Training School # 14

BioImage Analysis for Early Career Investigators
"Image processing and analysis using Fiji and ilastik"

Image filters and morphometric operators

Elnaz Fazeli

29.2-3.3, 2020
Centre Broca Nouvelle-Aquitaine, Bordeaux, France

Adopted from
Fabrice P. Cordelières slides
Christian Tischer
Paulo de Castro Aguiar
Olivier Burri, Romain Guiet & Arne Seitz

neubias
network of european bioimage analysts

During the lecture

1	How to use linear filtering	***Practice with ImageJ Fiji convolve tool*** ***Practice with filters***
2	How to use RANK filtering	***Practice with ImageJ Fiji RANK filters***
3	Practical exercises	***Trying uneven background subtraction*** ***Trying noise removal using filters***
4	Morphological operators	***Trying different morphological operators***

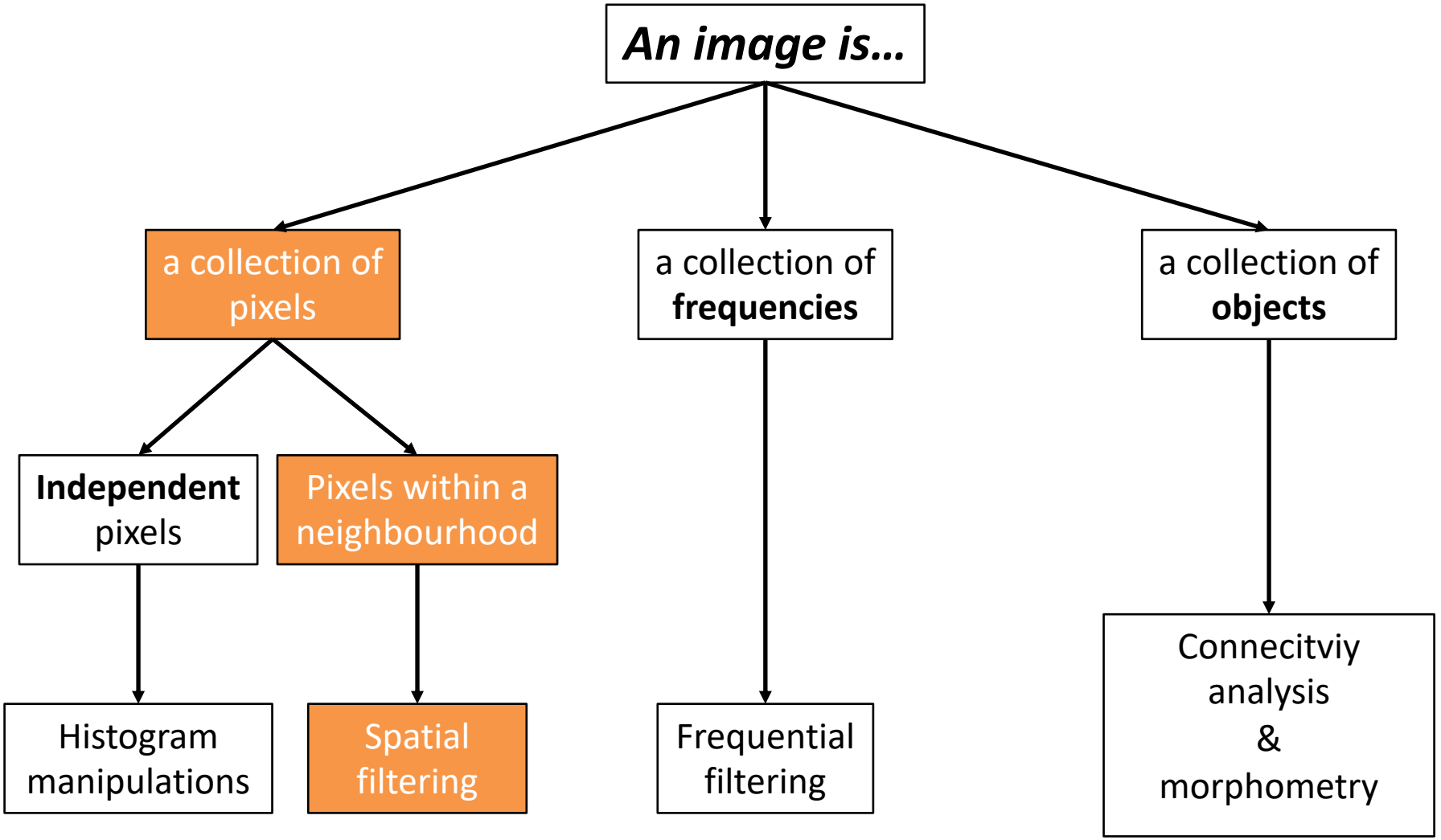
What are images

Quick recap of what are images

During the lecture

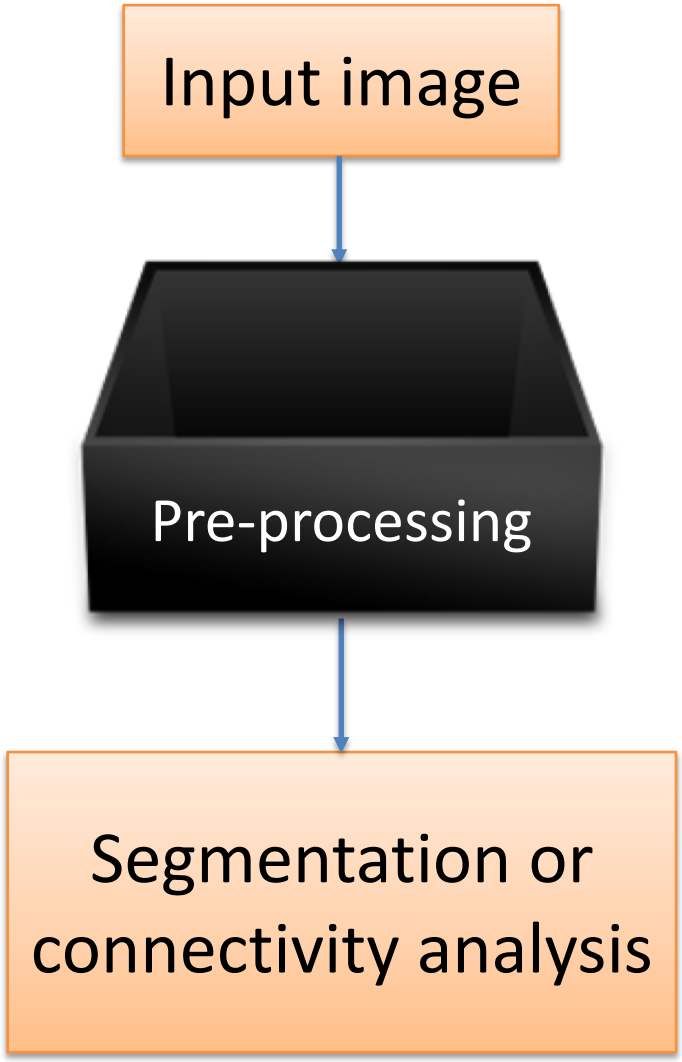
1	How to use linear filtering	***Practice with ImageJ Fiji convolve tool*** ***Practice with filters***

