Cancéropôle Grand Sud-Ouest
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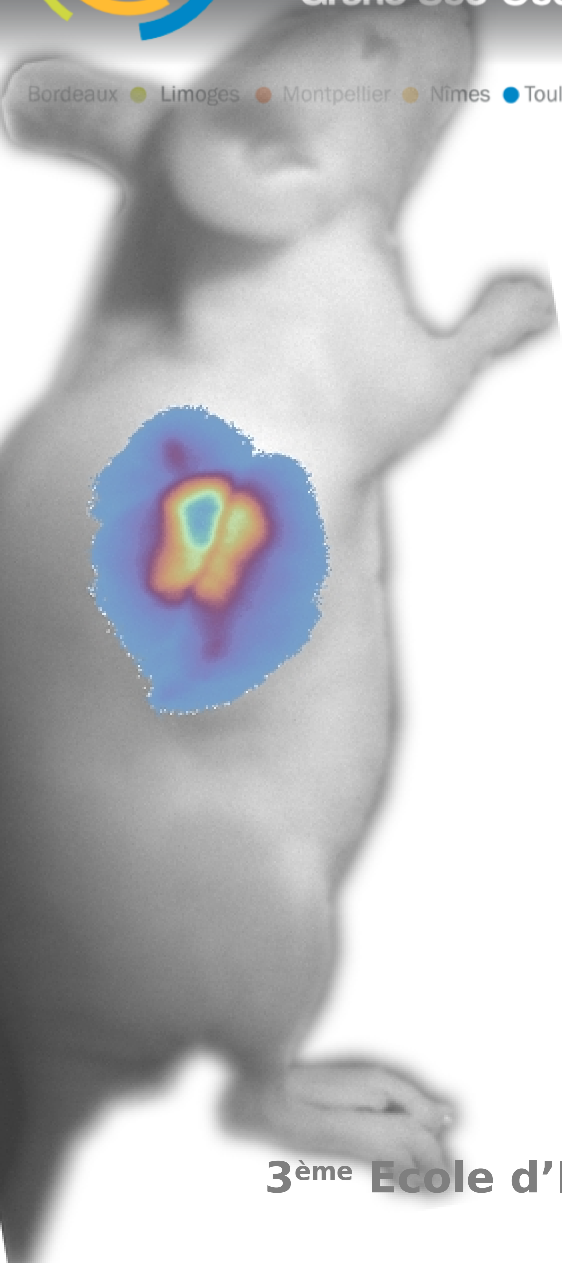
BioCampus Montpellier

IPAM
IMAGERIE DU PETIT ANIMAL DE MONTPELLIER



BIOLOGICAL IMAGE ANALYSIS
INTRODUCTION AND OVERVIEW

Volker Baecker

Montpellier RIO Imaging - TIGR
Biocampus Montpellier

volker.baecker@mri.cnrs.fr

3ème Ecole d'Imagerie du petit animal appliquée au cancer
27-30 Mai 2013 - Montpellier

OVERVIEW

1. Digital image
2. Basic Image Analysis
3. Advanced Image Analysis and Applications
4. Software Tools

WHAT IS A DIGITAL IMAGE? – EXAMPLES

Confocal scan of a Drosophila brain (ImageJ)

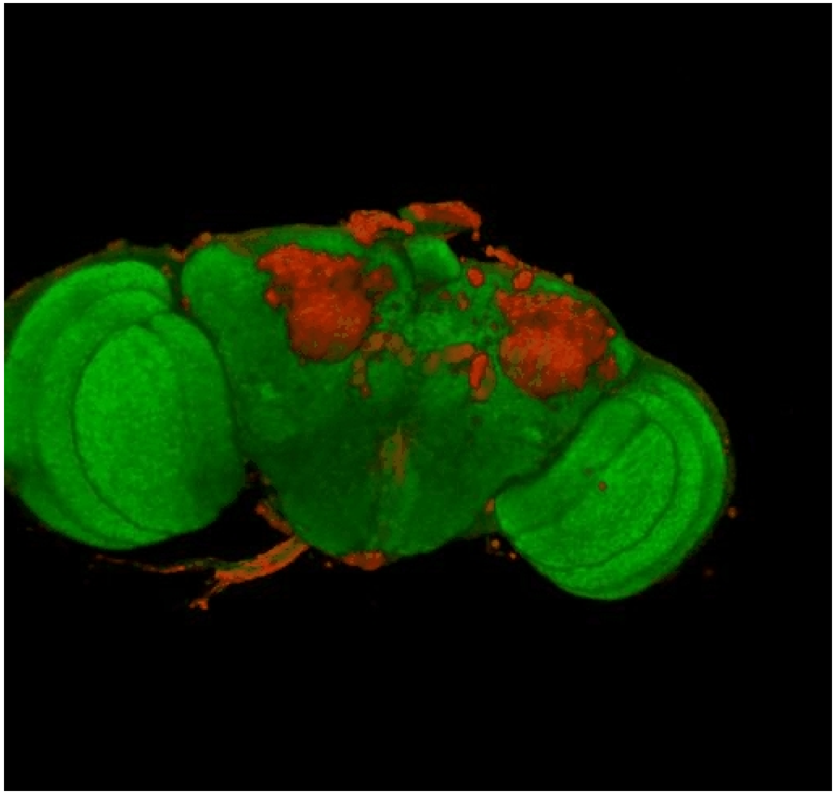


Image: Benjamin Schmid, Heisenberg lab in Wuerzburg

Embryonic heart of a 3 day old zebrafish (Imaris – Bitplane)

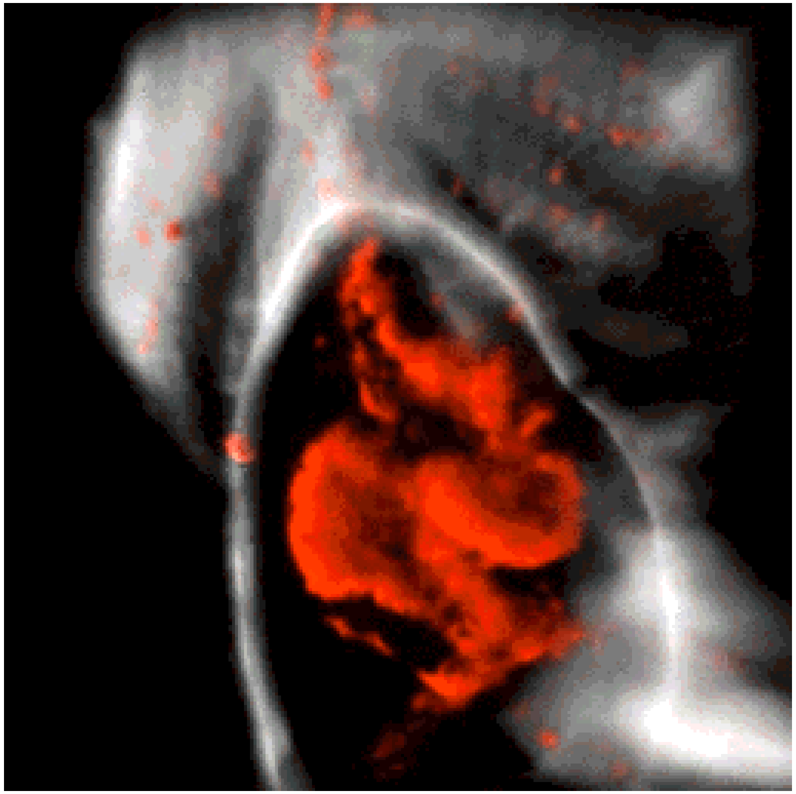


Image: Michael Liebling and Arian Forouhar, Caltech

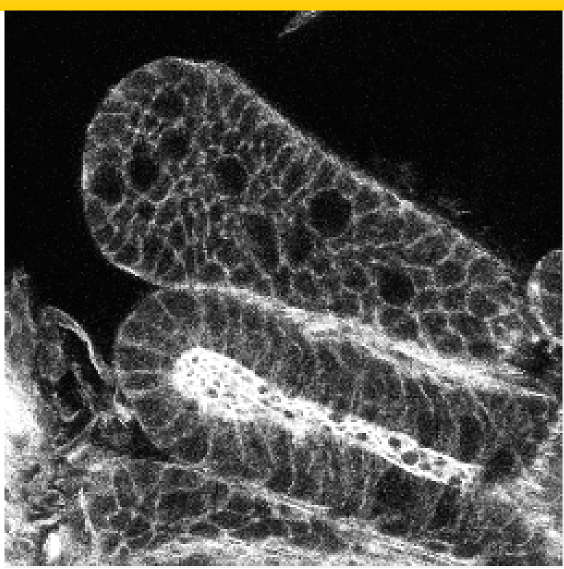
WHAT IS A DIGITAL IMAGE? – MATHEMATICAL POINT OF VIEW

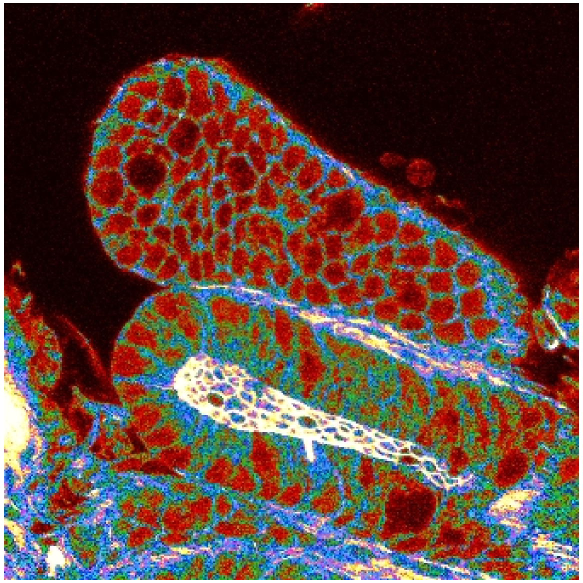
- matrix of sample values
  - finite number of samples
  - finite number of values per sample
- image dimensions
  - 1D, 2D, 2D+t, 3D, 3D+t, 3D+t+multispectral
  - $I_{(x,y,z,t)} \in W^n$

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

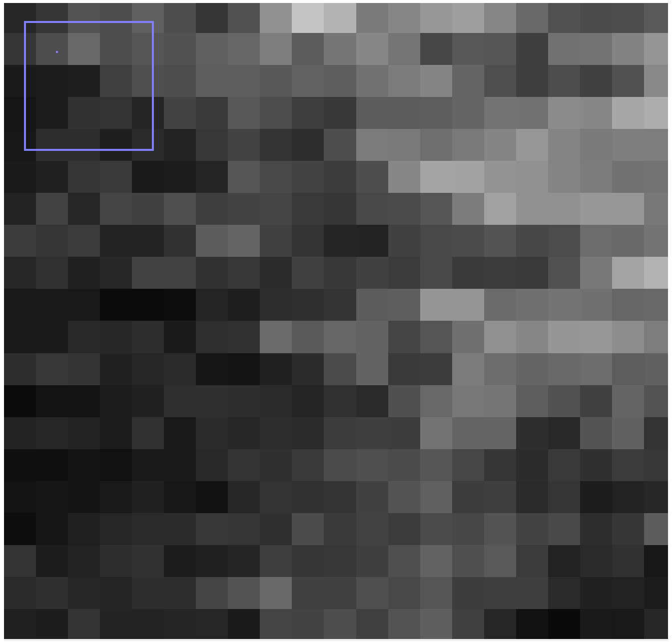




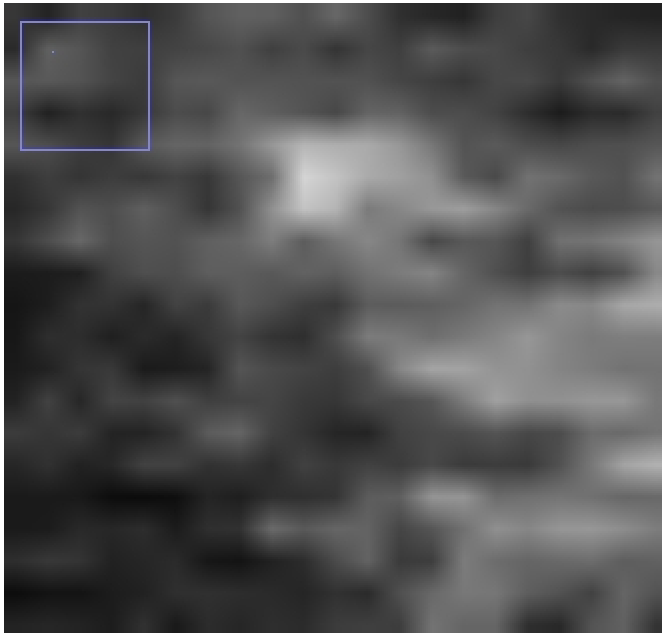
When I use a word," Humpty Dumpty said, in rather a scornful tone, "it means just what I choose it to mean—neither more nor less.

L. Carroll, Through the Looking-Glass

WHAT IS A DIGITAL IMAGE? – DISPLAYED BY THE COMPUTER



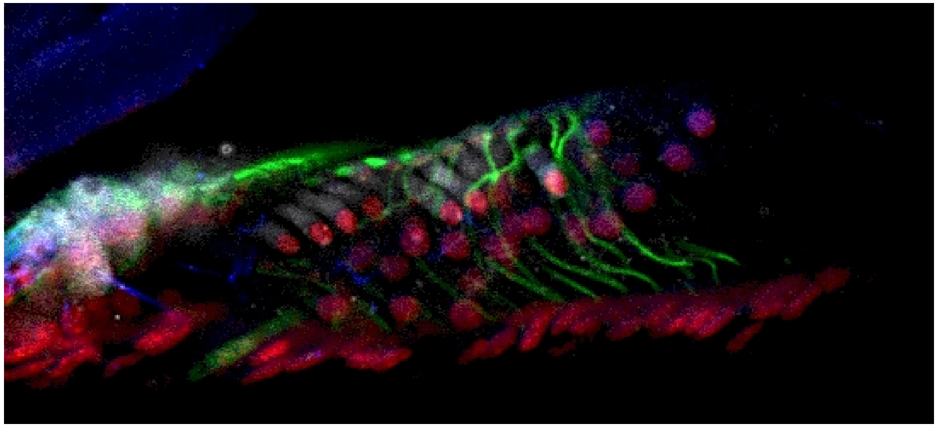
- mapping between sample grid and display grid
  - homogenous rectangles
  - interpolation

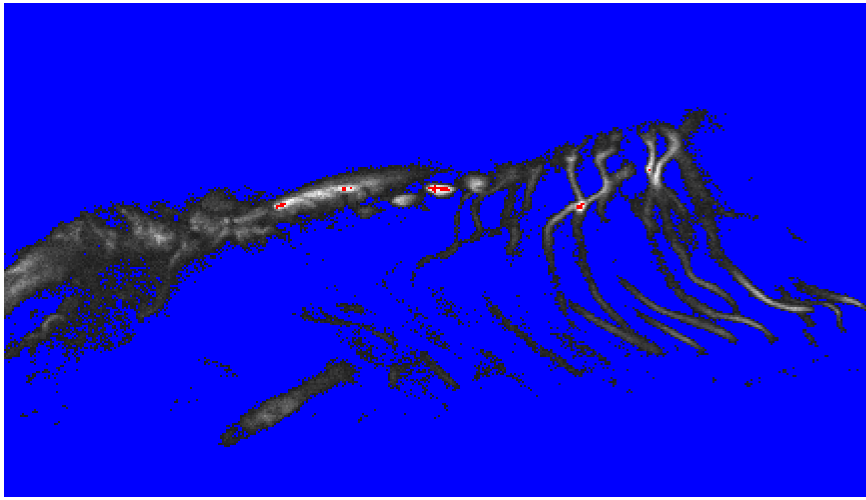


WHAT IS A DIGITAL IMAGE? – REPRESENTED IN MEMORY

Formats	Values	Interpretation
8-bit	0-255	unsigned integer
16-bit	0-65535	unsigned integer
32-bit	-3.4×10^{38} - $+3.4\times10^{38}$	6-7 decimal digits, NaN, Infinity, -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

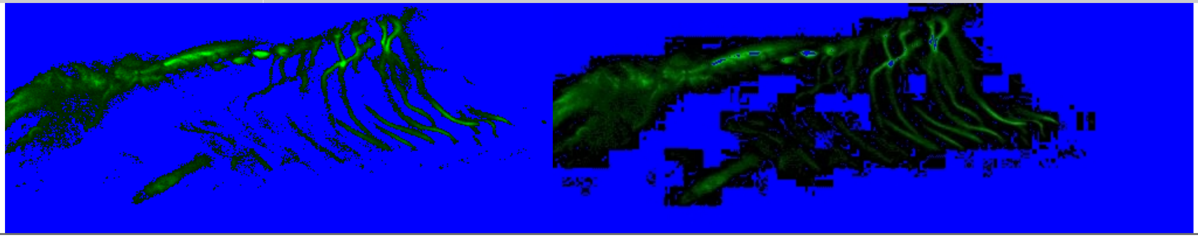
Label	Mean	Min	Max	IntDen
green	100.9	0	4095	13774198
10 x green	1009.0	0	40950	137741980
green 8bit	6.3	0	255	861340
10 x 8bit	6.3	0	255	861340

- 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

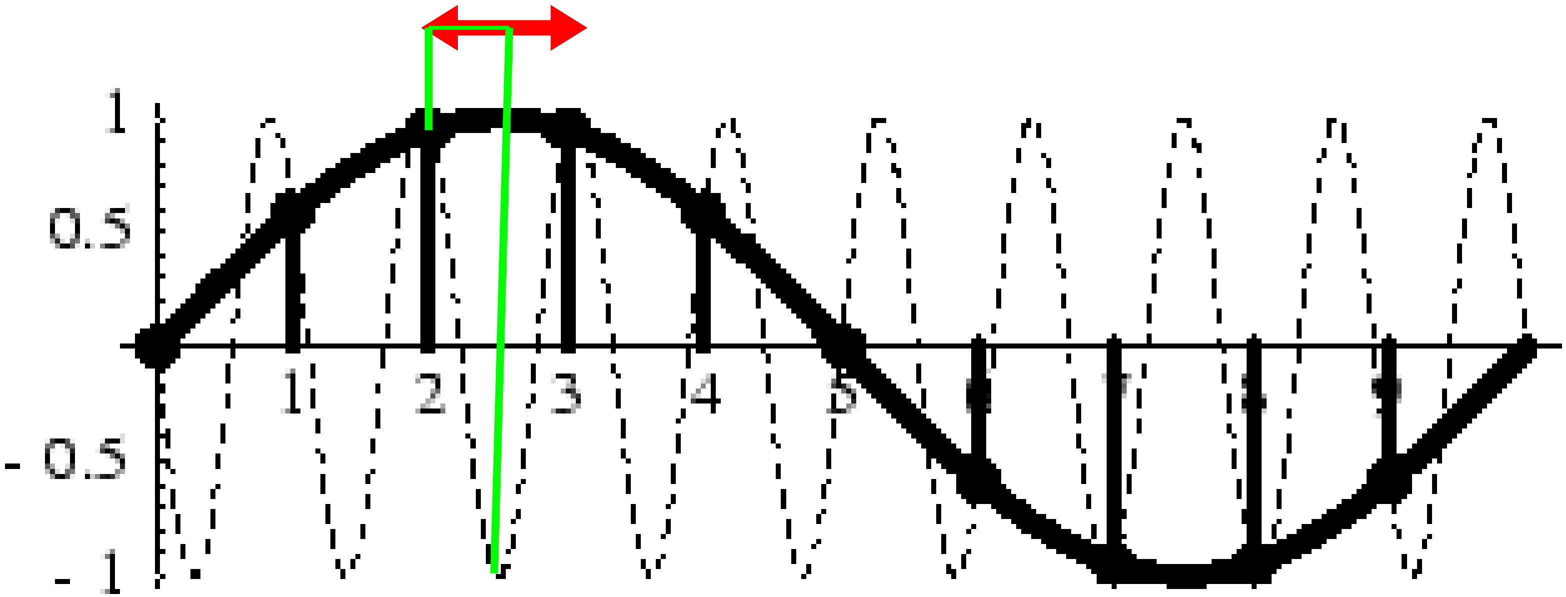


- artefacts from jpg-compression

WHAT IS A DIGITAL IMAGE? – THE IMAGE AND THE REAL WORLD

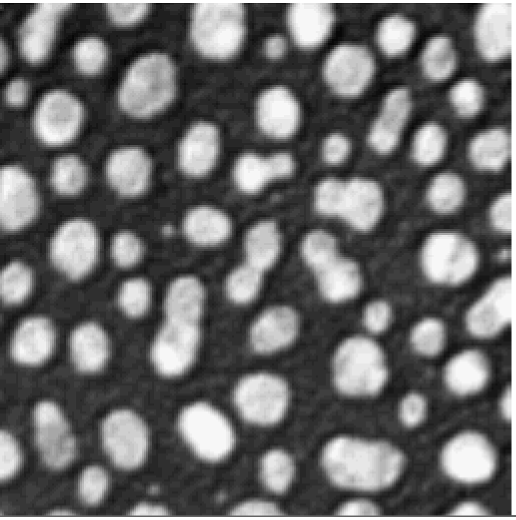
- sampling and resolution
  - digital image – finite number of samples
  - Nyquist-Shannon sampling theorem:

    “The sampling interval must be smaller than one-half the size of the smallest resolvable feature of the optical image”



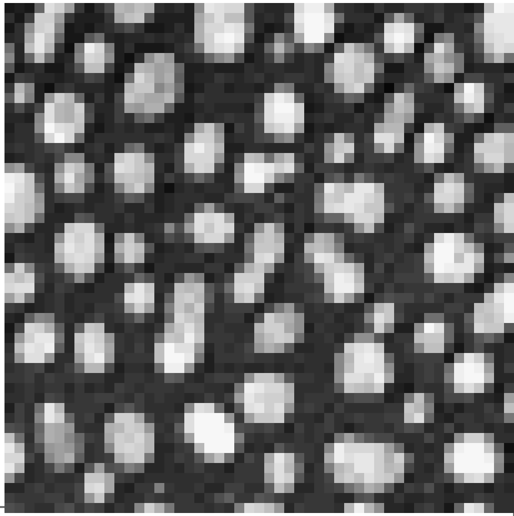
- sampling and resolution
  - resolution of an optical system
  - the smallest distance at which two objects can still be distinguished
  - given by the Rayleigh criterion
  - therefore the pixel size must be

$$r = \frac{0.61 * \lambda}{NA}$$



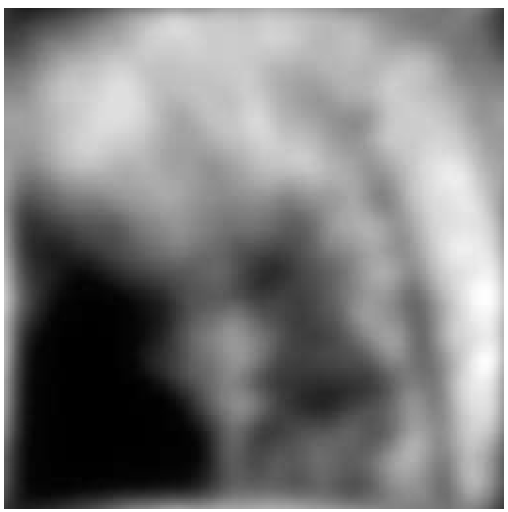
$$\Delta x < \frac{\lambda_{em}}{4 * NA}$$ for widefield

$$\Delta x < \frac{\lambda_{ex}}{8 * NA}$$ for confocal



WHAT IS A DIGITAL IMAGE? – THE IMAGE AND THE REAL WORLD

- point spread function (psf)
  - 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



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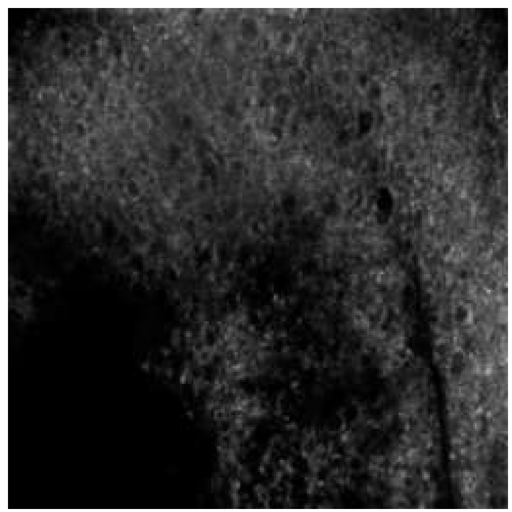

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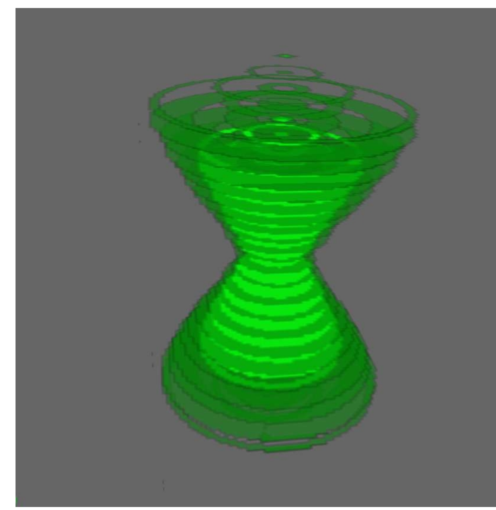
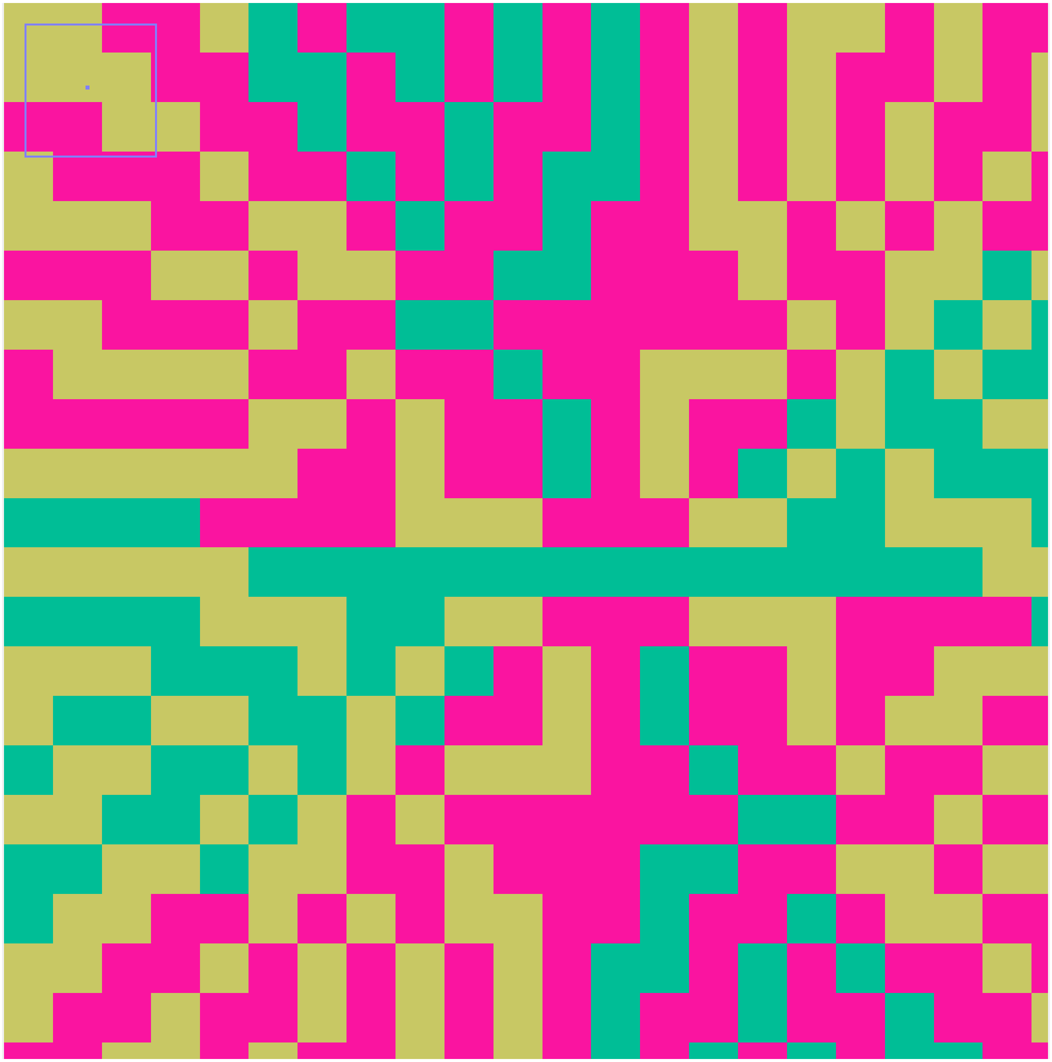


WHAT IS A DIGITAL IMAGE? – IMAGE AND PERCEPTION

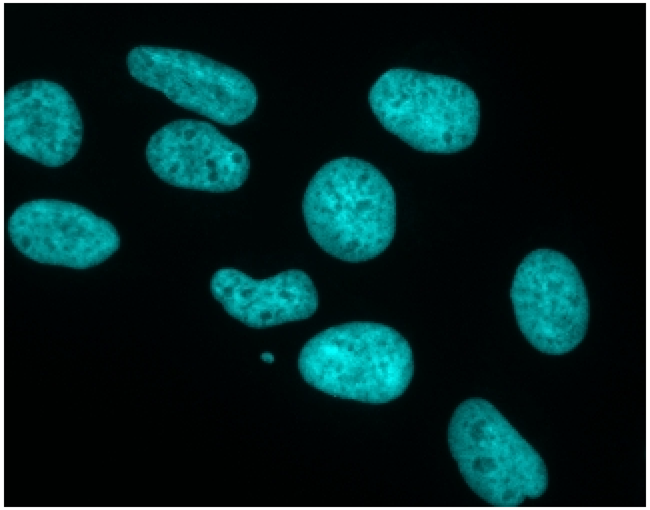
- How many colors do you see?



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

WHAT IS IMAGE ANALYSIS?

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



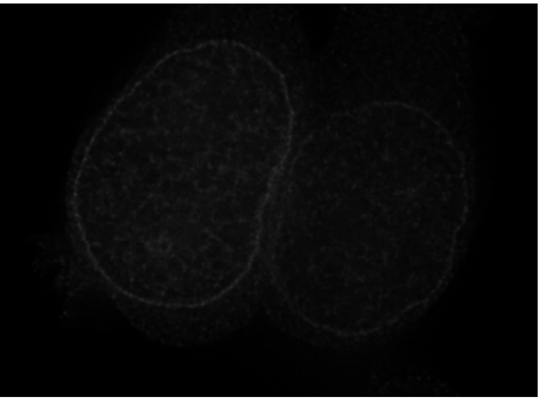
	Area	Perim.	
1	6101	353.061	
2	7047	329.120	
3	5455	292.392	
4	7524	328.191	
5	5653	300.978	
6	6178	304.392	
7	4583	296.392	
8	7312	333.120	
9	6820	343.345	

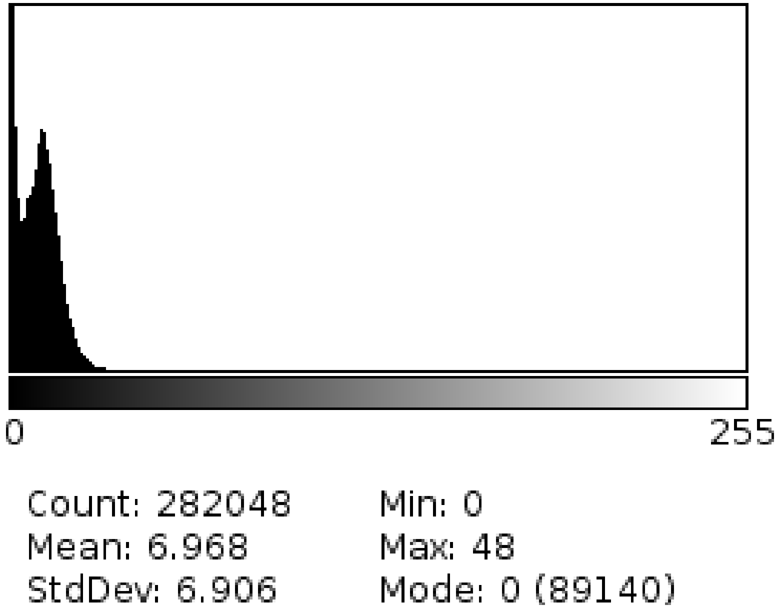
- IMAGE IN – FEATURES OUT

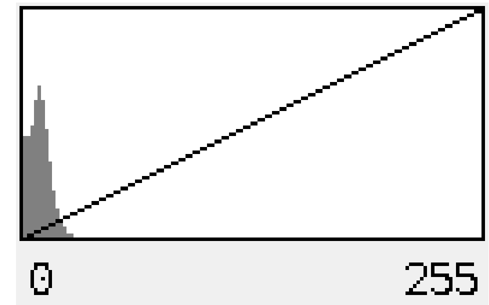
POINT OPERATIONS

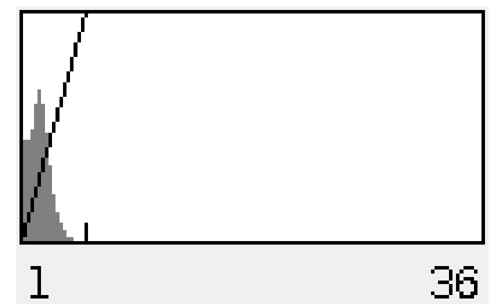
- global intensity transformations
  - intensity inversion
  - contrast and brightness adjustment
    - linear
    - gamma function
    - *histogram equalization*
  - pseudo-coloring
  - intensity thresholding

