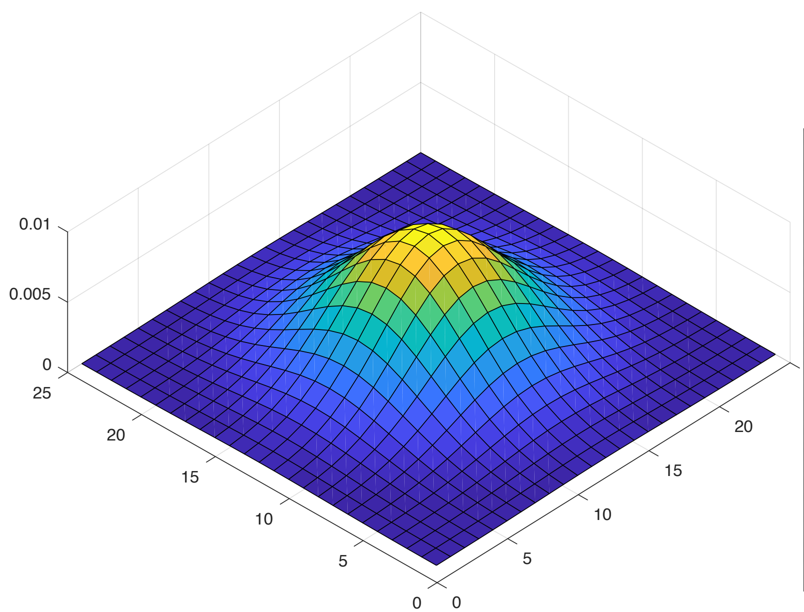
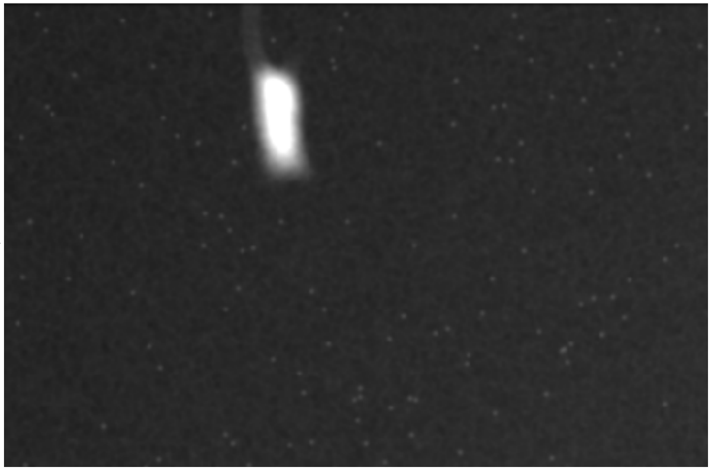
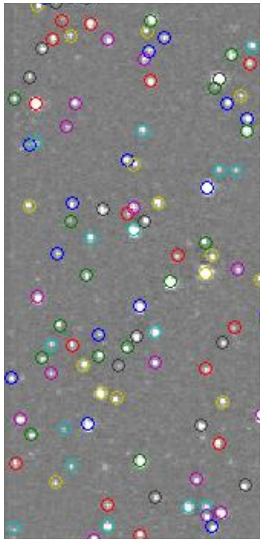
Lecture 12

Image/video and data analysis (2)

Assc. Prof. Halil Bayraktar
Molecular Biology and Genetics







Today,
Image histogram
Intensity profiles
Image convolution, low pass filter
1D, 2D filter

What is a digital image?

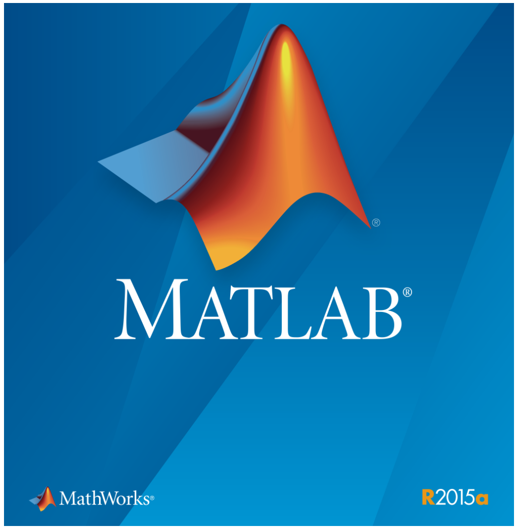
Image can be considered as a matrix that is composed by pixels.Color representation of data.

A number is assigned to each pixel that carry all the information.

What type of digital images are used?

1. Binary Image
2. Gray scale image
3. Color image
4. True color image

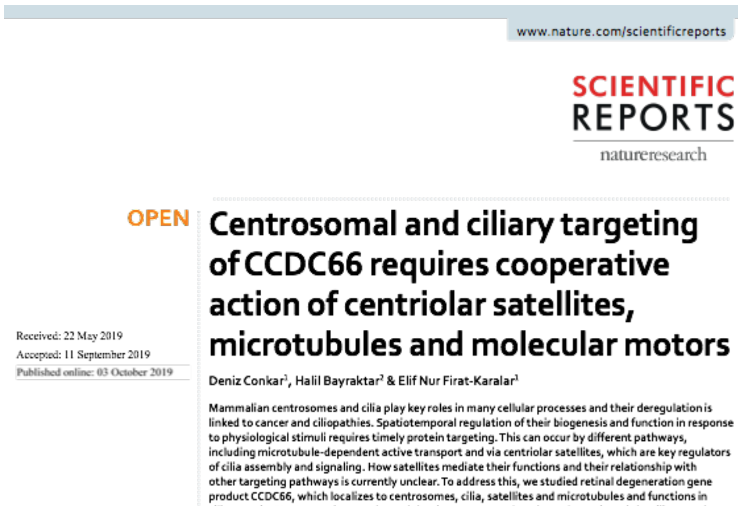
Custom programs for analysis of biological systems



Where do we use custom programming to determine the properties/dynamics of biological systems

1. Image/video processing
2. Genome analysis
3. Microarray analysis
4. Proteomics analysis
5. Advance graphics

Example: Understanding satellite dynamics leads us to develop new computer algorithms.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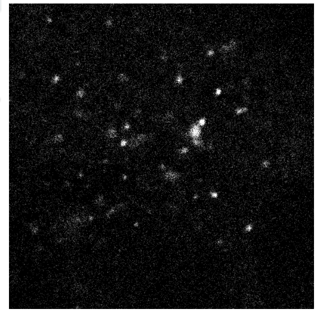
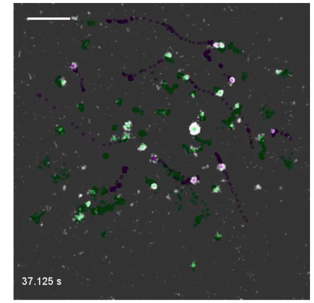
Centrosomal and ciliary targeting of CCDC66 requires cooperative action of centriolar satellites, microtubules and molecular motors

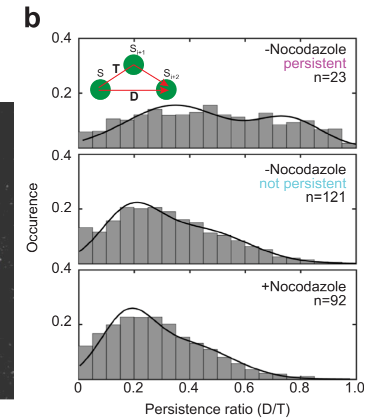
Deniz Conkar[1], Halil Bayraktar[2] & Elif Nur Firat-Karalar[1]

Mammalian centrosomes and cilia play key roles in many cellular processes and their deregulation is linked to cancer and ciliopathies. Spatiotemporal regulation of their biogenesis and function in response to physiological stimuli requires timely protein targeting. This can occur by different pathways, including microtubule-dependent active transport and via centriolar satellites, which are key regulators of cilia assembly and signaling. How satellites mediate their functions and their relationship with other targeting pathways is currently unclear. To address this, we studied retinal degeneration gene product CCDC66, which localizes to centrosomes, cilia, satellites and microtubules and functions in

Raw data







Challenges on the project:

1. Noisy images/videos
2. Moving objects
3. New parameters are needed to determine satellite dynamics
4. Advance graphics for data visualization

Our solutions was;

1. Noise free images
2. Build a custom tracking algorithms for moving objects (Satellites)
3. Compute new parameters : persistence, speed, distance, number etc.
4. Custom solutions for data visualization

Result: Analysis demonstrated that satellites can be distinguished based on their persistence ration and around centrosome they move both diffusively and persistently

Reading tif file name

- Spfile=dir('*.tif')

5x1 struct with 6 fields

Fields	name	folder	date	bytes	isdir	datenum
1	'Mark_and_Find 001_Position007_t000_R...	'/Users/...	'23-Jan-...	5532654	0	7.3781e+05
2	'cellimage.tif'	'/Users/...	'15-May...	5322953	0	7.3793e+05
3	'example1.tif'	'/Users/...	'13-May...	210949	0	7.3792e+05
4	'example2.tif'	'/Users/...	'13-May...	185639	0	7.3792e+05
5	'example3.tif'	'/Users/...	'13-May...	193645	0	7.3792e+05
6						
7						
8						

Each image comes with a metadata that demonstrates camera software, image properteis, where and how the image was generated.

