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Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Fast Optical Microscopy in Life Science Research

Content

Content:

- Localization of small objects by (super-resolution) fluorescence microscopy
- Scattering-based approach for rapid localization experiments, interferometric scattering microscopy (iSCAT).
- Single molecule tracking
- Single molecule assembly / disassembly
- Mass photometry

Date: June 21st

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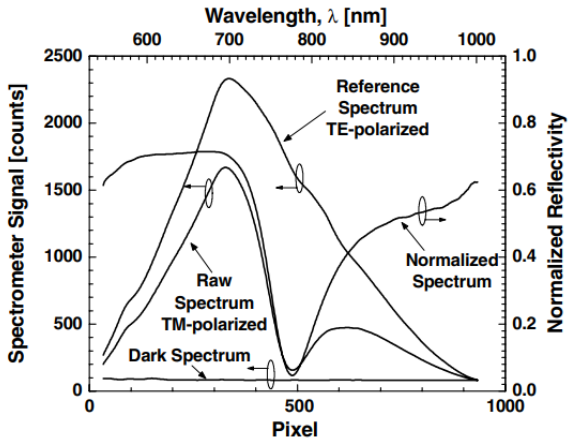
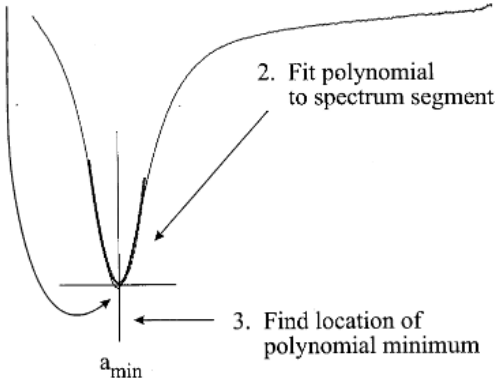
Fluorescence-Based Localization

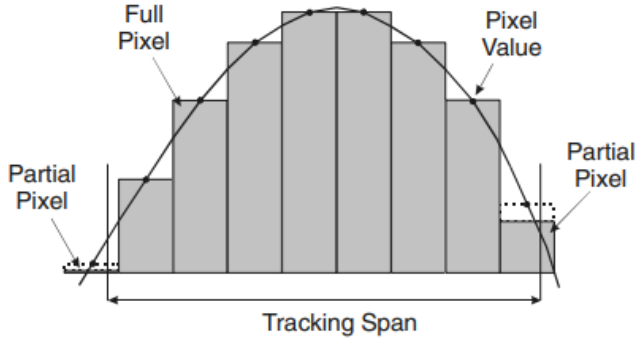
Centroid vs Fitting of SPR Spectra

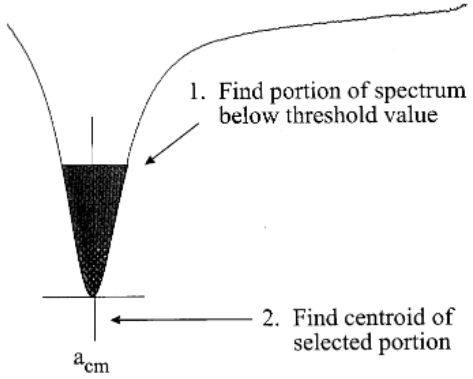


1. Pick segment of reflection spectrum surrounding minimum pixel



$$\frac{i\,(x_{thresh} - x_i)}{(x_{thresh} - x_i)}$$

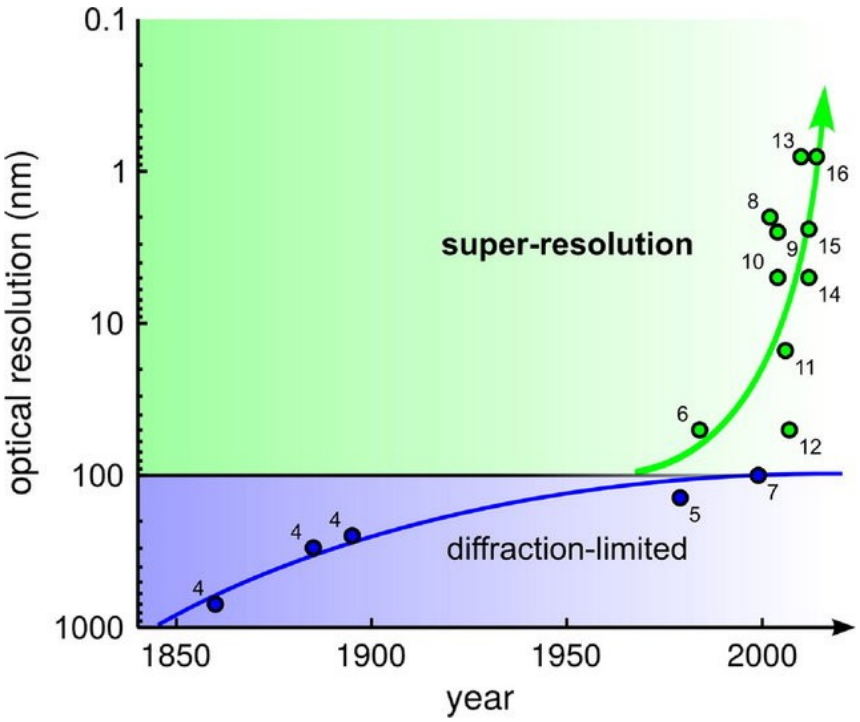




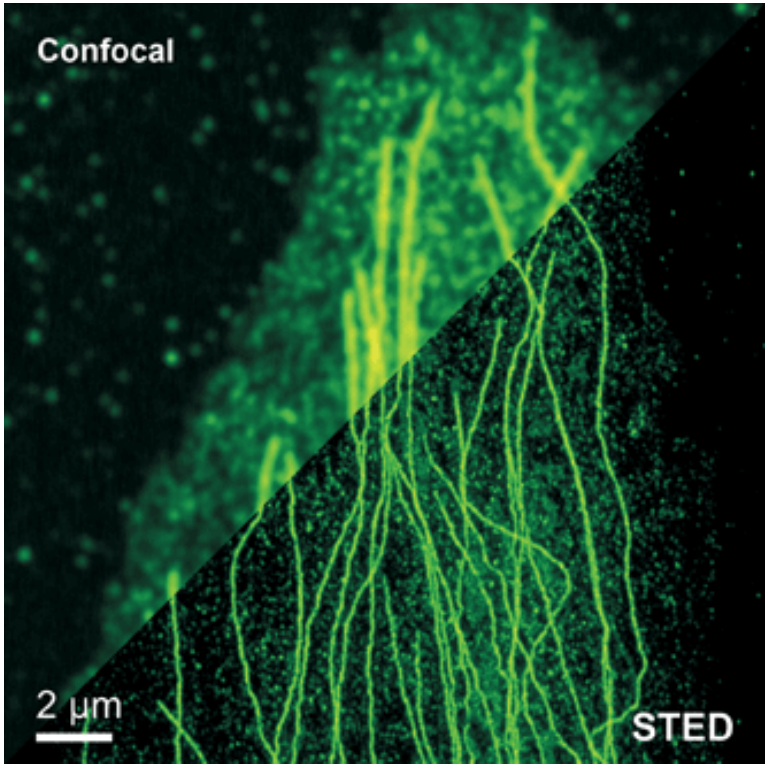
Garet G Nenninger *et al* 2002 *Meas. Sci. Technol.* **13** 2038

