Bioimage Informatics

Lecture 3, Spring 2012

Fundamentals of Light Microscopy (II)

Practical Issues in Bioimage Informatics

Outline

- Contrast generation in microscopy
- Practical considerations in fluorescence microscopy

- Useful software tools for microscopy images
- Basic image analysis concepts
- References
- Reading assignment 1

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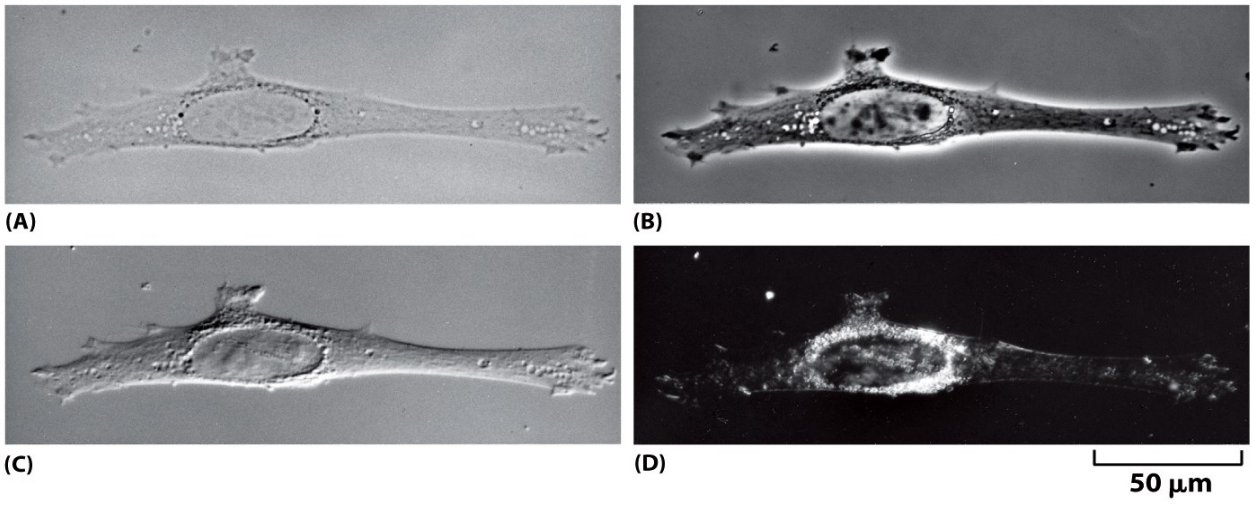
Contrast Generation in Light Microscopy

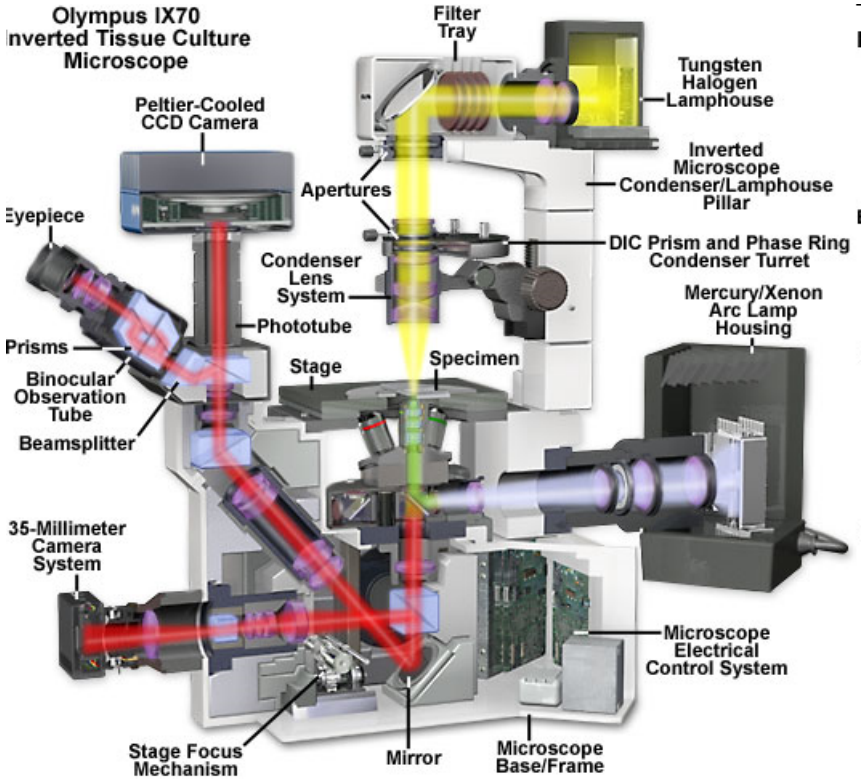
- Two fundamental roles of any microscope
  - To provide adequate contrast
  - To provide adequate resolution.

- Contrast generation
  - Transmitted light illumination vs reflected light illumination
  - Bright-field vs dark-field
  - Phase contrast
  - Fluorescent microscopy