This is useful when analyzing images and videos

```
Spfile(1).name
a = imfinfo(Spfile(1).name)
```

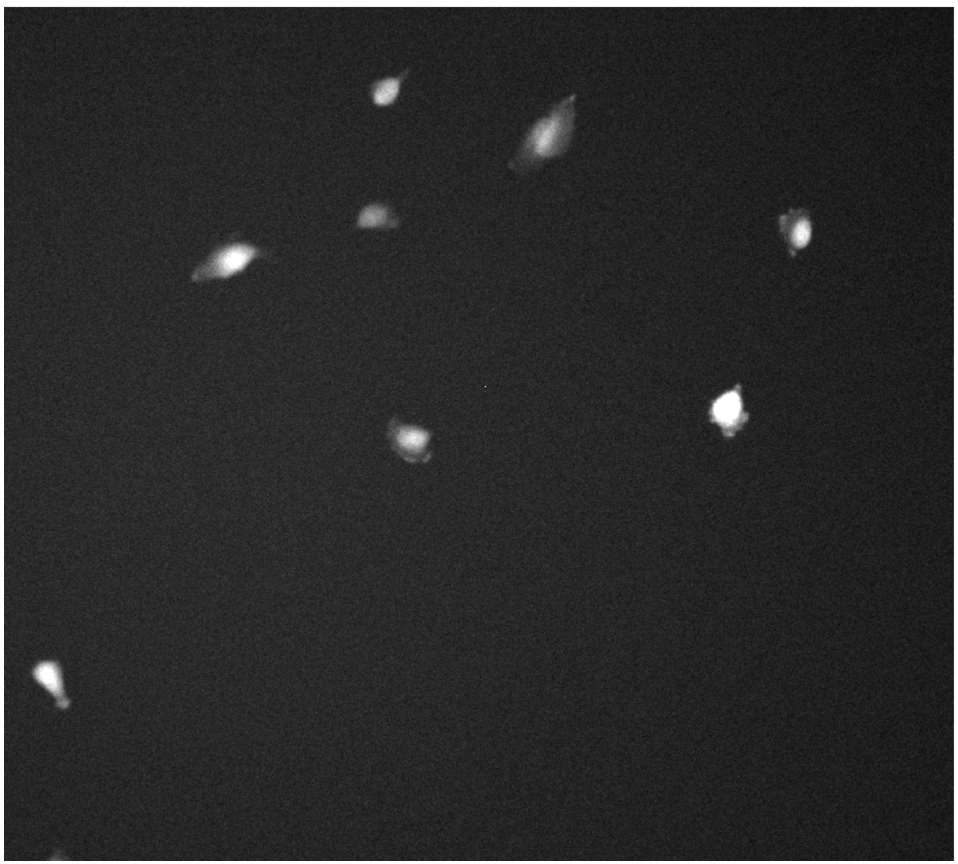
Field	Value
Filename	'/Users/halilbayraktar/Documents/Teaching/Scientific Computation...
FileModDate	'23-Jan-2020 16:04:24'
FileSize	5532654
Format	'tif'
FormatVersion	[ ]
Width	1920
Height	1440
BitDepth	16
ColorType	'grayscale'
FormatSignature	[73,73,42,0]
ByteOrder	'little-endian'
NewSubFileType	0
BitsPerSample	16
Compression	'Uncompressed'
PhotometricInte...	'BlackIsZero'
StripOffsets	1x360 double
SamplesPerPixel	1
RowsPerStrip	4
StripByteCounts	1x360 double
XResolution	1.5422e+04
YResolution	1.5422e+04
ResolutionUnit	'Centimeter'
Colormap	[ ]
PlanarConfigura...	'Chunky'
TileWidth	[ ]
TileLength	[ ]
TileOffsets	[ ]
TileByteCounts	[ ]
Orientation	1
FillOrder	1
GrayResponseUnit	0.0100
MaxSampleValue	65535
MinSampleValue	0
Thresholding	1

Size of an image

```
xsize = a(1).Width;
ysize = a(1).Height;
```

1920 pixels

1440 pixels



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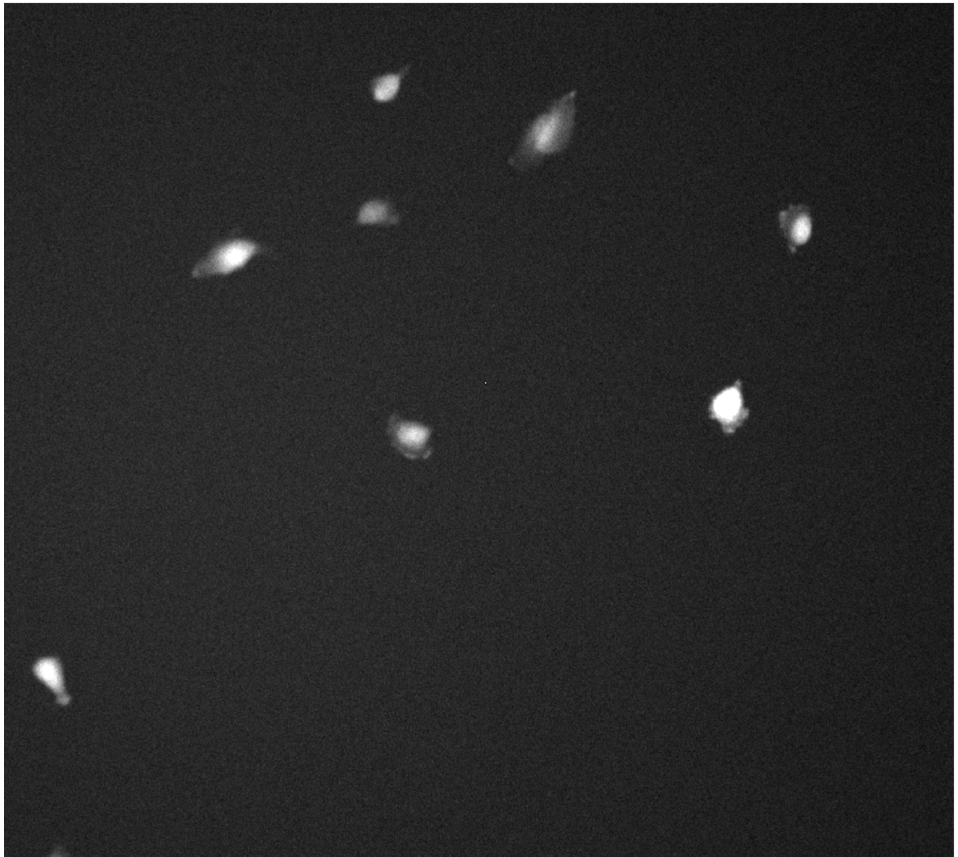
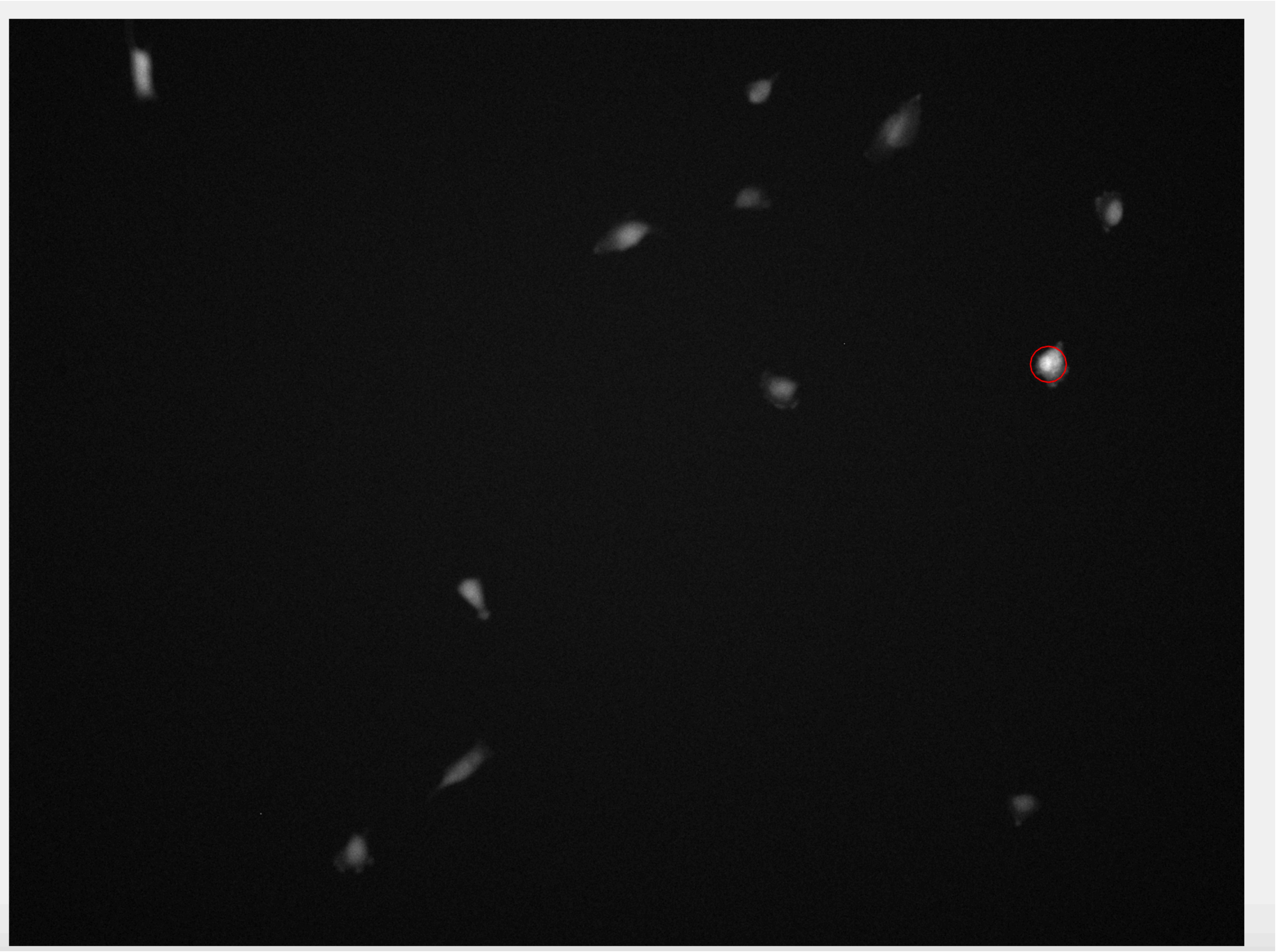
YResolution	1.5422e+04
ResolutionUnit	'Centimeter'
Colormap	[]
PlanarConfigura...	'Chunky'
TileWidth	[]
TileLength	[]
TileOffsets	[]
TileByteCounts	[]
Orientation	1
FillOrder	1
GrayResponseUnit	0.0100
MaxSampleValue	65535
MinSampleValue	0
Thresholding	1