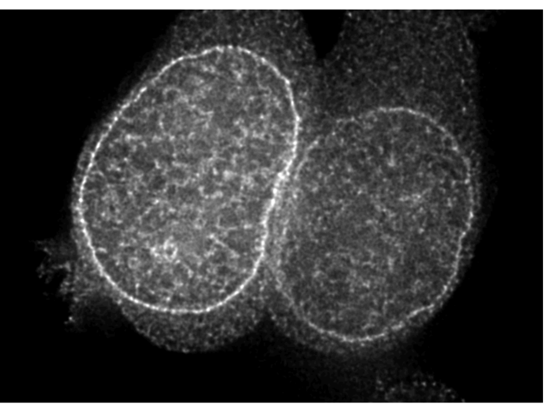
POINT OPERATIONS – CONTRAST STRETCHING

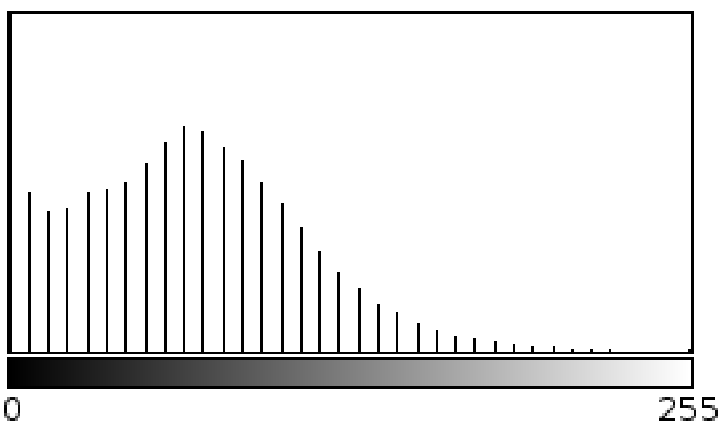






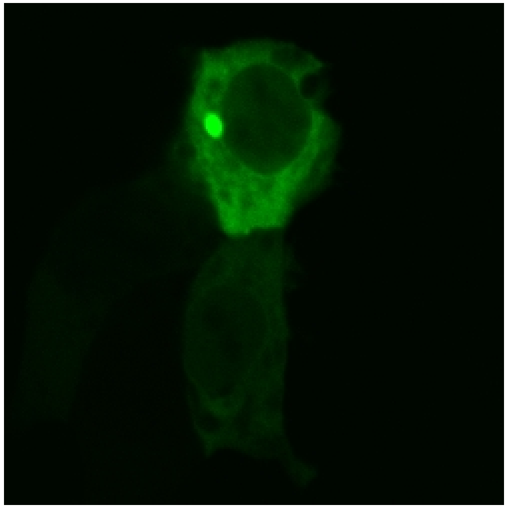


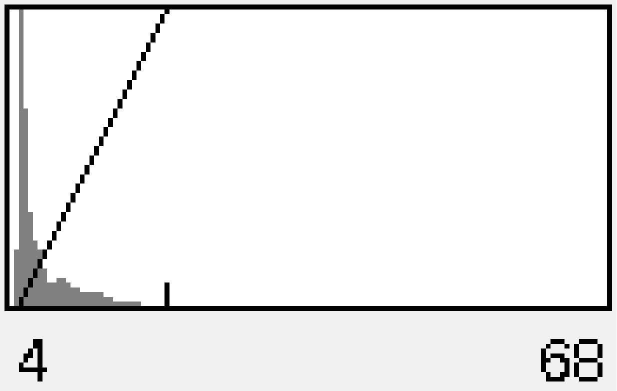


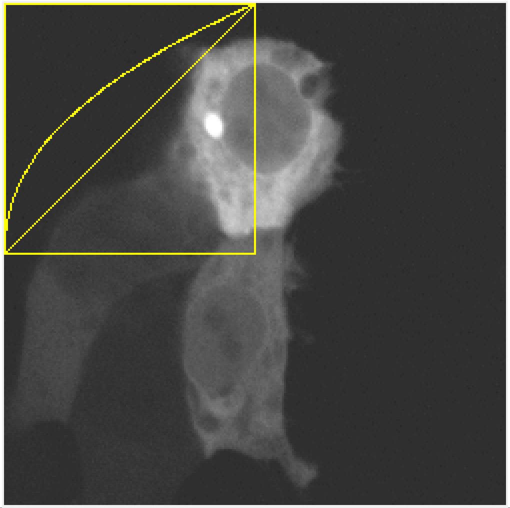


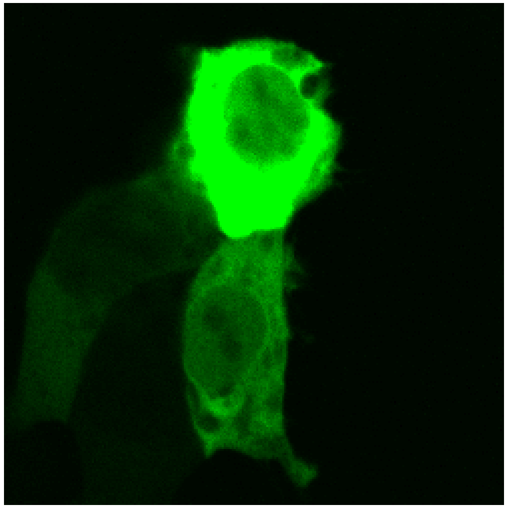
POINT OPERATIONS – GAMMA FUNCTION

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



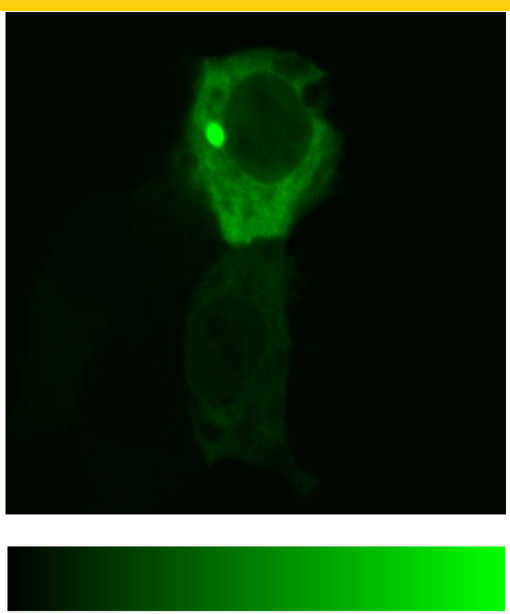


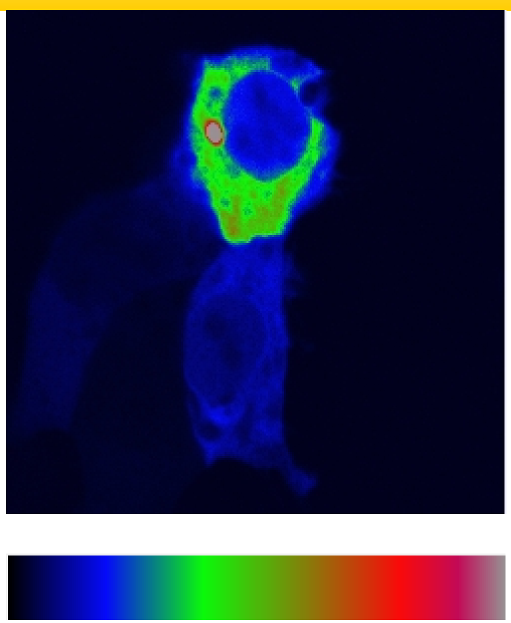


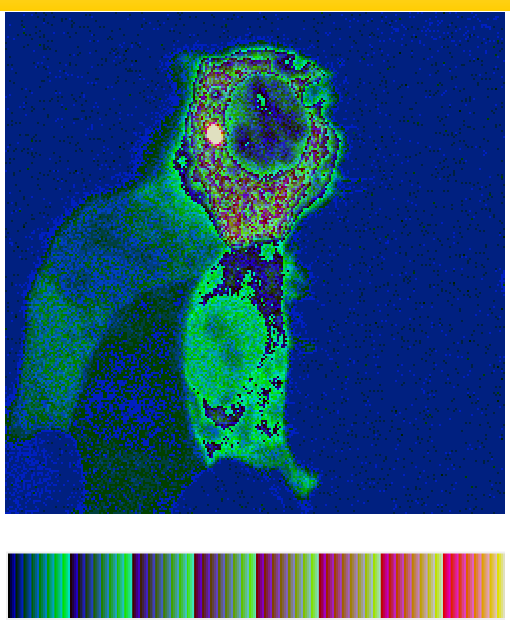


$$f(i)=\left(\frac{i}{255}\right)^{\frac{1}{\gamma}}\cdot 255$$

POINT OPERATIONS – LOOKUP TABLES

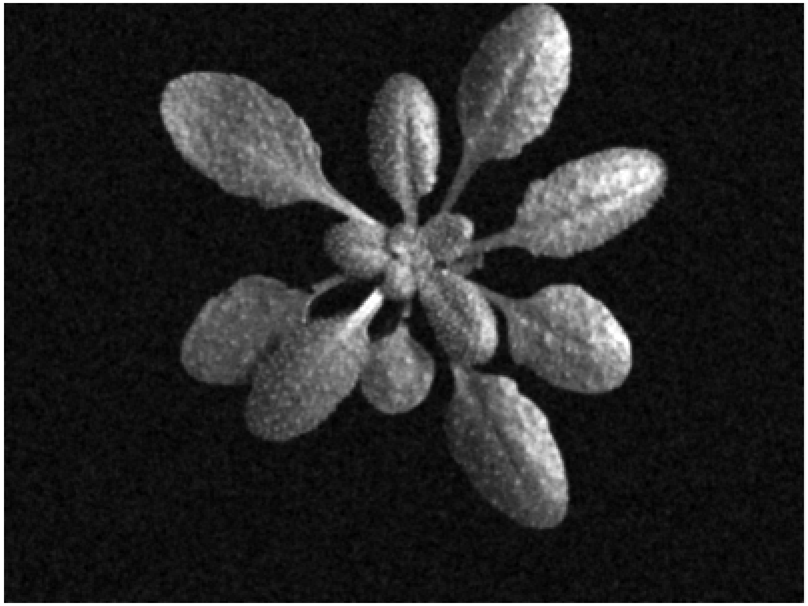


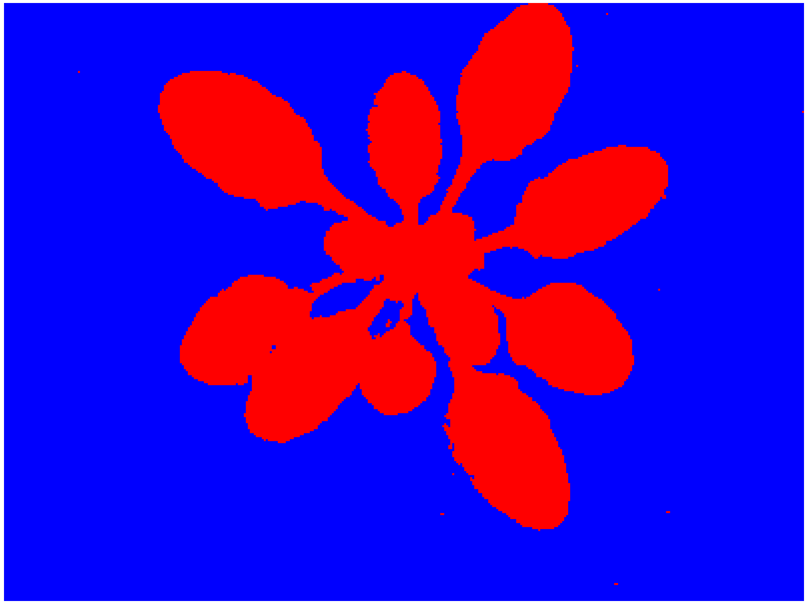




Index	Red	Green	Blue
0	0	0	0
1	0	0	5
2	0	0	10
3	0	1	15
4	0	1	20
5	0	1	25

POINT OPERATIONS – THRESHOLDING





- segmentation – separate objects from background
- can be done by applying a global threshold

- what threshold value?
- 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

LOCAL FILTERING – CONVOLUTION FILTER

kernel

input image

result image

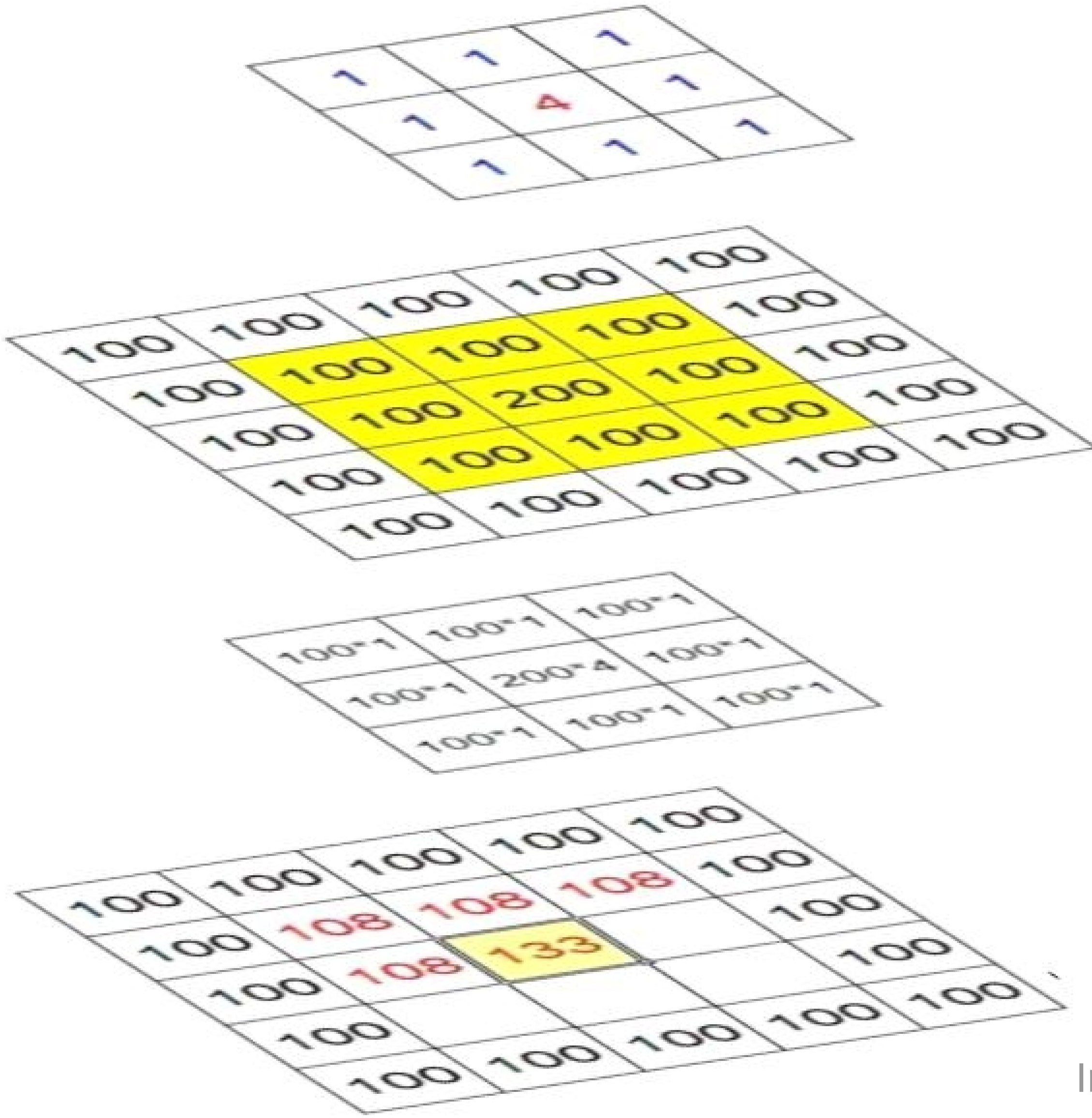
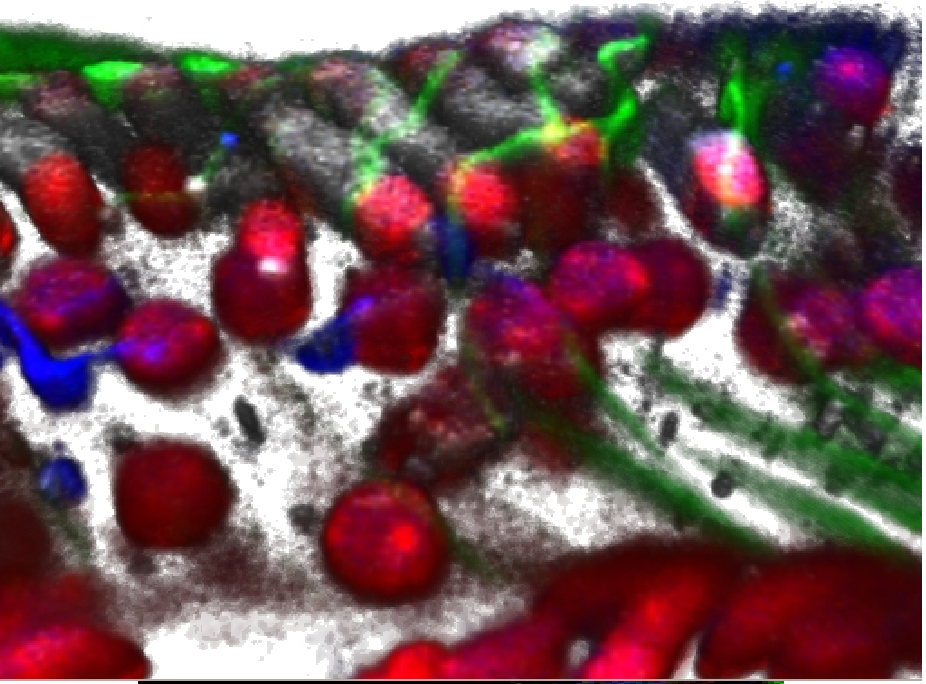


