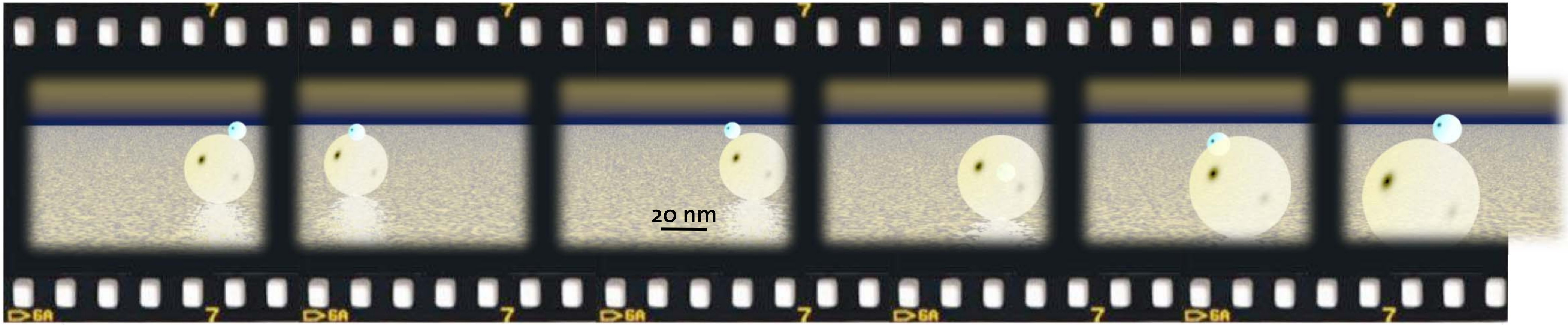
Optical microscopy at the limit

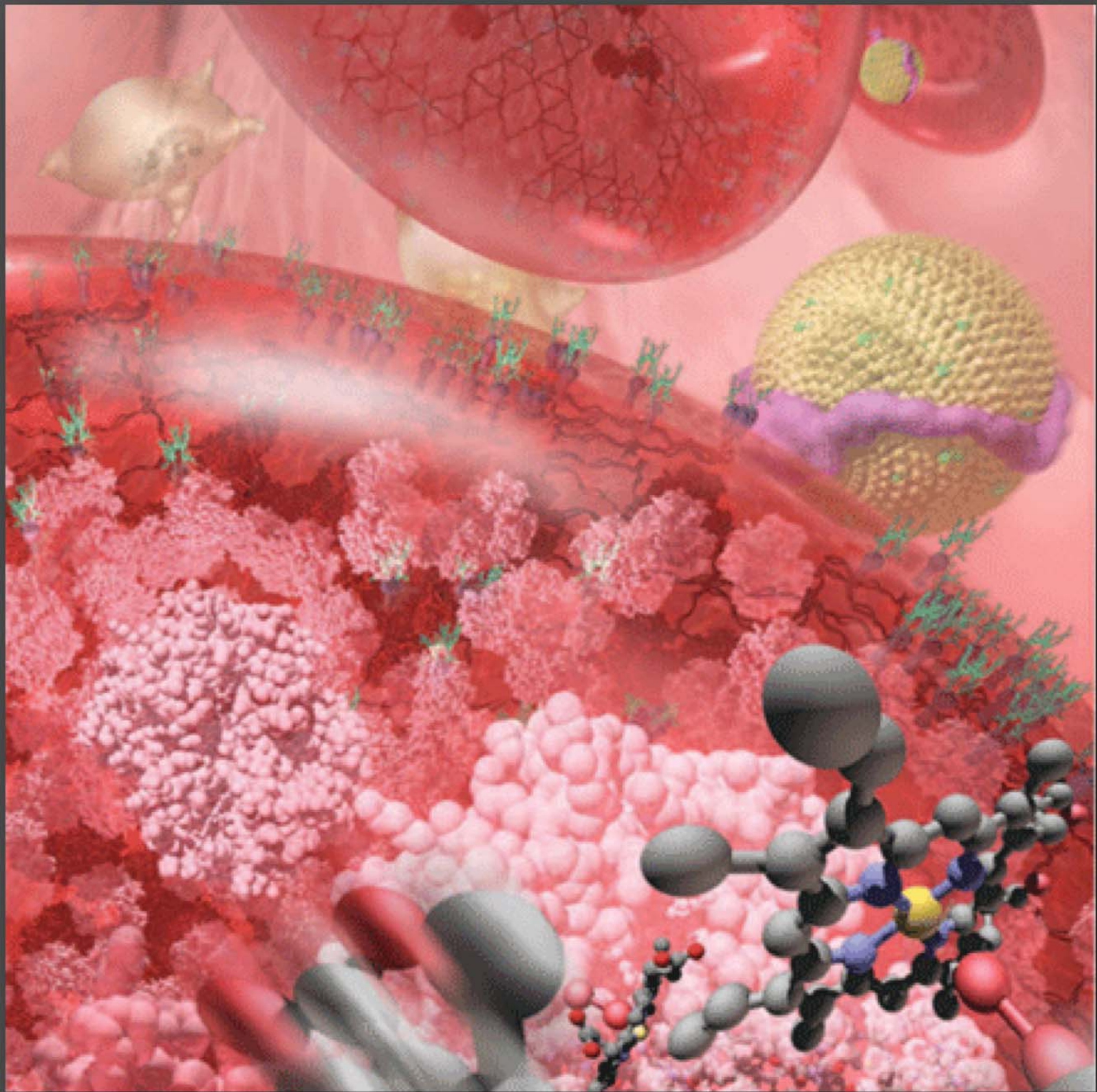


Philipp Kukura,[1] Helge Ewers,[2] Christian Müller,[1] Alois Renn,[1] Ari Helenius[2] and Vahid Sandoghdar[1]

