Fluorescence Imaging On The Nanoscale

FIONA – Fluorescence imaging with 1 nm accuracy

NALMS – Nanometer localized multiple single molecule fluorescence

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Seminar: Recent Progress in Nanooptics & Photonics

FIONA

- fluorescence imaging with one nanometer precision
- Rayleigh criterion: $d_r = 0{,}61 \frac{\lambda}{N.A.}$

d_r…spatial resolution
λ…wavelength of the collected photons
N.A.…numerical aperture

- Point spread function (PSF) – Airy Disk
- 2D-Gaussian fit: $N_{xy} = B + N_{00} \exp\left[-\frac{(x - x_0)^2}{2 s_x^2} - \frac{(y - y_0)^2}{2 s_y^2} \right]$

(x_0,y_0)…centroid of the point source
N_{xy}, N_{00}…counts at pixel (x,y) and at centroid pixel (x_0,y_0) on the CCD
S_x,S_y…width of the PSF in x- and y-direction
B…baseline (CCD background, read-out-noise, residual scattered light ,…)

PSF of several CY3 dyes

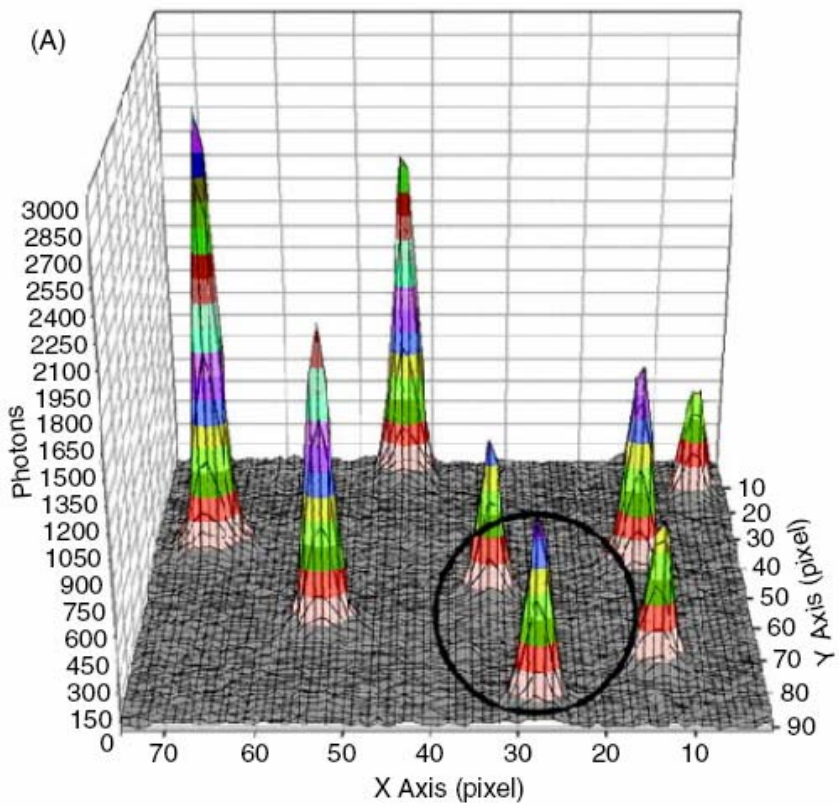

Fig 1: The intensity of each peak varies due to nonuniform illumination

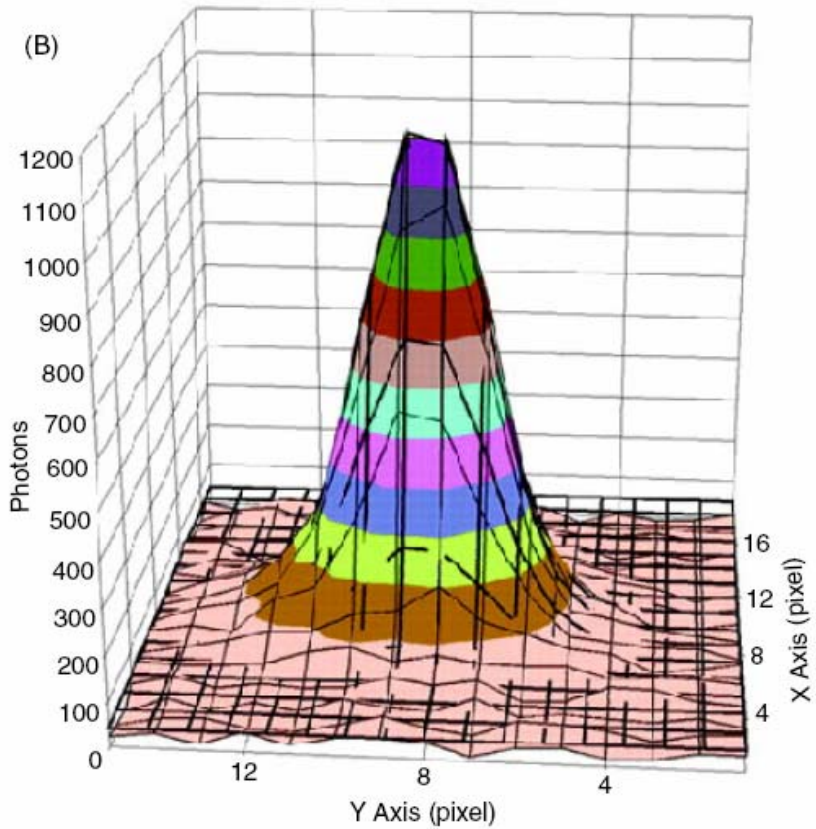

Fig 2: A Gaussian curve-fit (solid lines) to the PSF circled in (A) fits the PSF very well ($r^2 = 0.994$), enabling the center to be determined to 1.3 nm

Standard error of the PSF (theory)

$$\sigma_{\mu_i} = \sqrt{\frac{s_i^2}{N} + \frac{a^2}{12N} + \frac{8\pi s_i^4 b^2}{a^2 N^2}}$$

s…with of the fitted gaussian in direction i (standard deviation)
N…number of collected photons
a…pixel size of the detector
b…background