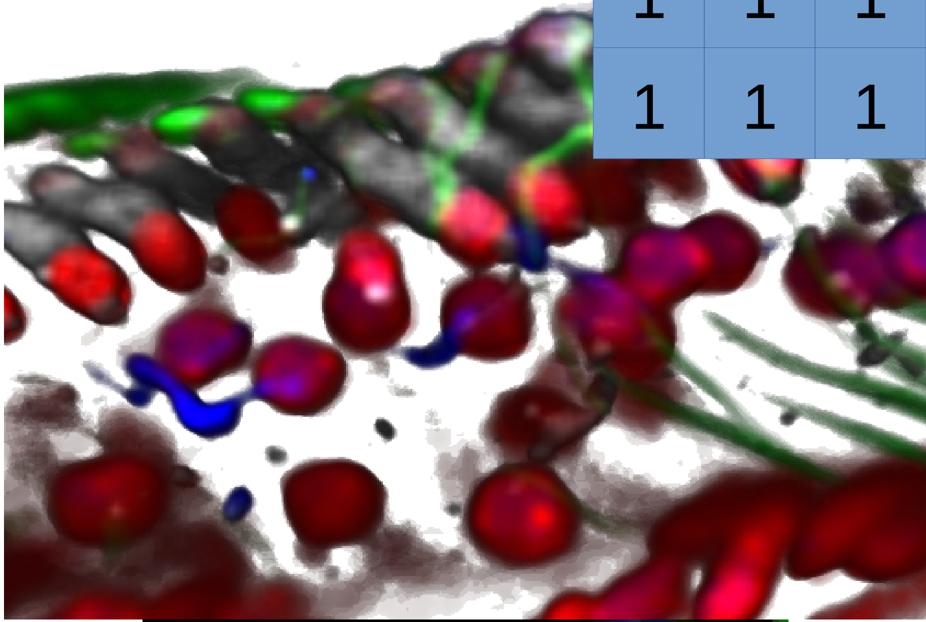
Image: Marc Lartaud
MRI-CIRAD

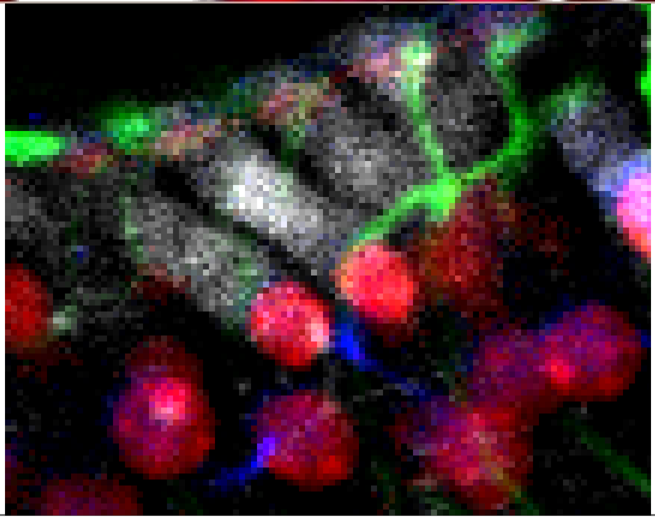
CONVOLUTION FILTER – SMOOTHING

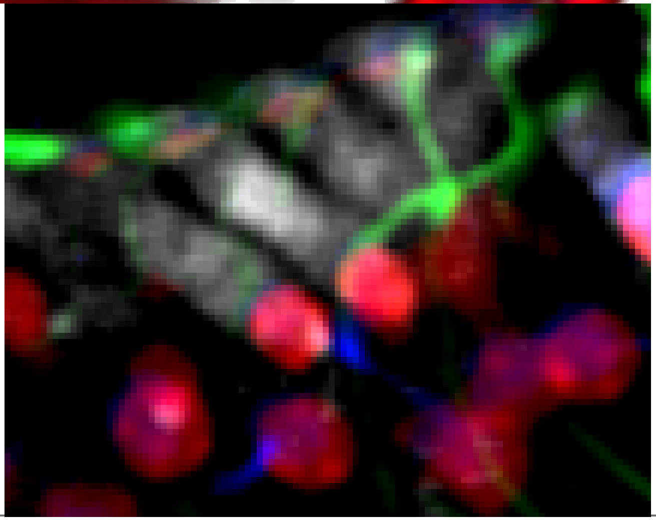
mean

1	1	1
1	1	1
1	1	1

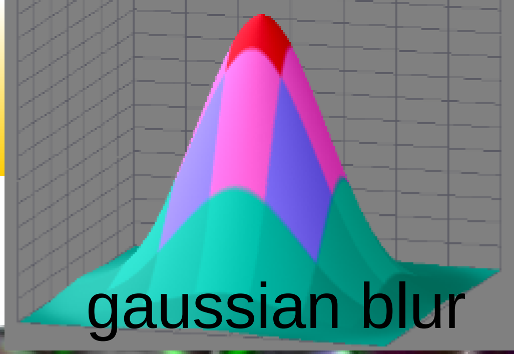


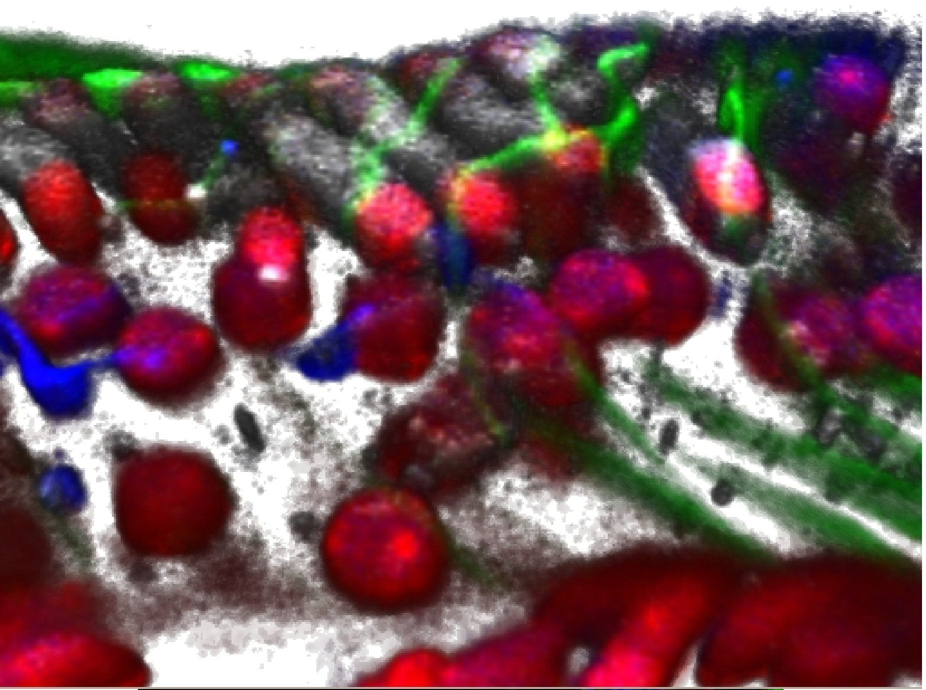


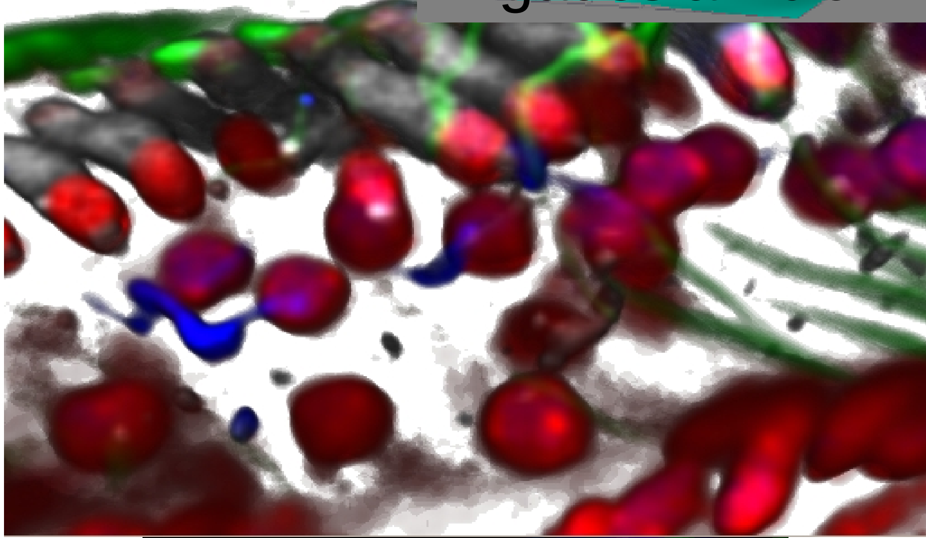


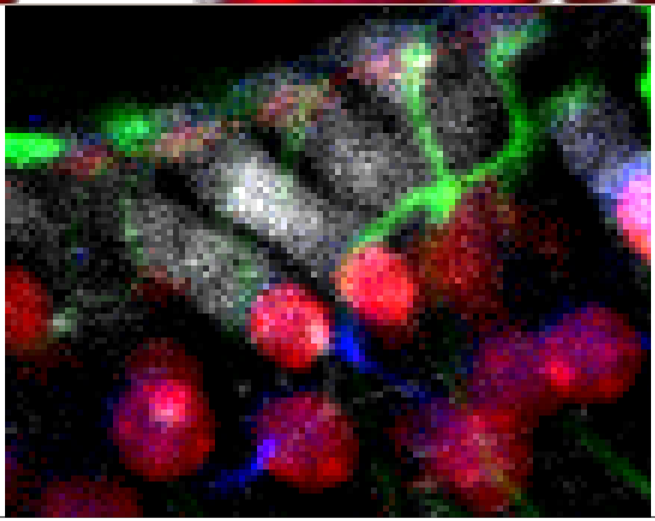


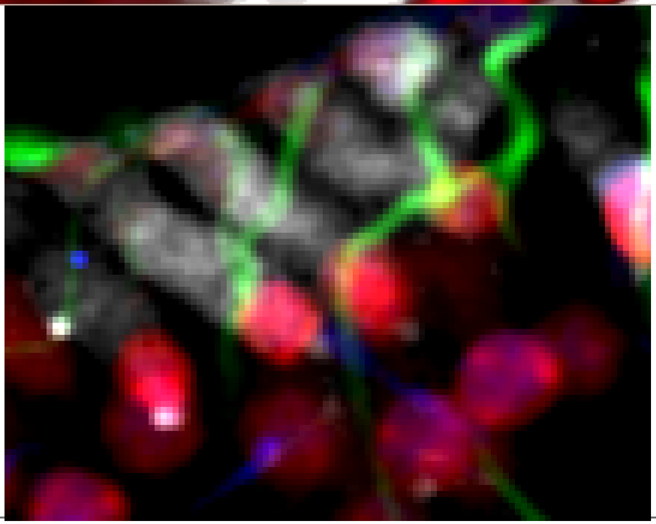
CONVOLUTION FILTER – SMOOTHING











CONVOLUTION FILTER – EDGE DETECTION

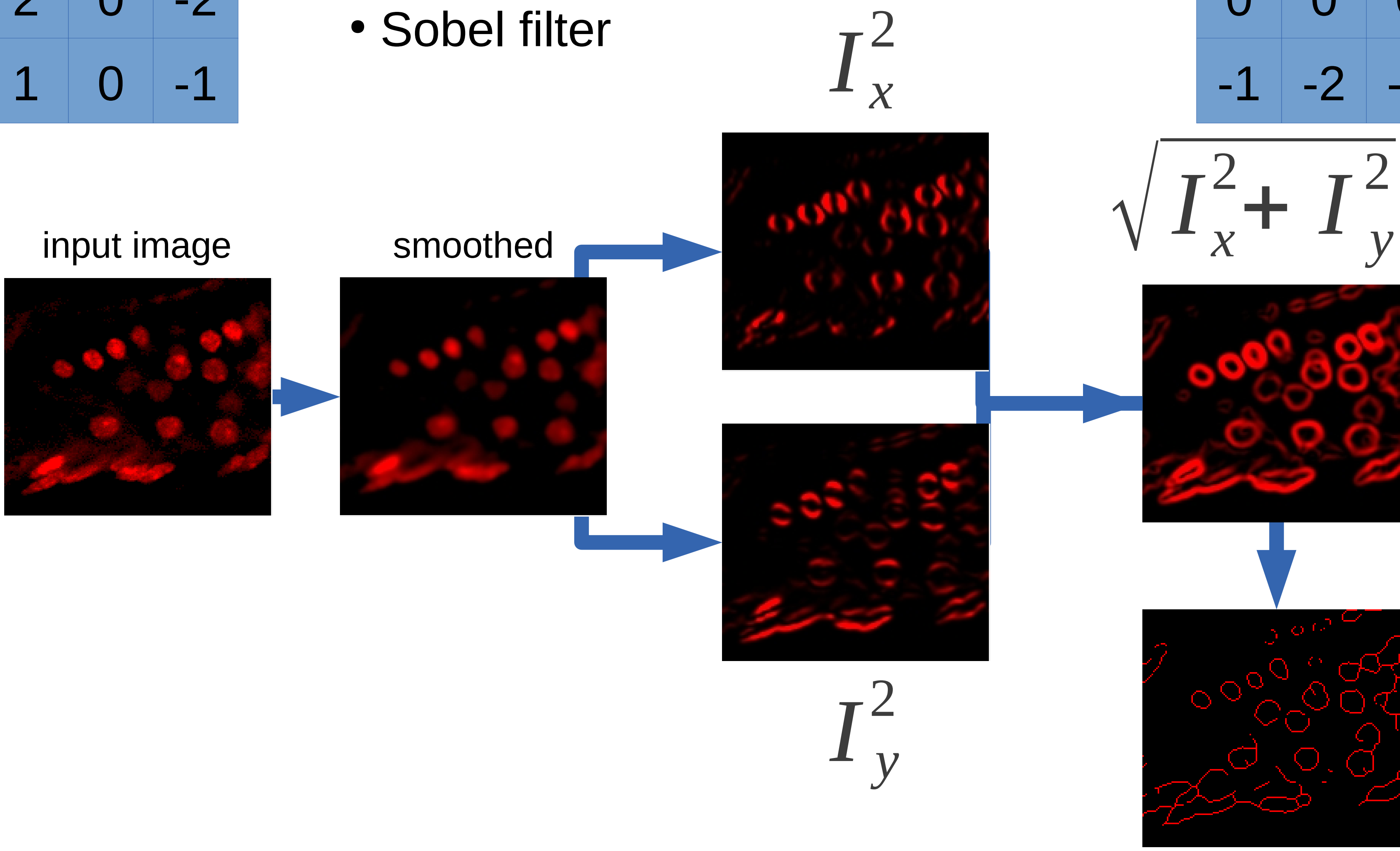
x-derivative

1	0	-1
2	0	-2
1	0	-1

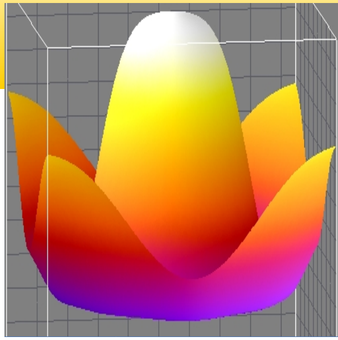
y-derivative

1	2	1
0	0	0
-1	-2	-1

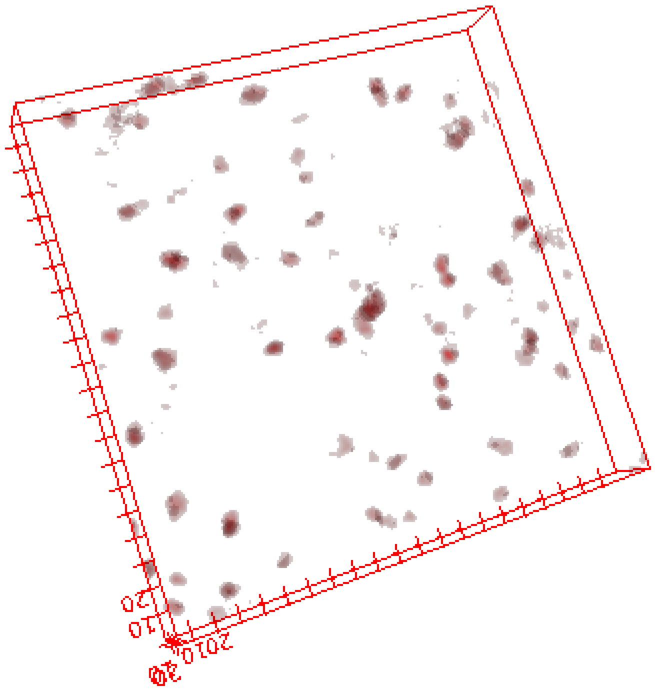
- Sobel filter

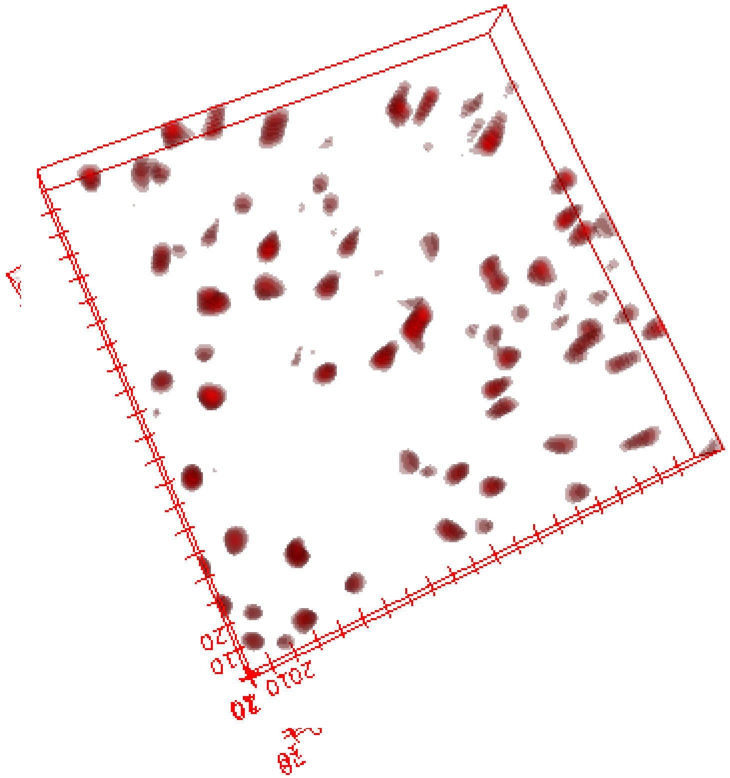


CONVOLUTION FILTER – SPOT DETECTION



- Laplacian filter enhaces 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

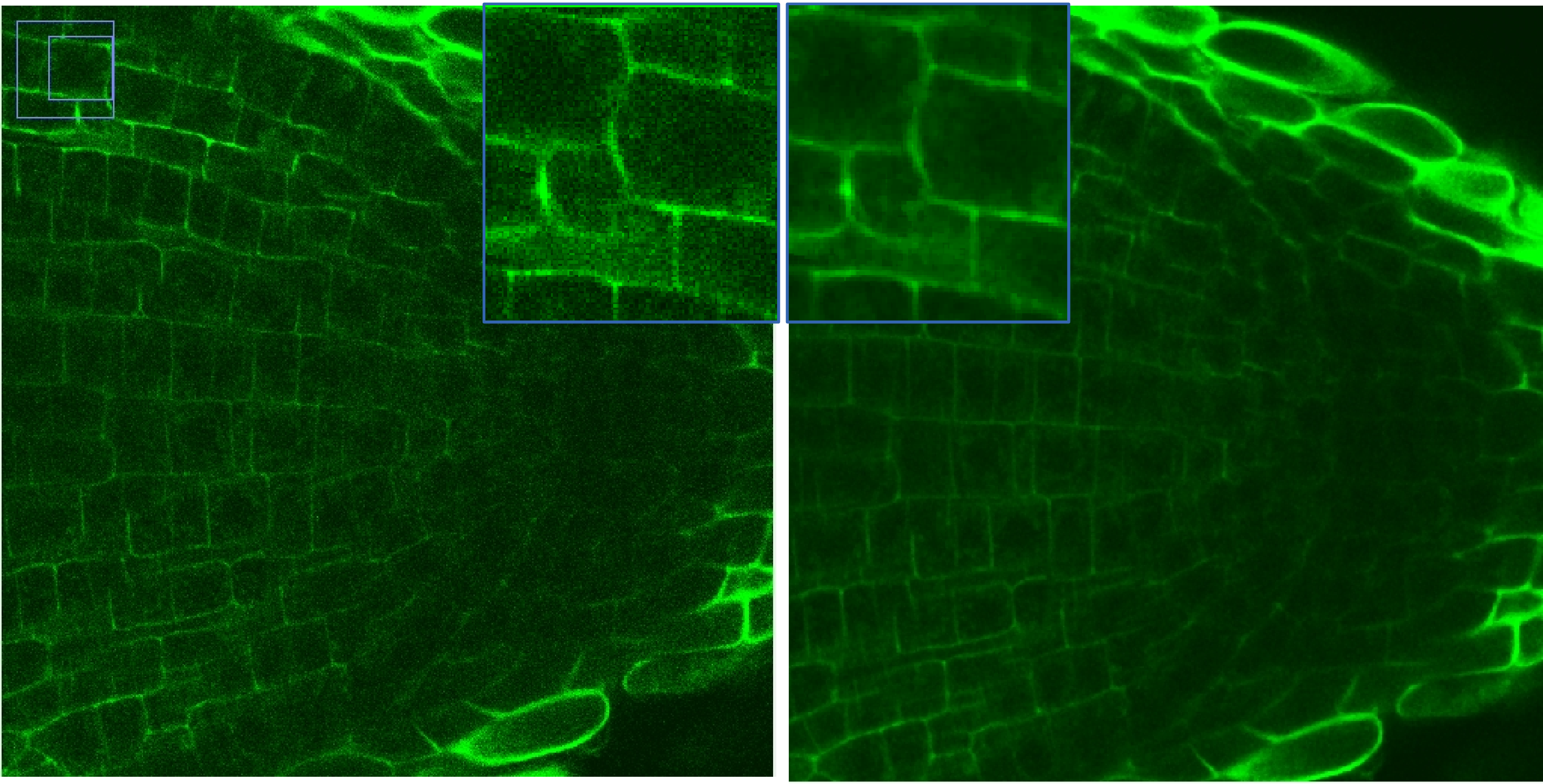
→

15	18	14
29	18	13
12	19	21

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

RANKING FILTER – MEDIAN FILTER

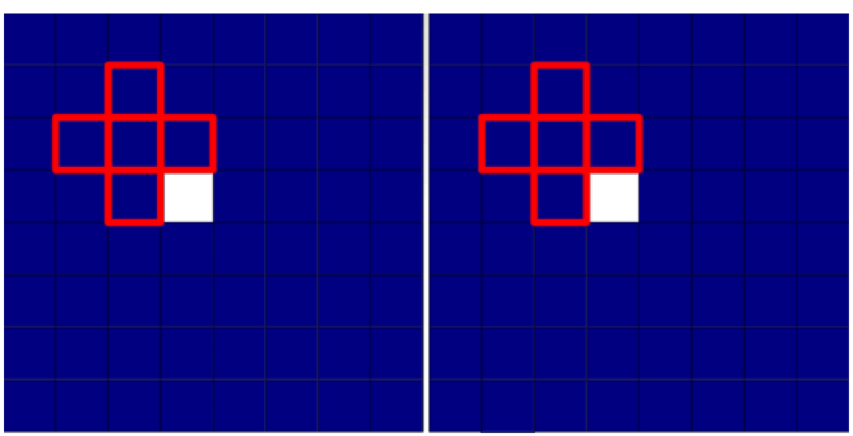
- \+ stable against outliers
- \- can be long to calculate



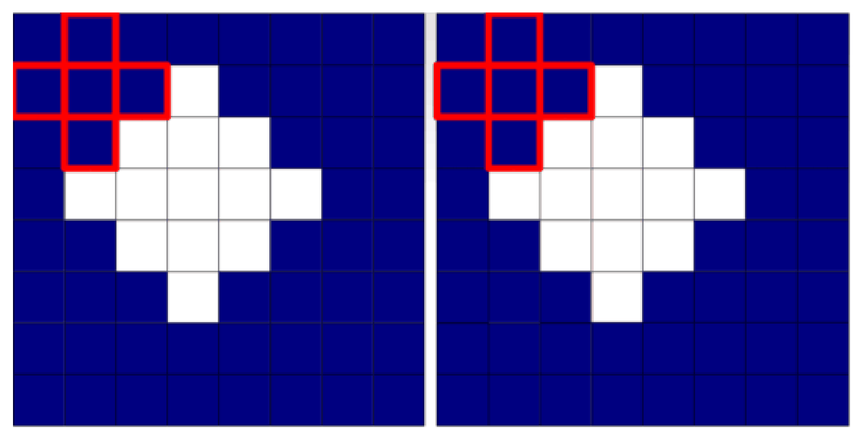
LOCAL FILTERING – BINARY MORPHOLOGY

- correct segmentation, measure features, granulometry, edge detection, skeletonization, reconstruct objects
- work on a mask (a binary image)
- move the structuring element (SE) along the image
- two basic operations

1. dilate (enlarge objects):
one if SE touches object



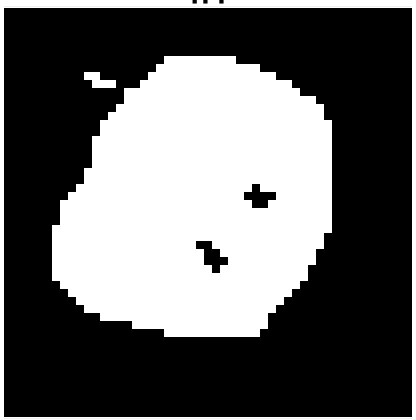
1. erode (shrink objects):
one if SE completely within object



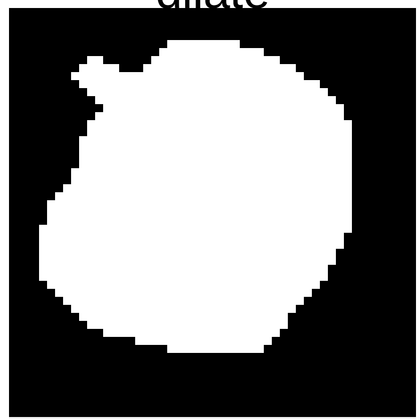
BINARY MORPHOLOGY – OPEN AND CLOSE

- close(X) = dilate(erode(X)) close holes in objects
- open(X) = erode(dilate(X)) remove small objects

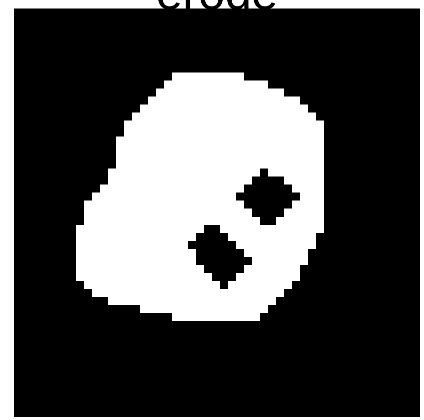
in

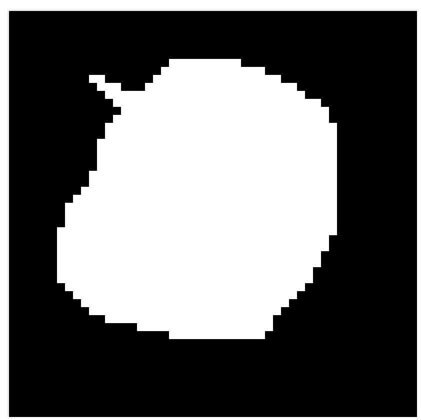


dilate

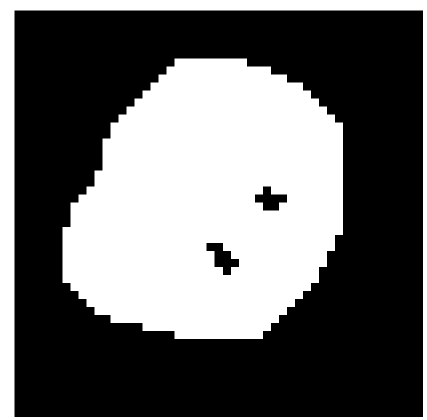


erode





close

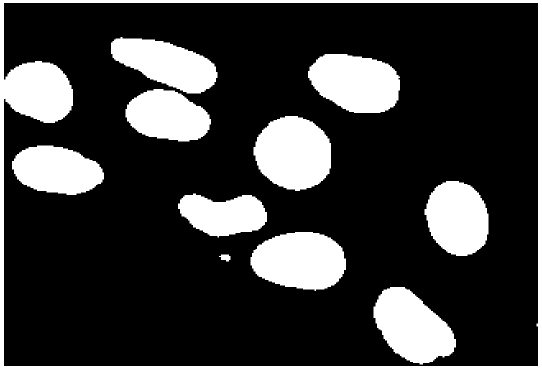


open

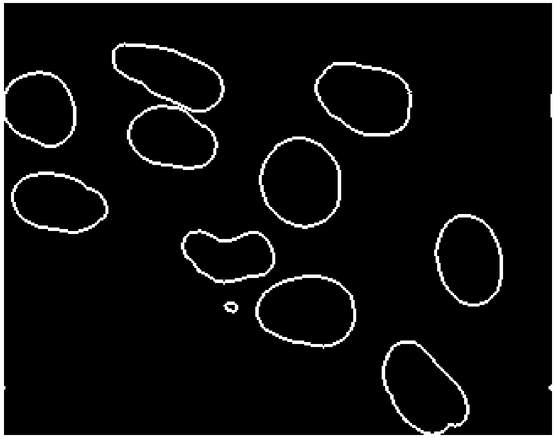
BINARY MORPHOLOGY – APPLICATIONS

edge detection

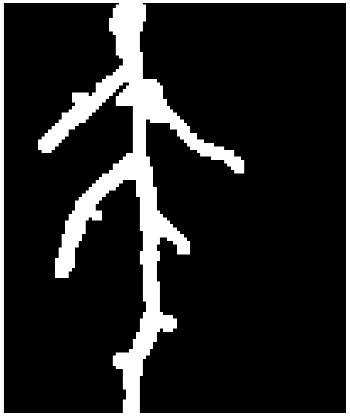
I

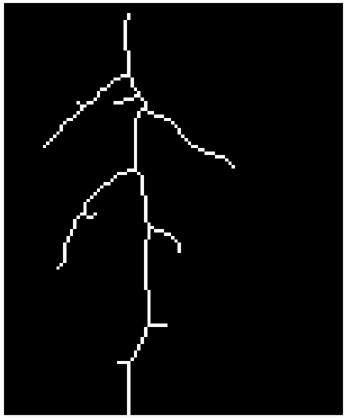


dilate(I) - erode(I)

