

Essentials of Biological Image Analysis

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Overview

1. Digital image

2. Basic Image Analysis

1. Point Operations
2. Local Filtering
3. Filtering in the frequency domain
4. Image Restoration
5. Segmentation

3. Advanced Image Analysis and Applications

1. Geometrical Transformations
2. Visualization
3. Colocalization
4. Filament Tracing
5. Particle Tracking
6. Cell Tracking

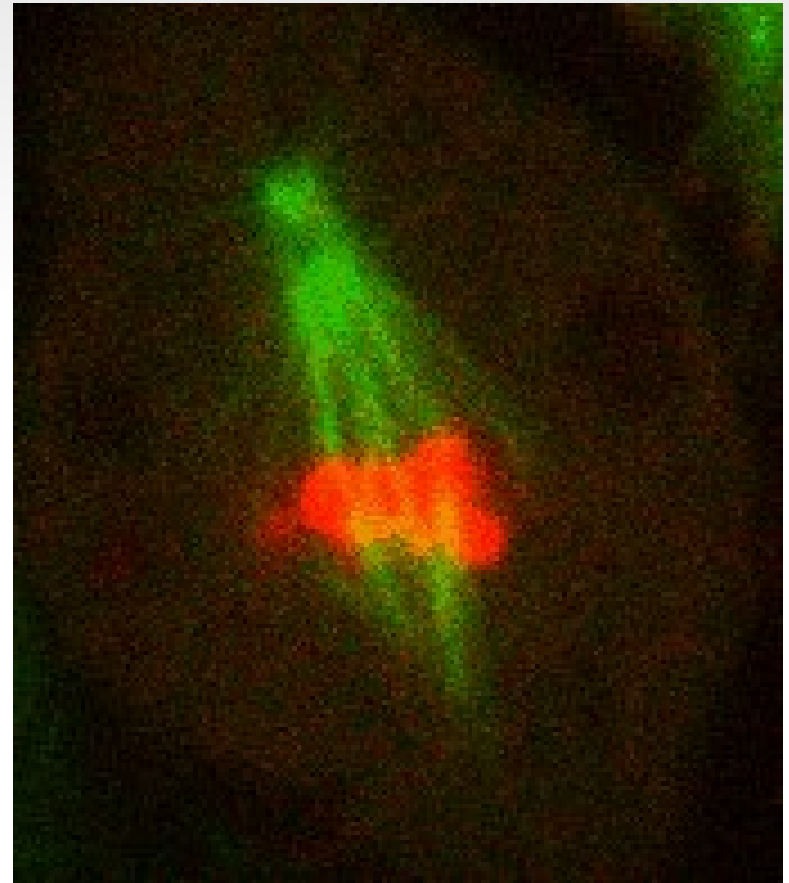
4. Software Tools

What is a digital image - examples

Example 1: Fly Brain example (ImageJ)



Example 2: Mitosis (ImageJ)



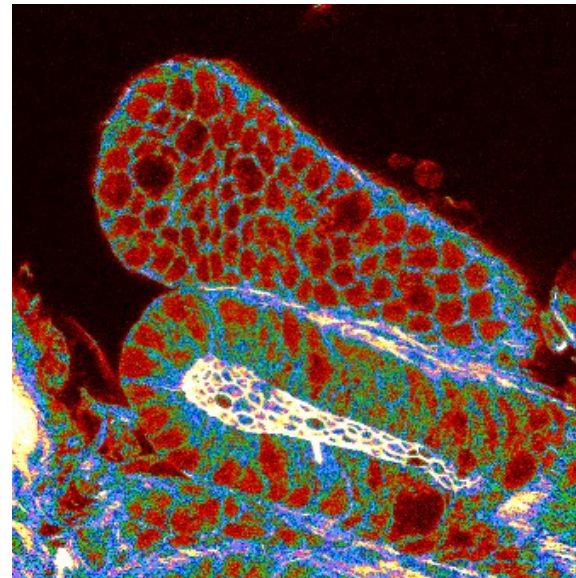
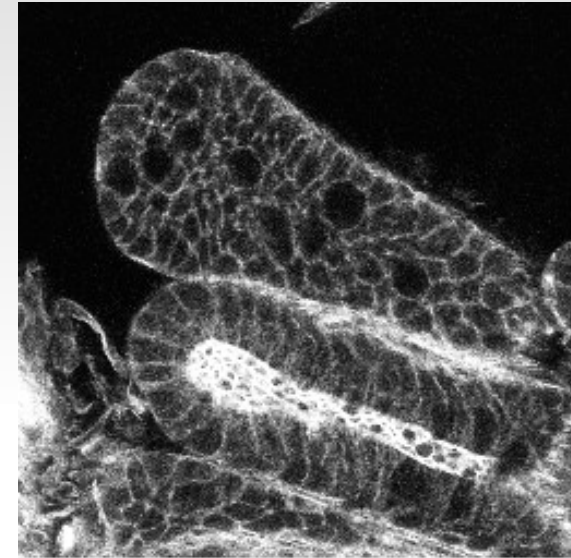
What is a digital image – mathematical point of view

- Matrix of sample values
 - finite number of samples
 - finite number of values per sample
- Image dimensions
 - 1D, 2D, 2D + t, 3D, 3D + t, 3D + t + multispectral
 - $I_{(x,y,z,t)} \in W^n$

0	0	0	0	0	2	3	7	10	12	13	14	11	5	1	0	0	0	0
0	0	0	1	1	7	14	17	25	30	30	29	26	16	7	2	0	0	0
0	0	0	4	8	18	26	37	48	52	52	53	41	30	18	9	3	0	0
0	0	3	8	19	29	44	60	72	76	73	67	61	45	30	19	7	0	0
0	0	5	14	27	45	61	79	94	102	98	88	70	59	43	26	13	5	0
0	1	7	18	35	56	79	99	111	114	108	94	80	67	53	36	19	8	0
0	4	12	23	40	62	87	105	123	124	111	97	83	73	59	45	28	12	2
0	4	12	23	41	62	89	108	120	117	103	96	88	75	63	47	29	13	2
0	3	10	21	37	54	80	102	108	103	96	88	80	67	56	41	21	6	0
0	2	8	17	28	44	62	75	84	88	87	80	62	53	43	27	11	3	0
0	0	4	12	19	31	43	52	63	65	67	56	49	41	28	15	5	0	0
0	0	1	6	9	16	25	34	39	45	40	38	32	25	15	5	0	0	0
0	0	0	1	4	8	13	18	22	20	22	18	16	8	3	1	0	0	0
0	0	0	0	1	2	4	5	9	6	7	5	3	0	0	0	0	0	0

What is a digital image – displayed by the computer

- mapping between sample values and display colors
 - bright means high values
 - bright means low values
 - brightness / contrast adjustments
 - lookup tables

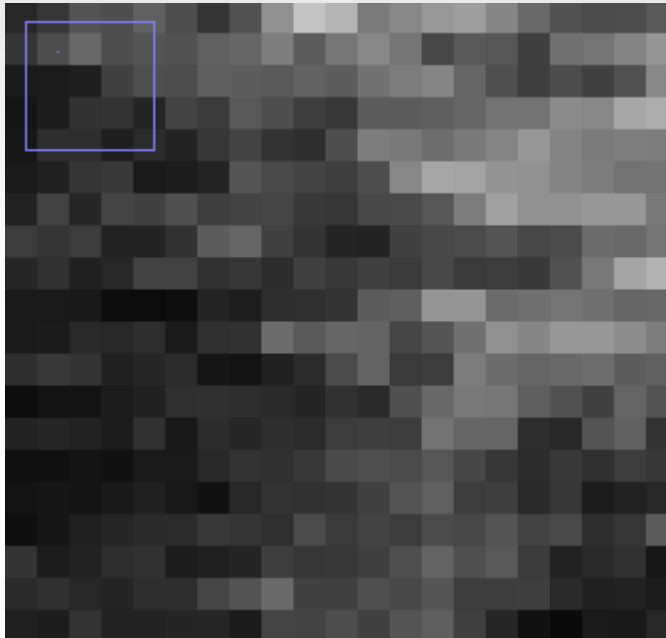


When I use a word,” Humpty Dumpty said, in rather a scornful tone, “it means just what I choose it to mean—neither more nor less.

L. Carroll, Through the Looking-Glass

What is a digital image – displayed by the computer

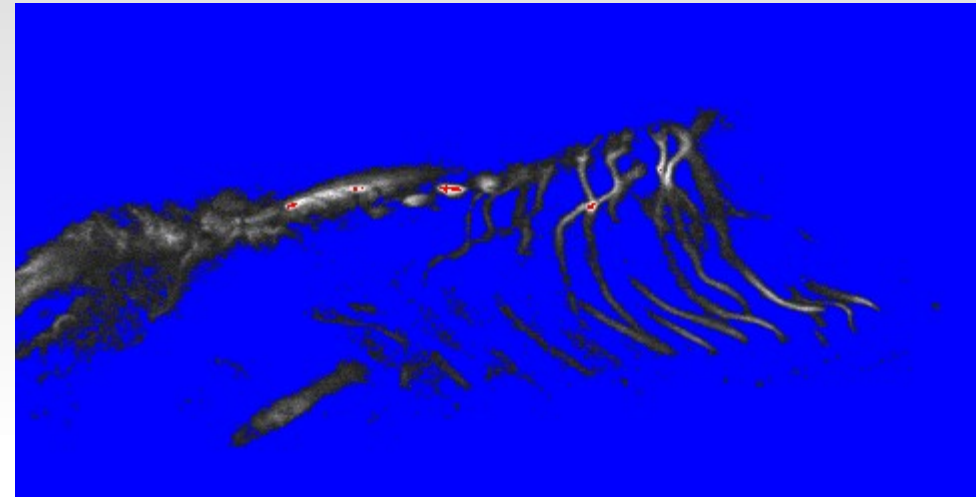
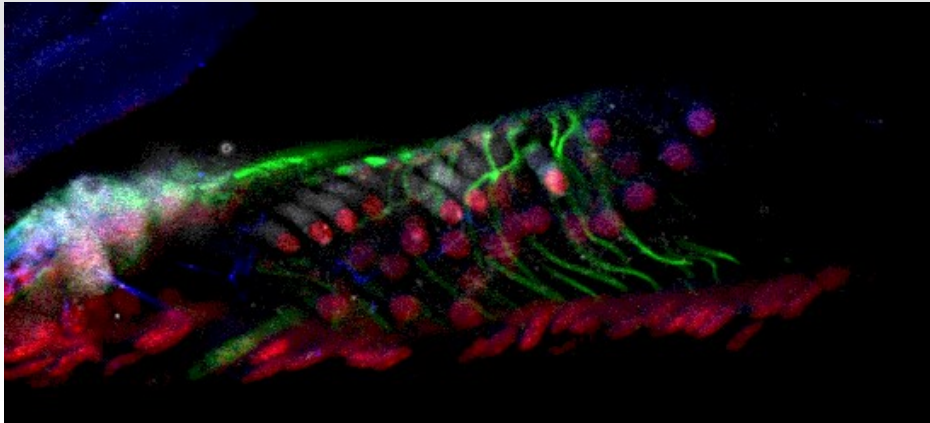
- mapping between sample grid and display grid
 - homogenous rectangles
 - interpolation



What is a digital image – represented in memory

Formats	Values	Interpretation
8-bit	0-255	unsigned integer
16-bit	0-65535	unsigned integer
32-bit	-3.4×10^{38} - $+3.4 \times 10^{38}$	precision 6-7 decimal digits, special values like NaN for “Not a Number”, Infinity and -Infinity
8-bit + lookup table	0-255	indexed color
24-bit	3 times 0-255	RGB
hyperstack	n channels of 8, 16 or 32 bit	3d + time + n channels

What is a digital image – conversion traps



- look at green channel
- multiply by ten
- convert both to 8-bit
- compare total intensity before and after

Label	Mean	Min	Max	IntDen
green	100.9	0	4095	13774198
10 x green	1009.0	0	40950	137741980
green 8bit	6.3	0	255	861340
10 x 8bit	6.3	0	255	861340

- conversion is done by linearly scaling from min–max to 0–255

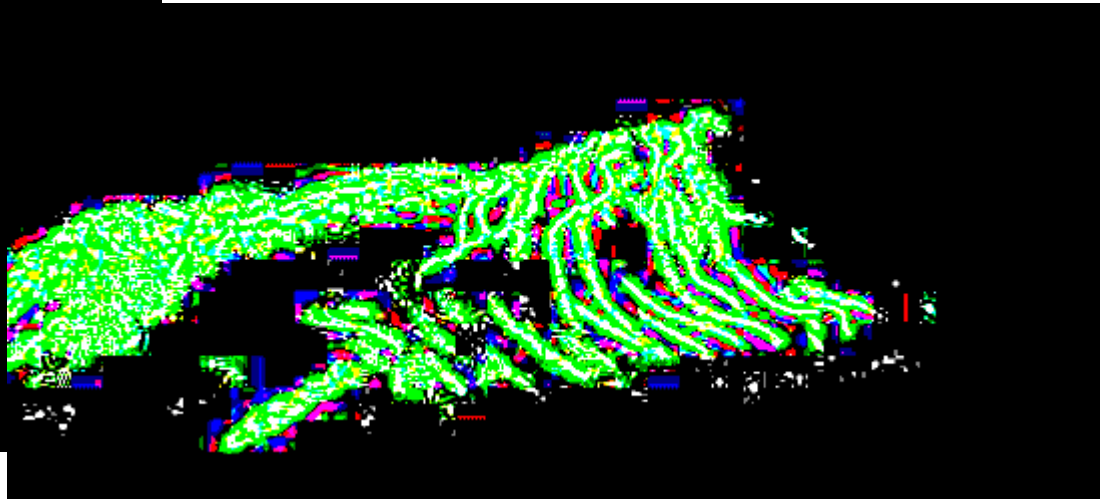
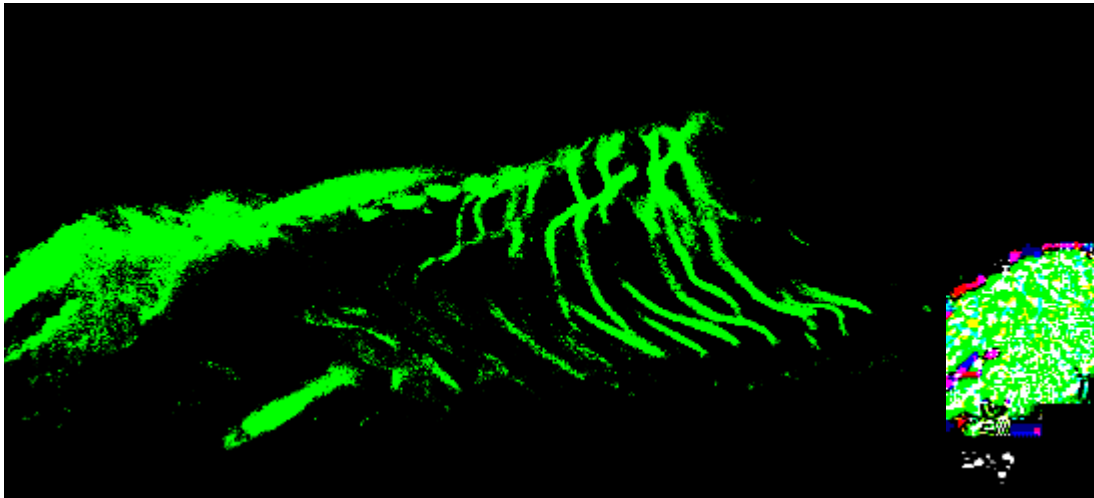
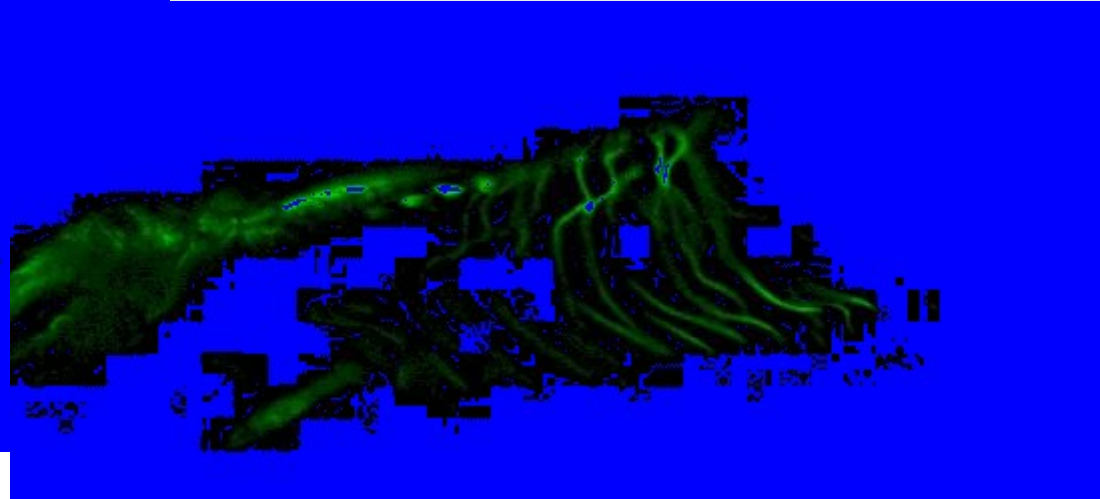
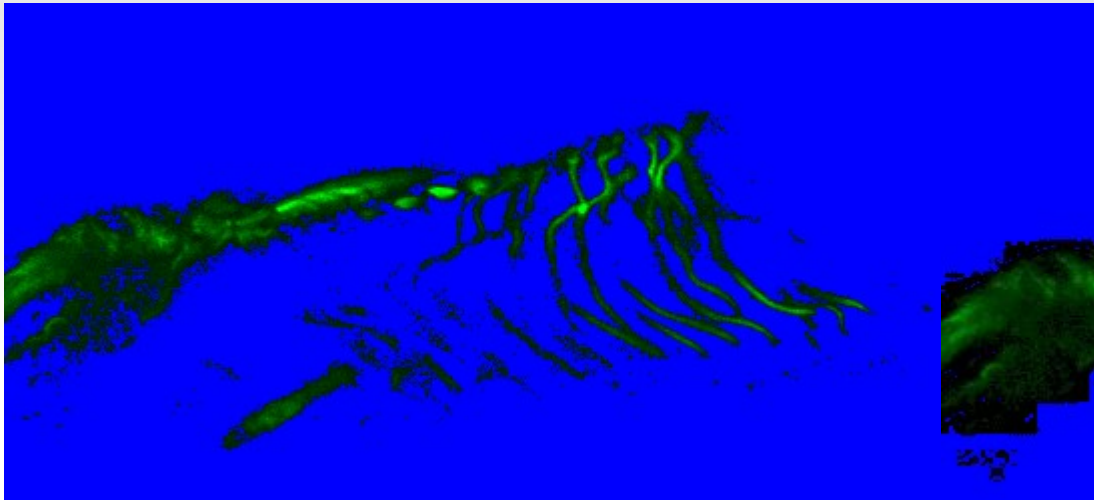
What is a digital image – stored on a disk

- data (sample values) + meta-data in header
- different organization of data and meta-data
- different possibilities / restraints

format	name	provider	properties
tiff	Tagged image file format	Adobe	lossless / metadata
ome-tiff	Open microscopy environment-tiff	OME	tiff with ontology for microscopy metadata
jpeg exif	Joint Photographic Experts Group - exchangeable image file format	ISO	lossy data compression / minimal metadata
lsm, stk	Laser scanning microscope file	Zeiss	extensions of tiff
lif	Leica image file format	Leica	can contain multiple images in one file

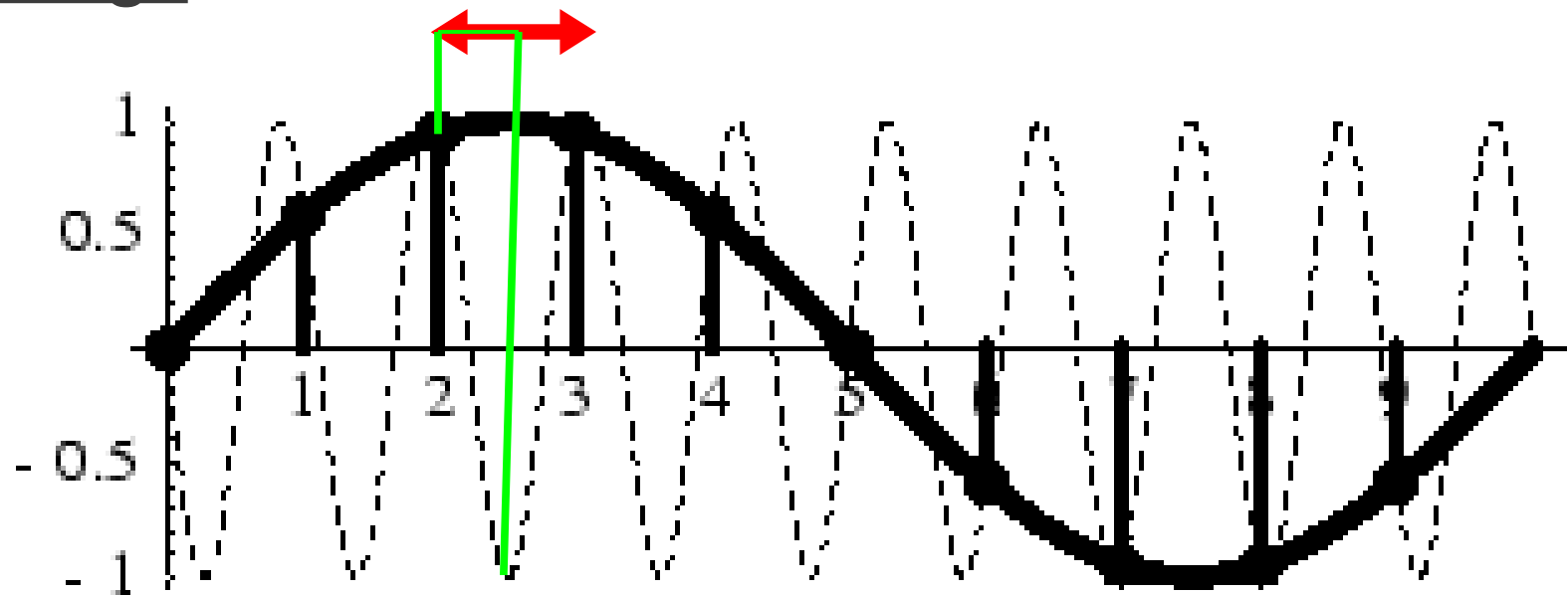
What is a digital Image – stored on a disk

