Bioimage Informatics

Lecture 10, Spring 2012

Bioimage Data Analysis (II):

Applications of Point Feature Detection Techniques:

Super Resolution Fluorescence Microscopy

Bioimage Data Analysis (III):

Edge Detection

List of Groups

Group ID	Group Member 01	Group Member 02	Group Member 03
1	Fangming Lu	Wei Zhou	Ruiqin Li
2	Xiaoyi Fu	Martin Jaszewski	Frank Yeh
3	Sombeet Sahu	Madhumitha Raghu	Arun Sampath
4	Divya Kagoo	Sowmya Aggarwal	Hao-Chih Lee
5	Samantha Horvath	Akanksha Bhardwaj	Jaclyn Brackett
6	Yutong Li	Dolsarit Somseang	Weilong Wang

Outline

- Overview of super-resolution fluorescence microscopy (SRFM)
- SRFM by random fluorophore switching
- SRFM by deterministic fluorophore switching
- SRFM by structural illumination

- Introduction to edge detection

- Overview of super-resolution fluorescence microscopy (SRFM)
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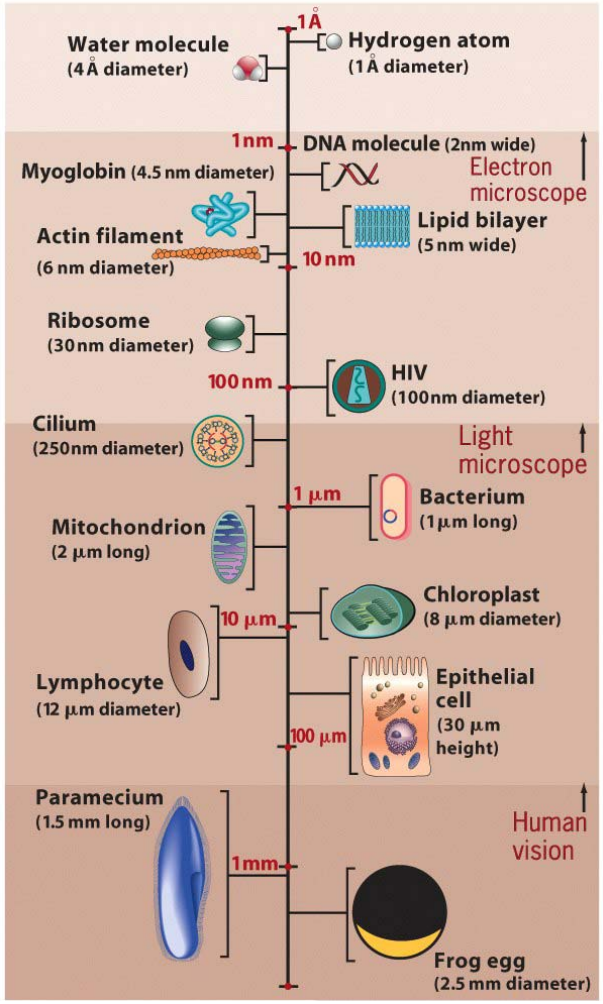
Why We Need Super Resolution Fluorescence Microscopy (I)



- Crystallography, NMR, spectroscopy
  - Resolution: ≤1 nm
  - Live/physiological condition: **NO**
    → Samples must be specially prepared
- Electron microscopy
  - Resolution: between 1nm &100nm
  - Live/physiological condition: **NO**
    → Samples must be fixed; limited specificity
- Light microscopy
  - Resolution: ≥ 100nm
  - Live/physiological condition: **YES**

Why We Need Super Resolution Fluorescence Microscopy (II)

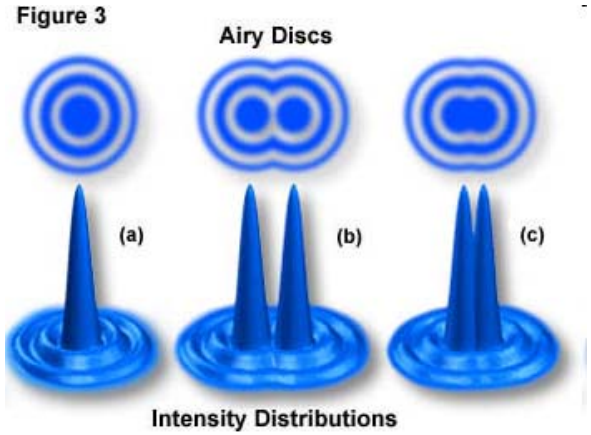
- Most cellular components are smaller than the diffraction limit of visible light.
- Fluorescence microscopy allows the visualization of cellular processes live under physiological conditions.
  - Not available under electron microscopy
- Fluorescence microscopy can achieve high specificity.
  - Not available under electron microscopy
- Fluorescence microscopy can achieve multiplexity (i.e. simultaneous multi-color imaging).
  - Not available under electron microscopy

Performance Metrics of a Fluorescence Microscope

- Resolution:

  - Rayleigh limit: $D = \frac{0.61\lambda}{NA}$

  - Sparrow limit: $D = \frac{0.47\lambda}{NA}$

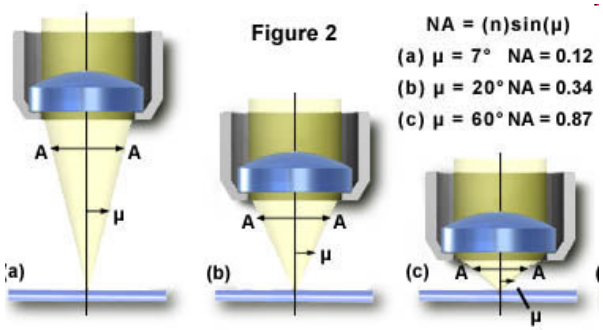


- Numerical aperture (NA)

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

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

μ: half of the angular aperture



- Water n=1.33
- Immersion oil n=1.51

There are Important Exceptions

- Well separated particles can be localized with an accuracy much smaller than the diffraction limit.

$$\sigma_\mu = \sqrt{\left(\frac{s_i^2}{N} + \frac{a^2/12}{N} + \frac{8\pi s_i^4 b^2}{a^2 N^2} \right)}$$

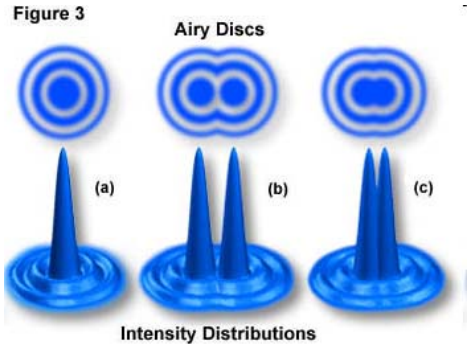
$$s_i = \frac{1}{2.2} \cdot \frac{0.61\lambda}{NA}$$

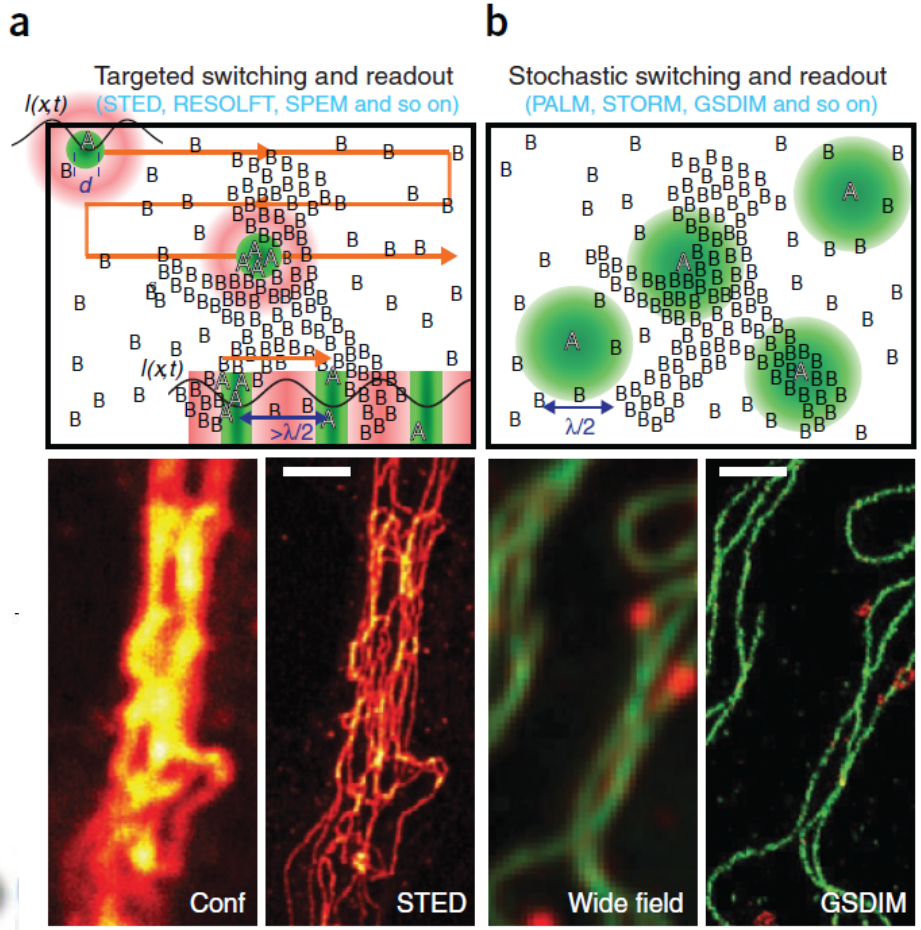
a: pixel size

b: background (in photons)

N: total number of photons

Basic Ideas of SRFM (I): Fluorophore Switching





Microscope Image Formation

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

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

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

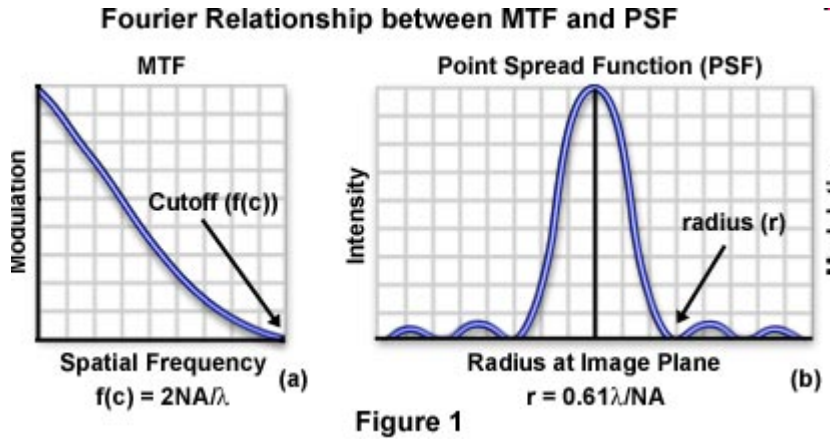
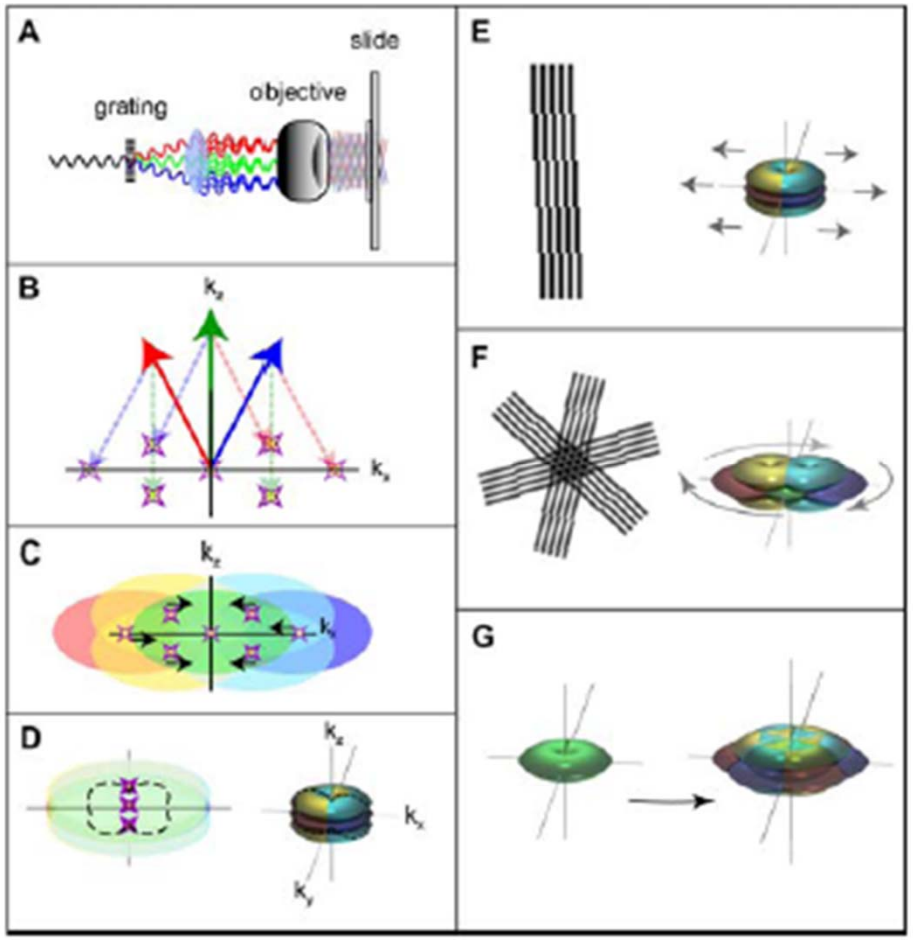


Figure 1

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

Basic Ideas of SRFM (II): Structural Illumination



- Overview of super-resolution fluorescence microscopy (SRFM)
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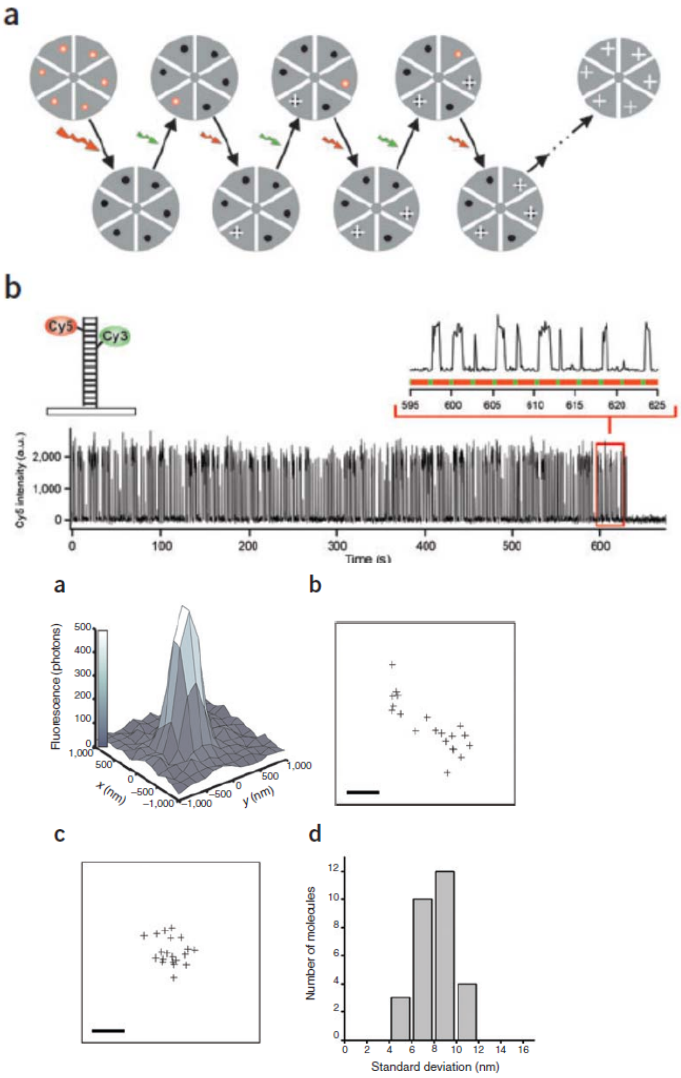
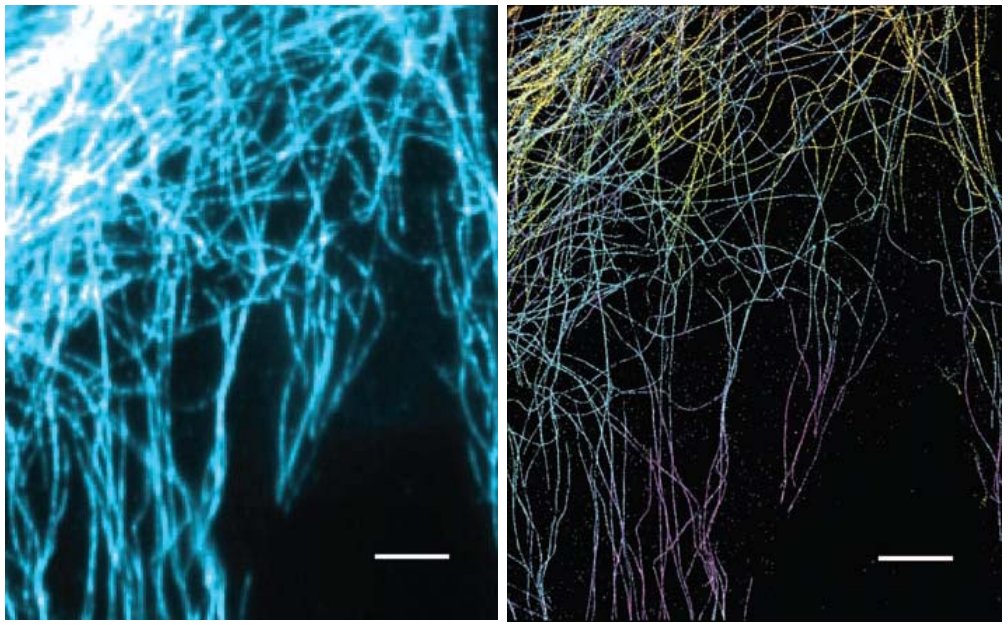
Stochastic Optical Reconstruction Microscopy (STORM)





Huang et al, Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy, Science, 319:810-813, 2008

M. J. Rust et al, Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nature Methods, 10:793-795, 2006.

Photoactivation Localization Microscopy (PALM)

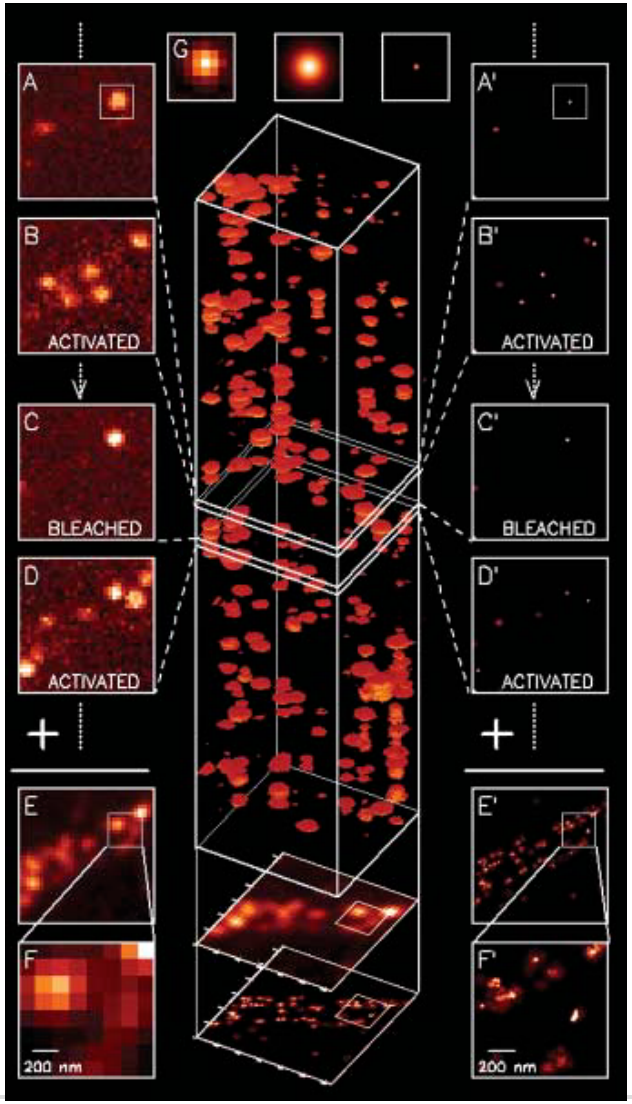
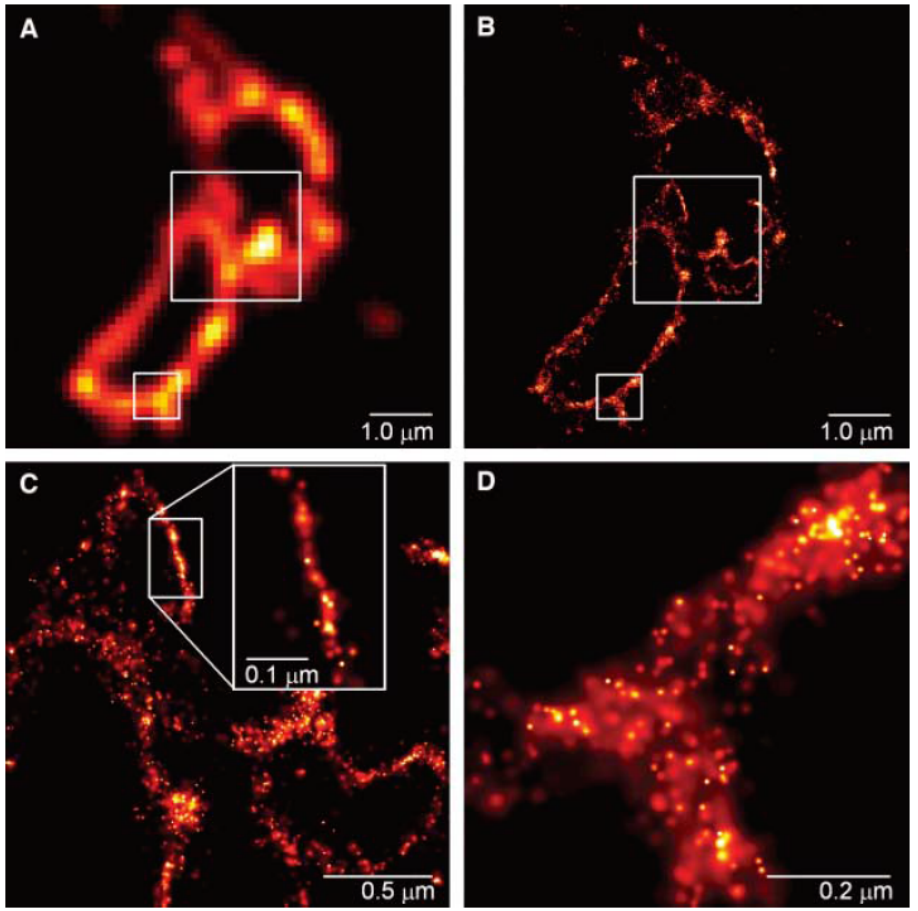


Fig. 1. The principle behind PALM. A sparse subset of PA-FP molecules that are attached to proteins of interest and then fixed within a cell are activated (**A** and **B**) with a brief laser pulse at λ_{act} = 405 mm and then imaged at λ_{exc} = 561 mm until most are bleached (**C**). This process is repeated many times (**C** and **D**) until the population of inactivated, unbleached molecules is depleted. Summing the molecular images across all frames results in a diffraction-limited image (**E** and **F**). However, if the location of each molecule is first determined by fitting the expected molecular image given by the PSF of the microscope [(**G**), center] to the actual molecular image [(G), left], the molecule can be plotted [(G), right] as a Gaussian that has a standard deviation equal to the uncertainty $\sigma_{x,y}$ in the fitted position. Repeating with all molecules across all frames (**A'** through **D'**) and summing the results yields a superresolution image (**E'** and **F'**) in which resolution is dictated by the uncertainties $\sigma_{x,y}$ as well as by the density of localized molecules. Scale: 1 × 1 μm in (F) and (F'), 4 × 4 μm elsewhere.



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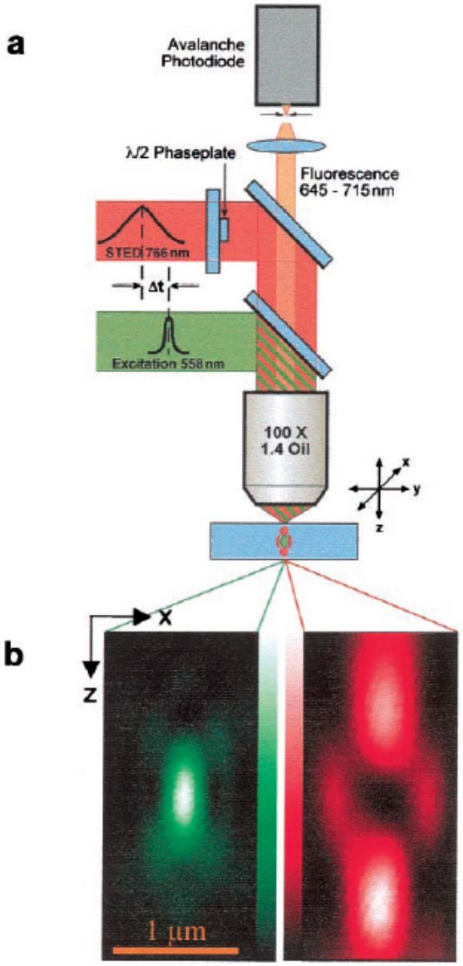
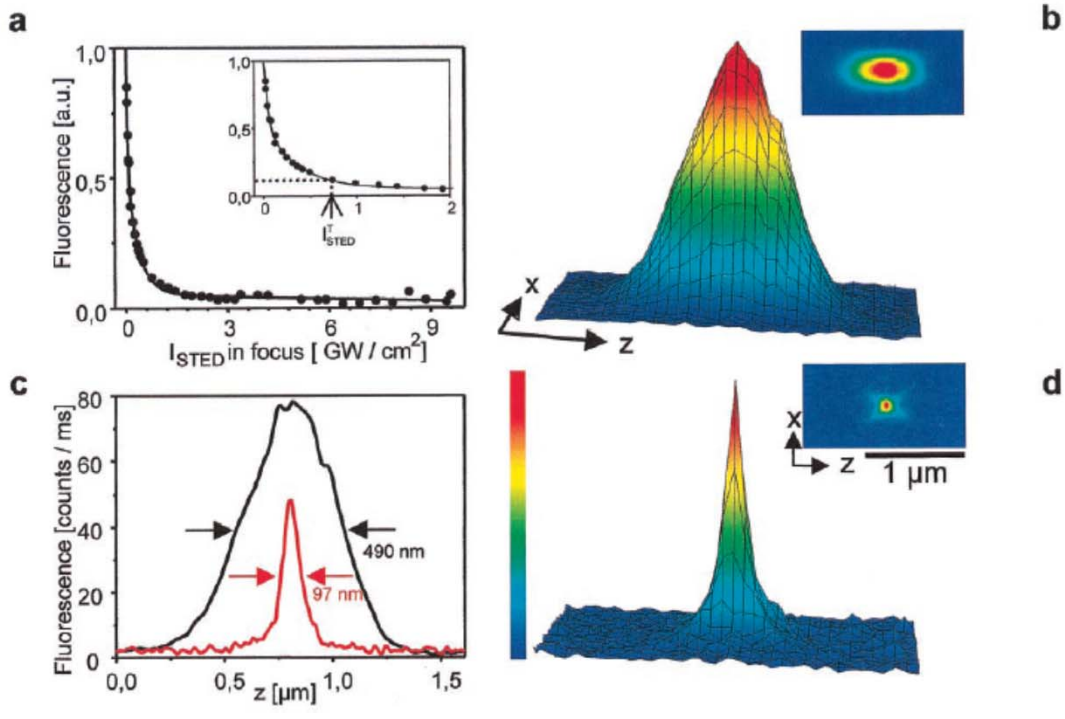
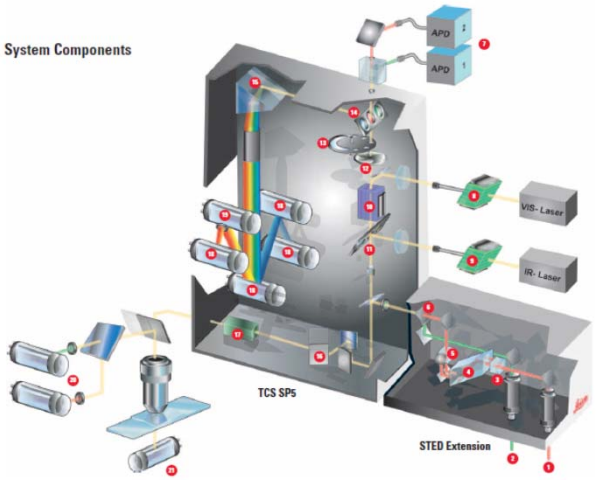
Stimulated Emission Depletion (I)



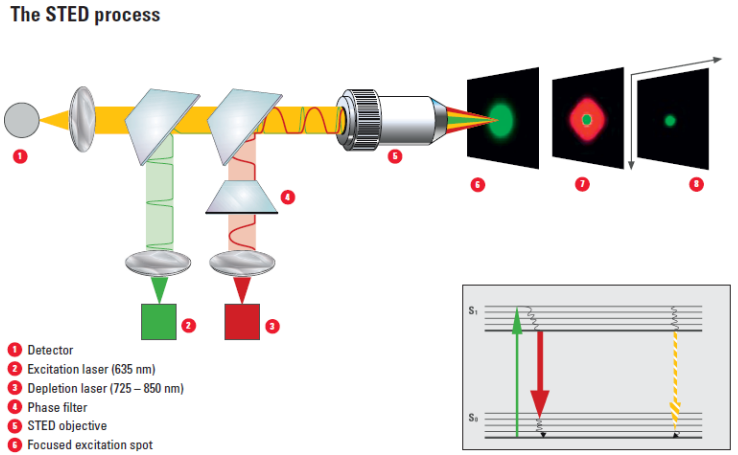


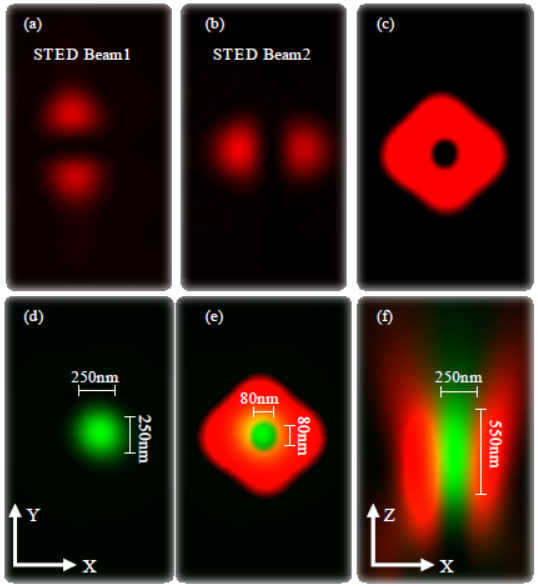
T. A. Klar et al, Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission, PNAS, 97:8206–8210, 2000.

Stimulated Emission Depletion (II)



1. Incoupling IR laser for STED
2. Incoupling 635 nm laser
3. Beam splitting prism
4. Phase filter
5. Beam combining prism
6. Dichroic LP650
7. Avalanche Photo Detectors (APD)
8. Visible range AOTF
9. IR EOM
10. AOBS
11. Multi function port
12. Confocal detection pinhole
13. Filter- and polarizer wheel
14. X1 – emission port
15. Spectrophotometer prism
16. Tandem Scanner
17. Field rotation optics
18. Photomultiplier 1,2,3 & 5
19. Photomultiplier 4 (STED)
20. Reflected light nondescanned detectors
21. Transmitted light detector





Comments

- STED
  - Photodamage
  - Deterministic; Live imaging possible
- STORM/PALM
  - Less photodamage
  - Stochastic; Live imaging challenging
  - Possible artifacts
- New possibilities for quantitative image analysis

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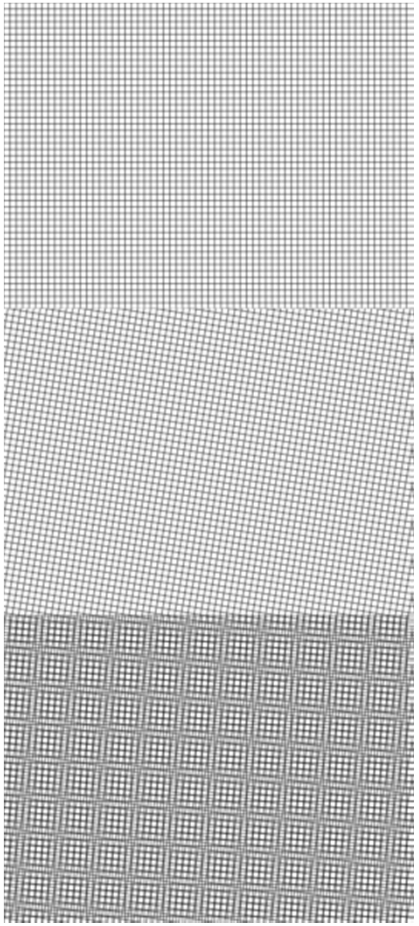
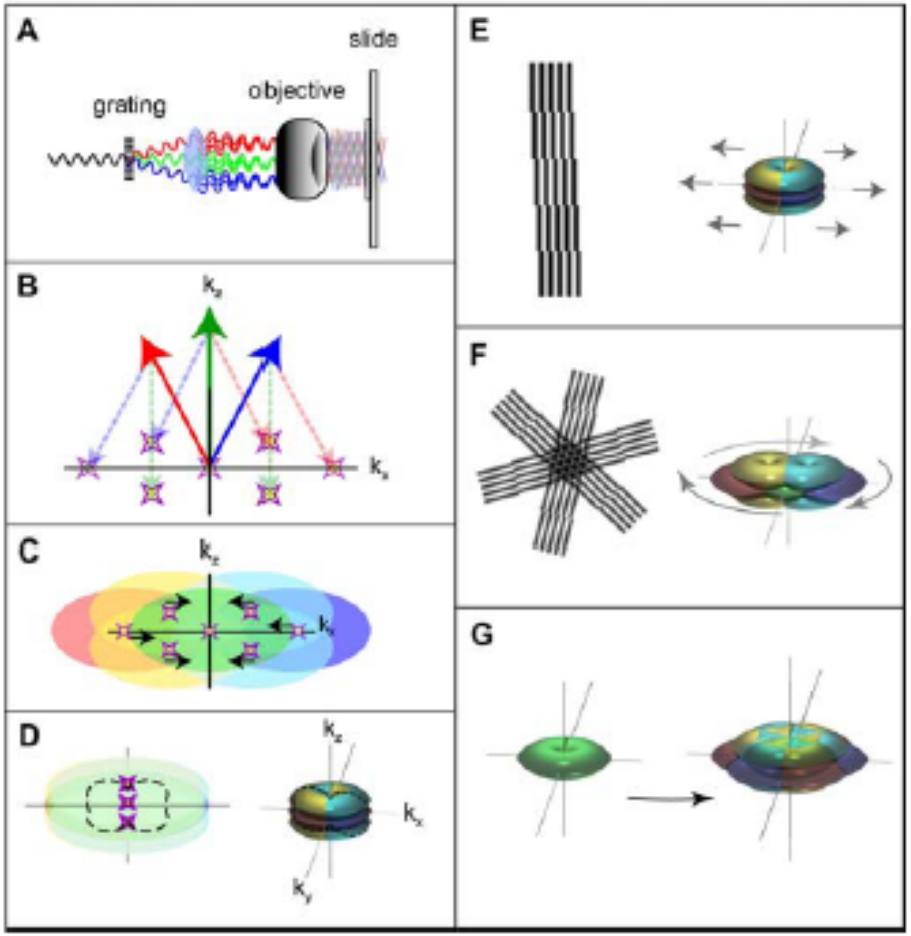
Moire Pattern



Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy, Lothar Schermelleh, et al, *Science* Vol. 320, 1332 (2008)

3D Structural Illumination Microscopy

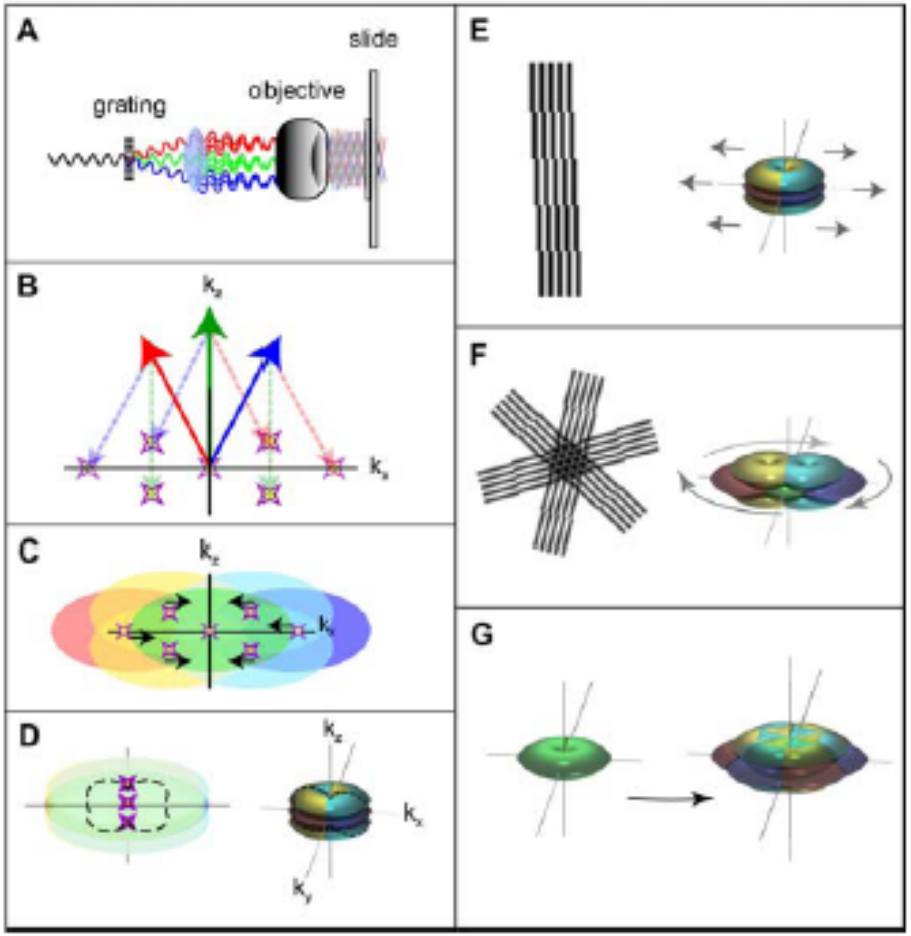
- The basic idea is to collect more information by extending the support in frequency domain.
- Use structural illumination
- Use three illumination angles
- Combination of imaging and computation



Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy, Lothar Schermelleh, et al, *Science* Vol. 320, 1332 (2008)

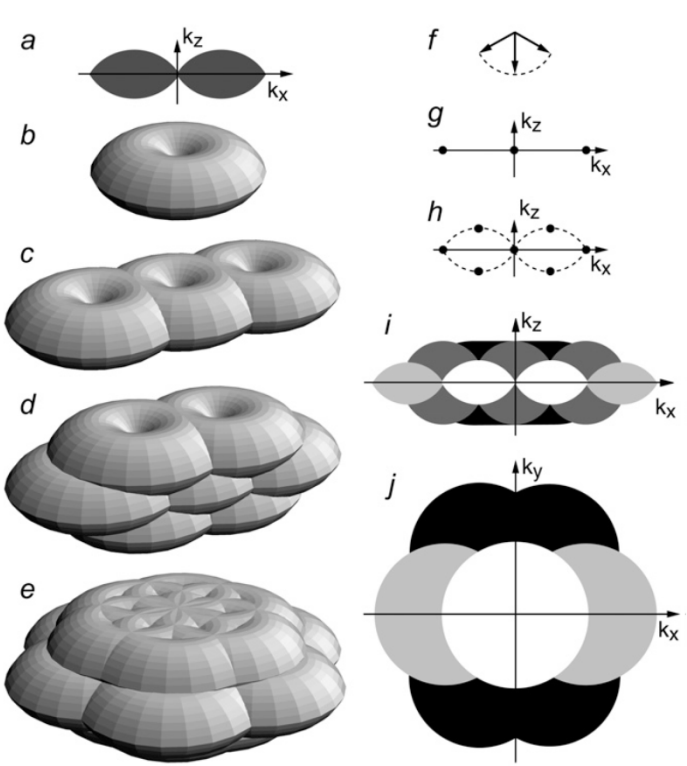
3D Structural Illumination Microscopy

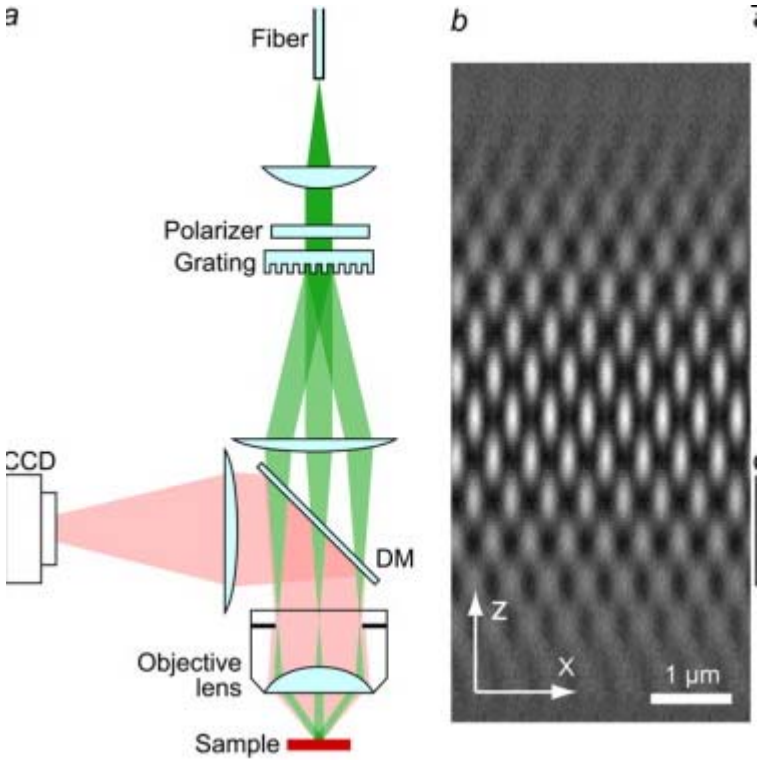
- To use structural illumination to capture image information at higher-spatial frequency.
- Three illumination angles; Five frames per angle.
- Final images are generated by computational reconstruction.



Subdiffraction Multicolor Imaging of the Nuclear Periphery with 3D Structured Illumination Microscopy, Lothar Schermelleh, et al, *Science* Vol. 320, 1332 (2008)

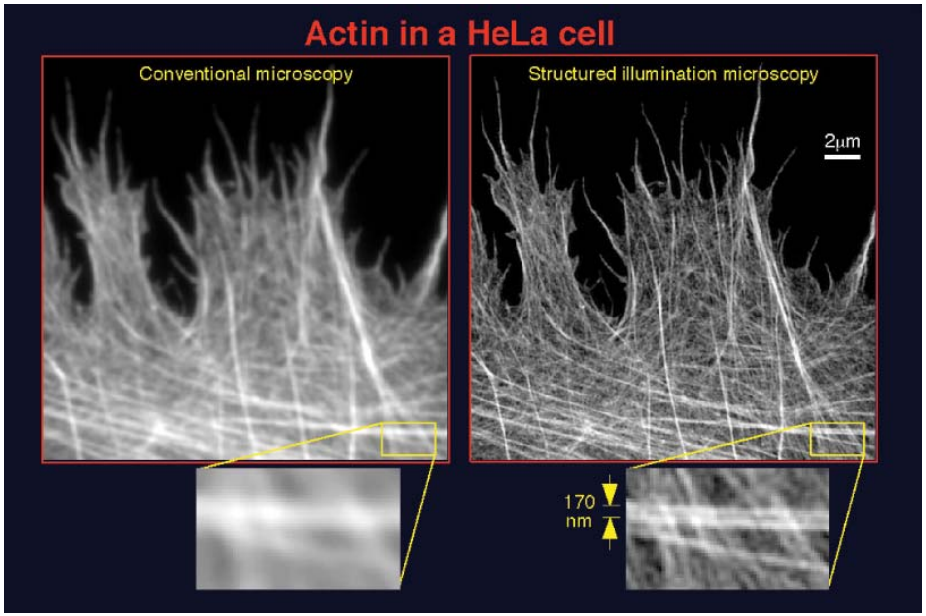
Structural Illumination Scheme

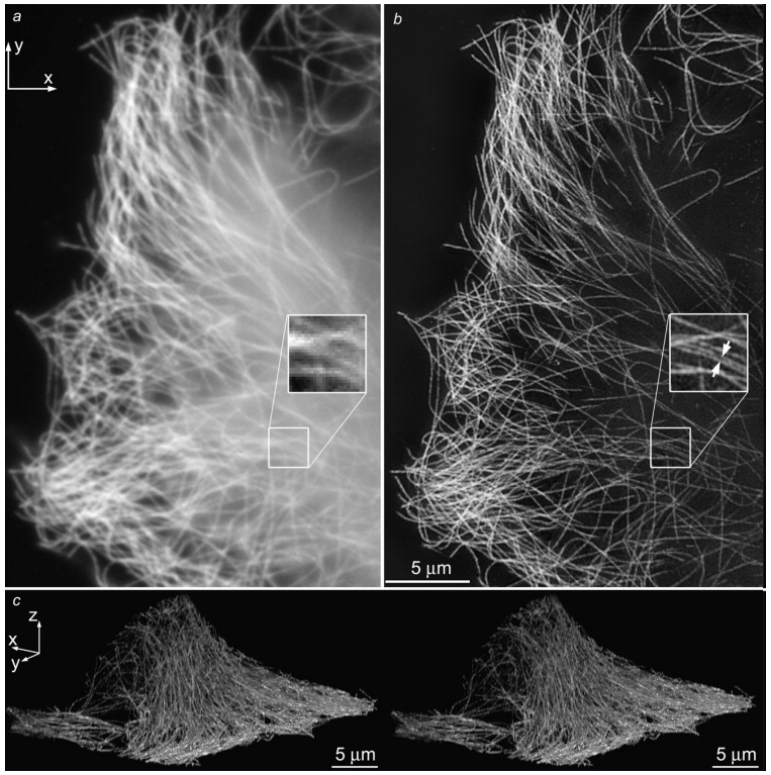




Three-Dimensional Resolution Doubling in Wide-Field Fluorescence Microscopy by Structured Illumination, Mats G. Gustafsson, et al. *Biophysical Journal* Vol. 94, 4957-4970, 2008

3D Structural Illumination Microscopy





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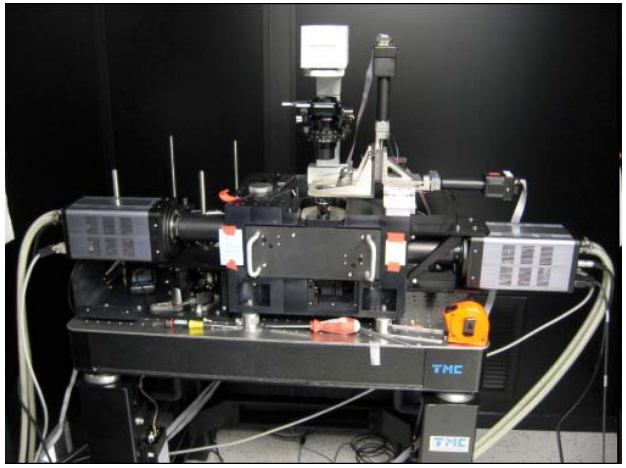
Commercial System From AppliedPrecision

DeltaVision OMX Specifications

General Information

- Three-dimensional Imaging and Analysis System
- High spatial resolution beyond the diffraction limit
  - ~100 nm lateral (depending on wavelength and optics)
  - ~200 nm axial (depending on wavelengths and optics)
- Fast temporal resolution truly simultaneous live-cell imaging
- Up to four-channels per data set
- Super resolution beyond the cover slip using 3D-SIM Structured Illumination Microscopy (per technology inventors Sedat, Agard and Gustafsson at UCSF)
- High speed imaging at up to 65 frames/second in each of four channels
- Applied Precision certified optics
- Includes personalDV widefield deconvolution system for integrated target ID and standard widefield imaging
- Custom Applied Precision transilluminator with ultra-white LED
- Choice of large field-of-view or high-speed camera options
- Laser fluorescence illumination
- Conventional and Structured Illumination light paths
- Image acquisition software
- Full suite of image processing and analysis tools
- Stage mapping between personalDV and DeltaVision OMX systems
- Proprietary image restructuring software
- Time-lapse
- 3-D Modeling and analysis
- Includes toolkit, calibration slides, alignment optics, and immersion oil kits.



References

- Sedat Lab

  http://msg.ucsf.edu/sedat/

- Agard Lab

  http://www.msg.ucsf.edu/agard/

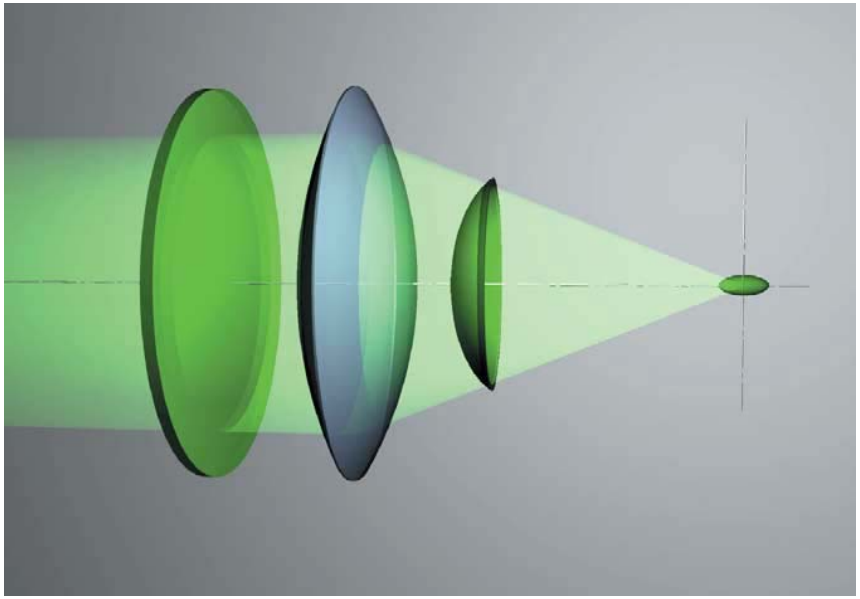
- Gustafsson Lab

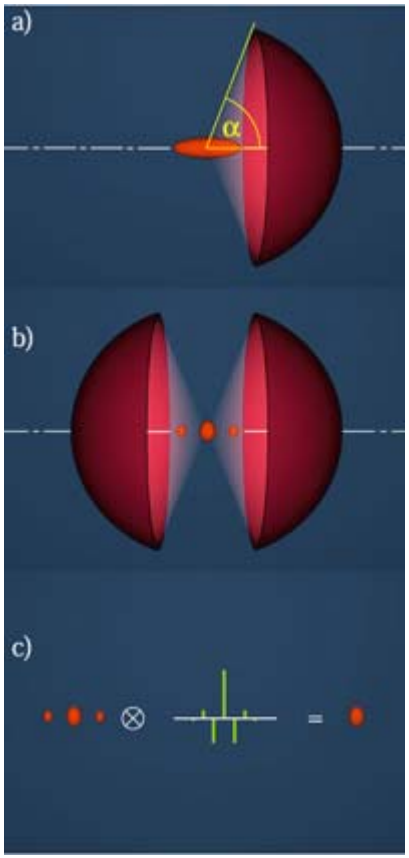
  http://www.msg.ucsf.edu/gustafsson/index.html

  http://www.hhmi.org/research/groupleaders/gustafsson_bio.html

4 Pi Microscopy (I)

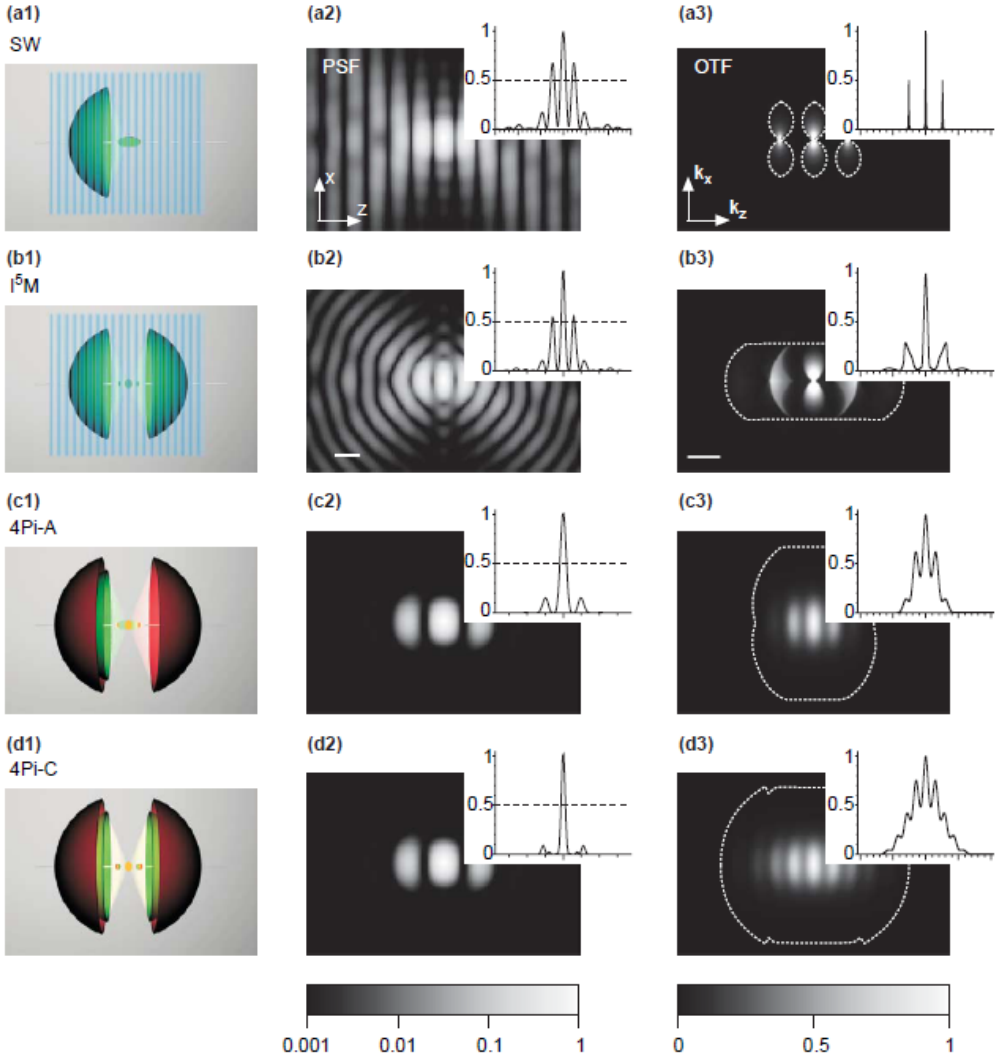
- A technique to improve axial resolution by several folds.





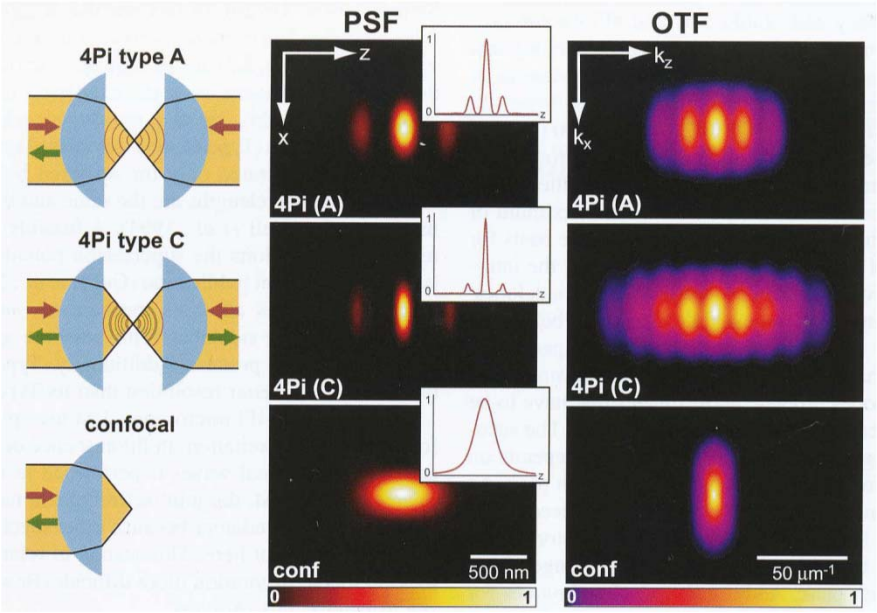
Egner & Hell, Fluorescence microscopy with super-resolved optical sections, *Trends in Cell Biology*, 15:207-215, 2005.

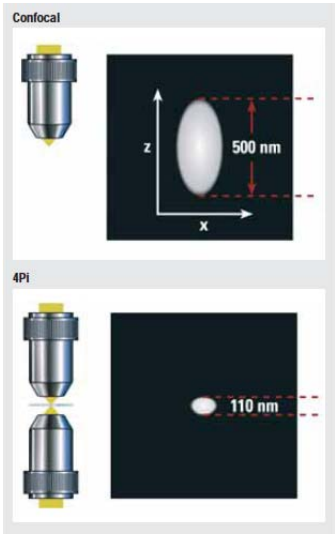
4 Pi Microscopy (II)

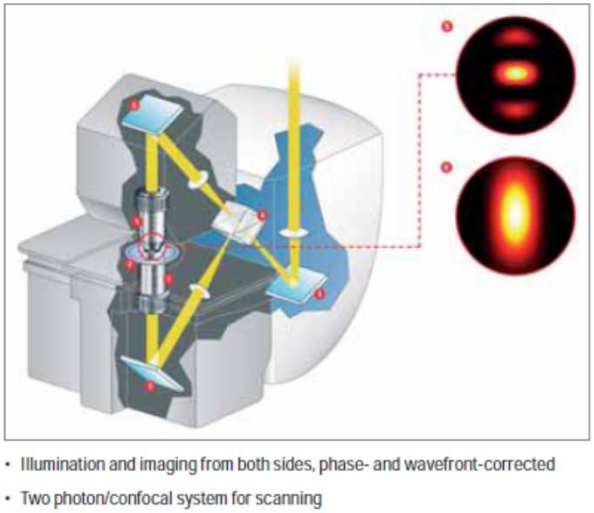


4 Pi Microscopy (III)

- A technique to improve axial resolution by several folds.

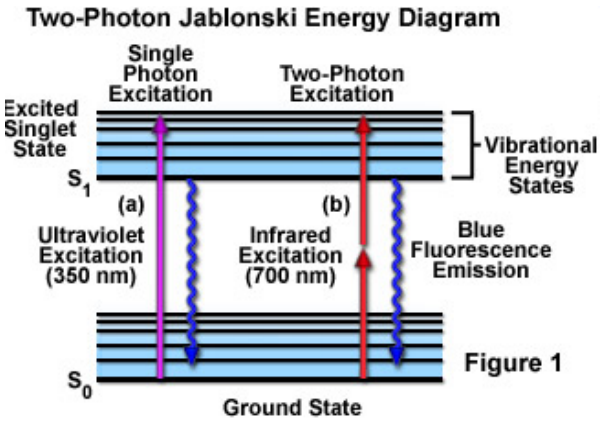






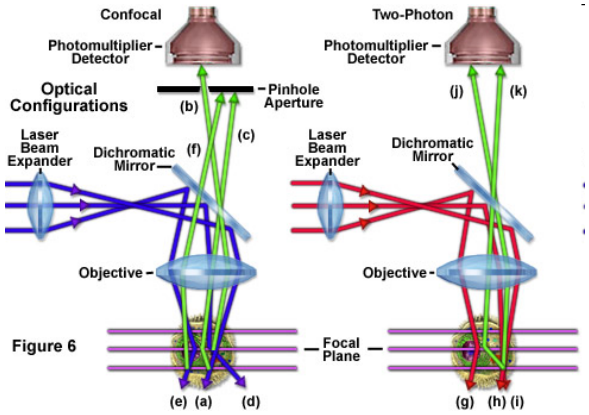
4 Pi Microscopy (IV)

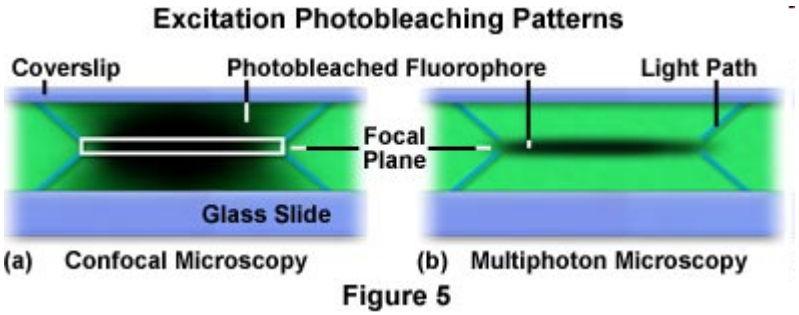
Maria Göppert-Mayer



Absorption of two photons must happen within 10^{-15}-10^{-18} sec.

$$E = h\nu = h\frac{c}{\lambda}$$



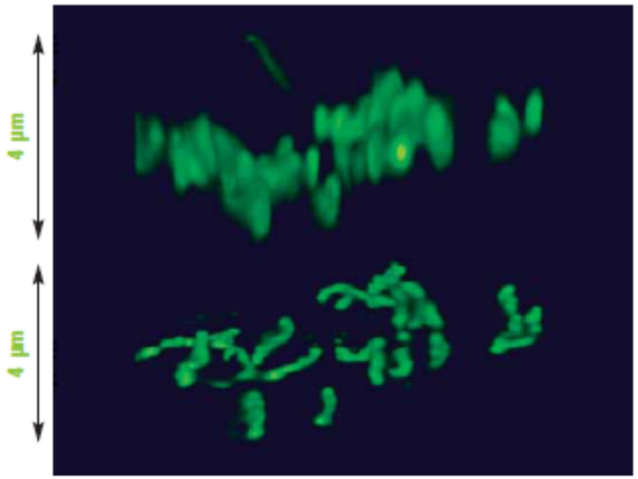


http://www.olympusmicro.com/primer/techniques/fluorescence/multiphoton/multiphotonintro.html

http://www.microscopyu.com/articles/fluorescence/multiphoton/multiphotonintro.html

http://www.fz-juelich.de/isb/isb-1/Two-Photon_Microscopy/

4 Pi Microscopy (V)



4pi microscopy – spectacular 3D resolution

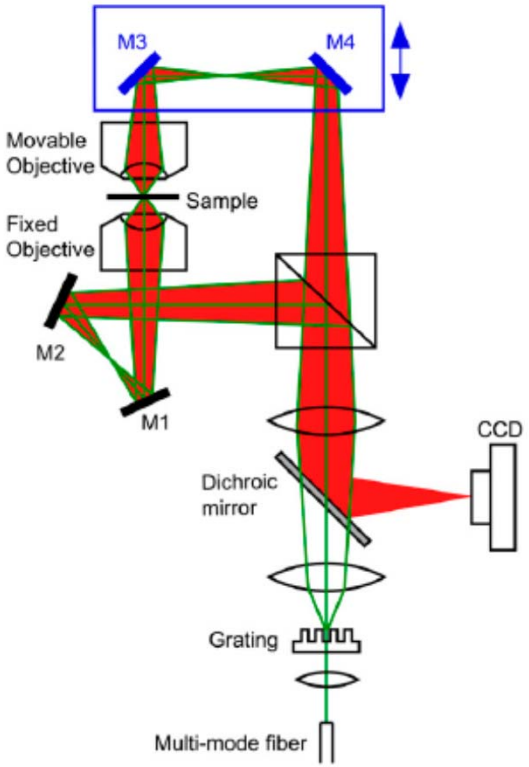
- Live yeast cell – mitochondrial protein distribution
  Label: GFP
  Objective: 63x1.2 W

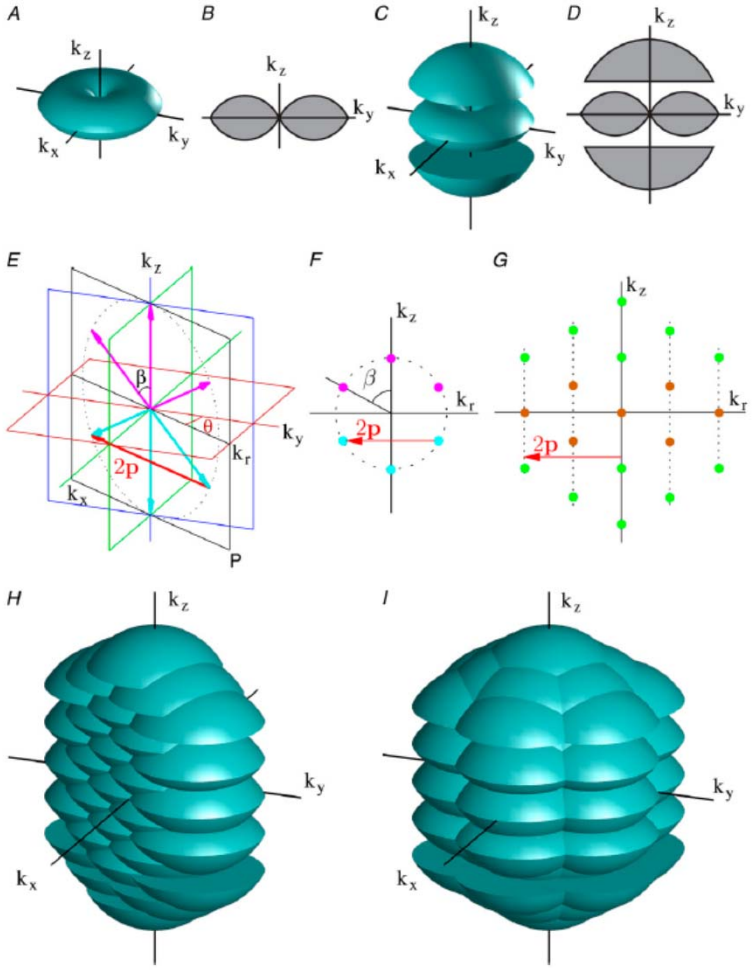
Nagorni, M. & Hell, S.W. 4Pi-confocal microscopy provides three-dimensional images of the microtubule network with 100- to 150-nm resolution. *J. Struct. Biol.* **123**, 236–247 (1998).

4 Pi Microscopy (VI)

- Advantages
  - Substantial improvement in axial resolution
- Disadvantages
  - No improvement in lateral resolution
  - Artifacts due to side lobes
  - Complexity, sample restriction, cost

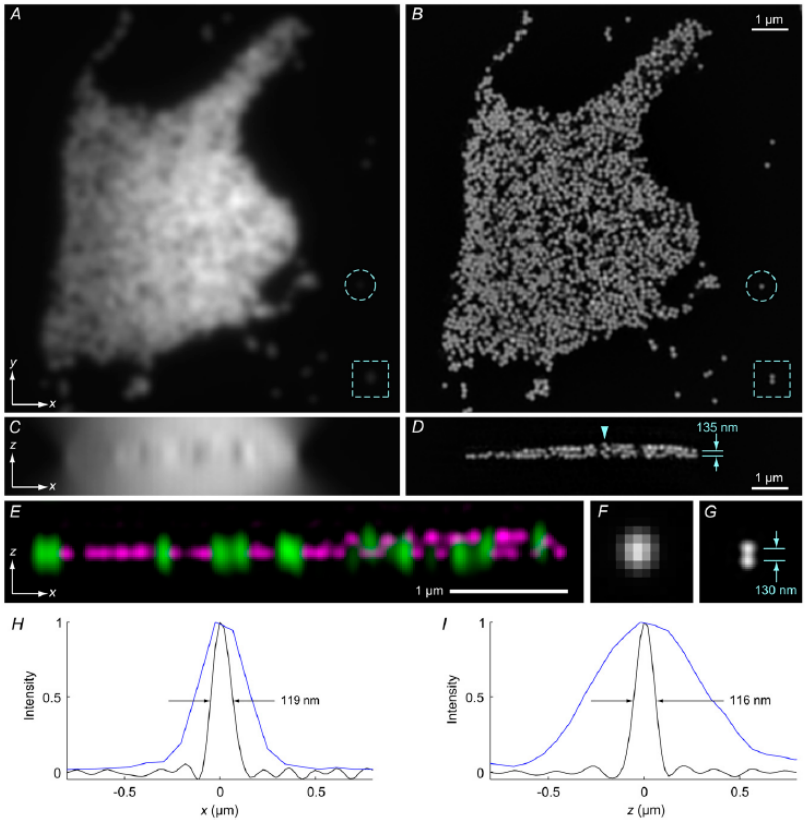
I^{5}M Microscopy (I)

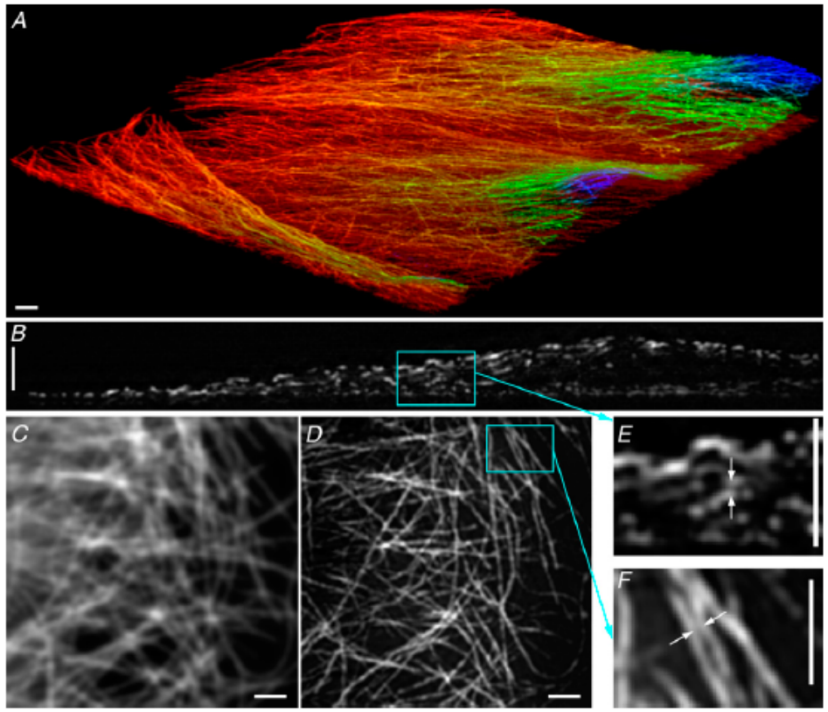




Shao et al, I^{5}S: wide-field light microscopy with 100-nm-scale resolution in three dimensions,Biophysical Journal, 94:4971-4983, 2008.

I^{5}M Microscopy (II)





Shao et al, I^{5}S: wide-field light microscopy with 100-nm-scale resolution in three dimensions, Biophysical Journal, 94:4971-4983, 2008.

Some References

[1] S. W. Hell, Microscopy and its focal switch, *Nature Methods*, vol. 6, no.1, pp. 24-32, 2009.

[2] J. Lippincott-Schwartz & S. Manley, Putting super-resolution fluorescence microscopy to work, *Nature Methods*, vol.6, no.1, pp. 21-23, 2009.

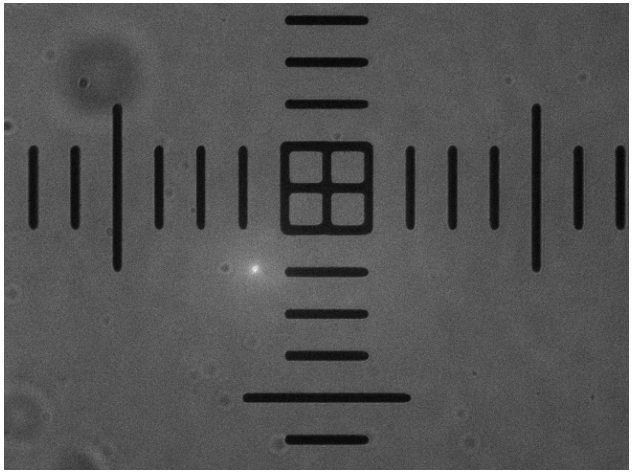
- Overview of super-resolution fluorescence microscopy (SRFM)
- SRFM by random fluorophore switching
- SRFM by deterministic fluorophore switching
- SRFM by structural illumination

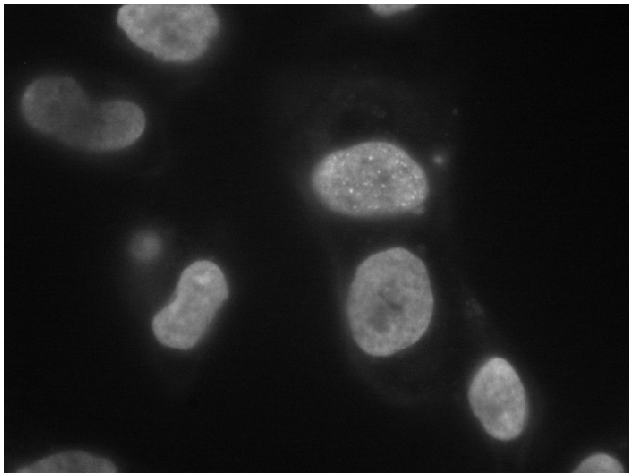
- Introduction to edge detection

Edge Detection

- What is an edge?

  An edge point, or an edge, is a pixel at or around which the image values undergo a sharp change.

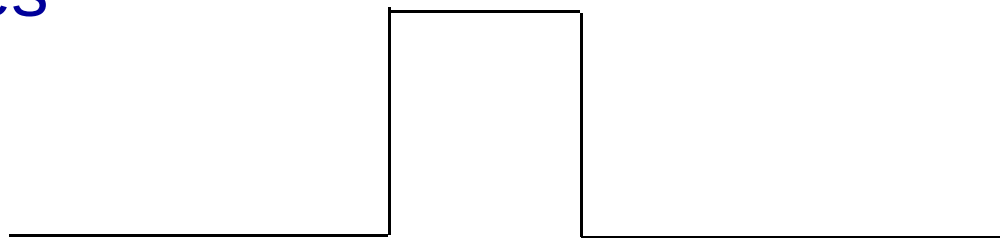




Typical Cases of Edges

- Type I: Steps

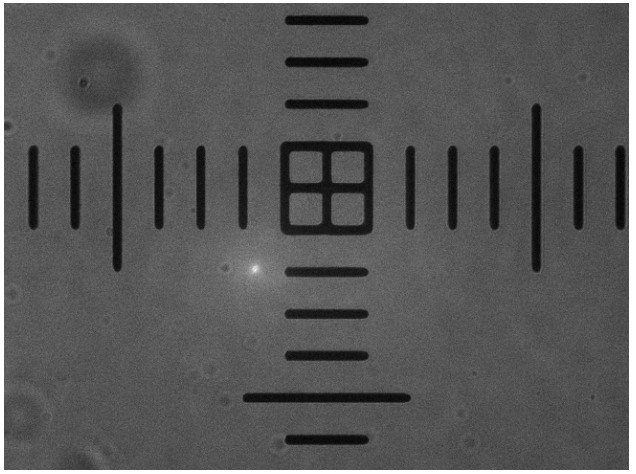
- Type II: Ridges

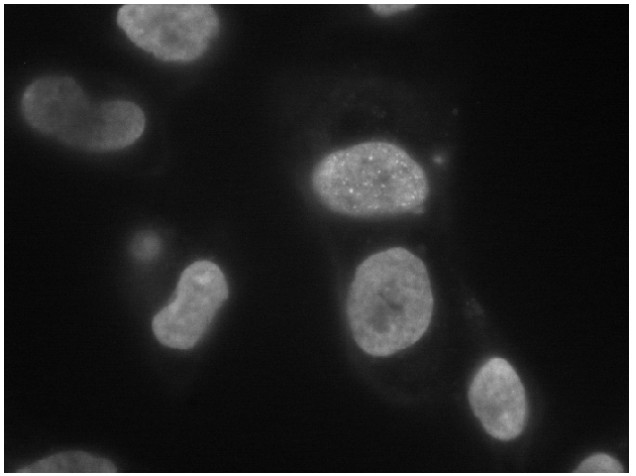


- The edge can be thought of as a 1D signal when observed under the normal direction.

Edge Detection Procedure

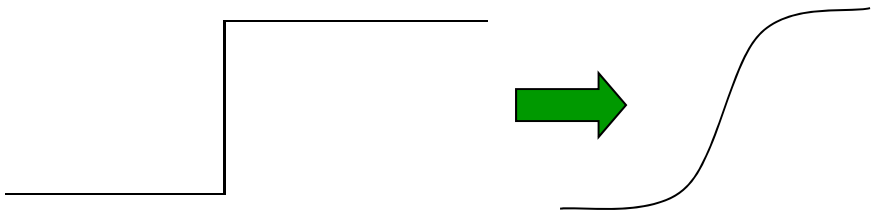
- Step I: noise suppression
- Step II: edge enhancement
- Step III: Edge localization





Noise Suppression

- Low pass filter using a Gaussian kernel



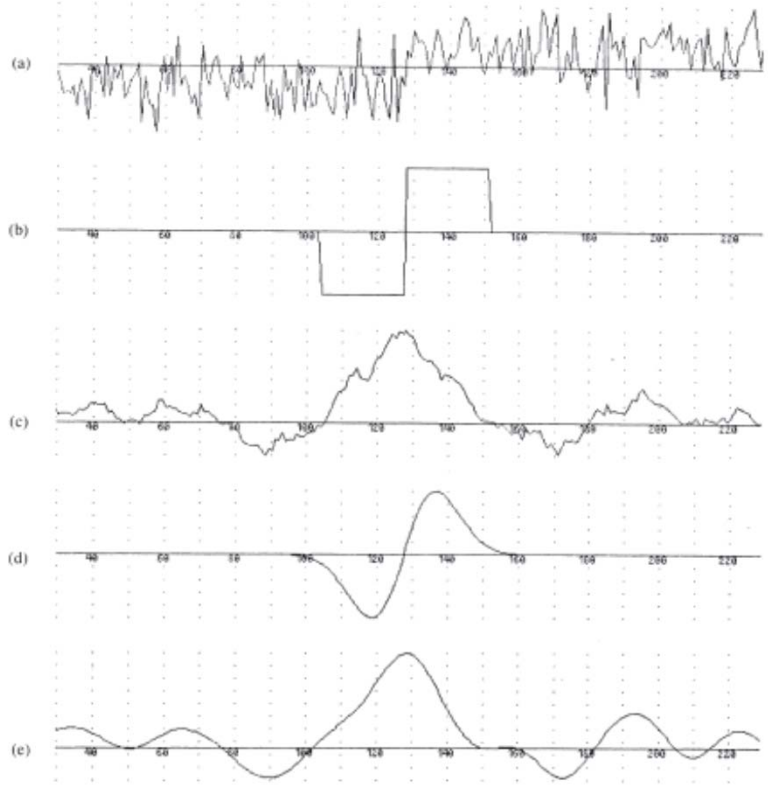


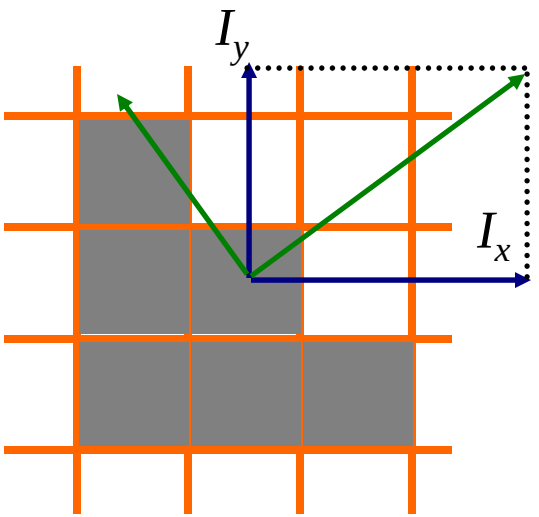
Fig. 1. (a) A noisy step edge. (b) Difference of boxes operator. (c) Difference of boxes operator applied to the edge. (d) First derivative of Gaussian operator. (e) First derivative of Gaussian applied to the edge.

Canny, IEEE *TPAMI*, 679-698, 1986.

Canny Edge Enhancement

- Step I: For each pixel $I(x_0,y_0)$, calculate the gradient

$$\left.\frac{\partial I}{\partial x}\right|_{x=x_0} \qquad \left.\frac{\partial I}{\partial y}\right|_{y=y_0}$$



- Step II: Estimate edge strength

$$E_s(x_0,y_0) = \sqrt{I_x^2(x_0,y_0) + I_y^2(x_0,y_0)}$$

- Step III: Estimation edge direction

$$E_o(x_0,y_0) = \arctan\frac{I_y(x_0,y_0)}{I_x(x_0,y_0)}$$

Combination of Noise Suppression and Gradient Estimation (I)

- A basic property of convolution

$$\frac{\partial\left(G * I\right)}{\partial x} \overset{\text{DEF}}{=} GI_x = \frac{\partial G}{\partial x} * I \qquad \frac{\partial\left(G * I\right)}{\partial y} \overset{\text{DEF}}{=} GI_y = \frac{\partial G}{\partial y} * I$$

$$E_s\left(x_0, y_0\right) = \sqrt{GI_x^2\left(x_0, y_0\right) + GI_y^2\left(x_0, y_0\right)}$$

$$E_o\left(x_0, y_0\right) = \arctan\frac{GI_y\left(x_0, y_0\right)}{GI_x\left(x_0, y_0\right)}$$

Combination of Noise Suppression and Gradient Estimation (II)

- Gaussian kernel in 1D

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

- First order derivative

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

- Second order derivative

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

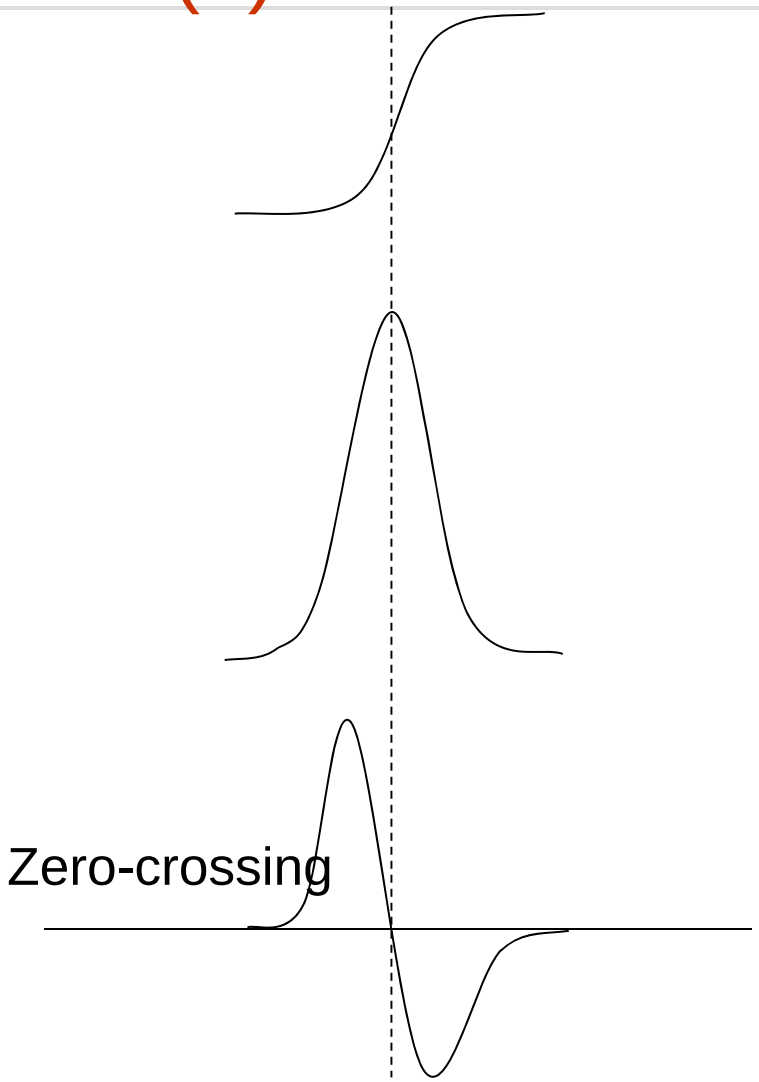
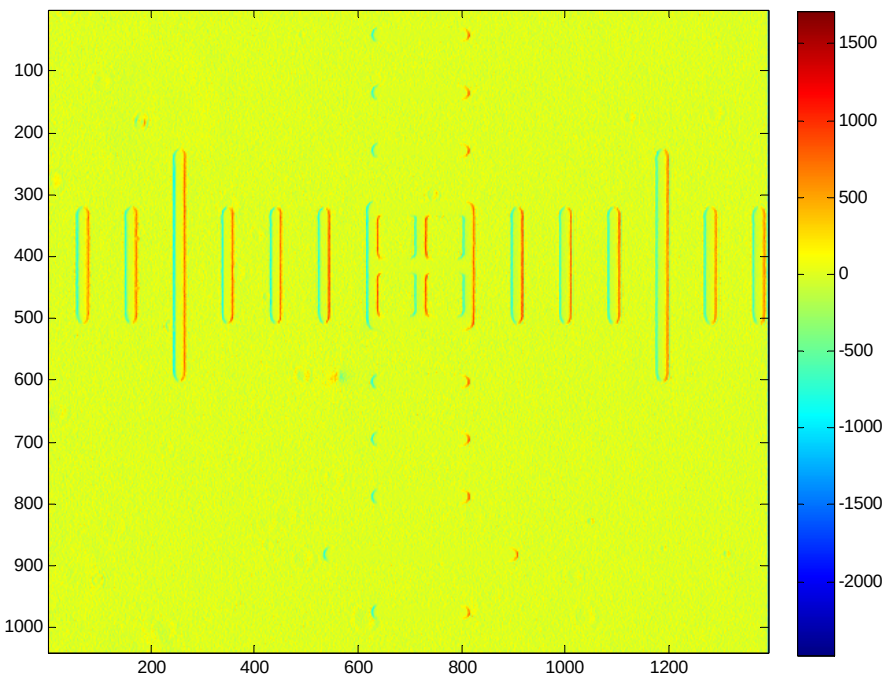
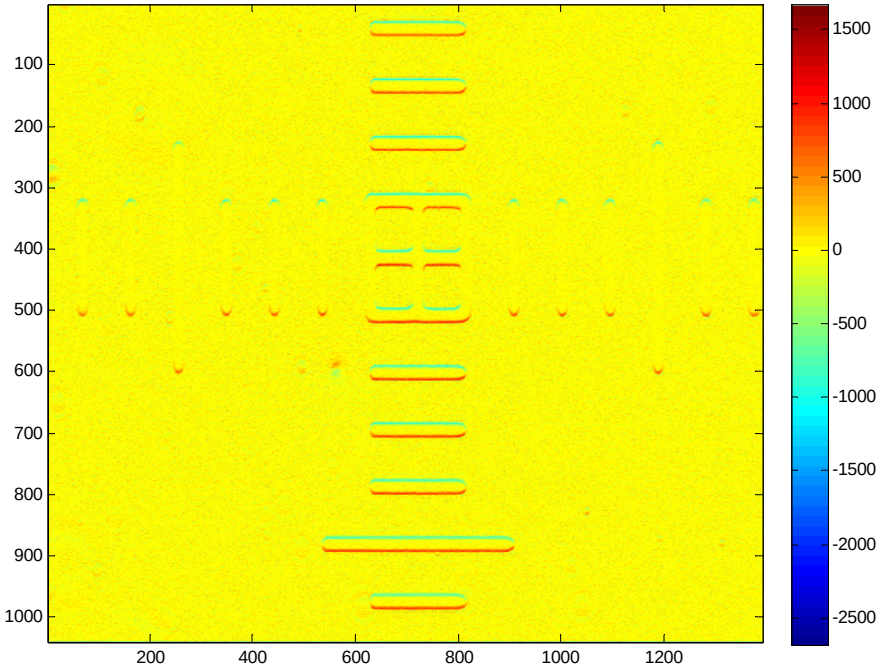
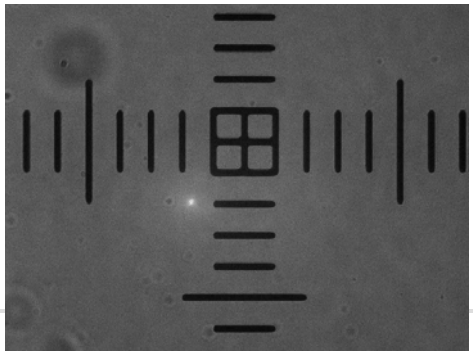


Image Gradient



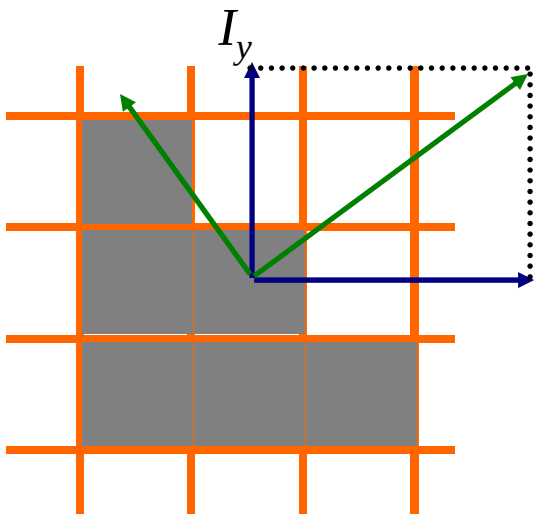




Non-Maximum Suppression

- For each pixel $I(x_0,y_0)$,compare the edge strength along the direction perpendicular to the edge

- An edge point must have its edge strength no less than its two neighbors.



Hysteresis Thresholding

- The main purpose is to link detected edge points while minimizing breakage.

- Basic idea

  - Using two thresholds T_L and T_H

  - Starting from a point where edge gradient magnitude higher than T_H

  - Link to neighboring edge points with edge gradient magnitude higher than T_L

Influence of Scale Selection on Edge Detection

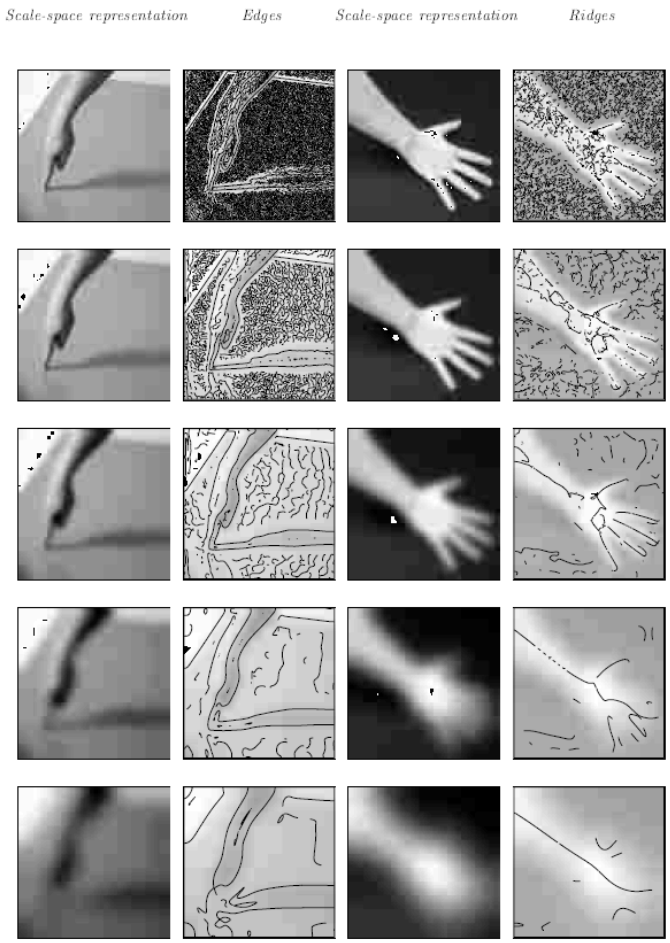


Figure 1.4: *Edges and ridges computed at different scales in scale-space (scale levels t = 1.0, 4.0, 16.0, 64.0 and 256.0 from top to bottom) using a differential geometric edge detector and ridge detector, respectively. (Image size: 256*256 pixels.)*

Lindeberg (1999) `` Principles for automatic scale selection'', in: B. J"ahne (et al., eds.), Handbook on Computer Vision and Applications, volume 2, pp 239--274, Academic Press.

Comments

- Gradient-based edge detection is effective and provides good localization accuracy.

- Gradient-based edge detection is sensitive to noise.

- How to achieve robust detection

  - Set robustness as a main goal in algorithm design
  - Integrate information at different scales
  - Integrate information from different sources

Questions?