1. Photon noise: $\sigma_\mu = 1.02$ nm
2. Pixelation: $\sigma_\mu = 0.02$ nm
3. Background: $\sigma_\mu = 0.20$ nm

=> $\sigma_\mu = 1.24$ nm

Molecular Motors – Kinesin and Myosin

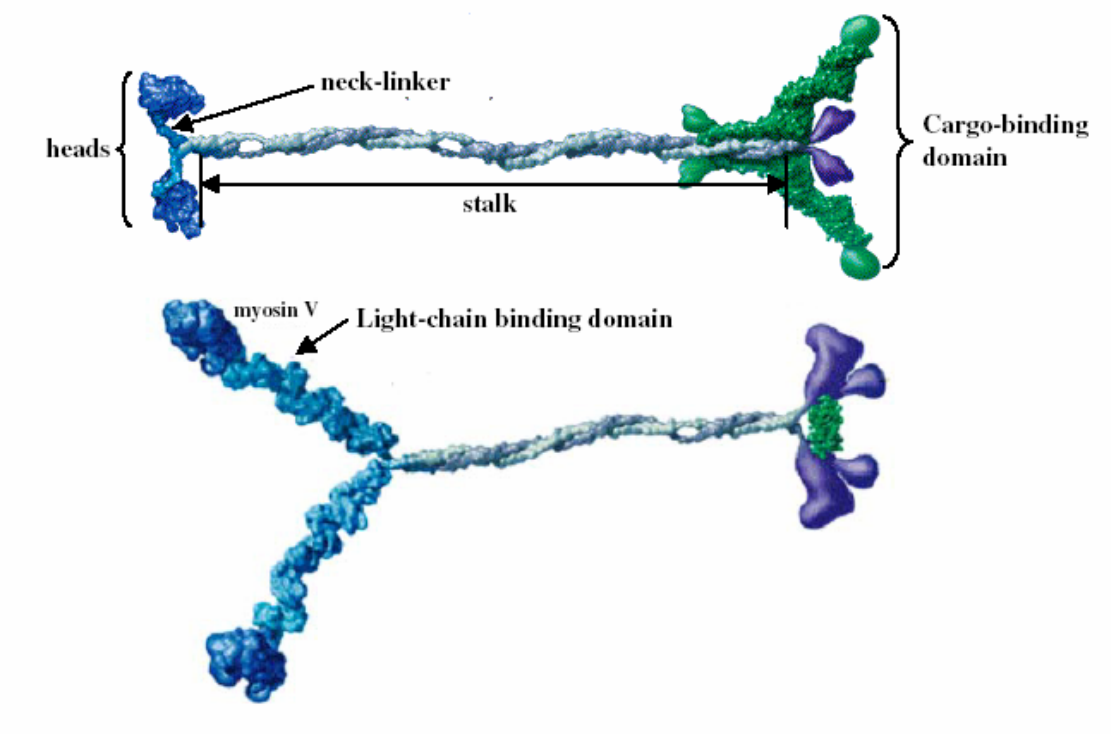

Fig 3: Both kinesin and myosin V are dimeric proteins that walk processively on their tracks. The dark blue regions are the motor domains capable of nucleotide hydrolysis and force production. The coiled-coil region, the stalk, links the head regions to the cargo binding domains

Myosin V – hoh vs. iw model

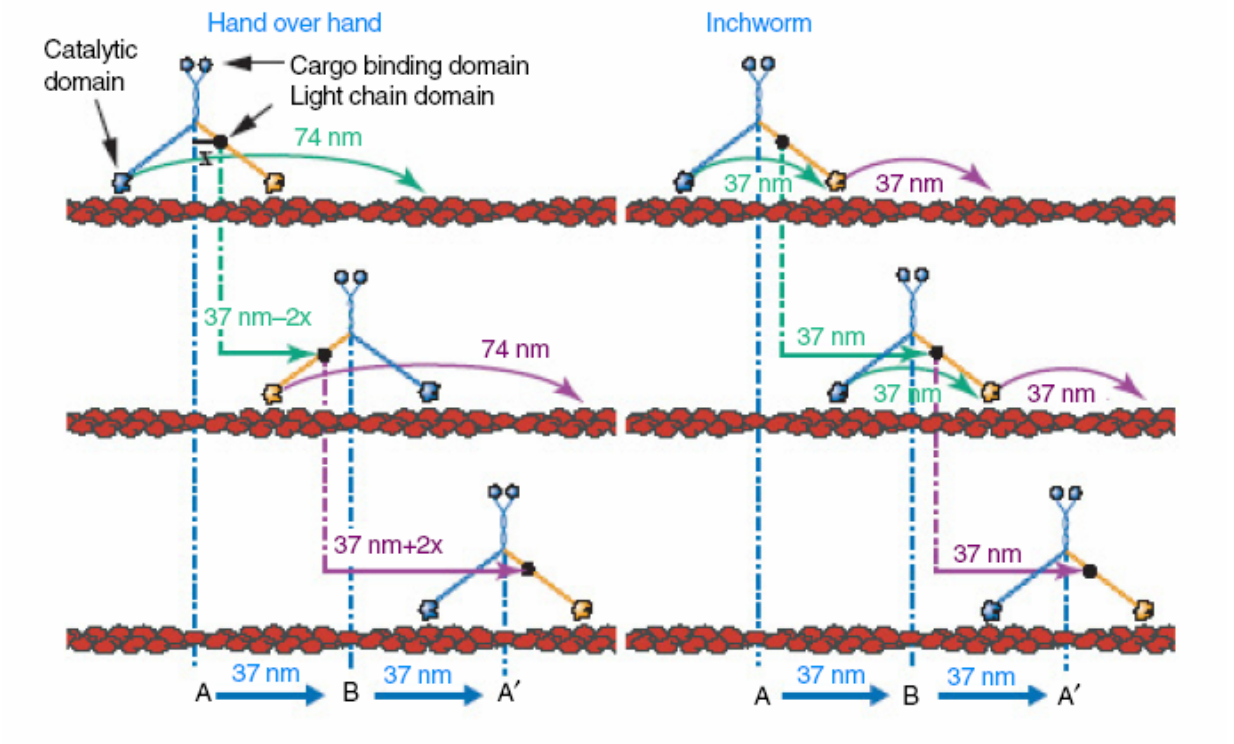

Fig 4: Hand-over-hand (hoh) vs. inchworm model (iw) of myosin V motility. A calmodulin light chain is labeled with a single fluorescent dye and exchanged into the myosin V light chain domain, where it binds in one of several possible positions (black dot). In the hoh-model, the rear head moves 74 nm forward but the front head does not move, the stalk moves 37 nm, and the dye takes alternating 37 ± 2x steps. In the iw-model, the marker moves a constant 37 nm in each step regardless of its position on the molecule

