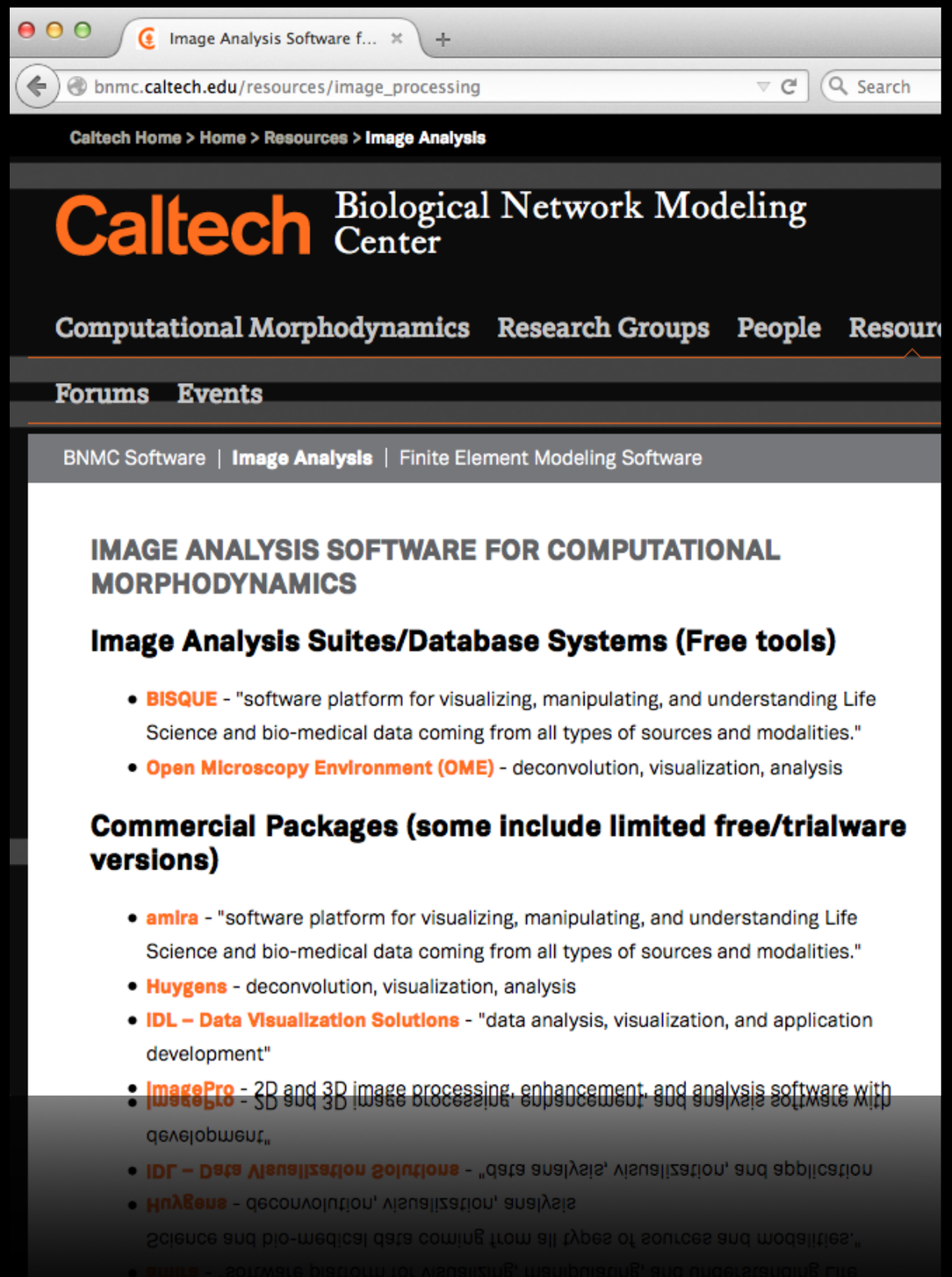


Image formats

raster : storing as a grid
vector : storing shapes

compression/no compression
reducing irrelevant/redundant information

TIFF	compression metadata annotation multiple frames
PNG	alpha channel compression
JPEG	compression RGB storage