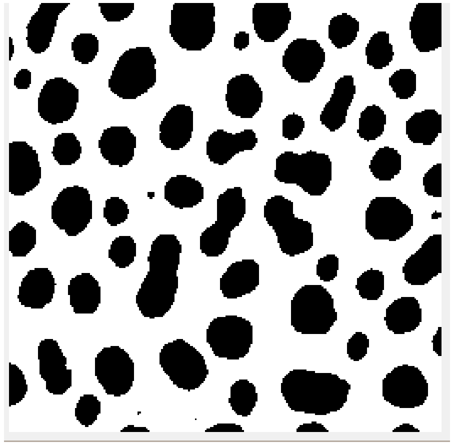


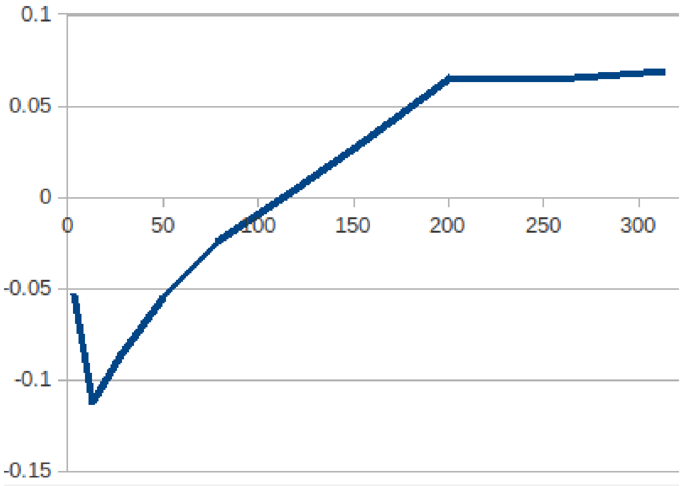
skeletonization





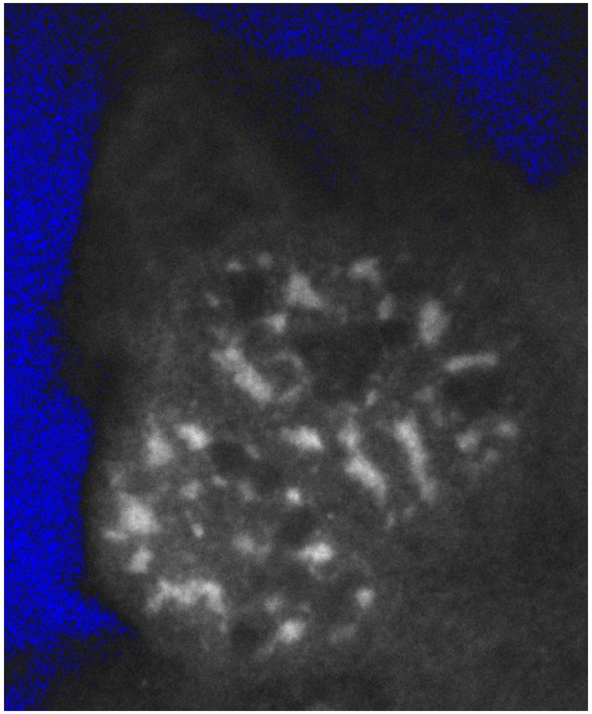
granulometry

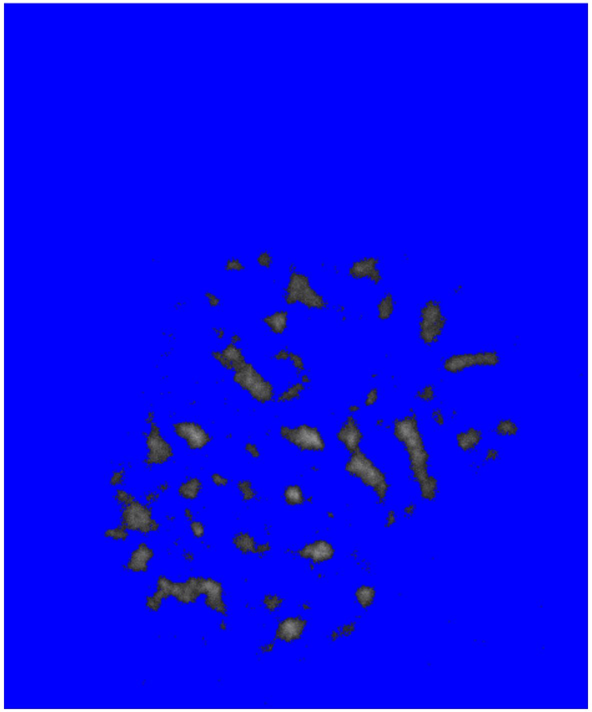




GRAYSCALE MORPHOLOGY

- dilate – max over structuring element
- erode – min over structuring element
- Example: grayscale top-hat filter (I-open(I))



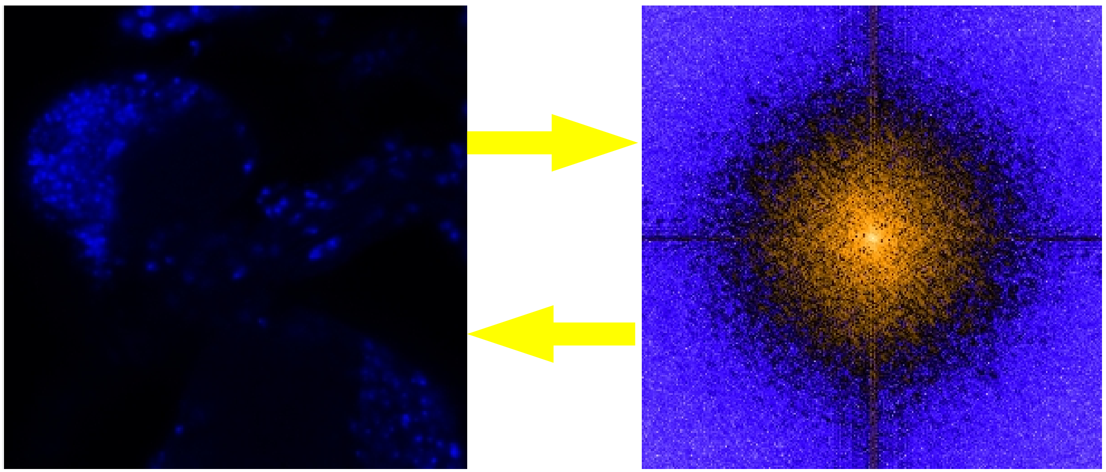


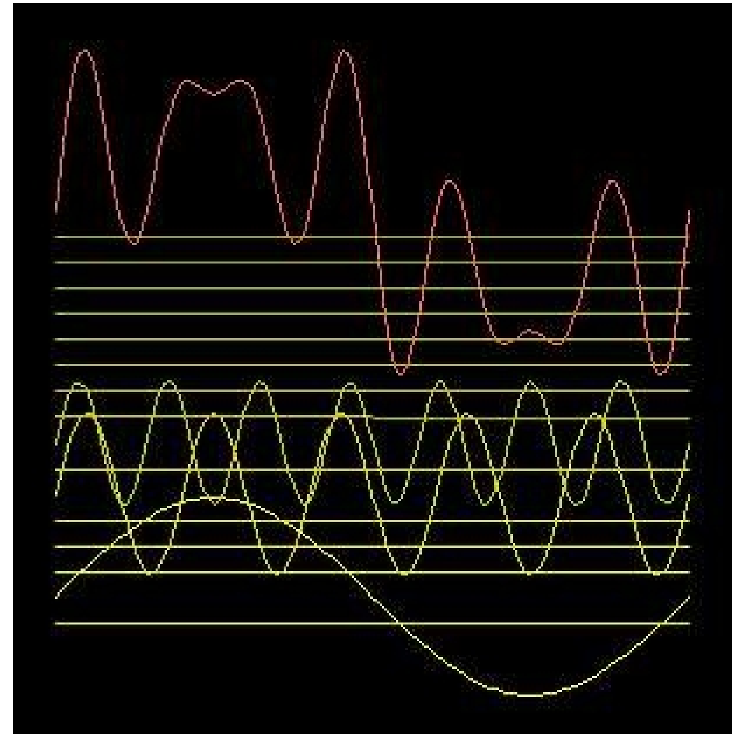
FILTERING IN THE FREQUENCY DOMAIN

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

FILTERING IN THE FREQUENCY DOMAIN – FOURIER TRANSFORM

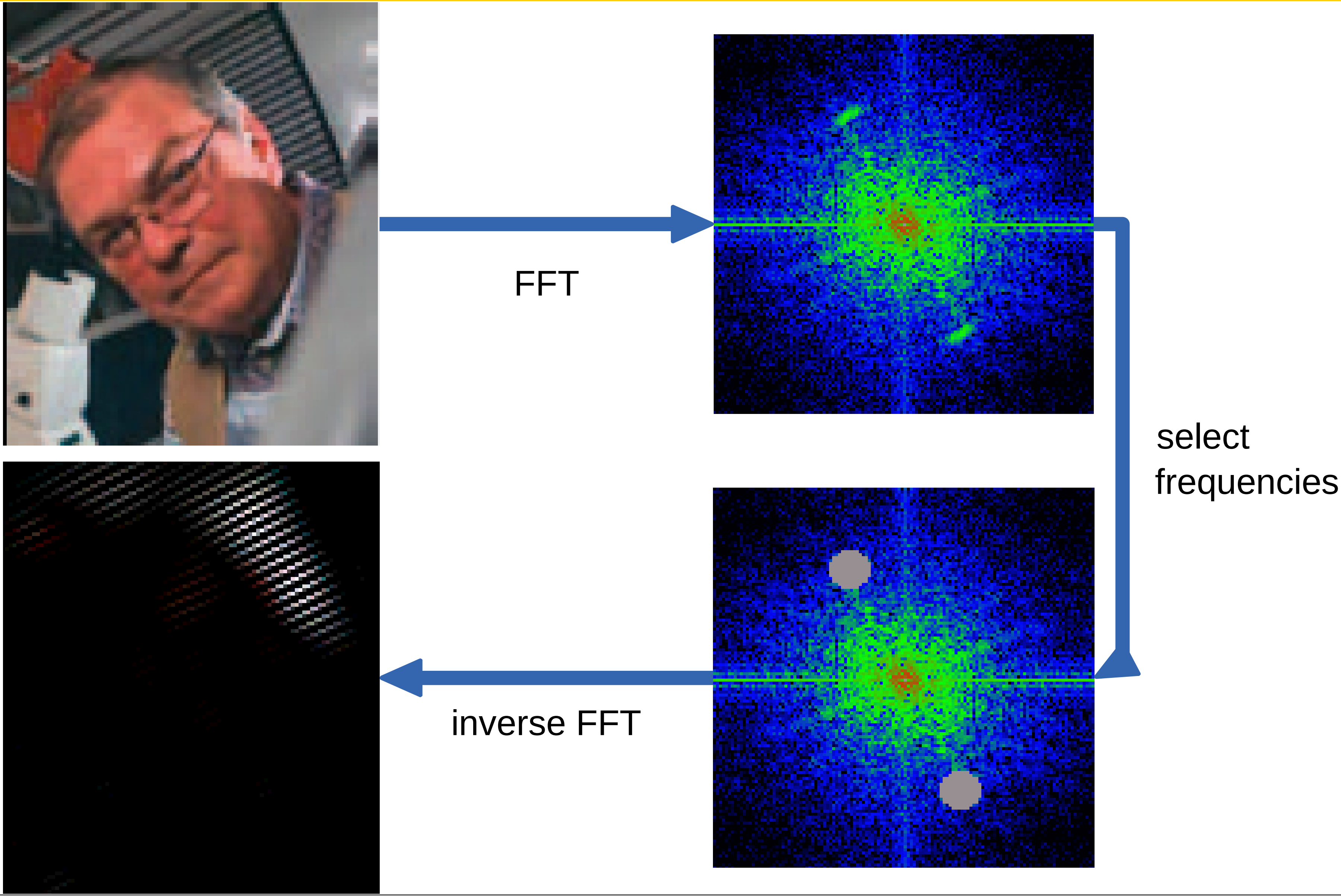
$$F(\nu)=\int f(x)\,e^{-i2\pi\nu x}\,dx$$



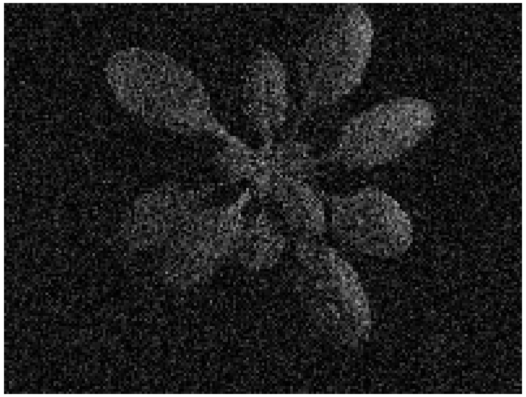


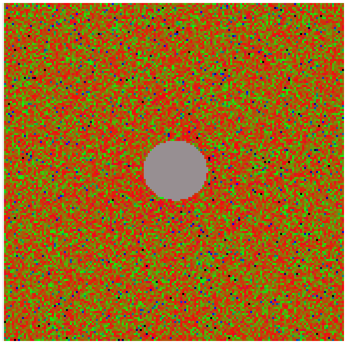
- signal can be represented as sum of sinoids
- FT transforms from spatial to frequency domain