Stepping traces of myosin V

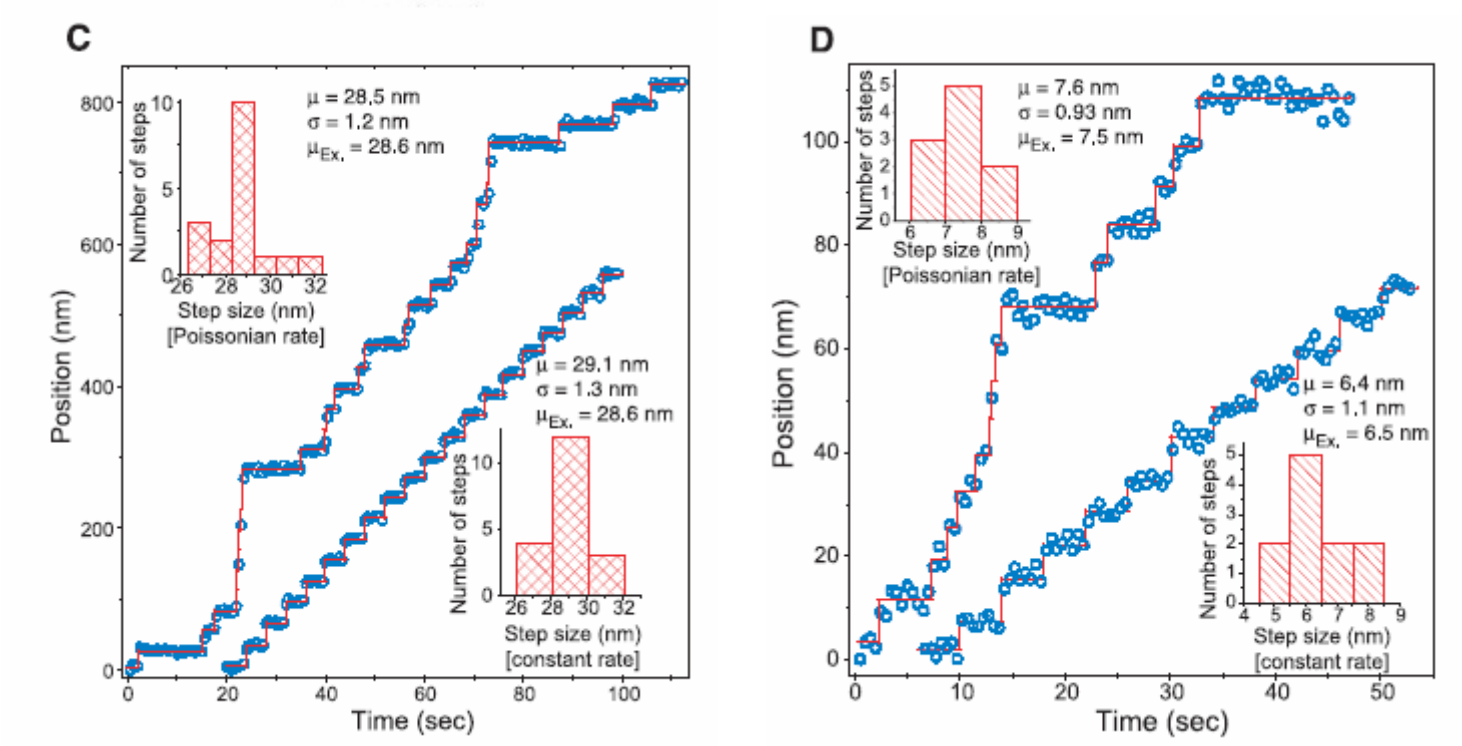

Fig 5 + 6: 30 nm and approximately 7 nm steps are observed upon moving the coverslip (at constant rate or Poisson distributed) with a nanometric stage and plotting the PSF vs time. Red lines show the average position between each step. The precision (σ) is about 1 nm and the accuracy (μ-μ_{ex}) is better than 1 nm

3 different myosin V molecules

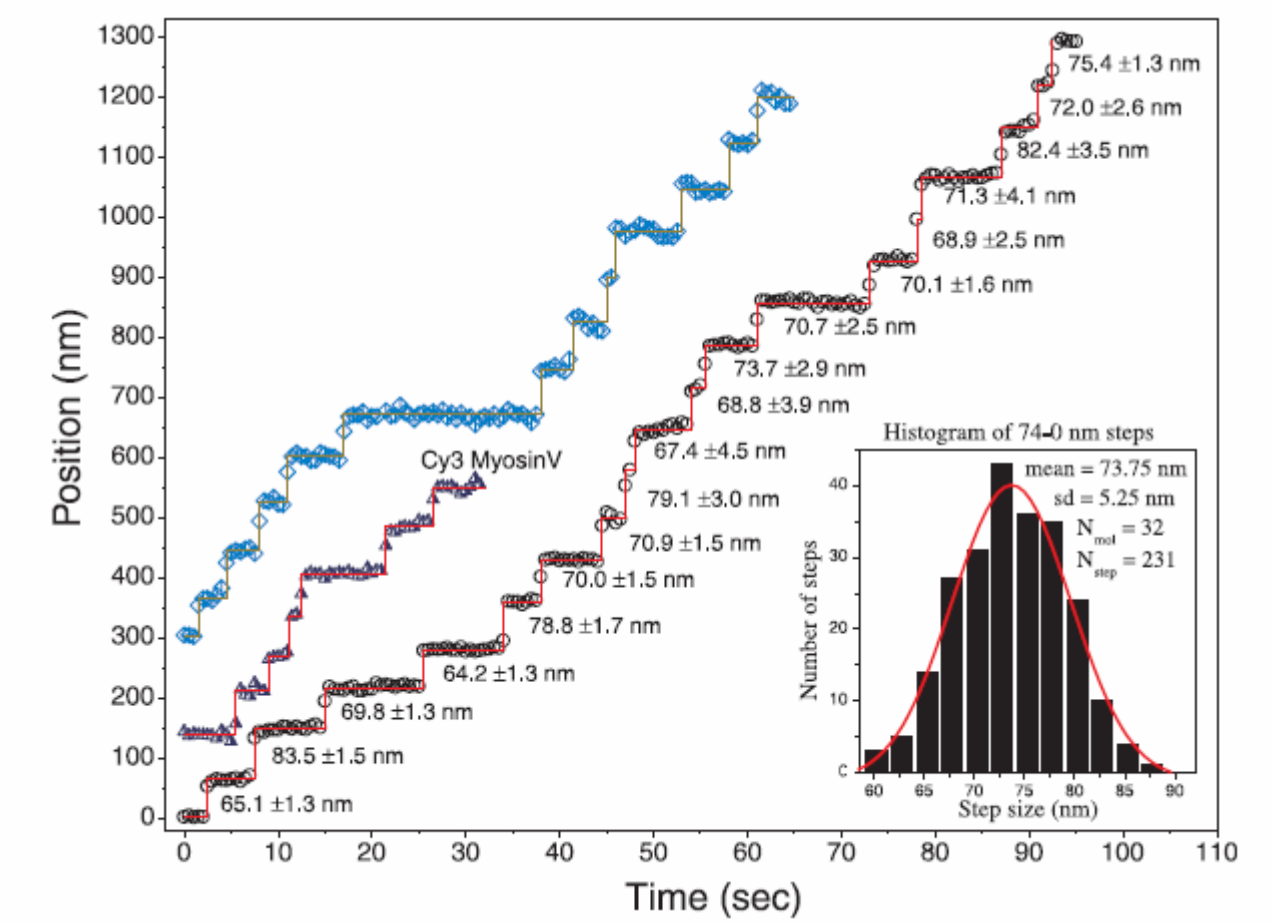

Fig 7: Stepping traces of 3 different myosin V molecules displaying 74 nm steps and a histogram of a total of 32 myosin V´s taking 231 steps

