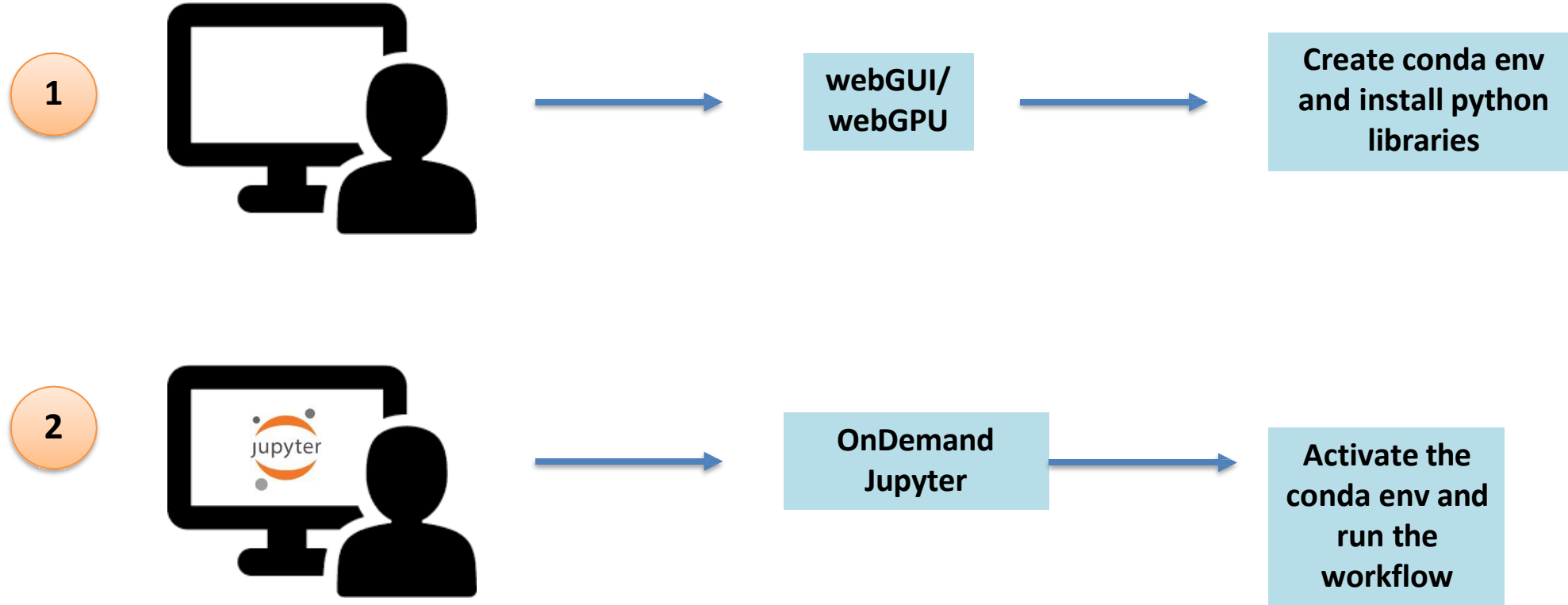

Image processing with Python

[web] portal.biohpc.swmed.edu
[email] biohpc-help@utsouthwestern.edu

Python image processing workflows



https://portal.biohpc.swmed.edu/media/filer_public/18/86/18864d7a-28ca-4ae5-be84-3e94b7c3bc4b/software_installation_2023_09_13.pdf

Python Libraries + Modules used in this training

Docs:

- os : <https://docs.python.org/3/library/os.html>
- matplotlib : <https://matplotlib.org/>
- scipy :
 - General : <https://docs.scipy.org>
 - ndimage : <https://docs.scipy.org/doc/scipy/reference/ndimage.html>
- skimage : <https://scikit-image.org/>
- sklearn : <https://scikit-learn.org/stable/>
- numpy :
 - General : <https://numpy.org/doc/stable/index.html>
 - ndarrays : <https://numpy.org/doc/stable/reference/arrays.ndarray.html#id1>

Already installed in Jupyter/JupyterLab OnDemand.