- artefacts from jpg-compression



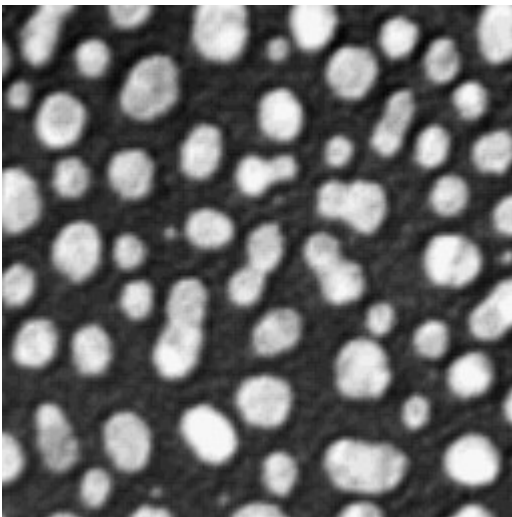
What is a digital image - the image and the real world

- sampling and resolution
- digital image – finite number of samples
- Nyquist-Shannon sampling theorem:
The sampling interval must be smaller than one-half the size of the smallest resolvable feature of the optical image

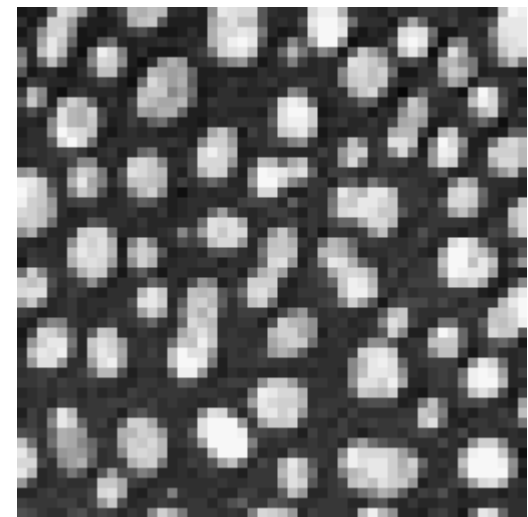


What is a digital image - the image and the real world

- sampling and resolution
- resolution of an optical system
 - the smallest distance at which two objects can still be distinguished
 - given by the Rayleigh criterion $r = \frac{0.61 * \lambda}{NA}$
- therefore the pixel size must be

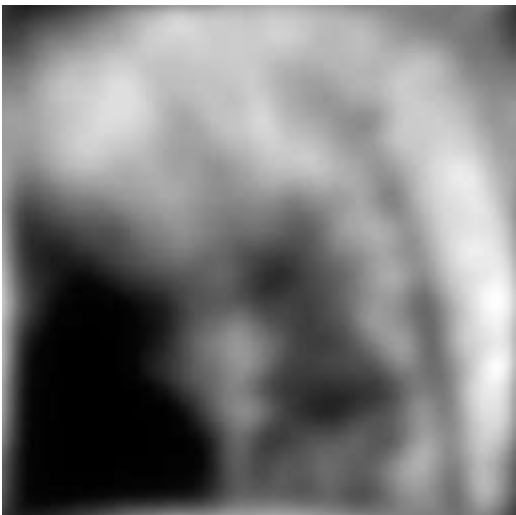


$$\Delta x < \frac{\lambda_{em}}{4 * NA} \quad \text{for widefield}$$
$$\Delta x < \frac{\lambda_{ex}}{8 * NA} \quad \text{for confocal}$$

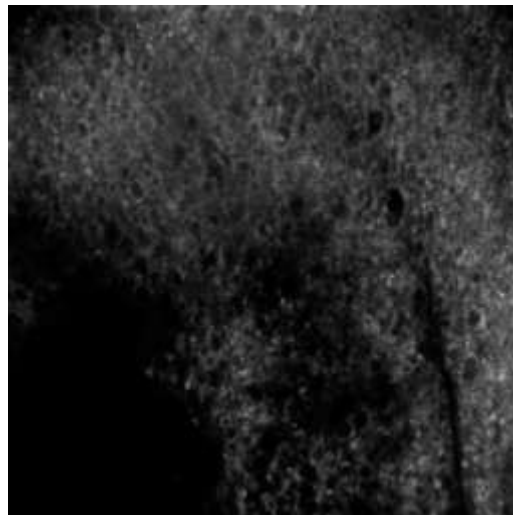


What is a digital image - the image and the real world

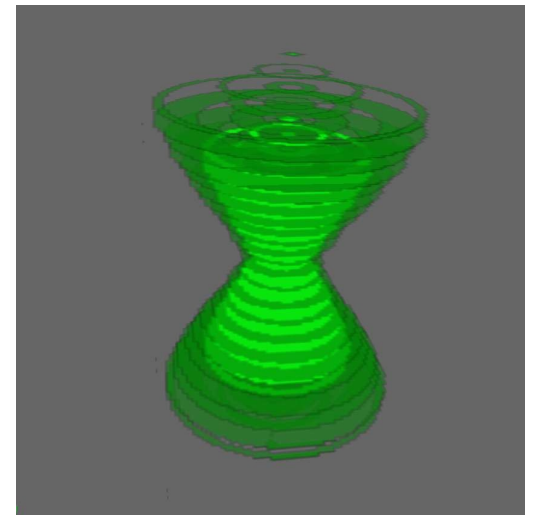
- point spread function
 - the way an optical system images one point
 - a point = an object at the limit of the resolution
 - acquired image = object function **convolved with** psf



=

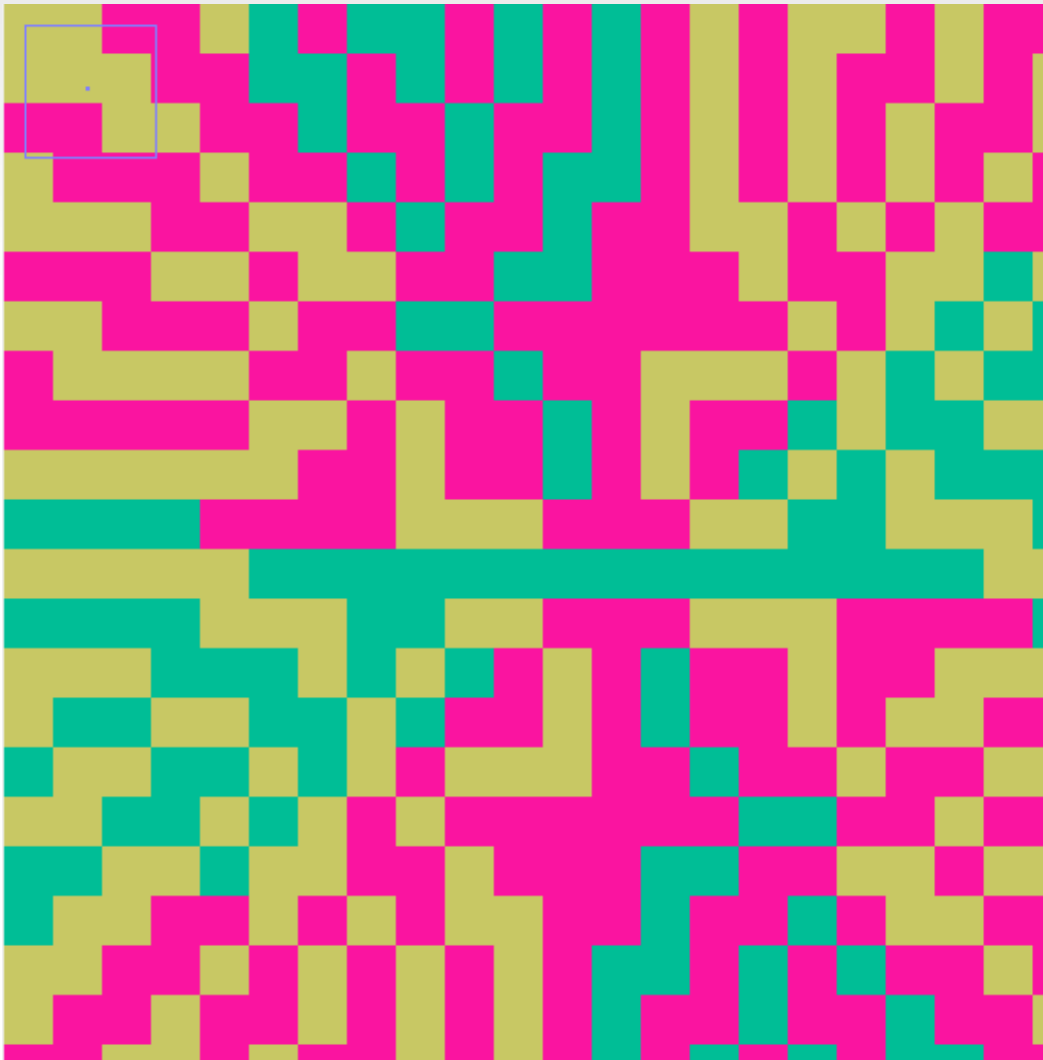


*



What is a digital image - image and perception

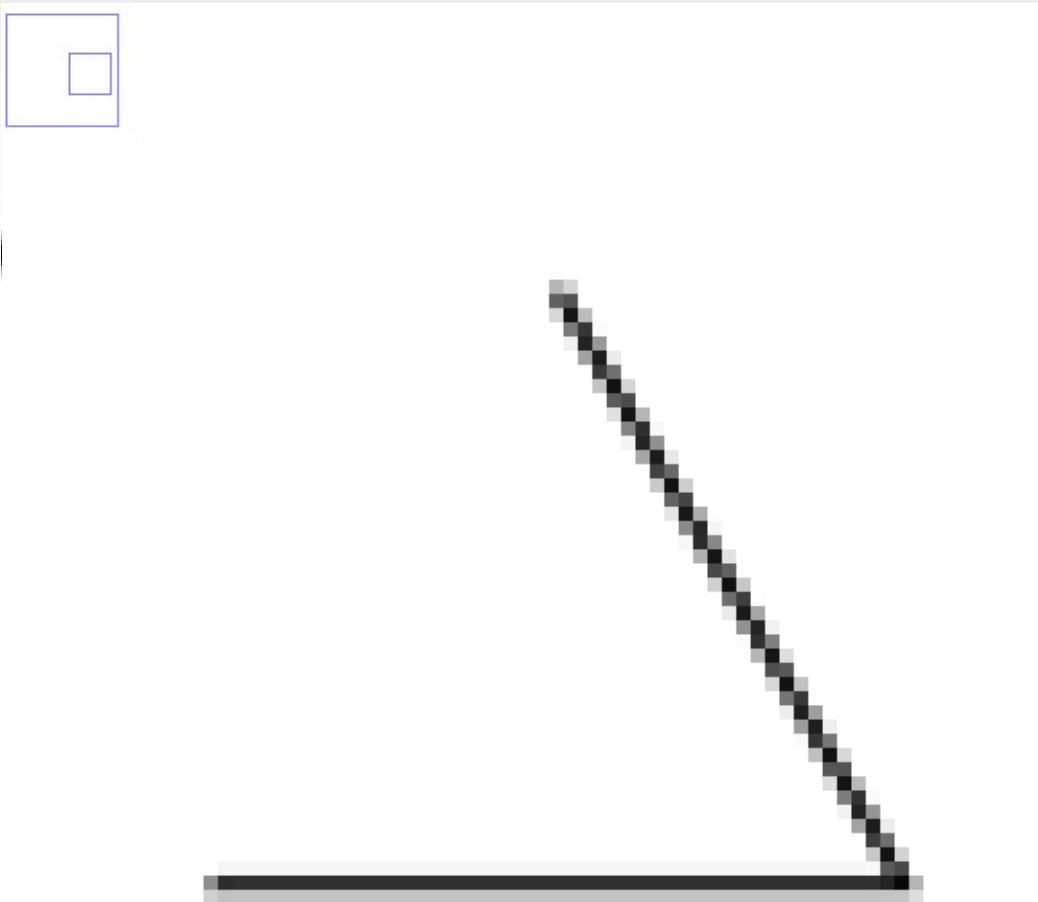
- How many colors do you see?



- the image contains 3 different colors
- the brain interprets color according to the background

What is a digital image - image and perception

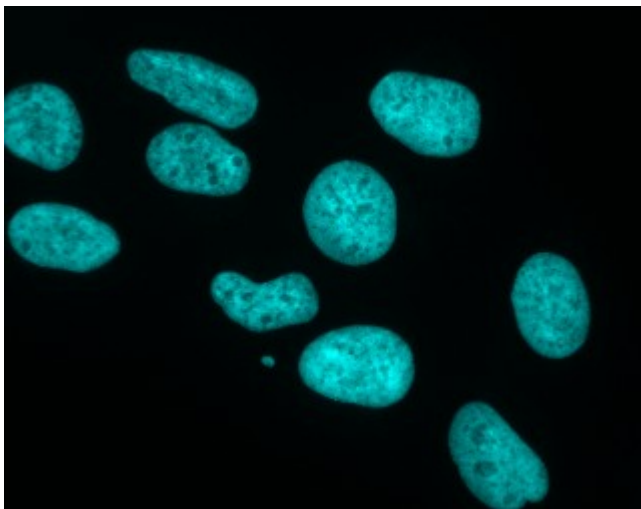
- Can you see the white triangle, standing on its head?



- the borders of the triangle don't exist
- the brain connects the points and interprets the scene as one triangle on top of another one

What is image analysis?

- Wikipedia
 - "Image analysis is the extraction of meaningful information from images; mainly from digital images by means of digital image processing techniques."



	Area	Perim.	
1	6101	353.061	
2	7047	329.120	
3	5455	292.392	
4	7524	328.191	
5	5653	300.978	
6	6178	304.392	
7	4583	296.392	
8	7312	333.120	
9	6820	343.345	

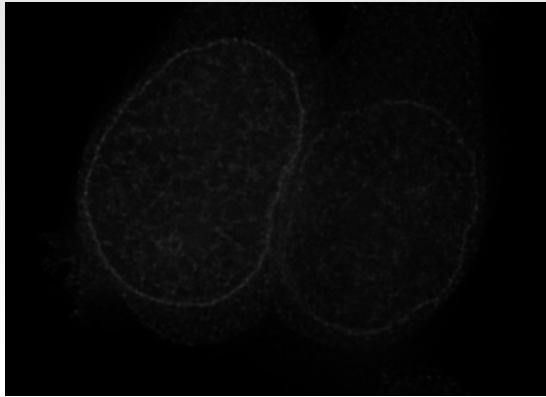
- IMAGE IN – FEATURES OUT

Point operations

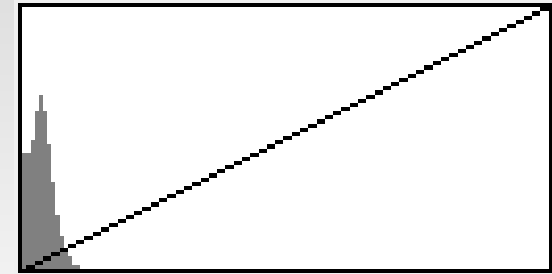
- global intensity transformations
 - intensity inversion
 - contrast and brightness adjustment
 - linear
 - gamma function
 - *histogram equalization*
 - pseudo-coloring
 - intensity thresholding



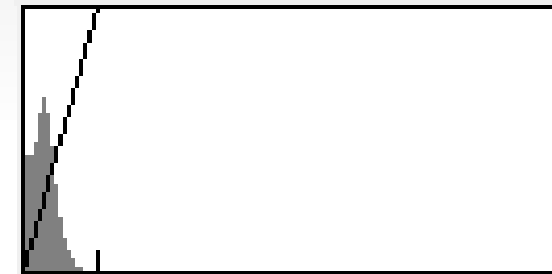
Point operations – contrast stretching



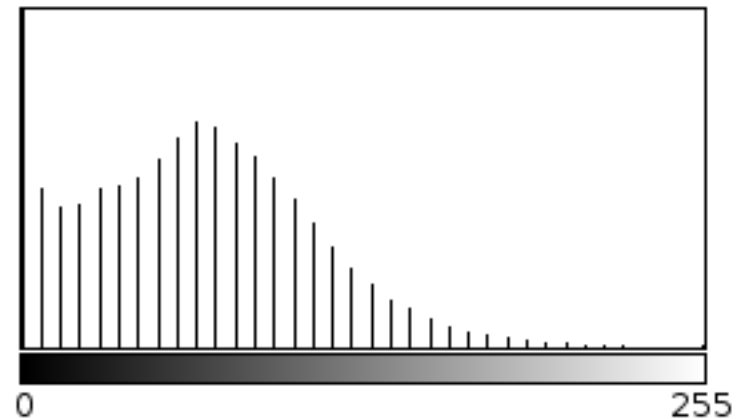
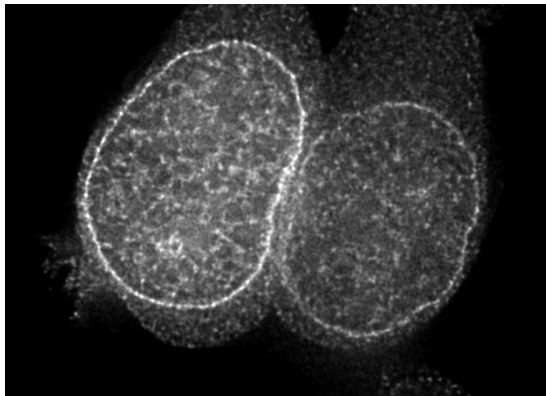
Count: 282048
Mean: 6.968
StdDev: 6.906
Min: 0
Max: 48
Mode: 0 (89140)



0 255

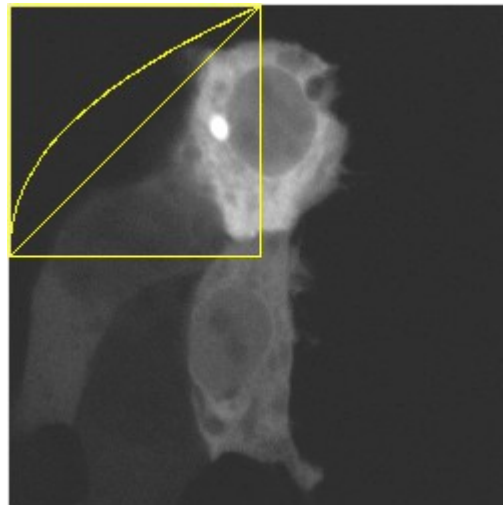
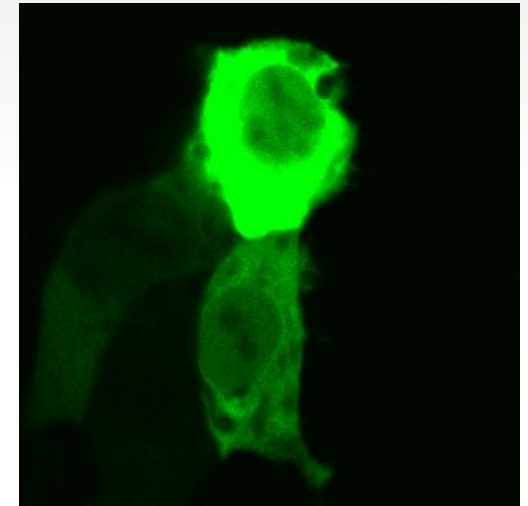
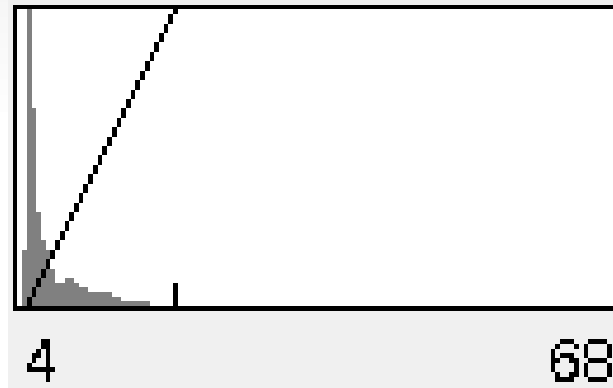
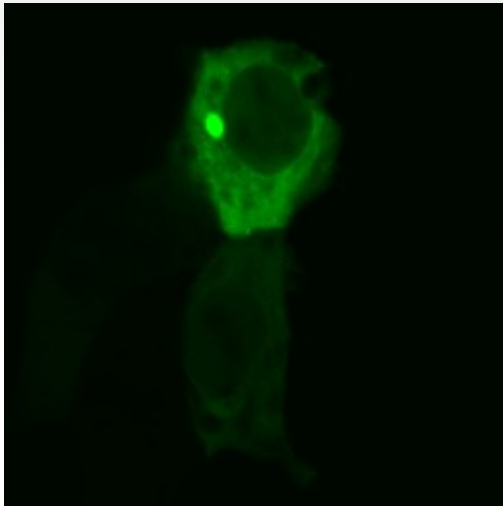


1 36



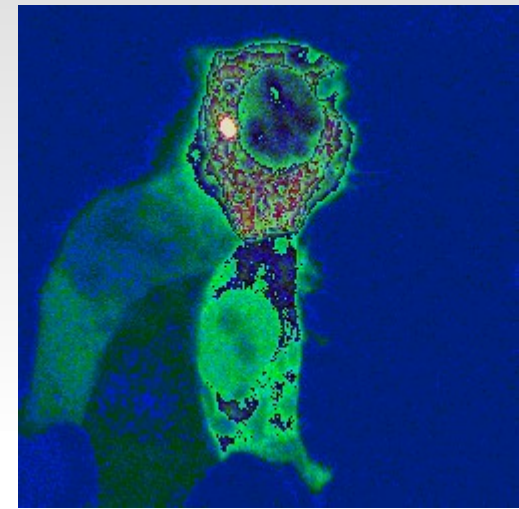
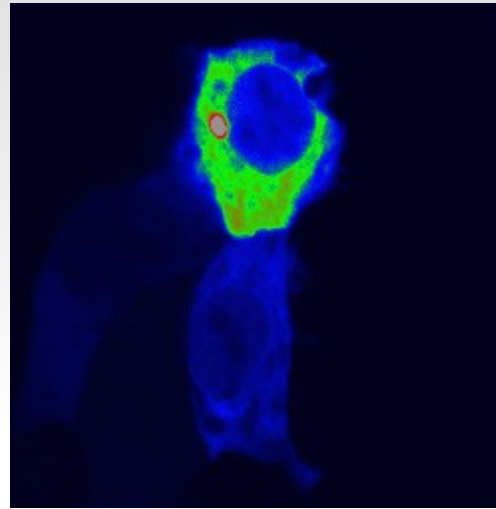
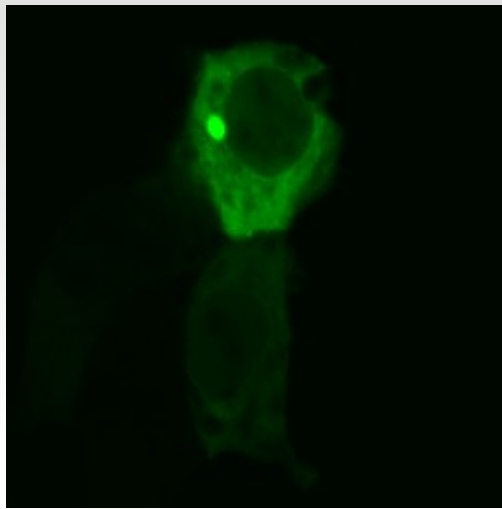
Point operations – gamma function