FILTERING IN THE FREQUENCY DOMAIN

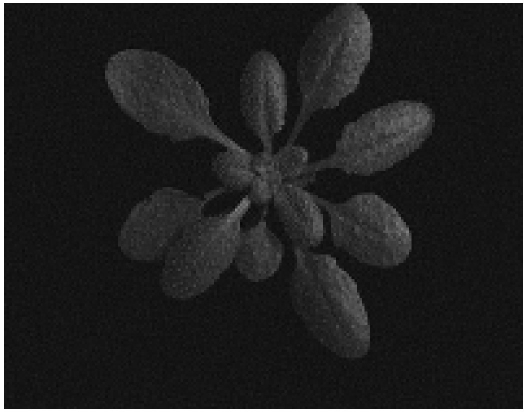


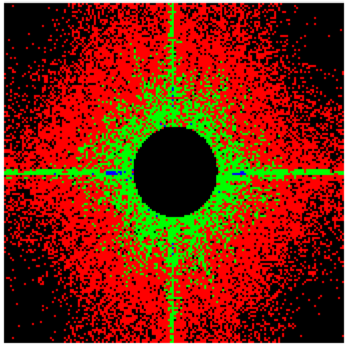
FILTERING IN THE FREQUENCY DOMAIN



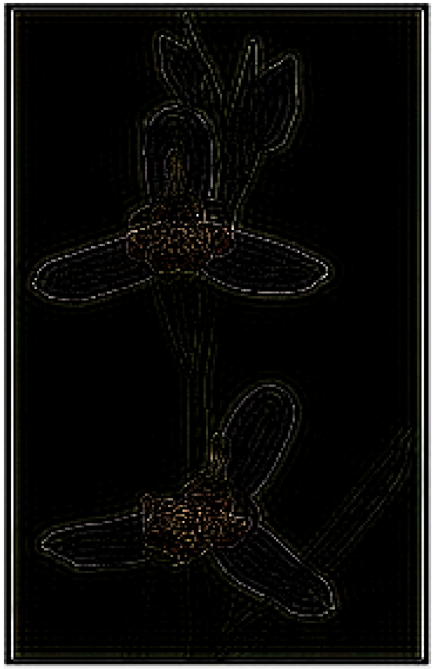


low pass filter

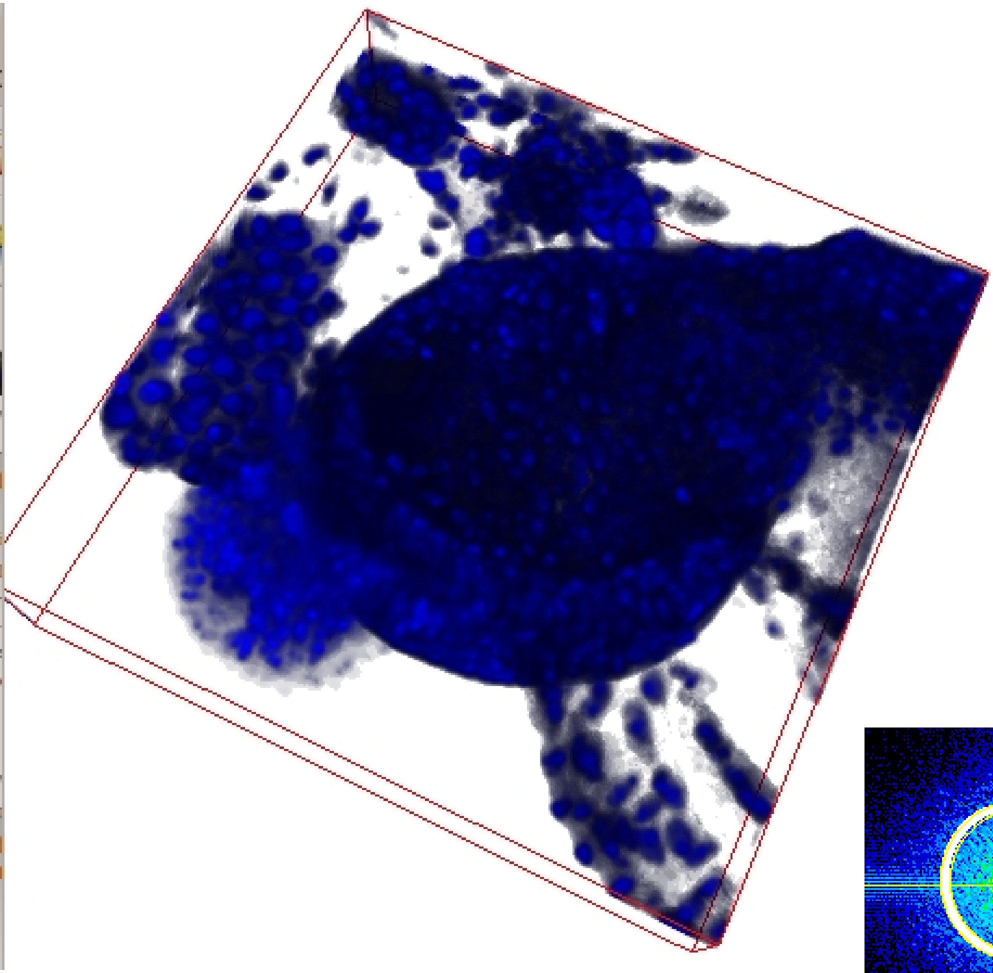


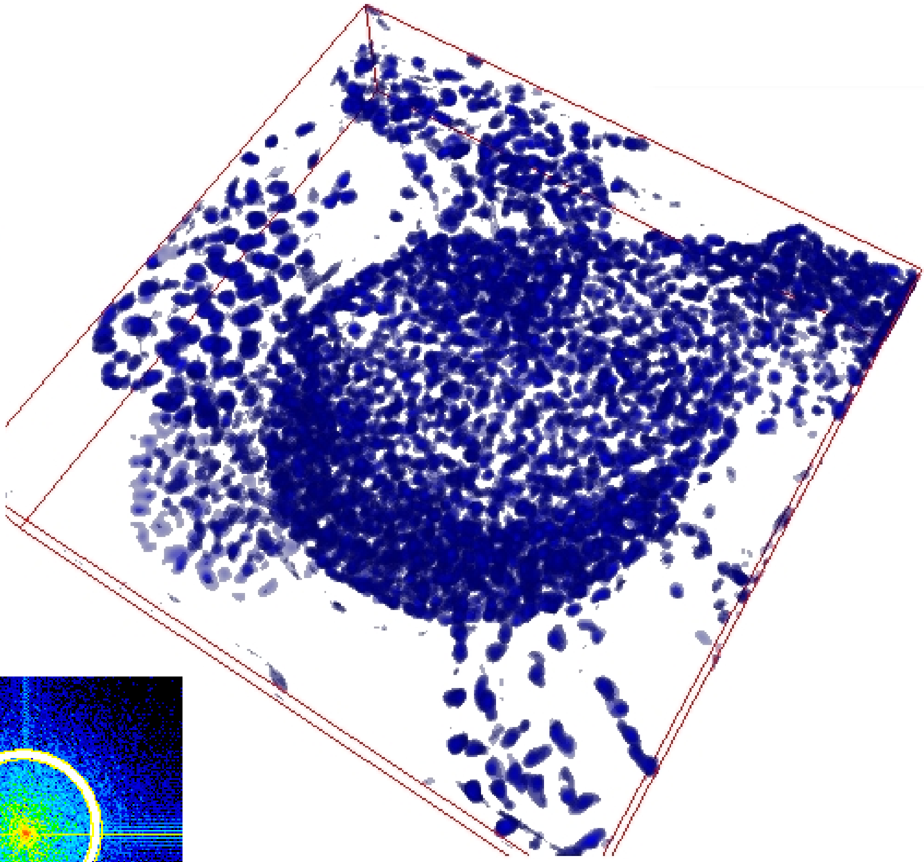


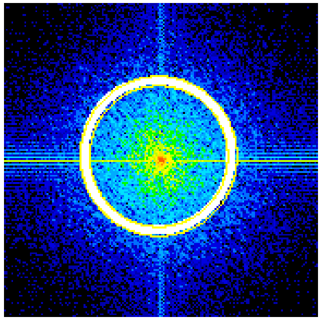
high pass filter



FILTERING IN THE FREQUENCY DOMAIN







band pass filter

IMAGE RESTORATION

- image degraded
  - noise
    - quantum nature of light (poisson distribution)
    - imperfect electronics (gaussian distribution)
  - ➔ filter

  - background
    - imperfect illumination
  - blur
    - out of focus light

IMAGE RESTORATION – BACKGROUND SUBTRACTION

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

- correct inhomogeneous illumination
  - correct with image of background
  - if not available: estimate background image

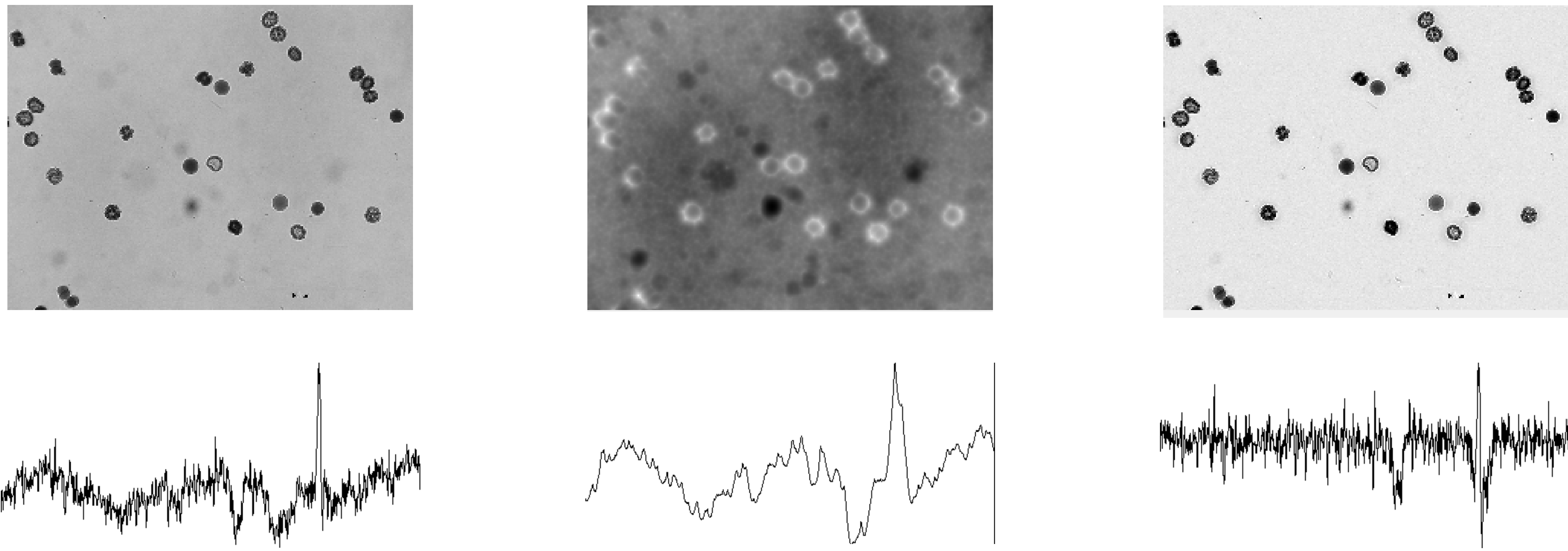
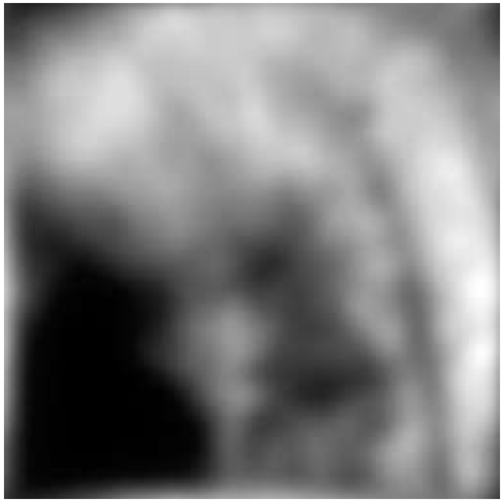
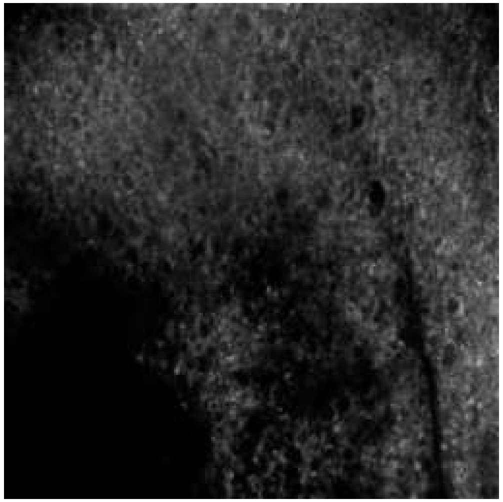


IMAGE RESTORATION – DECONVOLUTION

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



=



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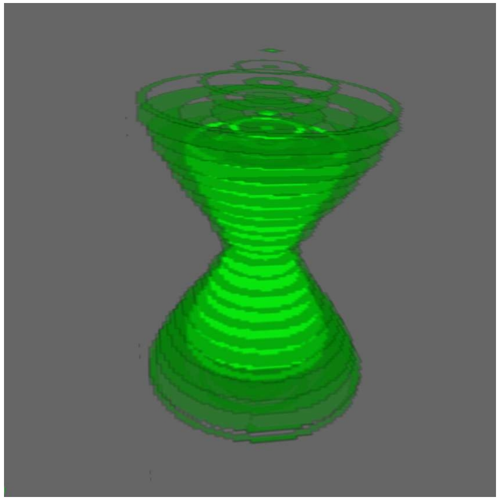
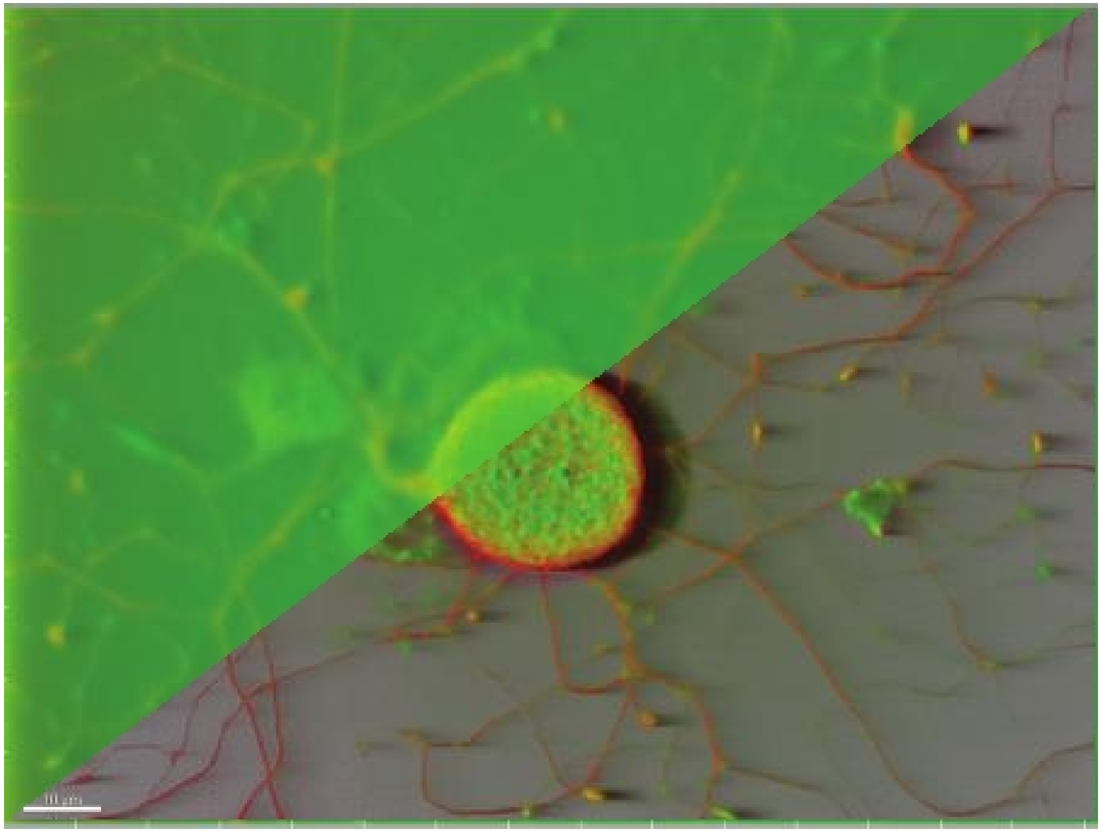
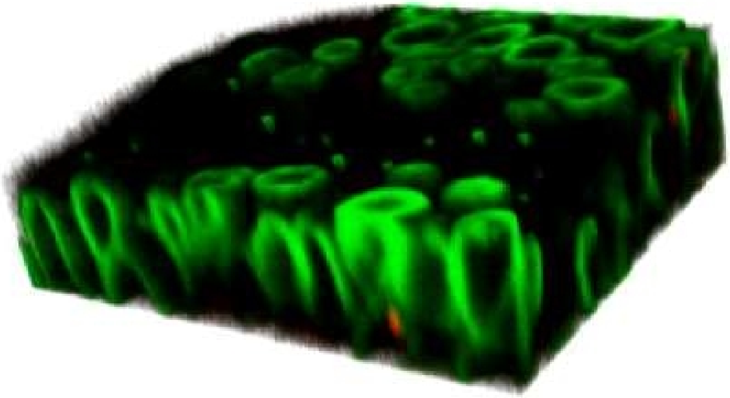