How a digital image is stored on a computer



```
08 02 22 97 38 15 00 40 00 75 04 05 07 18 32 12 50 77 17 29
49 49 99 40 17 81 18 57 60 87 17 40 98 43 65 44 11 54 62 00
81 49 31 73 55 79 14 29 93 71 40 67 54 11 30 03 49 13 36 65
52 70 95 23 04 60 11 42 63 14 85 56 01 32 56 71 37 02 36 91
22 31 16 71 51 47 43 59 41 92 36 54 22 40 40 28 66 33 13 80
24 67 33 80 99 03 45 02 44 75 33 53 78 36 84 20 35 17 12 50
12 98 81 28 64 25 67 10 26 38 40 67 59 54 70 66 18 38 64 70
67 26 20 68 02 62 12 20 95 63 94 39 63 08 40 91 66 49 94 21
24 55 58 05 46 73 99 26 97 17 78 78 96 83 14 88 34 89 63 72
21 36 23 09 75 00 76 44 20 45 35 14 00 61 33 97 34 31 33 95
78 17 55 28 22 75 31 67 15 94 03 80 04 42 14 14 09 53 56 92
16 39 05 42 96 35 31 47 55 58 88 24 00 17 54 24 36 29 85 57
86 56 00 48 35 71 89 07 05 44 44 37 44 40 21 58 31 54 17 58
19 80 81 68 05 94 47 69 28 73 92 13 86 52 17 77 04 89 55 40
04 52 08 83 97 35 99 16 07 97 57 32 16 26 24 79 33 27 88 66
14 14 68 87 57 62 20 72 03 46 33 67 46 55 12 32 63 93 53 69
04 42 16 73 56 51 38 11 24 94 72 18 08 46 29 32 40 62 76 36
20 49 36 41 72 30 33 83 34 25 69 82 67 59 85 74 04 36 14
20 73 35 29 78 31 90 01 74 31 49 71 16 14 85 16 23 57 05 54
01 70 54 72 83 51 54 69 16 82 33 48 61 43 52 01 89 27 61 48
```

What the computer sees

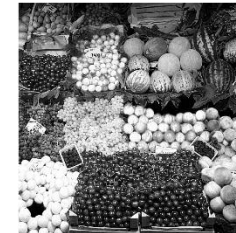
RGB



R



Red



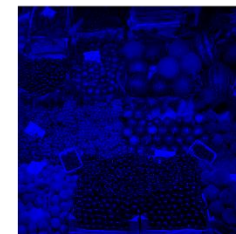
G



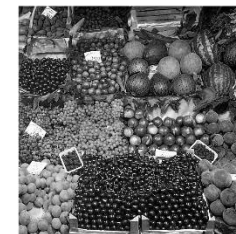
Green



B



Blue



Source - <http://cs231n.github.io/>

Images as Arrays

Different Python libraries have different array implementations

- array
- **numpy**
 - ndarray
- **openCV**
 - cv::Mat

Common data types for image pixels:

- **bool** (binary)- [0,1]
- **int8** (signed integer 8 bit) – numbers in the range: [-128 : 127]
- **float** (double-precision floating point) – Decimal numbers (e.g. 2.2251e-308, 0.4, 0.33...)
- **uint8** (unsigned 8-bit) – [0,255]
- **uint16** (unsigned 16-bit) – [0,65535]

Python Array Indexing

- Python starts counting indexes from 0, and arranges coordinates like C does (row-major)
- Array elements can be access in two ways:
 - By forward indexing
 - By backward indexing

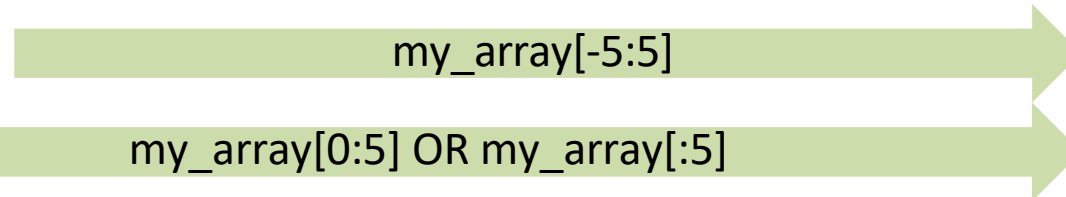
```
my_array = numpy.array([127, 128, 129, 130, 131, 132],  
dtype=np.int8)
```