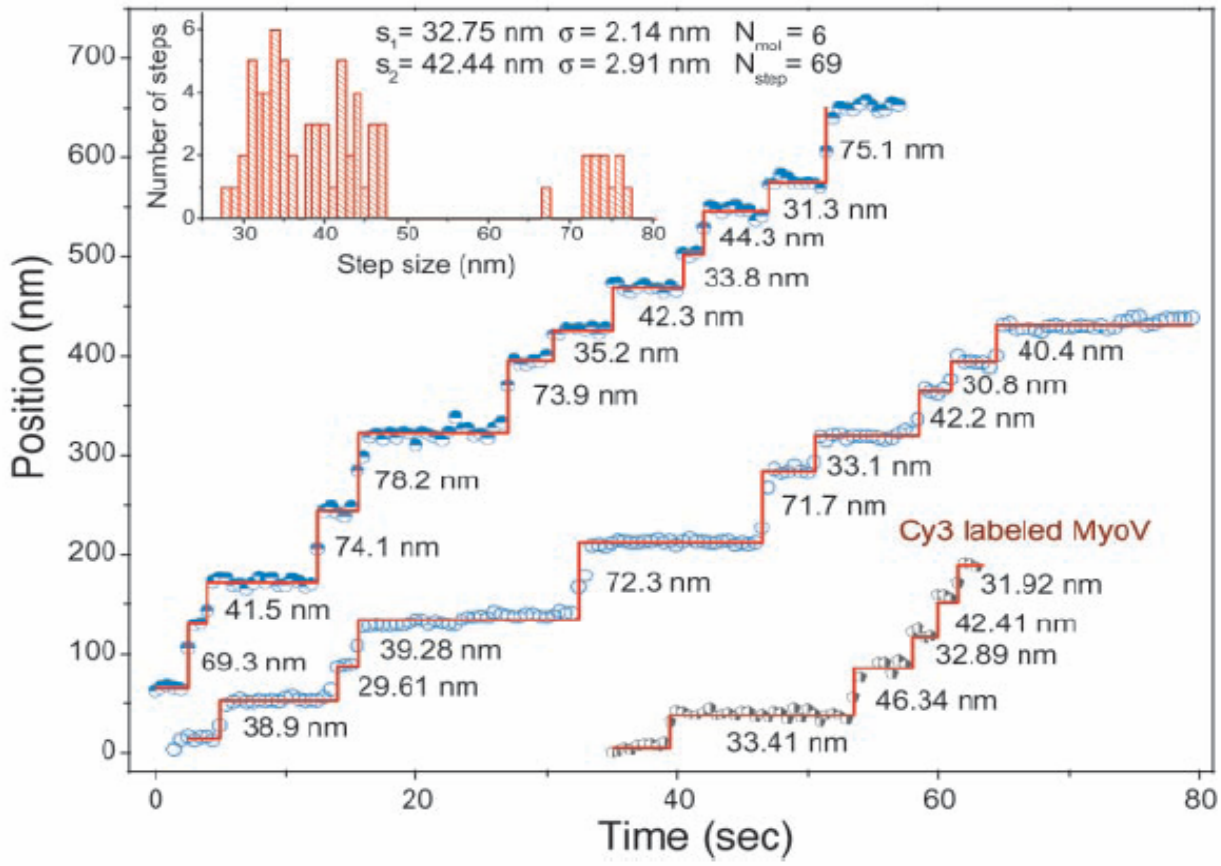

Fig 8: a

Stepping traces of 3 different myosin V molecules displaying alternating 42-33 steps and histogram of a total of 6 myosin V`s taking 69 steps. These step sizes indicate that the dye is 2-3 nm from the center of mass along the direction of motion. The bottom right trace is for a Cy3-labeled myosin V, whereas the other 2 are for BR-labeled myosin V`s. Due to the 0,5 s time resolution of measurements some steps are missed and yield 74 nm apparent steps (the sum of 2 steps)

Dwell time histogram for myosin V

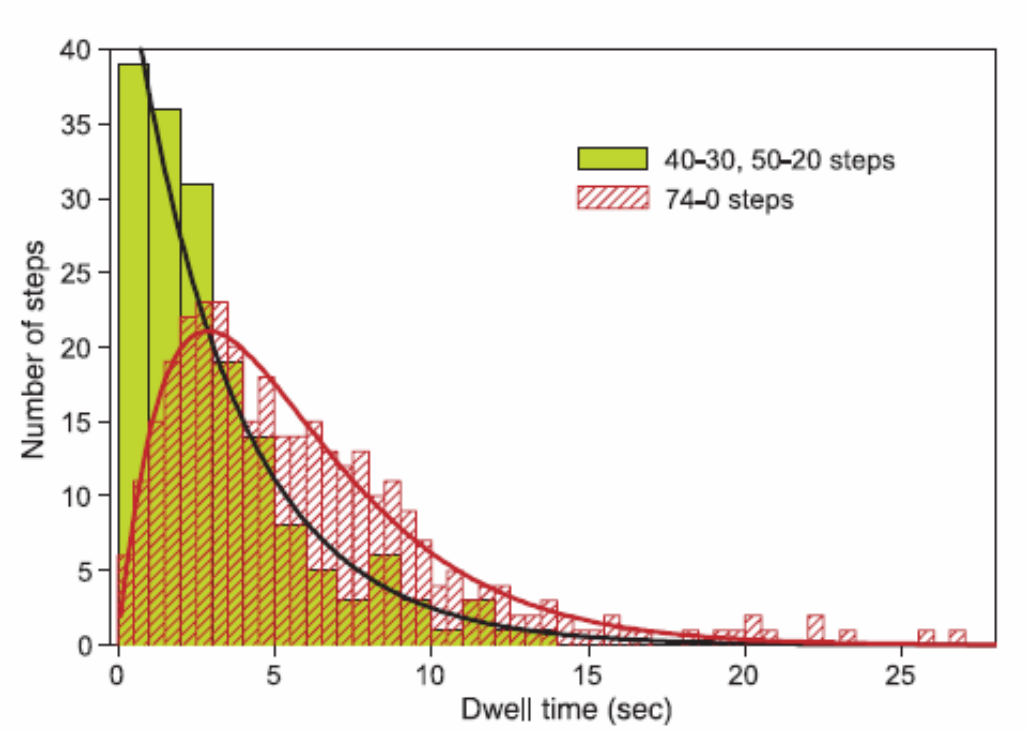

Fig 9: Dwell time histograms for myosin V´s displaying 74 nm steps (red cross hatched with solid red curve), or 52-23 and 42-33 combined (green with solid black curve fit). A 74 nm step in the 52-23 or 42-33 data is assumed to be 2 rapid steps in the 0- to 0,5 s bin. The curve fits are based on the kinetic hoh-model

BR-labeled myosin V

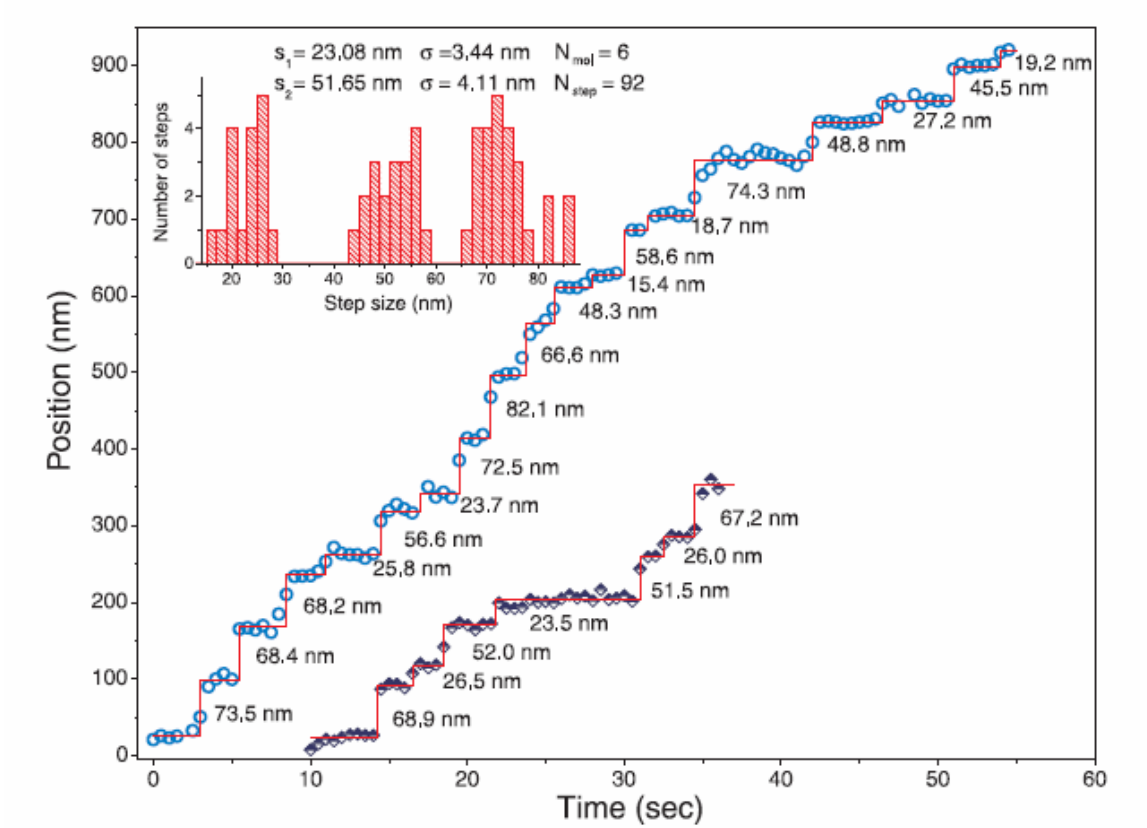

Fig 10: Stepping traces of 2 different myosin V molecules displaying alternating 52-23 steps and a histogram of a total of 6 myosin V`s taking 92 steps. Due to the 0,5 s time resolution of measurements some steps are missed and yield 74 nm apparent steps, the sum of 2 steps. On the basis of the lterating step size the dye is 7 nm from the center of mass along the direction of motion