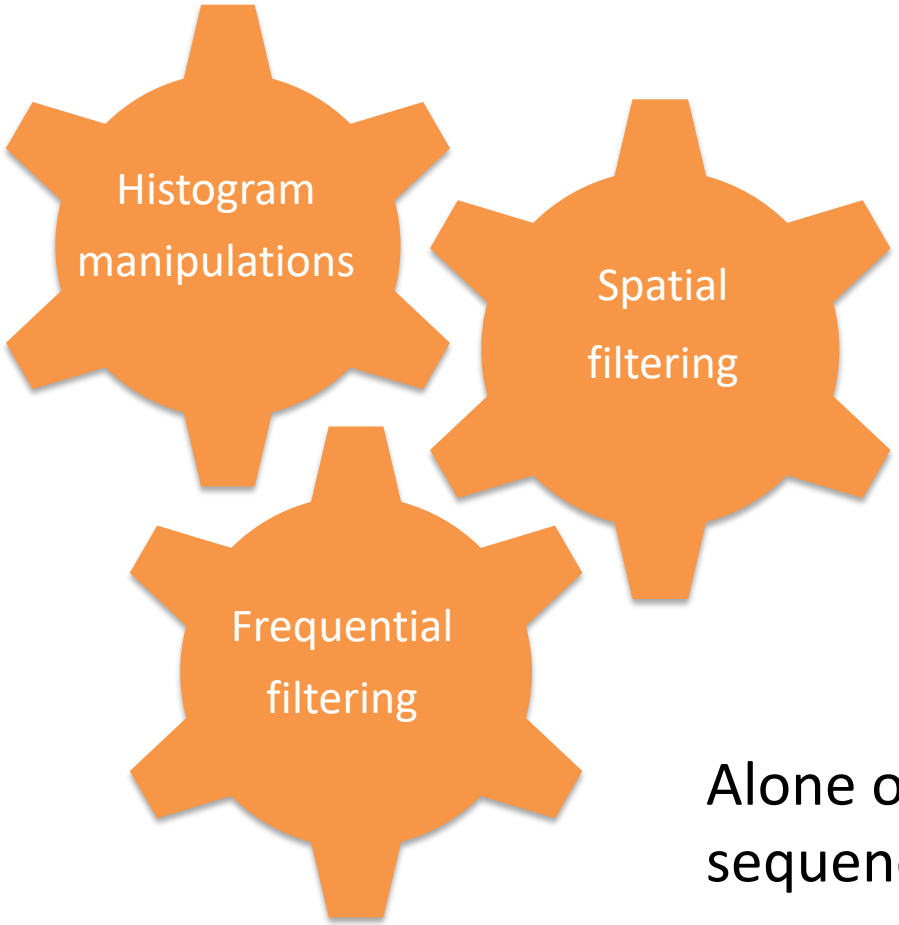
What are images, as Fabrice also said



neubias
network of european bioimage analysts

Images as a collection of pixels





Alone or as a sequence

Image filtering

Enhancing image features prior to image analysis.

The purpose is to improve the segmentation accuracy and efficiency, as well as ”beautifying images!”.

It is important to know what you are doing as you are changing the original data by performing any filters.

Linear spatial filters

- Linear: every new pixel is a linear combination of the corresponding pixel in the original image and its neighbors

Simple image matrix

1	0	1	1	0
0	0	1	0	1
1	1	1	1	1
0	1	0	1	0
1	0	1	0	1

Averaging kernel

1/9	1/9	1/9
1/9	1/9	1/9
1/9	1/9	1/9

Linear spatial filters

Simple image matrix

1 x1/9	0 x1/9	1 x1/9	1	0
0 x1/9	0 x1/9	1 x1/9	0	1
1 x1/9	1 x1/9	1 x1/9	1	1
0	1	0	1	0
1	0	1	0	1

Output matrix

0.67		

(1x1/9) + (0x1/9) + (1x1/9) + (0x1/9) +
(0x1/9) + (1x1/9) + (1x1/9) + (1x1/9) +
(1x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0 x1/9	1 x1/9	1 x1/9	0
0	0 x1/9	1 x1/9	0 x1/9	1
1	1 x1/9	1 x1/9	1 x1/9	1
0	1	0	1	0
1	0	1	0	1

Output matrix

0.67	0.67	

(1x1/9) + (0x1/9) + (1x1/9) + (0x1/9) +
(0x1/9) + (1x1/9) + (1x1/9) + (1x1/9) +
(1x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0	1 x1/9	1 x1/9	0 x1/9
0	0	1 x1/9	0 x1/9	1 x1/9
1	1	1 x1/9	1 x1/9	1 x1/9
0	1	0	1	0
1	0	1	0	1

Output matrix

0.67	0.67	0.78

(1x1/9) + (1x1/9) + (0x1/9) + (1x1/9) +
(0x1/9) + (1x1/9) + (1x1/9) + (1x1/9) +
(1x1/9) = 7/9 = 0.78

Linear spatial filters

Simple image matrix

1	0	1	1	0
0 x1/9	0 x1/9	1 x1/9	0	1
1 x1/9	1 x1/9	1 x1/9	1	1
0 x1/9	1 x1/9	0 x1/9	1	0
1	0	1	0	1

Output matrix

0.67	0.67	0.78
0.56		

(0x1/9) + (0x1/9) + (1x1/9) + (1x1/9) +
(1x1/9) + (1x1/9) + (0x1/9) + (1x1/9) +
(0x1/9) = 5/9 = 0.56

Linear spatial filters

Simple image matrix

1	0	1	1	0
0	0 x1/9	1 x1/9	0 x1/9	1
1	1 x1/9	1 x1/9	1 x1/9	1
0	1 x1/9	0 x1/9	1 x1/9	0
1	0	1	0	1

Output matrix

0.67	0.67	0.78
0.56	0.67	

(0x1/9) + (1x1/9) + (0x1/9) + (1x1/9) +
(1x1/9) + (1x1/9) + (1x1/9) + (0x1/9) +
(1x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0	1	1	0
0	0	1 x1/9	0 x1/9	1 x1/9
1	1	1 x1/9	1 x1/9	1 x1/9
0	1	0 x1/9	1 x1/9	0 x1/9
1	0	1	0	1

Output matrix

0.67	0.67	0.78
0.56	0.67	0.67

(1x1/9) + (0x1/9) + (1x1/9) + (1x1/9) +
(1x1/9) + (1x1/9) + (0x1/9) + (1x1/9) +
(0x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0	1	1	0
0	0	1	0	1
1 x1/9	1 x1/9	1 x1/9	1	1
0 x1/9	1 x1/9	0 x1/9	1	0
1 x1/9	0 x1/9	1 x1/9	0	1

Output matrix

0.67	0.67	0.78
0.56	0.67	0.67
0.67		

(1x1/9) + (1x1/9) + (1x1/9) + (0x1/9) +
(1x1/9) + (0x1/9) + (1x1/9) + (0x1/9) +
(1x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0	1	1	0
0	0	1	0	1
1	1 x1/9	1 x1/9	1 x1/9	1
0	1 x1/9	0 x1/9	1 x1/9	0
1	0 x1/9	1 x1/9	0 x1/9	1

Output matrix

0.67	0.67	0.78
0.56	0.67	0.67
0.67	0.67	

(1x1/9) + (1x1/9) + (1x1/9) + (1x1/9) +
(0x1/9) + (1x1/9) + (0x1/9) + (1x1/9) +
(0x1/9) = 6/9 = 0.67

Linear spatial filters

Simple image matrix

1	0	1	1	0
0	0	1	0	1
1	1	1 x1/9	1 x1/9	1 x1/9
0	1	0 x1/9	1 x1/9	0 x1/9
1	0	1 x1/9	0 x1/9	1 x1/9