- linear function
 - changes small and high values in the same way



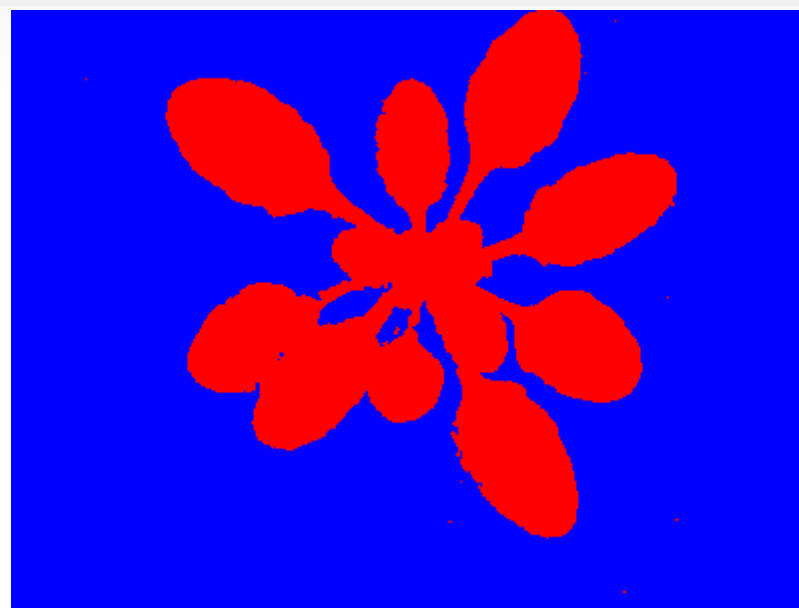
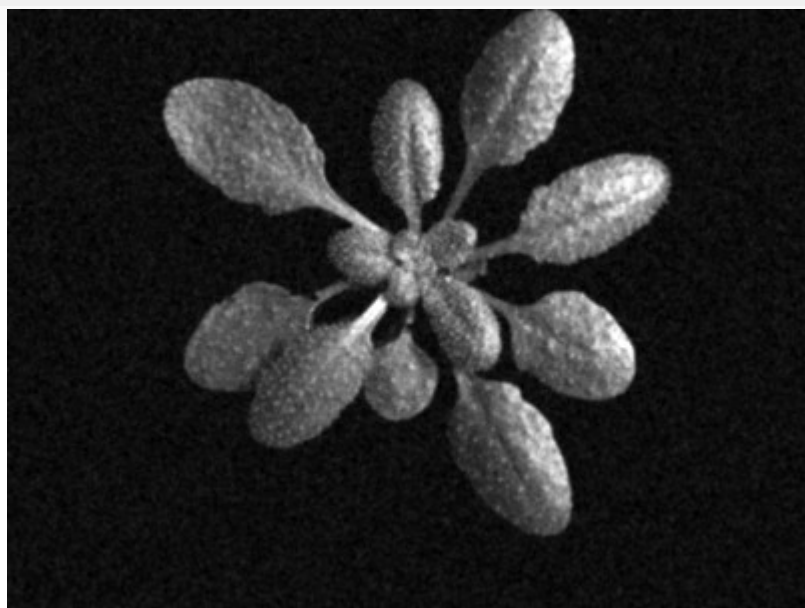
$$f(i) = \left(\frac{i}{255}\right)^{\frac{1}{\gamma}} \cdot 255$$

Point operations - lookup tables



Index	Red	Green	Blue
0	0	0	0
1	0	0	5
2	0	0	10
3	0	1	15
4	0	1	20
5	0	1	25

Point operations – thresholding



- segmentation – separate objects from background
- can be done by applying a global threshold
- what threshold value?
- the same for all images?

Local Filtering

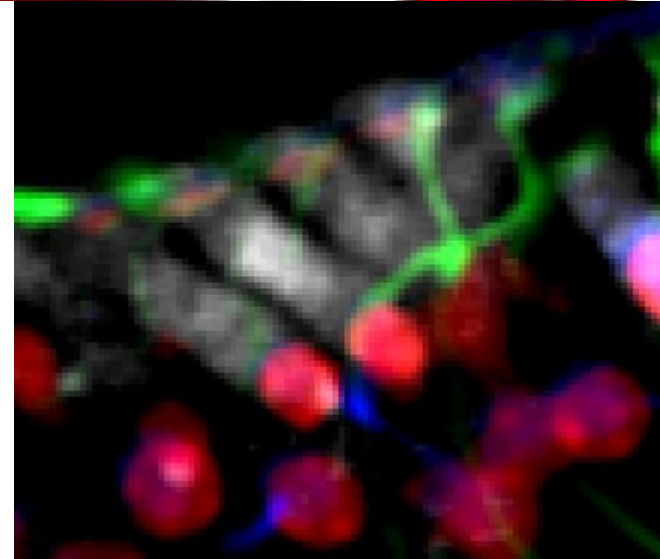
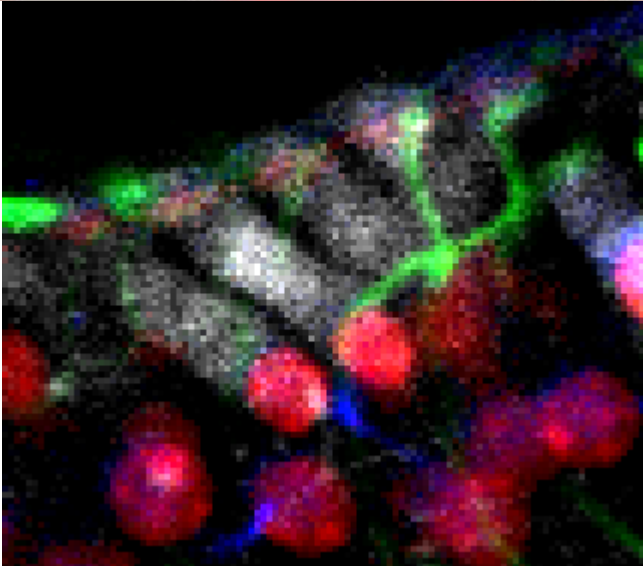
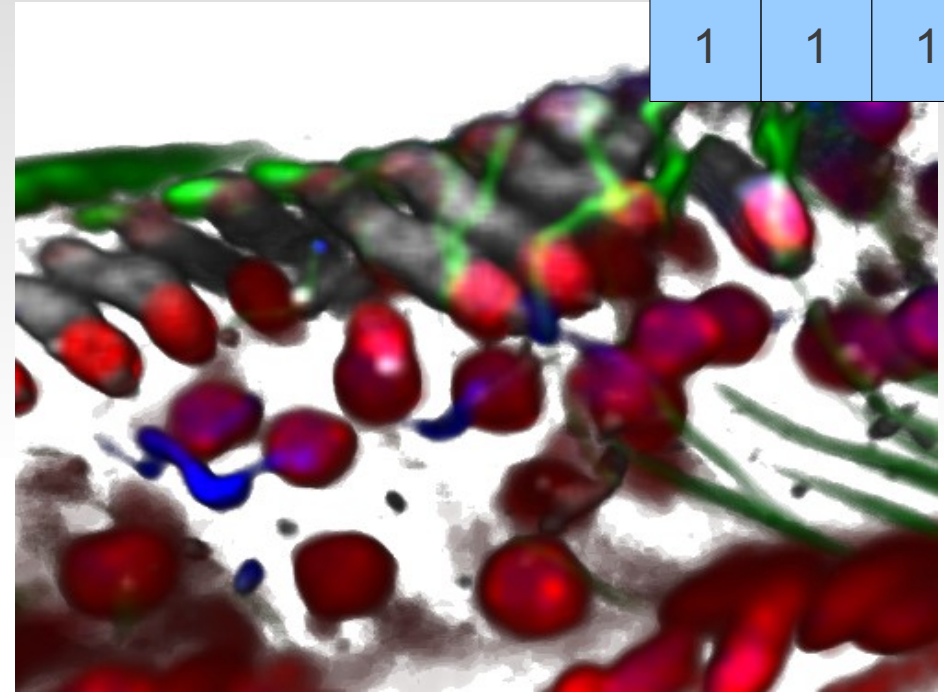
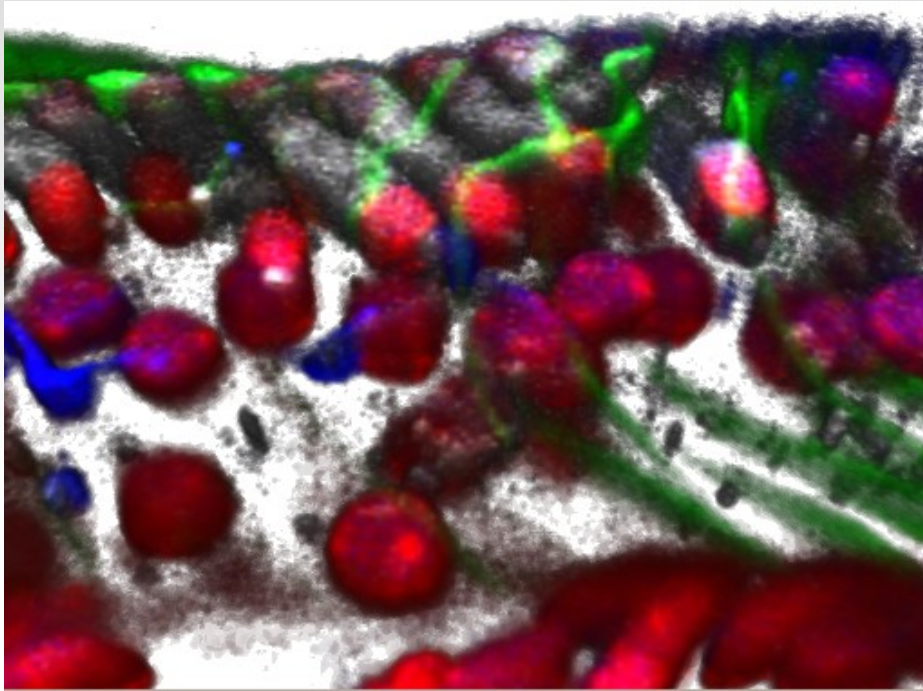
- convolution filter (linear filtering)
 - smoothing
 - mean filter
 - gaussian blur filter
 - edge detection
 - sobel filter
 - spot detection
 - Laplacian of Gaussian (Mexican Hat Filter)
- ranking filter
 - median, min, max
- mathematical morphology
 - post processing
 - erode, dilate, open, close, top hat, granulometry

The new value of a pixel is calculated from the values in the local neighborhood of the pixel

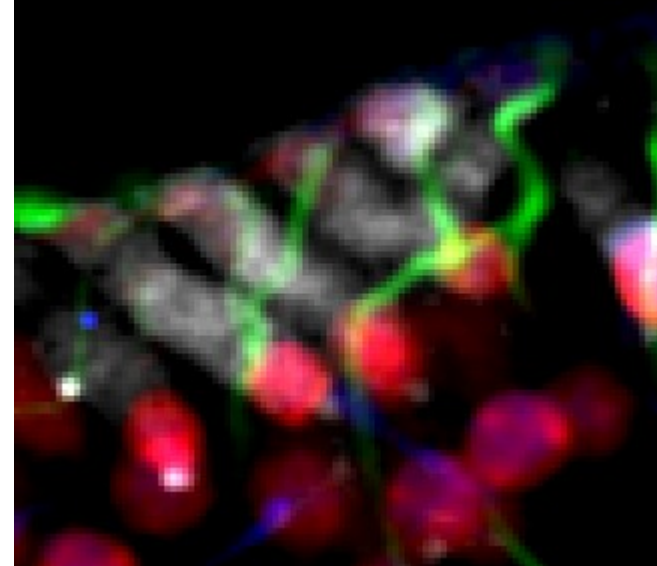
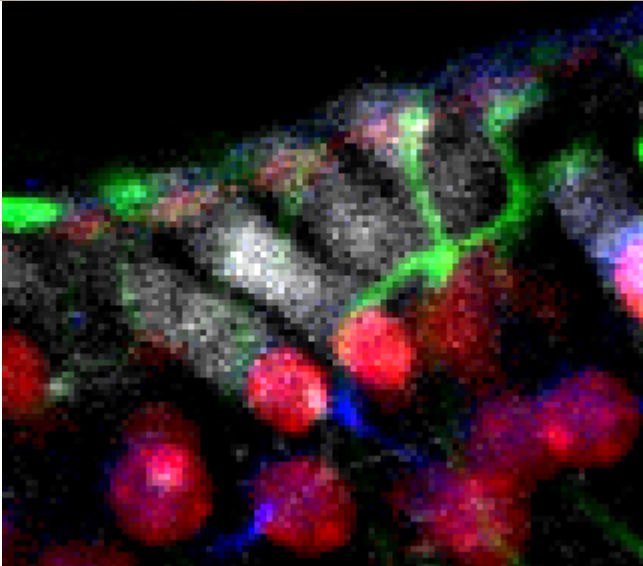
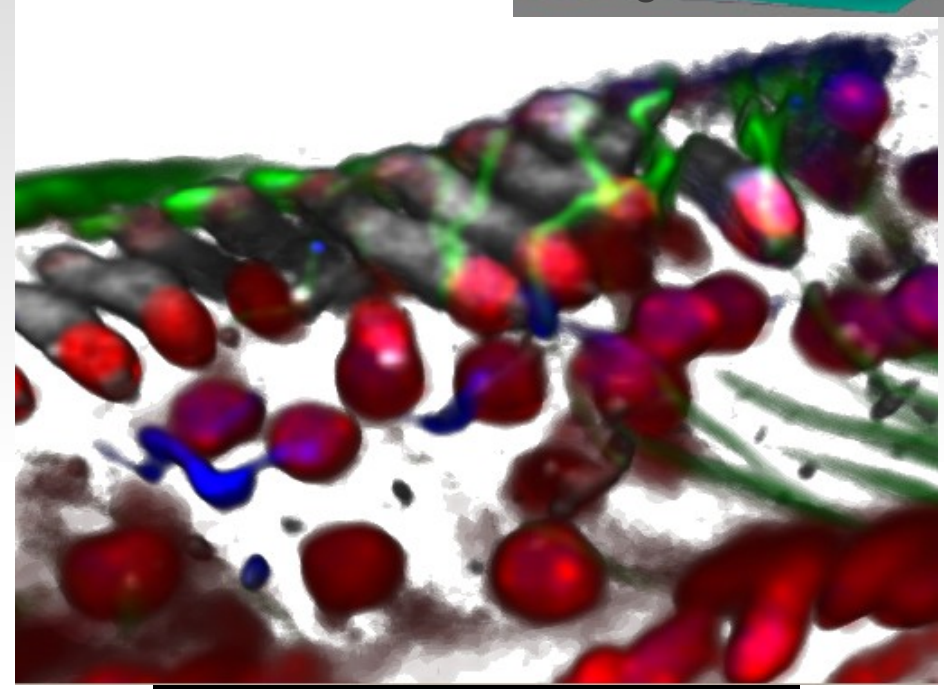
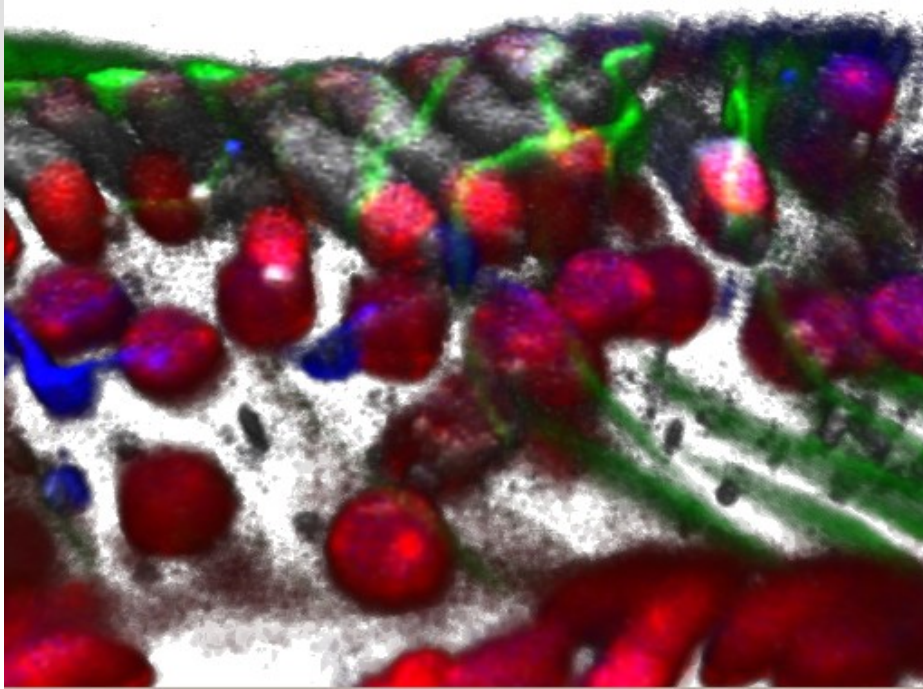
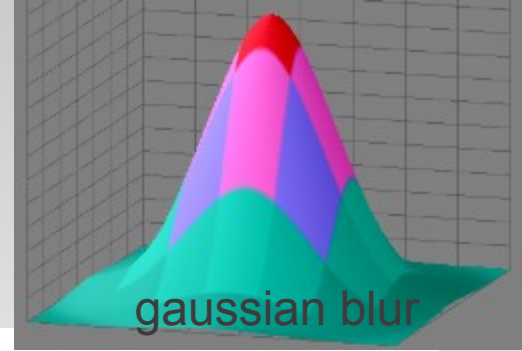
Convolution filter - smoothing

mean

1	1	1
1	1	1
1	1	1



Convolution filter smoothing



x-derivative

y-derivative

Convolution filter - edge detection

1	0	-1
---	---	----

2	0	-2
---	---	----

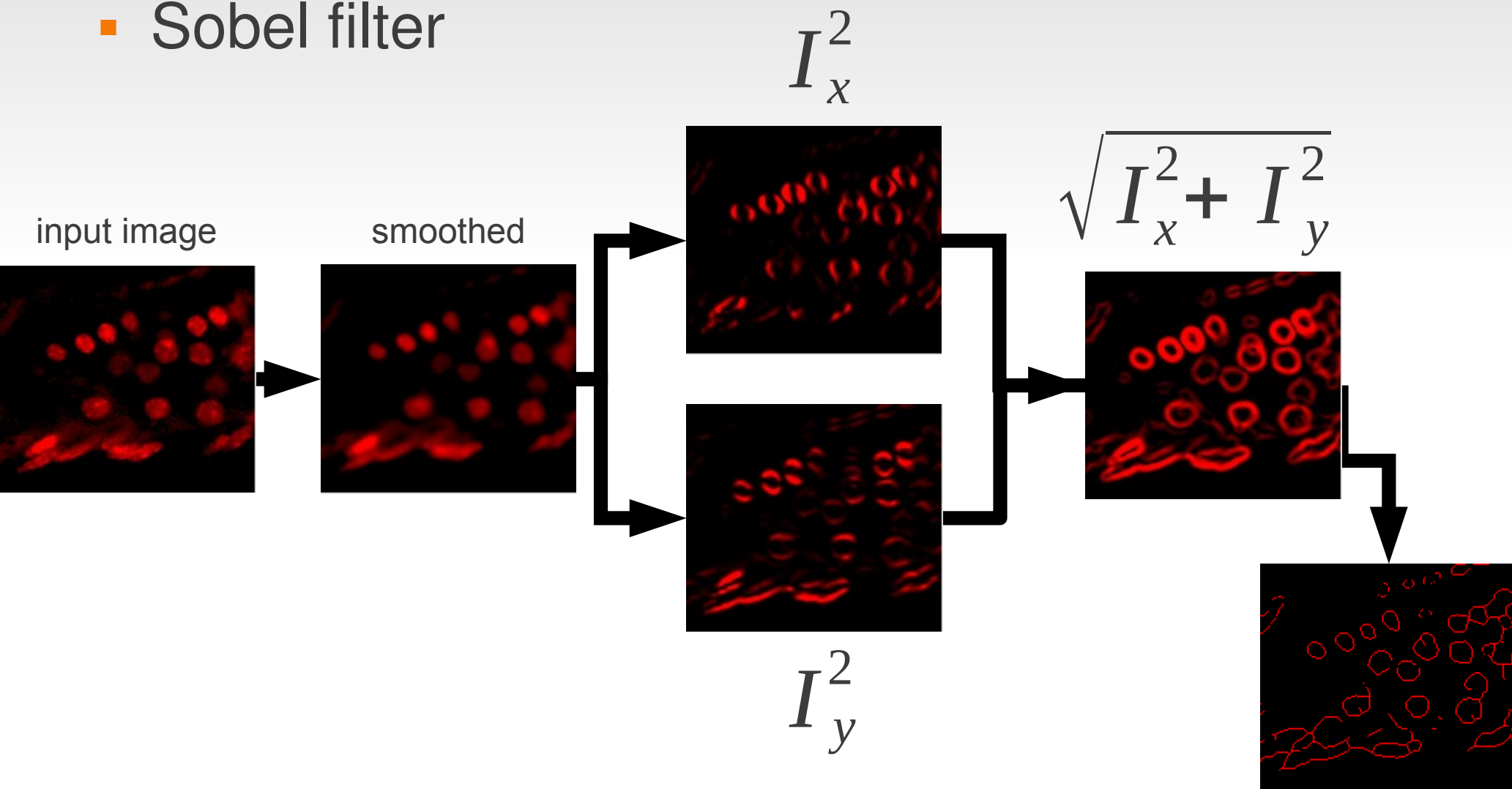
1	0	-1
---	---	----

1	2	1
---	---	---

0	0	0
---	---	---

-1	-2	-1
----	----	----

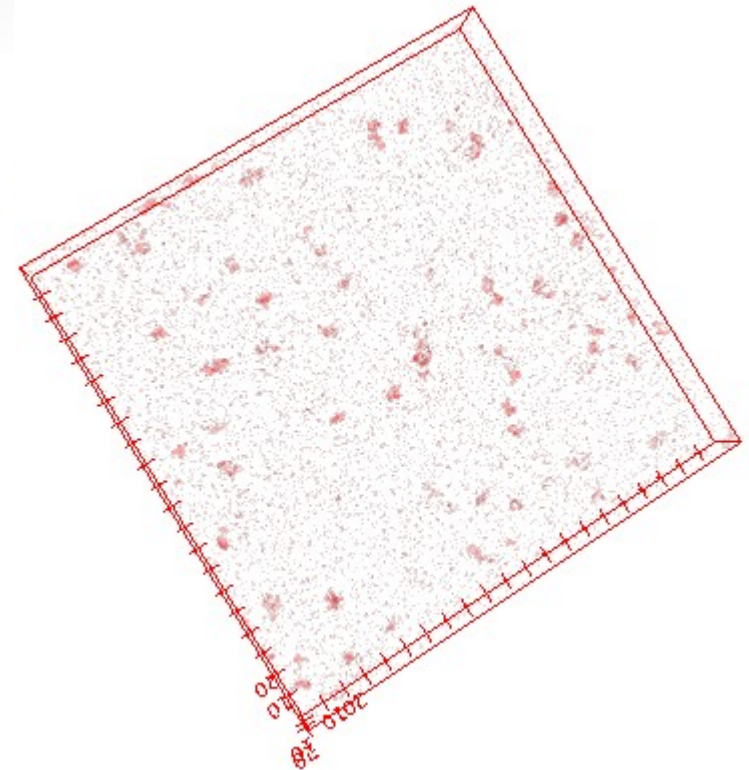
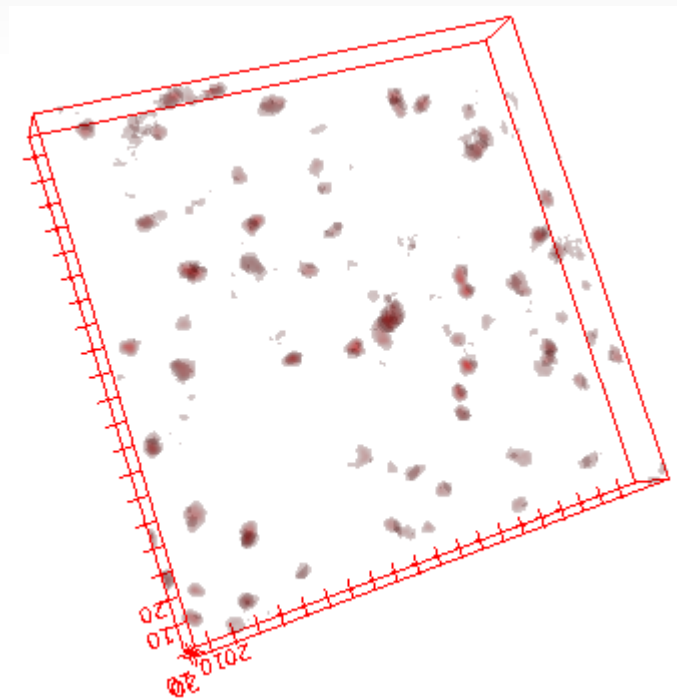
- Sobel filter