.M.Chinowsky et al.:Sensors and Actuators B54 (1999) 89 – 9

Super-resolution Microscopy



•10.1080/00107514.2015.1026557

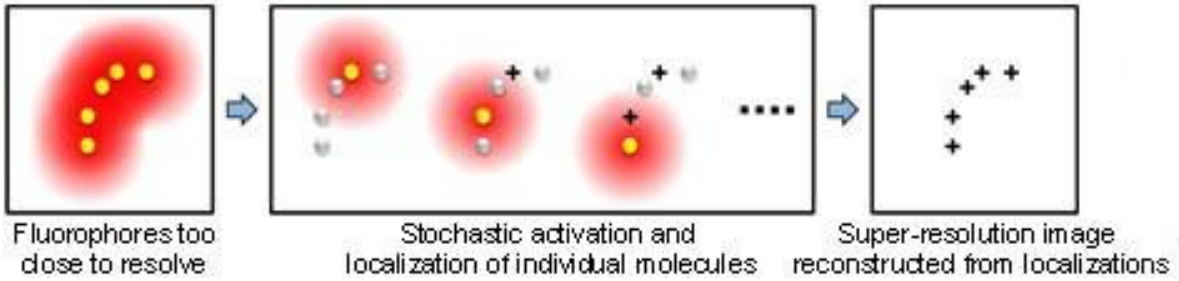


The images produced by STED microscopy show fine structures and features that are concealed in the confocal image. The example shows tubulin structures in a Vero cell labeled with Abberior STAR 635p.

https://www.photonics.com/Articles/STED_Microscopy_Made_Easy/a57011

Stochastic Optical Reconstruction Microscopy (STORM)

http://huanglab.ucsf.edu/STORM.html



- STORM (also named PALM) is a type of super-resolution optical microscopy technique based on stochastic switching of single-molecule fluorescence signal.
- Relies on fitting the point spread function (PSF) of diffraction limited spot corresponding to individual molecules.

Fluorescence Microscopy-based Localization

- Optics give advantage of the possibility of observation in compatible environment (unlike e.g. electron microscopy).
- Powerful toolbox available for localization of individual fluorophore emitters for imaging biological specimen, takes advantage of genetically encodable fluorescence proteins (Nobel prize 2008).
- Super resolution microscopy is rather slow, tracking of fast processes is difficult due to stochastic nature of fluorescence emission and bleaching / blinking
- Needed collection of $>10^4$ photons needed for nanometer localization by fitting the PSF, localization time > 1 sec.

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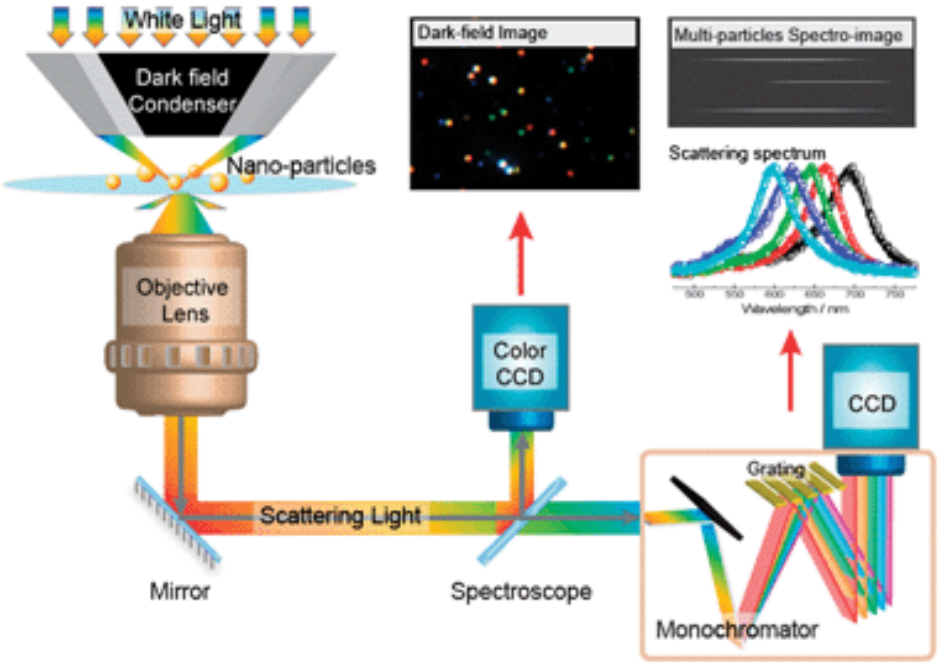
Optical Scattering-Based Localization

Direct Detection of Scattering



https://doi.org/10.1039/C1CS15143F

- Dark field microscopy enables rather easily observing individual plasmonic nanoparticles.

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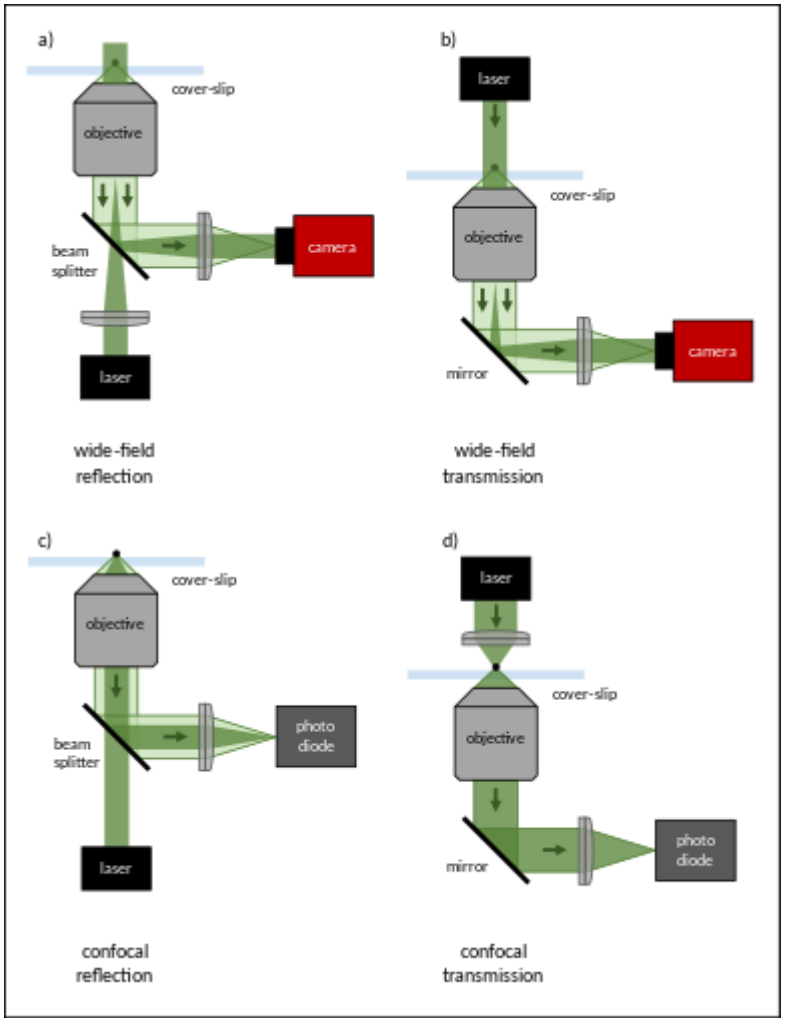
Scattering by Miniature Dielectric Particle



https://en.wikipedia.org/wiki/Interferometric_scattering_microscopy

Rayleigh scattering:

$$\sigma_{\mathrm{sca}} = \frac{8}{3}\pi^2 |\alpha|^2 (\lambda_m)^{-4}$$

Polarizibility of (dielectric) particle

$$\alpha = 3\epsilon_{\mathrm{m}} V \left(\frac{\epsilon_{\mathrm{p}} - \epsilon_{\mathrm{m}}}{\epsilon_{\mathrm{p}} + 2\epsilon_{\mathrm{m}}} \right)$$

