

# Image Analysis

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# Overview

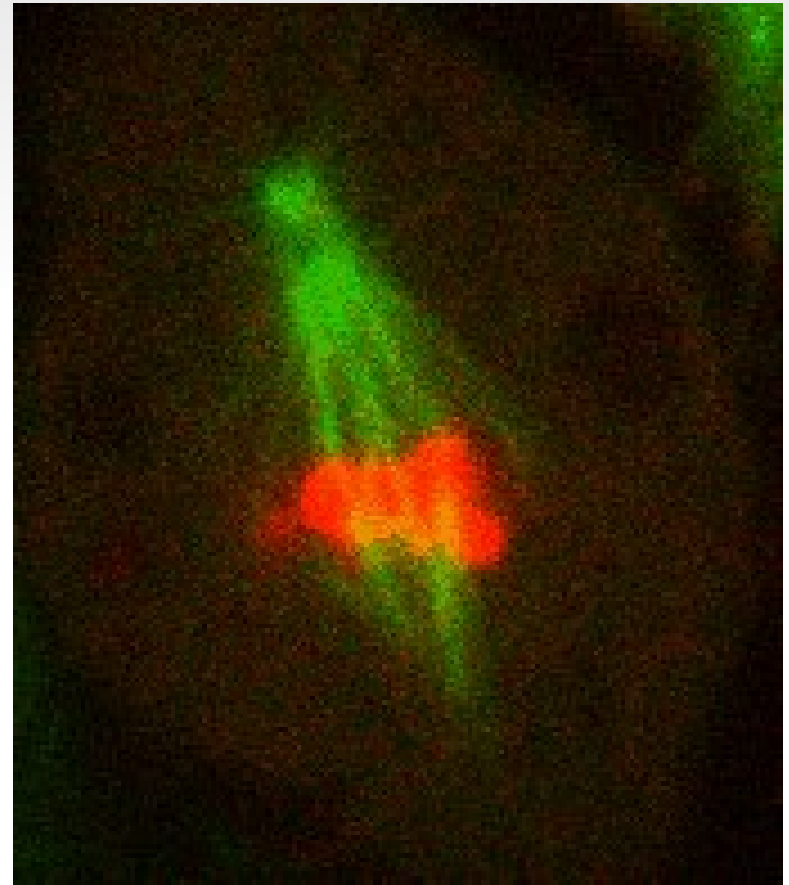
1. Digital image
2. Image Analysis
3. Point Operations
4. Local Filtering
5. Filtering in the frequency domain
6. Image Restoration
7. Segmentation
8. Geometrical Transformations
9. Visualization
10. Colocalization
11. Filament Tracing
12. Particle Tracking
13. Cell Tracking
14. Software Tools

# What is a digital image - examples

Example 1: Fly Brain example (ImageJ)



Example 2: Mitosis (ImageJ)



Example 3: Embryonic Zebrafish Heart Development

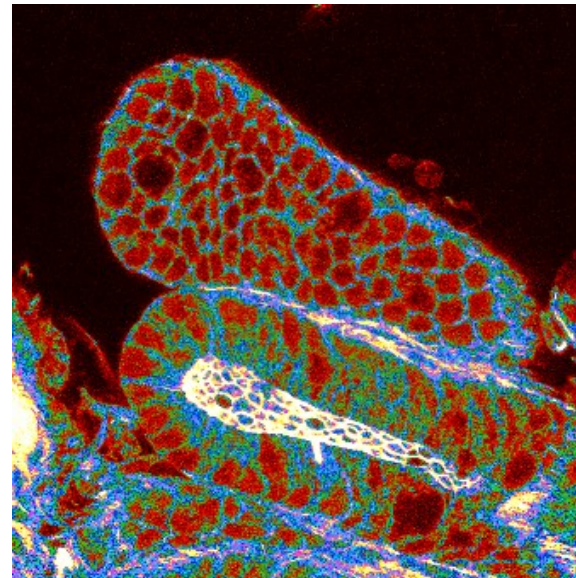
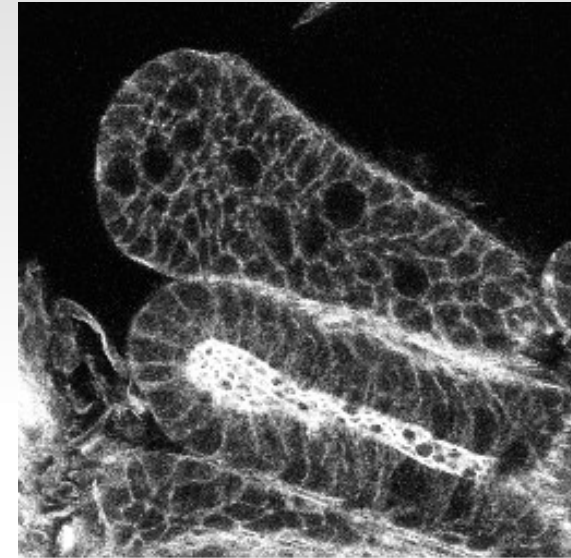
# What is a digital image - mathematical point of view

- 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

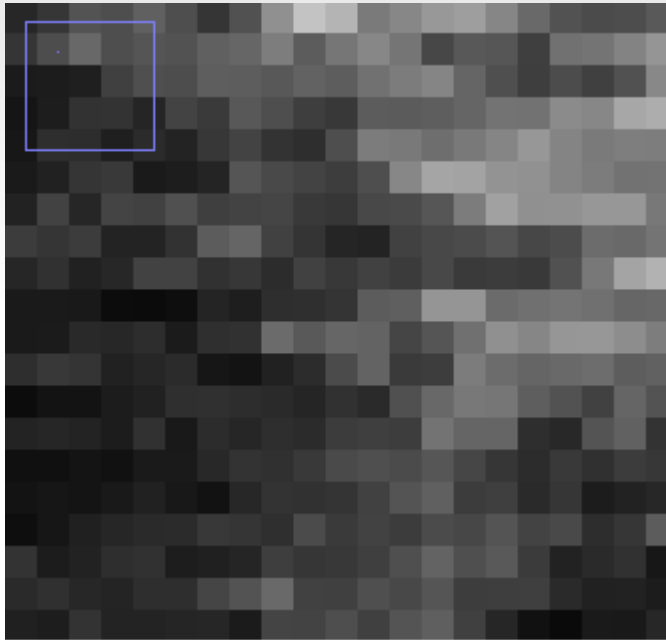


When I use a word," Humpty Dumpty said, in rather a scornful tone, "it means just what I choose it to mean—neither more nor less.

L. Carroll, Through the Looking-Glass

# What is a digital image - displayed by the computer

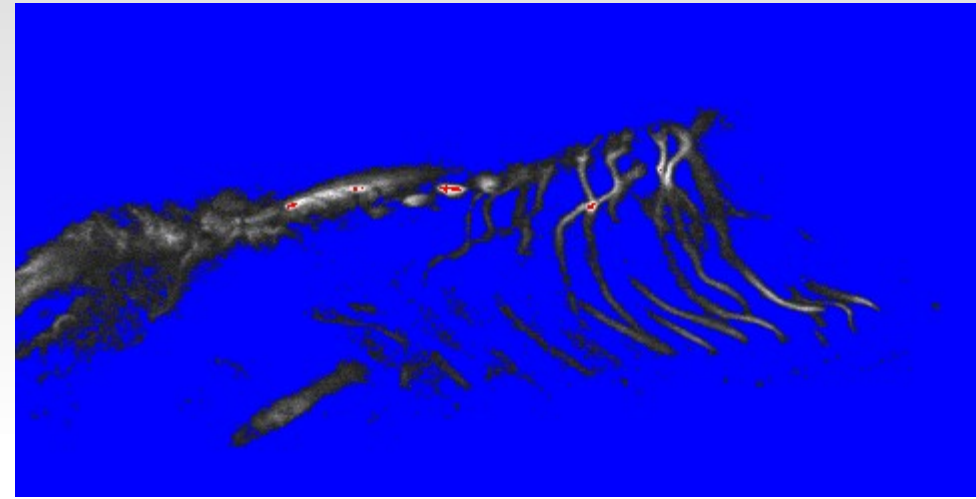
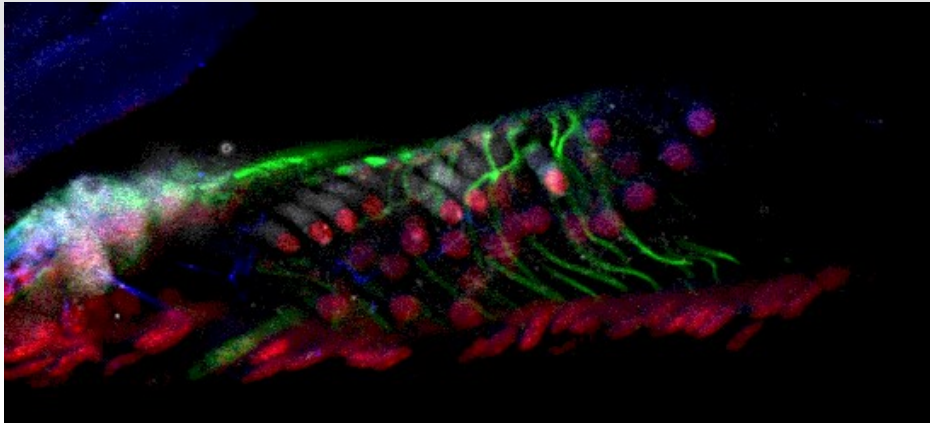
- mapping between sample grid and display grid
  - homogenous rectangles
  - interpolation



# 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	$-3.4 \times 10^{38}$ - $+3.4 \times 10^{38}$	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

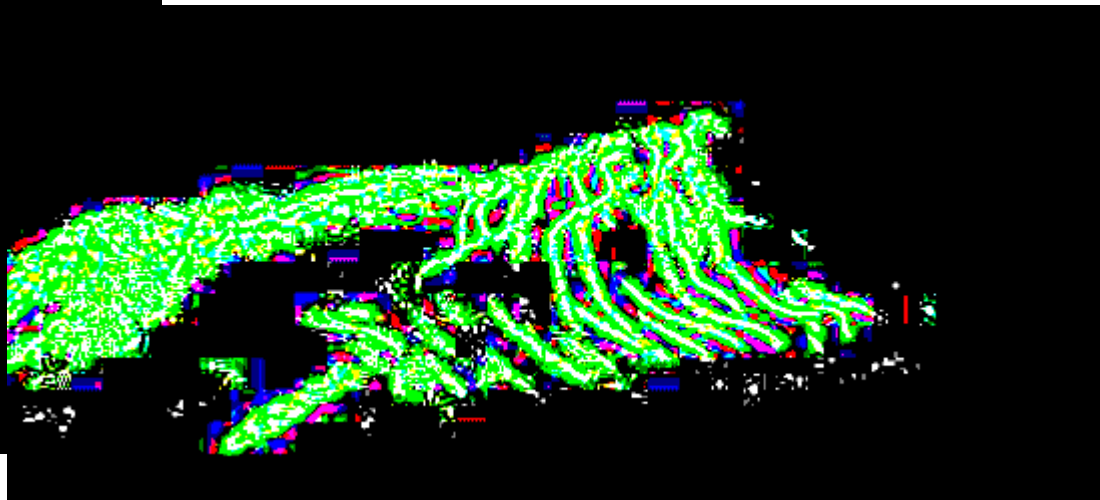
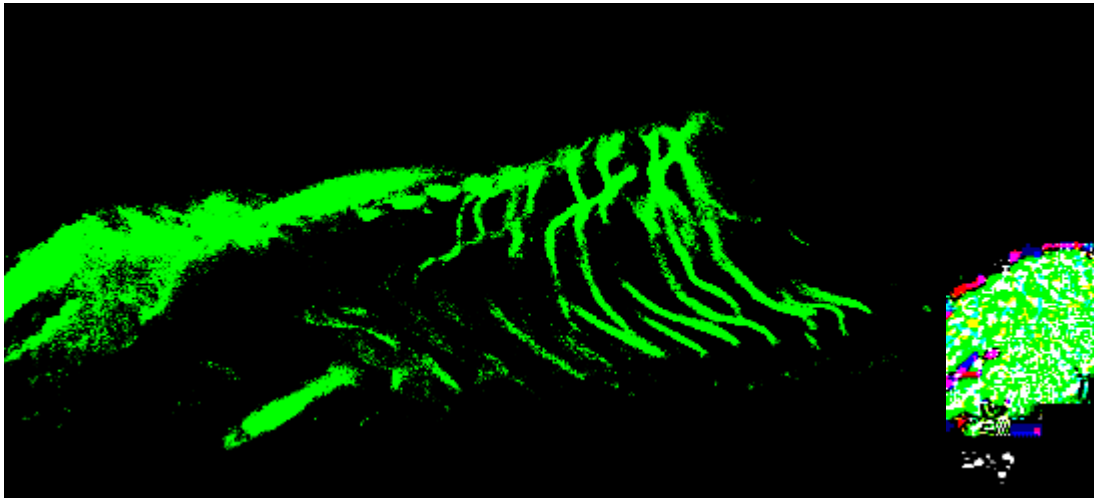
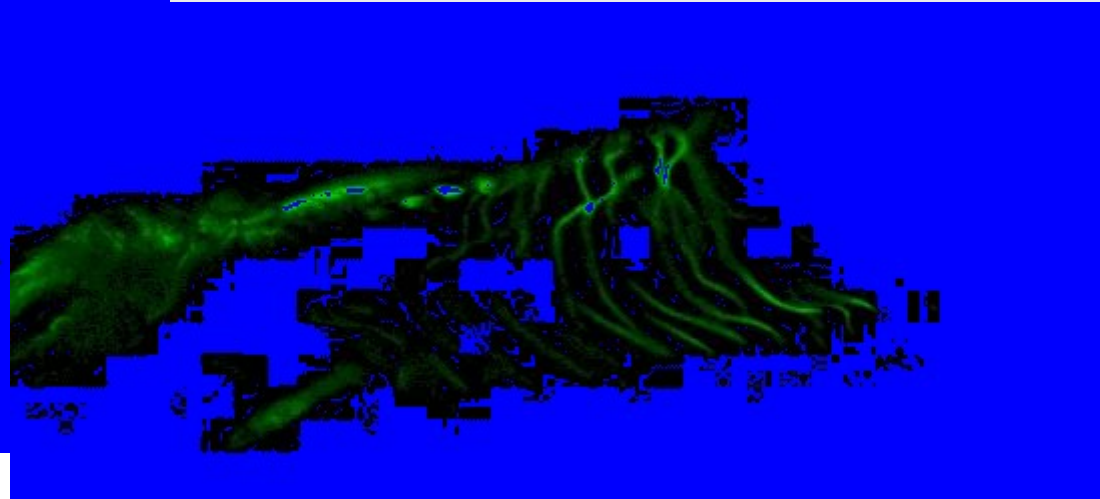
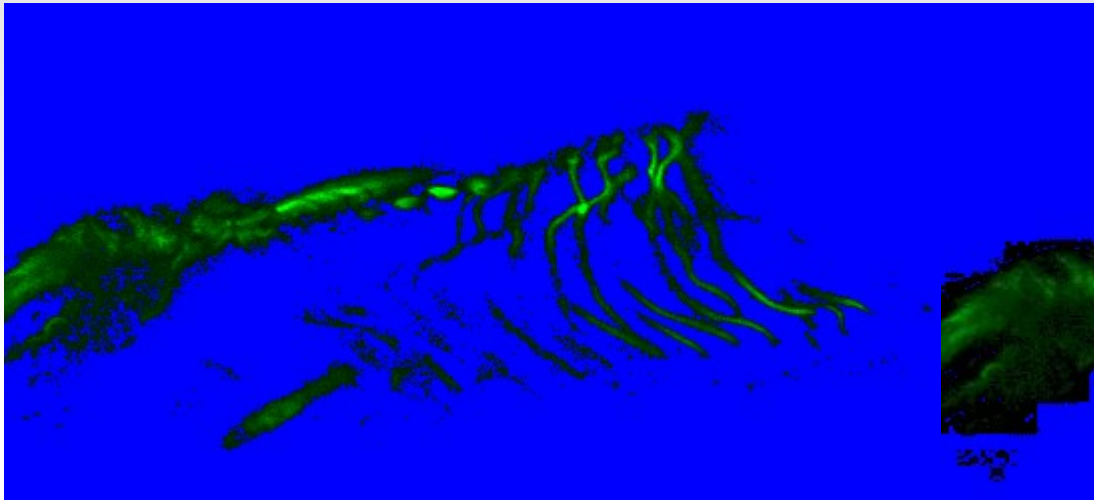
# What is a digital Image - stored on a disk

- some Ism-metadata

BitsPerPixel	8	DC#1 Pinhole Diameter	896.0
DimensionOrder	XYCZT	DC#1 Pinhole Name	PH1
IsInterleaved	false	DC#1 SPI Wavelength End	798.88
IsRGB	false	DC#1 SPI Wavelength Start	456.48
LittleEndian	true	...	
PixelType	uint8		
Series 0 Name	cafe		
SizeC	2		
SizeT	1		
SizeX	1320		
SizeY	1320		
SizeZ	1		
BeamSplitter #1 Acquire	true		
BeamSplitter #1 Filter	None		
BeamSplitter #1 Filter Set	FW1		
BeamSplitter #1 Name	FW1		

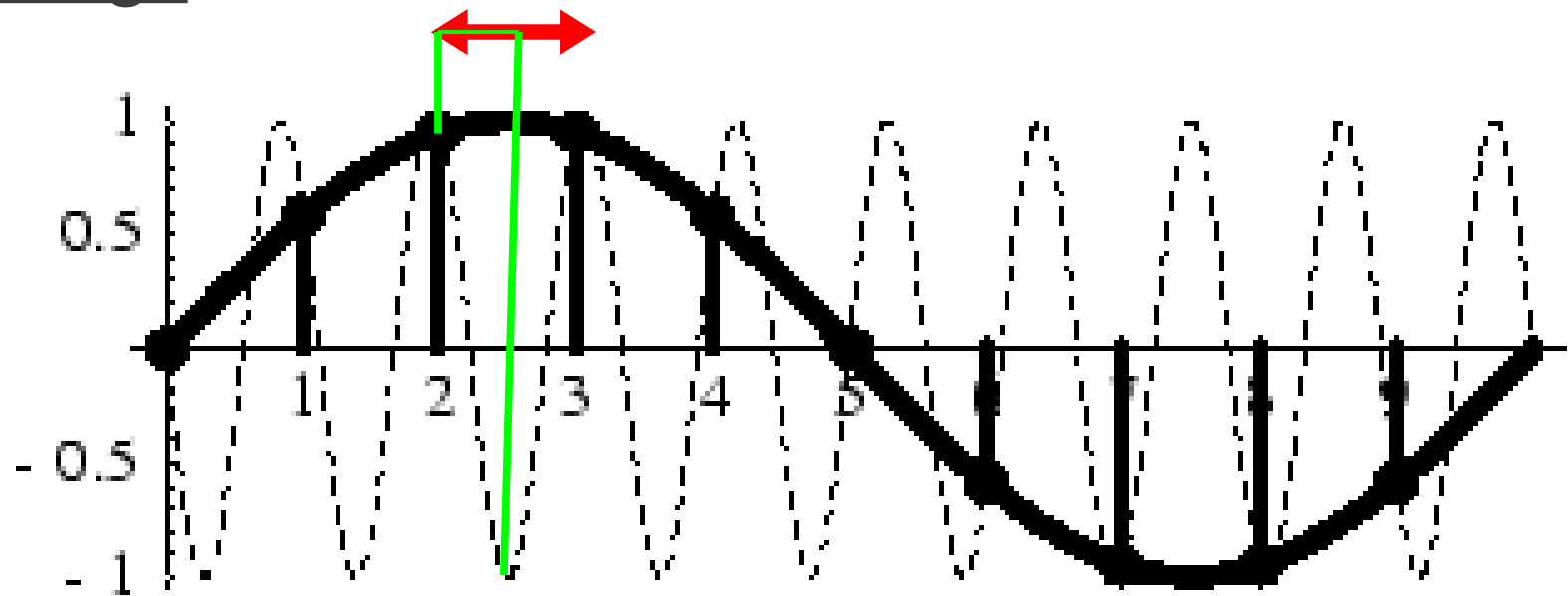
# What is a digital Image - stored on a disk

- artefacts from jpg-compression



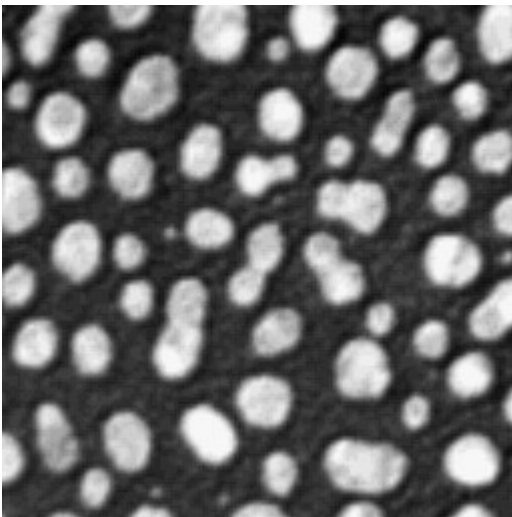
# What is a digital image - the image and the real world

- 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

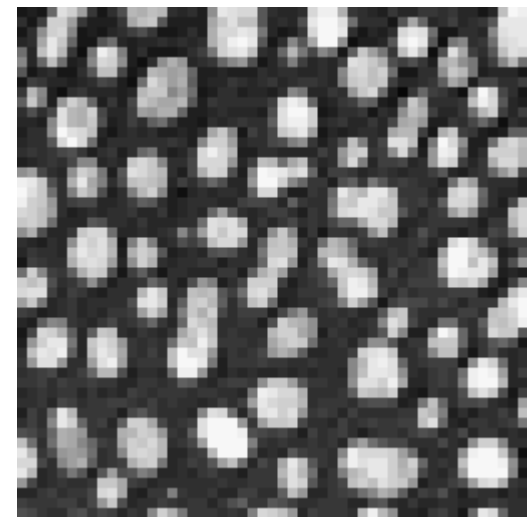


# What is a digital image - the image and the real world

- 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

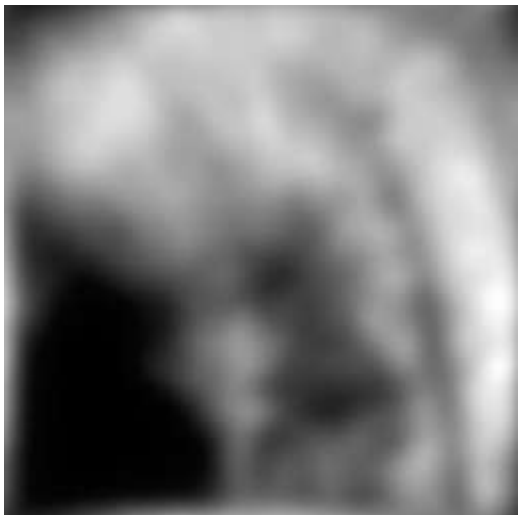


$$\Delta x < \frac{\lambda_{em}}{4 * NA} \quad \text{for widefield}$$
$$\Delta x < \frac{\lambda_{ex}}{8 * NA} \quad \text{for confocal}$$

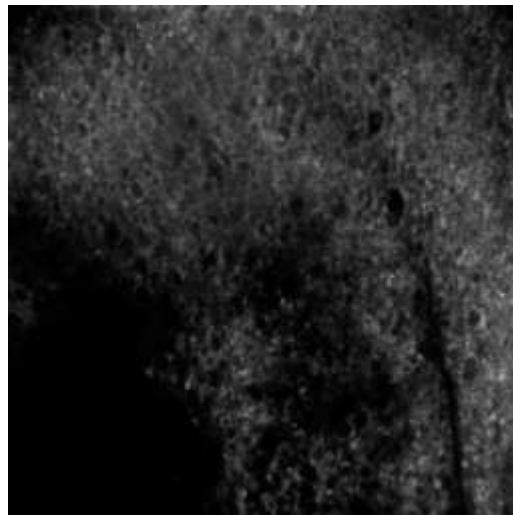


# What is a digital image - the image and the real world

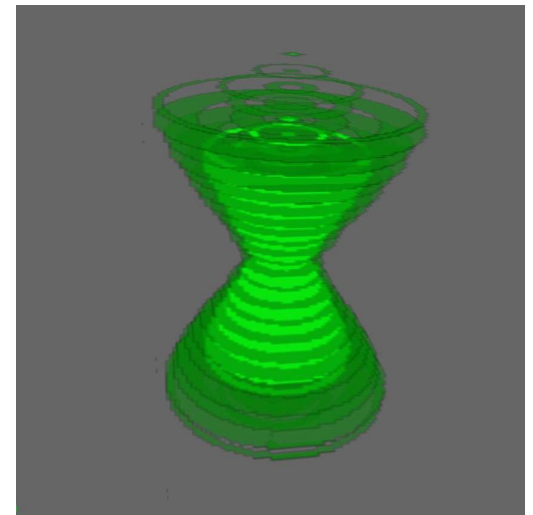
- point spread function
  - the way an optical system images one point
    - a point = an object at the limit of the resolution
  - acquired image = object function **convolved with** psf



=

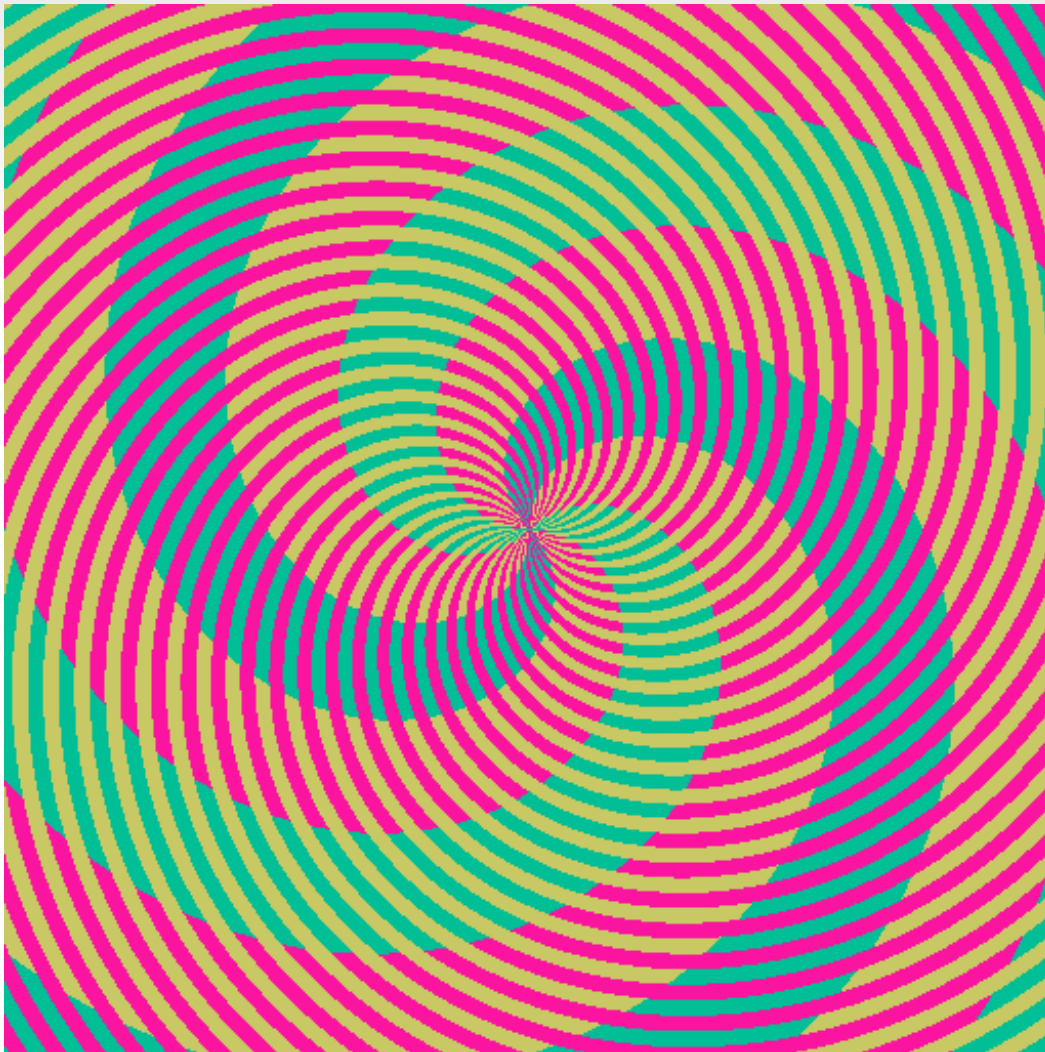


\*

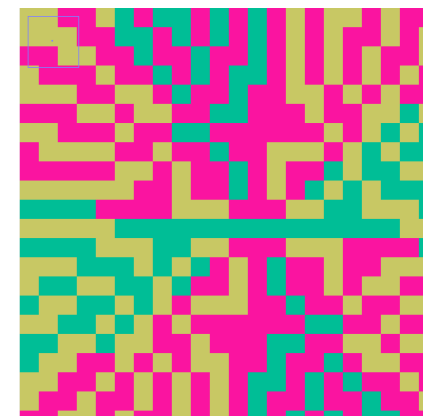


# What is a digital image - image and perception

- How many colors do you see?

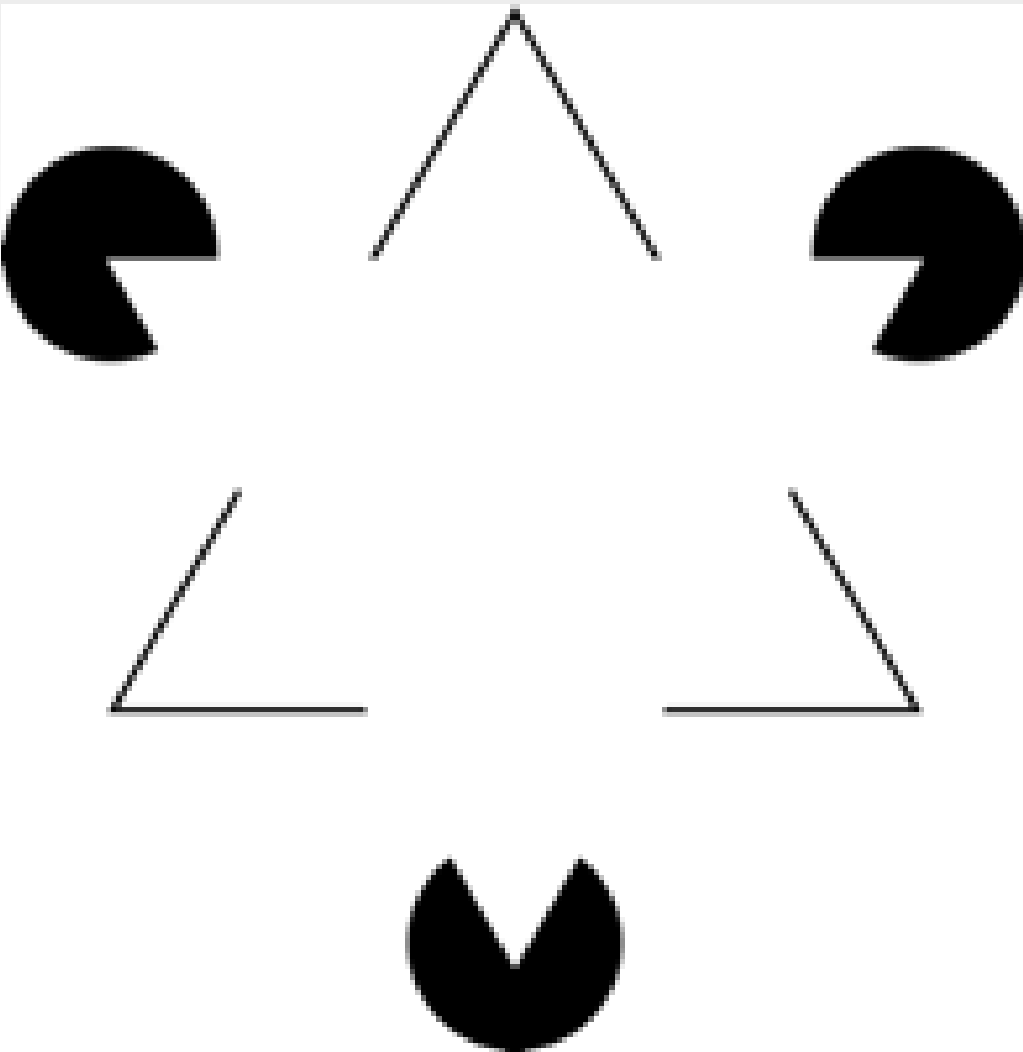


- the image contains 3 different colors
- the brain interprets color according to the background

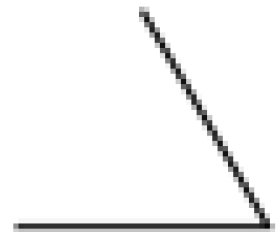


# What is a digital image - image and perception

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



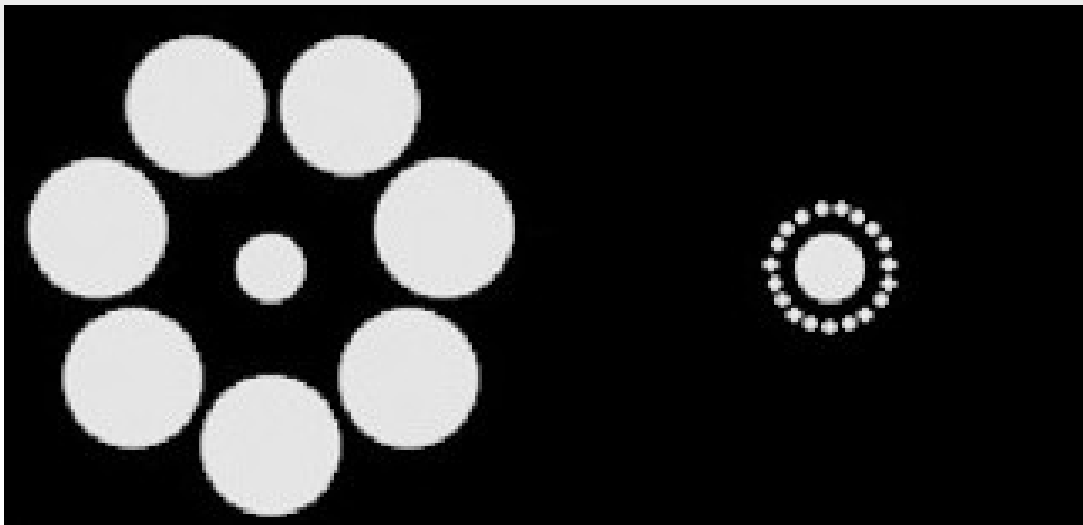
- the borders of the triangle don't exist
- the brain connects the points and interprets the scene as one triangle on top of another one





# What is a digital image - image and perception

- Which of the two central spots is bigger?



- both have the same size
- again the interpretation is relative to the environment

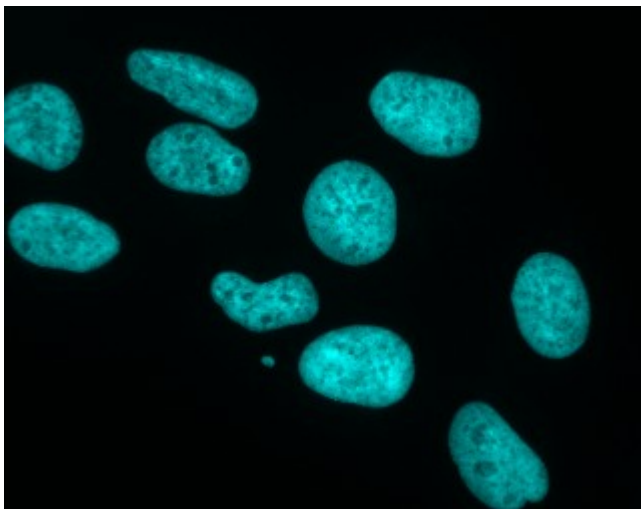
- conclusions

- we can not always trust our senses when comparing images
- quantification needed



# 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."

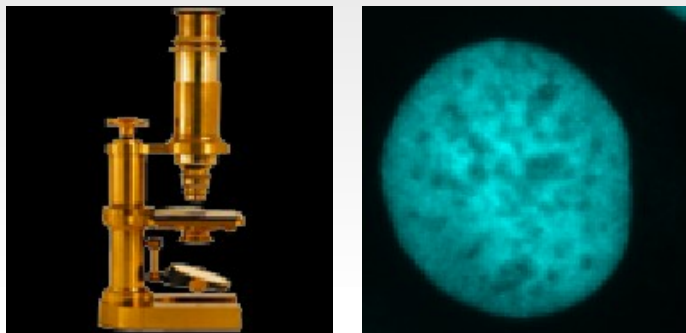


	Area	Perim.	
1	6101	353.061	
2	7047	329.120	
3	5455	292.392	
4	7524	328.191	
5	5653	300.978	
6	6178	304.392	
7	4583	296.392	
8	7312	333.120	
9	6820	343.345	

- IMAGE IN – FEATURES OUT

# Image analysis and related concepts

image acquisition



object in → image out

image processing

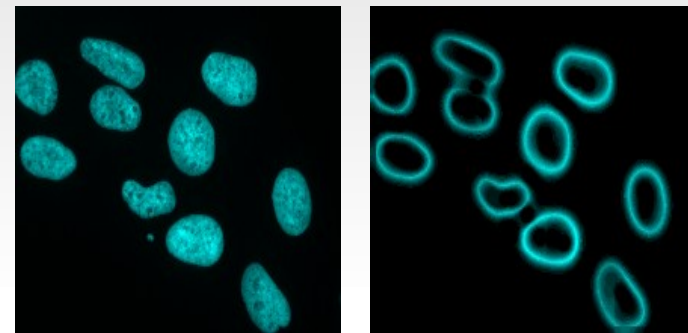
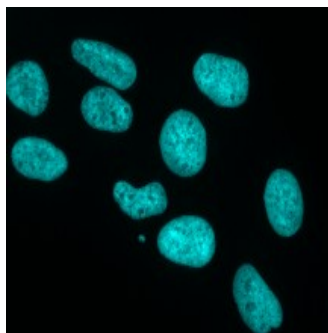


image in → image out

image analysis



	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

visualization

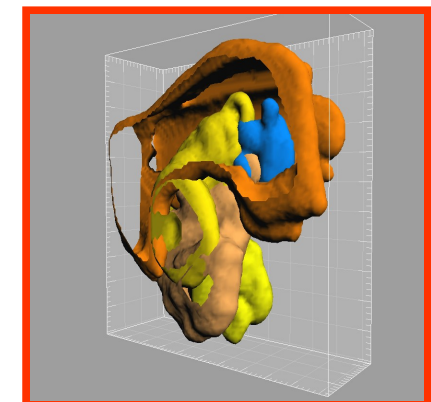
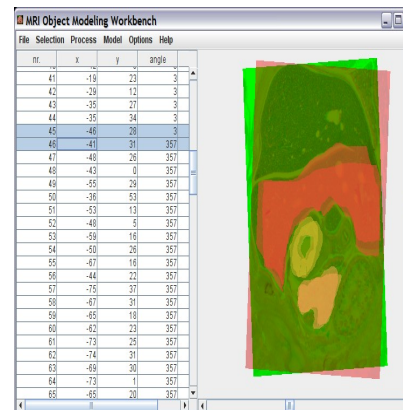
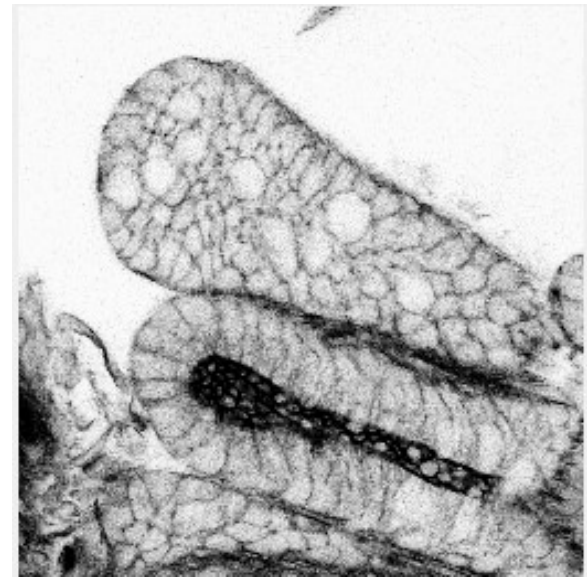
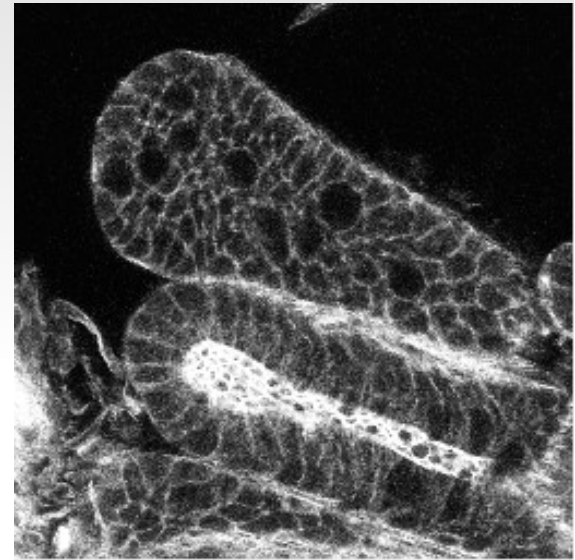


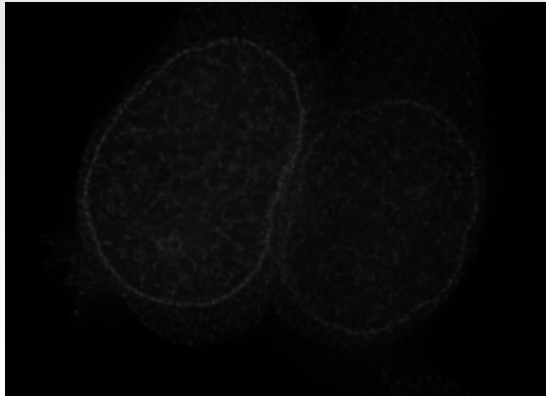
image in → representation out

# Point operations

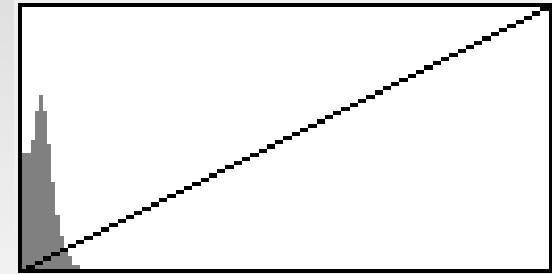
- global intensity transformations
  - intensity inversion
  - contrast and brightness adjustment
    - linear
    - gamma function
    - histogram equalization
  - pseudo-coloring
  - intensity thresholding



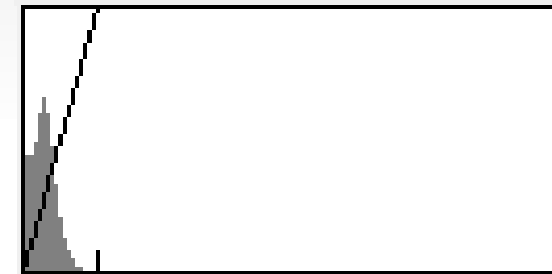
# Point operations - contrast stretching



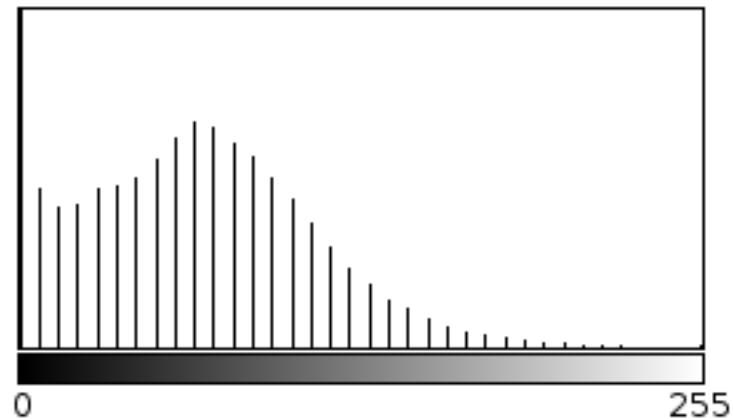
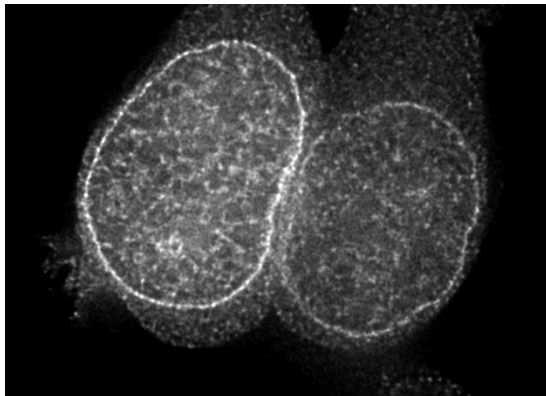
Count: 282048  
Mean: 6.968  
StdDev: 6.906  
Min: 0  
Max: 48  
Mode: 0 (89140)



0 255

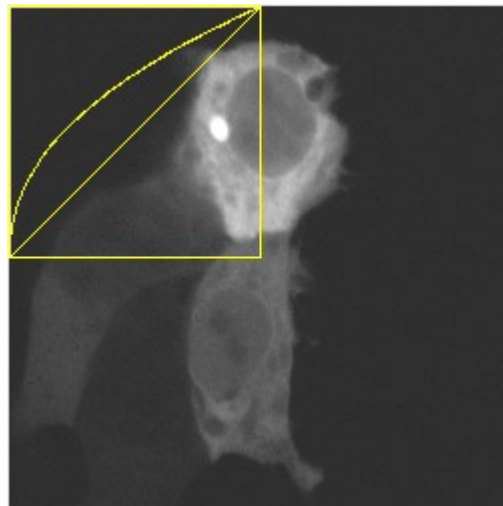
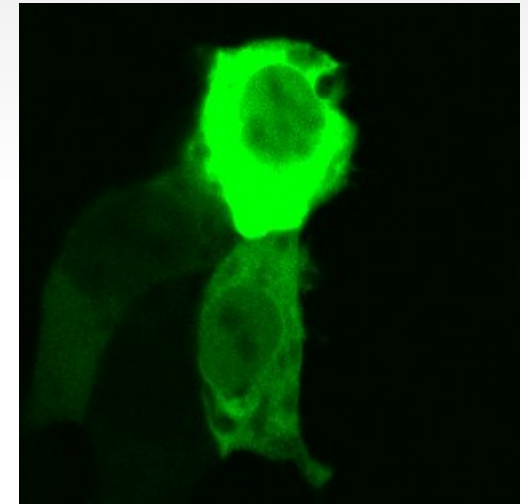
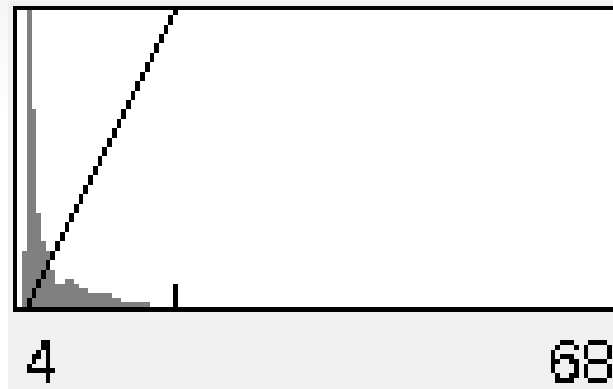
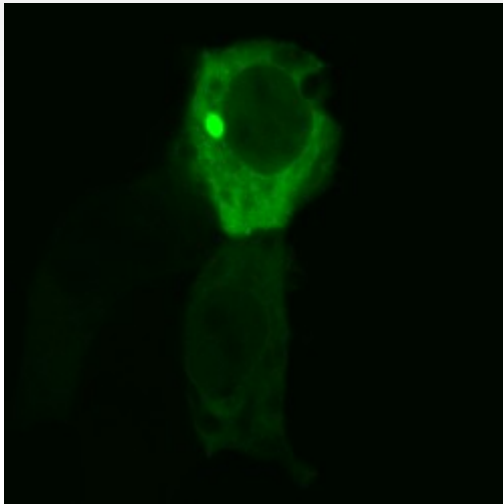


1 36



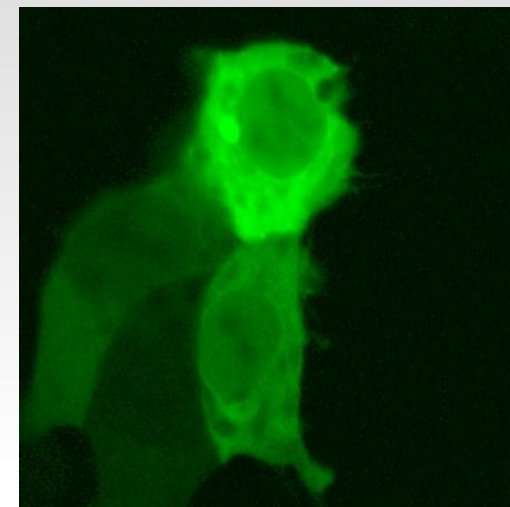
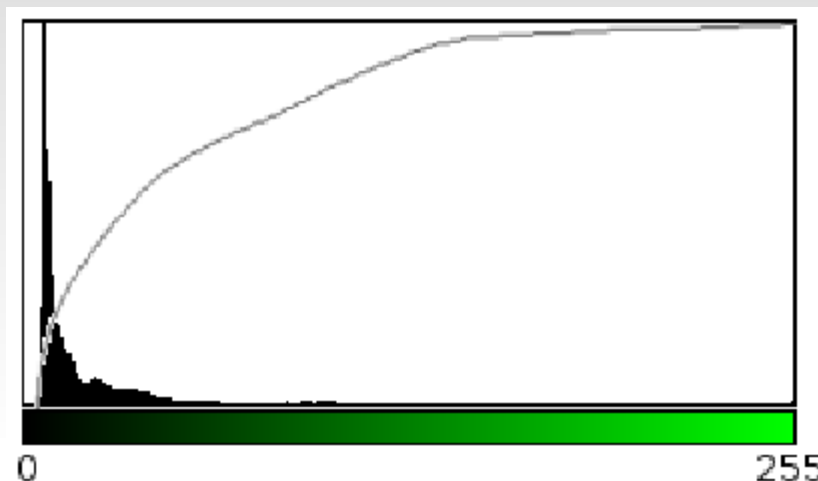
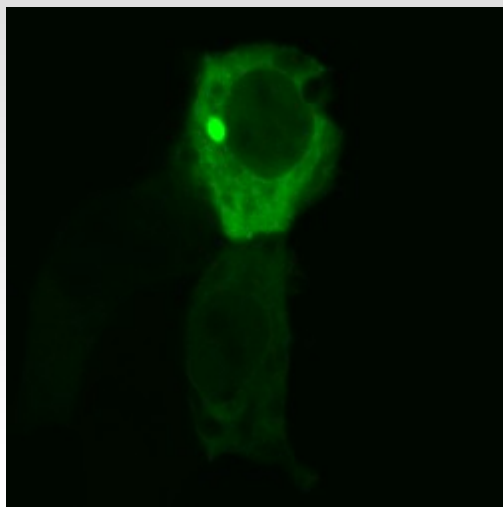
# Point operations - gamma function

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



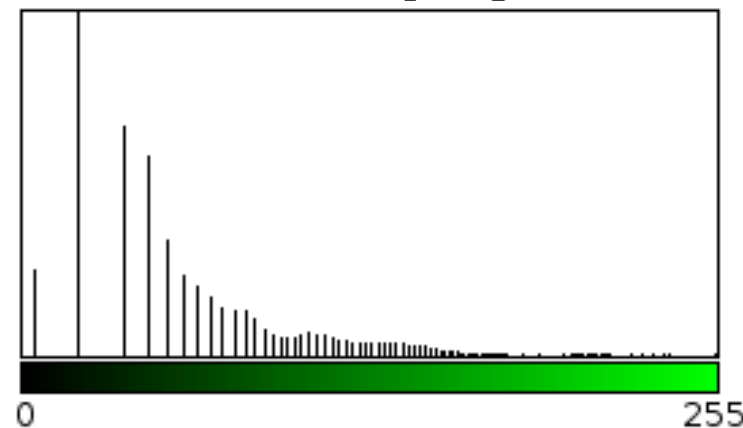
$$f(i) = \left(\frac{i}{255}\right)^{\frac{1}{\gamma}} \cdot 255$$

