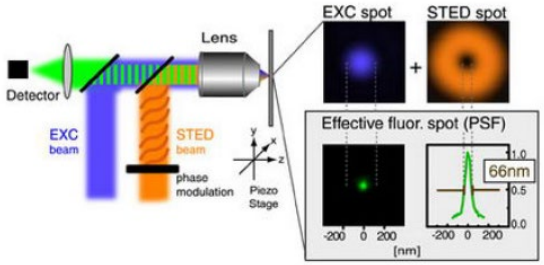
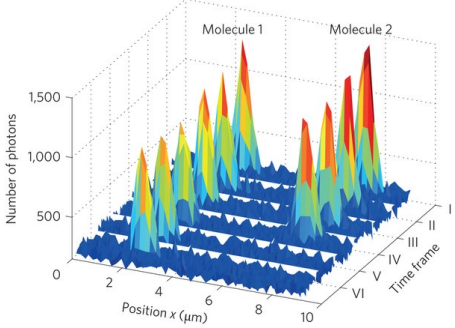
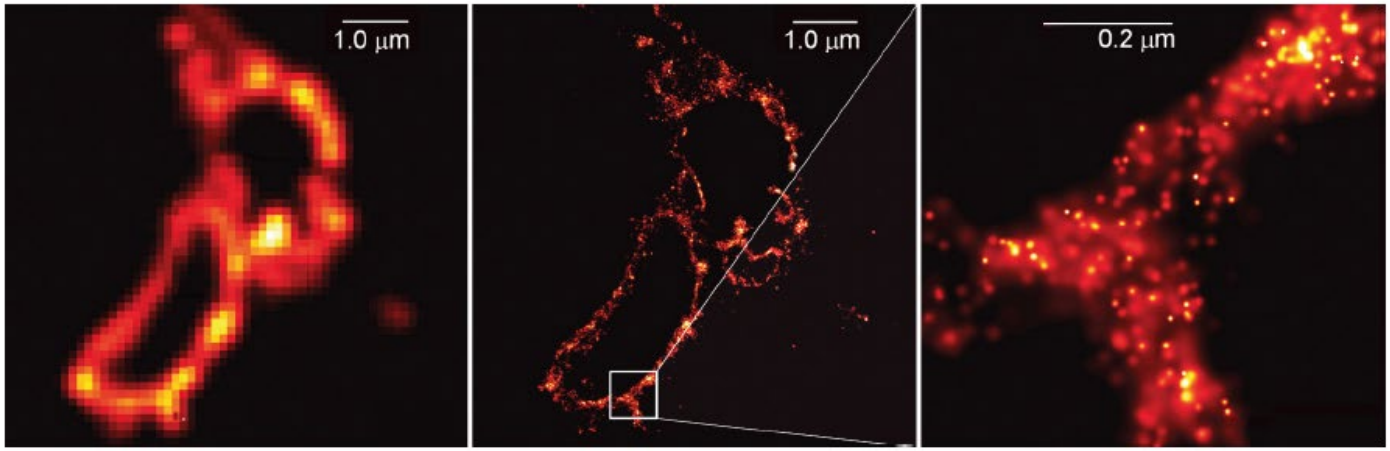




Nobel Prize in Chemistry 2014: development of super-resolved fluorescence microscopy



Winners 2014

Eric Betzig

Janelia Research Campus,
Howard Hughes Medical Institute,
Ashburn, VA, USA

Stefan Hell

Max-Planck Institute for Biophysical Chemistry, Gottingen, Germany;
German Cancer Research Center,
Heidelberg, Germany

W. E. Moerner

Stanford U, Palo Alto, CA, USA

See Nobel lectures detailing their discoveries (~30-35 min):

http://www.nobelprize.org/nobel_prizes/chemistry/laureates/2014/moerner-lecture.html

Optical Microscopy

The invention of the microscope was not sufficiently documented in its time to permit a definitive conclusion as regards date and inventor, but the first illustration of a recognizable microscope dates back to 1625. In the 17th century, "compound" microscopes consisting of a combination of lenses in two groups: objective (close to the object) and eyepiece (what you look through) were used by many observers, but the image quality was poor.

There was an alternative: the "simplex" microscope consisting of a single lens, like the magnifiers that are still in use, but of much higher power. This was the type of instrument used by Anthonie van Leeuwenhoek around 1700 and at the same magnification, this yielded a much better image than did the compound microscope then.

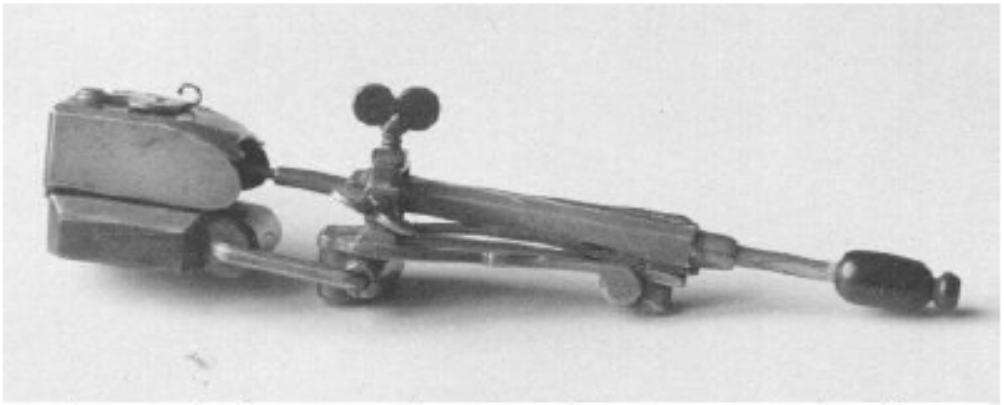
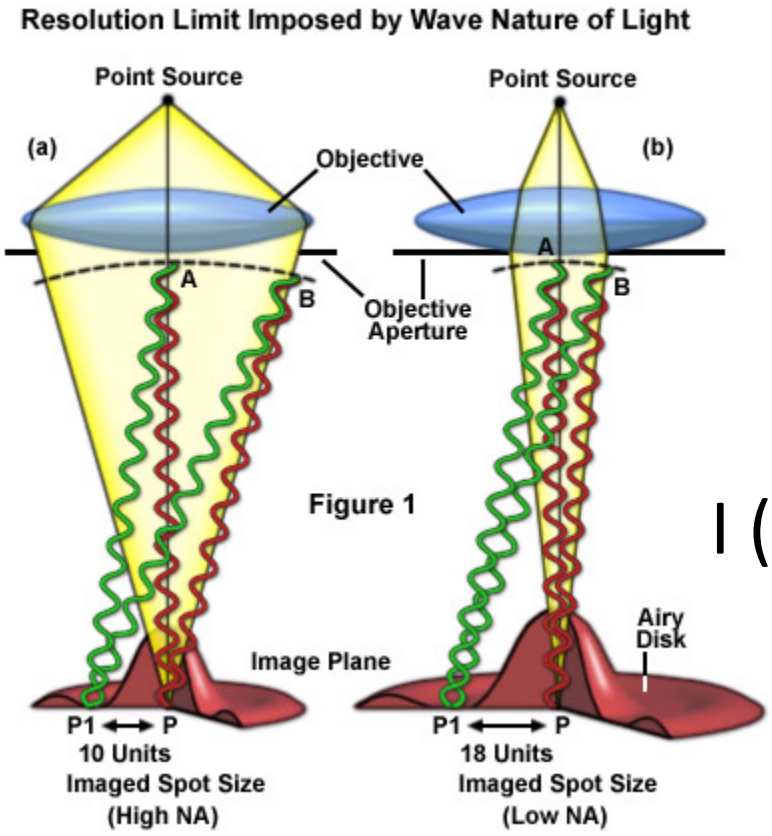
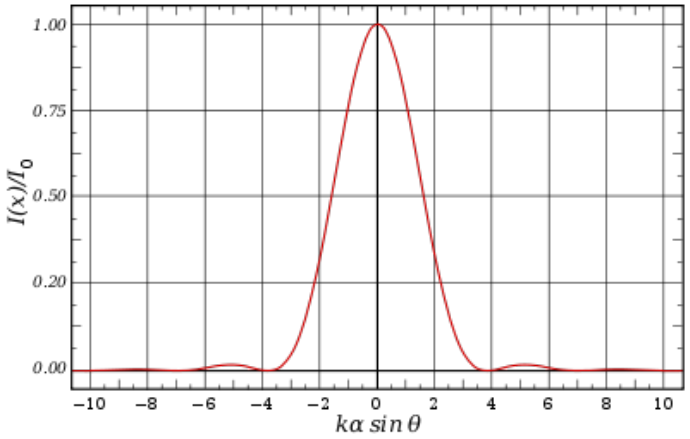


Fig. 6. "Simplex" type microscope by van Musschenbroek, late 17th century.

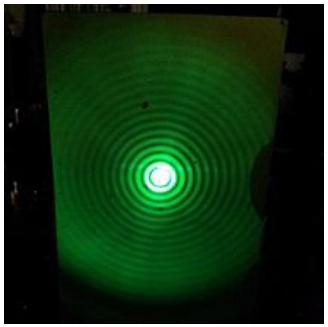
Compound microscope made by John Cuff in 1750

Recall diffraction on a circular aperture



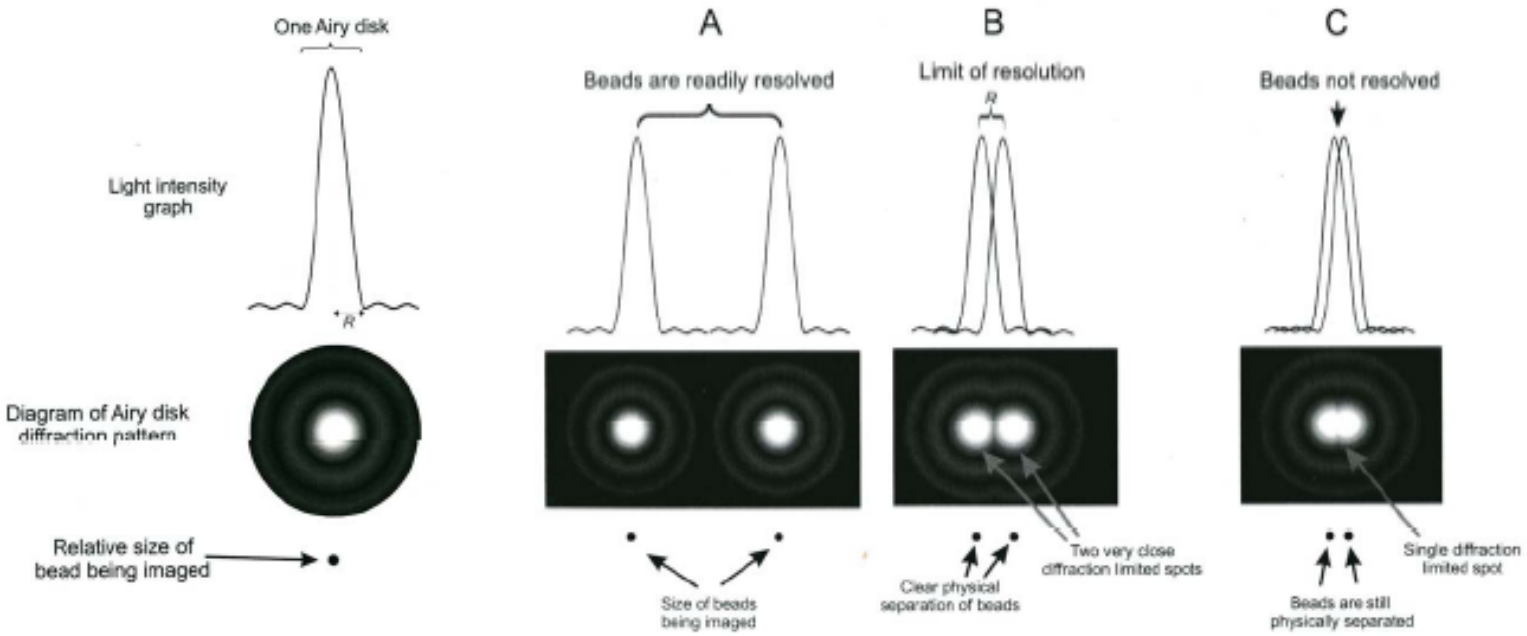


$$I(u) \sim [J_1(u)/u]^2$$



http://microscopyu.com/articles/superresolution/diffractionbarrier.html

Resolution limited by wavelength of light (diffraction)



$$R = \frac{1.22\lambda}{NA_{objective} + NA_{condenser}} = \frac{1.22\lambda}{2NA_{objective}}$$

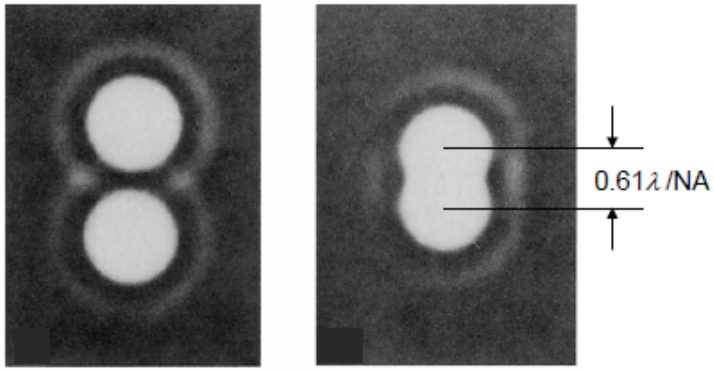
NA: numerical aperture

Numerical aperture and resolution

Rayleigh criterion:

resolution ~ 0.61λ /NA

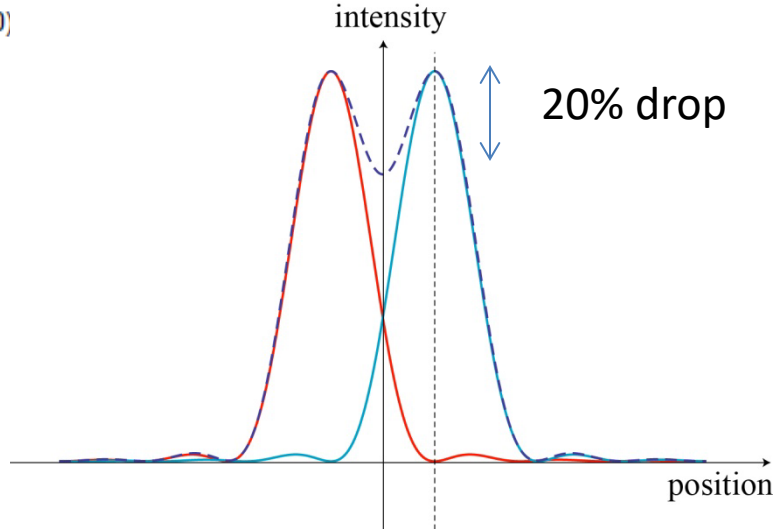
For dry samples, NA < 1.0



clearly resolved resolution limit

Ref: M. Born and E.Wolf, *Principles of Optics*, 6th ed. (Pergamon, Oxford, 1980)

Rayleigh criterion:



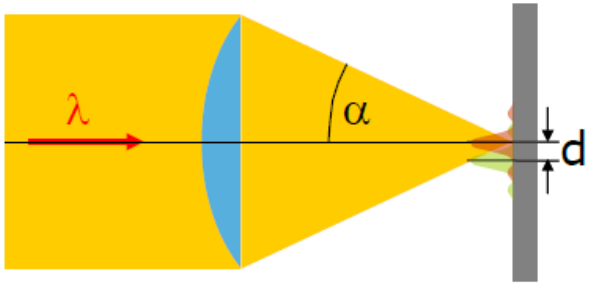
"lateral" resolution

Resolution

Ernst Abbe
(1872)

diffraction limit

structures smaller than half a wavelength cannot be resolved.



$$d_{min} \approx 0.61 \frac{\lambda}{n \sin \alpha}$$

wavelength (λ)

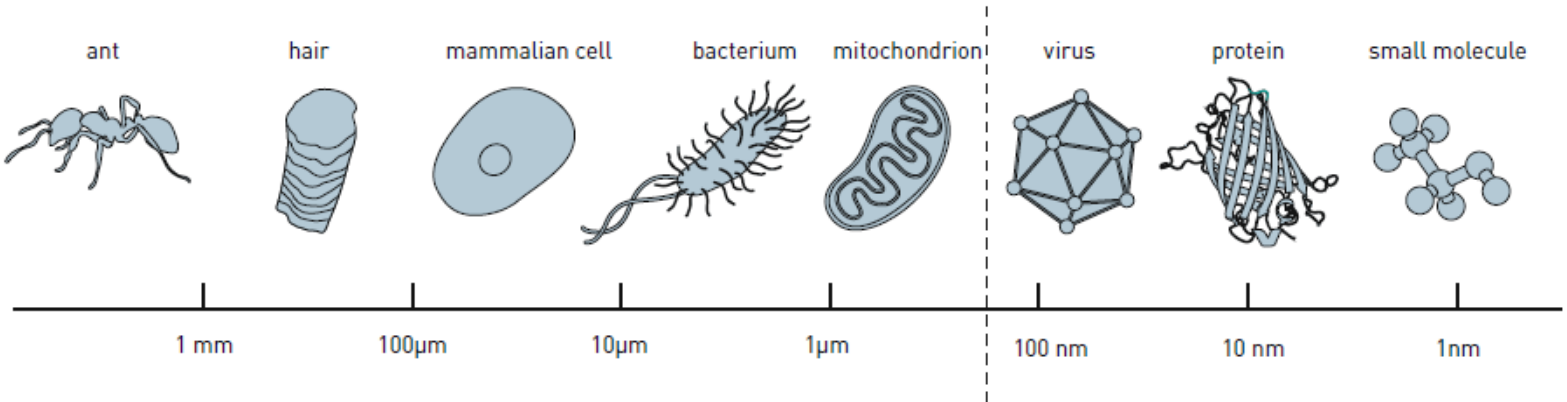
refractive index (n)

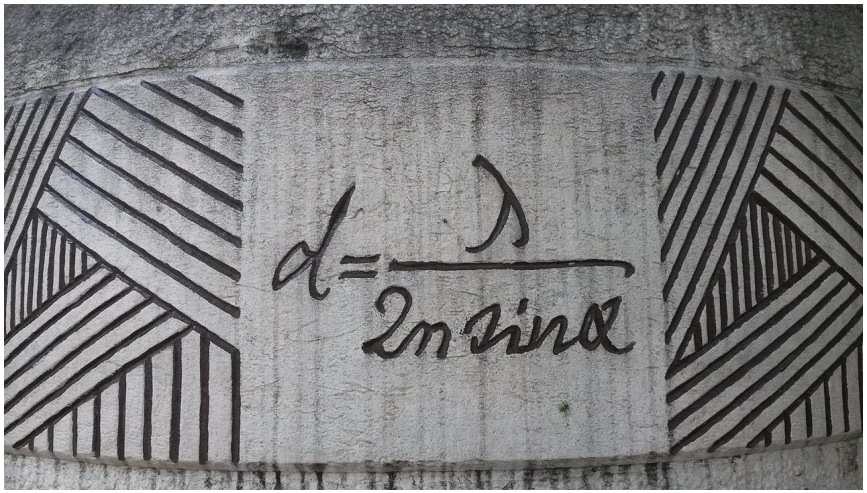
aperture angle (α)

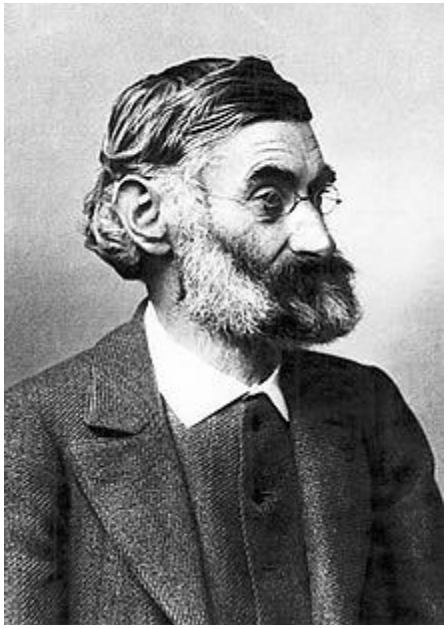
numerical aperture (NA) ($n \sin \alpha$)

source	λ	d_{min}
light	~ 500 nm	~ 250 nm
X-ray	~ 2 nm	~ 25 nm
electron	~ 0.001 nm	~ 0.1 nm (>2 nm)

(size of a cell ~ 10 µm)







Ernst Karl Abbe (1840-1905)

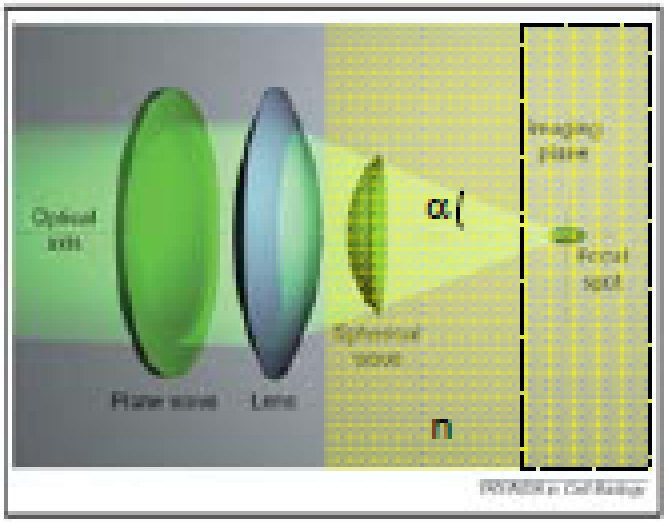
THE (1st) FOCUSING PROBLEM

- Focusing of light results in asymmetric focal spot
- Axial spot is longer than lateral
- For aberration-free lens