+ index	0	1	2	3	4	5
Element	127	128	129	130	131	132
- index	-6	-5	-4	-3	-2	-1

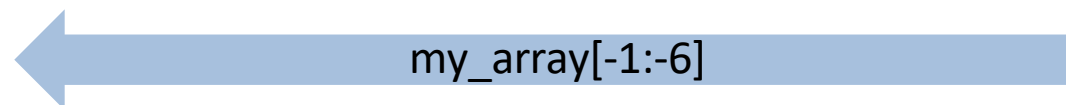
Slice indexes are defined by [Start:Stop] or [Start:Stop:Step] (Stop not included)

Python Array Indexing

```
my_array = numpy.array([127, 128, 129, 130, 131, 132], dtype=np.int8)
```



+ index	0	1	2	3	4	5
Element	127	128	129	130	131	132
- index	-6	-5	-4	-3	-2	-1

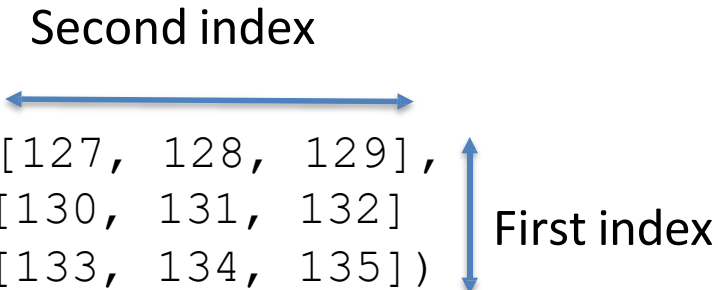


Multi-dimensional arrays – numpy arrays

Python counts in 'row-major' ordering, and orders dimensions like C does.

- Multidimensional arrays are 'lists of lists'
- This is in fact how elements are stored in memory

```
my_2D_array = numpy.array([[127, 128, 129],  
                           [130, 131, 132],  
                           [133, 134, 135]])
```

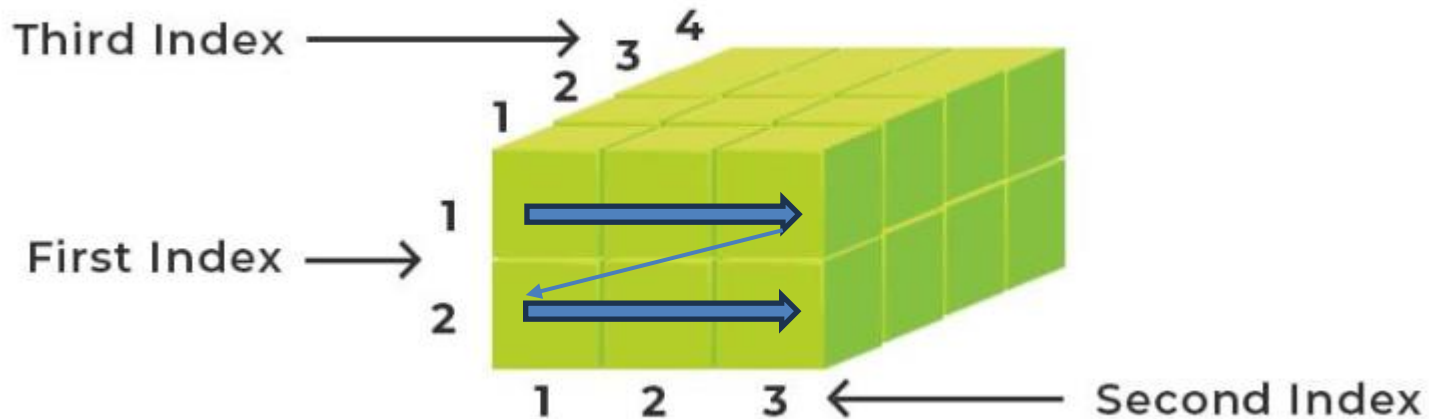


```
my_2D_array[1][0:1] = [130, 131]  
my_2D_array[-1][:] = [133, 134, 135]
```


