Bioimage Informatics

Lecture 5, Spring 2012

Fundamentals of Fluorescence Microscopy (II)

Bioimage Data Analysis (I): Basic Operations

Outline

- Performance metrics of a microscope

- Basic image analysis: open sources of images
- Basic image analysis: image filtering
- Basic image analysis: image intensity derivative calculation
- Project assignment 1

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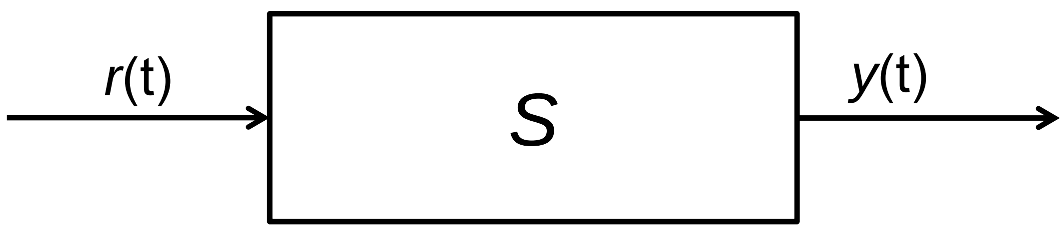
Performance Metrics of a Light Microscope

- Resolution: the smallest feature distance that can be resolved.
- Field of view: the area of a specimen that can be observed and recorded in an image.
- Depth-of-field: the axial distance (depth) range in the specimen that appears in focus in an image.
- Light collection power: determines image brightness.

Basic Concept of a Linear System

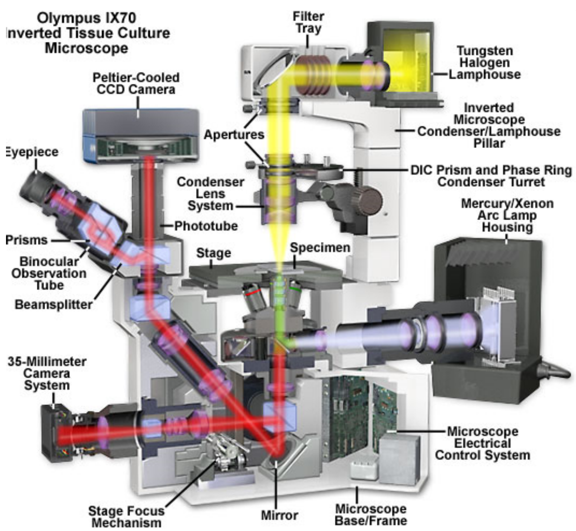
- A system is said to be linear if it satisfies the following two conditions
  - Homogeneity
  - Additivity

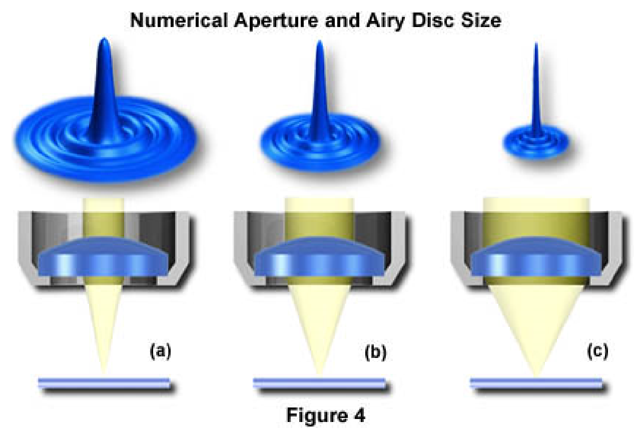


- A linear system can be characterized in the time domain by its impulse response.

- A properly built and aligned microscope can be accurately modeled as a linear system.

Microscope as a Linear System





- A light microscope is a linear system whose impulse response is an Airy disk.

