PoL
Physics of Life
TU Dresden

CENTER FOR
SYSTEMS BIOLOGY
DRESDEN

Image Processing: Filters

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With material from

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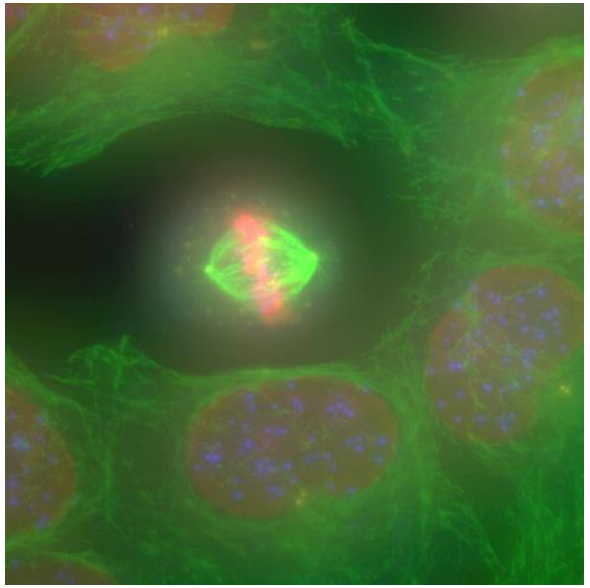
Filters

An image processing filter is an operation on an image.
It takes an image and produces a new image out of it.
Filters change pixel values.

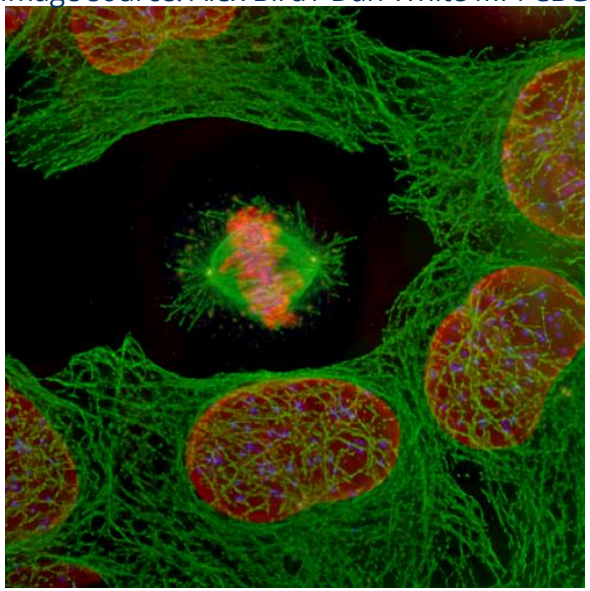
There is no “best” filter. Which filter fits your needs, depends on the context.
Filters do not do magic. They can not make things visible which are not in the image.

Application examples
- Noise-reduction
- Artefact-removal
- Contrast enhancement
- Correct uneven illumination



Filter

Image source: Alex Bird / Dan White MPI CBG



Effects harming image quality

Noise is a general term for unwanted modifications that a signal may suffer during capture, storage, transmission, processing, or conversion.

In microscopy, image quality suffers from

- shot noise: Statistical variation of the photons arriving at the camera
- dark noise: Statistical variation of how many electrons are generated if a photon arrives in a camera pixel (temperature dependent).
- read out noise: introduced by the electronics, especially the analog-digital-converter
- Physical/optical effects: aberrations, defocus
- Biological/physiological/structural effects: motion, diffusion

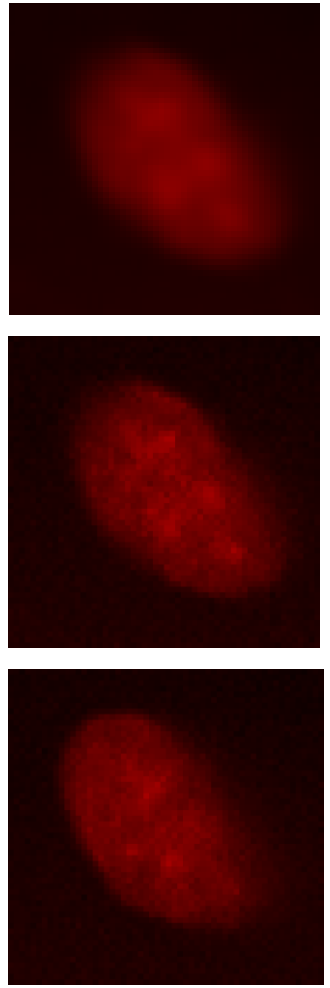


Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Noise

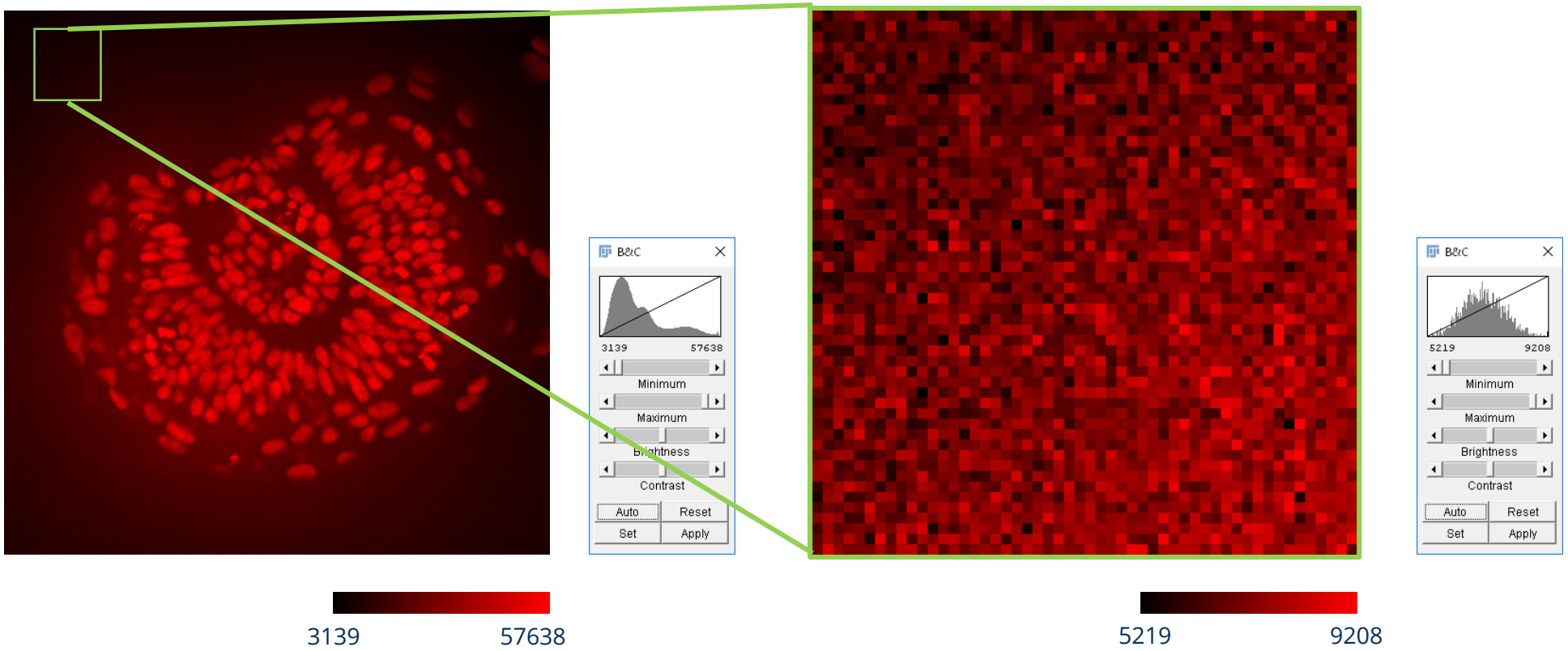
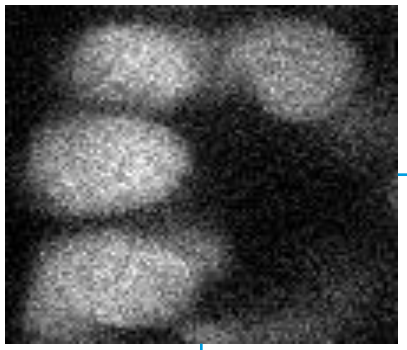