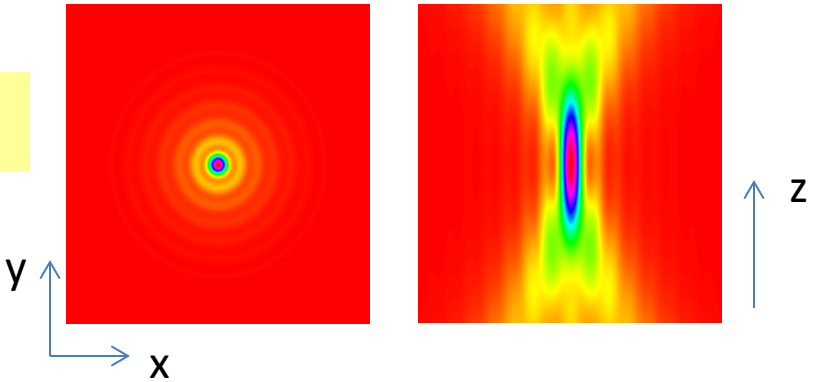
$$d_{lateral} = \frac{0.61}{NA}\lambda$$

$$d_{axial} = \frac{2n}{(NA)^2}\lambda$$

ex: λ=500 nm, NA =1.4, n =1.51
$d_{lateral}$ = 200 nm; d_{axial} = 770 nm

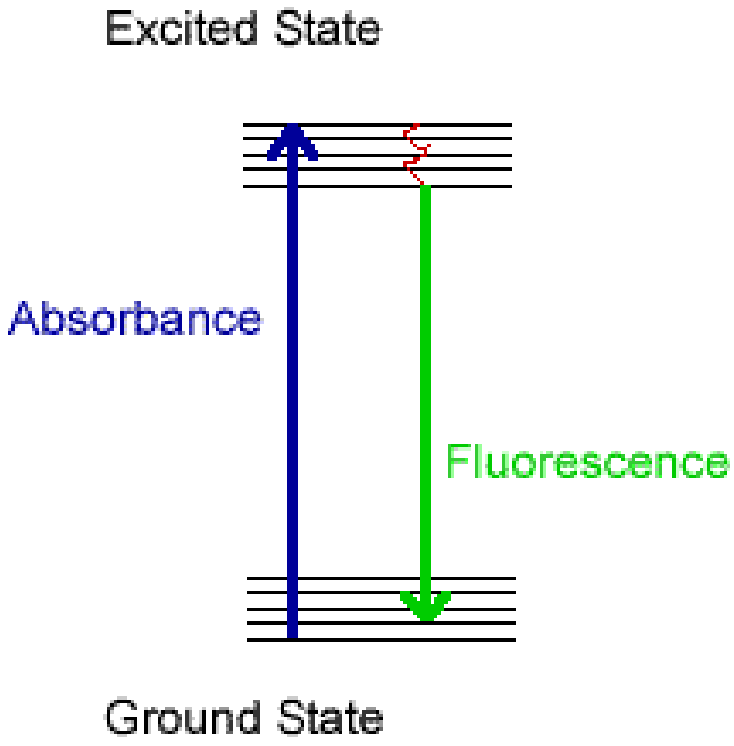


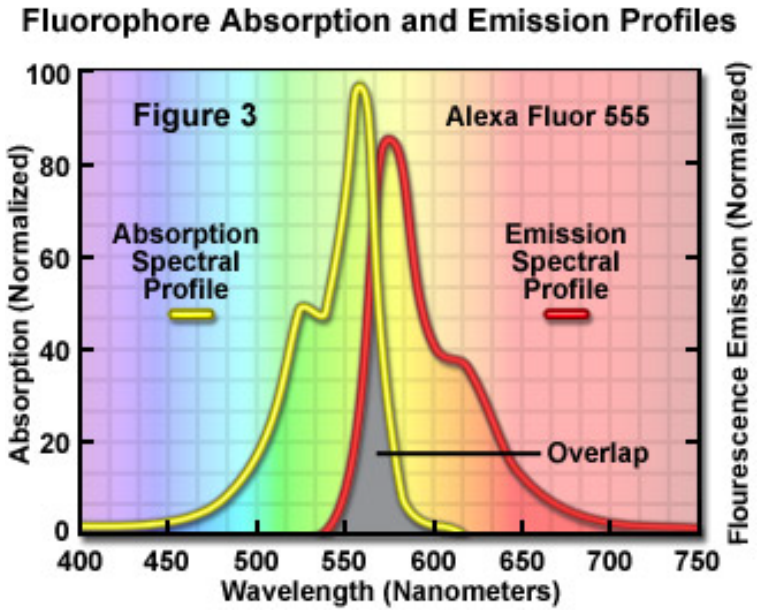
Egner, A. and Hell, S., Trends in Cell Biology 15, 207 (2005).

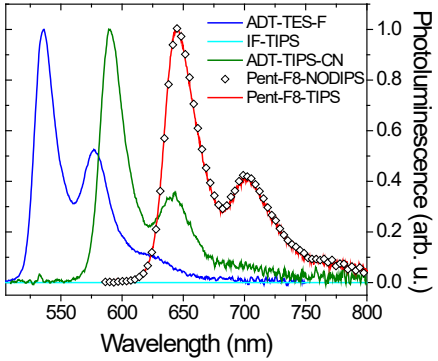


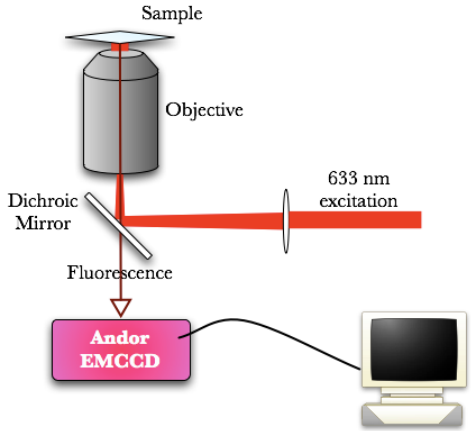
- **Super-resolved** = beat the diffraction limit = make it possible to resolve features smaller than d
- Minimize the point spread function (PSF) (response of the imaging system to a point source)

Fluorescence

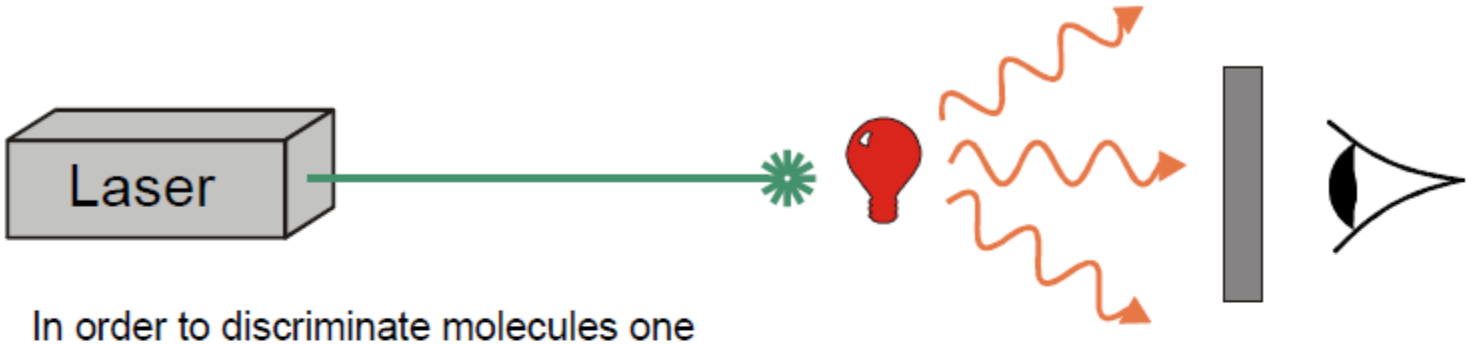






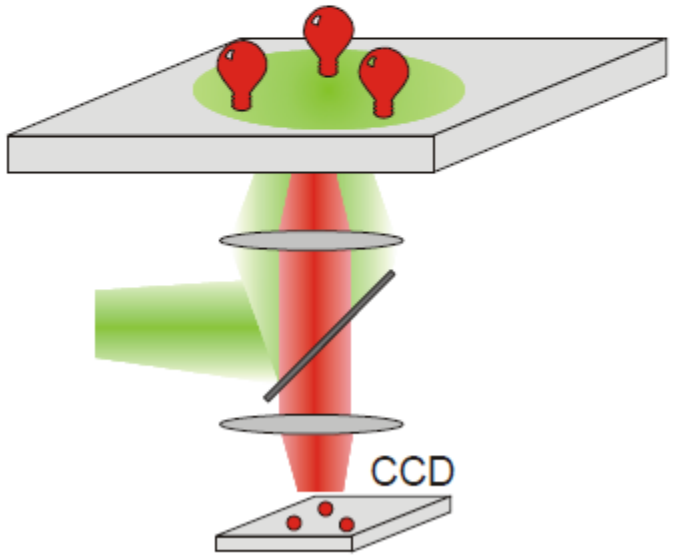


Measurement principle: Fluorescence microscopy



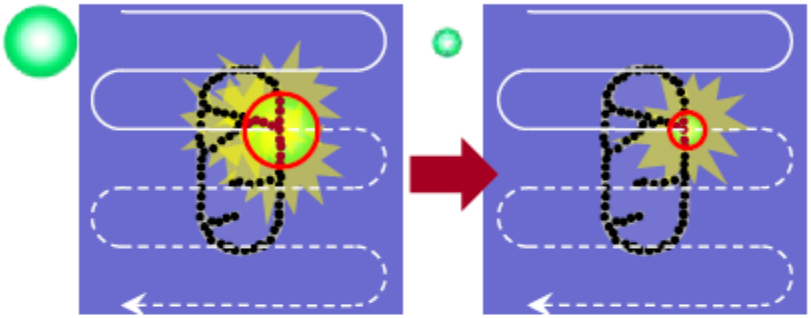
In order to discriminate molecules one from each others, it is necessary to dilute the concentration of fluorophores to such an extent that the average distance between two fluorophores is larger than the resolution of the microscope (confocal or wide-field). Depending on the experiment, the dilution will occur:

- in a cell membrane
- in an artificial membrane
- on a biomodified surface
- in a thin polymer sheet spin coated on a glass surface



Two Approaches for Superresolution

Point-Spread-Function Engineering



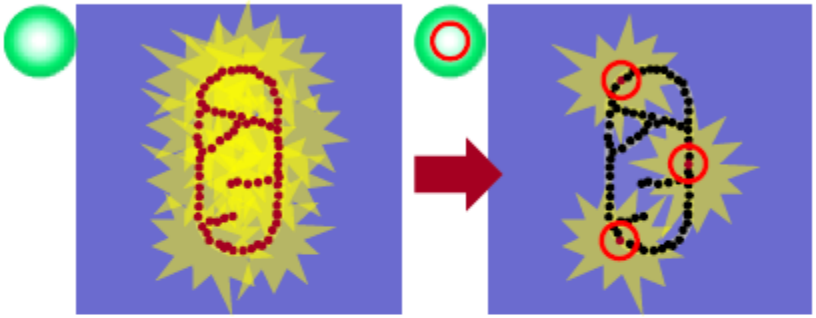
4Pi, I^{5}M, struct. illumination, …

Breaking the diffraction limit:

Targeted optical switching /non-linearity

STED, GSD, saturation, …

Single Molecule Localization

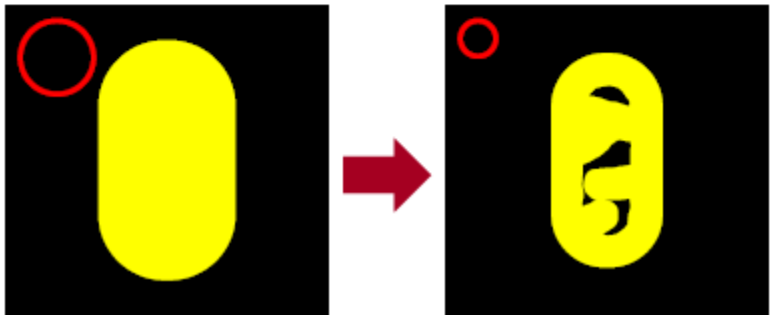


Related to particle tracking

Breaking the diffraction limit:

Stochastic optical switching

PALM, FPALM, STORM, …



W. E. Moerner

1989-200x – single molecule approach; then PSF engineering;
Currently combines the PSF engineering with single molecule methods

- Born 1953, Pleasanton, CA, USA
- Grew up in San Antonio, TX
- B.Sc. (1975) from Washington U in St. Louis (Physics, Math, EE)
- M.Sc. (1978) and Ph.D. (1982) in Physics from Cornell U
- 1981-1995 IBM Almaden, San Jose, CA
- 1995-1998 Professor, Dept. of Chemistry and Biochemistry, UCSD
- since 1998 Professor, Dept. of Chemistry, Stanford U

From W.E.'s publication list (>450 papers total):

7. A. R. Chraplyvy, W. E. Moerner, and A. J. Sievers, "High-Resolution Spectroscopy of Matrix-Isolated ReO4- Molecules," *Opt. Lett*. **6**, 254 (1981).

13. P. Pokrowsky, W. E. Moerner, F. Chu, and G. C. Bjorklund, "Reading and Writing of Photochemical Holes Using GaAlAs Diode Lasers," *Opt. Lett.* **8**, 280 (1983).

24. W. E. Moerner and M. D. Levenson, "Can Single-Photon Processes Provide Useful Materials for Frequency Domain Optical Storage?" *J. Opt. Soc. Amer. B: Opt. Phys.* **2**, 915 (1985).

43. W. E. Moerner and T. P. Carter, "Statistical Fine Structure in Inhomogeneously Broadened Absorption Lines," *Phys. Rev. Lett.*, **59**, 2705 (1987).

48. W. E. Moerner and L. Kador, "Optical Detection and Spectroscopy of Single Molecules in a Solid," *Phys. Rev. Lett.* **62**, 2535 (1989).

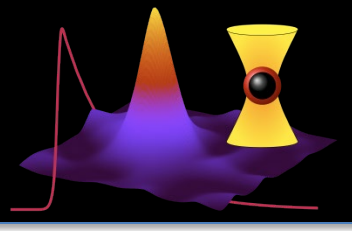
IBM time period – development of high-density optical storage

Erwin Schrödinger (1887-1961)

"…we never experiment with just one electron or atom or a small molecule. In thought-experiments we sometimes assume that we do; this invariably entails ridiculous consequences… In the first place it is fair to state that we are not experimenting with single particles, any more than we can raise Ichthyosauria in the zoo."

"Are there quantum jumps?" British J. for the Philosophy of Science 3, 233 (1952)

Light-matter interactions: two-level system



state 2 ——— E_2 $\quad \Psi_2(r,t) = \psi_2(r)\exp(-i\frac{E_2}{\hbar}t)$

state 1 ——— E_1 $\quad \Psi_1(r,t) = \psi_1(r)\exp(-i\frac{E_1}{\hbar}t)$

Transition rate:

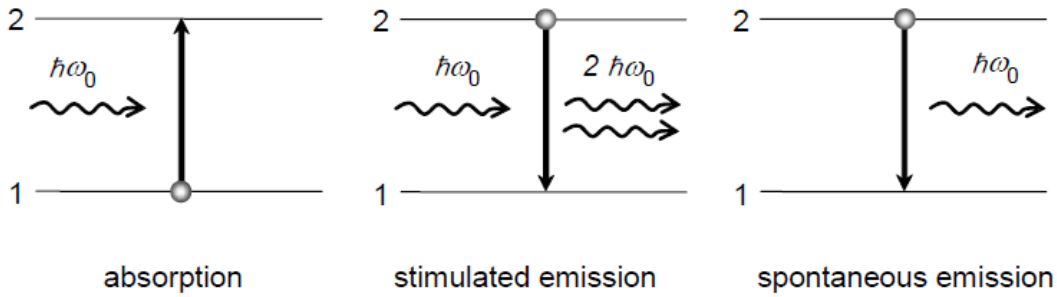
$$\Gamma_{12} = \frac{\pi}{\varepsilon_0 \hbar^2}|\mu_{12}|^2 W(\omega_0)$$

$$\Gamma_{12} = B_{12} W(\omega_0)$$

$$A = \frac{\hbar\omega^3}{\pi^2 c^3} B_{12}$$

Transition dipole moment:

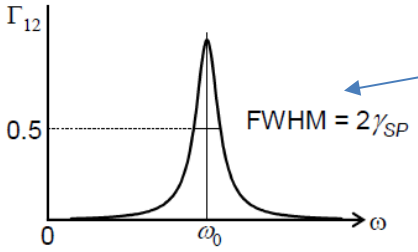
$$|\mu_{21}| = e\int \psi_1^* x \psi_2 dr$$



A, B – Einstein coefficients

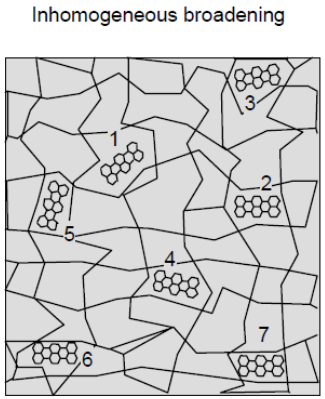
Linewidth

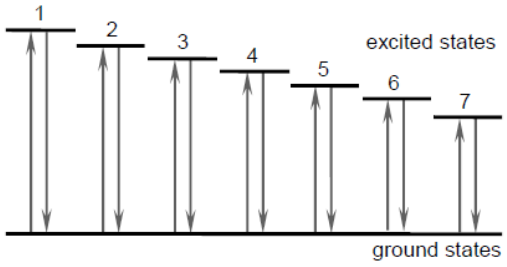
$$\Gamma_{12} = \frac{\pi}{2\hbar^2} E_0^2 |\mu_{12}|^2 \delta(\omega_0 - \omega) \quad \Rightarrow \quad \Gamma_{12} = \frac{\pi}{2\hbar^2} E_0^2 |\mu_{12}|^2 \frac{\gamma_{SP}/\pi}{(\omega_0 - \omega)^2 + \gamma_{SP}^2}$$

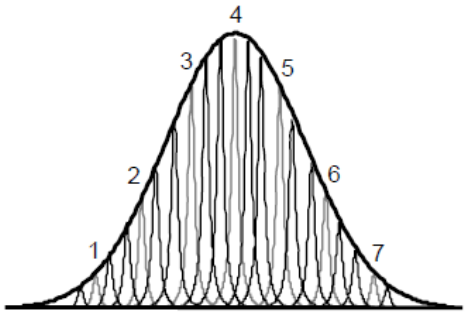


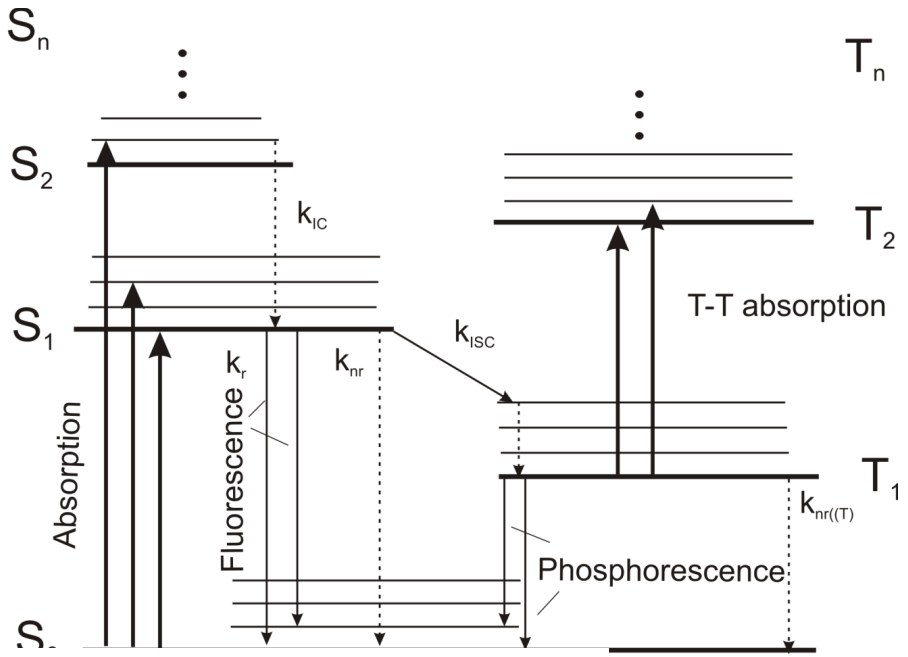
Lorentzian (natural linewidth)

- Homogeneous broadening: Lorentzian (interactions with phonons)
- Inhomogeneous broadening: Gaussian (mechanical strain, electrostatic interactions)