(A) Bright-field
(B) Phase
(C) DIC
(D) Dark-field



Reflected Light vs Transmitted Light

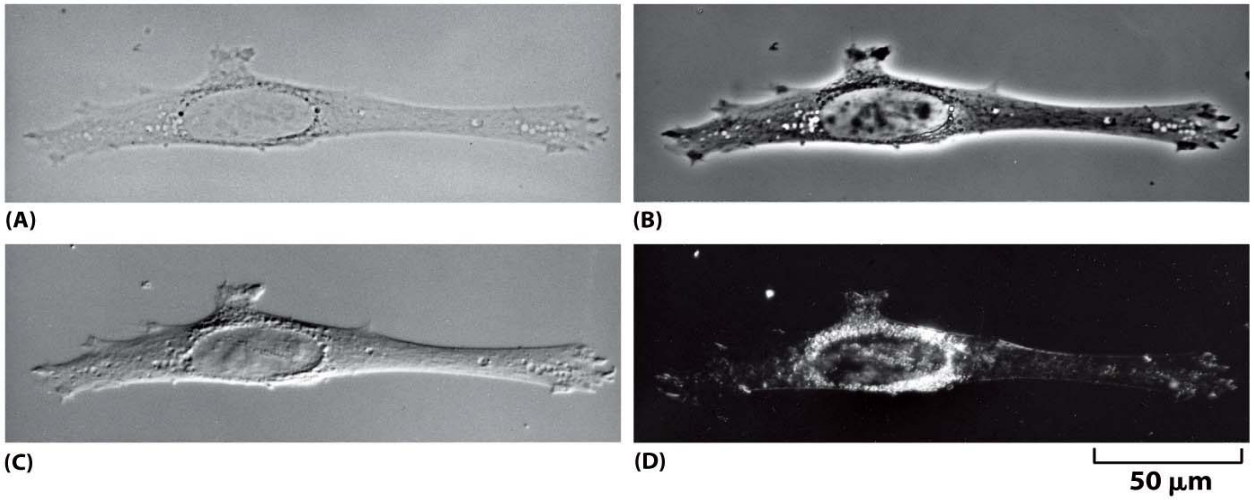


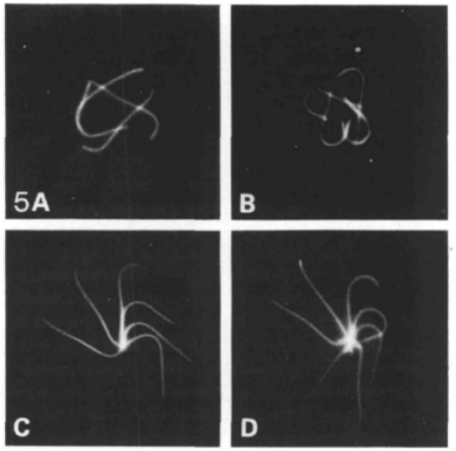
http://www.olympusmicro.com/primer/java/lightpaths/ix70fluorescence/ix70.html

Bright-field vs Dark-field (I)

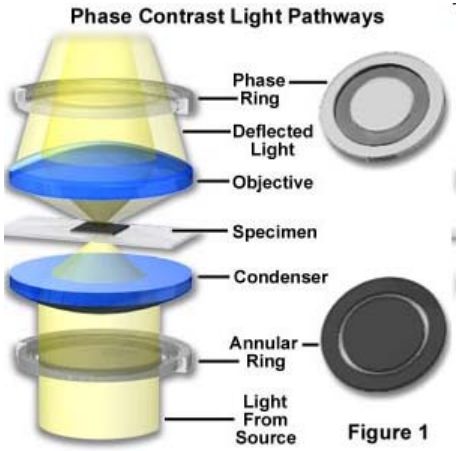
- Under bright-field contrast, the specimen appears dark against a bright background.

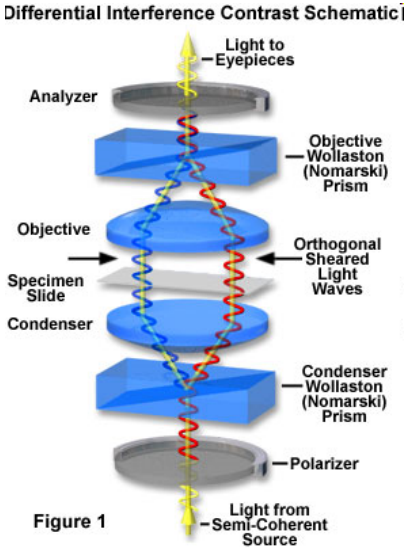
- Dark-field contrast is particularly useful when imaging thin filaments or small particles.

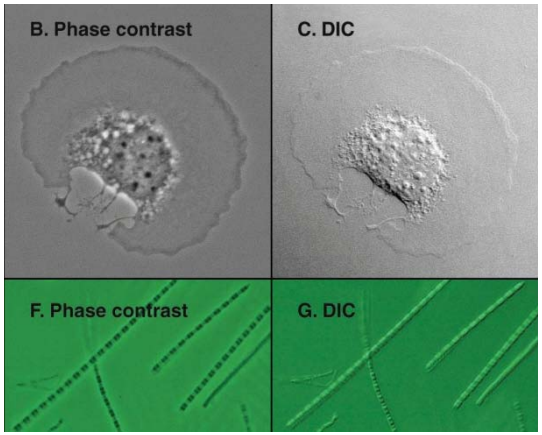


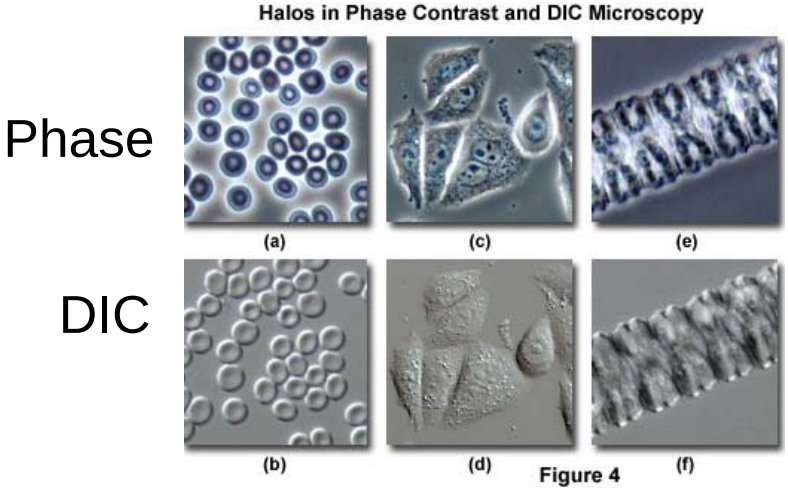


Phase Contrast & DIC (II)