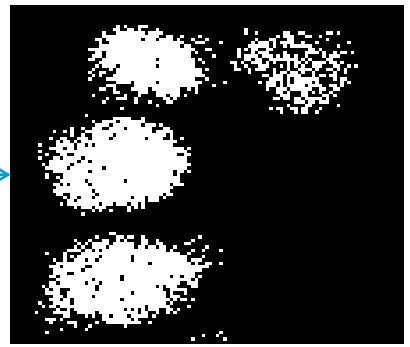
Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Image correction / noise removal

We need to remove the noise to help the computer *interpreting* the image

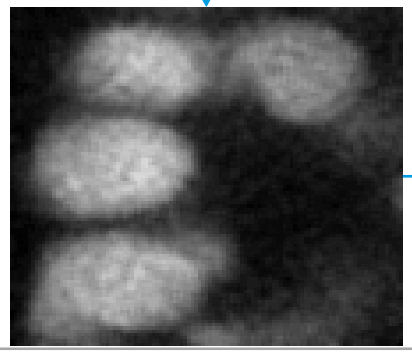


Thresholding

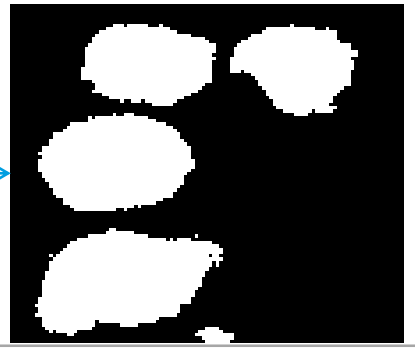


Oh no! I see thousands of tiny white objects!

Blurring



Thresholding



Ok, it's just 4 objects.

Image correction / noise removal

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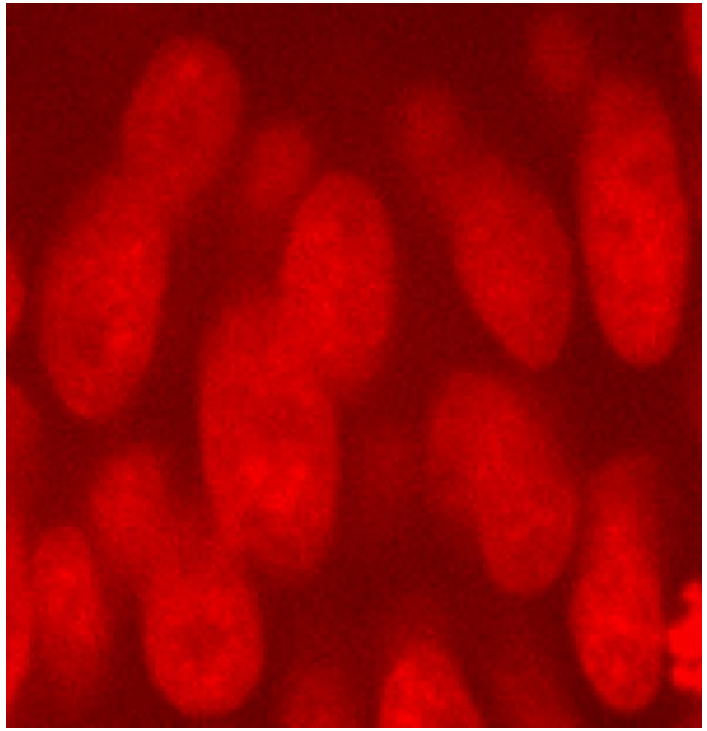
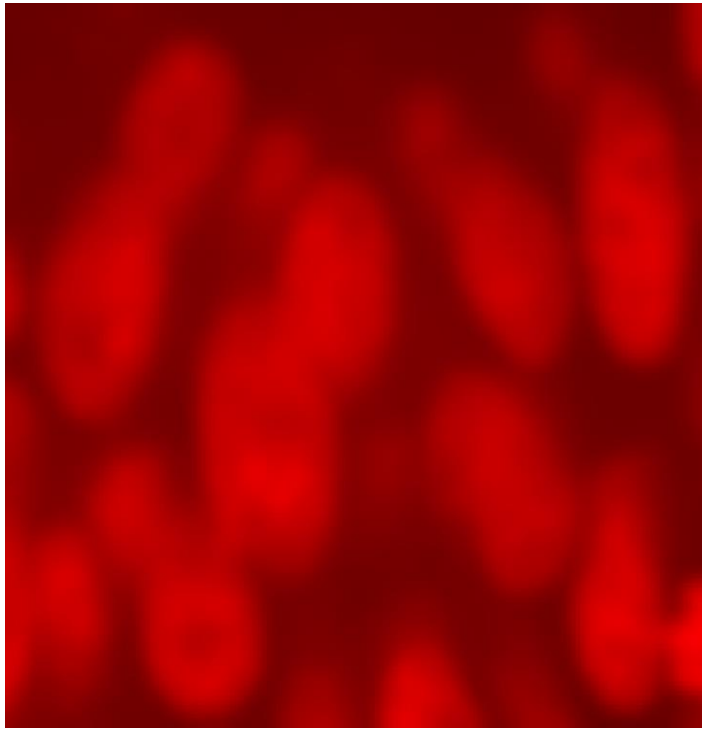
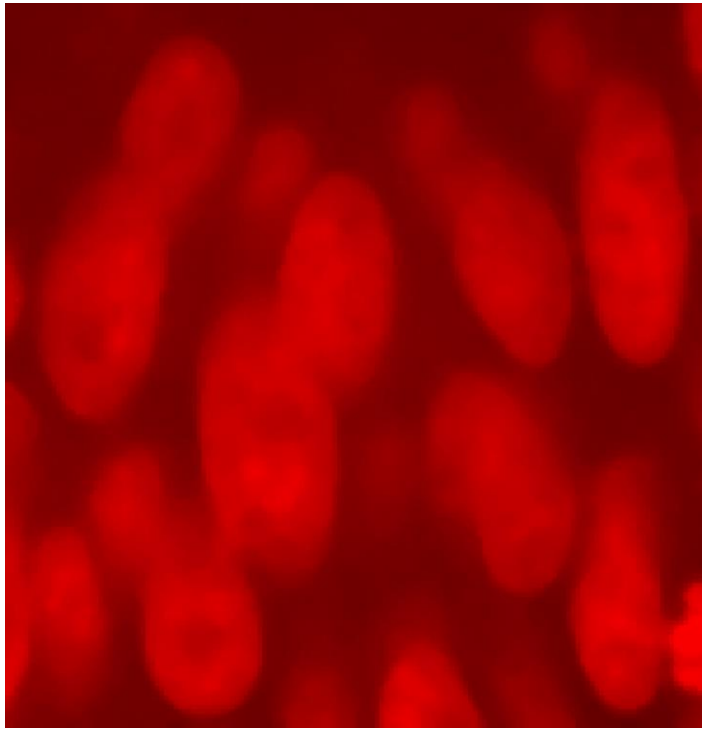
Filters can remove noise