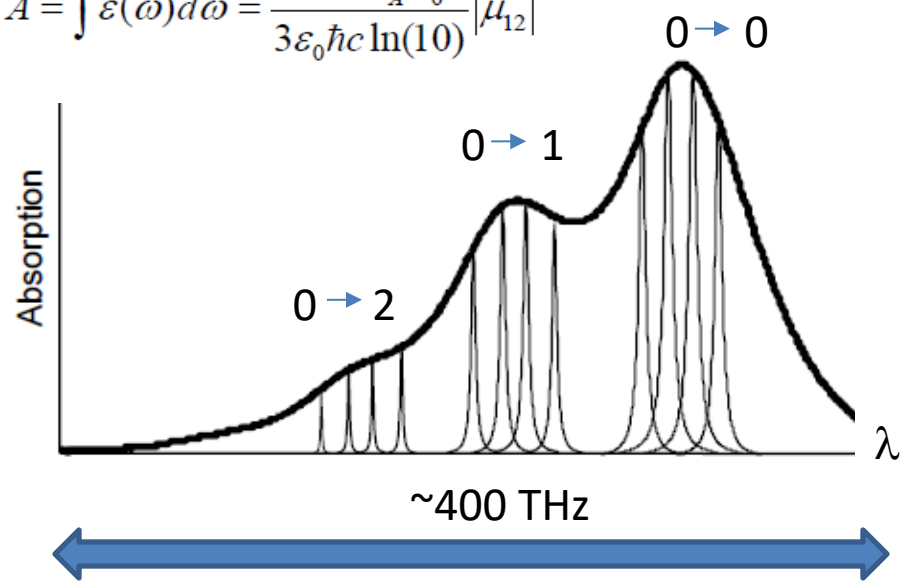


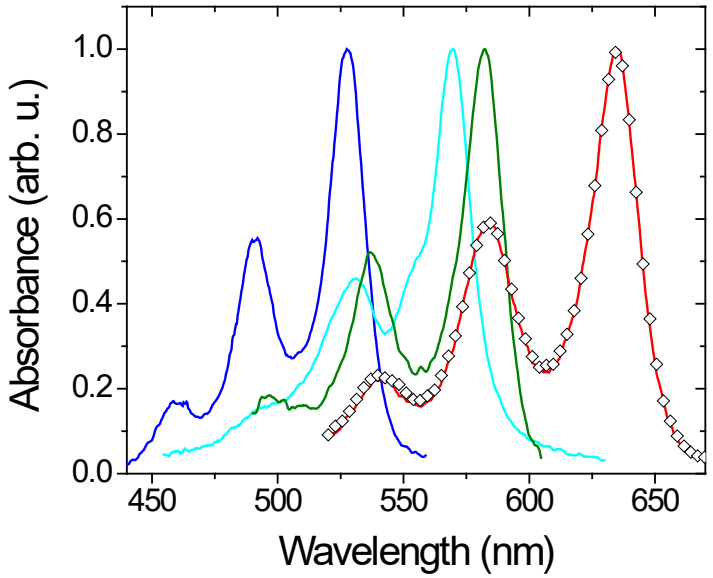






$$A = \int \varepsilon(\omega)d\omega = \frac{2\pi^2 N_A \omega_0}{3\varepsilon_0 \hbar c \ln(10)} |\mu_{12}|^2$$

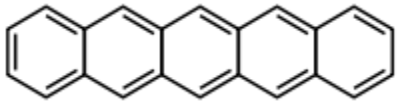


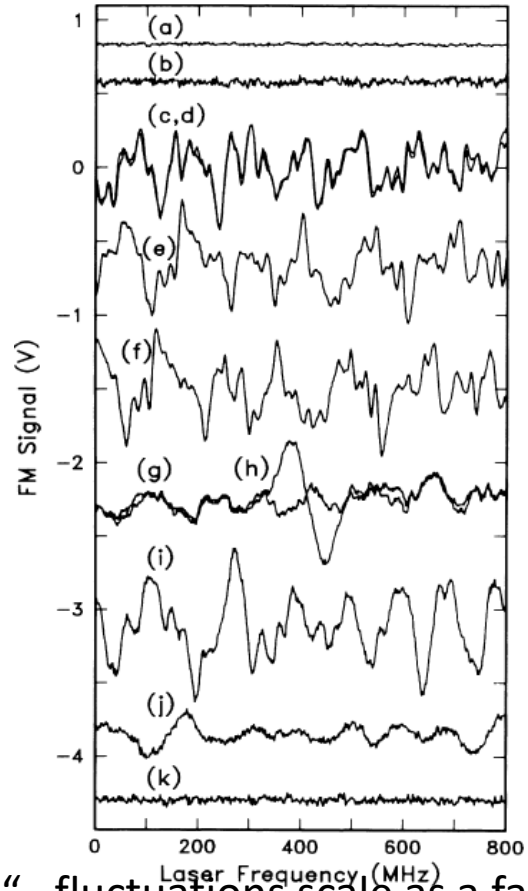


VOLUME 59, NUMBER 23 PHYSICAL REVIEW LETTERS 7 DECEMBER 1987

Statistical Fine Structure of Inhomogeneously Broadened Absorption Lines

W. E. Moerner and T. P. Carter[a]
IBM Almaden Research Center, San Jose, California 95120
(Received 31 July 1987)





Frequency-modulated absorption spectroscopy

10-5 – 10-7 mol/mol

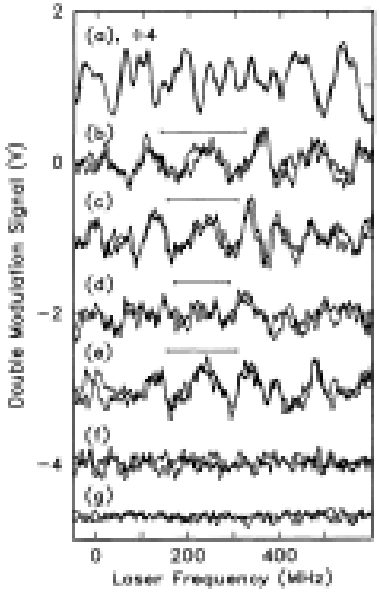
About 10^4-10^6 molecules; particular insertion sites (as "homogeneous" environment as possible; 1.4 K)

Linewidth ~ 7.8 MHz

"…fluctuations scale as a factor of N^1/2…If this signal comes from 1000 molecules, it means that the sensitivity of our apparatus need only to be increased by a factor of 32 to get to the single molecule limit. Therefore, it was going to be possible."

Optical Detection and Spectroscopy of Single Molecules in a Solid

W. E. Moerner and L. Kador[a]
IBM Research Division, Almaden Research Center, San Jose, California 95120
(Received 17 March 1989)



tained. Future development of techniques for recording SMD spectra at lower intensity and higher SNR should allow additional study of single local environments in solids without averaging over large numbers of "equivalent" molecular configurations. Such work would open up a new frontier of spectroscopy of single defect centers in solids where no Doppler, recoil, or multicenter averaging effects are present.

VOLUME 65, NUMBER 21 PHYSICAL REVIEW LETTERS 19 NOVEMBER 1990

Single Pentacene Molecules Detected by Fluorescence Excitation in a *p*-Terphenyl Crystal

M. Orrit and J. Bernard

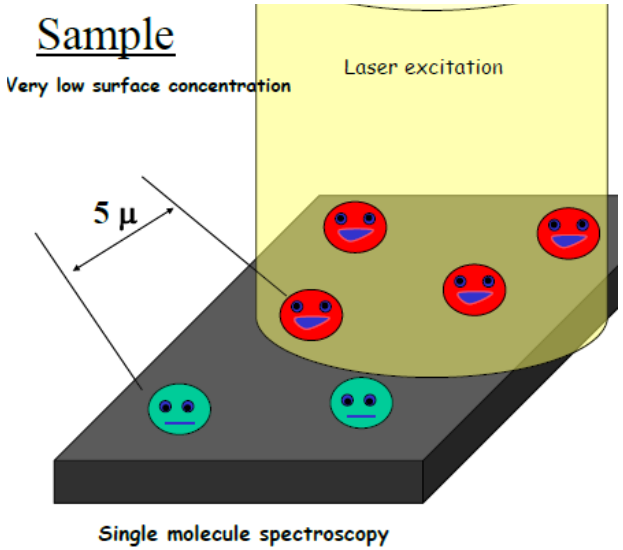
Centre de Physique Moléculaire Optique et Hertzienne, Centre National de la Recherche Scientific et Université de Bordeaux I, 351, Cours de la Libération, F-33405 Talence CEDEX, France

(Received 9 July 1990)

"If there were a fourth Nobel Prize, it should have gone to Orrit."
W. E. Moerner at the plenary lecture, APS meeting 2015

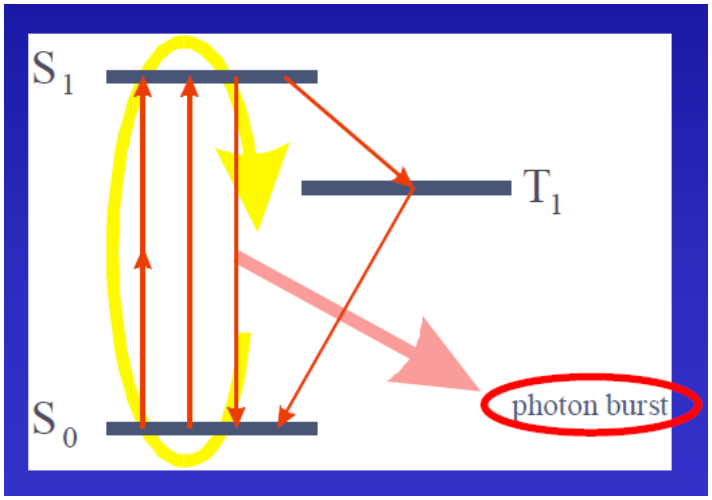
1990 – official beginning of single-molecule fluorescence microscopy

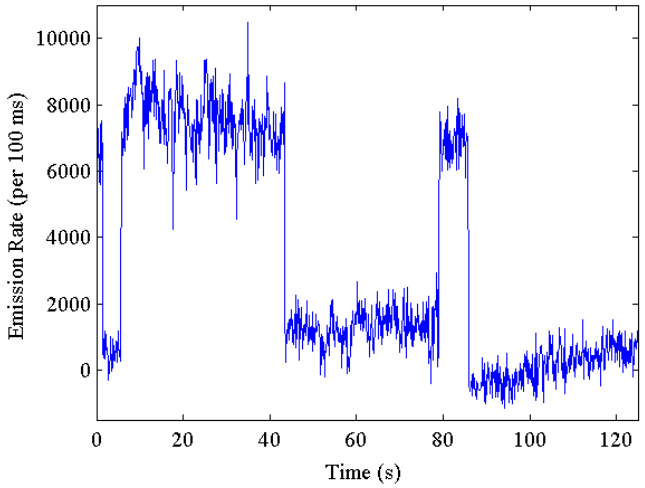
Single molecule microscopy



Use low concentration of molecules

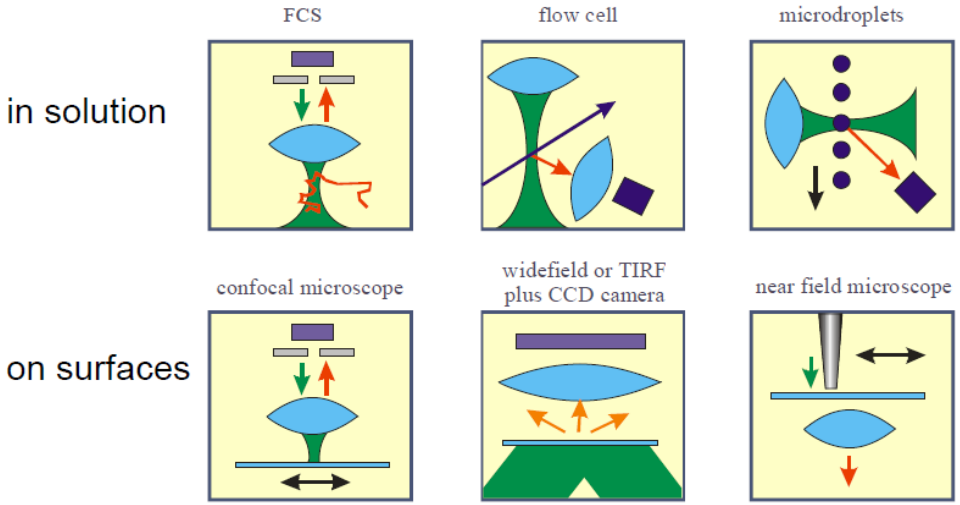
Video from
Rebecca Grollman





Single molecule microscopy

- Study inhomogeneous environments



  - Track position of the molecule in space and time
  - Detect conformational changes
  - Characterize charge transfer states
  - Characterize triplet states
  - Establish molecular alignment

Ensemble averaging allows one to see forests without getting lost in their trees…

It becomes more interesting when ensemble averaging goes wrong. Consider a room full of young children. About half of them have learned to walk, and about half of them are still crawling on all fours: on the average, children walk on three limbs! This of course is too simple minded; the naïve average masks a meaningful bimodal distribution.

Steve Granick, UIUC

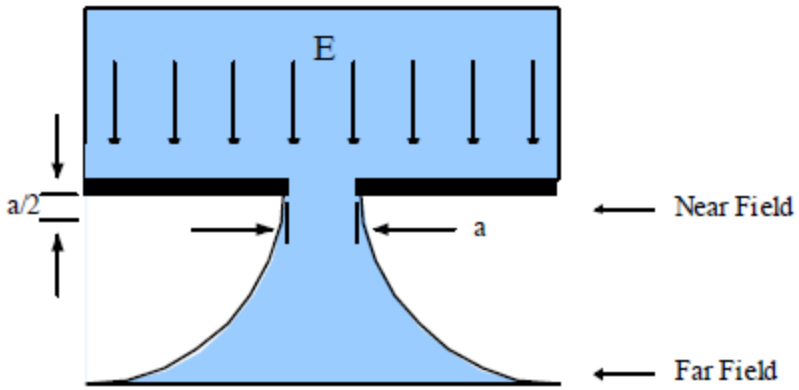
Journal of Polymer Science: Part B: Polymer Physics, Vol. 48, 2542–2543 (2010)

Eric Betzig

1990 – PSF engineering, then single molecules; now both

- Born in 1960 in Ann Arbor, Michigan
- B.Sc. (1983) in Physics from Caltech
- M. Sc. (1985) and Ph. D. in Applied and Engineering Physics, Cornell U
- 1988-1994 Bell Labs
- 1994 – founded NSOM Enterprises
- 1996 – abandoned microscopy; vice president of R&D at his father's machine tool firm, Ann Arbor, MI
- 2002 – founded New Millenium Research in Okemos, MI
- 2005-2017 – Janelia Farm Research Campus of the Howard Hughes Medical Institute (VA)
- since 2017 – UC Berkeley and LBNL

Near Field Optics



Resolution is only a function of aperture size!

1928: Proposal of concept (E. Synge, Phil. Mag. 6, 356, 1928)

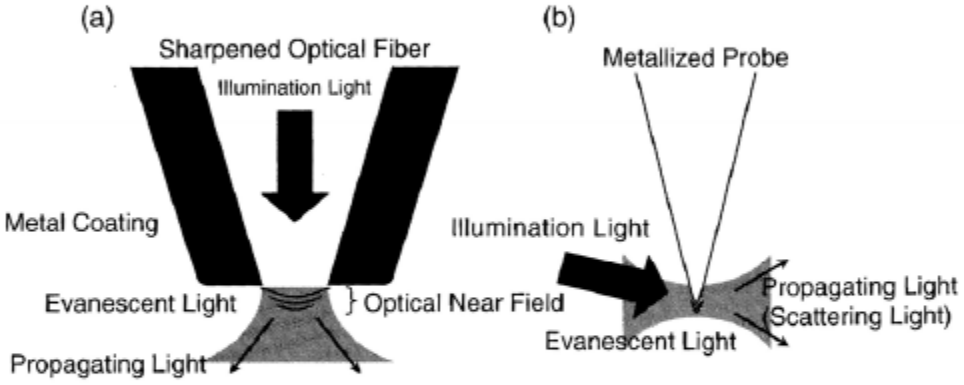
1944: Calculation of sub wavelength aperture coupling (H. Bethe, Phys. Rev. 66, 163, 1944)
Correct by Bouwkamp

1972: demonstration using microwaves (Ash et al., Nature 237, 510, 1972)

1980's Work by Pohl and Lewis

Near-field Scanning Optical Microscope (NSOM)

Principle of NSOM: Can be simply modeled by the electromagnetic interaction of two very closely positioned nano-objects, which represent a probe and sample



Aperture-type

- Nanoscale light spot same as aperture size
- Aperture-sample distance is regulated at < 10 nm

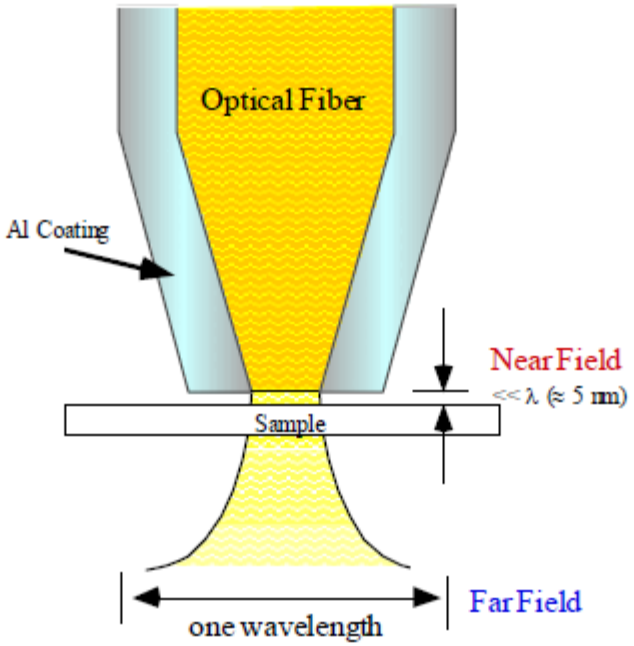
Scattering-type

- Sharpened homogeneous metal tip, with enhanced electric field
- Spatial resolution defined by apex diameter

Review paper: Lewis A., *Nature Biotech.* **21**, 1378 (2003)

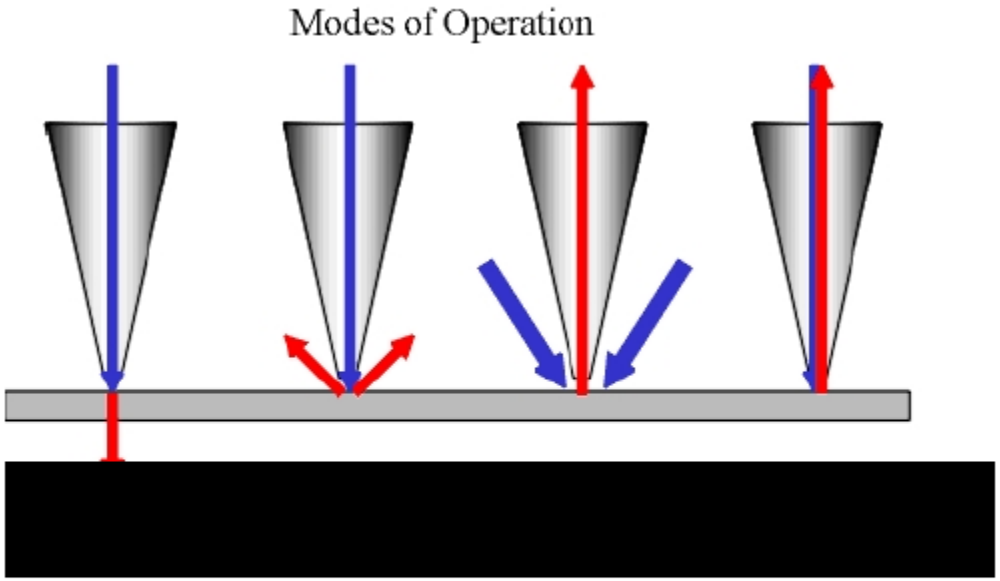
How do you break the diffraction limit?