ε_p Permittivity of particle

ε_m Permittivity of surrounding medium

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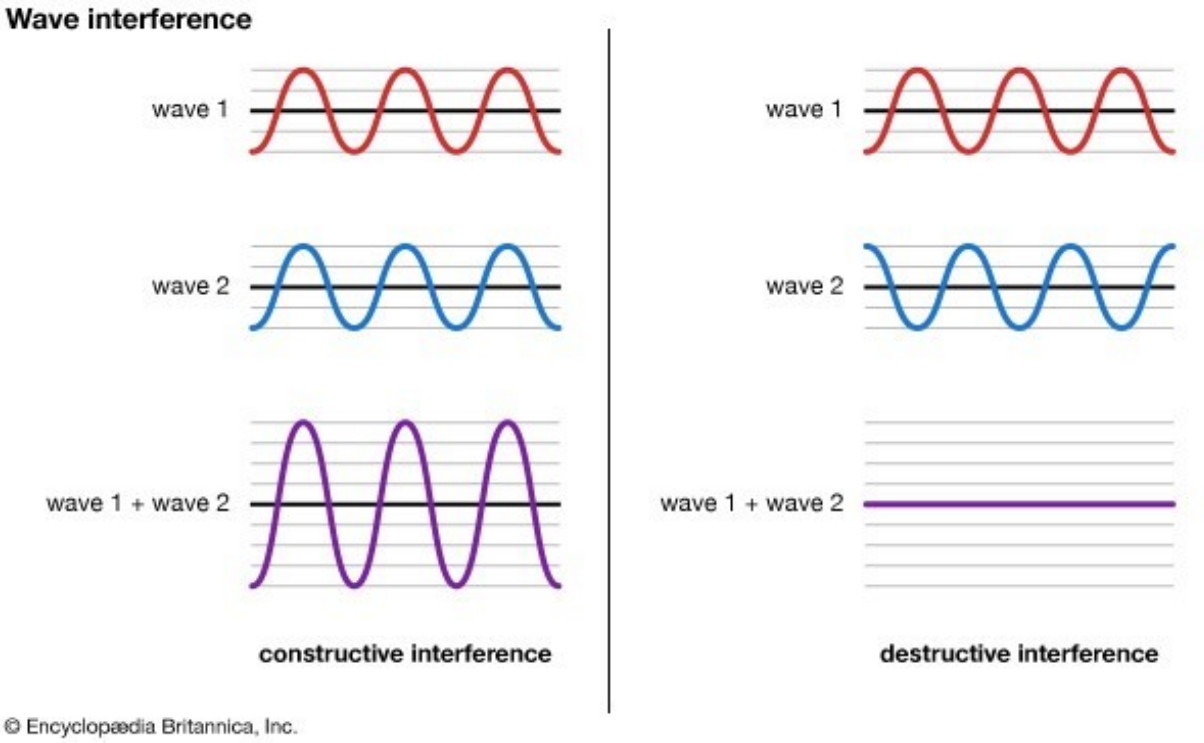
ISCAT – Interferometric Scattering Microscopy

10.1021/acs.nanolett.9b01822

Interferometric Scattering Microscopy



https://en.wikipedia.org/wiki/Interferometric_scattering_microscopy

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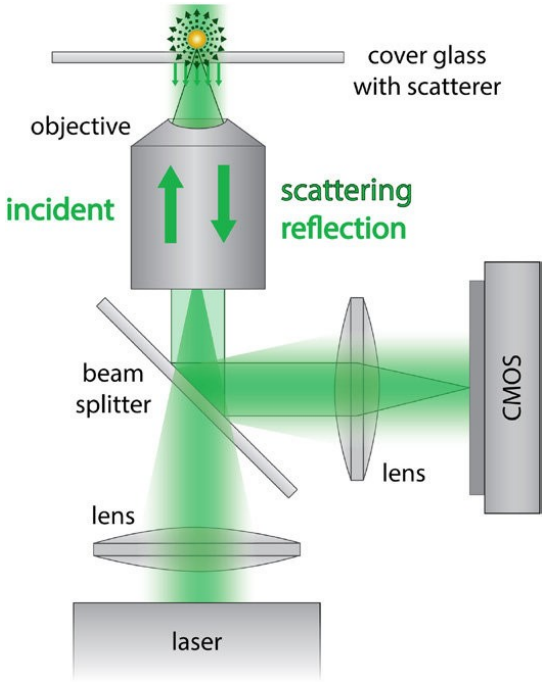
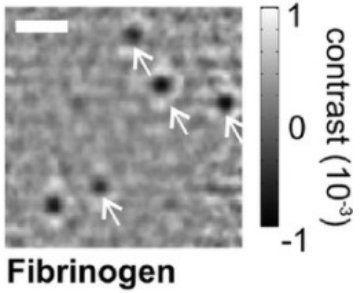
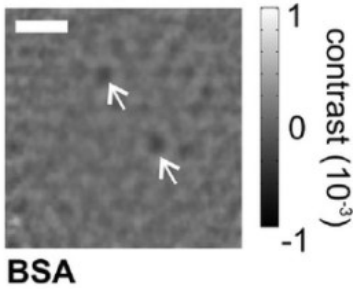
Interferometric Scattering Microscopy







$$s = \eta \varepsilon_m \pi \frac{D^3}{2} \frac{\varepsilon_p - \varepsilon_m}{\varepsilon_p + 2\varepsilon_m}$$

$$I_{det} = I_{inc}[r^2 + |s|^2 + 2r|s|\cos(\varphi)]$$

- For small objects, the interference term gives much higher optical signal
- Observed as a dark (diffraction limited) spot on bright background