Kinesin

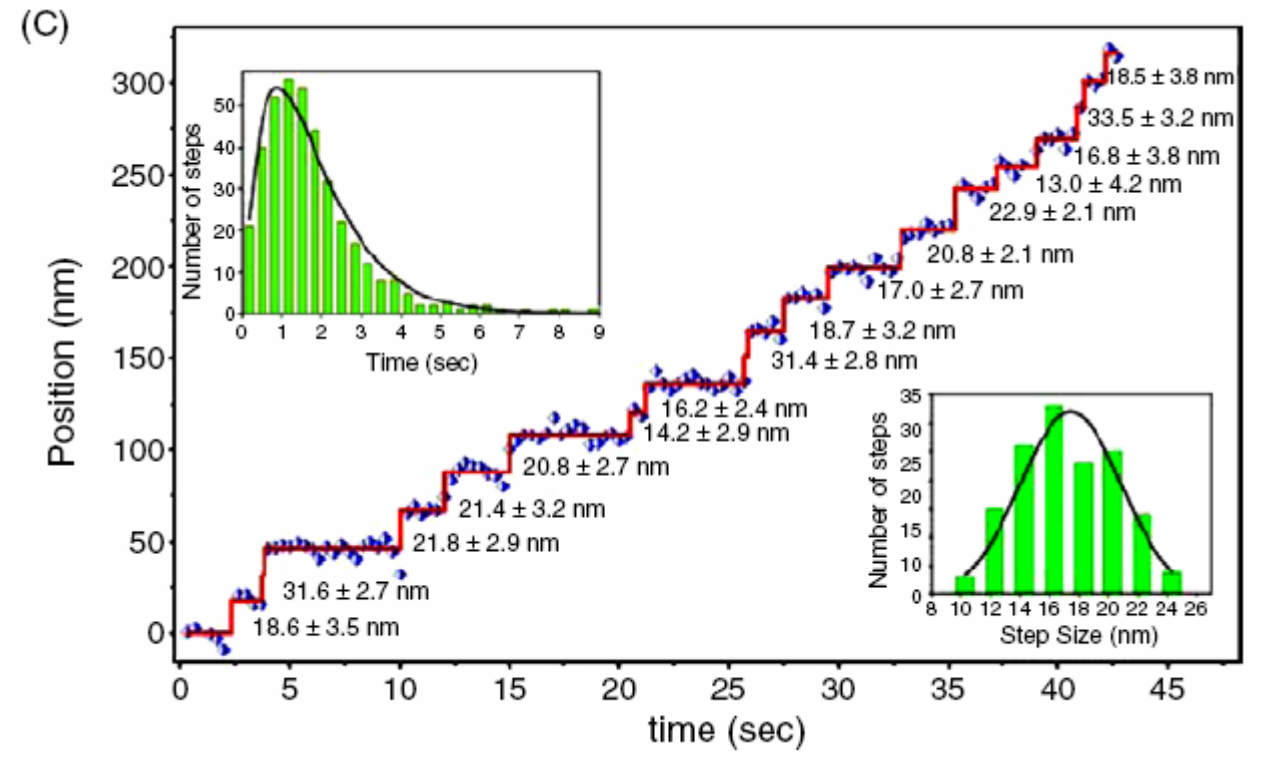

Fig 11: Stepping pattern of an individual head of kinesin. The average stepsize of 17 nm and the kinetic analysis of the dwell time histogram, which is a convolution of 0 and 16 nm steps, strongly suggest the hoh-model