Near-Field Optics



Resolution determined by size of aperture

To this day, NSOM remains the only diffraction-unlimited imaging method that does not rely on switching…



NSOM modes of operation

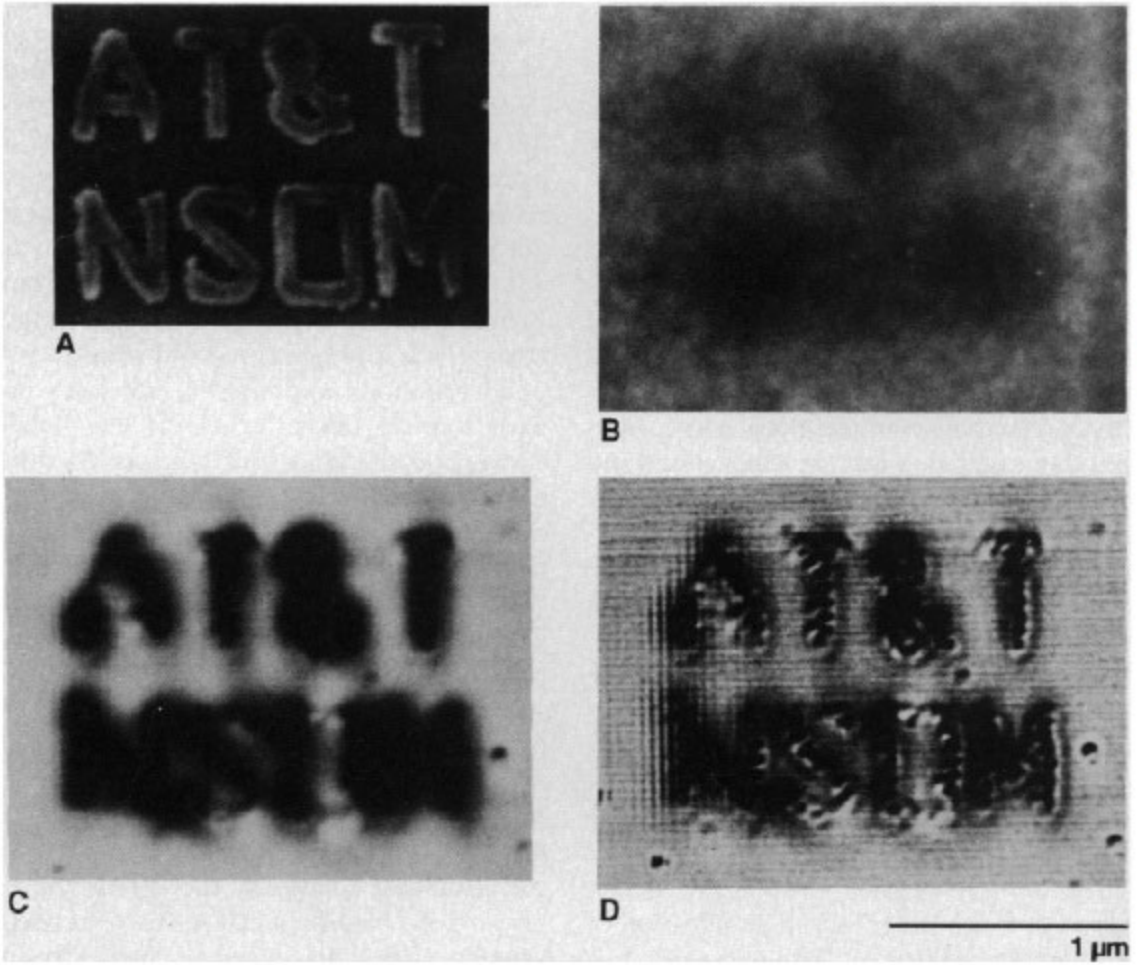
From L to R : Tranmission mode, Reflection Mode, Collection Mode and Illumination/Collection mode.

http://www.nanonics.co.il/main/twolevels_item1.php?ln=en&item_id=34&main_id=14

Breaking the Diffraction Barrier: Optical Microscopy on a Nanometric Scale

Science_251_1468 (1991)

E. BETZIG*, J. K. TRAUTMAN, T. D. HARRIS, J. S. WEINER, R. L. KOSTELAK



A. SEM

B. Optical microscopy (NA = 0.9)

C. NSOM

D. NSOM after deconvolution

Single Molecules Observed by Near-Field Scanning Optical Microscopy

Eric Betzig and Robert J. Chichester

Science_262_1422 (1993)

Interactions with evanescent fields

Map out E-fields

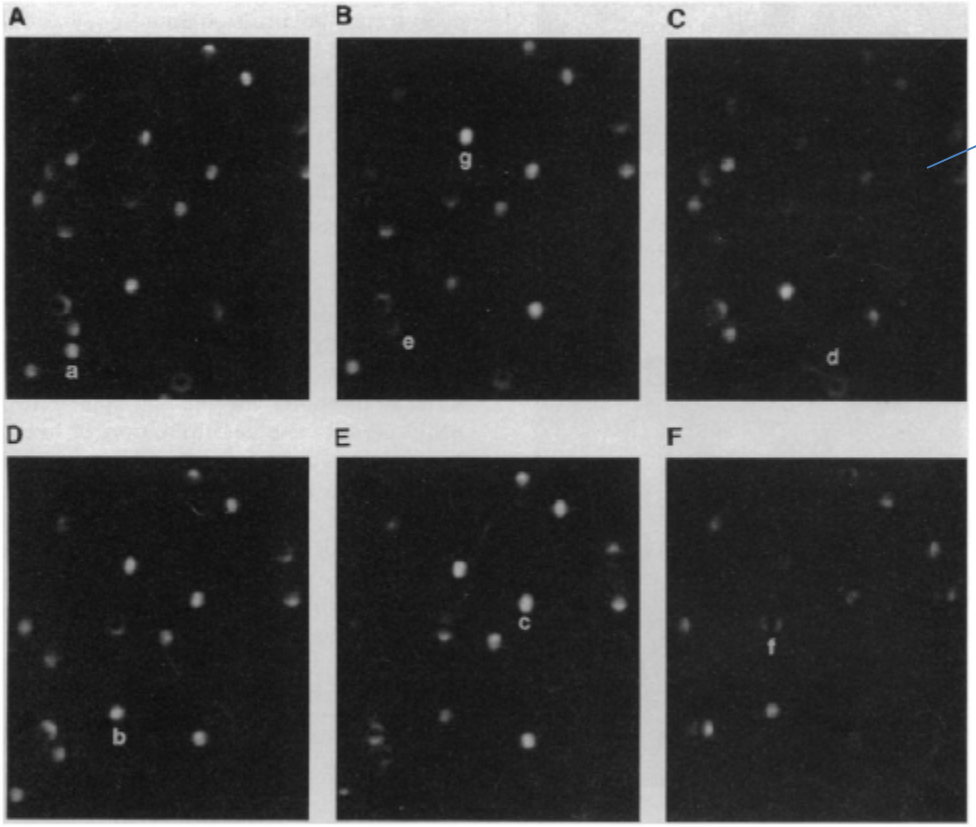


Fig. 1. Six sequential images of the exact same field of individual carbocyanine dye molecules as detected by near-field optical fluorescence microscopy. The excitation polarization is random in (**A**) through (**D**) and linear along *y* and *x*, respectively, in (**E**) and (**F**). The emission polarization is measured along *y* and *x* in (**B**) and (**C**), respectively, and not measured otherwise. Certain molecules have been labeled for discussion in the text.

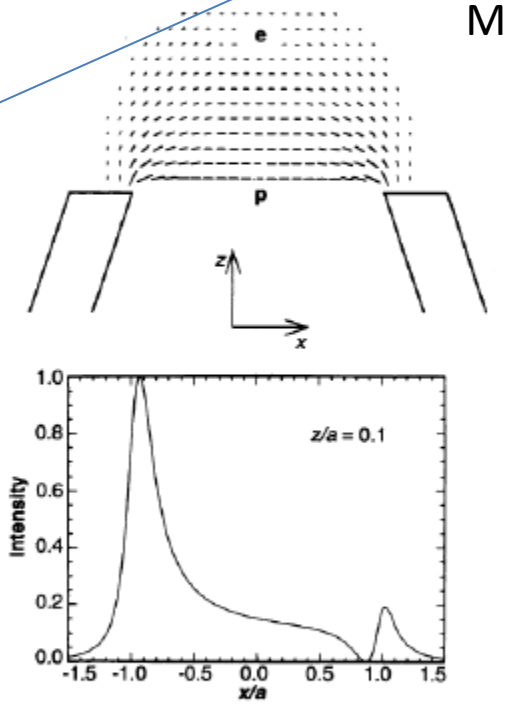


Fig. 2. (**Top**) Schematic view of a molecular dipole **p** (arrow) at a particular orientation within the electric field pattern **E** of a subwavelength aperture. (**Bottom**) Resulting intensity *I* versus *x* for this particular orientation, proportional to the square of the component of **E** along **p** ($I \propto |\mathbf{p}\cdot\mathbf{E}|^2$).

Use Bethe's theory to determine orientations and positions with 12 nm xy and 6 nm in z

"I am sick of science. I hate academia. I quit."

Eric Betzig, 1994

Limitations of NSOM became incredibly obvious… It was clear that there is no way I could realize my dream of looking at live cells with electron microscope resolution…"

February 1, 1995 / Vol. 20, No. 3 / OPTICS LETTERS

Proposed method for molecular optical imaging

E. Betzig

NSOM Enterprises, 17 Webster Drive, Berkeley Heights, New Jersey 07922

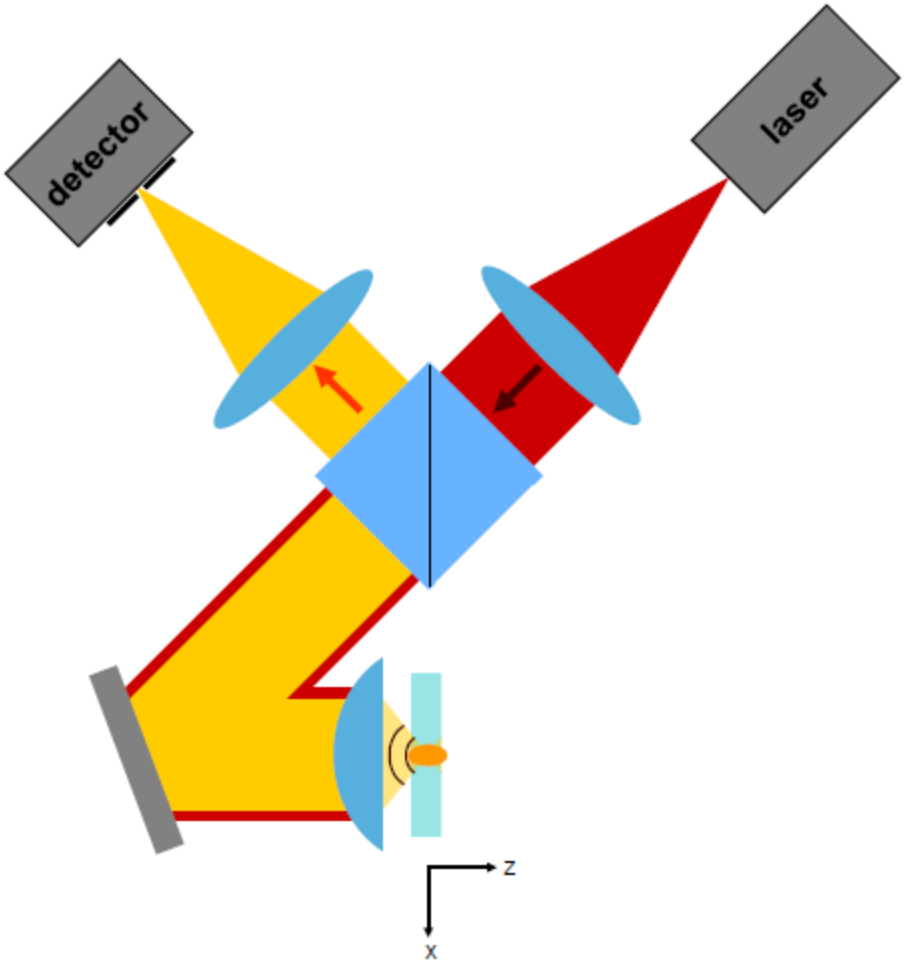
"…Isolate molecules by some properties and image them separately…I put forward a general concept and left it at that…"

Stefan Hell

PSF engineering

- Born 1962 in Arad, Romania
- Diploma in Physics (1987) and Ph. D. (1990) from U of Heidelberg, Germany
- 1991-1993 – postdoc at European Molecular Biology lab in Heidelberg, Germany
- 1993-1996 – lead scientist in the laser microscopy group, U of Turku, Finland
- since 1997 – at Max Planck Institute for Biophysical Chemistry, Gottingen, Germany (since 2002 – Director)

Confocal Microscopy



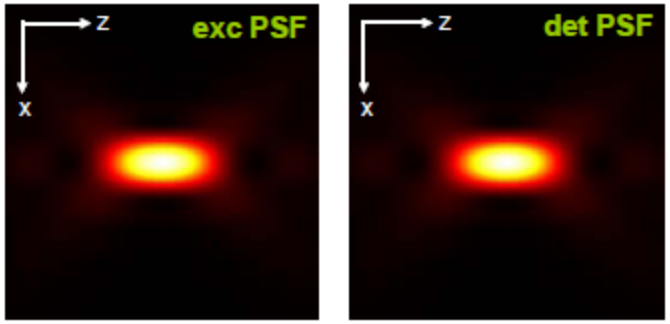
diffraction limit:

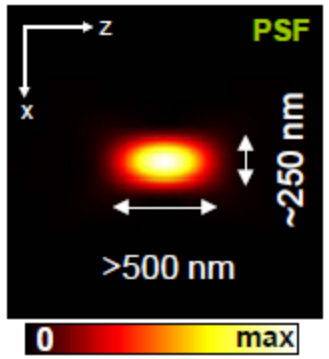
$$d_{\min} \approx 0.61 \frac{\lambda}{n \sin \alpha}$$

wavelength

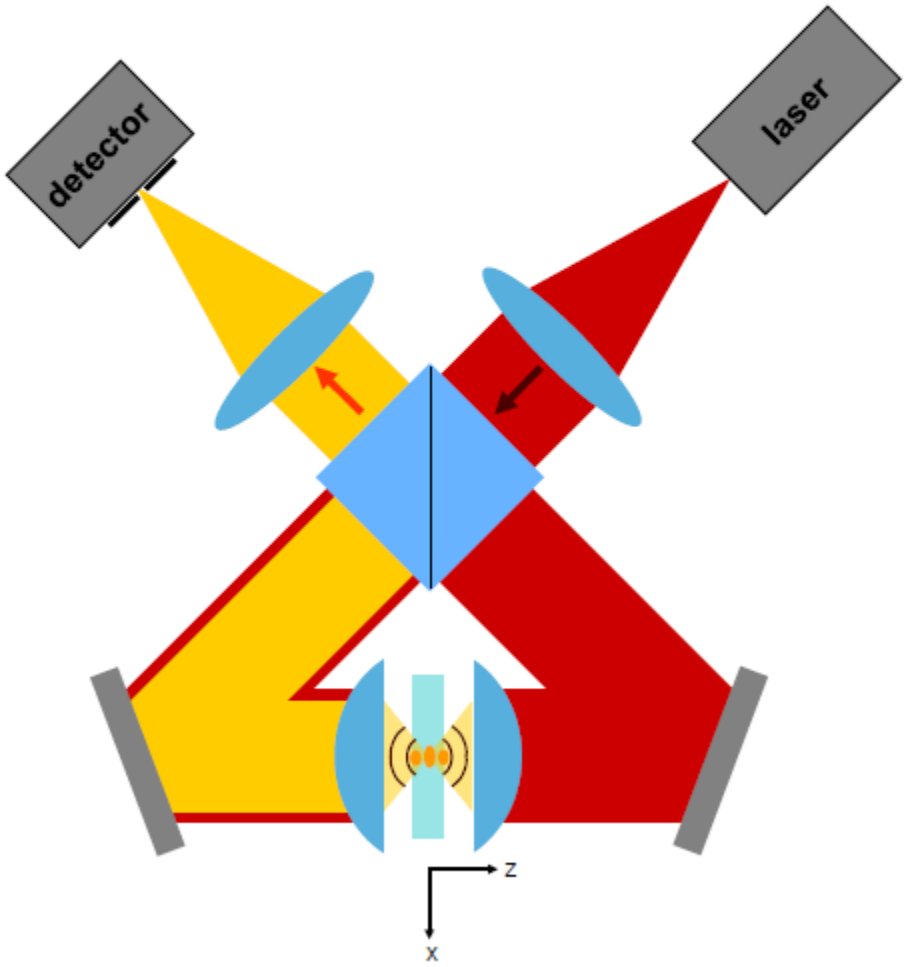
numerical aperture

aperture angle



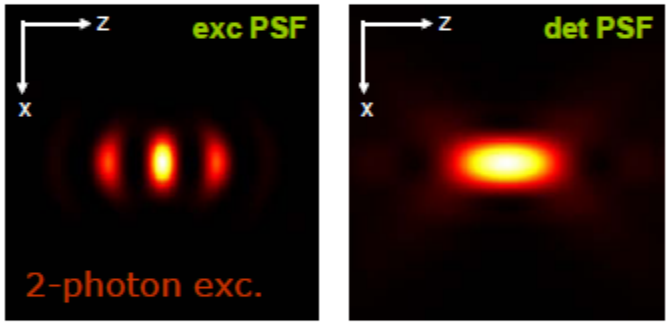


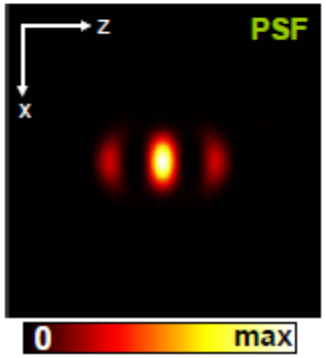
4Pi Microscopy



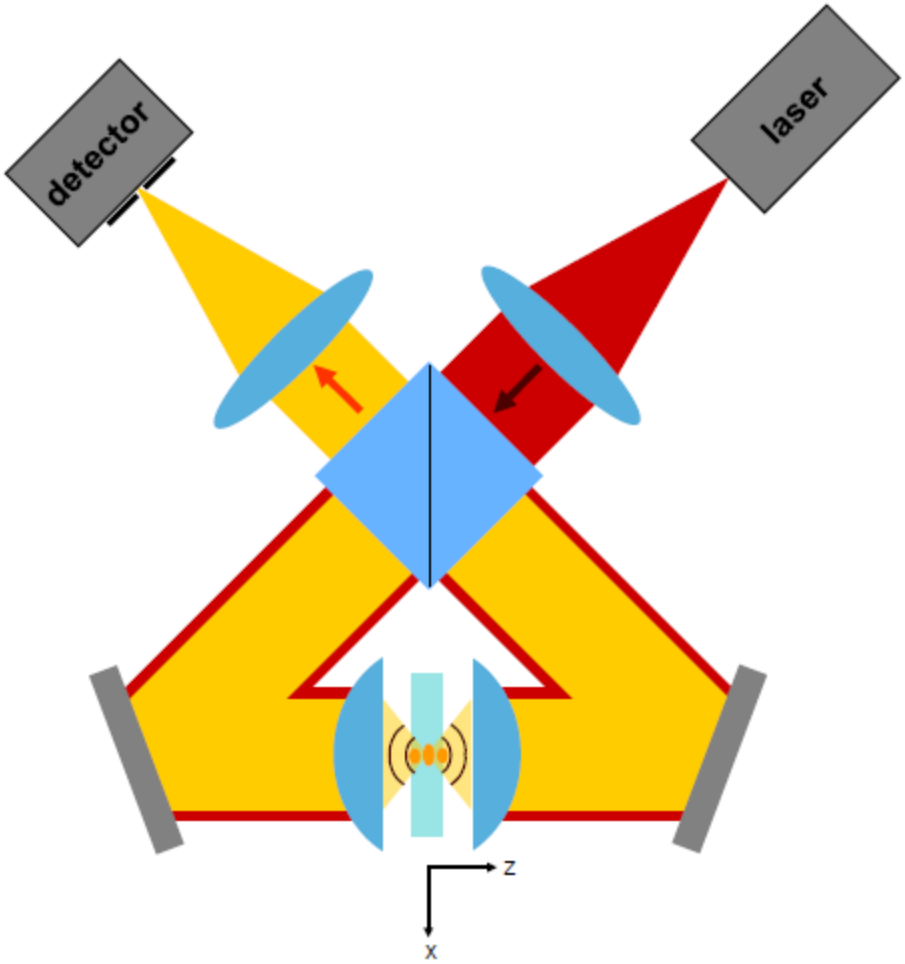
Stefan Hell 1990, 1992

Coherent excitation with two opposing objectives





Type C 4Pi Microscopy

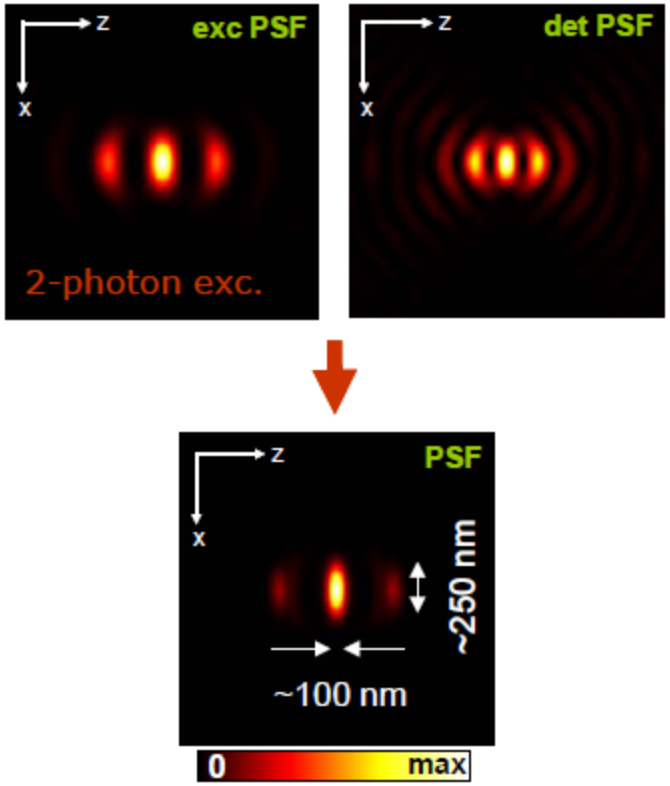


Type A 4Pi

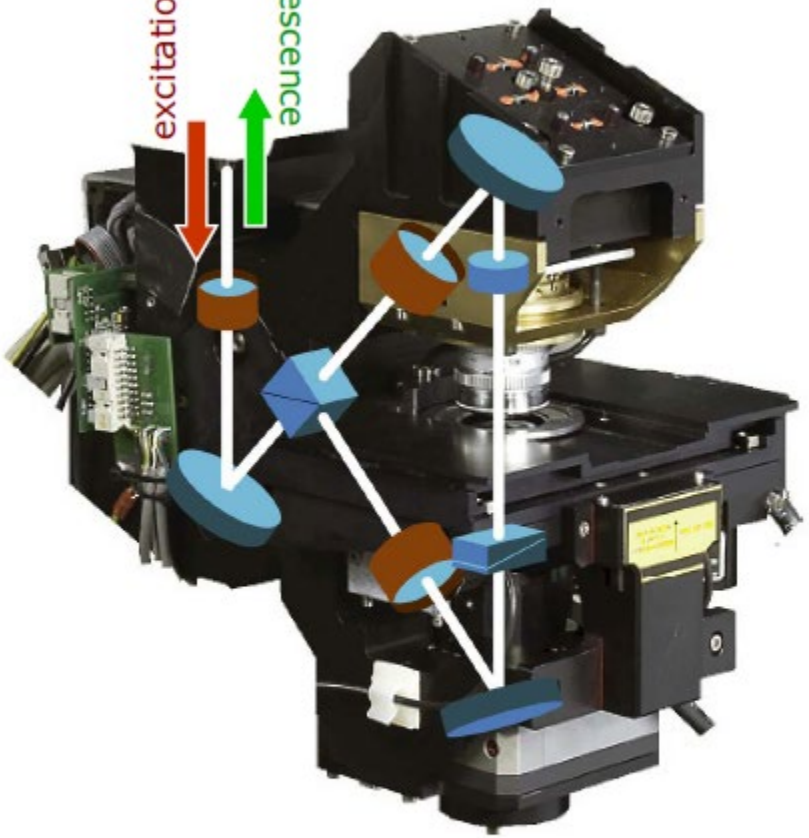
coherent excitation & regular conf. detection