•10.1088/0022-3727/49/27/274002

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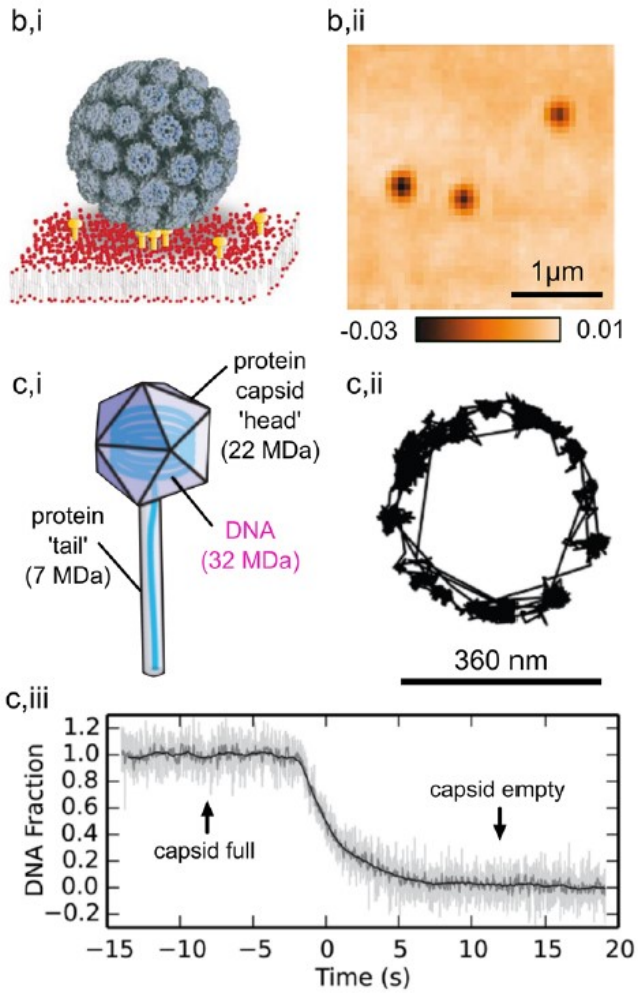
Scattering-based Observation of Virus Particles



- Direct label-free observation of time dependent rotation of attached virus.
- Release of DNA cargo from the virus.

Figure 2 Illustration of a SV40 virus bound to ganglioside (GM1)-tagged lipids in an artificial lipid bilayer (b,i). An iSCAT image of single SV40 virions attached to a lipid bilayer on a coverslip (b,ii), adapted from ref 22. Illustration of a bacteriophage showing head and tail geometry (c,i). A trajectory from the head of a single bacteriophage whereas its tail is adsorbed to the surface (c,ii). Following stimulation, the DNA content of the capsid head is ejected over time (c,iii), as determined through the diminishing iSCAT contrast. Adapted from ref 44.

Kukura, P.; Ewers, H.; Müller, C.; Renn, A.; Helenius, A.; Sandoghdar, V. Highspeed nanoscopic tracking of the position and orientation of a single virus. Nat. Methods 2009, 6, 923–927.

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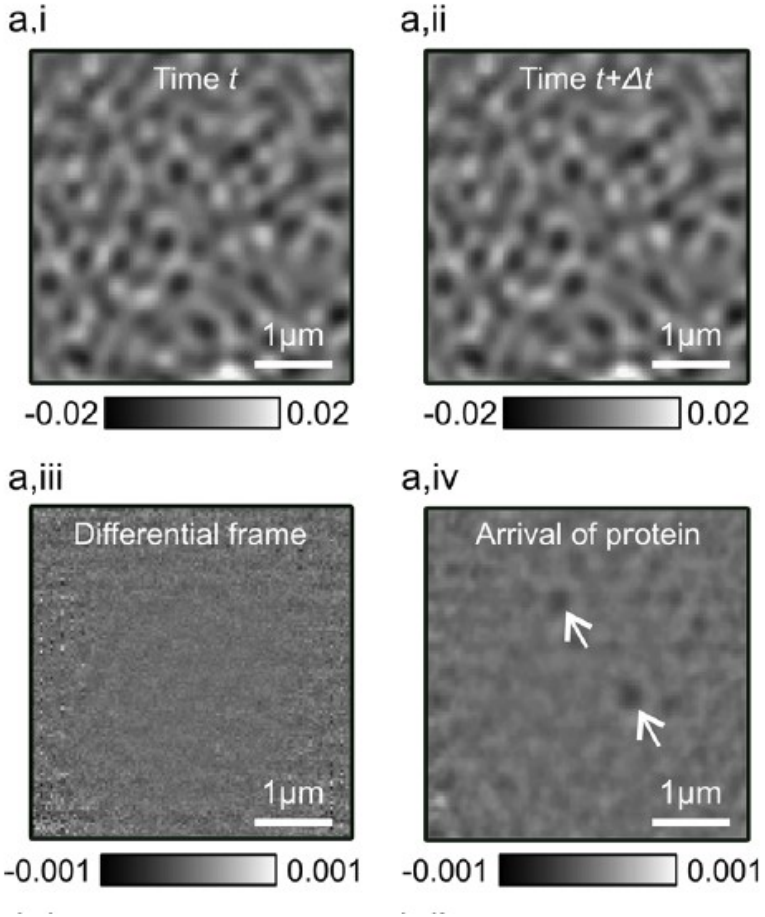
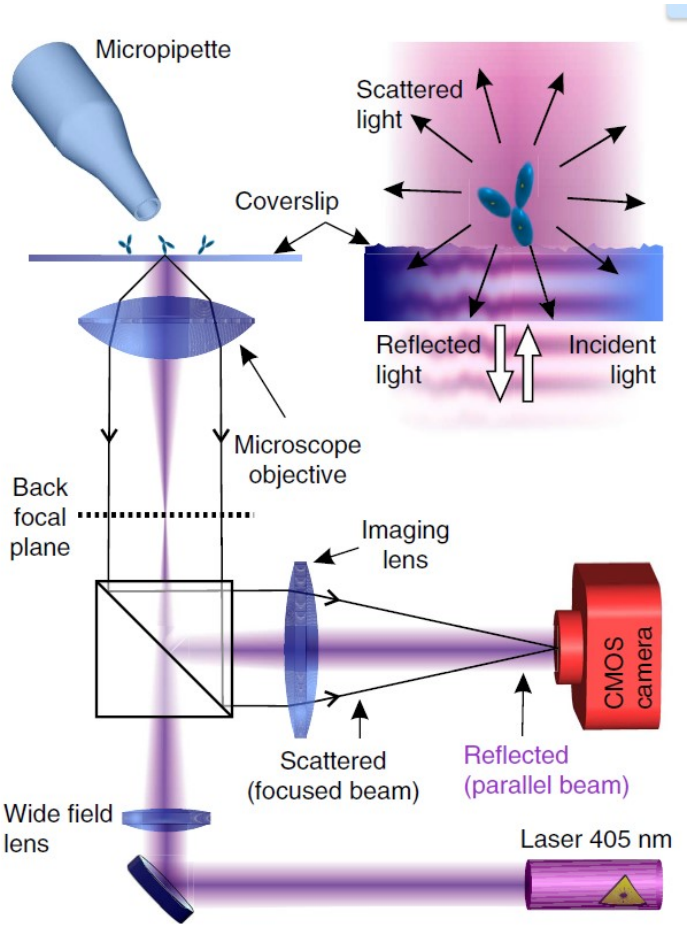
Scattering by Individual Protein Molecules





