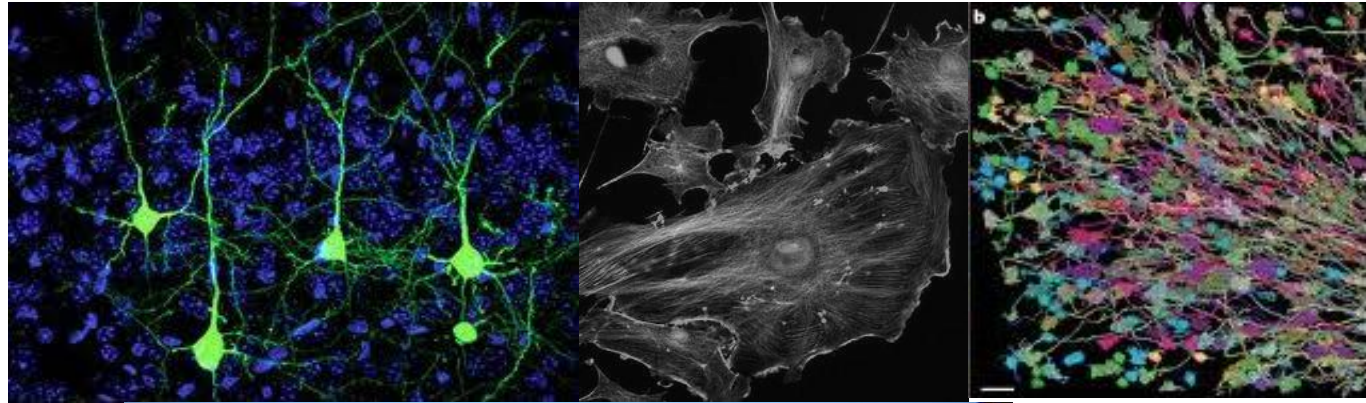


Week 8-9

Image Data Collection in Biology and Camera Systems



What is a digital image?

Image can be considered as a matrix that is composed by pixels.

Color representation of information (data).

Pixel carry all the information as a number.

What type of images are used in biology?

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

In computers, the numbers are represented by bits, 0 or 1.

Computers use binary system to represent and process any information.

In mathematics, we use decimal system 0 to 9 to represent the numbers.

Black-and-White Images

It is a 1 bit image. The pixel can carry either 0 (black) or 1 (white).

That is also called binary image.

No gray levels from black to white is present.

Binary Image

RGB Image





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



Gray Scale Images:

It is an image where the intensity values are scaled between black and white.

If only two color is available for pixels. It is a 1-bit picture- Pixels are 0 or 1

If 256 colors it is 8 bit (1byte) picture from 0 to 255. 8 bit or 1 Byte image has a 256 shades of gray if a gray scale image used.





6000x4000 pixels

Numbers represents the 256 shades of gray intensity values from 0 up to 255.

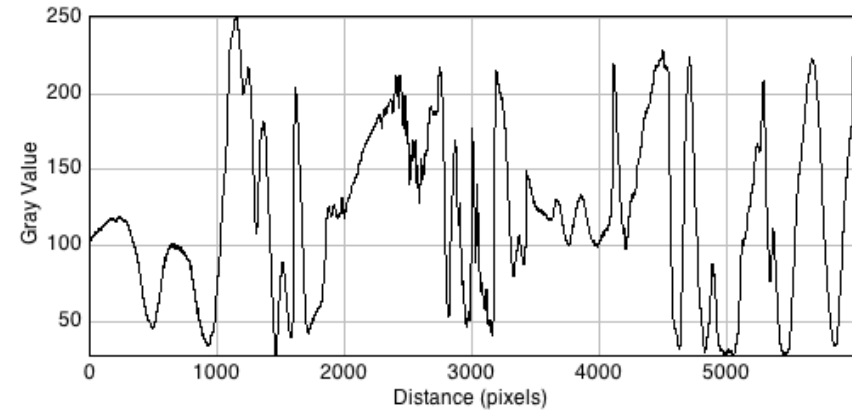
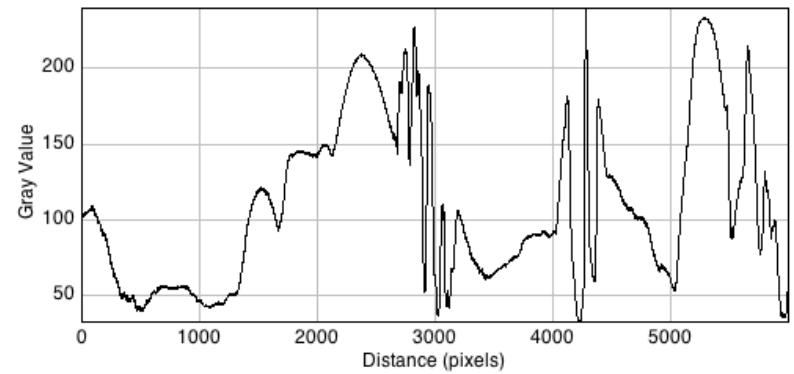
1 byte = 8 bit image

$2^8 = 256$ different levels

10 by 10 pixel – upper corner of the image

85	78	75	79	82	81	80	81	81	89
74	65	57	56	55	52	49	50	71	74
73	63	56	56	58	57	58	61	66	62
64	56	52	55	59	61	63	66	61	54
68	60	54	56	57	55	53	54	53	47
73	64	57	56	57	54	53	54	51	49
66	55	45	43	45	47	50	54	51	54
80	66	52	47	47	49	54	60	47	55
83	73	59	49	48	53	58	60	55	52
74	67	61	58	53	48	49	54	54	52

Intensity Profiles



Histogram of the image



Frequency



Count: 23976000 Min: 0
Mean: 112.693 Max: 255
StdDev: 62.426 Mode: 48 (166632)

Color Images

It carries more information and allow us to distinguish more details.

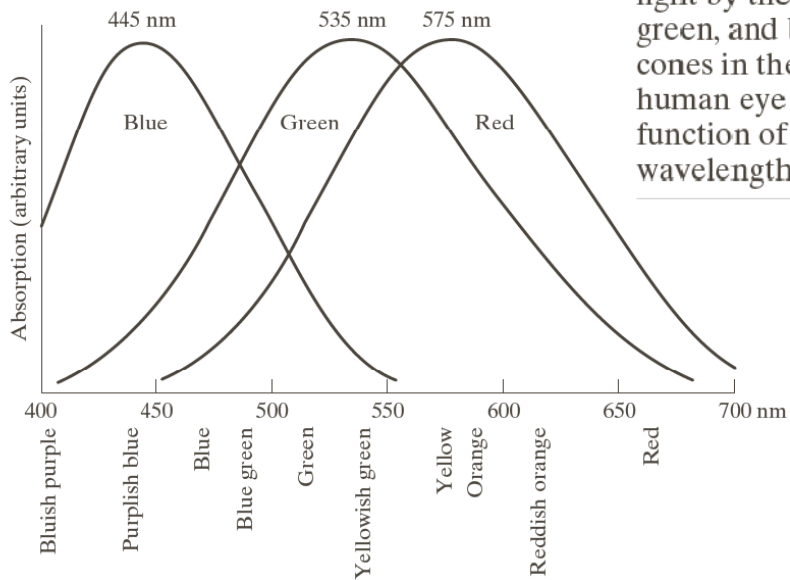
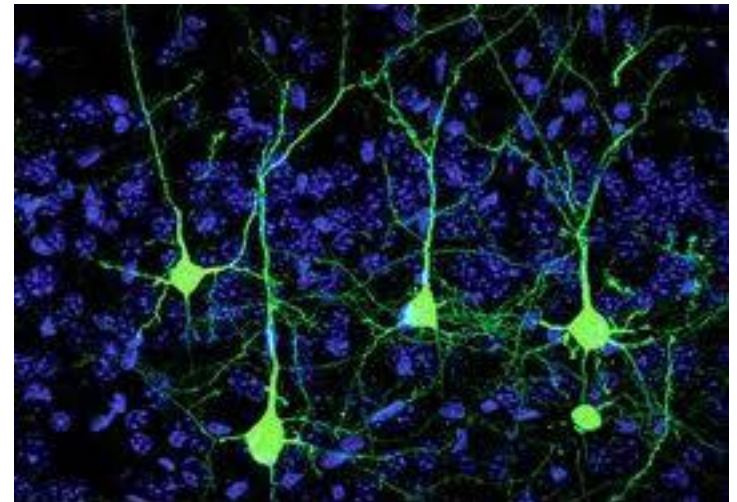
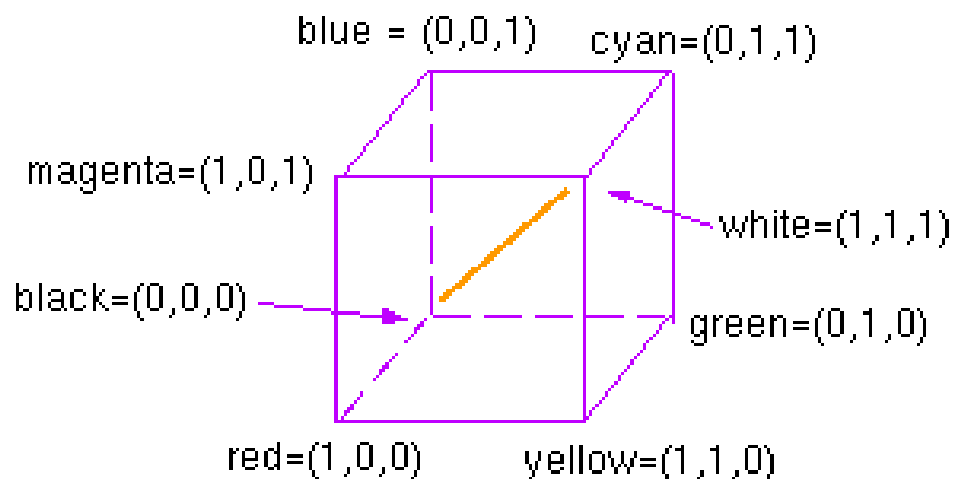
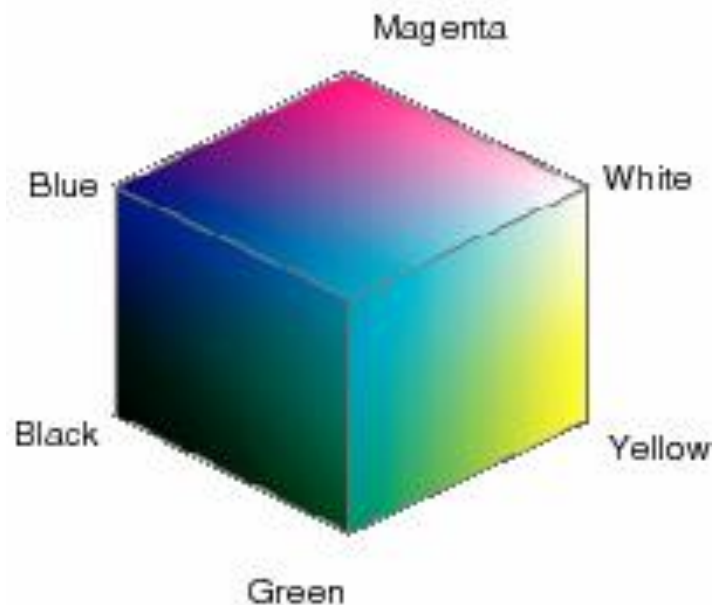


FIGURE 6.3
Absorption of light by the red, green, and blue cones in the human eye as a function of wavelength.