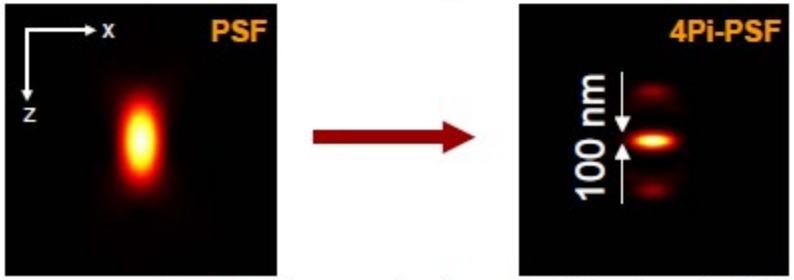
Type C 4Pi

coherent excitation & coherent detection



4Pi Microscopy

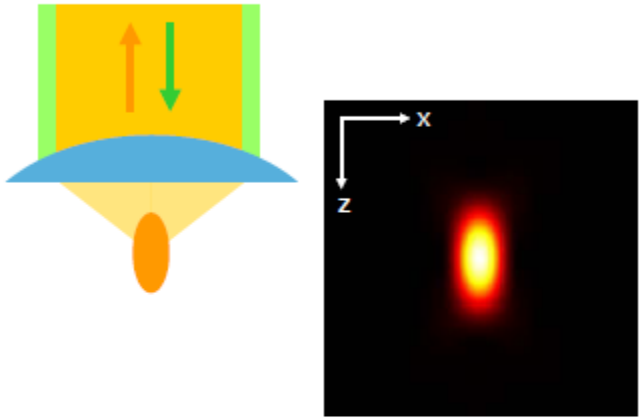




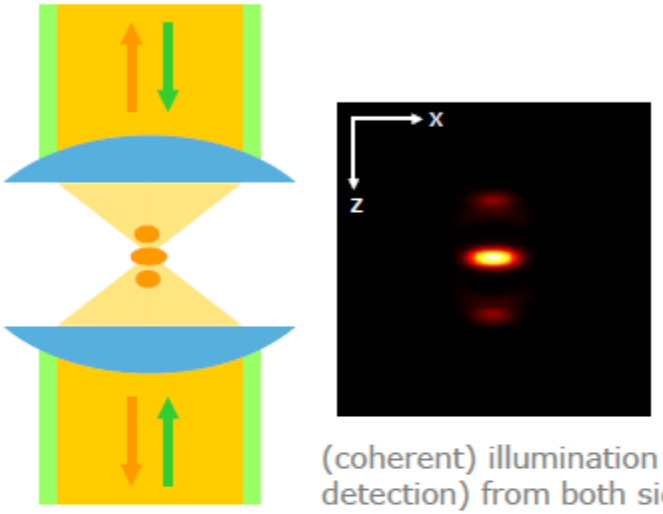
Gugel, Bewersdorf et al., *Biophys. J.* 2004

4Pi Microscopy Enhances the Depth Resolution

(confocal) Laser Scanning Microscope

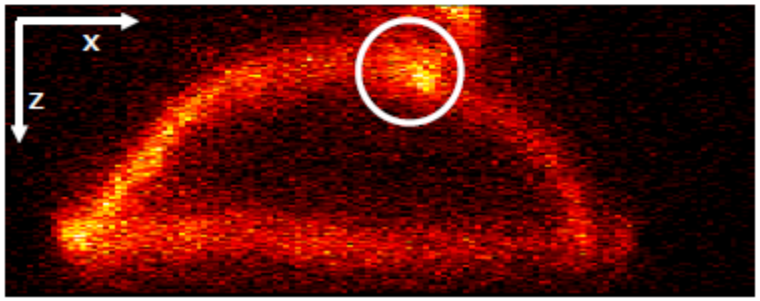


4Pi Microscope

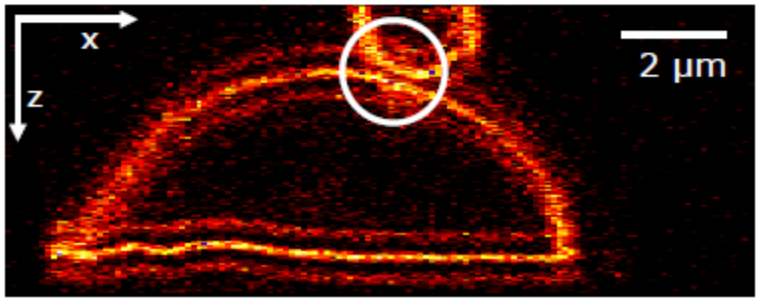


(coherent) illumination (and detection) from both sides

Depth resolution ca. 600 nm
Lateral resolution ca. 250 nm

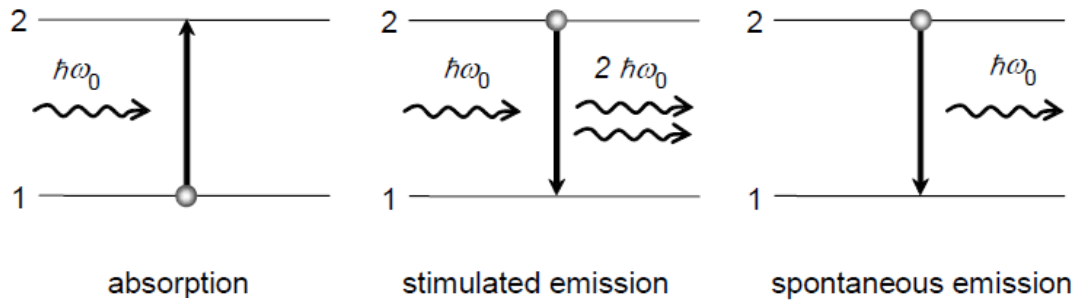


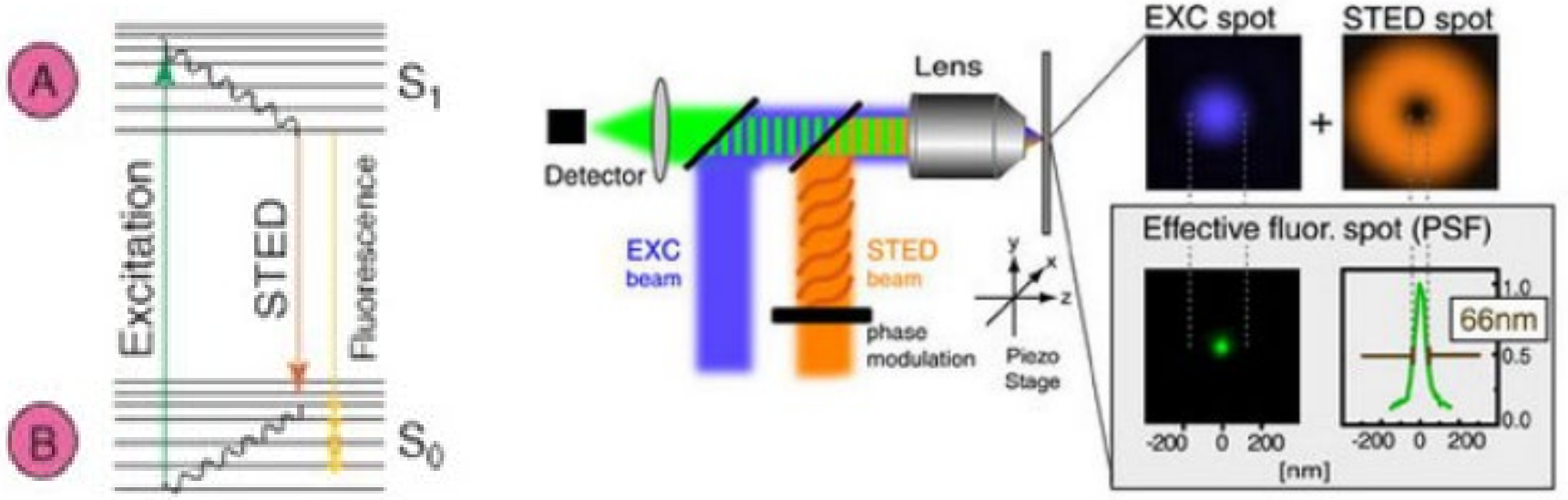
Depth resolution ca. 100 nm - 6x better
Lateral resolution ca. 250 nm - equal



STED microscopy

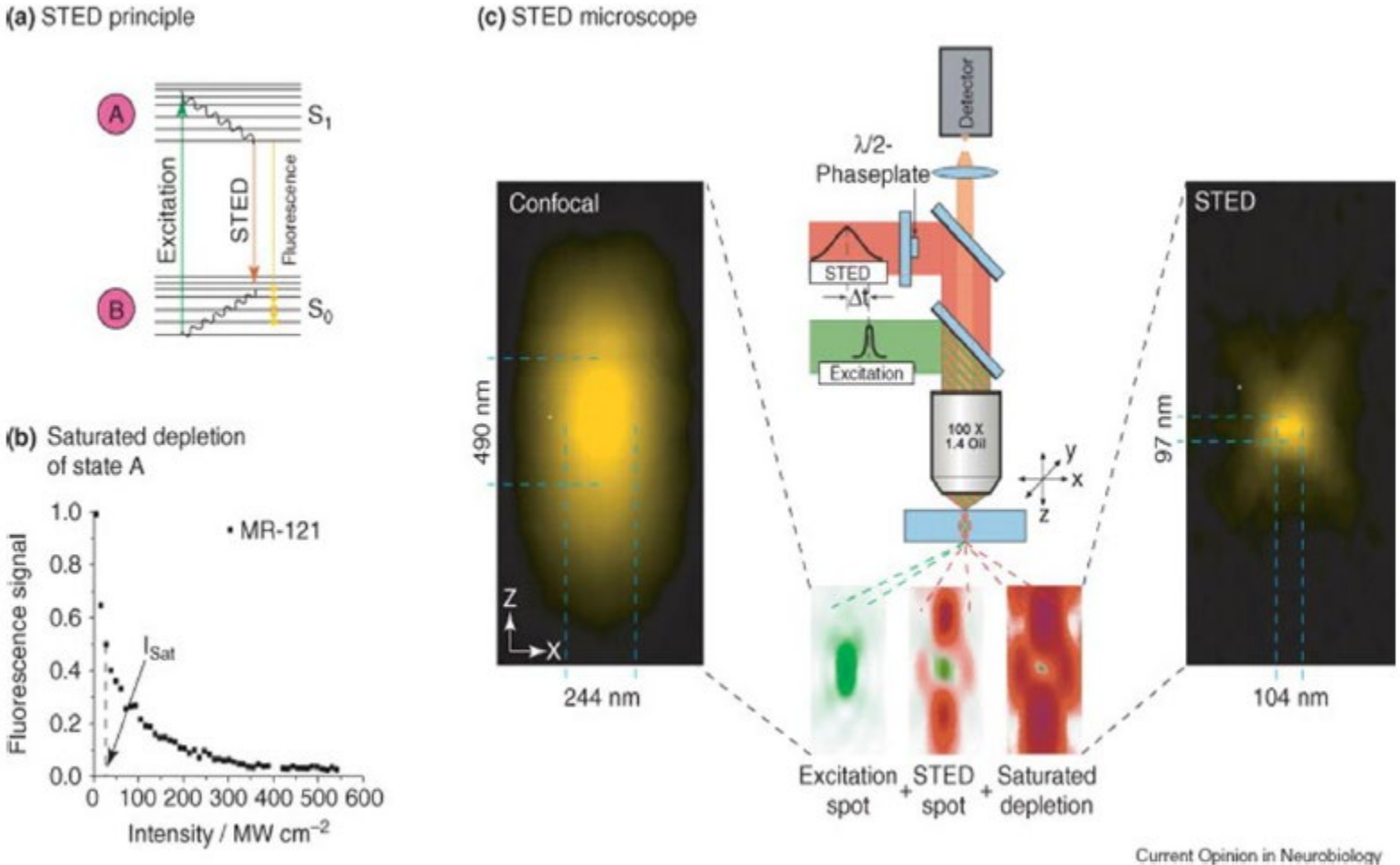
STED = stimulated emission depletion





- STED beam arrives before fluorescence is emitted
- fluorescence is emitted from a considerably smaller spot

$$\Delta_{min} \approx \frac{\lambda}{2n \sin \alpha (\sqrt{1 + I_0 / I_{sat}})}$$

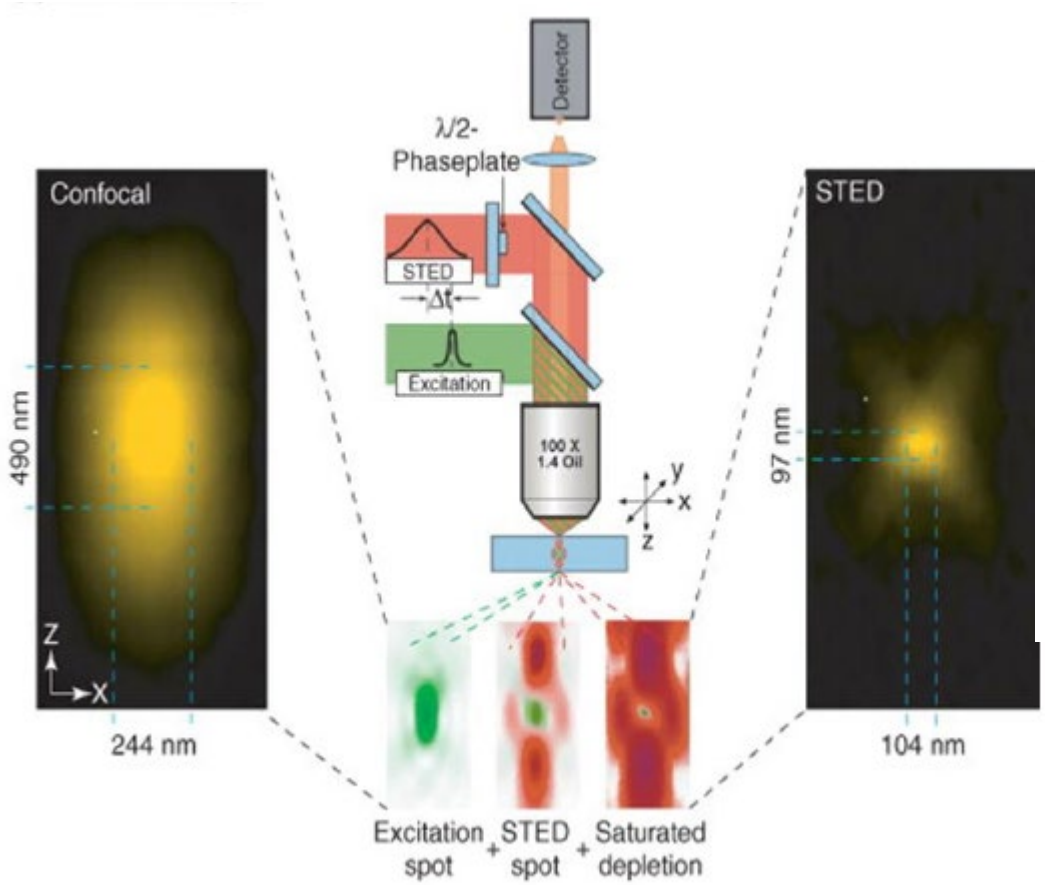


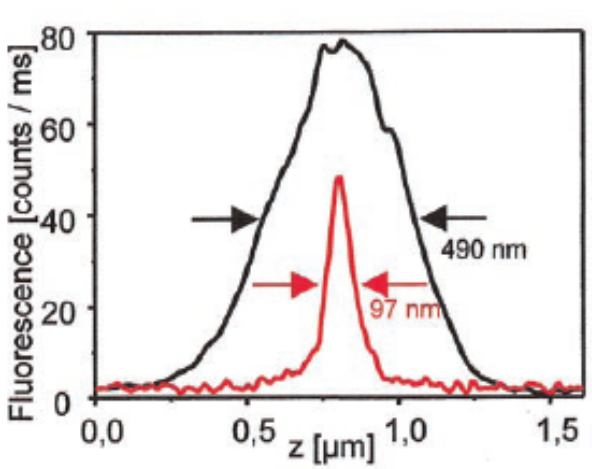
8206–8210 | PNAS | July 18, 2000 | vol. 97 | no. 15

Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission

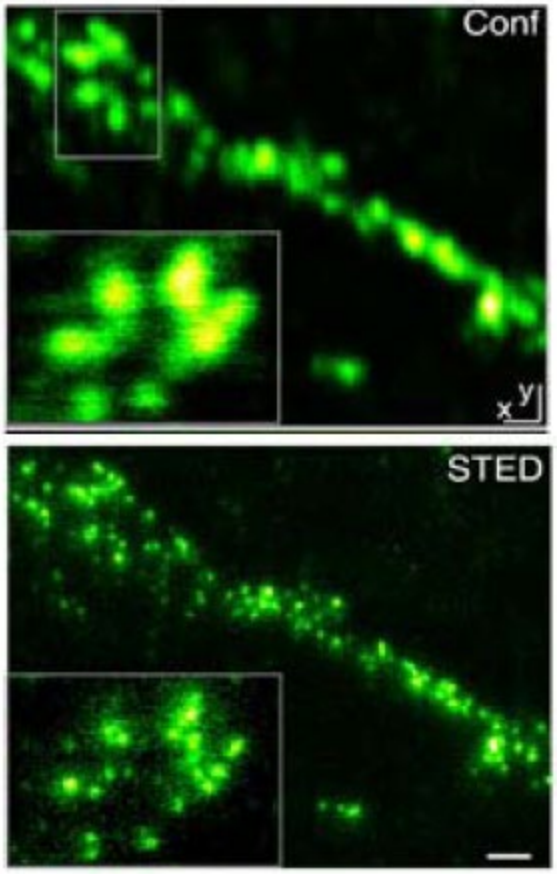
Thomas A. Klar, Stefan Jakobs, Marcus Dyba, Alexander Egner, and Stefan W. Hell†

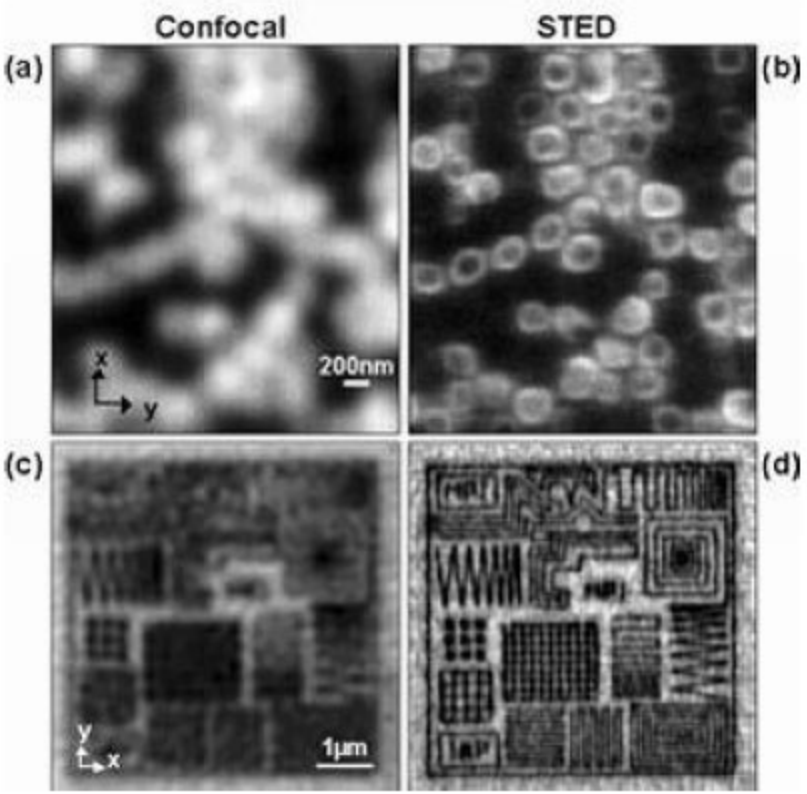
Max-Planck-Institute for Biophysical Chemistry, High Resolution Optical Microscopy Group, 37070 Göttingen, Germany