DECONVOLUTION – EXAMPLES



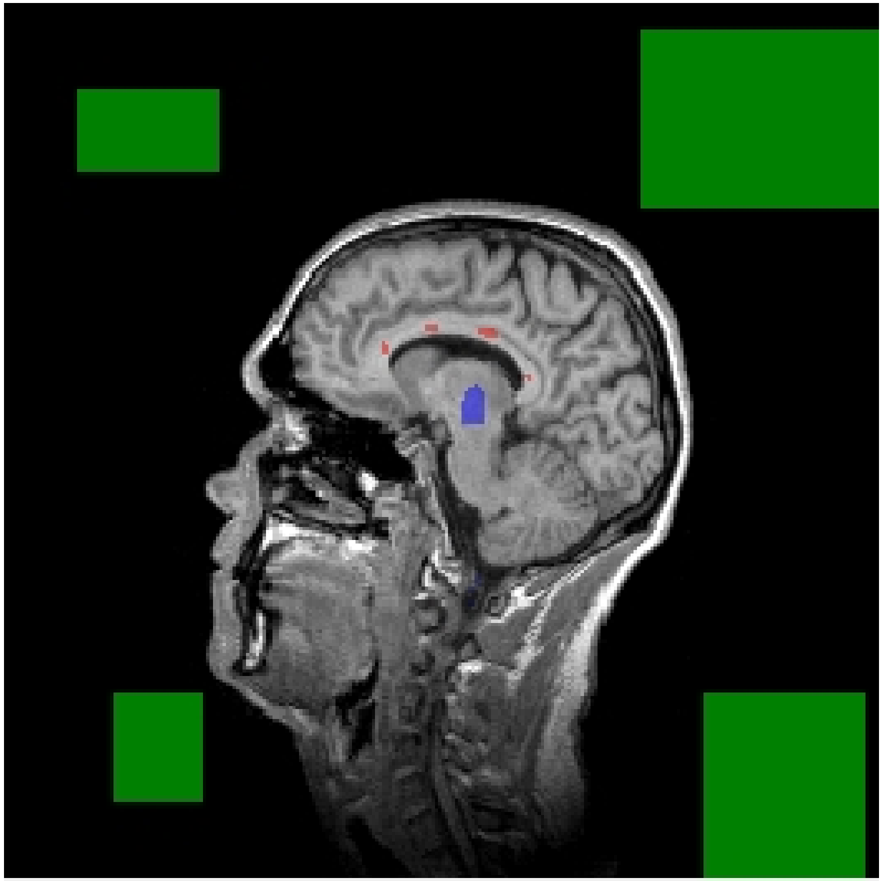


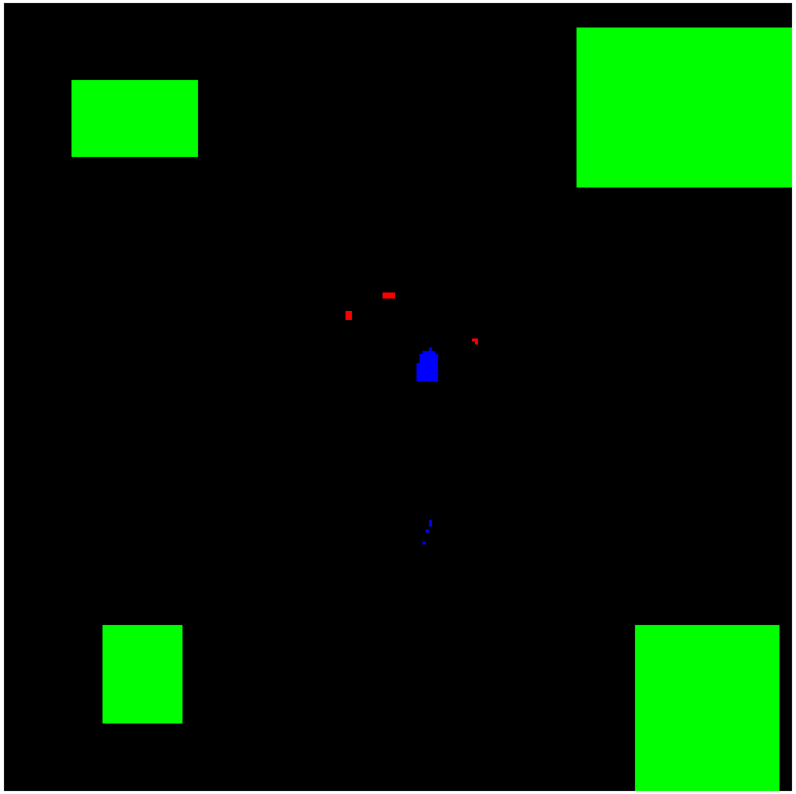
SEGMENTATION – REGION GROWING

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

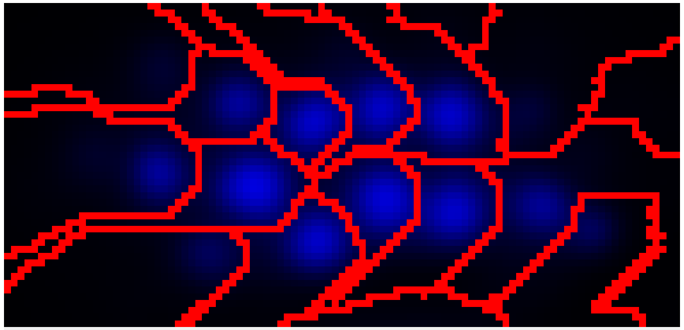
SEGMENTATION

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

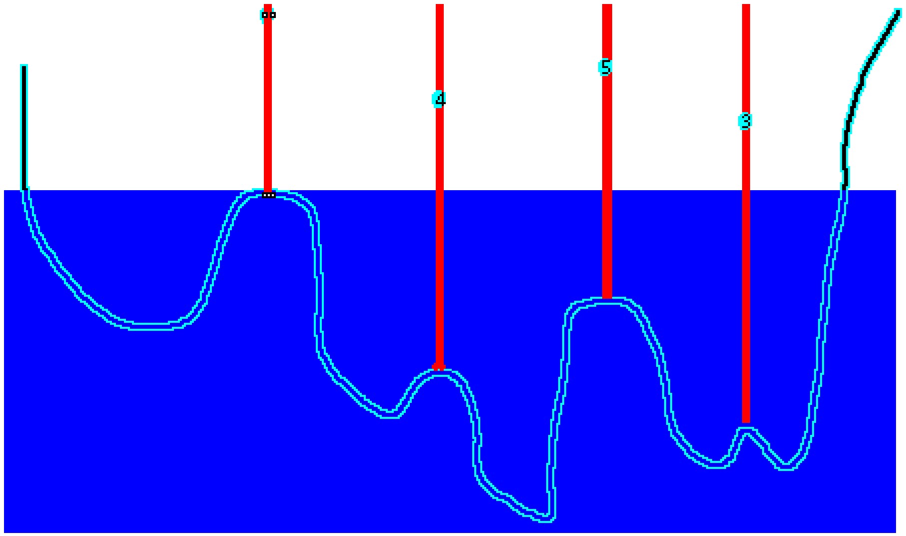


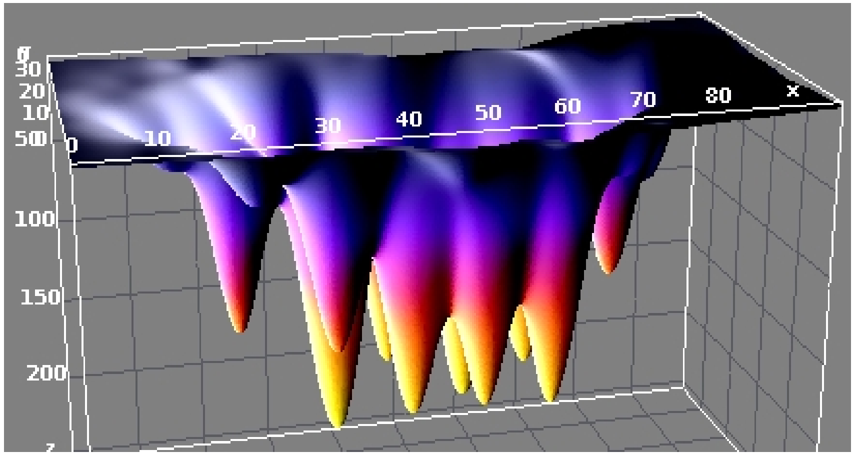


SEGMENTATION – WATERSHED



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





- problem: over-segmentation
  – possible solution: seeded watershed

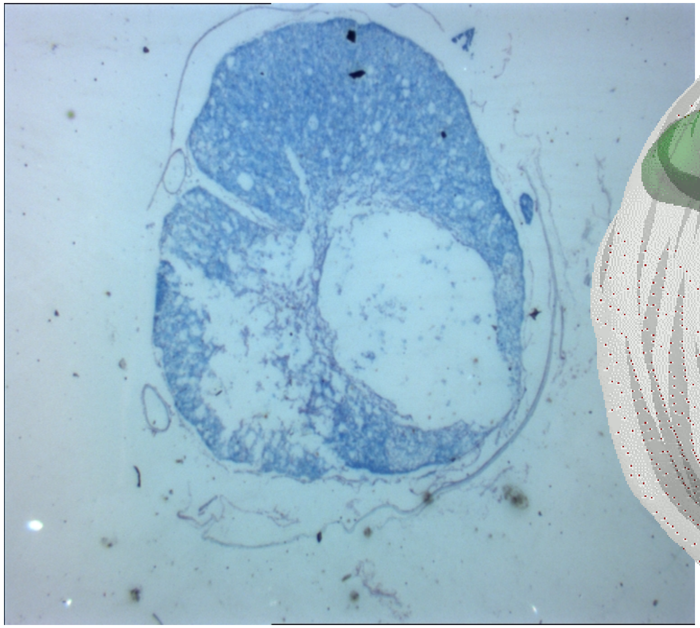
GEOMETRICAL TRANSFORMATION

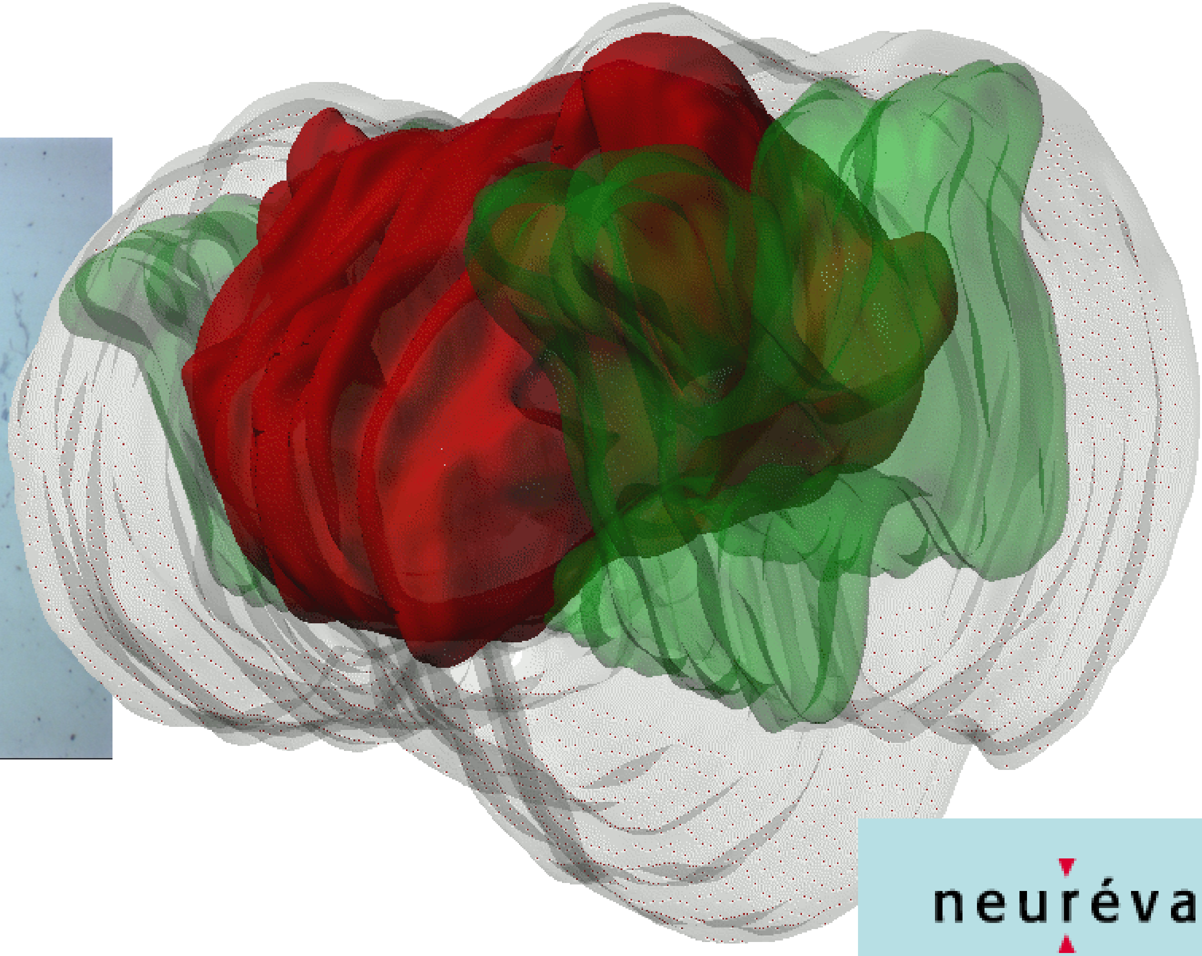
- problem:
  - image is spatially distorted or
    - mismatch between channels due to chromatic aberration
    - barrel distortion or pincushion distortion
    - specimen moved during acquisition
  - lacks spatial correspondence
    - histological slices
    - combining images from different sources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

IMAGE REGISTRATION

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

EXAMPLE REGISTRATION





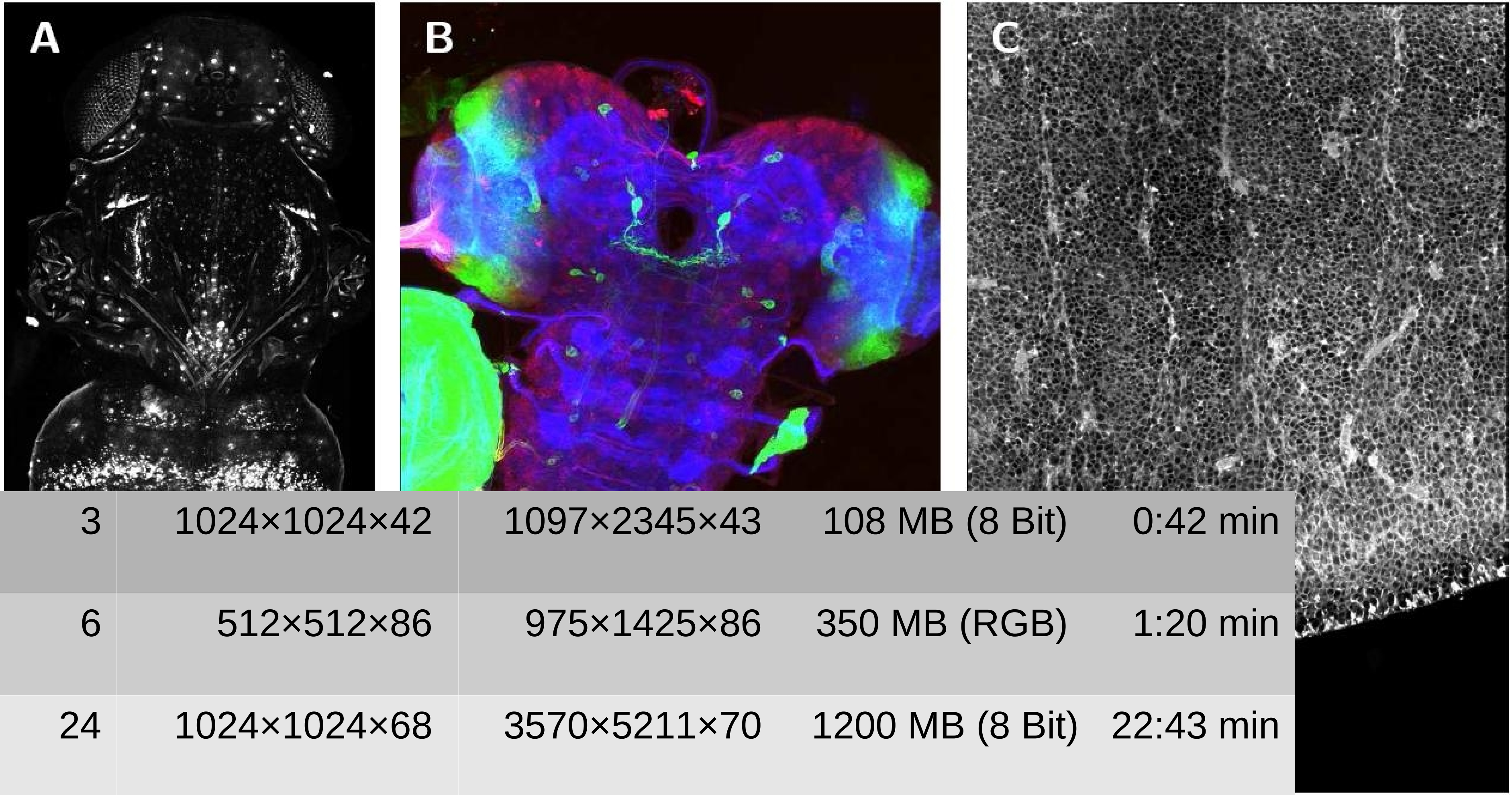
spinal cord

grey matter

traumatic lesion

neuréva

EXAMPLE STITCHING



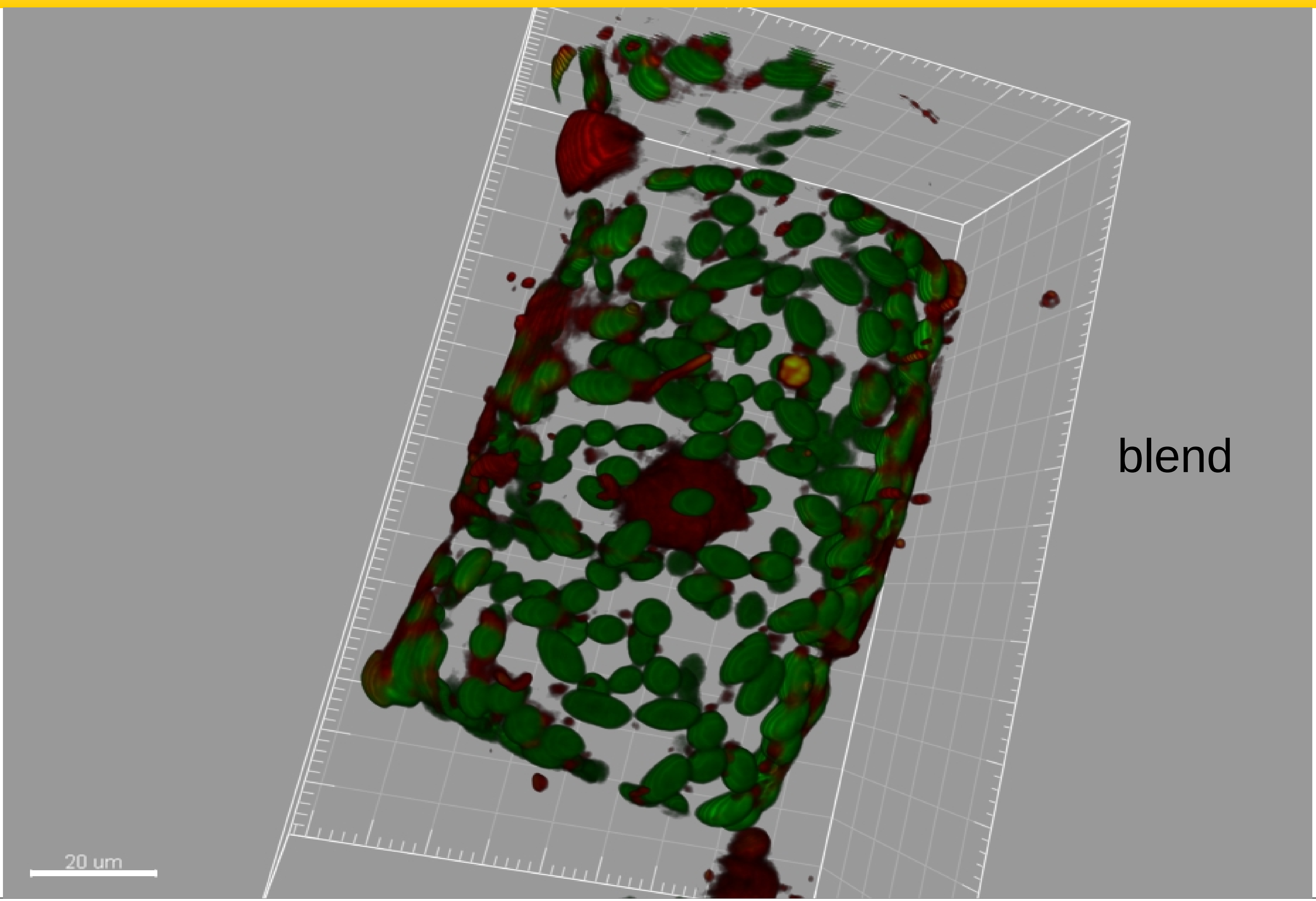
3	1024×1024×42	1097×2345×43	108 MB (8 Bit)	0:42 min
6	512×512×86	975×1425×86	350 MB (RGB)	1:20 min
24	1024×1024×68	3570×5211×70	1200 MB (8 Bit)	22:43 min

http://fiji.sc/wiki/index.php/Stitching#Stitch_Image_Grid_Sequence

VISUALIZATION

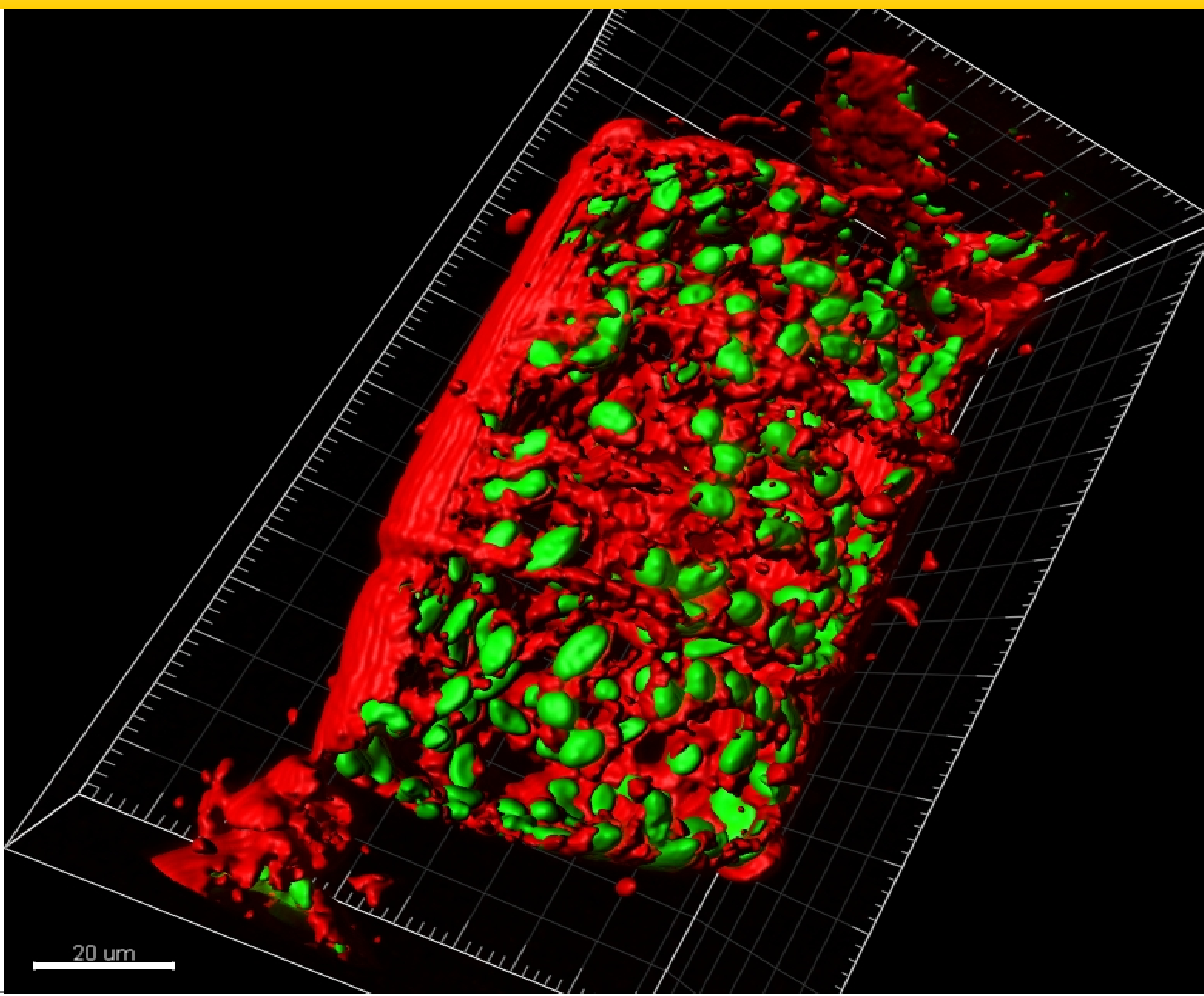
- how to understand multidimensional data?
  - reduce dimensionality in a sensible way
- methods
  - volume rendering
    - methods that use the raw data directly without geometrical representation
      - ray tracing
        - maximum intensity projection (MIP)
        - blend (calculated from all information 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