Output matrix

0.67	0.67	0.78
0.56	0.67	0.67
0.67	0.67	0.67

(1x1/9) + (1x1/9) + (1x1/9) + (0x1/9) +
(1x1/9) + (0x1/9) + (1x1/9) + (0x1/9) +
(1x1/9) = 6/9 = 0.67

Linear spatial filters

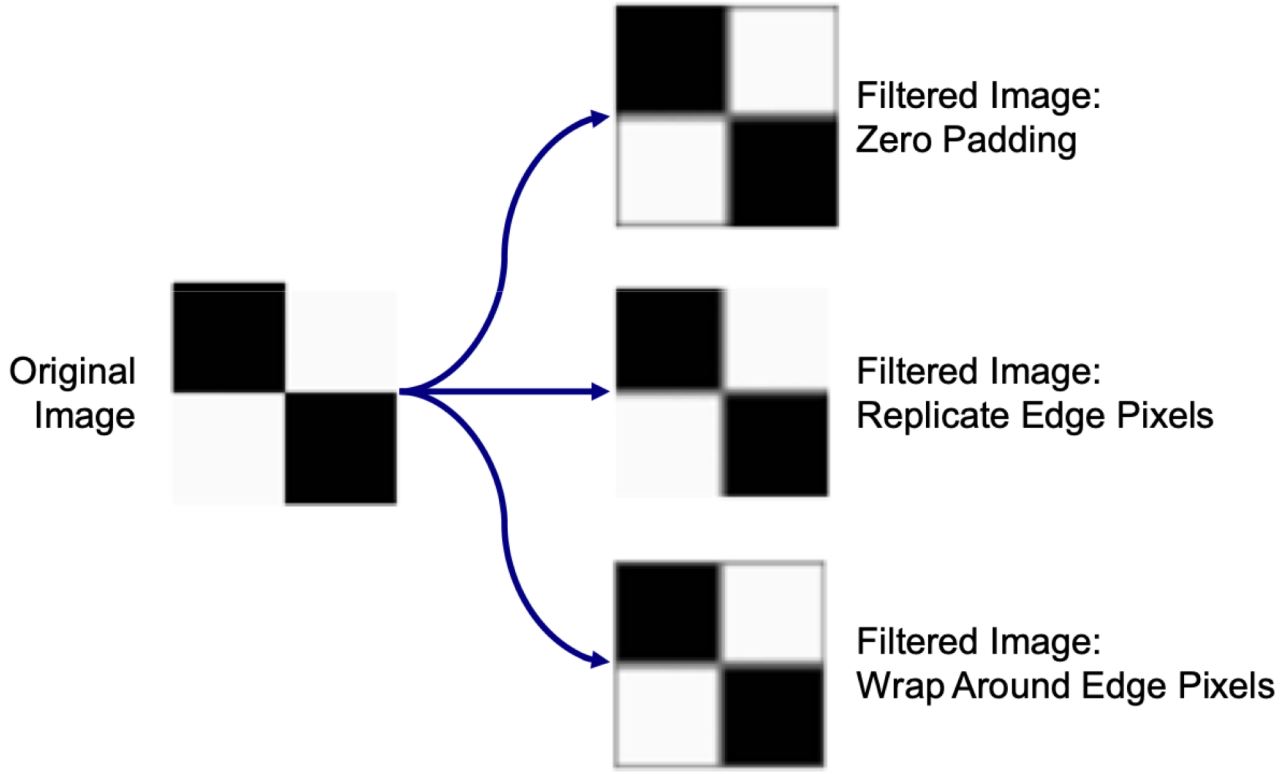
Simple image matrix

1	0	1	1	0
0	0	1	0	1
1	1	1	1	1
0	1	0	1	0
1	0	1	0	1

Output matrix

0.67	0.67	0.78
0.56	0.67	0.67
0.67	0.67	0.67

Linear spatial filters-padding



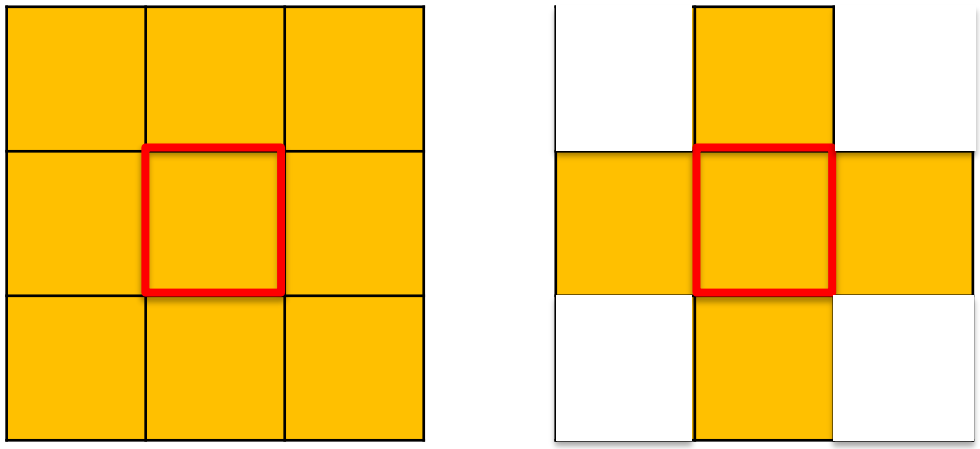
C. Nikou – Digital Image Processing (E12)

What type of padding does imageJ have?

Images/2-paddingTest.txt

Linear spatial filters-Additional consideration

Filter kernel



In some image processing filters, the operation is only performed using the central pixel and strong neighbors. You will see an example of this in morphological operations when you have 8 or 4 connectivity.

Linear spatial filters- Most frequently used ones

Low-pass filters:
High spatial frequesncies of the image: details or noise

Process -> Smooth
Process -> Filters -> mean…
Process -> Filters -> Gaussian blur...

High-pass filters:
Process -> Filters -> Unsharp mask…

Edge detection and enhancement:
Process -> Find edges
Process -> Filters -> variance

Linear spatial filters- averaging vs gaussian blur

Original Averaging Gaussian

Linear spatial filters- Most frequently used ones

Gaussian (σ=1)