LSM, LEI, OIB	encapsulated TIFF images
many others : BMP, GIF, RAW, XWD, …	

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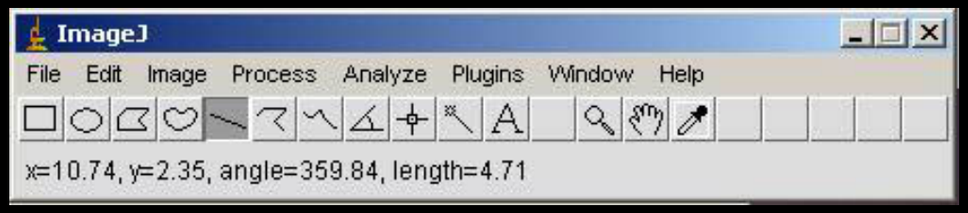
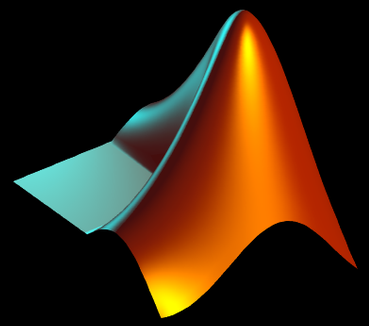
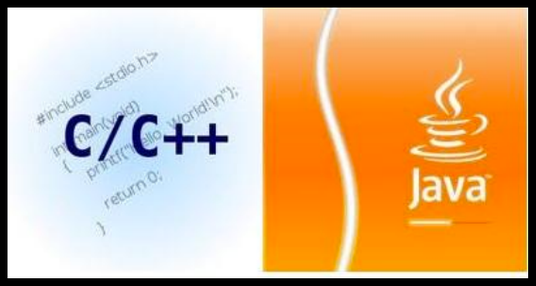
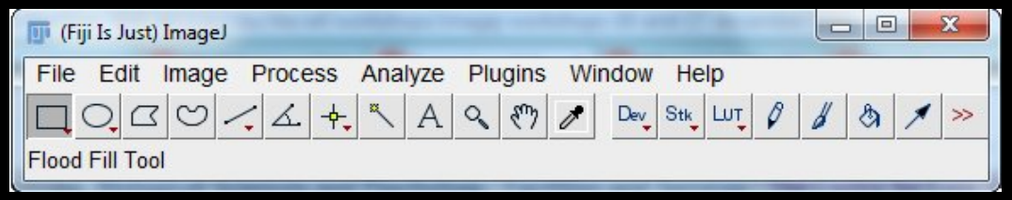
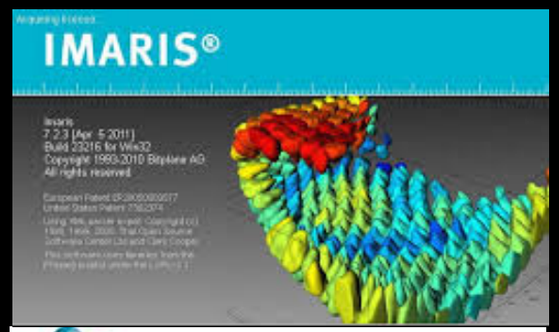
Available softwares











Amira	Bio-Formats	Bitplane Imaris	CellProfiler
Comstat2	Endrov	Farsight	Fiji
FocalPoint	i3dcore	IDL	ImageJ
ImageMagick	Imago	ImagePro	Imglib
Iqm	ITK-VTK	Macnification	Metamorph
MIPAV	PIL	Qu-Matlab	V3D
VisAD	VisBio	XuvTools	Bisque
Omero	Morphographix	OpenCV	

List compiled by Dr. Alphan Altinok

Questions