[1]Nano-optics group, Laboratory of Physical Chemistry, ETH Zurich

[2]Institute of Biochemistry, ETH Zurich

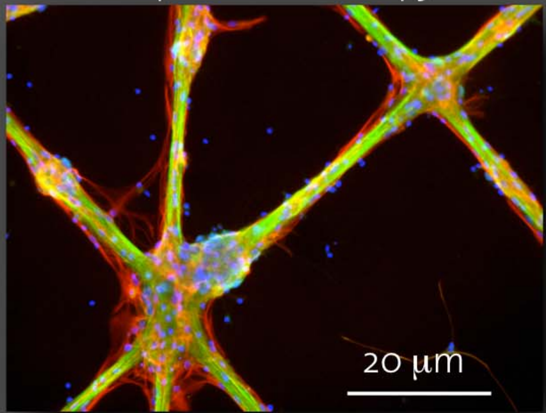
The desire to see small



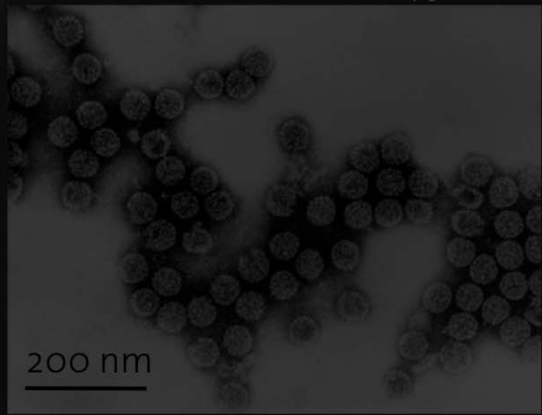
Different ways to see small

Using electromagnetic radiation

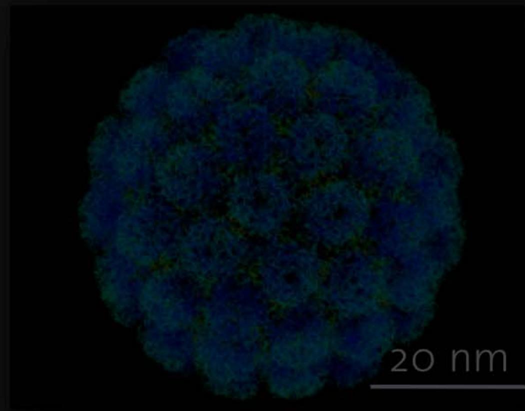
Optical Microscopy



Electron Microscopy

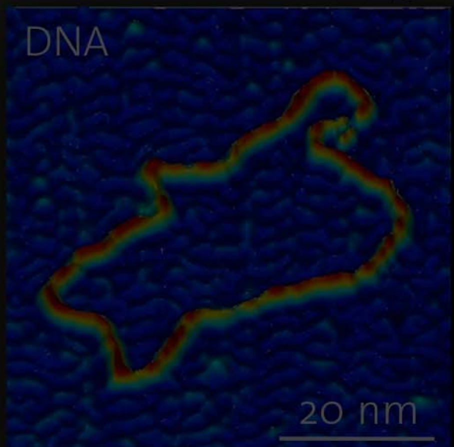


X-Ray Crystallography



Using a scanning probe

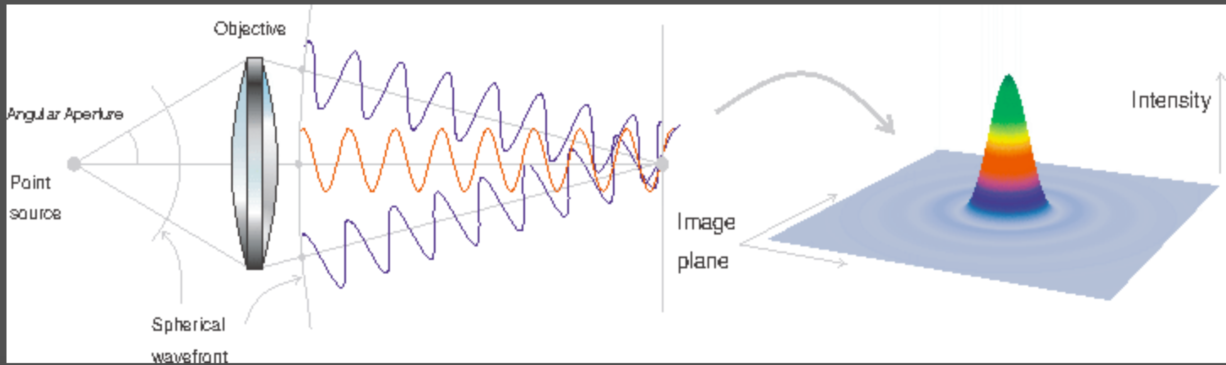
Atomic Force Microscopy

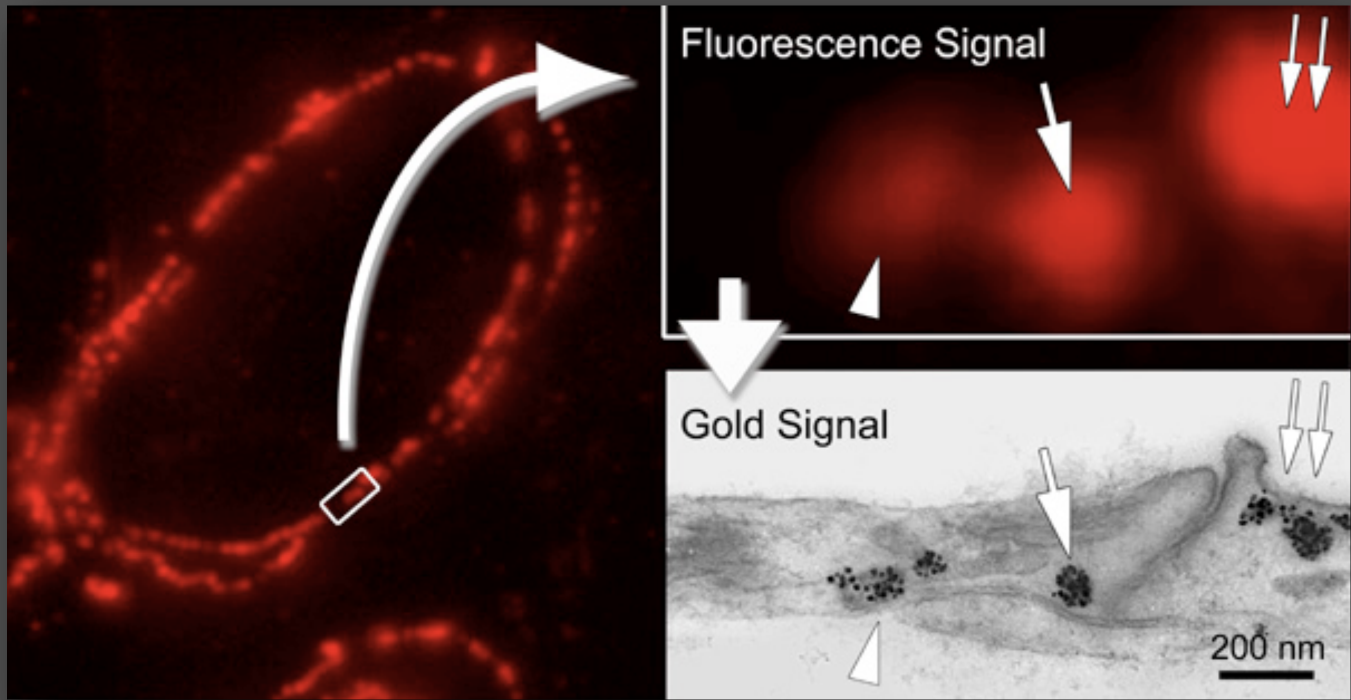


Scanning Tunneling Microscopy

For real time imaging of live specimens – optical microscopy wins

One big problem: the diffraction limit





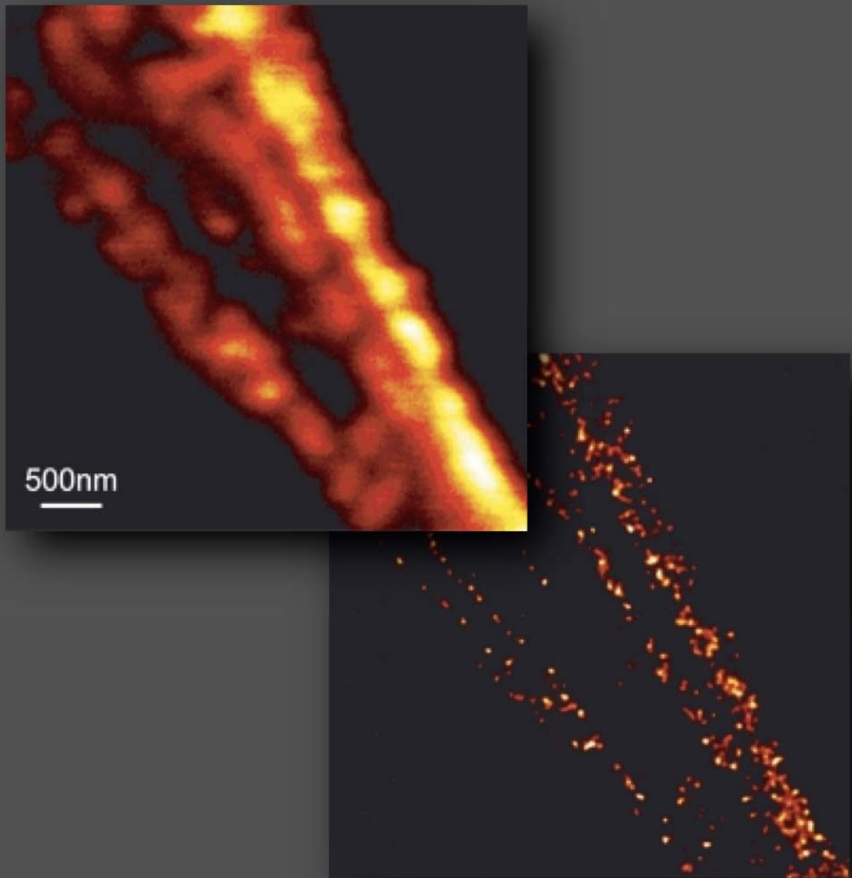
www.nanoprobes.com

No matter how small the light source – it looks (at least) as big as λ/2: > 200 nm