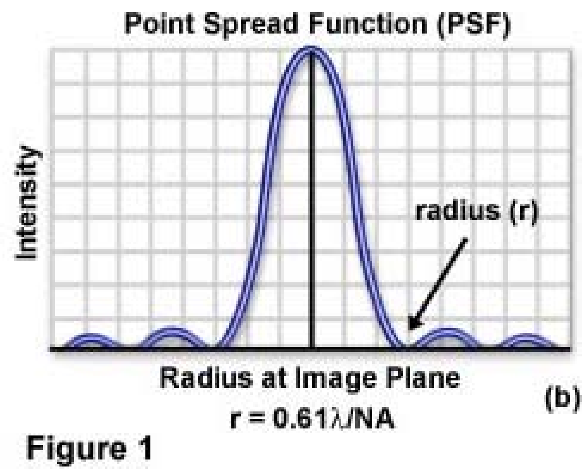
  http://micro.magnet.fsu.edu/primer/java/imageformation/airydiskformation/index.html

Airy Disk

- Airy (after George Biddell Airy) disk is the diffraction pattern of a point feature under a circular aperture.

- It has the following form

$$I(\theta) = I_0 \left[\frac{2J_1(r)}{r} \right]^2$$



$J_1(x)$ is a Bessel function of the first kind.

- Detailed derivation is given in

  Born & Wolf, Principles of Optics, 7th ed., pp. 439-441.

Microscope Image Formation: PSF & OTF

- The impulse response of the microscope is called its point spread function (PSF).

- The transfer function of a microscope is called its optical transfer function (OTF).

- The PSF of a properly built and aligned microscopy is an Airy Disk.

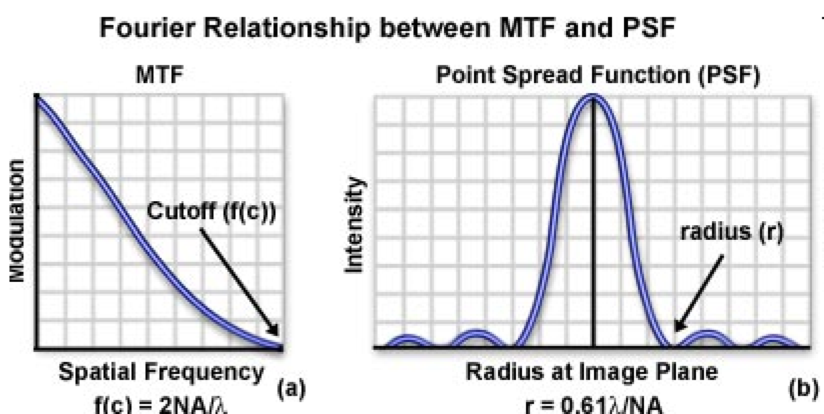
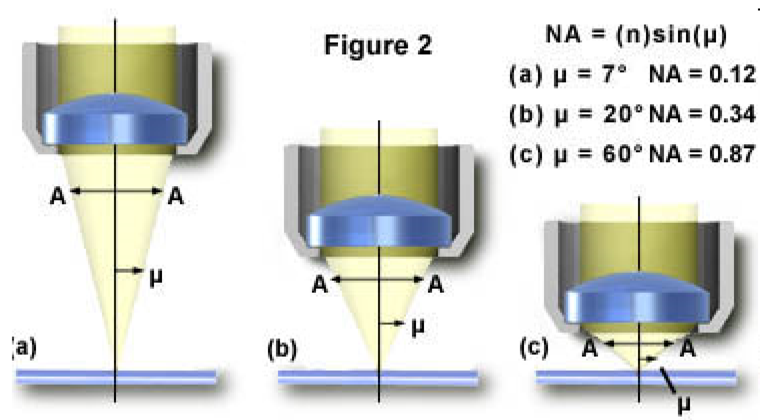


Figure 1

Numerical Aperture

- Numerical aperture (NA) determines microscope resolution and light collection power.



$$NA = n \cdot \sin \mu$$

n: refractive index of the medium between the lens and the specimen

μ: half of the angular aperture

Microscope Image Formation

- Microscope image formation can be modeled as a convolution with the PSF.