# Point operations - histogram equalization

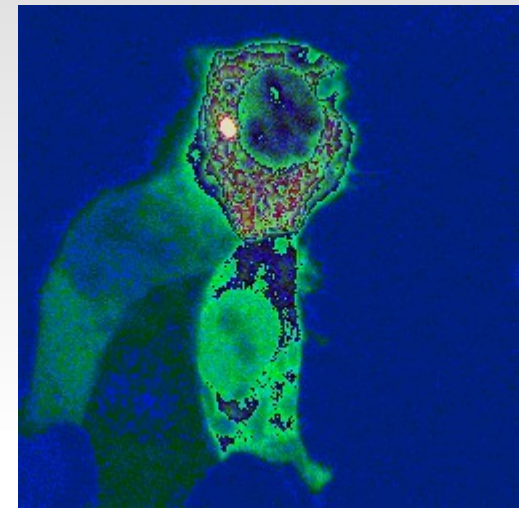
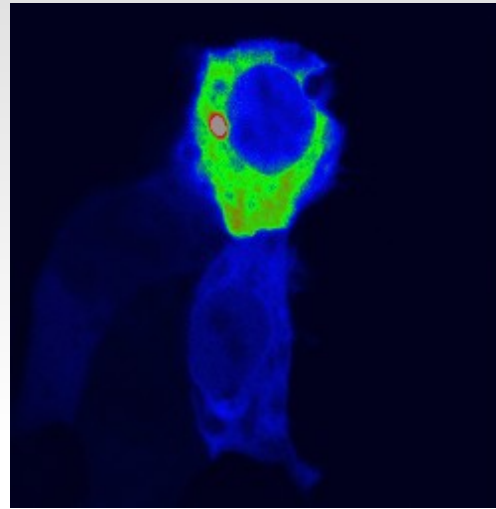
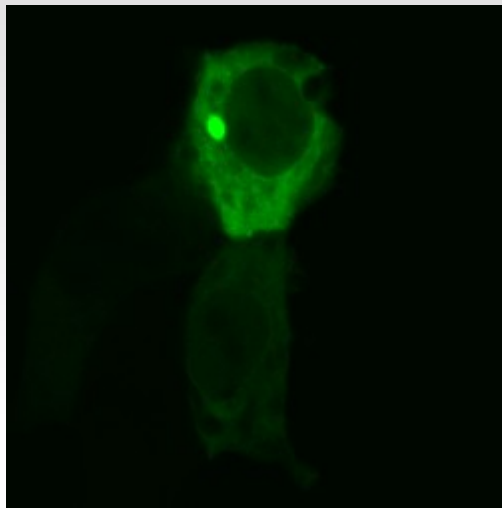


- calculate cumulative histogram
- normalize to range 0-255

$$h'(n) = \sum_{i=0}^n h(i)$$



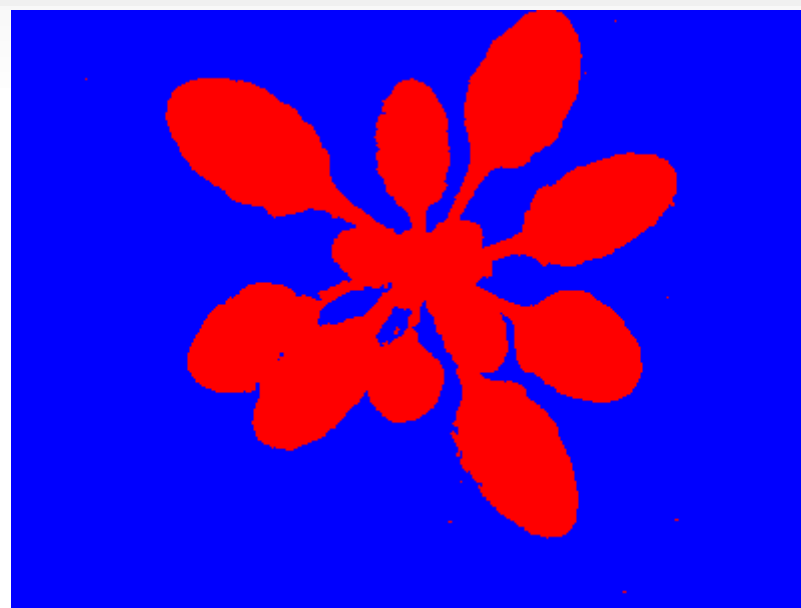
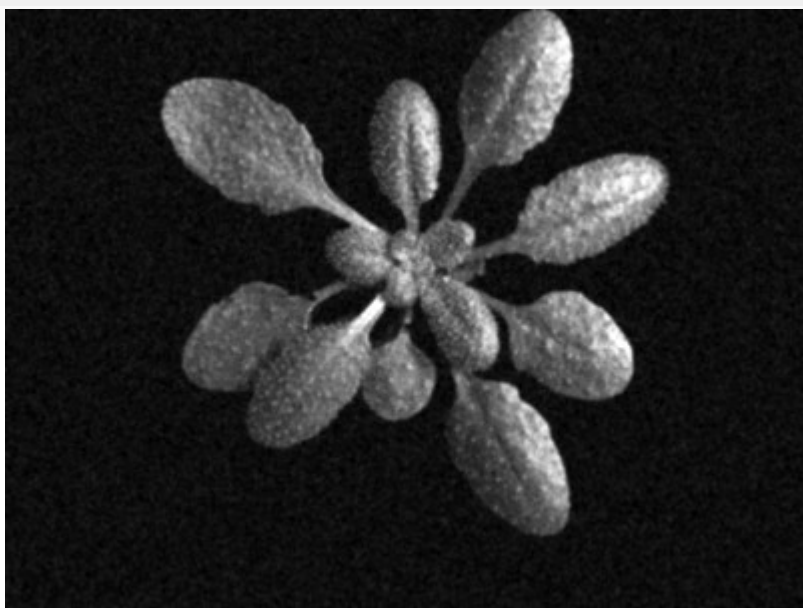
# Point operations - lookup tables



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

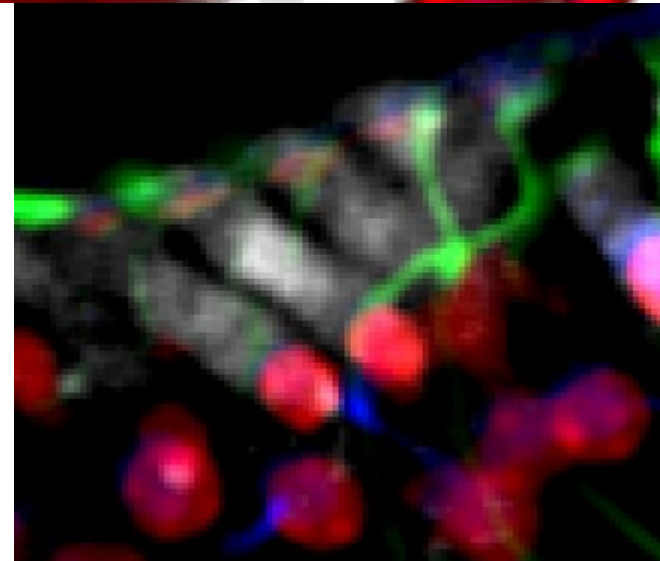
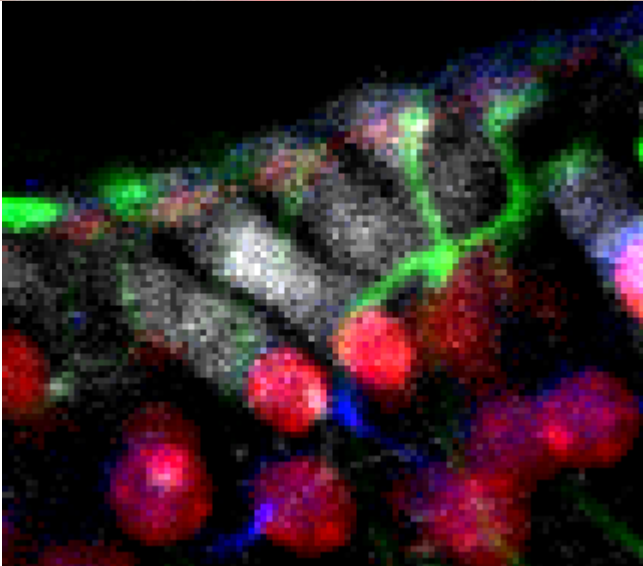
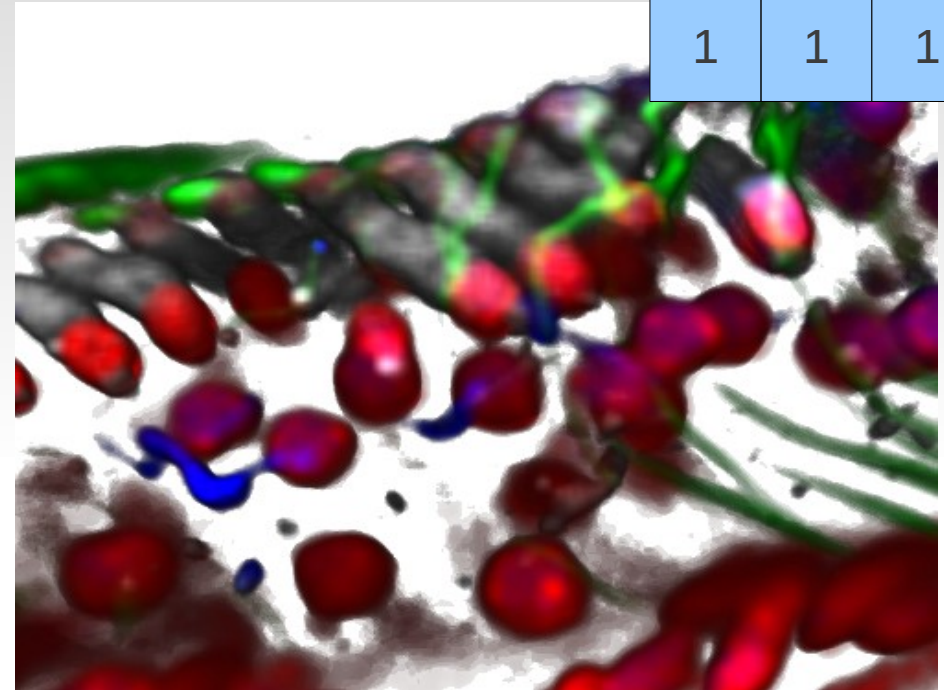
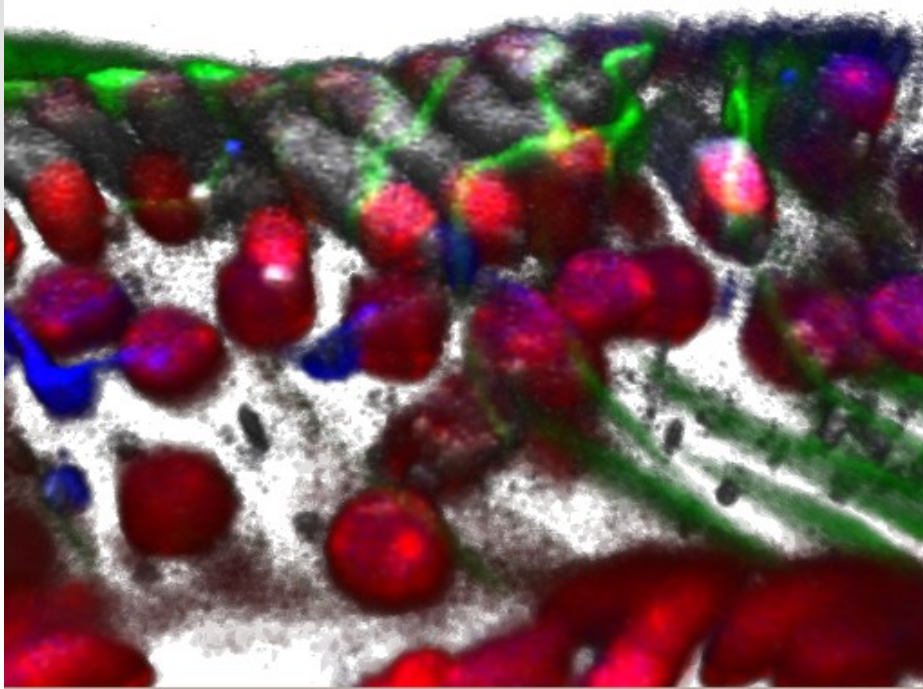
The new value of a pixel is calculated from the values in the local neighborhood of the pixel



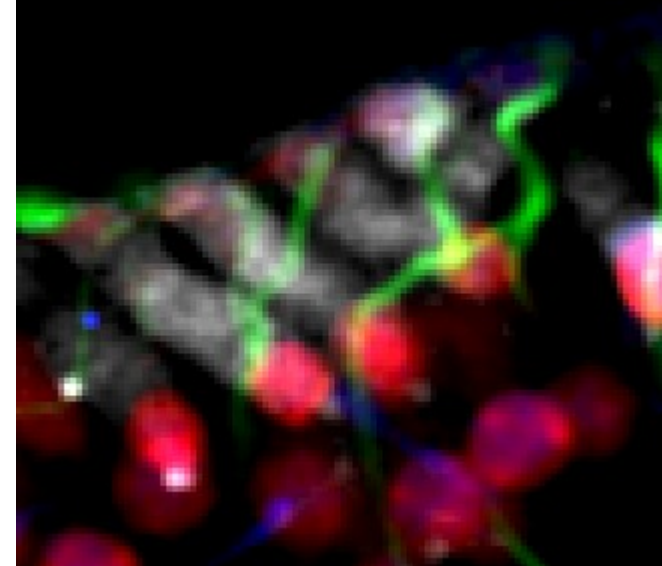
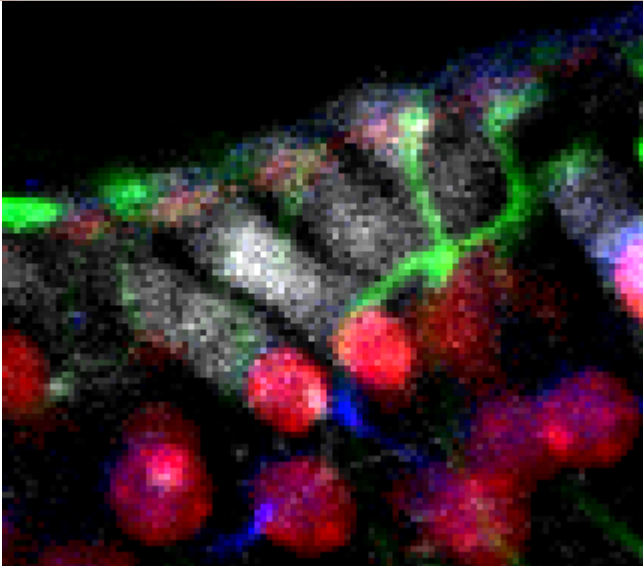
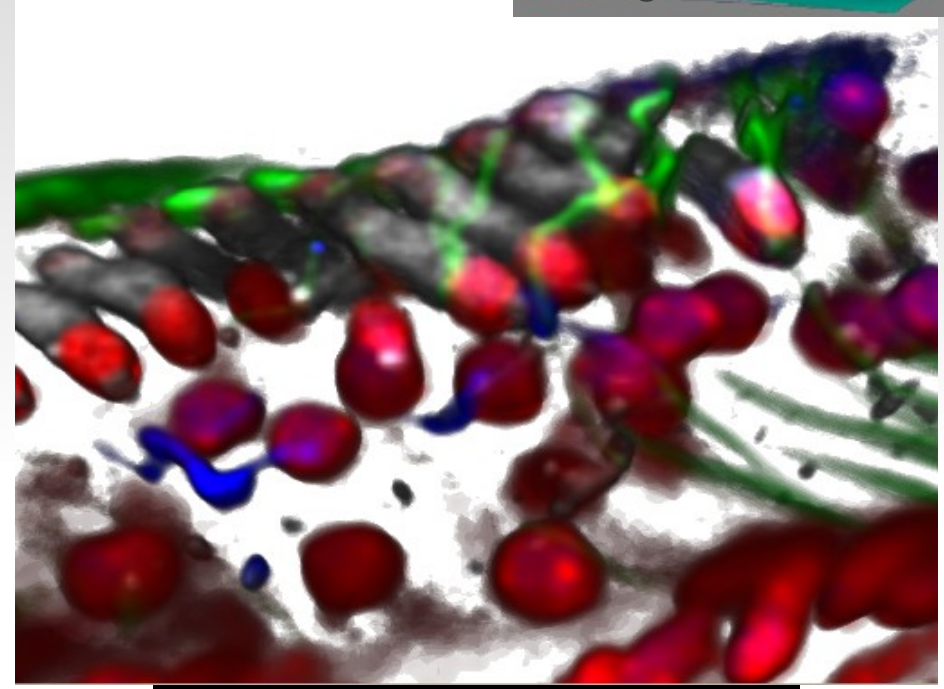
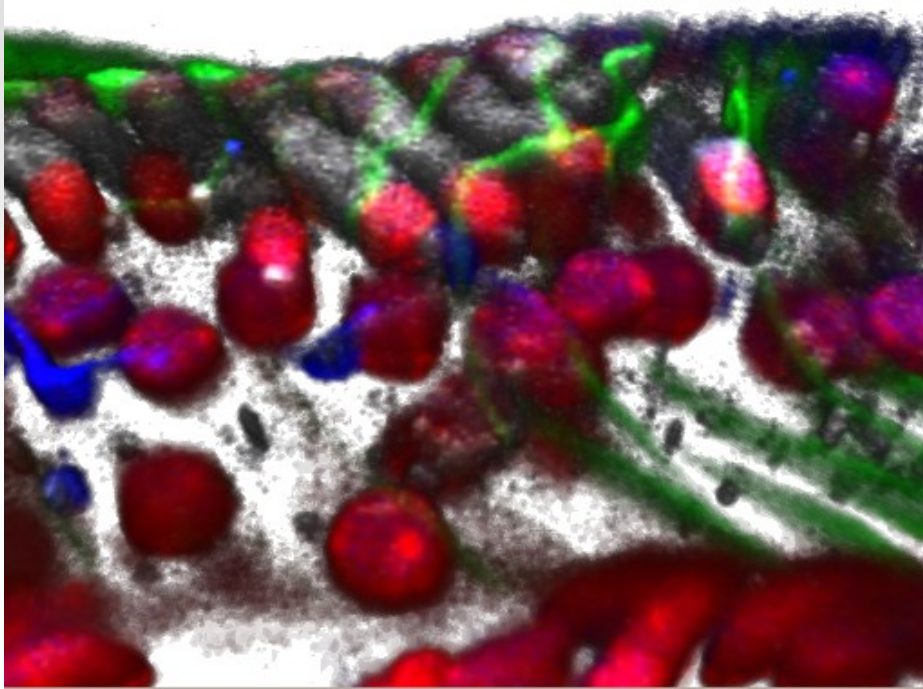
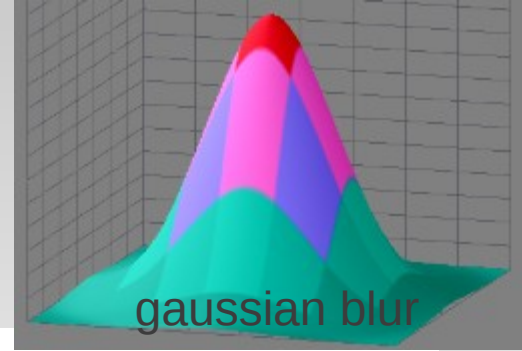
# Convolution filter - smoothing

mean

1	1	1
1	1	1
1	1	1



# Convolution filter smoothing



x-derivative

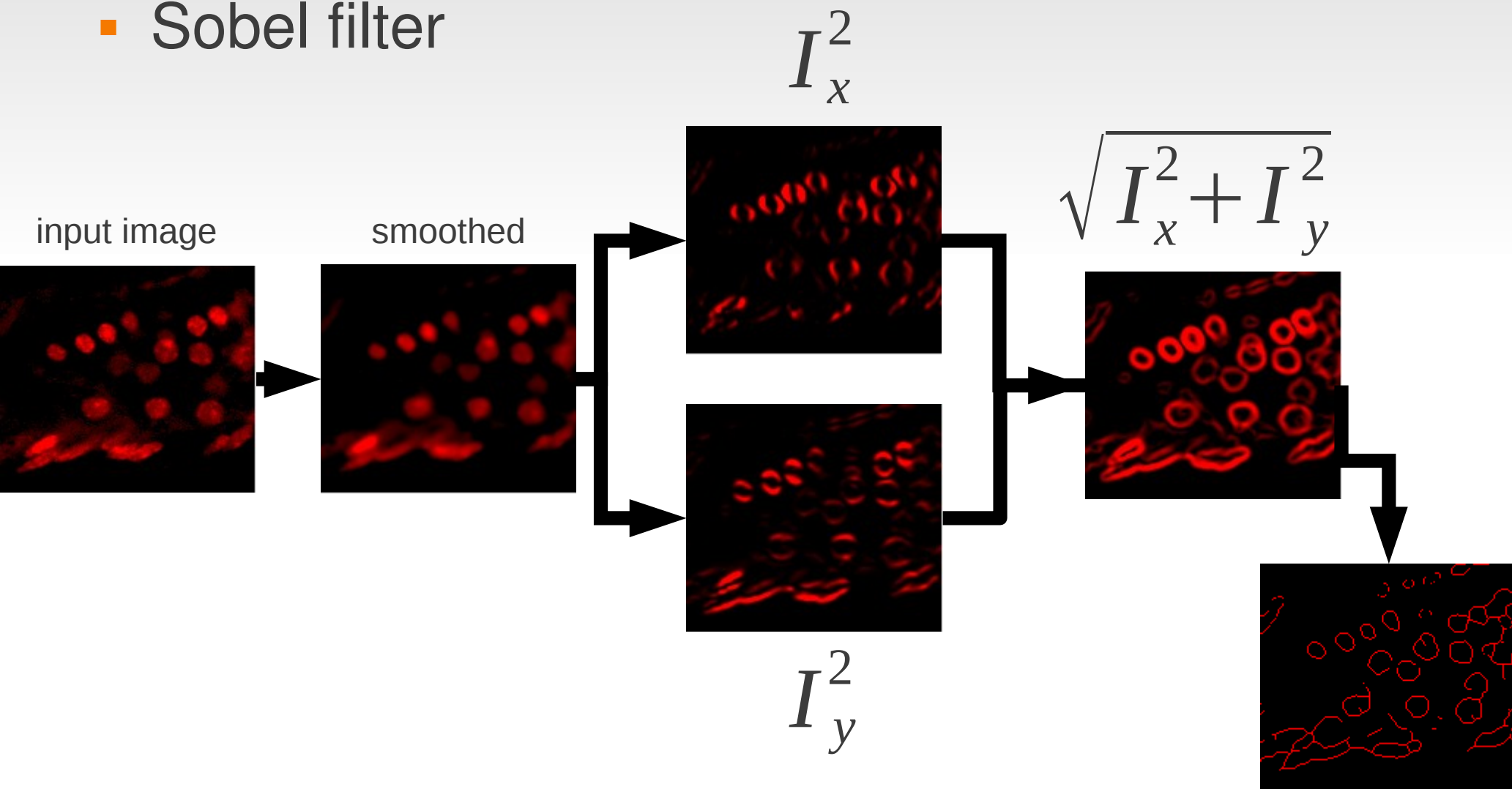
y-derivative

# Convolution filter - edge detection

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

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

- Sobel filter

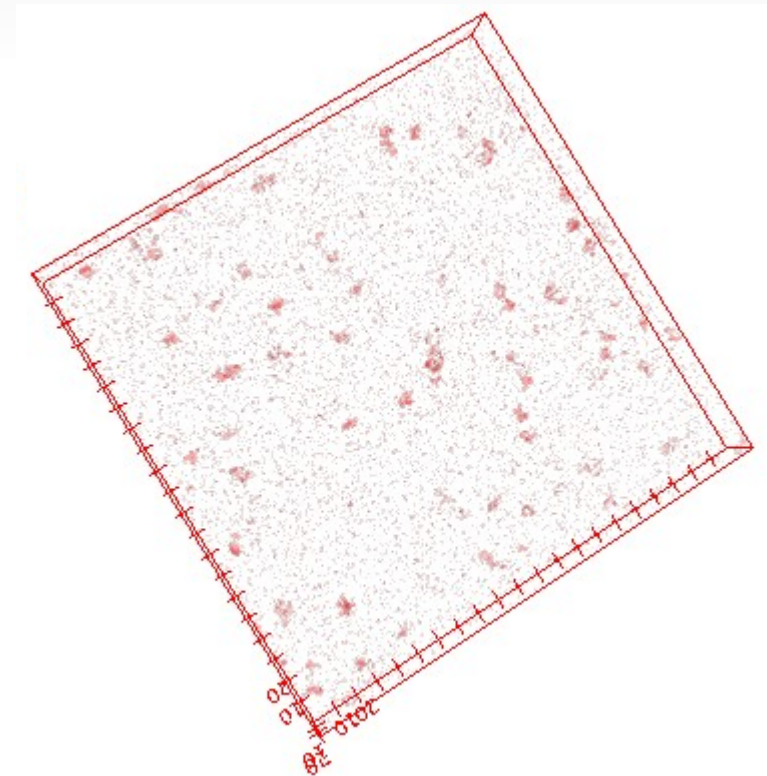
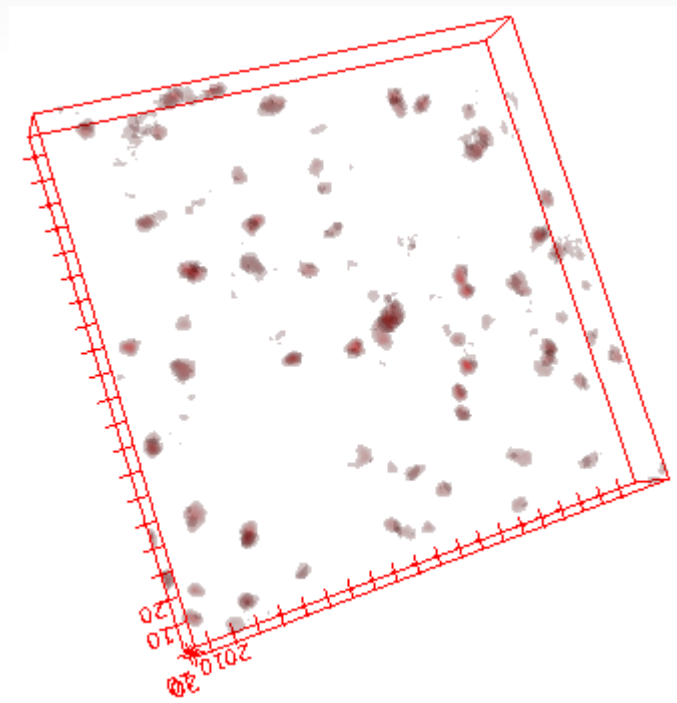


# Convolution filter - spot detection

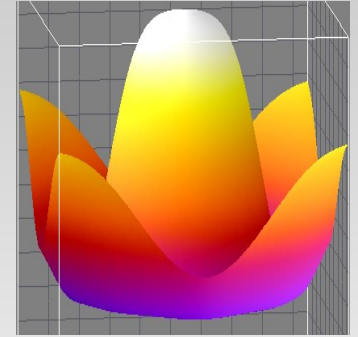
laplacian

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

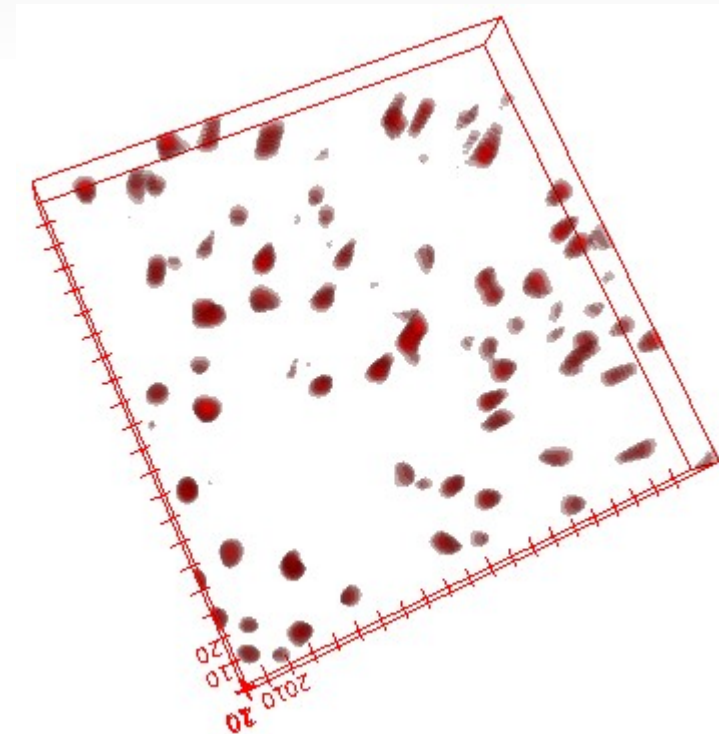
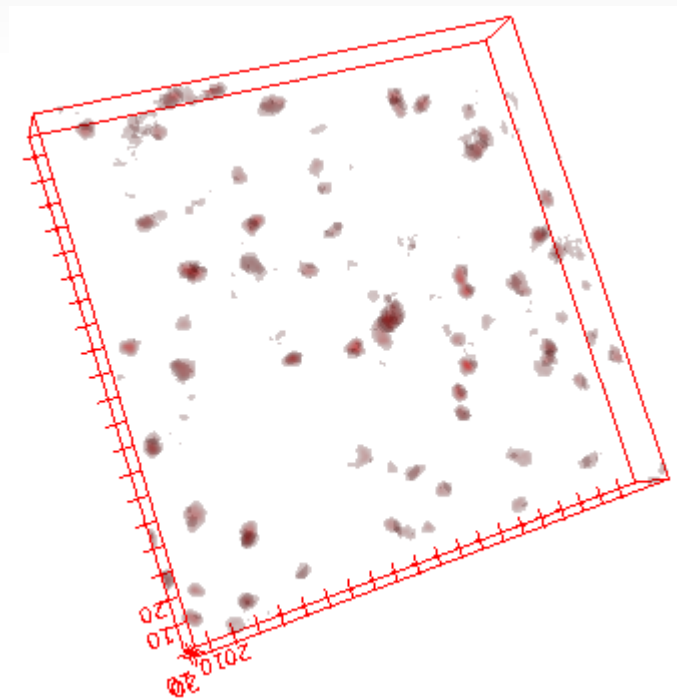
- Laplacian filter enhances spots but augments noise
- use 'Laplacian of Gaussian (LoG)' to enhance spots in noisy images



# Convolution filter - spot detection



- 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

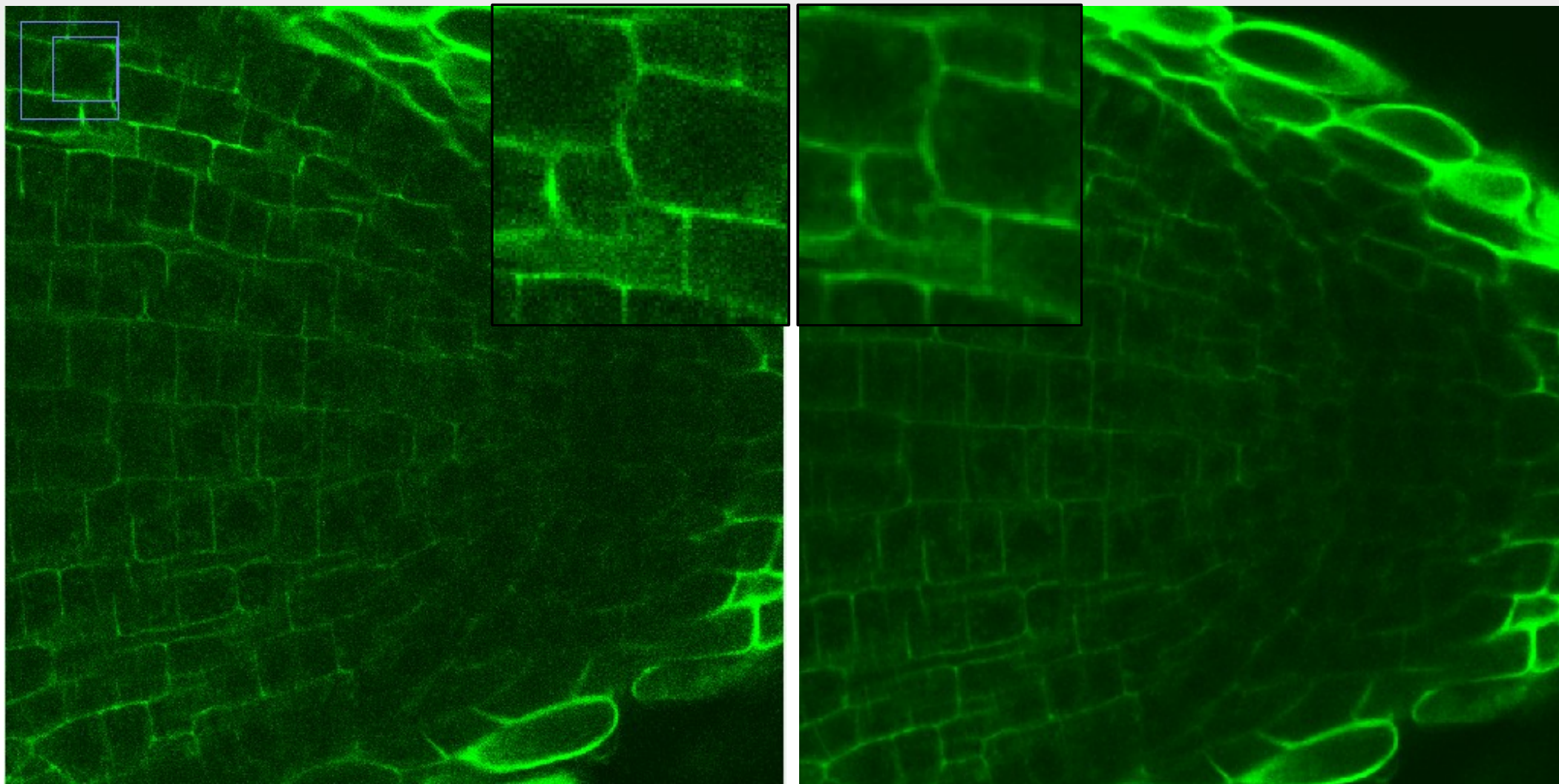


15	18	14
29	18	13
12	19	21

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

# Ranking filter - median filter

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



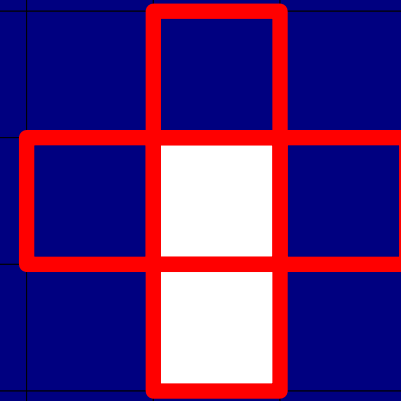
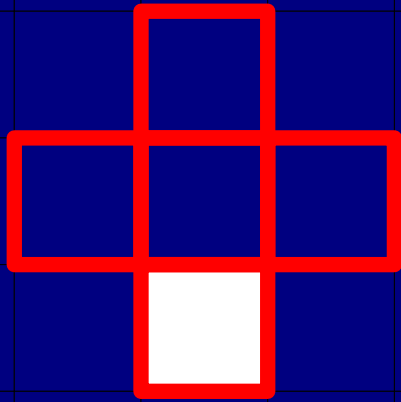
# Local Filtering

## binary morphology

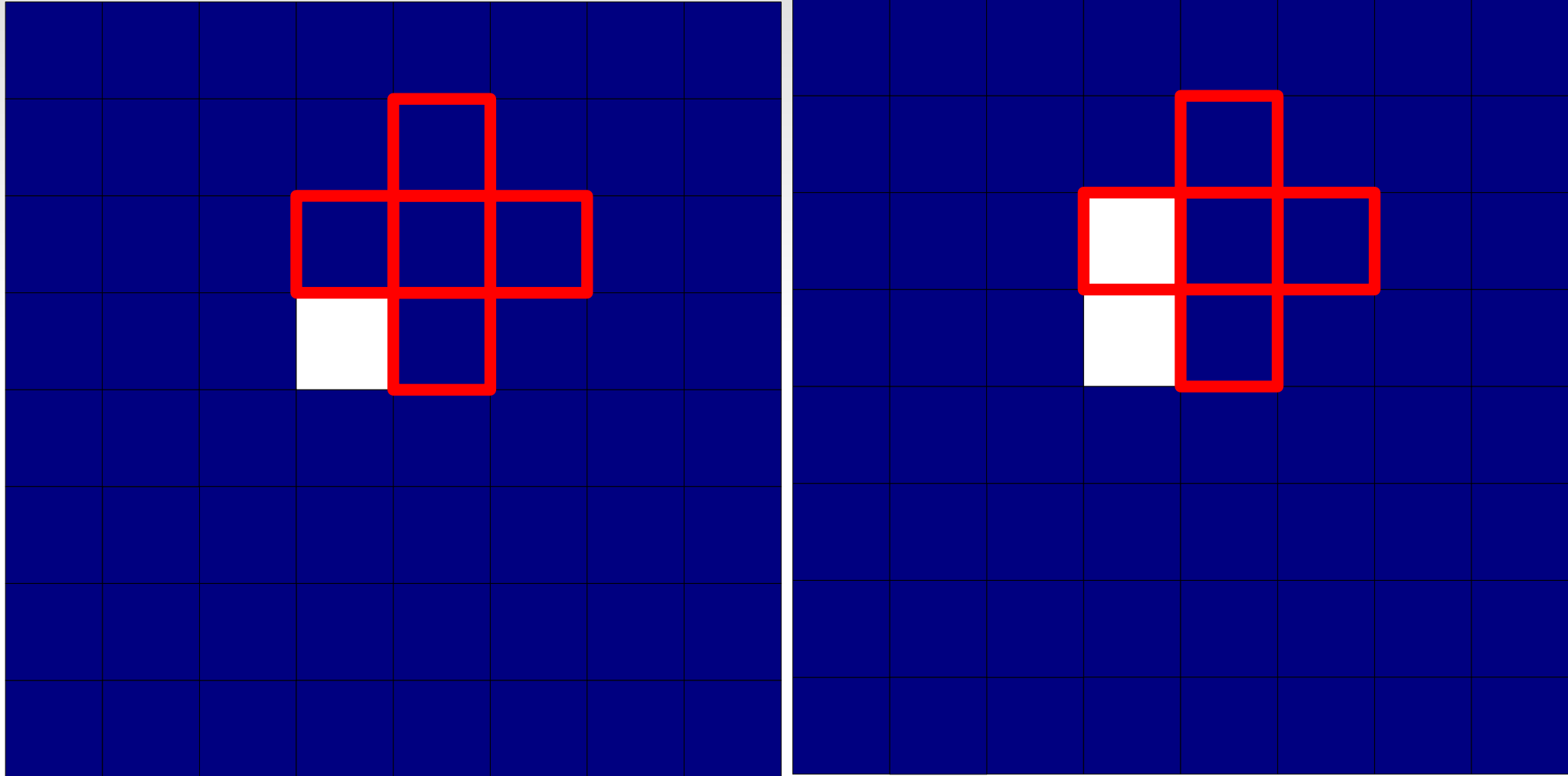
- correct segmentation, measure features, granulometry, edge detection, skeletonization, reconstruct objects
- work on a mask (a binary image)
- move the structuring element along the image
- two basic operations
  - dilate (enlarge objects):
    - current pixel is 1 if the SE touches a 1 in the image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



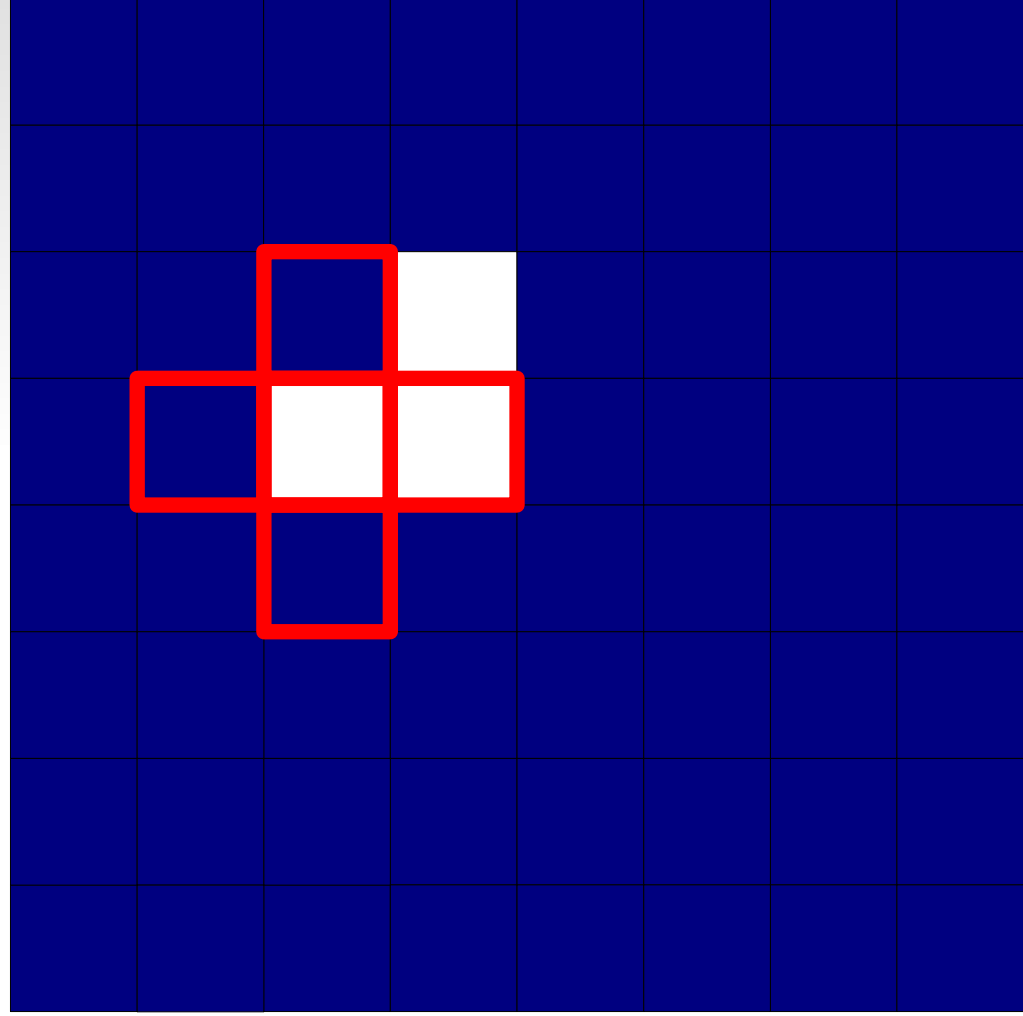
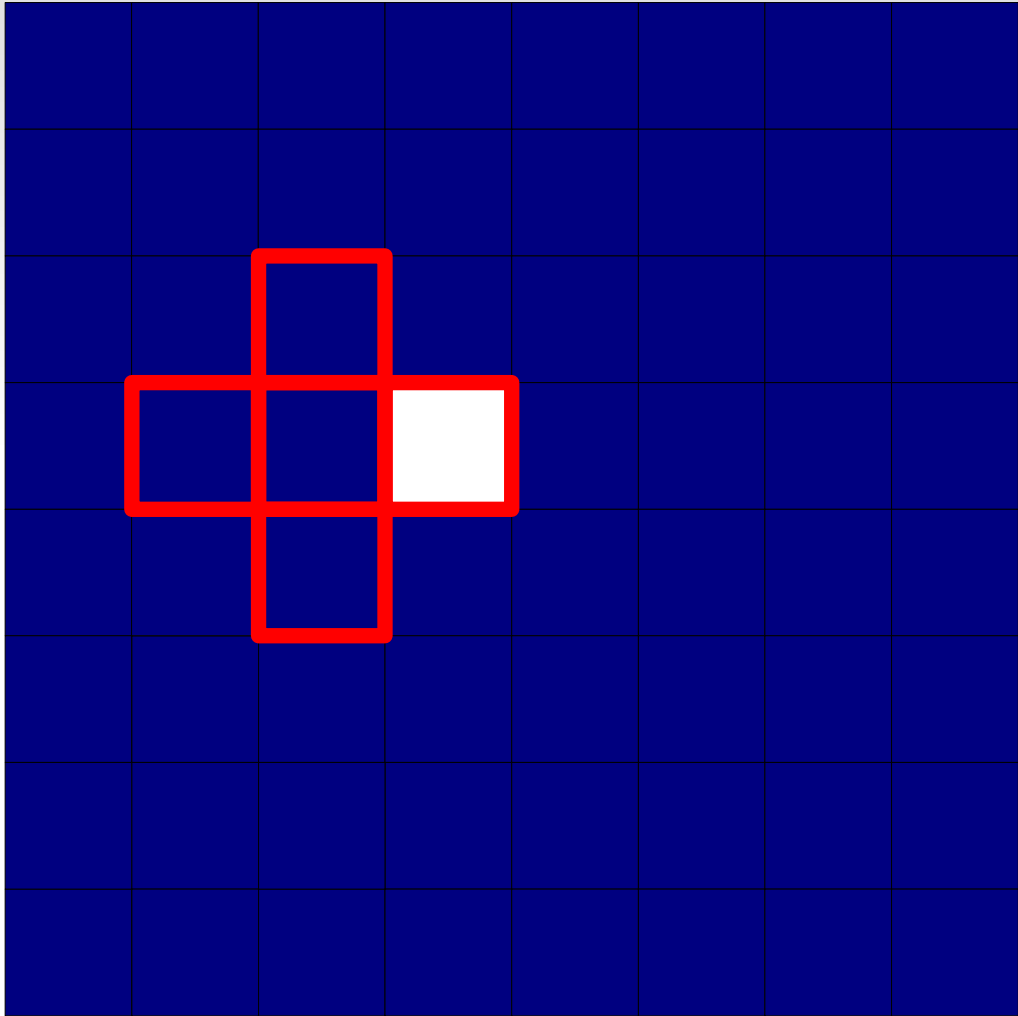
# Binary Morphology - dilation



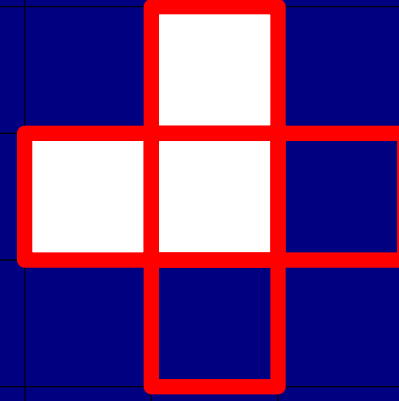
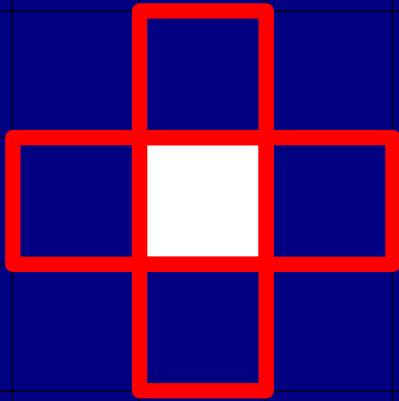
# Binary Morphology - dilation



# Binary Morphology - dilation



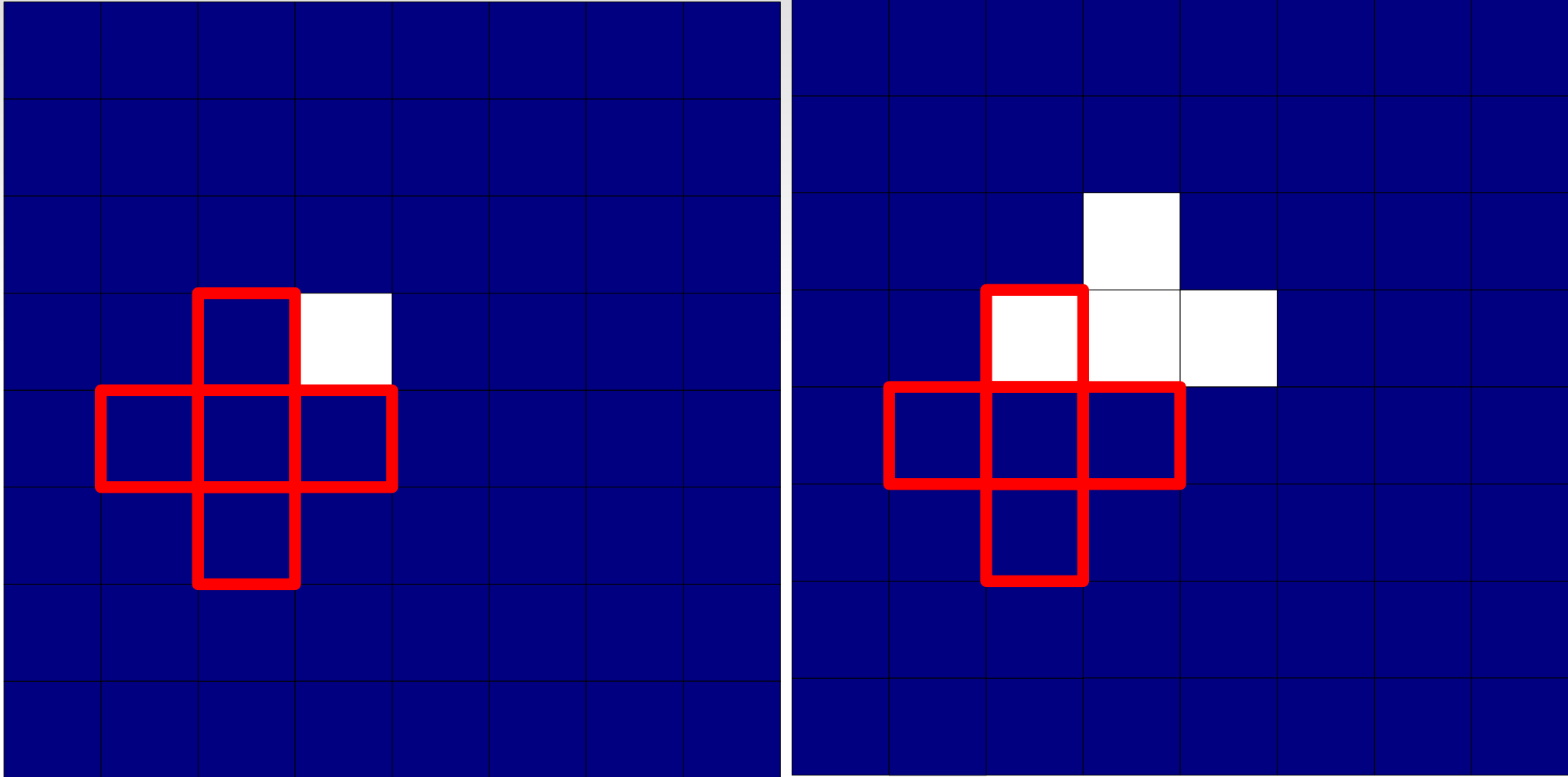
# Binary Morphology - dilation



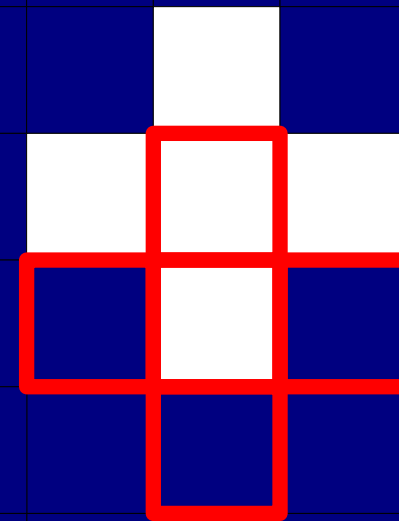
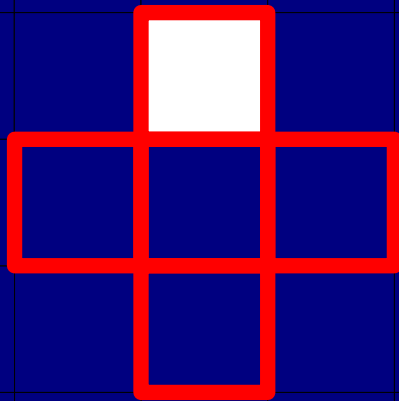




