



Nanoscopy Oxford



UNIVERSITY OF
OXFORD

Image Processing

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- Introduction to Image Processing
- Recap image formation
- Linear and non-linear filters
- Edge filters
- Filter banks
- Applied examples
- Deconvolution
- Conclusion



The MRC Weatherall Institute of Molecular Medicine is a strategic alliance between the Medical Research Council and the University of Oxford



What is image processing

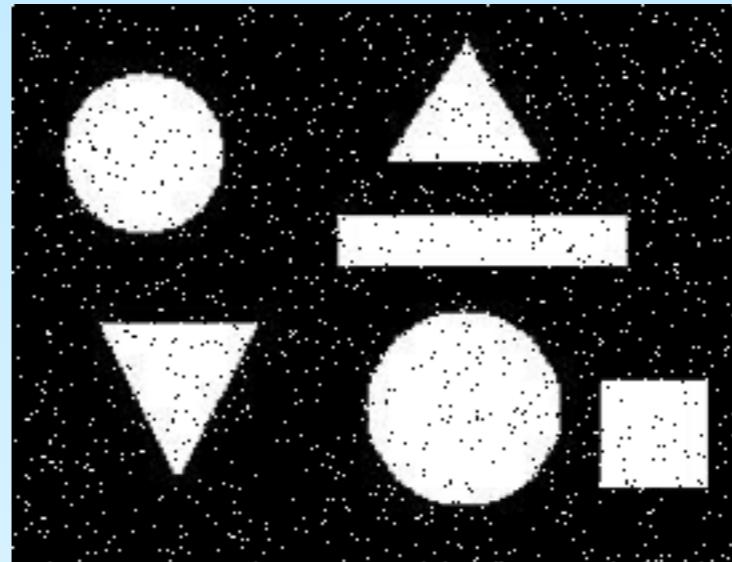
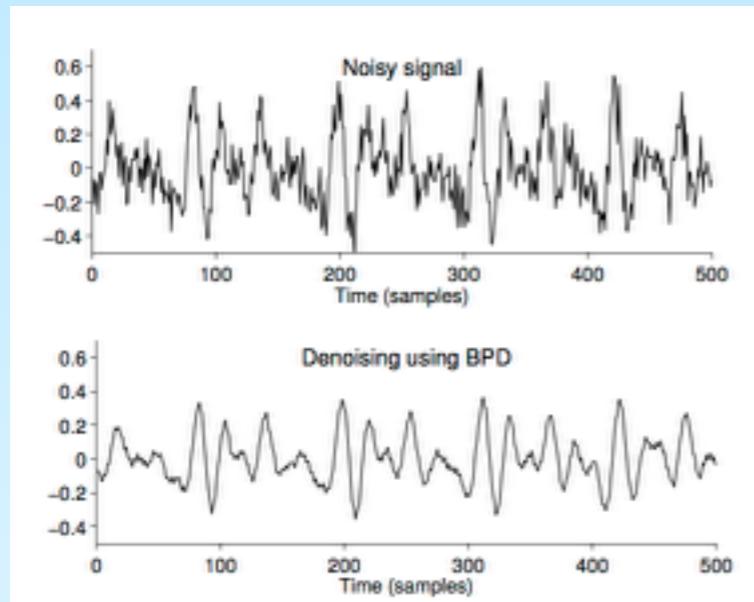


Image Processing usually involves a 2-D or 3-D images but has its roots in Signal Processing.

Signal processing is the science of transferring information contained in many different physical, symbolic, or abstract formats.

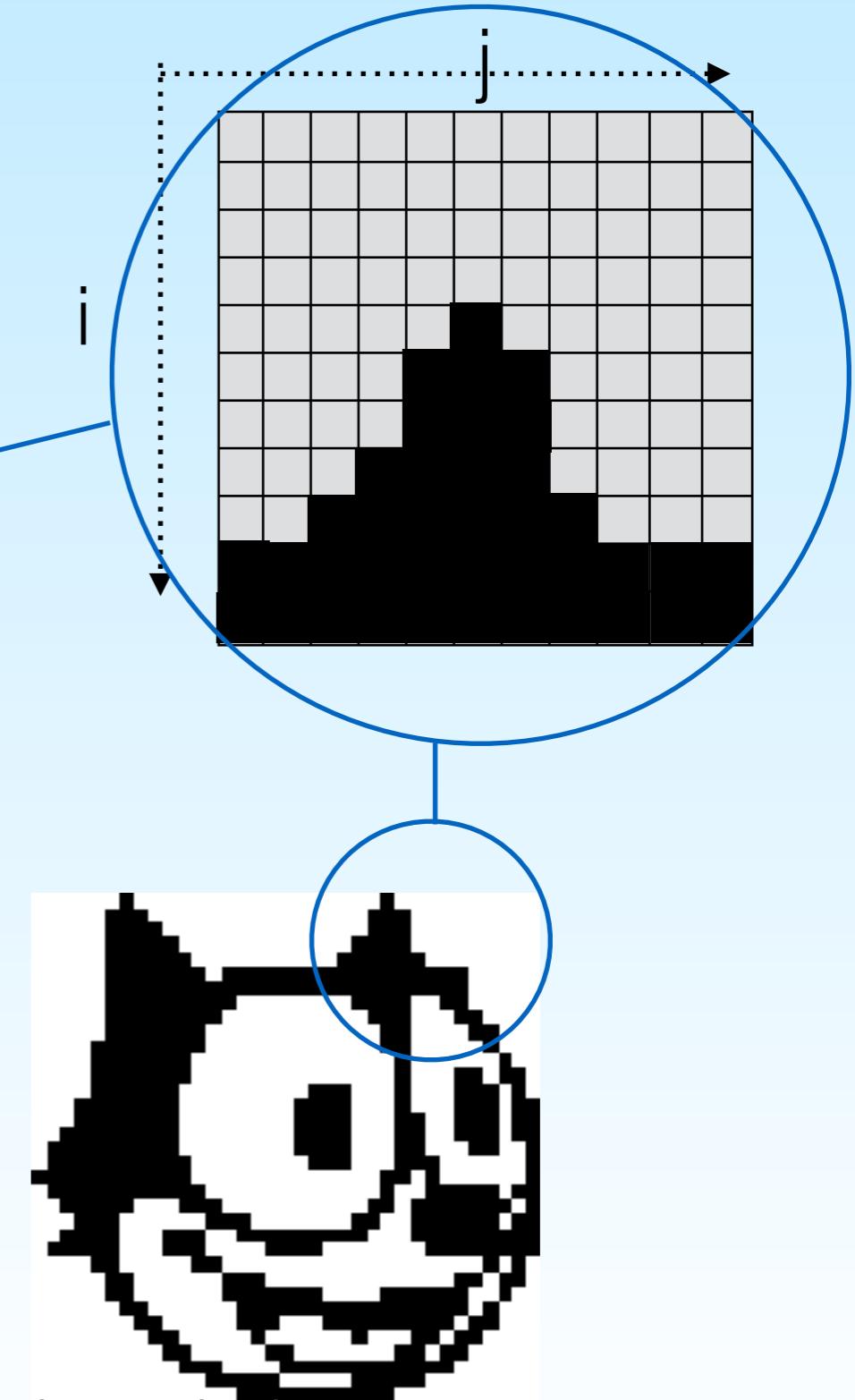
Usually we are trying to extract some type of meaning from our data, we process it to declutter our signal or focus on a particular aspect.

Source: http://eeweb.poly.edu/iselesni/lecture_notes/sparsity_intro/bpd.png
<http://photos1.blogger.com/blogger/82/1614/1600/fig1.2.jpg>

Image representation recap: Binary Image

Computer images are 2D arrays of numbers:

```
0,0,0,0,0,0,0,0,0,0,0  
0,0,0,0,0,0,0,0,0,0,0  
0,0,0,0,0,1,0,0,0,0,0  
0,0,0,0,1,1,1,0,0,0,0  
0,0,0,0,1,1,1,0,0,0,0  
0,0,0,0,1,1,1,0,0,0,0  
0,0,0,0,1,1,1,0,0,0,0  
0,0,0,1,1,1,1,0,0,0,0  
0,0,1,1,1,1,1,1,0,0,0  
1,1,1,1,1,1,1,1,1,1,1  
1,1,1,1,1,1,1,1,1,1,1
```



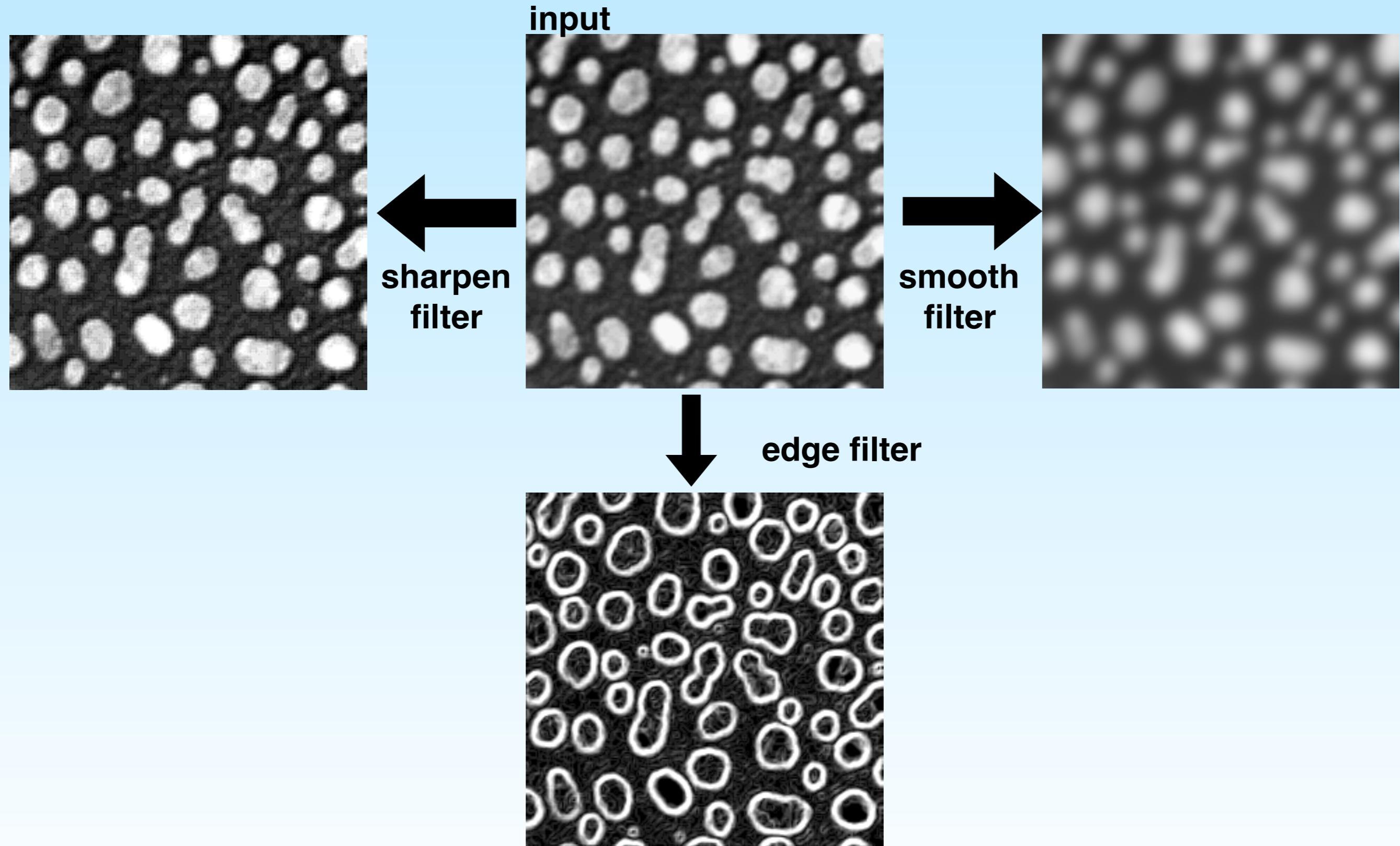
Source: http://www.uff.br/cdme/matrix/matrix-html/matrix_boolean/matrix_boolean_en.html

Image representation recap: Grayscale Image

Computer images are 2D arrays of numbers:

8	7	7	5	5	6	6	6	5	3	3	4	3	1	5	6	4	6	6	7	5	7	4	5	4	9	7	7	7	6	6	8	4	4	3	3	2	2		
9	9	9	10	7	10	8	7	7	6	6	2	5	4	4	3	4	3	5	6	2	6	5	4	9	5	6	12	6	7	7	5	3	4	4	4	5	2	2	
7	9	7	6	14	11	8	9	6	5	7	3	4	5	4	6	4	4	2	3	4	8	6	6	8	11	7	6	6	5	5	3	7	6	4	4	3	3		
12	10	10	7	11	9	8	7	5	6	5	3	3	5	4	7	6	5	4	4	4	9	8	8	6	7	7	8	8	9	6	6	5	5	8	5	7	5	4	
11	5	8	10	8	11	9	8	5	3	4	6	6	4	5	5	7	4	6	5	7	6	9	6	6	5	9	8	6	9	5	6	7	4	6	4	3	3		
8	10	11	10	5	8	6	10	7	6	5	9	7	7	5	5	6	6	8	6	6	9	8	7	7	9	7	9	7	8	4	4	5	5	2	3	7			
10	10	9	8	7	6	5	5	7	4	7	5	6	7	12	6	6	11	8	10	8	12	7	7	11	7	9	5	6	8	6	10	3	6	7	4	7	4		
8	10	10	9	12	8	8	5	5	7	4	7	7	6	10	8	9	10	10	7	9	13	13	11	8	10	12	6	12	6	8	4	7	6	5	4	3	6		
10	11	13	10	9	8	6	5	4	5	5	6	5	7	8	10	11	10	16	16	15	17	12	18	8	11	9	11	9	7	7	9	6	6	7	10	2	6	5	6
12	12	8	7	9	6	9	6	6	11	7	7	7	9	10	20	17	19	24	27	20	19	19	13	14	11	9	8	10	11	5	7	5	7	4	8	3			
16	12	9	8	10	9	8	9	6	4	3	7	9	8	17	20	19	27	34	35	31	36	27	15	19	14	9	7	6	8	6	5	7	5	7	4	8	3		
11	14	9	10	15	7	7	5	8	7	7	7	15	11	21	24	33	39	32	41	32	33	32	23	15	16	11	10	6	18	6	6	5	3	4	7	6	6		
16	15	13	13	11	11	6	8	6	5	6	7	13	14	17	26	31	44	51	50	45	48	36	39	31	22	10	16	11	11	8	8	6	5	6	4	4	1		
18	14	18	18	16	9	8	7	6	8	7	12	12	9	22	28	39	57	59	59	47	49	56	45	34	28	22	28	16	7	6	7	6	6	7	5				
19	16	17	12	14	8	8	10	12	7	9	9	14	16	28	37	48	68	58	66	57	68	56	55	48	34	28	15	18	12	6	8	8	7	8	4	7	6		
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17	21	15	18	13	12	10	12	8	11	10	9	12	28	22	46	52	67	85	85	88	84	76	68	41	37	25	23	20	14	9	12	9	7	8	5	8	6		
25	17	15	16	18	11	13	9	8	8	6	10	13	25	26	37	61	75	83	95	75	78	78	55	44	36	28	19	19	14	11	10	8	8	5	7	6	5	3	
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16	17	10	10	11	12	9	11	13	8	13	13	17	18	24	34	48	71	70	82	63	55	48	30	34	28	17	14	9	7	7	9	9	9	8	9	12	10		
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11	18	10	7	9	11	7	9	10	8	16	12	11	15	20	26	31	40	46	37	36	18	16	17	15	11	18	7	13	9	11	7	8	12	13	13	16	8		
13	18	15	12	13	10	11	10	8	9	8	7	11	8	13	22	22	30	31	36	31	19	25	25	13	12	9	11	14	9	12	8	9	18	11	10	14	17	9	
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13	8	10	12	11	9	13	9	12	12	13	9	7	11	14	13	15	18	18	16	18	14	16	18	11	9	11	18	12	9	10	8	9	11	11	14	12			
13	8	12	10	12	11	13	13	12	15	15	12	8	11	9	15	14	18	8	12	14	13	8	11	9	12	11	18	13	11	11	12	13	16	12	11	12	18	15	
12	18	12	8	12	10	12	17	13	11	9	10	9	18	11	12	13	11	11	11	10	9	12	17	17	18	13	13	19	16	14	11	14	11	9	18	18			
12	17	8	11	10	14	14	14	9	15	14	9	12	15	11	15	18	9	12	11	7	9	8	14	10	13	18	22	26	18	18	19	16	15	13	13	17	22		
11	15	13	11	10	10	12	11	15	13	15	12	9	14	16	13	13	9	9	11	12	0	10	13	17	12	15	20	18	19	10	13	16	10	14	12	14	15	19	
15	11	10	10	12	15	16	19	14	12	20	12	19	21	10	12	12	12	0	10	10	8	11	15	18	20	10	20	22	15	19	15	14	14	17	21	16			
12	11	11	9	10	12	16	19	22	17	16	15	24	20	19	21	15	16	9	9	10	15	13	14	19	1														

Image processing: Image Filtering



Linear and non-linear filtering.

Linear Filters

- mean filter
- Gaussian filter
- Sobel filter
- Prewitt filter
- Haar-like filter
- Gabor filter
- Difference of Gaussians

non-linear filters

- median filter
- percentile filter
- maximum filter
- minimum filter

(addition) $\tilde{\mathbf{L}}(\mathbf{f} + \mathbf{g}) = \tilde{\mathbf{L}}\mathbf{f} + \tilde{\mathbf{L}}\mathbf{g}$

(scalar multiplication) $\tilde{\mathbf{L}}(t\mathbf{f}) = t\tilde{\mathbf{L}}\mathbf{f}.$

Definition of a linear operator:
pair of functions **f** and **g**
(for example two images)
and scalar **s** and operator **L**

Convolution - Is a linear filter.

continuous convolution operator

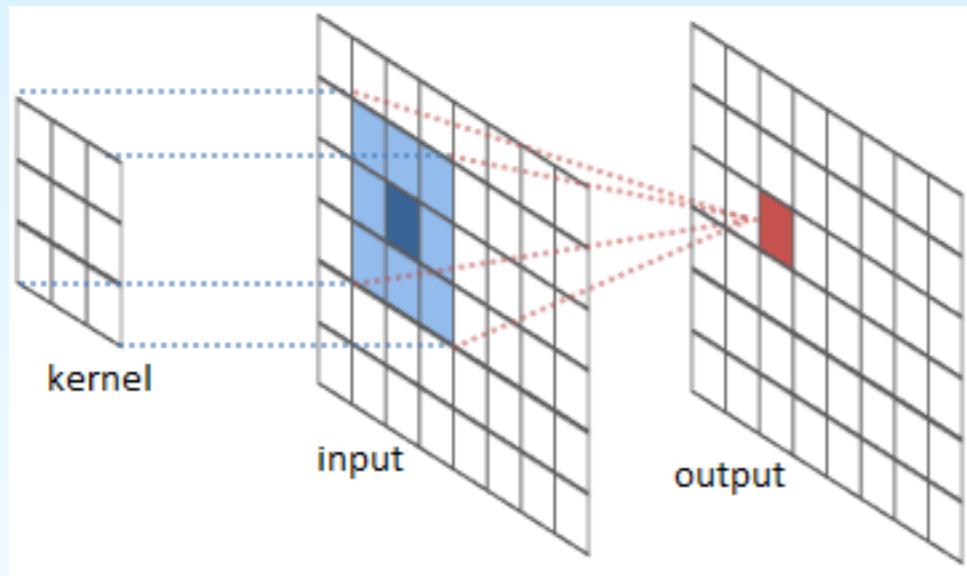
$$(f * g)(t) \stackrel{\text{def}}{=} \int_{-\infty}^{\infty} f(\tau) g(t - \tau) d\tau$$

discrete convolution operator

$$w(x, y) \star f(x, y) = \sum_{s=-a}^a \sum_{t=-b}^b w(s, t) f(x + s, y + t)$$

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

kernel: 3x3 mean filter



$$\begin{aligned} & (a_{-1-1} * b_{-1-1}) + \\ & (a_{-1 0} * b_{-1 0}) + \\ & (a_{-1 1} * b_{-1 1}) + \quad = \text{output} \\ & (a_{0 -1} * b_{0 -1}) + \\ & (a_{0 0} * b_{0 0}) + \\ & \text{etc, etc} \end{aligned}$$

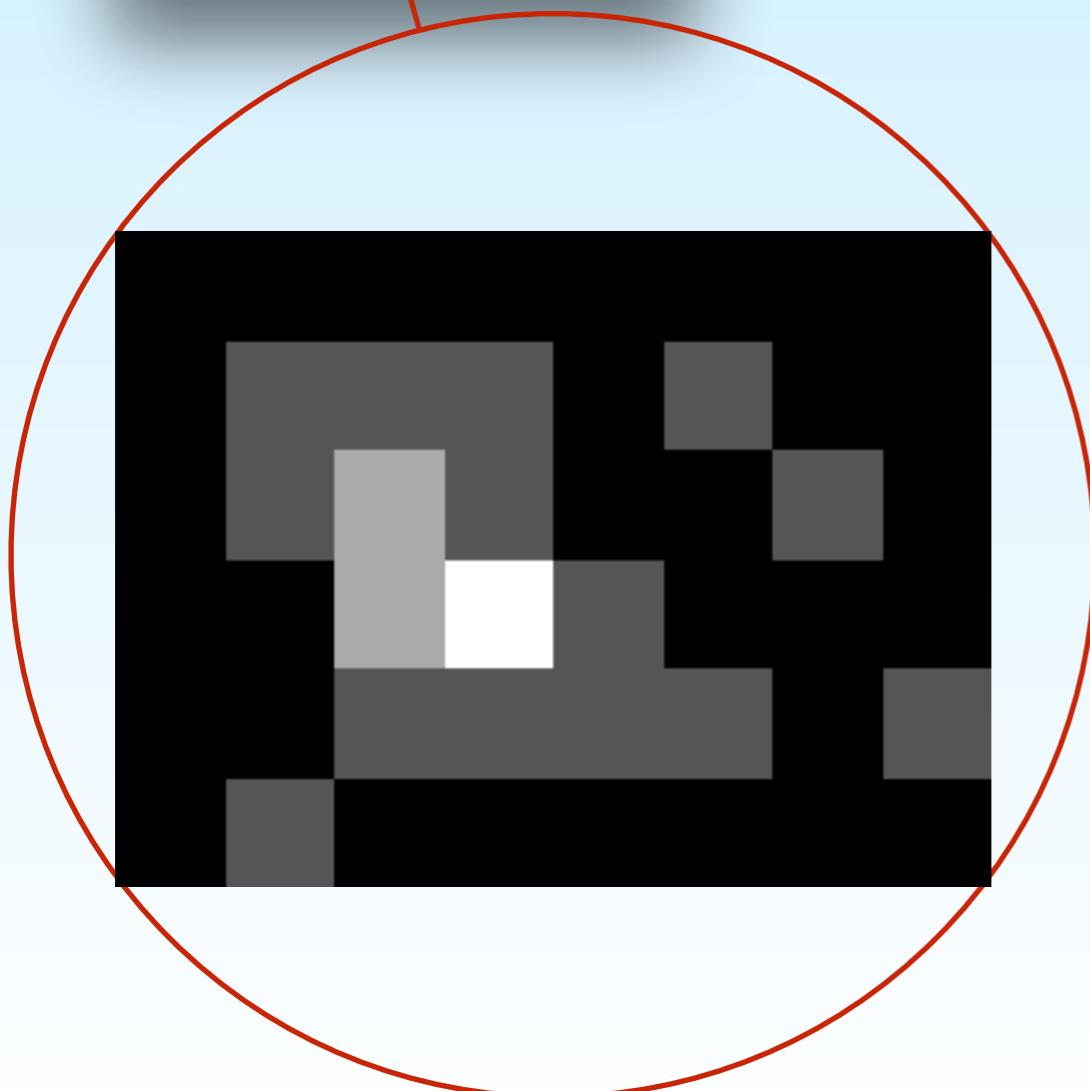
Convolution is the weighted sum of the pixel neighbourhood.
The kernel defines the weights.