$$I(x,y) = O(x,y) \otimes psf(x,y)$$

$$F\{I(x,y)\} = F\{O(x,y)\} \cdot F\{psf(x,y)\}$$

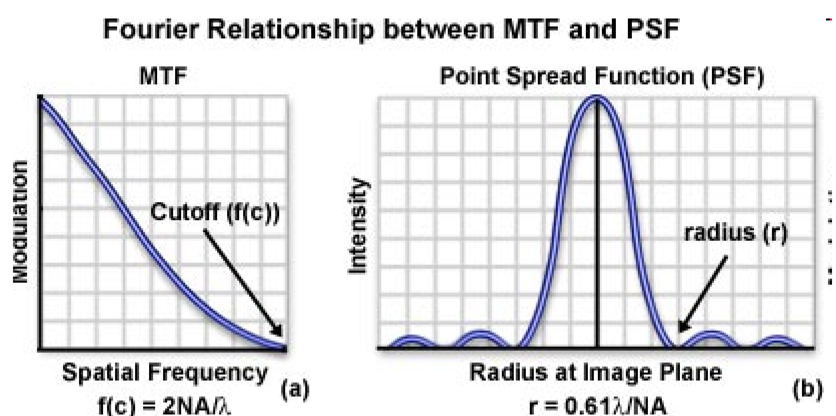


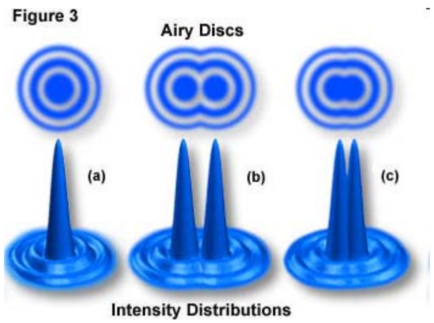
Figure 1

http://micro.magnet.fsu.edu/primer/java/mtf/airydisksize/index.html

Different Definition of Light Microscopy Resolution Limit (Demo)

- Rayleigh limit

$$D = \frac{0.61\lambda}{NA}$$



- Sparrow limit

$$D = \frac{0.47\lambda}{NA}$$

http://www.microscopy.fsu.edu/primer/java/imageformation/rayleighdisks/index.html

Field of View (Demo)

- Field of view: the region that is visible under a microscope

- If characterized in diameter

$$D \propto \frac{\text{Field diaphragm diameter}}{M}$$

- If characterized in area

$$S \propto \frac{\text{Field diaphragm diameter}^2}{M^2}$$

http://micro.magnet.fsu.edu/primer/java/microscopy/diaphragm/index.html

Depth-of-Field

- Depth-of-field: the axial distance (depth) in the specimen that appears in focus in the image.