Multi-dimensional arrays – numpy arrays

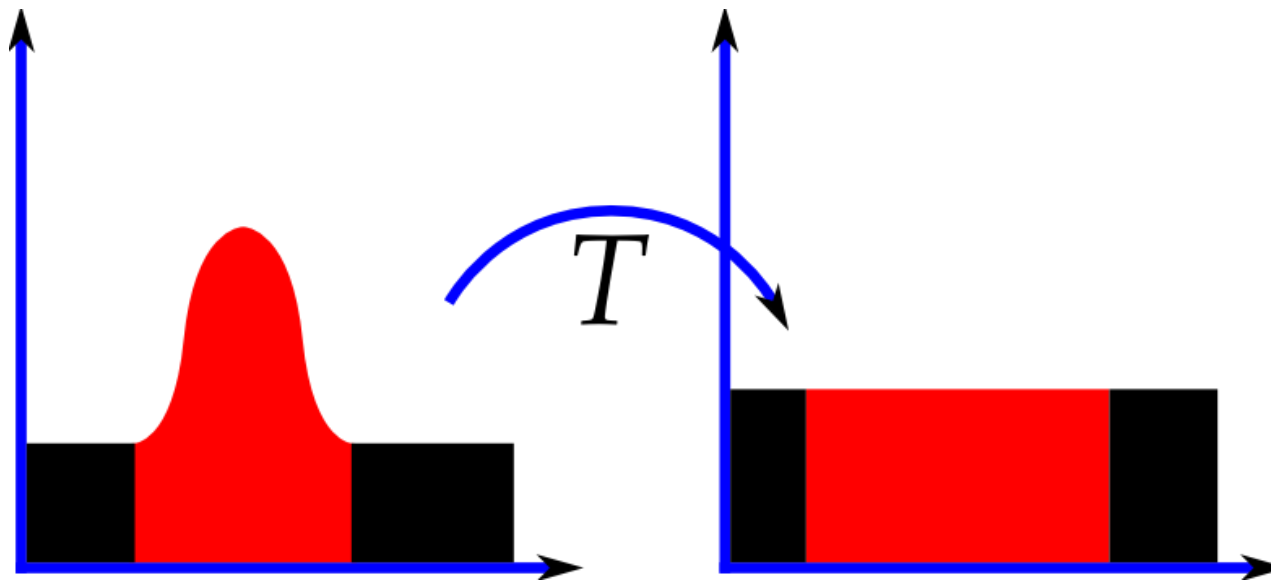
Python counts in 'row-major' ordering, and orders dimensions like C does.

- Multidimensional arrays are 'lists of lists'
- This is in fact how elements are stored in memory