Convolution filter - spot detection

-1	-1	-1
-1	8	-1
-1	-1	-1


- Laplacian filter enhances spots but augments noise
- use 'Laplacian of Gaussian (LoG)' to enhance spots in noisy images



Local Filtering - Ranking filter

- for each pixel:
 - sort the values in the neighborhood
 - take the value at a given position
 - first = min filter enlarge dark regions
 - middle = median filter filter noise
 - last = max filter enlarge bright regions

15	18	14
29	27	13
12	19	21

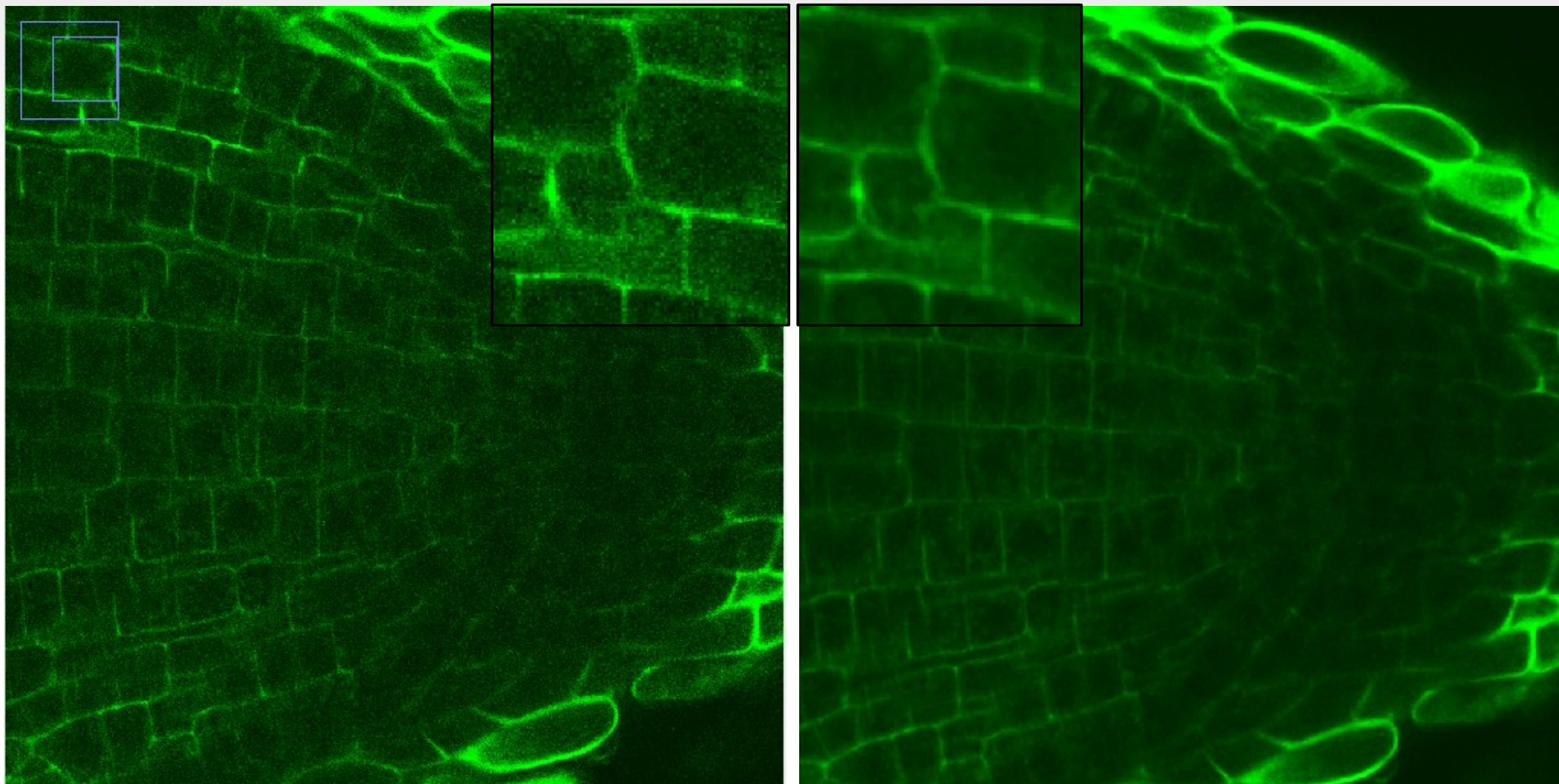


15	18	14
29	18	13
12	19	21

12, 13, 14, 15, 18, 19, 21, 27, 29

Ranking filter - median filter

- + stable against outliers
- - can be long to calculate

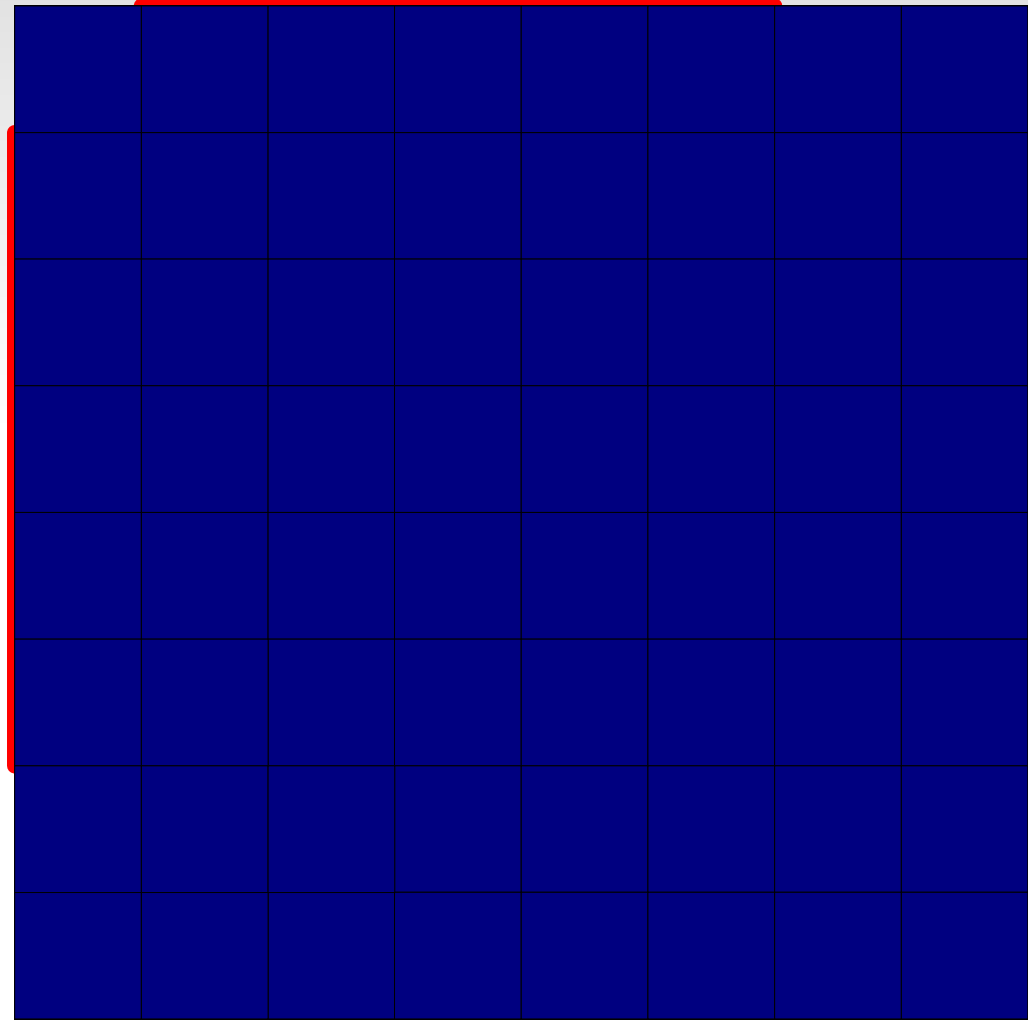
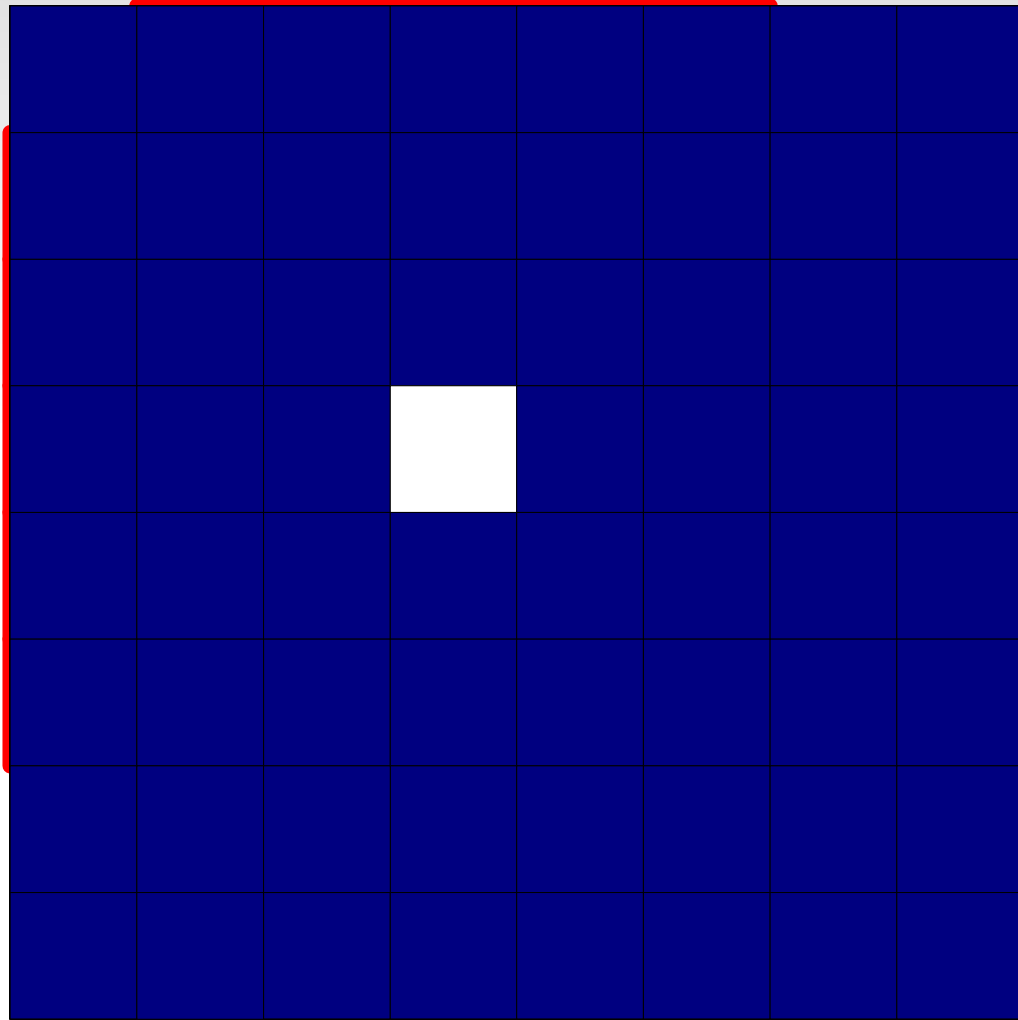


Local Filtering

binary morphology

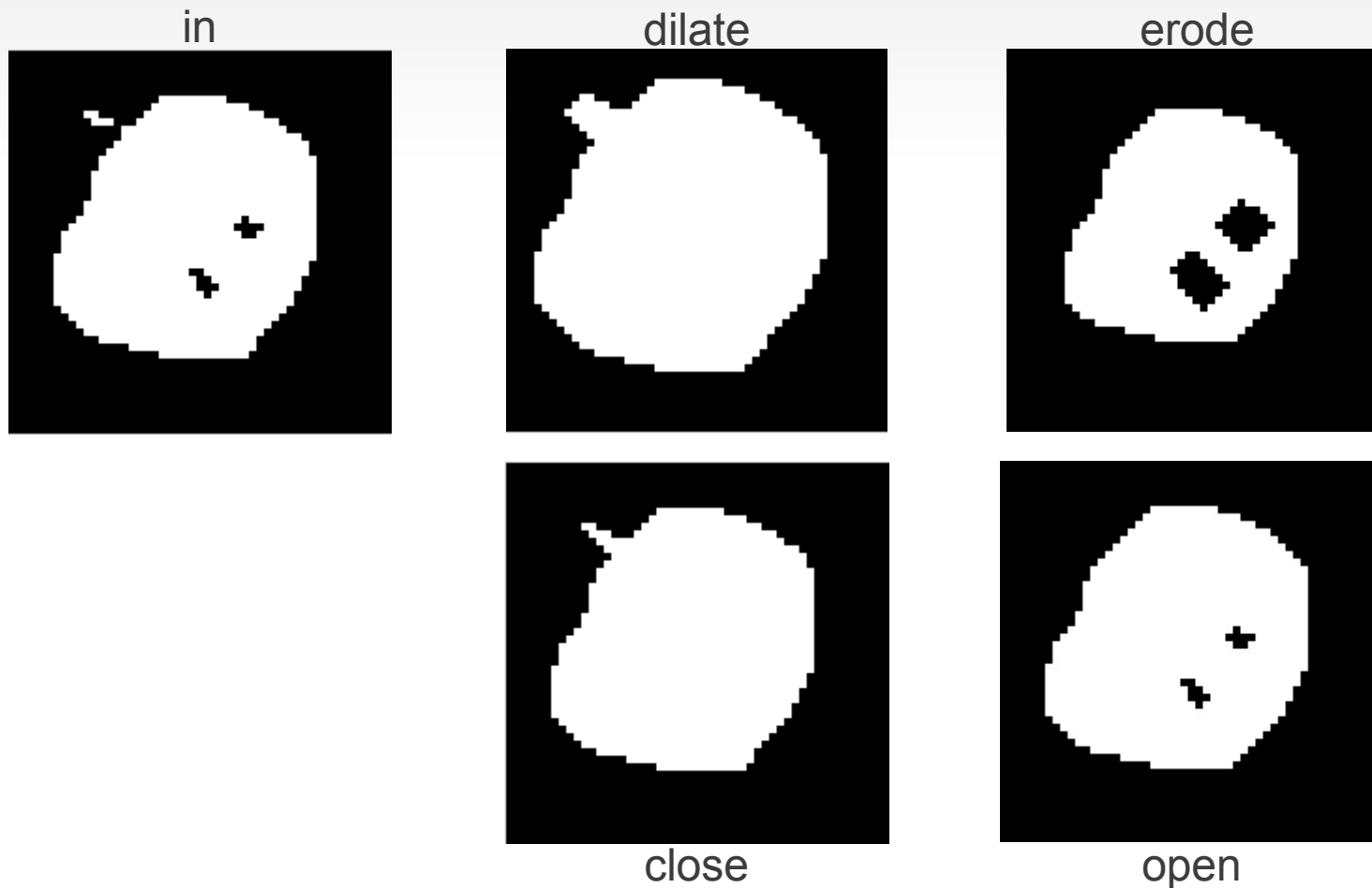
- correct segmentation, measure features, granulometry, edge detection, skeletonization, reconstruct objects
- work on a mask (a binary image)
- move the structuring element along the image
- two basic operations
 - dilate (enlarge objects):
 - current pixel is 1 if the SE touches a 1 in the image
 - erode (shrink objects):
 - current pixel is 1 if no 1 in the SE touches a zero in the image

Binary Morphology - erosion



Binary Morphology - open and close

- $\text{close}(X) = \text{dilate}(\text{erode}(X))$ close holes in objects
- $\text{open}(X) = \text{erode}(\text{dilate}(X))$ remove small objects



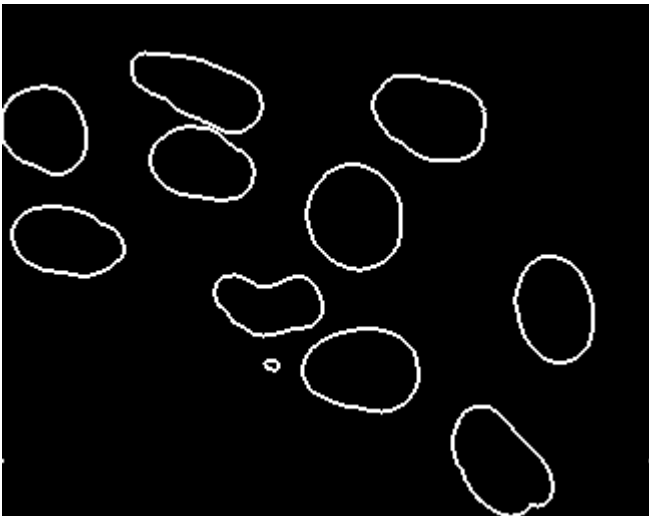
Binary Morphology - applications

edge detection

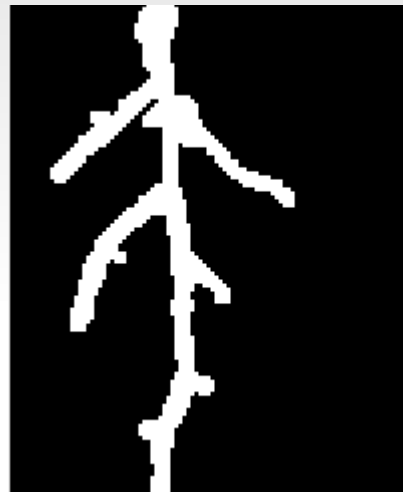
I



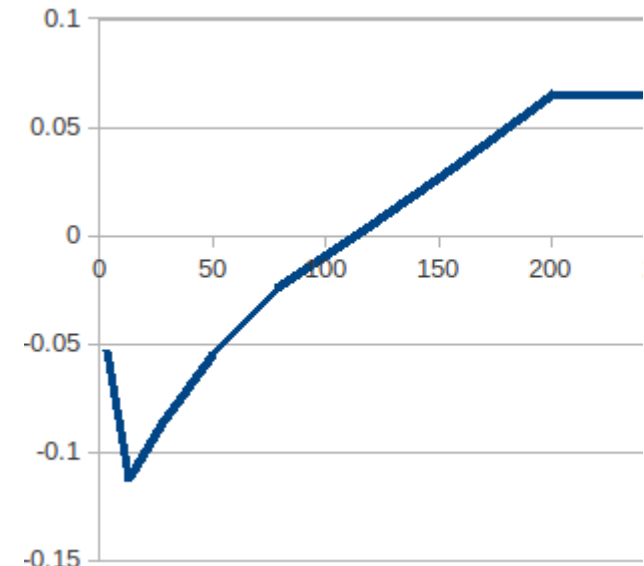
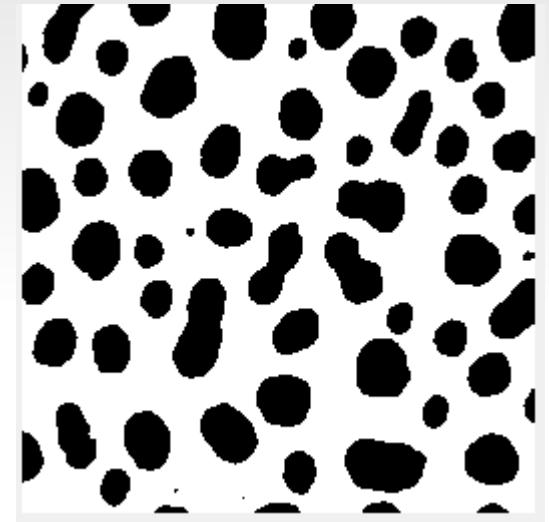
dilate(I) - erode(I)



skeletonization

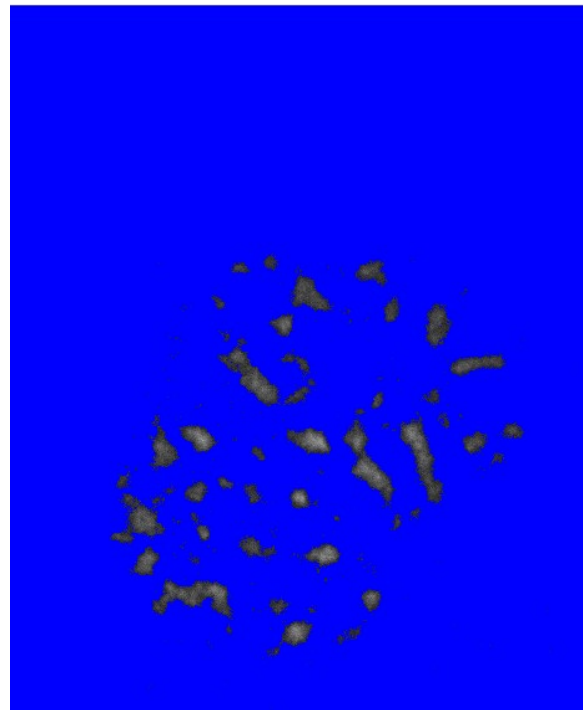
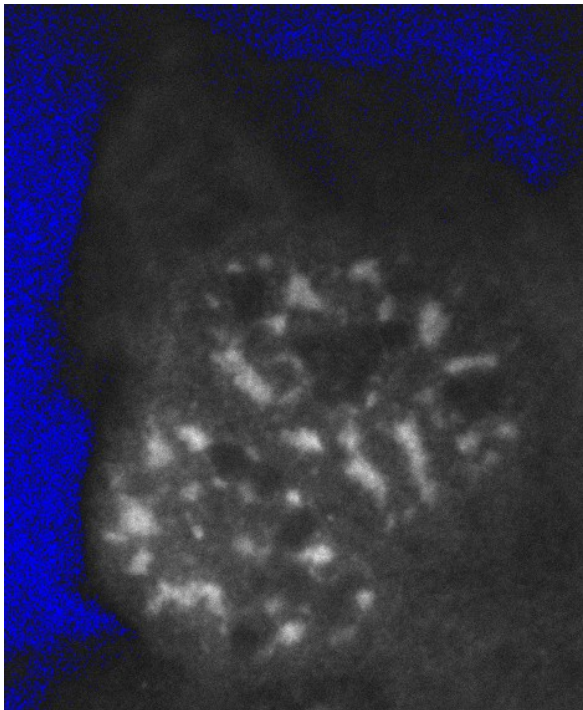


granulometry



grayscale morphology

- dilate – max over structuring element
- erode – min over structuring element
- Example: grayscale top-hat filter ($I - \text{open}(I)$)