Resolution Enhancement using STED





www.physorg.com

Focal Spots of Size $\lambda/23$ Open Up Far-Field Florescence Microscopy at 33 nm Axial Resolution

Marcus Dyba and Stefan W. Hell*

High Resolution Optical Microscopy Group, Max-Planck-Institute for Biophysical Chemistry, 37070 Göttingen, Germany

(Received 19 September 2001; published 4 April 2002)

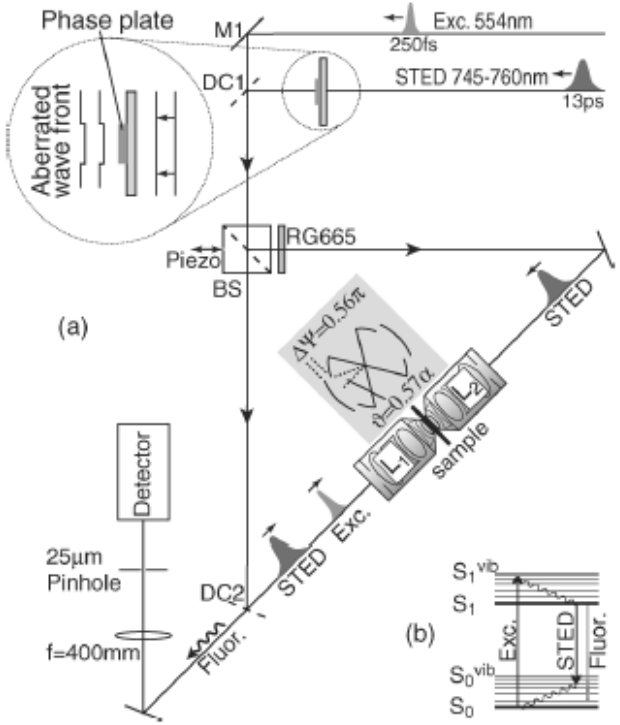
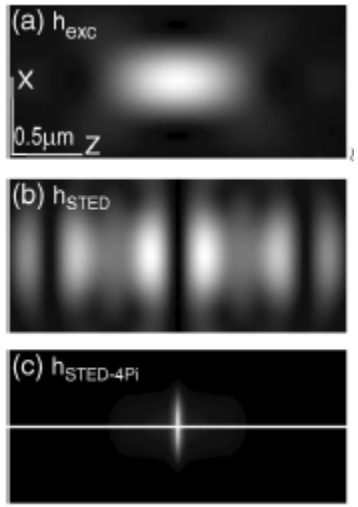
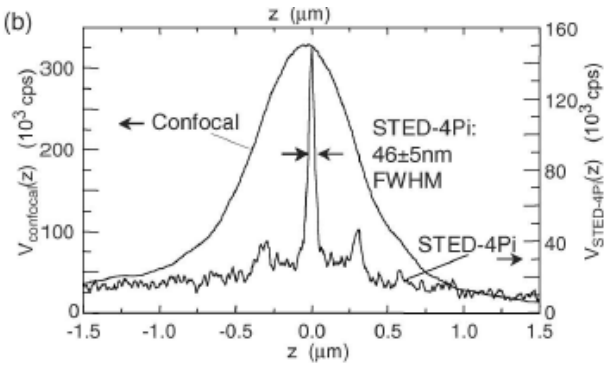


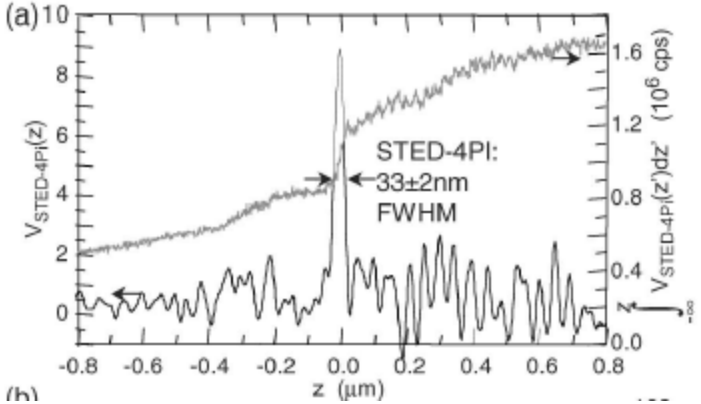
FIG. 1. STED-4Pi microscope. (a) Fluorescence excitation and detection occur via lens L_1, whereas stimulated emission is generated by the light field of counterpropagating, aberrated wave fronts of L_1 and L_2. Imaging is accomplished by scanning the sample through the sub-diffraction-sized spot of the two lenses. The inserted sketches depict the aberration induced by the phase plate on the counterpropagating STED-beam wave fronts. (b) Fluorophore energy levels.



water



oil



W. E. Moerner

- Born 1953, Pleasanton, CA, USA
- Grew up in San Antonio, TX
- B.Sc. (1975) from Washington U in St. Louis (Physics, Math, EE)
- M.Sc. (1978) and Ph.D. (1982) in Physics from Cornell U
- 1981-1995 IBM Almaden, San Jose, CA
- 1995-1998 Professor, Dept. of Chemistry and Biochemistry, UCSD
- since 1998 Professor, Dept. of Chemistry, Stanford U

From W.E.'s publication list (>450 papers total):

148. R. M. Dickson, A. B. Cubitt, R. Y. Tsien, and W. E. Moerner, "On/Off Blinking and Switching Behavior of Single Green Fluorescent Protein Molecules," *Nature* **388**, 355 (1997).

265. S. R. P. Pavani*, M. A. Thompson*, J. S. Biteen, S. J. Lord, N. Liu, R. J. Twieg, R. Piestun, and W. E. Moerner, (*equal contributions), "Three-Dimensional Single-Molecule Fluorescence Imaging Beyond the Diffraction Limit Using a Double-Helix Point Spread Function," *Proc. Nat. Acad. Sci. (USA)* **106**, 2995-2999 (2009), published online 11 February 2009.

336. Yoav Shechtman, Steffen J. Sahl, Adam S. Backer, and W. E. Moerner, "Optimal Point Spread Function Design for 3D Imaging," *Phys. Rev. Lett*. **113**, 133902 (2014), (DOI: 10.1103/PhysRevLett.113.133902, published online September 26, 2014)

letters to nature

On/off blinking and switching behaviour of single molecules of green fluorescent protein

Robert M. Dickson*, Andrew B. Cubitt†, Roger Y. Tsien‡ & W. E. Moerner*

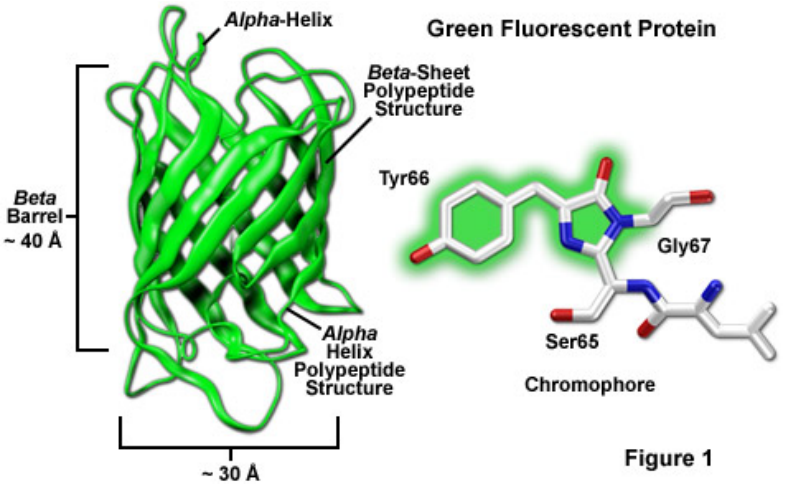
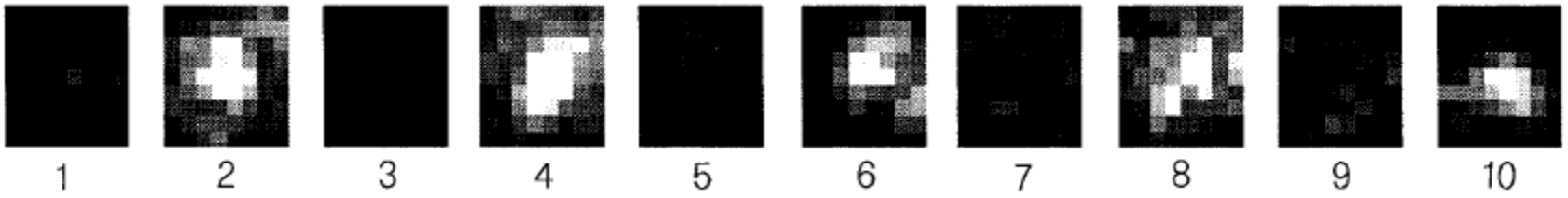
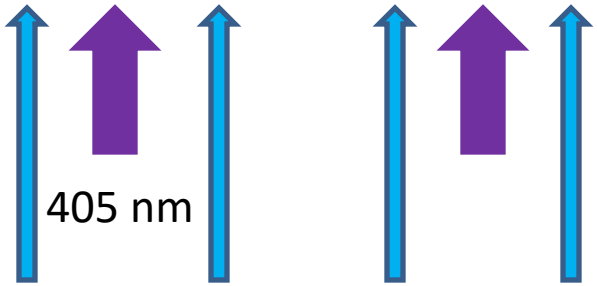
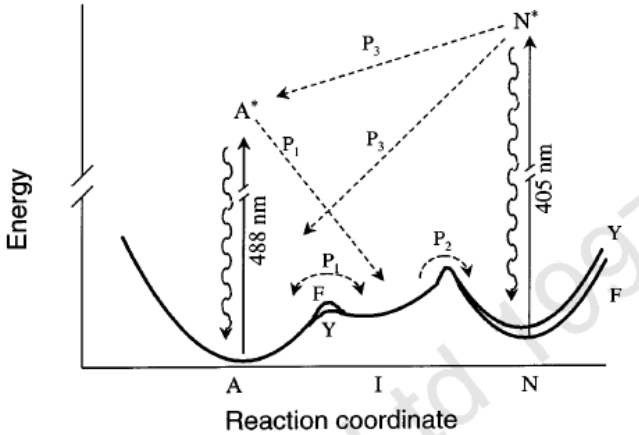


Figure 1





488 nm 488 nm



Fluorescent proteins

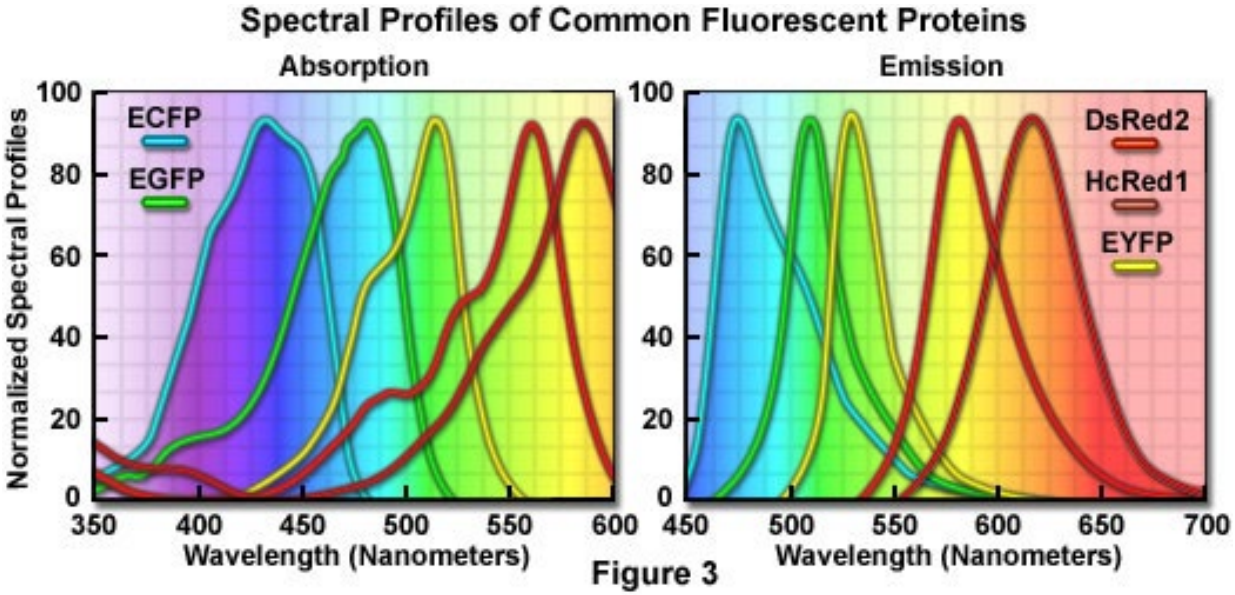
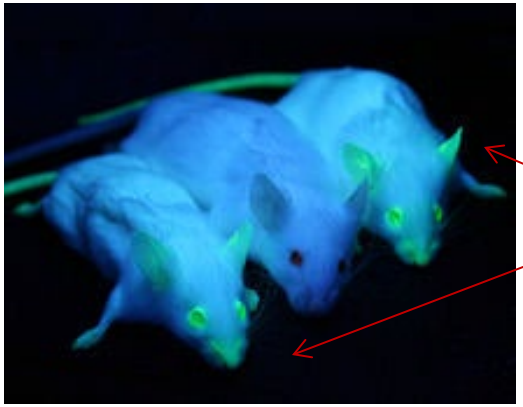
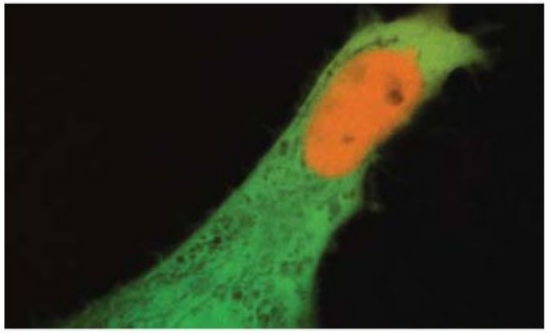


Figure 3



Transgenic mice



Green-to-red photoconversion of Dendra2 in cell nucleus.

"When I heard about it, my jaw was down for a week…" Betzig

Eric Betzig

No publications between 1998 and 2005

- Born in 1960 in Ann Arbor, Michigan
- B.Sc. (1983) in Physics from Caltech
- M. Sc. (1985) and Ph. D. in Applied and Engineering Physics, Cornell U
- 1988-1994 Bell Labs
- 1994 – founded NSOM Enterprises
- 1996 – abandoned microscopy; vice president of R&D at his father's machine tool firm, Ann Arbor, MI
- 2002 – founded New Millenium Research in Okemos, MI
- 2005 -2017 – at Janelia Farm Research Campus of the Howard Hughes Medical Institute, Ashburn, VA

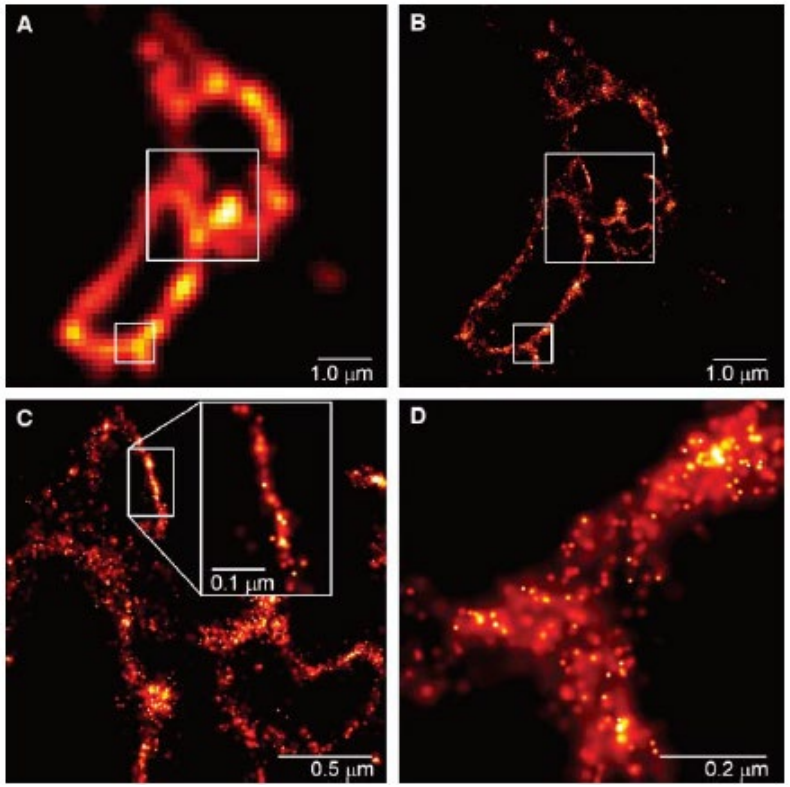
Also: very outspoken – check out his Nobel Lecture

15 SEPTEMBER 2006 VOL 313 SCIENCE

Imaging Intracellular Fluorescent Proteins at Nanometer Resolution

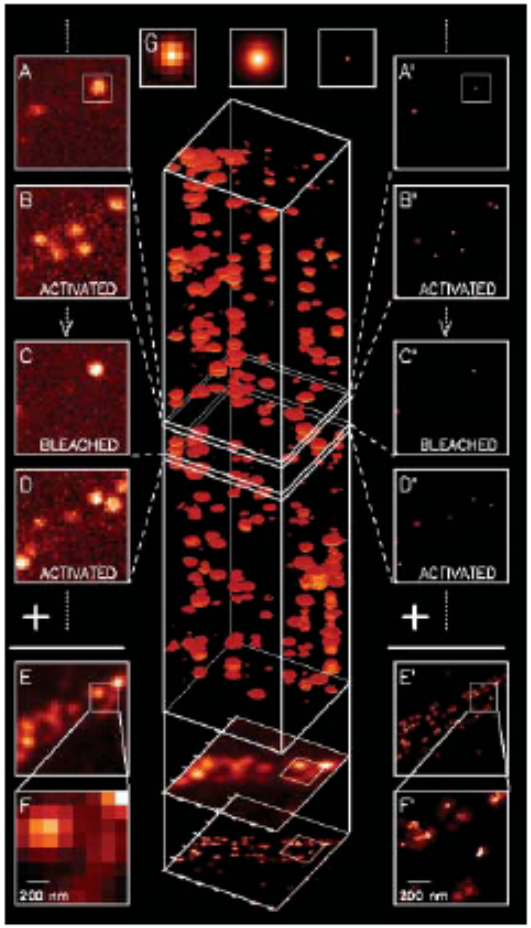
Eric Betzig,[1,2]*† George H. Patterson,[3] Rachid Sougrat,[3] O. Wolf Lindwasser,[3] Scott Olenych,[4] Juan S. Bonifacino,[3] Michael W. Davidson,[4] Jennifer Lippincott-Schwartz,[3] Harald F. Hess[5]*

confocal PALM



PALM = photoactivated localization microscopy

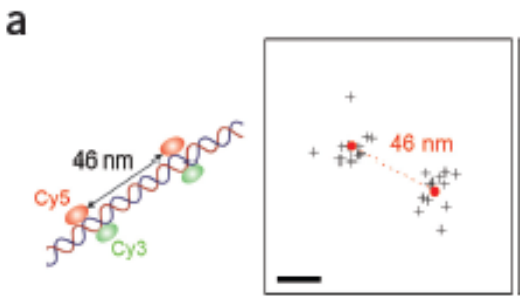
- Activate small subset of molecules at 405 nm
- Image at 561 nm until they photobleach
- Activate another subset at 405 nm
- …
- Fit image from each molecule using expected PSF to determine position and uncertainty (sub-nm accuracy)
- Resolution depends on the uncertainty and density of the molecules