1	4	7	4	1
4	16	26	16	4
7	26	41	26	7
4	16	26	16	4
1	4	7	4	1

Sobel Horizontal

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

Sobel Vertical

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

Sharpen

-1	-1	-1
-1	12	-1
-1	-1	-1

Laplacian 4-connected

0	-1	0
-1	4	-1
0	-1	0

Laplacian 8-connected

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

Prewitt horizontal

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

Prewitt vertical

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

Try your own filter

Images/3-cat.tif

Linear spatial filters- The effect of kernel size and shape on final image

3x3

1	1	1
1	1	1
1	1	1

5x5

1	1	1	1	1
1	1	1	1	1
1	1	1	1	1
1	1	1	1	1
1	1	1	1	1

7x7

1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1
1	1	1	1	1	1	1

Square

1	1	1	1	1
1	1	1	1	1
1	1	1	1	1
1	1	1	1	1
1	1	1	1	1

Diamond

0	0	1	0	0
0	1	1	1	0
1	1	1	1	1
0	1	1	1	0
0	0	1	0	0

Circle

0	1	1	1	0
1	1	1	1	1
1	1	1	1	1
1	1	1	1	1
0	1	1	1	0

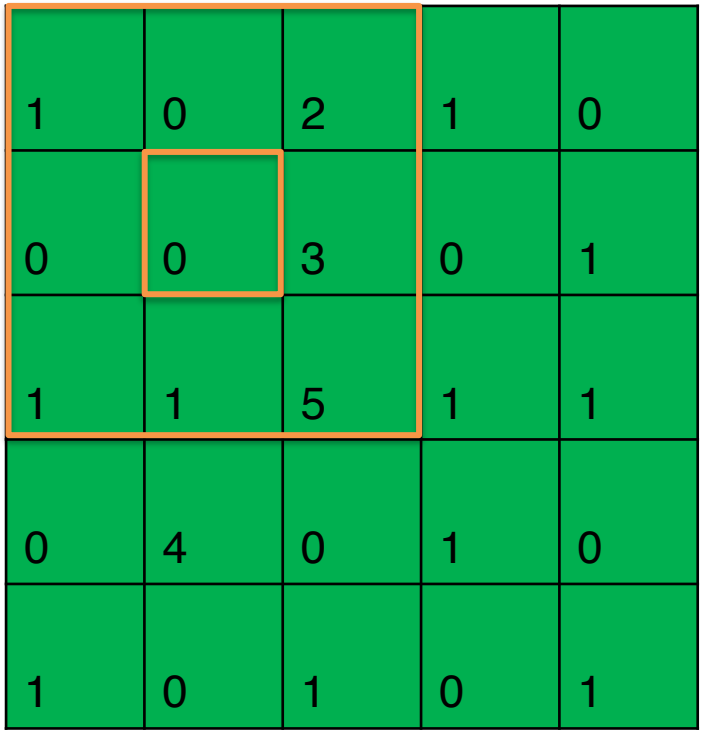
Images/3-cat.tif

During the lecture

1	How to use linear filtering	***Practice with ImageJ Fiji convolve tool*** ***Practice with filters***
2	How to use RANK filtering	***Practice with ImageJ Fiji RANK filters***

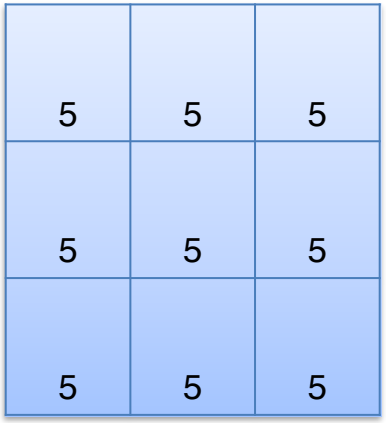
Spatial RANK filters

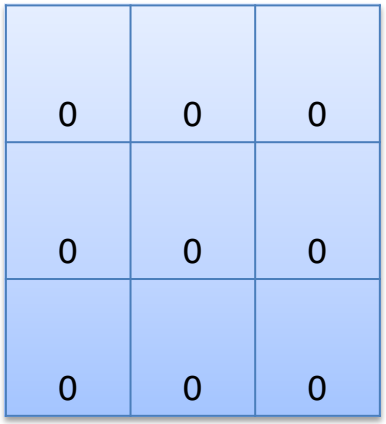
Simple image matrix

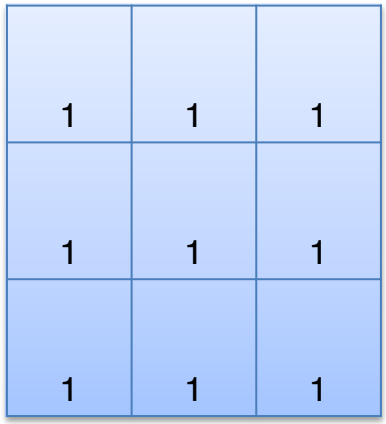


Min, Max and Median filters

Output matrix







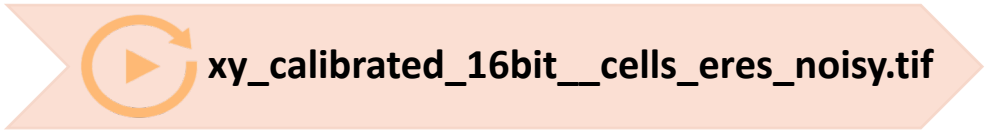
neubias network of european bioimage analysts

Spatial RANK filters

A mean filter reduces the intensity of the extreme values but spreads out their influence, while a small median filter is capable of removing them completely with a minimal effect upon the rest of the image.

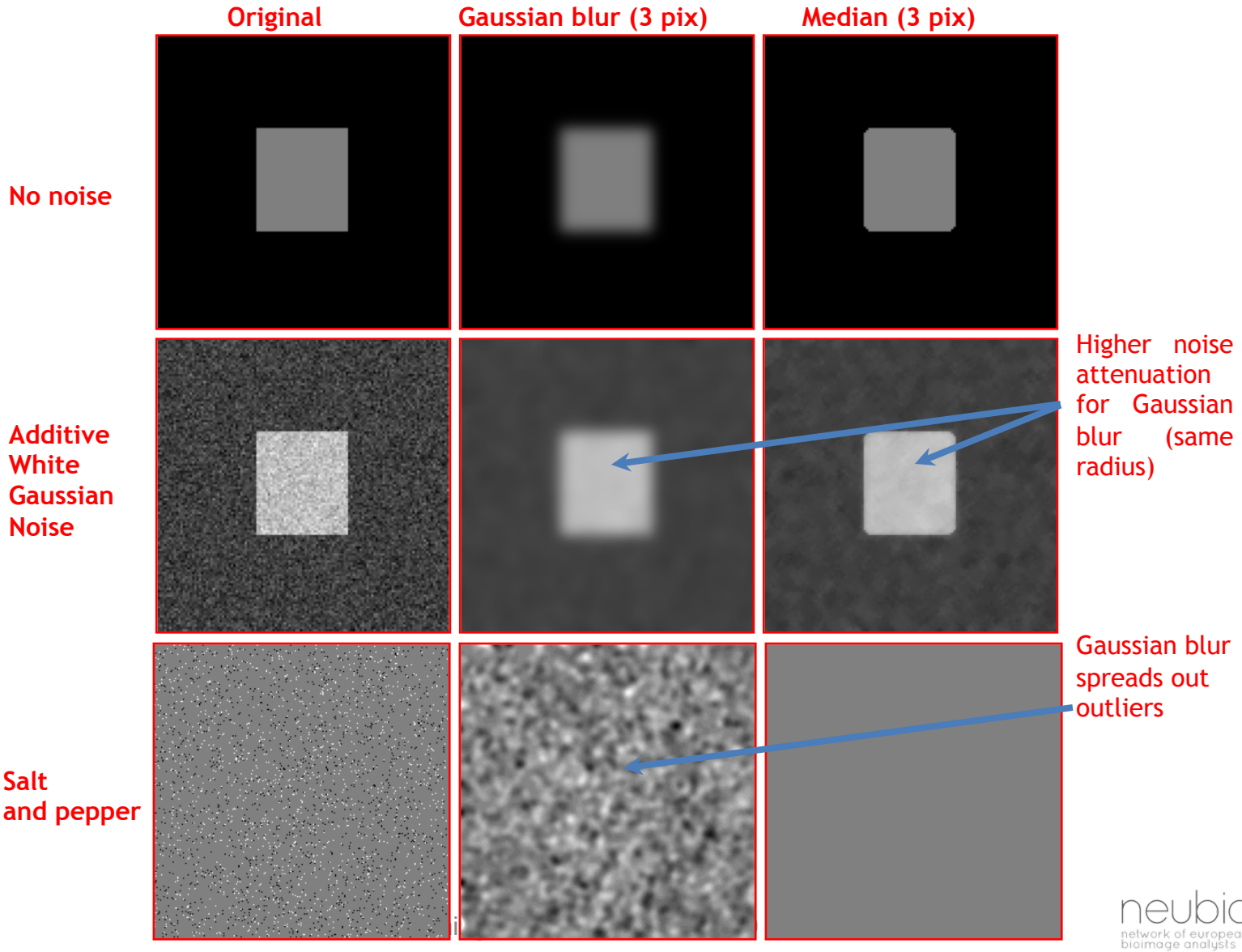
Minimum and maximum filters can be used for uneven background removal.