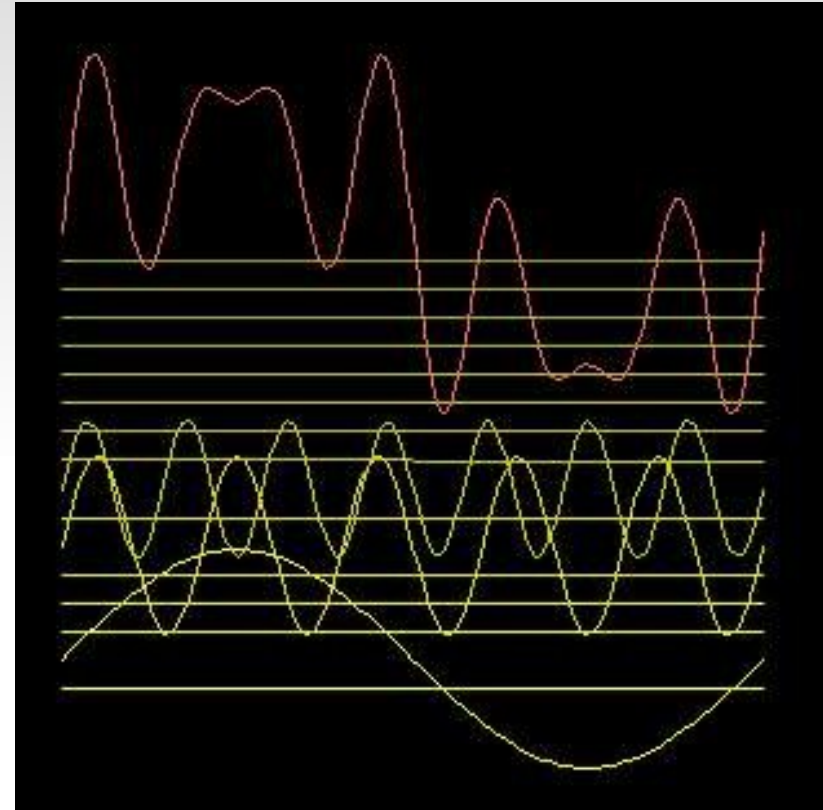
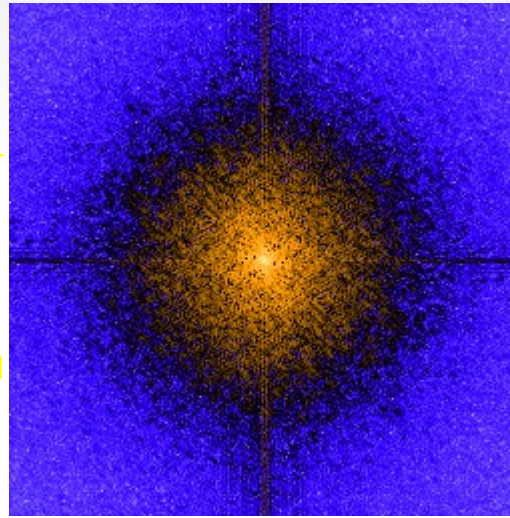
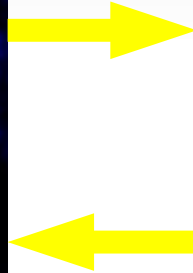
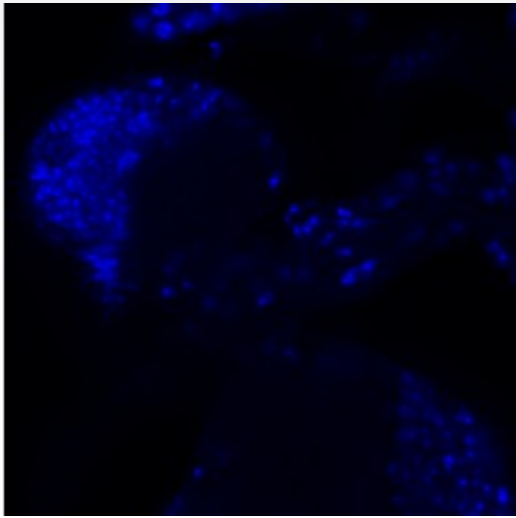


filtering in the frequency domain

- Fourier Transform
- low-pass
- high-pass
- band-pass
- *correlation*
- *convolution*

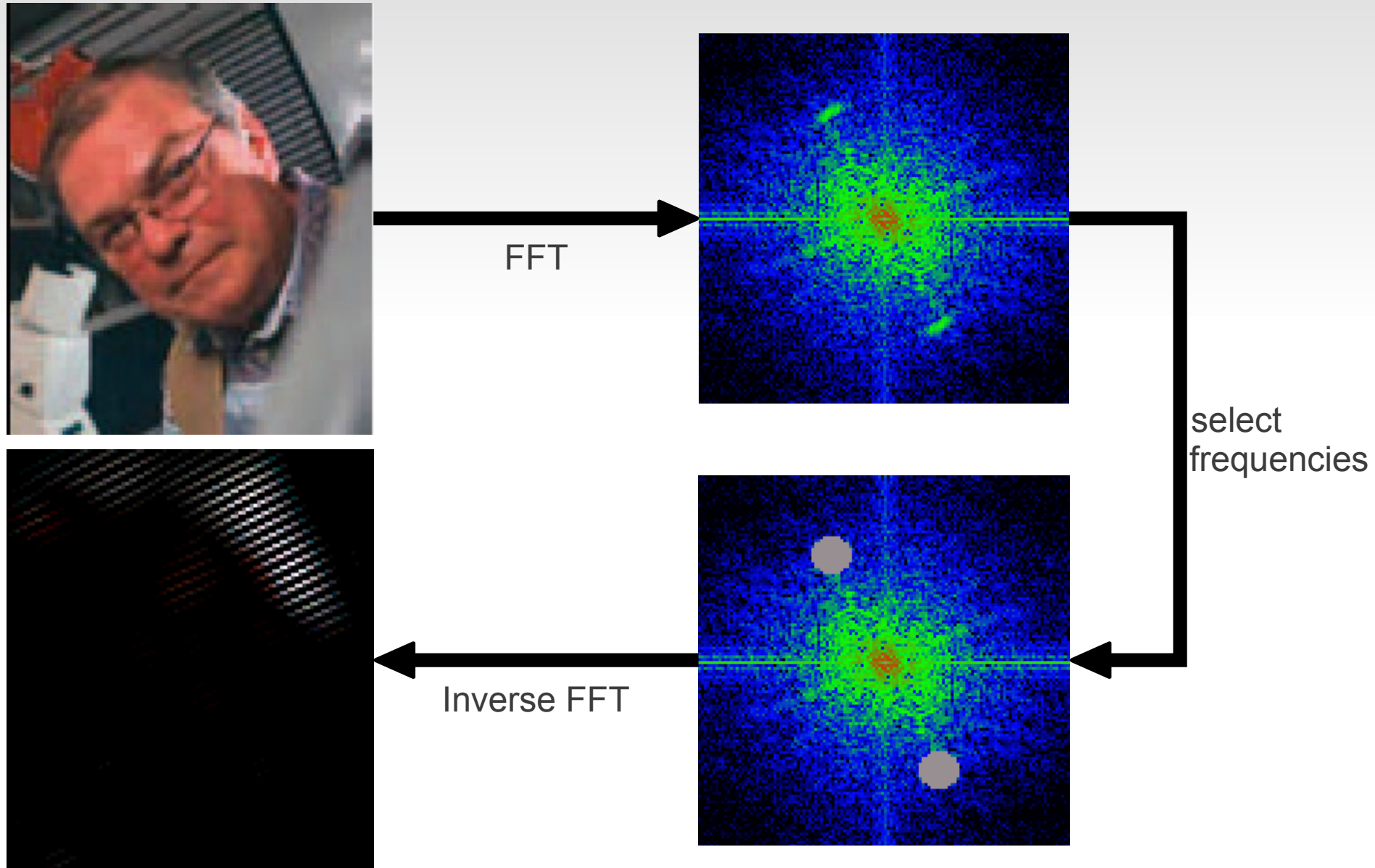
filtering in the frequency domain – fourier transform

$$F(\nu) = \int f(x) e^{-i2\pi\nu x} dx$$

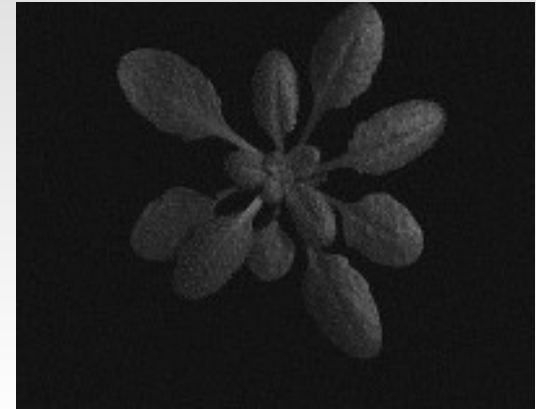
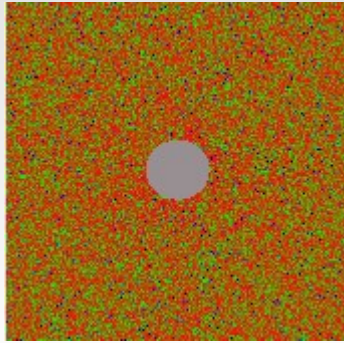
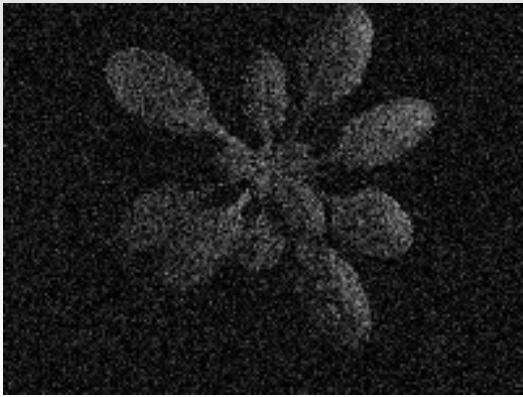


- signal can be represented as sum of sinoids
- FT transforms from spatial to frequency domain

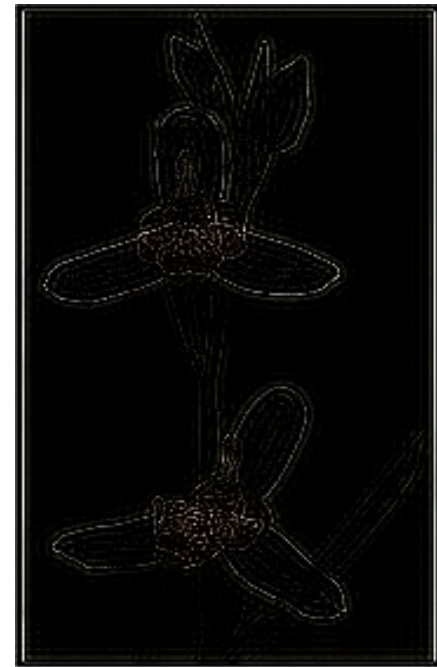
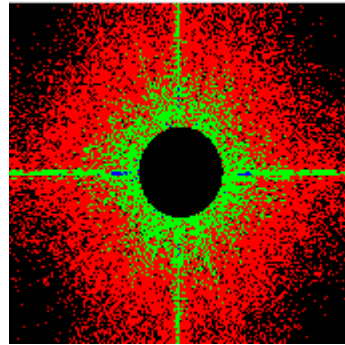
Filtering in the frequency domain



Filtering in the frequency domain



Low pass filter



High pass filter

Filtering in the frequency domain

Band pass filter

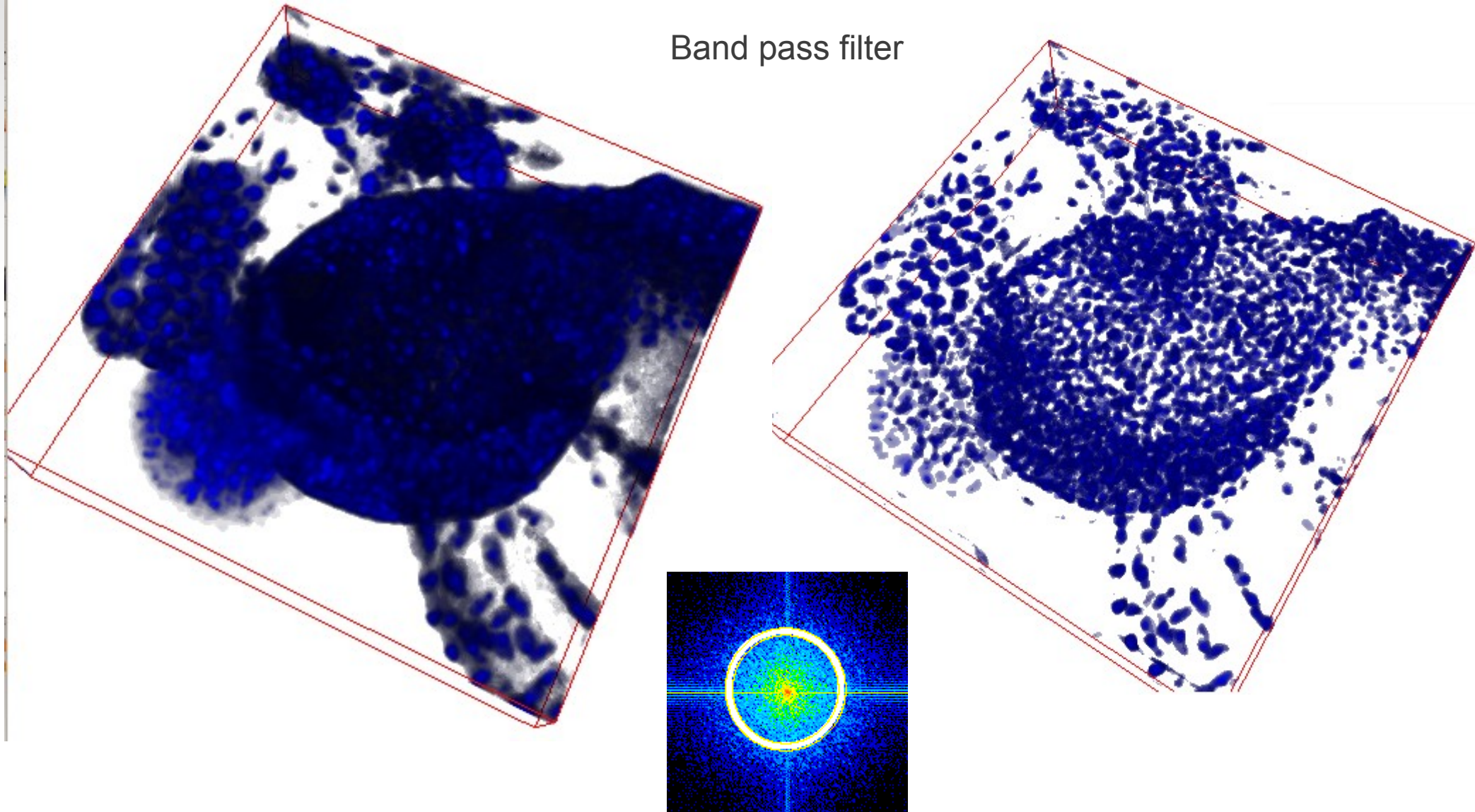
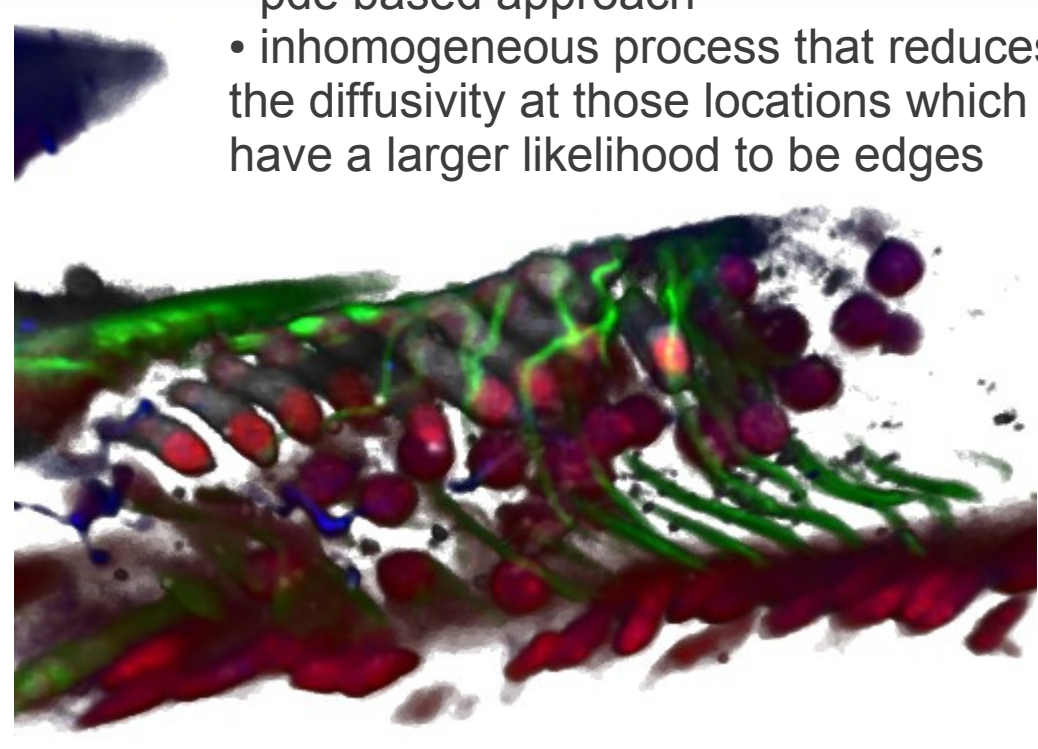
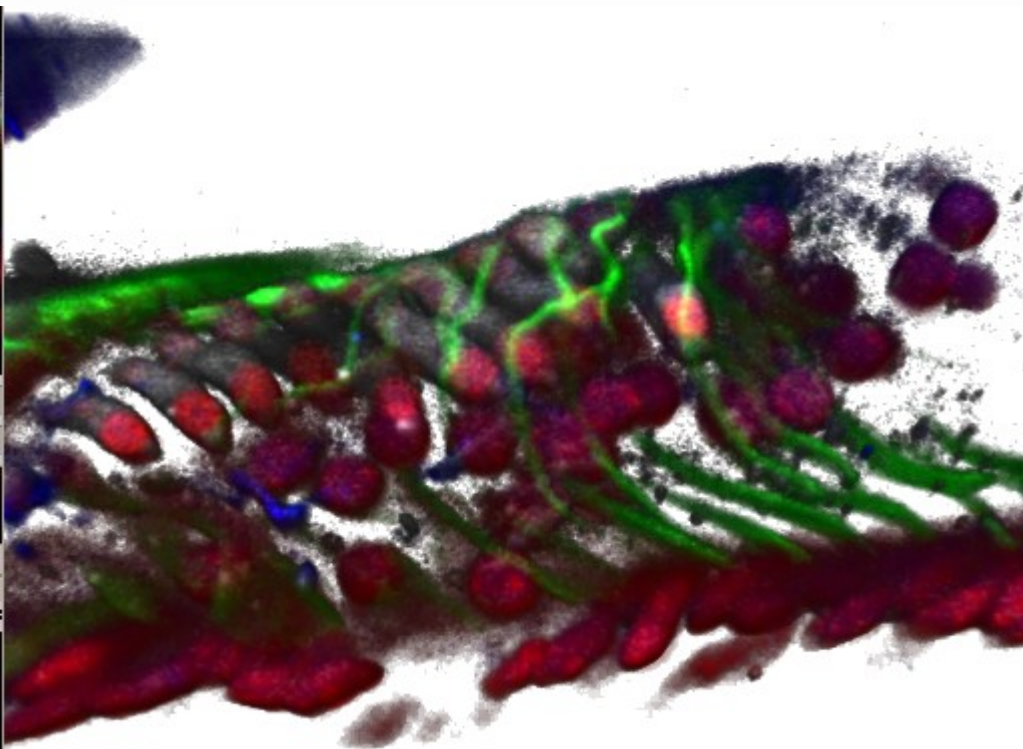