Original image



Gaussian blur filtered



Median filtered

Image source: Mauricio Rocha Martins (Norden/Myers lab, MPI CBG)

Linear Filters

- Linear filters replace each pixel value w pixels

- Kernels are matrices describing a linear filter

Mean filter 3x3 kernel = $\begin{bmatrix} 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \end{bmatrix}$

Kernel

32	48	64	96	120	128	144	152	152
40	72	96	128	160	176	184	184	184
72	104	136	160	176	184	192	192	184
104	136	168	184	192	200	200	192	184
136	160	184	184	192	192	192	184	184
168	184	192	192	184	184	176	176	176
192	192	192	192	184	184	176	176	176
208	200	184	184	184	184	176	176	168

New_value = (1/9)***32** + (1/9)***48** + (1/9)***64** + (1/9)***40** + (1/9)***72** + (1/9)***96** + (1/9)***72** + (1/9)***104** + (1/9)***136**

New_value = 73.8

What operation is this?

Linear Filters

- Linear filters replace each pixel value w pixels

- Kernels are matrices describing a linear filter

Mean filter 3x3 kernel =

$$\begin{bmatrix} 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \\ 1/9 & 1/9 & 1/9 \end{bmatrix}$$

Kernel

32	48	64	96	120	128	144	152	152
40	72	96	128	160	176	184	184	184
72	104	136	160	176	184	192	192	184
104	136	168	184	192	200	200	192	184
136	160	184	184	192	192	192	184	184
168	184	192	192	184	184	176	176	176
192	192	192	192	184	184	176	176	176
208	200	184	184	184	184	176	176	168

New_value = (1/9)***48** + (1/9)***64** + (1/9)***96** + (1/9)***72** + (1/9)***96** + (1/9)***128** + (1/9)***104** + (1/9)***136** + (1/9)***160**

New_value = 100.4

What operation is this?

Linear Filters

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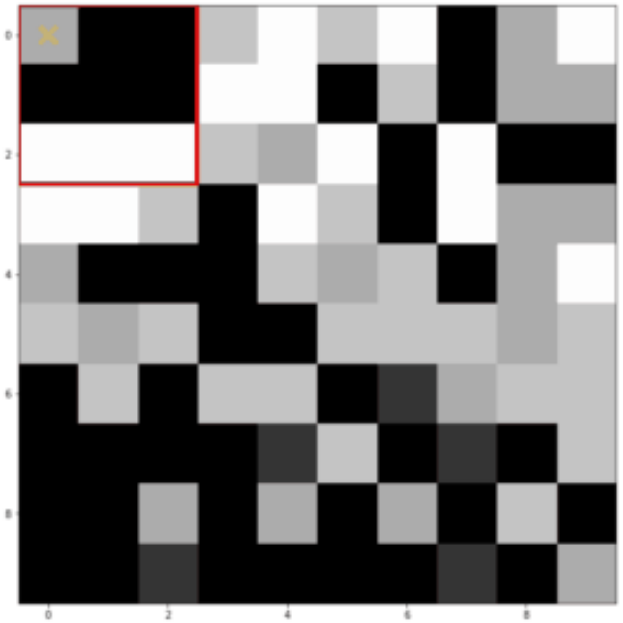
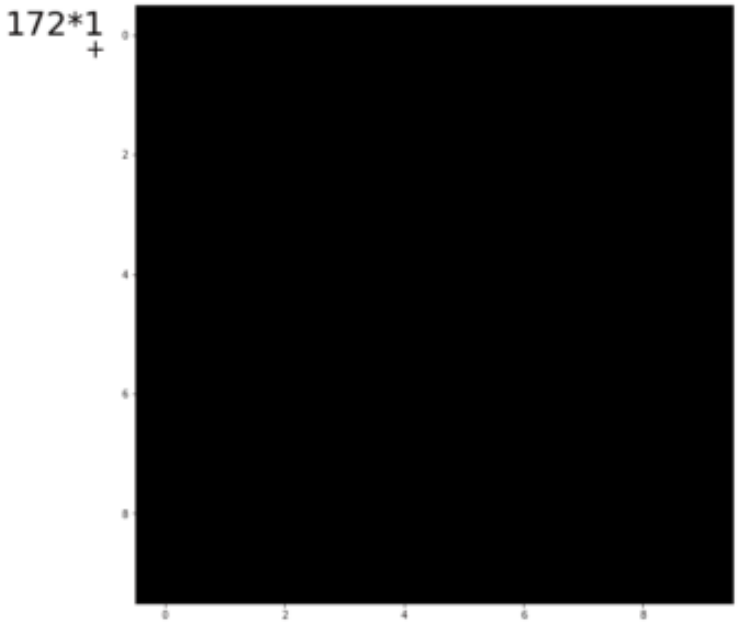
- Basically, linear filtering is a Convolution
- It needs a kernel (weight template)
- Result: new image where each pixel is replaced by the weighted sum of pixel values in the neighborhood



Image



Output_image

Terminology:

- "We convolve an image with a kernel."
- Convolution operator: *

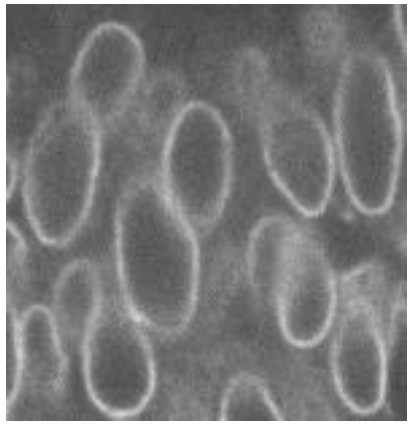
Output_image = Image * Kernel

Animation source: Dominic Waithe, Oxford University
https://github.com/dwaithe/generalMacros/tree/master/convolution_ani