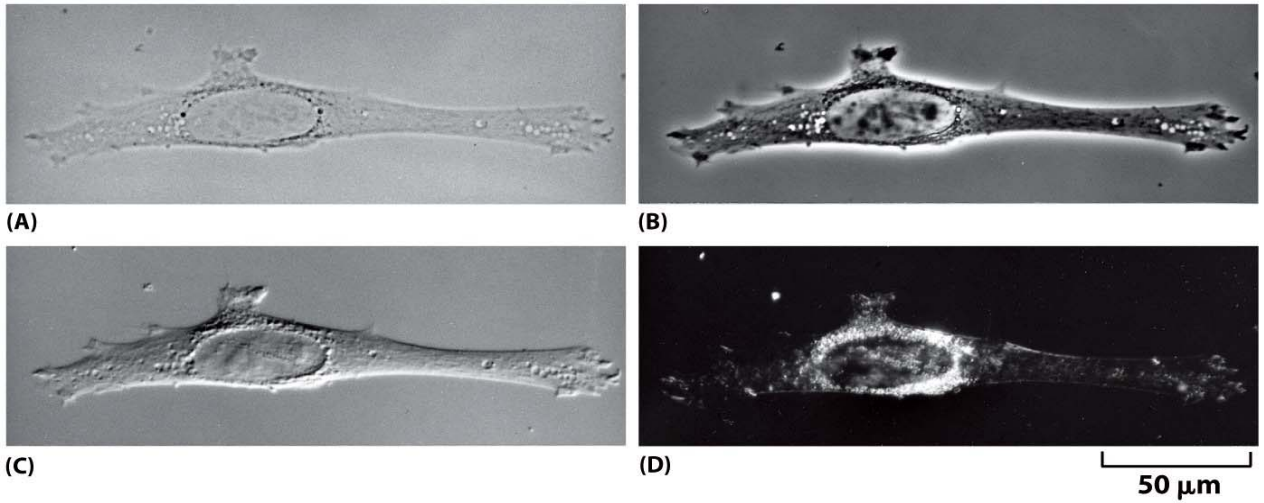


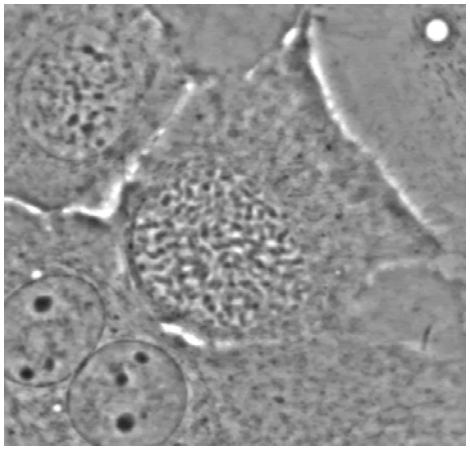


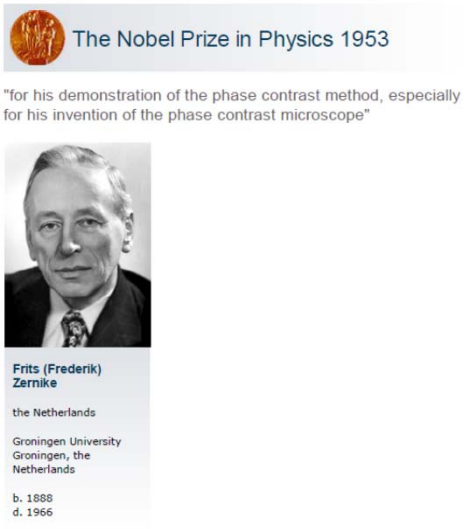




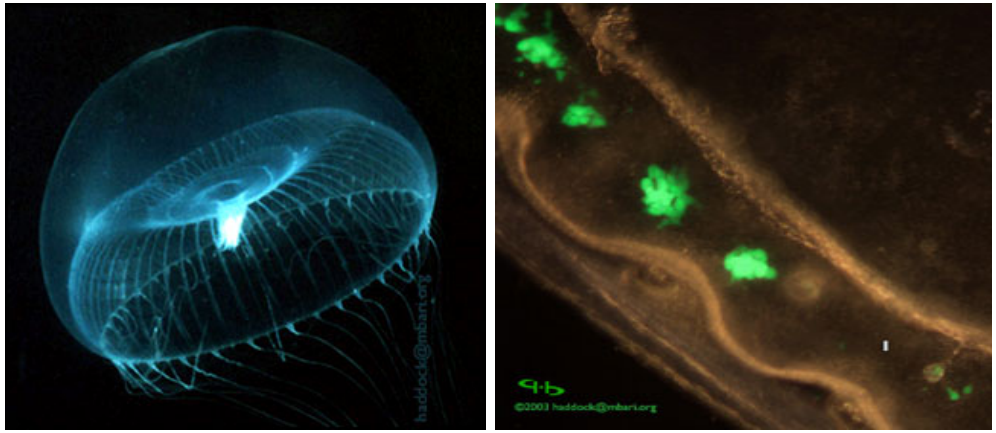
What is wrong with ALL the cell images?