Recent developments to get around it

STED

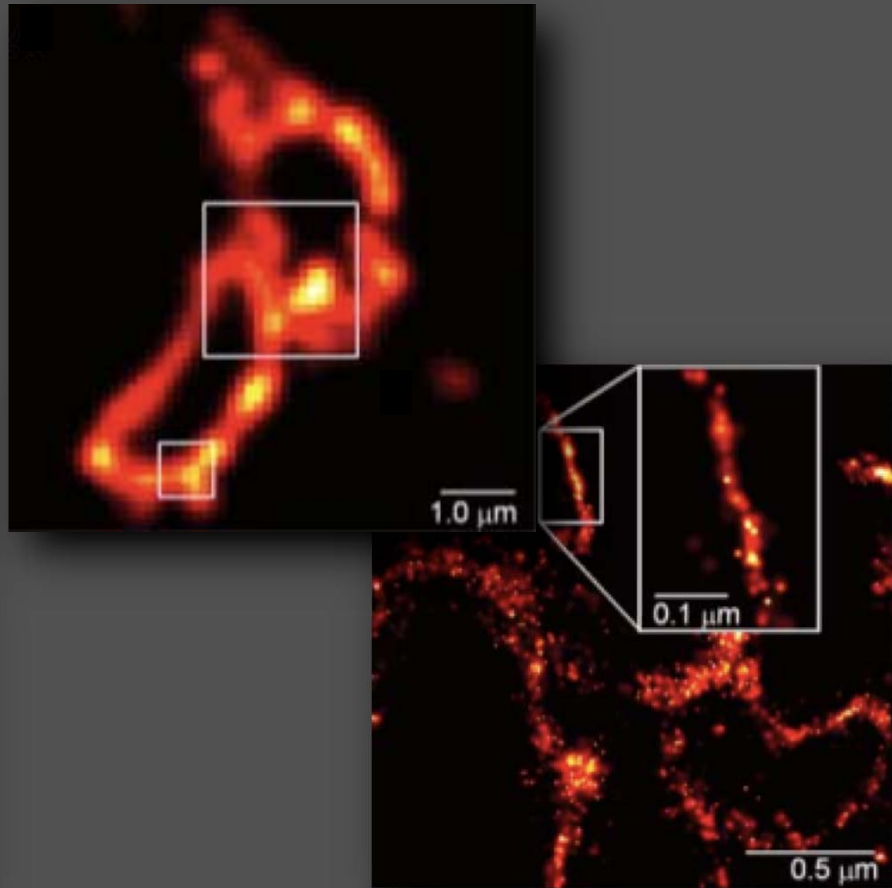
S. W. Hell, *Science* **316**, 1153 (2007)



Nonlinear contrast improvement

PALM, STORM, etc.

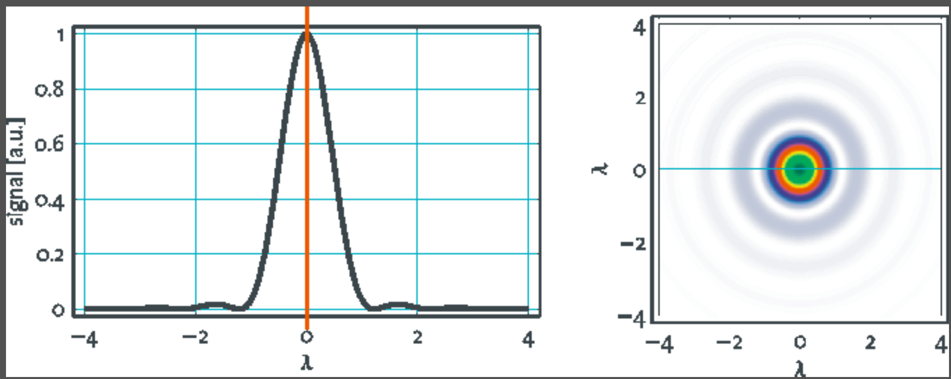
E. Betzig et al., *Science* **313**, 1643 (2006)
M. J. Rust et al., *Nat. Methods* **3**, 793 (2006)
S. Hess et al.; *Biophys. J.* **91**, 4258 (2006)

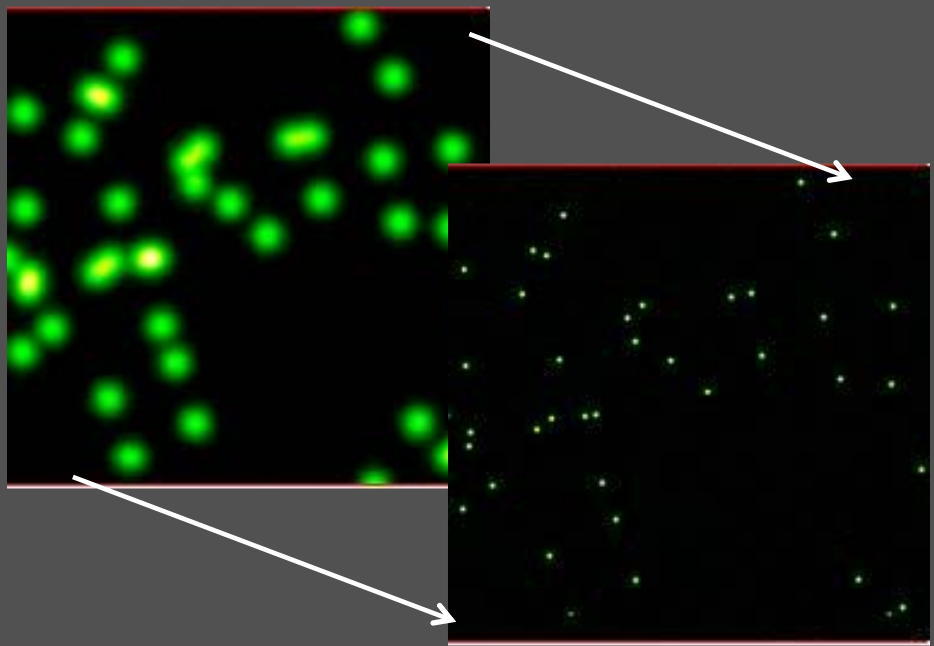


Single molecule localization

Single molecule localization

Determine center through statistical fit of ideal point spread function

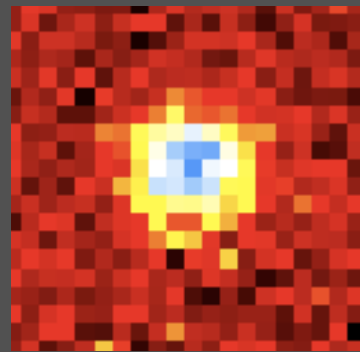




Accuracy scales with the number of detected photons, *N*:

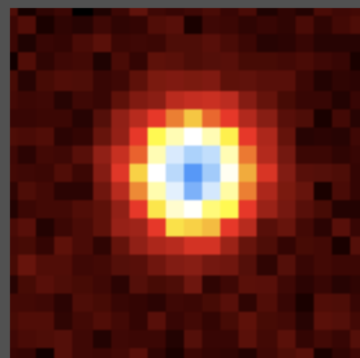
$N^{-(1/2)}$

N = 100



σ = ~ 10 nm

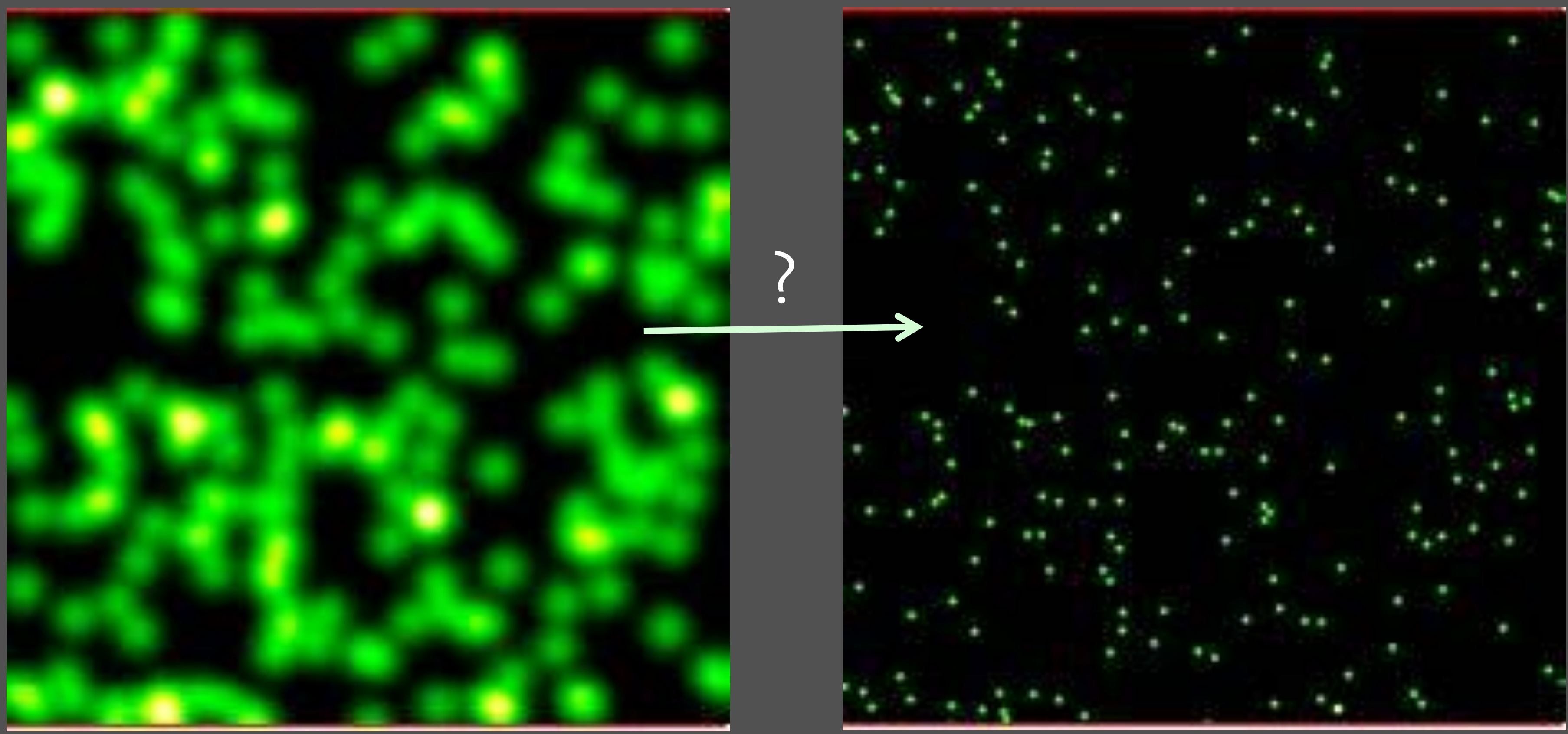
N = 10000



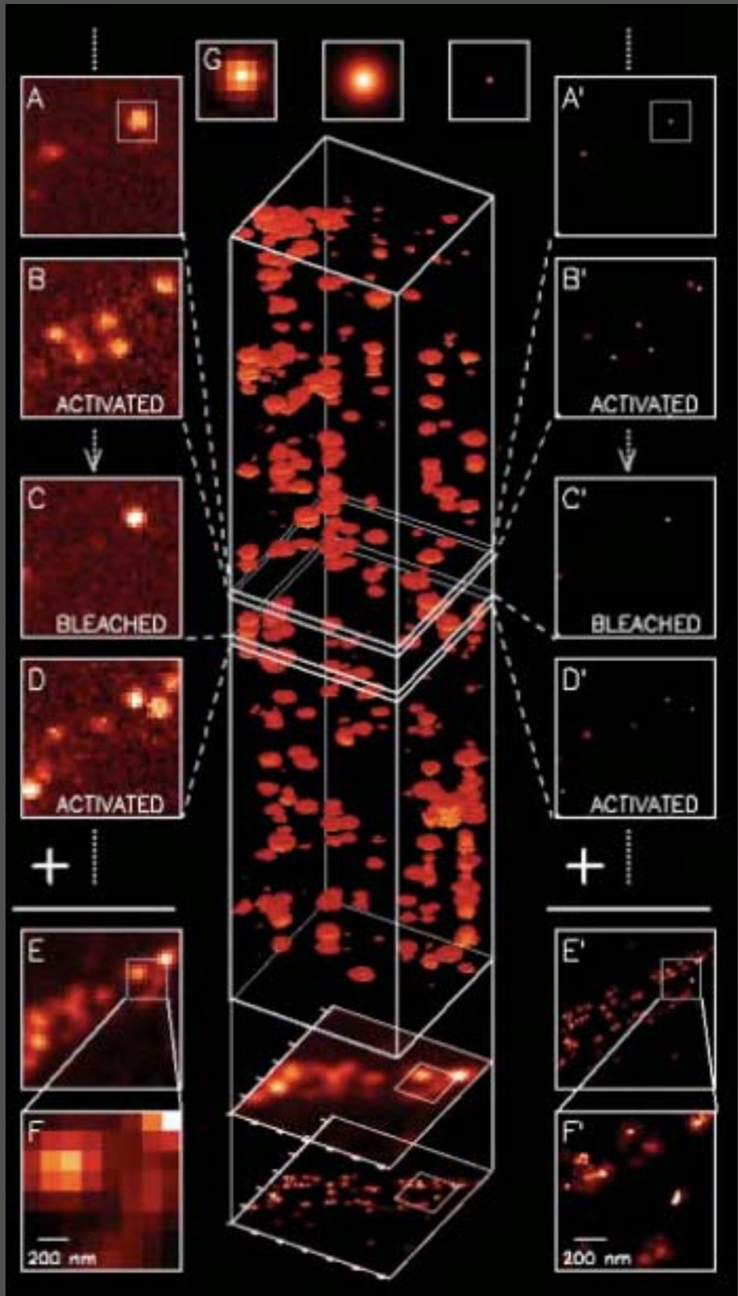
σ = ~ 1 nm

The trouble with *co*localization

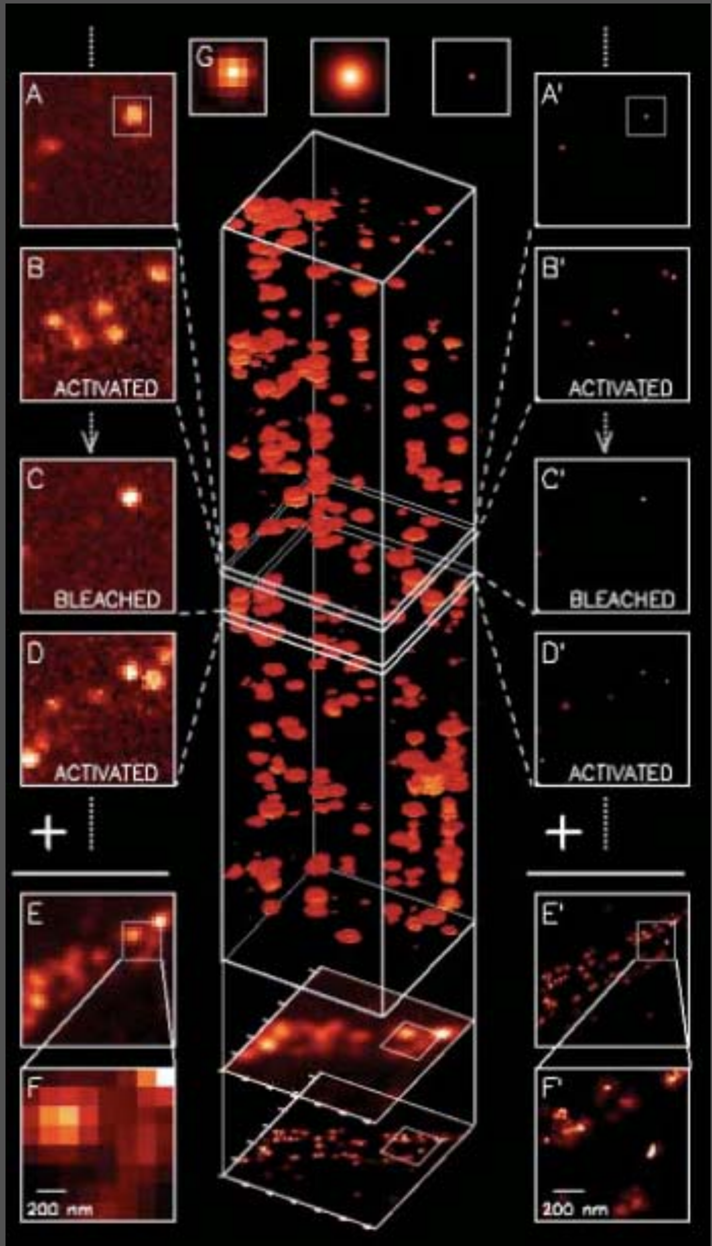
<u>What to do if the density becomes too high?</u>