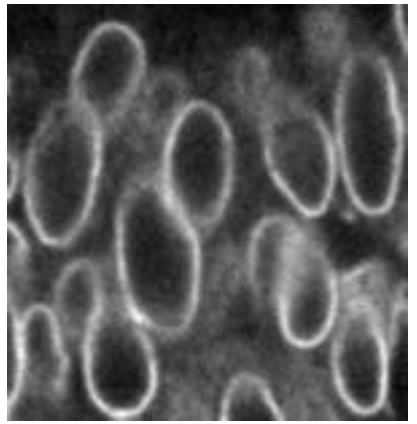
Linear filters

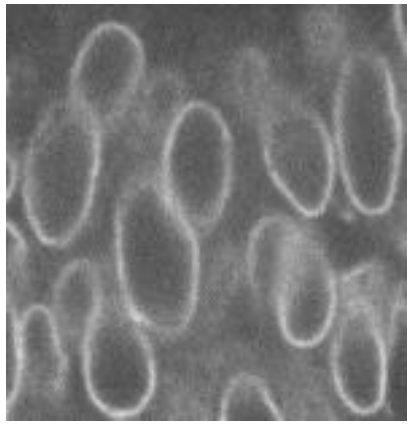
Different linear filters use different kernels

- Other Examples
  - Mean
  - Gaussian blur
  - Sobel-operator
  - Laplace-filter



$$* \begin{bmatrix} 1 & 1 & 1 \\ 1 & 8 & 1 \\ 1 & 1 & 1 \end{bmatrix} =$$





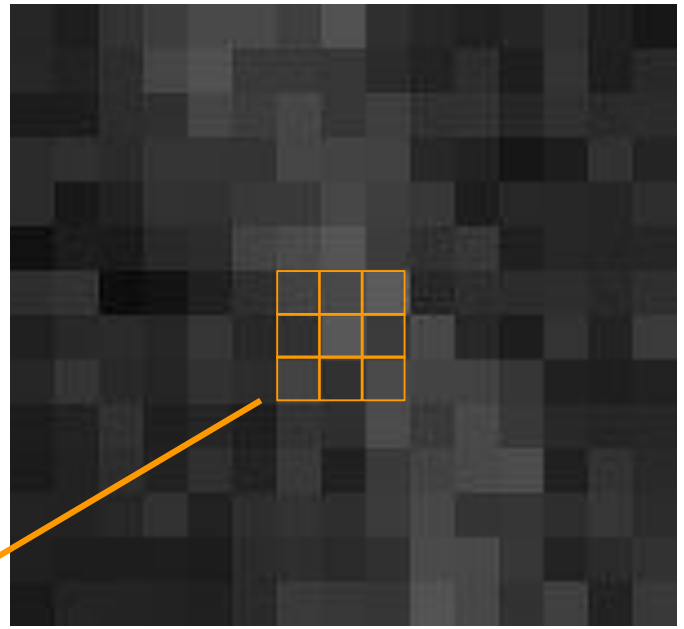
$$* \begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix} =$$

Nonlinear Filters

- Nonlinear filters also replace pixel value inside as rolling window but in a non linear function.

Examples: order statistics filters

- Min
- Median
- Max
- Variance
- Standard deviation



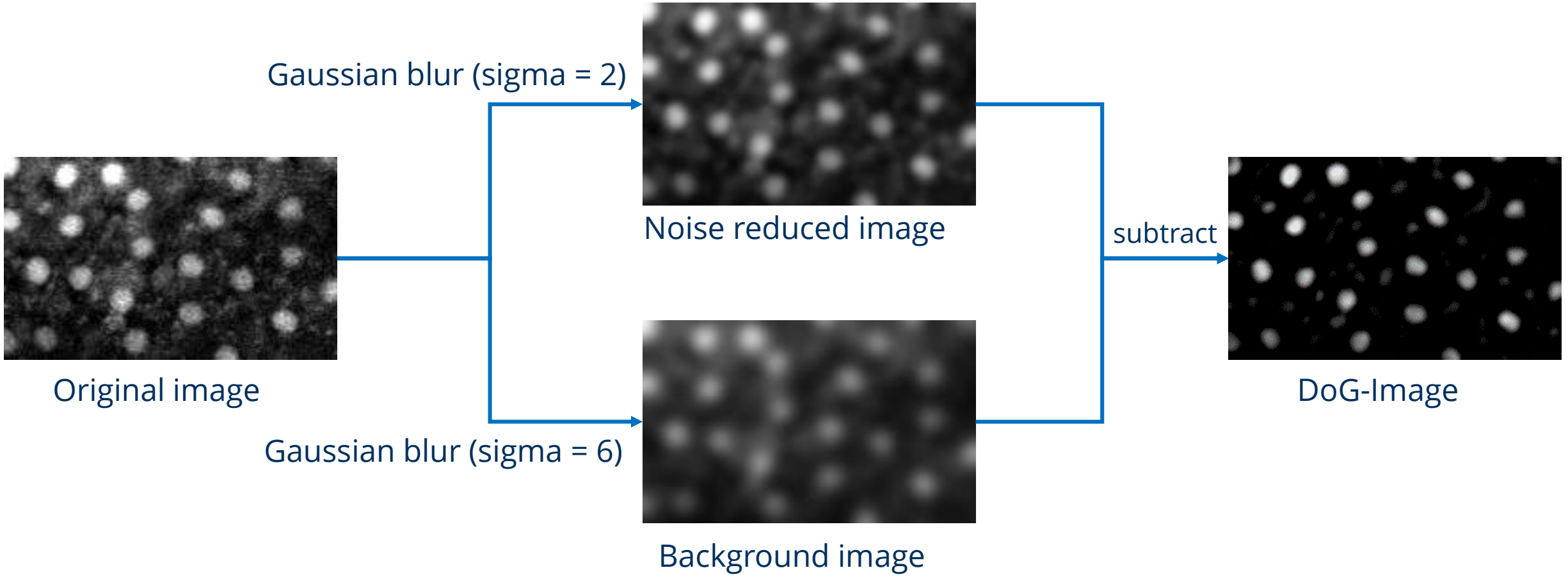
75	85	60
67	73	91
50	88	59

50 59 60 67 73 75 85 88 91

Min Median Max

Difference-of-Gaussian (DoG)

Improve image in order to detect bright objects.