# Binary Morphology - dilation

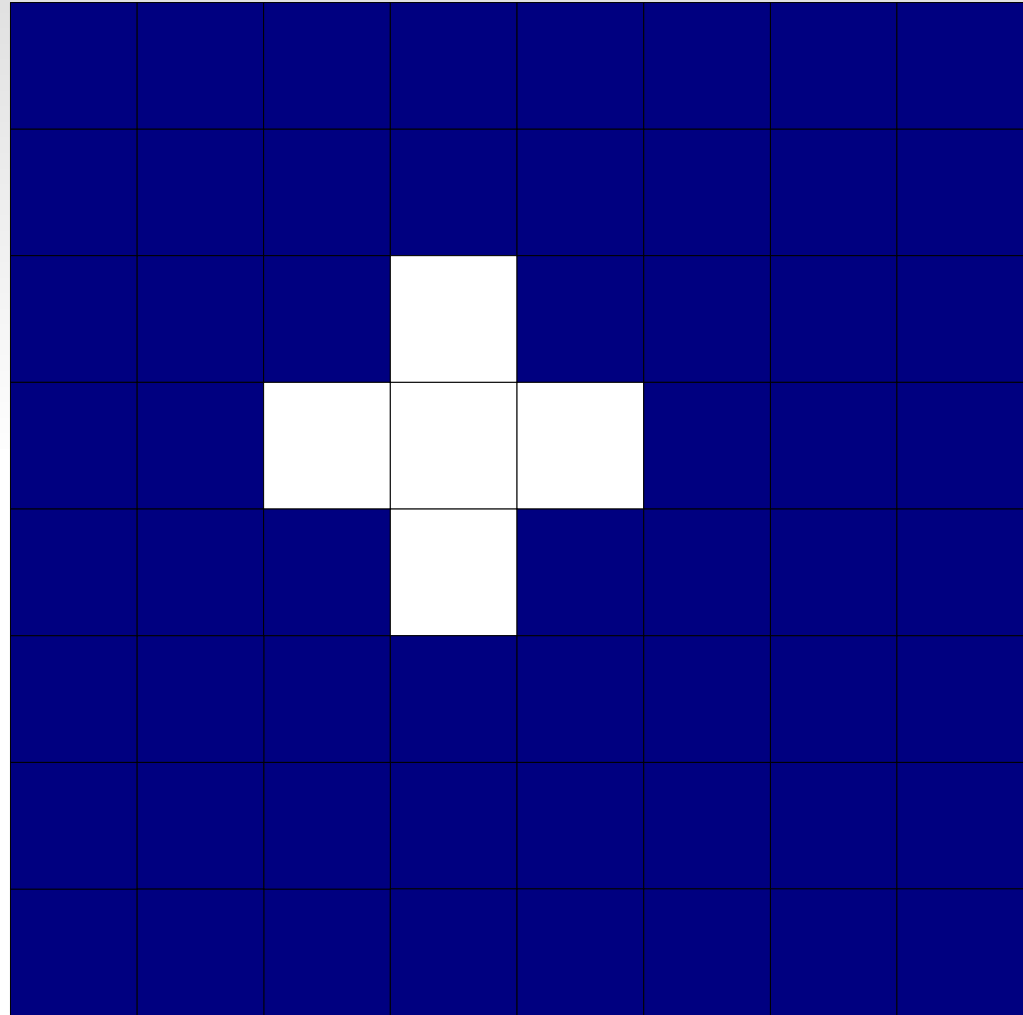
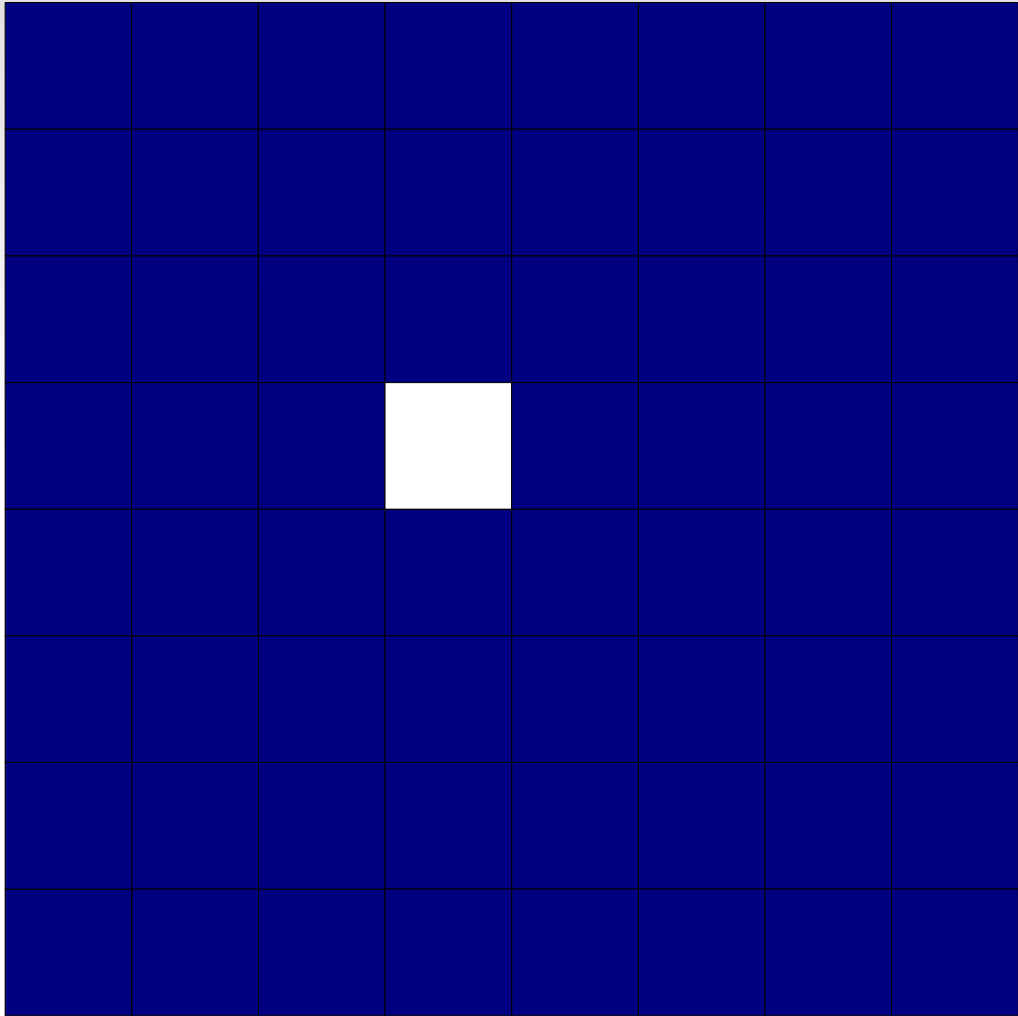


# Binary Morphology - dilation

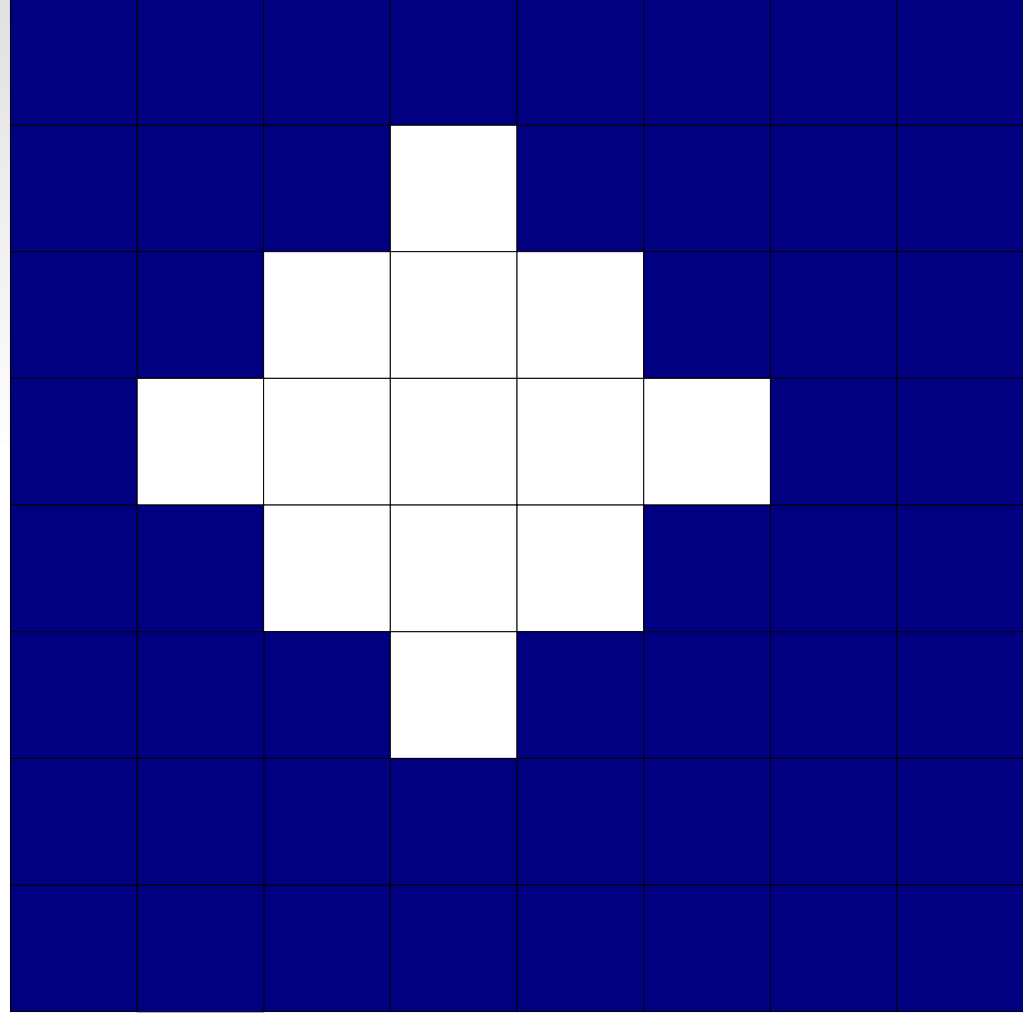
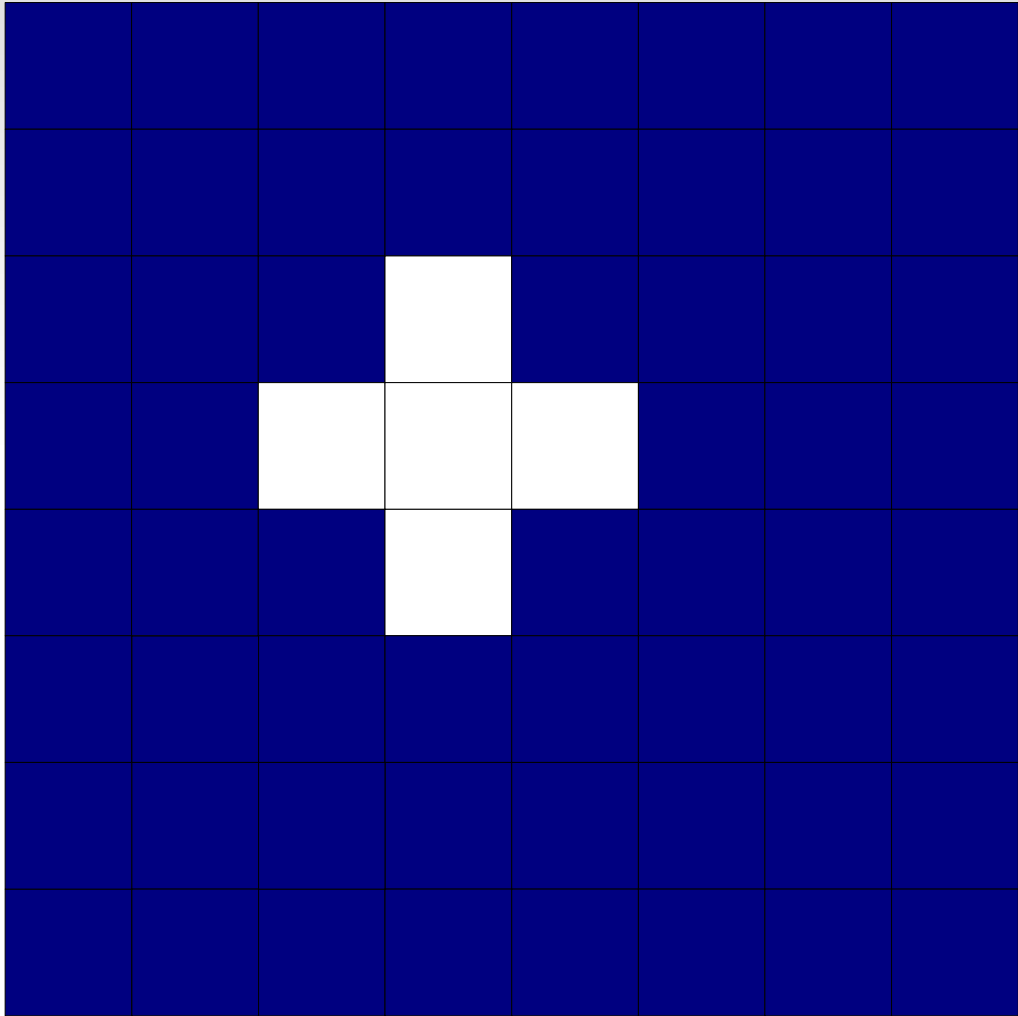




# Binary Morphology - dilation

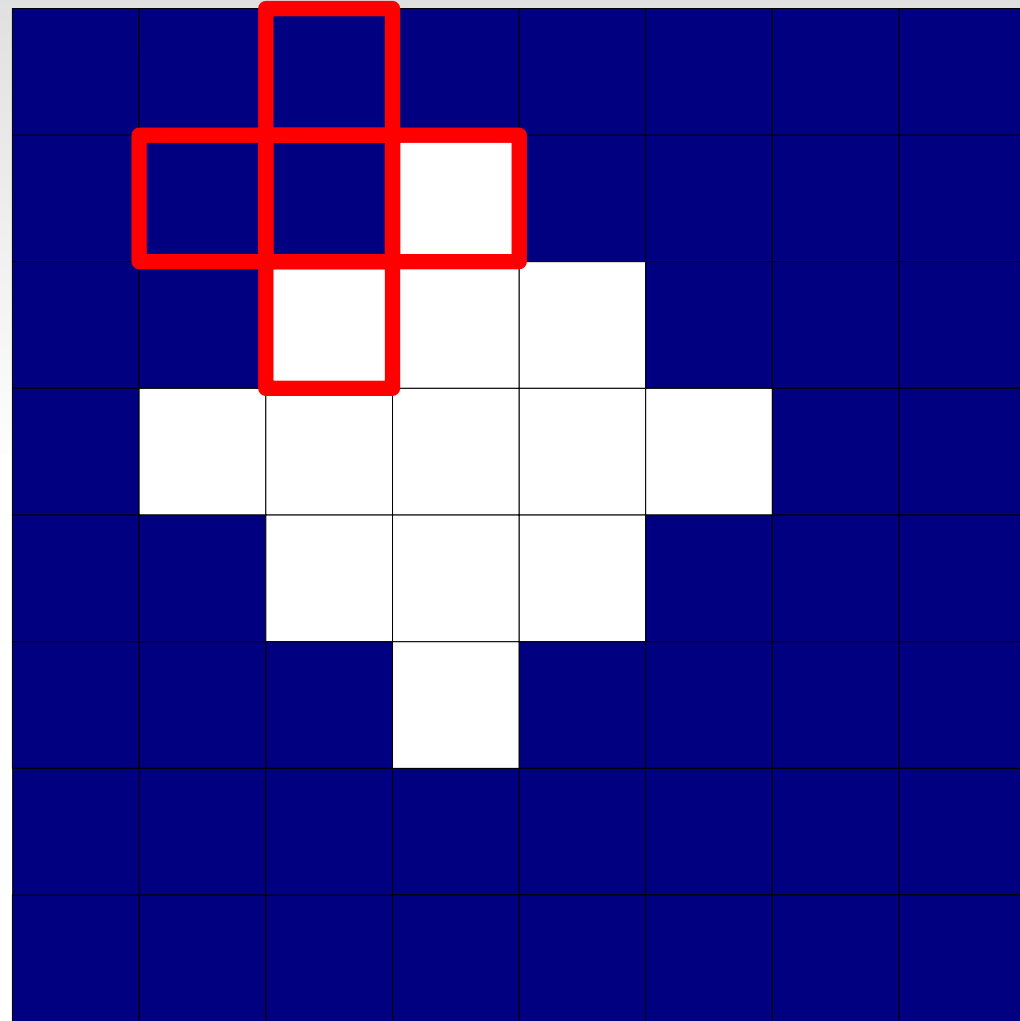
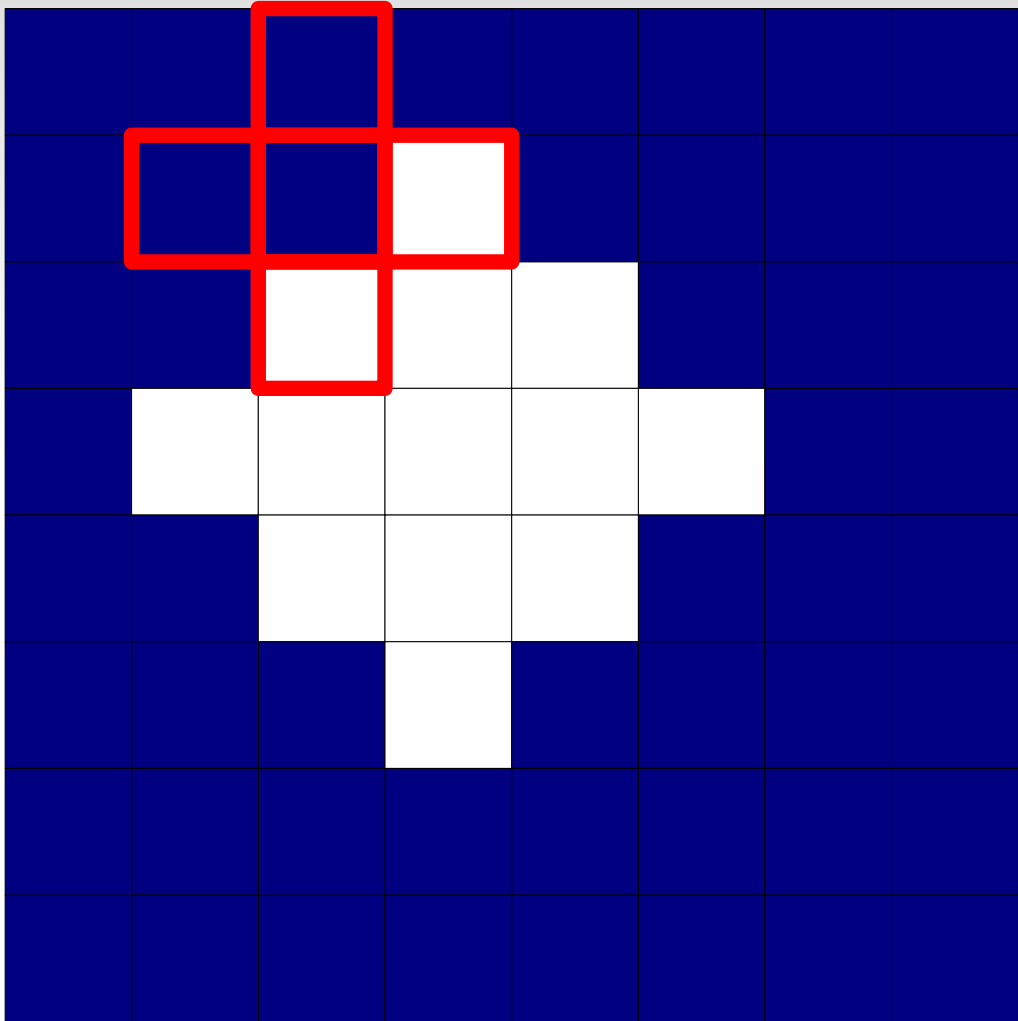


# Binary Morphology - dilation



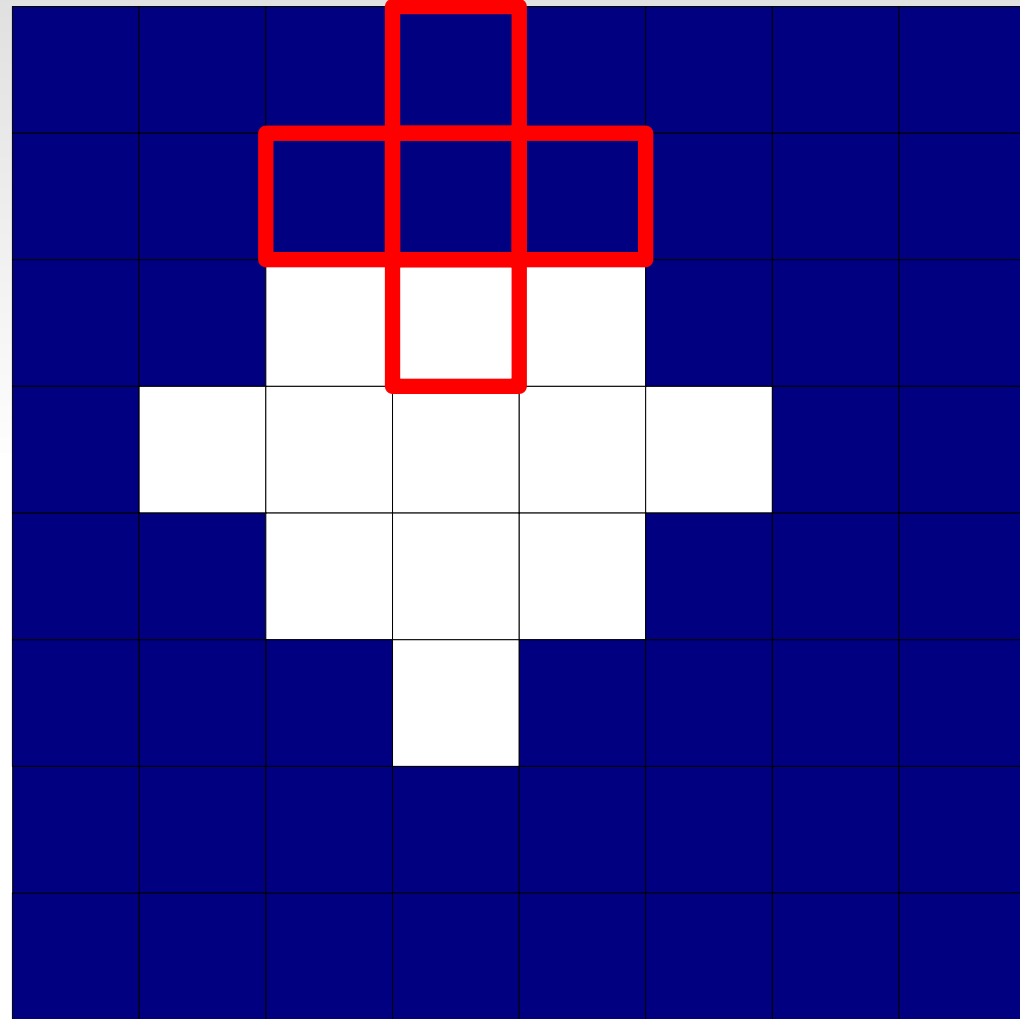
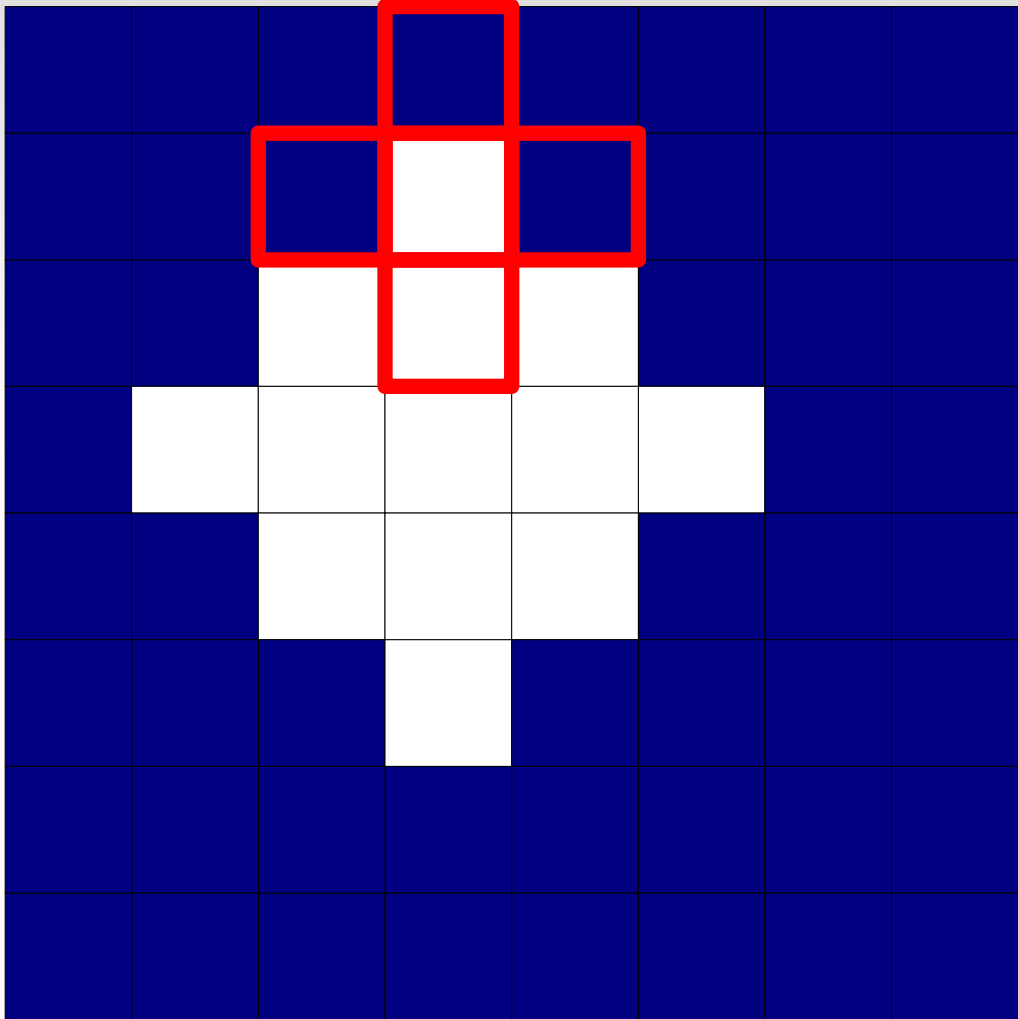


# Binary Morphology - erosion

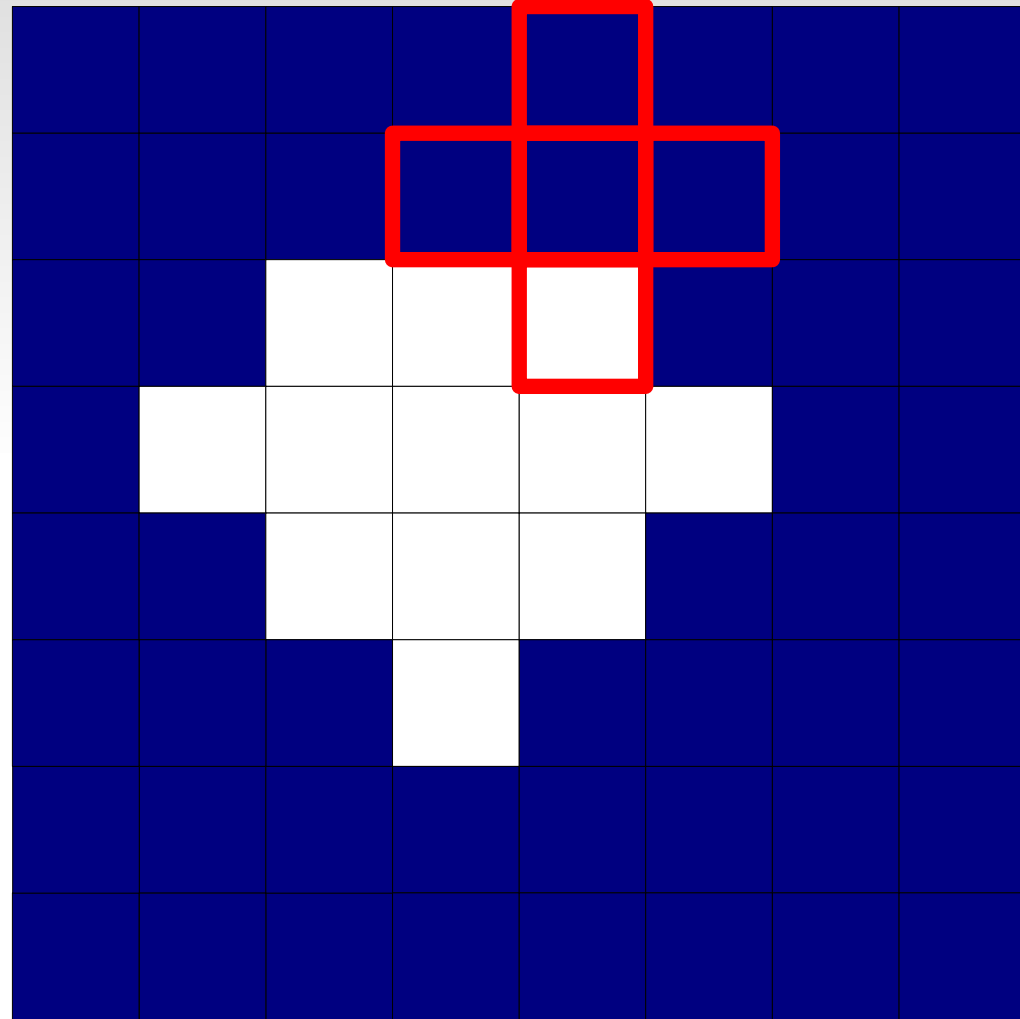
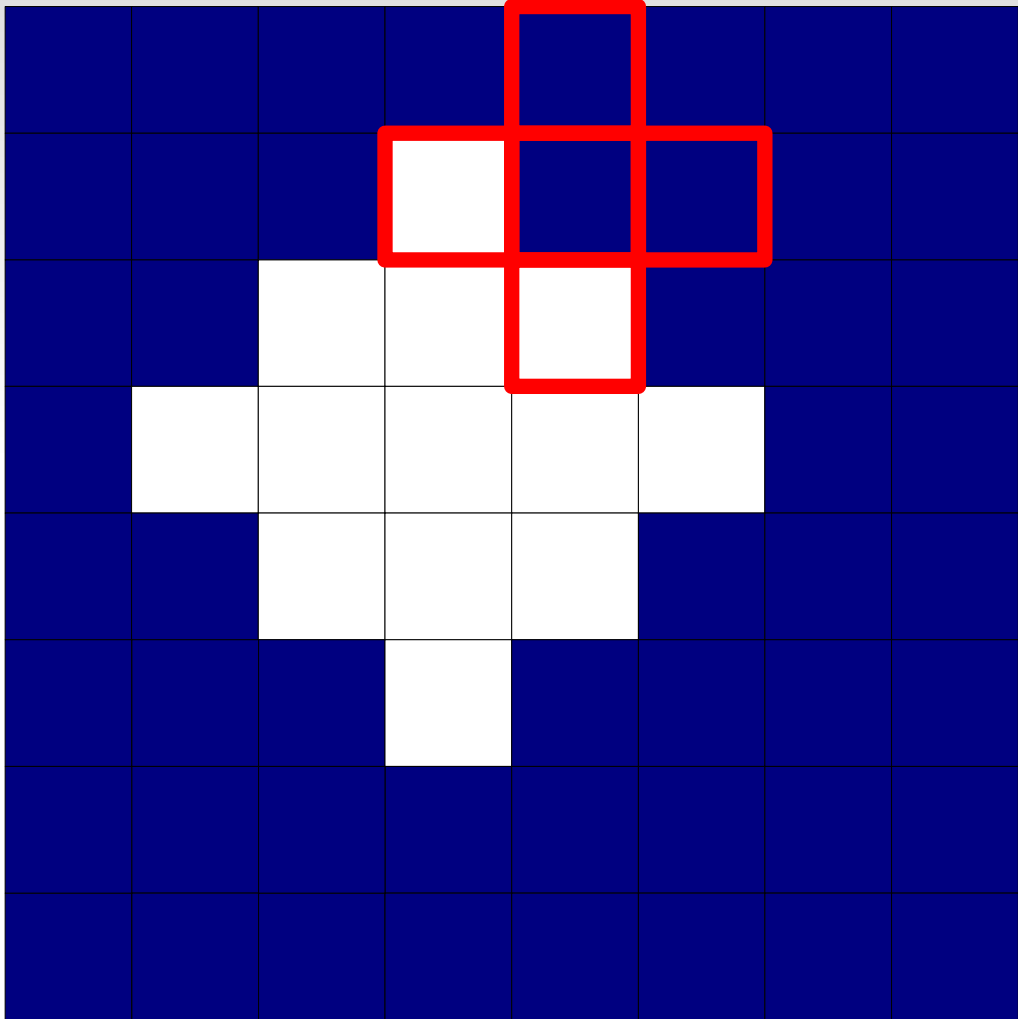




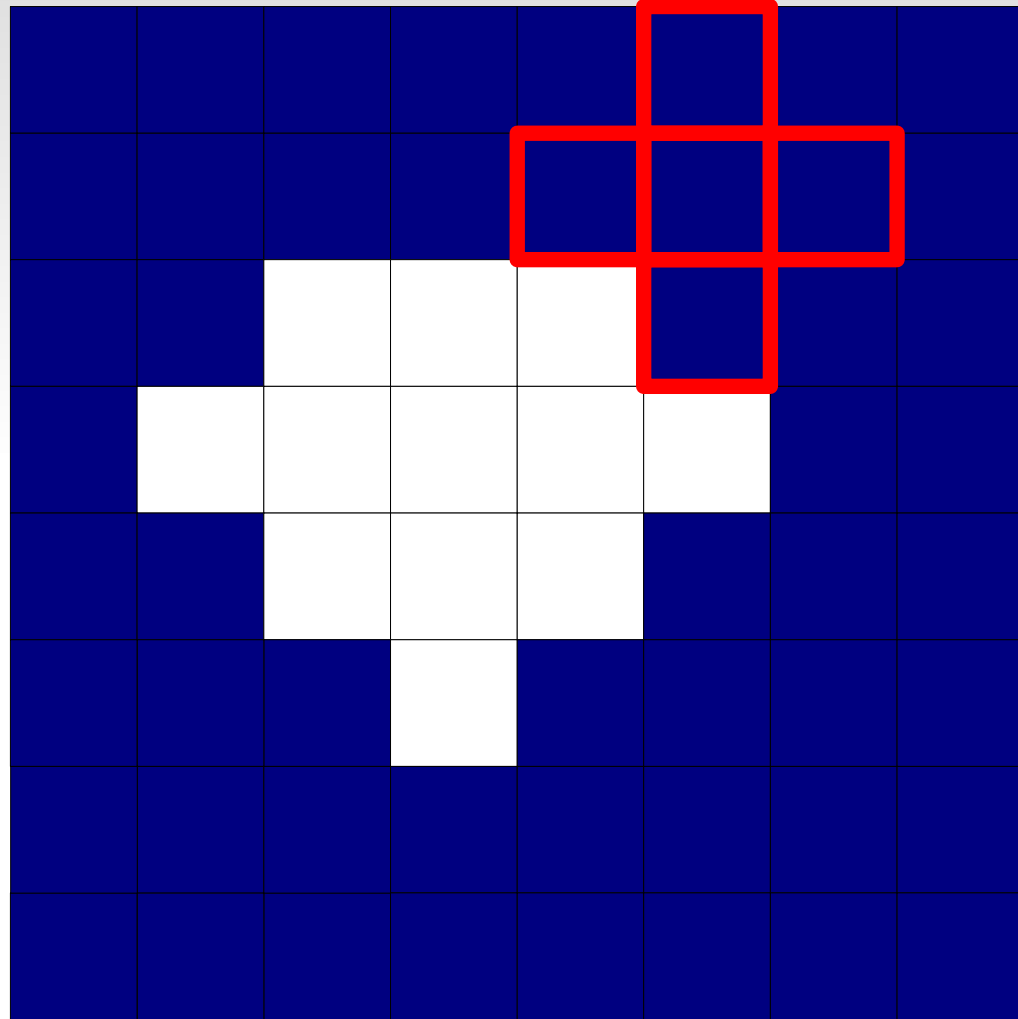
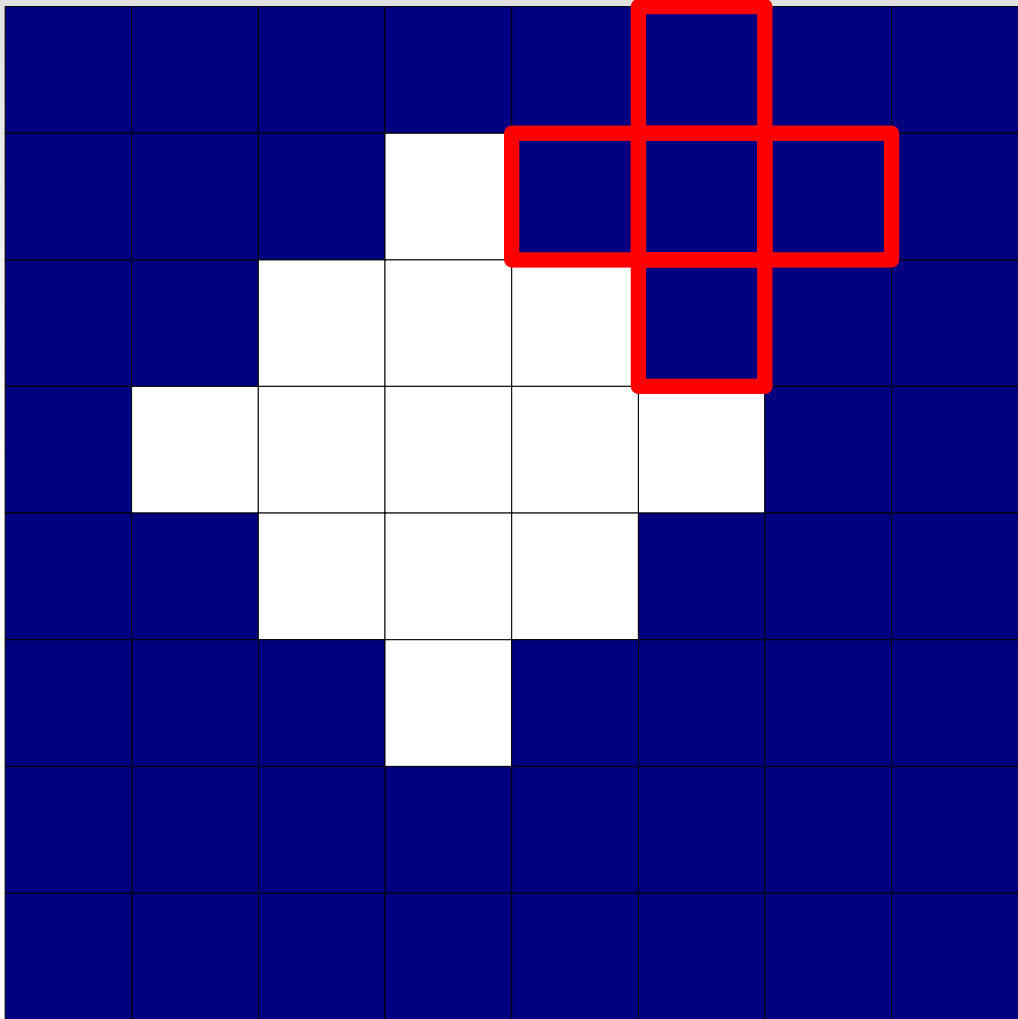
# Binary Morphology - erosion



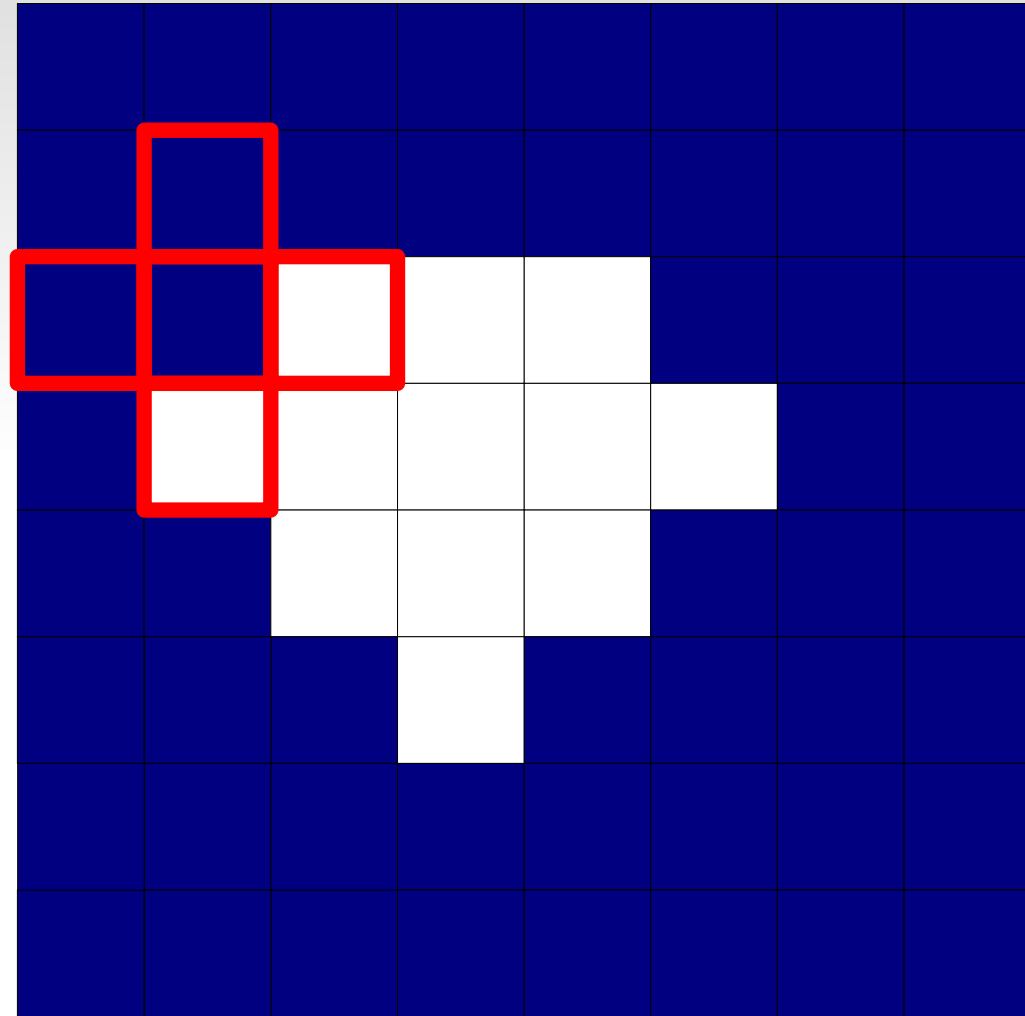
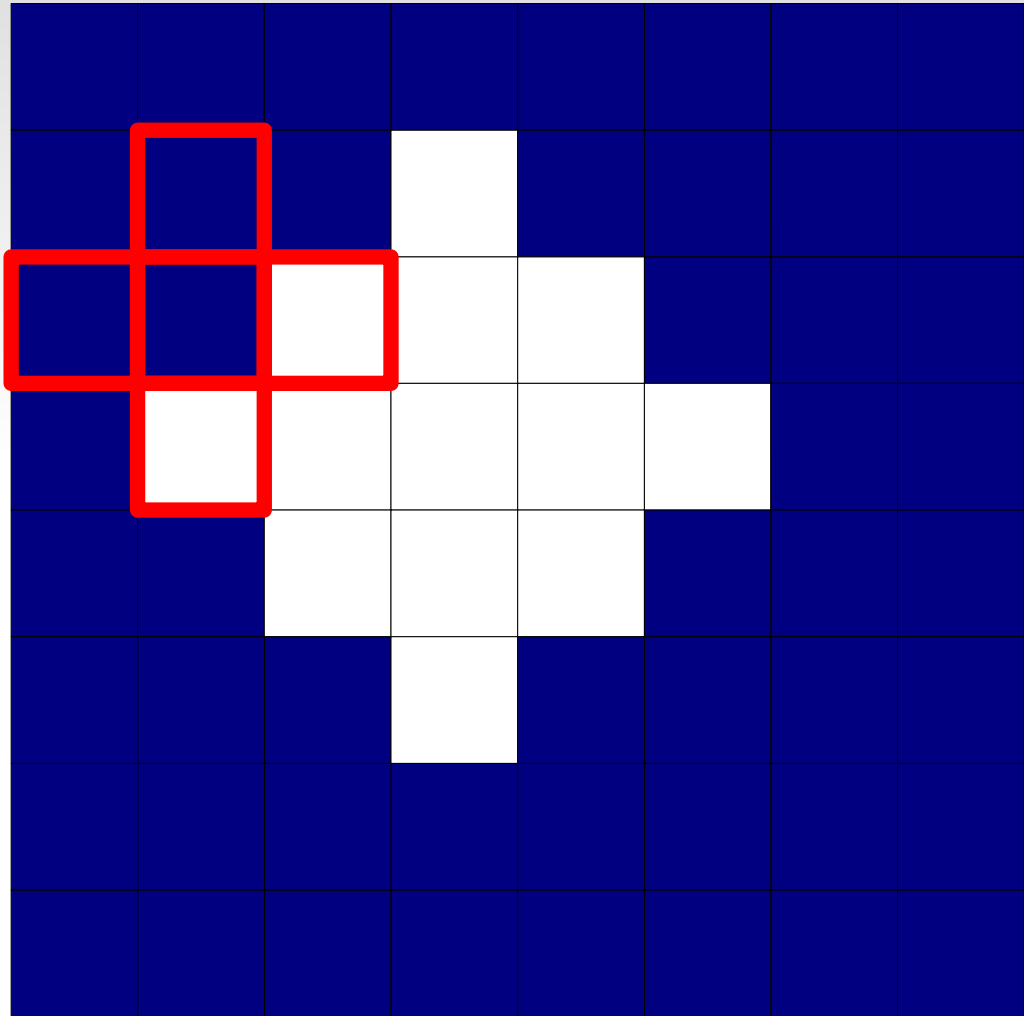
# Binary Morphology - erosion



# Binary Morphology - erosion

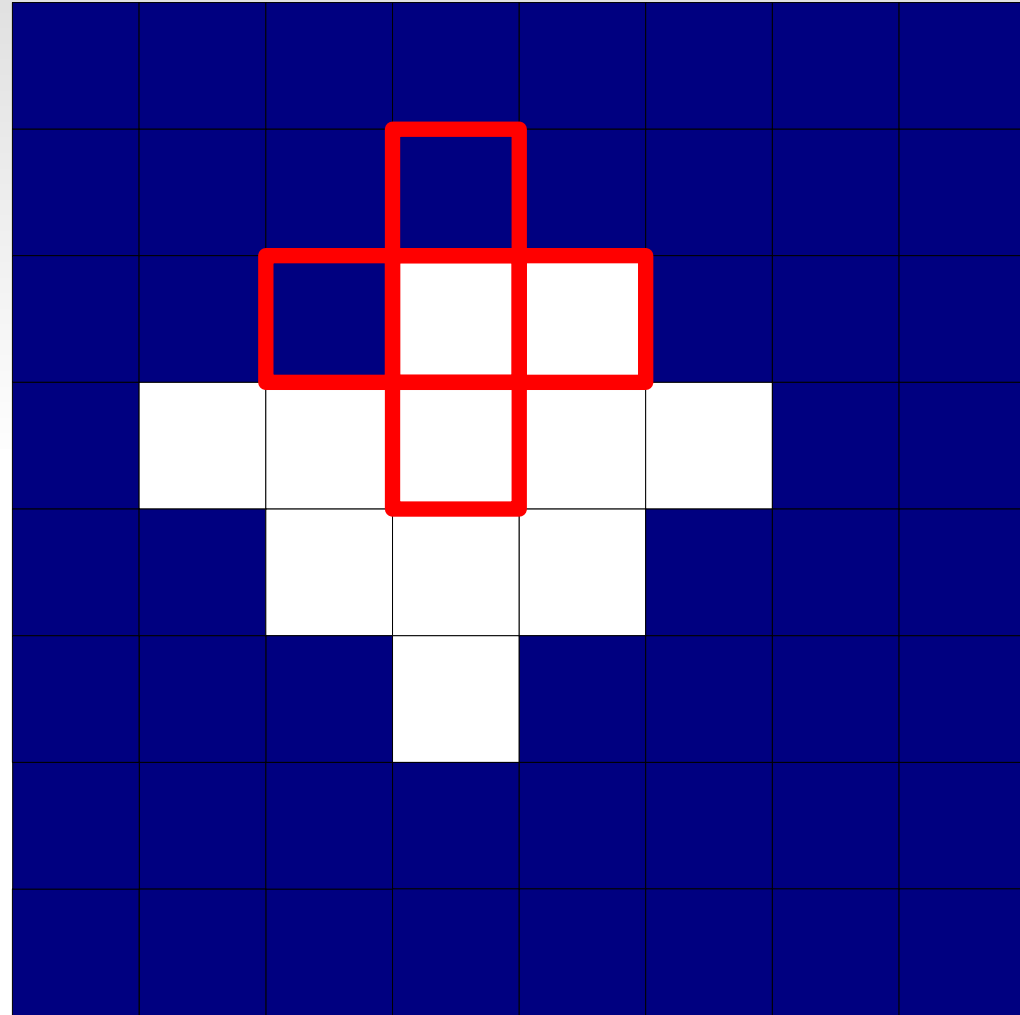
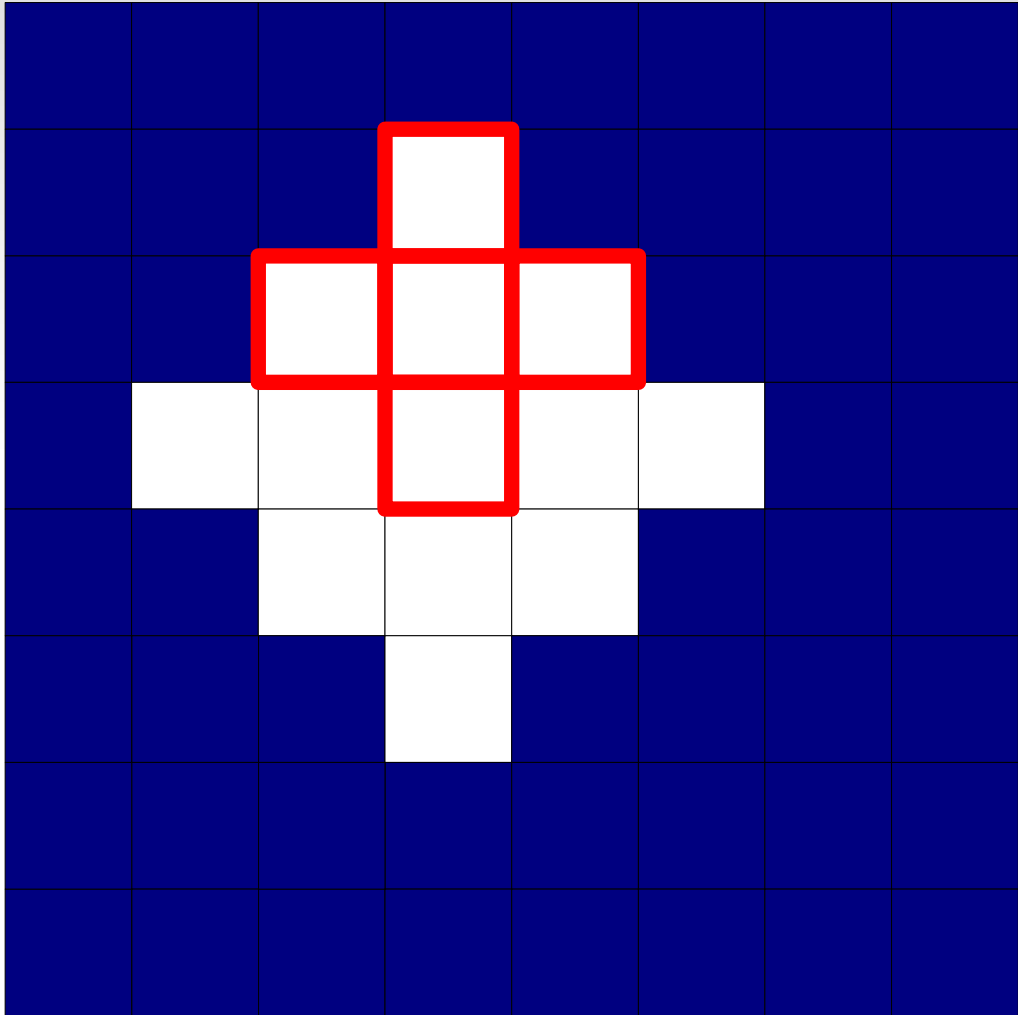