$$d_{tot} = \frac{\lambda \cdot n}{NA^2} + \frac{n}{M \cdot NA} e$$

n: refractive index of the medium between the lens and the specimen

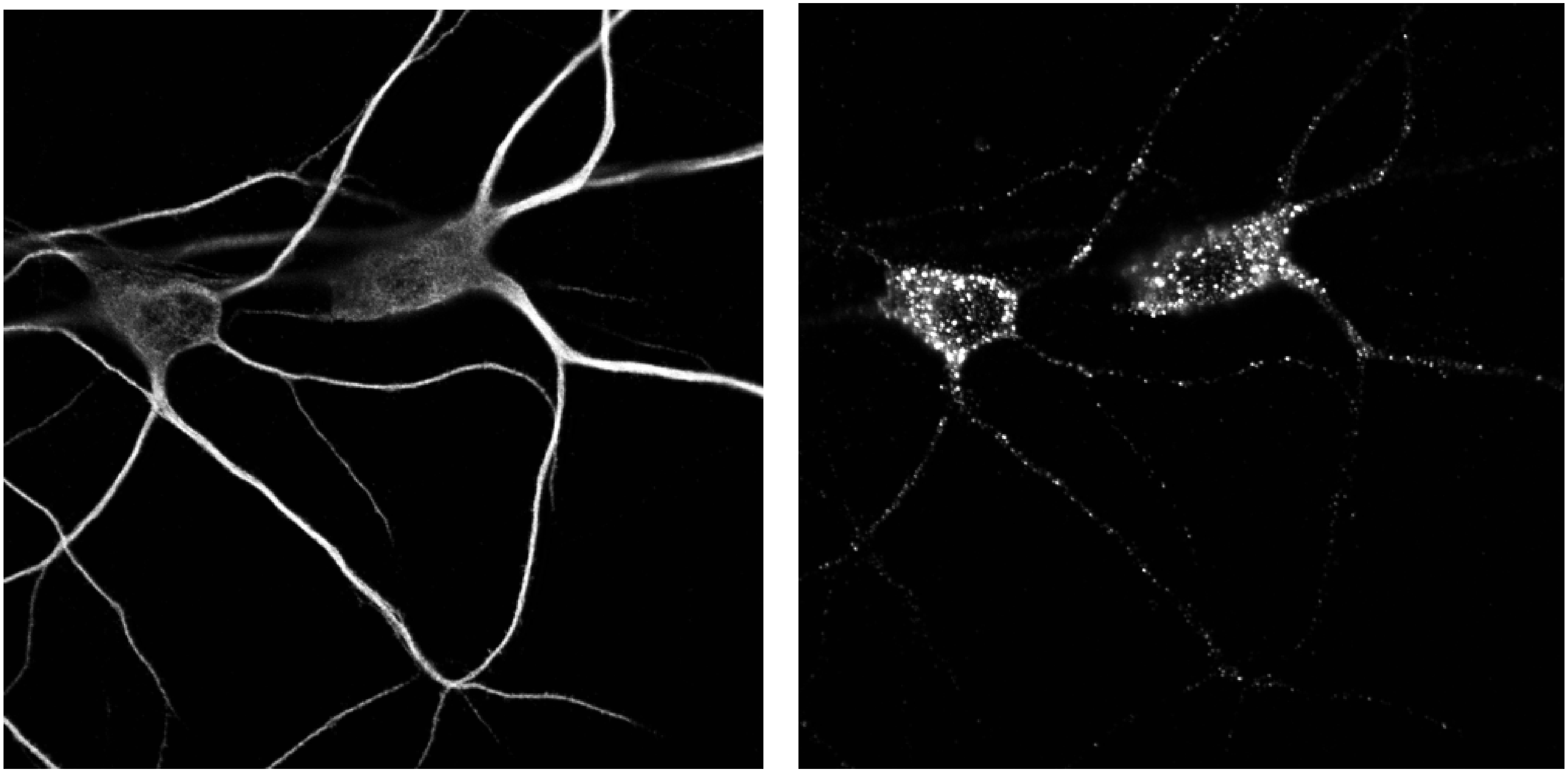
λ: emission wavelength

M: magnification

NA: numerical aperture

e: smallest resolvable distance in the image plane

Example: Depth-of-Field



Smaug1 mRNA-silencing foci respond to NMDA and modulate synapse formation, M. Baez, et al, JCB, 195:1141-1157, 2011