Laplace-filter

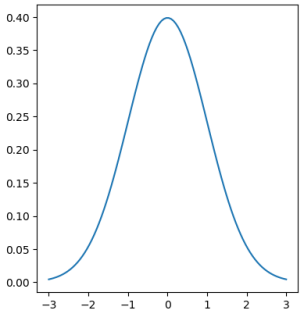
Second derivative of a Gaussian blur filter

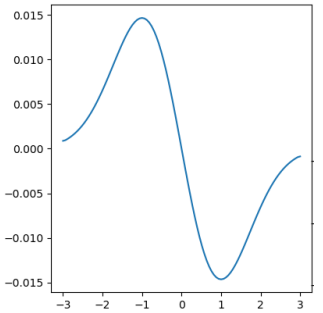
Used for edge-detection and edge enhanceme

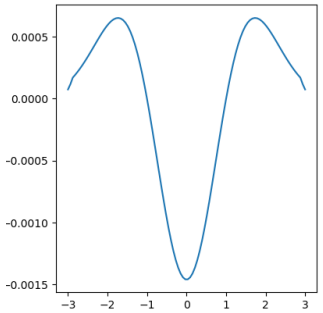
Also known as the Mexican-hat-filter

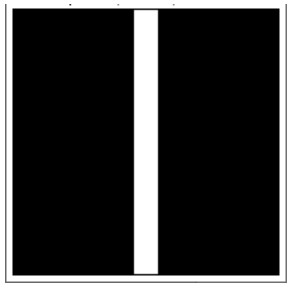
1st derivative

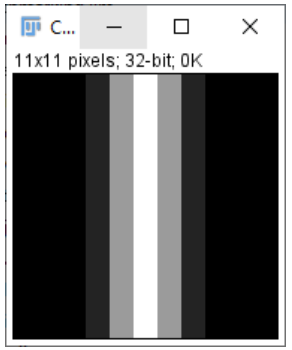
2nd derivative

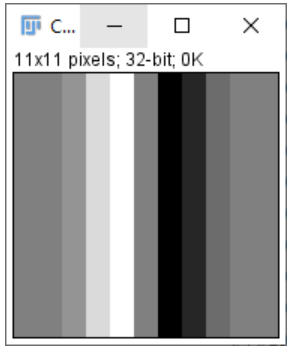


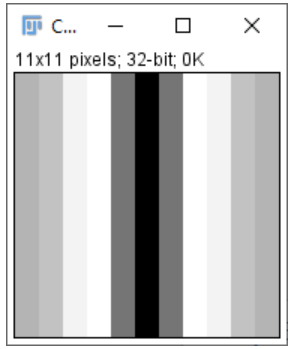


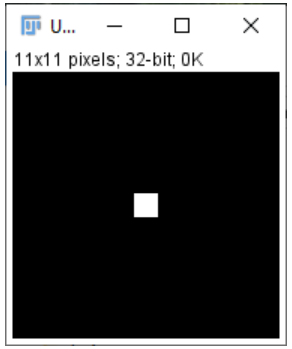


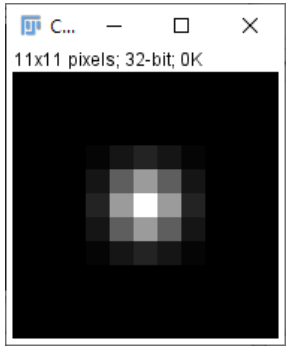


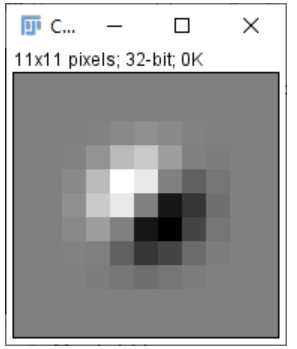


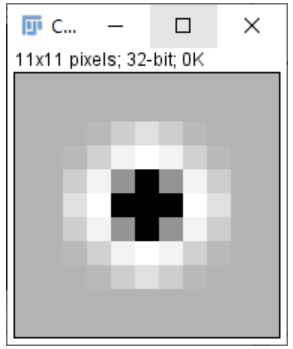












Laplacian-of-Gaussian (LoG)

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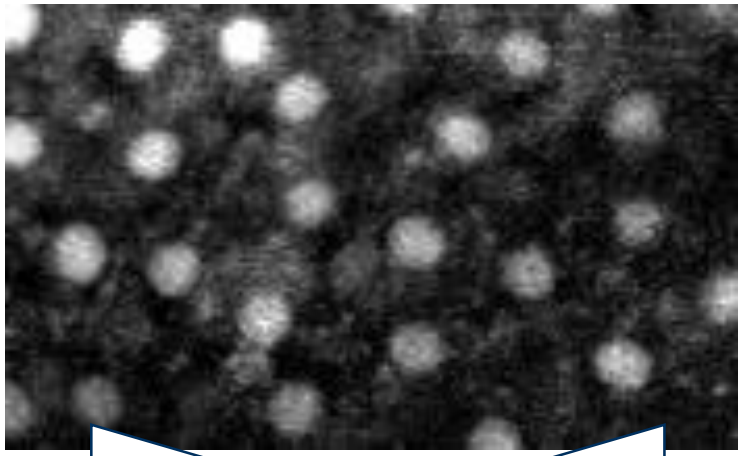
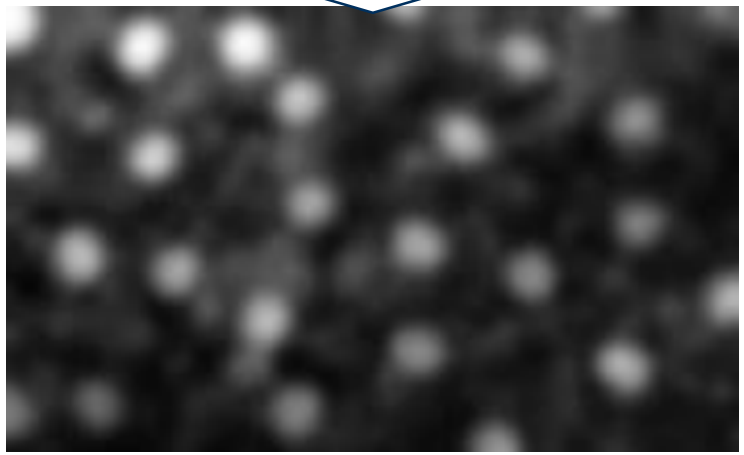
Laplace filter