Read images and show it in the figure

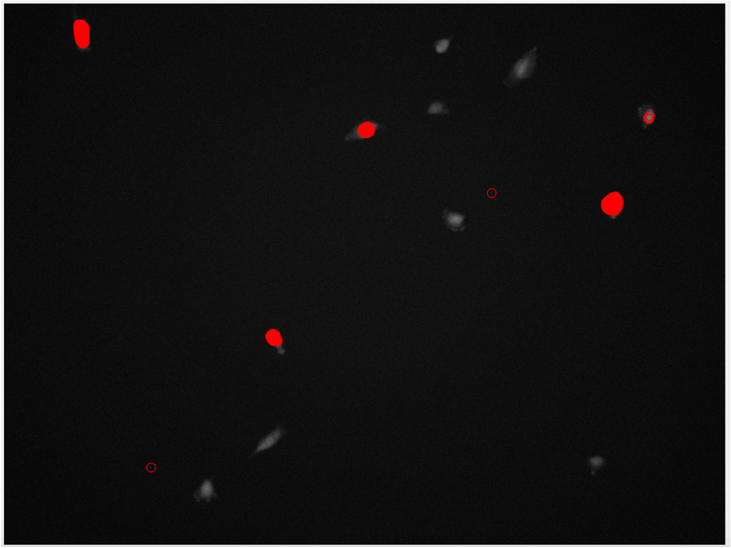
```
datB = imread(Spfile(1).name,
'tif', 1);

figure(1)
ax=imshow(datB,[min(min(datB)) max(max(datB))/3])
```