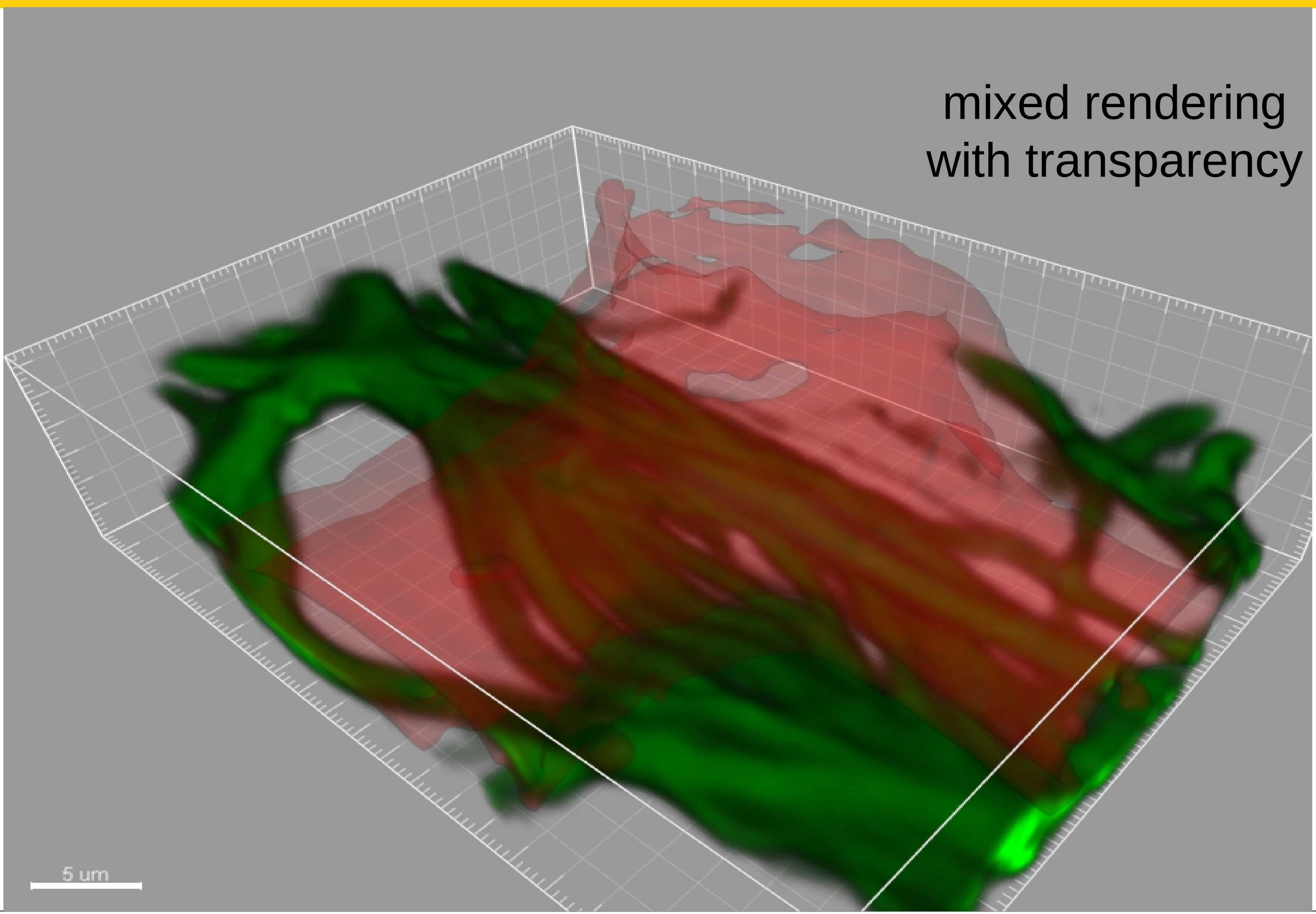
blend

VISUALIZATION – SURFACE RENDERING

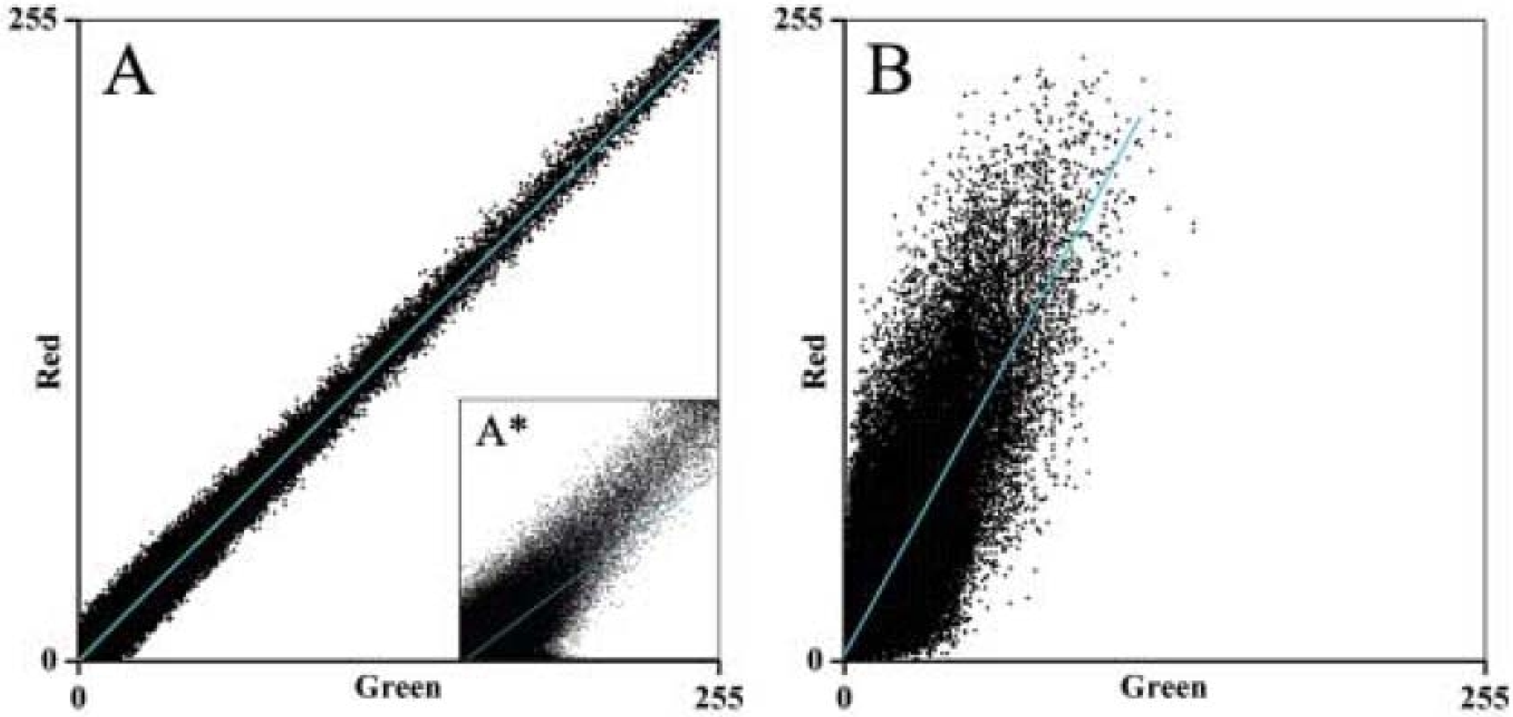


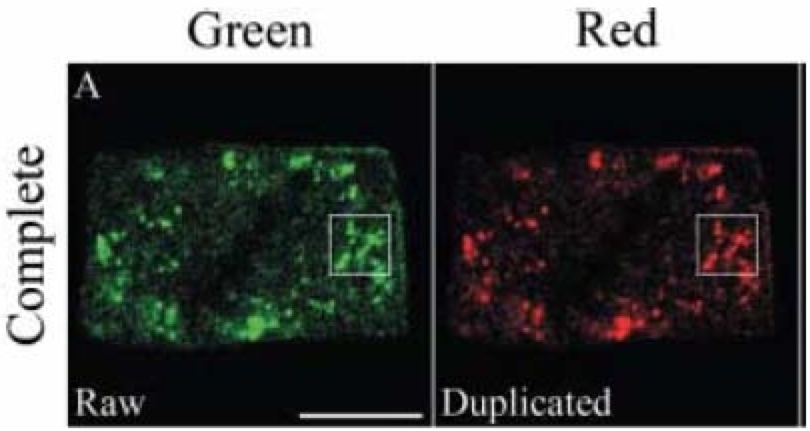
surfaces

VISUALIZATION – MIXED RENDERING



COLOCALIZATION ANALYSIS





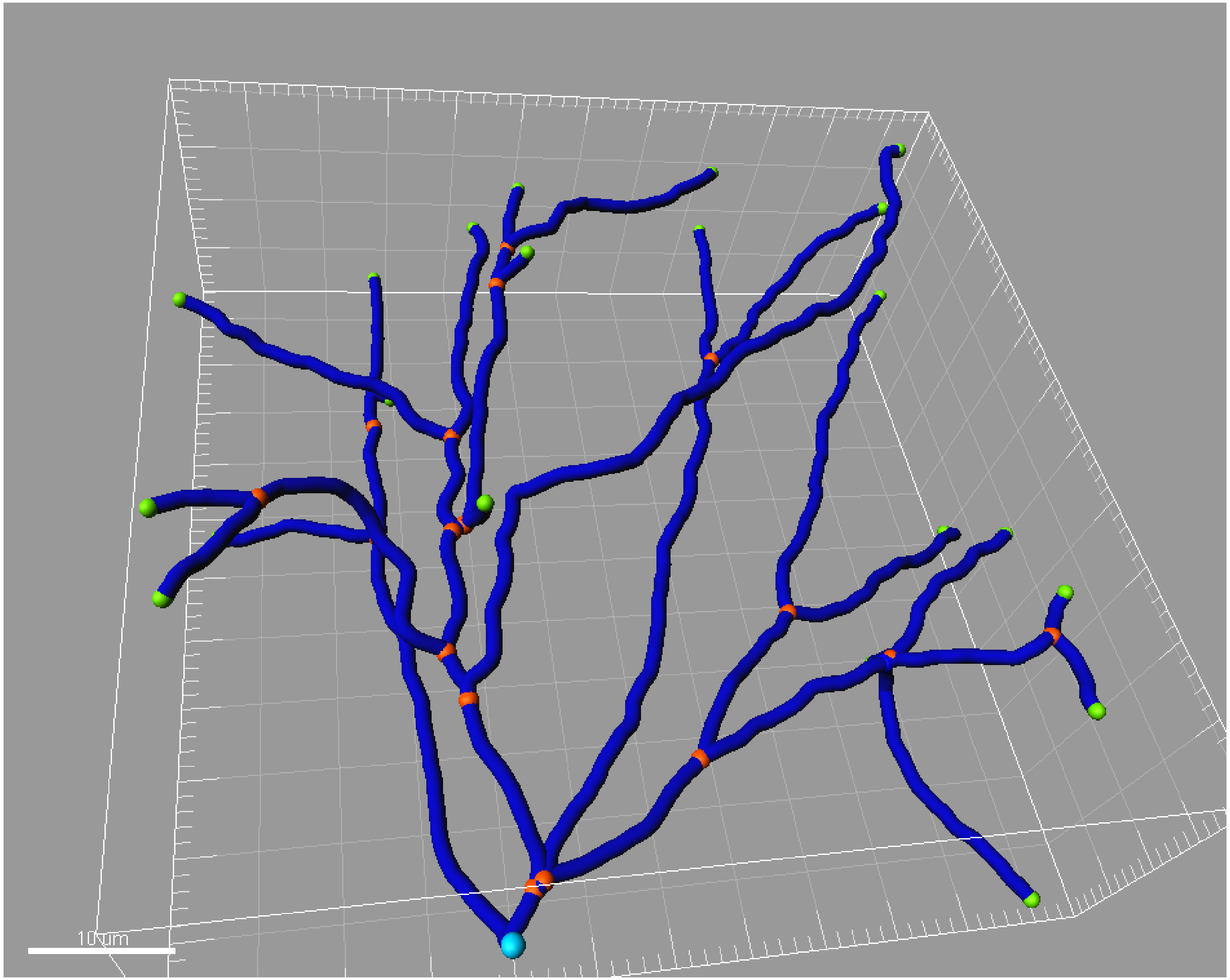
Pearson's Correlation Coefficient

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

FILAMENT TRACING AND ANALYSIS

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

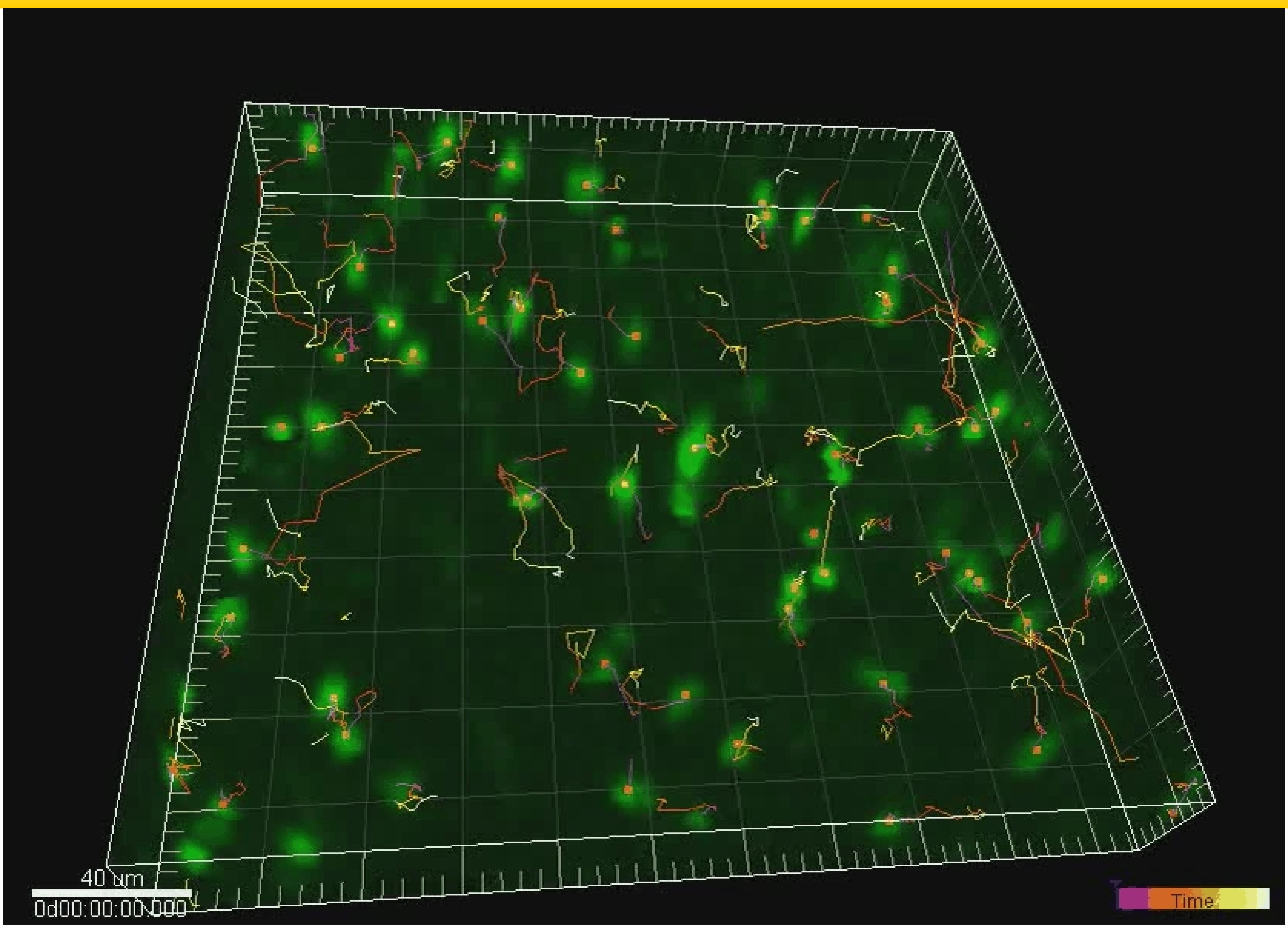
FILAMENT TRACING AND ANALYSIS – EXAMPLE



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

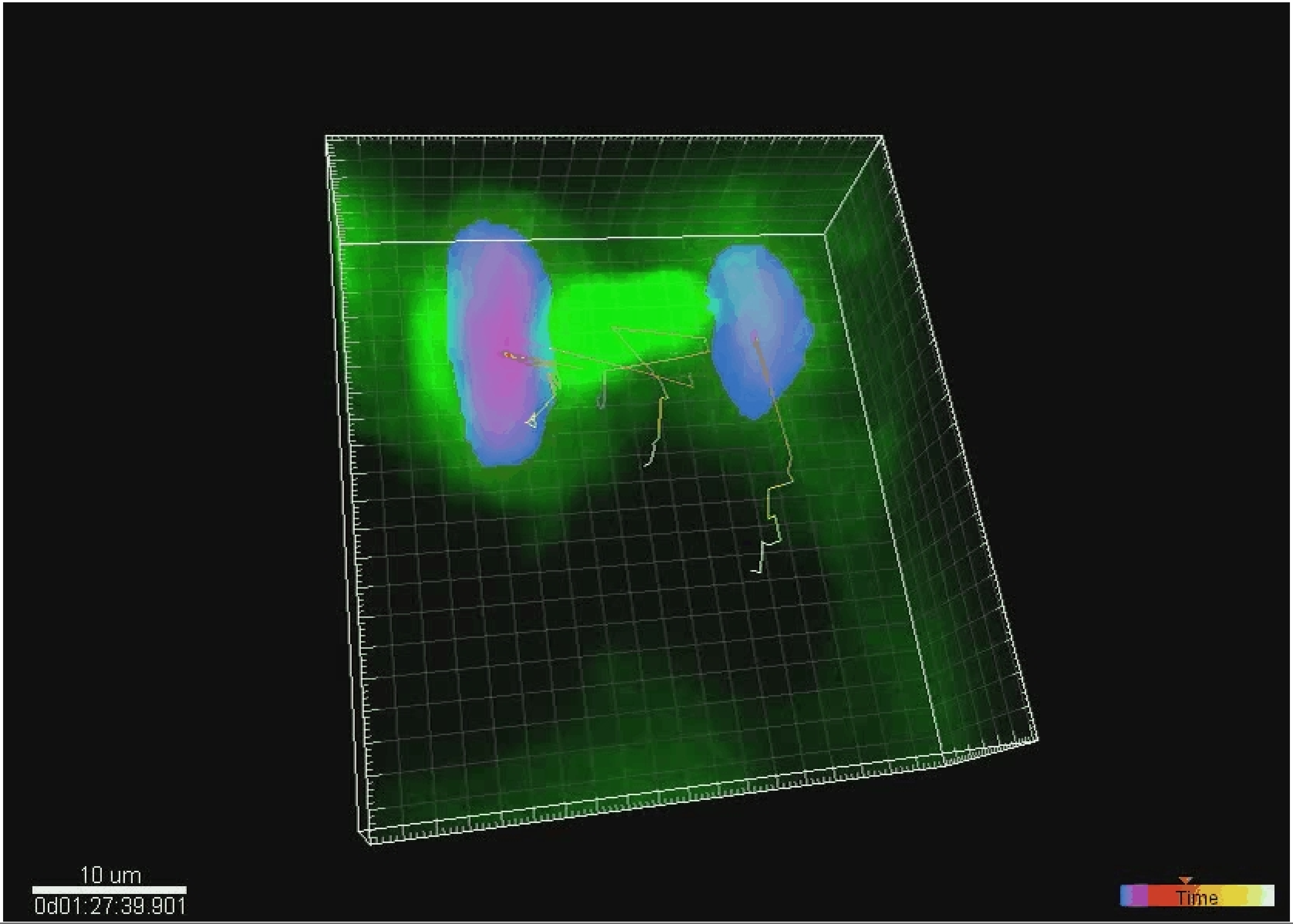
PARTICLE DETECTION AND TRACKING



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

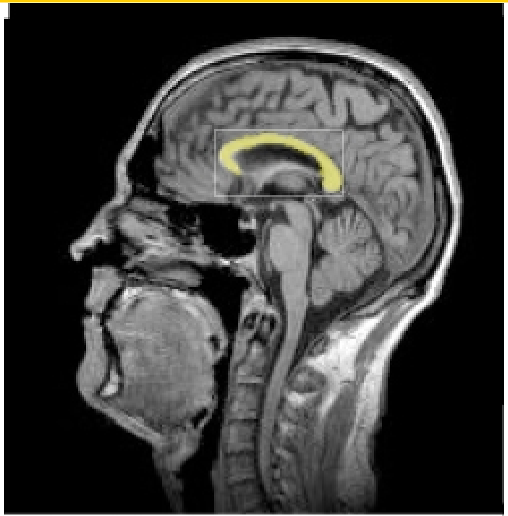
CELL SEGMENTATION AND TRACKING



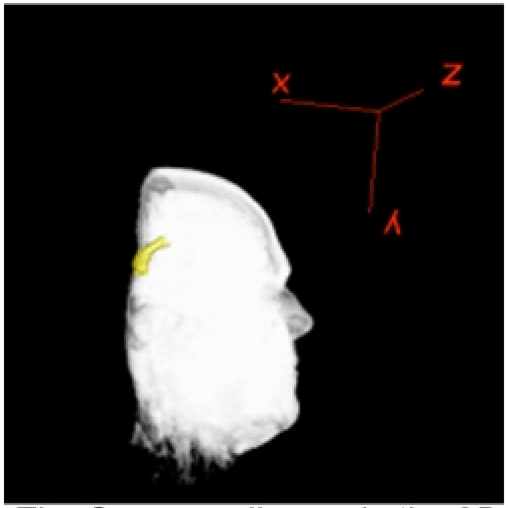
SOFTWARE TOOLS

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

- Nature Methods, July 2012, Volume 9 No 7, Focus on Bioimage Informatics
- Biological Image Analysis Primer, E. Meijering and G. van Cappellen, Erasmus MC, October 2006



Segmentation on a 2d slice of the Corpus callosum



The Corpus callosum in the 3D Viewer

THANK YOU

- Questions?

BioCampus Montpellier

MRI
Technologies Informatique et Gestion des Ressources
TIGR