Intensity enhancement

- Contrast stretching
- Histogram equalization
- Adaptive equalization

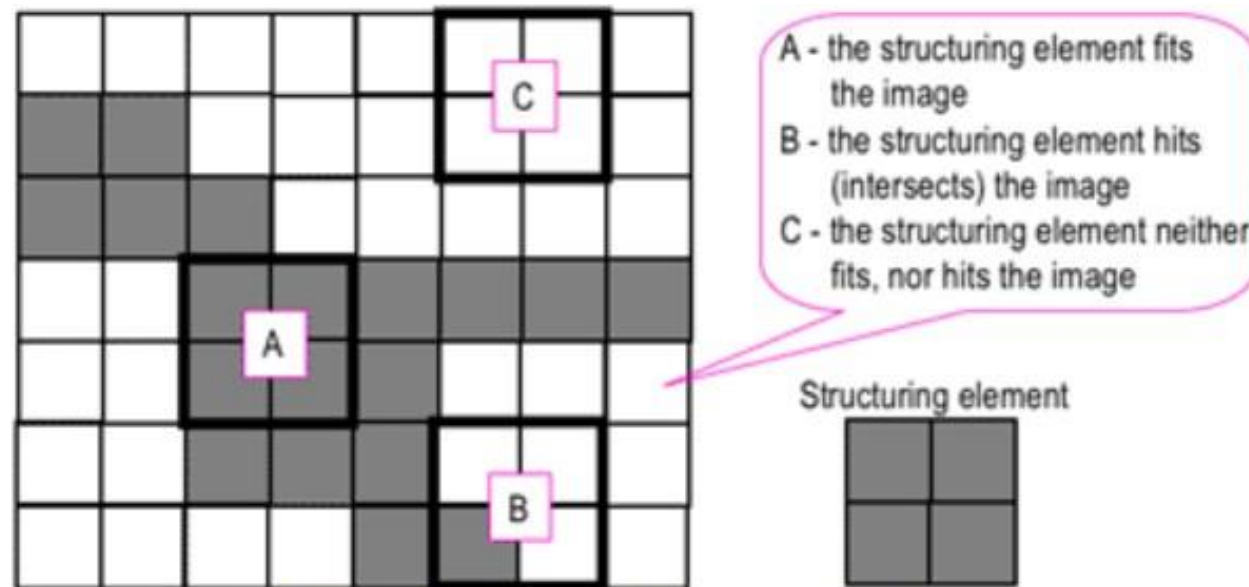


https://en.wikipedia.org/wiki/Histogram_equalization

Morphological operations: Structuring element

The structuring element is a small binary image or matrix such that:

- The matrix dimensions specify the size of the structuring element.
- The pattern of ones and zeros specifies the shape of the structuring element.



Probing of an image with a structuring element
(white and grey pixels have zero and non-zero values, respectively).

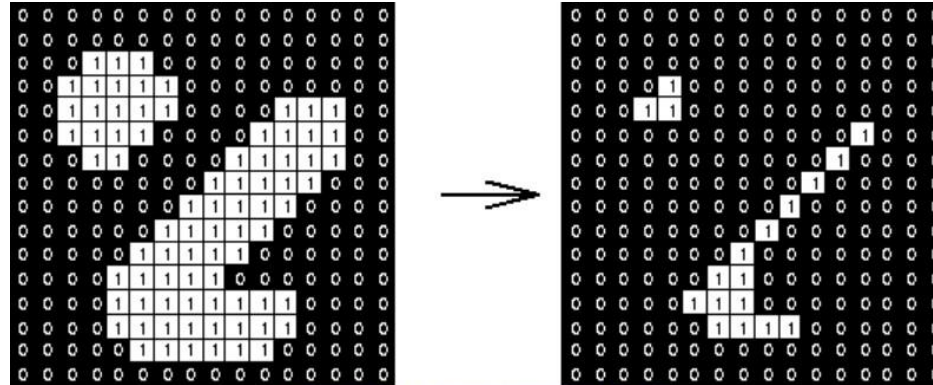
<https://www.cs.auckland.ac.nz/courses/compsci773s1c/lectures/ImageProcessing-html/topic4.htm>

Mathematical Morphology - Grayscale

- Grayscale images can be treated similarly, but with a slightly modified interpretation of 'hit or miss'
- Dilation will result in a pixel taking on the max value defined by the moving window of the strel.
- Erosion will result in a pixel taking on the min value defined by the moving window of the strel.

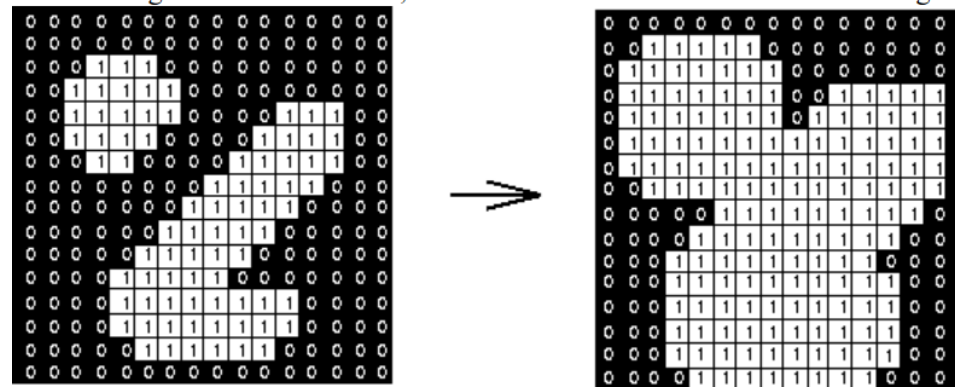
Morphological operations: Dilation and Erosion

Erosion:



Erosion: a 3×3 square structuring element
(www.cs.princeton.edu/~pshilane/class/mosaic/).