# Binary Morphology - erosion

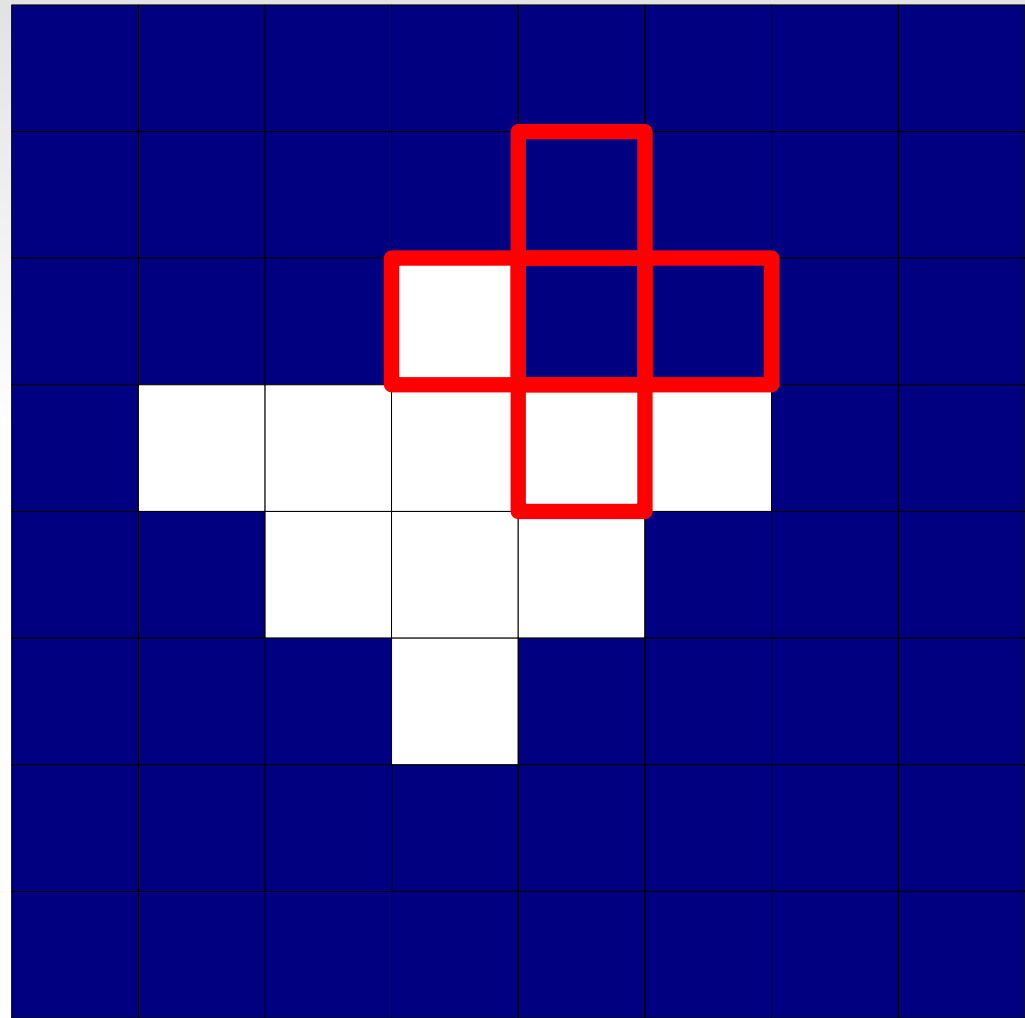
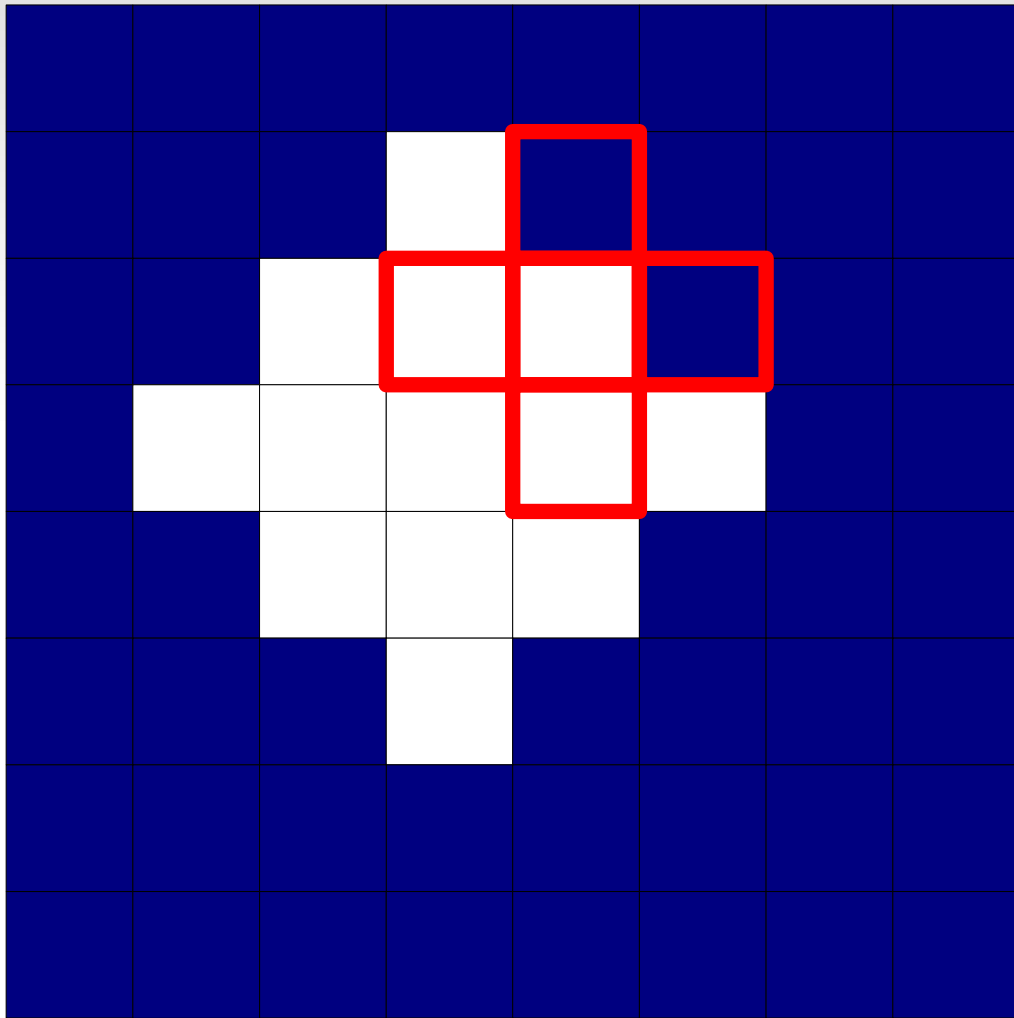




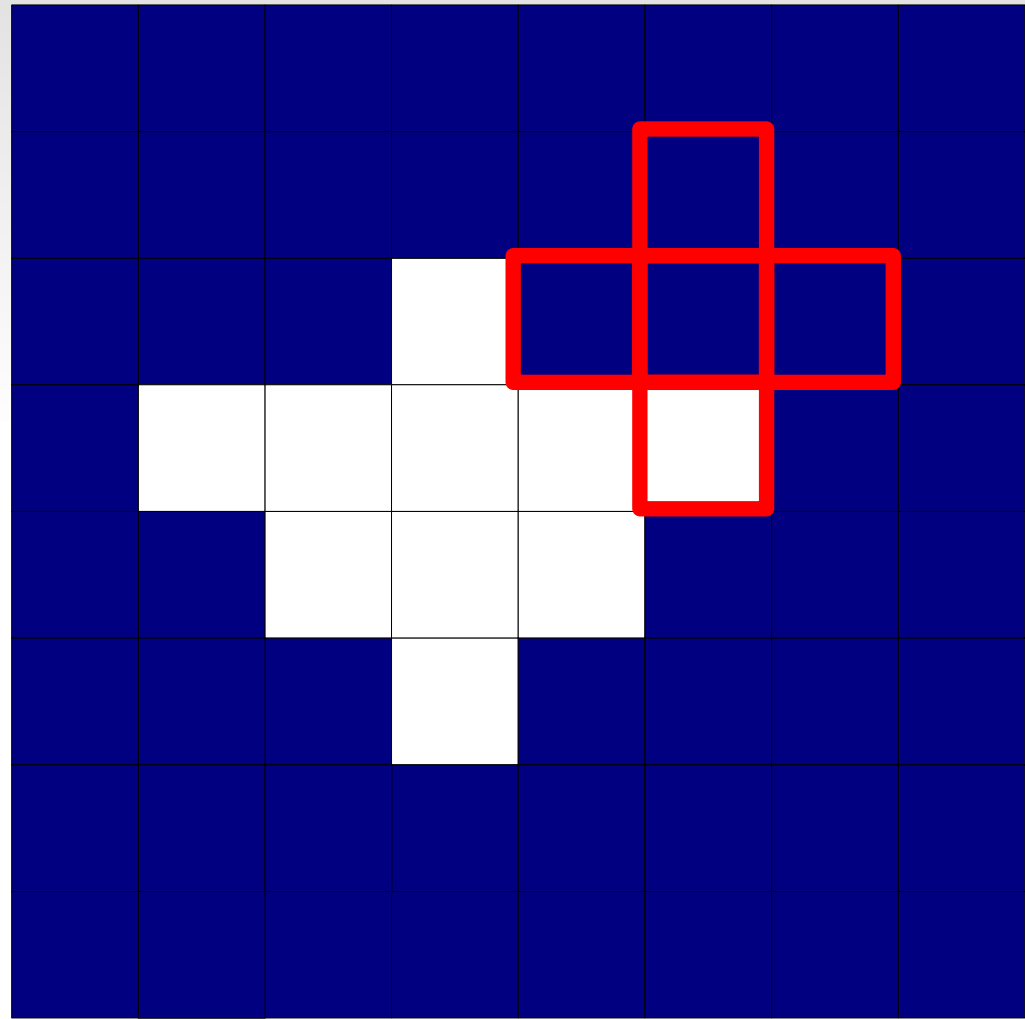
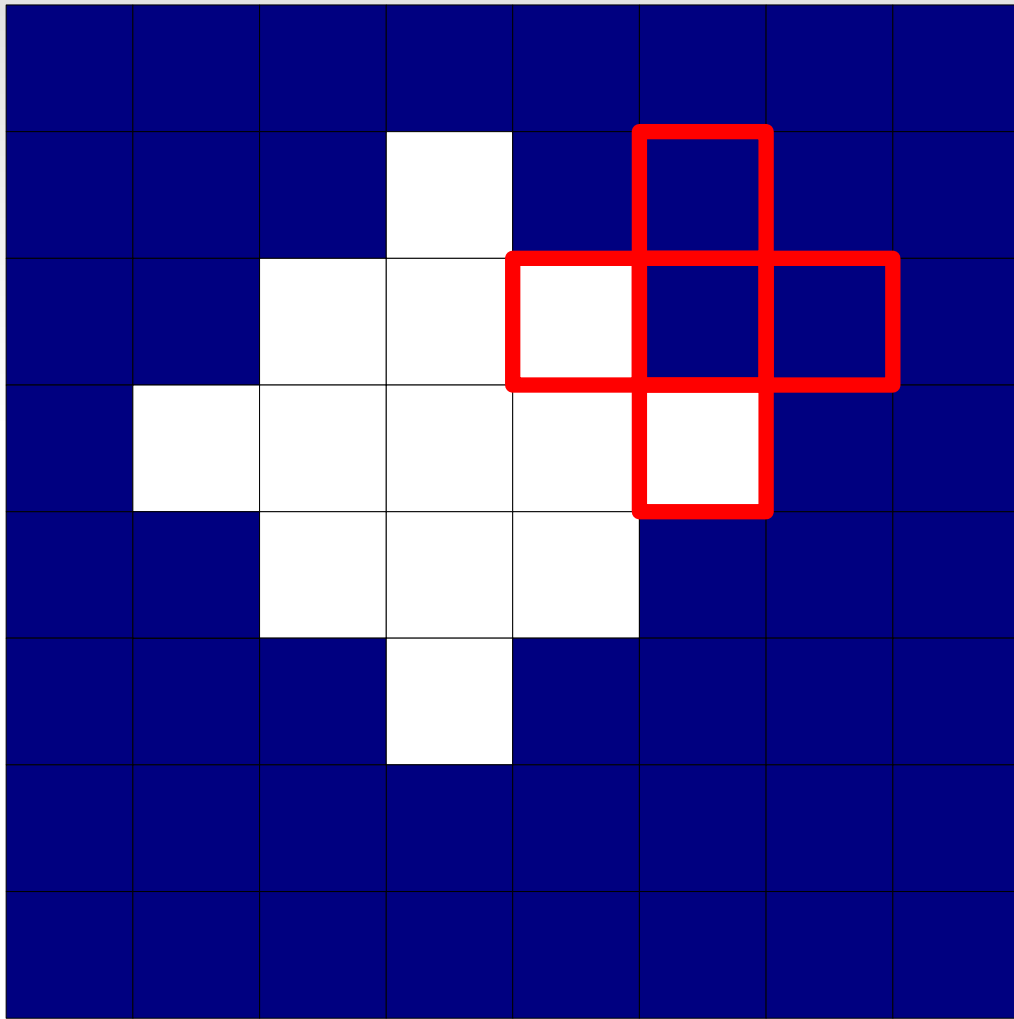
# Binary Morphology - erosion



# Binary Morphology - erosion

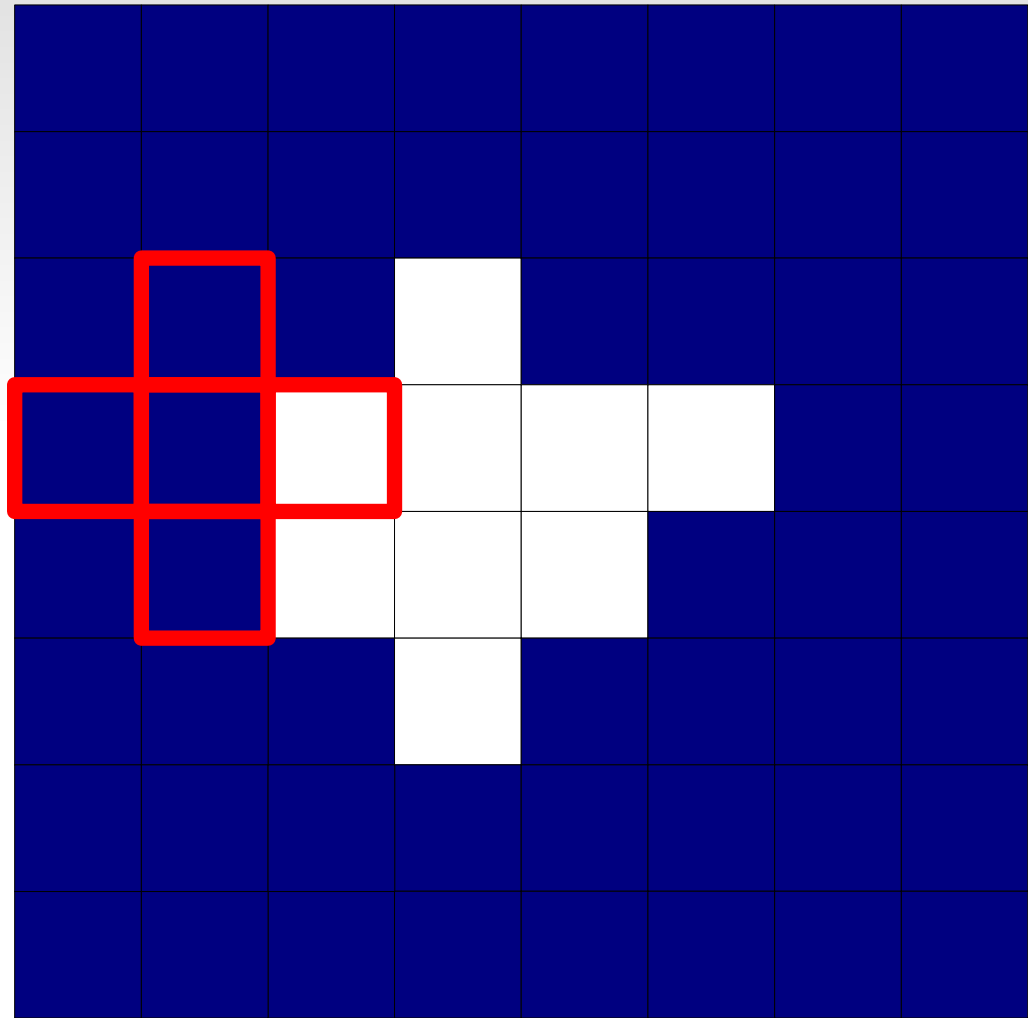
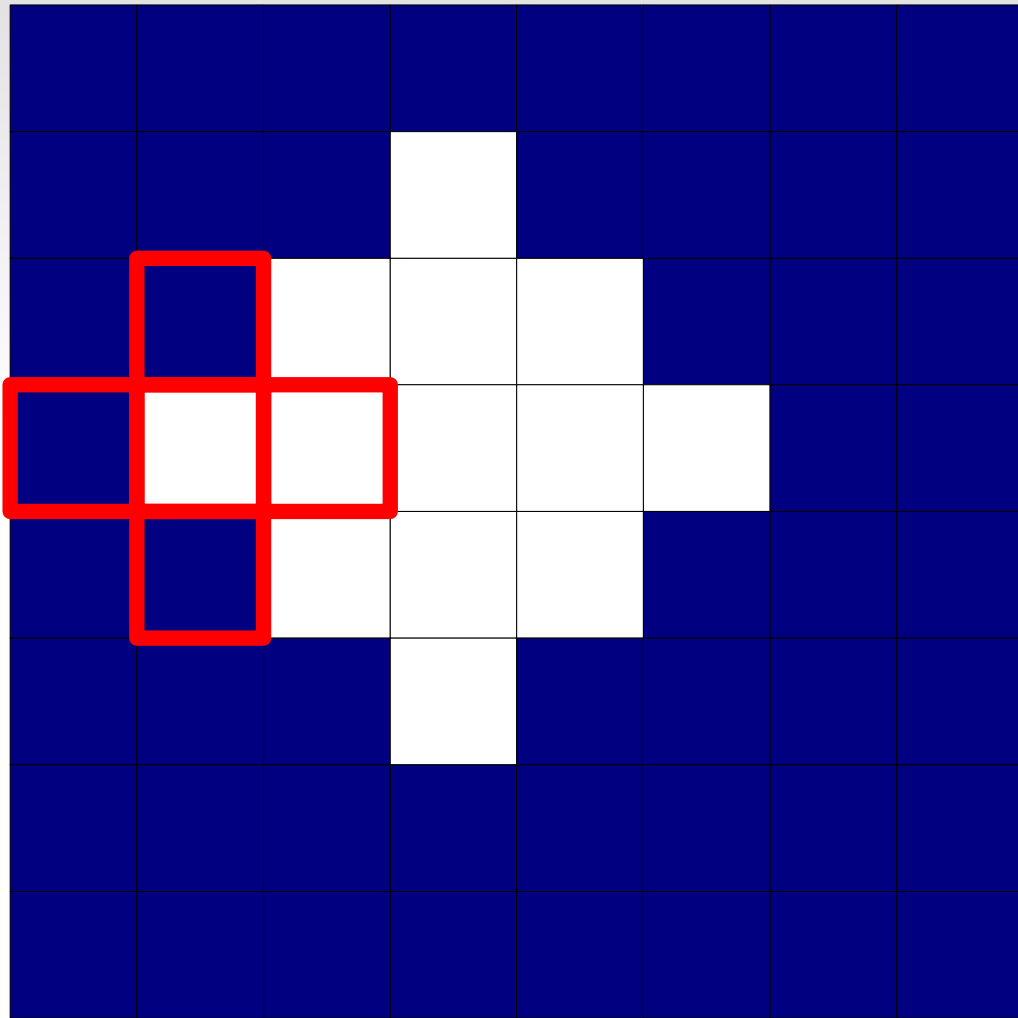


# Binary Morphology - erosion

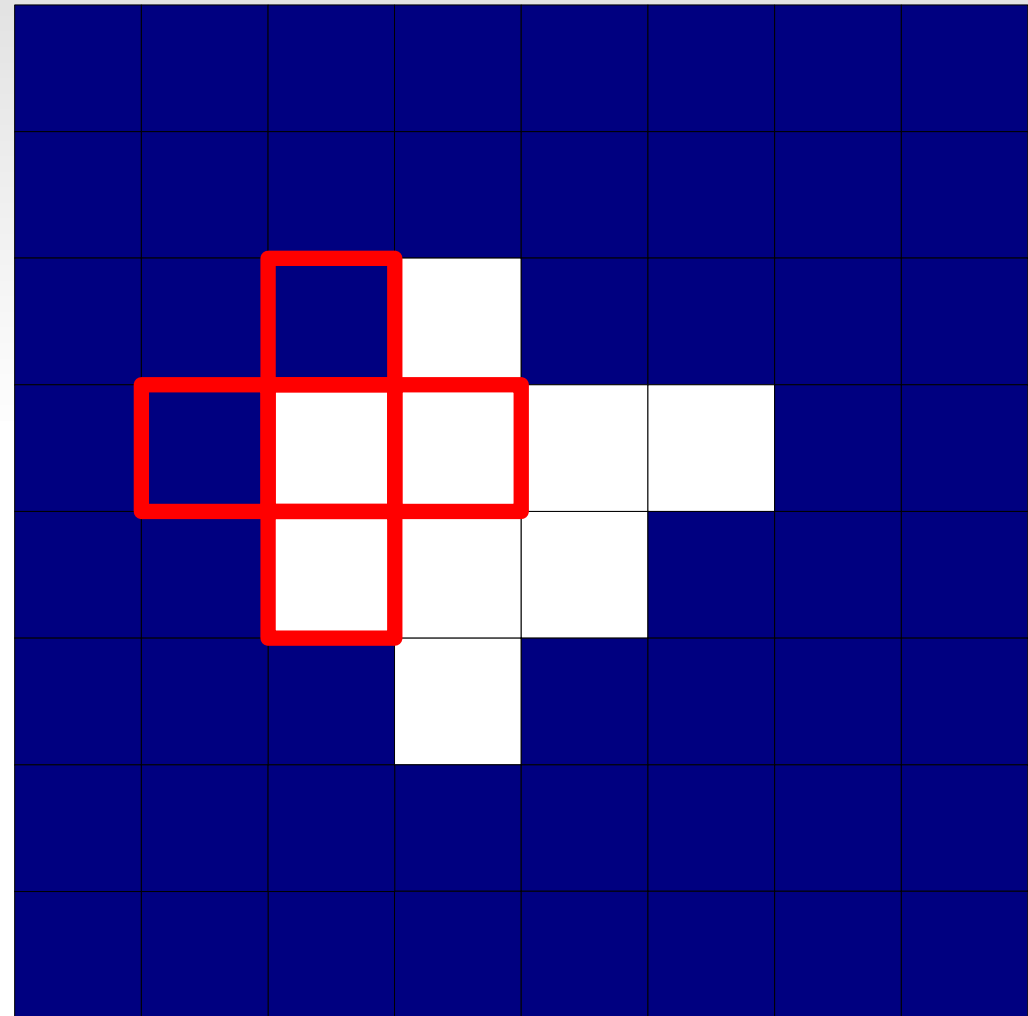
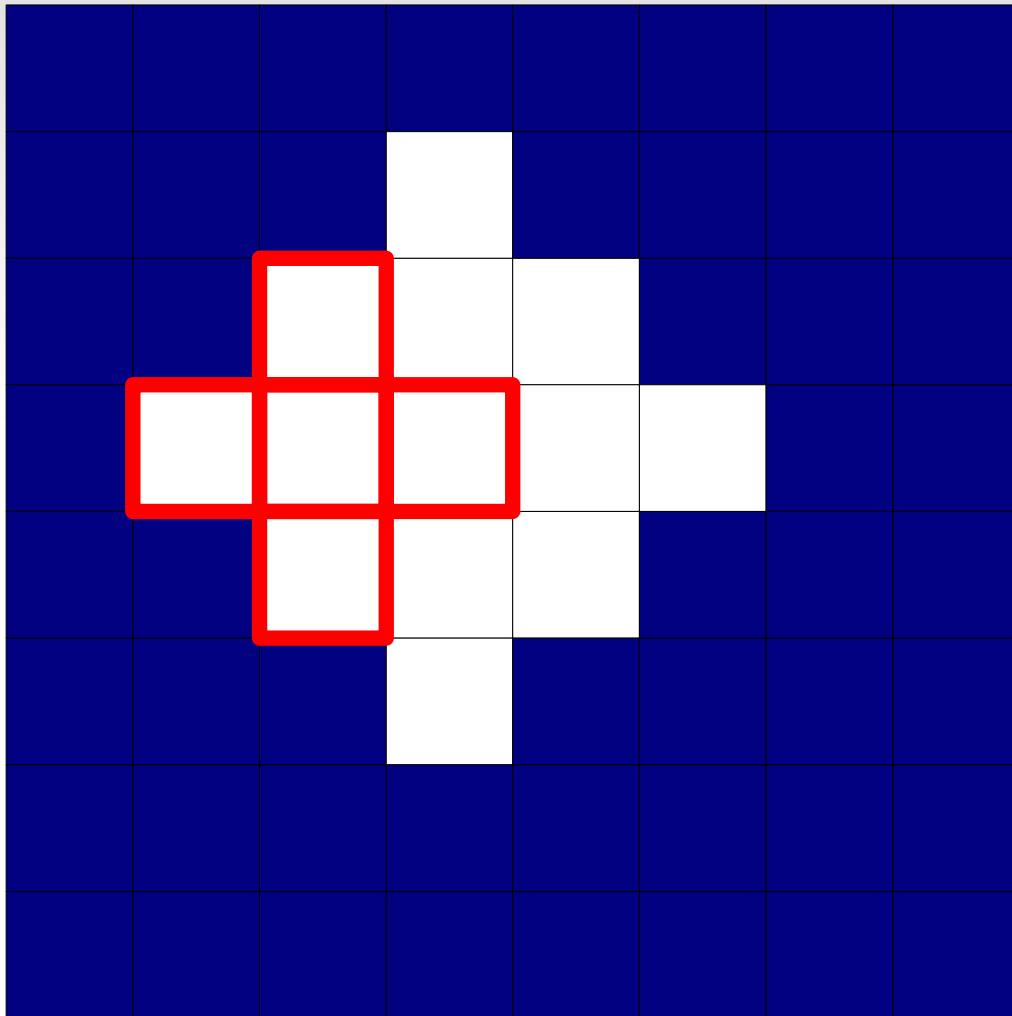




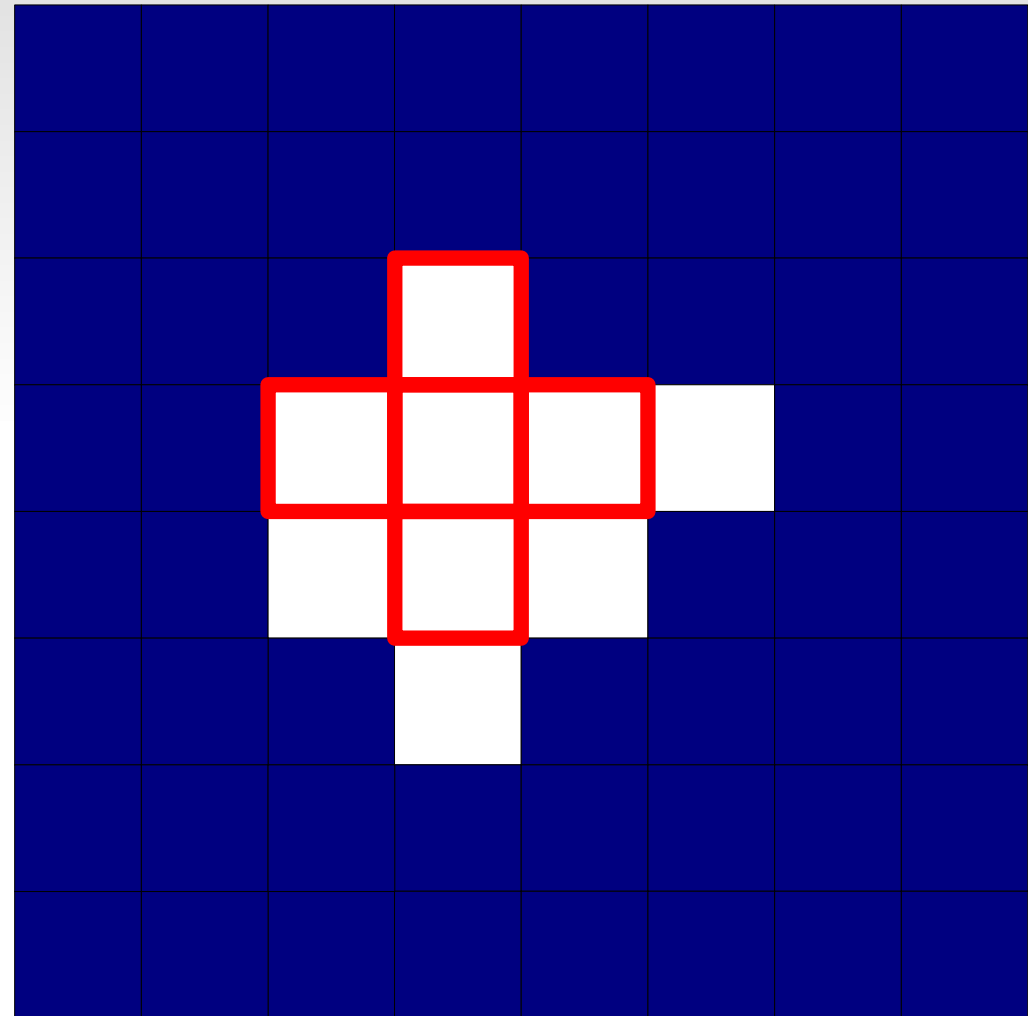
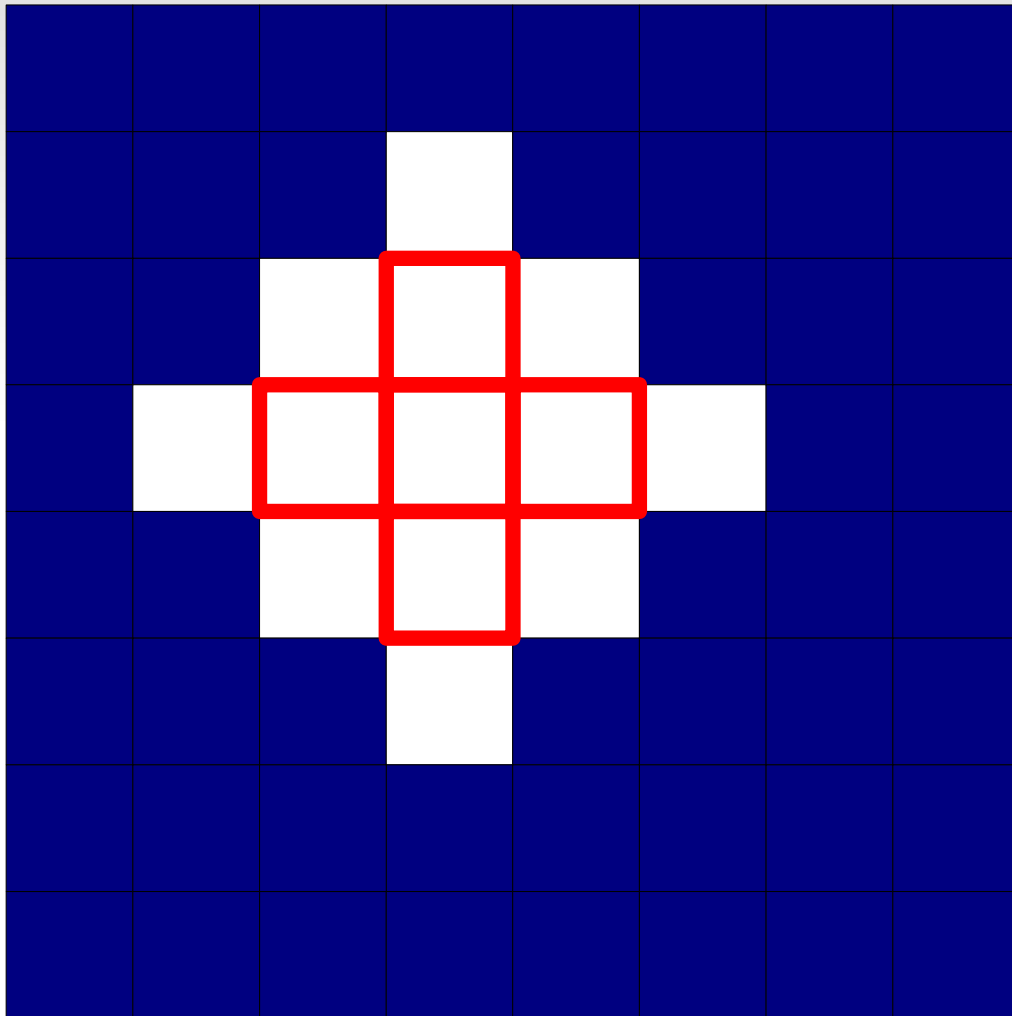
# Binary Morphology - erosion



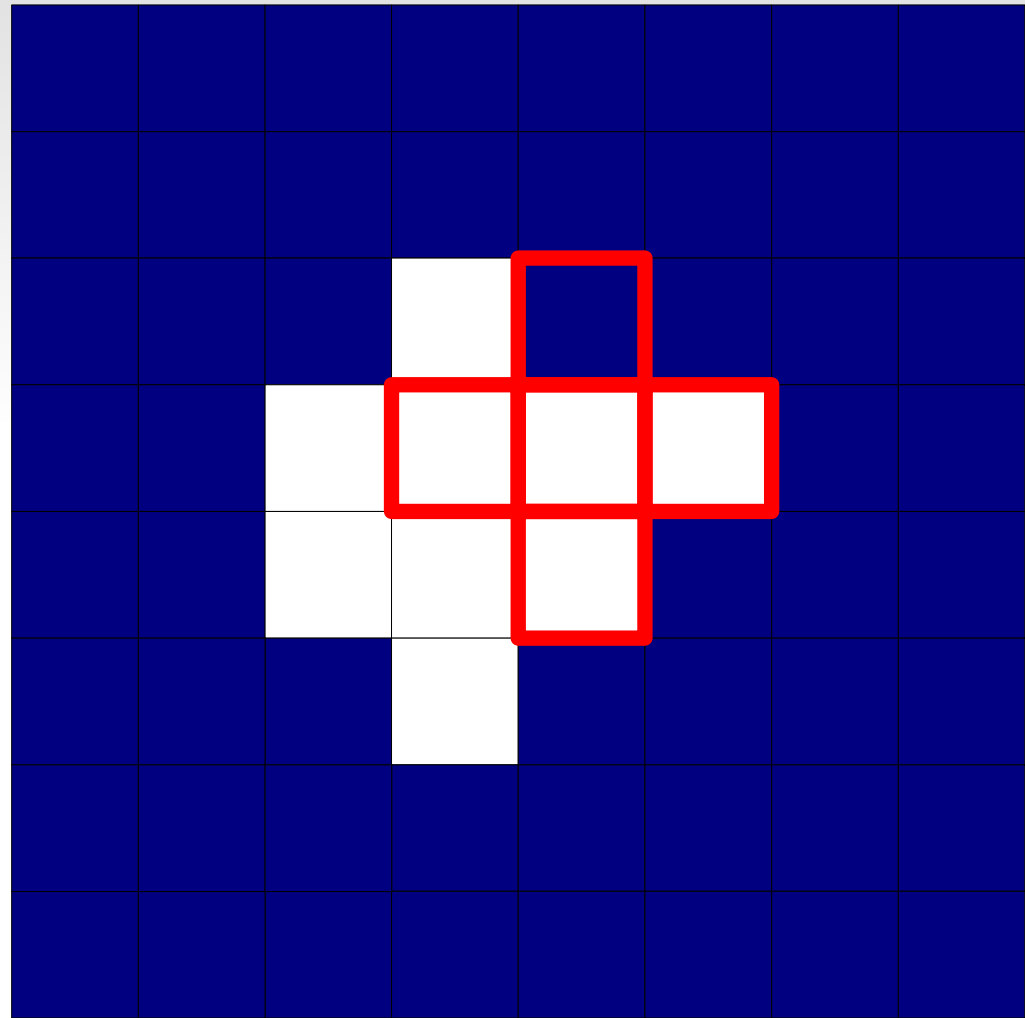
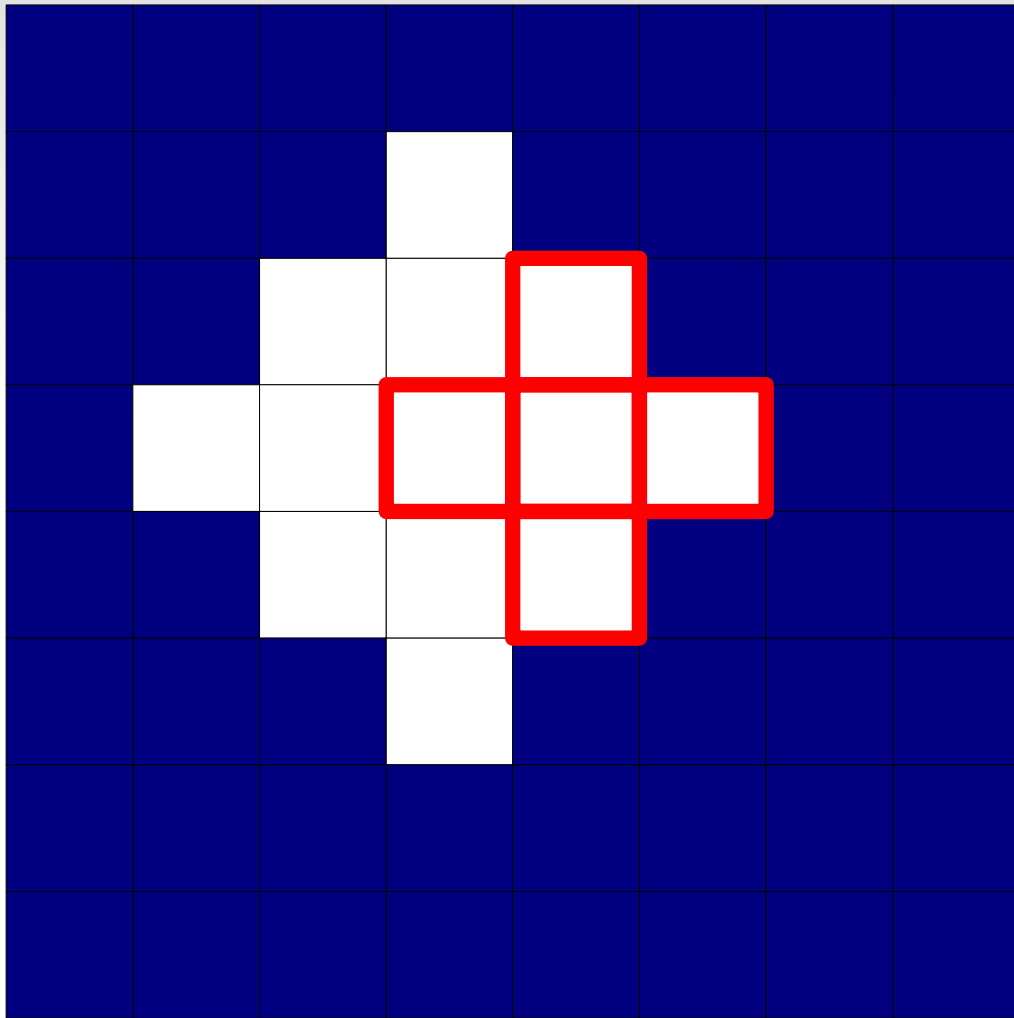
# Binary Morphology - erosion



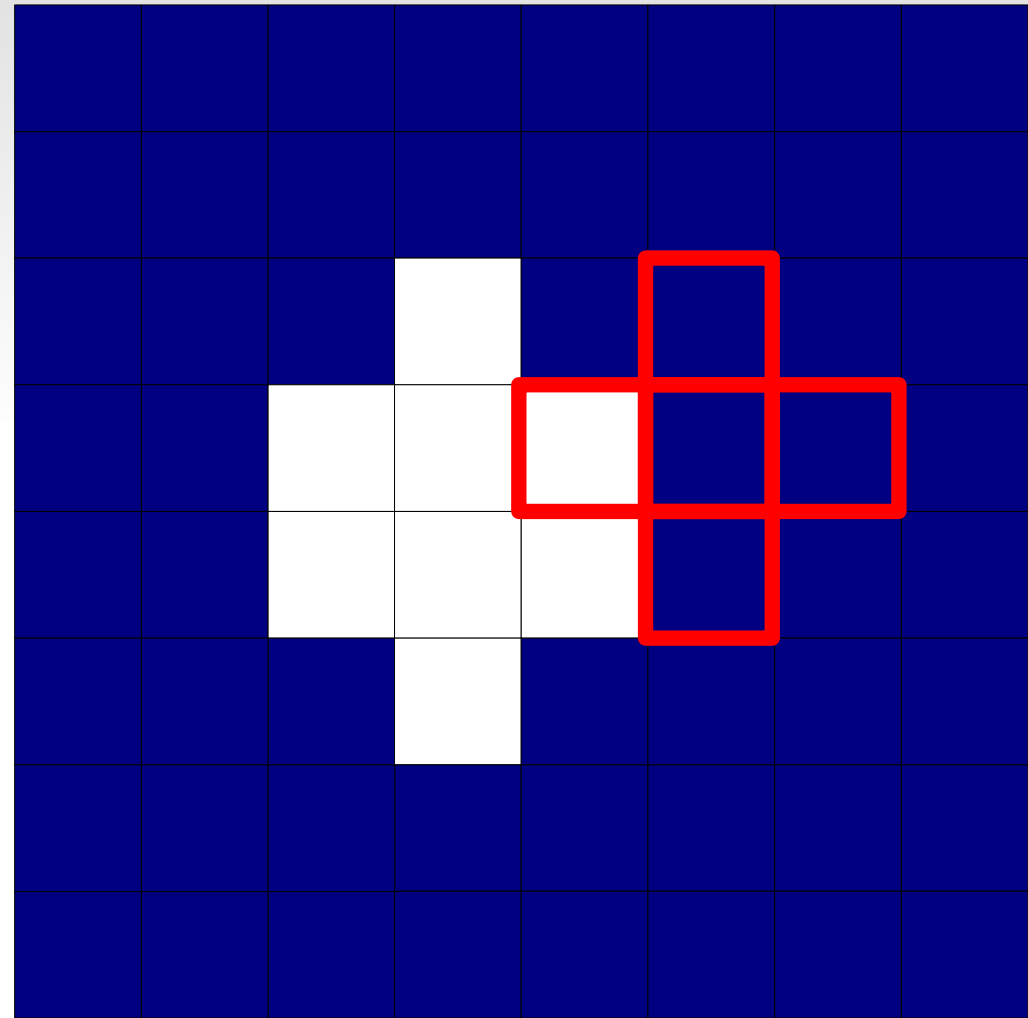
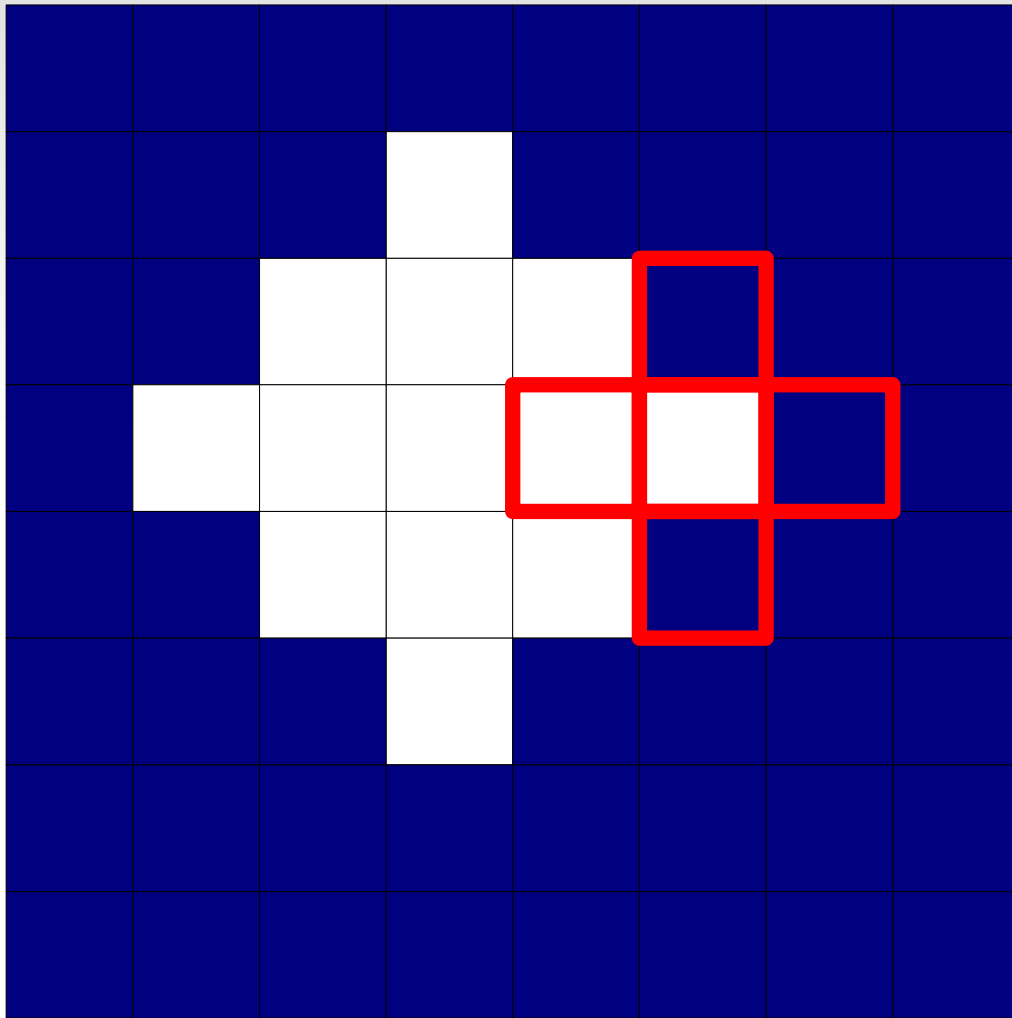
# Binary Morphology - erosion



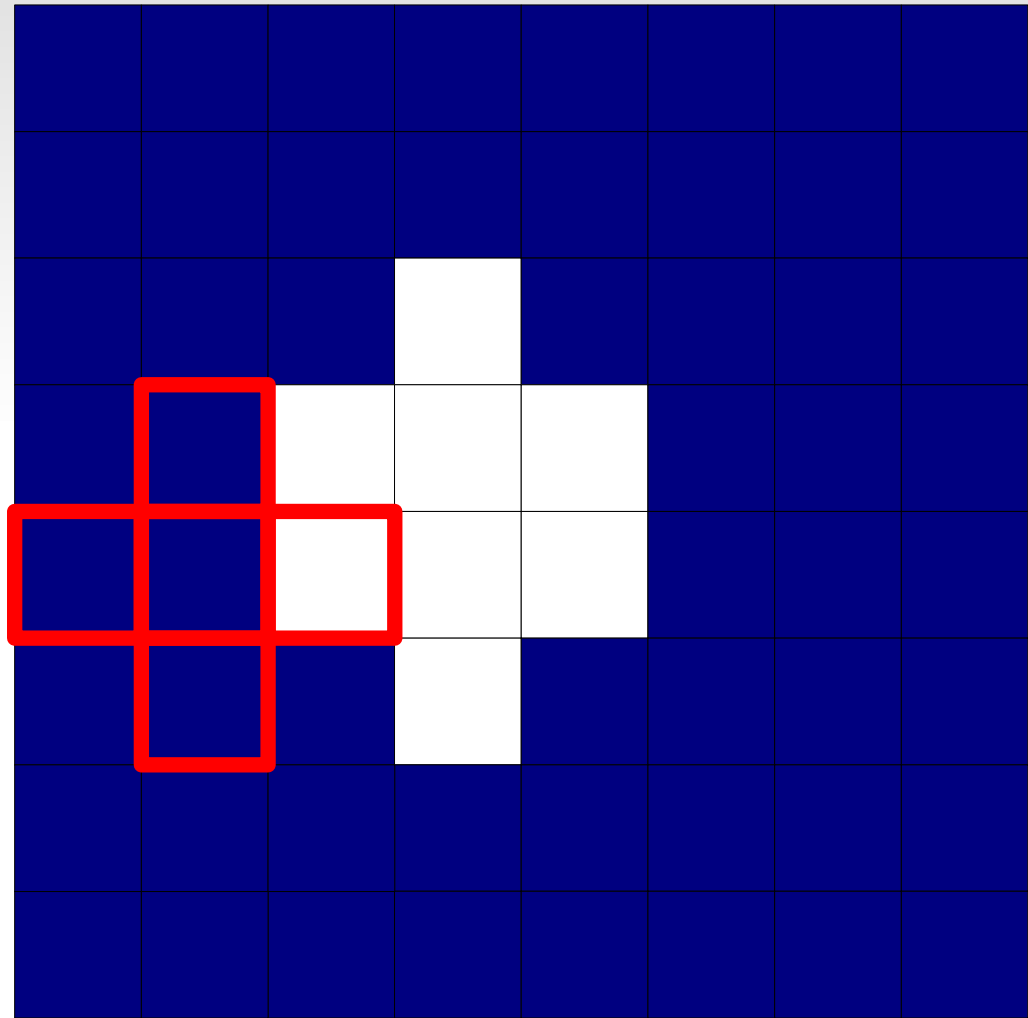
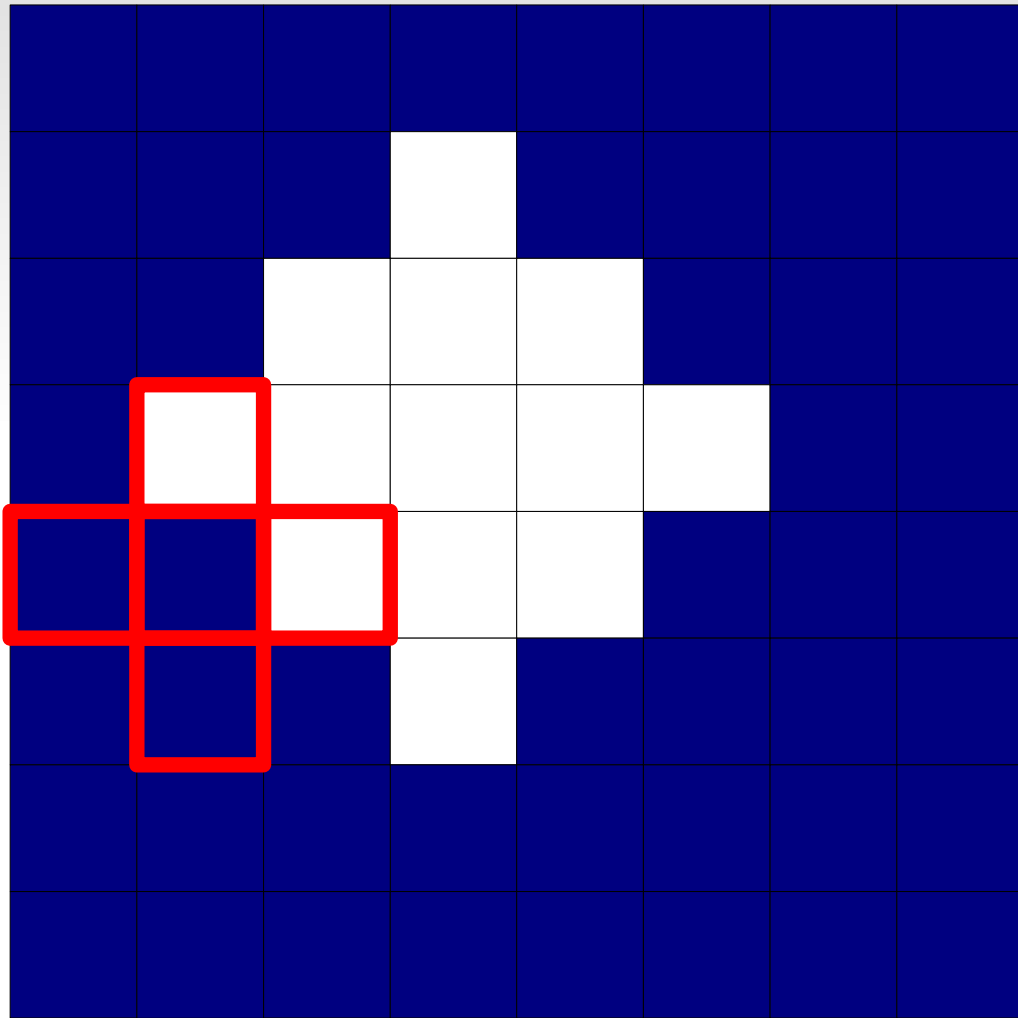
# Binary Morphology - erosion