Linear filter: Mean filter



$$\begin{array}{ccc} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \end{array}$$

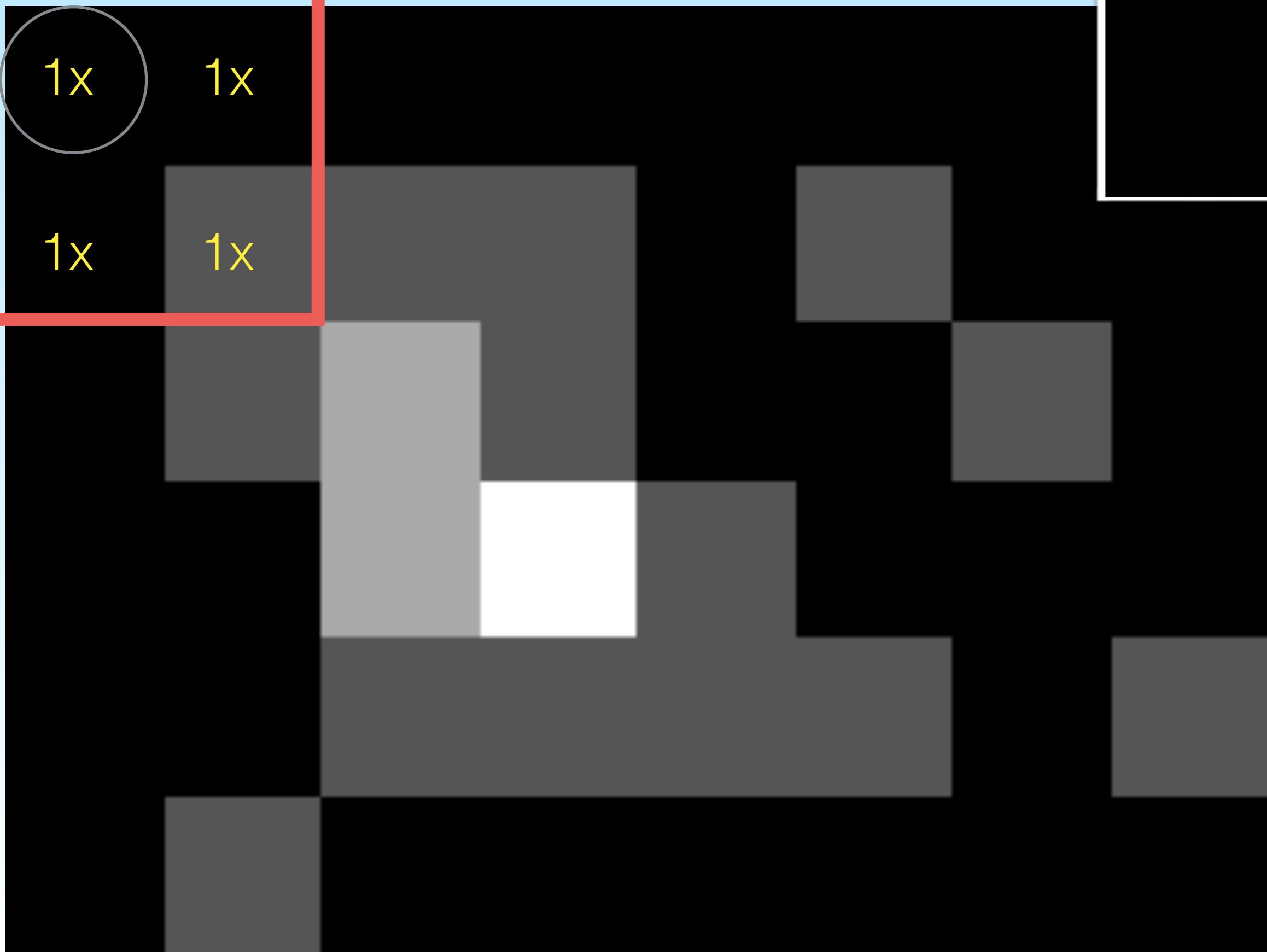
kernel: 3x3 mean filter



```
[[1, 1, 1, 1, 1, 1, 1, 1, 1],  
 [1, 2, 2, 2, 1, 2, 1, 1],  
 [1, 2, 3, 2, 1, 1, 2, 1],  
 [1, 1, 3, 4, 2, 1, 1, 1],  
 [1, 1, 2, 2, 2, 2, 1, 2],  
 [1, 2, 1, 1, 1, 1, 1, 1]]
```