Image Restoration

- Image degraded
 - Noise
 - quantum nature of light (poisson distribution)
 - imperfect electronics (gaussian distribution)
 - Background
 - imperfect illumination
 - Blur
 - out of focus light

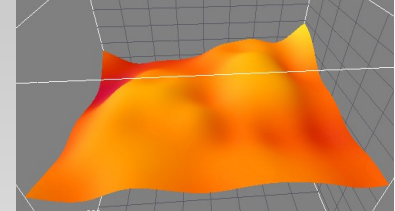
Image Restoration – Noise reduction

- Mean Filter, Gaussian filter, Median filter
- Nonlinear diffusion filtering (anisotropic diffusion, Perona–Malik)
 - Smooth noise while keeping edges



- pde based approach
- inhomogeneous process that reduces the diffusivity at those locations which have a larger likelihood to be edges

Image Restoration - Background subtraction



- correct inhomogeneous illumination

- correct with image of background

$$\frac{I}{B} \cdot \text{mean}(B)$$

- if not available: estimate background image

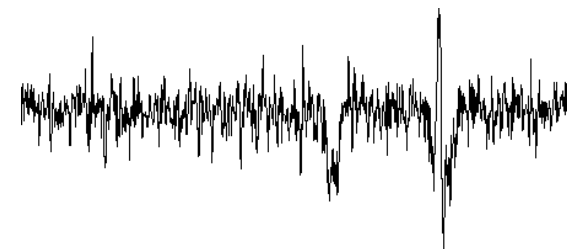
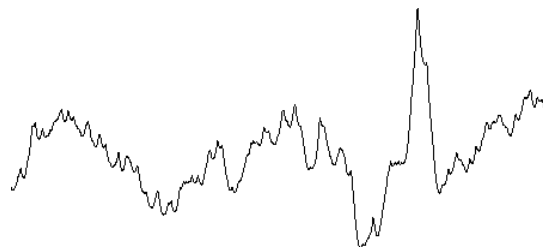
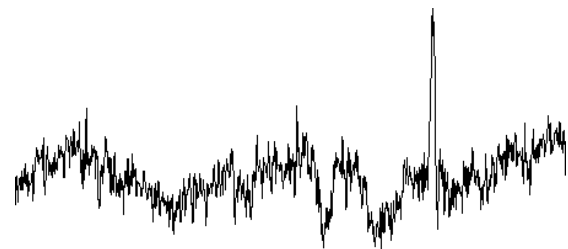
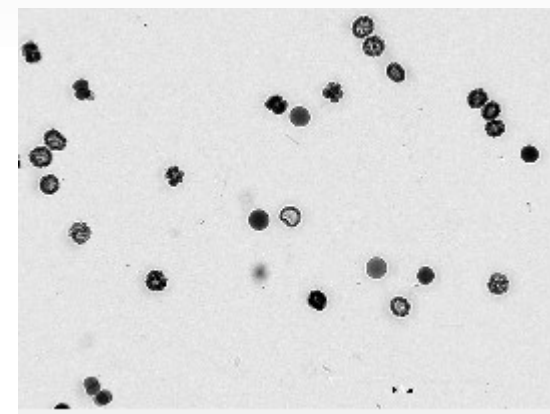
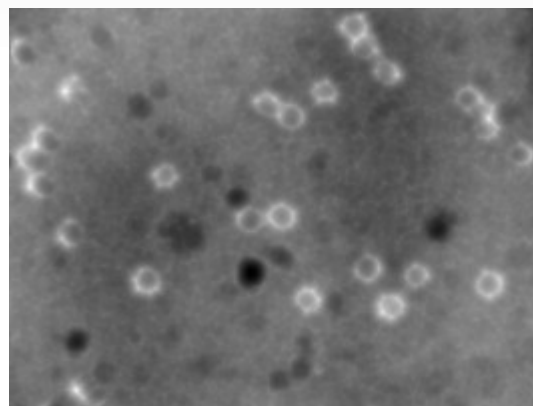
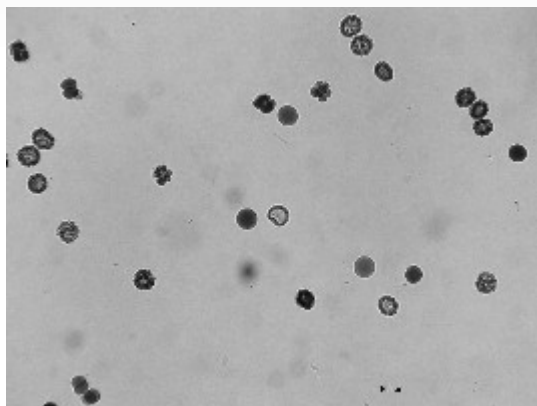
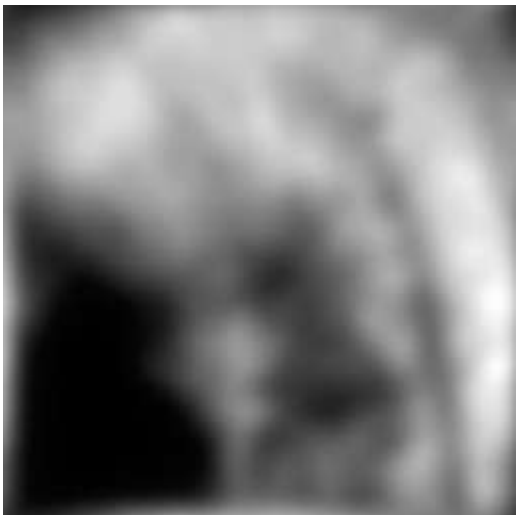
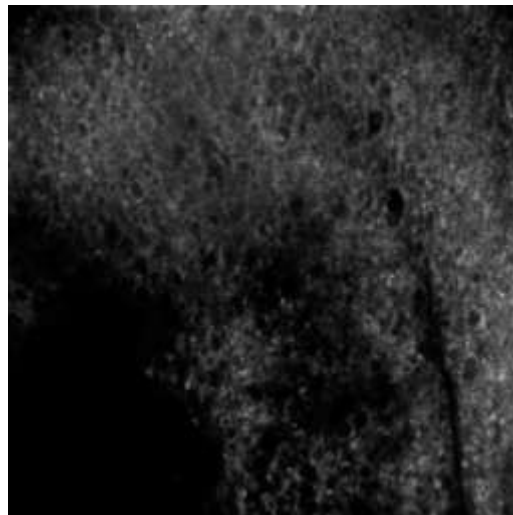


Image Restoration - Deconvolution

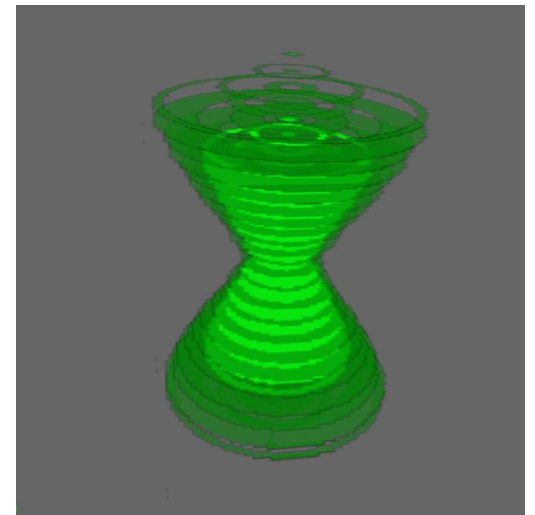
- blur
 - diffraction
 - out-of-focus light
- acquired image = object function **convolved with** psf



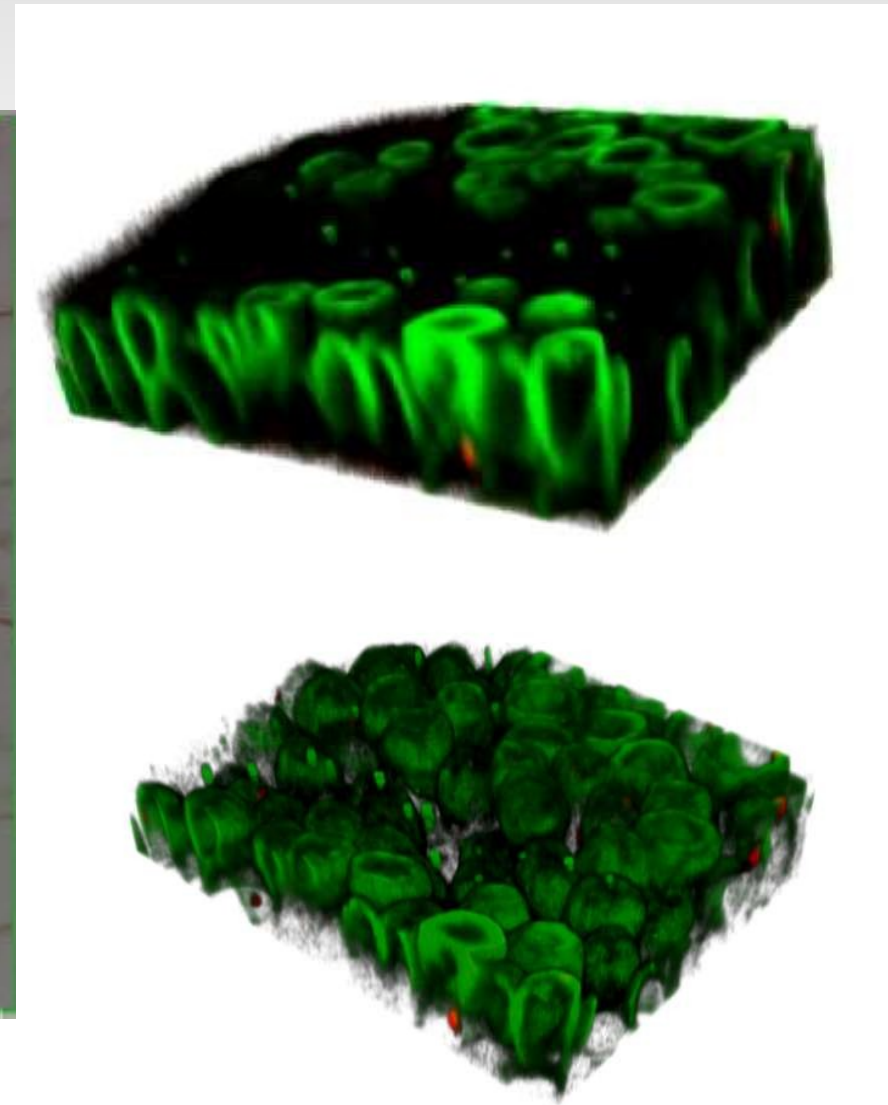
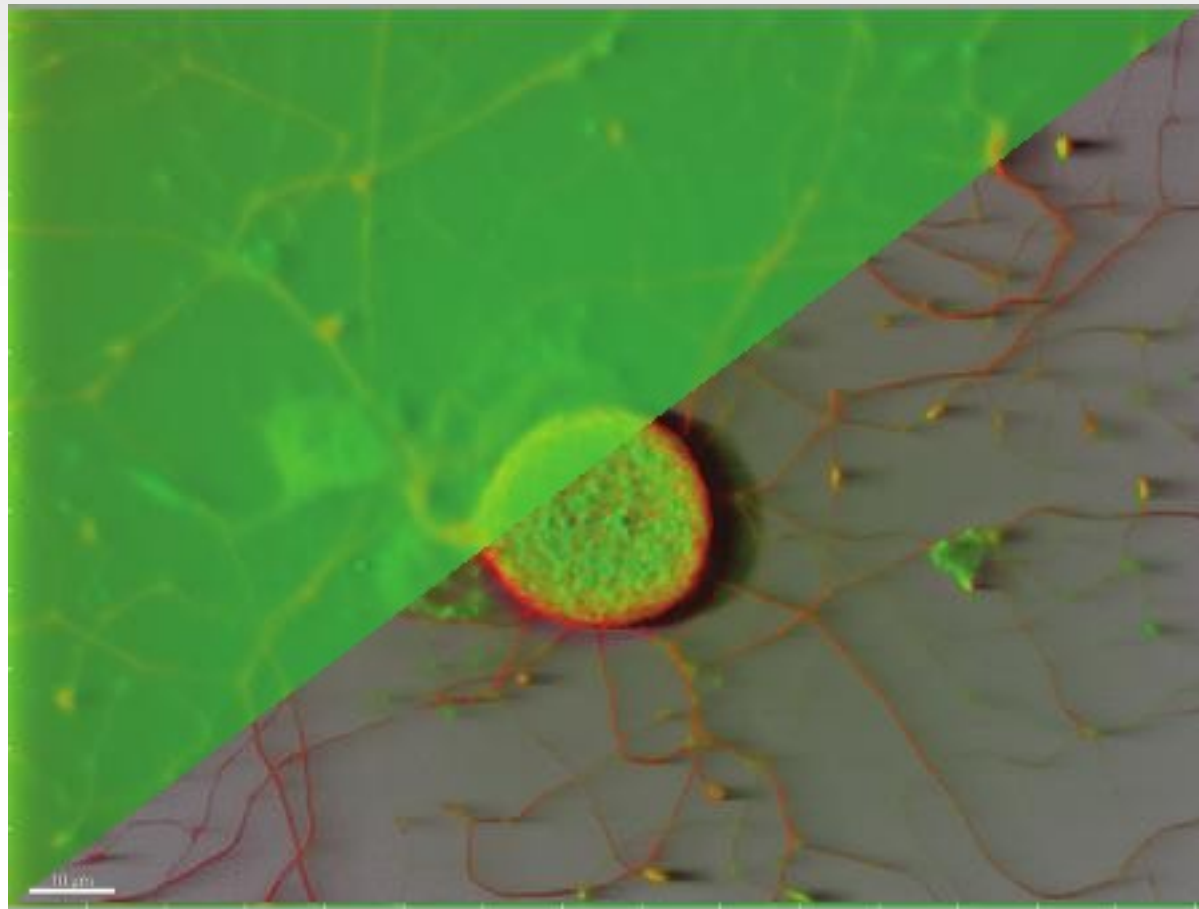
=



*



Deconvolution - examples

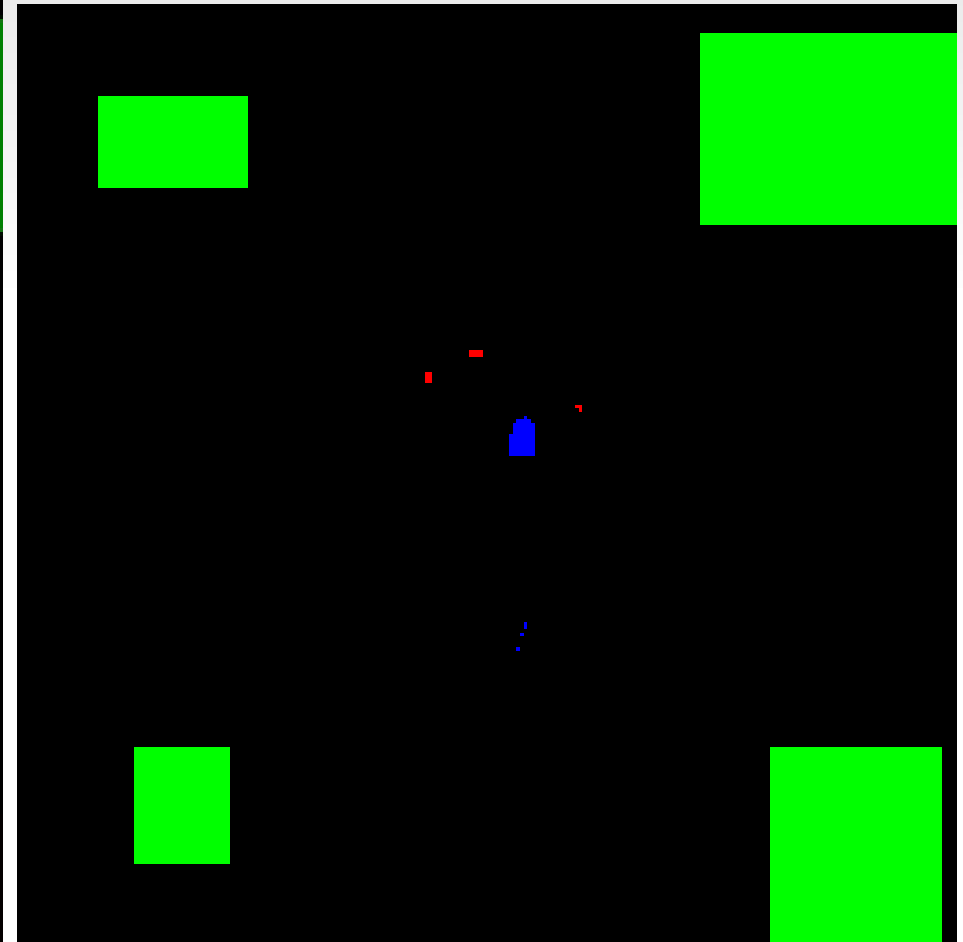
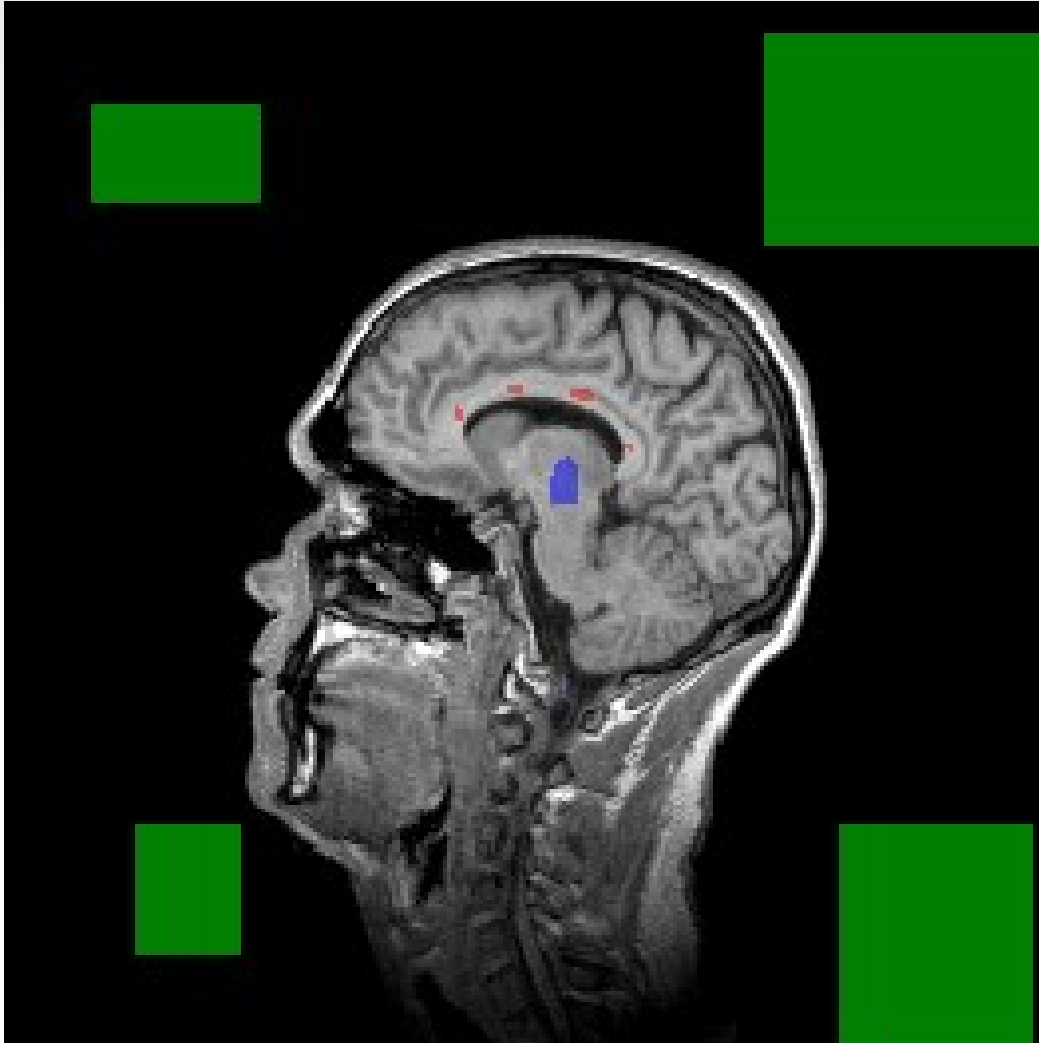


Segmentation

- separate objects from background and objects from each other
 - region growing
 - *clustering*
 - watershed transform