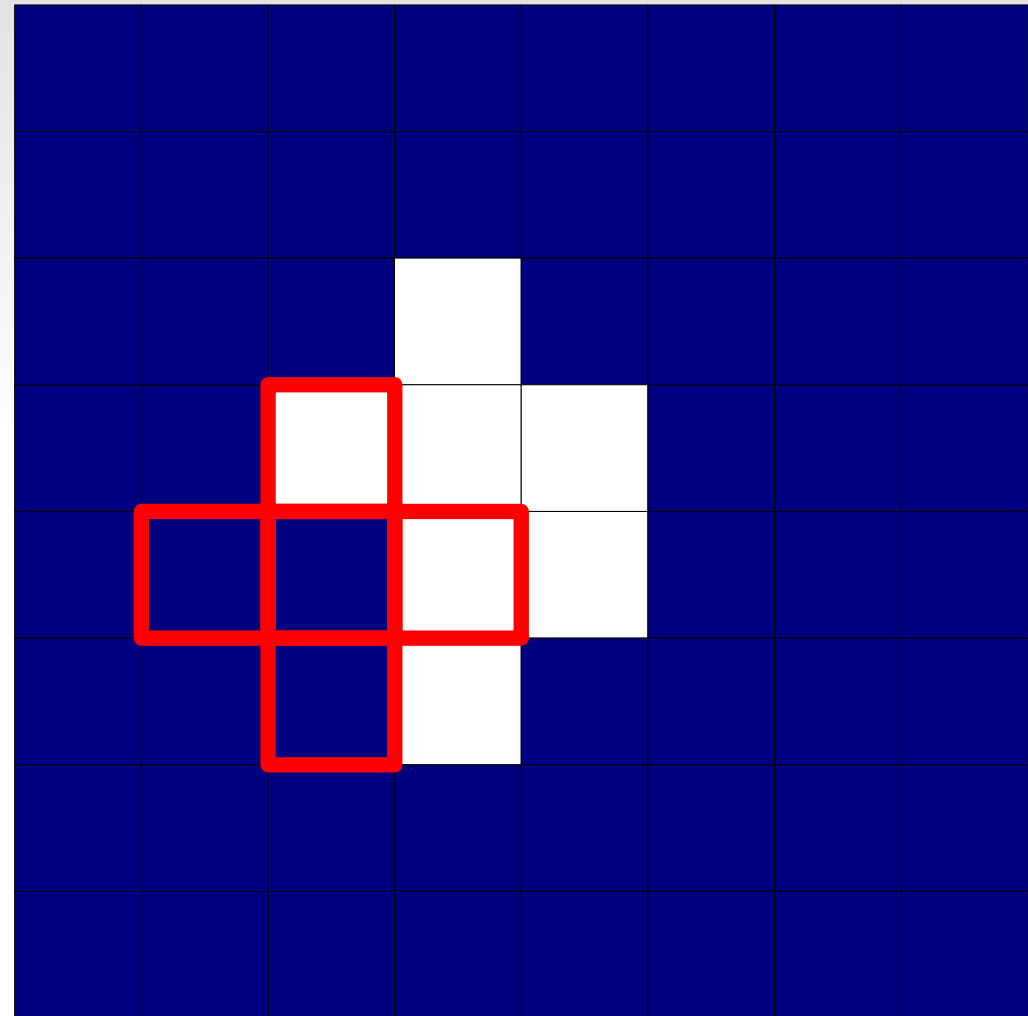
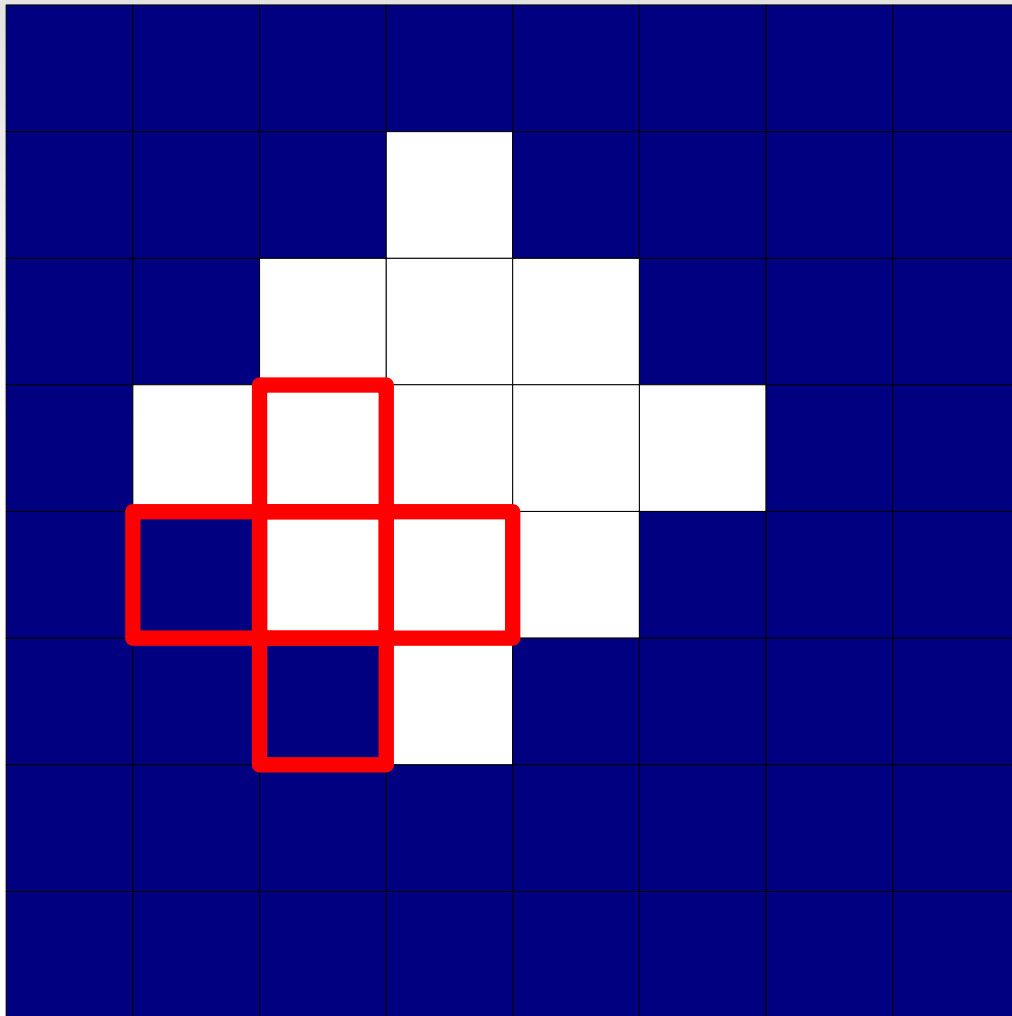
# Binary Morphology - erosion



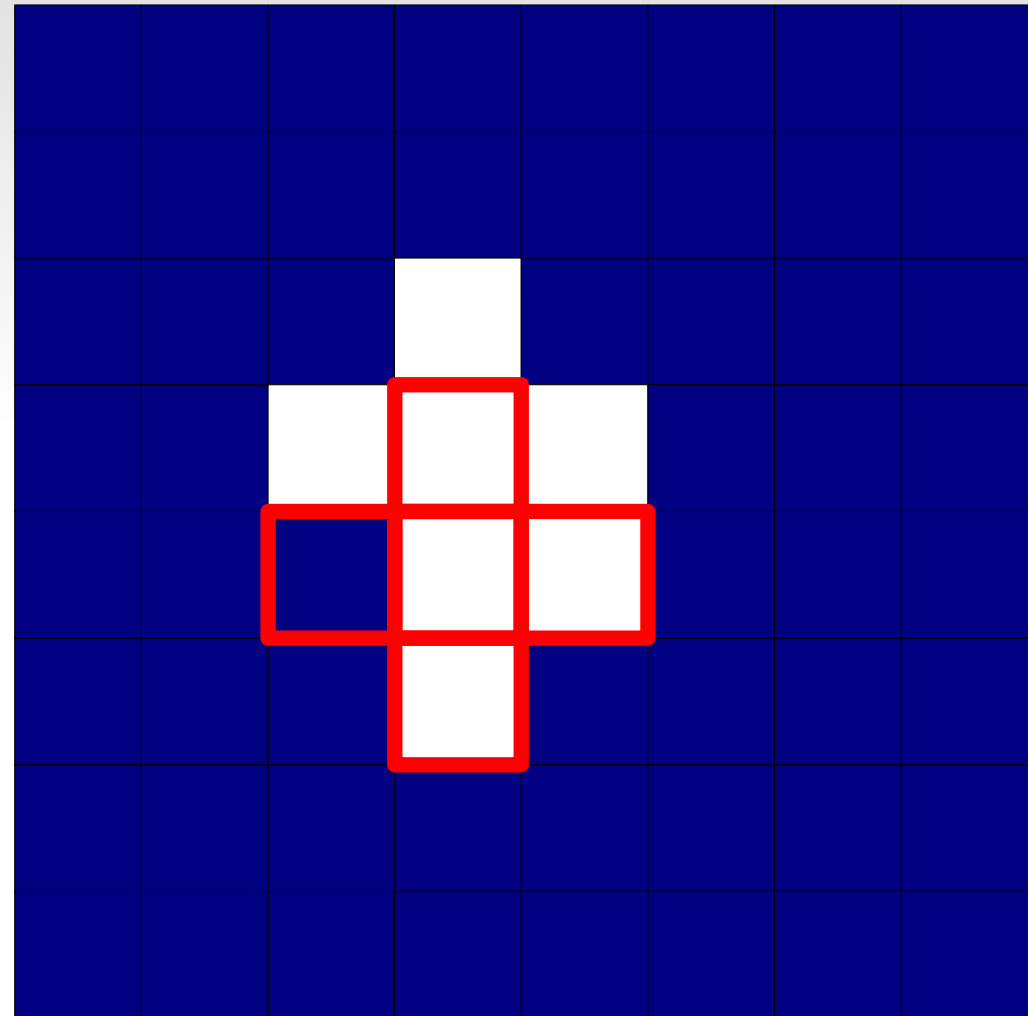
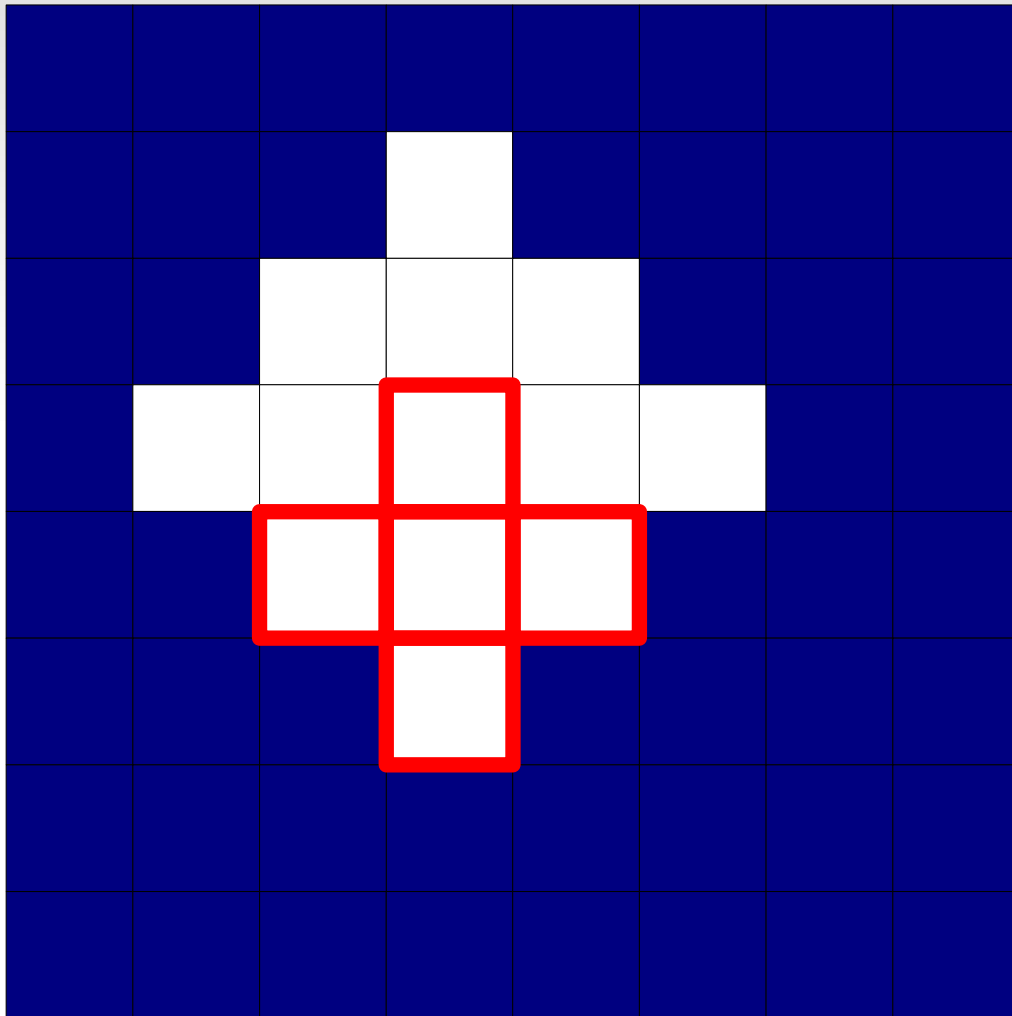
# Binary Morphology - erosion



# Binary Morphology - erosion

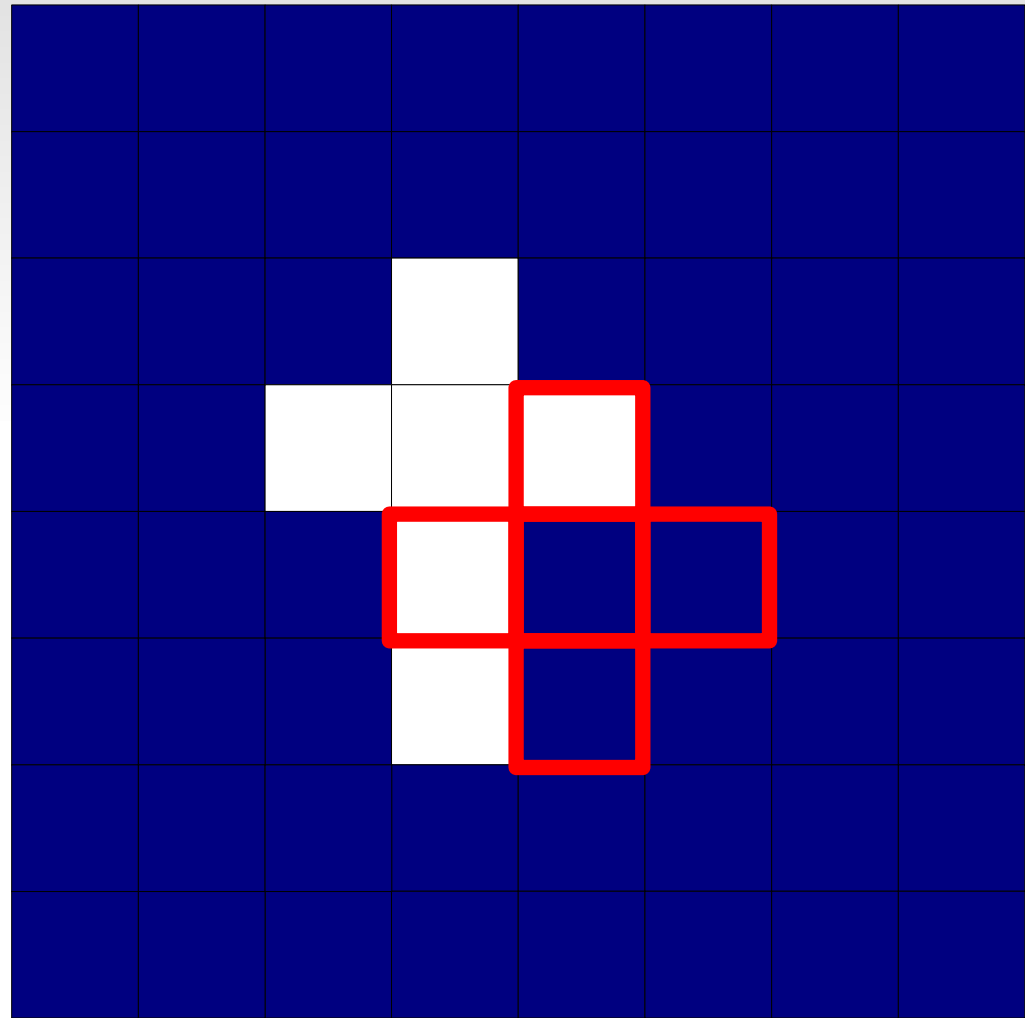
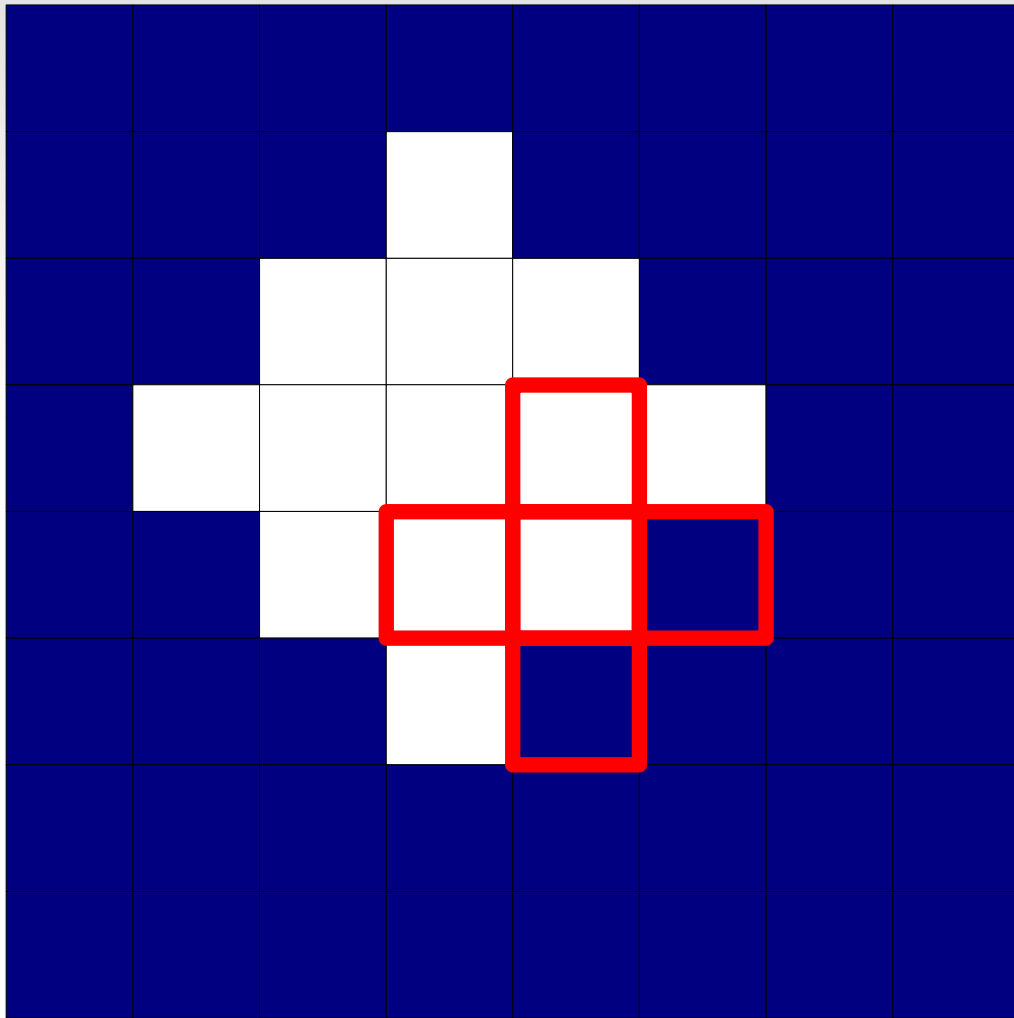


# Binary Morphology - erosion

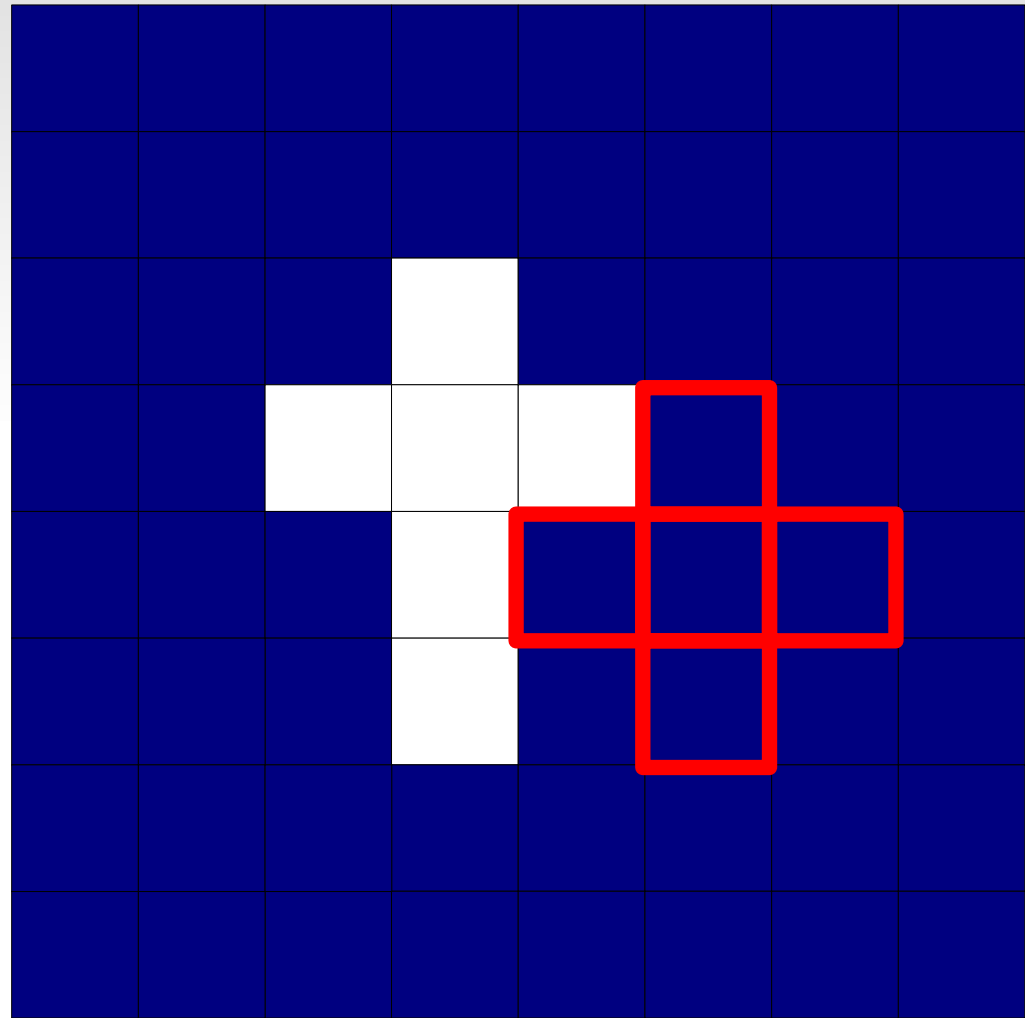
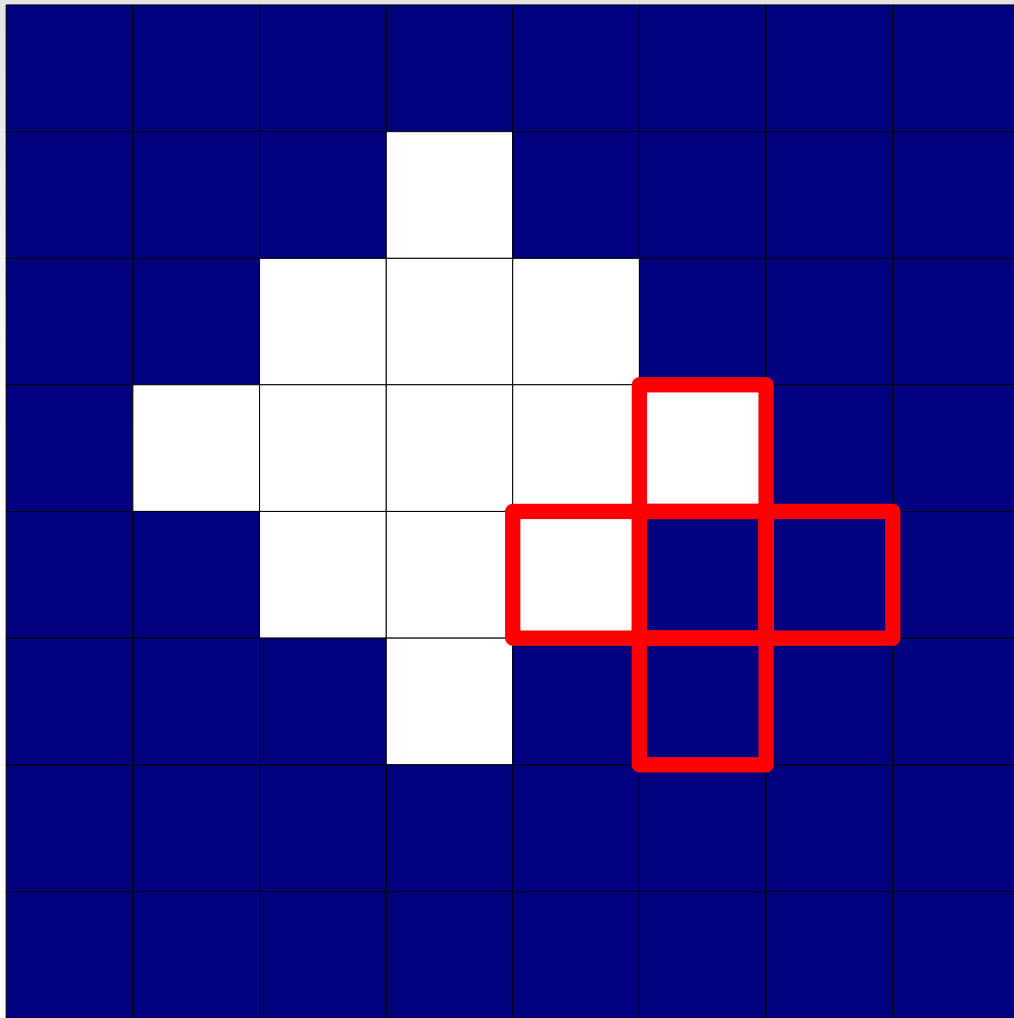




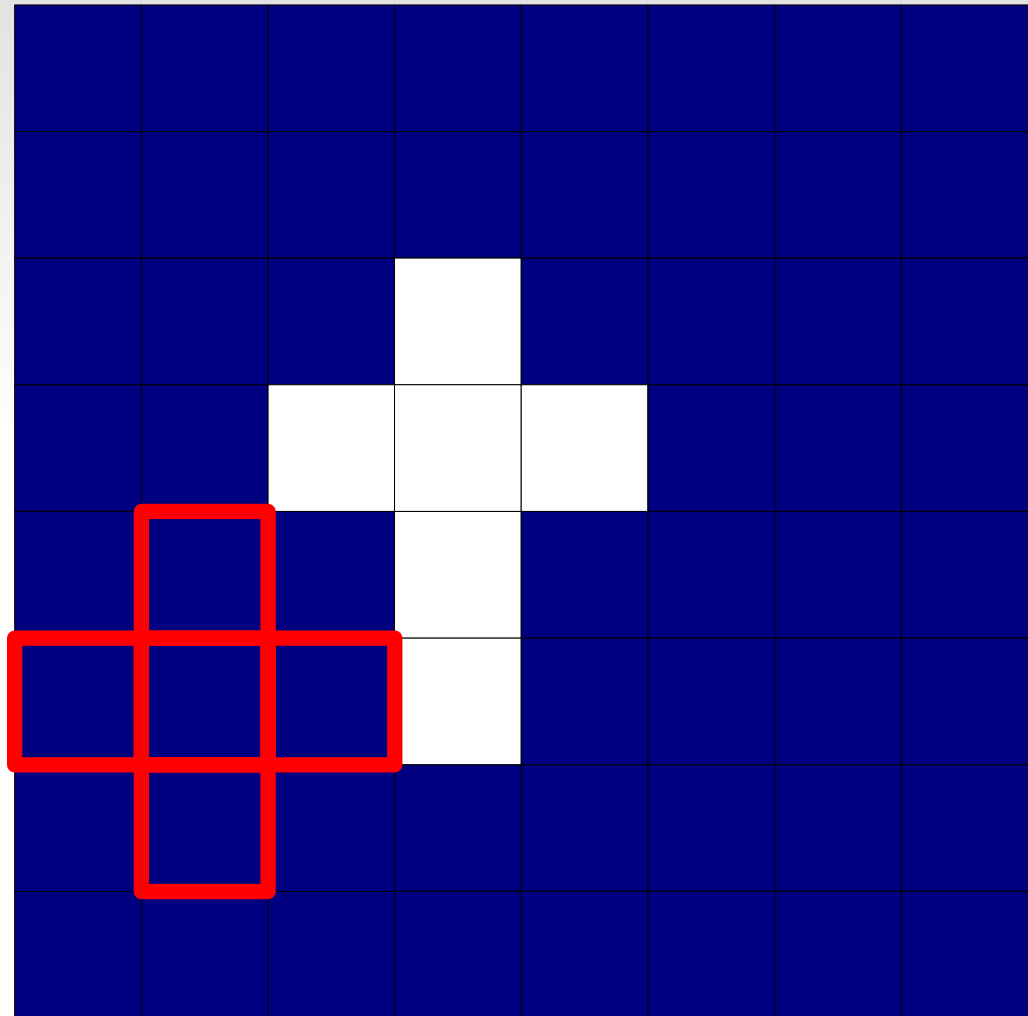
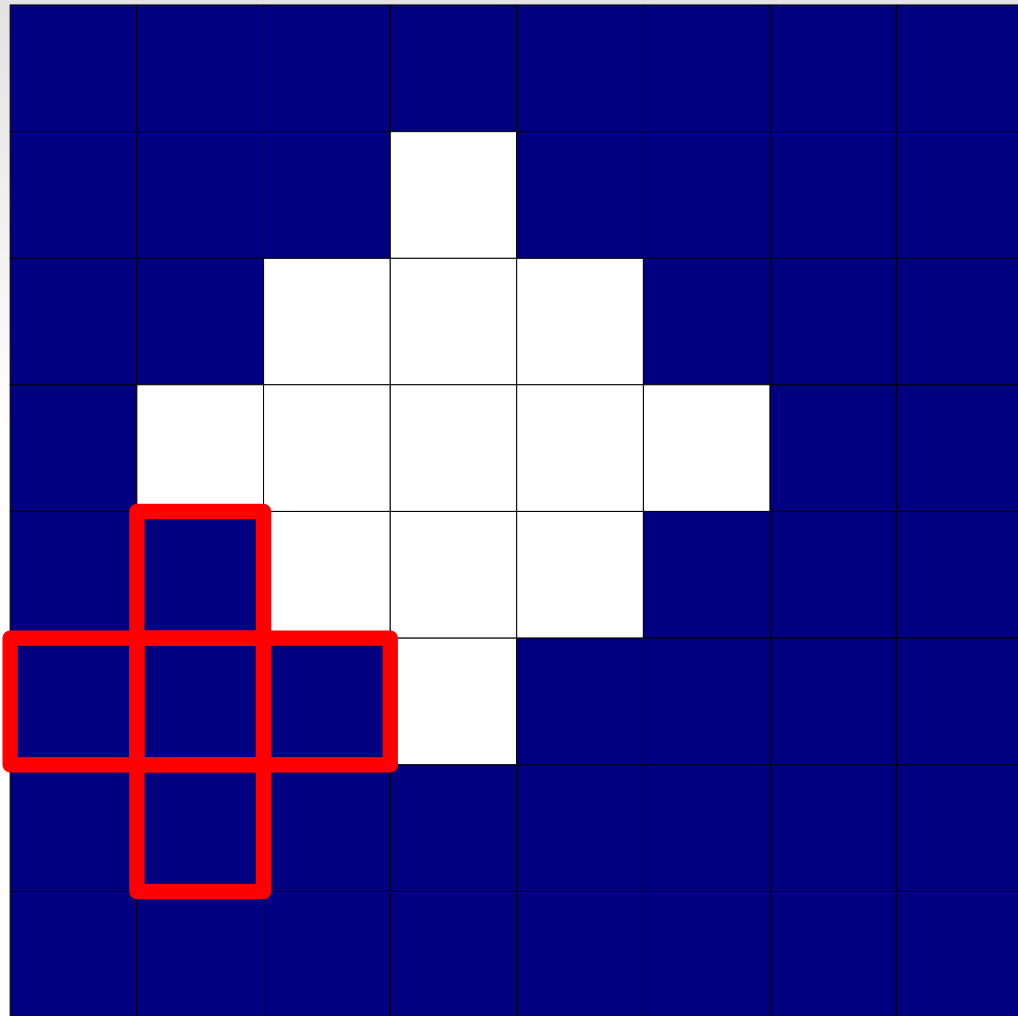
# Binary Morphology - erosion



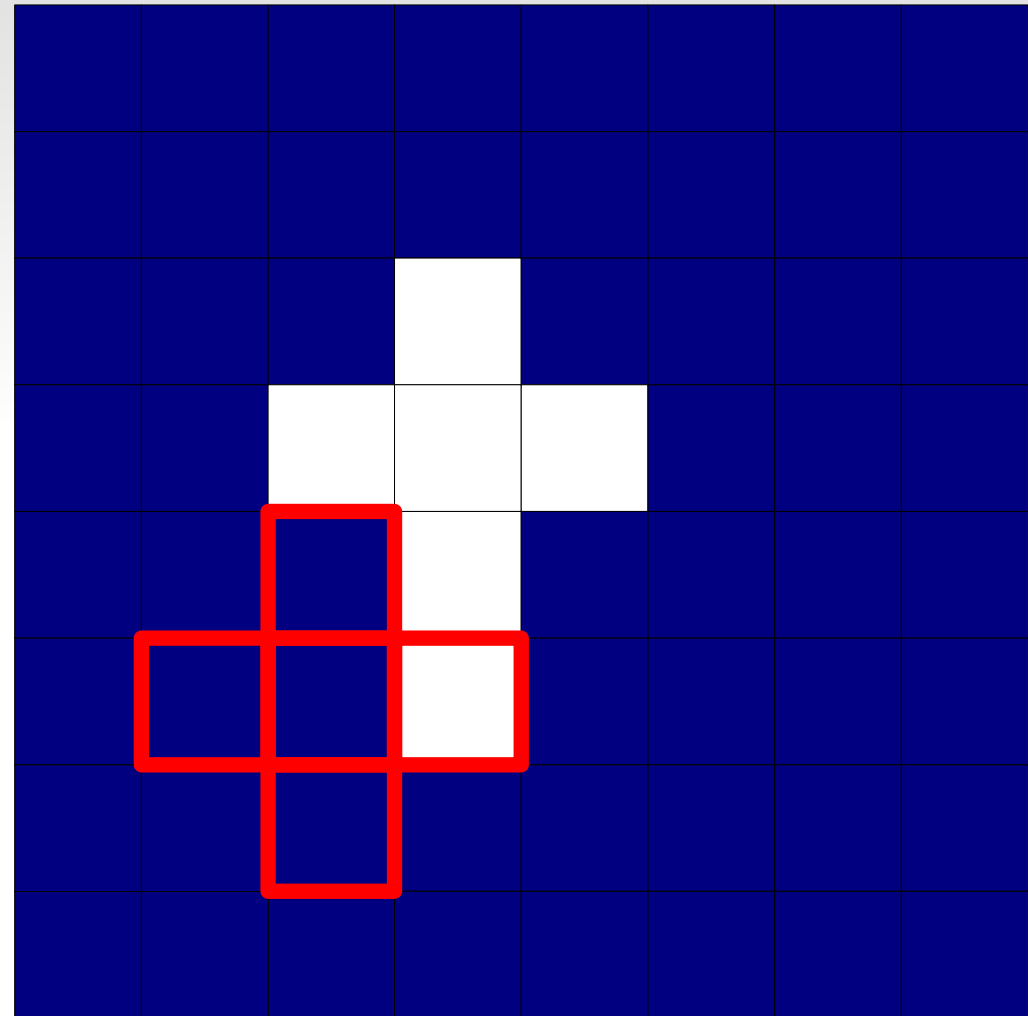
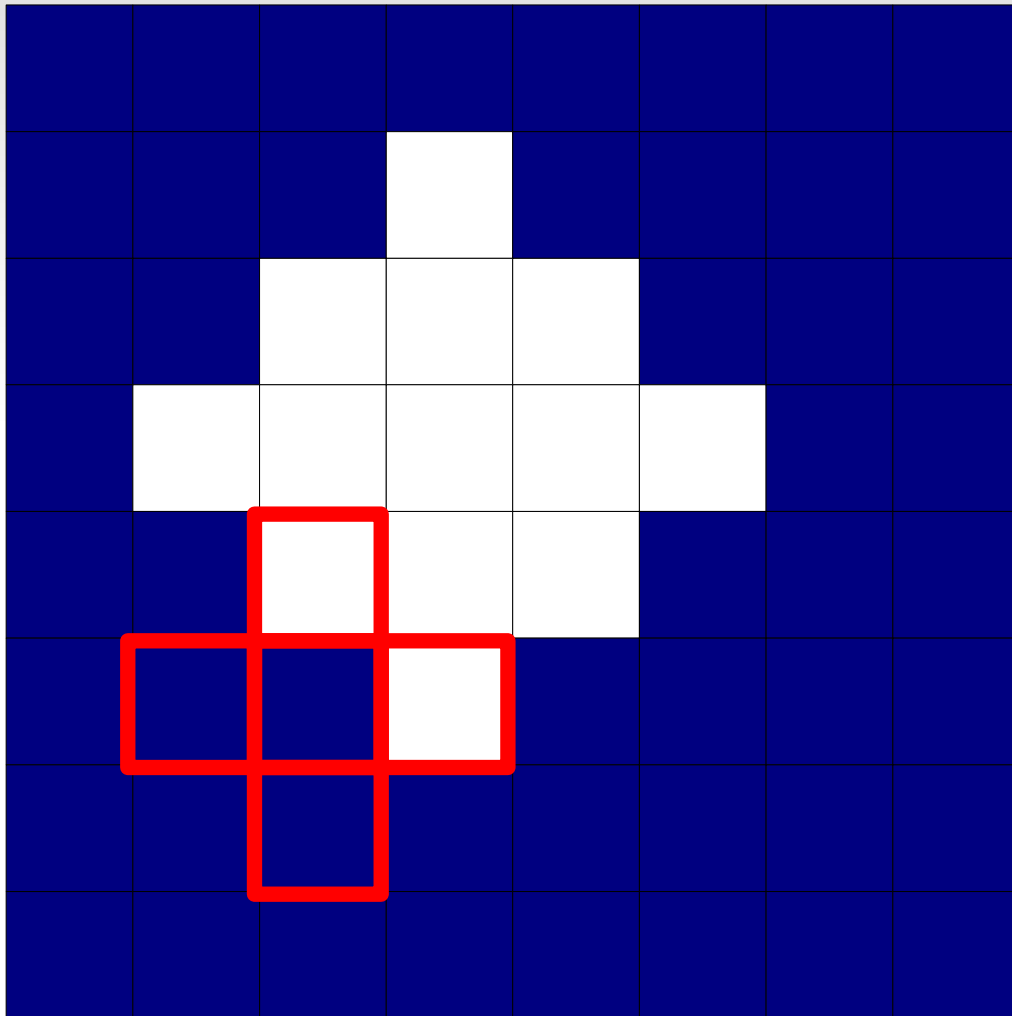
# Binary Morphology - erosion



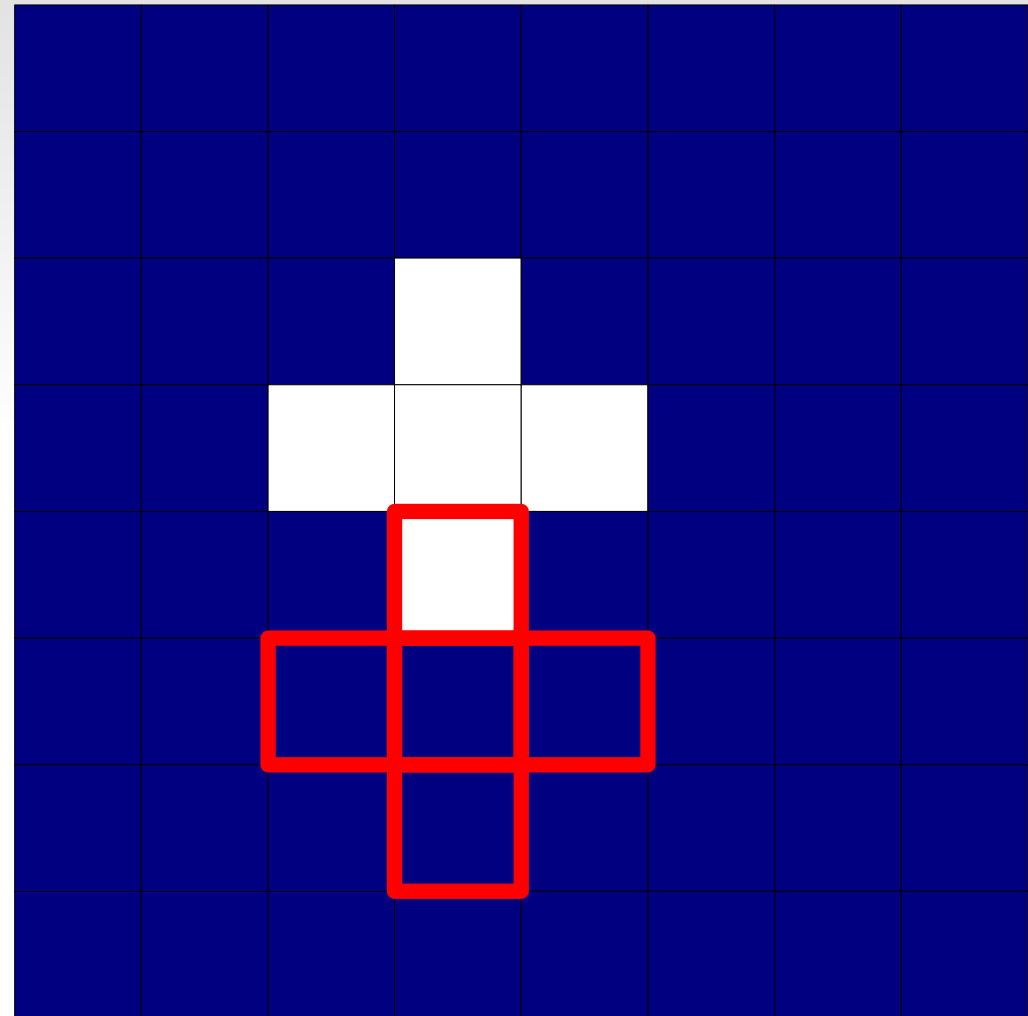
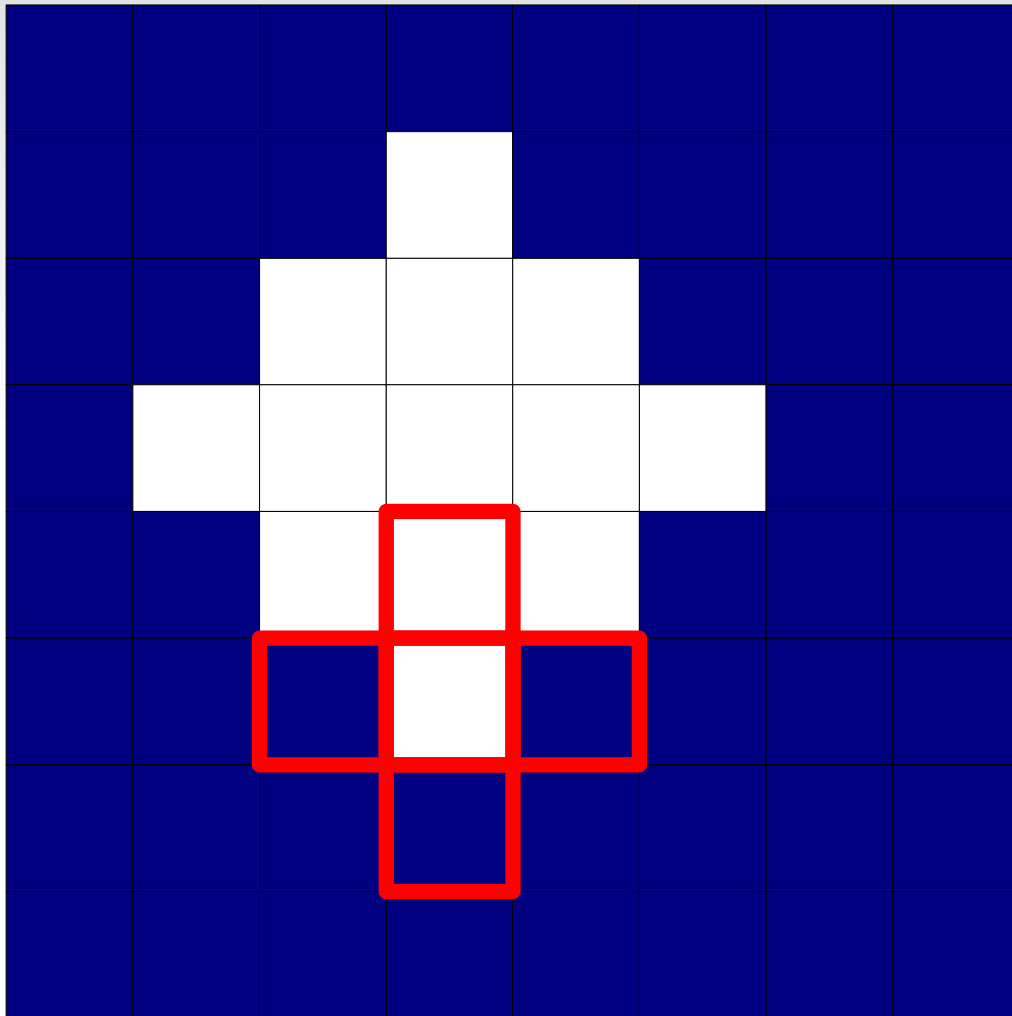
# Binary Morphology - erosion



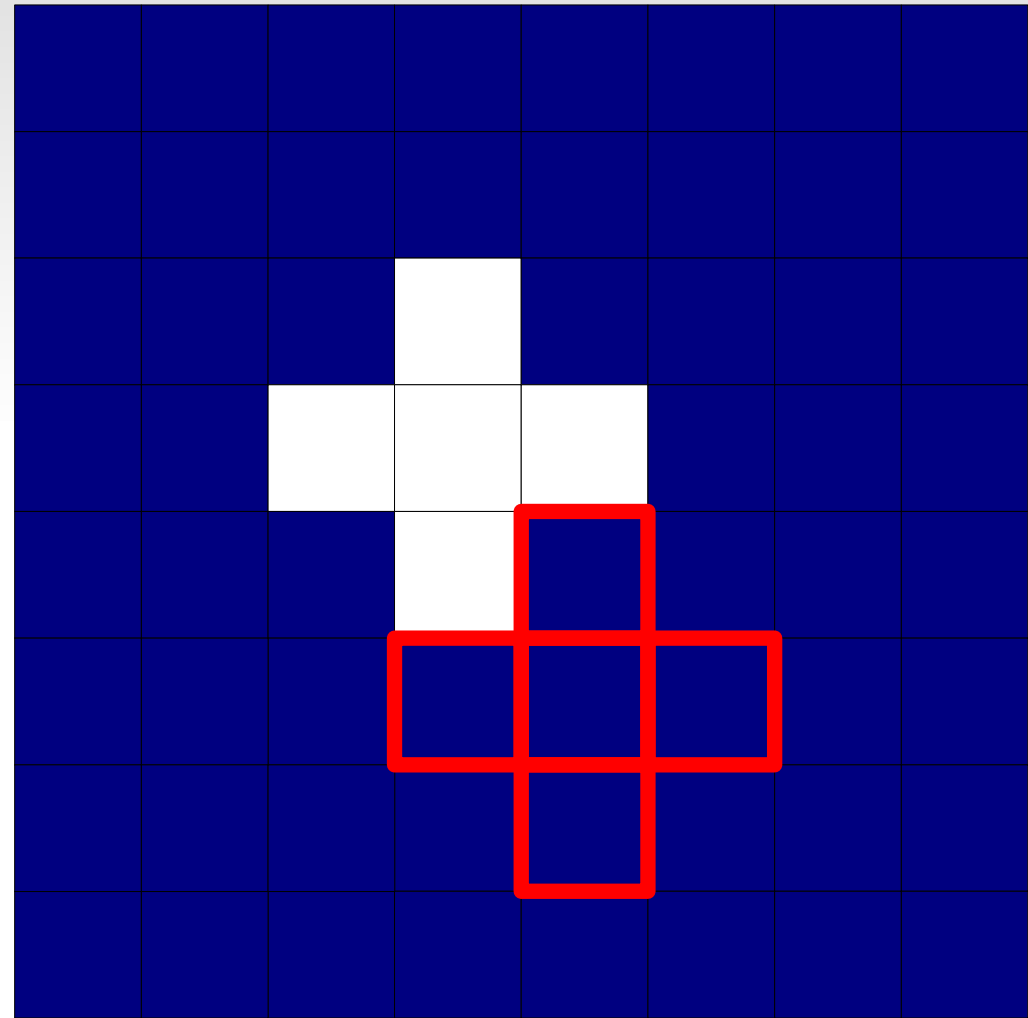
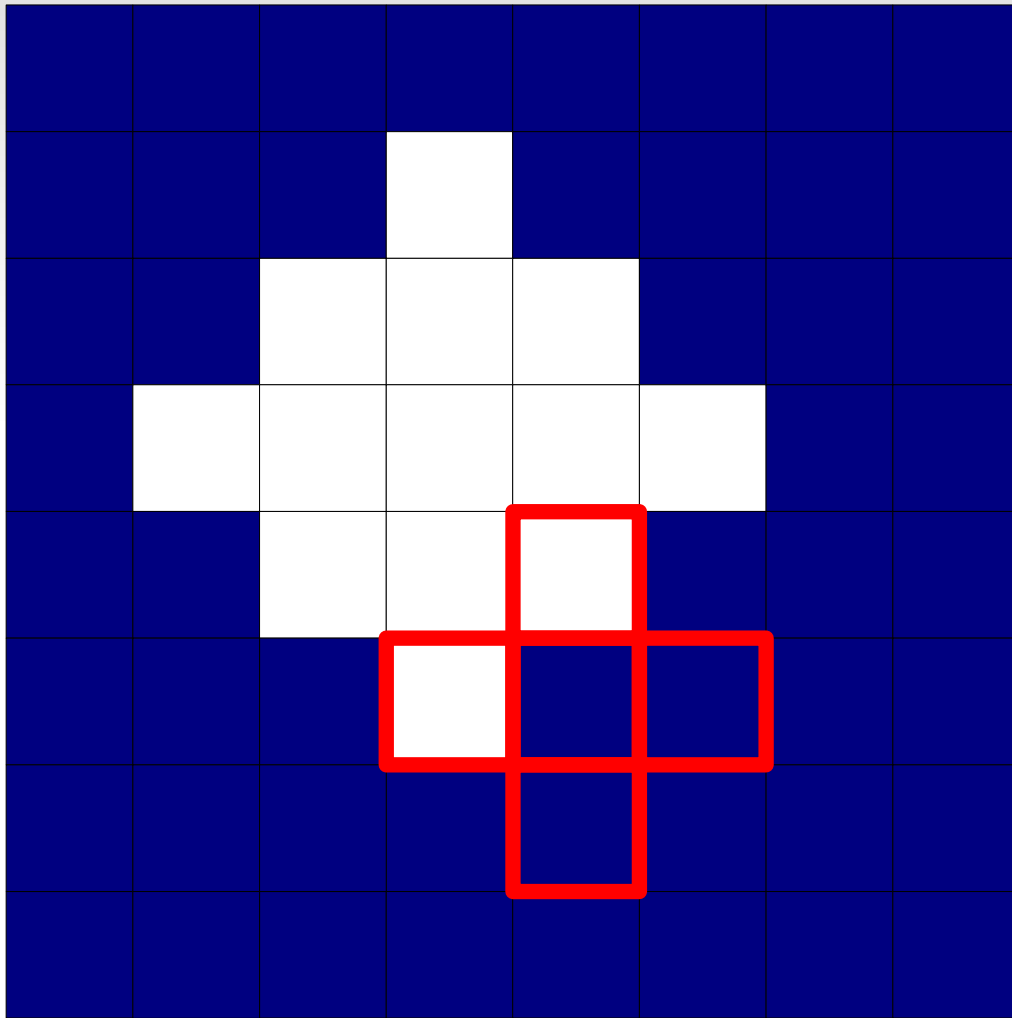
# Binary Morphology - erosion