Image region

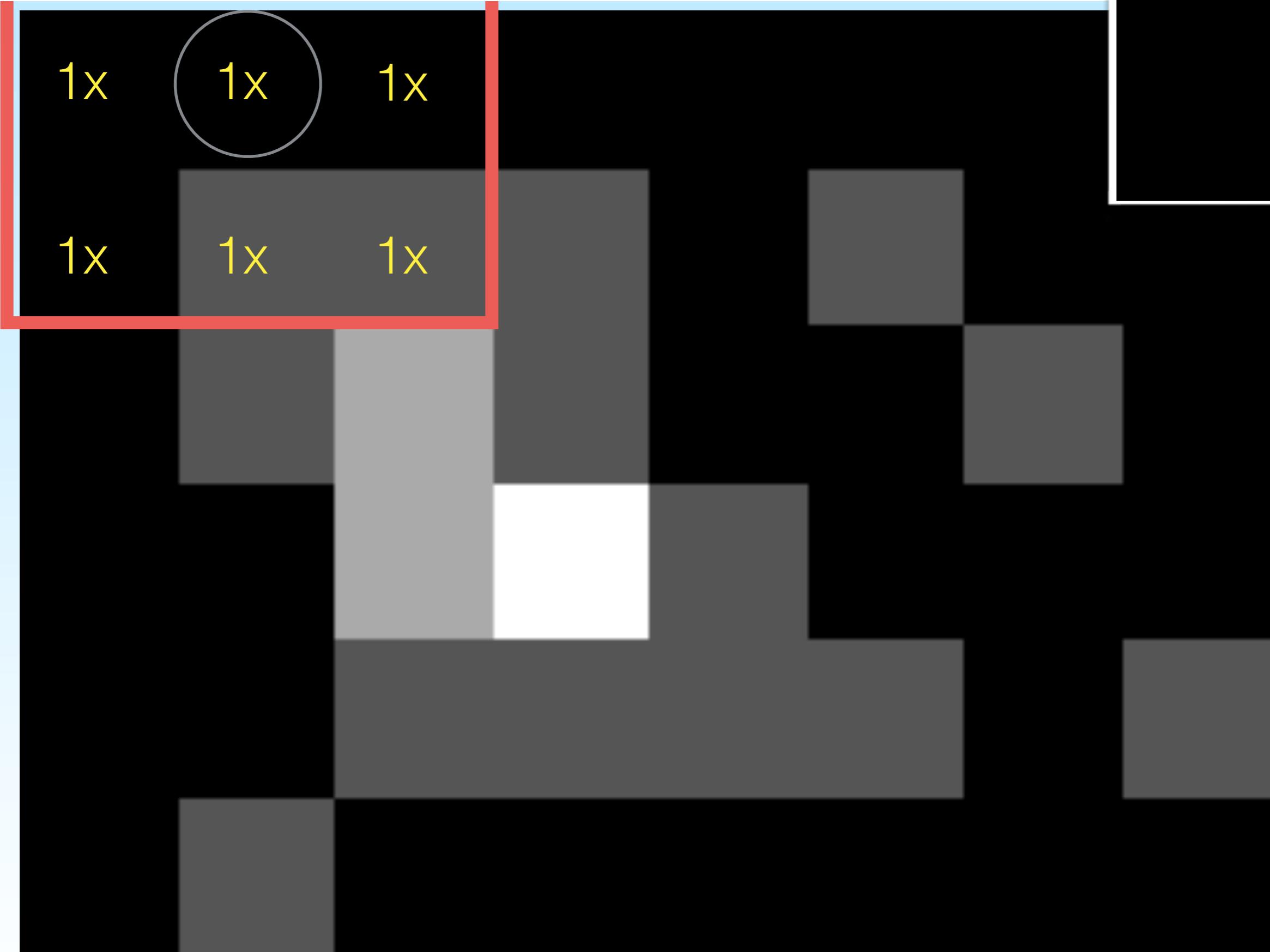
Mean Filter



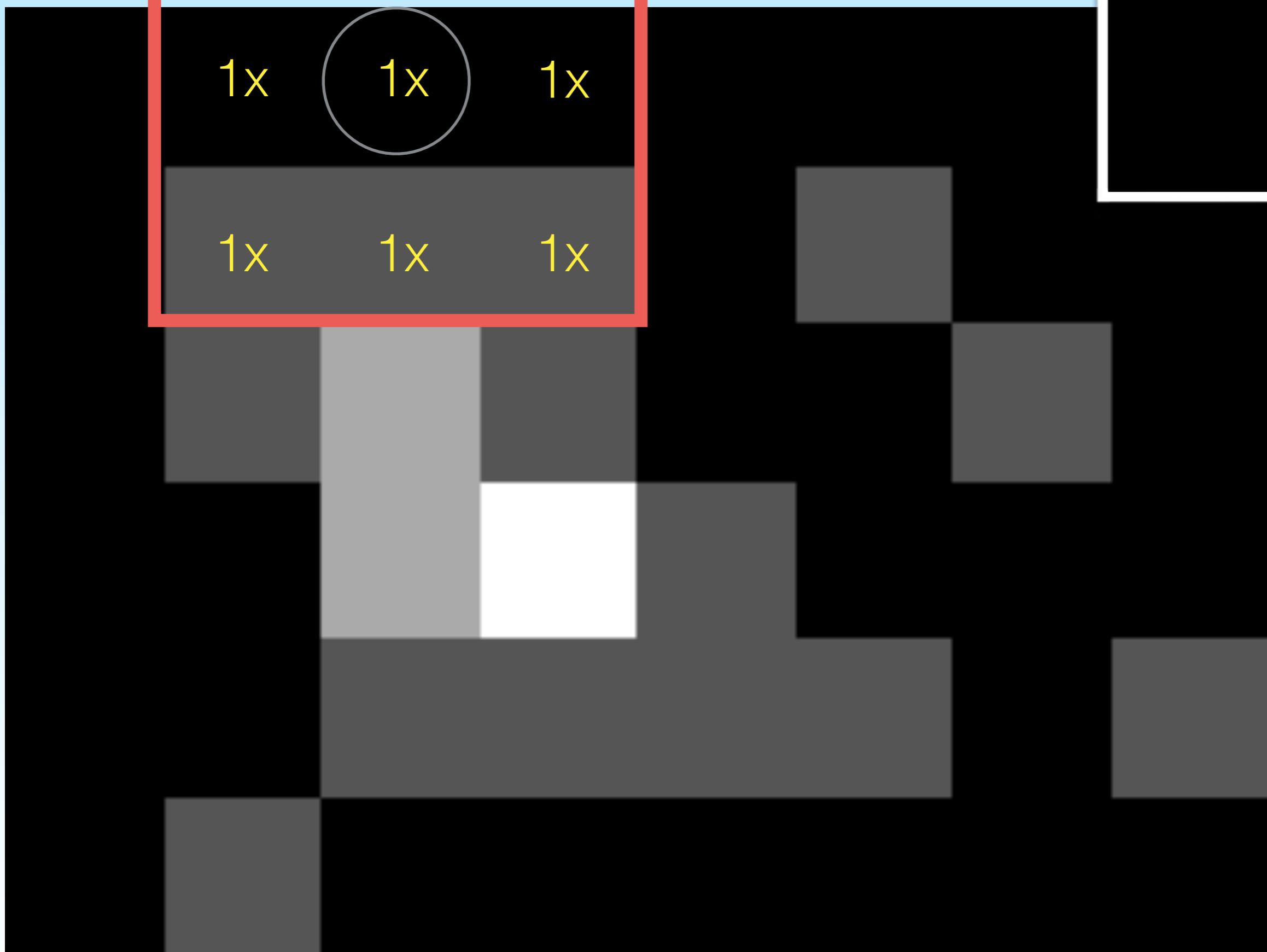
output



Mean Filter



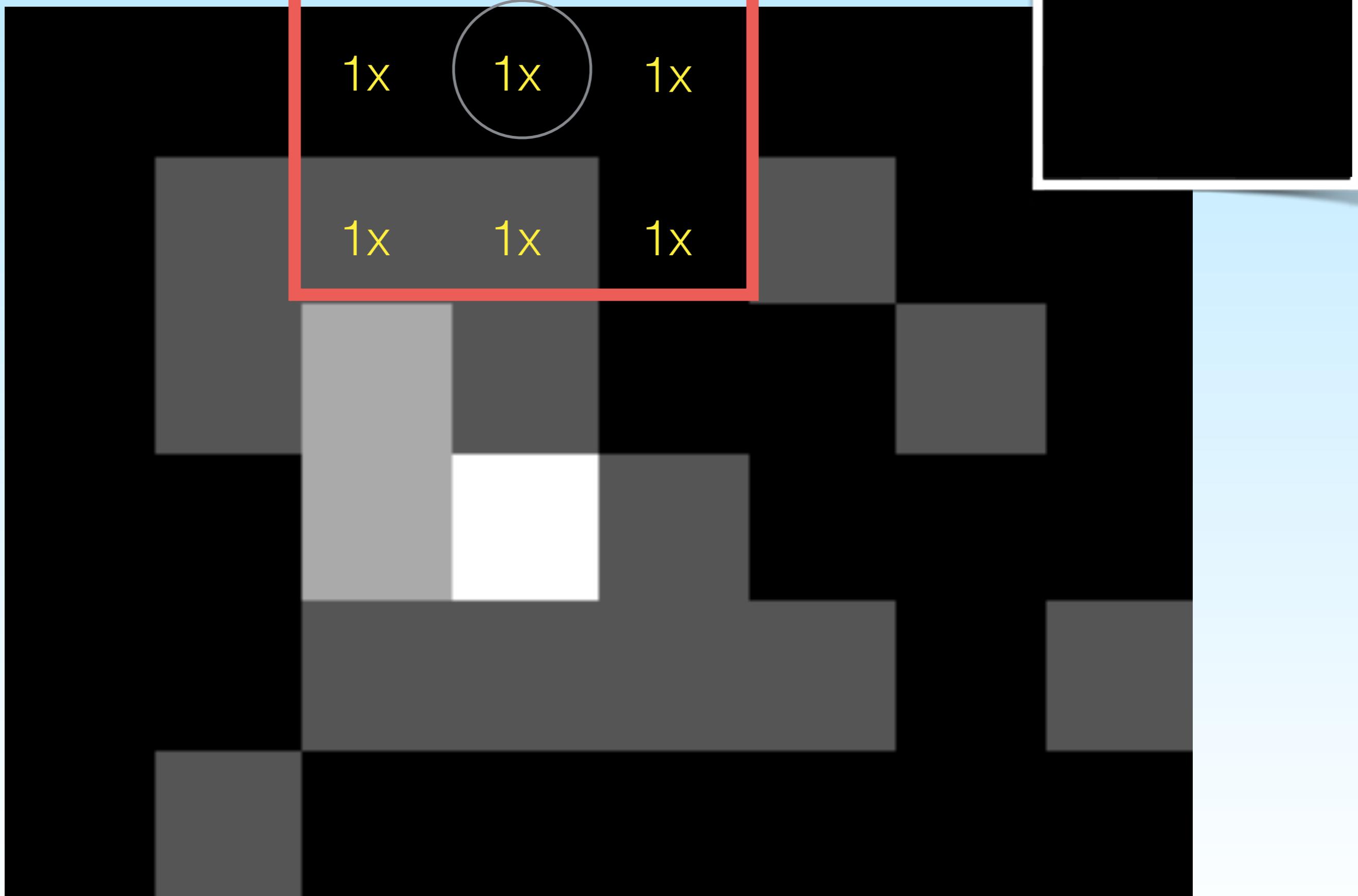
Mean Filter



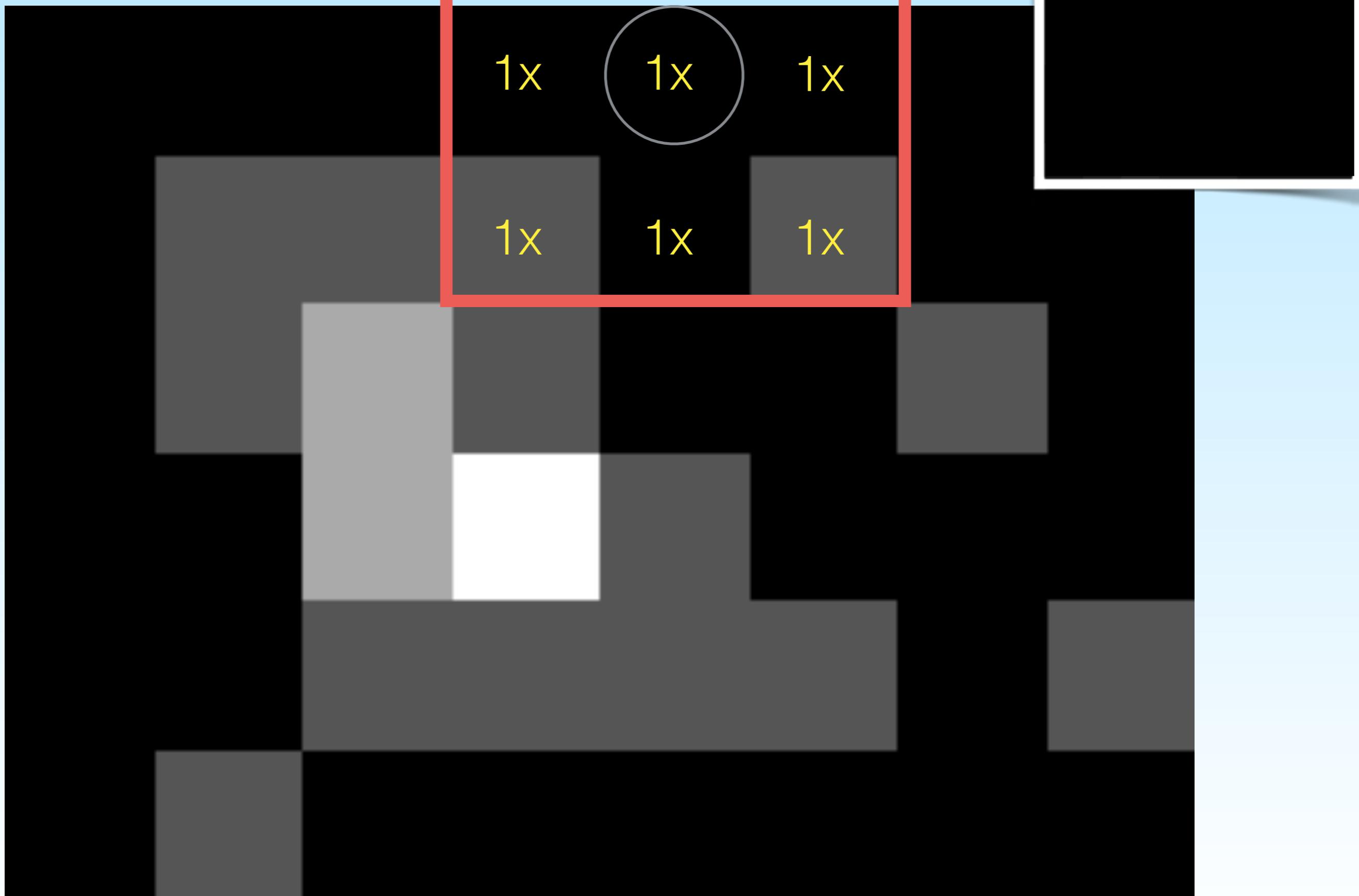
output



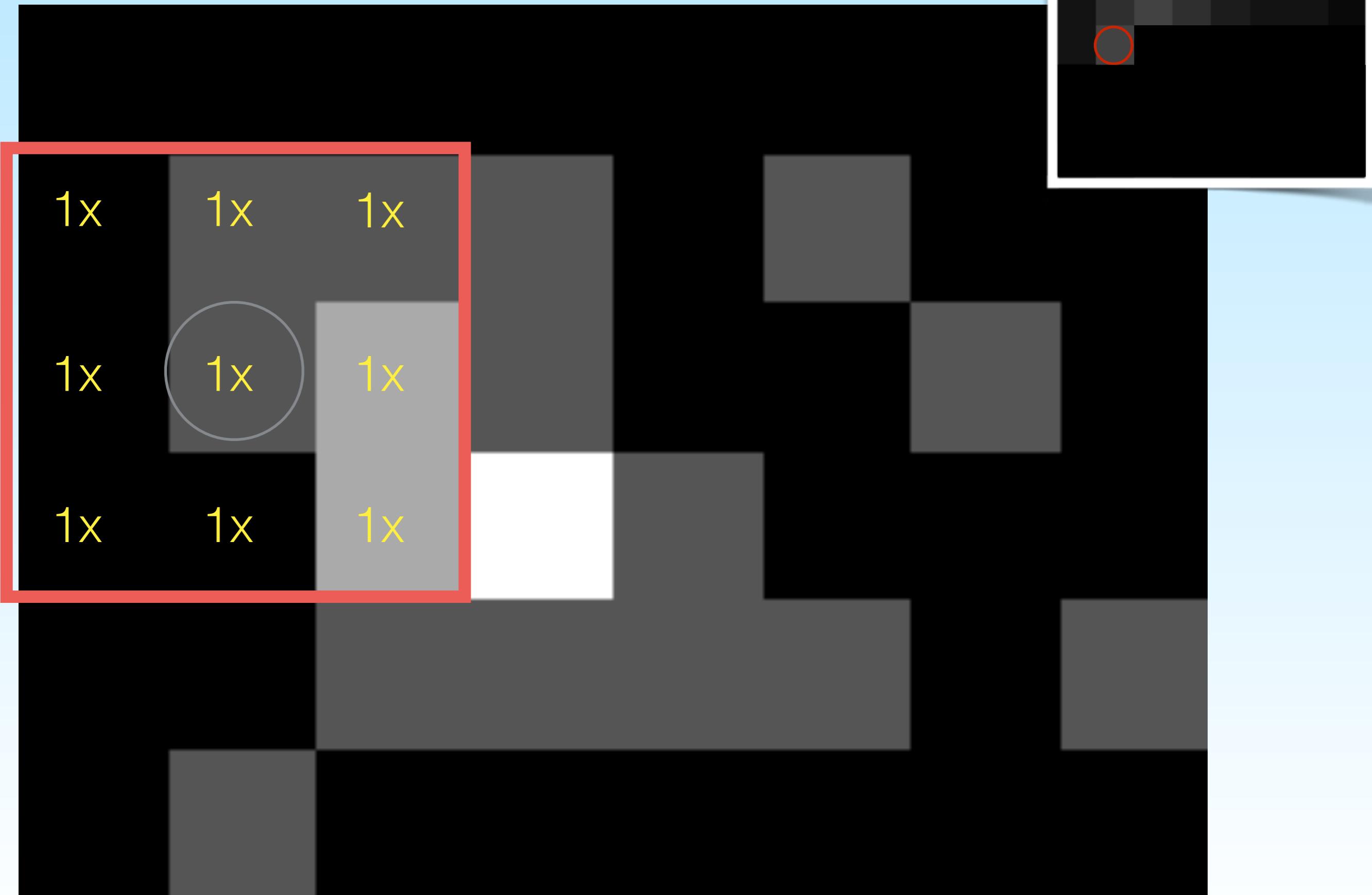
Mean Filter



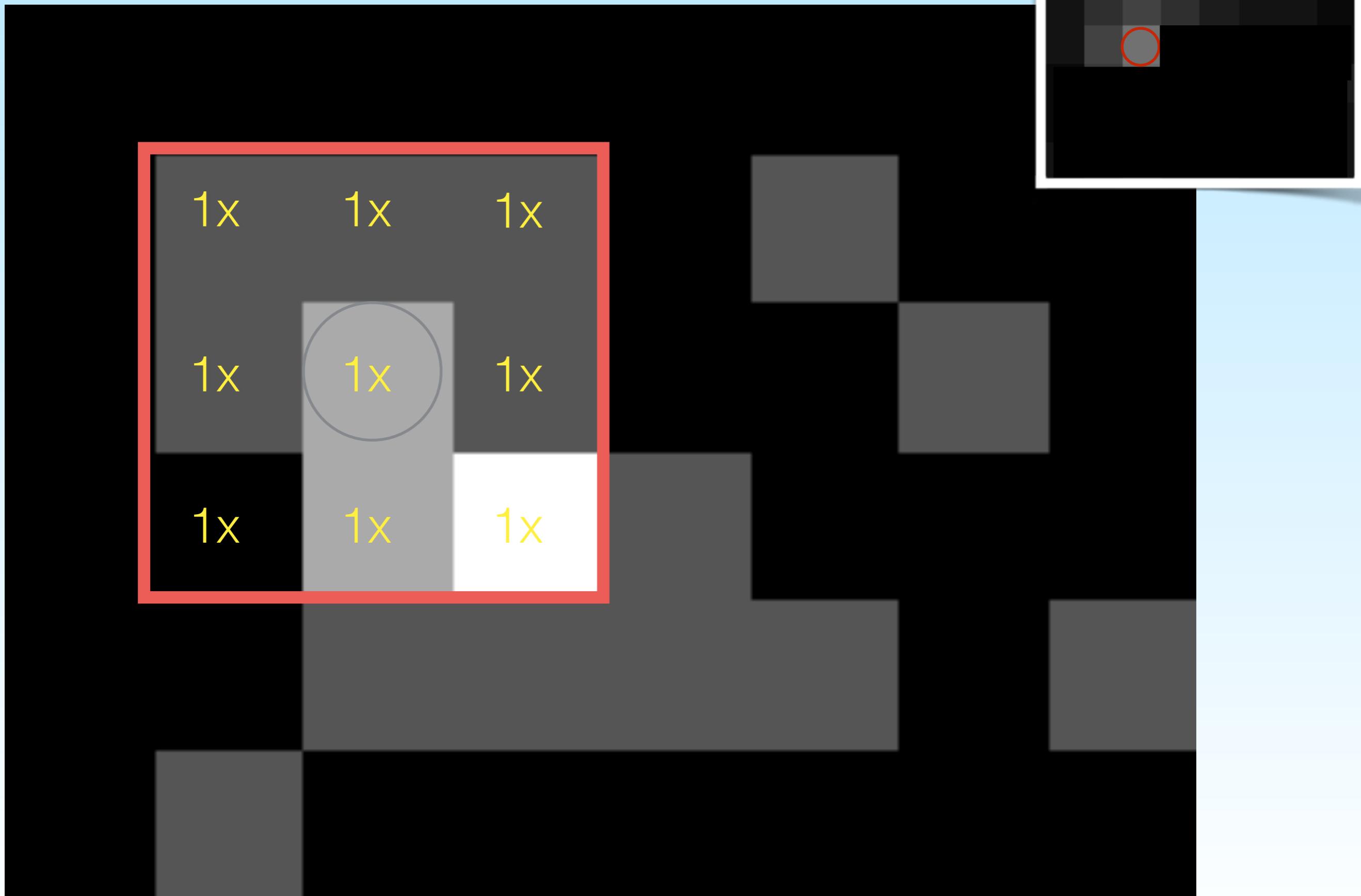
Mean Filter



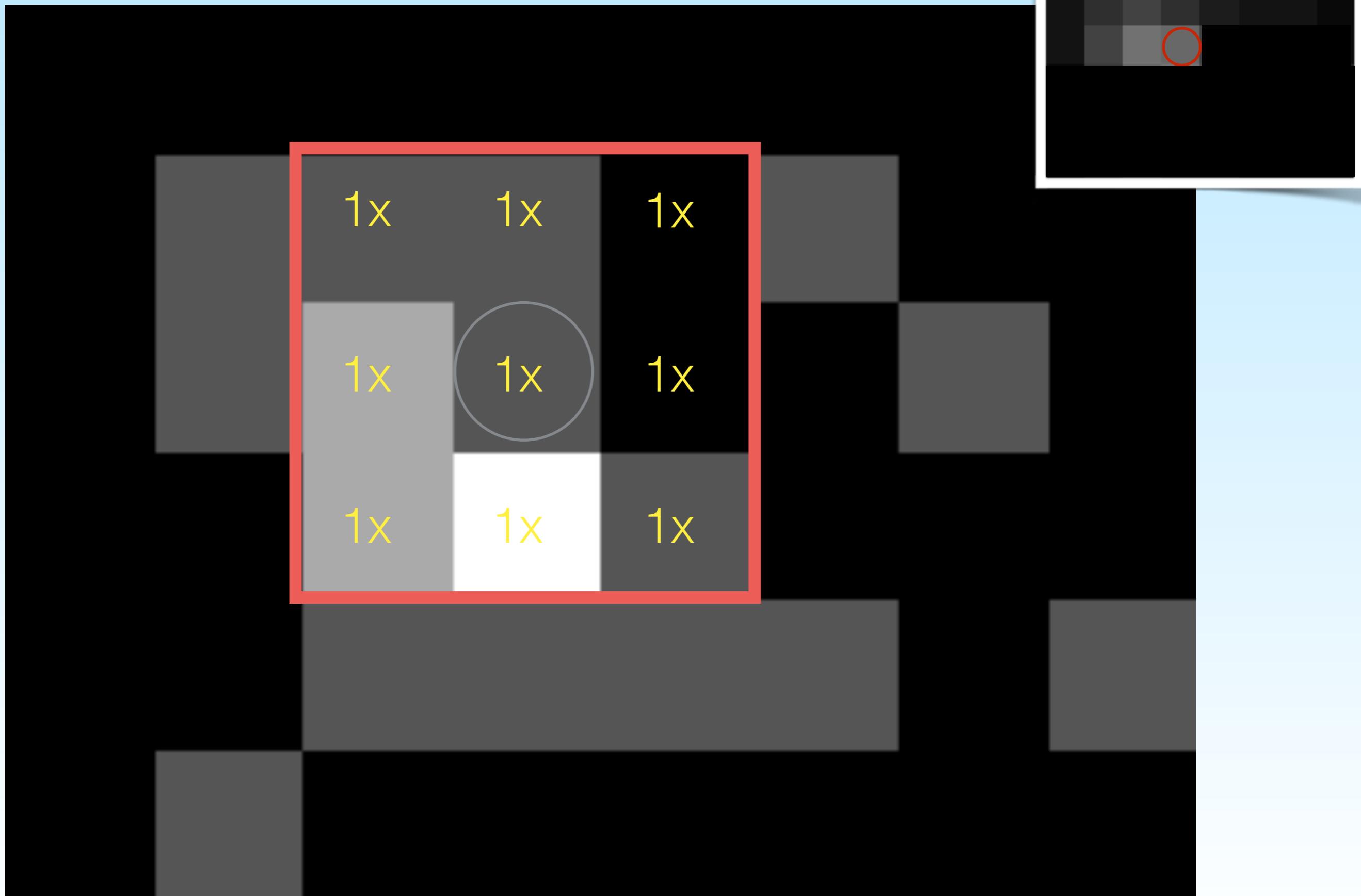
Mean Filter



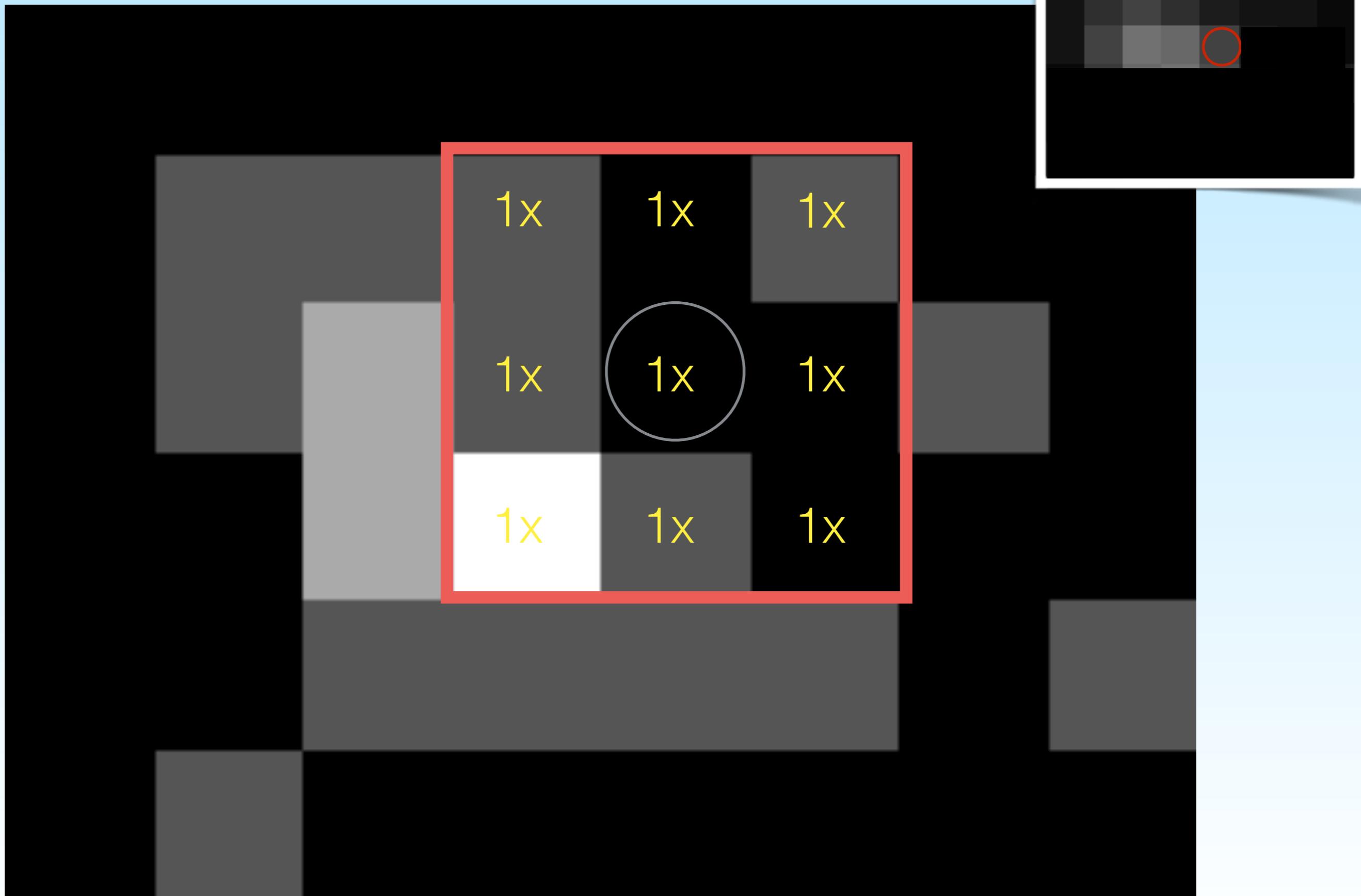
Mean Filter



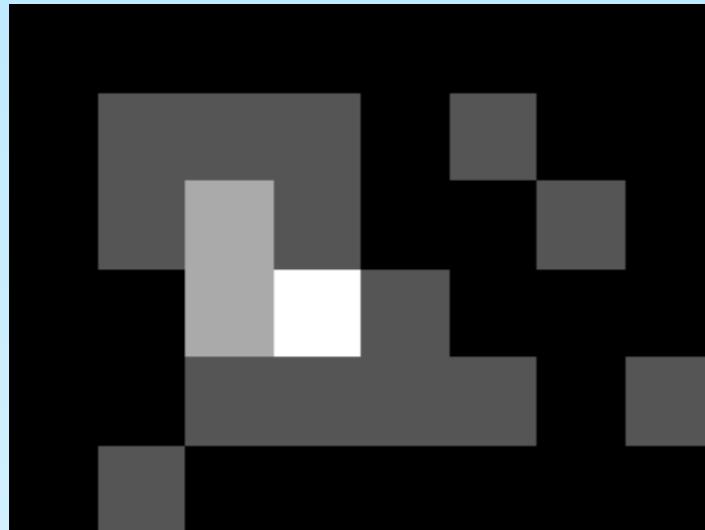
Mean Filter



Mean Filter



Result:

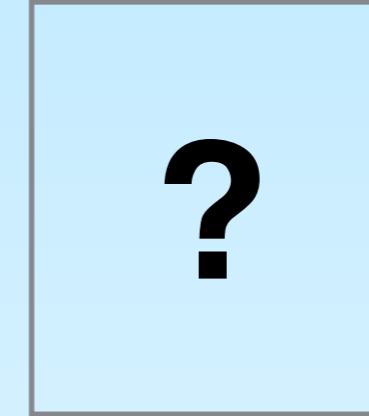
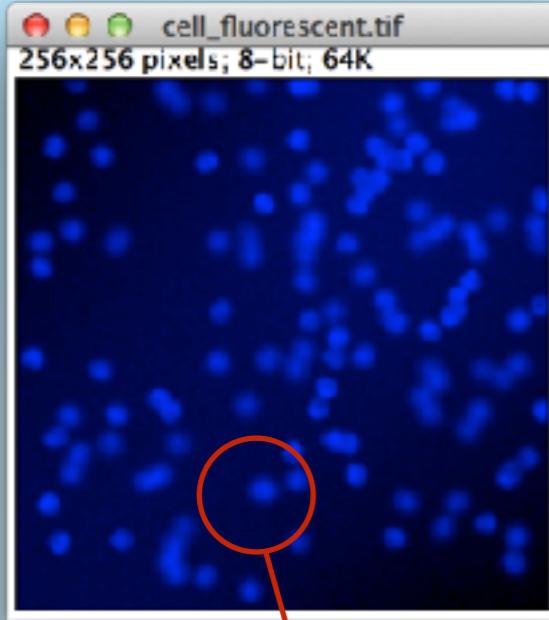


The output is a smoothed representation of the input.

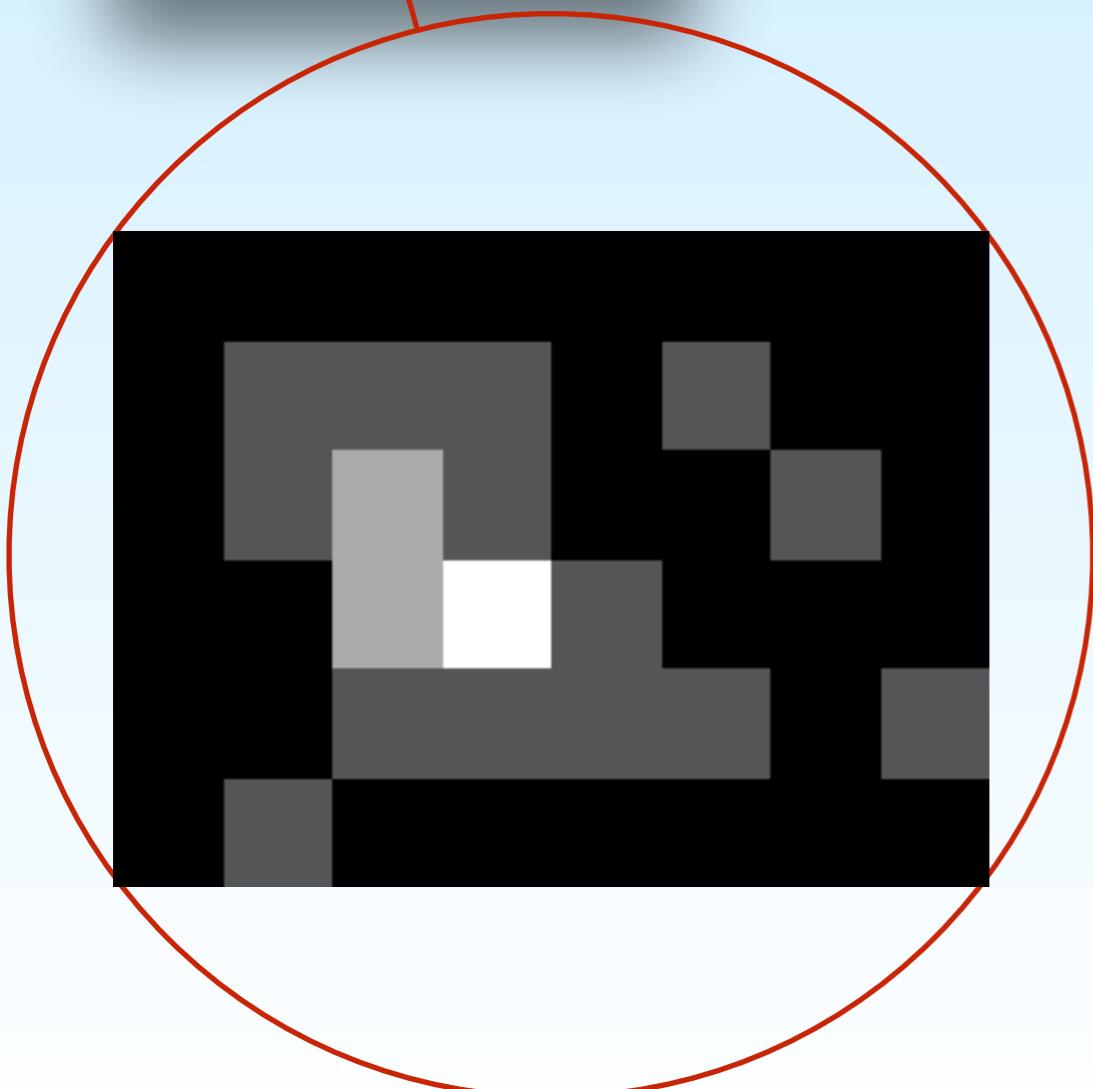


Source:

Non-linear filter: Maximum filter



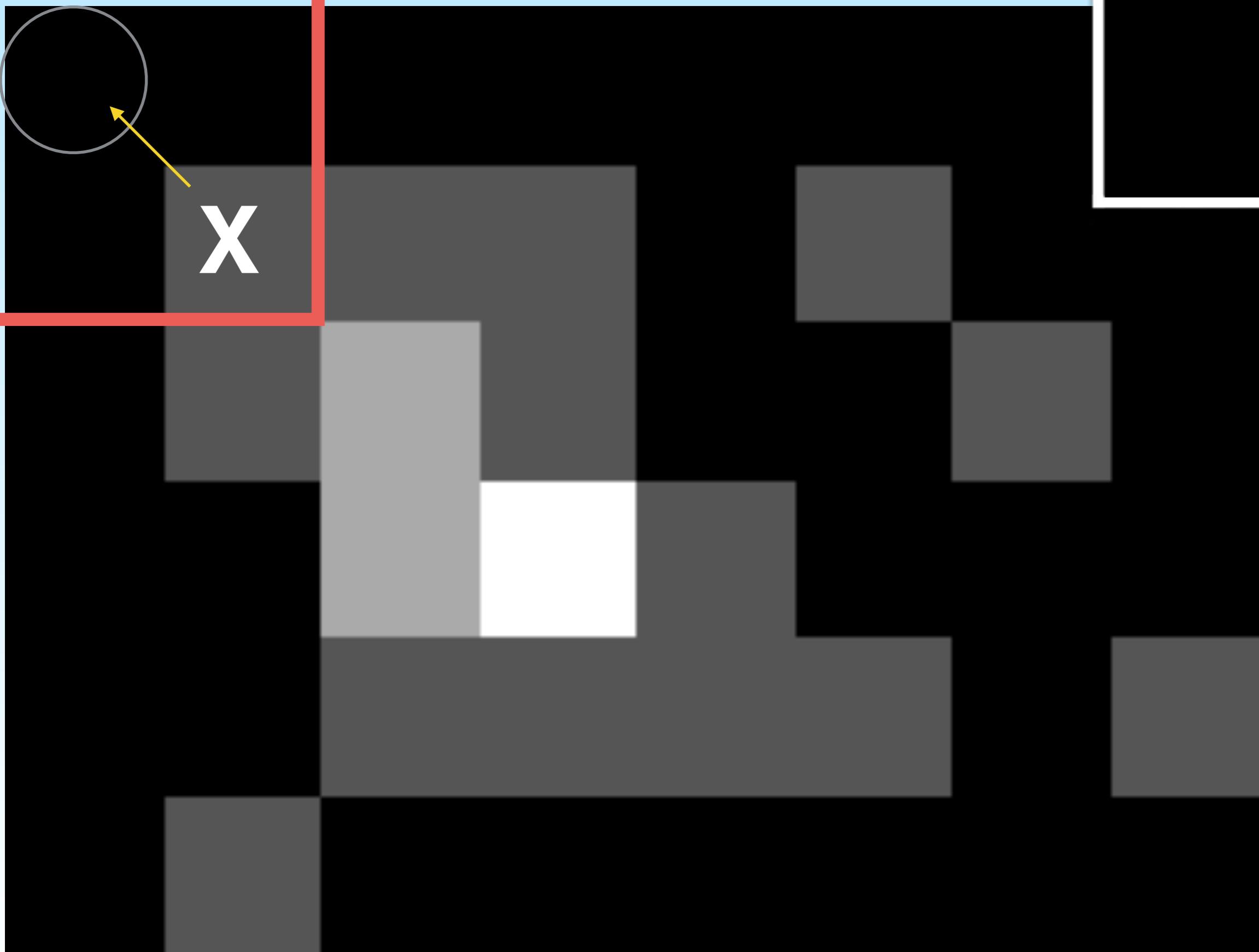
kernel can not be expressed as is non-linear



```
[[1, 1, 1, 1, 1, 1, 1, 1, 1],  
 [1, 2, 2, 2, 1, 2, 1, 1],  
 [1, 2, 3, 2, 1, 1, 2, 1],  
 [1, 1, 3, 4, 2, 1, 1, 1],  
 [1, 1, 2, 2, 2, 2, 1, 2],  
 [1, 2, 1, 1, 1, 1, 1, 1]]
```

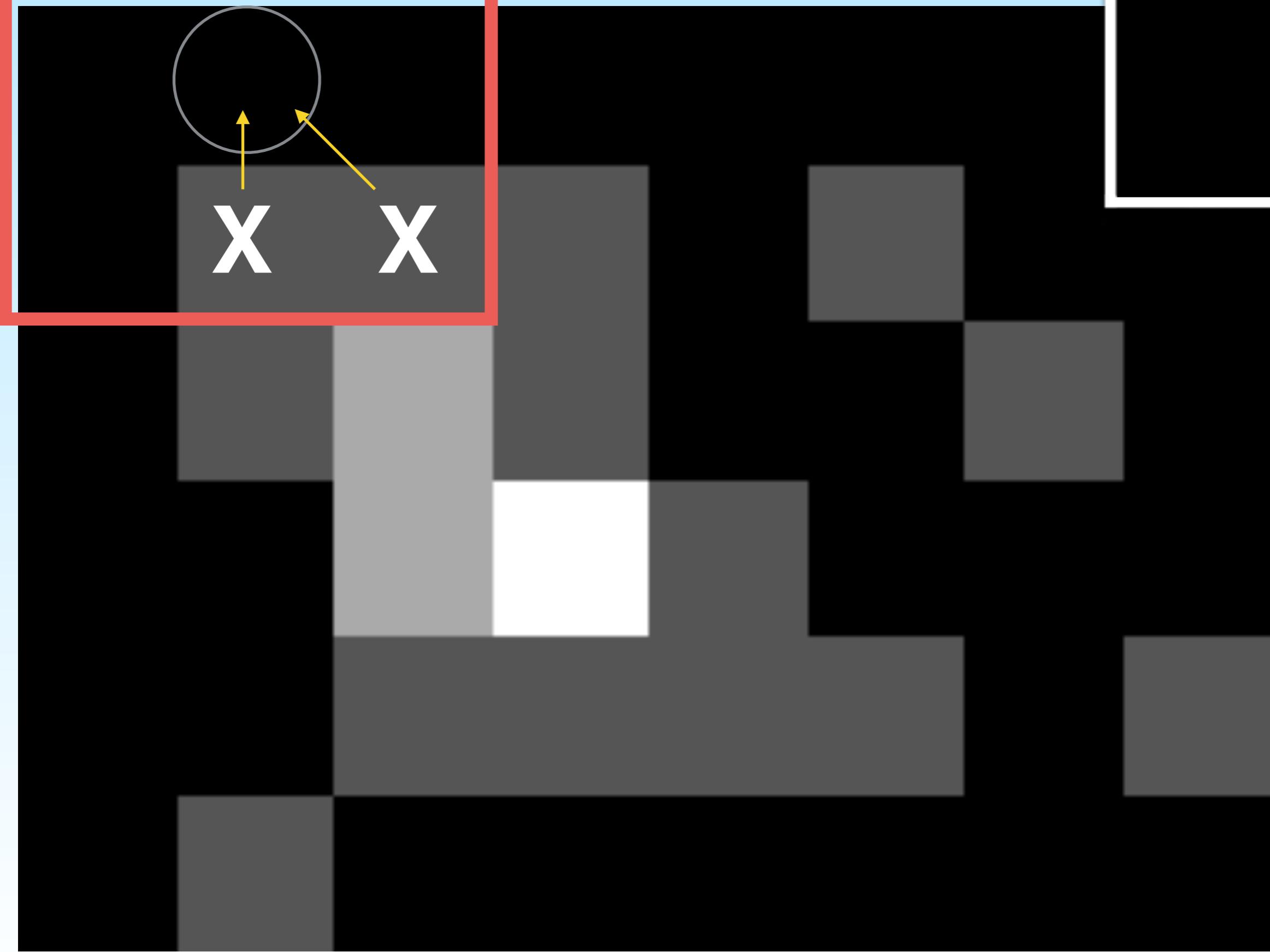
Non-linear filter: Maximum Filter

output

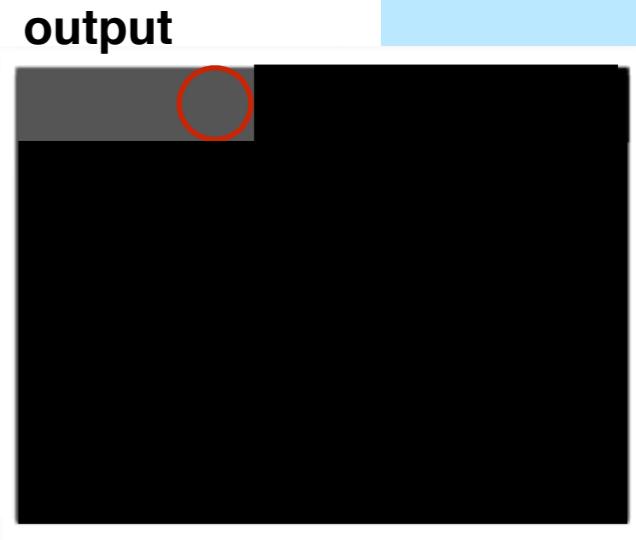
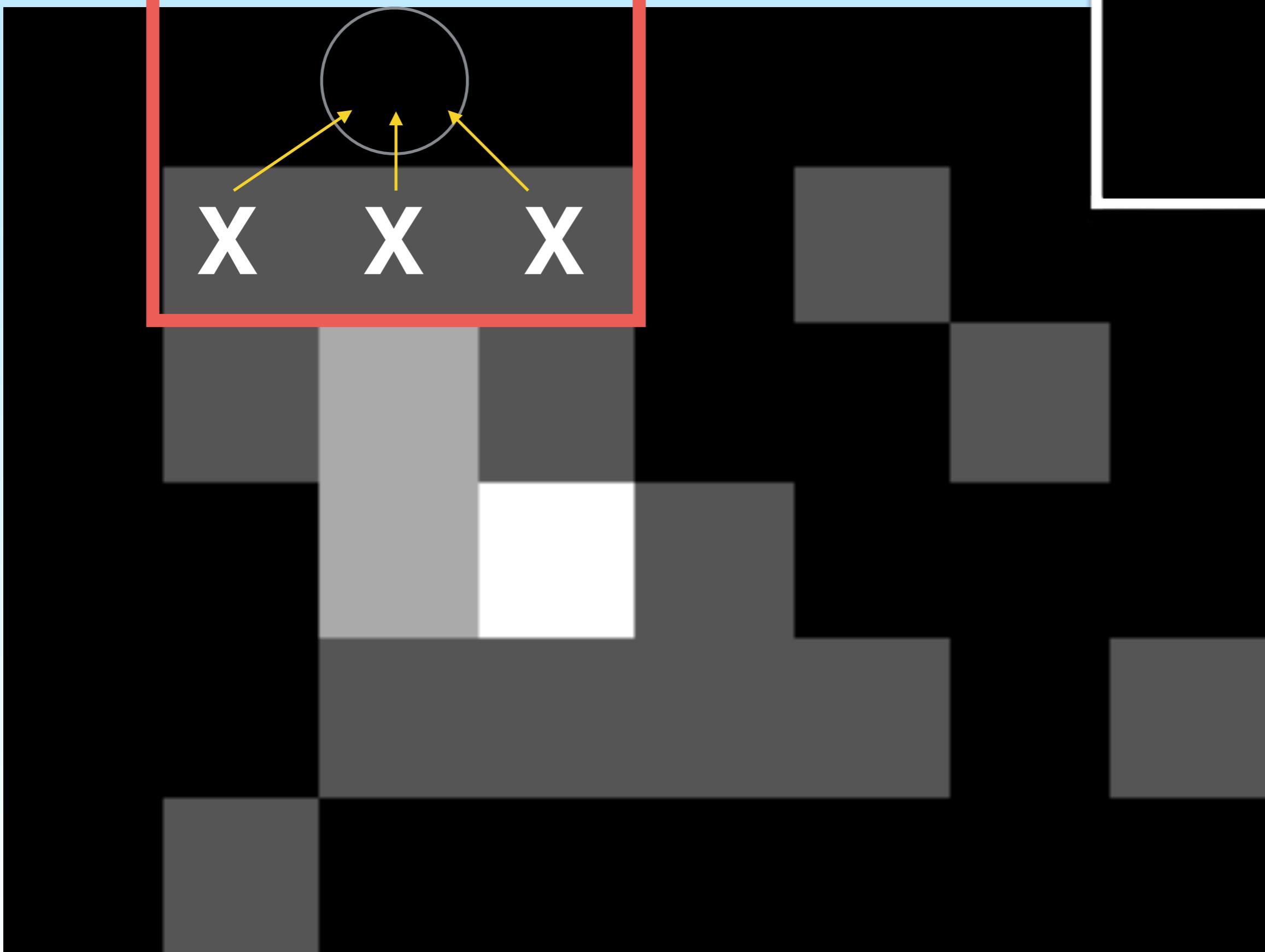