10.1038/ncomms5495

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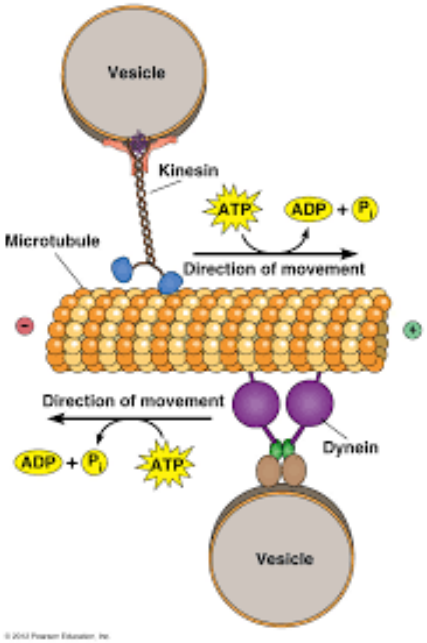
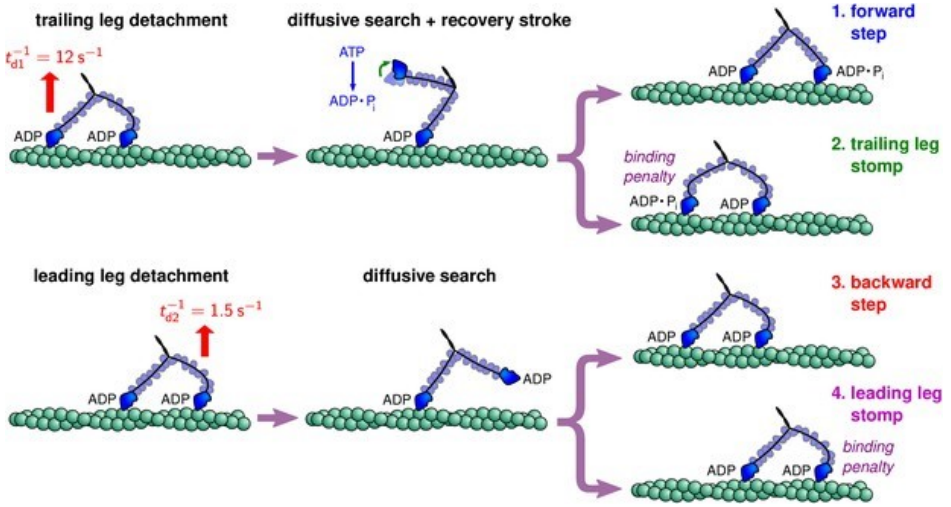
Single Molecule Tracking

Molecular Machines

- Myosin 5 walking along actine fibers.
- Kinesine movement along microtubuli.





https://www.mechanobio.info/cytoskeleton-dynamics/what-is-the-cytoskeleton/what-are-microtubules/

iSCAT with Gold Nanoparticle labels

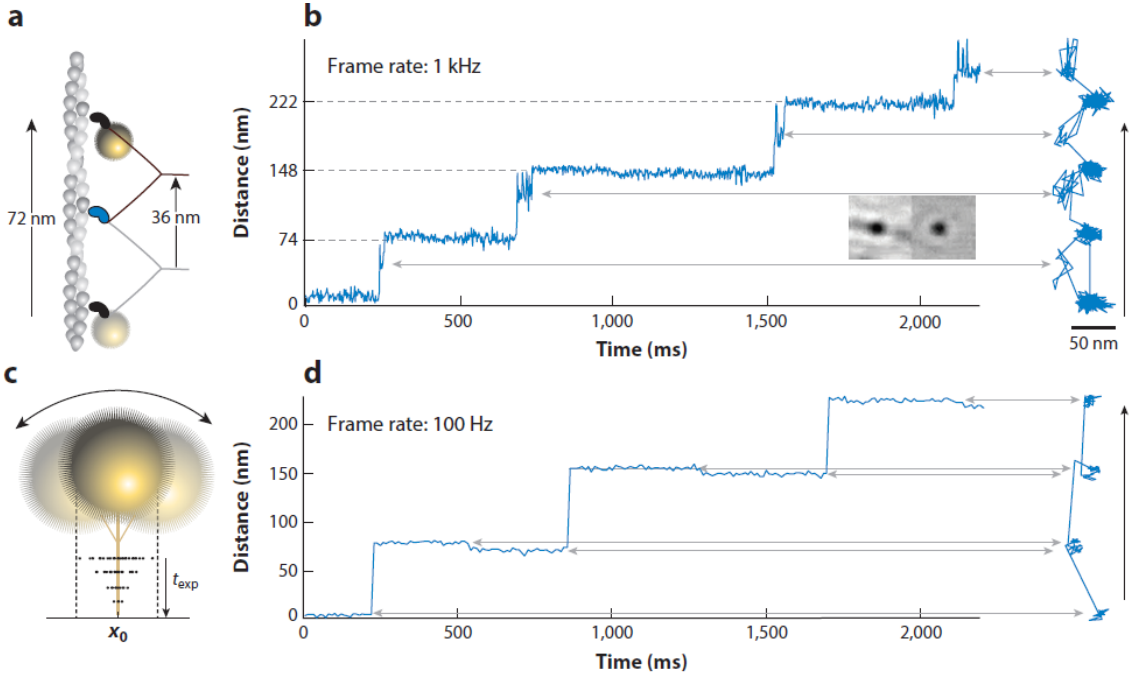


Figure 2

Tracking of molecular motors with gold nanoparticle labeling with high spatial precision and temporal resolution. (*a*) Schematic of a gold-labeled myosin 5 motor stepping along an actin filament. (*b*) Distance-time trace and accompanying XY trajectory of a myosin 5 molecule stepping along actin, imaged at 1 kHz. The transient state when the motor takes a step is localized to one side of the actin filament. (*Inset*) Image of a 20-nm gold nanoparticle label before and after background subtraction. (*c*) Schematic of the concept of temporal averaging to improve localization precision, through averaging the motion of the scattering label. (*d*) Distance-time trace and accompanying XY trajectory of a myosin 5 molecule stepping along actin, after frame averaging to 100 Hz. A small substep can be observed while the labeled head is bound to actin. Panels *a*, *b*, and *d* adapted from Reference 45.

https://doi.org/10.1146/annurev-physchem-050317-021247

- Actin filament immobilized, kinesin moving.

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iSCAT without Gold Nanoparticle labels