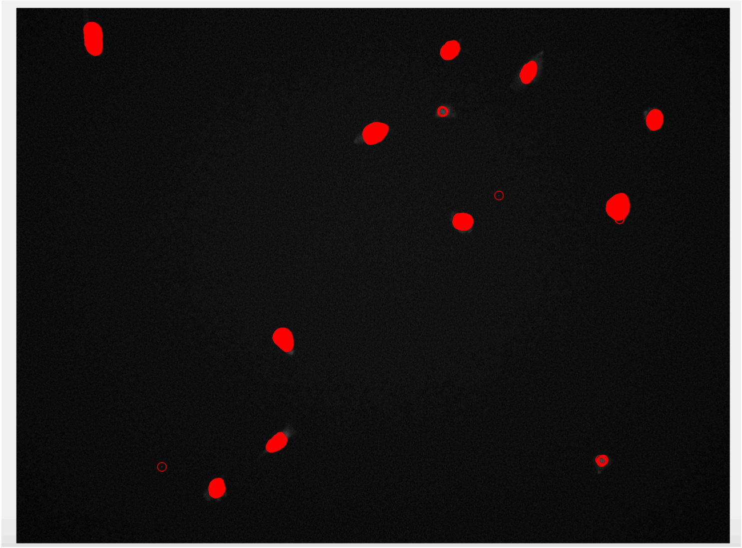


Finding brightest cell in the image



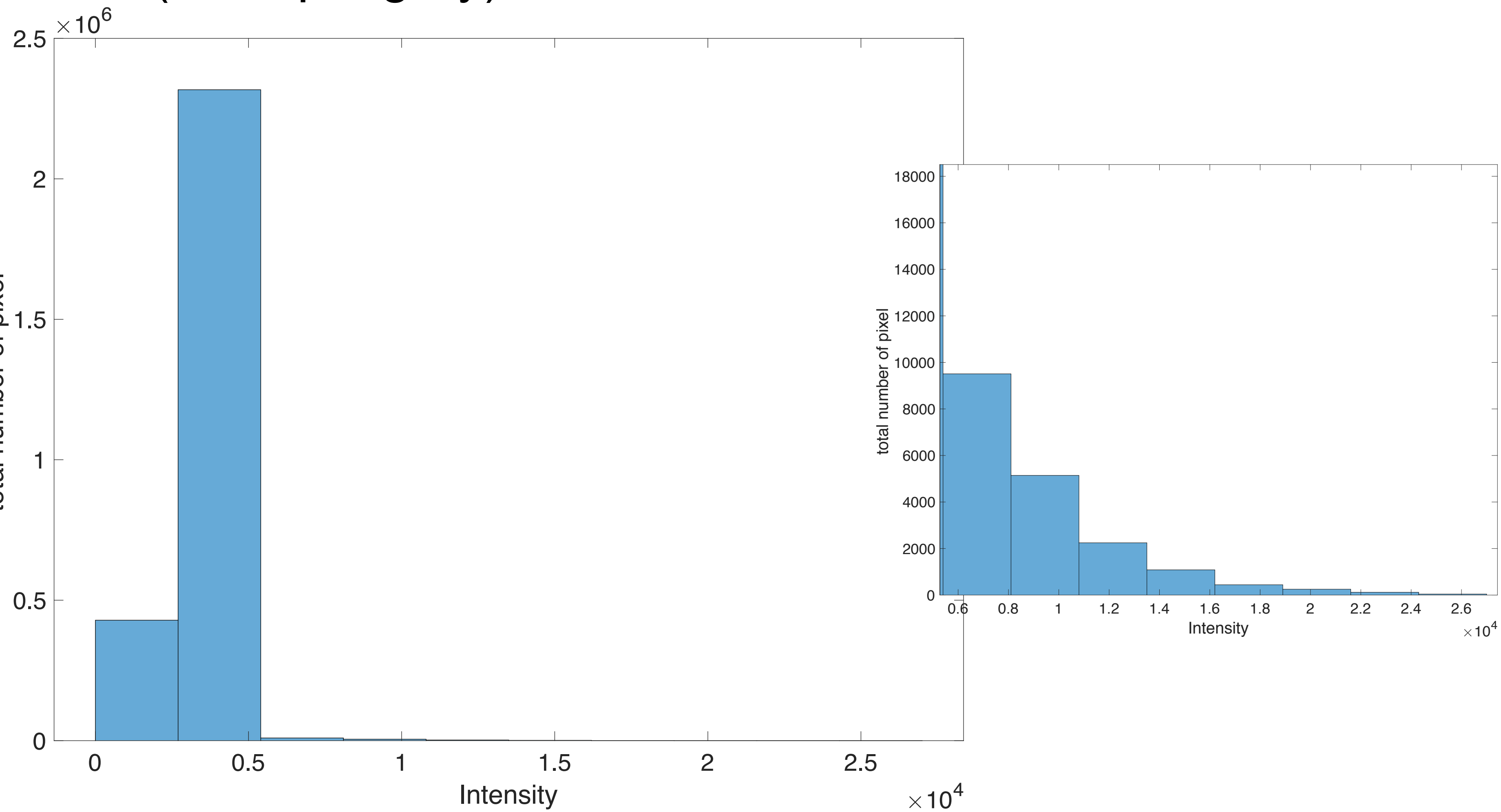
Finding all cells in the image



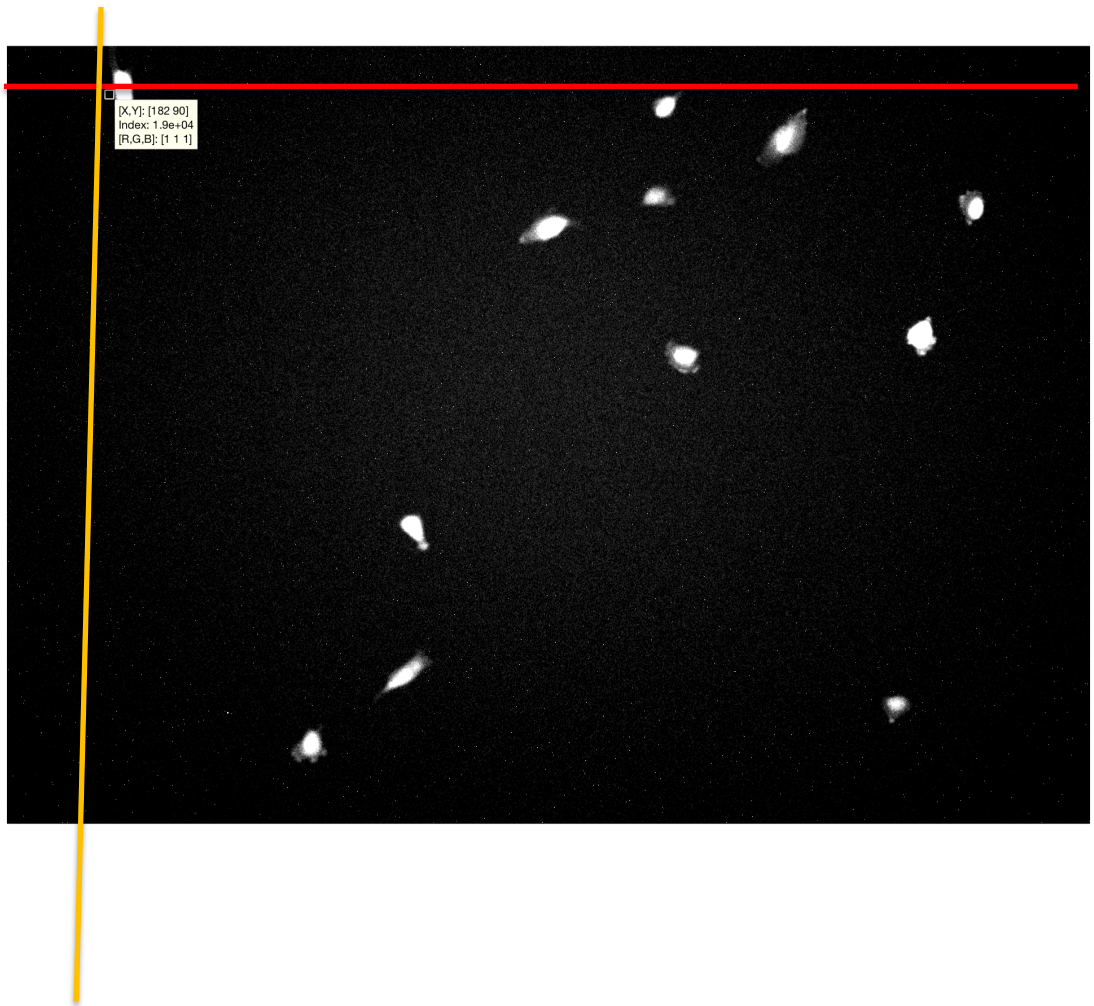


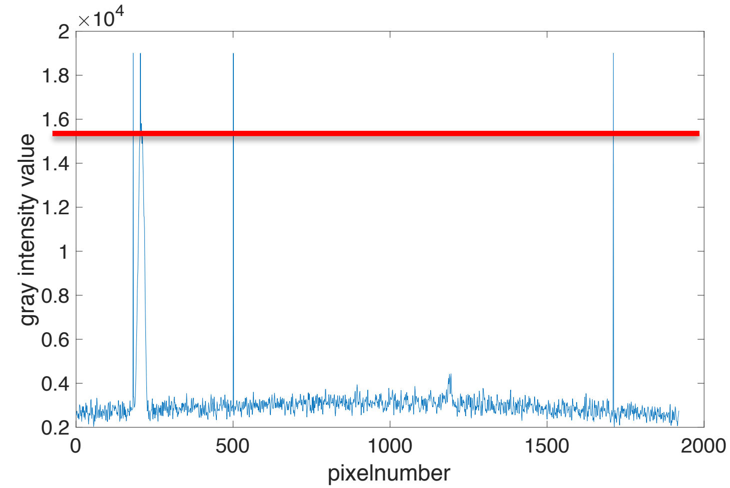
Intensity histogram

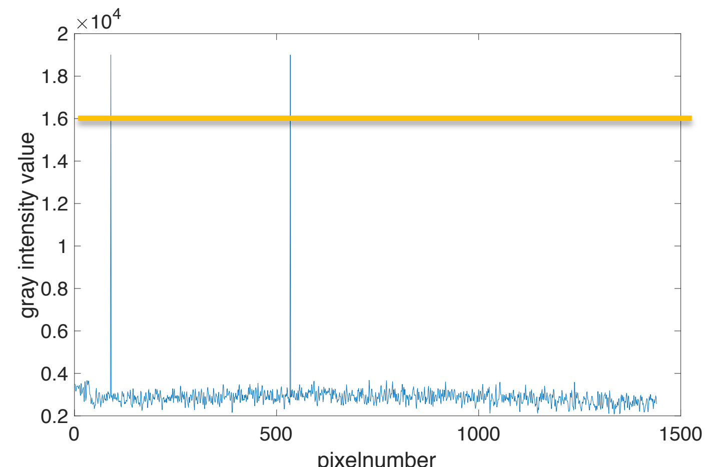
It is the number of pixels as a function of color shade scale (example gray)



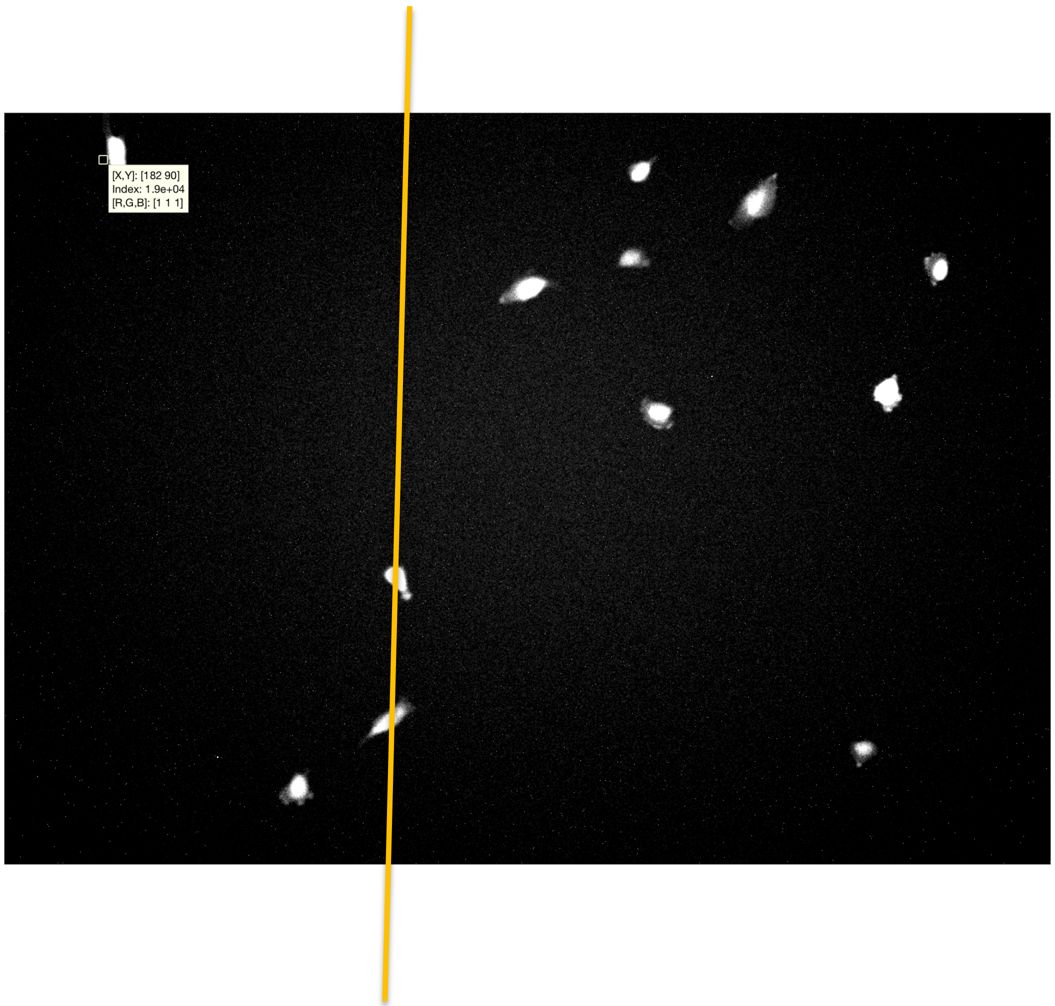
Intensity profiles from images

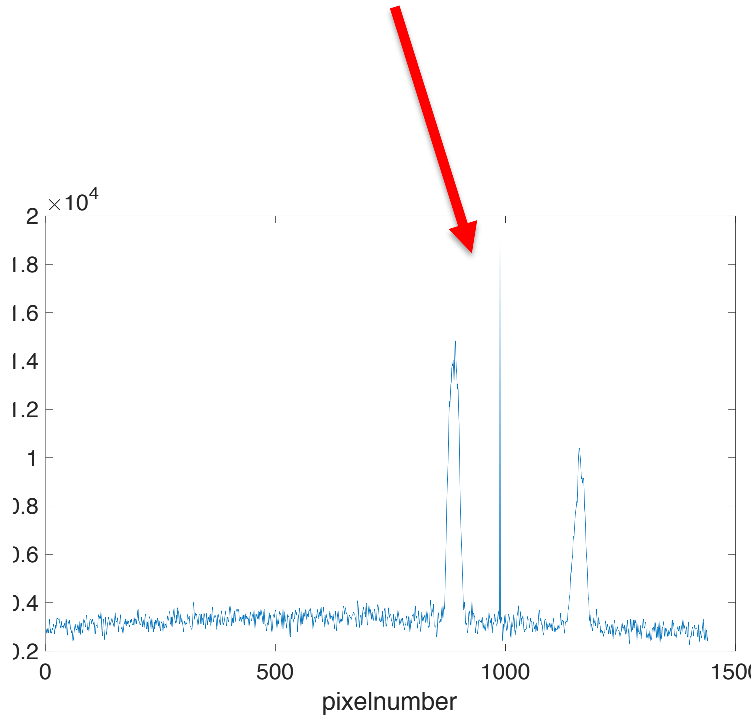






Intensity profiles from images





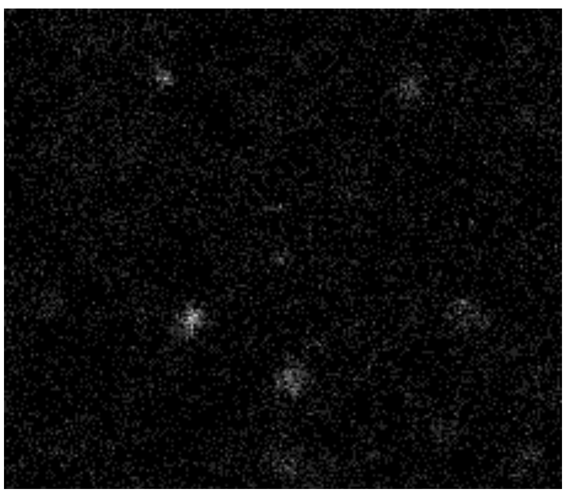
High frequency noise

Example 2, analysis of satellites around centrosomes

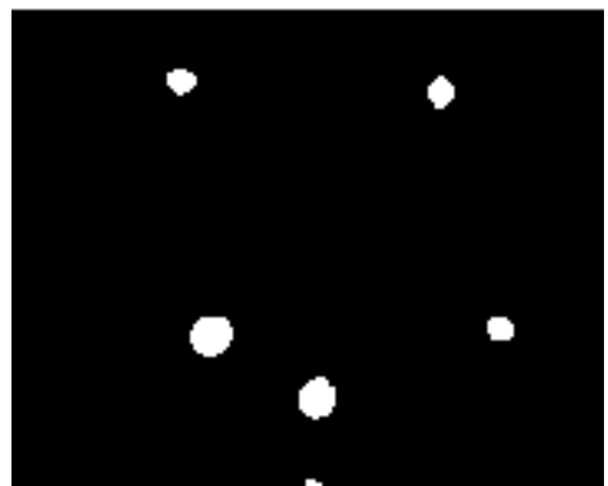
Technical term:

1. Low pass filters removes high frequency pixel noise in images.

All small pixels (3 or less) were removed from images.



Noisy images, signals are not clear.



Noise free images, biomacromolecules are clearl identified

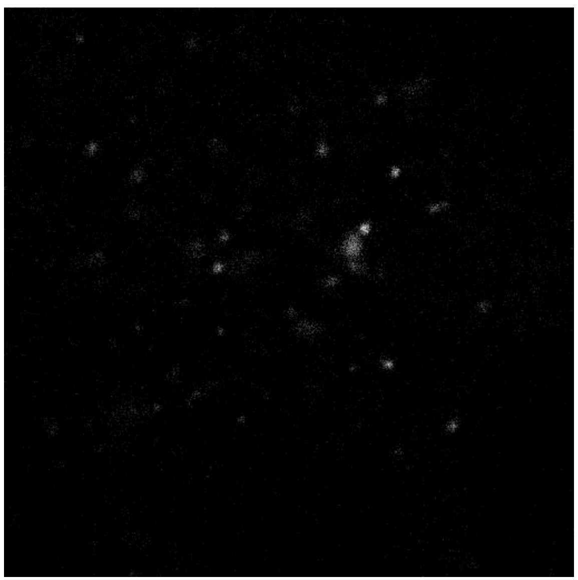
Challenges in Fluorescence Microscopy

Although we use high-end and super expensive microscopes, they are not perfect.

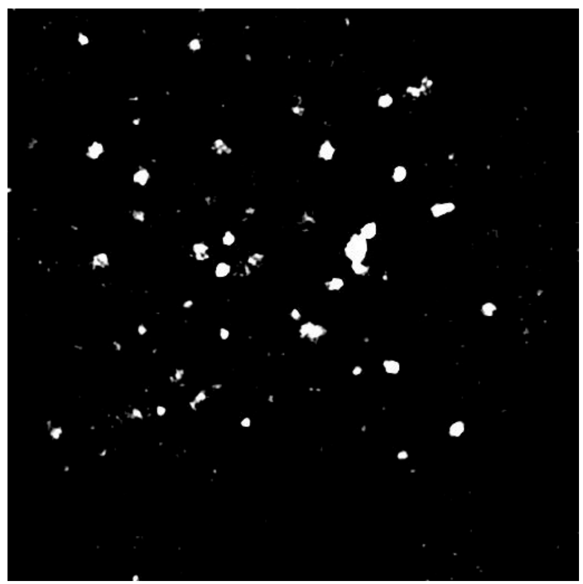
- Low signal to noise ratio
- Some issues: Blur images, pixel noise, focus loss, diffraction issues etc.

- Solution: Post-processing of image/videos

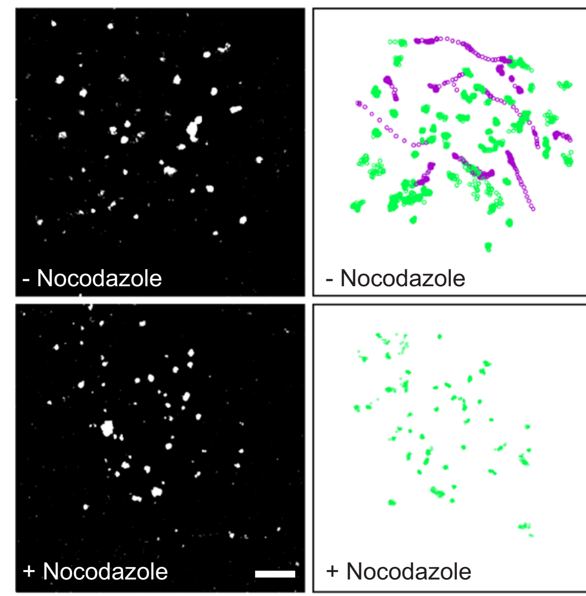
Technical term: Bandpass (low-pass) filter was used to remove noise.



Raw data:Pre-processing



Post-processing

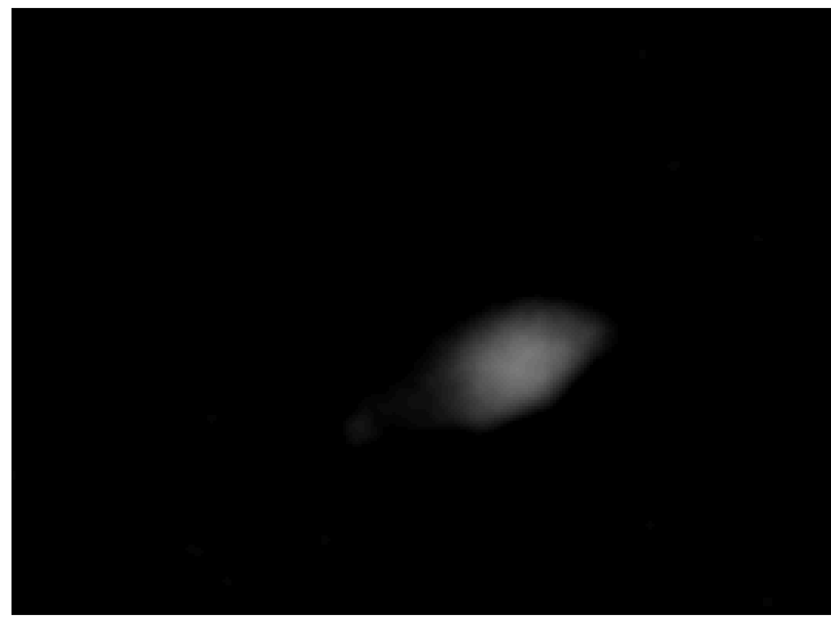


Conkar et al. scientific reports 2019

Salt pepper noise

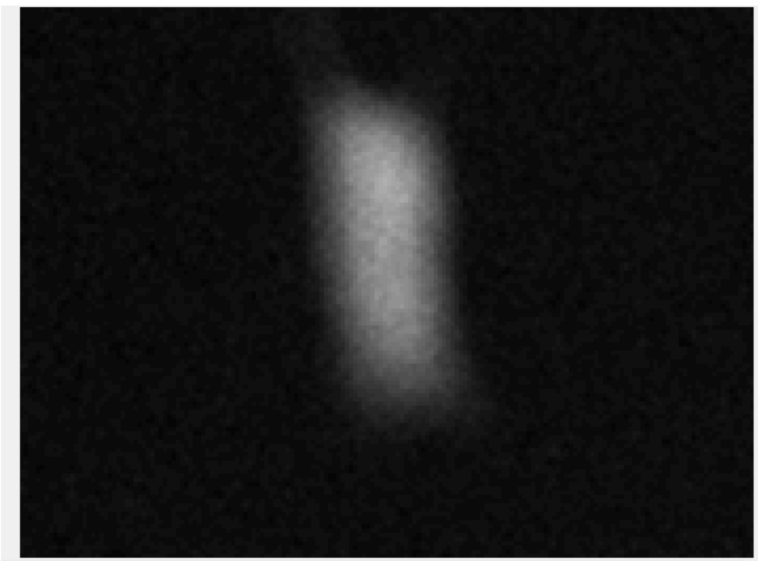
- Small white noise (dots) on the image is known as
- The cause of white noise is either from camera due to heating during data acquisition or emitted light from surorndings is captured by the camera.

Raw data with with noise



White noise is removed by using convolution

Image convolution



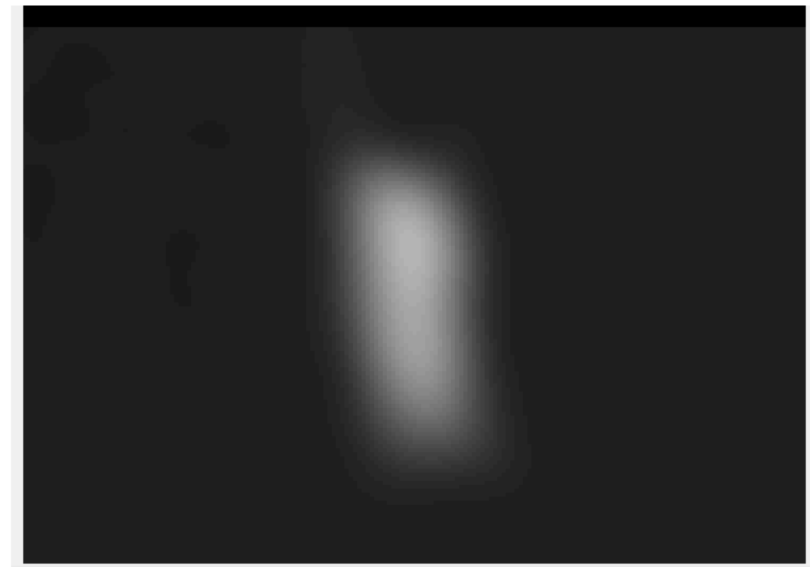


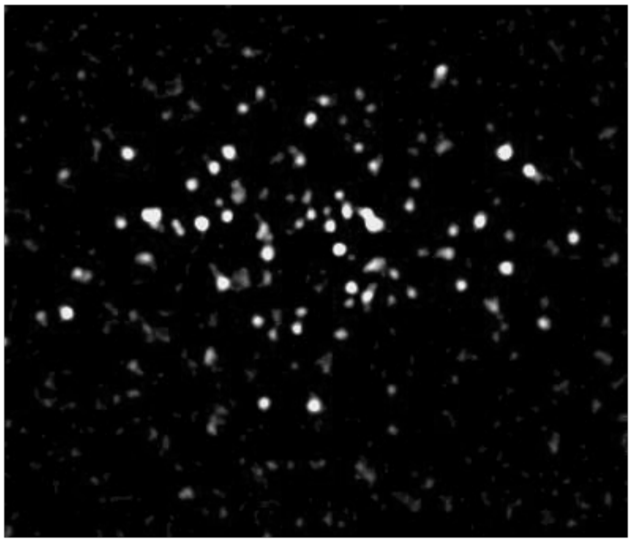
Image convolution is a process where image is filtered for noise reduction, bluring, sharpening or edge detection

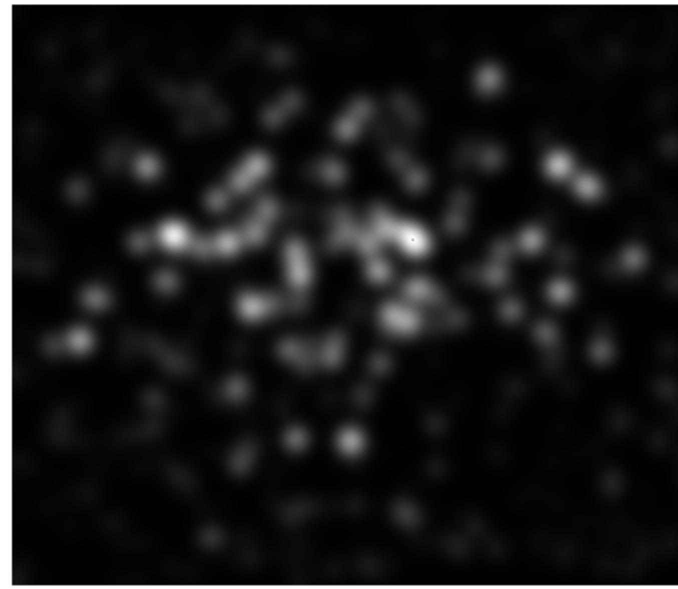
- output is a new modified filtered image

$$g(x,y) = f(x,y) * h(x,y)$$

We apply 2-dimensional convolution

It is used to blur the images to remove noise and some details

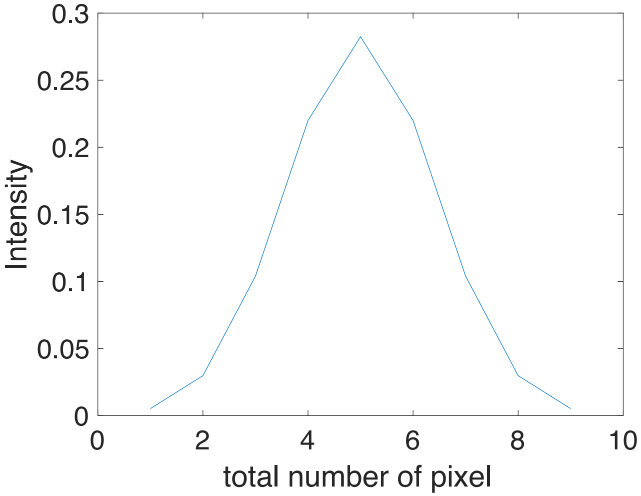


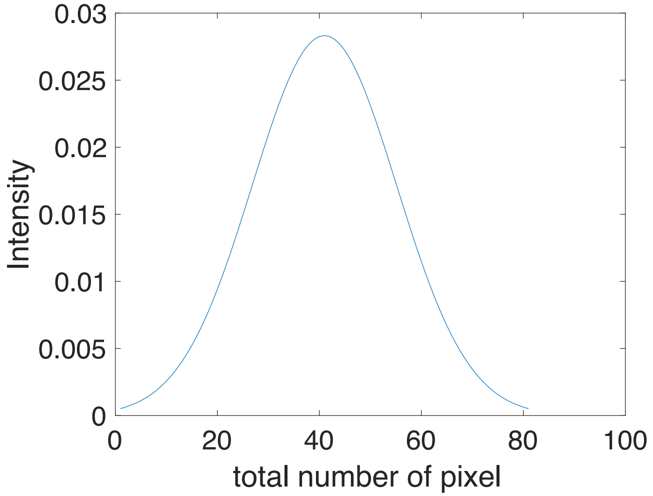


$$G(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}}$$

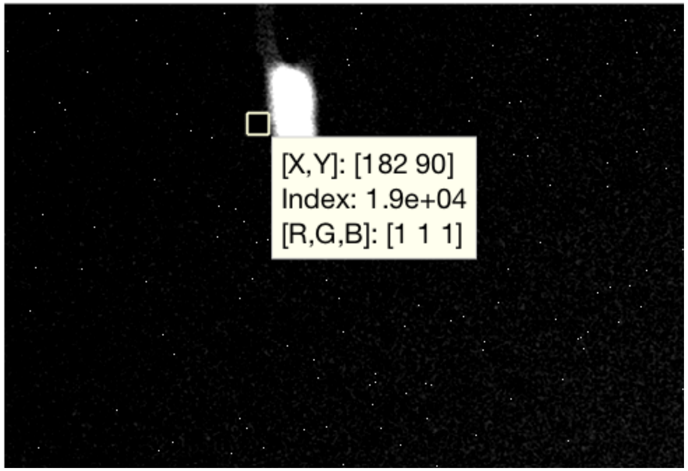
Step 1. Create a convolution matrix

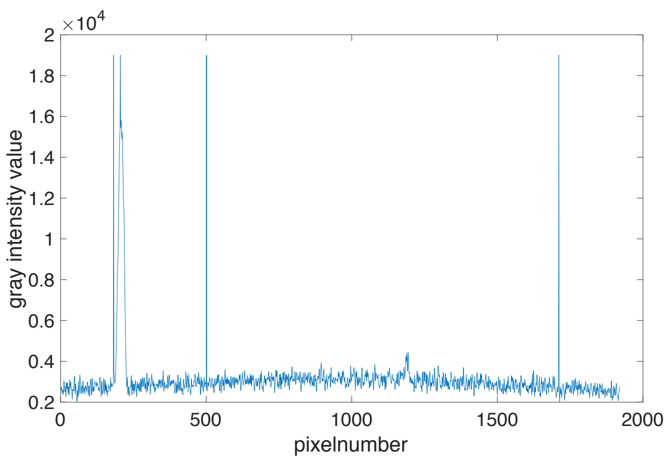
$$G(x) = \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{x^2}{2\sigma^2}}$$

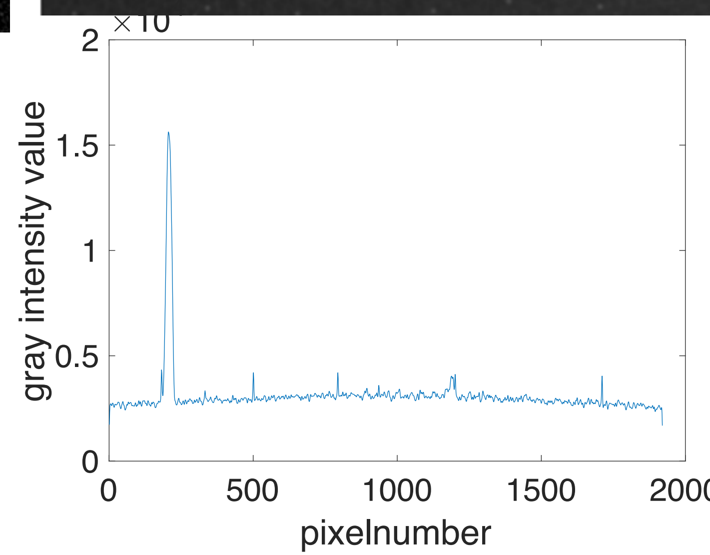




Step 2. multiply convolution matrix with image








```
convimage =
conv2(noisyimage',gaussianconv,'same');
```

Properties of convolution function

Convolution function can be represented by a two dimensional matrix.
The size of the function is 3x3, 5x5, 7x7 etc.
Size is an odd number, if not, you cannot find the middle of the mask. The convolution works by determining the value of a central pixel by adding the weighted values of all its neighbors together.

How to perform convolution?

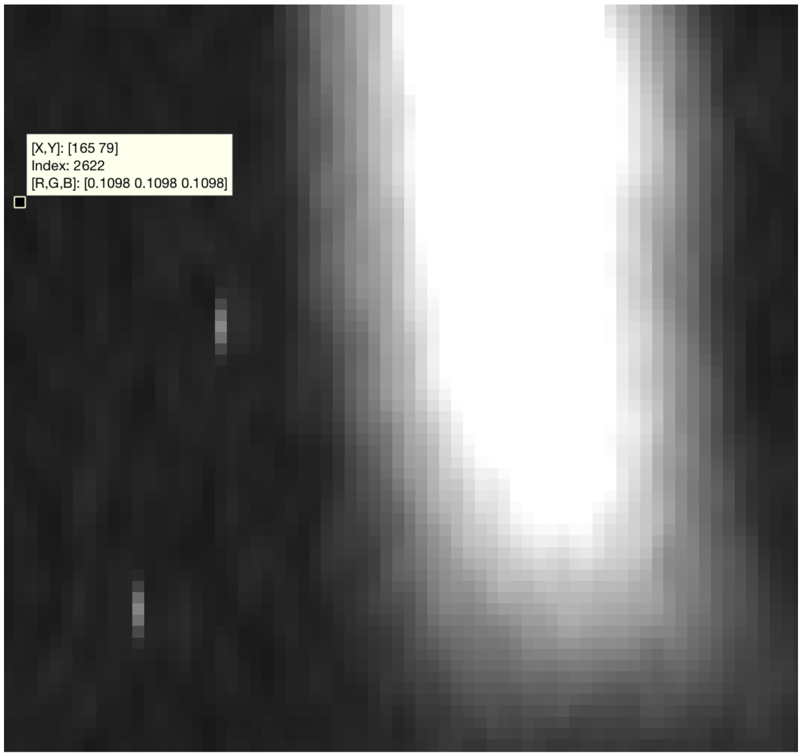
- Prepare your funcion
- For each pixel, slide the function over the image.
- Multiply the corresponding elements and then add them all together
- Repeat until all values of the image has been calculated.

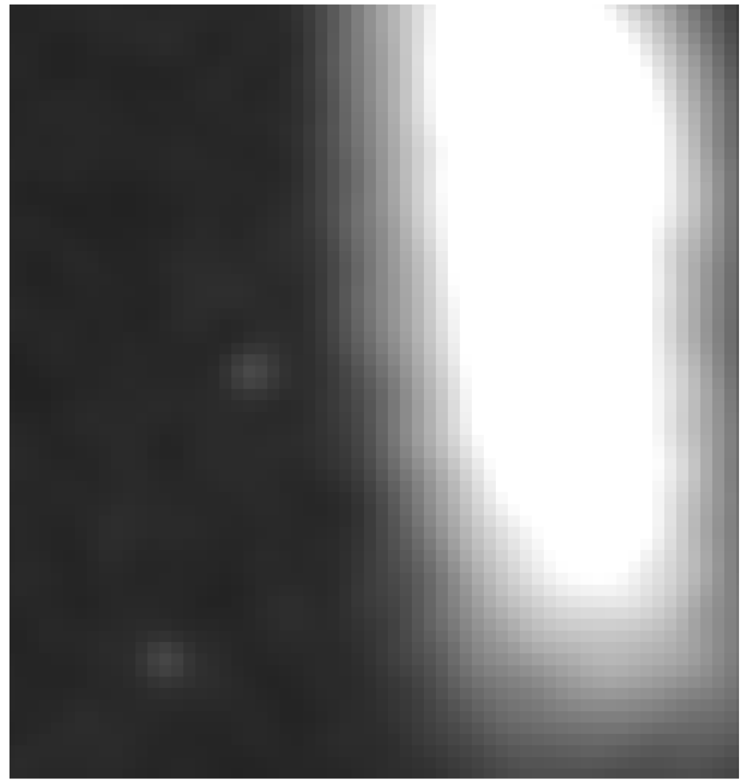
```
convimage =
conv2(noisyimage',gaussianconv,'same');
```

Noisyimage: image to be convoluted or smoothed
Gaussianconv: vector or matrix used to convolve with A
Same: it return the same matrix size As A

Convolution with 1d matrix

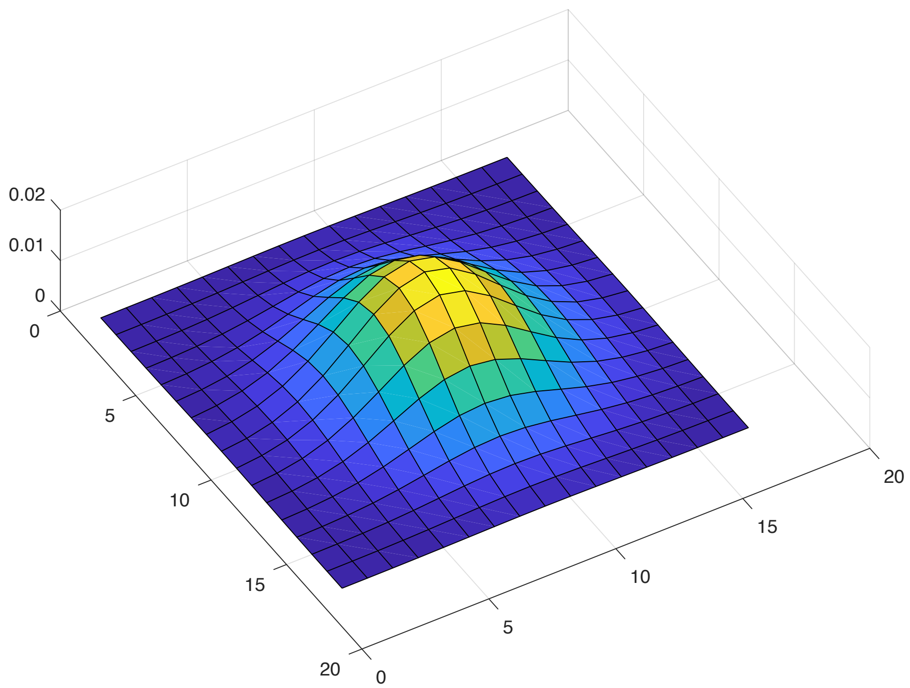
2d convolution occurs with two step if you use a vector

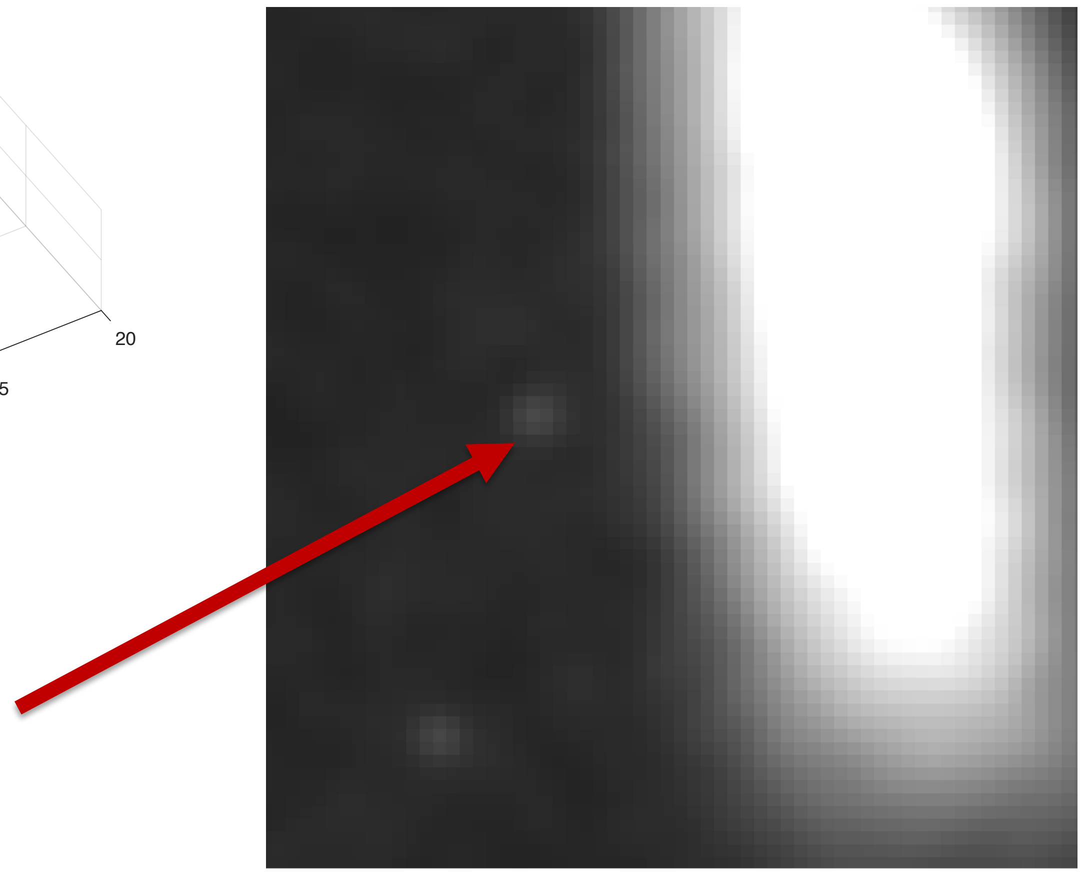




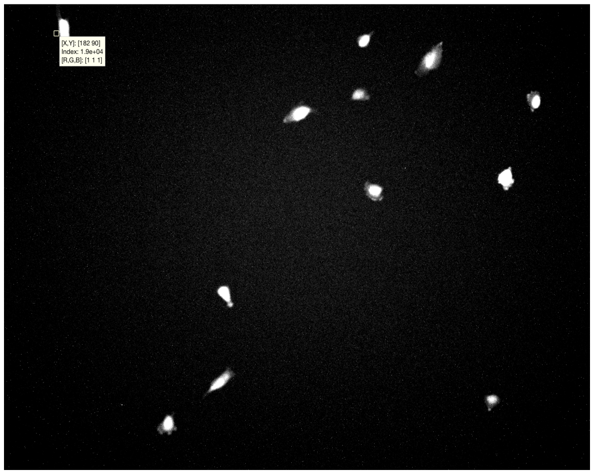
Convolution with 2d matrix

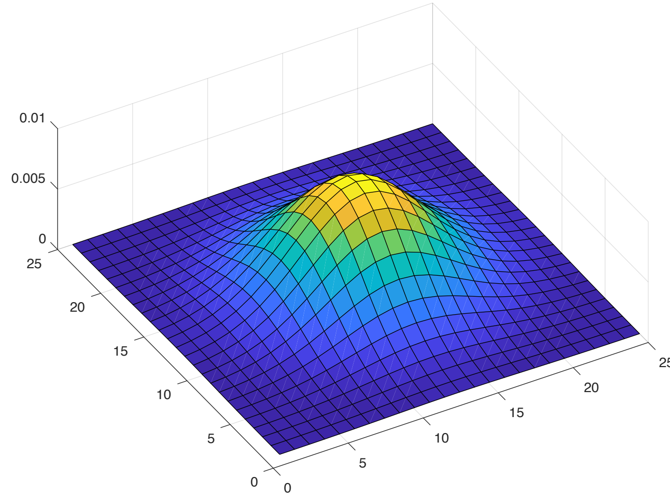
```
gauss3D=gaussianconv(1,1:size(gaussianconv,2)).
*gaussianconv(1,1:size(gaussianconv,2))'
```

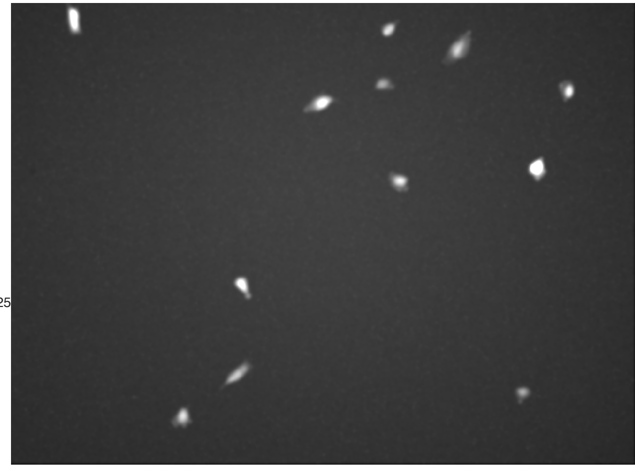




2D filter is applied to convolve an image







$$g(x,y) = f(x,y) * h(x,y)$$