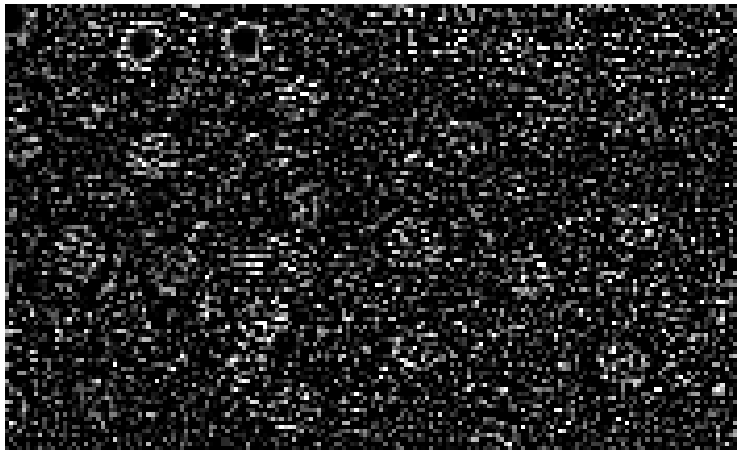


Gaussian blur

Laplacian of Gaussian filter

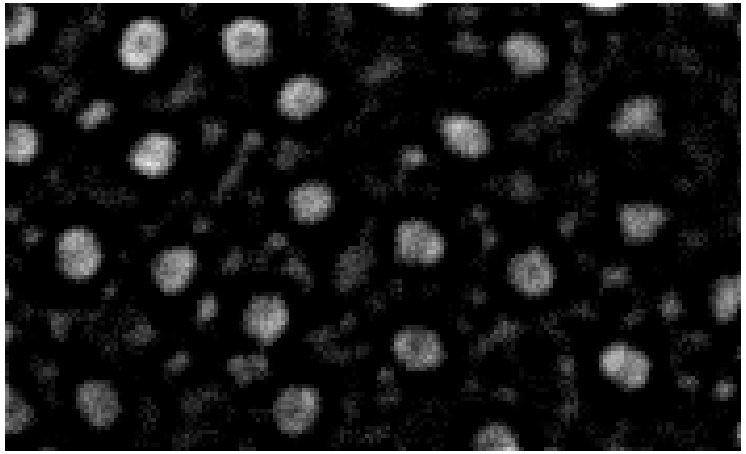


$$* \begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix} =$$



Laplace filtered image

$$* \begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix} =$$



LoG image

Filters

In python, for linear filters, we could apply a kernel to an image like this:

```
kernel = np.array(
    [[1/9, 1/9, 1/9],
     [1/9, 1/9, 1/9],
     [1/9, 1/9, 1/9]])
```

```
from scipy.ndimage import convolve

output = convolve(image, kernel)
```

But scikit-image already has several of these filters implemented and optimized.

```
from skimage.filters import gaussian

output = gaussian(image, sigma = 1)
```

Then there is no need to provide a kernel. And you can also directly apply non-linear filters.

```
from skimage.filters import median

output = median(image)
```

Available filters can be searched like this:

```
from skimage import filters

filters.
```

```
LPIFilter2D
median
meijering
prewitt
prewitt_h
prewitt_v
rank
rank_order
ridges
roberts
```

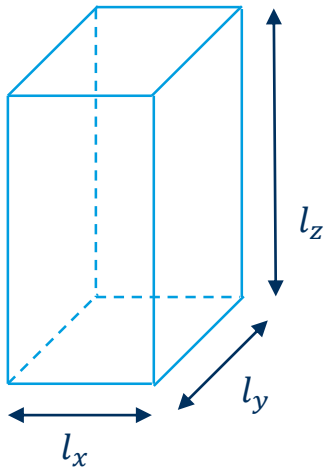
Volumetric Data

Voxel: “Volume element”, usually anisotropic

An-iso-tropy, from Greek:
- ἀν- (not)
- ἴσος *isos (equal)*
- τρόπος *tropos (rotation, direction)*

Not the same in all directions

Usually in 3D image processing:



$$l_x = l_y \neq l_z$$

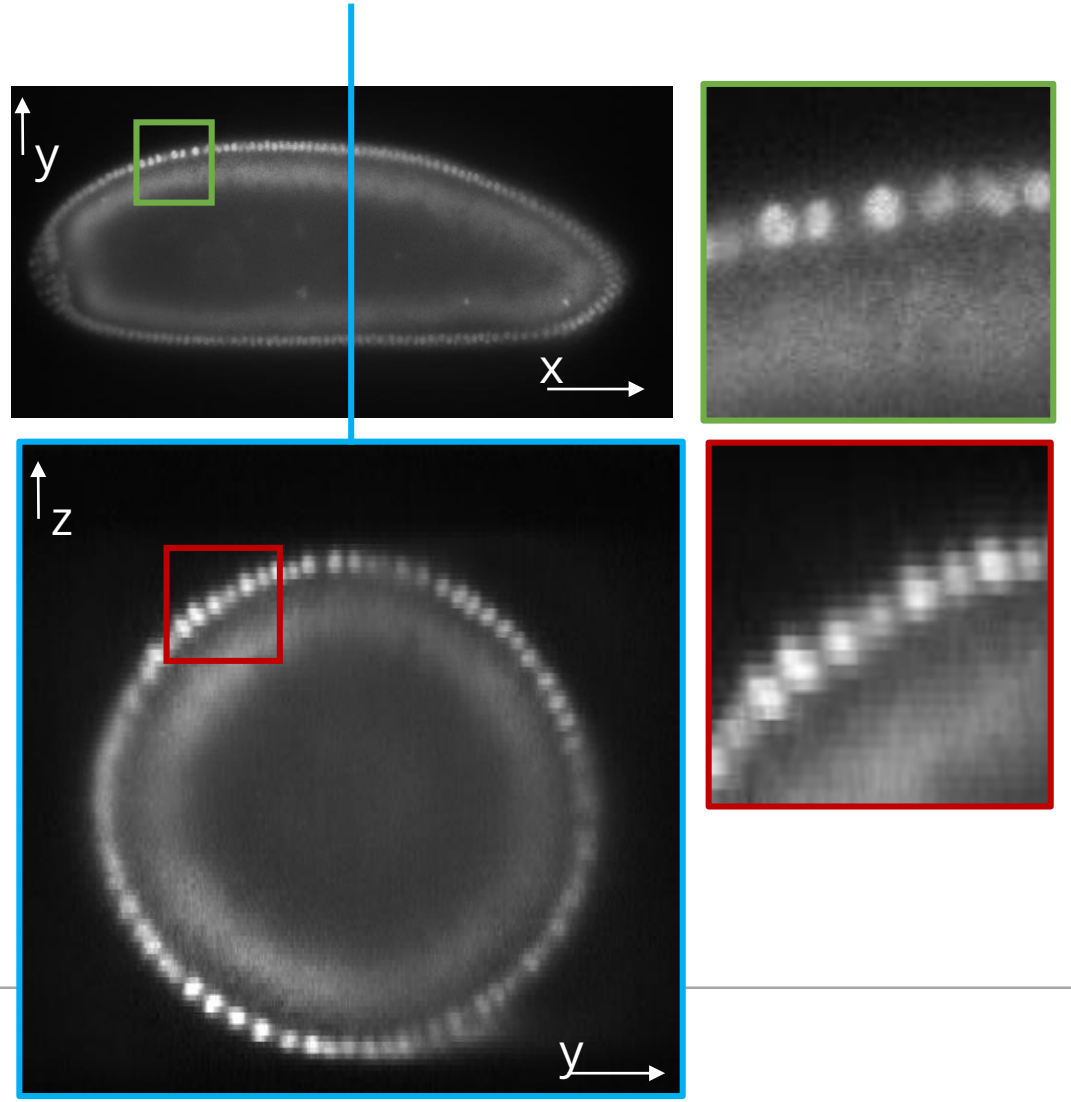
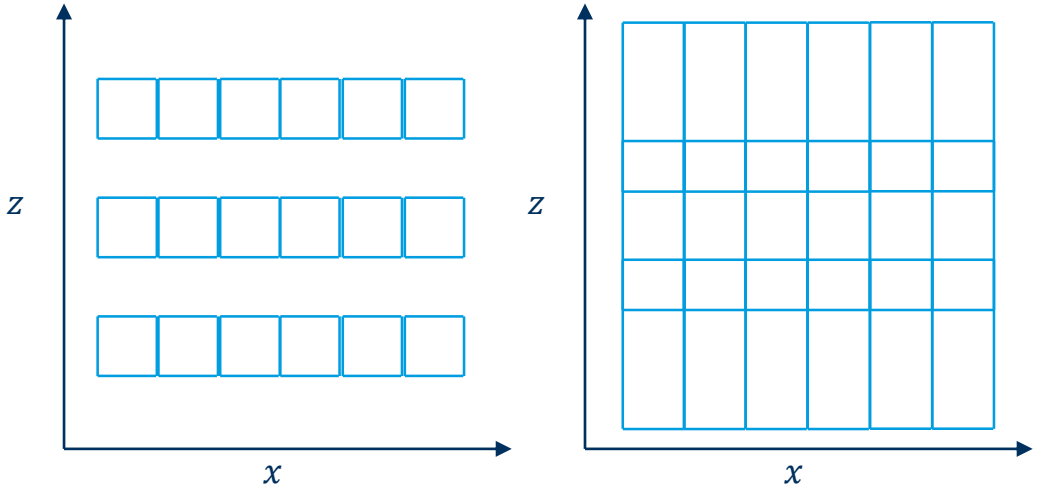