Non-linear filter: Maximum Filter

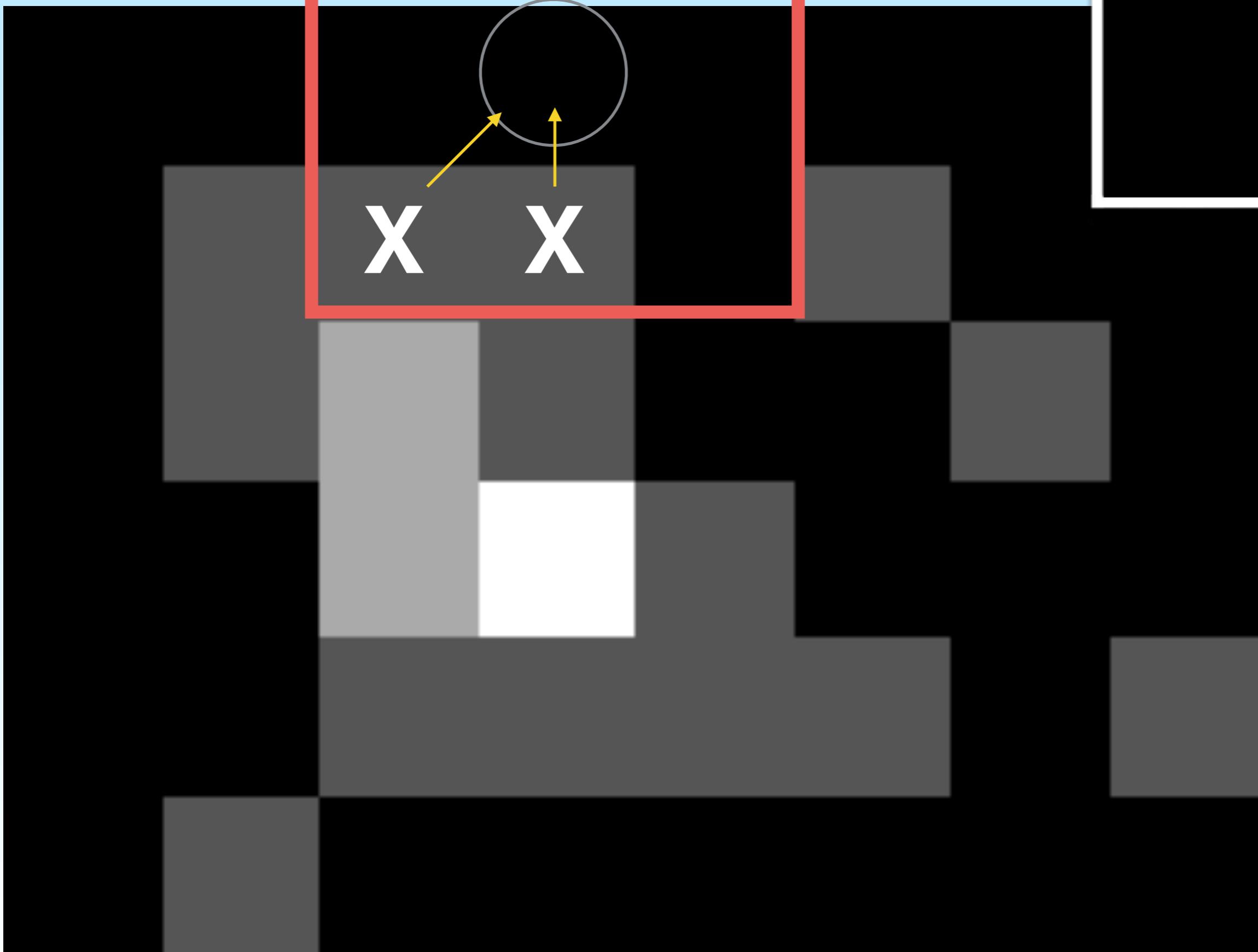
output



Non-linear filter: Maximum Filter



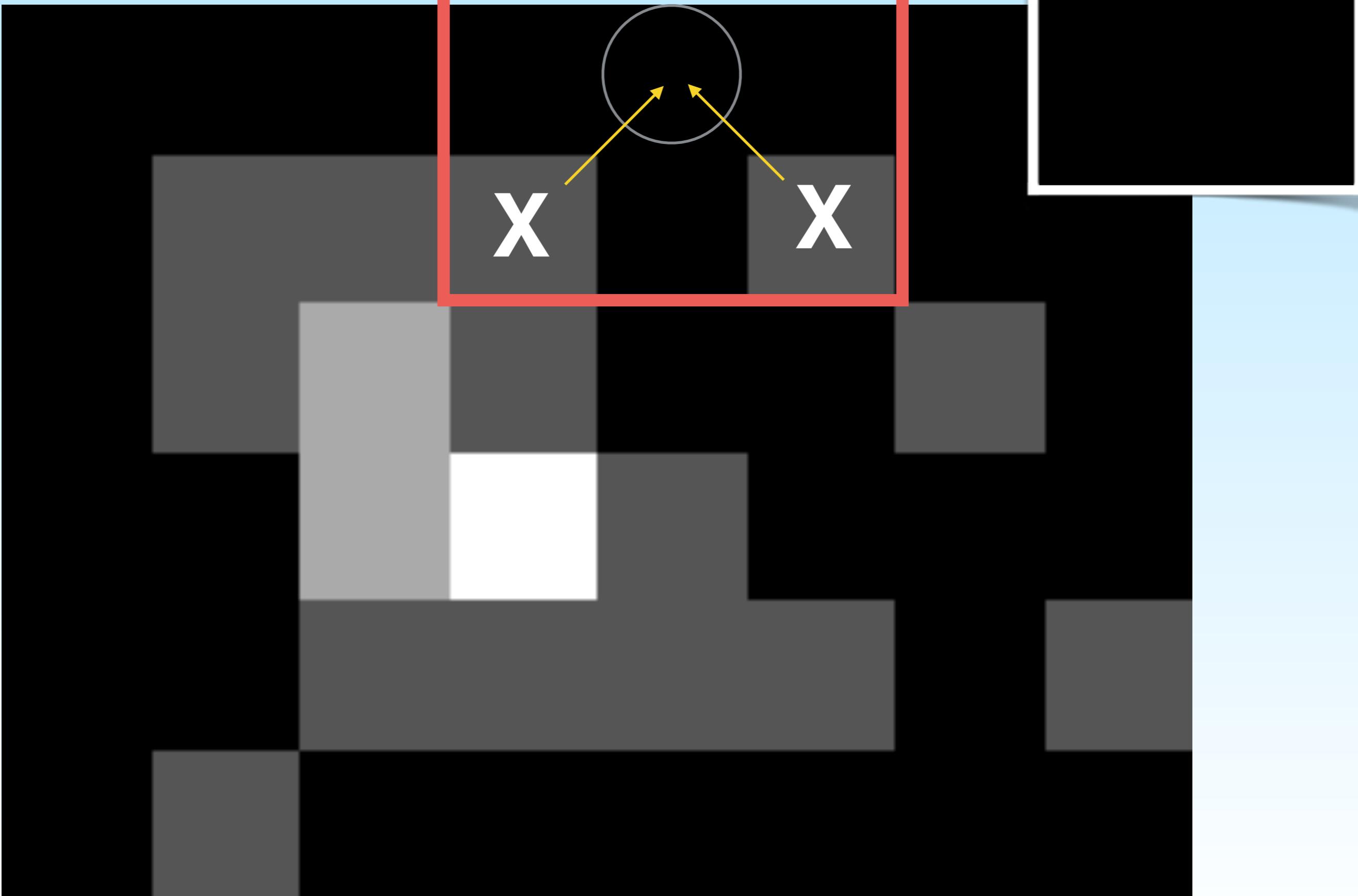
Non-linear filter: Maximum Filter



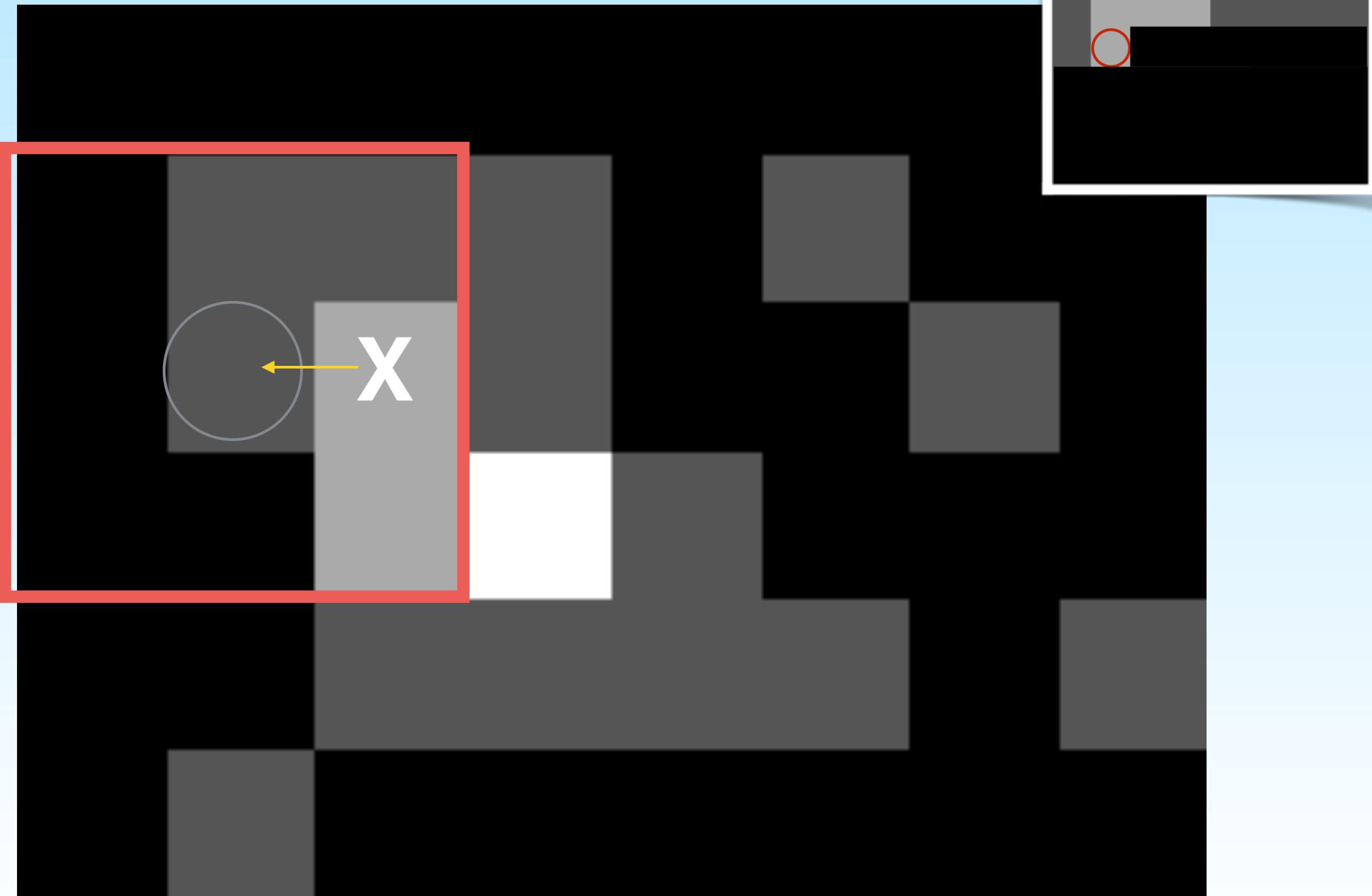
output

Non-linear filter: Maximum Filter

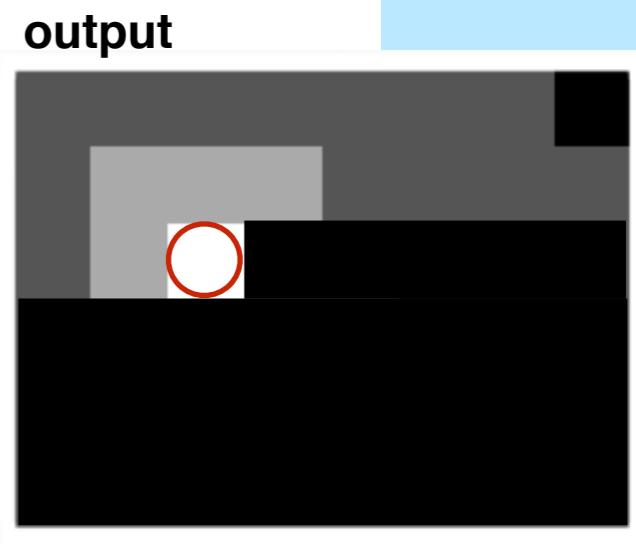
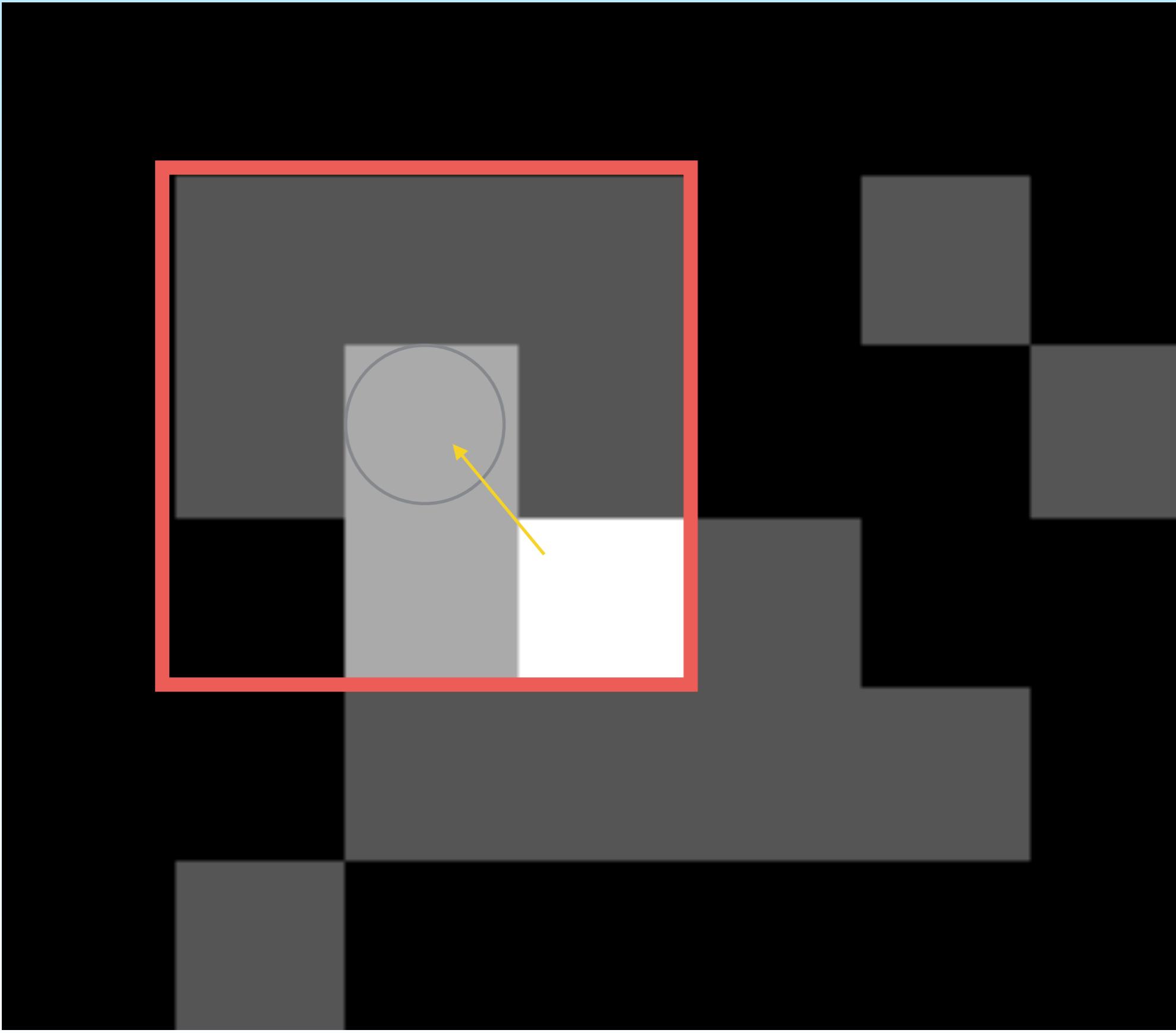
output



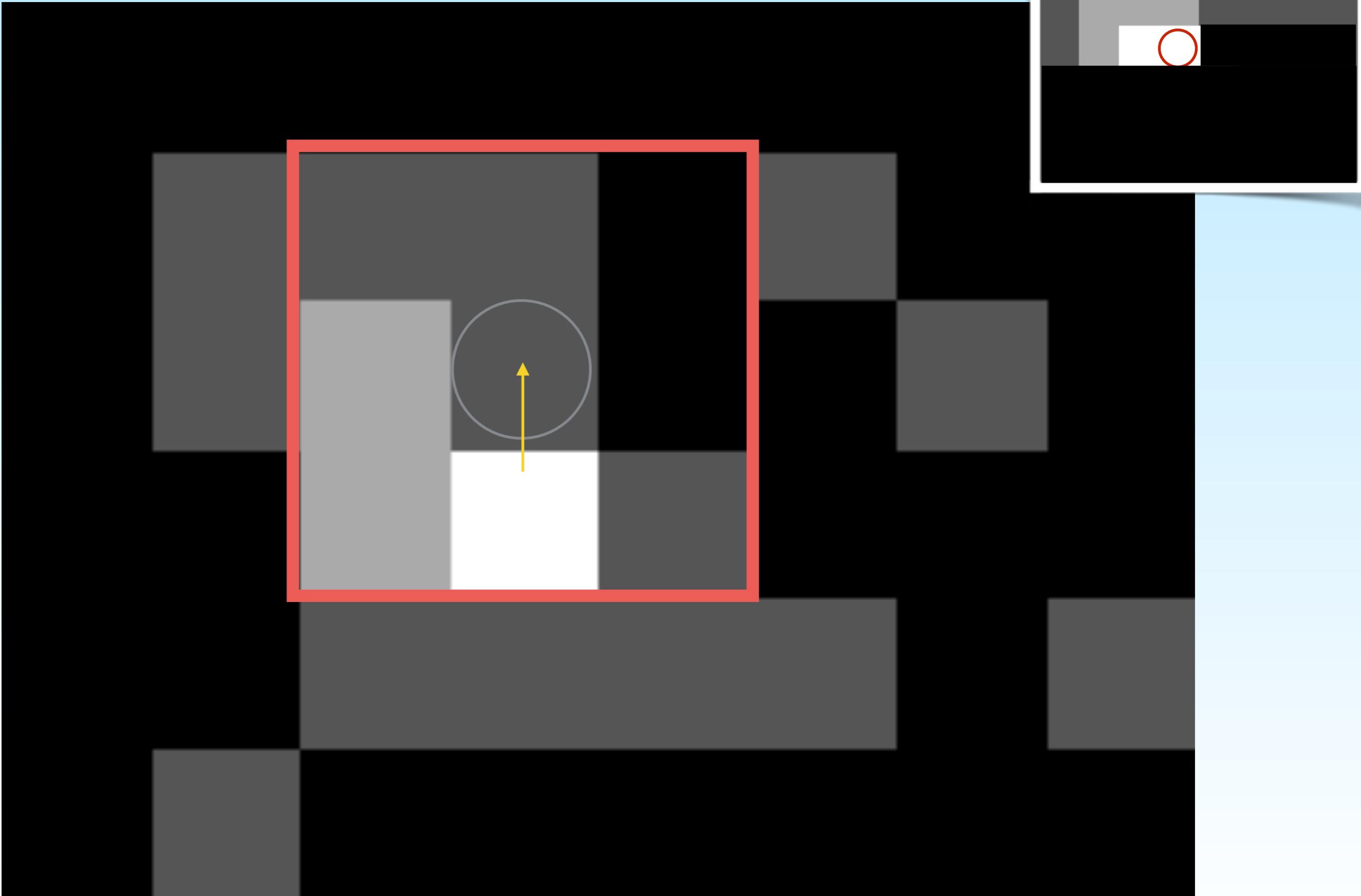
Non-linear filter: Maximum Filter



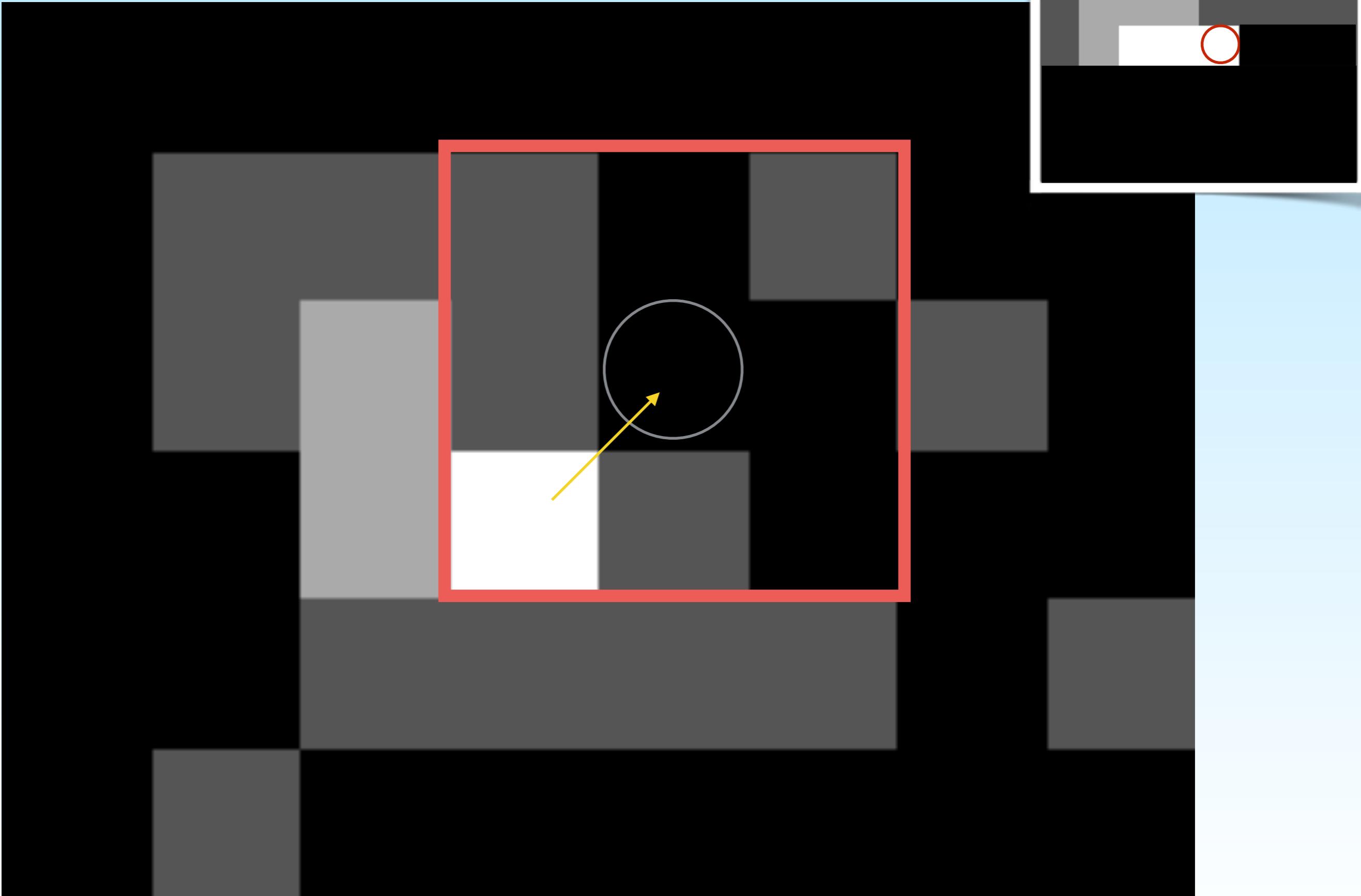
Non-linear filter: Maximum Filter



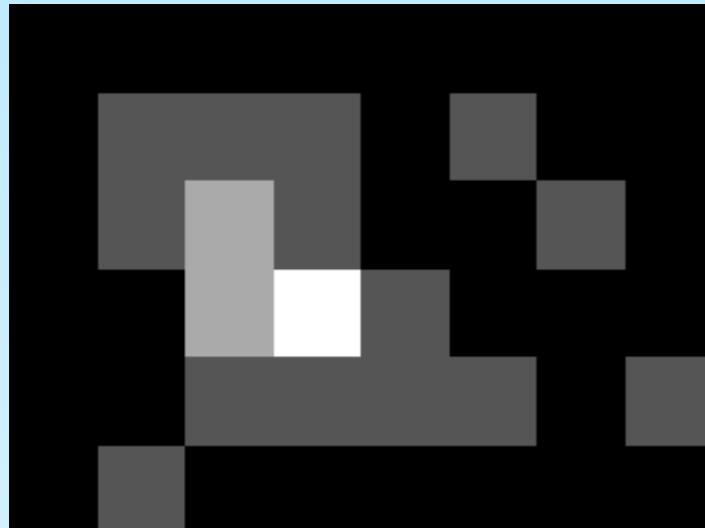
Non-linear filter: Maximum Filter



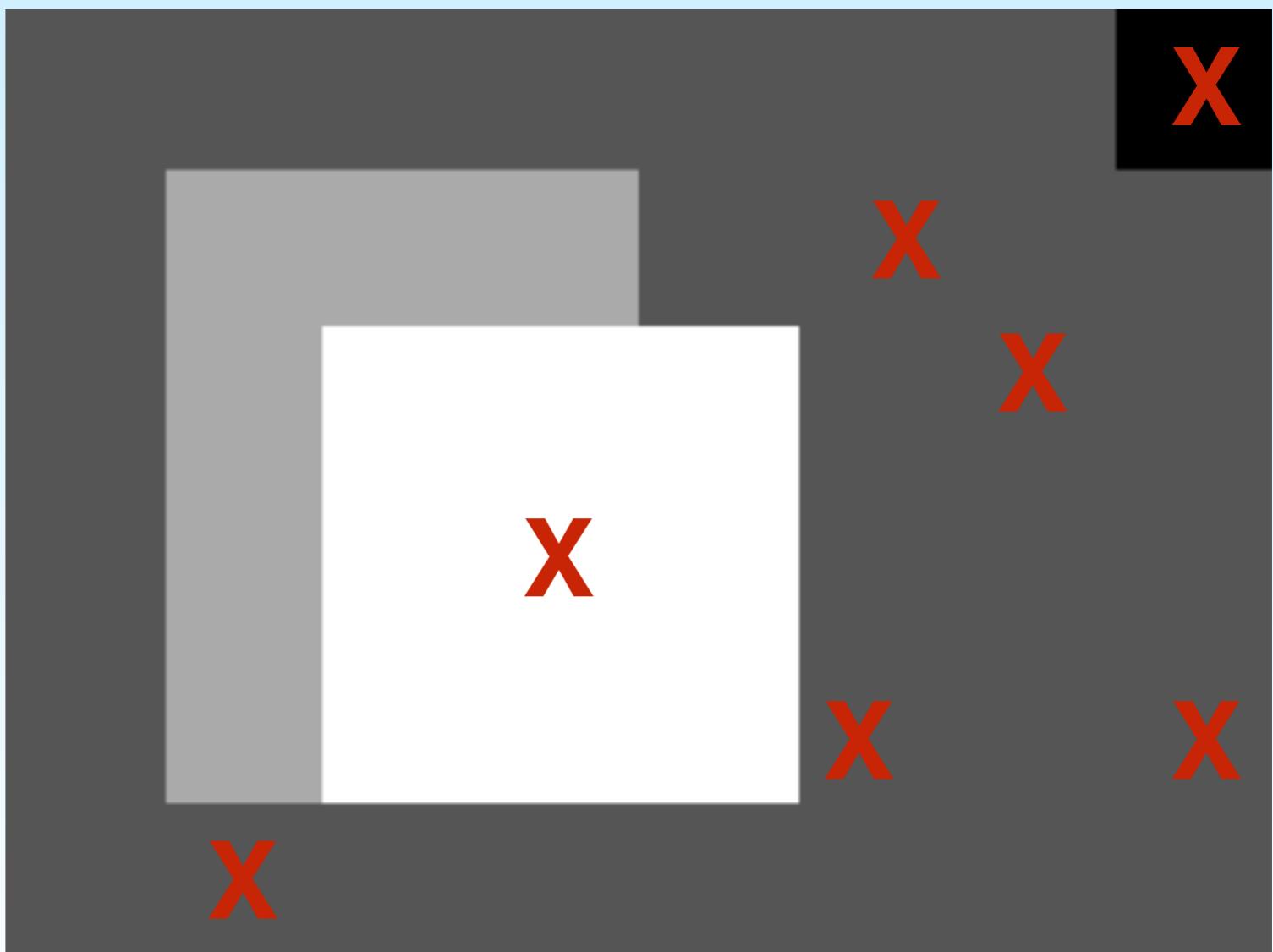
Non-linear filter: Maximum Filter



Result:



Where the pixel is the same in the input as the maximum filtered image you find your local maxima



Source:

Convolution - edge filters

1st derivative filter

kernel

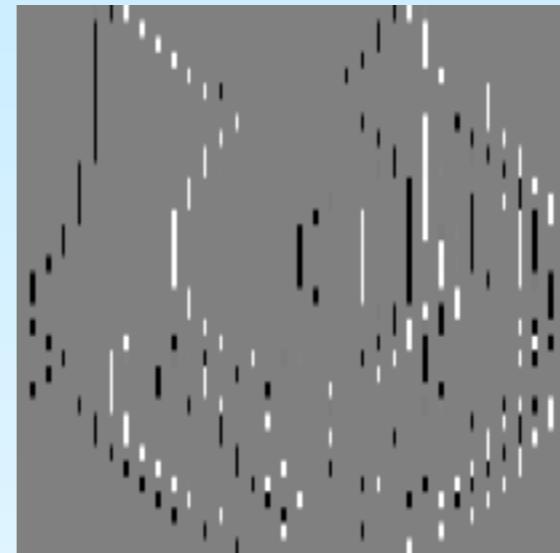
$$\begin{bmatrix} -1 \\ 0 \\ 1 \end{bmatrix}$$

$$\frac{\partial f}{\partial x} = f(x + 1) - f(x)$$

input

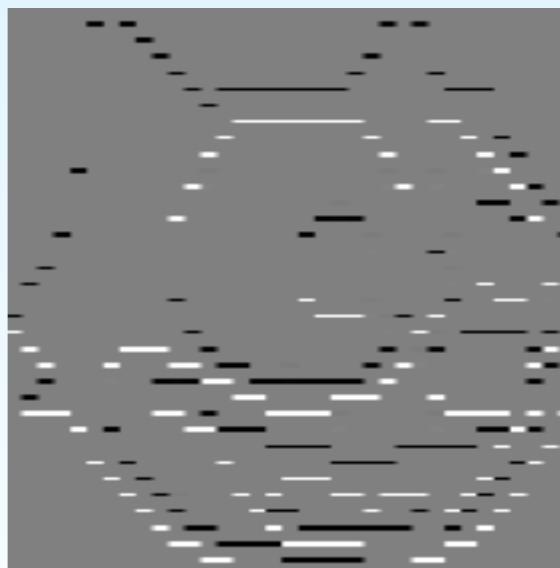


output (convolved)



other direction

$$\begin{bmatrix} -1 & 0 & 1 \end{bmatrix}$$



Extracts edges in a particular direction

**black is negative
white is positive**

Source: http://www.uff.br/cdme/matrix/matrix-html/matrix_boolean/matrix_boolean_en.html

Convolution - edge filters

1st derivative filter 2d filter

kernel

$$\begin{bmatrix} -1 \\ 0 \\ 1 \end{bmatrix}$$

+

$$\begin{bmatrix} -1 & 0 & 1 \end{bmatrix}$$

=

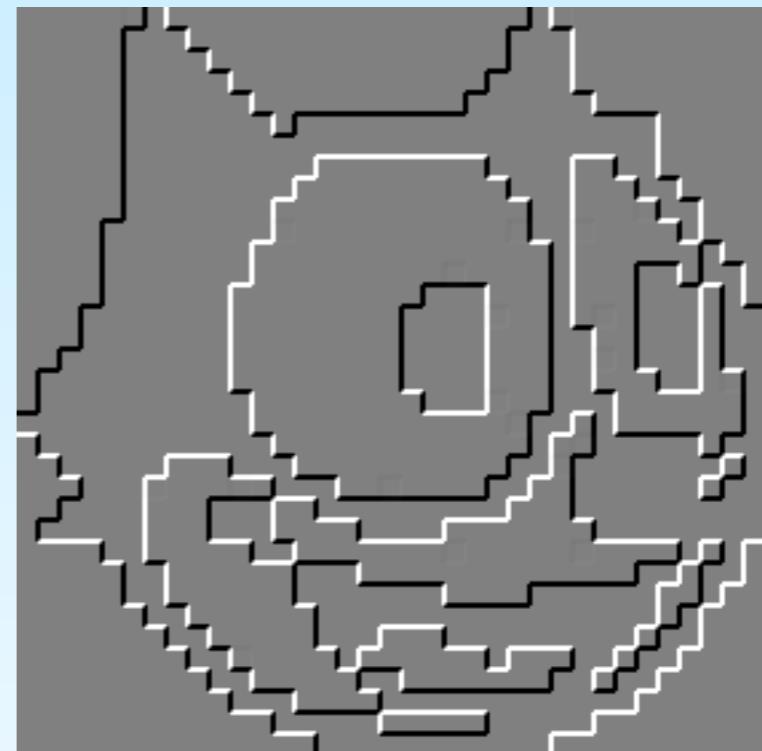
$$\begin{bmatrix} 0 & -1 & 0 \\ -1 & 0 & 1 \\ 0 & -1 & 0 \end{bmatrix}$$

*



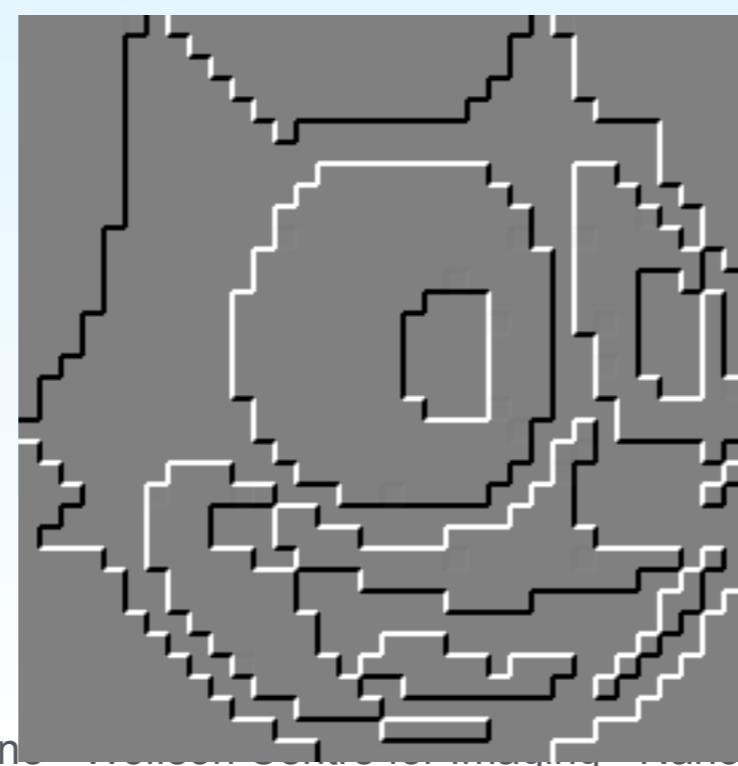
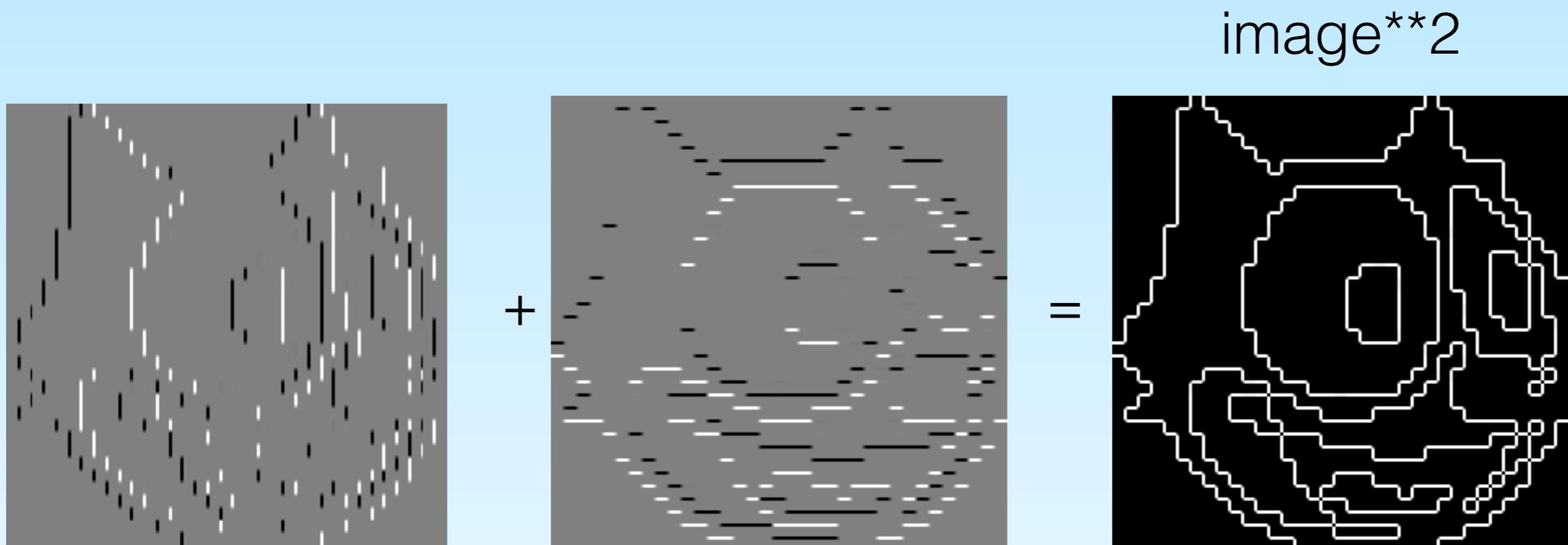
2D filter

output (convolved)



black is negative
white is positive

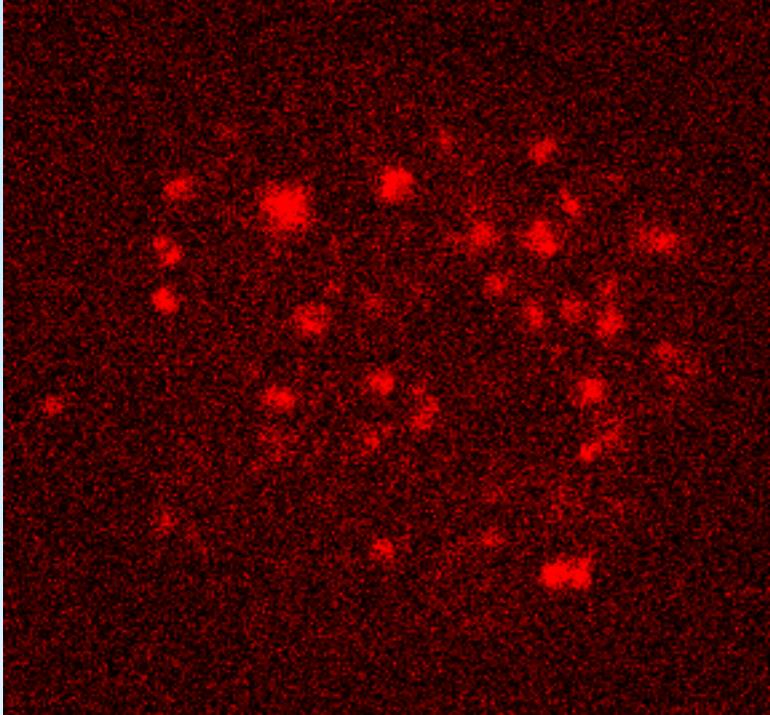
Convolution - Gradient magnitude gives edges



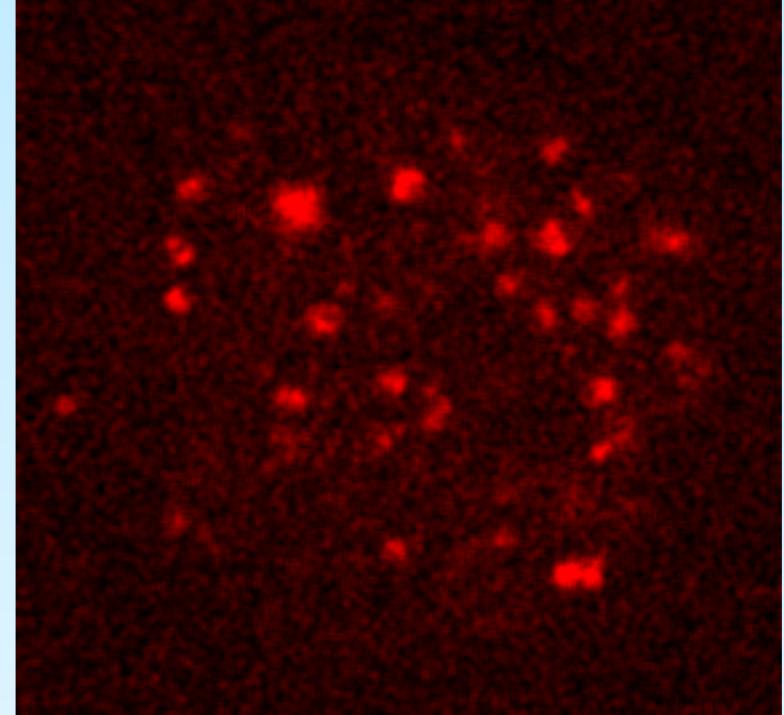
Source:

Mean Filter

input noisy image

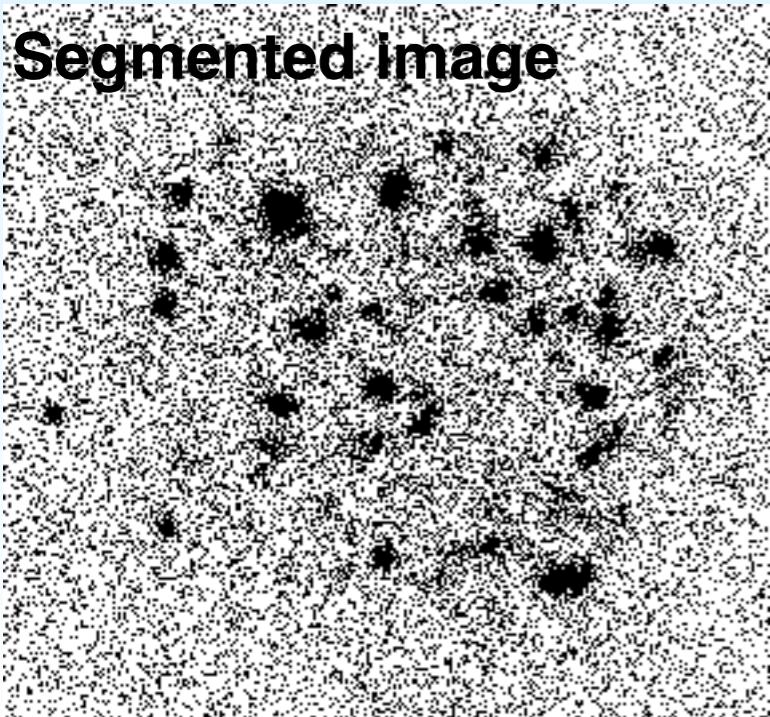


smoothed image

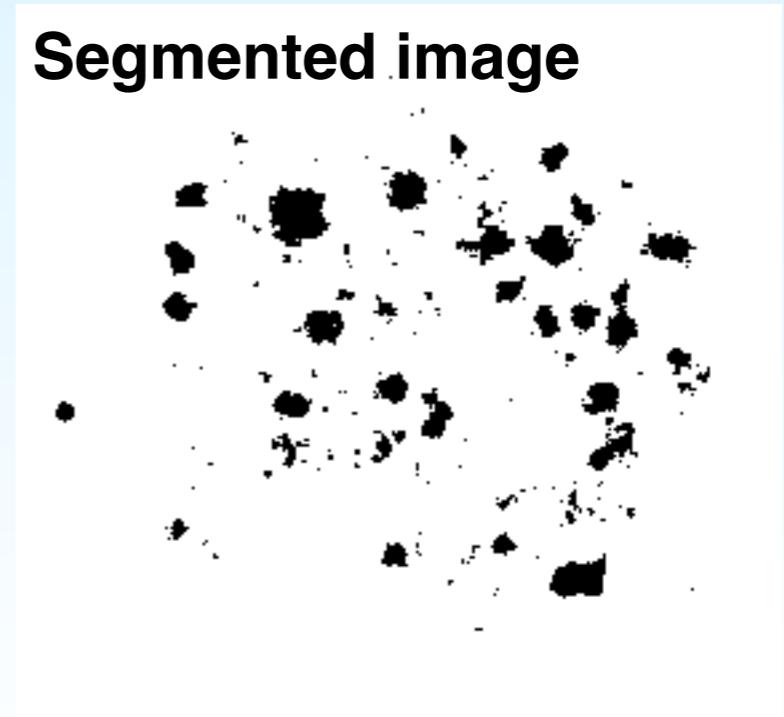


$$\begin{matrix} & 1 & 1 & 1 \\ * & 1 & 1 & 1 & = \\ & 1 & 1 & 1 \end{matrix}$$

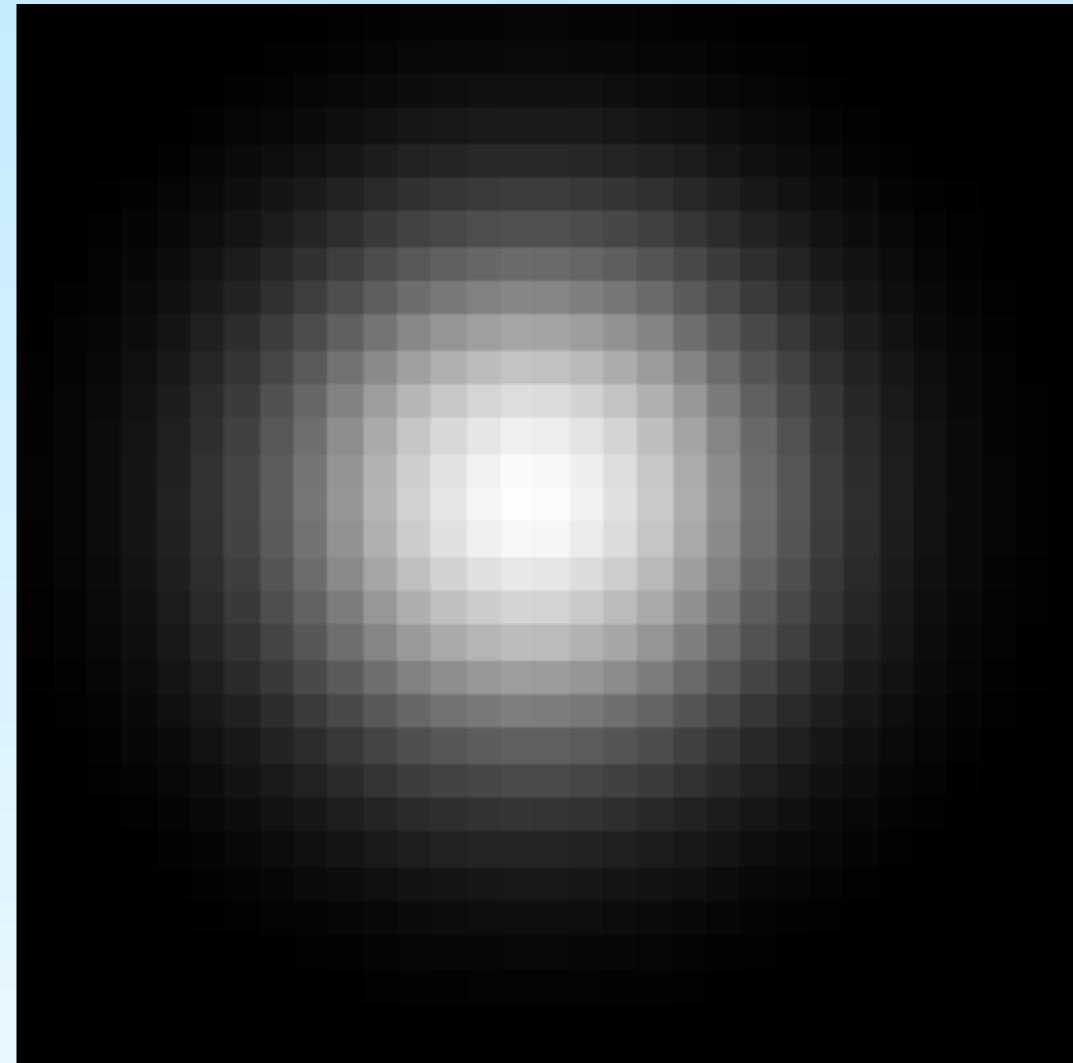
Segmented image



Segmented image



Gaussian filter.

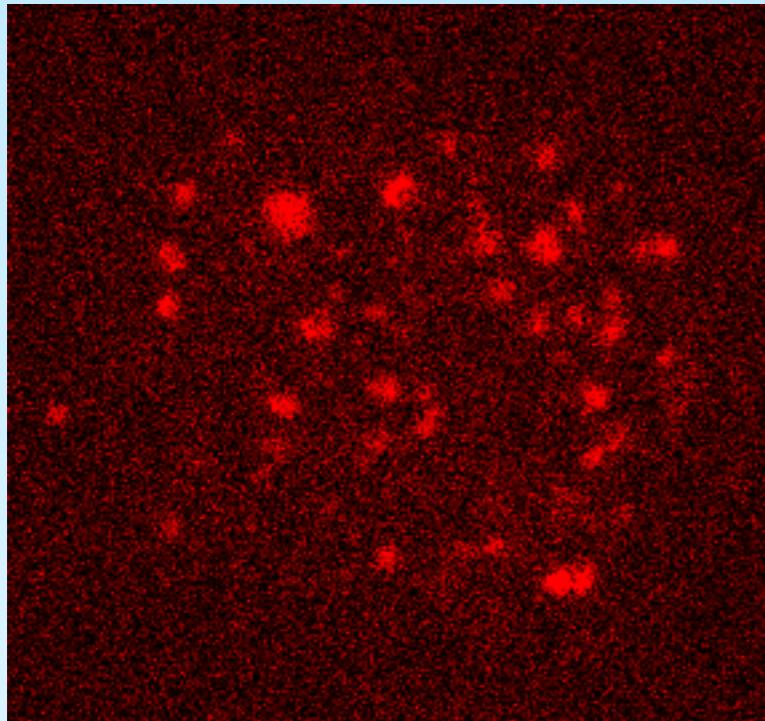


$$f(x, y) = A \exp\left(-\left(\frac{(x - x_o)^2}{2\sigma_x^2} + \frac{(y - y_o)^2}{2\sigma_y^2}\right)\right)$$

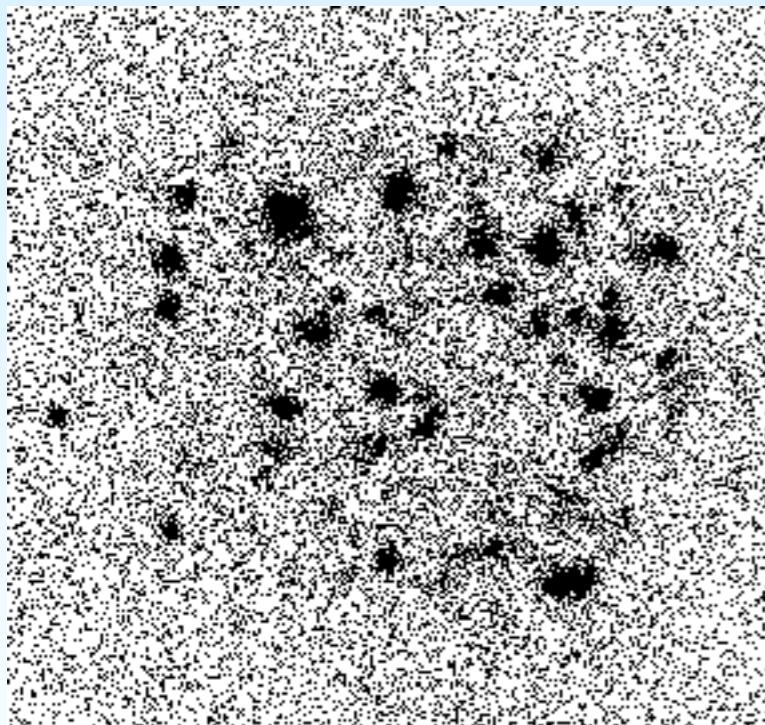
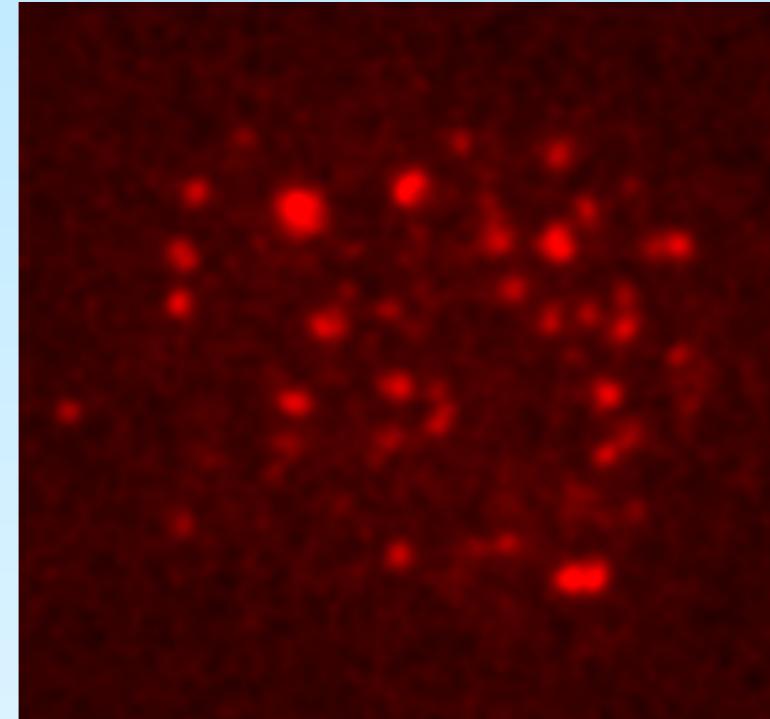
x, y (pixel coordinates)
sigma of kernel
x0,y0 center of Gaussian

We can use this to remove the image background.

Denoising through Gaussian deconvolution



$$\begin{matrix} \text{*} \\ \text{ } \end{matrix} = \begin{matrix} \text{ } \\ \text{ } \end{matrix}$$



Weights pixels nearer centre more

Convolution - Laplacian filter

2nd derivate filter

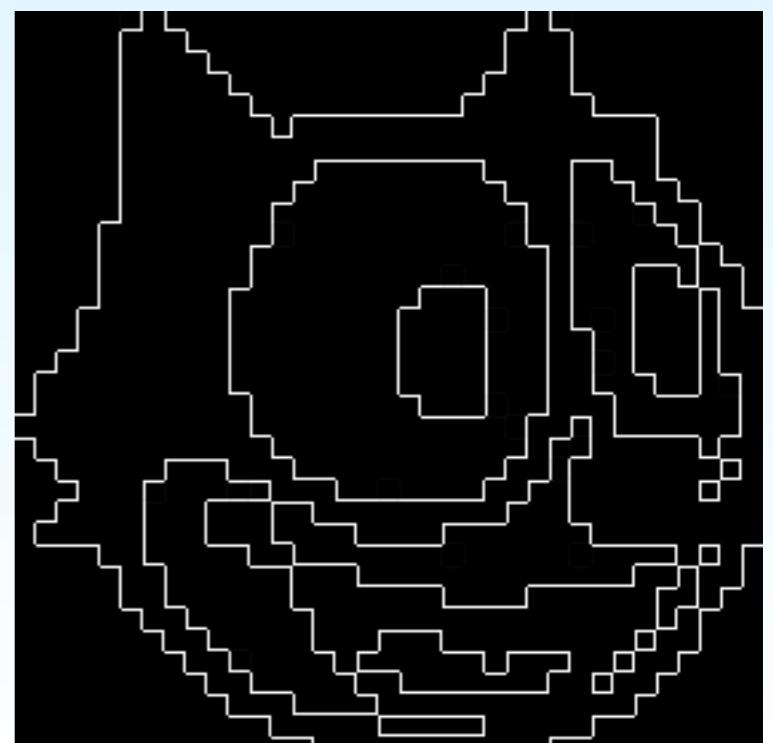
$$\Delta f = \frac{\partial^2 f}{\partial x^2} + \frac{\partial^2 f}{\partial y^2}$$

1D filter: $\vec{D}_x^2 = [1 \quad -2 \quad 1],$

2D filter: $\mathbf{D}_{xy}^2 = \begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}.$

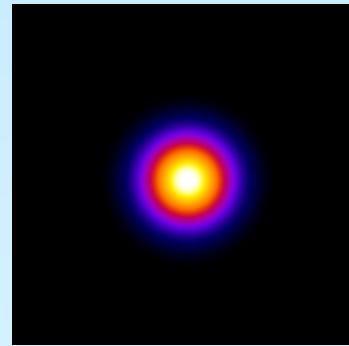
2D filter: $\mathbf{D}_{xy}^2 = \begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 1 \\ 0 & 1 & 0 \end{bmatrix}.$

2-D laplacian filter detects change no matter in which direction. Wherever the rate of change is large.