Image Intensity: Light Collecting Power

- For transmitted light

$$I \propto \frac{\mathrm{NA}^2}{M^2}$$

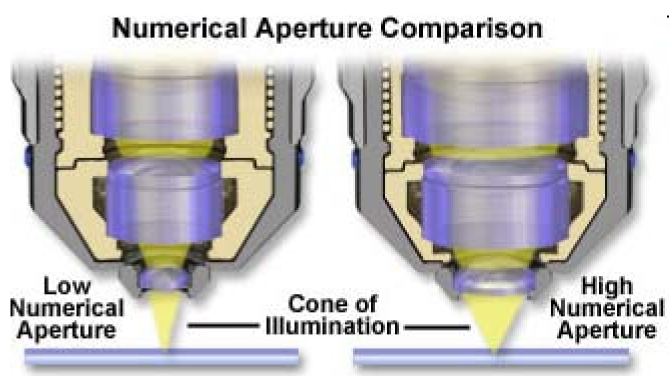
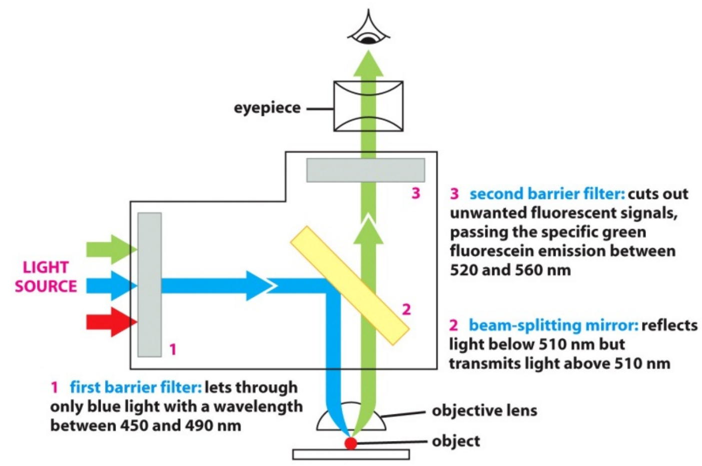


Figure 2

- For epi-fluorescence

$$I \propto \frac{\mathrm{NA}^4}{M^2}$$

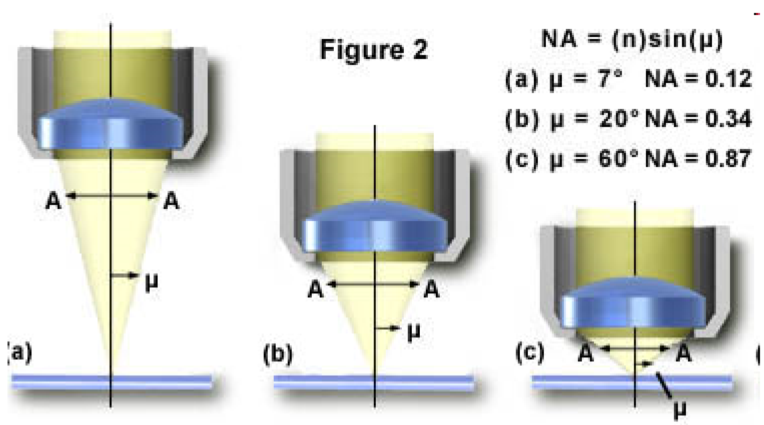


http://micro.magnet.fsu.edu/primer/anatomy/imagebrightness.html

Working Distance

- The distance between the objective lens and the specimen.

- Working distance does not directly influence imaging but may determine how images can be collected.



Summary: High Resolution Microscopy

- Size of cellular features are typically on the scale of a micron or smaller.

$$D = \frac{0.61\lambda}{NA}$$

- To resolve such features require
  - Shorter wavelength (e.g. electron microscopy)
  - High numerical aperture (for resolution)
  - High magnification (for spatial sampling)

Summary: High Resolution Microscopy

- Higher magnification and higher numerical aperture mean

  - Smaller field of view $$S \propto \frac{\text{Field diaphragm diameter}^2}{M^2}$$

  - Smaller depth of field $$d_{tot} = \frac{\lambda \cdot n}{NA^2} + \frac{n}{M \cdot NA} e$$

  - Lower light collection power $$I \propto \frac{\text{NA}^2}{M^2}$$

  - Smaller working distance

- Performance metrics of a microscope
- Basic image analysis: open sources of images
- Basic image analysis: image filtering
- Basic image analysis: image intensity derivative calculation
- Project assignment 1

A Few Words about MATLAB

- There are many excellent tutorials online.
- There are many excellent reference books.
- It is worthwhile to invest some time on learning MATLAB.
- Please bring your questions to our teaching assistant.