Myosin VI

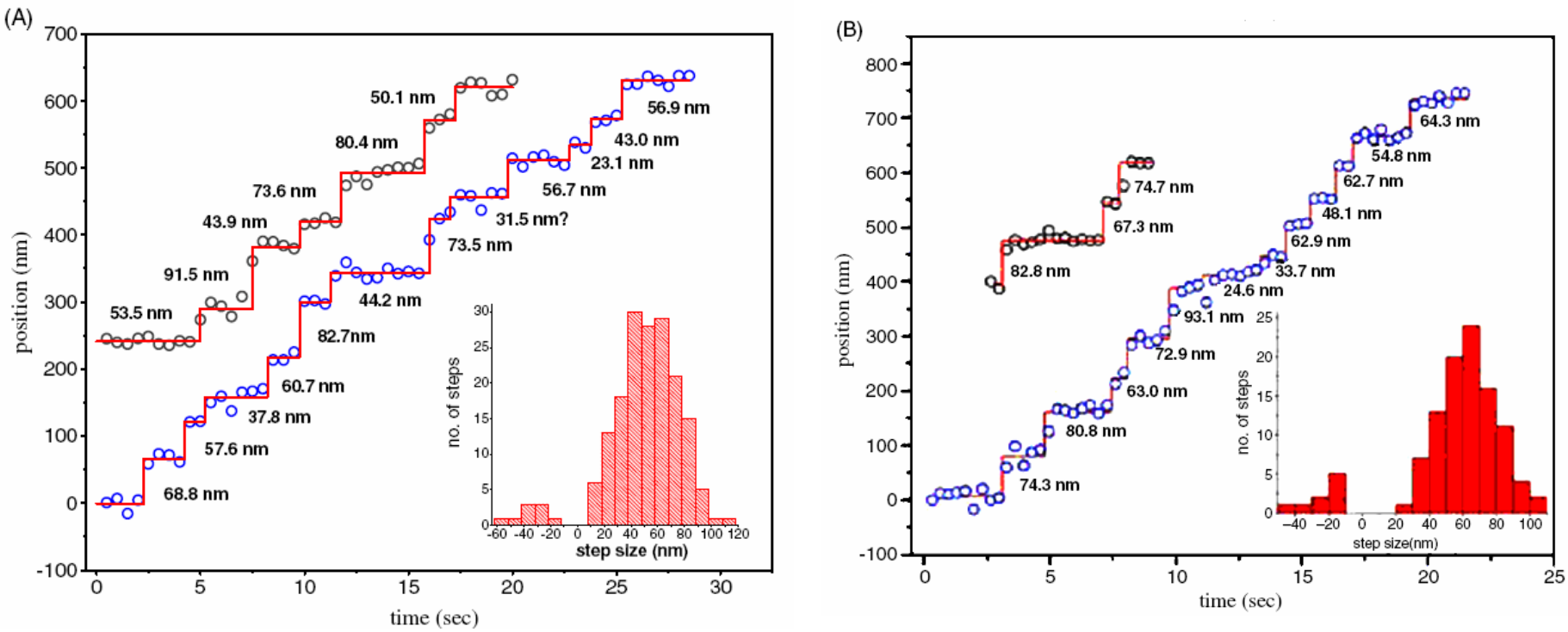

Fig 12: Stepping traces of myosin VI labeled with (A) cy3 and (B) eGFP molecules

Symmetric hoh-model for Myosin VI

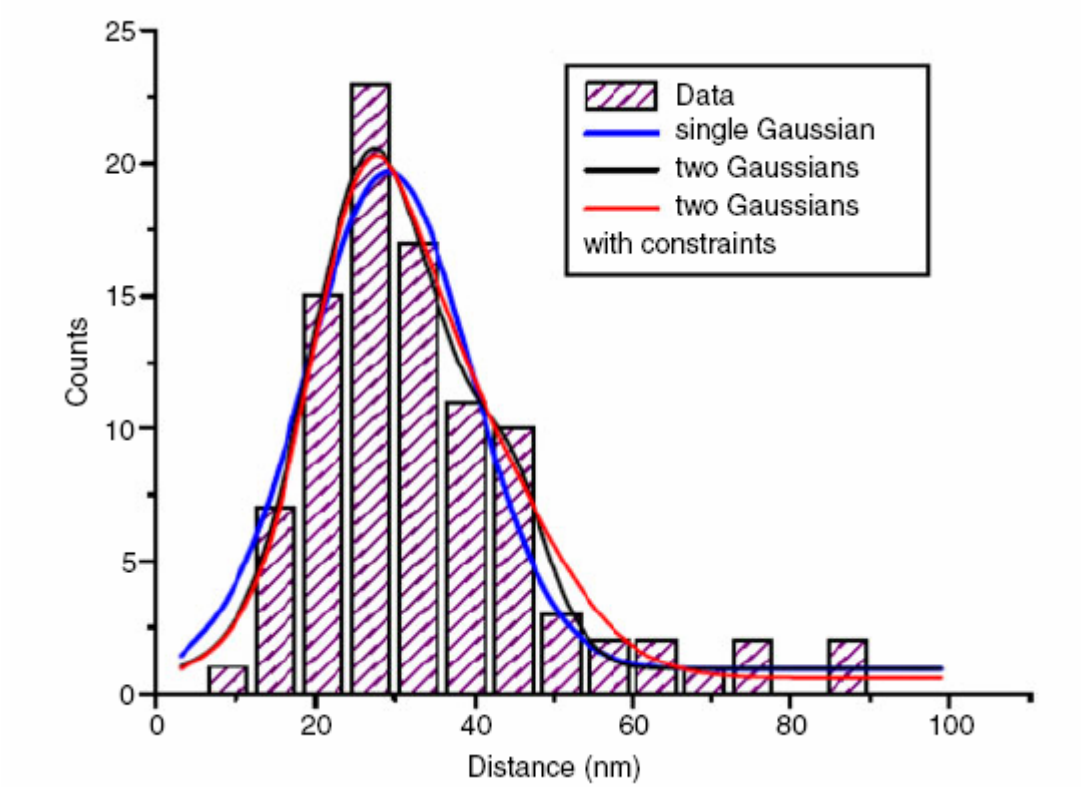

Fig 13: Histogram of measured separations between the myosin VI heads and 3 Gaussian fits to the data. The blue curve is a single Gaussian fit representing the hoh-model, the black curve is an unconstrained fit of 2 Gaussians and the red curve is a fit of 2 Gaussians with constraints

NALMS

- Nanometer Localized Multiple Single Molecule Fluorescence Microscopy
- Several objects within 1 diffraction-limited spot
- Use of centroid localization & Exploits photobleaching

=> Achivement of nanometer scale localization of single-molecule fluorophores

NALMS

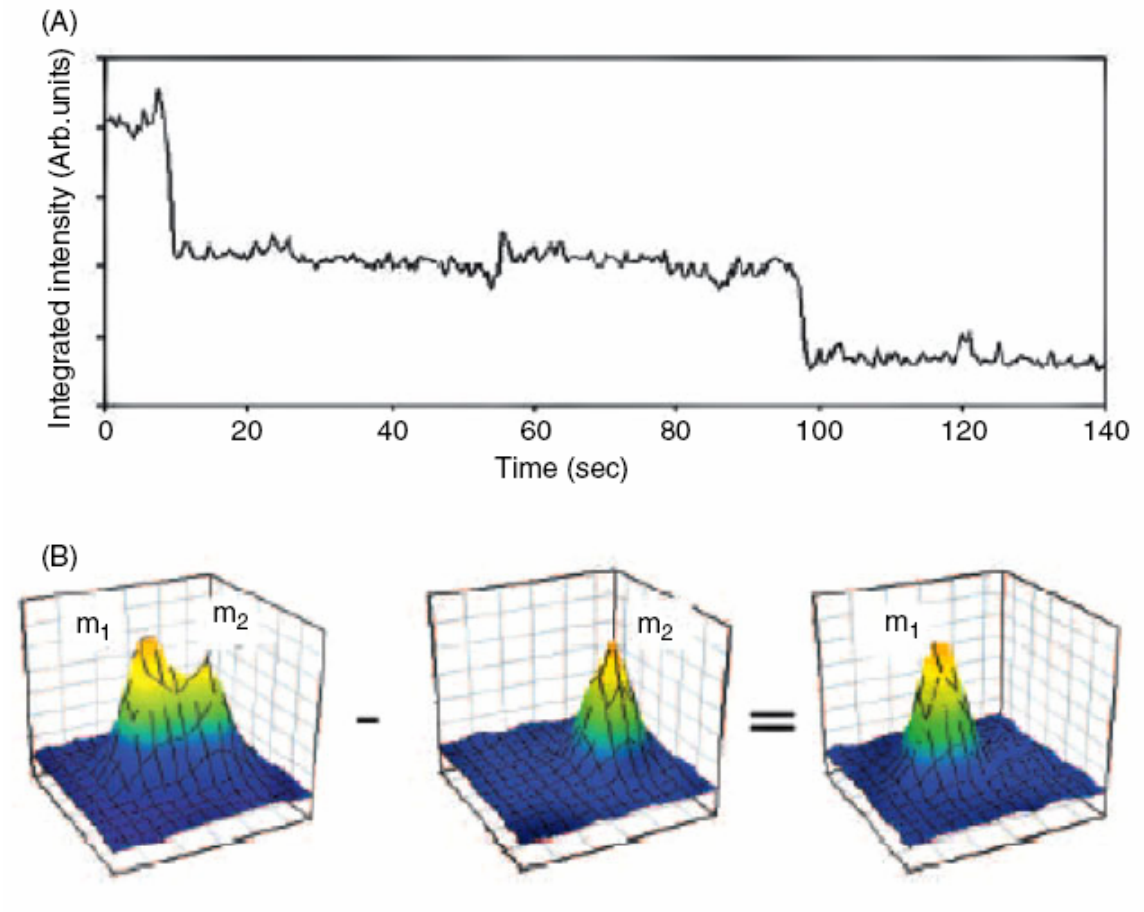

Fig 14: A plot of total integrated intensity vs. time for 2 closely spaced Cy3 molecules, showing a 2 step photobleaching behaviour. The PSF of the dye that photobleached first is calculated from the total PSF and the PSF of the dye that bleached second

2D Gaussian fit

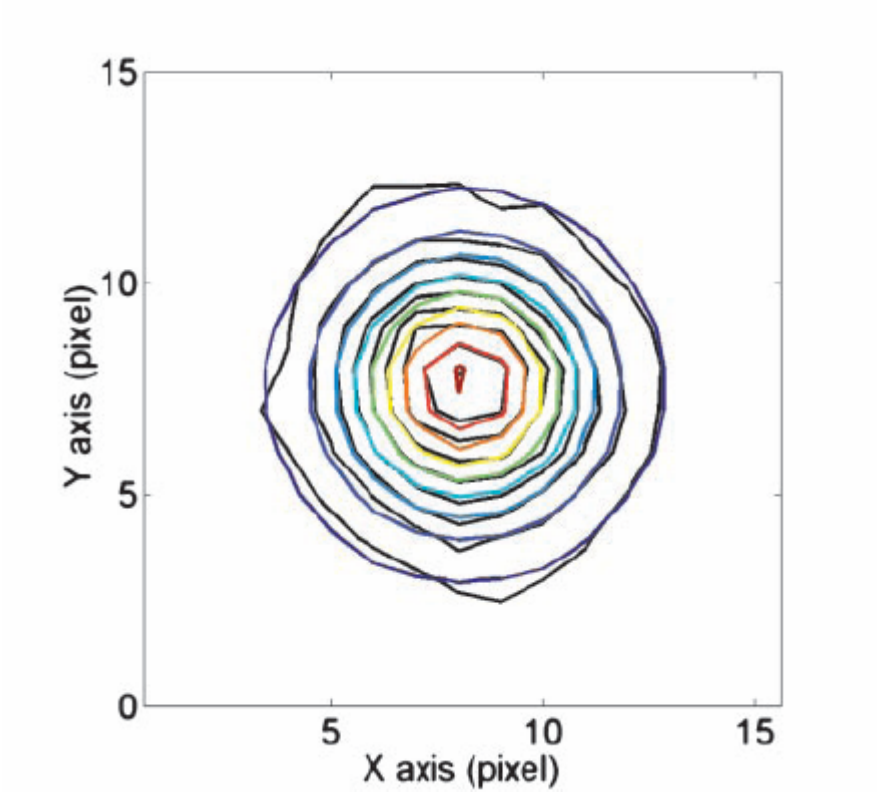

Fig 15: Contour plots of the CCD image of a Cy3 dye molecule (black) and a 2D Gaussian function (color), which is used as a PSF to fit the image