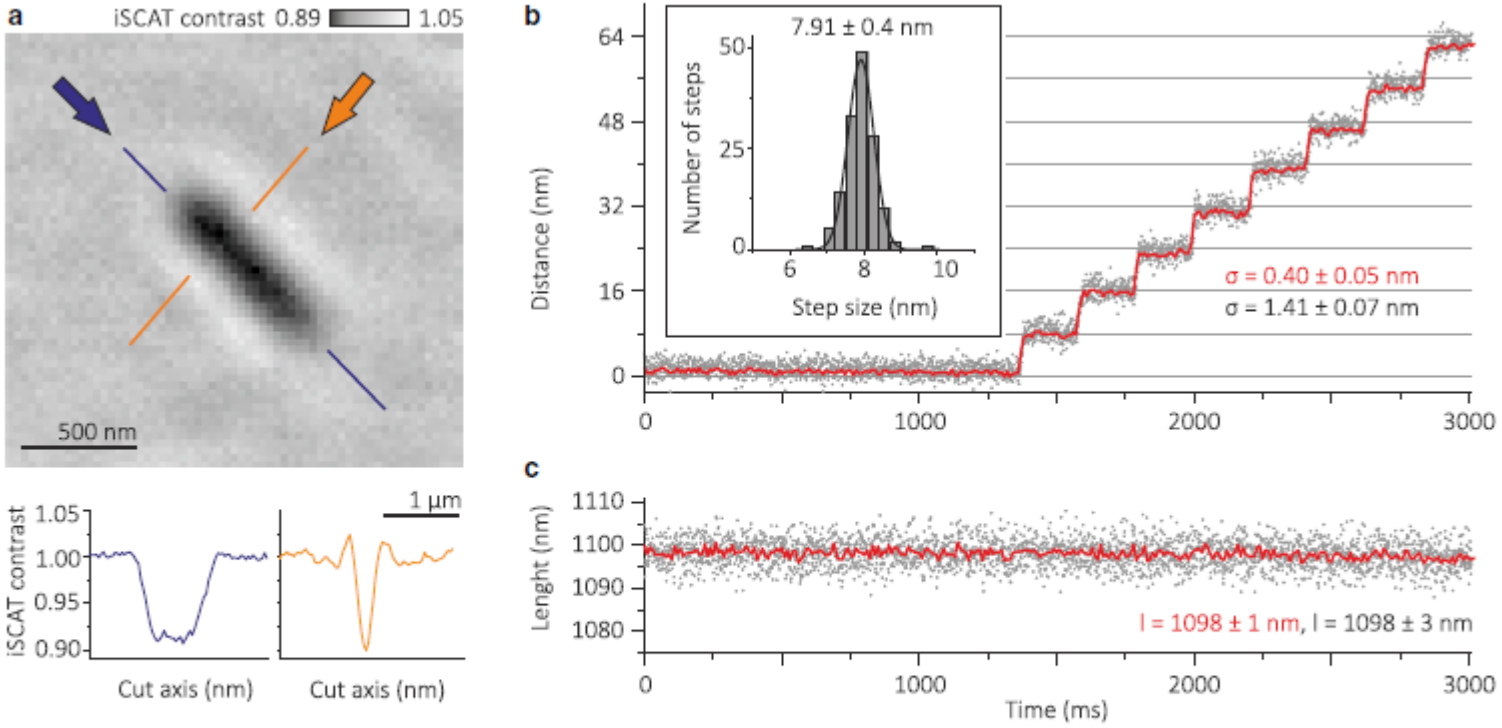

FIGURE 1 Label-free imaging and tracking of a single microtubule (MT) with interferometric scattering microscopy. (*a*) iSCAT image of a single MT, including cross sections along and across (*blue* and *orange arrows*, respectively) its long axis. (*b*) Artificial steps performed by moving an MT bound to a cover glass with a nanometric stage in 8 nm steps. The tracking precision was determined by calculating the positional fluctuation, σ, which varies with imaging speed, but is independent of MT orientation relative to the motion of the nanometric stage. (*c*) MT length as determined by fitting the iSCAT images to an elongated Gaussian as described in the text. Raw data were acquired at an imaging speed of 1000 frames/s (*gray dots*) and averaged to 100 frames/s (*red line*). To see this figure in color, go online.

- Kinesin immobilized, actin filament moving.

http://dx.doi.org/10.1016/j.bpj.2015.10.055

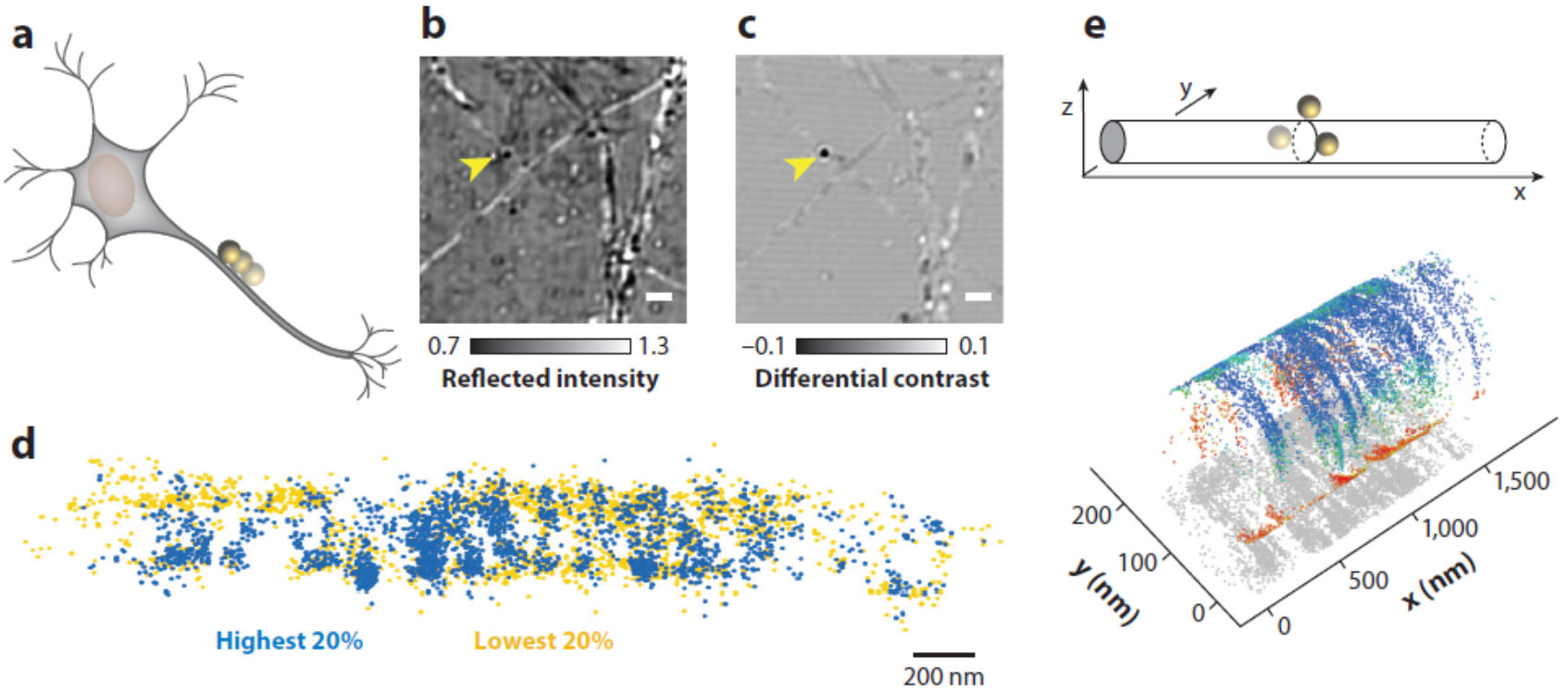

Figure 3

Interferometric scattering microscopy (iSCAT) tracking on cells. (*a*) Schematic of gold nanoparticle (GNP) tracking on an axon of a neuronal cell. (*b*) Raw iSCAT image of 40-nm GNP-labeled membrane protein on a neuronal cell. The yellow arrow marks the GNP. Scale bar: 2 μm. (*c*) Background-subtracted image of 40-nm GNP-labeled membrane protein on a neuronal cell; a yellow arrow marks the GNP. Scale bar: 2 μm. (*d*) Selected localizations from 2-kHz trajectory of 40-nm GNP-labeled glycosylphosphatidylinositol-green fluorescent protein on an axon. The 20% of all localizations exhibiting the highest confinement are shown in blue, whereas the 20% exhibiting the lowest confinement are shown in yellow. (*e*) Schematic of a three-dimensional reconstruction from the geometry of the neurite and example three-dimensional trajectory. Panels *b*–*e* adapted from Reference 54 with permission from Elsevier.

- Applicable also within whole cell.