# Binary Morphology - erosion

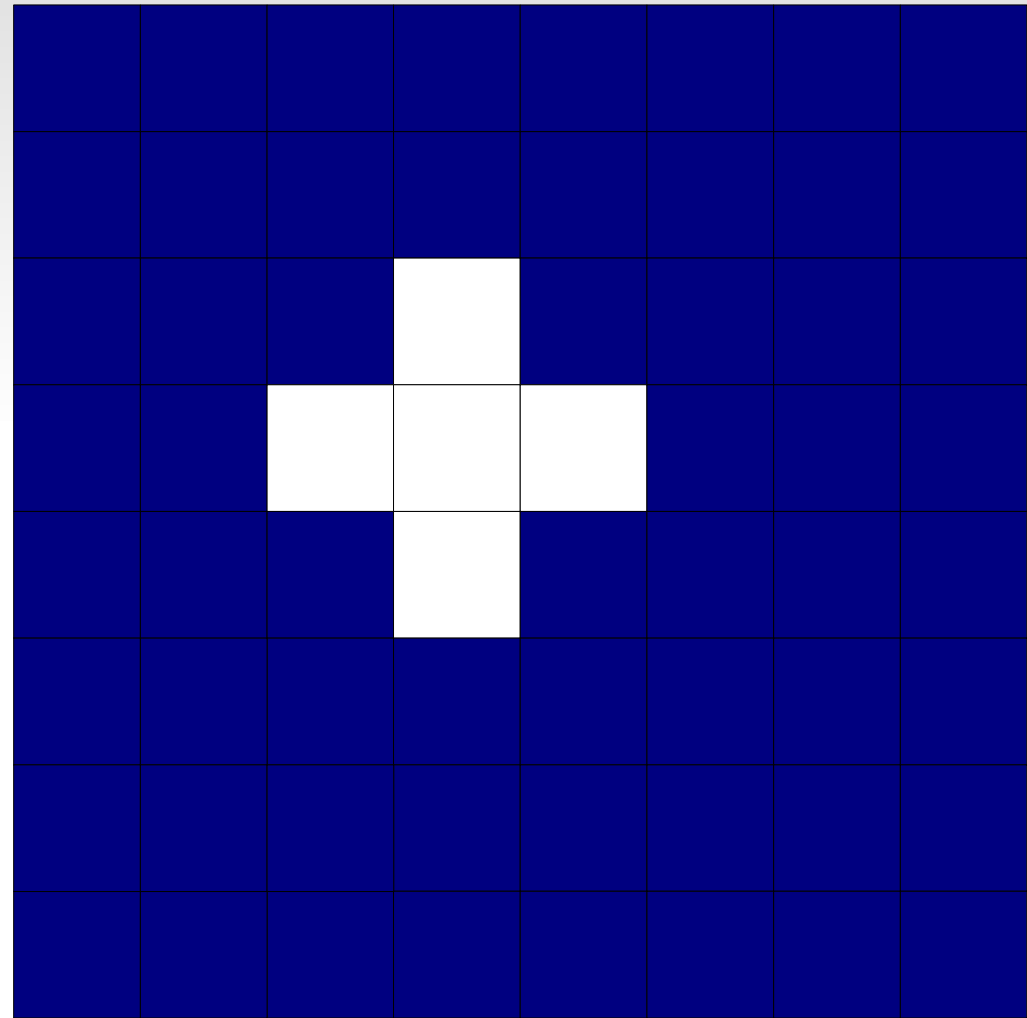
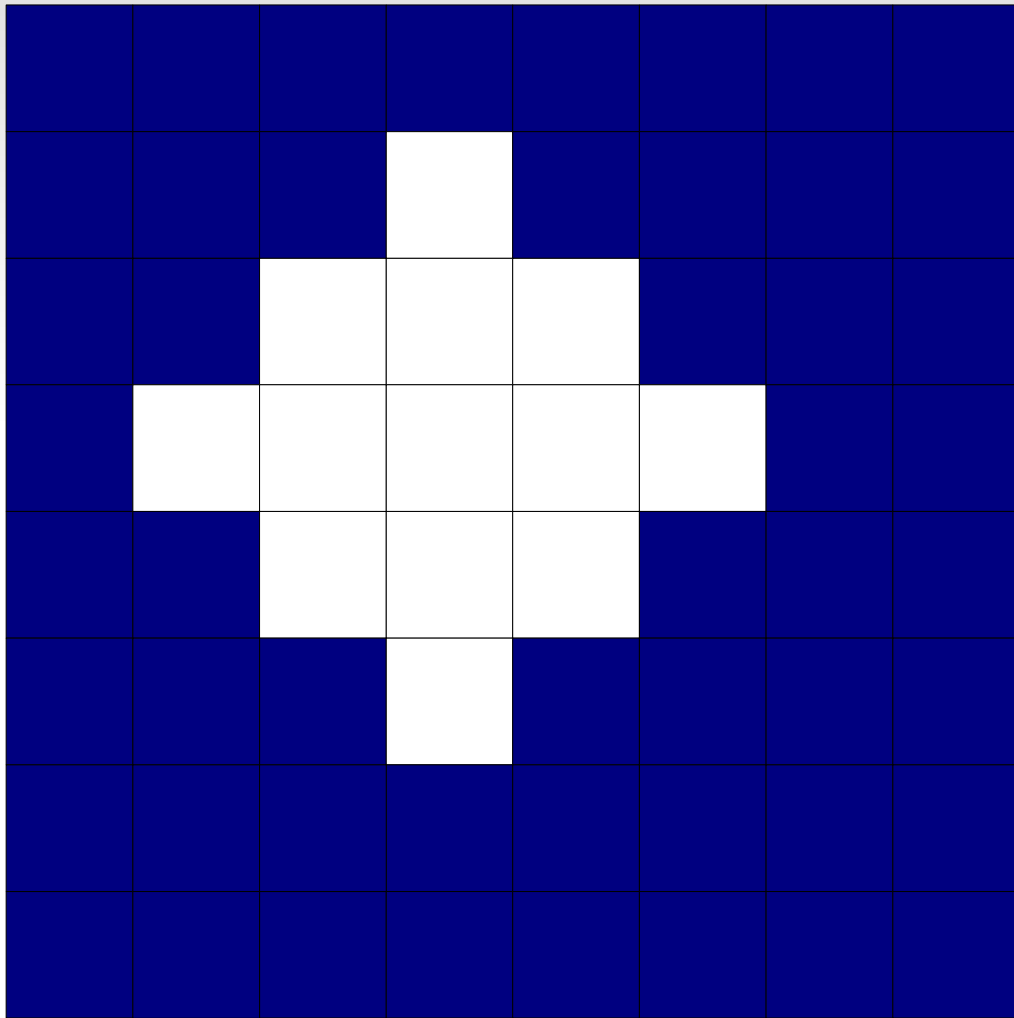


# Binary Morphology - erosion



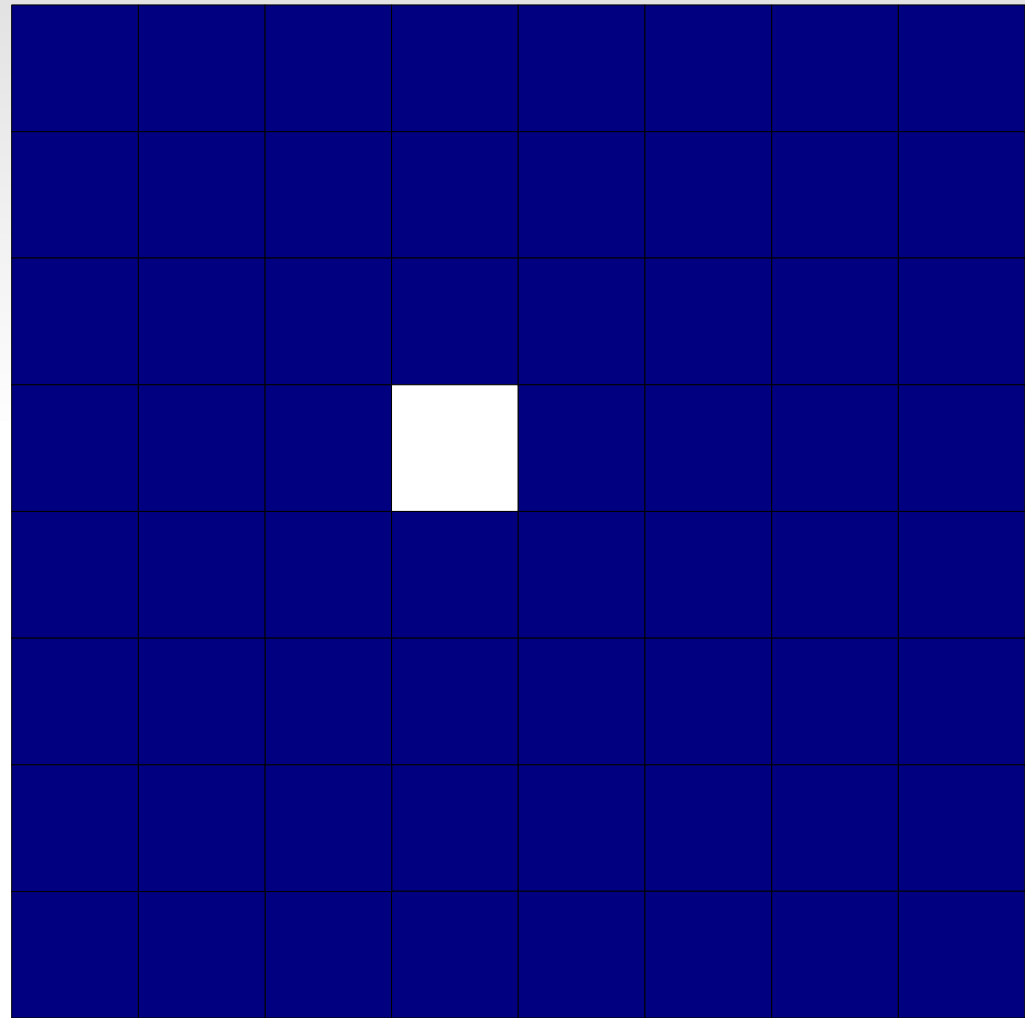
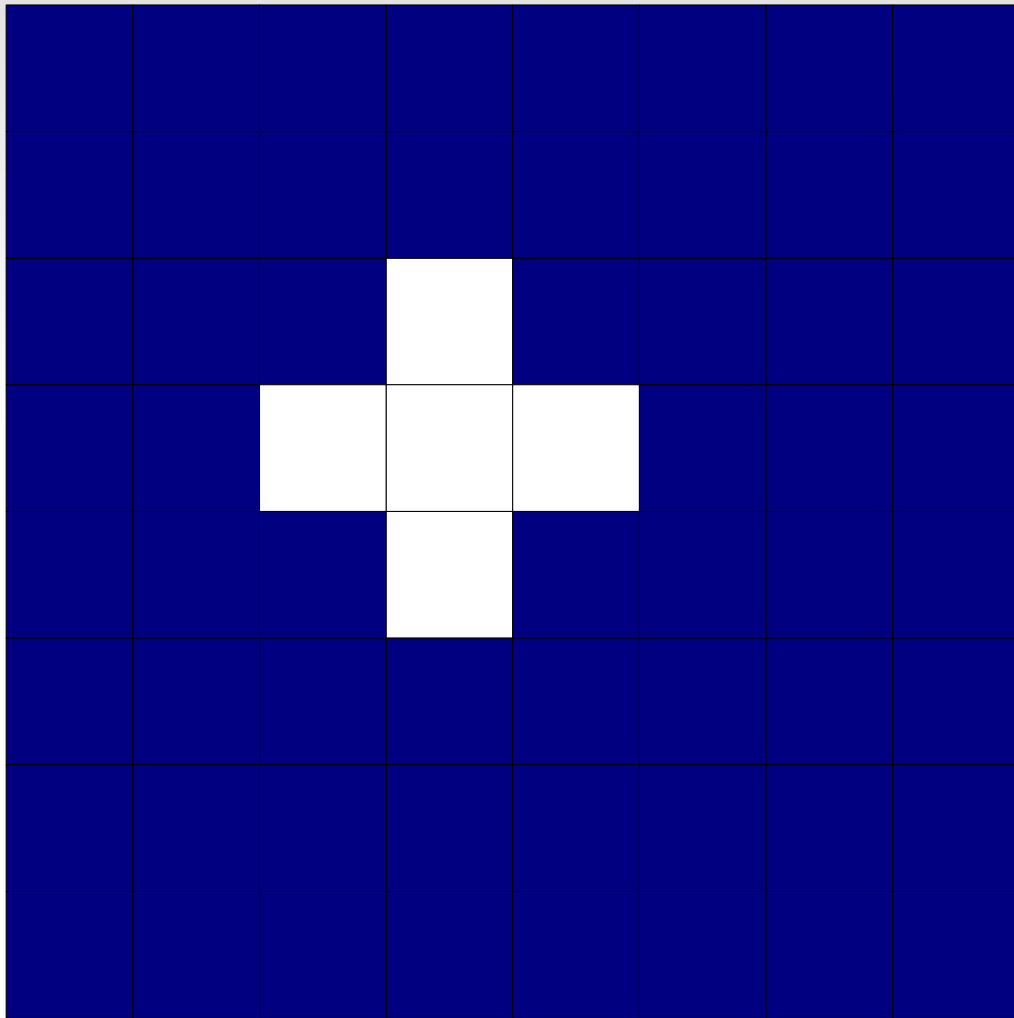


# Binary Morphology - erosion

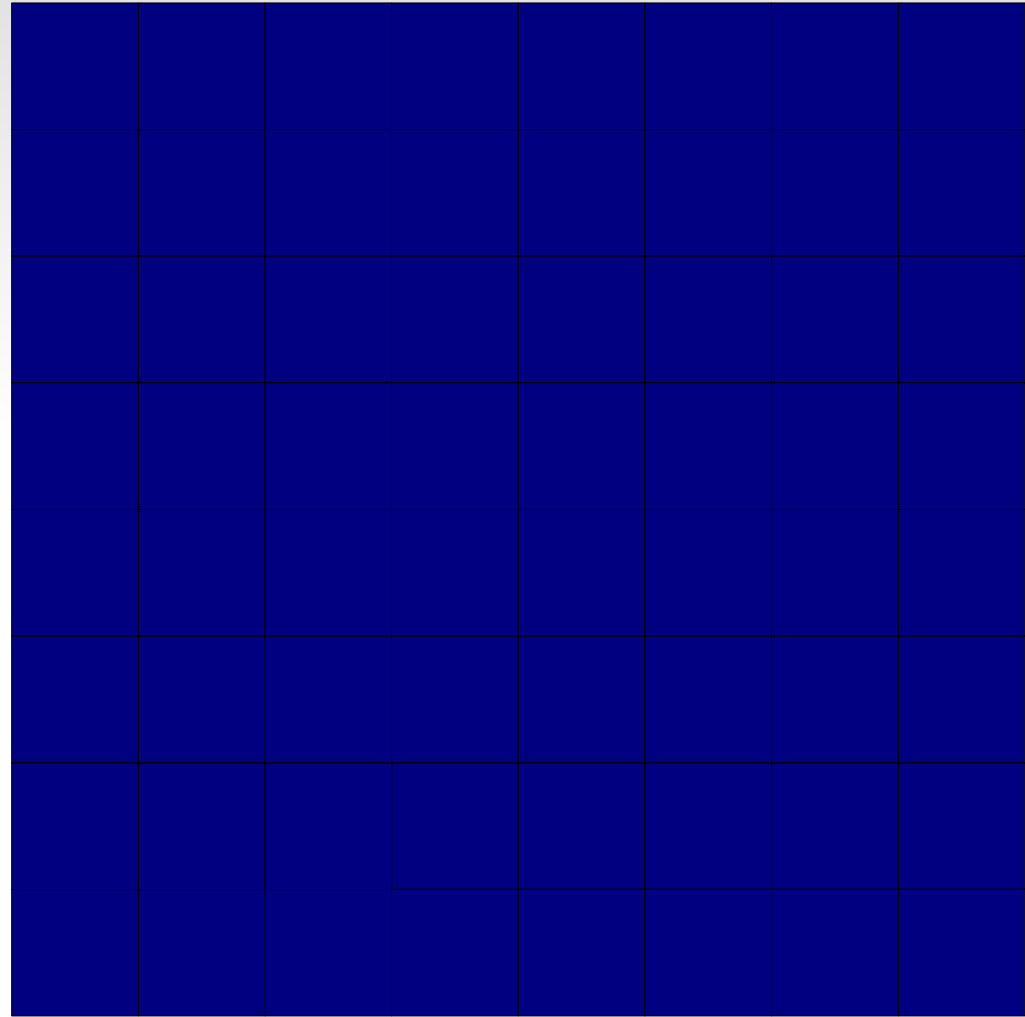
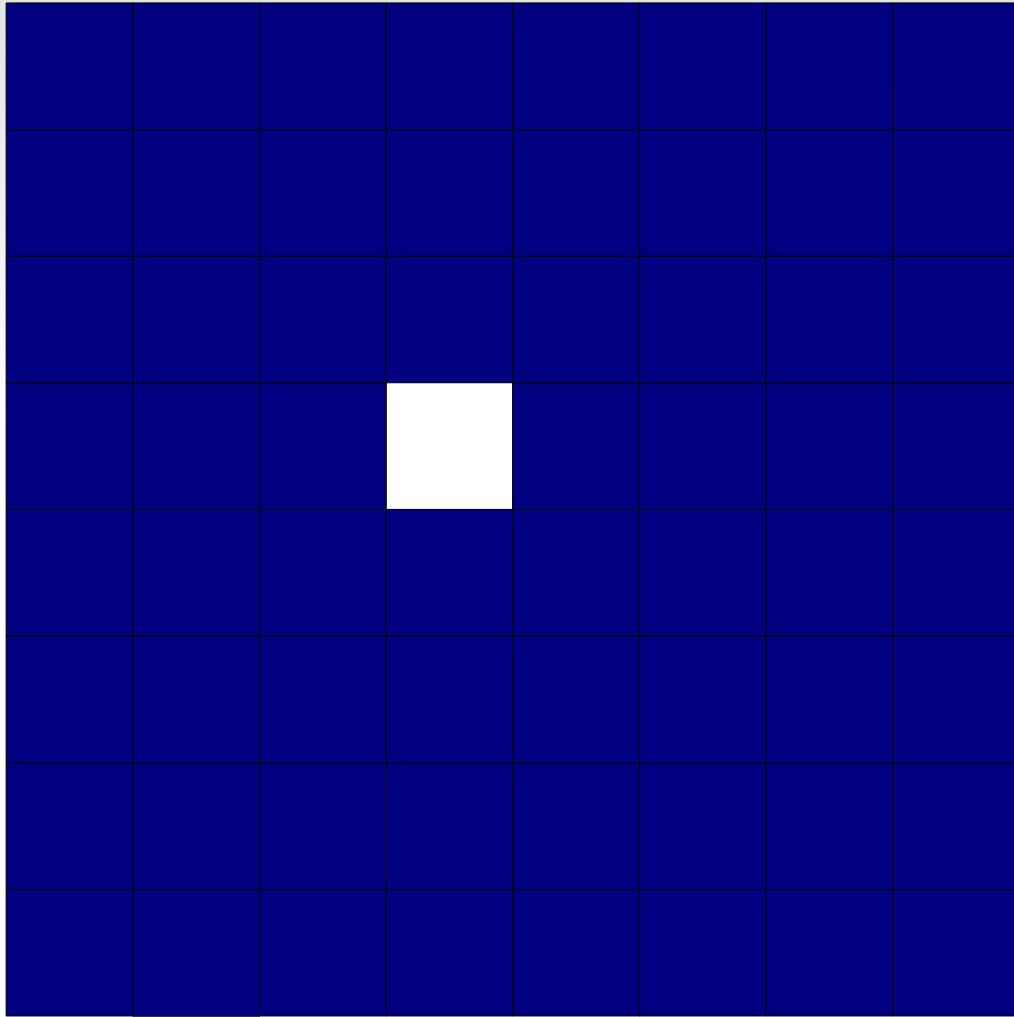




# Binary Morphology - erosion

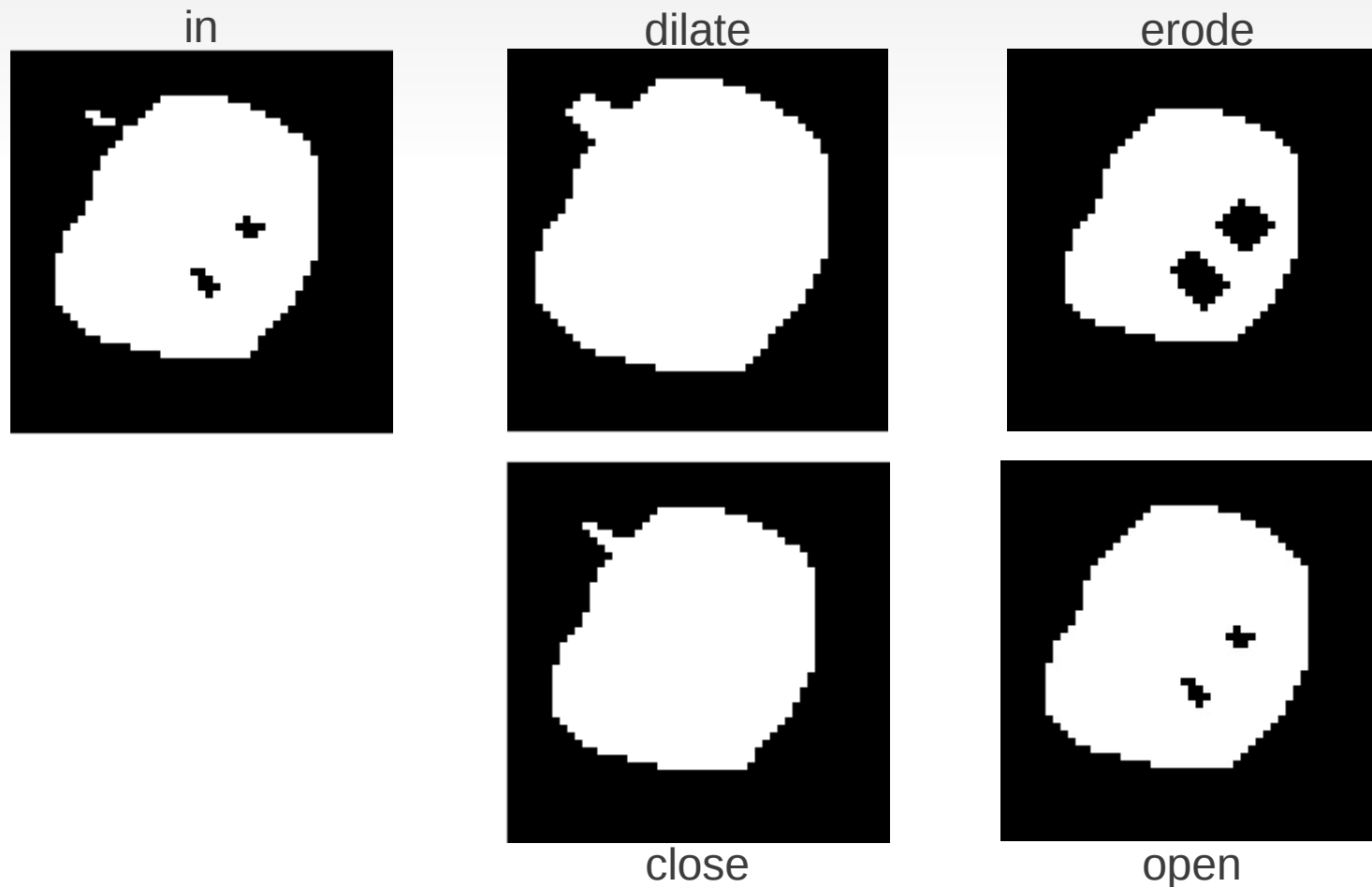


# Binary Morphology - erosion



# 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



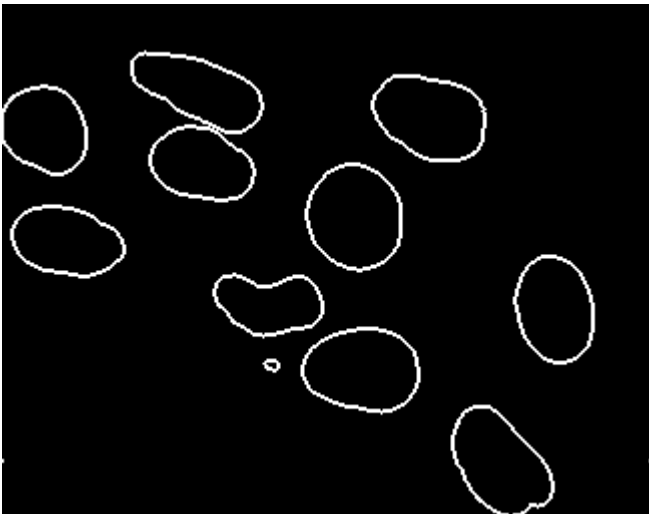
# Binary Morphology - applications

edge detection

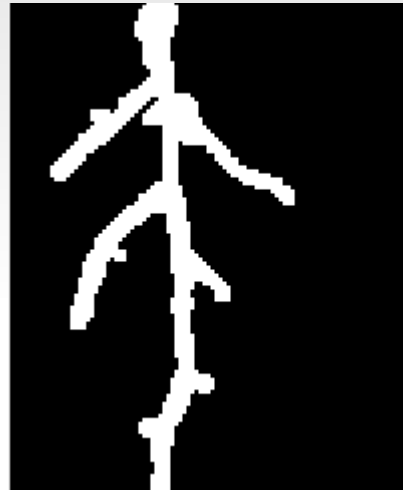
I



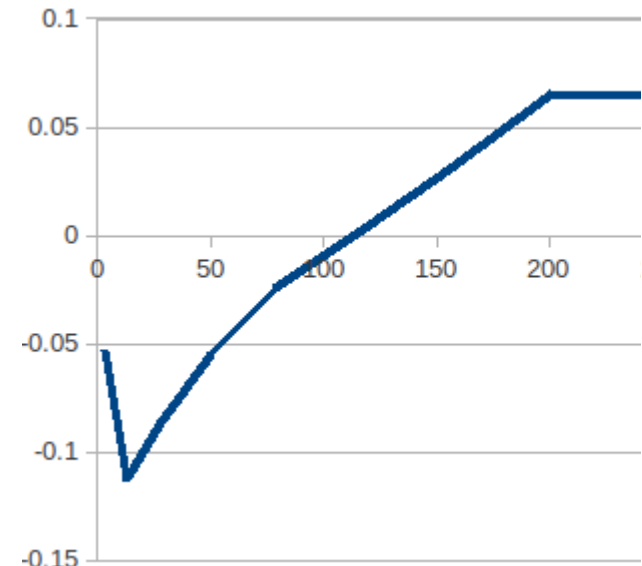
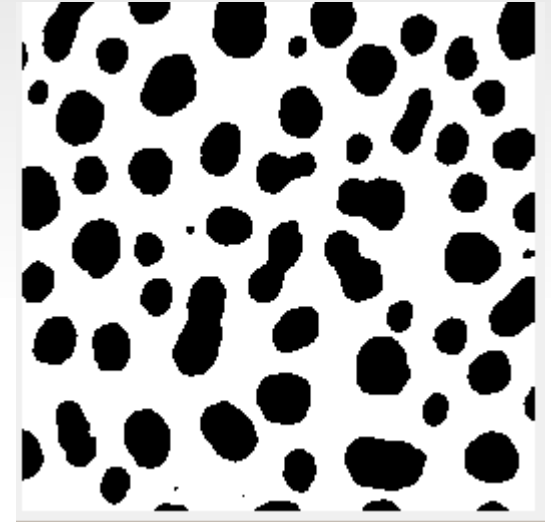
dilate(I) - erode(I)



skeletonization

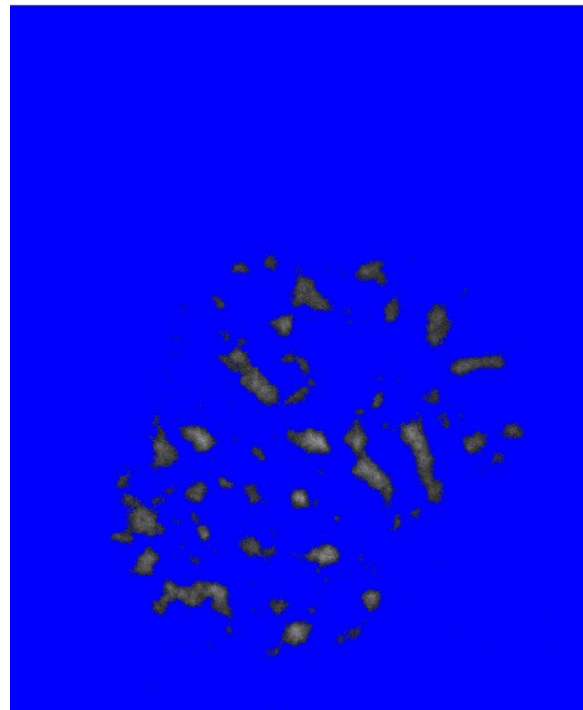
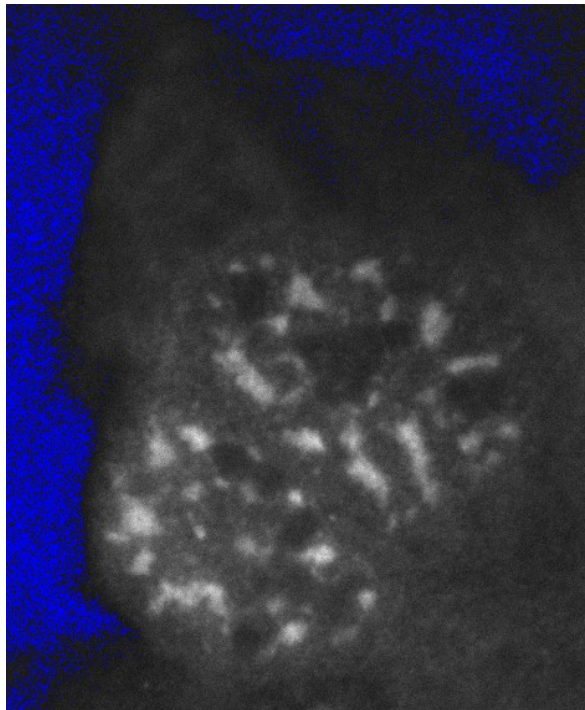


granulometry



# grayscale morphology

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

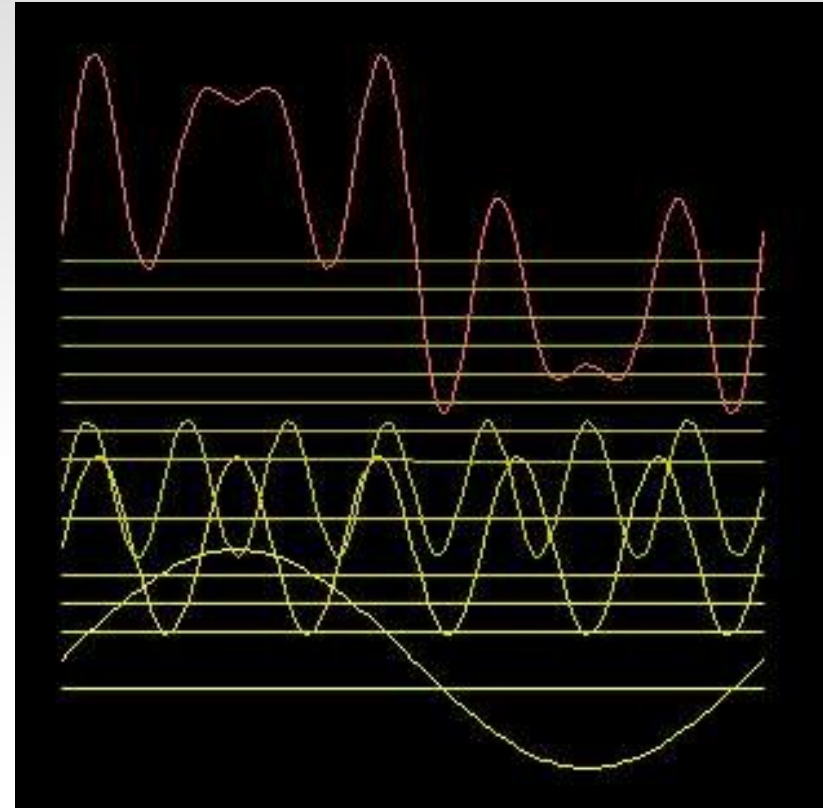
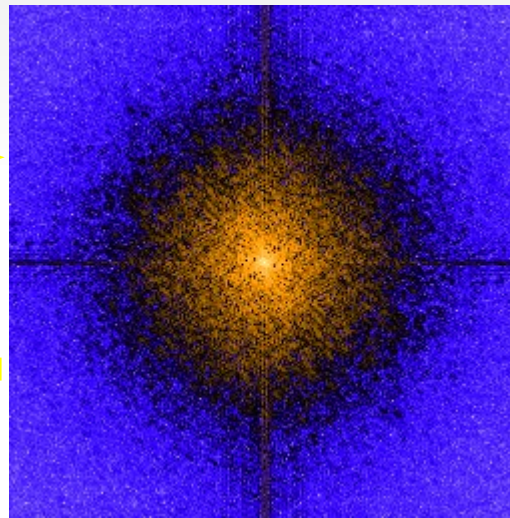
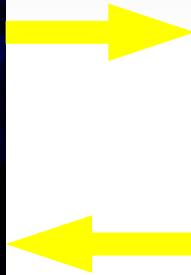
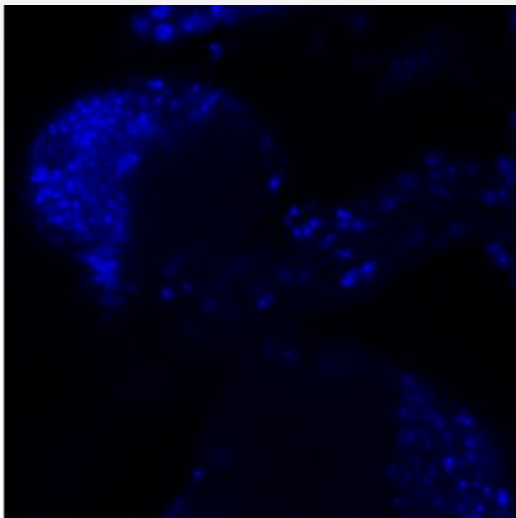


# filtering in the frequency domain

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

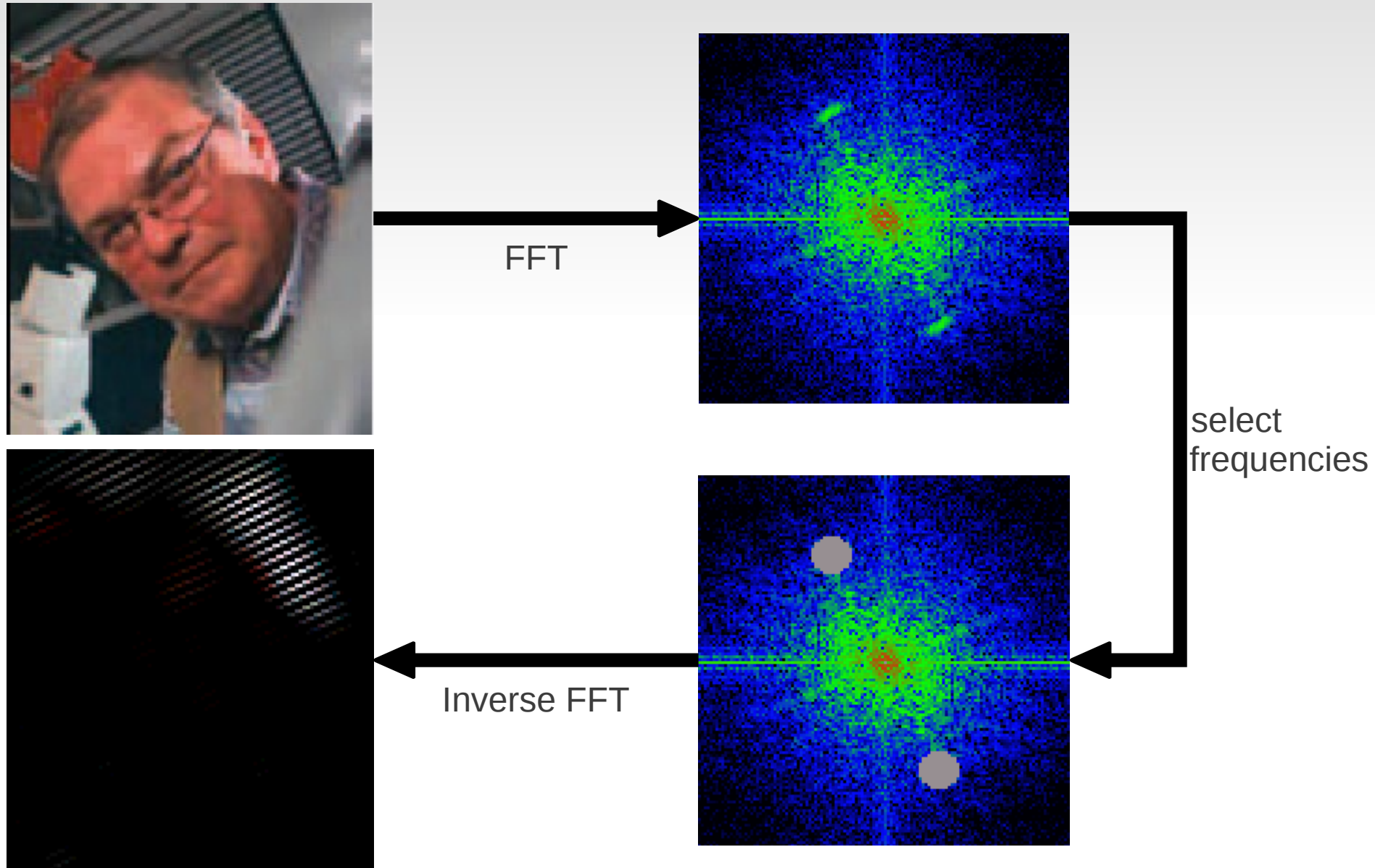
# filtering in the frequency domain - fourier transform

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



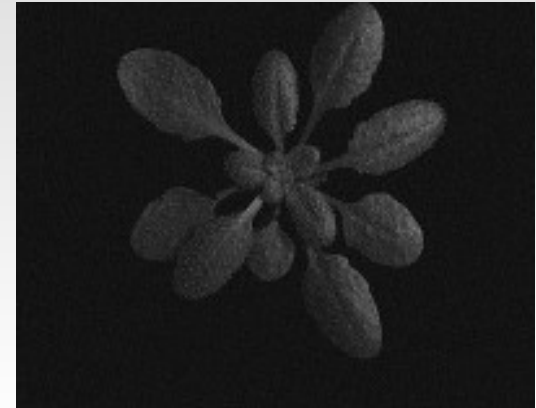
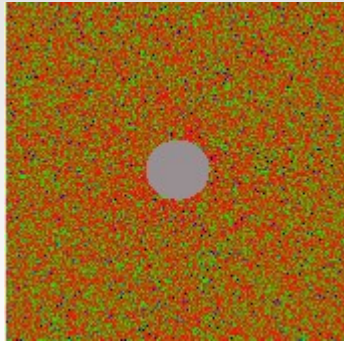
- signal can be represented as sum of sinoids
- FT transforms from spatial to frequency domain

# Filtering in the frequency domain

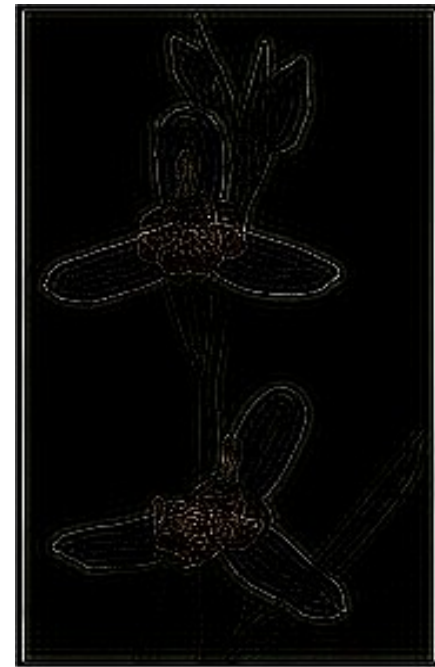
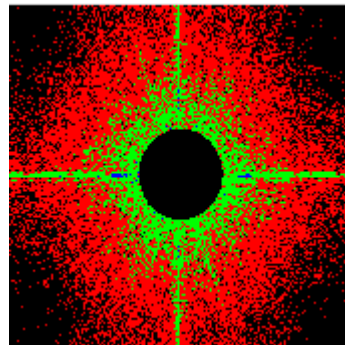




# Filtering in the frequency domain



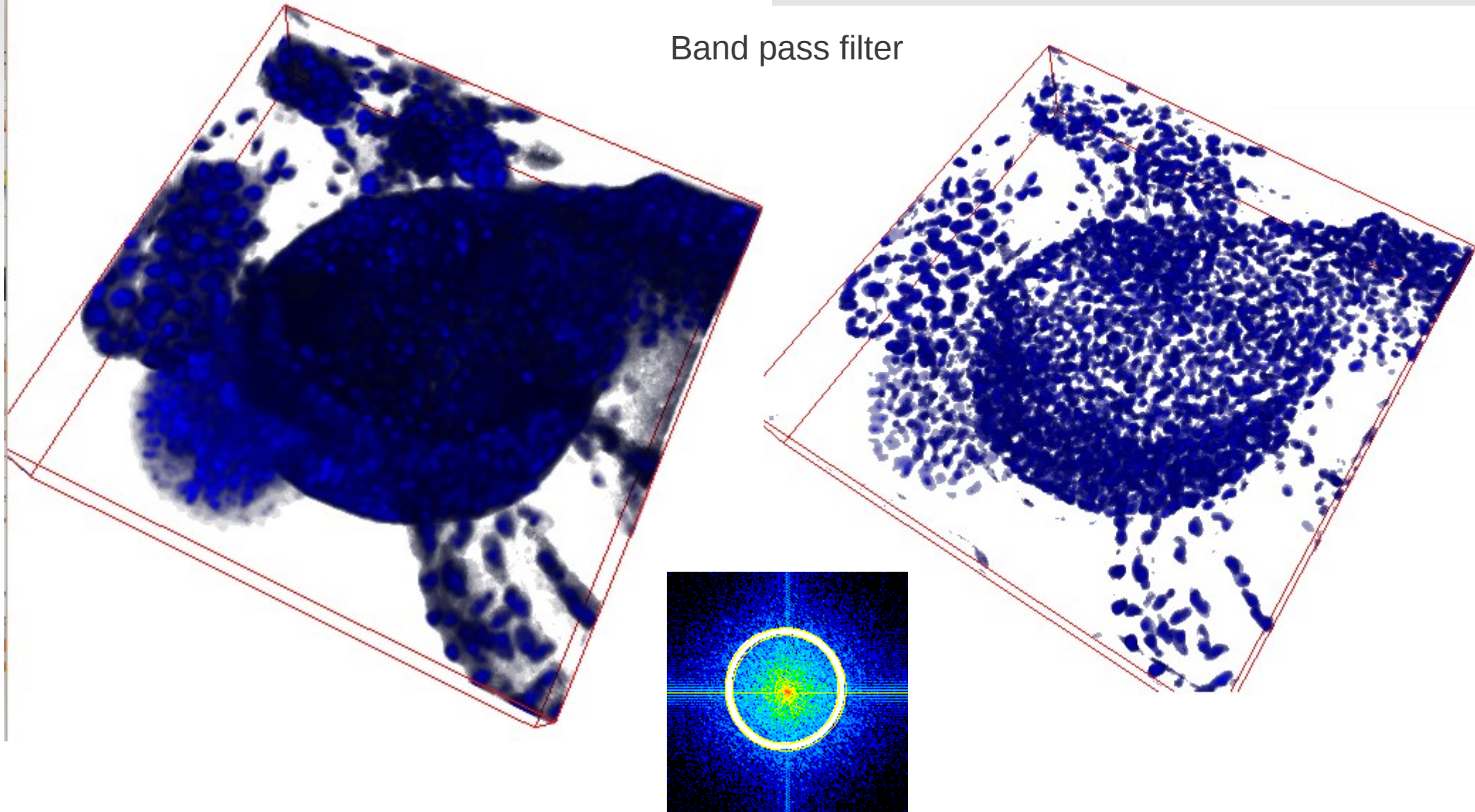
Low pass filter



High pass filter

# Filtering in the frequency domain

Band pass filter

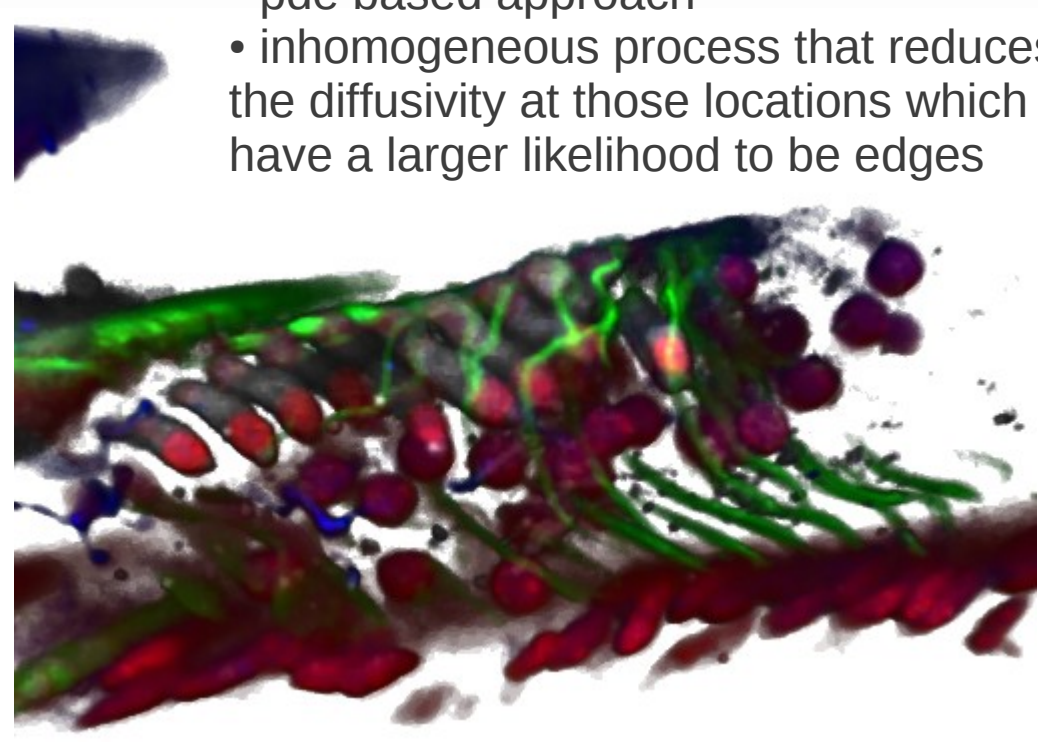
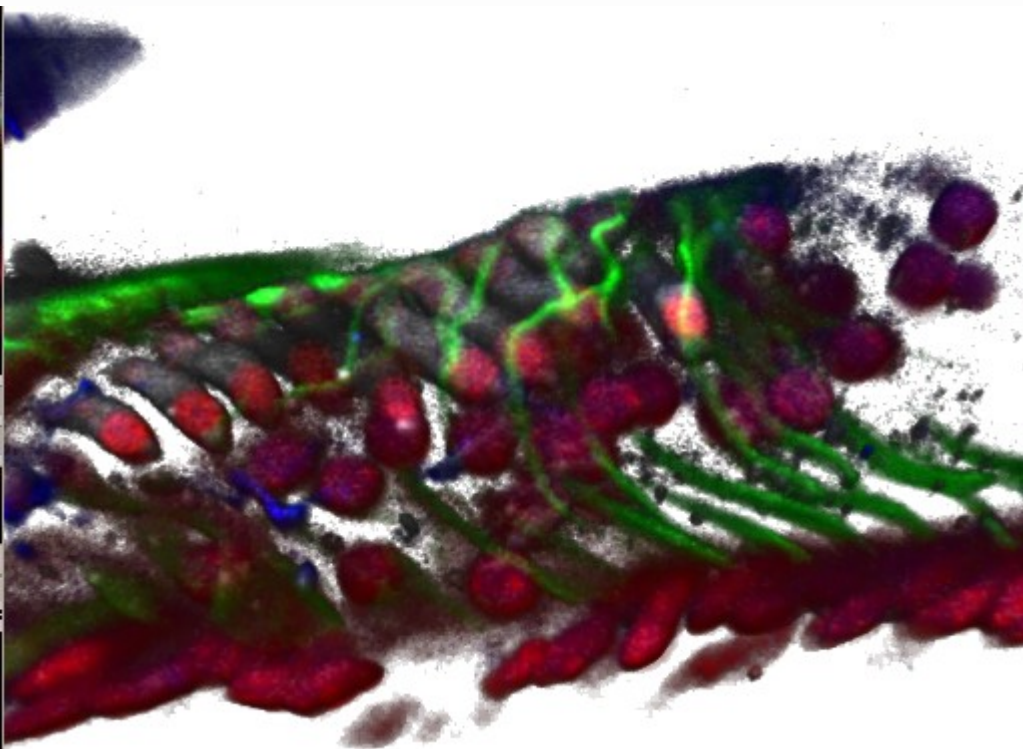


# Image Restoration

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

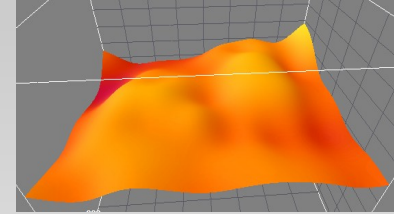
# Image Restoration - Noise reduction

- 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

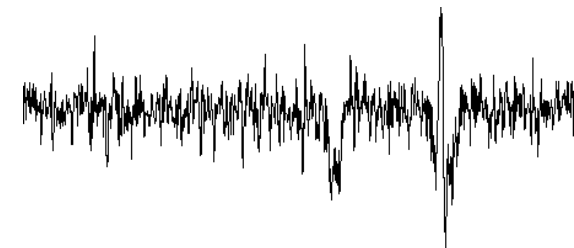
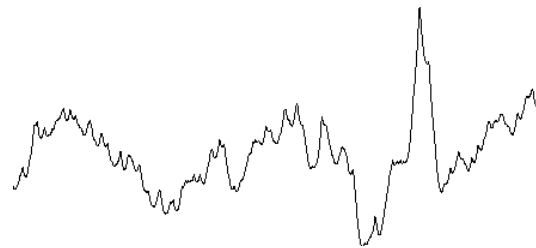
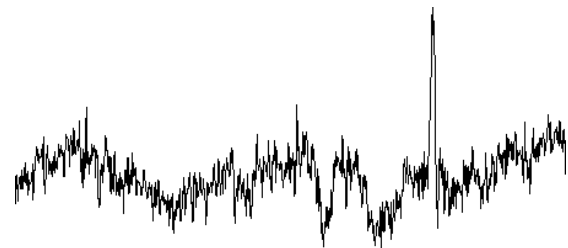
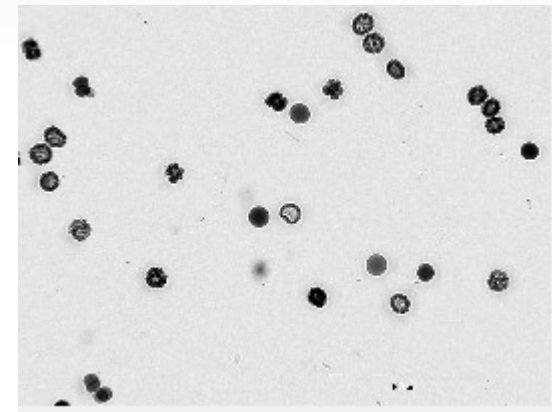
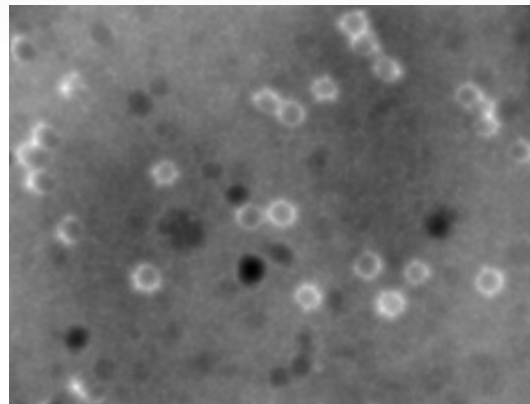
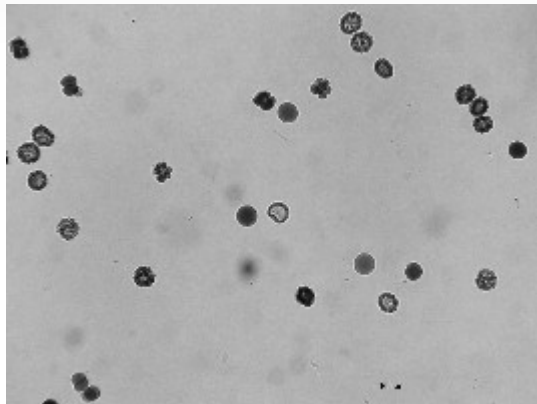


- correct inhomogeneous illumination

- correct with image of background

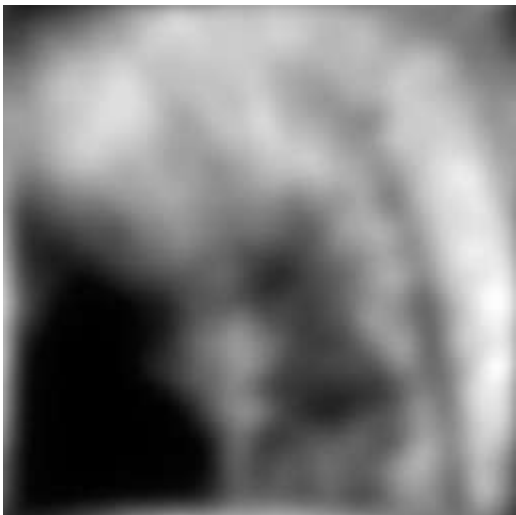
$$\frac{I}{B} \cdot \text{mean}(B)$$

- if not available: estimate background image

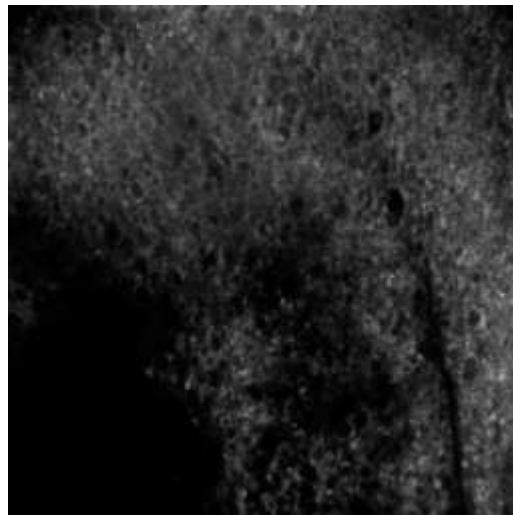


# Image Restoration - Deconvolution

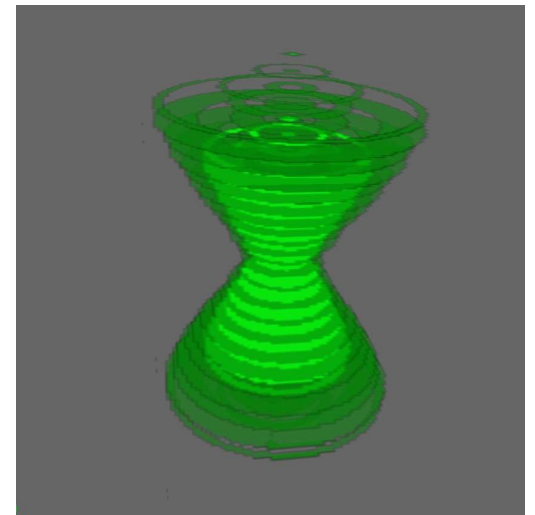
- blur
  - diffraction
  - out-of-focus light
- acquired image = object function **convolved with** psf



=

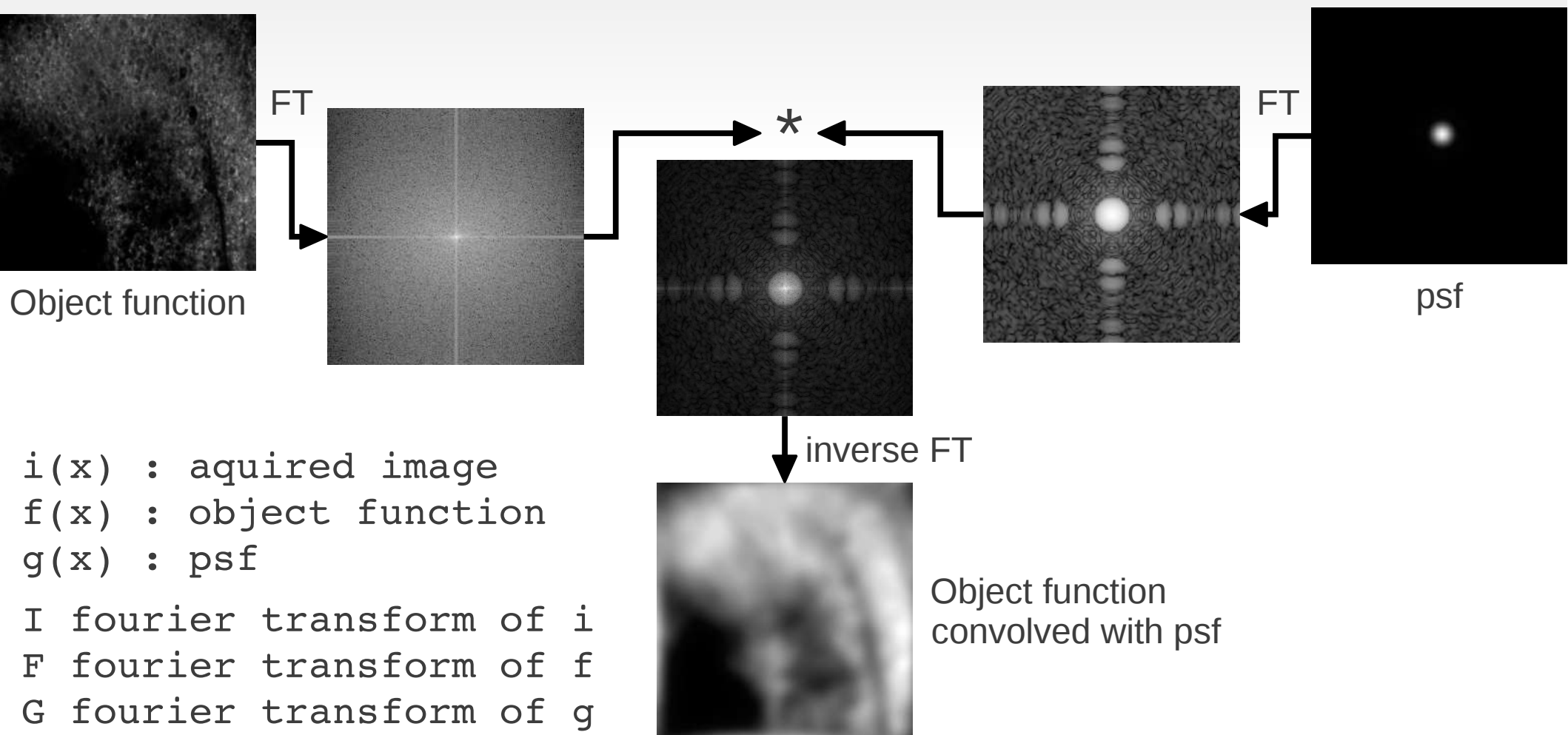


\*



# Deconvolution - Convolution theorem

$$i(x) = \int f(x') * g(x - x') dx \quad \Leftrightarrow \quad I = F * G$$



# Deconvolution

$$i(x) = \int f(x') * g(x - x') dx'$$

$\Leftrightarrow$

$$I = F * G$$

⇒ Deconvolution:

find object function  $f$  for given image  $i$  and psf  $g$

⇒ Unfortunately it is not practicable to compute

$$F = \frac{I}{G}$$

- $G$  has zeros outside certain regions
  - Setting  $F$  zero for these would create artefacts
- In practice there is noise
  - $N/G$  would amplify noise

$$I = F * G + N$$

⇒ **It's not possible to reconstruct the real object function**



# *Deconvolution algorithms*

## ⇒ Solution

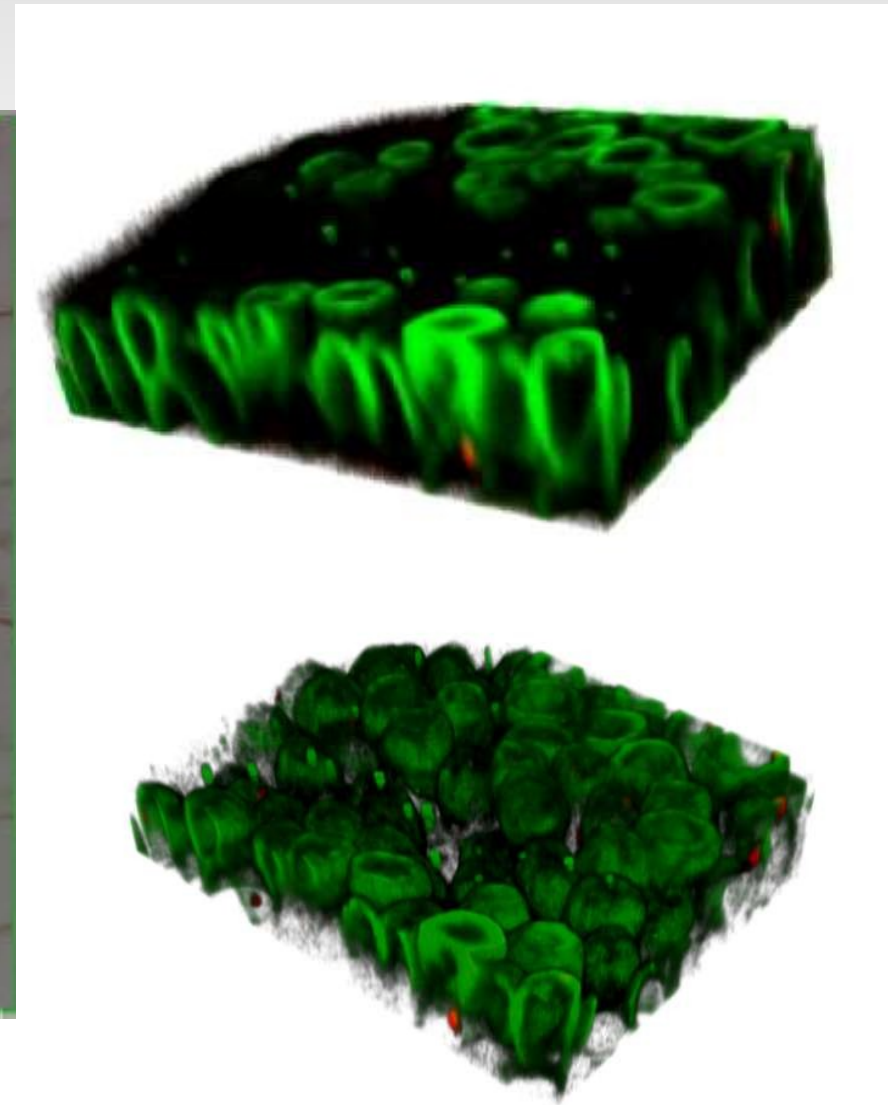
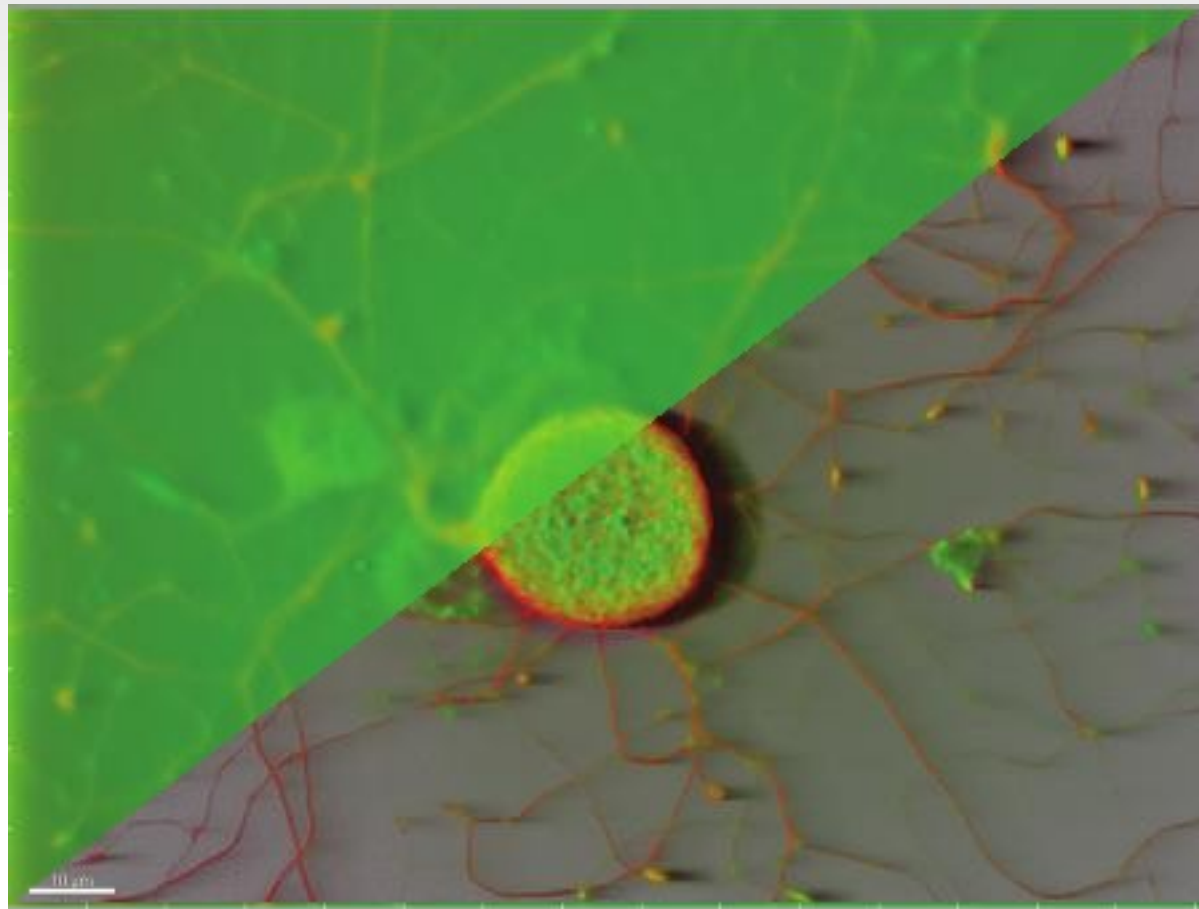
- Find an algorithm that computes a function  $f'$  so that
  - $f'$  estimates  $f$  as good as possible
  - works in the presence of noise

## ⇒ Different deconvolution algorithms exist

## ⇒ In general best for fluorescent microscopy:

- (Classical) Maximum Likelihood Estimation - MLE

# Deconvolution - examples

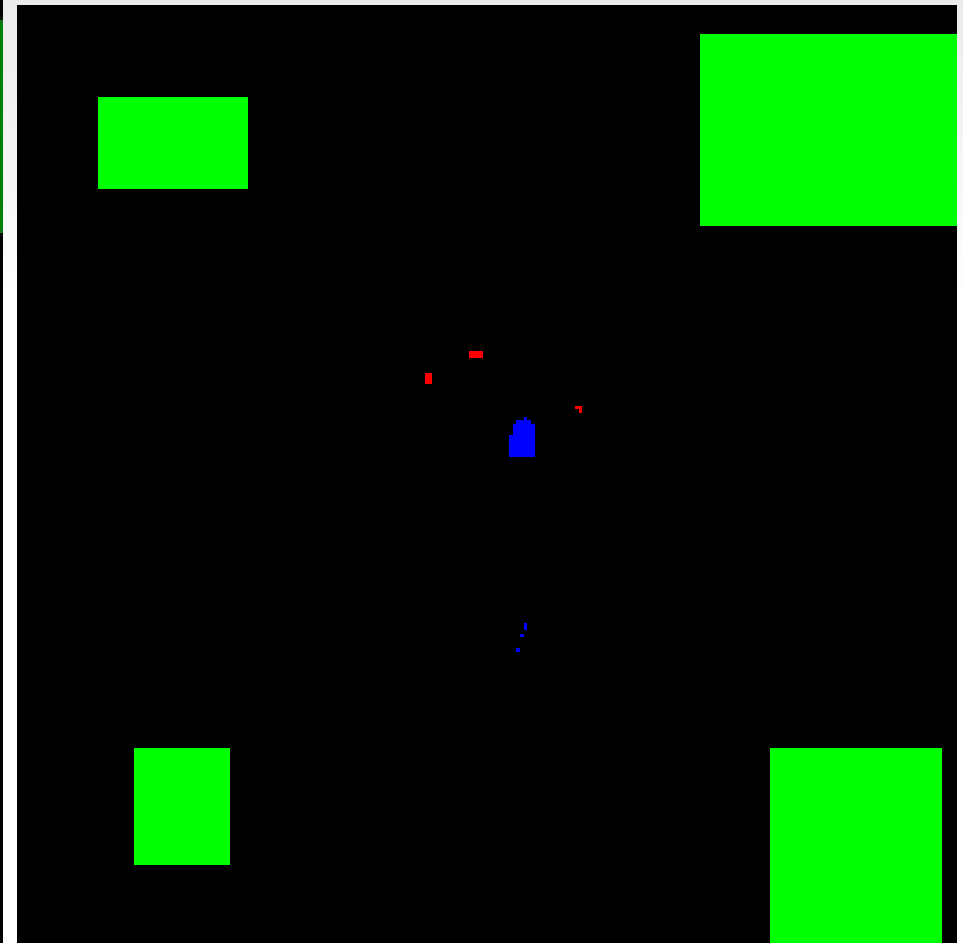
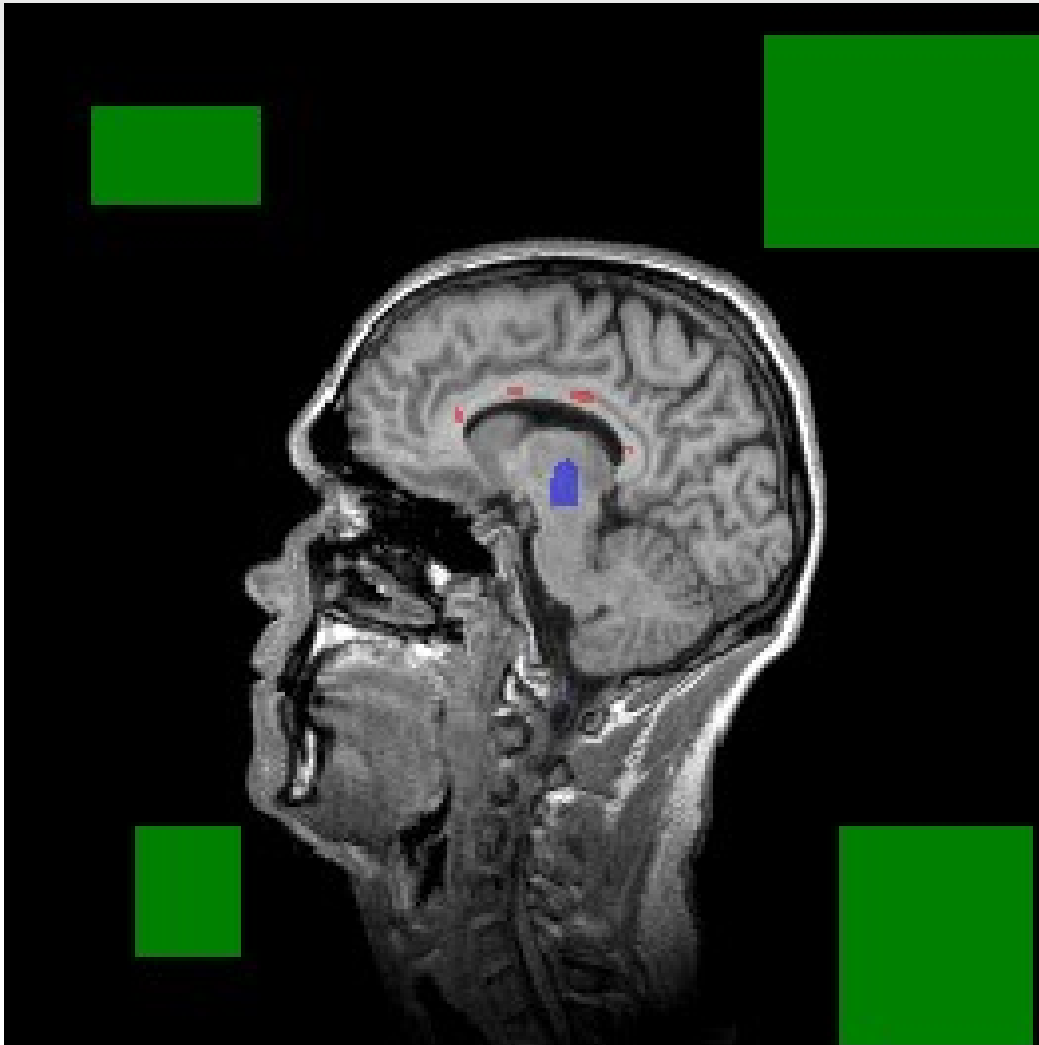


# Segmentation

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

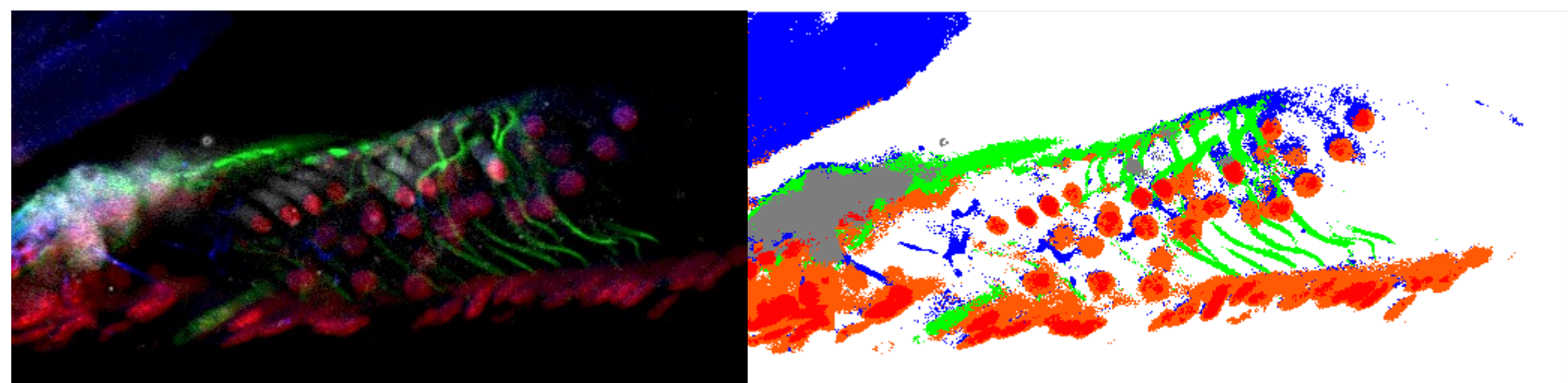
# Segmentation - region growing

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



# Segmentation - clustering

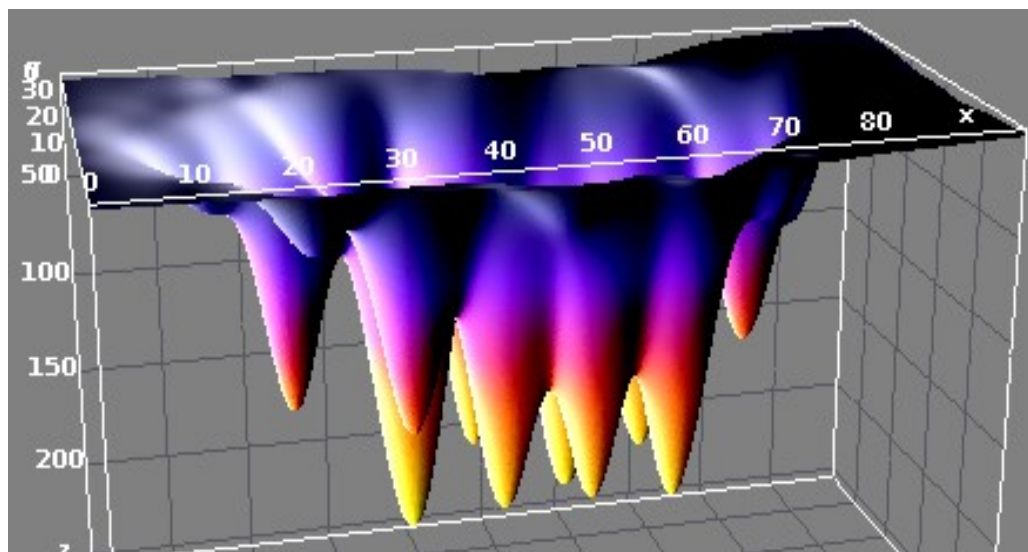
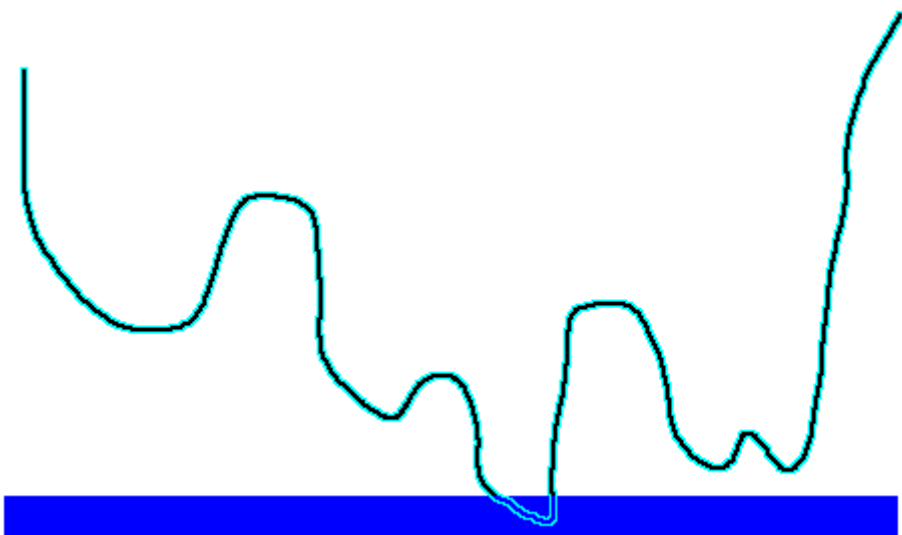
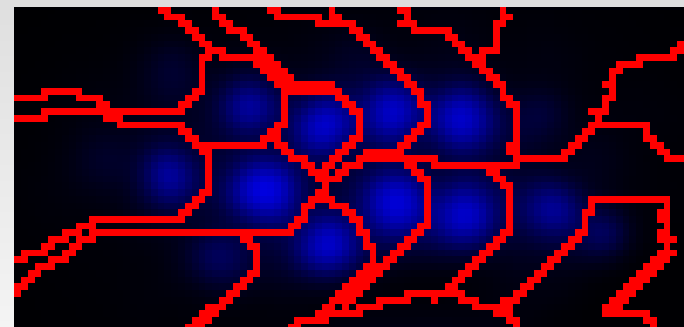
- k-means clustering
  - interpret channel values as coordinate vectors in space
  - partition space around mean values into k-clusters
  - iterative, optimization algorithm



6 clusters

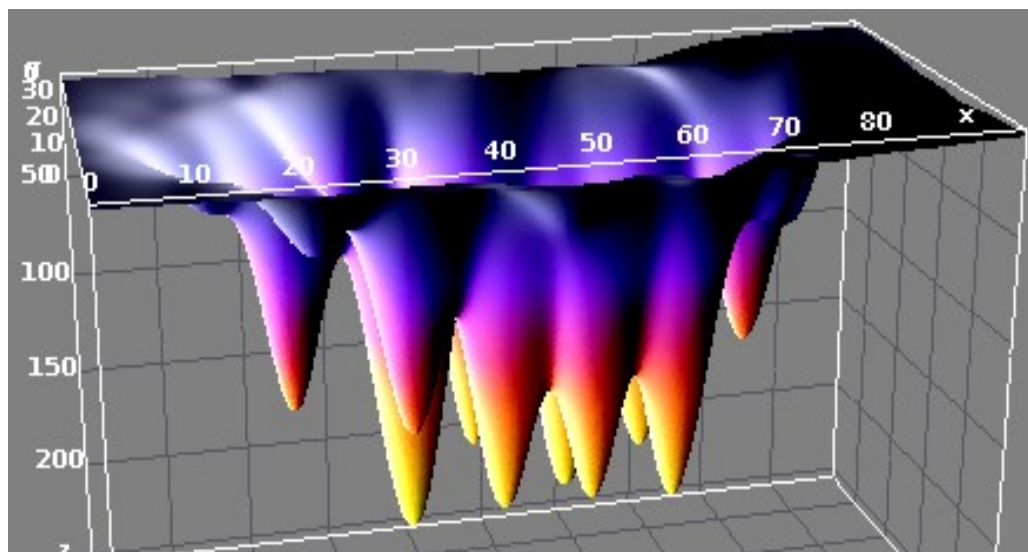
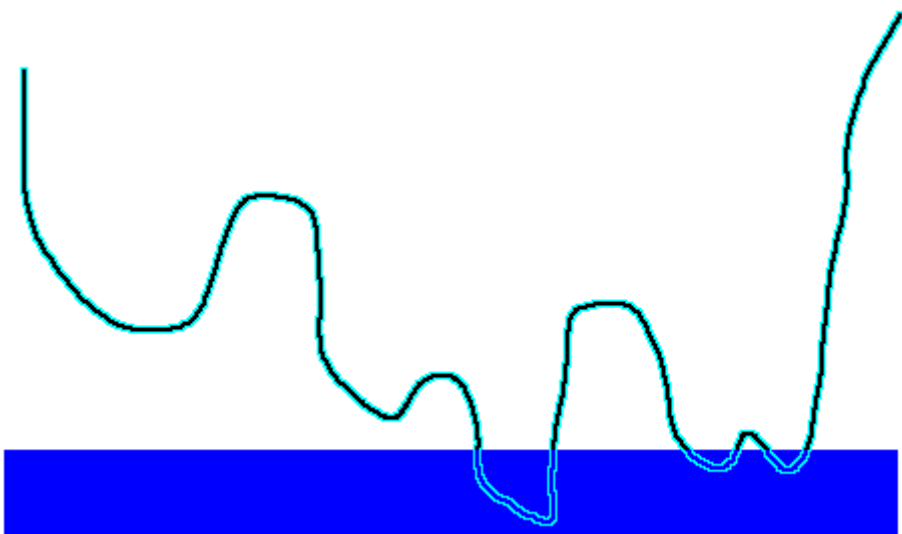
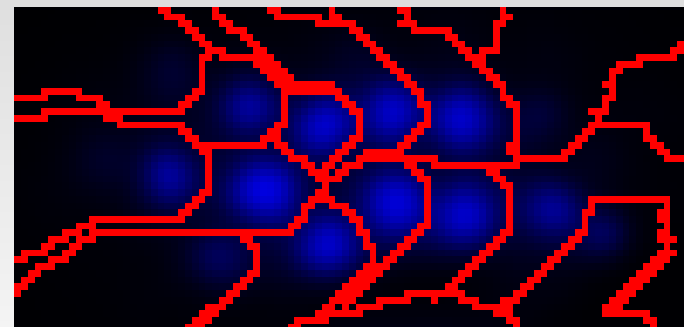
# Segmentation - Watershed

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



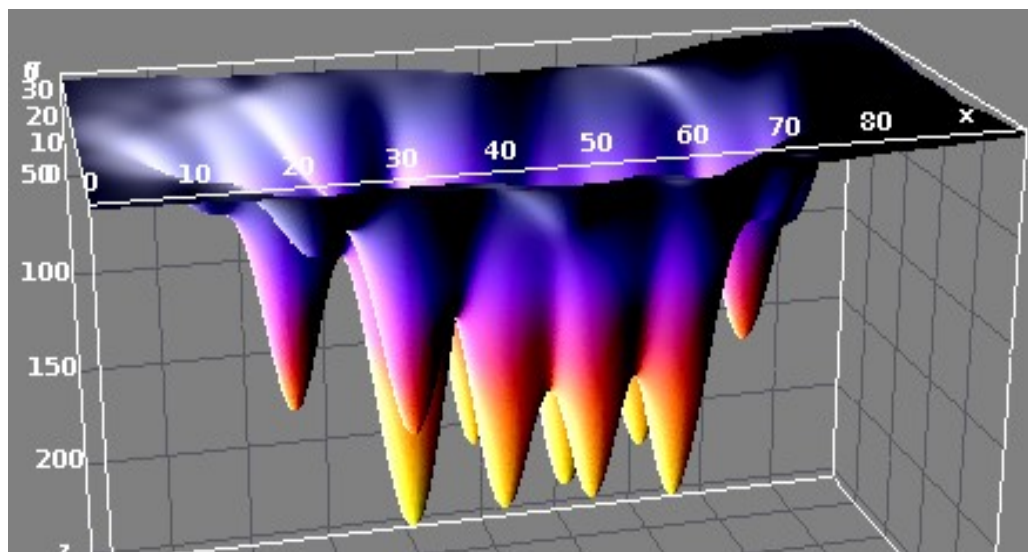
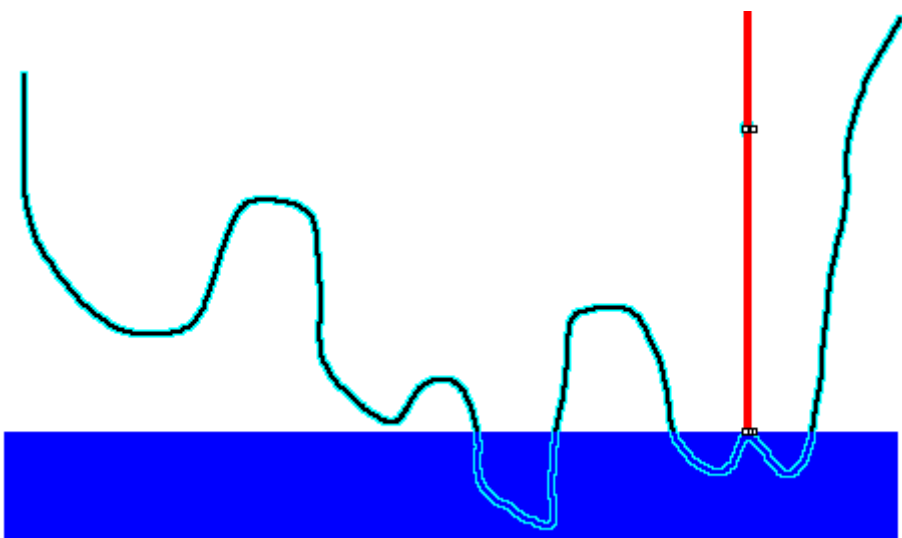
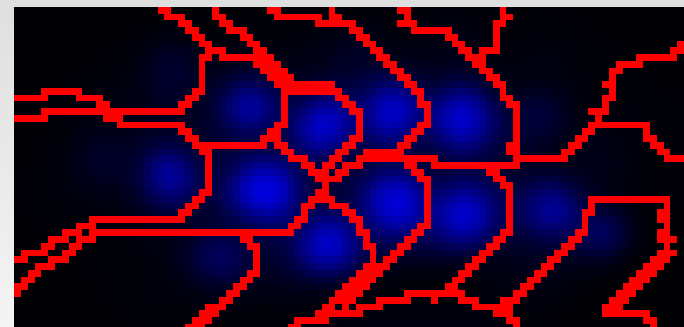
# Segmentation - Watershed

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



# Segmentation - Watershed

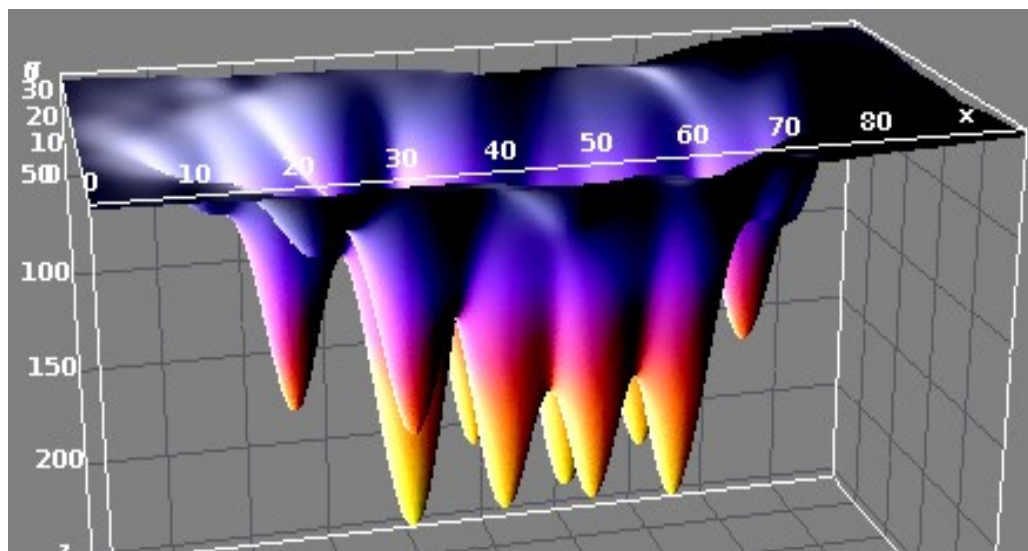
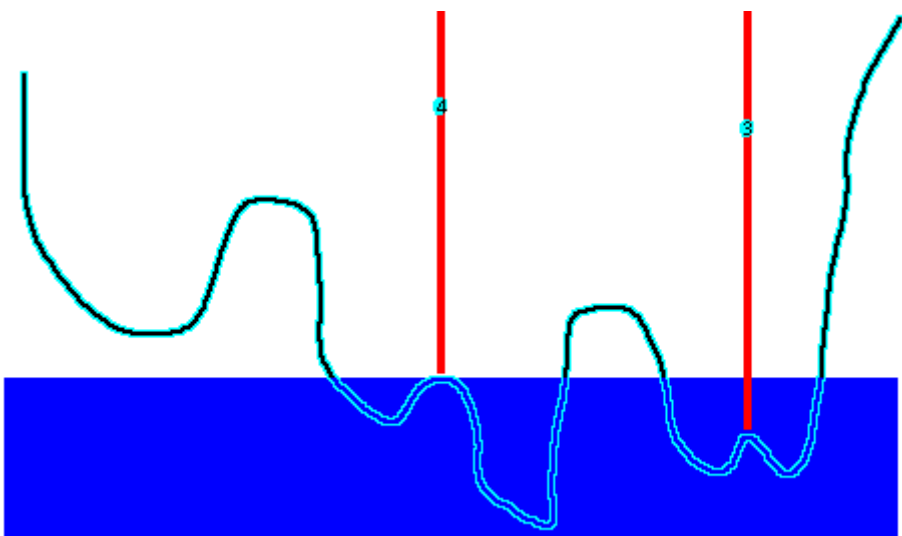
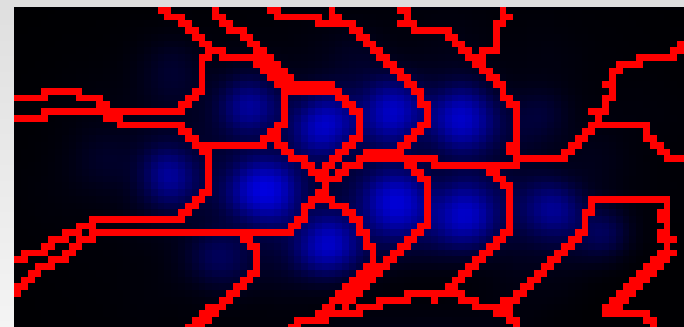
- interpret intensity as valleys
- fill slowly with rising water
- whenever two basins join create a separation





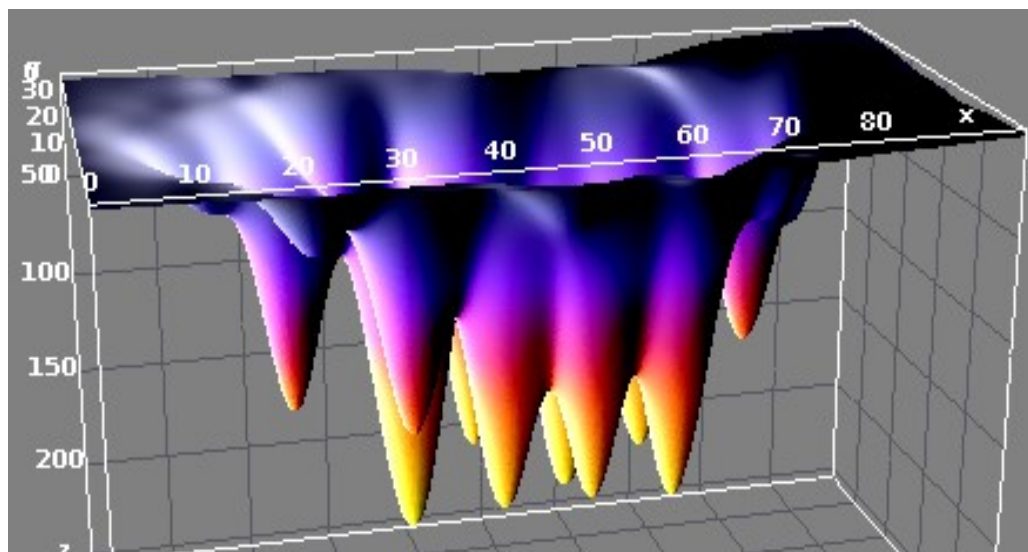
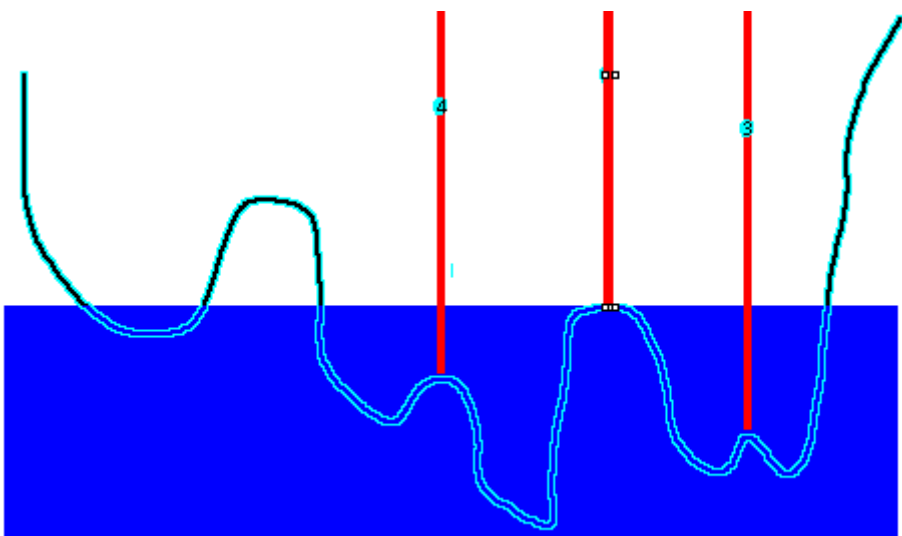
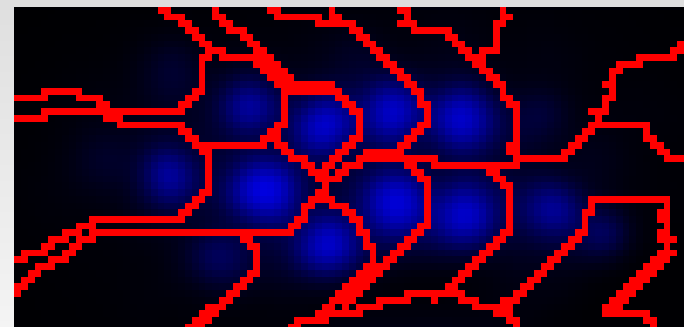
# Segmentation - Watershed

- interpret intensity as valleys
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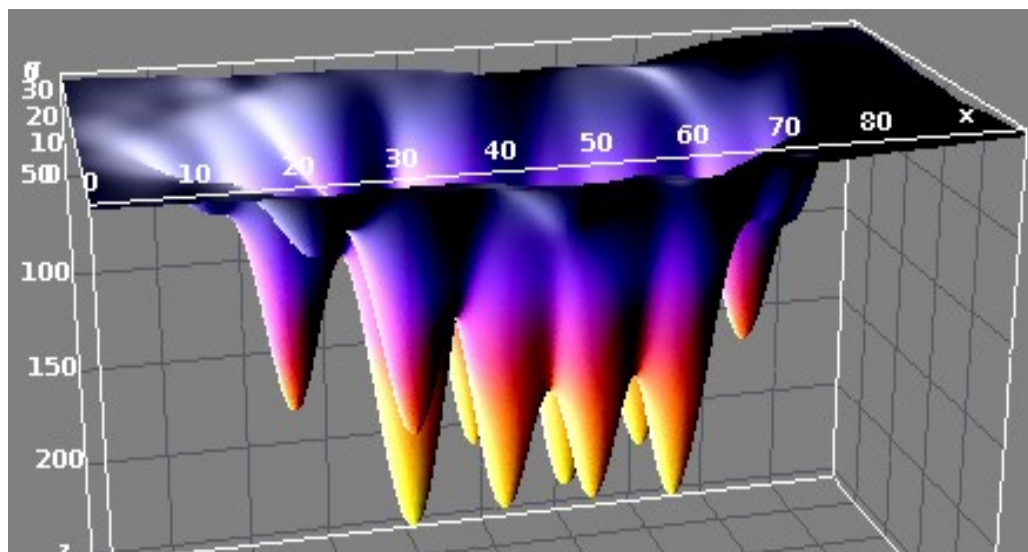
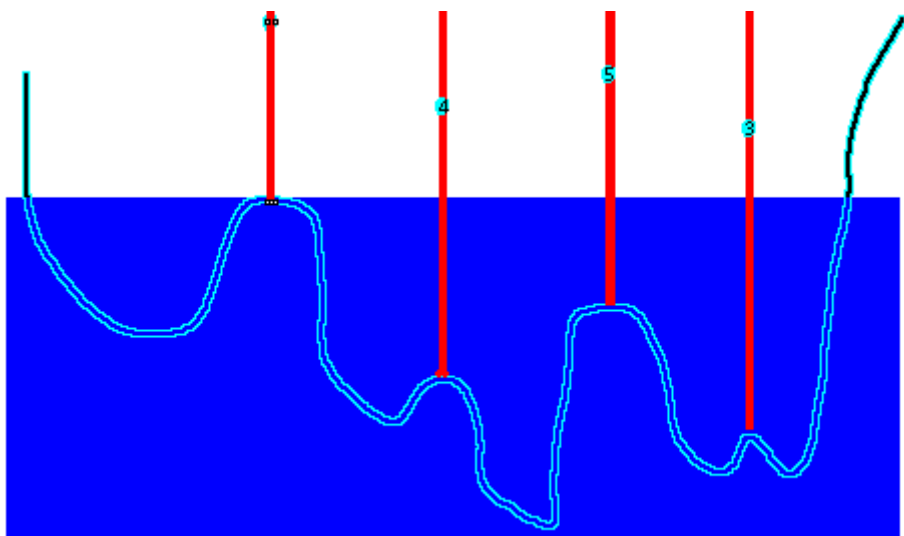
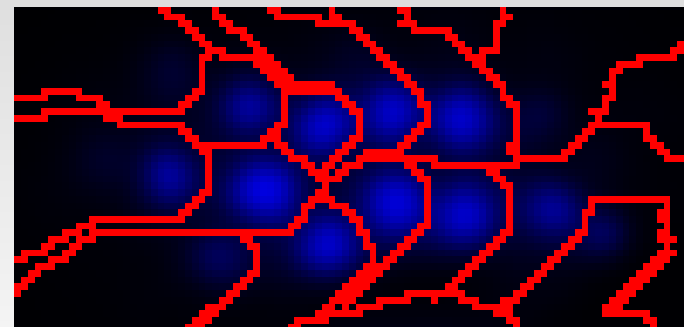
# Segmentation - Watershed

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



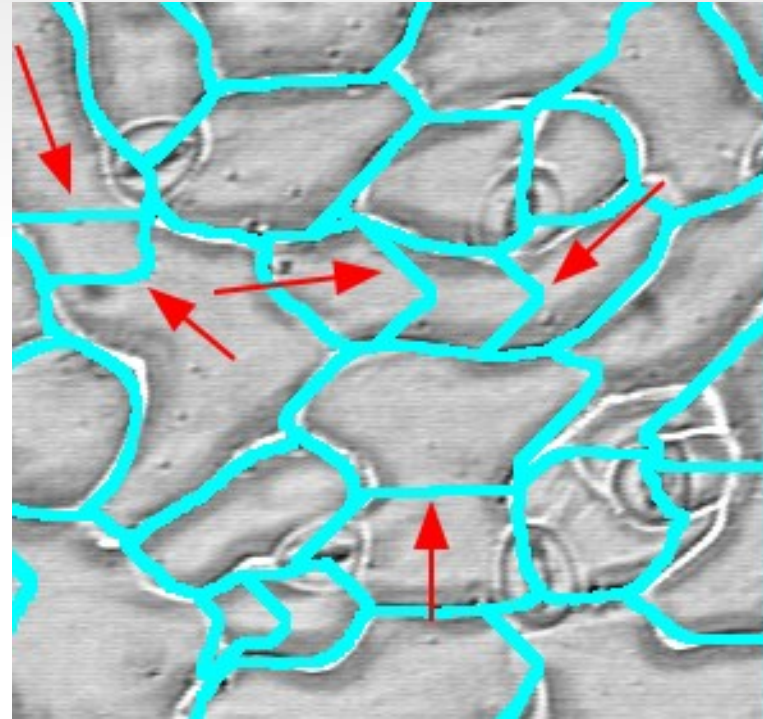
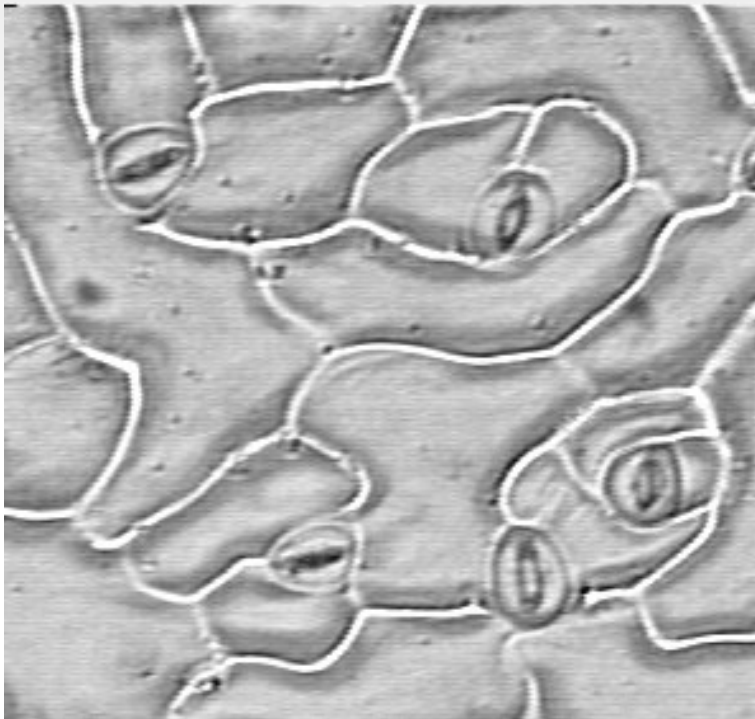
# Segmentation - Watershed