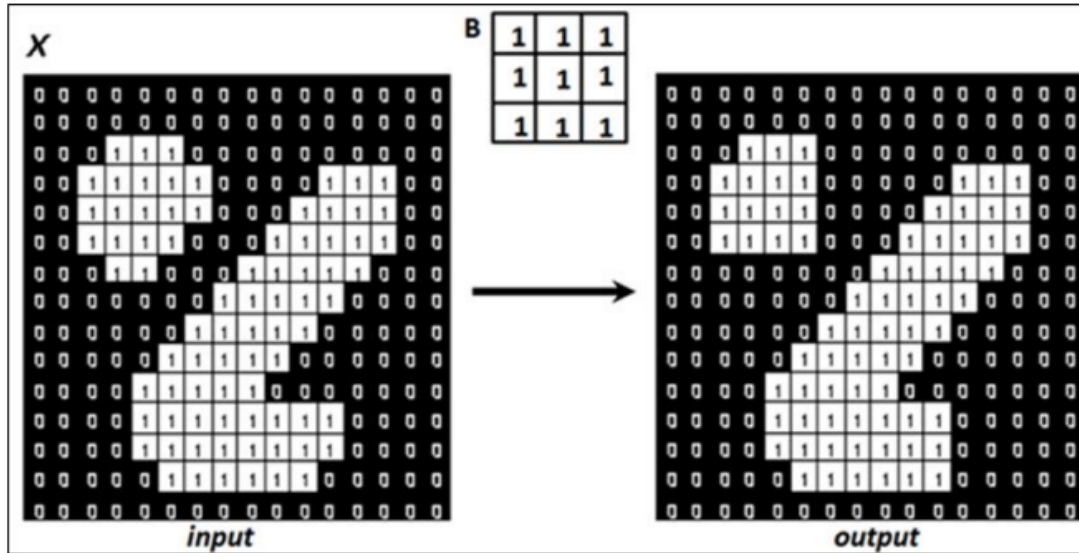
Dilation:



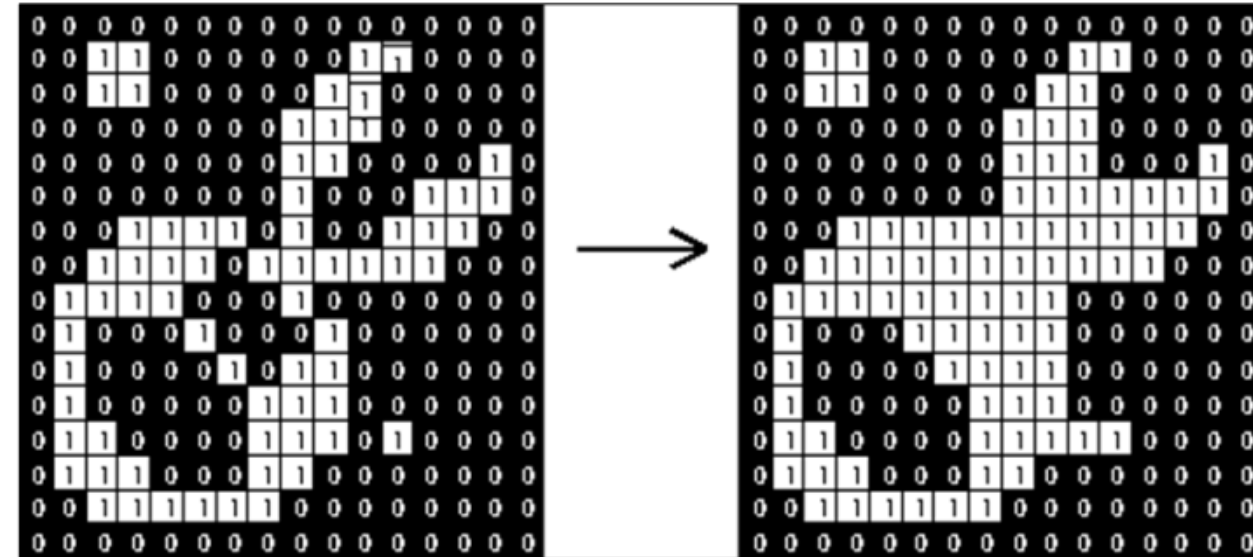
Dilation: a 3×3 square structuring element
(www.cs.princeton.edu/~pshilane/class/mosaic/).