Photobleaching – time trace

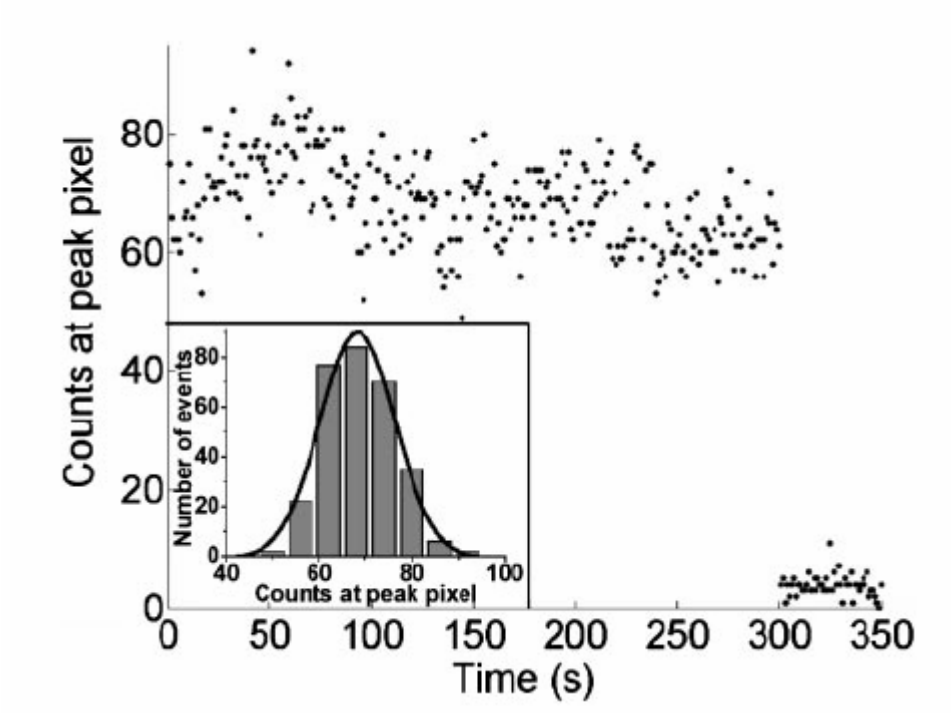

Fig 16: Time trace of a single Cy3 dye molecule. The counts at the peak pixel of the CCD image are plotted against time. The dye exhibits sudden photobleaching at 300 s and the counts drop to a background level afterward. Histogram of the collected counts at the peak pixel before photobleaching

Time trace analysis of Cy3

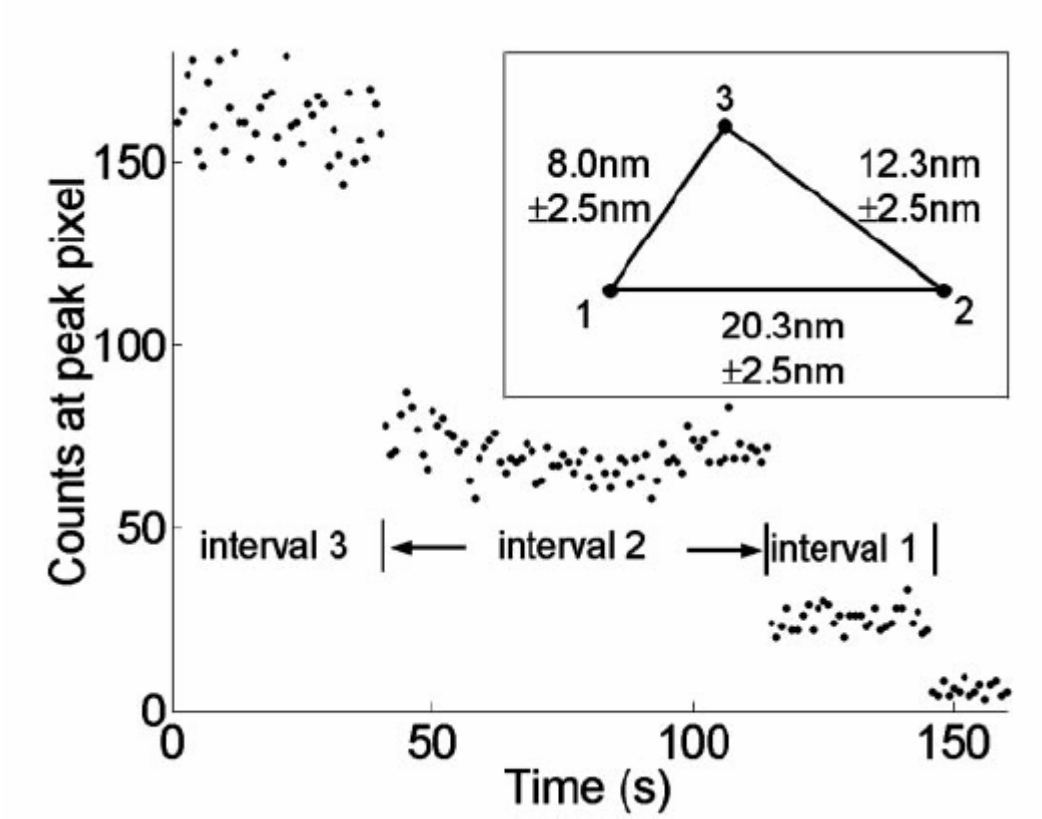

Fig 17: Time trace of 3 Cy3 dyes within the PSF of the microscope. The number of counts collected at the peak pixel of the CCD image is essentially constant over time with 3 sudden photobleaching events