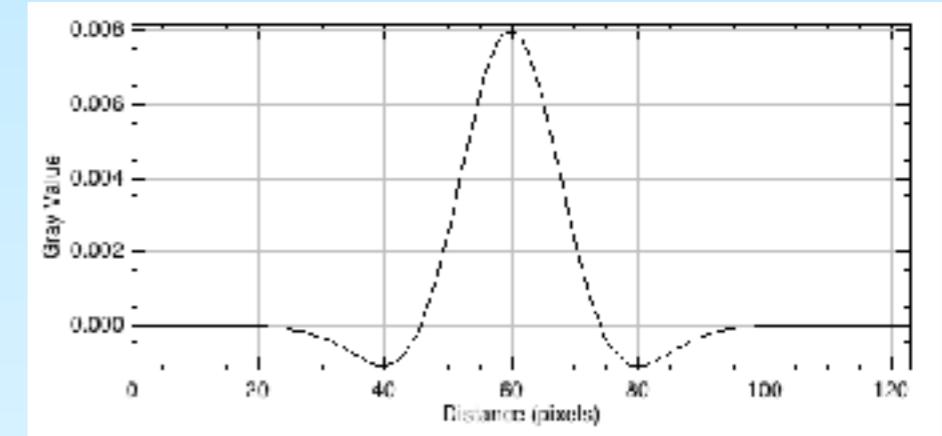
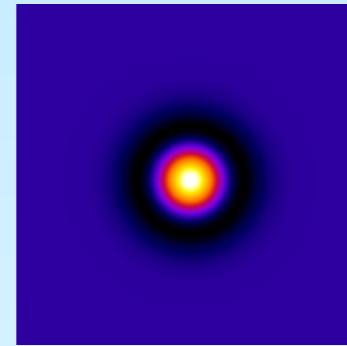


Gaussian of laplacian filters

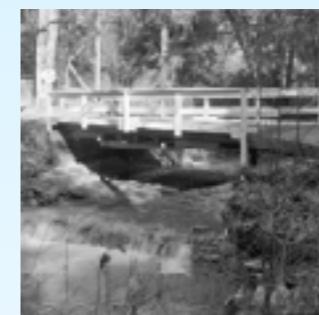
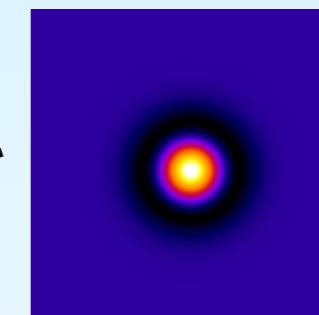
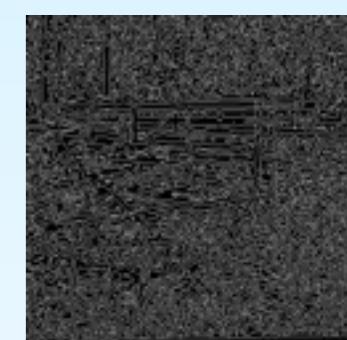
In practice discrete filters are noisy.



$$\begin{matrix} 0 & -1 & 0 \\ * & -1 & 4 & -1 \\ 0 & -1 & 0 \end{matrix} =$$



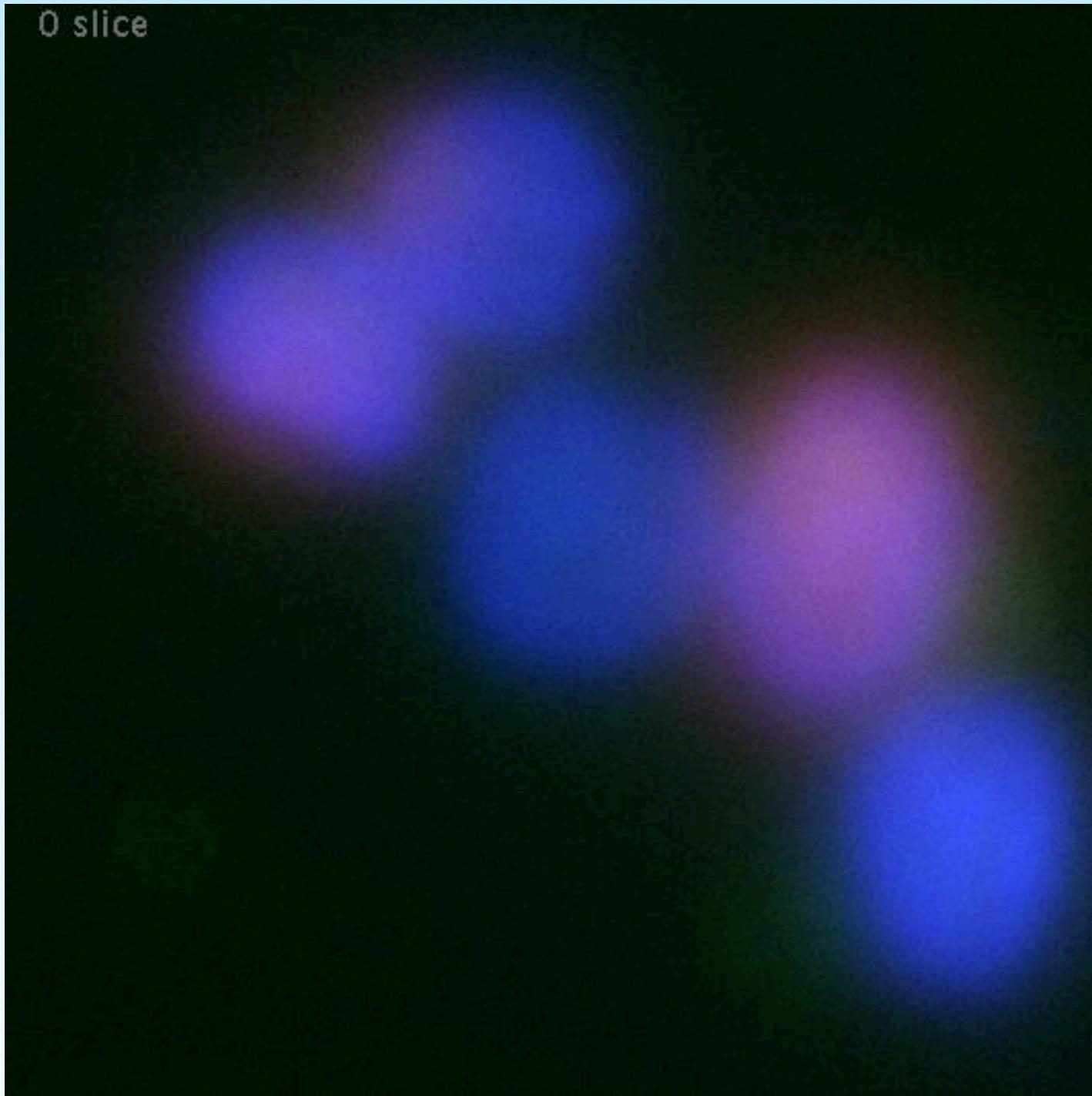
$$\begin{matrix} 0 & -1 & 0 \\ * & -1 & 4 & -1 \\ 0 & -1 & 0 \end{matrix} =$$

 $*$  $=$ 

zero crossing

We can prevent the effects of this by convolving our filter (or our image first with a Gaussian filter).

Motivation for Analysis



Acquired on a Deltavision wide-field microscope.

Multiple z-slices.

The data is very rich, 3-D.

We want to find the colocalisation of the ATRX protein (Green) and the YH2Ax protein (Red)

Source: David Clynes

Motivation for Analysis

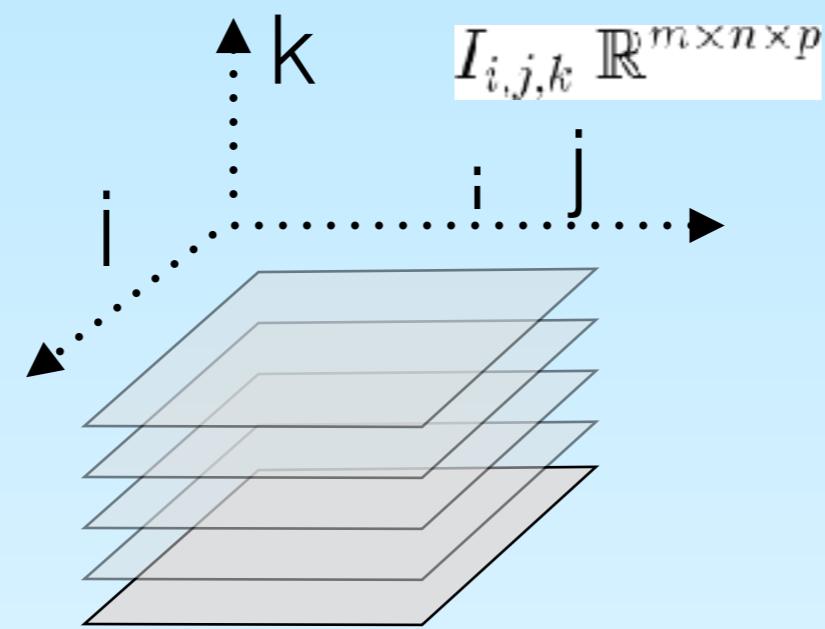
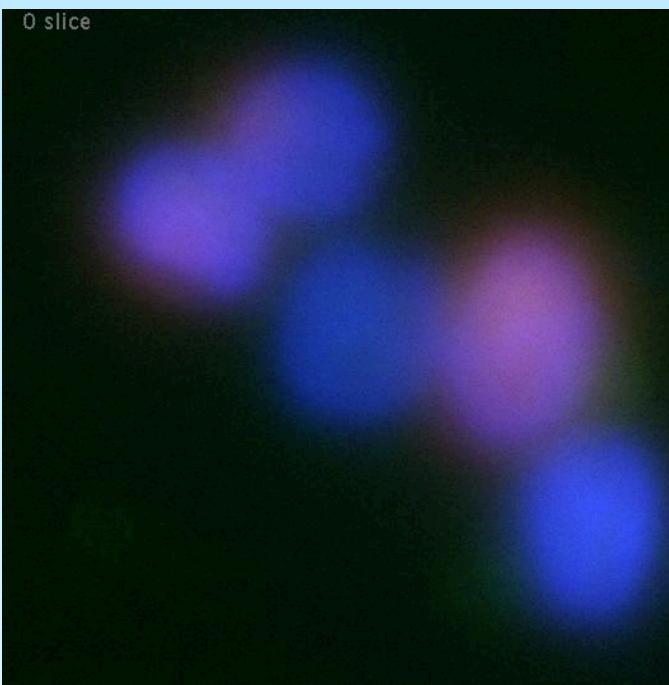
Early in your experimental pipeline you need to decide what the role of analysis is in your work.

- Is your research focus about developing novel analysis to solve a problem.
- Is your research about using analysis to investigate a biological question.

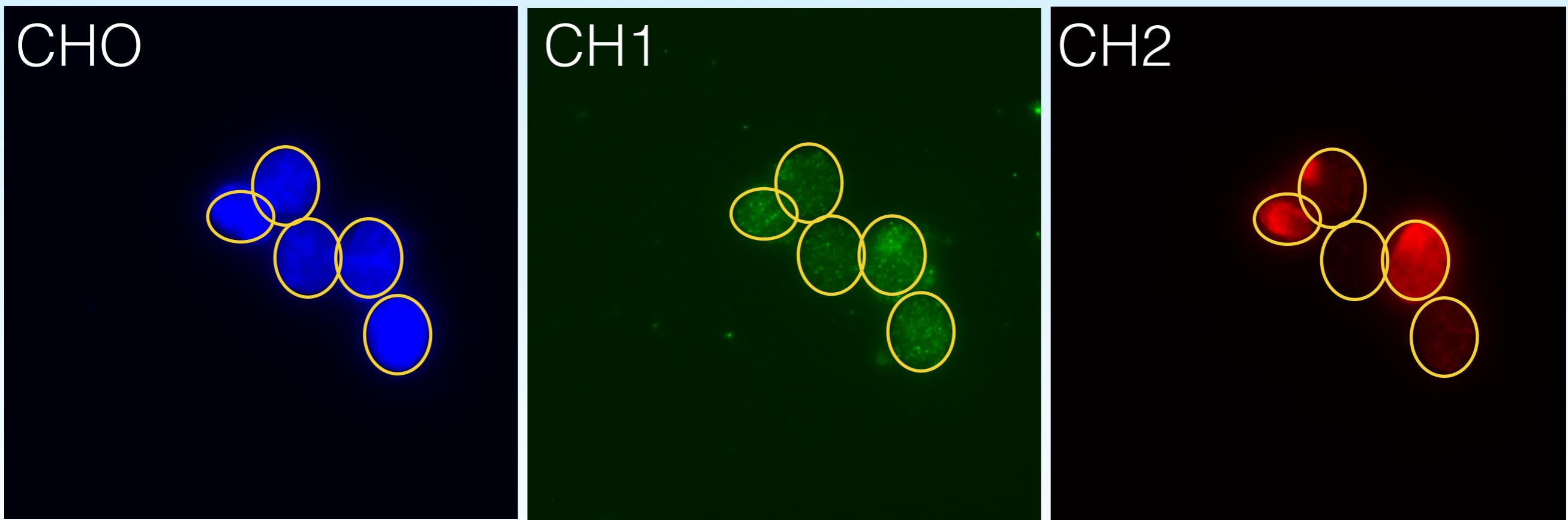
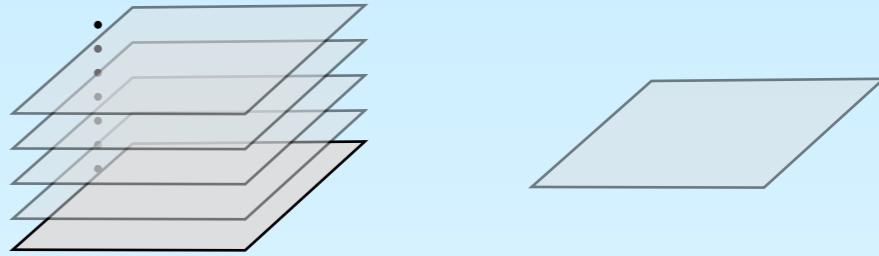
In this case we are just interested in creating a read-out for the biological experiment which is efficient and robust.

Source:

Typical imaging experiment

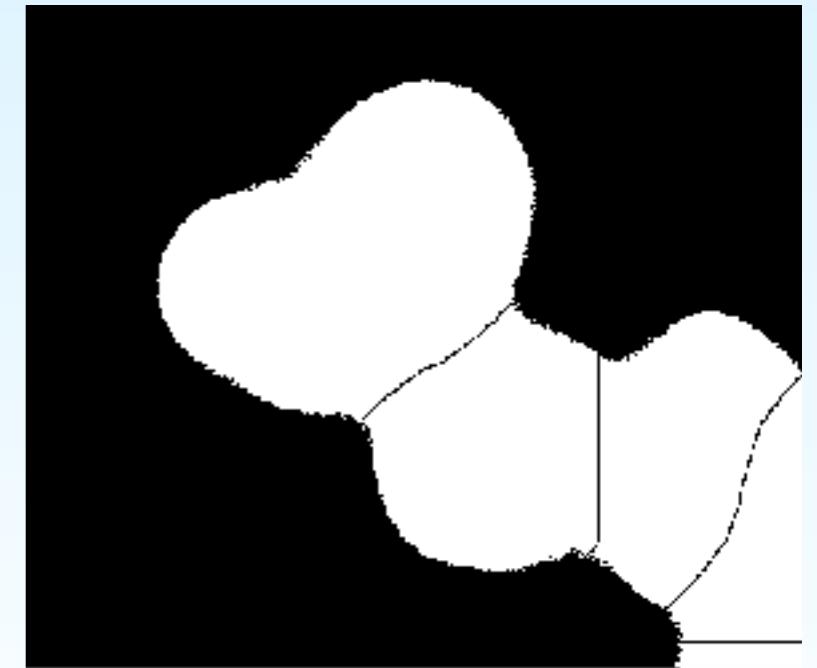
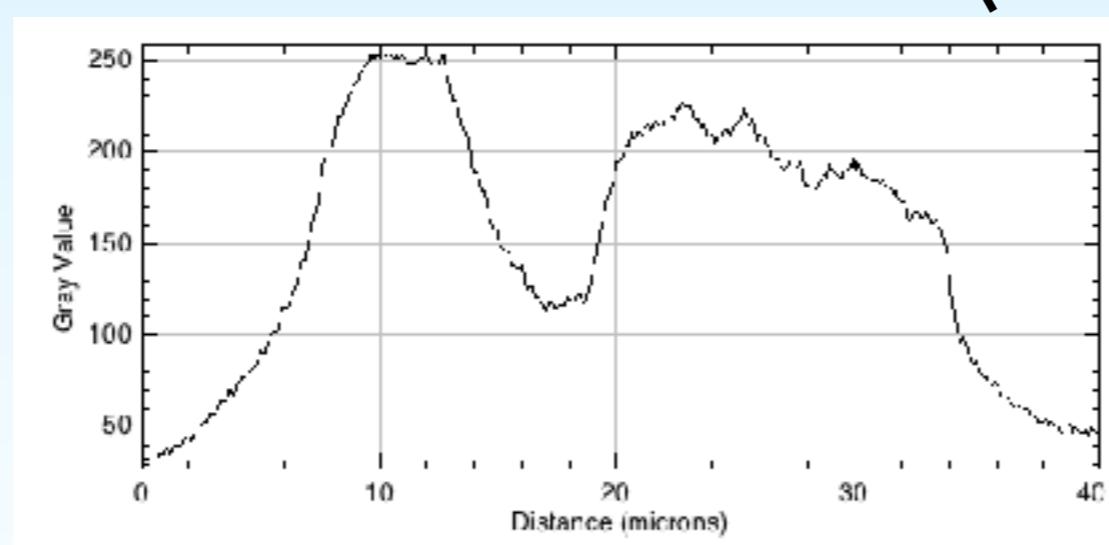
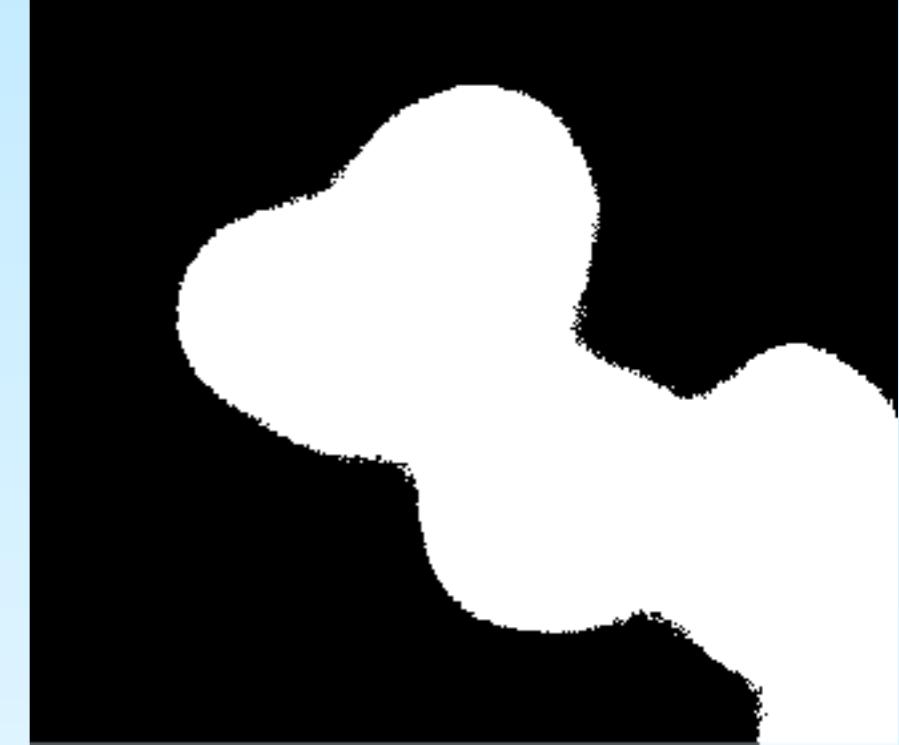
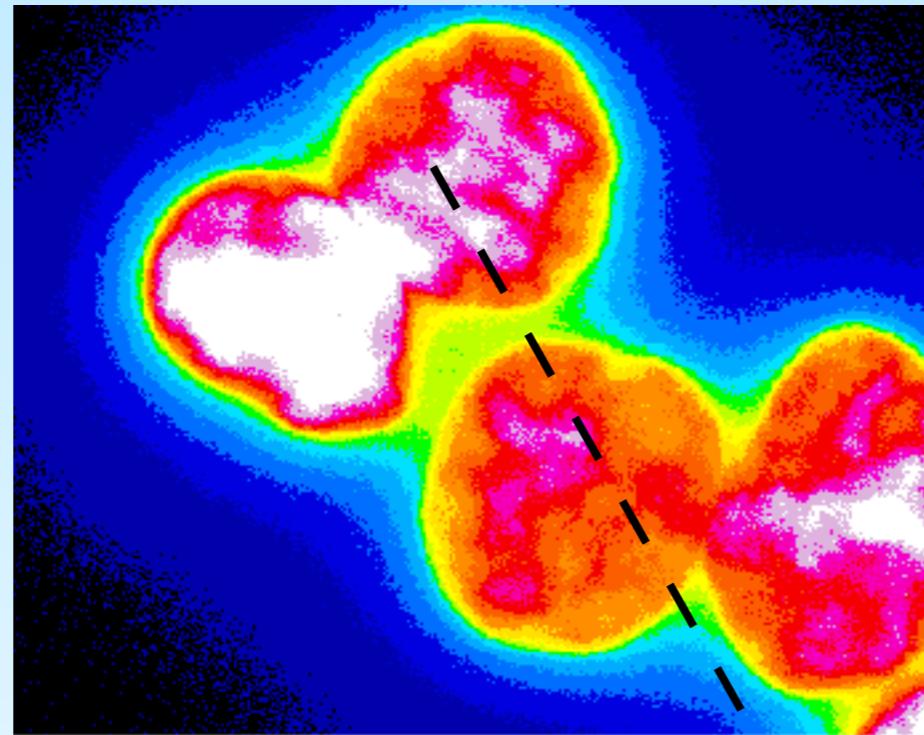
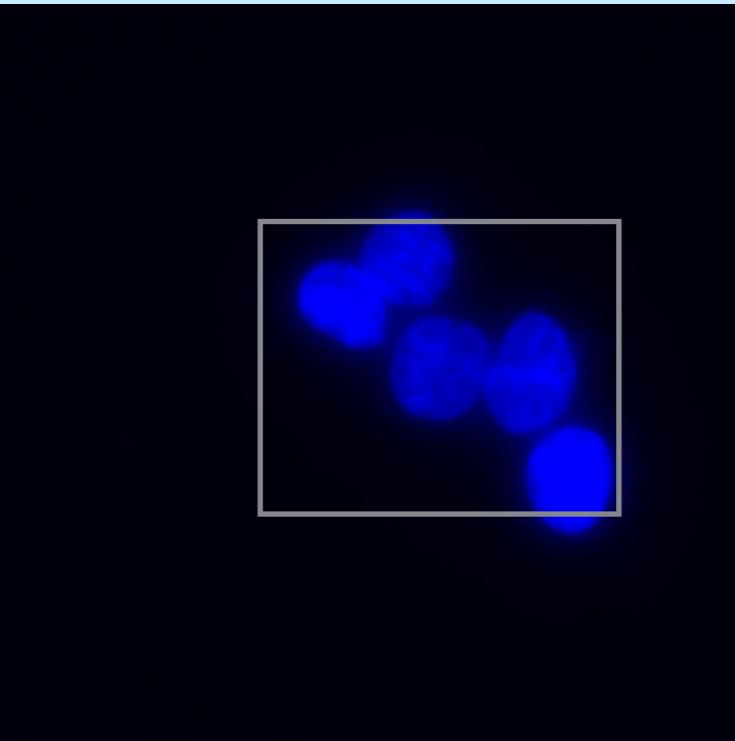


$$I_{i,j} = \max_{k=1}^P(I_{i,j,k})$$



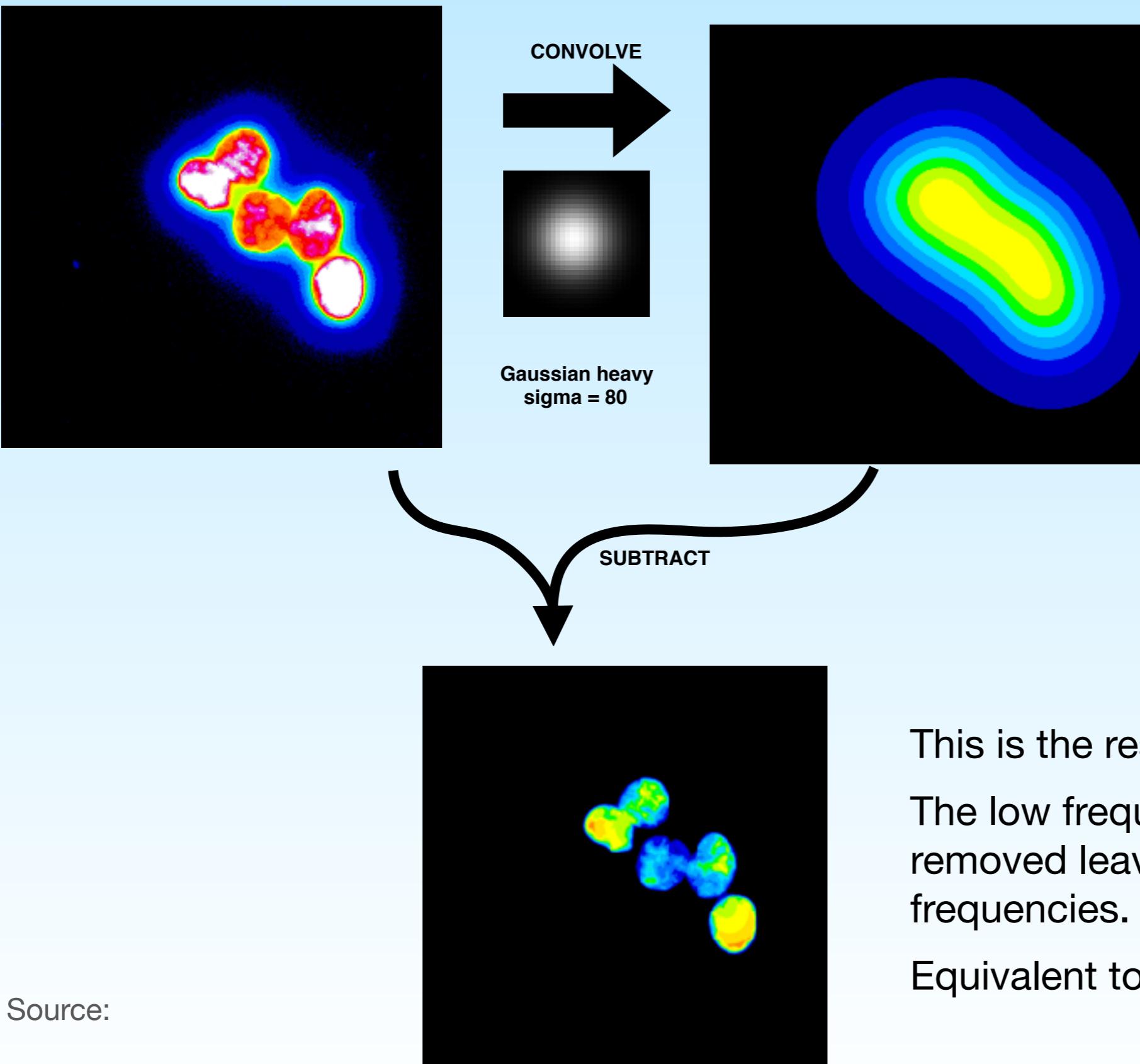
We want to isolate image regions into individual cells and then make measurements.