Task: Try, minimum, maximum, minimum followed by maximum, gaussian blur, and median filters with different radius



Good resource:
https://petebankhead.gitbooks.io/imagej-intro/content/chapters/filters/filters.html

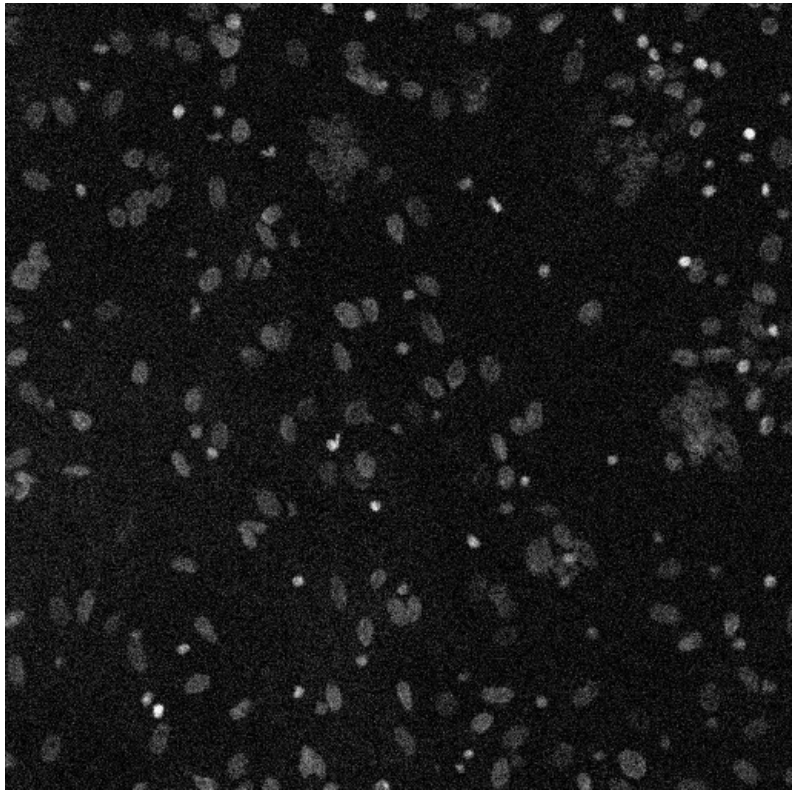
Linear spatial filters- median vs gaussian blur



During the lecture

1	How to use linear filtering	***Practice with ImageJ Fiji convolve tool*** ***Practice with filters***
2	How to use RANK filtering	***Practice with ImageJ Fiji RANK filters***
3	Practical exercises	***Trying uneven background subtraction*** ***Trying noise removal using filters***

When do we use filters? Noise removal

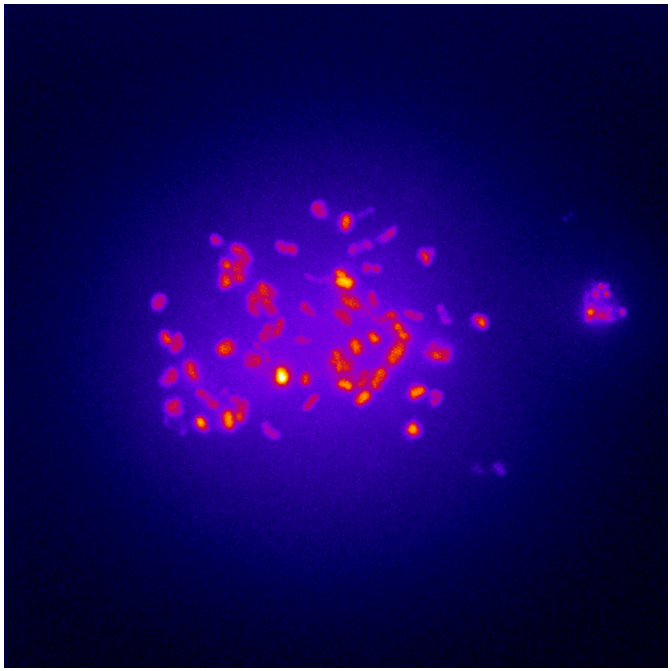


Task: Segment the nuclei in the image
Correct background and remove the noise

nuclei-noisy.tif

MAX_nuclei_noisy-2.tif

When do we use filters? Uneven background correction



- Small details (kinetochores, width: 5-6 pixels)
- Image from Fabrice

Task: Segment the spots in the image

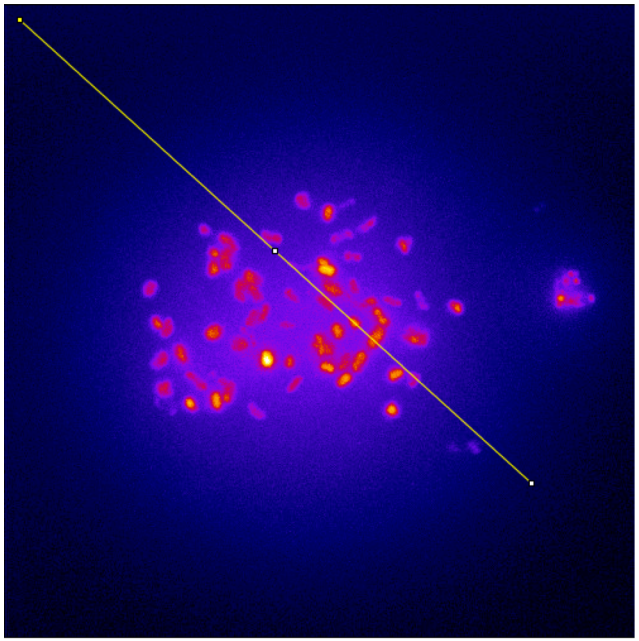
Let's look at the image intensity profile first and try what you have learnt yesterday

Use linear filters to remove the background first
Use RANK filters to remove the background

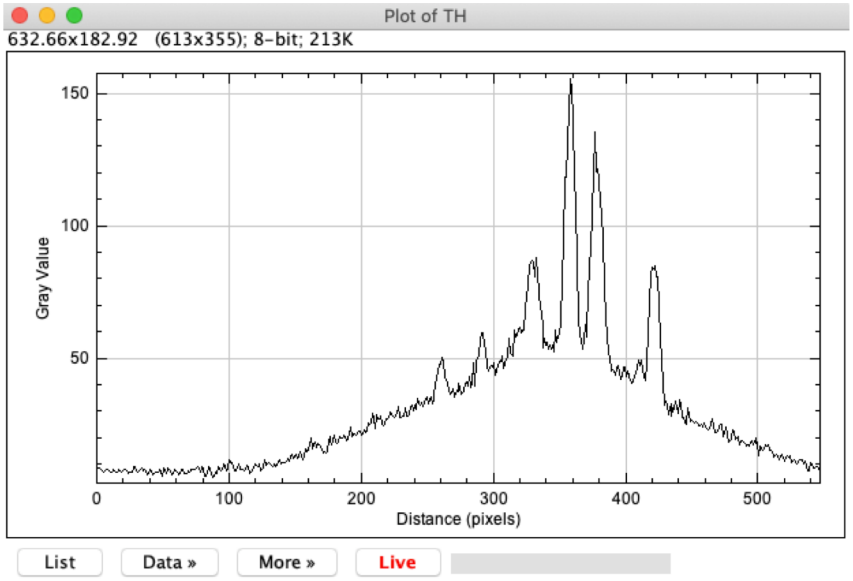
Good sample preparation and image acquisition is always the best way to start!