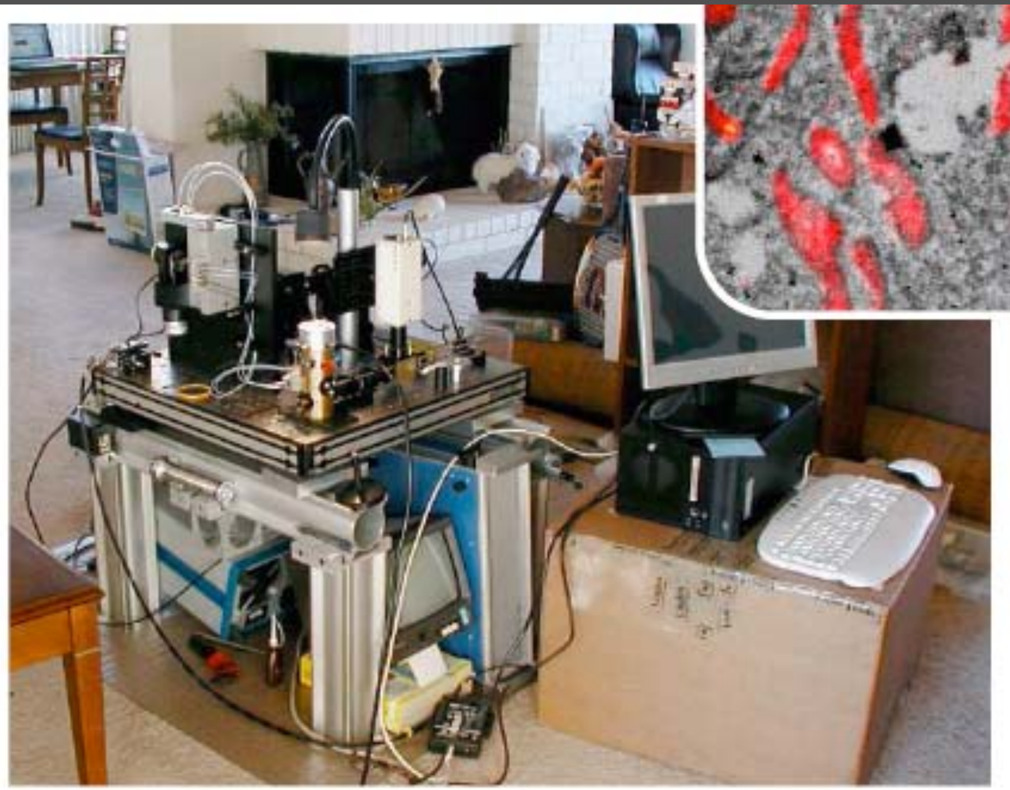


Photoactivation



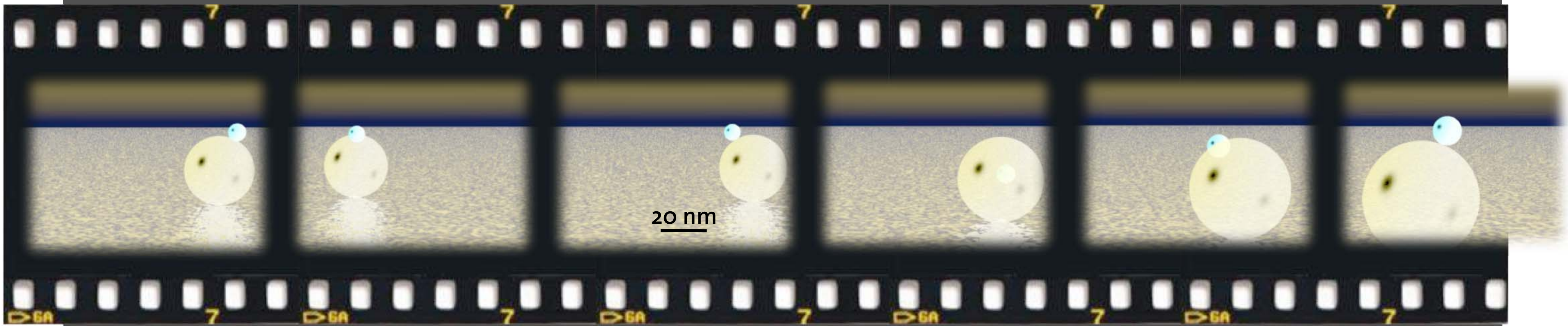
Photoactivation





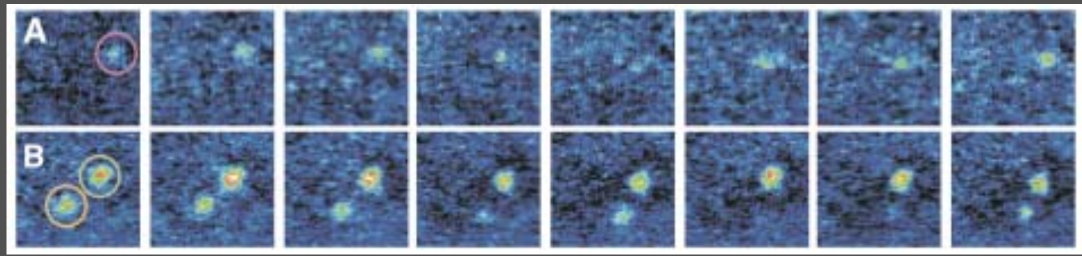
Up close. A high-tech microscope, assembled in a living room (*above*), revealed molecules (red, *inset*) nanometers apart inside a cell's mitochondria.

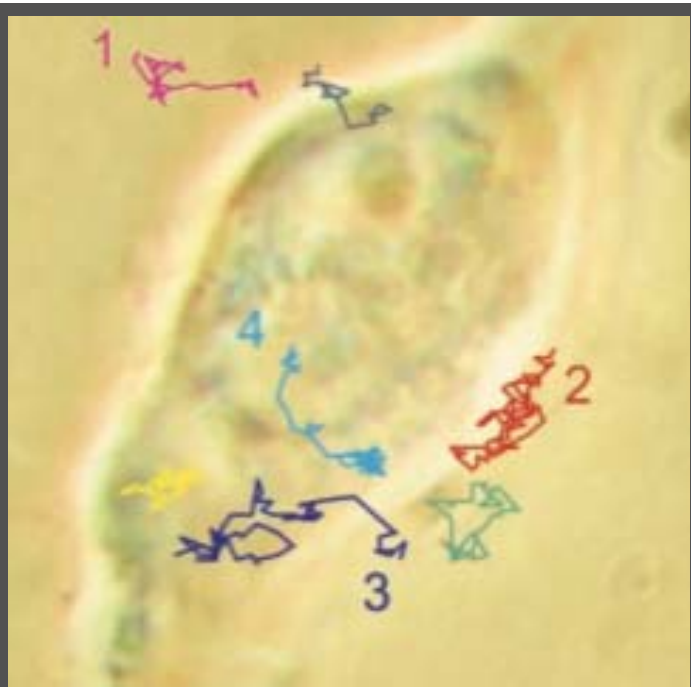
What about dynamics?