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Where & How to Get Image Data

- The number of open image repositories is constantly increasing.
- OME: open microscopy environment

  http://www.openmicroscopy.org/
- JCB DataViewer
- ASCB Cell Image Library

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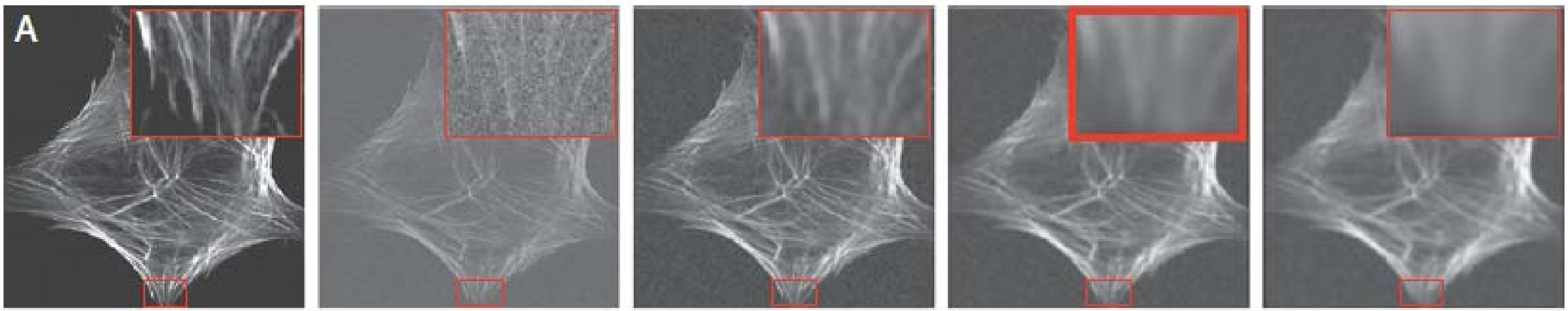
Basic Concept of Image Filtering (I)

- Application I: noise suppression



original | noise added | σ=2 | σ=10 | σ=20

Basic Concept of Image Filtering (II)

- Application II: image conditioning

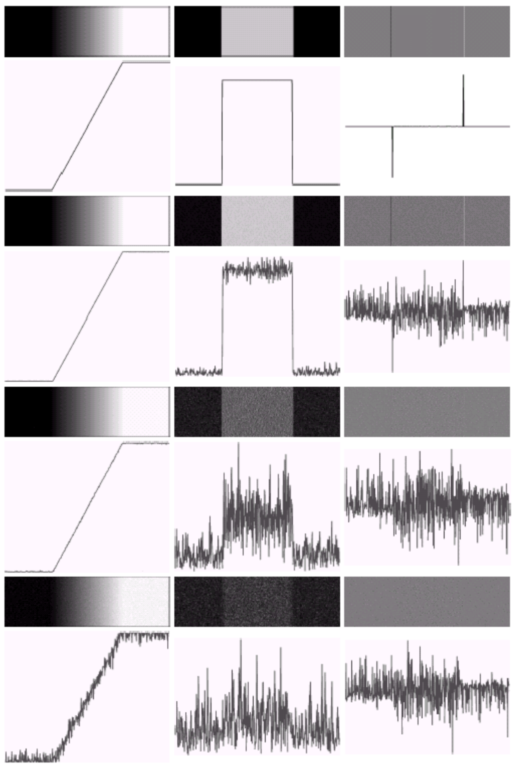


FIGURE 10.7 First column: images and gray-level profiles of a ramp edge corrupted by random Gaussian noise of mean 0 and $\sigma = 0.0, 0.1, 1.0$, and 10.0, respectively. Second column: first-derivative images and gray-level profiles. Third column: second-derivative images and gray-level profiles.

