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
• Performance metrics of a microscope

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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
  
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.
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
- \( \mu \): half of the angular aperture
Microscope Image Formation

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

\[
I(x, y) = O(x, y) \otimes psf(x, y)
\]

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

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
- \( \lambda \): 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

\[ I \propto \frac{NA^2}{M^2} \]

- For epi-fluorescence

\[ I \propto \frac{NA^4}{M^2} \]

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.

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

\[ D = \frac{0.61\lambda}{NA} \]
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
    \[ d_{\text{tot}} = \frac{\lambda \cdot n}{NA^2} + \frac{n}{M \cdot NA} e \]
  - Lower light collection power
    \[ I \propto \frac{NA^2}{M^2} \]
  - Smaller working distance
• Performance metrics of a microscope

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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 Image](image1)
![Noise Added](image2)
![σ=2](image3)
![σ=10](image4)
![σ=20](image5)
Basic Concept of Image Filtering (II)

• Application II: image conditioning

Basic Concept of Image Filtering (III)

**FIGURE 3.28** The mechanics of linear spatial filtering using a $3 \times 3$ 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
  \[ G(x; \sigma) = \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{x^2}{2\sigma^2}} \]

- 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 kernel in 2D
  \[ G(x, y; \sigma_x, \sigma_y) = \frac{1}{2\pi\sigma_x\sigma_y} e^{-\left( \frac{x^2}{2\sigma_x^2} + \frac{y^2}{2\sigma_y^2} \right)} \]
Gaussian Filters (II)

- Some basic properties of a Gaussian filter
  - It is a low pass filter
    
    \[
    \frac{1}{\sqrt{2\pi\sigma}} e^{-\frac{x^2}{2\sigma^2}} \xrightarrow{F} e^{-\frac{\sigma^2 \omega^2}{2}} \frac{1}{\sqrt{2\pi}}
    \]
  
  - It is separable

\[
G(x, y; \sigma_x, \sigma_y) = \frac{1}{2\pi\sigma_x\sigma_y} e^{-\frac{x^2}{2\sigma_x^2} + \frac{y^2}{2\sigma_y^2}} = \frac{1}{\sqrt{2\pi\sigma_x}} e^{-\frac{x^2}{2\sigma_x^2}} \cdot \frac{1}{\sqrt{2\pi\sigma_y}} e^{-\frac{y^2}{2\sigma_y^2}}
\]
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• **Basic image analysis: image intensity derivative calculation**

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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

\[ \frac{\partial (G \ast J)}{\partial x} = \frac{\partial I}{\partial x} = I_x = \frac{\partial G}{\partial x} \ast J \]

\[ \frac{\partial (G \ast J)}{\partial y} = \frac{\partial I}{\partial y} = I_y = \frac{\partial G}{\partial y} \ast J \]
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Basic Image Operations

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