Tracking single molecules

Visualizing the infection pathway of an adeno-associated virus



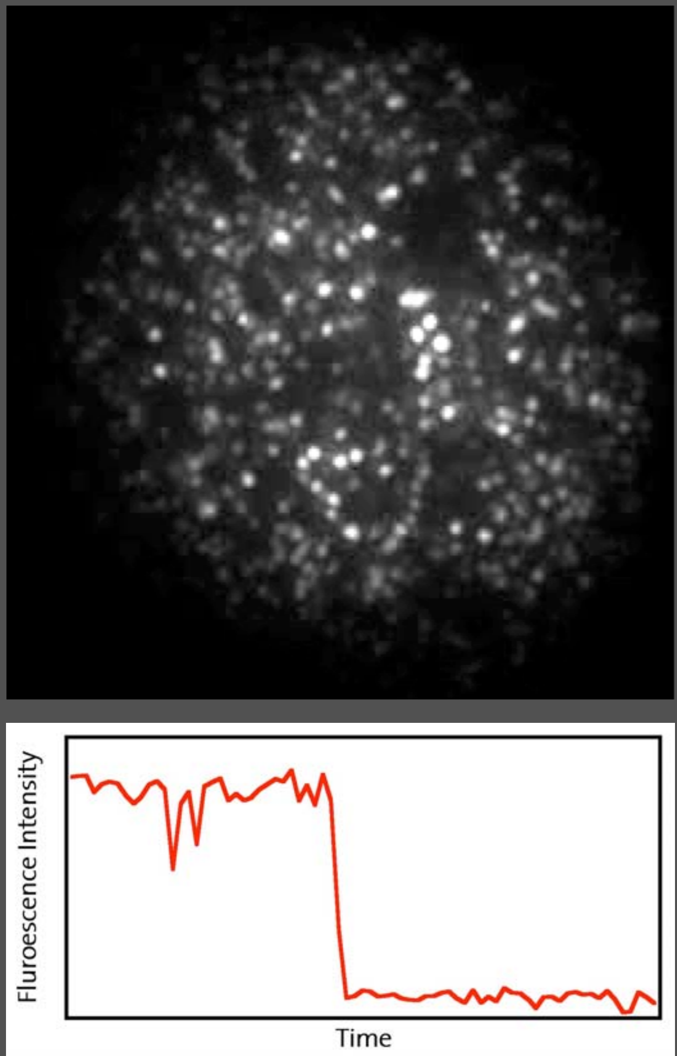


Localize virus in each frame
Connect positions to create trajectories

G. Seisenberger et al., *Science* **294**, 1929 (2001)

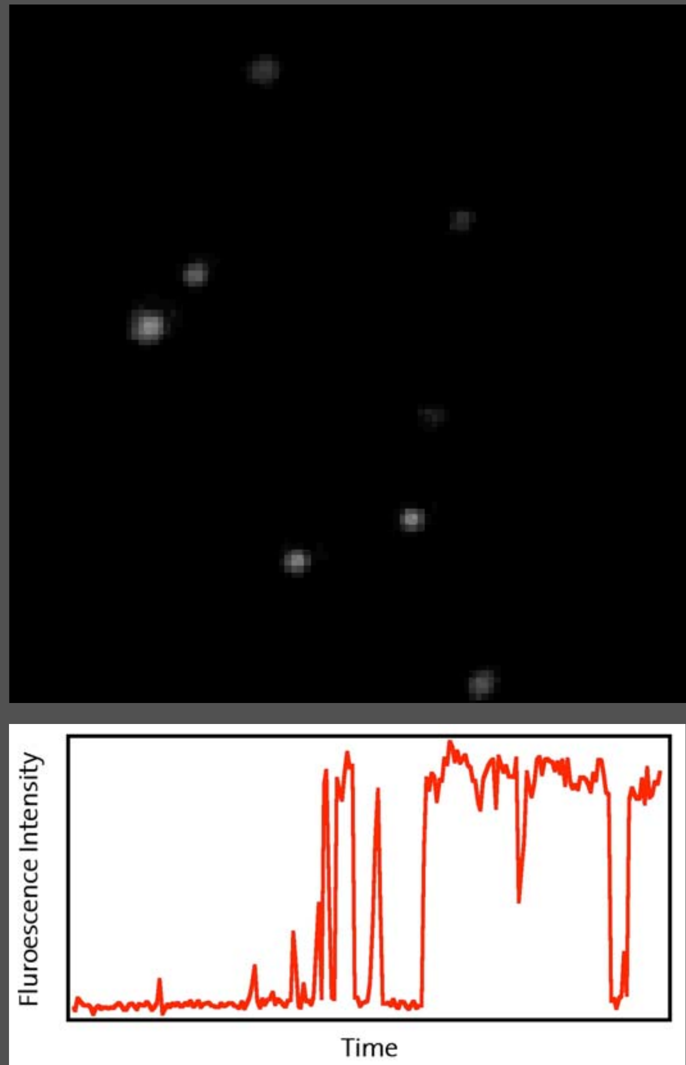
Single emitters: light sources with ticks

Single Cy3 molecules



„Bleaching“

Single Quantum Dots



„Blinking“

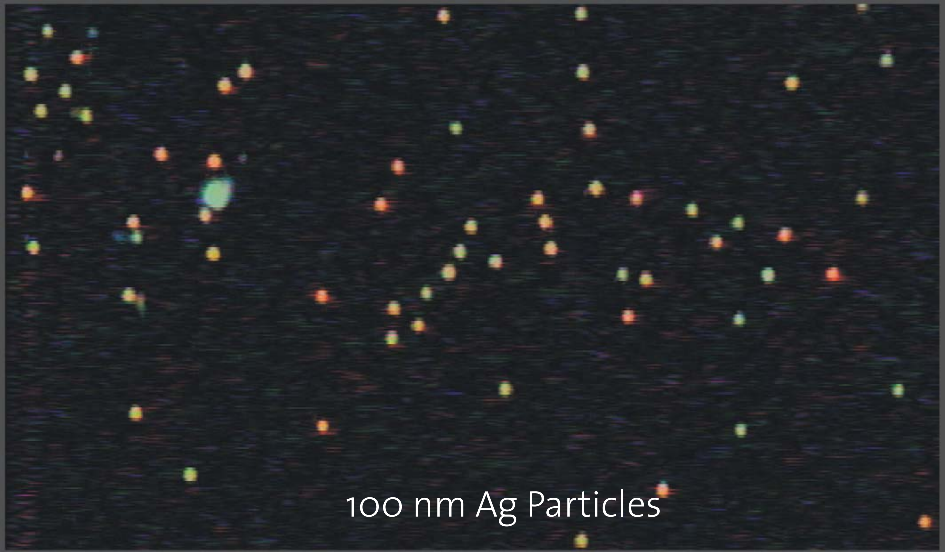
An alternative: light scattering

Milk does not blink or bleach!

Scattering detection of tiny particles

via scattering intensity: need to eliminate background scattering

⟶ dark-field illumination, total internal reflection



scattering amplitude, s(λ)

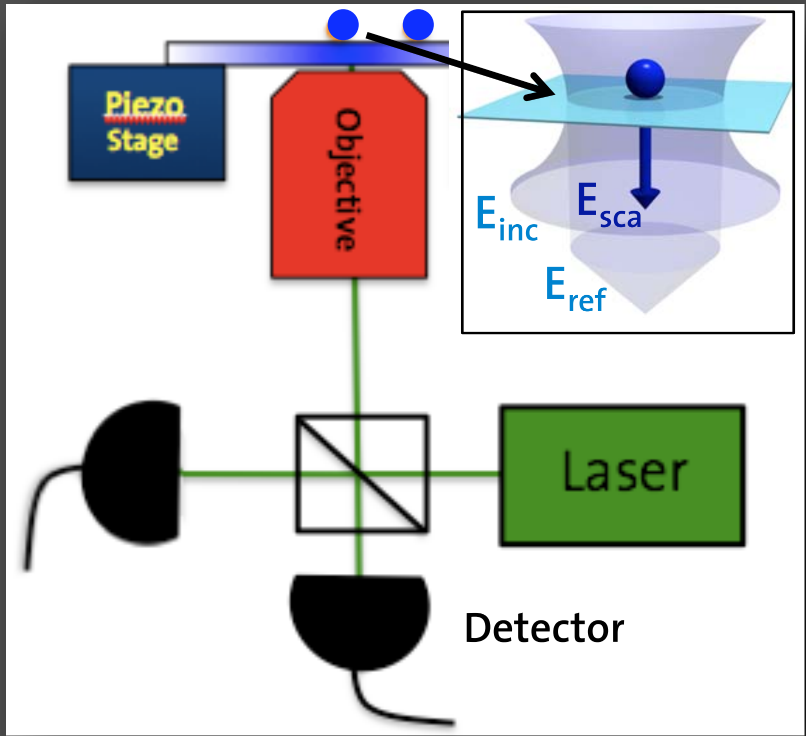
$$s(\lambda) = \eta\alpha(\lambda) = \eta\varepsilon_{med}(\lambda)\frac{\pi D^3}{2}\frac{\varepsilon_{part}(\lambda) - \varepsilon_{med}(\lambda)}{\varepsilon_{part}(\lambda) + 2\varepsilon_{med}(\lambda)}$$

The scattering cross section scales as D^6 thus rapidly drops below the noise

Detection limit: ~30 nm Au

interferometric scattering detection: iSCAT

Simplified experimental setup



Scattered field: Reflected field:

$E_{ref} \sim r e^{-i\pi/2} E_{inc}$ $E_{sca} \sim |\alpha| e^{-i\phi} E_{inc}$

$$I_{det} = |E_{ref}|^2 + |E_{sca}|^2 + 2\mathrm{Re}\{E_{ref}^* E_{sca}\}$$

$$I_{det} = I_{ref} + I_{sca} + I_{int}$$

o

Scattering contrast:

$$S = \frac{I_{int}}{I_{ref}}$$

Background:

$$B = \frac{1}{N^{-(1/2)}}$$

Signal-to-noise ratio:

$$SNR = \frac{S}{B} = S \times N^{(1/2)}$$

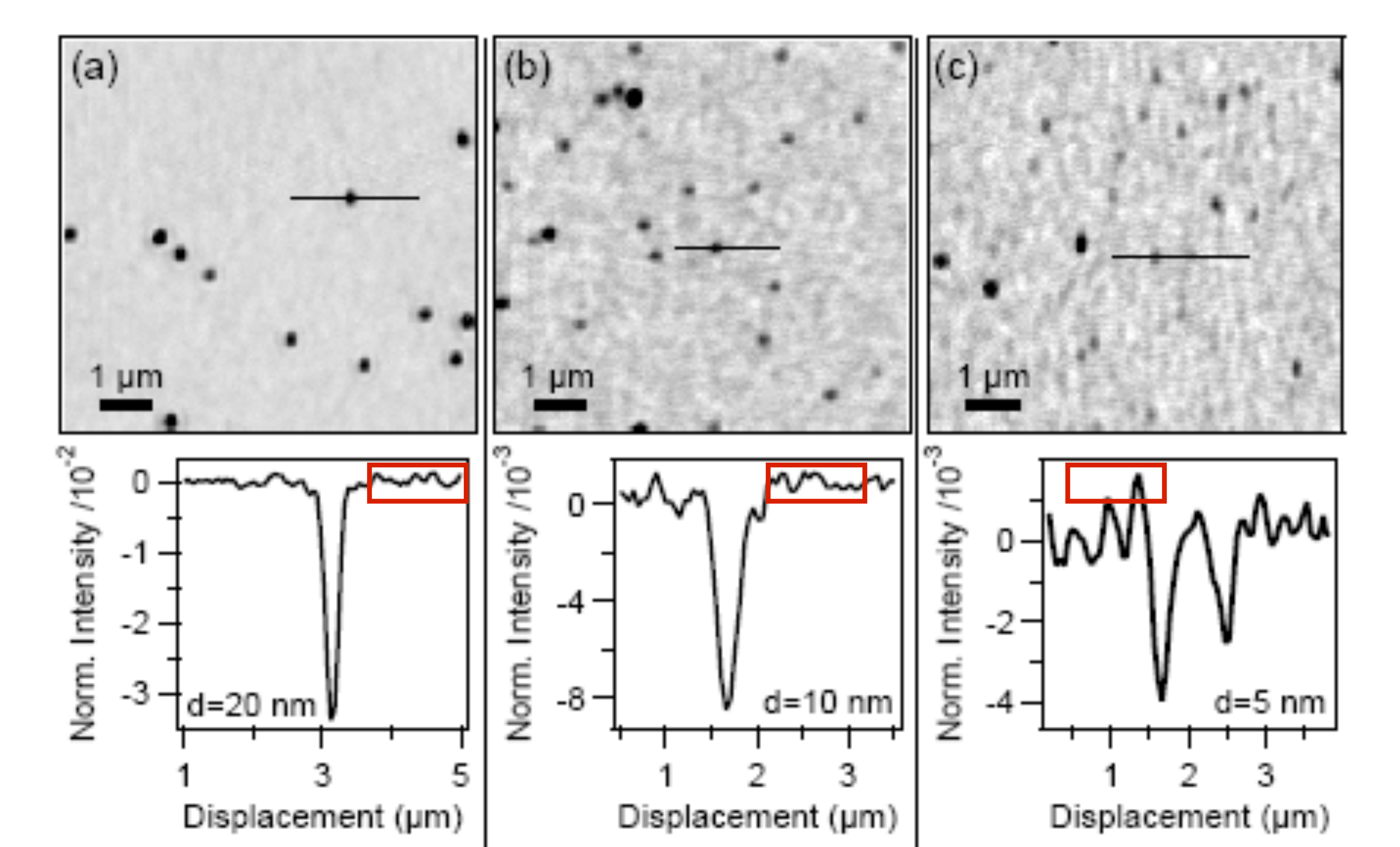
Interferometric detection much less sensitive to particle size: D^3 *vs* D^6

SNR scales with the number of incident photons

K. Lindfors, T. Kalkbrenner, P. Stoller and V. Sandoghdar, *Phys. Rev. Lett.* **93**, 037401 (2004)

Detection of *nearly* molecular sized scatterers

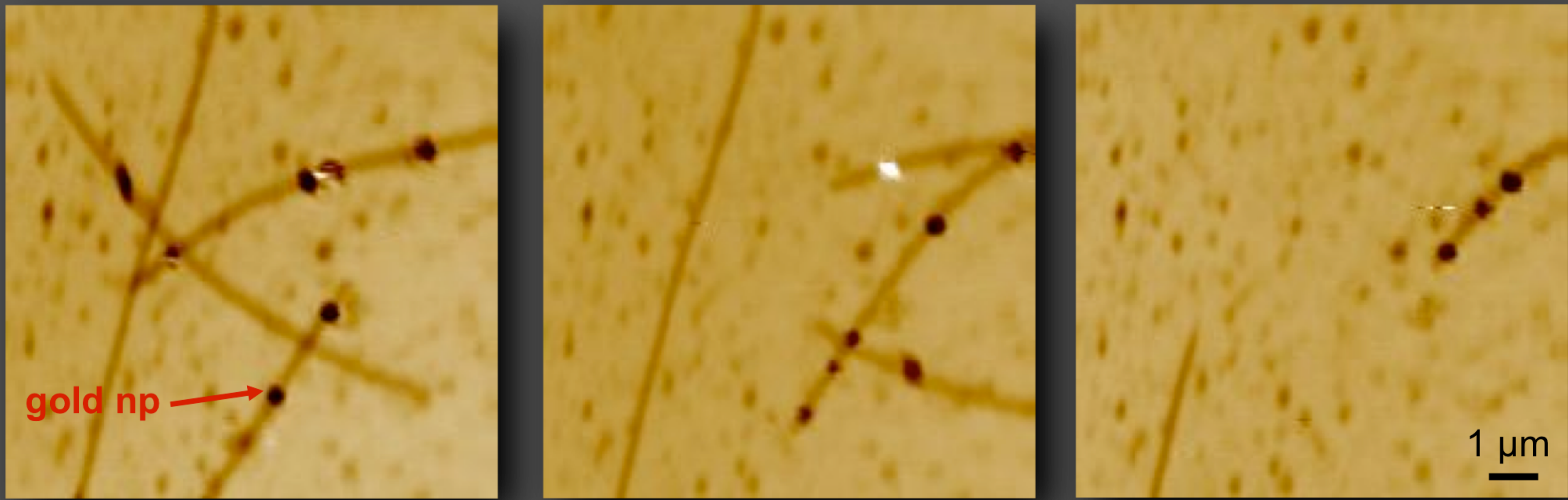
Piezo raster scan of gold nanoparticles on glass in water



V. Jacobsen, P. Stoller, C. Brunner, V. Vogel, V. Sandoghdar, *Opt. Exp.* **14**, 405 (2006).

Goldnanoparticles as biological labels

Microtubules labeled with 40 nm gold particles

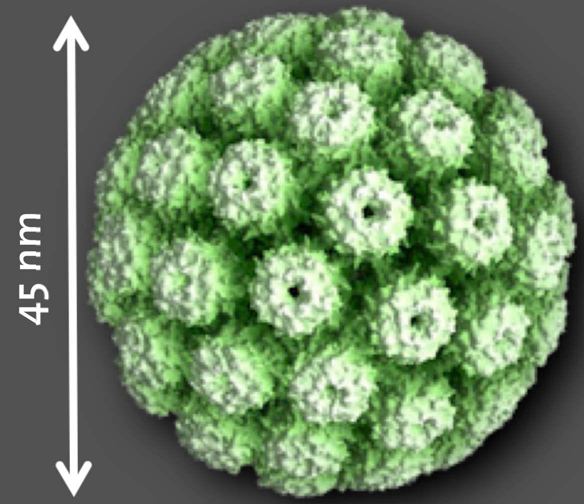


$$s(\lambda) = \eta\alpha(\lambda) = \eta\varepsilon_{med}(\lambda)\frac{\pi D^3}{2}\frac{\varepsilon_{part}(\lambda) - \varepsilon_{med}(\lambda)}{\varepsilon_{part}(\lambda) + 2\varepsilon_{med}(\lambda)}$$