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Single Molecule Assembly / Disassembly

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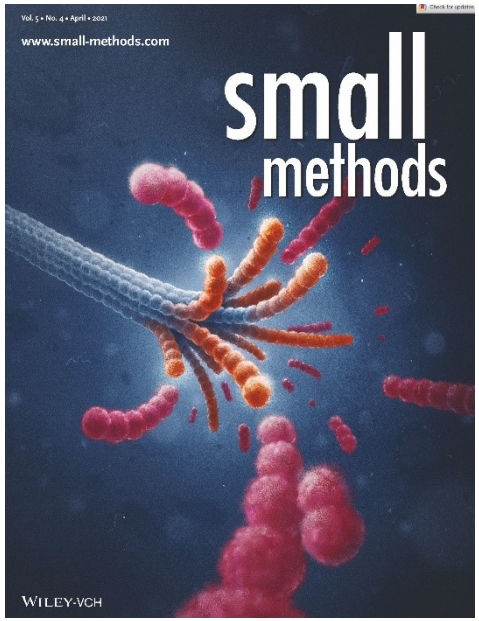
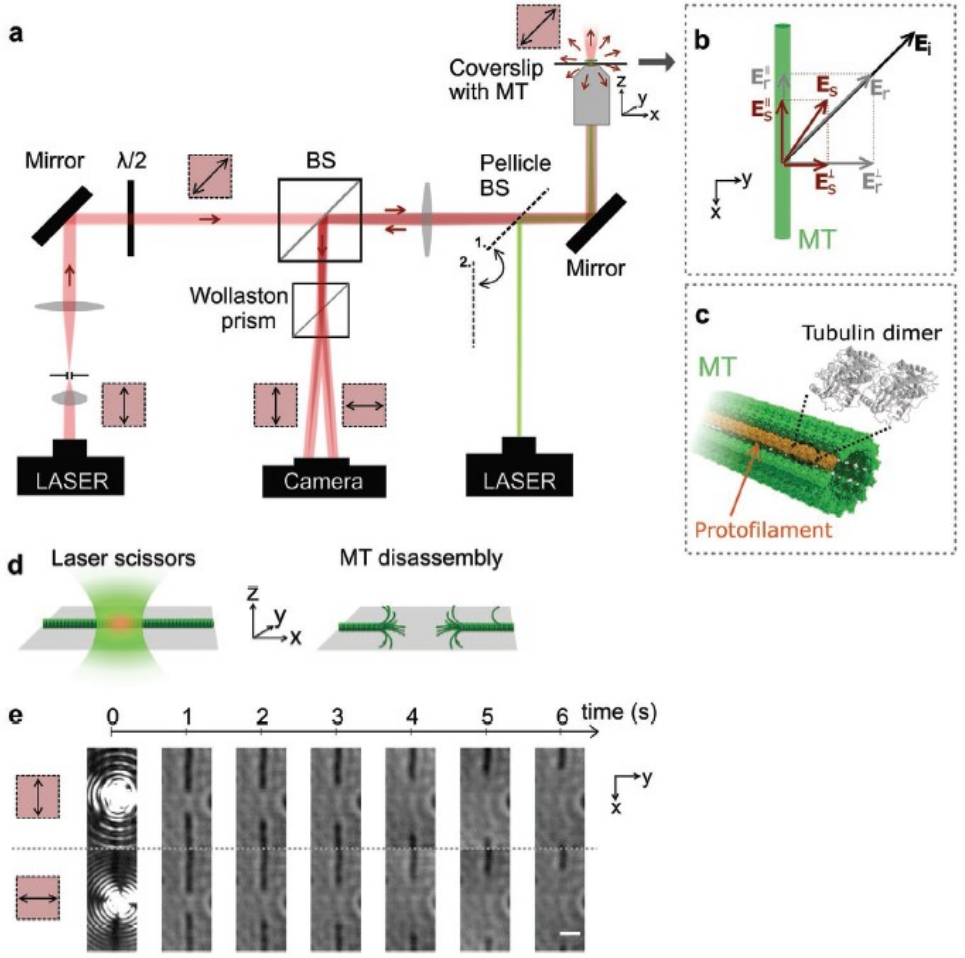
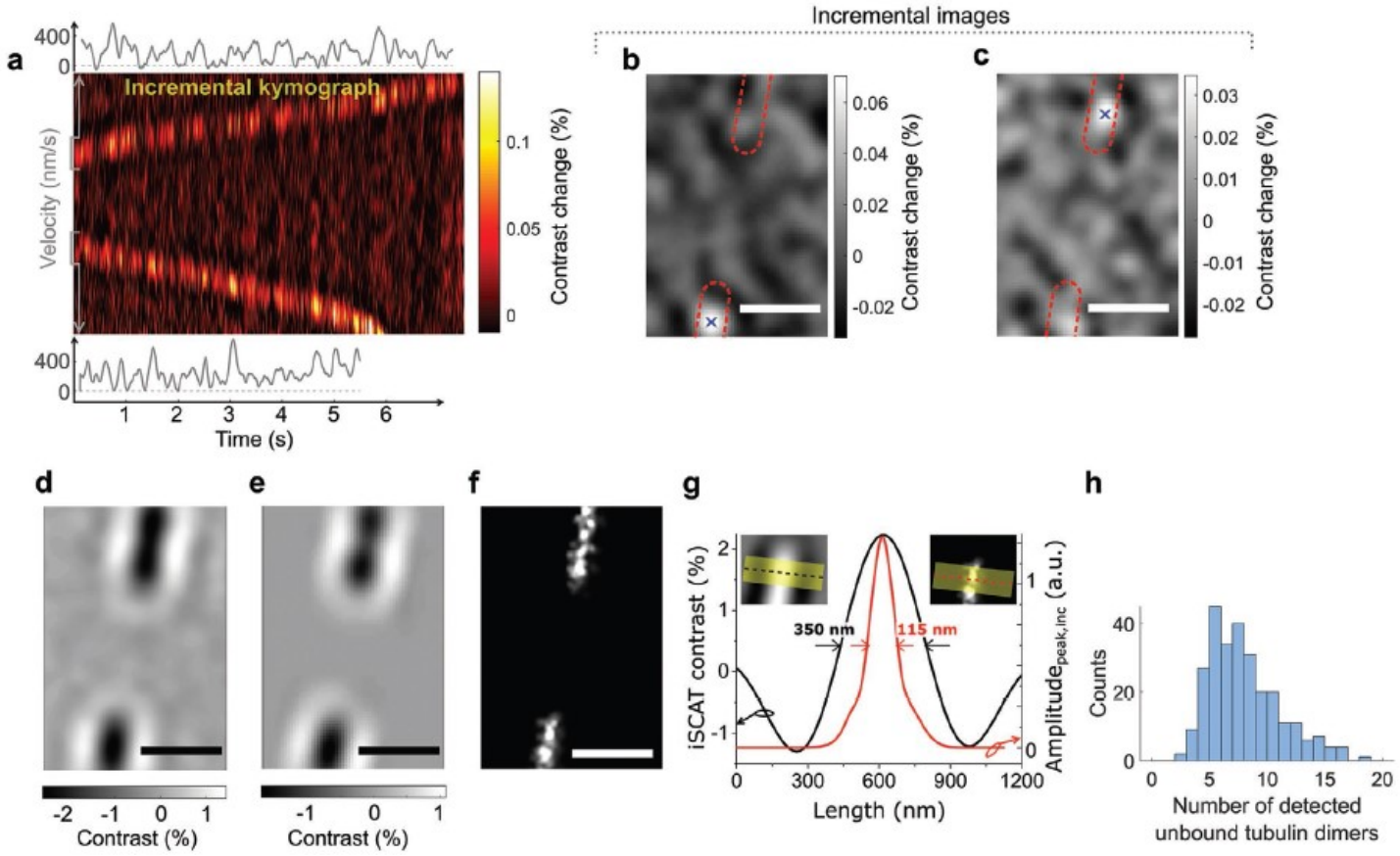
iSCAT with Polarization Interrogation