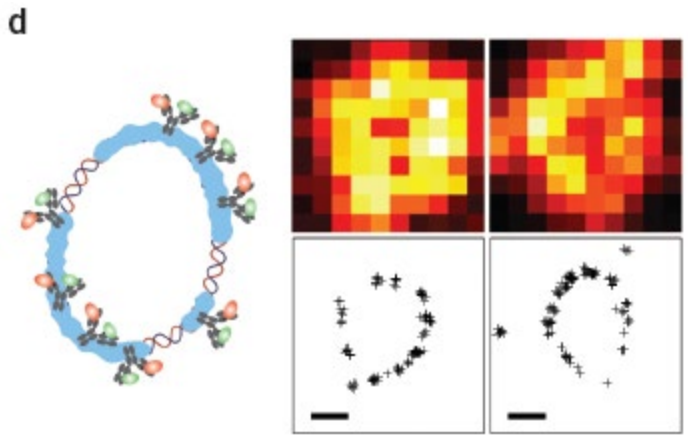


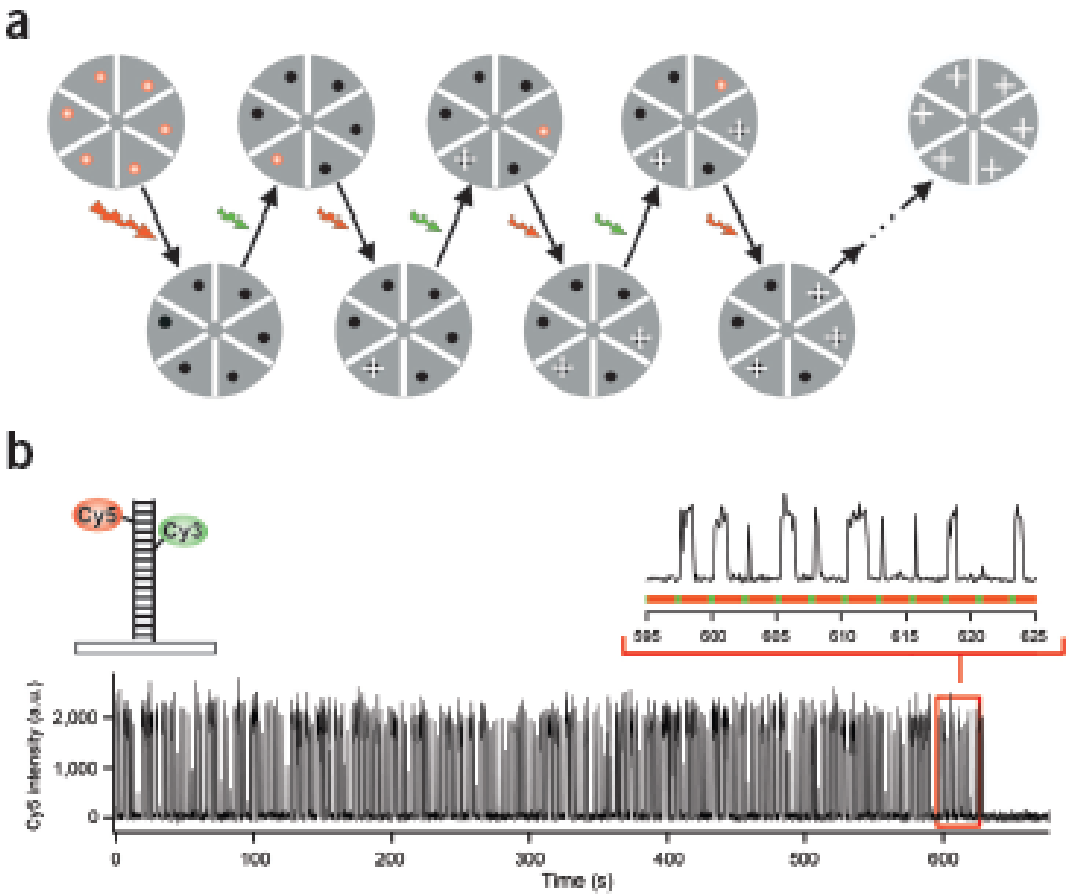
NATURE METHODS

Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)

Michael J Rust[1,5], Mark Bates[2,5] & Xiaowei Zhuang[1,3,4]







Nature Methods 3, 793 (2006)

Vol 466|29 July 2010|doi:10.1038/nature09163 nature

LETTERS

Subnanometre single-molecule localization, registration and distance measurements

Alexandros Pertsinidis[1,2], Yunxiang Zhang[1,2] & Steven Chu[1,2,3,4]†

Here we report a distance resolution of s_{reg} = 0.50 nm and an absolute accuracy of $s_{distance}$ = 0.77 nm in a measurement of the separation between differently colored fluorescent molecules using conventional far-field fluorescence imaging in physiological buffer conditions. The statistical uncertainty in the mean for an ensemble of identical single-molecule samples is limited only by the total number of collected photons, to s_{loc}<0.3 nm, which is $3x10^{-3}$ times the size of the optical PSF. Our method may also be used to improve the resolution of many subwavelength, far-field imaging methods such as those based on co-localization of molecules that are stochastically switched on in space. The improved resolution will allow the structure of large, multi-subunit biological complexes in biologically
relevant environments to be deciphered at the single-molecule level.

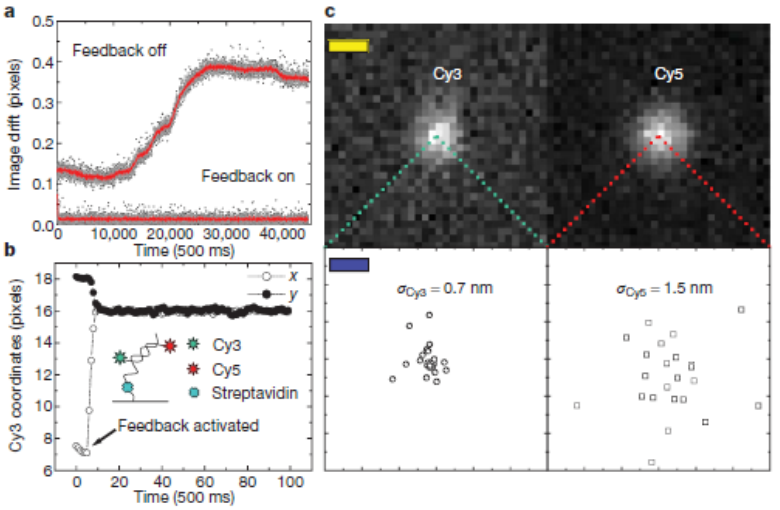


Figure 1 | Active feedback control. **a**, Performance of the actively stabilized imaging system. Black symbols: 2D registration of the green and red images at a rate of 500 ms^{-1} over 6.5 h; red lines: 64-point-average (1/10 points plotted). With feedback turned off, relative drift is ~0.3 pixels, or 19 nm, whereas with feedback on, the long-term registry is maintained to <0.01 pixels, or 0.64 nm. **b**, Feedback control on the position of single Cy3 molecules. The position noise is limited only by the number of collected photons in each frame (~3,500): $s_{loc} \approx$ 2–3 nm. **c**, Molecule-to-molecule reproducibility for the Cy3–Cy5 20-base-pair (bp) dsDNA. Top panels: images of single Cy3 and Cy5 molecules; scale bar, 320 nm. Bottom panels: each symbol represents the average position of a separate molecule ($N = 25$), averaged over all the frames during which the Cy3 was locked and before the Cy5 photobleached (typically 10–100 frames or 5–50 s). σ_{Cy3} and σ_{Cy5} are the standard deviations of the positions over the set of Cy3 and Cy5 molecules, respectively. Scale bar, 3.2 nm.

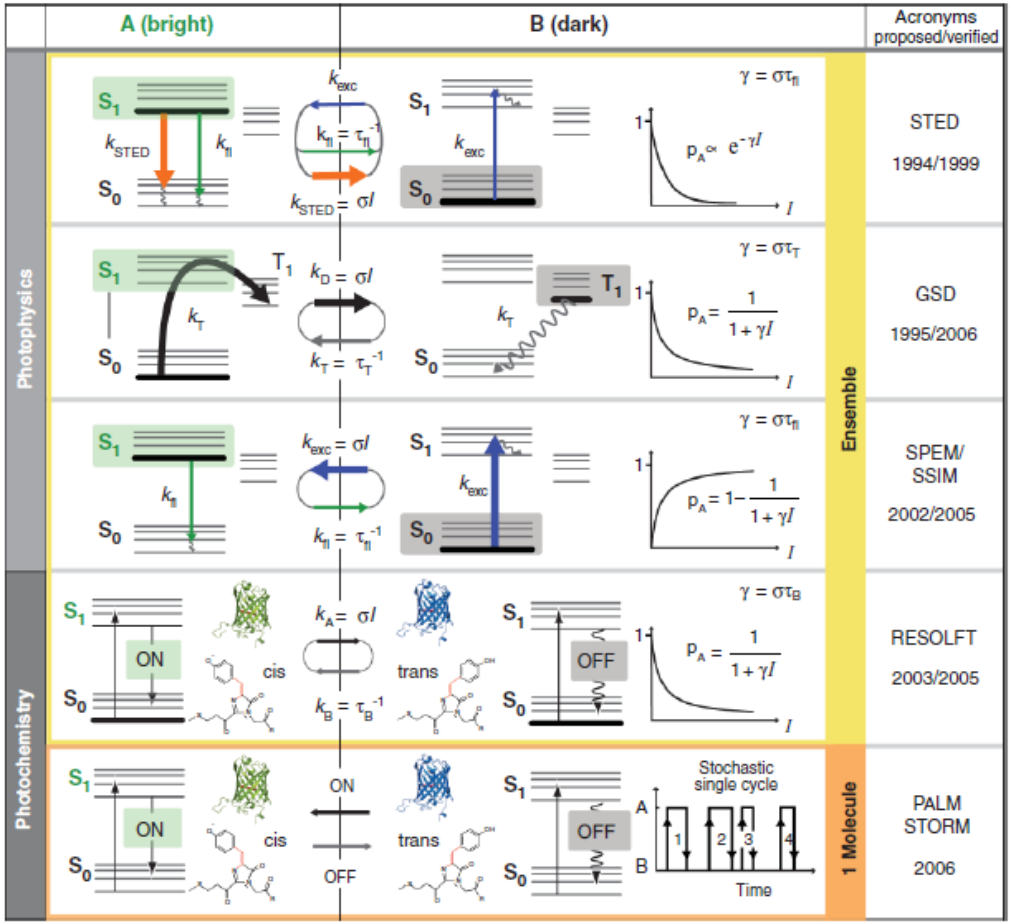
Nobel Prize in Physics 1997

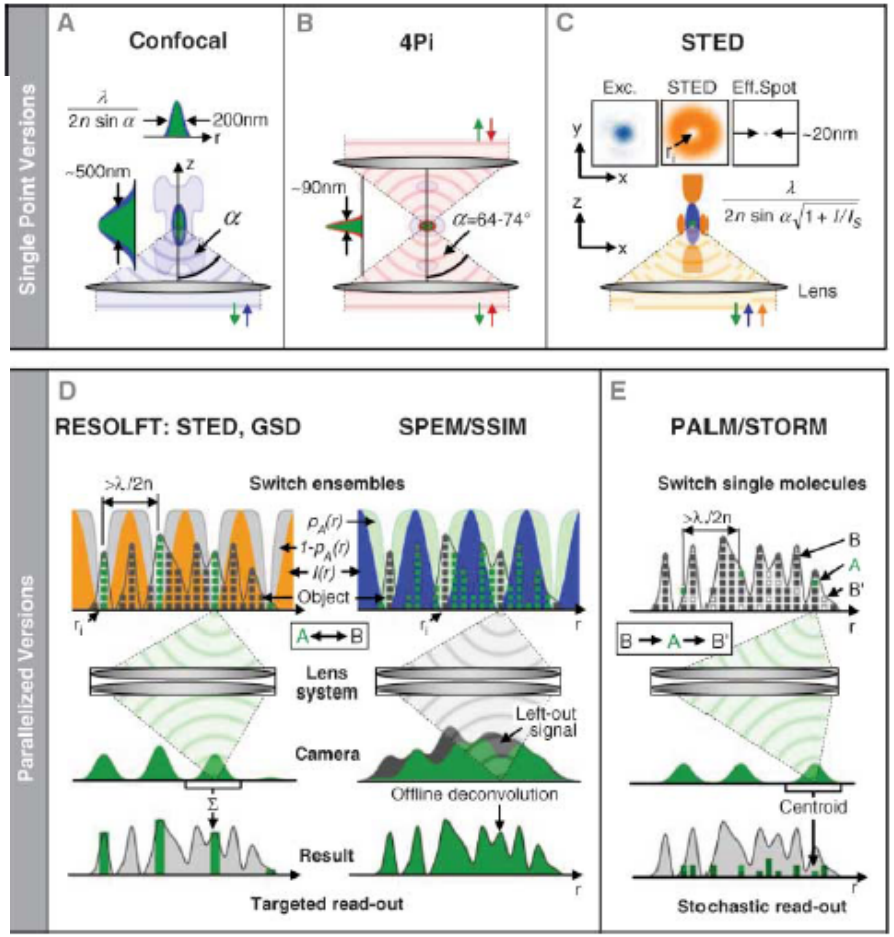
The Nobel Prize in Physics 1997 was awarded jointly to Steven Chu (Stanford U), Claude Cohen-Tannoudji (École Normale Supérieure, Paris, France) and William D. Phillips (NIST) *"for development of methods to cool and trap atoms with laser light"*.

Far-Field Optical Nanoscopy

Stefan W. Hell

In 1873, Ernst Abbe discovered what was to become a well-known paradigm: the inability of a lens-based optical microscope to discern details that are closer together than half of the wavelength of light. However, for its most popular imaging mode, fluorescence microscopy, the diffraction barrier is crumbling. Here, I discuss the physical concepts that have pushed fluorescence microscopy to the nanoscale, once the prerogative of electron and scanning probe microscopes. Initial applications indicate that emergent far-field optical nanoscopy will have a strong impact in the life sciences and in other areas benefiting from nanoscale visualization.





Eric Betzig

"…from rags to riches"

- Born in 1960 in Ann Arbor, Michigan
- B.Sc. (1983) in Physics from Caltech
- M. Sc. (1985) and Ph. D. in Applied and Engineering Physics, Cornell U
- 1988-1994 Bell Labs
- 1994 – founded NSOM Enterprises
- 1996 – abandoned microscopy; vice president of R&D at his father's machine tool firm, Ann Arbor, MI
- 2002 – founded New Millenium Research in Okemos, MI
- 2005-2017 – at Janelia Farm Research Campus of the Howard Hughes Medical Institute, Ashburn, VA
- since 2017 – at UC Berkeley and LBNL

W. E. Moerner

- Born 1953, Pleasanton, CA, USA
- Grew up in San Antonio, TX
- B.Sc. (1975) from Washington U in St. Louis (Physics, Math, EE)
- M.Sc. (1978) and Ph.D. (1982) in Physics from Cornell U
- 1981-1995 IBM Almaden, San Jose, CA
- 1995-1998 Professor, Dept. of Chemistry and Biochemistry, UCSD
- since 1998 Professor, Dept. of Chemistry, Stanford U

WEMpire

Moerner lab 2002

Moerner lab 2014

Fond memories

- Safety first, especially at pool parties
- Support and protect your lab members
- Always label your optics and your drawers
- Lists are good. Lists of lists are better
- Make friends with the people in the lab upstairs…

and check their plumbing often

Laser Safety

Because you only get two chances

WEMpire

- Pool parties at W. E.’s house

- Music parties at W. E.’s house

- Mandatory water rocket launch…
  or tennis ball launch… or cork launch

- Wine and cheese parties at group meetings

From W.E.'s publication list (>450 papers total):

...

265. S. R. P. Pavani*, M. A. Thompson*, J. S. Biteen, S. J. Lord, N. Liu, R. J. Twieg, R. Piestun, and W. E. Moerner, (*equal contributions), "Three-Dimensional Single-Molecule Fluorescence Imaging Beyond the Diffraction Limit Using a Double-Helix Point Spread Function," *Proc. Nat. Acad. Sci. (USA)* **106**, 2995-2999 (2009), published online 11 February 2009.

336. Yoav Shechtman, Steffen J. Sahl, Adam S. Backer, and W. E. Moerner, "Optimal Point Spread Function Design for 3D Imaging," *Phys. Rev. Lett*. **113**, 133902 (2014), (DOI: 10.1103/PhysRevLett.113.133902, published online September 26, 2014)

NANO LETTERS

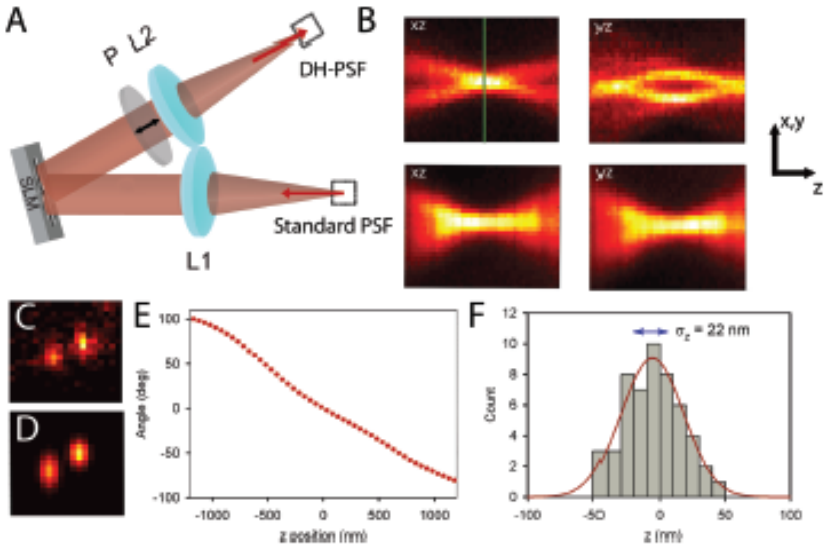
Nano Lett. 2010, 10, 211-218

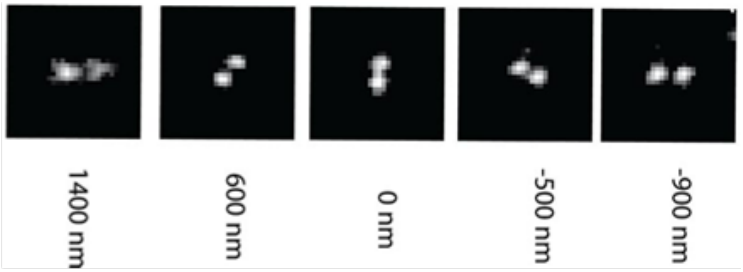
pubs.acs.org/NanoLett

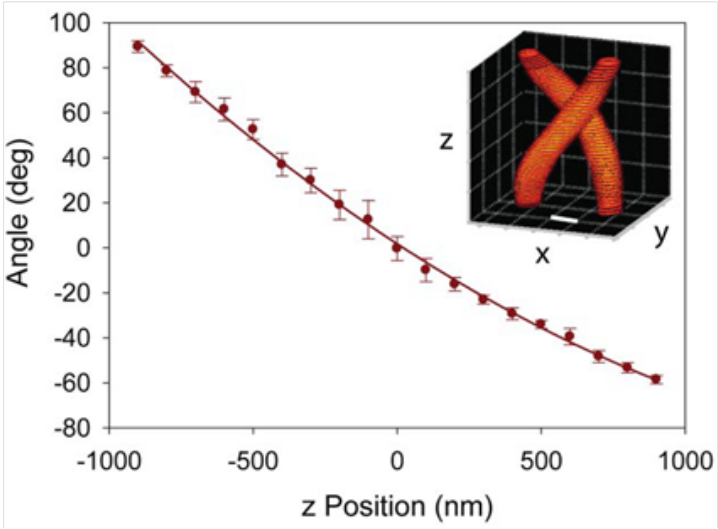
Localizing and Tracking Single Nanoscale Emitters in Three Dimensions with High Spatiotemporal Resolution Using a Double-Helix Point Spread Function

Michael A. Thompson,[†,§] Matthew D. Lew,[†,⊥,§] Majid Badieirostami,[†] and W. E. Moerner*[,†]

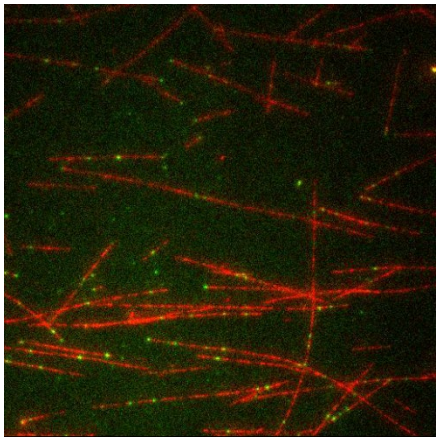
[†]Departments of Chemistry and [⊥]Electrical Engineering, Stanford University, Stanford, California 94305

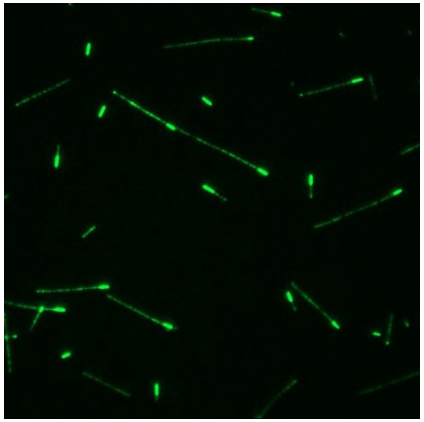






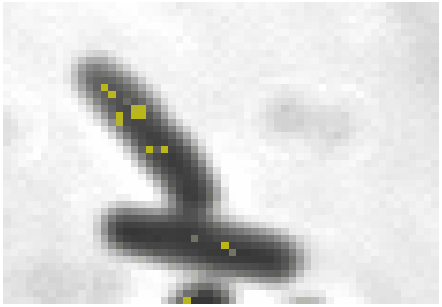
Applications: biophysics





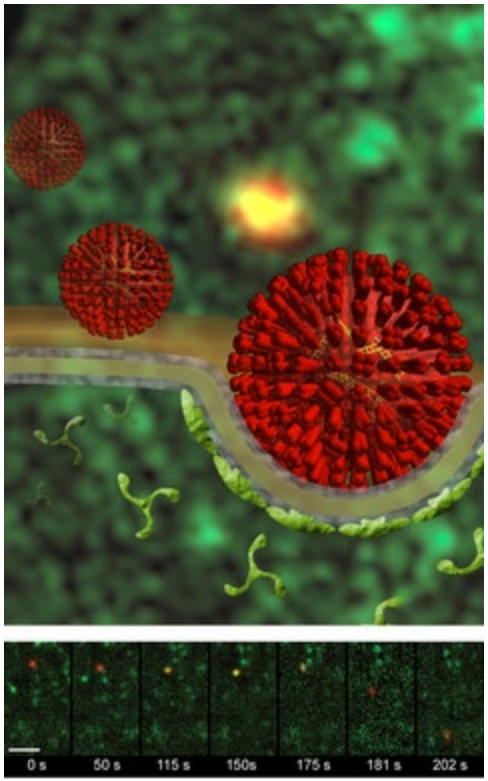
• Molecular motors (cargo transport in cells)