High background signal



Cells will be hard to separate. We need to remove this smooth background signal. This background signal is low-frequency image signal.

Background removal, using filtering

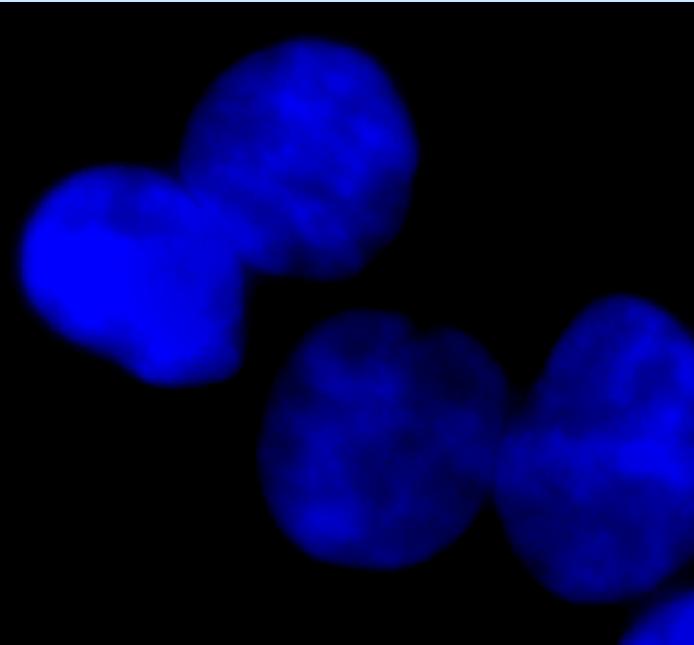


Low frequencies of
image.
equivalent to a low
pass filter

This is the resulting image.
The low frequencies have been
removed leaving all the other
frequencies.
Equivalent to a high pass filter.

Source:

Segmentation much improved.



Filtered image



Segmented image



Watershedded image

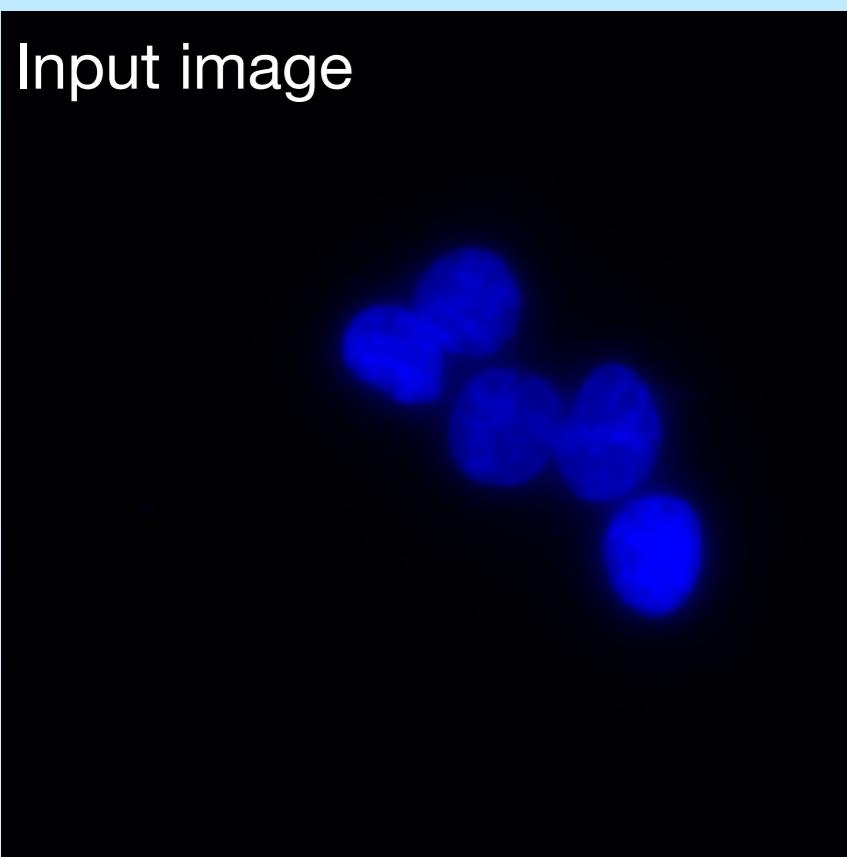


Segmented cells

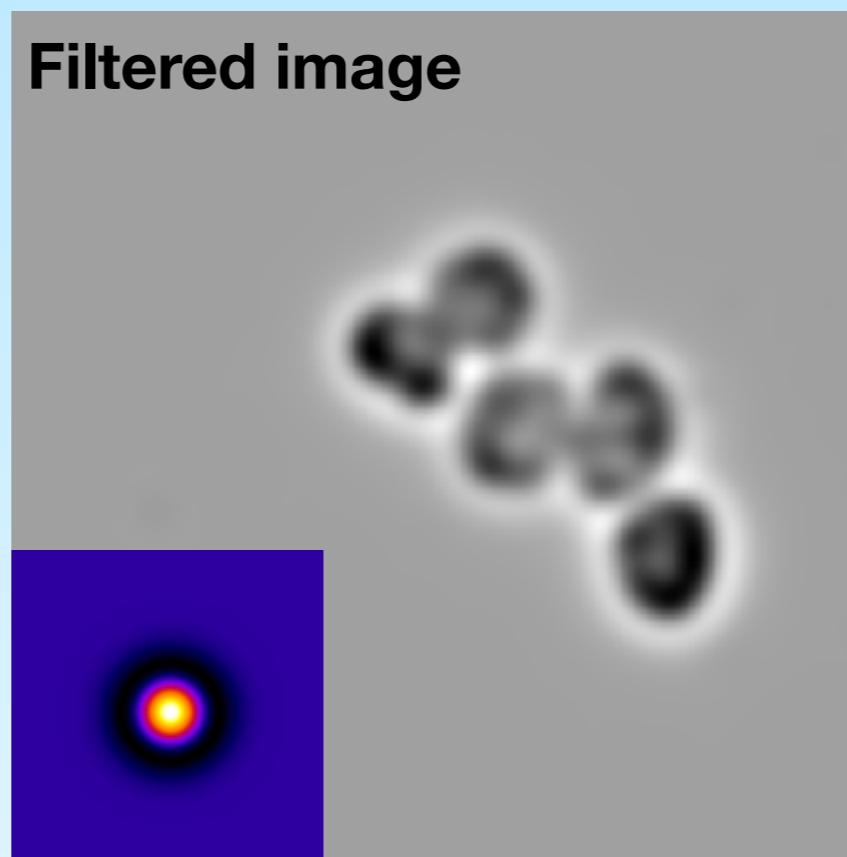
Source:

Smoothed laplacian filter

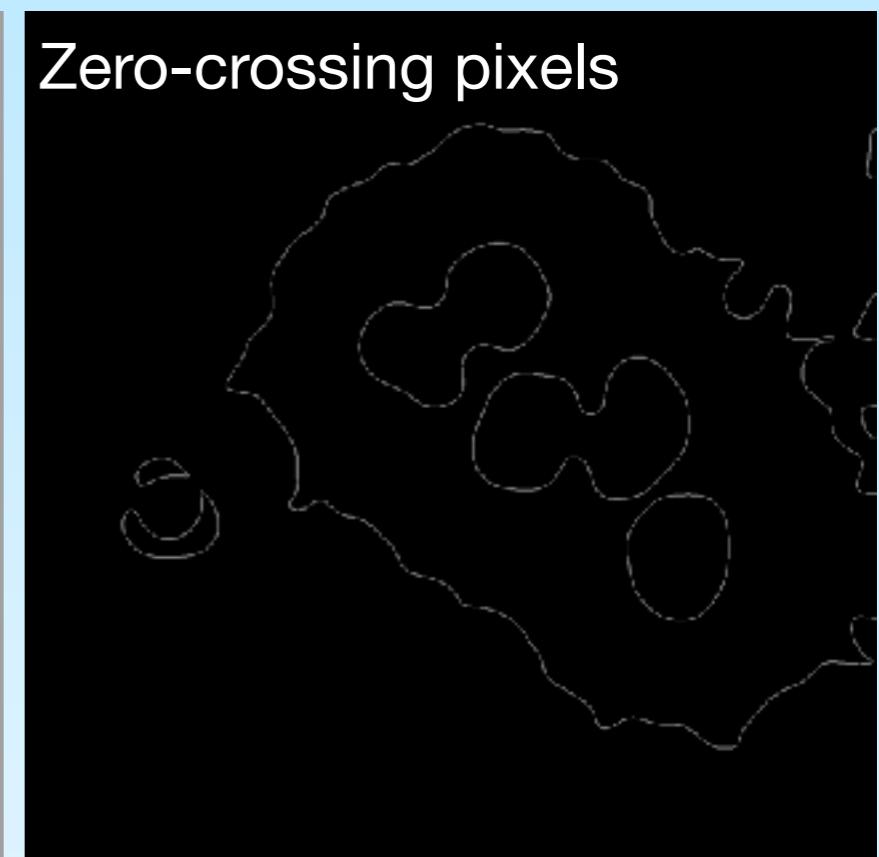
Input image



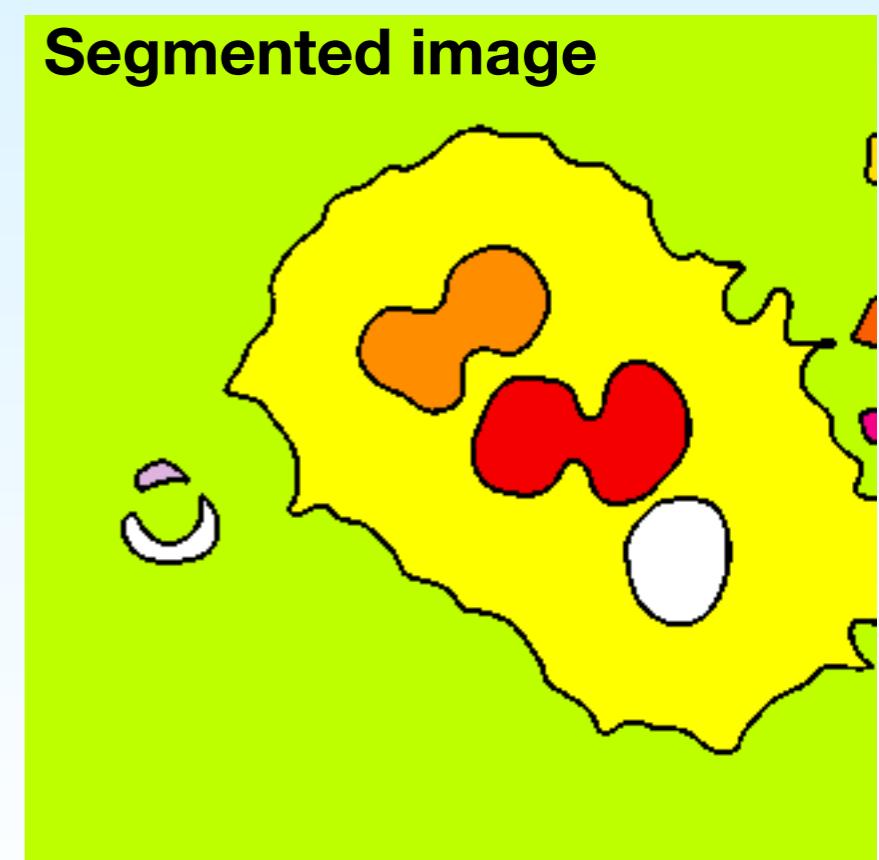
Filtered image



Zero-crossing pixels



Segmented image

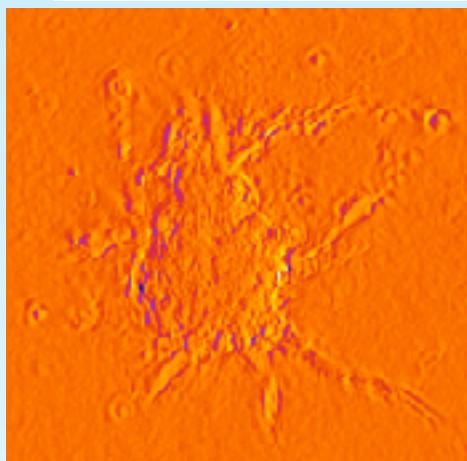


Source:

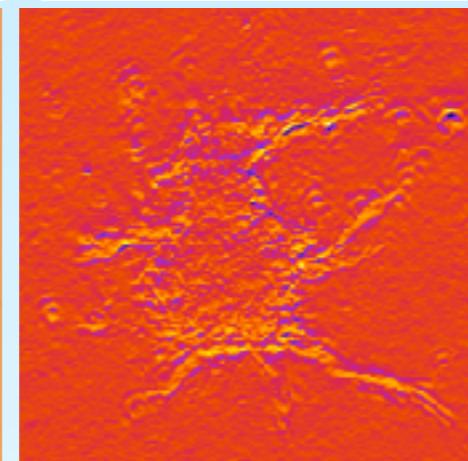
Feature calculation

If you filter and image with

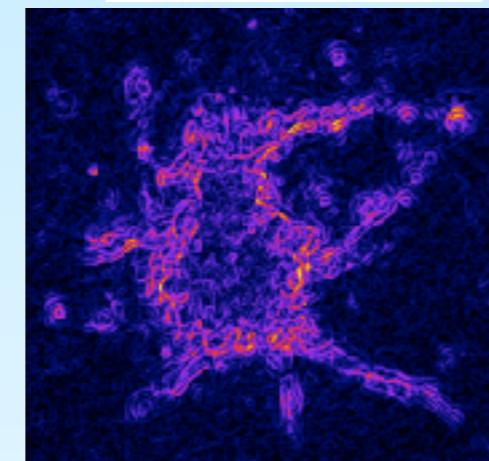
derivative-X



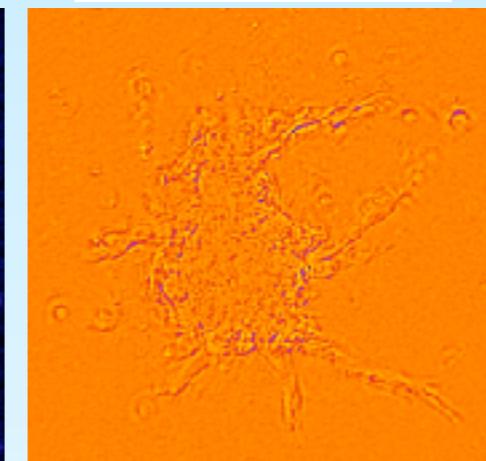
derivative-Y



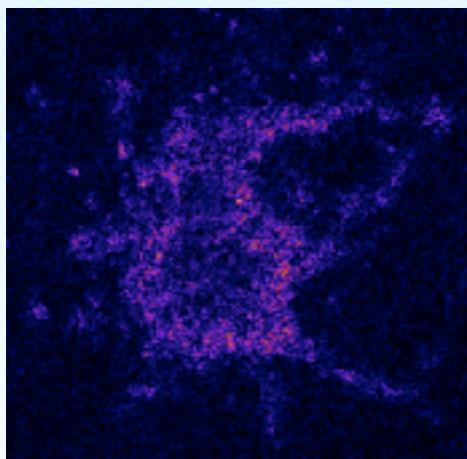
Edge mag.



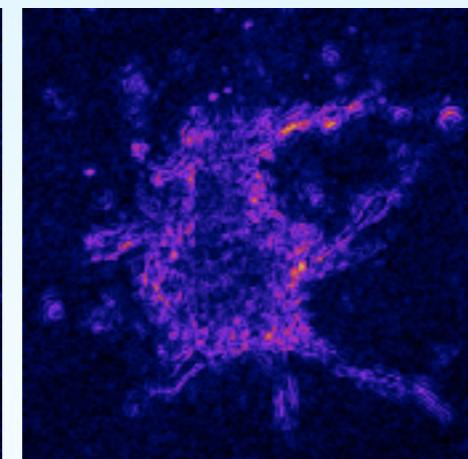
Laplacian



Hessian min

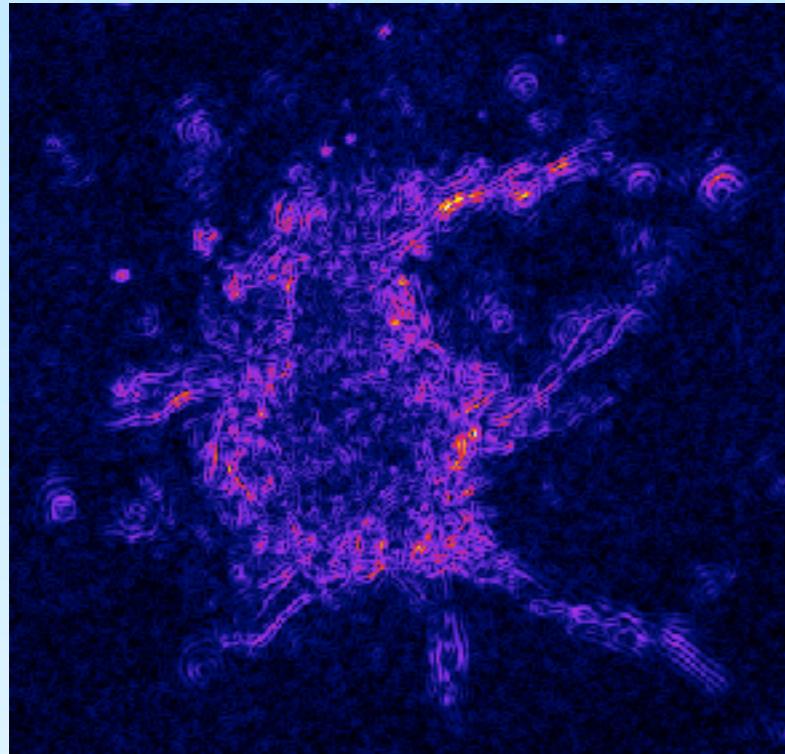


Hessian max

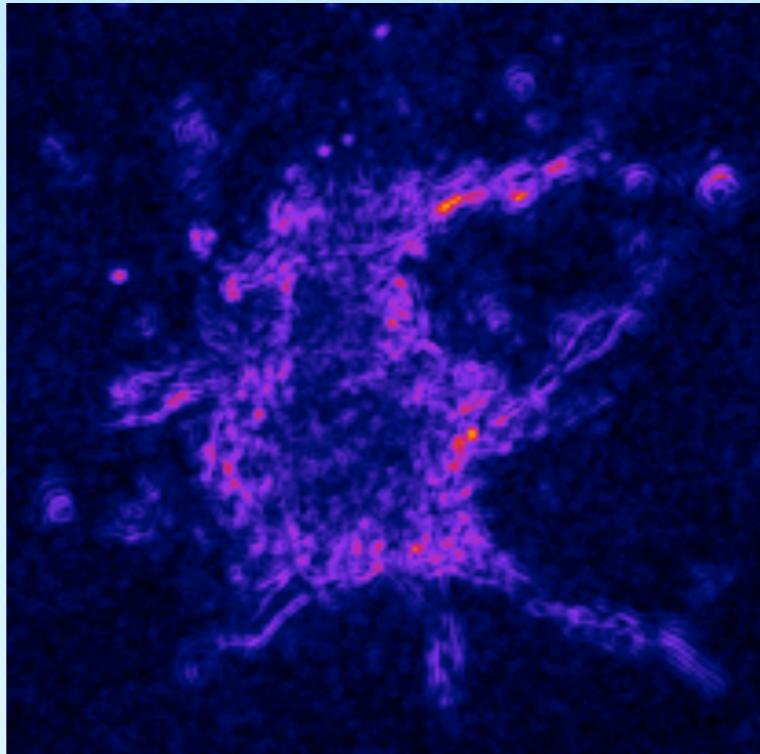


Source:FeatureJ

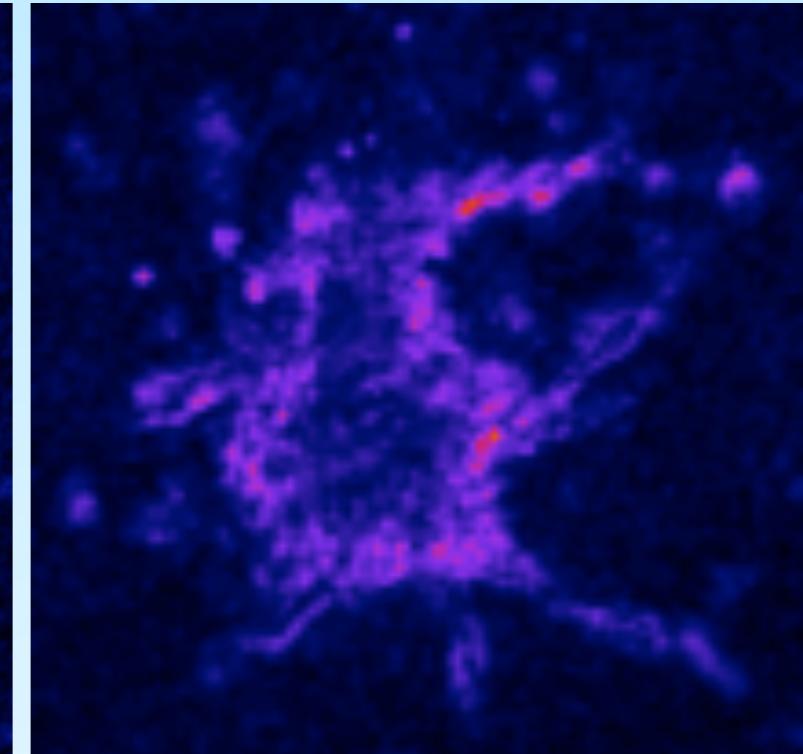
Feature Calculation different scales



sigma = 1.0

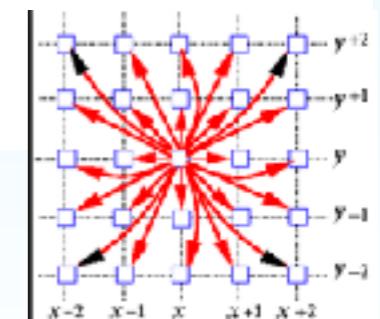


sigma = 4.0



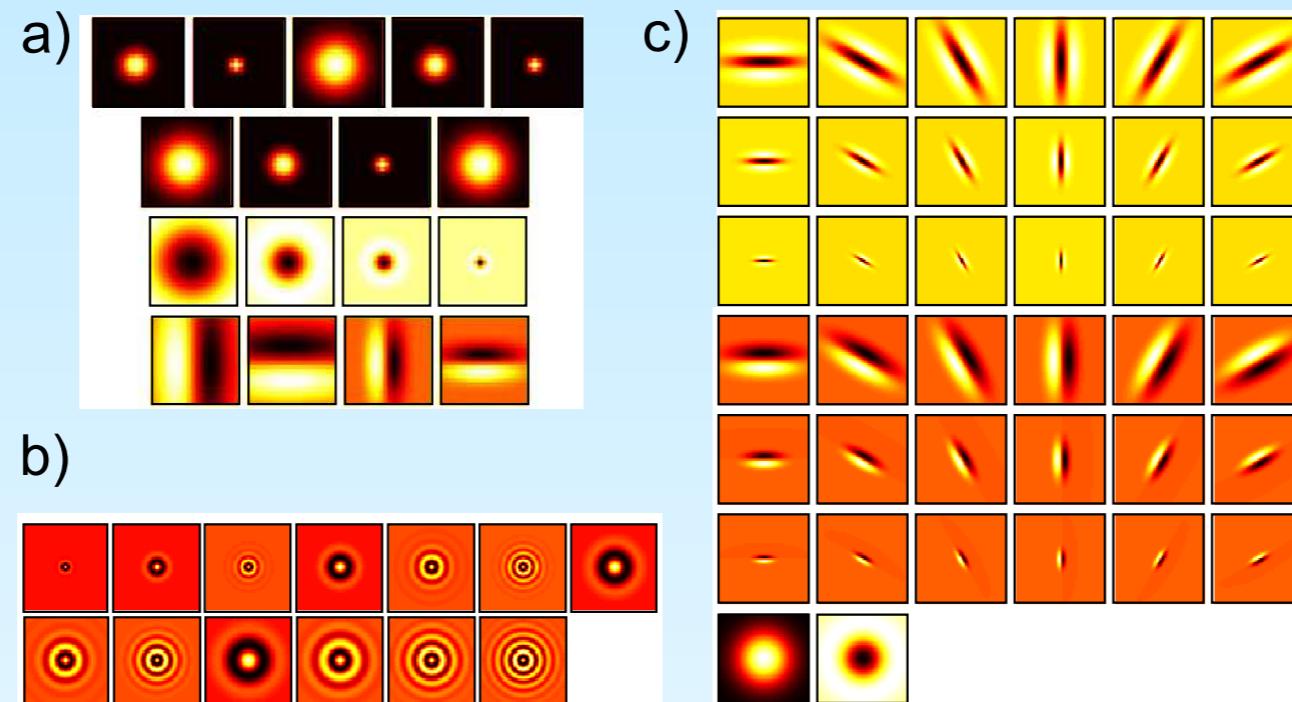
sigma = 8.0

By blurring the image and applying the different filters you get a sense of the greater neighbourhood. Many scales can be used in combination.