DOI: 10.1002/smtd.202000985

iSCAT with Polarization Interrogation



DOI: 10.1002/smtd.202000985

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Single Molecule Mass Photometry

iSCAT Contrast Evaluation

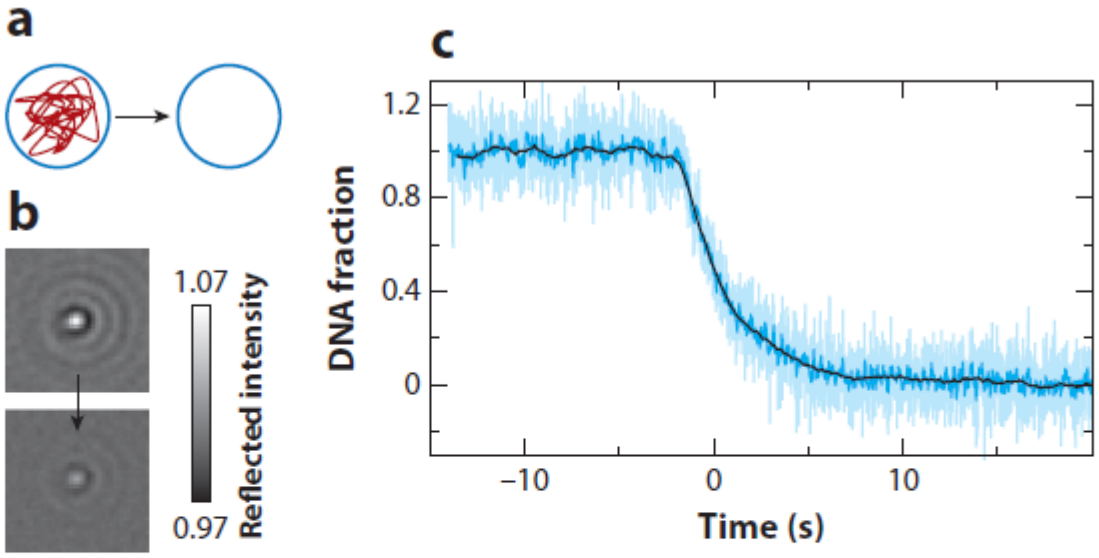

Figure 6

Viral capsid dynamics. (*a*) Schematic of the difference between a viral capsid with packaged DNA and after DNA ejection. (*b*) Interferometric scattering microscopy (iSCAT) images of full and empty bacteriophages. (*c*) Time trace of DNA content of an immobilized bacteriophage upon addition of LamB, as measured by iSCAT contrast: raw (100 Hz) trace (*light blue*), trace after applying a tenfold moving average (*dark blue*), and trace after a 100-fold moving average (*black*). Panels *b* and *c* adapted with permission from Reference 67; copyright 2016 American Chemical Society.

- Unloading of DNA from a virus manifested as a decrease in the contrast.

https://doi.org/10.1146/annurev-physchem-050317-021247

iSCAT Contrast Evaluation

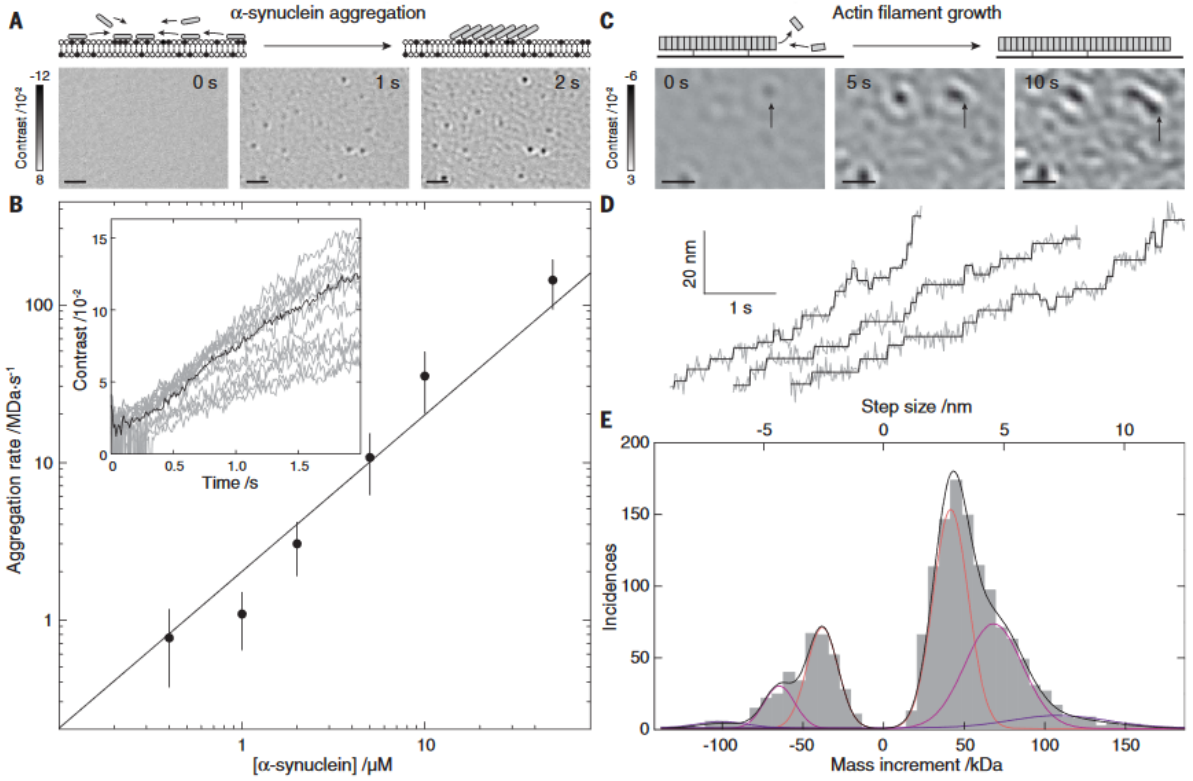

Fig. 4. Mass imaging of mesoscopic dynamics. (**A**) Schematic and iSCAMS images of α-synuclein (1 μM) aggregation on a negatively charged bilayer membrane. (**B**) Initial growth rate versus α-synuclein concentration, shown with the best fit assuming first-order kinetics. Error bars denote SEM for different particles. Inset, individual (gray) and average (black) growth trajectories for 21 particles from (A). (**C**) Schematic and iSCAMS images of actin polymerization. The arrow highlights a growing filament. (**D**) Representative traces of actin filament tip position (gray) and corresponding detected steps (black). (**E**) Step and mass histogram from 1523 steps and 33 filaments, including a fit to a Gaussian mixture model (black) and individual contributions (colored according to fig. S10G). Scale bars, 1 μm. In these experiments, background correction involved removal of the static background before acquisition, rather than continuous differential imaging as in Figs. 2 and 3 (supplementary materials).

- Contrast of detected features can be attributed to the increasing mass.

https://doi.org/10.1146/annurev-physchem-050317-021247