Videos from Weihong Qiu (OSU)



• Cell division (stochastically-triggered phenotype change); enzymatic activity; gene expression

Video from S. Xie (Harvard U)

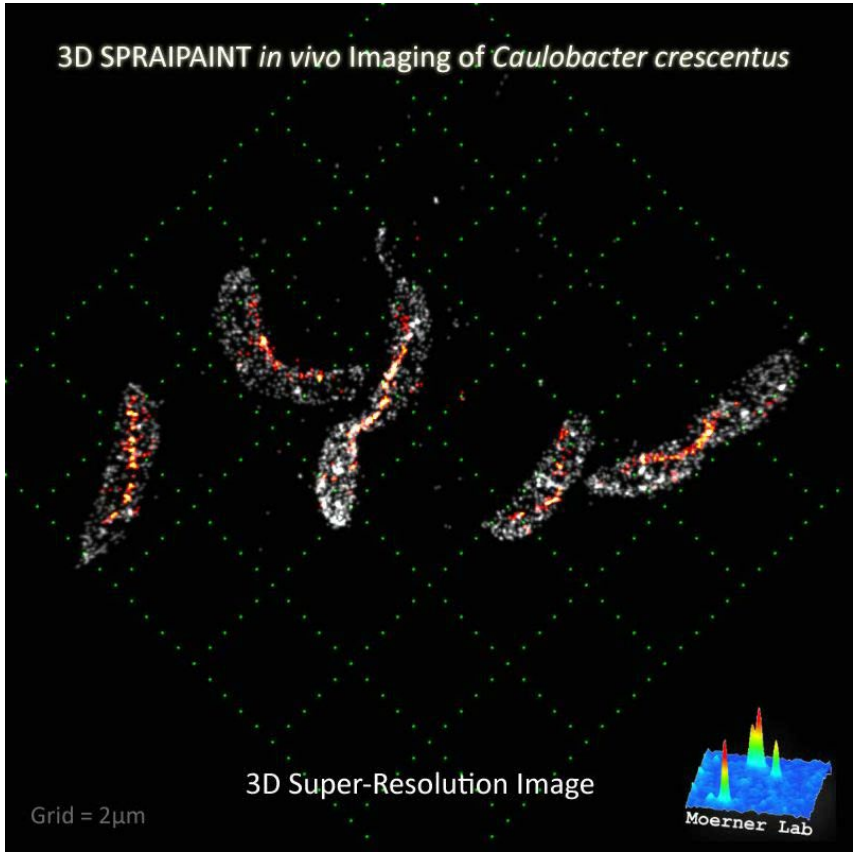


• Single virus tracking in live cells
Image from X. Zhuang (Harvard U)

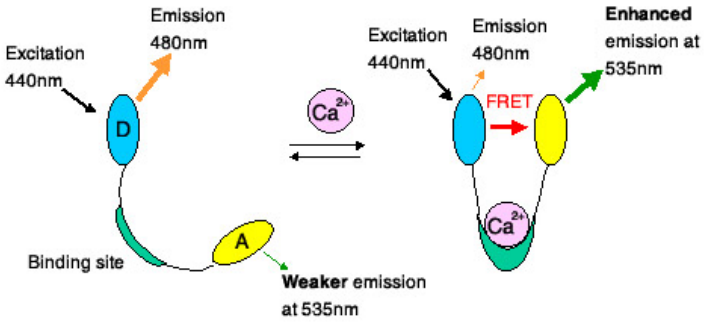
Applications: biophysics

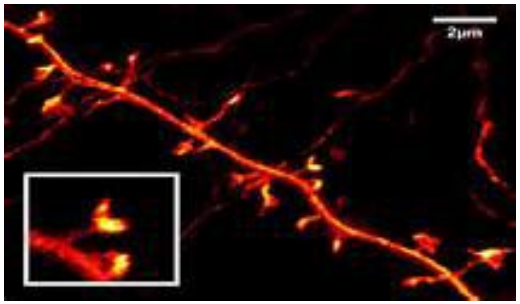
- In vivo super-resolution imaging

Video from W.E. Moerner (Stanford U)



- Protein folding

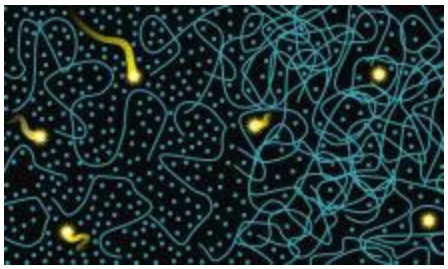




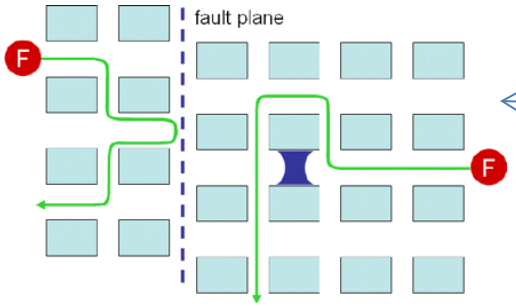
- Neuron function (mouse brain)

Image from S. Hell (Max Planck Institute)

Applications: materials science

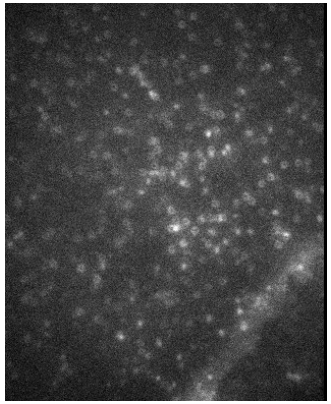


Polymer physics:
- Single molecule diffusion patterns probes heterogeneity in polymerization
- nonlinear rheology
- electrophoresis



- Catalysis:

Tracking of single molecule diffusion in porous materials = obstructions/faults = catalysis



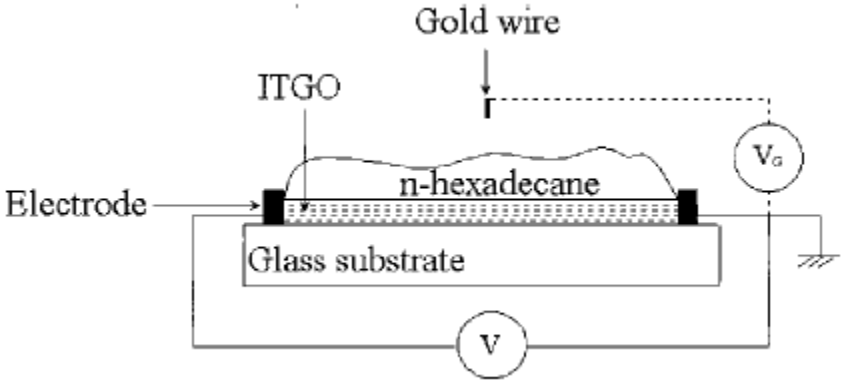
- Identifying micro-cracks in a crystal

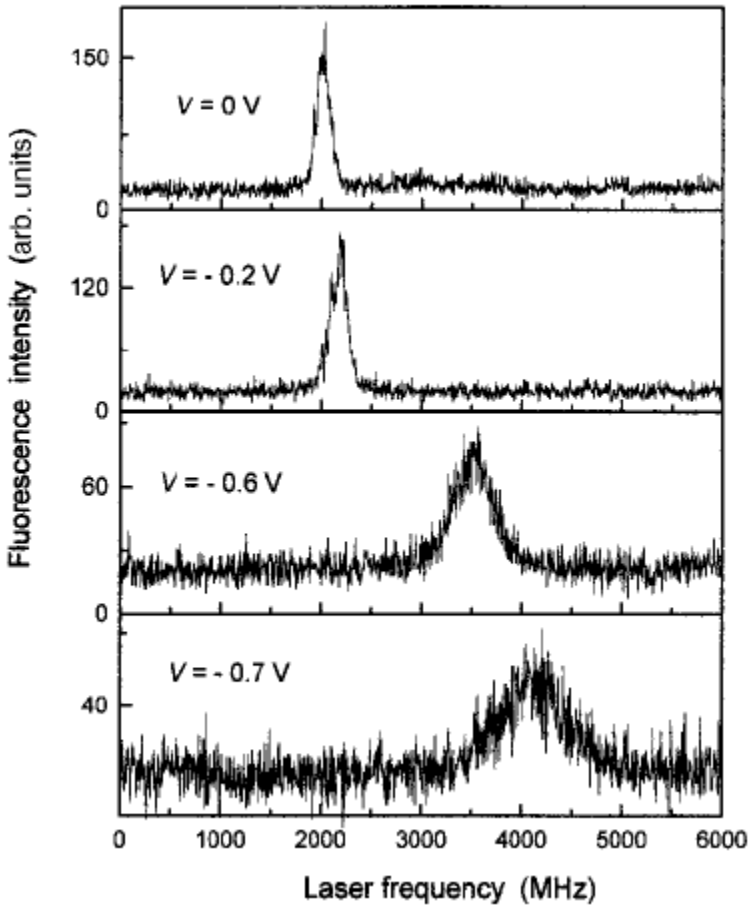
Video from W.E. Moerner

Applications: materials science

Probing local currents in semiconductors with single molecules

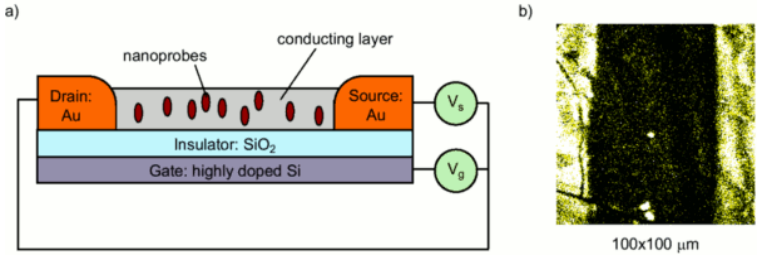
Jean-Michel Caruge and Michel Orrit

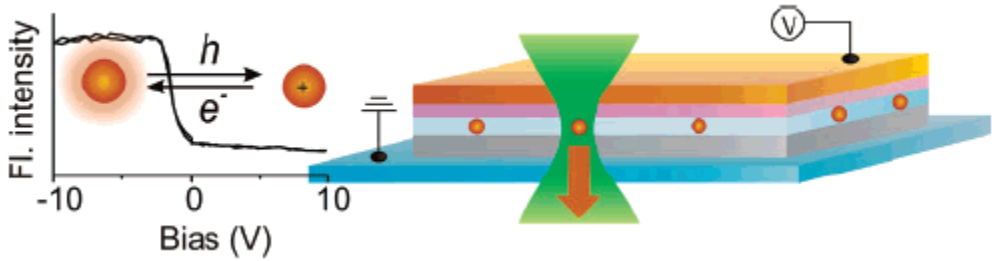




- E-field distribution
- Local thermometer (detect hot spots)

Applications: materials science





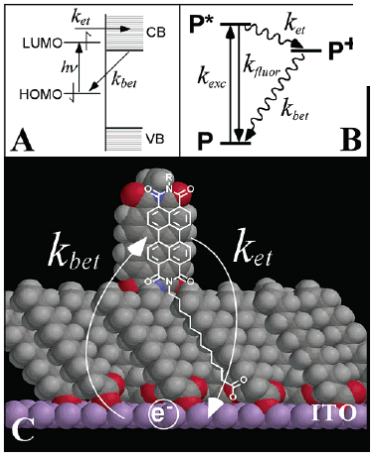
- In-situ nanoscale imaging of processes in organic thin-film transistors or solar cells

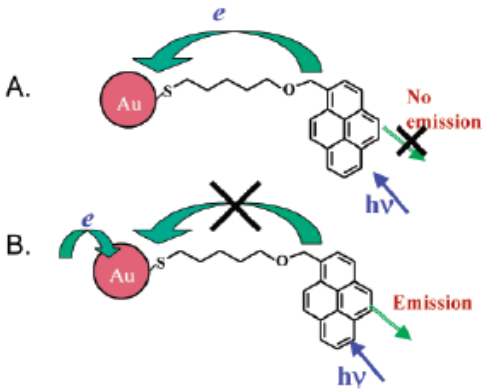
Images from M. Orrit and P. Barbara

- Charge transfer processes in quantum dots, molecules, nanoparticles

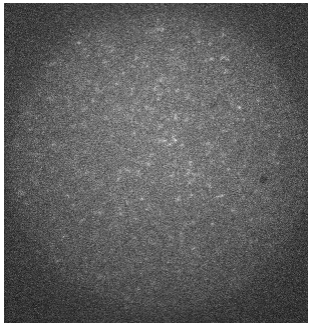
Single-Molecule Spectroscopy of Interfacial Electron Transfer

Michael W. Holman, Ruchuan Liu, and David M. Adams*



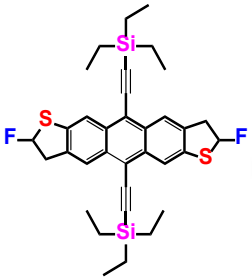


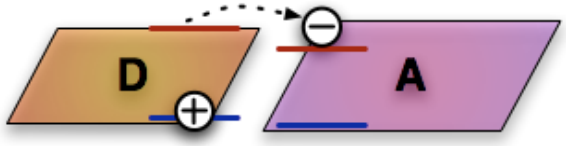
Applications: materials science

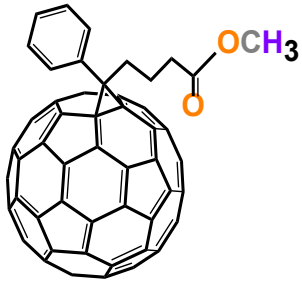


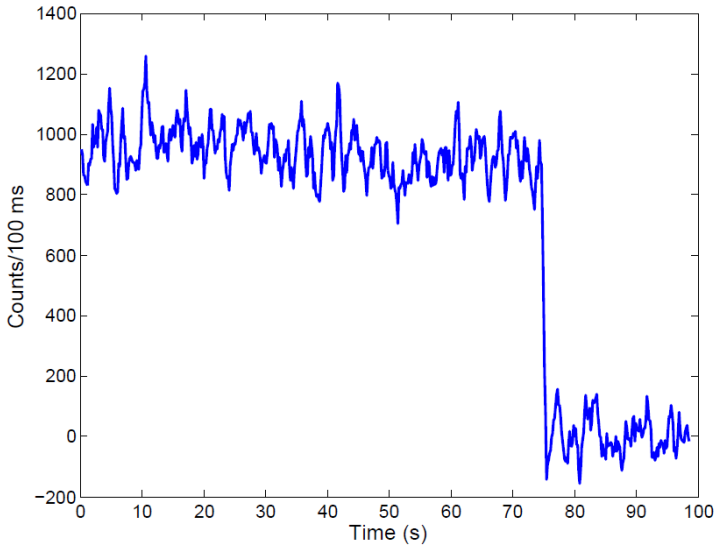
- Charge transfer in organic bulk heterojunctions

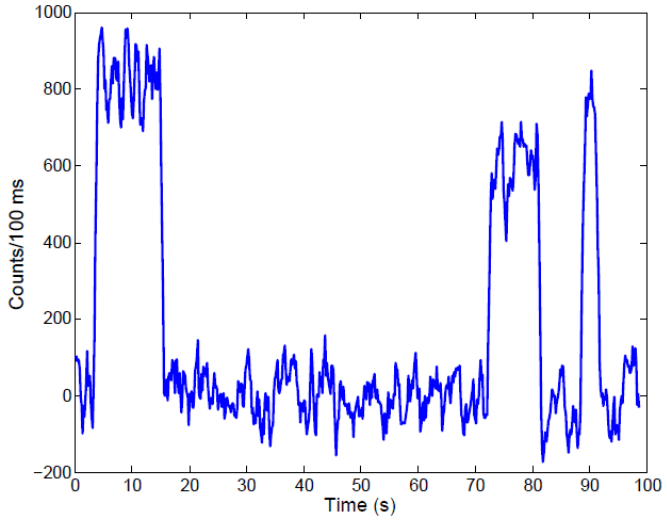
Videos from Rebecca Grollman





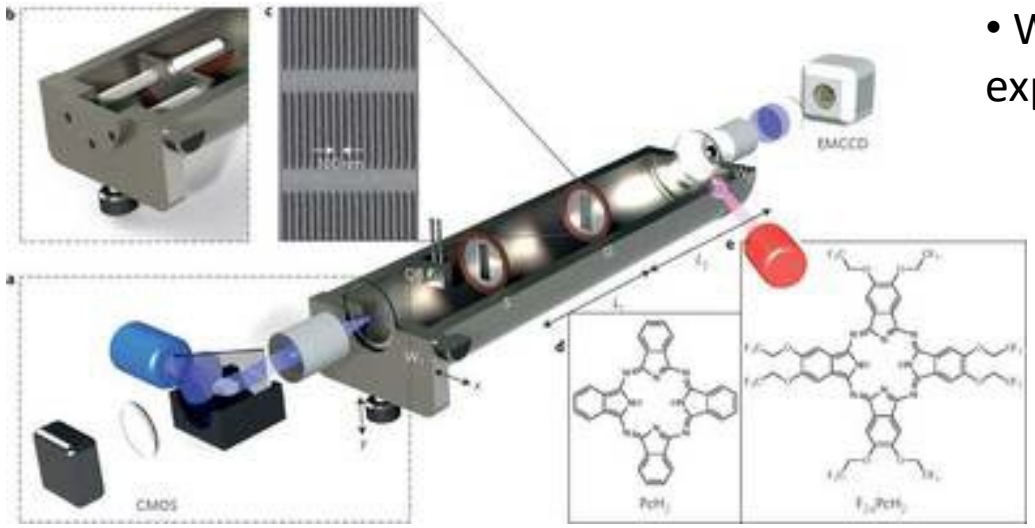




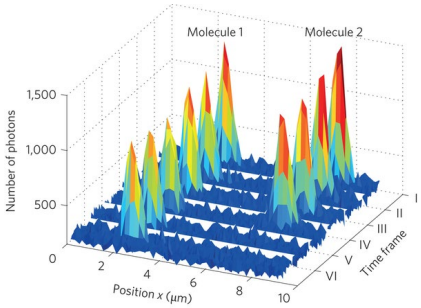


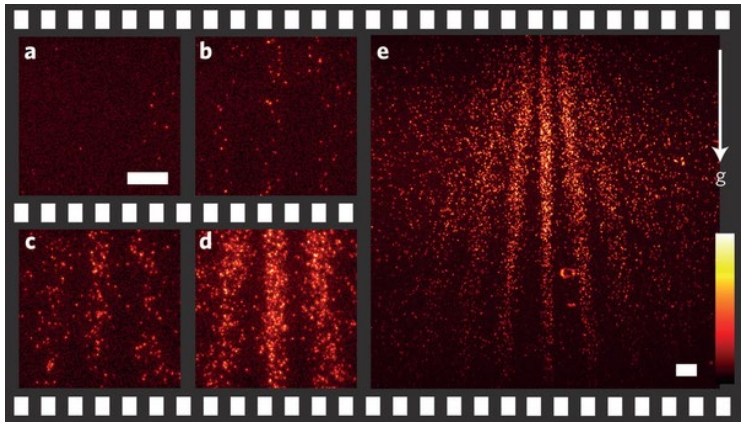
Applications: optics and quantum mechanics

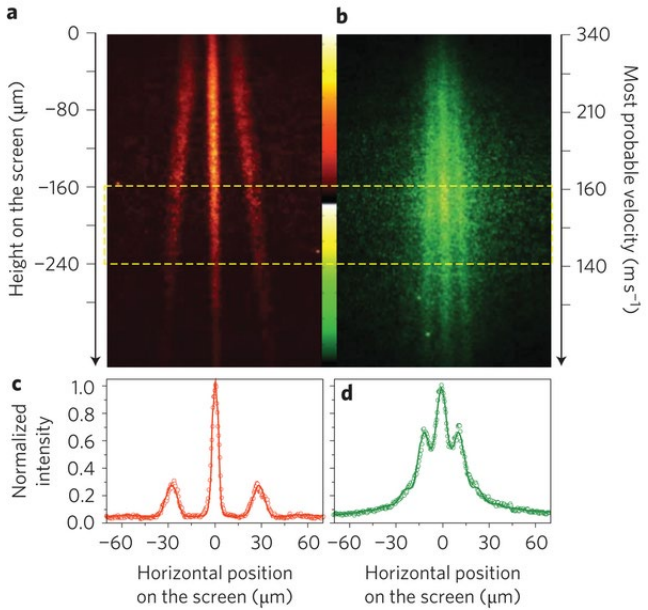
Nature Nanotech. 7, 297 (2012)



- Single photon sources
- Wave nature of matter (double-slit experiment with molecules)







Mw = 514 AMU, 1298 AMU

Future

- New photoswitchable molecules and photoswitching mechanisms
- New concepts for 3D/4D imaging (like double-helix microscope, passive pulse splitter to increase time resolution, etc.)

Driving forces:

- Living cells – need to collect lots of photons (kW-GW/cm2 – what are you doing to a poor cell?)
- high-resolution 3D imaging is slow
- need to look at cells inside the organism (adaptive optics)

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Epilogue

A lot of what you heard here is about taking risks…But you are hearing this from guys whose risks paid off...It's not a risk unless you fail most of the time. I want to dedicate my talk to any unknown people from any walk of life who have gambled their fortunes, their careers, and their reputations to try to take the risk but in the end failed… They should remember that it's the struggle itself that it's its own reward and the satisfaction that you gave it all you have to make a world a better place.

Eric Betzig, Nobel Prize in Chemistry 2014