FLUORESCENT PROBES

(i) normal probes: Texas Red
FITC, TRITC, Rhodamine

(ii) caged fluorophores

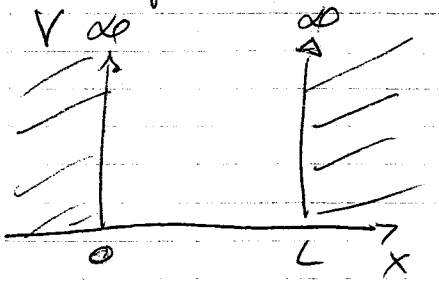
(iii) GFP: - green fluorescent protein
- can be coexpressed w/ protein of interest
- can be used in living cells
- labeling w/ other (normal) fluorophores can alter properties and lead to artifacts (rhodamine - phalloidin labeled actin)
- by now also YFP, etc. (evtl. see add. pages)

(iv) Quantum Dots

(see also pages 20-22)

e^- in box model

simple QM system to illustrate



$$V(x) = \begin{cases} 0 & 0 \le x \le L \\ \infty & \text{otherwise} \end{cases}$$

$$\text{SE}: -\frac{\hbar^2}{2m}\frac{d^2\psi}{dx^2} + V(x)\psi = E\psi$$

$\psi(x) = 0$ outside $0 \le x \le L$

$$\text{SE}: -\frac{\hbar^2}{2m}\frac{d^2\psi}{dx^2} = E\psi$$

solutions: $\psi(x) = A\sin kx + B\cos kx$

boundary cond.: $\psi(0) = \psi(L) = 0$

$\psi(x) = A \sin kx \qquad k = \frac{n\pi}{L}, \quad n = 1, 2, \ldots$

normalization: $\int_0^L \psi^2(x)\,dx = \frac{A^2 L}{2} \stackrel{!}{=} 1$

$$A = \sqrt{\frac{2}{L}}$$

$$\psi(x) = \sqrt{\frac{2}{L}} \sin \frac{n\pi x}{L}$$

$k = \frac{h}{2\pi}$

$$E_k = \frac{\hbar^2 k^2}{2m} = \frac{n^2\pi^2\hbar^2}{2mL^2} = \frac{n^2 h^2}{8mL^2} = E_n$$

$$\Delta E = E_{n+1} - E_n = \left[(n+1)^2 - n^2\right] \frac{h^2}{8mL^2}$$

$$= (2n+1)\frac{h^2}{8mL^2}$$

$$\Delta E \sim \frac{1}{L^2}$$

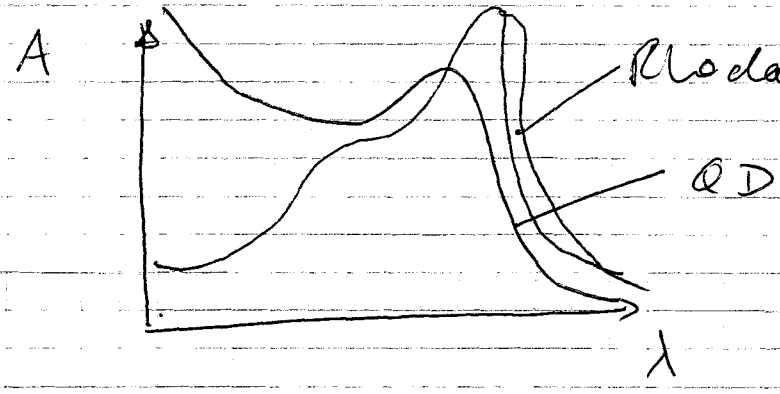
⇒ size of QD determines $\Delta E = h\nu$

the larger the QD the "redder" the color

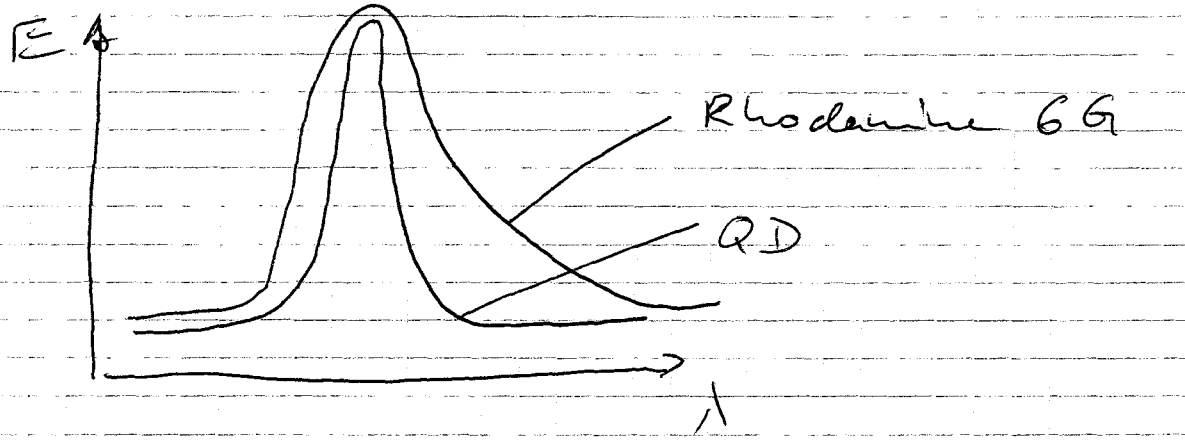
Additional benefits:

- high photostability!
- reduced photobleaching
- can be designed to suit needs by material properties + size

QD cont.

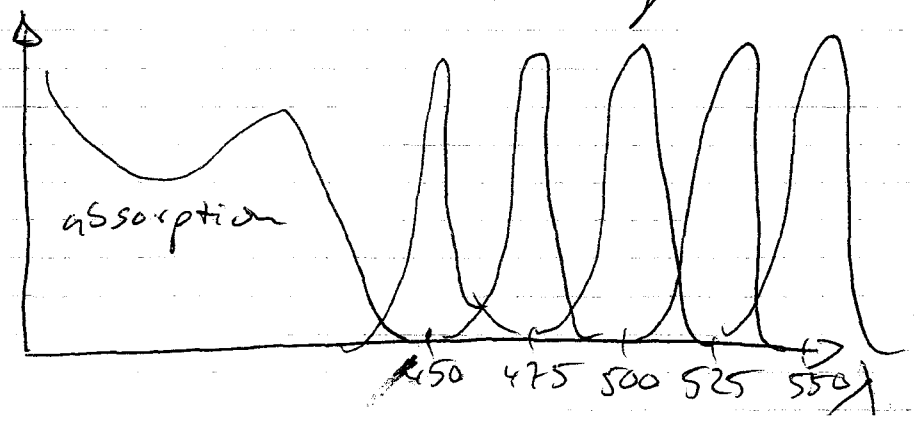


advantage of QD over conventional dye
much broader absorption spectrum



Conventional dyes: - assymmetric emission spectrum
- broad emission spectrum
- number of resolvable probes simultaneously is limited

QD: - very narrow, symmetric em. spectra
- many emission colors simultaneously
- excited w/ single short λ laser



QD's advantages?

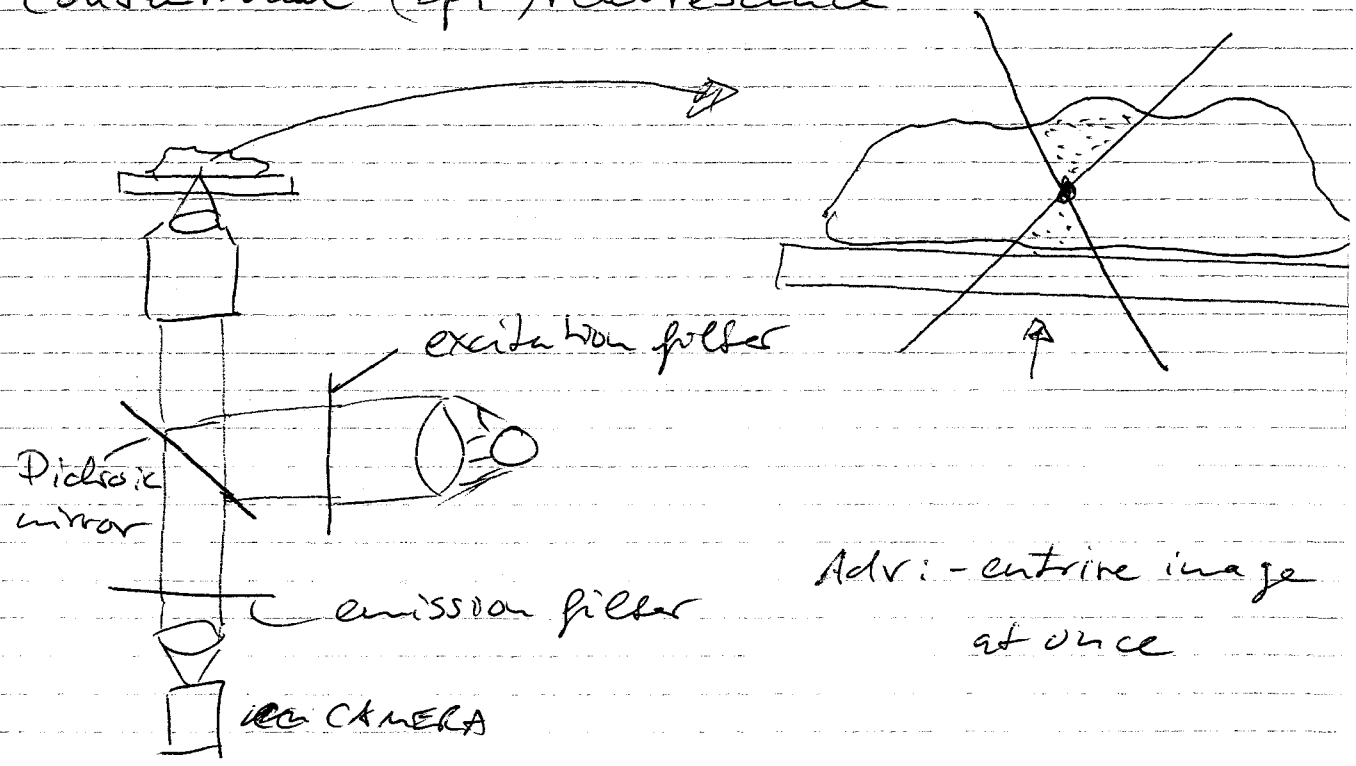
- "blinking", QR effect
- cytotoxicity (Cd, Se, ...)
  ⇒ need for good capsules
- ~~toxic~~ unknown long-term effects of nanoparticles in living organisms
- nanoparticles can get anywhere!

(literature "popular": "Prey" by Michael Crichton

⇒ soon new Prof. Wolfgang Parak

ADVANCED FLUORESCENCE MICROSCOPY TECHNIQUES

Conventional (Epi-) Fluorescence



Adv: - entire image at once

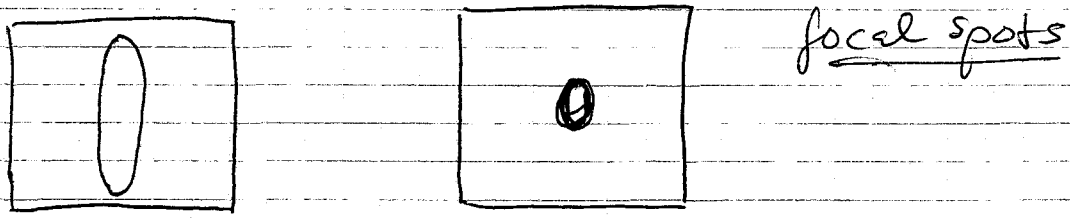
Problems: - camera resolution ↔ sensitivity ↔ speed
- out of focus fluorescence leads to blurred images
- limited axial resolution

lateral resolution: $HWB_L = \frac{0.4 \times \lambda}{NA}$

$NA = n \sin\alpha$

axial resolution: $HWB_A = \frac{0.45 \times \lambda}{n(1-\cos\alpha)}$

$\Rightarrow$ use short λ, high NA



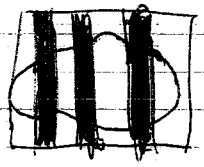
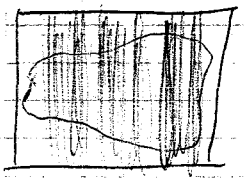
40x, NA=0.9 60x, NA=1.4

NA affects axial resolution much more than lateral resolution

NUMERICAL / COMPUTATIONAL APPROACHES

Structured Illumination Microscopy (SIM)

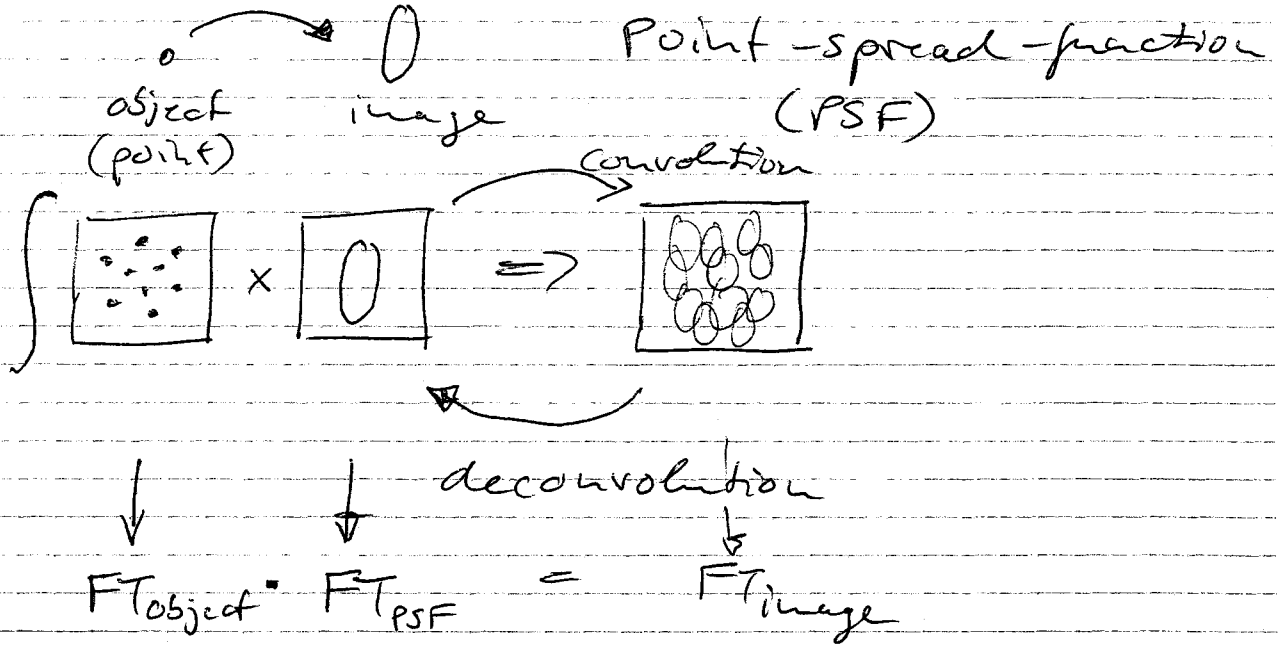
- project stripe pattern into focus

in focus:  out-of-focus: 

- move pattern across image
- remove computationally all information that is not sharp

Deconvolution microscopy

- remove out-of-focus blur by deconvolution
- a point will never be imaged as a point

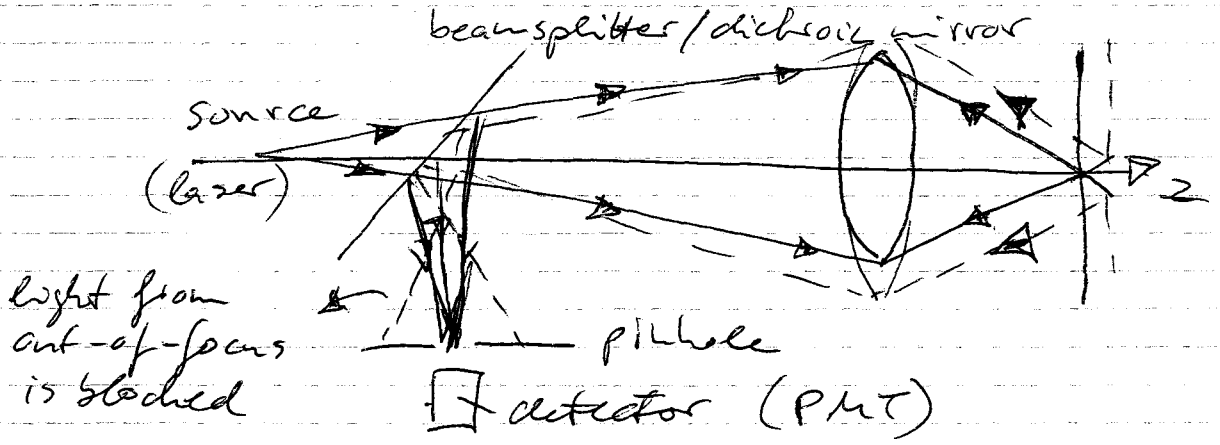


$$\text{Inverse FT}\left(\frac{FT_{image}}{FT_{PSF}}\right) \rightarrow \text{much clearer picture}$$

- usually iterative numerical procedure
- uses constraints such as smoothness etc.
- problem: takes time + computational power

CONFOCAL MICROSCOPY

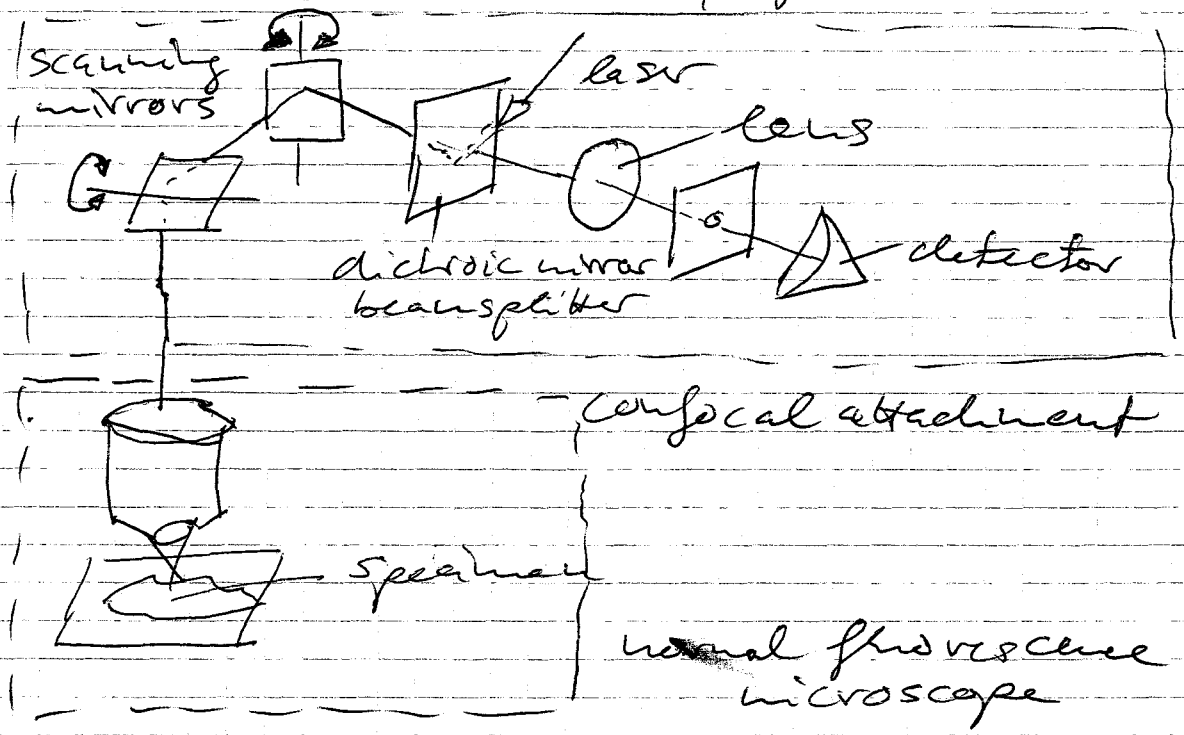
- removes out-of-focus blur by use of pinhole



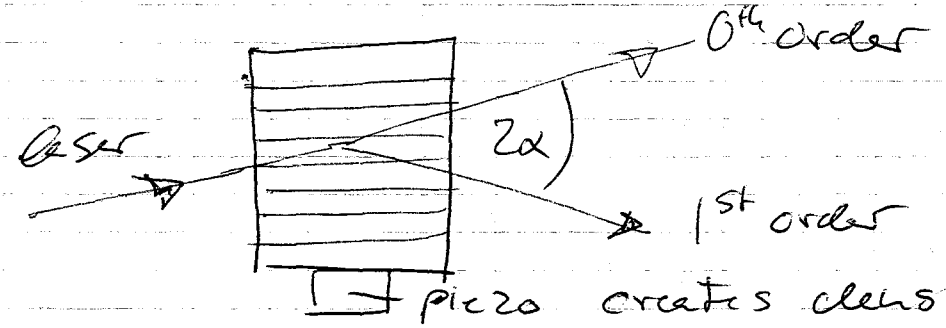
- light only from the focal spot
  → no image
- image is constructed by scanning either
  a) the object by moving the stage
  b) the pinhole
  c) the laser beam

a) and b) is too slow for x-y

c) scanning laser beam by galvanic mirrors



or using two acousto-optic modulators



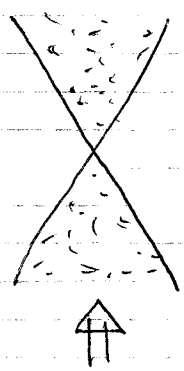
creates density standing wave in transparent crystal

deflection of laser beam from periodic structure

- deflection of laser beams by periodic density variation similar to Bragg-reflection of X-rays on crystal planes
- deflection efficiency ~ piezo-power
  deflection angle α ~ λ of light and Λ of sound wave
- Λ can be changed electronically very fast
- with two perpendicular AODs a laser beam can be scanned over an area
- much faster than mirrors, but more expensive, and smaller area
- image in 2D (x-y plane) usually consists of 512 x 512 pixels or 1024 x 1024 pixels
- each pixel has 8, 10, or 12 bit depth
- for third dimension usually the stage or objective is moved up and down

=> 3D reconstruction of objects w/ voxels
=> video-frame-rate (30 fps) for fast models (a stack of 30 sections in 1 s)

Problem still:



fluorescence from entire cone of illumination even though out-of-focus light is rejected
=> photobleaching