The mixture of primary colors red, green, and blue are used to make all color in the RGB image



$$x = \frac{X}{X+Y+Z} \quad y = \frac{Y}{X+Y+Z}$$

$$z = \frac{Z}{X+Y+Z} = 1 - x - y$$

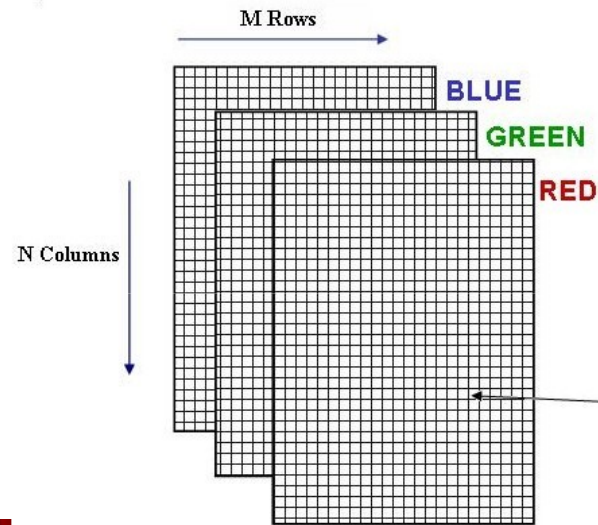
Blue
channel

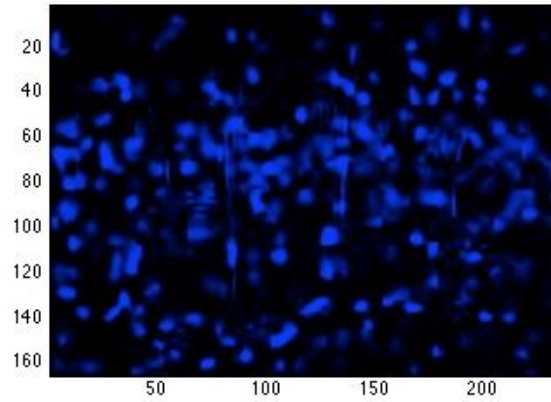
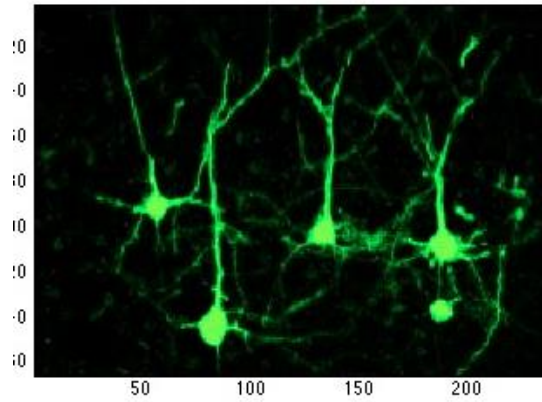
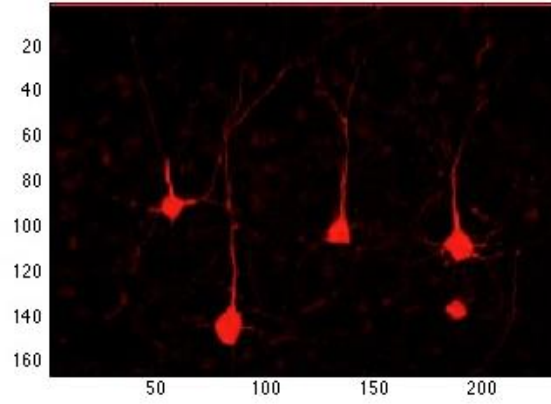
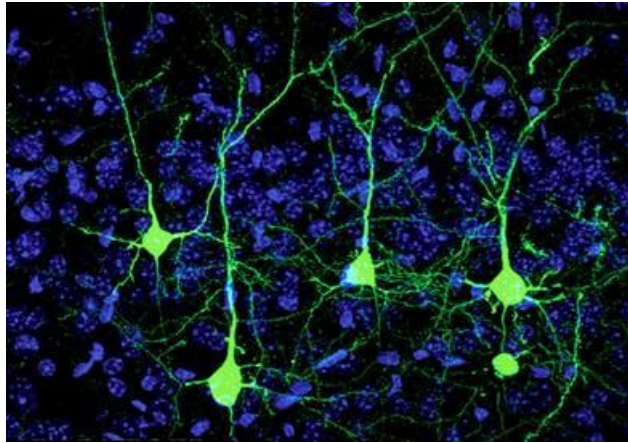


Green
channel

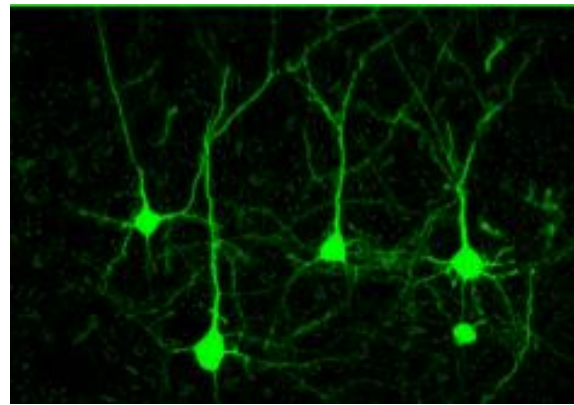


Red
channel

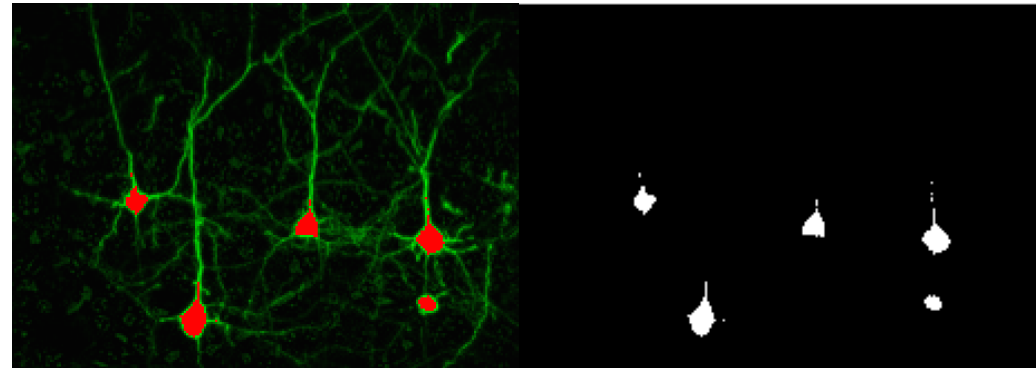




How many neurons are present? Where is the neurons?



Threshold filter



Intensity > threshold

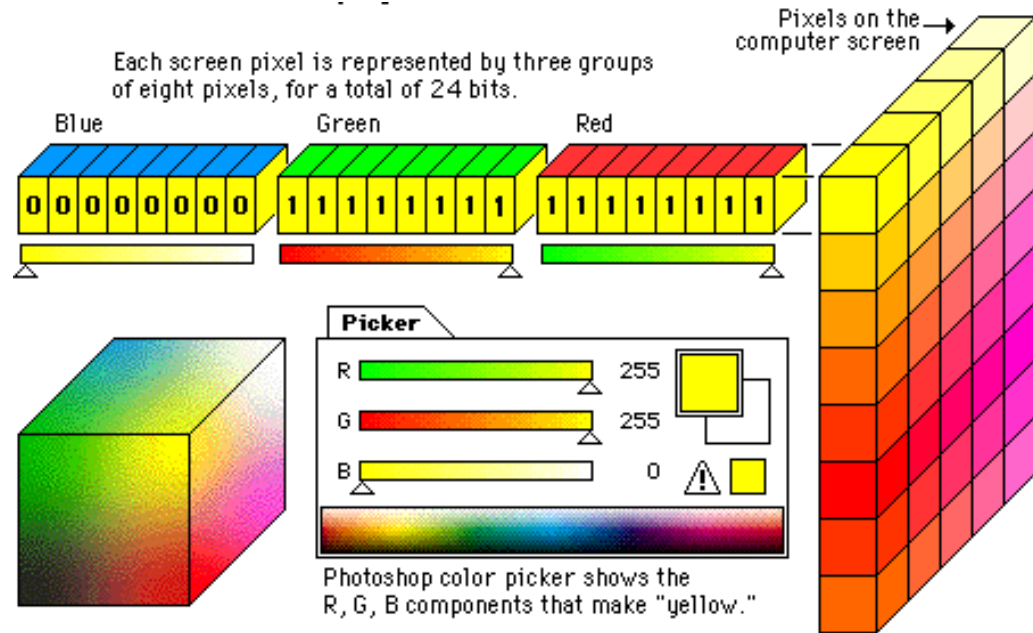
Size filter
(pixels)



1	67	244.552	231	255	56.642	88.736
2	81	246.444	232	255	133.458	99.893
3	109	245.514	230	255	188.591	105.818
4	132	246.311	231	255	82.823	141.115
5	38	245.289	231	255	187.224	134.800

What is the true-Color Image?

8-bit x 3 RGB image = 24 bits = 3 bytes

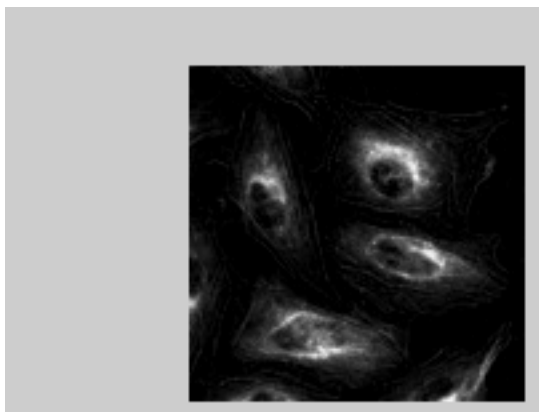


A resolution of: 6000 * 4000 pixel.

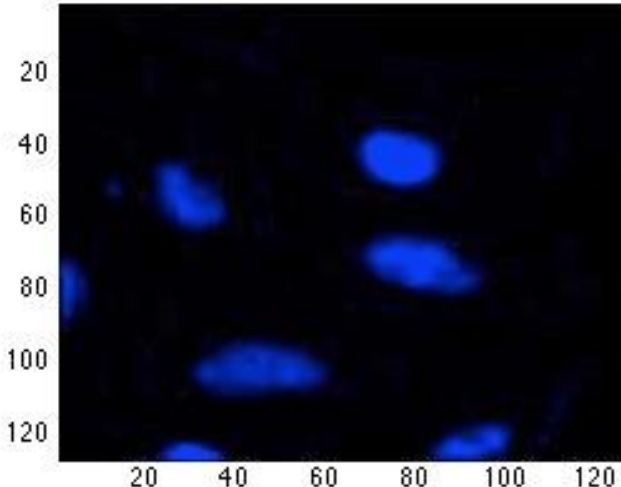
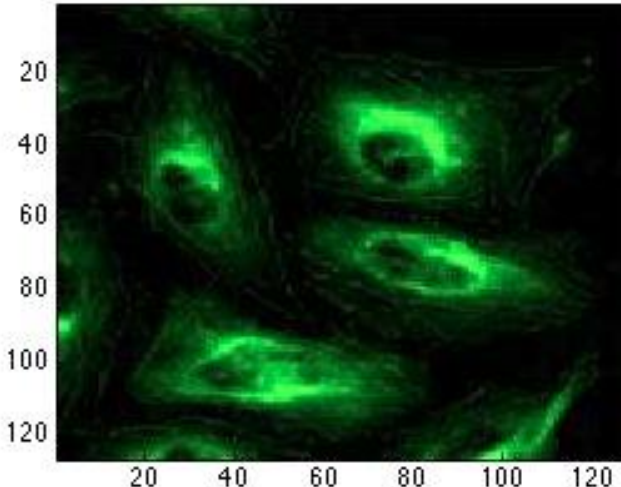
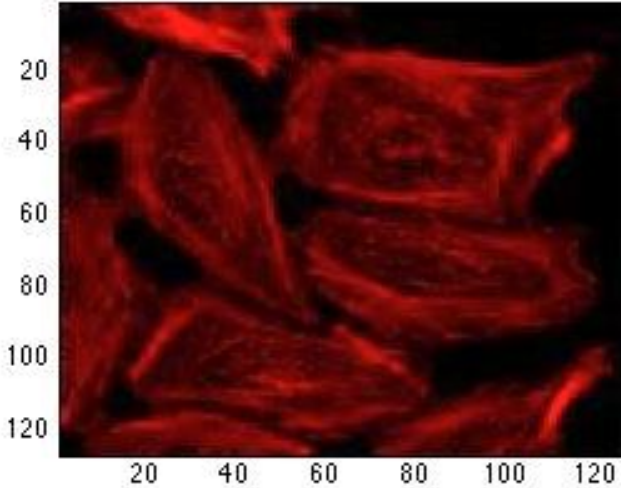
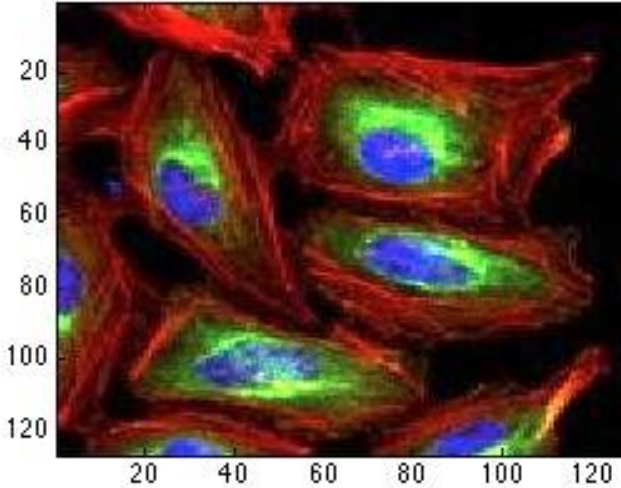
What is the size of the image?