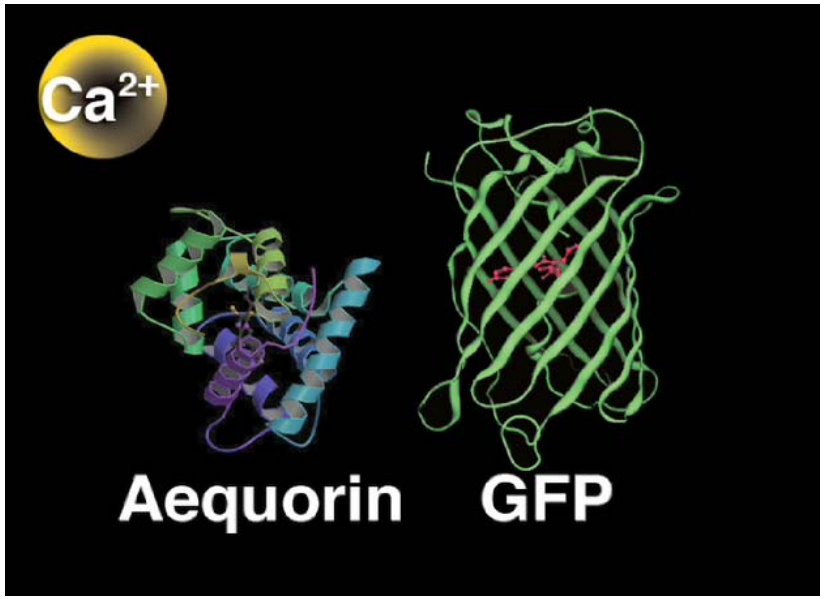




Green Fluorescence Protein

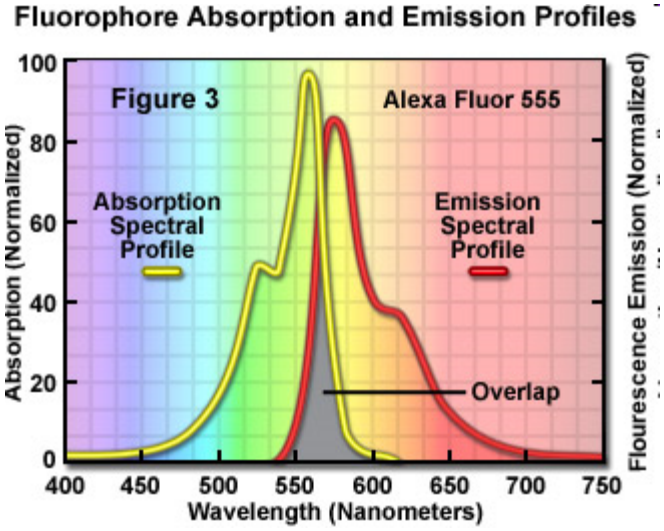


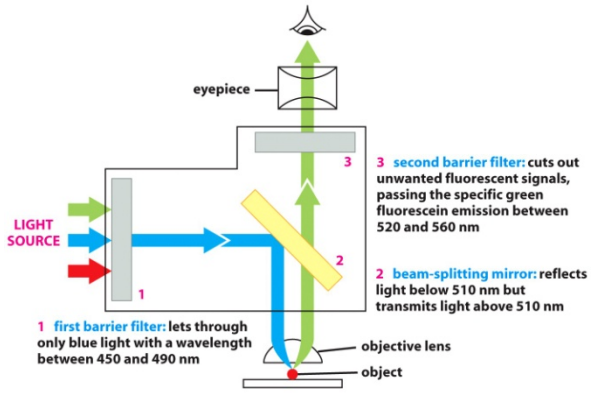
Jellyfish: *Aequorea victoria*

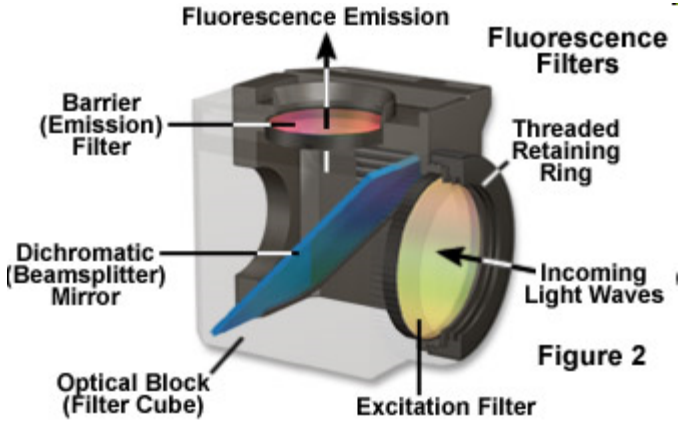


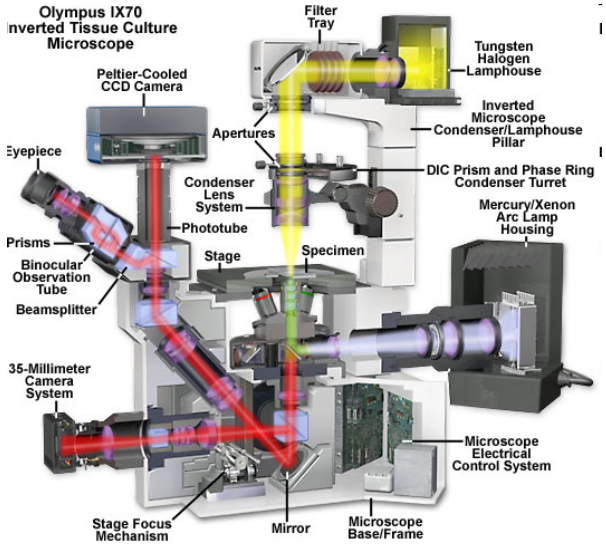
http://gfp.conncoll.edu/GFP-1.htm

Fluorescence Microscopy (I)