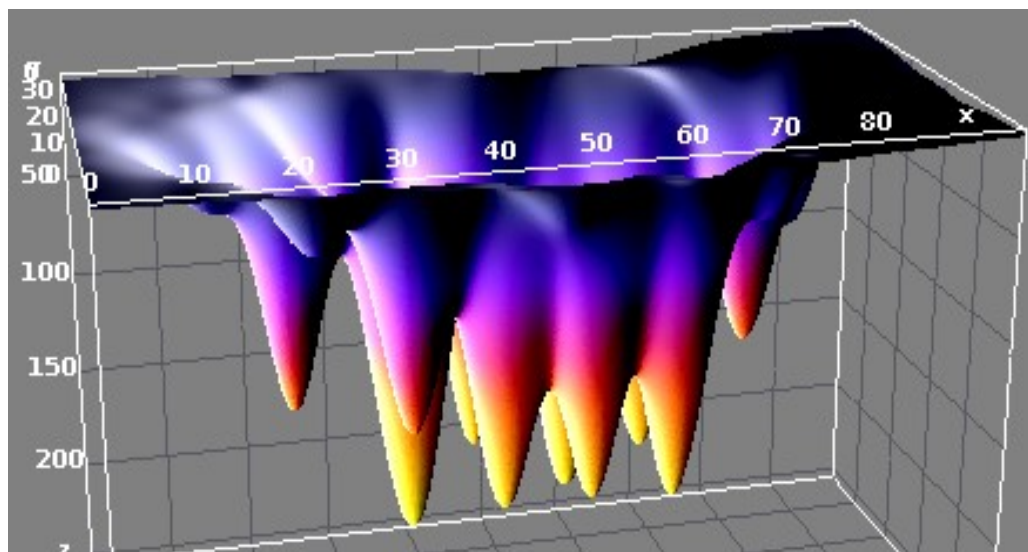
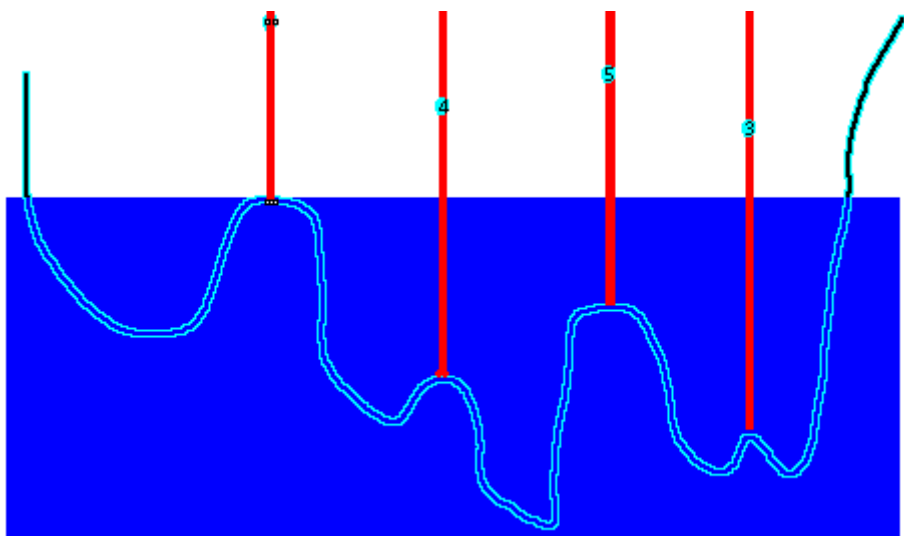
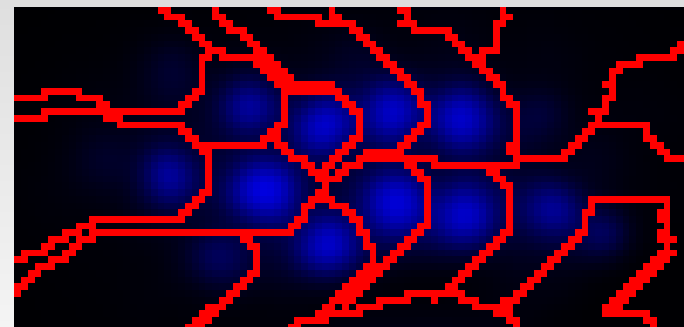
Segmentation - region growing

- start from seed-points
- simultaneously grow regions
- stop according to a homogeneity criterium



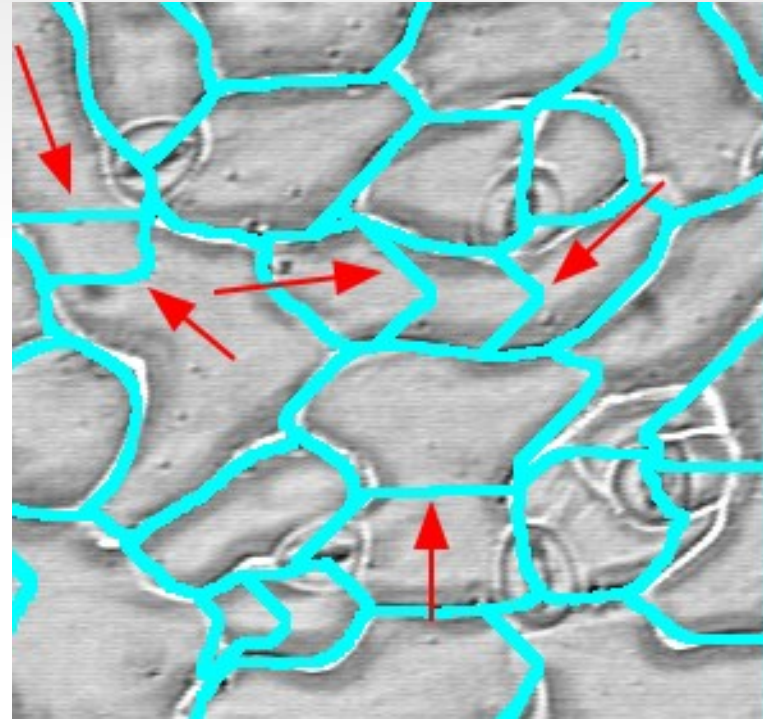
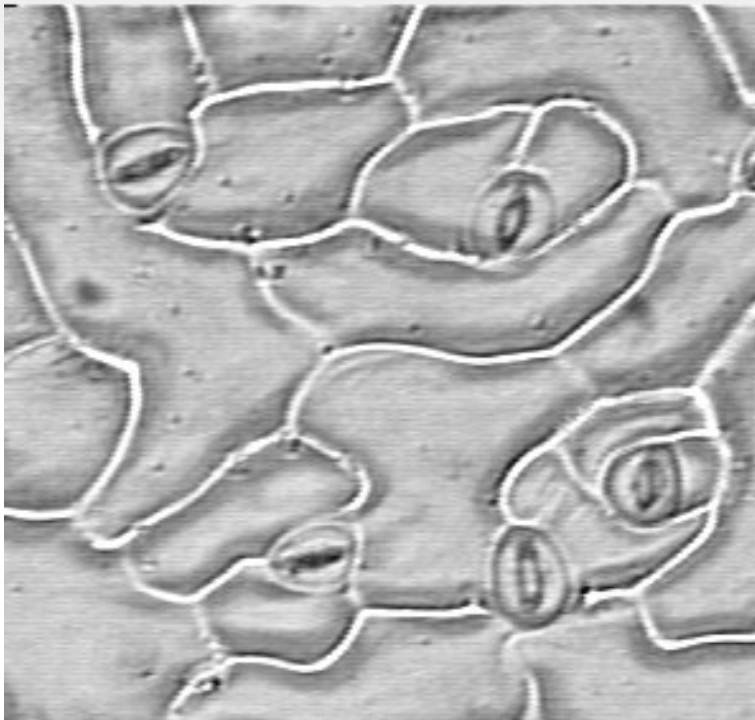
Segmentation - Watershed

- interpret intensity as valleys
- fill slowly with rising water
- whenever two basins join create a separation



Segmentation - Watershed

- problem: over-segmentation



possible solution: seeded watershed

- number of final basins = number of seeds

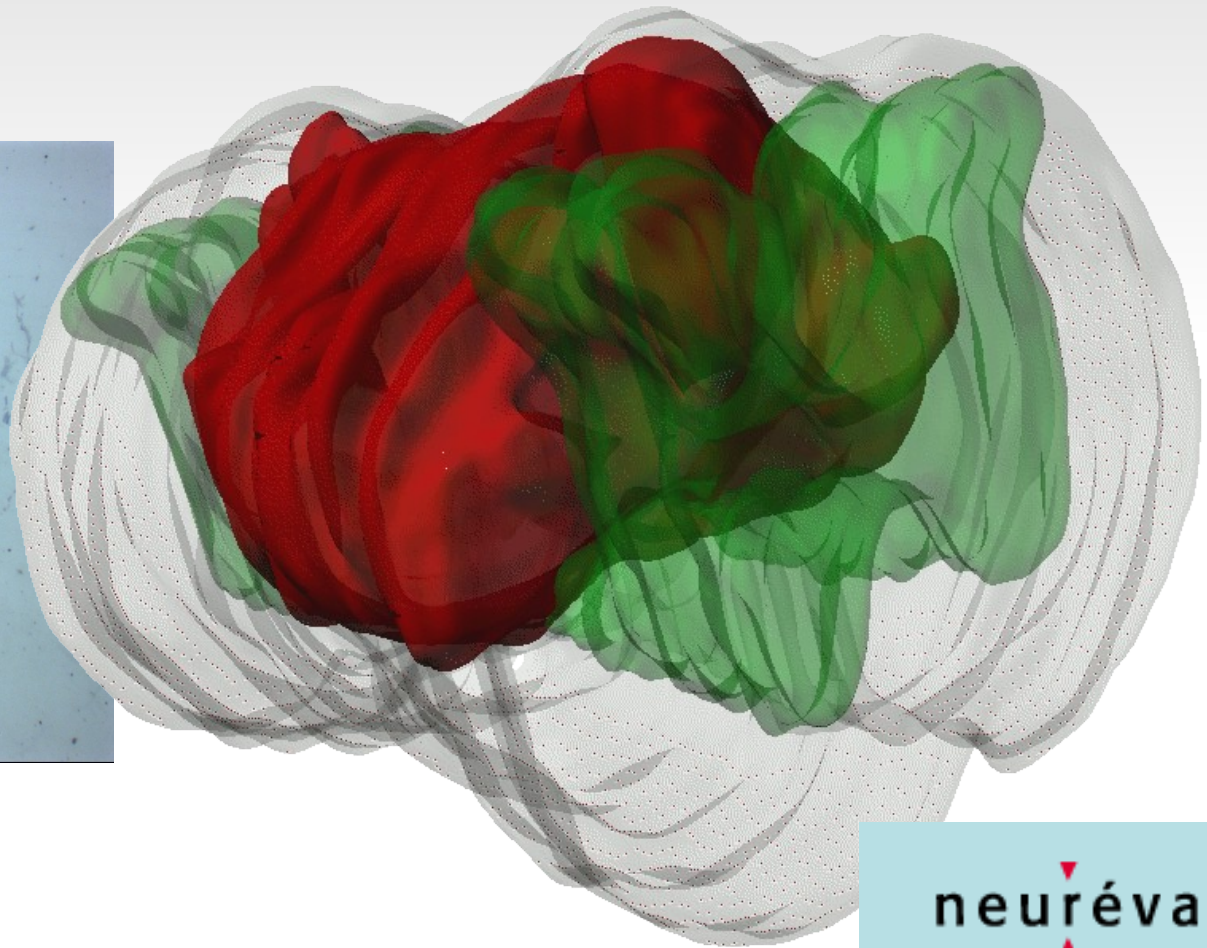
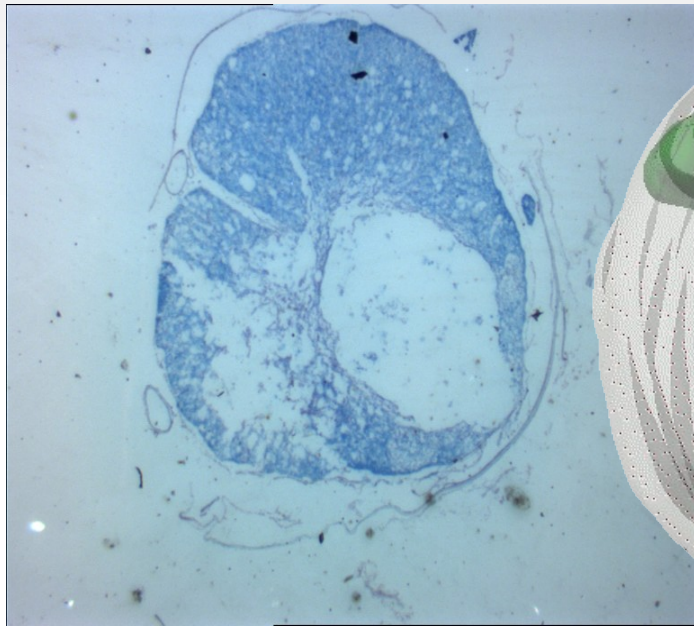
Geometrical Transformation

- problem:
 - image is spatially distorted or
 - mismatch between channels due to chromatic aberration
 - barrel distortion or pincushion distortion
 - specimen moved during acquisition
 - lacks spatial correspondence
 - histological slices
 - combining images from different sources
 - stitching of images of a mosaic
- solution:
 - image registration or alignment

Image Registration

- Image registration
 - coordinate transformation
 - landmark based
 - manually selected
 - automatically extracted
 - intensity based
 - calculate match between images
 - possible transformations
 - rigid, affine, curved
 - resampling
 - interpolation
 - nearest neighbor, linear, cubic spline

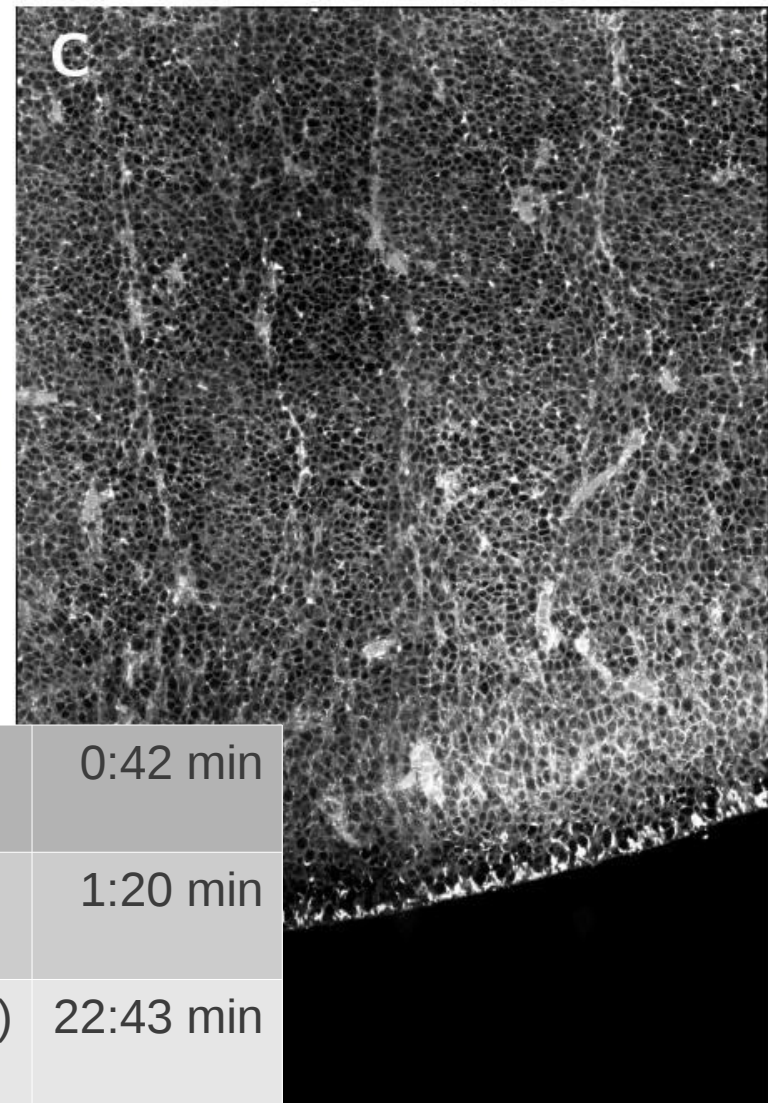
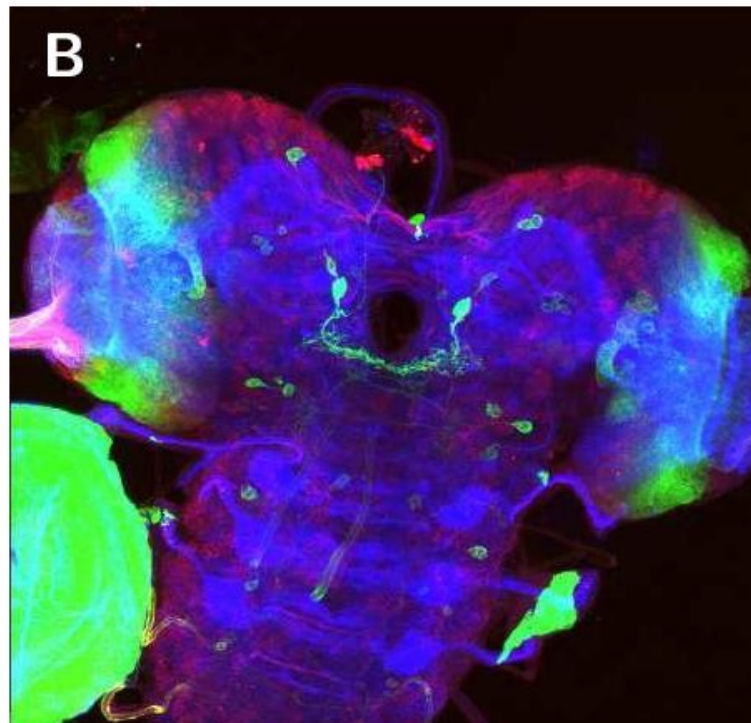
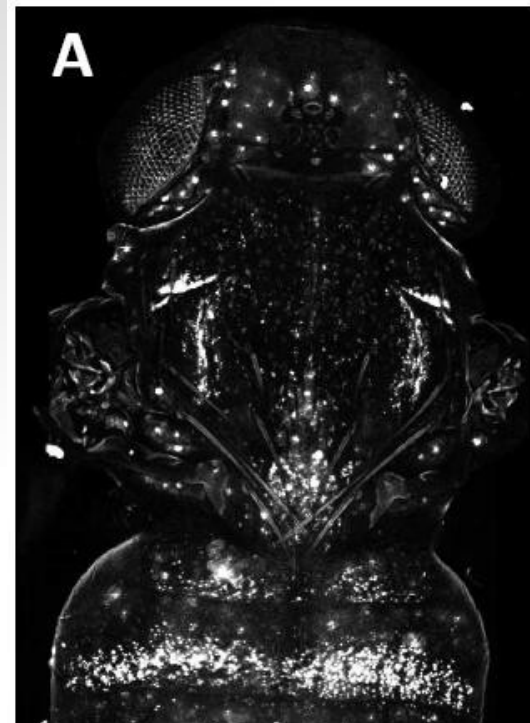
Example Registration



spinal cord
grey matter
traumatic lesion

neuréva

Example Stitching

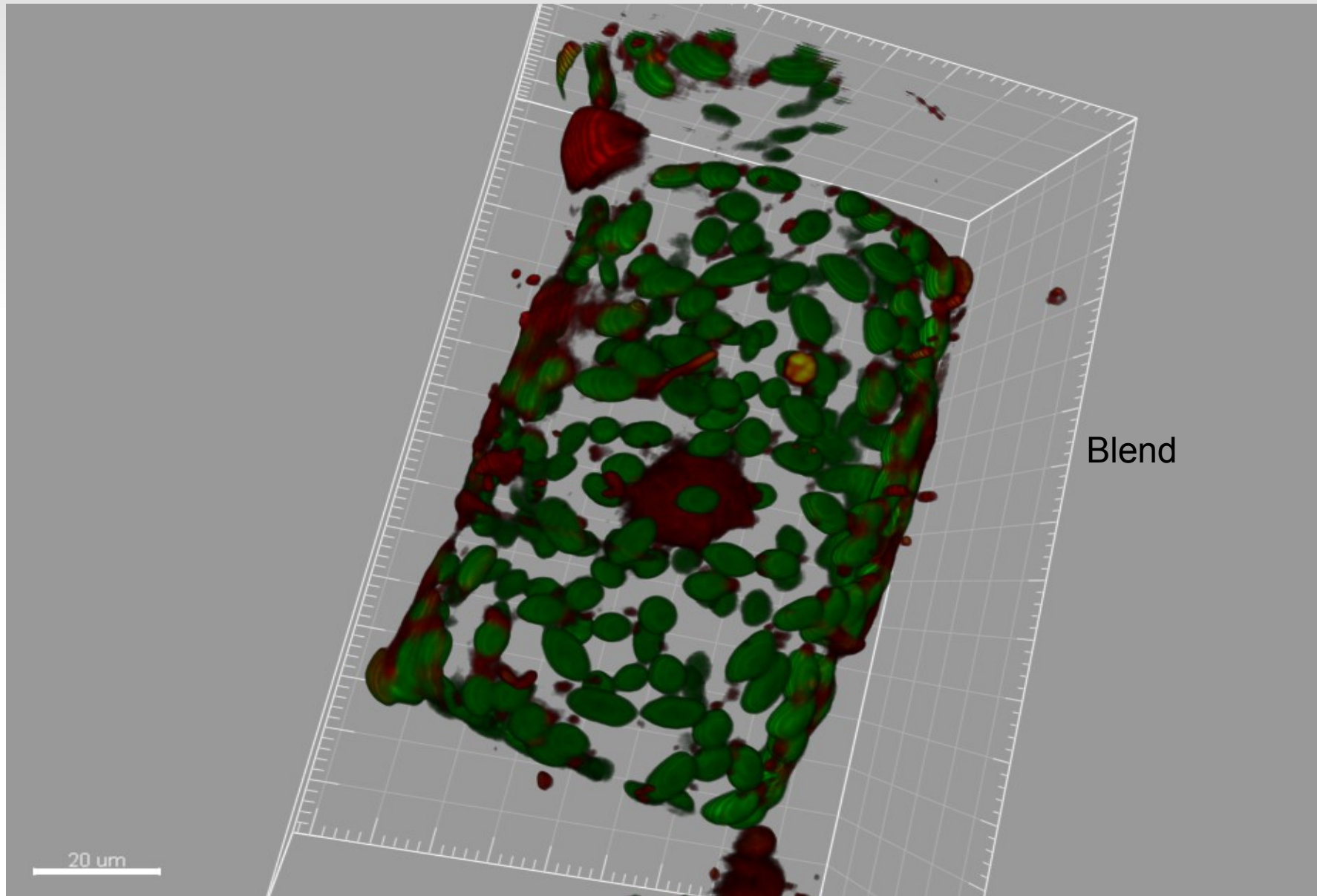


3	1024×1024×42	1097×2345×43	108 MB (8 Bit)	0:42 min
6	512×512×86	975×1425×86	350 MB (RGB)	1:20 min
24	1024×1024×68	3570×5211×70	1200 MB (8 Bit)	22:43 min