DNA molecules as nanoscale rulers

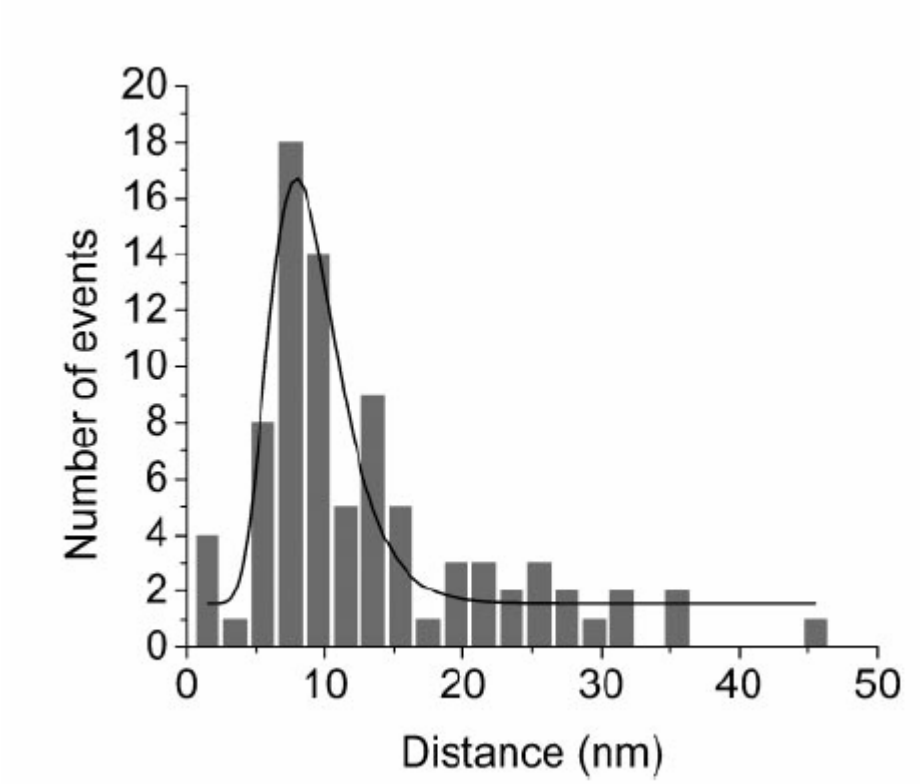

Fig 18: Histogram of the end-to-end distance measurements of a 24-base DNA ruler (labeled at both ends with Cy3 dyes). The Lognormal fit gives the end-to-end distance as [8 - 2, 8 + 3] nm where 8 nm is the location of the center of the lognormal fit, and 2nm and 3 nm are the widths of the fitted function for the region to the left and to the right of the center

Nanoscale rulers

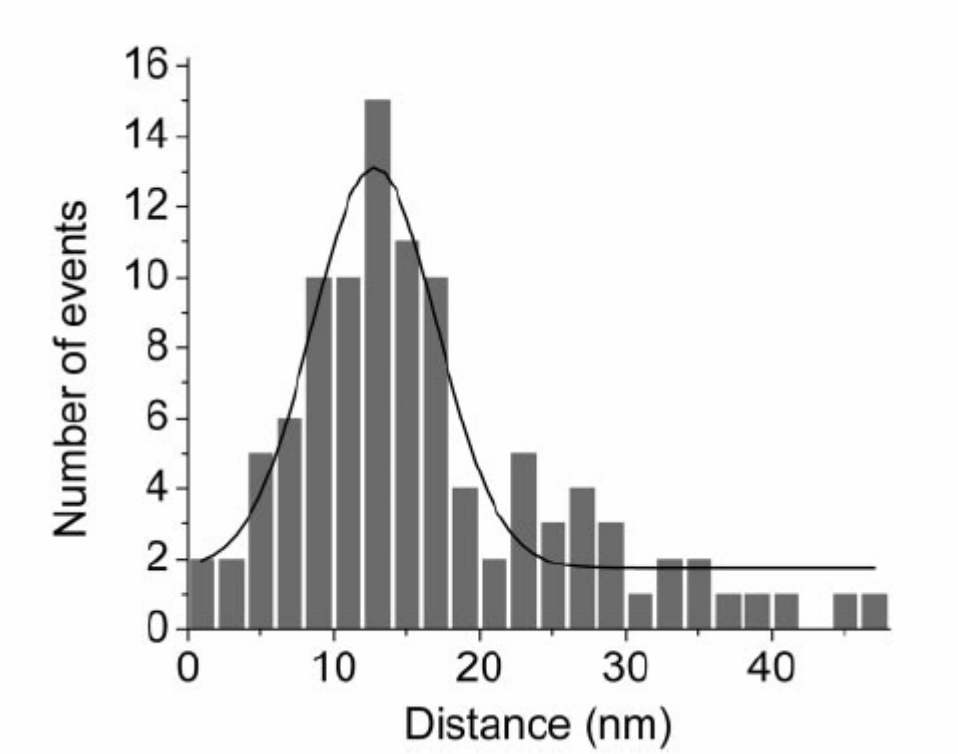

Fig 19: Histogram of end-to-end distance measurements of a 40-base DNA ruler. The Gaussian fit gives the end-to-end distance as 13 ±4 nm, where 13 nm is the location of the center of the Gaussian fit and 4 nm is the width of the fit

DNA mapping with NALMS microscopy

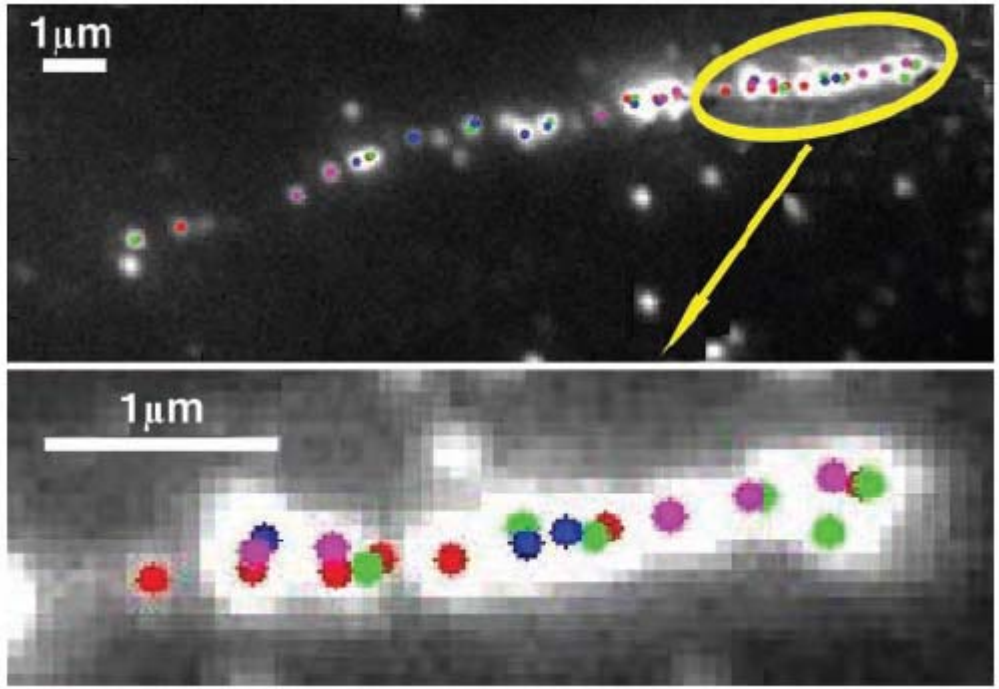

Fig 20: The grayscale was chosen so that single molecules can be seen. The right part of the image is saturated because many bis-PNA molecules are bound to this region. A series of images was taken at a rate of 1 frame/s until all of the fluorophores photobleached. Only the centroid localization results for fluorophores photobleached within the first 4 frames of images are shown (to avoid creating an overcrowded picture). The colored dots show the centroids of the localized fluorophores (same color for photobleaching in the same acquisition frame)

References

- Yildiz, et al. – „Myosin V Walks Hand-Over-Hand: Single Fluorophore Imaging with 1.5 nm Localization“ , Science 300, 2061 (2003)
- Yildiz, et al. – „Kinesin Walks Hand-Over-Hand“, Science 303, 676 (2004)
- Qu, Wu, Mets and Scherer – „Nanometer-localized multiple single-molecule fluorescence microscopy“, Pnas (2004)
- Kural, et al. – „Molecular motors one at a time: FIONA to the rescue“, J.Phys.: Condens. Matter 17 (2005)
- Kural, et al. – „Kinesin and Dynein Move a Peroxisome in Vivo: A Tug-of-War or Coordinated Movement ?“, Science 308, 1469 (2005)