TH.tif

When do we use filters? Uneven background correction



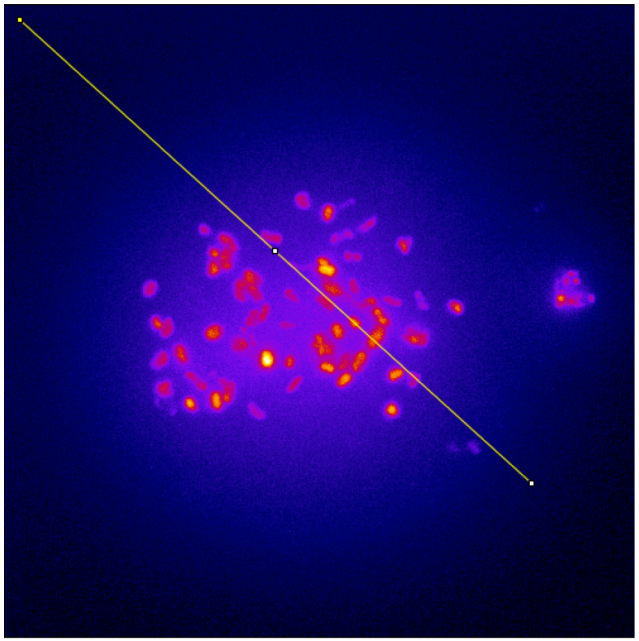
Plot profile



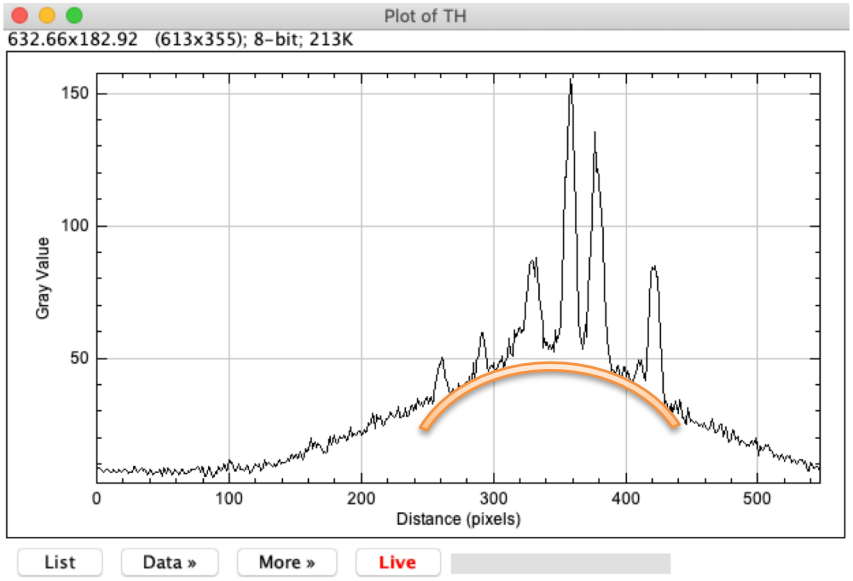
TH.tif

neubias network of european bioimage analysts

When do we use filters? Uneven background correction



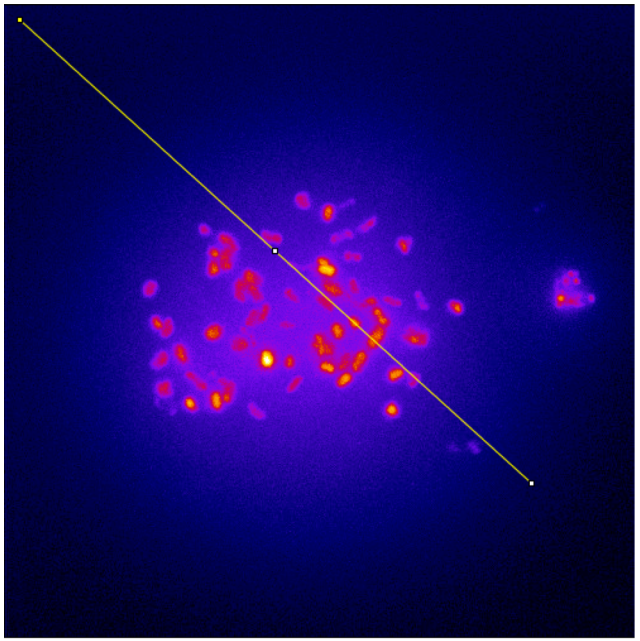
Plot profile



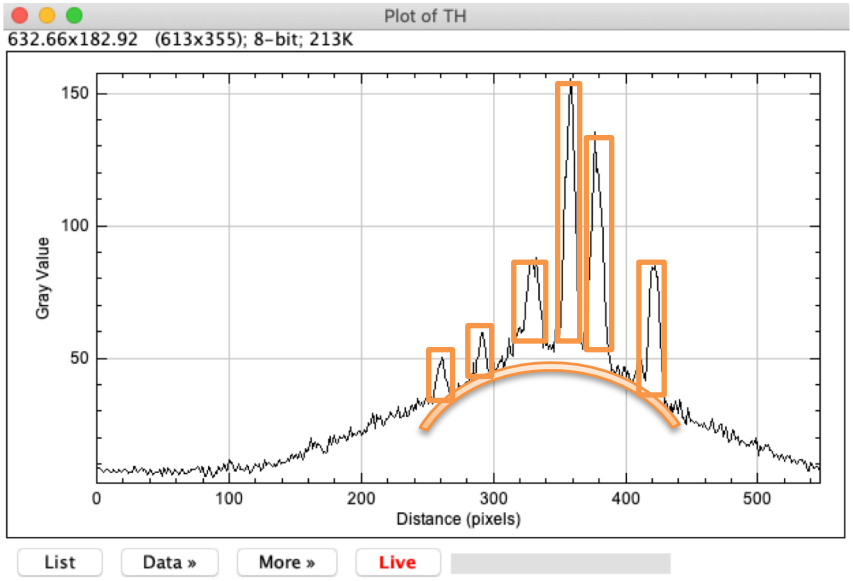
TH.tif

neubias
network of european bioimage analysts

When do we use filters? Uneven background correction

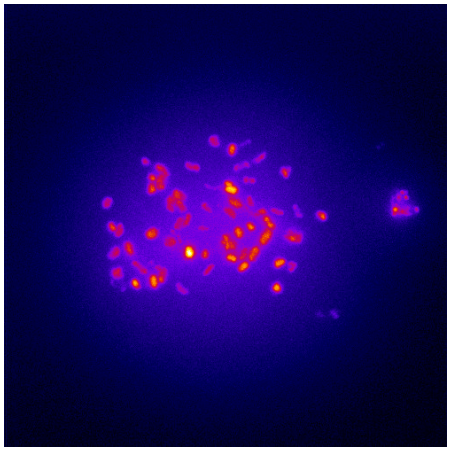


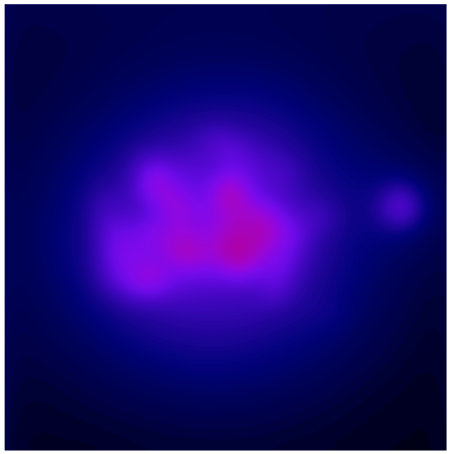
Plot profile



TH.tif

When do we use filters? Uneven background correction



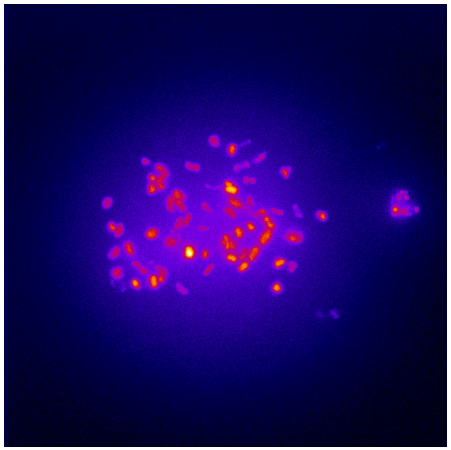


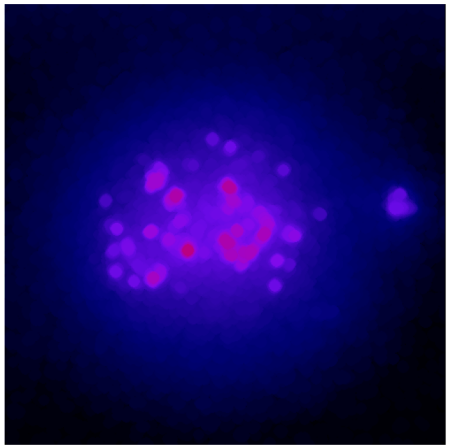
Task: Segment the spots in the image
Use linear filters to remove the background first

1. Duplicate the image
2. Blur the image using one of bluring techniques you learned
3. Subtract the image from the blurred image
4. Plot profile

TH.tif

When do we use filters? Uneven background correction



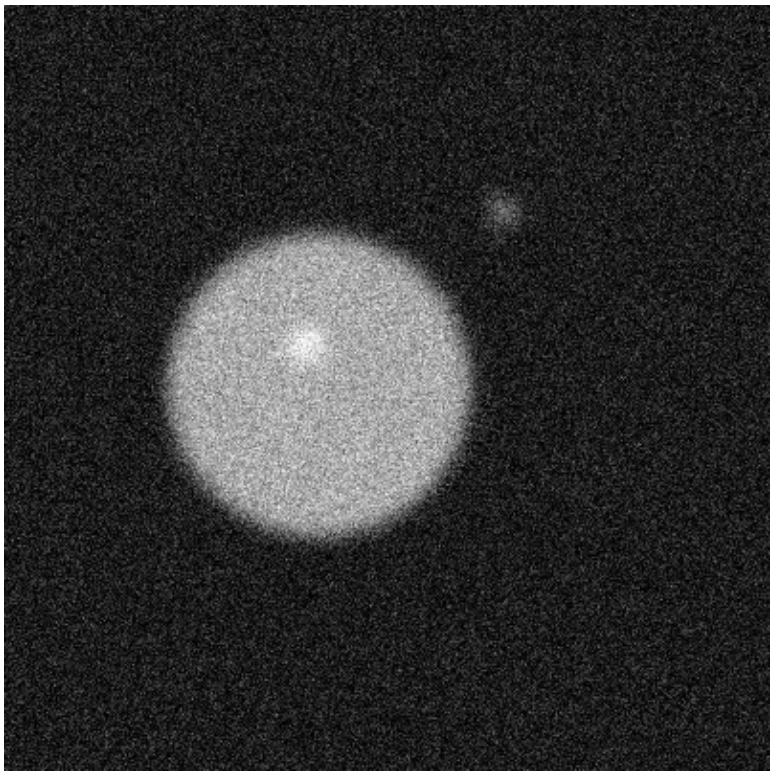