a b c d

Gonzalez & Woods, DIP 2/e

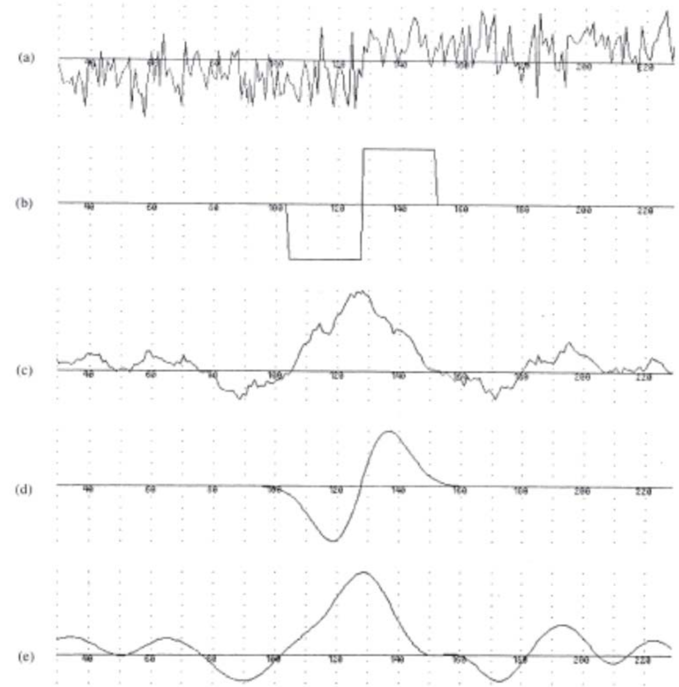


Fig. 1. (a) A noisy step edge. (b) Difference of boxes operator. (c) Difference of boxes operator applied to the edge. (d) First derivative of Gaussian operator. (e) First derivative of Gaussian applied to the edge.

Canny, J., *A Computational Approach To Edge Detection*, IEEE Trans. Pattern Analysis and Machine Intelligence, 8(6):679–698, 1986.

Basic Concept of Image Filtering (III)