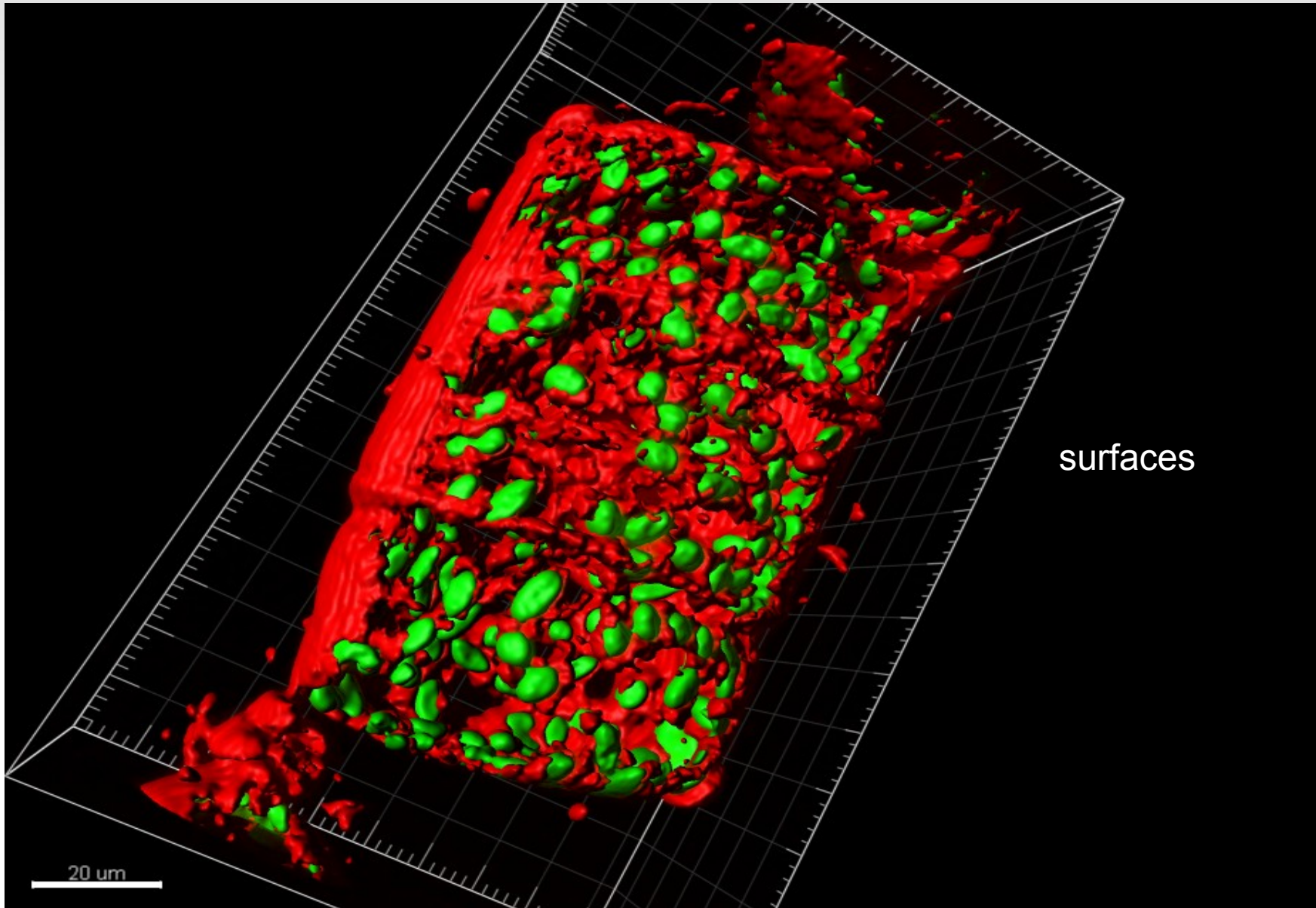
Visualization

- how to understand multidimensional data?
 - reduce dimensionality in a sensible way
- methods
 - volume rendering
 - methods that use the raw data directly without geometrical representation
 - ray tracing
 - maximum intensity projection (MIP)
 - blend (calculated from all information along the ray)
 - surface rendering
 - take into account only surfaces of objects
 - needs a description of the object in terms of geometrical entities

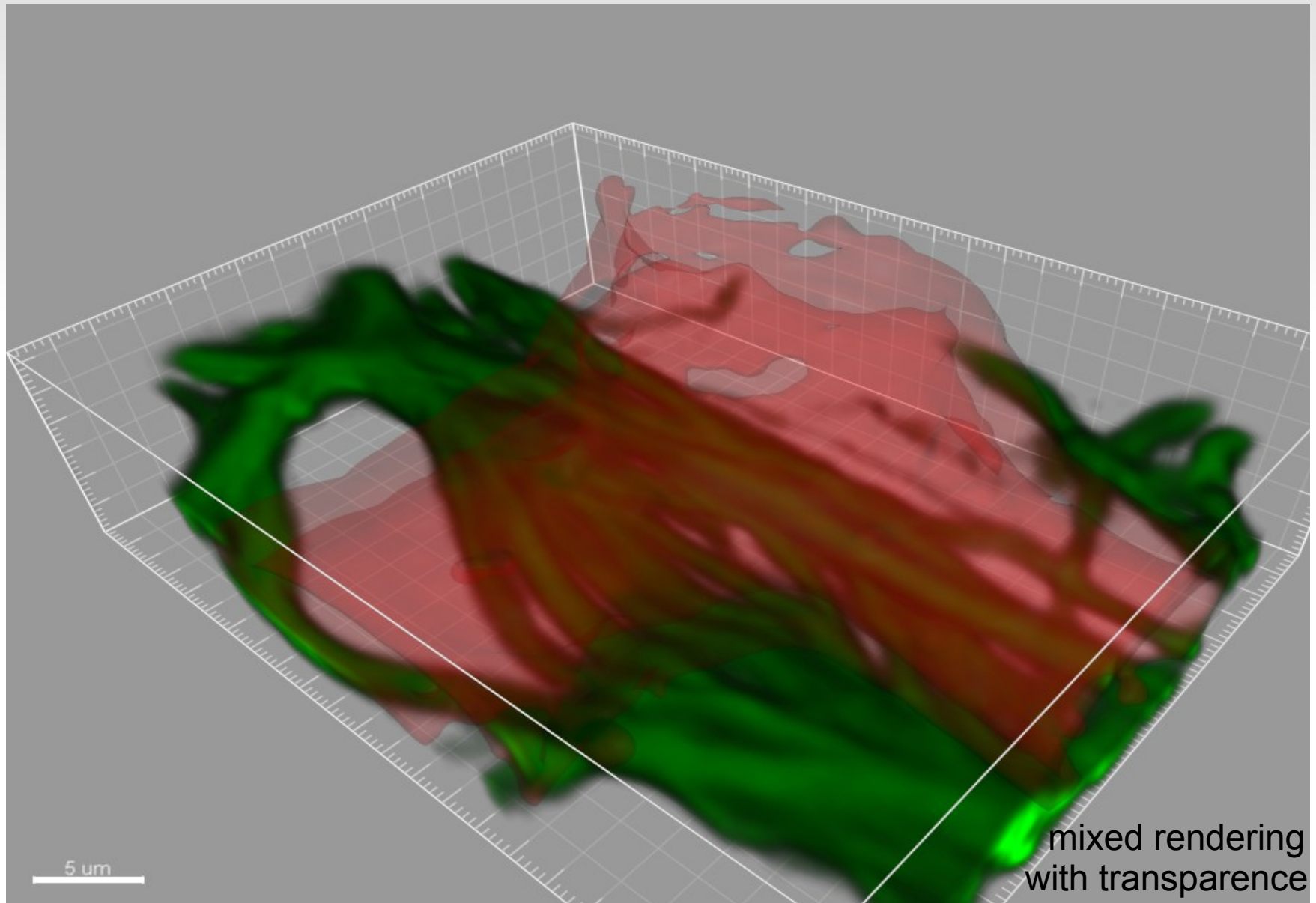
Visualization - Volume Rendering



Visualization - Surface Rendering



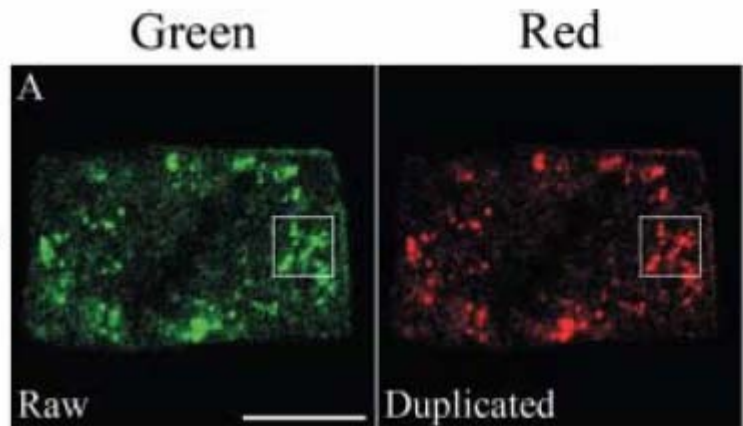
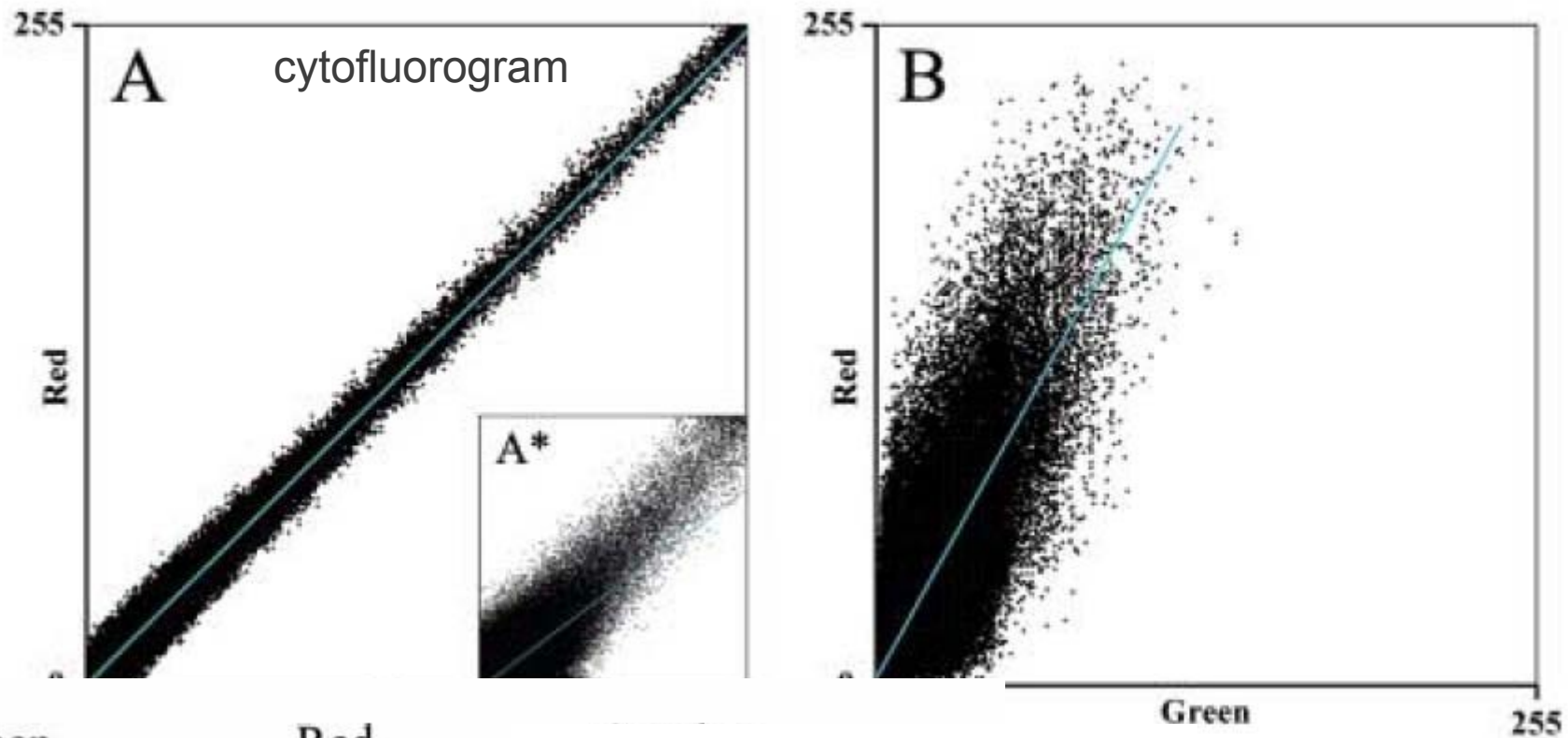
Visualization - Mixed Rendering



Colocalization Analysis

- Wikipedia:
"colocalization refers to observation of the spatial overlap between two (or more) different fluorescent labels, each having a separate emission wavelength, to see if the different "targets" are located in the same area of the cell or very near to one another. "
- "correlation, ... indicative of a biological interaction"

Colocalization Analysis

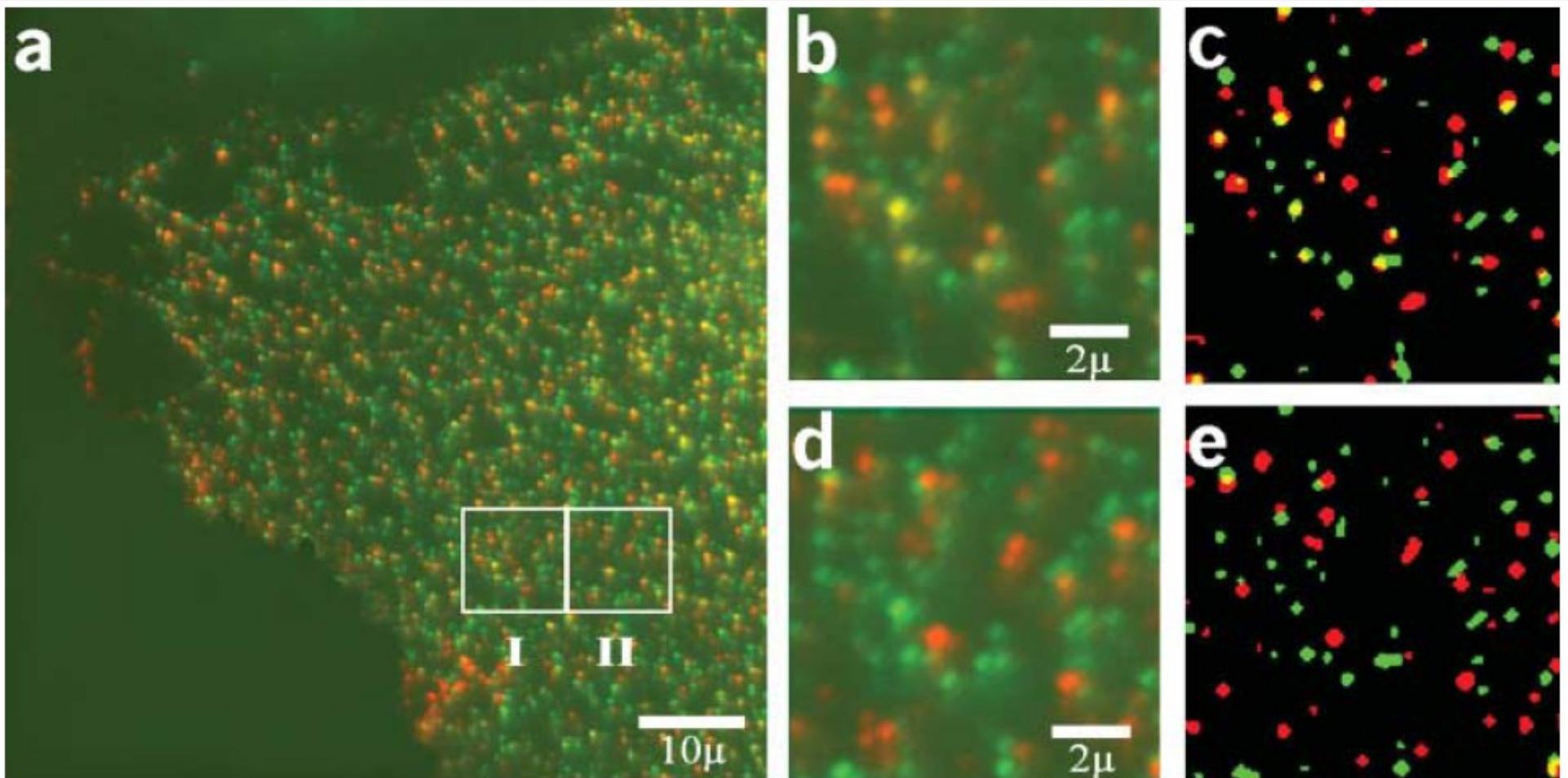


Pearson's Correlation Coefficient

$$r_p = \frac{\sum_i (A_i - a) \times (B_i - b)}{\sqrt{\sum_i (A_i - a)^2 \times \sum_i (B_i - b)^2}}$$

Colocalization Analysis

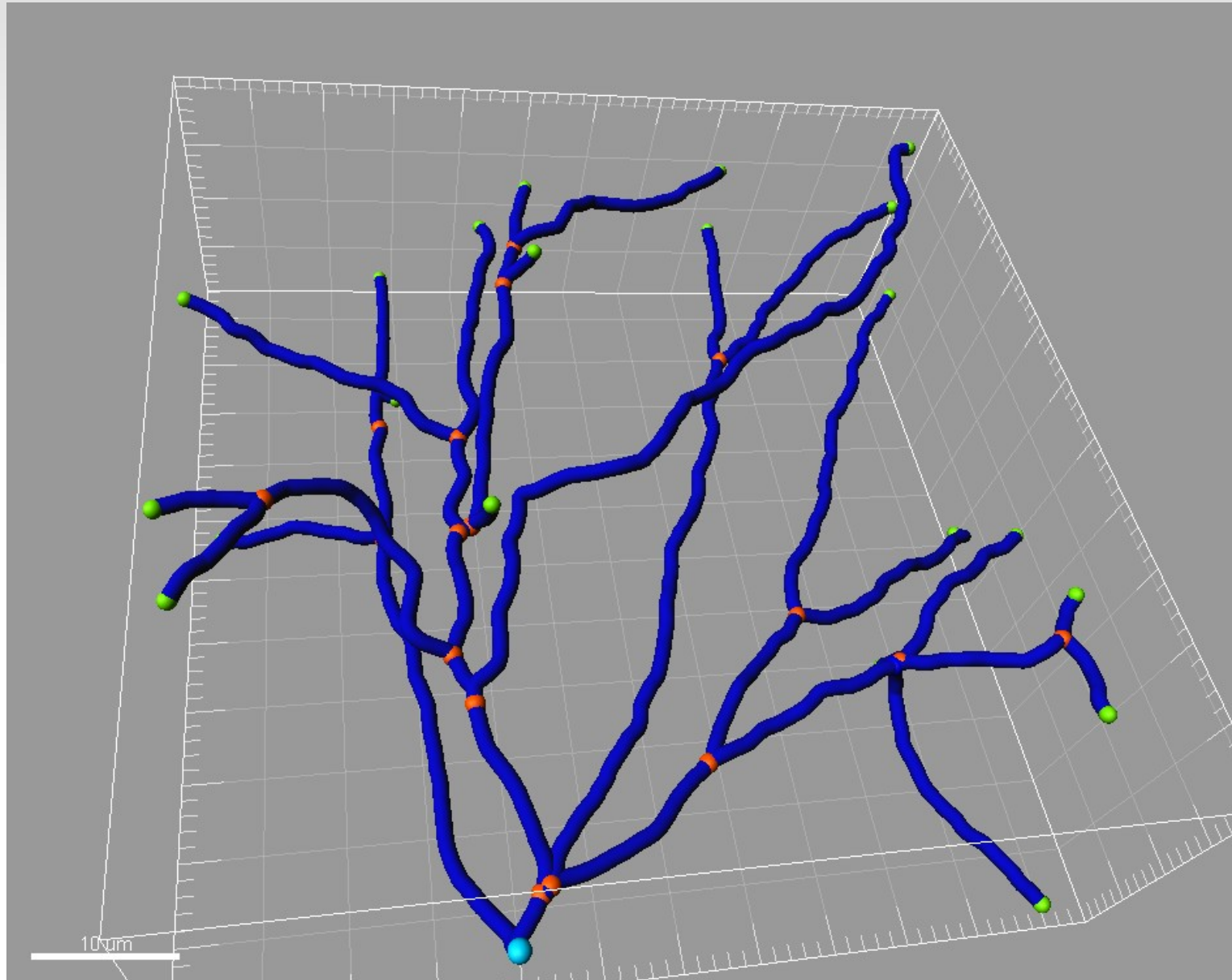
- object based colocalization
- test if distance between centroids is at resolution limit



Filament tracing and analysis

- possible approach
 - second order derivatives (hessian matrix)
 - cost image
 - shortest paths
- automatic or semi-interactive
- spine detection

Filament tracing and analysis



Particle detection and tracking

- 2 steps
- detection of particles (spots) per time-frame
 - least-squares fitting of a gaussian mixture model to the image data
- linking of particles in successive frames
 - problem: number not constant over time

Particle detection and tracking



Cell segmentation and tracking

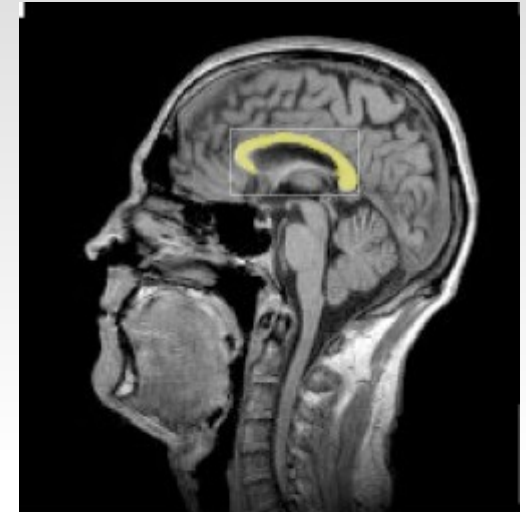
- cells have a distinct shape
- shape may change over time
- use active contours (snakes) to detect cells
 - active surfaces in 3D
 - shape constraint fitting to image data
- tracking
 - use contour of cell at $t=n$
as initial contour for cell at $t=n+1$

Cell segmentation and tracking

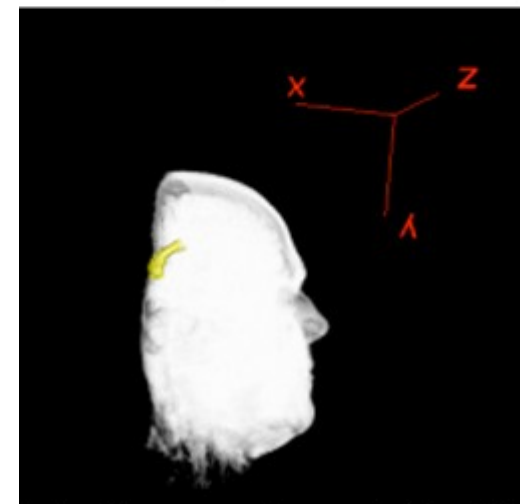


Software Tools

- Imaris (bitplane)
- Volocity (PerkinElmer)
- Avizo (vsg)
- FIJI (open source)
- ImageJ (open source)
- Matlab (MathWorks),
Octave
- huygens (svi)
 - hrm (open source)



*Segmentation on a 2d slice of
the Corpus callosum*



*The Corpus callosum in the 3D
Viewer*

Thank you

- Questions?