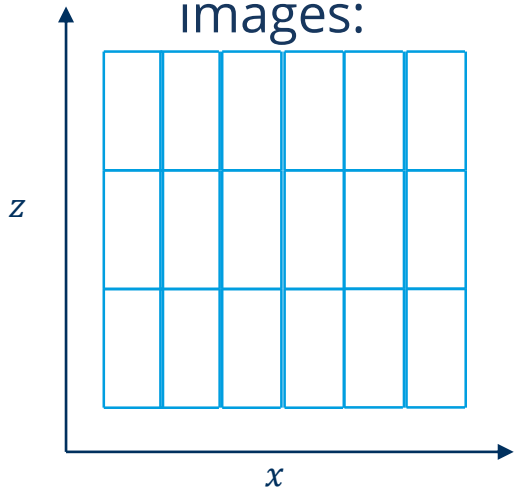
Image analysts *love* to have isotropic voxels, but it’s often not possible.

Slice distance versus slice thickness

What you may have measured using imaging:



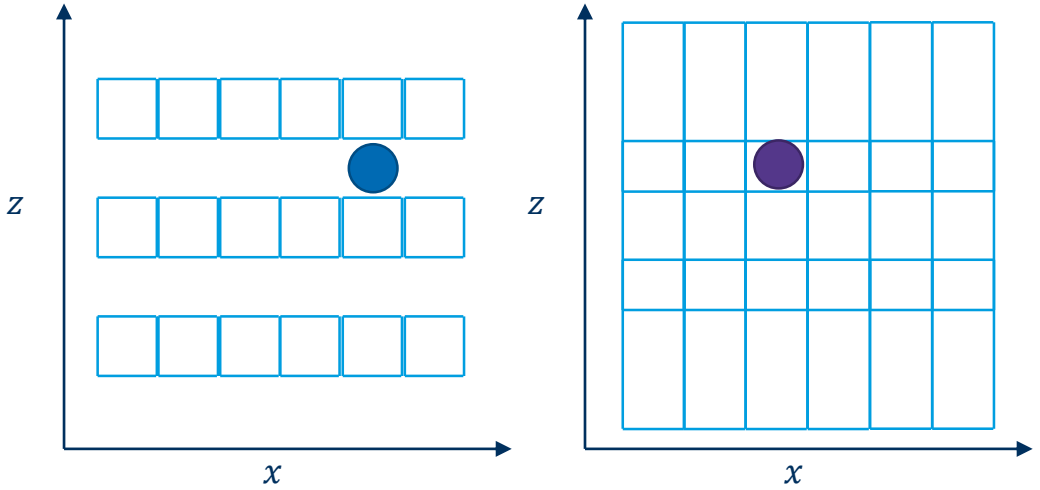
What you see when processing 3D images:



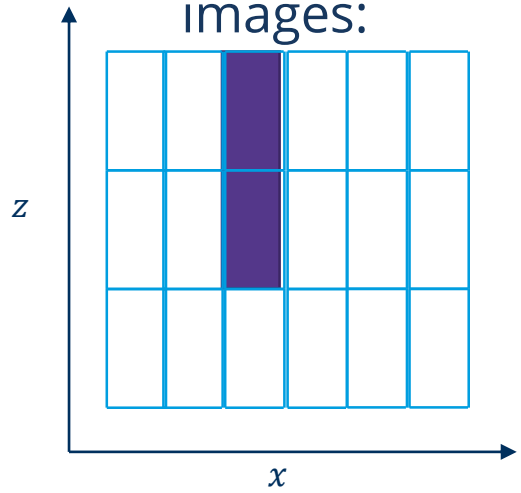
- Slice distance and slice thickness may be different, but
- many image processing tools ignore that.

Slice distance versus slice thickness

What you may have measured using imaging:



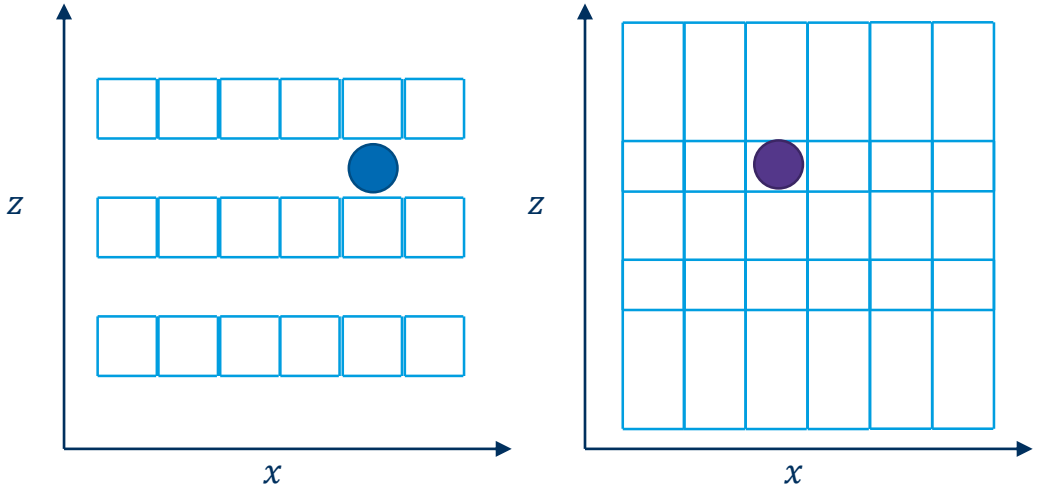
What you see when processing 3D images:



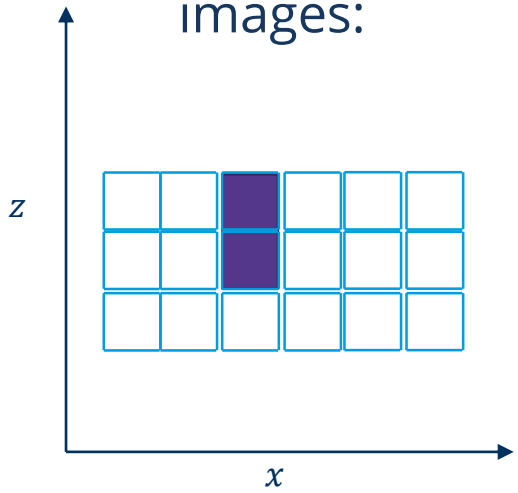
- Slice distance and slice thickness may be different, but
- many image processing tools ignore that!