Task: Segment the spots in the image
Use RANK filters to remove the background

1. Duplicate the image
2. Use minimum filter and subsequently maximum filter (top hat filter)
3. Subtract 1 from 2
4. Plot profile

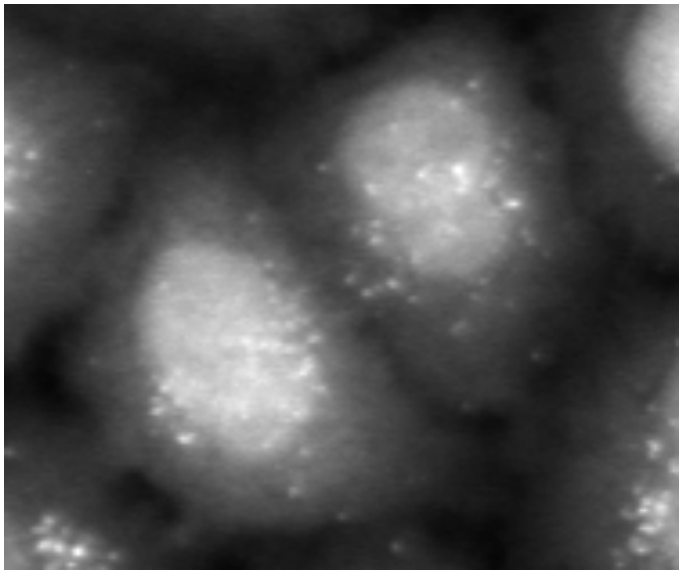
TH.tif

When do we use filters? Uneven background correction



Remember these images from Tischi's lecture?

Let's try removing the background using the two methods you just learned



uneven-background-test-image.tif
Autophagosomes.tif

During the lecture

1	How to use linear filtering	***Practice with ImageJ Fiji convolve tool*** ***Practice with filters***
2	How to use RANK filtering	***Practice with ImageJ Fiji RANK filters***
3	Practical exercises	***Trying uneven background subtraction*** ***Trying noise removal using filters***
4	Morphological operators	***Trying different morphological operators***

Morphological operations

Goal: Improve the binary mask obtained by the segmentation to correctly perform morphological analysis.
Need binary image as input.