Morphological operations: Open and Close

Opening: erosion followed by a dilation



Closing: dilation followed by a erosion



- Opening an image smooths its contour, fractures narrow isthmuses, making items more separated
- Closing fills in small gaps/holes and brings items closer, also smooths the contour

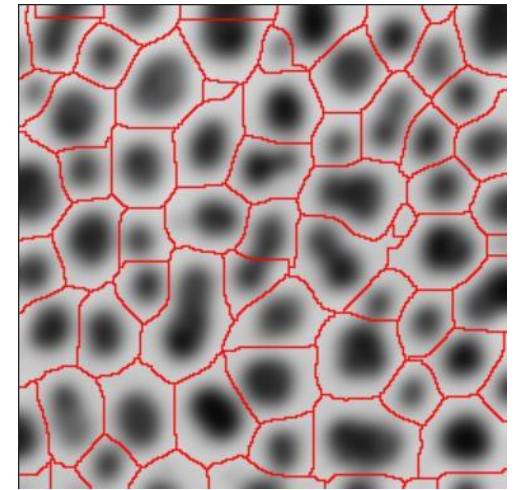
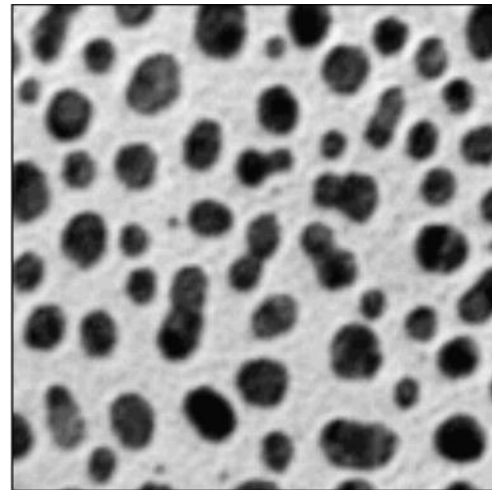
Segmentation – Separating an image into parts

Most basic: Manual thresholding

- Bright or dark background with a dark or bright foreground, respectively.
- Choose a cutoff value, threshold.
- Using global thresholds may miss important elements

More complex:

- Adaptive thresholds
- Morphological segmentation
- Clustering
- Machine learning methods

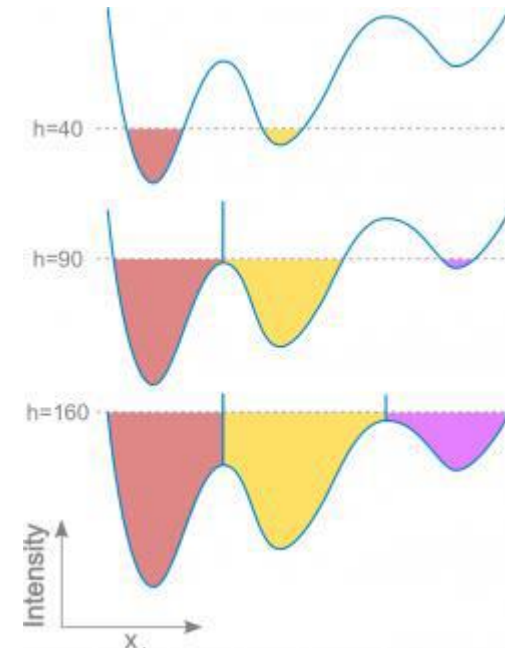


Morphological Segmentation: Watershed method

- Consider grey levels as altitudes
- Identify local minima
- Flood basins starting from minima
- Separate the basins by a “dam” → the watershed

Steps for performing the watershed method:

1. Segment objects of interest
2. Convert the mask into an intensity profile using the distance transform
3. Run the watershed algorithm



More useful materials

- Previous image processing using Matlab slides (More intro on filters)
https://portal.biohpc.swmed.edu/media/filer_public/61/34/6134df89-c5b8-4efd-9f60-fbc1c5005bb0/training_matlab_2022_10_19.pdf
- Image Processing with Python <https://datacarpentry.org/image-processing/>

Grayscale Histograms

We will start with grayscale images, and then move on to colour images. We will use this image of a plant seedling as an example:



```
Show me the solution ^
```

First, let's work through the process for one image:

```
PYTHON < >
```

```
import imageio.v3 as iio
import ipyml
import matplotlib.pyplot as plt
import numpy as np
import skimage as ski

%matplotlib widget

bacteria_image = iio.imread(uri="data/colonies-01.tif")

# display the image
fig, ax = plt.subplots()
plt.imshow(bacteria_image)
```