$6000 * 4000 * 1 \text{ byte} * 3 \text{ (RGB)} = 72 \text{ MByte}$
 $= 2.4 \text{ MB of image.}$

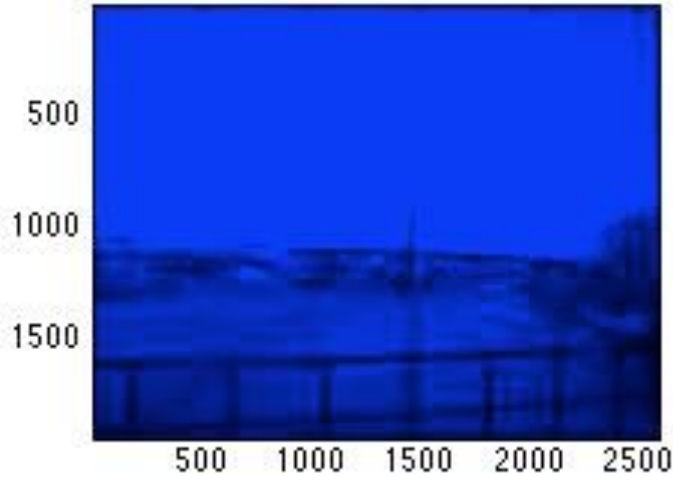
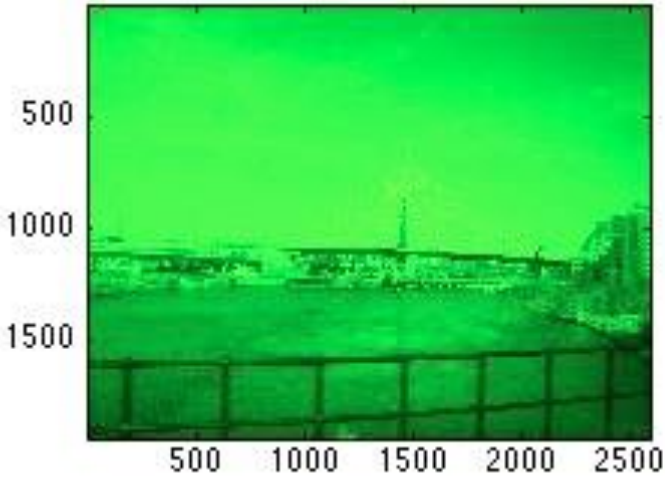
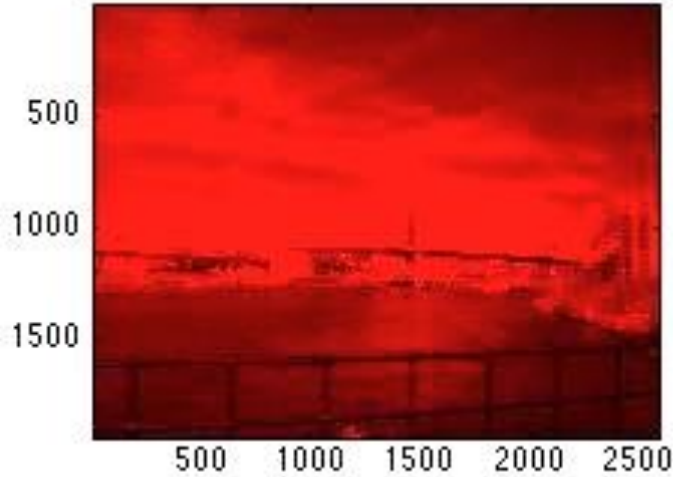
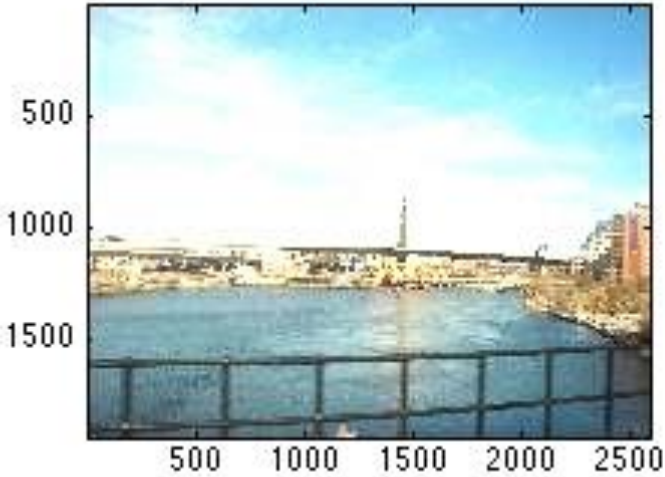
51	44	32	21	6	1
66	57	44	30	15	9
50	46	41	37	32	28
40	37	38	40	38	35
55	54	45	42	29	32
70	65	54	52	44	50



Separating Images into RGB channels



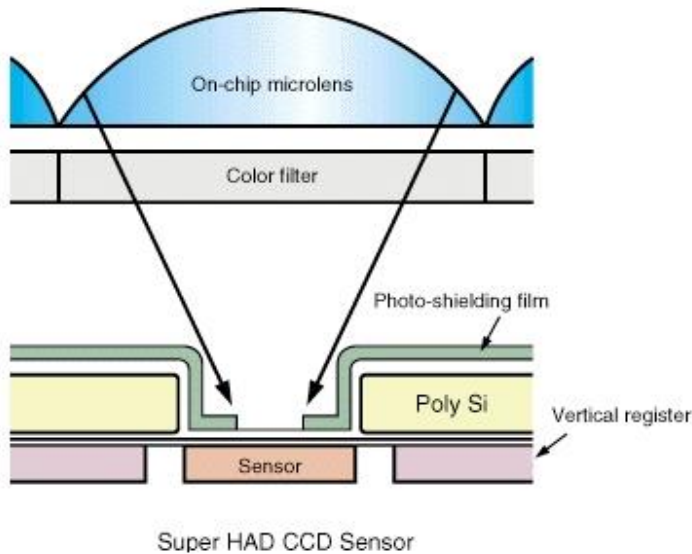
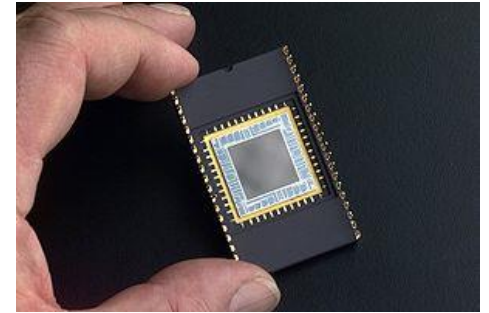
This image can not be separated as good as cell image.



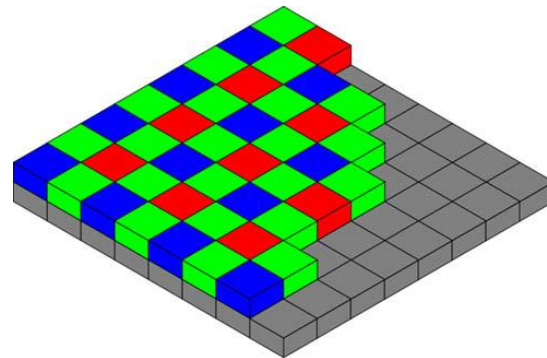
Cameras and Light Sensors

There are two kind of cameras available on the market

- 1) CCD = Charge Coupled Dedectors
- 2) CMOS camera = Complementary metal–oxide–semiconductor



If more CCD sensors requires the smaller the size of the CCD pixels, the higher the resolution of the resulting image. So rich information can be recorded.



CCD

It is a widely used technology and very well established

It provides a low noise and high quality images.

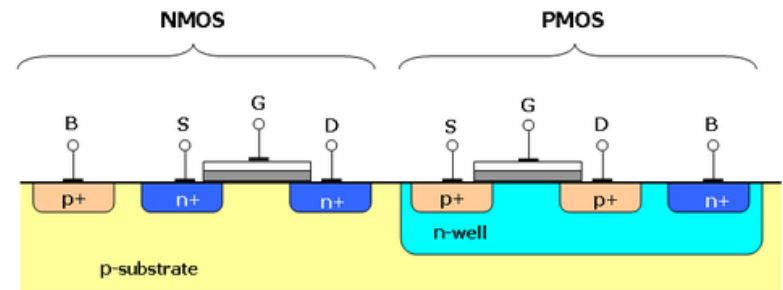
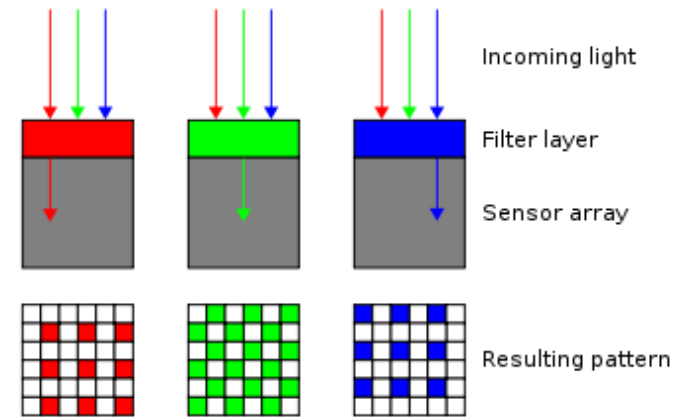
It is a very chip technology

CMOS

It is not very expensive

It is not as good as CCD camera

It provides more flexibility in term of integrating new features such as image detection or recognition



Basics of CCD image capturing.

An image is projected through a lens onto the capacitor array.

Each capacitor starts to accumulate a charge between plates.

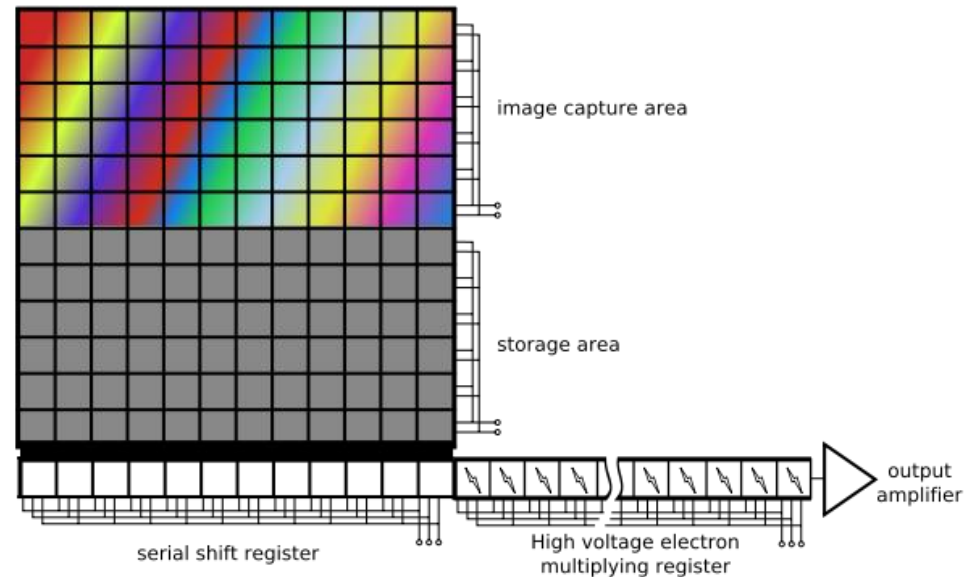
Each capacitor to transfer its contents to its neighbor (operating as a shift register).

The last capacitor in the array dumps its charge into a charge amplifier which converts the change into a voltage.

EMCCD Technology

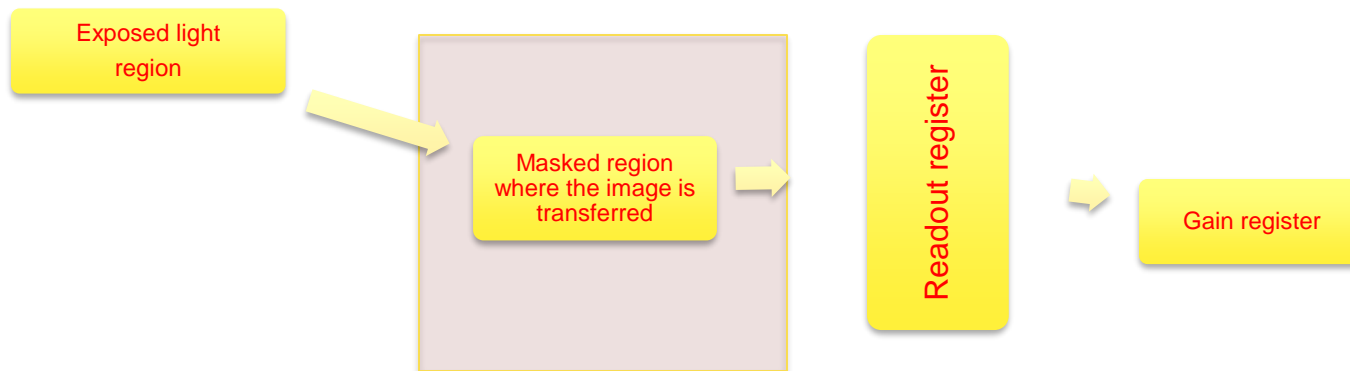
The electrons are multiplied in areas inside gain register by impact ionization. The gain register is composed by into a large number of stages.

iXon Back-Illuminated EMCCD



It is a regular CCD with shift register has additional multiplication and gain register.

Impact ionization cause generation of new electrons



14 bit analog to digital converter

SMB ext input is TTL level, CMOS has 470 ohm impedance

Internal: You can send signal fire pulse , from smb fire connector

External : ext smb connector, waits signal from external source

External start trigger: Data series will not be acquired until the system has received an external signal from external source

Avalanche Photodiodes (APD)

Very sensitive light sensor

Have a quantum efficiency as high as 95 %.



Images comes with different formats such as TIFF, GIF, JPEG AND PNG

TIFF = Tagged Image File Format

It can be used various systems

It is standard method used in image analysis and storage. It can be in the form of gray scale or color images

JPEG = Joint Photo Experts Group

It is a format for photography. It has a true color picture.

Not good for icon images, sharp contrast

Very good if the image has smooth edge features, if you taking a tissue image or cell image

PNG = Portable Network Graphics

-It has 24 bit and gray scale format, very similar to GIF, web use

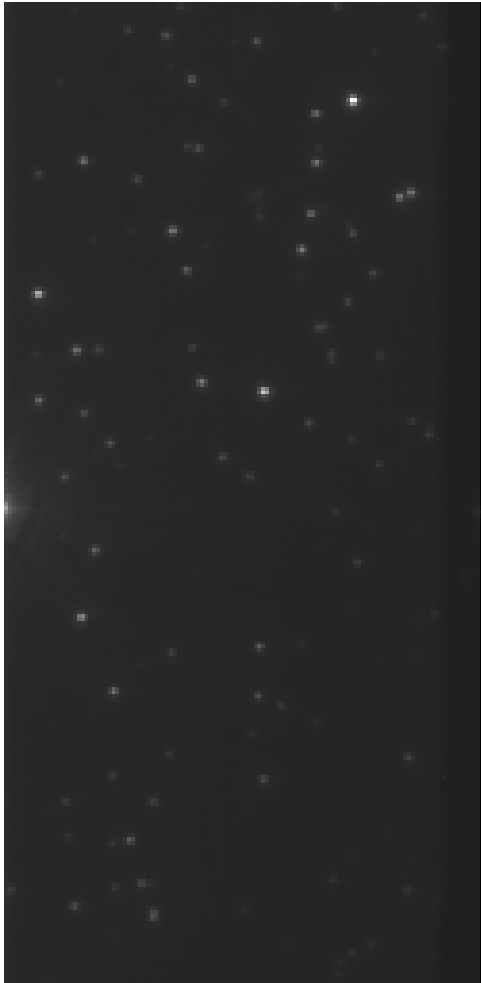
GIF = Graphics Interchange Format, suitable for graphics and icons

Used for web applications, image can be compressed by Lemper-Ziv-Welch (LZW) compression, It has limited upto certain colors so can not be used for true color images. It has certain limitations.

Water Shading in image Analysis

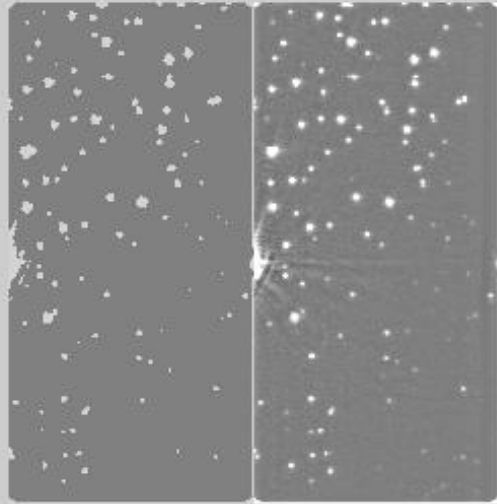
Color images usually are difficult choice for image analysis in neuroscience. Color images have to be converted to gray scale and then *thresholding* is used to realize binary images.

Image Processing helps to get information in a noisy data.



Using a mask will help to get rid of background in images.

Binary images are very useful for finding information in images



Binary image has only two values for each pixel- 1bit

Matlab is great for making binary images, you can use relational operators

Such as

Syntax

$A < B$

$A > B$

$A \leq B$

$A \geq B$

$A == B$

$A \sim= B$

It returns a logical array of the same size, with elements set to logical 1 (true) where the relation is true, and elements set to logical 0 (false) where it is not

```
>> thresh = 80;
```

```
>> binary = image > thresh;
```

How many particles in the image? 105 particles

Bwlabel = it returns the connected objects in the binary image.

```
>>L = bwlabel(BW, n)
```

```
>>[L, num] = bwlabel(BW, n)
```

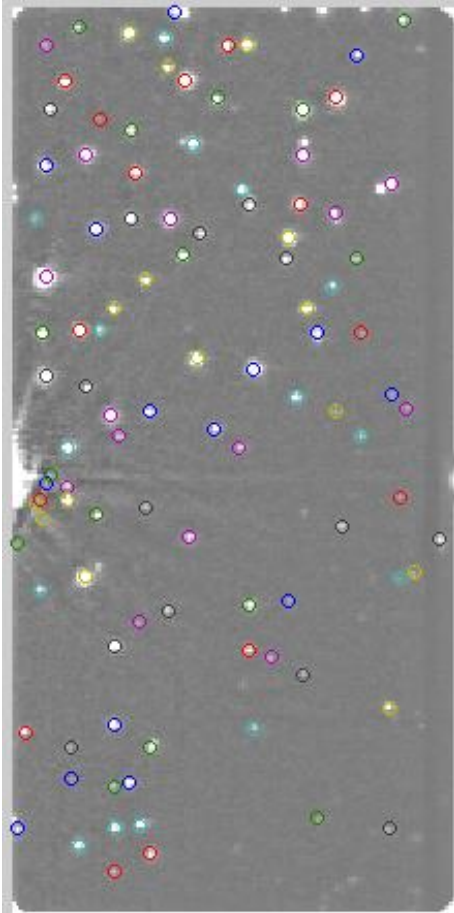
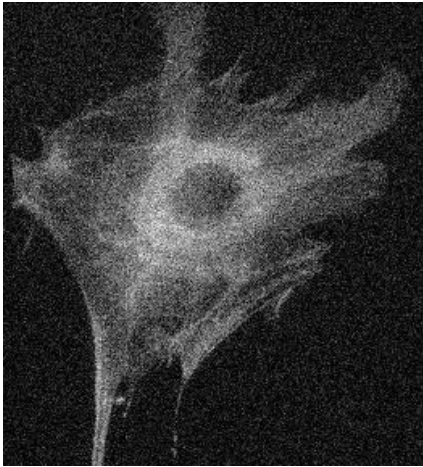
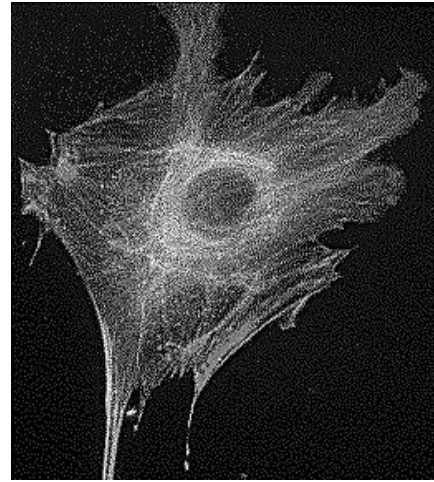


image degradation occurs due to

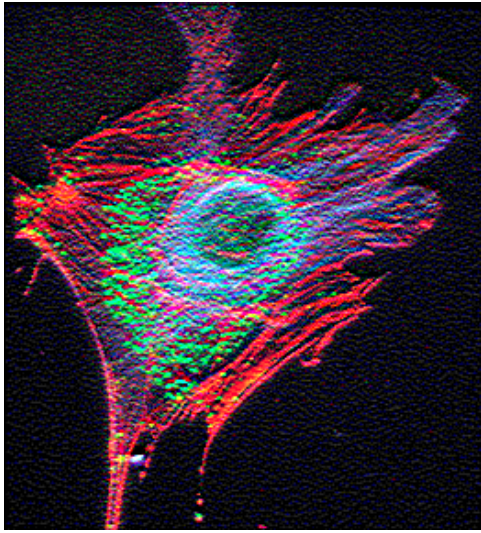
- Noise
- Scatter
- Glare
- Blur



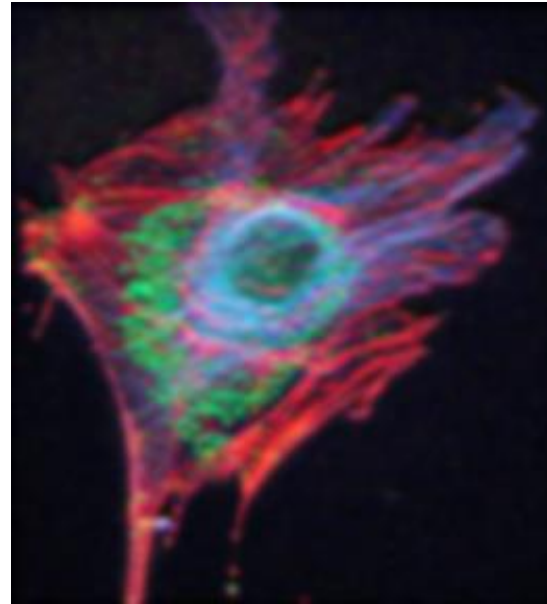
Noise in the image



Scattering in the image



Glare in the image



Blurring in the image

The source of noise in the images

Quasi random disagreement of details in image is called noise.
It appears in the forms of white noise or salt-and-pepper noise

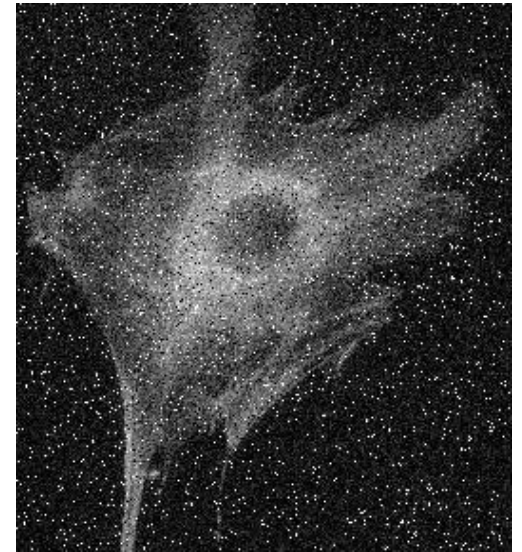
Statistical distribution of the noise can be predicted if the source is known so called quasi random.

Source of noise;

- a. Signal itself photon shot noise
- b. Dark noise due to digital imaging system

Signal dependent noise has a poisson distribution.

Dark noise has a gaussian distribution



Salt-Pepper Noise

This noise can be removed easily from the images using image filter in the deconvolution software

The source of scatter in the images

- Random disturbance of light due to heterogeneous refractive index within the specimen
- It is a random disturbance of image detail
- It is hard to predict the scattering in a given specimen. It depends on
 - a. Thickness of the sample
 - b. Optic properties of specimen
 - c. The material surrounding the sample

It increase with the increasing thickness of the sample and heterogeneity of the refractive index in the sample

Glaring in the images

- In the optical path, lenses, filters, prism can cause the random disturbance of the light.
- Antireflective coatings, glass formulations and preparation reduce the levels of glare in microscopes and images.

Blurring in the images

- The main source of blurring in the image is diffraction, the resolution is limited by blurring is known as diffraction-limited imaging. Limit in image resolution is determined by this intrinsic phenomena occurs during the microscopy.
- Spreading of light while travelling through the imaging system in optical path can cause severe blurring in the images.

Digital images taken in the microscopy can be deconvoluted to reduce the blurring effect. It can be effectively used if the blurring model is known.

Out of focus blur can be removed with several ways

- Optical methods.
 - a. Confocal
 - b. Two photon
 - c. 4pi
 - d. STED, PALM, STORM

Computationally

Deconvolution – few different
method exists to remove blurring

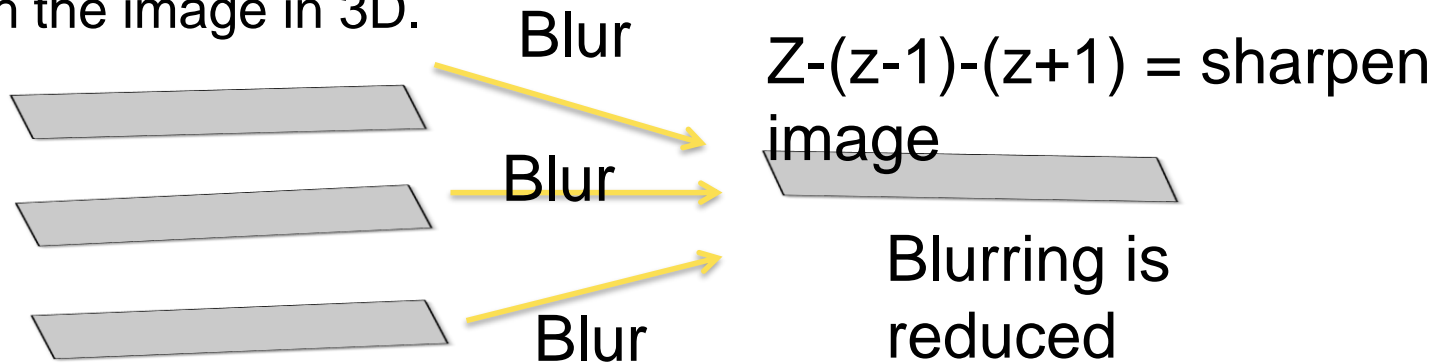
Optical and computational methods are together

Algorithms for deblurring of images

- Some deblurring algorithms are nearest-neighbor, unsharp masking, multi neighbor, no neighbor.
- The way it is applied is to collect images in various different focal planes $Z+1$, Z and $Z-1$.

Then, the planes that cause blurring in the focal plane can be subtracted, that is called nearest neighbor masking.

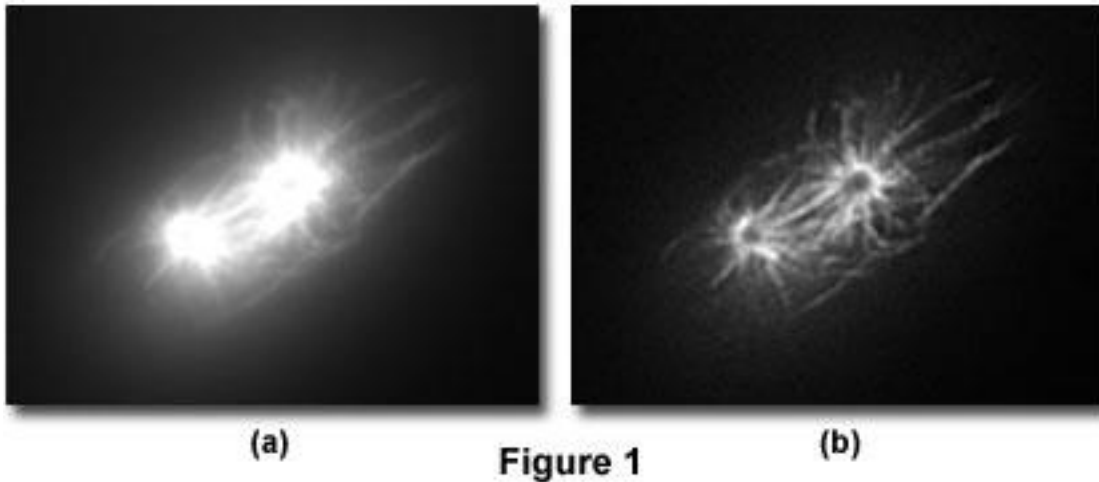
You can also use multineighbor masking to remove blur in every z axis through the image in 3D.



Deblurring algorithms use the PSF to estimate blur. then subtract it using computational operation to each optical section in z image stack.

Neighbor Deconvolution

Before and After Nearest Neighbor Deconvolution Analysis

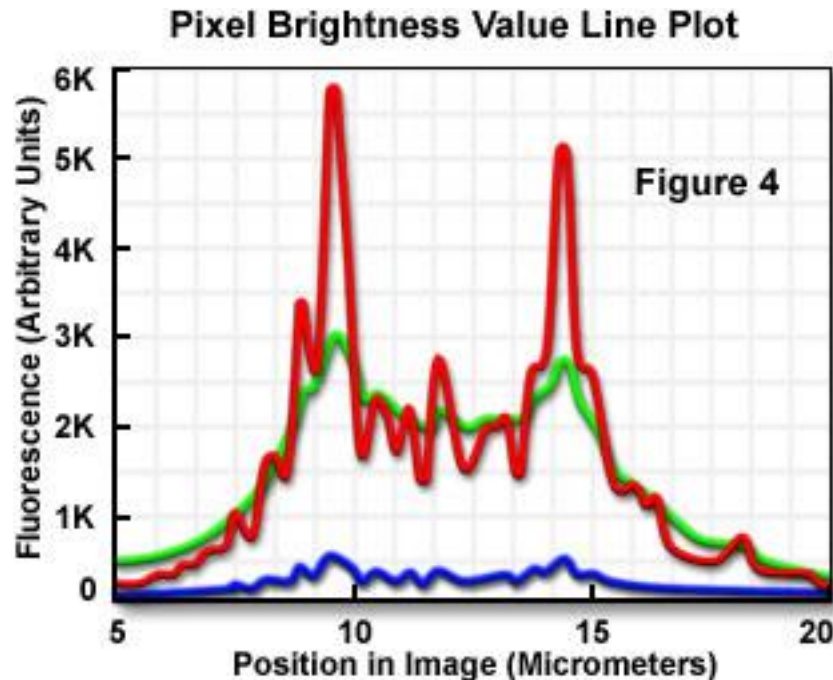


before processing Figure 1a, and after deconvolution by a nearest-neighbor algorithm Figure 1b.

Image Restoration with Computers

Deblurring and image restoration is the most commonly utilized algorithms for deconvolution in optical microscopy.

- Image restoration algorithms are properly termed "three-dimensional" because they operate simultaneously on every pixel in a three-dimensional image stack.
- Deblurring algorithms are fundamentally two-dimensional, because they apply an operation plane-by-plane to each two-dimensional plane of a three-dimensional image stack.



Statistical Iterative Algorithms

- algorithms uses probabilistic error criteria borrowed from statistical theory

Maximum likelihood estimation is used and maximize expected image.

Commercially available algorithms by SVI, Bitplane, Improvision, Carl Zeiss and Autoquant.

Maximum Likelihood Estimation

- It is a very important statistical tool with many applications in science.

used for predicting diseases states, flight information, location of photons etc.

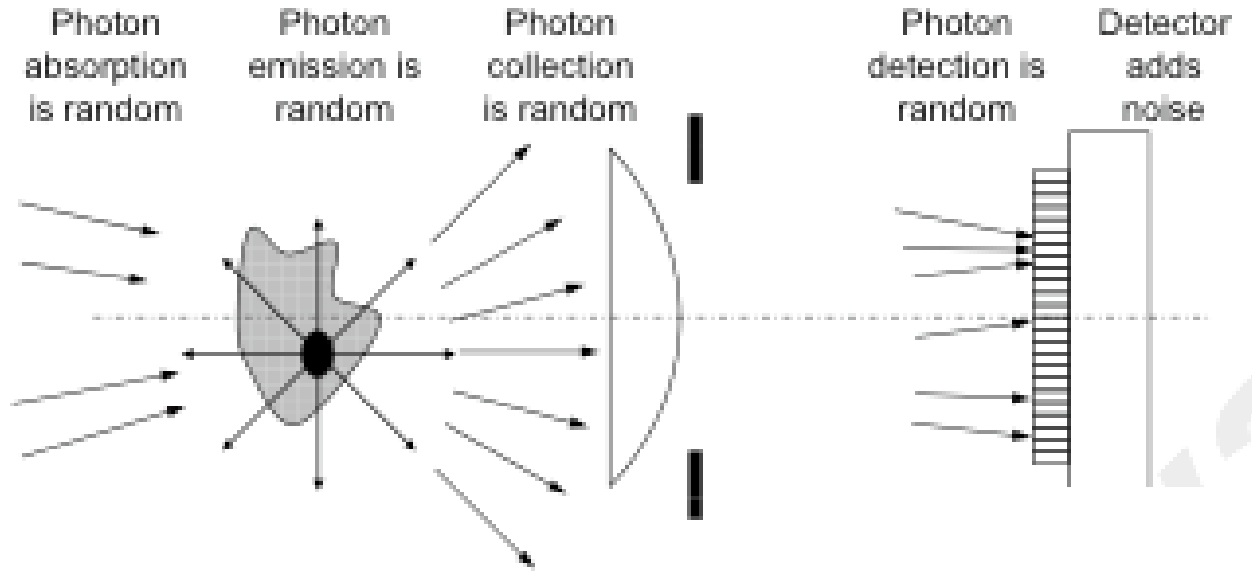
It is very computationally intensive than classical methods.

It takes more time to reach conclusion.

Their restoration capacity is superior to classical algorithms

They impose constraints on the expected noise statistics (poisson or Gaussian distribution)

Images can be restored using statistical approaches



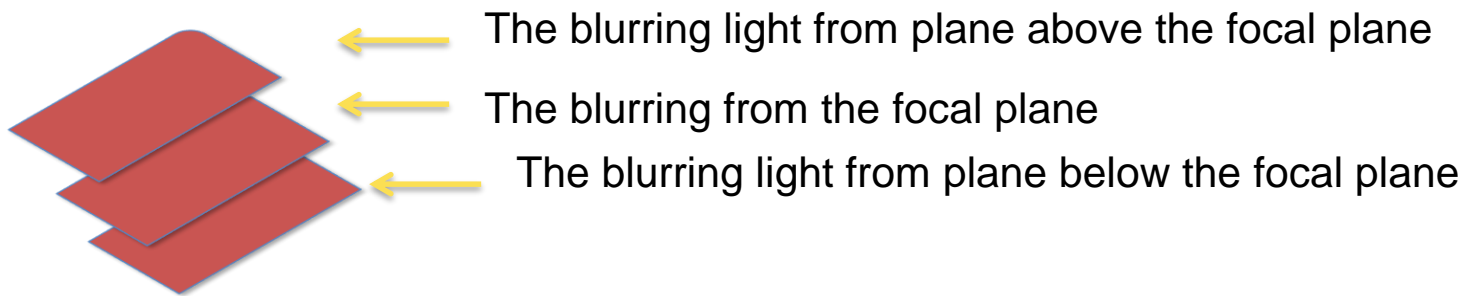
Deconvolution = Statistical estimation

Convolution and Deconvolution

The blurring process in the microscopy can be mathematically modeled as convolution (remember kernel matrix).

The way it work is to apply the point spread function to every point in the image. Then light emitted from each point in the object is convolved with the point spread function to produce final image. This convolution causes points in the specimen to become blurred regions in the image.

Point spread function is 3Dim. So blurring with point spread function is inherently 3D phenomenon. The image is formed by a convolution of the specimen with the point spread function.

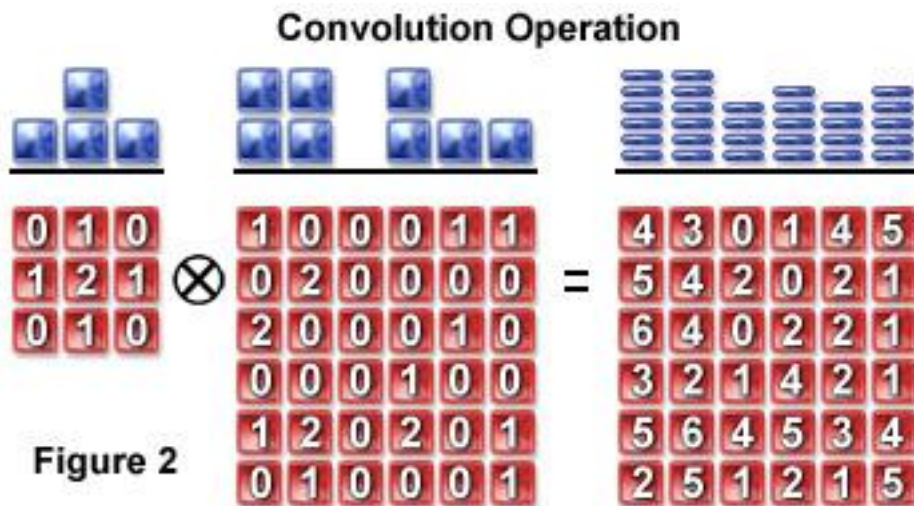


Deconvolution reverses this process and attempts to reconstruct the specimen from a blurred image.

Relation between specimen and its optical image

Each point object in the specimen is represented by a blurred image of the object (point spread function)

Remember pixel blurring using kernel.



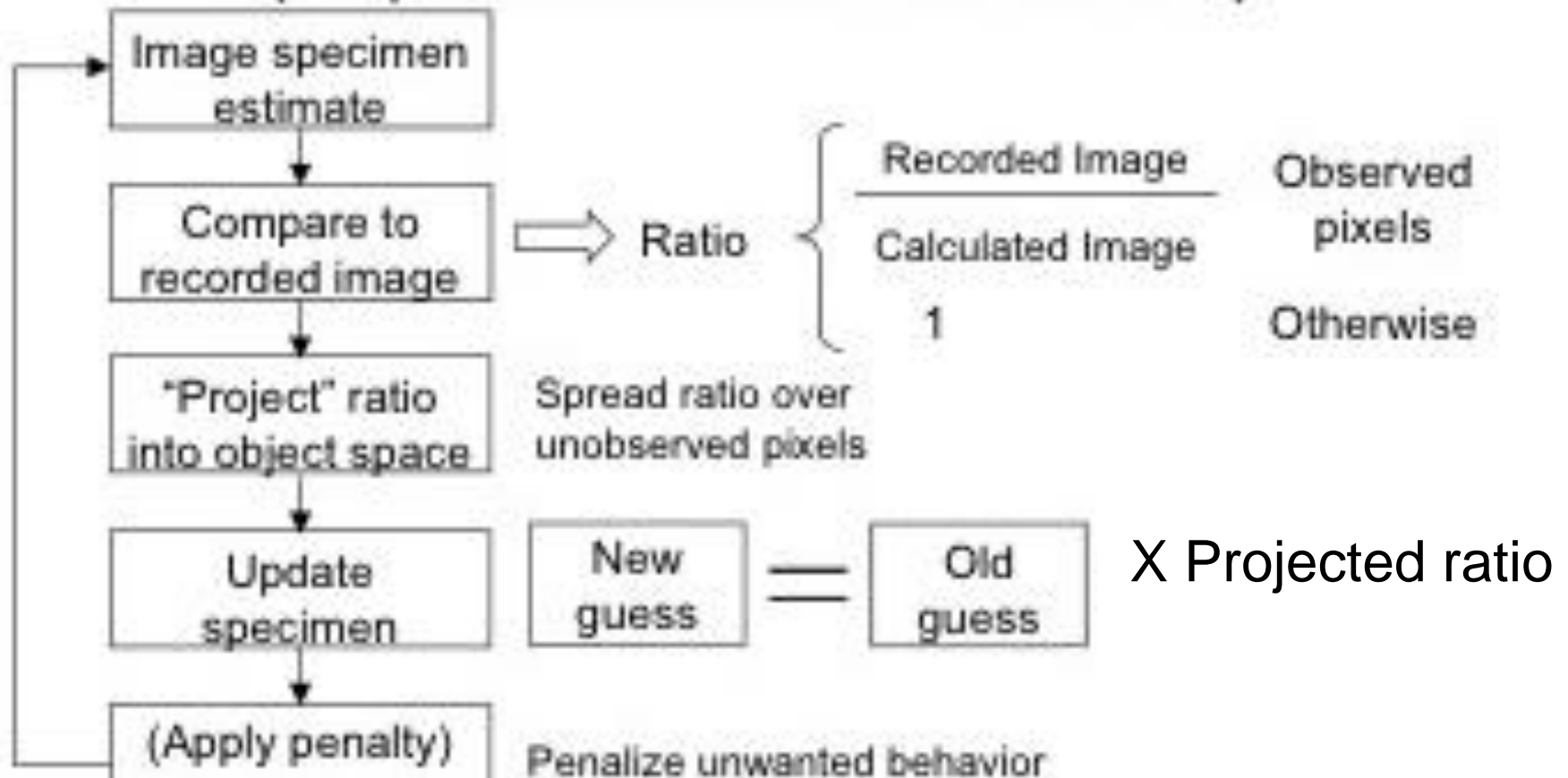
Expectation-maximization algorithms has several

- Collecting extra images above and below the focal plane is not needed.

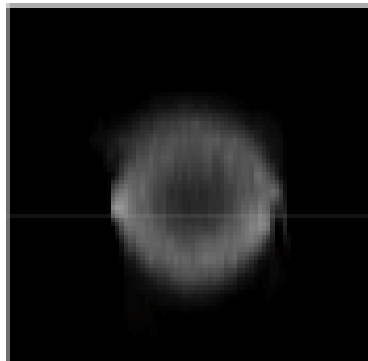
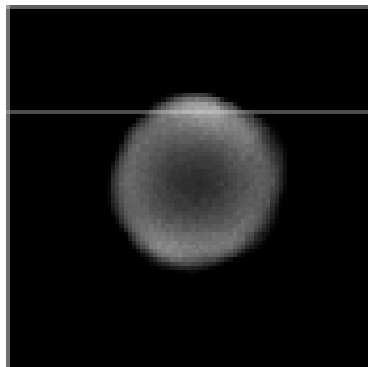
Some filters such as wiener filter, JvC or inverse filter need empty planes above and below specimen. These are planes have out of focus light but contain no fluorescent parts.

- Remove Bad pixels on CCD
- The image reformed may have a smaller pixel than original image.

(Expectation-maximization)



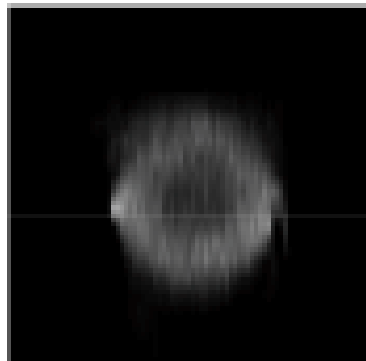
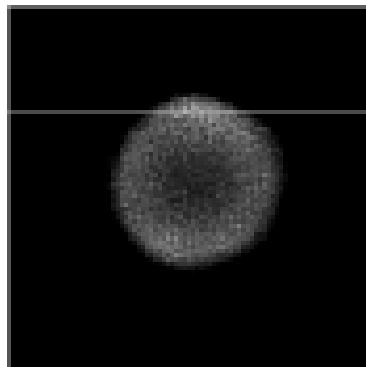
High SNR



3000

14 41

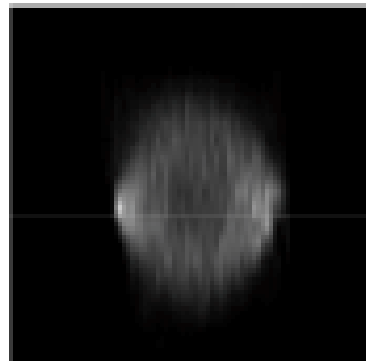
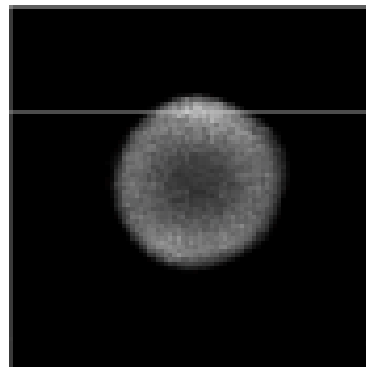
Midium SNR



3000

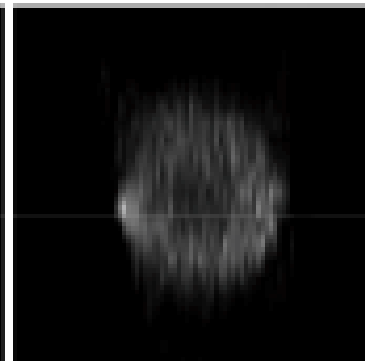
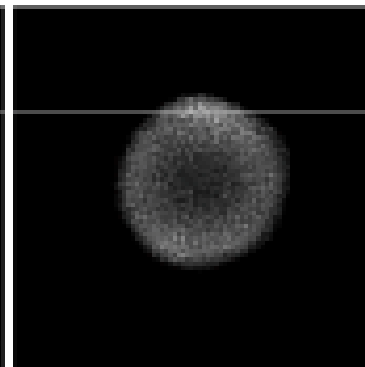
14 41

Low SNR



1000

14 41

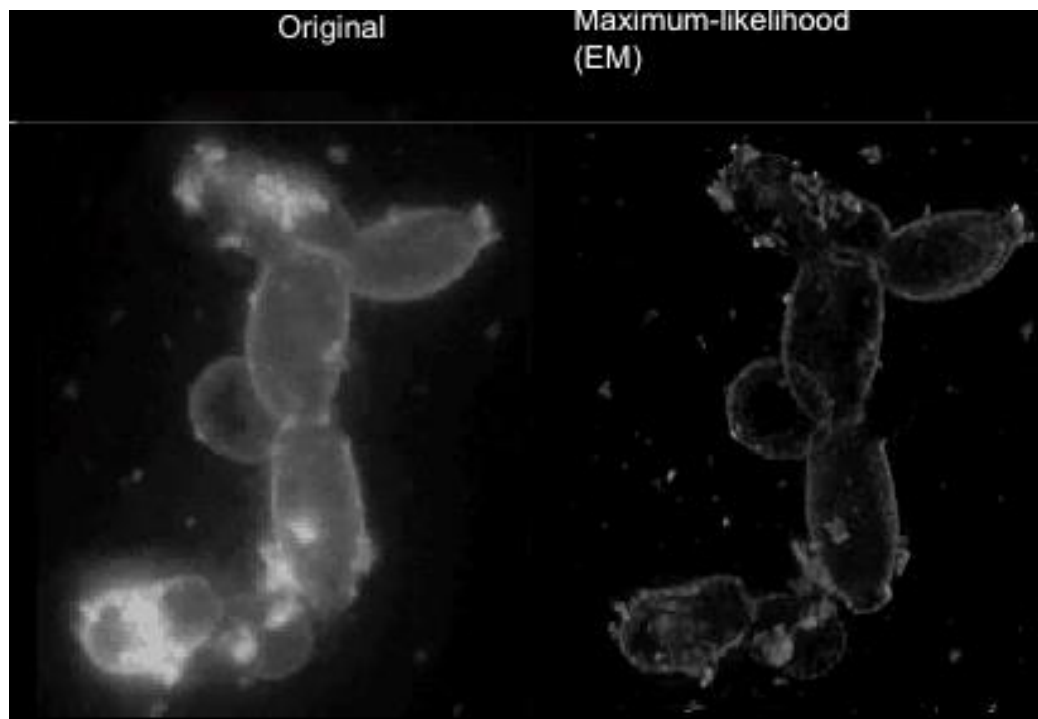


3000

14 41

Original

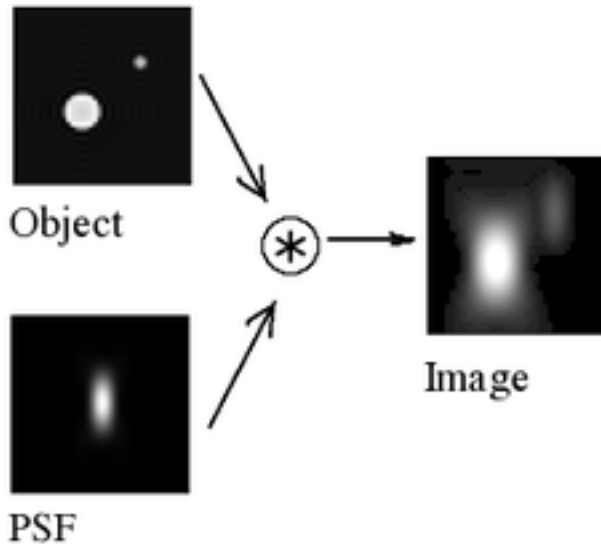
Maximum-likelihood
(EM)



How do you find PSF?

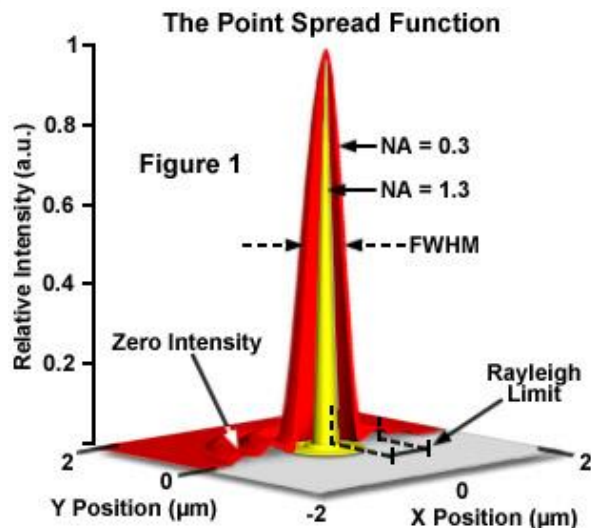
- Measure it experimentally –image of fluorescent microsphere
- Theoretically computed-use a mathematical model to find PSF
- During the deconvolution it can be estimated.

Point Spread Function (PSF)



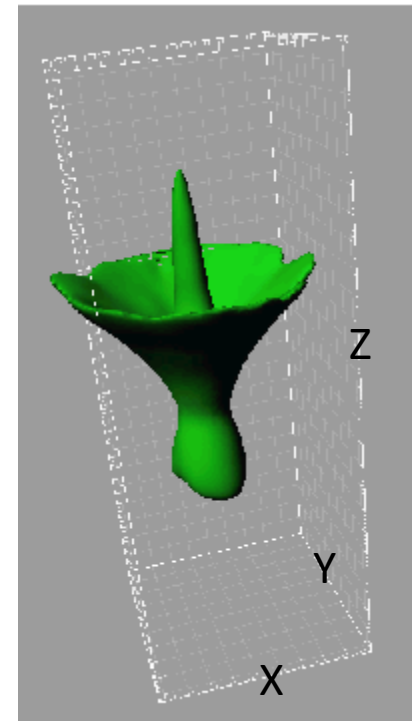
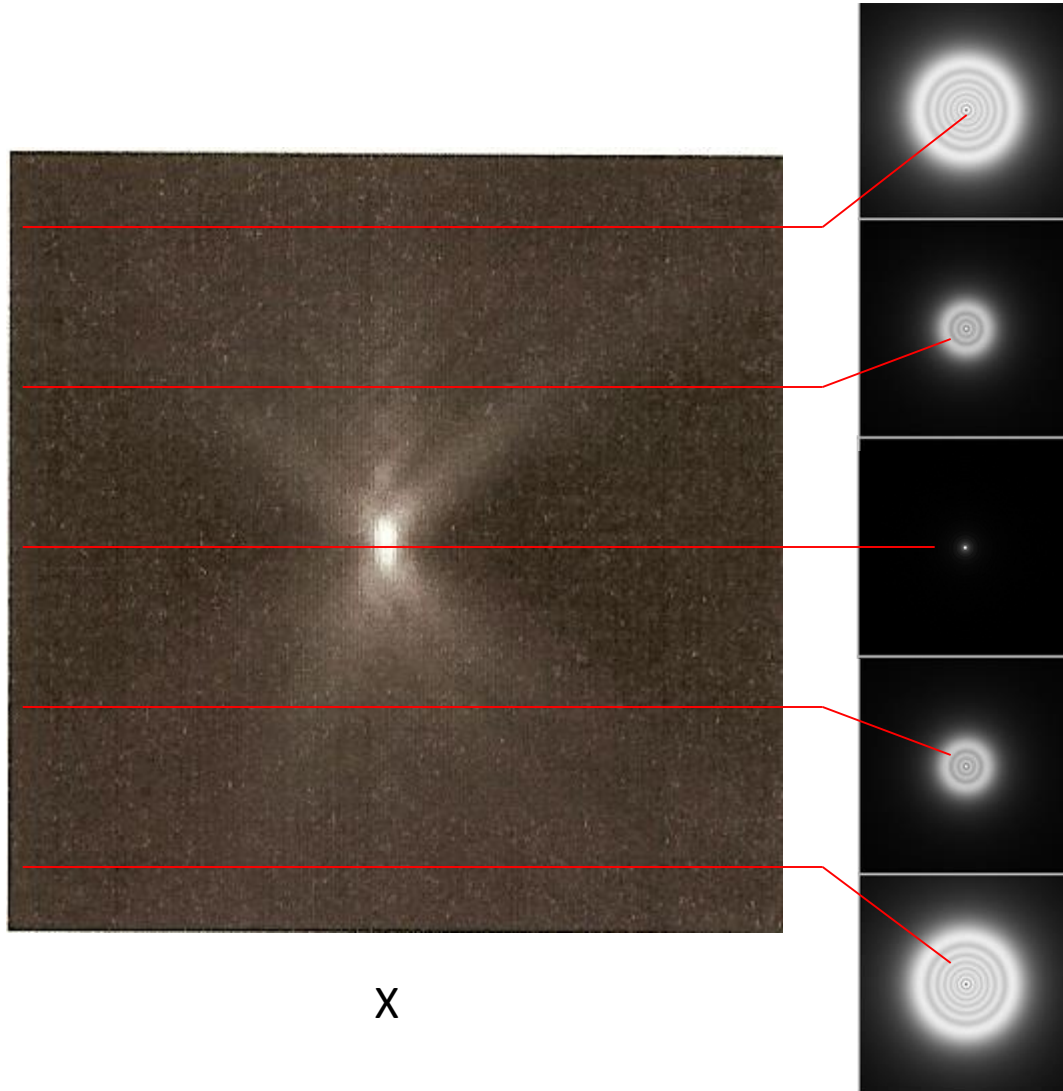
When the image is captured by a camera through a lens and objective, A real point object produces an image that is only an approximation of the object. The lens transforms the object of discrete dimensions shown on the left.

The point is no longer a point. the resultant object on the image is called a point spread function (PSF). Since an object of any form and size is just a collection of points, we should understand how a lens (microscope) distorts a single point.



It is the three-dimensional diffraction pattern of light emitted from an infinitely small point source and transmitted to the image plane through a high numerical aperture objective.

Point Spread Function



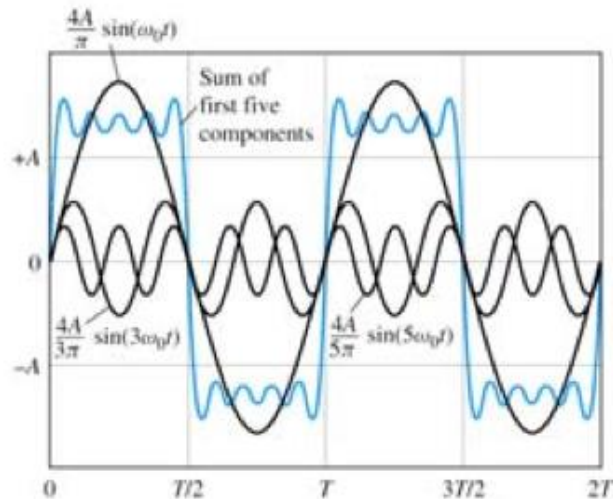
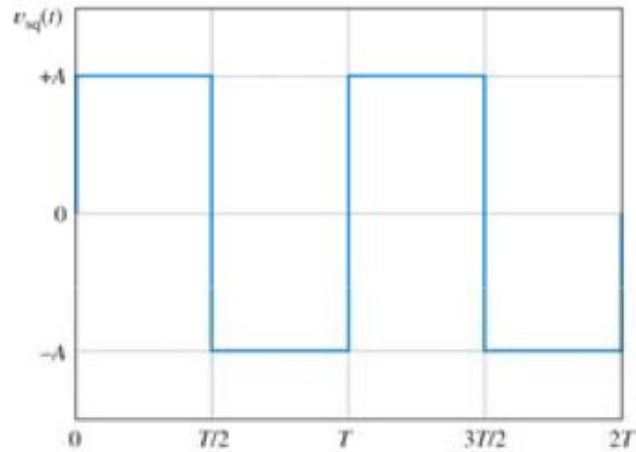
Deblurring -Deconvolution

Point Spread function is the Fourier transform of a filter. PSP says how much blurring there will be in trying to image a point.

$$\text{FFT}(\text{Unblurred Image}) * \text{FFT}(\text{Point Spread Function}) = \text{FFT}(\text{Blurred Image})$$

$$\text{Unblurred Image} = \text{FFT}^{-1} [\text{FFT}(\text{Blurred Image}) / \text{FFT}(\text{Point Spread Function})]$$

A square wave can be divided into many sine waves

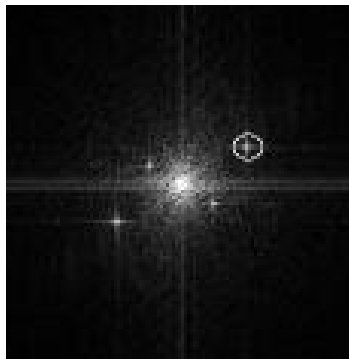


(b) Several of the sinusoidal components and the sum of the first five components

FFT in Image Processing : Removing noise in the image



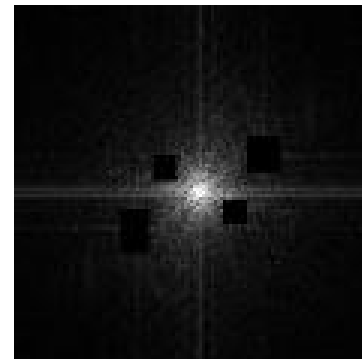
FFT



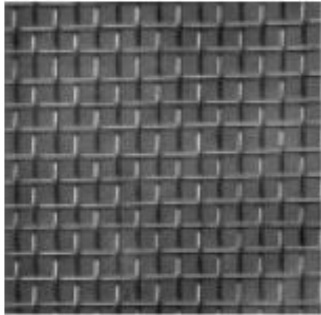
There are four spikes that cause the noise



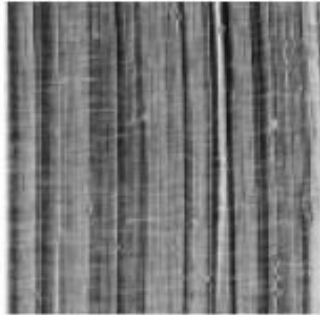
Inverse
FFT



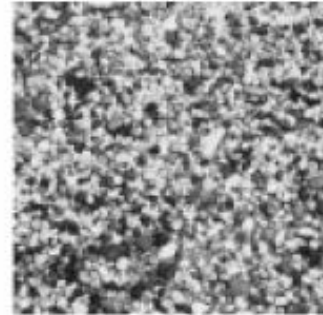
The spikes are removed from the image



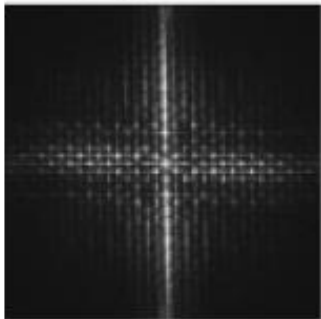
(a) A periodic texture (D1).



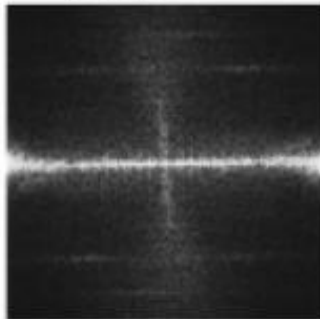
(b) A directional texture (D106).



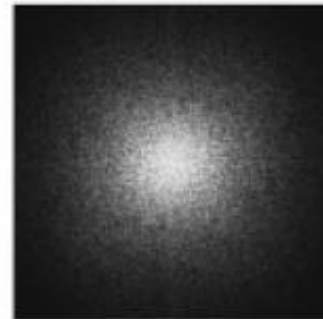
(c) A random texture (D106).



(d) The Fourier spectrum of (a).

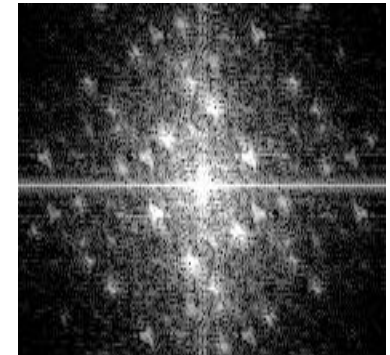
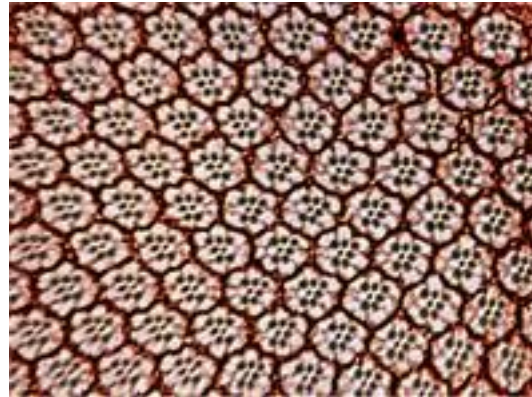
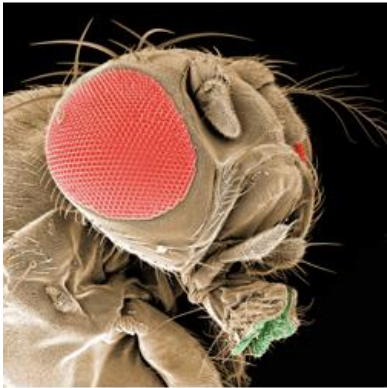


(e) The Fourier spectrum of (b).

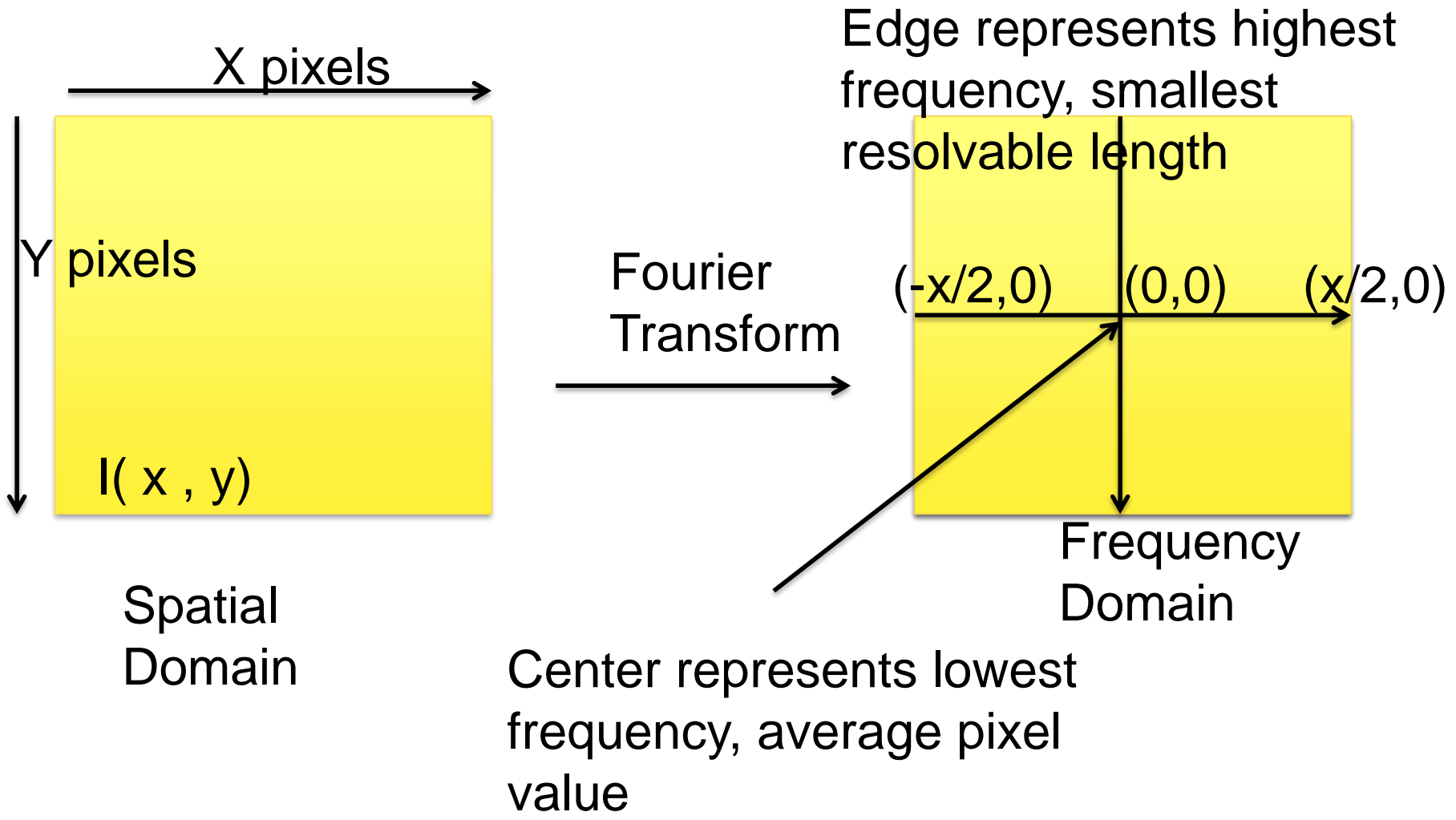


(f) The Fourier spectrum of (c).

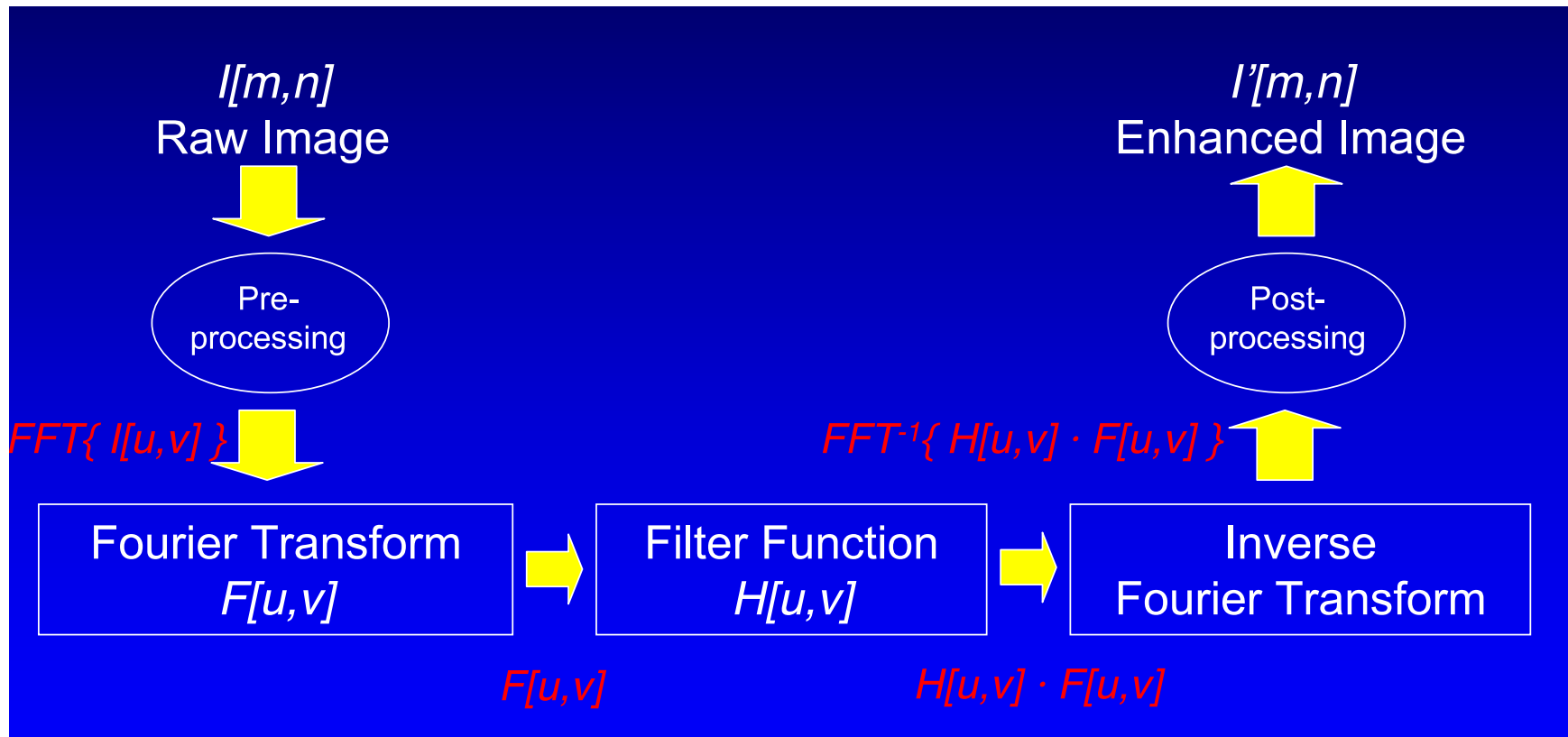
Hexagonal closed pack structure can be identified using FFT



Discrete Fourier Transform 2D

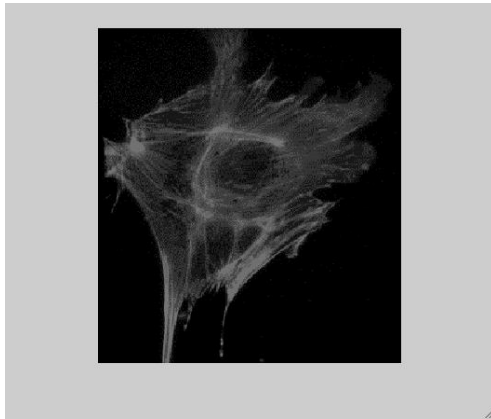


FFT: Filtering in the frequency domain

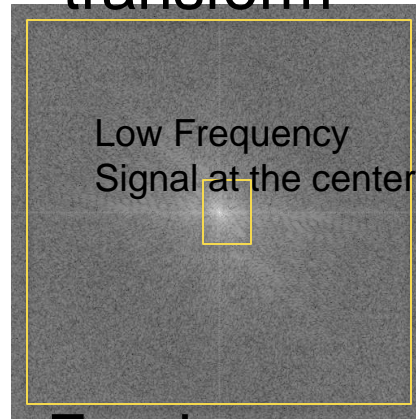


Steps to remove blurring in the images

- Step1



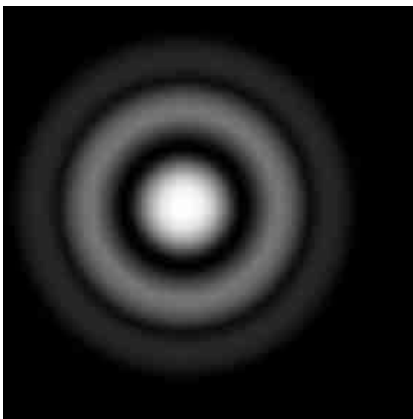
Fourier
transform



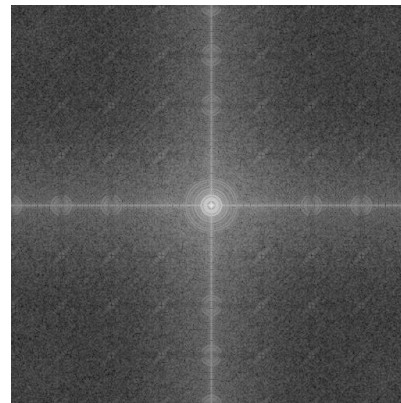
High Frequency signal at the edges

Low Frequency
Signal at the center

- Step2

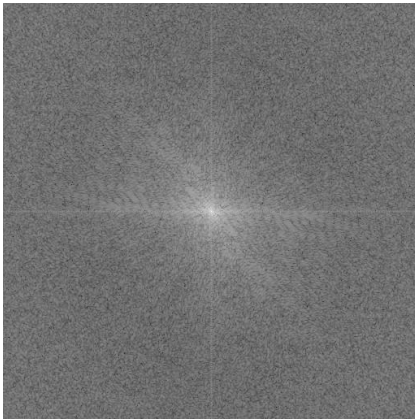


Fourier
transform

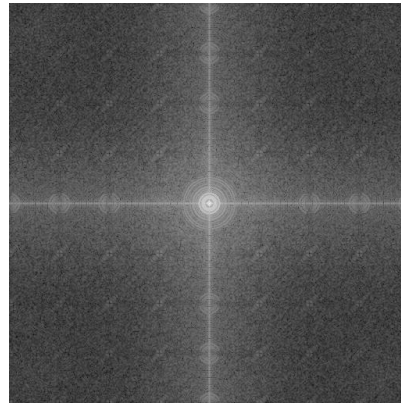


Deconvolution

- Step 3. The iamges are divided to each other and apply inverse FFT to obtain sharpen image.



/



= unblurred image

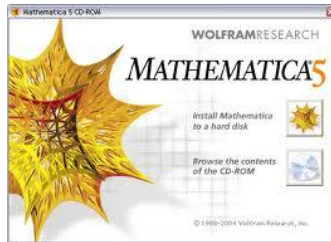
Using different Filters in the frequency domain

- High Pass Filter
- Low Pass Filter

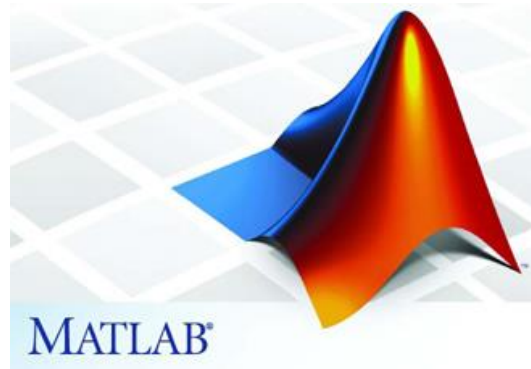
Summary of FFT

- Fourier Analysis is a powerful tool for removing the noise in the images.
- Discrete Fourier Transforms are appropriate for computation
- Interpretation of the complex Fourier Transform is not always straightforward. To restore or enhance images, convolution and deconvolution
- Many other image processing methods such as making JPEG images uses the Discrete Cosine Transform that is a special kind of Fourier transform

Several Computer Programs for FFT



It perform symbolic or numerical
FFT analysis



>> fft(x)

Signal processing toolbox or write your
own code.



fft(f(x))

Image J