Some of the morphological operators (morphomaths):

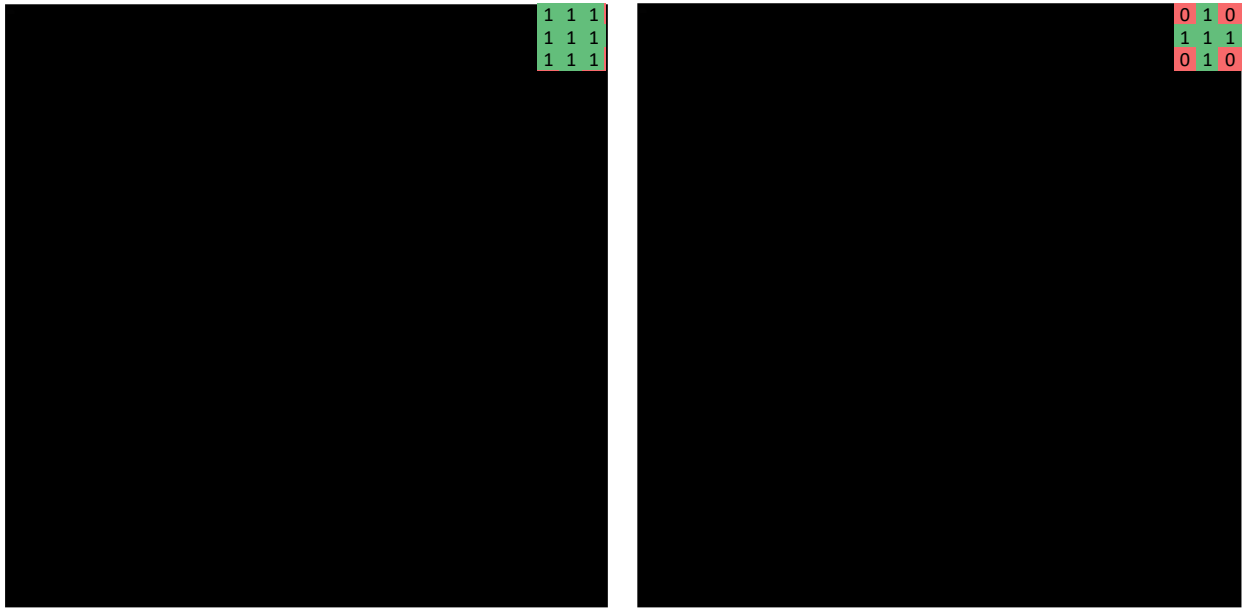
- Dilation
- Erosion
- Opening
- Closing
- Watershed
- Fill holes
- …

Double check Edit -> Options -> Colors…

Morphological operations

Dilation: When the intensity of the center pixel is 1, all the connected pixels are converted to 1.
Gray value equivalent: Maximum

Dilation

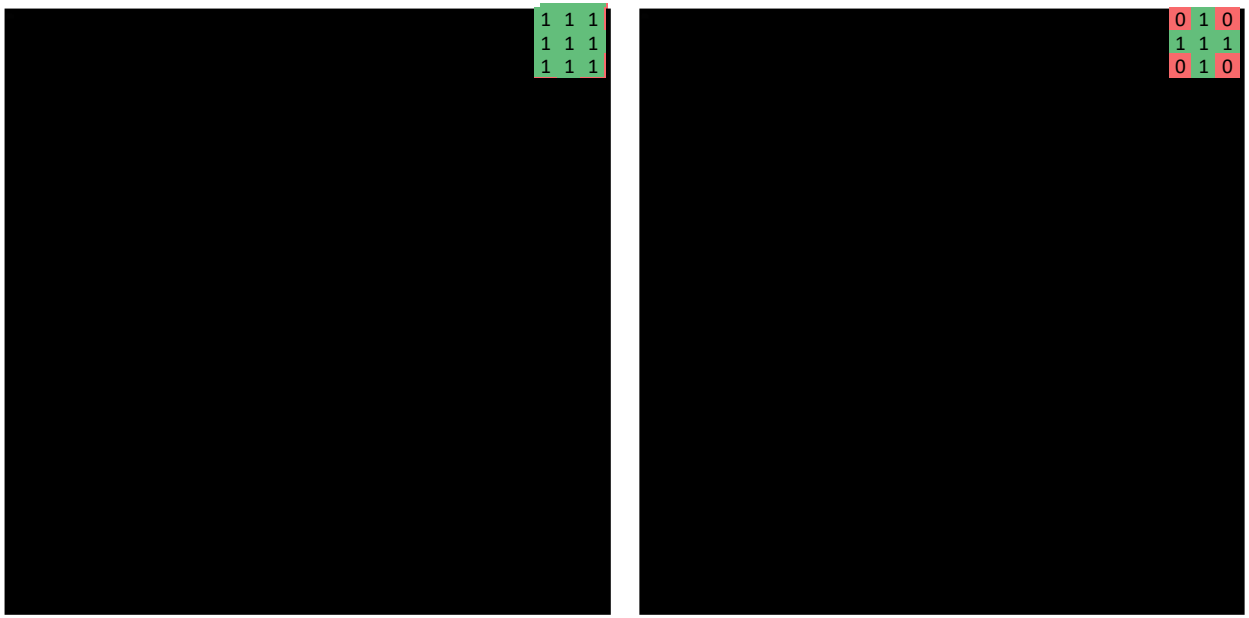


https://www.edx.org/course/image-processing-and-analysis-for-life-scientists

Morphological operations

Erosion: When the intensity of the center pixel is 0, all the connected pixels are converted to 0.
Gray value equivalent: Minimum

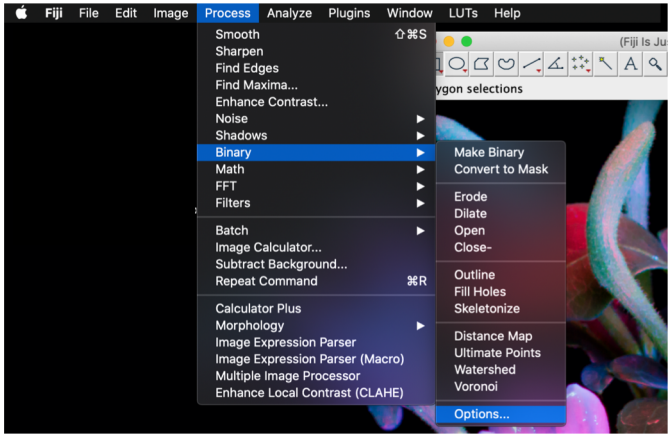
Erosion

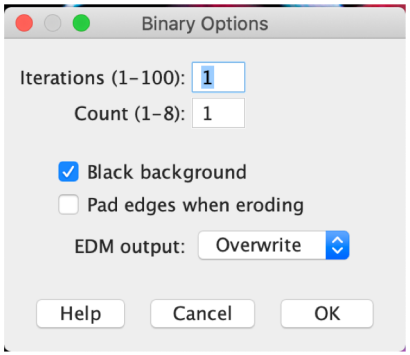


Morphological operations

Opening: Erosion followed by Dilation
Removes small objects and separates components.

Closing: Dilation followed by Erosion
Removes small holes and merges components.





Images/Binary.tif

https://imagej.nih.gov/ij/docs/guide/146-29.html

neubias network of european bioimage analysts

Morphological operations

Task: Using morphological operators, make an outline of the image with the thickness of one pixel

Images/Binary.tif

Morphological operations

Watershed:
Erodes the objects to find the ultimate erode point: objects -> single points
Dilates the points until it reaches the border of the object or the border of another growing object
Good for: homogenous objects with minimal overlap

Images/Binary.tif

Images/Binary2.tif

Morphological operations

Fil holes:
Good step before watershed
It can also fills the space between objects ☹

Task: Combine morphological operators to successfully watershed Binary3.tif

Images/Binary.tif

Images/Binary3.tif