Slice distance versus slice thickness

What you may have measured using imaging:



What you see when processing 3D images:

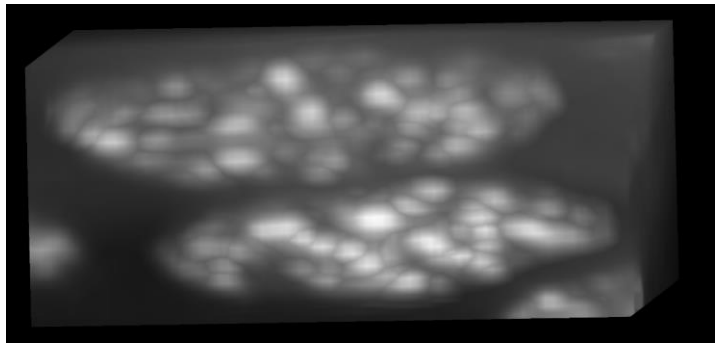


- Slice distance and slice thickness may be different, but
- many image processing tools ignore that!

Isotropic Images

The visualization can be fixed in napari by providing a ‘scale’ argument.

```
viewer.add_image(image, name = 'image')
```



```
image.shape
```

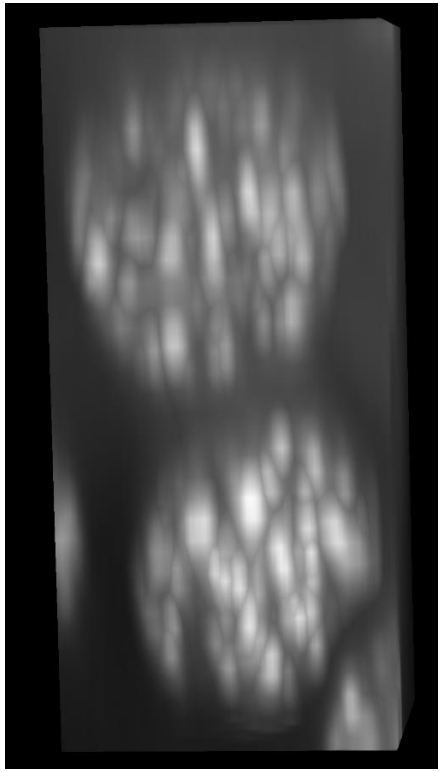
```
(32, 61, 74)
```

```
reference_size = voxel_size_z

factor_z = voxel_size_z / reference_size
factor_y = voxel_size_y / reference_size
factor_x = voxel_size_x / reference_size
```

Voxel_size_z = 1 µm
Voxel_size_y = 0.2 µm
Voxel_size_x = 0.2 µm

```
viewer.layers['image'].scale = [factor_z, factor_y, factor_x] # Z, Y, X order
```



```
image.shape
```

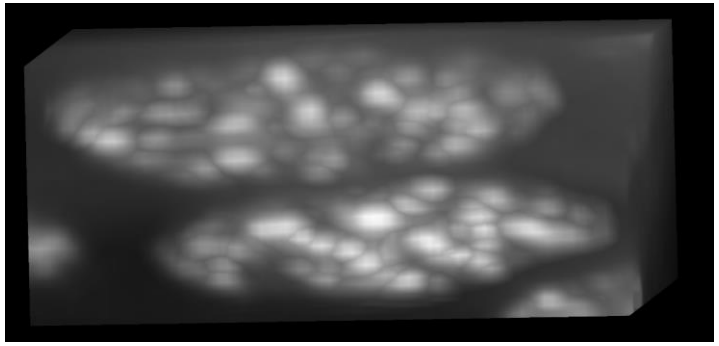
```
(32, 61, 74)
```

But that does not change voxel size, just adjusts visualization.

Filters in 3D Images

The functions are typically the same, but many of them may not account by default different voxel sizes.

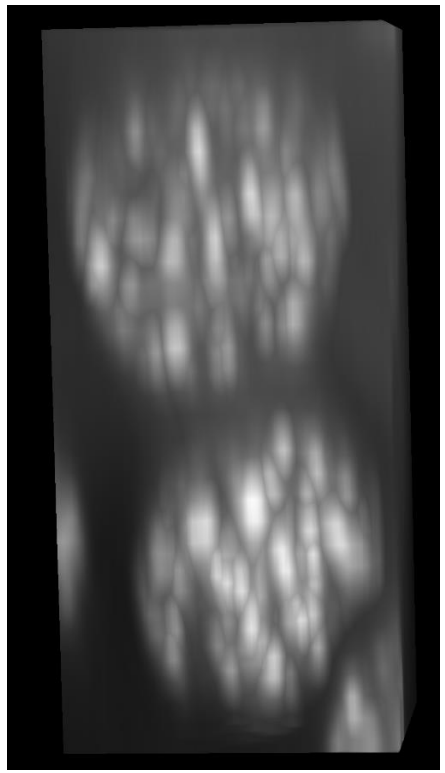
If the filter does not take voxel size into account, we would need to rescale the image before applying a filter.



```
image.shape
```

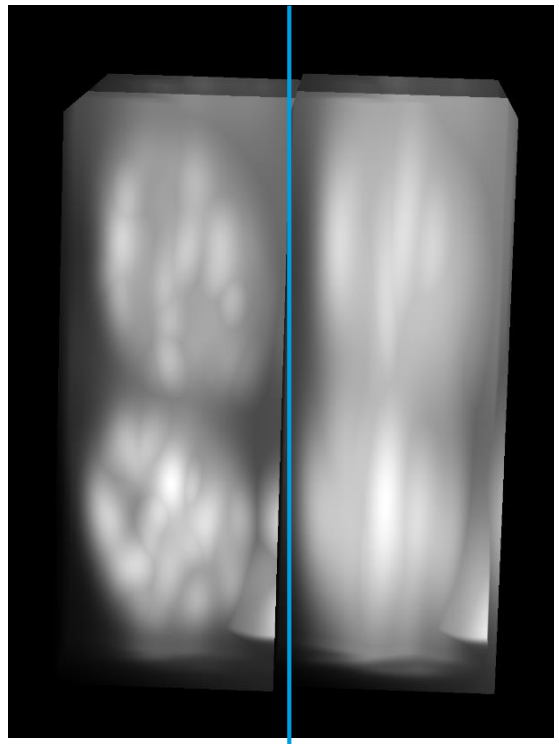
```
(32, 61, 74)
```

rescale



```
image.shape
```

```
(158, 61, 74)
```



Gaussian blur on rescaled image

Gaussian blur on image