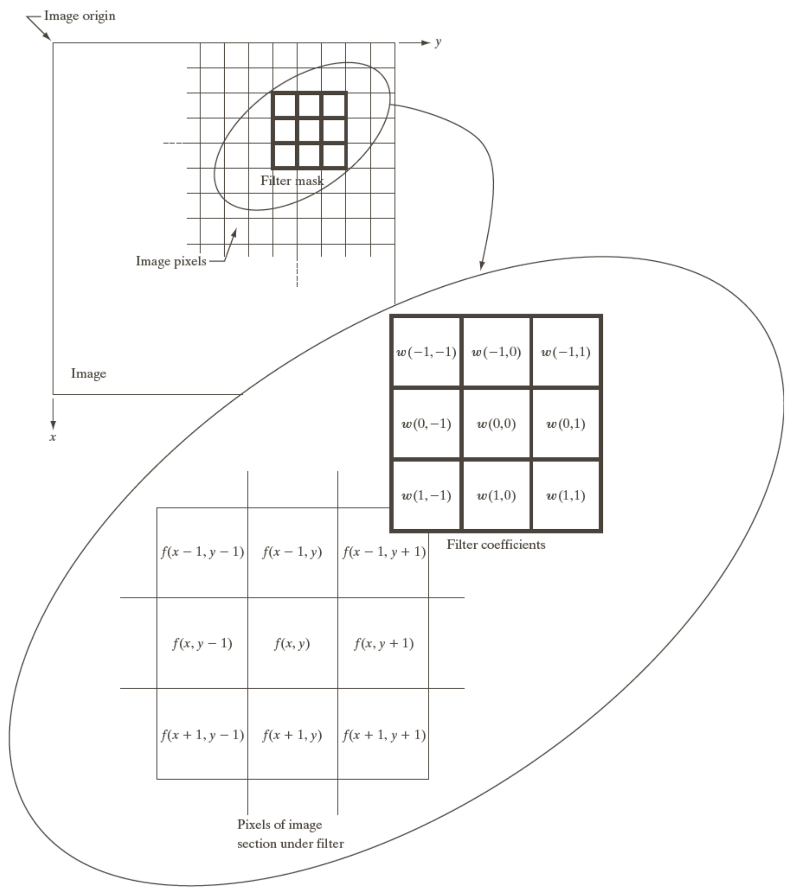


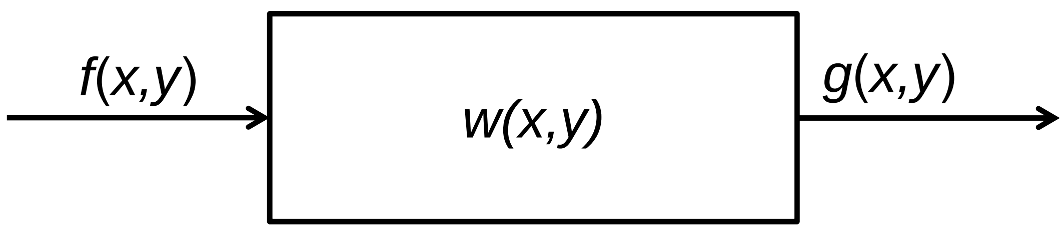
FIGURE 3.28 The mechanics of linear spatial filtering using a 3 × 3 filter mask. The form chosen to denote the coordinates of the filter mask coefficients simplifies writing expressions for linear filtering.

Gonzalez & Woods, DIP 3/e

Basic Concept of Image Filtering (IV)

- Image filtering in the spatial domain

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



$$g(x,y) = w(x,y)\otimes f(x,y)$$

$$G(u,v) = W(u,v)\cdot F(u,v)$$

http://www.imageprocessingplace.com/

Gaussian Filter (I)

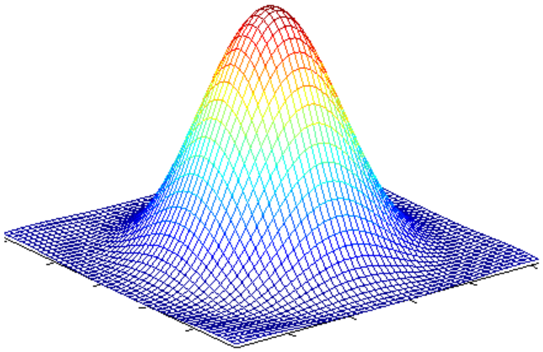
- Gaussian kernel in 1D

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

$$G(x,y;\sigma_x,\sigma_y) = \frac{1}{2\pi\sigma_x\sigma_y} e^{-\left(\frac{x^2}{2\sigma_x{}^2}+\frac{y^2}{2\sigma_y{}^2}\right)}$$

- First order derivative

$$G'(x;\sigma) = \frac{-x}{\sqrt{2\pi}\sigma^3} e^{-\frac{x^2}{2\sigma^2}}$$



- Second order derivative

$$G''(x;\sigma) = \frac{-x}{\sqrt{2\pi}\sigma^3} e^{-\frac{x^2}{2\sigma^2}} \left[1-\frac{x^2}{\sigma^2}\right]$$

Gaussian Filters (II)

- Some basic properties of a Gaussian filter
  - It is a low pass filter

$$\frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} \frac{e^{-\frac{\sigma^2\omega^2}{2}}}{\sqrt{2\pi}}$$

  - It is separable

$$G(x, y; \sigma_x, \sigma_y) = \frac{1}{2\pi\sigma_x\sigma_y} e^{-\left(\frac{x^2}{2\sigma_x^2} + \frac{y^2}{2\sigma_y^2}\right)} = \frac{1}{\sqrt{2\pi}\sigma_x} e^{-\frac{x^2}{2\sigma_x^2}} \cdot \frac{1}{\sqrt{2\pi}\sigma_y} e^{-\frac{y^2}{2\sigma_y^2}}$$

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Combination of Noise Suppression and Gradient Estimation (I)

- Implementation

$$I_x(i,j) = \frac{I(i+1,j) - I(i-1,j)}{2}$$

$$I_y(i,j) = \frac{I(i,j+1) - I(i,j-1)}{2}$$

- Notation:
  J: raw image;
  I: filtered image after convolution with Gaussian kernel G.

- A basic property of convolution

$$\frac{\partial(G*J)}{\partial x} = \frac{\partial I}{\partial x} = I_x = \frac{\partial G}{\partial x} * J \qquad \frac{\partial(G*J)}{\partial y} = \frac{\partial I}{\partial y} = I_y = \frac{\partial G}{\partial y} * J$$

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Basic Image Operations

- Reading an imaging
- Accessing individual pixels
- Setting a region of interest (ROI)
- Writing an image

Questions?