Source:

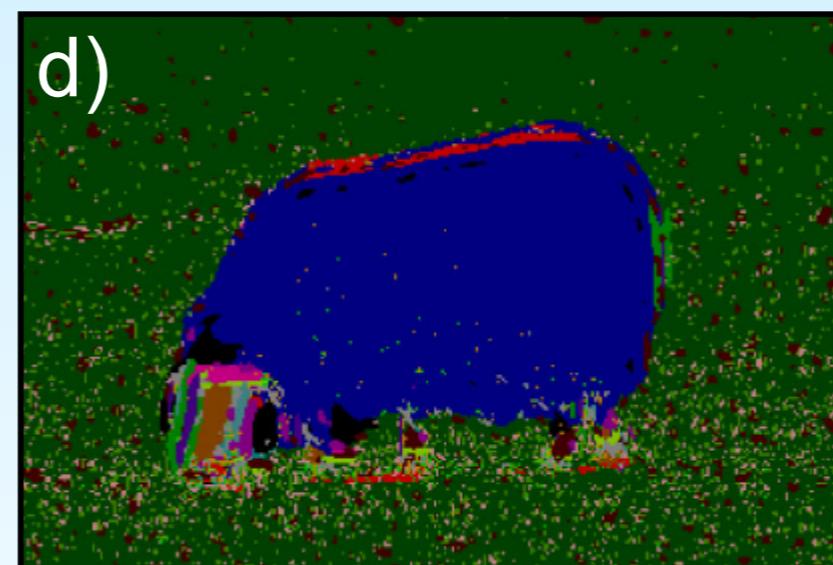
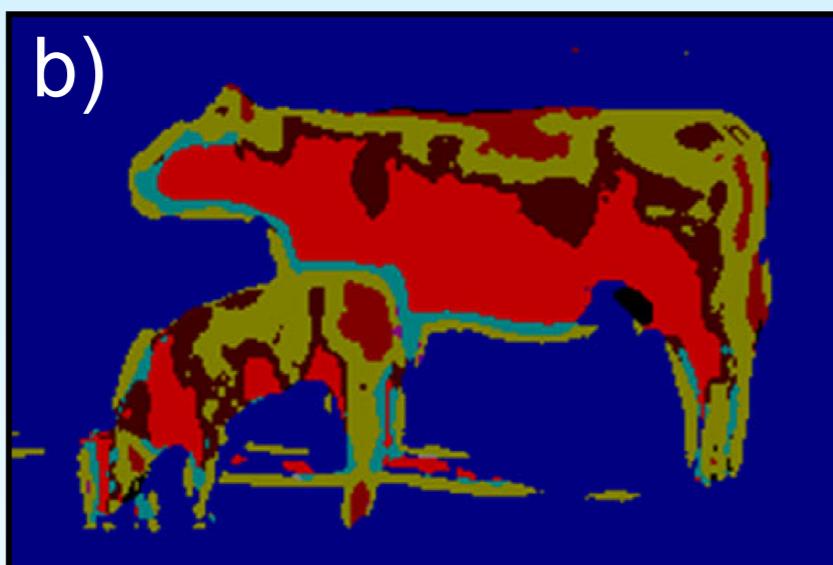
Filter banks



- a) A combination of Gaussians, derivatives of Gaussians, Laplacians of Gaussians
- b) Some rotationally invariant filters.
- c) The maximum response MR8 filter bank

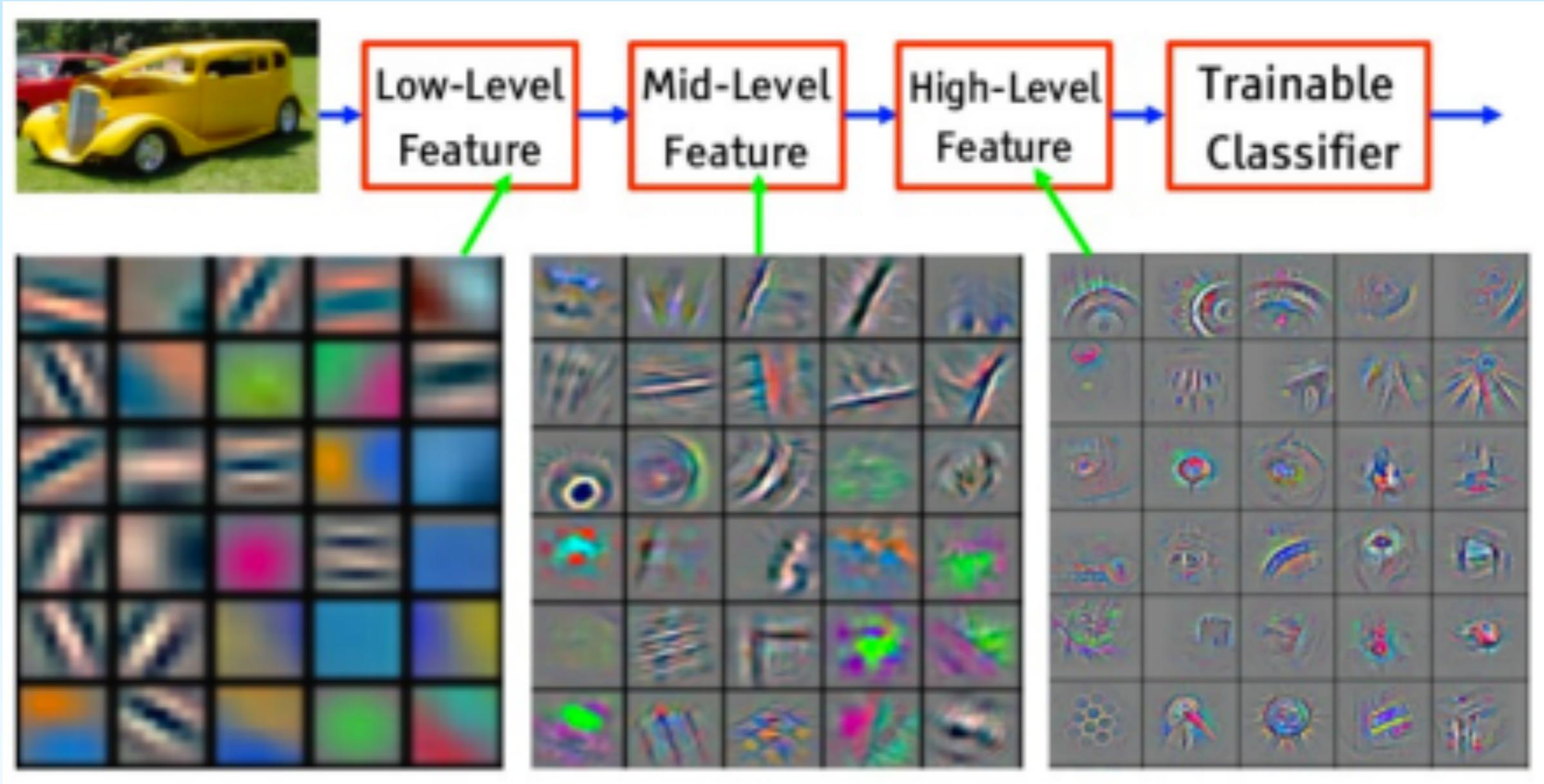
Source: Computer Vision, Simon Prince

Filters banks used for semantic segmentation



Source: Computer Vision, Simon Prince

These days filters banks are learnt using deep learning



You will cover this in more detail later in the week.

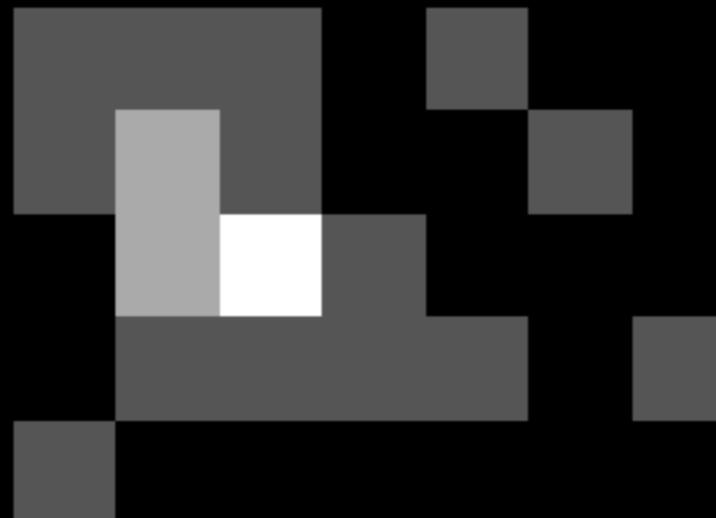
Source: www.quora.com%2FWhat-is-a-convolutional-neural-network

Deconvolution

$$f * g = h$$

convolution

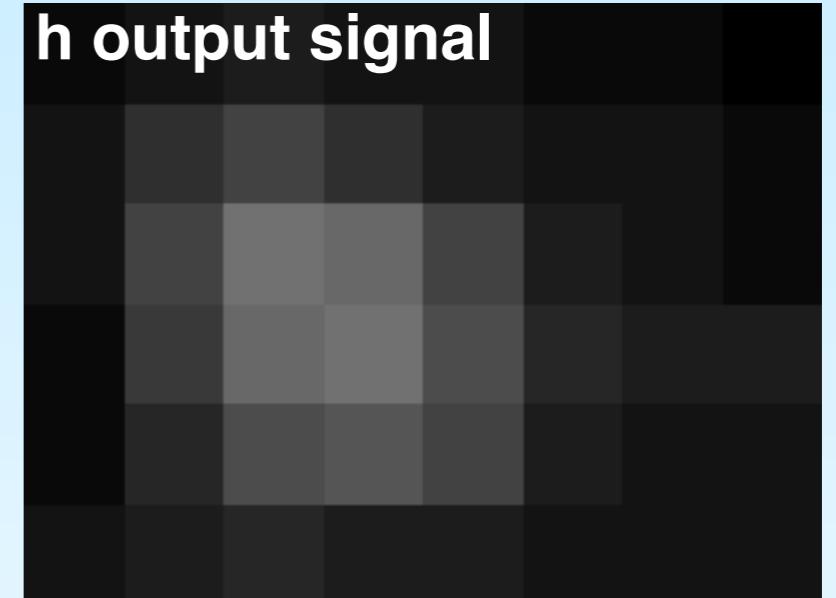
f the signal we are after



1	1	1
1	1	1
1	1	1

kernel: 3x3 mean filter

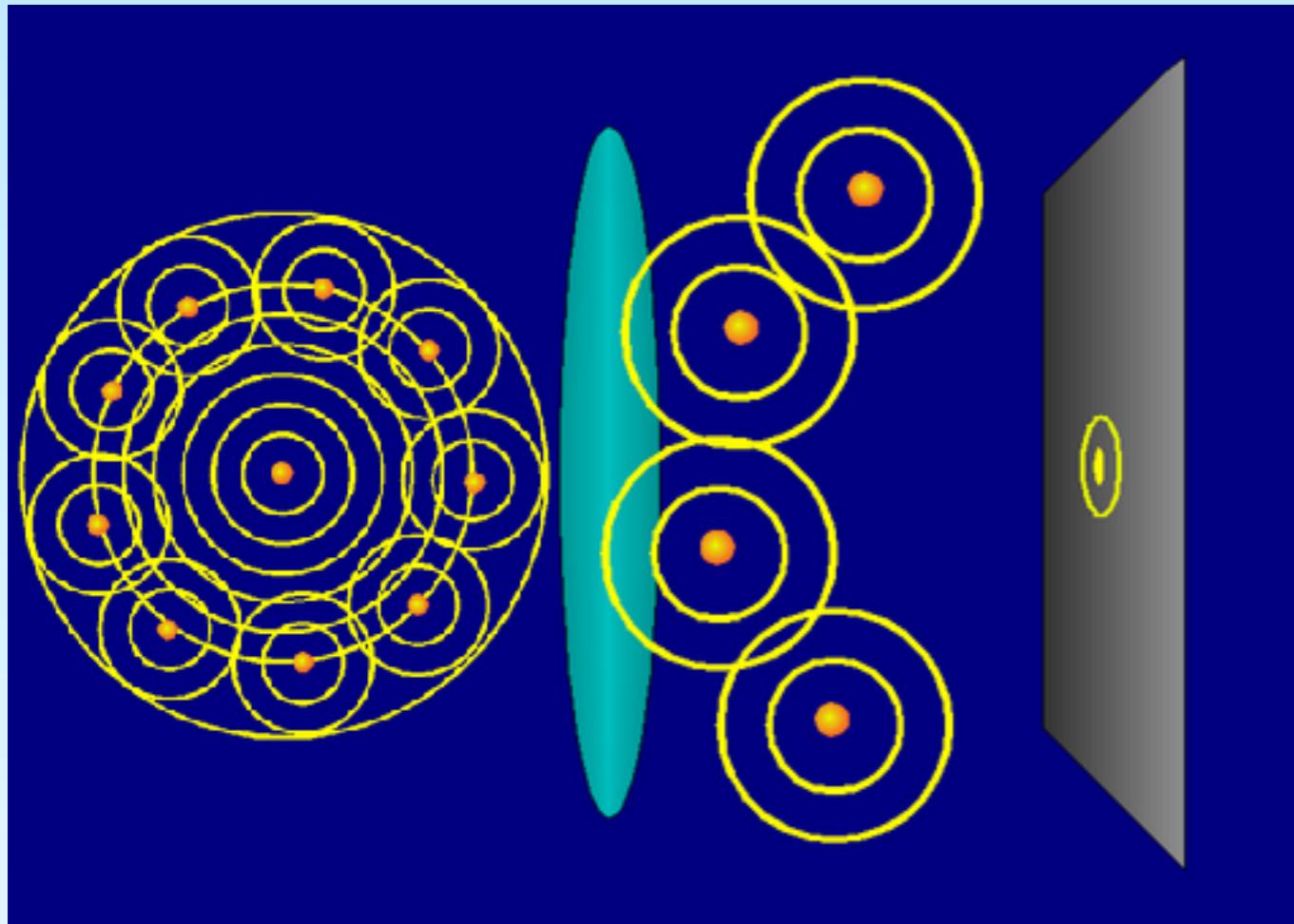
h output signal



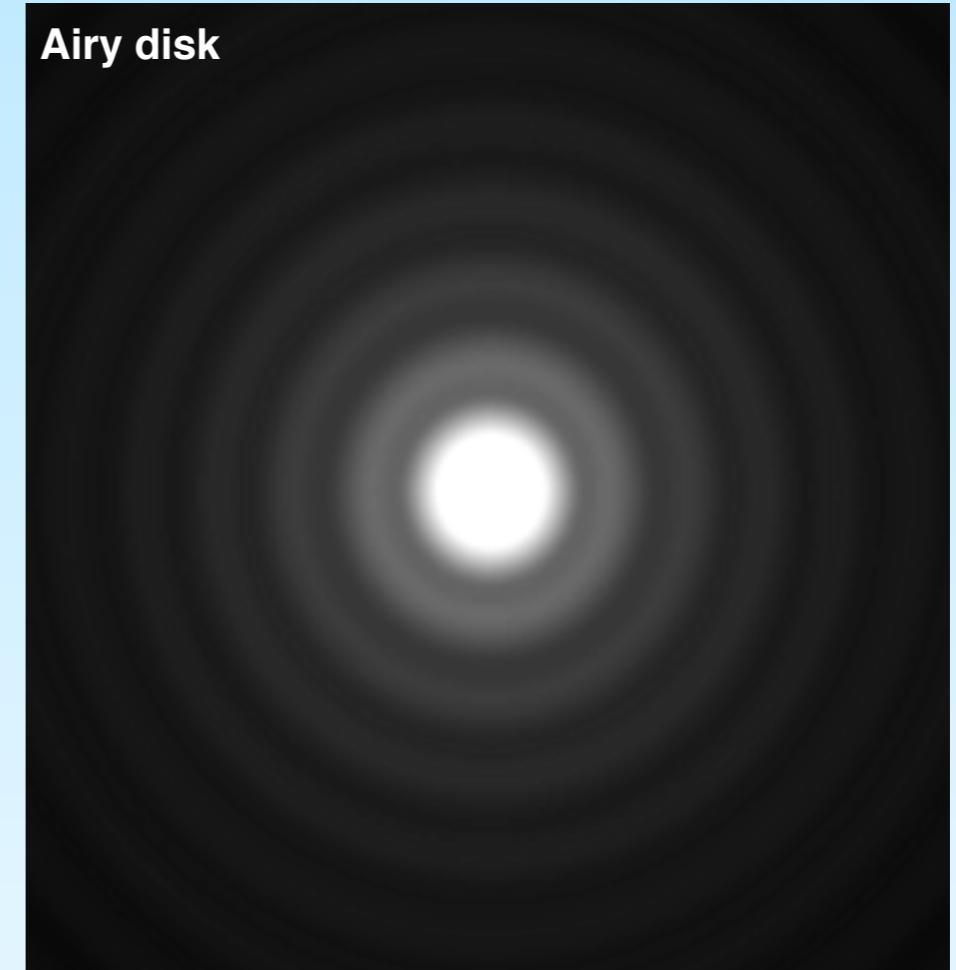
deconvolution

The goal of deconvolution is to find f given the kernel g and the output signal h

Deconvolution: Why we deconvolve?



Airy disk

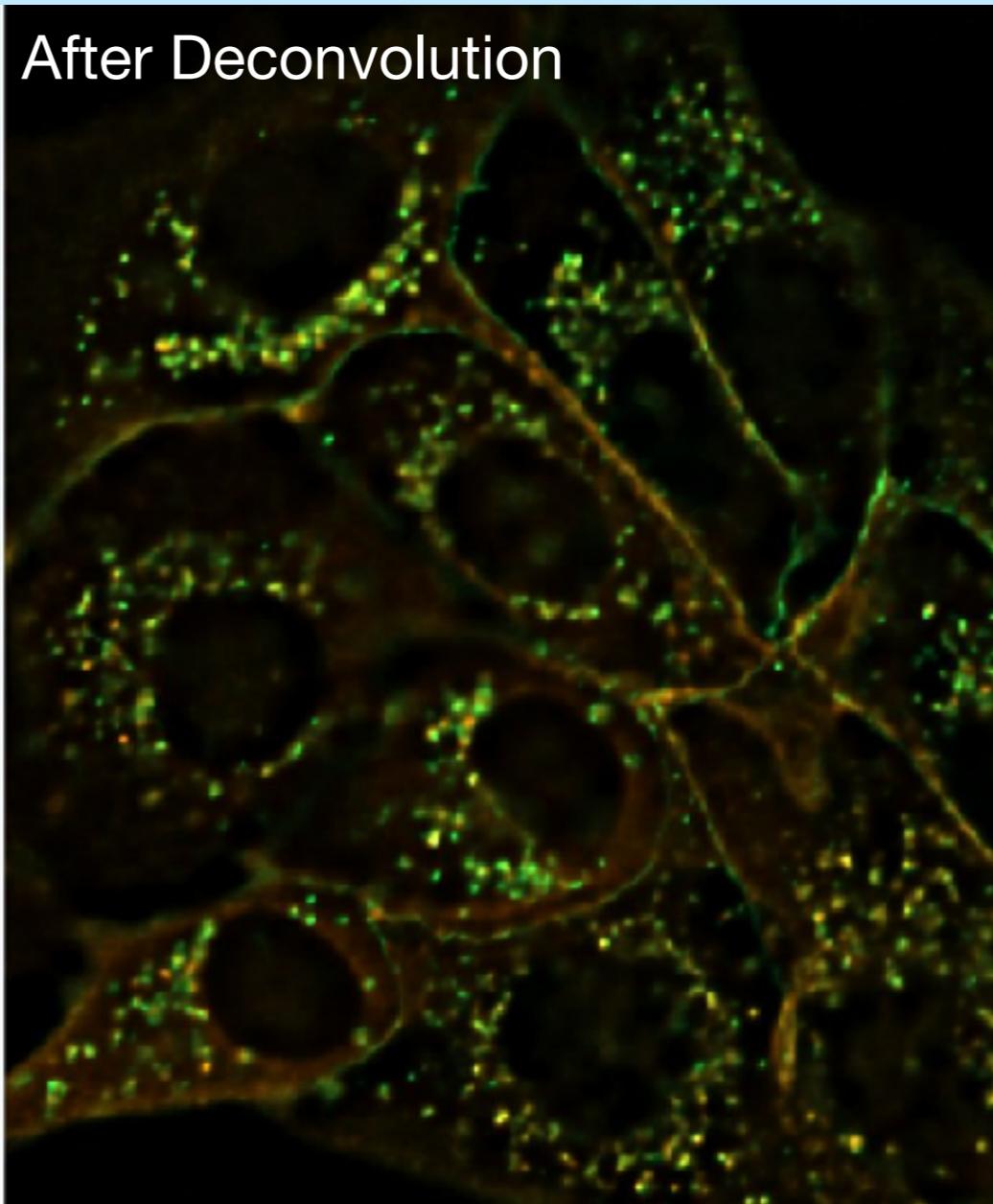
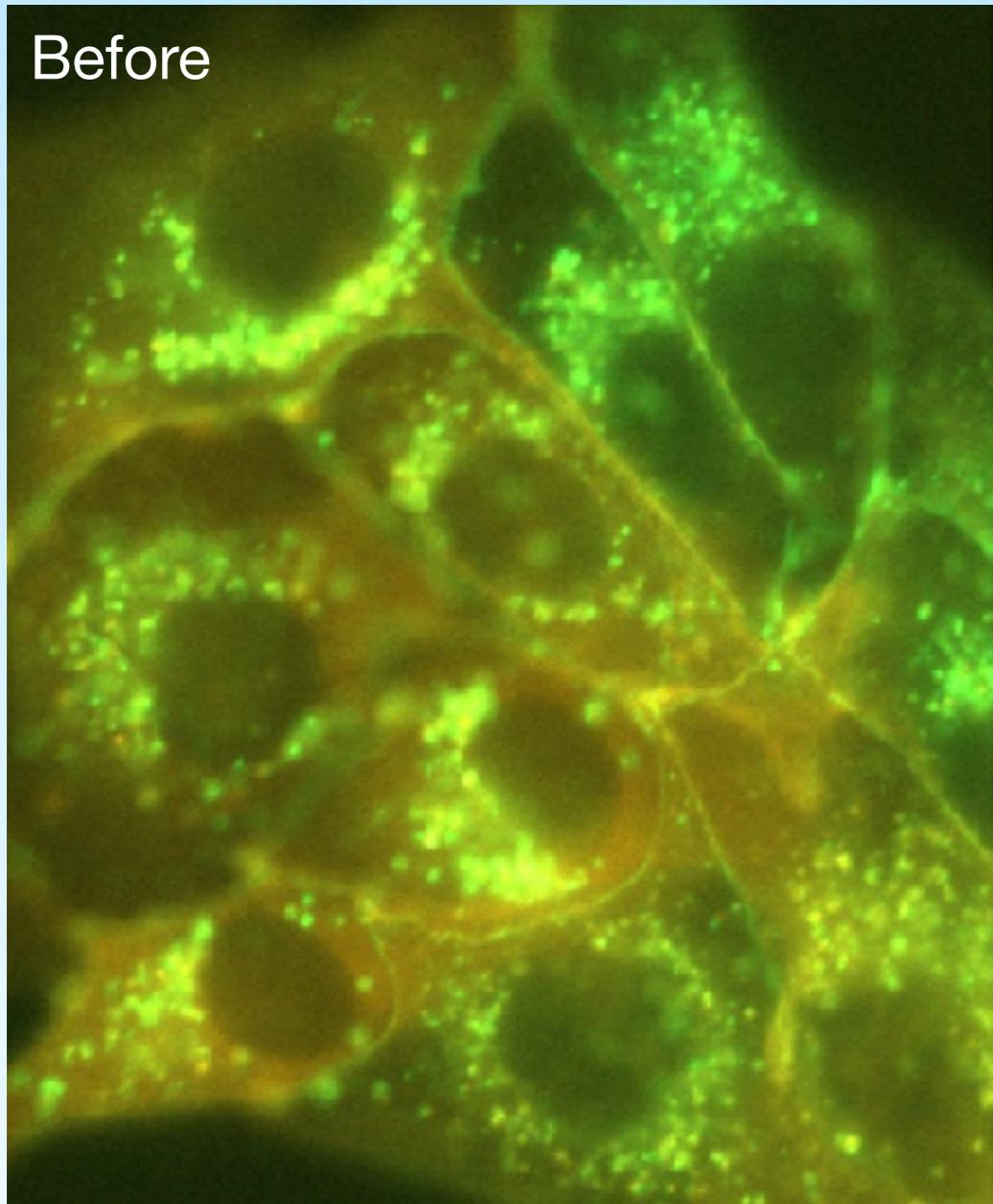


We have out-of-focus light contributing to our image plane, but we also have an artefact of the collection process influencing our final image.

Deconvolution seeks to remedy this issues.

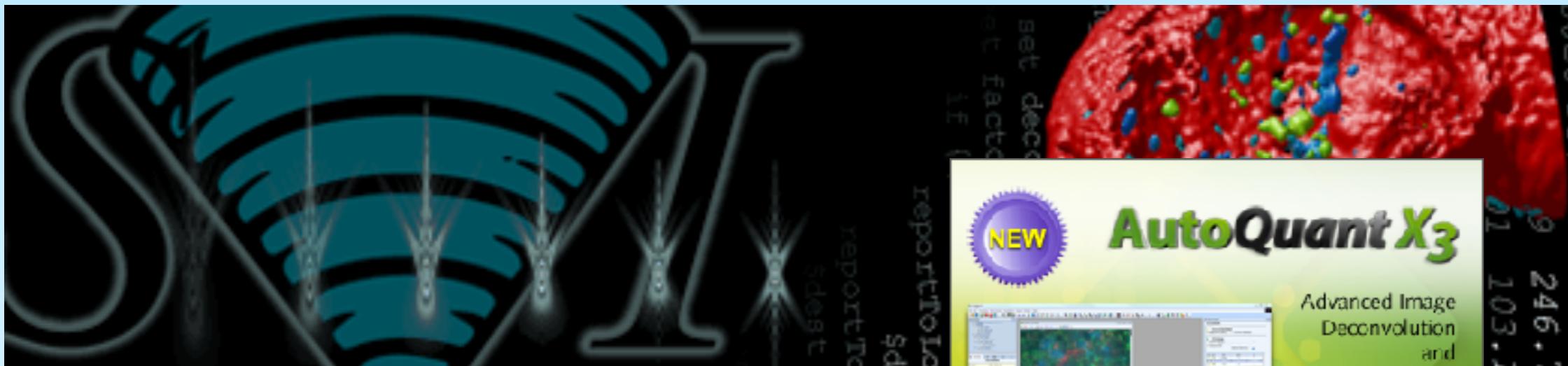
Source: www.huygens.org

Huygens software



Deconvolution of cell-cell junctions of MDCK cells.
Nikon Ti widefield microscope (Objective 40x; 1.3 NA oil lens).

Deconvolution software



Deconvolution

- Image restoration
- Volume Visualisation
- Some analysis

Expensive

WIMM CBRG has the 2nd fastest Huygens server in the world.



Source: <http://www.svi.nl/HuygensSoftware>, <http://www.mediacy.com/index.aspx?page=AutoQuant>, <http://api.gehealthcare.com/api/softworx-suite.asp>

Conclusions

- Image processing is fundamental to image analysis
- Filtering has many uses including noise removal
- Filtering can be linear or non-linear and plays a role in many different types of processing.
- Using many filters in coordination allows texture analysis of objects and pixels.

Thank you for time

<https://twitter.com/dwaithe>



<https://github.com/dwaithe>



dominic.waithe@imm.ox.ac.uk



Practical content and lectures

Doctoral Training Centre (MPLS)
Resources /Modules/ 2017-18 /
Michaelmas term / Week 9-10: Foundations
of Image Analysis / day01_practicals.html

Summary of course: Timetable Template 2017-18.pdf

Practicals: day01_practicals.html

Source: