The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific
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International Atomic
Energy Agency

SMR: 1643/7

WINTER COLLEGE ON OPTICS ON OPTICS AND PHOTONICS
IN NANOSCIENCE AND NANOTECHNOLOGY

(7 - 18 February 2005)

"Biophotonics at the Nanoscale" - 1

presented by:

L. Novotny
The Institute of Optics
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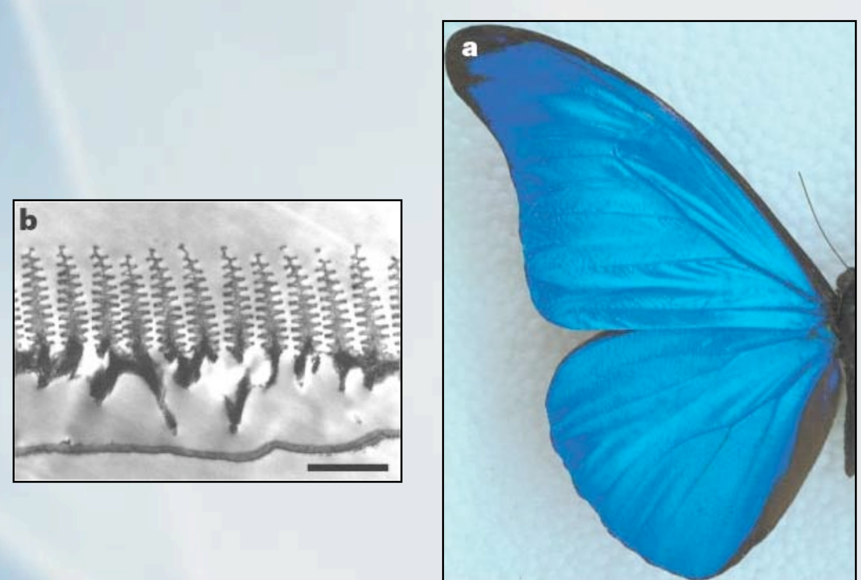
NANOBIOPHOTONICS



Lukas Novotny
The Institute of Optics, University of Rochester, Rochester, NY, 14627.

.. application of optical science and technology to the study of nanoscale biological processes.

Basic Sciences . . . Optics . . . Technical Sciences

↓ ↓ ↓

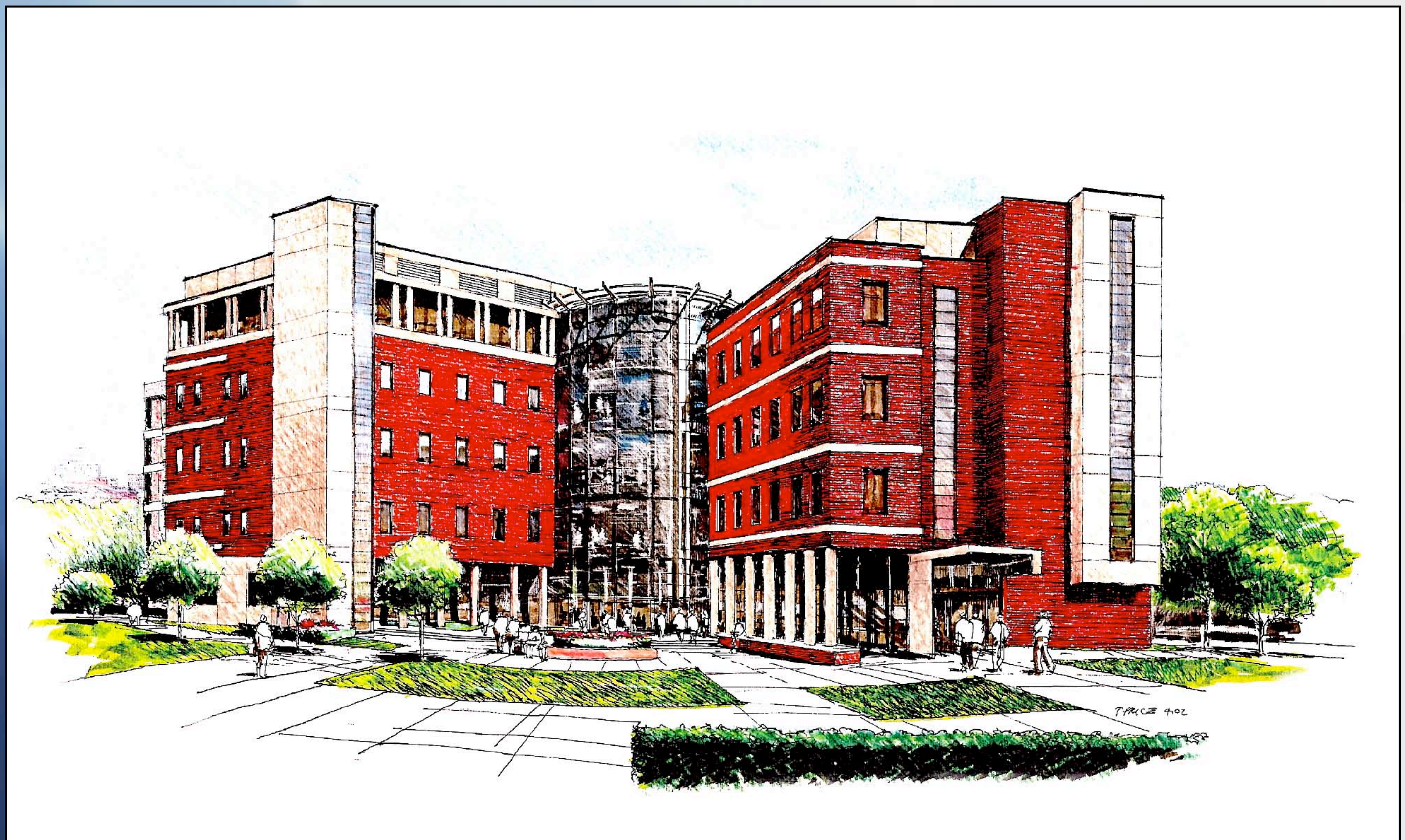
Nanoscience . . . **NANO-OPTICS** . . . Nanotechnology

Nano-Optics is the study of optical phenomena and techniques near or beyond the diffraction limit.

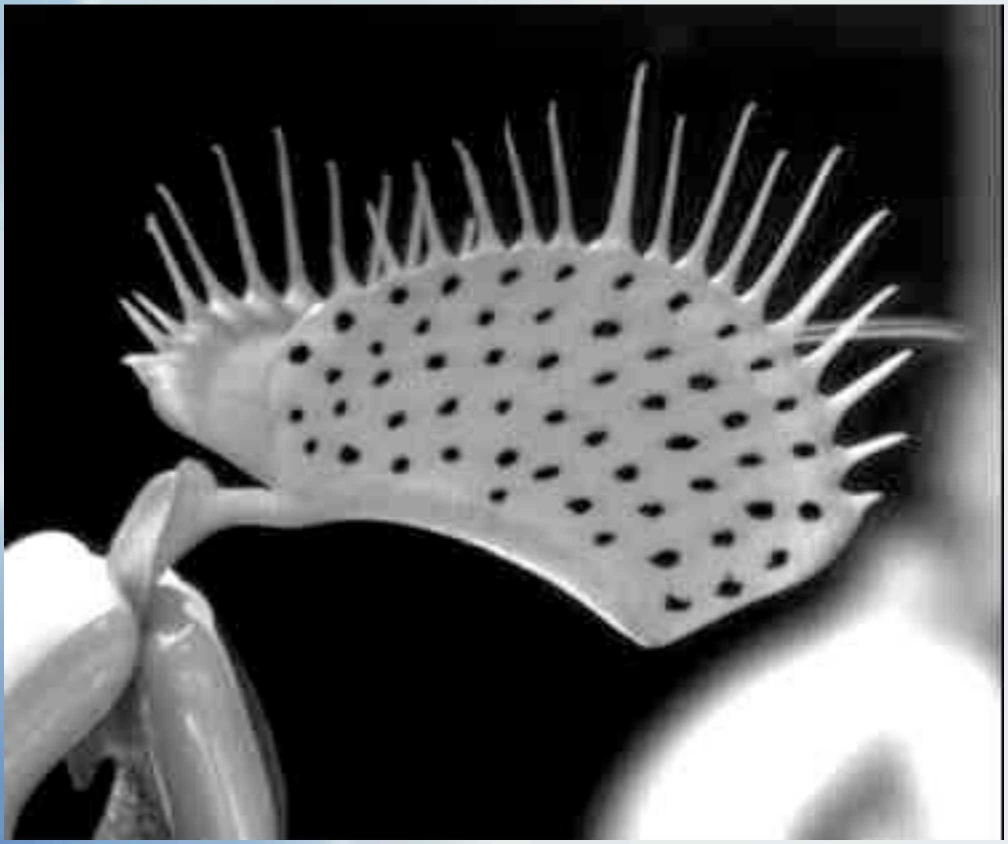
NANO-OPTICS @ ROCHESTER

www.nano-optics.org

OPTICS - BIOMEDICAL ENGINEERING BUILDING PROJECT

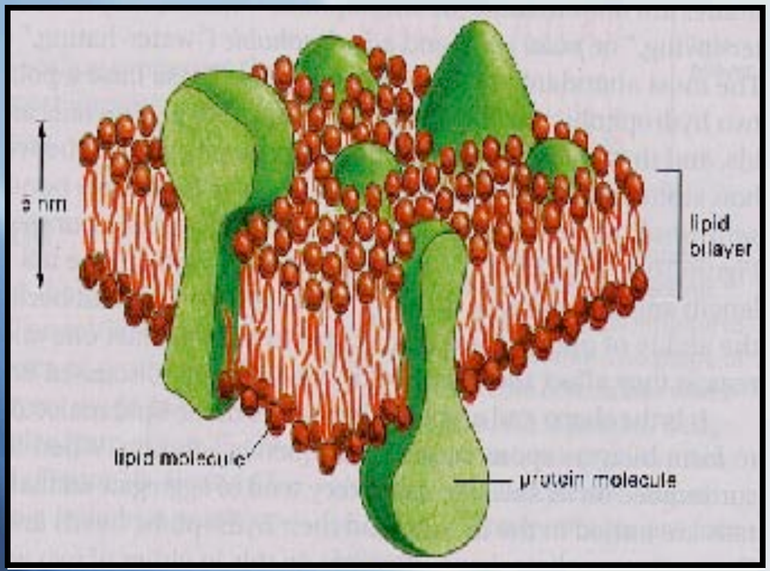


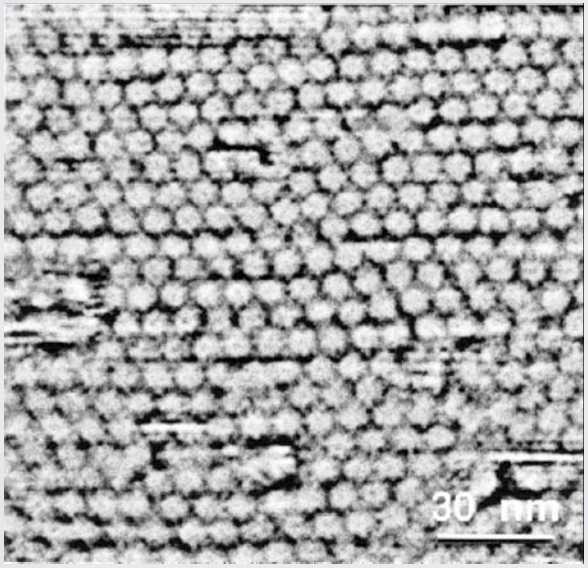
PROTEINS WORKING IN SYNCHRONY



Cell signaling and coordination are true marvels of nature !

BIOLOGICAL BUILDING BLOCKS





Biology functions through highly coordinated and complex interactions among nanostructures. Biology is Nanobiotechnology !

STRUCTURE OF NANOBIOPHOTONICS

BIOLOGY

PHOTONICS

Photonics for Biology:

- Bioimaging (Microscopy)
- Spectroscopy

Bioinspired Photonics:

- Photonic Bandgap Materials (→ Opal, Butterfly)
- Solar cells (→ Photosynthesis)

THE BIOTECH CYCLE

ADVANCES IN TECHNIQUES
(microscopy, spectroscopy, ..)

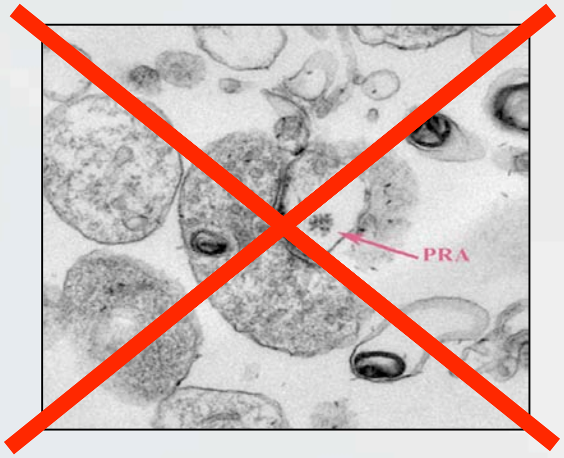
ADVANCES IN BIOLOGY
(cell signaling, protein folding, DNA sequencing, ..)

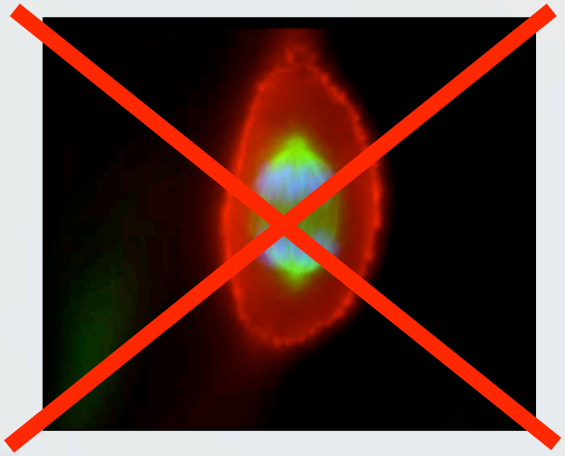
ADVANCES IN BIOTECHNOLOGY
(sensors, materials, ..)

"New Truths become evident when new tools become available"
(Rosalyn Yalow, Nobel Laureate)

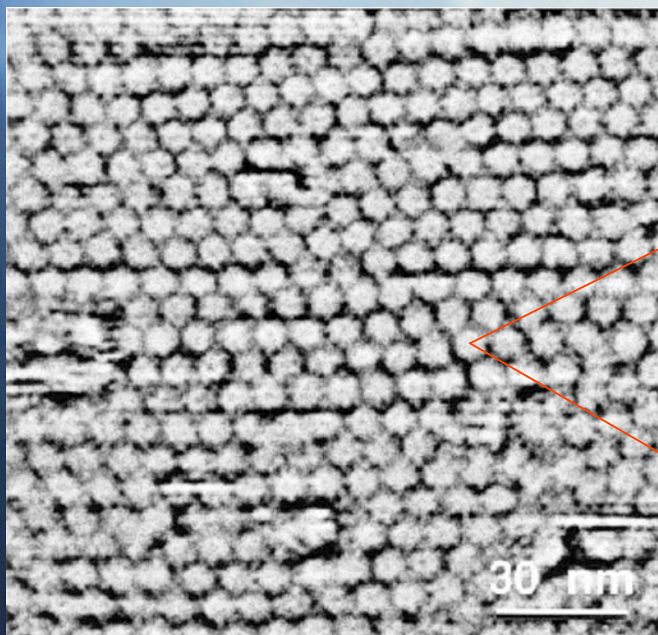
IMAGINE ..

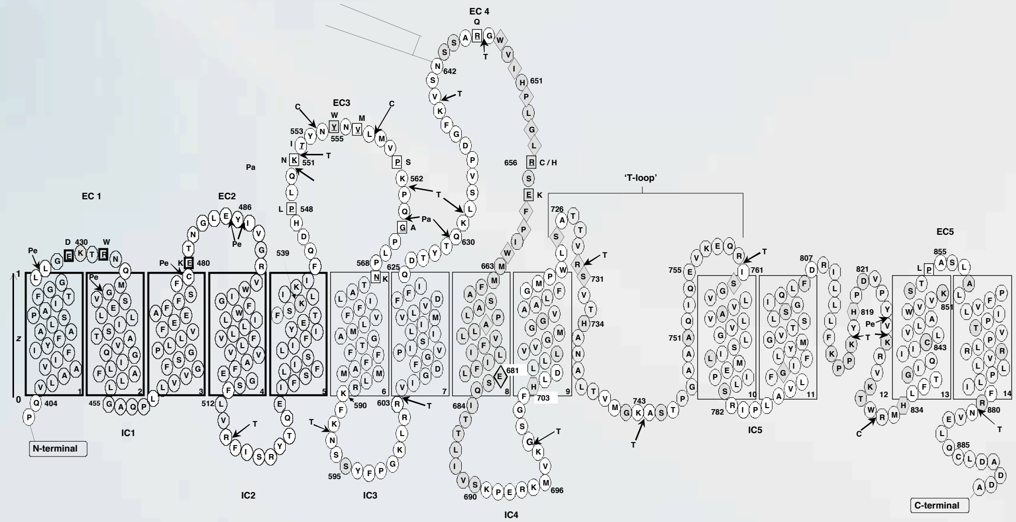
… if there were no light microscopes

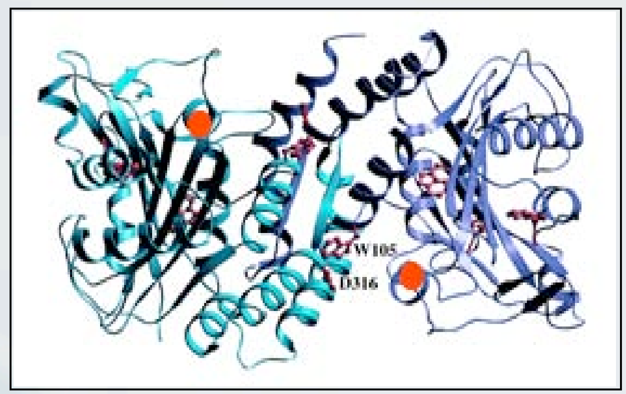




From DNA to AMINOACIDS to PROTEINS

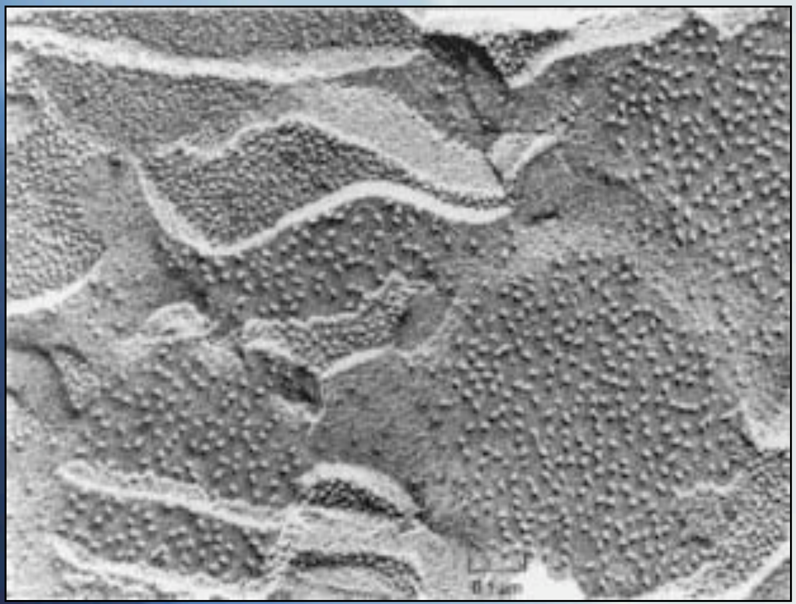


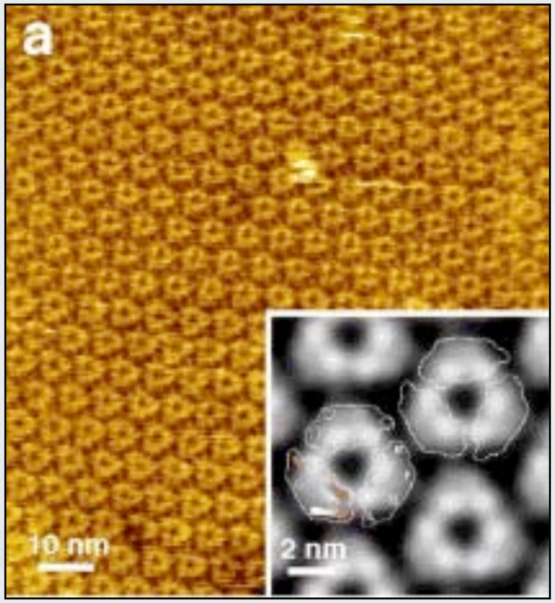




Protein ~ smallest functional unit !

HOW TO MEASURE PROTEINS ?



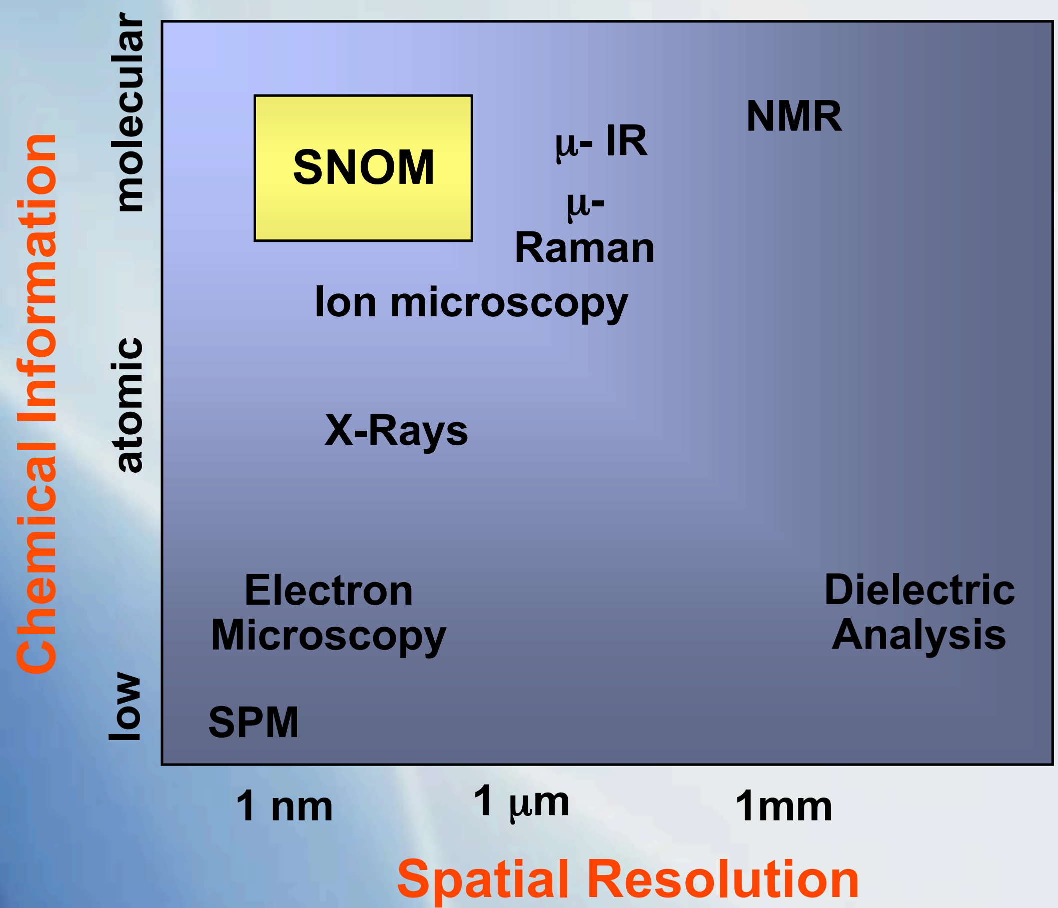


Freeze-fracture electron micrograph of the thylakoid membranes from the chloroplast of a plant cell.

AFM topographs of purple membrane from Halobacterium salinarium.

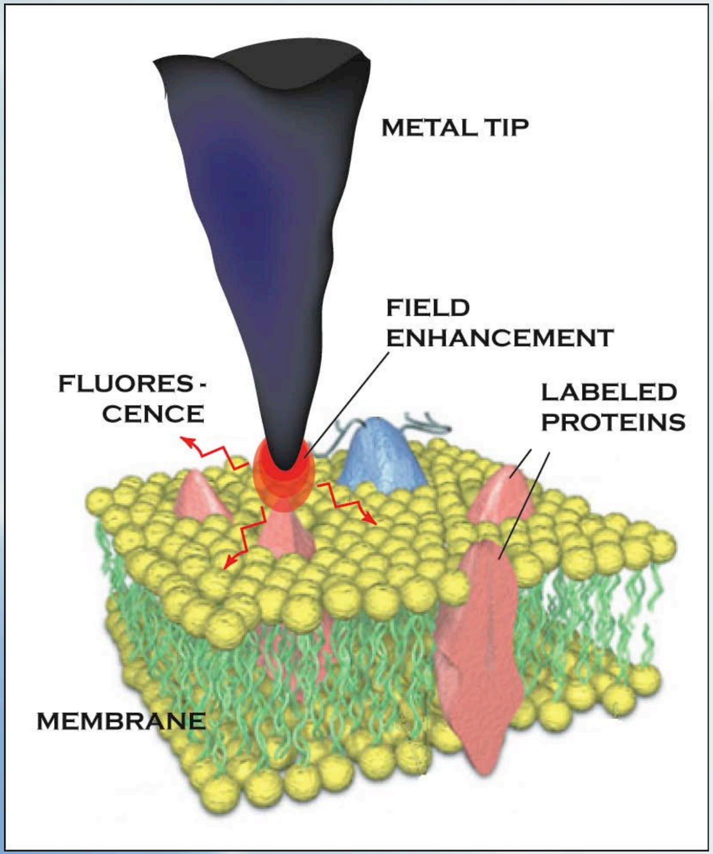
A. Engel, MSB Biozentrum, Basel, Switzerland.

SPATIAL RESOLUTION VS.CHEMICAL INFORMATION



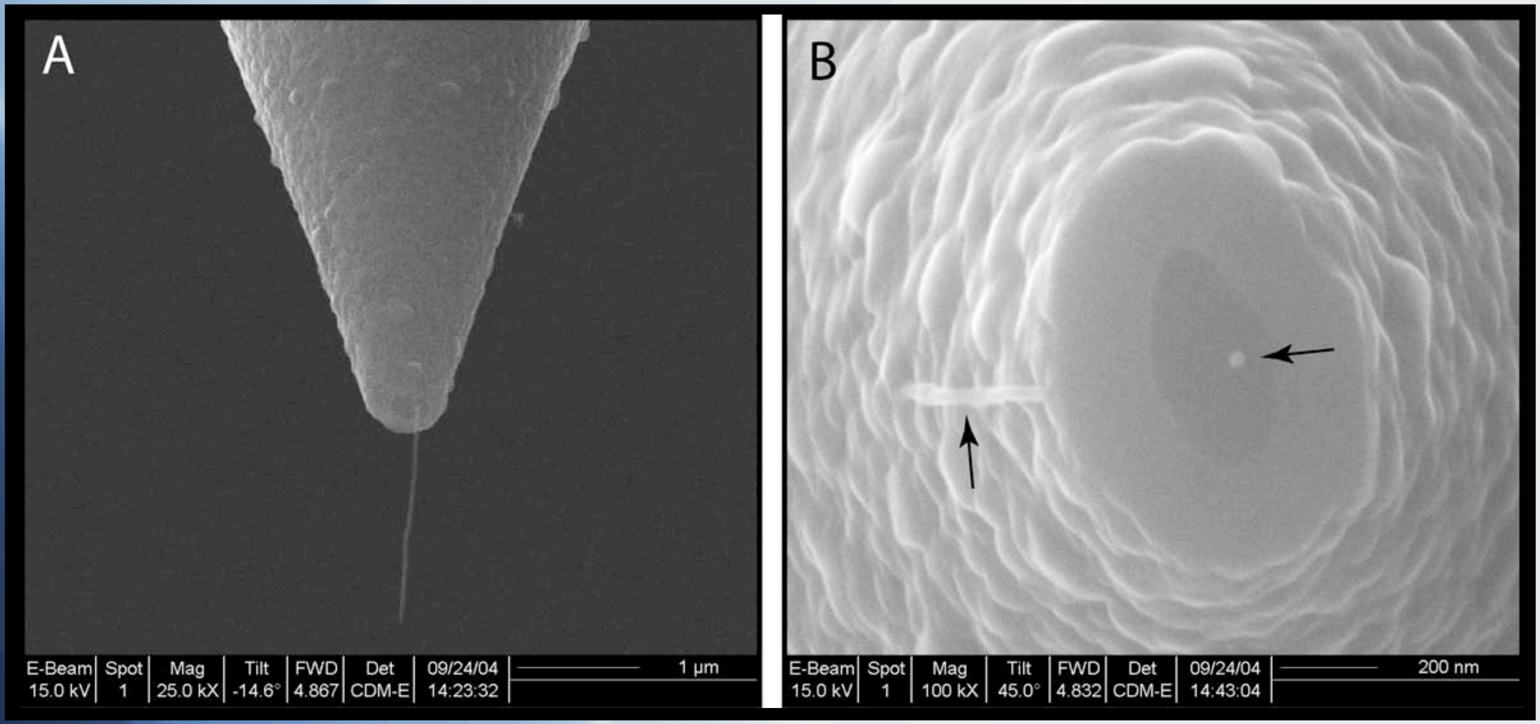
S. Stranick, NIST

HOW TO MEASURE PROTEINS ?

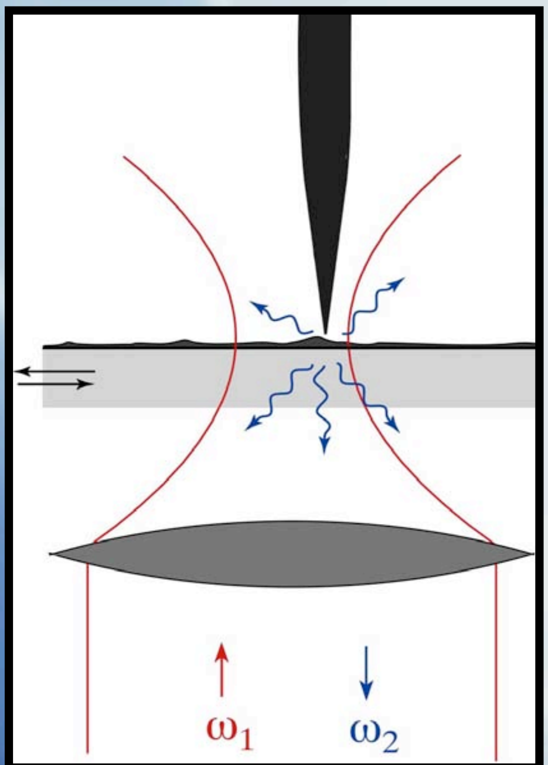


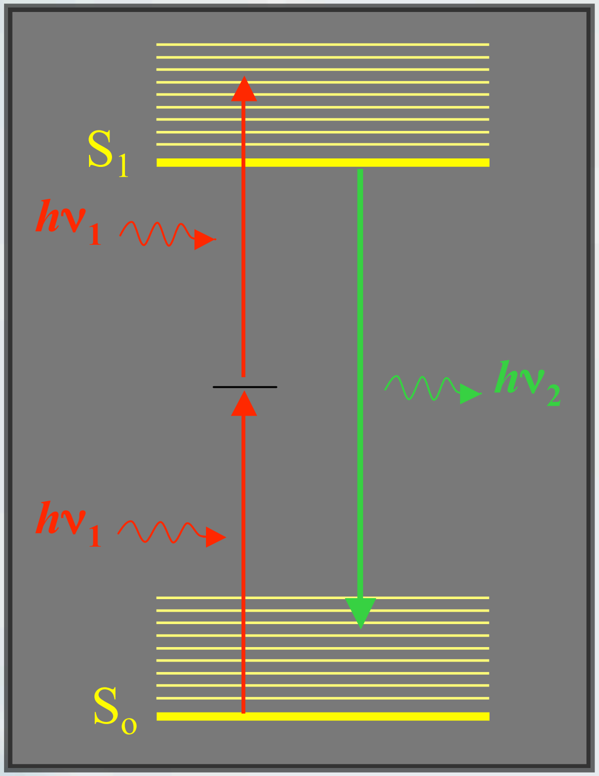
DOES IT WORK ?

NEW GENERATION

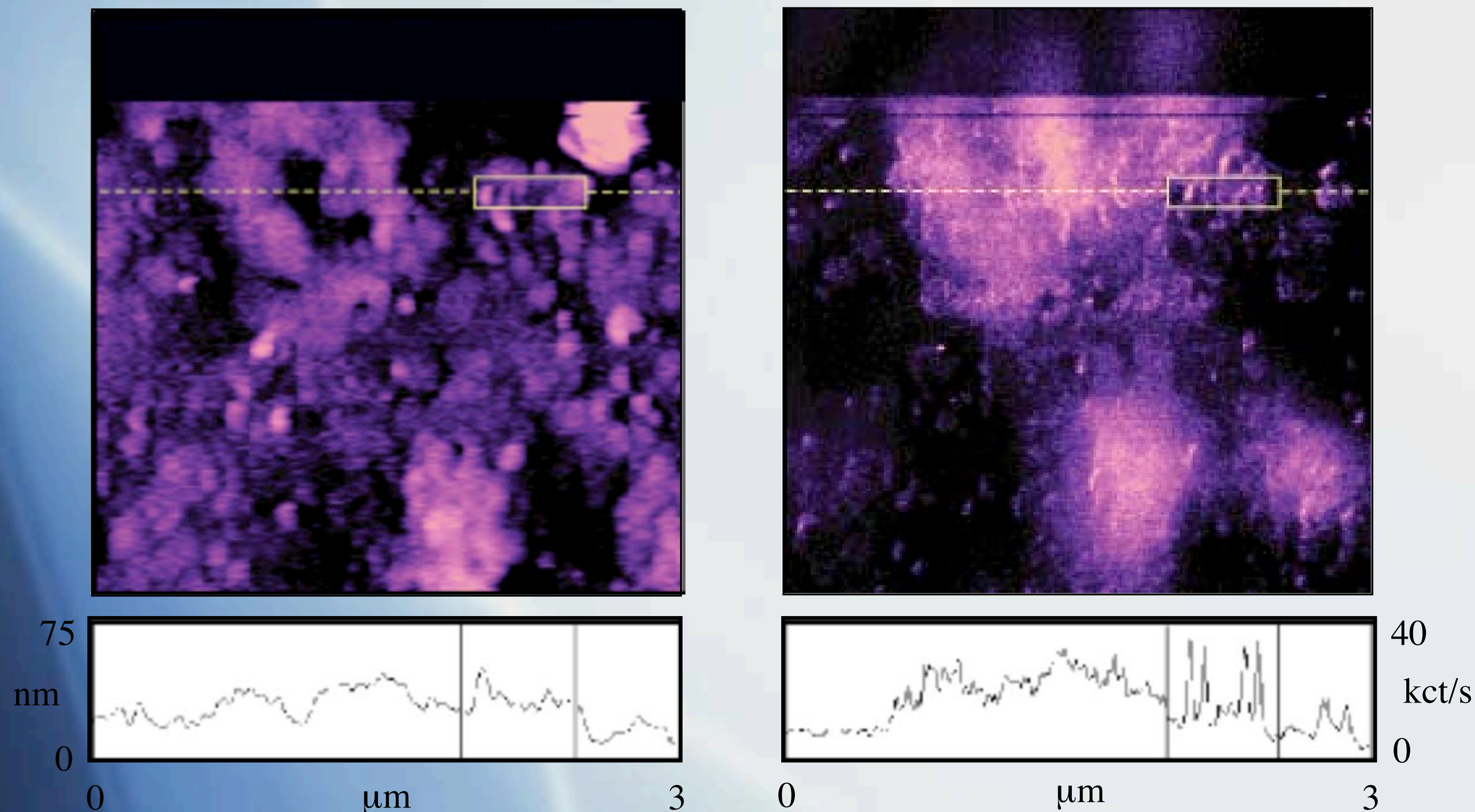


NEAR-FIELD TWO-PHOTON EXCITED FLUORESCENCE



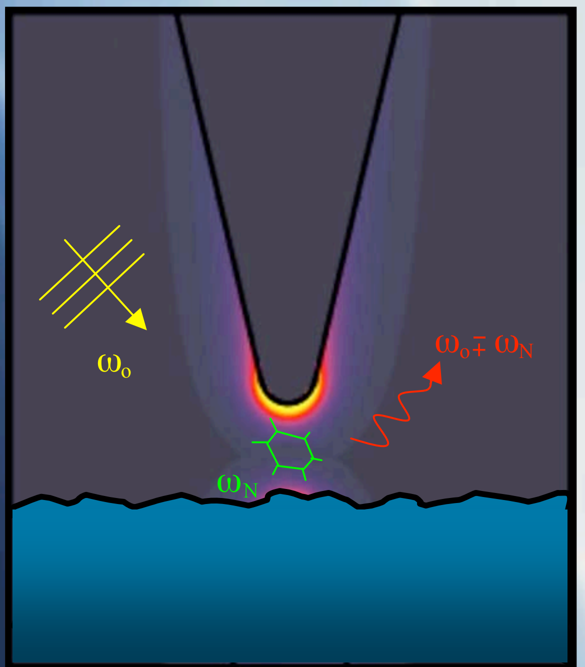


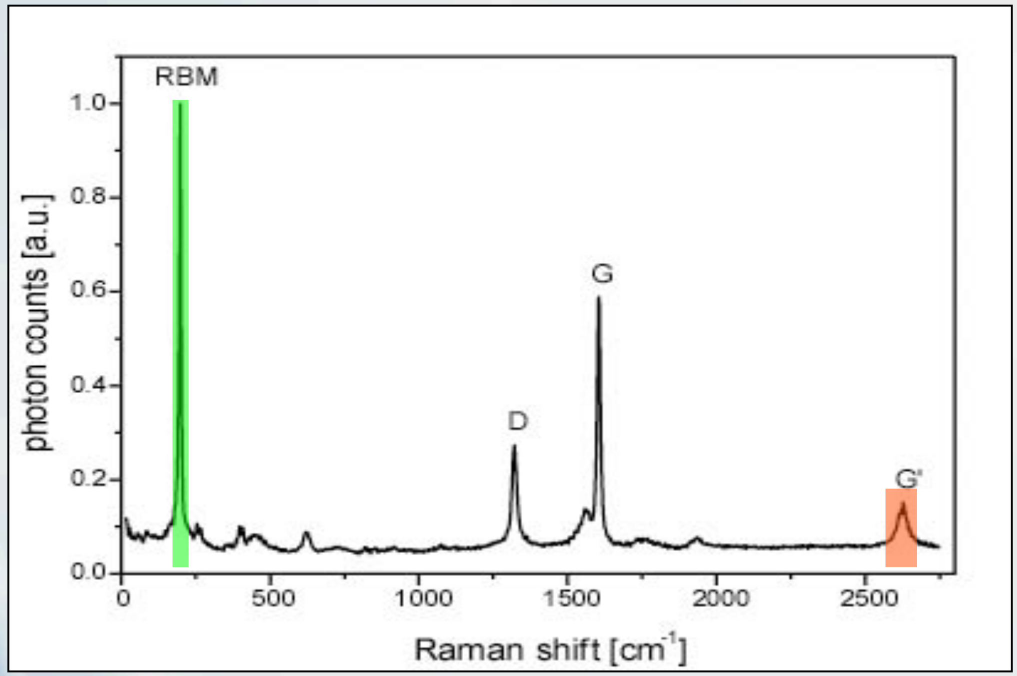
PHOTOSYNTHETIC MEMBRANE



PRL **82**, 4014 (1999).

NEAR-FIELD RAMAN SCATTERING ?





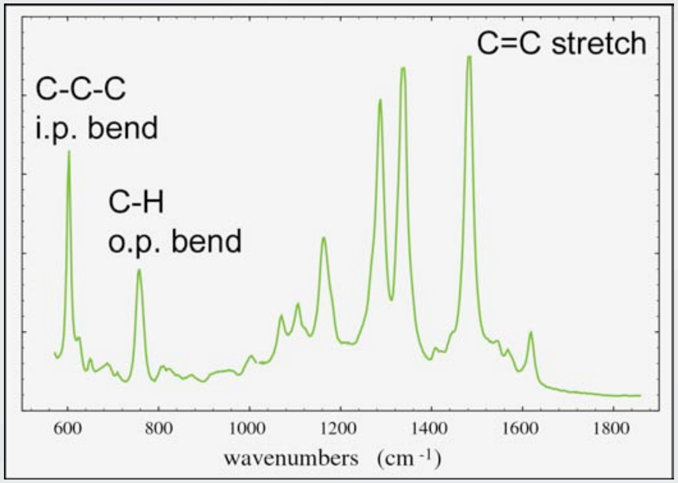
PRL **90**, 95503 (2003)

WHY RAMAN SCATTERING ?

Chemically specific ("fingerprint")

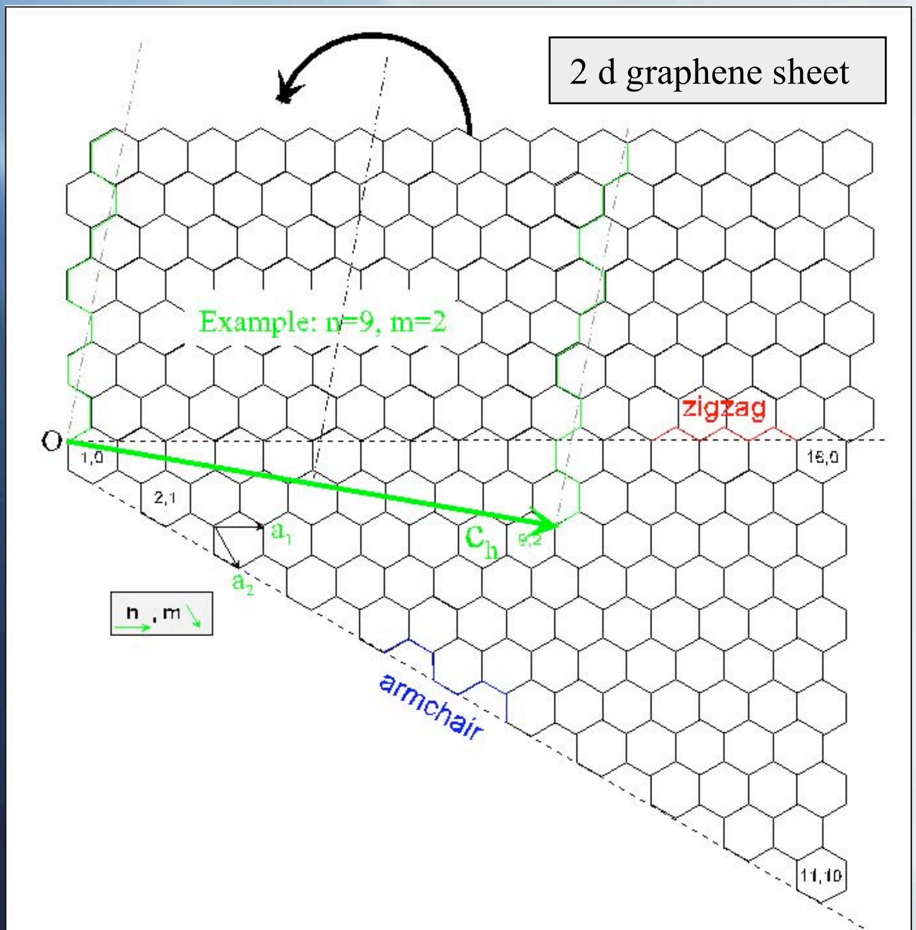
Intrinsic properties of molecular structures (no need for labels)

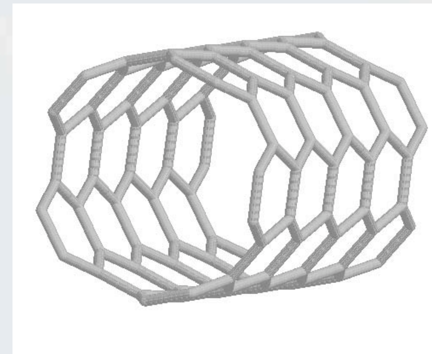
"No" photo-bleaching.



Raman signals can be locally enhanced by up to 10^{14} (SERS).

LEARN WITH EFFICIENT MOLECULES ..





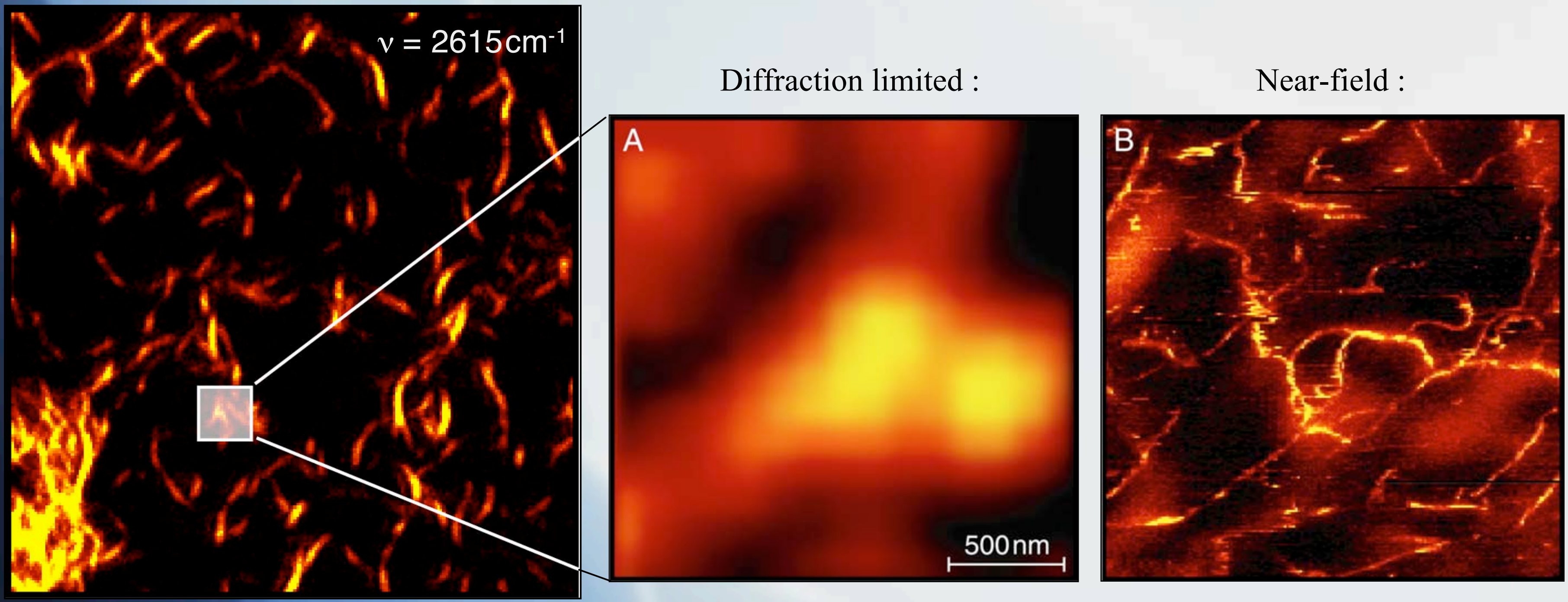
diameter: 0.7 - 2nm
length: up to several 10 µm

→ one dimensional systems

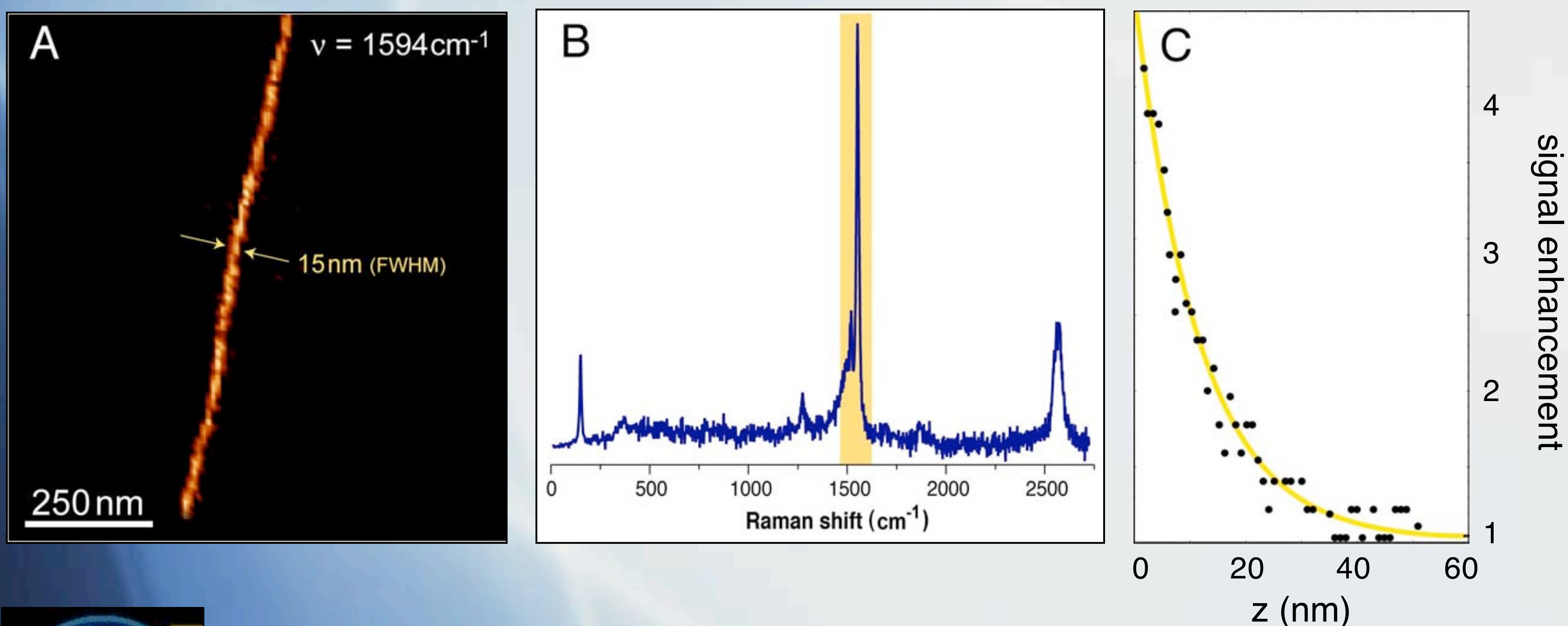
- well defined topography
- large σ_{Raman}
- resonance enhancement

THE ACTORS

NEAR-FIELD RAMAN SCATTERING

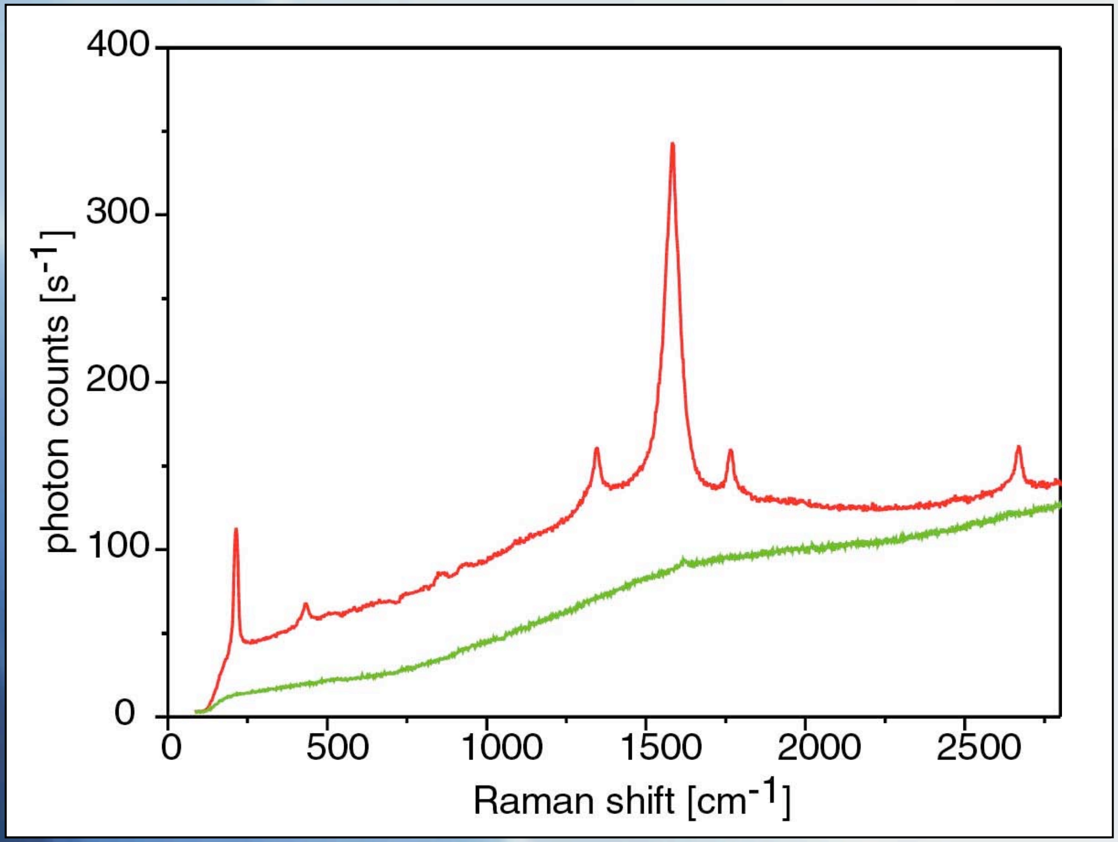


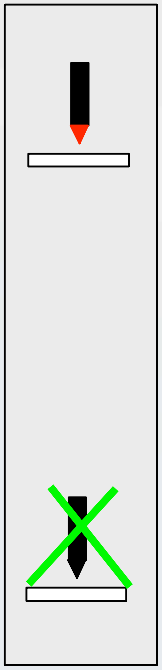
HIGH-RESOLUTION OPTICAL SPECTROSCOPY



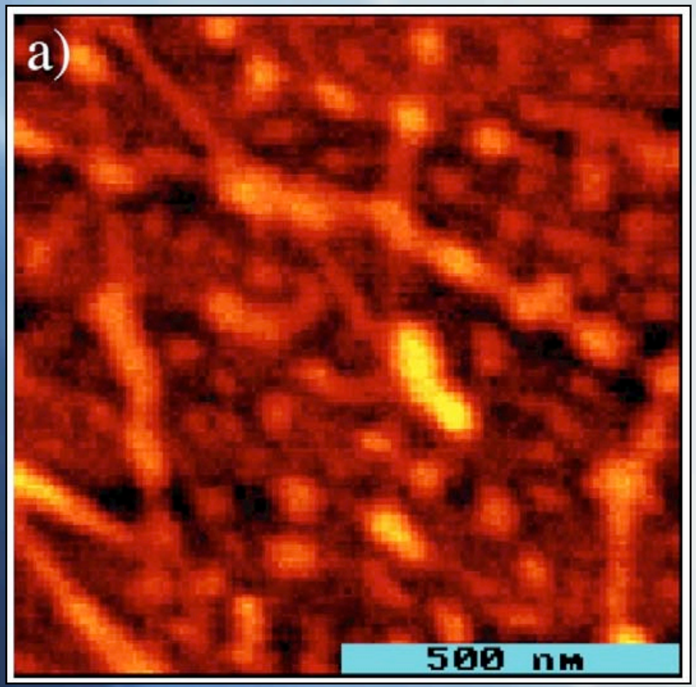
Featured in 2005 Guinness Book of World Records.

NEAR-FIELD SIGNAL ENHANCEMENT

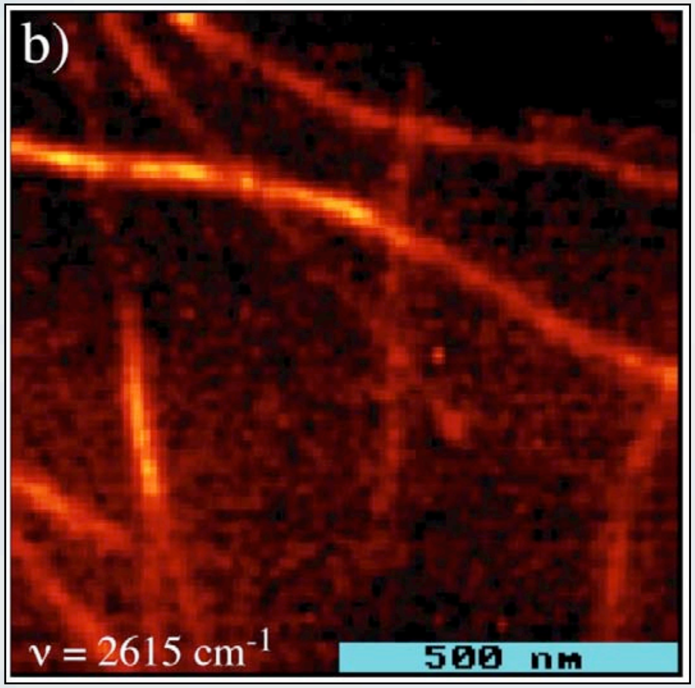




CHEMICAL SPECIFICITY



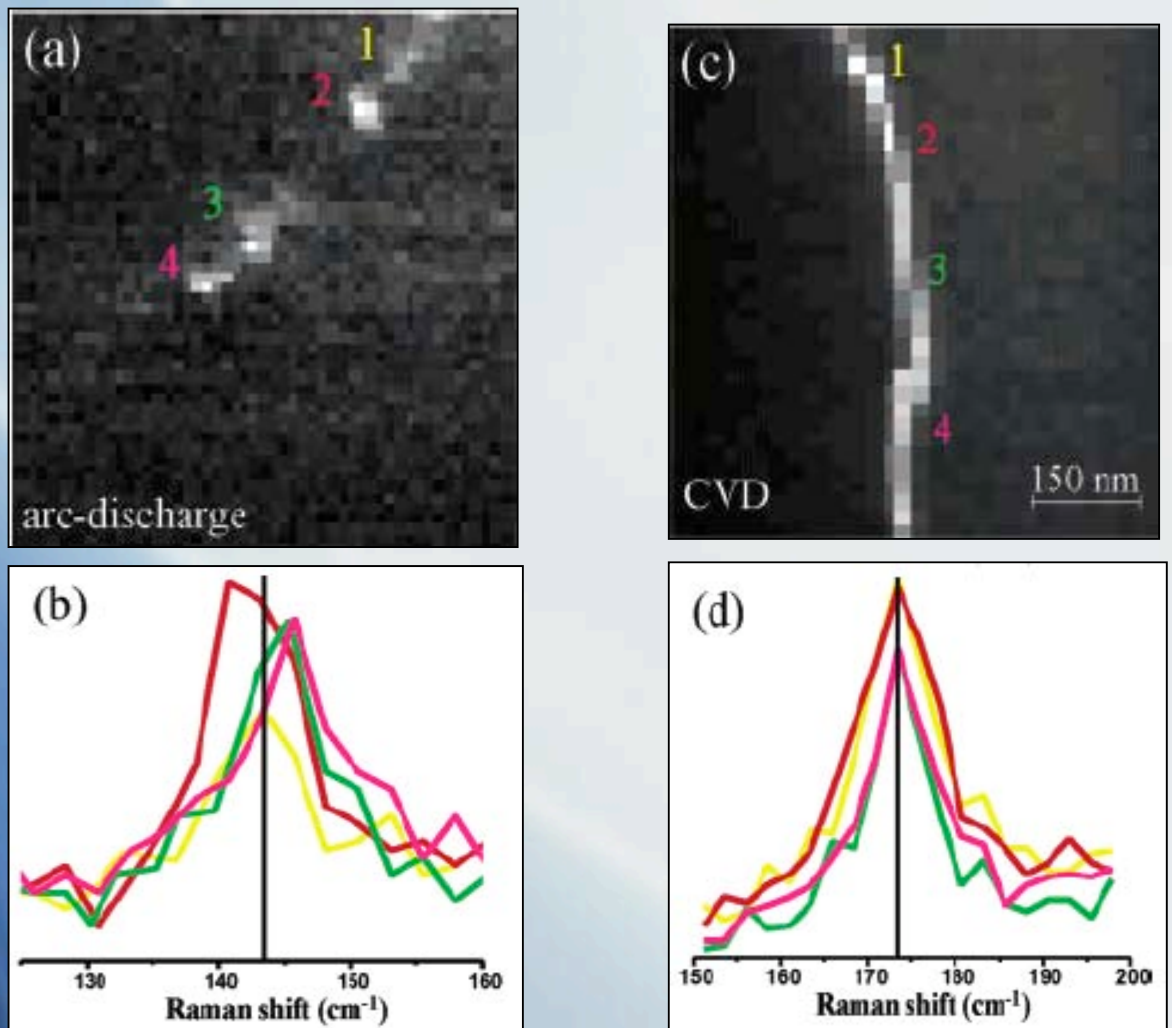
Topography



Raman scattering

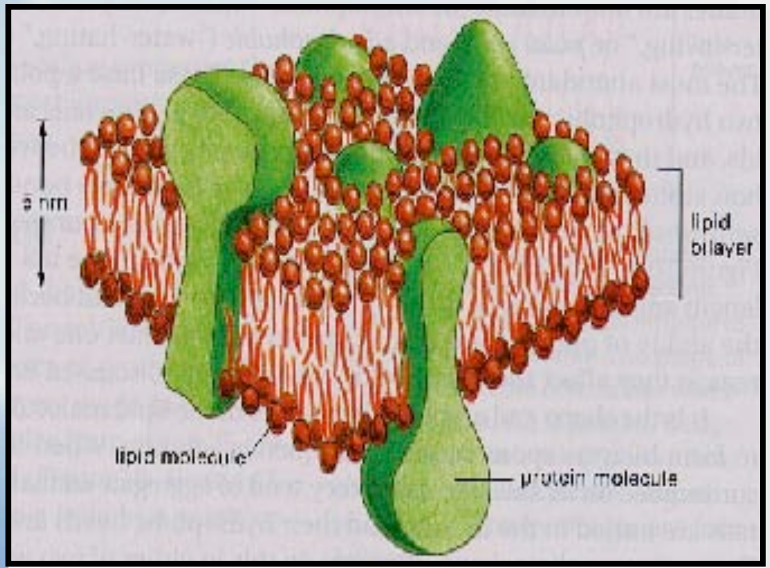
PRL **90**, 95503 (2003)

LOCALIZATION OF DEFECTS AND DOPANTS



JACS (in print)

SO WHAT ABOUT PROTEINS ??

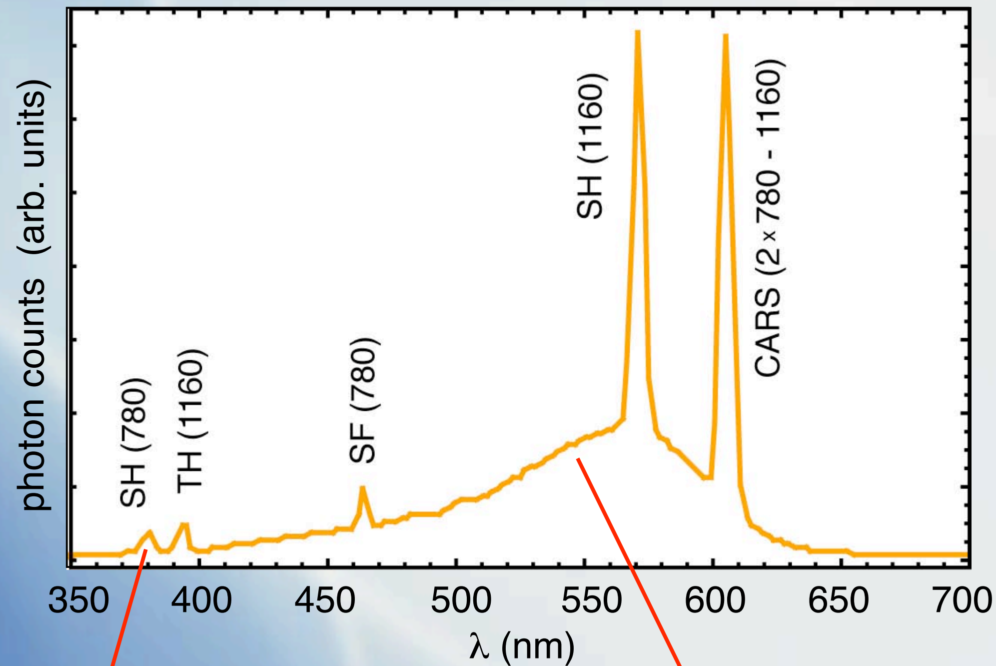


?

WHERE ARE THE MISSING **10** ORDERS OF MAGNITUDE ?

DIFFERENT APPROACH

METAL TIP AS A LOCALIZED PHOTON SOURCE

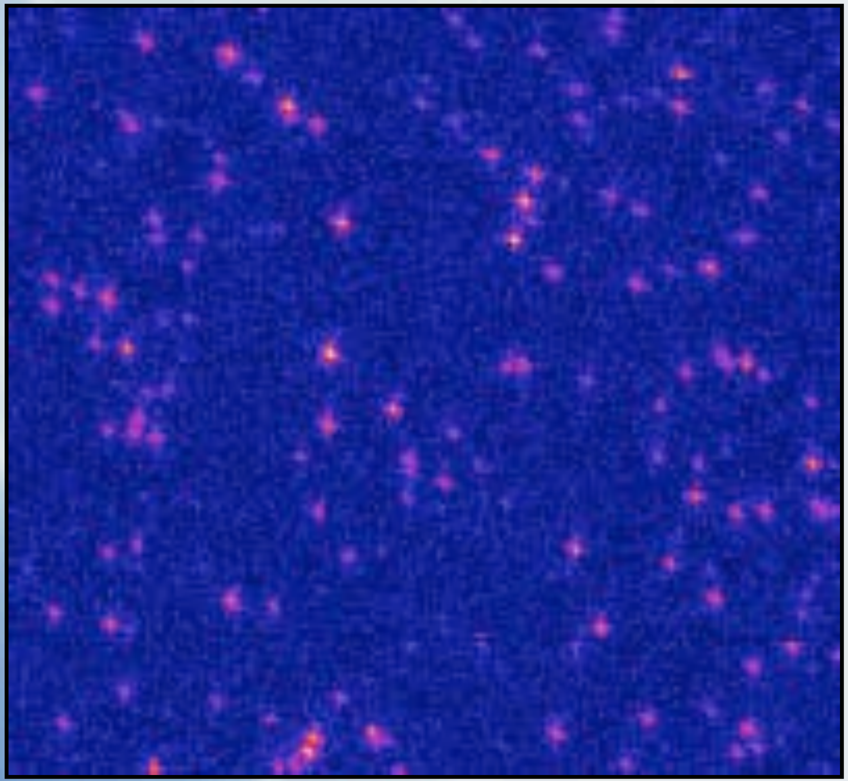


PRL **90**, 013903 (2003)

PRB **90**, 013903 (2003)

LET'S BE REALISTIC ..

FLUORESCENT LABELING



TEXTBOOK (Cambridge Univ. Press)

PRINCIPLES
OF
NANO-OPTICS

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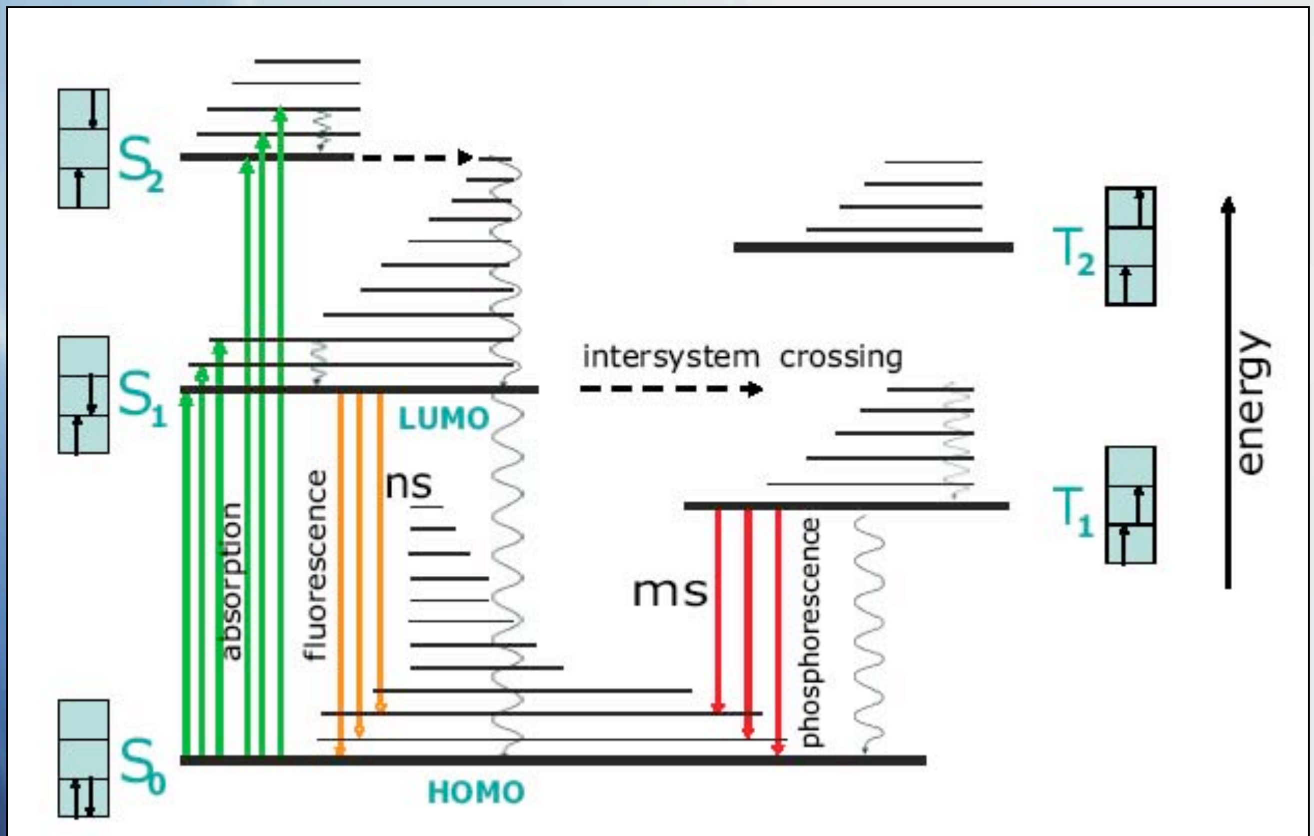
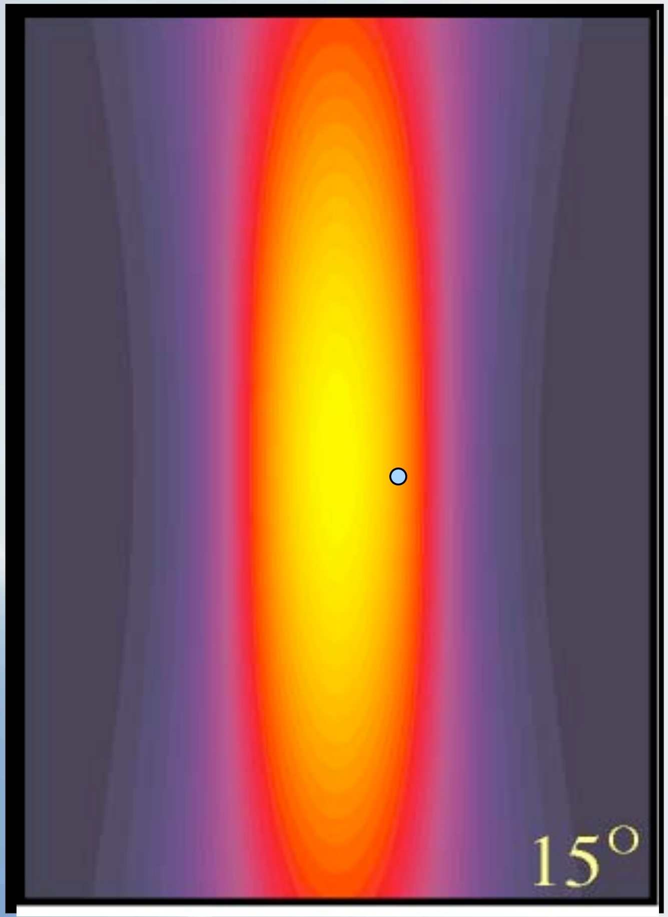
FLUORESCENT MOLECULES

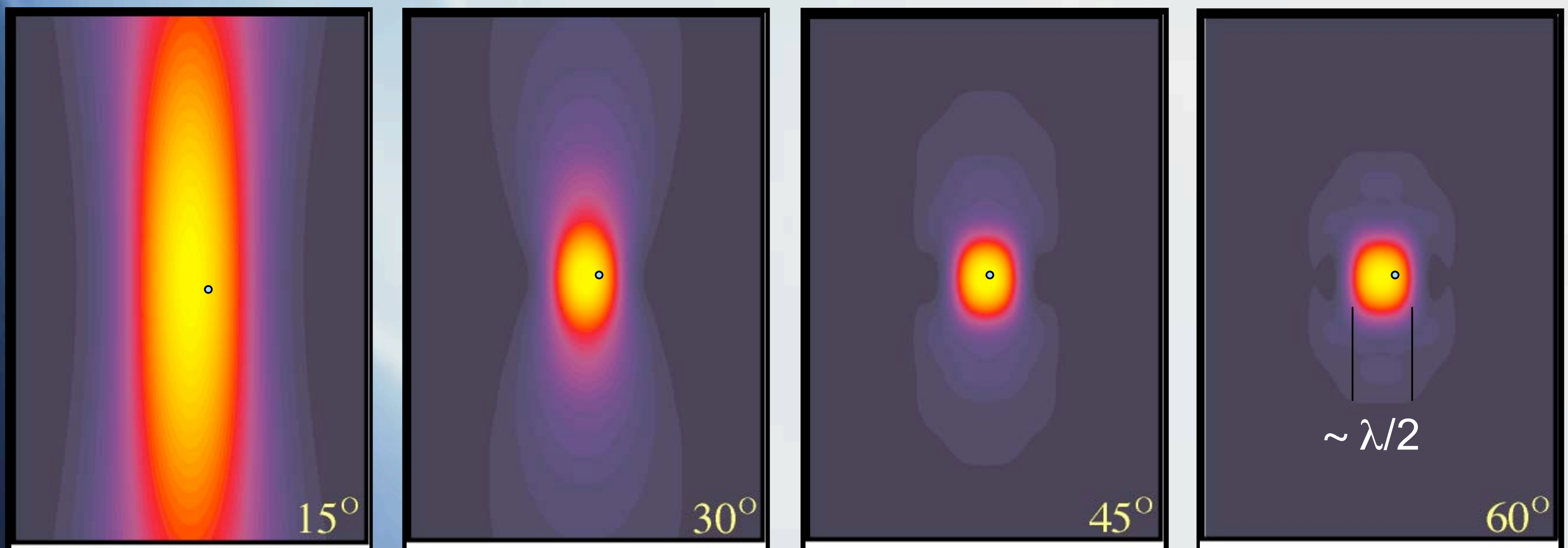


Excitation rate : $\sim |\boldsymbol{\mu} \cdot \mathbf{E}|^2$

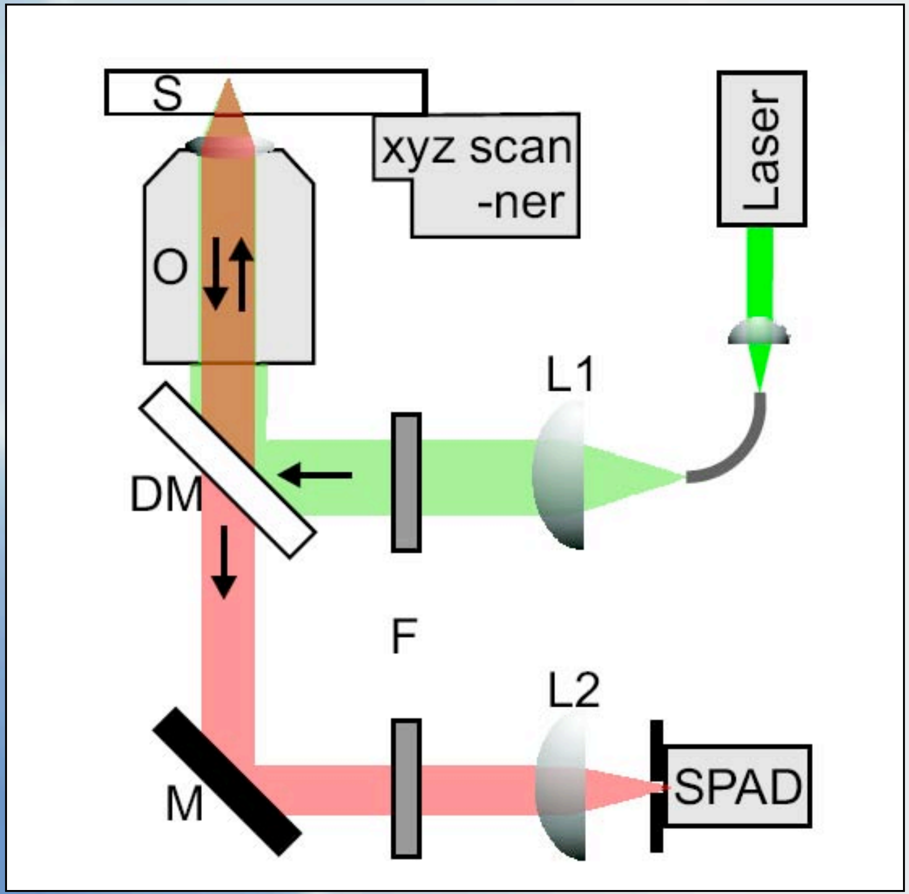
EXCITATION/DETECTION OF SINGLE MOLECULES



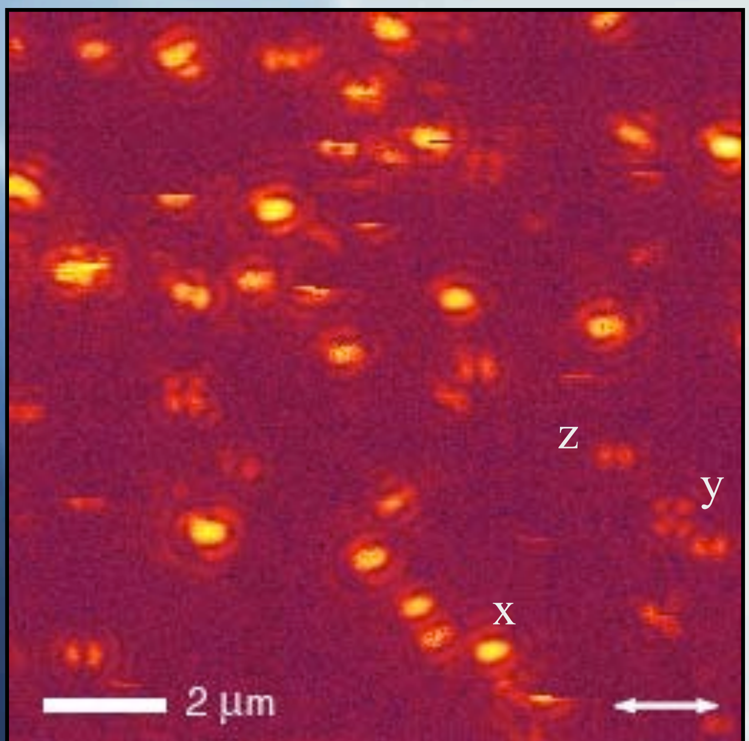
REDUCING INTERACTION VOLUME



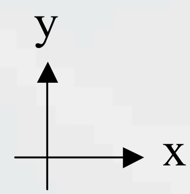
SINGLE MOLECULE DETECTION



EXAMPLE



Nile Blue molecules



fluorescence rate ~ excitation rate

$$\text{contrast} \sim |\,\boldsymbol{\mu}\cdot\mathbf{E}(x,y;z_o)|^2$$

FIELDS NEAR A STRONGLY FOCUSED BEAM ?!

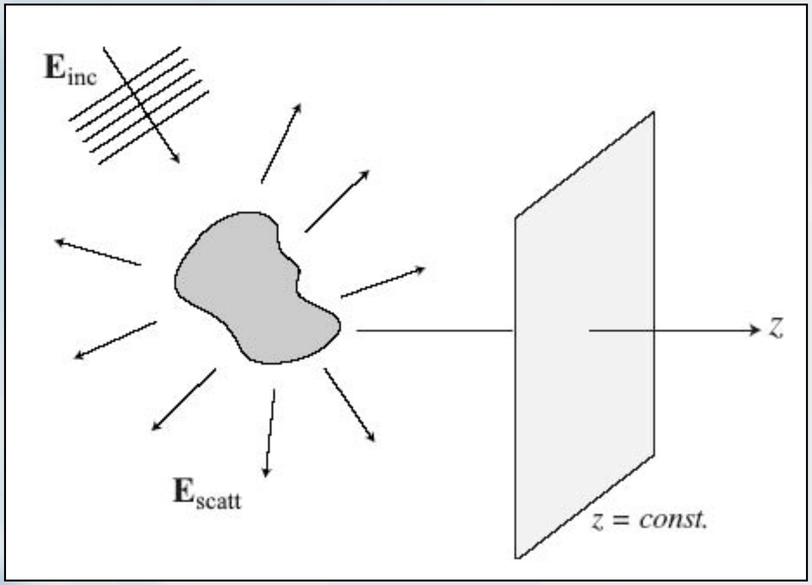
PLANE WAVES

$$\mathbf{E}(x) = \mathbf{E}_o\, e^{i\, k_x x} \quad \longrightarrow \quad \mathbf{E}(x,t) = \mathrm{Re}\left\{\mathbf{E}(x)\, e^{-i\,\omega t}\right\}$$

$$\mathbf{E}(x,y,z) = \mathbf{E}_o\, e^{i\,(k_x x + k_y y + k_z z)} = \mathbf{E}_o\, e^{i\,\mathbf{k}\cdot\mathbf{r}}$$

$$k^2 = \frac{\omega^2}{c^2} = k_x^2 + k_y^2 + k_z^2$$!

ANGULAR SPECTRUM REPRESENTATION



$$\hat{\mathbf{E}}(k_x, k_y; z) = \frac{1}{4\pi^2} \iint_{-\infty}^{\infty} \mathbf{E}(x, y, z)\, e^{-i\,[k_x x + k_y y]}\, dx\, dy$$

$$\mathbf{E}(x, y, z) = \iint_{-\infty}^{\infty} \hat{\mathbf{E}}(k_x, k_y; z)\, e^{i\,[k_x x + k_y y]}\, dk_x\, dk_y$$

$$(\nabla^2 + k^2)\, \mathbf{E}(\mathbf{r}) = 0$$

!

ANGULAR SPECTRUM REPRESENTATION

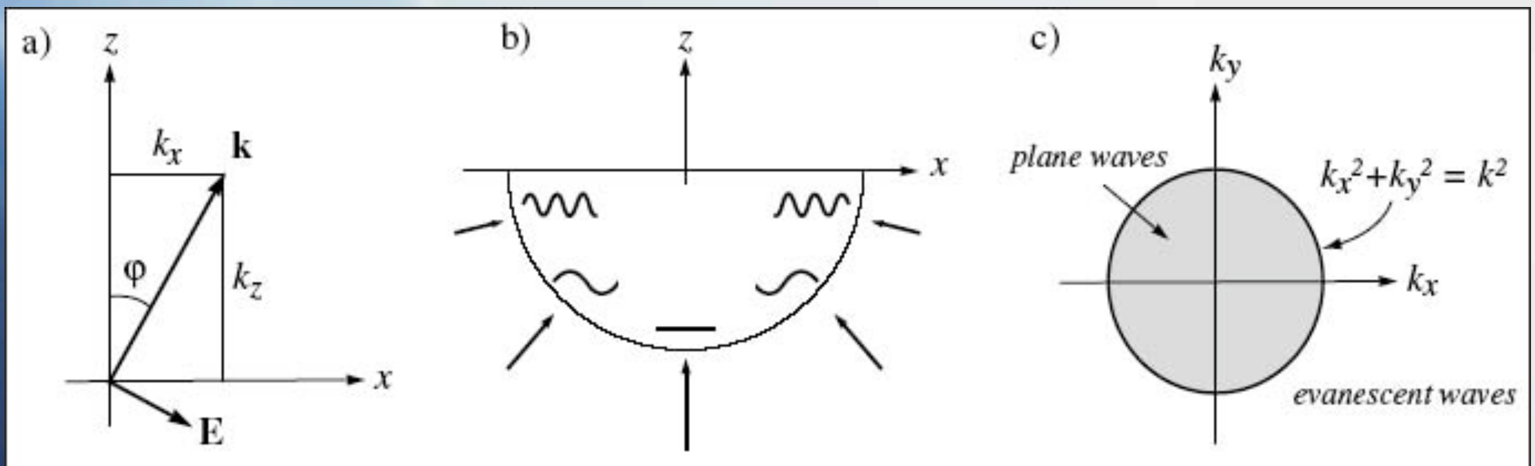
$$\hat{\mathbf{E}}(k_x, k_y;\, z) \;=\; \hat{\mathbf{E}}(k_x, k_y;\, 0)\; \mathrm{e}^{\pm i\, k_z\, z}$$

$$k_z \;\equiv\; \sqrt{(k^2 - k_x^2 - k_y^2)} \qquad with \quad Im\{k_z\} \geq 0$$

$$\mathbf{E}(x, y, z) \;=\; \iint_{-\infty}^{\infty} \hat{\mathbf{E}}(k_x, k_y\,;\, 0)\; \mathrm{e}^{i\,[k_x\, x \,+\, k_y\, y \,\pm\, k_z\, z]}\; dk_x\, dk_y$$

$$\begin{array}{rll} \text{Plane waves}: & \mathrm{e}^{i\,[k_x\,x\,+\,k_y\,y]}\;\mathrm{e}^{\pm i\,|k_z|\,z}, & k_x^2 + k_y^2 \leq k^2 \\ \text{Evanescent waves}: & \mathrm{e}^{i\,[k_x\,x\,+\,k_y\,y]}\;\mathrm{e}^{-|k_z|\,|z|}, & k_x^2 + k_y^2 > k^2 \end{array}$$

ANGULAR SPECTRUM REPRESENTATION



PARAXIAL APPROXIMATION / GAUSSIAN BEAMS

$$k_z = k\sqrt{1-(k_x^2+k_y^2)/k^2} \approx k - \frac{(k_x^2+k_y^2)}{2\,k}$$

$$\mathbf{E}(x',y',0) = \mathbf{E}_o\,\mathrm{e}^{-\frac{x'^2+y'^2}{w_o^2}} \longrightarrow \hat{\mathbf{E}}(k_x,k_y;0) = \mathbf{E}_o\,\frac{w_o^2}{4\pi}\,\mathrm{e}^{-(k_x^2+k_y^2)\,\frac{w_o^2}{4}}$$

$$\mathbf{E}(x,y,z) = \mathbf{E}_o\,\frac{w_o^2}{4\,\pi}\,\mathrm{e}^{i\,k\,z}\iint\limits_{-\infty}^{\infty}\mathrm{e}^{-(k_x^2+k_y^2)\,(\frac{w_o^2}{4}+\frac{i\,z}{2\,k})}\,\mathrm{e}^{i\,[k_x\,x\,+\,k_y\,y]}\,dk_x\,dk_y$$

PARAXIAL APPROXIMATION / GAUSSIAN BEAMS

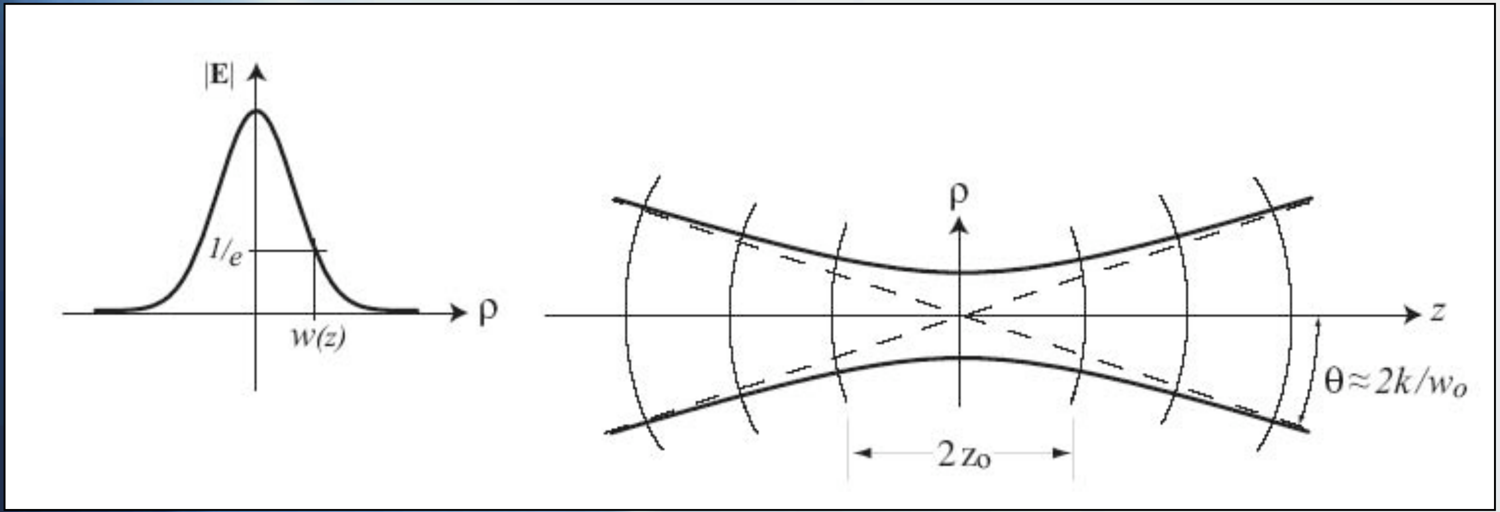
$$\mathbf{E}(\rho, z) = \mathbf{E}_o \frac{w_o}{w(z)} \, e^{-\frac{\rho^2}{w^2(z)}} \, e^{i[kz - \eta(z) + k\rho^2/2R(z)]}$$

$$z_o = \frac{k\, w_o^2}{2}$$

$$w(z) = w_o \,(1 + z^2/z_o^2)^{1/2} \qquad \textit{beam waist}$$

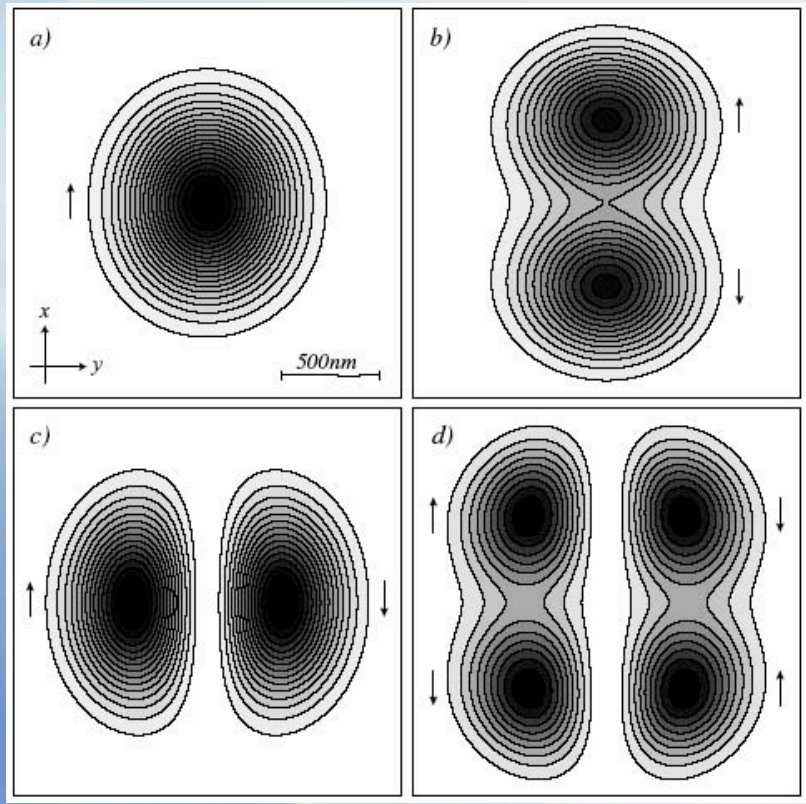
$$R(z) = z\,(1 + z_o^2/z^2) \qquad \textit{wavefront radius}$$

$$\eta(z) = \arctan z/z_o \qquad \textit{phase correction}$$



HIGHER-ORDER PARAXIAL BEAMS

$$\mathbf{E}^{H}_{nm}(x,y,z) = w_o^{n+m}\frac{\partial^n}{\partial x^n}\frac{\partial^m}{\partial y^m}\mathbf{E}(x,y,z)$$



FARFIELDS OF THE ANGULAR SPECTRUM

$$\mathbf{E}_\infty(s_x, s_y, s_z) = \lim_{kr\to\infty} \iint_{(k_x^2+k_y^2)\le k^2} \hat{\mathbf{E}}(k_x, k_y; 0)\; \mathrm{e}^{i\,kr\,[\frac{k_x}{k}s_x + \frac{k_y}{k}s_y \pm \frac{k_z}{k}s_z]}\, dk_x\, dk_y$$

$$\mathbf{s} = (s_x, s_y, s_z) = (\frac{x}{r}, \frac{y}{r}, \frac{z}{r})$$

Method of stationary phase:

$$\mathbf{E}_\infty(s_x, s_y, s_z) = -2\pi i k\, s_z\, \hat{\mathbf{E}}(ks_x, ks_y; 0)\, \frac{\mathrm{e}^{i\,kr}}{r}$$

$$\mathbf{s} = (s_x, s_y, s_z) = (\frac{k_x}{k}, \frac{k_y}{k}, \frac{k_z}{k})$$

Example:

$$\begin{aligned}\hat{\mathbf{E}}(k_x, k_y; 0) &= \frac{\mathbf{E}_o}{4\pi^2}\int_{-L_y}^{+L_y}\int_{-L_x}^{+L_x} \mathrm{e}^{-i\,[k_x x' + k_y y']}\, dx'\, dy' \\ &= \mathbf{E}_o\,\frac{L_x L_y}{\pi^2}\,\frac{\sin(k_x L_x)}{k_x L_x}\,\frac{\sin(k_y L_y)}{k_y L_y},\end{aligned}$$

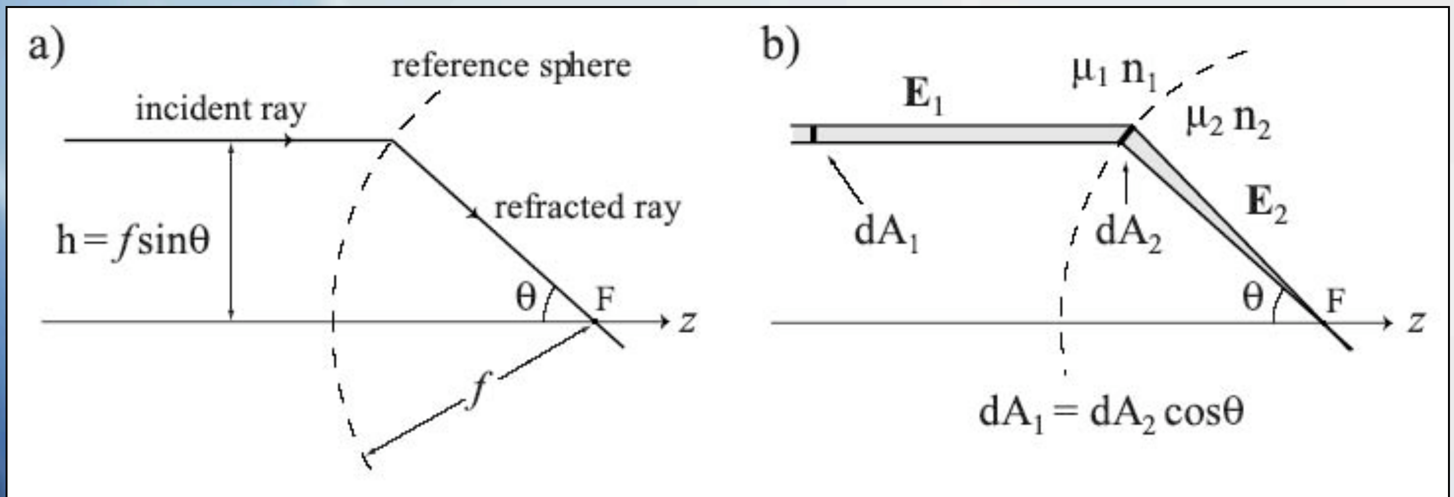
ANGULAR SPECTRUM IN TERMS OF FARFIELD

$$\hat{\mathbf{E}}(k_x, k_y; 0) = \frac{i r\, \mathrm{e}^{-i k r}}{2\pi k_z}\, \mathbf{E}_\infty(k_x, k_y)$$

$$\mathbf{E}(x, y, z) = \frac{i r\, \mathrm{e}^{-i k r}}{2\pi} \iint_{(k_x^2 + k_y^2) \le k^2} \mathbf{E}_\infty(k_x, k_y)\ \mathrm{e}^{i\,[k_x x + k_y y \pm k_z z]}\ \frac{1}{k_z}\, dk_x\, dk_y$$

For $k_z \sim k$: Fourier Optics !!

REFRACTION AT LENS



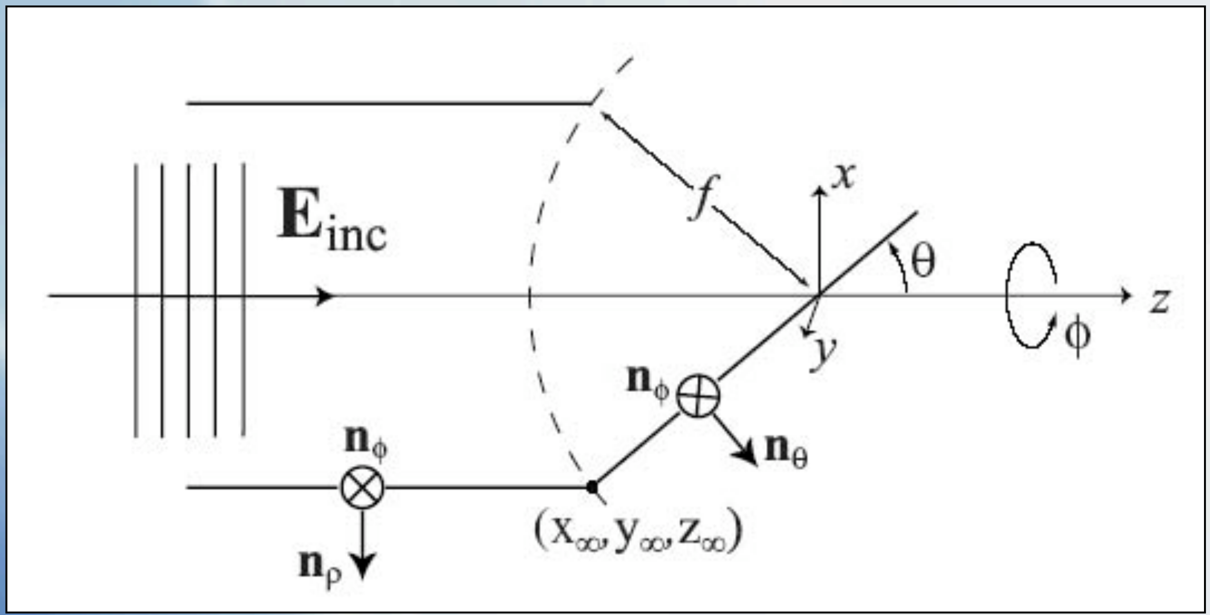
Sine Condition
(aplanatic system)

$$h = f\sin(\theta)$$

Ray Continuity
(energy conservation)

$$|\mathbf{E}_2| = |\mathbf{E}_1|\sqrt{\frac{n_1}{n_2}}\sqrt{\frac{\mu_2}{\mu_1}}\cos^{1/2}\theta$$

FOCUSING OF FIELDS



$$\mathbf{E}_\infty = \left[t^s \left[\mathbf{E}_{inc} \cdot \mathbf{n}_\phi \right] \mathbf{n}_\phi + t^p \left[\mathbf{E}_{inc} \cdot \mathbf{n}_\rho \right] \mathbf{n}_\theta \right] \sqrt{\frac{n_1}{n_2}} (\cos\theta)^{1/2}$$

EXPRESS IN TERMS OF ANGLES

$$\begin{aligned}\mathbf{n}_\rho &= \cos\phi\,\mathbf{n}_x + \sin\phi\,\mathbf{n}_y\ ,\\ \mathbf{n}_\phi &= -\sin\phi\,\mathbf{n}_x + \cos\phi\,\mathbf{n}_y\ ,\\ \mathbf{n}_\theta &= \cos\theta\,\cos\phi\,\mathbf{n}_x + \cos\theta\,\sin\phi\,\mathbf{n}_y - \sin\theta\,\mathbf{n}_z\end{aligned}$$

$$k_x = k\sin\theta\,\cos\phi, \quad k_y = k\sin\theta\,\sin\phi, \quad k_z = k\cos\theta$$

$$\frac{1}{k_z}\,dk_x\,dk_y = k\,\sin\theta\,d\theta\,d\phi$$

$$x = \rho\cos\varphi, \qquad y = \rho\sin\varphi$$

$$\mathbf{E}(\rho,\varphi,z) = \frac{i\,k\,f\,\mathrm{e}^{-i\,k\,f}}{2\pi}\int_0^{\theta_{max}}\int_0^{2\pi}\mathbf{E}_\infty(\theta,\phi)\,\mathrm{e}^{ikz\cos\theta}\,\mathrm{e}^{ik\rho\sin\theta\cos(\phi-\varphi)}\sin\theta\,d\phi\,d\theta$$

DEFINE INCIDENT FIELD

$$\mathbf{E}_{inc} = E_{inc}\,\mathbf{n}_x$$

$$t_\theta^s = t_\theta^p = 1$$

:

$$\mathbf{E}_\infty(\theta,\phi) = E_{inc}(\theta,\phi)\,[\cos\phi\,\mathbf{n}_\theta - \sin\phi\,\mathbf{n}_\phi]\,\sqrt{n_1/n_2}\,(\cos\theta)^{1/2}$$

$$= E_{inc}(\theta,\phi)\,\frac{1}{2}\begin{bmatrix}(1+\cos\theta) - (1-\cos\theta)\cos 2\phi \\ -(1-\cos\theta)\sin 2\phi \\ -2\cos\phi\,\sin\theta\end{bmatrix}\sqrt{\frac{n_1}{n_2}}\,(\cos\theta)^{1/2}$$

$(0,0)$ *mode* :

$$E_{inc} = E_o\,\mathrm{e}^{-(x_\infty^2+y_\infty^2)/w_o^2} = E_o\,\mathrm{e}^{-f^2\sin^2\theta/w_o^2}$$

INTEGRATE OVER ϕ

$$\int_0^{2\pi} \cos n\phi \; e^{ix\cos(\phi-\varphi)}\, d\phi = 2\pi(i^n)\, J_n(x)\, \cos n\varphi$$

$$\int_0^{2\pi} \sin n\phi \; e^{ix\cos(\phi-\varphi)}\, d\phi = 2\pi(i^n)\, J_n(x)\, \sin n\varphi\,.$$

SOLUTION

$(0,0)\ mode:$

$$\mathbf{E}(\rho,\varphi,z) = \frac{ikf}{2}\sqrt{\frac{n_1}{n_2}}\,E_o\,\mathrm{e}^{-ikf}\begin{bmatrix} I_{00}+I_{02}\cos 2\varphi \\ I_{02}\sin 2\varphi \\ -2i\,I_{01}\cos\varphi \end{bmatrix}$$

$$\mathbf{H}(\rho,\varphi,z) = \frac{ikf}{2Z_{\mu\varepsilon}}\sqrt{\frac{n_1}{n_2}}\,E_o\,\mathrm{e}^{-ikf}\begin{bmatrix} I_{02}\sin 2\varphi \\ I_{00}-I_{02}\cos 2\varphi \\ -2i\,I_{01}\sin\varphi \end{bmatrix}$$

$$f_w(\theta) = \mathrm{e}^{-\frac{1}{f_o^2}\frac{\sin^2\theta}{\sin^2\theta_{max}}}$$

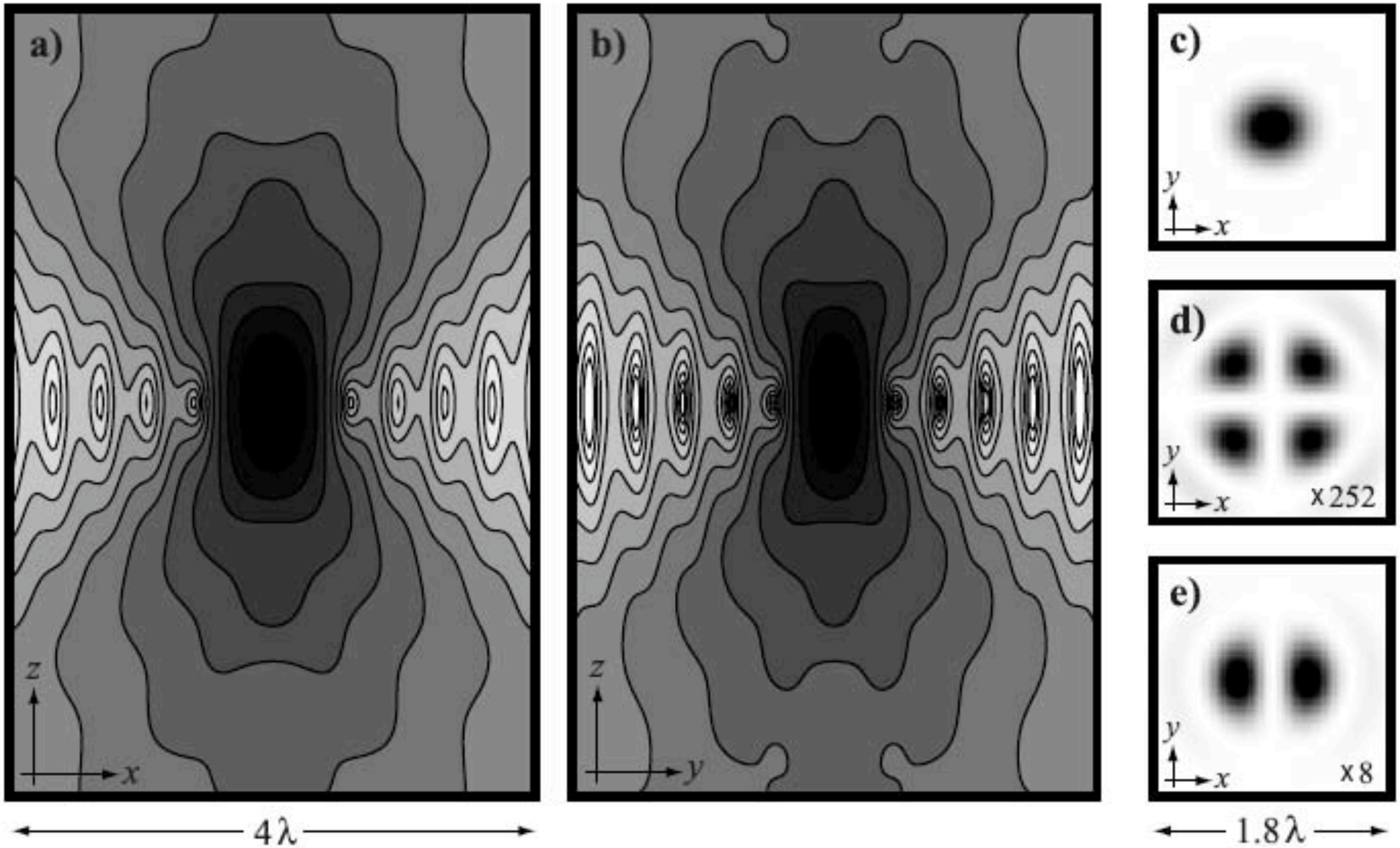
Fields given by 3 integrals :

$$I_{00} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin\theta\,(1+\cos\theta)\,J_0(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

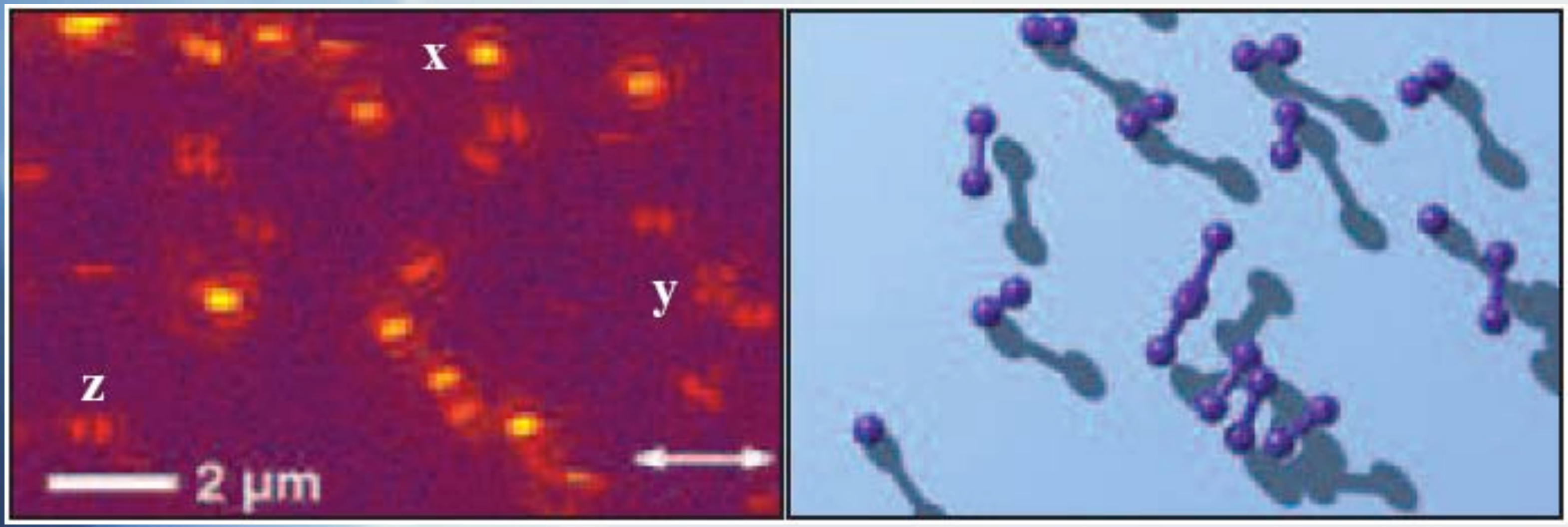
$$I_{01} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin^2\theta\,J_1(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

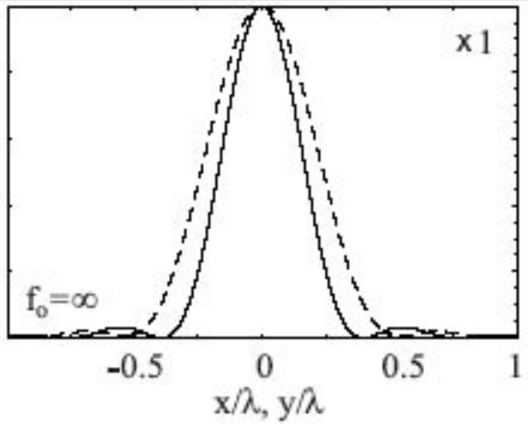
$$I_{02} = \int_0^{\theta_{max}} f_w(\theta)\,(\cos\theta)^{1/2}\sin\theta\,(1-\cos\theta)\,J_2(k\rho\sin\theta)\,\mathrm{e}^{ikz\cos\theta}\,d\theta$$

FOCAL FIELDS



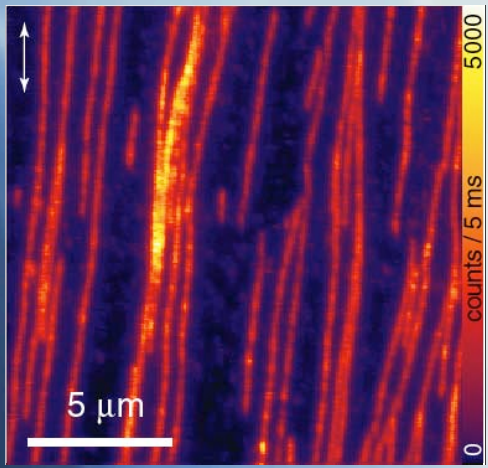
FOCAL FIELDS SAMPLED WITH SINGLE MOLECULES

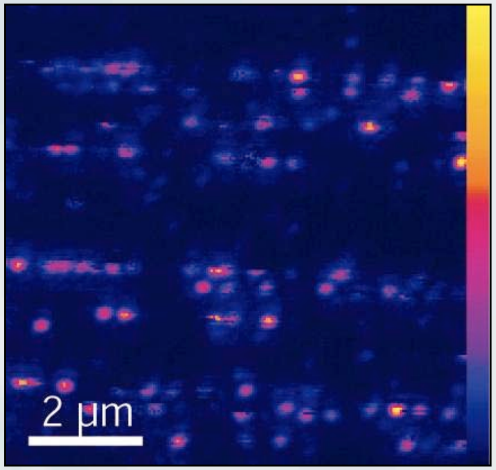


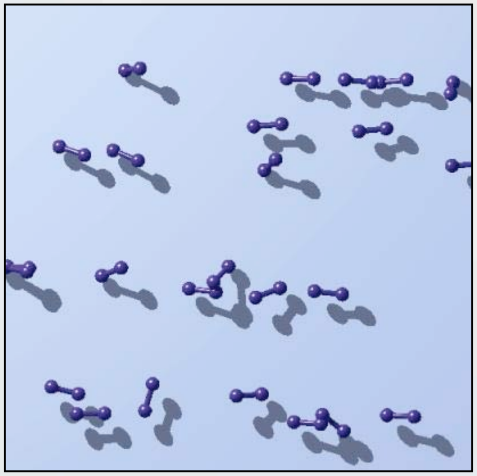


Elongated spot !!

λ-PHAGE DNA LABELED WITH YOYO-1







WEAKLY FOCUSED BEAMS

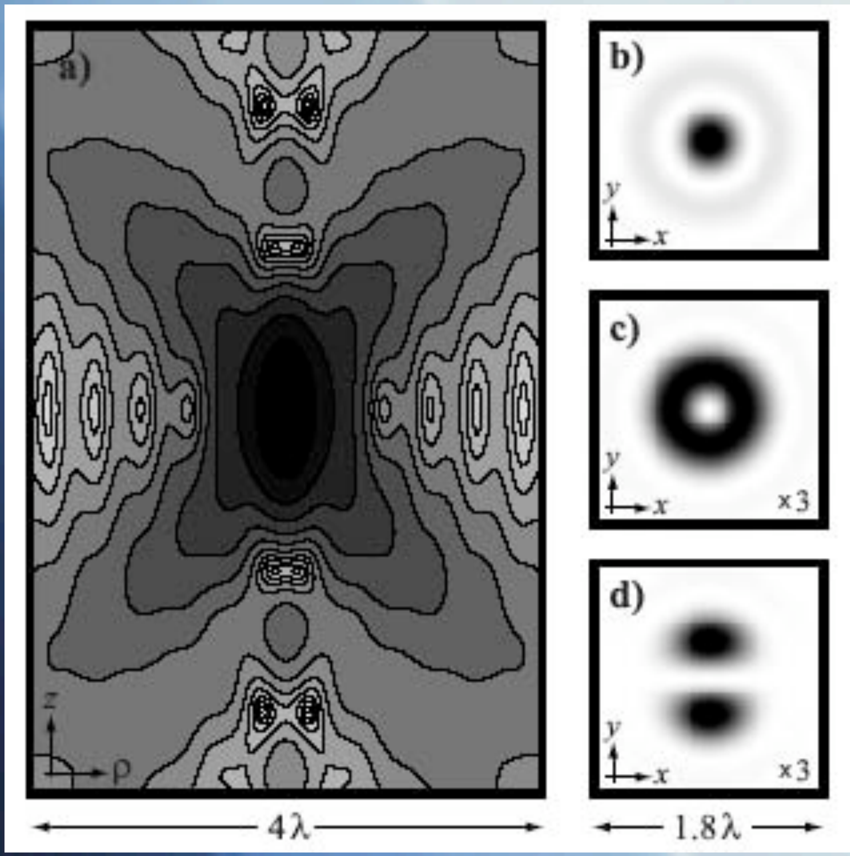
Focal plane (z=0):

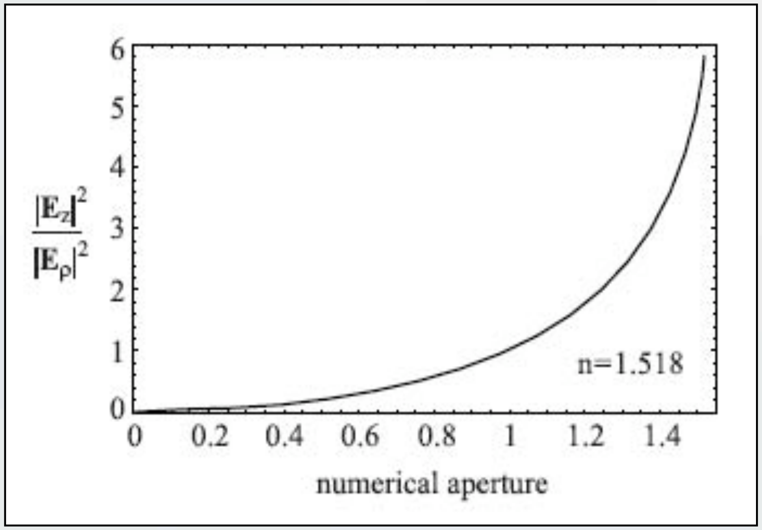
$$I_{00} \approx \frac{2}{k\rho} \int_0^{k\rho\theta_{max}} x\, J_0(x)\, dx = 2\theta_{max}^2 \frac{J_1(k\rho\theta_{max})}{k\rho\theta_{max}}$$

$$\mathbf{E} \approx i k f\, \theta_{max}^2\, E_o\, e^{-ikf}\, \frac{J_1(k\rho\theta_{max})}{k\rho\theta_{max}}\, \mathbf{n}_x$$

Not Gaussian !

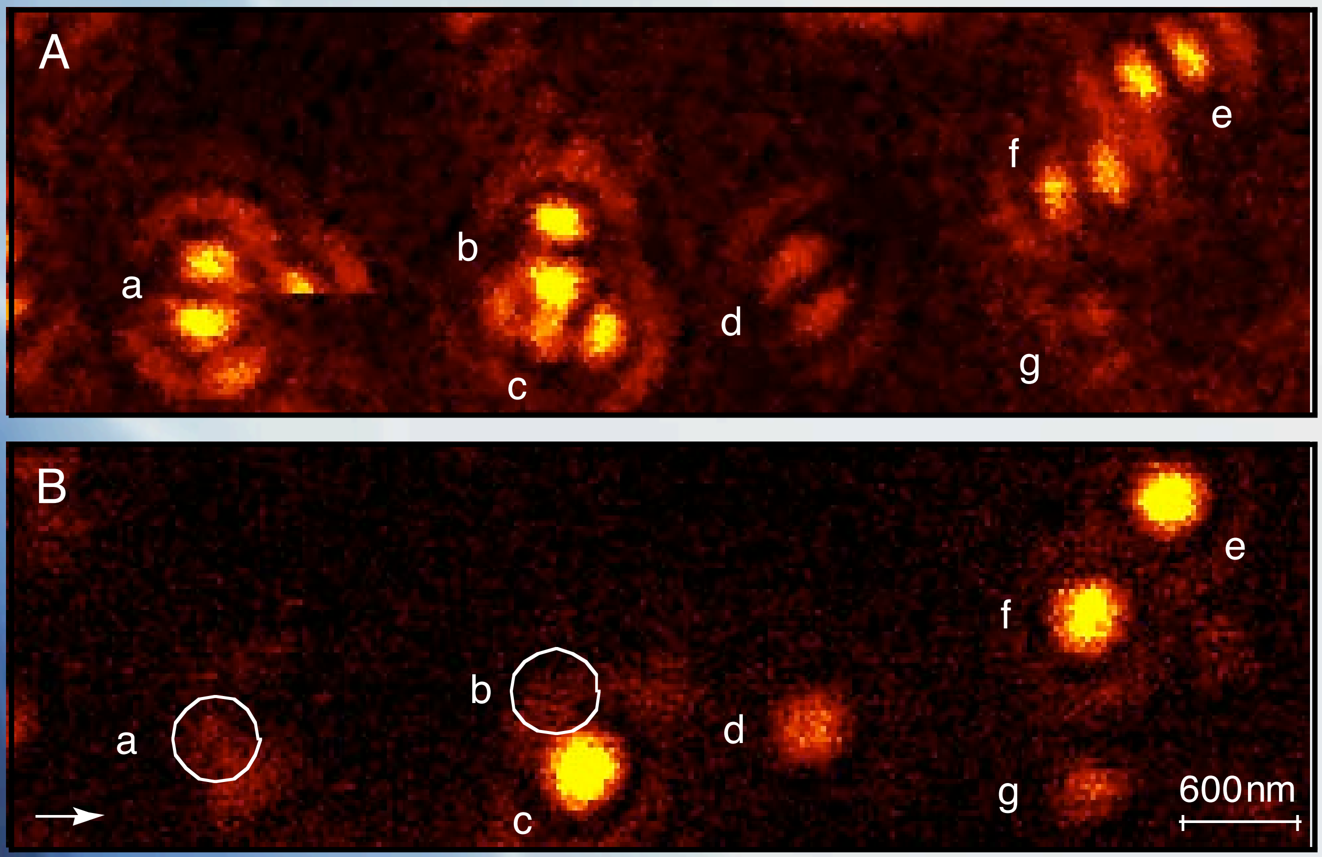
FOCUSED RADIALLY POLARIZED BEAMS



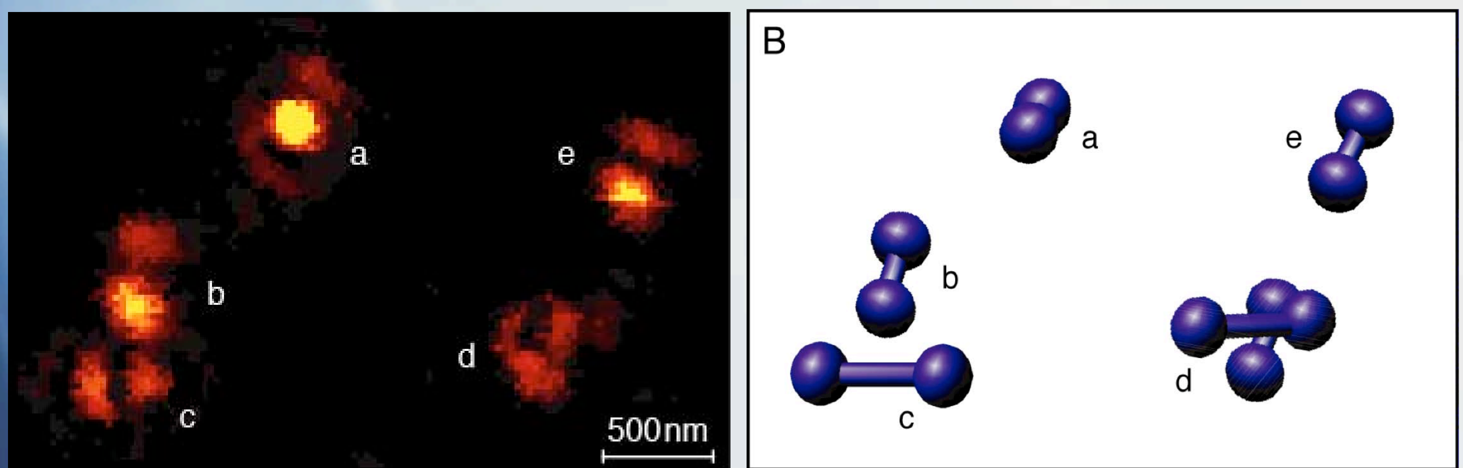


mostly longitudinal fields in focus !

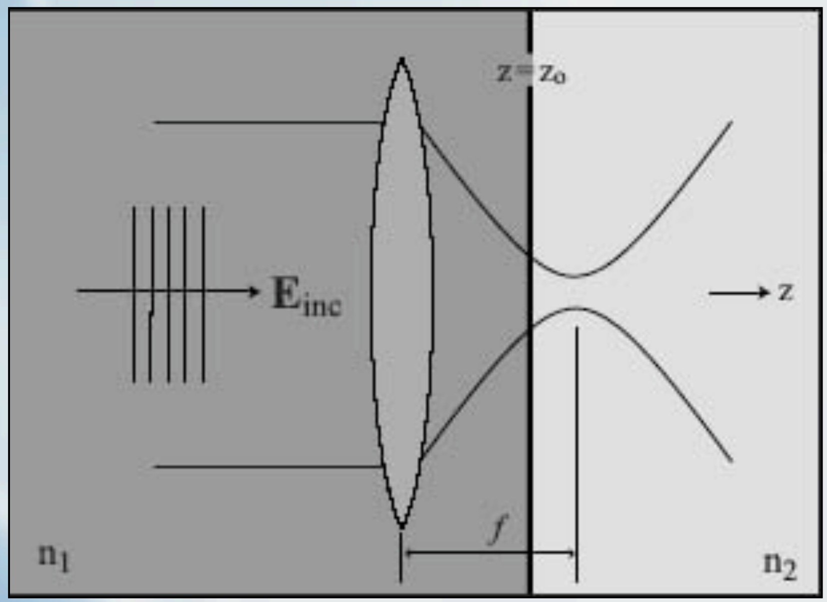
FOCUSED RADIALLY POLARIZED BEAMS



FOCUSED RADIALLY POLARIZED BEAMS

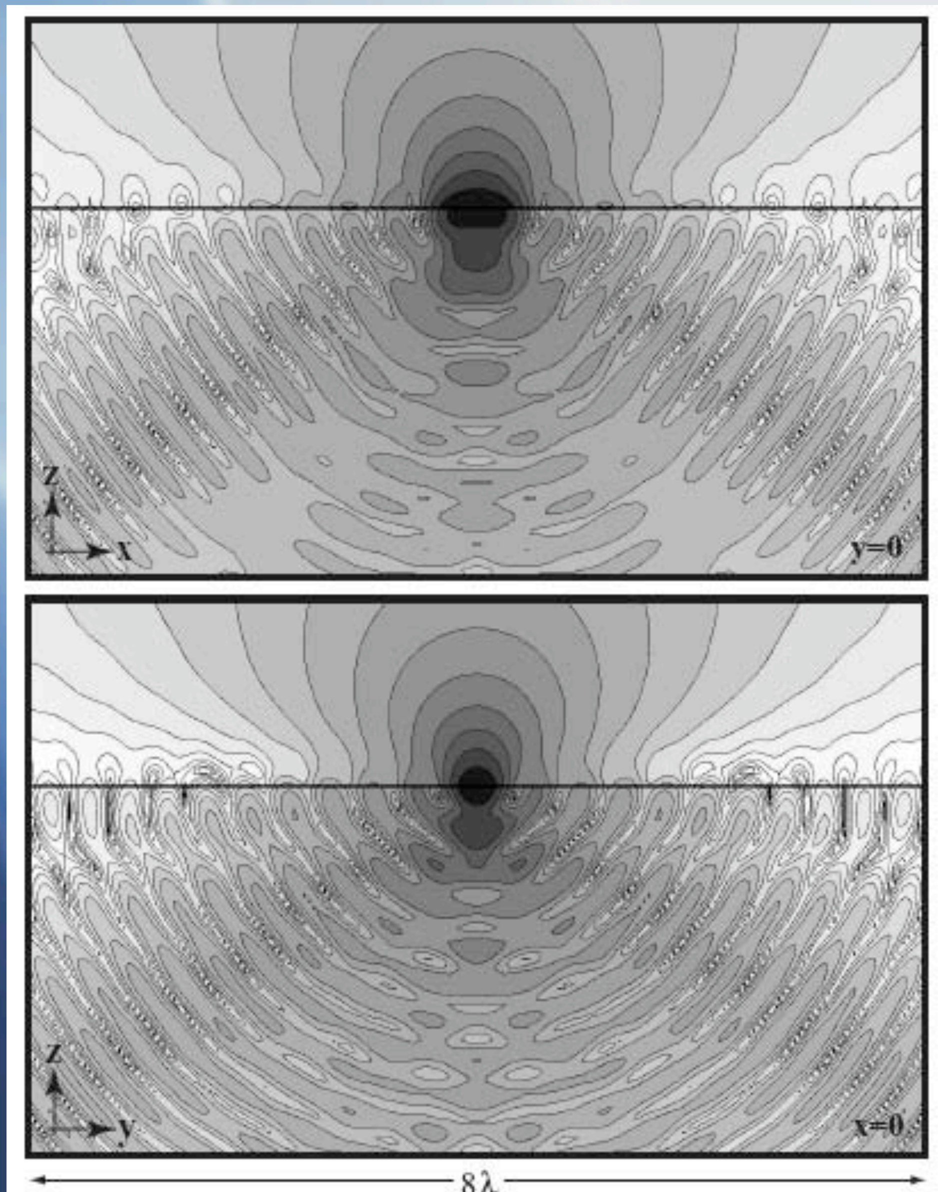


WHAT ABOUT INTERFACE ?



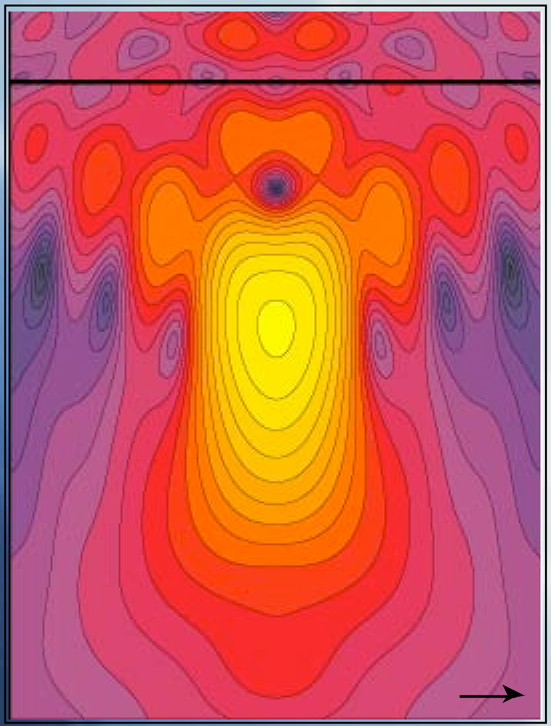
$$E = \begin{cases} E_f + E_r & : \quad z < z_o \\ E_t & : \quad z > z_o \end{cases}$$

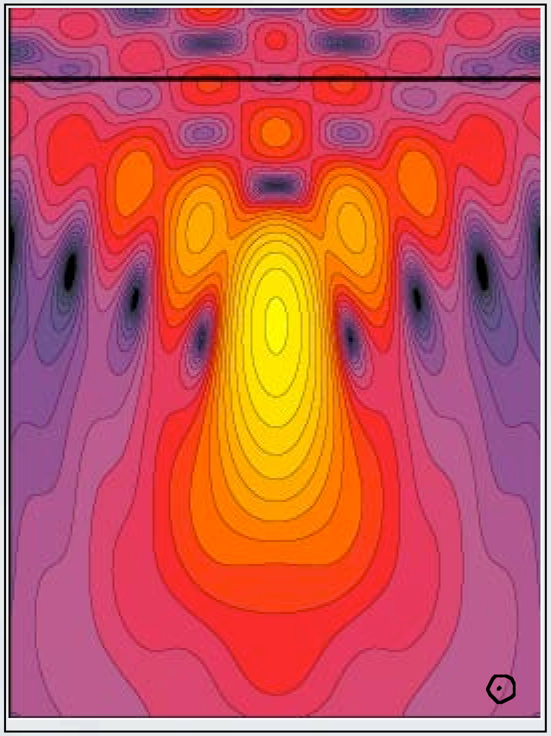
Angular spectrum representation with Fresnel reflection / transmission coefficients.



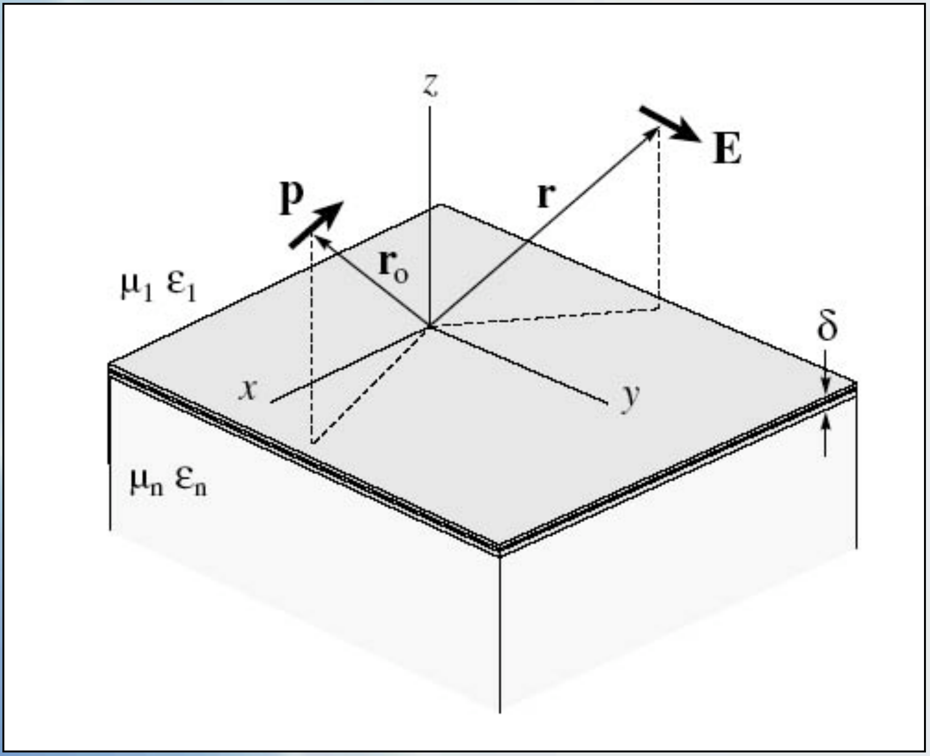
$$k_{z_2} = k_2 \sqrt{1 - \frac{k_1^2}{k_2^2} \sin^2 \theta}$$

EXAMPLE: LASER TWEEZERS



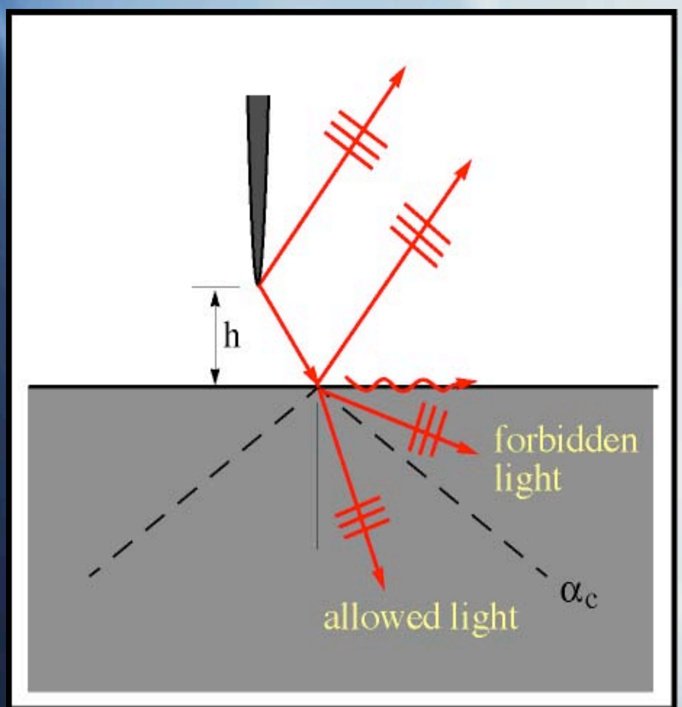


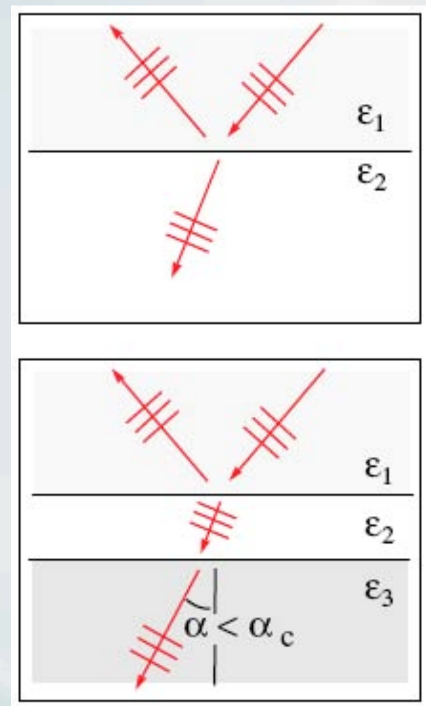
WHAT ABOUT EMISSION ?

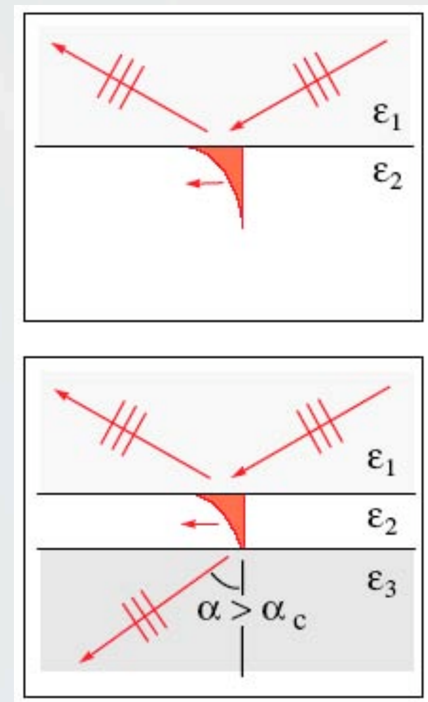


Molecules radiate like oscillating dipoles !!

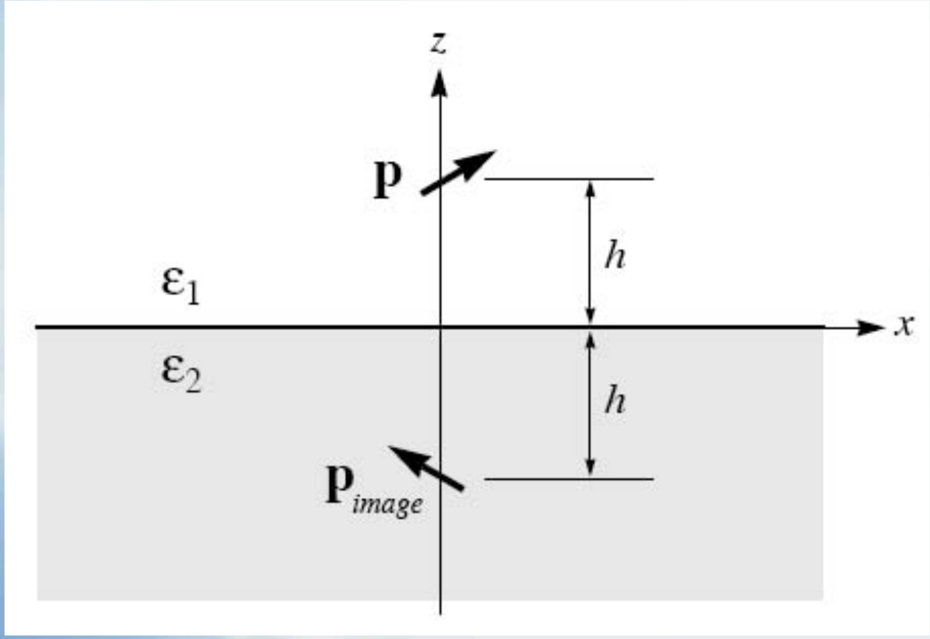
COUPLING OF EVANESCENT WAVES





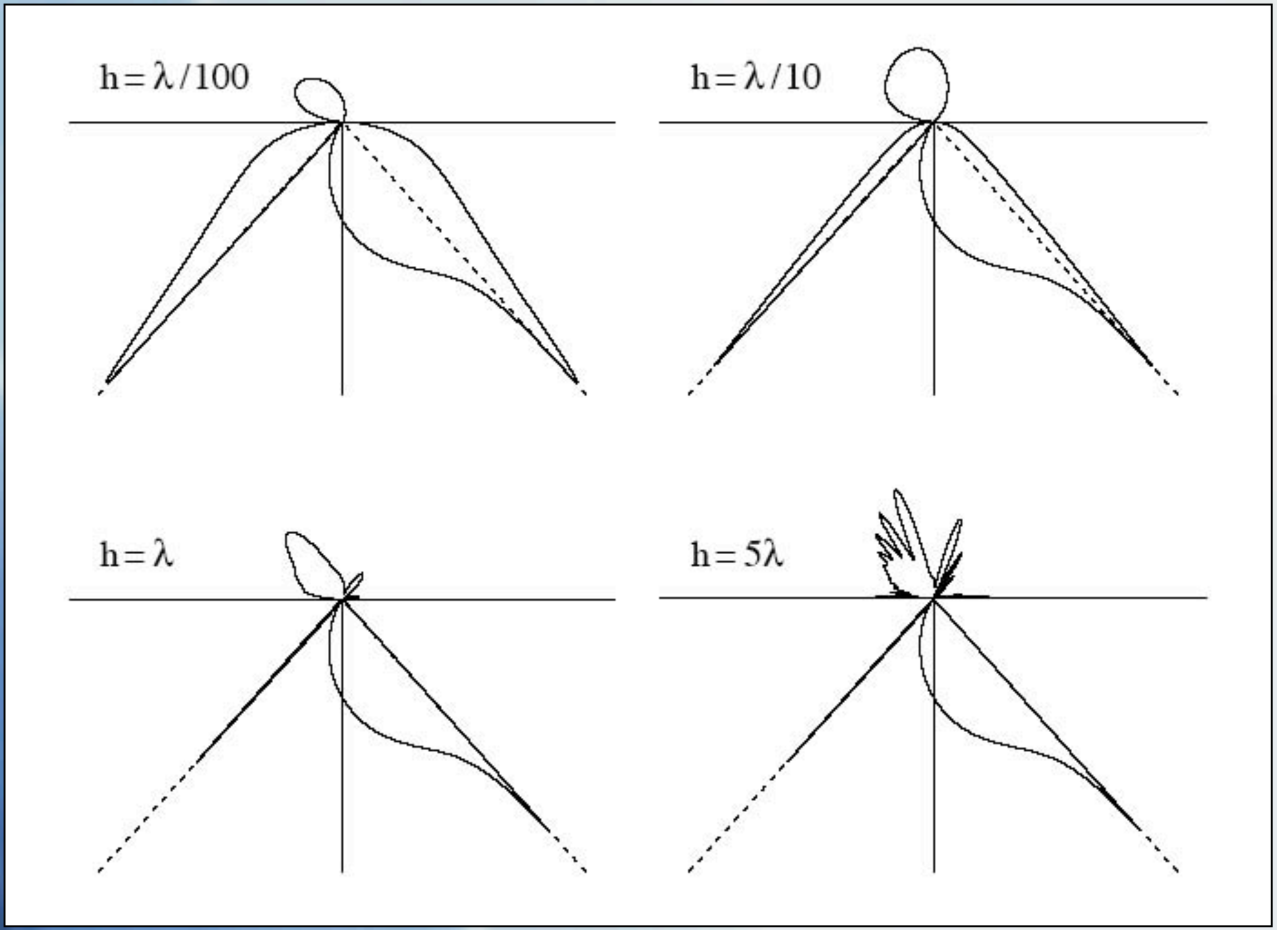


FARFIELD SOLUTION



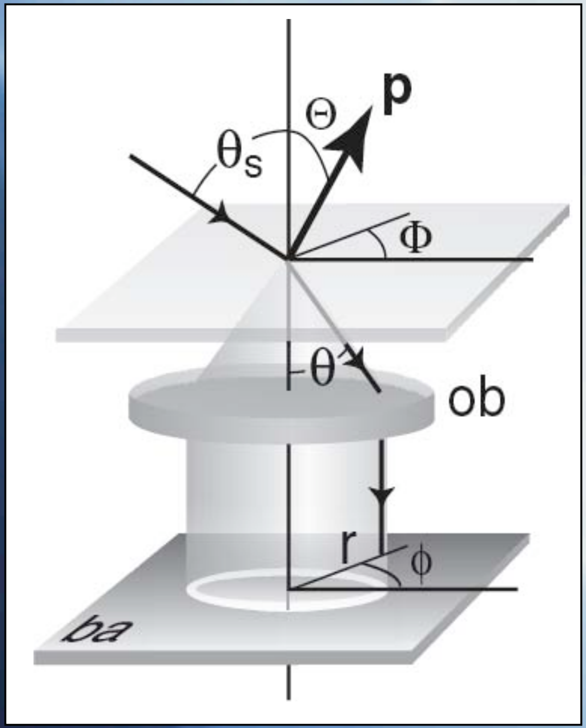
Exact solution = radiation from molecule and its image

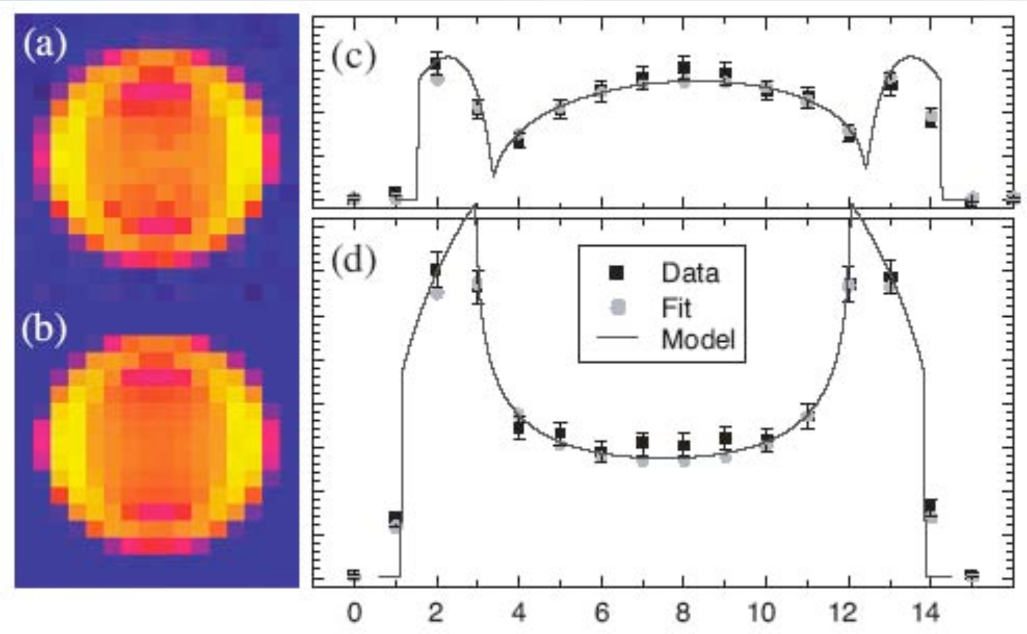
RADIATION PATTERNS



Need to sample the critical angle !!

DEMONSTRATION WITH SINGLE MOLECULES





JOSA B 21, 1210 (2004)

SUMMARY

BIOPHYSICAL PROCESSES ARE VISUALIZED USING SINGLE MOLECULES AS PROBES

EXCITATION OF SINGLE MOLECULES

- Angular Spectrum Representation
- Application to Strongly Focused Laser Beams
- Experimental Mapping using Single Molecules as Probe Dipoles

DETECTION OF SINGLE MOLECULES

- Molecule Radiates like Oscillating Dipole
- Interface Perturbs Radiation Pattern
- Collection Optics needs to include Critical Angle