Biological material also produces a signal since $\varepsilon_{part}(\lambda) \neq \varepsilon_{med}(\lambda)$

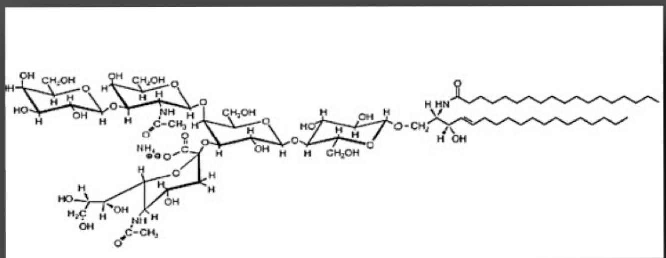
Label-free detection of single viruses

Simian virus 40

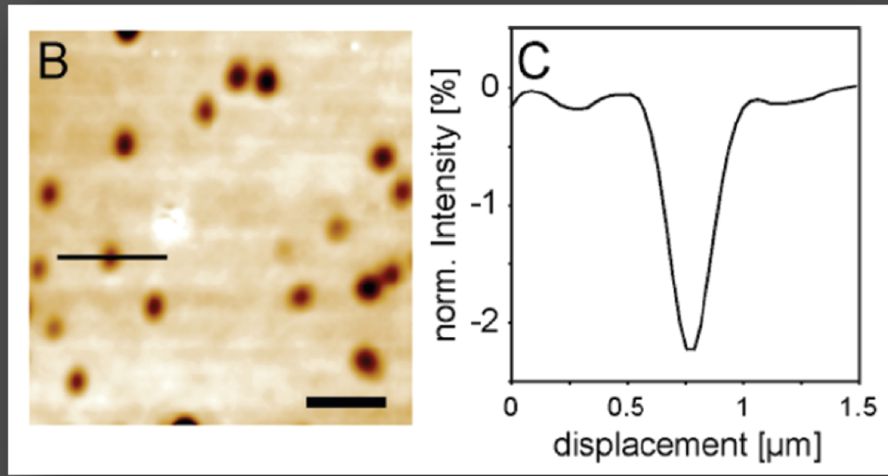


Liddington et al., *Nature* **354**, 6351 (1991)

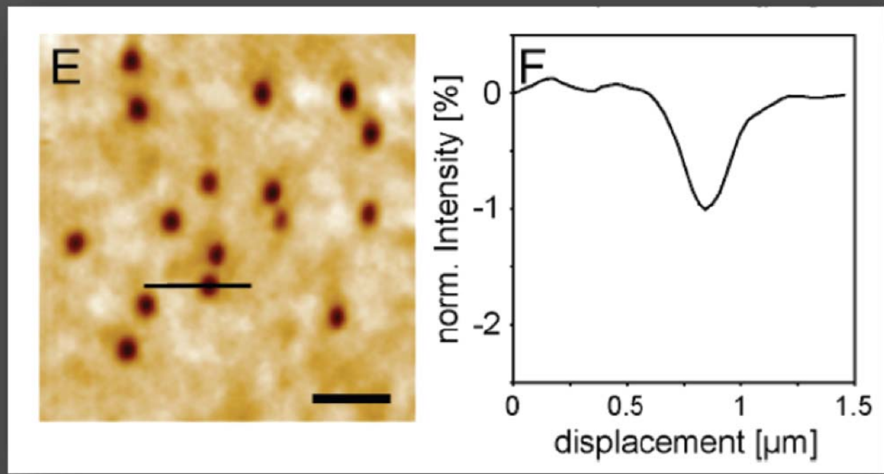
- Non-enveloped DNA tumor virus
- 72 pentamers of viral protein 1
- Cellular receptor: Glycol moiety of GM1



Single viruses bound to cover glass



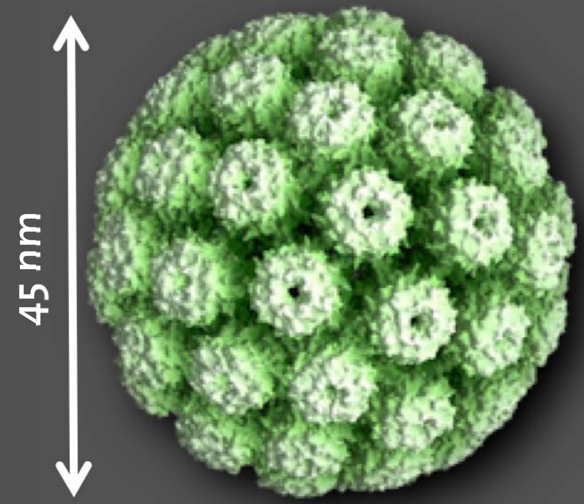
Virus like particles (empty protein shell)



H. Ewers, V. Jacobsen, E. Klotzsch, A. E. Smith, A. Helenius, V. Sandoghdar, *Nano Lett.* **7**, 2263 (2007).

Label-free detection of single viruses

Simian virus 40



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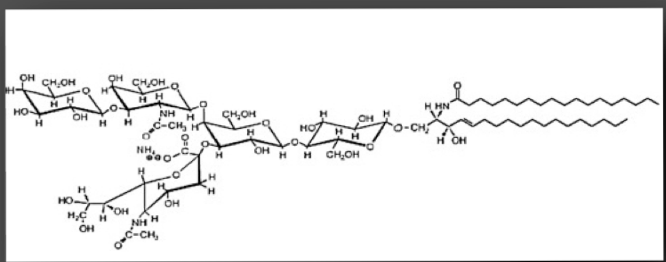
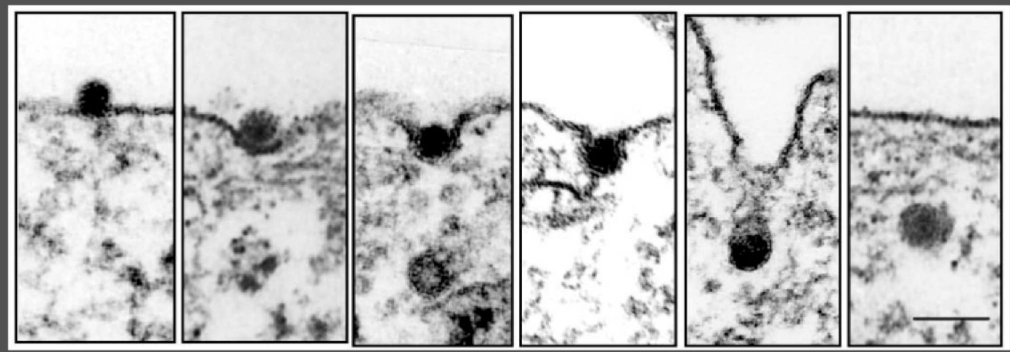


SV40 endocytosis



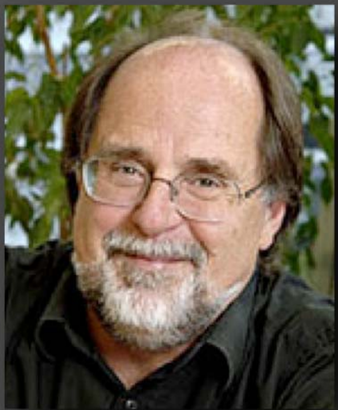
E. M. Damm, L. Pelkmans, J. Kartenbeck, A. Mezzacasa, T. Kurzckalia and A. Helenius, *J. Cell Biol.* **168**, 477 (2005)

M. J. Lehmann, N. M. Sherer, C. B. Marks, M. Pypaert and W. Mothes, *J. Cell Biol.* **170**, 317 (2005)

Acknowledgements

Institute of Biochemistry, ETH

Helge Ewers



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