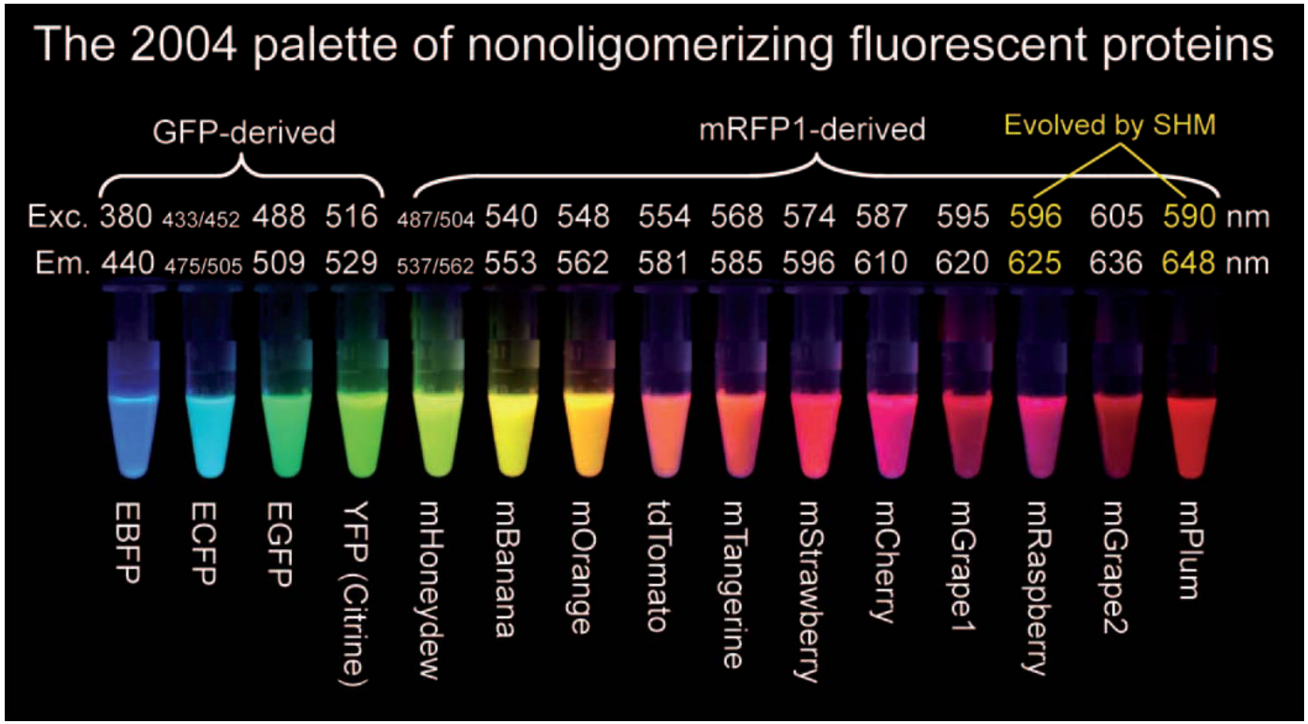






Fluorescence Microscopy (II)

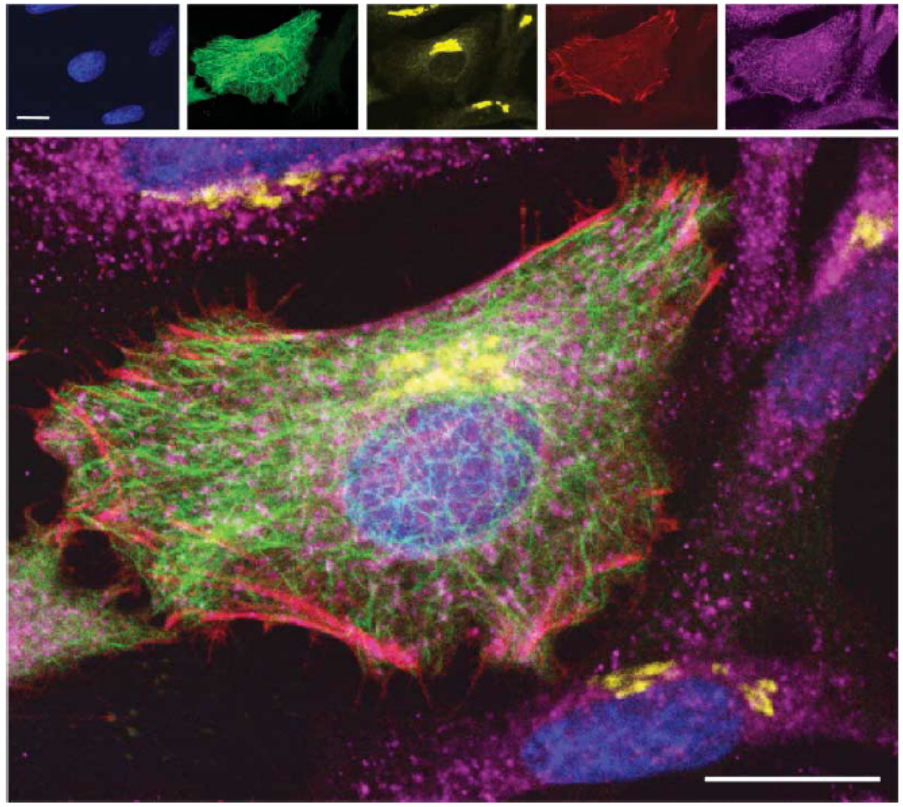
Fluorophores are available at many different colors



Roger Y. Tsien, 2009 Constructing and Exploiting the Fluorescent Protein Paintbox (Nobel Lecture). *Angew. Chem. Int. Ed.* 48**:** 5612 – 5626.

Fluorescence Microscopy (III)

Excitation (nm):	800 (2 photon)	488	432	568	637
Emission (nm):	410-490	500-530	555-565	580-620	>660
Fluorophore:	Hoechst	GFP	QD565	ReAsH	Cy5
Targeting:	direct affinity	genetic	immuno	genetic	immuno
Target:	DNA	α-tubulin	giantin	β-actin	Cytochrome c
Structure:	nuclei	microtubules	golgi	stress fibers	mitochondria



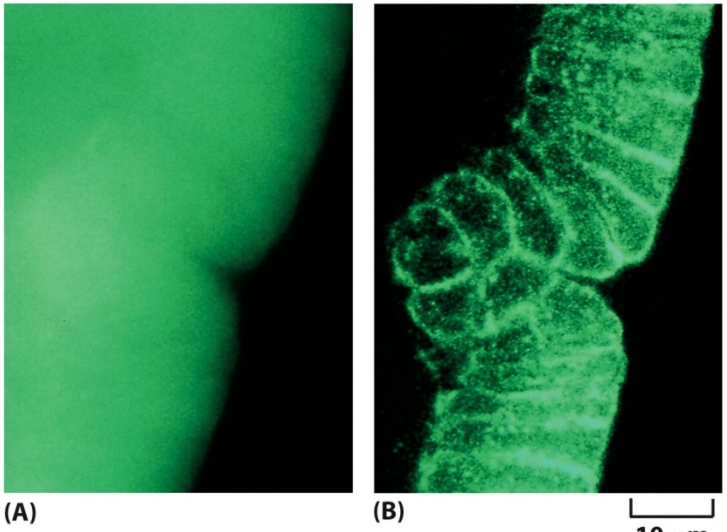
B. N. G. Giepmans, S. R. Adams, M. H. Ellisman & R. Y. Tsien 2006 The fluorescent toolbox for assessing protein location and function. *Science*. **312**: 217-224.

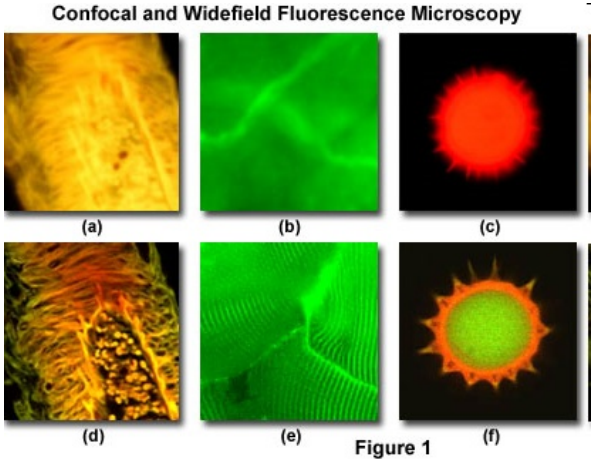
http://micro.magnet.fsu.edu/primer/techniques/fluorescence/gallery/cells/index.html

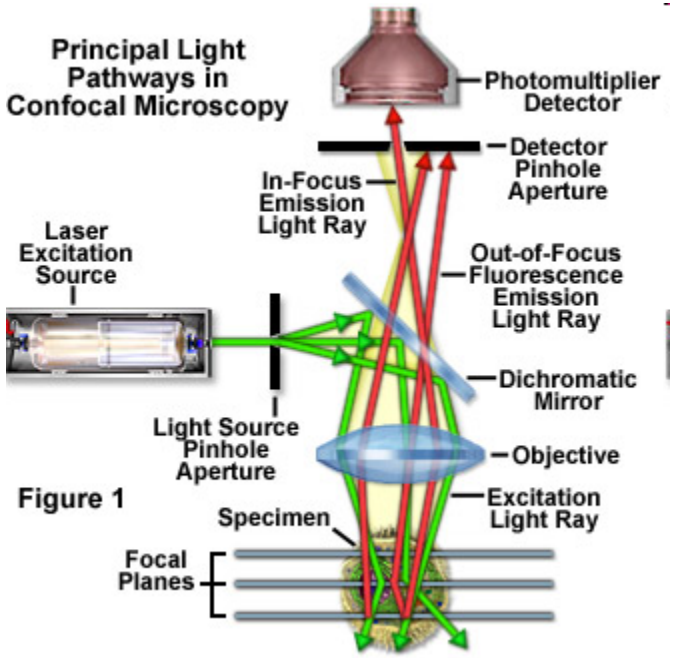
Fluorescence Microscopy (IV)

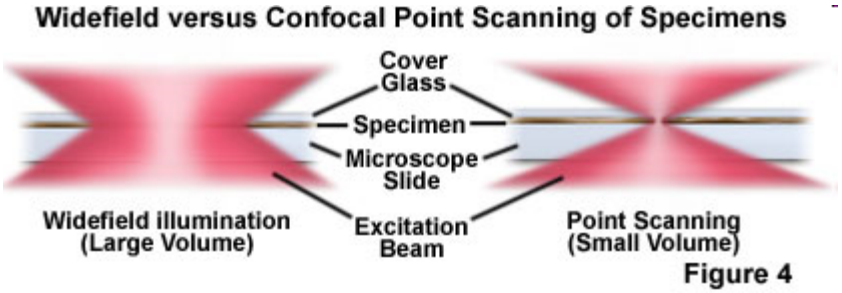
- There are four commonly used fluorescence modes
  - Widefield fluorescence microscopy (epifluorescence)
  - Confocal fluorescence microscopy
  - Total internal reflection fluorescence microscopy
  - Two photon fluorescence microscopy

Widefield vs Confocal Fluorescence Microscopy







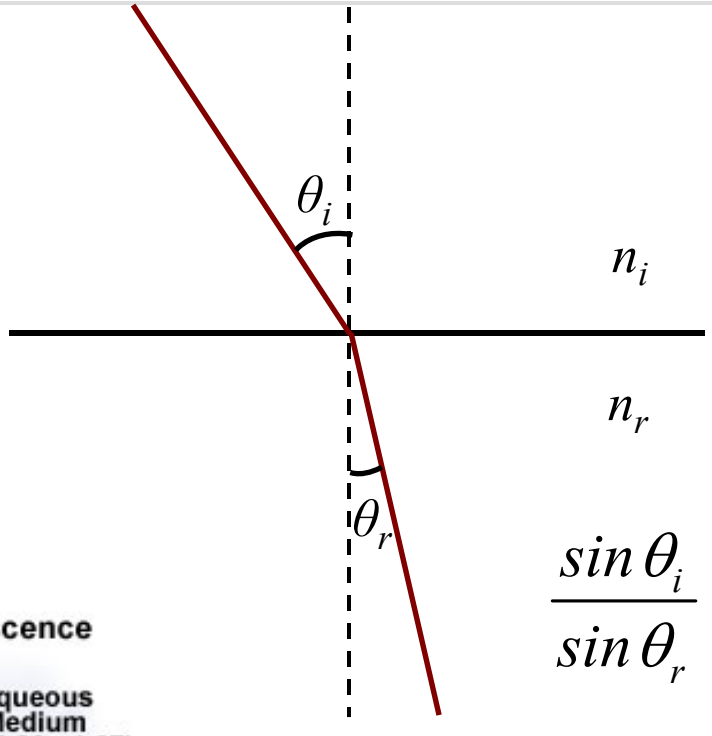


http://www.olympusfluoview.com/theory/confocalintro.html

Total Internal Reflection Microscopy (I)

- Typical thickness of the evanescent layer is less than 200nm

- Often used for imaging
  - membrane related cellular processes
  - single molecules



$$\frac{\sin\theta_i}{\sin\theta_r} = \frac{n_r}{n_i}$$

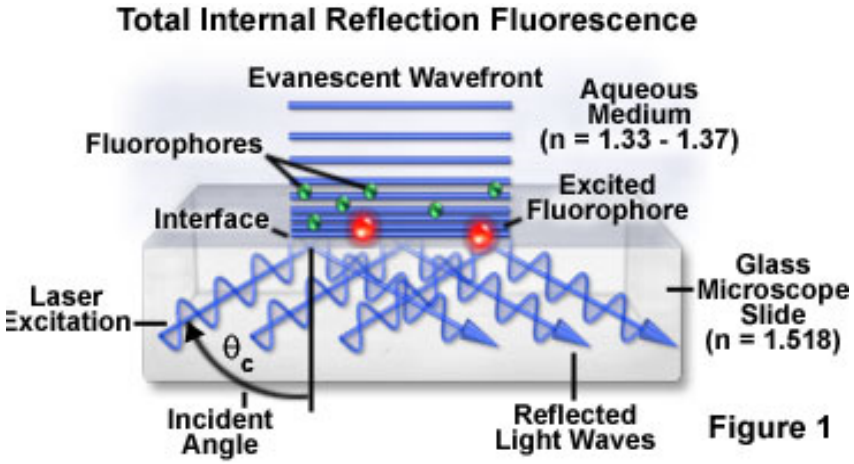
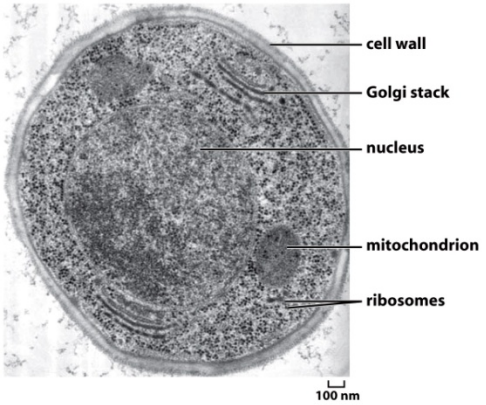
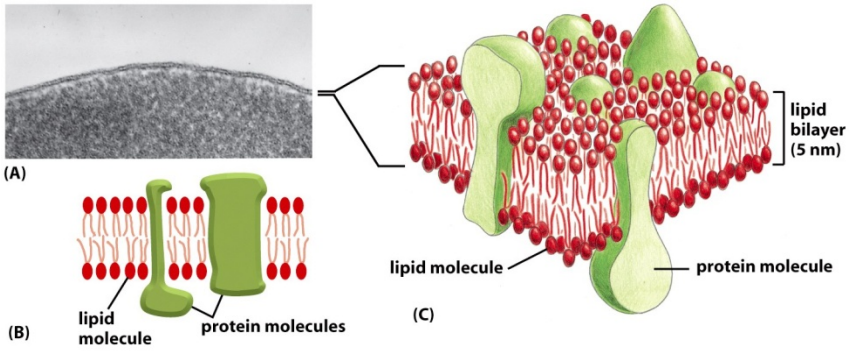


Figure 1

Total Internal Reflection Microscopy (II)

- TIRF is often used for imaging
  - membrane related cellular processes
  - single molecules





Two Photon Fluorescence Microscopy

- **Multi photon fluorescence for deep tissue imaging.**

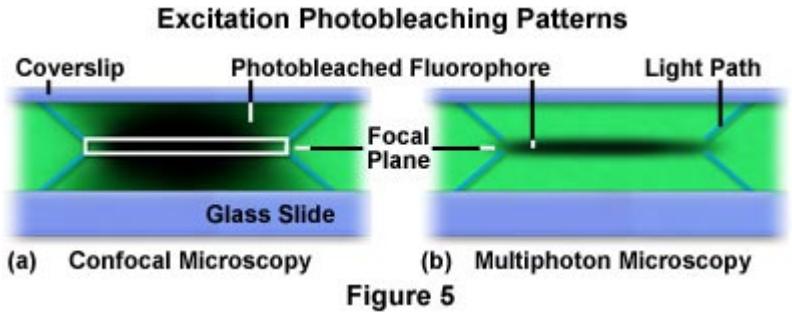
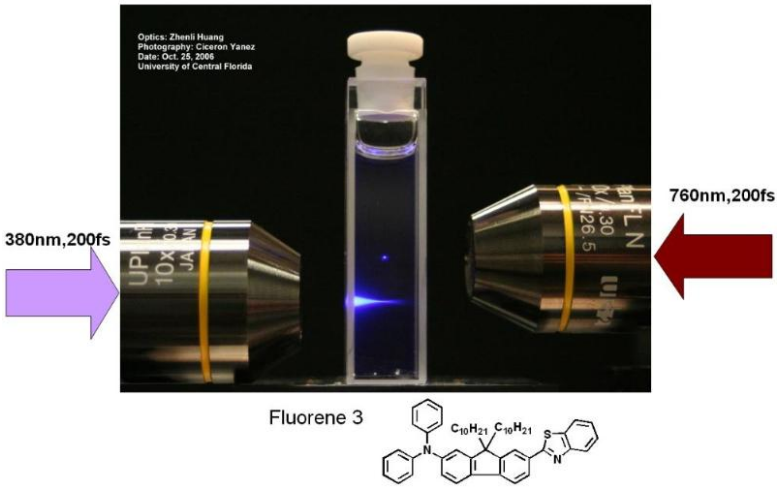


Figure 5



http://belfield.cos.ucf.edu/one%20vs%20two-photon%20excitation.html

First predicted by Maria Goeppert-Mayer in 1931 in her Ph.D. thesis; First observed in 1961.

Fluorescence Microscopy Summary

- High specificity
  - Chemical fluorophores (dyes)
  - Fluorescent proteins
- High sensitivity
  - Up to single molecules
- Multiplexity:
  - Multiple colors (channels)

- Contrast generation in microscopy
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Practical Considerations

- Photobleaching

  - Fluorophores gradually lose their ability of light emission.

  - This results in a continuous decrease in image intensity.

- Phototoxicity

  - Constant illumination generates free radicals that cause cell death.
  - This places a fundamental limit on how many frame of images can be collected.

References on Fluorescence Microscopy

- Lakowicz JR, *Principles of fluorescence spectroscopy*, Springer, 2006.

- Herman B, *Fluorescence microscopy*, 2nd ed., Taylor & Francis, 1998.

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Formats of Microscopy Images (I)

- Most commercial microscope is controlled by some kinds of control software.
  - Metamorph
    http://www.moleculardevices.com/pages/software/metamorph.html
  - Nikon Element
    http://www.nis-elements.com/
  - Micromanager
    http://www.micro-manager.org/
- Commercial software often uses proprietary image formats to save metadata.
- Free viewing software is sometimes available.

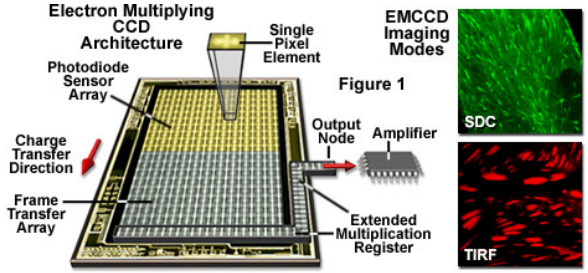
Formats of Microscopy Images (II)

- There are ~50 proprietary image formats.

  Swedlow et al, Bioimage informatics for experimental biology, *Ann. Rev. Biophys.* 2009, 38: 327-346.

- TIFF is the most commonly used format for image analysis. http://partners.adobe.com/public/developer/tiff/index.html

- For bioimages, bit depth is normally > 8



- In general, image compression that changes pixel values should be avoided.

Free Software For Viewing High Bit-Depth Images

- Irfanview

  http://www.irfanview.com/

- ImageJ

  - Web: http://rsbweb.nih.gov/ij/
  - Initially started at NIH ; Implemented using JAVA.
  - Provides bioimage view and analysis functions.
  - Many contributed plug-ins.

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Demo: Basic Image Manipulation Functions

- Image read: *imread*
- Image write: *imwrite*
- Image file information: *imfinfo*
- Image pixel informaiton: *impixelinfo*

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Image Processing vs Computer Vision

- Image processing normally refers to transformation from images to images.
  - Image enhancement
  - Image restoration
  - Image compression
  - Morphological image processing
  - …

- Computer vision aims to extract from images application-oriented information
  - Feature detection
  - Stereo vision
  - Robotic vision
  - Face recognition (HCI)
  - …

Some Related Journals

- IEEE Trans. Image Processing
- IEEE Trans. Pattern Analysis & Machine Intelligence (PAMI)
- International Journal of Computer Vision (IJCV)
- Computer Vision and Image Understanding
- Pattern Recognition

- IEEE Trans. Medical Imaging
- Medical Image Analysis

Some Microscopy Journals

- Journal of Microscopy

  http://www.wiley.com/bw/journal.asp?ref=0022-2720

- Biophysical Journal

  http://www.cell.com/biophysj/

- Nature Methods

  http://www.nature.com/nmeth/index.html

Literature Search Tools

- For image processing and computer vision references, use *IEEE xplore & ISI Web of Knowledge*.

- For microscopy and related biological application references, use *PubMed*.

http://www.ncbi.nlm.nih.gov/pubmed/

Open Source Software Packages & Public Image Libraries

- OpenCV

  http://opencv.willowgarage.com/wiki/Welcome

- ITK (Insight Toolkit)

  http://www.itk.org/

- JCB data viewer

  http://jcb-dataviewer.rupress.org/jcb/

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Reading Assignment 1

Comments on Reading Assignment 1

Questions?