- 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

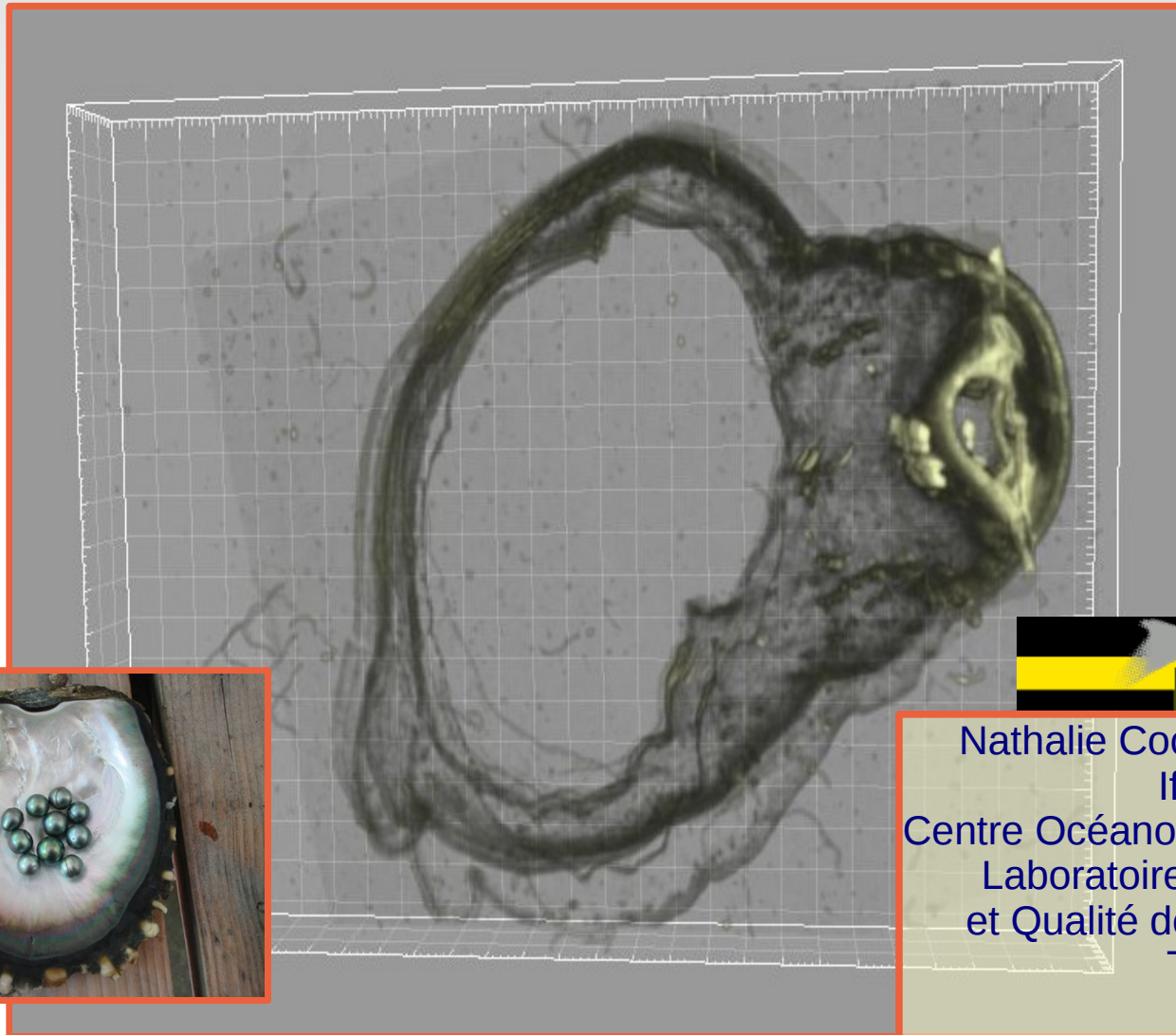
# Geometrical Transformation

- 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

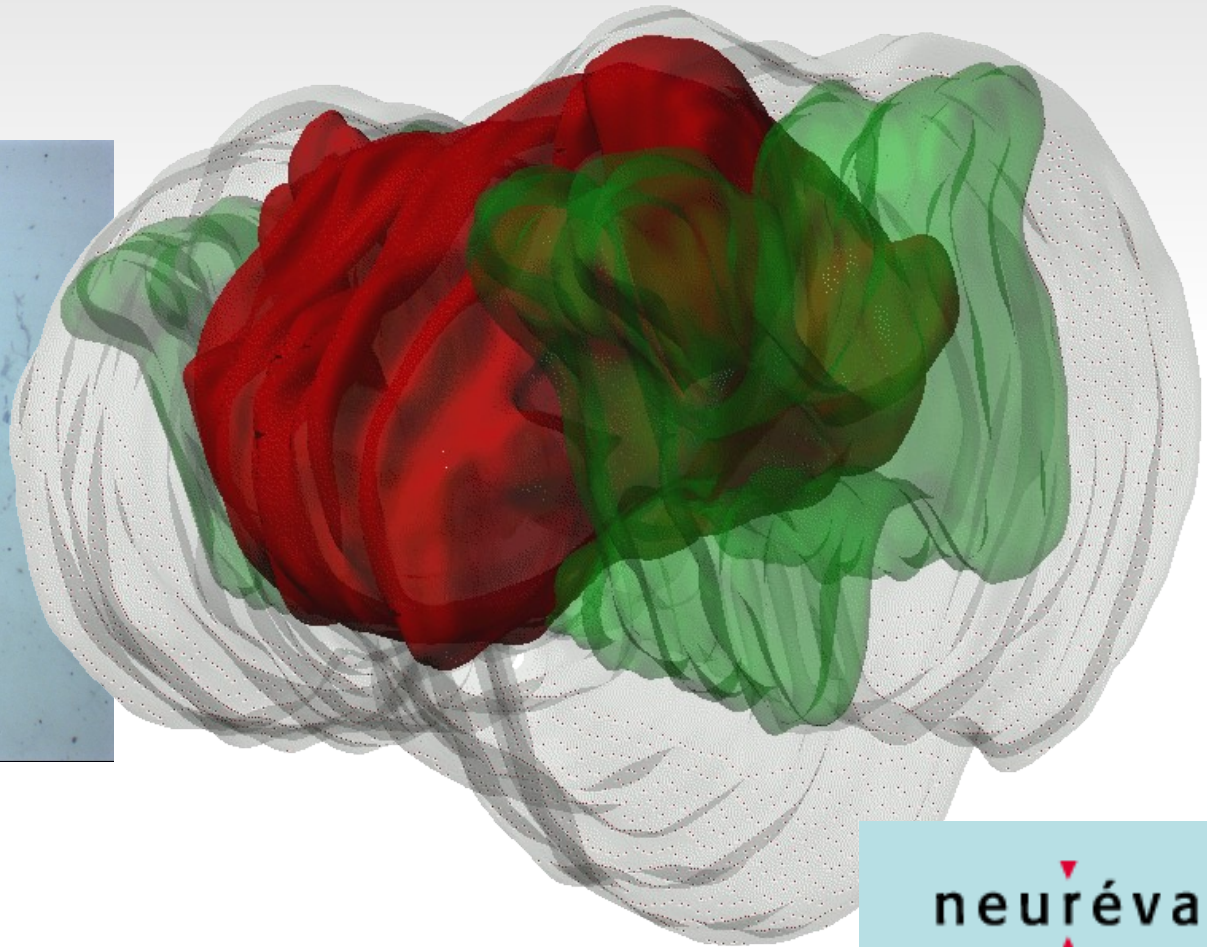
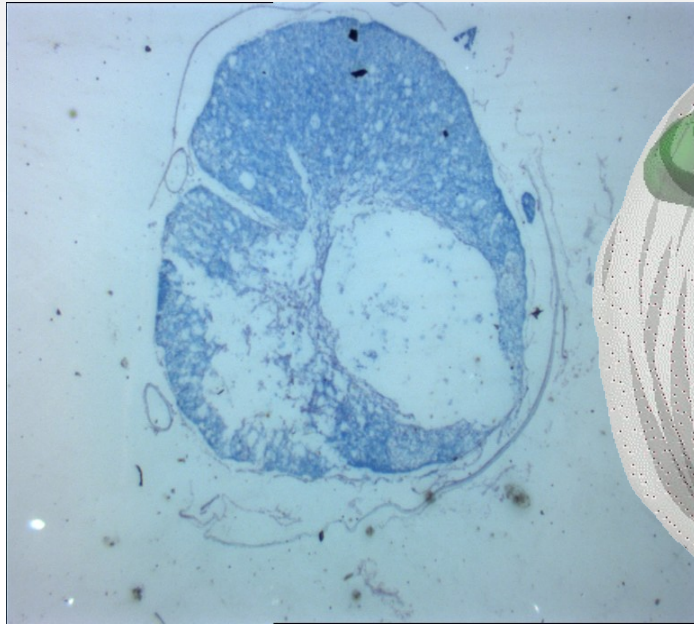
- Image registration
  - coordinate transformation
    - landmark based
      - manually selected
      - automatically extracted
    - intensity based
      - calculate match between images
    - possible transformations
      - rigid, affine, curved
  - resampling
    - interpolation
      - nearest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

# Example Registration



Nathalie Cochenec-Laureau  
Ifremer  
Centre Océanologique du Pacifique  
Laboratoire Biotechnologie  
et Qualité de la Perle (LBQP)  
Tahiti

# Example Registration

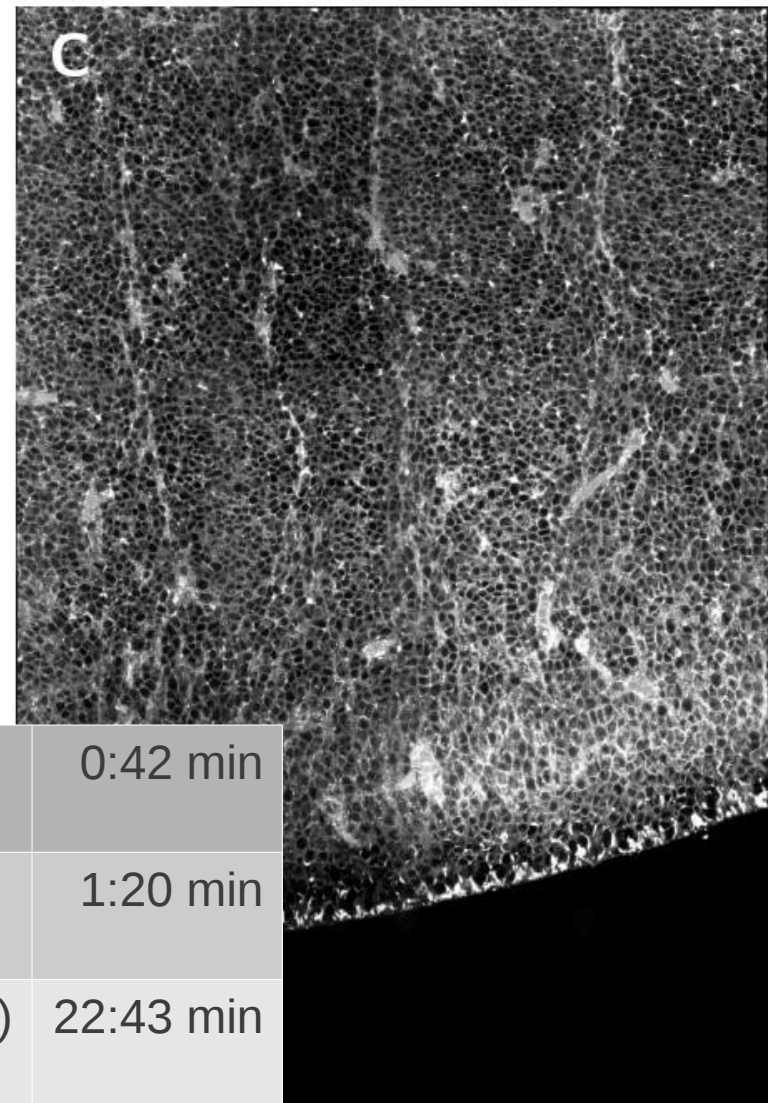
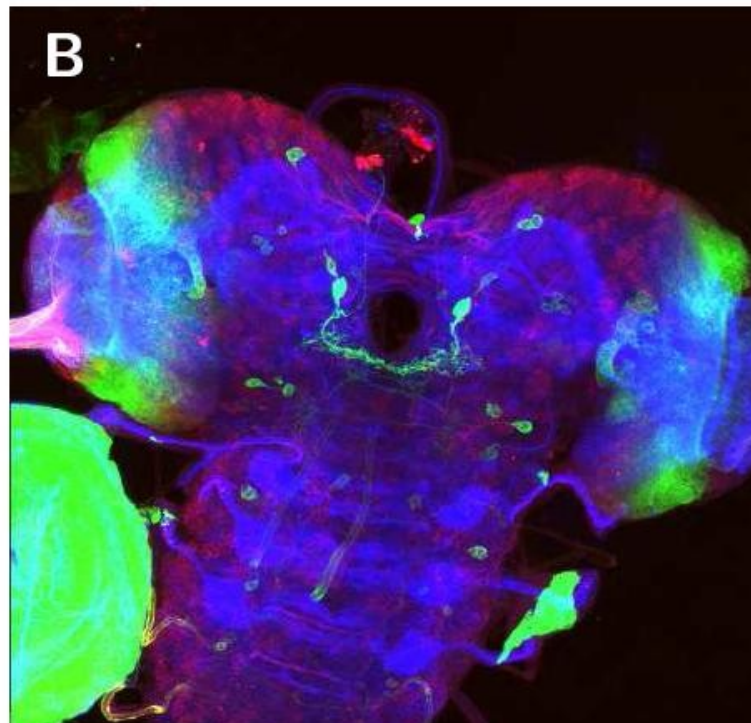
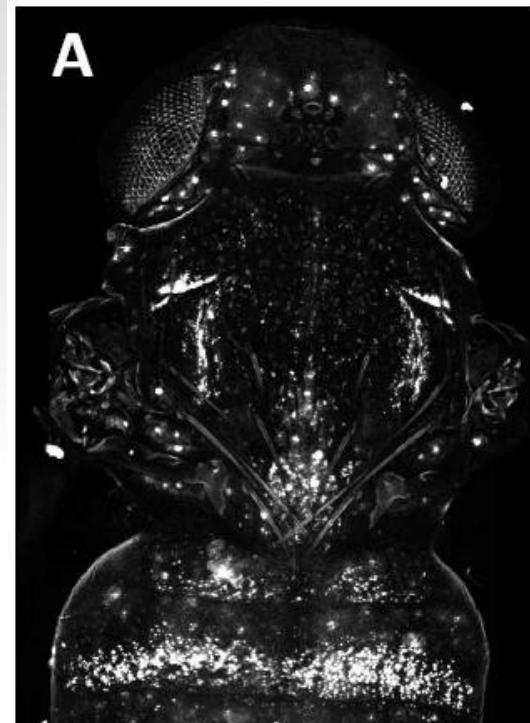


spinal cord  
grey matter  
traumatic lesion

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

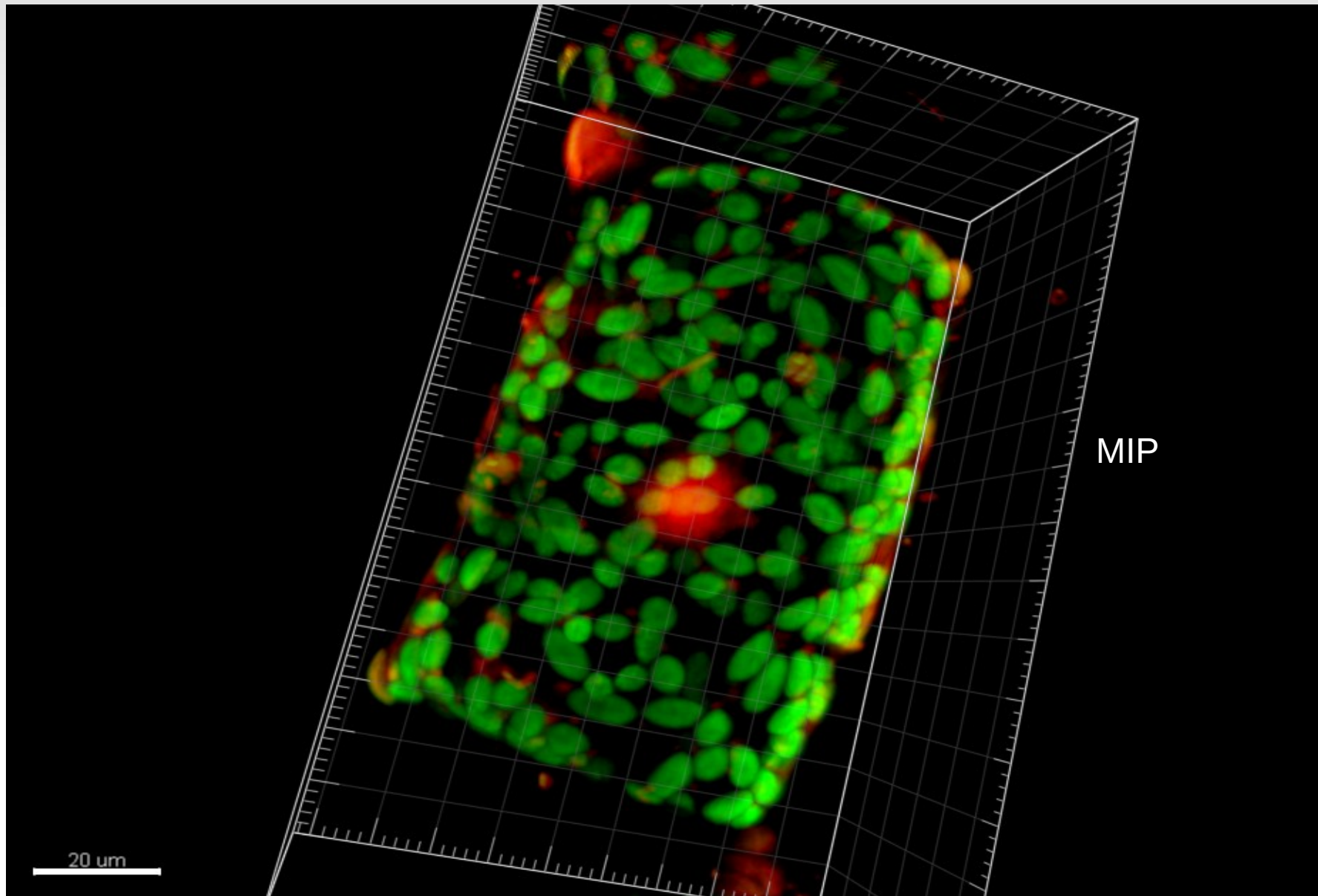
# Visualization

- 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

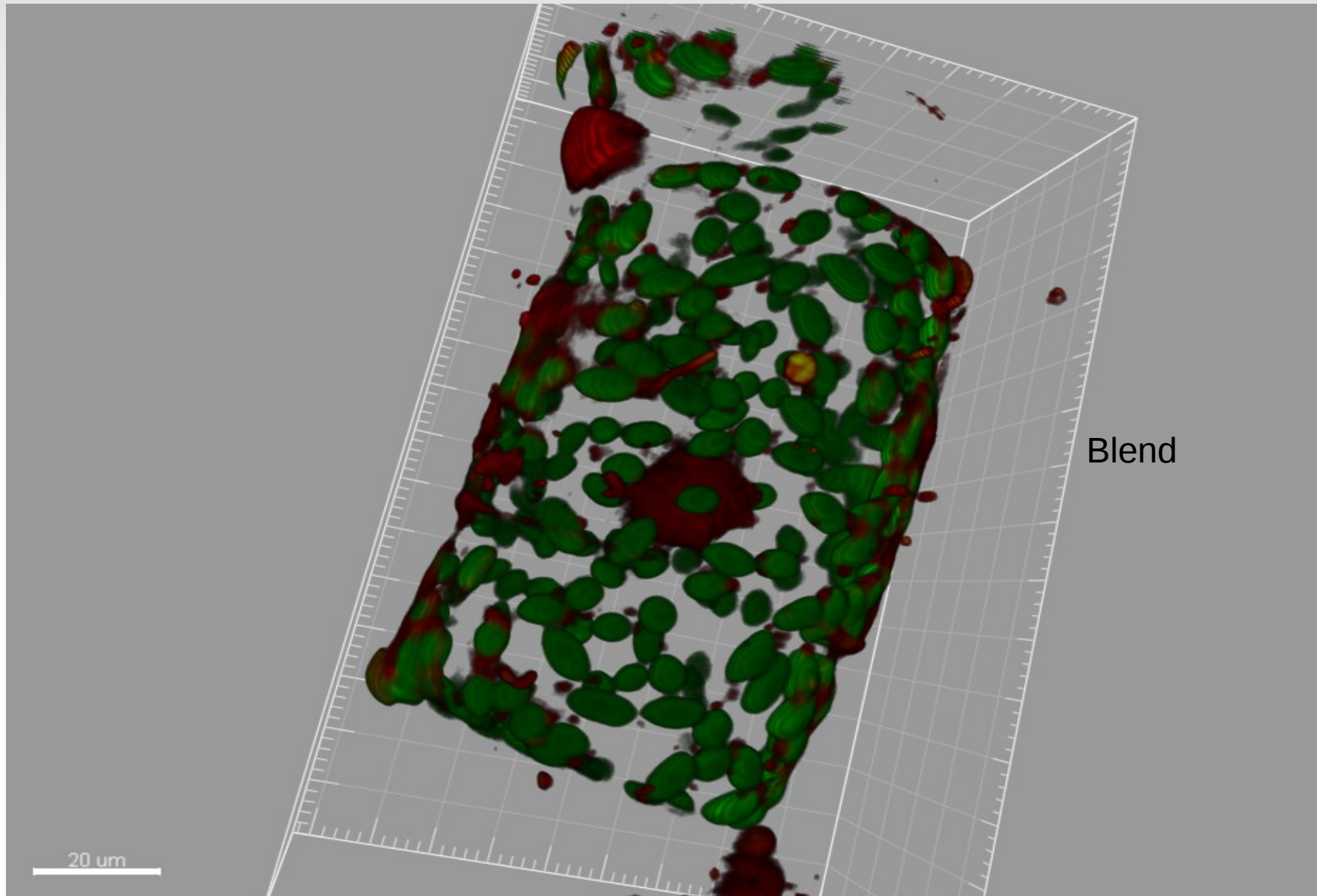
# Visualization - Volume Rendering

- how does the volume interact with a ray of light
  - given position and parameters of the light source
  - given the position of the observer

# Visualization - Volume Rendering



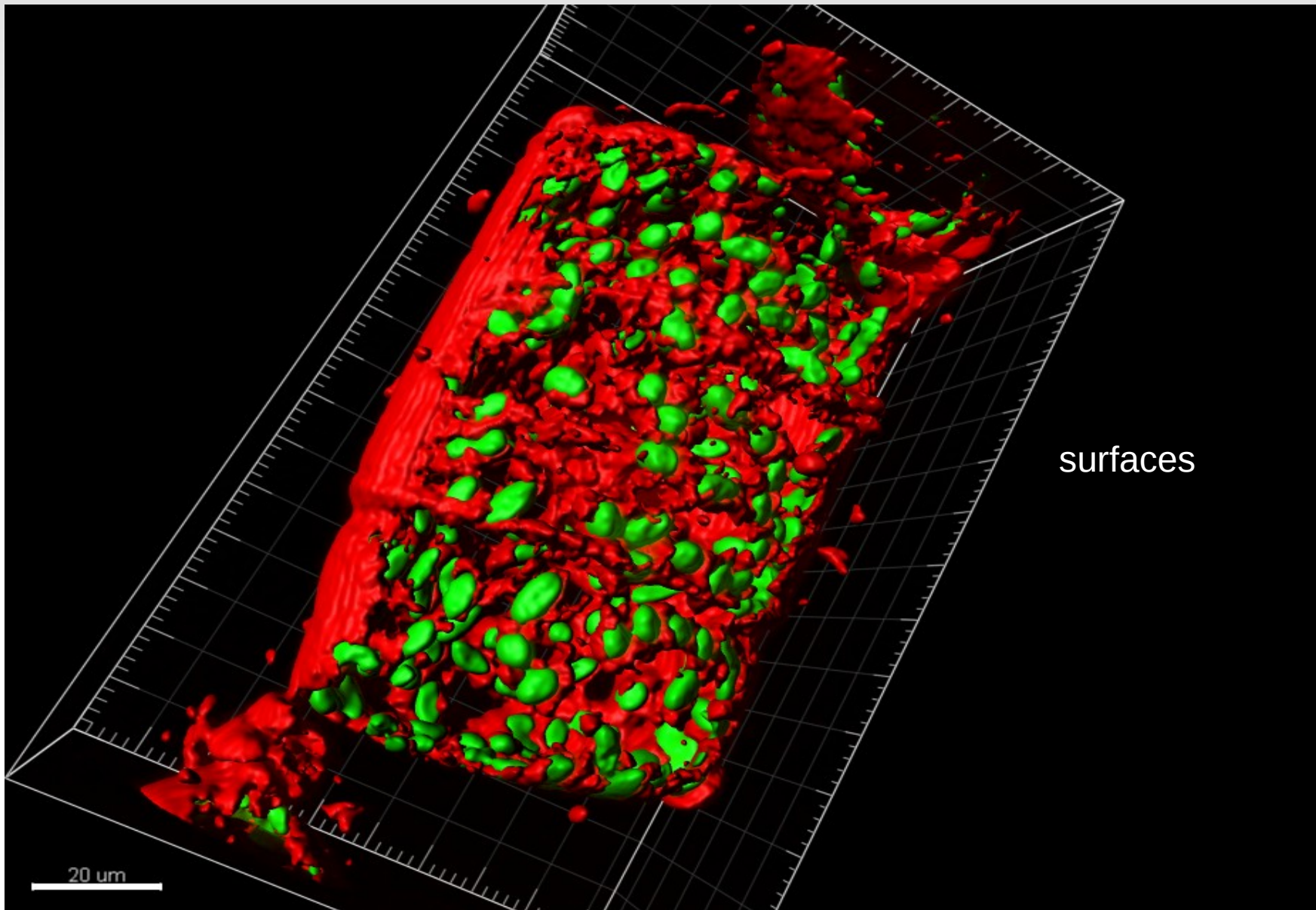
# Visualization - Volume Rendering



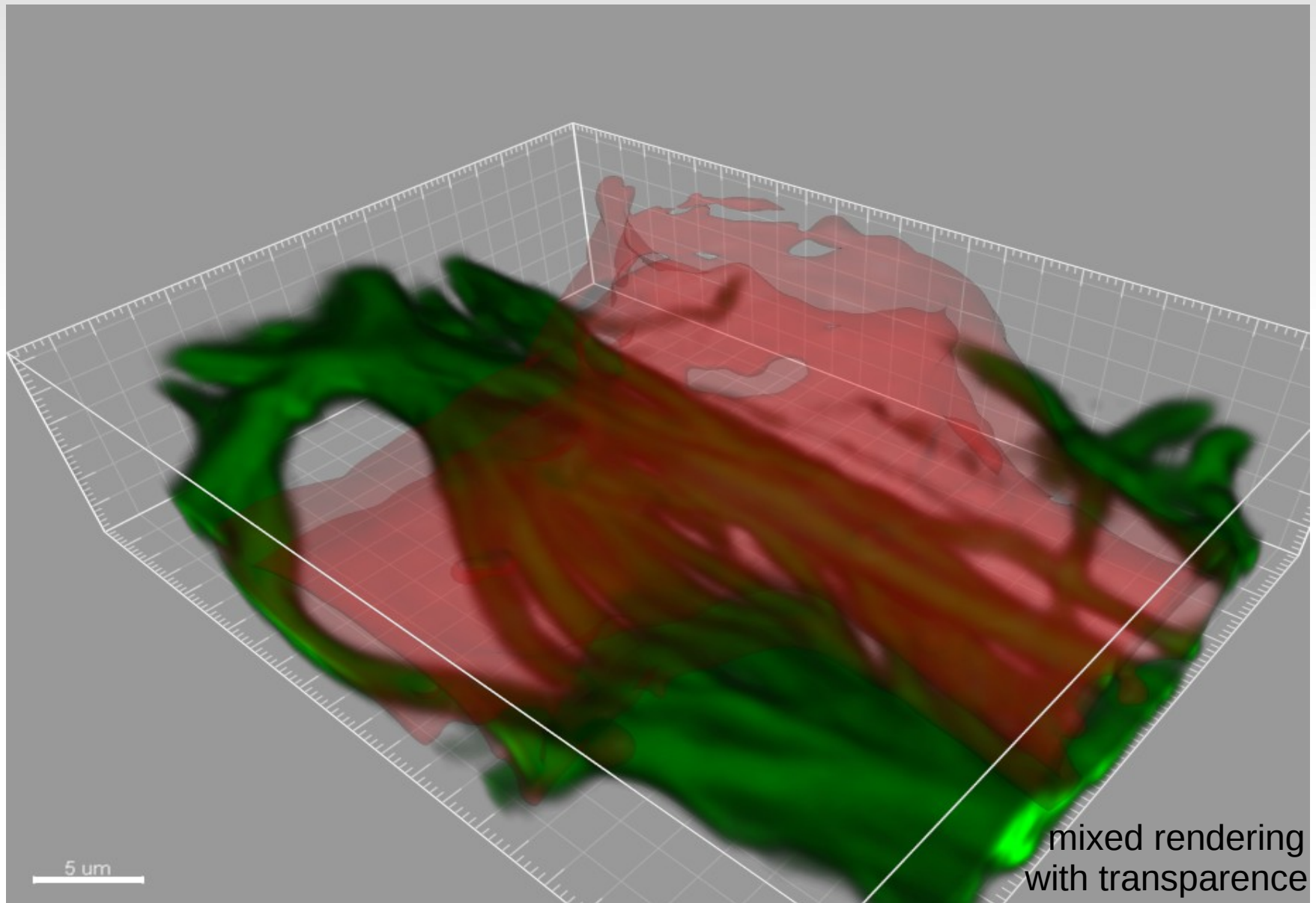
# Visualization - Surface Rendering

- segmentation of the object
- surface triangulation
  - marching cubes algorithm

# Visualization - Surface Rendering



# Visualization - Mixed Rendering





# Visualization - multi-scale data

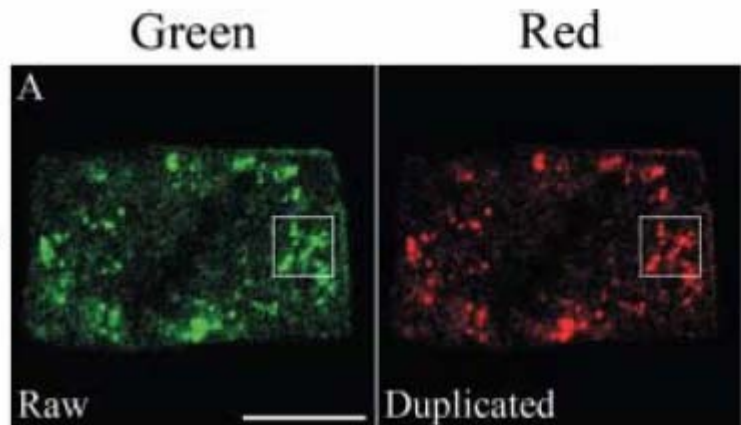
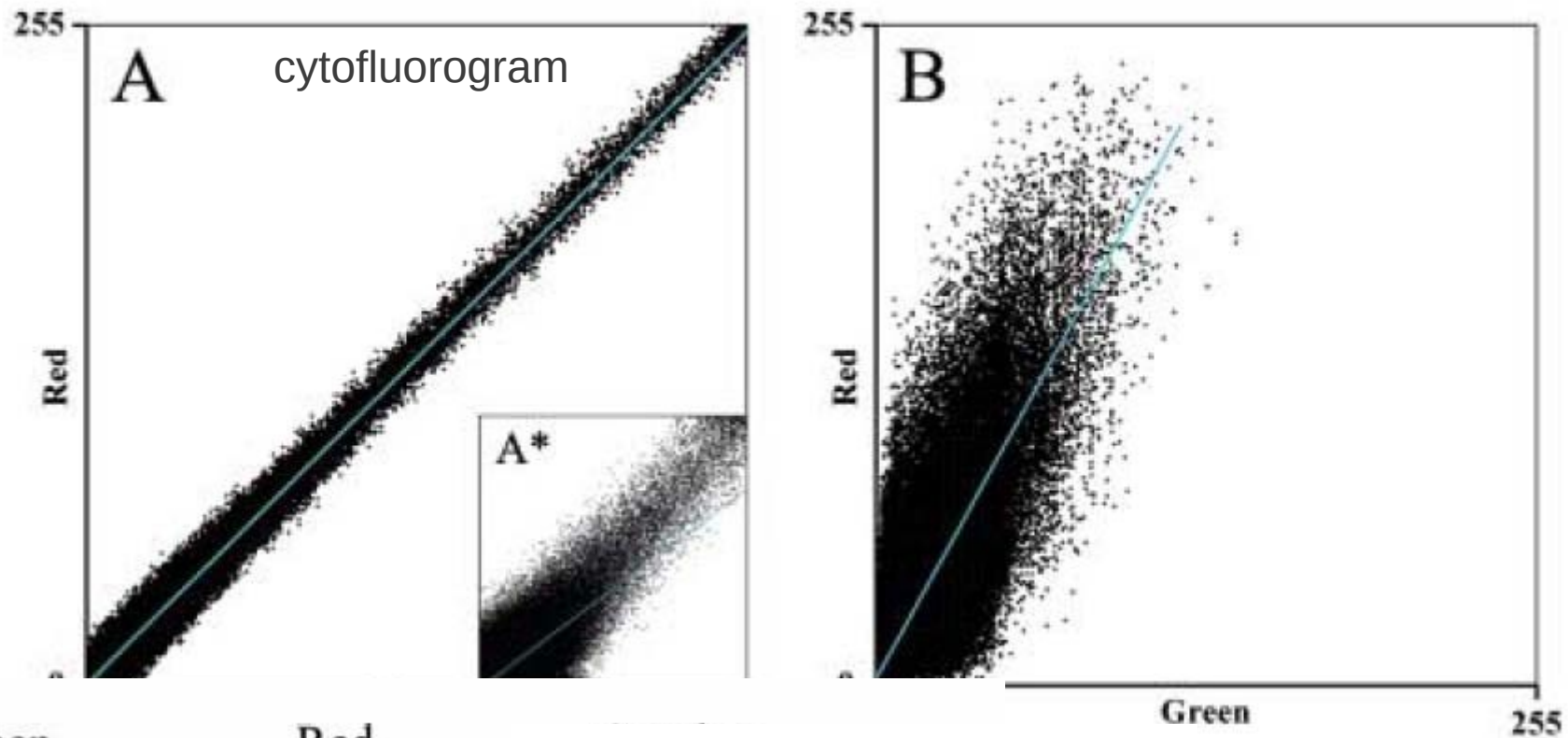
- imagine you could in one image
  - zoom smoothly from
    - organism to
    - organ to
    - tissue to
    - cells to
    - molecules
- see for example msv-project  
Multiscale Spatiotemporal Visualisation  
(<http://www.msv-project.eu>)



# 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

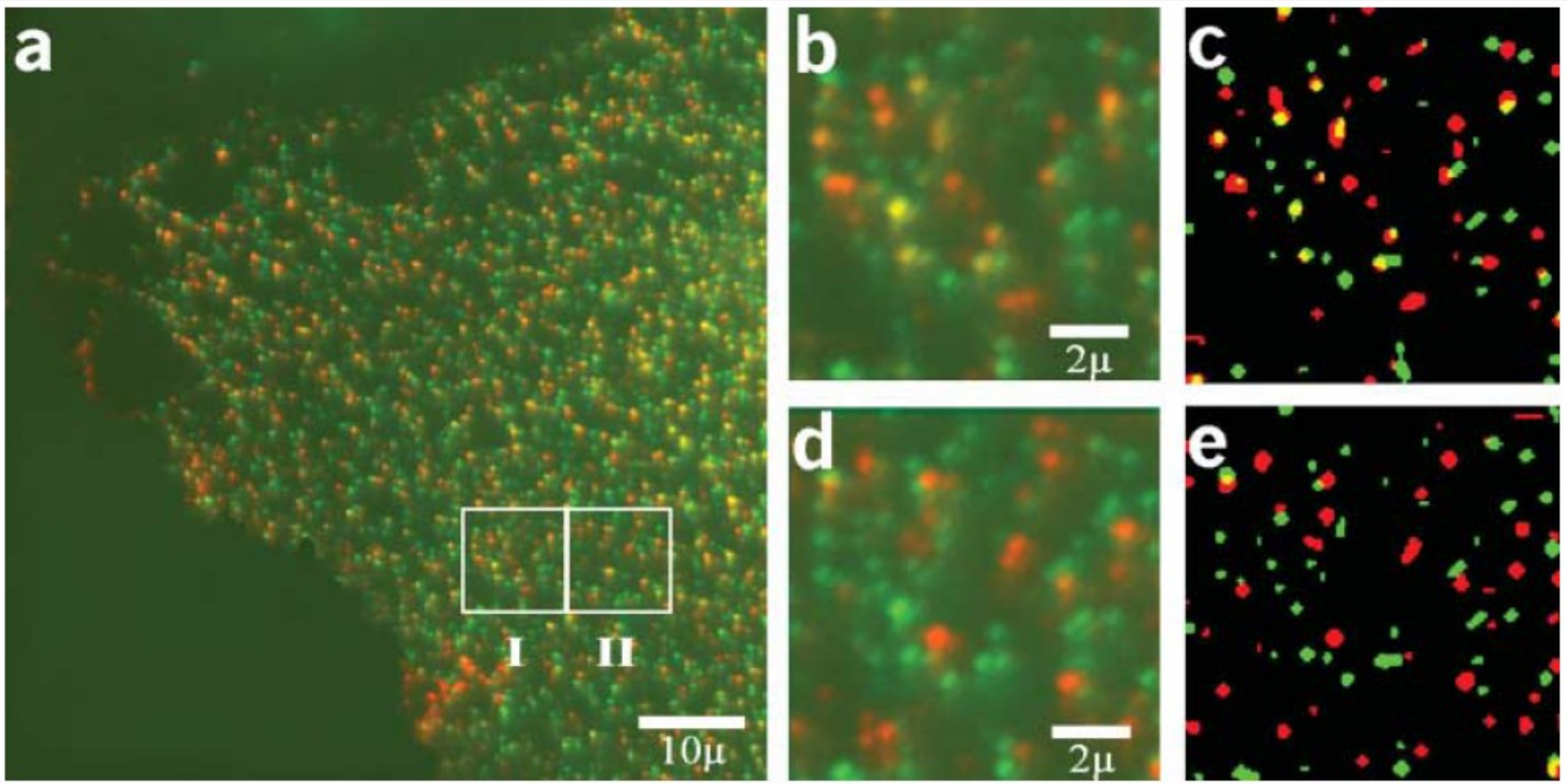


Pearson's Correlation Coefficient

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

# Colocalization Analysis

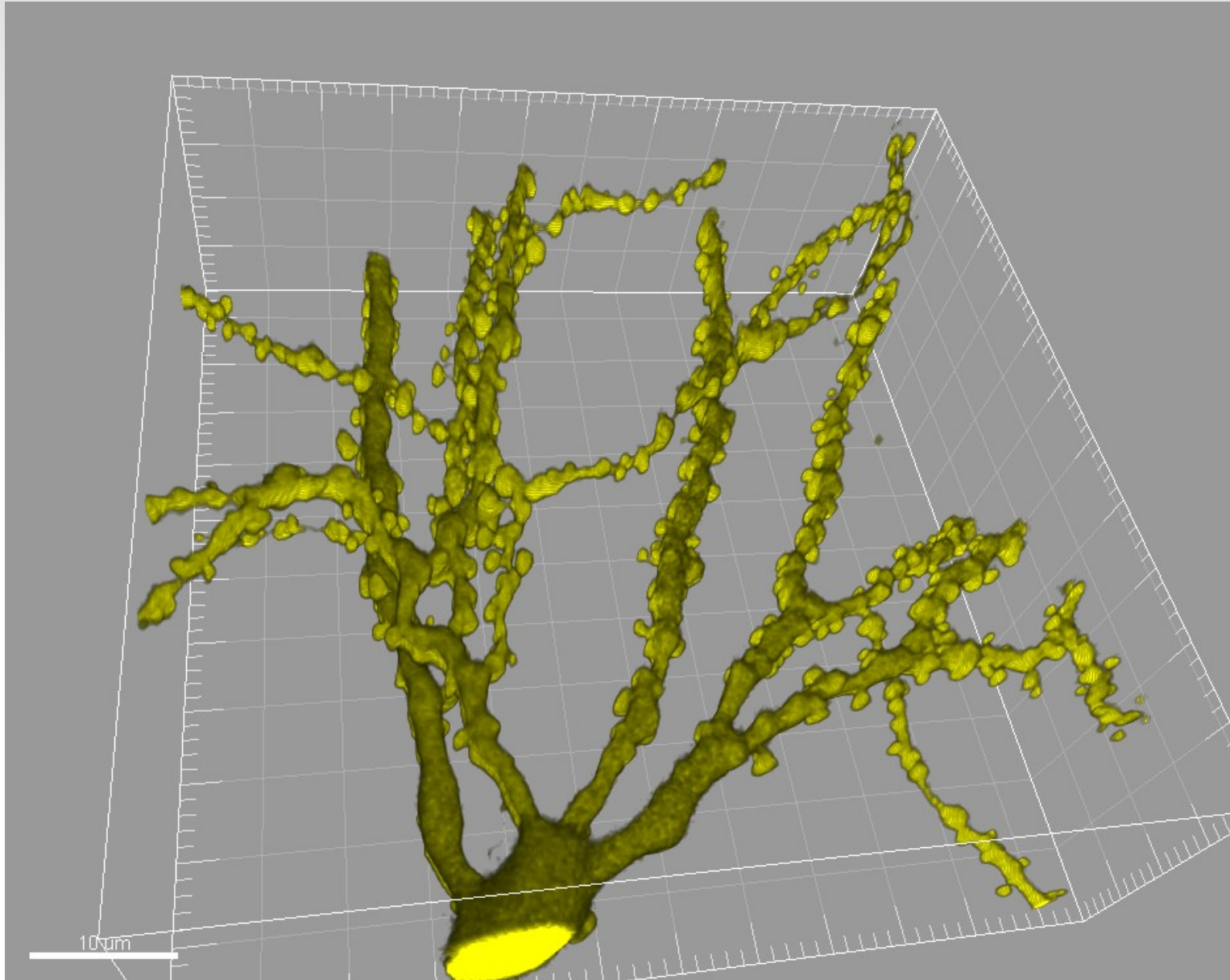
- object based colocalization
- test if distance between centroids is at resolution limit



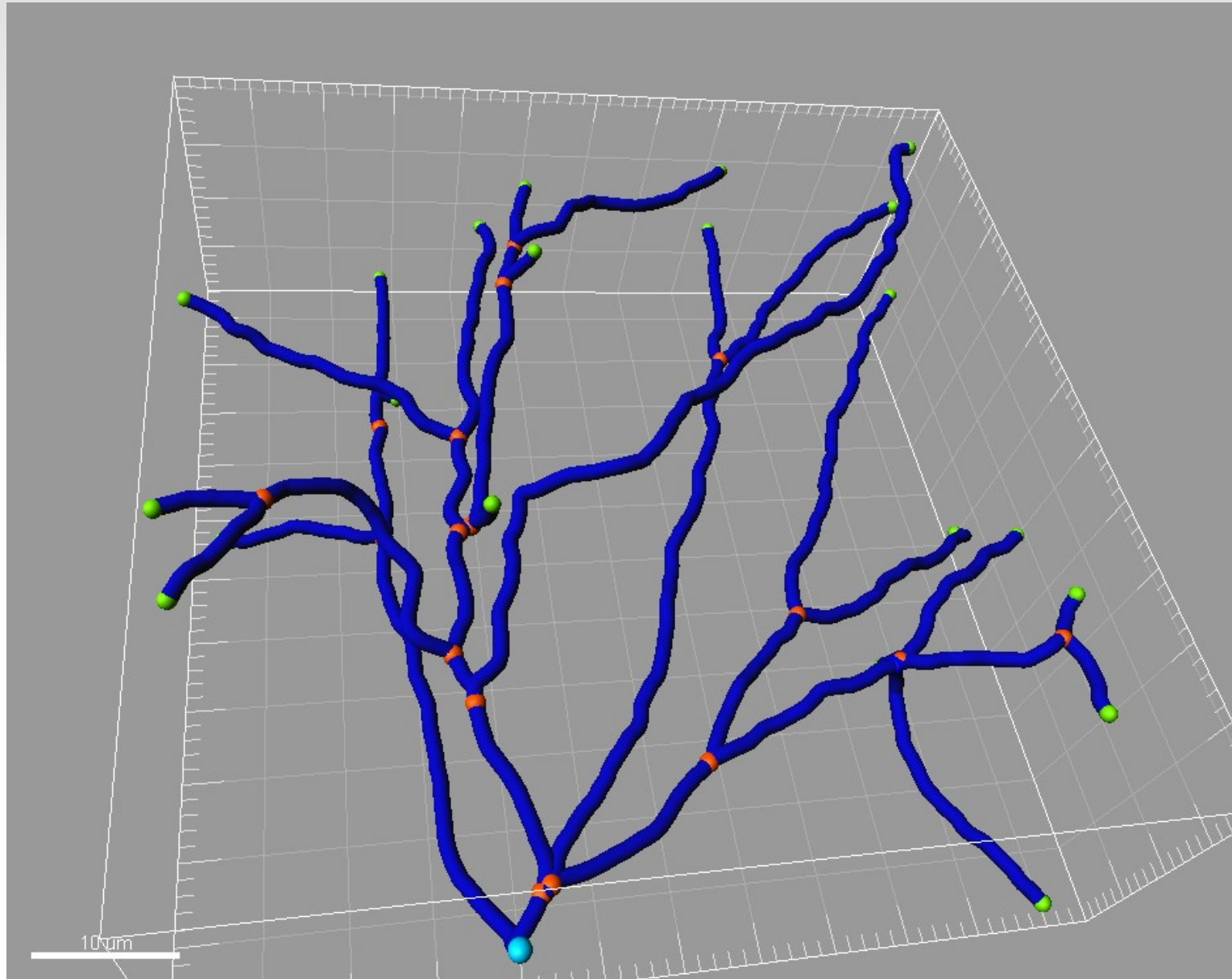
# Filament tracing and analysis

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

# Filament tracing and analysis



# Filament tracing and analysis

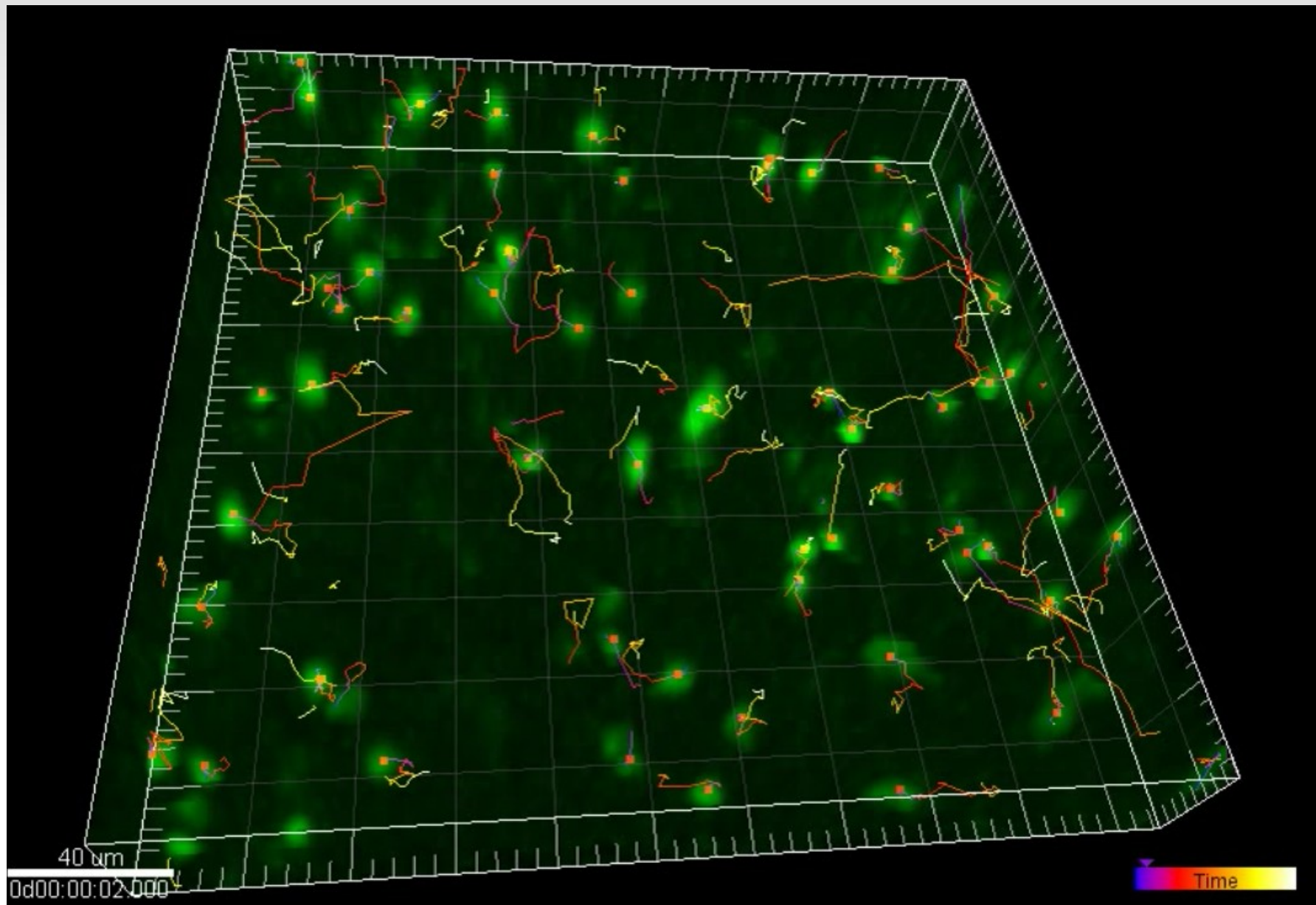


# Particle detection and tracking

- 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



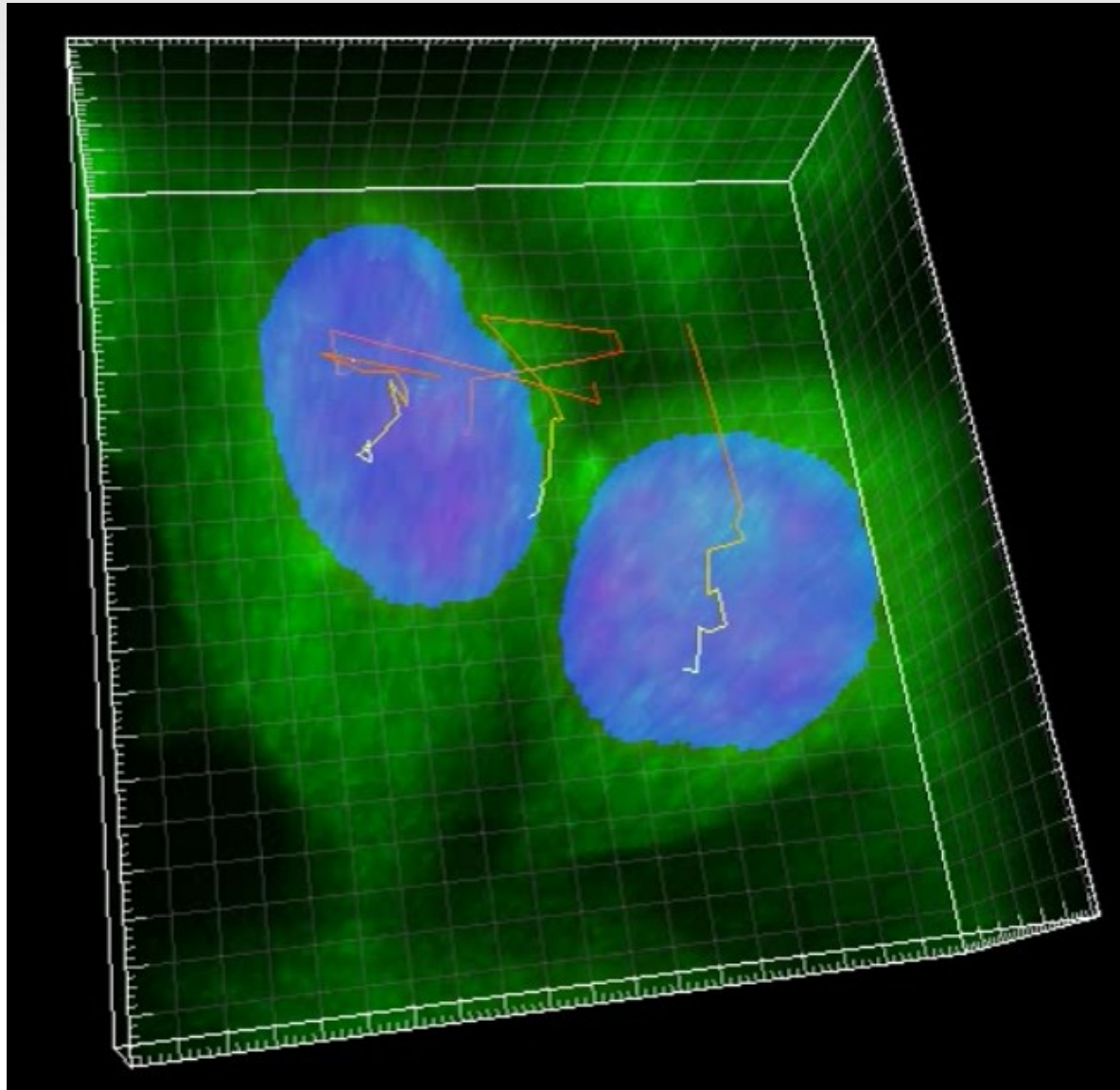
# Particle detection and tracking



# Cell segmentation and tracking

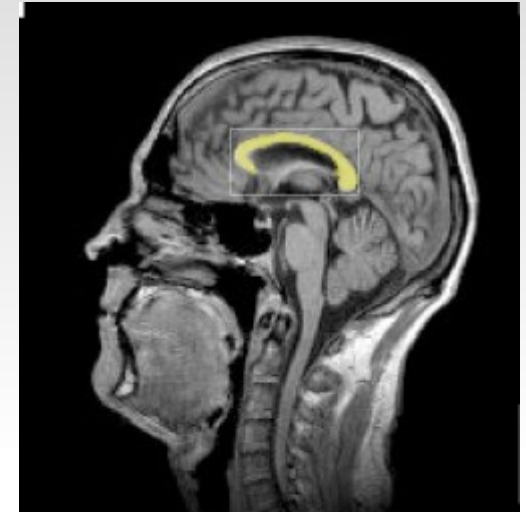
- cells have a distinct shape
- shape may change over time
- use active contours (snakes) to detect cells
  - active surfaces in 3D
  - shape constraint fitting to image data
- tracking
  - use contour of cell at  $t=n$   
as initial contour for cell at  $t=n+1$

# Cell segmentation and tracking

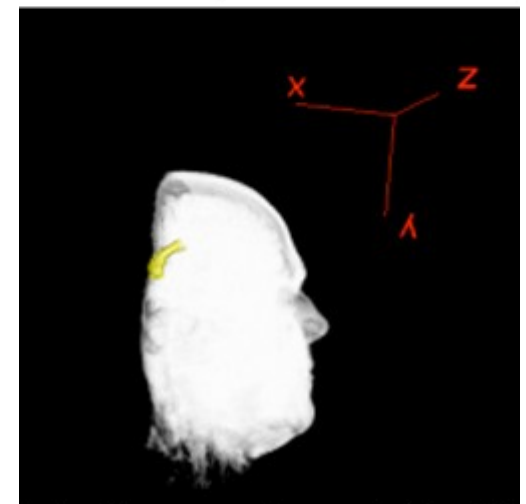


# Software Tools

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



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



*The Corpus callosum in the 3D Viewer*

# Thank you

- Questions?



# Literature

1. Meijering, E. & van Cappellen, G. **Biological Image Analysis Primer**. (Erasmus MC: 2006).  
at <<http://www.imagescience.org/meijering/publications/download/biap2006.pdf>>
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- 3 R. Fisher, S. Perkins, A. Walker and E. Wolfart. **The Hypermedia Image Processing Reference**  
at <<http://homepages.inf.ed.ac.uk/rbf/HIPR2/index.htm>>
4. Baecker, V. **Workshop: Image processing and analysis with ImageJ and MRI Cell Image Analyzer**  
at <<http://dev.mri.cnrs.fr/wiki/imagej-workshop>>